



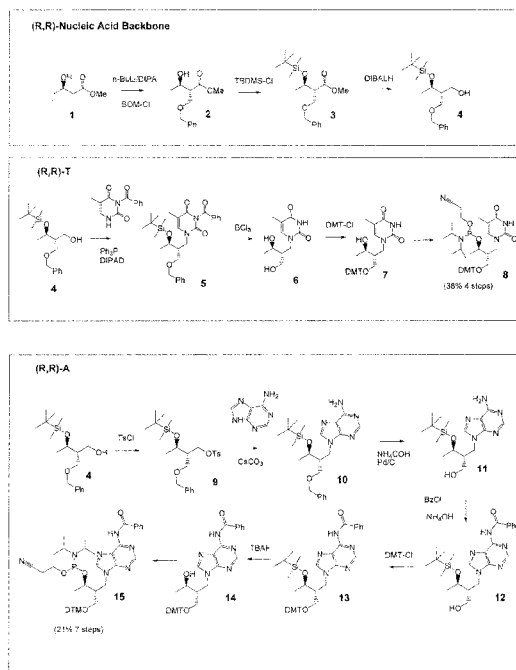
- (51) International Patent Classification: Not classified
- (21) International Application Number: PCT/US2014/025766
- (22) International Filing Date: 13 March 2014 (13.03.2014)
- (25) Filing Language: English
- (26) Publication Language: English
- (30) Priority Data: 61/778,961 13 March 2013 (13.03.2013) US
- (71) Applicant: ELITECH HOLDING B.V. [NL/NL]; Van Rensselaerweg 4, NL-6956 AV Spankeren (NL).
- (72) Inventors; and
- (71) Applicants (for US only): SCARR, Noah [US/US]; 12522 North Park Avenue N, Seattle, WA 98133 (US). LUKHTANOV, Eugeny, A. [US/US]; 21013 49th Ave, S.E., Bothell, WA 98021 (US).
- (74) Agents: BORRELLI, Sara K. et al.; Jackson Walker L.L.P., 901 Main Street, Suite 6000, Dallas, TX 75202 (US).
- (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, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, 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, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, 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, 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,

[Continued on next page]

(54) Title: ARTIFICIAL NUCLEIC ACIDS

(57) Abstract: Artificial nucleosides including 2-methyl-nucleobase-substituted butane- 1,3-diol nucleosides are disclosed. Four different stereoisomers of such nucleosides are possible. Oligonucleotides made up of the artificial nucleosides form homoduplexes of greater stability than DNA duplexes and have a reduced ability to hybridize to DNA or RNA.

Figure 2



TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

— *without international search report and to be republished upon receipt of that report (Rule 48.2(g))*

## ARTIFICIAL NUCLEIC ACIDS

### BACKGROUND

[0001] This application claims priority to U.S. Provisional Patent Application Serial No. 61/778,961, filed March 13, 2013, entitled "Artificial Nucleic Acids," the entire contents of which are hereby incorporated by reference.

[0002] This disclosure pertains to artificial nucleic acids and, more particularly, to artificial nucleic acid backbones which do not hybridize well to DNA or RNA, as well as their production and uses, including for diagnostic and chemotherapeutic purposes.

[0003] For about the last 20 years, a number of nucleic acid analogues have been synthesized to modify or improve nucleic acid hybridization characteristics. The properties of the nucleic acid analogues can be classified as those which hybridize to natural nucleic acids and those that hybridize only to themselves and not to natural nucleic acids. Peptide nucleic acids exhibit strong hybridization with DNA and RNA (Nielsen et al, *Science* 254: 1497-1500 (1991) and similarly locked nucleic acids show increased stability and discrimination properties when hybridized to nucleic acids (Koshkin et al, *Tetrahedron* 54: 3607-3630 (1998)). Other nucleic acid analogues with DNA and RNA binding properties include pyrrolidinyl peptide nucleic acid (Vilaivan et al, *Artificial DNA; PNA & XNA*, 2: 50-59 (2011)).

[0004] Pyranosyl nucleic acid (p-RNA), and 3- deoxyribofuranosyl nucleic acid (p-DNA) are polymers that preferentially pair with complementary pRNA or pDNA versus natural DNA and RNA sequences (Schlonvogt et al. *Helv. Chim. Acta* 79, 2316 (1996), Ashkerman et al. *Helv. Chim. Acta* 85, 1443-1462 (2002)). Pentopyranosyl nucleic acid preparation and use for the production of a therapeutic, diagnostic and/or electronic component has been described (US 6,506,896, US 7,153,955).

[0005] Acyclic phosphodiester nucleic acid backbones have been disclosed, for example, for GNA (Zang et al.), aTNA (Asanuma et al., *J. Am Chem Soc.*, 132:14702-14703 (2010) and UNA (Peterson et al., *Organic & Biomolecular Chemistry*, 9(10):3591-3597 (2011)). The (R)- and (S)-enantiomers of glycol nucleic acid (GNA) do not cross-pair with each other or with DNA; however, (S)-GNA cross-pairs with RNA (Johnson et al., *J.*

Org. Chem., 76:7964-74 (2011); Zhang et al., J. Amer. Chem. Soc., 127: 4174-4175 (2005)). Benzene-phosphate backbone (Ueno et al., Nucl. Acids Symposium Series., 51: 293-294 (2007)) is another preferably self-hybridizing backbone. A number of purine and pyrimidine acyclic nucleosides were disclosed and tested as antivirals (Guillarme et al., Tetrahedron, 59: 2177-2184(2003)).

[0006] Depurination is the cleavage of the glycosidic bond connecting the purine base to sugar during oligonucleotides synthesis and during the synthesis of the purine phosphoramidites. Limitation of depurination requires special protecting groups and reaction conditions (Froehler and Matteucci, Nucl. Acids Res., 11: 8031-8036 (1983), McBride et al., J. Amer. Chem. Soc., 108: 2040-2048 (1986)). For these reasons, it is therefore desirable to utilize artificial nucleic acids that lack the glycosidic bond.

[0007] The design of multiple nucleic acid sequences with the same  $T_m$  poses special challenges for use in applications such as microarrays and nano-fabrications. It is essential to prevent undesired hybridizations. It is also required that multiple nucleic acid sequences need to be designed that do not hybridize non-specifically with each other (Tanaka et al., Nucl. Acids Res., 33: 903-911(2005)). These so-called orthogonal nucleic acids can be designed as described in U.S. Application Publication No. 2012/0015358. The orthogonal nucleic acids with low affinity for DNA or/and not recognizable by DNA processing enzymes can be especially useful for labeling, barcoding or anchoring of multiple DNA-containing substrates co-existing in one mixture or on one array.

**SUMMARY**

[0008] The present disclosure relates to artificial nucleosides, or nucleoside analogues, and nucleotides and oligonucleotides formed from these artificial nucleoside monomer units, and to oligonucleotides formed from artificial nucleoside monomer units in which one or more of the units is functionalized, and to the use of these novel compositions for diagnostic and chemotherapeutic purposes.

[0009] The preferred artificial nucleosides can be characterized as 2-((nucleobase)methyl)butane-1,3-diols. Four different stereoisomers of the nucleoside analogues are possible. The nucleoside analogues can form oligomers that can include one or more phosphodiester bonds. Oligomers made up of the nucleoside analogues can also be utilized to form conjugates with oligomers having a natural nucleic acid backbone, different non-natural backbones, peptides, and the like.

**BRIEF DESCRIPTION OF THE DRAWINGS**

[0010] Figure 1 shows four possible stereoisomers of 2-((nucleobase)methyl)butane-1,3-diols;

[0011] Figure 2 shows a synthetic scheme for the preparation of (2R,3R)-2-((3,4-dihydro-5-methyl-2,4-dioxypyrimidin-1(2H)-yl)methyl)butane-1,3-diol and (2R,3R)-2-((6-amino-9H-purin-9-yl)methyl)butane-1,3-diol and their respective phosphoramidites;

[0012] Figure 3 shows a synthetic scheme for the preparation of (2R,3R)-2-((4-amino-2-oxypyrimidin-1(2H)-yl)methyl)butane-1,3-diol and (2R,3R)-2-((2-amino-1,6-dihydro-6-oxopurin-9-yl)methyl)butane-1,3-diol and their respective phosphoramidites;

[0013] Figure 4 shows a synthetic scheme for the preparation of (2S,3R)-2-((3,4-dihydro-5-methyl-2,4-dioxypyrimidin-1(2H)-yl)methyl)butane-1,3-diol and (2S,3R)-2-((2-amino-1,6-dihydro-6-oxopurin-9-yl)methyl)butane-1,3-diol and their respective phosphoramidites;

[0014] Figure 5 shows a synthetic scheme for the preparation of (2S,3R)-2-((4-amino-2-oxypyrimidin-1(2H)-yl)methyl)butane-1,3-diol and (2S,3R)-2-((7-adenyl)methyl)butane-1,3-diol and their respective phosphoramidites;

[0015] Figure 6 shows melting curves for matched homoduplexes (fully complementary duplexes with identical nucleic acid backbone) with variable A/T composition;

[0016] Figure 7 shows a summary of melting temperatures for homoduplexes with various nucleic acid backbones and A/T composition;

[0017] Figure 8 shows melting curves for matched heteroduplexes with (2R,3R)-, (2S,3R)- and (2S,3S) 2-methylbutane-1,3-diol (NNA-RR, NNA-SR and NNA-SS) and DNA backbones and variable A/T composition;

[0018] Figure 9 shows temperature dependence of absorption (268 nm) for various single stranded nucleic acids;

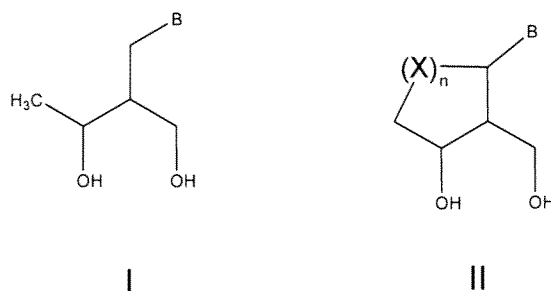
[0019] Figure 10 shows a synthetic scheme for the preparation of a cyclopentane-based thymidine analogue and its respective phosphoramidite;

[0020] Figure 11 shows a synthetic scheme for the preparation of a cyclopentane-based adenosine analogue and its respective phosphoramidite.

## DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

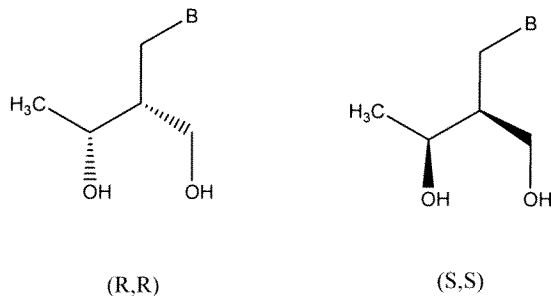
### I. General

[0021] The nucleoside analogues of the present disclosure can be generally represented by the following Formulas I and II:



wherein B is a nucleic acid base which may be natural or artificial, X is  $-\text{CH}_2-$  or substituted carbon, and n is 1 or 2.

[0022] Preferred nucleoside analogues can be represented by the formulas shown below:



wherein B is a nucleic acid base which may be natural or artificial.

[0023] The preferred R,R stereoisomer of the nucleosides, when incorporated into oligonucleotides, forms homoduplexes of greater stability than DNA oligonucleotides of the same sequences. Oligonucleotides made up of the preferred R,R stereoisomer nucleosides demonstrate a reduced ability to hybridize to DNA to form heteroduplexes. Oligonucleotides made up of the preferred S,S stereoisomer nucleosides, the mirror image of the R,R stereoisomers, demonstrate no ability to hybridize to DNA to form heteroduplexes because their orientation within the nucleic acid backbone is “left-handed” compared to a natural nucleic acid backbone. Furthermore, oligomers synthesized with the



S,S stereoisomers have the same self-hybridizing properties as the mirror image oligomers containing the R,R backbone.

## II. General Synthesis

[0024] 2-((Nucleobase)methyl)butane-1,3-diols were generally prepared by Mitsunobu condensation of properly protected stereochemically pure 2-(hydroxymethyl)butane-1,3-diols with nucleobases or base precursors. Four different stereoisomers of such nucleosides are possible (Figure 1).

[0025] The (R,R)-nucleosides were prepared starting from known (2R,3R)-2-((benzyloxy)methyl)-3-(tert-butyldimethylsiloxy)butan-1-ol (**4**, Figure 2) (W. H. Ham et al. J.Org. Chem. 2000, 65, 8372-8374). The alcohol was condensed with thymine in the presence of triphenylphosphine and diisopropylazodicarboxylate to give protected nucleoside **5**, which was then fully deprotected by a  $\text{BCl}_3$  treatment to yield thymidine **6**. DMT-protection of the primary hydroxyl followed with phosphitylation of the secondary hydroxyl group afforded thymidine phosphoramidite **8**. To make the respective adenine analogue (Figure 2), the alcohol **4** was, first, tosylated, and the resulting tosylate **9** was reacted with cesium salt of adenine generating protected adenosine **10**. Hydrogenation to remove the benzyl protection followed by benzoylation of the exocyclic gave intermediate **12**. DMT protection of the primary hydroxyl group and deprotection of the secondary hydroxyl with tetrabutylammonium fluoride yielded DMT-protected N-benzoyl adenosine **14**. Standard phosphitylation of the secondary hydroxyl afforded 2-cyanoethyl diisopropylphosphoramidite **15** completing the synthesis in 21% overall yield starting from **4**. To make a cytosine analogue with the same nucleic acid backbone structure, the alcohol **4** was, first, converted to protected uracil **16** (Figure 3). Debenzoylation to **17** followed by activation of the 4 position of uracil via the formation of the triazo intermediate **18** and reaction with ammonia gave protected cytidine **19**. The protecting groups of **19** were removed by a treatment with  $\text{BCl}_3$  to give fully deprotected cytidine **20**. The exocyclic aminogroup of **20** was selectively acetylated to yield N-acetylcytidine **21**, which was then protected by the DMT group and phosphitylated to afford cytidine phosphoramidite **23**. The guanosine phosphoramidite **29** was prepared from the alcohol **4** in 6 steps in 33% overall yield (Figure 3). The Mitsunobu condensation of **4** with 2-amino-6-chloropurine gave 2-amino-6-chloropurine nucleoside intermediate **24**. Treatment with

aqueous TFA replaced the chloro group with a hydroxyl group simultaneously removing the TBDMS protection. The resulting guanosine analogue **25** was protected at the exocyclic amine with a DMF group and hydrogenated to remove the benzyl protection freeing the primary hydroxyl. Standard DMT protection of **27** and phosphitylation of **28** completed the synthesis of guanosine phosphoramidite **29**.

[0026] The starting alcohol **4** was also used to produce the (S,R) versions of 2-((nucleobase)methyl)butane-1,3-diols. It was sequentially protected with a TBDMS group and debenzylated to afford alcohol **31**, starting material for the (S,R) nucleosides. The thymidine nucleoside **32** was obtained by Mitsunobu condensation of **31** with N-benzoylthymine. A treatment with TBAF removed both the TBDMS and benzoyl groups releasing the fully deprotected thymidine **33**. DMT protection of the primary and phosphitylation of the secondary hydroxyls afforded 2-cyanoethyl phosphoramidite **35**. The respective guanosine nucleoside phosphoramidite **40** (Figure 4) was prepared similarly to its (R,R) analogue except that it did not require the hydrogenation step for debenzylation. So was the case with the cytidine analogue **48**. The adenosine phosphoramidite **54** was synthesized in 7 steps. This time the initial condensation step was done using the Mitsunobu conditions, as oppose to alkylation using an activated tosylate in case of the (R,R) isomer (Figure 2), using alcohol **31** and 6-chloropurine. The resulting chloropurine nucleoside **49** was aminated with ammonia (compound **50**), benzoylated at the resulting exocyclic amine (compound **51**) and reacted with TBAF to deblock the hydroxyl groups and yield N-benzoyl adenosine **52**. Dimethoxytritylation (**53**) and the subsequent phosphitylation afforded the desired phosphoramidite **54**.

[0027] A combination of the methods utilized to synthesize the (R,R) and (R,S) nucleosides was also used to synthesize the respective (S,S) stereoisomers of A, T, C and G nucleosides of general structure I starting from the (2S,3S) stereoisomer of compound **4**. It has been shown that the (S,S) version of this nucleic acid backbone provides oligomers that form stable duplexes with complementary sequences of the same backbone (Figure 7) but do not hybridize to natural DNA (Figure 8). As expected, melting temperatures of the (S,S)-based and (R,R)-configured duplexes were essentially identical (Figure 7).

[0028] Cyclopentane-based nucleic acid monomers of the general structure II (X=CH<sub>2</sub>-, n=1) have also been synthesized. This structure can be viewed as a locked

(with a -CH<sub>2</sub>- bridging group) version of the nucleic acid backbone of structure I. The T and A nucleosides were prepared by Mitsunobu condensation of the suitably protected stereochemically pure (hydroxymethyl)cyclopentane-1,2-diol **55**, which had been prepared from penten-1-one as described in U.S. Patent Application Publication No. 2011/0251387, with a protected nucleobase (Figure 10) or a base precursor (Figure 11). The T monomer (compound **59**) was further furnished by removing the transient trityl and silyl protections from the primary and secondary hydroxyls and installing respective DMT and phosphoramidite groups. Analogously, the A monomer after having been aminated and protected was converted to the DMT phosphoramidite of structure **65**. The G and C monomers can be prepared by combination of the methods shown in Figures 3, 10 and 11.

[0029] The methods described in U.S. Patent Application Publication 2011/0251387, incorporated by reference, in combination with the known Mitsunobu-based stereo inversion of secondary hydroxyl group and the methods described herein provide synthetic routes to all 8 possible stereo configurations of nucleosides of Formula II (X= -CH<sub>2</sub>-, n=1) above.

