(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
24 February 2022 (24.02.2022)

WIPO | PCT

(10) International Publication Number
WO 2022/038539 A2

(51) International Patent Classification:
C07F 9/6561 (2006.01)

(21) International Application Number:
PCT/IB2021/057599

(22) International Filing Date:
18 August 2021 (18.08.2021)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:

276794	18 August 2020 (18.08.2020)	IL
63/085,218	30 September 2020 (30.09.2020)	US

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, IT, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:
— *without international search report and to be republished upon receipt of that report (Rule 48.2(g))*
— *with sequence listing part of description (Rule 5.2(a))*
— *in black and white; the international application as filed contained color or greyscale and is available for download from PATENTSCOPE*

(54) Title: ANTI-VIRAL AND ANTI-TUMORAL COMPOUNDS

(57) Abstract: Disclosed herein are 3, 4–didehydro- and 3′-deoxy-3, 4–didehydro-compounds and pharmaceutical compositions thereof. Methods of use of these pharmaceutical compositions include those for treating diseases including virus-induced diseases, cancer, autoimmune diseases, immune disorders, and bacterial-associated diseases or infections, or combinations thereof. Examples of viral-induced diseases include viral infections by RNA or DNA viruses, for example SAR-CoV-2, EBV, and BKV. P-595088-PC

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ANTI-VIRAL AND ANTI-TUMORAL COMPOUNDS

SEQUENCE LISTING

[001] The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on November 1, 2020, is named P-595088-PC-SQL-01NOV20.txt and is 1,589,425 bytes in size.

FIELD OF INTEREST

[002] Disclosed herein are 3, 4–didehydro- and 3´-deoxy-3, 4–didehydro- compounds, pharmaceutical compositions thereof, and uses thereof. Uses of the compounds and compositions disclosed include as anti-viral therapeutic agents against RNAnd DNA viruses.

BACKGROUND

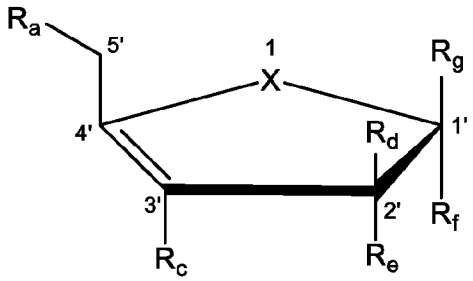
[003] It has been shown that the enzyme viperin from *Rattus norvegicus* (rVip) produces 3´-deoxy-3, 4–didehydro-CTP (ddhCTP) from CTP. ddhCTP acts as a chain terminator for certain viral RNA-dependent RNA polymerases, leading to inhibition of viral replication. In human cells, viperin's activity confers broad antiviral effects. To date, two additional eukaryotic viperins have been characterized, the human and fungal homologues. Similar to the rVip, both human and fungal viperins produce ddhCTP, while the fungal viperin can also produce 3´-deoxy-3, 4–didehydro-UTP (ddhUTP) from UTP. Currently, it is known that eukaryotic viperins can only produce a limited set of nucleotide analogues.

[004] Prokaryotic viperins (pVips) are newly discovered viperin homologs that have been demonstrated to take part in defense against phages. A total of 381 different pVips were computationally found and grouped in 7 different phylogenetic clades (International patent application No. PCT/IL2020/050377, filed March 29, 2020). From this set of pVips, 27 homologues were experimentally proven to protect bacteria from phage infection. LC-MS analysis of lysates from *E. coli* cultures overexpressing this set of 27 pVips demonstrated that many of them produce derivatives of one or multiple ddh-ribonucleotides, such as ddhCTP and ddhUTP (like the eukaryotic viperins) but also ddhGTP which was not previously reported as a product of eukaryotic viperins. These results revealed unprecedented insights into the substrate promiscuity of pVips, showing that pVips, in contrast to eukaryotic viperins, can accept multiple substrates.

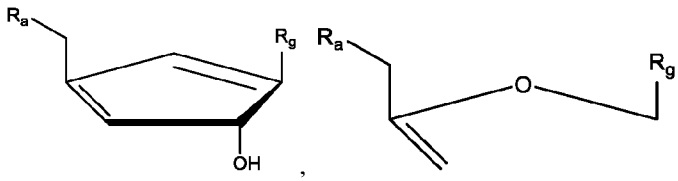
[005] Nucleotide/nucleoside analogs are crucial components of our medicinal chemistry arsenal, with more than 30 approved molecules in the market and many more currently in development. They are currently employed to treat a wide array of pathologies, including viral and microbial infections,

as well as to inhibit the proliferation of cancer cells. Moreover, it is known that minor changes in their chemical structure have profound effects on their activity against specific targets, as well as on potential undesired side-effects.

[006] Thus, substituted compounds (for example as presented herein) may mimic the overall structure of nucleotide/nucleoside analogs and may include: (1) heterocyclic nitrogen based ring or O-aryl or aryl and isomers therof attached to position 1' of the 5 membered ring (to the ddh or deoxy-ddh ribose sugar analog); and/or (2) different substitutions on the 5 member ribose sugar (position 2', 3', 4' and/or 5'); and/or (3) substitution of the O of the 5-member ring with N or CH_2 or CH or CCH_2; and/or (4) an open etheric ring instead of the 5 member ring, may comprise unique novel activities. Accordingly, these substituted compounds (substituted ddh or deoxy-ddh compounds) may add unique therapeutic compounds to the medicinal chemistry arsenal.



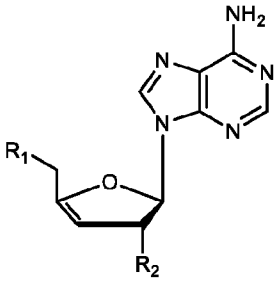
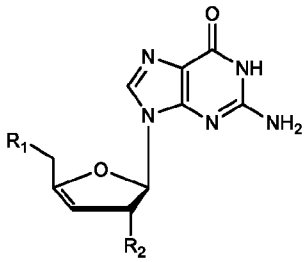
X is O, NH, CH_2, CH, CCH_2 ,

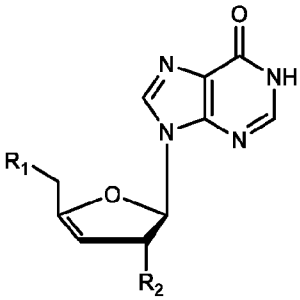
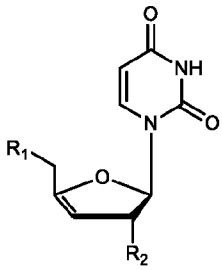


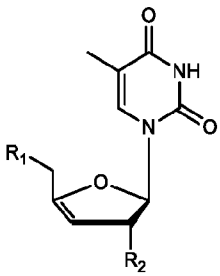
[007] There remains a need for targeted active compounds for treating diseases in a subject including viral and microbial infections, as well as to inhibit the proliferation of cancer cells Thus, there is a need for creating novel structural modifications of existing compound to generate new therapeutic variants with selective antiviral, antimicrobial, or anti-cancer activities, or a combination of activities thereof. The present disclosure describes novel ddh and deoxy-ddh variant compounds., for therapeutic use as antiviral, anti-tumoral, and/or antibacterial agents. Further, the present disclosure describes the utilization of pVips enzymes as a versatile platform for the synthesis of ddh and deoxy-ddh variants with novel antiviral, anti-tumoral, and/or antibacterial activities.

SUMMARY OF THE DISCLOSURE

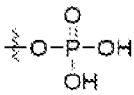
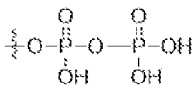
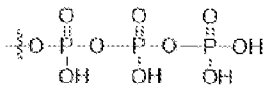
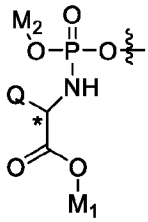
[008] In one aspect, provided herein is a compound represented by the structure of

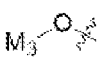
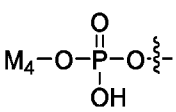
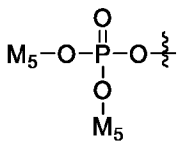
Formula IB:  , Formula IIB:  ,

Formula IIIB:  , Formula IVB:  , or

Formula VB:  ,

wherein

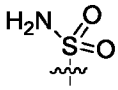
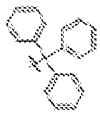
R_1 is OH,  ,  ,  ,  ,

 ,  ,  , or ;

Q is a side chain of an amino acid;

M_1 = an alkyl;

M_2 is an aryl, a substituted aryl, a heteroaryl or a substituted heteroaryl;

M_3 is  , ,  , or ;

M_4 is -(C_2-C_6)alkyl-O-(C_{10}-C_{20})alkyl;

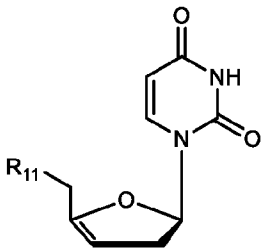
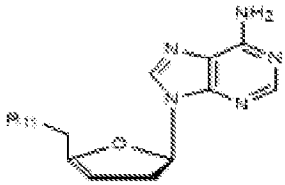
each M_5 is -$(CH_2)_n$-S-C(=O)-(C_1-C_8)alkyl;

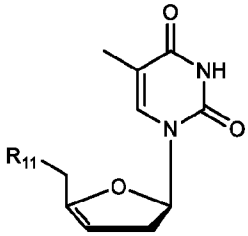
n is 1-4;

R_2 is -OH or -O-COO-alkyl; and

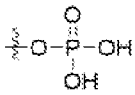
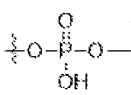
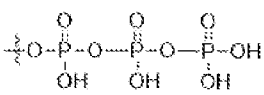
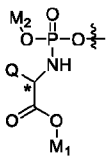
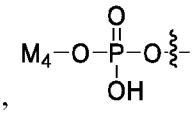
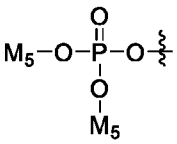
wherein if R_1 is OH, then R_2 is -O-COO-alkyl.

[009] In one aspect, provided herein is a compound represented by the structure of Formula VIB:

 , Formula VIIB:  , or

Formula VIIIB:  ,

wherein

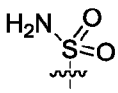
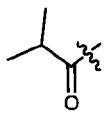
R_{11} is  ,  ,  ,  , ,  , 

or ;

Q is a side chain of an amino acid;

M_1 is an alkyl;

M_2 is an aryl, a substituted aryl, a heteroaryl or a substituted heteroaryl;

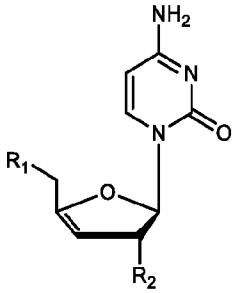
M_3 is  , , , or  ;

M_4 is -(C_2-C_6)alkyl-O-(C_{10}-C_{20})alkyl;

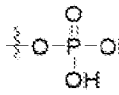
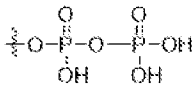
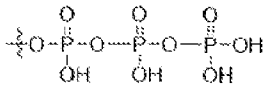
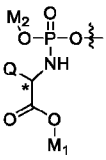
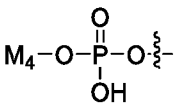
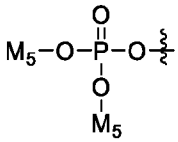
each M_5 is -$(CH_2)_n$-S-C(=O)-(C_1-C_8)alkyl; and

n is 1-4.

[0010] In one aspect, provided herein is a pharmaceutical composition comprising a compound represented by the structure of

Formula IXB:  ,

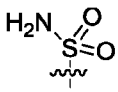
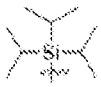
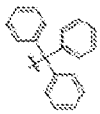
wherein

R_1 is OH,  ,  ,  ,  ,, ,  , or  ;

Q is a side chain of an amino acid;

M_1 is an alkyl;

M_2 is an aryl, a substituted aryl, a heteroaryl or a substituted heteroaryl;

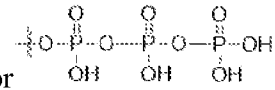
M_3 is  ,  ,  , or ;

M_4 is -(C_2-C_6)alkyl-O-(C_{10}-C_{20})alkyl;

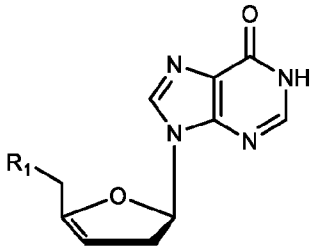
each M_5 is -$(CH_2)_n$-S-C(=O)-(C_1-C_8)alkyl;

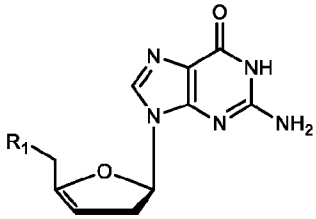
n is 1-4;

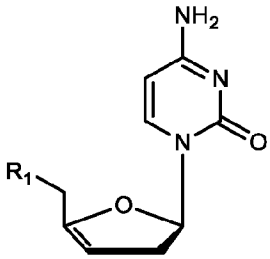
R_2 is -OH or -O-COO-alkyl; and

wherein if R_1 is OH or  , then R_2 is not OH.

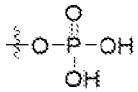
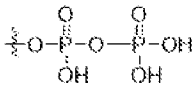
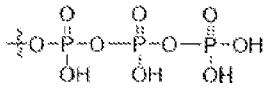
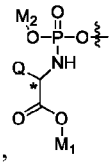
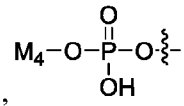
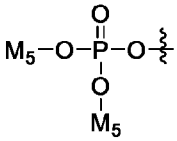
[0011] In one aspect, provided herein is a compound represented by the structure of Formula XB:



, Formula XIB:  , or

Formula XIIB:  ,

wherein

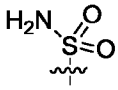
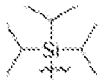
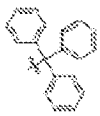
R_1 is OH,  ,  ,  ,  , ,  , 

or ;

Q is a side chain of an amino acid;

M_1 is an alkyl;

M_2 is an aryl, a substituted aryl, a heteroaryl or a substituted heteroaryl;

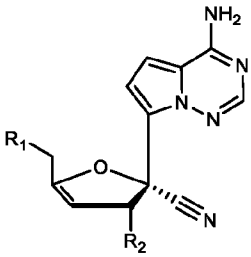
M_3 is  ,  ,  , or ;

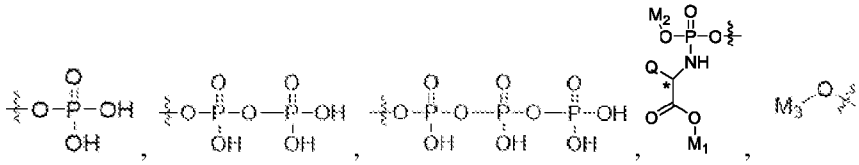
M_4 is -(C_2-C_6)alkyl-O-(C_{10}-C_{20})alkyl;

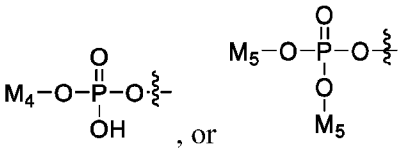
each M_5 is -$(CH_2)_n$-S-C(=O)-(C_1-C_8)alkyl; and

n is 1-4.

[0012] In one aspect, provided herein is a compound represented by the structure of formula XIIIB:

 , wherein

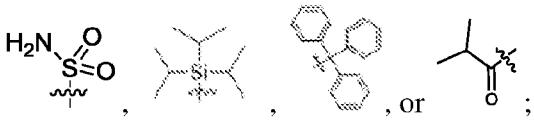
R_1 is OH, 



Q is a side chain of an amino acid;

M_1 is an alkyl;

M_2 is an aryl, a substituted aryl, a heteroaryl or a substituted heteroaryl;

M_3 is 

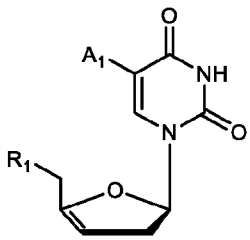
M_4 is -(C_2-C_6)alkyl-O-(C_{10}-C_{20})alkyl;

each M_5 is -$(CH_2)_n$-S-C(=O)-(C_1-C_8)alkyl;

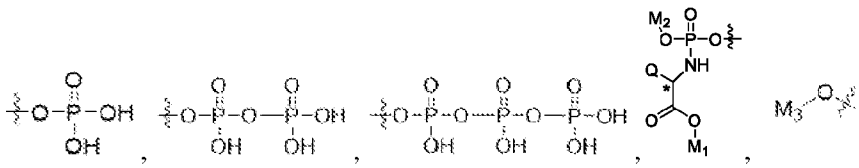
n is 1-4; and

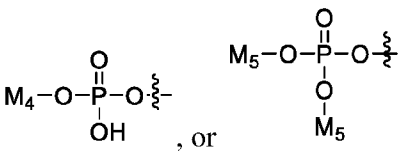
R_2 is -OH or –O-COO-alkyl.

[0013] In one aspect, provided herein is a compound represented by the structure of Formula XIVB:



, wherein

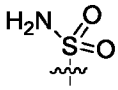
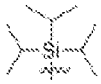
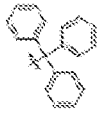
R_1 is OH, 



Q is a side chain of an amino acid;

M_1 is an alkyl;

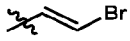
M_2 is an aryl, a substituted aryl, a heteroaryl or a substituted heteroaryl;

M_3 is  ,  ,  , or ;

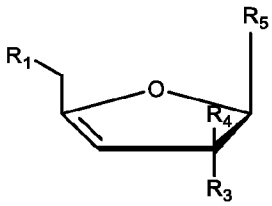
M_4 is -(C_2-C_6)alkyl-O-(C_{10}-C_{20})alkyl;

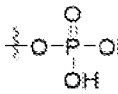
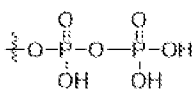
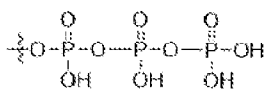
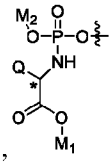
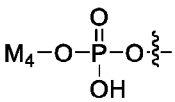
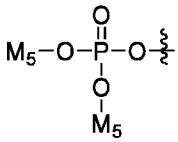
each M_5 is -$(CH_2)_n$-S-C(=O)-(C_1-C_8)alkyl;

n is 1-4; and

A_1 is a halo, a haloalkyl, an alkyl, or  .

[0014] In one aspect, provided herein is a compound represented by the structure of

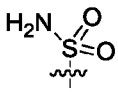
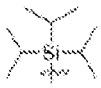
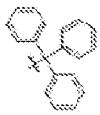
Formula XVB:  , wherein

R_1 is OH,  ,  ,  ,  , ,  ,  , or ;

Q is a side chain of an amino acid;

M_1 is an alkyl;

M_2 is an aryl, a substituted aryl, a heteroaryl or a substituted heteroaryl;

M_3 is  ,  ,  , or ;

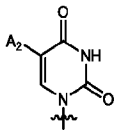
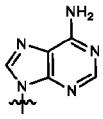
M_4 is -(C_2-C_6)alkyl-O-(C_{10}-C_{20})alkyl;

each M_5 is -$(CH_2)_n$-S-C(=O)-(C_1-C_8)alkyl;

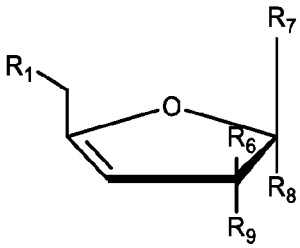
n is 1-4;

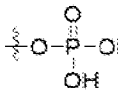
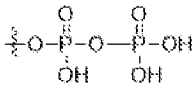
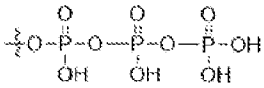
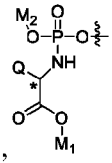
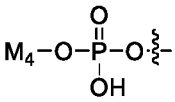
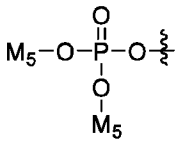
R_3 is H, a halo or an alkoxy;

R_4 is H, a halo or an alkyl;

R_5 is  , or  , wherein A_2 is H, a halo or an alkyl; and wherein R_3 is not the same as R_4.

[0015] In one aspect, provided herein is a compound represented by the structure of

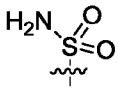
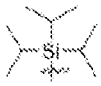
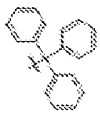
Formula XVIB:  , wherein

R_1 is OH,  ,  ,  ,  , ,  ,  , or ;

Q is a side chain of an amino acid;

M_1 is an alkyl;

M_2 is an aryl, a substituted aryl, a heteroaryl or a substituted heteroaryl;

M_3 is  ,  ,  , or ;

M_4 is -(C_2-C_6)alkyl-O-(C_{10}-C_{20})alkyl;

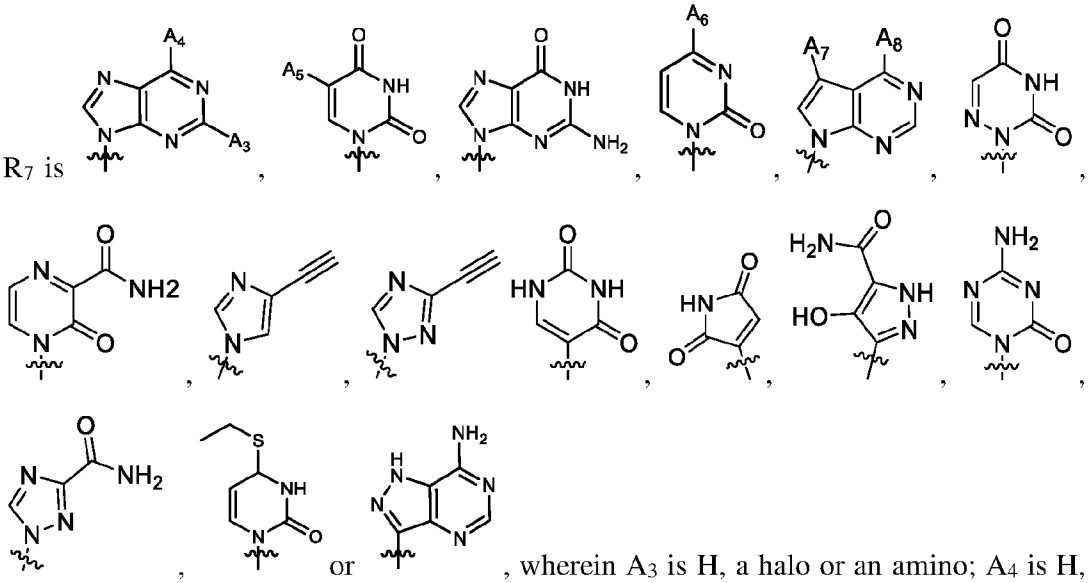
each M_5 is -$(CH_2)_n$-S-C(=O)-(C_1-C_8)alkyl;

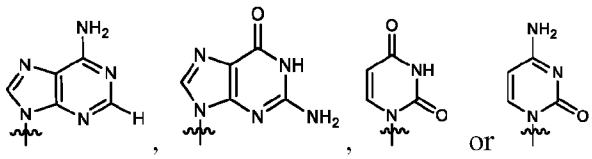
n is 1-4;

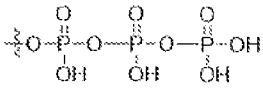
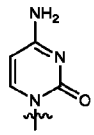
R_9 is H, OH, or -O-COO-alkyl;

R_6 is H, Me, -CCH or OH;

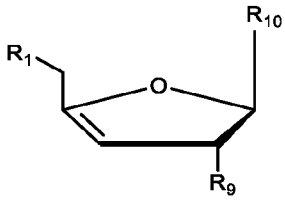
R_8 is H;

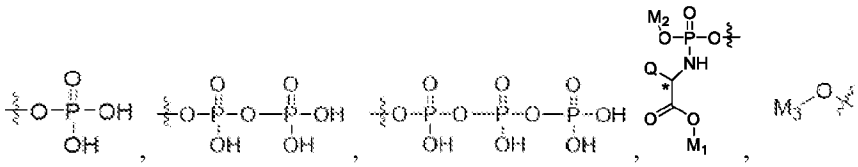
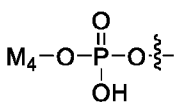
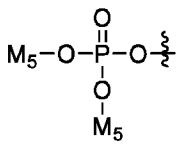
R_7 is  , wherein A_3 is H, a halo or an amino; A_4 is H, an amino, an alkoxy, or an alkyl; A_5 is H, a halo, a hydroxy or an alkyne; A_6 is H, an amino or a hydroxylamino; A_7 is H or an amido; A_8 is an amino or an alkyl;

wherein if R_1 is OH and R_7 is  , and R_9 is OH then R_6 is not H; and

wherein if R_1 is  and R_7 is  and R_9 is OH then R_6 is not H.

[0016] In one aspect, provided herein is a compound represented by the structure of Formula XVIIB,

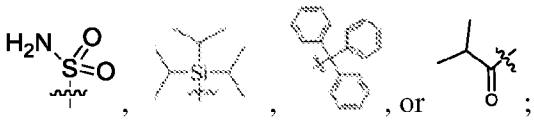
 , wherein

R_1 is OH,  ,  , or  ;

Q is a side chain of an amino acid;

M_1 is an alkyl;

M_2 is an aryl, a substituted aryl, a heteroaryl or a substituted heteroaryl;

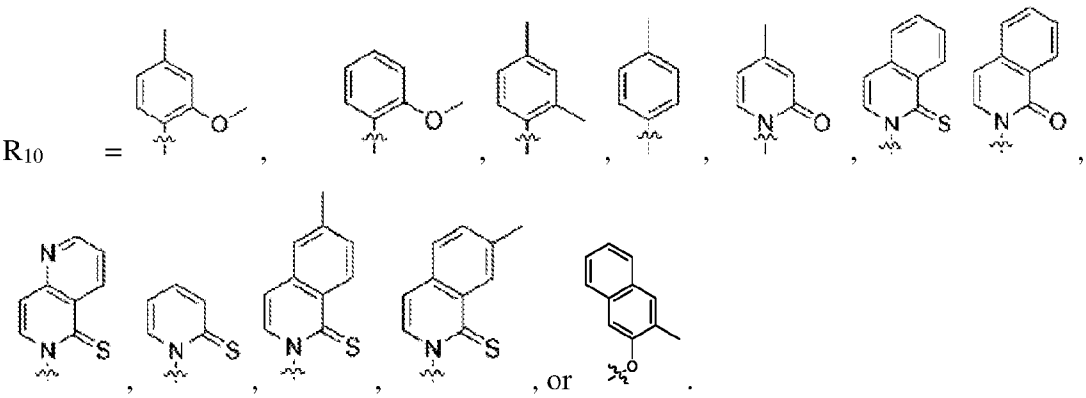
M_3 is  ;

M_4 is -(C_2-C_6)alkyl-O-(C_{10}-C_{20})alkyl;

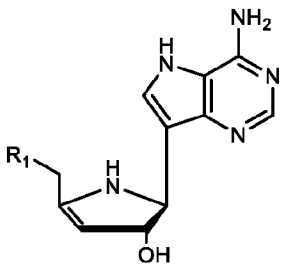
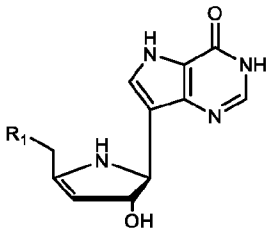
each M_5 is -$(CH_2)_n$-S-C(=O)-(C_1-C_8)alkyl;

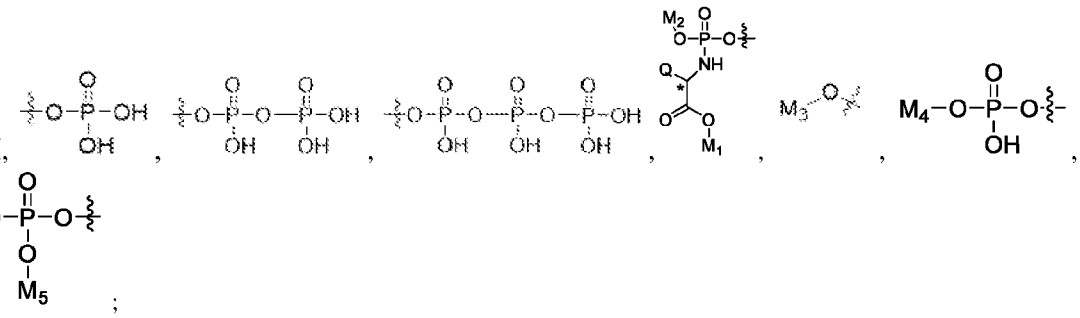
n is 1-4;

R_9 is H, OH or -O-COO-alkyl; and

R_{10} =  .

[0017] In one aspect, provided herein is a compound represented by the structure of Formula XVIIIB:

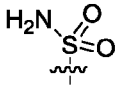
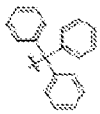
 , or Formula XXB:  , wherein

R_1 is OH,  ;

Q is a side chain of an amino acid;

M_1 is an alkyl;

M_2 is an aryl, a substituted aryl, a heteroaryl or a substituted heteroaryl;

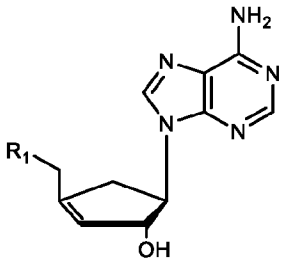
M_3 is  , ,  , or ;

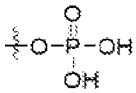
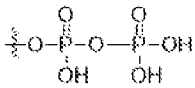
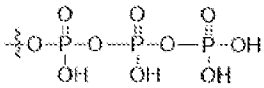
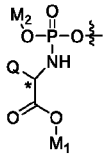
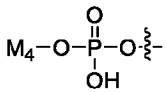
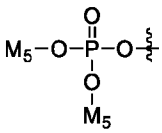
M_4 is -(C_2-C_6)alkyl-O-(C_{10}-C_{20})alkyl;

each M_5 is -$(CH_2)_n$-S-C(=O)-(C_1-C_8)alkyl; and

n is 1-4.

[0018] In one aspect, provided herein is a compound represented by the structure of Formula XIXB:

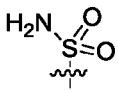
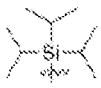
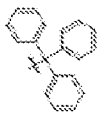
 , wherein

R_1 is OH,  ,  ,  ,  , ,  , or  ;

Q is a side chain of an amino acid;

M_1 is an alkyl;

M_2 is an aryl, a substituted aryl, a heteroaryl or a substituted heteroaryl;

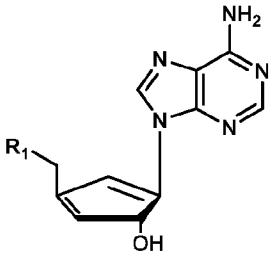
M_3 is  ,  ,  , or ;

M_4 is -(C_2-C_6)alkyl-O-(C_{10}-C_{20})alkyl;

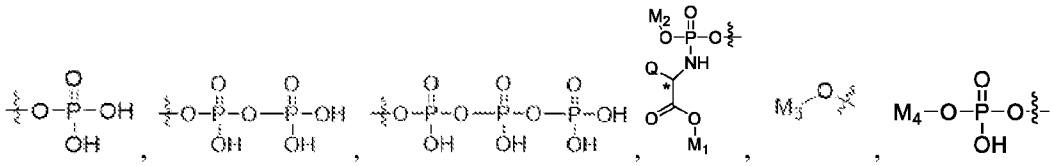
each M_5 is -$(CH_2)_n$-S-C(=O)-(C_1-C_8)alkyl; and

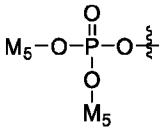
n is 1-4.

[0019] In one aspect, provided herein is a compound represented by the structure of Formula XXIB:



, wherein

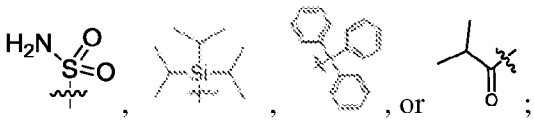
R_1 is OH, 

or  ;

Q is a side chain of an amino acid;

M_1 is an alkyl;

M_2 = an aryl, a substituted aryl, a heteroaryl or a substituted heteroaryl;

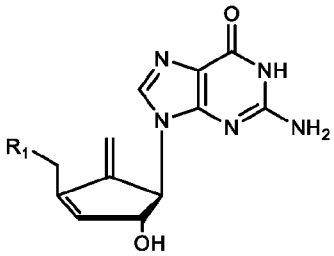
M_3 is  ;

M_4 is -(C_2-C_6)alkyl-O-(C_{10}-C_{20})alkyl;

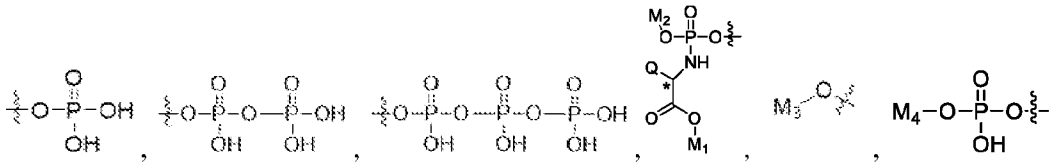
each M_5 is -$(CH_2)_n$-S-C(=O)-(C_1-C_8)alkyl; and

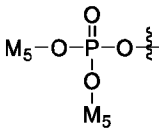
n is 1-4.

[0020] In one aspect, provided herein is a compound represented by the structure of Formula XXIIB:



, wherein

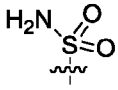
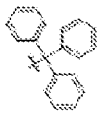
R_1 is OH, 

or  ;

Q is a side chain of an amino acid;

M_1 is an alkyl;

M_2 is an aryl, a substituted aryl, a heteroaryl or a substituted heteroaryl;

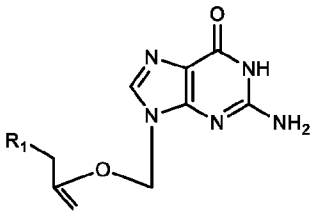
M_3 is  , ,  , or ;

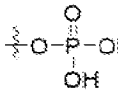
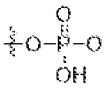
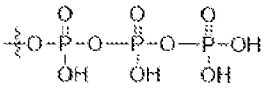
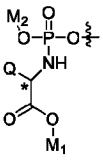
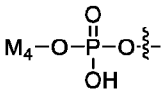
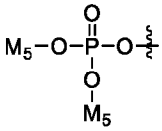
M_4 is -(C_2-C_6)alkyl-O-(C_{10}-C_{20})alkyl;

each M_5 is -$(CH_2)_n$-S-C(=O)-(C_1-C_8)alkyl; and

n is 1-4.

[0021] In one aspect, provided herein is a compound represented by the structure of Formula XXIIIB:

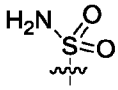
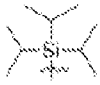
 , wherein

R_1 is OH,  ,  ,  ,  , ,  , or  ;

Q is a side chain of an amino acid;

M_1 is an alkyl;

M_2 is an aryl, a substituted aryl, a heteroaryl or a substituted heteroaryl;

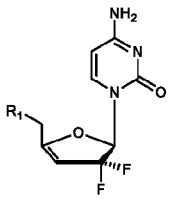
M_3 is  ,  , , or ;

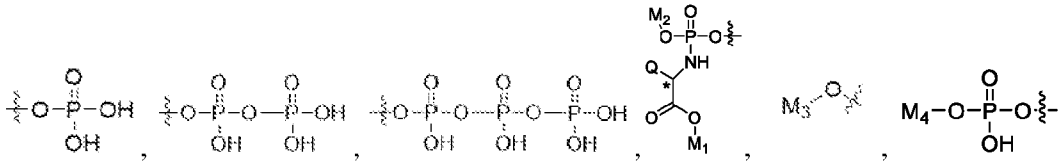
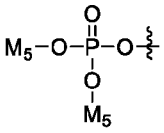
M_4 is -(C_2-C_6)alkyl-O-(C_{10}-C_{20})alkyl;

each M_5 is -$(CH_2)_n$-S-C(=O)-(C_1-C_8)alkyl; and

n is 1-4.

[0022] In one aspect, provided herein is a compound represented by the structure of Formula XXIVB:

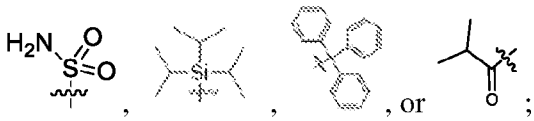
 , wherein

R_1 is OH, 
, or 
;

Q is a side chain of an amino acid;

M_1 is an alkyl;

M_2 is an aryl, a substituted aryl, a heteroaryl or a substituted heteroaryl;

M_3 is 
, or ;

M_4 is -(C_2-C_6)alkyl-O-(C_{10}-C_{20})alkyl;

each M_5 is -$(CH_2)_n$-S-C(=O)-(C_1-C_8)alkyl; and

n is 1-4.

[0023] In one aspect, provided herein is a pharmaceutical composition comprising a compound disclosed herein.

[0024] In one aspect, provided herein is a pharmaceutical composition comprising at least two compounds disclosed herein.

[0025] In one aspect, provided herein is a pharmaceutical composition comprising a compound disclosed herein, for use in the treatment of a disease in a subject in need thereof.

[0026] In a related aspect, the disease comprises a virus-induced disease, a cancer, an autoimmune disease, an immune disorder, a bacterial associated disease or infection, or a combination thereof. In a further related aspect, the disease is caused by a virus selected from the group consisting of norovirus, rotavirus, hepatitis virus A, B, C, D, or E, rabies virus, West Nile virus, enterovirus, echovirus, coxsackievirus, herpes simplex virus (HSV), varicella-zoster virus, mosquito-borne viruses, arbovirus, St. Louis encephalitis virus, California encephalitis virus, lymphocytic choriomeningitis virus, human immunodeficiency virus (HIV), poliovirus, zika virus, rubella virus, cytomegalovirus, human papillomavirus (HPV), enteovirus D68, severe acute respiratory syndrome (SARS) coronavirus, Middle East respiratory syndrome coronavirus (MERS-CoV), SARS coronavirus 2 (SARS-CoV-2), Epstein-Barr virus (EBV), influenza virus, influenza virus A2, influenza virus B, influenza virus A(H1N1), respiratory syncytical virus (RSV), polyoma viruses, BK virus, Tacaribe virus, Ebola virus, Dengue virus, and any combination thereof In another further

related aspect, the disease is COVID-19 caused by SARS-CoV-2.

[0027] In another related aspect, the method of use terminates polynucleotide chain synthesis in a cell. In a further related aspect, terminating polynucleotide chain synthesis increases termination of DNA chain synthesis, or increases termination of RNA chain synthesis, or a combination thereof. In another further related aspect, terminating polynucleotide chain synthesis confers viral resistance to said cell. In yet another further related aspect, the cell is a eukaryotic cell. In still another further related aspect, the eukaryotic cell is a tumor cell, or is a cell infected by a virus or a foreign DNA.

BRIEF DESCRIPTION OF THE DRAWINGS

[0028] The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawing(s) will be provided by the office upon request and payment of the necessary fee.

[0029] The subject matter disclosed describing ddh and deoxy-ddh compounds and uses thereof, is particularly pointed out and distinctly claimed in the concluding portion of the specification. The compounds, synthesis of, and use thereof, however, both as to organization and method of operation, together with objects, features, and advantages thereof, may best be understood by reference to the following detailed description when read with the accompanying drawings.

[0030] **Figure 1** shows an embodiment of the defensive genomic context of pVip genes. pVip genes are marked as red. Black arrows point to known anti-phage defense systems (bracketed by black brackets).

[0031] **Figure 2** shows an embodiment of the genomic neighborhood of pVip genes. The presence of diverse kinases, predicted to supply nucleotide substrates to the pVip, is observed in the neighborhood of pVip genes. pVip genes are represented in red. Black arrows point at genes annotated as nucleotide kinases.

[0032] **Figures 3A-3B** show phylogenetic trees of pVip genes. **Figure 3A** shows the phylogenetic tree of the pVip genes disclosed herein. Branch colors correspond to major clades. Filled circles represent presence of nucleotide kinases. Purple circles: predicted thymidilate kinases; brown circles: predicted cytidilate kinases; blue circles: predicted adenylate kinases. Stars represent pVip genes that we have experimentally showed to have anti-phage activity. Colors of stars represent different defense phenotypes for different pVips. Red stars: anti T7 and anti P1/lambdactivity; green star: anti T7 activity; light blue star: anti P1 and anti-lambdactivity. **Figure 3B** shows the phylogenetic tree of pVip genes including sequences extracted from metagenomes. Branch colors correspond to major

clades of **Figure 3A**. Black branches are sequences from metagenomes.

[0033] **Figure 4** shows the experimental approach used for functional validation of pVips. pVip gene candidates were synthetized and cloned in two different vectors under inducible promoters. *E. coli* and *B. subtilis* bacteria were transfected with these vectors and then tested for viral resistance against a collection of phages. Anti-viral activity of pVips was assessed in two types of assays: solid plaque assays and liquid infection assays.

[0034] **Figures 5A-5B** show that a strain with a knockout in the iscR gene (Keio ΔiscR) rescues pVips activity *in vivo*. **Figures 5A**nd **5B** show plaque assays of bacteria transformed with pVip9 (**Figure 5A**) or pVip10 (**Figure 5B**). The left panel shows WT MG1655 colonies. The right panel shows Keio ΔiscR colonies. Bacteria were challenged with phages SECPhi6, SECPhi17, SECPhi18, SECPhi27, SECPhi32, and T7 (dilutions from 10^{-3} to 10^{-8}). A star indicates phages in which pVip anti-viral activity was observed. Shown is an experiment representative of triplicates.

[0035] **Figures 6A-6Z** show plaque assays of multiple pVips cloned and expressed in Keio ΔiscR colonies indicating *in vivo* anti-viral activity of the pVips. Shown are plaque assays in which pVip expression was either non induced or induced by adding 0.004% arabinose, as indicated. Colonies were challenged with the following phages: P1, lambda vir, SECPhi6, T4, SECPhi27, T7, SECPhi4, SECPhi17, SECPhi18, T2, T5, and T6 as indicated. Phages were diluted from 10^{-1} to 10^{-6} of the original stock. Star indicates phages for which activity of pVip was observed. **Figure 6A** shows Keio ΔiscR control colonies transfected with MoaA. Three main defense phenotypes were observed for the different pVips: activity against P1 and lambda but not T7 (**Figures 6B-6H**) activity against T7 only (**Figures 6I-6M**), and activity against P1, lambdand T7 (**Figures 6N-6Z**). All experiments were performed at 37°C.

[0036] **Figures 7A-7B** show *in vivo* anti-viral activity of pVip7 in *B. subtilis*. **Figure 7A** shows *in vivo* anti-viral activity of pVip7 in solid plaque assays. The left panel shows colonies in which pVip7 expression was not induced. The right panel shows colonies in which pVip7 expression was induced by 1mM IPTG. *B. subtilis* colonies were challenged with the following phages: SBSphiC, SPO1, rho14, spbeta, SPR, phi3T (dilution from 10^{-1} to 10^{-6} of the original stock). A star indicates phages for which pVip7 anti-viral activity was observed. Shown here is an experiment representative of triplicates. **Figure 7B** shows *in vivo* anti-viral activity of pVip7 in a liquid infection assay using phage phi3T (MOI = 0.1). Grey = non-infected controls, salmon = phage-infected bacteria in which pVip7 expression was not induced, red = phage-infected bacteria in which pVip7 expression was induced. Shown here is one representative experiment of triplicates.

[0037] **Figures 8A-8G** shows T7 RNA polymerase (RNAP) susceptibility to pVips products. **Figure**

8A shows the experimental design of the assay. A GFP reporter operably linked to a T7 promoter was cloned into a plasmid and transfected to bacterial cells expressing the T7 RNAP. T7 polymerase is activated by a pLac promoter inducible by IPTG. pVips are activated by a pAra promoter inducible by arabinose. A plasmid cloned with MoaA instead of pVips was used as a control. Cells were first provided with arabinose and then IPTG, thus inducing first pVip and then T7 RNAP. The expressed T7 RNAP in turn transcribed GFP. It was reasoned that if T7 RNAP is sensitive to pVip products, the presumed chain terminator will be incorporated generating prematurely terminated transcripts, leading to reduced GFP translation and signal. **Figures 8B-8G** show the experimental results. **Figure 8B** shows that activation of the control plasmid, expressing MoaA, did not affect GFP expression. **Figures 8C-8G** show that co-expression of pVip8, pVip9, pVip37, pVip46, and pVip63, respectively, affected GFP expression. Graphs represent GFP divided by optical density (OD) (A.U). Grey curves indicate no GFP induction (no IPTG), green curves indicate GFP induction but no pVip induction (IPTG 0.01mM, no arabinose), pink curves indicate GFP and pVip induction (IPTG 0.01mM, arabinose 0.02%).

[0038] **Figures 9A-9B** show pVips produce a variety of modified ribonucleotides. **Figure 9A** shows extracted ion chromatogram for singly charged masses corresponding to ddhC (m/z 226.08223, retention time (RT) of 2.2 minutes), ddhCMP (m/z 306.04856, RT 9.7), ddhCTP (m/z 465.98122, RT 11.1), ddhUMP (m/z 307.03258, RT 8.7), ddhUTP (m/z 466.96524, RT 9.5), ddhGMP (m/z 266.08838, RT 9.8), and ddhGTP (m/z 505.98737, RT 10.6). X-axis depicts RT in minutes. Y axis, normalized ion intensity (arbitrary units). Normalization was performed on all pVips and MoaA samples, with maximal value set to 1.0. Representative of 3 replicates. **Figure 9B** shows production of ddh nucleotide derivatives by pVips. Colored boxes depict detected compounds. Lighter color corresponds to compounds detected in a smaller quantity.

[0039] **Figure 10** shows detection of ddhCTP and ddhCTP derivatives in cell lysates from an *E. coli* strain expressing the human viperin. Extracted ion chromatogram for singly charged masses corresponding to ddhC (m/z 226.08223, retention time (RT) of 2.2 minutes), ddhCMP (m/z 306.04856, RT 9.7), ddhCTP (m/z 465.98122, RT 11.1), ddhUMP (m/z 307.03258, RT 8.7), ddhUTP (m/z 466.96524, RT 9.5), ddhGMP (m/z 266.08838, RT 9.8), and ddhGTP (m/z 505.98737, RT 10.6). X-axis depicts RT in minutes. Y axis, normalized ion intensity (arbitrary units). Normalization was performed on all human viperin and MoaA samples, with maximal value set to 1.0. Three biological replicates are presented.

[0040] **Figure 11** shows detection of ddh nucleotides in lysates of cells that express pVips. Extracted ion chromatogram for singly charged masses corresponding to ddhC (m/z 226.08223, retention time

(RT) of 2.2 minutes), ddhCMP (m/z 306.04856, RT 9.7), ddhCTP (m/z 465.98122, RT 11.1), ddhUMP (m/z 307.03258, RT 8.7), ddhUTP (m/z 466.96524, RT 9.5), ddhGMP (m/z 266.08838, RT 9.8), and ddhGTP (m/z 505.98737, RT 10.6). X-axis depicts RT in minutes. Y axis, normalized ion intensity (arbitrary units). Normalization was performed on all pVips and MoaA samples, with maximal value set to 1.0. Three biological replicates are presented for each pVip.

[0041] **Figure 12** shows quantification of ddh cytidine in lysates of cells expressing pVips. Detection and quantification of ddhC was performed using LC-MS with a synthesized chemical standard. For MoaA, the measurement was under the limit of detection. Bar graph represents average of three replicates, with individual data points overlaid.

[0042] **Figures 13A-13F** are schematic representations of catalytic modifications of a diverse group of non-natural substrates by pVips. The 3' hydroxyl groups of the substrates are removed by pVips. **Figure 13A** shows enzymatic modification of non-natural substrate represented by the structure of a 5-member etheric ring (sugar ribose) substituted with hydroxy at 3' position to obtain a double bond at positions 3'-4' by pVips. **Figure 13B** shows enzymatic modification of non-natural substrate represented by the structure of a 5-membered nitrogen based ring (sugar ribose analog) substituted with hydroxy at 3' position, to obtain a double bond at positions 3'-4' by pVips. **Figure 13C** shows enzymatic modification of non-natural substrate represented by the structure of a 5-membered carbon based (sugar ribose analog) substituted with hydroxy at 3' position to obtain a double bond at positions 3'-4'by pVips. **Figure 13D** shows enzymatic modification of non-natural substrate represented by the structure of 5-membered carbon based ring contain a doble bond at positions 4'-1, substituted with hydroxy at positions 3' to obtain a doble bond at positions 3'-4' and 1-1' by pVips. **Figure 13E** shows enzymatic modification of non-natural substrate represented by the structure of 5-membered carbon based ring substituted at position 1 with $=CH_2$, substituted with hydroxy at positions 3' to obtain a doble bond at positions 3'-4' by pVips. **Figure 13F** shows enzymatic modification of non-natural substrate represented by the structure of an etheric chain which is terminally substituted with hydroxy to obtain a terminal double bond, by pVips.

[0043] **Figure 14** presents results showing pVips display broad substrate promiscuity. Each pVip was screened against a set of substrates and 5'-dA production was measure by HPLC. Black boxes depict reactions in which enhanced 5'-dA production by at least 2-fold was observed compared to control reactions (without substrate or dithionite), indicating substrate activation. Grey boxes represent negative reactions where the positive and negative control display similar 5'-dA levels. Outlined boxed indicate predicted pVip natural substrates from products detected in lysates overexpressing each pVip. White boxes depict untested conditions. * denotes results from Gizzi et

al., (2018) *A naturally occurring antiviral ribonucleotide encoded by the human genome.* Nature 558, 610–614 .

[0044] **Figures 15A-15B** shows LC-MS analysis indicating that pVips catalyze the conversion of UTP into ddhUTP. **Figure 15A** presents chromatographs showing the presence of a product with a mass corresponding to ddhUTP (negative mode) in reaction samples where the enzyme (here pVip8) was incubated with UTP as substrate. This product was not observed in control samples: dithionite (reducing agent) or UTP. **Figure 15B** presents MS/MS analysis of this product indicating the observed fragments result from ddhUTP. Potential fragments are indicated for each peak.

[0045] **Figures 16A-16B** shows LC-MS analysis indicating that pVips catalyze the conversion of CTP into ddhCTP. **Figure 16A** presents chromatographs showing the presence of a product with a mass corresponding to ddhCTP (negative mode) in reaction samples where the enzyme (here pVip6) was incubated with CTP as substrate. This product was not observed in control samples: dithionite (reducing agent) or CTP. **Figure 16B** presents MS/MS analysis of this product indicating the observed fragments result from ddhCTP. Potential fragments are indicated for each peak.

[0046] **Figures 17A-17B** shows LC-MS analysis indicating that pVips catalyze the conversion of ATP into ddhATP. **Figure 17A** presents chromatographs showing the presence of a product with a mass corresponding to ddhATP (negative mode) in reaction samples where the enzyme (here pVip6) was incubated with ATP as substrate. This product was not observed in control samples: dithionite (reducing agent) or ATP. **Figure 17B** presents MS/MS analysis of this product indicating the observed fragments result from ddhATP. Potential fragments are indicated for each peak.

[0047] **Figures 18A-18B** shows LC-MS analysis indicating that pVips catalyze the conversion of ITP into ddhITP. **Figure 18A** presents chromatographs showing the presence of a product with a mass corresponding to ddhITP (negative mode) in reaction samples where the enzyme (here pVip62) was incubated with ITP as substrate. This product was not observed in control samples: dithionite (reducing agent) or ITP. **Figure 18B** presents MS/MS analysis of this product indicating the observed fragments result from ddhITP. Potential fragments are indicated for each peak.

[0048] **Figures 19A-19B** shows LC-MS analysis indicating that pVips catalyze the conversion of dUTP into ddhdUTP. **Figure 19A** presents chromatographs showing the presence of a product with a mass corresponding to ddhdUTP (negative mode) in reaction samples where the enzyme (here pVip62) was incubated with dUTP as substrate. This product was not observed in control samples: dithionite (reducing agent) or dUTP. **Figure 19B** presents MS/MS analysis of this product indicating the observed fragments result from ddhdUTP. Potential fragments are indicated for each peak.

[0049] **Figures 20A-20B** shows LC-MS analysis indicating that pVips catalyze the conversion of

GTP into ddhGTP. **Figure 20A** presents chromatographs showing the presence of a product with a mass corresponding to ddhGTP (negative mode) in reaction samples where the enzyme (here pVip56) was incubated with GTP as substrate. This product was not observed in control samples: dithionite (reducing agent) or GTP. **Figure 20B** presents MS/MS analysis of this product indicating the observed fragments result from ddhGTP. Potential fragments are indicated for each peak.

[0050] **Figure 21.** RNA-primes RNA templates. Red and underlined nucleotides indicate position for the incorporation of the natural rNTP/dNTP or NTP analog.

[0051] **Figures 22A-22E.** Nsp12 and nsp8-7 concentration optimization. Analysis of the primer extension activity in the condition of different concentrations of nsp12 and nsp8-7. Serial dilutions of nsp12 are indicated on the top of the gels. **Figure 22A**. Serial dilutions of 0 mM:0 mM for nsp7-8. **Figure 22B**. Serial dilutions of 0.25 mM:0.05 mM for nsp7-8. **Figure 22C**. Serial dilutions of 0.5 mM:0.1 mM for nsp7-8. **Figure 22D**. Serial dilutions of 1 mM:0.2 mM for nsp7-8. **Figure 22E**. Serial dilutions of 2.5 mM:0.5 mM for nsp7-8.

[0052] **Figures 23A-23B.** Nsp12 and nsp8-7 concentration optimization. Analysis of the primer extension activity in the condition of different concentrations of nsp12 and nsp8-7. Serial dilutions of nsp12 are indicated on the top of the gels. **Figure 23A**. Serial dilutions of 5 mM:1 mM for nsp7-8. **Figure 23B**. Serial dilutions of 10 mM:2 mM for nsp7-8.

[0053] **Figure 24**. Incorporation of REM and ddhREM in primer extension assay catalyzed by SARS-CoV-2 RdRp. Reaction products from SARS-Cov2 RdRp-catalyzed incorporation of ATP and its analogs.

[0054] **Figure 25.** Chain termination effect of REM and ddhREM in primer extension assay catalyzed by SARS-CoV-2 RdRp. Analysis of the chain termination abilities of the ATP analogs.

[0055] **Figures 26A-26D.** Inhibition of SARS-Cov2 RdRp by REM-TP and ddhREM-TP. **Figure 26A and Figure 26C** - A representative image of the REM and ddhREM IC50 calculation for the inhibition of the RNA synthesis catalyzed by SARS-Cov2 RdRp. **Figure 26B and Figure 26D** - Quantitative analysis of REM-TP and ddhREM-TP. Product formation was quantified using ImageLab software (BioRad, California, USA). IC50 values were calculated by fitting the product formation using the following equation: Y = A+(D-A)/(1+([In]/C)^B), where Y represents SARS-Cov2 RdRp activity; [In] – concentration of inhibitor at mM; A – inhibition at inhibitor concentration equal zero; D – inhibition at the highest inhibitor concentration; B – slope of the at the mid-range concentration point; C – mid-range concentrate point (IC50).

[0056] **Figures 27A-27B** Analysis of incorporation and chain termination effect of ddhUTP in primer extension assay catalyzed by SARS-CoV-2 RdRp. **Figure 27A**. Incorporation of UTP and its

analogs, 3'-dUTP and ddhUTP into RNA template. **Figure 27B**. Chain termination ability of ddhUTP in primer extension reaction. 3'-dUTP was used as a positive control for chain termination.

[0057] **Figures 28A-28B.** Analysis of incorporation and chain termination effect of ddhGTP in primer extension assay catalyzed by SARS-CoV-2 RdRp. **Figure 28A**. Incorporation of GTP and its analogs, 3'-dGTP and ddhGTP into RNA template. **Figure 28B**. Chain termination ability of ddhGTP in primer extension reaction. 3'-dGTP was used as a positive control for chain termination.

[0058] **Figures 29A-29D.** Inhibition of SARS-Cov2 RdRp by ddhUTP and ddhGTP. Figure 29A and Figure 29C. A representative image of the ddhUTP and ddhGTP IC50 calculation for the inhibition of the RNA synthesis catalyzed by SARS-Cov2 RdRp. **Figure 29B and Figure 29D** . Quantitative analysis of ddhUTP and ddhGTP. Product formation was quantified using ImageLab software (BioRad, California, USA). IC50 values were calculated by fitting the product formation using the following equation: Y = A+(D-A)/(1+([In]/C)^B), where Y represents SARS-Cov2 RdRp activity; [In] – concentration of inhibitor at mM; A – inhibition at inhibitor concentration equal zero; D – inhibition at the highest inhibitor concentration; B – slope of the at the mid-range concentration point; C – mid-range concentrate point (IC50).

[0059] **Figures 30A-30B.** Inhibition of POLRMT by ddhREM-TP. **Figure 30A.** A representative image of the ddhREM-TP IC50 calculation for the inhibition of the DNA synthesis catalyzed by POLRMT. **Figure 30B**. Quantitative analysis of ddhREM-TP. Product formation was quantified using ImageLab software (BioRad, California, USA). IC50 values were calculated by fitting the product formation using the following equation: Y = A+(D-A)/(1+([In]/C)^B), where Y represents POLMRT activity; [In] – concentration of inhibitor at mM; A – inhibition at inhibitor concentration equal zero; D – inhibition at the highest inhibitor concentration; B – slope of the at the mid-range concentration point; C – mid-range concentrate point (IC50).

[0060] **Figures 31A-31B.** Inhibition of POLRMT by ddhUTP. **Figure 31A.** A representative image of the ddhUTP IC50 calculation for the inhibition of the DNA synthesis catalyzed by POLRMT. **Figure 31B**. Quantitative analysis of ddhUTP. Product formation was quantified using ImageLab software (BioRad, California, USA). IC50 values were calculated by fitting the product formation using the following equation: Y = A+(D-A)/(1+([In]/C)^B), where Y represents POLRMT activity; [In] – concentration of inhibitor at mM; A – inhibition at inhibitor concentration equal zero; D – inhibition at the highest inhibitor concentration; B – slope of the at the mid-range concentration point; C – mid-range concentrate point (IC50).

[0061] **Figures 32A-32D.** Inhibition of POLG1 by ddhREM-TP and ddhUTP. **Figure 32A and Figure 32C.** A representative image of the ddhREM-TP and ddhUTP IC50 calculation for the

inhibition of the DNA synthesis catalyzed by POLG1. **Figure 32B and Figure 32D** . Quantitative analysis of ddhREM-TP and ddhUTP. Product formation was quantified using ImageLab software (BioRad, California, USA). IC50 values were calculated by fitting the product formation using the following equation: Y = A+(D-A)/(1+([In]/C)^B), where Y represents POLG1 activity; [In] – concentration of inhibitor at mM; A – inhibition at inhibitor concentration equal zero; D – inhibition at the highest inhibitor concentration; B – slope of the at the mid-range concentration point; C – mid-range concentrate point (IC50).

[0062] **Figures 33A-33B.** Inhibition of PrimPol by ddhREM-TP and ddhUTP. **Figure 33A and Figure 33B.** A representative image of the ddhUTP and ddhUTP for the inhibition of the DNA synthesis catalyzed by PrimPol.

[0063] **Figures 34A-34B.** Inhibition of IMPDH by ddhUMP and ddhGMP. **Figure 34A.** MPA (purple dots) and RMP (blue dots) inhibition on IMPDH activity with IC50 of 76 ± 10 nM and 2.6 ± 1.8 mM, respectively. **Figure 34B**. ddhUMP (purple dots) and ddhGMP (red squares) inhibition on IMPDH activity with IC50 > 1mM.

DETAILED DESCRIPTION

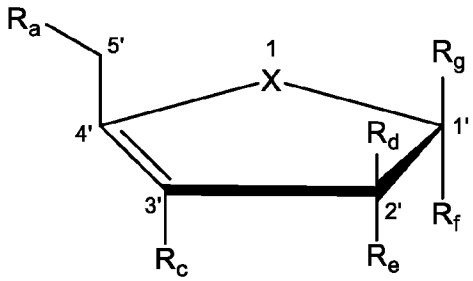
[0064] In the following detailed description, numerous specific details and embodiments are set forth in order to provide a thorough understanding of the anti-viral, anti-bacterial, and anti-tumoral chain terminator compounds disclosed herein, including descriptions of the 3, 4–didehydro 3´- (ddh) or deoxy-3, 4–didehydro (deoxy-ddh) compounds, methods of use thereof for terminating polynucleotide chain synthesis in a cell; methods of use thereof for treating a disease; and methods for producing these compounds including methods using pVip enzymes. In some instances, well-known methods, procedures, and components have not been described in detail so as not to obscure the present disclosure.

[0065] The substrate compounds from which the ddh- and deoxy-ddh-compounds may be synthesized chemically or produced enzymatically, may in certain embodiments be considered non-natural substrates of pVip enzymes. One skilled in the art would appreciate that the term "non-natural substrates" may encompass nucleotides/nucleosides derivatives or other substrates that are not the natural (*in vivo*) substrates of enzymatic reactions catalyzed by Vips or pVips *in vivo*.

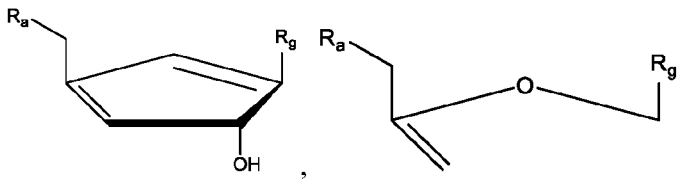
[0066] The substrate compounds from which the ddh- and deoxy-ddh-compounds may be synthesized chemically or produced enzymatically, may in certain embodiments be considered analogs. One skilled in the art would appreciate that the term "analog" may encompass a molecule having a structure similar to that of another molecule, but differing from it in respect to a certain

component. In some embodiments, the terms "analog", "structural analog", "chemical analog" and "substrate analog" are used herein interchangeably, having all the same qualities and meanings.

[0067] In some embodiments, the substrate compounds from which the ddh- and deoxy-ddh-compounds may be synthesized chemically or produced enzymatically, refer to compounds "A" in Table 1, Table 2, and in Figures 13A-13F. In certain embodiments, the substrate compounds are defined as non-natural substrate. A skilled artisan would appreciate that the ddh- and deoxy-ddh-compounds described in detail herein, encompass structures comprising compounds "B" in Table 1, Table 2, and Figures 13A-13F. In some embodiments, a ddh- and deoxy-ddh-compounds comprises a structure of a nucleotide/nucleoside analogs comprising substituted compounds (for example as presented herein) wherein the ddh- and deoxy-ddh-compounds mimics the overall structure of nucleotide/nucleoside analogs and may include: (1) heterocyclic nitrogen based ring or O-aryl or aryl and isomers thereof attached to position 1' of the 5 membered ring (to the ddh or deoxy-ddh ribose sugar analog); and/or (2) different substitutions on the 5 member ring (position 2', 3', 4' and/or 5'); and/or (3) substitution of the O of the 5-member ring with N or CH_2 or CH or CCH_2; and/or (4) an open etheric ring instead of the 5 member ring, may comprise unique novel activities. Accordingly, these substituted compounds (substituted ddh or deoxy-ddh compounds) may add unique therapeutic compounds to the medicinal chemistry arsenal.



X is O, NH, CH_2, CH, CCH_2



[0068] In some embodiments, ddh- and deoxy-ddh-compounds comprise a ddh- compound. In some embodiments, ddh- and deoxy-ddh-compounds comprise a deoxy-ddh-compound. In some embodiments, ddh- and deoxy-ddh-compounds comprise a prodrug of a ddh or deoxy-ddh-compound. In some embodiments, ddh- and deoxy-ddh-compounds comprise compounds wherein the 5-member ring (ribose sugar or analog) is substituted at position 2' with hydroxyl group. In some

embodiments, ddh- and deoxy-ddh-compounds comprise compounds wherein the 5-member ring (ribose sugar or ribose sugar analog) is not substituted at position 2' with hydroxyl group. In some embodiments, the 5-member ring is ribose sugar. In some embodiments, 5-member ring is ribose sugar analog. In some embodiments, the ribose sugar analog is 5-member oxygen or nitrogen or carbon based ring. In some embodiments, the 5-memberd ring is 5-member oxygen based ring. In another embodiment, the 5-member ring is dihydrofuran. In some embodiments, the 5-member ring is a 5-member nitrogen based ring. In another embodiments, the 5-member nitrogen based ring is dihydropyrrole. In some embodiments, the 5-member ring is 5-member carbon based ring. In another embodiments, the 5-member carbon based ring is cyclopentene In some embodiments, ddh- and deoxy-ddh-compounds do not contain a 5-member ring. In another embodiment, In some embodiments, ddh- and deoxy-ddh-compounds comprise an etheric chain.

[0069] The term "ddh- and deoxy-ddh-compound", "ddh- and deoxy-ddh-product", ddh- and deoxy-ddh-prodrug" and "a compound" may in some embodiments be used herein interchangeably, having all the same qualities and meanings. The term "deoxy-ddH-", "ddh-d-", and "ddh-deoxy-" may in some embodiments be used herein interchangeably, having all the same qualities and meanings.

[0070] In some embodiments, the substrate compounds from which the ddh- and deoxy-ddh-compounds may be synthesized chemically or produced enzymatically, comprise nucleotide/nucleoside analogs. One skilled in the art would appreciate that the term ddh- and deoxy-ddh-compounds may encompass compounds generated by the pVips or synthesized chemically to include 3, 4–didehydro 3´- (ddh) or deoxy-3, 4–didehydro (deoxy-ddh) compounds that can be used to treat a disease. In some embodiments, ddH- and deoxy-ddH- compounds may be used as DNA or RNA chain terminators. In some embodiment, the nucleotide/nucleoside analogs are generated by the pVips from non-natural substrates.

[0071] In some embodiments, while a molecule in the nucleotide form can be a DNA or RNA chain terminator, its corresponding nucleoside form, without any phosphate group, can cross cell membrane and enter into a cell. Once inside the cell, the nucleoside can be phosphorylated by one or more viral or cellular kinases to become a nucleotide. The nucleotide can be in the form of monophosphate, diphosphate or triphosphate. Each step of phosphorylation can be mediated by the same or different viral or cellular kinases. For example, the nucleoside is converted to monophosphate nucleotide by a first kinase, the monophosphate nucleotide is converted to diphosphate nucleotide by another kinase, and the diphosphate nucleotide is converted to triphosphate nucleotide by yet another kinase.

[0072] In some embodiments, the present disclosure describes the use of pVips to produce novel ddh- and deoxy-ddh compounds from non-natural substrates. Enzymatic reactions between pVips and the

non-natural substrates may in some embodiments, be performed *in vitro*. In some embodiments, the novel ddh- and deoxy-ddh compounds are chemically synthesized from non-natural substrates using methods known in the art. In some embodiments, the novel ddh- and deoxy-ddh compounds from non-natural substrates are provided in the form of a pro-drug. The substrates can have 0 to 3 phosphate groups, i.e. being non-phosphorylated, or in the form of monophosphate, diphosphate or triphosphate nucleotide. As described in the Examples below, results from the enzymatic reactions led to the discovery of a number of non-natural substrates that can be converted by the pVips into ddh- and deoxy-ddh compounds. These ddh- and deoxy-ddh compounds can then be tested to see whether they possess the function of DNA or RNA chain termination. The products generated from the non-natural substrates can have 0 to 3 phosphate groups, i.e. being non-phosphorylated, or in the form of monophosphate, diphosphate or triphosphate nucleotide. In other embodiments, the products generated from may be in the form of a pro-drug.

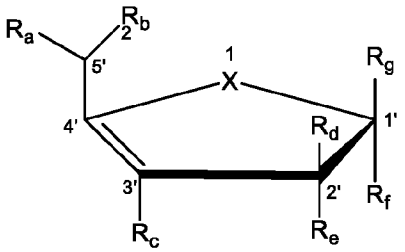
[0073] In some embodiments, the ddh- and deoxy-ddh compounds can be used to block cellular DNA or RNA replication. In some embodiments, the ddh- and deoxy-ddh compounds can be used to treat a disease in a subject in need thereof. Methods of these ddh- and deoxy-ddh compounds includes treating viral infections including RNAnd DNA virus.

[0074] In some embodiments, the ddh- and deoxy-ddh compounds or prodrugs thereof, can be synthesized or enzymatically produced, and administered directly to cells of a subject. Upon entering the cells, these ddh- and deoxy-ddh compounds or prodrugs thereof, can be phosphorylated by one or more viral or cellular kinases to produce active forms of the compound that can inhibit DNA/RNA replication.

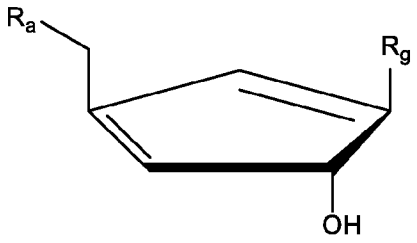
[0075] In certain embodiments, the ddh- and deoxy-ddh compounds or prodrugs thereof described herein can be used to block cellular DNA/RNA replication or treat a disease in a subject. In some embodiments, the ddh- and deoxy-ddh compounds or prodrugs thereof are administered to cells in a form that can enter the cells (e.g. nucleoside form or prodrug form). Once inside the cells, these the ddh- and deoxy-ddh compounds or prodrugs thereof can be converted (e.g. phosphorylation by one or more viral or cellular kinases).

Compounds

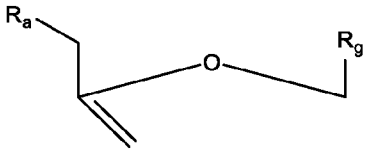
[0076] In some embodiments, provided herein is a compound represented by the structure of Formula B(i), Formula B(ii), Formula B(iii):



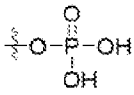
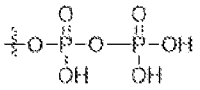
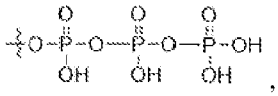
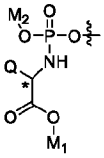
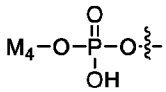
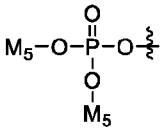
X is O, NH, CH_2, CH, CCH_2 Formula B(i),

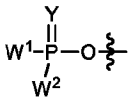


Formula B(ii),



Formula B(iii),

wherein R_a is OH,  ,  ,  ,  , ,  , or  ;-OC(=O)R^{12}, -OC(=O)OR^{12}, -OC(=O)N$R^{12}R^{13}$, -OC(=O)SR^{12}, -OS(O)R^{12}, -OS(O)$_2R^{12}$, -OS(O)(OR^{12}), -OS(O)$_2$(OR^{12}), -O$SO_2NR^{12}R^{13}$ or the group of Formula (Ic)



Formula Ic

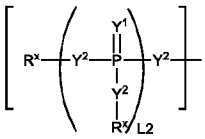
wherein

Y is O, S, NR, $^+$N(O)(R), N(OR), $^+$N(O)(OR), or N-NR;

W^1 and W^2, when taken together, are -$Y^3(C(R^y)_2)_3Y^3$-;

or one of W^1 and W^2 together with Rc is -Y^3- and the other of W^1 or W^2 is Formula Id;

or W^1 and W^2 are each, independently, a group of Formula Id:



Formula Id

Wherein

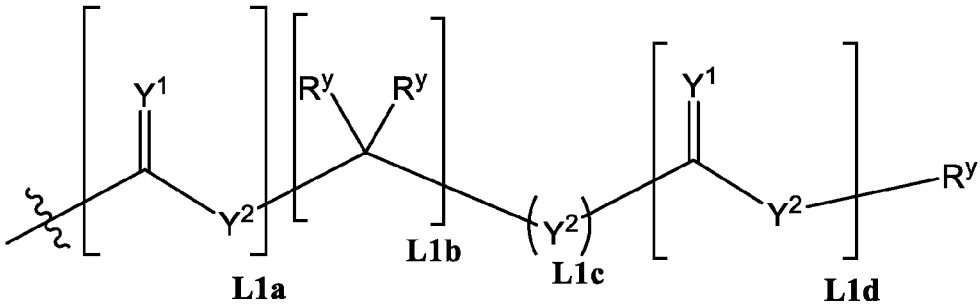
each Y^1 is independently O, S, NR, $^+N(O)(R)$, N(OR), $^+N(O)(OR)$, or N-NR_2;

each Y^2 is independently a bond, O, CR_2, NR, $^+N(O)(R)$, N(OR), $^+N(O)(OR)$, N-NR_2, S, S-S, S(O), or $S(O)_2$;

each Y^3 is independently O, S, or NR;

L2 is 0, 1, or 2;

Each R^x is a group of Formula Ie:



Formula Ie

wherein

each L1a, L1c, and L1d is independently O or 1;

L1b is 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 or 12;

each R^y is independently H, F, Cl, Br, I, -CN, $-N_3$, $-NO_2$, -OR, $-C(R)_2-O-C(R)_3$, $-C(=Y^1)R$, $C(=Y^1)R^{14}$, $-C(=Y^1)OR$, $-C(=Y^1)N(R)_2$, $-N(R)_2$, $-^+N(R)_3$, -SR, -S(O)R, $-S(O)_2R$, $-S(O)R^{14}$, $-S(O)_2R^{14}$, -S(O)(OR), $S(O)_2(OR)$, $-OC(=Y^1)R$, $-OC(=Y^1)OR$, $-OC(=Y^1)(N(R)_2)$, $-SC(=Y^1)R$, $-SC(=Y^1)OR$, $-SC(=Y^1)(N(R)_2)$, $-N(R)C(=Y^1)R$, $-N(R)C(=Y^1)OR$, $-N(R)C(=Y^1)N(R)_2$, $-SO_2NR_2$, (C_1-C_{18}) alkyl, (C_2-C_8) alkenyl, (C_2-C_8) alkynyl, (C_6-C_{20}) aryl, (C_3-C_{20}) cycloalkyl, (C_2-C_{20}) heterocyclyl, arylalkyl, or heteroarylalkyl,

wherein each (C_1-C_8) alkyl, (C_2-C_8) alkenyl, (C_2-C_8) alkynyl, (C_6-C_{20}) aryl, (C_3-C_{20}) cycloalkyl, (C_2-C_{20}) heterocyclyl, arylalkyl, or heteroarylalkyl is optinally susbstituted with 1-3 R^{15} groups; or when taken together, two R^y on the same carbon atom from a cycloalkyl ring of 3 to 7 carbon atoms;

each R is independently H, (C_1-C_8) alkyl, (C_2-C_8) alkenyl, (C_2-C_8) alkynyl, (C_6-C_{20}) aryl, (C_3-C_{20}) cycloalkyl, (C_2-C_{20}) heterocyclyl, or arylalkyl;

each R^{12} or R^{13} is independently H, (C_1-C_8) alkyl, (C_2-C_8) alkenyl, (C_2-C_8) alkynyl, (C_4-C_8) cycloalkylalkyl, (C_3-C_{20}) cycloalkyl, (C_2-C_{20}) heterocyclyl, optionally substituted aryl, optionally substituted heteroaryl, -C(=O)(C_1-C_8)alkyl, -S(O)$_n$(C_1-C_8)alkyl or aryl(C_1-C_8)alkyl; or R^{12} and R^{13} taken together with a nitrogen to which they are both attached form a 3-to 7-membered heterocyclic ring wherein any one carbon atom of said heterocyclic ring can optionally be replaced with -O-, -S-, or -NR^{16}-;

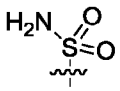
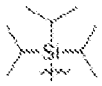
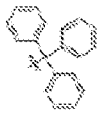
each R^{14} is independently a cycloalkyl or heterocycle optionally substituted with 1-3 R or R^{15} groups;

each R^{15} is independently, halogen, CN, -N_3, -N(R)$_2$, OR, -SR, -S(O)R, -S(O)$_2$R, -S(O)(OR), -S(O)$_2$(OR), -C(=Y^1)R, -C(=Y^1)OR, or -C(=Y^1)N(R)$_2$;

Q is a side group of an amino acid;

M_1 is an alkyl;

M_2 is an aryl, a substituted aryl, a heteroaryl or a substituted heteroaryl;

M_3 is  ,  ,  , or ;

M_4 is -(C_2-C_6)alkyl-O-(C_{10}-C_{20})alkyl;

each M_5 is -(CH_2)$_n$-S-C(=O)-(C_1-C_8)alkyl;

n is 1-4;

wherein each alkyl, alkenyl, alkynyl, aryl or heteroaryl of each of R, R^{12}, R^{13}, M_1 or M_2 is, independently, optionally substituted with 1 to 3 halo, hydroxy, CN, -N_3, N(R^{16})2 or OR^{16}; and wherein 1 to 3 of the non-terminal carbon atoms of each said (C_1-C_8) alkyl may be optionally replaced with -O-, -S-, or -NR^{16}-;

each R^{16} is independently H, (C_1-C_8)alkyl, (C_2-C_8)alkenyl, (C_2-C_8)alkynyl, aryl(C_1-C_8)alkyl, (C_4-C_8)cycloalkylalkyl, -C(=O)R^{12}, -C(=O)OR^{12}, -C(=O)N$R^{12}R^{13}$, -C(=O)SR^{12}, -S(O)R^{12}, -S(O)(OR^{12}), -S(O)$_2$(OR^{12}), or -$SO_2NR^{12}R^{13}$;

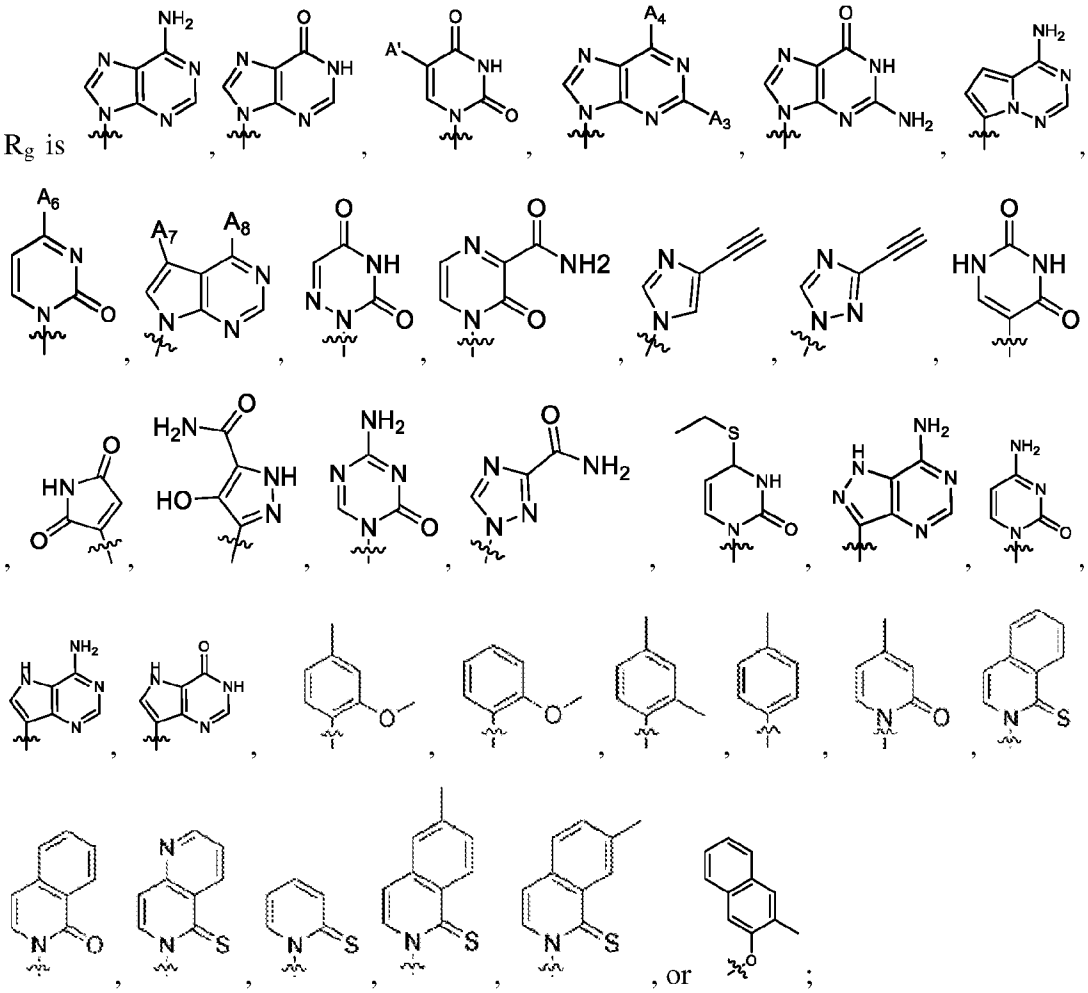
R_b is H or (C_1-C_6) alkyl;

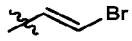
R_c is H, or (C_1-C_6) alkyl ;

R_d is H, a halo, an alkyl, an alkyne, or -OH;

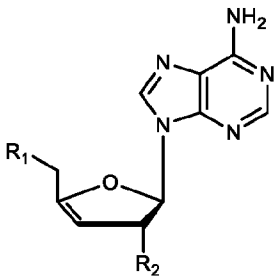
R_e is H, -OH, -O-COO-alkyl, a halo or an alkoxy ;

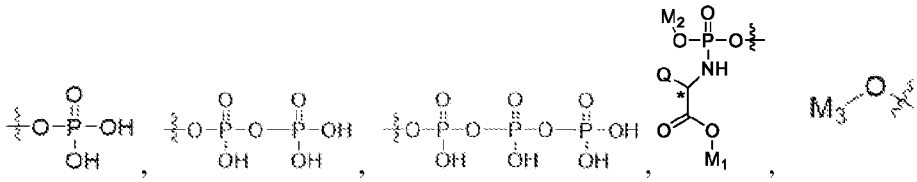
R_f is H or -CN;

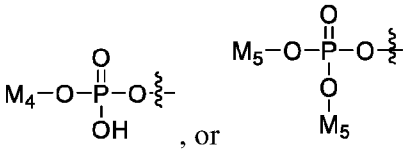
R_g is 

wherein A' is H, a halo, a haloalkyl, an alkyl, a hydroxy, an alkyne or  ;, A_3 is H, a halo or an amino; A_4 is H, an amino, an alkoxy or an alkyl;A_6 is H, an amino or an hydroxylamine; A_7 is H or an amido; A_8 is an amino or an alkyl.

[0077] In one embodiment, provided herein is a compound represented by the structure of Formula

IB:  , wherein

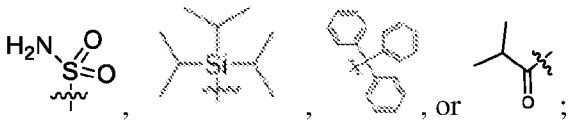
R_1 is OH, 



Q is a side chain of an amino acid;

M_1 is an alkyl;

M_2 is an aryl, a substituted aryl, a heteroaryl or a substituted heteroaryl;

M_3 is 

M_4 is -(C_2-C_6)alkyl-O-(C_{10}-C_{20})alkyl;

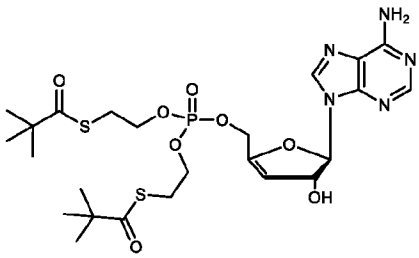
each M_5 is -$(CH_2)_n$-S-C(=O)-(C1-C8)alkyl;

n is 1-4;

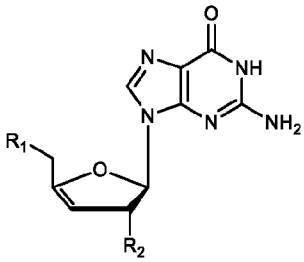
R_2 is -OH or -O-COO-alkyl; and

In another embodiment, in the compound of Formula IB, if R_1 is OH, then R_2 is -O-COO-alkyl.

In another embodiment, a compound of Formula IB, is represented by the structure of

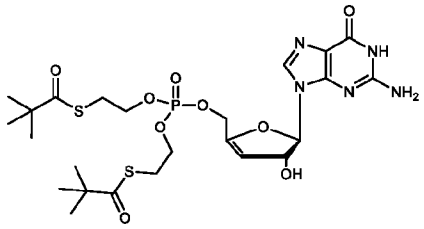
Compound 1 

[0078] In one embodiment, provided herein is a compound represented by the structure of Formula

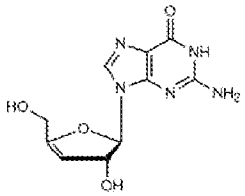
IIB: 

wherein R_1 and R_2 are as described in Formula IB. In another embodiment, in the compound of Formula IIB, if R_1 is OH, then R_2 is -O-COO-alkyl.

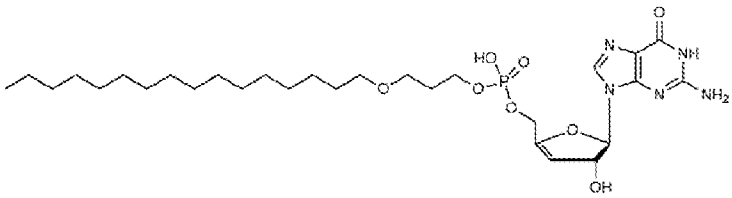
In another embodiment, a compound of Formula IIB, is represented by the structure of

Compound 2  .

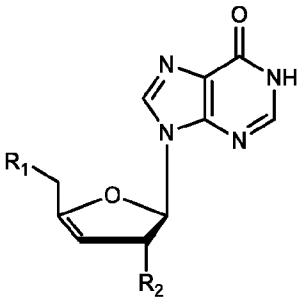
[0079] In another embodiment, a compound of Formula IIB, is represented by the structure of

Compound 31:  (AB21651).

[0080] In another embodiment, a compound of Formula IIB, is represented by the structure of Compound 103:

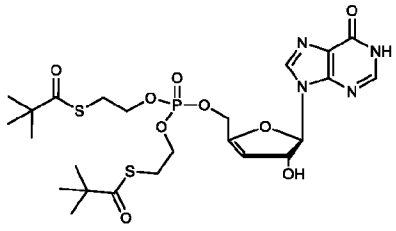
[0081]  (AB23040).

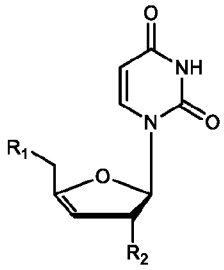
[0082] In one embodiment, provided herein is a compound represented by the structure of

Formula IIIB:  ,

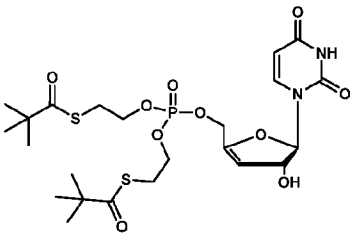
wherein R_1 and R_2 are as described in Formula IB. In another embodiment, in the compound of Formula IIIB, if R_1 is OH, then R_2 is -O-COO-alkyl.

In another embodiment, a compound of Formula IIIB, is represented by the structure of

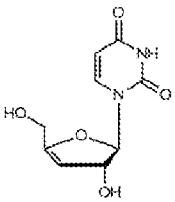
Compound 3  .

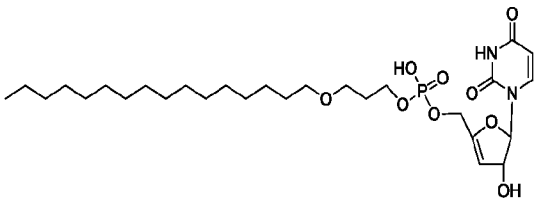
[0083] In one embodiment, provided herein is a compound, represented by the structure of Formula IVB:  ,

wherein R_1 and R_2 are as described in Formula IB. In another embodiment, in the compound of Formula IVB, if R_1 is OH, then R_2 is -O-COO-alkyl.

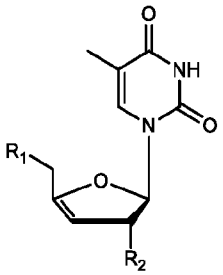
[0084] In another embodiment, a compound of Formula IVB, is represented by the structure of Compound 4  .

[0085] In another embodiment, a compound of Formula IVB, is represented by the structure of Compound 24:

 .

[0086] In another embodiment, a compound of Formula IVB, is represented by the structure of Compound 104:  (AB23039).

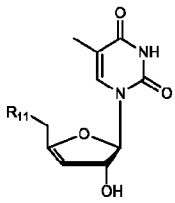
[0087] In one embodiment, provided herein is a compound represented by the structure of

Formula VB:  ,

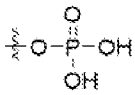
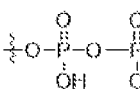
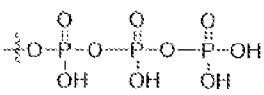
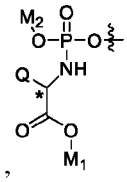
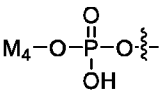
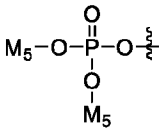
wherein R_1 and R_2 are as described in Formula IB. In another embodiment, in the compound of Formula VB, if R_1 is OH, then R_2 is -O-COO-alkyl.

[0088] In one embodiment, provided herein is a compound represented by the structure of Formula IB, IIB, IIIB, IVB, or VB, wherein R1 and R2 are as described in Formula IB. In another embodiment, in the compound represented by the structure of Formula IB, IIB, IIIB, IVB, or VB, if R_1 is OH, then R_2 is -O-COO-alkyl.

[0089] In one embodiment, provided herein is a compound represented by the structure of

Formula VB1: 

wherein

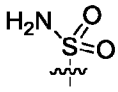
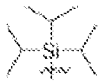
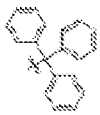
R_{11} is  ,  ,  ,  , ,  , 

or ;

Q is a side chain of an amino acid;

M_1 is an alkyl;

M_2 is an aryl, a substituted aryl, a heteroaryl or a substituted heteroaryl;

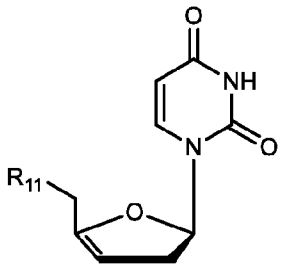
M_3 is  ,  ,  , or ;

M_4 is -(C_2-C_6)alkyl-O-(C_{10}-C_{20})alkyl;

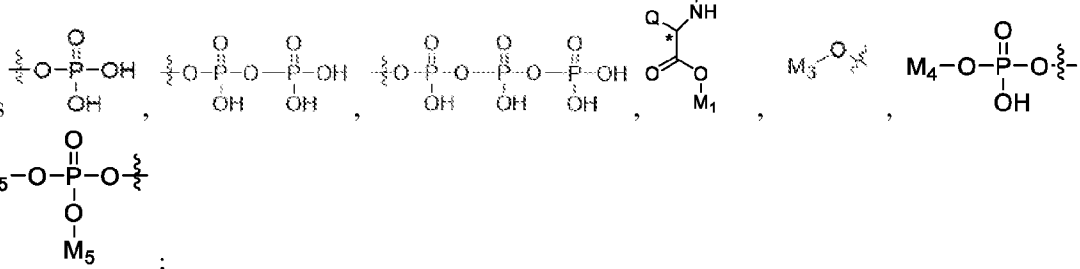
each M_5 is -$(CH_2)_n$-S-C(=O)-(C1-C8)alkyl; and

n is 1-4.

[0090] In one embodiment, provided herein is a compound represented by the structure of

Formula VIB:  ,

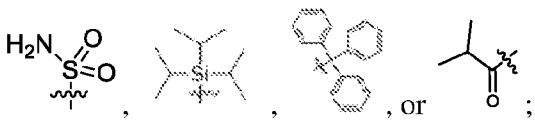
wherein

R_{11} is  ;

Q is a side chain of an amino acid;

M_1 is an alkyl;

M_2 is an aryl, a substituted aryl, a heteroaryl or a substituted heteroaryl;

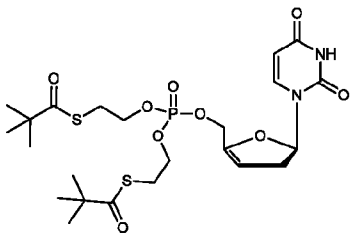
M_3 is  ;

M_4 is -(C_2-C_6)alkyl-O-(C_{10}-C_{20})alkyl;

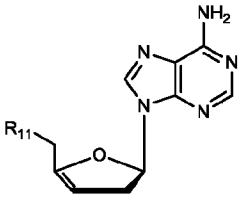
each M_5 is -$(CH_2)_n$-S-C(=O)-(C_1-C_8)alkyl; and

n is 1-4.

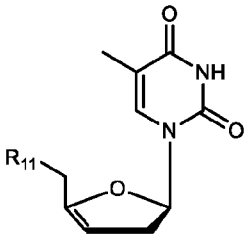
In another embodiment, a compound of Formula VIB, is represented by the structure of

Compound 5  .

[0091] In one embodiment, provided herein is a compound represented by the structure of

Formula VIIB:  , wherein R_{11} is as described in Formula VIB.

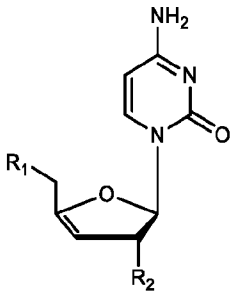
[0092] In one embodiment, provided herein is a compound represented by the structure of

Formula VIIIB:  ,

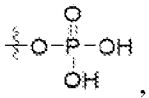
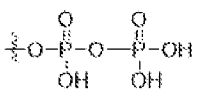
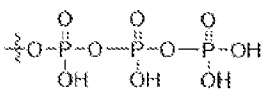
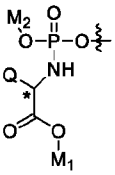
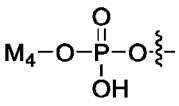
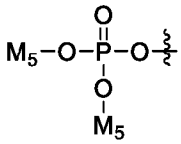
wherein R_{11} is as described in Formula VIB.

[0093] In some embodiments, provided herein is a compound represented by the structure of Formula VIB, Formula VIIB, or Formula VIIIB, wherein R_{11} is as described in Formula VIB.

[0094] In one embodiment, provided herein is a compound represented by the structure of

Formula IXB:  .

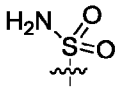
wherein

R_1 is OH,  ,  ,  ,  , ,  ,  , or ;

Q is a side chain of an amino acid;

M_1 is an alkyl;

M_2 is an aryl, a substituted aryl, a heteroaryl or a substituted heteroaryl;

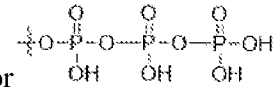
M_3 is  , , , or ;

M_4 is -(C_2-C_6)alkyl-O-(C_{10}-C_{20})alkyl;

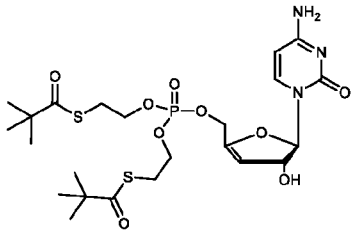
each M_5 is -$(CH_2)_n$-S-C(=O)-(C_1-C_8)alkyl;

n is 1-4; and

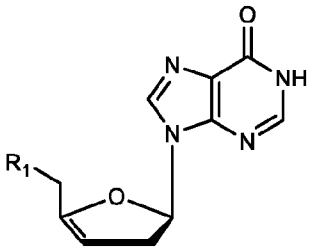
R_2 is -OH or -O-COO-alkyl.

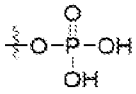
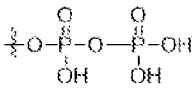
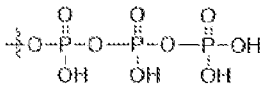
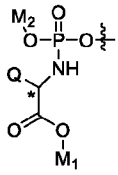
In another embodiment, in the compound of Formula IXB, if R_1 is OH or  , then R_2 is not OH.

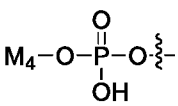
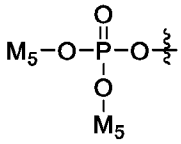
[0095] In another embodiment, a compound of Formula IXB, is represented by the structure of

Compound 6  .

[0096] In one embodiment, provided herein is a compound represented by the structure of

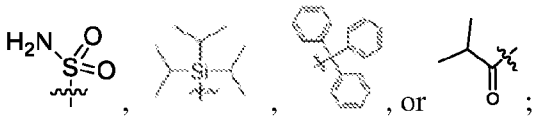
Formula XB:  , wherein

R_1 is OH,  ,  ,  ,  , ,

 , or  ;

Q is a side chain of an amino acid;M_1 is an alkyl;

M_2 is an aryl, a substituted aryl, a heteroaryl or a substituted heteroaryl;

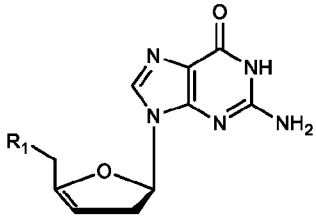
M_3 is  , or ;

M_4 is -(C_2-C_6)alkyl-O-(C_{10}-C_{20})alkyl;

each M_5 is -$(CH_2)_n$-S-C(=O)-(C_1-C_8)alkyl; and

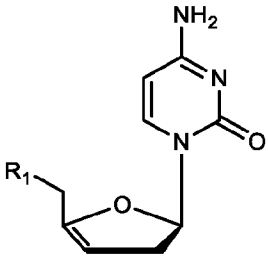
n is 1-4.

[0097] In one embodiment, provided herein is a compound, represented by the structure of

Formula XIB:  ,

wherein R_1 is as described in Formula XB.

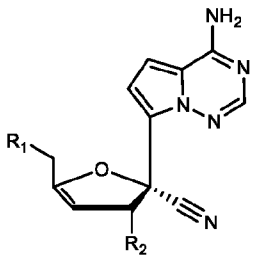
[0098] In one embodiment, provided herein is a compound, represented by the structure of

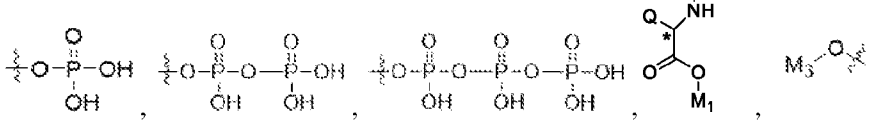
Formula XIIB:  ,

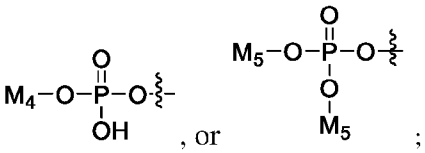
wherein R_1 is as described in Formula XB.

[0099] In one embodiment, provided herein is a compound, represented by the structure of Formula XB, Formula XIB, or Formula XIIB, wherein R_1 is as described in Formula XB.

[00100] In one embodiment, provided herein is a compound, represented by the structure of

formula XIIIB:  .

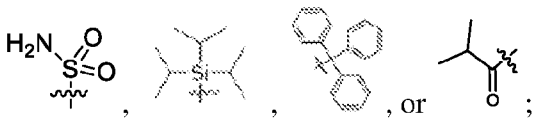
R_1 is OH,  ,



Q is a side chain of an amino acid;

M_1 is an alkyl;

M_2 is an aryl, a substituted aryl, a heteroaryl or a substituted heteroaryl;

M_3 is 

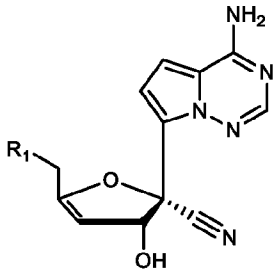
;

M_4 is -(C_2-C_6)alkyl-O-(C_{10}-C_{20})alkyl;

each M_5 is -$(CH_2)_n$-S-C(=O)-(C_1-C_8)alkyl;

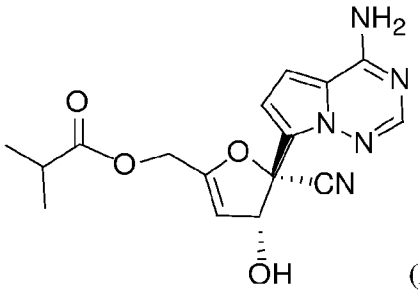
n is 1-4; and

R_2 is -OH or –O-COO-alkyl.

[00101] In another embodiment a compound of Formula XIIIB, is represented by the structure of Formula XIIIB1: 

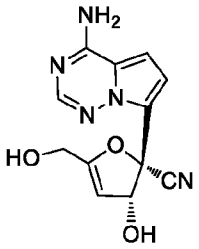
wherein R_1 is as defined in the structure of Formula XIIIB.

[00102] In another embodiment, a compound of Formula XIIIB1, is represented by the structure of Compound 100:

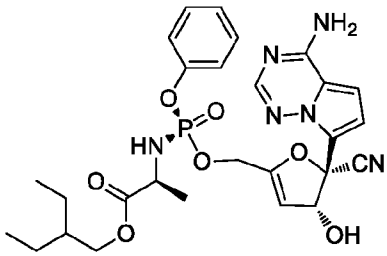


(AB23050).

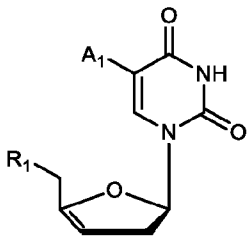
[00103] In another embodiment, a compound of Formula XIIIB1, is represented by the structure of Compound 101:

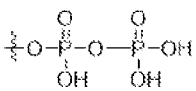
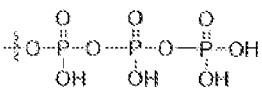
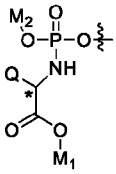
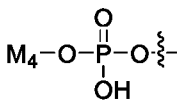
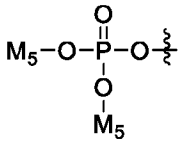

(AB23046).

[00104] In another embodiment, a compound of Formula XIIIB1, is represented by the structure of Compound 102:


(AB23048).

[00105] In one embodiment, provided herein is a compound represented by the structure of

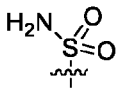
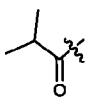
Formula XIVB  , wherein

R_1 is OH, ,  ,  ,  , ,  , or  ;

Q is a side chain of an amino acid;

M_1 is an alkyl;

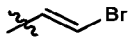
M_2 is an aryl, a substituted aryl, a heteroaryl or a substituted heteroaryl;

M_3 is  , , , or  ;

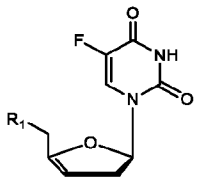
M_4 is -(C_2-C_6)alkyl-O-(C_{10}-C_{20})alkyl;

each M_5 is $-(CH_2)_n-S-C(=O)-(C_1-C_8)$alkyl;

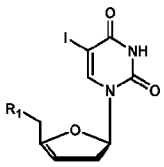
n is 1-4; and

A_1 is a halo a haloalkyl, an alkyl, or  .

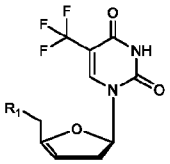
[00106] In another embodiment a compound of Formula XIVB, is represented by the structure of

Formula XIVB1:  wherein R_1 is as defined in the structure of Formula XIVB.

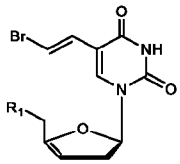
[00107] In another embodiment a compound of Formula XIVB, is represented by the structure of

Formula XIVB2:  , wherein R_1 is as defined in the structure of Formula XIVB.

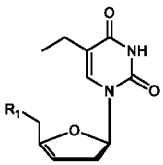
[00108] In another embodiment a compound of Formula XIVB, is represented by the structure of

Formula XIVB3:  , wherein R_1 is as defined in the structure of Formula XIVB.

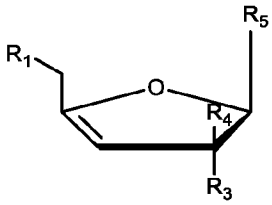
[00109] In another embodiment a compound of Formula XIVB, is represented by the structure of

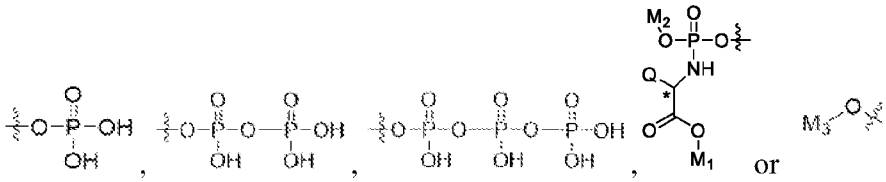
Formula XIVB4:  , wherein R_1 is as defined in the structure of Formula XIVB.

[00110] In one embodiment, provided herein is a compound of Formula XIVB, represented by the

structure of Formula XIVB5:  , wherein R1 is represented XIVB.

[00111] In one embodiment, provided herein is a compound represented by the structure of

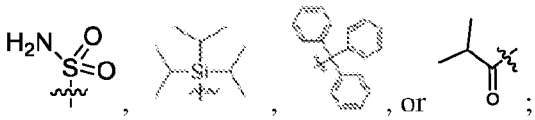
Formula XVB:  , wherein

R_1 is OH,  or ;

Q is a side chain of an amino acid;

M_1 is an alkyl;

M_2 is an aryl, a substituted aryl, a heteroaryl or a substituted heteroaryl;

M_3 is  ;

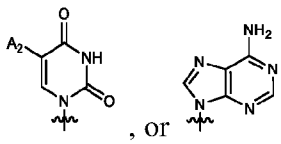
M_4 is -(C_2-C_6)alkyl-O-(C_{10}-C_{20})alkyl;

each M_5 is -$(CH_2)_n$-S-C(=O)-(C_1-C_8)alkyl;

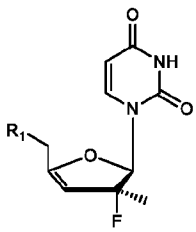
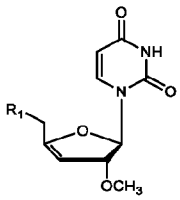
wherein n is 1-4;

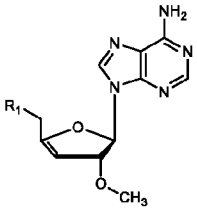
R_3 is H, a halo or an alkoxy;

R_4 is H, a halo or an alkyl;

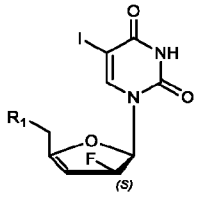
R_5 is  , and wherein A_2 is selected from the group consisting of H, a halo or an alkyl.

In another embodiment, in the compound of Formula XVB, R_3 is not the same as R_4.

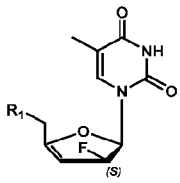
[00112] In another embodiment, a compound of Formula XVB, is represented by the structure of Formula XVB1:  , wherein R_1 is as defined in the structure of Formula XVB. In another embodiment, a compound of Formula XVB, is represented by the structure of Formula XVB2:  , wherein R_1 is as defined in the structure of Formula XVB. In another embodiment, a compound of Formula XVB, is represented by the structure of Formula XVB3:



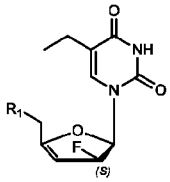
, wherein R_1 is as defined in the structure of Formula XVB. In another embodiment, a compound of Formula XVB, is represented by the structure of Formula XVB4:



, wherein R_1 is as defined in the structure of Formula XVB. In another embodiment, a compound of Formula XVB, is represented by the structure of Formula XVB5

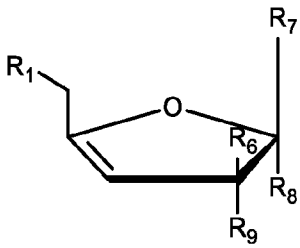


, wherein R_1 is as defined in the structure of Formula XVB. In another embodiment, a compound of Formula XVB, is represented by the structure of Formula XVB6



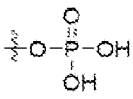
,wherein R_1 is as defined in the structure of Formula XVB.

[00113] In one embodiment, provided herein is a compound represented by the structure of Formula XVIB,

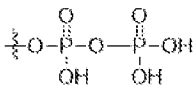


, wherein

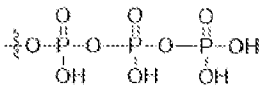
R_1 is of OH,



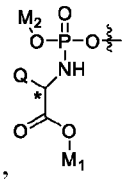
,



,

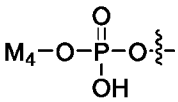


,

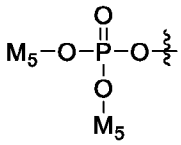


,

,



, or

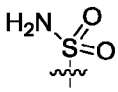
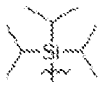
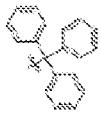


;

Q is a side chain of an amino acid;

M_1 is an alkyl;

M_2 is selected from the group consisting of an aryl, a substituted aryl, a heteroaryl or a substituted heteroaryl;

M_3 is  ,  ,  , or ;

M_4 is -(C_2-C_6)alkyl-O-(C_{10}-C_{20})alkyl;

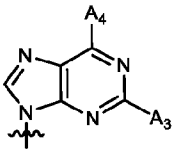
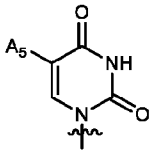
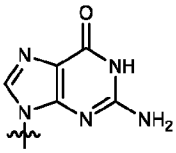
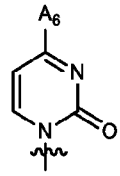
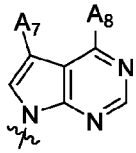
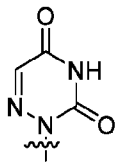
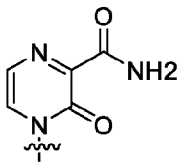
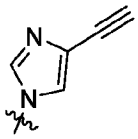
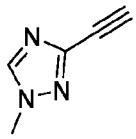
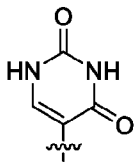
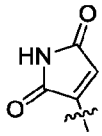
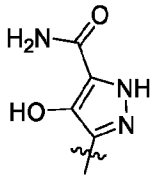
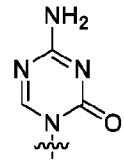
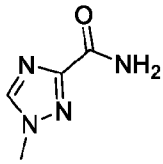
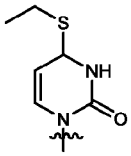
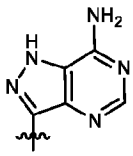
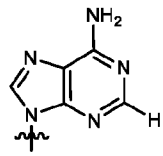
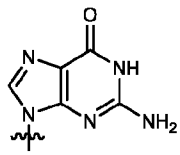
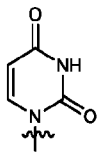
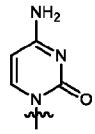
each M_5 is -$(CH_2)_n$-S-C(=O)-(C_1-C_8)alkyl;

n is 1-4;

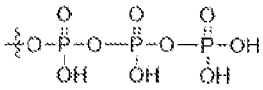
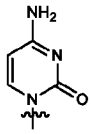
R_9 is H, OH, or -O-COO-alkyl;

R_6 is H, Me, -CCH or OH;

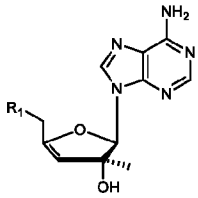
R_8 is H;

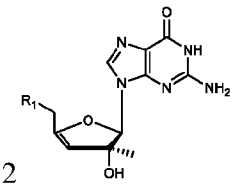
R_7 is  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  , or  , wherein A_3 is H, a halo or an amino; A_4 is H, an amino, an alkoxy, or an alkyl; A_5 is H, a halo, a hydroxy or an alkyne; A_6 is H, an amino or a hydroxylamino; A_7 is H or an amido; and A_8 is an amino or an alkyl. In another embodiment, in the compound of Formula XVIB if R_1 is OH and R_7 is  ,  ,  , or  ,

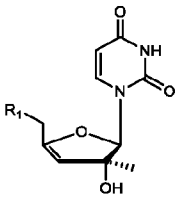
and R_9 is OH then R_6 is not H. In another embodiment, in the compound of Formula XVIB

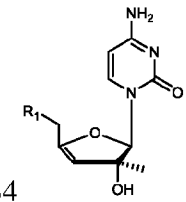
if R_1 is  and R_7 is  and R_9 is OH, then R_6 is not H.

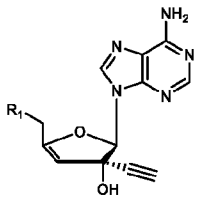
[00114] In another embodiment of formula XVIB, the compound is represented by the structure of

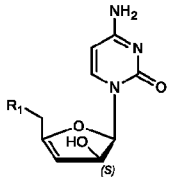
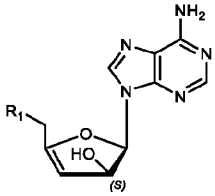
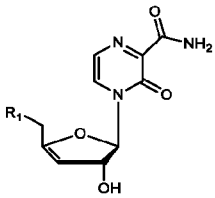
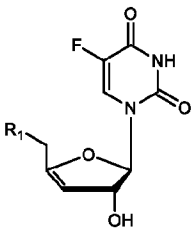
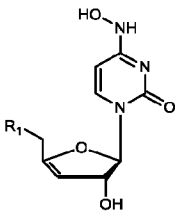
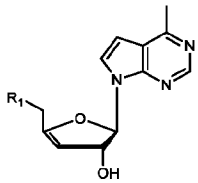
Formula XVIB1  wherein R_1 is as defined in the structure of Formula XVIB. In another embodiment a compound of Formula XVIB, is represented by the structure of Formula

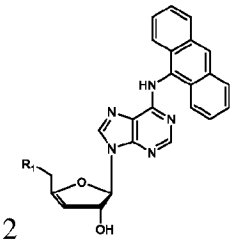
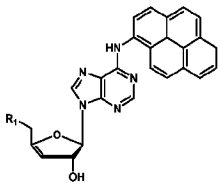
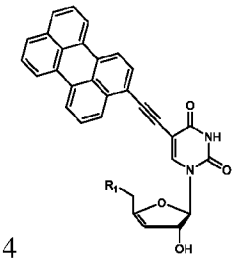
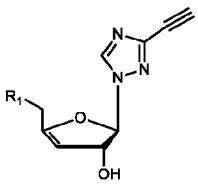
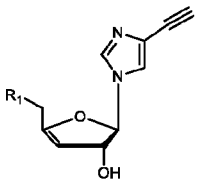
XVIB2  wherein R_1 is as defined in the structure of Formula XVIB. In another embodiment a compound of Formula XVIB, is represented by the structure of Formula XVIB3

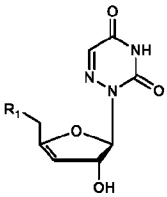
 wherein R_1 is as defined in the structure of Formula XVIB. In another embodiment a

compound of Formula XVIB, is represented by the structure of Formula XVIB4  wherein R_1 is as defined in the structure of Formula XVIB. In another embodiment a compound of

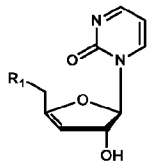
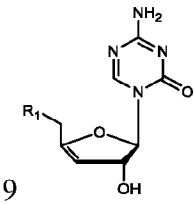
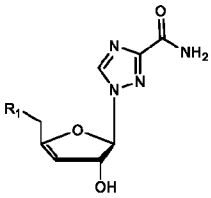
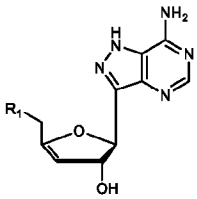
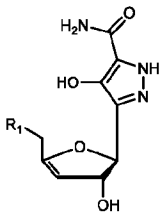
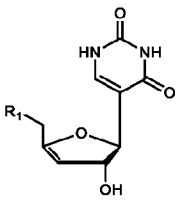
Formula XVIB, is represented by the structure of Formula XVIB5  wherein R_1 is as defined in the structure of Formula XVIB. In another embodiment a compound of Formula XVIB, is

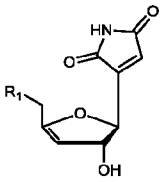
represented by the structure of Formula XVIB6  wherein R_1 is as defined in the structure of Formula XVIB. In another embodiment a compound of Formula XVIB, is represented by the structure of Formula XVIB7  wherein R_1 is as defined in the structure of Formula XVIB. In another embodiment a compound of Formula XVIB, is represented by the structure of Formula XVIB8  wherein R_1 is as defined in the structure of Formula XVIB. In another embodiment a compound of Formula XVIB, is represented by the structure of Formula XVIB9  wherein R_1 is as defined in the structure of Formula XVIB. In another embodiment a compound of Formula XVIB, is represented by the structure of Formula XVIB10  wherein R_1 is as defined in the structure of Formula XVIB. In another embodiment a compound of Formula XVIB, is represented by the structure of Formula XVIB11  wherein R_1 is as defined in the structure of Formula XVIB. In another embodiment a compound of

Formula XVIB, is represented by the structure of Formula XVIB12  wherein R_1 is as defined in the structure of Formula XVIB. In another embodiment a compound of Formula XVIB, is represented by the structure of Formula XVIB13  wherein R_1 is as defined in the structure of Formula XVIB. In another embodiment a compound of Formula XVIB, is represented by the structure of Formula XVIB14  wherein R_1 is as defined in the structure of Formula XVIB. In another embodiment a compound of Formula XVIB, is represented by the structure of Formula XVIB15  wherein R_1 is as defined in the structure of Formula XVIB. In another embodiment a compound of Formula XVIB, is represented by the structure of Formula XVIB16  wherein R_1 is as defined in the structure of Formula XVIB. In another embodiment a compound of Formula XVIB, is represented by the structure of Formula XVIB17

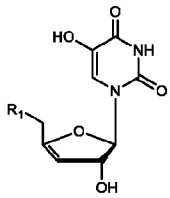


wherein R_1 is as defined in the structure of Formula XVIB. In another embodiment a

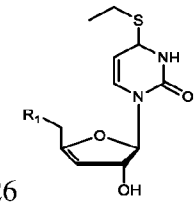
compound of Formula XVIB, is represented by the structure of Formula XVIB18 
wherein R_1 is as defined in the structure of Formula XVIB. In another embodiment a compound of Formula XVIB, is represented by the structure of Formula XVIB19 
wherein R_1 is as defined in the structure of Formula XVIB. In another embodiment a compound of Formula XVIB, is represented by the structure of Formula XVIB20 
wherein R_1 is as defined in the structure of Formula XVIB. In another embodiment a compound of Formula XVIB, is represented by the structure of Formula XVIB21 
wherein R_1 is as defined in the structure of Formula XVIB. In another embodiment a compound of Formula XVIB, is represented by the structure of Formula XVIB22 
wherein R_1 is as defined in the structure of Formula XVIB. In another embodiment a compound of Formula XVIB, is represented by the structure of Formula XVIB23 
wherein R_1 is as defined in the structure of Formula XVIB. In another embodiment a compound of Formula XVIB, is represented by the structure of Formula XVIB24



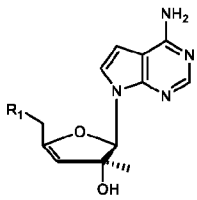
wherein R_1 is as defined in the structure of Formula XVIB. In another embodiment a compound of Formula XVIB, is represented by the structure of Formula XVIB25



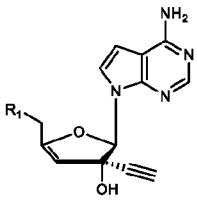
wherein R_1 is as defined in the structure of Formula XVIB. In another embodiment a compound of Formula XVIB, is represented by the structure of Formula XVIB26



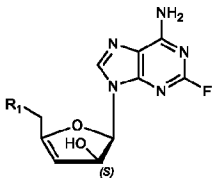
wherein R_1 is as defined in the structure of Formula XVIB. In another embodiment a compound of Formula XVIB, is represented by the structure of Formula XVIB27



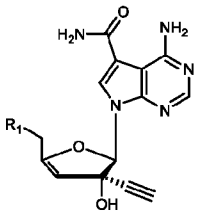
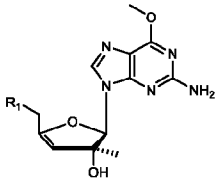
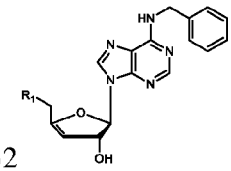
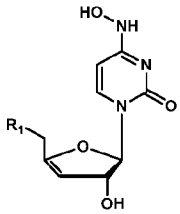
wherein R_1 is as defined in the structure of Formula XVIB. In another embodiment a compound of Formula XVIB, is represented by the structure of Formula XVIB28

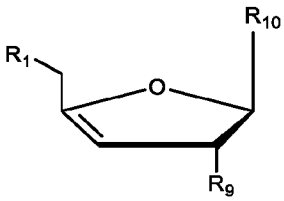


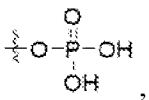
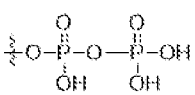
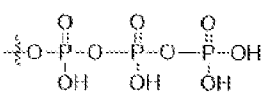
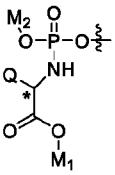
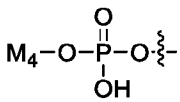
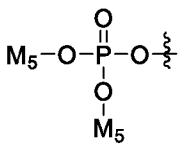
wherein R_1 is as defined in the structure of Formula XVIB. In another embodiment a compound of Formula XVIB, is represented by the structure of Formula XVIB29



wherein R_1 is as defined in the structure of Formula XVIB. In another embodiment a compound of Formula XVIB, is represented by the structure of Formula

XVIB30  wherein R_1 is as defined in the structure of Formula XVIB. In another embodiment a compound of Formula XVIB, is represented by the structure of Formula XVIB31  wherein R_1 is as defined in the structure of Formula XVIB. In another embodiment a compound of Formula XVIB, is represented by the structure of Formula XVIB32  wherein R_1 is as defined in the structure of Formula XVIB. In another embodiment a compound of Formula XVIB, is represented by the structure of Formula XVIB33  wherein R_1 is as defined in the structure of Formula XVIB.

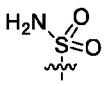
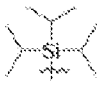
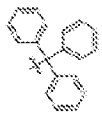
[00115] In one embodiment, provided herein is a compound represented by the structure of Formula XVIIB,  , wherein

R_1 is OH,  ,  ,  ,  , ,  , or  ;

Q is a side chain of an amino acid;

M_1 is an alkyl;

M_2 is an aryl, a substituted aryl, a heteroaryl or a substituted heteroaryl;

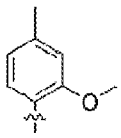
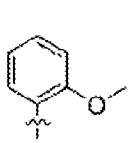
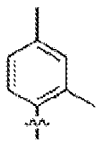
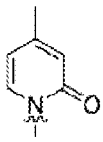
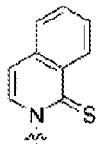
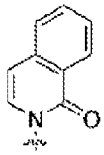
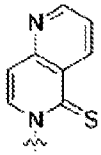
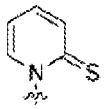
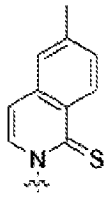
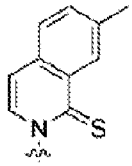
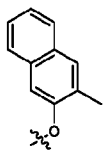
M_3 is  ,  ,  , or ;

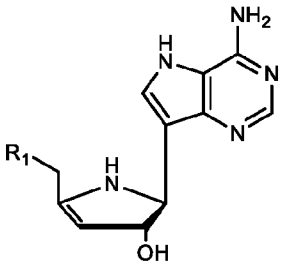
M_4 is -(C_2-C_6)alkyl-O-(C_{10}-C_{20})alkyl;

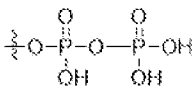
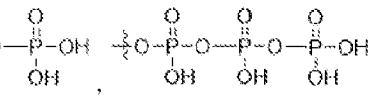
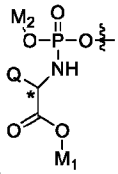
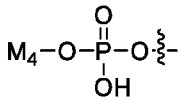
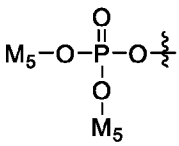
each M_5 is -$(CH_2)_n$-S-C(=O)-(C_1-C_8)alkyl;

n is 1-4;

R_9 is H, OH or -O-COO-alkyl; and

R_{10} is  ,  ,  , ,  ,  ,  ,  ,  ,  ,  , or  .

[00116] In one embodiment, provided herein is a compound, represented by the structure of Formula XVIIIB:  , wherein

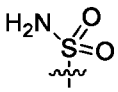
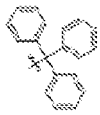
R_1 is OH, ,  ,  ,  , ,  , 

or ;

Q is a side chain of an amino acid;

M_1 is an alkyl;

M_2 is an aryl, a substituted aryl, a heteroaryl or a substituted heteroaryl;

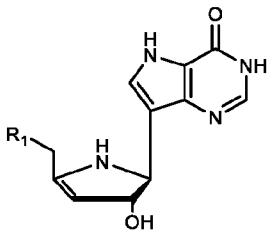
M_3 is  , ,  , or ;

M_4 is -(C_2-C_6)alkyl-O-(C_{10}-C_{20})alkyl;

each M_5 is -$(CH_2)_n$-S-C(=O)-(C_1-C_8)alkyl; and

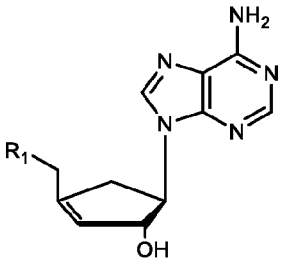
n is 1-4.

