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(54) Title: 6-OXOPURINE PHOSPHORIBOSYL TRANSFERASE INHIBITORS

(57) Abstract: The invention relates to compounds which are useful as inhibitors of 6-oxopurine phosphoribosyltransferases such as hypoxanthine-guanine-(xanthine) phosphoribosyltransferase (HG(X)PRT).



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6-OXOPURINE PHOSPHORIBOSYLTRANSFERASE INHIBITORS

Field of the invention

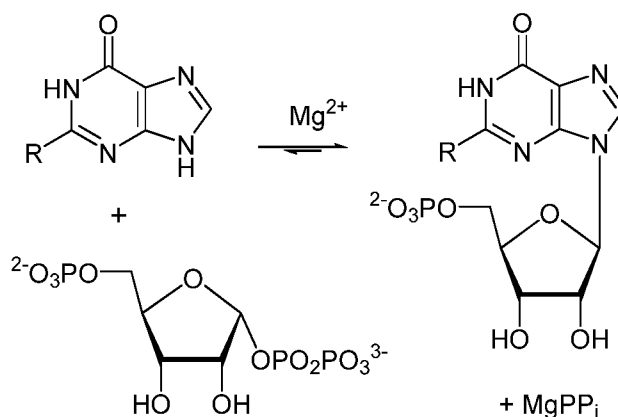
The invention relates to compounds which are useful as inhibitors of 6-oxopurine
5 phosphoribosyltransferases such as hypoxanthine-guanine-(xanthine)
phosphoribosyltransferase (HG(X)PRT). Inhibitors of these enzymes may be used in the
prevention or treatment of microbial infections, including infections caused by protozoal
and bacterial species. The compounds of the invention are particularly suited to the
prevention or treatment of microbial infections caused by *Plasmodium spp.*, which is
10 responsible for malaria, and infections caused by *Mycobacterium tuberculosis*.

Background of the invention

The 6-oxopurine phosphoribosyltransferases (PRTases) (EC:2.4.2.8 HG(X)PRT and/or
XGPRT EC:2.4.2.2) are necessary for both survival and reproduction of many
microorganisms (including protozoa and certain bacteria) because, unlike mammalian
15 cells, such microorganisms are auxotrophic for the purine ring. Thus, these
microorganisms depend on these enzymes for the synthesis of the 6-oxopurine nucleoside
monophosphates required for RNA/DNA production. On the other hand, humans possess
two metabolic pathways to synthesise nucleoside monophosphates: *de novo* and salvage.
Partial inhibition of the human enzyme should not have any serious side-effects. This is
20 based on the fact that humans with an inherited genetic defect which results in only 3% of
normal activity of this enzyme lead normal lives.

Accordingly, the 6-oxopurine PRTases represent a target with therapeutic potential. The
reactions catalysed by these enzymes are shown in Scheme 1.

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Scheme 1. The reaction catalysed by HG(X)PRT. R = -H (hypoxanthine); -NH₂ (guanine); -OH (xanthine).

- 5 6-Oxopurine phosphoribosyltransferase is the generic name for enzymes which add a phosphoribosyl group from PRib-PP onto the N9 atom of a 6-oxopurine to form a nucleoside monophosphate. The 6-oxopurine PRTases found in nature vary in their specificities for the three naturally occurring 6-oxopurines, hypoxanthine, guanine and xanthine. The specific names given to 6-oxopurine PRTases denote this specificity. For
- 10 example, the human enzyme is called hypoxanthine guanine PRTase (HGPRT) because it can efficiently use both hypoxanthine and guanine as substrates. The *Plasmodium falciparum* enzyme is called HGXPRT because it can use xanthine in addition to hypoxanthine and guanine as substrates.
- 15 Some organisms (including human, other mammals and *Plasmodium* species) encode and synthesize only one 6-oxopurine PRTase. Other organisms encode and synthesize two 6-oxopurine PRTases. Of these enzymes, the best characterized are *Escherichia coli* XGPRT and HPRT (Guddat, L.W. *et al.*, (2002) Crystal structures of free, IMP-, and GMP-bound *Escherichia coli* hypoxanthine phosphoribosyltransferase. *Protein Sci.* 11, 1626-1638). As
- 20 the abbreviations indicate, the former enzyme prefers guanine and xanthine while the latter prefers hypoxanthine.

The 6-oxopurine PRTases are members of purine salvage pathways present in all or virtually all species. These pathways contain a variety of enzymes. Their function is to

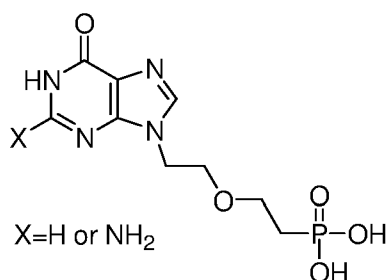
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make all of the required purine nucleotides (for RNA and DNA synthesis and for other purposes) using preformed purines.

Many organisms (including humans and many microorganisms) can produce purine bases from simple precursor molecules by the pathway known as *de novo* synthesis. Organisms which lack the *de novo* pathway and depend absolutely on the activity of one 6-oxopurine PRTase for the synthesis of purine nucleotides and which are human or animal pathogens are therefore the prime targets for 6-oxopurine PRTase inhibitors. Such organisms include several protozoan parasites including the *Plasmodium* species responsible for human malaria and *Helicobacter pylori*, the causative organisms of gastric ulcers. For bacterial strains, especially those resistant to current antibiotics, inhibitors of the 6-oxopurine PRTases are potential leads for the development of novel antibiotics. It is also proposed that combination therapy which includes the co-administration with inhibitors of the *de novo* pathway (such as azaserine) will be a successful therapy.

15

Acyclic nucleoside phosphonates (ANPs) are reverse transcriptase inhibitors and several ANP-based drugs are in current clinical use for the treatment of serious viral infections (*e.g.* Viread[®], Vistide[®], Hepsera[®]). These compounds consist of a nucleobase, either 6-aminopurine or pyrimidine, linked to a phosphonate group by an acyclic linker. 2-(Phosphonoethoxy)ethyl guanine (PEEG) and 2-(phosphonoethoxy)ethyl hypoxanthine (PEEHx) are good inhibitors of both human HGPRT and *Plasmodium falciparum* HGXPRT (*Pf*HGXPRT):



PEEG and PEEHx have K_i values for human HGPRT of 1 μ M and 3.6 μ M respectively, and 0.1 μ M and 0.3 μ M for *Pf*HGXPRT. ANPs are believed to be metabolically stable due to the presence of a phosphonate P-C linkage instead of a P-O phosphoester, making them resistant towards phosphomonoesterases and nucleotidases. ANPs are also believed to be

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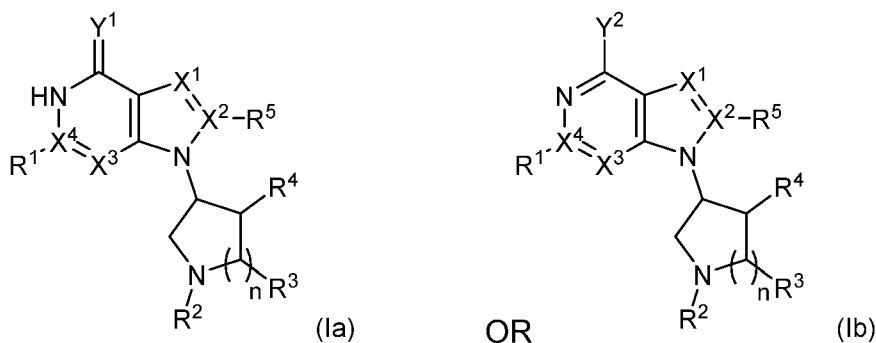
stable because the bond between the purine and the linker is stable, in contrast to the bond between the purine and the ribose in purine nucleotides.

There are four major species of *Plasmodium* that infect humans and result in symptoms of malaria: *falciparum*, *vivax*, *malariae* and *ovale*. *Plasmodium falciparum* (Pf) and *Plasmodium vivax* (Pv) are the most lethal and widespread with both species infecting around 500 million people per year, resulting in at least 1 million deaths per annum, mainly children. Drugs such as artemisinin and combination therapies, quinine, chloroquine, primaquine, are the only known treatments for malaria but, because of increasing resistance to these drugs as well as cost-effectiveness, there is an urgent need for the discovery of new targets and therapeutic leads for the development of potent anti-malarials. Likewise, there is an ongoing need for new agents that are effective against a range of other pathogenic microorganisms.

Summary of the invention

It has now been found that a new series of ANPs are potent inhibitors of 6-oxopurine phosphoribosyltransferases such as hypoxanthine-guanine-(xanthine) phosphoribosyltransferase (HG(X)PRT).

Accordingly, in one aspect the present invention provides a compound of formula (Ia) or (Ib):



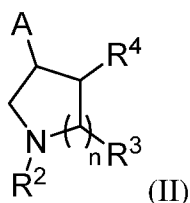
wherein:

R^1 is alkyl, aryl, heteroaryl, alkenyl, alkynyl, arylalkyl, heteroarylalkyl, NR^6_2 , halogen, OR^6 , H or NH_2 ;
 R^2 is H, $-C(O)L^1P(O)R^7R^8$ or $-C(O)L^2P(O)R^7R^8$;

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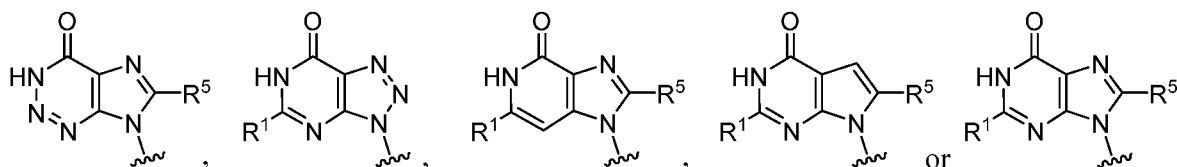
- the or each R^3 is independently selected from H, $-L^1OH$, $-L^1OR^6$, $-CH_2OC(O)L^1P(O)R^7R^8$, $-CH_2C(O)L^1P(O)R^7R^8$, $-OC(O)L^1P(O)R^7R^8$, and $-OL^1P(O)R^7R^8$;
- R^4 is $-OC(O)L^2P(O)R^7R^8$, $-OL^1P(O)R^7R^8$ or $-NHC(O)L^1P(O)R^7R^8$; or
- 5 R^4 is $-C(O)L^1P(O)R^7R^8$ when R^2 is $-C(O)L^2P(O)R^7R^8$; or
- R^4 is H when R^3 is other than H;
- R^5 is H, alkyl, aryl, heteroaryl, NR^6_2 , halogen or OR^6 ;
- R^7 and R^8 are independently selected from OH, OR^6 , SR^6 , NHR^6 , NR^6_2 , and R^p ;
- wherein R^p is a prodrug substituent and wherein the or each R^6 , where present, is
- 10 independently selected from alkyl, alkenyl, alkynyl, aryl, acyl and arylalkyl optionally containing one or more heteroatoms;
- L^1 is C_{1-4} alkylene, C_{2-4} alkenylene or C_{2-4} alkynylene;
- L^2 is C_{2-4} alkylene, C_{2-4} alkenylene or C_{2-4} alkynylene;
- wherein L^1 and L^2 are optionally substituted with one or more OH, C_{1-4} alkyl,
- 15 C_{2-4} alkenyl or C_{2-4} alkynyl;
- X^1 is N or CH;
- X^2 is C or X^2-R^5 forms N;
- X^3 is N or CH;
- X^4 is C or X^4-R^1 forms N;
- 20 Y^1 where present is S or O,
- Y^2 where present is halogen; and
- n is 1 or 2;
- or a pharmaceutically acceptable salt thereof.

- 25 In another aspect, the present invention provides a compound of formula (Ia) represented by formula (II):



wherein A is selected from one of the following:

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and wherein:

- 5 R^1 is alkyl, aryl, heteroaryl, alkenyl, alkynyl, arylalkyl, heteroarylalkyl, NR^6_2 , halogen, OR^6 , H or NH_2 ;
- R^2 is H, $-C(O)L^1P(O)R^7R^8$ or $-C(O)L^2P(O)R^7R^8$;
- the or each R^3 is independently selected from H, $-L^1OH$, $-L^1OR^6$, $-CH_2OC(O)L^1P(O)R^7R^8$, $-CH_2C(O)L^1P(O)R^7R^8$, $-OC(O)L^1P(O)R^7R^8$, and $-OL^1P(O)R^7R^8$;
- 10 R^4 is $-OC(O)L^2P(O)R^7R^8$, $-OL^1P(O)R^7R^8$ or $-NHC(O)L^1P(O)R^7R^8$; or R^4 is $-C(O)L^1P(O)R^7R^8$ when R^2 is $-C(O)L^2P(O)R^7R^8$; or R^4 is H when R^3 is other than H;
- R^5 is H, alkyl, aryl, heteroaryl, NR^6_2 , halogen or OR^6 ;
- R^7 and R^8 are independently selected from OH, OR^6 , SR^6 , NHR^6 , NR^6_2 , and R^p ;
- 15 wherein R^p is a prodrug substituent and wherein the or each R^6 , where present, is independently selected from alkyl, alkenyl, alkynyl, aryl, acyl and arylalkyl optionally containing one or more heteroatoms;
- L^1 is C_{1-4} alkylene, C_{2-4} alkenylene or C_{2-4} alkynylene;
- L^2 is C_{2-4} alkylene, C_{2-4} alkenylene or C_{2-4} alkynylene;
- 20 wherein L^1 and L^2 are optionally substituted with one or more OH, C_{1-4} alkyl, C_{2-4} alkenyl or C_{2-4} alkynyl; and
- n is 1 or 2;

or a pharmaceutically acceptable salt thereof.

- 25 In one aspect the invention provides the use of a compound of the invention, or a pharmaceutically acceptable salt thereof, in the inhibition of a 6-oxopurine phosphoribosyltransferase enzyme such as hypoxanthine-guanine-(xanthine) phosphoribosyltransferase (HG(X)PRT).

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In one aspect the invention provides the use of a compound of the invention, or a pharmaceutically acceptable salt thereof, in the manufacture of a medicament for the prevention or treatment of a microbial infection. In another aspect, the invention provides a method of preventing or treating a microbial infection comprising administering a therapeutically effective amount of a compound of the invention, or a pharmaceutically acceptable salt thereof, to a subject in need thereof. In a still further aspect the invention provides a compound according to the invention, or a pharmaceutically acceptable salt thereof, for use in the prevention or treatment of a microbial infection.

- 10 In yet a further aspect the invention provides a pharmaceutical composition comprising a therapeutically effective amount of a compound of the invention, or a pharmaceutically acceptable salt thereof, together with at least one pharmaceutically acceptable carrier or diluent. The invention also provides a combination comprising a therapeutically effective amount of a compound of the invention, or a pharmaceutically acceptable salt thereof, together with at least one other therapeutic agent.

In some embodiments, the compounds of the invention are selective inhibitors for *Pf*HGXPRT and/or *Pv*HGPRT over human HGPRT.

Detailed description of the invention

- 20 The new series of compounds described herein differ most significantly from other ANPs in that they comprise a nitrogen containing heterocyclic group such as pyrrolidine or piperidine which replaces the oxygen atom in the acyclic portion of the earlier molecules. Without wishing to be bound by theory, this heterocyclic group provides a branching point in the acyclic portion of the molecule such that the compound may bind at *three* closely spaced sub-sites of the target. This is believed to increase the binding potential of the compound which leads to a significant improvement in the affinity and/or selectivity for 6-oxopurine phosphoribosyltransferases such as hypoxanthine-guanine-(xanthine) phosphoribosyltransferase (HG(X)PRT). Additionally, the heterocyclic group provides rigidity between the purine base and the phosphonate groups which, when combined with the relative stereochemistry of the ring substituents, is believed improve orientation of the purine base and the phosphonate groups into the active site of the enzyme. The

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heterocyclic group also enables groups to be appended in a modular fashion, enabling the construction of a diverse range of molecular architectures with a diverse range of chemical groups, using a range of readily accessible synthetic techniques.

- 5 The compounds of the invention are particularly well suited to preventing or treating infections caused by those organisms that are auxotrophic for the purine ring present in nucleosides, and thus rely, or substantially rely, on the salvage pathway to provide the purine moiety for reproduction. In some embodiments such microorganisms possess no *de novo*, or substantially no *de novo*, pathway for purine synthesis.

10

In this specification a number of terms are used which are well known to a skilled addressee. Nevertheless, for the purposes of clarity a number of terms will be defined.

- For the compounds described herein R^p is taken to be a prodrug substituent. As used
15 herein the term prodrug refers to a masked form of a compound of the invention such that drug absorption and/or drug delivery into the target organism is typically enhanced. The highly polar phosphonate group (wherein R^2 and R^3 are OH), for example, may be masked with a more lipophilic group to enhance transport across cell membranes. The prodrug may be unmasked by cellular enzymes (including lipases, esterases, reductases, oxidases,
20 nucleases or amidases) or by chemical cleavage such as hydrolysis to release the compound of the invention after the prodrug has entered a cell in the target of interest.

- It has been found that the prodrugs of the compounds of the invention may provide certain advantages over the correspondingly unmasked compounds of the invention, such as
25 improved levels of uptake into the microorganism (prodrugs are not inhibitors of the enzymes activity or are weakly inhibitory). Examples of substituents which may be used to form prodrugs according to the invention include those substituents that may be cleaved *in vivo* to provide the compound of the invention with a phosphonate residue. As an example, a phosphoramidate moiety formed by condensing the amine group of an amino
30 acid with a phosphonate (or activated form thereof) may undergo hydrolysis *in vivo* to reform the phosphonate group. Accordingly, in some embodiments R^p may be an amino

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acid residue (including an ester derivative of an amino acid residue) or R^p may be an optionally substituted alkoxy group.

Suitable prodrug substituents include, but are not limited to: proteins; antibiotics (and
 5 antibiotic fragments); amino acids (D- and/or L-) including derivatives thereof (such as esters and amides), attached to the -P(O)- moiety in the compounds of the invention via a nitrogen atom or an oxygen atom; peptides (up to 10 amino acids) attached to the -P(O)- moiety in the compounds of the invention via a nitrogen atom or an oxygen atom; drug
 10 moieties attached to the -P(O)- moiety in the compounds of the invention via a nitrogen atom or an oxygen atom; steroids; cholesterol; folic acids; vitamins; polyamines; carbohydrates; polyethylene glycols (PEGs); cyclosaligenyls; substituted 4 to 8-membered rings, with or without heteroatom substitutions, with 1,3-phosphodiester, 1,3-phosphoramidate/phosphoester or 1,3-phosphoramidate attachments; acylthioethoxy, (SATE) $\text{RCOSCH}_2\text{CH}_2\text{O}-$; $\text{RCOSCH}_2\text{CH}_2\text{O-W-O}-$; $\text{RCOSCH}_2\text{CH}_2\text{O-W-S}-$;
 15 $\text{RCOSCH}_2\text{CH}_2\text{O-W-NH}-$; acyloxymethoxy, $\text{RCOOCH}_2\text{O}-$; $\text{RCOOCH}_2\text{O-W-O}-$; $\text{RCOOCH}_2\text{O-W-S}-$; $\text{RCOOCH}_2\text{O-W-NH}-$; alkoxycarbonyloxymethoxy, $\text{ROCOOCH}_2\text{O}-$; $\text{ROCOOCH}_2\text{O-W-O}-$; $\text{ROCOOCH}_2\text{O-W-S}-$; $\text{ROCOOCH}_2\text{O-W-NH}-$;
 acylthioethyldithioethoxy (DTE) $\text{RCOSCH}_2\text{CH}_2\text{SSCH}_2\text{CH}_2\text{O}-$;
 $\text{RCOSCH}_2\text{CH}_2\text{SSCH}_2\text{CH}_2\text{O-W-O}-$; $\text{RCOSCH}_2\text{CH}_2\text{SSCH}_2\text{CH}_2\text{O-W-S}-$;
 20 $\text{RCOSCH}_2\text{CH}_2\text{SSCH}_2\text{CH}_2\text{O-W-NH}-$; acyloxymethylphenylmethoxy (PAOB) $\text{RCO}_2\text{-C}_6\text{H}_4\text{-CH}_2\text{-O}-$; $\text{RCO}_2\text{-C}_6\text{H}_4\text{-CH}_2\text{-O-W-O}-$; $\text{RCO}_2\text{-C}_6\text{H}_4\text{-CH}_2\text{-O-W-S}-$; $\text{RCO}_2\text{-C}_6\text{H}_4\text{-CH}_2\text{-O-W-NH}-$; RCO_2- ; 1,2-*O*-diacyl-glyceroyloxy, $\text{RCOO-CH}_2\text{-CH(OCOR)-CH}_2\text{-O}-$; 1,2-*O*-dialkyl-glyceroyloxy, $\text{RO-CH}_2\text{-CH(OR)-CH}_2\text{-O}-$; 1,2-*S*-dialkyl-glyceroyloxy, $\text{RS-CH}_2\text{-CH(SR)-CH}_2\text{-O}-$; 1-*O*-alkyl-2-*O*-acyl-glyceroyloxy, $\text{RO-CH}_2\text{-CH(OCOR)-CH}_2\text{-O}-$; 1-*S*-alkyl-2-*O*-
 25 acyl-glyceroyloxy, $\text{RS-CH}_2\text{-CH(OCOR)-CH}_2\text{-O}-$; 1-*O*-acyl-2-*O*-alkyl-glyceroyloxy, $\text{RCOO-CH}_2\text{-CH(OR)-CH}_2\text{-O}-$; 1-*O*-acyl-2-*S*-alkyl-glyceroyloxy, $\text{RCOO-CH}_2\text{-CH(SR)-CH}_2\text{-O}-$; any substituent attached via a nitrogen or oxygen atom to the compound of the invention that liberates the phosphonate *in vivo*, wherein W is selected from alkyl, aryl, arylalkyl or a heterocycle and R is selected from alkyl, alkenyl, alkynyl, aryl, acyl and arylalkyl
 30 optionally containing one or more heteroatoms.

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Preferably the prodrug is a group attached to the -P(O)- moiety in the compounds of the invention via a nitrogen atom or an oxygen atom. Prodrug substituents can include a residue of an amino acid, including derivatives thereof (such as esters and amides). Especially preferred prodrugs of the compounds of the invention are phosphoramidates
5 formed from an alkyl ester of phenylalanine attached via a nitrogen atom to the -P(O)- moiety (such as the isopropyl or ethyl ester of phenylalanine, particularly (L)-phenylalanine) and phosphoesters formed from an alkyl group such as $\text{RCOSCH}_2\text{CH}_2\text{O-}$ (eg SATE), lipophilic esters (eg Hostetler esters) and pivaloyloxymethyl esters (POM).

10 The term "alkyl" includes saturated aliphatic groups, including straight-chain alkyl groups (e.g., methyl, ethyl, propyl, butyl, pentyl, hexyl, heptyl, octyl, nonyl, decyl, etc.) and branched-chain alkyl groups (isopropyl, tert-butyl, isobutyl, etc.). In some embodiments "alkyl" refers to straight chained alkyl. The expression " C_{x-y} alkyl", wherein x is 1-5 and y is 2-10 indicates an alkyl group (straight- or branched-chain) containing the specified
15 number of carbon atoms. For example, the expression C_{1-4} alkyl includes, but is not limited to, methyl, ethyl, propyl, butyl, isopropyl, tert-butyl and isobutyl. The term "alkylene" refers to a divalent alkyl group.

The term "alkenyl" includes unsaturated aliphatic groups analogous in length and possible
20 substitution to the alkyls described above, but which contain at least one double bond. For example, the term "alkenyl" includes straight-chain alkenyl groups (e.g., ethenyl, propenyl, butenyl, pentenyl, hexenyl, heptenyl, octenyl, nonenyl, decenyl, etc.) and branched-chain alkenyl groups. In some embodiments "alkenyl" refers to straight chained alkenyl. In certain embodiments, a straight chain or branched chain alkenyl group has 6 or
25 fewer carbon atoms in its backbone (e.g., $\text{C}_2\text{-C}_6$ for straight chain, $\text{C}_3\text{-C}_6$ for branched chain). Likewise, cycloalkenyl groups may have from 3-8 carbon atoms in their ring structure, and more preferably have 5 or 6 carbons in the ring structure. The term $\text{C}_2\text{-C}_6$ includes alkenyl groups containing 2 to 6 carbon atoms. The term "alkenylene" refers to a divalent alkenyl group.

30

The term "alkynyl" includes unsaturated aliphatic groups analogous in length and possible

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substitution to the alkyls described above, but which contain at least one triple bond. For example, the term "alkynyl" includes straight-chain alkynyl groups (e.g., ethynyl, propynyl, butynyl, pentynyl, hexynyl, heptynyl, octynyl, nonynyl, decynyl, etc.) and branched-chain alkynyl groups. In some embodiments "alkynyl" refers to straight chained
5 alkynyl. In certain embodiments, a straight chain or branched chain alkynyl group has 6 or fewer carbon atoms in its backbone (e.g., C₂-C₆ for straight chain, C₁-C₆ for branched chain). The term C₂-C₆ includes alkynyl groups containing 2 to 6 carbon atoms. The term "alkynylene" refers to a divalent alkynyl group.

10 The term "aryl" refers to aromatic monocyclic (eg phenyl) or polycyclic groups (e.g., tricyclic, bicyclic, e.g., naphthalene, anthryl, phenanthryl). Aryl groups can also be fused or bridged with alicyclic or heterocyclic rings which are not aromatic so as to form a polycycle (e.g., tetralin, methylenedioxyphenyl).