[0030] Cyclohexane-based nucleic acid monomers of the general structure II wherein X=-CH<sub>2</sub>- and n=2 can be synthesized analogously using cyclohexen-1-one in place of cyclopenten-1-one as the starting material.

## II. Definitions

[0031] Artificial bases include modified bases. Modified bases include naturally-occurring and synthetic modifications and analogues of the major bases such as, for example, hypoxanthine, 2-aminoadenine, 2-thiouracil, 2-thiothymine, inosine, 5-N<sup>4</sup>-ethenocytosine, 4-aminopyrazolo[3,4-d]pyrimidine and 6-amino-4-hydroxy-[3,4-d]pyrimidine. Any modified nucleotide or nucleotide analogue compatible with hybridization of probe with a target nucleic acid conjugate to a target sequence is useful, even if the modified nucleotide or nucleotide analogue itself does not participate in base-pairing, or has altered base-pairing properties compared to naturally-occurring nucleotides. Examples of modified bases are disclosed in U.S. Pat. Nos. 7,045,610; 5,824,796; 6,127,121; 5,912,340; and PCT Publications WO 01/38584; WO 01/64958, each of which is hereby incorporated herein by reference in its entirety. Preferred modified bases include

5-hydroxybutynyl uridine for uridine; 4-(4,6-Diamino-<sup>1</sup>H-pyrazolo[3,4-d]pyrimidin-3-yl)-but-3-yn-1-ol, 4-amino-<sup>1</sup>H-pyrazolo[3,4-d]pyrimidine, and 4-amino-<sup>1</sup>H-pyrazolo[3,4-d]pyrimidine for adenine; 5-(4-Hydroxy-but-1-ynyl)-1H-pyrimidine-2,4-dione for thymine; and 6-amino-<sup>1</sup>H-pyrazolo[3,4-d]pyrimidin-4(5H)-one for guanine. Particularly preferred modified bases are "Super A®: 4-(4,6-Diamino-1H-pyrazolo[3,4-d]pyrimidin-3-yl)-but-3-yn-1-ol," "Super G®: 4-hydroxy-6-amino pyrazolopyrimidine" ([www.elitechgroup.com](http://www.elitechgroup.com)) and "Super T®: 5-(4-hydroxy-but-1-ynyl)-1H-pyrimidine-2,4-dione". 3-Alkynyl pyrazolopyrimidine analogues as universal bases are disclosed in U.S. Patent Application Publication No. 2012/0244535, incorporated by reference.

[0032] The terms "oligonucleotide," "nucleic acid," and "polynucleotide" are used interchangeably herein. These terms refer to a compound comprising nucleic acid, nucleotide, or its polymer in either single- or double-stranded form, e.g., DNA, RNA, analogues of natural nucleotides, and hybrids thereof. The terms encompass polymers containing modified or non-naturally-occurring nucleotides, or to any other type of polymer capable of stable base-pairing to DNA or RNA including, but not limited to, peptide nucleic acids as described in Nielsen et al., *Science*, 254:1497-1500 (1991), bicyclo DNA oligomers as described in Bolli et al., *Nucleic Acids Res.*, 24:4660-4667 (1996), and related structures. Unless otherwise limited, the terms encompass known analogues of natural nucleotides that hybridize to nucleic acids in a manner similar to naturally-occurring nucleotides. Examples of such analogues include, without limitation, phosphorothioates, phosphoramidates, methyl phosphonates, chiral-methyl phosphonates, 2-O-methyl ribonucleotides, and peptide-nucleic acids (PNAs). A "subsequence" or "segment" refers to a sequence of nucleotides that comprise a part of a longer sequence of nucleotides.

[0033] The practice of the methods described herein will employ, unless otherwise indicated, conventional techniques in organic chemistry, biochemistry, oligonucleotide synthesis and modification, bioconjugate chemistry, nucleic acid hybridization, molecular biology, microbiology, genetics, recombinant DNA, and related fields as are within the skill of the art. These techniques are fully explained in the literature. See, for example, Sambrook, Fritsch & Maniatis, *MOLECULAR CLONING: A LABORATORY MANUAL*, Second Edition, Cold Spring Harbor Laboratory Press

(1989); Ausubel, et al., CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons (1987, 1988, 1989, 1990, 1991, 1992, 1993, 1994, 1995, 1996); Gait (ed.), OLIGONUCLEOTIDE SYNTHESIS: A PRACTICAL APPROACH, IRL Press (1984); and Eckstein (ed.), OLIGONUCLEOTIDES AND ANALOGUES: A PRACTICAL APPROACH, IRL Press (1991).

### EXAMPLES

[0034] The following examples are provided to illustrate, but not to limit, the subject matter described herein.

#### EXAMPLE 1

[0035] (2R,3R)-1-Benzyloxy-3-(*tert*-butyldimethylsiloxy)-2-((3-benzoyl-3,4-dihydro-5-methyl-2,4-dioxypyrimidin-1(2H)-yl)methyl)butane (**5**):

[0036] To a cold (ice/water bath) solution of (2R,3R)-2-((benzyloxy)methyl)-3-(*tert*-butyldimethylsiloxy)butan-1-ol (**4**) (W.-H. Ham et al. J.Org. Chem. **2000**, 65, 8372-8374) 1.5 g, 4.6 mmol), 3-benzoylthymine (1.5 g, 6.5 mmol) and triphenylphosphine (1.71 g, 6.5 mmol) in 50 ml of anhydrous THF was added dropwise via syringe with stirring (1.26 ml, 6.5 mmol) of diisopropylazodicarboxylate. The reaction was removed from the bath and stirred at room temperature for 2 hrs and then concentrated. The reaction was diluted with ether (50 ml) and hexane (50 ml) to precipitate some of triphenylphosphine oxide and the DIPADC by-product. The solids were filtered off and the filtrate concentrated. The resultant crude product, which still contained some Ph<sub>3</sub>PO, was chromatographed on silica eluting with 20% ethyl acetate in hexane. Concentration of the pure product fractions afforded 2.0 g (81%) of **5** as a colorless viscous syrup. <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 7.85 (d, J=8.4 Hz, 2H), 7.58 (t, J=7.5 Hz, 1H), 7.42 (t, J=7.8 Hz, 2H), 7.35-7.25 (m, 5H), 7.06 (d, J=1.2 Hz, 1H), 4.42 (s, 2H), 3.98 (m, 2H), 3.61 (m, 1H), 3.46 (d, J=5.7 Hz, 2H), 2.08 (m, 1H), 1.83 (s, 3H), 1.16 (d, J=6.6 Hz, 3H), 0.84 (s, 9H), 0.02 (s, 3H), 0.00 (s, 3H).

**EXAMPLE 2**

**[0037]** (2R,3R)-2-((3,4-Dihydro-5-methyl-2,4-dioxypyrimidin-1(2H)-yl)methyl)butane-1,3-diol (**6**):

**[0038]** To a cold (-77°C, acetone/dry ice bath) solution of **5** (2.0 g, 3.72 mmol) in 100 ml of anhydrous dichloromethane was added (via syringe) 38 ml of 1M BCl<sub>3</sub> in dichloromethane over 5 min. The reaction was stirred for 6 hrs until no more changes were observed by HPLC analysis and then quenched by adding a mixture of pyridine (26 ml) and methanol (54 ml) over 5 min. The reaction mixture was allowed to warm up to room temperature and concentrated. The resultant material was re-dissolved in methanol (100 ml) and co-evaporated twice with triethylamine (10 ml) to remove pyridine. The residue was suspended in dichloromethane and filtered to remove some triethylammonium chloride. The solid were washed with dichloromethane and the combined filtrates were concentrated and then chromatographed on silica eluting with 10% methanol in dichloromethane. Concentration of the pure product fractions afforded 0.6 g (70%) of nucleoside **6**. <sup>1</sup>H NMR (DMSO-d<sub>6</sub>): δ 11.19 (s, 1H), 7.46 (s, 1H), 4.56 (d, J=4.8 Hz, 1H), 4.50 (t, J=5.1 Hz, 1H), 3.83 (m, 1H), 3.73 (m, 1H), 3.57 (m, 1H), 3.38 (m, 2H), 1.75 (s, 3H), 1.73 (m, 1H), 1.10 (d, J=6.3 Hz, 3H).

**EXAMPLE 3**

**[0039]** (2R,3R)-1-Bis(4-methoxyphenyl)(phenyl)methoxy-2-((3,4-dihydro-5-methyl-2,4-dioxypyrimidin-1(2H)-yl)methyl)butan-3-ol (**7**):

**[0040]** To a stirred solution of **6** (0.6 g, 2.6 mmol) in 20 ml of anhydrous pyridine was added 1.05 g (3.1 mmol) of DMTCl. After being stirred at room temperature for 5 hrs the reaction was quenched with methanol (3 ml), concentrated and portioned between 10% citric acid and ethyl acetate. The organic phase was washed with saturated NaCl, dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated *in vacuo* to a solid foam. The crude product was chromatographed on silica eluting with ethyl acetate to afford 1.15 g (83%) of **7** as an amorphous white solid. <sup>1</sup>H NMR (DMSO-d<sub>6</sub>): δ 11.13 (s, 1H), 7.3 (m, 5H), 7.13 (m, 5H), 6.84 (m, 4H), 4.61 (d, 4.8 Hz, 1H), 3.75 (m, 2H), 3.73 (s, 6H), 3.65 (m, 1H), 3.10 (m, 1H), 2.88 (m, 1H), 2.00 (m, 1H), 1.66 (s, 3H), 1.01 (d, J=6.3 Hz, 3H).

**EXAMPLE 4**

[0041] (2R,3R)-1-Bis(4-methoxyphenyl)(phenyl)methoxy-2-((3,4-dihydro-5-methyl-2,4-dioxypyrimidin-1(2H)-yl)methyl)butan-3-yl 2-cyanoethyl N,N-diisopropylphosphoramidite (**8**):

[0042] To a stirred solution of **7** (1.1 g, 2.07 mmol) in 30 ml of anhydrous dichloromethane was added diisopropylammonium tetrazolide (0.37 g) followed by 0.8 g (2.66 mmol) of 2-cyanoethyl N,N,N',N'-tetraisopropylphosphordiamidite. After being stirred at room temperature overnight the reaction was concentrated, and the residue portioned between ethyl acetate and saturated NaHCO<sub>3</sub>. The organic phase was washed with saturated NaCl, dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated in vacuo. The crude phosphoramidite was precipitated twice by re-dissolving in small (~15 ml) amount of ethyl acetate, diluting with hexane (100 ml), allowing to settle as an oil and decanting the liquid. Drying in vacuo afforded 1.3 g (86%) of **8** as an amorphous white solid. <sup>31</sup>P NMR (DMSO-d<sub>6</sub>): δ 146.94, 145.89.

**EXAMPLE 5**

[0043] (2R,3R)-2-((Benzyloxy)methyl)-3-(*tert*-butyldimethylsiloxy)butan-1-yl 4-methylbenzenesulfonate (**9**):

[0044] *p*-Toluenesulfonyl chloride (1.4 g, 7.4 mmol) was added in one portion to a cold (ice/ water bath) stirred solution of **4** (2.0 g, 6.16 mmol) in anhydrous pyridine (25 ml). The reaction was allowed to warm up to room temperature and stirred for 6 hrs until no starting material was found by HPLC analysis. Ethyl acetate (100 ml) and 10% citric acid (enough to neutralize pyridine) were added. The organic phase was washed with saturated NaCl, saturated NaHCO<sub>3</sub> and dried over MgSO<sub>4</sub>. The crude tosylate **9** (2.9 g) was used in the next step without additional purification. <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 7.80 (d, J=8.1 Hz, 2H), 7.40-7.25 (m, 7H), 4.41 (d, J=3.6 Hz, 2H), 4.25 (m, 1H), 4.11 (m, 1H), 3.98 (m, 1H), 3.45 (m, 2H), 2.45 (s, 3H), 1.96 (m, 1H), 1.12 (d, J=6.3 Hz, 3H), 0.83 (s, 9H), 0.036 (s, 3H), 0.00 (s, 3H).

**EXAMPLE 6**

[0045] (2R,3R)-1-Benzoyloxy-3-(*tert*-butyldimethylsiloxy)-2-((6-amino-9H-purin-9-yl)methyl)butane (**10**):

[0046] To a suspension of adenine (1.22 g, 9.1 mmol) in 25 ml of anhydrous DMF was added 2.96 g of Ce<sub>2</sub>CO<sub>3</sub> (hydrate) and the mixture was stirred at 100°C for 15 min. A solution of 9 (2.9 g, ~7.4 mmol) in 20 ml of DMF was added in one portion. The reaction was stirred at 100°C for 4 hrs until no starting material was found by HPLC, then cooled and filtered to remove unreacted adenine and cesium salts. The filtrate was concentrated, re-dissolved in ethyl acetate, washed with water, saturated NaCl and dried under Na<sub>2</sub>SO<sub>4</sub>. The crude product obtained after solvent evaporation was chromatographed on silica eluting with a gradient of acetone (20-35%) in ethyl acetate. Concentration of the pure product fractions and drying in vacuo afforded 1.2 g (37%) of **10** as a pale yellow viscous syrup. <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 8.32 (s, 1H), 7.70 (s, 1H), 7.24 (m, 5H), 5.64 (s, 2H), 4.38 (m, 1H), 4.34 (d, 8.1 Hz, 2H), 4.16 (m, 1H), 4.02 (m, 1H), 3.32 (m, 2H), 2.23 (m, 1H), 1.14 (d, J=6.3 Hz, 3H), 0.85 (s, 9H), 0.015 (s, 3H), 0.00 (s, 3H).

**EXAMPLE 7**

[0047] (2R,3R)-3-(*tert*-Butyl)(dimethyl)siloxy-2-((6-amino-9H-purin-9-yl)methyl)butan-1-ol (**11**)

[0048] To a solution of **10** (1.2 g, 2.7 mmol) in 80 ml of MeOH were added 10% Pd/C (0.5 g) and ammonium formate (3 g). The mixture was refluxed for 2 days until no starting material was found by HPLC analysis. The reaction was filtered through Celite and concentrated to give **11** as a white amorphous solid (0.9 g, 95%), which was sufficiently pure to be used in the next step without additional purification. <sup>1</sup>H NMR (DMSO-d<sub>6</sub>): δ 8.12 (s, 1H), 8.07 (s, 1H), 7.17 (s, 2H), 4.61 (br s, 1H), 4.24 (m, 1H), 4.08 (m, 1H), 3.98 (m, 1H), 3.32 (m, 2H), 2.03 (m, 1H), 1.15 (d, J=6.6 Hz, 3H), 0.85 (s, 9H), 0.00 (s, 6H).



**EXAMPLE 8**

[0049] (2R,3R)-3-(*tert*-Butyldimethylsiloxy)-2-((6-benzamido-9H-purin-9-yl)methyl)butan-1-ol (**12**):

[0050] Compound **11** (0.9 g, 2.56 mmol) was dried by co-evaporation with anhydrous pyridine (20 ml), then re-dissolved in a fresh portion of pyridine (20 ml) and cooled to about 0°C (ice/water bath) and treated with 0.9 ml (7.3 mmol) of chlorotrimethylsilane. The reaction was stirred at 0°C for 1 hr. Benzoyl chloride 1.5 ml (12.3 mmol) was added with stirring over 2 min. After having been warmed up to room temperature the reaction was left stirring for 2 hrs and then treated with water (5 ml) and conc. NH<sub>4</sub>OH (10 ml). The deprotection was allowed to proceed for about 1 h until all bis-benzoyl intermediates were converted to the desired mono-benzoyl derivative **12**. The reaction was concentrated, re-suspended in ethyl acetate, filtered to remove some partially precipitated benzamide, washed with 10% citric acid, saturated NaCl and dried over Na<sub>2</sub>SO<sub>4</sub>. Concentration of the filtrate resulted in crystallization of the desired product, which was collected by re-suspending the crystals in small amount of ethyl acetate/hexane (1:1) and filtration (0.69 g). The solid obtained from concentration of the filtrate was chromatographed on silica eluting with a gradient of acetone (0-20%) in ethyl acetate yielding 0.14 g of **12**. Total yield - 0.83 g (71 %). <sup>1</sup>H NMR (DMSO-d<sub>6</sub>): δ 11.08 (s, 1H), 8.70 (s, 1H), 8.42 (s, 1H), 8.00 (d, J=8.4 Hz, 2H), 7.61 (m, 1H), 7.51 (t, J=7.5 Hz, 2H), 4.58 (t, J=4.8 Hz, 1H), 4.37 (m, 1H), 4.17 (m, 1H), 4.00 (m, 1H), 3.32 (m, 2H), 2.08 (m, 1H), 1.18 (d, J=6.6 Hz, 3H), 0.84 (s, 9H), 0.00 (s, 6H).

**EXAMPLE 9**

[0051] (2R,3R)-3-(*tert*-Butyldimethylsiloxy)-2-((6-benzamido-9H-purin-9-yl)methyl)-1-(bis(4-methoxyphenyl)(phenyl)methoxy)butane (**13**):

[0052] DMTCI (0.8 g, 2.37 mmol) was added in one portion to a stirred solution of **12** (0.82 g, 1.8 mmol) in 20 ml of anhydrous pyridine. The reaction was kept at room temperature for 20 hrs, concentrated and partitioned between ethyl acetate and 10% citric acid. The organic phase was washed with saturated NaCl, dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated. The resultant crude product was chromatographed on silica eluting with

50% ethyl acetate in hexane. Concentration of the pure product fractions afforded 1.2 g (88%) of **13** as a white amorphous solid. <sup>1</sup>H NMR (DMSO-d<sub>6</sub>): δ 11.12 (s, 1H), 8.70 (s, 1H), 8.38 (s, 1H), 8.05 (d, J=8.7 Hz, 2H), 7.65 (m, 1H), 7.55 (t, J=7.5 Hz, 2H), 7.18 (m, 5H), 7.04 (t, J=8.7, 4H), 6.77 (m, 4H), 4.36 (m, 1H), 4.24 (m, 1H), 4.02 (m, 1H), 3.60 (s, 6H), 3.12 (m, 1H), 2.95 (m, 1H), 2.39 (m, 1H), 1.14 (d, J=6.6 Hz, 3H), 0.74 (s, 9H), 0.00 (s, 3H), -0.10 (s, 3H).