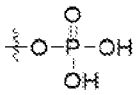
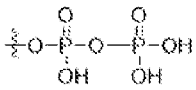
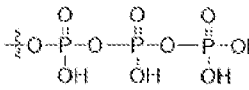
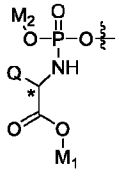
[00117] In one embodiment, provided herein is a compound represented by the structure of

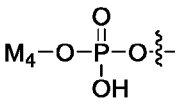
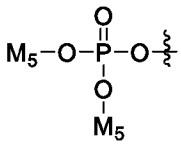
Formula XXB:  , wherein R_1 is as described in Formula XVIIIB.

[00118] In one embodiment, provided herein is a compound represented by the structure of Formula XVIIIB or Formula XXB, wherein R_1 is as described in Formula XVIIIB.

[00119] In one embodiment, provided herein is a compound represented by the structure of

Formula XIXB:  , wherein

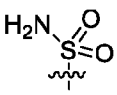
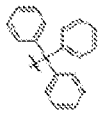
R_1 is OH,  ,  ,  ,  , ,

 , or  ;

Q is a side chain of an amino acid;

M_1 is an alkyl;

M_2 is an aryl, a substituted aryl, a heteroaryl or a substituted heteroaryl;

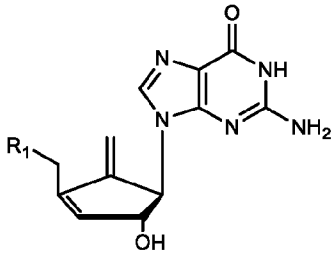
M_3 is  , ,  , or ;

M_4 is -(C_2-C_6)alkyl-O-(C_{10}-C_{20})alkyl;

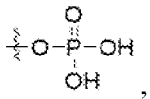
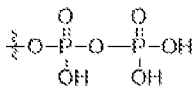
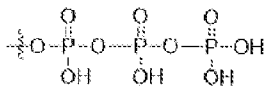
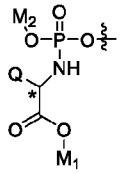
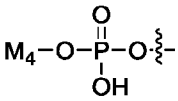
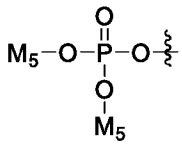
each M_5 is -$(CH_2)_n$-S-C(=O)-(C_1-C_8)alkyl; and

n is 1-4.

[00120] In one embodiment, provided herein is a compound represented by the structure of

Formula XXIIB:  ,

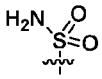
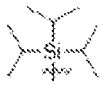
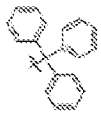
wherein

R_1 is OH,  ,  ,  ,  , ,  , or  ;

Q is a side chain of an amino acid;

M_1 is an alkyl;

M_2 is an aryl, a substituted aryl, a heteroaryl or a substituted heteroaryl;

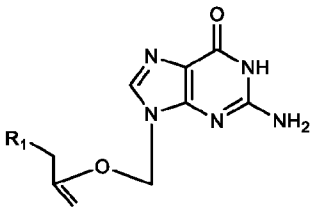
M_3 is  ,  ,  , or ;

M_4 is -(C_2-C_6)alkyl-O-(C_{10}-C_{20})alkyl;

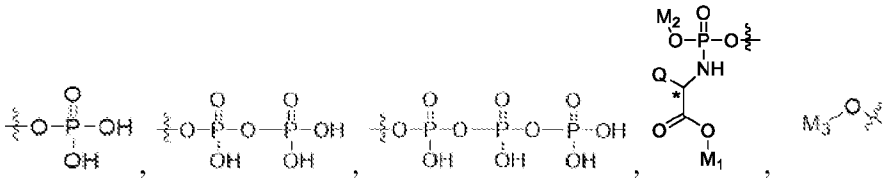
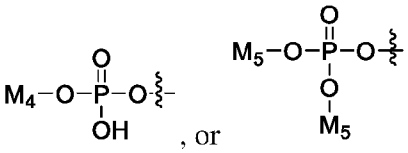
each M_5 is -$(CH_2)_n$-S-C(=O)-(C_1-C_8)alkyl; and

n is 1-4.

[00121] In one embodiment, provided herein is a compound represented by the structure of

Formula XXIIIB:  ,

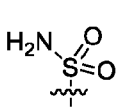
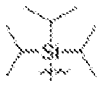
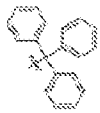
wherein

R_1 is OH,  ,  , or ;

Q is a side chain of an amino acid;

M_1 is an alkyl;

M_2 is an aryl, a substituted aryl, a heteroaryl or a substituted heteroaryl;

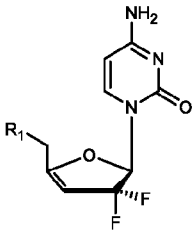
M_3 is  ,  ,  , or ;

M_4 is -(C_2-C_6)alkyl-O-(C_{10}-C_{20})alkyl;

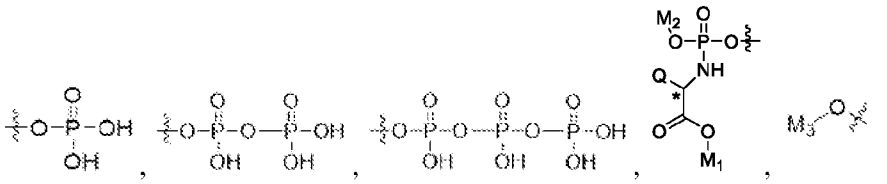
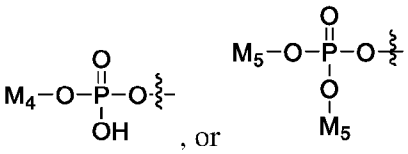
each M_5 is -$(CH_2)_n$-S-C(=O)-(C_1-C_8)alkyl; and

n is 1-4;

[00122] In one embodiment, provided herein is a compound represented by the structure of

Formula XXIVB:  ,

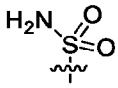
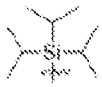
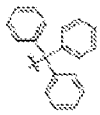
wherein

R_1 is OH,  ,  , or ;

Q is a side chain of an amino acid;

M_1 is an alkyl;

M_2 is an aryl, a substituted aryl, a heteroaryl or a substituted heteroaryl;

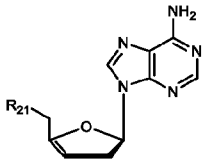
M_3 is  ,  ,  , or ;

M_4 is -(C_2-C_6)alkyl-O-(C_{10}-C_{20})alkyl;

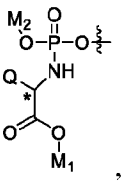
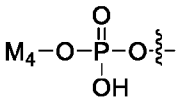
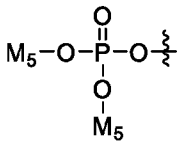
each M_5 is -$(CH_2)_n$-S-C(=O)-(C_1-C_8)alkyl; and

n is 1-4.

[00123] In one embodiment, provided herein is a compound represented by the structure of

Formula XXVB:  ,

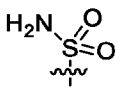
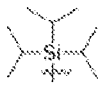
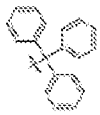
wherein

R_{21} is  , ,  , or  ;

Q is a side chain of an amino acid;

M_1 is an alkyl;

M_2 is an aryl, a substituted aryl, a heteroaryl or a substituted heteroaryl;

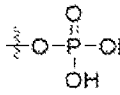
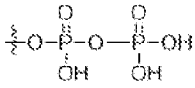
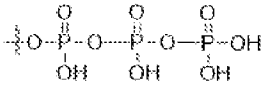
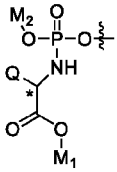
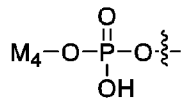
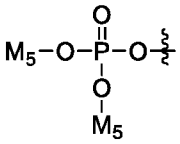
M_3 is  ,  ,  , or ;

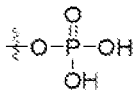
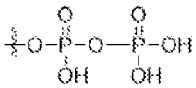
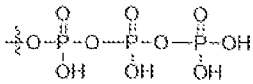
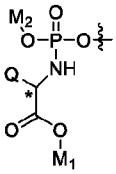
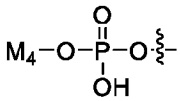
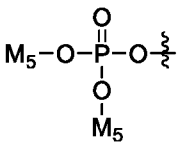
M_4 is -(C_2-C_6)alkyl-O-(C_{10}-C_{20})alkyl;

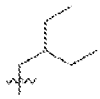
each M_5 is -$(CH_2)_n$-S-C(=O)-(C_1-C_8)alkyl; and

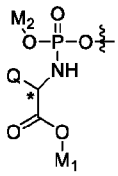
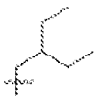
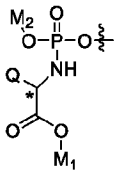
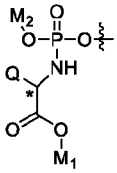
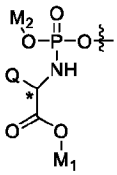
n is 1-4.

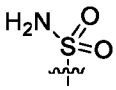
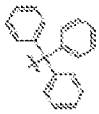
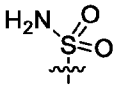
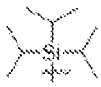
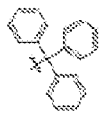
[00124] In one embodiment, R_1 of Formula IB, Formula IIB, Formula IIIB, Formula IVB, Formula VB, Formula IXB, Formula XB, Formula XIB, Formula XIIB, Formula XIIIB, Formula XIIIB1, Formula XIVB, Formula XIVB1, Formula XIVB2, Formula XIV3, Formula XIV4, Formula XIV5, Formula XVB, Formula XVB1, Formula XVB2, Formula XVB3, Formula XVB4, Formula XVB5, Formula XVB6, Formula XVIB, Formula XVIB1, Formula XVIB2, Formula XVIB3, Formula XVIB4, Formula XVIB5, Formula XVIB6, Formula XVIB7, Formula XVIB8, Formula XVIB9,

Formula XVIB10, Formula XVIB11, Formula XVIB12, Formula XVIB13, Formula XVIB14, Formula XVIB15, Formula XVIB16, Formula XVIB17, Formula XVIB18, Formula XVIB19, Formula XVIB20, Formula XVIB21, Formula XVIB22, Formula XVIB23, Formula XVIB24, Formula XVIB25, Formula XVIB26, Formula XVIB27, Formula XVIB28, Formula XVIB29, Formula XVIB30, Formula XVIB31, Formula XVIB32, Formula XVIB33, Formula XVIIB, Formula XVIIIB, Formula XXB, Formula XIXB, Formula XXIB, Formula XXIIB, Formula XXXIIIB, or Formula XXIVB is OH,  ,  ,  ,  , ,  ,  ; [00125]

In another embodiment, R_1 is OH. In another embodiment, R_1 is  . In another embodiment, R_1 is  . In another embodiment, R_1 is  . In another embodiment, R_1 is  . In another embodiment, R_1 is  . In another embodiment, R_1 is  . In one embodiment, Q of R_1 is a side chain of an amino acid.

[00126] In one embodiment, M_1 of R_1 is an alkyl. In another embodiment, M_1 is . In another embodiment, M_1 is . In another embodiment, M_1 is  . In one embodiment, M_2 of R_1, is an aryl, a substituted aryl, a heteroaryl or a substituted heteroaryl. In another embodiment, M_2 is . In another embodiment, M_2 is . In another embodiment, M_2 is an aryl. In another

embodiment, M_2 is a substituted aryl. In another embodiment, M_2 is a heteroaryl. In another embodiment, M_2 is an a substituted heteroaryl. In one embodiment, R_1 is  , wherein M_1 is  and M_2 is . In another embodiment, the chiral carbon of  is an S. In another embodiment, the chiral carbon of  is an R. In another embodiment, the chiral carbon of  is a racemate.

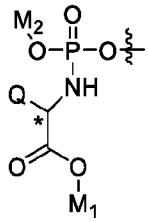
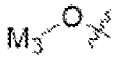
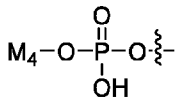
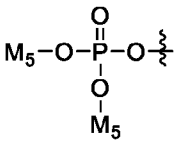
In one embodiment, M_3 of R_1 is  , ,  or .In another embodiment, M_3 is  . In another embodiment, M_3 is  . In another embodiment, M_3 is  . In another embodiment, M_3 is . In one embodiment, M_4 of R_1 is -(C_2-C_6)alkyl-O-(C_{10}-C_{20})alkyl. In another embodiment, M_4 is -$(CH_2)_3$-O-$(CH_2)_{15}CH_3$. In one embodiment, each M_5 of R_1, is -$(CH_2)_n$-S-C(=O)-(C_1-C_8)alkyl. In another embodiment, each M_5 is -$(CH_2)_2$-S-C(=O)-$C(CH_3)_3$. In one embodiment n of M_5 is 1-4. In another embodiment, n is 1. In another embodiment, n is 2. In another embodiment, n is 3. In another embodiment, n is 4.

[00127] In some embodiments, a compound described herein comprises a prodrug. In some embodiments, embodiment, the ddh or deoxy-ddh products or prodrugs thereof, or active metabolites thereof can be synthesized and administered directly to cells or a subject. Upon entering the cells, these ddh or deoxy-ddh products or prodrugs thereof can be phosphorylated by one or more viral or cellular kinases to produce the active metabolites that inhibit DNA/RNA replication. In one

embodiment, the ddh or deoxy-ddh products comprise a prodrug.

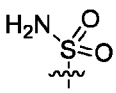
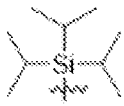
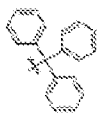
[00128] One skilled in the art would appreciate that the term "prodrug" may in certain embodiments, encompass any compound that when administered to a biological system could be converted into an active compound or metabolite thereof as a result of spontaneous chemical reaction(s), enzyme catalyzed chemical reaction(s), photolysis, and/or metabolic chemical reaction(s). The active compound or metabolites in the present disclosure are the DNA/RNA chain terminators, which in some embodiments comprise the products produced by the pVip or synthesized using methods known in the art, from non-natural substrates as described herein. pVips, the nucleotide sequence encoding them, and their activities are described in detail elsewhere in this application.

[00129] In some embodiments, a prodrug facilitates the crossing of the plasma membrane of a cell by the compound. In some embodiments, the prodrug form of a compound facilitates passive diffusion through the cell membrane by masking negative charge until the compound is within the cell.

[00130] In some embodiments, a prodrug comprises a protective chemical group. In some embodiments, a protective chemical group comprises a  ,  ,  , or  ;

; Q is a side chain of an amino acid; M_1 is an alkyl;

M_2 is an aryl, a substituted aryl, a heteroaryl or a substituted heteroaryl;

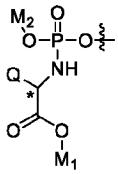
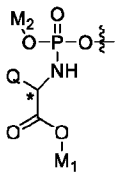
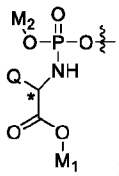
M_3 is  ,  ,  , or ,

M_4 is -(C_2-C_6)alkyl-O-(C_{10}-C_{20})alkyl;

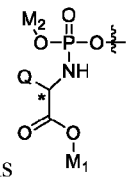
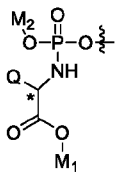
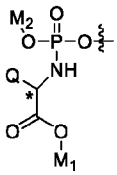
each M_5 is -$(CH_2)_n$-S-C(=O)-(C_1-C_8)alkyl;

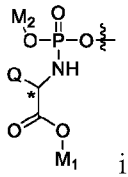
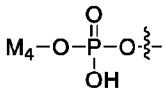
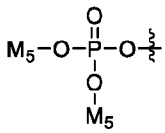
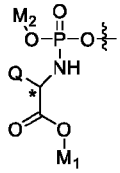
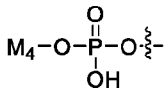
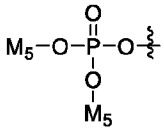
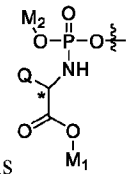
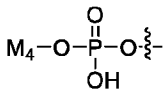
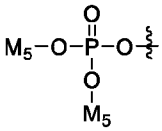
wherein n is 1-4;

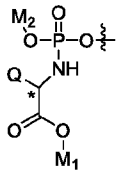
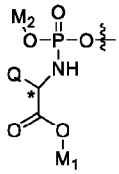
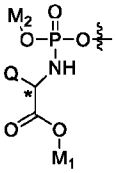
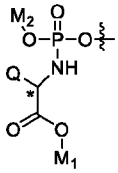
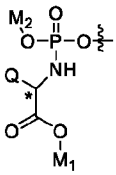
at the R_1, R_{11}, or R_{21} position in a compound disclosed herein.

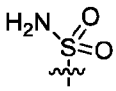
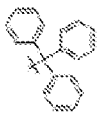
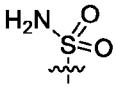
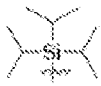
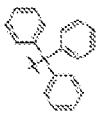
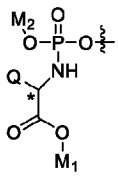
In another embodiment, the chiral carbon of  is an S. In another embodiment, the chiral carbon of  is an R. In another embodiment, the chiral carbon of  is a racemate.

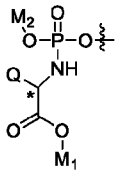
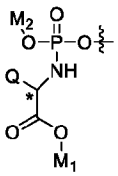
[00131] In some embodiments, the present disclosure includes all forms of prodrugs that are covalently modified analogs or latent forms of the therapeutically active metabolites (the DNA/RNA chain terminators). In one embodiment, the prodrugs comprise the ddh or deoxy-ddh compounds described herein or modified structures thereof. The prodrug form can serve to enhance solubility, absorption and lipophilicity to optimize drug delivery, bioavailability and efficacy of the comprise the ddh or deoxy-ddh compounds. In one embodiment, a prodrug comprises the comprise the ddh or deoxy-ddh compounds with a chemical structure that can be oxidized, reduced, aminated, deaminated, esterified, deesterified, alkylated, dealkylated, acylated, deacylated, phosphorylated, dephosphorylated, photolyzed, hydrolyzed, or other functional group change or conversion to produce the therapeutically active metabolite (the DNA/RNA chain terminators), or produce the active metabolite that can be transported across cell membrane. Enzymes which are capable of enzymatic activation of prodrugs include, but are not limited to, amidases, esterases, microbial enzymes, phospholipases, cholinesterases, and phosphases. Designs and uses of prodrugs are generally known in the art, e.g. Bundgaard, Hans, "Design and Application of Prodrugs" in *Textbook of Drug Design and Development* (1991), P. Krogsgaard-Larsen and H. Bundgaard, Eds. Harwood Academic Publishers.

In one embodiment, R_1 is  . In another embodiment, the chiral carbon of  is an S. In another embodiment, the chiral carbon of  is an R. In another embodiment, the chiral

carbon of  is a racemate. In another embodiment, R_1 is . In another embodiment, R_1 is  . In another embodiment, R_1 is  . In one embodiment, R_{11} is  . In another embodiment, R_{11} is . In another embodiment, R_{11} is  . In another embodiment, R_{11} is  . In one embodiment, R_{21} is  . In another embodiment, R_{21} is . In another embodiment, R_{21} is  . In another embodiment, R_{21} is  . In one embodiment, Q of R_1 is a side chain of an amino acid. In one embodiment, M_1 of R_1, R_{11}, R_{21} is an alkyl. In another embodiment, M_1 is . In another embodiment, M_1 is . In another embodiment, M_1 is . In another embodiment, M_1 is an alkyl. In one embodiment, M_2 of R_1, R_{11} or R_{21} is an aryl, a substituted aryl, a heteroaryl or a substituted heteroaryl. In another embodiment, M_2 is . In another embodiment, M_2 is . In another embodiment, M_2 is an aryl. In another embodiment, M_2 is a substituted aryl. In another embodiment, M_2 is a heteroaryl. In another embodiment, M_2 is an a substituted

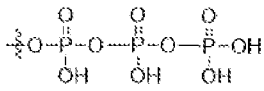
heteroaryl. In one embodiment, R_1, R_{11} or R_{21} is  , wherein M_1 is and M_2 is . In one embodiment, R_1, R_{11} or R_{21} is  , wherein M_1 is , M_2 is and Q is methyl. In one embodiment, Q of R_1, R_{11} or R_{21} is methyl. In one embodiment, the chiral carbon of  is an S. In another embodiment, the chiral carbon of  is an R. In another embodiment, the chiral carbon of  is a racemate.

In one embodiment, M_3 of R_1, R_{11}, or R_{21} is  , ,  or .In another embodiment, M_3 is  . In another embodiment, M_3 is  . In another embodiment, M_3 is  . In another embodiment, M_3 is . In one embodiment, M_4 of R_1, R_{11}, or R_{21} is -(C_2-C_6)alkyl-O-(C_{10}-C_{20})alkyl. In another embodiment, M_4 is -$(CH_2)_3$-O-$(CH_2)_{15}CH_3$. In one embodiment, each M_5 of R_1, R_{11}, or R_{21} is -$(CH_2)_n$-S-C(=O)-(C_1-C_8)alkyl. In another embodiment, each M_5 is -$(CH_2)_2$-S-C(=O)-$C(CH_3)_3$. In one embodiment n of M_5 is 1-4. In another embodiment, n is 1. In another embodiment, n is 2. In another embodiment, n is 3. In another embodiment, n is 4. In another embodiment, the chiral carbon of  of R_1, R_{11} or R_{21} is an S. In another embodiment,

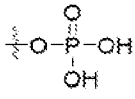
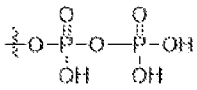
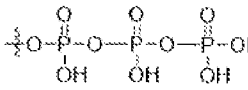
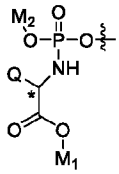
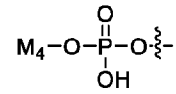
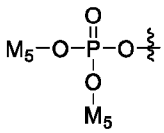
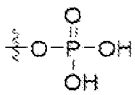
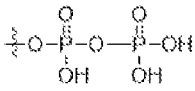
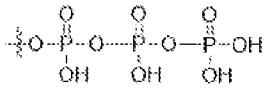
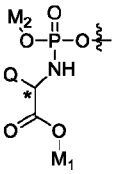
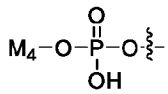
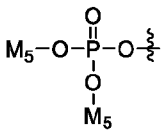
the chiral carbon of  of R_1, R_{11} or R_{21} is an R. In another embodiment, the chiral carbon of  of R_1, R_{11} or R_{21} is a racemate.

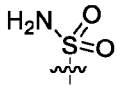
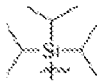
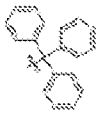
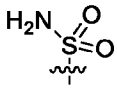
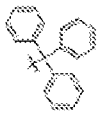
[00132] In one embodiment, R_2 of Formula IB, Formula IIB, Formula IIIB, Formula IVB, Formula VB, Formula IXB and Formula XIIIB is OH or -O-COO-alkyl. In another embodiment, R_2 is OH. In another embodiment, R_2 is -O-COO-alkyl.

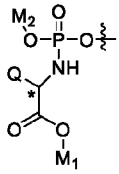
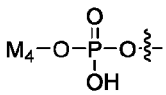
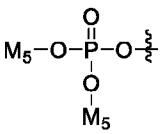
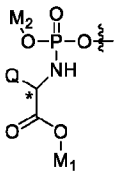
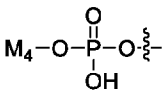
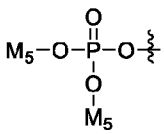
[00133] In one embodiment, if R_1 of Formula IB, Formula IIB, Formula IIIB, Formula IVB, Formula VB is OH, then R_2 of is -O-COO-alkyl.

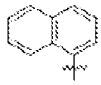
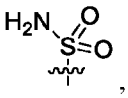
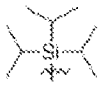
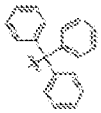
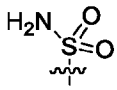
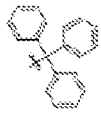
[00134] In one embodiment, if R_1 of Formula IXB is OH or  , then R_2 is not OH.

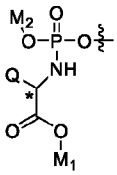
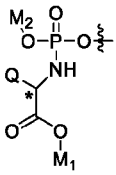
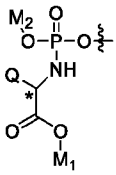
[00135]

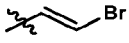
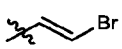
In one embodiment, R_{11} of Formula VB1, Formula VIB, Formula VIIB or Formula VIIIB is  ,  ,  ,  , ,  , or  . In another embodiment, R_{11} is  . In another embodiment, R_{11} is  . In another embodiment, R_{11} is  . In another embodiment, R_{11} is  . In another embodiment, R_{11} is . In another embodiment, R_{11} is  . In another embodiment, R_{11} is  .

acid. In one embodiment, M_1 of R_{11} is an alkyl. In another embodiment, M_1 is . In another embodiment, M_1 is . In another embodiment, M_1 is . In one embodiment, M_2 of R_{11} is an aryl, a substituted aryl, a heteroaryl or a substituted heteroaryl. In another embodiment, M_2 is . In another embodiment, M_2 is . In another embodiment, M_2 is an aryl. In another embodiment, M_2 is a substituted aryl. In another embodiment, M_2 is a heteroaryl. In another embodiment, M_2 is a substituted heteroaryl. In one embodiment, M_3 of R_{11} is  ,  ,  or .In another embodiment, M_3 is  . In another embodiment, M_3 is . In another embodiment, M_3 is  . In another embodiment, M_3 is . In one embodiment, M_4 of R_{11} is -(C_2-C_6)alkyl-O-(C_{10}-C_{20})alkyl. In another embodiment, M_4 is -$(CH_2)_3$-O-$(CH_2)_{15}CH_3$. In one embodiment, each M_5 of R_{21} is -$(CH_2)_n$-S-C(=O)-(C_1-C_8)alkyl. In another embodiment, each M_5 is -$(CH_2)_2$-S-C(=O)-$C(CH_3)_3$. In one embodiment n of M_5 is 1-4. In another embodiment, n is 1. In another embodiment, n is 2. In another embodiment, n is 3. In another embodiment, n is 4.

[00136] In one embodiment, R_{21} of Formula XXVB is  , ,  , or  . In another embodiment, R_{21} is  . In another embodiment, R_{21} is . In another embodiment, R_{21} is  . In another embodiment, R_{21} is  . In one embodiment, Q of R_{21} is a side chain of an amino acid. In one embodiment, M_1 of R_{21} is an alkyl. In

another embodiment, M_1 is . In another embodiment, M_1 is . In another embodiment, M_1 is . In one embodiment, M_2 of R_{21} is , or an aryl. In another embodiment, M_2 is . In another embodiment, M_2 is  . In another embodiment, M_2 is an aryl. In one embodiment, M_3 of R_{21} is  ,  ,  or .In another embodiment, M_3 is  . In another embodiment, M_3 is . In another embodiment, M_3 is  . In another embodiment, M_3 is . In one embodiment, M_4 of R_{21} is -(C_2-C_6)alkyl-O-(C_{10}-C_{20})alkyl. In another embodiment, M_4 is -$(CH_2)_3$-O-$(CH_2)_{15}CH_3$. In one embodiment, each M_5 of R_{21} is -$(CH_2)_n$-S-C(=O)-(C_1-C_8)alkyl. In another embodiment, each M_5 is -$(CH_2)_2$-S-C(=O)-$C(CH_3)_3$. In one embodiment n of M_5 is 1-4. In another embodiment, n is 1. In another embodiment, n is 2. In another embodiment, n is 3. In another embodiment, n is 4.

In another embodiment, the chiral carbon of  is an S. In another embodiment, the chiral carbon of  is an R. In another embodiment, the chiral carbon of  is a racemate.

[00137] In one embodiment, A_1 of Formula XIVB is a halo, a haloalkyl, an alkyl, or  . In another embodiment, A_1 is a halo. In another embodiment, A_1 is a haloalkyl. In another embodiment, A_1 is an alkyl. In another embodiment, A_1 is  .

[00138] In one embodiment, R_3 of Formula XVB is H, a halo or an alkoxy. In another embodiment, R_3 is H. In another embodiment, R_3 is a halo. In another embodiment, R_3 is an alkoxy.

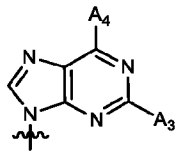
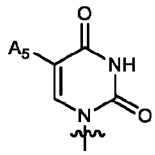
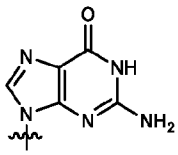
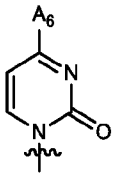
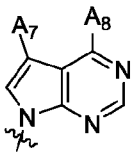
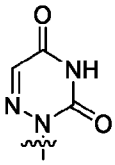
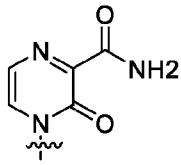
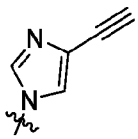
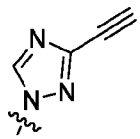
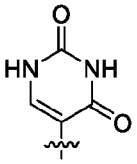
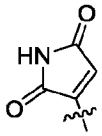
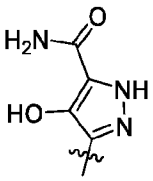
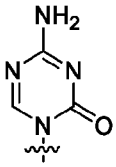
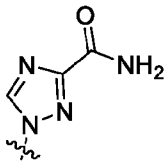
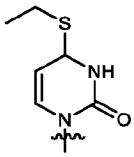
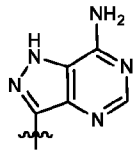
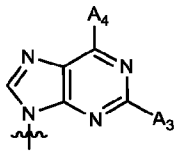
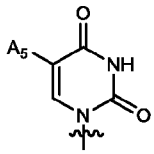
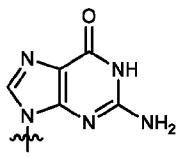
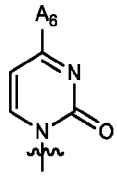
[00139] In one embodiment, R_4 of Formula XVB is H, a halo, or an alkyl. In another embodiment, R_4 is H. In another embodiment, R_4 is a halo. In another embodiment, R_4 is an alkyl.

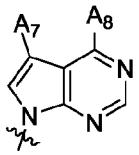
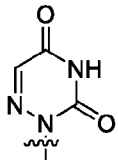
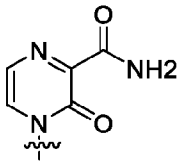
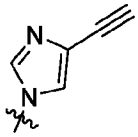
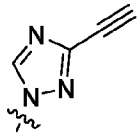
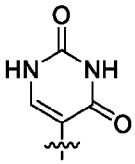
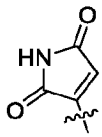
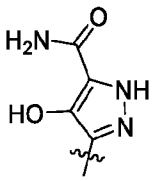
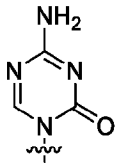
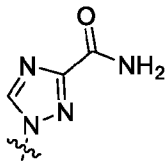
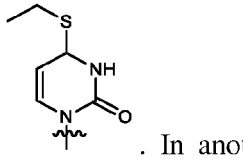
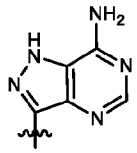
[00140] In one embodiment, R_3 of Formula XVB is not the same as R_4.

[00141] In one embodiment, R_9 of Formula XVIB and Formula XVIIB is H, OH, or -O-COO-alkyl. In another embodiment, R_9 is H. In another embodiment, R_9 is OH. In another embodiment, R_9 is -O-COO-alkyl.

[00142] In one embodiment, R_6 of Formula XVIB is H, OH, -CCH or Me. In another embodiment, R_6 is H. In another embodiment, R_6 is OH. In another embodiment, R_6 is -CCH. In another embodiment, R_6 is Me.

[00143] In one embodiment, R_8 of Formula XVIB is H or CN. In another embodiment, R_8 is H. In another embodiment, R_8 is CN.

[00144] In one embodiment, R_7 of Formula XVIB is  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  or  . In another embodiment, R_7 is  . In another embodiment, R_7 is  . In another embodiment, R_7 is  . In another embodiment, R_7 is  . In another embodiment,

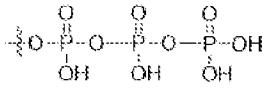
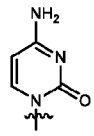
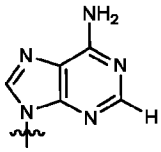
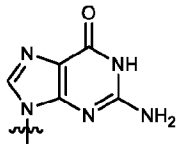
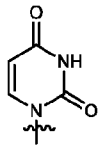
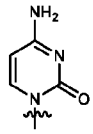
R_7 is  . In another embodiment, R_7 is  . In another embodiment, R_7 is  . In another embodiment, R_7 is  .In another embodiment, R_7 is  . In another embodiment, R_7 is  . In another embodiment, R_7 is  . In another embodiment, R_7 is  . In another embodiment, R_7 is  . In another embodiment, R_7 is  . In another embodiment, R_7 is  . In another embodiment, R_7 is . In another embodiment, R_7 is . In another embodiment, R_7 is  .

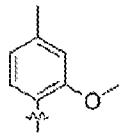
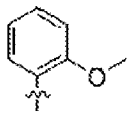
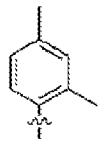
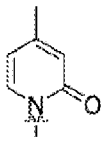
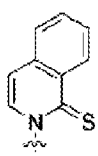
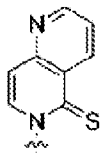
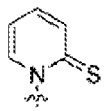
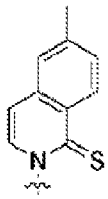
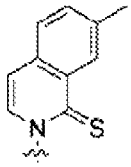
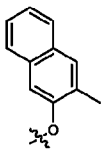
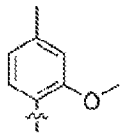
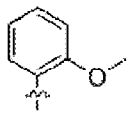
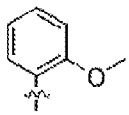
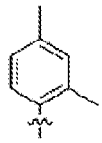
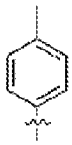
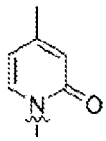
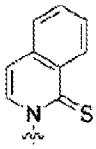
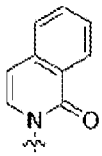
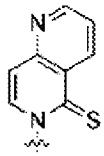
[00145] In one embodiment, A_3 of R_7 is H, a halo or an amino. In another embodiment, A_3 is H. In another embodiment, A_3 is a halo. In another embodiment, A_3 is an amino.

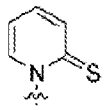
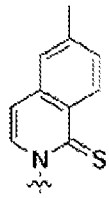
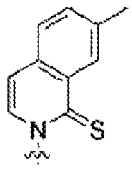
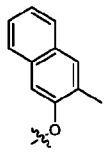
In one embodiment, A_4 of R_7 is H, an amino, or an alkyl. In another embodiment, A_4 is H. In another embodiment, A_4 is an amino. In another embodiment, A_4 is an alkoxy. In another embodiment, A_4 is an alkyl.

[00146] In one embodiment, A_5 of R_7 is H, a halo, a hydroxy or an alkyne. In another embodiment, A_5 is H. In another embodiment, A_5 is a halo. In another embodiment, A_5 is a hydroxy. In another embodiment, A_5 is an alkyne. In one embodiment, A_6 of R_7 is H, an amino, or hydroxylamino. In another embodiment, A_6 is H. In another embodiment, A_6 is an amino. In another embodiment, A_6 is a hydroxylamino. In one embodiment, A_7 of R_7 is H or an amino. In another embodiment, A_7 is H. In another embodiment, A_7 is an amido. In one embodiment, A_8 of R_7 is an amino or an alkyl. In another

embodiment, A_8 is an amino. In another embodiment, A_8 is an alkyl.

[00147] In one embodiment, if R_1 of Formula XVIB is  and R_7 is  and R_9 is OH, then R_6 is not H. In one embodiment, if R_1 Formula XVIB is OH and R_7 is  ,  ,  or  , and R_9 is OH, then R_6 is not H.

[00148] In one embodiment, R_{10} of a compound of Formula XVIIB is  ,  ,  , ,  ,  ,  ,  ,  ,  , or  . In another embodiment, R_{10} o is  .In another embodiment, R_{10} is  In another e embodiment, R_{10} is  .In another embodiment, R_{10} is  . In another embodiment, R_{10} is  . In another embodiment, R_{10} is  .In another embodiment, R_{10} is  . In another embodiment, R_{10} is  . In another embodiment, R_{10} is  . In

another embodiment, R_{10} is  . In another embodiment, R_{10} is  . In one embodiment, R_{10} is  . In another embodiment, R_{10} is  .

[00149] As used herein, the term alkyl, used alone or as part of another group, refers, in one embodiment, to a "C_1 to C_{18} alkyl" and denotes linear and branched, saturated or unsaturated (e.g., alkenyl, alkynyl) groups, the latter only when the number of carbon atoms in the alkyl chain is greater than or equal to two, and can contain mixed structures. Non-limiting examples are alkyl groups containing from 1 to 6 carbon atoms (C_1 to C_6 alkyls), or alkyl groups containing from 1 to 4 carbon atoms (C_1 to C_4 alkyls). Examples of saturated alkyl groups include, but are not limited to, methyl, ethyl, n-propyl, isopropyl, n-butyl, iso-butyl, sec-butyl, tert-butyl, amyl, tert-amyl and hexyl. Examples of alkenyl groups include, but are not limited to, vinyl, allyl, butenyl and the like. Examples of alkynyl groups include, but are not limited to, ethynyl, propynyl and the like. Similarly, the term "C_1 to C_{12} alkylene" denotes a bivalent radical of 1 to 12 carbons.

[00150] The alkyl group can be unsubstituted, or substituted with one or more substituents selected from the group consisting of halogen, hydroxy, alkoxy, aryloxy, alkylaryloxy, heteroaryloxy, oxo, cycloalkyl, phenyl, heteroaryls, heterocyclyl, naphthyl, amino, alkylamino, arylamino, heteroarylamino, dialkylamino, diarylamino, alkylarylamino, alkylheteroarylamino, arylheteroarylamino, acyl, acyloxy, nitro, carboxy, carbamoyl, carboxamide, cyano, sulfonyl, sulfonylamino, sulfinyl, sulfinylamino, thiol, alkylthio, arylthio, or alkylsulfonyl groups. Any substituents can be unsubstituted or further substituted with any one of these aforementioned substituents.

[00151] The term "haloalkyl" used herein alone or as part of another group, refers to, in some embodiments, to an alkyl group as defined above, which is substituted by one or more halogen atoms, e.g. by F, Cl, Br or I.

[00152] The term "alkoxy" used herein alone or as part of another group, refers to the –O-(alkyl) group, where the point of attachment is through the oxygen-atom and the alkyl group is as defined hereinbefore.

[00153] The term "alkyne" used herein alone or as part of another group, refers to an alkyl as

defined above with at least one triple bond. The "alkyne" in some embodiment, refer to have 2 to 20 carbon atoms (C_2-C_{18} alkyne). The Alkyne, in some embodiments, is unsubstituted. The Alkyne, in some embodiments, is substituted with one or more substituents selected from the group consisting of aryl, halogen, hydroxy, alkoxy, aryloxy, alkylaryloxy, heteroaryloxy, oxo, cycloalkyl, phenyl, heteroaryls, heterocyclyl, naphthyl, amino, alkylamino, arylamino, heteroarylamino, dialkylamino, diarylamino, alkylarylamino, alkylheteroarylamino, arylheteroarylamino, acyl, acyloxy, nitro, carboxy, carbamoyl, carboxamide, cyano, sulfonyl, sulfonylamino, sulfinyl, sulfinylamino, thiol, alkylthio, arylthio, or alkylsulfonyl groups. Any substituents can be unsubstituted or further substituted with any one of these aforementioned substituents.

[00154] "Alkenyl" is a hydrocarbon containing normal, secondary, tertiary or cyclic carbon atoms with at least one site of unsaturation, i.e., a carbon-carbon, sp^2 double bond. For example, an alkenyl group can have 2 to 20 carbon atoms (i.e., C_2-C_{20} alkenyl), 2 to 8 carbon atoms (i.e., C_2-C_8 alkenyl), 2 to 6 carbon atoms (i.e., C_2-C_6 alkenyl) or 2 to 4 carbon atoms (i.e., C_2-C_4 alkenyl). Examples of suitable alkenyl groups include, but are not limited to, ethenyl or vinyl (both having a structure -$CH{=}CH_2$), allyl (-$CH_2CH{=}CH_2$), cyclopentenyl (-C_5H_7), and 5-hexenyl (-$CH_2CH_2CH_2CH_2CH{=}CH_2$).

[00155] "Alkynyl" is a hydrocarbon containing normal, secondary, tertiary or cyclic carbon atoms with at least one site of unsaturation, i.e., a carbon-carbon, sp triple bond. For example, an alkynyl group can have 2 to 20 carbon atoms (i.e., C_2-C_{20} alkynyl), 2 to 8 carbon atoms (i.e., C_2-C_8 alkyne,), 2 to 6 carbon atoms (i.e., C_2-C_6 alkynyl), or 2 to 4 carbon atoms (i.e., C_2-C_4 alkynyl). Examples of suitable alkynyl groups include, but are not limited to, ethynyl or acetylenic (—C≡CH), propargyl (—CH_2C—≡CH), and the like.

[00156] "Alkylene" refers to a saturated, branched or straight chain or cyclic hydrocarbon radical having two monovalent radical centers derived by the removal of two hydrogen atoms from the same or two different carbon atoms of a parent alkane. For example, an alkylene group can have 1 to 20 carbon atoms, 1 to 10 carbon atoms, or 1 to 6 carbon atoms. Typical alkylene radicals include, but are not limited to, methylene (—CH_2—), 1,1-ethyl (—$CH(CH_3)$—), 1,2-ethyl (—CH_2CH_2—), 1,1-propyl (—$CH(CH_2CH_3)$—), 1,2-propyl (—$CH_2CH(CH_3)$—), 1,3-propyl (—$CH_2CH_2CH_2$—), 1,4-butyl (—$CH_2CH_2CH_2CH_2$—), and the like.

[00157] The term "aryl" used herein alone or as part of another group denotes an aromatic ring system containing from 6-14 ring carbon atoms. The aryl ring can be a monocyclic, bicyclic, tricyclic and the like. Non-limiting examples of aryl groups are phenyl, naphthyl including 1-naphthyl and 2-naphthyl, and the like. The aryl group can be unsubtituted or substituted through available carbon

atoms with one or more groups such as halogen, alkyl, aryl, hydroxy, alkoxy, aryloxy, alkylaryloxy, heteroaryloxy, oxo, cycloalkyl, phenyl, heteroaryls, heterocyclyl, naphthyl, amino, alkylamino, arylamino, heteroarylamino, dialkylamino, diarylamino, alkylarylamino, alkylheteroarylamino, arylheteroarylamino, acyl, acyloxy, nitro, carboxy, carbamoyl, carboxamide, cyano, sulfonyl, sulfonylamino, sulfinyl, sulfinylamino, thiol, alkylthio, arylthio,alkylsulfonyl -OCN, -SCN, -N=C=O, -NCS, -NO, $-N_3$, $-OP(=O)(OR^*)_2$, $-P(=O)(OR^*)_2$, $-P(=O)(O^-)_2$, $-P(=O)(OH)_2$, $-P(O)(OR^*)(O^-)$, $-C(=O)R^*$, -C(=O)X, $-C(S)R^*$, $-C(S)OR^*$, $-C(O)SR^*$, $-C(S)SR^*$, $-C(S)NR^*{}_2$ or $-C(=NR^*)NR^*{}_2$ groups, where each R^* is independently H, alkyl, aryl, arylalkyl, a heterocycle, or a protecting group or prodrug moiety groups. Any substituents can be unsubstituted or further substituted with any one of these aforementioned substituents.

[00158] The term "heteroaryl" refers to an aromatic ring system containing from 5-14 member ring having at least one heteroatom in the ring. Non-limiting examples of suitable heteroatoms which can be included in the aromatic ring include oxygen, sulfur, phospate and nitrogen. Non-limiting examples of heteroaryl rings include pyridinyl, pyrrolyl, oxazolyl, indolyl, isoindolyl, purinyl, furanyl, thienyl, benzofuranyl, benzothiophenyl, carbazolyl, imidazolyl, thiazolyl, isoxazolyl, pyrazolyl, isothiazolyl, quinolyl, isoquinolyl, pyridazyl, pyrimidyl, pyrazyl, etc. The heteroaryl group can be unsubtituted or substituted through available carbon atoms with one or more groups such as. halogen, alkyl, aryl, hydroxy, alkoxy, aryloxy, alkylaryloxy, heteroaryloxy, oxo, cycloalkyl, phenyl, heteroaryls, heterocyclyl, naphthyl, amino, amido, alkylamino, arylamino, heteroarylamino, dialkylamino, diarylamino, alkylarylamino, alkylheteroarylamino, arylheteroarylamino, acyl, acyloxy, nitro, carboxy, carbamoyl, carboxamide, cyano, sulfonyl, sulfonylamino, sulfinyl, sulfinylamino, thiol, alkylthio, arylthio, alkylsulfonyl, -OCN, -SCN, -N=C=O, -NCS, -NO, $-N_3$, $-OP(=O)(OR^*)_2$, $-P(=O)(OR^*)_2$, $-P(=O)(O^-)_2$, $-P(=O)(OH)_2$, $-P(O)(OR^*)(O^-)$, $-C(=O)R^*$, -C(=O)X, $-C(S)R^*$, $-C(S)OR^*$, $-C(O)SR^*$, $-C(S)SR^*$, $-C(S)NR^*{}_2$ or $-C(=NR^*)NR^*{}_2$ groups, where each R^* is independently H, alkyl, aryl, arylalkyl, a heterocycle, or a protecting group or prodrug moiety. Any substituents can be unsubstituted or further substituted with any one of these aforementioned substituents.

[00159] As used herein, the term "amino", used alone or as part of another group, refers to any primary, secondary, tertieary or quartenary amine each independently substituted with H, substituted or unsubstituted straight or branched $C_1 - C_{10}$ alkyl, straight or branched $C_2 - C_{10}$ alkenyl, straight or branched $C_2 - C_{10}$ alkynyl, substituted or unsubstituted carbocyclyl, substituted or unsubstituted aryl, substituted or unsubstituted heterocyclyl, etc. . In some embodiments, the primary, secondary and tertiary amines where the point of attachment is through the nitrogen-atom. In case of the secondary

or tertiary amines, the substituting groups on the nitrogen may be the same or different. Nonlimiting types of amino include $-NH_2$, $-N(alkyl)_2$, -NH(alkyl), $-N(carbocyclyl)_2$, -NH(carbocyclyl), $-N(heterocyclyl)_2$, -NH(heterocyclyl), $-N(aryl)_2$, -NH(aryl), -N(alkyl)(aryl), -N(alkyl)(heterocyclyl), -N(carbocyclyl)(heterocyclyl), -N(aryl)(heteroaryl), —N(alkyl)(heteroaryl), etc. The term "alkylamino" refers to an amino group substituted with at least one alkyl group. Nonlimiting examples of amino groups include $-NH_2$, $-NH(CH_3)$, $-N(CH_3)_2$, $-NH(CH_2CH_3)$, $-N(CH_2CH_3)_2$, -NH(phenyl), $-N(phenyl)_2$, -NH(benzyl), $-N(benzyl)_2$, etc. Substituted alkylamino refers generally to alkylamino groups, as defined above, in which at least one substituted alkyl, as defined herein, is attached to the amino nitrogen atom. Non-limiting examples of substituted alkylamino includes -NH(alkylene-C(O)-OH), -NH(alkylene-C(O)-O-alkyl), $-N(alkylene-C(O)—OH)_2$, $-N(alkylene-C(O)-O-alkyl)_2$, etc.

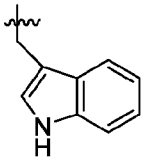
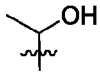
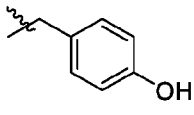
[00160] The term "halogen" or "halo" as used herein refers to -Cl, -Br, -F, or -I groups.

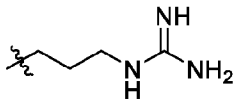
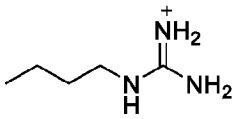
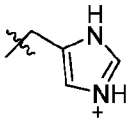
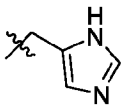
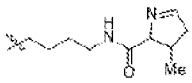
[00161] As used herein, the term "hydroxylamino", used alone or as part of another group, refers, in one embodiment, to an amino group as defined above, which is substituted by one or more hydroxyl groups.

[00162] As used herein, the term "amido", used alone or as part of another group, refers, to the formula -C(=O)NRR' or -NHCO-R or -N(R)-C(O)-R, wherein R and R' are each individually is H or an C_1 to C_{10} alkyl, aryl, cycloalkyl, heterocycle as defined above.

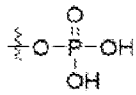
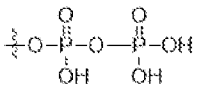
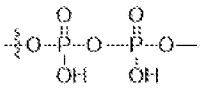
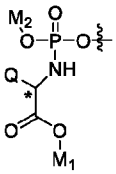
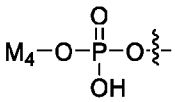
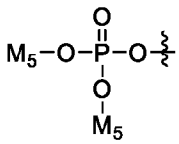
[00163] As used herein, the term "heterocyclic nitrogen based ring" refers to substituted or unsubstituted uracil or uracil derivative, substituted or unsubstituted cytosine or cytosine derivative, substituted or unsubstituted adenine or adenine derivative, substituted or unsubstituted guanine or guanine derivative, substituted or unsubstituted 5 to 6 member ring with between 1-3 nitrogen atoms, substituted or unsubstituted bicyclic rings with between 1-4 nitrogen atoms , substituted or unsubstituted fused rings with between 1-4 nitrogen atoms . The "heterocyclic nitrogen based ring" can be substituted with one or more groups such as halogen, hydroxy, alkoxy, aryloxy, alkylaryloxy, heteroaryloxy, aryl, oxo, cycloalkyl, phenyl, heteroaryls, heterocyclyl, naphthyl, amino, amido, alkylamino, arylamino, heteroarylamino, dialkylamino, diarylamino, alkylarylamino, alkylheteroarylamino, arylheteroarylamino, acyl, acyloxy, nitro, carboxy, carbamoyl, carboxamide, cyano, sulfonyl, sulfonylamino, sulfinyl, sulfinylamino, thiol, alkylthio, arylthio, or alkylsulfonyl groups. Any substituents can be unsubstituted or further substituted with any one of these aforementioned substituents. Some of the "Heterocyclic nitrogen based ring" is exemplified herein as R_7.

[00164] As used herein, the term "side chain of an amino acid" refers to the side group of each

amino acid, such as substituent that is specific to each amino acid, wherein the "side chain" is an organic substituent. The "side chain of an amino acid" comprises H refers to the side chain of Glycine, methyl refers to the side chain of Alanine, benzyl refers to the side chain of Phenylalanine, iso-propyl refers to the side chain of Valine, iso-butyl refers to the side chain of Leucine, sec-butyl refers to the side chain of Isoleucine, $-CH_2OH$ refers to the side chain of Serine, refers to the side chain of Methionine, refers to the side chain of Cysteine,  refers to the side chain of Tryptophan,  refers to the side chain of Threonine, - CH_2CONH_2 refers to the side chain of Asparagine,  refers to the side chain of Tyrosine, $-CH_2COOH$ or CH_2COO-refers to the side chain of Aspartic acid, - CH_2CH_2COOH or - $CH_2CH_2COO^-$ refers to the side chain of Glutamic acid,

, - $CH_2CH_2CONH_2$ refers to the side chain of Glutamine - $CH_2CH_2CH_2NH_2$ or - CH_2CH_2 $CH_2NH_3^+$ refer to the side chain of Lysine,  or  refer to the side chain of Arginine,  or  refer to the side chain of Histidine, - $CH_2CH_2CH_2$-connected to the N of the R_1 structure refer to the side chain of Proline, $-CH_2SeH$ refer to the side chain of Selenocysteine,  refer to the side chain of Pyrrolysine.

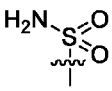
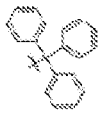
[00165] In one embodiment, the process for the preparation of compounds represented by the structure of Formula IB, Formula IIB, Formula IIIB, Formula IVB, Formula VB, Formula VB1, Formula VIB, Formula VIIB, Formula VIIIB, Formula IXB, Formula XB, Formula XIB, Formula XIIB, Formula XIIIB, Formula XIIIB1, Formula XIVB, Formula XIVB1, Formula XIVB2, Formula XIVB3, Formula XIVB4, Formula XIV5, Formula XVB, Formula XVB1, Formula XVB2, Formula XVB3, Formula XVB4, Formula XVB5, Formula XVB6, Formula XVIB, Formula XVIB1, Formula XVIB2, Formula XVIB3, Formula XVIB4, Formula XVIB5, Formula XVIB6, Formula XVIB7, Formula XVIB8, Formula XVIB9, Formula XVIB10, Formula XVIB11, Formula XVIB12, Formula XVIB13, Formula XVIB14, Formula XVIB15, Formula XVIB16, Formula XVIB17, Formula

XVIB18, Formula XVIB19, Formula XVIB20, Formula XVIB21, Formula XVIB22, Formula XVIB23, Formula XVIB24, Formula XVIB25, Formula XVIB26, Formula XVIB27, Formula XVIB28, Formula XVIB29, Formula XVIB30, Formula XVIB31, Formula XVIB32, Formula XVIB33, Formula XVIIB, Formula XVIIIB, Formula XXB, Formula XIXB, Formula XXIB, Formula XXIIB, Formula XXXIIIB, Formula XXIVB, or Formula XXVB comprises reacting the corresponding substrate of each compound by any known process known in the art, wherein the corresponding substrate are represented in **Table 1** and **Table 2**, and R_1 is OH,  ,  ,  ,  , ,  , or  ;

Q is a side chain of an amino acid;

M_1 is an alkyl;

M_2 is an aryl, a substituted aryl, a heteroaryl or a substituted heteroaryl;

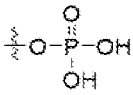
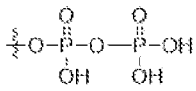
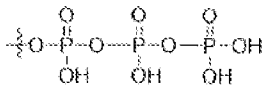
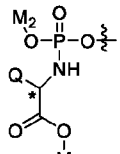
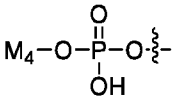
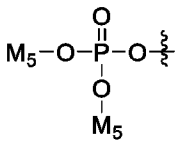
M_3 is  , ,  , or ;

M_4 is -(C_2-C_6)alkyl-O-(C_{10}-C_{20})alkyl;

each M_5 is -$(CH_2)_n$-S-C(=O)-(C1-C8)alkyl;

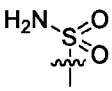
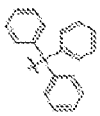
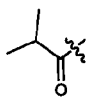
n is 1-4;

or

R_{11} is  ,  ,  ,  , ,  , or  ;

M_1 is an alkyl;

M_2 is an aryl, a substituted aryl, a heteroaryl or a substituted heteroaryl;

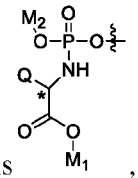
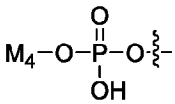
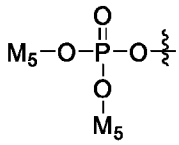
M_3 is  , ,  , or  .

M_4 is -(C_2-C_6)alkyl-O-(C_{10}-C_{20})alkyl;

each M_5 is -$(CH_2)_n$-S-C(=O)-(C1-C8)alkyl; and

n is 1-4;

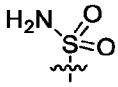
or

R_{21} is  , ,  , or  ;

Q is a side chain of an amino acid;

M_1 is an alkyl;

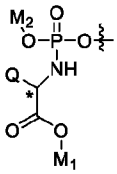
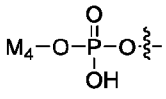
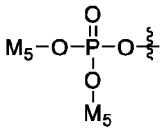
M_2 is an aryl, a substituted aryl, a heteroaryl or a substituted heteroaryl;

M_3 is  , , , or ;

M_4 is -(C_2-C_6)alkyl-O-(C_{10}-C_{20})alkyl;

each M_5 is -$(CH_2)_n$-S-C(=O)-(C_1-C_8)alkyl; and

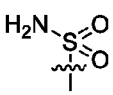
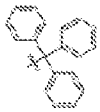
n is 1-4.

[00166] In another embodiment, the process comprises corresponding substrate wherein R_1 is  , ,  , or  ;

Q is a side chain of an amino acid;

M_1 is an alkyl;

M_2 is an aryl, a substituted aryl, a heteroaryl or a substituted heteroaryl;

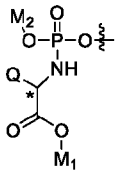
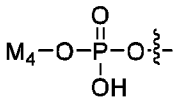
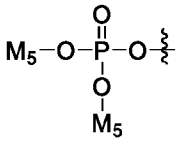
M_3 is  , ,  , or .

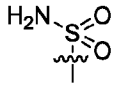
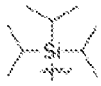
M_4 is -(C_2-C_6)alkyl-O-(C_{10}-C_{20})alkyl;

each M_5 is -$(CH_2)_n$-S-C(=O)-(C_1-C_8)alkyl;

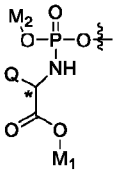
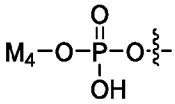
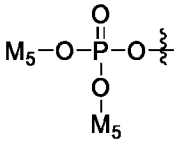
n is 1-4.

[00167] In another embodiment, the process comprises corresponding substrate wherein

R_{11} is  , ,  , or  ; Q is a side chain of an amino acid; M_1 an alkyl;

M_2 is an aryl, a substituted aryl, a heteroaryl or a substituted heteroaryl; M_3 is  ,  , , or ; M_4 is -(C_2-C_6)alkyl-O-(C_{10}-C_{20})alkyl; each M_5 is -$(CH_2)_n$-S-C(=O)-(C1-C8)alkyl; and n is 1-4

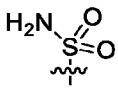
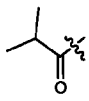
[00168] In another embodiment, the process comprises corresponding substrate wherein

R_{21} is  , ,  , or  ;

Q is a side chain of an amino acid;

M_1 is an alkyl;

M_2 is an aryl, a substituted aryl, a heteroaryl or a substituted heteroaryl;

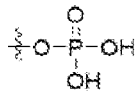
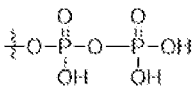
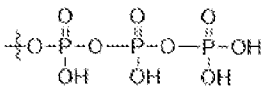
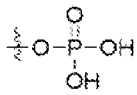
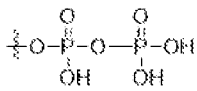
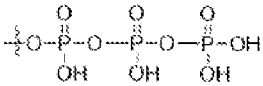
M_3 is  , , , or  ;

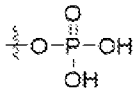
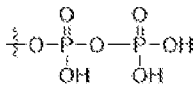
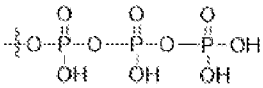
M_4 is -(C_2-C_6)alkyl-O-(C_{10}-C_{20})alkyl;

each M_5 is -$(CH_2)_n$-S-C(=O)-(C_1-C_8)alkyl; and

n is 1-4.

[00169] In one embodiment, the process for the preparation of compounds represented by the structure of Formula IB, Formula IIB, Formula IIIB, Formula IVB, Formula VB, Formula VB1, Formula VIB, Formula VIIB, Formula VIIIB, Formula IXB, Formula XB, Formula XIB, Formula XIIB, Formula XIIIB, Formula XIIIB1, Formula XIVB, Formula XIVB1, Formula XIVB2, Formula XIVB3, Formula XIVB4, Formula XIV5, Formula XVB, Formula XVB1, Formula XVB2, Formula XVB3, Formula XVB4, Formula XVB5, Formula XVB6, Formula XVIB, Formula XVIB1, Formula XVIB2, Formula XVIB3, Formula XVIB4, Formula XVIB5, Formula XVIB6, Formula XVIB7, Formula XVIB8, Formula XVIB9, Formula XVIB10, Formula XVIB11, Formula XVIB12, Formula XVIB13, Formula XVIB14, Formula XVIB15, Formula XVIB16, Formula XVIB17, Formula

XVIB18, Formula XVIB19, Formula XVIB20, Formula XVIB21, Formula XVIB22, Formula XVIB23, Formula XVIB24, Formula XVIB25, Formula XVIB26, Formula XVIB27, Formula XVIB28, Formula XVIB29, Formula XVIB30, Formula XVIB31, Formula XVIB32, Formula XVIB33, Formula XVIIB, Formula XVIIIB, Formula XXB, Formula XIXB, Formula XXIB, Formula XXIIB, Formula XXXIIIB, or Formula XXIVB, comprises reacting the corresponding substrate of each compound with pVip enzymes, wherein the corresponding substrate is described in **Table 1** and **Table 2**, wherein R_1 of each corresponding substrate is OH,  ,  ,  , or R_{11} is  ,  ,  .

[00170] In some embodiments, the process is *in vivo*. In other embodiments, the process is *in vitro*. In another embodiment, R1 of the corresponding substrate is OH. In another embodiment, R_1 or R_{11} of the corresponding substrate is  . In another embodiment, R_1 or R_{11} of the corresponding substrate is  . In another embodiment, R_1 or R_{11} of the corresponding substrate is  .

[00171] In another embodiment, the method comprises introducing and expressing a nucleic acid construct comprising and expressing a pVip gene, then purifying the expressed the pVip protein, then using the purified pVip protein to produce a ddh or deoxy-ddh compound from non-natural substrates *in vitro*. In some embodiments, when the pVip synthesizes a ddh or deoxy-ddh compound, said ddh or deoxy-ddh compound is de-phosphorylated. In some embodiments, when the pVip synthesizes a ddh or deoxy-ddh compound, said ddh or deoxy-ddh compound is phosphorylated. In some embodiments, when the pVip synthesizes a ddh or deoxy-ddh compound, said ddh or deoxy-ddh compound is modified to include a protective chemical group at the R_1 or R_{11} position in place of a hydroxyl or phosphate group(s).

[00172] As described above, ddh or deoxy-ddh compounds produced, for example from the non-natural substrates comprise a variant of the corresponding compounds lacking a 4′ hydrogen and a 3′ hydroxyl group. In another embodiment, the non-natural substrates are modified to have the 3'

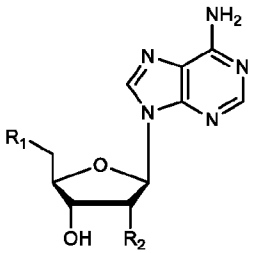
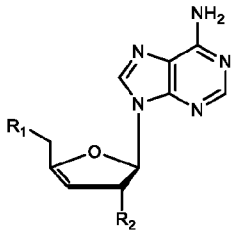
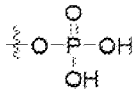
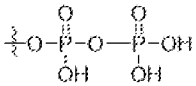
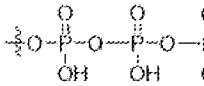
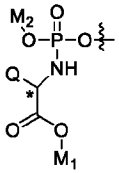
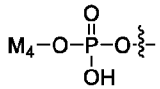
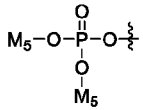
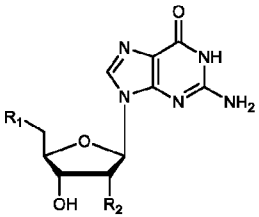
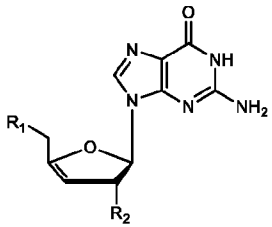
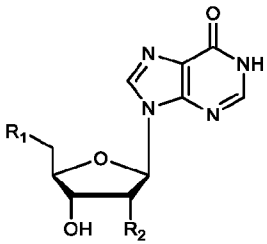
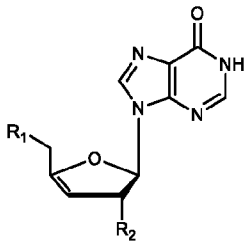
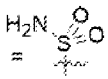
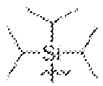
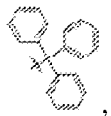
hydroxyl groups removed. In one embodiment, a ddh or deoxy-ddh compound comprises a dehydrated form of the corresponding substrate. In one embodiment, the dehydration positions are the 3' and 4' of the sugar molecule. In one embodiment, the sugar is a ribose. In another embodiment, the sugar is a deoxyribose. In one embodiment, a ddh or deoxy-ddh compound is in the 3′-deoxy-3′,4′-didehydro (deoxy-ddh) form. In one embodiment, a ddh or deoxy-ddh compound is in the 3′,4′-didehydro (ddh) form.

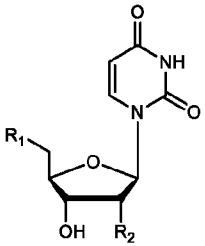
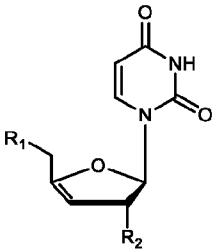
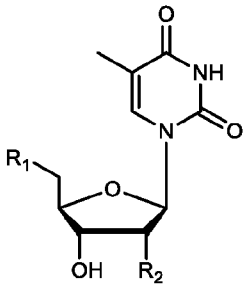
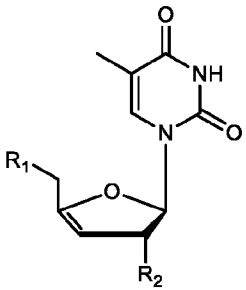
[00173] As described herein, a pVip may produce one or more kinds of a ddh or deoxy-ddh compound. In one embodiment, a pVip may produce one kind of a ddh or deoxy-ddh compound. In another embodiment, a pVip may produce multiple kinds of a ddh or deoxy-ddh compound analogs. In another embodiment, the DNA or RNA chain terminators, or anti-viral substances, anti-cancer, anti-tumor, or antibiotic produced by a pVip or synthesized using methods known in the art may not include a ribose or deoxy-ribose sugar.

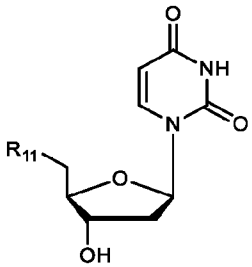
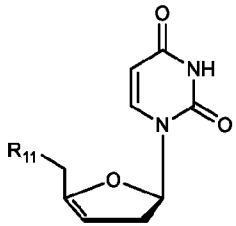
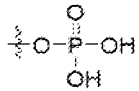
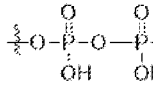
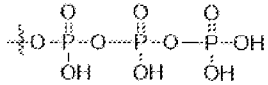
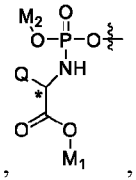
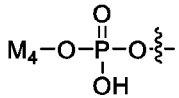
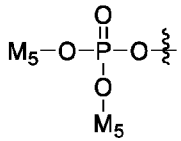
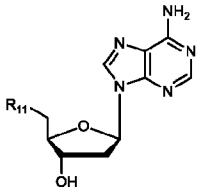
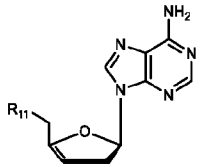
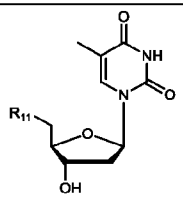
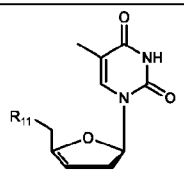
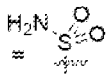
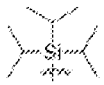
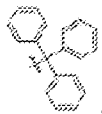
[00174] In another embodiment, the present disclosure provides a method of producing a ddh or deoxy-ddh compound from non-natural substrates, the method comprising: (a) introducing a pVip, or a nucleic acid construct encoding a pVip into a cell, wherein the pVip produces a ddh or deoxy-ddh compound from non-natural substrates; and (b) purifying the ddh or deoxy-ddh compound from the cell. In one embodiment, the pVip has the sequence of any one of SEQ ID NOs:409-789 or a homologue thereof comprising at least 80% homology to the amino acid sequence set forth in any one of SEQ ID NOs:409-789. In another embodiment, the pVip is encoded by a pVip gene comprising one of the sequences of SEQ ID Nos:3-408 or a homologue thereof comprising at least 80% identity to any one of SEQ ID Nos:3-408. In one embodiment, when the pVip in the above method produces a ddh or deoxy-ddh compound, the method further comprises dephosphorylating the ddh or deoxy-ddh compound. In one embodiment, the above method further comprises introducing into the cell pVip co-factors, or pVip substrates, or any combination thereof.

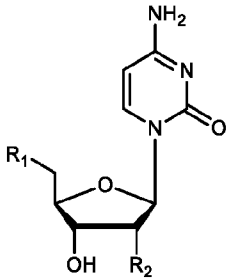
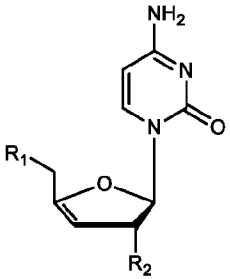
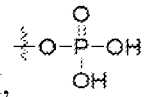
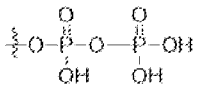
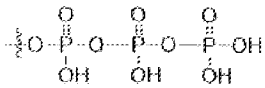
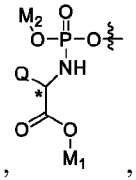
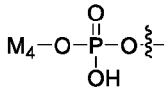
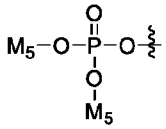
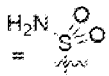
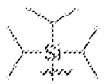
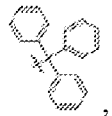
[00175] In another embodiment, the present disclosure provides a method of producing a ddh or deoxy-ddh compound *in vitro*, the method comprising: (a) providing an isolated prokaryotic viperin homolog (pVip) in vitro; (b) mixing the isolated pVip with a pVip non-natural substrate and co-factors; (c) purifying the ddh or deoxy-ddh compound produced in step (b), thereby producing the ddh or deoxy-ddh compound, or a combination thereof. In one embodiment, the amino acid sequence of the pVip is set forth in any one of SEQ ID NOs:409-789 or a homologue thereof comprising at least 80% homology to any one of SEQ ID NOs:409-789. In another embodiment, the pVip is encoded by a pVip gene comprising the sequence of one of SEQ ID Nos:3-408 or a homologue thereof comprising at least 80% identity to any one of SEQ ID Nos:3-408.

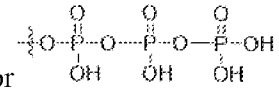
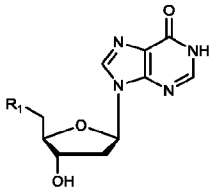
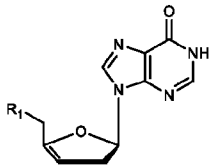
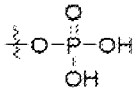
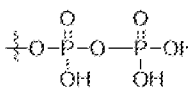
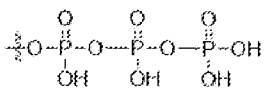
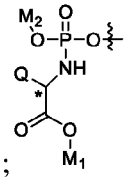
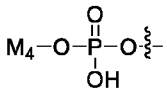
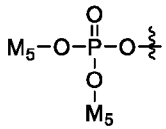
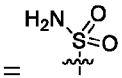
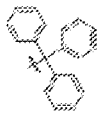
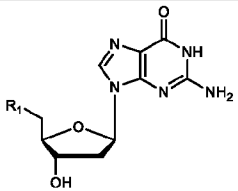
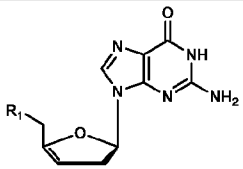
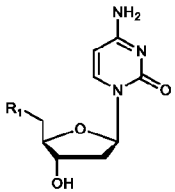
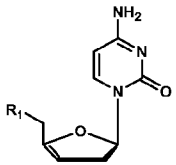
TABLE 1 – Markush Substrates and Products

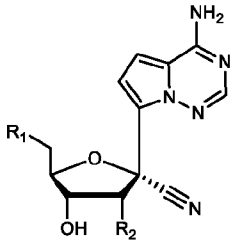
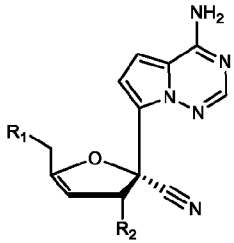
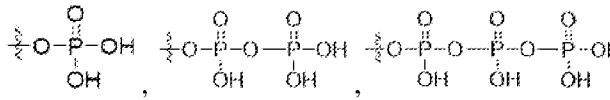
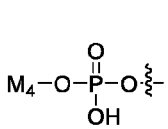
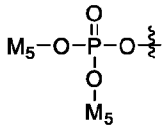
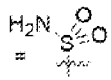
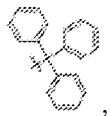
A	Markush Substrates	B	Markush Products	
IA		IB		R_1 = OH,  ,  ,  ,  , ,  , or  ;
IIA		IIB		Q is a side chain of an amino acid;
IIIA		IIIB		M_1 = an alkyl;
				M_2 = an aryl, a substituted aryl, a heteroaryl or a substituted heteroaryl;
				M_3 =  ,  ,  , or ;

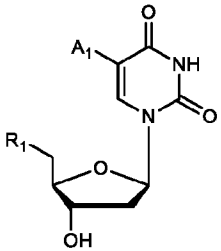
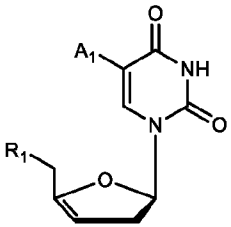
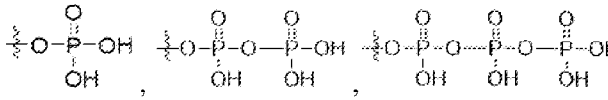
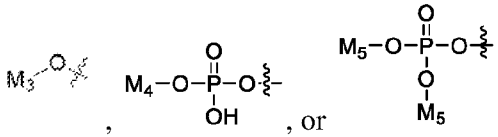
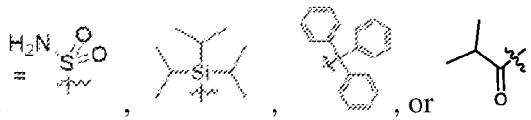
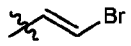
IVA		IVB		M_4 is -(C_2-C_6)alkyl-O-(C_{10}-C_{20})alkyl; each M_5 is -$(CH_2)_n$-S-C(=O)-(C1-C8)alkyl; n is 1-4; R_2 = -OH or -O-COO-alkyl; and
VA		VB		wherein if R_1 is OH, then R_2 is -O-COO-alkyl.

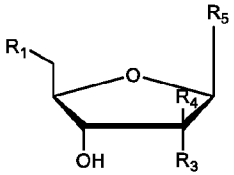
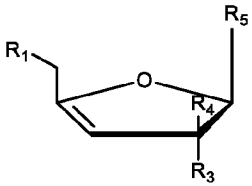
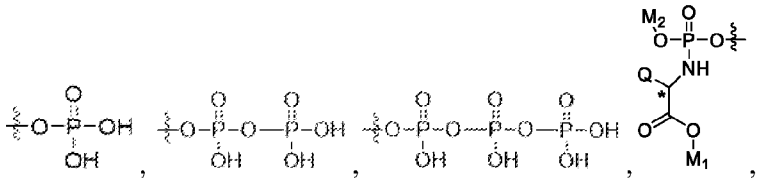
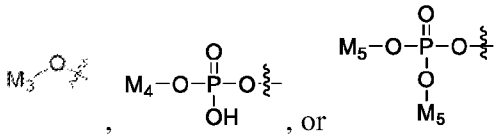
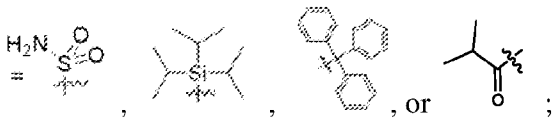
VIA		VIB		R_{11} =,  ,  ,  ,  , ,  , or  ;
VIIA		VIIB		Q is a side chain of an amino acid;
VIII A		VIIIB		M_1 = an alkyl;
				M_2 = an aryl, a substituted aryl, a heteroaryl or a substituted heteroaryl;
				M_3  ,  ,  , or ;
				M_4 is -(C_2-C_6)alkyl-O-(C_{10}-C_{20})alkyl;
				each M_5 is -$(CH_2)_n$-S-C(=O)-(C1-C8)alkyl; and
				n is 1-4

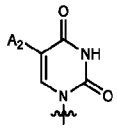
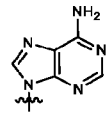
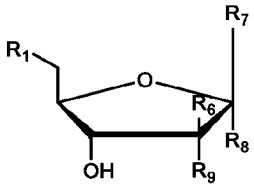
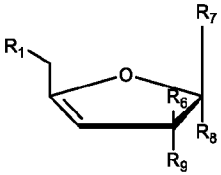
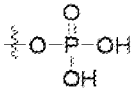
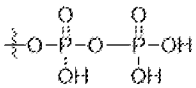
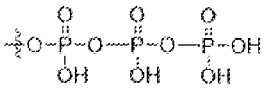
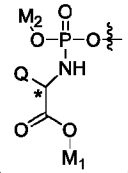
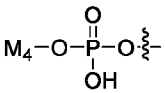
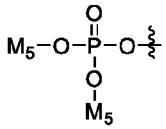
IXA		IXB		R_1 = OH,  ,  ,  ,  , ,  , or  ; Q is a side chain of an amino acid; M_1 = an alkyl; M_2 = an aryl, a substituted aryl, a heteroaryl or a substituted heteroaryl; M_3 =  ,  ,  , or ; M_4 = -(C_2-C_6)alkyl-O-(C_{10}-C_{20})alkyl; each M_5 = -$(CH_2)_n$-S-C(=O)-(C_1-C_8)alkyl; n is 1-4 R_2 = -OH or -O-COO-alkyl; and

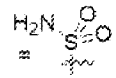
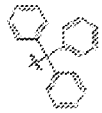
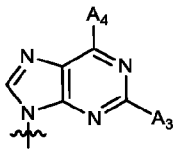
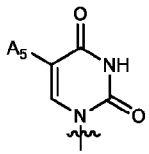
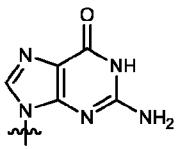
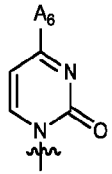
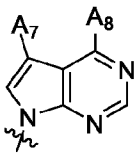
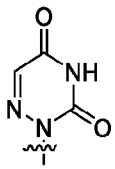
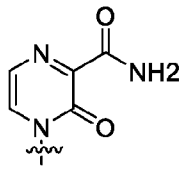
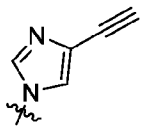
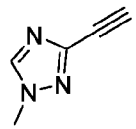
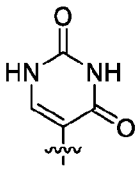
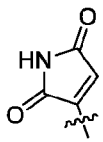
				wherein if R_1 is OH or  , then R_2 is not OH
XA		XB		R_1 = OH,  ,  ,  ;  , ,  , or  ; ; M_1 = an alknyl; M_2 = an aryl, a substituted aryl, a heteroaryl or a substituted heteroaryl; M_3 =  , ,  , or ; M_4 = -(C_2-C_6)alkyl-O-(C_{10}-C_{20})alkyl; each M_5 = -$(CH_2)_n$-S-C(=O)-(C_1-C_8)alkyl; a n is 1-4; and
XIA		XIB		
XIIA		XIIB		

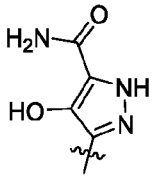
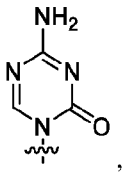
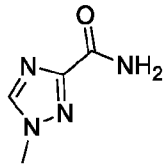
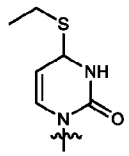
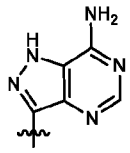
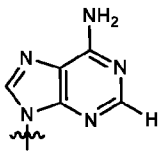
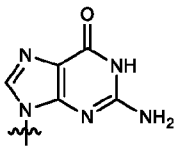
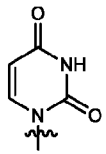
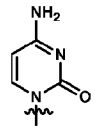
				Q is a side chain of an amino acid;
XIII A		XIIIB		R_1 = OH,  , ,  , or  ; Q is a side chain of an amino acid; M_1 = an alkyl; M_2 = an aryl, a substituted aryl, a heteroaryl or a substituted heteroaryl; M_3 =  , ,  , or ; M_4 = -(C_2-C_6)alkyl-O-(C_{10}-C_{20})alkyl; each M_5 = -$(CH_2)_n$-S-C(=O)-(C_1-C_8)alkyl; n is 1-4; and R_2 = -OH, -O-COO-alkyl

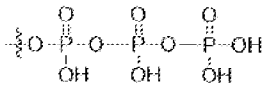
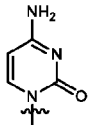
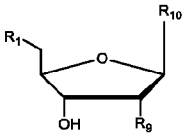
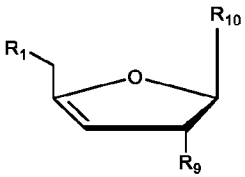
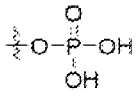
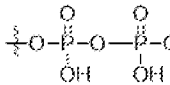
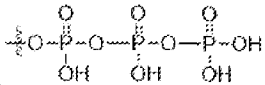
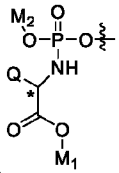
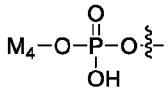
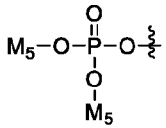
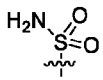
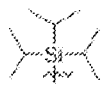
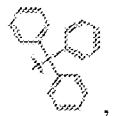
XIV A		XIVB		R_1 = OH,  ,  , or ; Q is a side chain of an amino acid; M_1 = an alkyl; M_2 = an aryl, a substituted aryl, a heteroaryl or a substituted heteroaryl; M_3 =  ; M_4 = -(C_2-C_6)alkyl-O-(C_{10}-C_{20})alkyl; each M_5 = -$(CH_2)_n$-S-C(=O)-(C_1-C_8)alkyl; n is 1-4; and A_1 = a halo, a haloalkyl, an alkyl, 

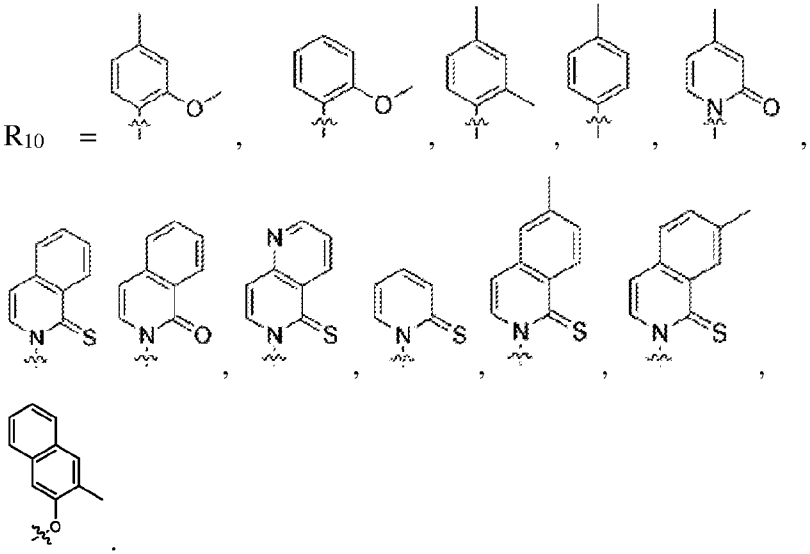
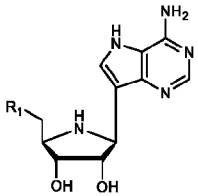
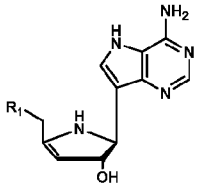
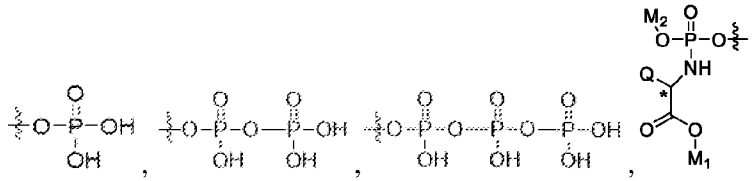
XVA		XVB		R_1 = OH,  ,  , or ; Q is a side chain of an amino acid; M_1 = an alkyl; M_2 = an aryl, a substituted aryl, a heteroaryl or a substituted heteroaryl; M_3 =  , or ; M_4 = -(C_2-C_6)alkyl-O-(C_{10}-C_{20})alkyl; each M_5 = -$(CH_2)_n$-S-C(=O)-(C_1-C_8)alkyl; n is 1-4; R_4 = H, a halo or an alkyl R_3 = H, a halo, an alkoxy

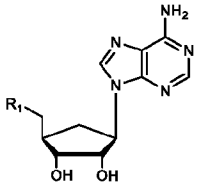
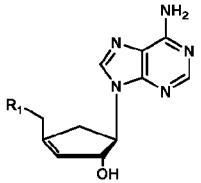
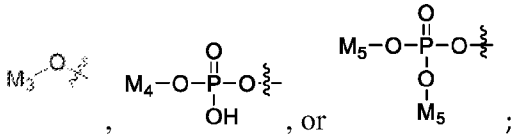
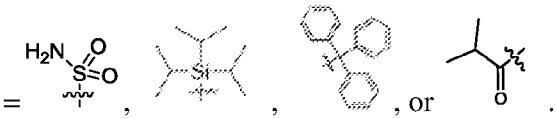
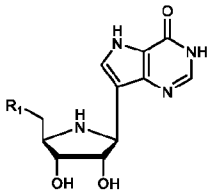
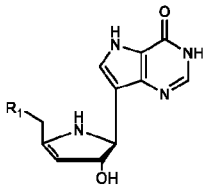
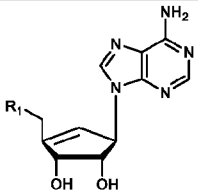
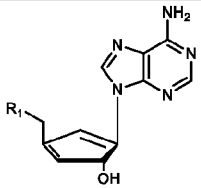
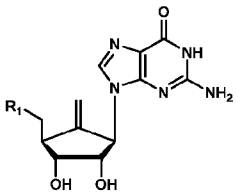
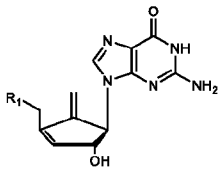
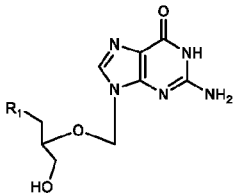
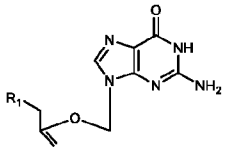
				R_5 =  or  ; A_2 = H, a halo or alkyl; and wherein R_3 is not the same as R_4
XVI A		XVIB		R_1 = OH,  ,  ,  ,  , ,  , or  ; Q is a side chain of an amino acid; M_1 = an alkyl; M_2 = an aryl, a substituted aryl, a heteroaryl or a substituted

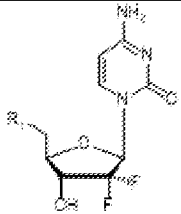
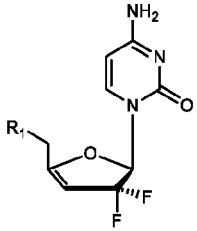
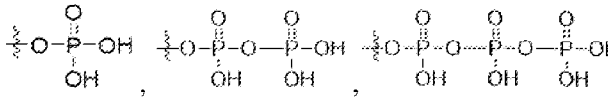
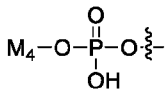
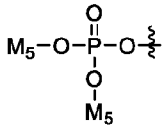
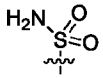
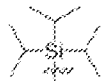
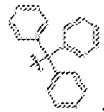
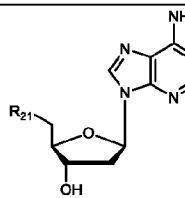
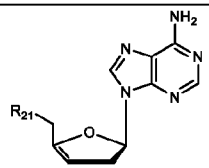
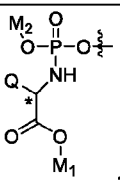
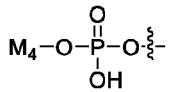
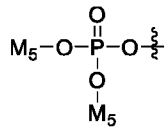
				heteroaryl; M_3  , ,  , or ; M_4 = -(C_2-C_6)alkyl-O-(C_{10}-C_{20})alkyl; each M_5 = -$(CH_2)_n$-S-C(=O)-(C_1-C_8)alkyl; n is 1-4; R_9 = H, OH or -O-COO-alkyl R_6 = H, Me, -CCH or OH R_8 = H R_7 =  ,  ,  ,  ,  , ,  ,  ,  ,  ,  ,  ,

				 ,  ,  ,  ,  or A_3 = H, a halo or an amino; A_4 = H, an amino, an alkoxy, or an alkyl; A_5 = H, a halo, a hydroxy or an alkyne; A_6 = H, an amino or a hydroxylamino; A_7 = H or an amido; A_8 = an amino or an alkyl wherein if R_1 is OH and R_7 is  ,  ,  or  , and R_9 is OH then R_6 is not H; and

				wherein if R_1 is  and R_7 is  and R_9 is OH, then R_6 is not H.
XVII A		XVII B		R_1 = OH,  ,  ,  ,  , ,  , or  ; Q is a side chain of an amino acid; M_1 = an alkyl, M_2 = an aryl, a substituted aryl, a heteroaryl or a substituted heteroaryl; M_3 =  ,  ,  , or ; M_4 = -(C_2-C_6)alkyl-O-(C_{10}-C_{20})alkyl;

				each M_5 = -$(CH_2)_n$-S-C(=O)-(C_1-C_8)alkyl; n is 1-4; R_9 = H, OH or -O-COO-alkyl. R_{10} = 
XVII IA		XVIII B		R_1 = OH, 

XIX A		XIXB		 Q is a side chain of an amino acid; M_1 = an alkyl; M_2 = an aryl, a substituted aryl, a heteroaryl or a substituted heteroaryl; M_3 =  M_4 = -(C_2-C_6)alkyl-O-(C_{10}-C_{20})alkyl; each M_5 = -$(CH_2)_n$-S-C(=O)-(C_1-C_8)alkyl; and n is 1-4.
XXA		XXB		
XXI A		XXIB		
XXII A		XXII B		
XXII IA		XXIII B		

XXI VA		XXI VB		R_1 = OH,  , ,  , or  ; Q is a side chain of an amino acid; M_1 = an alkyl, M_2 = an aryl, a substituted aryl, a heteroaryl or a substituted heteroaryl; M_3 =  ,  ,  , or ; M_4 = -(C_2-C_6)alkyl-O-(C_{10}-C_{20})alkyl; each M_5 = -$(CH_2)_n$-S-C(=O)-(C_1-C_8)alkyl; and n is 1-4
XXV A		XXV B		R_{21} =  , ,  , or  ;

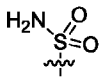
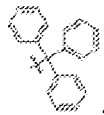
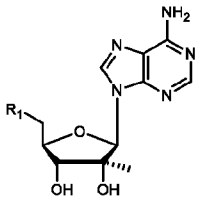
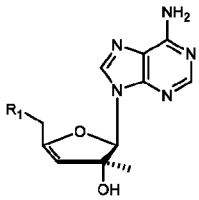
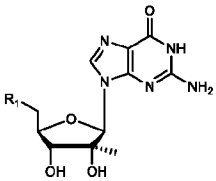
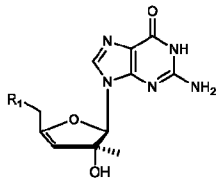
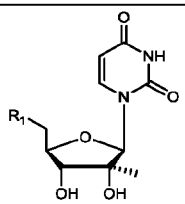
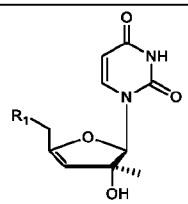
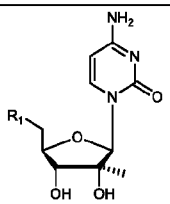
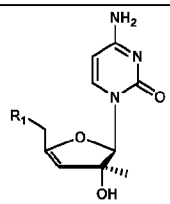
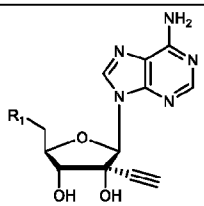
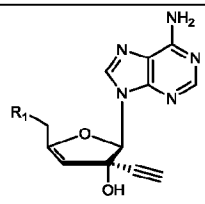
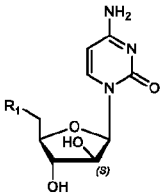
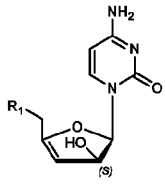
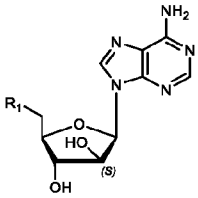
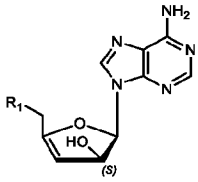
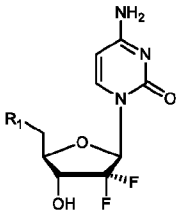
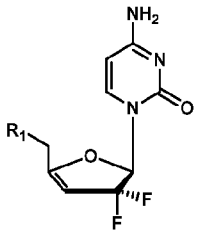
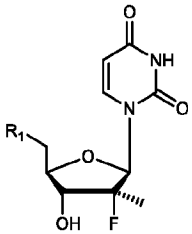
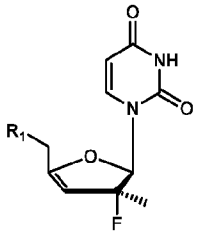
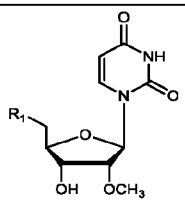
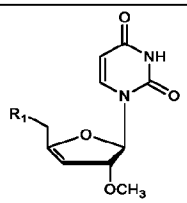
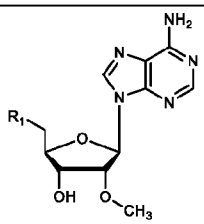
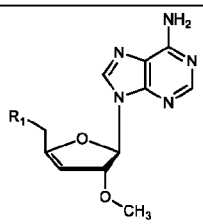
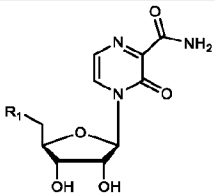
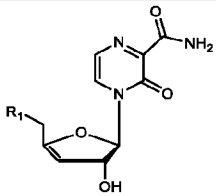
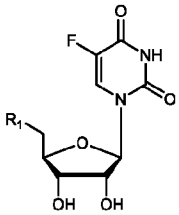
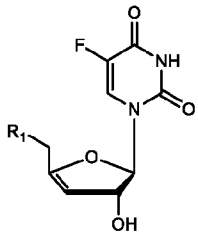
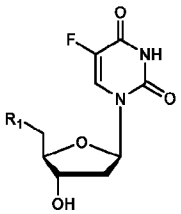
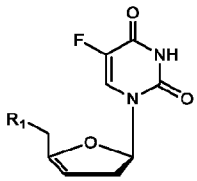
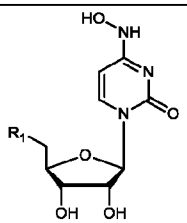
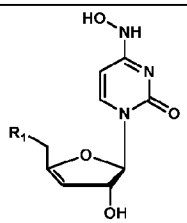
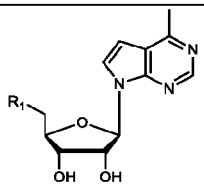
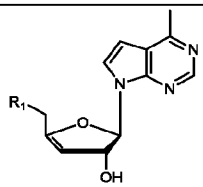
				Q is a side chain of an amino acid; M_1 = an alkyl; M_2 = an aryl, a substituted aryl, a heteroaryl or a substituted heteroaryl; M_3 =  , ,  , or ; M_4 = -(C_2-C_6)alkyl-O-(C_{10}-C_{20})alkyl; each M_5 = -$(CH_2)_n$-S-C(=O)-(C_1-C_8)alkyl; and n is 1-4

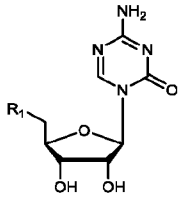
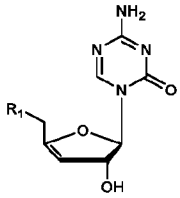
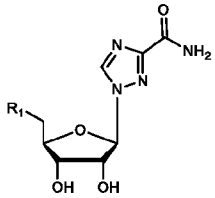
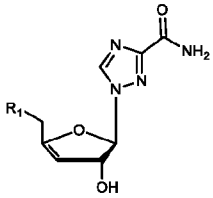
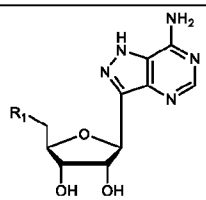
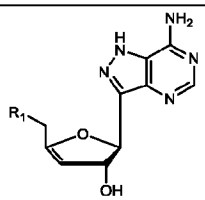
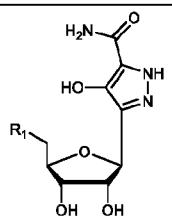
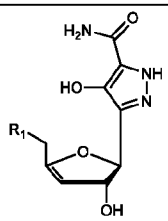
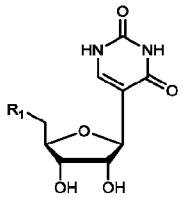
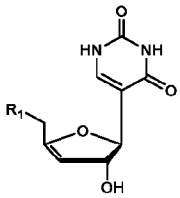
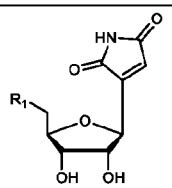
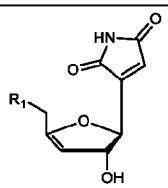
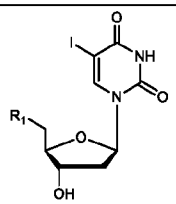
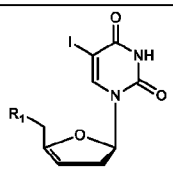
TABLE 2: Substrate and product structures

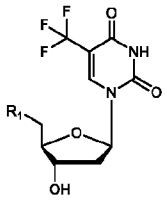
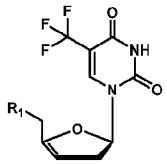
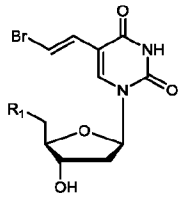
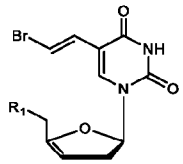
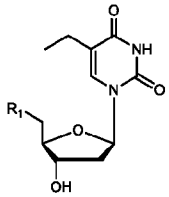
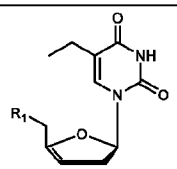
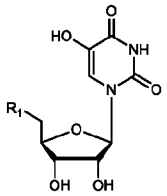
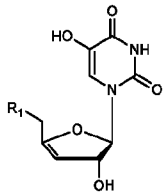
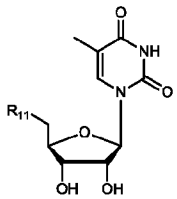
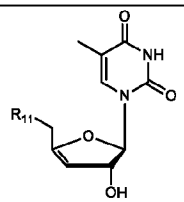
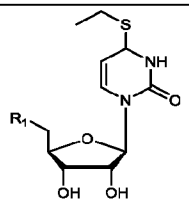
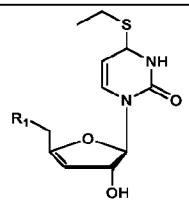
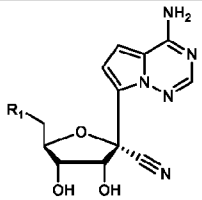
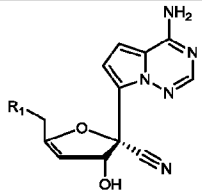
Substrate Name/ A	Substrate Structure	Product Name/B (Formula #)	Product Structure	Markush group
2'-C-methyladenosine		**XVIB1**		**XVIB**
2'-C-methylguanosine		**XVIB2**		**XVIB**
2'-C-methyluridine		**XVIB3**		**XVIB**
2'-C-Methylcytidine		**XVIB4**		**XVIB**
2'-C-ethynyladenosine		**XVIB5**		**XVIB**
Cytarabine (ara-C)		**XVIB6**		**XVIB**

ara-A (vidarabine)		**XVIB7**		**XVIB**
Gemcitabine hydrochloride		**XXIVB**		**XXIVB**
2'-Deoxy-2'-fluoro-2'-methyluridine		**XVB1**		**XVB**
2'OMe-Uridine		**XVB2**		**XVB**
2'OMe-Adenosine		**XVB3**		**XVB**
T-1106		**XVIB8**		**XVIB**
Fluorouridine 1-[(2*R*,3*R*,4*S*,5*R*)-3,4-dihydroxy-5-(hydroxymethyl)		5-fluoro-1-((2R,3R)-3-hydroxy-		**XVIB**

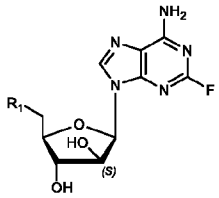
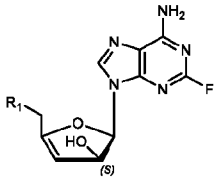
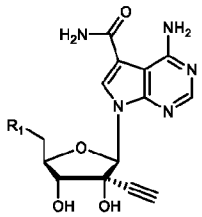
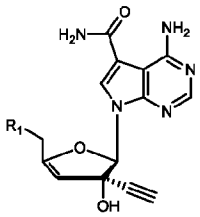
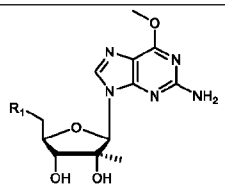
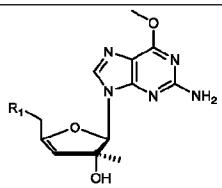
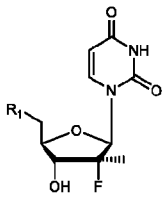
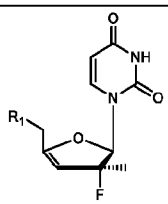
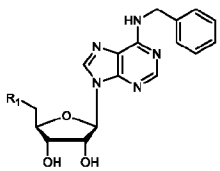
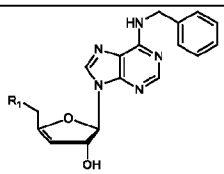
oxolan-2-yl]-5-fluoropyrimidine-2,4-dione		5-(hydroxymethyl)-2,3-dihydrofuran-2-yl)pyrimidine-2,4(1H,3H)-dione **XVIB9**		
5-Fluoro-deoxy-uridine		**XIVB1**		**XIVB**
3,4-dihydroxy-5-(hydroxymethyl) tetrahydrofuran-2-yl)-4-(hydroxyamino) pyrimidin-2(1H)-one		**XVIB10**		**XVIB**
6-Methyl-7-deazaadenosine		**XVIB11**		**XVIB**

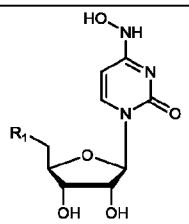
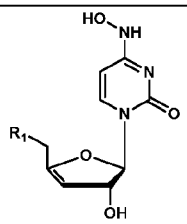
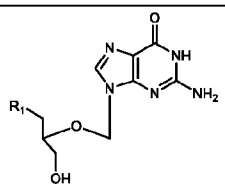
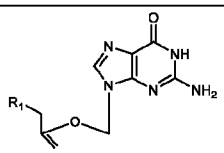
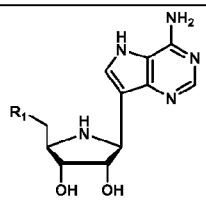
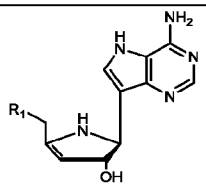
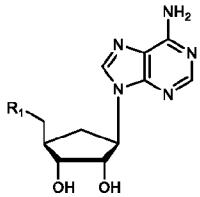
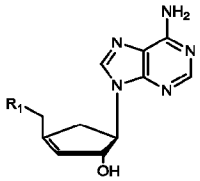
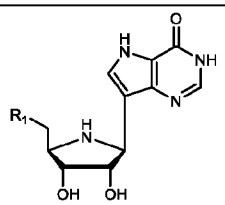
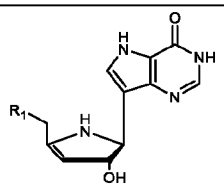
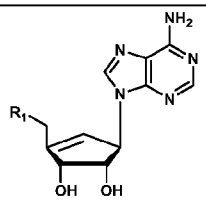
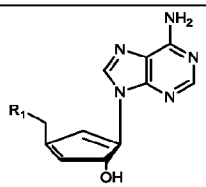
N6-(9-antranylmethyl) adenosine		**XVIB12**		**XVIB**
N6-(1-pyrenylmethyl) adenosine		**XVIB13**		**XVIB**
5-(Perylen-3-yl)ethynyl-arabino-uridine		**XVIB14**		**XVIB**
ETAR		**XVIB15**		**XVIB**
IM18		**XVIB16**		**XVIB**
6-Azauridine		**XVIB17**		**XVIB**
Zebularine		**XVIB18**		**XVIB**

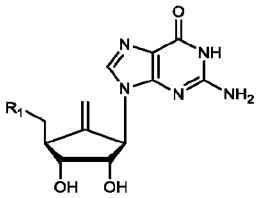
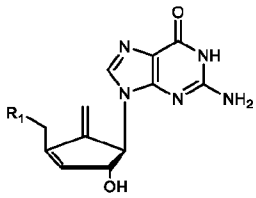
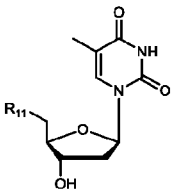
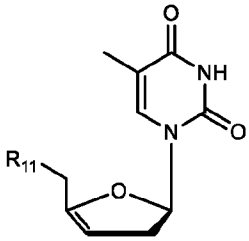
5-Azacytidine		**XVIB19**		**XVIB**
Ribavirin (Virazole)		**XVIB20**		**XVIB**
Formycin A		**XVIB21**		**XVIB**
Pyrazofurin		**XVIB22**		**XVIB**
Pseudouridine		**XVIB23**		**XVIB**
Showdomycin		**XVIB24**		**XVIB**
Idoxuridine		**XIVB2**		**XIVB**

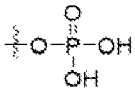
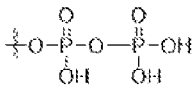
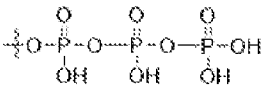
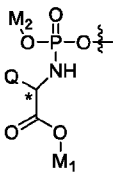
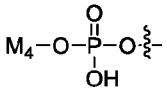
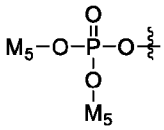
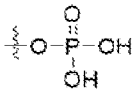
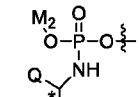
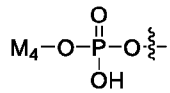
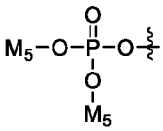
Trifluridine		**XIVB3**		**XIVB**
Brivudine		**XIVB4**		**XIVB**
Acedurid		**XIVB5**		**XIVB**
5-Hydroxy-Uridine		**XVIB25**		**XVIB**
5-Methyl-Uridine		**VB1**		**VB**
4-Thio-i-propyl-Uridine		**XVIB26**		**XVIB**
GS-441524		**XIIIB1**		**XIIIB**

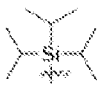
Remdesvir-nucleoside		**Compound 101**		
7-Deaza-2'-C-methyl-adenosine		**XVIB27**		**XVIB**
NITD008		**XVIB28**		**XVIB**
2'-Deoxy-2'-fluoro-arabinofuranosyl nucleoside FIAU		**XVB4**		**XVB**
2'-Deoxy-2'-fluoro-arabinofuranosyl nucleoside FMAU		**XVB5**		**XVB**
2'-Deoxy-2'-fluoro-arabinofuranosyl nucleoside FEAU		**XVB6**		**XVB**

Fludarabine (2-Fluoro-ara-Adenosine)		**XVIB29**		**XVIB**
NITD449		**XVIB30**		**XVIB**
2-(2-amino-6-methoxy-9H-purin-9-yl)-5-(hydroxymethyl)-3-methyltetrahydrofuran-3,4-diol		**XVIB31**		**XVIB**
3-fluoro-4-hydroxy-5-(hydroxymethyl)-3-methyltetrahydrofuran-2-yl)pyrimidine-2,4(1H,3H)-dione		**XVB1**		**XVB**
2-(6-(benzylamino)-9H-purin-9-yl)-5-(hydroxymethyl)		**XVIB32**		**XVIB**

tetrahydrofuran-3,4-diol				
3,4-dihydroxy-5-(hydroxymethyl) tetrahydrofuran-2-yl)-4-(hydroxyamino) pyrimidin-2(1H)-one		**XVIB33**		**XVIB**
Ganciclovir		**XXIIIB**		**XXIIIB**
BCX4430		**XVIIIB**		**XVIIIB**
Aristeromycin		**XIXB**		**XIXB**
Forodesine		**XXB**		**XXB**
Neplanocin A		**XXIB**		**XXIB**

Entecavir		**XXIIIB**		**XXIIB**
Telbivudine		**VIIIB**		**VIIIB**

[00176] In some embodiments R_1 OH,  ,  ,  ,  , ,  , or  ;. In some embodiments R_{11} is OH,  ,  , , , ,  , or  . In one embodiment, Q of R_1 or R_{11} is a side chain of an amino acid. In another embodiment, M_1 of R_1 or R_{11} is an alkyl. In another embodiment, M_2 is of R_1 or R_{11} an aryl, a substituted aryl, a heteroaryl or a substituted aryl. In another embodiment,

M_3 of R_1 or R_{11} is  , ***, or*** **.** In another embodiment, M_4 of R_1 or R_{11} is -(C_2-C_6)alkyl-O-(C_{10}-C_{20})alkyl. In another embodiment, M_5 of R_1 or R_{11} is -$(CH_2)_n$-S-C(=O)-(C_1-C_8)alkyl. In one embodiment, n of M_5 is 1, 2, 3 or 4.

Pharmaceutical Compositions

[00177] In one embodiment, provided herein is a pharmaceutical composition comprising any one of the compounds disclosed herein. In one embodiment, provided herein is a pharmaceutical composition comprising any one of the compounds disclosed herein and a pharmaceutically

acceptable carrier. In one embodiment, a pharmaceutical composition comprises a preparation of one or more of the ddh- or deoxy-ddh compounds, or prodrugs thereof, described herein with other chemical components, such as physiologically suitable carriers and excipients. The purpose of a pharmaceutical composition is to facilitate administration of a compound to an organism. In certain embodiments, a pharmaceutical composition provides the pharmaceutical dosage form of a drug.

[00178] In one embodiment, provided herein is a pharmaceutical composition comprising a compound represented by a the structure any one of the following compounds: Formula IB, Formula IIB, Formula IIIB, Formula IVB, Formula VB, Formula VB1, Formula VIB, Formula VIIB, Formula VIIIB, Formula IXB, Formula XB, Formula XIB, Formula XIIB, Formula XIIIB, Formula XIIIB1, Formula XIVB, Formula XIVB1, Formula XIVB2, Formula XIV3, Formula XIV4, Formula XIV5, Formula XVB, Formula XVB1, Formula XVB2, Formula XVB3, Formula XVB4, Formula XVB5, Formula XVB6, Formula XVIB, Formula XVIB1, Formula XVIB2, Formula XVIB3, Formula XVIB4, Formula XVIB5, Formula XVIB6, Formula XVIB7, Formula XVIB8, Formula XVIB9, Formula XVIB10, Formula XVIB11, Formula XVIB12, Formula XVIB13, Formula XVIB14, Formula XVIB15, Formula XVIB16, Formula XVIB17, Formula XVIB18, Formula XVIB19, Formula XVIB20, Formula XVIB21, Formula XVIB22, Formula XVIB23, Formula XVIB24, Formula XVIB25, Formula XVIB26, Formula XVIB27, Formula XVIB28, Formula XVIB29, Formula XVIB30, Formula XVIB31, Formula XVIB32, Formula XVIB33, Formula XVIIB, Formula XVIIIB, Formula XXB, Formula XIXB, Formula XXIB, Formula XXIIB, Formula XXXIIIB, Formula XXIVB, Formula XXVB or a combination thereof.

[00179] In one embodiment, provided herein is a pharmaceutical composition comprising a compound represented by the structure of Formula IB, Formula IIB, Formula IIIB, Formula IVB, Formula VB, Formula VIB, Formula VIIB, Formula VIIIB, Formula IXB, Formula XB, Formula XIB, Formula XIIB, Formula XIIIB, Formula XIVB, Formula XVB, Formula XVIB, Formula XVIIB, Formula XVIIIB, Formula XXB, Formula XIXB, Formula XXIB, Formula XXIIB, Formula XXXIIIB, Formula XXIVB, Formula XXVB or a combination thereof.

[00180] In one embodiment, provided herein is a pharmaceutical composition comprising a compound represented by the structure of Formula IB, Formula IIB, Formula IIIB, Formula IVB, Formula VB, or a combination thereof.

[00181] In one embodiment, provided herein is a pharmaceutical composition comprising a compound represented by the structure of Formula IB. In one embodiment, provided herein is a pharmaceutical composition comprising a compound represented by the structure of Formula IIB. In one embodiment, provided herein is a pharmaceutical composition comprising a compound represented by the structure of Formula IIIB. In one embodiment, provided herein is a

pharmaceutical composition comprising a compound represented by the structure of Formula IVB. In one embodiment, provided herein is a pharmaceutical composition comprising a compound represented by the structure of Formula VB. In one embodiment, provided herein is a pharmaceutical composition comprising a compound represented by the structure of Formula VB1.

[00182] In one embodiment, provided herein is a pharmaceutical composition comprising a compound represented by the structure of Formula VIB, Formula VIIB, Formula VIIIB or a combination thereof. In one embodiment, provided herein is a pharmaceutical composition comprising a compound represented by the structure of Formula VIB. In one embodiment, provided herein is a pharmaceutical composition comprising a compound represented by the structure of Formula VIIB. In one embodiment, provided herein is a pharmaceutical composition comprising a compound represented by the structure of Formula VIIIB. In one embodiment, provided herein is a pharmaceutical composition comprising a compound represented by the structure of Formula IXB. In one embodiment, provided herein is a pharmaceutical composition comprising a compound represented by the structure of Formula XB, Formula XIB, Formula XIIB, or a combination thereof. In one embodiment, provided herein is a pharmaceutical composition comprising a compound represented by the structure of Formula XB. In one embodiment, provided herein is a pharmaceutical composition comprising a compound represented by the structure of Formula XIB. In one embodiment, provided herein is a pharmaceutical composition comprising a compound represented by the structure of Formula XIIB. In one embodiment, provided herein is a pharmaceutical composition comprising a compound represented by the structure of Formula XIIIB. In one embodiment, provided herein is a pharmaceutical composition comprising a compound represented by the structure of Formula XIIIB1.

[00183] In one embodiment, provided herein is a pharmaceutical composition comprising a compound represented by the structure of Formula XIVB. In one embodiment, provided herein is a pharmaceutical composition comprising a compound represented by the structure of Formula XIVB1, Formula XIVB2, Formula XIVB3, Formula XIVB4, Formula XIVB5, or a combination thereof. In one embodiment, provided herein is a pharmaceutical composition comprising a compound represented by the structure of Formula XIVB1. In one embodiment, provided herein is a pharmaceutical composition comprising a compound represented by the structure of Formula XIVB2. In one embodiment, provided herein is a pharmaceutical composition comprising a compound represented by the structure of Formula XIVB3. In one embodiment, provided herein is a pharmaceutical composition comprising a compound represented by the structure of Formula XIVB4. In one embodiment, provided herein is a pharmaceutical composition comprising a compound represented by the structure of Formula XIVB5.

[00184] In one embodiment, provided herein is a pharmaceutical composition comprising a compound represented by the structure of Formula XVB. In one embodiment, provided herein is a pharmaceutical composition comprising a compound represented by the structure of Formula XVB1, Formula XVB2, Formula XVB3, Formula XVB4, Formula XVB5, Formula XVB6, or a combination thereof. In one embodiment, provided herein is a pharmaceutical composition comprising a compound represented by the structure of Formula XVB1. In one embodiment, provided herein is a pharmaceutical composition comprising a compound represented by the structure of Formula XVB2. In one embodiment, provided herein is a pharmaceutical composition comprising a compound represented by the structure of Formula XVB3. In one embodiment, provided herein is a pharmaceutical composition comprising a compound represented by the structure of Formula XVB4. In one embodiment, provided herein is a pharmaceutical composition comprising a compound represented by the structure of Formula XVB5. In one embodiment, provided herein is a pharmaceutical composition comprising a compound represented by the structure of Formula XVB6.

[00185] In one embodiment, provided herein is a pharmaceutical composition comprising a compound represented by the structure of Formula XVIB. In one embodiment, provided herein is a pharmaceutical composition comprising a compound represented by the structure of Formula XVIB1, Formula XVIB2, Formula XVIB3, Formula XVIB4, Formula XVIB5, Formula XVIB6, Formula XVIB7, Formula XVIB8, Formula XVIB9, Formula XVIB10, Formula XVIB11, Formula XVIB12, Formula XVIB13, Formula XVIB14, Formula XVIB15, Formula XVIB16, Formula XVIB17, Formula XVIB18, Formula XVIB19, Formula XVIB20, Formula XVIB21, Formula XVIB22, Formula XVIB23, Formula XVIB24, Formula XVIB25, Formula XVIB26, Formula XVIB27, Formula XVIB28, Formula XVIB29, Formula XVIB30 Formula XVIB31, Formula XVIB32, Formula XVIB33, or a combination thereof.

[00186] In one embodiment, provided herein is a pharmaceutical composition comprising a compound represented by the structure of Formula XVIB1. In one embodiment, provided herein is a pharmaceutical composition comprising a compound represented by the structure of Formula XVIB2. In one embodiment, provided herein is a pharmaceutical composition comprising a compound represented by the structure of Formula XVIB3. In one embodiment, provided herein is a pharmaceutical composition comprising a compound represented by the structure of Formula XVIB4. In one embodiment, provided herein is a pharmaceutical composition comprising a compound represented by the structure of Formula XVIB5. In one embodiment, provided herein is a pharmaceutical composition comprising a compound represented by the structure of Formula XVIB6. In one embodiment, provided herein is a pharmaceutical composition comprising a

compound represented by the structure of Formula XVIB7. In one embodiment, provided herein is a pharmaceutical composition comprising a compound represented by the structure of Formula XVIB8. In one embodiment, provided herein is a pharmaceutical composition comprising a compound represented by the structure of Formula XVIB9. In one embodiment, provided herein is a pharmaceutical composition comprising a compound represented by the structure of Formula XVIB10. In one embodiment, provided herein is a pharmaceutical composition comprising a compound represented by the structure of Formula XVIB11. In one embodiment, provided herein is a pharmaceutical composition comprising a compound represented by the structure of Formula XVIB12. In one embodiment, provided herein is a pharmaceutical composition comprising a compound represented by the structure of Formula XVIB13. In one embodiment, provided herein is a pharmaceutical composition comprising a compound represented by the structure of Formula XVIB14. In one embodiment, provided herein is a pharmaceutical composition comprising a compound represented by the structure of Formula XVIB15. In one embodiment, provided herein is a pharmaceutical composition comprising a compound represented by the structure of Formula XVIB16. In one embodiment, provided herein is a pharmaceutical composition comprising a compound represented by the structure of Formula XVIB17. In one embodiment, provided herein is a pharmaceutical composition comprising a compound represented by the structure of Formula XVIB18. In one embodiment, provided herein is a pharmaceutical composition comprising a compound represented by the structure of Formula XVIB19. In one embodiment, provided herein is a pharmaceutical composition comprising a compound represented by the structure of Formula XVIB20. In one embodiment, provided herein is a pharmaceutical composition comprising a compound represented by the structure of Formula XVIB21. In one embodiment, provided herein is a pharmaceutical composition comprising a compound represented by the structure of Formula XVIB22. In one embodiment, provided herein is a pharmaceutical composition comprising a compound represented by the structure of Formula XVIB23. In one embodiment, provided herein is a pharmaceutical composition comprising a compound represented by the structure of Formula XVIB24. In one embodiment, provided herein is a pharmaceutical composition comprising a compound represented by the structure of Formula XVIB25. In one embodiment, provided herein is a pharmaceutical composition comprising a compound represented by the structure of Formula XVIB26. In one embodiment, provided herein is a pharmaceutical composition comprising a compound represented by the structure of Formula XVIB27. In one embodiment, provided herein is a pharmaceutical composition comprising a compound represented by the structure of Formula XVIB28. In one embodiment, provided herein is a pharmaceutical composition comprising a compound represented by the structure of Formula XVIB29. In one embodiment, provided herein

is a pharmaceutical composition comprising a compound represented by the structure of Formula XVIB30. In one embodiment, provided herein is a pharmaceutical composition comprising a compound represented by the structure of Formula XVIB31. In one embodiment, provided herein is a pharmaceutical composition comprising a compound represented by the structure of Formula XVIB32. In one embodiment, provided herein is a pharmaceutical composition comprising a compound represented by the structure of Formula XVIB33.

[00187] In one embodiment, provided herein is a pharmaceutical composition comprising a compound represented by the structure of Formula XVIIB.

[00188] In one embodiment, provided herein is a pharmaceutical composition comprising a compound represented by the structure of Formula XVIIIB, Formula XXB or a combination thereof.

[00189] In one embodiment, provided herein is a pharmaceutical composition comprising a compound represented by the structure of Formula XVIIB. In one embodiment, provided herein is a pharmaceutical composition comprising a compound represented by the structure of Formula XXB. In one embodiment, provided herein is a pharmaceutical composition comprising a compound represented by the structure of Formula XIXB. In one embodiment, provided herein is a pharmaceutical composition comprising a compound represented by the structure of Formula XXIB. In one embodiment, provided herein is a pharmaceutical composition comprising a compound represented by the structure of Formula XXIIB. In one embodiment, provided herein is a pharmaceutical composition comprising a compound represented by the structure of Formula XXIIIB. In one embodiment, provided herein is a pharmaceutical composition comprising a compound represented by the structure of Formula XXIVB. In one embodiment, provided herein is a pharmaceutical composition comprising a compound represented by the structure of Formula XXVB

[00190] In one embodiment, provided herein is a pharmaceutical composition comprising at least two of the following compounds: Formula IB, Formula IIB, Formula IIIB, Formula IVB, Formula VB, Formula VB1, Formula VIB, Formula VIIB, Formula VIIIB, Formula IXB, Formula XB, Formula XIB, Formula XIIB, Formula XIIIB, Formula XIIIB1, Formula XIVB, Formula XIVB1, Formula XIVB2, Formula XIV3, Formula XIV4, Formula XIV5, Formula XVB, Formula XVB1, Formula XVB2, Formula XVB3, Formula XVB4, Formula XVB5, Formula XVB6, Formula XVIB, Formula XVIB1, Formula XVIB2, Formula XVIB3, Formula XVIB4, Formula XVIB5, Formula XVIB6, Formula XVIB7, Formula XVIB8, Formula XVIB9, Formula XVIB10, Formula XVIB11, Formula XVIB12, Formula XVIB13, Formula XVIB14, Formula XVIB15, Formula XVIB16, Formula XVIB17, Formula XVIB18, Formula XVIB19, Formula XVIB20,

Formula XVIB21, Formula XVIB22, Formula XVIB23, Formula XVIB24, Formula XVIB25, Formula XVIB26, Formula XVIB27, Formula XVIB28, Formula XVIB29, Formula XVIB30, Formula XVIB31, Formula XVIB32, Formula XVIB33, Formula XVIIB, Formula XVIIIB, Formula XXB, Formula XIXB, Formula XXIB, Formula XXIIB, Formula XXXIIIB, Formula XXIVB, or Formula XXVB.

[00191] In one embodiment, provided herein is a pharmaceutical composition comprising at least two of the following compounds: Formula IB, Formula IIB, Formula IIIB, Formula IVB, Formula VB, Formula VIB, Formula VIIB, Formula VIIIB, Formula IXB, Formula XB, Formula XIB, Formula XIIB, Formula XIIIB, Formula XIIIB1, Formula XIVB, Formula XVB, Formula XVIB, Formula XVIIB, Formula XVIIIB, Formula XXB, Formula XIXB, Formula XXIB, Formula XXIIB, Formula XXXIIIB, Formula XXIVB, or Formula XXVB.

[00192] In one embodiment, provided herein is a pharmaceutical composition comprising at least two of the following compounds: Formula VB1. Formula XIIIB1, Formula XIVB1, Formula XIVB2, Formula XIV3, Formula XIV4, Formula XIV5, Formula XVB1, Formula XVB2, Formula XVB3, Formula XVB4, Formula XVB5, Formula XVB6, Formula XVIB1, Formula XVIB2, Formula XVIB3, Formula XVIB4, Formula XVIB5, Formula XVIB6, Formula XVIB7, Formula XVIB8, Formula XVIB9, Formula XVIB10, Formula XVIB11, Formula XVIB12, Formula XVIB13, Formula XVIB14, Formula XVIB15, Formula XVIB16, Formula XVIB17, Formula XVIB18, Formula XVIB19, Formula XVIB20, Formula XVIB21, Formula XVIB22, Formula XVIB23, Formula XVIB24, Formula XVIB25, Formula XVIB26, Formula XVIB27, Formula XVIB28, Formula XVIB29, Formula XVIB30, Formula XVIB31, Formula XVIB32 or Formula XVIB33.

[00193] In one embodiment, the pharmaceutical composition comprises ddh or deoxy-ddh compounds that are in a prodrug form as described herein, comprising a protective chemical group.