15 The term "heteroaryl", as used herein, represents a monocyclic or bicyclic ring, typically of up to 7 atoms in each ring, wherein at least one ring is aromatic and contains from 1 to 4 heteroatoms selected from the group consisting of O, N and S. Heteroaryl groups within the scope of this definition include but are not limited to: benzimidazole (otherwise known as benzoimidazole), acridinyl, carbazolyl, cinnolinyl, quinoxalinyl, pyrazolyl, indolyl,
20 benzotriazolyl, furanyl, thienyl, benzothienyl, benzofuranyl, quinolinyl, isoquinolinyl, oxazolyl, isoxazolyl, indoiyl, pyrazinyl, pyridazinyl, pyridinyl, pyrimidinyl, pyrrolyl, tetrahydroquinoline. As with the definition of heterocycle below, "heteroaryl" is also understood to include the N-oxide derivative of any nitrogen-containing heteroaryl. In cases where the heteroaryl substituent is bicyclic and one ring is non-aromatic or contains
25 no heteroatoms, it is understood that attachment is via the aromatic ring or via the heteroatom containing ring, respectively.

The term "acyl" includes compounds and moieties which contain the acyl radical (CH₃CO-) or a carbonyl group such as CH₃CH₂CH₂CO-.

30

The term "alkoxy" includes substituted and unsubstituted alkyl, alkenyl, and alkynyl

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groups covalently linked to an oxygen atom. Examples of alkoxy groups include methoxy, ethoxy, isopropoxy (isopropoxy), propoxy, butoxy, and pentoxy groups and may include cyclic groups such as cyclopentoxy.

- 5 The term "ester" includes compounds and moieties that contain a carbon or a heteroatom bound to an oxygen atom that is bonded to the carbon of a carbonyl group. The term "ester" includes alkoxycarboxy groups such as methoxycarbonyl, ethoxycarbonyl, propoxycarbonyl, butoxycarbonyl, pentoxycarbonyl, etc. The alkyl, alkenyl, or alkynyl groups are as defined above.

10

The term "halogen" includes fluorine, chlorine, bromine and iodine. In some embodiments halogen refers to fluorine or chlorine.

The term "heteroatom" includes atoms of any element other than carbon or hydrogen.

- 15 Preferred heteroatoms are nitrogen, oxygen, sulfur and phosphorus. Particularly preferred heteroatoms are nitrogen and oxygen.

- As used herein, the term "optionally substituted" typically refers to where a hydrogen atom on a group has been substituted with a non-hydrogen group. Any optionally substituted
20 group may bear one, two, three or more optional substituents.

- Additionally, any number of the listed functional groups and molecules may be combined to create a larger molecular architecture. For example, the terms "phenyl," "carbonyl" (or " $=O$ "), "-O-," "-OH," and C_1 alkyl and C_3 alkylene (i.e., $-CH_3$ and $-CH_2CH_2CH_2-$) can be
25 combined to form a 3-methoxy-4-propoxybenzoic acid substituent. In another example the terms aryl and alkyl can be combined to form an arylalkyl group, an example of which is a phenylmethyl group, otherwise known as a benzylic group. It is to be understood that when combining functional groups and molecules to create a larger molecular architecture, hydrogens can be removed or added, as required to satisfy the valence of each atom.

30

It is to be understood that all of the compounds of the invention described above will

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further include bonds between adjacent atoms and/or hydrogens as required to satisfy the valence of each atom. That is, double bonds and/or hydrogen atoms are typically added to provide the following number of total bonds to each of the following types of atoms: carbon: four bonds; nitrogen: three bonds; oxygen: two bonds; and sulfur: two, four or six
5 bonds. It is also to be understood that definitions given to the variables of the generic formulae described herein will result in molecular structures that are in agreement with standard organic chemistry definitions and knowledge, e.g., valency rules.

It will be understood that the compounds of the invention may exist in a plurality of
10 equivalent tautomeric forms. For the sake of clarity the compounds have been depicted as single tautomers, despite all such tautomeric forms being considered within the scope of the invention.

It will be noted that the structures of some of the compounds of the invention include
15 asymmetric carbon atoms. It is to be understood accordingly that the isomers arising from such asymmetry (e.g., all enantiomers, stereoisomers, rotamers, tautomers, diastereomers, or racemates) are included within the scope of this invention. The present invention includes within its scope all of these stereoisomeric forms either isolated (in, for example, enantiomeric isolation), or in combination (including racemic mixtures and diastereomic
20 mixtures).

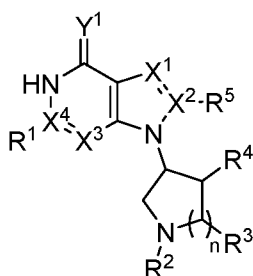
The invention thus also relates to compounds in substantially pure stereoisomeric form with respect to the asymmetric centres of the amino acid residues, e.g., greater than about 90% de, such as about 95% to 97% de, or greater than 99% de, as well as mixtures,
25 including racemic mixtures, thereof. The skilled person will appreciate that there are a range of techniques available to produce achiral compounds of the invention in racemic, enantioenriched or enantiopure forms. For example, enantioenriched or enantiopure forms of the compounds may be produced through stereoselective synthesis and/or through the use of chromatographic or selective recrystallisation techniques. In some embodiments the
30 compounds of the invention may be prepared by appending a natural or unnatural amino acid to the phosphonate group to form a prodrug. Accordingly, a racemic mixture of

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amino acids may be used to prepare a racemic mixture of a compound of the invention, an enantioenriched amino acid may be used to prepare an enantioenriched mixture of a compound of the invention and an enantiopure amino acid may be used to prepare an enantiopure compound of the invention.

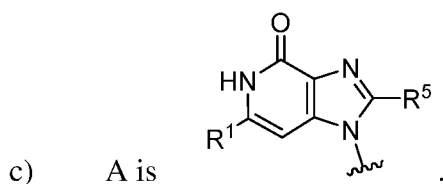
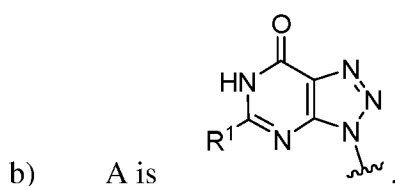
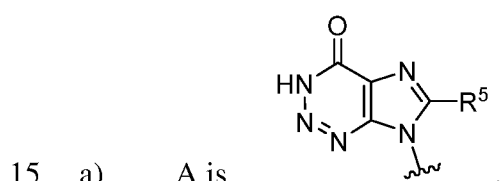
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In the compounds of the invention Y^1 where present is selected from S and O, and Y^2 where present is selected from halogen. In preferred embodiments the compound of the invention has the following formula where Y^1 is selected from S and O:

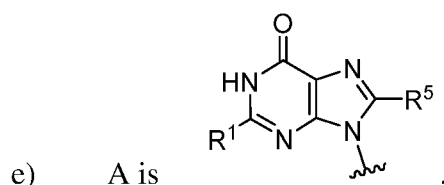
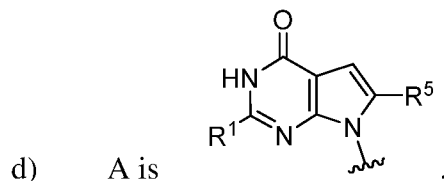


10 In even more preferred embodiments Y^1 is O.

In some embodiments of the invention, and with reference to the general formula (II), one or more of the following preferred embodiments apply:



- 15 -



5

f) R^1 is alkyl, aryl, heteroaryl, alkenyl, alkynyl, arylalkyl, heteroarylalkyl, NR^6_2 , halogen, OR^6 , H or NH_2 .

g) R^1 is H.

10

h) R^1 is NH_2 .

i) R^2 is H.

15 j) R^2 is $-C(O)L^1P(O)R^7R^8$.

k) R^2 is $-C(O)L^2P(O)R^7R^8$.

20 l) The or each R^3 is independently selected from H, $-L^1OH$, $-L^1OR^6$, $-CH_2OC(O)L^1P(O)R^7R^8$, $-CH_2C(O)L^1P(O)R^7R^8$, $-OC(O)L^1P(O)R^7R^8$, and $-OL^1P(O)R^7R^8$.

m) $n = 2$ wherein one R^3 is H and the other R^3 is H, $-L^1OH$, $-L^1OR^6$, $-CH_2OC(O)L^1P(O)R^7R^8$ or $-CH_2C(O)L^1P(O)R^7R^8$.

25

n) $n = 1$ and R^3 is H, $-L^1OH$, $-L^1OR^6$,

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$-\text{CH}_2\text{OC(O)L}^1\text{P(O)R}^7\text{R}^8$ or $-\text{CH}_2\text{C(O)L}^1\text{P(O)R}^7\text{R}^8$.

- o) $n = 1$ and R^3 is H.
- 5 p) R^4 is $-\text{OC(O)L}^2\text{P(O)R}^7\text{R}^8$, $-\text{OL}^1\text{P(O)R}^7\text{R}^8$ or $-\text{NHC(O)L}^1\text{P(O)R}^7\text{R}^8$.
- q) R^4 is $-\text{C(O)L}^1\text{P(O)R}^7\text{R}^8$ when R^2 is $-\text{C(O)L}^2\text{P(O)R}^7\text{R}^8$.
- r) R^4 is H when R^3 is other than H.
- 10 s) R^5 is H, alkyl, aryl, heteroaryl, NR^6_2 , halogen or OR^6 .
- t) R^5 is H.
- 15 u) R^7 and R^8 are independently selected from OH, OR^6 , SR^6 , NHR^6 , NR^6_2 , and R^p .
- v) the or each R^6 is independently selected from alkyl, alkenyl, alkynyl, aryl, acyl and arylalkyl optionally containing one or more heteroatoms.
- 20 w) R^7 and R^8 are independently selected from OH, OR^6 and R^p , wherein R^6 is C_{1-3} alkyl.
- x) R^7 and R^8 are OH.
- 25 y) R^7 and R^8 are R^p .
- z) R^p is an amino acid residue, or derivative or ester thereof, or an optionally substituted alkoxy group.
- 30 aa) R^p is a C_{1-3} alkyl ester derivative of phenylalanine or $-\text{OCH}_2\text{CH}_2\text{SC(O)R}$ (SATE) wherein R is C_{1-4} alkyl.

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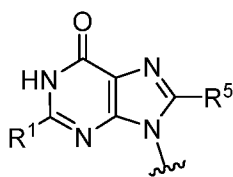
ab) L^1 C_{1-4} alkylene, C_{2-4} alkenylene or C_{2-4} alkynylene optionally substituted with one or more OH, C_{1-4} alkyl, C_{2-4} alkenyl or C_{2-4} alkynyl.

5 ac) L^1 is C_{1-4} alkylene.

ad) L^2 is C_{2-4} alkylene, C_{2-4} alkenylene or C_{2-4} alkynylene optionally substituted with one or more OH, C_{1-4} alkyl, C_{2-4} alkenyl or C_{2-4} alkynyl.

10 ae) L^2 is C_{2-4} alkylene.

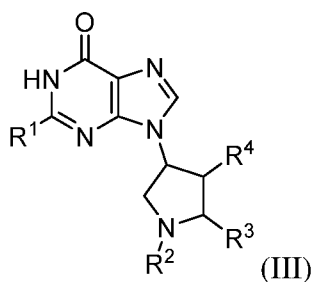
af) L^1 is methylene or ethylene and L^2 is ethylene.



In a preferred embodiment A is where R^5 is H.

15

Accordingly, in a further embodiment, the present invention provides compounds of the formula (II) represented by formula (III):



wherein:

20 R^1 is H or NH_2 ;

R^2 is H, $-C(O)L^1P(O)R^7R^8$ or $-C(O)L^2P(O)R^7R^8$;

R^3 is H;

R^4 is $-OC(O)L^2P(O)R^7R^8$, $-OL^1P(O)R^7R^8$ or $-NHC(O)L^1P(O)R^7R^8$; or

R^4 is $-C(O)L^1P(O)R^7R^8$ when R^2 is $-C(O)L^2P(O)R^7R^8$;

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R^7 and R^8 are independently selected from OH, OR^6 , SR^6 , NHR^6 , NR^6_2 , and R^p ;

R^p is a prodrug substituent;

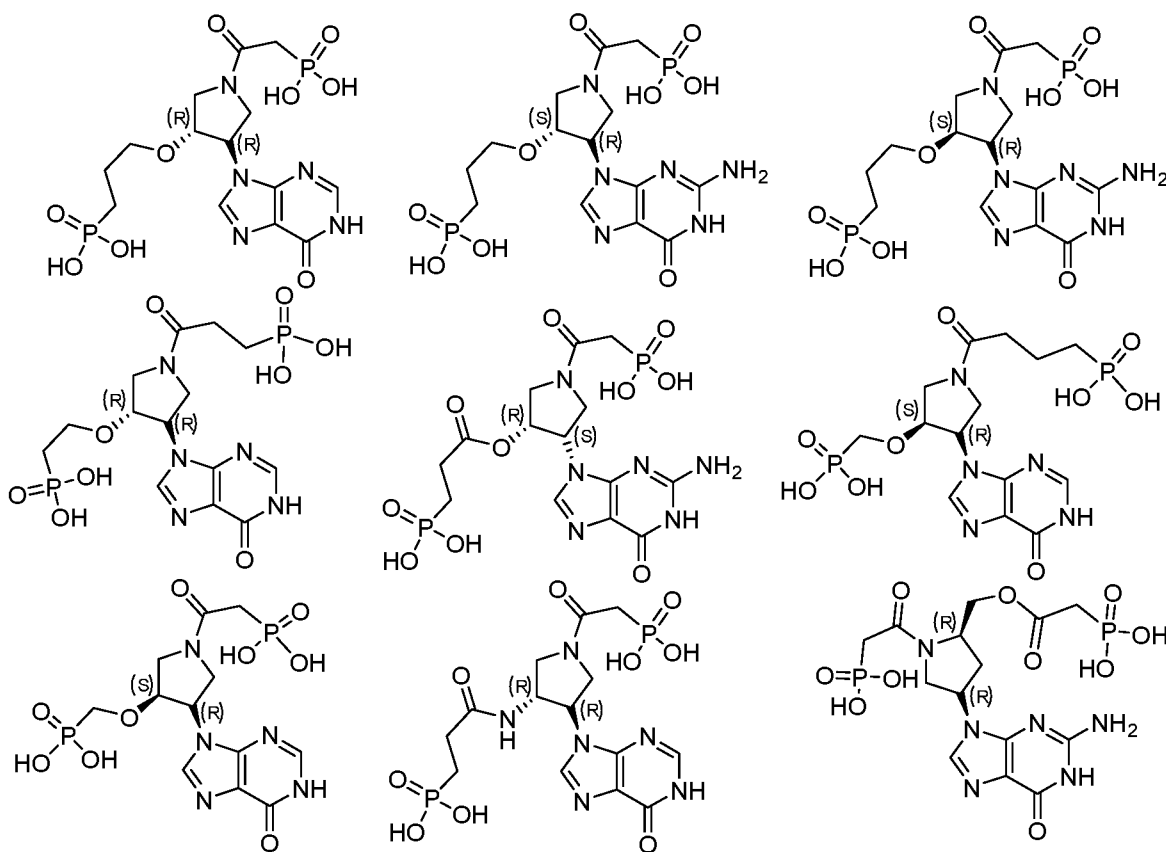
the or each R^6 , where present, is independently selected from alkyl, alkenyl, alkynyl, aryl, acyl and arylalkyl optionally containing one or more heteroatoms;

5 L^1 is C_{1-4} alkylene; and

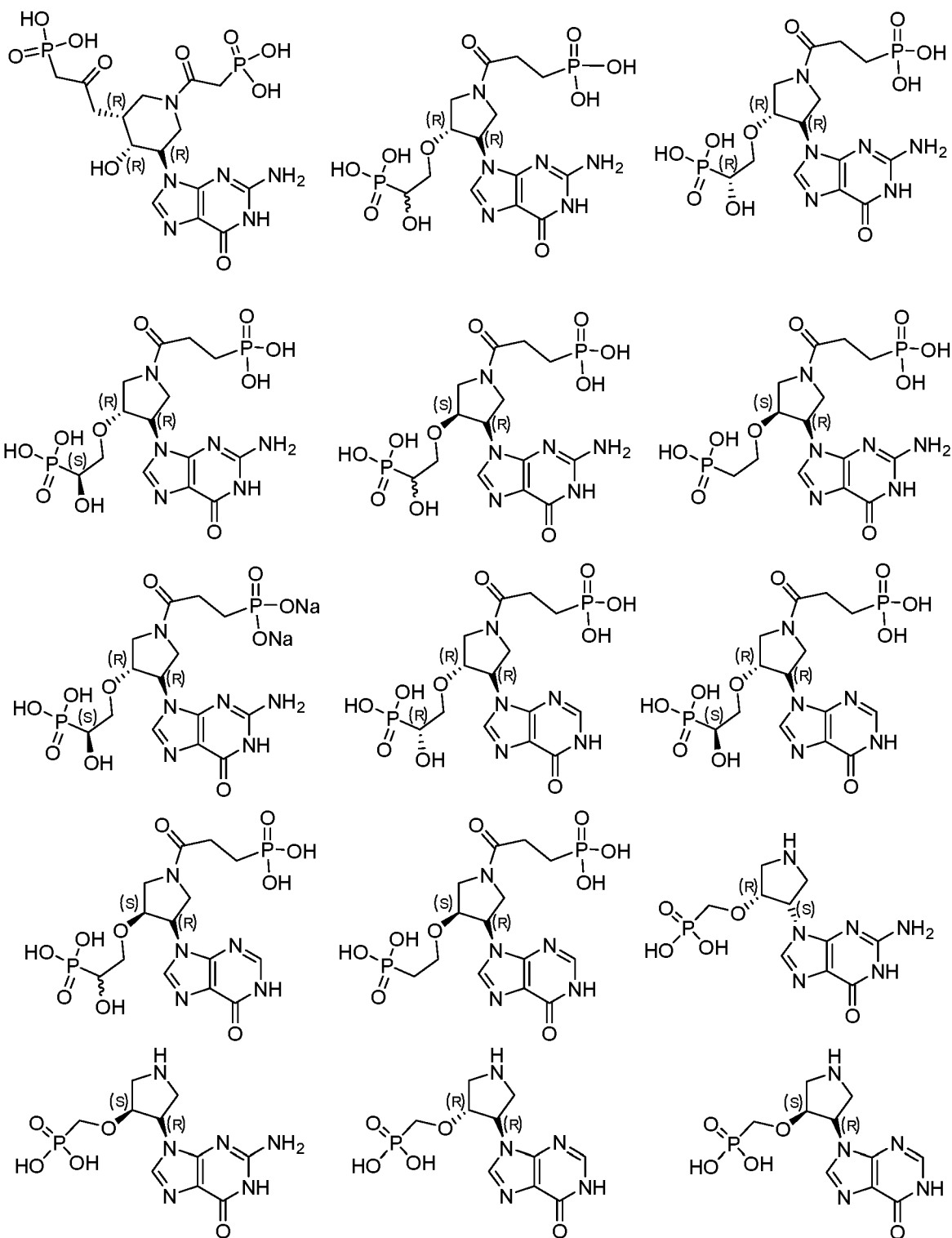
L^2 is C_{2-4} alkylene; or

or a pharmaceutically acceptable salt thereof.

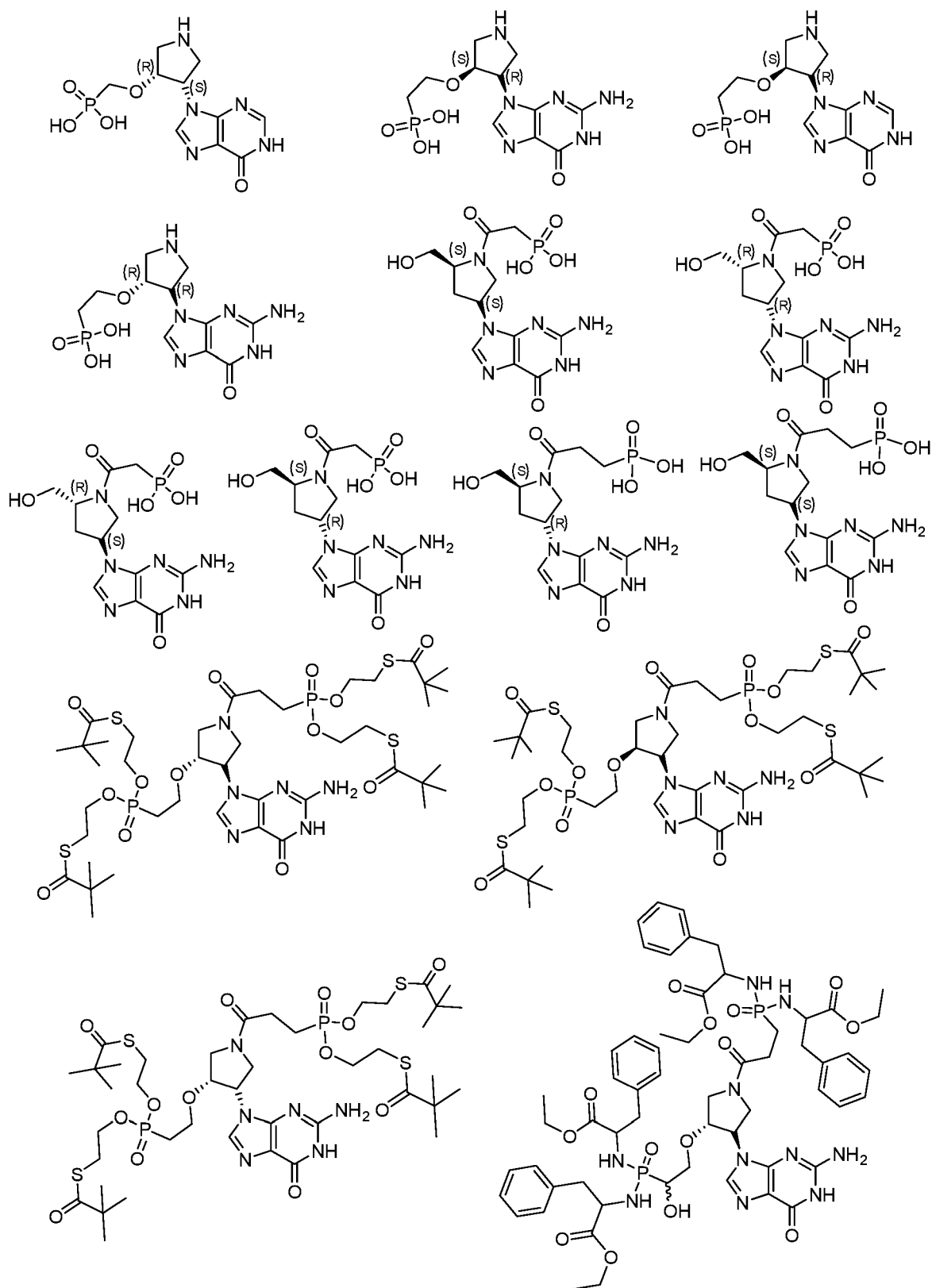
In a preferred embodiment, the compound of formula (Ia) is selected from:



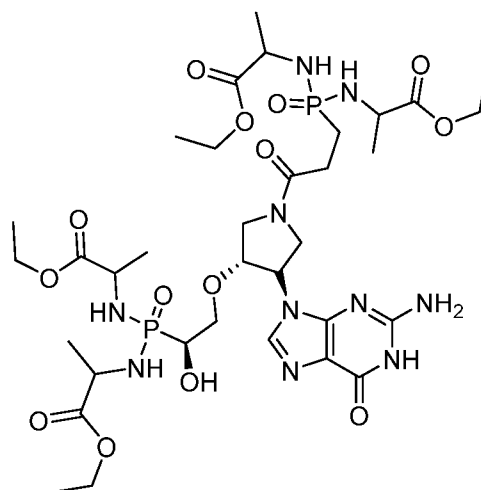
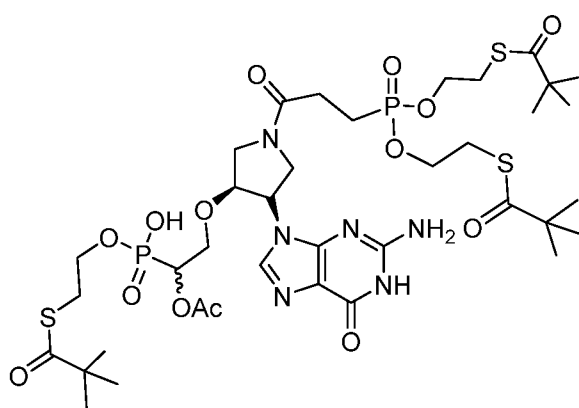
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The compounds and methods of the present invention may be used in the treatment and/or prevention of a range of microbial infections. As used herein, treatment may include
 5 alleviating or ameliorating the symptoms, diseases or conditions associated with the microbial infection being treated, including reducing the severity and/or frequency of the microbial infection. As used herein, prevention may include preventing or delaying the onset of, inhibiting the progression of, or halting or reversing altogether the onset or progression of the particular symptoms, disease or condition associated with a microbial
 10 infection.

The terms "microbial", "microorganism", etc includes any microscopic organism or taxonomically related macroscopic organism within the categories algae, bacteria, fungi and protozoa or the like. Preferably the microorganism is a bacteria or protozoa, most
 15 preferably a protozoa. In this respect the present invention is predicated, in part, on the susceptibility of microorganisms to inhibition of 6-oxopurine phosphoribosyltransferases such as the hypoxanthine-guanine-(xanthine) phosphoribosyltransferase (HG(X)PRT) enzyme involved in the purine salvage pathway. Accordingly the compounds of the invention are particularly well suited to those organisms that are auxotrophic for the purine
 20 ring present in nucleosides, and thus rely, or substantially rely, on the salvage pathway to provide the purine moiety for reproduction. To this end it is understood that there are known techniques (such as genomic sequencing or gene deletion experiments) that may be used to determine whether a microorganism is reliant, or substantially reliant, on the

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salvage pathway for reproduction. In some embodiments the compounds of the invention are particularly well suited to preventing or treating a microbial infection caused by a microorganism that cannot sustain itself in the absence of the salvage pathway. In some embodiments such microorganisms possess no *de novo*, or substantially no *de novo*,
5 pathway for purine synthesis.