#### EXAMPLE 10

[0053] (2R,3R)-2-((6-Benzamido-9H-purin-9-yl)methyl)-1-(bis(4-methoxyphenyl)(phenyl)methoxy)butan-3-ol (**14**):

[0054] A solution of tetrabutylammonium fluoride 1M in THF (3 ml) was added to a solution of **13** (1.1 g, 1.45 mmol) in 10 ml of THF. The reaction was kept at room temperature for 2 days until no starting material was seen by HPLC analysis. The reaction was concentrated and partitioned between water and ethyl acetate. The organic phase was washed with saturated NaCl, dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated. The resulting material was chromatographed on silica eluting with a gradient of acetone (0-40%) in ethyl acetate. Concentration of the pure product fractions afforded 0.9 g (96%) of **14** as a white amorphous solid. <sup>1</sup>H NMR (DMSO-d<sub>6</sub>): δ 11.12 (s, 1H), 8.70 (s, 1H), 8.34 (s, 1H), 8.05 (d, J=8.7 Hz, 2H), 7.65 (m, 1H), 7.56 (t, J=7.5 Hz, 2H), 7.18 (m, 5H), 7.04 (m, 4H), 6.77 (m, 4H), 4.79 (d, J=4.8 Hz, 1H), 4.39 (m, 2H), 3.80 (m, 1H), 3.71 (s, 6H), 3.09 (m, 1H), 2.88 (m, 1H), 2.31 (m, 1H), 1.03 (d, J=6.3 Hz, 3H).

#### EXAMPLE 11

[0055] (2R,3R)-1-(Bis(4-methoxyphenyl)(phenyl)methoxy)-2-((6-benzamido-9H-purin-9-yl)methyl)butan-3-yl 2-cyanoethyl N,N-diisopropylphosphoramidite (**15**):

[0056] To a stirred solution of **14** (0.9 g, 1.4 mmol) in 20 ml of anhydrous dichloromethane was added diisopropylammonium tetrazolide (0.25 g) followed by 0.5 g (1.66 mmol) of 2-cyanoethyl N,N,N',N'-tetraisopropylphosphordiamidite. In about 5 hrs another 0.04 g of 2-cyanoethyl N,N,N',N'-tetraisopropylphosphordiamidite was added. The reaction was allowed to proceed for total 20 hrs and then concentrated. The resultant semi-solid was portioned between ethyl acetate and saturated NaHCO<sub>3</sub>. The organic phase

was washed with saturated NaCl, dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated in vacuo. The crude phosphoramidite was precipitated twice by re-dissolving in small (~5-10 ml) amount of ethyl acetate, diluting with hexane (100 ml), allowing to settle as an oil and decanting the liquid. Drying in vacuo afforded 1.1 g (93%) of **15** as a white amorphous solid. <sup>31</sup>P NMR (DMSO-d<sub>6</sub>): δ 147.11, 146.16.

### EXAMPLE 12

[0057] (2R,3R)-1-Benzyloxy-3-(*tert*-butyldimethylsiloxy)-2-((3-benzoyl-3,4-dihydro-2,4-dioxypyrimidin-1(2H)-yl)methyl)butane (**16**):

[0058] To a cold (ice/water bath) solution of **4** (2.3 g, 7.09 mmol), 3-benzoyluracil (2.1 g, 10 mmol) and triphenylphosphine (3.8 g, 14.5 mmol) in 75 ml of anhydrous THF was added dropwise via syringe with stirring (2.94 ml, 15 mmol) of diisopropylazodicarboxylate. The reaction was removed from the bath and stirred at room temperature for 2 hrs and then concentrated. The resulting mixture was chromatographed on silica eluting with a gradient of ethyl acetate (20-33%) in hexane. Concentration of the product-containing fractions afforded 4.5 g of crude **16** which was still contained about 2 eqv. of hydrazine N,N'-bis(isopropyl)carbamate, the DIPADC by-product. <sup>1</sup>H NMR (DMSO-d<sub>6</sub>): δ 7.87 (d, J=8.4 Hz, 2H), 7.82 (d, J=8.1 Hz, 1H), 7.76 (t, J=8.3 Hz, 1H), 7.54 (t, J=8.1 Hz, 2H), 7.31 (m, 5H), 5.78 (d, J=8.1 Hz, 1H), 4.42 (d, J=4.8 Hz, 2H), 3.95 (m, 2H), 3.73 (m, 1H), 3.45 (d, J=5.7 Hz, 2H), 2.09 (m, 1H), 1.13 (d, J=5.7 Hz, 3H), 0.83 (s, 9H), 0.02 (s, 3H), 0.00 (s, 3H).

### EXAMPLE 13

[0059] (2R,3R)-1-Benzyloxy-3-(*tert*-butyldimethylsiloxy)-2-((3,4-dihydro-2,4-dioxypyrimidin-1(2H)-yl)methyl)butane (**17**):

[0060] To a solution of **16** (about 4.6 mmol) in 100 ml of MeOH was added 2 ml of 25% NaOCH<sub>3</sub> in MeOH. After being kept at room temperature for 15 hrs the reaction was quenched by adding 1.5 g of solid triethylammonium chloride and concentrated. The resulting material was partitioned between water and ethyl acetate. The organic phase was washed with saturated NaCl, dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated to an oil, which was then chromatographed on silica eluting with a gradient of ethyl acetate (33-

50%) in hexane. Concentration of the pure product fractions afforded 1.7 g (about 88%) of **17** as a white crystalline solid. <sup>1</sup>H NMR (DMSO-d<sub>6</sub>): δ 11.18 (s, 1H), 7.52 (d, J=7.8 Hz, 1H), 7.28 (m, 5H), 5.47 (d, J=7.8 Hz, 1H), 4.38 (s, 2H), 3.97 (m, 1H), 3.84 (m, 1H), 3.62 (m, 1H), 3.38 (m, 2H), 2.02 (m, 1H), 1.13 (d, J=5.7 Hz, 3H), 0.84 (s, 9H), 0.01 (s, 3H), 0.00 (s, 3H).

#### EXAMPLE 14

[0061] (2R,3R)-1-Benzyloxy-3-(*tert*-butyldimethylsiloxy)-2-((2-oxo-4-(1H-1,2,4-triazol-1-yl)pyrimidin-1(2H)-yl)methyl)butane (**18**):

[0062] To a cold (ice/water bath) solution of **17** (1.7 g, 4.06 mmol) and 1,2,4-triazole (3.9 g, 56.4 mmol) in 25 ml of anhydrous pyridine was added dropwise over 5 min 2.8 g (11.4 mmol) of 4-chlorophenyldichlorophosphate (via syringe). The reaction was allowed to warm up and kept at room temperature for 3 days until all starting material and some intermediates converted to the desired triazolide **18** (monitored by HPLC). The reaction was concentrated and partitioned between ethyl acetate (150 ml) and water (150 ml). The organic phase was washed with 10% citric acid, saturated NaCl and dried over Na<sub>2</sub>SO<sub>4</sub>. The crude material obtained after concentration of the extract was chromatographed on silica eluting with a gradient of ethyl acetate (33-50%) in hexane. Concentration of the pure product fractions afforded 1.5 g (79%) of **18** as a light tan crystalline solid. <sup>1</sup>H NMR (DMSO-d<sub>6</sub>): δ 9.34 (s, 1H), 8.35 (s, 1H), 8.34 (d, J=7.2 Hz, 1H), 7.17 (m, 5H), 6.79 (d, J=7.2 Hz), 4.30 (s, 2H), 4.10 (m, 1H), 4.00 (m, 1H), 3.82 (m, 1H), 3.42 (d, J=5.7 Hz, 2H), 2.19 (m, 1H), 1.14 (d, J=6.3 Hz, 3H), 0.83 (s, 9H), 0.01 (s, 3H), 0.00 (s, 3H).

#### EXAMPLE 15

[0063] (2R,3R)-1-Benzyloxy-3-(*tert*-butyldimethylsiloxy)-2-((4-amino-2-oxo-pyrimidin-1(2H)-yl)methyl)butane (**19**):

[0064] A solution of **18** (1.5 g, 3.2 mmol) in 100 ml of 6 M NH<sub>3</sub>/MeOH was heated at 100°C in a Parr bomb for 2 days. The reaction was cooled, concentrated and chromatographed on silica eluting with 10% MeOH in ethyl acetate. Concentration of the product-containing fractions afforded 1.27 g (95%) of **19** as an off-white amorphous solid.

<sup>1</sup>H NMR (DMSO-d<sub>6</sub>): δ 7.43 (d, J=7.2 Hz, 1H), 7.29 (m, 5H), 6.97 (br s, 2H), 5.58 (d, J=7.2 Hz), 4.39 (d, J=1.8 Hz, 2H), 3.98 (m, 1H), 3.83 (m, 1H), 3.51 (m, 1H), 3.35 (m, 2H), 2.06 (m, 1H), 1.14 (d, J=6.3 Hz, 3H), 0.85 (s, 9H), 0.02 (s, 3H), 0.00 (s, 3H).

#### EXAMPLE 16

[0065] (2R,3R)-2-((4-Amino-2-oxo-pyrimidin-1(2H)-yl)methyl)butane-1,3-diol (**20**):

[0066] To a cold (~-77°C, acetone/dry ice bath) solution of **19** (1.2 g, 2.87 mmol) in 50 ml of anhydrous dichloromethane was added via syringe 19 ml (19 mmol) of 1 M BCl<sub>3</sub>/CH<sub>2</sub>Cl<sub>2</sub>. The reaction was stirred for 10 hrs and quenched with a mixture of methanol (27 ml) and pyridine (13 ml). The reaction was warmed up, concentrated, co-evaporated with a triethylamine/MeOH mixture to remove pyridine and chromatographed on C18 (5x25 cm) column eluting with a gradient of acetonitrile (0-15%). Concentration of the pure product fractions afforded 0.5 g (82%) of nucleoside **20** as a white solid. <sup>1</sup>H NMR (DMSO-d<sub>6</sub>): δ 7.52 (d, J=7.2 Hz, 1H), 7.01 (br s, 2H), 5.65 (d, J=7.2 Hz), 4.70 (d, J=4.8 Hz, 1H), 4.62 (t, J=5.4 Hz, 1H), 3.84 (m, 1H), 3.63 (m, 2H), 3.34 (m, 1H), 3.25 (m, 1H), 1.68 (m, 1H), 1.08 (d, J=6.3 Hz, 3H).

#### EXAMPLE 17

[0067] (2R,3R)-2-((4-Acetamido-2-oxo-pyrimidin-1(2H)-yl)methyl)butane-1,3-diol (**21**):

[0068] To a solution of **20** (0.5 g, 2.34 mmol) in 12 ml of anhydrous DMF was added dropwise with stirring 0.243 ml (2.57 mmol) of acetic anhydride. The reaction was kept at room temperature for 3 days and then concentrated. The oily residue was dried by co-evaporation with xylene and then in vacuo to afford 0.65 g (108%) of **21** (contaminated with some DMF) as a white solid, which was sufficiently pure to be used in the next step without additional purification. <sup>1</sup>H NMR (DMSO-d<sub>6</sub>): δ 10.81 (s, 1H), 8.00 (d, J=7.2 Hz, 1H), 7.13 (d, J=7.2 Hz, 1H), 4.62 (d, J=5.1 Hz, 1H), 4.53 (t, J=4.8 Hz, 1H), 4.02 (m, 1H), 3.73 (m, 2H), 3.53 (m, 2H), 2.09 (s, 3H), 1.80 (m, 1H), 1.10 (d, J=6.3 Hz, 3H).

## EXAMPLE 18

[0069] (2R,3R)-1-(Bis(4-methoxyphenyl)(phenyl)methoxy)-2-((4-acetamido-2-oxo-pyrimidin-1(2H)-yl)methyl)butan-3-ol (**22**):

[0070] DMTCI (0.9 g, 2.66 mmol) was added to a stirred solution of **21** (0.6 g, 2.35 mmol) in 25 ml of anhydrous pyridine. After being stirred at room temperature for 4 hrs the reaction was quenched with MeOH (2 ml) and concentrated to an oil. The oil was partition between dichloromethane and 10% citric acid. The organic phase was washed with saturated NaCl, dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated. The resulting material was chromatographed on silica eluting with a gradient of methanol (5-7.5%) in ethyl acetate. Concentration of the pure product fractions afforded 1.2 g (91%) of **22** as a white amorphous solid. <sup>1</sup>H NMR (DMSO-d<sub>6</sub>): δ 10.78 (s, 1H), 7.77 (d, J=7.2 Hz, 1H), 7.27 (m, 5H), 6.97 (d, J=7.2 Hz, 1H), 6.84 (m, 4H), 4.66 (d, J=4.8 Hz, 1H), 4.01 (m, 1H), 3.78 (m, 2H), 3.73 (s, 6H), 3.73 (m, 1H), 3.09 (m, 1H), 2.89 (m, 1H), 2.12 (m, 1H), 2.09 (s, 3H), 1.02 (d, J=6.3 Hz, 3H).

## EXAMPLE 19

[0071] (2R,3R)-1-(Bis(4-methoxyphenyl)(phenyl)methoxy)-2-((4-acetamido-2-oxo-pyrimidin-1(2H)-yl)methyl)butan-3-yl 2-cyanoethyl N,N-diisopropylphosphoramidite (**23**):

[0072] To a stirred solution of **22** (1.0 g, 1.79 mmol) in 25 ml of anhydrous dichloromethane was added diisopropylammonium tetrazolide (0.35 g) followed by 0.8 g (2.66 mmol) of 2-cyanoethyl N,N,N',N'-tetraisopropylphosphordiamidite. After being stirred at room temperature for 20 hrs the reaction was concentrated, and the residue portioned between ethyl acetate and saturated NaHCO<sub>3</sub>. The organic phase was washed with saturated NaCl, dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated in vacuo. The crude phosphoramidite was precipitated twice by re-dissolving in small (~10 ml) amount of ethyl acetate, diluting with hexane (100 ml), allowing precipitate as an oil and decanting the liquid. Drying in vacuo afforded 1.2 g (88%) of **23** as an amorphous white solid. <sup>31</sup>P NMR (DMSO-d<sub>6</sub>): δ 148.03, 147.19.

**EXAMPLE 20**

[0073] (2R,3R)-1-Benzoyloxy-3-(*tert*-butyldimethylsiloxy)-2-((2-amino-6-chloro-9H-purin-9-yl)methyl)butane (**24**):

[0074] To a cold (~0°C, ice/water bath) mixture of 2-amino-6-chloropurine (2.2 g, 13.2 mmol), **4** (3.9 g, 12 mmol), triphenylphosphine (3.52 g, 13.4 mmol) in 50 ml of anhydrous THF was added with stirring 2.75 ml (13.9 mmol) of diisopropylazodicarboxylate. The reaction was stirred at room temperature for 5 hrs. The solvent was removed in vacuo and the residue chromatographed on silica eluting with a gradient of ethyl acetate (20-66%) in hexane. The product-containing fractions were pooled and concentrated to afford a semi-crystalline material, which was re-suspended in 20% ethyl acetate/hexane and filtered to remove insoluble hydrazine bis(isopropyl)carbamate. Concentration of the filtrate and drying in vacuo afforded 4.3 g (75%) of **24** as a white solid. <sup>1</sup>H NMR (DMSO-d<sub>6</sub>): δ 8.09 (s, 1H), 7.4-7.2 (m, 5H), 6.85 (s, 2H), 4.36 (s, 2H), 4.19 (m, 1H), 4.06 (m, 1H), 3.96 (m, 1H), 3.38 (m, 2H), 2.33 (m, 1H), 1.16 (d, J=6 Hz, 3H), 0.85 (s, 9H), 0.17 (s, 3H), 0.00 (s, 3H).

**EXAMPLE 21**

[0075] (2R,3R)-1-Benzoyloxy-2-((2-amino-1,6-dihydro-6-oxopurin-9-yl)methyl)butan-3-ol (**25**):

[0076] A solution of **24** (4.3 g, 9.03 mmol) in a mixture of TFA (60 ml) and water (20 ml) was kept at room temperature for 2 days until almost no starting **24** was seen by HPLC analysis. The reaction was concentrated, co-evaporated with acetonitrile (3x100 ml) to remove most of TFA and water and re-dissolved in THF (100 ml). triethylamine (2 ml) was added to neutralize residual TFA followed by 12 ml of 1 M tetrabutylammonium fluoride in THF. The reaction was allowed to proceed for 2 hrs (monitored by HPLC), concentrated and treated with water (70 ml) and ethyl acetate. At this point crystallization of **25** started as a gelatinous solid. After being allowed the solid to form for several hours the mixture was filtered to collect the solid, which was washed with 33% ethyl acetate in hexane and dried to afford 1.65 g of **25**. The aqueous phase was extracted with ethyl acetate. All ethyl acetate extracts, including the very first one, were combined,

concentrated and chromatographed on silica in a gradient of MeOH (10-20%) in dichloromethane to afford 0.75 g of **25** (combined yield - 2.4 g, 77%). <sup>1</sup>H NMR (DMSO-d<sub>6</sub>): δ 10.53 (s, 1H), 7.57 (s, 1H), 7.30 (m, 5H), 6.42 (s, 2H), 4.80 (d, J=4.8 Hz, 1H), 4.36 (d, J=1.5 Hz, 2H), 4.11 (m, 1H), 3.99 (m, 1H), 3.67 (m, 1H), 3.32 (m, 2H), 2.10 (m, 1H), 1.09 (d, J=6.3 Hz, 3H).