[00194] In some embodiments, a pharmaceutical composition comprises any one of the compounds represented by the structures disclosed herein, and a pharmaceutically acceptable carrier. In some embodiments, a pharmaceutical composition comprises at least one of the compounds represented by the structures disclosed herein, and a pharmaceutically acceptable carrier. In some embodiments, a pharmaceutical composition comprises at least 2, 3, 4, etc, of the compounds represented by the structures disclosed herein, and a pharmaceutically acceptable carrier. In one embodiment, provided herein is a pharmaceutical composition comprising a compound represented by a the structure any one of the following compounds: Formula IB, Formula IIB, Formula IIIB, Formula IVB, Formula VB, Formula VB1, Formula VIB, Formula VIIB, Formula VIIIB, Formula IXB, Formula XB, Formula XIB, Formula XIIB, Formula XIIIB,

Formula XIIIB1, Formula XIVB, Formula XIVB1, Formula XIVB2, Formula XIV3, Formula XIV4, Formula XIV5, Formula XVB, Formula XVB1, Formula XVB2, Formula XVB3, Formula XVB4, Formula XVB5, Formula XVB6, Formula XVIB, Formula XVIB1, Formula XVIB2, Formula XVIB3, Formula XVIB4, Formula XVIB5, Formula XVIB6, Formula XVIB7, Formula XVIB8, Formula XVIB9, Formula XVIB10, Formula XVIB11, Formula XVIB12, Formula XVIB13, Formula XVIB14, Formula XVIB15, Formula XVIB16, Formula XVIB17, Formula XVIB18, Formula XVIB19, Formula XVIB20, Formula XVIB21, Formula XVIB22, Formula XVIB23, Formula XVIB24, Formula XVIB25, Formula XVIB26, Formula XVIB27, Formula XVIB28, Formula XVIB29, Formula XVIB30, Formula XVIB31, Formula XVIB32, Formula XVIB33, Formula XVIIB, Formula XVIIIB, Formula XXB, Formula XIXB, Formula XXIB, Formula XXIIB, Formula XXXIIIB, Formula XXIVB, or Formula XXVB, or a combination thereof, and a pharmaceutically acceptable carrier.

[00195] In some embodiments, a composition with an appropriate physiologically acceptable carrier may be formulated into preparations in solid, semi-solid, liquid or gaseous forms, such as tablets, capsules, powders, granules, ointments, solutions, suppositories, injections, inhalants, gels, microspheres, and aerosols. In addition, other pharmaceutically active ingredients and/or suitable excipients such as salts, buffers and stabilizers may, but need not, be present within the composition. As used herein, the term “pharmaceutically acceptable carrier” may in some embodiments be used interchangeably with the terms “physiological carrier”, “physiologically acceptable carrier”, “pharmaceutically acceptable diluent” or “pharmaceutically acceptable excipient” having all the same qualities and meanings.

[00196] Administration of a pharmaceutical composition disclosed herein may be achieved by a variety of different routes, including oral, parenteral, nasal, intravenous, intradermal, subcutaneous or topical. In some embodiments, modes of administration depend upon the nature of the condition to be treated or prevented. In some embodiments, an amount that, following administration, reduces, inhibits, prevents or delays the progression and/or metastasis of a cancer is considered effective. In some embodiments, an amount that, following administration, reduces, inhibits, prevents or delays the progression of a viral infection or disease associated with a viral infection is considered effective. In some embodiments, an amount that, following administration, reduces, inhibits, prevents or delays the progression of a bacterial infection or disease associated with a bacterial infection is considered effective. In some embodiments, an amount that, following administration, reduces, inhibits, prevents or delays the progression of an immune disease or disorder is considered effective. In some embodiments, an amount that, following administration, reduces, inhibits, prevents or delays the progression of an autoimmune disease or disorder is

considered effective. A skilled artisan would appreciate that the term "physiologically acceptable carrier, diluent or excipient", may in some embodiments be used interchangeably with the term "pharmaceutically acceptable carrier" having all the same means and qualities.

[00197] A pharmaceutical composition may be in the form of a solid or liquid. In some embodiments, the pharmaceutically acceptable carrier(s) are particulate, so that the compositions are, for example, in tablet or powder form. The pharmaceutically acceptable carrier(s) may be liquid, with the compositions being, for example, an oral oil, injectable liquid or an aerosol, which is useful in, for example, inhalatory administration. When intended for oral administration, the pharmaceutical composition is preferably in either solid or liquid form, where semi-solid, semi-liquid, suspension and gel forms are included within the forms considered herein as either solid or liquid.

[00198] As a solid composition for oral administration, the pharmaceutical composition may be formulated into a powder, granule, compressed tablet, pill, capsule, chewing gum, wafer or the like. Such a solid composition will typically contain one or more inert diluents or edible pharmaceutically acceptable carriers. In addition, one or more of the following may be present: binders such as carboxymethylcellulose, ethyl cellulose, microcrystalline cellulose, gum tragacanth or gelatin; excipients such as starch, lactose or dextrins, disintegrating agents such as alginic acid, sodium alginate, Primogel, corn starch and the like; lubricants such as magnesium stearate or Sterotex; glidants such as colloidal silicon dioxide; sweetening agents such as sucrose or saccharin; a flavoring agent such as peppermint, methyl salicylate or orange flavoring; and a coloring agent. When the pharmaceutical composition is in the form of a capsule, for example, a gelatin capsule, it may contain, in addition to materials of the above type, a liquid pharmaceutically acceptable carrier such as polyethylene glycol or oil.

[00199] The pharmaceutical composition may be in the form of a liquid, for example, an elixir, syrup, solution, emulsion or suspension. The liquid may be for oral administration or for delivery by injection, as two examples. When intended for oral administration, preferred composition contain, in addition to the present compounds, one or more of a sweetening agent, preservatives, dye/colorant and flavor enhancer. In a composition intended to be administered by injection, one or more of a surfactant, preservative, wetting agent, dispersing agent, suspending agent, buffer, stabilizer and isotonic agent may be included.

[00200] The liquid pharmaceutical compositions, whether they be solutions, suspensions or other like form, may include one or more of the following adjuvants: sterile diluents such as water for injection, saline solution, preferably physiological saline, Ringer's solution, isotonic sodium chloride, fixed oils such as synthetic mono or diglycerides which may serve as the solvent or

suspending medium, polyethylene glycols, glycerin, propylene glycol or other solvents; antibacterial agents such as benzyl alcohol or methyl paraben; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose. The parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic. Physiological saline is a preferred adjuvant. An injectable pharmaceutical composition is preferably sterile.

[00201] A liquid pharmaceutical composition intended for either parenteral or oral administration should contain an amount of a ddh- or deoxy-ddh-compound or prodrug thereof as herein disclosed, such that a suitable dosage will be obtained.

[00202] The pharmaceutical composition may be intended for topical administration, in which case the pharmaceutically acceptable carrier may suitably comprise a solution, emulsion, ointment or gel base. The base, for example, may comprise one or more of the following: petrolatum, lanolin, polyethylene glycols, bee wax, mineral oil, diluents such as water and alcohol, and emulsifiers and stabilizers. Thickening agents may be present in a pharmaceutical composition for topical administration. If intended for transdermal administration, the composition may include a transdermal patch or iontophoresis device. The pharmaceutical composition may be intended for rectal administration, in the form, for example, of a suppository, which will melt in the rectum and release the drug. The composition for rectal administration may contain an oleaginous base as a suitable nonirritating excipient. Such bases include, without limitation, lanolin, cocoa butter and polyethylene glycol.

[00203] The pharmaceutical composition may include various materials, which modify the physical form of a solid or liquid dosage unit. For example, the composition may include materials that form a coating shell around the active ingredients. The materials that form the coating shell are typically inert, and may be selected from, for example, sugar, shellac, and other enteric coating agents. Alternatively, the active ingredient or prodrug thereof (a ddh- or deoxy-ddh compound or prodrug thereof) may be encased in a gelatin capsule. The pharmaceutical composition in solid or liquid form may include an agent that binds to the ddh or deoxy-ddh compounds as disclosed herein, and thereby assists in the delivery of the compound. Suitable agents that may act in this capacity include monoclonal or polyclonal antibodies, one or more proteins or a liposome. The pharmaceutical composition may consist essentially of dosage units that can be administered as an aerosol. The term aerosol is used to denote a variety of systems ranging from those of colloidal nature to systems consisting of pressurized packages. Delivery may be by a liquefied or compressed gas or by a suitable pump system that dispenses the active ingredients. Aerosols may

be delivered in single phase, bi-phasic, or tri-phasic systems in order to deliver the active ingredient(s). Delivery of the aerosol includes the necessary container, activators, valves, subcontainers, and the like, which together may form a kit. One of ordinary skill in the art, without undue experimentation may determine preferred aerosols.

[00204] The pharmaceutical compositions may be prepared by methodology well known in the pharmaceutical art. For example, a pharmaceutical composition intended to be administered by injection can be prepared by combining a composition that comprises a ddh- or deoxy-ddh-compound or prodrug thereof as described herein, and optionally, one or more of salts, buffers and/or stabilizers, with sterile, distilled water so as to form a solution. A surfactant may be added to facilitate the formation of a homogeneous solution or suspension. Surfactants are compounds that non-covalently interact with the ddh- or deoxy-ddh composition so as to facilitate dissolution or homogeneous suspension of ddh- or deoxy-ddh compound in the aqueous delivery system.

[00205] The compositions may be administered in a therapeutically effective amount, which will vary depending upon a variety of factors including the activity of the ddh- or deoxy-ddh compound employed; the metabolic stability and length of action of the ddh- or deoxy-ddh compound; the age, body weight, general health, sex, and diet of the patient; the mode and time of administration; the rate of excretion; the drug combination; the severity of the particular allergic or respiratory disorder or condition; and the subject undergoing therapy.

[00206] In some embodiments, a pharmaceutically acceptable carrier may be liquid, semi-liquid or solid. Solutions or suspensions used for parenteral, intradermal, subcutaneous or topical application may include, for example, a sterile diluent (such as water), saline solution, fixed oil, polyethylene glycol, glycerin, propylene glycol or other synthetic solvent; antimicrobial agents (such as benzyl alcohol and methyl parabens, phenols or cresols, mercurials, chlorobutanol, methyl and propyl p-hydroxybenzoic acid esters, thimerosal, benzalkonium chloride and benzethonium chloride); antioxidants (such as ascorbic acid and sodium bisulfite; methionine, sodium thiosulfate, platinum, catalase, citric acid, cysteine, thioglycerol, thioglycolic acid, thiosorbitol, butylated hydroxyanisol, butylated hydroxytoluene, and/or propyl gallate) and chelating agents (such as ethylenediaminetetraacetic acid (EDTA)); buffers (such as acetates, citrates and phosphates). If administered intravenously, suitable pharmaceutically acceptable carriers include physiological saline or phosphate buffered saline (PBS), and solutions containing thickening and solubilizing agents, such as glucose, polyethylene glycol, polypropylene glycol and mixtures thereof.

[00207] The compositions comprising a ddh- or deoxy-ddh compound as described herein, may be prepared with pharmaceutically acceptable carriers that protect the ddh- or deoxy-ddh compound against rapid elimination from the body, such as time release formulations or coatings.

Such pharmaceutically acceptable carriers include controlled release formulations, such as, but not limited to, implants and microencapsulated delivery systems, and biodegradable, biocompatible polymers, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, polyorthoesters, polylactic acid and others known to those of ordinary skill in the art.

Prokaryotic Viperin Homologs (pVips)

[00208] In some embodiments, disclosed herein are prokaryotic viperin homologs (pVips). Viperin is a protein found in eukaryotic cells, usually localized in the endoplasmic reticulum where it is anchored via its N-terminal domain, though it is also found in other cell compartments. The presence of viperin in a cell was reported to inhibit replication of many DNAnd RNA viruses in the cell, viruses including by not limited to chikungunya, human cytomegalovirus (HCV), hepatitis C virus, dengue, West Nile virus, sindbis virus, influenza, HIV LAI strain, and others. Viperin expression can be induced by the release of inflammatory signals, such as IFN-γ. Viperin was reported to down-regulate the concentration of viral structural proteins essential for viral assembling and maturation.

[00209] In eukaryotes, viperin catalyzes the conversion of the nucleotide cytidine triphosphate (CTP) to 3′-deoxy-3′,4′-didehydro-CTP (ddhCTP) via SAM- dependent radical mechanism. This RNA nucleotide analog lacks 4′ hydrogen and the 3′ hydroxyl group compared to CTP, and acts as a new type of polynucleotide chain terminator for viral RNA dependent polymerases. In vertebrate genomes, the kinase cytidylate monophosphate kinase 2 (CMPK2) is adjacent to the viperin. This kinase phosphorylates cytidine monophosphate (CMP) to CTP thus generating the substrate of vertebrate viperins. When tested as an anti-viral agent, 3′-deoxy-3′,4′-didehydro-C (ddhC), was applied to cells, where it was phosphorylated by endogenous proteins producing ddhCTP, and directly inhibited replication of Zika virus in vivo.

[00210] In some embodiments, disclosed herein are prokaryotic enzymes showing sequence similarity to vertebrate viperin, and that produce modified nucleotides that function as anti-viral chain terminators. In some embodiments, disclosed herein are methods to identify such prokaryotic enzymes out of other prokaryotic enzymes that show sequence similarity to the vertebrate viperin but do not have anti-viral activities. In some embodiments, bacterial and archeal enzymes showing sequence or functional similarity to eukaryotic viperin are referred to herein as "prokaryotic viperin homologs" or "pVips".

[00211] While prokaryotic homologs of viperins share some sequence similarity with eukaryotic viperins, an initial similarity-based search revealed a very large number of enzymes. Only by using the method disclosed herein, it was possible to predict the defense score of these enzymes, and to reduce considerably the number of proteins to find true viperin homologs. The *in vivo* verification

of the activity of such enzymes required a complex strategy to heterologously express enzymes in model organisms (including the use of a specific strains to increase iron-sulfur cluster production) and test them against a wide array of bacteriophages.

[00212] A skilled artisan would recognize that immune genes from eukaryotes, such a viperin gene, are expected to be different from immune genes in prokaryotes. This is corroborated, for example, by the almost absence of immune systems present in both eukaryotes and prokaryotes. Only the pAgo proteins have been described as being involved in both RNA interference in eukaryotes and plasmid restriction in prokaryotes. This stresses the unexpectedness to discover prokaryotic viperin homologs (pVips). The fact that no prokaryotic defense systems similar to the disclosed herein is known, i.e. a defense system comprising enzymes generating chain terminators, further highlights the unexpectedness of the of the present disclosure.

[00213] A skilled artisan will recognize that, in some embodiments, prokaryotes or prokaryotic cells comprise unicellular organisms lacking a membrane-restricted nucleus, mitochondria, or other eukaryotic-specific organelle. In some embodiments a prokaryote comprises one of Euryarchaeota, Proteobacteria, Firmicutes, Bacteriodetes, or cyanobacteria.

[00214] In some embodiments, a prokaryote comprises a microbial cell such as bacteria, e.g., Gram-positive or Gram-negative bacteria. In some embodiments, a bacteria comprise Gram-negative bacteria or Negativicutes that stain negative in Gram stain. In some embodiments, a bacteria comprises gram-positive bacteria, gram-negative bacteria, or archaea.

[00215] In some embodiments, Gram-negative bacteria can be *Acinetobacter calcoaceticus, Actinobacillus actinomycetemcomitans, Aeromonas hydrophila, Alcaligenes xylosoxidans, Bacteroides, Bacteroides fragilis, Bartonella bacilliformis, Bordetella spp., Borrelia burgdorferi, Branhamella catarrhalis, Brucella spp., Campylobacter spp., Chalmydia pneumoniae, Chlamydia psittaci, Chlamydia trachomatis, Chromobacterium violaceum, Citrobacter spp., Eikenella corrodens, Enterobacter aerogenes, Escherichia coli, Flavobacterium meningosepticum, Fusobacterium spp., Haemophilus influenzae, Haemophilus spp., Helicobacter pylori, Klebsiella spp., Legionella spp., Leptospira spp., Moraxella catarrhalis, Morganella morganii, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Pasteurella multocida, Plesiomonas shigelloides, Prevotella spp., Proteus spp., Providencia rettgeri, Pseudomonas aeruginosa, Pseudomonas spp., Rickettsia prowazekii, Rickettsia rickettsii, Rochalimaea spp., Salmonella spp., Salmonella typhi, Serratia marcescens, Shigella spp., Treponema carateum, Treponema pallidum, Treponema pallidum endemicum, Treponema pertenue, Veillonella spp., Vibrio cholerae, Vibrio vulnificus, Yersinia enterocolitica*, or *Yersinia pestis*.

[00216] In some embodiments, the bacteria comprise gammaproteobacteria (e.g. *Escherichia*

coli, pseudomonas, vibrio and klebsiella) or *Firmicutes* (belonging to class *Negativicutes* that stain negative in Gram stain).

[00217] In some embodiments, Gram-positive bacteria can be *Actinomyces spp., Bacillus anthracis, Bifidobacterium spp., Clostridium botulinum, Clostridium perfringens, Clostridium spp., Clostridium tetani, Corynebacterium diphtheriae, Corynebacterium jeikeium, Enterococcus faecalis, Enterococcus faecium, Erysipelothrix rhusiopathiae, Eubacterium spp., Gardnerella vaginalis, Gemella morbillorum, Leuconostoc spp., Mycobacterium abcessus, Mycobacterium avium complex, Mycobacterium chelonae, Mycobacterium fortuitum, Mycobacterium haemophilium, Mycobacterium kansasii, Mycobacterium leprae, Mycobacterium marinum, Mycobacterium scrofulaceum, Mycobacterium smegmatis, Mycobacterium terrae, Mycobacterium tuberculosis, Mycobacterium ulcerans, Nocardia spp., Peptococcus niger, Peptostreptococcus spp., Proprionibacterium spp., Staphylococcus aureus, Staphylococcus auricularis, Staphylococcus capitis, Staphylococcus cohnii, Staphylococcus epidermidis, Staphylococcus haemolyticus, Staphylococcus hominis, Staphylococcus lugdanensis, Staphylococcus saccharolyticus, Staphylococcus saprophyticus, Staphylococcus schleiferi, Staphylococcus similans, Staphylococcus warneri, Staphylococcus xylosus, Streptococcus agalactiae (group B streptococcus), Streptococcus anginosus, Streptococcus bovis, Streptococcus canis, Streptococcus equi, Streptococcus milleri, Streptococcus mitior, Streptococcus mutans, Streptococcus pneumoniae, Streptococcus pyogenes (group A streptococcus), Streptococcus salivarius,* or *Streptococcus sanguis.*

[00218] In some embodiments, the bacteria can be from a species of *Escherichia, Shigella, Salmonella, Erwinia, Yersinia, Bacillus, Vibrio, Legionella, Pseudomonas, Neisseria, Bordetella, Helicobacter, Listeria, Agrobacterium, Staphylococcus, Streptococcus, Enterococcus, Clostridium, Corynebacterium, Mycobacterium, Treponema, Borrelia, Francisella, Brucella, Campylobacter, Klebsiella, Frankia, Bartonella, Rickettsia, Shewanella, Serratia, Enterobacter, Proteus, Providencia, Brochothrix,* or *Brevibacterium.*

[00219] In some embodiments, a prokaryote comprises archaea. In some embodiments, the archaea can be: *Archaeoglobi, Methanobacteria, Methanococci, Methanomicrobia, Methanopyri, Nanohaloarchaea, Thermococci, Thermoplasmata, Thermoprotei, Aeropyrum pernix, Cenarchaeum symbiosum, Haladaptatus paucihalophilus, Haloarcula quadrata, Halobacterium salinarum, Halobiforma haloterrestris, Haloferax larsenii, Haloferax volcanii, Haloquadratum walsbyi, Halorubrum salsolis, Metallosphaera sedula, Methanobrevibacter curvatus, Methanobrevibacter cuticularis, Methanobrevibacter filiformis, Methanobrevibacter gottschalkii, Methanobrevibacter oralis, Methanobrevibacter smithii, Methanobrevibacter thaueri,*

Methanobrevibacter woesei, Methanobrevibacter wolinii, Methanocella paludicola, Methanococcoides methylutens, Methanogenium boonei, Methanogenium frigidum, Methanogenium marinum, Methanosarcinacetivorans, Methanosarcina thermophila, Methanosphaera stadtmaniae, Methanothrix soehngenii, Methylosphaera hansonii, Nanoarchaeum equitans, Palaeococcus helgesonii, Picrophilus oshimae, Picrophilus torridus, Pyrococcus abyssi, Pyrococcus furiosus, Pyrococcus horikoshii, Pyrococcus woesei, Pyrodictium abyssi, Pyrolobus fumarii, Saccharolobus shibatae, Salinirubellus salinus, Thermococcus alcaliphilus, Thermococcus barophilus, Thermococcus celer, Thermococcus chitonophagus, Thermococcus gammatolerans, Thermococcus hydrothermalis, Thermococcus kodakarensis, Thermococcus litoralis, Thermococcus profundus, or *Thermococcus stetteri.*

[00220] In some embodiments, a pVip comprises a prokaryotic protein comprising an amino acid sequence homologous to the sequence of a vertebrate viperin, for example but not limited to NCBI accession NP_542388.2 (SEQ ID NO: 2) or SEQ ID NOs 826-828.

[00221] A skilled artisan will recognize that there are several methods that can be used to determine sequence homology and/or sequence identity. Such techniques are thoroughly explained in the literature. See, for example, "A survey of sequence alignment algorithms for next-generation sequencing", Li H et al. Brief Bioinform. 2010 Sep;11(5):473-83; or "Sequence Alignment" Altschul SF et al in SourceHandbook of Discrete and Combinatorial Mathematics. 2017 Nov. 20.

[00222] In some embodiments, a pVip comprises an amino acid sequence comprising at least 10% sequence identity to eukaryotic viperin. In some embodiments, a pVip comprises an amino acid sequence comprising at least 20% sequence identity to eukaryotic viperin. In some embodiments, a pVip comprises an amino acid sequence comprising at least 25% sequence identity to eukaryotic viperin. In some embodiments, a pVip comprises an amino acid sequence comprising at least 30% sequence identity to eukaryotic viperin. In some embodiments, a pVip comprises an amino acid sequence comprising at least 35% sequence identity to eukaryotic viperin. In some embodiments, a pVip comprises an amino acid sequence comprising at least 40% sequence identity to eukaryotic viperin. In some embodiments, a pVip comprises an amino acid sequence comprising at least 45% sequence identity to eukaryotic viperin. In some embodiments, a pVip comprises an amino acid sequence comprising at least 50% sequence identity to eukaryotic viperin.

[00223] In some embodiments, a pVip comprises an amino acid sequence comprising at least 55% sequence identity to eukaryotic viperin. In some embodiments, a pVip comprises an amino acid sequence comprising at least 60% sequence identity to eukaryotic viperin. In some embodiments, a pVip comprises an amino acid sequence comprising at least 65% sequence identity to eukaryotic viperin. In some embodiments, a pVip comprises an amino acid sequence comprising

at least 70% sequence identity to eukaryotic viperin. In some embodiments, a pVip comprises an amino acid sequence comprising at least 75% sequence identity to eukaryotic viperin. In some embodiments, a pVip comprises an amino acid sequence comprising at least 80% sequence identity to eukaryotic viperin. In some embodiments, a pVip comprises an amino acid sequence comprising at least 85% sequence identity to eukaryotic viperin. In some embodiments, a pVip comprises an amino acid sequence comprising at least 90% sequence identity to eukaryotic viperin. In some embodiments, a pVip comprises an amino acid sequence comprising at least 95% sequence identity to eukaryotic viperin. A skilled artisan would recognize that, in some embodiments, the terms "sequence identity" and "sequence homology" are used herein interchangeably having all the same qualities and meanings.

[00224] In some embodiments, a pVip comprises an amino acid sequence comprising between about 15% to about 25% sequence identity to eukaryotic viperin. In some embodiments, a pVip comprises an amino acid sequence comprising between about 25% to about 35% sequence identity to eukaryotic viperin. In some embodiments, a pVip comprises an amino acid sequence comprising between about 35% to about 45% sequence identity to eukaryotic viperin. In some embodiments, a pVip comprises an amino acid sequence comprising between about 45% to about 15% sequence identity to eukaryotic viperin. In some embodiments, a pVip comprises an amino acid sequence comprising between about 55% to about 65% sequence identity to eukaryotic viperin. In some embodiments, a pVip comprises an amino acid sequence comprising between about 65% to about 75% sequence identity to eukaryotic viperin. In some embodiments, a pVip comprises an amino acid sequence comprising between about 75% to about 85% sequence identity to eukaryotic viperin. In some embodiments, a eukaryotic viperin is a human viperin.

[00225] In some embodiments, pVips are clustered according to their homology across prokaryotic species into pVip clusters. In some embodiments, a defense score is calculated for a pVip cluster. In some embodiments, pVip clusters have a "defense score" above a pre-determined threshold. In some embodiments, a defense score above a pre-determined threshold is indicative that a cluster of genes comprises pVips. As used herein, "defense score" is a value computed for a cluster of homologous genes, that is useful in predicting whether the genes of said cluster have antiviral functions. The computation of defense scores is detailed in Doron, S. et al. Systematic discovery of antiphage pVips in the microbial pangenome. Science (80). 4120, eaar4120 (2018), and WO 2018/220616 A2, which are incorporated herein by reference. Briefly, the neighborhood of a gene of interest (+/- 10 genes) is screened for known defense genes. In some embodiments, enrichment of known defense genes in the vicinity of genes of a cluster is a predictor that said genes of said cluster perform anti-viral functions.

[00226] In some embodiments, a defense score is calculated for a cluster of genes comprising homology to a viperin. In some embodiments, a defense score comprises a first score indicating the proportion of genes with defensive neighborhood, termed also "Score 1". In some embodiments a defense score comprises a second score indicating the average number of defense genes in the neighborhood of the genes of said cluster, termed also "Score 2". In some embodiments, a defense score comprises a Score 1 and a Score 2.

[00227] In some embodiments, the enrichment of known defense genes in the vicinity to the genes of a cluster predicts that the cluster comprises pVips. In some embodiments, enrichment of known defense genes in the vicinity of genes of the cluster can be calculated as statistically significant enrichment beyond the background expected by chance. In some embodiments, enrichment of known defense genes in the vicinity of genes of the cluster, or a Score 1, can be calculated as a fraction of the total genes in the cluster that are found in the vicinity of known defense genes, wherein this fraction is above the fraction expected by chance.

[00228] In some embodiments, a fraction of at least 40% of the genes of a cluster predicts that the cluster comprises pVips. In some embodiments, a fraction of at least 50% of the genes of a cluster predicts that the cluster comprises pVips. In some embodiments, a fraction of at least 75% of the genes of a cluster predicts that the cluster comprises pVips. In some embodiments, a fraction of at least 100% of the genes of a cluster predicts that the cluster comprises pVips.

[00229] In some embodiments, the average number of known defense genes in the vicinity of the genes of a cluster, or a Score 2, provides an additional support to the prediction that the cluster comprises pVips. In some embodiments, an average of at least 0.75, 1, 1.5, 2, 3, 4, or 5 known defense genes in the vicinity to the genes of a cluster predicts that the cluster comprises pVips. In some embodiments, an average of between 0.75 and 1 known defense genes in the vicinity to the genes of a cluster predicts that the cluster comprises pVips. In some embodiments, an average of between 1 and 2 known defense genes in the vicinity to the genes of a cluster predicts that the cluster comprises pVips. In some embodiments, an average of between 2 and 5 known defense genes in the vicinity to the genes of a cluster predicts that the cluster comprises pVips.

[00230] In some embodiments, a gene encoding a pVip is located in the vicinity of a gene encoding a nucleotide kinase. In some embodiments, proximity to a nucleotide kinase gene predicts that a gene of interest is a pVip. In some embodiments, said nucleotide kinase is selected from a group comprising a Cytidine/Uridine Monophosphate Kinase 2 (CMPK2), a cytidylate kinase, a thymidylate kinase, a guanylate kinase, and an adenylate kinase. In some embodiments, the substrate of the nucleotide kinases is a ribonucleoside or a ribonucleotide. In some embodiments, the substrate of the nucleoside kinases is a deoxy-ribonucleoside or a deoxy-

ribonucleotide.

[00231] As described below, the pVips are found to have wider substrate promiscuity as compared to the eukaryotic Vips. Based on the substrate promiscuity of pVips as shown herein, it is expected that the pVips would act on various non-natural substrates and generate novel structural modifications on multiple nucleotide derivatives and other molecules.

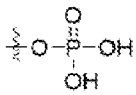
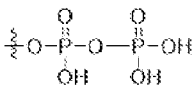
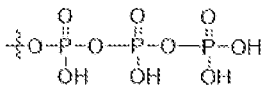
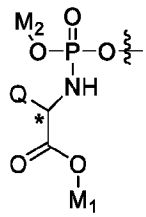
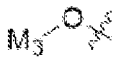
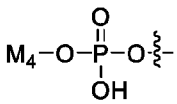
[00232] In one embodiment, the modification catalyzed by pVips on a non-natural substrate is the dehydration of the 3' carbon in the ribose moiety of a nucleotide derivatives (**Figure 13A**). In one embodiment, the modification catalyzed by pVips on a non-natural substrate is the dehydration of the 3' carbon in the5 member nitrogen based ring (**Figure 13B**). In one embodiment, the modification catalyzed by pVips on a non-natural substrate is the dehydration of the 3' carbon in the 5 member carbon based ring) (**Figures 13C, 13D and 13E**). In one embodiment, the modification catalyzed by pVips on a non-natural substrate is the dehydration of the terminal hydroxyl group on an etheric chain (**Figure 13F**).

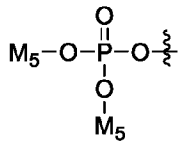
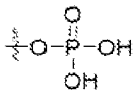
[00233] In certain embodiments, the product produced is a ddh-compound. In certain embodiments, the product produced is a deoxy-ddh-compound. In one embodiment, the products generated from the non-natural substrates by the pVips provide novel therapeutic properties. In one embodiment, the pVips would be able to modify a large set of non-natural substrates as disclosed herein, and one or more of the products of these modifications are useful in treating various diseases, such as viral infection, bacterial infection, a bacterial associated disease, a virus-induced disease, an autoimmune disease, an immune disorder, or cancer, or a combination thereof.

[00234] In some embodiments, the non-natural substrates comprise the compounds represented by the structure of Formula IA, Formula IIA, Formula IIIA, Formula IVA, Formula VA, Formula VIA, Formula VIIA, Formula VIIIA, Formula IXA, Formula XA, Formula XIA, Formula XIIA, Formula XIIIA, Formula XIVA, Formula XVA, Formula XVIA, Formula XVIIA, Formula XVIIIA, Formula XIXA, Formula XXA, Formula XXIA, Formula XXIIA, Formula XXIIIA, Formula XXIVA, Formula XXVA, as disclosed in Table 1 and having the variants as listed therein. In one embodiment, a non-natural substrate comprises a compound represented by the structure of Formula IA, as disclosed in Table 1 and having the variants as listed therein. In one embodiment, a non-natural substrate comprises a compound represented by the structure of Formula IIA, as disclosed in Table 1 and having the variants as listed therein. In one embodiment, a non-natural substrate comprises a compound represented by the structure of Formula IIIA, as disclosed in Table 1 and having the variants as listed therein. In one embodiment, a non-natural substrate comprises a compound represented by the structure of Formula IVA, as disclosed in Table 1 and having the variants as listed therein. In one embodiment, a non-natural substrate comprises a compound

represented by the structure of Formula VA, as disclosed in Table 1 and having the variants as listed therein. In one embodiment, a non-natural substrate comprises a compound represented by the structure of Formula VIA, as disclosed in Table 1 and having the variants as listed therein. In one embodiment, a non-natural substrate comprises a compound represented by the structure of Formula VIIA, as disclosed in Table 1 and having the variants as listed therein. In one embodiment, a non-natural substrate comprises a compound represented by the structure of Formula VIIIA, as disclosed in Table 1 and having the variants as listed therein. In one embodiment, a non-natural substrate comprises a compound represented by the structure of Formula IXA, as disclosed in Table 1 and having the variants as listed therein. In one embodiment, a non-natural substrate comprises a compound represented by the structure of Formula XA, as disclosed in Table 1 and having the variants as listed therein. In one embodiment, a non-natural substrate comprises a compound represented by the structure of Formula XIA, as disclosed in Table 1 and having the variants as listed therein. In one embodiment, a non-natural substrate comprises a compound represented by the structure of Formula XIIA, as disclosed in Table 1 and having the variants as listed therein. In one embodiment, a non-natural substrate comprises a compound represented by the structure of Formula XIIIA, as disclosed in Table 1 and having the variants as listed therein. In one embodiment, a non-natural substrate comprises a compound represented by the structure of Formula XIVA, as disclosed in Table 1 and having the variants as listed therein. In one embodiment, a non-natural substrate comprises a compound represented by the structure of Formula XVA, as disclosed in Table 1 and having the variants as listed therein. In one embodiment, a non-natural substrate comprises a compound represented by the structure of Formula XVIA, as disclosed in Table 1 and having the variants as listed therein. In one embodiment, a non-natural substrate comprises a compound represented by the structure of Formula XVIIA, as disclosed in Table 1 and having the variants as listed therein. In one embodiment, a non-natural substrate comprises a compound represented by the structure of Formula XVIIIA, as disclosed in Table 1 and having the variants as listed therein. In one embodiment, a non-natural substrate comprises a compound represented by the structure of Formula XIXA, as disclosed in Table 1 and having the variants as listed therein. In one embodiment, a non-natural substrate comprises a compound represented by the structure of Formula XXA, as disclosed in Table 1 and having the variants as listed therein. In one embodiment, a non-natural substrate comprises a compound represented by the structure of Formula XXIA, as disclosed in Table 1 and having the variants as listed therein. In one embodiment, a non-natural substrate comprises a compound represented by the structure of Formula XXIIA, as disclosed in Table 1 and having the variants as listed therein. In one embodiment, a non-natural substrate comprises a compound represented by the structure of

Formula XXIIIA, as disclosed in Table 1 and having the variants as listed therein. In one embodiment, a non-natural substrate comprises a compound represented by the structure of Formula XXIVA, as disclosed in Table 1 and having the variants as listed therein. In one embodiment, a non-natural substrate comprises a compound represented by the structure of Formula XXVAs disclosed in Table 1 and having the variants as listed therein.

[00235] In some embodiments, the non-natural substrates comprise the compounds represented by the structure of 2'-C-methyladenosine, 2'-C-methylguanosine, 2'-C-methyluridine, 2'-C-Methylcytidine, 2'-C-ethynyladenosine, Cytarabine (ara-C), ara-A (vidarabine), Gemcitabine hydrochloride, 2'-Deoxy-2'-fluoro-2'-methyluridine, 2'OMe-Uridine, 2'OMe-Adenosine, T-1106, Fluorouridine, 1-[(2R,3R,4S,5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-5-fluoropyrimidine-2,4-dione, 5-Fluoro-deoxy-uridine, 3,4-dihydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)-4-(hydroxyamino)pyrimidin-2(1H)-one, 6-Methyl-7-deazaadenosine, N6-(9-antranylmethyl) adenosine, N6-(1-pyrenylmethyl) adenosine, 5-(Perylen-3-yl)ethynyl-arabino-uridine, ETAR, IM18, 6-Azauridine, Zebularine, 5-Azacytidine, Ribavirin (Virazole), Formycin A, Pyrazofurin, Pseudouridine, Showdomycin, Idoxuridine, Trifluridine, Brivudine, Acedurid, 5-Hydroxy-Uridine, 5-Methyl-Uridine, 4-Thio-i-propyl-Uridine, GS-441524, 7-Deaza-2'-C-methyl-adenosine, NITD008, 2'-Deoxy-2'-fluoro-arabinofuranosyl nucleoside, FIAU, 2'-Deoxy-2'-fluoro-arabinofuranosyl nucleoside, FMAU, 2'-Deoxy-2'-fluoro-arabinofuranosyl nucleoside, FEAU, Fludarabine (2-Fluoro-ara-Adenosine), NITD449, 2-(2-amino-6-methoxy-9H-purin-9-yl)-5-(hydroxymethyl)-3-methyltetrahydrofuran-3,4-diol, 3-fluoro-4-hydroxy-5-(hydroxymethyl)-3-methyltetrahydrofuran-2-yl)pyrimidine-2,4(1H,3H)-dione, 2-(6-(benzylamino)-9H-purin-9-yl)-5-(hydroxymethyl)tetrahydrofuran-3,4-diol, 3,4-dihydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)-4-(hydroxyamino)pyrimidin-2(1H)-one, Ganciclovir, BCX4430, Aristeromycin, Forodesine, Neplanocin A, Entecavir, or Telbivudine, as disclosed in Table 2, wherein R_1 of each corresponding substrate is OH,  ,  ,  ,  ,  ,  , or


; or R_{11} is

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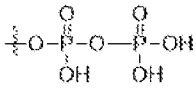
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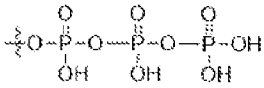
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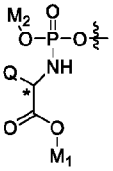
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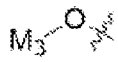
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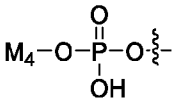
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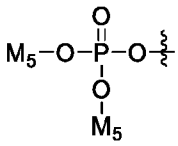
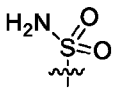

;

wherein Q of R_1 or R_{11} is a side chain of an amino acid; M_1 of R_1 or R_{11} is an alkyl;

M_2 of R_1 or R_{11} is an aryl, a substituted aryl, a heteroaryl or a substituted heteroaryl;

M_3 of R_1 or R_{11} is

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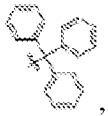
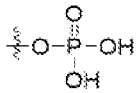
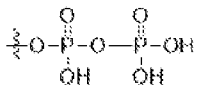
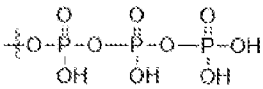
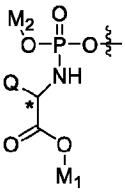
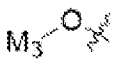
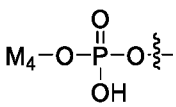
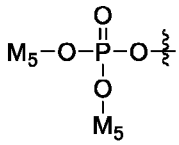

, or

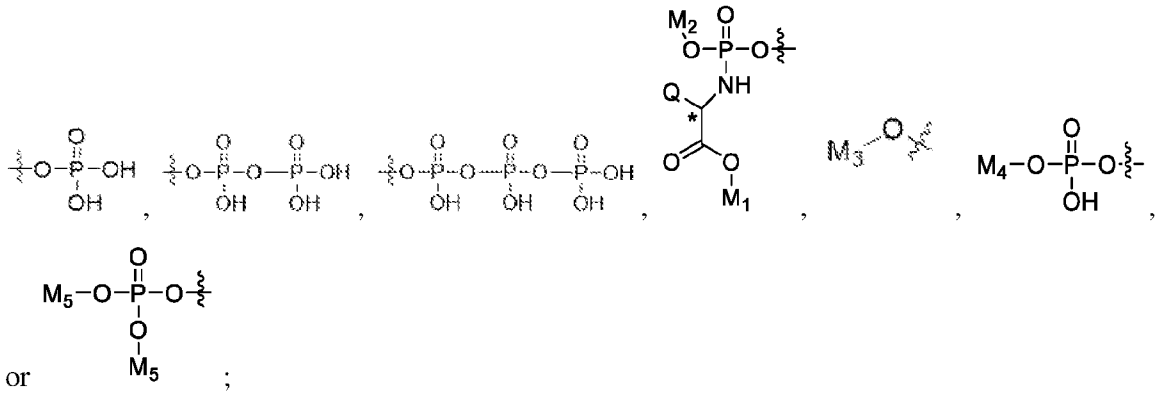
;

M_4 of R_1 or R_{11} is -(C_2-C_6)alkyl-O-(C_{10}-C_{20})alkyl; each M_5 of R_1 or R_{11} is -$(CH_2)_n$-S-C(=O)-(C_1-C_8)alkyl; and n of M_5 is 1-4.

[00236] In one embodiment, a non-natural substrate comprises a compound represented by the structure of 2'-C-methyladenosine. In one embodiment, a non-natural substrate comprises a compound represented by the structure of 2'-C-methylguanosine. In one embodiment, a non-natural substrate comprises a compound represented by the structure of 2'-C-methyluridine. In one embodiment, a non-natural substrate comprises a compound represented by the structure of 2'-C-Methylcytidine. In one embodiment, a non-natural substrate comprises a compound represented by the structure of 2'-C-ethynyladenosine. In one embodiment, a non-natural substrate comprises a compound represented by the structure of Cytarabine (ara-C). In one embodiment, a non-natural substrate comprises a compound represented by the structure of ara-A (vidarabine). In one embodiment, a non-natural substrate comprises a compound represented by the structure of Gemcitabine hydrochloride. In one embodiment, a non-natural substrate comprises a compound represented by the structure of 2'-Deoxy-2'-fluoro-2'-methyluridine. In one embodiment, a non-natural substrate comprises a compound represented by the structure of 2'OMe-Uridine. In one embodiment, a non-natural substrate comprises a compound represented by the structure of 2'OMe-Adenosine. In one embodiment, a non-natural substrate comprises a compound represented by the structure of T-1106. In one embodiment, a non-natural substrate comprises a compound represented by the structure of Fluorouridine. In one embodiment, a non-natural substrate comprises a compound represented by the structure of 1-[(2R,3R,4S,5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-5-fluoropyrimidine-2,4-dione. In one embodiment, a non-natural

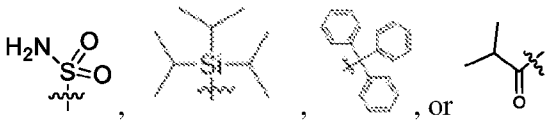
substrate comprises a compound represented by the structure of 5-Fluoro-deoxy-uridine. In one embodiment, a non-natural substrate comprises a compound represented by the structure of 3,4-dihydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)-4-(hydroxyamino)pyrimidin-2(1H)-one. In one embodiment, a non-natural substrate comprises a compound represented by the structure of 6-Methyl-7-deazaadenosine. In one embodiment, a non-natural substrate comprises a compound represented by the structure of N6-(9-antranylmethyl) adenosine. In one embodiment, a non-natural substrate comprises a compound represented by the structure of N6-(1-pyrenylmethyl) adenosine. In one embodiment, a non-natural substrate comprises a compound represented by the structure of 5-(Perylen-3-yl)ethynyl-arabino-uridine. In one embodiment, a non-natural substrate comprises a compound represented by the structure of ETAR. In one embodiment, a non-natural substrate comprises a compound represented by the structure of IM18. In one embodiment, a non-natural substrate comprises a compound represented by the structure of 6-Azauridine. In one embodiment, a non-natural substrate comprises a compound represented by the structure of Zebularine. In one embodiment, a non-natural substrate comprises a compound represented by the structure of 5-Azacytidine. In one embodiment, a non-natural substrate comprises a compound represented by the structure of Ribavirin (Virazole). In one embodiment, a non-natural substrate comprises a compound represented by the structure of Formycin A. In one embodiment, a non-natural substrate comprises a compound represented by the structure of Pyrazofurin. In one embodiment, a non-natural substrate comprises a compound represented by the structure of Pseudouridine. In one embodiment, a non-natural substrate comprises a compound represented by the structure of Showdomycin. In one embodiment, a non-natural substrate comprises a compound represented by the structure of Idoxuridine. In one embodiment, a non-natural substrate comprises a compound represented by the structure of Trifluridine. In one embodiment, a non-natural substrate comprises a compound represented by the structure of Brivudine. In one embodiment, a non-natural substrate comprises a compound represented by the structure of Acedurid. In one embodiment, a non-natural substrate comprises a compound represented by the structure of 5-Hydroxy-Uridine. In one embodiment, a non-natural substrate comprises a compound represented by the structure of 5-Methyl-Uridine. In one embodiment, a non-natural substrate comprises a compound represented by the structure of 4-Thio-i-propyl-Uridine. In one embodiment, a non-natural substrate comprises a compound represented by the structure of GS-441524. In one embodiment, a non-natural substrate comprises a compound represented by the structure of 7-Deaza-2'-C-methyl-adenosine. In one embodiment, a non-natural substrate comprises a compound represented by the structure of NITD008. In one embodiment, a non-natural substrate comprises a compound represented by the structure of 2'-Deoxy-2'-fluoro-arabinofuranosyl nucleoside. In one

embodiment, a non-natural substrate comprises a compound represented by the structure of FIAU. In one embodiment, a non-natural substrate comprises a compound represented by the structure of 2'-Deoxy-2'-fluoro-arabinofuranosyl nucleoside. In one embodiment, a non-natural substrate comprises a compound represented by the structure of FMAU. In one embodiment, a non-natural substrate comprises a compound represented by the structure of 2'-Deoxy-2'-fluoro-arabinofuranosyl nucleoside. In one embodiment, a non-natural substrate comprises a compound represented by the structure of FEAU. In one embodiment, a non-natural substrate comprises a compound represented by the structure of Fludarabine (2-Fluoro-ara-Adenosine). In one embodiment, a non-natural substrate comprises a compound represented by the structure of NITD449. In one embodiment, a non-natural substrate comprises a compound represented by the structure of 2-(2-amino-6-methoxy-9H-purin-9-yl)-5-(hydroxymethyl)-3-methyltetrahydrofuran-3,4-diol. In one embodiment, a non-natural substrate comprises a compound represented by the structure of 3-fluoro-4-hydroxy-5-(hydroxymethyl)-3-methyltetrahydrofuran-2-yl)pyrimidine-2,4(1H,3H)-dione. In one embodiment, a non-natural substrate comprises a compound represented by the structure of 2-(6-(benzylamino)-9H-purin-9-yl)-5-(hydroxymethyl)tetrahydrofuran-3,4-diol. In one embodiment, a non-natural substrate comprises a compound represented by the structure of 3,4-dihydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)-4-(hydroxyamino)pyrimidin-2(1H)-one. In one embodiment, a non-natural substrate comprises a compound represented by the structure of Ganciclovir. In one embodiment, a non-natural substrate comprises a compound represented by the structure of BCX4430. In one embodiment, a non-natural substrate comprises a compound represented by the structure of Aristeromycin. In one embodiment, a non-natural substrate comprises a compound represented by the structure of Forodesine. In one embodiment, a non-natural substrate comprises a compound represented by the structure of Neplanocin A. In one embodiment, a non-natural substrate comprises a compound represented by the structure of Entecavir. In one embodiment, a non-natural substrate comprises a compound represented by the structure of Telbivudine. In some embodiments, for the non-natural substrates disclosed herein from Table 2, R_1 of each corresponding substrate is OH,  ,  ,  ,  ,  ,  , or  ; or R_{11} is



wherein Q of R_1 or R_{11} is a side chain of an amino acid; M_1 of R_1 or R_{11} is an alkyl;

M_2 of R_1 or R_{11} is an aryl, a substituted aryl, a heteroaryl or a substituted heteroaryl;

M_3 of R_1 or R_{11} is  ;

M_4 of R_1 or R_{11} is -(C_2-C_6)alkyl-O-(C_{10}-C_{20})alkyl; each M_5 is -$(CH_2)_n$-S-C(=O)-(C_1-C_8)alkyl; and n of M_5 is 1-4

[00237] In some embodiment, any one of the above non-natural substrates can be modified by the pVips disclosed herein to generate ddh or deoxy-ddh compounds that can be used as DNA/RNA chain terminators. In some embodiments, these non-natural substrates are modified by the pVips to produce their 3′,4′-didehydro (ddh) derivates. In other embodiments, these non-natural substrates are modified by the pVips to become the 3′-deoxy-3′,4′-didehydro (deoxy-ddh) derivates.

[00238] In some embodiments, a pVip comprises any of the pVips provided in Table 3, Table 4, or Table 5 (Tables 3, 4, and 5 are provided below). In some embodiments, a pVip comprises an amino acid sequence having at least 80% sequence identity to any one of amino acid sequences of SEQ ID NOs: 409-789. In some embodiments, a pVip comprises any one of the amino acid sequences set forth in SEQ ID NOs: 409-789. In some embodiments, a pVip comprises an amino acid sequence with at least 20%, at least 30%, at least 40%, at least 50%, or at least 60% sequence identity to SEQ ID NO: 2. In some embodiments, a pVip comprises an amino acid sequence with at least 20%, at least 30%, at least 40%, at least 50%, or with at least 60% sequence identity to a vertebrate viperin.

[00239] **TABLE 6**: Examples of Protein and Gene Sequences of Eukaryotic Viperins

SEQ ID No	description	NCBI Accession number
1	human viperin gene	AF442151.1
2	human viperin protein	NP_542388.2
826	rat viperin protein	NP_620236.1
827	mouse viperin protein	NP_067359.2
828	zebra fish viperin protein	NP_001020727.1

[00240] In some embodiments, the terms "prokaryotic viperin homolog", "pVip", "pVip protein", and "pVip polypeptide" may be used herein interchangeably having all the same qualities and meanings.

[00241] In some embodiments, a pVip comprises an amino acid sequence encoded by one of the polynucleotide sequences of SEQ ID NOs: 3-383. In some embodiments, a pVip comprises an amino acid sequence encoded by one of the polynucleotide sequences of SEQ ID NOs: 384-408. In some embodiments, a pVip comprises an amino acid sequence encoded by a polynucleotide sequence comprising at least 80% identity to a polynucleotide sequence selected from SEQ ID NOs: 3-383. In some embodiments, a pVip comprises an amino acid sequence encoded by a polynucleotide sequence comprising at least 80% identity to a polynucleotide sequence selected from SEQ ID NOs: 384-408.

[00242] In some embodiments, a pVip gene comprises a gene encoding a pVip. In some embodiments, a pVip gene comprises a gene encoding a pVip, wherein said pVip amino acid sequence is set forth in any one of SEQ ID NOs: 409-789. In some embodiments, said pVip gene comprises a sequence with at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, or at least 70% to SEQ ID NO: 1.

[00243] In some embodiments, a pVip comprises a fragment or a functional domain of any one of SEQ ID NOs: 409-789.

[00244] pVips and viperins are radical-SAM enzymes that contain an iron sulfur cluster 4Fe-4S8. For such enzymes, the 4Fe-4S cluster is built by a complex of proteins and then carried into the apoenzyme making it an active holoenzyme. This metabolic step can require some specific interactions between the proteins that build the iron sulfur cluster and the pVip. Heterologous expression of iron-sulfur cluster enzymes such as viperins can thus be devoid of catalytic activity, if the cell in which the viperin is expressed does not express the iron sulfur clusters to high enough levels.

[00245] A skilled artisan would recognize that catalytic activity of metaloenzymes in heterologous hosts can be promoted by a number of strategies. For example, synthesis of iron sulfur cluster in the host can be promoted by deleting the regulator iscR in E. coli. Further, heterologous iron sulfur cluster operons can be expressed to promote iron sulfur cluster synthesis, for example by transfection with plasmids as pDB1282, which encodes the isc operon from *Azotobacter vinelandii*. A further strategy comprises expressing the protein in a more closely related organism from a phylogenetic point of view. Given the sensitivity to oxygen of iron-sulfur cluster proteins, growth in anaerobic conditions, as well as engineering electron transfer pathways into the host cells, are avenues that can also be followed to improve metaloenzymes activities. Further methods can be found, for example, in Shomar H, "Producing high-value chemicals in Escherichia coli through synthetic biology and metabolic engineering", ISBN number 978-90-8593-386-1.

[00246] Table 3, which is displayed at the end of this specification, shows 381 pVip genes, each with its correspondent IMG_id number, metagenome genome IMG_id number, genome or metagenome name, nucleic acid sequence, the clade to which it was clustered (see Example 2, and **Figures 3A**nd **3B**), and whether a kinase was found in its genomic neighborhood, and its SEQ ID NO. "IMG_id" refers to an identification number in the "Integrated Microbial Genomes and Metagenomes" database, https://img.jgi.doe.gov/.

[00247] Table 4, which is displayed at the end of this specification, shows 25 pVips experimentally shown to have anti-viral activity, each with its correspondent IMG_id number, metagenome or genome IMG_id number, genome or metagenome name, the codon-optimized sequence used for its expression (see Example 4), the clade to which it was clustered (see Example 2, and **Figures 3A**nd **3B**), whether a kinase was found in its genomic neighborhood, and its SEQ ID No.

[00248] Table 5, which is displayed at the end of this specification, shows 381 pVip proteins, each with its correspondent IMG_id number, metagenome or genome IMG_id number, genome or metagenome name, amino acid sequence, and SEQ ID No.

Nucleic Acid Constructs Encoding pVips

[00249] In some embodiments, disclosed herein is a nucleic acid construct encoding a pVip. In some embodiments, the pVip construct comprises any one of the pVip genes provided in Table 3 or Table 4. In some embodiments, the pVip construct comprises any one of SEQ ID NOs: 3-408. In some embodiments, the pVip construct comprises a nucleic acid sequence comprising at least 80% identity to one of SEQ ID NOs: 3-408. In some embodiments, a pVip construct comprises a fragment of any one of SEQ ID NOs: 409-789.

[00250] In some embodiments, provided herein is a nucleic acid construct encoding a pVip, said nucleic acid construct comprising a pVip gene and a non-naturally occurring regulatory element operably linked. In some embodiments, said regulatory element comprises a cis-acting regulatory element for directing expression of said pVip gene, a transmissible element for directing transfer of said pVip gene from one cell to another, or a recombination element for integrating said pVip gene into a genome of a cell transfected with said construct, or an element providing episomal maintenance of said construct within a cell transfected with said construct, or any combination thereof.

[00251] In some embodiment, the nucleic acid sequence of the regulatory element is from the same species of the pVip gene. In some embodiment, the nucleic acid sequence of the regulatory element is not from the same species as the pVip gene. In some embodiment, the nucleic acid sequence of the regulatory element is not from the donor species of the pVip gene. In some embodiment, when a host cell comprises a pVip gene, the nucleic acid sequence of the regulatory element is from the host species.

[00252] In some embodiments, cis-acting regulatory elements include those that direct constitutive expression of a nucleic acid sequence. In some embodiments, cis-acting regulatory elements comprise those that direct inducible expression of the nucleic acid sequence only under certain conditions.

[00253] Constitutive promoters suitable for use with some embodiments of the nucleic acid constructs disclosed herein are promoter sequences which are active under most environmental conditions and most types of cells such as those from the cytomegalovirus (CMV) and Rous sarcoma virus (RSV). Inducible promoters suitable for use with some embodiments of pVip constructs disclosed herein include, but are not limited to the tetracycline-inducible promoter (Zabala M, et al., Cancer Res. 2004, 64(8): 2799-804) or pathogen-inducible promoters. Such promoters include those from pathogenesis-related proteins (PR proteins), which are induced following infection by a pathogen.

[00254] A non-limiting example of a gene activator protein is the catabolite activator protein (CAP), which helps initiate transcription of the lac operon in Escherichia coli (Raibaud et al. (1984) Annu. Rev. Genet. 18:173). Regulated expression can therefore be either positive or negative, thereby either enhancing or reducing transcription. Other examples of positive and negative regulatory elements are well known in the art. Various promoters that can be included in the protein expression system include, but are not limited to, a T7/LacO hybrid promoter, a trp promoter, a T7 promoter, a lac promoter, and a bacteriophage lambda promoter.

[00255] Any suitable promoter can be used with the pVips disclosed herein, including the native

promoter or a heterologous promoter. In some embodiments, the promoter is a naturally occurring pVip promoter. In some embodiments, the promoter is a non-naturally occurring, or a heterologous pVip promoter. Heterologous promoters can be constitutively active or inducible. A non-limiting example of a heterologous promoter is given in U.S. Pat. No. 6,242,194 to Kullen and Klaenhammer, which is incorporated herein in full. In some embodiments, the promoter comprises a pARA promoter. In some embodiments, the promoter comprises a pHypraspank promoter. In some embodiments, a pARA promoter is induced by arabinose. In some embodiments, a pHypraspank promoter is induced by IPTG.

[00256] Sequences encoding metabolic pathway enzymes provide particularly useful promoter sequences. Examples include promoter sequences derived from sugar metabolizing enzymes, such as galactose, lactose (lac) (Chang et al. (1987) Nature 198:1056), and maltose. Additional examples include promoter sequences derived from biosynthetic enzymes such as tryptophan (trp) (Goeddel et al. (1980) Nucleic Acids Res. 8:4057; Yelverton et al. (1981) Nucleic Acids Res. 9:731; U.S. Pat. No. 4,738,921; EPO Publication Nos. 36,776 and 121,775). The beta-lactamase (bla) promoter system (Weissmann, (1981) "The Cloning of Interferon and Other Mistakes," in Interferon 3 (ed. I. Gresser); bacteriophage lambda PL (Shimatake et al. (1981) Nature 292:128); the arabinose-inducible araB promoter (U.S. Pat. No. 5,028,530); and T5 (U.S. Pat. No. 4,689,406) promoter systems also provide useful promoter sequences. See also Balbas (2001) Mol. Biotech. 19:251-267, where E. coli expression systems are discussed.

[00257] In addition, synthetic promoters that do not occur in nature also function as bacterial promoters. For example, transcription activation sequences of one bacterial or phage promoter can be joined with the operon sequences of another bacterial or phage promoter, creating a synthetic hybrid promoter (U.S. Pat. No. 4,551,433). For example, the tac (Amann et al. (1983) Gene 25:167; de Boer et al. (1983) Proc. Natl. Acad. Sci. 80:21) and trc (Brosius et al. (1985) J. Biol. Chem. 260:3539-3541) promoters are hybrid trp-lac promoters comprised of both trp promoter and lac operon sequences that are regulated by the lac repressor. The tac promoter has the additional feature of being an inducible regulatory sequence. Thus, for example, expression of a coding sequence operably linked to the tac promoter can be induced in a cell culture by adding isopropyl-1-thio-β-D-galactoside (IPTG). Furthermore, bacterial promoter can include naturally occurring promoters of non-bacterial origin that have the ability to bind bacterial RNA polymerase and initiate transcription. A naturally occurring promoter of non-bacterial origin can also be coupled with a compatible RNA polymerase to produce high levels of expression of some genes in prokaryotes. The phage T7 RNA polymerase/promoter system is an example of a coupled promoter system (Studier et al. (1986) J. Mol. Biol. 189:113; Tabor et al. (1985) Proc. Natl. Acad.

Sci. 82:1074). In addition, a hybrid promoter can also be comprised of a phage promoter and an E. coli operator region (EPO Publication No. 267,851).

[00258] The nucleic acid construct can additionally contain a nucleic acid sequence encoding the repressor or the inducer for that promoter. For example, an inducible construct can regulate transcription from the Lac operator (LacO) by expressing the nucleotide sequence encoding the LacI repressor protein. Other examples include the use of the lexA gene to regulate expression of pRecA, and the use of trpO to regulate ptrp. Alleles of such genes that increase the extent of repression (e.g., lacIq) or that modify the manner of induction (e.g., lambda CI857, rendering lambda pL thermo-inducible, or lambda CI+, rendering lambda pL chemo-inducible) can be employed.

[00259] In the construction of the construct, in some embodiments, the promoter is positioned approximately the same distance from the heterologous transcription start site as it is from the transcription start site in its natural setting. As is known in the art, however, some variation in this distance can be accommodated without loss of promoter function.

[00260] According to some embodiments, the nucleic acid construct includes a promoter sequence for directing transcription of the nucleic acid sequence in the cell in a constitutive or inducible manner. In some embodiments, the expression of the pVip genes disclosed herein can be transient or consistent, episomal or integrated into the chromosome of a host cell. According to some embodiments, the expression is on a transmissible genetic element.

[00261] The nucleic acid construct disclosed herein may further include additional sequences which render this construct suitable for replication and integration in prokaryotes, eukaryotes, or both (e.g., shuttle vectors). In some embodiments, the nucleic acid construct comprises a recombination element for integrating the pVip gene into the genome of a cell transfected with the construct. A skilled artisan would appreciate that the term “recombination element” encompasses a nucleic acid sequence that allows the integration of the polynucleotide in the genome of a cell (e.g. bacteria) transfected with the construct.

[00262] In some embodiments, the nucleic acid construct comprises an element providing episomal maintenance of said construct within a cell transfected with said construct.

[00263] In some embodiments, a construct may also contain a transcription and translation initiation sequence, transcription and translation terminator and a polyadenylation signal. By way of example, such constructs will typically include a 5' LTR, a tRNA binding site, a packaging signal, an origin of second-strand DNA synthesis, and a 3' LTR or a portion thereof.

[00264] In some embodiments, the nucleic acid construct further comprises a transmissible element for directing transfer of said nucleic acid sequence from one cell to another. In some

embodiments, a pVip gene is on a transmissible genetic element. In some embodiments, a pVip gene selected from a gene provided in Table 1, Table 2, or comprising any one of SEQ ID NOs: 3-408 is on a transmissible genetic element.

[00265] A skilled artisan would appreciate that the term “transmissible element” or “transmissible genetic element”, which are interchangeably used, encompasses a polynucleotide that allows the transfer of the nucleic acid sequence from one cell to another, e.g. from one bacterium to another.

[00266] According to some embodiments, a transmissible genetic element comprises a conjugative genetic element or mobilizable genetic element. In some embodiments, a transmissible genetic element comprises a conjugative genetic element. In some embodiments, a transmissible genetic element comprises a mobilizable genetic element. The skilled artisan would appreciate that a “conjugative plasmid” encompasses a plasmid that is transferred from one cell (e.g. bacteria) to another during conjugation, and the term “mobilizable element” encompasses a transposon, which is a DNA sequence that can change its position within the genome.

[00267] In some embodiments, a nucleic acid construct disclosed herein comprises an expression vector. In some embodiments, an "expression vector" or a “vector”, used interchangeably herein, comprises and expresses a pVip gene encoding a pVip disclosed herein. In some embodiments, expression comprises transient expression. In some embodiments, expression comprises constitutive expression. In some embodiments, expression is from an episomal nucleic acid sequence. In some embodiments, expression is from a nucleic acid sequence integrated into the chromosome of the cell. According to specific embodiments, the expression is on a transmissible genetic element.

[00268] In some embodiments, provided herein is a transmissible genetic element comprising a nucleic acid construct encoding a pVip. In some embodiments, disclosed herein is an expression vector comprising a nucleic acid construct encoding a pVip.

[00269] According to some embodiment, the nucleic acid construct comprises a plurality of cloning sites for ligating a nucleic acid sequence of a pVip gene, such that it is under transcriptional regulation of the regulatory elements.

[00270] Selectable marker genes that ensure maintenance of a construct in a host cell can also be included in the construct. In some embodiments, selectable markers include those which confer resistance to drugs such as ampicillin, chloramphenicol, erythromycin, kanamycin (neomycin), and tetracycline (Davies et al. (1978) Annu. Rev. Microbiol. 32:469). Selectable markers can also allow a cell to grow on minimal medium, or in the presence of toxic metabolite and can include biosynthetic genes, such as those in the histidine, tryptophan, and leucine biosynthetic pathways.

[00271] Other than containing the necessary elements for the transcription and translation of the inserted coding sequence, the expression construct of some embodiments can also include sequences engineered to enhance stability, production, purification, yield or toxicity of the expressed polypeptide. Where appropriate, the nucleic acid sequences may be optimized for increased expression in the transformed organism. For example, the nucleic acid sequences can be synthesized using preferred codons for improved expression.

Introduction of pVips Into Cells

[00272] Various methods known within the art can be used to introduce a pVip into a cell. In some embodiments, introducing a pVip into a cell comprises introducing a pVip polypeptide into a cell. In some embodiments, introducing a pVip into a cell comprises introducing a nucleic acid construct encoding a pVip gene into a cell. Methods for introducing a nucleic acid construct or a polypeptide into a cell are generally described in Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Springs Harbor Laboratory, New York (1989, 1992), in Ausubel et al., Current Protocols in Molecular Biology, John Wiley and Sons, Baltimore, Md. (1989), Chang et al., Somatic Gene Therapy, CRC Press, Ann Arbor, Mich. (1995), Vega et al., Gene Targeting, CRC Press, Ann Arbor Mich. (1995), Vectors: A Survey of Molecular Cloning Vectors and Their Uses, Butterworths, Boston Mass. (1988) and Gilboa et at. [Biotechniques 4 (6): 504-512, 1986] and include, for example, stable or transient transfection, natural or induced transformation, lipofection, electroporation and infection with recombinant viral vectors. In addition, see U.S. Pat. Nos. 5,464,764 and 5,487,992 for positive-negative selection methods, which are incorporated herein.

[00273] Introduction of nucleic acids by phage infection offers several advantages over other methods such as transformation, since higher transfection efficiency can be obtained due to the infectious nature of phages. These methods are especially useful for rendering bacteria more sensitive to phage attack for antibiotics purposes as further described hereinbelow.

[00274] It will be appreciated that a pVip can be introduced directly into the cell (e.g., bacterial cell) and not via recombinant expression to confer viral resistance. Thus, according to some embodiments, disclosed herein are isolated pVips or functional fragments thereof as described herein.

[00275] In some embodiments, a pVip can be introduced directly into the cell (e.g., bacterial cell) and not via recombinant expression, for example to confer viral resistance. In some embodiments, said pVip comprises a pVip provided in Table 5, or any of SEQ ID NOs: 409-789. In some embodiments, viral resistance comprises resistance to foreign nucleic acid invasion, to at least one phage infection, resistance to plasmid transformation, resistance to entry of a conjugative

element, or any combination thereof.

[00276] In some embodiments, a pVip or a pVip gene is introduced into a cell together with co-factors. In some embodiments, these co-factors are needed for pVip proper functioning. In some embodiments, said co-factors comprise an s-adenosyl methionine. In some embodiments, said co-factors comprise the pVip specific substrate. In some embodiments, the specific substrate can be a non-natural substrate having any one of the structures of as disclosed herein, for example as provided in Table 1 and Table 2, or any combination thereof.

[00277] In some embodiments, the cell to which a pVip is introduced is a eukaryotic cell. In some embodiments, the eukaryotic cell is a tumor cell. In some embodiments, the cell to which a pVip is introduced is a prokaryotic cell, for example, a bacterium or achaea. In some embodiments, the bacterium is a gram-positive bacterium or a gram-negative bacterium.

Isolated Cells Comprising Prokaryotic Viperin Homologs (pVips)

[00278] In some embodiments, provided herein are isolated cells comprising an ectopic prokaryotic viperin homolog (pVip). In some embodiments, provided herein are cells genetically modified to express a pVip or a fragment thereof. pVips have been described in detail herein. In some embodiments, a pVip comprises a pVip provided in Table 5, or any one of SEQ ID NOs: 409-789. In some embodiments, a pVip comprises an amino acid sequence with at least 80% homology to pVip provided in Table 5, or any one of SEQ ID NOs: 409-789. In some embodiments, the isolated cell comprises more than one pVip.

[00279] In some embodiments, the cell comprises an ectopic pVip gene. In some embodiments, the cell comprises one or more of the genes provided in Table 3, Table 4, or comprising one or more of SEQ ID NOs: 3-408. In some embodiment the cell comprises more than one ectopic pVip gene. In some embodiments, the cell comprises endogenous pVip co-factors. In some embodiments, pVip co-factors are ectopically provided.

[00280] In some embodiments, a cell is genetically modified to express a pVip gene. In some embodiments, a cell is genetically modified to express a combination of more than one pVip gene. In some embodiments, the cell comprises anti-phage, anti-plasmid, or anti-phage and anti-plasmid resistance provided by pVip genes. In some embodiments, multiple pVips are comprised in a single nucleic acid construct. In some embodiments, multiple pVips are comprised in multiple nucleic acid constructs.

[00281] In some embodiments, a cell (e.g., a bacterial cell) does not express an endogenous pVip. In some embodiments, the cell expresses an endogenous pVip which is different than the ectopically expressed pVip. In some embodiments, the cell expresses an endogenous pVip similar to the ectopically expressed pVip. In some embodiments, when an endogenous pVip is similar to

the ectopically expressed pVip, expression of the ectopic pVip increases the concentration of said pVip in the cell.

Uses of Prokaryotic Viperin Homolog (pVip)

[00282] Structural elements, such as amino acid sequences of prokaryotic viperin homologs (pVips) have been described in detail above, as well as the genes that encode these pVips. Uses of pVips have been described above as well. Further details for uses of pVips is presented herein and exemplified in the Examples section below. In some embodiments, methods of using a pVip disclosed herein comprise use of a pVip, or a pVip gene. In some embodiments, the pVip comprises a pVip provided in Table 5, or any one of SEQ ID NOs: 409-789. In some embodiments, the pVip comprises an amino acid comprising at least 80% homology to a pVip provided in Table 5, or to any one of SEQ ID Nos: 409-789. In some embodiments, methods of use of pVip comprise use of a combination of pVips. In some embodiments, the pVips is encoded by a polynucleotide having at least 80% identity to a gene provided in Table 3, Table 4, or to any one of SEQ ID NOs: 3-408.

[00283] In some embodiments, the present disclosure provides methods of using ddh or deoxy-ddh compounds generated by the pVips from non-natural substrates disclosed herein. In some embodiments, methods of using these using ddh or deoxy-ddh compounds include methods of protecting eukaryotic cells from viral infection, methods for decreasing viral replication in eukaryotic cells, and methods of decreasing RNA transcription, for example for viruses with RNA genomes. In some embodiments, methods of using ddh or deoxy-ddh compounds disclosed herein include methods of increasing termination of DNA synthesis, methods of increasing termination of RNA synthesis, methods of decreasing proliferation in a cell, methods of conferring tumor resistance to a cell. In some embodiments, methods of using ddh or deoxy-ddh compounds disclosed herein include methods of treating an autoimmune disease, an immune disorder, or a disease or disorder associated with bacterial infection in a cell. In some embodiments, the cell is a eukaryotic cell. In some embodiment, the eukaryotic cell comprises a human cell.

[00284] In some embodiments, methods of using these using ddh or deoxy-ddh compounds described herein include, methods of treating a disease in a subject in need. In some embodiments, methods of using ddh or deoxy-ddh compounds described herein comprise treating a disease in a subject in need, wherein said disease comprises a virus-induced disease, a viral infection, a cancer or a tumor, an autoimmune disease, an immune disorder, or a disease or disorder associated with a bacterial infection, or any combination thereof. In some embodiments methods of using ddh or deoxy-ddh compounds described herein comprise treating a disease in a subject in need, wherein said disease comprises a virus-induced disease. In some embodiments methods of using ddh or

deoxy-ddh compounds described herein comprise treating a disease in a subject in need, wherein said disease comprises a cancer or a tumor. In some embodiments methods of using ddh or deoxy-ddh compounds described herein comprise treating a disease in a subject in need, wherein said disease comprises an autoimmune disease. In some embodiments methods of using ddh or deoxy-ddh compounds described herein comprise treating a disease in a subject in need, wherein said disease comprises an immune disorder. In some embodiments methods of using ddh or deoxy-ddh compounds described herein comprise treating a disease in a subject in need, wherein said disease comprises a disease or disorder associated with a bacterial infection, or any combination thereof. In some embodiment, the subject in need is a human.

[00285] In some embodiments, a virus induced disease comprises a viral infection. In some embodiments, a viral induced disease comprises a viral infection, wherein said virus is selected from the group consisting of norovirus, rotavirus, hepatitis virus A, B, C, D, or E, rabies virus, West Nile virus, enterovirus, echovirus, coxsackievirus, herpes simplex virus (HSV), varicella-zoster virus, mosquito-borne viruses, arbovirus, St. Louis encephalitis virus, California encephalitis virus, lymphocytic choriomeningitis virus, human immunodeficiency virus (HIV), poliovirus, zika virus, rubella virus, cytomegalovirus, human papillomavirus (HPV), enteovirus D68, severe acute respiratory syndrome (SARS) coronavirus, Middle East respiratory syndrome coronavirus (MERS-CoV), SARS coronavirus 2 (SARS-CoV-2), Epstein-Barr virus (EBV), influenza virus, influenza virus A2, influenza virus B, influenza virus A(H1N1), respiratory syncytical virus (RSV), polyoma viruses, BK virus, Tacaribe virus, Ebola virus, Dengue virus, and any combination thereof

[00286] In some embodiments, the viral induced disease comprises COVID19 as a result of a SARS-CoV-2 infection. In some embodiments, the viral induced disease comprises COVID19 as a result of a SARS-CoV-2 infection. In some embodiments, the viral induced disease comprises infectious mononucleosis; non-malignant, premalignant, and malignant Epstein–Barr virus-associated lymphoproliferative diseases such as Burkitt lymphoma, hemophagocytic lymphohistiocytosis; Hodgkin's lymphoma; non-lymphoid malignancies such as gastric cancer and nasopharyngeal carcinoma; or conditions associated with human immunodeficiency virus such as hairy leukoplakiand central nervous system lymphomas, as a result of a EBV infection. In some embodiments, the viral induced disease occurs in an immunocompromised or immunosuppressed subject, as a result of a BKV infection. .

[00287] In some embodiments, activity of the ddh- and deoxy-ddh compounds terminating polynucleotide chain synthesis confers viral resistance in a cell, wherein said cell is a eukaryotic cell. In some embodiments, the eukaryotic cell is a tumor cell, a cancer cell, or is a cell infected by

a virus. or foreign DNA.

[00288] A skilled artisan would appreciate that the while the ddh or deoxy-ddh compounds described herein may in certain embodiments be generated by the pVips described herein or homologs thereof from non-natural pVip substrates, in other embodiments, the ddh or deoxy-ddh compounds could be synthesized using chemical synthetic methods known in the art.

[00289] In one embodiment, methods of use of a pVip described herein include but are not limited to methods of producing modified nucleosides or modified nucleotides, methods for the discovery of nucleotide chain terminator molecules, methods to produce nucleotide analogs, methods to produce nucleoside analogs, methods to produce anti-viral compounds, and methods to produce antibiotic compounds. In some embodiments, the ddh or deoxy-ddh compounds are generated by the pVips from non-natural substrates having the substrate structures as described in detail herein, for example in Table 1 and Table 2 above.

[00290] In one embodiment, a ddh or deoxy-ddh compound is produced by the pVips from a non-natural substrate that is described in Table 1. In one embodiment, a ddh or deoxy-ddh compound is produced by the pVips from a non-natural substrates that is described in Table 2. In one embodiment, such ddh or deoxy-ddh compound is used in methods described herein. In one embodiment, such ddh or deoxy-ddh compound is used in methods described herein. In one embodiment, combinations of such ddh or deoxy-ddh compounds are used in methods described herein. In one embodiment, combinations of such ddh or deoxy-ddh compound are used in methods described herein. In one embodiment, a composition comprising 2 or more such ddh or deoxy-ddh compound are used. In one embodiment, a composition comprising 2 or more such ddh or deoxy-ddh compound are used. In one embodiment, a composition comprising 3 or more such ddh or deoxy-ddh compound are used. In one embodiment, a composition comprising 3 or more such ddh or deoxy-ddh compound are used.

[00291] In one embodiment, any one of the non-natural substrates described herein can be modified by the pVips to generate ddh or deoxy-ddh compound that can be used as DNA/RNA chain terminators. These ddh or deoxy-ddh compound can be applied in the various methods of uses as described herein. In one embodiment, a pVip may produce one kind of ddh or deoxy-ddh compound from the non-natural substrates. In another embodiment, a pVip may produce multiple kinds of ddh or deoxy-ddh compounds from the non-natural substrates. For example, a pVip may produce two kinds of ddh or deoxy-ddh compounds, or a pVip may produce three kinds ddh or deoxy-ddh compounds, etc.

Methods for Treating A Disease

[00292] In one embodiment, the present disclosure provides a pharmaceutical composition comprising one or more ddh- or deoxy-ddh compounds as disclosed herein, for use in the treatment of a disease in a subject in need thereof. The ddh- or deoxy-ddh compounds may in certain embodiments, be used to treat a disease as a result of a viral infection. In some embodiments, the ddh- or deoxy-ddh compounds disclosed herein comprise antiviral activity.

[00293] In some embodiments, the viral infection comprises infection by an RNA virus. In some embodiments, the viral infection comprises infection by a DNA virus.

[00294] The ddh- or deoxy-ddh compounds may in certain embodiments, be produced by a prokaryotic homolog of viperin (pVip) from non-natural substrates, wherein the pVip comprises the amino acid sequence of one of SEQ ID NOs:409-789. In another embodiment, the pVip comprises an amino acid having at least 80% homology to a pVip provided in Table 3, or having at least 80% homology to any one of SEQ ID NOs: 409-789. In some embodiments, the ddh- or deoxy-ddh compounds are produced synthetically using methods known in the art.

[00295] In one embodiment, the non-natural substrates, for example as described in detail herein, can have one of the structures of wherein the non-natural substrates each comprises 0, 1, 2, or 3 phosphate groups.

[00296] In some embodiments, a non-natural substrate comprises 0, 1, 2, or 3 phosphate groups. In some embodiments, a non-natural substrate comprises 0 phosphate groups. In some embodiments, a non-natural substrate comprises 1 phosphate group. In some embodiments, a non-natural substrate comprises 2 phosphate group. In some embodiments, a non-natural substrate comprises 3 phosphate group.

[00297] In some embodiments, the ddh- or deoxy-ddh compounds produced from the non-natural substrates comprise a variant of the corresponding ddh- or deoxy-ddh compounds lacking a 4′ hydrogen and a 3′ hydroxyl group. In some embodiments, a ddh- or deoxy-ddh compounds comprises 0, 1, 2, or 3 phosphate groups. In some embodiments, a ddh- or deoxy-ddh compound comprises 0 phosphate groups. In some embodiments, a ddh- or deoxy-ddh compound comprises 1 phosphate group. In some embodiments, a ddh- or deoxy-ddh compound comprises 2 phosphate group. In some embodiments, a ddh- or deoxy-ddh compound comprises 3 phosphate group.

[00298] In some embodiments provided herein is a ddh- or deoxy-ddh compound wherein the compound is represented by the structure of Formula IB, Formula IIB, Formula IIIB, Formula IVB, Formula VB, Formula VB1, Formula VIB, Formula VIIB, Formula VIIIB, Formula IXB, Formula XB, Formula XIB, Formula XIIB, Formula XIIIB, Formula XIIIB1, Formula XIVB, Formula XIVB1, Formula XIVB2, Formula XIV3, Formula XIV4, Formula XIV5, Formula XVB,

Formula XVB1, Formula XVB2, Formula XVB3, Formula XVB4, Formula XVB5, Formula XVB6, Formula XVIB, Formula XVIB1, Formula XVIB2, Formula XVIB3, Formula XVIB4, Formula XVIB5, Formula XVIB6, Formula XVIB7, Formula XVIB8, Formula XVIB9, Formula XVIB10, Formula XVIB11, Formula XVIB12, Formula XVIB13, Formula XVIB14, Formula XVIB15, Formula XVIB16, Formula XVIB17, Formula XVIB18, Formula XVIB19, Formula XVIB20, Formula XVIB21, Formula XVIB22, Formula XVIB23, Formula XVIB24, Formula XVIB25, Formula XVIB26, Formula XVIB27, Formula XVIB28, Formula XVIB29, Formula XVIB30, Formula XVIB31, Formula XVIB32, Formula XVIB33, Formula XVIIB, Formula XVIIIB, Formula XXB, Formula XIXB, Formula XXIB, Formula XXIIB, Formula XXXIIIB, Formula XXIVB, or Formula XXVB for use in the treatment of a disease in a subject in need thereof.

[00299] In some embodiments, provided herein is a compound wherein the compound is represented by the structure of Formula IB, Formula IIB, Formula IIIB, Formula IVB, Formula VB, Formula VB1, Formula VIB, Formula VIIB, Formula VIIIB, Formula IXB, Formula XB, Formula XIB, Formula XIIB, Formula XIIIB, Formula XIIIB1, Formula XIVB, Formula XIVB1, Formula XIVB2, Formula XIV3, Formula XIV4, Formula XIV5, Formula XVB, Formula XVB1, Formula XVB2, Formula XVB3, Formula XVB4, Formula XVB5, Formula XVB6, Formula XVIB, Formula XVIB1, Formula XVIB2, Formula XVIB3, Formula XVIB4, Formula XVIB5, Formula XVIB6, Formula XVIB7, Formula XVIB8, Formula XVIB9, Formula XVIB10, Formula XVIB11, Formula XVIB12, Formula XVIB13, Formula XVIB14, Formula XVIB15, Formula XVIB16, Formula XVIB17, Formula XVIB18, Formula XVIB19, Formula XVIB20, Formula XVIB21, Formula XVIB22, Formula XVIB23, Formula XVIB24, Formula XVIB25, Formula XVIB26, Formula XVIB27, Formula XVIB28, Formula XVIB29, Formula XVIB30, Formula XVIB31, Formula XVIB32, Formula XVIB33, Formula XVIIB, Formula XVIIIB, Formula XXB, Formula XIXB, Formula XXIB, Formula XXIIB, Formula XXXIIIB, Formula XXIVB or Formula XXVB for use in the treatment of a disease comprises a virus-induced disease, a cancer, an autoimmune disease, an immune disorder, a bacterial associated disease or infection, or a combination thereof, in a subject in need thereof. In another embodiment, the disease is a virus-induced disease. In another embodiment, the disease is a cancer. In another embodiment, the disease is an autoimmune disease. In another embodiment, the disease is an immune disorder. In another embodiment, the disease is a bacterial associated disease.

[00300] In another embodiment, methods of use treat a disease comprising an infection. In another embodiment, methods of use disclosed herein treat COVID19 because of of SARS-CoV-2 infection.

[00301] Subjects infected with EBV have an increased the risk for the development of several cancers and autoimmune diseases. Diseases associate with EBV infection included but are not limited to infectious mononucleosis, hemophagocytic lymphohistiocytosis, non-malignant or premalignant or malignant lymphoproliferative diseases such as Burkitt lymphoma, Hodgkin's lymphoma, non-lymphoid malignancies such as gastric cancer and nasopharyngeal carcinoma, hairy leukoplakia, central nervous system lymphomas, and multiple sclerosis. (*See*, Drosu et al., (2020) *Tenofovir prodrugs potently inhibit Epstein–Barr virus lytic DNA replication by targeting the viral DNA polymerase*. PNAS 117(22):12368-12374.) In some embodiment, methods of use disclosed herein treat an EBV infection-associated disease comprising infectious mononucleosis, hemophagocytic lymphohistiocytosis, non-malignant or premalignant or malignant lymphoproliferative diseases such as Burkitt lymphoma, Hodgkin's lymphoma, non-lymphoid malignancies such as gastric cancer and nasopharyngeal carcinoma, hairy leukoplakia, central nervous system lymphomas, and multiple sclerosis.

[00302] Double-stranded (ds) DNA virus infections often occur concomitantly in immunocompromised patients. In another embodiment, methods of use disclosed herein treat a viral induced disease occurring in an immunocompromised or immunosuppressed subject. In some embodiments, methods of use treat an immunocompromised patient infected with a BK virus, an adenovirus, a herpesvirus including Epstein-Barr virus, a poxvirus, or a polyoma virus including BK virus or JC virus (human polyomavirus 2)). Patients undergoing solid organ transplantation or allogeneic hematopoietic cell transplant (allo-HCT) are susceptible to viral infection, due to immunosuppressive environment created to support the transplant. In some embodiments, these patients are suffering from diseases including but not limited to nephropathy or hemorrhagic cystitis, etc. These transplant patients are particularly susceptible to dsDNA viral infections, for example EBV infections or polyoma viral infection including BKV and JCV infections.

[00303] In some embodiments, methods of treating disclosed herein, treat a disease associated with a dsDNA viral infection. In some embodiments, diseases associated dsDNA viral infections include diseases associated with a hematopoietic cell transplantation including nephropathy, hemorrhagic cystitis, etc.

[00304] In some embodiments, methods of use treat an immunosuppressed transplant patient, for example a subject undergoing a solid organ transplantation or a hematopoietic cell transplantation, wherein said patient has a dsDNA viral infection. In some embodiments, methods of use treat an immunosuppressed transplant patient, for example a subject undergoing a solid organ transplantation or a hematopoietic cell transplantation, wherein said patient has an EBV, BKV, herpes virus-6, adenovirus, CMV, or JCV infection. (*See* for example, Chemaly et al., (2019)

In vitro comparison of currently available and investigational antiviral agents against pathogenic human double-stranded DNA viruses: A systematic literature review. Antiviral Research 163: 50–58.*)* In some embodiments, methods of use treat an immunosuppressed transplant patient with an EBV infection. In some embodiments, methods of use treat an immunosuppressed transplant patient with an BKV infection. In some embodiments, methods of use treat an immunosuppressed transplant patient with a herpes virus-6 infection. In some embodiments, methods of use treat an immunosuppressed transplant patient with an adenovirus infection. In some embodiments, methods of use treat an immunosuppressed transplant patient with a CMV infection. In some embodiments, methods of use treat an immunosuppressed transplant patient with a JCV infection.

[00305] In another embodiment, a disease treated by methods disclosed herein is caused by a viral infection, wherein the virus is selected from the group consisting of norovirus, rotavirus, hepatitis virus A, B, C, D, or E, rabies virus, West Nile virus, enterovirus, echovirus, coxsackievirus, herpes simplex virus (HSV), varicella-zoster virus, mosquito-borne viruses, arbovirus, St. Louis encephalitis virus, California encephalitis virus, lymphocytic choriomeningitis virus, human immunodeficiency virus (HIV), poliovirus, zika virus, rubella virus, cytomegalovirus, human papillomavirus (HPV), enteovirus D68, severe acute respiratory syndrome (SARS) coronavirus, Middle East respiratory syndrome coronavirus (MERS-CoV), SARS coronavirus 2 (SARS-CoV-2), Epstein-Barr virus (EBV), influenza virus, influenza virus A2, influenza virus B, influenza virus A(H1N1), respiratory syncytical virus (RSV), polyoma viruses, BK virus, Tacaribe virus, Ebola virus, Dengue virus, and any combination thereof.

[00306] In another embodiment, the disease is caused by an EBV infection. In another embodiment, the disease is caused by an BKV infection. In another embodiment, the disease is caused by a SAR-CoV-2 infection.

[00307] In some embodiments provided herein is a pharmaceutical composition comprising a compound wherein the compound is represented by the structure of Formula IB, Formula IIB, Formula IIIB, Formula IVB, Formula VB, Formula VB1, Formula VIB, Formula VIIB, Formula VIIIB, Formula IXB, Formula XB, Formula XIB, Formula XIIB, Formula XIIIB, Formula XIIIB1, Formula XIVB, Formula XIVB1, Formula XIVB2, Formula XIV3, Formula XIV4, Formula XIV5, Formula XVB, Formula XVB1, Formula XVB2, Formula XVB3, Formula XVB4, Formula XVB5, Formula XVB6, Formula XVIB, Formula XVIB1, Formula XVIB2, Formula XVIB3, Formula XVIB4, Formula XVIB5, Formula XVIB6, Formula XVIB7, Formula XVIB8, Formula XVIB9, Formula XVIB10, Formula XVIB11, Formula XVIB12, Formula XVIB13, Formula XVIB14, Formula XVIB15, Formula XVIB16, Formula XVIB17, Formula XVIB18, Formula XVIB19, Formula XVIB20, Formula XVIB21, Formula XVIB22, Formula XVIB23, Formula

XVIB24, Formula XVIB25, Formula XVIB26, Formula XVIB27, Formula XVIB28, Formula XVIB29, Formula XVIB30, Formula XVIB31, Formula XVIB32, Formula XVIB33, Formula XVIIB, Formula XVIIIB, Formula XXB, Formula XIXB, Formula XXIB, Formula XXIIB, Formula XXXIIIB, Formula XXIVB, or Formula XXVB or a combination thereof, for use in the treatment of a disease in a subject in need thereof.

[00308] In some embodiments provided herein is a pharmaceutical composition comprising a compound wherein the compound is represented by the structure of Formula IB, Formula IIB, Formula IIIB, Formula IVB, Formula VB, Formula VIB, Formula VIIB, Formula VIIIB, Formula IXB, Formula XB, Formula XIB, Formula XIIB, Formula XIIIB, Formula XIIIB1, Formula XIVB, Formula XIVB1, Formula XIVB2, Formula XIV3, Formula XIV4, Formula XIV5, Formula XVB, Formula VB1, Formula XVB1, Formula XVB2, Formula XVB3, Formula XVB4, Formula XVB5, Formula XVB6, Formula XVIB, Formula XVIB1, Formula XVIB2, Formula XVIB3, Formula XVIB4, Formula XVIB5, Formula XVIB6, Formula XVIB7, Formula XVIB8, Formula XVIB9, Formula XVIB10, Formula XVIB11, Formula XVIB12, Formula XVIB13, Formula XVIB14, Formula XVIB15, Formula XVIB16, Formula XVIB17, Formula XVIB18, Formula XVIB19, Formula XVIB20, Formula XVIB21, Formula XVIB22, Formula XVIB23, Formula XVIB24, Formula XVIB25, Formula XVIB26, Formula XVIB27, Formula XVIB28, Formula XVIB29, Formula XVIB30, Formula XVIB31, Formula XVIB32, Formula XVIB33, Formula XVIIB, Formula XVIIIB, Formula XXB, Formula XIXB, Formula XXIB, Formula XXIIB, Formula XXXIIIB, Formula XXIVB, or Formula XXVB or a combination thereof, for use in the treatment of a disease comprises a virus-induced disease, a cancer, an autoimmune disease, an immune disorder, a bacterial associated disease or infection, or a combination thereof, in a subject in need thereof. In another embodiment, the disease is a virus-induced disease. In another embodiment, the disease is a cancer. In another embodiment, the disease is an autoimmune disease. In another embodiment, the disease is an immune disorder. In another embodiment, the disease is a bacterial associated disease. In another embodiment, the disease is an infection. In another embodiment, the disease is COVID19 caused by a SARS-CoV-2 infection. In another embodiment, the disease is caused by an EBV infection. In another embodiment, the disease is caused by an BKV infection.

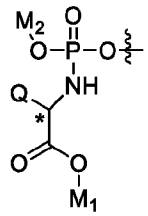
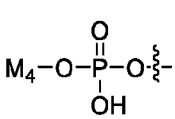
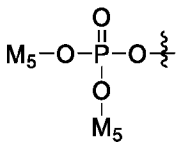
[00309] In another embodiment, the disease is caused by a virus selected from the group consisting of norovirus, rotavirus, hepatitis virus A, B, C, D, or E, rabies virus, West Nile virus, enterovirus, echovirus, coxsackievirus, herpes simplex virus (HSV), varicella-zoster virus, mosquito-borne viruses, arbovirus, St. Louis encephalitis virus, California encephalitis virus, lymphocytic choriomeningitis virus, human immunodeficiency virus (HIV), poliovirus, zika virus,

rubella virus, cytomegalovirus, human papillomavirus (HPV), enteovirus D68, severe acute respiratory syndrome (SARS) coronavirus, Middle East respiratory syndrome coronavirus (MERS-CoV), SARS coronavirus 2 (SARS-CoV-2), Epstein-Barr virus (EBV), influenza virus, influenza virus A2, influenza virus B, influenza virus A(H1N1), respiratory syncytical virus (RSV), polyoma viruses, BK virus, Tacaribe virus, Ebola virus, Dengue virus, and any combination thereof.

[00310] In some embodiments, the methods of treating comprising use of a ddh- or deoxy-ddh compound disclosed herein, terminates polynucleotide chain synthesis in a cell.

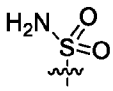
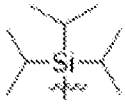
[00311] As it is generally known in the art, in order to function as DNA or RNA chain terminators *in vivo*, a ddh- or deoxy ddh compound would have to be converted by one or more viral or cellular kinases into their 5'-triphosphate form before they can compete with the natural substrates (dNTPs for DNA synthesis and NTPs for RNA synthesis) in the DNA or RNA polymerization reaction. Thus, the "active metabolite" for the purpose of DNA or RNA chain termination is the ddh- or deoxy ddh compound in a 5'-triphosphate form. In other words, the products generated from the non-natural substrates by the pVips, or any other means, for example chemical synthesis, comprise active metabolites as DNA or RNA chain terminators, and these products or active metabolites are in 5'-triphosphate form. However, in order to administer these products or active metabolites to the cells and allow transport across cell membrane, these products or active metabolites need to be made into a form without a phosphate group.

[00312] In another embodiment, terminating polynucleotide chain synthesis increases termination of DNA chain synthesis, or increases termination of RNA chain synthesis, or a combination thereof. In another embodiment, terminating polynucleotide chain synthesis increases termination of DNA chain synthesis. In another embodiment, the terminating polynucleotide chain synthesis increases termination of RNA chain synthesis.

[00313] In some embodiments, these ddh or deoxy-ddh products or active metabolites thereof comprise a pro-drug functional group:  , ,  , or  ;

Q is a side chain of an amino acid; M_1 is an alkyl;

M_2 is an aryl, a substituted aryl, a heteroaryl or a substituted heteroaryl;

M_3 is  ,  , , or ;

M_4 is -(C_2-C_6)alkyl-O-(C_{10}-C_{20})alkyl;

each M_5 is -$(CH_2)_n$-S-C(=O)-(C_1-C_8)alkyl; and

n is 1-4

at the R_1,R_{11} or R_{21} positions.

[00314] In one embodiment, the above compositions comprising one or more ddh or deoxy-ddh compounds can be provided to the subject with additional active agents to achieve an improved therapeutic effect as compared to treatment with each agent by itself. In another embodiment, additional active agents can be anti-viral agents or anti-cancer drugs or antibiotics.

[00315] In another embodiment, the present disclosure provides a composition comprising one or more non-natural substrates of pVip for use in the treatment of a disease in a subject in need thereof. The subject has been or is concurrently treated to express the pVip. To express the pVip in the subject, the subject can be treated prior or concurrently with a composition comprising the pVip. Alternatively, the subject can be treated prior or concurrently with a composition comprising nucleotide sequences encoding the pVip. The non-natural substrates are recognized as substrates by a pVip that comprises the amino acid sequence of one of SEQ ID NOs:409-789. In another embodiment, the pVip comprises an amino acid having at least 80% homology to a pVip provided in Table 5, or having at least 80% homology to any one of SEQ ID NOs: 409-789. In one embodiment, the non-natural substrates have been described in detail herein.

[00316] In one embodiment, the non-natural substrates are administered to cells or a subject in a form that can enter the cells (e.g. non-phosphorylated form). Once inside the cells, these non-natural substrates can be converted (e.g. phosphorylation by one or more viral or cellular kinases) to a form that can be recognized as substrates by the pVip. pVip expressed in the cells would then convert these non-natural substrates to produce ddh or deoxy-ddh compounds that can inhibit DNA/RNA replication. In one embodiment, the non-natural substrates can be modified and administered in "prodrug" form as described above. In one embodiment, a prodrug comprises a non-natural substrate with a chemical structure that can be oxidized, reduced, aminated, deaminated, esterified, deesterified, alkylated, dealkylated, acylated, deacylated, phosphorylated, dephosphorylated, photolyzed, hydrolyzed, or other functional group change or conversion to produce the non-natural substrates that can be recognized by pVip as substrate, or produce the non-

natural substrates that can be transported across cell membrane. In one embodiment, the non-natural substrate catalyzed by the pVip can be modified by adding a protective chemical group and thereby becoming a prodrug.

[00317] In one embodiment, the above composition comprising one or more non-natural substrates can be provided to the subject with additional active agents to achieve an improved therapeutic effect as compared to treatment with each agent by itself. In one embodiment, additional active agents can be anti-viral agents or anti-cancer drugs or antibiotics.

[00318] In another embodiment, the present disclosure provides a method of use of a composition, wherein the composition comprises one or more ddh or deoxy-ddh compounds as described herein, for use in the treatment of a disease in a subject in need thereof.

[00319] In one embodiment, the disease can be a virus-induced disease, a cancer or a tumor, an autoimmune disease, an immune disorder, or a disease or disorder associated with a bacterial infection, or a combination thereof.

[00320] In one embodiment, a viral infection is caused by viruses in the Baltimore classification Group I group of viruses: double-stranded DNA viruses (e.g. Adenoviruses, Herpesviruses including Epstein-Barr virus, Poxviruses, Polyoma viruses including BK virus and JC virus (human polyomavirus 2)). In another embodiment, the viral infection is caused by viruses in the Baltimore classification Group II group of viruses: single-stranded (or "sense") DNA viruses (e.g. Parvoviruses). In another embodiment, the viral infection is caused by viruses in the Baltimore classification Group III group of viruses: double-stranded RNA viruses (e.g. Reoviruses). In another embodiment, the viral infection is caused by viruses in the Baltimore classification Group IV group of viruses: single-stranded (sense) RNA viruses (e.g. Picornaviruses, Togaviruses, Coronavirus including SARS-CoV-2). In another embodiment, the viral infection is caused by viruses in the Baltimore classification Group V of viruses: single-stranded (antisense) RNA viruses (e.g. Orthomyxoviruses, Rhabdoviruses). In another embodiment, the viral infection is caused by viruses in the Baltimore classification Group VI group of viruses: single-stranded (sense) RNA viruses with DNA intermediate in life-cycle (e.g. Retroviruses). In another embodiment, the viral infection is caused by viruses in the Baltimore classification Group VII group of viruses: double-stranded DNA viruses with RNA intermediate in life-cycle (e.g. Hepadnaviruses).

[00321] In one embodiment, the virus-induced disease can be respiratory viral infection (e.g. common cold, seasonal influenzas), gastrointestinal viral infection, liver viral infection, nervous system viral infection, skin viral infection, sexually transmitted viral infection, placental viral infection, or fetal viral infection.

[00322] In one embodiment, examples of viral induced disease include, but are not limited to,

gastroenteritis, keratoconjunctivitis, pharyngitis, croup, pharyngoconjunctival fever, pneumonia, cystitis (Adenovirus), Hand, foot and mouth disease, pleurodynia, aseptic meningitis, pericarditis, myocarditis (Coxsackievirus), infectious mononucleosis, Burkitt's lymphoma, Hodgkin's lymphoma, nasopharyngeal carcinoma (Epstein–Barr virus), acute hepatitis, chronic hepatitis, hepatic cirrhosis, hepatocellular carcinoma, herpes labialis, cold sores, gingivostomatitis in children, tonsillitis & pharyngitis in adults, skin vesicles, mucosal ulcers, oral and/or genital ulcers, Aseptic meningitis (Herpes simplex virus, type 2), Cytomegalic inclusion disease, liver, lung and spleen diseases in the newborn, congenital seizures in the newborn (Cytomegalovirus), Kaposi sarcoma, multicentric Castleman disease, primary effusion lymphoma (Human herpesvirus, type 8), AIDS (HIV), influenza, Reye syndrome (Influenza virus), measles, postinfectious encephalomyelitis (Measles virus), mumps, hyperplastic epithelial lesions (common, flat, plantar and anogenital warts, laryngeal papillomas, epidermodysplasia verruciformis), cervical carcinoma, squamous cell carcinomas (Human papillomavirus), bronchiolitis, common cold (Parainfluenza virus), poliomyelitis (Poliovirus), rabies, influenza-like syndrome, severe bronchiolitis with pneumonia (Respiratory syncytial virus), congenital rubella, German measles (Rubella virus), chickenpox, herpes zoster, Congenital varicella syndrome (Varicella-zoster virus).

[00323] In one embodiment, the disease is caused by one or more of the following viruses: norovirus, rotavirus, hepatitis virus A, B, C, D, or E, rabies virus, West Nile virus, enterovirus, echovirus, coxsackievirus, herpes simplex virus (HSV), varicella-zoster virus, mosquito-borne viruses, arbovirus, St. Louis encephalitis virus, California encephalitis virus, lymphocytic choriomeningitis virus, human immunodeficiency virus (HIV), poliovirus, zika virus, rubella virus, cytomegalovirus, human papillomavirus (HPV), enteovirus D68, severe acute respiratory syndrome (SARS) coronavirus, Middle East respiratory syndrome coronavirus (MERS-CoV), SARS coronavirus 2 (SARS-CoV-2), Epstein-Barr virus (EBV), influenza virus, influenza virus A2, influenza virus B, influenza virus A(H1N1), respiratory syncytical virus (RSV), polyoma viruses, BK virus, Tacaribe virus, Ebola virus, and Dengue virus.

[00324] In one embodiment, the disease is COVID19 caused by SARS coronavirus 2. In one embodiment, the disease is the result of an EBV infection. In one embodiment, the disease is the result of an BKV infection.

[00325] In one embodiment, the viral infection is caused by viruses of human or non-human origin. In some embodiments, the viral infection is caused by modified or unmodified viruses that originate from animals or any foreign organism, for example, infection caused by SARS coronavirus, SARS-CoV-2, etc.

[00326] In some embodiments, treating a viral infection comprises protecting an organism from

foreign nucleic acid invasion. In some embodiments, treating a viral infection comprises decreasing viral nucleic acid replication.

[00327] In one embodiment, the above-described composition comprising one or more ddh or deoxy-ddh compounds generated by the pVips from non-natural substrates or synthesized using methods known in the art, can be used in the treatment of cancer or a tumor. Representative examples of cancer include, but are not limited to, carcinoma, sarcoma, lymphoma, leukemia, germ cell tumor, blastoma, chondrosarcoma, Ewing's sarcoma, malignant fibrous histiocytoma of bone, osteosarcoma, rhabdomyosarcoma, heart cancer, brain cancer, astrocytoma, glioma, medulloblastoma, neuroblastoma, breast cancer, medullary carcinoma, adrenocortical carcinoma, thyroid cancer, Merkel cell carcinoma, eye cancer, gastrointestinal cancer, colon cancer, gallbladder cancer, gastric (stomach) cancer, gastrointestinal carcinoid tumor, hepatocellular cancer, pancreatic cancer, rectal cancer, bladder cancer, cervical cancer, endometrial cancer, ovarian cancer, renal cell carcinoma, prostate cancer, testicular cancer, urethral cancer, uterine sarcoma, vaginal cancer, head cancer, neck cancer, nasopharyngeal carcinoma, hematopoetic cancer, Non-Hodgkin lymphoma, skin cancer, basal-cell carcinoma, melanoma, small cell lung cancer, non-small cell lung cancer, or any combination thereof.

[00328] In one embodiment, the above-described composition comprising one or more ddh or deoxy-ddh compounds generated by the pVips from non-natural substrates or synthesized using methods known in the art, can be used in the treatment of autoimmune disease. Representative examples of autoimmune disease include, but are not limited to, achalasia, amyloidosis, ankylosing spondylitis, anti-gbm/anti-tbm nephritis, antiphospholipid syndrome, arthritis, autoimmune angioedema, autoimmune encephalomyelitis, autoimmune hepatitis, autoimmune myocarditis, autoimmune oophoritis, autoimmune orchitis, autoimmune pancreatitis, autoimmune retinopathy, autoimmune urticaria, Behcet's disease, celiac disease, chagas disease, chronic inflammatory demyelinating polyneuropathy, Cogan's syndrome, congenital heart block, Crohn's disease, dermatitis, dermatomyositis, discoid lupus, Dressler's syndrome, endometriosis, fibromyalgia, fibrosing alveolitis, granulomatosis with polyangiitis, Graves' disease, Guillain-Barre syndrome, herpes gestationis, immune thrombocytopenic purpura, interstitial cystitis, juvenile arthritis, juvenile diabetes (type 1 diabetes), juvenile myositis, Kawasaki disease, Lambert-Eaton syndrome, lichen planus, lupus, Lyme disease, multiple sclerosis, myasthenia gravis, myositis, neonatal lupus, neutropenia, palindromic rheumatism, peripheral neuropathy, polyarteritis nodosa, polymyalgia rheumatica, polymyositis, postmyocardial infarction syndrome, postpericardiotomy syndrome, primary biliary cirrhosis, primary sclerosing cholangitis, progesterone dermatitis, psoriasis, psoriatic arthritis, reactive arthritis, retroperitoneal fibrosis, rheumatic fever, rheumatoid arthritis,

sarcoidosis, Schmidt syndrome, scleritis, scleroderma, Sjögren's syndrome, thrombocytopenic purpura, type 1 diabetes, ulcerative colitis, uveitis, vasculitis, and vitiligo.

[00329] In one embodiment, the above-described composition comprising one or more ddh or deoxy-ddh compounds generated by the pVips from non-natural substrates or synthesized using methods known in the art, can be used in the treatment of immune disorders.

[00330] In one embodiment, the above-described composition comprising one or more ddh or deoxy-ddh compounds generated by the pVips from non-natural substrates or synthesized using methods known in the art, can be used in the treatment of bacterial infections and diseases or disorders associated with bacterial infections. Bacterial infections can be caused by numerous bacterial pathogens. In general, bacterial pathogens may be classified as either Gram-positive or Gram-negative pathogens. In some embodiments, the ddh or deoxy-ddh compounds described herein may comprise effective activity against either a Gram-positive bacterium or a Gram-negative bacteria, or both. In some embodiments, the ddh or deoxy-ddh compounds described herein comprise a broad-spectrum antibiotic activity. For example, but not limited to, in some embodiments, a bacterial infection may be the result of infection from a *Streptococcus pneumoniae; Staphylococcus aureus; Haemophilus influenza, Myoplasma species*, or *Moraxella catarrhalis*.

[00331] In some embodiments, disclosed herein is a method for treating a disease in a subject in need thereof, the method comprising administering to said subject

[00332] In some embodiments, disclosed herein is a method for treating a disease in a subject in need thereof, the method comprising administering to said subject a composition comprising a nucleic acid construct comprising pVip gene. In some embodiments, disclosed herein is a method for treating a disease in a subject in need thereof, the method comprising administering to said subject a composition comprising a nucleic acid construct comprising pVip gene and a non-natural substrate as described herein. In some embodiments, disclosed herein is a method for treating a disease in a subject in need thereof, the method comprising administering to said subject a composition comprising a cell comprising a pVip gene. In some embodiments, disclosed herein is a method for treating a disease in a subject in need thereof, the method comprising administering to said subject a composition comprising a cell comprising a pVip gene, wherein administering further includes providing a non-natural substrate as described herein. In some embodiments, a non-natural substrate is provided following administration of a composition comprising a nucleic acid construct comprising a pVip gene or a cell comprising a pVip gene. The later administration of the non-natural substrate provides a window of time for the medical professional to access expression of the pVip gene prior to administration of the non-natural substrate.

[00333] Suitable routes of administration may, for example, include oral, rectal, transmucosal, especially transnasal, intestinal or parenteral delivery, including intramuscular, subcutaneous and intramedullary injections as well as intrathecal, direct intraventricular, intracardiac, e.g., into the right or left ventricular cavity, into the common coronary artery, intravenous, intraperitoneal, intranasal, or intraocular injections.

[00334] Determination of a therapeutically effective amount is well within the capability of those skilled in the art. For any preparation used in the methods disclosed herein, the therapeutically effective amount or dose can be estimated initially from *in vitro* assays. For example, a dose can be formulated in animal models and such information can be used to more accurately determine useful doses in humans.

[00335] Toxicity and therapeutic efficacy of the active ingredients described herein can be determined by standard pharmaceutical procedures in vitro, in cell cultures or experimental animals. The data obtained from these *in vitro* and cell culture assays and animal studies can be used in formulating a range of dosage for use in human. The dosage may vary depending upon the dosage form employed and the route of administration utilized. The exact formulation, route of administration and dosage can be chosen by the individual physician in view of the patient's condition. [See e.g., Fingl, et al., (1975) "The Pharmacological Basis of Therapeutics", Ch. 1 p.1].

[00336] The amount of a composition to be administered will, of course, be dependent on e.g. the subject being treated, the severity of the affliction, the manner of administration, the judgment of the prescribing physician, etc.

[00337] In another embodiment, terminating polynucleotide chain synthesis confers viral resistance to said cell.

[00338] In another embodiment, the cell is a eukaryotic cell. In another embodiment, said eukaryotic cell is a tumor cell, or is infected by a virus or a foreign DNA. In another embodiment, said eukaryotic cell is a tumor cell. In another embodiment, said eukaryotic cell is infected by a virus or a foreign DNA.

[00339] In some embodiments, the cell in which termination of polynucleotide chain synthesis is desired is a eukaryotic cell. In some embodiments, the eukaryotic cell is a tumor cell.

[00340] In some embodiments, termination of polynucleotide chain synthesis confers viral resistance to a cell. In some embodiments, termination of polynucleotide chain synthesis decreases DNA replication in a cell. In some embodiments, termination of polynucleotide chain synthesis decreases RNA transcription in a cell.

[00341] In one embodiment, the present disclosure provides a method of terminating polynucleotide chain synthesis in a cell, the method comprises contacting the cell with a

composition comprising one or more ddh or deoxy-ddh compounds. In one embodiment, the present disclosure provides a method of terminating polynucleotide chain synthesis in a cell, the method comprises contacting the cell with a composition comprising one or more ddh or deoxy-ddh compounds comprising a protective chemical group. In one embodiment, the present disclosure provides a method of terminating polynucleotide chain synthesis in a cell, the method comprises contacting the cell with a composition comprising one or more ddh or deoxy-ddh compounds that are derived from products produced by a prokaryotic homolog of viperin (pVip) or by synthetic methods known in the art from non-natural substrates, wherein the pVip comprises the amino acid sequence of one of SEQ ID NOs:409-789. In one embodiment, the pVip comprises an amino acid having at least 80% homology to a pVip provided in Table 5, or having at least 80% homology to any one of SEQ ID NOs: 409-789. In one embodiment, the non-natural substrates for the pVips have been described in detail herein.

[00342] In one embodiment, the ddh or deoxy-ddh compounds can be applied in a prodrug form as described above. Various forms of ddh or deoxy-ddh compounds can be applied as described herein, for example, a compound is in the 3′-deoxy-3′,4′-didehydro (ddh) form. In one embodiment, the ddh or deoxy-ddh compound can be administered to the cells in the form that can enter the cells (e.g. non-phosphorylated form). Once inside the cells, these nucleoside analogs can be converted by one or more viral or cellular kinases to the active form that can inhibit DNA/RNA replication.

[00343] In another embodiment, the present disclosure provides a method of terminating polynucleotide chain synthesis in a cell, the method comprising contacting the cell with a composition comprising one or more non-natural substrates of prokaryotic homolog of viperin (pVip). The cell has been or is concurrently treated to express the pVip. To express the pVip in the cell, the cell can be treated prior or concurrently with a composition comprising the pVip. Alternatively, the cell can be treated prior or concurrently with a composition comprising nucleotide sequences encoding the pVip. The non-natural substrates are recognized as substrates by a pVip that comprises the amino acid sequence of one of SEQ ID NOs:409-789. In one embodiment, the pVip comprises an amino acid having at least 80% homology to a pVip provided in Table 3, or having at least 80% homology to any one of SEQ ID NOs: 409-789. In one embodiment, the non-natural substrates for the pVips have been described in detail herein. In one embodiment, the method is carried out in vitro.

[00344] In one embodiment, the non-natural substrates are administered to cells in a form that can enter the cells (e.g. nucleoside form, or non-phosphorylated form). Once inside the cells, these non-natural substrates can be converted (e.g. phosphorylation by one or more viral or cellular

kinases) to a form that can be recognized as substrates by the pVip. pVip expressed in the cells would then convert these non-natural substrates to produce nucleotide/nucleoside analogs that can inhibit DNA/RNA replication. In one embodiment, the non-natural substrates can be modified and administered in "prodrug" form as described above. In one embodiment, the non-natural substrate catalyzed by the pVip can be modified by adding a protective chemical group and thereby becoming a prodrug.

[00345] In some embodiments, the cell in which termination of polynucleotide chain synthesis is desired is a eukaryotic cell. In some embodiments, the eukaryotic cell is a tumor cell or a cancer cell.

[00346] In some embodiments, termination of polynucleotide chain synthesis confers viral resistance to a cell. In some embodiments, termination of polynucleotide chain synthesis decreases DNA replication in a cell. In some embodiments, termination of polynucleotide chain synthesis decreases RNA transcription in a cell.

[00347] In some embodiments, termination of polynucleotide chain synthesis comprises increased termination of DNA chain synthesis. In some embodiments, termination of polynucleotide chain synthesis comprises increased termination of RNA chain synthesis. In some embodiments, termination of polynucleotide chain synthesis decreases proliferation of a cell. In some embodiments, termination of polynucleotide chain synthesis comprises an anti-tumor activity.

[00348] In some embodiments, terminating polynucleotide chain synthesis in a cell comprises reducing polynucleotide chain synthesis in a cell by at least 1%, by at least 2%, by at least 3%, by at least 4%, by at least 5%, by at least 6%, by at least 7%, by at least 8%, by at least 9%, by at least 10%, by at least 20%, by at least 30%, by at least 40%, by at least 50%, by at least 60%, by at least 70%, by at least 80%, by at least 90%, or by 100%.

[00349] In some embodiments, terminating polynucleotide chain synthesis in a cell comprises reducing viral DNA replication. In some embodiments, terminating polynucleotide chain synthesis in a cell comprises reducing viral RNA chain synthesis. In some embodiments, terminating polynucleotide chain synthesis in a cell comprises reducing viral DNA or RNA chain synthesis without modifying DNA replication of the host cell.

[00350] In some embodiments, terminating polynucleotide chain synthesis in a cell comprises reducing eukaryotic DNA replication. In some embodiments, the eukaryotic cell is a tumor cell.

[00351] In some embodiments, terminating polynucleotide chain synthesis in a cell comprises reducing polynucleotide chain synthesis in a cell by between about 0% and about 10%, between about 10% and about 20%, between about 20% and about 30%, between about 30% and about

40%, between about 40% and about 50%, between about 50% and about 60%, between about 60% and about 70%, between about 70% and about 80%, between about 80% and about 90%, or between about 90% and about 100%.

[00352] In some embodiments, provided herein is a method for treating a disease wherein the method comprises administration of a pharmaceutical composition described herein.

[00353] In some embodiments, provided herein is a method for treating a disease wherein the method comprises administration of a compound described herein.

Methods of Protecting A Cell from Viral Infection

[00354] In some embodiments, disclosed herein is a method of protecting a cell from viral infection, said method comprising a step of introducing into said cell a prokaryotic viperin homolog (pVip), or a pVip gene. In some embodiments, a method of protecting a cell from viral infection comprises a step of introducing into said cell a pVip gene selected from a gene provided in Table 3, Table 4, or comprising any one of SEQ ID NOs: 3-408. In some embodiments, a method of protecting a cell from viral infection comprises a step of introducing into said cell a pVip gene encoding for a protein with an amino acid sequence of one of those provided in Table 5, or comprising any one of SEQ ID NOs: 409-789. In another embodiment, the pVip comprises an amino acid having at least 80% homology to a pVip provided in Table 5, or having at least 80% homology to any one of SEQ ID NOs: 409-789.

[00355] In some embodiments, a method of protecting a cell from viral infection comprises a step of introducing into said cell a composition comprising one or more ddh or deoxy-ddh compounds, or prodrug forms thereof, as described herein, which may be generated by the pVips from the non-natural substrates described herein or as synthesized using methods known in the art. In some embodiments, the cell comprises a human cell. In some embodiments, the cell comprises a tumor cell or a cancer cell. In some embodiments, the cell has been infected by a virus. In one embodiment, the non-natural substrates are modified by the pVips to have the 3' hydroxyl groups removed. In another embodiment, these non-natural substrates are modified by the pVips to become the 3′-deoxy-3′,4′-didehydro (ddh) derivates. In some embodiments, the ddh or deoxy-ddh compounds are modified to have the 3' hydroxyl groups removed. In some embodiments, the ddh or deoxy-ddh compounds that are modified become the 3′-deoxy-3′,4′-didehydro (ddh) derivates. In one embodiment, the ddh or deoxy-ddh compounds are modified to include a protective chemical group, wherein the modified compound comprises a prodrug. In some embodiments, the viral infection comprises infection with a phage. In some embodiments, the viral infection comprises infection with a virus. Examples of viruses or viral infections have been described above.

[00356] As used herein the term “about” refers to ± 10 %. As used herein, the singular form "a", "an" and "the" include plural references unless the context clearly dictates otherwise. For example, the term "a compound" or "at least one compound" may include a plurality of compounds, including mixtures thereof.

[00357] Throughout this application, various embodiments are disclosed that may be presented in a range format. It should be understood that the description in range format is merely for convenience and brevity and should not be construed as an inflexible limitation on the scope. Accordingly, the description of a range should be considered to have specifically disclosed all the possible subranges as well as individual numerical values within that range. For example, description of a range such as from 1 to 6 should be considered to have specifically disclosed subranges such as from 1 to 3, from 1 to 4, from 1 to 5, from 2 to 4, from 2 to 6, from 3 to 6 etc., as well as individual numbers within that range, for example, 1, 2, 3, 4, 5, and 6. This applies regardless of the breadth of the range.

[00358] Whenever a numerical range is indicated herein, it is meant to include any cited numeral (fractional or integral) within the indicated range. The phrases “ranging/ranges between” a first indicate number and a second indicate number and “ranging/ranges from” a first indicate number “to” a second indicate number are used herein interchangeably and are meant to include the first and second indicated numbers and all the fractional and integral numerals therebetween.

[00359] As used herein the term "method" refers to manners, means, techniques and procedures for accomplishing a given task including, but not limited to, those manners, means, techniques and procedures either known to, or readily developed from known manners, means, techniques and procedures by practitioners of the chemical, pharmacological, biological, biochemical and medical arts.

EXAMPLES

[00360] Reference is now made to the following examples, which together with the above descriptions illustrate some embodiments in a non-limiting fashion.

[00361] Generally, the nomenclature used herein, and the laboratory procedures utilized, include molecular, biochemical, microbiological and recombinant DNA techniques. Such techniques are thoroughly explained in the literature. See, for example, "Molecular Cloning: A laboratory Manual" Sambrook et al., (1989); "Current Protocols in Molecular Biology" Volumes I-III Ausubel, R. M., ed. (1994); Ausubel et al., "Current Protocols in Molecular Biology", John Wiley and Sons, Baltimore, Maryland (1989); Perbal, "A Practical Guide to Molecular Cloning", John Wiley & Sons, New York (1988); Watson et al., "Recombinant DNA", Scientific American Books,

New York; Birren et al. (eds) "Genome Analysis: A Laboratory Manual Series", Vols. 1-4, Cold Spring Harbor Laboratory Press, New York (1998); methodologies as set forth in U.S. Pat. Nos. 4,666,828; 4,683,202; 4,801,531; 5,192,659 and 5,272,057; "Cell Biology: A Laboratory Handbook", Volumes I-III Cellis, J. E., ed. (1994); "Culture of Animal Cells - A Manual of Basic Technique" by Freshney, Wiley-Liss, N. Y. (1994), Third Edition; "Current Protocols in Immunology" Volumes I-III Coligan J. E., ed. (1994); Stites et al. (eds), "Basic and Clinical Immunology" (8th Edition), Appleton & Lange, Norwalk, CT (1994); Mishell and Shiigi (eds), "Selected Methods in Cellular Immunology", W. H. Freeman and Co., New York (1980); available immunoassays are extensively described in the patent and scientific literature, see, for example, U.S. Pat. Nos. 3,791,932; 3,839,153; 3,850,752; 3,850,578; 3,853,987; 3,867,517; 3,879,262; 3,901,654; 3,935,074; 3,984,533; 3,996,345; 4,034,074; 4,098,876; 4,879,219; 5,011,771 and 5,281,521; "Oligonucleotide Synthesis" Gait, M. J., ed. (1984); "nucleic Acid Hybridization" Hames, B. D., and Higgins S. J., eds. (1985); "Transcription and Translation" Hames, B. D., and Higgins S. J., eds. (1984); "Animal Cell Culture" Freshney, R. I., ed. (1986); "Immobilized Cells and Enzymes" IRL Press, (1986); "A Practical Guide to Molecular Cloning" Perbal, B., (1984) and "Methods in Enzymology" Vol. 1-317, Academic Press; "PCR Protocols: A Guide To Methods And Applications", Academic Press, San Diego, CA (1990); Marshak et al., "Strategies for Protein Purification and Characterization - A Laboratory Course Manual" CSHL Press (1996); all of which are incorporated by reference as if fully set forth herein. Other general references are provided throughout this document. The procedures therein are well known in the art and are provided for the convenience of the reader. All the information contained therein is incorporated herein by reference.

Example 1 – Materials and Methods

Bacterial strains and growth conditions

[00362] *Escherichia coli* strains (MG1655, Keio ΔiscR, DH5α) were grown in LB or LB agar at 37 °C unless mentioned otherwise. Whenever applicable, media were supplemented with ampicillin (100 μgml^{-1}), kanamycin (50 μgml^{-1}) or tetracycline (10 $ugmL^{-1}$) to ensure the maintenance of plasmids. *Bacillus subtilis* strain BEST7003 and its derivatives were grown in LB or LB agar at 37 °C. Whenever applicable, media were supplemented with spectinomicin (100 μgml^{-1}). Expression from pArand pHypraspank promoters was induced by the addition of respectively arabinose (0.2%) or IPTG (1mM).

Plasmids and strain construction

[00363] pVip genes were codon optimized and synthetized by Twist Bioscience (pVips 6-10,

and 12) or by Genscript (all other pVips). Synthetized pVip are shown in Table 2. Each candidate sequence was cloned in two plasmids: pDR111 and pBad/His A (Thermofisher, Catalog number 43001). For pVips 6-12, PCR fragments were joined using Gibson assembly®. The primers used in these experiments are shown in Table 5. For other candidates, cloning was performed by Genscript. Candidate pVip plasmids were first cloned and propagated in DH5α. pBad/HisA derivatives were further transformed in relevant strains (MG1655, Keio ΔiscR). pDR111 derivatives were integrated in the amyE locus of the BEST strains. pAGG encodes a GFP under a T7 promoter and a module with T7 lyzozyme to limit the leakiness of RNAP in strain BL21-DE3. The pAGG plasmid was obtained through two consecutives Gibbson assemblies, the first to generate pAG (insert pDR111 primers OG630, OG631, vector pACYc, primers OG629, OG628) and then a second to generate pAGG (insert pLysS primers AB55, AB56, vector pAG, primers AB53, AB54) (Table 7).

TABLE 7 – Primers

Name	SEQ ID NO:
AB_Vip1-gibbson_coli_vector_F	790
AB_Vip2-gibbson_coli_vector_R	791
AB_Vip3-gibbson_coli_insert_F	792
AB_Vip4-gibbson_coli_insert_R	793
AB_Vip5-res_coli_vector_F	794
AB_Vip6-res_coli_vector_R	795
AB_Vip7-gibbson_subtilis_vector_F	796
AB_Vip8-gibbson_subtilis_vector_R	797
AB_Vip19-gibbson_subtilis_insert_pVip6_F	798
AB_Vip20-gibbson_subtilis_insert_pVip6_R	799
AB_Vip21-gibbson_subtilis_insert_pVip7_F	800
AB_Vip22-gibbson_subtilis_insert_pVip7_R	801
AB_Vip23-gibbson_subtilis_insert_pVip8_F	802
AB_Vip24-gibbson_subtilis_insert_pVip8_R	803
AB_Vip25-gibbson_subtilis_insert_pVip9_F	804
AB_Vip26-gibbson_subtilis_insert_pVip9_R	805
AB_Vip27-gibbson_subtilis_insert_pVip10_F	806
AB_Vip28-gibbson_subtilis_insert_pVip10_R	807
AB_Vip31-gibbson_subtilis_insert_pVip12_F	808
AB_Vip32-gibbson_subtilis_insert_pVip12_R	809
AB_Vip37-sequencing_primer_coli_1	810

Name	SEQ ID NO:
AB_Vip38-sequencing_primer_coli_2	811
AB_Vip39-sequencing_primer_subtilis_1	812
AB_Vip40-sequencing_primer_subtilis_2	813
AB_Vip41-pVip_control_coli_vector_F	814
AB_Vip42-pVip_control_coli_vector_R	815
AB_Vip43-pVip_control_coli_insert_F	816
AB_Vip44-pVip_control_coli_insert_R	817
AB53	818
AB54	819
AB55	820
AB56	821
OG628	822
OG629	823
OG630	824
OG631	825

Phage propagation

[00364] Phages were propagated on either *E. coli* MG1655, *E. coli* MG1655 F+ or *B. subtilis* BEST7003 using the plate lysate method as described in Fortier, L.C. et al. Phage Production and Maintenance of Stocks, Including Expected Stock Lifetimes; in "Bacteriophages: Methods and Protocols, Vol 1: Isolation, Characterization, and Interactions" (eds. Clokie, M. R. J. & Kropinski, A. M.) 203–219 (Humana Press, 2009). Lysate titer was determined using the small drop plaque assay method as described in Kropinski et al. Enumeration of Bacteriophages by Double Agar Overlay Plaque Assay; in "Bacteriophages: Methods and Protocols, Volume 1: Isolation, Characterization, and Interactions" (eds. Clokie, M. R. J. & Kropinski, A. M.) 69–76 (Humana Press, 2009). Phages used in this study are presented in Table 8.