The bacterial infection may be caused by one or more species selected from one or more of the Gram-negative bacterial genera: *Acinetobacter*; *Actinobacillus*; *Bartonella*; *Bordetella*; *Brucella*; *Burkholderia*; *Campylobacter*; *Cyanobacteria*; *Enterobacter*; *Erwinia*;
10 *Escherichia*; *Francisella*; *Helicobacter*; *Hemophilus*; *Klebsiella*; *Legionella*; *Moraxella*; *Morganella*; *Mycobacterium*; *Neisseria*; *Pasteurella*; *Proteus*; *Providencia*; *Pseudomonas*; *Salmonella*; *Serratia*; *Shigella*; *Stenotrophomonas*; *Treponema*; *Vibrio*; and *Yersinia*. Specific examples include, but are not limited to, infections caused by *Helicobacter pylori* and uropathogenic *Escherichia coli*.

15

The bacterial infection may be caused by one or more species selected from one or more of the Gram-positive bacterial genera: *Actinobacteria*; *Bacillus*; *Clostridium*; *Corynebacterium*; *Enterococcus*; *Listeria*; *Nocardia*; *Staphylococcus*; and *Streptococcus*.

20 Protozoal infections include, but are not limited to, infections caused by *Leishmania*, *Toxoplasma*, *Plasmodia* (which are understood to be the causative agent(s) of malarial infection), *Theileria*, *Anaplasma*, *Giardia*, *Tritrichomonas*, *Trypanosoma*, *Schistosoma*, *Coccidia*, and *Babesia*. Specific examples include *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium malariae*, *Plasmodium knowlesi*, *Plasmodium ovale* and *Giardia*
25 *lamblia*.

In this respect, it is believed that the compounds of the invention are particularly well suited to the prevention or treatment of infections caused by *Plasmodia* spp. (especially *Plasmodium falciparum* and *Plasmodium vivax*), *Giardia* spp. (especially *Giardia lamblia*),
30 *Trypanosoma* spp., *Schistosoma* spp., *Helicobacter* spp. (such as *Helicobacter pylori*), *Mycobacterium tuberculosis*, and certain uropathogenic *Escherichia coli*.

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It will be understood that certain nucleosides may modulate the activity of one or more kinases, including certain nucleosides bearing saccharide and/or polyphosphate moieties (for example diphosphate or triphosphate moieties). To the extent that it is preferable that
5 the compounds of the invention do not adversely modulate the activity of one or more kinases, such compounds do not fall within the scope of the invention.

Examples of microbial infections include bacterial or fungal wound infections, mucosal infections, enteric infections, septic conditions, pneumonia, trachoma, ornithosis,
10 trichomoniasis, fungal infections and salmonellosis, such as in veterinary practice. The compounds of the invention may also be used for the treatment of resistant microbial species or in various fields where antiseptic treatment or disinfection of materials is required, for example, surface disinfection.

15 The term "subject" is intended to include organisms such as mammals, *e.g.* humans, dogs, cows, horses, pigs, sheep, goats, cats, mice, rabbits, rats, and transgenic non-human animals. In certain embodiments, the subject is a human, *e.g.* a human suffering from, at risk of suffering from, or potentially capable of suffering from a microbial infection. In another embodiment, the subject is a cell.

20 The compounds of the invention may be in crystalline form or as solvates (*e.g.* hydrates) and it is intended that both forms are within the scope of the present invention. The term "solvate" is a complex of variable stoichiometry formed by a solute (in this invention, a compound of the invention) and a solvent. Such solvents should preferably not interfere
25 with the biological activity of the solute. Solvents may be, by way of example, water, acetone, ethanol or acetic acid. Methods of solvation are generally known within the art.

The present invention also provides a pharmaceutical composition comprising a therapeutically effective amount of a compound as hereinbefore defined, or a
30 pharmaceutically acceptable salt thereof, together with at least one pharmaceutically acceptable carrier or diluent.

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Pharmaceutically acceptable acid addition salts may be prepared from inorganic and organic acids. Examples of inorganic acids include hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid, phosphoric acid and the like. Examples of organic acids include acetic acid, propionic acid, glycolic acid, pyruvic acid, oxalic acid, malic acid, malonic acid, succinic acid, maleic acid, fumaric acid, tartaric acid, citric acid, benzoic acid, cinnamic acid, mandelic acid, methanesulfonic acid, ethanesulfonic acid, p-toluenesulfonic acid, salicylic acid and the like. For example, the nitrogen atom in the acyclic portion of the compounds of the invention may undergo reaction with an acid to form the acid addition salt.

10

Pharmaceutically acceptable base addition salts may be prepared from inorganic and organic bases. Corresponding counterions derived from inorganic bases include the sodium, potassium, lithium, ammonium, calcium and magnesium salts. Organic bases include primary, secondary and tertiary amines, substituted amines including naturally-occurring substituted amines, and cyclic amines, including isopropylamine, trimethylamine, diethylamine, triethylamine, tripropylamine, ethanolamine, 2-dimethylaminoethanol, tromethamine, lysine, arginine, histidine, caffeine, procaine, hydrabamine, choline, betaine, ethylenediamine, glucosamine, N-alkylglucamines, theobromine, purines, piperazine, piperidine, and N-ethylpiperidine. For example, where the compound of the invention possesses a phosphonate group the compound may undergo reaction with a base to form the base addition salt.

20

Acid/base addition salts tend to be more soluble in aqueous solvents than the corresponding free acid/base forms.

25

The term "composition" is intended to include the formulation of an active ingredient with encapsulating material as carrier, to give a capsule in which the active ingredient (with or without other carrier) is surrounded by carriers.

30

While the compounds as hereinbefore described, or pharmaceutically acceptable salt thereof, may be the sole active ingredient administered to the subject, the administration of

- 25 -

other active ingredient(s) with the compound is within the scope of the invention. For example, the compound could be administered with one or more therapeutic agents in combination. The combination may allow for separate, sequential or simultaneous administration of the compound as hereinbefore described with the other active
5 ingredient(s). The combination may be provided in the form of a pharmaceutical composition.

As will be readily appreciated by those skilled in the art, the route of administration and the nature of the pharmaceutically acceptable carrier will depend on the nature of the
10 condition and the mammal to be treated. It is believed that the choice of a particular carrier or delivery system, and route of administration could be readily determined by a person skilled in the art. In the preparation of any formulation containing the compound care should be taken to ensure that the activity of the compound is not destroyed in the process and that the compound is able to reach its site of action without being destroyed.
15 In some circumstances it may be necessary to protect the compound by means known in the art, such as, for example, micro encapsulation or coating (such as the use of enteric coating). Similarly the route of administration chosen should be such that the compound reaches its site of action.

20 Those skilled in the art may readily determine appropriate formulations for the compounds of the present invention using conventional approaches. Identification of preferred pH ranges and suitable excipients, for example antioxidants, is routine in the art. Buffer systems are routinely used to provide pH values of a desired range and include carboxylic acid buffers for example acetate, citrate, lactate and succinate. A variety of antioxidants
25 are available for such formulations including phenolic compounds such as BHT or vitamin E, reducing agents such as methionine or sulphite, and metal chelators such as EDTA.

The compounds as hereinbefore described, or pharmaceutically acceptable salt thereof, may be prepared in parenteral dosage forms, including those suitable for intravenous,
30 intrathecal, and intracerebral or epidural delivery. The pharmaceutical forms suitable for injectable use include sterile injectable solutions or dispersions, and sterile powders for the

extemporaneous preparation of sterile injectable solutions. They should be stable under the conditions of manufacture and storage and may be preserved against reduction or oxidation and the contaminating action of microorganisms such as bacteria or fungi.

- 5 The solvent or dispersion medium for the injectable solution or dispersion may contain any of the conventional solvent or carrier systems for the compound, and may contain, for example, water, ethanol, polyol (for example, glycerol, propylene glycol and liquid polyethylene glycol, and the like), suitable mixtures thereof, and vegetable oils. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by
- 10 the maintenance of the required particle size in the case of dispersion and by the use of surfactants. The prevention of the action of microorganisms can be brought about where necessary by the inclusion of various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal and the like. In many cases, it will be preferable to include agents to adjust osmolarity, for example, sugars or sodium
- 15 chloride. Preferably, the formulation for injection will be isotonic with blood. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminium monostearate and gelatin. Pharmaceutical forms suitable for injectable use may be delivered by any appropriate route including intravenous, intramuscular, intracerebral, intrathecal, epidural
- 20 injection or infusion.

- Sterile injectable solutions are prepared by incorporating the active compound in the required amount in the appropriate solvent with various of the other ingredients such as those enumerated above, as required, followed by filtered sterilization. Generally,
- 25 dispersions are prepared by incorporating the various sterilised active ingredient into a sterile vehicle which contains the basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, preferred methods of preparation are vacuum drying or freeze-drying of a previously sterile-filtered solution of the active ingredient plus
- 30 any additional desired ingredients.

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Other pharmaceutical forms include oral and enteral formulations of the present invention, in which the active compound may be formulated with an inert diluent or with an assimilable edible carrier, or it may be enclosed in hard or soft shell gelatin capsule, or it may be compressed into tablets, or it may be incorporated directly with the food of the diet. For oral therapeutic administration, the active compound may be incorporated with excipients and used in the form of ingestible tablets, buccal or sublingual tablets, troches, capsules, elixirs, suspensions, syrups, wafers, and the like. The amount of active compound in such therapeutically useful compositions is such that a suitable dosage will be obtained.

10

The tablets, troches, pills, capsules and the like may also contain the components as listed hereafter: a binder such as gum, acacia, corn starch or gelatin; excipients such as dicalcium phosphate; a disintegrating agent such as corn starch, potato starch, alginic acid and the like; a lubricant such as magnesium stearate; and a sweetening agent such a sucrose, lactose or saccharin may be added or a flavouring agent such as peppermint, oil of wintergreen, or cherry flavouring. When the dosage unit form is a capsule, it may contain, in addition to materials of the above type, a liquid carrier. Various other materials may be present as coatings or to otherwise modify the physical form of the dosage unit. For instance, tablets, pills, or capsules may be coated with shellac, sugar or both. A syrup or elixir may contain the active compound, sucrose as a sweetening agent, methyl and propylparabens as preservatives, a dye and flavouring such as cherry or orange flavour. Of course, any material used in preparing any dosage unit form should be pharmaceutically pure and substantially non-toxic in the amounts employed. In addition, the active compound may be incorporated into sustained-release preparations and formulations, including those that allow specific delivery of the active compound to specific regions of the gut.

Liquid formulations may also be administered enterally via a stomach or oesophageal tube.

30 Enteral formulations may be prepared in the form of suppositories by mixing with appropriate bases, such as emulsifying bases or water-soluble bases. It is also possible, but

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not necessary, for the compounds of the present invention to be administered topically, intranasally, intravaginally, intraocularly and the like.

The present invention also extends to any other forms suitable for administration, for example topical application such as creams, lotions and gels, or compositions suitable for
5 inhalation or intranasal delivery, for example solutions, dry powders, suspensions or emulsions.

The compounds of the present invention may be administered by inhalation in the form of
10 an aerosol spray from a pressurised dispenser or container, which contains a propellant such as carbon dioxide gas, dichlorodifluoromethane, nitrogen, propane or other suitable gas or combination of gases. The compounds may also be administered using a nebuliser.

Pharmaceutically acceptable vehicles and/or diluents include any and all solvents,
15 dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents and the like. The use of such media and agents for pharmaceutical active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active ingredient, use thereof in the therapeutic compositions is contemplated. Supplementary active ingredients can also be incorporated into the
20 compositions.

It is especially advantageous to formulate the compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the mammalian subjects to be
25 treated; each unit containing a predetermined quantity of active material calculated to produce the desired therapeutic effect in association with the required pharmaceutically acceptable vehicle. The specification for the novel dosage unit forms of the invention are dictated by and directly dependent on (a) the unique characteristics of the active material and the particular therapeutic effect to be achieved, and (b) the limitations inherent in the
30 art of compounding active materials for the treatment of disease in living subjects having a diseased condition in which bodily health is impaired as herein disclosed in detail.

As mentioned above the principal active ingredient may be compounded for convenient and effective administration in therapeutically effective amounts with a suitable pharmaceutically acceptable vehicle in dosage unit form. A unit dosage form can, for example, contain the principal active compound in amounts ranging from 0.25 μ g to about 200 mg. Expressed in proportions, the active compound may be present in concentrations ranging from about 0.25 μ g to about 200 mg/mL of carrier. In the case of compositions containing supplementary active ingredients, the dosages are determined by reference to the usual dose and manner of administration of the said ingredients.

10

As used herein, the term "effective amount" refers to an amount of compound which, when administered according to a desired dosing regimen, provides the desired therapeutic activity. Dosing may occur once, or at intervals of minutes or hours, or continuously over any one of these periods. Suitable dosages may lie within the range of about 0.1 ng per kg of body weight to 1 g per kg of body weight per dosage. A typical dosage is in the range of 1 μ g to 1 g per kg of body weight per dosage, such as is in the range of 1 mg to 1 g per kg of body weight per dosage. In one embodiment, the dosage may be in the range of 1 mg to 500 mg per kg of body weight per dosage. In another embodiment, the dosage may be in the range of 1 mg to 250 mg per kg of body weight per dosage. In yet another embodiment, the dosage may be in the range of 1 mg to 100 mg per kg of body weight per dosage, such as up to 50 mg per body weight per dosage.

15

Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise", and variations such as "comprises" and "comprising", will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integers or steps.

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The reference in this specification to any prior publication (or information derived from it), or to any matter which is known, is not, and should not be taken as an acknowledgment or admission or any form of suggestion that that prior publication (or information derived

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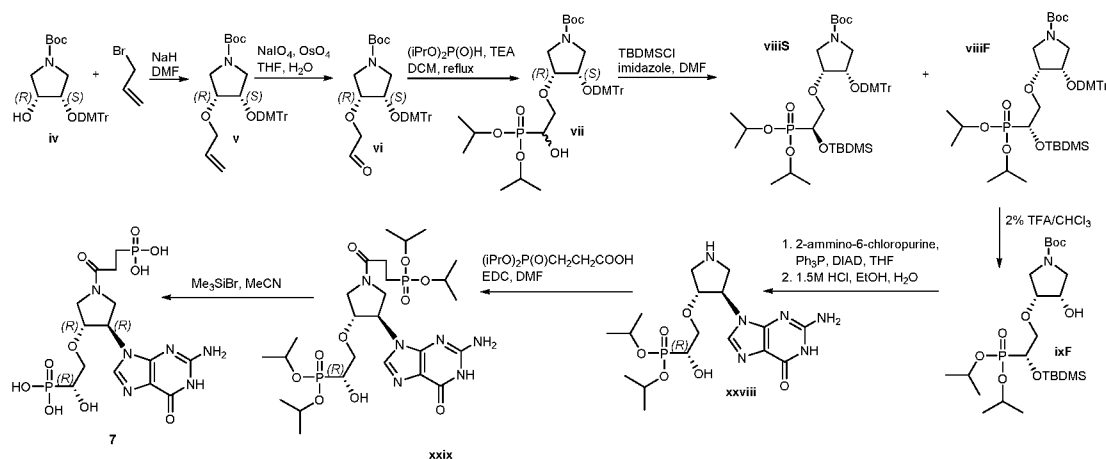
from it) or known matter forms part of the common general knowledge in the field of endeavour to which this specification relates.

The invention will now be described with reference to some specific examples and drawings. However, it is to be understood that the particularity of the following description is not to supersede the generality of the invention as hereinbefore described.

Examples

10 *Methods for Preparing Compounds of the General Formula (Ia) and (Ib).*

The following examples are representative of the present invention, and provide detailed methods for preparing exemplary compounds of the present invention.



15 **Scheme 1**

One general synthetic approach to prepare compounds of the invention is shown above in Scheme 1 with respect to Compound 7. As can be seen, synthesis began with partially protected dihydroxypyrrolidine derivative **iv**, which was alkylated with allyl bromide in DMF in the presence of sodium hydride. Subsequently, obtained allyl derivative **v** is oxidized with NaIO₄/OsO₄ system to aldehyde **vi**. Addition of diisopropyl phosphite to aldehyde **vi** affords a mixture of diastereomeric phosphonates **vii**. The mixture is silylated with *tert*-butyldimethylsilyl chloride under imidazole catalysis affording after silica gel column chromatography separation of both diastereoisomers **viiiS** and **viiiF** in pure form.

DMTr protecting group from **viiiF** is removed by treatment with 2% TFA in chloroform. Obtained derivative **ixF** undergoes Mitsunobu alkylation with 2-amino-6-chloropurine and subsequently acid hydrolysis with hydrochloric acid resulting in guanine derivative **xxvii**. Condensation with diisopropyl phosphonopropionic acid catalyzed by EDC affords
5 intermediate **xxix**. In the final step, all phosphonate ester groups are removed by reaction with bromotrimethylsilane affording final product **7**.

General methods:

Unless stated otherwise, all solvents used were anhydrous. TLC was performed on silica
10 gel pre-coated aluminium plates Silica gel/TLC-cards, UV 254 (Fluka) and compounds were detected by UV light (254 nm), by heating (detection of dimethoxytrityl group; orange color), by spraying with 1% solution of ninhydrine to visualize amines, or by spraying with 1% solution of 4-(4-nitrobenzyl)pyridine in ethanol followed by heating and treating with gaseous ammonia (blue color of mono- and diesters of phosphonic acid).
15 Preparative column chromatography was carried out on silica gel (40-60 μ m; Fluka) and elution was performed at the flow rate of 40 ml/min. The following solvent systems were used for TLC and preparative chromatography: toluene-ethyl acetate 1:1 (T); chloroform-ethanol 9:1 (C1); ethyl acetate-acetone-ethanol-water 6:1:1:0.5 (H3); ethyl acetate-acetone-ethanol-water 4:1:1:1 (H1). The concentrations of solvent systems are
20 stated in volume percent (% v/v). Purity of prepared compounds was determined by LC-MS performed on Waters Auto Purification System with 2545 Quaternary Gradient Module and 3100 Single Quadrupole Mass Detector using LUNA C18 column (Phenomenex, 100 x 4.6 mm, 3 μ m) at a flow rate of 1 ml/min. Typical conditions were: mobile phase, A - 50mM NH_4HCO_3 ; B - 50 mM NH_4HCO_3 in 50% aq. CH_3CN ; C –
25 CH_3CN ; A $\rightarrow$ B/10 min, B $\rightarrow$ C/10 min, C/5 min. Preparative reverse phase HPLC (rpHPLC) was performed on a LC5000 Liquid Chromatograph (INGOS-PIKRON, CR) using a Luna C18 (2) column (250 x 21.2 mm, 5 μ m) at a flow rate of 10 ml/min by gradient elution of methanol in 0.1M TEAB pH 7.5 (A = 0.1M TEAB; B = 0.1M TEAB in 50% aq. methanol; C = methanol) or without buffer. Final compounds were lyophilized
30 from water. Mass spectra were recorded on LTQ Orbitrap XL (Thermo Fisher Scientific) using ESI ionization. NMR spectra were measured on Bruker AVANCE 400 (^1H at 400

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MHz, ^{13}C at 100.6 MHz), Bruker AVANCE 500 and Varian UNITY 500 (^1H at 500 MHz, ^{13}C at 125.8 MHz) spectrometers. D_2O (reference (dioxane) = ^1H 3.75 ppm, ^{13}C 69.3 ppm. Chemical shifts (in ppm, δ scale) were referenced to TMS as an internal standard; coupling constants (J) are given in Hz. All intermediates were determined by LC-MS.

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1-N-Boc-3-dimethoxytrityloxy-4-hydroxypyrrolidines (all configurations) were prepared according to literature procedure (Rejman D., *et al*, Synthesis of diastereomeric 3-hydroxy-4-pyrrolidinyl derivatives of nucleobases, *Tetrahedron*. 2007; 63(5):1243-53).

10 General procedures

General procedure A – removal of esters from phosphonates

Me_3SiBr (0.66 ml, 5 mmol) was added to the dialkyl phosphonate (1 mmol) in MeCN (10 ml) under strictly anhydrous conditions. The reaction mixture was stirred at rt under argon atmosphere overnight. The reaction mixture was concentrated *in vacuo*, dissolved in 0.5M TEAB (5 ml) and concentrated *in vacuo*. Final product was obtained by preparative rpHPLC, converted to its sodium salt by passing through a column of Dowex 50 in Na^+ form and lyophilized from water.

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General procedure B – removal of esters from bisphosphonates

Me_3SiBr (0.92 ml, 7 mmol) was added to the tetraalkyl bisphosphonate (1 mmol) in MeCN (10 ml) under strictly anhydrous conditions. The reaction mixture was stirred at rt under an atmosphere of argon overnight. The reaction mixture was concentrated *in vacuo*, dissolved in 0.5M TEAB (5 ml) and concentrated *in vacuo*. Final product was obtained by preparative rpHPLC, converted to its sodium salt by passing through a column of Dowex 50 in Na^+ form and lyophilized from water.

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General procedure C – Mitsunobu nucleosidation with 6-chloropurine

DIAD (0.79 ml, 4 mmol) was added to the solution of Ph_3P (1.1 g, 4 mmol) in THF (10 ml) at rt under argon atmosphere. The reaction mixture was stirred until a white precipitate appeared (~20 min). A mixture of 6-chloropurine (0.23 g, 1.5 mmol) and OH component (1 mmol) in THF (10 ml) was added and the reaction mixture was stirred at rt

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under an atmosphere of argon overnight. The reaction mixture was concentrated *in vacuo*, and the desired product was obtained by chromatography on silica gel using linear gradient of ethanol in CHCl_3 .

5 General procedure D – Mitsunobu nucleosidation with 2-amino-6-chloropurine

DIAD (0.79 ml, 4 mmol) was added to the solution of Ph_3P (1.1 g, 4 mmol) in THF (10 ml) at rt under an argon atmosphere. The reaction mixture was stirred until a white precipitate appeared (~20 min). A mixture of 2-amino-6-chloropurine (0.25 g, 1.5 mmol) and OH component (1 mmol) in THF (10 ml) was added and the reaction mixture was
10 stirred at rt under an atmosphere of argon overnight. The reaction mixture was concentrated *in vacuo*, and the desired product was obtained by chromatography on silica gel using linear gradient of ethanol in CHCl_3

General procedure E – Removal of Boc protecting group and hydrolysis of 6-chloropurine
15 or 2-amino-6-chloropurine derivative to hypoxanthine or guanine derivative respectively
Boc protected 6-chloropurine or 2-amino-6-chloropurine pyrrolidine intermediate (1 mmol) was dissolved in ethanol (15 ml) and 3M aq. HCl (15 ml) was added. The reaction mixture was stirred overnight at 75 °C. The mixture was diluted with water (30 ml) and applied on a column of Dowex 50 in H^+ form (50 ml). The column was washed with
20 ethanol:water (1:1) mixture (100 ml) and crude product was eluted with 3% NH_3 in ethanol:water (1:1) (~150 ml, UV absorption was monitored). The crude material was purified by rpHPLC using a linear gradient of methanol in water.

General procedure F – Attachment of phosphonoalkylcarboxylic acid to 1-N nitrogen atom
25 of a pyrrolidine derivative

EDC (0.77 g, 4 mmol) was added to the mixture of diisopropyl 2-phosphonoacetic (0.29 g, 1.2 mmol) or 3- phosphonopropionic (0.27 g, 1.2 mmol) acid and pyrrolidine intermediate (1 mmol) in DMF (10 ml). The reaction mixture was stirred at 80 °C for 2h, concentrated *in vacuo*, and the desired product was obtained by chromatography on silica gel using
30 linear gradient of H1 in ethyl acetate.

General procedure G – Alkylation of hydroxyl group of protected dihydroxypyrrolidine with diethyl 3-bromopropylphosphonate

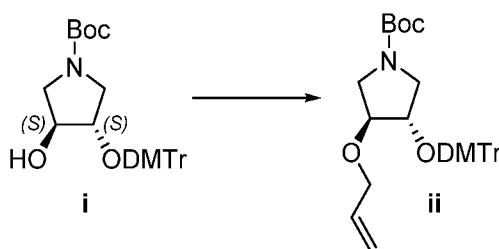
Sodium hydride (60 mg, 1.5 mmol) was added to the mixture of protected dihydroxypyrrolidine (1 mmol) and diethyl 3-bromopropylphosphonate (0.39 g, 1.5 mmol) in DMF (10 ml) under an argon atmosphere. The reaction mixture was stirred overnight, cooled to 0 °C and acetic acid (86 µl, 1.5 mmol) was added drop wise. The mixture was stirred at rt for 30 min, evaporated and the product was obtained by chromatography on silica gel using linear gradient of ethyl acetate in toluene.

General procedure H – removal of DMTr and Boc protecting groups from pyrrolidine phosphonates

Protected pyrrolidine (1 mmol) in 20% TFA in DCM (10 ml) was stirred overnight. The reaction mixture was diluted with chloroform (10 ml) and extracted with water (20 ml). The aqueous phase was washed with chloroform (3x 10 ml) and applied on a column of Dowex 50 in H⁺ form (20 ml/mmol). The resin was washed with a water/ethanol (1:1) mixture and the product was eluted with 3% ammonia in water/ethanol (1:1). The crude product obtained after evaporation *in vacuo* was used without further purification.