### EXAMPLE 22

[0077] (2R,3R)-1-Benzyloxy-2-((2-(N,N-dimethylformamidino)-1,6-dihydro-6-oxopurin-9-yl)methyl)butan-3-ol (**26**):

[0078] A solution of **25** (0.65 g, 1.9 mmol) in 150 ml of MeOH was hydrogenated at 60 psi in the presence of 0.4 10% Pd/C for 3 days. The reaction was filtered through Celite to remove the catalyst and then concentrated. The resulting solid was re-suspended in ethyl acetate, collected by filtration and dried in vacuo to afford 0.42 g (87%) of **26** as an off-white solid. <sup>1</sup>H NMR (DMSO-d<sub>6</sub>): δ 10.54 (s, 1H), 7.61 (s, 1H), 6.46 (s, 2H), 4.73 (d, J=4.8 Hz, 1H), 4.59 (t, J=5 Hz, 1H), 4.10 (m, 1H), 3.96 (m, 1H), 3.63 (m, 1H), 3.25 (m, 2H), 1.83 (m, 1H), 1.10 (d, J=6.3 Hz, 3H).

### EXAMPLE 23

[0079] (2R,3R)-2-((2-(N,N-dimethylformamidino)-1,6-dihydro-6-oxopurin-9-yl)methyl)butane-1,3-diol (**27**):

[0080] To a suspension of **26** (0.41 g, 1.61 mmol) in 6 ml of anhydrous DMF was added 0.6 ml (4.5 mmol) N,N-dimethylformamide dimethyl acetal. The reaction was stirred for several minutes before clear solution was obtained. The reaction was allowed to proceed for 5 hrs, concentrated, co-evaporated twice with xylene and re-dissolved in MeOH (15 ml). After being kept at room temperature for 1 hrs, the reaction was concentrated on a rotary evaporator. The resulting material was dried by co-evaporation with MeOH and ethyl acetate until white solid (0.5 g, 100%) was obtained. <sup>1</sup>H NMR (DMSO-d<sub>6</sub>): δ 11.20 (s, 1H), 8.52 (s, 1H), 7.74 (s, 1H), 4.69 (d, J=4.8 Hz, 1H), 4.60 (t, J=5 Hz, 1H), 4.16 (m, 1H), 4.01 (m, 1H), 3.69 (m, 1H), 3.37 (m, 1H), 3.27 (m, 1H), 3.14 (s, 3H), 3.03 (s, 3H), 1.87 (m, 1H), 1.14 (d, J=6.3 Hz, 3H).



**EXAMPLE 24**

[0081] (2R,3R)-1-(Bis(4-methoxyphenyl)(phenyl)methoxy)-2-((2-(N,N-dimethylformamidino)-1,6-dihydro-6-oxopurin-9-yl)methyl)butan-3-ol (**28**):

[0082] DMTCl (0.62 g, 1.8 mmol) was added to a solution of **27** (0.5 g, 1.6 mmol) in 20 ml of anhydrous pyridine. The reaction was stirred at room temperature overnight, quenched with MeOH (2 mL), concentrated in vacuo and partitioned between ethyl acetate and 10% citric acid. The organic phase was washed with saturated NaCl dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated. The resultant material was chromatographed on silica eluting with a gradient of MeOH (10-15%) in ethyl acetate. Concentration of the product-containing fractions afforded 0.76 g (78%) of **28** as a white amorphous solid. <sup>1</sup>H NMR (DMSO-d<sub>6</sub>): δ 11.24 (s, 1H), 8.42 (s, 1H), 7.67 (s, 1H), 7.22 (m, 5H), 7.11 (m, 4H), 6.77 (d, J=8.1 Hz, 4H), 4.72 (d, J=4.8 Hz, 1H), 4.17 (m, 2H), 3.75 (m, 1H), 3.72 (s, 6H), 3.05 (m, 1H), 3.01 (s, 3H), 2.96 (s, 3H), 2.89 (m, 1H), 2.18 (m, 1H), 0.99 (d, J=6.3 Hz, 3H).

**EXAMPLE 25**

[0083] (2R,3R)-1-(Bis(4-methoxyphenyl)(phenyl)methoxy)-2-((2-(N,N-dimethylformamidino)-1,6-dihydro-6-oxopurin-9-yl)methyl)butan-3-yl 2-cyanoethyl N,N-diisopropylphosphoramidite (**29**)

[0084] To a stirred solution of **28** (0.75 g, 1.22 mmol) in 20 ml of anhydrous dichloromethane was added diisopropylammonium tetrazolide (0.26 g) followed by 0.66g (2.19 mmol) of 2-cyanoethyl N,N,N',N'-tetraisopropylphosphordiamidite. After being stirred at room temperature for 20 hrs the reaction was concentrated, and the residue portioned between ethyl acetate and saturated NaHCO<sub>3</sub>. The organic phase was washed with saturated NaCl, dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated in vacuo. The crude phosphoramidite was precipitated twice by re-dissolving in small (~10 ml) amount of ethyl acetate, diluting with hexane (100 ml), allowing precipitate as an oil and decanting the liquid. Drying in vacuo afforded 0.95 g (96%) of **29** as an amorphous white solid. <sup>31</sup>P NMR (DMSO-d<sub>6</sub>): δ 146.97, 145.66.

**EXAMPLE 26**

[0085] (2S,3R)-2-((Benzyloxy)methyl)-3-(*tert*-butyl)dimethylsiloxy)butoxy)(*tert*-butyl)dimethylsilane (**30**):

[0086] To a solution of **4** (17.9 g, 55.3 mmol) in 200 ml of anhydrous DMF was added imidazole (5.3 g) followed by *tert*-butyldimethylsilyl chloride (10.6 g, 70.3 mmol). The reaction was kept at room temperature for 2 h and concentrated on a rotary evaporator to an oil. The oil was partitioned between water (200 ml) and ethyl acetate (300 ml). The organic phase was washed with water, saturated NaCl, and dried over MgSO<sub>4</sub>. The crude product obtained after concentration of the extract was chromatographed on silica (~600 g) eluting with 0 to 5% ethyl acetate in hexane to afford 23.4 g (96%) of **30** as a colorless liquid. <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 7.27 (m, 5H), 4.45 (d, J=1.5 Hz, 2H), 4.02 (m, 1H), 3.72 (m, 1H), 3.62 (m, 1H), 3.48 (m, 2H), 1.81 (m, 1H), 1.09 (d, J=6.3 Hz, 3H), 0.85 (s, 9H), 0.83 (s, 9H), 0.00 (s, 12H).

**EXAMPLE 27**

[0087] (2S,3R)-2-(((*tert*-Butyl)dimethylsiloxy)methyl)-3-((*tert*-butyl)dimethylsiloxy)butan-1-ol (**31**):

[0088] A solution of **30** (4.5 g, 10.2 mmol) in 100 ml of anhydrous ethanol was hydrogenated at 60 psi in the presence of 10% Pd/C for 3 hrs. The catalyst was removed by filtration through Celite and washed with ethanol. The filtrate was concentrated, co-evaporated with ethyl acetate to remove residual ethanol and dried in vacuo to afford 3.4 g (96%) of **31** as a colorless oil. <sup>1</sup>H NMR (DMSO-d<sub>6</sub>): δ 4.28 (t, J=5 Hz, 1H), 3.96 (m, 1H), 3.62 (m, 2H), 3.41 (m, 1H), 3.33 (m, 1H), 1.52 (m, 1H), 1.08 (d, J=6.3 Hz, 3H), 0.84 (s, 9H), 0.83 (s, 9H), 0.00 (s, 12H).

**EXAMPLE 28**

[0089] (2S,3R)-1,3-Bis((*tert*-Butyl)dimethylsiloxy)-2-((3-benzoyl-3,4-dihydro-5-methyl-2,4-dioxypyrimidin-1(2H)-yl)methyl)butane (**32**)

[0090] To a cold (ice/water bath) solution of **31** (1.7 g, 4.9 mmol), 3-benzoylthymine (1.5 g, 6.5 mmol) and triphenylphosphine (1.71 g, 6.5 mmol) in 50 ml of

anhydrous THF was added dropwise via syringe with stirring 1.26 ml (6.5 mmol) of diisopropylazodicarboxylate. The reaction was removed from the bath and stirred at room temperature for 15 hrs and then concentrated. The reaction was diluted with ethyl acetate (~10 ml) to precipitate some of triphenylphosphine oxide and the DIPADC by-product. The solids were filtered off and the filtrate concentrated. The resultant crude product, which still contained some Ph<sub>3</sub>PO, was chromatographed on silica eluting with 10% ethyl acetate in hexane. Concentration of the pure product fractions afforded 1.9 g (69%) of **32** as a colorless viscous syrup. <sup>1</sup>H NMR (DMSO-d<sub>6</sub>): δ 7.89 (d, J=6.9 Hz, 2H), 7.77 (t, J=7.5 Hz, 1H), 7.67 (d, J=1 Hz, 1H), 7.57 (t, J=8.1 Hz, 2H), 3.99 (m, 1H), 3.80 (m, 1H), 3.60 (m, 3H), 2.07 (m, 1H), 1.81 (s, 3H), 1.12 (d, J=6.3 Hz, 3H), 0.84 (s, 9H), 0.83 (s, 9H), 0.01 (s, 6H), 0.00 (s, 6H).

#### EXAMPLE 29

[0091] (2S,3R)-2-((3,4-Dihydro-5-methyl-2,4-dioxopyrimidin-1(2H)-yl)methyl)butane-1,3-diol (**33**):

[0092] To a solution of **32** (1.9 g, 3.38 mmol) in 20 ml of THF was added 6 ml of 1 M TBAF in THF. After being kept at room temperature for 2 hrs the reaction was diluted with methanol (100 ml), water (100 ml) and treated with 10 ml of 1 M NaOH. The reaction was allowed to proceed overnight, then combined with 10 g of Dowex 66 and 20 g of Dowex 50wx8x200 and stirred for 1 h. The resin was removed by filtration, washed with 50% methanol and the combined filtrates concentrated to afford 0.6 g (77%) of **33** as a white solid. This material contained some impurities but was considered to be pure enough for further use without additional purification.

#### EXAMPLE 30

[0093] (2S,3R)-1-(Bis(4-methoxyphenyl)(phenyl)methoxy)-2-((3,4-dihydro-5-methyl-2,4-dioxopyrimidin-1(2H)-yl)methyl)butan-3-ol (**34**):

[0094] To a stirred solution of **33** (0.6 g, 2.6 mmol) in 20 ml of anhydrous pyridine was added 0.9 g (2.66 mmol) of DMTCl. After being stirred at room temperature for 5 hrs the reaction was quenched with methanol (3 ml), concentrated and portioned between 10% citric acid and ethyl acetate. The organic phase was washed with saturated

NaCl, dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated *in vacuo* to a solid foam. The crude product was chromatographed on silica eluting with ethyl acetate to afford 0.95 g (69%) of **34** as an amorphous white solid. <sup>1</sup>H NMR (DMSO-d<sub>6</sub>): δ 11.16 (s, 1H), 7.28 (m, 6H), 7.21 (m, 4H), 6.85 (m, 4H), 4.59 (d, J=5Hz, 1H), 3.76 (m, 2H), 3.74 (s, 6H), 3.57 (m, 1H), 3.10 (m, 1H), 2.95 (m, 1H), 2.11 (m, 1H), 1.67 (s, 3H), 1.02 (d, J=6.3 Hz, 3H).

### EXAMPLE 31

[0095] (2S,3R)-1-(Bis(4-methoxyphenyl)(phenyl)methoxy)-2-((3,4-dihydro-5-methyl-2,4-dioxypyrimidin-1(2H)-yl)methyl)butan-3-yl 2-cyanoethyl N,N-diisopropylphosphoramidite (**35**):

[0096] To a stirred solution of **34** (0.95 g, 1.79 mmol) in 25 ml of anhydrous dichloromethane was added diisopropylammonium tetrazolide (0.25 g) followed by 0.7 g (2.32 mmol) of 2-cyanoethyl N,N,N',N'-tetraisopropylphosphordiamidite. After being stirred at room temperature for 18 hrs the reaction was concentrated, and the residue portioned between ethyl acetate and saturated NaHCO<sub>3</sub>. The organic phase was washed with saturated NaCl, dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated *in vacuo*. The crude phosphoramidite was precipitated twice by re-dissolving in small (~15 ml) amount of ethyl acetate, diluting with hexane (100 ml), allowing to settle as an oil and decanting the liquid. Drying *in vacuo* afforded 0.95 g (72%) of **35** as an amorphous white solid. <sup>31</sup>P NMR (DMSO-d<sub>6</sub>): δ 145.81, 144.94.

### EXAMPLE 32

[0097] (2S,3R)-1,3-Bis((*tert*-Butyl)dimethylsiloxy)-2-((2-amino-6-chloro-9H-purin-9-yl)methyl)butane (**36**):

[0098] To a cold (~0°C, ice/water bath) partial solution of 2-amino-6-chloropurine (0.89 g, 5.26 mmol), **31** (1.7 g, 4.87 mmol) and triphenylphosphine (1.5 g, 5.7 mmol) was added dropwise with stirring 1.25 ml (6.4 mmol) of diisopropylazodicarboxylate. The reaction was stirred at room temperature overnight and concentrated. The residue was co-evaporated with xylene to remove residual DMF and chromatographed on silica eluting with 20% to 25% ethyl acetate in hexane. Concentration of the pure product fractions afforded 1.8 g (74%) of **36** as a white solid. <sup>1</sup>H NMR

(DMSO-d<sub>6</sub>):  $\delta$  8.05 (s, 1H), 6.76 (s, 2H), 4.08 (m, 1H), 3.98 (m, 2H), 3.56 (m, 2H), 2.31 (m, 1H), 1.16 (d, J=6.3 Hz, 3H), 0.81 (s, 9H), 0.78 (s, 9H), 0.00 (s, 3H), -0.025 (s, 3H), -0.095 (s, 3H), -0.10 (s, 3H).

### EXAMPLE 33

[0099] (2S,3R)-2-((2-Amino-1,6-dihydro-6-oxopurin-9-yl)methyl)butane-1,3-diol (**37**):

[0100] A solution of **36** (1.8 g, 3.6 mmol) in a mixture of TFA (60 ml) and water (20 ml) was kept at room temperature for 2 days until almost no starting material was found by HPLC analysis. The reaction was concentrated, co-evaporated with water (3x100 ml) and re-dissolved in ~5 ml of acetonitrile. Ethyl ether (~50 ml) was added to precipitate 0.84 g of **37** as a TFA salt. The salt was dissolved in ~7 ml of ethanol, treated with 0.5 ml of triethylamine and allowed to crystallize at -20°C for 2 hrs. The crystals were collected by filtration, washed with ethyl ether and dried in vacuo to afford 0.64 g (70%) of **37** as a white solid. <sup>1</sup>H NMR (DMSO-d<sub>6</sub>):  $\delta$  10.55 (s, 1H), 7.63 (s, 1H), 6.46 (s, 2H), 4.63 (d, J=5 Hz, 1H), 4.55 (t, J=5 Hz, 1H), 3.97 (d, J=7.5 Hz, 2H), 3.64 (m, 1H), 3.41 (m, 2H), 1.92 (m, 1H), 1.10 (d, J=6.3 Hz, 3H).

### EXAMPLE 34

[0101] (2S,3R)-2-((2-(N,N-Dimethylformamidino)-1,6-dihydro-6-oxopurin-9-yl)methyl)butane-1,3-diol (**38**):

[0102] To a suspension of **37** (0.64 g, 2.5 mmol) in 10 ml of anhydrous DMF was added 1.0 ml (7.5 mmol) of N,N-dimethylformamide dimethyl acetal. A clear solution was obtained after about 5 min of stirring. The solution was kept at room temperature for 3 hrs and concentrated in vacuo to an oil, which was then re-dissolved in 25 ml of methanol and left at room temperature overnight. The reaction was concentrated and dried in vacuo to afford 0.78 g (100%) of **38** as a white solid, which contained some residual DMF. <sup>1</sup>H NMR (DMSO-d<sub>6</sub>):  $\delta$  11.12 (br s, 1H), 8.52 (s, 1H), 7.73 (s, 1H), 4.62 (d, J=5 Hz, 1H), 4.54 (t, J=5 Hz, 1H), 4.05 (d, J=6.6 Hz, 2H), 3.66 (m, 1H), 3.43 (m, 2H), 3.13 (s, 3H), 3.02 (s, 3H), 1.95 (m, 1H), 1.11 (d, J=6.3 Hz, 3H).

**EXAMPLE 35**

[0103] (2S,3R)-1-(Bis(4-methoxyphenyl)(phenyl)methoxy)-2-((2-(N,N-dimethylformamidino)-1,6-dihydro-6-oxopurin-9-yl)methyl)butan-3-ol (**39**):

[0104] DMTCl (1.0 g, 2.96 mmol) was added to a solution of **38** (0.78 g, 2.5 mmol) in 30 ml of anhydrous pyridine. The reaction was stirred at room temperature overnight, quenched with MeOH (2 mL), concentrated in vacuo and partitioned between ethyl acetate and 10% citric acid. The organic phase was washed with saturated NaCl dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated. The resultant material was chromatographed on silica eluting with a gradient of MeOH (10-15%) in ethyl acetate. Concentration of the product-containing fractions afforded 1.15 g (75%) of **39** as a white amorphous solid. <sup>1</sup>H NMR (DMSO-d<sub>6</sub>): δ 11.24 (s, 1H), 8.41 (s, 1H), 7.64 (s, 1H), 7.26 (m, 5H), 7.14 (m, 4H), 6.79 (m, 4H), 4.66 (d, J=5 Hz, 1H), 4.20 (m, 1H), 4.03 (m, 1H), 3.74 (m, 1H), 3.71 (s, 6H), 3.12 (m, 1H), 3.00 (s, 3H), 2.94 (m, 1H), 2.88 (s, 3H), 2.26 (m, 1H), 1.00 (d, J=6.3 Hz, 3H).