TABLE 8 - Phages used in these experiments

Phage	Host	Taxonomy	Accession number
SBSphi28-4	*B. subtilis*	Siphoviridae	N/A
SP82G	*B. subtilis*	Myoviridae	N/A
phi105	*B. subtilis*	Siphoviridae	HM072038.1
SPP1	*B. subtilis*	Siphoviridae	NC_004166.2
Phi3T	*B. subtilis*	Siphoviridae	KY030782.1
SPBeta	*B. subtilis*	Siphoviridae	AF020713.1
SPR	*B. subtilis*	Siphoviridae	N/A

Phage	Host	Taxonomy	Accession number
Rho14	*B. subtilis*	Siphoviridae	N/A
SPO1	*B. subtilis*	Myoviridae	NC_011421.1
phi29	*B. subtilis*	Podoviridae	NC_011048.1
SBSphiC	*B. subtilis*	Myoviridae	LT960610.1
SBSphiJ	*B. subtilis*	Myoviridae	LT960608.1
SECphi18	*E. coli*	Siphoviridae	LT960609.1
SECphi27	*E. coli*	Siphoviridae	LT961732.1
SEC32-2	*E. coli*	Siphoviridae	N/A
Lambda_VIR	*E. coli*	Siphoviridae	NC_001416.1
SECphi17	*E. coli*	Microviridae	LT960607.1
SECphi6_1	*E. coli*	Siphoviridae	N/A
P1	*E. coli*	Myoviridae	AF234172.1
T2	*E. coli*	Myoviridae	LC348380.1
T4	*E. coli*	Myoviridae	AF158101.6
T5	*E. coli*	Siphoviridae	AY543070.1
T6	*E. coli*	Myoviridae	MH550421.1
T7	*E. coli*	Podoviridae	NC_001604.1

Plaque assays

[00365] Plaque assays were performed as previously described in Kropinski, AM et al. Enumeration of Bacteriophages by Double Agar Overlay Plaque Assay. in Bacteriophages: Methods and Protocols, Volume 1: Isolation, Characterization, and Interactions (eds. Clokie, M. R. J. & Kropinski, A. M.) 69–76 (Humana Press, 2009). doi:10.1007/978-1-60327-164-6_7. Bacteria from overnight cultures were mixed with MMB agar (LB + 0.1 mM MnCl2 + 5 mM MgCl2 + 5 mM CaCl2 + 0.5% agar), and serial dilutions of phage lysate in MMB agar were dropped on top of them. After the drops dried up, plates were incubated overnight at room temperature for *B. subtilis* phages and for *E. coli* phages SECphi6, SECphi17, SECphi18, SECphi27, SECphi32, and T7, or at 37°C for *E. coli* phages P1, T2, T4, T5, T6, λvir, Qbeta, M13, Fd, and MS2. Efficiency of plating (EOP) was measured by performing small drop plaque assay with the same phage lysate on control and induced bacteria, and comparing the ratio of plaque formation.

Liquid infection assays

[00366] Bacteria were grown for one hour at 37°C. Inducer (arabinose or IPTG) was added and cells were incubated one hour at room temperature. Cells were infected with phages within 96-well plates. OD was monitored using Tecan Plate reader.

Example 2 – Sequence Homology-Based Discovery of Prokaryotic Homologs of Viperins

Search for viperin homologs in prokaryotic genomes

[00367] The human viperin protein sequence (NCBI accession NP_542388.2 (SEQ ID NO: 2)) was used as a seed for a MMseqs search (v6-f5a1c, default parameters, 3 iterations) on the IMG database (https://img.jgi.doe.gov/ downloaded October 2017, 38183 genomes). MMseqs (Many-against-Many sequence searching) is a software suite for fast and deep clustering and searching of large datasets. MMseqs is open-source software available at https://github.com/soedinglab/MMseqs. The search yielded 2150 hits, that show between 25%-41% sequence identity to the human viperin. Genes with an e-value higher than 10^{-5} were discarded, leaving 1724 genes. This dataset was clustered using MMseqs (v6-f5a1c, default parameter, coverage 60%, sensitivity 7.5) and redundancy was removed resulting in 17 clusters, among which 5 clusters had more than 10 genes (Table 1). For each cluster, defense scores were computed as described in Doron, S. et al. Systematic discovery of antiphage pVips in the microbial pangenome. Science (80). 4120, eaar4120 (2018).

[00368] Some of these bacterial and archaeal genes distantly homologous to the human viperin may function in anti-phage activities in prokaryotes. However, it was not trivial to predict which of these homologs is indeed an anti-phage gene. In prokaryotes, genes involved in anti-viral function co-localize on the genome forming "defense islands". Enrichment next to known defense genes can be a predictor that this group of genes performs anti-viral functions. Briefly, neighborhood of the selected gene (+/- 10 genes) was screened for known defense genes. A first score corresponds to the proportion of genes in the cluster which exhibit at least one defense gene in its neighborhood. A second score corresponds to the average number of defense genes found in the neighborhood of the genes of the cluster. Only one of viperin-homolog clusters obtained showed high propensity for being enriched next to known defense systems (Table 7). Manual examination of the genomic context of genes of this cluster confirmed the presence of many known anti-phage defense genes in its vicinity (**Figure 1**). This cluster (of 134 genes) showed high defense scores (0.602 and 1.687 respectively), and was selected for further analysis. Given that the online IMG database is constantly growing (31242 additional genomes since the download on October 2017), additional candidate prokaryotic viperin homologs (pVips) were searched manually using the "top IMG homologs" function in IMG. This added 84 genes to the cluster. Finally, a MMseqs search using genes of this cluster as seeds was performed on a metagenomes database (downloaded from IMG in October 2017, comprising 9769 metagenomes altogether, scaffolds with less than 21 genes were removed). Hits were filtered to cover at least 200aand hit at least 20 target genes from the pVip cluster. This added 163 genes, resulting in a total of 381 pVips (Table 3 and Table 5).

[00369] Table 9 below shows clusters (sized at least 10 genes) of hits of homologs search. The first column indicates the number of genes in the cluster. Second and third columns show defense scores (proportion of genes in the cluster with known anti-phage defense genes in their vicinity; average number of known defense genes in neighborhood).

TABLE 9 - Clusters of genes retrieved in the homology-based search

	Number of genes	Proportion of genes with defensive neighborhood (score 1)	Average number of Defense Genes in neighborhood (score 2)
Cluster 1	855	0.061	0.094
Cluster 2	134	0.602	1.687
Cluster 3	54	0.2	0.32
Cluster 4	21	0.077	0.077
Cluster 5	17	0.077	0.077

Example 3 – Diversity of pVips

[00370] Examination of the genomic context of pVips revealed the presence of nucleoside kinases or nucleotide kinases in their vicinity, an observation reminiscent of the organization of the human system, in which viperin is located close to CMPK2 (**Figure 2**). In vertebrates, CMPK2 phosphorylates cytidine monophosphate (CMP) to generate cytidine tri-phosphate (CTP), which is the viperin substrate that is converted by the viperin to ddhCTP. The adjacent kinases might therefore be indicative of the potential substrate of the nearby viperin. In total, 15% of the pVips encode a kinase in their neighborhood. Some pVip-associated kinases are annotated as cytidylate kinase pointing at a potentially identical substrate as CMPK2, namely that the substrate of these pVips is predicted to be CTP. However, many other pVips are found next to nucleoside kinases or nucleotide kinases annotated as thymidylate, guanylate or adenylate kinases (**Figure 2**). This suggests that the substrate of some pVips may be nucleotides other than CTP, and that they can thus generate new chain terminators that were not described previously. For example, pVips found next to thymidylate or guanylate kinases may generate ddhUTP or ddhGTP or derivatives thereof. Moreover, some of these kinases are annotated as kinases of deoxy-nucleosides or deoxy-nucleotides, namely the DNA form of the nucleoside or nucleotide rather than the RNA form that is modified by the eukaryotic viperins. In this case, the relevant pVips can generate deoxy form of ddh nucleosides or nucleotides, leading to new DNA chain terminator molecules rather than RNA chain terminator molecules.

[00371] The sequences of pVips are highly diverse with on average 37% identity at the protein level when compared to one another. pVips were found in 94 genera of diverse phyla including *Euryarchaeota*, *Proteobacteria*, *Firmicutes*, and *Bacteriodetes*. To better understand this diversity and phylogenetic relationship with eukaryotic viperins, a phylogenetic tree of the protein family was built (**Figure 3A**).

[00372] The Molybdenum cofactor biosynthesis protein (MoaA) is known to be a structural homolog of Viperin, but MoaA does not participate in defense against viruses and does not generate antiviral chain terminator nucleotide analogs (Santamaria-Araujo JA et al. (2004) J Biol Chem. 279(16):15994-9; Fenwick MK et al. (2017) Proc Natl Acad Sci U S A. 114(26):6806-6811). Hence, the MoaA gene can be used as an outgroup for phylogenetic analyses. Eukaryotic sequences of viperins were chosen to represent a diversity of species for the tree building and are provided in attached files. Prokaryotic viperins, eukaryotic viperins and MoaA sequences were aligned using mafft (v7.402, default parameters). The tree was computed with IQ-TREE multicore v.1.6.5 under model LG+I+G4. This model gave the lowest Bayesian Information Criterion (BIC) among all models available for both trees (option –m TEST in IQ-TREE). 1000 ultra-fast bootstraps were made in order to evaluate node support (options –bb 1000 –wbtl in IQ-TREE). Phylogenetic trees figures were designed using ITOL.

[00373] It was found that pVips are grouped in 7 major clades (**Figure 3A**) that partly correspond to major prokaryotic phyla. For example, clade 2 encompasses many archaeand cyanobacteria versions while clades 5, 6, 7 mainly encode pVips from Proteobacteria. Interestingly, all eukaryotic viperins are found in one clade within the tree, with a closest common ancestor with pVips from clade 2. This specific place of eukaryotic viperins in the pVip tree suggests that the evolutionary origin of all eukaryotic viperins was a pVip from clade 2. This also means that pVips encode higher diversity than eukaryotic viperins, suggesting again that pVips would produce a variety of polynucleotide chain terminators other than ddhCTP. While some clades encode exclusively one type of kinases, like clade 7 (thymidylate kinases) some encode diverse kinases like clade 5 (both thymidylate and adenylate) (**Figure 3A**).

[00374] To fully capture the diversity of this protein family, homologs search was extended to metagenomes. Sequences from the initial cluster were used as a seed for a MMseqs search on a database of 9769 metagenomes that were downloaded from IMG in October 2017 as described in Example 2. This search added, after filtering (coverage of at least 200aand hit at least 20 target genes from the pVips cluster), 163 sequences to the pVips dataset yielding 381 homologs in total. These additional 163 pVips identified within metagenomes also had a high propensity to be found next to known defense genes (85 of the 163, 52%), suggesting that these set of genes also functions

in antiviral defense. A second phylogenetic tree was built that includes the pVips from isolate genomes as well as these 163 additional genes (**Figure 3B**). Sequences found in metagenomes do not change the topology of the initial tree, with still seven major clades and eukaryotic viperins being embedded in one of the prokaryotic clades. These observations suggest that the dataset of 381 pVips is representative of the diversity of the protein family.

[00375] Altogether, these results indicate the existence of a diverse family of pVips. While quite rare among microbial genomes, they are present in phylogenetically very distant organisms suggesting an ancient evolutionary origin. Their genomic context is indicative of a potential anti-viral activity. Presence of nearby nucleoside kinases or nucleotide kinases with diverse predicted substrates suggest a diversity of substrates and subsequently of products generated by the pVips which are predicted to be other than the known ddhCTP produced by the eukaryotic viperins.

Example 4 – pVips provide anti-viral activity *in vivo*.

[00376] The objective of this study was testing whether prokaryotic homologs of viperins (pVips) provide defense against bacteriophages in vivo. 25 genes that span across the pVip phylogenetic tree were selected to assess activity of diverse representatives of the family. MoaA from *E. coli*, structurally similar to viperins but with a demonstrated function in metabolism and not in antiviral activity, was used as a negative control. The sequences of these genes were codon optimized for expression in lab bacteria (*E. coli*), resulting in the codon-optimized sequences presented in (SEQ ID NOs: 384-408), and cloned in vectors for *E. coli* and *B. subtilis* under the control of inducible promoters (pAra for *E. coli*, pHypraspank for *B. subtilis*) to avoid potential toxicity effects (**Figure 4**).

[00377] pVips, as well as eukaryotic viperins, are Radical-SAM enzymes that contain an iron sulfur cluster 4Fe-4S. For such enzymes, the 4Fe-4S cluster is built by a complex of proteins and then carried into the apoenzyme making it an active holoenzyme. This metabolic step can require some specific interactions between the proteins that build the iron sulfur cluster and the protein that receive it, in this case the pVip. Heterologous expression of iron-sulfur cluster enzymes such as viperins can thus lead to loss of catalytic activity, if the cell in which the viperin is expressed does not express the iron sulfur clusters to high enough levels.

[00378] Some of the tested pVip candidates could be inactive *in vivo* in *E. coli* or in *B. subtilis* because of this limitation. Several strategies have been employed to circumvent this issue for other iron-sulfur cluster proteins, such as the expression of an exogenous set of genes responsible for iron sulfur cluster formation or the endogenous overexpression of the iron sulfur cluster

metabolism genes of *E. coli* through deletion of the endogenous repressor of these genes, iscR, in *E. coli.* In the current study we used the second approach, and pVips were cloned into an *E. coli* strain from the Keio collection deleted for iscR. As a control, *E. coli* Keio ΔiscR were transfected with MoaA.

[00379] To test if pVips have antiviral activities, their expression (as well as the expression of the MoaA control) was induced with 0.004% arabinose. A reduction in plaque numbers as compared to MoaA control was observed for the 25 pVips including pVip6, pVip7, pVip8, pVip9, pVip10, pVip12, pVip15, pVip19, pVip21, pVip27, pVip32, pVip34, pVip39, pVip42, pVip44, pVip46, pVip47, pVip48, pVip50, pVip56, pVip57, pVip58, pVip60, pVip62, and pVip63 provided defense against phages in the strain Keio ΔiscR (**Figures 5A**nd **5B, Figures 6A-6Z, Table 4,** and **Table 10**). Phages P1, lambda vir, T7, SecPhi4, SecPhi6, SecPhi17, and SecPhi18 were found susceptible to pVips. At least one viperin from each major clade of the protein family characterized showed activity against phages (**Figure 3A**, **Table 4** and **Table 10**). Three main defense phenotypes were observed for the different pVips: strong activity against T7 only (**Figures 6I-6M**), strong activity against P1 and lambda but not T7 (**Figures 6B-6H**) and strong activity against P1, lambdand T7 (**Figures 6N-6Z**). While clades 1, 2 and 6 seem to encode pVips with strong activity against P1 and lambda but not against T7, pVips with strong activity against T7 only are restricted to clade 3, and pVips with strong activity against P1, lambdand T7 are found in clades 3, 4, 5, and 7 (**Figure 3A**). Given the homology with the eukaryotic viperins, it was hypothesized that the mechanism of defense involved synthesis of small anti-viral molecules, most probably chain terminators. These different phenotypes against the same phages suggest the existence of several different pVip products. These products could be, for example, nucleotide analogs other than ddhCTP; deoxy versions of ddh nucleotides; or other chain terminator nucleotide analogs.

[00380] Table 10 shows candidate pVips that were found to be active in protecting *E. coli* bacteriagainst phage infection

[00381]

TABLE 10: pVips found to protect bacteriagainst phage infection

pVip_number	IMG gene identifier	Genome name	Clade
6	2624749465	Selenomonas ruminatium S137	1
7	2739066738	Fibrobacter sp. UWT3	5
8	2521798317	Psychrobacter lutiphocae DSM 21542	4
9	2574301464	Vibrio porteresiae DSM 19223	7
10	2720695169	Vibrio vulnificus ATL 6-1306	7
12	2698137626	Ruegeria intermedia DSM 29341	6
15	646713396	Coraliomargaritakajimensis DSM 45221	3

19	2506475787	Methanoplanus limicola M3, DSM 2279	2
21	2515428782	Lewinella persica DSM 23188	3
27	2574506394	Desulfovibrio senezii DSM 8436	6
32	2609132705	Phormidium sp. OSCR GFM (version 2)	5
34	2619892213	Cryomorphaceae bacterium EBPR_Bin_135	3
39	2634960437	Burkholderiales-76 (UID4002)	6
42	2639213731	Planktothricoides sp. SR001	2
44	2648875132	Chondromyces crocatus Cm c5	3
46	2649993803	Photobacterium swingsii CAIM 1393	7
47	2651203508	Flammeovirga pacifica WPAGA1	3
48	2651490945	Vibrio crassostreae J5-19	7
50	2661858798	Methanogenic archaeon ISO4-H5	2
56	2701115162	Fibrobacter sp. UWH6	5
57	2718503187	Flavobacterium lacus CGMCC 1.12504	3
58	2721736750	Pseudoalteromonas ulvae TC14	7
60	2733913669	Lacinutrix sp. JCM 13824	3
62	2743907592	Fibrobacteria bacterium GUT31 IN01_31	5
63	2744633848	Pseudoalteromonas sp. XI10	7

Example 5 –pVips Provide Defense in *B. subtilis*

[00382] Next it was tested if pVips could provide anti-viral activity in bacteria other than *E. Coli.* We cloned pVip7 from *Fibrobacter sp. UWT3* in Bacillus subtilis BEST7003 and tested it against an array of 12 different phages (detailed in Example 1).

[00383] pVip7 showed protection in *B. subtilis* against two phages: phi3T and spbeta (**Figure 7A**). They both belong to the spBeta group of phages (Siphovridae). Protection against these two phages was very strong (more than 10,000 fold, which is the limit of detection of the assay used). Protection against phi3T was confirmed with liquid infection assays, where the population in which the pVip expression was induced fully survived the phage infection, while the non-induced collapsed due to phage infection (**Figure 7B**). Temperature was found to be another important parameter. While pVip7 was fully active at 25°C in B. subtilis, it did not show a strong defense phenotype at 37°C in liquid assays.

Example 6 – T7 RNA polymerase is susceptible to some of the products of pVips

[00384] Given that some pVips provide defense against phage T7, it was hypothesized that T7 polymerase-dependent RNA synthesis might be affected by the nucleotide chain terminators produced by pVips. Therefore, it was tested if expression of a reporter gene (GFP) by the T7 polymerase was impacted by different pVips activities

[00385] To do so, a collection of strains derivatives of BL21-DE3, which encodes a T7 RNA polymerase (RNAP) under the control of a lac promoter, was created. The derivative strains bore the reporter plasmid pAGG encoding a GFP under the control of T7 promoter, and a module with T7 lyzozyme to limit basal expression of T7 RNAP. Further derivative strains bore a pVip candidate under the control of arabinose promoter. In these consturcts, the T7 RNA polymerase is induced by the addition of IPTG, thus activating the T7 promoter and inducing GFP transcription. We hypothesized that upon arabinose addition, pVips would be expressed inducing synthesis of polynucleotide chain terminators, which would terminate GFP transcription prematurely (**Figure 8A**).

[00386] Cells were grown to OD600 0.1 overnight and pVips were induced by addition of arabinose 0.02%. After 45 minutes T7 RNAP expression was induced by addition of IPTG 0.01mM (**Figure 8A**). GFP and OD were monitored with a plate reader (Tecan, Switzerland).

[00387] It was observed that induction of pVip8, pVip9, pVip37, pVip46, and pVip63 prevented or substantially inhibited the expression of GFP by T7 polymerase (**Figures 8B-8G**). However, co-expression of MoaA, which is structurally similar to pVip, did not inhibit GFP expression. This suggests that the pVip product inhibits T7-RNAP-dependent expression of GFP by a chain terminator that interrupts the nascent GFP mRNA.

Example 7 – Production of New Chain Terminators

[00388] The pVips disclosed herein can be used in order to produce chain terminators, including (but not limited to) ddhUTP, ddhATP, ddhGTP, ddhCTP, ddh-deoxy-GTP, and ddh-deoxy-ATP, ddh-deoxy-TTP, and ddh-deoxy-CTP. For this, the pVip protein would first be expressed in a heterologous expression system (e.g., in bacteria such a *E. coli* or *B. subtilis*, or in a eukaryotic expression system). Then, the expressed pVip will be purified, and then supplied with the necessary cofactors (e.g., s-adenosyl methionine) and the substrate (e.g., CTP, TTP etc, depending on the substrate of the specific pVip).

[00389] The pVip will produce the chain terminator, which will then be purified from the reaction and used for the proper application. Example 4 shows the importance of iron sulfur cluster metabolism for expression of functional pVips. Therefore, protein expression for pVips should be performed in strains such as ΔiscR or that contain plasmids like pDB1282, that encodes the iscR operon from *Azotobacter vinelandii*, or in another strain that allows expression of iron-sulfur cluster genes. Given the sensitive nature of iron sulfur cluster enzymes to oxygen, protein purification should preferentially be performed in anaerobic conditions.

[00390] While nucleotide analogs are actual chain terminators *in vivo*, nucleoside analogs, which is the version without phosphate groups, are the molecules generally used as drugs. The phosphate groups of the nucleotides may prevent entry to the cell due to its charge. Once nucleoside analogs enter the cells, they can be phosphorylated by endogenous enzymes or enzymes of the phage, and thus generate the cognate nucleotide analogs. Such an approach was used to show the efficiency of ddhC as an anti-viral molecule by Gizzi, A. S. et al. A naturally occurring antiviral ribonucleotide encoded by the human genome. Nature 558, 610–614 (2018). Upon entry to the cell, ddhC is phosphorylated to become ddhCTP and provides anti-viral activity against for example Zika virus. Similarly, cognate nucleoside analogs to the modified nucleotides produced by the pVips may be for example (but not limited to): ddhT, ddh-deoxy-G, ddh-deoxy-A, etc. Chemical strategies can be used to synthetize such types of nucleosides and could be applied to obtain these molecules.

Example 8 –pVips and Products Thereof

[00391] Examples 1-6 reveal the existence of a new family of prokaryotic anti-viral genes, pVips. A homology-based search in 69425 prokaryotic genomes followed by a detailed and quantitative analysis of gene neighborhoods allowed to discriminate potential anti-viral genes among a wider family of radical-SAM enzymes. The pVips family was further enriched with similar genes extracted from a database of 9769 metagenomes. The analysis of the evolutionary history of pVips and the eukaryotic viperin (a known anti-viral enzyme which produces ddhCTP, a chain terminator) suggests that eukaryotic viperins has evolutionarily originated from pVips and represent only a small fraction of the diversity of the protein family. Furthermore, the analysis of pVip accessory genes (nucleoside kinases or nucleotide kinases) suggests the existence of diverse substrate for the pVips, suggesting a diversity of pVips chain terminator products.

[00392] An experimental approach to screen active pVips *in vivo* was developed. After selection, codon optimization and synthesis of diverse pVips, strains encoding pVips were screened against a diverse collection of phages. It was found that the use of a specific strain of E. coli, where iron sulfur cluster auxiliary genes are more highly expressed, greatly improves pVips activity.

[00393] Products of the pVip enzymes may include nucleotide analogs or nucleoside analogs. These can include, for example, ddhUTP, ddhGTP, ddhATP, ddhCTP, ddh-deoxy-GTP, ddh-deoxy-ATP, ddh-deoxy-TTP, ddh-deoxy-CTP, as well as modified versions of these modified nucleotides that can be used as new anti-viral or anti-tumor drugs functioning as DNA or RNA chain terminators.

Example 9 –pVips Produce Diverse Anti-Viral Molecules

Material and Methods

Cell lysates preparation

[00394] Overnight cultures of Keio ΔiscR encoding pVips, MoaA or the human viperin were diluted 1:100 in 100 ml LB medium and grown at 37 °C (250 r.p.m.) for 1 hour and 45 minutes. The expression of viperin or MoaA was induced by the addition of arabinose (final concentration 0.2%) and cells were further incubated at 37 °C (250 r.p.m.) for one hour. Cells were then centrifuged at 3,900*g* for 10 min at 4 °C and samples kept on ice throughout the cell lysate preparation. Pellets were resuspended in 600 µl PBS buffer containing 100 mM sodium phosphate (pH 7.4). The resuspended pellet was supplemented with 1 µl of hen-lysozyme (Merck) (final hen-lysozyme concentration of 10 µg/ml). The resuspended cells were then mixed with Lysing matrix B (MP) beads and cells were disrupted mechanically using a FastPrep-24 bead-beater device (MP) (2 cycles of 40 s, 6 m s^{-1}, at 4 °C). Cell lysates were then centrifuged at 12,000*g* for 10 min at 4 °C and the supernatant was loaded onto a 3-kDa filter Amicon Ultra-0.5 centrifugal filter unit (Merck) and centrifuged at 14,000*g* for 30 min at 4 °C. The resulting flow-through, containing substances smaller than 3 kDa, was used as the lysate sample for evaluating the presence of ddh nucleotides by LC-MS.

Detection of ddh-nucleotides

[00395] Sample analysis was carried out by MS-Omics (Vedbæk, Denmark) as follows. Samples where diluted 1:1 in 10 mM ammonium acetate in 90% acetonitrile. The analysis was carried out using a UHPLC system (Vanquish, Thermo Fisher Scientific, US) coupled with a high-resolution quadrupole-orbitrap mass spectrometer (Q Exactive™ HF Hybrid Quadrupole-Orbitrap, Thermo Fisher Scientific). An electrospray ionization interface was used as ionization source. Analysis was performed in positive ionization mode. The UPLC was performed using a slightly modified version of a previously described protocol. Peak areas were extracted using Compound Discoverer 2.0 (Thermo Scientific).

Quantification of 3'-deoxy-3',4'-didehydro cytidine (ddhC)

[00396] The 3'-deoxy-3',4'-didehydro cytidine molecule was synthesized by Jena Bioscience (Jena, Germany) and was used as a standard for ddC quantification in cell lysates using LC-MS. Sample analysis was carried out by MS-Omics (Vedbæk, Denmark) as follows. Samples were diluted 1:1 in 10 mM ammonium formate and 0.1% formic acid in ultra-pure water. The analysis was carried out using the LC-MS setup described above. An electrospray ionization interface was

used as ionization source performed in positive ionization mode. The UHPLC method is based on Waters Application note 2011, 720004042en (Waters Corporation, Milford, US). Peak areas of 3'-deoxy-3',4'-didehydrocytidine (ddhC) were extracted using Trace Finder™ Version 4.1 (Thermo Fisher Scientific, US) and quantified using an external calibration with the standard.

Results

[00397] The animal viperin catalyzes the production of ddhCTP. Whether pVips produce ddhCTP and/or other types of modified nucleotides was examined. For this, pVips were expressed in *E. coli* and the fraction of small molecules was extracted from the cell lysates, presuming that the pVip-produced molecule would be present in that fraction. These lysates were analyzed with liquid chromatography followed by mass spectrometry (LC-MS) using an untargeted approach. As a positive control, cell lysates from cells expressing the human viperin protein were similarly analyzed. As expected, a compound conforming with the mass of ddhCTP was readily detected in lysates from cells expressing the human viperin but not in the negative control lysates that were derived from MoaA-expressing cells (**Figure 10**). Additional compounds found in the human viperin sample matched the masses of ddh-cytidine (ddhC) and ddh-cytidine monophosphate (CMP), possibly derived from natural decay of ddhCTP as also known to occur for CTP in neutral or acidic pH. Analysis of fragment ions using MS-MS further supported that the identified masses are ddhCTP, ddhCMP and ddhC with additional confirmation attained by subjecting synthesized ddhC standard to MS-MS analysis (**Figure 12**). These results confirm that the human viperin actively produces ddhCTP when expressed in *E. coli*, explaining its observed anti-phage activity.

[00398] The small molecule fractions from lysates of cells expressing 27 pVips that were found to have an anti-phage activity were then analyzed. Derivatives of ddhCTP were detected by LC-MS in the lysate of pVip50, a protein derived from a methanogenic archaeon that belongs to clade 2 of the pVips tree, verifying that pVips are indeed functional homologs of the human viperin that produce similar antiviral molecules. Moreover, other masses that were markedly enriched in the lysates of cells expressing pVips and absent from the negative control lysate were also examined. For several of the pVips it was found masses that conform with 3′-deoxy-3′,4′-didehydro-guanosine-triphosphate (ddhGTP) and 3′-deoxy-3′,4′-didehydro-guanosine-diphosphate (ddhGDP), and for other pVips other molecules were found with masses matching 3′-deoxy-3′,4′-didehydro-uridine triphosphate (ddhUTP) and 3′-deoxy-3′,4′-didehydro-uridine monophosphate (ddhUMP) (**Figures 9And 9B**, **Figure 11**). These results suggest that pVips produce new types of antiviral ribonucleotides that were not observed before in nature.

[00399] For most of the pVips, predicted derivatives of a single modified nucleotide were observed in the lysate (either ddhCTP, ddhGTP or ddhUTP). However, seven of the pVips were

found to produce derivatives of multiple ddh ribonucleotides. For example, in lysates derived from pVip8-expressing cells, it was found both ddhCTP and ddhUTP, and in lysates from pVip58 cells, ddhCTP, ddhUTP, ddhGTP and their derivatives were detected (**Figure 11**). These results suggest that throughout evolution some pVips may have become more promiscuous and can modify more than one ribonucleotide to its ddh antiviral form. Presumably such pVips may have an advantage when encountering phages that can overcome one of these antiviral molecules but not the other two.

[00400] For seven of the tested pVips, no ddh nucleotide or its derivatives were detected in the cell lysates, despite a clear antiviral activity conferred by these pVips (**Figure 9A**). It is possible that these pVips produce a different antiviral molecule that could not have been detected via the LC-MS protocol, or, alternatively, that these pVips have evolved to confer defense by another mechanism of action that does not involve production of antiviral molecules.

[00401] The identity of the molecules produced by the various pVips is largely consistent with their phylogenetic relatedness. pVips from clades 4-7 were predicted to produce ddhUTP, with some of these also producing additional ddh ribonucleotides. In clade 1 and clade 2, which resides together with the eukaryotic viperins on the same super-clade, certain pVips were found to produce ddhCTP. Clade 3 includes pVips that were predicted to generate either ddhGTP or ddhUTP (**Figure 9B**).

Example 10 – Anti-Viral Activities of ddh-Compounds

[00402] The present example examines the antiviral activities for ddhC (compound AB21650), ddhU (compound AB21649) and ddhG (compound AB21651).

[00403] The compounds were tested against a panel of 17 viruses: adenovirus-5 (Ad5), acaribe virus (TCRV), Rift Valley fever virus (RVFV), SARS-CoV, dengue virus-2 (DV-2), Japanese encephalitis virus (JEV), Powassan virus (POWV), West Nile virus (WNV), Yellow fever virus (YFV), Zika virus, Influenza(H1N1), Influenza(H5N1), Influenza B, RSV, poliovirus-1 (POV-1), enterovirus-68 (EV-68), and Venezuelan equine encephalitis virus (VEEV). Cell types used were A549 for Ad5; Vero E6 for TCRV; Huh7 for DV-2 and YFV; BHK-21 for POWV; RD for EV-68; MA-104 for RSV; MDCK for influenza viruses; and Vero 76 for all other viruses.

[00404] The compounds were solubilized in DMSO to prepare a 400 mM stock solution. The compounds were then serially diluted using eight half-log dilutions in test medium (MEM supplemented with 2% FBS and 50 μg/mL gentamicin) so that the starting (high) test concentration was 2 mM. Each dilution was added to 5 wells of a 96-well plate with 80-100% confluent cells.

Three wells of each dilution were infected with virus, and two wells remained uninfected as toxicity controls. Six wells were infected and untreated as virus controls, and six wells were uninfected and untreated as cell controls. The viruses were prepared to achieve the lowest possible multiplicity of infection (MOI) that would yield >80% cytopathic effect (CPE) within 3-7 days. Positive control compounds were tested in parallel for each virus tested. Plates infected with EV-68 were incubated at 33±2°C, 5% CO_2; all other plates were incubated at 37±2°C, 5%CO_2.

[00405] On day 3-7 post-infection, once untreated virus control wells reached maximum CPE, the plates were stained with neutral red dye for approximately 2 hours (±15 minutes). Supernatant dye was removed and the wells were rinsed with PBS, and the incorporated dye was extracted in 50:50 Sorensen citrate buffer/ethanol for >30 minutes and optical density was read on a spectrophotometer at 540 nm. Optical densities were converted to percent of cell controls and normalized to the virus control, then the concentration of test compound required to inhibit CPE by 50% (EC_{50}) was calculated by regression analysis. The concentration of compound that would cause 50% cell death in the absence of virus was similarly calculated (CC_{50}). The selective index (SI) is the CC_{50} divided by EC_{50}.

[00406] The results are shown in Table 11. It is found that ddhG exhibits antiviral activity against Influenza (H1N1) and Influenza (H5N1); ddhU exhibits antiviral activity against Influenza B and Influenza (H1N1 and H5N1); ddhC exhibits some activity against enterovirus EV-68.

TABLE 11. *In vitro* antiviral activity of AB21650 (ddhC), AB21651 (ddhG), and AB21649 (ddhU).

		AB21650			AB21651			AB21649			Positive Control		
	Positive Control	EC_{50}	CC_{50}	SI	EC_{50}	CC_{50}	SI	EC_{50}	CC_{50}	SI	EC_{50}	CC_{50}	SI
Ad5	2-3 Dideoxycytidine	>2	>2	0	>2	>2	0	>2	>2	0	1.8	>100	>56
TCRV	Ribavirin	>2	>2	0	>2	>2	0	>1.2	1.2	0	13	820	63
RVFV	Ribavirin	>2	>2	0	>2	>2	0	>1.1	1.1	0	14	870	62
SARS-CoV	M128533	>2	>2	0	>2	>2	0	>1.5	1.5	0	0.075	>100	>1300
SARS-CoV-2	M128533	>2	>2	0	>2	>2	0	>2	>2	0	0.33	>100	>300
DV-2	Infergen	>2	>2	0	>2	>2	0	>2	>2	0	0.13	>10	>77
JEV	Infergen	>2	>2	0	>2	>2	0	>1.7	1.7	0	0.043	>10	>230
POWV	Infergen	>2	>2	0	>2	>2	0	>2	>2	0	0.0051	>10	>2000
WNV	Infergen	>2	>2	0	>2	>2	0	>1.4	1.4	0	0.12	>10	>83
YFV	Infergen	>2	>2	0	>2	>2	0	>2	>2	0	0.012	>10	>830
VEEV	Infergen	>2	>2	0	>2	>2	0	>1.1	1.1	0	0.17	>10	>59
Zika	NITD008	>2	>2	0	>2	>2	0	>1.5	1.5	0	1.8	44	24
Influenza A(H1N1)	Ribavirin	>2	>2	0	0.44	>2	>4.5	0.56	1.4	2.5	4.6	>1000	>220
Influenza A(H5N1)	Ribavirin	>2	>2	0	1.3	>2	>1.5	0.8	1.3	1.6	1.8	>1000	>560
Influenza B	Ribavirin	>2	>2	0	>2	>2	0	0.31	1.3	4.2	1.4	>1000	>710
RSV	Ribavirin	>2	>2	0	>2	>2	0	>1.9	1.9	0	7.3	42	5.8
POV-1	Enviroxime	>2	>2	0	>2	>2	0	>1.0	1.0	0	0.0095	3.3	350
EV-68	Pirodavir	0.91	1.8	2	>1.1	1.1	0	>0.39	0.39	0	0.039	4.3	110

Units are in mM for test compounds, ng/mL for Infergen™, and µg/mL for all other positive control compounds

EC_{50}: 50% effective antiviral concentration

CC_{50}: 50% cytotoxic concentration of compound without virus added

SI = CC_{50}/EC_{50}

M128533 positive control is Z-Leu-Gln(NMe2)-FMK (Zhang et al. (2006) J. Med. Chem. 2006, 49, 1198-1201).

Example 11 – Synthesis of Novel Synthetic Nucleotide Analogs

[00407] To test whether pVips can modify substrates that are non-natural to their native activities *in vivo*, the catalytic activities of pVips against a battery of substrates were explored by performing *in vitro* enzymatic assays with purified enzymes. The results show that pVips accept multiple nucleotides as substrates *in vitro* and catalyze the production of 3′-deoxy-3′,4′-didehydro forms of ATP, GTP, CTP, UTP and ITP, as well deoxy-UTP, even when these products are not produced by these enzymes *in vivo*. For example, pVip6 was shown *in vivo* to generate the product ddhCTP; but *in vitro*, it also produces ddhUTP, ddhATP, ddhGTP and ddhITP (**Figure 14**). These results show that pVips, as opposed to eukaryotic viperins, are promiscuous enzymes that can perform their enzymatic activities (removal of the OH from the 3' carbon) on multiple different substrates.

[00408] In addition to the previous identified products - ddhCTP, ddhUTP and ddhGTP - the production of three novel nucleotide analogs by pVips was detected *in vitro*: ddhATP, ddhITP and ddhdUTP. These results demonstrate that pVips can produce a wide range of nucleotide analogs and display a wider substrate promiscuity as compared to eukaryotic homologues.

[00409] Based on the proven substrate promiscuity of pVips, it is predicted that these enzymes would generate novel structural modifications on multiple non-natural nucleotide derivatives and other molecules. In one embodiment, the modification done by pVips is the dehydration of the 3' carbon in the ribose moiety of the nucleotide, and these non-natural products of pVips could confer novel therapeutic properties. In one embodiment, the pVips would be able to modify a large set of non-natural nucleotides as disclosed herein, and one or more of the products of these modifications could have potential therapeutic properties. For instance, pVips could be harnessed to generate 3′-deoxy-3′,4′-didehydro variants (*see* **Figure 13**) of existing synthetic nucleotide analogs such as Ribavirin, 6-Azauridine, Gemcitabine or Remdesivir. Moreover, given the observed pVips promiscuity, it is predicted that these enzymes could catalyze other types of nucleotide modifications – other than the 3'-dehydroxylation – leading to the generation of additional novel analogs.

[00410] The utilization of pVips to modify clinically relevant non-natural nucleotide analogs could lead to the discovery of new compounds with improved therapeutic properties that display, for instance, enhanced antiviral/anti-tumoral/antibacterial potency, reduced toxicity, or improved bioconversion and pharmacokinetic properties. Moreover, given the advantages that bio-based production methods offer over chemical synthesis methods - which are time-consuming, costly

and polluting - the enzymatic generation of non-natural nucleotide analogs using pVips could accelerate the discovery and production of novel therapeutic variants as well as their deployment to the clinic.

Materials and Methods

Strains and Growth Conditions

[00411] *E. coli* strains BL21 (NEB®) and BL21-ΔiscR were used for protein production and strain DH10β (NEB®) for molecular cloning. Unless otherwise noted, all *E. coli* strains were grown in LB medium supplemented with antibiotics kanamycin (25 μ g/mL), ampicillin (50 μ g/mL) or chloramphenicol (17.5 μ g/mL) when appropriate for selective plasmid propagation. The strain BL21-ΔiscR was constructed from *E. coli* BL21 by knocking out the chromosomal *iscR* gene through P1 transduction using phages propagated from the Keio ΔiscR strain, followed by several rounds of kanamycin selection. Successful deletion of *iscR* was verified by PCR and sequencing.

Plasmid Construction

[00412] All plasmids were constructed using Gibson assembly in *E. coli* DH10β. Codon-optimized pVip genes were amplified by PCR from pAB_Vip expression vectors (Sorek lab) and cloned into the aTc-inducible expression vector pASG-IBA143 (IBA Lifesciences) for fusion of a Twin-Strep-tag® to the C terminus of the pVips. To construct the suf operon expression vector "pSuf", the complete operon (*sufABCDSE*) amplified from *E. coli* MG1655 genomic DNAnd the arabinose expression system from pAB_Vip vectors were cloned into the pACYC-184 (NEB®) backbone. The resulting expression vector is arabinose-inducible and contains a chloramphenicol resistance cassette as well as a p15A origin of replication.

Protein Expression

[00413] BL21-ΔiscR or BL21 pSuf cells freshly transformed with plasmids encoding the tagged pVips were used. Transformants were grown overnight on selective LB agar plates at 37°C. Individual colonies of engineered strains were picked into 5 mL selective LB medium and incubated overnight with shaking at 37°C. From overnight cultures, protein production cultures were seeded at an initial OD600 of ~0.06 either in 1-2 L of selective LB medium or in 1-2 L of selective M9 medium supplemented with 4 g L^{-1} D-glucose. Cultures were incubated at 37°C with shaking. Cultures of strains carrying the pSuf vector were supplemented with 100 μM $FeCl_3$, 100 μM L-cysteine and induced with 0.2% arabinose at OD_{600} = 0.2-0.3. For all cultures, pVip expression was induced with 50 ng per mL aTc when OD_{600} reached 0.6–0.8. BL21-ΔiscR strains were also supplemented with 100 μM $FeCl_3$, 100 μM L-cysteine at OD_{600} = 0.6–0.8. After induction with aTc, cultures grown in LB were incubated at 37°C with shaking for 3-4h and pellets

were harvested by centrifugation. After induction with aTc, cultures grown in M9 were placed at 4 °C for 1h, pellets were harvested by centrifugation after incubation overnight at 18 °C with shaking. Pellets were stored at -20 °C.

Protein Purification

[00414] Frozen cell pellets were thawed, resuspended in lysis buffer (50 mM Tris·HCl, 500 mM NaCl, 5 mM dithiothreitol (DTT), 0.5 M arginine, and 20% glycerol), and sonicated with a Branson Sonifier (15 sec ON, 45 sec OFF, 10 min total ON, 30% amplitude) on ice. Lysates were subjected to centrifugation for 30 min at 17,000 g and 4 °C (Avanti J-20 XP centrifuge; JA-25.50 rotor). The lysate was loaded onto a StrepTactin Superflow High Capacity (50% suspension; IBA #2-1208-025) column previously equilibrated with 20 column volumes of Buffer W (100 mM Tris-HCl pH 8, 300 mM NaCl, 5 mM DTT, 10% glycerol). The column was washed twice with 10 column volumes of Buffer W and eluted with buffer E (50 mM Tris-HCl pH 8, 300 mM NaCl, 5 mM DTT, 2.5 mM desthiobiotin, 20% glycerol). The presence of the pVip proteins in the resulting fractions was confirmed by SDS-PAGE. Purified proteins were frozen in liquid nitrogen and stored at – 80°C.

Protein Reconstitution

[00415] Purified protein solutions were thawed on ice and introduced into in a customized MBraun anaerobic chamber maintained at <0.1 ppm oxygen. All subsequent steps were performed in anaerobic conditions at 12 °C. Proteins were incubated for 1 hour with 50 mM DTT with gentle shaking. Protein solutions were supplemented with 8-fold molar excess $Fe(NH_4)_2(SO_4)_2$, incubated for 15 min with gentle shaking, followed by adding 8-fold molar excess of Na2S droplet by droplet. After incubation for 3-4h to overnight with slow shaking, the reconstituted pVips were transferred to the Reaction Buffer (50 mM HEPES pH 7.5, 150 mM KCl, 5 mM DTT, 20% Glycerol) using PD-10 desalting columns (GE Healthcare) and concentrated using an Amicon Ultra centrifugal 10 kDa filter (Merck) to a final protein concentration of 20-50 μM. Proteins were then flash-freezed with liquid nitrogen and stored at – 80°C.

Enzymatic Assay

[00416] Reactions were performed in a total volume of 100 μL containing: 20-50 μM protein in Reaction buffer, 2 mM S-Adenosyl methionine (SAM), 1 mM of nucleotide substrate, and 5 mM sodium dithionite. Reactions were carried out inside the anaerobic chamber maintained at <0.1 ppm oxygen. Reaction mixtures without dithionite were incubated at 37 °C for 5 minutes. A 10 μL aliquot was removed from the reaction mixture (sample before reaction). Reactions were then initiated with sodium dithionite and incubated at 37 °C for 1-2 h. After incubation, samples were taken out of the anaerobic chamber and stored at -80 °C until analysis.

HPLC Method

[00417] High-performance liquid chromatography (HPLC) with detection of UV absorbance (280 nm wavelength) was conducted at 23°C with a constant flow of 0.5 mL per minute. The mobile phase was composed of solvent A (0.1% formic acid, 5% methanol) and solvent B (100% acetonitrile). Samples in volume of 1 µL were fed onto an Agilent Eclipse Plus C18 RRHD column equilibrated with 100 % solvent A. Samples were separated with a gradient from 0% to 5% of solvent B in 0.5 min, followed by a gradient from 5% to 20% for 1.5 min, then from 20% to 50% solvent B for 1 min, and then held at 50% solvent B for 0.5 min before returning to 0% solvent B for equilibration for 5.5 min. Quantification of 5'-dA in reaction samples was performed using a standard curves generated with 5'dA purchased from Sigma-Aldrich.

LC-MS Method

[00418] LC-MS measurements were performed with a Thermo Scientific Q Exactive Orbitrap mass spectrometry system equipped with a Dionex Ultimate 3000 UHPLC system. The software Thermo Xcalibur was used for instrument control and data processing. Prior to analysis, 10 µL of samples from enzymatic assays were mixed with 40 µL of acetonitrile:methanol organic mixture (5:3 v/v ratio). The mixtures were vortexed, centrifuged at 17,000g for 2 min and 3 µL of supernatant were injected onto an SeQuant® ZIC®-pHILIC 5µm polymeric 100 x 2.1 mm HPLC column. The mobile phase was composed of 20 mM ammonium carbonate pH 9.5 (solvent A) and 100% acetonitrile (solvent B). Samples were separated using a constant flow rate of 0.2 mL/min, 80% solvent B was held for 2 min, followed by a gradient from 80% to 20% of solvent B for 15 min, before immediately returning to 80% solvent B for equilibration for 9 min. Datanalysis was performed using the Thermo Scientific FreeStyle software.

In Vitro Production of 3'-deoxy-3',4'-didehydro-GTP

[00419] To obtain sufficient amounts of ddhGTP for MS/MS analysis, enzymatic reactions were performed in a total volume of 1ml containing: 113 mM pVip56, 2mM SAM, 2mM GTP and 5mM dithionite in Reaction Buffer. Reactions were carried out in anaerobic conditions as previously described and incubated at 37°C for 3 hours. To remove the protein, 10K centrifugal filters were used. The flow through was diluted 2-fold into cold 10 mM ammonium bicarbonate buffer pH 9.0 (buffer A), then loaded onto Capto™ HiRes Q 5/50 (GE Healthcare) pre-equilibrated with buffer A. The column was washed with 25 mL of buffer And elution was performed using linear elution gradient (100 mL) of 200 mM to 800 mM ammonium bicarbonate, pH 9. The purified product was lyophilized and resuspended in water prior LC-MS analysis.

[00420] *Results*

[00421] The substrate selectivity and catalytic potential of pVips were investigated by

performing *in vitro* biochemical studies with purified enzymes from different clades. To this purpose, vectors were constructed for overexpressing the 27 codon-optimized pVips of interest with a Strep II-tag fused to their C-terminus. Individual tagged pVips were overexpressed in *E. coli* and purified by Strep II-tag affinity purification. After reconstitution of the enzyme's [4Fe-4S] cluster, the ability of each pVip to catalyze reductive cleavage of SAM into 5′-deoxyadenosine (5′-dA) was measured in the presence of a specific substrate. Enhanced 5′-dA production under reducing conditions is a characteristic indicator of substrate activation of radical SAM enzymes, and widely employed for substrate identification. Each pVip was screened against a set of substrate candidates corresponding to most members of the natural nucleotide triphosphate pool: ribonucleotides (ATP, GTP, UTP, CTP, ITP) and deoxyribonucleotides (dATP, dGTP, dUTP, dCTP, dTTP). For each pVip, the production of 5′-dA was measured in the presence of a substrate, as compared to control reactions without nucleotide or activating reducing agent (condition before reaction).

[00422] It was found that pVips from different evolutionary clades accept up to 6 natural nucleotides (ATP, CTP, GTP, ITP, UTP and dUTP) as substrates (**Figure 14**). Furthermore, the reaction samples were analyzed by LC-MS in order to identify the products generated by the pVips. It was first confirmed that pVips catalyze the generation of a product with a mass that conforms to that of 3′-deoxy-3′,4′-didehydro-nucleotides (ddhNTPs). The production of ddhUTP (**Figures 15A-15B**), ddhCTP (**Figures 16A-16B**), ddhATP (**Figures 17A-17B**), ddhITP (**Figures 18A-18B**) and ddhdUTP (**Figures 19A-19B**), and ddhGTP (**Figures 20A-20B**) by the pVips was further supported by the fragmentation spectrum of ions obtained via MS-MS, which displays masses corresponding to potential structures obtained from ddhNTPs.

[00423] The data reveal that pVips display, *in vitro,* a wider substrate range than the one predicted by *in vivo* analysis (*see* **Figure 14**). For instance, only derivatives of ddhCTP were detected in lysates derived from pVip6-expressing cells (clade 1), suggesting that this enzyme could only catalyze the transformation of CTP. However, enzymatic assays presented herein demonstrate that CTP, UTP, ATP, GTP and ITP can all act as substrates. In the same manner, *in vitro* assays with pVip56 and pVip62 from clade 5, as well as pVip8 from clade 4, revealed that these enzymes accept more nucleotide substrates than reported by previous *in vivo* data. In conclusion, these *in vitro* results reveal that the substrate specificity of pVips is greater than the one reported for any other Viperin homologue from eukaryotic organisms.

[00424] Overall, the data presented herein demonstrate that pVips exhibit uniquely broad substrate promiscuity and catalyze *in vitro* the production of 3′-deoxy-3′,4′-didehydro-variants of diverse natural nucleotide substrates. It was also shown that purified pVips can produce new

molecules that were not described before, e.g. the production of the novel compounds ddhATP, ddhITP and ddhdUTP. These novel non-natural nucleotide analogs generated by pVips could hold new antiviral/anti-tumoral/antibacterial properties that can be harnessed to develop new therapies.

Example 12 – ANTIVIRAL ASSESSMENT OF NUCLEOSIDE ANALOGS

[00425] *Objective*: The objective of this study was to test ddh- and ddh-deoxy-compounds against Herpes simplex virus 1 (HSV-1), Human cytomegalovirus (HCMV), Epstein-Barr virus (EBV), BK virus (BKV), and JC virus (JCV).

[00426] *Methods*:

[00427] Table 12 sets forth the compounds tested.

Table 12: ddh- and ddh-deoxy-Compounds

Name	MW	Amt
SATE-ddhU Compound 4	594.64	5.4mg
SATE-ddhC Compound 6	593.65	4.3mg
SATE-ddhG Compound 2	633.67	4.7mg
SATE-ddhA Compound 1	617.68	4.4mg
SATE-ddhI Compound 3	618.88	4.9mg
SATE-ddhdeoxyU Compound 5	578.15	9.5mg

[00428] All compounds were solubilized by adding DMSO to each, based on quantities and molecular weights specified to achieve a final 30mM concentration. These stocks were stored at 20°C. The working test compound solutions (1.5mM) were prepared by diluting the 30mM DMSO stocks 1:20 in warmed MEM + 2%FBS. Positive and negative control drugs were prepared with working solutions at 1.5 and 1.0 mM in MEM + 2%FBS.

[00429] Cells were infected with given virus and cultured in the presence of ddh- and ddh-deoxy compounds or controls. Cell viability was analyzed using a commercial assay according to the manufacturer's instructions (Promega,USA; CellTiter-Glo® Luminescent Cell Viability Assay), in cells infected with HSV-1, HCMV, EBV, BKV, or JCV.

[00430] **Cells culture and virus strains.** Human foreskin fibroblast (HFF) cells prepared from human foreskin tissue were obtained. The tissue was incubated at 4°C for 4 h in Clinical Medium consisting of minimum essential media (MEM) with Earl's salts supplemented with 10% fetal bovine serum (FBS) (Hyclone, Inc. Logan UT), L-glutamine, fungizone, and vancomycin. Tissue

is then placed in phosphate buffered saline (PBS), minced, rinsed to remove the red blood cells, and resuspended in trypsin/EDTA solution. The tissue suspension is incubated at 37°C and gently agitated to disperse the cells, which are collected by centrifugation. Cells are resuspended in 4 ml Clinical Medium and placed in a 25 cm2 flask and incubated at 37°C in a humidified CO_2 incubator for 24 h. The media is then replaced with fresh Clinical Medium and the cell growth is monitored daily until a confluent monolayer has formed. The HFF cells are then expanded through serial passages in standard growth medium of MEM with Earl's salts supplemented with 10% FBS, L-glutamine, penicillin, and gentamycin. The cells are passaged routinely and used for assays at or below passage 10. COS7 and C-33 A, Guinea Pig Lung, and Mouse embryo fibroblast cells were obtained from ATCC and maintained in standard growth medium of MEM with Earl's salts supplemented with 10% FBS, L-glutamine, penicillin, and gentamycin.

[00431] Akata cells were kindly provided by John Sixbey (Louisiana State University, Baton Rouge, LA). BCBL-1 cells were obtained through the NIH AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, NIH. Molt-3 cells were obtained from Scott Schmid at the Centers for Disease Control and Prevention, Atlanta, GA. Lymphocytes were maintained routinely in RPMI 1640 (Mediatech, Inc., Herndon, VA) with 10% FBS, L-glutamine and antibiotics and passaged twice a week.

[00432] The E-377 strain of HSV-1 was a gift of Jack Hill (Burroughs Wellcome). The HCMV strain AD169, HSV-2 strain G, AdV5 strain Adenoid 75, GP CMV strain 11211 and MCMV strain were obtained from the American Type Culture Collection (ATCC, Manassas, VA). The Copenhagen strain of VACV and Brighton strain, CPXV were kindly provided by John W. Huggins (Department of Viral Therapeutics, Virology Division, United States Army Medical Research Institute of Infections Disease). VZV, strain Ellen, the BK virus Gardner strain and JC virus MAD4 strain were obtained from the ATCC. Akata cells latently infected with EBV were obtained from John Sixbey. The Z29 strain of HHV-6B was a gift of Scott Schmid at the Centers for Disease Control and Prevention, Atlanta GA. HHV-8 was obtained as latently infected BCBL-1 cells through the NIH AIDS Research and Reference Reagent Program.

[00433] **Antiviral Assays:** Each experiment that evaluated the antiviral activity of the compounds included both positive and negative control compounds to ensure the performance of each assay. Concurrent assessment of cytotoxicity was also performed for each study in the same cell line and with the same compound exposure (see below).

[00434] **CPE assays for HSV-1, HSV-2, VZV, HCMV, MCMV, GPCMV, AdV, VACV, CPXV** Assays were performed in monolayers as described (Hartline CB, Keith KA, Eagar J, Harden EA, Bowlin TL, Prichard MN. Antiviral Res. 2018. A standardized approach to the

evaluation of antivirals against DNA viruses: Orthopox-, adeno-, and herpesviruses. Nov;159:104-112.). Briefly, cells were seeded in 384 well plates and incubated for 24h to allow the formation of confluent monolayers. Dilutions of test drug were prepared directly in the plates and the monolayers infected at a predetermined MOI based on virus used. After incubation, cytopathology was determined by the addition of CellTiter-Glo (CTG) reagent. Concentrations of test compound sufficient to reduce CPE by 50% (EC50) or decrease cell viability by 50% (CC50) were interpolated using standard methods in Microsoft excel.

[00435] **Plaque reduction assays for HSV-1, HSV-2, VZV, HCMV, MCMV, GPCMV, VACV, CPXV.** Monolayers of HFF cells were prepared in six-well plates and incubated at 37°C for 2 d to allow the cells to reach confluency. Media was then aspirated from the wells and 0.2 ml of virus was added to each of three wells to yield 20-30 plaques in each well. The virus was allowed to adsorb to the cells for 1 h and the plates were agitated every 15 minutes. Compounds were diluted in assay media consisting of MEM with Earl's salts supplemented with 2% FBS, L-glutamine, penicillin, and gentamycin. Diluted drug was added to duplicate wells and the plates were incubated for various times, depending on the virus used. For HSV-1 and -2, the monolayers were then stained with 1% crystal violet in 20% methanol and the unbound dye removed by washing with dH_20. For all other assays, the cell monolayer was stained with 1% Neutral Red solution for 4 h then the stain was aspirated and the cells were washed with PBS. For all assays, plaques were enumerated using a stereomicroscope and the concentration of compound that reduced plaque formation by 50% (EC_{50}) was interpolated from the experimental data.

[00436] **Yield reduction assays for HSV-1, HSV-2, HCMV, VACV, CPXV and AdV.** Monolayers of HFF cells were prepared in 96-well plates and incubated at 37°C for 1 d to allow the cells to reach confluency. Media was then aspirated from the wells and cells infected at a high MOI. At 1 h following infection, the inocula were removed and the monolayers rinsed with fresh media. Compounds were then diluted in assay media consisting of MEM with Earl's salts supplemented with 2% FBS, L-glutamine, penicillin, and gentamycin. Drug dilutions were added to the wells and the plates were incubated for various times, depending on the virus used and represents a single replication cycle for the virus. A duplicate set of dilutions were also performed but remained uninfected to serve as a cytotoxicity control and received equal compound exposure. Supernatants from each of the infected wells were subsequently titered in a TCID50 assay to quantify the progeny virus. For the cytotoxicity controls, cytotoxicity was assed using CTG according to the manufacturer's suggested protocol. For all assays, the concentration of compound that reduced virus titer by 90% (EC_{90}) was interpolated from the experimental data.

[00437] **Assays for EBV, HHV-6B, and HHV-8.** Assays for EBV, HHV-6B and HHV-8 were

performed by methods we reported previously (Keith KA, Hartline CB, Bowlin TL, Prichard MN. Antiviral Res. 2018. A standardized approach to the evaluation of antivirals against DNA viruses: Polyomaviruses and lymphotropic herpesviruses.). Akata cells were induced to undergo a lytic infection with 50 μg/ml of a goat anti-human IgG antibody. Experimental compounds were diluted within plates; the cells were added and incubated for 72 h. For HHV-6 assays, compounds were serially diluted plates then uninfected Molt-3 cells were added to each well. Infection was initiated by adding HHV-6B infected Molt-3 cells, at a ratio of approximately 1 infected cell for every 10 uninfected cells. Assay plates were incubated for seven days at 37°C. Assays for HHV-8 were performed in BCBL-1 cells. Similar plates were initiated without virus induction/addition and used for measuring cytotoxicity by the addition of CTG. For all assays, the replication of the virus was assessed by the quantification of viral DNA by PCR. Compound concentrations sufficient to reduce genome copy number by 50% were calculated from experimental datas well as compound cytotoxicity.

[00438] **Assays for BK virus and JC virus.** Primary assays for BKV and JCV were performed by methods we reported previously (Keith et al. 2018; *ibid*). For BKV, compound dilutions were prepared in plates containing cells, subsequently infected and incubated for 7d. Total DNA was prepared and genome copy number was quantified by real time PCR (Leung AY, Suen CK, Lie AK, Liang RH, Yuen KY, Kwong YL. Quantification of polyoma BK viruria in hemorrhagic cystitis complicating bone marrow transplantation. Blood. 2001;98(6):1971-8.). Plasmid pMP526 serves as the DNA standard for quantification purposes. Compounds that were positive in this assay were confirmed in a similar assay in 96-well plates according to established laboratory protocols with the compounds added 1h post infection to identify compounds that inhibit early stages of replication including adsorption and penetration. Genome copy number was determined by methods described above.

[00439] Primary evaluation of compounds against JC virus were also performed by methods similar to those for BK virus primary assays but were done in COS7 cells and utilized the 1-4 strain of JCV in COS7 cells. Viral DNA was quantified using PCR. Secondary assays against JCV were also performed in COS7 cells by methods similar to those for BK virus to identify compounds that inhibited adsorption or penetration of the virus.

[00440] **Assays for HPV**

[00441] Primary assay: An HPV11 replicon assay was developed and expresses the essential E1 and E2 proteins from the native promoter. The E2 origin binding protein interacts with the virus origin of replication and recruits the E1 replicative helicase which unwinds the DNA and helps to recruit the cellular DNA replication machinery (including DNA polymerases, type I and type II

topoisomerases, DNA ligase, single-stranded DNA binding proteins, proliferating cell nuclear antigen). The replication complex then drives the amplification of the replicon which can be assessed by the expression of a destabilized NanoLuc reporter gene carried on the replicon. In this assay, the replicon (pMP619) is transfected into C-33 A cells grown as monolayers in 384-well plates. At 48 h post transfection, the enzymatic activity of the destabilized NanoLuc reporter is assessed with NanoGlo reagent. The reference compound for this assay is PMEG and its EC50 value is within the prescribed range of 2 - 9.2 μM and is similar to a compound as reported previously (Beadle JR, Valiaeva N, Yang G, Yu JH, Broker TR, Aldern KA, et al. Synthesis and Antiviral Evaluation of Octadecyloxyethyl Benzyl 9-[(2-Phosphonomethoxy)ethyl]guanine (ODE-Bn-PMEG), a Potent Inhibitor of Transient HPV DNAmplification. J Med Chem. 2016;59(23):10470-8.).

[00442] *Results*:

[00443] There were no problems with solubility of the compounds in DMSO or in MEM + 2% FBS. For each assay using virus infected cells, the concentrations of ddh- and ddh-deoxy-compounds ranged from 0.048-150 μM (HSV-1; HCMV; EBV (also tested drug concentrations of 0.032-100); BKV; and JCV). Similarly, control drug concentration ranged from 0.048-150 μM. The results are presented in Tables 13, 14, 15, 16, and 17 below:

[00444] Assay against Herpes Simplex Virus 1 (HSV-1)

	Strain	**Cell Line**	**Incubation**	**Assay Name**
HSV-1	E-377	HFF	5 d	CellTiter-Glo *(Cytopathic effect/Toxicity)*

TABLE 13: Effect of ddh- and ddh-deoxy-compounds in cells infected with HSV-1

Drug	**Activity**	**EC_{50}**	**EC_{90}**	**CC_{50}**	**SI_{50}**	**SI_{90}**
ACV	-	2.32	>150.00	>150.00	>65	1
TNV	-	>150.00	>150.00	>150.00	1	1
SATE-ddhU Compound 4	NA	>150.00	>150.00	>150.00	1	1
SATE-ddhC Compound 6	NA	>150.00	>150.00	>150.00	1	1
SATE-ddhG Compound	NA	>150.00	>150.00	>150.00	1	1

2						
SATE-ddhA Compound 1	NA	>30.00	>30.00	82.43	<3	<3
SATE-ddhI Compound 3	NA	>150.00	>150.00	>150.00	1	1
SATE-ddhdeoxyU Compound 5	NA	>150.00	>150.00	>150.00	1	1

ACV - Acyclovir - positive control, TNV - Tenofovir - negative control, NA (Not Active) SI_{50} = 0 - 3.9, LO (Slightly Active) SI_{50} = 4 - 9.9, MOD (Moderately Active) SI_{50} = 10 - 49.9, HI (Highly Active) SI_{50} = ≥50.

[00445] Assay against Human cytomegalovirus (HCMV)

	Strain	Cell Line	Incubation	Assay Name
HCMV	AD169	HFF	14 d	CellTiter-Glo *(Cytopathic effect/Toxicity)*

TABLE 14: Effect of ddh- and ddh-deoxy-compounds in cells infected with HCMV

Drug	Activity	EC_{50}	EC_{90}	CC_{50}	SI_{50}	SI_{90}
GCV	-	0.81	>150.00	>150.00	>186	1
TNV	-	>150.00	>150.00	>150.00	1	1
SATE-ddhU Compound 4	NA	>150.00	>150.00	>150.00	1	1
SATE-ddhC Compound 6	NA	>150.00	>150.00	>150.00	1	1
SATE-ddhG Compound 2	NA	>30.00	>30.00	93.16	<3	<3
SATE-ddhA Compound 1	NA	>30.00	>30.00	84.56	<3	<3
SATE-ddhI Compound	NA	>150.00	>150.00	>150.00	1	1

3						
SATE-ddhdeoxyU Compound 5	NA	>150.00	>150.00	>150.00	1	1

GCV - Ganciclovir - positive control, TNV - Tenofovir - negative control, NA (Not Active) SI_{50} = 0 - 3.9, LO (Slightly Active) SI_{50} = 4 - 9.9, MOD (Moderately Active) SI_{50} = 10 - 49.9, HI (Highly Active) SI_{50} = ≥50.

[00446] Assay against Epstein-Barr virus (EBV)

	Strain	Cell Line	Incubation	Assay Name
EBV	Akata	Akata	3 d	Quantitative Polymerase Chain Reaction *(DNA)*/ CellTiter-Glo *(Toxicity)*

TABLE 15: Effect of ddh- and ddh-deoxy-compounds in cells infected with EBV

Drug	Activity	EC_{50}	EC_{90}	CC_{50}	SI_{50}	SI_{90}
CDV	-	9.23	29.89	>100.00	>11	>3
TNV	-	45.76	>150.00	>150.00	>3	1
SATE-ddhU Compound 4	NA	>30.00	>30.00	108.58	<4	<4
SATE-ddhC Compound 6	MOD	18.76	>30.00	123.78	7	<4
SATE-ddhG Compound 2	HI	2.98	>30.00	105.71	35	<4
SATE-ddhA Compound 1	MOD	12.89	26.79	55.82	4	2
SATE-ddhI Compound 3	MOD	18.75	81.93	>150.00	>8	>2
SATE-ddhdeoxyU Compound 5	NA	79.81	>150.00	>150.00	>2	1

CDV - Cidofovir - positive control, TNV - Tenofovir - negative control, NA (Not Active) SI_{50} = 0

- 3.9, MOD (Moderately Active) SI_{50} = 4 - 9.9, HI (Highly Active) SI_{50} = ≥10.

[00447] Assay against BK virus (BKV)

	Strain	Cell Line	Incubation	Assay Name
BKV	Gardner	HFF	7 d	Quantitative Polymerase Chain Reaction *(DNA)*/ CellTiter-Glo *(Toxicity)*

TABLE 16: Effect of ddh- and ddh-deoxy-compounds in cells infected with BKV

Drug	Activity	EC_{50}	EC_{90}	CC_{50}	SI_{50}	SI_{90}
CDV	-	4.30	24.05	>150.00	>35	>6
TNV	-	>150.00	>150.00	>150.00	1	1
SATE-ddhU Compound 4	LO	21.28	>150.00	>150.00	>7	1
SATE-ddhC Compound 6	NA	96.04	>150.00	>150.00	>2	1
SATE-ddhG Compound 2	HI	5.32	5.86	>150.00	>28	>26
SATE-ddhA Compound 1	NA	>30.00	>30.00	89.65	<3	<3
SATE-ddhI Compound 3	NA	115.28	>150.00	>150.00	>1	1
SATE-ddhdeoxyU Compound 5	NA	111.30	>150.00	>150.00	>1	1

CDV - Cidofovir - positive control, TNV - Tenofovir - negative control, NA (Not Active) SI_{50} = 0 - 3.9, LO (Slightly Active) SI_{50}=4-9.9, MOD (Moderately Active) SI_{50} = 10 - 19.9, HI (Highly Active) SI_{50} = ≥20.

[00448] Assay against JC virus (JCV)

	Strain	Cell Line	Incubation	Assay Name
JCV	MAD-4	COS7	7 d	Quantitative Polymerase Chain Reaction *(DNA)*/ CellTiter-Glo *(Toxicity)*

TABLE 17: Effect of ddh- and ddh-deoxy-compounds in cells infected with JCV

Drug	Activity	EC_{50}	EC_{90}	CC_{50}	SI_{50}	SI_{90}
CDV	-	5.56	117.63	>150.00	>27	>1
TNV	-	>150.00	>150.00	>150.00	1	1
SATE-ddhU Compound 4	NA	116.01	>150.00	>150.00	>1	1
SATE-ddhC Compound 6	NA	>150.00	>150.00	>150.00	1	1
SATE-ddhG Compound 2	NA	>150.00	>150.00	>150.00	1	1
SATE-ddhA Compound 1	NA	>30.00	>30.00	89.65	<3	<3
SATE-ddhI Compound 3	NA	>150.00	>150.00	>150.00	1	1
SATE-ddhdeoxyU Compound 5	NA	116.53	>150.00	>150.00	>1	1

CDV - Cidofovir - positive control, TNV - Tenofovir - negative control, NA (Not Active) $SI_{50} = 0$ - 3.9, LO (Slightly Active) SI_{50}=4-9.9, MOD (Moderately Active) SI_{50} = 10 - 19.9, HI (Highly Active) $SI_{50} = \geq 20$.

[00449] *Summary*: SATE-ddhA (Compound 1) exhibits cytotoxicity in all cell lines; SATE-ddhG (Compound 2) was toxic in HFF cells (skin fibroblasts) in HCMV assay (14 d); SATE-ddhU (Compound 4) and SATE-ddhC (Compound 6) showed toxicity in Akata cells (lymphocyte cell line) in addition to the previously mentioned compounds. Although it was only a 3-day assay, there is typically more cytotoxicity with the Akata cell line.

[00450] None of the compounds showed antiviral efficacy in the HSV-1, HCMV or JCV assays. SATE-ddhC (Compound 6), SATE-ddhA (Compound 1), and SATE-ddhI (Compound 3) were

moderately active against EBV. SATE-ddhG was highly active against both EBV and BK virus.

Example 13 – *In Vitro* Antiviral Activity against Influenza Viruses

[00451] *Objective*: The objective of this study was to test ddh- and ddh-deoxy-compounds against influenza viruses including SARS-CoV-2, MERS-CoV, Influenza(H1N1)pdm09, Influenza B, RSV, A2, Enterovirus-68, Tacaribe virus, and Dengue virus.

[00452] *Methods*:

[00453] Compounds tested included SATE-ddhU (Compound 4), SATE-ddhC (Compound 6), SATE-ddhG (Compound 2), SATE-ddhA (Compound 1), and SATE-ddhI (Compound 3). These compounds were tested against a panel of viruses including SARS-CoV-2 (USA-WA1/2020), MERSCoV (EMC), influenza/California/07/2009 (H1N1)pdm09, Influenza B/Florida/4/2006 (Yamagata), enterovirus-68 (EV-68, US/DY/14-18953), respiratory syncytial virus (RSV, A2), Tacaribe virus (TCRV, TRVL-11573), and dengue virus-2 (DENV-2, New Guinea C). For SARS-CoV-2 and MERS-CoV, Vero 76 cells were used, and test media was MEM supplemented with 2% FBS and 50 μg/mL gentamicin. MDCK cells were used for influenza viruses and test media was MEM supplemented with 10 IU/mL trypsin, 1 μg/mL EDTA, and gentamicin. MA-104 cells were used for RSV and test media was MEM with 5% FBS and gentamicin. RD cells were used for EV68 and test media was MEM with 2% FBS, 25 μg/mL MgCl2, and gentamicin. Vero cells were used for TCRV and test media was MEM with 2% FBS and gentamicin. Huh7 cells were used for DENV and test media was MEM with 5% FBS and gentamicin.

[00454] SATE-ddhU (Compound 4), SATE-ddhC (Compound 6), SATE-ddhG (Compound 2), SATE-ddhA (Compound 1), and SATE-ddhI (Compound 3) were in powder form. Compounds were solubilized in DMSO to prepare 200 mM stock solutions. Compounds were then serially diluted using eight half-log dilutions in test medium so that the starting (high) test concentration was 100 μM for EV-68 and MERS-CoV and 1000 μM for all other viruses.

[00455] Each dilution was added to 5 wells of a 96-well plate with 60-100% confluent cells. Three wells of each dilution were infected with virus, and two wells remained uninfected as toxicity controls. Six wells were infected and untreated as virus controls, and six wells were uninfected and untreated as cell controls. Viruses were prepared to achieve the lowest possible multiplicity of infection (MOI) that would yield >80% cytopathic effect (CPE) within 3-6 days. A positive control compound was tested in parallel for each virus tested.

[00456] Plates were incubated at 37±2°C and 5% CO_2 for all viruses except EV-68, which was

incubated at 33±2°C and 5% CO_2.

[00457] On day 3-6 post-infection, once untreated virus control wells reached maximum CPE, plates were stained with neutral red dye for approximately 2 hours (±15 minutes). Supernatant dye was removed and wells rinsed with PBS, and the incorporated dye was extracted in 50:50 Sorensen citrate buffer/ethanol for >30 minutes and the optical density was read on a spectrophotometer at 540 nm. Optical densities were converted to percent of cell controls and normalized to the virus control, then the concentration of test compound required to inhibit CPE by 50% (EC_{50}) was calculated by regression analysis. The concentration of compound that would cause 50% cell death in the absence of virus was similarly calculated (CC_{50}). The selective index (SI) is the CC_{50} divided by EC_{50}.

[00458] *Results*:

[00459] *In vitro* antiviral results are shown in Tables 18-22. SATE-ddhU (Compound 4) was slightly active against SARS-CoV-2 (SI=3.5). SATE-ddhU (Compound 4), SATE-ddhC (Compound 6), SATE-ddhG (Compound 2), SATE-ddhA (Compound 1), SATE-ddhI (Compound 3) were not active against the other viruses tested. All compounds formed precipitate at 1000 μM when diluted in test media, and SATE-ddhG (Compound 2) also formed precipitate at 320 μM. The positive control compounds performed as expected (Table 23).

TABLE 18: *In vitro* antiviral activity of SATE-ddhU (Compound 4), **against a panel of viruses**

Virus	Positive Control	SATE-ddhU EC50	CC50	SI50
SARS-CoV-2	Z-LEU-GLN(NME2)-FMK	22	76	3.5
MERS-CoV	Z-LEU-GLN(NME2)-FMK	>100	>100	0
Influenza(H1N1)	Ribavirin	>560	560	0
Influenza B	Ribavirin	>560	560	0
EV-68	Enviroxime	>100	>100	0
RSV	Ribavirin	>180	180	0
TCRV	Ribavirin	>170	170	0
DENV-2	Infergen	>160	160	0

Units are in gM
EC50: 50% effective antiviral concentration
CC50: 50% cytotoxic concentration of compound without virus added
SI = CC50/EC50

TABLE 19: *In vitro* antiviral activity of SATE-ddhC (Compound 6), **against a panel of viruses**

Virus	Positive Control	SATE-ddhC EC50	CC50	SI
SARS-CoV-2	Z-LEU-GLN(NME2)-FMK	>410	410	0
MERS-CoV	Z-LEU-GLN(NME2)-FMK	>100	>100	0
Influenza(H1N1)	Ribavirin	>550	550	0
Influenza B	Ribavirin	>1000	>1000	0

EV-68	Enviroxime	>100	>100	0
RSV	Ribavirin	>540	540	0
TCRV	Ribavirin	>210	210	0
DENV-2	Infergen	>71	71	0

Units are in gM
EC50: 50% effective antiviral concentration
CC50: 50% cytotoxic concentration of compound without virus added
SI = CC50/EC50

TABLE 20: ***In vitro*** **antiviral activity of** SATE-ddhG Compound 2 **against a panel of viruses**

Virus	Positive Control	SATE-ddhG EC50	CC50	SI
SARS-CoV-2	Z-LEU-GLN(NME2)-FMK	>1000	>1000	0
MERS-CoV	Z-LEU-GLN(NME2)-FMK	>100	>100	0
Influenza(H1N1)	Ribavirin	>1000	>1000	0
Influenza B	Ribavirin	>1000	>1000	0
EV-68	Enviroxime	>83	83	0
RSV	Ribavirin	>1000	>1000	0
TCRV	Ribavirin	>610	610	0
DENV-2	Infergen	>150	150	0

Units are in gM
EC50: 50% effective antiviral concentration
CC50: 50% cytotoxic concentration of compound without virus added
SI = CC50/EC50

TABLE 21: ***In vitro*** **antiviral activity of** SATE-ddhA Compound 1 **against a panel of viruses**

Virus	Positive Control	SATE-ddhA EC50	CC50	SI50
SARS-CoV-2	Z-LEU-GLN(NME2)-FMK	>49	49	0
MERS-CoV	Z-LEU-GLN(NME2)-FMK	>100	>100	0
Influenza(H1N1)	Ribavirin	>180	180	0
Influenza B	Ribavirin	>180	180	0
EV-68	Enviroxime	>2	2	0
RSV	Ribavirin	>170	170	0
TCRV	Ribavirin	>58	58	0
DENV-2	Infergen	>14	14	0

Units are in gM
EC50: 50% effective antiviral concentration
CC50: 50% cytotoxic concentration of compound without virus added
SI = CC50/EC50

TABLE 22: ***In vitro*** **antiviral activity of** SATE-ddhI Compound 3 **against a panel of viruses**

Virus	Positive Control	SATE-ddhI EC50	CC50	SI50
SARS-CoV-2	Z-LEU-GLN(NME2)-FMK	>530	530	0
MERS-CoV	Z-LEU-GLN(NME2)-FMK	>100	>100	0
Influenza(H1N1)	Ribavirin	>1000	>1000	0

Influenza B	Ribavirin	>1000	>1000	0
EV-68	Enviroxime	>100	>100	0
RSV	Ribavirin	>1000	>1000	0
TCRV	Ribavirin	>900	900	0
DENV-2	Infergen	>150	150	0

Units are in gM for test compounds; gg/mL Ribavirin, Z-LEU-GLN(NME2)-FMK, and Enviroxime; and ng/mL for Infergen
EC50: 50% effective antiviral concentration
CC50: 50% cytotoxic concentration of compound without virus added
SI = CC50/EC50

TABLE 23: *In vitro* antiviral activity of positive controls against a panel of viruses

Virus	Positive Control	Positive Control EC50	CC50	SI50
SARS-CoV-2	Z-LEU-GLN(NME2)-FMK	0.089	>100	>1100
MERS-CoV	Z-LEU-GLN(NME2)-FMK	8	>100	>13
Influenza(H1N1)	Ribavirin	7.6	>1000	>130
Influenza B	Ribavirin	7.7	>1000	>130
EV-68	Enviroxime	0.12	5.5	46
RSV	Ribavirin	19	310	16
TCRV	Ribavirin	5.3	>1000	>190
DENV-2	Infergen	0.045	>10	>220

Units are jug/mL Ribavirin, Z-LEU-GLN(NME2)-FMK, and Enviroxime; and ng/mL for Infergen
EC50: 50% effective antiviral concentration
CC50: 50% cytotoxic concentration of compound without virus added
SI = CC50/EC50

Example 14 – *In Vitro* Antiviral Activity against SARS-CoV-2

[00460] *Objective*: The objective of this study was to test ddh-compounds SATE-ddhU (Compound 4) and SATE-ddhC (Compound 6) against SARS-CoV-2.

[00461] *Methods*:

[00462] SARS-CoV-2 (USA-WA1/2020) stocks were prepared by growing virus in Vero 76 cells. Test media was MEM supplemented with 2% FBS and 50 μg/mL gentamicin.

[00463] SATE-ddhU (Compound 4) and SATE-ddhC (Compound 6) were in powder form. The compounds were solubilized in DMSO to prepare a 200 mM stock solution. The compounds were then serially diluted using eight half-log dilutions in test medium so that the starting (high) test concentration was 100 μM. Each dilution was added to 5 wells of a 96-well plate with 80-100% confluent cells. Three wells of each dilution were infected with virus, and two wells remained uninfected as toxicity controls. Six wells were infected and untreated as virus controls, and six wells were uninfected and untreated as cell controls. SARS-CoV-2 was prepared to achieve the

lowest possible multiplicity of infection (MOI) that would yield >80% cytopathic effect (CPE) within 5 days. A positive control compound was tested in parallel for each virus tested. Plates were incubated at 37±2°C and 5% CO_2.

[00464] The test was set up as described above in two replicates. For one test, all media was removed from plates and replaced with fresh compound on day 1 and 2 post-infection (p.i.). The plates were stained on day 3 p.i. as described below. For the second test, media was not replaced daily, but the plate was stained and virus yield reduction (VYR) assay was performed as described below on day 2 p.i.

[00465] On day 3 p.i., once untreated virus control wells reached maximum CPE, plates were stained with neutral red dye for approximately 2 hours (±15 minutes). Supernatant dye was removed and wells rinsed with PBS, and the incorporated dye was extracted in 50:50 Sorensen citrate buffer/ethanol for >30 minutes and the optical density was read on a spectrophotometer at 540 nm. Optical densities were converted to percent of cell controls and normalized to the virus control, then the concentration of test compound required to inhibit CPE by 50% (EC_{50}) was calculated by regression analysis. The concentration of compound that would cause 50% cell death in the absence of virus was similarly calculated (CC_{50}). The selective index (SI) is the CC_{50} divided by EC_{50}.

[00466] For VYR, the supernatant fluid from each compound concentration was collected on day 2 p.i., before neutral red staining (3 wells pooled) and tested for virus titer using a standard endpoint dilution CCID50 assay and titer calculations using the Reed-Muench (1948) equation. The concentration of compound required to reduce virus yield by 1 log10 (EC90) was calculated by regression analysis.

[00467] *Results*:

[00468] *In vitro* antiviral results are shown in Tables 24-25. SATE-ddhU (Compound 4) was slightly active against SARS-CoV- 2 when media was replaced with fresh compound daily (SI>6.7, Table 24). SATE-ddhU did not inhibit SARS-CoV-2 replication on day 2 when assayed for virus yield reduction (SI=0, Table 25).

[00469] The positive control compound performed as expected.

TABLE 24: *In vitro* antiviral activity of SATE-ddhU against SARS-CoV-2 when compound was replenished daily.

Compound	EC50	CC50	SI50
SATE-ddhU	15	>100	>6.7
Z-LEU-GLN(NME2)-FMK	0.38	>100	>260

Units are in pM for test compound and pg/mL for Z-LEU-GLN(NME2)-FMK
EC50: 50% effective antiviral concentration
CC50: 50% cytotoxic concentration of compound without virus added SI = CC50/EC50

TABLE 25: *In vitro* antiviral activity of SATE-ddhC against SARS-CoV-2 when compound was replenished daily.

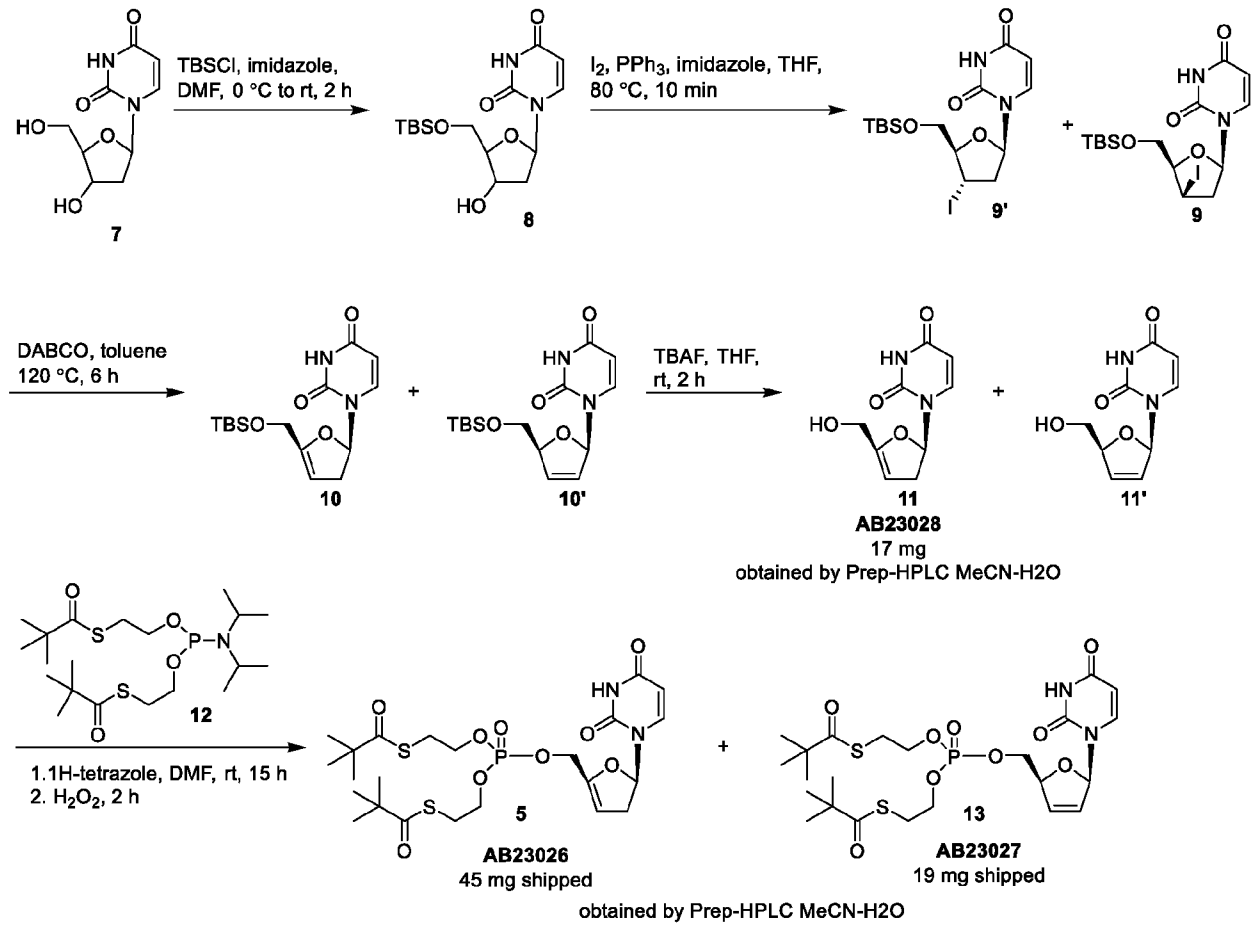
Compound	EC90	CC50	SI90
SATE-ddhC Compound 6	>100	>100	0
Z-LEU-GLN(NME2)-FMK	0.15	>100	>680

Units are in gM for test compound and gg/mL for Z-LEU-GLN(NME2)-FMK
EC50: 50% effective antiviral concentration
CC50: 50% cytotoxic concentration of compound without virus added
EC90: Calculated concentration to reduce virus yield by 1 log (90%)
SI = CC50/EC50

Example 15 – Experimental Procedures for the Synthesis of Compounds

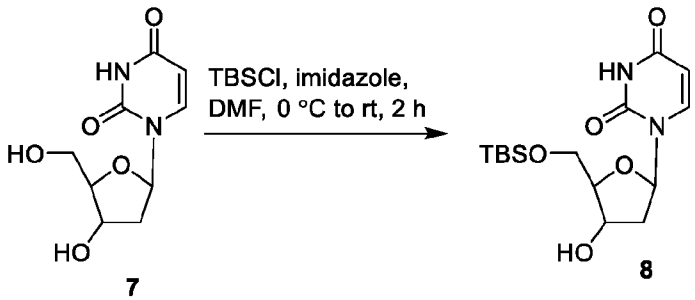
[00470] *Objective*: To synthesize embodiments of prodrug compounds disclosed herein.

[00471] **Synthetic Approach for Compounds 5, 13 and 11**



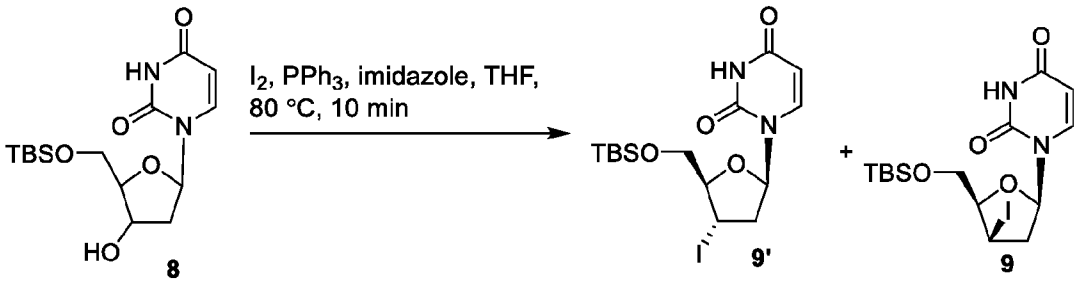
[00472] **Procedure**

[00473] ***General Procedure for the Preparation of Compound 8***



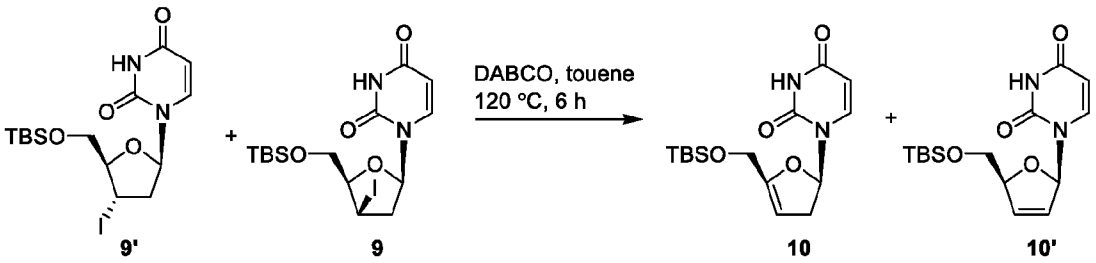
[00474] To a solution of compound **7** (10.0 g, 43.8 mmol, 1.0 eq) in dry DMF (100 mL) at 0 °C was added imidazole (14.9 g, 219 mmol, 5.0 eq) and TBSCl (9.9 g, 65.7 mmol, 1.5 eq) sequentially. The mixture was allowed to warm to room temperature. After being stirred at room temperature for 2 h, the mixture was poured into water and extracted with EtOAc. After evaporation of the solvent, the residue was purified by flash column chromatography (dichloromethane/methanol, 10/1) to afford **8** (14.5 g, 97%) as a white solid.

[00475] ***General Procedure for the Preparation of Compound 9 and 9′***



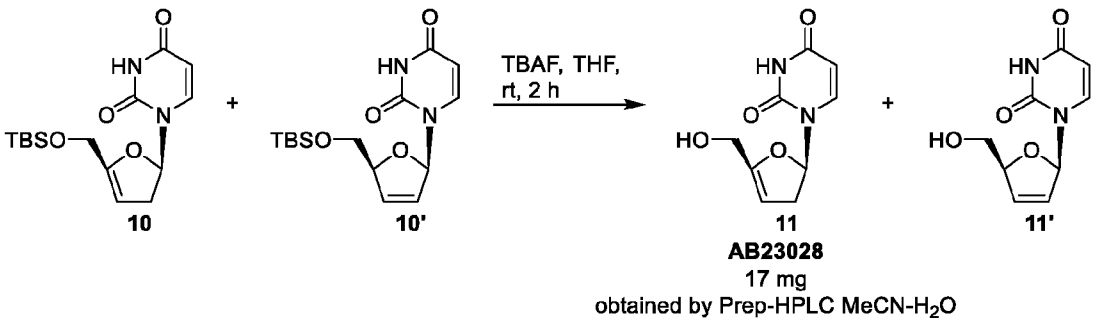
[00476] To a solution of compound **8** (5.4 g, 15.8 mmol, 1.0 eq) in dry THF (100 mL) at room temperature was added imidazole (2.15 g, 31.6 mmol, 2.0 eq), PPh_3 (6.2 g, 23.7 mmol, 1.5 eq) and I_2 (6.0 g, 23.7 mmol, 1.5 eq) sequentially. Then the mixture was heated to 80 °C. After being stirred at 80 °C for 10 min, the reaction mixture was cooled to room temperature, quenched with saturated aqueous $Na_2S_2O_3$ and extracted with EtOAc. The organic layer was further washed with saturated aqueous Na_2HCO_3 and water. After evaporation of the solvent, the residue was purified by flash column chromatography (petroleum ether/EtOAc, 1/1) to afford **9** and **9′** (3.29 g) as white foams.

[00477] ***General Procedure for the Preparation of Compound 10 and 10′***



[00478] A solution of compound **9** and **9′** (3.23 g, 7.14 mmol, 1.0 eq) and DABCO (3.20 g, 28.6 mmol, 4.0 eq) in toluene (40 mL) was stirred at 120 °C for 6 h. Then the mixture was heated to 80 °C. After being stirred at 80 °C for 10 min, the reaction mixture was cooled to room temperature, quenched with saturated aqueous NH_4Cl and extracted with EtOAc. The organic layer was further washed with saturated aqueous $NaHCO_3$ and water. After evaporation of the solvent, the residue was purified by flash column chromatography (petroleum ether/EtOAc, 1/1) to afford and (1.61 g, **10/10′** = 2:1 determined by H-NMR) as white foams.

[00479] ***General Procedure for the Preparation of Compound 11 and 11′***



[00480] A solution of compound **10** and **10′** (3.2 g, 9.86 mmol, 1.0 eq) and TBAF (1.0 M, 19.7 mL, 2.0 eq) in dry THF (20 mL) was stirred at room temperature for 2 h. Then the reaction mixture was poured into water, extracted with EtOAc, and concentrated. After a crude purification with flash column chromatography, the residue was further purified by Biotage to afford crude **11** and **11′** (2.38 g) as white foams. The crude product (**11** and **11′**, 0.40 g) was further purified on reverse phase HPLC (MeCN-H_2O) to afford **11** (17 mg) as a white solid.

TLC: Dichloromethane/Methanol, 10/1

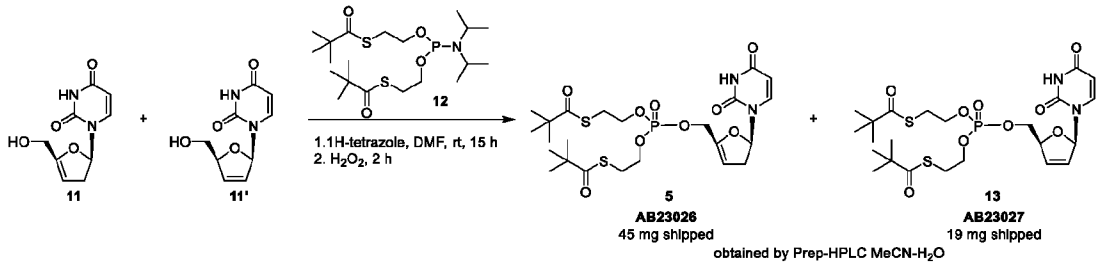
R_f (Compound **10/10′**) = 0.95

Rf (Product **11/11′**) = 0.5

LC-MS: 233.00 $[M+Na]^+$

^{1}H NMR (400 MHz, D_2O) δ 7.62 (d, *J* = 8.1 Hz, 1H), 6.67 (d, *J* = 9.4 Hz, 1H), 5.87 (d, *J* = 7.9 Hz, 1H), 5.21 (s, 1H), 4. 30–4.12 (m, 2H), 3.28 (dd, *J* = 17.3, 9.6 Hz, 1H), 2.76 (d, *J* = 17.7 Hz, 1H).

[00481] ***General Procedure for the Preparation of Compound 5 and 13***



[00482] To a solution of alcohols **11** and **11′** (0.40 g, 1.90 mmol, 1.0 eq) in dry DMF (20 mL) was added 1H-tetrazole (0.27 g, 3.80 mmol, 2.0 eq) and **12** (1.72 g, 3.80 mmol, 2.0 eq) sequentially. The mixture was stirred at room temperature for 15 h. The reaction was cooled to 0 ºC and hydrogen peroxide (30% in water, 1.91 mL, 19.0 mmol, 10.0 eq) was added. After stirring for 0.5 h, the mixture was purified by Prep-HPLC to afford **5** (45 mg, 4%) and **13** (19 mg, 1.7%) as white solids.

TLC: Dichloromethane/Methanol, 20/1

Rf (Compound **11/11′**) = 0.1

Rf (Product **5/13**) = 0.5

5:

LC-MS: 579.10$[M+H]^+$

^{1}H NMR (400 MHz, $CDCl_3$) δ 9.20 (s, 1H), 7.34 (d, *J* = 7.4 Hz, 1H), 6.74 (d, *J* = 6.3 Hz, 1H), 5.78 (d, *J* = 7.4 Hz, 1H), 5.23 (s, 1H), 4.64 (br, 2H), 4.10 (d, *J* = 5.7 Hz, 4H), 3.30 (dd, *J* = 16.0, 9.9 Hz, 1H), 3.12 (br, 4H), 2.65 (d, *J* = 17.2 Hz, 1H), 1.22 (s, 18H).

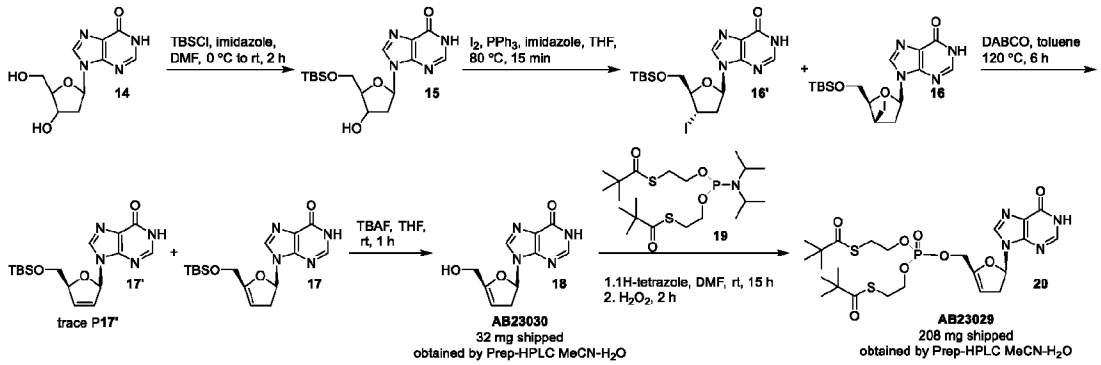
^{31}P NMR (162 MHz, $CDCl_3$) δ -1.78.

13: LC-MS: $[M+H]^+$ 579.10

^{1}H NMR (399 MHz, $CDCl_3$) δ 8.45 (s, 1H), 7.51 (d, *J* = 8.0 Hz, 1H), 7.02 (s, 1H), 6.37 (d, *J* = 5.1 Hz, 1H), 5.92 (d, *J* = 4.6 Hz, 1H), 5.75 (d, *J* = 8.0 Hz, 1H), 5.01 (s, 1H), 4.26 (br, 2H), 4.09 (dd, *J* = 13.6, 6.6 Hz, 4H), 3.12 (br, 4H), 1.23 (s, 18H).

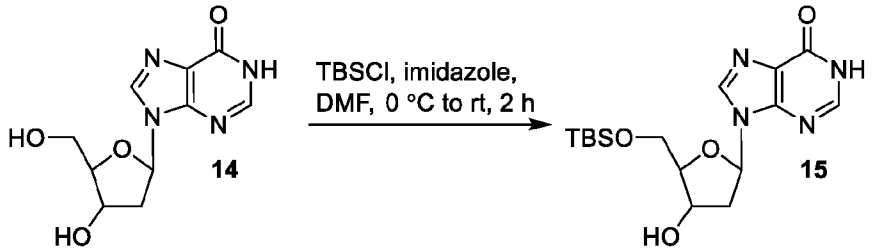
^{31}P NMR (162 MHz, $CDCl_3$) δ -1.60.

[00483] **Synthetic Approach for Compounds 20 and 18**



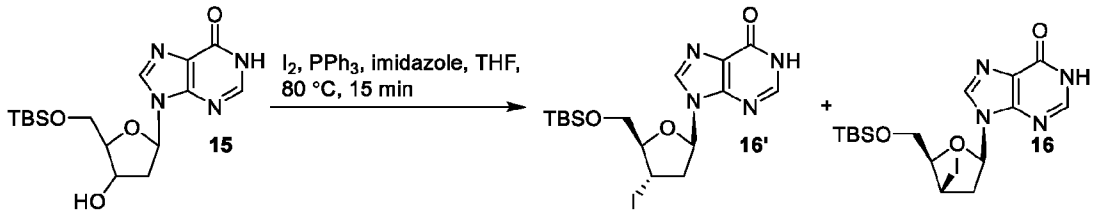
[00484] **Procedure**

[00485] ***General Procedure for the Preparation of Compound 15***



[00486] To a solution of compound **14** (10.0 g, 39.6 mmol, 1.0 eq) in dry DMF (100 mL) at 0 °C was added imidazole (13.5 g, 198 mmol, 5.0 eq) and TBSCl (8.96 g, 59.4 mmol, 1.5 eq) sequentially. The mixture was allowed to warm to room temperature. After being stirred at room temperature for 2 h, the mixture was poured into water and extracted with EtOAc. After evaporation of the solvent, the residue was purified by flash column chromatography (dichloromethane/methanol, 10/1) to afford **15** (12.8 g, 88%) as a white solid.

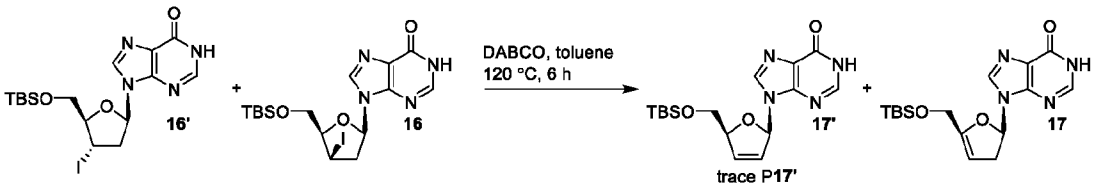
[00487] ***General Procedure for the Preparation of Compound 16 and 16 ′***



[00488] To a solution of compound **15** (7.58 g, 20.7 mmol, 1.0 eq) in dry THF (100 mL) at room temperature was added imidazole (2.82 g, 41.4 mmol, 2.0 eq), PPh_3 (8.16 g, 31.1 mmol, 1.5 eq) and I_2 (7.89 g, 31.1 mmol, 1.5 eq) sequentially. Then the mixture was heated to 80 °C. After being stirred at 80 °C for 15 min, the reaction mixture was cooled to room temperature, quenched with saturated aqueous $Na_2S_2O_3$ and extracted with EtOAc. The organic layer was further washed with saturated aqueous Na_2HCO_3 and water. After evaporation of the solvent, the residue was purified

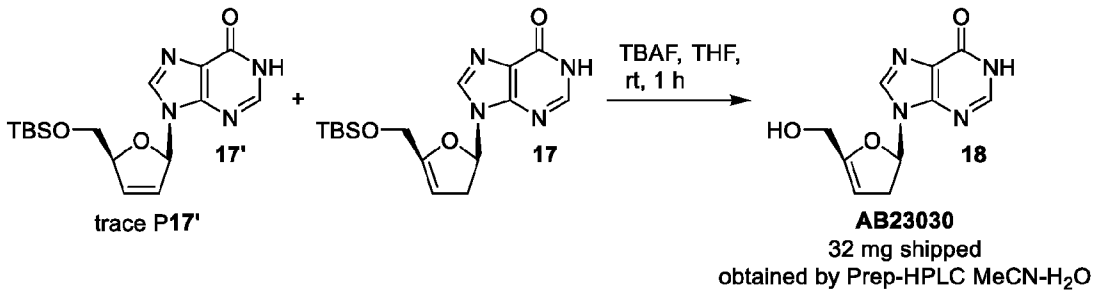
by flash column chromatography (petroleum ether/EtOAc, 1/1) to afford **16** and **16′** (5.97 g) as white foams.

[00489] ***General Procedure for the Preparation of Compound 17 and 17′***



[00490] A solution of compound **16** and **16′** (5.97 g, 12.5 mmol, 1.0 eq) and DABCO (5.61 g, 50.0 mmol, 4.0 eq) in toluene (40 mL) was stirred at 120 °C for 6 h. Then the reaction mixture was cooled to room temperature, quenched with saturated aqueous NH_4Cl and extracted with EtOAc. The organic layer was further washed with saturated aqueous $NaHCO_3$ and water. After evaporation of the solvent, the residue was purified by flash column chromatography (petroleum ether/EtOAc, 1/1) to afford main product **17** and including trace product **17′** (2.38 g) as white foams.

[00491] ***General Procedure for the Preparation of Compound 18***



[00492] A solution of compound **17** (2.38 g, 6.82 mmol, 1.0 eq) and TBAF (1.0 M, 13.6 mL, 2.0 eq) in dry THF (20 mL) was stirred at room temperature for 1 h. Then the reaction mixture was poured into water, extracted with EtOAc, and concentrated. After a crude purification with flash column chromatography, the residue was further purified by Biotage to afford **18** (0.3 g, 18%) as a white solid. Another crude product **18** (0.30 g) was further purified on reverse phase HPLC (MeCN-H_2O) to afford **18** (32 mg) as a white solid.

TLC: Dichloromethane/Methanol, 10/1

R_f (Compound **17**) = 0.95

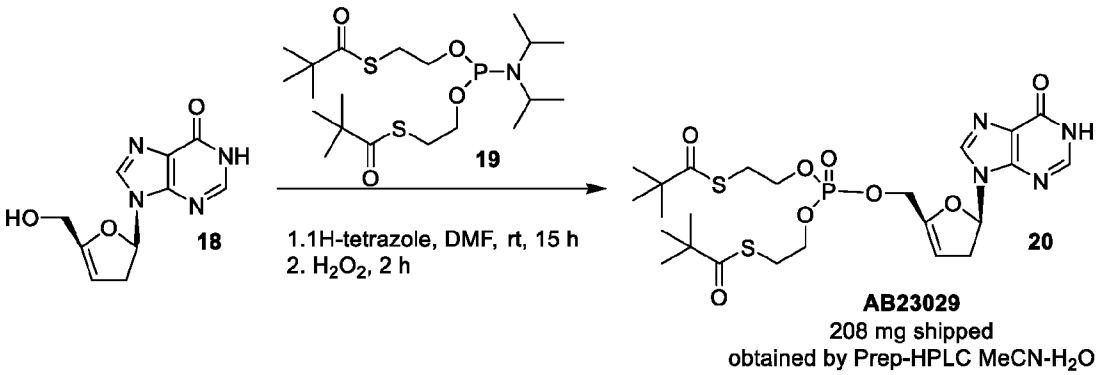
R_f (Product **18**) = 0.5

LC-MS: 235.15 $[M+H]^+$

^{1}H NMR (400 MHz, D_2O) δ 8.26 (s, 1H), 8.22 (s, 1H), 6.85 (d, J = 7.6 Hz, 1H), 5.34 (s, 1H),

4.20 (s, 2H), 3.43 (dd, J = 17.6, 9.0 Hz, 1H), 3.10 (d, J = 17.5 Hz, 1H).

[00493] ***General Procedure for the Preparation of Compound 20***



[00494] To a solution of alcohol **18** (0.30 g, 1.28 mmol, 1.0 eq) in dry DMF (20 mL) was added 1H-tetrazole (0.18 g, 2.56 mmol, 2.0 eq) and **19** (1.16 g, 2.56 mmol, 2.0 eq) sequentially. The mixture was stirred at room temperature for 15 h. The reaction was cooled to 0 °C and hydrogen peroxide (30% in water, 1.28 mL, 12.8 mmol, 10.0 eq) was added. After stirring for 0.5 h, the mixture was purified by Prep-HPLC to afford **20** (208 mg, 27%) as a white solid.

TLC: Dichloromethane/Methanol, 20/1

Rf (Compound **18**) = 0.1

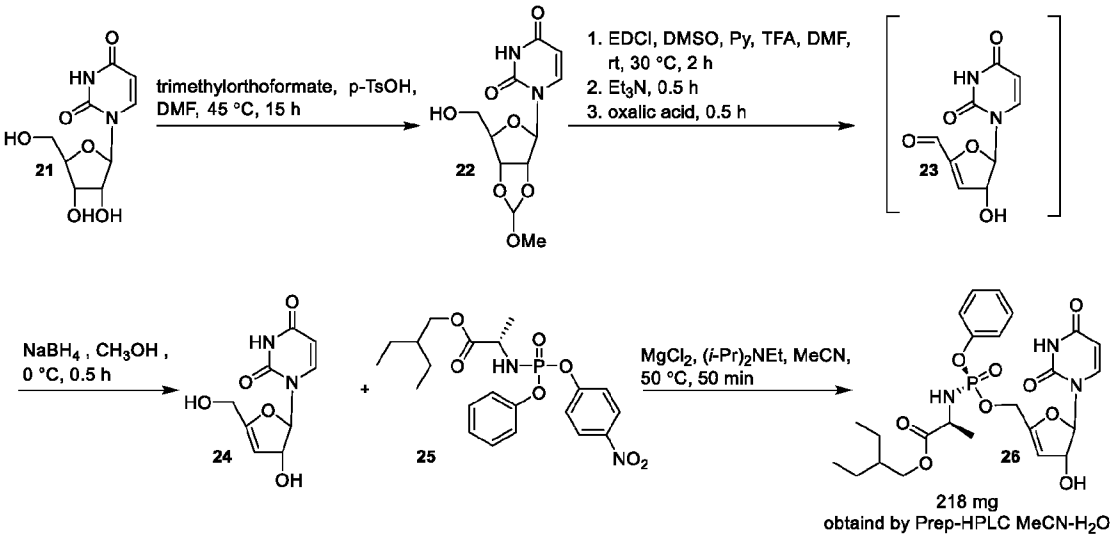
Rf (Product **20**) = 0.5

LC-MS: 603.15 $[M+H]^+$

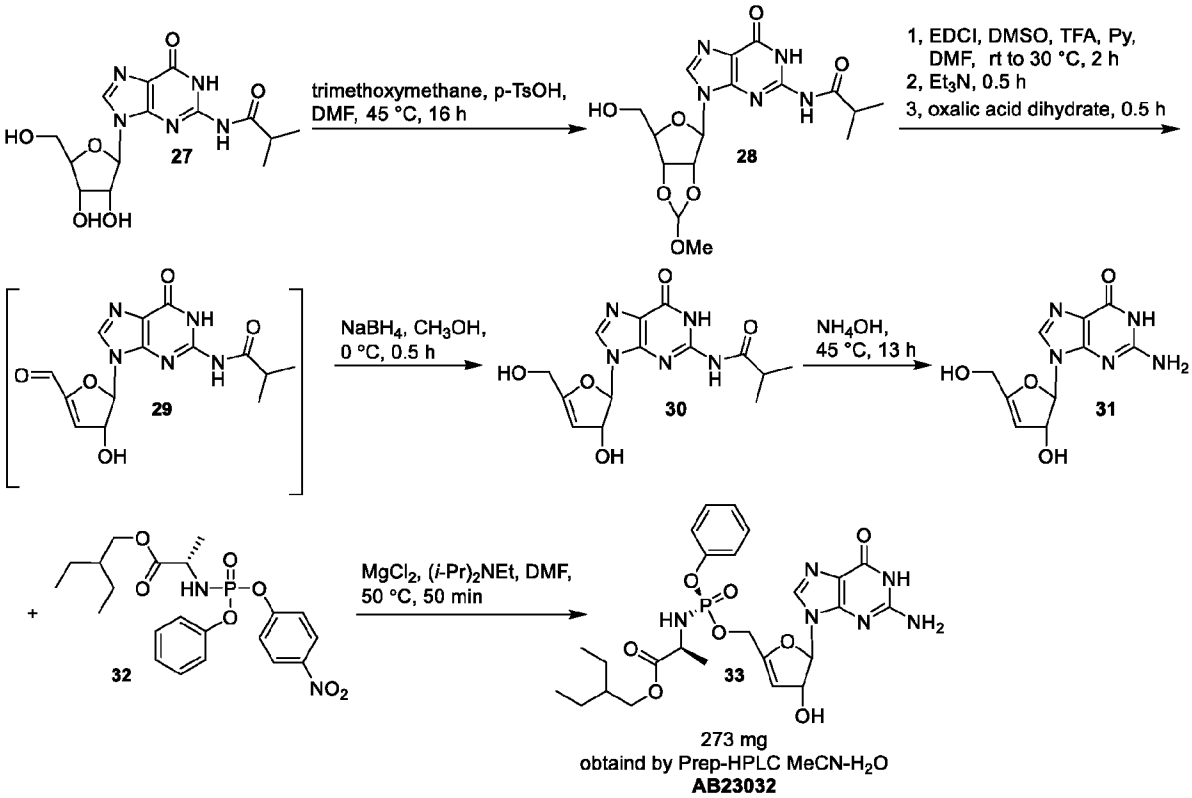
^{1}H NMR (400 MHz, $CDCl_3$) δ 12.38 (br, 1H), 8.09 (s, 1H), 8.00 (s, 1H), 6.82 (d, J = 5.4 Hz, 1H), 5.36 (s, 1H), 4.66 (d, J = 9.0 Hz, 2H), 4.23–3.99 (m, 4H), 3.44 (dd, J = 16.8, 8.7 Hz, 1H), 3.13 (q, J = 6.8 Hz, 4H), 3.05 (d, J = 17.9 Hz, 1H), 1.22 (s, 18H).

^{31}P NMR (162 MHz, $CDCl_3$) δ -1.74.

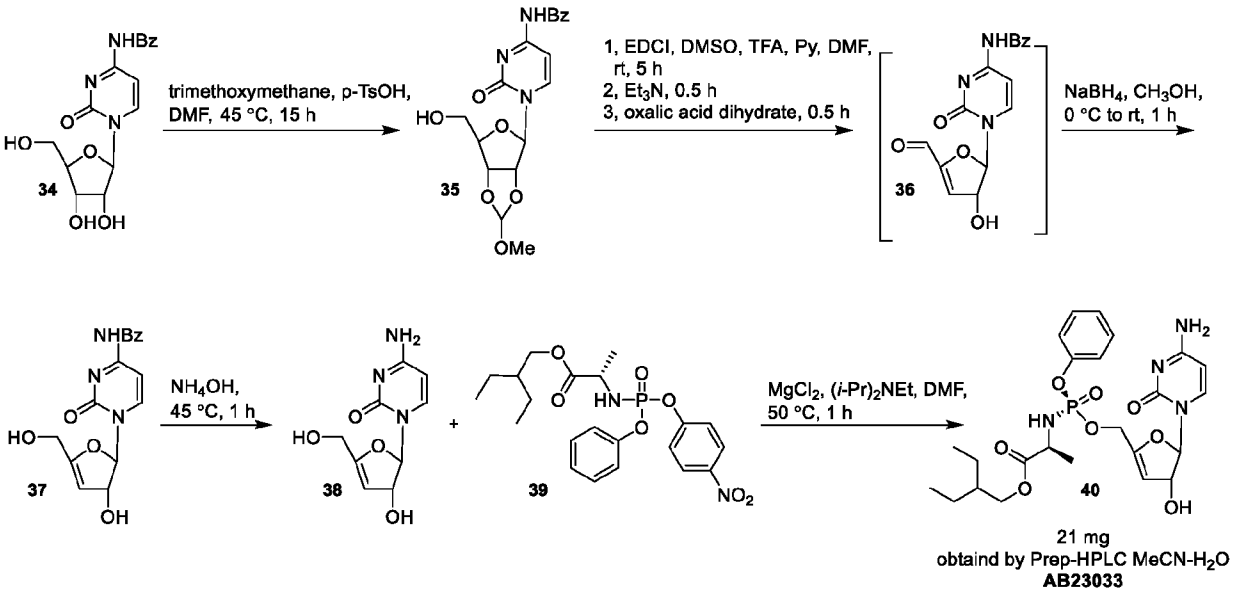
[00495] **Synthetic Approach for Compound 26**



[00496] **Synthetic Approach for Compound 33**

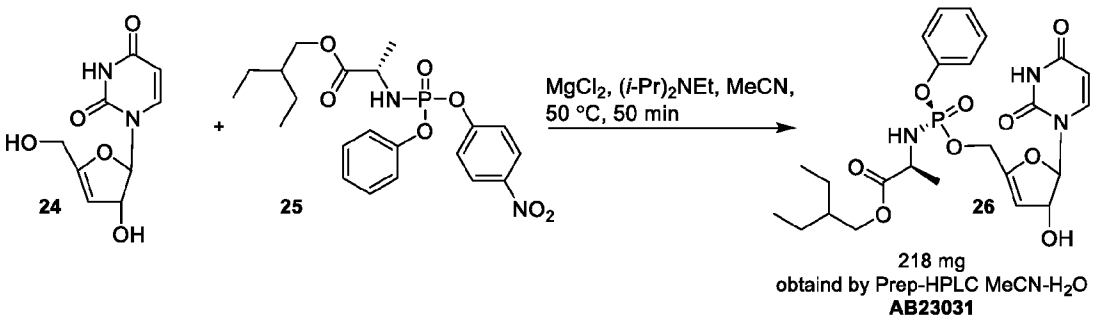


[00497] **Synthetic Approach for Compound 40**



[00498] **Procedure**

[00499] ***General Procedure for the Preparation of Compound 26***



[00500] To a solution of compound **24** (0.77 g, 3.40 mmol, 1.0 eq) in MeCN (60 mL) at room temperature was added **25** (1.84 g, 4.08 mmol, 1.2 eq) and $MgCl_2$ (0.32 g, 3.40 mmol, 1.0 eq) sequentially. Then the mixture was heated to 50 °C for 10 min, and *N,N*-diisopropylethylamine (1.40 mL, 8.50 mmol, 2.5 eq) was added. After being stirred at 50 °C for another 50 min, the reaction mixture was cooled to room temperature, quenched with diluted HCl (1 M) and extracted with EtOAc. The organic layer was further washed with saturated aqueous Na_2HCO_3 and water. After evaporation of the solvent, the residue was purified by flash column chromatography (dichloromethane/methanol, 20/1) and further purified by prep-HPLC (MeCN-H_2O) to afford **26** (218 mg, 12%) as a white solid.

TLC: Dichloromethane/Methanol, 20/1

R_f (Compound **24**) = 0.1

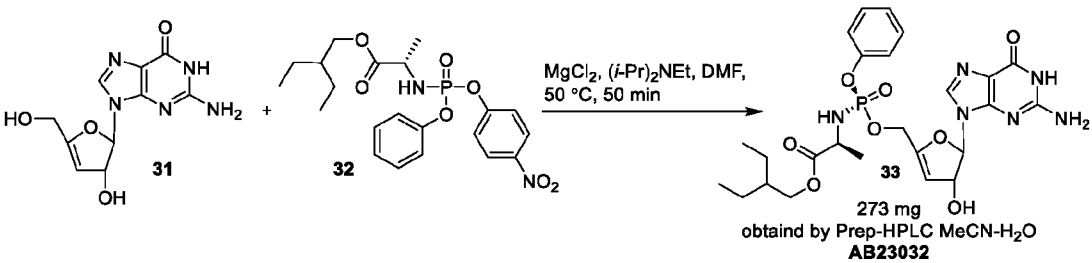
R_f (Product **26**) = 0.5

LC-MS: 538.15 $[M+H]^+$

^{1}H NMR (400 MHz, $CDCl_3$) δ 9.19 (br, 1H), 7.32 (d, *J* = 7.5 Hz, 2H), 7.19 (dd, *J* = 25.7, 8.0 Hz, 5H), 6.28 (s, 1H), 5.70 (d, *J* = 8.0 Hz, 1H), 5.34 (s, 1H), 4.89 (s, 1H), 4.70 (d, *J* = 8.2 Hz, 2H), 4.21–3.93 (m, 4H), 3.78 (s, 1H), 1.55–1.48 (m, 1H), 1.40–1.25 (m, 7H), 0.88 (t, *J* = 7.2 Hz, 6H).

^{31}P NMR (162 MHz, $CDCl_3$) δ 2.41.

[00501] ***General Procedure for the Preparation of Compound 33***



[00502] To a solution of compound **31** (0.65 g, 2.45 mmol, 1.0 eq) in DMF (20 mL) at room temperature was added **32** (1.32 g, 2.94 mmol, 1.2 eq) and $MgCl_2$ (0.23 g, 2.45 mmol, 1.0 eq) sequentially. Then the mixture was heated to 50 °C for 10 min, and *N,N*-diisopropylethylamine (1.01 mL, 6.13 mmol, 2.5 eq) was added. After being stirred at 50 °C for another 50 min, the reaction mixture was cooled to room temperature, quenched with diluted HCl (1 M) and extracted with EtOAc. The organic layer was further washed with saturated aqueous Na_2HCO_3 and water. After evaporation of the solvent, the residue was purified by flash column chromatography (dichloromethane/methanol, 20/1) and further purified by prep-HPLC (MeCN-H_2O) to afford **33** (273 mg, 19%) as a white solid.

TLC: Dichloromethane/Methanol, 20/1

R_f (Compound **31**) = 0.1

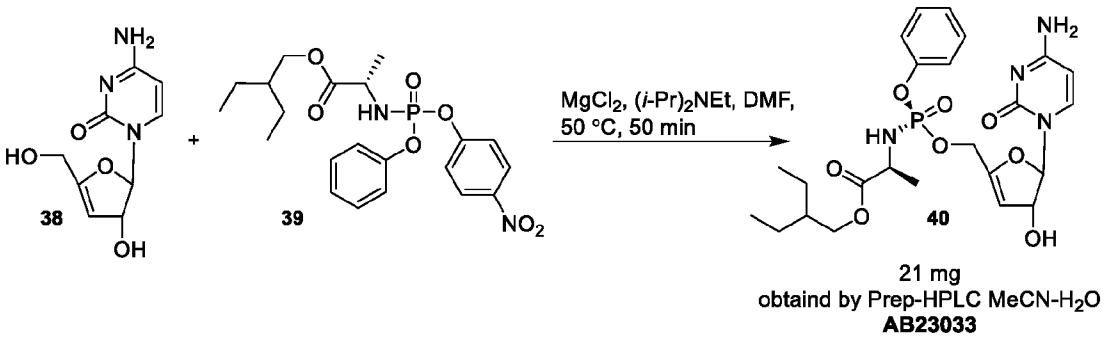
R_f (Product **33**) = 0.5

LC-MS: 577.15 $[M+H]^+$

^{1}H NMR (400 MHz, DMSO-*d6*) δ 10.69 (s, 1H), 7.66 (s, 1H), 7.33 (t, *J* = 7.3 Hz, 2H), 7.18–7.14 (m, 3H), 6.55 (s, 2H), 6.11–6.07 (m, 2H), 5.85 (d, *J* = 5.5 Hz, 1H), 5.41–5.38 (m, 1H), 5.11 (s, 1H), 4.59 (d, *J* = 6.9 Hz, 2H), 4.04–3.75 (m, 3H), 1.51–1.34 (m, 1H), 1.23 (q, *J* = 13.1 Hz, 7H), 0.80 (t, *J* = 7.2 Hz, 6H).

^{31}P NMR (162 MHz, DMSO-*d6*) δ 3.49.

[00503] ***General Procedure for the Preparation of Compound 40***



[00504] To a solution of compound **38** (0.51 g, 2.26 mmol, 1.0 eq) in DMF (20 mL) at room temperature was added **39** (1.22 g, 2.71 mmol, 1.2 eq) and $MgCl_2$ (0.22 g, 2.26 mmol, 1.0 eq) sequentially. Then the mixture was heated to 50 °C for 10 min, and *N,N*-diisopropylethylamine (0.93 mL, 5.65 mmol, 2.5 eq) was added. After being stirred at 50 °C for another 50 min, the reaction mixture was cooled to room temperature, quenched with diluted HCl (1 M) and extracted with EtOAc. The organic layer was further washed with saturated aqueous Na_2HCO_3 and brine. After evaporation of the solvent, the residue was purified by flash column chromatography

(dichloromethane/methanol, 20/1) and further purified by prep-HPLC (MeCN-H_2O) to afford **40** (21 mg, 1.7%) as a white solid.

TLC: Dichloromethane/Methanol, 20/1

R_f (Compound **38**) = 0.1

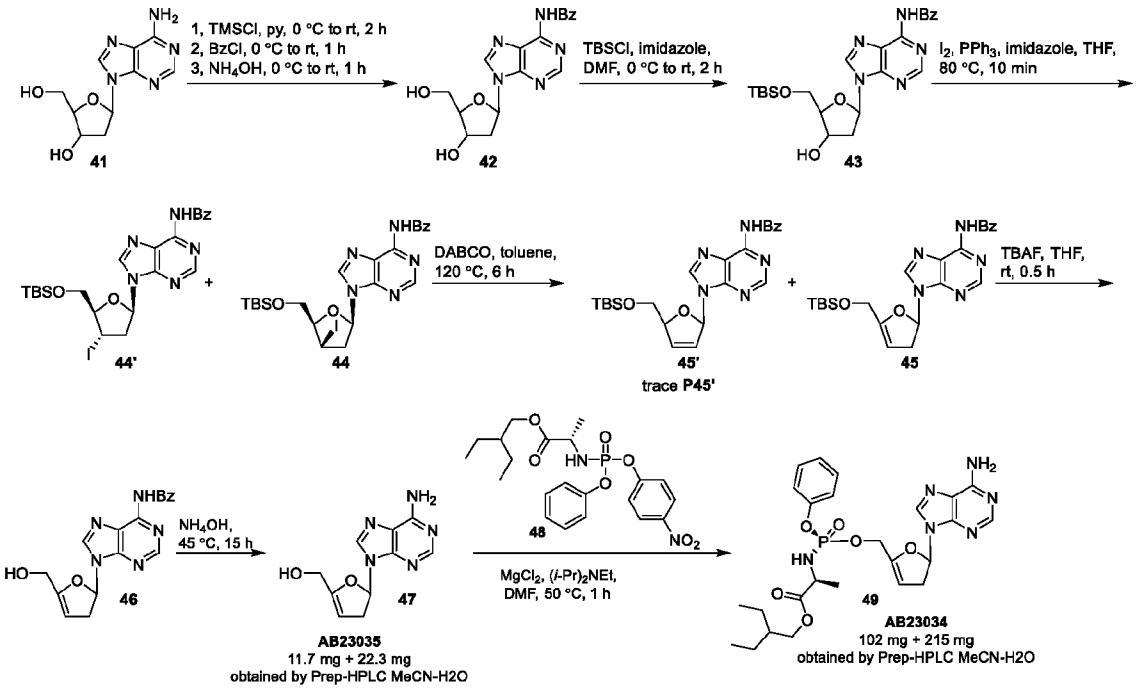
R_f (Product **40**) = 0.5

LC-MS: 537.15 $[M+H]^+$

^{1}H NMR (400 MHz, DMSO-*d6*) δ 7.36-7.34 (m, 3H), 7.21-7.20 (m, 5H), 6.21 (s, 1H), 6.15 – 6.09 (m, 1H), 5.72 (t, *J* = 7.1 Hz, 1H), 5.28 (s, 1H), 4.71 (s, 1H), 4.60 (d, *J* = 6.9 Hz, 2H), 4.07 – 3.76 (m, 3H), 1.44 (s, 1H), 1.24 (d, *J* = 6.8 Hz, 9H), 0.81 (t, *J* = 7.0 Hz, 6H).

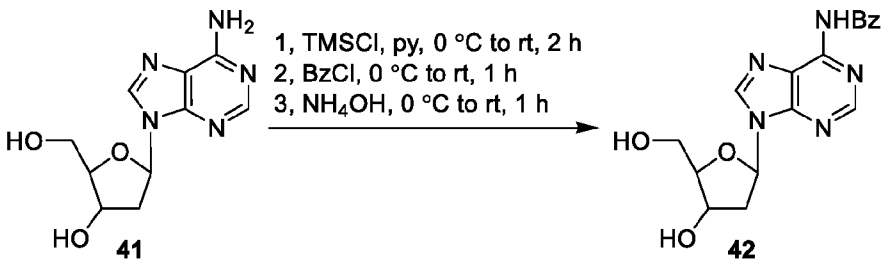
^{31}P NMR (162 MHz, DMSO-*d6*) δ 3.47.