Synthesis of Precursors

Example 1. [3S,4S]-1-N-Boc-3-allyloxy-4-dimethoxytrityloxypyrrolidine (ii)



Sodium hydride (2.3 g, 57.84 mmol) was added to a solution of [S,R]-1-N-Boc-3-dimethoxytrityloxy-4-hydroxypyrrolidine (9.7 g, 19.28 mmol) and 3-bromoprop-1-ene (2.5 ml, 28.92 mmol) in DMF (150 ml) at 0 °C under an atmosphere of argon. The reaction mixture was warmed to rt and stirred for 3 h. The reaction mixture was cooled to 0 °C and acetic acid (3.3 ml, 57.84 mmol) was added. The reaction mixture was warmed to rt,

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concentrated *in vacuo* and final product **ii** was obtained by column chromatography on silica gel using a linear gradient of ethyl acetate in toluene in 88% yield (9.26 g, 16.97 mmol). The final product was obtained as an ~ 5:4 mixture of two rotamers.

5 ¹H NMR (500.0 MHz, CDCl₃): 1.42 (s, 9H, (CH₃)₃C-A); 1.47 (s, 9H, (CH₃)₃C-B); 2.85 (dd, 1H, *J*_{gem} = 11.9, *J*_{5b,4} = 1.7, H-5b-A); 3.02 (dd, 1H, *J*_{gem} = 11.9, *J*_{5a,4} = 4.8, H-5a-A); 3.20 (ddd, 1H, *J*_{3,2} = 4.3, 1.5, *J*_{3,4} = 2.0, H-3-B); 3.24 (dd, 1H, *J*_{gem} = 12.0, *J*_{5b,4} = 2.0, H-5b-B); 3.27 (dd, 1H, *J*_{gem} = 12.0, *J*_{5a,4} = 4.7, H-5a-B); 3.30 (dd, 1H, *J*_{gem} = 11.9, *J*_{2b,3} = 1.5, H-2b-B); 3.35 (dd, 1H, *J*_{gem} = 11.7, *J*_{2b,3} = 1.5, H-2b-A); 3.39 (ddd, 1H, *J*_{3,2} = 4.4, 1.5, *J*_{3,4} = 2.0, H-3-A); 3.50 (dd, 1H, *J*_{gem} = 11.9, *J*_{2a,3} = 4.3, H-2a-B); 3.56 (dd, 1H, *J*_{gem} = 11.7, *J*_{2a,3} = 4.4, H-2a-A); 3.61 (ddt, 1H, *J*_{gem} = 12.7, *J*_{vic} = 5.4, ⁴*J* = 1.5, CH_aH_bO-B); 3.64 (ddt, 1H, *J*_{gem} = 12.7, *J*_{vic} = 5.4, ⁴*J* = 1.5, CH_aH_bO-B); 3.69 (ddt, 1H, *J*_{gem} = 12.7, *J*_{vic} = 5.4, ⁴*J* = 1.5, CH_aH_bO-A); 3.78 (ddt, 1H, *J*_{gem} = 12.7, *J*_{vic} = 5.4, ⁴*J* = 1.5, CH_aH_bO-A); 3.787, 3.792 (2 × s, 2 × 6H, CH₃O-DMTr-A,B); 4.07 (ddd, 1H, *J*_{4,5} = 4.8, 1.7, *J*_{4,3} = 2.0, H-4-A); 4.10 (dt, 1H, *J*_{4,5} = 4.7, 2.0, *J*_{4,3} = 2.0, H-4-B); 5.06 – 5.21 (m, 4H, =CH₂-A,B); 5.59 – 5.82 (m, 2H, =CH-A,B); 6.81-6.86 (m, 8H, H-*m*-C₆H₄OMe-DMTr); 7.15 – 7.33 (m, 6H, H-*m,p*-C₆H₅-DMTr); 7.32-7.38 (m, 8H, H-*o*-C₆H₄OMe-DMTr); 7.42 – 7.48 (m, 4H, H-*o*-C₆H₅-DMTr).

13C NMR (125.7 MHz, CDCl₃): 28.42 ((CH₃)₃C-A); 28.50 ((CH₃)₃C-B); 48.78 (CH₂-2-A); 49.61 (CH₂-2-B); 50.74 (CH₂-5-A); 50.83 (CH₂-5-B); 55.18 (CH₃O-DMTr-A,B); 70.08 (CH₂O-B); 70.21 (CH₂O-A); 75.04 (CH-4-B); 75.85 (CH-4-A); 79.06 (C(CH₃)₃-A); 79.14 (C(CH₃)₃-B); 80.95 (CH-3-A); 81.63 (CH-3-B); 86.94 (C-DMTr-A); 87.10 (C-DMTr-B); 113.18 (CH-*m*-C₆H₄OMe-DMTr-A,B); 116.73 (=CH₂-B); 116.97 (=CH₂-A); 126.93 (CH-*p*-C₆H₅-DMTr-A,B); 127.86 (CH-*m*-C₆H₅-DMTr-A,B); 128.22 (CH-*o*-C₆H₅-DMTr-A); 128.27 (CH-*o*-C₆H₅-DMTr-B); 130.13 (CH-*o*-C₆H₄OMe-DMTr-A,B); 134.39 (=CH-A); 134.43 (=CH-B); 136.30, 136.34, 136.39, 136.46 (C-*i*-C₆H₄OMe-DMTr-A,B); 145.11 (C-*i*-C₆H₅-DMTr-A); 145.17 (C-*i*-C₆H₅-DMTr-B); 154.66 (NCO-B); 154.78 (NCO-A); 158.60 (C-*p*-C₆H₄OMe-DMTr-B); 158.65 (C-*p*-C₆H₄OMe-DMTr-A).

IR ν_{max}(CHCl₃) 3087 (w), 3062 (w), 2980 (m), 2935 (m), 2912 (w), 2880 (w), 2839 (w), 1687 (s), 1675 (s, sh), 1645 (w, sh), 1608 (m), 1582 (w), 1509 (vs), 1494 (w, sh), 1478 (m), 1464 (m), 1456 (m), 1446 (m), 1442 (m), 1415 (vs), 1393 (m), 1367 (m), 1301 (m),

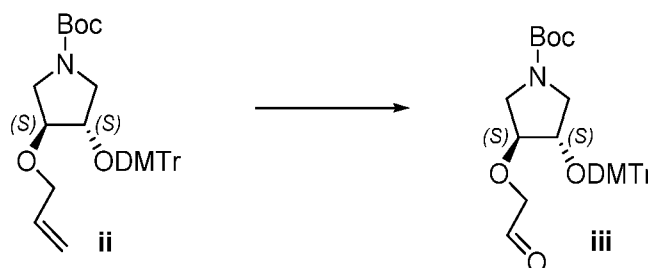
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1252 (vs), 1180 (s, sh), 1175 (vs), 1154 (m), 1119 (s), 1090 (m, sh), 1081 (m), 1036 (s), 1010 (w), 1002 (w), 997 (w), 913 (vw), 829 (m), 705 (w), 700 (w), 639 (vw), 625 (vw), 596 (w), 583 (m), 525 (vw), 460 (vw).

HRMS (ESI+) for $C_{33}H_{39}NO_6Na$ ($M+Na$)⁺: calcd 568.26696, found 568.26714.

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Example 2. [3*S*,4*S*]-1-*N*-Boc-4-dimethoxytrityloxy-3-(2-oxoethyl)pyrrolidine (iii)



A mixture of **ii** (9.78 g, 19.9 mmol), NaIO₄ (19 g, 90 mmol), OsO₄ (1 ml of 2.5% in tBuOH), H₂O (40 ml) and THF (180 ml) was stirred overnight at rt. The reaction mixture
10 was filtered and concentrated *in vacuo*. The final product **iii** was obtained by column chromatography on silica gel using a linear gradient of ethyl acetate in toluene in 53% (5.2 g, 9.5 mmol) yield, as an ~8:7 mixture of two rotamers.

¹H NMR (500.0 MHz, CDCl₃): 1.44 (s, 9H, (CH₃)₃C-A); 1.48 (s, 9H, (CH₃)₃C-B); 3.03
15 (bd, 1H, *J*_{4,5a} = 4.0, H-4-B); 3.07 (d, 1H, *J*_{gem} = 12.0, H-2b-A); 3.197 (bd, 1H, *J*_{4,5a} = 4.3, H-4-B); 3.20 (dd, 1H, *J*_{gem} = 12.0, *J*_{2a,3} = 4.7, H-2a-A); 3.34 – 3.47 (m, 4H, H-2,5b-B, H-5b-A); 3.52 (dd, 1H, *J*_{gem} = 12.0, *J*_{2a,3} = 4.0, H-5a-B); 3.56 (dd, 1H, *J*_{gem} = 12.2, *J*_{2a,3} = 4.3, H-5a-A); 3.92 (dd, 1H, *J*_{gem} = 17.7, *J*_{vic} = 0.7, CH_aH_bO-A); 3.64 (dd, 1H, *J*_{gem} = 17.7, *J*_{vic} = 0.7, CH_aH_bO-A); 3.65 (dd, 1H, *J*_{gem} = 17.7, *J*_{vic} = 0.9, CH_aH_bO-A); 3.785, 3.790 (2 × d, 2
20 × 6H, CH₃O-DMTr-A,B); 3.80 (dd, 1H, *J*_{gem} = 17.7, *J*_{vic} = 0.9, CH_aH_bO-A); 4.09 – 4.12 (m, 2H, H-3-A,B); 6.79 – 6.86 (m, 8H, H-*m*-C₆H₄OMe-DMTr); 7.20 – 7.25 (m, 2H, H-*p*-C₆H₅-DMTr); 7.26 – 7.32 (m, 4H, H-*m*-C₆H₅-DMTr); 7.32 – 7.36 (m, 8H, H-*o*-C₆H₄OMe-DMTr); 7.42 – 7.45 (m, 4H, H-*o*-C₆H₅-DMTr); 9.45 (s, 1H, CHO-B); 9.51 (s, 1H, CHO-A).

25 ¹³C NMR (125.7 MHz, CDCl₃): 28.43 ((CH₃)₃C-A); 28.48 ((CH₃)₃C-B); 48.69 (CH₂-5-A); 49.58 (CH₂-5-B); 50.91 (CH₂-2-A,B); 55.21 (CH₃O-DMTr-A,B); 74.47 (CH₂O-B); 74.50 (CH-3-B); 74.54 (CH₂O-A); 75.45 (CH-3-A); 79.39 (C(CH₃)₃-A); 79.44 (C(CH₃)₃-B);

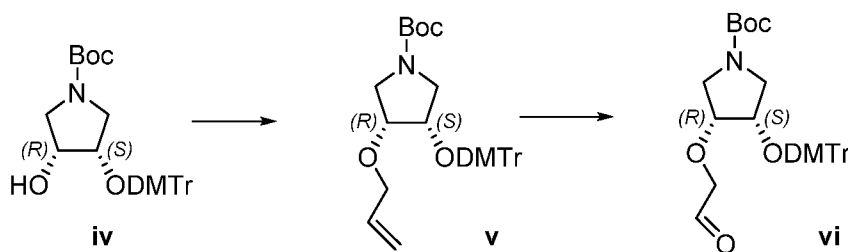
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82.42 (CH-4-A); 83.02 (CH-4-B); 87.20 (C-DMTr-A); 87.33 (C-DMTr-B); 113.23, 113.24, 113.27 (CH-*m*-C₆H₄OMe-DMTr-A,B); 127.07 (CH-*p*-C₆H₅-DMTr-A,B); 127.94 (CH-*m*-C₆H₅-DMTr-A,B); 128.18, 128.21 (CH-*o*-C₆H₅-DMTr-A,B); 130.07, 130.10, 130.11 (CH-*o*-C₆H₄OMe-DMTr-A,B); 136.06, 136.13, 136.17, 136.27 (C-*i*-C₆H₄OMe-DMTr-A,B); 144.91, 144.97 (C-*i*-C₆H₅-DMTr-A,B); 154.63 (NCO-B); 154.76 (NCO-A); 158.69 (C-*p*-C₆H₄OMe-DMTr-B); 158.72 (C-*p*-C₆H₄OMe-DMTr-A); 199.84 (CHO-A); 200.08 (CHO-B).

IR $\nu_{\max}$ (KBr) 3059 (w), 3035 (vw), 2974 (m), 2933 (w), 2877 (w), 2837 (w), 1695 (vs), 1648 (w, sh), 1608 (m), 1582 (w), 1509 (vs), 1492 (w, sh), 1478 (w), 1456 (m), 1446 (m), 1408 (s), 1393 (m, sh), 1366 (m), 1301 (m), 1252 (vs), 1175 (s), 1154 (m, sh), 1114 (m), 1083 (m), 1035 (m), 1010 (w), 928 (w), 828 (m), 769 (w), 727 (w), 703 (w), 638 (vw), 626 (vw), 596 (w), 584 (w), 525 (vw), 464 (vw).

HRMS (ESI+) for C₃₂H₃₇NO₇Na (M+Na)⁺: calcd 570.24622, found 570.24620.

15 **Example 3. [3R,4S]-1-N-Boc-4-dimethoxytrityloxy-3-(2-oxoethyl)pyrrolidine (vi)**



Sodium hydride (3 g, 74.16 mmol) was added to a solution of [3S,4R]-1-N-Boc-3-dimethoxytrityloxy-4-hydroxypyrrolidine (12.5 g, 24.72 mmol) and 3-bromoprop-1-ene (3.2 ml, 37.08 mmol) in DMF (200 ml) at 0 °C under an atmosphere of argon. The reaction mixture was warmed to rt and stirred for 3 h. The reaction mixture was cooled to 0 °C and acetic acid (4.3 ml, 74.16 mmol) was added. The reaction mixture was warmed to rt, concentrated *in vacuo*, dissolved in EtOAc (200 ml) and washed with sat. soln. NaHCO₃ (2x100 ml). The organic phase was dried over Na₂SO₄ and concentrated *in vacuo*. The allyl derivative **v** was obtained by column chromatography on silica gel using a linear gradient of ethyl acetate in toluene in 84% yield (HRMS (ESI+) for C₃₃H₃₉NO₆Na (M+Na)⁺: calcd 568.26696, found 568.26644.) and used without further characterization for the next step.

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A mixture of **v** (3.85 g, 7 mmol), NaIO₄ (7.5 g, 35 mmol), OsO₄ (1.5 ml of 2.5% in tBuOH), H₂O (15 ml) and THF (60 ml) was stirred at rt for 5h. The reaction mixture was diluted with ethyl acetate (150 ml), washed with sat. soln. NaHCO₃ (2x100 ml), dried over
 5 Na₂SO₄, filtered and concentrated *in vacuo*. The final product **vi** was obtained by column chromatography on silica gel using a linear gradient of ethyl acetate in toluene in 59% (2.28 g, 4.16 mmol) yield as an ~ 10:9 mixture of two rotamers.

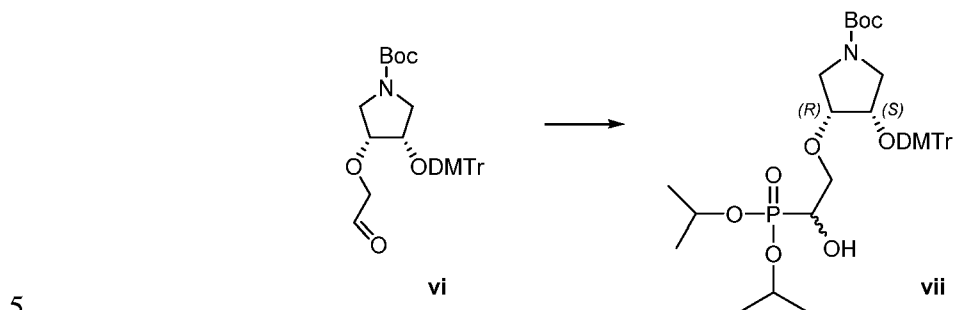
¹H NMR (500.0 MHz, CDCl₃): 1.41 (s, 18H, (CH₃)₃C-A,B); 2.79 (t, 1H, *J*_{4,3} = *J*_{4,5b} = 3.5,
 10 H-4-B); 3.01 – 3.13 (m, 4H, H-2b,4,5b-A, H-5b-B); 3.22 (dd, 1H, *J*_{gem} = 10.2, *J*_{2a,3} = 9.5, H-2a-A); 3.34 (dd, 1H, *J*_{gem} = 12.5, *J*_{5a,4} = 0.7, H-5a-B); 3.38 – 3.46 (m, 2H, H-2b-B, H-5a-A); 3.51 (dd, 1H, *J*_{gem} = 10.2, *J*_{2a,3} = 7.6, H-2a-B); 3.790, 3.795, 3.796 (3 × d, 12H, CH₃O-DMTr-A,B); 3.92 (dd, 1H, *J*_{gem} = 17.9, *J*_{vic} = 1.0, CH_aH_bO-B); 3.98 (dd, 1H, *J*_{gem} = 17.6, *J*_{vic} = 1.1, CH_aH_bO-A); 3.98 – 4.09 (m, 4H, H-3-A,B, CH_aH_bO-A,B); 6.79 – 6.88 (m,
 15 8H, H-*m*-C₆H₄OMe-DMTr); 7.14 – 7.32 (m, 6H, H-*m,p*-C₆H₅-DMTr); 7.33 – 7.42 (m, 8H, H-*o*-C₆H₄OMe-DMTr); 7.43 – 7.51 (m, 4H, H-*o*-C₆H₅-DMTr); 9.65 (t, 1H, *J*_{vic} = 1.0, CHO-B); 9.70 (t, 1H, *J*_{vic} = 1.0, CHO-A).

¹³C NMR (125.7 MHz, CDCl₃): 28.40 ((CH₃)₃C-A,B); 47.01 (CH₂-2-B); 47.55 (CH₂-2-A); 48.95 (CH₂-5-A); 50.06 (CH₂-5-B); 55.24 (CH₃O-DMTr-A,B); 73.42 (CH-3-B);
 20 73.76(CH-3-A); 75.66 (CH₂O-A); 75.91 (CH₂O-B); 78.45 (CH-4-A); 78.79 (CH-4-B); 79.43 (C(CH₃)₃-B); 79.44 (C(CH₃)₃-A); 87.00 (C-DMTr-A); 87.02 (C-DMTr-B); 113.29, 113.34 (CH-*m*-C₆H₄OMe-DMTr-A,B); 127.11 (CH-*p*-C₆H₅-DMTr-A); 127.16 (CH-*p*-C₆H₅-DMTr-B); 128.01, 128.06 (CH-*o,m*-C₆H₅-DMTr-A,B); 130.00, 130.02, 130.03 (CH-*o*-C₆H₄OMe-DMTr-A,B); 135.96, 136.09, 136.16 (C-*i*-C₆H₄OMe-DMTr-A,B); 144.87 (C-*i*-C₆H₅-DMTr-B); 144.93 (C-*i*-C₆H₅-DMTr-A); 154.18 (NCO-B); 154.53 (NCO-A);
 25 158.76 (C-*p*-C₆H₄OMe-DMTr-B); 158.80 (C-*p*-C₆H₄OMe-DMTr-A); 201.33 (CHO-A,B).

IR $\nu_{\max}$ (CHCl₃) 3088 (vw), 3060 (vw), 2980 (m), 2936 (m), 2839 (w), 1736 (m), 1689 (vs), 1608 (s), 1582 (w), 1509 (vs), 1495 (m, sh), 1477 (m), 1464 (m), 1456 (m), 1446 (m), 1442 (m), 1414 (vs), 1393 (m), 1367 (s), 1336 (vw), 1302 (m), 1252 (vs), 1175 (vs), 1153
 30 (m), 1105 (s), 1035 (s), 1013 (w), 912 (w), 831 (m), 700 (w), 636 (vw), 629 (vw), 595 (w), 585 (m), 466 (vw).

HRMS (ESI+) for $C_{32}H_{37}NO_7Na$ ($M+Na$)⁺: calcd 570.24622, found 570.24617.

Example 4. Diisopropyl ((*RS*)-1-hydroxy-2-(((3*R*,4*S*)-1-*N*-boc-4-dimethoxytrityloxypyrrolidin-3-yl)oxy)ethyl)phosphonate (vii).



Intermediate **vi** was dissolved in DCM (120 ml) and diisopropyl phosphite (2.58 g, 15.54 mmol) and triethylamine (0.84 ml, 6.48 mmol) were added and the reaction mixture was refluxed for 8 h. The reaction mixture was concentrated *in vacuo* and final product **vii** was obtained by column chromatography on silica gel using a linear gradient of ethyl acetate in
 10 68% overall yield (6 g, 8.75 mmol) in a 1:1:1:1 mixture of four isomers (two times two epimeric rotamers).

¹H NMR (500.0 MHz, CDCl₃): 1.29-1.35 (m, 48H, (CH₃)₂CH-A,B); 1.387, 1.390, 1.396 (3 × s, 36H, (CH₃)₃CO-A,B); 2.60, 2.73 (2 × bm, 2 × 1H, H-3-A); 2.81-3.07 (m, 8H, H-2b-A,B, H-3,5b-B); 3.11-3.18 (m, 2H, H-5a-B); 3.25-3.83 (m, 40H, H-2a-A,B, H-5-A, OCH₂-A,B, CH₃O-DMTr-A,B); 3.88 – 4.08 (m, 8H, H-4-A,B, CHOH-A,B); 4.69-4.81 (m, 8H, (CH₃)₂CH-A,B); 6.80-6.87 (m, 16H, H-*m*-C₆H₄-DMTr); 7.19-7.24 (m, 4H, H-*p*-C₆H₅-DMTr); 7.26-7.32 (m, 8H, H-*m*-C₆H₅-DMTr); 7.35-7.42 (m, 16H, H-*o*-C₆H₄-DMTr); 7.46-7.51 (m, 8H, H-*m*-C₆H₅-DMTr).

20 ¹³C NMR (125.7 MHz, CDCl₃): 23.92-24.18 (m, (CH₃)₂CH-A,B); 28.39 ((CH₃)₃CO-A,B); 47.16, 47.19 (CH₂-5-B); 47.60 (CH₂-5-A); 48.68, 48.83, 49.43, 49.66 (CH₂-2-A,B); 55.20 (CH₃O-DMTr-A,B); 67.72 (d, *J*_{C,P} = 163.8), 67.76 (d, *J*_{C,P} = 164.0), 67.92 (d, *J*_{C,P} = 166.0), 68.07 (d, *J*_{C,P} = 166.5, OCH₂CH-OH-A,B); 69.78-70.07 (m, OCH₂-A,B); 71.03-71.37 (m, (CH₃)₂CH-A,B); 73.07, 73.13, 73.57, 73.60 (CH-4-A,B); 77.94, 78.07, 78.30, 78.36 (CH-3-A,B); 79.27, 79.32, 79.33, 79.34 ((CH₃)₃CO-A,B); 86.92, 86.95, 86.99 (C-DMTr-A,B); 113.27, 113.31, 113.34 (CH-*m*-C₆H₄-DMTr-A,B); 127.00, 127.03 (CH-*p*-C₆H₅-DMTr-

25

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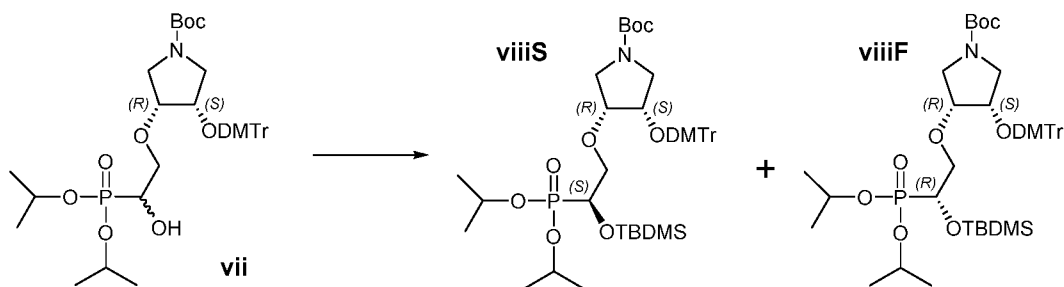
A,B); 127.96, 127.98, 128.02 (CH-*o,m*-C₆H₅-DMTr-A,B); 129.95, 129.98, 129.99, 130.01 (CH-*o*-C₆H₄-DMTr-A,B); 136.09, 136.10, 136.19, 136.21, 136.32, 136.34 (C-*i*-C₆H₄-DMTr-A,B); 144.99, 145.01, 145.03, 145.04 (C-*i*-C₆H₅-DMTr-A,B); 154.17, 154.20, 154.47, 154.53 (CO-A,B); 158.66, 158.72 (C-*p*-C₆H₄-DMTr-A,B).

5 ³¹P{¹H} NMR (202.3 MHz, CDCl₃): 20.04, 20.06, 20.44, 20.53.

IR ν_{max}(CHCl₃) 3562 (w, br), 3088 (vw), 3060 (vw), 2983, 2930 (m), 2878 (w), 2839 (w), 1689 (s), 1608 (m), 1582 (w), 1509 (vs), 1494 (m), 1476 (m), 1465 (m), 1455 (m), 1447 (m), 1442 (m), 1414 (s), 1392 (m), 1387 (m), 1376 (m), 1368 (m), 1347 (w), 1336 (w), 1302, 1251 (vs), 1179 (s), 1175 (s), 1153 (m), 1143 (m), 1104 (vs), 1035 (s), 1013 (s, sh),
10 994 (vs), 912 (w), 885 (w), 831 (m), 702 (w), 636 (vw), 629 (vw), 596 (w), 584 (w).

HRMS (ESI+) for C₃₂H₃₇NO₇Na (M+Na)⁺: calcd, found.