**EXAMPLE 36**

[0105] (2S,3R)-1-(Bis(4-methoxyphenyl)(phenyl)methoxy)-2-((2-(N,N-dimethylformamidino)-1,6-dihydro-6-oxopurin-9-yl)methyl)butan-3-yl 2-cyanoethyl N,N-diisopropylphosphoramidite (**40**):

[0106] To a stirred solution of **39** (1.1 g, 1.8 mmol) in 30 ml of anhydrous dichloromethane was added diisopropylammonium tetrazolide (0.3 g) followed by 0.78g (2.6 mmol) of 2-cyanoethyl N,N,N',N'-tetraisopropylphosphordiamidite. After being stirred at room temperature for 20 hrs the reaction was concentrated, and the residue portioned between ethyl acetate and saturated NaHCO<sub>3</sub>. The organic phase was washed with saturated NaCl, dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated in vacuo. The crude phosphoramidite was p twice by re-dissolving in small (~10 ml) amount of ethyl acetate, diluting with hexane (100 ml), allowing precipitate as an oil and decanting the liquid. Drying in vacuo afforded 1.1 g (75%) of **40** as an amorphous white solid. <sup>31</sup>P NMR (DMSO-d<sub>6</sub>): δ 146.17, 144.42.

**EXAMPLE 37**

[0107] (2S,3R)-1,3-Bis(*tert*-butyl)dimethylsiloxy)-2-((3-benzoyl-3,4-dihydro-2,4-dioxypyrimidin-1(2H)-yl)methyl)butane (**41**):

[0108] To a cold (ice/water bath) solution of **31** (6.6 g, 18.9 mmol), 3-benzoyluracil (4.9 g, 22.7 mmol) and triphenylphosphine (7.2 g, 27.5 mmol) in 100 ml of anhydrous THF was added dropwise via syringe with stirring (5.5 ml, 27.9 mmol) of diisopropylazodicarboxylate. The reaction was removed from the bath and stirred at room temperature for 3 hrs and then concentrated. The residue was dried by co-evaporation with xylene and chromatographed on silica eluting with a gradient of ethyl acetate (10-20%) in hexane. Concentration of the product-containing fractions afforded 8.7 g (84%) of **41** as a colorless oil. <sup>1</sup>H NMR (DMSO-d<sub>6</sub>): δ 7.92 (d, J=8.1 Hz, 2H), 7.78 (m, 2H), 7.59 (t, J=8.1 Hz, 2H), 5.84 (d, J=7.8 Hz, 1H), 4.01 (m, 1H), 3.84 (m, 1H), 3.69 (m, 1H), 3.59 (m, 2H), 2.08 (m, 1H), 1.12 (d, J=6 Hz, 3H), 0.85 (s, 18H), 0.02 (s, 12H).

**EXAMPLE 38**

[0109] (2S,3R)-1,3-Bis(*tert*-butyl)dimethylsiloxy)-2-((3,4-dihydro-2,4-dioxypyrimidin-1(2H)-yl)methyl)butane (**42**):

[0110] Sodium methoxide, 25% in MeOH (6 ml) was added to a solution of **41** (8.7 g, 15.9 mmol) in 300 ml of MeOH. The reaction was stirred at 50°C for 3 hrs, then treated with 5.3 g of solid triethylammonium chloride and concentrated. The obtained residue was partitioned between water and ethyl acetate. The organic phase was washed with saturated NaCl and dried over Na<sub>2</sub>SO<sub>4</sub>. Concentration of the extract gave an oil. The oil was chromatographed on silica eluting with 20 to 30% ethyl acetate in hexane to afford a crude product, which was re-crystallized from hexane (~30 ml) at -20°C to yield 5.4 g (76%) of **42** as white crystals. <sup>1</sup>H NMR (DMSO-d<sub>6</sub>): δ 11.18 (s, 1H), 7.49 (d, J=7.5 Hz, 1H), 5.51 (d, J=7.5 Hz, 1H), 3.99 (m, 1H), 3.75 (m, 1H), 3.55 (m, 3H), 2.03 (m, 1H), 1.12 (d, J=6 Hz, 3H), 0.84 (s, 18H), 0.02 (s, 6H), 0.00 (s, 6H).

**EXAMPLE 39**

[0111] (2S,3R)-1,3-Bis(*tert*-butyl)dimethylsiloxy)-2-((2-oxo-4-(1H-1,2,4-triazol-1-yl)pyrimidin-1(2H)-yl)methyl)butane (**43**):

[0112] To a cold (ice/water bath) solution of **42** (2.7 g, 6.08 mmol) and 1,2,4-triazole (5.8 g, 84 mmol) in 37 ml of anhydrous pyridine was added dropwise over 5 min 4.2 g (17.11 mmol) of 4-chlorophenyldichlorophosphate (via syringe). The reaction was allowed to warm up and kept at room temperature for 3 days. The reaction was concentrated and partitioned between ethyl acetate (150 ml) and water (150 ml). The organic phase was washed with 10% citric acid, saturated NaCl and dried over Na<sub>2</sub>SO<sub>4</sub>. The crude material (brown oil) obtained after concentration of the extract was chromatographed on silica eluting with a gradient of ethyl acetate (50-66%) in hexane. Concentration of the pure product fractions afforded 2.0 g (66%) of **43** as a light tan solid. <sup>1</sup>H NMR (DMSO-d<sub>6</sub>): δ 9.40 (s, 1H), 8.38 (s, 1H), 8.37 (d, J=7 Hz, 1H), 6.90 (d, J=7 Hz, 1H), 4.05 (m, 2H), 3.83 (m, 1H), 3.60 (d, J=6.3 Hz, 2H), 2.22 (m, 1H), 1.15 (d, J=6 Hz, 3H), 0.81 (s, 9H), 0.79 (s, 9H), 0.01 (s, 6H), -0.04 (s, 6H).

**EXAMPLE 40**

[0113] (2S,3R)-1,3-Bis(*tert*-butyl)dimethylsiloxy)-2-((4-amino-2-oxo-pyrimidin-1(2H)-yl)methyl)butane (**44**):

[0114] A solution of **43** (2.0 g, 4.05 mmol) in 60 ml of 6 M NH<sub>3</sub>/MeOH was heated at 100°C in a Parr bomb for 2 days. The reaction was cooled, concentrated and chromatographed on silica eluting, first, with ethyl acetate and, second, with 5% MeOH in ethyl acetate. Concentration of the product-containing fractions afforded 1.2 g (67%) of **44** as a white solid. <sup>1</sup>H NMR (DMSO-d<sub>6</sub>): δ 7.39 (d, J=7 Hz, 1H), 6.94 (br s, 2H), 5.60 (d, J=7 Hz, 1H), 4.00 (m, 1H), 3.65 (m, 1H), 3.53 (m, 3H), 2.02 (m, 1H), 1.13 (d, J=6 Hz, 3H), 0.86 (s, 9H), 0.84 (s, 9H), 0.04 (s, 6H), -0.01 (s, 3H), -0.02 (s, 3H).

**EXAMPLE 41**

[0115] (2S,3R)-2-((4-Amino-2-oxo-pyrimidin-1(2H)-yl)methyl)butane-1,3-diol (**45**):



[0116] To a solution of **44** (1.2 g, 2.7 mmol) in 15 ml of anhydrous DMF was added triethylamine (0.45 ml) followed by 0.31 ml (3.3 mmol) of acetic anhydride. The reaction was kept at room temperature for 1 day, then 3 hrs at 50C and concentrated. The oily residue was dried by co-evaporation with xylene and then re-dissolved in ethyl acetate. The solution was washed with saturated NaHCO<sub>3</sub> (2x100 ml), saturated NaCl, dried over MgSO<sub>4</sub>. Concentration of the extract and drying in vacuo afforded 1.3 g (99%) of **45** as an off-white amorphous solid. <sup>1</sup>H NMR (DMSO-d<sub>6</sub>): δ 10.79 (s, 1H), 7.94 (d, J=7 Hz, 1H), 7.12 (d, J=7 Hz, 1H), 4.01 (m, 1H), 3.87 (m, 1H), 3.69 (m, 1H), 3.56 (d, J=6 Hz, 2H), 2.14 (m, 1H), 2.08 (s, 3H), 1.14 (d, J=6 Hz, 3H), 0.84 (s, 9H), 0.82 (s, 9H), 0.02 (s, 6H), -0.02 (s, 3H), -0.03 (s, 3H).

#### EXAMPLE 46

[0117] (2S,3R)-2-((4-Acetamido-2-oxo-pyrimidin-1(2H)-yl)methyl)butane-1,3-diol (**46**):

[0118] To a solution of **45** (1.3 g, 2.7 mmol) in 38 ml of THF was added 2.3 ml of 1M TBAF in THF. The reaction was stirred for 24 hrs, concentrated and chromatographed on silica eluting with, first, 10% MeOH in dichloromethane and, second, with 15% MeOH in dichloromethane. Concentration of the pure product fractions afforded 0.5 g (72%) of **46** as white amorphous solid. <sup>1</sup>H NMR (DMSO-d<sub>6</sub>): δ 10.81 (s, 1H), 7.98 (d, J=7 Hz, 1H), 7.12 (d, J=7 Hz, 1H), 4.58 (d, J=5 Hz, 1H), 4.48 (t, J=5 Hz, 1H), 3.82 (d, J=7 Hz, 2H), 3.67 (m, 1H), 3.46 (m, 1H), 3.38 (m, 1H), 2.09 (s, 3H), 1.89 (m, 1H), 1.09 (d, J=6 Hz, 3H).

#### EXAMPLE 43

[0119] (2S,3R)-1-(Bis(4-methoxyphenyl)(phenyl)methoxy)-2-((4-acetamido-2-oxo-pyrimidin-1(2H)-yl)methyl)butan-3-ol (**47**):

[0120] DMTCI (0.79 g, 2.34 mmol) was added to a stirred solution of **46** (0.5 g, 1.96 mmol) in 20 ml of anhydrous pyridine. After being stirred at room temperature overnight the reaction was quenched with MeOH (2 ml) and concentrated to an oil. The oil was partition between dichloromethane and 10% citric acid. The organic phase was washed with saturated NaCl, dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated. The resulting material

was chromatographed on silica eluting with a gradient of methanol (5-7.5%) in ethyl acetate. Concentration of the pure product fractions afforded 0.99 g (90%) of **47** as a white amorphous solid. <sup>1</sup>H NMR (DMSO-d<sub>6</sub>): δ 10.78 (s, 1H), 7.73 (d, J=7 Hz, 1H), 7.27 (m, 5H), 7.16 (m, 4H), 6.98 (d, J=7 Hz, 1H), 6.82 (m, 4H), 4.60 (d, J=5 Hz, 1H), 3.84 (d, J=7 Hz, 2H), 3.76 (m, 1H), 3.72 (s, 6H), 3.08 (m, 1H), 2.99 (m, 1H), 2.20 (m, 1H), 2.09 (s, 3H), 1.03 (d, J=6 Hz, 3H).

#### EXAMPLE 44

[0121] (2S,3R)-1-(Bis(4-methoxyphenyl)(phenyl)methoxy)-2-((4-acetamido-2-oxo-pyrimidin-1(2H)-yl)methyl)butan-3-yl 2-cyanoethyl N,N-diisopropylphosphoramidite (**48**):

[0122] To a stirred solution of **47** (0.98 g, 1.75 mmol) in 30 ml of anhydrous dichloromethane was added diisopropylammonium tetrazolide (0.3 g) followed by 0.8 g (2.66 mmol) of 2-cyanoethyl N,N,N',N'-tetraisopropylphosphordiamidite. After being stirred at room temperature for 20 hrs the reaction was concentrated, and the residue portioned between ethyl acetate and saturated NaHCO<sub>3</sub>. The organic phase was washed with saturated NaCl, dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated in vacuo. The crude phosphoramidite was purified twice by re-dissolving in small (~10 ml) amount of ethyl acetate, diluting with hexane (100 ml), allowing precipitate as an oil and decanting the liquid. Drying in vacuo afforded 1.1 g (83%) of **48** as an amorphous white solid. <sup>31</sup>P NMR (DMSO-d<sub>6</sub>): δ 145.84, 144.94.

#### EXAMPLE 45

[0123] (2S,3R)-1,3-Bis((*tert*-butyl)dimethylsiloxy)-2-((6-chloro-9H-purin-9-yl)methyl)butane (**49**):

[0124] To a cold (~0°C, ice/water bath) partial solution of 6-chloropurine (2.2 g, 14.2 mmol), **31** (4.1 g, 11.76 mmol) and triphenylphosphine (4.8 g, 18.3 mmol) was added dropwise with stirring 3.6 ml (18.3 mmol) of diisopropyl azodicarboxylate. The reaction was stirred at room temperature for 4 hrs and concentrated. The residue was co-evaporated with xylene to remove residual DMF and chromatographed on silica eluting with 10% to 20% ethyl acetate in hexane. Concentration of the pure product fractions

afforded 4.2 g (73%) of **49** as a viscous oil. <sup>1</sup>H NMR (DMSO-d<sub>6</sub>): δ 8.75 (s, 1H), 8.66 (s, 1H), 4.38 (m, 1H), 4.25 (m, 1H), 4.03 (m, 1H), 3.58 (m, 2H), 2.40 (m, 1H), 1.10 (d, J=6 Hz, 3H), 0.74 (s, 9H), 0.73 (s, 9H), -0.06 (s, 6H), -0.13 (s, 6H).

#### EXAMPLE 46

[0125] (2S,3R)-1,3-Bis((*tert*-butyl)dimethylsiloxy)-2-((6-amino-9H-purin-9-yl)methyl)butane (**50**):

[0126] A solution of **49** (4.2 g, 8.65 mmol) in 60 ml of 6M NH<sub>3</sub>/MeOH was sealed in a 100 ml Parr bomb and heated with stirring for 20 hrs. The reaction was cooled and concentrated to a white solid, which was re-crystallized from 10% ethyl acetate/hexane to afford 2.7 g (67%) of **50** as white crystals. <sup>1</sup>H NMR (DMSO-d<sub>6</sub>): δ 8.10 (s, 1H), 8.02 (s, 1H), 7.16 (s, 2H), 4.18 (m, 1H), 4.06 (m, 1H), 4.00 (m, 1H), 3.53 (m, 2H), 2.29 (m, 1H), 1.16 (d, J=6 Hz, 3H), 0.84 (s, 9H), 0.79 (s, 9H), 0.01 (s, 6H), -0.10 (s, 6H).

#### EXAMPLE 47

[0127] (2S,3R)-1,3-Bis((*tert*-butyl)dimethylsiloxy)-2-((6-benzamido-9H-purin-9-yl)methyl)butane (**51**):

[0128] To a cold (~0C, ice/water bath) solution of **50** (2.65 g, 5.7 mmol) in 50 ml of anhydrous pyridine was added dropwise with stirring 1.0 ml (8.6 mmol) of benzoyl chloride (via a syringe). The reaction was allowed to warm up to room temperature, stirred for about 5 hrs until no starting material was found by HPLC analysis, and then treated with 10 ml of 6M NH<sub>3</sub>/MeOH. After being stirred for about 3 hrs and no more of the bis-benzoylated intermediate found by HPLC, the reaction was concentrated, re-suspended in dichloromethane (~50 ml) and filtered to remove poorly insoluble benzamide. The filtrate was concentrated, washed with 10% citric acid, saturated NaCl, dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated again. The crude product was crystallized from 50% ethyl acetate/hexane (~20 ml) to afford 2.4 g (74%) of **51** as white crystals. <sup>1</sup>H NMR (DMSO-d<sub>6</sub>): δ 11.15 (s, 1H), 8.70 (s, 1H), 8.40 (s, 1H), 8.02 (d, J=7.2 Hz, 2H), 7.63 (m, 1H), 7.54 (t, J=7.8 Hz, 2H), 4.33 (m, 1H), 4.21 (m, 1H), 4.04 (m, 1H), 3.58 (m, 2H), 2.38 (m, 1H), 1.19 (d, J=6 Hz, 3H), 0.84 (s, 9H), 0.79 (s, 9H), 0.01 (s, 3H), 0.00 (s, 3H), -0.10 (s, 6H).

**EXAMPLE 48**

[0129] (2S,3R)-2-((6-Benzamido-9H-purin-9-yl)methyl)butane-1,3-diol (**52**):

[0130] To a solution of **51** (2.38 g, 4.18 mmol) in 60 ml of THF was added 3.7 ml of 1M TBAF in THF. The reaction was stirred for 24 hrs, concentrated and chromatographed on silica eluting with, first, 10% MeOH in dichloromethane and, second, with 15% MeOH in dichloromethane. Concentration of the pure product fractions afforded 1.2 g (84%) of **52** as white amorphous solid. <sup>1</sup>H NMR (DMSO-d<sub>6</sub>): δ 11.14 (s, 1H), 8.74 (s, 1H), 8.42 (s, 1H), 8.04 (d, J=7.2 Hz, 2H), 7.65 (m, 1H), 7.55 (t, J=7.8 Hz, 2H), 4.69 (d, J=5 Hz, 1H), 4.59 (t, J=5 Hz, 1H), 4.32 (d, J=7.2 Hz, 2H), 3.68 (m, 1H), 3.45 (m, 2H), 2.13 (m, 1H), 1.13 (d, J=6 Hz, 3H).