[00505] **Synthetic Approach for Compounds 49 and 47**



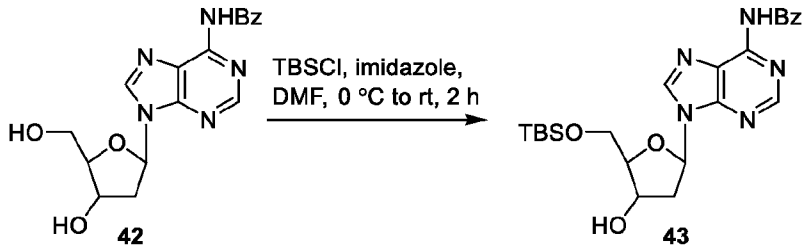
[00506] **Procedure**

[00507] ***General Procedure for the Preparation of Compound 42***



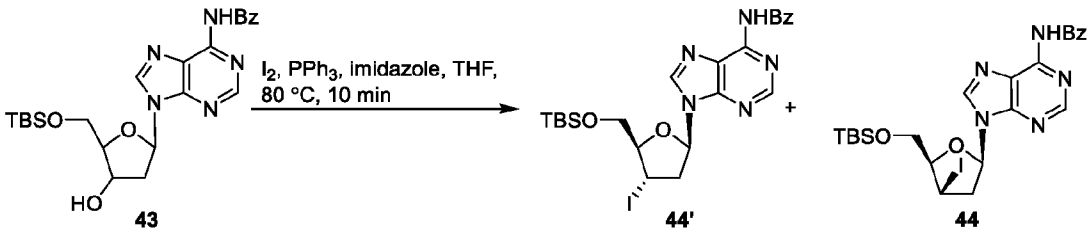
[00508] To a solution of compound **41** (11.0 g, 43.8 mmol, 1.0 eq) in pyridine (160 mL) at 0 °C was added TMSCl (27.8 mL, 219 mmol, 5.0 eq). The mixture was allowed to warm to room temperature. After being stirred at room temperature for 2 h, BzCl (5.56 mL, 48.2 mmol, 1.1 eq) was added to the reaction mixture at 0 °C. After being stirred at room temperature for another 1 h, H_2O (40 mL) and NH_4OH (80 mL) was added to the reaction mixture at 0 °C. After being stirred at room temperature for one more 1 h, the mixture was poured into water and extracted with EtOAc. After evaporation of the solvent, the residue was purified by flash column chromatography (dichloromethane/methanol, 9/1) to afford **42** (7.47 g, 48%) as a white solid.

[00509] ***General Procedure for the Preparation of Compound 43***



[00510] To a solution of compound **42** (6.78 g, 19.1 mmol, 1.0 eq) in dry DMF (100 mL) at 0 °C was added imidazole (6.50 g, 95.5 mmol, 5.0 eq) and TBSCl (4.32 g, 28.7 mmol, 1.5 eq) sequentially. The mixture was allowed to warm to room temperature. After being stirred at room temperature for 15 h, the mixture was poured into water and extracted with EtOAc. After evaporation of the solvent, the residue was purified by flash column chromatography (dichloromethane/methanol, 10/1) to afford **43** (7.17 g, 80%) as a white solid.

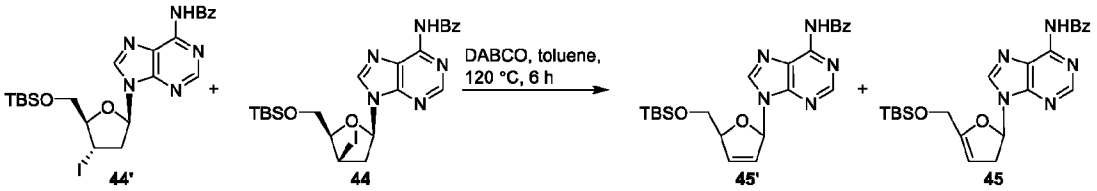
[00511] ***General Procedure for the Preparation of Compound 44 and 44′***



[00512] To a solution of compound **43** (5.18 g, 11.0 mmol, 1.0 eq) in dry THF (100 mL) at room temperature was added imidazole (1.50 g, 22.0 mmol, 2.0 eq), PPh_3 (4.32 g, 16.5 mmol, 1.5 eq) and I_2 (4.17 g, 16.5 mmol, 1.5 eq) sequentially. Then the mixture was heated to 80 °C. After being stirred at 80 °C for 10 min, the reaction mixture was cooled to room temperature, quenched with saturated aqueous $Na_2S_2O_3$ and extracted with EtOAc. The organic layer was further washed with saturated aqueous Na_2HCO_3 and water. After evaporation of the solvent, the residue was purified by flash column chromatography (petroleum ether/EtOAc, 1/1) to afford **4** and **44′** (5.74 g) as

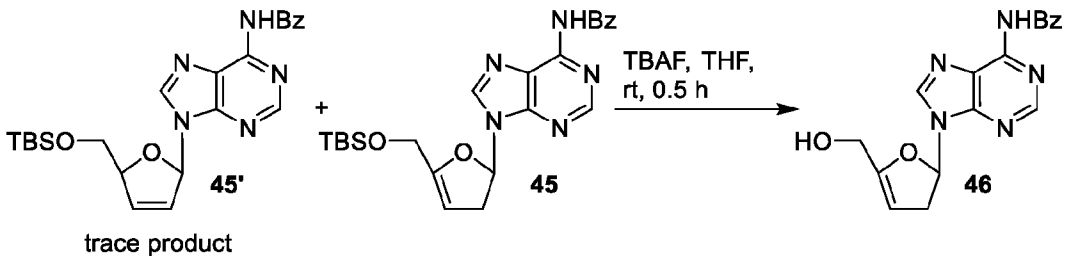
white foams.

[00513] ***General Procedure for the Preparation of Compound 45 and 45 ′***



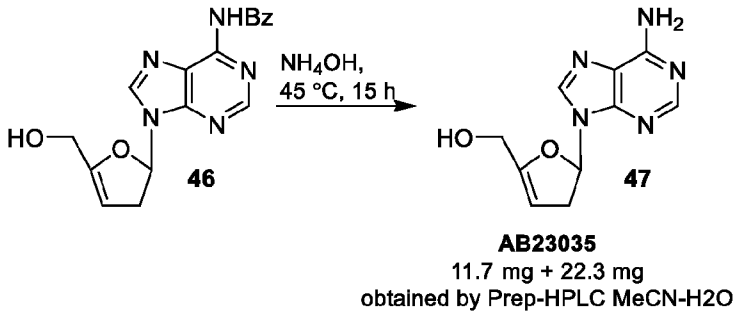
[00514] A solution of compound **44** and **44′** (7.88 g, 13.6 mmol, 1.0 eq) and DABCO (6.10 g, 54.4 mmol, 4.0 eq) in toluene (60 mL) was stirred at 120 °C for 6 h. Then the reaction mixture was cooled to room temperature, quenched with saturated aqueous NH_4Cl and extracted with EtOAc. The organic layer was further washed with saturated aqueous $NaHCO_3$ and water. After evaporation of the solvent, the residue was purified by flash column chromatography (dichloromethane/methanol, 20/1) to afford crude product **45** (3.45 g) including trace product **45′** as white foams.

[00515] ***General Procedure for the Preparation of Compound 46***



[00516] A solution of compound **45** (3.45 g, 7.64 mmol, 1.0 eq) and TBAF (1.0 M, 15.3 mL, 2.0 eq) in dry THF (20 mL) was stirred at room temperature for 1 h. Then the reaction mixture was poured into water, extracted with EtOAc, and concentrated. After evaporation of the solvent, the residue was purified by flash column chromatography (dichloromethane/methanol, 20/1) to afford crude product **46** (2.51 g) as a white foam.

[00517] ***General Procedure for the Preparation of Compound 47***



[00518] A solution of **46** (2.51 g, 7.44 mmol, 1.0 eq) in NH_4OH (20 mL) was stirred at 45 °C for 15 h. The result mixture was concentrated under reduced pressure, the residue was purified by Biotage to afford **47** (0.69 g, 40%) as a white solid. Another crude product **47** (0.12 g) was further purified on reverse phase HPLC (MeCN-H_2O) to afford **47** (34 mg) as a white solid.

TLC: Dichloromethane/Methanol, 5/1

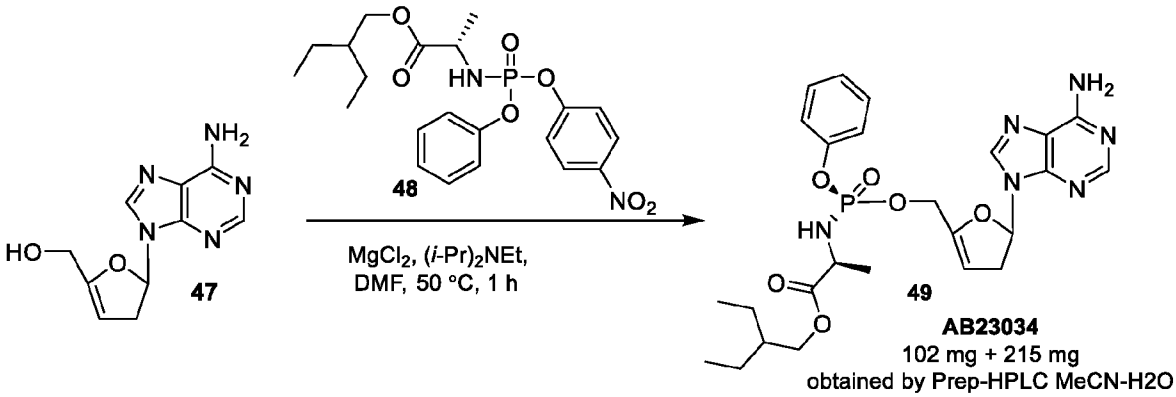
Rf (Compound **46**) = 0.95

Rf (Product **47**) = 0.5

LC-MS: 234.05 $[M+H]^+$

^{1}H NMR (400 MHz, DMSO-*d6*) δ 8.17 (d, *J* = 7.2 Hz, 2H), 7.31 (s, 2H), 6.76 (d, *J* = 6.0 Hz, 1H), 5.11 (d, *J* = 17.6 Hz, 2H), 3.96 (s, 2H), 3.22 (d, *J* = 9.3 Hz, 1H), 3.07 (d, *J* = 16.6 Hz, 1H).

[00519] ***General Procedure for the Preparation of Compound 49 (AB23034)***



[00520] To a solution of compound **47** (0.57 g, 2.44 mmol, 1.0 eq) in DMF (20 mL) at room temperature was added **48** (1.32 g, 2.93 mmol, 1.2 eq) and $MgCl_2$ (0.23 g, 2.44 mmol, 1.0 eq) sequentially. Then the mixture was heated to 50 °C for 10 min, and *N,N*-diisopropylethylamine (1.01 mL, 6.10 mmol, 2.5 eq) was added. After being stirred at 50 °C for another 50 min, the reaction mixture was cooled to room temperature, quenched with diluted HCl (1 M) and extracted

with EtOAc. The organic layer was further washed with saturated aqueous Na_2HCO_3 and brine. After evaporation of the solvent, the residue was purified by flash column chromatography (dichloromethane/methanol, 20/1) and further purified by prep-HPLC (MeCN-H_2O) to afford **49** (317 mg, 24%) as a white solid.

TLC: Dichloromethane/Methanol, 20/1

Rf (Compound **47**) = 0.1

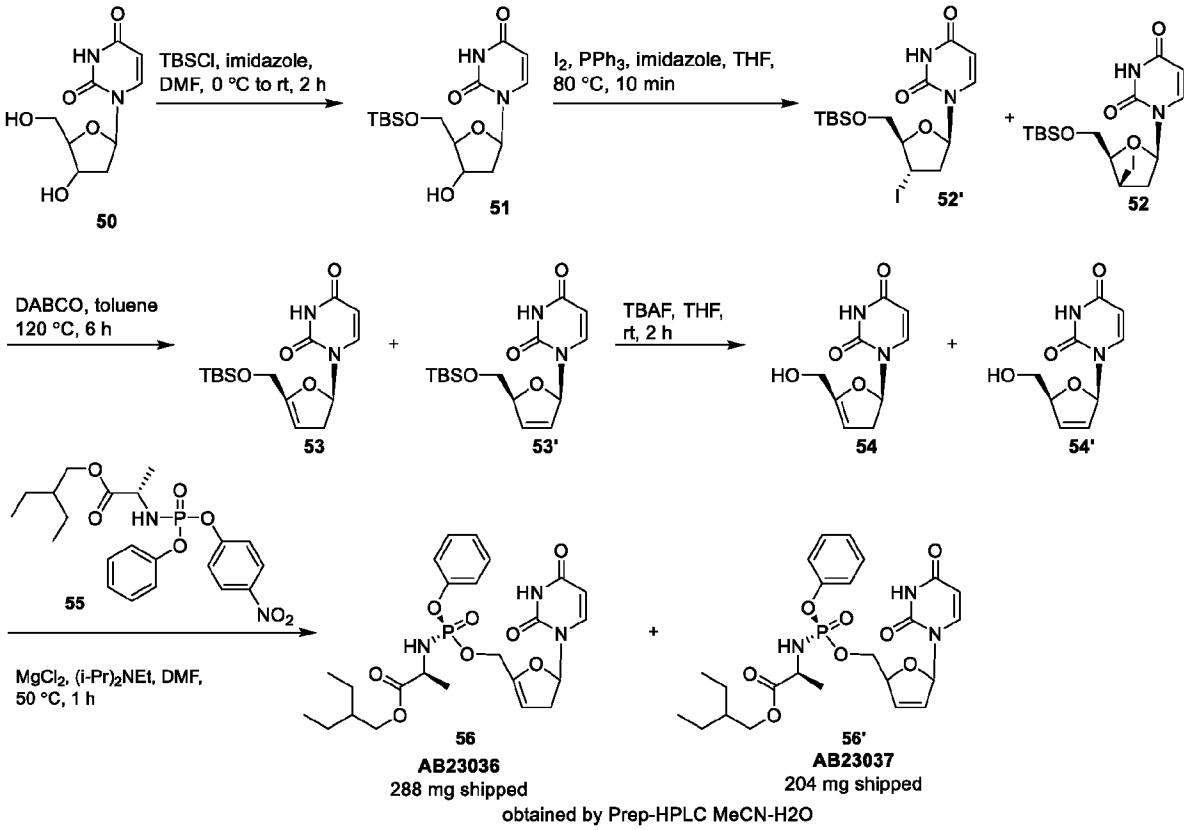
Rf (Product **49**) = 0.5

LC-MS: 545.30 $[M+H]^+$

^{1}H NMR (400 MHz, DMSO-*d6*) δ 8.17 (d, *J* = 9.3 Hz, 2H), 7.46–7.24 (m, 4H), 7.14 (d, *J* = 7.3 Hz, 3H), 6.79 (d, *J* = 5.5 Hz, 1H), 6.14–5.97 (m, 1H), 5.33 (s, 1H), 4.56 (s, 1H), 3.90 (d, *J* = 5.5 Hz, 1H), 3.84 (d, *J* = 5.7 Hz, 1H), 3.13 (d, *J* = 17.4 Hz, 1H), 1.39 (d, *J* = 6.0 Hz, 1H), 1.23 (t, *J* = 8.9 Hz, 7H), 0.78 (t, *J* = 7.3 Hz, 6H).

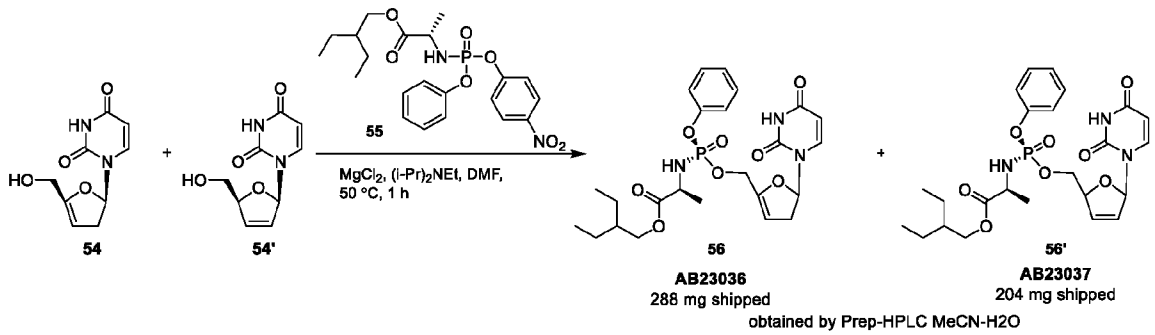
^{31}P NMR (162 MHz, DMSO) δ 3.47.

[00521] **Synthetic Approach for Compounds 56 and 56'**



[00522] **Procedure**

[00523] ***General Procedure for the Preparation of Compound 7 and Compound 7'***



[00524] To a solution of alcohols **54** and **54'** (0.48 g, 2.28 mmol, 1.0 eq) in DMF (20 mL) at room temperature was added **55** (1.23 g, 2.74 mmol, 1.2 eq) and $MgCl_2$ (0.22 g, 2.28 mmol, 1.0 eq) sequentially. Then the mixture was heated to 50 °C for 10 min, and *N,N*-diisopropylethylamine (0.94 mL, 5.70 mmol, 2.5 eq) was added. After being stirred at 50 °C for another 50 min, the reaction mixture was cooled to room temperature, quenched with diluted HCl (1 M) and extracted with EtOAc. The organic layer was further washed with saturated aqueous Na_2HCO_3 and water. After evaporation of the solvent, the residue was purified by flash column chromatography (dichloromethane/methanol, 20/1) and further purified by prep-HPLC (MeCN-H_2O) to afford **56** (288 mg, 24%) as a yellow solid and **56'** (204 mg, 17%) as a yellow solid.

TLC: Dichloromethane/Methanol, 20/1

R_f (Compound **54**) = 0.1

R_f (Product **56/56'**) = 0.5

56:

LC-MS: 522.15 $[M+H]^+$

^{1}H NMR (400 MHz, DMSO-*d6*) δ 11.41 (s, 1H), 7.37 (dd, *J* = 16.9, 8.0 Hz, 3H), 7.18 (dd, *J* = 13.6, 7.4 Hz, 3H), 6.58 (d, *J* = 6.2 Hz, 1H), 6.09 (t, *J* = 11.5 Hz, 1H), 5.60 (d, *J* = 8.0 Hz, 1H), 5.23 (s, 1H), 4.57 (d, *J* = 7.1 Hz, 2H), 4.00–3.83 (m, 3H), 3.16 (dd, *J* = 16.9, 9.6 Hz, 1H), 2.66 (d, *J* = 17.4 Hz, 1H), 1.52–1.38 (m, 1H), 1.36–1.14 (m, 7H), 0.82 (t, *J* = 7.3 Hz, 6H).

^{31}P NMR (162 MHz, DMSO) δ 3.45.

56':

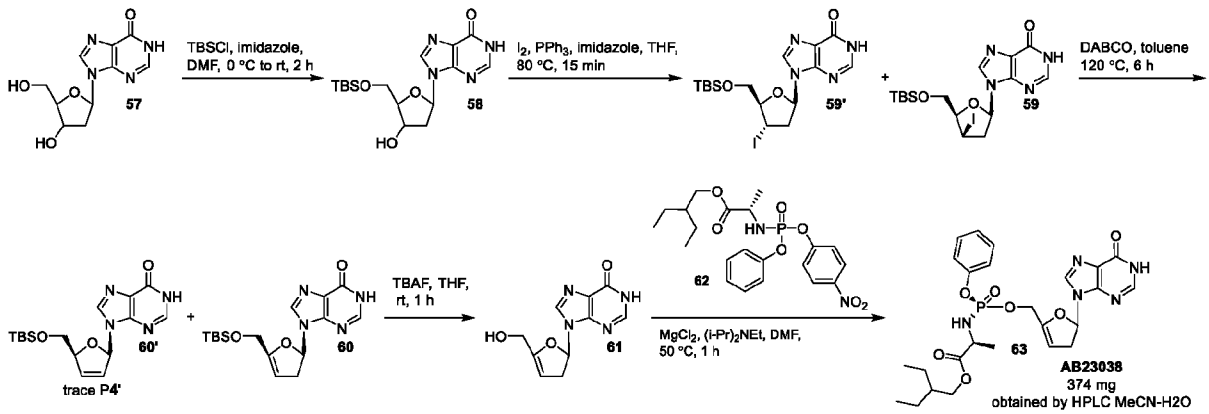
LC-MS: 522.15 $[M+H]^+$

^{1}H NMR (400 MHz, DMSO-*d6*) δ 11.36 (s, 1H), 7.45 (d, *J* = 7.9 Hz, 1H), 7.35 (d, *J* = 7.4 Hz, 2H), 7.19 (d, *J* = 7.2 Hz, 3H), 6.82 (s, 1H), 6.39 (d, *J* = 4.4 Hz, 1H), 6.13 – 5.93 (m, 2H), 5.45

(d, J = 8.1 Hz, 1H), 4.96 (s, 1H), 4.14 (s, 2H), 4.04 – 3.74 (m, 3H), 1.44 (s, 1H), 1.36 – 1.13 (m, 8H), 0.82 (t, J = 7.2 Hz, 7H).

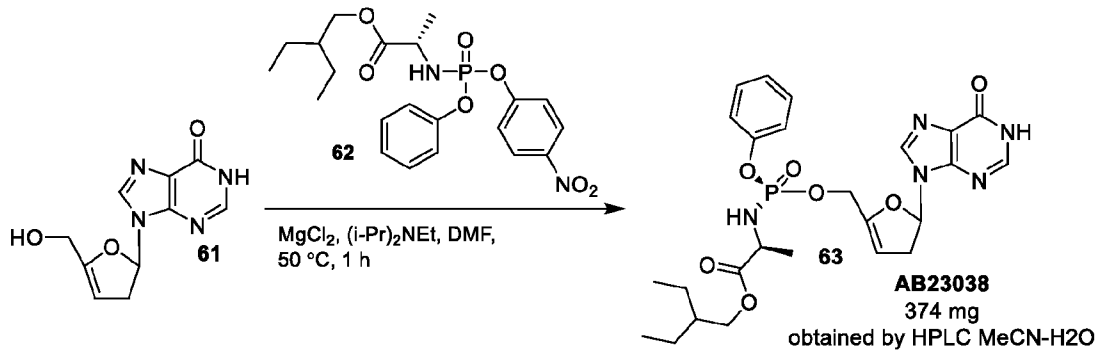
^{31}P NMR (162 MHz, DMSO-*d6*) δ 3.60.

[00525] **Synthetic Approach for Compound 63**



[00526] **Procedure**

[00527] ***General Procedure for the Preparation of Compound 63***



[00528] To a solution of alcohol **61** (0.60 g, 2.56 mmol, 1.0 eq) in DMF (20 mL) at room temperature was added **62** (1.38 g, 3.07 mmol, 1.2 eq) and $MgCl_2$ (0.24 g, 2.56 mmol, 1.0 eq) sequentially. Then the mixture was heated to 50 °C for 10 min, and N,N-diisopropylethylamine (1.06 mL, 6.40 mmol, 2.5 eq) was added. After being stirred at 50 °C for another 50 min, the reaction mixture was cooled to room temperature, quenched with diluted HCl (1 M) and extracted with EtOAc. The organic layer was further washed with saturated aqueous Na_2HCO_3 and water. After evaporation of the solvent, the residue was purified by flash column chromatography (dichloromethane/methanol, 20/1) and further purified by Prep-HPLC (MeCN-H_2O) to afford **63** (374 mg, 27%) as a white solid.

TLC: Dichloromethane/Methanol, 20/1

R_f (Compound **61**) = 0.1

R_f (Product **63**) = 0.5

LC-MS: 546.15 $[M+H]^+$

^{1}H NMR (400 MHz, DMSO-*d6*) δ 12.42 (s, 1H), 8.11 (d, *J* = 20.9 Hz, 2H), 7.32 (t, *J* = 7.1 Hz, 2H), 7.15 (d, *J* = 6.9 Hz, 4H), 6.77 (d, *J* = 6.1 Hz, 1H), 6.05 (t, *J* = 11.5 Hz, 1H), 5.34 (s, 1H), 4.56 (br, 2H), 4.05 – 3.71 (m, 3H), 3.08 (d, *J* = 16.6 Hz, 1H), 1.41 (s, 1H), 1.22 (d, *J* = 6.5 Hz, 7H), 0.79 (t, *J* = 6.7 Hz, 6H).

^{31}P NMR (162 MHz, DMSO-*d6*) δ 3.47.

Example 16 –ANTIVIRAL ACTIVITY - Materials and Methods

[00529] *Objective*: To provide the general methods used in assaying effect of ddh-compounds on viral activity. Specific and additional details are provided in the Examples below.

[00530] *Methods*:

[00531] **Cells culture and virus strains.** Human foreskin fibroblast (HFF) cells prepared from human foreskin tissue were obtained from the University of Alabama at Birmingham tissue procurement facility with approval from its IRB. The tissue was incubated at 4°C for 4 h in Clinical Medium consisting of minimum essential media (MEM) with Earl's salts supplemented with 10% fetal bovine serum (FBS) (Hyclone, Inc. Logan UT), L-glutamine, fungizone, and vancomycin. Tissue is then placed in phosphate buffered saline (PBS), minced, rinsed to remove the red blood cells, and resuspended in trypsin/EDTA solution. The tissue suspension is incubated at 37°C and gently agitated to disperse the cells, which are collected by centrifugation. Cells are resuspended in 4 ml Clinical Medium and placed in a 25 cm2 flask and incubated at 37°C in a humidified CO2 incubator for 24 h. The media is then replaced with fresh Clinical Medium and the cell growth is monitored daily until a confluent monolayer has formed. The HFF cells are then expanded through serial passages in standard growth medium of MEM with Earl's salts supplemented with 10% FBS, L-glutamine, penicillin, and gentamycin. The cells are passaged routinely and used for assays at or below passage 10 (1,2). COS7 and C-33 A, Guinea Pig Lung, and Mouse embryo fibroblast cells were obtained from ATCC and maintained in standard growth medium of MEM with Earl's salts supplemented with 10% FBS, L-glutamine, penicillin, and gentamycin.

[00532] Akata cells were kindly provided by John Sixbey (Louisiana State University, Baton Rouge, LA). BCBL-1 cells were obtained through the NIH AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, NIH. Molt-3 cells were obtained from Scott Schmid at the Centers for Disease Control and Prevention, Atlanta, GA. Lymphocytes were maintained routinely in RPMI 1640 (Mediatech, Inc., Herndon, VA) with 10% FBS, L-glutamine and antibiotics and passaged twice a week, as described previously (3, 4).

[00533] The E-377 strain of HSV-1 was a gift of Jack Hill (Burroughs Wellcome). The HCMV strain AD169, HSV-2 strain G, AdV5 strain Adenoid 75, GP CMV strain 11211 and MCMV strain

were obtained from the American Type Culture Collection (ATCC, Manassas, VA). The Copenhagen strain of VACV and Brighton strain, CPXV were kindly provided by John W. Huggins (Department of Viral Therapeutics, Virology Division, United States Army Medical Research Institute of Infections Disease). VZV, strain Ellen, the BK virus Gardner strain and JC virus MAD4 strain were obtained from the ATCC. Akata cells latently infected with EBV were obtained from John Sixbey. The Z29 strain of HHV-6B was a gift of Scott Schmid at the Centers for Disease Control and Prevention, Atlanta GA. HHV-8 was obtained as latently infected BCBL-1 cells through the NIH AIDS Research and Reference Reagent Program.

[00534] **Antiviral Assays:** Each experiment that evaluated the antiviral activity of the compounds included both positive and negative control compounds to ensure the performance of each assay. Concurrent assessment of cytotoxicity was also performed for each study in the same cell line and with the same compound exposure (see below).

[00535] **CPE assays for HSV-1, HSV-2, VZV, HCMV, MCMV, GPCMV, AdV, VACV, CPXV**

[00536] Assays were performed in monolayers. Briefly, cells were seeded in 384 well plates and incubated for 24h to allow the formation of confluent monolayers. Dilutions of test drug were prepared directly in the plates and the monolayers infected at a predetermined MOI based on virus used. After incubation, cytopathology was determined by the addition of CellTiter-Glo (CTG) reagent. Concentrations of test compound sufficient to reduce CPE by 50% (EC50) or decrease cell viability by 50% (CC50) were interpolated using standard methods in Microsoft excel.

[00537] **Plaque reduction assays for HSV-1, HSV-2, VZV, HCMV, MCMV, GPCMV, VACV, CPXV.** Monolayers of HFF cells were prepared in six-well plates and incubated at 37°C for 2 d to allow the cells to reach confluency. Media was then aspirated from the wells and 0.2 ml of virus was added to each of three wells to yield 20-30 plaques in each well. The virus was allowed to adsorb to the cells for 1 h and the plates were agitated every 15 minutes. Compounds were diluted in assay media consisting of MEM with Earl's salts supplemented with 2% FBS, L-glutamine, penicillin, and gentamycin. Diluted drug was added to duplicate wells and the plates were incubated for various times, depending on the virus used. For HSV-1 and -2, the monolayers were then stained with 1% crystal violet in 20% methanol and the unbound dye removed by washing with dH20. For all other assays, the cell monolayer was stained with 1% Neutral Red solution for 4 h then the stain was aspirated and the cells were washed with PBS. For all assays, plaques were enumerated using a stereomicroscope and the concentration of compound that reduced plaque formation by 50% (EC50) was interpolated from the experimental data.

[00538] **Yield reduction assays for HSV-1, HSV-2, HCMV, VACV, CPXV and AdV.** Monolayers of HFF cells were prepared in 96-well plates and incubated at 37°C for 1 d to allow the cells to reach confluency. Media was then aspirated from the wells and cells infected at a high MOI. At 1 h following infection, the inocula were removed and the monolayers rinsed with fresh media. Compounds were then diluted in assay media consisting of MEM with Earl's salts supplemented with 2% FBS, L-glutamine, penicillin, and gentamycin. Drug dilutions were added to the wells and the plates were incubated for various times, depending on the virus used and represents a single replication cycle for the virus. A duplicate set of dilutions were also performed but remained uninfected to serve as a cytotoxicity control and received equal compound exposure. Supernatants from each of the infected wells were subsequently titered in a TCID50 assay to quantify the progeny virus. For the cytotoxicity controls, cytotoxicity was assed using CTG according to the manufacturer's suggested protocol. For all assays, the concentration of compound that reduced virus titer by 90% (EC90) was interpolated from the experimental data.

[00539] **Assays for EBV, HHV-6B, and HHV-8.** Assays for EBV, HHV-6B and HHV-8 were performed by methods we reported previously (3). Akata cells were induced to undergo a lytic infection with 50 μg/ml of a goat anti-human IgG antibody. Experimental compounds were diluted within plates; the cells were added and incubated for 72 h. For HHV-6 assays, compounds were serially diluted plates then uninfected Molt-3 cells were added to each well. Infection was initiated by adding HHV-6B infected Molt-3 cells, at a ratio of approximately 1 infected cell for every 10 uninfected cells. Assay plates were incubated for seven days at 37°C. Assays for HHV-8 were performed in BCBL-1 cells. Similar plates were initiated without virus induction/addition and used for measuring cytotoxicity by the addition of CTG. For all assays, the replication of the virus was assessed by the quantification of viral DNA. For EBV, primers 5'-CCC AGG AGT CCC AGT AGT CA-3' and 5'-CAG TTC CTC GCCTTAGGTTG-3 amplified a fragment corresponding to coordinates 96802–97234 in EBV genome (AJ507799). For HHV-8, 5'-TTC CCC AGA TAC ACG ACA GAA TC-3', reverse primer 5'-CGG AGC GCA GGC TAC CT-3', and probe 5'-(FAM) CCT ACG TGT TCG TCG AC (TAMRA)-3'. Plasmid pMP218 containing a DNA sequence corresponding to nucleotides 14120-14182 (AF148805.2) was used to provide absolute quantification of viral DNA. For HHV-6B, 5'-GTT AGG GTA TAC CGA TGT GCG TGA T-3' 5'-TAC AGA TAC GGA GGC AAT AGA TTC G-3' and 5'-(FAM) TCC GAA ACA ACT GTC TGA CTG GCA AAA-3' were used to quantify virus DNA. Compound concentrations sufficient to reduce genome copy number by 50% were calculated from experimental data as well as compound cytotoxicity.

[00540] **Assays for BK virus and JC virus.** Primary assays for BKV and JCV were performed by methods we reported previously (3). For BKV, compound dilutions were prepared in plates containing cells, subsequently infected and incubated for 7d. Total DNA was prepared, and genome copy number was quantified by real time PCR using the primers 5'- AGT GGA TGG GCA GCC TAT GTA-3', 5'- TCA TAT CTG GGT CCC CTG GA-3' and probe 5'-6-FAM AGG TAG AAG AGG TTA GGG TGT TTG ATG GCA CAG TAMRA-3'. Plasmid pMP526 serves as the DNA standard for quantification purposes. Compounds that were positive in this assay were confirmed in a similar assay in 96-well plates according to established laboratory protocols with the compounds added 1h post infection to identify compounds that inhibit early stages of replication including adsorption and penetration. Genome copy number was determined by methods described above. Primary evaluation of compounds against JC virus were also performed by methods similar to those for BK virus primary assays but were done in COS7 cells and utilized the 1-4 strain of JCV in COS7 cells. Viral DNA was quantified using primers 5'-CTG GTC ATG TGG ATG CTG TCA-3' and 5'-GCC AGC AGG CTG TTG ATA CTG-3' and probe 5'-6-FAM-CCC TTT GTT TGG CTG CT-TAMRA-3 together with the plasmid pMP508 to provide a standard curve for absolute quantification. Secondary assays against JCV were also performed in COS7 cells by methods similar to those for BK virus to identify compounds that inhibited adsorption or penetration of the virus.

[00541] **Assays for HPV**

[00542] Primary assay: An HPV11 replicon assay was developed and expresses the essential E1 and E2 proteins from the native promoter. The E2 origin binding protein interacts with the virus origin of replication and recruits the E1 replicative helicase which unwinds the DNA and helps to recruit the cellular DNA replication machinery (including DNA polymerases, type I and type II topoisomerases, DNA ligase, single stranded DNA binding proteins, proliferating cell nuclear antigen). The replication complex then drives the amplification of the replicon which can be assessed by the expression of a destabilized NanoLuc reporter gene carried on the replicon. In this assay, the replicon (pMP619) is transfected into C-33 A cells grown as monolayers in 384-well plates. At 48 h post transfection, the enzymatic activity of the destabilized NanoLuc reporter is assessed with NanoGlo reagent. The reference compound for this assay is PMEG and its EC50 value is within the prescribed range of 2 - 9.2 μM and is similar to a compound we have reported previously.

[00543] Secondary Assay: HPV genome replication will be done by methods similar to HPV 11 but will be done in a plasmid system utilizing HPV16 or HPV18. Additional replicates will also be tested to ensure that the estimates of EC_{50} and EC_{90} values are more precise. Recent publications

demonstrate our ability to perform these studies.

Example 17 – ANTIVIRAL ASSESSMENT OF NATURAL NUCLEOSIDE ANALOGS

[00544] *Objective*: To test 6 ddh-compounds against Herpes simplex virus 1 (HSV-1), Human cytomegalovirus (HCMV), Epstein-Barr virus (EBV), BK virus (BKV) and JC virus (JCV).

[00545] *Methods*:

[00546] Compounds tested:

Name
AB23021 (compound 4)
AB23022 (compound 6)
AB23023 (compound 2)
AB23024 (compound 1)
AB23025 (compound 3)
AB23026 (compound 5)

[00547] All compounds were solubilized in the submitted vials by adding DMSO to each, based on quantities and molecular weights specified to achieve a final 30mM concentration. These stocks were stored at -20°C.

[00548] There were no problems with solubility of the compounds in DMSO.

[00549] The working test compound solutions (1.5mM) were prepared by diluting the 30mM DMSO stocks 1:20 in warmed MEM + 2%FBS.

[00550] There were no problems with solubility of the compounds in 2% MEM.

[00551] Positive and negative control drugs were prepared as per usual with working solutions at 1.5 and 1.0 mM in MEM + 2%FBS.

[00552] *Results*:

Herpes Simplex Virus 1 (HSV-1)

	Isolate	**Cell Line**	**Incubation**	**Assay Name**
HSV-1	E-377	HFF	5 d	CellTiter-Glo *(Cytopathic effect/Toxicity)*

[00553] Test Drug Concentration: 0.048-150 μM. Control Drug Concentration: 0.048-150 μM.

TABLE 26: HSV-1 Assays Results

Drug	Activity	EC_{50}	EC_{90}	CC_{50}	SI_{50}	SI_{90}
ACV	-	2.32	>150.00	>150.00	>65	1
TNV	-	>150.00	>150.00	>150.00	1	1
AB23021	NA	>150.00	>150.00	>150.00	1	1
AB23022	NA	>150.00	>150.00	>150.00	1	1
AB23023	NA	>150.00	>150.00	>150.00	1	1
AB23024	NA	>30.00	>30.00	82.43	<3	<3
AB23025	NA	>150.00	>150.00	>150.00	1	1
AB23026	NA	>150.00	>150.00	>150.00	1	1

ACV - Acyclovir - positive control
TNV - Tenofovir - negative control
NA (Not Active) $SI_{50} = 0 - 3.9$
LO (Slightly Active) $SI_{50} = 4 - 9.9$
MOD (Moderately Active) $SI_{50} = 10 - 49.9$
HI (Highly Active) $SI_{50} = \geq 50$

[00554] Human Cytomegalovirus (HCMV)

	Isolate	**Cell Line**	**Incubation**	**Assay Name**
HCMV	AD169	HFF	14 d	CellTiter-Glo *(Cytopathic effect/Toxicity)*

[00555] Test Drug Concentration: 0.048-150 μM.

[00556] Control Drug Concentration: 0.048-150 μM.

TABLE 27:HCMV Assay Results

Drug	Activity	EC_{50}	EC_{90}	CC_{50}	SI_{50}	SI_{90}
GCV	-	0.81	>150.00	>150.00	>186	1
TNV	-	>150.00	>150.00	>150.00	1	1
AB23021	NA	>150.00	>150.00	>150.00	1	1
AB23022	NA	>150.00	>150.00	>150.00	1	1
AB23023	NA	>30.00	>30.00	93.16	<3	<3
AB23024	NA	>30.00	>30.00	84.56	<3	<3
AB23025	NA	>150.00	>150.00	>150.00	1	1
AB23026	NA	>150.00	>150.00	>150.00	1	1

GCV - Ganciclovir - positive control
TNV - Tenofovir - negative control
NA (Not Active) $SI_{50} = 0 - 3.9$
LO (Slightly Active) $SI_{50} = 4 - 9.9$
MOD (Moderately Active) $SI_{50} = 10 - 49.9$
HI (Highly Active) $SI_{50} = \geq 50$

[00557] Epstein-Barr virus (EBV)

	Isolate	Cell Line	Incubation	Assay Name
EBV	Akata	Akata	3 d	Quantitative Polymerase Chain Reaction *(DNA)*/ CellTiter-Glo *(Toxicity)*

[00558] Test Drug Concentration: 0.032-100; 0.048-150 μM.

[00559] Control Drug Concentration: 0.048-150 μM.

TABLE 28: EBV Assay Results

Drug	Activity	EC_{50}	EC_{90}	CC_{50}	SI_{50}	SI_{90}
GCV	-	0.81	>150.00	>150.00	>186	1
TNV	-	>150.00	>150.00	>150.00	1	1
AB23021	NA	>150.00	>150.00	>150.00	1	1
AB23022	NA	>150.00	>150.00	>150.00	1	1
AB23023	NA	>30.00	>30.00	93.16	<3	<3
AB23024	NA	>30.00	>30.00	84.56	<3	<3
AB23025	NA	>150.00	>150.00	>150.00	1	1
AB23026	NA	>150.00	>150.00	>150.00	1	1

CDV - Cidofovir - positive control
TNV - Tenofovir - negative control
NA (Not Active) $SI_{50} = 0 - 3.9$
MOD (Moderately Active) $SI_{50} = 4 - 9.9$
HI (Highly Active) $SI_{50} = \geq 10$

[00560] BK Virus (BKV)

	Isolate	Cell Line	Incubation	Assay Name
EBV	Akata	Akata	3 d	Quantitative Polymerase Chain Reaction *(DNA)*/ CellTiter-Glo *(Toxicity)*

[00561] Test Drug Concentration: 0.032-100; 0.048-150 μM.

[00562] Control Drug Concentration: 0.048-150 μM.

TABLE 29: BKV Assay Results

Drug	Activity	EC_{50}	EC_{90}	CC_{50}	SI_{50}	SI_{90}
CDV	-	9.23	29.89	>100.00	>11	>3
TNV	-	45.76	>150.00	>150.00	>3	1
AB23021	NA	>30.00	>30.00	108.58	<4	<4
AB23022	MOD	18.76	>30.00	123.78	7	<4
AB23023	HI	2.98	>30.00	105.71	35	<4
AB23024	MOD	12.89	26.79	55.82	4	2
AB23025	MOD	18.75	81.93	>150.00	>8	>2
AB23026	NA	79.81	>150.00	>150.00	>2	1

CDV - Cidofovir - positive control
TNV - Tenofovir - negative control
NA (Not Active) SI_{50} = 0 - 3.9
LO (Slightly Active) SI_{50} = 4 - 9.9
MOD (Moderately Active) SI_{50} = 10 - 19.9
HI (Highly Active) SI_{50} = ≥20

[00563] JC Virus

	Isolate	**Cell Line**	**Incubation**	**Assay Name**
JCV	MAD-4	COS7	7 d	Quantitative Polymerase Chain Reaction *(DNA)*/ CellTiter-Glo *(Toxicity)*

[00564] Test Drug Concentration: 0.048-150 μM.

[00565] Control Drug Concentration: 0.048-150 μM.

TABLE 30: JCV Assay Results

Drug	Activity	EC_{50}	EC_{90}	CC_{50}	SI_{50}	SI_{90}
CDV	-	5.56	117.63	>150.00	>27	>1
TNV	-	>150.00	>150.00	>150.00	1	1
AB23021	NA	116.01	>150.00	>150.00	>1	1
AB23022	NA	>150.00	>150.00	>150.00	1	1
AB23023	NA	>150.00	>150.00	>150.00	1	1
AB23024	NA	>30.00	>30.00	89.65	<3	<3
AB23025	NA	>150.00	>150.00	>150.00	1	1
AB23026	NA	116.53	>150.00	>150.00	>1	1

CDV - Cidofovir - positive control

TNV - Tenofovir - negative control
NA (Not Active) SI_{50} = 0 - 3.9
LO (Slightly Active) SI_{50} = 4 - 9.9
MOD (Moderately Active) SI_{50} = 10 - 19.9
HI (Highly Active) SI_{50} = ≥20.

[00566] *Summary*: AB23024 (compound 1; Formula IB) exhibits cytotoxicity in all cell lines; AB23023 (compound 2; Formula IIB) was toxic in HFF cells in HCMV assay (14 d); AB23021 (compound 4; Formula IVB) and AB23022 (compound 6; Formula IXB) showed toxicity in Akata cells (lymphocyte cell line) in addition to the previously mentioned compounds.

[00567] None of the compounds showed antiviral efficacy in the HSV-1, HCMV or JCV assays. AB23022, AB23024 and AB23025 (compound 3; Formula IIIB) were moderately active against EBV. AB23023 was highly active against both EBV and BK virus.

Example 18 – LIVER S9 STABILITY AND PLASMA STABILITY DETERMINATIONS

[00568] *Objective*: To examine the stability and half-life of ddh-test compounds and metabolites thereof.

[00569] *Methods:* Test compound working solutions:

Compound	MW	Stock Conc. (mM)	Metabolite
AB23039 (compound 104; Formula IVB)	588.70	10	AB21649 (compound 24; Formula IVB)
AB23040 (compound 103; Formula IIB)	627.70	10	AB21651 (compound 31; Formula IIB)

[00570] Liver S9 working solutions

Species			Test compound =	3	
			Working solution Vol. =	2623.5	µL
	Liver S9 stock Conc. (mg/mL)	Liver S9 stock Vol. (µL)	Working solution Conc. (mg/mL)	Final Conc. in incubation (mg/mL)	Phosphate buffer Vol. (µL)
Human	20	83	0.629	0.5	2541
Species			Test compound =	3	
			Working solution Vol. =	2623.5	µL

	Liver S9 stock Conc. (mg/mL)	Liver S9 stock Vol. (µL)	Working solution Conc. (mg/mL)	Final Conc. in incubation (mg/mL)	Phosphate buffer Vol. (µL)
Rat	20	83	0.629	0.5	2541

[00571] *Results:*

TABLE 31

Compound	Human			Rat		
	$T_{1/2}$ (min)	*In vitro* CL_{int} (µL/min/mg protein)	CL_{hep} (mL/min/kg)	$T_{1/2}$ (min)	*In vitro* CL_{int} (µL/min/mg protein)	CL_{hep} (mL/min /kg)
AB23039	264.7	5.2	9.1	347.1	4.0	14.3
AB23040	198.7	7.0	10.6	237.1	5.8	18.7
Dextromethorphan	94.0	14.7	14.3	58.8	23.6	37.2

TABLE 32: Peak area ratio measured for parent and its Metabolite:

Compound	Analyst	Human						Rat					
		Peak area ratio						Peak area ratio					
		With NADPH				Without NADPH		With NADPH				Without NADPH	
		0 min	15 min	60 min	180 min	0 min	180 min	0 min	15 min	60 min	180 min	0 min	180 min
AB23039	AB23039	3.92E-01	3.41E-01	3.09E-01	2.34E-01	3.84E-01	3.28E-01	4.30E-01	3.61E-01	3.25E-01	2.81E-01	3.76E-01	3.27E-01
	AB21649	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00
AB23040	AB23040	1.29E-01	1.33E-01	1.05E-01	7.13E-02	1.31E-01	8.41E-02	1.37E-01	1.16E-01	1.11E-01	7.68E-02	1.20E-01	8.34E-02
	AB21651	7.04E-04	1.03E-03	1.50E-03	3.03E-03	6.17E-04	3.06E-03	7.08E-04	6.93E-04	7.68E-04	1.30E-03	6.03E-04	2.39E-03

TABLE 33: Stability without NADPH

Compound	Stability (Yes/No)		$T_{1/2}$ (min)	
	Human	Rat	Human	Rat
AB23039	Yes	Yes	--	--
AB23040	No	No	281.52	342.91

[00572] **Summary Plasma Stability**

Compound	$T_{1/2}$ (min)	
	Human	Rat
AB23039	>559.1	>559.1
AB23040	>559.1	>559.1
Propantheline	16.8	--
Diltiazem	--	124.8

[00573] **Peak Area Ratio Measured for parent and metabolite in plasma stability measurements.**

Compound	Analyst	Human				Rat			
		Peak area ratio				Peak area ratio			
		0 min	15 min	60 min	180 min	0 min	15 min	60 min	180 min
AB23039	AB23039	3.13E-01	3.19E-01	3.08E-01	3.15E-01	3.11E-01	2.91E-01	2.98E-01	3.03E-01
	AB21649	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	2.76E-03	2.85E-03	3.08E-03
AB23040	AB23040	6.76E-02	6.03E-02	5.81E-02	6.50E-02	7.25E-02	7.28E-02	8.13E-02	7.58E-02
	AB21651	5.52E-04	4.09E-04	5.47E-04	3.83E-04	8.33E-04	1.22E-03	1.02E-03	5.08E-04

Example 19 – ANALYSIS OF REMDESIVIR AND ddh-REMDESIVIR ACTIVITIES

[00574] *Objective*: To examine Remdesivir (AB2570) and ddh-Remdesivir (AB23046; compound 101), and triphosphate analogs thereof, for antiviral activities, for example but not limited to nucleotide incorporation and inhibition of RNA-Dependent RNA polymerase (RdRp) activity as they relates with SARS-CoV-2 and components thereof.

[00575] *Methods*:

[00576] **Nsp12 protein expression and purification**

[00577] SARS-CoV-2 nsp12 gene (amino acid 4393-5324 Uniprot: P0DTD1) was synthesized *de novo* by GenScript (Nanjing, China) and constructed onto pET28A vector between *BamHI* and *XhoI* sites. Nsp12 expressed with a C-terminal 6 His-tag in BL21 (DE3) at 37 ºC. 2.5 mM $MgCl_2$ and 0.1 mM $ZnSO_4$ were supplemented in the culture during induction with 0.25 mM of IPTG. After 2 hours, cells were harvested and lysed by Branson sonifier in buffer containing 50 mM Tris pH 7.5, 500 mM NaCl, 1 mM $MgCl_2$, 20% glycerol, 5 mM BME and 25 mM imidazole. After centrifugation at 4000 rpm, Nsp12 was purified by Ni-Sepharose resin (GE Healthcare) using 20-mL gravity-flow column. Nsp12 was eluted at 200 mM imidazole and fractions were combined, concentrated and diluted with buffer containing 20 mM HEPES pH 7.5, 150 mM NaCl, 1 mM $MgCl_2$, 20% glycerol, 1 mM Tris(2-carboxyethyl)phosphine and stored at -80 ºC. The protein concentration was determined by A280 using the extinction coefficient calculated with ProtParam.

[00578] **Nsp7 and Nsp8 protein expression and purification**

[00579] SARS-CoV-2 nsp8 gene (nucleotide 12092-12685, strain, GenBank: MN908947.3) and SARS-CoV-2 nsp7 gene (nucleotide 11846-12091, GenBank: MN908947.3) were synthesized de novo by GenScript (Nanjing, China) and cloned into a pET28A vector. Nsp7 and 8 were expressed with N-terminal 6 His-tag in BL21 (DE3) at 37 ºC and induced with 0.5 mM of IPTG for 2 hours. Next, cells were harvested and lysed by Branson sonifier in buffer containing 50 mM Tris pH 7.5, 500 mM NaCl and 25mM imidazole. Cell extract was clarified by centrifugation at 4000 rpm for 10 min at 4 ºC. Both proteins were purified by Nickel-affinity chromatography followed by ion-

exchange chromatography (using HisTrap FF and Superdex 200 increase 10/300 GL columns, respectively, GE Healthcare). After first column peak fractions were combined, concentrated to 1 mM and injected onto second column in a buffer containing 20 mM HEPES pH 7.5, 150 mM NaCl, 5mM Glycerol and 1 mM Tris(2-carboxyethyl)phosphine. Peaks were combined and concentrated to 500 mM and stored at -80 °C. The protein concentration was determined by A280 using the extinction coefficient calculated with ProtParam.

[00580] **Expression and purification of mitochondrial RNA polymerase (POLRMT)**

[00581] POLRMT D141 gene (amino acids 142-1230, GenBank: AAH98387.1) was synthesized by GenScript ((Nanjing, China) and cloned into a pET28A vector. POLRMT D141 was expressed with N-terminal 6 His-tag in BL21 (DE3) at 18 °C and induced with 0.25 mM of IPTG for 18 hours. On the next day, cells were harvested and lysed by Branson sonifier in buffer containing 50 mM Tris pH 7.5, 500 mM NaCl, 1 mM $MgCl_2$, 20% glycerol, 5 mM BME. After centrifugation at 4000 rpm, Nsp12 was purified by Ni-Sepharose resin (GE Healthcare) using 20-mL gravity-flow column. After elution at 250 mM imidazole, fractions were combined and concentrated then diluted with buffer containing 50 mM Tris pH 8, 500 mM NaCl, 20% glycerol, 10 mM BME, 500 mM EDTA and stored at -80 °C. The protein concentration was determined by A280 using the extinction coefficient calculated with ProtParam.

[00582] **Expression and purification of primase/polymerase protein isoform 1 (PrimPol)**

[00583] PrimPol gene (amino acids 2-559, GenBank: NP_001332824.1) was synthesized by GenScript (Nanjing, China) and cloned into a pET28A vector. PrimPol was expressed with N-terminal 6 His-tag in BL21 (DE3) at 18 °C and induced with 0.5 mM of IPTG for 24 hours. On the next day, cells were harvested and lysed by Branson sonifier in buffer containing 50 mM Tris pH 7.5, 500 mM NaCl, 1 mM $MgCl_2$, 20% glycerol, 5 mM BME. After centrifugation at 4000 rpm, Nsp12 was purified by Ni-Sepharose resin (GE Healthcare) using 20-mL gravity-flow column. After elution at 250 mM imidazole, fractions were combined and concentrated then diluted with buffer containing 50 mM Tris pH 8, 500 mM NaCl, 20% glycerol, 10 mM BME, 500 mM EDTA and stored at -80 °C. The protein concentration was determined by A280 using the extinction coefficient calculated with ProtParam.

[00584] **Primer and template annealing for SARS-Cov2 RdRp polymerization**

[00585] To generate RNA primer-template complexes for primer extension assay of SARS-Cov-2 RdRp, 1 mM fluorescently (Cy5.5) labeled RNA primer and 10 mM unlabeled RNA template (Table 1) were mixed in 50 mM NaCl in nuclease-free water, incubated at 98 °C for 10 min and slowly cooled to room temperature. The annealed primer and template complexes (P/Ts) were stored at -20 °C before use in primer extension assays.

[00586] **Primer extension assay**

[00587] Primer extension assay was performed. In brief, P/Ts were prepared by annealing Cy5/5 labeled RNA primer and unlabeled RNA template (described above). Primer extension reaction was performed in a 10 mL reaction mixture containing reaction buffer (20 mM HEPES pH 7.5, 5mM MgCl2, 10 mM DTT, 0.01% Tween 20 and 1 kU/ml RNase Inhibitor), 200 nM P/T and 2 mM Nsp12 and Nsp8, and 10 mM Nsp7. The reaction was initiated by addition of rNTP at a final concentration of 100 mM, followed by incubation for 1 hour at 37 °C. Reactions were quenched by addition of 20 mL of stopping solution (8M Urea, 90 mM Tris base, 29 mM Taurine, 10 mM EDTA, 0.02% SDS, 0.1% bromophenol blue). The quenched samples were denaturated at 95 °C for 10 min and primer extensions products were separated using 15% denaturing polyacrylamide gel electrophoresis (Urea-PAGE) in TTE buffer (90 mM Tris base, 29 mM Taurine, 0.5 mM EDTA). Gels were scanned using ChemiDoc MP imaging system (Bio-Rad, California, USA).

[00588] **Analysis of chain termination ability of nucleotide analogs**

[00589] Nucleotide analog incorporation assay was performed using same concentrations as described above in primer extension assay reactions: 200 nM P/T and 2 mM Nsp12 and Nsp8, and 10 mM Nsp7. Reaction started with addition of the natural rNTP (the first nucleotide to be incorporated) and tested nucleotide analog (the second nucleotide to be incorporated) and incubated for 30 min at 37 °C before addition stopping solution. For chain termination assay: Incorporation of nucleotide analogs was performed as described above and then two natural rNTP (third and fourth nucleotide to be incorporated) were added to the reaction mixture and samples were incubated at 37 °C for another 30 min before addition stopping solution. The quenched samples were denatured at 95 °C for 10 min and primer extensions products were separated using 15% denaturing polyacrylamide gel electrophoresis (Urea-PAGE) as described above. After electrophoresis gels were scanned using ChemiDoc MP imaging system.

[00590] **SARS -Cov2 RdRp inhibition assay**

[00591] In reaction mixture containing 20 mM HEPES pH 7.5, 5mM MgCl2, 10 mM DTT, 0.01% Tween 20, 1 kU/ml RNase Inhibitor, 200 nM P/T, 2 mM Nsp12 and Nsp8 and 10 mM Nsp7 was incubated with natural nucleotide (first nucleotide to be incorporated) for 30 min at 37 °C. After first incorporation, nucleotide analogs were added using 12-step serial dilution in 2- and 5-fold increments and preincubated at 30 °C for 10 min. The reaction was initiated with addition of 50 mM of natural rNTP at 30 °C for 30 min and quenched with stopping solution. The quenched samples were treated as described above and gels were scanned using ChemiDoc MP imaging system. The images were analyzed and quantified using ImageLab™ software (Bio-Rad, California, USA).

[00592] **Inhibition of human mitochondrial RNA polymerase (POLRMT)**

[00593] To generate RNA primer-DNA template complexes for inhibition assay of mitochondrial RNA polymerases (POLRMP), 10 mM fluorescently (Cy5.5) labeled RNA primer and 10 mM unlabeled DNA template were mixed in 50 mM NaCl in nuclease-free water, incubated at 95 ºC for 5 min and slowly cooled to room temperature. The annealed primer and template complexes (P/Ts) were stored at -20 ºC before use in primer extension assays. Reaction mixtures contained 50 mM Tris-HCl pH 7.5, 100 mM NaCl, 2.5 mM MgCl2, 2 mM DTT, 0.05mg/ml BSA, 1 mM primer-template and 1 mM of POLRMT. Enzyme was preincubated with the P/T at 37 ºC for 10 min and nucleotide analogs were added using 10-step serial dilution in 2- and 5-fold increments followed immediately by addition of 100 mM of rNTP. All samples were incubated for 30 min at 30 ºC. Reaction mixtures were quenched and treated as described above. The images were analyzed and quantified using ImageLab™ software (Bio-Rad, California, USA).

[00594] **Inhibition of mitochondrial DNA polymerase (POLG1)**

[00595] Inhibition assay of POLG1 was conducted in the similar manner as described above except that the concentration of POLG1 was modified to 100 nM and the incubation temperature to 37 ºC.

[00596] **Inhibition of primase/polymerase protein isoform 1 (PrimPol)**

[00597] Inhibition assay of PrimPol was conducted in the similar manner as described in the paragraph above except that the concentration of PrimPol was 1 mM, the incubation time - one hour and temperature - 25 ºC.

[00598] **Single nucleotide incorporation by mitochondrial DNA polymerase (POLG1)**

[00599] A mixture of buffer 50 mM Tris-HCl pH 7.5, 100 mM NaCl, 2.5 mM MgCl2, 2 mM DTT, 0.05mg/ml BSA, 200 nM P6/T6 or P6/T7 (Table 1) and 100 nM POLG1 was preincubated at 37 ºC for 5 min. The reaction was started by addition 100 mM of dGTP/dUTP (depending on the template in use) for 30 min at 30 ºC. For the next step, 500 mM of NTP analog was added at 37 ºC and 10 uL of reaction was removed and quenched with stopping solution at 0, 2, 5, 10, 20, 30, 60 and 90 min. The quenched samples were denaturated at 95 ºC for 10 min and primer extensions products were separated using 15% denaturing polyacrylamide gel electrophoresis (Urea-PAGE) in TTE buffer (90 mM Tris base, 29 mM Taurine, 0.5 mM EDTA).

[00600] **IMPDH inhibitor screening assay**

[00601] IMPDH inhibition screening assay kit was purchased for BioVision (California, USA).

TABLE 34. RNA and DNA oligonucleotides used in this study.

Oligo	Sequence
P1	Cy5.5 – 5'- GUCAUUCUCCUAAGAAGCUAUUAAAAUCAC – 3'
P6	Cy5.5 – 5' – UUUUGCCGCGCC – 3'
P9	Cy5.5 – 5' – TTTTGCCGCGCC – 3'
*T2	5'- AUUAUUGC**U**AGUGAUUUUAAUAGCUUCUUAGGAGAAUGAC – 3'
T3	5'- AUUAUUGU**C**AGUGAUUUUAAUAGCUUCUUAGGAGAAUGAC – 3'
T4	5'- AUUAUUGC**A**UGUGAUUUUAAUAGCUUCUUAGGAGAAUGAC – 3'
dT1	5' – GGGAATGCA**T**GGCGCGGC – 3'
dT2	5' – GGGAATCAT**G**GGCGCGGC – 3'
dT3	5' - GGGAATGTA**C**GGCGCGGC – 3'
dT4	5' – GGGAATTGC**A**GGCGCGGC – 3'
dT6	5' – GCAAATTAGTGGT**A**CCGGCGCGGC – 3'
dT7	5' – GCAAATTAGTGGT**C**AAGGCGCGGC – 3'

*Bold and underlined nucleotides indicate position for the incorporation of the natural rNTP/dNTP or NTP analog.

[00602] *Results:*

[00603] **Expression, purification, and activity measurements of SARS-Cov2 Nsp12-Nsp7-Nsp8**

[00604] Nsp12, Nsp7, and Nsp8 were successfully expressed and purified *from E. coli*. The functional nsp12-nap8-nsp7 complex was assembled by simply mixing nsp12, nsp7 and nsp8 together *in vitro* as described before. For activity measurements of purified complex, a recently developed a primer extension assay was used employing an RNA template (40-mer RNA corresponding to sequence of 3'-end of SARS-Cov2 RNA genome) and a fluorescently labeled RNA primer (30-mer) (Table 34) . In this report, three RNA templates (Table 34, T2-T4) were used to form three different P1/T scaffolds (**Figure 21**) for testing incorporation of different nucleotide analogs.

[00605] For optimal nsp7-nsp8-nsp12 concentrations we used an enzyme dilution assay with different concentrations of proteins. The results showed that nsp12 alone did not possess any RNA synthesis ability (**Figure 22**). Maximum activity was observed in the in the presence of all components with following concentration ratio: 10 mM nsp7, 2 mM nsp8 and nsp12 (**Figure 23**).

[00606] **Nucleotide incorporation of Remdesivir-TP (REM-TP) and ddhRemdesivir (ddhREM-TP) by SARS-Cov2 RdRp**

[00607] It was shown that REM-Triphosphate (REM-TP) can incorporate into RNA and this incorporation causes delayed chain termination. Here, the incorporation of ddhREM-TP was evaluated and compare with incorporation of REM-TP into 30-mer P1/T2 template (Table 34) that was designed for testing ATP analogs. P1/T2 template requires first incorporation of UTP and second incorporation of ATP analog, in this case – REM-TP and ddhREM-TP. The results showed that REM-TP and ddhREM-TP incorporated into RNA as ATP analogs at concentration of 100 uM (**Figure 24**, lane 4 and 5). After addition GTP and CTP (third and fourth nucleotides to be incorporated), if incorporation of ATP analog leads to chain termination, the band will not extend. Incorporation of REM-TP did not cause chain termination while ddhREM-TP caused termination of RNA synthesis, since the elongation continued to the position of n+10 (**Figure 25**, Lane 4 and 5).

[00608] **Inhibition of SARS-Cov2 RdRp by REM-TP and ddhREM-TP**

[00609] To interrogate the inhibitory effect of ddhREM-TP versus REM-TP, half-maximal inhibitory concentration (IC50) ddhREM-TP and REM-TP was calculated against RdRp. As summarized in Table 35, IC50 of ddhREM-TP was 1.32 ± 0.42 mM while IC50 of REM was 65 ± 21 mM mM (**Figure 26**). These results indicate that ddhREM-TP with its mechanism of chain termination is a potent inhibitor of RNA replication of SARS-Cov2 RdRp while REM-TP shows inhibitory effect only in high concentrations.

TABLE 35. Inhibition of SARS-Cov2 RdRp mad human polymerases by NTP analogs

Enzyme	**REM-TP IC50 (mM)**	**ddhREM-TP IC50 (mM)**	**ddhUTP IC50 (mM)**	**ddhGTP IC50 (mM)**
SARS-Cov2 RdRp	65 ± 0.42	1.32 ± 0.42	1.9 ± 0.1	0.29 ± 0.07
POLRMT	> 200[a]	> 600	> 150	ND
POLG1	> 200[a]	> 250	> 250	ND
PrimPol		> 250	> 250	ND

[00610] [a]Taken from the literature.

[00611] **Nucleotide incorporation and inhibition of SARS-Cov2 RdRp by ddhUTP and ddhGTP**

[00612] To test the incorporation of ddhUTP and ddhGTP, 30-mer P1/T4 template for UTP

analogs incorporation and 30-mer P1/T3 for GTP analogs incorporation were used (Table 34). P1/T4 template requires first incorporation of ATP and second incorporation of UTP analog. P1/T3 template requires first incorporation of UTP and second incorporation of GTP analogs. The results showed that ddhUTP incorporated onto RNA as UTP analog at concentration of 100 mM (**Figure 27A**, lane 9 and 10). The incorporation of the natural nucleotide and known chain terminator - 3'-deoxyuracil triphosphate (3'-dUTP) that was used as a positive control, demonstrated the same level of incorporation as ddhUTP (**Figure 27A**, lane 5-6 and 7- 8). Likewise, ddhGTP also incorporates onto RNA as GTP analog at the same concentration (**Figure 28A**, lane 5) the same as natural nucleotide and its positive control, 3'-dGTP (**Figure 28A**, lane 3 and 4).

[00613] After incorporation of natural nucleotide, the addition of GTP and CTP (third and fourth nucleotides to be incorporated), proceeds RNA elongation up to 10th position. However, the incorporation of ddhUTP and ddhGTP caused chain termination of RNA synthesis, similarly as canonical chain terminator, 3'-dUTP and 3'-dGTP (**Figures 27B**, lanes 7-10 and **Figure 28B** lanes 4, 5). Both ddh nucleotides were able to terminate the polymerization of RNA at concentration of 100 mM.

[00614] ddhUTP and ddhGTP inhibitory effect on SARS-Cov2 RdRp was analyzed by calculating the half-maximal inhibitory concentration (IC50) of ddhUTP and ddhGTP against RdRp. IC50 of 1.9 ± 0.1 and 0.29 ± 0.07 mM for ddhUTP and ddhGTP, respectively (Table 25, **Figure 29**) indicates that both compounds are a potent inhibitors of RNA replication of SARS-Cov2 RdRp.

[00615] **Inhibition of human DNA and RNA polymerases**

[00616] To assess the potential for off-target effect we tested the inhibition effect of potent inhibitors - ddhREM-TP and ddhUTP with the potential molecular targets of toxicity - POLG1, POLRMT and PrimPol. For ddhREM-TP toxicity, P6/dT1 primer/template complex requires 1st incorporation of analog –and then 2nd and 3rd incorporation of UTP and GTP. For ddhUTP toxicity, P6/dT4 primer/template complex requires 1st incorporation of analog –and then 2nd and 3rd incorporation of GTP and CTP. **Figures 30-31** show that none of the ddh analogs inhibited POLRMT in concentration of 100 mM with IC50 of >200 mM. **Figures 32 and 33** demonstrate inhibitory effect of POLG1 and PrimPol, respectively by ddhREM-TP and ddhUTP (Table 35). These results indicate that ddhREM-TP and ddhUTP are poor substrates for POLG1, POLRMT and PrimPol.

[00617] **Human inosine-5'-monophosphate dehydrogenase 2 (IMPDH2) inhibitor screening assay**

[00618] IMPDH2 is a rate-limiting enzyme in *de novo* guanine nucleotide biosynthesis.

IMPDH2 oxidizes inosine 5'-monophosphate (IMP) to xanthine 5'-monophopshate (XMP) using NAD as a cofactor. This enzyme is critical in cell growth and recognized as a validated target of some antiviral agents. Here, the goal was to examine if monophosphates metabolites of ddhUTP and ddhGTP can bind IMPDH and block its activity. To test the inhibitory effect of ddhUMP and ddhGMP toward IMPDH a BioVision IMPDH inhibitor screening assay was used, where IMP oxidized by IMPDH producing series of intermediates, which react with the probe to generated colorimetric signal (OD 450 nm). The signal is directly proportional to the IMPDH activity. Mycophenolic acid (MPA), a known IMPDH inhibitor was used as a positive control. Ribavirin 5'- monophosphate (RMP), that also known as IMPDH inhibitor that inhibits viral DNA and RNA replication through guanosine triphosphate synthesis was used as another positive control and antiviral agent. MPA and RMP demonstrated strong inhibition of IMPDH with IC50 of 76 ± 10 nM and 2.6 ± 1.8 mM, respectively (**Figure 34A**). ddhUMP and ddhGMP both failed decrease IMPDH activity - IC50 > 1mM (**Figure 34B**). These results indicates that monophosphate form of ddhUTP and ddhGTP do not inhibit IMPDH at concentration that were used here.

Example 20 – ANTIVIRAL ACTIVITY OF ddh-COMPOUNDS IN hCMV ASSAY

[00619] *Objective*: To examine the anti-hCMV activity of ddh-Compounds: AB21649 (compound 24; Formula IVB), AB23031 (compound 26; Formula IVB), AB23039 (compound 104; Formula IVB); AB21651 (compound 31; Formula IIB), AB23032 (compound 33; Formula IIB), and AB23040 (compound 103; Formula IIB).

[00620] *Methods*:

[00621] **HCMV Plaque Reduction Assay**

[00622] *General Description of HCMV Plaque Reduction Assay*

[00623] MRC-5 cells were seeded at 1 x 105 cells/well in 24 well plates using MRC-5 growth medium. The plates were incubated overnight at 37°C and 5% CO_2. The following day, media is aspirated and approximately 100 plaque forming units (pfu) of HCMV AD169 is added to 21 wells of each plate in a volume of 200 µL of assay medium (MRC-5 growth medium containing 2% FBS rather than 10% FBS). The remaining three wells of each plate serve as cellular control wells and receive 200 µL of assay medium without virus. The virus is allowed to adsorb onto the cells for 1 hr at 37°C and 5% CO2.

[00624] Compounds were prepared by diluting them in assay medium containing 0.5% Methylcellulose. After the incubation period, 1 mL of each drug dilution is added to triplicate

[00625] wells of a plate (without aspirating the virus inoculum). Assay medium (without drug)

containing 0.5% Methylcellulose is added to the three cell control wells and to three virus control wells on each plate. Table 36 represents the standard plate format for evaluating the efficacy of compounds at 6 (triplicate) concentrations using a representative high-test concentration of 100 μM.

TABLE 36: Plate Format for Human Cytomegalovirus Plaque Reduction Assays

Triplicate (6 dilutions)

	1	2	3	4	5	6
A	Cell Control			Virus Control		
B						
C	Drug 1 High-Test 100 μM	Drug 1 32 μM	Drug 1 10 μM	Drug 1 3.2 μM	Drug 1 1.0 μM	Drug 1 Low-Test 0.32 μM
D						

[00626] The plates were incubated for 6 days to allow for plaque formation. Cultures were examined microscopically, and compound precipitation and toxicity were noted. The media is then aspirated from the wells and the cells were fixed and stained using 20% Methanol containing Crystal Violet. Plaques were enumerated by microscopic inspection and the data is plotted as percent of virus control.

[00627] *MRC-5 Cell Culture (ATCC CCL-171)*

[00628] MRC-5 cells (Embryonal lung fibroblast, diploid, male, Human) were obtained from the American Type Culture Collection (ATCC, Rockville, Maryland) and were grown in Dulbecco's Modified Eagle's Medium DMEM) supplemented with 10% fetal bovine serum (FBS), 0.1 mM non-essential amino acids, 1.0 mM sodium pyruvate, 2.0 mM L-Glutamine, 100 units/ml Penicillin and 100 μg/ml Streptomycin ("MRC-5 growth medium"). Cells were sub-cultured twice a week at a split ratio of 1:2 using standard cell culture techniques.

[00629] *HCMV Strain AD169 (ATCC VR-538)*

[00630] HCMV Strain AD169 was obtained from the ATCC. Virus stocks were prepared by infecting approximately 80% confluent monolayers of MRC-5 cells at a minimal multiplicity of infection in MRC-5 growth medium containing a reduced FBS concentration (2%). Monolayers were incubated at 37°C and 5% CO2 until 90-95% viral cytopathic effect (CPE) was observed (6 days). Culture medium was then collected from the cells, centrifuged at low speed to remove

cellular debris, aliquoted in 1 ml volumes and frozen/stored at -80°C as stock virus. The virus titer of the stock was determined by infecting MRC-5 cells with serial dilutions of the stock virus using an overlay medium. Plaques were enumerated in the cultures where the dilutions of virus allowed for the formation of individual plaques and the titer was determined based upon the number of plaques counted, the dilution of virus used and the volume of virus used to infect the cells.

[00631] *MRC-5 Cytotoxicity Assay*

[00632] MRC-5 cells were seeded at 1 x 104 cells per well in 96 well plates using MRC-5 growth medium. The plates were incubated overnight at 37°C and 5% CO2. The following day, compounds were prepared in assay medium. Growth medium is removed from the plates and replaced with the prepared test compounds. Each dilution of compound is tested in triplicate. Each toxicity plate contains the necessary cell control and compound color controls. Table 37 represents the standard plate format for evaluating toxicity of compounds at 6 (triplicate) concentrations using a representative high-test concentration of 100 μM.

TABLE 37: Plate Format for Human Cytomegalovirus Cytotoxicity Assays

Triplicate (6 dilutions)

	1	2	3	4	5	6	7	8	9	10	11	12
A	Reagent Background Control Wells (Media plus MTS, no cells)											
B	Color 1 100 μM	Drug 1 Tox High-Test 100 μM			Drug 2 Tox High-Test 100 μM			Drug 3 Tox High-Test 100 μM			Cell Control	Reagent Background Control Wells (Media plus MTS, no cells)
C	Color 1 32 μM	Tox 1 32 μM			Tox 2 32 μM			Tox 3 32 μM				
D	Color 1 10 μM	Tox 1 10 μM			Tox 2 10 μM			Tox 3 10 μM				
E	Color 1 3.2 μM	Tox 1 3.2 μM			Tox 2 3.2 μM			Tox 3 3.2 μM				
F	Color 1 1.0 μM	Tox 1 1.0 μM			Tox 2 1.0 μM			Tox 3 1.0 μM				
G	Color 1 0.32 μM	Drug 1 Tox Low-Test 0.32 μM			Drug 2 Tox Low-Test 0.32 μM			Drug 3 Tox Low-Test 0.32 μM				
H	Color 2 100 μM	Color 2 32 μM	Color 2 10 μM	Color 2 3.2 μM	Color 2 1.0 μM	Color 2 0.32 μM	Color 2 100 μM	Color 2 32 μM	Color 2 10 μM	Color 2 3.2 μM	Color 2 1.0 μM	Color 2 0.32 μM

Cells labeled as "Tox 1", "Tox 2", or "Tox 3" = Cells + Drug 1, Drug 2, or Drug 3, respectively (toxicity tested in triplicate)
Cells labeled as "Color 1" "Color 2", or "Color 3" = Media + Drug 1, Drug 2, or Drug 3, respectively (colorimetric background, no cells)

[00633] After a six day incubation period, cell viability is determined using CellTiter 96 Aqueous One Solution (Promega Corporation). This solution contains MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium) which is converted by viable cells into an intensely colored formazan product. The solution is added to the

[00634] wells of the 96 well plate in a volume resulting in a final 10% concentration (volume:volume) in each well.

[00635] Plates were incubated for an additional 4 hours at 37°C. Adhesive plate sealers were

used in place of the lids, the sealed plates were inverted several times to mix the soluble formazan product and the plate is read spectrophotometrically at 490 and 650 nm with a Molecular Devices SpectraMax i3 plate reader. In addition to MTS dye reduction, the visual cytotoxicity of each concentration of test compound is visually assessed directly on the plates used for plaque assay. In this case, the appearance of cells in wells that contain both test article and virus were compared to the appearance of cells in the virus control. Visual cytotoxicity (tox) is scored as follows: 0 = 0% tox, 1 = monolayer is disturbed, 2 = monolayer is up to 50% gone, 3 = significant loss of monolayer, and 4 = 100% tox (no cells).

[00636] *Data Analysis*

[00637] The minimum inhibitory drug concentration that reduces plaque formation by 50% (EC50) and the minimum drug concentration that inhibits cell growth by 50% (CC50) were calculated. A selectivity index (SI) for each active compound is determined by dividing the CC50 by the EC50. Raw data for both antiviral activity and toxicity with a graphical representation of the data were provided in a printout summarizing the individual compound activity.

[00638] *Results:*

TABLE 38: hCMV assay (MRC5 cells, AD169 strain of hCMV)

Test Article	EC_{50} (μM)	CC_{50} (μM)	Selectivity Index (CC_{50}/EC_{50})
AB21649	>100	>100	N/A
AB23031	>100	>100	N/A
AB23039	18.1	42.2	2.33
AB21651	>100	>100	N/A
AB23032	>100	>100	N/A
AB23040	22.1	>100	> 4.52
Ganciclovir	7.36	>100	>13.6

Plaque assay, 6-days duration

[00639] *Summary:* The data presented above for AB23039 (Hostetler prodrug of ddhU) and AB23040 (Hostetler prodrug of ddhG) indicate that these ddh-compounds show impressive antiviral activity against hCMV.

Example 21 –ANTIVIRAL ASSESSMENT OF NATURAL NUCLEOSIDE ANALOGS

[00640] *Objective*: To examine the antiviral (BCV, JCV, and hCMV) activity, and effects on HFF and MOLT-3 proliferation of ddh-Compounds: AB23031 (compound 26; Formula IVB), AB23039 (Formula IVB); AB23032 (compound 33; Formula IIB), and AB23040 (Formula IIB).

[00641] *Methods*:

[00642] Compounds were solubilized by adding DMSO to each, based on quantities and molecular weights specified to achieve a final 30mM concentration. These stocks were stored at -20°C. There were no problems with solubility of the compounds in DMSO.

[00643] The working test compound solutions (1.5; 1.0mM) were prepared by diluting the DMSO stocks in warmed MEM + 2%FBS. There were no problems with solubility of the compounds in 2% MEM.

[00644] Positive and negative control drugs were prepared as per usual with working solutions at 1.5 and 1.0 mM in MEM + 2%FBS.

[00645] **Cell Proliferation Assay – Human Foreskin Fibroblast (HFF) Cells**

[00646] HFF cells were seeded in 6-well plates at a concentration of 2.5 x 10^4 cells per well. The plates were incubated for 24 h and the media removed prior to adding compound dilutions in growth media. The cells were incubated for 72 h at 37°C, then the cells were removed with trypsin/EDTA and viable cells in duplicate wells were enumerated with a Coulter Counter. Average cell number in untreated control cultures was used to calculate the concentration of compound sufficient to reduce cell number by 50% (IC_{50}).

[00647] **Cell Proliferation Assay – Human Lymphoblastoid MOLT-3 Cells**

[00648] Drug dilutions were prepared in a 384 well spheroid plate as per usual. MOLT-3 cells were dispensed into each well at a concentration of 2.0 x 10^3 cells per well and the plate was incubated for 7d at 37°C. After incubation, CellTiter-Glo was added and the relative light units determined using a luminometer. The concentration of compound sufficient to reduce cell number by 50% was calculated as the CC_{50}.

[00649] **Secondary JCV Assay**

[00650] COS7 cells in MEM with 10% FBS were plated into 96 well plates and incubated overnight in a CO_2 incubator. The media was then removed and replaced with 2% MEM. Drugs at the appropriate starting concentrations were added and then serially diluted 1:3 for 12 dilutions. Virus or media, for toxicity determinations, was then added and the plates incubated for 7 days. After 7 days, the DNA was extracted and quantified by qPCR using the primers and probes previously given for JC virus. EC_{50} and EC_{90} values were then calculated.

[00651] **Secondary BKV Assay**

[00652] Human foreskin fibroblast cells in media with 10% FBS were plated into 96 well plates and incubated overnight in a CO_2 incubator. The media was then removed and replaced with 2% MEM. Drugs at the appropriate starting concentrations were added and then serially diluted 1:3 for 12 dilutions. Virus or media, for toxicity determinations, was then added and the plates

incubated for 7 days. After 7 days, the DNA was extracted and quantified by qPCR using the primers and probes previously given for BK virus. EC_{50} and EC_{90} values were then calculated.

[00653] **Virus Yield Reduction (VYR) Assay – Human Cytomegalovirus**

[00654] Monolayers of HFF cells were prepared in 96-well plates (Source Plate) and incubated at 37°C for 1 d to allow the cells to reach confluency. Medium was aspirated from the wells and cells infected at a high MOI with wild-type and drug resistant isolates of HCMV. At 1 h following infection, the inoculum was removed and the monolayers rinsed with fresh media. Compounds were diluted in assay media consisting of MEM with Earl's salts supplemented with 2% FBS, L-glutamine, penicillin, and gentamicin. Drug dilutions were prepared and added to the wells and the plates were incubated for 4 d. A duplicate set of dilutions were also performed but remained uninfected to serve as cytotoxicity/cell controls and received equal compound exposure or medium. Supernatants from each of the infected wells were subsequently transferred to 384 well plates containing HFF cells and these plates incubated for 14 d. Viral yield in the supernatants from the infected cells were titered using qPCR with HCMV primers 5'- AGG TCT TCA AGG AAC TCA GCA AGA-3', 5'-CGG CAA TCG GTT TGT TGT AAA-3', and probe 5'-CCG TCA GCC ATT CTC TCG GC-3' and data analyzed in a $TCID_{50}$ assay to quantify the progeny virus. For cytotoxicity controls, drug toxicity was assessed (Source Plate - incubated for 7d) using CTG according to the manufacturer's suggested protocol. The concentration of compound that reduced virus titer by 90% (EC_{90}) or compound sufficient to reduce cell number by 50% (calculated as the CC_{50}) was interpolated from the experimental data.

[00655] *Results*

[00656] *Secondary JCV Results*

[00657] Test drug concentration 0.001-100 μM.

[00658] Control drug concentration 0.001-100 μM.

Drug	Activity	EC_{50}	EC_{90}	CC_{50}	SI_{50}	SI_{90}
AB23031	NA	93.82	>100.00	>100.00	>1	1
AB23032	NA	>100.00	>100.00	>100.00	1	1

NA (Not Active) SI_{50} = 0 - 3.9
LO (Slightly Active) SI_{50} = 4 - 9.9
MOD (Moderately Active) SI_{50} = 10 - 19.9
HI (Highly Active) SI_{50} = ≥20

Drug	Activity	EC_{50}	EC_{90}	CC_{50}	SI_{50}	SI_{90}
AB13039	MOD	3.07	9.44	46.77	15	5
AB23040	HI	0.37	18.55	31.28	85	2

NA (Not Active) SI_{50} = 0 - 3.9
LO (Slightly Active) SI_{50} = 4 - 9.9
MOD (Moderately Active) SI_{50} = 10 - 19.9
HI (Highly Active) SI_{50} = ≥20

[00659] *Secondary BKV Results*

[00660] Test drug concentration 0.001-100 μM.

[00661] Control drug concentration 0.001-100 μM.

Drug	Activity	EC_{50}	EC_{90}	CC_{50}	SI_{50}	SI_{90}
AB23031	NA	>100.00	>100.00	>100.00	1	1
AB23032	NA	>100.00	>100.00	>100.00	1	1

NA (Not Active) SI_{50} = 0 - 3.9
LO (Slightly Active) SI_{50} = 4 - 9.9
MOD (Moderately Active) SI_{50} = 10 - 19.9
HI (Highly Active) SI_{50} = ≥20

Drug	Activity	EC_{50}	EC_{90}	CC_{50}	SI_{50}	SI_{90}
AB23039	NA	22.83	32.80	46.44	2	1
AB23040	NA	>20.00	>20.00	50.05	<3	<3

NA (Not Active) SI_{50} = 0 - 3.9
LO (Slightly Active) SI_{50} = 4 - 9.9
MOD (Moderately Active) SI_{50} = 10 - 19.9
HI (Highly Active) SI_{50} = ≥20

[00662] *Summary:*

[00663] Proliferation assays were performed using AB23039 and AB23040 in human foreskin fibroblast (HFF) cells and human lyphoblastoid MOLT-3 cells (4 assays total). AB23039 showed no inhibition of cell growth at the highest concentration tested (IC_{50} = >100 μM) in the proliferation assay using HFF cells. The same was noted for this compound at the highest concentration tested (CC_{50} = >150 μM) in the proliferation assay using MOLT-3 cells. AB23040 yielded values of 65.90 μM and 112.04 μM in the HFF proliferation and the MOLT-3 assay, respectively. Positive and negative controls drugs performed as expected in these assays.

[00664] Secondary testing results against JCV demonstrate moderate (AB23039) and high (AB23040) antiviral activity for these compounds, as well as a measure of toxicity for each: AB23039 - CC_{50} = 46.77 μM; AB23040 - CC_{50} = 31.28 μM in COS7 cells.

[00665] Compounds AB23031 and AB23032 presented neither antiviral nor cytotoxic effects against JCV in COS7 cells. Secondary results against BKV showed no significant antiviral activity for any of the 4 compounds tested, but similar cytotoxic effects for AB23039 and AB23040—CC_{50} = 46.44 μM and CC_{50} = 50.05 μM, respectively—were seen using HFF cells in this assay. (Total assays tested = 8)

[00666] Secondary testing results against HCMV wild-type and drug resistant isolates were performed using virus yield reductions assays. While positive control drugs performed as expected against both virus types, no antiviral activity was demonstrated for AB23031, AB23032, AB23039 or AB23040. Cytotoxicity was observed for AB23039 (CC_{50} = 50.72 μM) and AB23040 (CC_{50} =

45.70 μM) only.

Example 22 –ANTIVIRAL ASSESSMENT OF NATURAL NUCLEOSIDE ANALOGS

[00667] *Objective*: To examine compounds AB23039 (compound 104; Formula IVB) and AB23040 (compound 103; Formula IIB)) in primary assays against Herpes simplex virus 1 (HSV-1), Human cytomegalovirus (HCMV), Epstein-Barr virus (EBV), BK virus (BKV) and JC virus (JCV) (10 assays total). Brincidofovir (BCV) was added as an additional positive control drug to be used against all of the viruses tested in the primary assays.

[00668] *Methods*:

[00669] Compounds AB23031 (compound 26; Formula IVB) and AB23039 were solubilized by adding DMSO to each, based on quantities and molecular weights specified to achieve a final 30mM concentration. These stocks were stored at -20°C. AB2304 was solubilized to a final 25mM DMSO stock concentration and was also stored at -20°C after use.

[00670] There were no problems with solubility of the compounds in DMSO.

[00671] The working test compound solutions (1.5mM) were prepared by diluting the DMSO stocks in warmed MEM + 2%FBS.

[00672] There were no problems with solubility of the compounds in 2% MEM.

[00673] Positive and negative control drugs were prepared as per usual with working solutions at 1.5, 1.0 and 0.1 mM in MEM + 2%FBS.

[00674] See Example 20 above.

[00675] *Results:*

[00676] **Primary Assay Results**

[00677] *Herpes Simplex Virus 1 (HSV-1)*

[00678] Test Drug Concentration: 0.048-150 μM.

[00679] Control Drug Concentration: 0.048-150; 0.003-10 μM.

TABLE 39: HSV-1 Assay Results

Drug	Activity	EC_{50}	EC_{90}	CC_{50}	SI_{50}	SI_{90}
ACV	-	0.66	1.59	>150.00	>227	>94
TNV	-	>150.00	>150.00	>150.00	1	1
BCV	-	0.06	>10.00	>10.00	>167	1
AB23039	NA	>30.00	>30.00	87.34	<3	<3
AB23040	NA	>30.00	>30.00	72.10	<2	<2

NA (Not Active) SI_{50} = 0 - 3.9

LO (Slightly Active) SI_{50} = 4 - 9.9

MOD (Moderately Active) SI_{50} = 10 - 49.9

HI (Highly Active) SI_{50} = ≥50

[00680]

[00681] *Human Cytomegalovirus (hCMV)*

[00682] Test Drug Concentration: 0.048-150 μM.

[00683] Control Drug Concentration: 0.048-150; 0.0003-1 μM.

TABLE 40: hCMV Assay Results

Drug	Activity	EC_{50}	EC_{90}	CC_{50}	SI_{50}	SI_{90}
GCV	-	0.20	1.07	>150.00	>750	>140
TNV	-	>150.00	>150.00	>150.00	1	1
BCV	-	0.001	>1.00	>1.00	>1000	1
AB23039	NA	>30.00	>30.00	78.03	<3	<3
AB23040	NA	>6.00	>6.00	13.89	<2	<2

NA (Not Active) SI_{50} = 0 - 3.9

LO (Slightly Active) SI_{50} = 4 - 9.9

MOD (Moderately Active) SI_{50} = 10 - 49.9

HI (Highly Active) SI_{50} = ≥50

[00684] *Epstein-Barr Virus (EBV)*

[00685] Test Drug Concentration: 0.048-150 μM.

[00686] Control Drug Concentration: 0.032-100; 0.003-10 μM.

TABLE 41: EBV Assay Results

Drug	Activity	EC_{50}	EC_{90}	CC_{50}	SI_{50}	SI_{90}
CDV	-	6.58	62.89	>100.00	>15	>2
TNV	-	96.37	>100.00	>100.00	>1	1
BCV	-	0.91	7.17	>10.00	>11	>1
AB23039	NA	>6.00	>6.00	24.33	<4	<4
AB23040	NA	>30.00	>30.00	38.09	<1	<1

NA (Not Active) SI_{50} = 0 - 3.9

MOD (Moderately Active) SI_{50} = 4 - 9.9

HI (Highly Active) SI_{50} = ≥10

[00687] *BK Virus (BKV)*

[00688] Test Drug Concentration: 0.048-150 μM.

[00689] Control Drug Concentration: 0.048-150; 0.003-10 μM.

TABLE 42: BKV Assay Results

Drug	Activity	EC_{50}	EC_{90}	CC_{50}	SI_{50}	SI_{90}
CDV	-	0.22	25.63	>150.00	>682	>26
TNV	-	28.82	>150.00	>150.00	>5	1
BCV	-	0.06	0.39	>10.00	>167	>26
AB23039	HI	0.86	7.76	90.90	106	12
AB23040	LO	4.97	>6.00	25.12	5	<4

NA (Not Active) SI_{50} = 0 - 3.9

LO (Slightly Active) SI_{50} = 4 - 9.9

MOD (Moderately Active) SI_{50} = 10 - 19.9

HI (Highly Active) SI_{50} = ≥20

[00690] *JC Virus (JCV)*

[00691] Test Drug Concentration: 0.048-150 μM.

[00692] Control Drug Concentration: 0.048-150; 0.003-10 μM.

TABLE 43: JCV Assay Results

Drug	Activity	EC_{50}	EC_{90}	CC_{50}	SI_{50}	SI_{90}
CDV	-	5.66	106.69	>150.00	>27	>1
TNV	-	>150.00	>150.00	>150.00	1	1
BCV	-	0.74	1.85	9.25	12	5
AB23039	HI	0.21	23.40	71.79	342	3
AB23040	LO	8.85	27.98	80.71	9	3

NA (Not Active) SI_{50} = 0 - 3.9
LO (Slightly Active) SI_{50} = 4 - 9.9
MOD (Moderately Active) SI_{50} = 10 - 19.9
HI (Highly Active) SI_{50} = ≥20

[00693] Secondary assay results examined proliferation effects and antiviral (hCMV & BKV) of AB23031, which indicated that AB23031 did not inhibit cell growth using HFF but provided some inhibition with MOLT-3 cells. (data not shown) Antiviral activity against hCMV or BKV was not confirmed using compounds AB23031 (data not shown).

[00694] *Summary*:

[00695] All primary assays have been performed, with AB23039 exhibiting high activity against BK and JC viruses but shown to be inactive against HSV-1, HCMV, and EBV. Low activity was noted for AB23040 against both BK and JC viruses, but this compound was also inactive against HSV-1, HCMV, and EBV. As expected, BCV was active against all viruses in the appropriate test range with high or moderate activity.

[00696] Proliferation assays were performed using AB23031 (compound 26; Formula IVB) in human foreskin fibroblast (HFF) cells and human lyphoblastoid MOLT-3 cells (2 assays total). AB23031 showed no inhibition of cell growth at the highest concentration tested (IC_{50} = >100 μM) in the proliferation assay using HFF cells, but yielded a CC_{50} of 55.40 μM in the MOLT-3 cells.

[00697] Secondary (confirmatory) testing was requested using AB23031 against HCMV and BKV, with and without the addition of 100 μM Guanosine (4 assays total). The standard secondary HCMV assay tested this compound against a wild-type (AD169) and a drug-resistant isolate (GDGr K17). These secondary assays for AB23031 have been performed and activity was not confirmed against either HCMV or BKV. The addition of 100 μM Guanosine showed no effect regarding activity or cytotoxicity.

Example 23 –ANTIVIRAL ASSESSMENT OF NATURAL NUCLEOSIDE ANALOGS

[00698] *Objective*: To examine three compounds: AB23032 (compound 26; Formula IVB), AB23032 (compound 33; Formula IIB), and AB23036 (compound 56; Formula VIB) in primary assays against compounds against Herpes simplex virus 1 (HSV-1), Human cytomegalovirus (HCMV), Epstein-Barr virus (EBV), BK virus (BKV), and JC virus (JCV).

[00699] *Methods*:

[00700] All compounds were solubilized by adding DMSO to each, based on quantities and molecular weights specified to achieve a final 30mM concentration. These stocks were stored at -20°C. There were no problems with solubility of the compounds in DMSO.

[00701] The working test compound solutions (1.5mM) were prepared by diluting the 30mM DMSO stocks 1:20 in warmed MEM + 2%FBS. There were no problems with solubility of the compounds in 2% MEM.

[00702] Positive and negative control drugs were prepared as per usual with working solutions at 1.5 and 1.0 mM in MEM + 2%FBS.

[00703] Viral assays were performed as described above.

[00704] *Results*:

[00705] *Herpes Simplex Virus (HSV-1)*

[00706] Test Drug Concentration: 0.048-150 μM.

[00707] Control Drug Concentration: 0.048-150 μM.

TABLE 44: HSV-1 Assay Results

Drug	Activity	EC_{50}	EC_{90}	CC_{50}	SI_{50}	SI_{90}
ACV	-	0.55	4.18	>150.00	>273	>36
TNV	-	>150.00	>150.00	>150.00	1	1
AB23031	NA	>150.00	>150.00	>150.00	1	1
AB23032	NA	>150.00	>150.00	>150.00	1	1
AB23036	NA	>150.00	>150.00	>150.00	1	1

NA (Not Active) SI_{50} = 0 - 3.9

LO (Slightly Active) SI_{50} = 4 - 9.9

MOD (Moderately Active) SI_{50} = 10 - 49.9

HI (Highly Active) SI_{50} = ≥50

[00708] *Human Cytomegalovirus (hCMV)*

[00709] Test Drug Concentration: 0.048-150 μM.

[00710] Control Drug Concentration: 0.048-150 μM.

TABLE 45: hCMV Assay Results

Drug	Activity	EC_{50}	EC_{90}	CC_{50}	SI_{50}	SI_{90}
GCV	-	0.19	>150.00	>150.00	>785	1
TNV	-	>150.00	>150.00	>150.00	1	1
AB23031	HI	0.15	>150.00	>150.00	>1034	1
AB23032	NA	>150.00	>150.00	>150.00	1	1
AB23036	NA	>150.00	>150.00	>150.00	1	1

NA (Not Active) SI_{50} = 0 - 3.9

LO (Slightly Active) SI_{50} = 4 - 9.9

MOD (Moderately Active) SI_{50} = 10 - 49.9

HI (Highly Active) SI_{50} = ≥50

[00711] *Epstein-Barr Virus (EBV)*

[00712] Test Drug Concentration: 0.048-150 μM.

[00713] Control Drug Concentration: 0.032-100 μM.

TABLE 46: EBV Assay Results

Drug	Activity	EC_{50}	EC_{90}	CC_{50}	SI_{50}	SI_{90}
CDV	-	8.79	76.77	>100.00	>11	>1
TNV	-	>100.00	>100.00	>100.00	1	1
AB23031	NA	>150.00	>150.00	>150.00	1	1
AB23032	NA	>150.00	>150.00	>150.00	1	1
AB23036	NA	>150.00	>150.00	>150.00	1	1

NA (Not Active) SI_{50} = 0 - 3.9

MOD (Moderately Active) SI_{50} = 4 - 9.9

HI (Highly Active) SI_{50} = ≥10

[00714] *BK Virus (BKV)*

[00715] Test Drug Concentration: 0.048-150 μM.

[00716] Control Drug Concentration: 0.048-150 μM.

TABLE 47: BKV Assay Results

Drug	Activity	EC_{50}	EC_{90}	CC_{50}	SI_{50}	SI_{90}
CDV	-	4.62	106.45	>150.00	>32	>1
TNV	-	>150.00	>150.00	>150.00	1	1
AB23031	HI	5.12	>150.00	>150.00	>29	1
AB23032	NA	77.21	149.16	>150.00	>2	>1
AB23036	NA	108.88	>150.00	>150.00	>1	1

NA (Not Active) SI_{50} = 0 - 3.9
LO (Slightly Active) SI_{50} = 4 - 9.9
MOD (Moderately Active) SI_{50} = 10 - 19.9
HI (Highly Active) SI_{50} = ≥20

[00717] *JC Virus (JCV)*

[00718] Test Drug Concentration: 0.048-150 μM.

[00719] Control Drug Concentration: 0.048-150 μM.

TABLE 48: JCV Assay Results

Drug	Activity	EC_{50}	EC_{90}	CC_{50}	SI_{50}	SI_{90}
CDV	-	4.91	33.09	>150.00	>31	>5
TNV	-	>150.00	>150.00	>150.00	1	1
AB23031	NA	99.65	>150.00	>150.00	>2	1
AB23032	NA	>150.00	>150.00	>150.00	1	1
AB23036	NA	107.59	>150.00	>150.00	>1	1

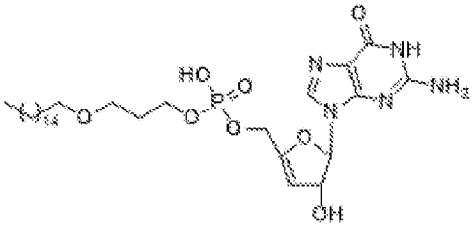
NA (Not Active) SI_{50} = 0 - 3.9
LO (Slightly Active) SI_{50} = 4 - 9.9
MOD (Moderately Active) SI_{50} = 10 - 19.9
HI (Highly Active) SI_{50} = ≥20

[00720] *Summary:*

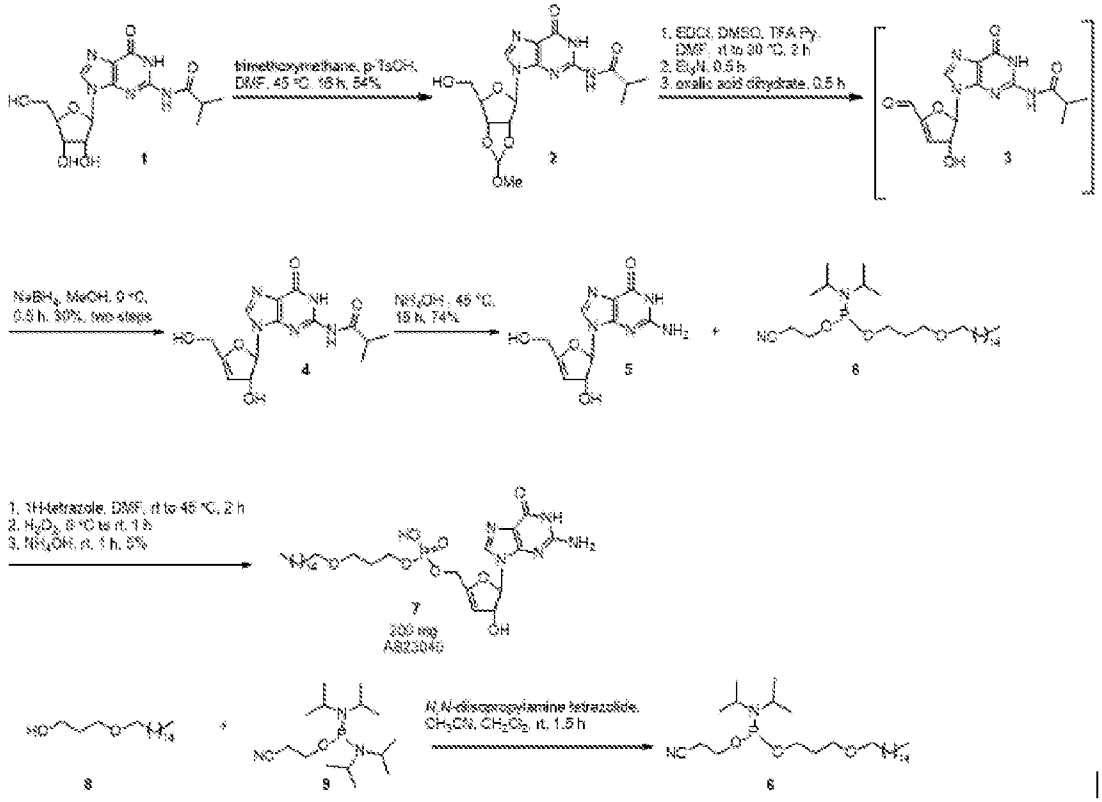
[00721] No toxicity was detected in the 3 new compounds. AB23031 is highly active against HCMV and BKV.

Example 24 – Synthetic Approach for AB23040 (Compound 103; Formula IIB)

[00722] *Objective*: To provide a synthetic scheme for compound 103

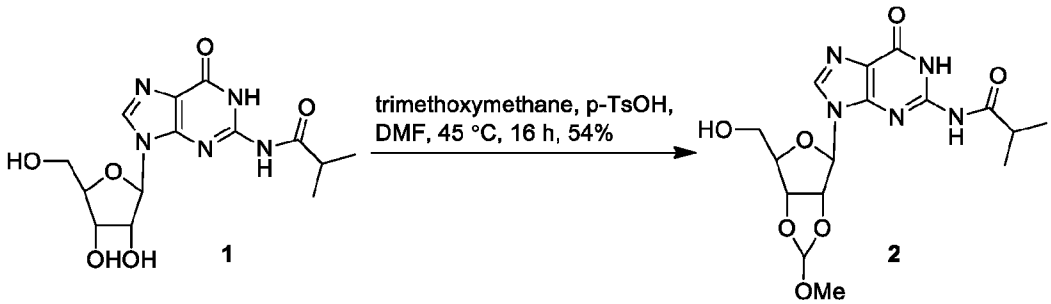


[00723] *Scheme*:



[00724] Procedure

[00725] *General Procedure for the Preparation of Compound 2*:

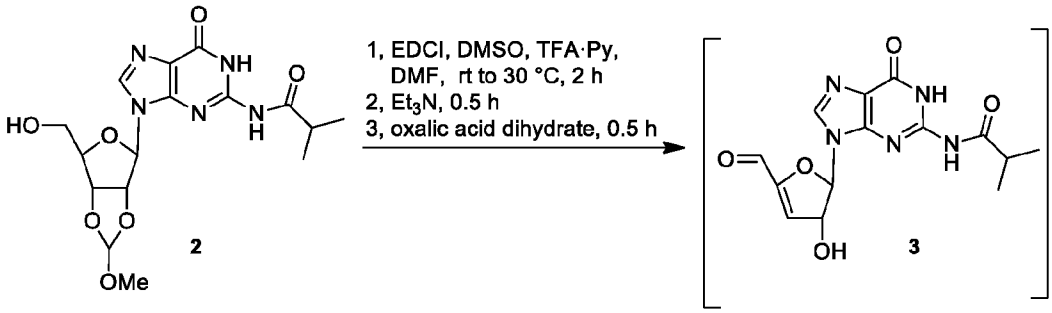


[00726]

[00727] To a solution of compound **1** (50 g, 0.14 mol, 1.0 eq) in dry DMF (120 mL) was added trimethoxymethane (154.2 mL, 1.4 mol, 10.0 eq) and *p*-TsOH (24.2 g, 0.14 mol, 1.0 eq) sequentially. The mixture was stirred at 45 °C for 15 h. The mixture was quenched with amberlyst® A-21 and triethylamine. After the evaporation of the solvent, the residue was purified by flash column chromatography (dichloromethane : methanol, 9:1) to afford compound **2** (30 g , 54%) as a white foam.

[00728] *General Procedure for the Preparation of Compound 3*

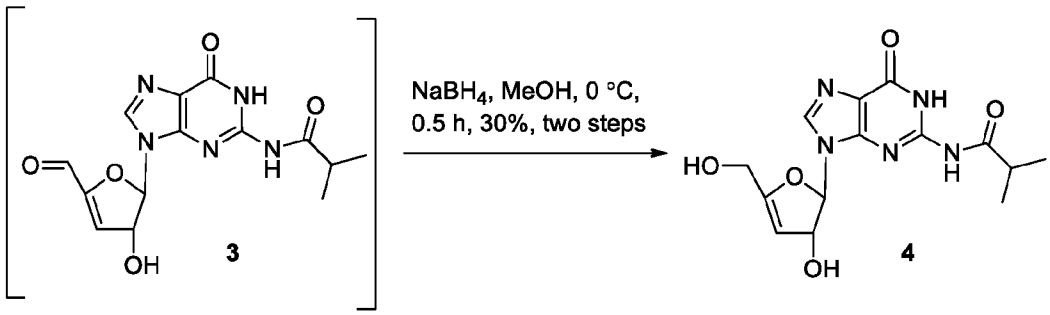
[00729]



[00730] To a solution of **2** (30 g, 75.9 mmol, 1.0 eq) in dry DMF (180 mL) was added dimethyl sulfoxide (32.3 mL, 455.4 mmol, 6.0 eq), EDCI (43.7 g, 227.7 mmol, 3.0 eq) and TFA•py (7.32 g, 37.95 mmol, 0.5 eq) sequentially. The mixture was stirred at 30 °C for 2 h. Then triethylamine (42.2 mL, 303.6 mmol, 4.0 eq) was added to the reaction mixture, after stirring for 0.5 h, oxalic acid dihydrate (19.1 g, 151.8 mmol, 2.0 eq) was added. After stirring for another 30 min at room temperature, the mixture was concentrated under reduce pressure and purified by chromatography over silica gel (dichloromethane/methanol, 50/1 to 10/1) to afford crude compound **3** (40 g) as a yellow solid, which was used for the next step directly without further purification.

[00731] *General Procedure for the Preparation of Compound 4*

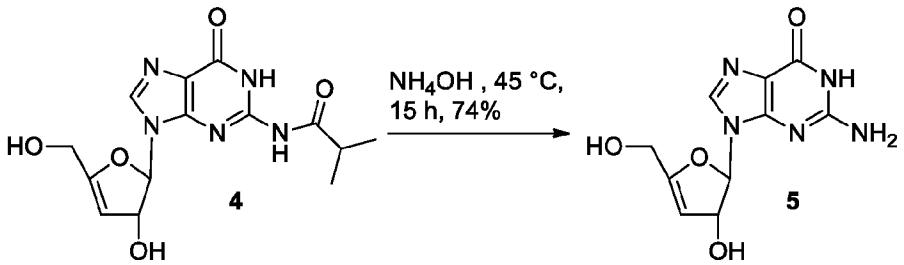
[00732]



[00733] The crude compound **3** (40 g, 120.1 mmol, 1.0 eq) was dissolved in methanol (200 mL). The reaction mixture was cooled to 0 °C and $NaBH_4$ (2.27 g, 60.05 mmol, 0.50 eq) was added. After stirring at 0 °C for 1 h, the result mixture was quenched with diluted aqueous NH_4Cl (5 M, 20 mL). After evaporation of the solvent, the residue was purified by a flash column chromatography (dichloromethane/methanol, 8/1) to afford **4** (7.5 g, 30%, two steps) as a white

solid.

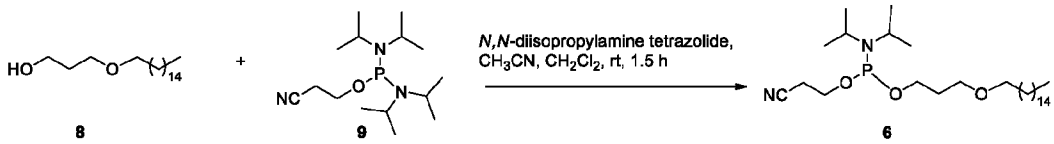
[00734] *General Procedure for the Preparation of Compound 5*

[00735] 

[00736] A solution of **4** (7.5 g, 22.3 mmol, 1.0 eq) in NH_4OH (100 mL) was stirred at 45 °C for 13 h. The result mixture was concentrated under reduced pressure, the residue was purified by triturated with methanol to afford **5** (4.4 g, 74%) as white solid.

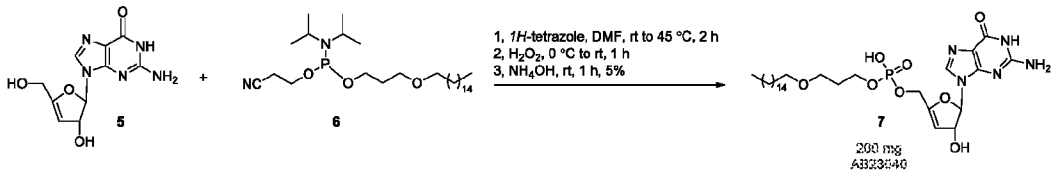
[00737] ^{1}H NMR (400 MHz, DMSO-*d6*) δ 9.22 (s, 1H), 7.60 (s, 1H), 6.53 (s, 2H), 6.00 (d, J = 2.4 Hz, 1H), 5.66 (d, J = 4.9 Hz, 1H), 5.26–5.12 (m, 2H), 5.02 (s, 1H), 3.97 (s, 2H).

[00738] *General Procedure for the Preparation of Compound 6*

[00739] 

[00740] A mixture of 3-(hexadecyloxy)propan-1-ol (8 g, 26.6 mmol) and *N,N*-diisopropylammonium tetrazolide (3.06 g, 17.8 mmol) was coevaporated with DCM-acetonitrile mixture (50:50, 10 mL) 3 times. To the dried mixture in DCM (50 mL) was added compound **9** (17.9 ml, 56.4 mmol). After being stirred for 1 hour at room temperature, methanol (5 mL) was added and stirred for 15 minutes. Then the reaction was concentrated under vacuum; diluted with 10% TEA solution in EtOAc (300 mL) and washed with 10% $NaHCO_3$ solution (2 x 100 mL) and water (2 x 100 mL); dried over anhydrous Na_2SO_4; filtered and evaporated. The crude product was purified with column chromatography using petroleum ether:EtOAc : TEA (10:1:0.05) to afford compound **6** (11 g, 82%) as a pale yellow oil.

[00741] *General Procedure for the Preparation of Compound AB23040*

[00742] 

[00743] To a solution of **5** (1.77 g, 6.6 mmol, 1.0 eq) in dry DMF (50 mL) was added *1H*-tetrazole (0.93 g, 13.2 mmol, 2.0 eq) and compound **6** (3.3 g, 6.6 mmol, 1.0 eq) sequentially. The mixture was stirred at 45 °C for 2 h. The reaction mixture was cooled to 0 °C and H_2O_2 (30%, 10 mL) was added. Then the reaction mixture was allowed to warm to room temperature. After stirring

for 1 h, NH_4OH (10 mL) was added. After stirring for another 1 h at room temperature, the residue was purified by reverse-phase flash chromatography (C18, using a 5% → 60 % ~ 90 % gradient of ACN in water) and further purified with reverse phase HPLC (ACN and water). After lyophilization of the desired fractions, the desired product **AB23040** was obtained as a white solid (0.20 g, 5%).

[00744] **LC-MS**: 628.30 $[M+H]^+$

[00745] **HPLC**: Ret Time 8.629 min; Purity @ 220 nm, 99.4%, @254 nm, 100.0%.

[00746] ^{1}H NMR (400 MHz, CD_3OD) δ 7.74 (s, 1H), 6.24 (d, J = 2.1 Hz, 1H), 5.46–5.42 (m, 1H), 5.10 (br, 1H), 4.47 (d, J = 6.9 Hz, 2H), 3.94 (q, J = 6.3 Hz, 2H), 3.59–3.48 (m, 3H), 3.40 (dd, J = 14.2, 7.4 Hz, 3H), 3.22 (s, 1H), 3.12 (s, 1H), 2.70 (s, 2H), 2.29 (s, 1H), 2.21 (s, 1H), 1.91–1.81 (m, 2H), 1.58–1.47 (m, 3H), 1.33–1.28 (m, 40H), 0.90 (t, J = 6.7 Hz, 4H).

[00747] ^{31}P NMR (162 MHz, CD_3OD) δ 0.47.