Example 5. Diisopropyl ((*S*)-1-*tert*-butyldimethylsilyloxy-2-(((3*R*,4*S*)-1-*N*-*boc*-4-dimethoxytrityloxypyrrolidin-3-yl)oxy)ethyl)phosphonate (viiiS) and diisopropyl ((*R*)-1-*tert*-butyldimethylsilyloxy-2-(((3*R*,4*S*)-1-*N*-*boc*-4-dimethoxytrityloxypyrrolidin-3-yl)oxy)ethyl)phosphonate (viiiF)
15



TBDMSCl (1.97 g, 13.1 mmol) was added to a mixture of **vii** (6 g, 8.75 mmol) and
20 imidazole (0.9 g, 13.1 mmol) in DMF. The reaction mixture was stirred at rt under an atmosphere of argon for 3 days. The reaction mixture was concentrated *in vacuo*, dissolved in CHCl₃ (150 ml) and washed with sat soln. NaHCO₃. The organic phase was dried over Na₂SO₄, filtered and evaporated. The obtained diastereoisomers were separated by column chromatography on silica gel using gradient of ethyl acetate in toluene. The
25 fractions containing both diastereoisomers were evaporated and chromatography was repeated. The faster eluting isomer **viiiF** was obtained in 32% yield (2.31 g, 2.79 mmol),

the slower eluting isomer **viiiS** was obtained in 30% yield (2.15 g, 2.6 mmol), and remaining mixture of the isomers in 5% (0.33 g, 0.4 mmol)

viiiS (obtained as an ~ 1:1 mixture of two rotamers)

- 5 ^1H NMR (500.0 MHz, CDCl_3): 0.128, 0.135, 0.142 ($3 \times \text{s}$, 12H, CH_3Si); 0.91, 0.92 ($2 \times \text{s}$, $2 \times 9\text{H}$, $(\text{CH}_3)_3\text{CSi}$); 1.25-1.31 (m, 24H, $(\text{CH}_3)_2\text{CH}$); 1.38, 1.39 ($2 \times \text{s}$, $2 \times 9\text{H}$, $(\text{CH}_3)_3\text{CO}$); 2.78 (t, 1H, $J_{3,2b} = J_{3,4} = 3.7$, H-3); 2.81 (dd, 1H, $J_{\text{gem}} = 10.2$, $J_{5b,4} = 7.4$, H-5b); 3.00 (dd, 1H, $J_{\text{gem}} = 12.3$, $J_{2b,3} = 3.7$, H-2b); 3.04 (dd, 1H, $J_{\text{gem}} = 12.3$, $J_{2b,3} = 4.0$, H-2b); 3.08 – 3.14 (m, 2H, H-3,5a); 3.26 (dd, 1H, $J_{\text{gem}} = 12.3$, $J_{2a,3} = 0.6$, H-2a); 3.30-3.38 (m, 4H, H-2a,5, $\text{OCH}_a\text{H}_b\text{CH-OTBDMS}$); 3.47 (m, 1H, $\text{OCH}_a\text{H}_b\text{CH-OTBDMS}$); 3.78, 3.79 ($2 \times \text{s}$, $2 \times 6\text{H}$, $\text{CH}_3\text{O-DMTr}$); 3.86 – 4.04 (m, 6H, H-4, $\text{OCH}_a\text{H}_b\text{CH-OTBDMS}$); 4.63 – 4.79 (m, 4H, $(\text{CH}_3)_2\text{CH}$); 6.79 – 6.85 (m, 8H, H-*m*- $\text{C}_6\text{H}_4\text{OMe-DMTr}$); 7.18 – 7.24 (m, 2H, H-*p*- $\text{C}_6\text{H}_5\text{-DMTr}$); 7.25 – 7.31 (m, 4H, H-*m*- $\text{C}_6\text{H}_5\text{-DMTr}$); 7.32 – 7.42 (m, 8H, H-*o*- $\text{C}_6\text{H}_4\text{OMe-DMTr}$); 7.46 – 7.52 (m, 4H, H-*o*- $\text{C}_6\text{H}_5\text{-DMTr}$).
- 15 ^{13}C NMR (125.7 MHz, CDCl_3): -5.02, -4.99, -4.68, -4.64 (CH_3Si); 18.22, 18.27 ($(\text{CH}_3)_3\text{CSi}$); 24.01 (d, $J_{\text{C,P}} = 4.9$, $(\text{CH}_3)_2\text{CH}$); 24.05 (d, $J_{\text{C,P}} = 3.8$, $(\text{CH}_3)_2\text{CH}$); 24.12 (d, $J_{\text{C,P}} = 4.6$, $(\text{CH}_3)_2\text{CH}$); 24.28 (d, $J_{\text{C,P}} = 3.2$, $(\text{CH}_3)_2\text{CH}$); 25.73, 25.79 ($(\text{CH}_3)_3\text{CSi}$); 28.39, 28.42 ($(\text{CH}_3)_3\text{CO}$); 47.06, 47.44 ($\text{CH}_2\text{-5}$); 49.67, 50.39 ($\text{CH}_2\text{-2}$); 55.19 ($\text{CH}_3\text{O-DMTr}$); 70.58 (d, $J_{\text{C,P}} = 7.4$, $(\text{CH}_3)_2\text{CH}$); 70.67, 70.76 (d, $J_{\text{C,P}} = 168.6$, $\text{OCH}_2\text{CH-OTBDMS}$);
- 20 71.04, 71.07 ($2 \times \text{d}$, $J_{\text{C,P}} = 6.8$, $(\text{CH}_3)_2\text{CH}$); 72.39, 72.50 ($2 \times \text{d}$, $J_{\text{C,P}} = 10.1$, $\text{OCH}_2\text{CH-OTBDMS}$); 73.82, 74.32 (CH-4); 78.36, 78.75 (CH-3); 79.07, 79.12 ($(\text{CH}_3)_3\text{CO}$); 87.00 (C-DMTr); 113.18, 113.20, 113.23 (CH-*m*- $\text{C}_6\text{H}_4\text{OMe-DMTr}$); 126.88, 126.90 (CH-*p*- $\text{C}_6\text{H}_5\text{-DMTr}$); 127.89 (CH-*m*- $\text{C}_6\text{H}_5\text{-DMTr}$); 128.11, 128.17 (CH-*o*- $\text{C}_6\text{H}_5\text{-DMTr}$); 130.08, 130.14, 130.17 (CH-*o*- $\text{C}_6\text{H}_4\text{OMe-DMTr}$); 136.28, 136.31, 136.40, 136.43 (C-*i*- $\text{C}_6\text{H}_4\text{OMe-DMTr}$); 145.18, 145.21 (C-*i*- $\text{C}_6\text{H}_5\text{-DMTr}$); 154.20, 154.49 (CO); 158.58, 158.64, 158.66 (C-*p*- $\text{C}_6\text{H}_4\text{OMe-DMTr}$).
- 25 $^{31}\text{P}\{^1\text{H}\}$ NMR (202.3 MHz, CDCl_3): 20.28, 20.38.

HRMS (ESI+) for $\text{C}_{44}\text{H}_{66}\text{NO}_{10}\text{NaPSi}$ ($\text{M}+\text{Na}$) $^+$: calcd 850.40858, found 850.40864.

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viiiF (obtained as an ~ 6:5 mixture of two rotamers)

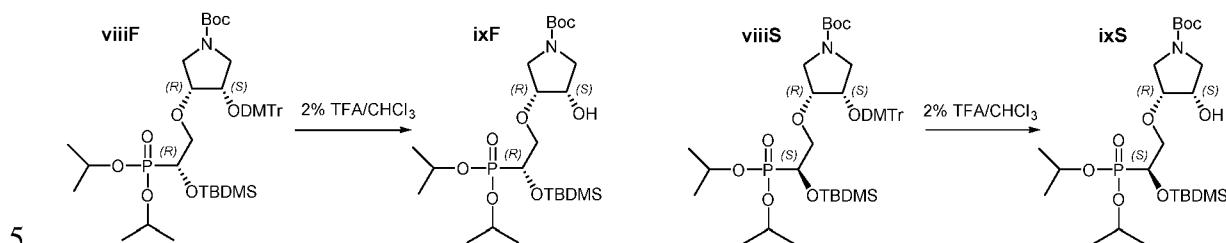
¹H NMR (500.0 MHz, CDCl₃): 0.13, 0.19, 0.20 (3 × s, 12H, CH₃Si-A,B); 0.90 (s, 18H, (CH₃)₃CSi-A,B); 1.24-1.32 (m, 24H, (CH₃)₂CH-A,B); 1.39 (s, 9H, (CH₃)₃CO-B); 1.40 (s, 9H, (CH₃)₃CO-A); 2.51 (bm, 1H, H-3-B); 2.68 (bm, 1H, H-3-A); 2.92 (dd, 1H, $J_{\text{gem}} =$
 5 12.3, $J_{2b,3} = 3.7$, H-2b-B); 2.94 (dd, 1H, $J_{\text{gem}} = 12.3$, $J_{2b,3} = 3.7$, H-2b-A); 3.08 (dd, 1H, $J_{\text{gem}} = 10.1$, $J_{5b,4} = 7.7$, H-5b-A); 3.20 (dd, 1H, $J_{\text{gem}} = 10.1$, $J_{5a,4} = 9.5$, H-5a-A); 3.26 - 3.50 (m, 8H, H-2a-A,B, H-5-B, OCH₂CH-OTBDMS-A,B); 3.77 (s, 6H, CH₃O-DMTr-B); 3.78 (s, 6H, CH₃O-DMTr-A); 3.91 (ddd, 1H, $J_{4,5} = 9.5$, 7.7, $J_{4,3} = 4.0$, H-4-A); 3.93 (ddd, 1H, $J_{4,5} = 9.5$, 7.7, $J_{4,3} = 4.0$, H-4-B); 4.08 - 4.15 (m, 2H, OCH₂CH-OTBDMS-A,B); 4.64 -
 10 4.79 (m, 4H, (CH₃)₂CH); 6.80 - 6.85 (m, 8H, H-*m*-C₆H₄OMe-DMTr); 7.18 - 7.23 (m, 2H, H-*p*-C₆H₅-DMTr); 7.25 - 7.41 (m, 12H, H-*m*-C₆H₅-DMTr, H-*o*-C₆H₄OMe-DMTr); 7.47 - 7.52 (m, 4H, H-*o*-C₆H₅-DMTr).

¹³C NMR (125.7 MHz, CDCl₃): -5.06, -5.04, -4.84, -4.81 (CH₃Si); 18.18 ((CH₃)₃CSi-B); 18.20 ((CH₃)₃CSi-A); 23.78-24.27 (m, (CH₃)₂CH-A,B); 25.65 ((CH₃)₃CSi-B); 25.68
 15 ((CH₃)₃CSi-A); 28.35 ((CH₃)₃CO-B); 28.36 ((CH₃)₃CO-A); 47.34 (CH₂-5-B); 47.83 (CH₂-5-A); 48.19 (CH₂-2-A); 49.15 (CH₂-2-B); 55.14 (CH₃O-DMTr); 70.18 (d, $J_{\text{C,P}} = 170.4$, OCH₂CH-OTBDMS-B); 70.34 (d, $J_{\text{C,P}} = 170.3$, OCH₂CH-OTBDMS-A); 70.74 (d, $J_{\text{C,P}} = 7.5$, (CH₃)₂CH-A,B); 71.23 (d, $J_{\text{C,P}} = 12.5$, OCH₂CH-OTBDMS-A); 71.34 (d, $J_{\text{C,P}} = 7.1$, (CH₃)₂CH-A,B); 71.39 (d, $J_{\text{C,P}} = 12.7$, OCH₂CH-OTBDMS-B); 71.50 (d, $J_{\text{C,P}} = 6.7$,
 20 (CH₃)₂CH-A,B); 72.97 (CH-4-B); 73.30 (CH-4-A); 77.54 (CH-3-A); 78.07 (CH-3-B); 79.18 ((CH₃)₃CO-B); 79.27 ((CH₃)₃CO-A); 86.55 (C-DMTr-A); 86.57 (C-DMTr-B); 113.13, 113.15, 113.17 (CH-*m*-C₆H₄OMe-DMTr-A,B); 126.84, 126.85 (CH-*p*-C₆H₅-DMTr-A,B); 127.82 (CH-*m*-C₆H₅-DMTr-A,B); 127.97, 128.03 (CH-*o*-C₆H₅-DMTr-A,B); 129.92, 129.94 (CH-*o*-C₆H₄OMe-DMTr-A,B); 136.32, 136.42, 136.48 (C-*i*-C₆H₄OMe-
 25 DMTr-A,B); 145.18 (C-*i*-C₆H₅-DMTr-B); 145.23 (C-*i*-C₆H₅-DMTr-A); 154.17 (CO-B); 154.50 (CO-A); 158.52, 158.57 (C-*p*-C₆H₄OMe-DMTr-A,B).

³¹P{¹H} NMR (202.3 MHz, CDCl₃): 19.63 (A); 19.79 (B).

HRMS (ESI+) for C₄₄H₆₆NO₁₀NaPSi (M+Na)⁺: calcd 850.40858, found 850.40861.

Example 6. Diisopropyl ((S)-1-tert-butyldimethylsilyloxy-2-(((3R,4S)-1-N-boc-4-hydroxypyrrolidin-3-yl)oxy)ethyl)phosphonate (ixS) and diisopropyl ((R)-1-tert-butyldimethylsilyloxy-2-(((3R,4S)-1-N-boc-4-hydroxypyrrolidin-3-yl)oxy)ethyl)phosphonate (ixF)



100 ml of 2% TFA in CHCl₃ was added to the solution of **viiiS** (6.54 g, 7.9 mmol) or **viiiF** (5.27 g, 6.36 mmol) in 100 ml of CHCl₃. The reaction mixture was stirred for 15 min at rt. Solid NaHCO₃ (10 g) and MeOH (20 ml) was added. The suspension was stirred until
 10 neutral pH (30 min), filtered, concentrated *in vacuo* and the desired product was obtained by column chromatography on silica gel using a linear gradient of ethanol in chloroform.

ixS (70% yield (2.91 g, 5.54 mmol), obtained as an ~ 3:2 mixture of two rotamers).

¹H NMR (500.0 MHz, CDCl₃): 0.10, 0.13, 0.14 (3 × s, 12H, CH₃Si-A,B); 0.89 (s, 9H, (CH₃)₃CSi-A); 0.90 (s, 9H, (CH₃)₃CSi-B); 1.30-1.34 (m, 24H, (CH₃)₂CH-A,B); 1.43 (s, 18H, (CH₃)₃CO-A,B); 3.28 – 3.40 (m, 4H, H-2b,5b-A,B); 3.43 – 3.59 (m, 4H, H-2a,5a-A,B); 3.67 – 3.83 (m, 4H, OCH₂CH-OTBDMS-A,B); 3.82 – 3.94 (m, 2H, H-3-A,B); 3.97 – 4.05 (m, 2H, OCH₂CH-OTBDMS-A,B); 4.28-4.34 (m, 2H, H-4-A,B); 4.66 – 4.85 (m, 4H, (CH₃)₂CH-A,B).

¹³C NMR (125.7 MHz, CDCl₃): -5.01, -4.86 (CH₃Si-A,B); 18.12 ((CH₃)₃CSi-A,B); 23.90 (d, *J*_{C,P} = 5.3, (CH₃)₂CH-A,B); 24.07 (d, *J*_{C,P} = 3.8, (CH₃)₂CH-A,B); 24.15 (d, *J*_{C,P} = 4.6, (CH₃)₂CH-A,B); 24.28 (d, *J*_{C,P} = 2.8, (CH₃)₂CH-A,B); 25.62 ((CH₃)₃CSi-A,B); 28.44 ((CH₃)₃CO-A,B); 47.09 (CH₂-2-A); 47.60 (CH₂-2-B); 50.53 (CH₂-5-B); 51.06 (CH₂-5-A); 68.79 (CH-4-B); 69.00 (d, *J*_{C,P} = 171.6, OCH₂CH-OTBDMS-A); 69.17 (d, *J*_{C,P} = 171.5, OCH₂CH-OTBDMS-B); 69.42 (CH-4-A); 70.92 (d, *J*_{C,P} = 2.4, OCH₂CH-OTBDMS-A); 71.01 (d, *J*_{C,P} = 7.4, (CH₃)₂CH-A); 71.04 (d, *J*_{C,P} = 7.3, (CH₃)₂CH-B); 71.24 (d, *J*_{C,P} = 2.7,

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OCH₂CH-OTBDMS-B); 71.42 (d, $J_{C,P}$ = 7.0, (CH₃)₂CH-A,B); 79.33 ((CH₃)₃CO-B); 79.39 ((CH₃)₃CO-A); 79.44 (CH-3-A); 80.13 (CH-3-B); 154.38 (CO-B); 154.52 (CO-A).

³¹P{¹H} NMR (202.3 MHz, CDCl₃): 21.49 (B); 21.76 (A).

IR $\nu_{\max}$ (CHCl₃) 3550 (vw, vbr), 3487 (vw, vbr), 3335 (w, vbr), 2982 (s), 2956 (s), 2932 (s),
 5 2896 (m, sh), 2885 (m), 2860 (m), 1690 (vs), 1473 (m), 1464 (m), 1415 (vs), 1392 (s),
 1387 (s), 1375 (m), 1367 (s), 1252 (s), 1172 (s), 1141 (s), 1102 (vs), 1068 (w, sh), 1012
 (vs, sh), 1002 (vs), 990 (vs, sh), 939 (w), 887 (m), 839 (s).

HRMS (ESI+) for C₄₄H₆₆NO₁₀NaPSi (M+Na)⁺ : calcd 850.40858, found 850.40864.

10 **ixF** (70% yield (2.23 g, 4.43 mmol), obtained as an ~ 3:2 mixture of two rotamers).

¹H NMR (500.0 MHz, CDCl₃): 0.09 (s, 3H, CH₃Si-A); 0.10 (s, 3H, CH₃Si-B); 0.12 (s, 3H,
 CH₃Si-A); 0.13 (s, 3H, CH₃Si-B); 0.896 (s, 9H, (CH₃)₃CSi-A); 0.904 (s, 9H, (CH₃)₃CSi-
 B); 1.28-1.35 (m, 24H, (CH₃)₂CH-A,B); 1.43 (s, 9H, (CH₃)₃CO-A); 1.44 (s, 9H,
 (CH₃)₃CO-B); 3.29 (dd, 1H, J_{gem} = 11.0, $J_{2b,3}$ = 6.9, H-2b-B); 3.34 (dd, 1H, J_{gem} = 11.1,
 15 $J_{2b,3}$ = 6.7, H-2b-A); 3.38 (dd, 1H, J_{gem} = 11.9, $J_{5b,4}$ = 3.2, H-5b-B); 3.41 (dd, 1H, J_{gem} =
 11.9, $J_{5b,4}$ = 3.2, H-5b-A); 3.45 (dd, 1H, J_{gem} = 11.9, $J_{5a,4}$ = 5.3, H-5a-A); 3.50 (dd, 1H, J_{gem}
 = 11.9, $J_{5a,4}$ = 5.2, H-5a-B); 3.55 (dd, 1H, J_{gem} = 11.0, $J_{2a,3}$ = 6.6, H-2a-B); 3.59 (dd, 1H,
 J_{gem} = 11.1, $J_{2a,3}$ = 6.6, H-2a-A); 3.68 – 3.82 (m, 4H, OCH₂CH-OTBDMS-A,B); 3.83 –
 3.87 (m, 2H, H-3-A,B); 4.07 – 4.12 (m, 2H, OCH₂CH-OTBDMS-A,B); 4.29-4.33 (m, 2H,
 20 H-4-A,B); 4.68 – 4.77, 4.77-4.86 (2 × m, 2 × 2H, (CH₃)₂CH-A,B).

¹³C NMR (125.7 MHz, CDCl₃): -4.91, -4.90 (CH₃Si-A,B); 18.08 ((CH₃)₃CSi-A,B); 23.78
 (d, $J_{C,P}$ = 5.0, (CH₃)₂CH-A); 23.82 (d, $J_{C,P}$ = 5.0, (CH₃)₂CH-B); 24.04 (d, $J_{C,P}$ = 4.5,
 (CH₃)₂CH-B); 24.23 (d, $J_{C,P}$ = 3.1, (CH₃)₂CH-A,B); 25.58 ((CH₃)₃CSi-A,B); 28.44
 ((CH₃)₃CO-A); 28.46 ((CH₃)₃CO-B); 47.06 (CH₂-2-A); 47.46 (CH₂-2-B); 50.69 (CH₂-5-
 25 B); 51.22 (CH₂-5-A); 68.43 (CH-4-B); 69.12 (CH-4-A); 69.77 (d, $J_{C,P}$ = 169.4, OCH₂CH-
 OTBDMS-A); 69.79 (d, $J_{C,P}$ = 169.5, OCH₂CH-OTBDMS-B); 70.88 (d, $J_{C,P}$ = 7.4,
 (CH₃)₂CH-A); 70.95 (d, $J_{C,P}$ = 7.3, (CH₃)₂CH-B); 70.99 (d, $J_{C,P}$ = 1.4, OCH₂CH-
 OTBDMS-A); 71.10 (d, $J_{C,P}$ = 1.7, OCH₂CH-OTBDMS-B); 71.39 (d, $J_{C,P}$ = 6.9,
 (CH₃)₂CH-B); 71.45 (d, $J_{C,P}$ = 6.8, (CH₃)₂CH-A); 78.55 (CH-3-A); 79.37 ((CH₃)₃CO-
 30 A,B); 79.49 (CH-3-B); 154.37 (CO-B); 154.46 (CO-A).

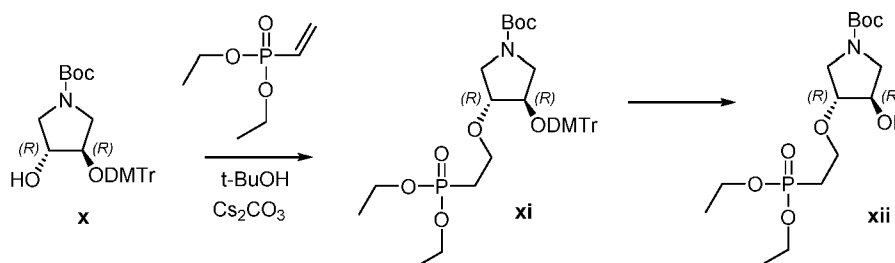
³¹P{¹H} NMR (202.3 MHz, CDCl₃): 21.67 (B); 21.93 (A).

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IR $\nu_{\max}$ (CHCl₃) 3491 (w, vbr), 3330 (w, vbr), 2982 (s), 2955 (m), 2932 (s), 2898 (m, sh), 2886 (m), 2859 (m), 1690 (vs), 1473 (m), 1464 (m), 1415 (s), 1392 (s), 1387 (s), 1375 (m), 1367 (s), 1253 (s), 1172 (s), 1141 (s), 1103 (vs), 1075 (m, sh), 1012 (vs), 996 (vs), 948 (m), 940 (m, sh), 889 (w), 839 (s), 831 (s).

5 HRMS (ESI⁺) for C₄₄H₆₆NO₁₀NaPSi (M+Na)⁺ : calcd 850.40858, found 850.40861.

Example 7. Diethyl ((3*R*,4*R*)-4-hydroxypyrrolidin-3-yl)oxyethylphosphonate (xii)



A mixture of [3*R*,4*R*]-1-*N*-*boc*-3-dimethoxytrityloxy-4-hydroxypyrrolidine (4.45 g, 8.8 mmol), diethyl vinylphosphonate (2 ml, 13.2 mmol), cesium carbonate (2.86 g, 8.8 mmol) and KOH (60 mg, 1.1 mmol) in *t*BuOH (9 ml) was stirred overnight. The reaction mixture was concentrated *in vacuo*, dissolved in EtOAc (200 ml) and washed with sat. soln. NaHCO₃ (2x100 ml). The organic phase was dried over Na₂SO₄ and concentrated *in vacuo*. The residue was dissolved in 2% TFA in DCM (150 ml) and methanol (20 ml) and stirred at rt for 2h. Solid NaHCO₃ (10 g) was added and the reaction mixture was stirred for additional 30 min. The reaction mixture was filtered, concentrated *in vacuo* and the final product **xii** was obtained by column chromatography on silica gel using a linear gradient of ethanol in chloroform in 59% yield (1.9 g, 5.17 mmol) as an ~1:1 mixture of two rotamers.

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¹H NMR (500.0 MHz, CDCl₃): 1.31 (t, 12H, *J*_{vic} = 7.1, CH₃CH₂O); 1.43 (s, 18H, (CH₃)₃C); 2.06 (dt, 4H, *J*_{H,P} = 18.2, *J*_{vic} = 7.0, CH₂P); 3.21 – 3.35 (m, 4H, H-2b,5b); 3.52–3.56 (m, 4H, H-2a,5a); 3.71–3.79 (m, 4H, CH₂O); 3.79–3.84 (m, 2H, H-3); 3.88, 3.97 (2 × bs, 2 × 1H, OH); 4.00 – 4.15 (m, 8H, CH₃CH₂O); 4.22 (dt, 2H, *J*_{4,5} = 5.6, 3.5, *J*_{4,3} = 3.5, H-4).

25

¹³C NMR (125.7 MHz, CDCl₃): 16.34, 16.39 (CH₃CH₂O); 26.90, 26.97 (2 × d, *J*_{C,P} = 140.7, CH₂P); 28.44 ((CH₃)₃C); 48.44, 49.22 (CH₂-2); 51.10, 51.30 (CH₂-5); 61.65, 61.71

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(2 × d, $J_{C,P} = 5.1$, CH₃CH₂O); 61.89, 61.97 (2 × d, $J_{C,P} = 6.3$, CH₃CH₂O); 63.55, 63.57 (CH₂O); 72.54, 73.39 (CH-4); 79.45 (C(CH₃)₃); 82.76, 83.37 (CH-3); 154.65 (CO).

³¹P{¹H} NMR (202.3 MHz, CDCl₃): 29.41, 29.62.

IR ν_{max} (CHCl₃) 3610 (w), 3359 (w, vbr), 2983 (s), 2932 (m), 2885 (m), 1688 (vs), 1495
5 (w, sh), 1478 (m), 1456 (m), 1415 (vs), 1393 (s), 1368 (s), 1245 (s), 1167 (s), 1109 (s), 1056 (vs), 1030 (vs), 965 (s), 461 (vw).