**EXAMPLE 49**

[0131] (2S,3R)-1-(Bis(4-methoxyphenyl)(phenyl)methoxy)-2-((6-benzamido-9H-purin-9-yl)methyl)butan-3-ol (**53**):

[0132] DMTCI (0.58 g, 1.71 mmol) was added to a stirred solution of **52** (0.5 g, 1.46 mmol) in 20 ml of anhydrous pyridine. After being stirred at room temperature overnight the reaction was quenched with MeOH (2 ml) and concentrated to an oil. The oil was partition between dichloromethane and 10% citric acid. The organic phase was washed with saturated NaCl, dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated. The resulting material was chromatographed on silica eluting with acetone (30 to 40%) in ethyl acetate. Concentration of the pure product fractions afforded 0.7 g (74%) of **53** as a white amorphous solid. <sup>1</sup>H NMR (DMSO-d<sub>6</sub>): δ 11.14 (s, 1H), 8.71 (s, 1H), 8.31 (s, 1H), 8.03 (d, J=7.2 Hz, 2H), 7.65 (m, 1H), 7.56 (t, J=7.8 Hz, 2H), 7.21 (m, 5H), 7.08 (t, J=8.7 Hz, 4H), 6.80 (m, 4H), 4.73 (d, J=5 Hz, 1H), 4.32 (m, 2H), 3.77 (m, 1H), 3.14 (m, 1H), 2.96 (m, 1H), 2.43 (m, 1H), 1.05 (d, J=6 Hz, 3H).

**EXAMPLE 50**

[0133] (2S,3R)-1-(Bis(4-methoxyphenyl)(phenyl)methoxy)-2-((6-benzamido-9H-purin-9-yl)methyl)butan-3-yl 2-cyanoethyl N,N-diisopropylphosphoramidite (**54**):

[0134] To a stirred solution of **53** (0.7 g, 1.08 mmol) in 20 ml of anhydrous dichloromethane was added diisopropylammonium tetrazolide (0.18 g) followed by 0.52 g (1.72 mmol) of 2-cyanoethyl N,N,N',N'-tetraisopropylphosphordiamidite. After being stirred at room temperature for 20 hrs the reaction was concentrated, and the residue portioned between ethyl acetate and saturated NaHCO<sub>3</sub>. The organic phase was washed with saturated NaCl, dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated in vacuo. The crude phosphoramidite was p twice by re-dissolving in small (~10 ml) amount of ethyl acetate, diluting with hexane (100 ml), allowing precipitate as an oil and decanting the liquid. Drying in vacuo afforded 0.9 g (99%) of **54** as an amorphous white solid. <sup>31</sup>P NMR (DMSO-d<sub>6</sub>): δ 145.96, 145.03.

### EXAMPLE 51

[0135] The melting curves of the oligonucleotides of the disclosure were determined as follows: oligonucleotides having the sequences shown in Figure 6 were combined in equimolar 2 μM concentrations in buffer containing 980 mM NaCl and 20 mM Na-PIPES (pH 7). The solutions in 1 cm cuvettes were brought to 80 °C briefly then the temperature lowered to 15 °C. Measurements were conducted on a Cary Bio 400 UV-Vis spectrophotometer equipped with a thermal peltier cell block and temperature probe. The temperature was ramped at a rate of 1 °C/min from 15 to >75 °C with the wavelength monitored at 268 nm. Melting curves for matched homoduplexes (fully complementary duplexes with identical nucleic acid backbone of either DNA, R,R stereoisomer of the preferred nucleoside (NNA-RR), S,R stereoisomer of the preferred nucleosides (NNA-SR), or pDNA) with variable A/T composition are shown in Figure 6. The melting temperature was calculated as the midpoint between the baselines of the associated and dissociated portions of the melting curve. Figure 7 shows a summary of melting temperatures for the homoduplexes with various nucleic acid backbones and A/T composition defined in Figure 6. Figure 8 shows the melting curves for the matched heteroduplexes with NNA-RR and DNA backbones and variable A/T composition defined in Figure 6. Figure 9 shows the temperature dependence of absorption (268 nm) for various single stranded nucleic acids defined in Figure 6.

## EXAMPLE 52

[0136] 3-Benzoyl-1-((1S,2R,3S)-3-((tert-butyldiphenylsilyloxy)-2-((trityloxy)methyl)cyclopentyl)-5-methylpyrimidine-2,4(1H,3H)-dione (**56**):

[0137] Diisopropyl azodicarboxylate (0.396 g, 0.386 ml, 1.96 mmol) was added dropwise over 5 min to a stirred cold (0-3°C) solution of compound **55** (US Appl. 20110251387) (1.0 g, 1.63 mmol), N3-benzoyl-thymine (0.451 g, 1.96 mmol), and triphenylphosphine (0.514 g, 1.96 mmol) in anhydrous DMF (24 ml). The resultant solution was stirred under argon overnight at room temperature, then concentrated and the obtained residue carefully chromatographed on silica eluting with a gradient (3-5%) of ethyl acetate in dichloromethane to afford 0.49g (37% yield) of compound **56** as a white solid. <sup>1</sup>H NMR (DMSO-d<sub>6</sub>): δ7.75 (bt, J=7.5 Hz, 1H), 7.67 (bd, J=7.7 Hz, 2H), 7.54-7.18 (m, 28H), 5.08 (q, J=7.8, 1H), 4.18 (q, J=6.0 Hz, 1H), 3.06 (dd, J=9.6, J=4.5, 1H), 2.68-2.55 (m, 1H), 2.31 (t, J=9.6, 1H), 2.20-2.10 (m, 1H), 1.95-1.77 (m, 2H), 1.62-1.50 (m, 1H), 1.52 (s, 3H), 0.92 (s, 9H).

## EXAMPLE 53

[0138] 3-Benzoyl-1-((1S,2R,3S)-3-hydroxy-2-(hydroxymethyl)cyclopentyl)-5-methylpyrimidine-2,4(1H,3H)-dione (**57**):

[0139] Compound **56** (0.45 g, 0.55 mmol) was dissolved in mixture of TFA (15 ml) and water (5 ml). The solution was stirred under argon for 1 h, then concentrated, co-evaporated 2 times with water to remove residual TFA and chromatographed on silica eluting with a gradient (5-7%) of MeOH in dichloromethane to yield nucleoside **57** (0.140 g, 75% yield) as a white solid. <sup>1</sup>H NMR (DMSO-d<sub>6</sub>): δ7.95-7.92 (m, 2H), 7.80-7.74 (m, 2H), 7.57 (t, J=7.7 Hz, 2H), 4.96-4.86 (m, 1H), 4.81 (d, J=5.1 Hz, 1H), 4.62 (t, J=4.0, 1H), 3.95-3.88 (m, 1H), 3.50-3.45 (m, 1H), 3.35-3.25 (m, 1H), 2.12-1.98 (m, 4H), 1.86 (s, 3H), 1.48-1.38 (m, 1H).

**EXAMPLE 54**

[0140] 3-Benzoyl-1-((1S,2R,3S)-2-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-3-hydroxycyclopentyl)-5-methylpyrimidine-2,4(1H,3H)-dione (**58**):

[0141] Dimethoxytrityl chloride (0.325 g, 0.958 mmol) was added to a solution of nucleoside **57** (0.30 g, 0.871 mmol) in anhydrous pyridine (5 ml); the reaction was stirred overnight at room temperature and then concentrated. The obtained residue was diluted with ethyl acetate, washed twice with 10% citric acid, saturated sodium bicarbonate, brine and dried over MgSO<sub>4</sub>. The extract was concentrated and the resulting oil chromatographed on silica eluting with a gradient (10-15%) of ethyl acetate in dichloromethane to afford DMT-protected thymidine analogue **58** (0.29 g, 51% yield) as a solid foam. <sup>1</sup>H NMR (DMSO-d<sub>6</sub>): δ7.74-7.70 (m, 3H), 7.53-7.43 (m, 3H), 7.37-7.16 (m, 9H), 6.86-6.80 (m, 4H), 5.1-5.0 (m, 1H), 4.96-4.86 (m, 1H), 4.87 (d, J=5.4 Hz, 1H), 4.04-4.00 (m, 1H), 3.72 (s, 3H), 3.71 (s, 3H), 3.14 (dd, J=9.8 Hz, J=4.0 Hz, 1H), 2.40-2.28 (m, 1H), 2.25-1.83 (m, 3H), 1.56 (s, 3H), 1.50-1.40 (m, 1H).

**EXAMPLE 55**

[0142] (1S,2R,3S)-3-(3-Benzoyl-5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-2-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)cyclopentyl (2-cyanoethyl) diisopropylphosphoramidite (**59**):

[0143] 2-Cyanoethyl N,N,N',N'- tetraisopropylphosphordiamidite (0.189 g, 0.626 mmol) was added to mixture of compound **58** (0.27 g, 0.417 mmol) and diisopropylammonium tetrazolide (0.071 g, 0.417 mmol) in dry DCM (5 ml) under argon. The reaction was stirred overnight then diluted with saturated sodium bicarbonate and extracted with ethyl acetate. The organic phase was separated, washed with brine, dried over Na<sub>2</sub>SO<sub>4</sub>, and concentrated. The obtained material was re-precipitated by dissolving in a small amount of ethyl acetate and adding excess hexane followed by decanting the liquid phase. Drying in vacuo afforded phosphoramidite **59** as a light tan viscous oil. <sup>31</sup>P NMR (CDCl<sub>3</sub>): δ148.12, 147.41.

**EXAMPLE 56**

[0144] 9-((1S,2R,3S)-3-((tert-Butyldiphenylsilyl)oxy)-2-((trityloxy)methyl)cyclopentyl)-6-chloro-9H-purine (**60**):

[0145] Diisopropyl azodicarboxylate (0.726 g, 0.707 ml, 3.59 mmol) was added dropwise over 5 min to a stirred cold (0-3°C) solution of compound **55** (2.0 g, 13.26 mmol), 6-chloropurine (0.555 g, 3.593 mmol), and triphenylphosphine (0.942 g, 3.59 mmol) in anhydrous DMF (24 ml). The reaction was stirred under argon for 22 h and then concentrated in vacuo. The resultant residue was chromatographed on silica eluting with 20% ethyl acetate in hexane to afford nucleoside **60** (1.24 g, 51% yield) as a white solid. <sup>1</sup>H NMR (DMSO-d<sub>6</sub>): δ8.45 (s, 1H), 8.36 (s, 1H), 7.49-7.39 (m, 6H), 7.36-7.20 (m, 4H), 7.15-7.06 (m, 9H), 6.96-6.88 (m, 6H), 5.56-5.47 (m, 1H), 4.43-4.34 (m, 1H), 3.04 (dd, J=9.5, J=4.4, 1H), 2.88-2.82 (m, 1H), 2.38-2.28 (m, 1H), 2.05-1.94 (m, 2H), 1.80-1.67 (m, 2H), 0.94 (s, 9H).

**EXAMPLE 57**

[0146] 9-((1S,2R,3S)-3-((tert-Butyldiphenylsilyl)oxy)-2-((trityloxy)methyl)cyclopentyl)-9H-purin-6-amine (**61**):

[0147] A solution of chloropurine intermediate **60** (1.2 g, 1.6 mmol) in 60 ml of 7 N ammonia in methanol was heated at 100°C in a Parr bomb for 18 h. The reaction was cooled, concentrated and the residue partitioned between ethyl acetate and water. The organic phase was washed with brine, dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated. The obtained residue was chromatographed on silica eluting with 3% triethylamine in ethyl acetate to yield adenosine analogue **61** (1.0 g, 85% yield) as a light tan solid foam. <sup>1</sup>H NMR (DMSO-d<sub>6</sub>): δ7.85 (s, 1H), 7.80 (s, 1H), 7.50-7.20 (m, 10H), 7.14-6.98 (m, 17H), 5.38-5.29 (m, 1H), 4.50-4.42 (m, 1H), 3.00 (dd, J=9.3, J=4.8, 1H), 2.76-2.70 (m, 1H), 2.32-2.22 (m, 1H), 2.05-1.84 (m, 3H), 1.67-1.55 (m, 1H), 0.92 (s, 9H).

**EXAMPLE 58**

[0148] N-(9-((1S,2R,3S)-3-((tert-Butyldiphenylsilyl)oxy)-2-((trityloxy)methyl)cyclopentyl)-9H-purin-6-yl)benzamide (**62**):



[0149] Benzoyl chloride (0.28 g, 0.231 ml, 2.0 mmol) was added dropwise to a cooled (ice-water bath) solution of compound **61** (0.97 g, 1.33 mmol) in anhydrous pyridine (10 ml). The bath was removed and reaction was stirred overnight, then cooled (ice-water bath) and treated with 2.5 ml of 7 N NH<sub>3</sub>/MeOH (added over 5 min). The reaction was removed from the bath, stirred under argon for 3 h to decompose the bis-benzoylated by-product and then concentrated in vacuo. The resultant residue was diluted with EtOAc and washed with 10% citric acid, saturated NaHCO<sub>3</sub>, saturated NaCl, dried over MgSO<sub>4</sub> and concentrated in vacuo. The obtained material was chromatographed on silica eluting with 1:1 ethyl acetate: dichloromethane to give N-benzamide **62** (0.98 g, 88% yield) as a white solid foam.

[0150] <sup>1</sup>H NMR (DMSO-d<sub>6</sub>): δ 11.06 (s, 1H), 8.30 (s, 1H), 8.19 (s, 1H), 8.05-8.01 (m, 2H), 7.65-7.20 (m, 13H), 7.17-7.10 (m, 9 H), 7.02-6.95 (m, 6H), 5.55-5.45 (m, 1H), 4.52-4.45 (m, 1H), 3.05 (dd, J=9.6, J=4.5, 1H), 2.85-2.78 (m, 1H), 2.40-2.30 (m, 1H), 2.05-1.92 (m, 2H), 1.89-1.67 (m, 2H), 0.94 (s, 9H).

#### EXAMPLE 59

[0151] N-(9-((1S,2R,3S)-3-Hydroxy-2-(hydroxymethyl)cyclopentyl)-9H-purin-6-yl)benzamide (**63**):

[0152] A solution of **62** (0.95 g, 1.139 mmol) in a mixture of TFA (15 ml) and water (5 ml) was stirred under argon for 10 min, then concentrated and co-evaporated twice with water to remove residual TFA. The obtained residue was purified by column chromatography (silica gel, gradient 10-20% MeOH in dichloromethane) to afford N-benzoyl adenosine analogue **63** (0.36 g, 89% yield) as a white solid.

[0153] <sup>1</sup>H NMR (DMSO-d<sub>6</sub>): δ 11.10 (s, 1H), 8.71 (s, 1H), 8.46 (s, 1H), 8.06-8.02 (m, 2H), 7.67-7.52 (m, 3H), 5.27 (q, J=8.1 Hz, 1H), 4.88 (d, J=4.8 Hz, 1H), 4.32 (t, J=4.6, 1H), 4.28-4.20 (m, 1H), 3.29-3.23 (m, 1H), 2.95-2.90 (m, 1H), 2.36-2.20 (m, 4H), 1.63-1.55 (m, 1H).

## EXAMPLE 60

[0154] N-(9-((1S,2R,3S)-2-((Bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-3-hydroxycyclopentyl)-9H-purin-6-yl)benzamide (**64**):

[0155] A solution of **63** (0.33 g, 0.93 mmol) in anhydrous pyridine (5 ml) and dimethoxytrityl chloride (0.348 g, 1.03 mmol) was kept at room temperature overnight, then concentrated and re-dissolved in ethyl acetate. The solution was washed twice with 10% citric acid, once with sodium bicarbonate, brine, dried over MgSO<sub>4</sub>, and concentrated. The resultant mixture was chromatographed on silica eluting with 3% MeOH in dichloromethane to afford desired DMT derivative **64** (0.27 g, 44% yield) as a white solid.

[0156] <sup>1</sup>H NMR (DMSO-d<sub>6</sub>): δ 11.11 (s, 1H), 8.52 (s, 1H), 8.29 (s, 1H), 8.06-8.03 (m, 2H), 7.67-7.61 (m, 1H), 7.58-7.52 (m, 2H), 7.21-7.08 (m, 5H), 6.94-6.86 (m, 4H), 6.75-6.68 (m, 4H), 5.49-5.43 (m, 1H), 4.94 (d, J=5.7 Hz, 1H), 4.25-4.17 (m, 1H), 3.69 (s, 3H), 3.68 (s, 3H), 3.06 (dd, J=9.3 Hz, J=4.2 Hz, 1H), 2.58-2.50 (m, 1H), 2.45-2.05 (m, 4H), 1.63-1.55 (m, 1H).

## EXAMPLE 61

[0157] (1S,2R,3S)-3-(6-Benzamido-9H-purin-9-yl)-2-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)cyclopentyl (2-cyanoethyl) diisopropylphosphoramidite (**65**):

[0158] 2-Cyanoethyl N,N,N',N'- tetraisopropylphosphordiamidite (0.179 g, 0.595 mmol) was added to mixture of compound **64** (0.27 g, 0.417 mmol) and diisopropylammonium tetrazolide (0.078 g, 0.457 mmol) in dry dichloromethane (5 ml) under argon. The reaction was stirred for 15 h and treated with another portion (55 mg) of 2-cyanoethyl N,N,N',N'- tetraisopropylphosphordiamidite to complete the phosphoramidite formation. Saturated sodium bicarbonate solution and ethyl acetate were added. The organic phase was separated, washed with brine, dried over Na<sub>2</sub>SO<sub>4</sub>, and concentrated. The obtained material was re-precipitated by dissolving in a small amount of ethyl acetate and adding excess hexane followed by decanting the liquid phase. Drying in vacuo afforded phosphoramidite **65** (0.32 g, 82% yield) as a white solid foam.

[0159]  $^{31}\text{P}$  NMR ( $\text{CDCl}_3$ ):  $\delta$ 147.80, 147.57.

**REFERENCES CITED**

The following documents and publications are hereby incorporated by reference.