TABLE 3
pVip Genes

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3	2624749465	6	2623620517
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5	2521798317	8	2521172648
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7	2720695169	10	2718218250
8	2698137626	12	2695420938
9	646713396	15	646564524
10	2506475787	19	2506381025
11	2515428782	21	2515154070
12	2574506394	27	2574179788
13	2609132705	32	2608642208
14	2619892213	34	2619618891
15	2634960437	39	2634166261
16	2639213731	42	2636416084
17	2648875132	44	2648501185
18	2649993803	46	2648501459
19	2651203508	47	2648501771
20	2651490945	48	2648501840
21	2661858798	50	2660238307
22	2701115162	56	2700988679
23	2718503187	57	2718217692
24	2721736750	58	2718218507
25	2733913669	60	2731957952
26	2743907592	62	2740892545
27	2744633848	63	2744054527
28	2695043264	1	2693429896
29	2684559953	2	2681813561
30	2507146842	3	2506783068
31	2632766730	11	2630968672
32	2744653400	13	2744054531
33	2654783232	14	2654587543
34	2504625218	17	2504557017
35	2506474236	18	2506381025
36	2509664214	20	2509601008
37	2518436022	22	2518285547
38	2522341593	23	2522125098
39	2524269675	24	2524023156
40	2525334630	25	2524614668
41	2557036911	26	2556921023
42	2574517928	28	2574179790
43	2582805913	29	2582580599

SEQ ID NO	IMG ID	pVip #	Metagenome genome IMG ID
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46	2618018523	33	2617270916
47	2631333032	36	2630968323
48	2632937107	37	2630968711
49	2633985761	38	2630968972
50	2635314107	40	2634166348
51	2637497700	41	2636415666
52	2641427518	43	2639762959
53	2649163162	45	2648501251
54	2651585264	49	2648501863
55	2665950188	51	2663763173
56	2674184607	52	2671180787
57	2684813341	53	2684622550
58	2693697599	54	2693429564
59	2694112273	55	2693429660
60	2728147792	59	2724679805
61	2741341560	61	2740891962
62	2741409035	64	2740891993
63	2504129180	65	2503982047
64	637160692	66	637000327
65	637364324	67	637000336
66	637468954	68	637000337
67	637586319	69	637000206
68	637752529	70	637000204
69	639797708	71	639633052
70	640805406	72	640753033
71	640830189	73	640753049
72	641096015	74	640963011
73	641147750	75	640963027
74	641288534	76	641228507
75	643461066	77	643348574
76	646369858	78	646311927
77	646419713	79	646311963
78	647622404	80	647533121
79	649804297	81	649633054
80	650410387	82	650377991
81	650419199	83	650377942
82	650463340	84	650377984
83	650537321	85	650377925
84	650742368	86	650716002
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86	2501733929	88	2501651210

SEQ ID NO	IMG ID	pVip #	Metagenome genome IMG ID
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89	2512440669	91	2512047059
90	2519473577	92	2519103099
91	2519473579	93	2519103099
92	2519484486	94	2519103103
93	2519815572	95	2519103180
94	2521802859	96	2521172649
95	2522303848	97	2522125086
96	2524107537	98	2524023060
97	2525610838	99	2524614740
98	2525930338	100	2524614816
99	2528325157	101	2528311002
100	2531202617	102	2529293096
101	2532381218	103	2531839141
102	2532646932	104	2531839206
103	2538932271	105	2537561856
104	2540642849	106	2540341105
105	2540668036	107	2540341115
106	2540825991	108	2540341170
107	2541039228	109	2540341248
108	2541315631	110	2541046975
109	2546450678	111	2545824694
110	2546738312	112	2545824767
111	2547718745	113	2547132187
112	2551476655	114	2551306039
113	2551491916	115	2551306042
114	2551562099	116	2551306058
115	2551596444	117	2551306067
116	2553401559	118	2551306520
117	2553886541	119	2551306646
118	2558097217	120	2556921621
119	2559286049	121	2558860239
120	2559416375	122	2558860277
121	2562001279	123	2561511079
122	2563081558	124	2562617115
123	2563230595	125	2562617155
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125	2565702223	127	2563367170
126	2566542256	128	2565956643
127	2566736970	129	2565956698
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129	2574423613	131	2574179766

SEQ ID NO	IMG ID	pVip #	Metagenome genome IMG ID
130	2574578667	132	2574179802
131	2577747326	133	2576861245
132	2577787495	134	2576861258
133	25804401517	135	2579778656
134	25810324187	136	2579778800
135	2581542389	137	2579778918
136	2582293224	138	2579779100
137	2582959978	139	2582580668
138	2583671671	140	2582580861
139	2584203718	141	2582580995
140	2585240392	142	2582581301
141	2587265930	143	2585427937
142	2589217693	144	2588253911
143	2597063350	145	2596583606
144	2600497862	146	2600254970
145	2600833866	147	2600255071
146	2609594859	148	2609459643
147	2609930410	149	2609459764
148	2611345001	150	2609460080
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150	2612132826	152	2609460245
151	2617465221	153	2617270765
152	2617538802	154	2617270789
153	2619647987	155	2619618818
154	2619760352	156	2619618853
155	2620549291	157	2619619052
156	2621169600	158	2619619266
157	2623278845	159	2622736530
158	2632746825	160	2630968667
159	2642232622	161	2639763156
160	2644760915	162	2643221740
161	2645912334	163	2645727543
162	2647434260	164	2645727892
163	2649993012	165	2648501459
164	2651793160	166	2648501913
165	2652273697	167	2651869653
166	2654809173	168	2654587547
167	2658339966	169	2657245169
168	2667505054	170	2663763602
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170	2668144532	172	2667527434
171	2668847476	173	2667527626
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174	2677278474	176	2675903261
175	2682061458	177	2681812894
176	2684092807	178	2681813425
177	2688794699	179	2687453440
178	2693209812	180	2690316327
179	2694949528	181	2693429874
180	2700499480	182	2698536835
181	2701140257	183	2700988686
182	2701911183	184	2700989248
183	2705695255	185	2703719122
184	2706043000	186	2703719236
185	2712662546	187	2711768198
186	2714077658	188	2713896747
187	2719376594	189	2718217925
188	2719498267	190	2718217953
189	2719828580	191	2718218033
190	2722236530	192	2721755284
191	2727845415	193	2724679709
192	2728971251	194	2728369061
193	2729066335	195	2728369080
194	2730169305	196	2728369366
195	2731232863	197	2728369654
196	2735939253	198	2734482289
197	2740266671	199	2739367982
198	2741408272	200	2740891993
199	2742412079	201	2740892189
200	2742415354	202	2740892190
201	2743908240	203	2740892545
202	2751139676	204	2747843223
203	2752652723	205	2751185612
204	2753090639	206	2751185737
205	2753093587	207	2751185738
206	2753363234	208	2751185801
207	2753367132	209	2751185802
208	2753371117	210	2751185803
209	2753755176	211	2751185895
210	2758508848	212	2757320913
211	2758538137	213	2757320982
212	2758668677	214	2758568024
213	2766104288	215	2765235962
214	2770832229	216	2767802753
215	2558444101	217	2558309039

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217	2620553354	219	2619619052
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223	2061766007 assembled _HiSeq_08062520	225	2061766007
224	2061766007 assembled _HiSeq_12004210	226	2061766007
225	2061766007 assembled _HiSeq_13805260	227	2061766007
226	2061766007 assembled _HiSeq_17035850	228	2061766007
227	2061766007 assembled _HiSeq_22354030	229	2061766007
228	3300000553 assembled TBL_comb47_HYPODRAFT_100 0031312	230	3300000553
229	3300000558 assembled Draft_1000017819	231	3300000558
230	3300000558 assembled Draft_1020415419	232	3300000558
231	3300000568 assembled Draft_1000864417	233	3300000568
232	3300000970 assembled BBAY66_100003029	234	3300000970
233	3300001102 assembled BBAY67_1000022226	235	3300001102
234	3300001200 assembled BBAY65_1000011634	236	3300001200
235	3300001348 assembled JGI20154J14316_1000097623	237	3300001348
236	3300001450 assembled JGI24006J15134_1000007033	238	3300001450
237	3300001450 assembled JGI24006J15134_1000007151	239	3300001450
238	3300001598 assembled EMG_100002329	240	3300001598
239	3300001749 assembled JGI24025J20009_1000044120	241	3300001749
240	3300001750 assembled JGI24023J19991_100005742	242	3300001750
241	3300001835 assembled shallow_100084433	243	3300001835
242	3300002119 assembled JGI20170J26628_1000030318	244	3300002119
243	3300002165 assembled JGI24527J20359_100014812	245	3300002165
244	3300002180 assembled JGI24724J26744_1000065020	246	3300002180
245	3300002219 assembled SCADCLC_1000381914	247	3300002219

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246	3300002219 assembled SCADCLC_1000709320	248	3300002219
247	3300002220 assembled MLSBCLC_100183129	249	3300002220
248	3300002220 assembled MLSBCLC_1002228019	250	3300002220
249	3300002462 assembled JGI24702J35022_1000091311	251	3300002462
250	3300002518 assembled JGI25134J35505_1000001183	252	3300002518
251	3300002835 assembled B570J40625_1000006467	253	3300002835
252	3300003765 assembled Ga0056911_100030025	254	3300003765
253	3300003767 assembled Ga0056908_1000061101	255	3300003767
254	3300004166 assembled Ga0066427_100005916	256	3300004166
255	3300004173 assembled Ga0066412_100001438	257	3300004173
256	3300004173 assembled Ga0066412_100011719	258	3300004173
257	3300004178 assembled Ga0066410_100009118	259	3300004178
258	3300004197 assembled Ga0066420_100001947	260	3300004197
259	3300004197 assembled Ga0066420_100010317	261	3300004197
260	3300004202 assembled Ga0066418_100009418	262	3300004202
261	3300004203 assembled Ga0066419_100000529	263	3300004203
262	3300004203 assembled Ga0066419_100003817	264	3300004203
263	3300004230 assembled Ga0066452_100000937	265	3300004230
264	3300004250 assembled Ga0066472_1000237	266	3300004250
265	3300004253 assembled Ga0066464_100004618	267	3300004253
266	3300004253 assembled Ga0066464_100006643	268	3300004253
267	3300004806 assembled Ga0007854_100000246	269	3300004806
268	3300005080 assembled Ga0069611_1000016445	270	3300005080
269	3300005124 assembled Ga0070424_1100226	271	3300005124
270	3300005125 assembled Ga0070411_1062712	272	3300005125
271	3300005144 assembled Ga0068711_100038117	273	3300005144
272	3300005286 assembled Ga0065721_1000460410	274	3300005286
273	3300005326 assembled Ga0074195_10008286	275	3300005326
274	3300005531 assembled	276	3300005531

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276	3300005588 assembled Ga0070728_1000125023	278	3300005588
277	3300005589 assembled Ga0070729_10000081117	279	3300005589
278	3300005589 assembled Ga0070729_1000129613	280	3300005589
279	3300005609 assembled Ga0070724_1000012829	281	3300005609
280	3300005609 assembled Ga0070724_1000028613	282	3300005609
281	3300005609 assembled Ga0070724_1000048517	283	3300005609
282	3300005675 assembled Ga0074424_10021430	284	3300005675
283	3300005915 assembled Ga0075122_100007968	285	3300005915
284	3300005920 assembled Ga0070725_1000012429	286	3300005920
285	3300005920 assembled Ga0070725_1000027223	287	3300005920
286	3300005920 assembled Ga0070725_100003449	288	3300005920
287	3300005986 assembled Ga0075152_1000034111	289	3300005986
288	3300006056 assembled Ga0075163_1000220113	290	3300006056
289	3300006104 assembled Ga0007882_1000004313	291	3300006104
290	3300006104 assembled Ga0007882_1000014836	292	3300006104
291	3300006182 assembled Ga0075033_10000633	293	3300006182
292	3300006226 assembled Ga0099364_100017018	294	3300006226
293	3300006243 assembled Ga0099348_1001723	295	3300006243
294	3300006417 assembled Ga0069787_1004128015	296	3300006417
295	3300006417 assembled Ga0069787_1005605520	297	3300006417
296	3300006417 assembled Ga0069787_1005688918	298	3300006417
297	3300006417 assembled Ga0069787_1021696324	299	3300006417
298	3300006417 assembled Ga0069787_1113807921	300	3300006417
299	3300006736 assembled Ga0098033_1000001464	301	3300006736
300	3300006738 assembled Ga0098035_100006013	302	3300006738
301	3300006789 assembled Ga0098054_10000219	303	3300006789
302	3300006790 assembled Ga0098074_100033128	304	3300006790

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305	3300006927 assembled Ga0098034_100013824	307	3300006927
306	3300006929 assembled Ga0098036_100012625	308	3300006929
307	3300006987 assembled Ga0098063_100010810	309	3300006987
308	3300006988 assembled Ga0098064_10002211	310	3300006988
309	3300007344 assembled Ga0070745_100033022	311	3300007344
310	3300007346 assembled Ga0070753_100014333	312	3300007346
311	3300007462 assembled Ga0099934_110520	313	3300007462
312	3300007485 assembled Ga0099929_1008119	314	3300007485
313	3300007516 assembled Ga0105050_1000139429	315	3300007516
314	3300007640 assembled Ga0070751_1000004111	316	3300007640
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317	3300008050 assembled Ga0098052_10001839	319	3300008050
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319	3300008224 assembled Ga0105350_100000945	321	3300008224
320	3300009093 assembled Ga0105240_100005042	322	3300009093
321	3300009169 assembled Ga0105097_1000009945	323	3300009169
322	3300009175 assembled Ga0073936_1000120334	324	3300009175
323	3300009415 assembled Ga0115029_100184931	325	3300009415
324	3300009419 assembled Ga0114982_10001831	326	3300009419
325	3300009488 assembled Ga0114925_1000023517	327	3300009488
326	3300009488 assembled Ga0114925_100003506	328	3300009488
327	3300009508 assembled Ga0115567_1000068222	329	3300009508
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329	3300009546 assembled Ga0099799_100233	331	3300009546
330	3300009669 assembled Ga0116148_10010742	332	3300009669
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334	3300010028 assembled Ga0134115_1006245	336	3300010028
335	3300010160 assembled Ga0114967_100001146	337	3300010160
336	3300010162 assembled Ga0131853_1000011621	338	3300010162
337	3300010162 assembled Ga0131853_1000234120	339	3300010162
338	3300010162 assembled Ga0131853_1000511220	340	3300010162
339	3300010270 assembled Ga0129306_100025163	341	3300010270
340	3300010313 assembled Ga0116211_100026028	342	3300010313
341	3300010373 assembled Ga0134128_1000050820	343	3300010373
342	3300010379 assembled Ga0136449_1000153745	344	3300010379
343	3300010396 assembled Ga0134126_1000011835	345	3300010396
344	3300010430 assembled Ga0118733_10000149451	346	3300010430
345	3300010430 assembled Ga0118733_10000158731	347	3300010430
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347	3300012103 assembled Ga0136578_1000209	349	3300012103
348	3300012533 assembled Ga0138256_1000042615	350	3300012533
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354	3300013131 assembled Ga0172373_100005744	356	3300013131
355	3300014491 assembled Ga0182014_100007864	357	3300014491
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363	3300018033 assembled Ga0187867_1000087624	365	3300018033
364	3300018038 assembled Ga0187855_1000057816	366	3300018038
365	3300018042 assembled Ga0187871_100009711	367	3300018042
366	3300018080 assembled Ga0180433_1001105911	368	3300018080
367	3300018428 assembled Ga0181568_1000115027	369	3300018428
368	3300018475 assembled Ga0187907_1000663212	370	3300018475
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370	3300018475 assembled Ga0187907_1000859111	372	3300018475
371	3300018493 assembled Ga0187909_1000543313	373	3300018493
372	3300018494 assembled Ga0187911_1000586113	374	3300018494
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374	3300018495 assembled Ga0187908_1000576413	376	3300018495
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TABLE 4
pVip-Encoding Polynucleotides

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398	44
399	46
400	47
401	48
402	50
403	56
404	57
405	58
406	60
407	62
408	63

TABLE 5
pVip Proteins

SEQ ID NO	IMG id	pVip #	Clade	Metagenome genome IMG ID	Genome Metagenome Name	Kinase
409	2624749465	6	1	2623620517	Selenomonas ruminatium S137	No
410	2739066738	7	5	2738541339	Fibrobacter sp. UWT3	No
411	2521798317	8	4	2521172648	Psychrobacter lutiphocae DSM 21542	No
412	2574301464	9	7	2574179732	Vibrio porteresiae DSM 19223	Yes
413	2720695169	10	7	2718218250	Vibrio vulnificus ATL 6-1306	Yes
414	2698137626	12	6	2695420938	Ruegeria intermedia DSM 29341	No
415	646713396	15	3	646564524	Coraliomargaritakajimensis DSM 45221	No
416	2506475787	19	2	2506381025	Methanoplanus limicola M3, DSM 2279	No
417	2515428782	21	3	2515154070	Lewinella persica DSM 23188	No
418	2574506394	27	6	2574179788	Desulfovibrio senezii DSM 8436	No
419	2609132705	32	5	2608642208	Phormidium sp. OSCR GFM (version 2)	Yes
420	2619892213	34	3	2619618891	Cryomorphaceae bacterium EBPR_Bin_135	No
421	2634960437	39	6	2634166261	Burkholderiales-76 (UID4002)	No
422	2639213731	42	2	2636416084	Planktothricoides sp. SR001	Yes
423	2648875132	44	3	2648501185	Chondromyces crocatus Cm c5	No
424	2649993803	46	7	2648501459	Photobacterium swingsii CAIM 1393	Yes
425	2651203508	47	3	2648501771	Flammeovirga pacifica WPAGA1	No
426	2651490945	48	7	2648501840	Vibrio crassostreae J5-19	No
427	2661858798	50	2	2660238307	Methanogenic archaeon ISO4-H5	No
428	2701115162	56	5	2700988679	Fibrobacter sp. UWH6	No
429	2718503187	57	3	2718217692	Flavobacterium lacus CGMCC 1.12504	No
430	2721736750	58	7	2718218507	Pseudoalteromonas ulvae TC14	No
431	2733913669	60	3	2731957952	Lacinutrix sp. JCM 13824	No
432	2743907592	62	5	2740892545	Fibrobacteria bacterium GUT31 IN01_31	No
433	2744633848	63	6	2744054527	Pseudoalteromonas sp. XI10	Yes

SEQ ID NO	IMG id	pVip #	Clade	Metagenome genome IMG ID	Genome Metagenome Name	Kinase
434	2695043264	1	3	2693429896	Lutibacter oricola DSM 24956	No
435	2684559953	2	3	2681813561	Chryseobacterium gambrini DSM 18014	Yes
436	2507146842	3	2	2506783068	Methanofollis liminatans GKZPZ, DSM 4140	No
437	2632766730	11	7	2630968672	Shewanella baltica OS678	No
438	2744653400	13	6	2744054531	Marinobacter sp. YWL01	No
439	2654783232	14	6	2654587543	Pseudomonas nitroreducens B	No
440	2504625218	17	7	2504557017	Marinomonas sp GOBB3-320	No
441	2506474236	18	2	2506381025	Methanoplanus limicola M3, DSM 2279	No
442	2509664214	20	2	2509601008	Methanomethylovorans hollandica DSM 15978	No
443	2518436022	22	3	2518285547	Pelobacter carbinolicus Bd1, GraBd1	No
444	2522341593	23	5	2522125098	Tolumonas lignilytica BRL6-1	No
445	2524269675	24	4	2524023156	Conchiformibius kuhniae DSM 17694	No
446	2525334630	25	2	2524614668	Methanocorpusculum bavaricum DSM 4179	No
447	2557036911	26	7	2556921023	Pseudoalteromonas sp. H105 PacBio methylation	No
448	2574517928	28	7	2574179790	Endozoicomonas numazuensis DSM 25634	No
449	2582805913	29	3	2582580599	Composite genome from Lake Mendota Epilimnion pan-assembly MEint.metabat.6813	No
450	2582946381	30	3	2582580664	Composite genome from Trout Bog Hypolimnion pan-assembly TBhypo.metabat.2746	No
451	2596421479	31	3	2595698251	Kibdelosporangium aridum DSM 43828	No
452	2618018523	33	6	2617270916	Marinobacter zhejiangensis CGMCC 1.7061	No
453	2631333032	36	7	2630968323	Nitrincola sp. A-D6	No
454	2632937107	37	7	2630968711	Shewanella sp. cp20	No
455	2633985761	38	2	2630968972	Methanococcoides methylutens DSM 2657	Yes
456	2635314107	40	3	2634166348	Actinomadura echinospora DSM 43163	No
457	2637497700	41	7	2636415666	Photobacterium leiognathi mandapamensis KNH6	No
458	2641427518	43	6	2639762959	Actinobacteria bacterium OK074	No
459	2649163162	45	7	2648501251	Moritella viscosa 06/09/139	Yes

SEQ ID NO	IMG id	pVip #	Clade	Metagenome genome IMG ID	Genome Metagenome Name	Kinase
460	2651585264	49	6	2648501863	Aeromonas caviae CECT 4221	No
461	2665950188	51	6	2663763173	Legionella santicrucis SC-63-C7	No
462	2674184607	52	6	2671180787	Pseudomonas stutzeri C2	No
463	2684813341	53	6	2684622550	Aquabacterium parvum B6	No
464	2693697599	54	7	2693429564	Vibrio metoecus YB4D01	No
465	2694112273	55	4	2693429660	Helicobacter bilis Missouri	No
466	2728147792	59	6	2724679805	Shimia sagamensis DSM 29734	No
467	2741341560	61	3	2740891962	Marine group II.A Euryarchaeotarchaeon SCGC AG-487_M08 (contamination screened)	No
468	2741409035	64	2	2740891993	Candidatus Heimdallarchaeotarchaeon LC_3	No
469	2504129180	65	2	2503982047	Anabaena cylindrica PCC 7122	Yes
470	637160692	66	1	637000327	Treponema denticolaTCC 35405	No
471	637364324	67	7	637000336	Vibrio vulnificus CMCP6	Yes
472	637468954	68	7	637000337	Vibrio vulnificus YJ016	Yes
473	637586319	69	7	637000206	Photobacterium profundum SS9	Yes
474	637752529	70	3	637000204	Pelobacter carbinolicus Bd1, GraBd1	No
475	639797708	71	7	639633052	Psychromonas ingrahamii 37	No
476	640805406	72	7	640753033	Marinomonas sp. MWYL1	No
477	640830189	73	7	640753049	Shewanella baltica OS185	No
478	641096015	74	4	640963011	Beggiatoa sp. PS	No
479	641147750	75	6	640963027	Marinobacter algicola DG893	No
480	641288534	76	7	641228507	Shewanella baltica OS195	No
481	643461066	77	7	643348574	Shewanella baltica OS223	No
482	646369858	78	5	646311927	Fibrobacter succinogenes S85	No
483	646419713	79	3	646311963	Thermomonospora curvata DSM 43183	No
484	647622404	80	4	647533121	Campylobacterales sp. GD 1	No
485	649804297	81	4	649633054	Helicobacter felis CS1, ATCC 49179	No
486	650410387	82	6	650377991	Marinobacter adhaerens HP15	No

SEQ ID NO	IMG id	pVip #	Clade	Metagenome genome IMG ID	Genome Metagenome Name	Kinase
487	650419199	83	5	650377942	Fibrobacter succinogenes S85	No
488	650463340	84	7	650377984	Vibrio furnissii 2510/74, NCTC 11218	No
489	650537321	85	1	650377925	Coprococcus catus GD/7	No
490	650742368	86	6	650716002	Acidiphilium multivorum AIU301	No
491	650921542	87	3	650716044	Lacinutrix sp. 5H-3-7-4	No
492	2501733929	88	7	2501651210	Photobacterium profundum 3TCK	Yes
493	2502233141	89	2	2502171154	Thermoplasmatales archaeon BRNA1	No
494	2509552219	90	1	2509276055	Treponema saccharophilum PB, DSM 2985	No
495	2512440669	91	4	2512047059	Haemophilus haemolyticus M21621	No
496	2519473577	92	2	2519103099	Methanolobus psychrophilus R15	No
497	2519473579	93	2	2519103099	Methanolobus psychrophilus R15	No
498	2519484486	94	1	2519103103	Brachyspira pilosicoli B2904	No
499	2519815572	95	6	2519103180	Curvibacter lanceolatus ATCC 14669	No
500	2521802859	96	6	2521172649	Rheinheimera perlucida DSM 18276	No
501	2522303848	97	1	2522125086	Succinimonas amylolytica DSM 2873	No
502	2524107537	98	7	2524023060	Ferrimonas kyonanensis DSM 18153	No
503	2525610838	99	6	2524614740	Pseudomonas stutzeri MF28	No
504	2525930338	100	5	2524614816	Halodesulfovibrio aestuarii DSM 10141	No
505	2528325157	101	6	2528311002	Comamonas testosteroni ZNC0007	No
506	2531202617	102	4	2529293096	Sulfurimonas gotlandica GD1	No
507	2532381218	103	4	2531839141	Kingella kingae PYKK081	No
508	2532646932	104	6	2531839206	Thauera sp. 63	No
509	2538932271	105	1	2537561856	Brachyspira hampsonii 30446	No
510	2540642849	106	2	2540341105	Methanoculleus bourgensis MS2	No
511	2540668036	107	2	2540341115	Candidatus Methanomethylophilus alvus Mx1201	No
512	2540825991	108	3	2540341170	Pseudodesulfovibrio piezophilus C1TLV30	No
513	2541039228	109	1	2540341248	Ruminococcus flavefaciens AE3010	No

SEQ ID NO	IMG id	pVip #	Clade	Metagenome genome IMG ID	Genome Metagenome Name	Kinase
514	2541315631	110	1	2541046975	Treponema medium ATCC 700293	No
515	2546450678	111	6	2545824694	Marinobacter santoriniensis NKSG1	No
516	2546738312	112	7	2545824767	Bacteriovorax sp. DB6_IX	Yes
517	2547718745	113	4	2547132187	Acinetobacter sp. MDS7A	No
518	2551476655	114	7	2551306039	Vibrio harveyi ZJ0603	No
519	2551491916	115	7	2551306042	Vibrio genomosp. F10 ZF-129	No
520	2551562099	116	7	2551306058	Vibrio splendidus 12E03	No
521	2551596444	117	7	2551306067	Vibrio rumoiensis 1S-45	No
522	2553401559	118	7	2551306520	Aliivibrio logei ATCC 35077	No
523	2553886541	119	7	2551306646	Vibrio harveyi AOD131	No
524	2558097217	120	4	2556921621	Acinetobacter towneri DSM 14962	No
525	2559286049	121	1	2558860239	Spiroplasma culicicolaES-1	No
526	2559416375	122	1	2558860277	Treponema primitia ZAS-1	No
527	2562001279	123	1	2561511079	Selenomonas sp. FC4001	No
528	2563081558	124	3	2562617115	Myxococcus hansupus DSM 436	No
529	2563230595	125	4	2562617155	Helicobacter bilis ATCC 43879	No
530	2565569616	126	7	2563367142	Vibrio halioticoli NBRC 102217	No
531	2565702223	127	4	2563367170	Helicobacter bilis WiWa	No
532	2566542256	128	4	2565956643	Acinetobacter parvus NIPH 1103	No
533	2566736970	129	4	2565956698	Acinetobacter towneri DSM 14962	No
534	2569938648	130	7	2568526421	Vibrio parahaemolyticus TUMSAT_H10_S6	Yes
535	2574423613	131	3	2574179766	Thiomonas sp. FB-Cd, DSM 25617	No
536	2574578667	132	5	2574179802	Sulfitobacter mediterraneus KCTC 32188	No
537	2577747326	133	7	2576861245	Vibrio parahaemolyticus VIP4-0444	Yes
538	2577787495	134	7	2576861258	Pseudoalteromonas haloplanktis TB25	No
539	25804401517	135	7	2579778656	Pseudoalteromonas haloplanktis AC163	No
540	25810324187	136	7	2579778800	Vibrio metoecus PPCK-2014	No

SEQ ID NO	IMG id	pVip #	Clade	Metagenome genome IMG ID	Genome Metagenome Name	Kinase
541	2581542389	137	7	2579778918	Vibrio harveyi E385	No
542	2582293224	138	7	2579779100	Vibrio parahaemolyticus VIP4-0430	Yes
543	2582959978	139	3	2582580668	Composite genome from Trout Bog Hypolimnion pan-assembly TBhypo.metabat.3004	No
544	2583671671	140	7	2582580861	Pseudoalteromonas sp. TAE56	No
545	2584203718	141	7	2582580995	Vibrio parahaemolyticus TUMSAT_DE2_S2	Yes
546	2585240392	142	6	2582581301	Janthinobacterium sp. RA13	No
547	2587265930	143	7	2585427937	Pseudoalteromonas sp. 520P1	No
548	2589217693	144	3	2588253911	Chondromyces apiculatus DSM 436	No
549	2597063350	145	5	2596583606	Fibrobacter succinogenes elongatus HM2	No
550	2600497862	146	6	2600254970	Pseudomonas sp. 1-7	No
551	2600833866	147	7	2600255071	Vibrio ezurae NBRC 102218	No
552	2609594859	148	6	2609459643	Janthinobacterium sp. OK676	No
553	2609930410	149	6	2609459764	Marinobacter sp. ES.048	No
554	2611345001	150	3	2609460080	Hyalangium minutum DSM 14724	No
555	2611749855	151	6	2609460164	Acidithiobacillus thiooxidans Licanantay	No
556	2612132826	152	6	2609460245	Delftia tsuruhatensis 391	No
557	2617465221	153	6	2617270765	Marinobacter mobilis CGMCC 1.7059	No
558	2617538802	154	3	2617270789	Flavobacterium omnivorum CGMCC 1.2747	No
559	2619647987	155	7	2619618818	Pseudidiomarina donghaiensis CGMCC 1.7284	No
560	2619760352	156	6	2619618853	Betaproteobacteria sp. genome_bin_13	No
561	2620549291	157	3	2619619052	Unclassified Chloroflexi bacterium bin152	No
562	2621169600	158	7	2619619266	Photobacterium phosphoreum ANT220	Yes
563	2623278845	159	6	2622736530	Roseovarius lutimaris DSM 28463	No
564	2632746825	160	3	2630968667	Nonlabens ulvanivorans JCM 19297	No
565	2642232622	161	6	2639763156	Aeromonas sobria CECT 4245	No
566	2644760915	162	3	2643221740	Chryseobacterium sp. Leaf201	Yes
567	2645912334	163	7	2645727543	Aeromonas tecta CECT 7082	No

SEQ ID NO	IMG id	pVip #	Clade	Metagenome genome IMG ID	Genome Metagenome Name	Kinase
568	2647434260	164	6	2645727892	Comamonas testosteroni KF712	No
569	2649993012	165	7	2648501459	Photobacterium swingsii CAIM 1393	No
570	2651793160	166	6	2648501913	Pseudomonas nitroreducens DPB	No
571	2652273697	167	6	2651869653	Rubrivivax sp. AAP121	No
572	2654809173	168	6	2654587547	Achromobacter spanius CGMCC9173	No
573	2658339966	169	2	2657245169	Methanoculleus sp. EBM-46	No
574	2667505054	170	6	2663763602	Pseudomonas hussainii JCM 19513	No
575	2667963948	171	3	2667527390	Fabibacter pacificus CGMCC 1.12402	No
576	2668144532	172	6	2667527434	Pseudomonas oryzae KCTC 32247	No
577	2668847476	173	7	2667527626	Vibrio parahaemolyticus S164	Yes
578	2672407511	174	7	2671180348	Vibrio tritonius AM2	Yes
579	2674782375	175	7	2671180928	Vibrio parahaemolyticus CFSAN007447	Yes
580	2677278474	176	2	2675903261	Anabaena sp. 4-3	Yes
581	2682061458	177	6	2681812894	Sphaerotilus natans ATCC 13338	No
582	2684092807	178	2	2681813425	Methanoculleus sp. MAB1	No
583	2688794699	179	6	2687453440	Aeromonas veronii TH0426	No
584	2693209812	180	7	2690316327	Vibrio parahaemolyticus S165	Yes
585	2694949528	181	3	2693429874	Olleya namhaensis DSM 28881	No
586	2700499480	182	sp	2698536835	Microgenomates bacterium JGI CrystG Apr02-3-G15 (contamination screened)	No
587	2701140257	183	5	2700988686	Fibrobacter sp. UWH9	No
588	2701911183	184	7	2700989248	Vibrio parahaemolyticus CFSAN007448	Yes
589	2705695255	185	5	2703719122	unclassified Deltaproteobacteria bin 1	No
590	2706043000	186	5	2703719236	Fibrobacter sp. UWB7	No
591	2712662546	187	6	2711768198	Arsukibacterium ikkense GCM72	No
592	2714077658	188	7	2713896747	Vibrio alginolyticus V2	No
593	2719376594	189	7	2718217925	Alteromonas sp. Mex14	No
594	2719498267	190	6	2718217953	Marinobacter salinus Hb8	No

SEQ ID NO	IMG id	pVip #	Clade	Metagenome genome IMG ID	Genome Metagenome Name	Kinase
595	2719828580	191	3	2718218033	Lutibacter sp. LPB0138	No
596	2722236530	192	6	2721755284	Gammaproteobacteria bacterium GWF2_41_13	No
597	2727845415	193	3	2724679709	Saccharicrinis carchari DSM 27040	No
598	2728971251	194	7	2728369061	Aliivibrio wodanis CL7	No
599	2729066335	195	6	2728369080	Dechloromonas denitrificans ATCC BAA-841	No
600	2730169305	196	3	2728369366	Tenacibaculum sp. LPB0136	No
601	2731232863	197	7	2728369654	Vibrio sp. JCM 19061	Yes
602	2735939253	198	5	2734482289	Sulfitobacter mediterraneus DSM 12244	No
603	2740266671	199	5	2739367982	Oceanospirillales bacterium JGI 01_G13_750m (contamination screened)	No
604	2741408272	200	2	2740891993	Candidatus Heimdallarchaeotarchaeon LC_3	No
605	2742412079	201	6	2740892189	Marinobacter sp. EN3	No
606	2742415354	202	4	2740892190	Acinetobacter sp. COS3	No
607	2743908240	203	5	2740892545	Fibrobacteria bacterium GUT31 IN01_31	Yes
608	2751139676	204	6	2747843223	Janthinobacterium sp. 64	No
609	2752652723	205	2	2751185612	Bacteroidales bacterium Bact_07	No
610	2753090639	206	7	2751185737	Salinivibrio sp. DV	No
611	2753093587	207	7	2751185738	Salinivibrio sp. BNH	No
612	2753363234	208	7	2751185801	Aliivibrio sp. 1S128	Yes
613	2753367132	209	7	2751185802	Aliivibrio sp. 1S165	No
614	2753371117	210	7	2751185803	Aliivibrio sp. 1S175	No
615	2753755176	211	4	2751185895	Haemophilus quentini MP1	No
616	2758508848	212	6	2757320913	Diaphorobacter polyhydroxybutyrativorans SL-205	No
617	2758538137	213	3	2757320982	Winogradskyella sp. PC-19	No
618	2758668677	214	2	2758568024	Thermococcus siculi RG-20	No
619	2766104288	215	4	2765235962	Neisseria sp. 10023	No
620	2770832229	216	3	2767802753	Cystobacter ferrugineus Cbfe23	No
621	2558444101	217	sp	2558309039	Megasphaera elsdenii T81	Yes

SEQ ID NO	IMG id	pVip #	Clade	Metagenome genome IMG ID	Genome Metagenome Name	Kinase
622	2620552401	218	3	2619619052	Unclassified Chloroflexi bacterium bin152	No
623	2620553354	219	3	2619619052	Unclassified Chloroflexi bacterium bin152	No
624	2671326339	220	sp	2671180039	Streptomyces rubidus CGMCC 4.2026	No
625	2722096198	221	sp	2721755233	Nitrospirae bacterium GWD2_57_9	No
626	2725246328	222	7	2724679053	Photobacterium kishitanii 201212X	Yes
627	2049941002 assembled LHMISPF_00252280	223	3	2049941002	Sinkhole freshwater microbial communities from Lake Huron, US, Sample 419 (*) (MER-FS) (assembled)	
628	2061766007 assembled _HiSeq_03538890	224	2	2061766007	Bovine rumen microbial communities fromthe University of Illinois at Urbana-Champaign, USA, that are switchgrass associated - Sample 470 (*) (MER-FS) (assembled)	
629	2061766007 assembled _HiSeq_08062520	225	5	2061766007	Bovine rumen microbial communities fromthe University of Illinois at Urbana-Champaign, USA, that are switchgrass associated - Sample 470 (*) (MER-FS) (assembled)	
630	2061766007 assembled _HiSeq_12004210	226	2	2061766007	Bovine rumen microbial communities fromthe University of Illinois at Urbana-Champaign, USA, that are switchgrass associated - Sample 470 (*) (MER-FS) (assembled)	
631	2061766007 assembled _HiSeq_13805260	227	1	2061766007	Bovine rumen microbial communities fromthe University of Illinois at Urbana-Champaign, USA, that are switchgrass associated - Sample 470 (*) (MER-FS) (assembled)	
632	2061766007 assembled _HiSeq_17035850	228	5	2061766007	Bovine rumen microbial communities fromthe University of Illinois at Urbana-Champaign, USA, that are switchgrass associated - Sample 470 (*) (MER-FS) (assembled)	
633	2061766007 assembled _HiSeq_22354030	229	1	2061766007	Bovine rumen microbial communities fromthe University of Illinois at Urbana-Champaign, USA, that are switchgrass associated - Sample 470 (*) (MER-FS) (assembled)	
634	3300000553 assembled TBL_comb47_HYPOD RAFT_1000031312	230	1	3300000553	Trout Bog Lake May 28 2007 Hypolimnion (Trout Bog Lake Combined Assembly 47 Hypolimnion Samples, Aug 2012 Assem) (*) (MER-FS) (assembled)	
635	3300000558 assembled Draft_1000017819	231	6	3300000558	Wastewater microbial communities from Syncrude, Ft. McMurray, Alberta - West In Pit SyncrudeMLSB2011 (*) (MER-FS) (assembled)	
636	3300000558 assembled Draft_1020415419	232	2	3300000558	Wastewater microbial communities from Syncrude, Ft. McMurray, Alberta - West In Pit SyncrudeMLSB2011 (*) (MER-FS) (assembled)	
637	3300000568 assembled Draft_1000864417	233	2	3300000568	Tailings pond microbial communities from Northern Alberta -Short chain hydrocarbon degrading methanogenic enrichment culture SCADC: (*) (MER-FS) (assembled)	

SEQ ID NO	IMG id	pVip #	Clade	Metagenome genome IMG ID	Genome Metagenome Name	Kinase
638	3300000970 assembled BBAY66_100003029	234	3	3300000970	Macroalgal surface ecosystem from Botany Bay, Sydney, Australia - BBAY66 (*) (MER-FS) (assembled)	
639	3300001102 assembled BBAY67_1000022226	235	3	3300001102	Macroalgal surface ecosystem from Botany Bay, Sydney, Australia - BBAY67 (*) (MER-FS) (assembled)	
640	3300001200 assembled BBAY65_1000011634	236	3	3300001200	Macroalgal surface ecosystem from Botany Bay, Sydney, Australia - BBAY65 (*) (MER-FS) (assembled)	
641	3300001348 assembled JGI20154J14316_1000097623	237	2	3300001348	Pelagic Microbial community sample from North Sea - COGITO 998_met_04 (*) (MER-FS) (assembled)	
642	3300001450 assembled JGI24006J15134_1000007033	238	6	3300001450	Marine viral communities from the Pacific Ocean - LP-53 (*) (MER-FS) (assembled)	
643	3300001450 assembled JGI24006J15134_1000007151	239	6	3300001450	Marine viral communities from the Pacific Ocean - LP-53 (*) (MER-FS) (assembled)	
644	3300001598 assembled EMG_100002329	240	sp	3300001598	Elephant fecal microbiome from Asian Elephant in Hamburg Zoo, Germany (*) (MER-FS) (assembled)	
645	3300001749 assembled JGI24025J20009_1000044120	241	7	3300001749	Oil polluted marine microbial communities from Coal Oil Point, Santa Barbara, California, USA - Sample 3 (*) (MER-FS) (assembled)	
646	3300001750 assembled JGI24023J19991_1000005742	242	3	3300001750	Oil polluted marine microbial communities from Coal Oil Point, Santa Barbara, California, USA - Sample 1 (*) (MER-FS) (assembled)	
647	3300001835 assembled shallow_100084433	243	7	3300001835	Hydrothermal vent plume microbial communities from the Mid Cayman Rise - Shallow Sites - gte4kb (*) (MER-FS) (assembled)	
648	3300002119 assembled JGI20170J26628_1000030318	244	1	3300002119	Nasutitermes corniger P3 segment microbial communities from Max Planck Institute, Germany - Nc150P3 (*) (MER-FS) (assembled)	
649	3300002165 assembled JGI24527J20359_1000014812	245	7	3300002165	Marine viral communities from the Subarctic Pacific Ocean - LP-52 (*) (MER-FS) (assembled)	
650	3300002180 assembled JGI24724J26744_1000065020	246	3	3300002180	Oil polluted marine microbial communities from Coal Oil Point, Santa Barbara, California, USA - Sample 7 (*) (MER-FS) (assembled)	
651	3300002219 assembled SCADCLC_1000381914	247	6	3300002219	Tailings pond microbial communities from Northern Alberta -Short chain hydrocarbon degrading methanogenic enrichment culture SCADC: (*) (MER-FS)	

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					(assembled)	
652	3300002219 assembled SCADCLC_1000709320	248	2	3300002219	Tailings pond microbial communities from Northern Alberta -Short chain hydrocarbon degrading methanogenic enrichment culture SCADC: (*) (MER-FS) (assembled)	
653	3300002220 assembled MLSBCLC_100183129	249	6	3300002220	Wastewater microbial communities from Syncrude, Ft. McMurray, Alberta - West In Pit SyncrudeMLSB2011 (*) (MER-FS) (assembled)	
654	3300002220 assembled MLSBCLC_1002228019	250	2	3300002220	Wastewater microbial communities from Syncrude, Ft. McMurray, Alberta - West In Pit SyncrudeMLSB2011 (*) (MER-FS) (assembled)	
655	3300002462 assembled JGI24702J35022_1000091311	251	2	3300002462	Termite gut P4 segment microbial communities from Max Planck Institute, Germany - Th196 (*) (MER-FS) (assembled)	
656	3300002518 assembled JGI25134J35505_1000001183	252	6	3300002518	Marine viral communities from the Pacific Ocean - ETNP_6_100 (*) (MER-FS) (assembled)	
657	3300002835 assembled B570J40625_1000006467	253	3	3300002835	Freshwater microbial communities from Lake Mendota, WI - (Lake Mendota Combined Ray assembly, ASSEMBLY_DATE=20140605) (*) (MER-FS) (assembled)	
658	3300003765 assembled Ga0056911_100030025	254	3	3300003765	Wastewater treatment Type I Accumulibacter community from EBPR Bioreactor in Madison, WI, USA - Reactor 2_5/13/2013_ DNA (*) (MER-FS) (assembled)	
659	3300003767 assembled Ga0056908_1000061101	255	3	3300003767	Wastewater treatment Type I Accumulibacter community from EBPR Bioreactor in Madison, WI, USA - Reactor 1_10/4/2010_ DNA (*) (MER-FS) (assembled)	
660	3300004166 assembled Ga0066427_100005916	256	3	3300004166	Freshwater sediment methanotrophic microbial communities from Lake Washington under simulated oxygen tension - Sediment Metagenome 39_LOW7 (*) (MER-FS) (assembled)	
661	3300004173 assembled Ga0066412_100001438	257	3	3300004173	Freshwater sediment methanotrophic microbial communities from Lake Washington under simulated oxygen tension - Sediment Metagenome 16_LOW5 (*) (MER-FS) (assembled)	
662	3300004173 assembled Ga0066412_100011719	258	3	3300004173	Freshwater sediment methanotrophic microbial communities from Lake Washington under simulated oxygen tension - Sediment Metagenome 16_LOW5 (*) (MER-FS) (assembled)	
663	3300004178 assembled Ga0066410_100009118	259	3	3300004178	Freshwater sediment methanotrophic microbial communities from Lake Washington under simulated oxygen tension - Sediment Metagenome 14_LOW5 (*) (MER-FS) (assembled)	
664	3300004197 assembled Ga0066420_100001947	260	3	3300004197	Freshwater sediment methanotrophic microbial communities from Lake Washington under simulated oxygen tension - Sediment Metagenome 28_LOW6 (*) (MER-FS) (assembled)	

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665	3300004197 assembled Ga0066420_100010317	261	3	3300004197	Freshwater sediment methanotrophic microbial communities from Lake Washington under simulated oxygen tension - Sediment Metagenome 28_LOW6 (*) (MER-FS) (assembled)	
666	3300004202 assembled Ga0066418_100009418	262	3	3300004202	Freshwater sediment methanotrophic microbial communities from Lake Washington under simulated oxygen tension - Sediment Metagenome 26_LOW6 (*) (MER-FS) (assembled)	
667	3300004203 assembled Ga0066419_100000529	263	3	3300004203	Freshwater sediment methanotrophic microbial communities from Lake Washington under simulated oxygen tension - Sediment Metagenome 27_LOW6 (*) (MER-FS) (assembled)	
668	3300004203 assembled Ga0066419_100003817	264	3	3300004203	Freshwater sediment methanotrophic microbial communities from Lake Washington under simulated oxygen tension - Sediment Metagenome 27_LOW6 (*) (MER-FS) (assembled)	
669	3300004230 assembled Ga0066452_100000937	265	6	3300004230	Freshwater sediment methanotrophic microbial communities from Lake Washington under simulated oxygen tension - Sediment Metagenome 76_LOW10 (*) (MER-FS) (assembled)	
670	3300004250 assembled Ga0066472_1000237	266	6	3300004250	Freshwater sediment methanotrophic microbial communities from Lake Washington under simulated oxygen tension - Sediment Metagenome 106_HOW12 (*) (MER-FS) (assembled)	
671	3300004253 assembled Ga0066464_100004618	267	6	3300004253	Freshwater sediment methanotrophic microbial communities from Lake Washington under simulated oxygen tension - Sediment Metagenome 94_HOW11 (*) (MER-FS) (assembled)	
672	3300004253 assembled Ga0066464_100006643	268	6	3300004253	Freshwater sediment methanotrophic microbial communities from Lake Washington under simulated oxygen tension - Sediment Metagenome 94_HOW11 (*) (MER-FS) (assembled)	
673	3300004806 assembled Ga0007854_100000246	269	3	3300004806	Freshwater microbial communities from Crystal Bog, Wisconsin, USA - CBH12Aug08 (*) (MER-FS) (assembled)	
674	3300005080 assembled Ga0069611_1000016445	270	3	3300005080	Combined Assembly of Gp0111534, Gp0111535, Gp0111536, Gp0111537, Gp0111539, Gp0111540, Gp0111541, Gp0111542, Gp0111543 (*) (MER-FS) (assembled)	
675	3300005124 assembled Ga0070424_1100226	271	3	3300005124	Active sludge cell enrichment microbial communities from Klosterneuburg, Austria - Nitrospira DOME DR08B08 (*) (MER-FS) (assembled)	
676	3300005125 assembled Ga0070411_1062712	272	3	3300005125	Active sludge cell enrichment microbial communities from Klosterneuburg, Austria - Nitrosomonas DOME CR02B12 (*) (MER-FS) (assembled)	
677	3300005144 assembled Ga0068711_100038117	273	2	3300005144	Enrichment culture microbial communities from Arthur Kill intertidal strait, New Jersey, USA, that are MTBE-degrading - MTBE-AKM (Arthur Kill Methanogenic) MetaG (*) (MER-FS) (assembled)	

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678	3300005286 assembled Ga0065721_1000460410	274	6	3300005286	Mesophilic microbial community from rice straw/compost enrichment Sample: eDNA_1 (*) (MER-FS) (assembled)	
679	3300005326 assembled Ga0074195_10008286	275	2	3300005326	Bioremediated contaminated groundwater from EPA Superfund site, New Mexico - Sample HSE6-23 (*) (MER-FS) (assembled)	
680	3300005531 assembled Ga0070738_1000151042	276	3	3300005531	Surface soil microbial communities from Centralia Pennsylvania, which are recovering from an underground coalmine fire - Coalmine Soil_Cen12_06102014_R2 (*) (MER-FS) (assembled)	
681	3300005588 assembled Ga0070728_1000021436	277	3	3300005588	Marine sediment microbial communities from the Atlantic coast under amendment with organic carbon and nitrate - tdDd47.1 (*) (MER-FS) (assembled)	
682	3300005588 assembled Ga0070728_1000125023	278	3	3300005588	Marine sediment microbial communities from the Atlantic coast under amendment with organic carbon and nitrate - tdDd47.1 (*) (MER-FS) (assembled)	
683	3300005589 assembled Ga0070729_1000008111 7	279	3	3300005589	Marine sediment microbial communities from the Atlantic coast under amendment with organic carbon and nitrate - tdDd47.2 (*) (MER-FS) (assembled)	
684	3300005589 assembled Ga0070729_1000129613	280	3	3300005589	Marine sediment microbial communities from the Atlantic coast under amendment with organic carbon and nitrate - tdDd47.2 (*) (MER-FS) (assembled)	
685	3300005609 assembled Ga0070724_1000012829	281	7	3300005609	Marine sediment microbial communities from the Atlantic coast under amendment with organic carbon and nitrate - tdDd00.1 (*) (MER-FS) (assembled)	
686	3300005609 assembled Ga0070724_1000028613	282	3	3300005609	Marine sediment microbial communities from the Atlantic coast under amendment with organic carbon and nitrate - tdDd00.1 (*) (MER-FS) (assembled)	
687	3300005609 assembled Ga0070724_1000048517	283	3	3300005609	Marine sediment microbial communities from the Atlantic coast under amendment with organic carbon and nitrate - tdDd00.1 (*) (MER-FS) (assembled)	
688	3300005675 assembled Ga0074424_10021430	284	3	3300005675	Enhanced biological phosphorus removal bioreactor viral communities from the University of Queensland, Australia - SBR4-V90806 Phage Sequencing (*) (MER-FS) (assembled)	
689	3300005915 assembled Ga0075122_100007968	285	2	3300005915	Saline lake microbial communities from Ace Lake, Antarctica - Antarctic Ace Lake Metagenome 02UKB (*) (MER-FS) (assembled)	
690	3300005920 assembled Ga0070725_1000012429	286	7	3300005920	Marine sediment microbial communities from the Atlantic coast under amendment with organic carbon and nitrate - tdDd00.2 (*) (MER-FS) (assembled)	
691	3300005920 assembled Ga0070725_1000027223	287	3	3300005920	Marine sediment microbial communities from the Atlantic coast under amendment with organic carbon and nitrate - tdDd00.2 (*) (MER-FS) (assembled)	
692	3300005920 assembled Ga0070725_100003449	288	3	3300005920	Marine sediment microbial communities from the Atlantic coast under amendment with organic carbon and nitrate - tdDd00.2 (*) (MER-FS) (assembled)	
693	3300005986 assembled Ga0075152_1000034111	289	3	3300005986	Wastewater effluent complex algal communities from Wisconsin, to seasonally profile nutrient transformation and Carbon sequestration - JI 6/11/14 C2 DNA (*) (MER-FS) (assembled)	

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694	3300006056 assembled Ga0075163_1000220113	290	3	3300006056	Wastewater effluent complex algal communities from Wisconsin, to seasonally profile nutrient transformation and Carbon sequestration - JI 10/23/14 1A DNA (*) (MER-FS) (assembled)	
695	3300006104 assembled Ga0007882_1000004313	291	3	3300006104	Freshwater microbial communities from Crystal Bog, Wisconsin, USA - CBH12Aug09.1 (*) (MER-FS) (assembled)	
696	3300006104 assembled Ga0007882_1000014836	292	3	3300006104	Freshwater microbial communities from Crystal Bog, Wisconsin, USA - CBH12Aug09.1 (*) (MER-FS) (assembled)	
697	3300006182 assembled Ga0075033_10000633	293	6	3300006182	Synthetic microbial communities from Ohio, USA - SynthPrep_5_END_DS10_MetaG (*) (MER-FS) (assembled)	
698	3300006226 assembled Ga0099364_100017018	294	3	3300006226	Termite gut P3 segment microbial communities from Max Planck Institute, Germany - Th196 (*) (MER-FS) (assembled)	
699	3300006243 assembled Ga0099348_1001723	295	4	3300006243	Human buccal mucosa microbial communities from NIH, USA - visit 2, subject 370425937 (*) (MER-FS) (assembled)	
700	3300006417 assembled Ga0069787_1004128015	296	3	3300006417	Combined Assembly of Gp0110018, Gp0110022, Gp0110020 (*) (MER-FS) (assembled)	
701	3300006417 assembled Ga0069787_1005605520	297	3	3300006417	Combined Assembly of Gp0110018, Gp0110022, Gp0110020 (*) (MER-FS) (assembled)	
702	3300006417 assembled Ga0069787_1005688918	298	6	3300006417	Combined Assembly of Gp0110018, Gp0110022, Gp0110020 (*) (MER-FS) (assembled)	
703	3300006417 assembled Ga0069787_1021696324	299	3	3300006417	Combined Assembly of Gp0110018, Gp0110022, Gp0110020 (*) (MER-FS) (assembled)	
704	3300006417 assembled Ga0069787_1113807921	300	3	3300006417	Combined Assembly of Gp0110018, Gp0110022, Gp0110020 (*) (MER-FS) (assembled)	
705	3300006736 assembled Ga0098033_1000001464	301	6	3300006736	Marine viral communities from the Subarctic Pacific Ocean - 1_ETSP_OMZ_AT15124 metaG (*) (MER-FS) (assembled)	
706	3300006738 assembled Ga0098035_100006013	302	6	3300006738	Marine viral communities from the Subarctic Pacific Ocean - 3_ETSP_OMZ_AT15126 metaG (*) (MER-FS) (assembled)	
707	3300006789 assembled Ga0098054_10000219	303	6	3300006789	Marine viral communities from the Subarctic Pacific Ocean - 16_ETSP_OMZ_AT15313 metaG (*) (MER-FS) (assembled)	
708	3300006790 assembled Ga0098074_100033128	304	6	3300006790	Marine viral communities from the Gulf of Mexico - 32_GoM_OMZ_CsCl metaG (*) (MER-FS) (assembled)	
709	3300006810 assembled Ga0070754_1000007993	305	3	3300006810	Aqueous microbial communities from the Delaware River and Bay under freshwater to marine salinity gradient to study organic matter cycling in a time-series - Viral MetaG DEL_Sep_01 (*) (MER-FS) (assembled)	
710	3300006879 assembled Ga0079226_100011884	306	3	3300006879	Agricultural soil microbial communities from Georgia to study Nitrogen management - Poultry litter 2014 (*) (MER-FS) (assembled)	

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711	3300006927 assembled Ga0098034_100013824	307	6	3300006927	Marine viral communities from the Subarctic Pacific Ocean - 2_ETSP_OMZ_AT15125 metaG (*) (MER-FS) (assembled)	
712	3300006929 assembled Ga0098036_100012625	308	6	3300006929	Marine viral communities from the Subarctic Pacific Ocean - 4_ETSP_OMZ_AT15127 metaG (*) (MER-FS) (assembled)	
713	3300006987 assembled Ga0098063_100010810	309	6	3300006987	Marine viral communities from the Gulf of Mexico - 24_WHOI_OMZ metaG (*) (MER-FS) (assembled)	
714	3300006988 assembled Ga0098064_10002211	310	6	3300006988	Marine viral communities from the Gulf of Mexico - 24B_WHOI_OMZ_CsCl metaG (*) (MER-FS) (assembled)	
715	3300007344 assembled Ga0070745_100033022	311	7	3300007344	Aqueous microbial communities from the Delaware River and Bay under freshwater to marine salinity gradient to study organic matter cycling in a time-series - Viral MetaG DEL_Mar_4 (*) (MER-FS) (assembled)	
716	3300007346 assembled Ga0070753_100014333	312	7	3300007346	Aqueous microbial communities from the Delaware River and Bay under freshwater to marine salinity gradient to study organic matter cycling in a time-series - Viral MetaG DEL_Aug_31 (*) (MER-FS) (assembled)	
717	3300007462 assembled Ga0099934_110520	313	3	3300007462	Active sludge microbial communities from Klosterneuburg, Austria - Klosterneuburg WWTP active sludge D35_HANv2 (*) (MER-FS) (assembled)	
718	3300007485 assembled Ga0099929_1008119	314	3	3300007485	Active sludge microbial communities from Klosterneuburg, Austria - Klosterneuburg WWTP active sludge D02_HANv2 (*) (MER-FS) (assembled)	
719	3300007516 assembled Ga0105050_1000139429	315	3	3300007516	Freshwater microbial communities from Lake Fryxell liftoff mats and glacier meltwater in Antarctica - FRY-01 (*) (MER-FS) (assembled)	
720	3300007640 assembled Ga0070751_1000004111	316	7	3300007640	Aqueous microbial communities from the Delaware River and Bay under freshwater to marine salinity gradient to study organic matter cycling in a time-series - Viral MetaG DEL_Aug_28 (*) (MER-FS) (assembled)	
721	3300007961 assembled Ga0079305_100003992	317	2	3300007961	Deep subsurface shale carbon reservoir microbial communities from Ohio, USA - LMS_cellobiose_enrichment (*) (MER-FS) (assembled)	
722	3300007963 assembled Ga0110931_100009625	318	6	3300007963	Marine viral communities from the Subarctic Pacific Ocean - 4_ETSP_OMZ_AT15127 metaG (version 2) (*) (MER-FS) (assembled)	
723	3300008050 assembled Ga0098052_10001839	319	6	3300008050	Marine viral communities from the Subarctic Pacific Ocean - 15_ETSP_OMZ_AT15312 metaG (*) (MER-FS) (assembled)	
724	3300008050 assembled Ga0098052_100026416	320	3	3300008050	Marine viral communities from the Subarctic Pacific Ocean - 15_ETSP_OMZ_AT15312 metaG (*) (MER-FS) (assembled)	
725	3300008224 assembled Ga0105350_100000945	321	3	3300008224	Methane-oxidizing microbial communities from mesocosms in the Hudson Canyon - EN1E Hudson Canyon (*) (MER-FS) (assembled)	
726	3300009093 assembled Ga0105240_100005042	322	3	3300009093	Corn rhizosphere microbial communities from Kellogg Biological Station, Michigan, USA - KBS C5-4 metaG (*) (MER-FS) (assembled)	
727	3300009169 assembled	323	3	3300009169	Freshwater sediment microbial communities from Prairie Pothole Lake near	

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	Ga0105097_1000009945				Jamestown, North Dakota, USA - PPLs Lake P7 Core (1) Depth 10-12cm May2015 (*) (MER-FS) (assembled)	
728	3300009175 assembled Ga0073936_1000120334	324	3	3300009175	Freshwater lake bacterial and archeal communities from Alinen Mustajarvi, Finland, to study Microbial Dark Matter (Phase II) - Alinen Mustajarvi 5m metaG (*) (MER-FS) (assembled)	
729	3300009415 assembled Ga0115029_100184931	325	3	3300009415	Marine algal microbial communities from Sidmouth, United Kingdom - Sidmouth_Asex1 metaG (*) (MER-FS) (assembled)	
730	3300009419 assembled Ga0114982_10001831	326	3	3300009419	Subsurface microbial communities from deep shales in Ohio, USA - Utica-3 well 1 S input2 FT (*) (MER-FS) (assembled)	
731	3300009488 assembled Ga0114925_1000023517	327	2	3300009488	Deep subsurface microbial communities from Indian Ocean to uncover new lineages of life (NeLLi) - Sumatra_00607 metaG (*) (MER-FS) (assembled)	
732	3300009488 assembled Ga0114925_100003506	328	2	3300009488	Deep subsurface microbial communities from Indian Ocean to uncover new lineages of life (NeLLi) - Sumatra_00607 metaG (*) (MER-FS) (assembled)	
733	3300009508 assembled Ga0115567_1000068222	329	7	3300009508	Pelagic marine microbial communities from North Sea - COGITO_mtgs_120412 (*) (MER-FS) (assembled)	
734	3300009512 assembled Ga0115003_100022198	330	3	3300009512	Marine microbial communities from western Arctic Ocean - ArcticOcean_MG_CB11_88 (*) (MER-FS) (assembled)	
735	3300009546 assembled Ga0099799_100233	331	3	3300009546	Marine eukaryotic communities from CALCOFI LINE 67, Pacific Ocean - CN11_C50_N6_SortLC_1 (*) (MER-FS) (assembled)	
736	3300009669 assembled Ga0116148_10010742	332	3	3300009669	Active sludge microbial communities of municipal wastewater-treating anaerobic digesters from USA - AD_UKC055_MetaG (*) (MER-FS) (assembled)	
737	3300009779 assembled Ga0116152_100003906	333	3	3300009779	Active sludge microbial communities of municipal wastewater-treating anaerobic digesters from Hong Kong - AD_UKC119_MetaG (*) (MER-FS) (assembled)	
738	3300009788 assembled Ga0114923_1000042134	334	2	3300009788	Deep subsurface microbial communities from Indian Ocean to uncover new lineages of life (NeLLi) - Sumatra_00157 metaG (*) (MER-FS) (assembled)	
739	3300009838 assembled Ga0116153_100010806	335	6	3300009838	Active sludge microbial communities of municipal wastewater-treating anaerobic digesters from USA - AD_UKC028_MetaG (*) (MER-FS) (assembled)	
740	3300010028 assembled Ga0134115_1006245	336	3	3300010028	Active sludge microbial communities from wastewater treatment plant in Klosterneuburg, Austria - C35_LANv3 (*) (MER-FS) (assembled)	
741	3300010160 assembled Ga0114967_100001146	337	3	3300010160	Freshwater microbial communities from Lake Montjoie, Canada to study carbon cycling - M_130628_MF_MetaG (*) (MER-FS) (assembled)	
742	3300010162 assembled Ga0131853_1000011621	338	3	3300010162	Termite gut microbial communities from Petit-Saut, French Guiana - Lab288P1 metaG (version 2) (*) (MER-FS) (assembled)	
743	3300010162 assembled Ga0131853_1000234120	339	3	3300010162	Termite gut microbial communities from Petit-Saut, French Guiana - Lab288P1 metaG (version 2) (*) (MER-FS) (assembled)	
744	3300010162 assembled	340	3	3300010162	Termite gut microbial communities from Petit-Saut, French Guiana - Lab288P1	

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	Ga0131853_1000511220				metaG (version 2) (*) (MER-FS) (assembled)	
745	3300010270 assembled Ga0129306_100025163	341	1	3300010270	Capybara group fecal microbial communities from Wisconsin, USA - P827 metagenome (*) (MER-FS) (assembled)	
746	3300010313 assembled Ga0116211_100026028	342	6	3300010313	Hot spring microbial communities from South Africa to study Microbial Dark Matter (Phase II) - Sagole hot spring metaG (*) (MER-FS) (assembled)	
747	3300010373 assembled Ga0134128_1000050820	343	3	3300010373	Terrestrial soil microbial communities with excess Nitrogen fertilizer from Kellogg Biological Station, Michigan, USA - KB3-175-4 (*) (MER-FS) (assembled)	
748	3300010379 assembled Ga0136449_1000153745	344	2	3300010379	Sb_50d combined assembly (*) (MER-FS) (assembled)	
749	3300010396 assembled Ga0134126_1000011835	345	3	3300010396	Terrestrial soil microbial communities with excess Nitrogen fertilizer from Kellogg Biological Station, Michigan, USA - KB3-175-2 (*) (MER-FS) (assembled)	
750	3300010430 assembled Ga0118733_10000149451	346	3	3300010430	Marine sediment microbial communities from Gulf of Thailand under amendment with organic carbon and nitrate - JGI co-assembly of 8 samples (*) (MER-FS) (assembled)	
751	3300010430 assembled Ga0118733_10000158731	347	6	3300010430	Marine sediment microbial communities from Gulf of Thailand under amendment with organic carbon and nitrate - JGI co-assembly of 8 samples (*) (MER-FS) (assembled)	
752	3300010430 assembled Ga0118733_10000628422	348	3	3300010430	Marine sediment microbial communities from Gulf of Thailand under amendment with organic carbon and nitrate - JGI co-assembly of 8 samples (*) (MER-FS) (assembled)	
753	3300012103 assembled Ga0136578_1000209	349	6	3300012103	Saline lake microbial communities from Deep lake, Antarctica - Metagenome #190 (*) (MER-FS) (assembled)	
754	3300012533 assembled Ga0138256_1000042615	350	6	3300012533	Active sludge microbial communities from wastewater in Klosterneuburg, Austria - KNB2014incub_MG (*) (MER-FS) (assembled)	
755	3300012950 assembled Ga0163108_1000095519	351	3	3300012950	Marine microbial communities from the Central Pacific Ocean - Fk160115 155m metaG (*) (MER-FS) (assembled)	
756	3300012979 assembled Ga0123348_1000024225	352	5	3300012979	Fecal eukaryotic communites from dung pellets of Tule Elk in California, USA - Elk Dung B1 Day 1 Metagenome (*) (MER-FS) (assembled)	
757	3300012983 assembled Ga0123349_1000049625	353	5	3300012983	Fecal eukaryotic communites from dung pellets of Tule Elk in California, USA - Elk Dung C2 Day 2 Metagenome (*) (MER-FS) (assembled)	
758	3300013088 assembled Ga0163200_1000002129	354	6	3300013088	Freshwater microbial communities from Powell Lake, British Columbia, Canada to study Microbial Dark Matter (Phase II) - PL_2010_200m (*) (MER-FS) (assembled)	
759	3300013092 assembled Ga0163199_1000006211	355	6	3300013092	Freshwater microbial communities from Powell Lake, British Columbia, Canada to study Microbial Dark Matter (Phase II) - PL_2010_150m (*) (MER-FS) (assembled)	

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760	3300013131 assembled Ga0172373_100005744	356	3	3300013131	Freshwater microbial communities from Kabuno Bay, South-Kivu, Congo ? kab_092012_10m (*) (MER-FS) (assembled)	
761	3300014491 assembled Ga0182014_100007864	357	2	3300014491	Permafrost microbial communities from Stordalen Mire, Sweden - 612S2D metaG (*) (MER-FS) (assembled)	
762	3300014499 assembled Ga0182012_100003757	358	3	3300014499	Permafrost microbial communities from Stordalen Mire, Sweden - 612S2S metaG (*) (MER-FS) (assembled)	
763	3300017795 assembled Ga0189288_1022816	359	6	3300017795	Marine microbial communities from the Costa Rica Dome - UW105 mini metaG (*) (MER-FS) (assembled)	
764	3300017798 assembled Ga0189289_1026116	360	6	3300017798	Marine microbial communities from the Costa Rica Dome - UW106 mini metaG (*) (MER-FS) (assembled)	
765	3300017805 assembled Ga0189287_100018226	361	6	3300017805	Marine microbial communities from the Costa Rica Dome - UW86 mini metaG (*) (MER-FS) (assembled)	
766	3300017990 assembled Ga0180436_1000345026	362	3	3300017990	Hypersaline lake sediment archaeal communities from the Salton Sea, California, USA - SS_3_S_2 metaG (*) (MER-FS) (assembled)	
767	3300018018 assembled Ga0187886_100041240	363	2	3300018018	Peatland microbial communities from SPRUCE experiment site at the Marcell Experimental Forest, Minnesota, USA - June2016WEW_20_150 (*) (MER-FS) (assembled)	
768	3300018018 assembled Ga0187886_100069122	364	2	3300018018	Peatland microbial communities from SPRUCE experiment site at the Marcell Experimental Forest, Minnesota, USA - June2016WEW_20_150 (*) (MER-FS) (assembled)	
769	3300018033 assembled Ga0187867_1000087624	365	3	3300018033	Peatland microbial communities from SPRUCE experiment site at the Marcell Experimental Forest, Minnesota, USA - June2016WEW_13_10 (*) (MER-FS) (assembled)	
770	3300018038 assembled Ga0187855_1000057816	366	3	3300018038	Peatland microbial communities from SPRUCE experiment site at the Marcell Experimental Forest, Minnesota, USA - June2016WEW_8_10 (*) (MER-FS) (assembled)	
771	3300018042 assembled Ga0187871_100009711	367	3	3300018042	Peatland microbial communities from SPRUCE experiment site at the Marcell Experimental Forest, Minnesota, USA - June2016WEW_16_10 (*) (MER-FS) (assembled)	
772	3300018080 assembled Ga0180433_1001105911	368	3	3300018080	Hypersaline lake sediment archaeal communities from the Salton Sea, California, USA - SS_1_D_1 metaG (*) (MER-FS) (assembled)	
773	3300018428 assembled Ga0181568_1000115027	369	3	3300018428	Coastal salt marsh microbial communities from the Groves Creek Marsh, Skidaway Island, Georgia - 101404AT metaG (megahit assembly) (*) (MER-FS) (assembled)	
774	3300018475 assembled Ga0187907_1000663212	370	1	3300018475	Goat fecal pellet fungal communities from Santa Barbara, California, USA ? pellet 1 (*) (MER-FS) (assembled)	
775	3300018475 assembled	371	1	3300018475	Goat fecal pellet fungal communities from Santa Barbara, California, USA ? pellet	

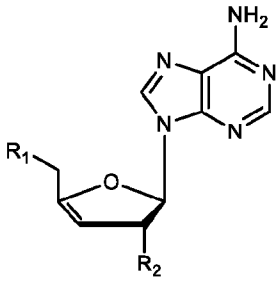
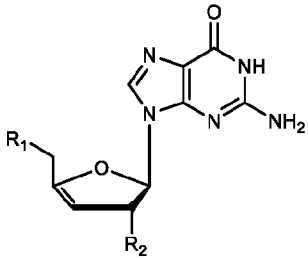
SEQ ID NO	IMG id	pVip #	Clade	Metagenome genome IMG ID	Genome Metagenome Name	Kinase
	Ga0187907_100078053				1 (*) (MER-FS) (assembled)	
776	3300018475 assembled Ga0187907_1000859111	372	1	3300018475	Goat fecal pellet fungal communities from Santa Barbara, California, USA ? pellet 1 (*) (MER-FS) (assembled)	
777	3300018493 assembled Ga0187909_1000543313	373	1	3300018493	Goat fecal pellet fungal communities from Santa Barbara, California, USA ? pellet 3 (*) (MER-FS) (assembled)	
778	3300018494 assembled Ga0187911_1000586113	374	1	3300018494	Goat fecal pellet fungal communities from Santa Barbara, California, USA ? diluted pellet 2 (*) (MER-FS) (assembled)	
779	3300018494 assembled Ga0187911_1001224520	375	1	3300018494	Goat fecal pellet fungal communities from Santa Barbara, California, USA ? diluted pellet 2 (*) (MER-FS) (assembled)	
780	3300018495 assembled Ga0187908_1000576413	376	1	3300018495	Goat fecal pellet fungal communities from Santa Barbara, California, USA ? pellet 2 (*) (MER-FS) (assembled)	
781	3300018495 assembled Ga0187908_1000603814	377	1	3300018495	Goat fecal pellet fungal communities from Santa Barbara, California, USA ? pellet 2 (*) (MER-FS) (assembled)	
782	3300018495 assembled Ga0187908_100073603	378	1	3300018495	Goat fecal pellet fungal communities from Santa Barbara, California, USA ? pellet 2 (*) (MER-FS) (assembled)	
783	3300018878 assembled Ga0187910_1000693112	379	1	3300018878	Goat fecal pellet fungal communities from Santa Barbara, California, USA ? diluted pellet 1 (*) (MER-FS) (assembled)	
784	3300018878 assembled Ga0187910_1000711113	380	1	3300018878	Goat fecal pellet fungal communities from Santa Barbara, California, USA ? diluted pellet 1 (*) (MER-FS) (assembled)	
785	3300018878 assembled Ga0187910_100083003	381	1	3300018878	Goat fecal pellet fungal communities from Santa Barbara, California, USA ? diluted pellet 1 (*) (MER-FS) (assembled)	
786	3300018878 assembled Ga0187910_1000906015	382	sp	3300018878	Goat fecal pellet fungal communities from Santa Barbara, California, USA ? diluted pellet 1 (*) (MER-FS) (assembled)	
787	3300019373 assembled Ga0187895_100043618	383	1	3300019373	Goat fecal pellet enrichment culture fungal communities from Isla Vista, California, USA - Alfalfa, Gen0, Rep 3 (*) (MER-FS) (assembled)	
788	3300019457 assembled Ga0193932_1007821	384	sp	3300019457	Sorted cell/s from Southern Trench hydrothermal vent microbial mat, Guaymas Basin, Mexico ? 6X_4868_18_01 (*) (MER-FS) (assembled)	
789	3300019750 assembled Ga0194000_100000539	385	1	3300019750	Sediment microbial communities from the Broadkill River, Lewes, Delaware, United States ? FLT_6-7_MG (*) (MER-FS) (assembled)	

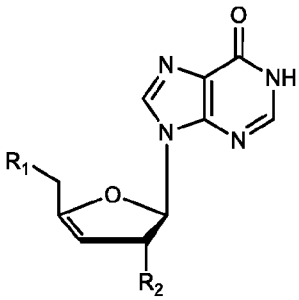
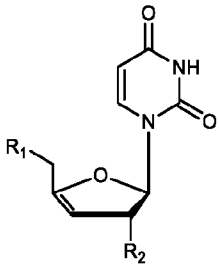
[00748] While certain features disclosed have been illustrated and described herein, many modifications, substitutions, changes, and equivalents will now occur to those of ordinary skill in the art. It is, therefore, to be understood that the appended claims are intended to cover all such modifications and changes as fall within the true spirit of the compounds and methods of use disclosed herein.

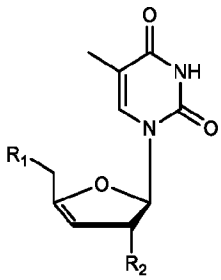
CLAIMS

What is claimed is:

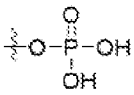
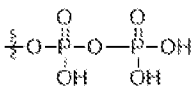
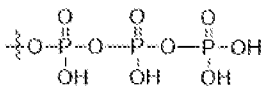
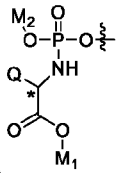
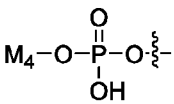
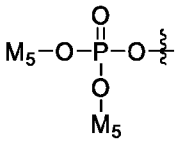
1. A compound represented by the structure of

Formula IB:  , Formula IIB:  ,

Formula IIIB:  , Formula IVB:  , or

Formula VB:  ,

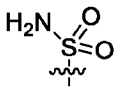
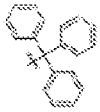
wherein

R_1 is OH,  ,  ,  ,  , ,  , or  ;

Q is a side chain of an amino acid;

M_1 is an alkyl;

M_2 is an aryl, a substituted aryl, a heteroaryl or a substituted heteroaryl;

M_3 is  , ,  , or ;

M_4 is -(C_2-C_6)alkyl-O-(C_{10}-C_{20})alkyl;

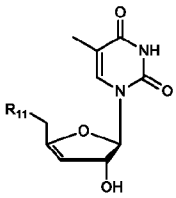
each M_5 is -$(CH_2)_n$-S-C(=O)-(C_1-C_8)alkyl;

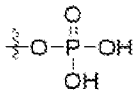
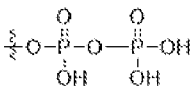
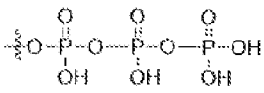
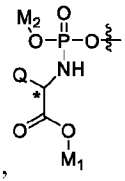
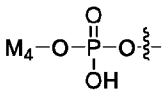
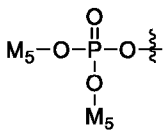
n is 1-4;

R_2 is -OH or -O-COO-alkyl; and

wherein if R_1 is OH, then R_2 is -O-COO-alkyl.

2. The compound of claim 1, wherein said compound has the structure of Formula

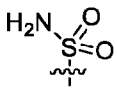
VB1:  wherein

R_{11} is  ,  ,  ,  , ,  , or  ;

Q is a side chain of an amino acid;

M_1 is an alkyl;

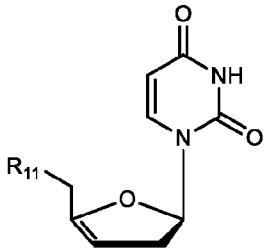
M_2 is an aryl, a substituted aryl, a heteroaryl or a substituted heteroaryl;

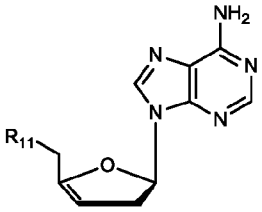
M_3 is  , , , or ;

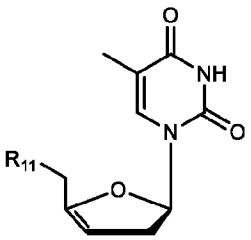
M_4 is -(C_2-C_6)alkyl-O-(C_{10}-C_{20})alkyl;

each M_5 is -$(CH_2)_n$-S-C(=O)-(C_1-C_8)alkyl; and

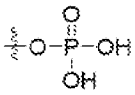
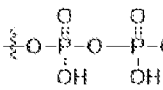
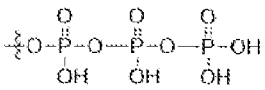
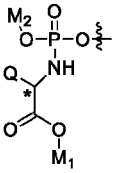
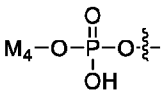
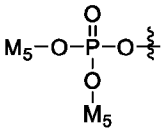
n is 1-4.

3. A compound represented by the structure of Formula VIB: 

, Formula VIIB:  , or

Formula VIIIB:  ,

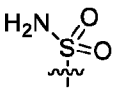
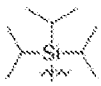
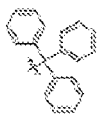
wherein

R_{11} is  ,  ,  ,  , ,  , or  ;

Q is a side chain of an amino acid;

M_1 is an alkyl;

M_2 is an aryl, a substituted aryl, a heteroaryl or a substituted heteroaryl;

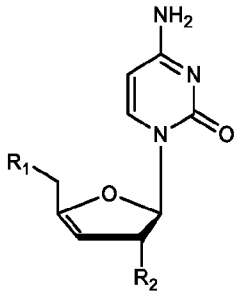
M_3 is  ,  ,  , or ;

M_4 is -(C_2-C_6)alkyl-O-(C_{10}-C_{20})alkyl;

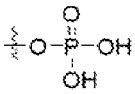
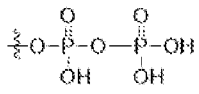
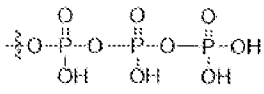
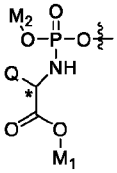
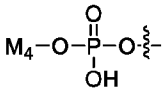
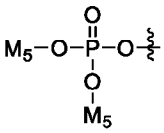
each M_5 is -$(CH_2)_n$-S-C(=O)-(C_1-C_8)alkyl; and

n is 1-4

4. A compound represented by the structure of

Formula IXB:  ,

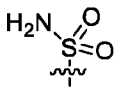
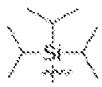
wherein

R_1 is OH,  ,  ,  ,  , ,  , or  ;

Q is a side chain of an amino acid;

M_1 is an alkyl;

M_2 is an aryl, a substituted aryl, a heteroaryl or a substituted heteroaryl;

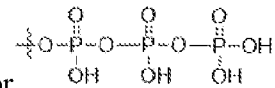
M_3 is  ,  , , or ;

M_4 is -(C_2-C_6)alkyl-O-(C_{10}-C_{20})alkyl;

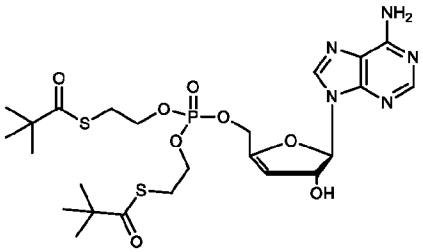
each M_5 is -$(CH_2)_n$-S-C(=O)-(C_1-C_8)alkyl;

n is 1-4;

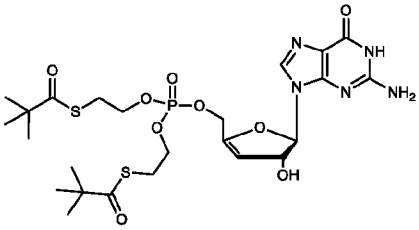
R_2 is -OH or -O-COO-alkyl; and

wherein if R_1 is OH or  , then R_2 is not OH.

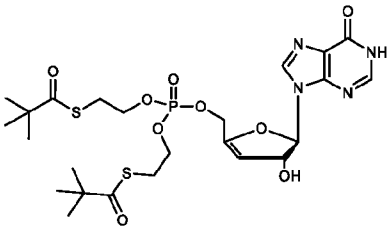
5. The compound of any one of claims 1-4, wherein said compound has the structure of



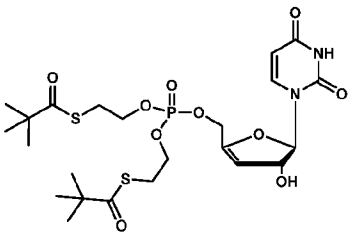
Compound 1,



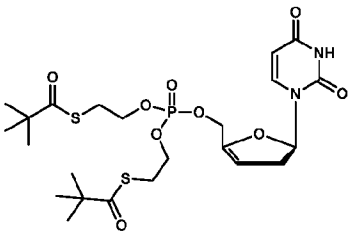
Compound 2,



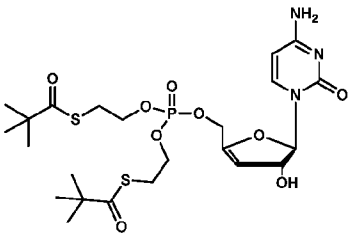
Compound 3,



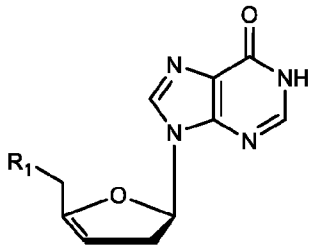
Compound 4,

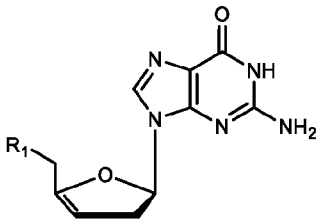


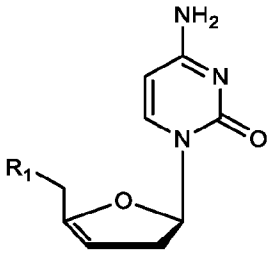
Compound 5, or

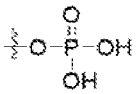
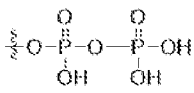
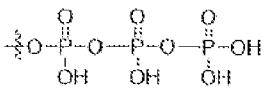
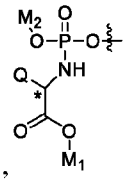
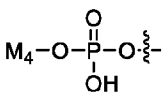
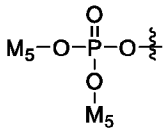


Compound 6.

6. A compound represented by the structure of Formula XB:  ,

Formula XIB:  , or

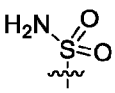
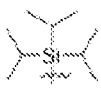
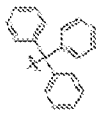
Formula XIIB:  , wherein

R_1 is OH,  ,  ,  ,  , ,  , or  ;

Q is a side chain of an amino acid;

M_1 is an alkyl;

M_2 is an aryl, a substituted aryl, a heteroaryl or a substituted heteroaryl;

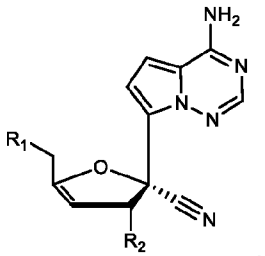
M_3 is  ,  ,  , or ;

M_4 is -(C_2-C_6)alkyl-O-(C_{10}-C_{20})alkyl;

each M_5 is -$(CH_2)_n$-S-C(=O)-(C_1-C_8)alkyl; and

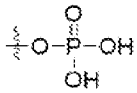
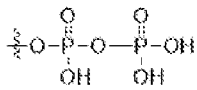
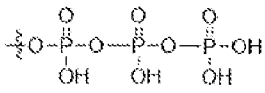
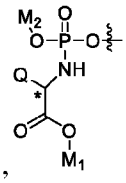
n is 1-4.

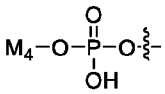
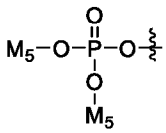
7. A compound represented by the structure of formula XIIIB:



,

wherein

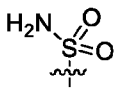
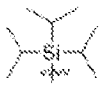
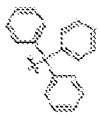
R_1 is OH,  ,  ,  ,  , ,

 , or  ;

Q is a side chain of an amino acid;

M_1 is an alkyl;

M_2 is an aryl, a substituted aryl, a heteroaryl or a substituted heteroaryl;

M_3 is  ,  ,  , or ;

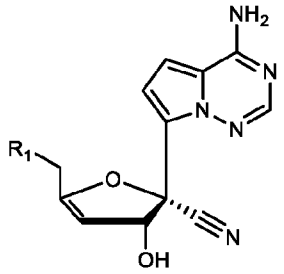
M_4 is -(C_2-C_6)alkyl-O-(C_{10}-C_{20})alkyl;

each M_5 is -$(CH_2)_n$-S-C(=O)-(C_1-C_8)alkyl;

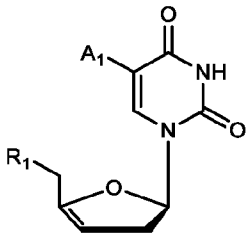
n is 1-4; and

R_2 is -OH or –O-COO-alkyl.

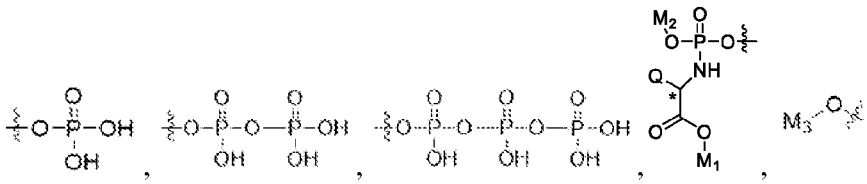
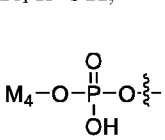
8. The compound of claim 7, wherein said compound has the structure of Formula

XIIIB1:  , wherein R_1 is as defined in claim 7.

9. A compound represented by the structure of Formula XIVB:

 ,

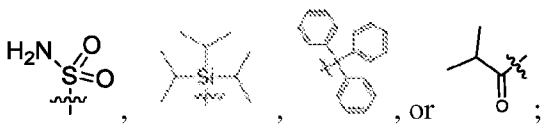
wherein

R_1 is OH,  ,  ; or

Q is a side chain of an amino acid;

M_1 is an alkyl;

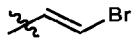
M_2 is an aryl, a substituted aryl, a heteroaryl or a substituted heteroaryl;

M_3 is  , or ;

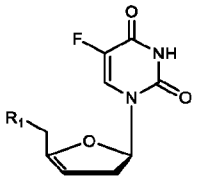
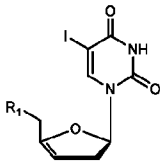
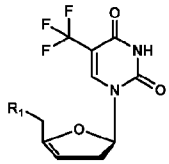
M_4 is -(C_2-C_6)alkyl-O-(C_{10}-C_{20})alkyl;

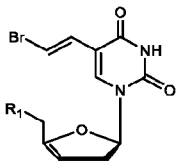
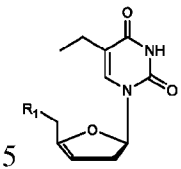
each M_5 is -$(CH_2)_n$-S-C(=O)-(C_1-C_8)alkyl;

n is 1-4; and

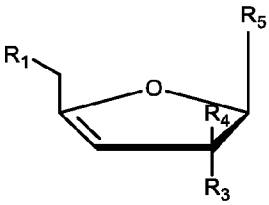
A_1 is a halo, a haloalkyl, an alkyl, or  .

10. The compound of claim 9, wherein said compound has the structure of Formula XIVB1

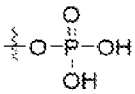
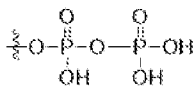
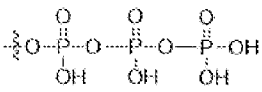
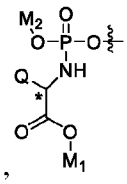
 , Formula XIVB2  , Formula XIVB3  ,

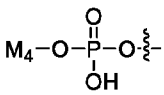
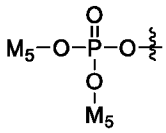
Formula XIVB4  , or Formula XIVB5  , or a combination thereof; wherein R_1 is as defined in claim 9.

11. A compound represented by the structure of

Formula XVB:  ,

wherein

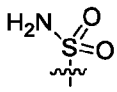
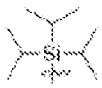
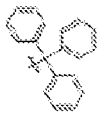
R_1 is OH,  ,  ,  ,  , ,

 , or  ;

Q is a side chain of an amino acid;

M_1 is an alkyl;

M_2 is an aryl, a substituted aryl, a heteroaryl or a substituted heteroaryl;

M_3 is  ,  ,  , or ;

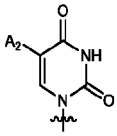
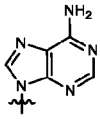
M_4 is -(C_2-C_6)alkyl-O-(C_{10}-C_{20})alkyl;

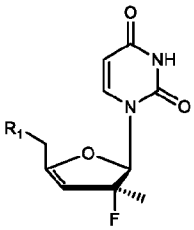
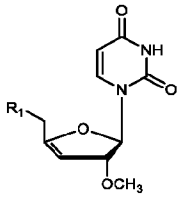
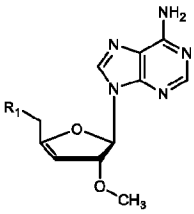
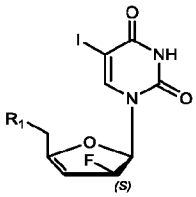
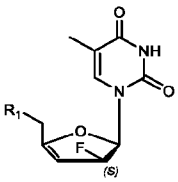
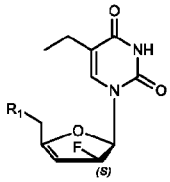
each M_5 is -$(CH_2)_n$-S-C(=O)-(C_1-C_8)alkyl;

n is 1-4;

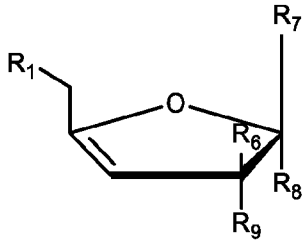
R_3 is H, a halo or an alkoxy;

R_4 is H, a halo or an alkyl;

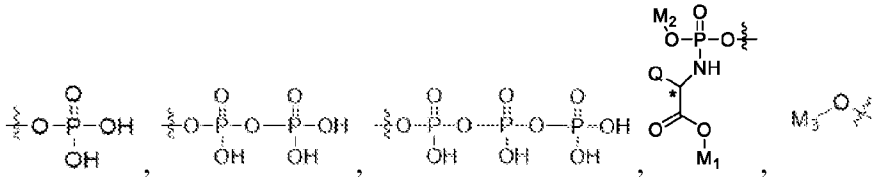
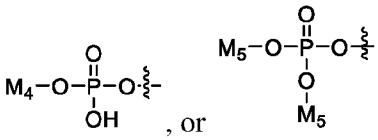
R_5 is  , or  , wherein A_2 is H, halo or alkyl; and wherein R_3 is not the same as R_4.

12. The compound of claim 11, represented by the structure of Formula XVB1  , Formula XVB2  , Formula XVB3  , Formula XVB4  , Formula XVB5  , or Formula XVB6  , wherein R_1 is as defined in claim 11.

13. A compound represented by the structure of

Formula XVIB:  ,

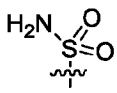
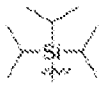
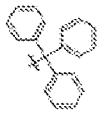
wherein

R_1 is OH,  ,  , or ;

Q is a side chain of an amino acid;

M_1 is an alkyl;

M_2 is an aryl, a substituted aryl, a heteroaryl or a substituted heteroaryl;

M_3 is  ,  ,  , or ;

M_4 is -(C_2-C_6)alkyl-O-(C_{10}-C_{20})alkyl;

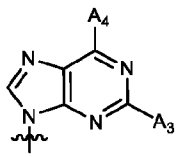
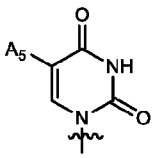
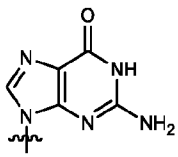
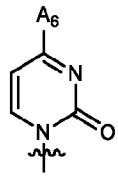
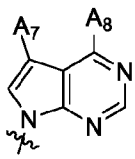
each M_5 is -$(CH_2)_n$-S-C(=O)-(C_1-C_8)alkyl;

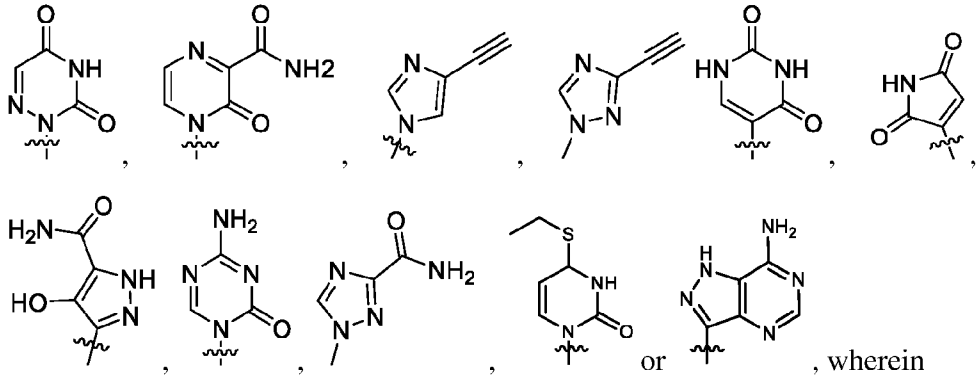
n is 1-4;

R_9 is H, OH, or -O-COO-alkyl;

R_6 is H, Me, -CCH or OH;

R_8 is H

R_7 is  ,  ,  ,  ,  ,



, wherein

A_3 is H, a halo or an amino;

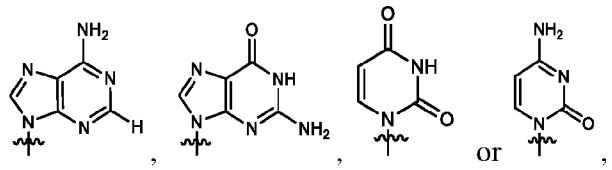
A_4 is H, an amino, an alkoxy, or an alkyl;

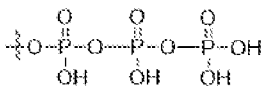
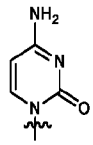
A_5 is H, a halo, a hydroxy or an alkyne;

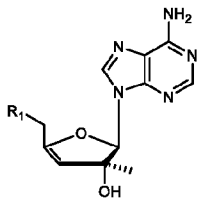
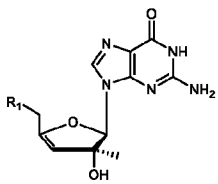
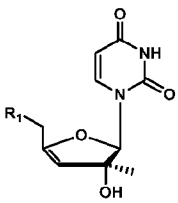
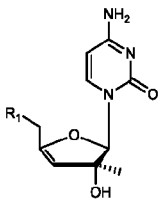
A_6 is H, an amino or a hydroxylamino;

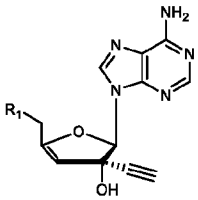
A_7 is H or an amido;

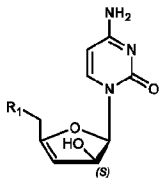
A_8 is an amino or an alkyl;

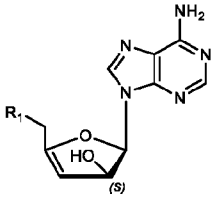
wherein if R_1 is OH and R_7 is  , and R_9 is OH, then R_6 is not H; and

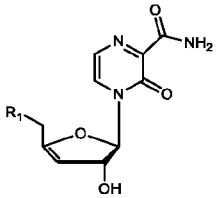
wherein if R_1 is  and R_7 is  and R_9 is OH then R_6 is not H.

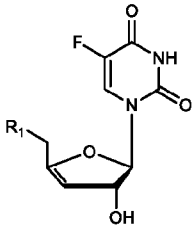
14. The compound of claim 13, wherein said compound has the structure of Formula XVIB1  , Formula XVIB2  , Formula XVIB3  , Formula XVIB4  , Formula XVIB5

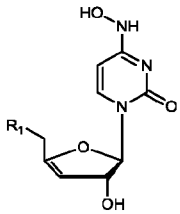

, Formula XVIB6

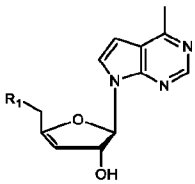

, Formula XVIB7

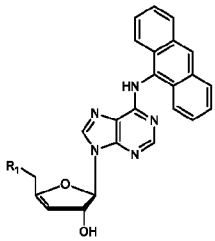

, Formula XVIB8

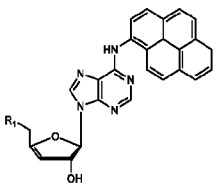

, Formula XVIB9

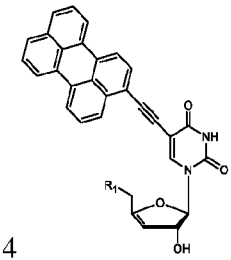

, Formula XVIB10

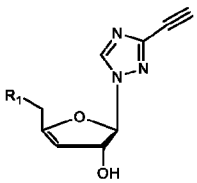

, Formula XVIB11

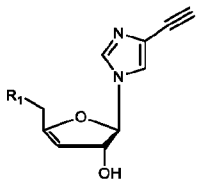

, Formula XVIB12

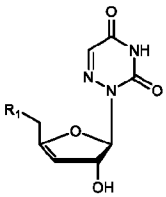

, Formula XVIB13

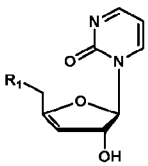

, Formula XVIB14

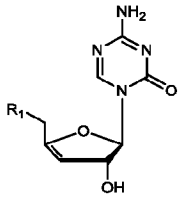

, Formula XVIB15


, Formula XVIB16


, Formula XVIB17


, Formula XVIB18


, Formula XVIB19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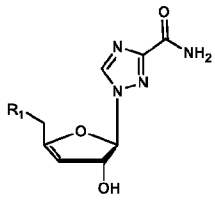
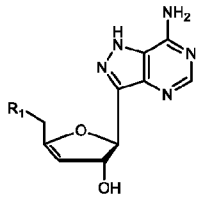
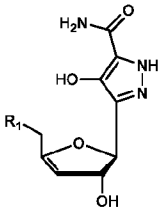
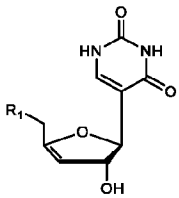
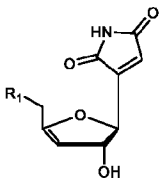
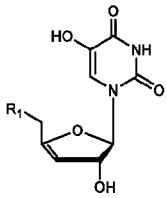
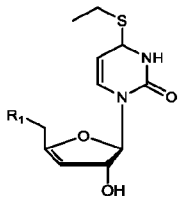
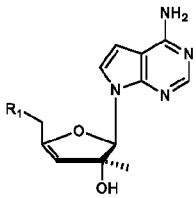
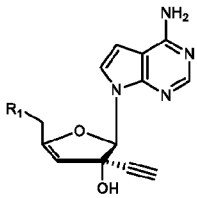
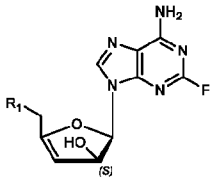
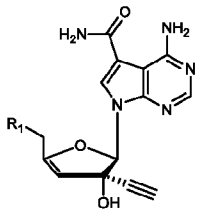
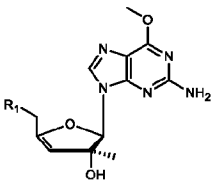
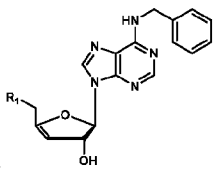
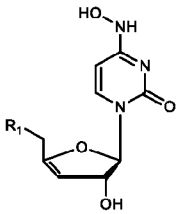
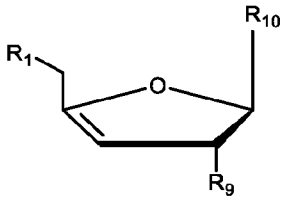
Formula XVIB20  , Formula XVIB21  , Formula XVIB22  , Formula XVIB23  , Formula XVIB24  , Formula XVIB25  , Formula XVIB26  , Formula XVIB27  , Formula XVIB28  , Formula XVIB29  , Formula XVIB30  , Formula XVIB31  , Formula XVIB32  , or Formula XVIB33  ,

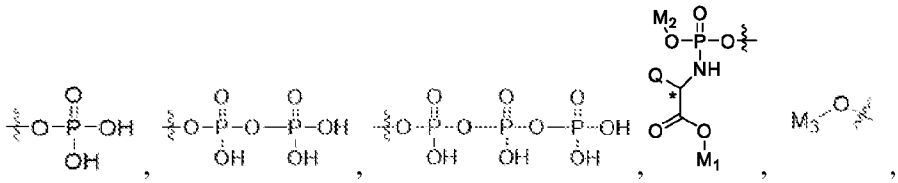
wherein R_1 is as defined in claim 13.

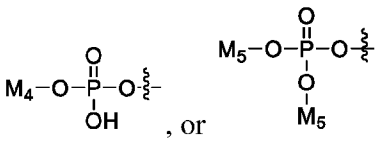
15. A compound represented by the structure of Formula XVIIB,



,

wherein

R_1 is OH, 

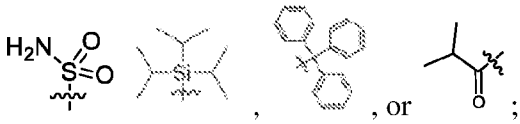


, or ;

Q is a side chain of an amino acid;

M_1 is an alkyl;

M_2 is an aryl, a substituted aryl, a heteroaryl or a substituted heteroaryl;

M_3 is 

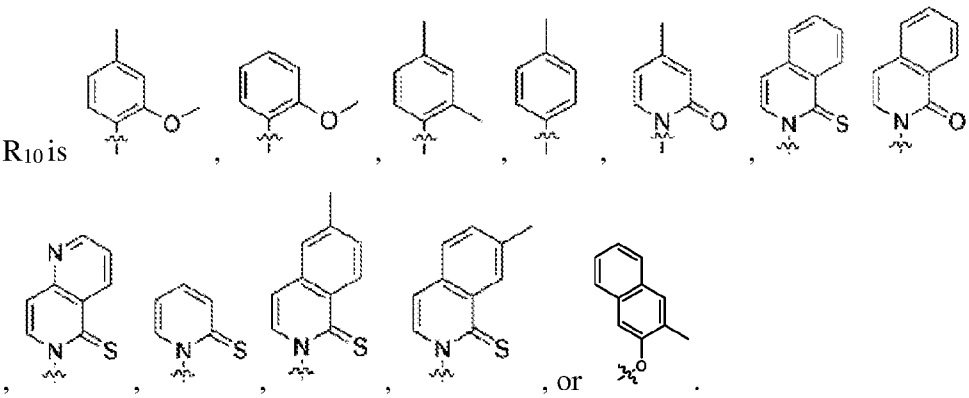
, or ;

M_4 is -(C_2-C_6)alkyl-O-(C_{10}-C_{20})alkyl;

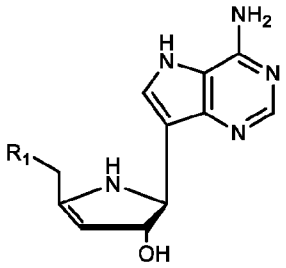
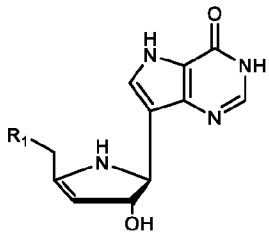
each M_5 is -$(CH_2)_n$-S-C(=O)-(C_1-C_8)alkyl;

n is 1-4;

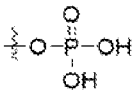
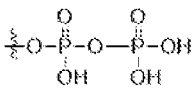
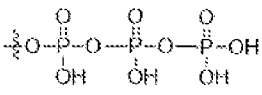
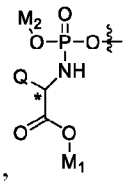
R_9 is H, OH or -O-COO-alkyl; and

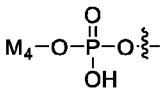
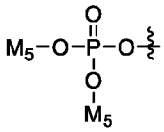
R_{10} is 

, or .

16. A compound represented by the structure of Formula XVIIIB:  , or Formula XXB:  ,

wherein

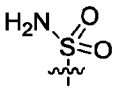
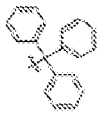
R_1 is OH,  ,  ,  ,  , ,

 , or  ;

Q is a side chain of an amino acid;

M_1 is an alkyl;

M_2 is an aryl, a substituted aryl, a heteroaryl or a substituted heteroaryl;

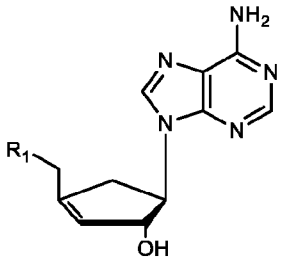
M_3 is  , ,  , or ;

M_4 is -(C_2-C_6)alkyl-O-(C_{10}-C_{20})alkyl;

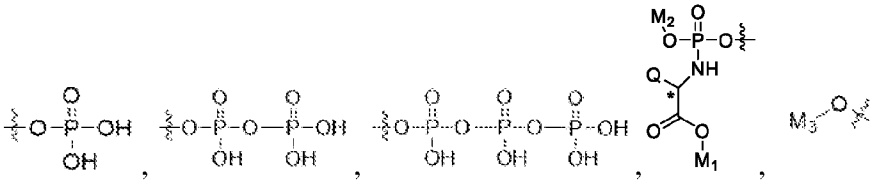
each M_5 is -$(CH_2)_n$-S-C(=O)-(C_1-C_8)alkyl; and

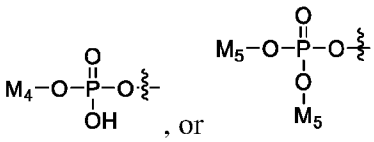
n is 1-4.

17. A compound represented by the structure of Formula XIXB:



, wherein

R_1 is OH, 

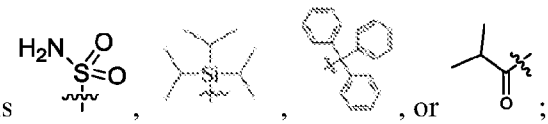


, or ;

Q is a side chain of an amino acid;

M_1 is an alkyl;

M_2 is an aryl, a substituted aryl, a heteroaryl or a substituted heteroaryl;

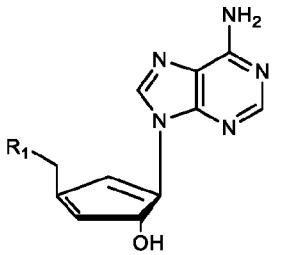
M_3 is 

M_4 is -(C_2-C_6)alkyl-O-(C_{10}-C_{20})alkyl;

each M_5 is -$(CH_2)_n$-S-C(=O)-(C_1-C_8)alkyl; and

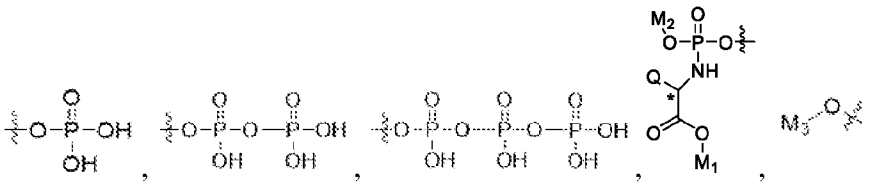
n is 1-4.

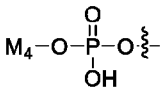
18. A compound represented by the structure of Formula XXIB:

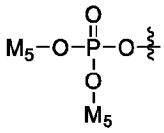


,

wherein

R_1 is OH, 

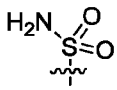


, or


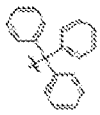
;

Q is a side chain of an amino acid;
M_1 is an alkyl;

M_2 is an aryl, a substituted aryl, a heteroaryl or a substituted heteroaryl;

M_3 is 

,

, 
, or

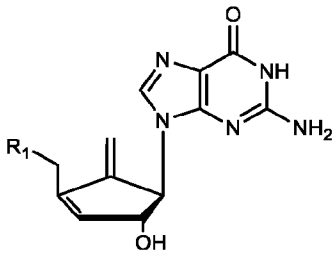
;

M_4 is -(C_2-C_6)alkyl-O-(C_{10}-C_{20})alkyl;

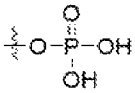
each M_5 is -$(CH_2)_n$-S-C(=O)-(C_1-C_8)alkyl; and

n is 1-4.

19. A compound represented by the structure of Formula XXIIB:



, wherein

R_1 is OH, 

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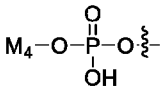
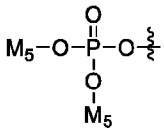
,

,

,

,

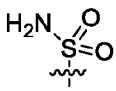


, or


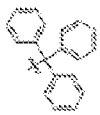
;

Q is a side chain of an amino acid;
M_1 is an alkyl;

M_2 is an aryl, a substituted aryl, a heteroaryl or a substituted heteroaryl;

M_3 is , 

,

, 
, or

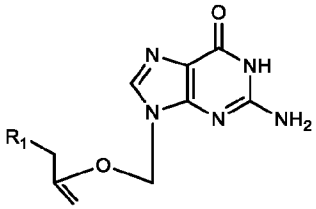
;

M_4 is -(C_2-C_6)alkyl-O-(C_{10}-C_{20})alkyl;

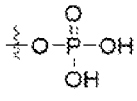
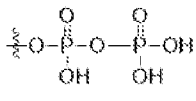
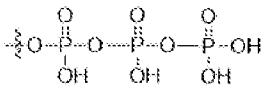
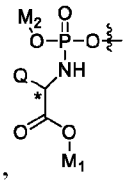
each M_5 is -$(CH_2)_n$-S-C(=O)-(C_1-C_8)alkyl; and

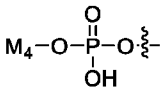
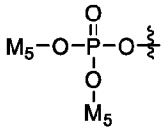
n is 1-4.

20. A compound represented by the structure of Formula XXIIIB:



, wherein

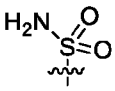
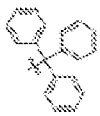
R_1 is OH,  ,  ,  ,  , ,

 , or  ;

Q is a side chain of an amino acid;

M_1 is an alkyl;

M_2 is an aryl, a substituted aryl, a heteroaryl or a substituted heteroaryl;

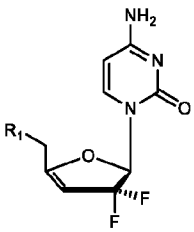
M_3 is  , ,  , or ;

M_4 is -(C_2-C_6)alkyl-O-(C_{10}-C_{20})alkyl;

each M_5 is -$(CH_2)_n$-S-C(=O)-(C_1-C_8)alkyl; and

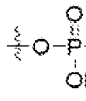
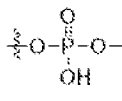
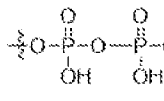
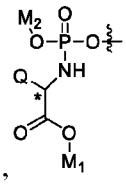
n is 1-4.

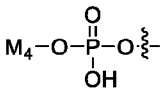
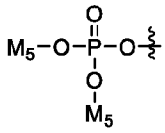
21. A compound represented by the structure of Formula XXIVB:



,

wherein

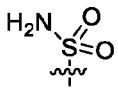
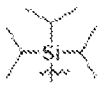
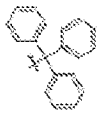
R_1 is OH,  ,  ,  ,  , ,

 , or  ;

Q is a side chain of an amino acid;

M_1 is an alkyl;

M_2 is an aryl, a substituted aryl, a heteroaryl or a substituted heteroaryl;

M_3 is  ,  ,  , or ;

M_4 is -(C_2-C_6)alkyl-O-(C_{10}-C_{20})alkyl;

each M_5 is -$(CH_2)_n$-S-C(=O)-(C_1-C_8)alkyl; and

n is 1-4.

22. A pharmaceutical composition comprising the compounds of any one of claims 1-21.

23. A pharmaceutical composition comprising at least two of the compounds of any one of claims 1-21.

24. The pharmaceutical composition of claim 22 or claim 23, wherein said composition comprises a pharmaceutically acceptable carrier.

25. A method of treating a disease in a subject in need thereof, comprising administering the pharmaceutical composition of claim 22 or claim 23.

26. The method of claim 25, wherein said disease comprises a virus-induced disease, a cancer, an autoimmune disease, an immune disorder, a bacterial associated disease or infection, or a combination thereof.

27. The method of claim 26, wherein said disease is caused by a virus selected from the group consisting of norovirus, rotavirus, hepatitis virus A, B, C, D, or E, rabies virus, West Nile virus, enterovirus, echovirus, coxsackievirus, herpes simplex virus (HSV), varicella-zoster virus, mosquito-borne viruses, arbovirus,

St. Louis encephalitis virus, California encephalitis virus, lymphocytic choriomeningitis virus, human immunodeficiency virus (HIV), poliovirus, zika virus, rubella virus, cytomegalovirus, human papillomavirus (HPV), enterovirus D68, severe acute respiratory syndrome (SARS) coronavirus, Middle East respiratory syndrome coronavirus (MERS-CoV), SARS coronavirus 2 (SARS-CoV-2), Epstein-Barr virus (EBV), influenza virus, influenza virus A2, influenza virus B, influenza virus A(H1N1), respiratory syncytial virus (RSV), polyoma viruses, BK virus, Tacaribe virus, Ebola virus, Dengue virus, and any combination thereof.

28. The method of claim 26, wherein said virus-induced disease is COVID19 caused by SARS coronavirus 2 infection.

29. The method of claim 25, wherein said treating a disease terminates polynucleotide chain synthesis in a cell.

30. The method of claim 29, wherein terminating polynucleotide chain synthesis increases termination of DNA chain synthesis, or increases termination of RNA chain synthesis, or a combination thereof.

31.The method of claim 29, wherein terminating polynucleotide chain synthesis confers viral resistance to said cell.

32. The method of claim 29, wherein said cell is a eukaryotic cell.

33. The method of claim 32, wherein said eukaryotic cell is a tumor cell, or is a cell infected by a virus or a foreign DNA.

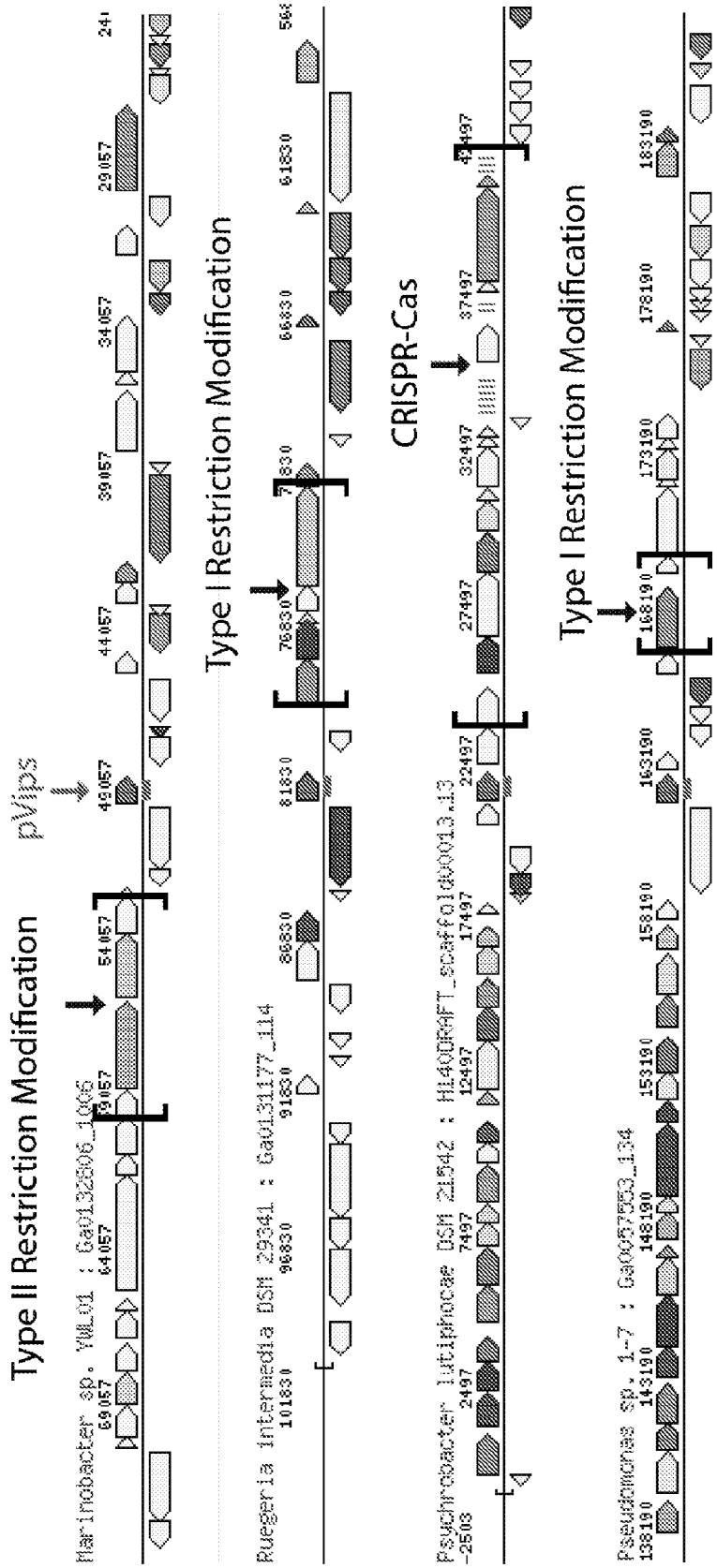

Type II Restriction Modification
pVips
Type I Restriction Modification
CRISPR-Cas
Type I Restriction Modification
Marinobacter sp. YWL01 : Ga0132806_1005
Ruegeria intermedia DSM 29341 : Ga0131177_114
Psychrobacter lutiphocae DSM 21542 : HL40DRAFT_scaffold00013.13
Pseudomonas sp. 1-7 : Ga0057553_134

Figure 1

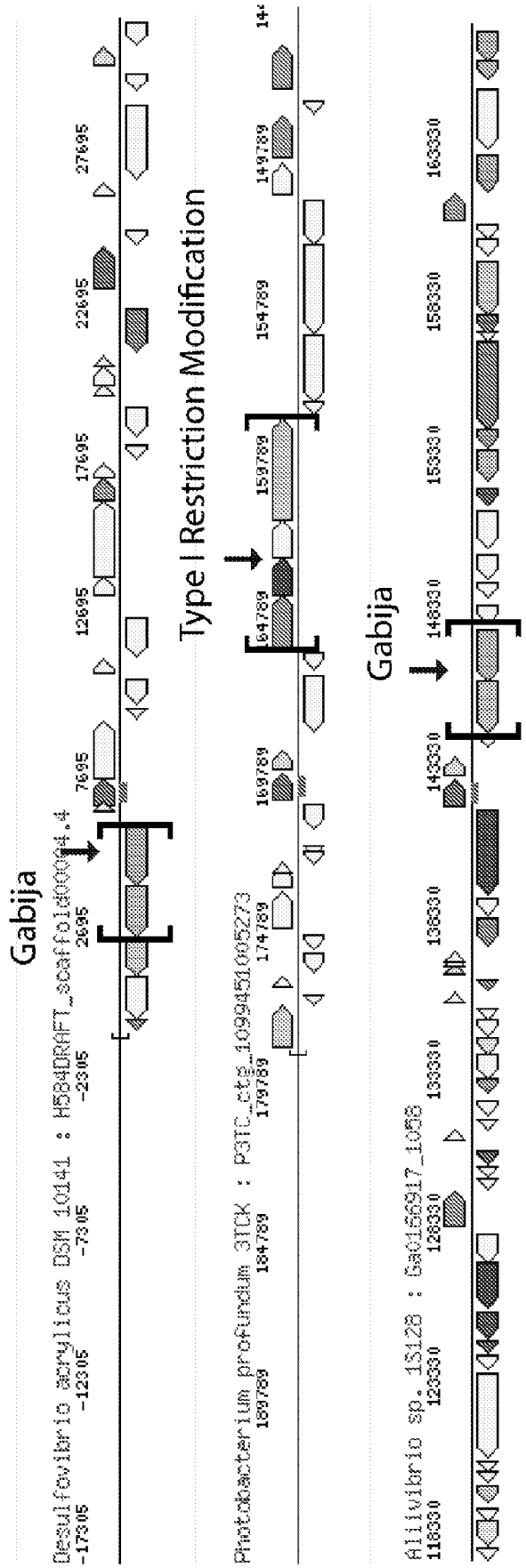


Figure 1 (cont.)