HRMS (ESI+) for C₁₅H₃₁NO₇P (M+H)⁺ : calcd 368.18327, found 368.18315.

Diethyl ((3*S*,4*S*)-4-hydroxypyrrolidin-3-yl)oxyethylphosphonate (**xiii**) and diethyl
10 ((3*R*,4*S*)-4-hydroxypyrrolidin-3-yl)oxyethylphosphonate (**xiv**) where also obtained using the above methodology.

Diethyl ((3*S*,4*S*)-4-hydroxypyrrolidin-3-yl)oxyethylphosphonate (**xiii**)

¹H NMR (600.1 MHz, D₂O, 25 °C), ¹³C NMR (150.9 MHz, D₂O, 25 °C), and ³¹P{¹H}
15 NMR (202.3 MHz, D₂O, 25 °C) data were identical to those for enantiomeric **xii**

IR ν_{max} (CHCl₃) 3610 (w), 3359 (m, br), 2985 (s), 2932 (m), 1688 (vs), 1478 (m), 1456 (m), 1415 (vs), 1394 (s), 1368 (s), 1245 (s), 1167 (s), 1109 (s), 1055 (vs), 1030 (vs), 964 (s).

HRMS (ESI+) for C₁₅H₃₁NO₇P (M+H)⁺ : calcd 368.18327, found 368.18309.

20

Diethyl ((3*R*,4*S*)-4-hydroxypyrrolidin-3-yl)oxyethylphosphonate (**xiv**, obtained as an ~1:1 mixture of two rotamers).

¹H NMR (500.0 MHz, DMSO-*d*₆): 1.22 (t, 12H, $J_{vic} = 7.1$, CH₃CH₂O); 1.38 (s, 18H, (CH₃)₃C); 2.02-2.17 (m, 4H, $J_{H,P} = 18.2$, $J_{vic} = 7.0$, CH₂P); 3.07 – 3.13 (m, 4H, H-2b,5b);
25 3.27-3.39 (m, 4H, H-2a,5a); 3.60-3.77 (m, 4H, CH₂O); 3.83-3.87 (m, 2H, H-3); 3.93 – 4.05 (m, 8H, CH₃CH₂O); 4.13-4.18 (m, 2H, H-4); 4.92, 4.93 (2 × d, 2 × 1H, $J_{OH,4} = 4.7$, OH);

¹³C NMR (125.7 MHz, DMSO-*d*₆): 16.42, 16.47 (CH₃CH₂O); 26.26 (d, $J_{C,P} = 137.3$, CH₂P); 28.35 ((CH₃)₃C); 47.79, 48.19 (CH₂-2); 50.81, 51.21 (CH₂-5); 61.30 (d, $J_{C,P} = 6.2$, CH₃CH₂O); 61.37, 61.39 (2 × d, $J_{C,P} = 6.3$, CH₃CH₂O); 63.55, 63.58 (2 × d, $J_{C,P} = 5.8$,
30 CH₃CH₂O);

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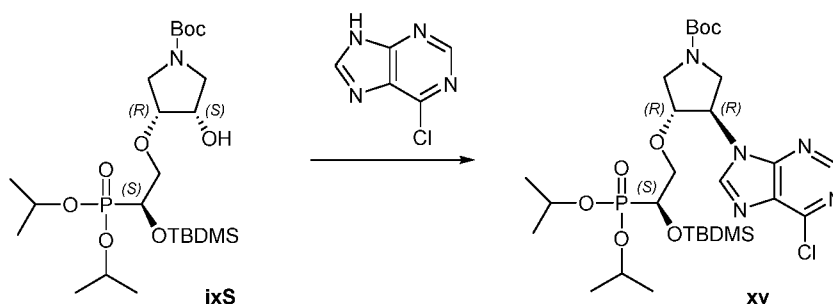
CH₂O); 68.26, 68.89 (CH-4); 78.66 (CH-3); 78.61 (C(CH₃)₃); 78.66 (CH-3); 153.88, 153.89 (CO).

³¹P{¹H} NMR (202.3 MHz, DMSO-*d*₆): 30.70, 30.79.

HRMS (ESI+) for C₁₅H₃₁NO₇P (M+H)⁺ : calcd 368.18327, found 368.18321.

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Example 8. Diisopropyl ((*S*)-1-*tert*-butyldimethylsilyloxy-2-(((3*R*,4*R*)-1-*N*-*boc*-4-(6-chloropurin-9-yl)pyrrolidin-3-yl)oxy)ethyl)phosphonate (xv)



DIAD (3.1 ml, 15.9 mmol) was added to the solution of Ph₃P (4.2 g, 15.9 mmol) in THF (50 ml) at rt under an atmosphere of argon. The reaction mixture was stirred until a white precipitate appeared (~20 min). A mixture of 6-chloropurine (1.23 g, 7.95 mmol) and **ixS** (0.87 g, 3.97 mmol) in THF (50 ml) was added and the reaction mixture was stirred at rt under an argon atmosphere overnight. The reaction mixture was concentrated *in vacuo* and the desired product was obtained by chromatography on silica gel using a linear gradient of ethanol in CHCl₃ in 31% yield (0.82 g, 1.24 mmol) in the form of grey foam as an ~1:1 mixture of two rotamers.

¹H NMR (500.0 MHz, CDCl₃): 0.08, 0.12 (2 × s, 2 × 6H, CH₃Si); 0.88 (s, 18H, (CH₃)₃CSi); 1.27-1.34 (m, 24H, (CH₃)₂CH); 1.49 (s, 18H, (CH₃)₃CO); 3.47 - 3.59 (bm, 2H, H-5'b); 3.60-3.72 (bm, 3H, H-5'a, OCH_aH_bCH-OTBDMS); 3.77 - 3.87 (bm, 2H, H-5'a, OCH_aH_bCH-OTBDMS); 3.87 - 3.96 (bm, 2H, H-2'b, OCH_aH_bCH-OTBDMS); 3.9 - 4.08 (bm, 5H, H-2'a,2'b, OCH₂CH-OTBDMS); 4.33, 4.36 (2 × bm, 2 × 1H, H-4'); 4.63 - 4.80 (m, 4H, (CH₃)₂CH); 5.07 - 5.15 (bm, 2H, H-3'); 8.08 (s, 2H, H-8); 8.75 (s, 2H, H-2).

¹³C NMR (125.7 MHz, CDCl₃): -4.88, -4.77 (CH₃Si); 18.18 ((CH₃)₃CSi); 23.93 (d, *J*_{C,P} = 4.9, (CH₃)₂CH); 24.02 (d, *J*_{C,P} = 3.6, (CH₃)₂CH); 24.10 (d, *J*_{C,P} = 4.6, (CH₃)₂CH); 24.24 (d, *J*_{C,P} = 3.4, (CH₃)₂CH); 25.64 ((CH₃)₃CSi); 28.38 ((CH₃)₃CO); 47.56, 48.21 (CH₂-

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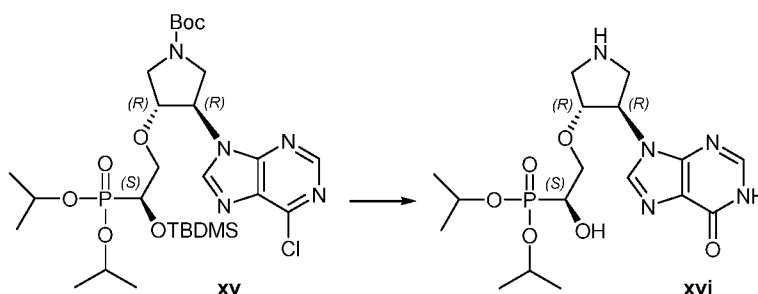
2'); 49.16, 49.63 (CH₂-5'); 58.00, 58.85 (CH-3'); 69.74 (d, $J_{C,P}$ = 170.2, OCH₂CH-OTBDMS); 71.02 (d, $J_{C,P}$ = 7.4, (CH₃)₂CH); 71.44 (bd, $J_{C,P}$ = 5.6, (CH₃)₂CH); 71.57 (d, $J_{C,P}$ = 10.2, OCH₂CH-OTBDMS); 80.36 (CH-4'); 80.75 ((CH₃)₃CO); 81.44 (CH-4'); 131.89 (C-5); 142.95, 143.03 (CH-8); 151.46, 151.49 (C-4,6); 152.06 (CH-2); 153.91, 5 154.12 (CO).

³¹P{¹H} NMR (202.3 MHz, CDCl₃): 18.87, 18.98.

IR ν_{max} (CHCl₃) 3118 (w, br), 3066 (vvw), 2983 (s), 2959 (m), 2932 (m), 2899 (m), 2886 (m), 1694 (vs), 1591 (s), 1563 (s), 1486 (m), 1473 (m), 1464 (m), 1456 (m), 1437 (m), 1426 (m, sh), 1408 (s, sh), 1399 (s), 1393 (s, sh), 1388 (s), 1376 (m), 1369 (m), 1340 (m), 10 1251 (s), 1239 (s, sh), 1195 (m), 1180 (m, sh), 1165 (s), 1150 (m), 1143 (m), 1105 (s), 1012 (s, sh), 1005 (s, sh), 993 (vs), 941 (m), 886 (w), 838 (m), 648 (w), 637 (w).

HRMS (ESI+) for C₂₈H₅₀N₅O₇ClPSi (M+H)⁺: calcd 662.29002, found 662.29023.

Example 9. Diisopropyl ((S)-1-hydroxy-2-(((3R,4R)-4-(6-oxopurin-9-yl)pyrrolidin-3-yl)oxy)ethyl)phosphonate (xvi)



Intermediate **xv** (0.82 g, 1.24 mmol) was dissolved in ethanol (20 ml) and 3M aq. HCl (20 ml) was added. The reaction mixture was stirred overnight at 75 °C. The mixture was 20 diluted with water (30 ml) and applied on a column of Dowex 50 in H⁺ form (70 ml). The column was washed with an ethanol:water mixture (1:1, 150 ml) and the crude product was eluted with 3% NH₃ in ethanol:water (1:1) (300 ml, UV absorption was monitored). The crude material was purified by rpHPLC using a linear gradient of methanol in water affording desired product **xvi** in 60% yield (0.32 g, 0.75 mmol).

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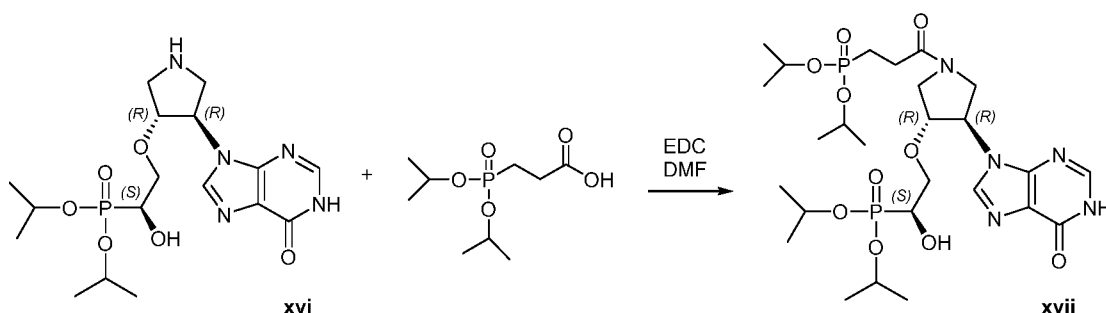
^1H NMR (500.0 MHz, CD_3OD): 1.308, 1.312, 1.316, 1.319 (4 $\times$ d, 4 $\times$ 3H, $J_{\text{vic}} = 6.2$, $(\text{CH}_3)_2\text{CH}$); 3.08 (dd, 1H, $J_{\text{gem}} = 12.5$, $J_{5'b,4'} = 3.6$, H-5'b); 3.25 (dd, 1H, $J_{\text{gem}} = 12.5$, $J_{2'b,3'} = 5.5$, H-2'b); 3.46 (dd, 1H, $J_{\text{gem}} = 12.5$, $J_{5'a,4'} = 5.9$, H-5'a); 3.55 (dd, 1H, $J_{\text{gem}} = 12.5$, $J_{2'a,3'} = 8.0$, H-2'a); 3.61 (ddd, 1H, $J_{\text{gem}} = 10.6$, $J_{\text{vic}} = 7.9$, $J_{\text{H,P}} = 5.7$, $\text{OCH}_a\text{H}_b\text{CH}(\text{OH})\text{P}$); 3.84 (ddd, 1H, $J_{\text{gem}} = 10.6$, $J_{\text{H,P}} = 9.4$, $J_{\text{vic}} = 3.0$, $\text{OCH}_a\text{H}_b\text{CH}(\text{OH})\text{P}$); 4.00 (ddd, 1H, $J_{\text{H,P}} = 10.8$, $J_{\text{vic}} = 7.9$, 3.0, $\text{CH}(\text{OH})\text{P}$); 4.44 (ddd, 1H, $J_{4',5'} = 5.9$, 3.6, $J_{4',3'} = 3.0$, H-4'); 4.63 – 4.78 (m, 4H, $\text{CH}(\text{CH}_3)_2$); 5.04 (ddd, 1H, $J_{3',2'} = 8.0$, 5.5, $J_{3',4'} = 3.0$, H-3'); 8.06 (s, 1H, H-2); 8.18 (s, 1H, H-8).

^{13}C NMR (125.7 MHz, CD_3OD): 24.21, 24.23 (2 $\times$ d, $J_{\text{C,P}} = 4.9$, $(\text{CH}_3)_2\text{CH}$); 24.33, 24.40 (2 $\times$ d, $J_{\text{C,P}} = 3.7$, $(\text{CH}_3)_2\text{CH}$); 52.25 (CH_2 -2'); 52.86 (CH_2 -5'); 63.48 (CH -3'); 68.89 (d, $J_{\text{C,P}} = 166.0$, $\text{CH}(\text{OH})\text{P}$); 71.43 (d, $J_{\text{C,P}} = 8.9$, $\text{OCH}_2\text{CH}(\text{OH})\text{P}$); 73.02, 73.27 (2 $\times$ d, $J_{\text{C,P}} = 7.4$, $(\text{CH}_3)_2\text{CH}$); 87.27 (CH -4'); 125.76 (C-5); 141.05 (CH -8); 146.59 (CH -2); 149.84 (C-4); 159.02 (C-6).

$^{31}\text{P}\{^1\text{H}\}$ NMR (202.3 MHz, CD_3OD): 21.98.

HRMS (ESI+) for $\text{C}_{17}\text{H}_{29}\text{N}_5\text{O}_6\text{P}$ ($\text{M}+\text{H}$) $^+$: calcd 430.18500, found 430.18501.

Example 10. [3*R*,4*R*]-4-hypoxanthin-9-yl-3-(diisopropyl phosphonoethoxy)-1-*N*-(diisopropyl phosphonopropionyl)pyrrolidine (xvii)

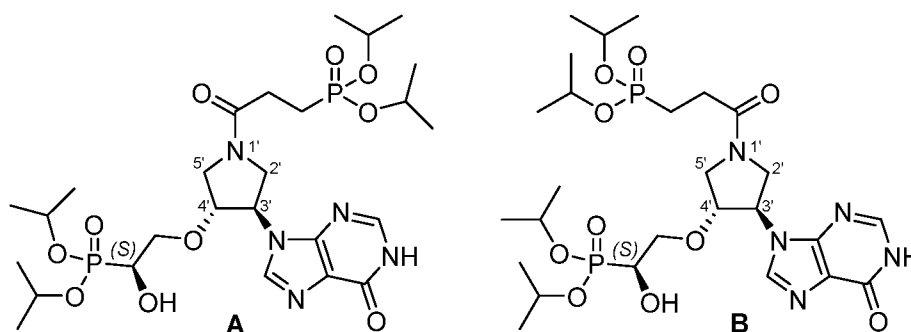


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EDC (0.36 g, 0.61 mmol) was added to a mixture of diisopropyl 3- phosphonopropionic acid (0.15 g, 1.2 mmol) and **xvi** (0.2 g, 0.47 mmol) in DMF (5 ml). The reaction mixture was stirred at 80 °C for 2h, concentrated *in vacuo*, and the desired product was obtained by chromatography on silica gel using a linear gradient of H1 in ethyl acetate in 74% yield (0.23 g, 0.35 mmol) in the form of a colorless glass as an ~ 4:5 mixture of rotamers A:B:

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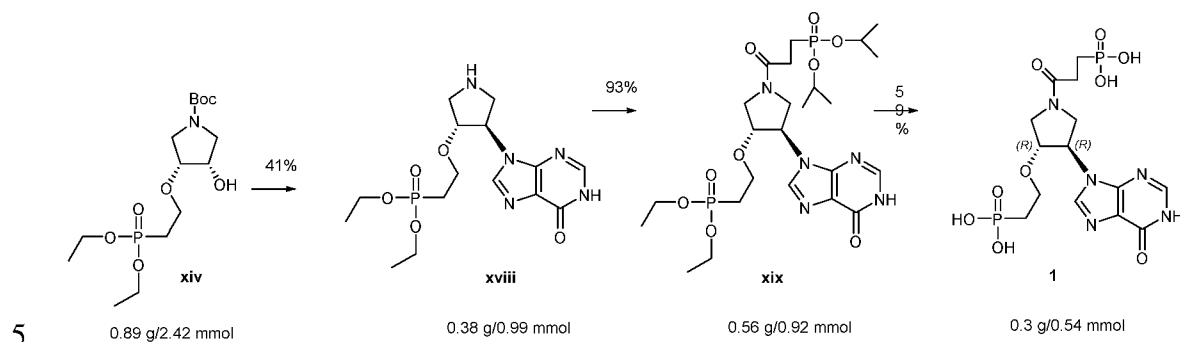


¹H NMR (500.0 MHz, CD₃OD): 1.30 - 1.36 (m, 48H, (CH₃)₂CH-A,B); 2.03 - 2.19 (m, 4H, COCH₂CH₂P-A,B); 2.55 - 2.72 (m, 4H, COCH₂CH₂P-A,B); 3.64 (dd, 1H, *J*_{gem} = 13.0, *J*_{5'b,4'} = 4.1, H-5'b-A); 3.66 - 3.74 (m, 3H, H-5'b-B, OCH_aH_bCH(OH)P-A,B); 3.84 - 3.92
 5 (m, 3H, H-5'a-A, OCH_aH_bCH(OH)P-A,B); 3.97 - 4.03 (m, 2H, CH(OH)P-A,B); 4.03 - 4.08 (m, 2H, H-2'b,5'a-B); 4.13 (dd, 1H, *J*_{gem} = 13.1, *J*_{2'a,3'} = 7.5, H-2'a-B); 4.16 (dd, 1H, *J*_{gem} = 11.5, *J*_{2'b,3'} = 5.2, H-2'b-A); 4.27 (dd, 1H, *J*_{gem} = 11.5, *J*_{2'a,3'} = 7.6, H-2'a-A); 4.58 (dt, 1H, *J*_{4',5'} = 6.0, 4.1, *J*_{4',3'} = 4.1, H-4'-A); 4.64 (dt, 1H, *J*_{4',5'} = 6.0, 4.1, *J*_{4',3'} = 4.1, H-4'-B); 4.64 - 4.76 (m, 8H, CH(CH₃)₂-A,B); 5.18 (dt, 1H, *J*_{3',2'} = 7.5, 5.2, *J*_{3',4'} = 4.1, H-3'-B); 5.27
 10 (dt, 1H, *J*_{3',2'} = 7.5, 5.2, *J*_{3',4'} = 4.1, H-3'-A); 8.06 (s, 1H, H-2-B); 8.07 (s, 1H, H-2-A); 8.09 (s, 1H, H-8-B); 8.16 (s, 1H, H-8-B).

¹³C NMR (125.7 MHz, CD₃OD): 22.54 (d, *J*_{C,P} = 145.8, COCH₂CH₂P-B); 22.55 (d, *J*_{C,P} = 145.8, COCH₂CH₂P-A); 24.15 - 24.47 (m, (CH₃)₂CH-A,B); 28.21 (d, *J*_{C,P} = 3.3, COCH₂CH₂P-A); 28.34 (d, *J*_{C,P} = 3.2, COCH₂CH₂P-B); 49.23 (CH₂-2'-B); 49.72 (CH₂-2'-
 15 A); 50.63 (CH₂-5'-A); 51.28 (CH₂-5'-B); 59.06 (CH-3'-B); 60.37 (CH-3'-A); 68.85 (d, *J*_{C,P} = 165.8, CH(OH)P-A,B); 71.89 (d, *J*_{C,P} = 9.1, OCH₂CH(OH)P-A); 72.01 (d, *J*_{C,P} = 9.6, OCH₂CH(OH)P-B); 72.43 (d, *J*_{C,P} = 6.7, (CH₃)₂CH-A,B); 73.06, 73.27, 73.31 (d, *J*_{C,P} = 7.4, (CH₃)₂CH-A,B); 81.68 (CH-4'-A); 83.02 (CH-4'-B); 125.78 (C-5-A); 125.80 (C-5-B); 140.34 (CH-8-B); 140.46 (CH-8-A); 146.75 (CH-2-B); 146.78 (CH-2-A); 150.16 (C-4-
 20 A,B); 158.89 (C-6-A,B); 172.22 (d, *J*_{C,P} = 16.4, NCO-A); 172.30 (d, *J*_{C,P} = 16.7, NCO-B).

³¹P{¹H} NMR (202.3 MHz, CD₃OD): 21.2 (PCH(OH)CH₂O-B); 21.75 (PCH(OH)CH₂O-A); 31.29 (PCH₂CH₂CO-A); 31.30 (PCH₂CH₂CO-B).

HRMS (ESI+) for C₂₆H₄₅N₅O₁₀ P₂Na (M+Na)⁺ : calcd 672.25339, found 672.25344.

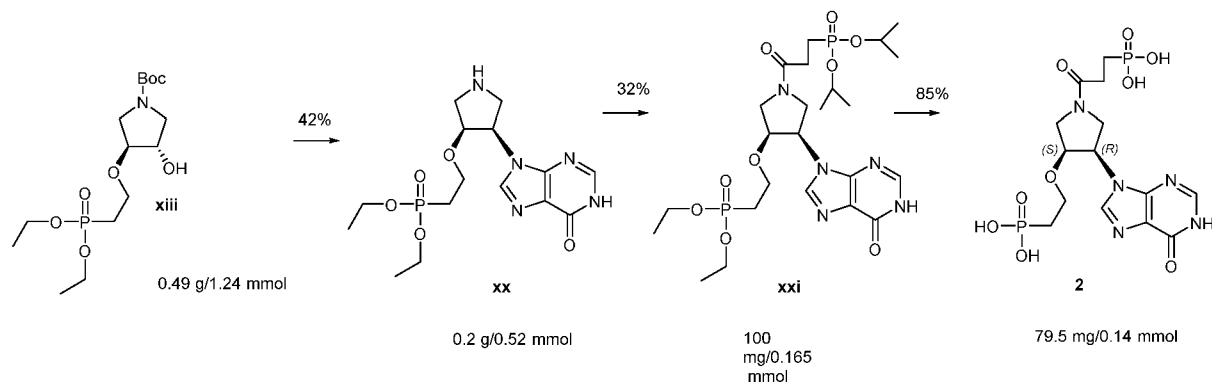
Synthesis of Compounds of the Invention**Example 11. [3*R*,4*R*]-4-hypoxanthin-9-yl-3-phosphonoethoxy-1-*N*-(phosphonopropionyl)pyrrolidine (1)**

Precursor **xiv** (0.89 g, 2.42 mmol) was converted via the sequence of general methods C, E, F, and B to Compound **1** in 22% overall yield (0.3 g, 0.54 mmol) in the form of a white amorphous lyophilizate as an ~ 6:7 mixture of two rotamers.