**U.S. and Foreign Patent Documents**

US 7,153,955

US 6,506,896

U.S. 7,045,610

US 5,824,796

US 6,127,121

US 5,912,340

U.S. Patent Application Publication No. 2012/0015358

U.S. Patent Application Publication No. 2012/0244535

PCT Publication WO 01/38584

PCT Publication WO 01/64958

**Non-Patent References**

Nielsen et al, *Science* 254: 1497-1500 (1991)

Koshkin et al, *Tetrahedron* 54: 3607-30 (1998)

Viaivan et al, *Artificial DNA; PNA & XNA*, 2: 50-59 (2011)

Ueno et al., *Nucl. Acids Symposium Series.*, 51: 293-294 (2007)

Devi and Ganesh, *Artificial DNA; PNA & XNA*, 1;2; 1-8 (2010)

Johnson et al., *J. Org. Chem.*, 76:7964-74 (2011)

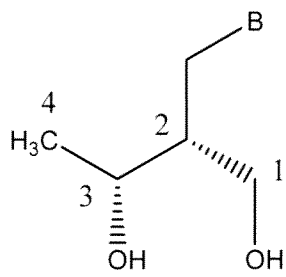
Zhang et al., *J. Amer. Chem. Soc.*, 127: 4174-4175 (2005)

Asanuma et al., *J Am Chem Soc.*, 132:14702-14703 (2010)

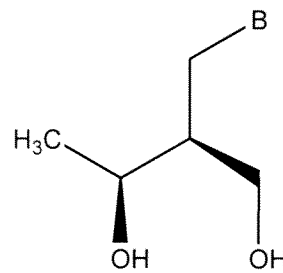
- Peterson et al., *Organic & Biomolecular Chemistry*, 1, 3293-3296 (2003)
- Guillarme et al., *Tetrahedron*, 59: 2177-2184(2003)
- Froehler and Matteucci, *Nucl. Acids Res.*, 11: 8031-8036 (1983)
- McBride et al., *J. Amer. Chem. Soc.*, 108: 2040 (1986)
- Tanaka et al., *Nucl. Acids Res.*, 33: 903-911 (2005)
- W. H. Ham et al. *J.Org. Chem.* 2000, 65, 8372-8374
- Bolli et al., *Nucleic Acids Res.*, 24:4660-4667 (1996)
- Sambrook, Fritsch & Maniatis, *MOLECULAR CLONING: A LABORATORY MANUAL*, Second Edition, Cold Spring Harbor Laboratory Press (1989)
- Ausubel, et al., *CURRENT PROTOCOLS IN MOLECULAR BIOLOGY*, John Wiley & Sons (1987, 1988, 1989, 1990, 1991, 1992, 1993, 1994, 1995, 1996)
- Gait (ed.), *OLIGONUCLEOTIDE SYNTHESIS: A PRACTICAL APPROACH*, IRL Press (1984)
- Eckstein (ed.), *OLIGONUCLEOTIDES AND ANALOGUES: A PRACTICAL APPROACH*, IRL Press (1991)



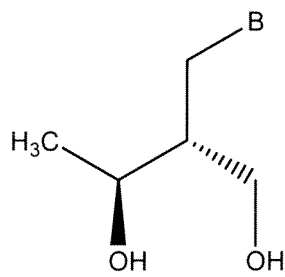
Figure 1



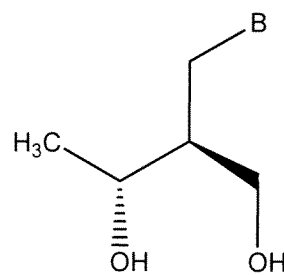
(R,R)



(S,S)



(R,S)



(S,R)

Figure 2

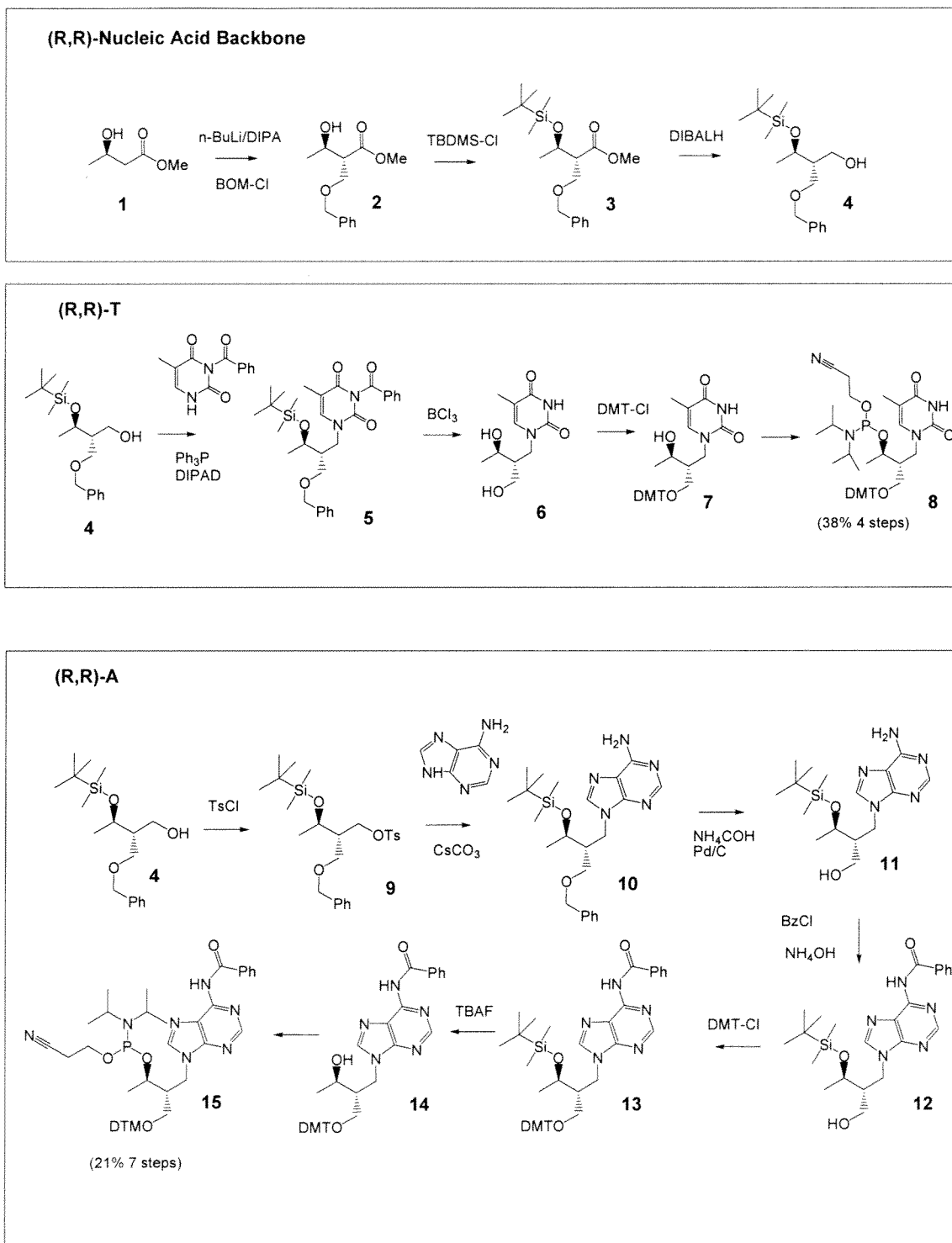




Figure 3

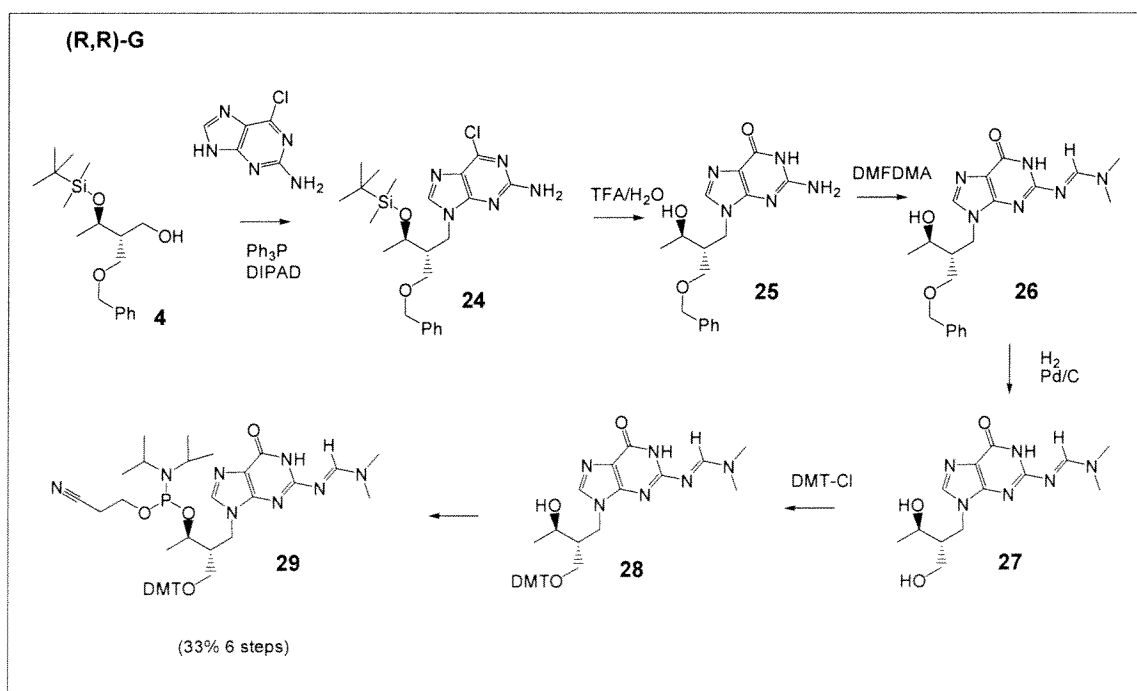
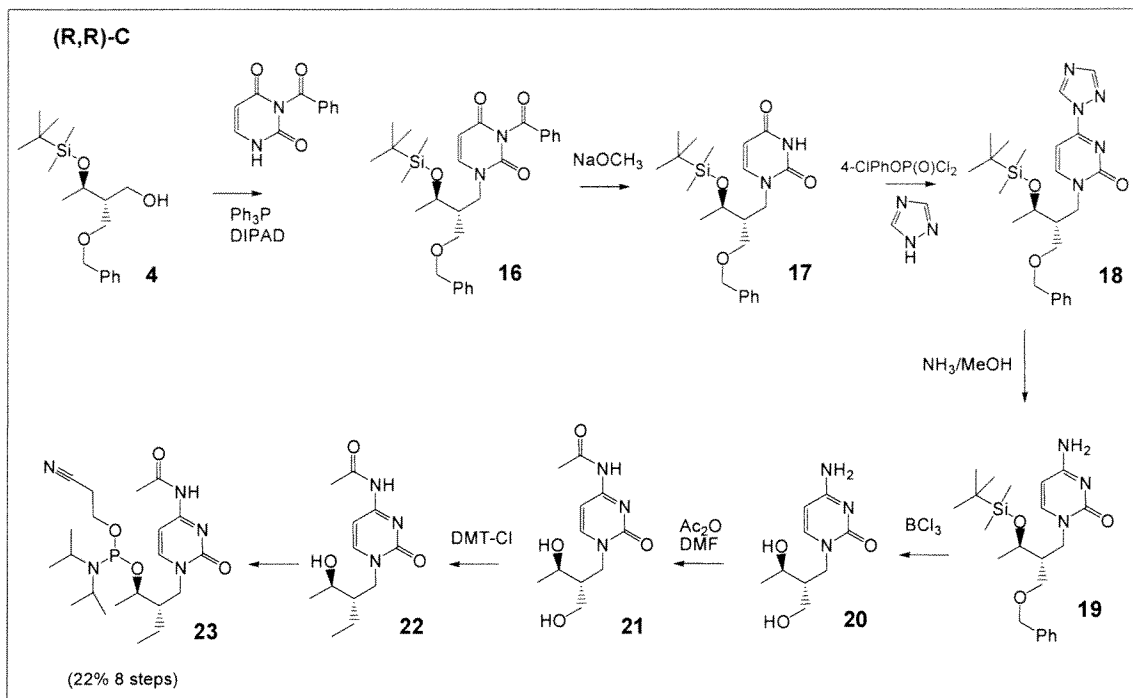