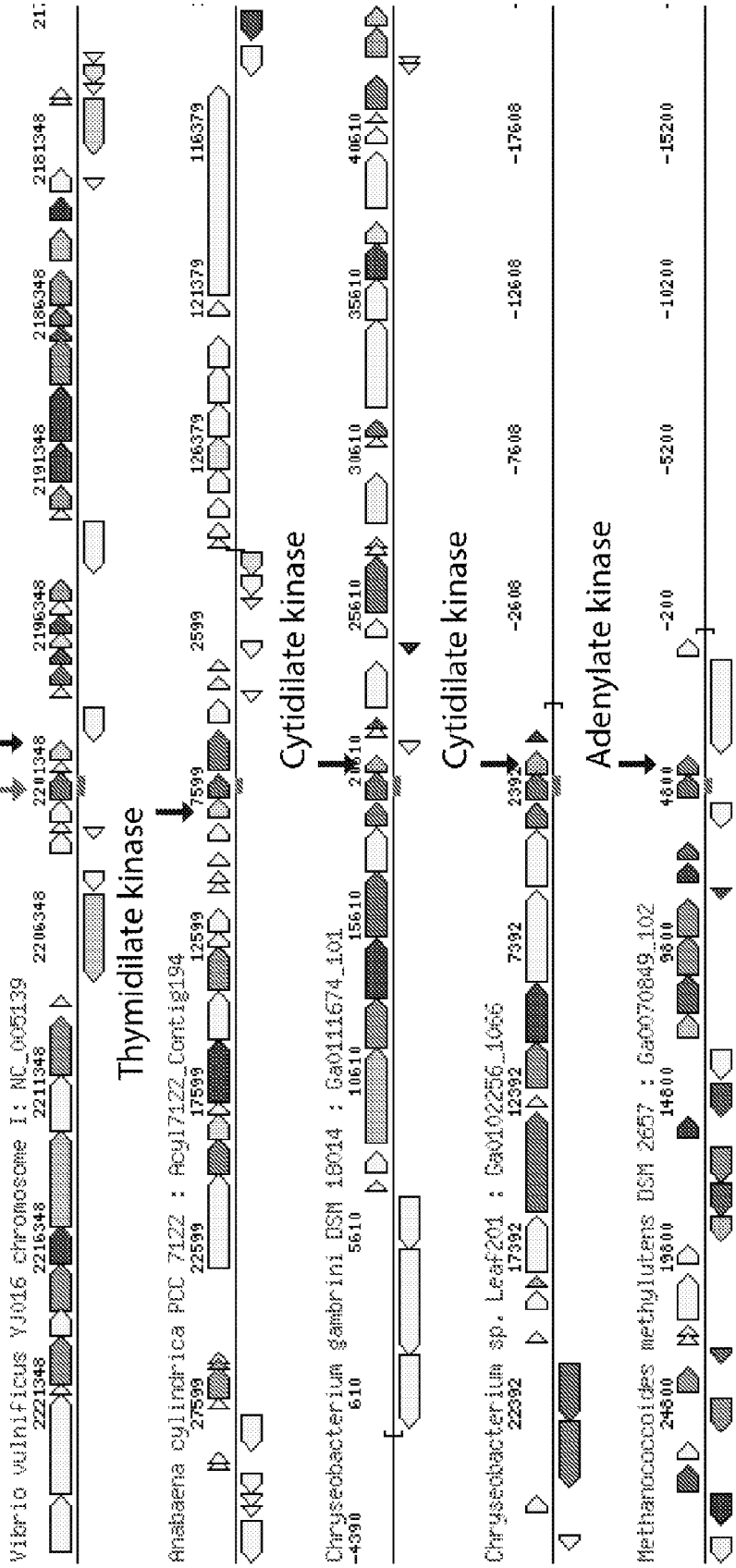

pVips
Thymidilate kinase
Thymidilate kinase
Cytidilate kinase
Cytidilate kinase
Adenylate kinase
Vibrio vulnificus YJ016 chromosome I: NC_005139
Anabaena cylindrica PCC 7122 : Acyl7122_Contig194
Chryseobacterium gambrini DSM 18014 : Ga0111674_101
Chryseobacterium sp. Leaf201 : Ga0102256_1066
Methanococcoides methylutens DSM 2657 : Ga0070849_102

Figure 2

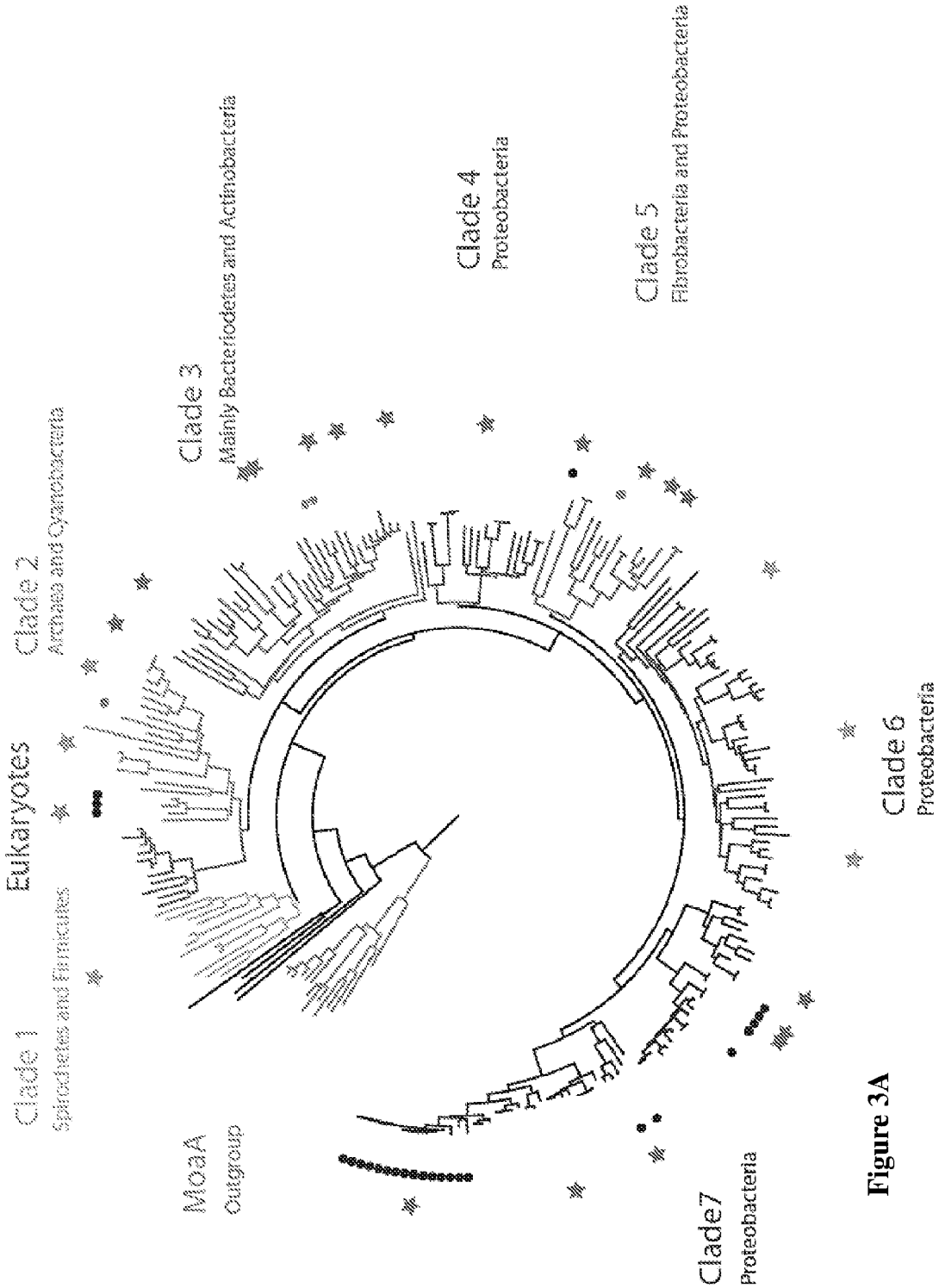

Clade 1
Spirochetes and Firmicutes
Eukaryotes
Clade 2
Archaea and Cyanobacteria
Clade 3
Mainly Bacteriodetes and Actinobacteria
Clade 4
Proteobacteria
Clade 5
Fibrobacteria and Proteobacteria
Clade 6
Proteobacteria
Clade7
Proteobacteria
MoaA
Outgroup

Figure 3A

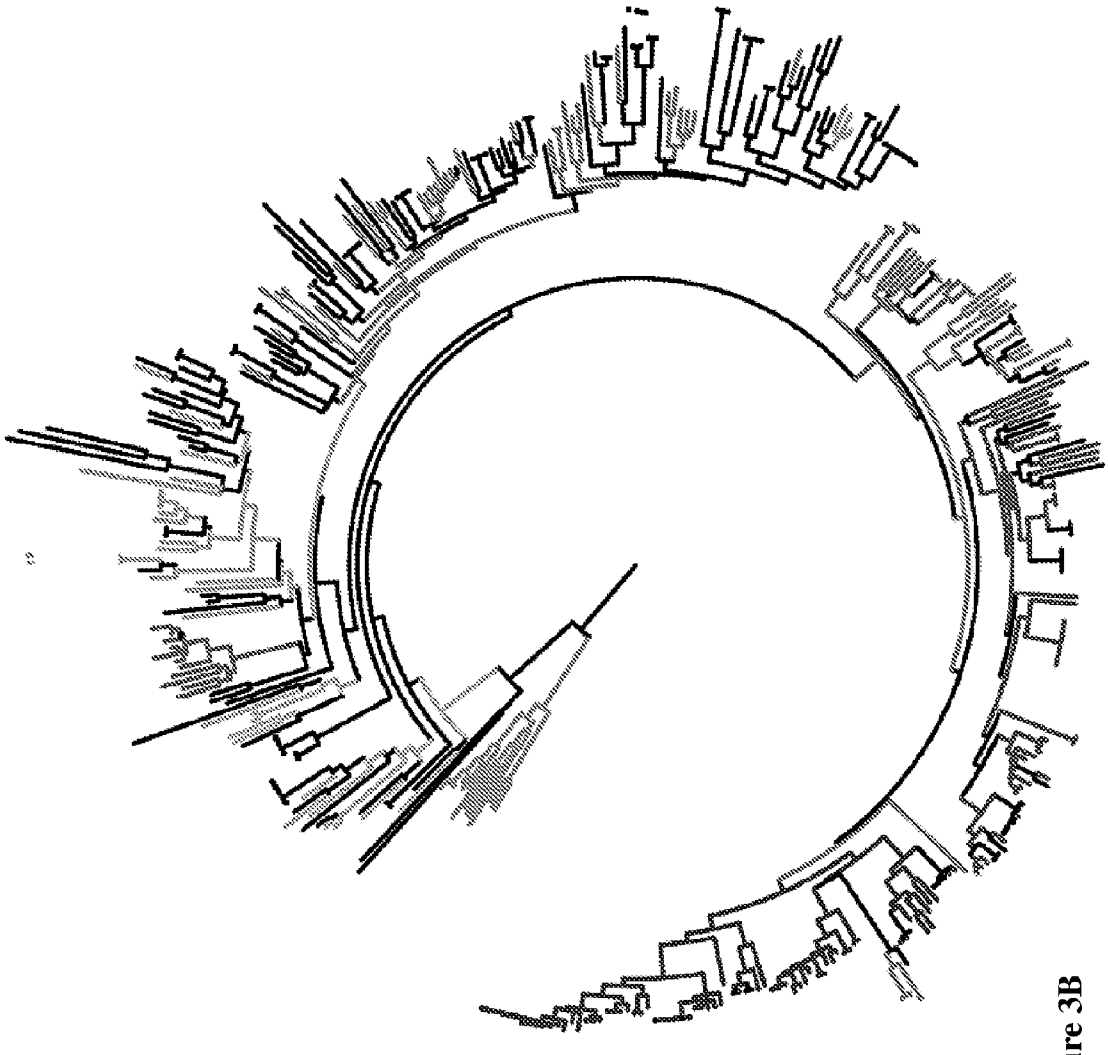


Figure 3B

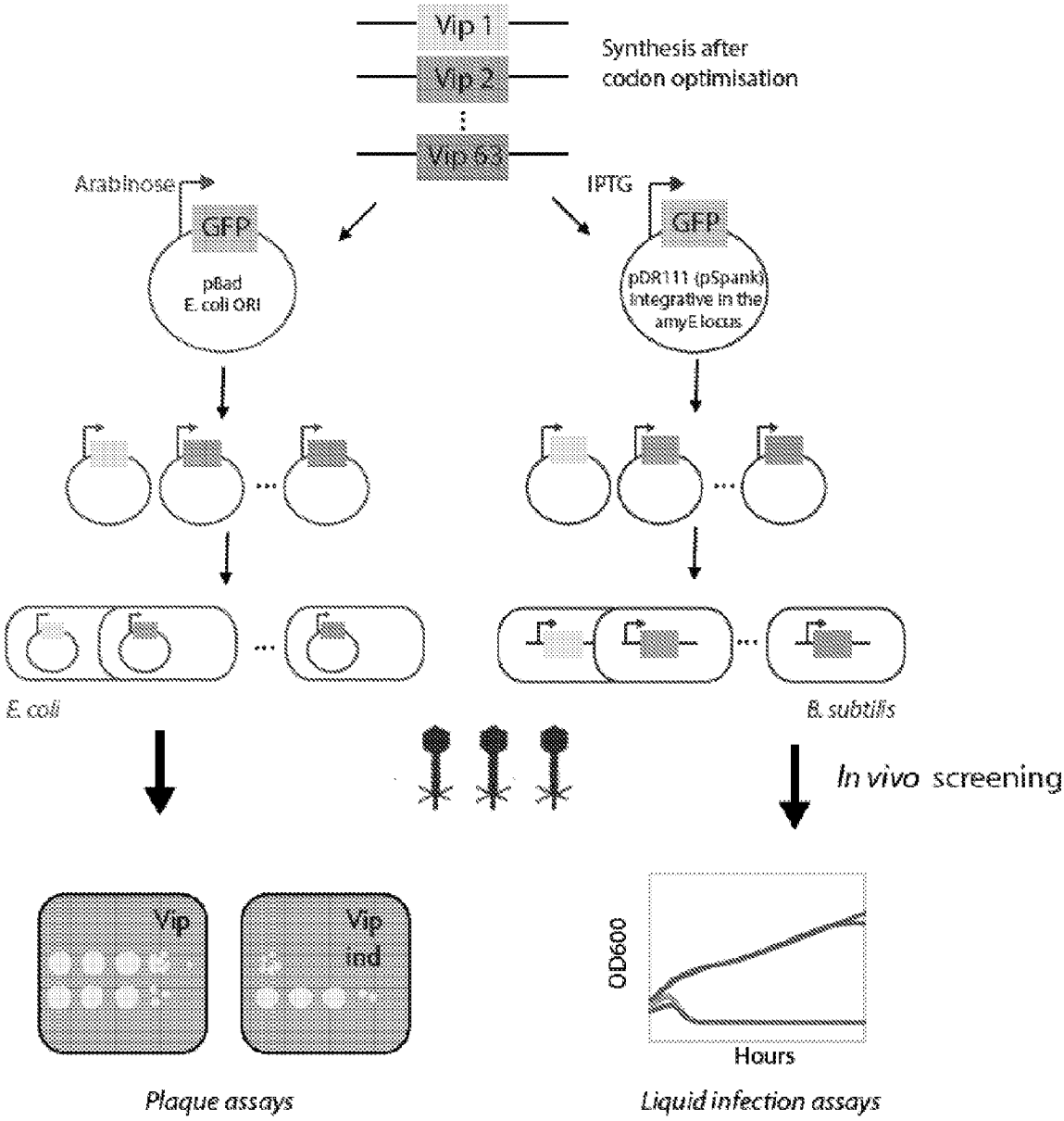

Vip 1
Vip 2
Vip 63
Synthesis after
codon optimisation
Arabinose
GFP
pBad
E. coli ORI
IPTG
GFP
pDR111 (pSpank)
integrative in the
amyE locus
E. coli
B. subtilis
In vivo screening
Vip
Vip
ind
OD600
Hours
Plaque assays
Liquid infection assays

Figure 4

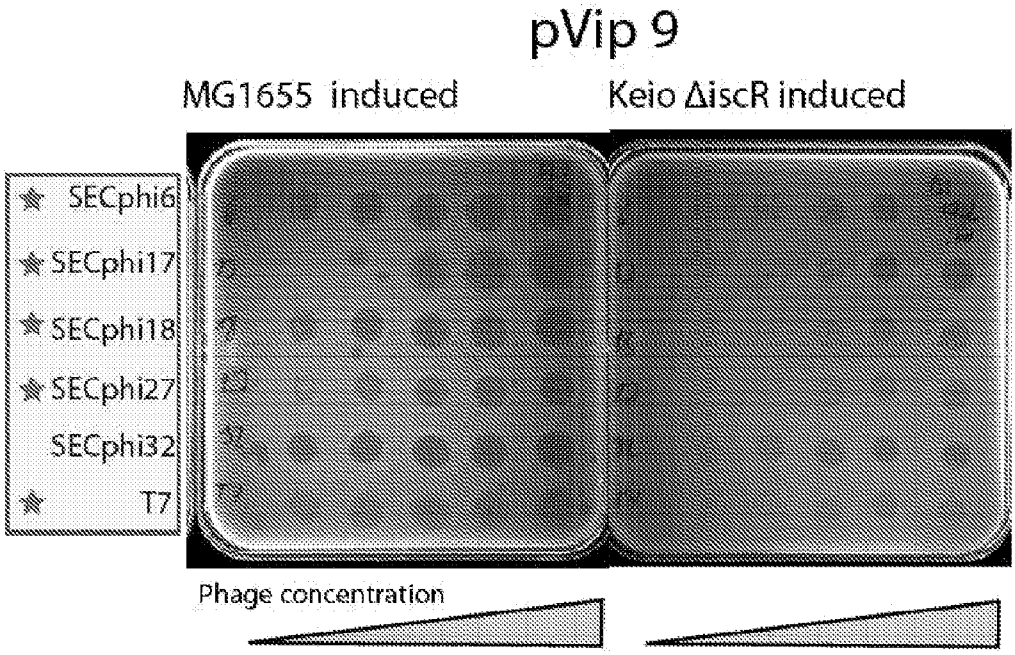


Figure 5A

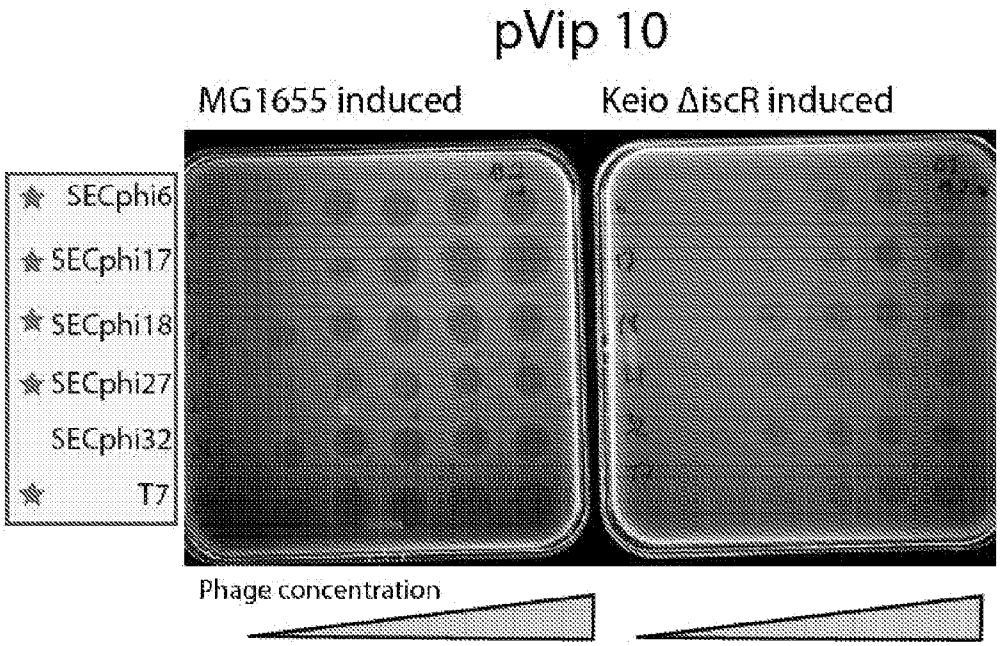


Figure 5B

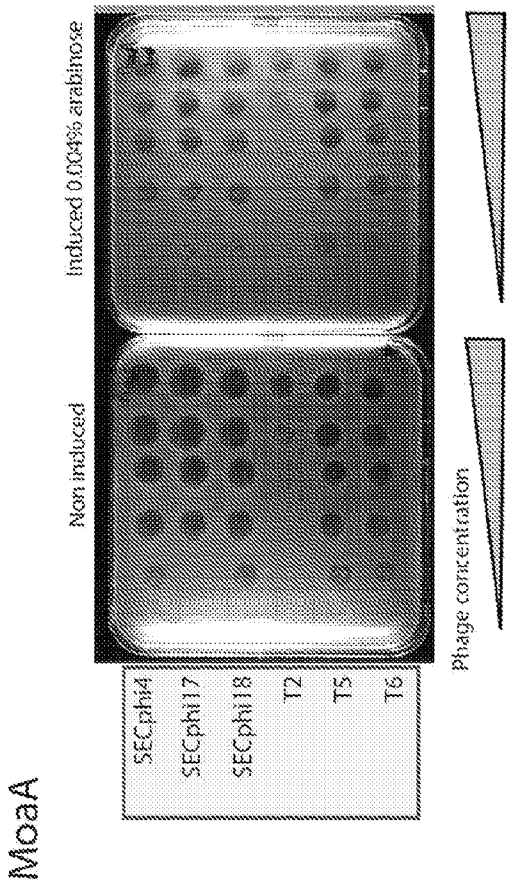


Figure 6A

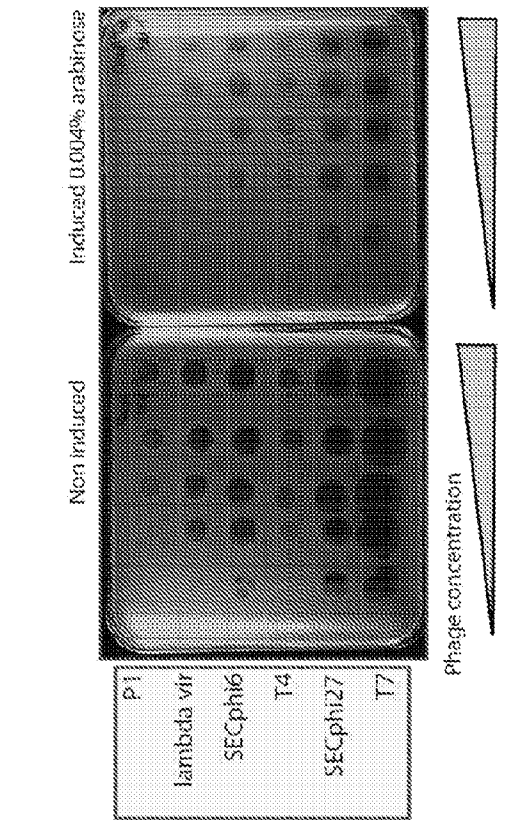


Figure 6B

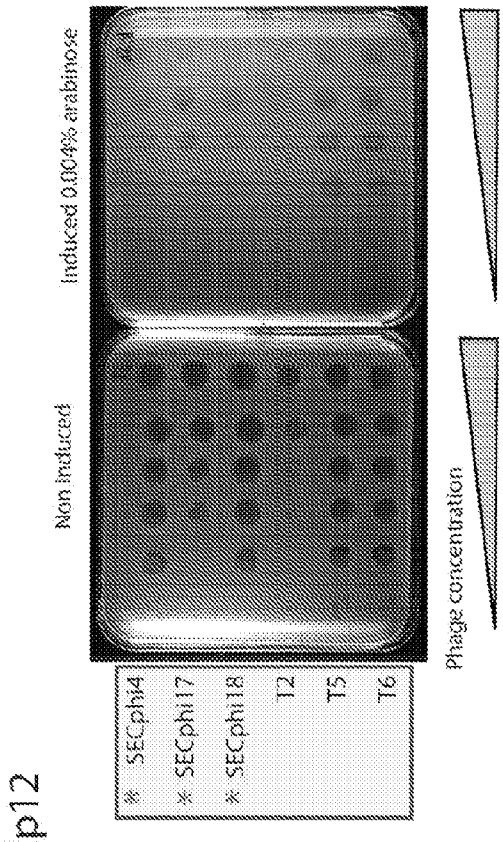


Figure 6C

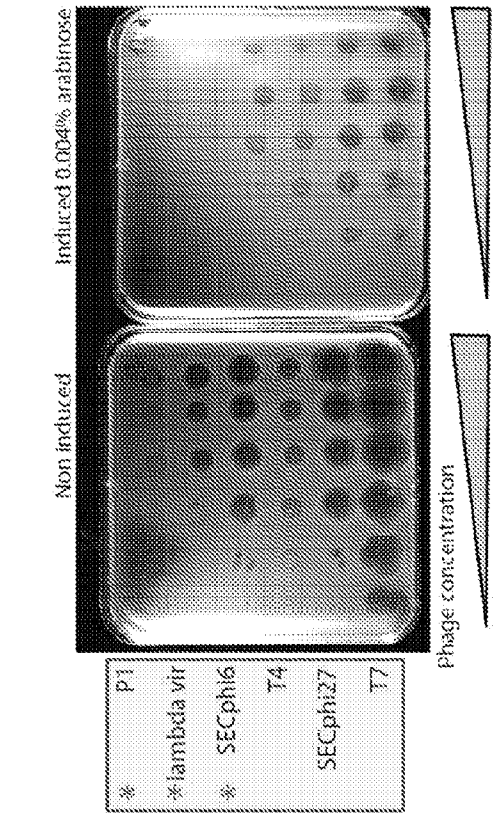


Figure 6D

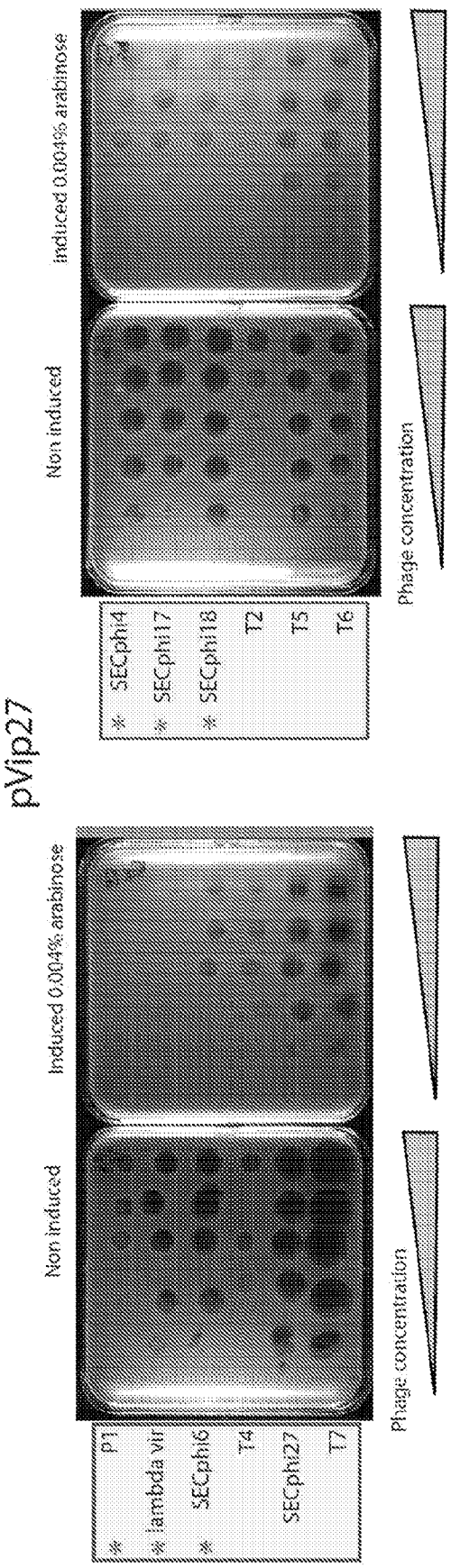


Figure 6E

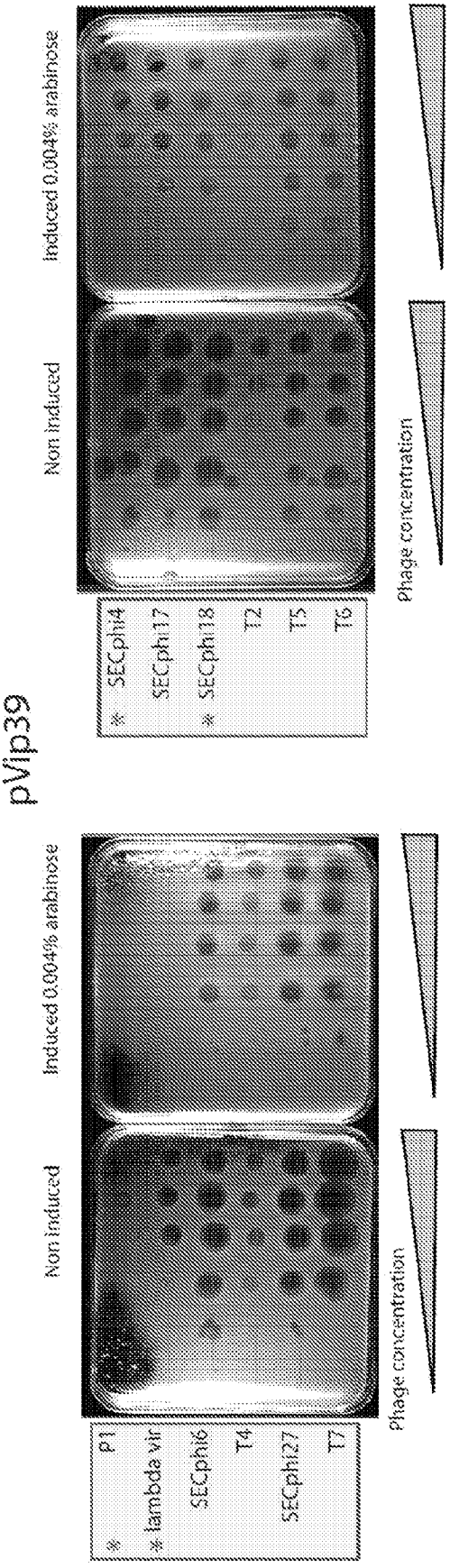


Figure 6F

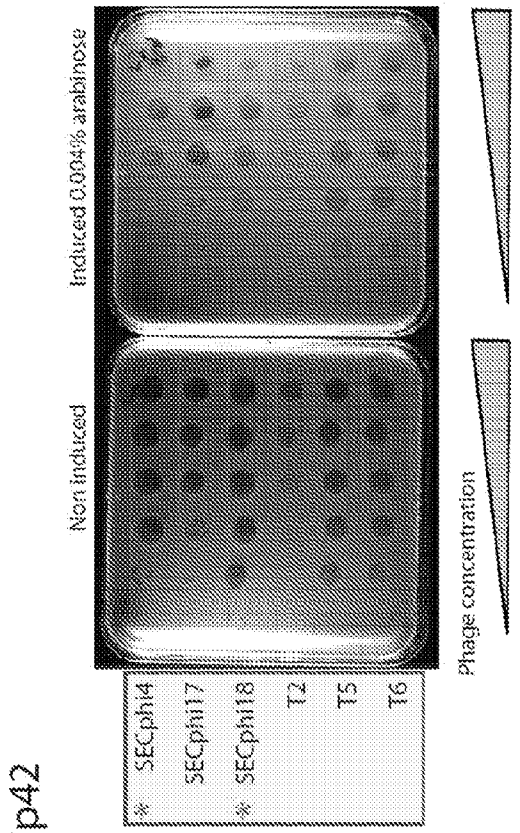


Figure 6G

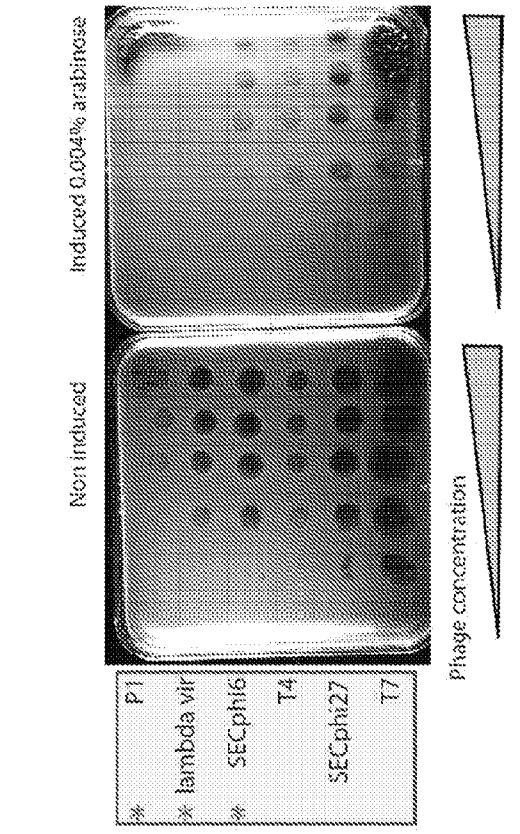


Figure 6H

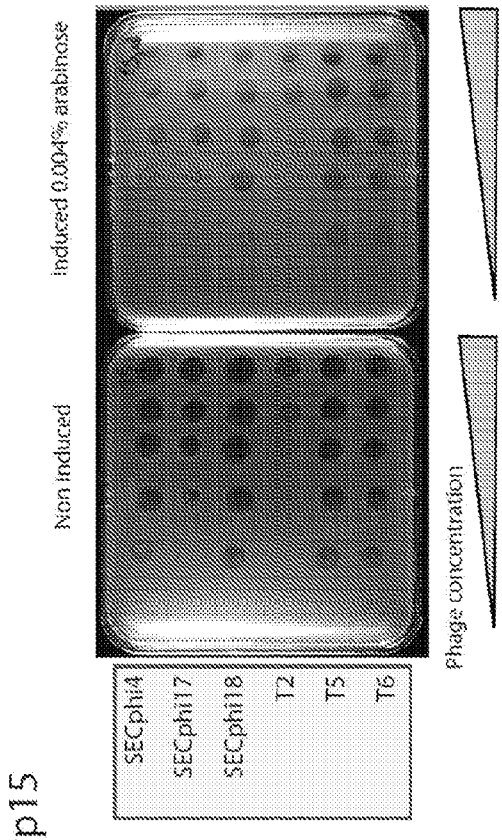


Figure 6I

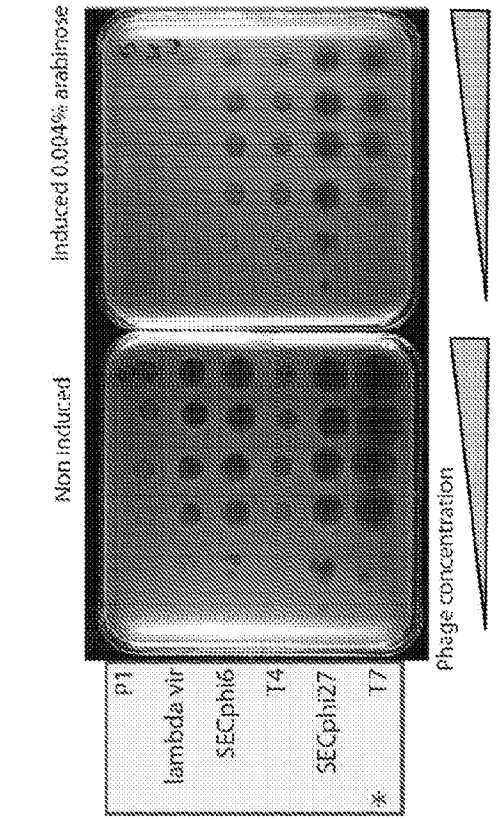


Figure 6J

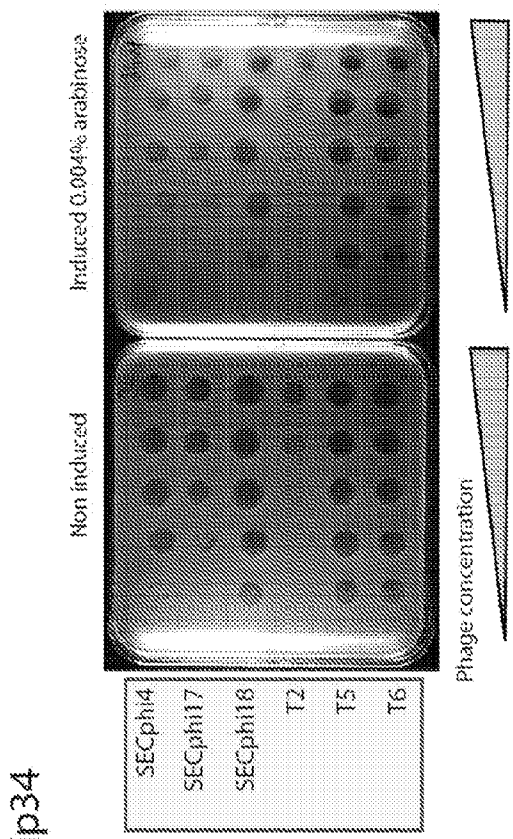


Figure 6K

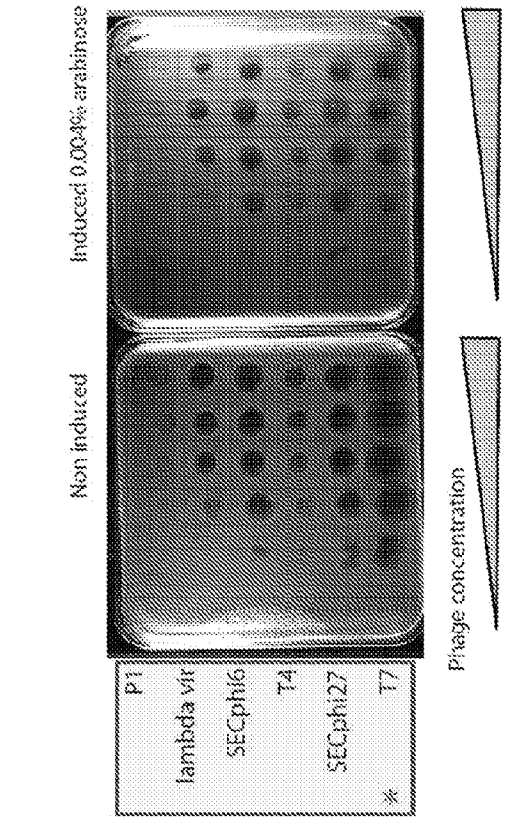
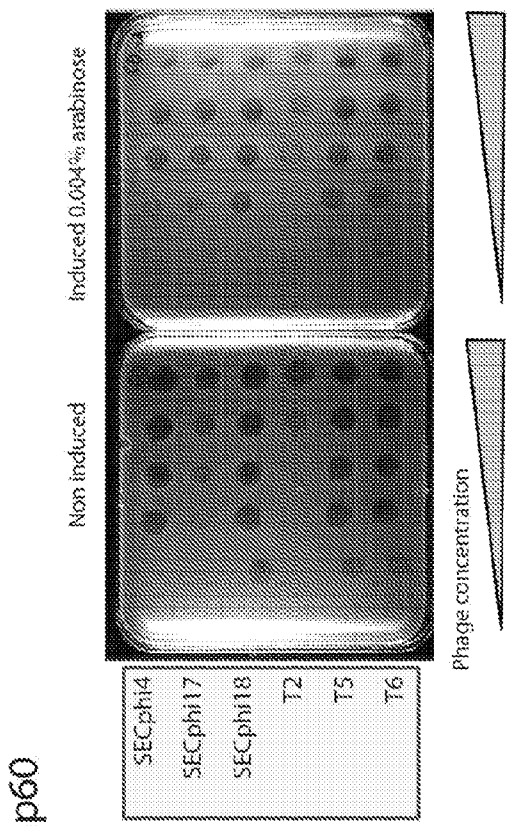


Figure 6L



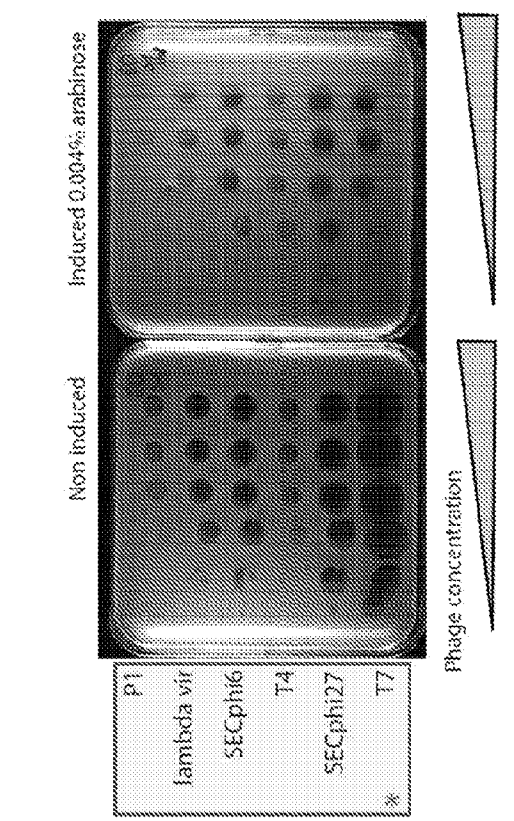


Figure 6M

Figure 6N

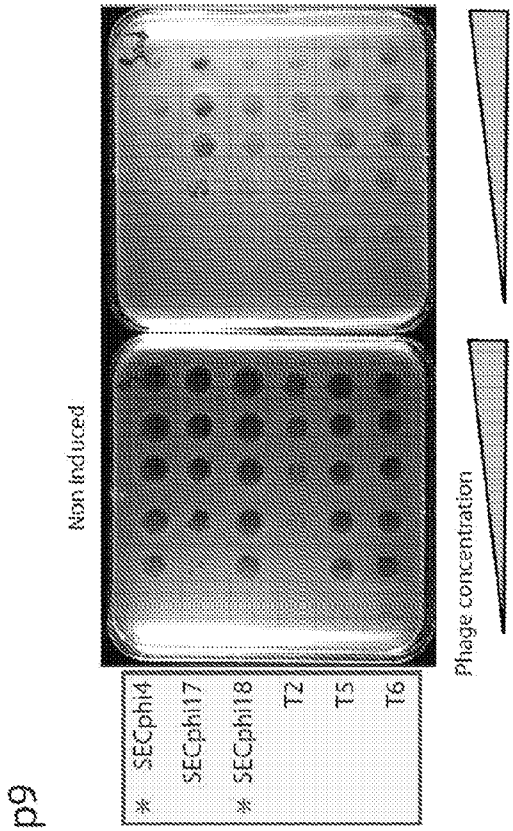


Figure 6O

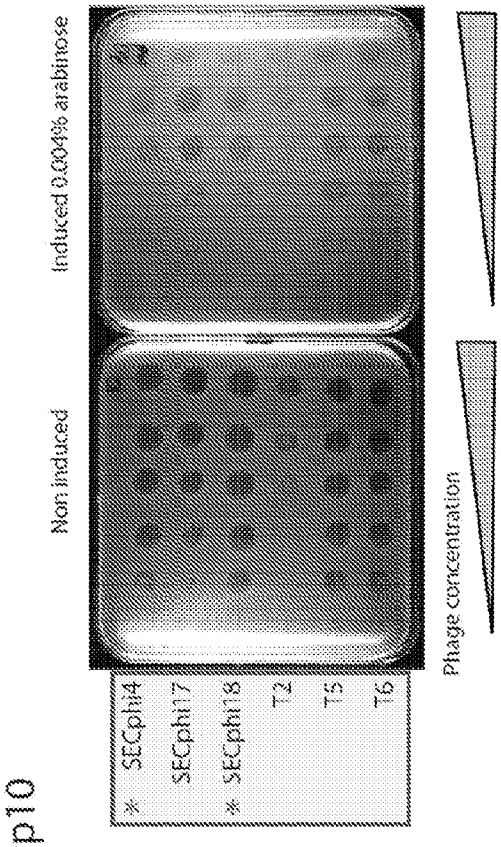
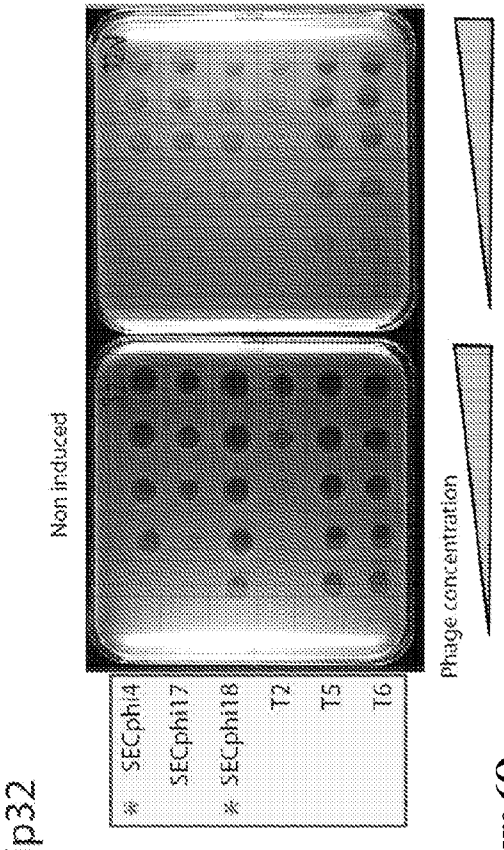
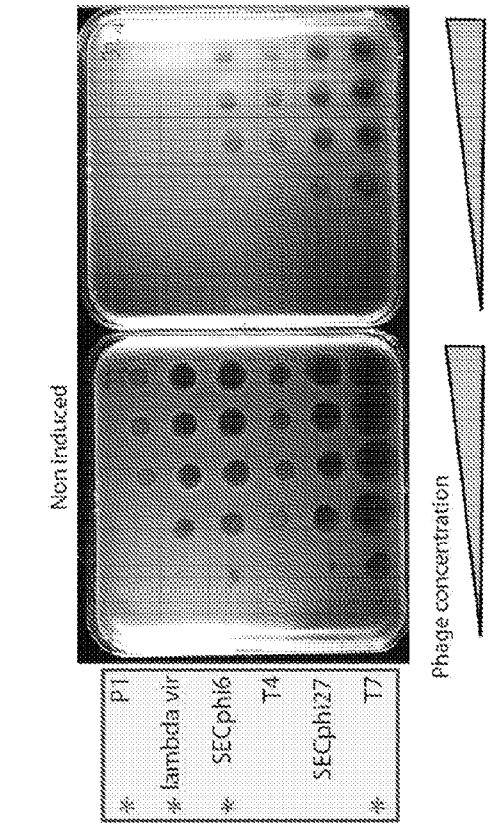


Figure 6P





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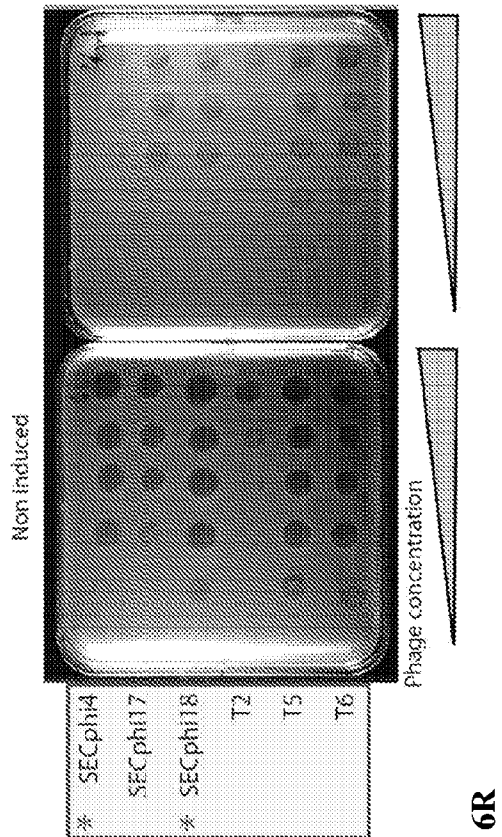

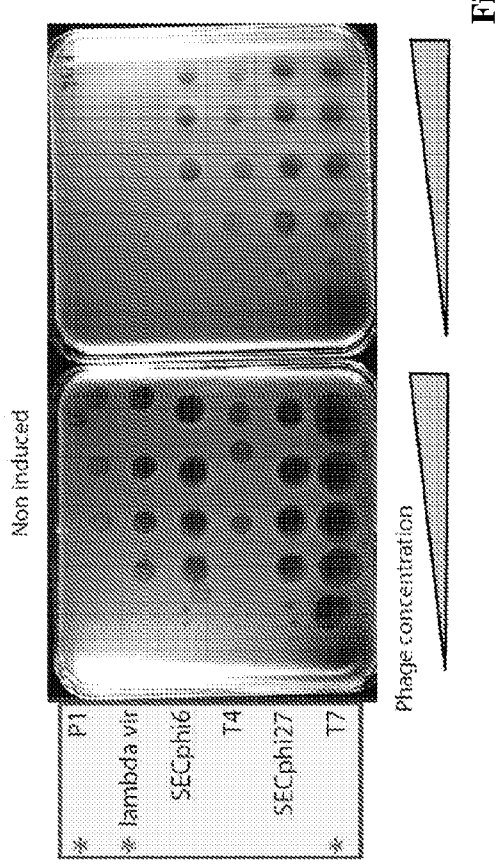

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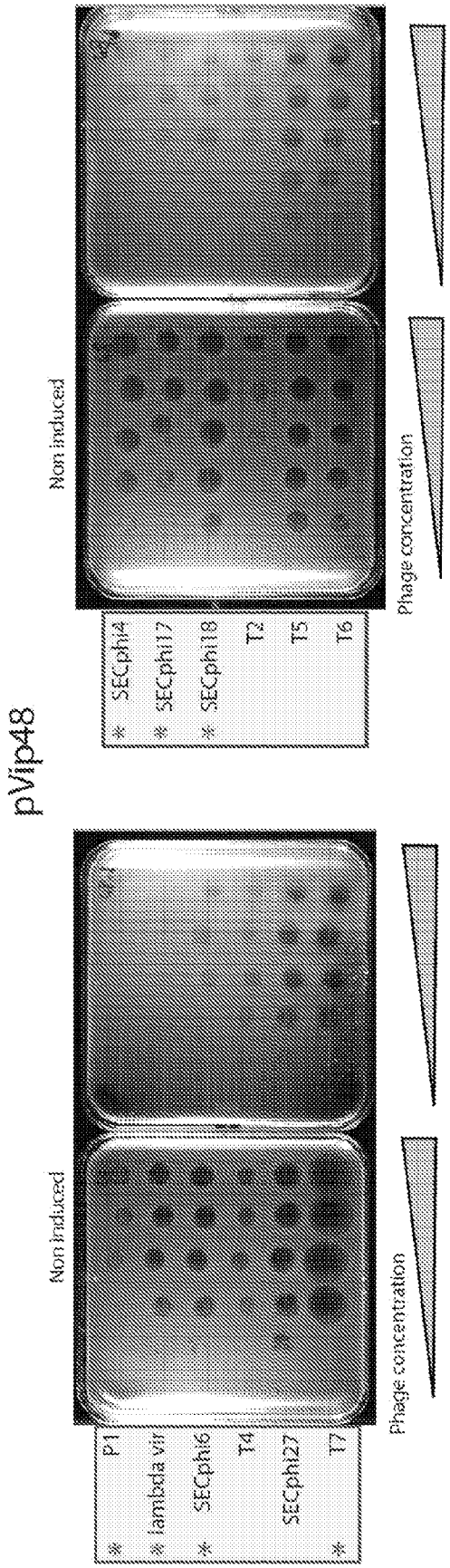

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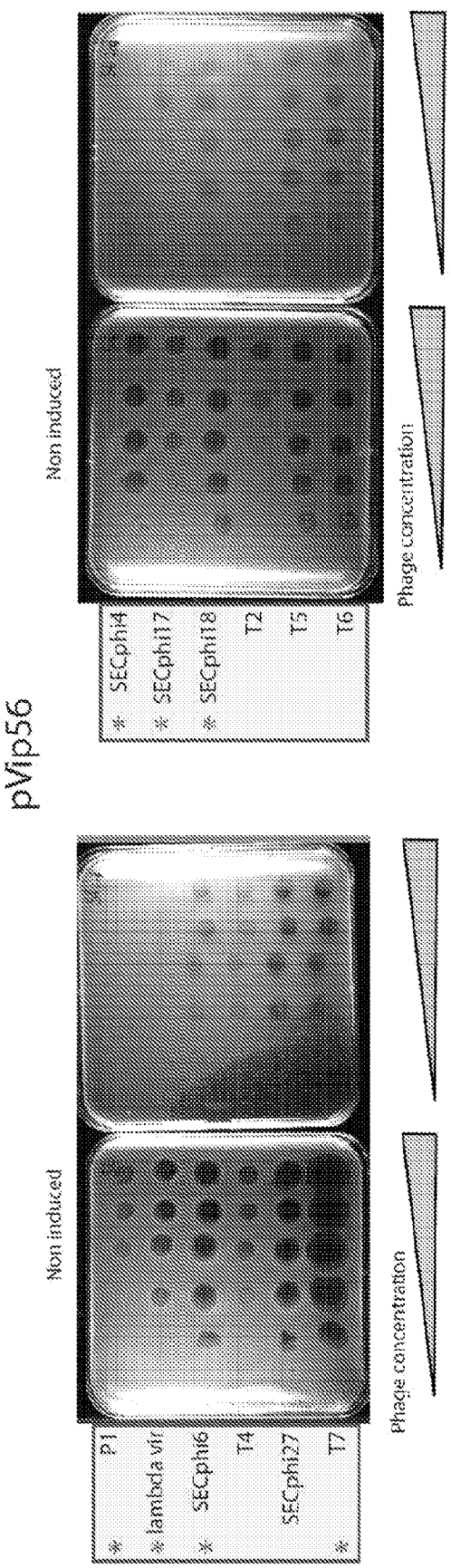

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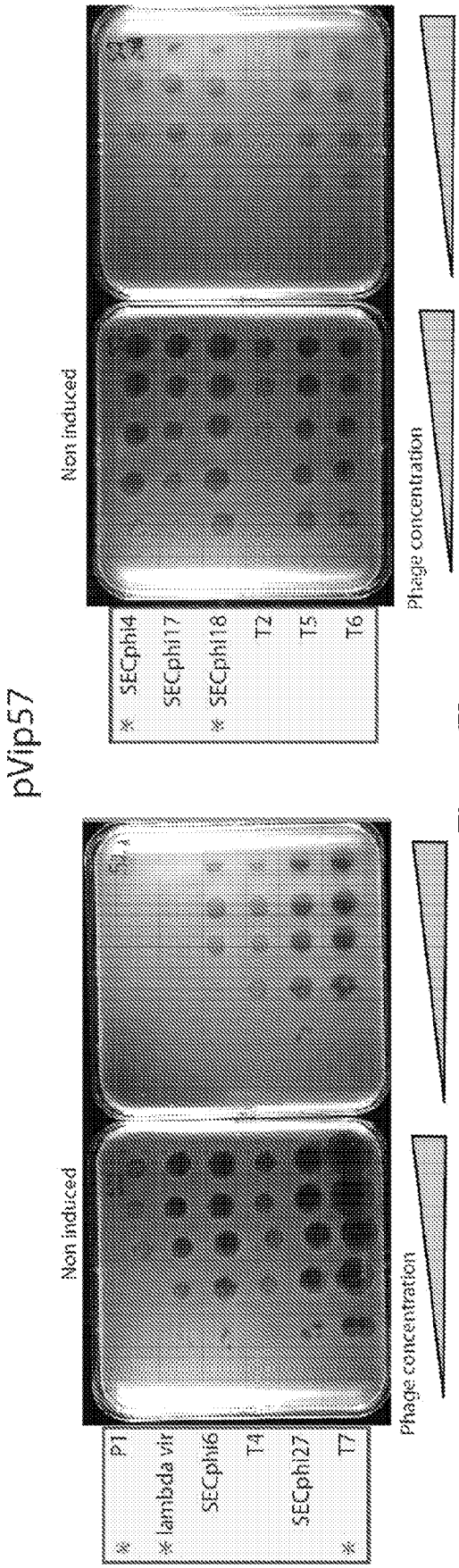

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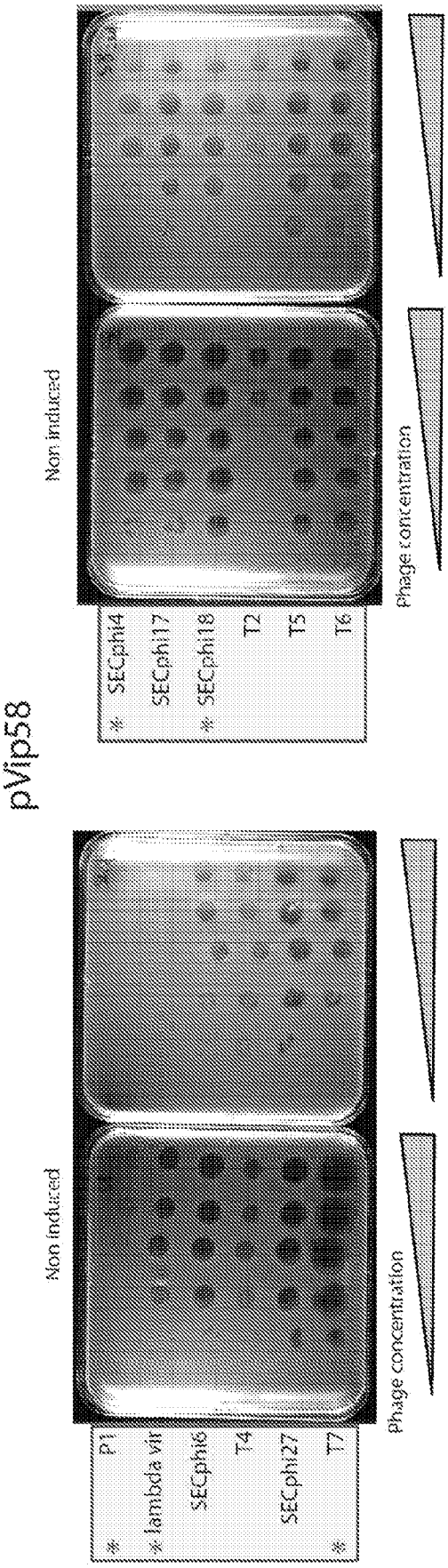

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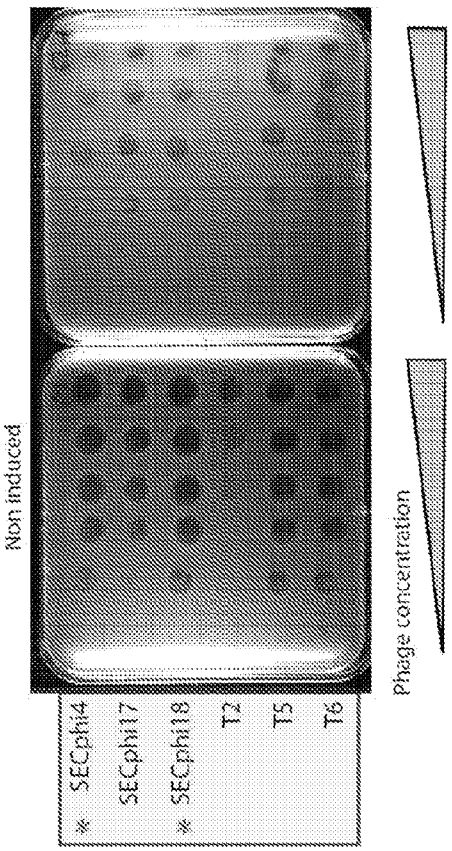
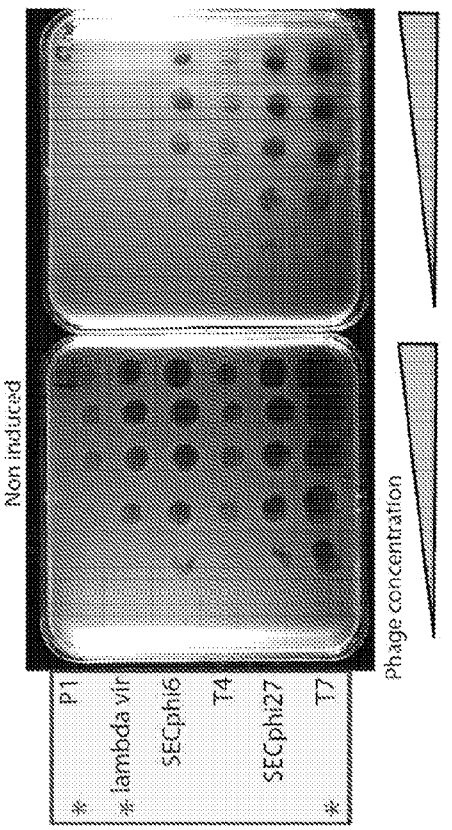
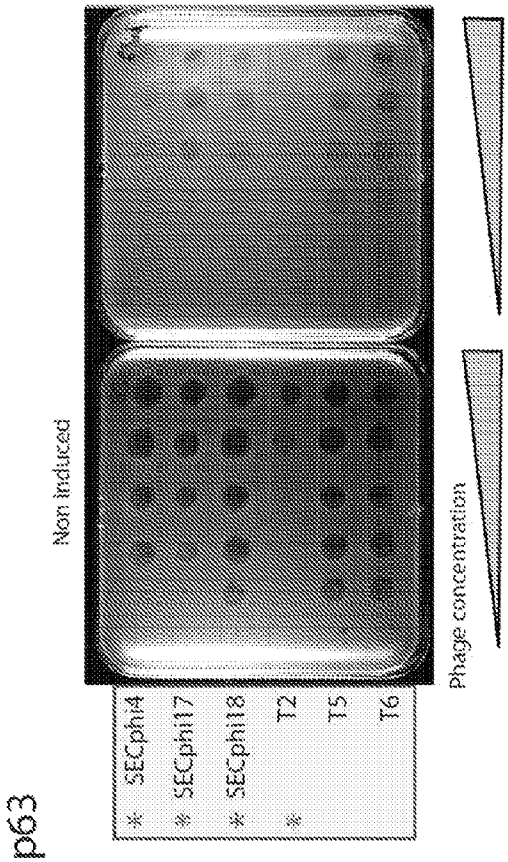


Figure 6W





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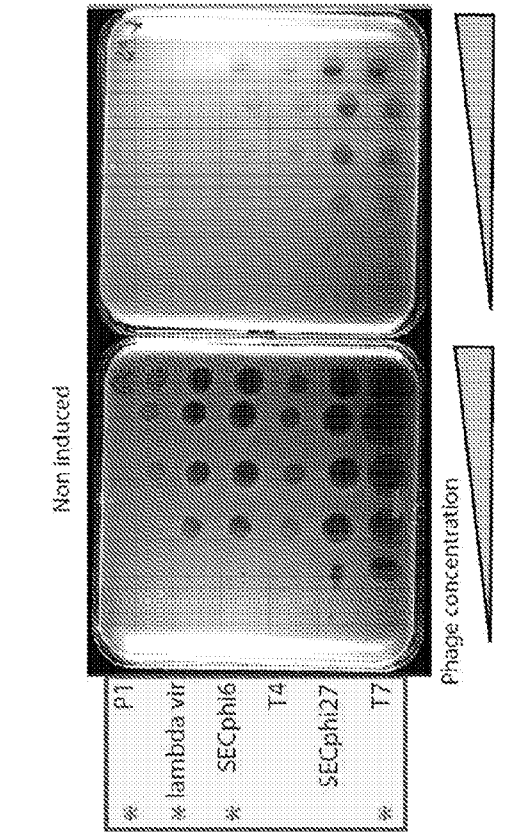


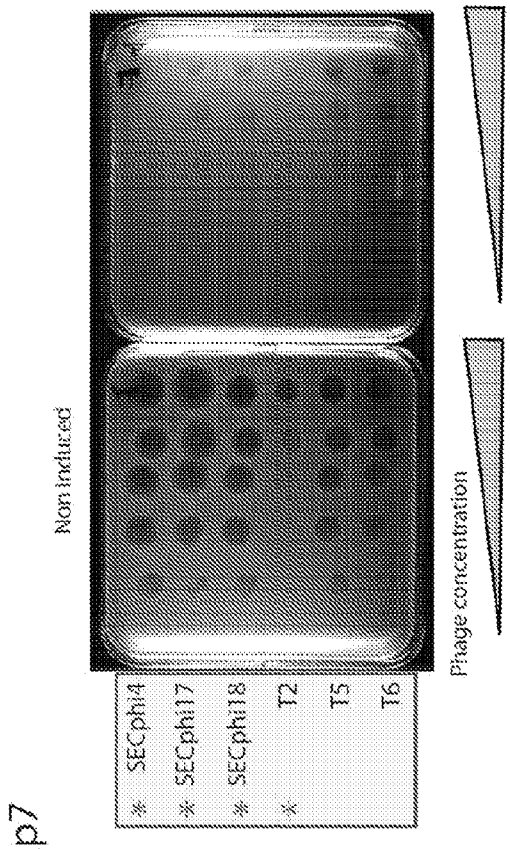


Figure 6Y

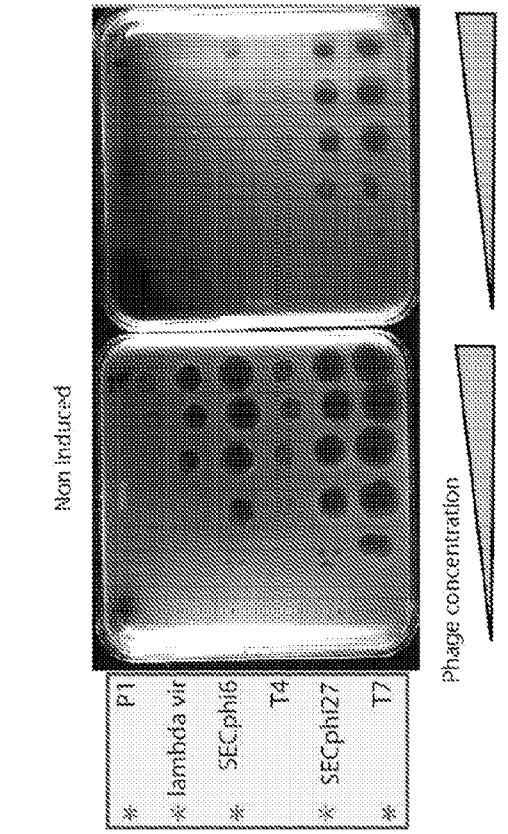


Figure 6Z

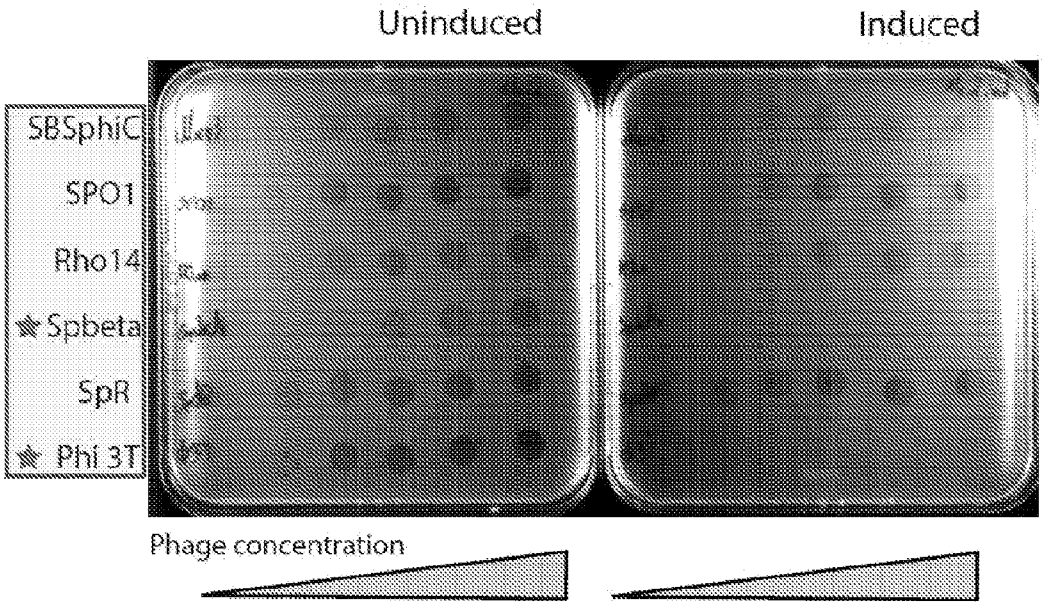


Figure 7A

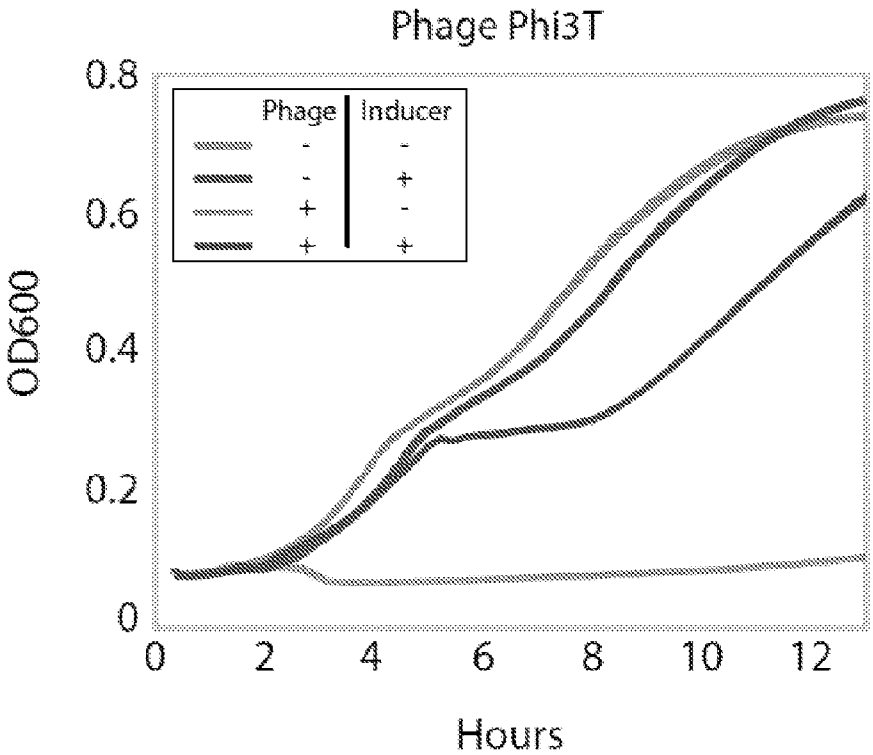


Figure 7B

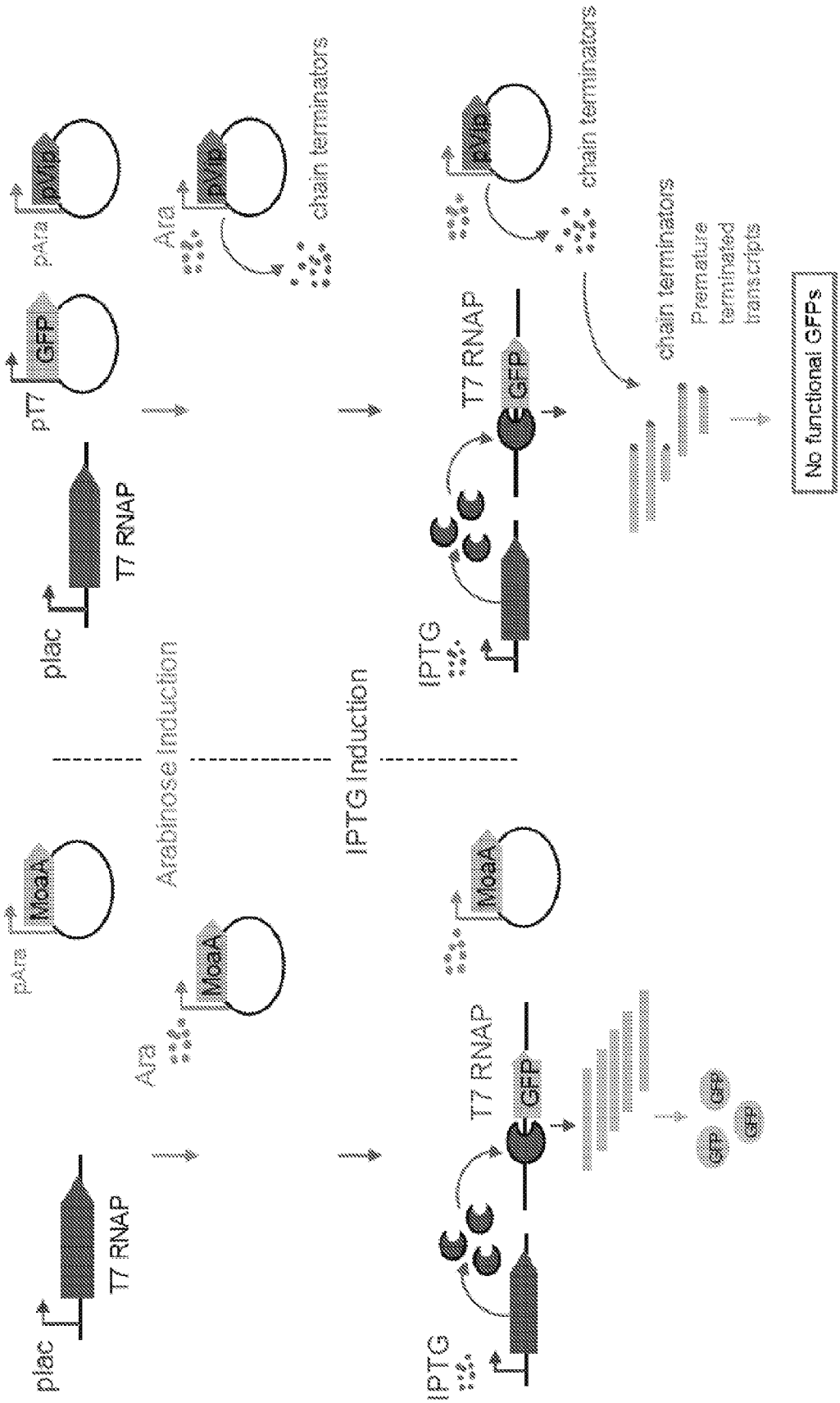

plac
T7 RNAP
pT7
GFP
pAra
pVia
MoaA
pAra
Arabinose Induction
Ara
MoaA
pVia
chain terminators
IPTG Induction
plac
T7 RNAP
IPTG
T7 RNAP
GFP
MoaA
T7 RNAP
GFP
IPTG
pVia
chain terminators
chain terminators
Premature terminated transcripts
No functional GFPs
GFP
GFP
GFP

Figure 8A

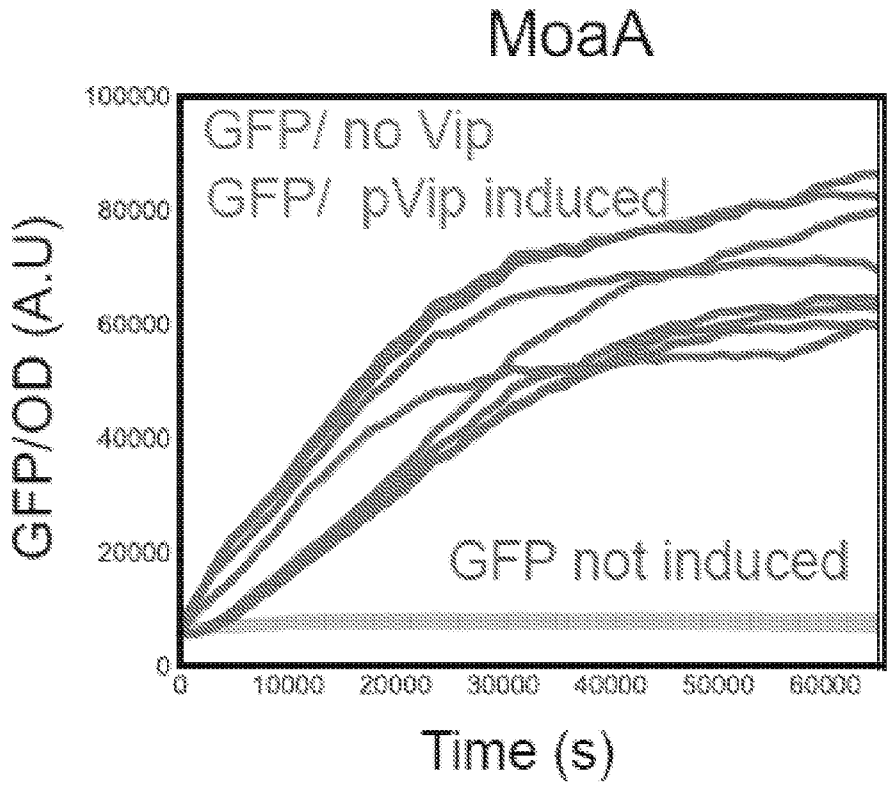


Figure 8B

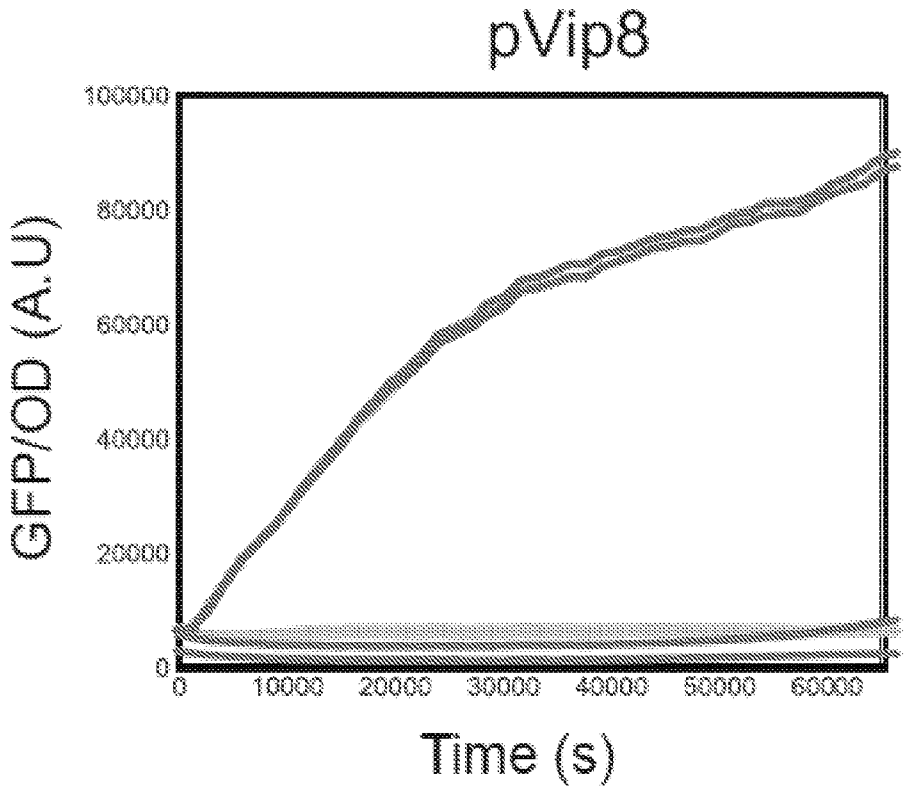


Figure 8C

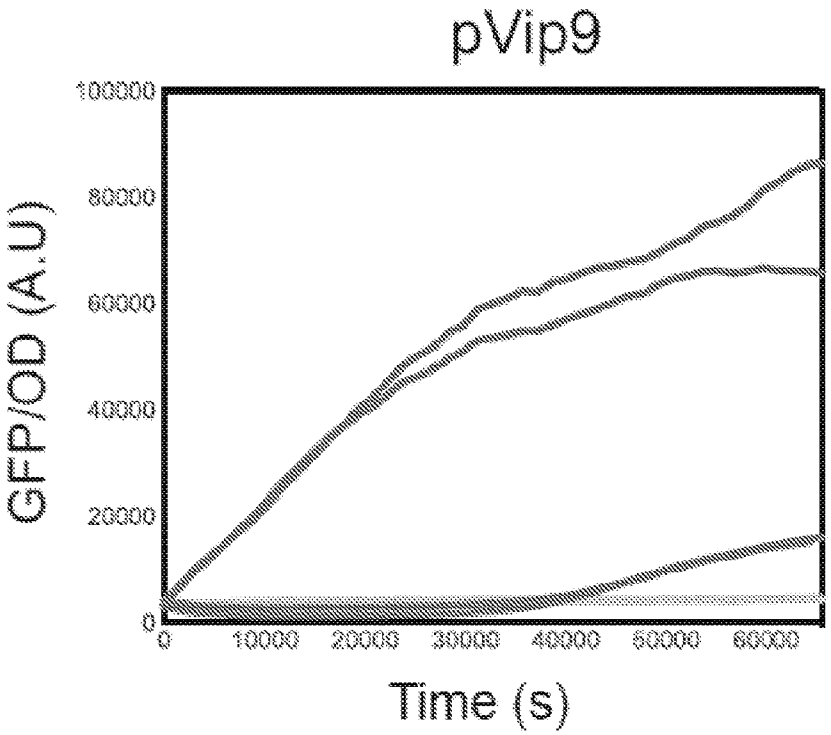


Figure 8D

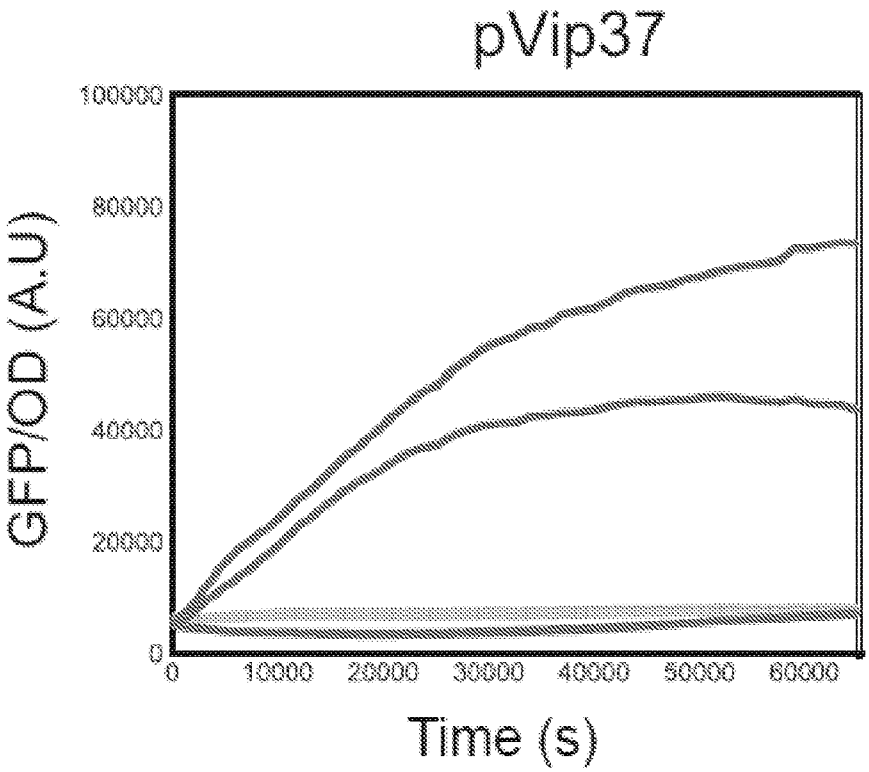


Figure 8E

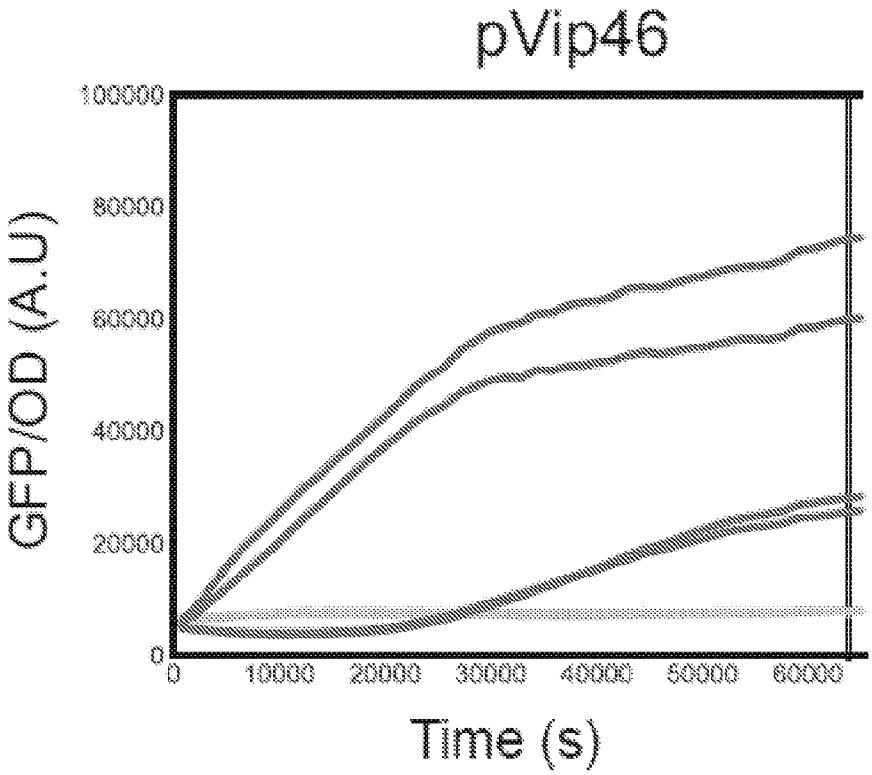


Figure 8F

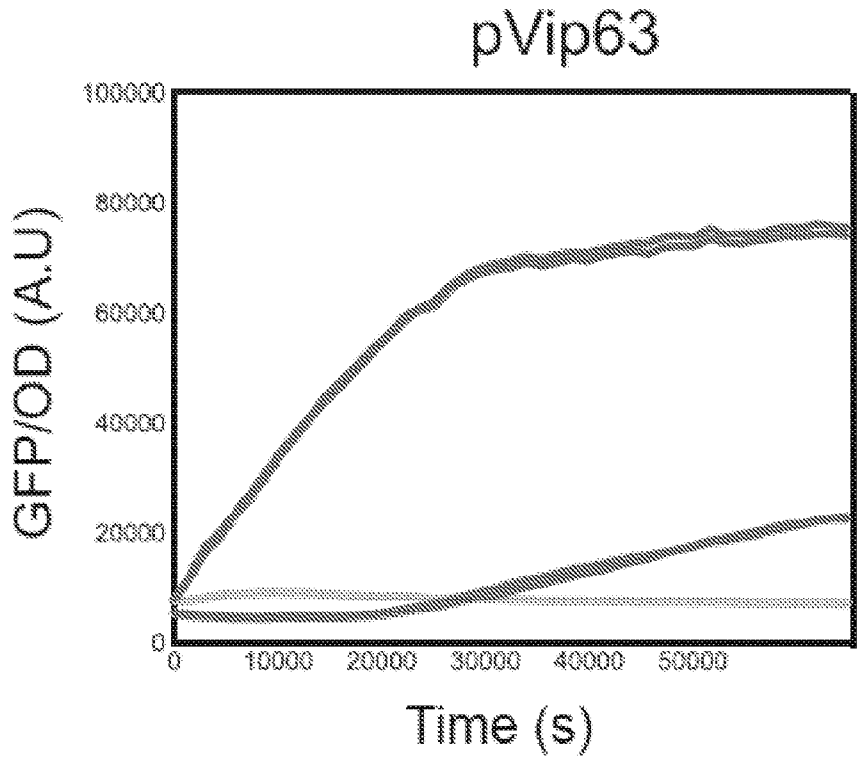


Figure 8G

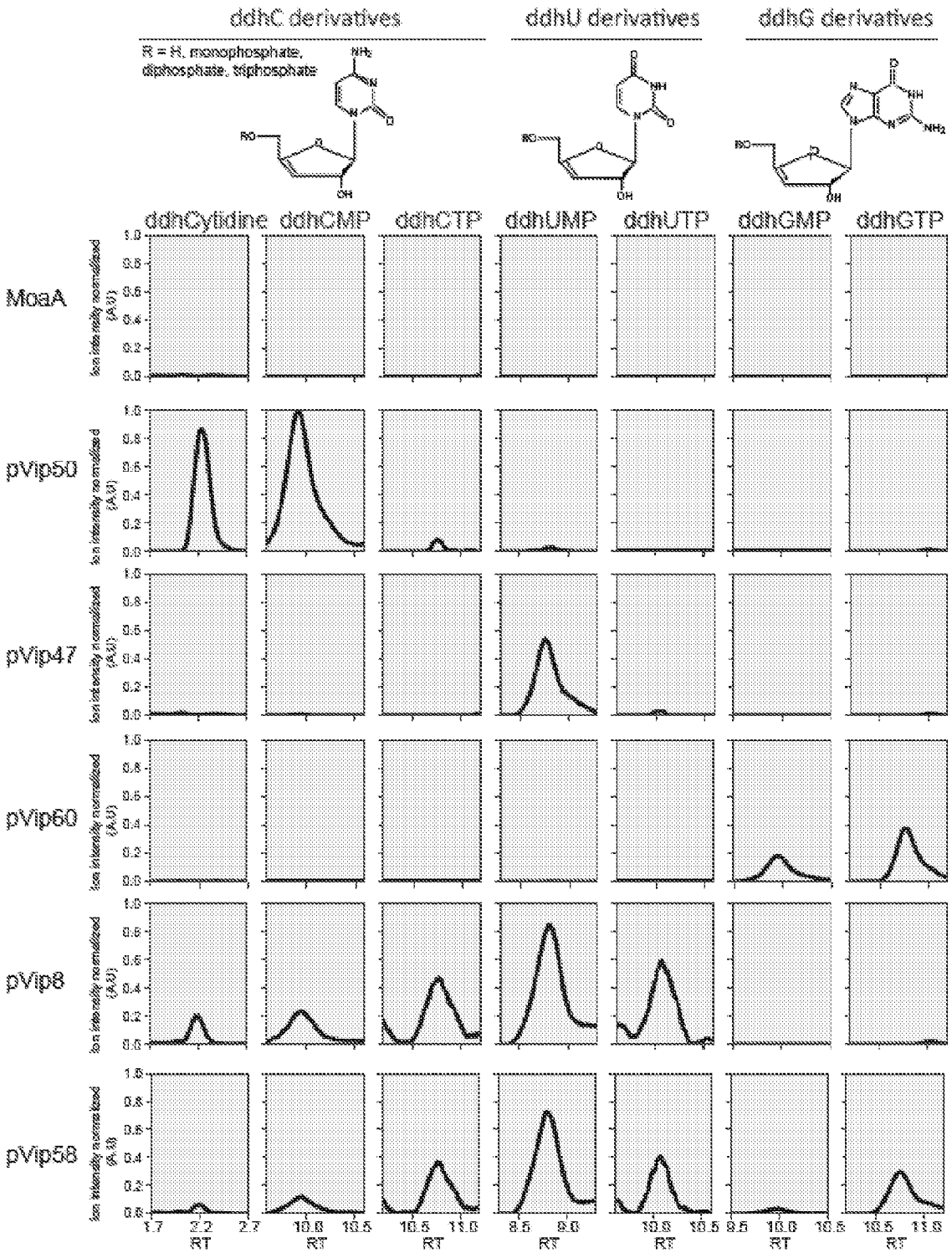

ddhC derivatives
ddhU derivatives
ddhG derivatives
R = H, monophosphate, diphosphate, triphosphate
ddhCytidine
ddhCMP
ddhCTP
ddhUMP
ddhUTP
ddhGMP
ddhGTP
MoaA
pVip50
pVip47
pVip60
pVip8
pVip58
Ion intensity normalized (A.U)
RT

Figure 9A

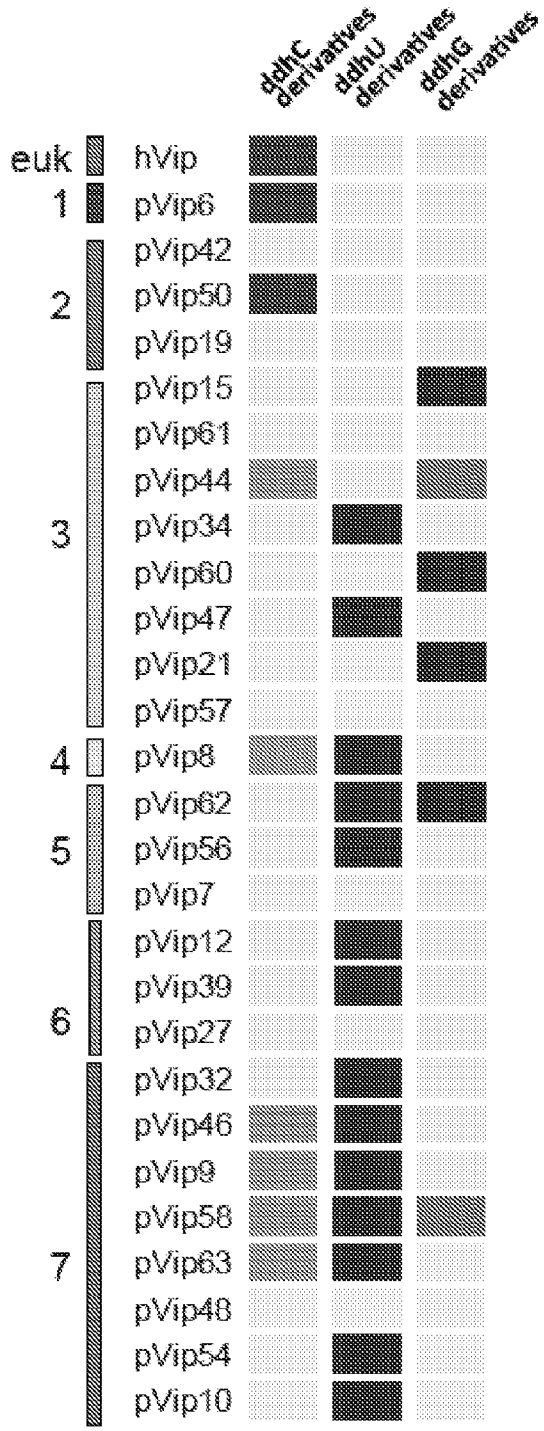

ddhC derivatives
ddhU derivatives
ddhG derivatives
euk
1
2
3
4
5
6
7
hVip
pVip6
pVip42
pVip50
pVip19
pVip15
pVip61
pVip44
pVip34
pVip60
pVip47
pVip21
pVip57
pVip8
pVip62
pVip56
pVip7
pVip12
pVip39
pVip27
pVip32
pVip46
pVip9
pVip58
pVip63
pVip48
pVip54
pVip10

Figure 9B

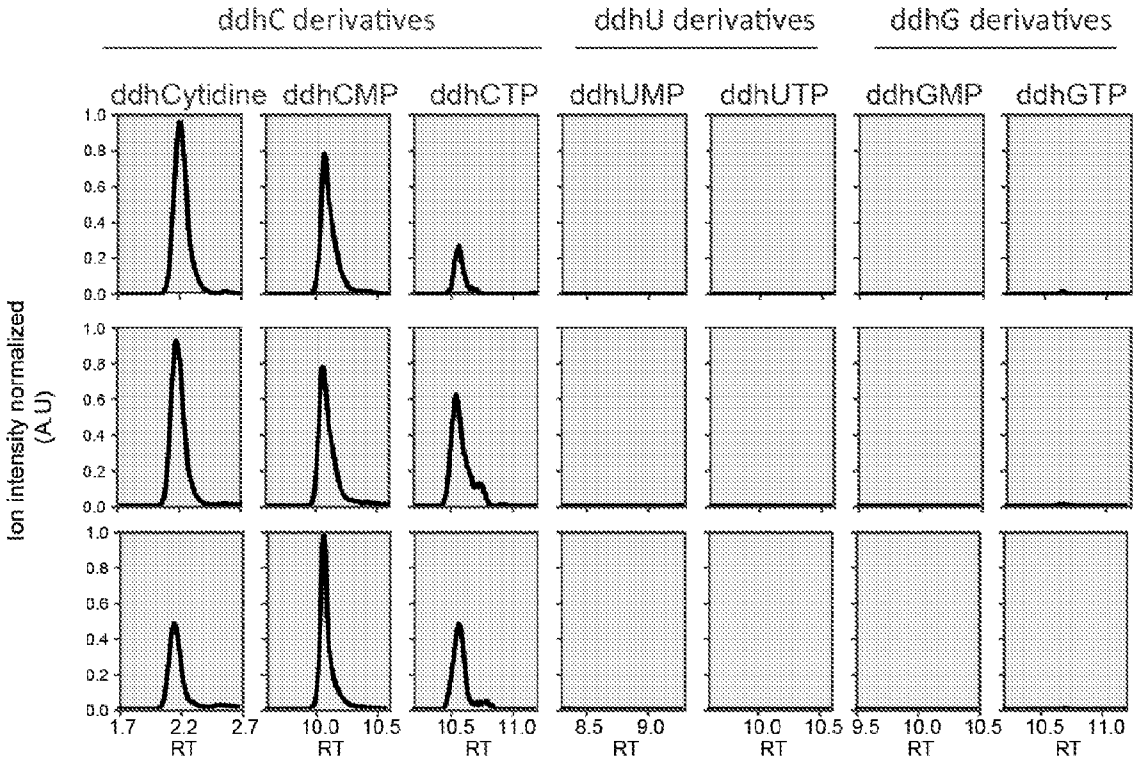

ddhC derivatives
ddhU derivatives
ddhG derivatives
ddhCytidine
ddhCMP
ddhCTP
ddhUMP
ddhUTP
ddhGMP
ddhGTP
Ion intensity normalized (A.U)
1.0
0.8
0.6
0.4
0.2
0.0
1.7
2.2
2.7
10.0
10.5
11.0
8.5
9.0
9.5
RT

Figure 10

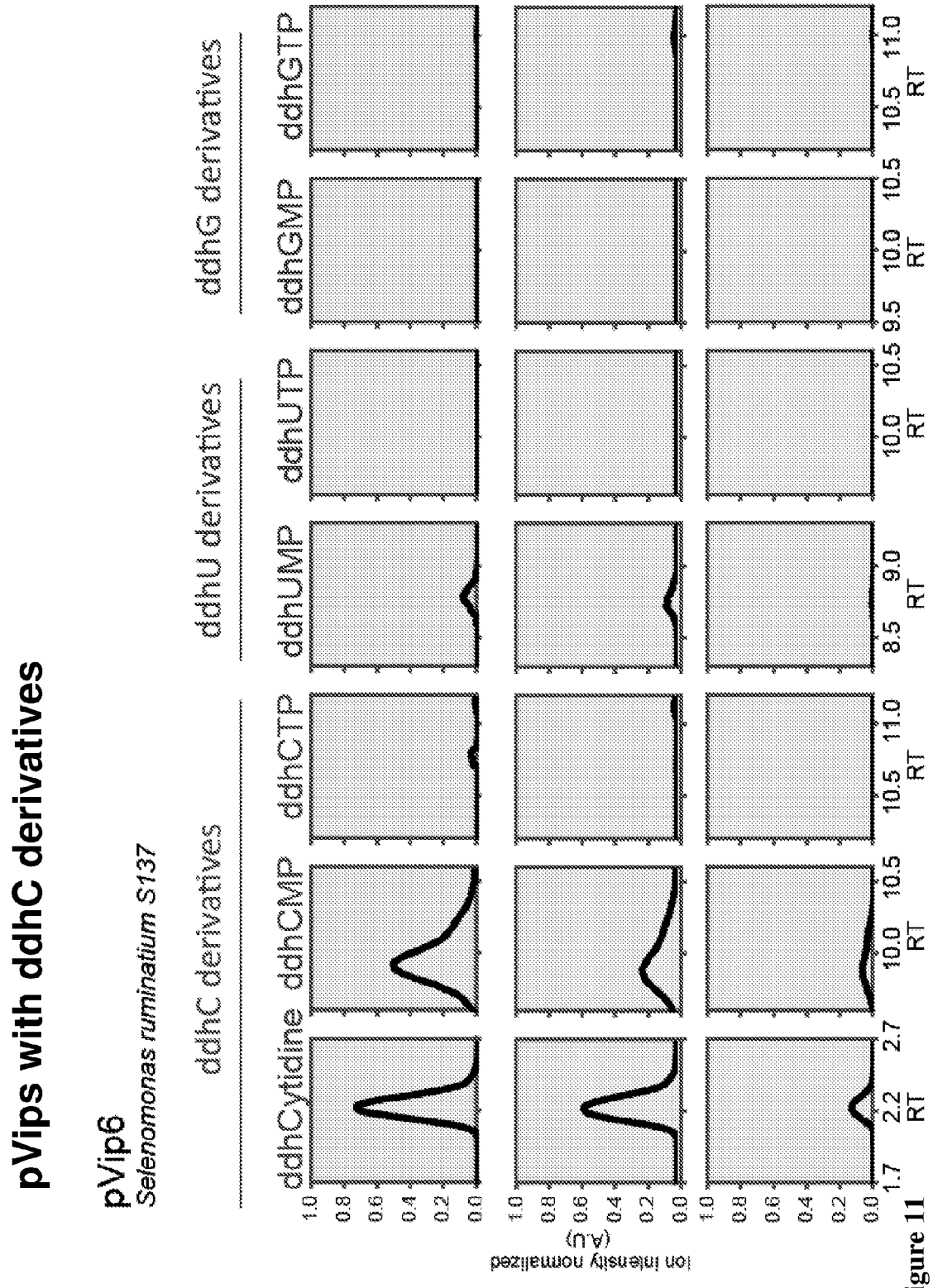

pVips with ddhC derivatives
pVip6
Selenomonas ruminatium S137
ddhC derivatives
ddhU derivatives
ddhG derivatives
ddhCytidine
ddhCMP
ddhCTP
ddhUMP
ddhUTP
ddhGMP
ddhGTP
ion intensity normalized (A.U)
RT

Figure 11

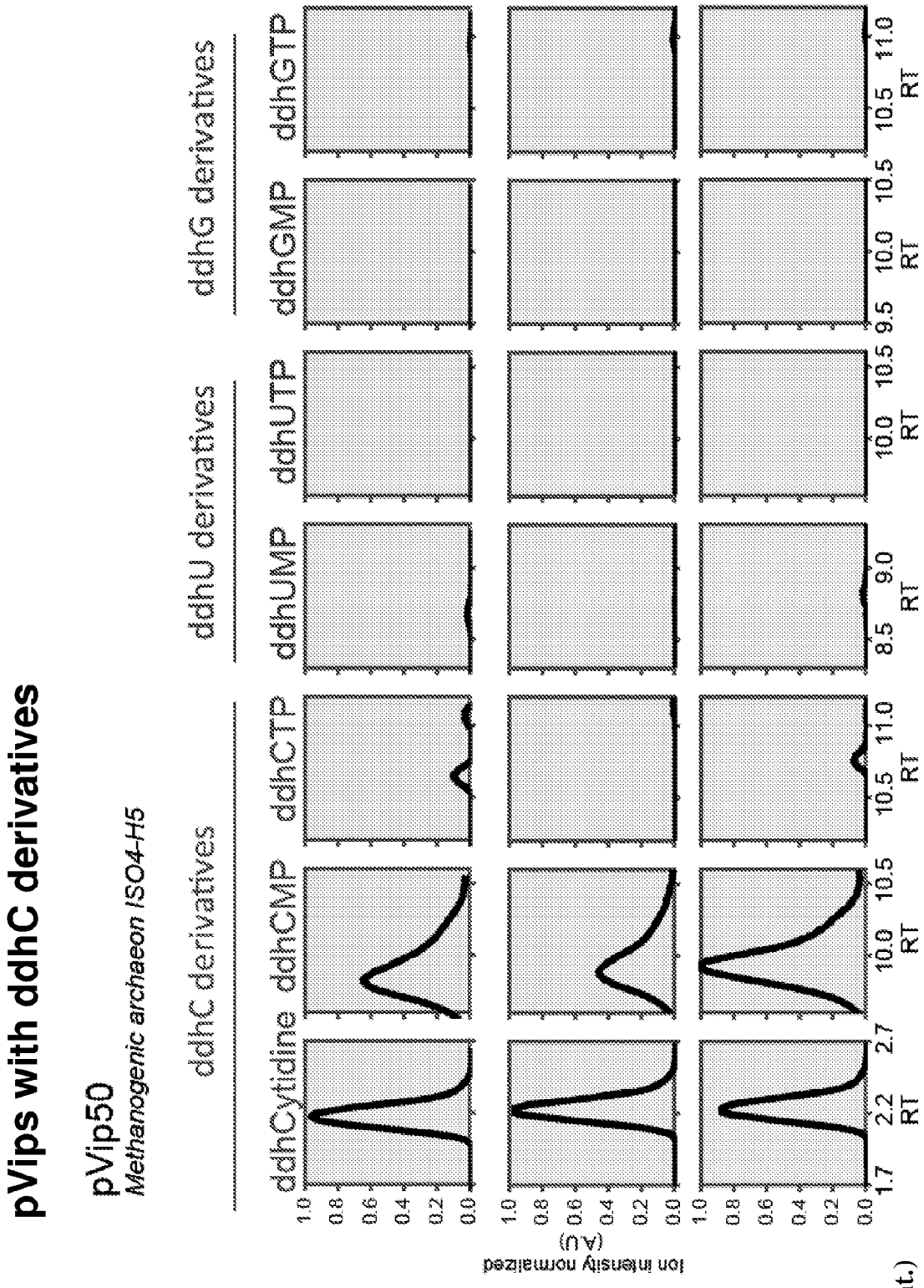


Figure 11 (cont.)

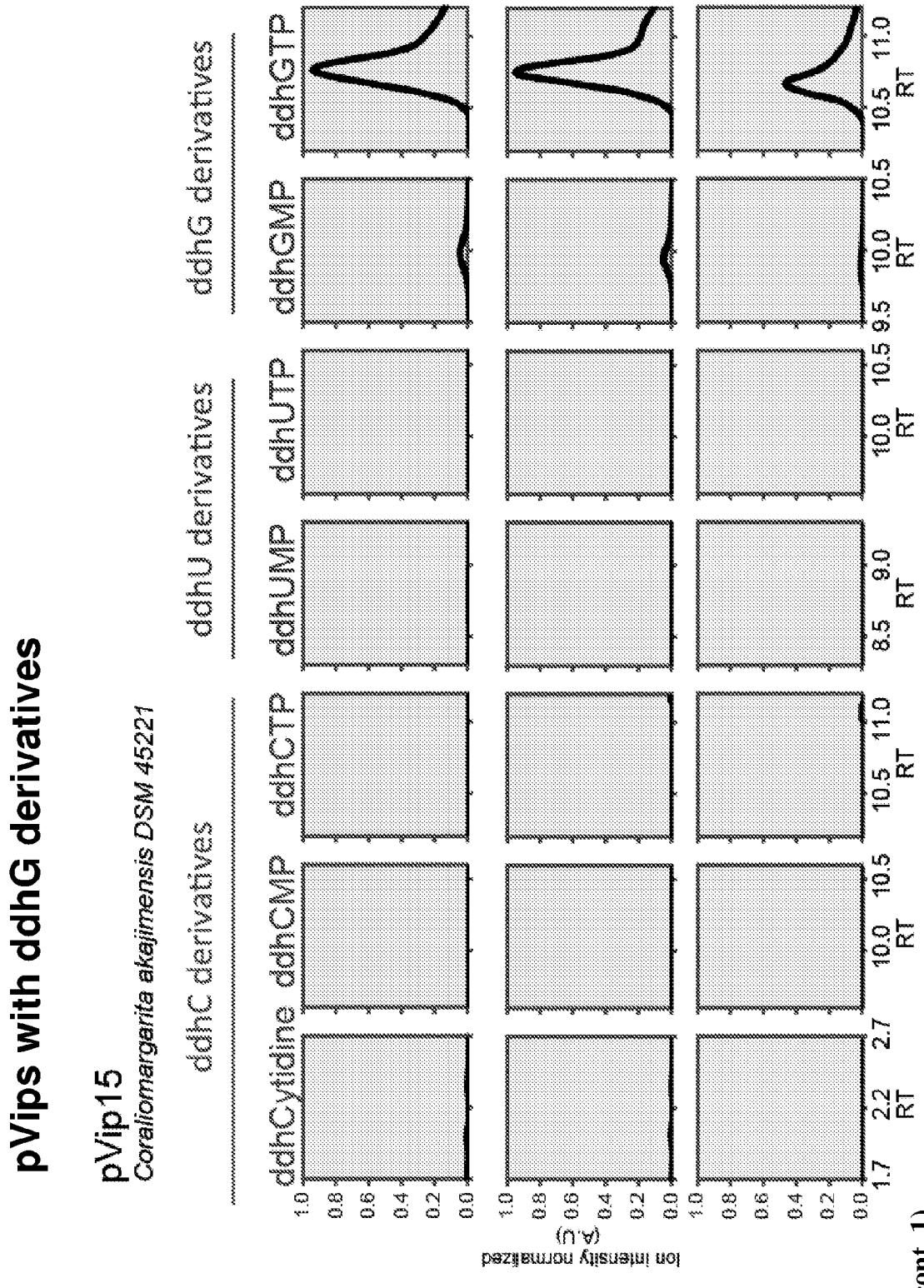


Figure 11 (cont. 1)

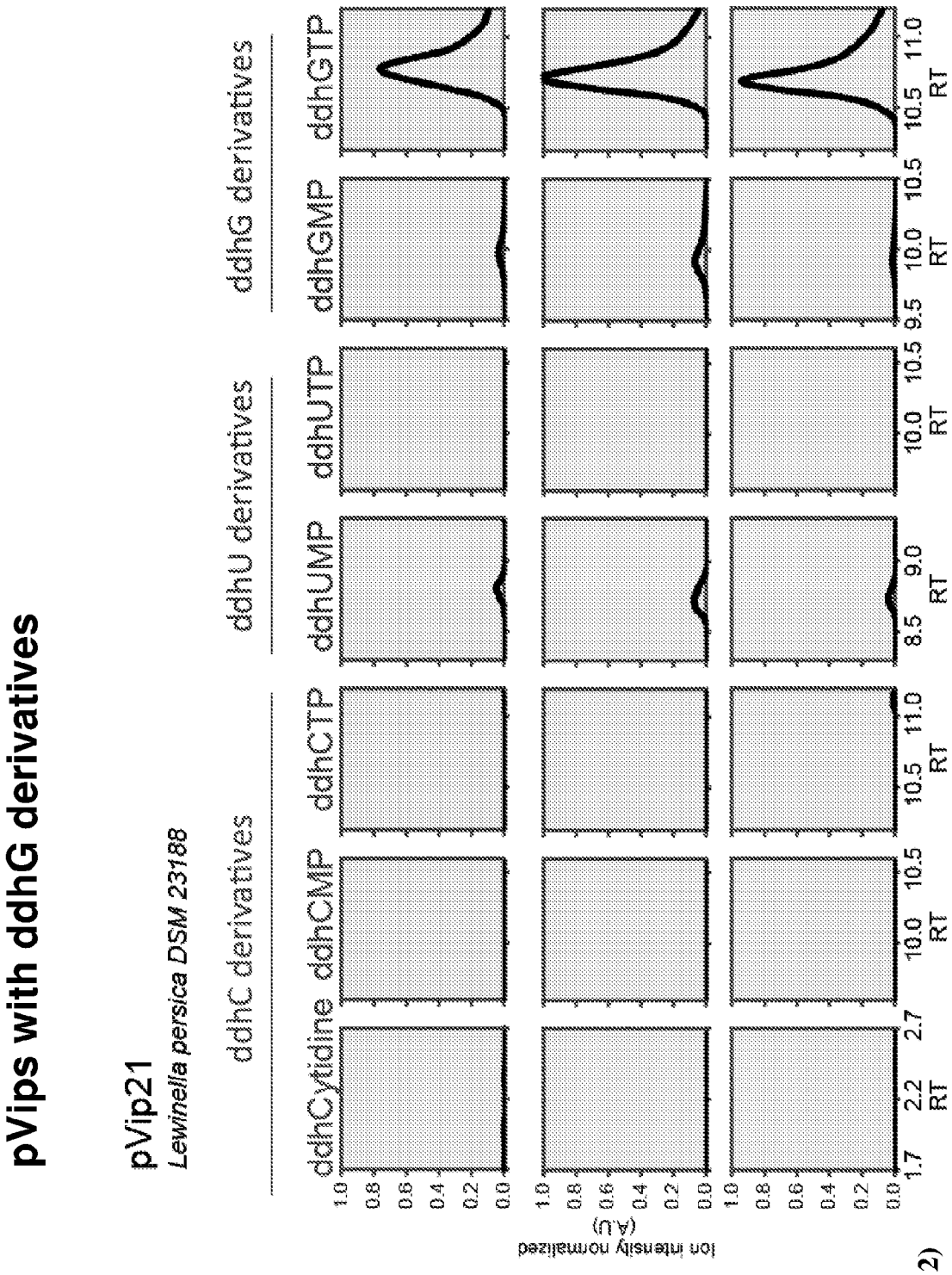


Figure 11 (cont. 2)

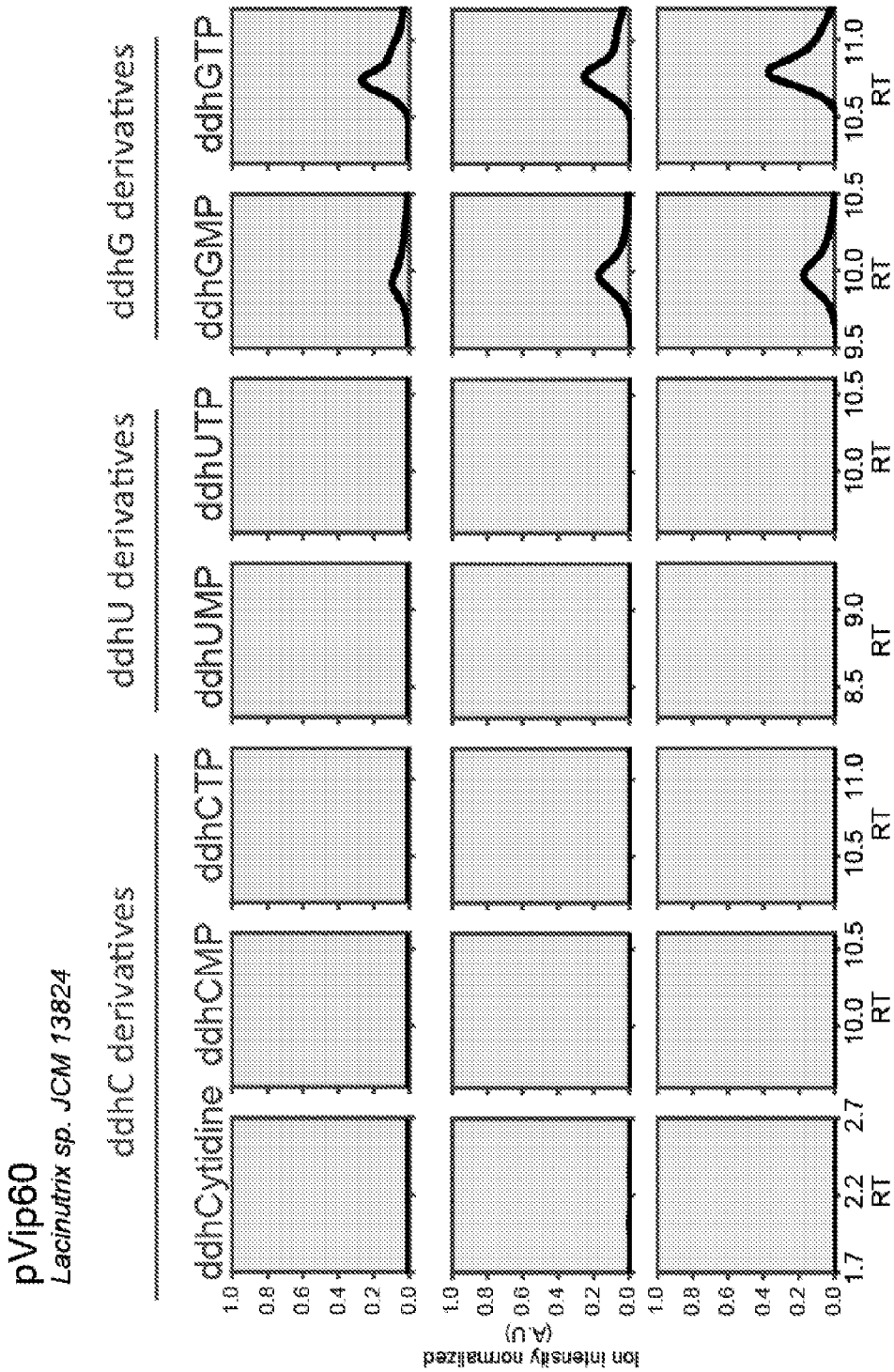
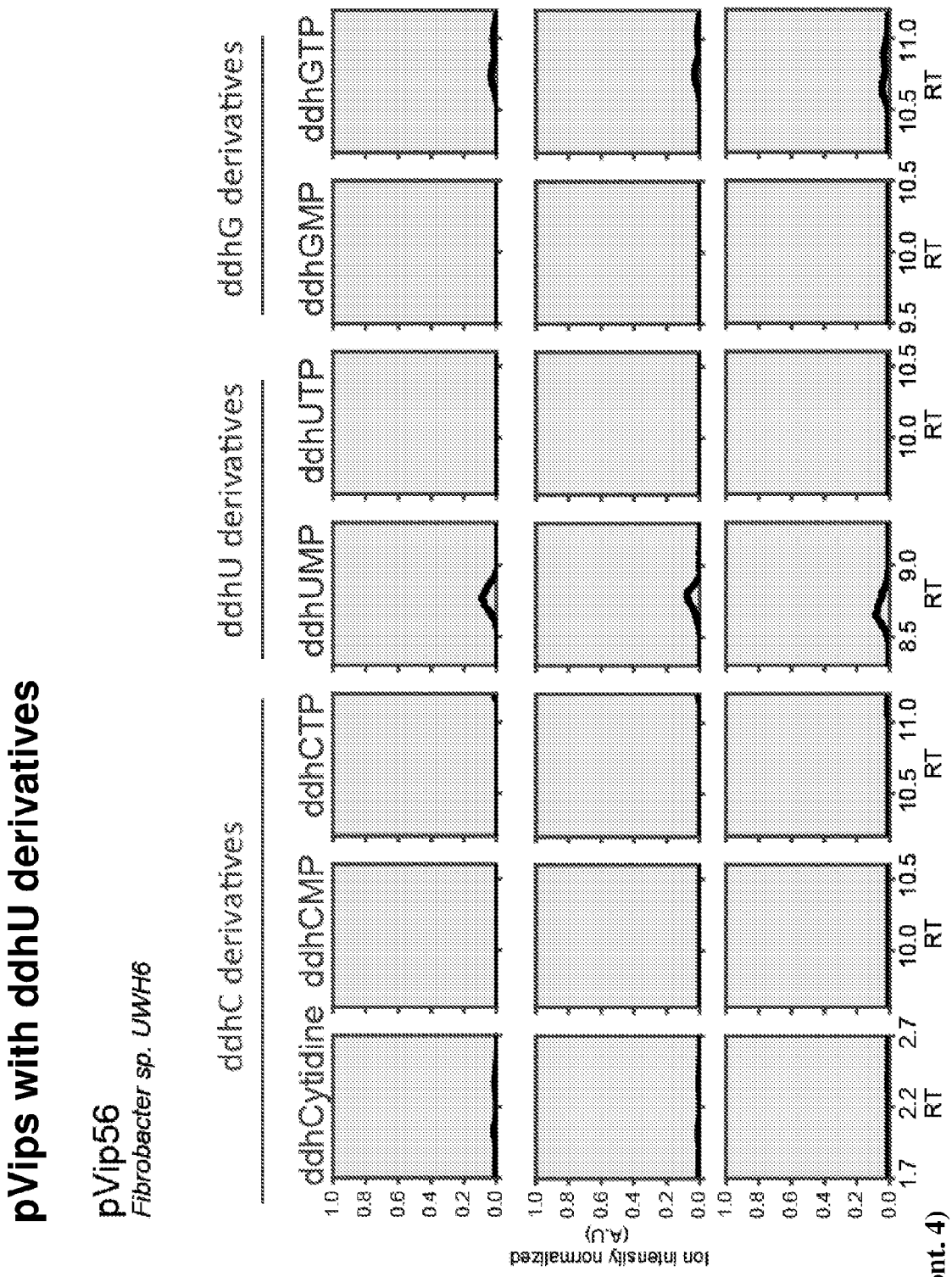


Figure 11 (cont. 3)



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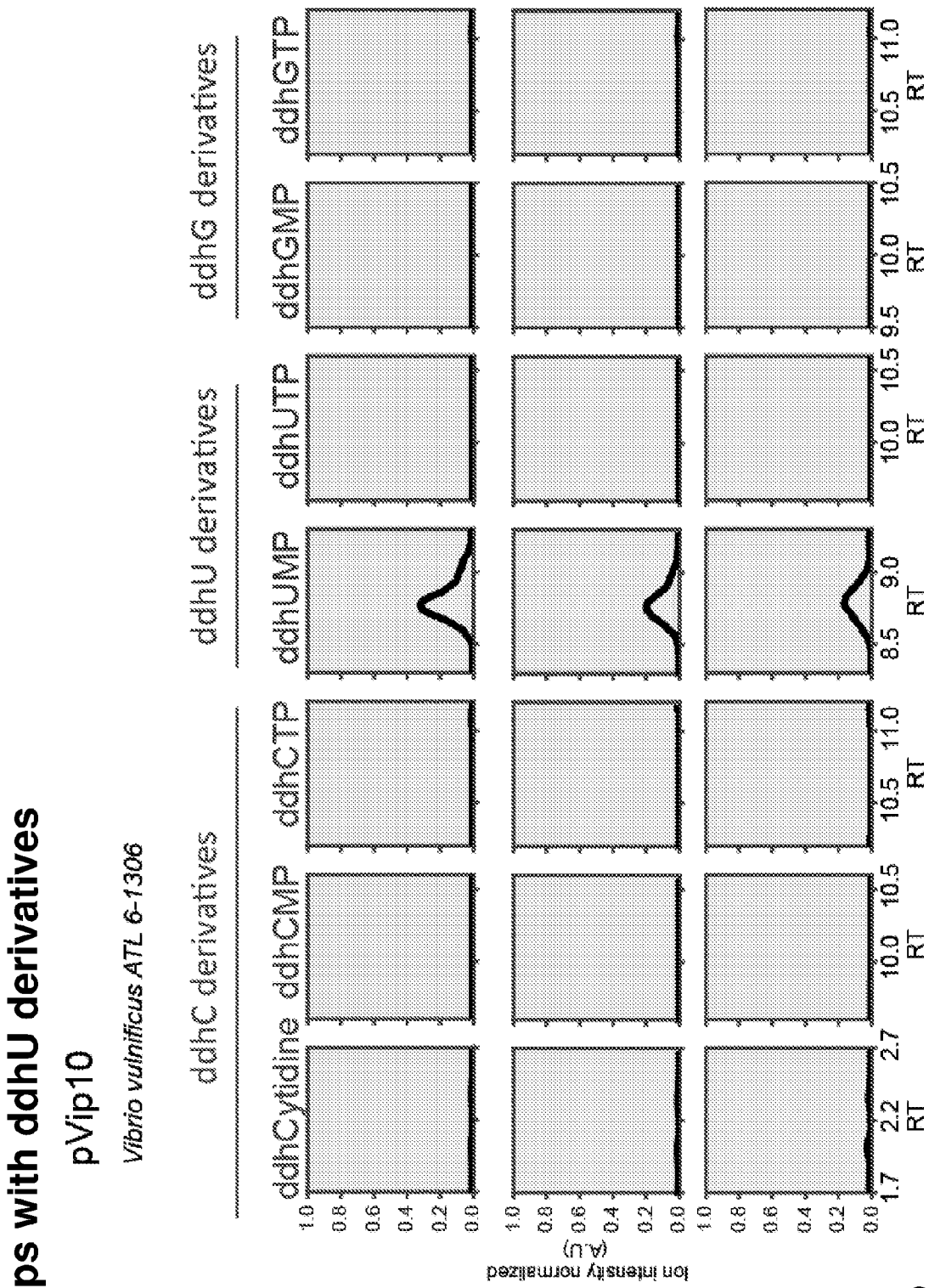

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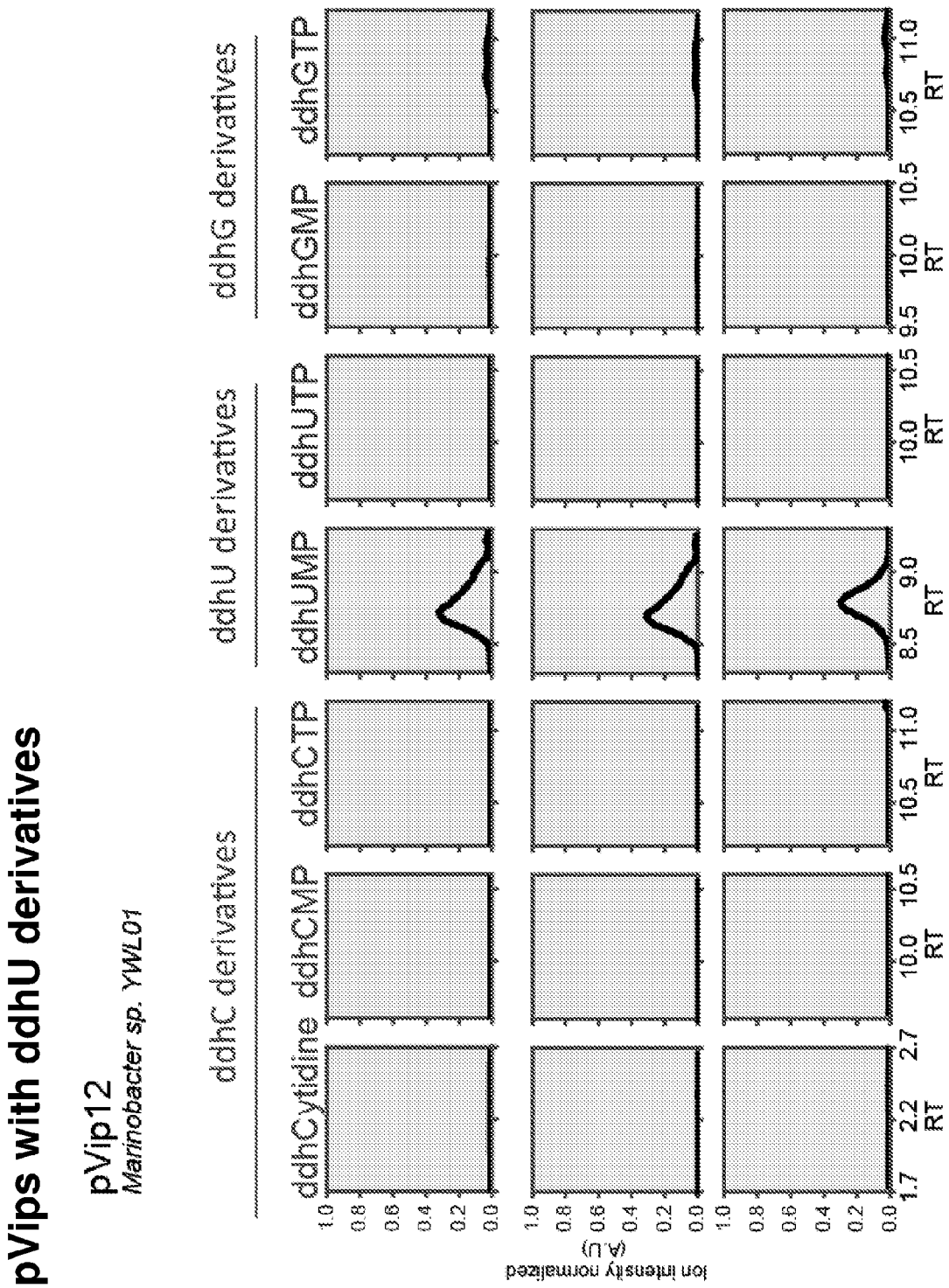
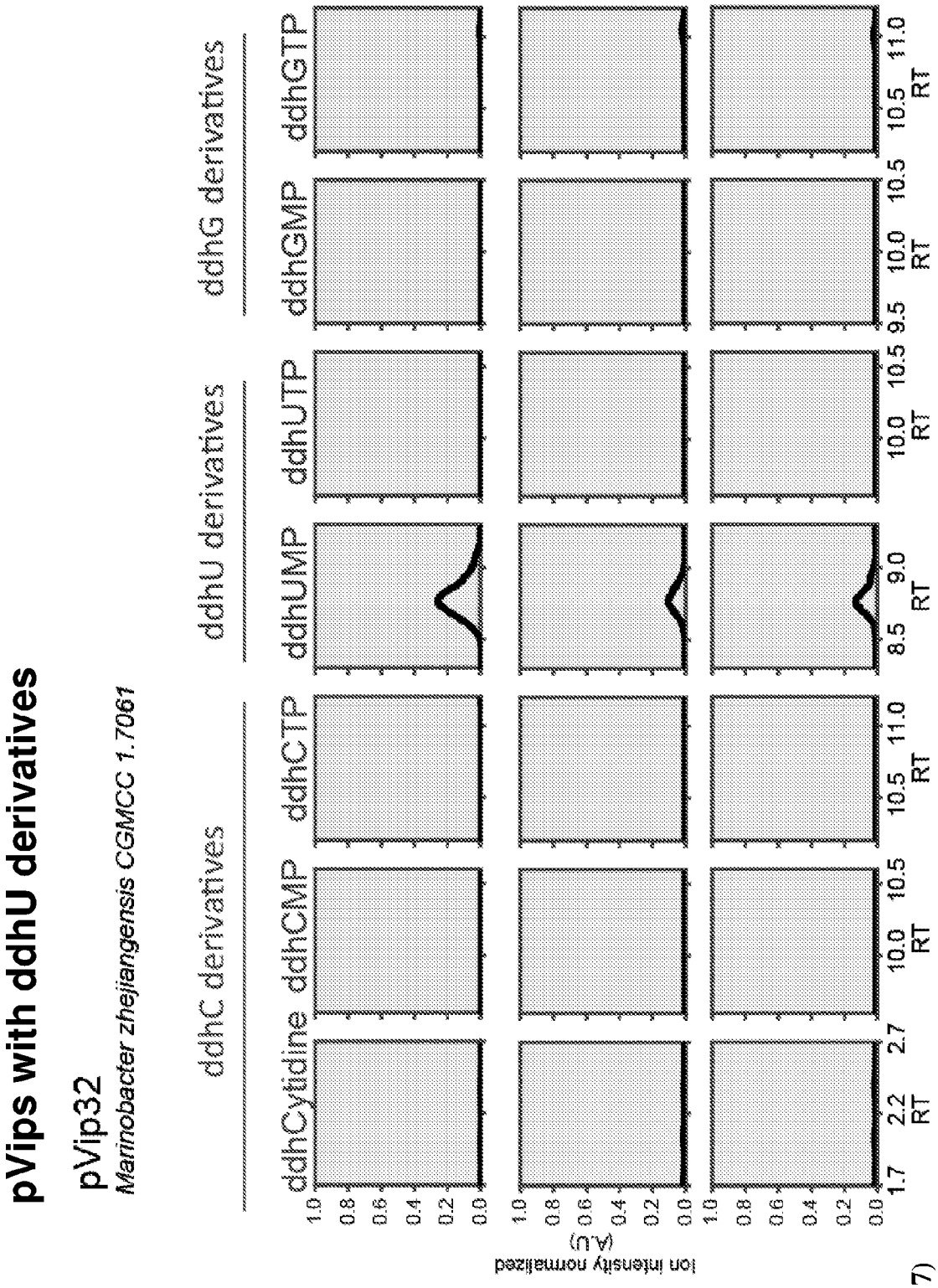


Figure 11 (cont. 6)



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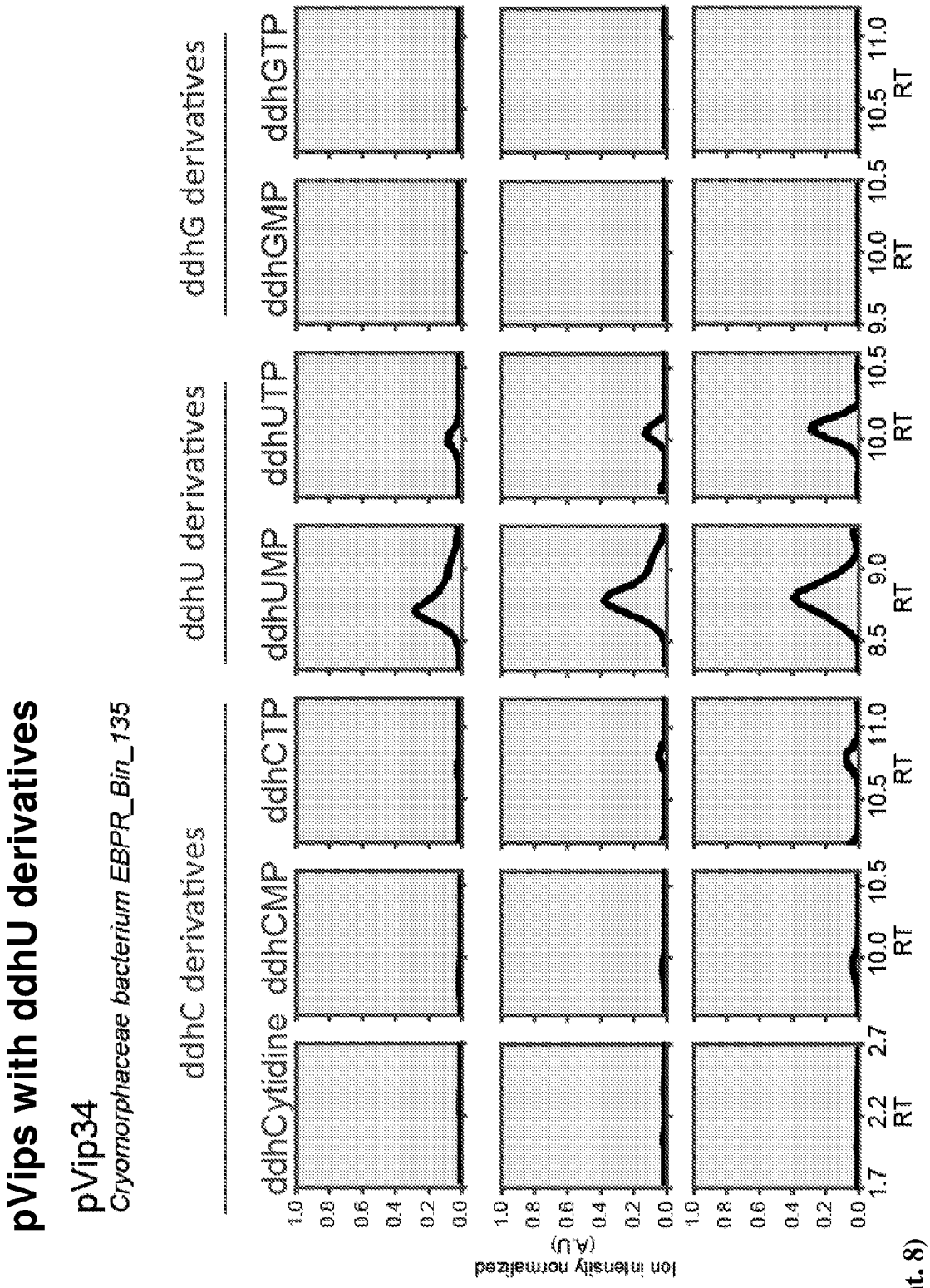

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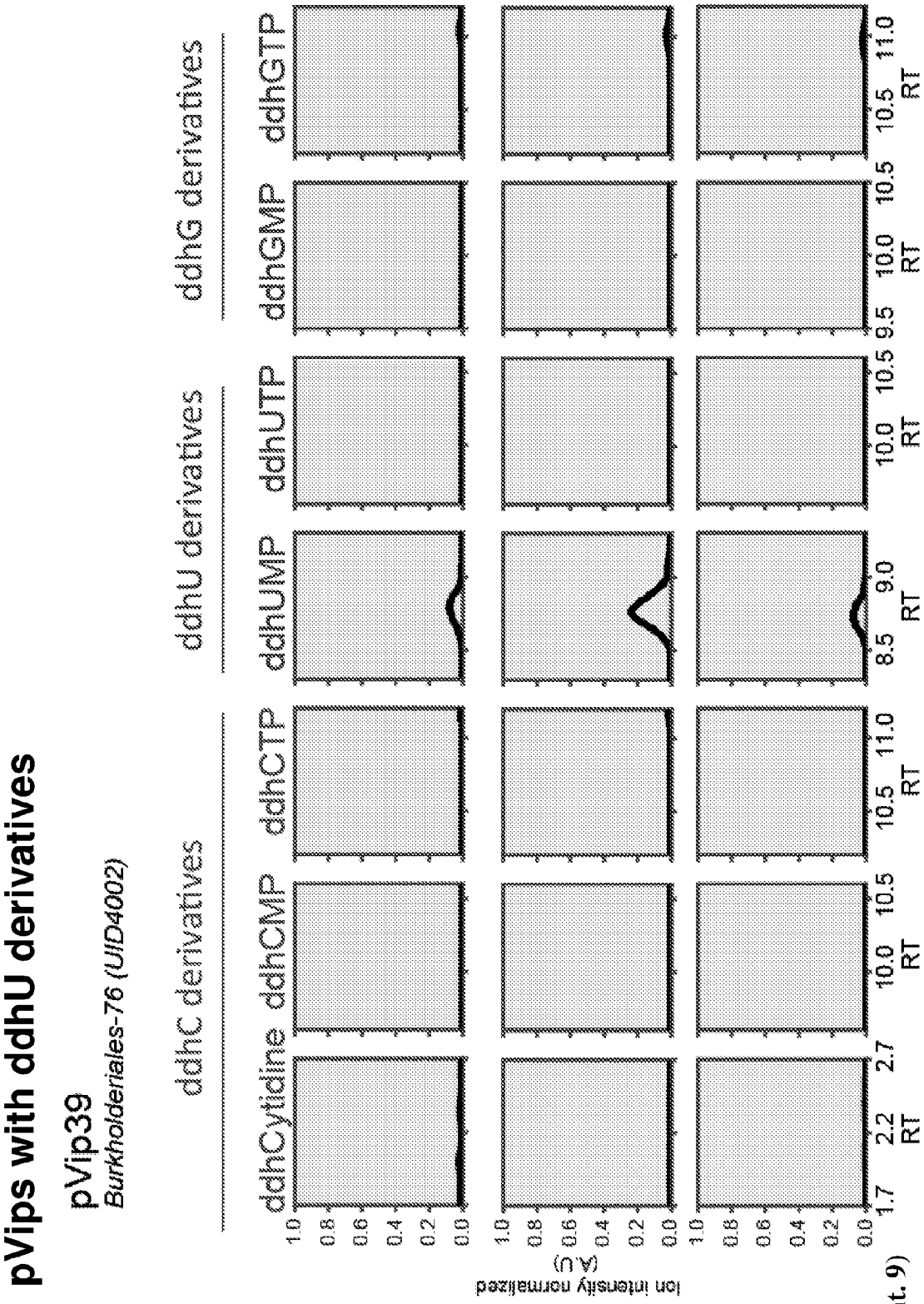

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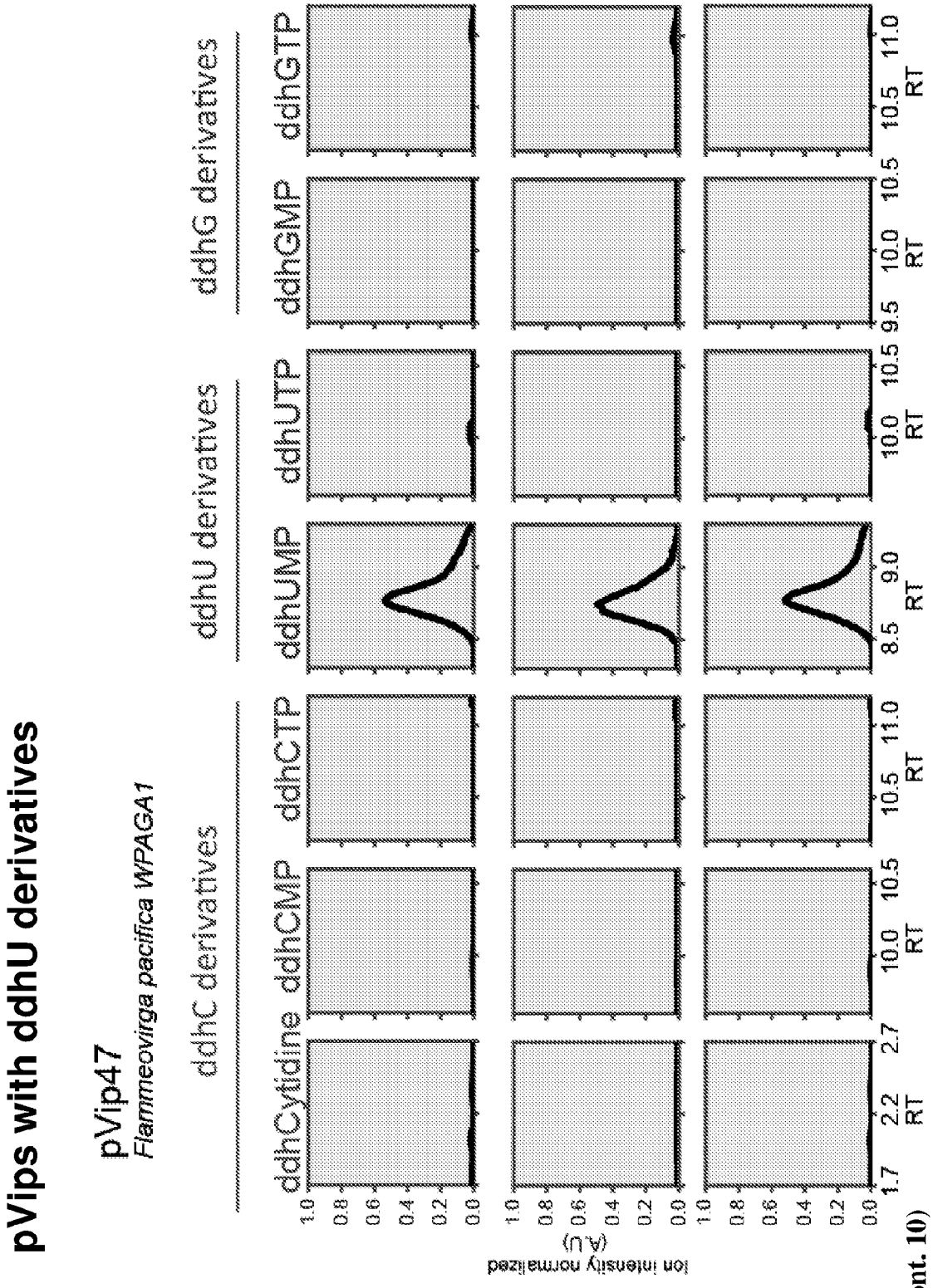