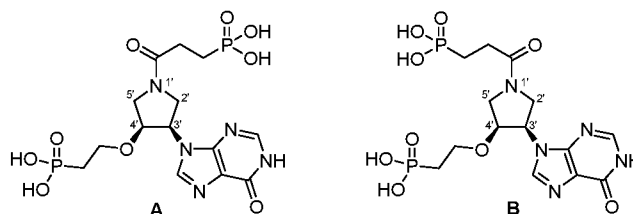
- 10 ^1H NMR (500.0 MHz, D_2O , ref (dioxane) = 3.75 ppm, 25 °C): 1.82 – 2.00 (m, 8H, $\text{COCH}_2\text{CH}_2\text{P-A,B}$, $\text{OCH}_2\text{CH}_2\text{P-A,B}$); 2.58 – 2.69 (m, 4H, $\text{COCH}_2\text{CH}_2\text{P-A,B}$); 3.63 (dd, 1H, $J_{\text{gem}} = 13.1$, $J_{5'b,4'} = 3.7$, H-5'b-A); 3.75 – 3.88 (m, 5H, H-5'b-B, $\text{OCH}_2\text{CH}_2\text{P-A,B}$); 3.94 (dd, 1H, $J_{\text{gem}} = 13.1$, $J_{5'a,4'} = 5.9$, H-5'a-A); 4.06 (dd, 1H, $J_{\text{gem}} = 13.3$, $J_{2'b,3'} = 4.7$, H-2'b-B); 4.09 – 4.18 (m, 2H, H-2'a,5'a-B); 4.20 (dd, 1H, $J_{\text{gem}} = 12.1$, $J_{2'b,3'} = 4.9$, H-2'b-A);
- 15 4.34 (dd, 1H, $J_{\text{gem}} = 12.1$, $J_{2'a,3'} = 7.5$, H-2'a-A); 4.57 (ddd, 1H, $J_{4'5'} = 5.9$, 3.7, $J_{4'3'} = 4.3$, H-4'-A); 4.62 (dt, 1H, $J_{4'5'} = 5.8$, 4.0, $J_{4'3'} = 4.0$, H-4'-B); 5.23 (ddd, 1H, $J_{3'2'} = 7.4$, 4.7, $J_{3'4'} = 4.0$, H-3'-B); 5.31 (ddd, 1H, $J_{3'2'} = 7.5$, 4.9, $J_{3'4'} = 4.3$, H-3'-A); 8.13 (s, 1H, H-8-B); 8.17 (s, 1H, H-8-A); 8.20 (s, 1H, H-2-B); 8.21 (s, 1H, H-2-A).
- ^{13}C NMR (125.7 MHz, D_2O , ref (dioxane) = 69.30 ppm, 25 °C): 25.89 (d, $J_{\text{C,P}} = 134.9$, $\text{COCH}_2\text{CH}_2\text{P-A,B}$); 31.25 (d, $J_{\text{C,P}} = 2.5$, $\text{COCH}_2\text{CH}_2\text{P-A}$); 31.36 (d, $J_{\text{C,P}} = 2.5$, $\text{COCH}_2\text{CH}_2\text{P-B}$); 31.81 (d, $J_{\text{C,P}} = 129.8$, $\text{OCH}_2\text{CH}_2\text{P-B}$); 31.82 (d, $J_{\text{C,P}} = 129.8$, $\text{OCH}_2\text{CH}_2\text{P-A}$); 50.72 ($\text{CH}_2\text{-2'-B}$); 51.59 ($\text{CH}_2\text{-2'-A}$); 52.17 ($\text{CH}_2\text{-5'-A}$); 53.06 ($\text{CH}_2\text{-5'-B}$); 60.01 (CH-3'-B); 61.18 (CH-3'-A); 68.75 (d, $J_{\text{C,P}} = 1.2$, $\text{OCH}_2\text{CH}_2\text{P-A}$); 68.81 (d, $J_{\text{C,P}} = 1.2$, $\text{OCH}_2\text{CH}_2\text{P-B}$); 82.14 (CH-4'-A); 83.40 (CH-4'-B); 126.48 (C-5-A); 126.51 (C-5-B);
- 20 142.67 (CH-8-B); 142.80 (CH-8-A); 148.52 (CH-2-A,B); 151.69 (C-4-A,B); 161.40 (C-6-A,B); 177.06 (d, $J_{\text{C,P}} = 17.8$, NCO-A); 177.15 (d, $J_{\text{C,P}} = 17.8$, NCO-B).

$^{31}\text{P}\{^1\text{H}\}$ NMR (202.3 MHz, D_2O , ref (external H_3PO_4) = 0 ppm, 25 °C): 20.71 (PCH₂CH₂O-A); 20.74 (PCH₂CH₂O-B); 24.10 (PCH₂CH₂CO-A); 24.15 (PCH₂CH₂CO-B). HRMS (ESI-) for C₁₄H₂₀N₅O₉P₂ (M-H)⁻: calcd 464.07417, found 464.07388.

5 Example 12. [3*S*,4*R*]-4-hypoxanthin-9-yl-3-phosphonoethoxy-1-*N*-(phosphonopropionyl)pyrrolidine (2)



Precursor **xiii** (0.49 g, 1.24 mmol) was converted via the sequence of general methods C, E, F, and B to Compound **2** in 11% overall yield (79.5 mg, 0.14 mmol) in the form of white amorphous lyophilizate as an ~ 6:7 mixture of rotamers A:B:



^1H NMR (500.0 MHz, D_2O , ref (dioxane) = 3.75 ppm, 25 °C): 1.58-1.81 (m, 4H, OCH₂CH₂P-A,B); 1.81 – 1.91 (m, 4H, COCH₂CH₂P-A,B); 2.59 – 2.70 (m, 4H, COCH₂CH₂P-A,B); 3.46-3.61 (m, 2H, OCH_aCH_bCH₂P-A,B); 3.68-3.78 (m, 3H, H-5'b-A, OCH_aCH_bCH₂P-A,B); 3.82 (dd, 1H, $J_{\text{gem}} = 11.9$, $J_{5'b,4'} = 4.3$, H-5'b-B); 3.84 (dd, 1H, $J_{\text{gem}} = 13.2$, $J_{5'a,4'} = 5.4$, H-5'a-A); 4.05 (dd, 1H, $J_{\text{gem}} = 12.6$, $J_{2'b,3'} = 6.9$, H-2'b-B); 4.07 (dd, 1H, $J_{\text{gem}} = 11.9$, $J_{5'a,4'} = 5.5$, H-5'a-B); 4.13 (dd, 1H, $J_{\text{gem}} = 12.6$, $J_{2'b,3'} = 7.7$, H-2'a-B); 4.24 (dd, 1H, $J_{\text{gem}} = 11.2$, $J_{2'b,3'} = 7.6$, H-2'b-A); 4.29 (dd, 1H, $J_{\text{gem}} = 11.2$, $J_{2'a,3'} = 7.6$, H-2'a-A); 4.50 (ddd, 1H, $J_{4',5'} = 5.4$, 3.7, $J_{4',3'} = 4.7$, H-4'-A); 4.56 (ddd, 1H, $J_{4',5'} = 5.5$, 4.3, $J_{4',3'} = 4.8$, H-

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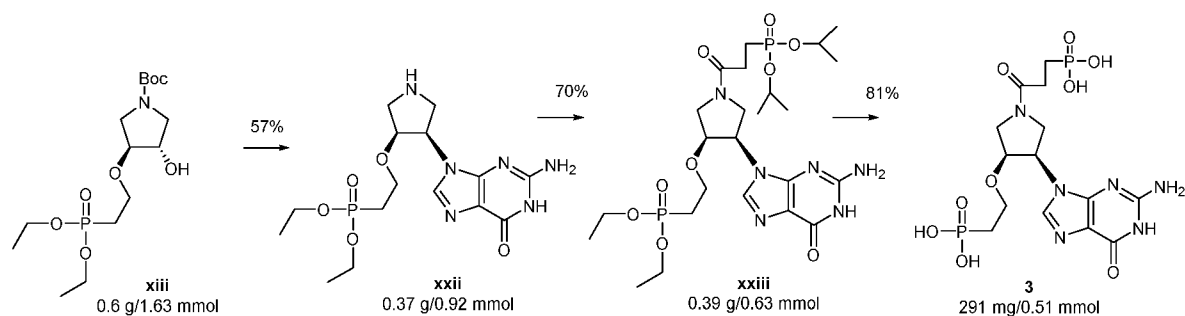
4'-B); 5.40 (ddd, 1H, $J_{3',2'} = 7.6, 6.9, J_{3',4'} = 4.8$, H-3'-B); 5.21 (td, 1H, $J_{3',2'} = 7.6, J_{3',4'} = 4.6$, H-3'-A); 8.22 (s, 2H, H-2-A,B); 8.25 (s, 1H, H-8-B); 8.28 (s, 1H, H-8-A).

^{13}C NMR (125.7 MHz, D_2O , ref(dioxane) = 69.30 ppm, 25 °C): 25.95 (d, $J_{\text{C,P}} = 134.7$, $\text{COCH}_2\text{CH}_2\text{P-B}$); 25.99 (d, $J_{\text{C,P}} = 134.6$, $\text{COCH}_2\text{CH}_2\text{P-A}$); 31.28 (d, $J_{\text{C,P}} = 2.4$, $\text{COCH}_2\text{CH}_2\text{P-B}$); 31.36 (d, $J_{\text{C,P}} = 2.4$, $\text{COCH}_2\text{CH}_2\text{P-A}$); 31.70 (d, $J_{\text{C,P}} = 129.1$, $\text{OCH}_2\text{CH}_2\text{P-B}$); 31.71 (d, $J_{\text{C,P}} = 129.1$, $\text{OCH}_2\text{CH}_2\text{P-A}$); 50.25 ($\text{CH}_2\text{-2'-B}$); 50.92 ($\text{CH}_2\text{-2'-A}$); 52.13 ($\text{CH}_2\text{-5'-A}$); 52.82 ($\text{CH}_2\text{-5'-B}$); 56.87 (CH-3'-B); 57.90 (CH-3'-A); 69.13 (d, $J_{\text{C,P}} = 2.4$, $\text{OCH}_2\text{CH}_2\text{P-A}$); 69.32 (d, $J_{\text{C,P}} = 2.5$, $\text{OCH}_2\text{CH}_2\text{P-B}$); 78.93 (CH-4'-A); 80.09 (CH-4'-B); 125.79 (C-5-A); 125.84 (C-5-B); 143.74 (CH-8-B); 143.89 (CH-8-A); 148.55 (CH-2-A,B); 152.04 (C-4-A); 152.09 (C-4-B); 161.41 (C-6-A,B); 177.21 (d, $J_{\text{C,P}} = 17.9$, NCO-A); 177.29 (d, $J_{\text{C,P}} = 17.9$, NCO-B).

$^{31}\text{P}\{^1\text{H}\}$ NMR (202.3 MHz, D_2O , ref (external H_3PO_4) = 0 ppm, 25 °C): 19.53 ($\text{PCH}_2\text{CH}_2\text{O-A}$); 19.58 ($\text{PCH}_2\text{CH}_2\text{O-B}$); 23.20 ($\text{PCH}_2\text{CH}_2\text{CO-A}$); 23.29 ($\text{PCH}_2\text{CH}_2\text{CO-B}$). IR ν_{max} (KBr) 2360 (w, vbr), 1691 (vs), 1634 (vs), 1590 (m), 1516 (w), 1458 (m), 1420 (w), 1341 (w), 1219 (m), 1073 (m, sh), 1154 (m, br), 1051 (s), 979 (w), 986 (m), 792 (w), 649 (w).

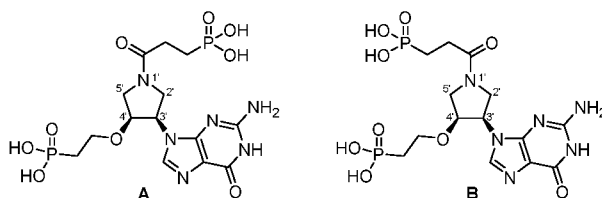
HRMS (ESI-) for $\text{C}_{14}\text{H}_{20}\text{N}_5\text{O}_9\text{P}_2$ (M-H^-): calcd 464.07417, found 464.07333.

Example 13. [3*S*,4*R*]-4-guanin-9-yl-3-phosphonoethoxy-1-*N*-(phosphonopropionyl)pyrrolidine (**3**)



Precursor **xiii** (0.6 g, 1.63 mmol) was converted via the sequence of general methods D, E, F, and B to title compound **3** in 31% overall yield (291 mg, 0.51 mmol) in the form of white amorphous lyophilizate as an ~ 6:7 mixture of rotamers A:B:

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^1H NMR (500.0 MHz, D_2O , ref (dioxane) = 3.75 ppm, 25 °C): 1.64-1.83 (m, 4H, $\text{OCH}_2\text{CH}_2\text{P-A,B}$); 1.83 – 1.91 (m, 4H, $\text{COCH}_2\text{CH}_2\text{P-A,B}$); 2.60 – 2.67 (m, 4H, $\text{COCH}_2\text{CH}_2\text{P-A,B}$); 3.52-3.83 (m, 7H, H-5'-A, H-5'-B, $\text{OCH}_2\text{CH}_2\text{P-A,B}$); 3.96 (dd, 1H, $J_{\text{gem}} = 12.6$, $J_{2'b,3'} = 7.1$, H-2'-b-B); 4.02 (dd, 1H, $J_{\text{gem}} = 11.9$, $J_{5'a,4'} = 5.4$, H-5'-a-B); 4.07 (dd, 1H, $J_{\text{gem}} = 12.6$, $J_{2'b,3'} = 7.7$, H-2'-a-B); 4.15 (dd, 1H, $J_{\text{gem}} = 11.2$, $J_{2'b,3'} = 7.6$, H-2'-b-A); 4.23 (dd, 1H, $J_{\text{gem}} = 11.2$, $J_{2'a,3'} = 7.6$, H-2'-a-A); 4.44 (ddd, 1H, $J_{4',5'} = 5.1$, 3.7, $J_{4',3'} = 4.6$, H-4'-A); 4.50 (dt, 1H, $J_{4',5'} = 5.4$, 4.6, $J_{4',3'} = 4.6$, H-4'-B); 5.15 (ddd, 1H, $J_{3',2'} = 7.7$, 7.1, $J_{3',4'} = 4.6$, H-3'-B); 5.21 (td, 1H, $J_{3',2'} = 7.6$, $J_{3',4'} = 4.6$, H-3'-A); 7.89 (s, 1H, H-8-B); 7.93 (s, 1H, H-8-A).

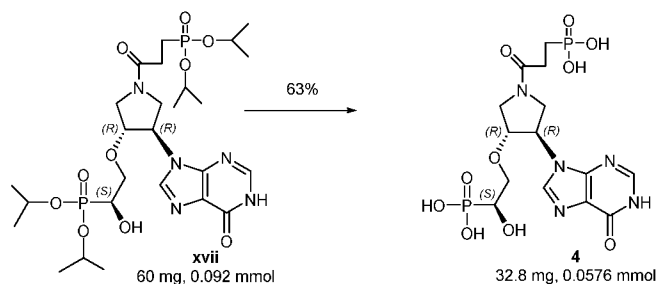
^{13}C NMR (125.7 MHz, D_2O , ref (dioxane) = 69.30 ppm, 25 °C): 25.92 (d, $J_{\text{C,P}} = 134.7$, $\text{COCH}_2\text{CH}_2\text{P-B}$); 25.95 (d, $J_{\text{C,P}} = 134.7$, $\text{COCH}_2\text{CH}_2\text{P-A}$); 31.21 (d, $J_{\text{C,P}} = 2.4$, $\text{COCH}_2\text{CH}_2\text{P-B}$); 31.32 (d, $J_{\text{C,P}} = 2.4$, $\text{COCH}_2\text{CH}_2\text{P-A}$); 31.65 (d, $J_{\text{C,P}} = 129.0$, $\text{OCH}_2\text{CH}_2\text{P-B}$); 31.67 (d, $J_{\text{C,P}} = 129.0$, $\text{OCH}_2\text{CH}_2\text{P-A}$); 50.08 ($\text{CH}_2\text{-2'-B}$); 50.82 ($\text{CH}_2\text{-2'-A}$); 52.11 ($\text{CH}_2\text{-5'-A}$); 52.76 ($\text{CH}_2\text{-5'-B}$); 56.02 (CH-3'-B); 57.13 (CH-3'-A); 69.17 (d, $J_{\text{C,P}} = 2.5$, $\text{OCH}_2\text{CH}_2\text{P-A}$); 69.37 (d, $J_{\text{C,P}} = 25.0$, $\text{OCH}_2\text{CH}_2\text{P-B}$); 78.88 (CH-4'-A); 80.05 (CH-4'-B); 118.26 (C-5-A); 118.29 (C-5-B); 141.14 (CH-8-B); 141.34 (CH-8-A); 154.71 (C-4-A); 154.76 (C-4-B); 156.48 (C-2-A,B); 161.68 (C-6-A,B); 177.13 (d, $J_{\text{C,P}} = 17.8$, NCO-A); 177.17 (d, $J_{\text{C,P}} = 17.8$, NCO-B).

$^{31}\text{P}\{^1\text{H}\}$ NMR (202.3 MHz, D_2O , ref (external H_3PO_4) = 0 ppm, 25 °C): 19.73 ($\text{PCH}_2\text{CH}_2\text{O-A}$); 19.75 ($\text{PCH}_2\text{CH}_2\text{O-B}$); 23.32 ($\text{PCH}_2\text{CH}_2\text{CO-A}$); 23.39 ($\text{PCH}_2\text{CH}_2\text{CO-B}$).

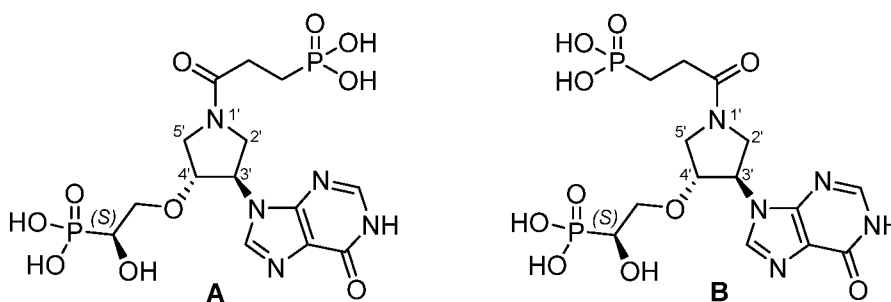
IR ν_{max} (KBr) 3120 (m, br), 2775 (m, br), 2365 (w, vbr), 1691 (vs), 1632 (vs), 1605 (s, sh), 1570 (m, sh), 1534 (w), 1478 (m), 1415 (w), 1169 (m), 1148 (m, sh), 1067 (m), 1052 (m, sh), 979 (w), 898 (m), 782 (w), 640 (w).

HRMS (ESI-) for $\text{C}_{14}\text{H}_{21}\text{N}_6\text{O}_9\text{P}_2$ (M-H)⁻: calcd 479.08507, found 479.08478.

Example 14. [3*R*,4*R*]-4-hypoxanthin-9-yl-3-((*S*)-2-hydroxy-2-phosphonoethyl)oxy-1-*N*-(phosphonopropionyl)pyrrolidine (4**)**



Precursor **xvii** (60 mg, 0.092 mmol) was converted using general method B to title compound **4** in 63% yield (32.8 mg, 0.058 mmol) in the form of white amorphous lyophilizate as an ~ 6:7 mixture of rotamers A:B:



- ¹H NMR (500.0 MHz, D₂O, ref (dioxane) = 3.75 ppm, 25 °C): 1.80 – 1.93 (m, 4H, COCH₂CH₂P-A,B); 2.60 – 2.69 (m, 4H, COCH₂CH₂P-A,B); 3.66 – 3.79 (m, 3H, H-5'b-A, OCH_aH_bCH(OH)P-A,B); 3.83 (dd, 1H, *J*_{gem} = 12.1, *J*_{5'b,4'} = 3.4, H-5'b-B); 3.86-4.02 (m, 5H, H-5'a-A, CH(OH)P-A,B, OCH_aH_bCH(OH)P-A,B); 4.06 – 4.19 (m, 3H, H-2',5'a-B,); 4.22 (dd, 1H, *J*_{gem} = 12.2, *J*_{2'b,3'} = 4.5, H-2'b-A); 4.37 (dd, 1H, *J*_{gem} = 12.2, *J*_{2'a,3'} = 7.3, H-2'a-A); 4.58 (dt, 1H, *J*_{4',5'} = 5.8, 3.8, *J*_{4',3'} = 3.8, H-4'-A); 4.63 (dt, 1H, *J*_{4',5'} = 5.7, 3.4, *J*_{4',3'} = 3.4, H-4'-B); 5.27 (ddd, 1H, *J*_{3',2'} = 7.2, 3.9, *J*_{3',4'} = 3.4, H-3'-B); 5.34 (ddd, 1H, *J*_{3',2'} = 7.2, 4.8, *J*_{3',4'} = 3.8, H-3'-A); 8.12 (s, 1H, H-8-B); 8.16 (s, 1H, H-8-A); 8.20 (s, 2H, H-2-A,B).
- ¹³C NMR (125.7 MHz, D₂O, ref (dioxane) = 69.30 ppm, 25 °C): 25.95 (d, *J*_{C,P} = 134.8, COCH₂CH₂P-A,B); 31.36 (d, *J*_{C,P} = 2.4, COCH₂CH₂P-A); 31.46 (d, *J*_{C,P} = 2.4, COCH₂CH₂P-B); 50.81 (CH₂-2'-B); 51.68 (CH₂-2'-A); 52.29 (CH₂-5'-A); 53.16 (CH₂-5'-B); 60.03 (CH-3'-B); 61.24 (CH-3'-A); 71.74 (d, *J*_{C,P} = 150.1, CH(OH)P-B); 71.76 (d, *J*_{C,P} = 150.1, CH(OH)P-A); 74.20 (d, *J*_{C,P} = 11.1, OCH₂CH(OH)P-A); 74.26 (d, *J*_{C,P} = 11.2,

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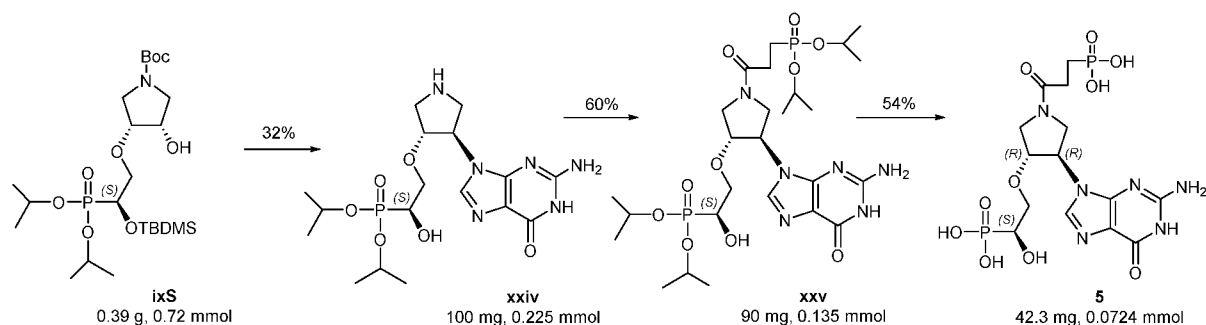
OCH₂CH(OH)P-B); 82.68 (CH-4'-A); 83.97 (CH-4'-B); 126.45 (C-5-A); 126.49 (C-5-B); 142.67 (CH-8-B); 142.81 (CH-8-A); 148.49 (CH-2-A,B); 151.68 (C-4-A,B); 161.40 (C-6-A,B); 177.17 (d, $J_{C,P}$ = 18.0, NCO-A); 177.25 (d, $J_{C,P}$ = 17.9, NCO-B).

³¹P{¹H} NMR (202.3 MHz, D₂O, ref (external H₃PO₄) = 0 ppm, 25 °C): 15.08 (PCH(OH)CH₂O-A); 15.09 (PCH(OH)CH₂O-B); 23.24 (PCH₂CH₂CO-A); 23.30 (PCH₂CH₂CO-B).

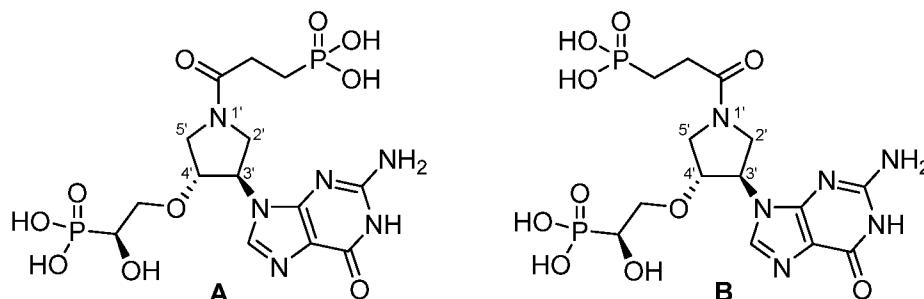
IR $\nu_{\max}$ (KBr) 1690 (s), 1632 (vs), 1590 (m, sh), 1551 (w), 1516 (w), 1460 (w), 1419 (w), 1342 (w), 1221 (w), 1175 (w), 1140 (w, sh), 1069 (w, sh), 1047 (w), 973 (w), 896 (m, br), 791 (w), 647 (w).

10 HRMS (ESI-) for C₁₄H₂₀N₅O₁₀P₂ (M-H)⁻: calcd 480.06909, found 480.06808.

Example 15. [3*R*,4*R*]-4-guanin-9-yl-3-((*S*)-2-hydroxy-2-phosphonoethyl)oxy-1-*N*-(phosphonopropionyl)pyrrolidine (5**)**



Precursor **ixS** (0.39 g, 0.72 mmol) was converted via the sequence of general methods D, E, F, and B to title compound **5** in 10% overall yield (42.3 mg, 72.4 μ mol) in the form of white amorphous lyophilizate as an ~ 1:1 mixture of rotamers A:B:



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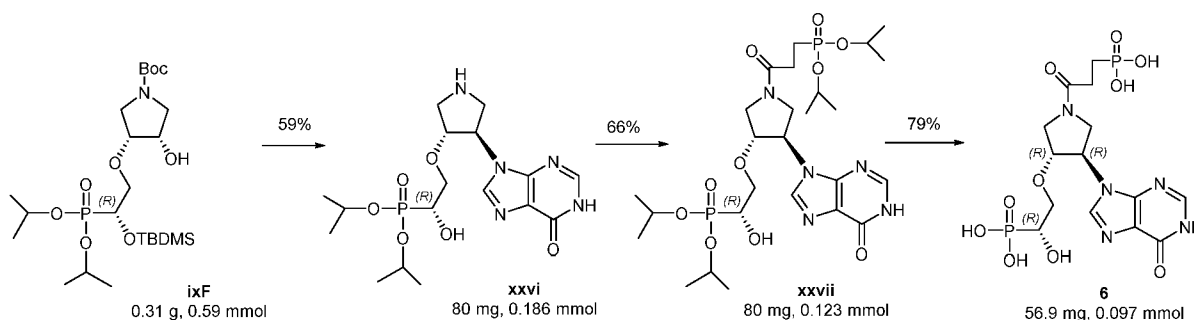
¹H NMR (500.0 MHz, D₂O, ref (dioxane) = 3.75 ppm, 25 °C): 1.76 – 1.93 (m, 4H, COCH₂CH₂P-A,B); 2.49 – 2.70 (m, 4H, COCH₂CH₂P-A,B); 3.45 (dd, 1H, *J*_{gem} = 12.4, *J*_{5'b,4'} = 6.9, H-5'b-A); 3.57 – 3.69 (m, 3H, H-5'b-B, OCH₄H₆CH(OH)P-A,B); 3.77-3.89 (m, 4H, CH(OH)P-A,B, OCH₄H₆CH(OH)P-A,B); 4.03 (dd, 1H, *J*_{gem} = 12.5, *J*_{2'b,3'} = 9.5, H-2'b-B); 4.16 (dd, 1H, *J*_{gem} = 12.5, *J*_{2'b,3'} = 8.2, H-2'a-B); 4.19 (dd, 1H, *J*_{gem} = 11.2, *J*_{2'b,3'} = 9.2, H-2'b-A); 4.27 (dd, 1H, *J*_{gem} = 12.4, *J*_{5'a,4'} = 7.5, H-5'a-A); 4.36 (dd, 1H, *J*_{gem} = 11.2, *J*_{2'a,3'} = 8.4, H-2'a-A); 4.40 (dd, 1H, *J*_{gem} = 11.2, *J*_{5'a,4'} = 7.5, H-5'a-B); 5.09-5.23 (m, 4H, H-3',4'-A,B); 7.97, 7.98 (2 × s, 2 × 1H, H-8-B).