Figure 4

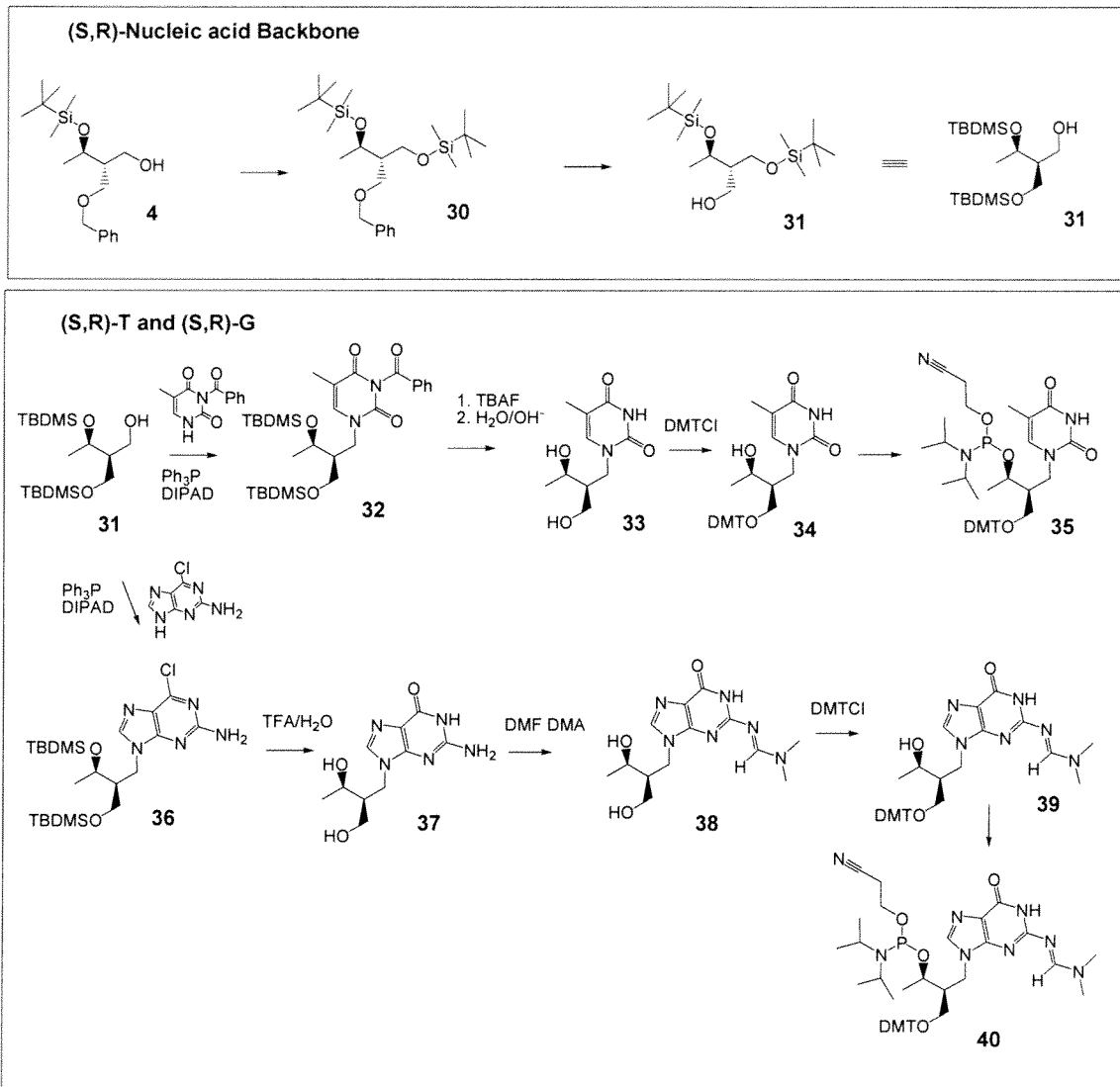


Figure 5

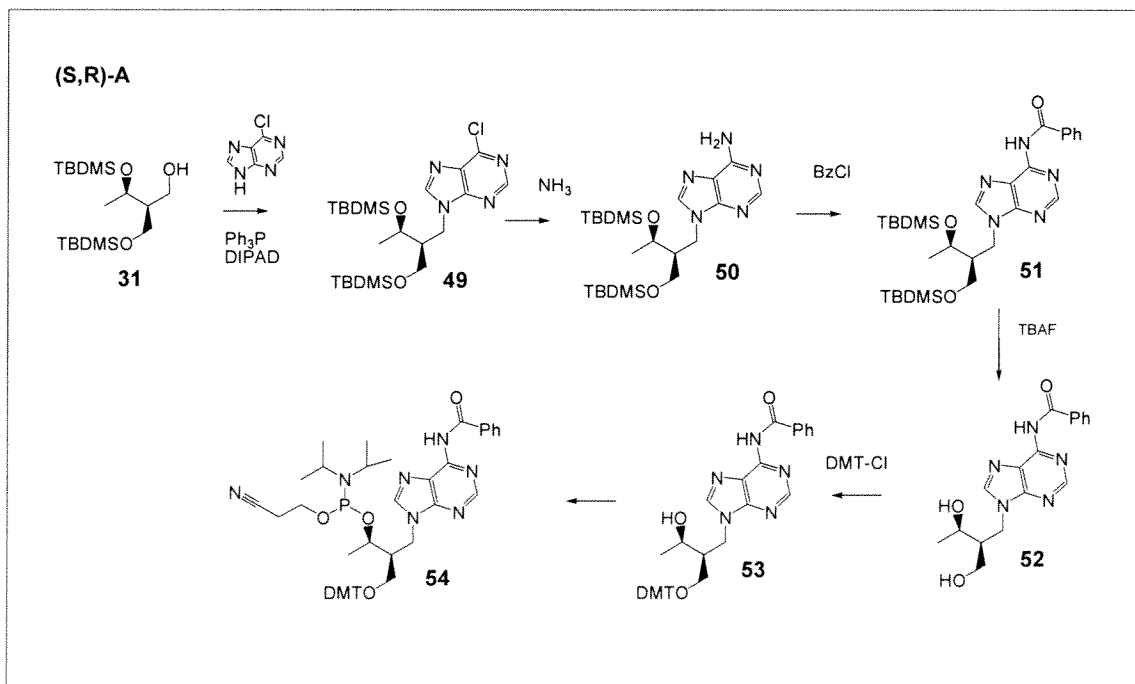
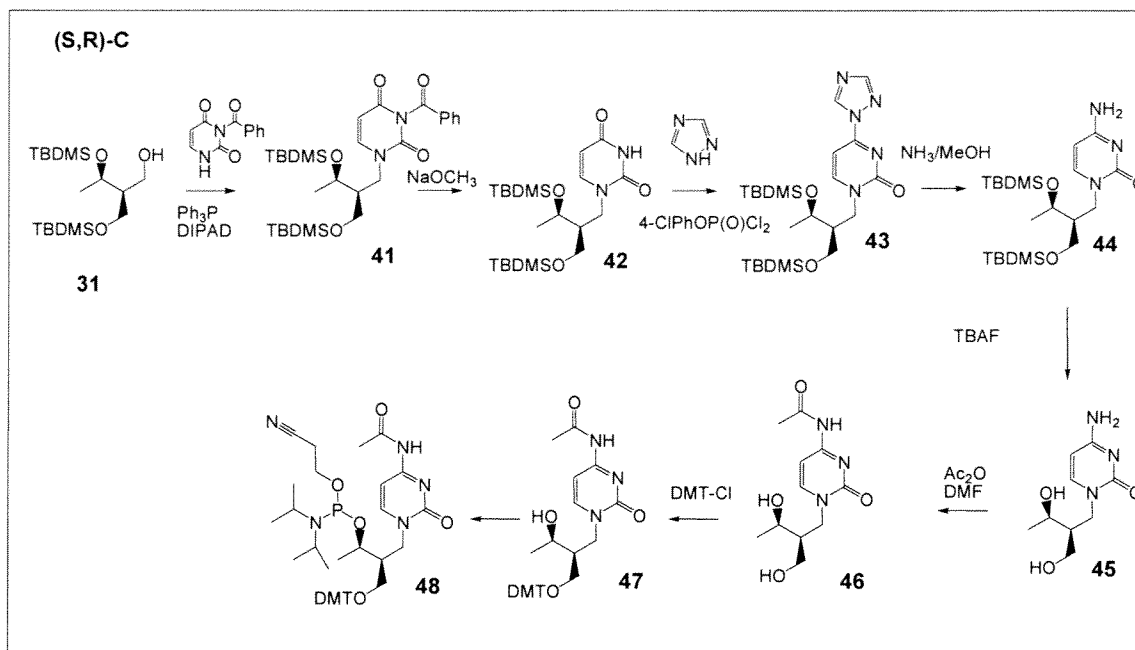
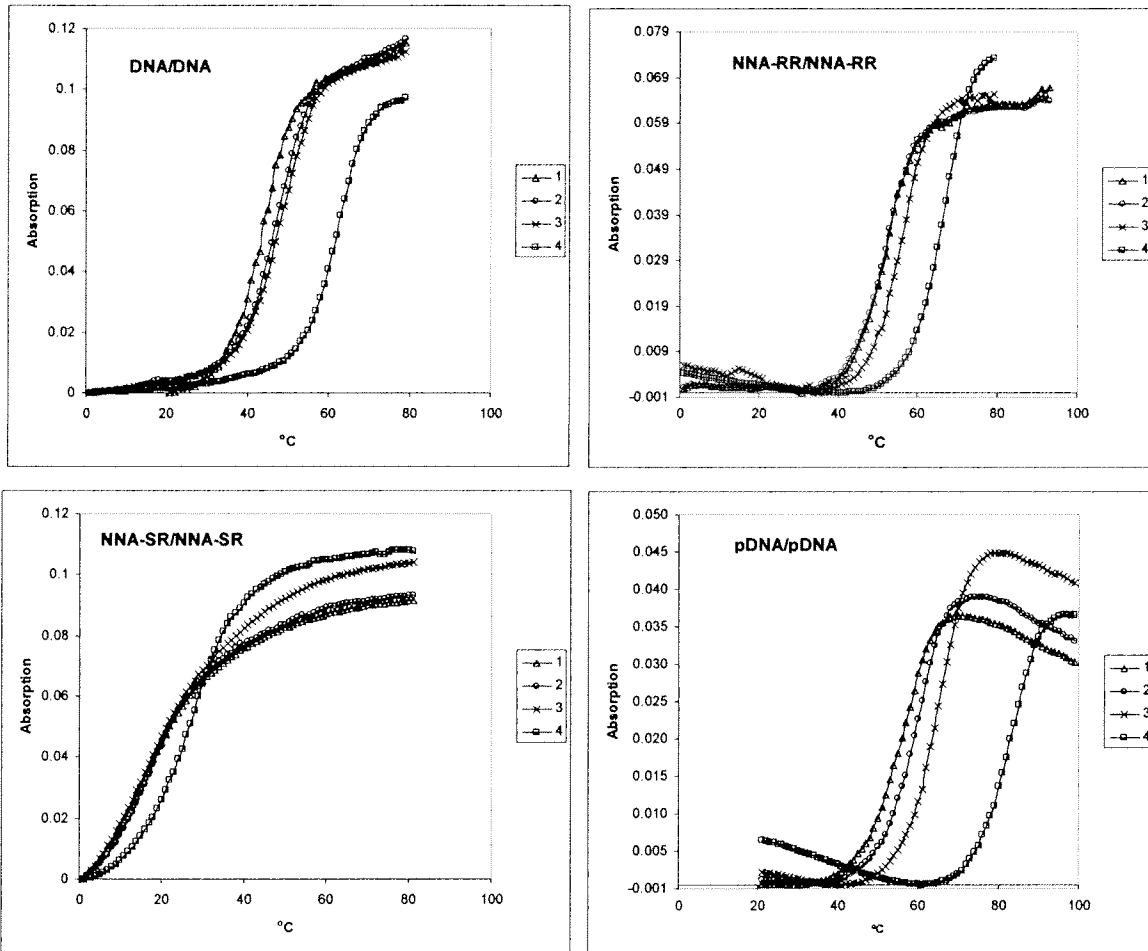


Figure 6



Seq#	Sequence (5'-3')	Seq#	Sequence (5'-3')	%AT
1	TACAAGATTTAT-H	5 (complement to 1)	ATAAATCTTGTA-H	83.33
2	TACAAGATTTAC-H	6 (complement to 2)	GTAAATCTTGTA-H	75
3	TTCAAGATGTAC-H	7 (complement to 3)	GTACATCTTGAA-H	66.67
4	TCCACCGTCGAG-H	8 (complement to 4)	CTCGACGGTGGA-H	33.33

H –is a hexanol linker

Figure 7

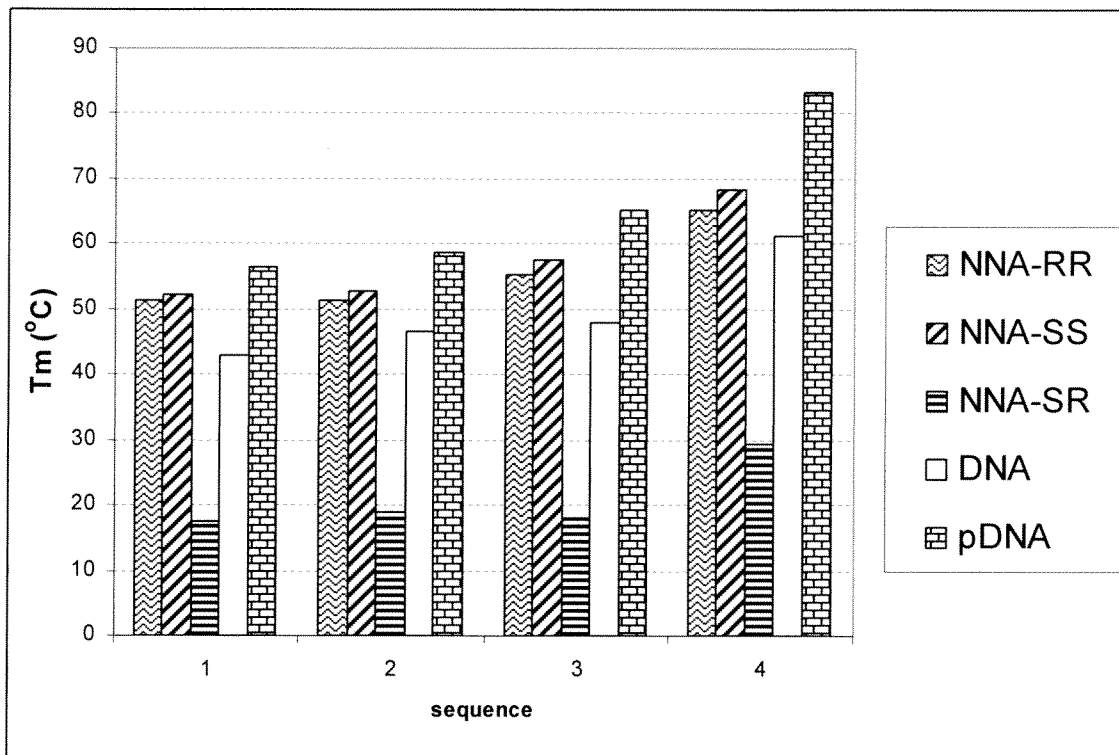


Figure 8

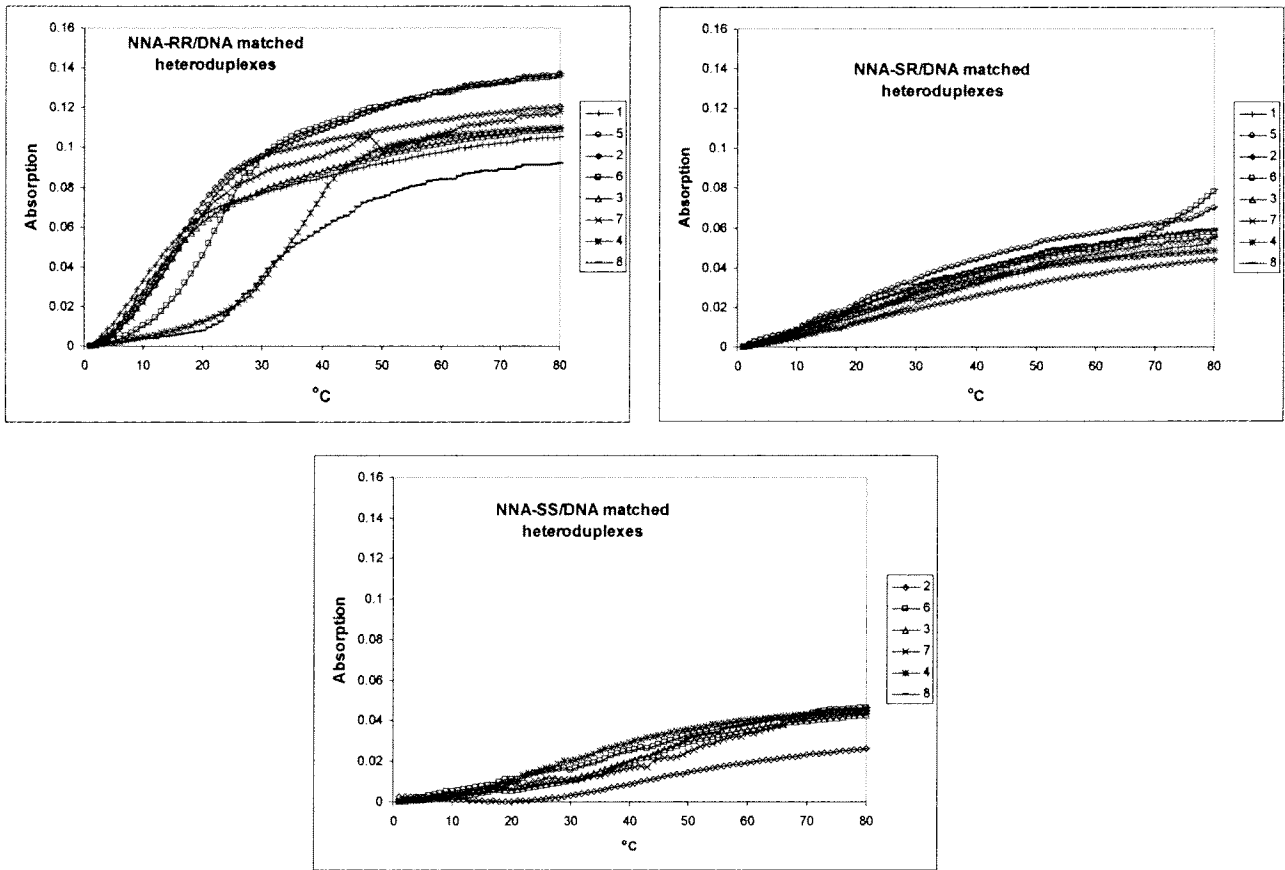


Figure 9

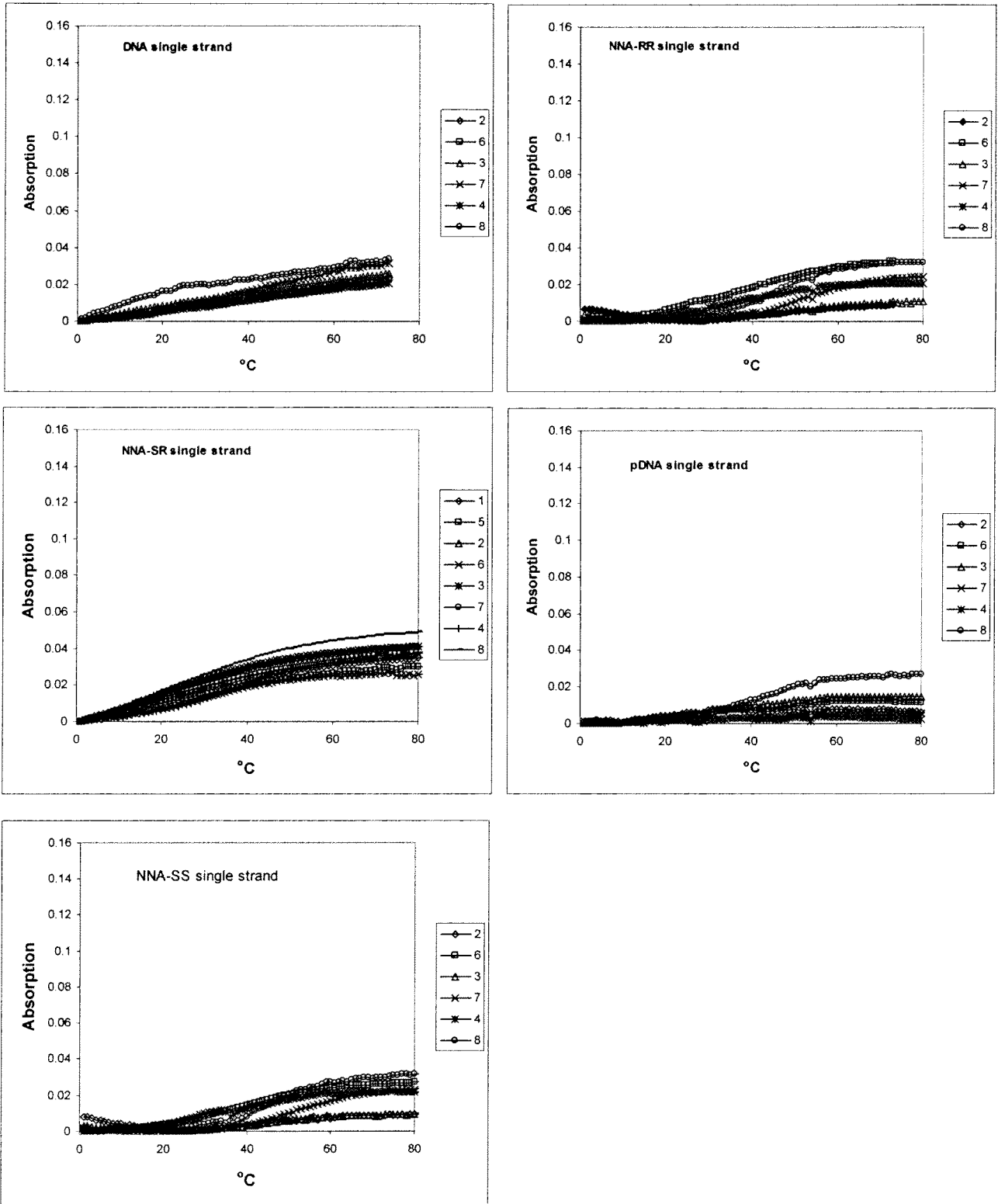


Figure 10

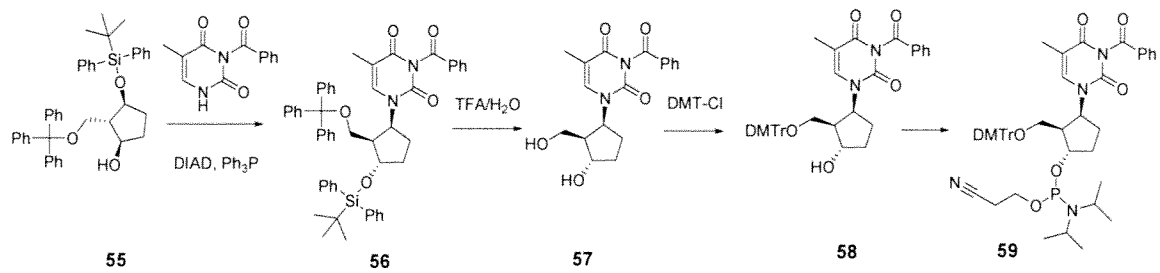




Figure 11