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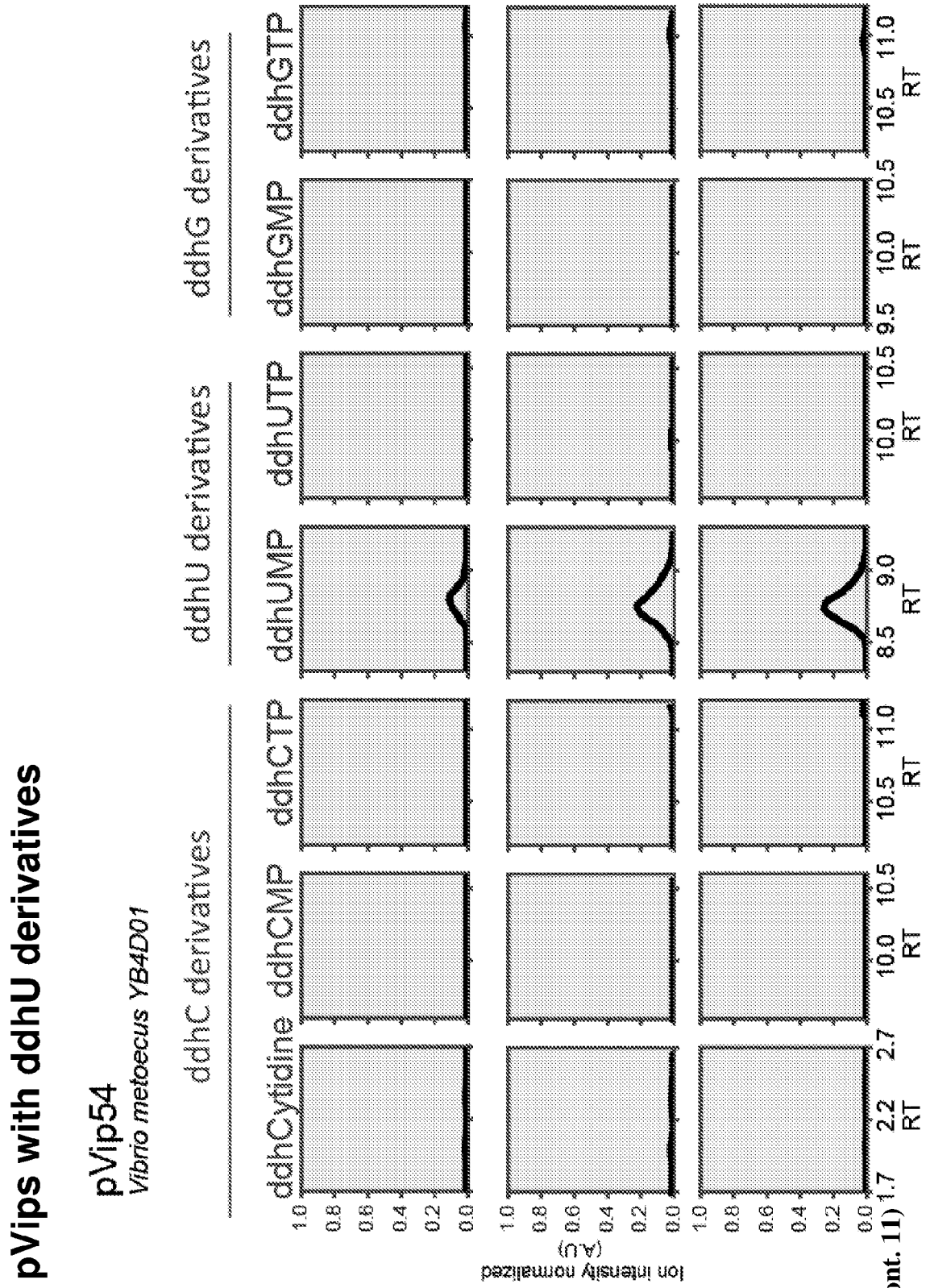

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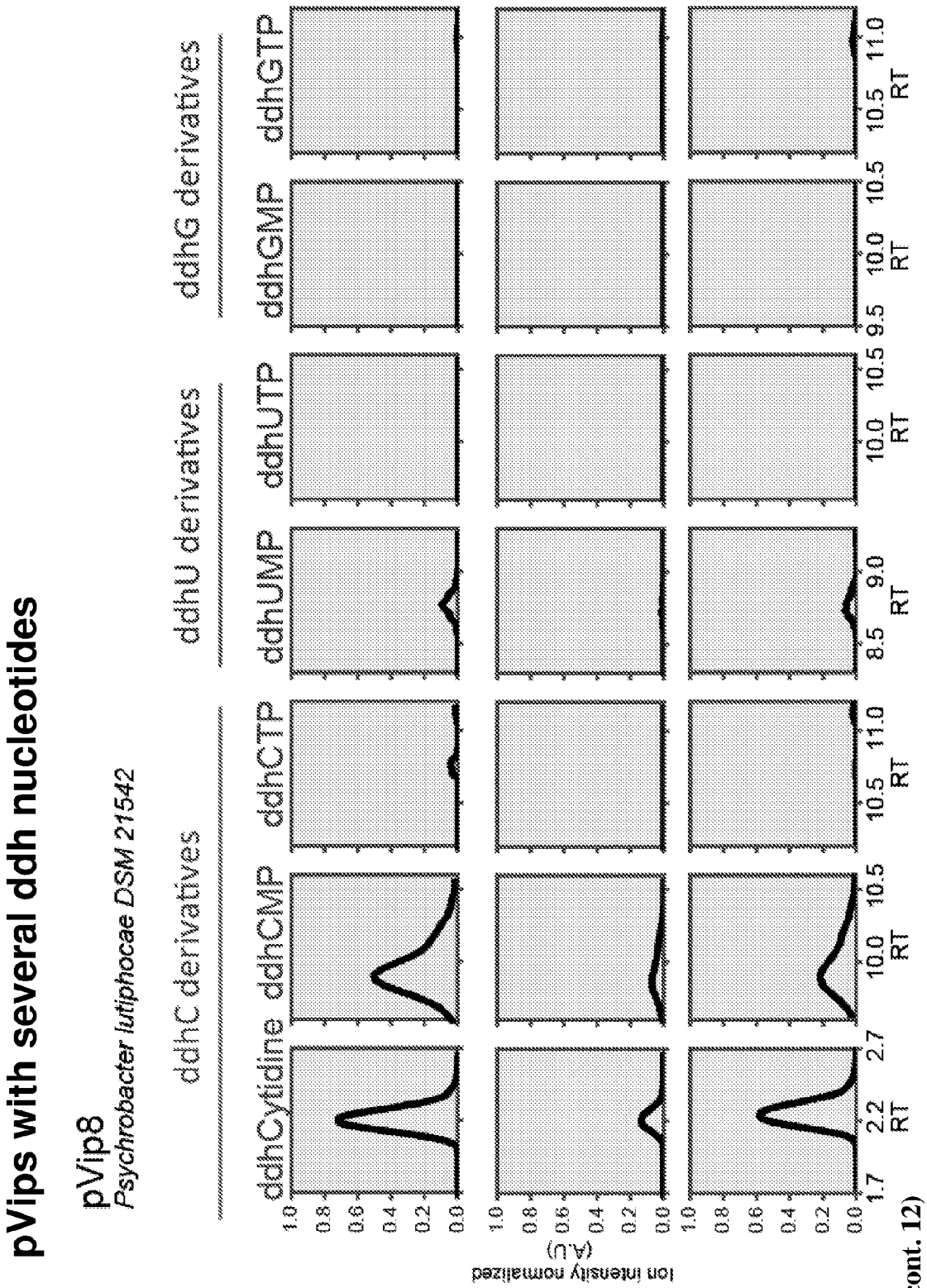

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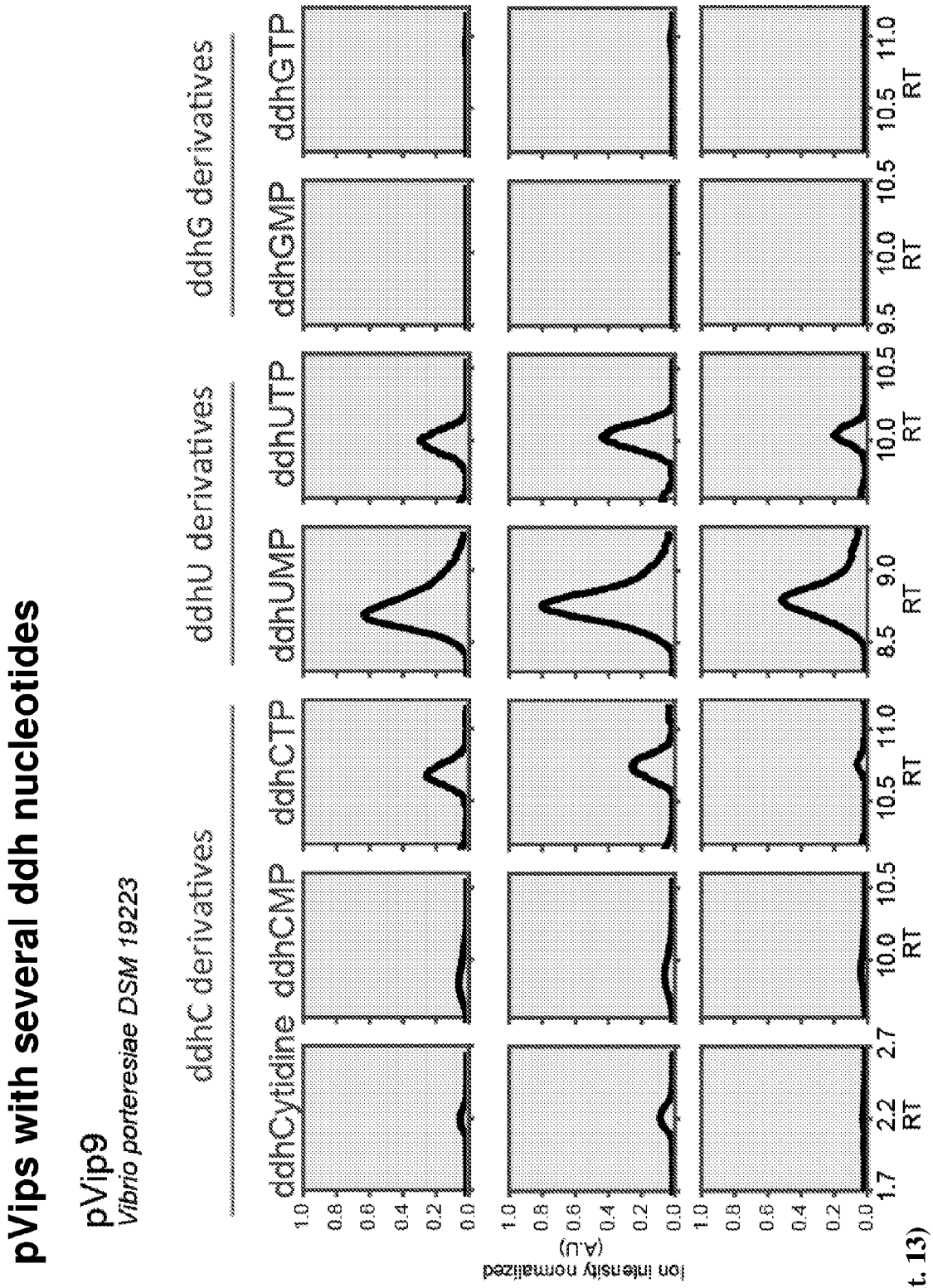

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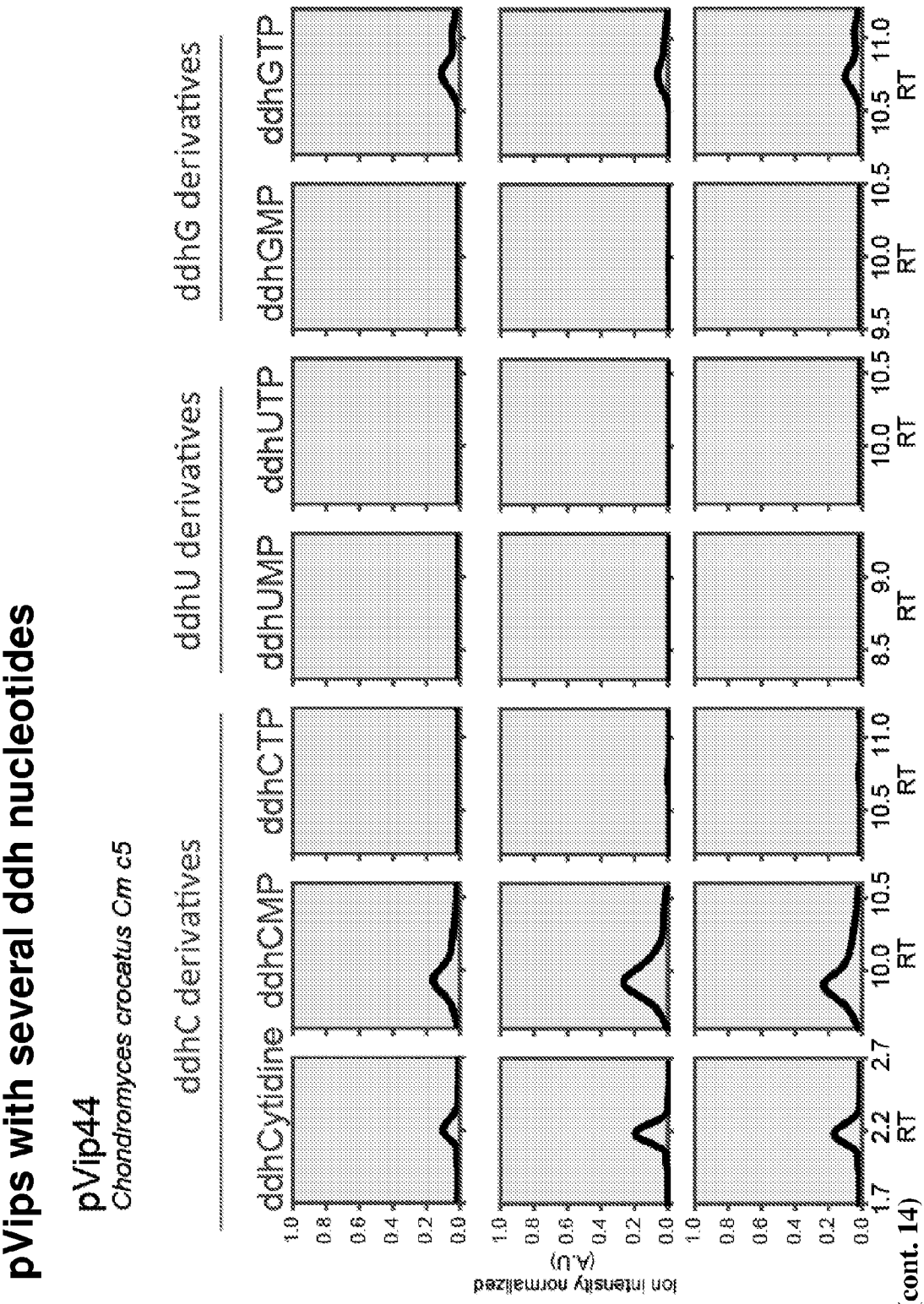

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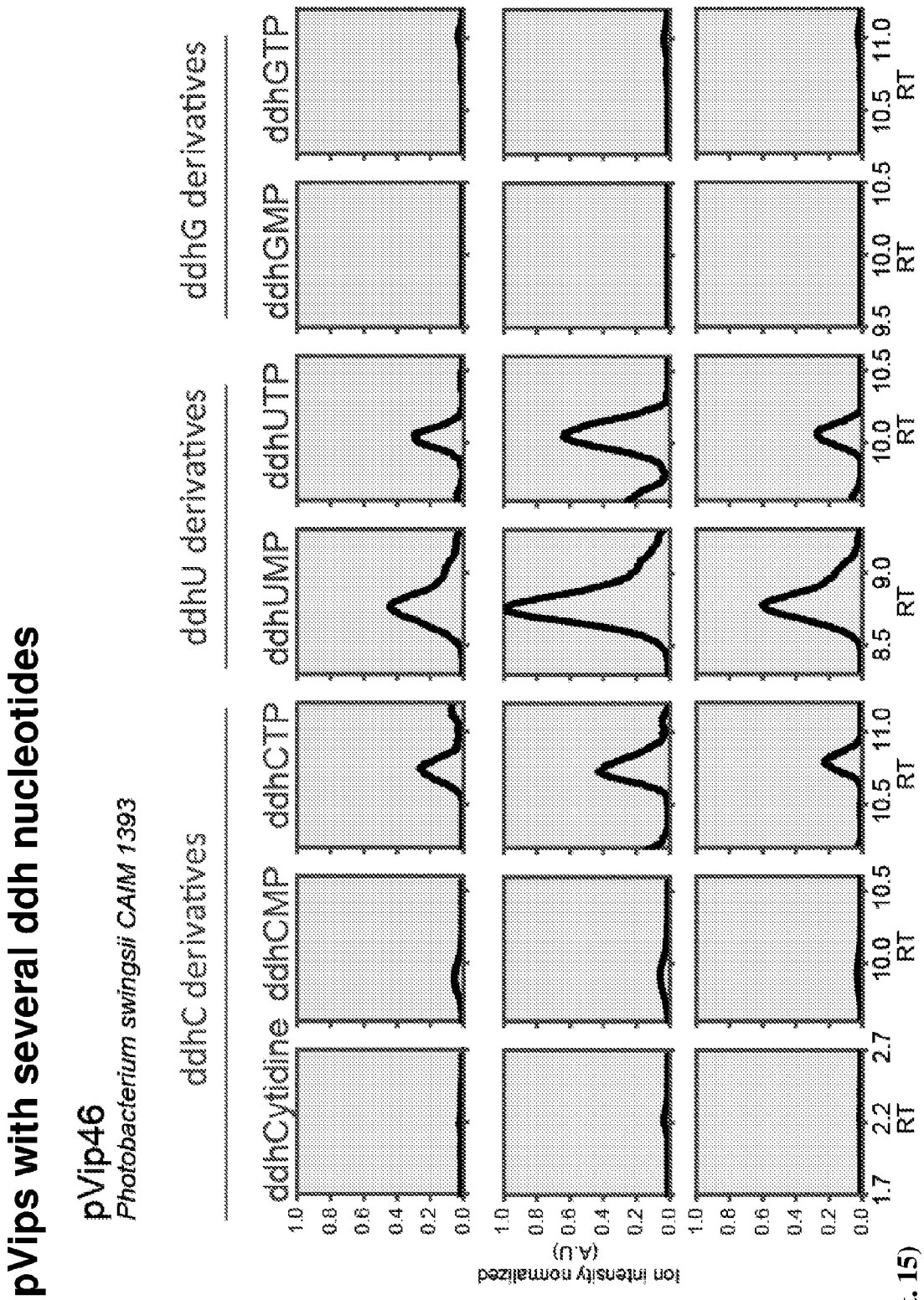

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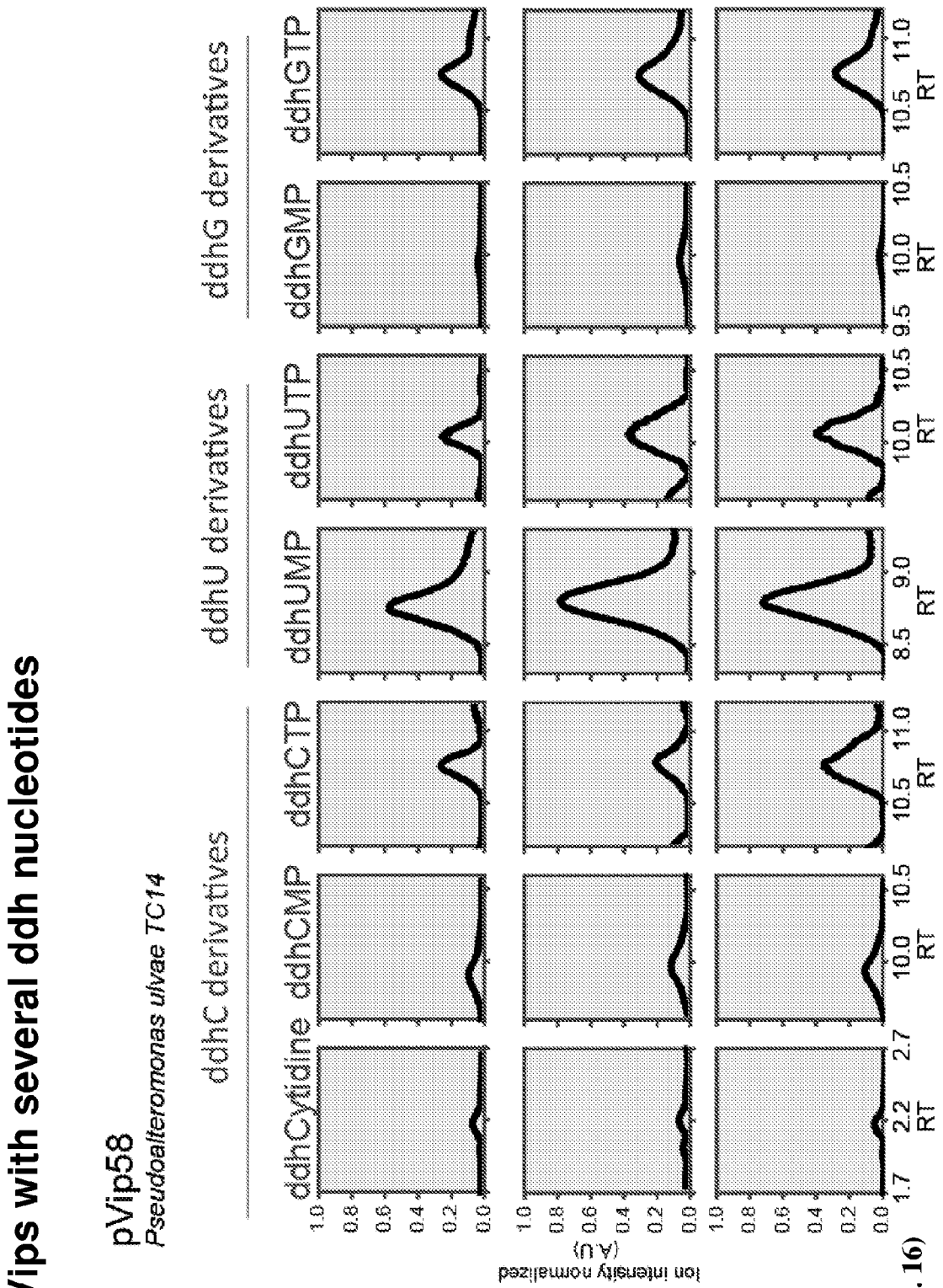

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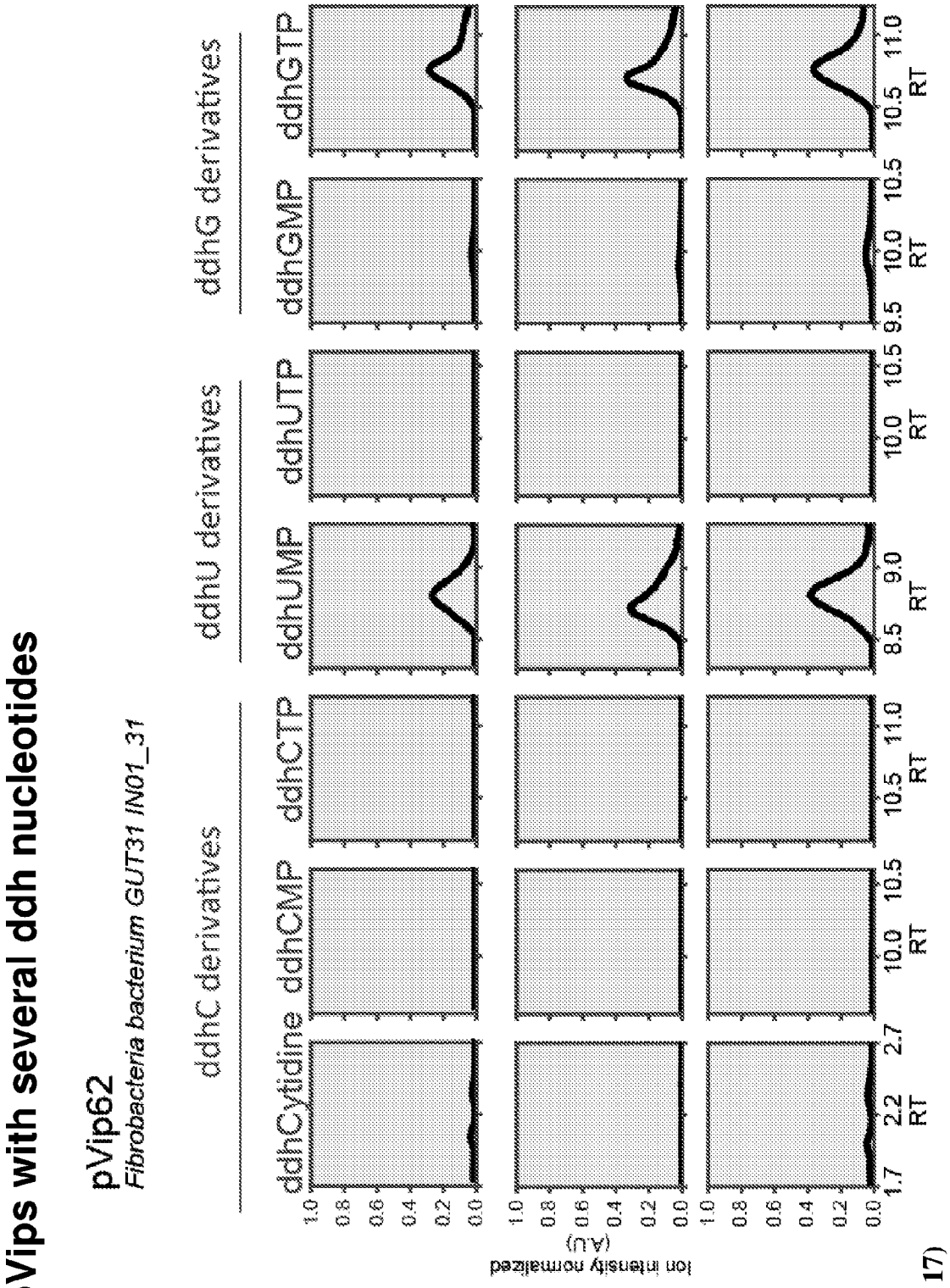

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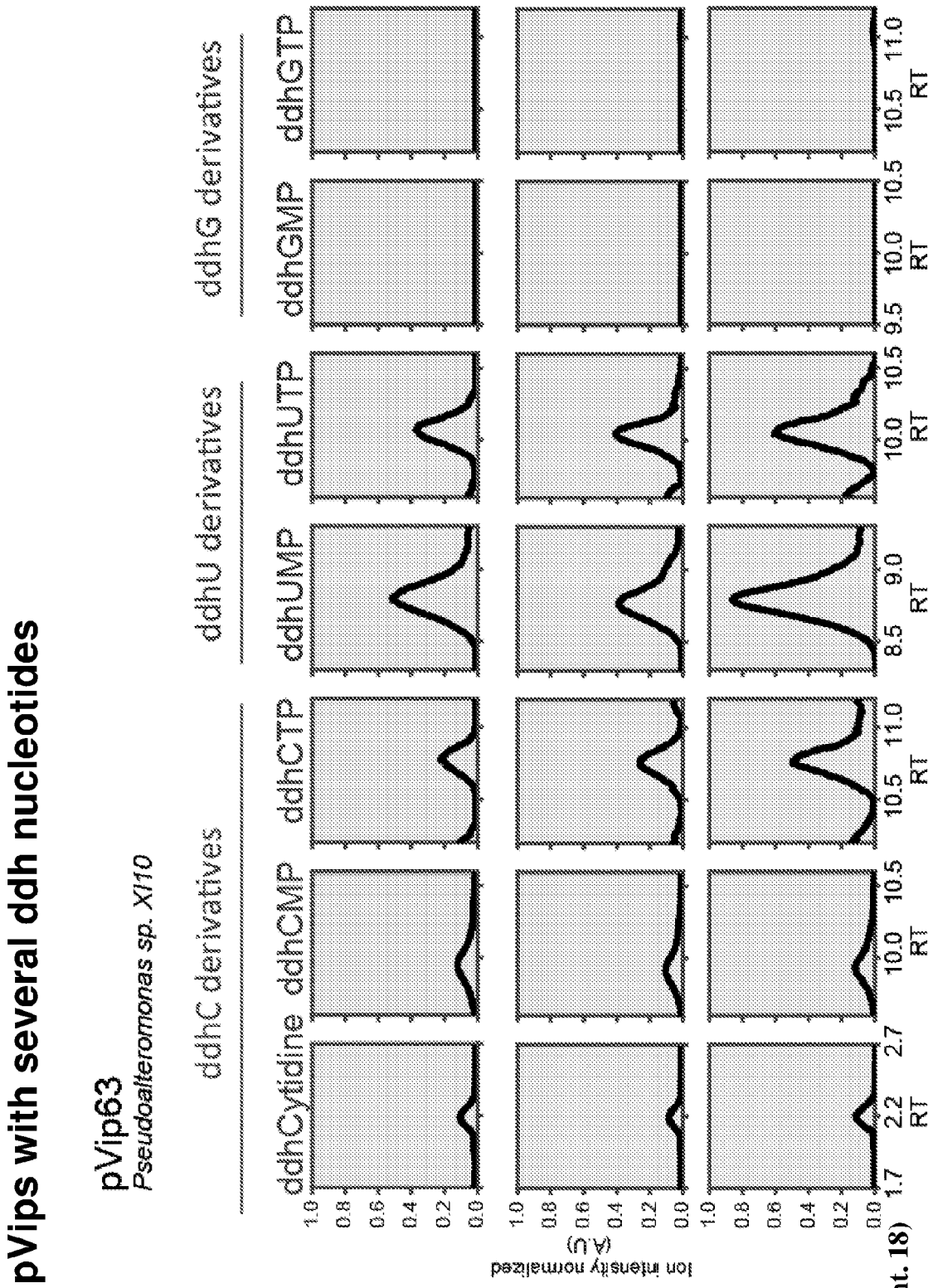


Figure 11 (cont. 18)

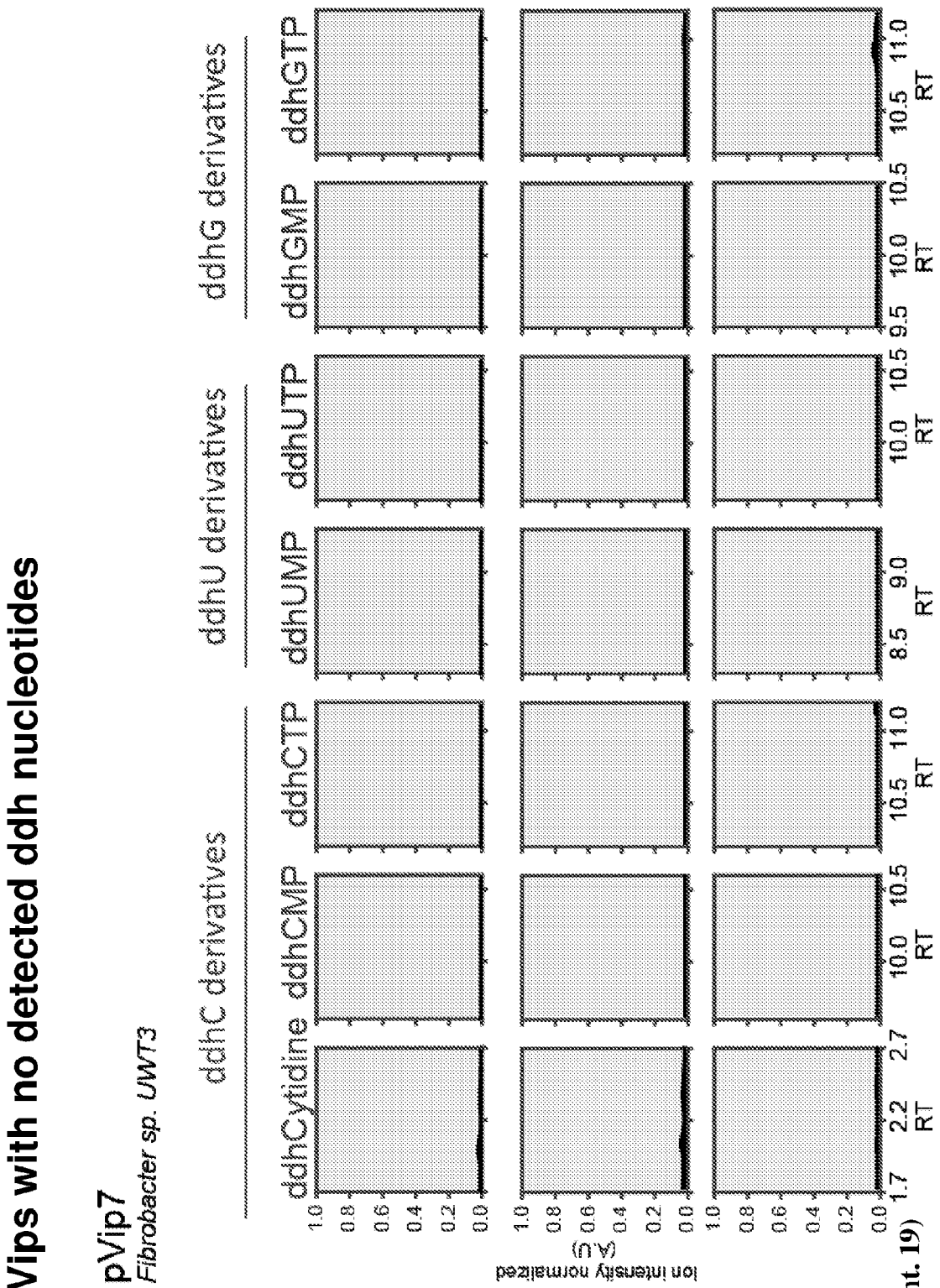


Figure 11 (cont. 19)

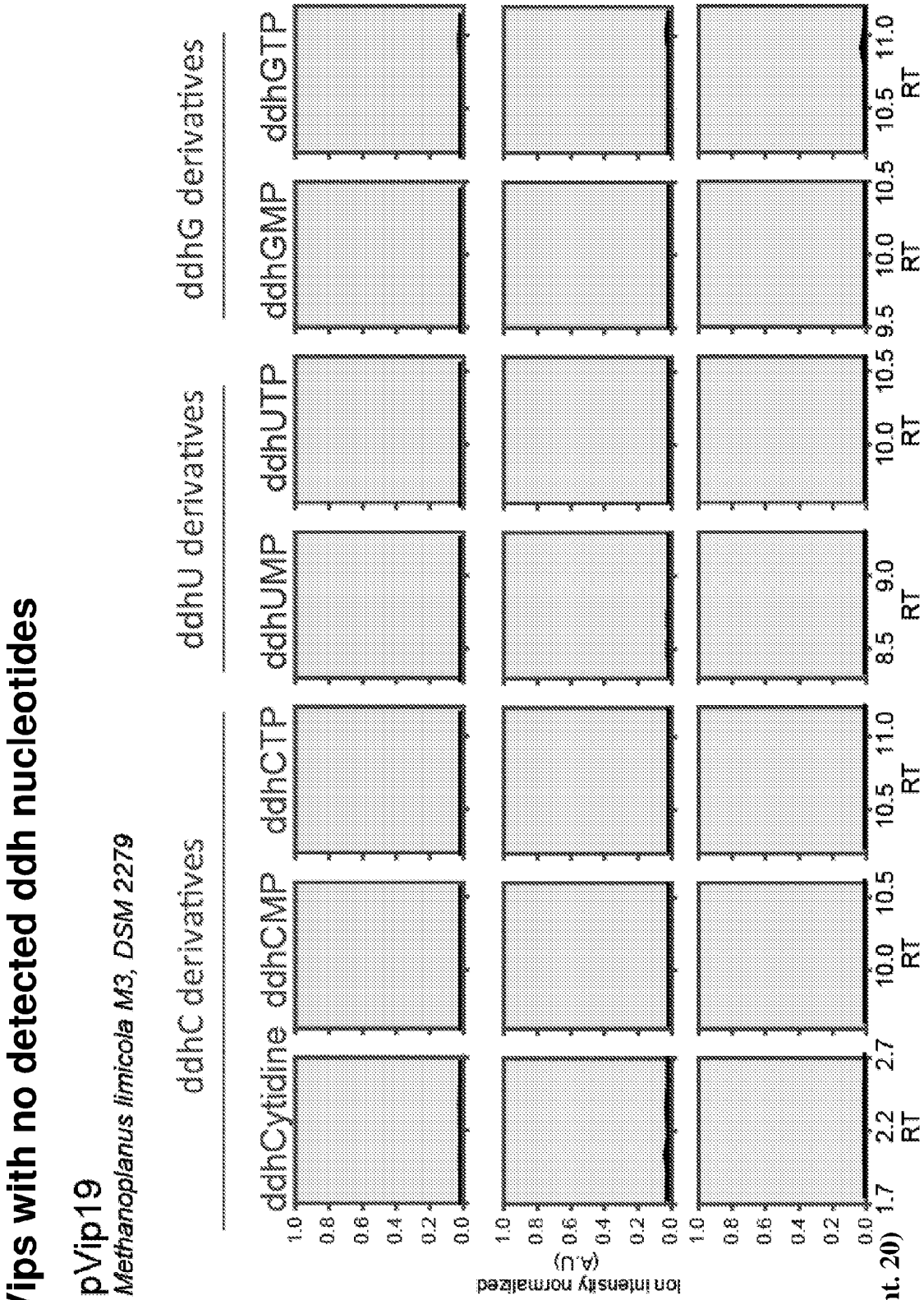


Figure 11 (cont. 20)

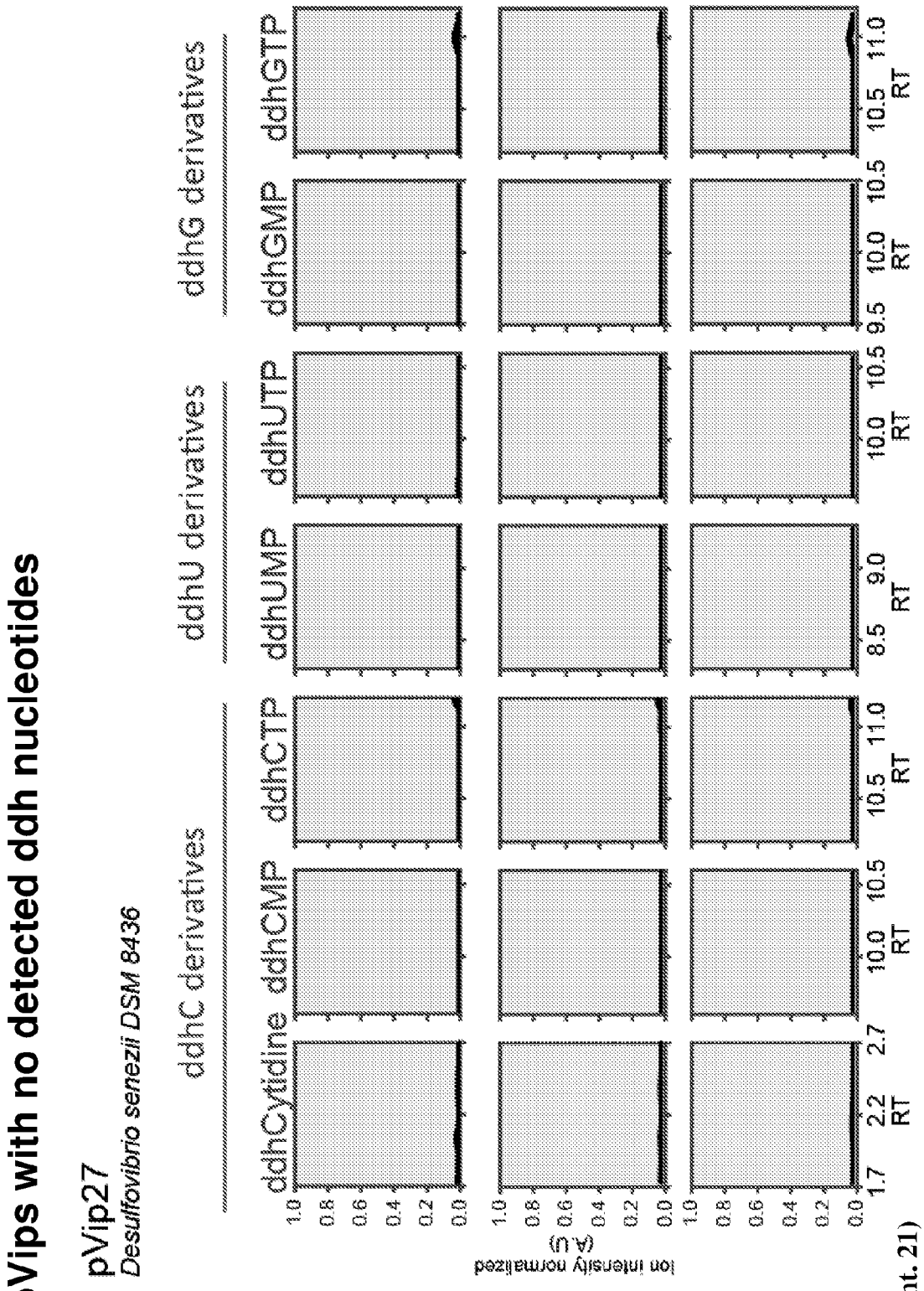


Figure 11 (cont. 21)

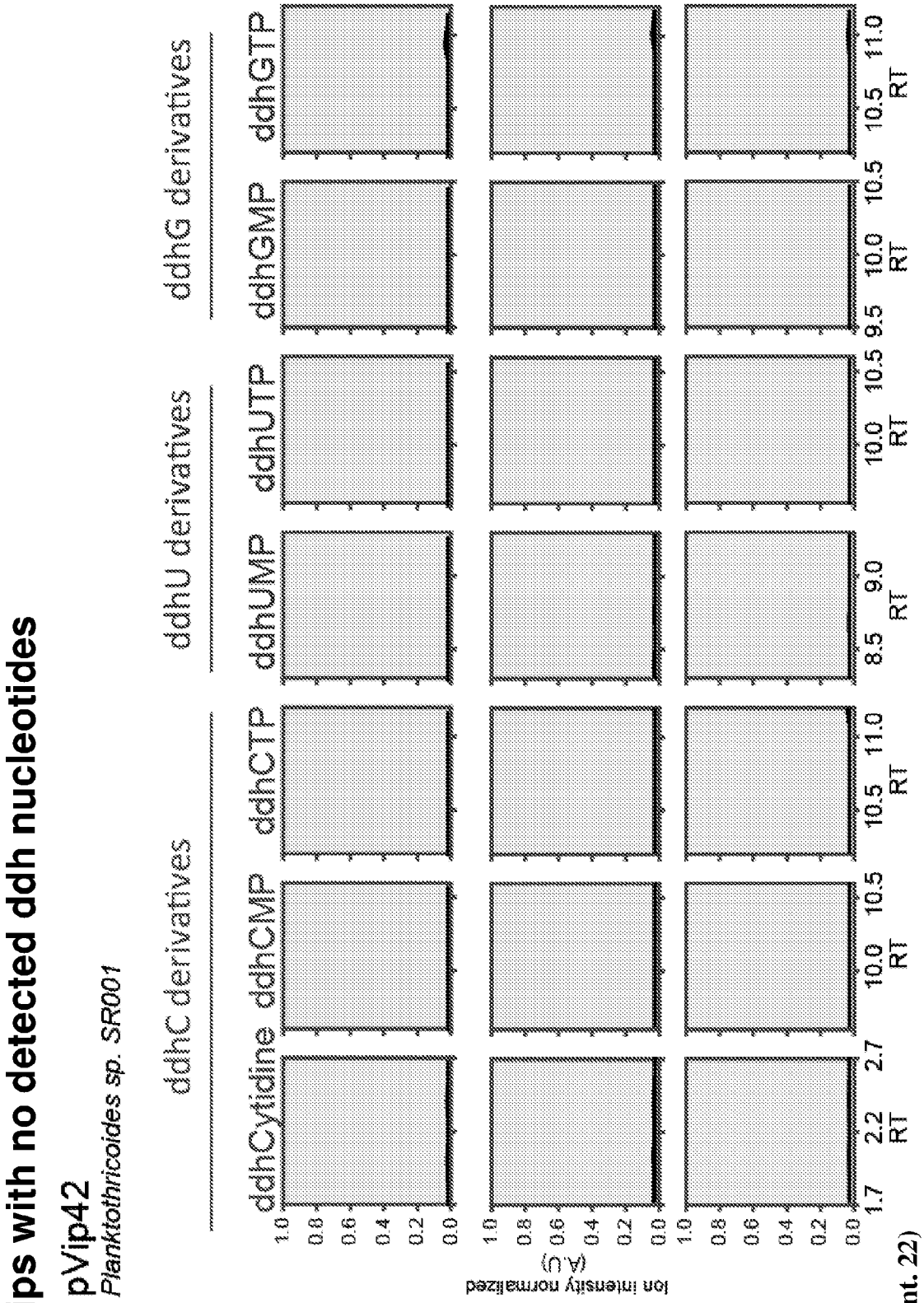
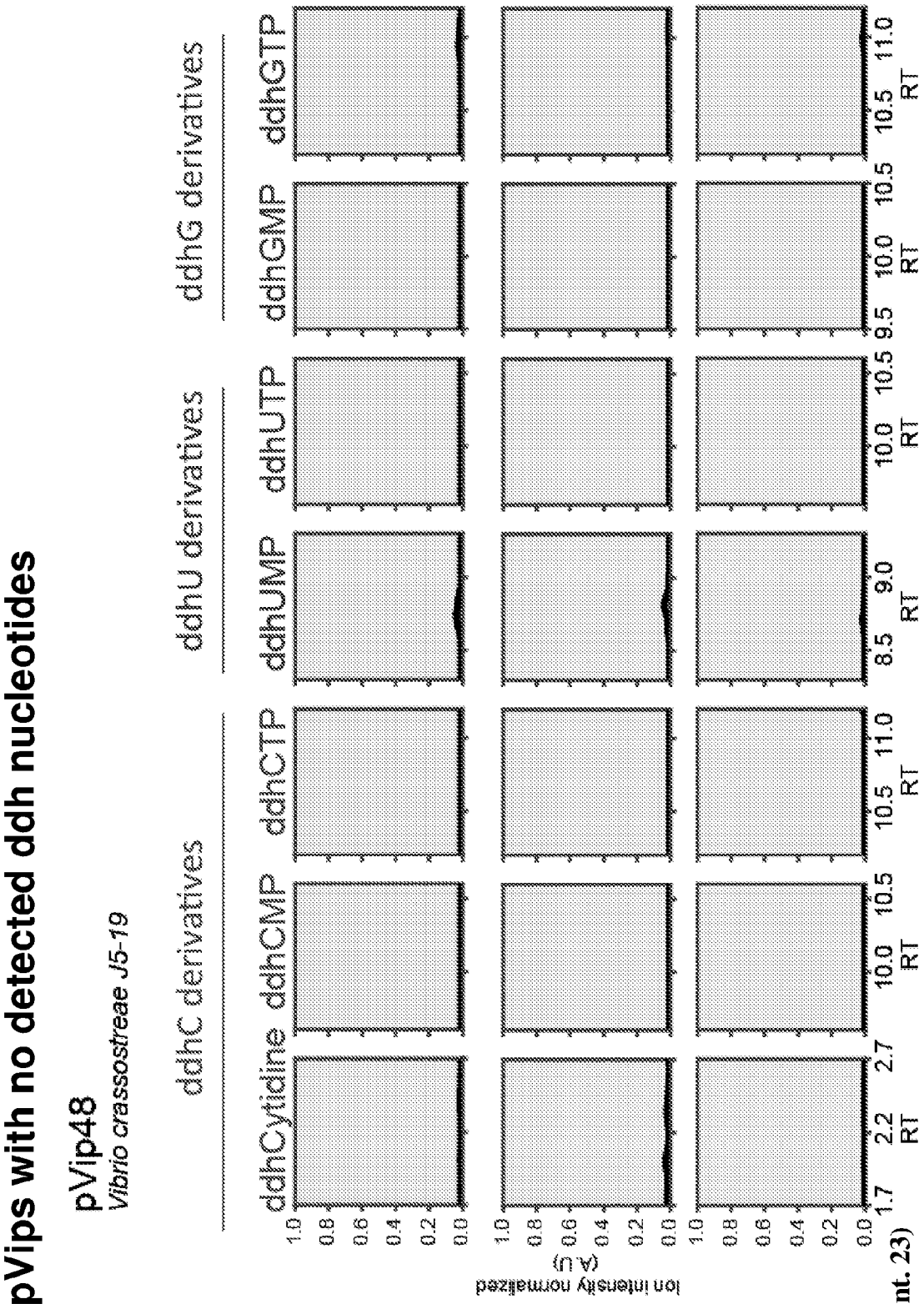


Figure 11 (cont. 22)



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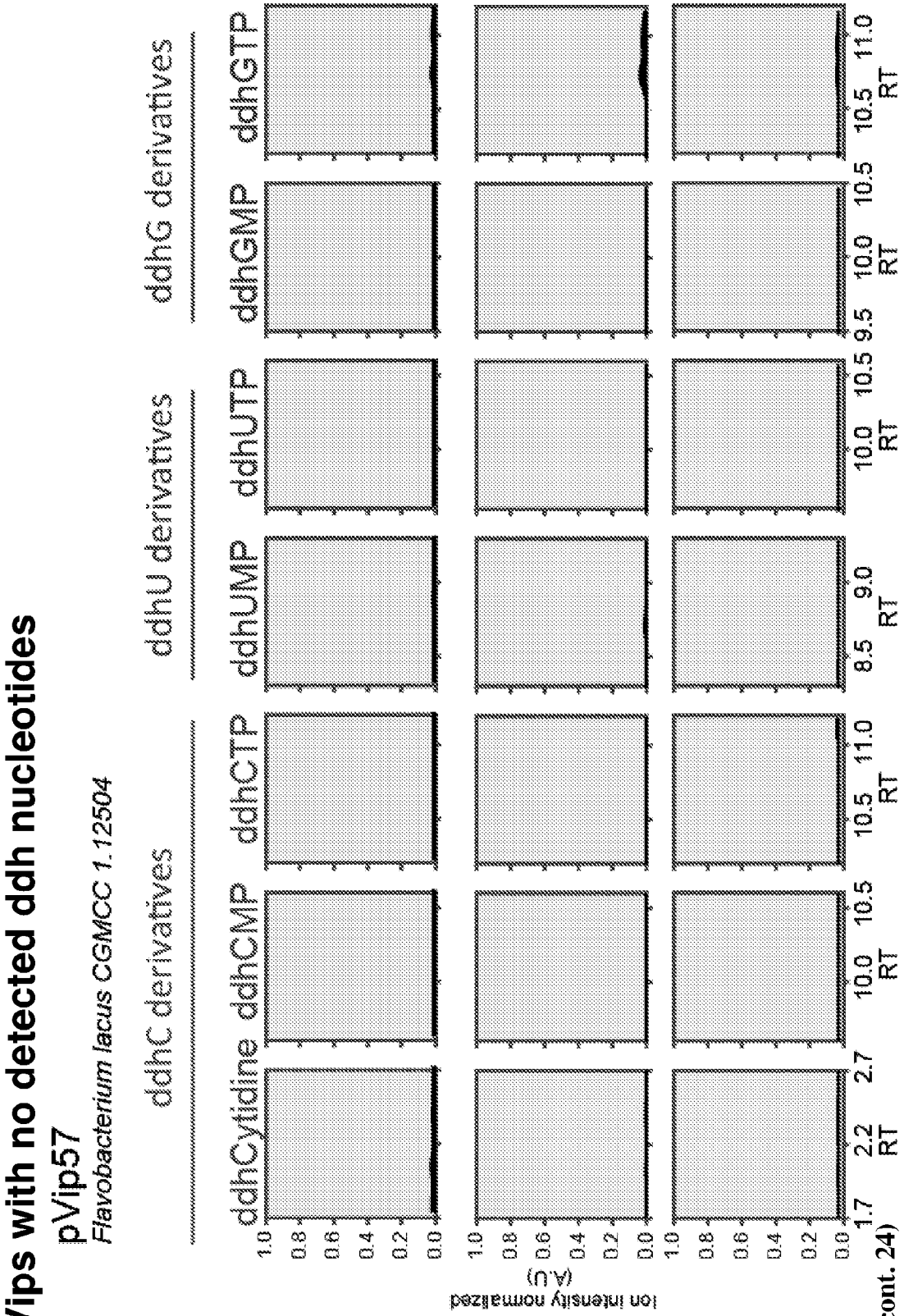

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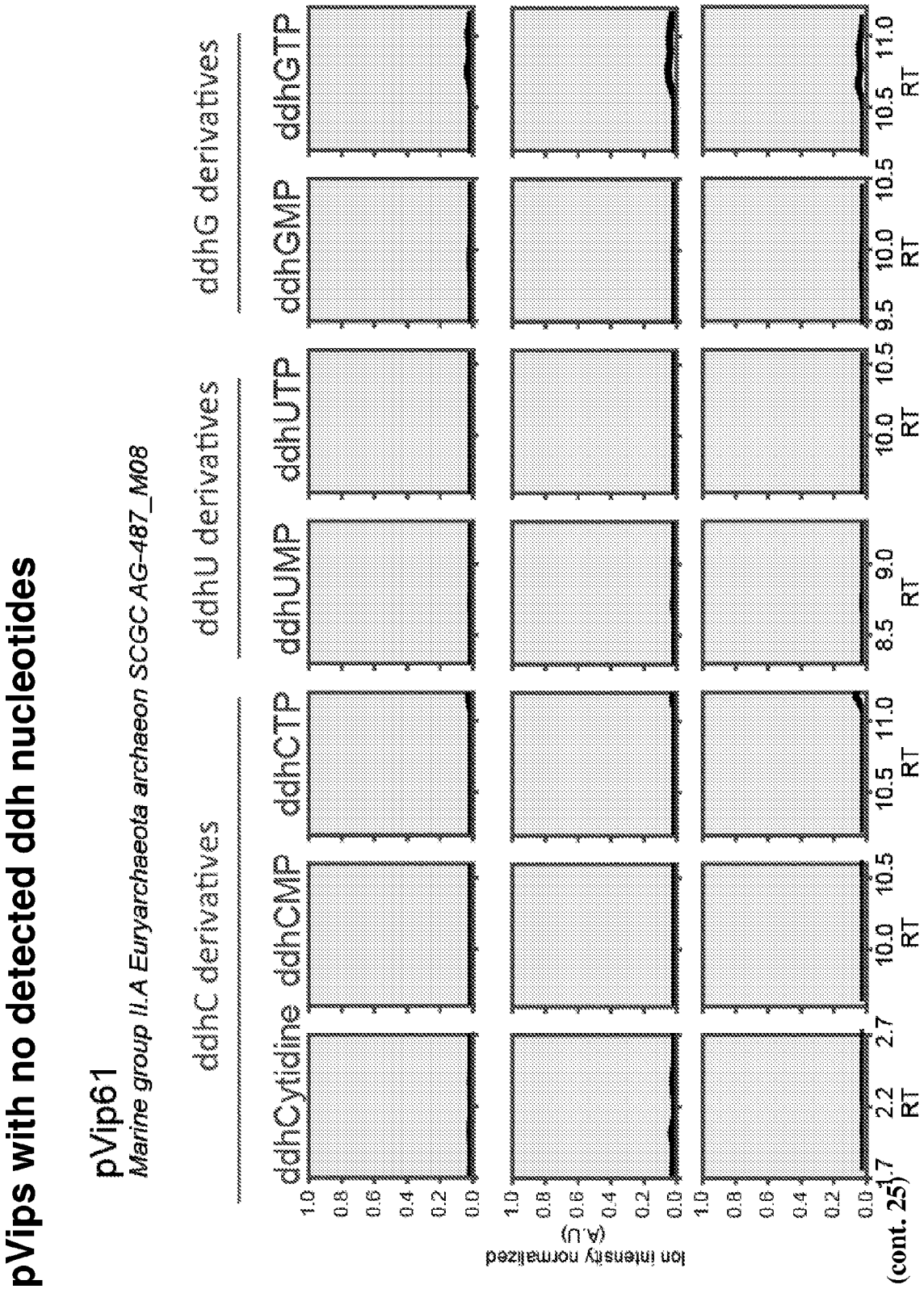


Figure 11 (cont. 25)

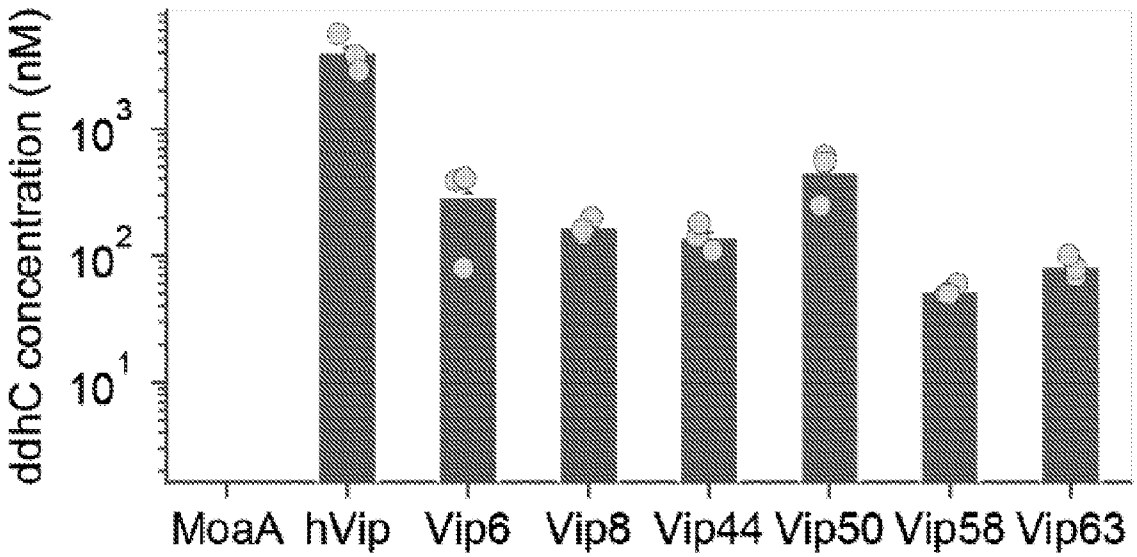
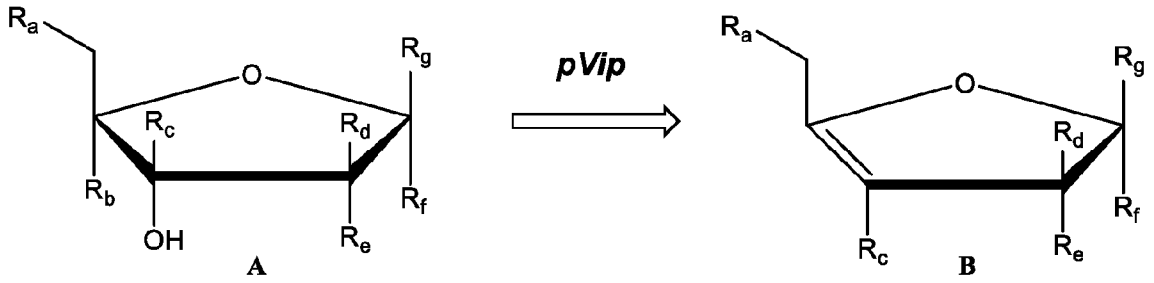
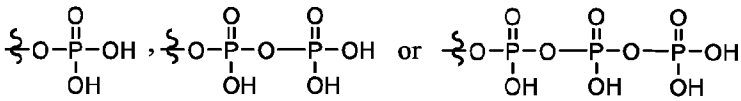

ddhC concentration (nM)
10^3
10^2
10^1
MoaA
hVip
Vip6
Vip8
Vip44
Vip50
Vip58
Vip63

Figure 12



Ra = OH, 

Rg = Heterocyclic Nitrogen based ring, O-aryl or aryl and isomers therof

Figure 13A

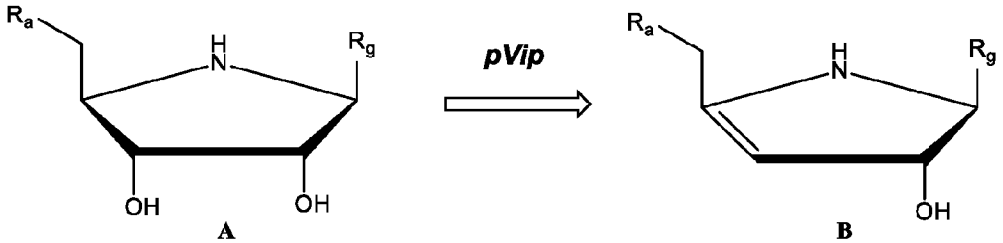


Figure 13B

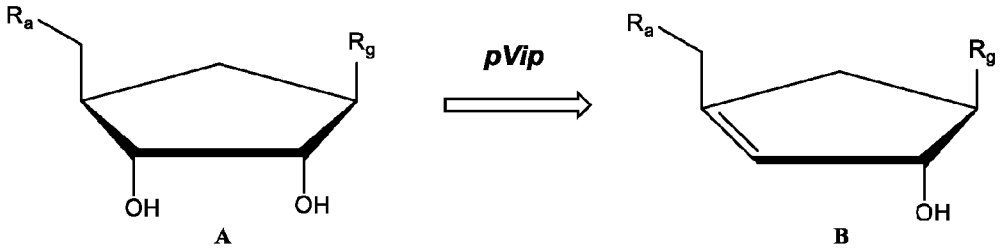


Figure 13C

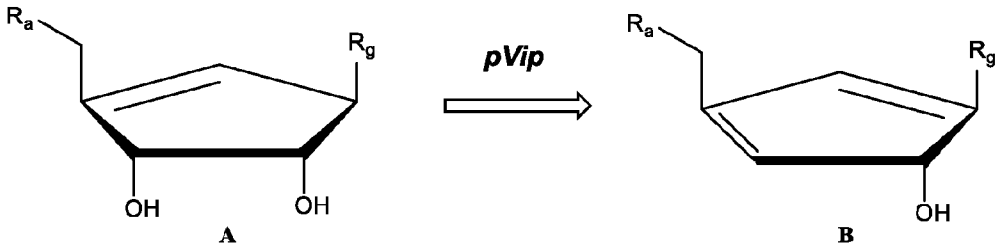


Figure 13D

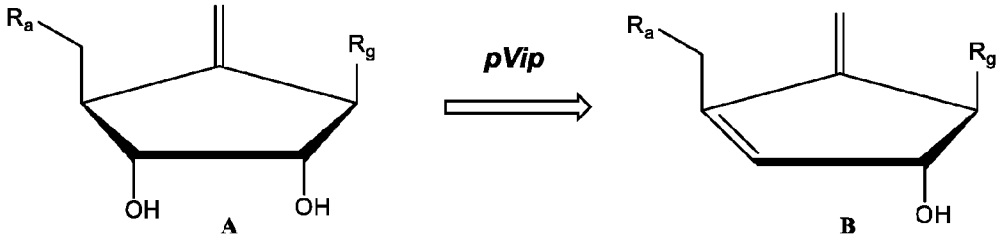


Figure 13E

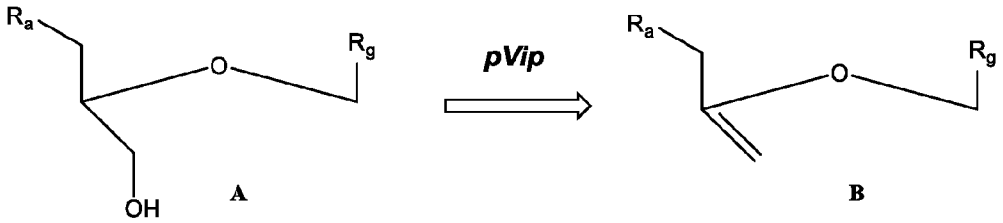


Figure 13F

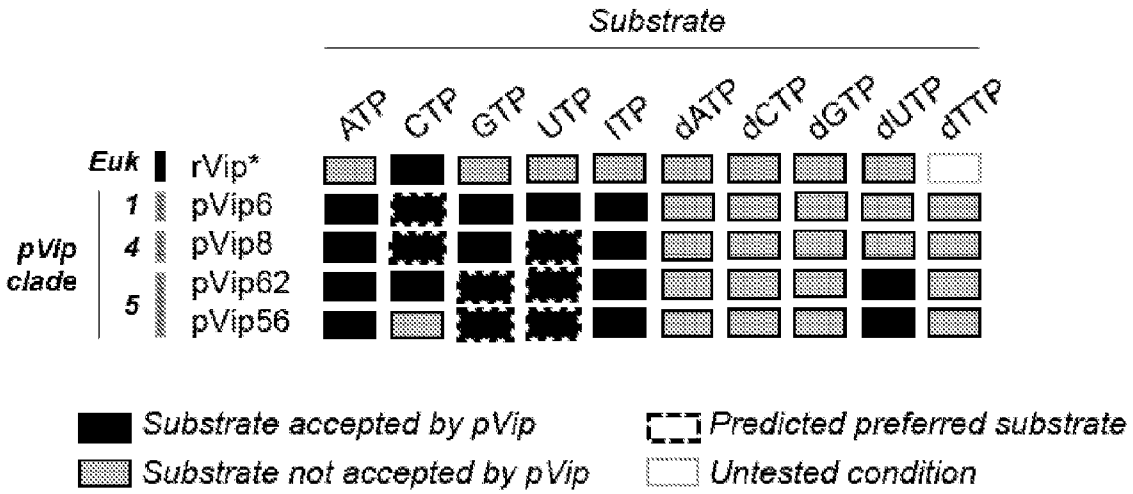


Figure 14

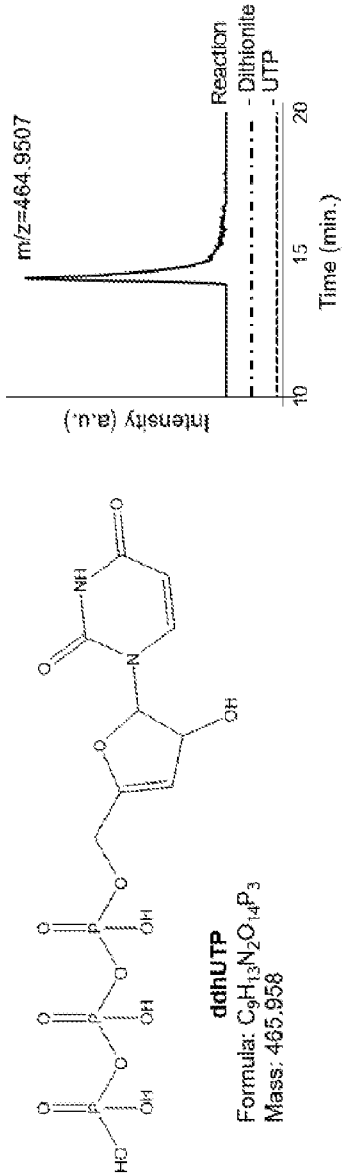


Figure 15A

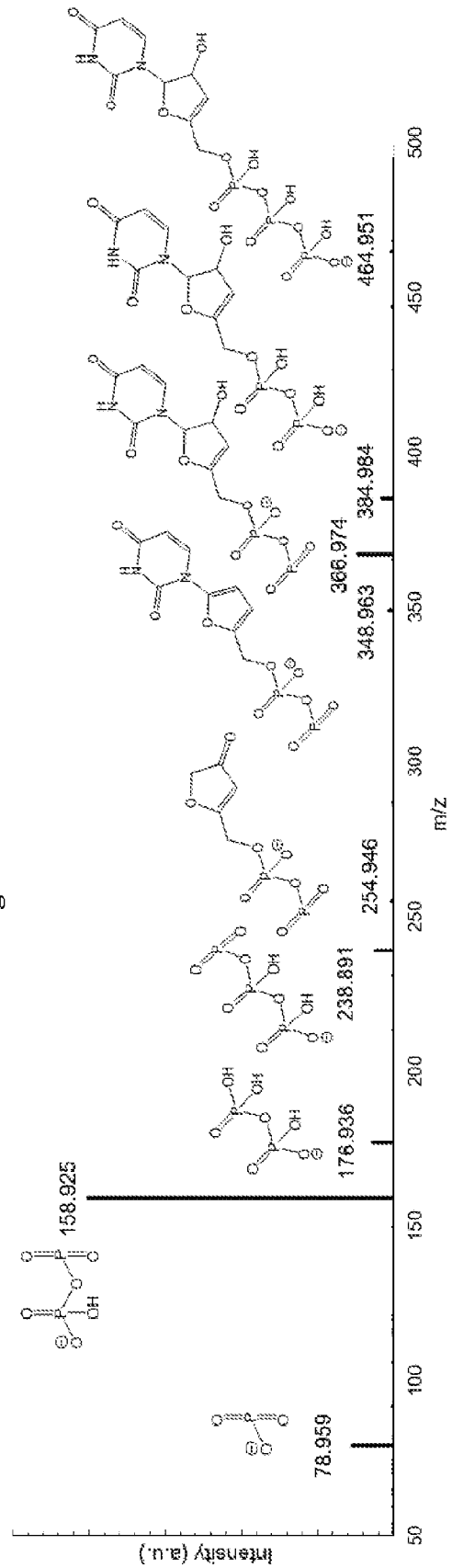


Figure 15B

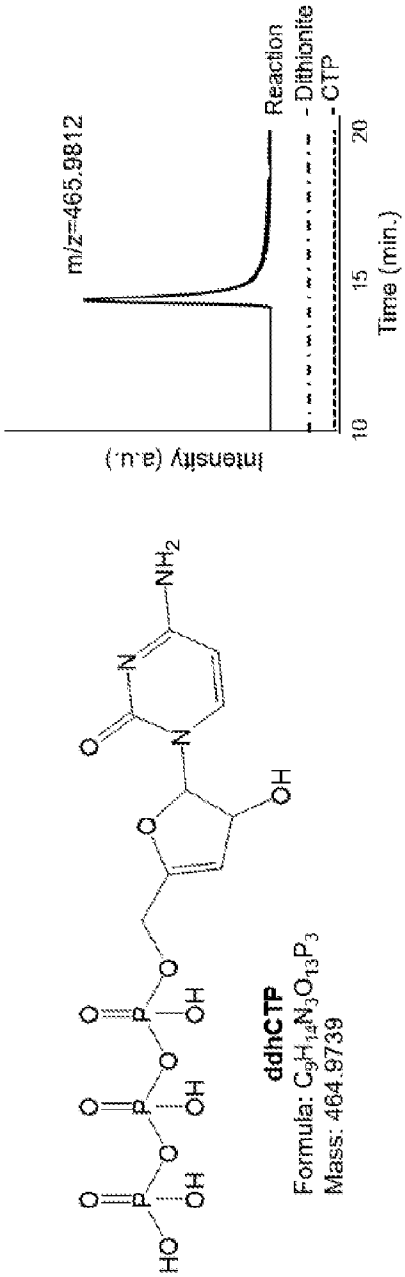


Figure 16A

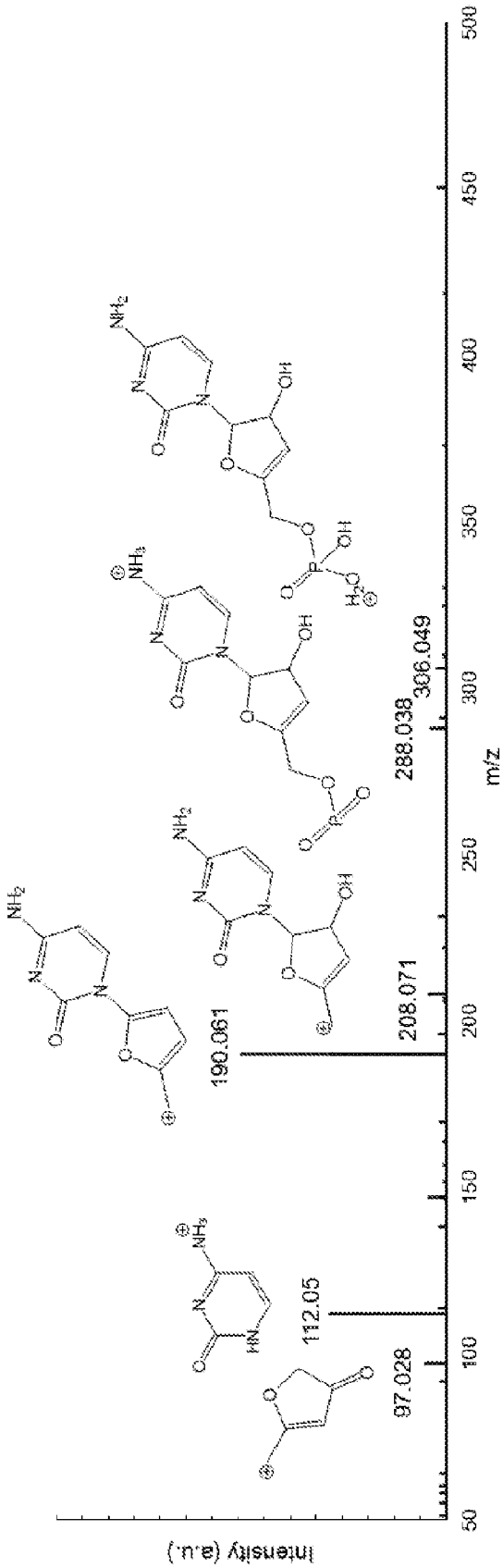


Figure 16B

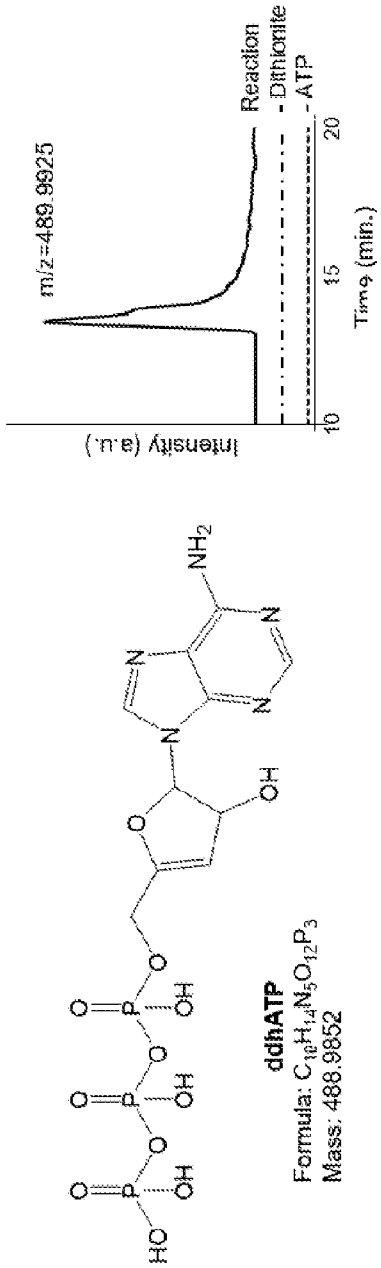


Figure 17A

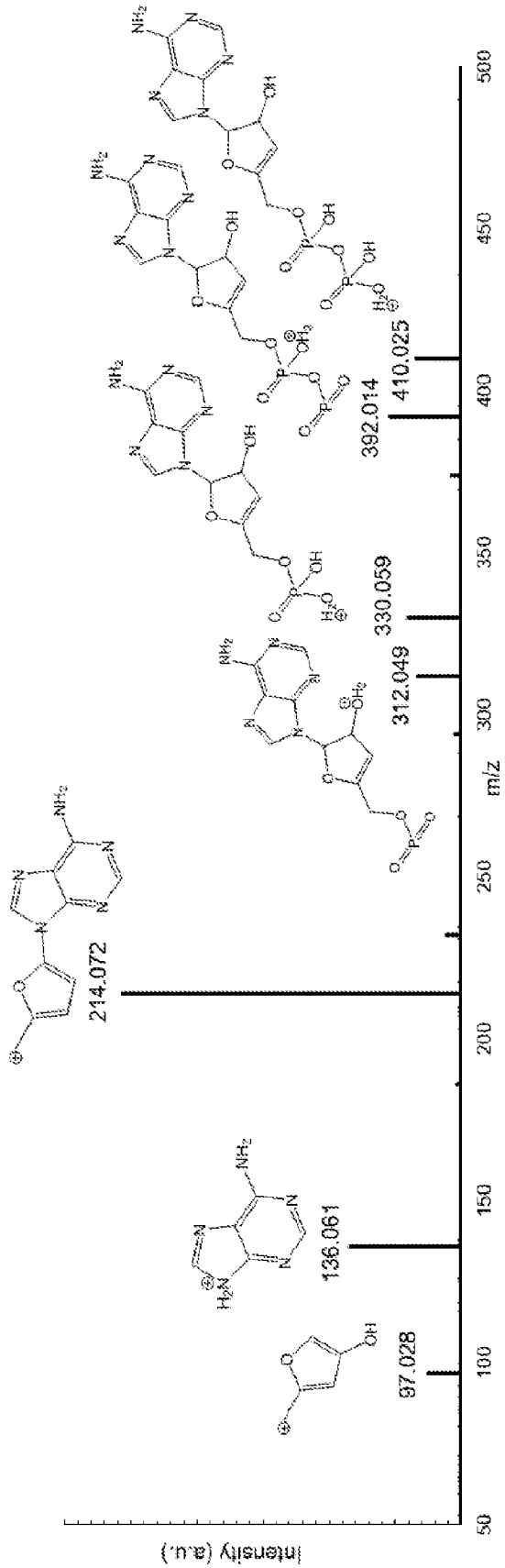


Figure 17B

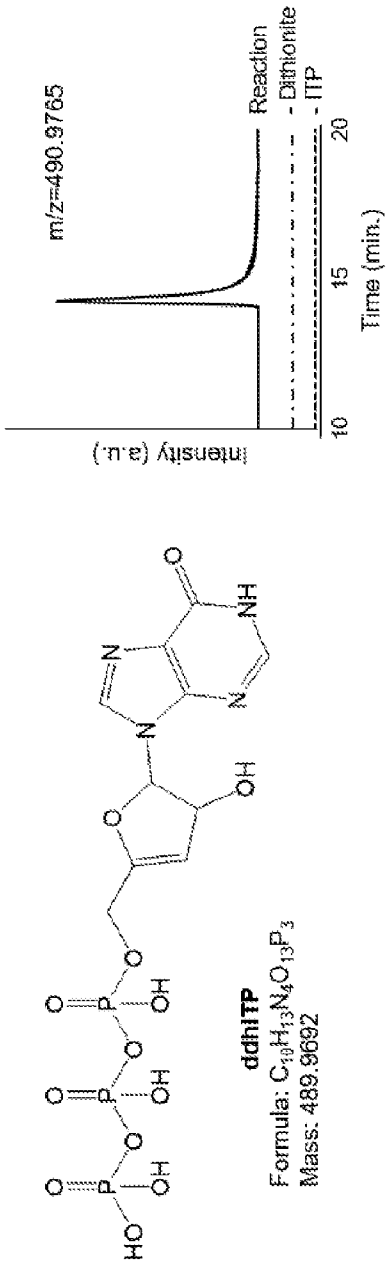


Figure 18A

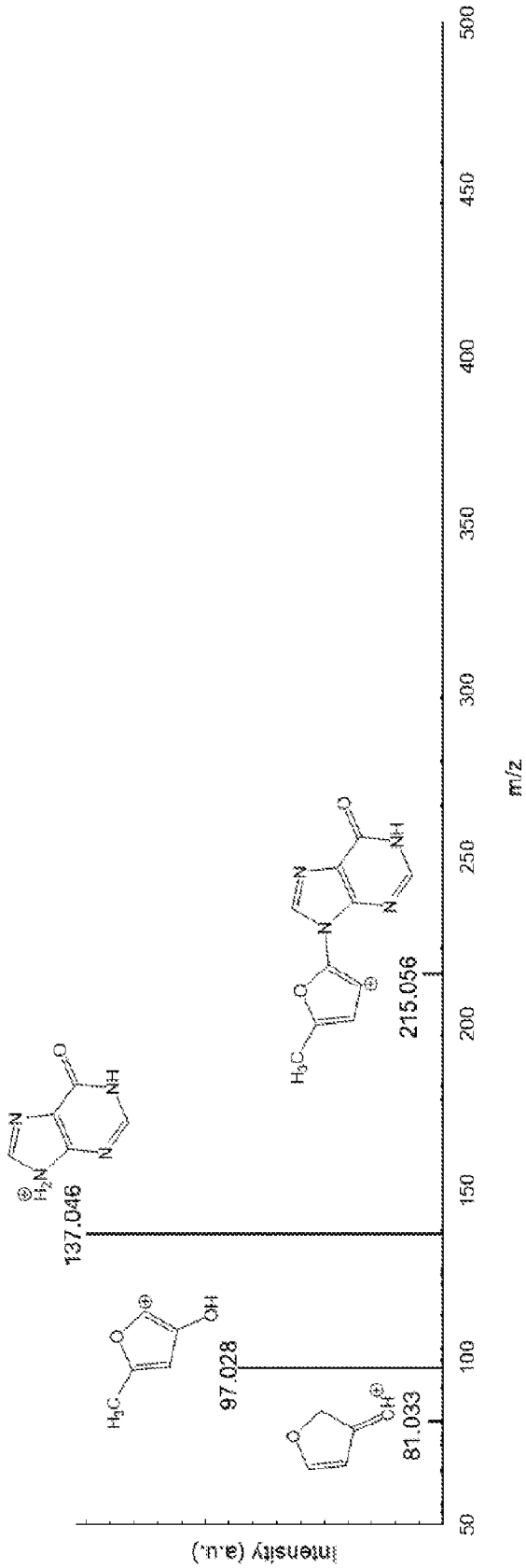


Figure 18B

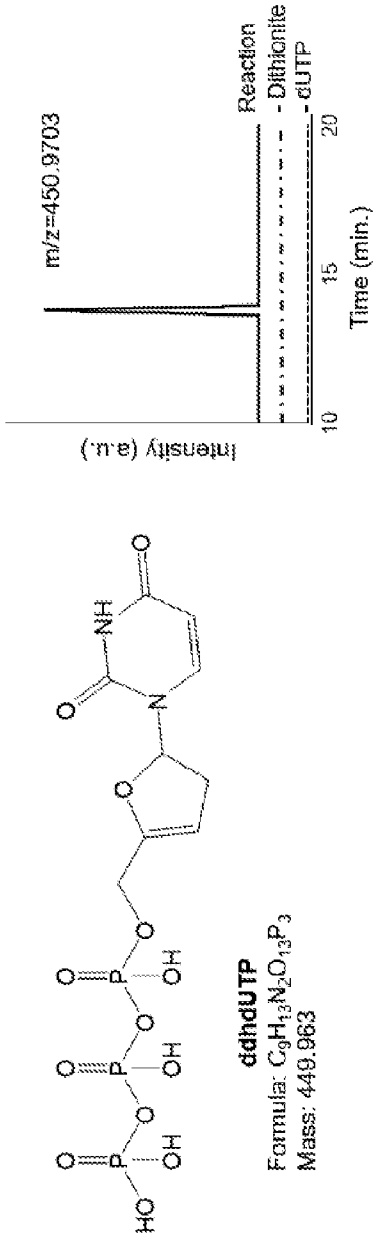


Figure 19A

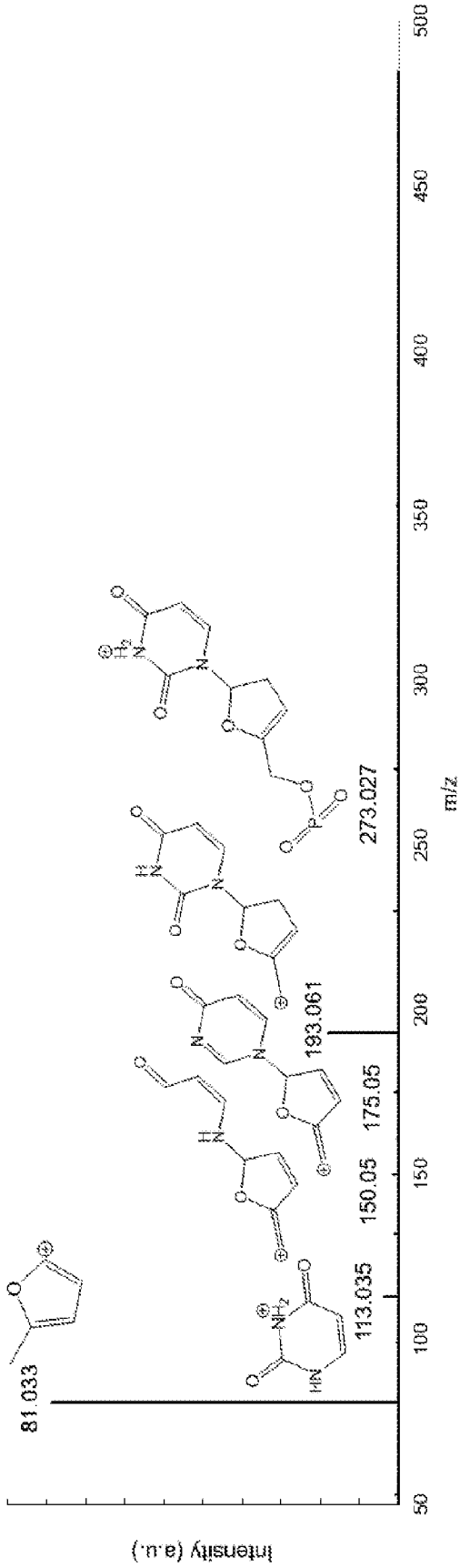


Figure 19B

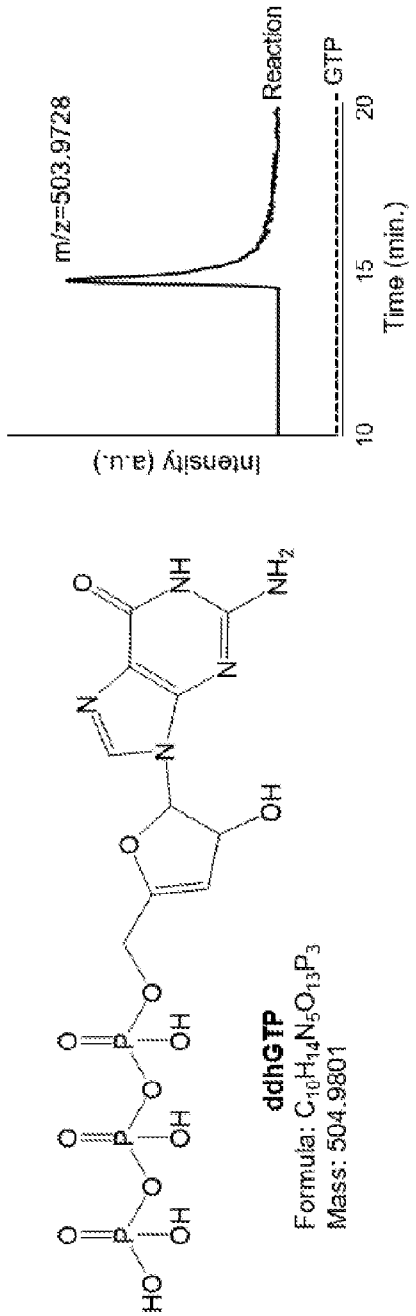


Figure 20A

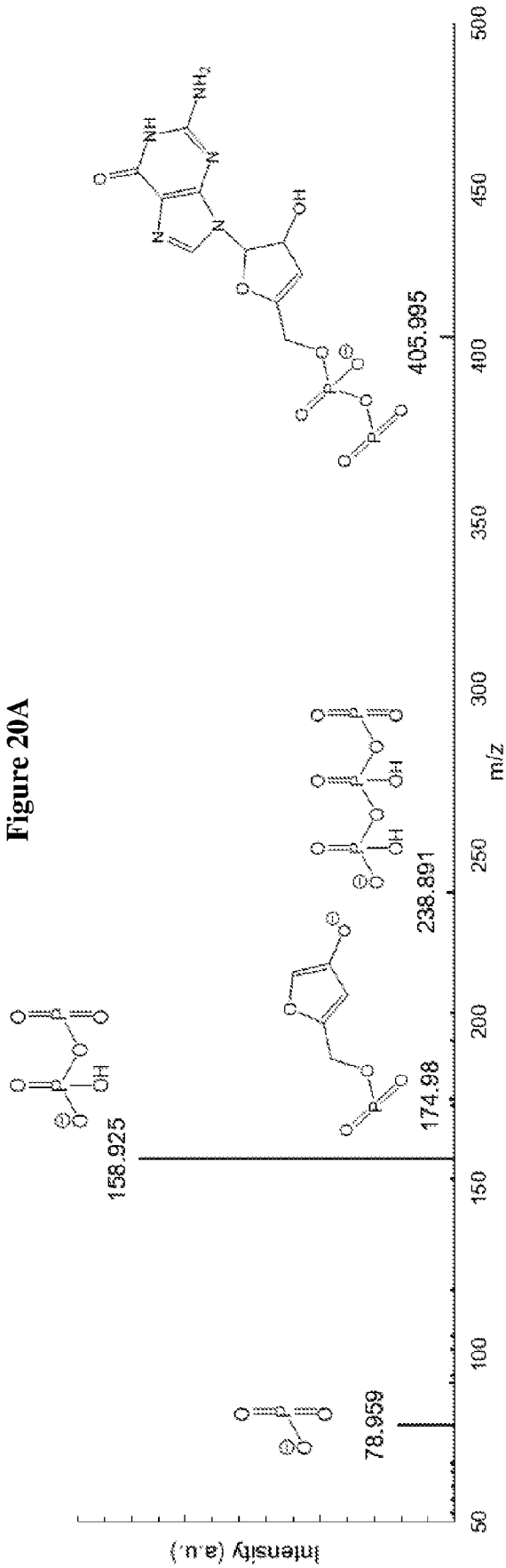


Figure 20B

CACUAAAAUUAUCGAAGAAUCCUCUUACUG
5′- AUUAUUGCUAGUGAUUUUAAUAGCUUCUUAGGAGAAUGAC – 3′

CACUAAAAUUAUCGAAGAAUCCUCUUACUG
5′- AUUAUUGCCAGUGAUUUUAAUAGCUUCUUAGGAGAAUGAC – 3′

CACUAAAAUUAUCGAAGAAUCCUCUUACUG
5′- AUUAUUGCAUGUGAUUUUAAUAGCUUCUUAGGAGAAUGAC – 3′

Figure 21

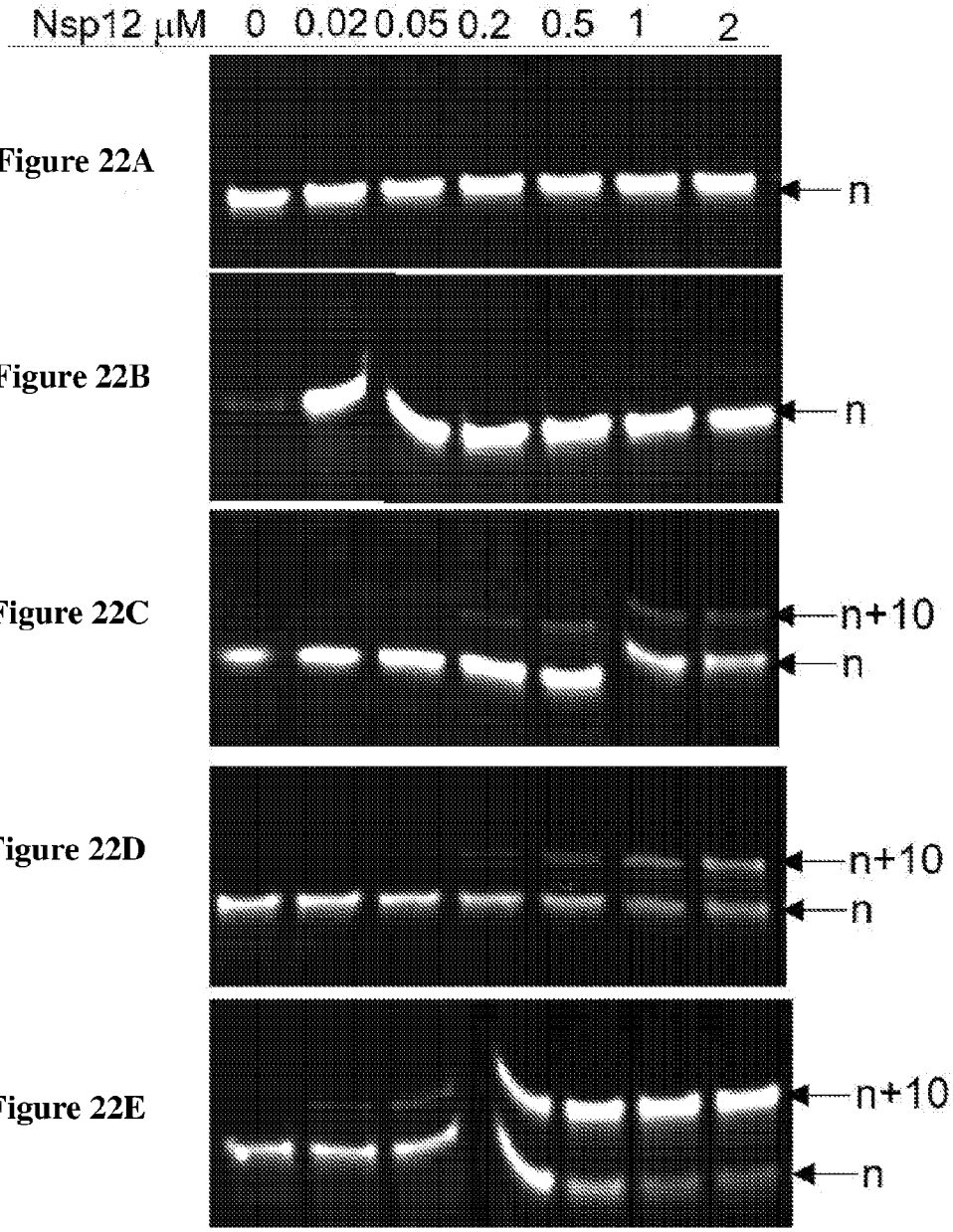


Figure 22A

Figure 22B

Figure 22C

Figure 22D

Figure 22E

Figure 23A

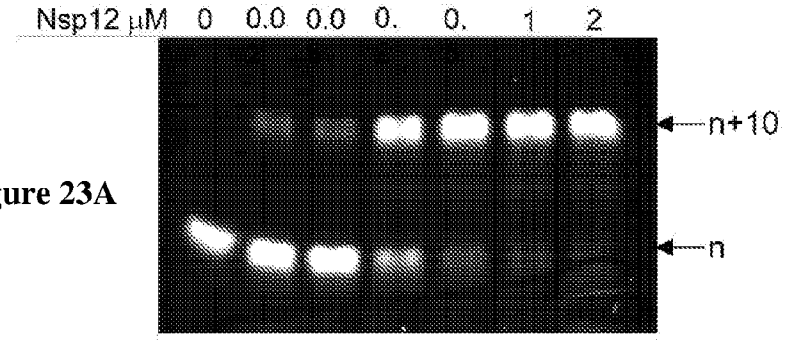
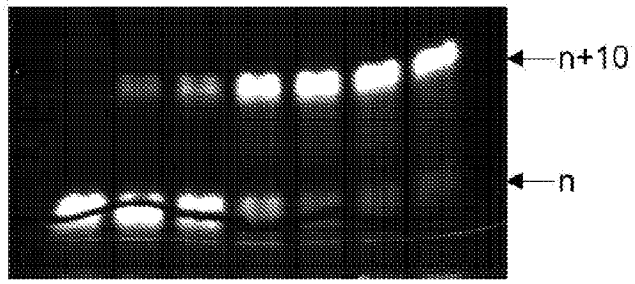


Figure 23BA



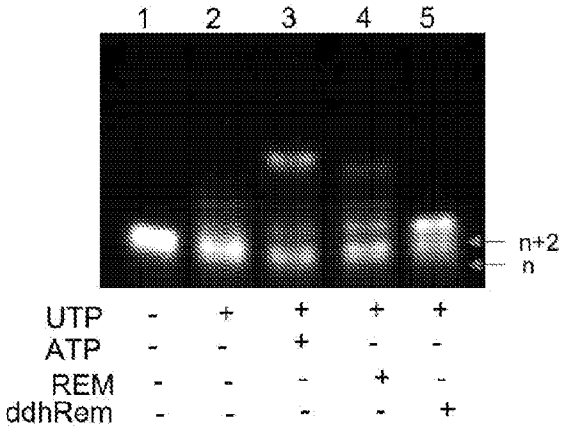


Figure 24

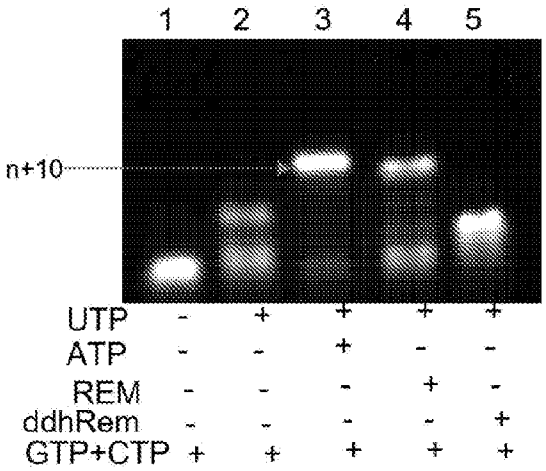


Figure 25

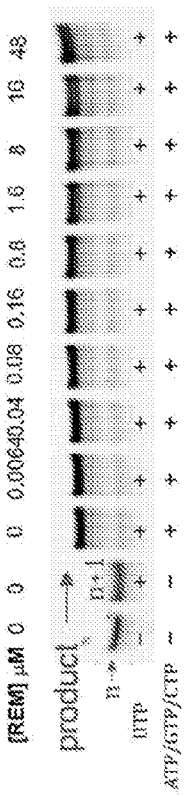


Figure 26A

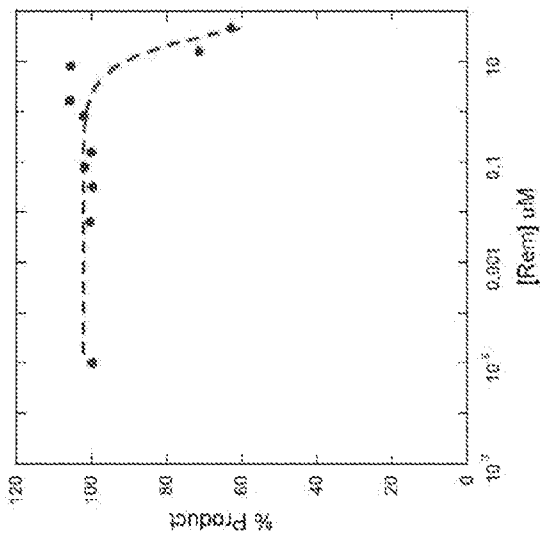


Figure 26B

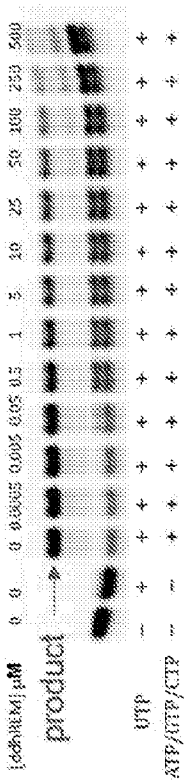


Figure 26C

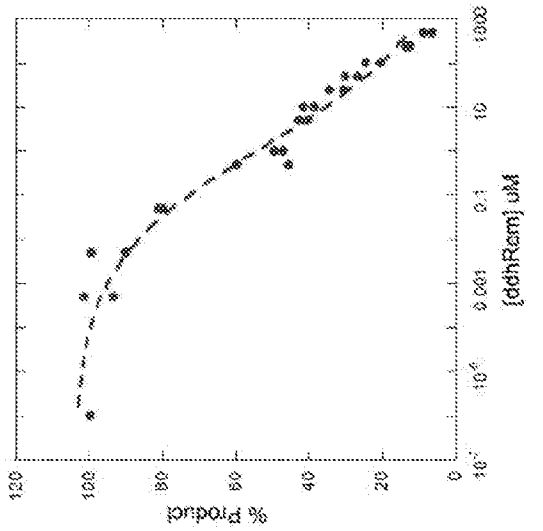


Figure 26D

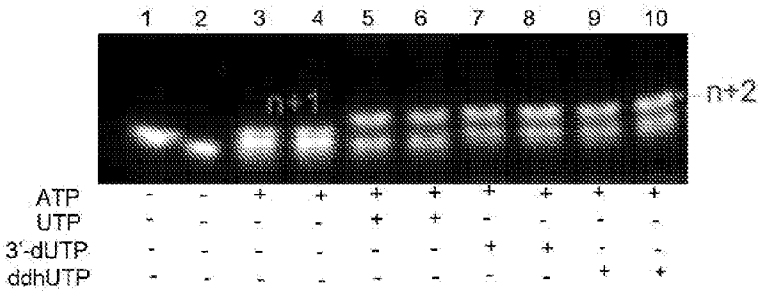


Figure 27A

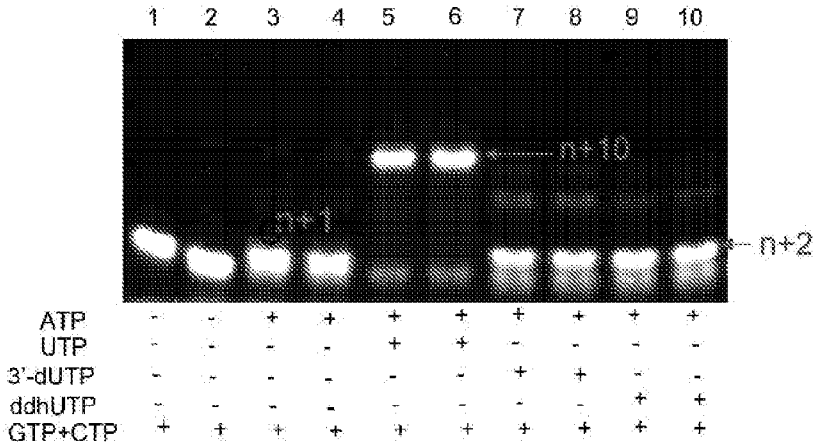


Figure 27B

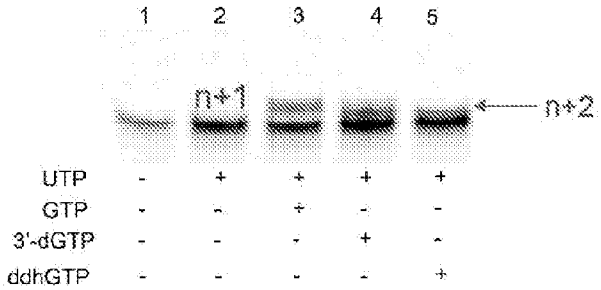


Figure 28A

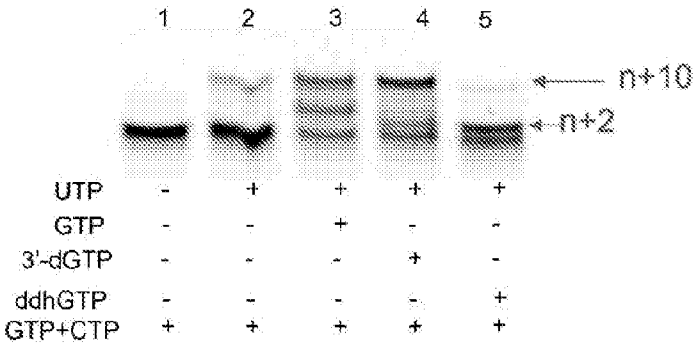


Figure 28B

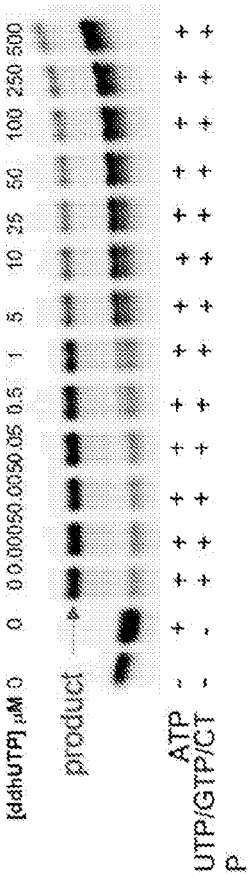


Figure 29A

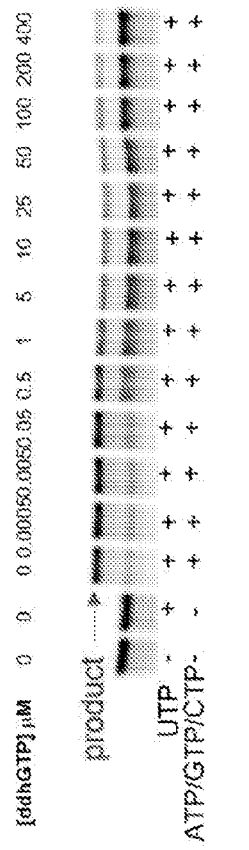


Figure 29C

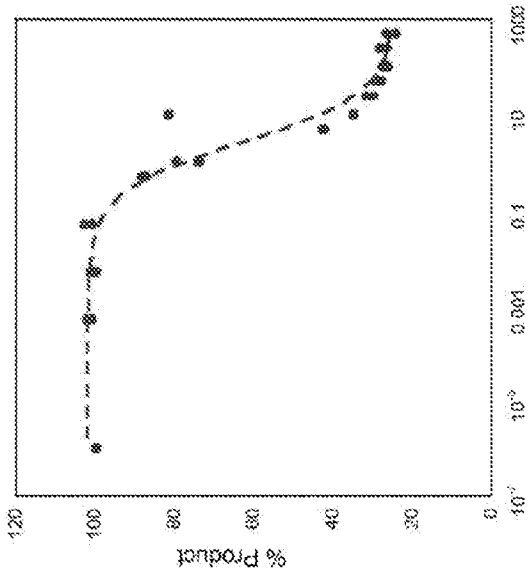


Figure 29B

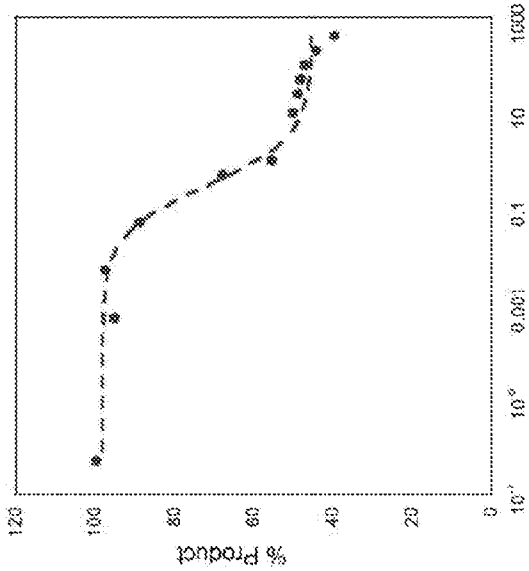


Figure 29D

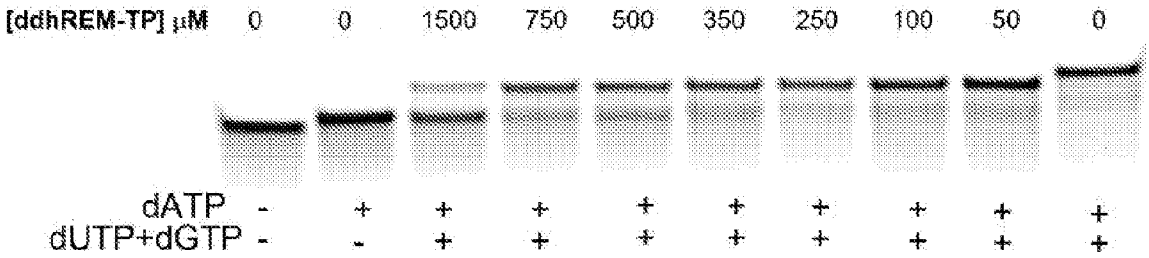


Figure 30A

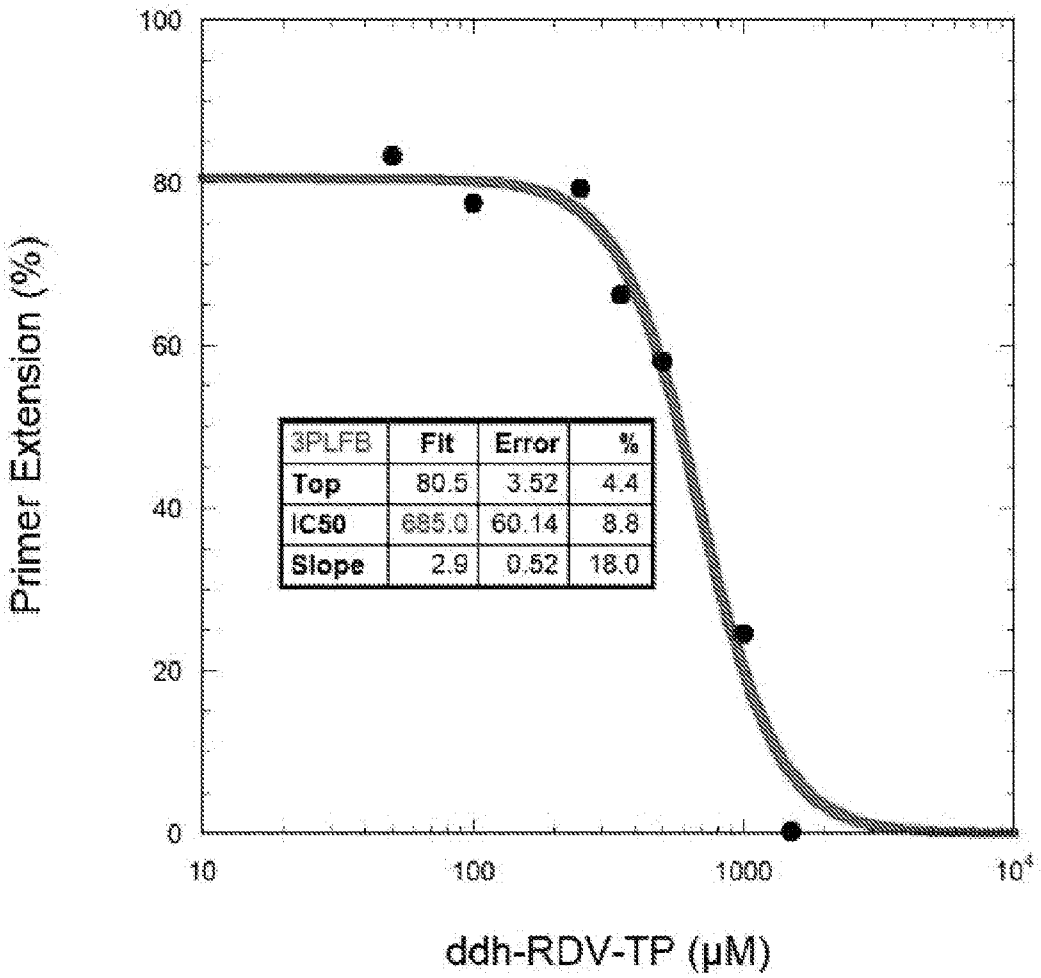


Figure 30B

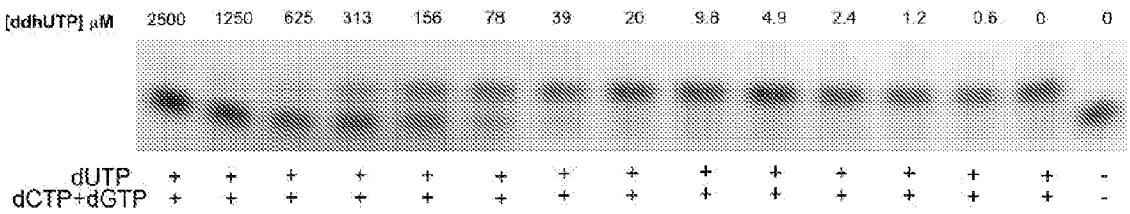


Figure 31A

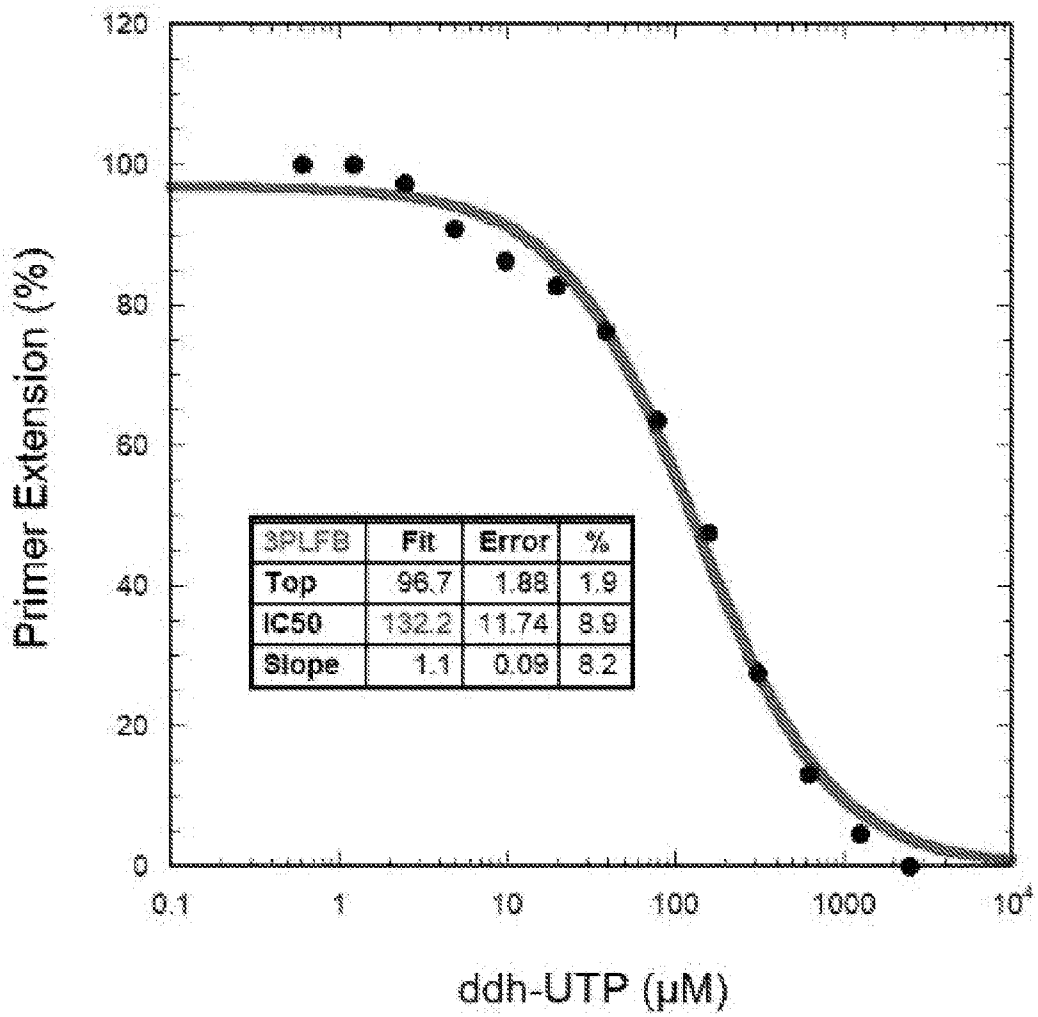


Figure 31B

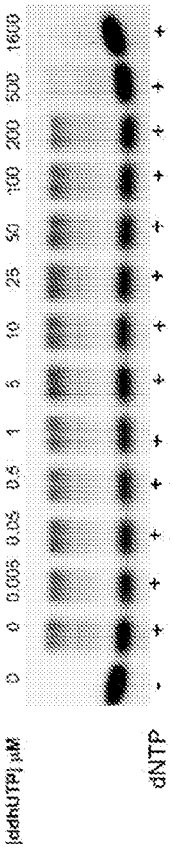


Figure 32C

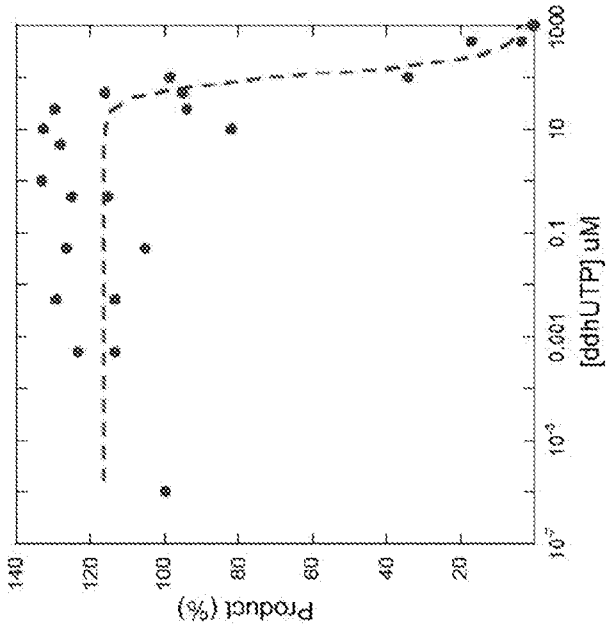


Figure 32D

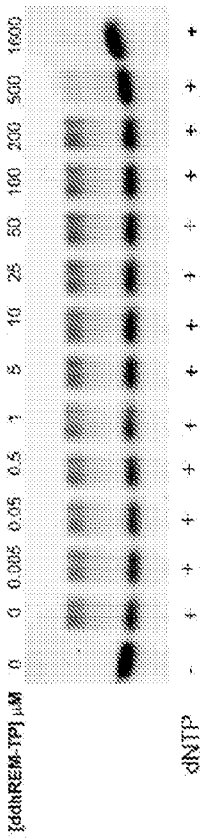


Figure 32A

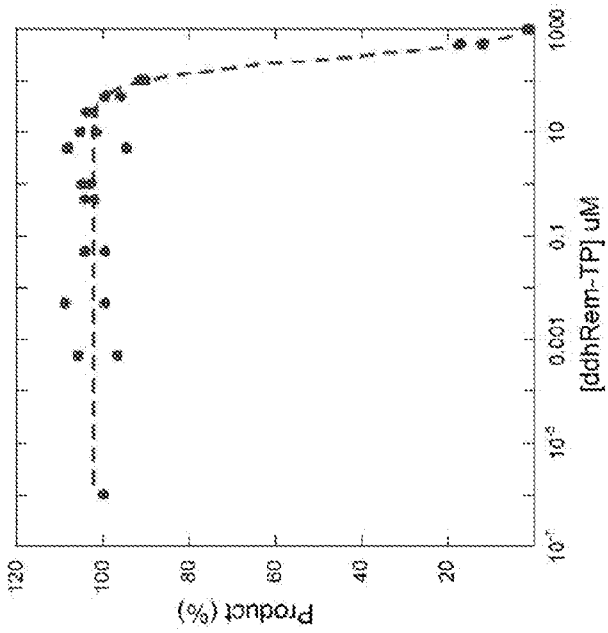


Figure 32B

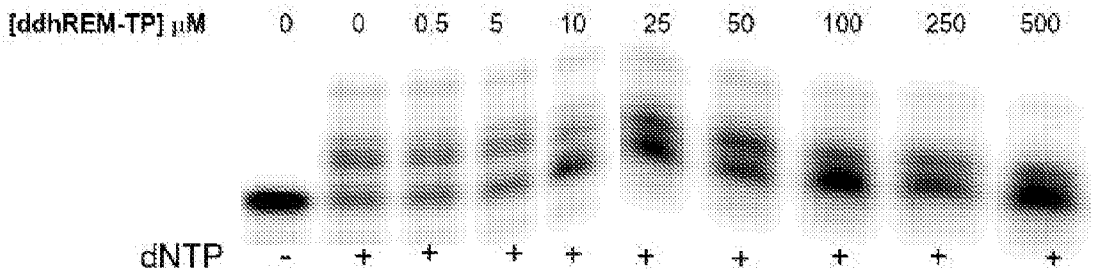


Figure 33A

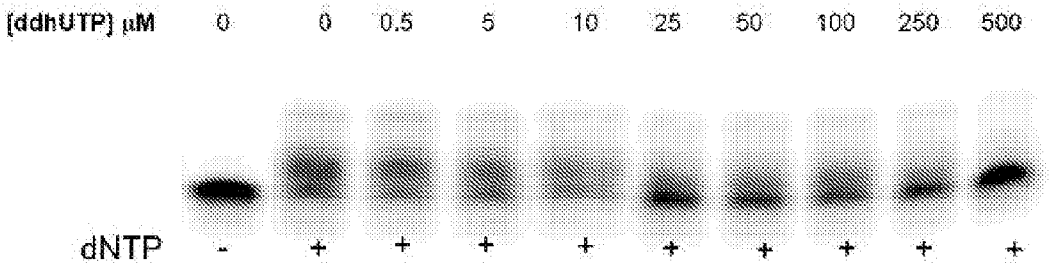


Figure 33B

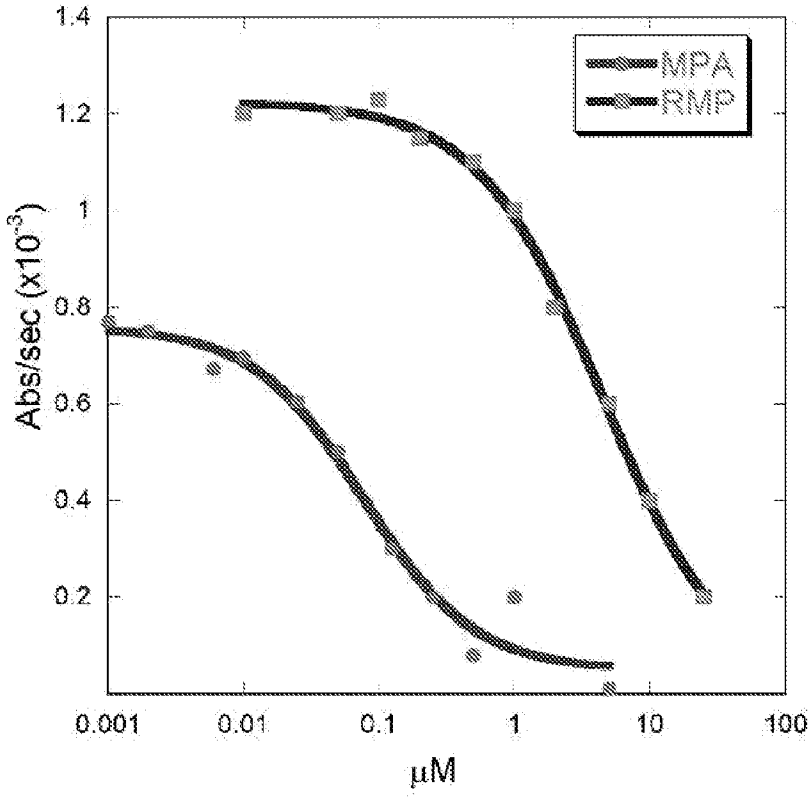


Figure 34A

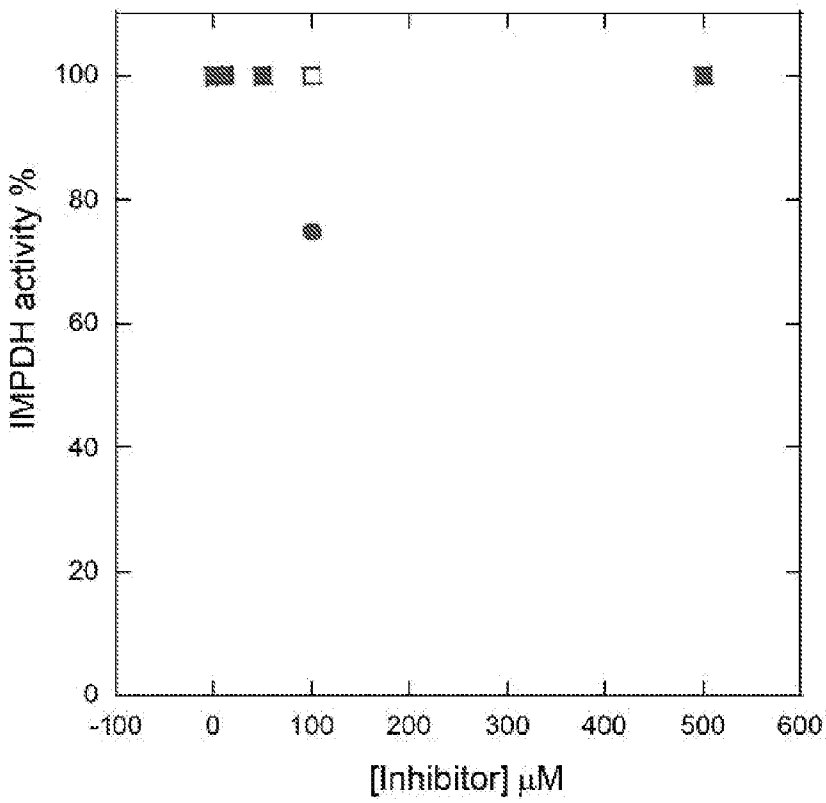


Figure 34B