¹³C NMR (125.7 MHz, D₂O, ref (dioxane) = 69.30 ppm, 25 °C): 25.85, 25.87 (d, *J*_{C,P} = 135.0, COCH₂CH₂P-A,B); 30.87 (d, *J*_{C,P} = 2.2, COCH₂CH₂P-A); 30.96 (d, *J*_{C,P} = 2.4, COCH₂CH₂P-B); 47.81 (CH₂-2'-B); 48.76 (CH₂-2'-A); 51.75 (CH₂-5'-A); 52.97 (CH₂-5'-B); 63.32 (CH-3'-B); 64.20 (CH-3'-A); 71.50 (d, *J*_{C,P} = 152.6, CH(OH)P-A,B); 74.23, 74.29 (2 × d, *J*_{C,P} = 10.4, OCH₂CH(OH)P-A,B); 80.69 (CH-4'-A); 81.67 (CH-4'-B); 114.36, 114.37 (C-5-A,B); 142.68 (CH-8-A,B); 149.79 (C-4-A,B); 157.41, 157.46 (C-2-A,B); 165.31 (C-6-A,B); 176.91, 176.99 (2 × d, *J*_{C,P} = 17.8, NCO-A,B)

³¹P{¹H} NMR (202.3 MHz, D₂O, ref (external H₃PO₄) = 0 ppm, 25 °C): 16.37 (PCH(OH)CH₂O-A,B); 24.26, 24.33 (PCH₂CH₂CO-A,B).

HRMS (ESI-) for C₁₄H₂₁N₆O₁₀P₂ (M-H)⁻: calcd 495.07999, found 495.07951.

Example 16. [3*R*,4*R*]-4-hypoxanthin-9-yl-3-((*R*)-2-hydroxy-2-phosphonoethyl)oxy-1-*N*-(phosphonopropionyl)pyrrolidine (6)

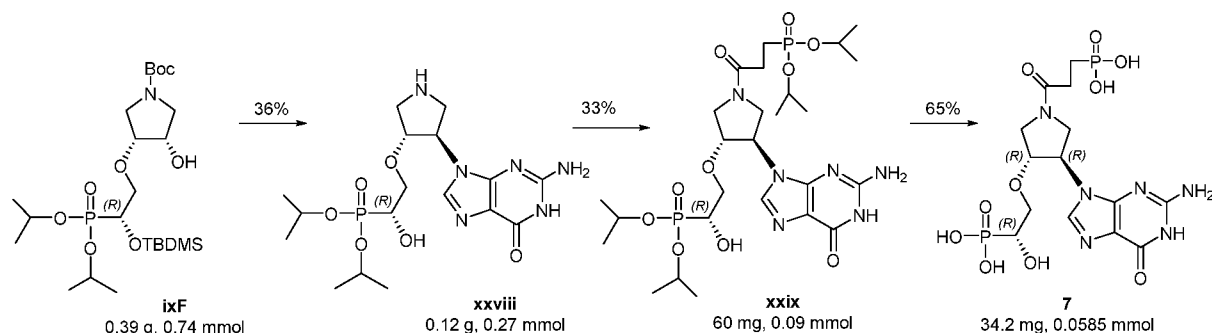


Precursor **ixF** (0.31 g, 0.59 mmol) was converted via the sequence of general methods C, E, F, and B to title compound **6** in 16% overall yield (56.9 mg, 97 μmol) in the form of white amorphous lyophilizate as an ~ 6:7 mixture of rotamers A:B.

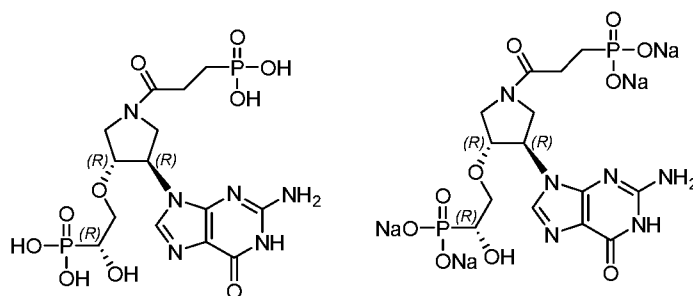
- 58 -

- ¹H NMR (500.0 MHz, D₂O, ref (dioxane) = 3.75 ppm, 25 °C): 1.81 – 1.94 (m, 4H, COCH₂CH₂P-A,B); 2.60 – 2.71 (m, 4H, COCH₂CH₂P-A,B); 3.64 – 3.78 (m, 3H, H-5'b-A, OCH_aH_bCH(OH)P-A,B); 3.84 (dd, 1H, *J*_{gem} = 12.1, *J*_{5'b,4'} = 3.6, H-5'b-B); 3.85-4.03 (m, 5H, H-5'a-A, CH(OH)P-A,B, OCH_aH_bCH(OH)P-A,B); 4.08 (dd, 1H, *J*_{gem} = 13.3, *J*_{2'b,3'} = 4.5, H-2'b-B); 4.10-4.21 (m, 2H, H-2'a,5'a-B); 4.22 (dd, 1H, *J*_{gem} = 12.2, *J*_{2'b,3'} = 4.7, H-2'b-A); 4.37 (dd, 1H, *J*_{gem} = 12.2, *J*_{2'a,3'} = 7.3, H-2'a-A); 4.60 (dt, 1H, *J*_{4',5'} = 5.9, 4.0, *J*_{4',3'} = 4.0, H-4'-A); 4.65 (ddd, 1H, *J*_{4',5'} = 5.9, 3.6, *J*_{4',3'} = 3.9, H-4'-B); 5.25 (ddd, 1H, *J*_{3',2'} = 7.2, 4.5, *J*_{3',4'} = 3.9, H-3'-B); 5.33 (ddd, 1H, *J*_{3',2'} = 7.3, 4.7, *J*_{3',4'} = 4.0, H-3'-A); 8.12 (s, 1H, H-8-B); 8.17 (s, 1H, H-8-A); 8.20 (s, 2H, H-2-A,B).
- ¹³C NMR (125.7 MHz, D₂O, ref (dioxane) = 69.30 ppm, 25 °C): 25.93 (d, *J*_{C,P} = 134.8, COCH₂CH₂P-A,B); 31.31 (d, *J*_{C,P} = 2.4, COCH₂CH₂P-A); 31.41 (d, *J*_{C,P} = 2.4, COCH₂CH₂P-B); 50.79 (CH₂-2'-B); 51.67 (CH₂-2'-A); 52.15 (CH₂-5'-A); 53.03 (CH₂-5'-B); 60.04 (CH-3'-B); 61.22 (CH-3'-A); 71.60 (d, *J*_{C,P} = 150.7, CH(OH)P-A,B); 74.10 (d, *J*_{C,P} = 11.0, OCH₂CH(OH)P-A); 74.19 (d, *J*_{C,P} = 11.0, OCH₂CH(OH)P-B); 82.59 (CH-4'-A); 83.88 (CH-4'-B); 126.45 (C-5-A); 126.49 (C-5-B); 142.64 (CH-8-B); 142.78 (CH-8-A); 148.52 (CH-2-A,B); 151.67 (C-4-A,B); 161.37 (C-6-A,B); 177.12 (d, *J*_{C,P} = 17.9, NCO-A); 177.21 (d, *J*_{C,P} = 17.9, NCO-B).
- ³¹P{¹H} NMR (202.3 MHz, D₂O, ref (external H₃PO₄) = 0 ppm, 25 °C): 15.17 (PCH(OH)CH₂O-A); 15.19 (PCH(OH)CH₂O-B); 23.33 (PCH₂CH₂CO-A); 23.39 (PCH₂CH₂CO-B).
- IR *v*_{max}(KBr) 1690 (s), 1632 (vs), 1590 (m, sh), 1551 (w), 1516 (w), 1459 (w), 1419 (w), 1342 (w), 1219 (w), 1172 (w, br), 1140 (w, sh), 1068 (w, sh), 1051 (w, br), 974 (w), 898 (w, br), 792 (w), 647 (w).
- HRMS (ESI-) for C₁₄H₂₀N₅O₁₀P₂ (M-H)⁻ : calcd 480.06909, found 480.06833.

Example 17. [3*R*,4*R*]-4-guanin-9-yl-3-((*R*)-2-hydroxy-2-phosphonoethyl)oxy-1-*N*-(phosphonopropionyl)pyrrolidine (7**)**



- 5 Precursor **ixF** (0.39 g, 0.74 mmol) was converted via the sequence of general methods D, E, F, and B to title compound **7** in 8% overall yield (34.2 mg, 59 μ mol) in the form of white amorphous lyophilizate as an ~ 6:7 mixture of rotamers A:B:



- ¹H NMR (500.0 MHz, D₂O, ref (dioxane) = 3.75 ppm, 25 °C): 1.74 – 1.92 (m, 4H, COCH₂CH₂P-A,B); 2.54 – 2.70 (m, 4H, COCH₂CH₂P-A,B); 3.64 – 3.78 (m, 3H, H-5'b-A, OCH_aH_bCH(OH)P-A,B); 3.81 (dd, 1H, *J*_{gem} = 12.4, *J*_{5'b,4'} = 3.1, H-5'b-B); 3.87 (dd, 1H, *J*_{gem} = 13.4, *J*_{5'a,4'} = 5.6, H-5'a-A); 3.90 – 3.98 (m, 2H, CH(OH)P-A,B); 4.00 – 4.13 (m, 5H, H-2',5'a-B, OCH_aH_bCH(OH)P-A,B); 4.15 (dd, 1H, *J*_{gem} = 12.2, *J*_{2'b,3'} = 4.0, H-2'b-A); 4.31 (dd, 1H, *J*_{gem} = 12.2, *J*_{2'a,3'} = 7.1, H-2'a-A); 4.50 (dt, 1H, *J*_{4',5'} = 5.6, 4.0, *J*_{4',3'} = 4.0, H-4'-A); 4.55 (m, 1H, H-4'-B); 5.04 (dt, 1H, *J*_{3',2'} = 7.2, 3.9, *J*_{3',4'} = 3.9, H-3'-B); 5.12 (dt, 1H, *J*_{3',2'} = 7.1, 4.0, *J*_{3',4'} = 4.0, H-3'-A); 7.74 (s, 1H, H-8-B); 7.80 (s, 1H, H-8-A).

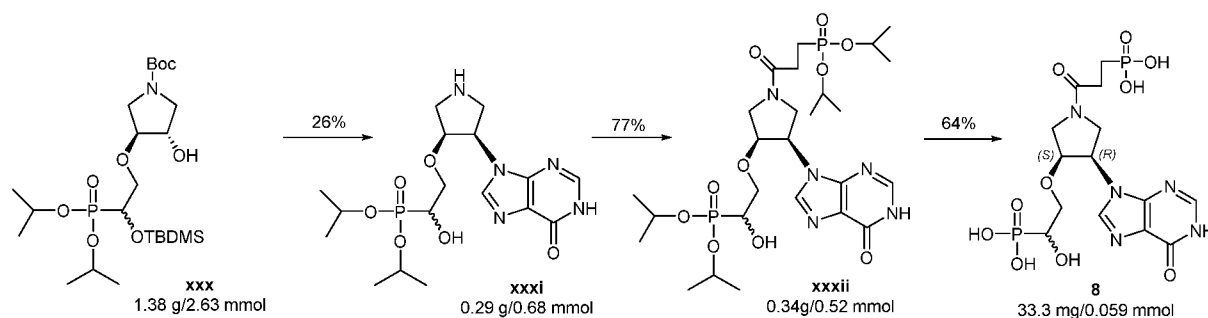
- ¹³C NMR (125.7 MHz, D₂O, ref (dioxane) = 69.30 ppm, 25 °C): 25.98 (d, *J*_{C,P} = 134.6, COCH₂CH₂P-B); 25.99 (d, *J*_{C,P} = 134.6, COCH₂CH₂P-A); 31.42 (d, *J*_{C,P} = 2.4, COCH₂CH₂P-A); 31.51 (d, *J*_{C,P} = 2.4, COCH₂CH₂P-B); 50.55 (CH₂-2'-B); 51.54 (CH₂-2'-A); 52.28 (CH₂-5'-A); 53.08 (CH₂-5'-B); 59.11 (CH-3'-B); 60.39 (CH-3'-A); 71.88 (d, *J*_{C,P} = 149.0, CH(OH)P-A,B); 74.30 (d, *J*_{C,P} = 11.6, OCH₂CH(OH)P-A); 74.40 (d, *J*_{C,P} = 11.6,

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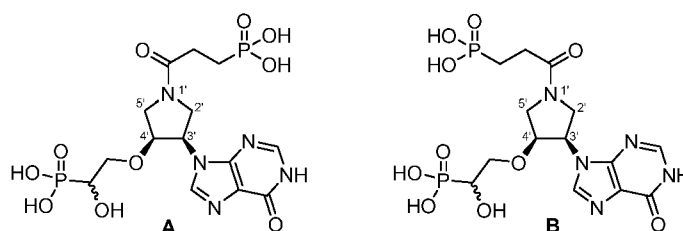
OCH₂CH(OH)P-B); 82.61 (CH-4'-A); 83.93 (CH-4'-B); 118.89 (C-5-A); 118.91 (C-5-B); 139.77 (CH-8-B); 140.02 (CH-8-A); 154.49 (C-4-A); 154.51 (C-4-B); 156.48 (C-2-A); 156.51 (C-2-B); 161.77 (C-6-A,B); 177.15 (d, $J_{C,P}$ = 17.9, NCO-A); 177.24 (d, $J_{C,P}$ = 17.9, NCO-B).

- 5 ³¹P{¹H} NMR (202.3 MHz, D₂O, ref (external H₃PO₄) = 0 ppm, 25 °C): 14.89 (PCH(OH)CH₂O-A); 14.92 (PCH(OH)CH₂O-B); 23.13 (PCH₂CH₂CO-A); 23.20 (PCH₂CH₂CO-B). IR $\nu_{\max}$ (KBr) 3128 (m, vbr), 2770 (w, vbr, sh), 2360 (w, vbr), 1693 (vs), 1632 (vs, br), 1534 (w), 1477 (w), 1413 (w), 1174 (m), 1070 (m, br), 974 (w), 900 (w, br), 780 (w), 724 (w), 637 (w).
- 10 HRMS (ESI-) for C₁₄H₂₁N₆O₁₀P₂ (M-H)⁻ : calcd 495.07999, found 495.07932.

Example 18. [3*S*,4*R*]-4-hypoxanthin-9-yl-3-((*RS*)-2-hydroxy-2-phosphonoethyl)oxy-1-*N*-(phosphonopropionyl)pyrrolidine (8**)**



Precursor **xxx** (1.38 g, 2.63 mmol) was converted via the sequence of general methods C, E, F, and B to title compound **8** in 13% overall yield (33.3 mg, 59 μ mol) in the form of white amorphous lyophilizate as an ~ 1:1:1:1 mixture of four isomers (two epimeric rotamers A and two epimeric rotamers B).



¹H NMR (500.0 MHz, D₂O, ref (dioxane) = 3.75 ppm, 25 °C): 1.81-1.91 (m, 8H, COCH₂CH₂P-A,B); 2.59-2.69 (m, 8H, COCH₂CH₂P-A,B); 3.43-3.52 (m, 2H, OCH_aH_bCH(OH)P-A); 3.59-3.93 (m, 16H, H-5'-A, H-5'-b-B, OCH₂CH(OH)P-A,B,

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OCH_aH_bCH(OH)P-A, OCH₂CH(OH)P-B); 3.99-4.09 (m, 2H, H-2'b,5'a-B); 4.10-4.17 (m, 2H, H-2'a-B); 4.19-4.26 (m, 2H, H-2'b-A); 4.26-4.32 (m, 2H, H-2'a-A); 4.50-4.54 (m, 2H, H-4'-A); 4.56-4.61 (m, 2H, H-4'-B); 5.36-5.42 (m, 2H, H-3'-B); 5.42-5.48 (m, 2H, H-3'-A); 8.208 (s, 2H, H-2-B); 8.213 (s, 2H, H-2-A); 8.28, 8.32, 7.36 (3 × s, 4H, H-8-A,B).

- 5 ¹³C NMR (125.7 MHz, D₂O, ref (dioxane) = 69.30 ppm, 25 °C): 26.00, 26.04 (d, *J*_{C,P} = 134.4, COCH₂CH₂P-A,B); 31.26, 31.29 (d, *J*_{C,P} = 2.4, COCH₂CH₂P-A); 31.43 (d, *J*_{C,P} = 2.4, COCH₂CH₂P-B); 50.30, 50.36 (CH₂-2'-B); 50.98, 51.03 (CH₂-2'-A); 52.10, 52.29 (CH₂-5'-A); 52.80, 53.05 (CH₂-5'-B); 56.89, 56.90 (CH-3'-B); 57.86, 57.89 (CH-3'-A); 71.72, 71.75, 71.82, 71.88 (d, *J*_{C,P} = 149.7, CH(OH)P-A,B); 74.28, 74.46, 74.52, 74.69 (d, *J*_{C,P} = 12.0, OCH₂CH(OH)P-A,B); 79.30, 79.40 (CH-4'-A); 80.54, 80.57 (CH-4'-B); 125.70, 125.73, 125.76, 125.79 (C-5-A,B); 143.89, 144.04, 144.12, 144.28 (CH-8-A,B); 148.48, 148.54 (CH-2-A,B); 152.06, 152.11, 152.15 (C-4-A,B); 161.43, 161.44 (C-6-A,B); 177.28 (d, *J*_{C,P} = 17.9, NCO-A); 177.36 (d, *J*_{C,P} = 18.0, NCO-B).

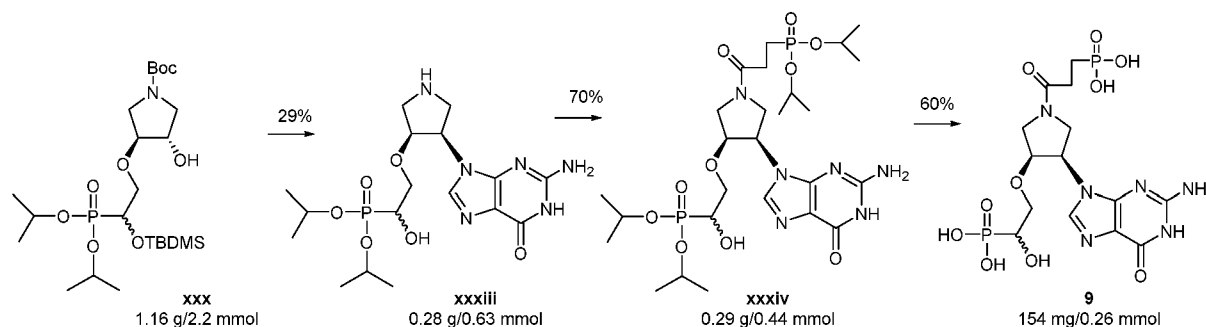
- 15 ³¹P{¹H} NMR (202.3 MHz, D₂O, ref (external H₃PO₄) = 0 ppm, 25 °C): 14.87, 14.90, 15.03, 15.05 (PCH(OH)CH₂O-A,B); 23.09, 23.10, 23.20, 23.21 (PCH₂CH₂CO-A,B).

IR *v*_{max}(KBr) 2360 (w, vbr), 1690 (s), 1633 (vs), 1590 (m, sh), 1551 (w), 1516 (w), 1459 (w), 1420 (w), 1342 (w), 1218 (m), 1175 (m), 1141 (m), 1070 (m), 1053 (m), 972 (w), 895 (w), 792 (w), 647 (w).

HRMS (ESI-) for C₁₄H₂₀N₅O₁₀P₂ (M-H)⁻: calcd 480.06909, found 480.06848.

20

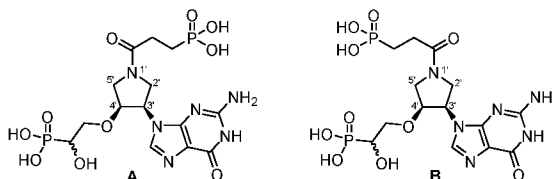
Example 19. [3*S*,4*R*]-4-guanin-9-yl-3-((*RS*)-2-hydroxy-2-phosphonoethyl)oxy-1-*N*-(phosphonopropionyl)pyrrolidine (9)



- 25 Precursor xxx (1.16 g, 2.2 mmol) was converted via the sequence of general methods C, E, F, and B to title compound 9 in 12% overall yield (154 mg, 260 μmol) in the form of white

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amorphous lyophilizate as an ~ 1:1:1:1 mixture of four isomers (two epimeric rotamers A and two epimeric rotamers B):



^1H NMR (500.0 MHz, D_2O , ref (dioxane) = 3.75 ppm, 25 °C): 1.82 – 1.93 (m, 8H, $\text{COCH}_2\text{CH}_2\text{P-A,B}$); 2.59 – 2.69 (m, 8H, $\text{COCH}_2\text{CH}_2\text{P-A,B}$); 3.47-3.56 (m, 2H, $\text{OCH}_a\text{H}_b\text{CH(OH)P-A}$); 3.60 – 3.90 (m, 16H, H-5'-A, H-5'b-B, $\text{OCH}_2\text{CH(OH)P-A,B}$, $\text{OCH}_a\text{H}_b\text{CH(OH)P-A}$, $\text{OCH}_2\text{CH(OH)P-B}$); 3.90-3.99 (m, 2H, H-2'b-B); 3.99-4.05 (m, 2H, H-5'a-B); 4.05-4.11 (m, 2H, H-2'a-B); 4.11-4.19 (m, 2H, H-2'b-A); 4.20-4.27 (m, 2H, H-2'a-A); 4.44-4.49 (m, 2H, H-4'-A); 4.50 – 4.55 (m, 2H, H-4'-B); 5.12-5.18 (m, 2H, H-3'-B); 5.18-5.24 (m, 2H, H-3'-A); 7.93, 7.97, 7.98, 8.02 ($4 \times \text{s}$, $4 \times 1\text{H}$, H-8-A,B).

^{13}C NMR (125.7 MHz, D_2O , ref (dioxane) = 69.30 ppm, 25 °C): 25.89, 25.92 (d, $J_{\text{C,P}} = 134.9$, $\text{COCH}_2\text{CH}_2\text{P-A,B}$); 31.12, 31.15 (d, $J_{\text{C,P}} = 3.0$, $\text{COCH}_2\text{CH}_2\text{P-A}$); 31.28, 31.30 (d, $J_{\text{C,P}} = 1.8$, $\text{COCH}_2\text{CH}_2\text{P-B}$); 50.16, 50.20 ($\text{CH}_2\text{-2'-B}$); 50.89, 50.92 ($\text{CH}_2\text{-2'-A}$); 52.02, 52.28 ($\text{CH}_2\text{-5'-A}$); 52.65, 53.00 ($\text{CH}_2\text{-5'-B}$); 56.08, 56.11 (CH-3'-B); 57.15 (CH-3'-A); 71.46, 71.52 (d, $J_{\text{C,P}} = 151.7$, CH(OH)P-A,B); 74.11, 74.22, 74.36, 74.49 (d, $J_{\text{C,P}} = 12.0$, $\text{OCH}_2\text{CH(OH)P-A,B}$); 79.36, 79.45 (CH-4'-A); 80.57, 80.65 (CH-4'-B); 118.15, 118.19, 118.21, 118.25 (C-5-A,B); 141.30, 141.51, 141.56, 141.78 (CH-8-A,B); 154.71, 154.76, 154.78 (C-4-A,B); 156.48 (C-2-A); 156.51 (C-2-B); 161.68, 161.69 (C-6-A,B); 177.11 (d, $J_{\text{C,P}} = 17.9$, NCO-A); 177.18 (d, $J_{\text{C,P}} = 17.9$, NCO-B).

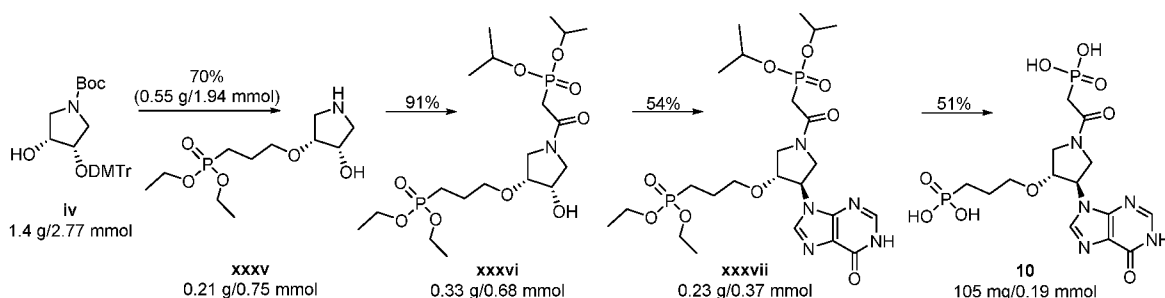
$^{31}\text{P}\{^1\text{H}\}$ NMR (202.3 MHz, D_2O , ref (external H_3PO_4) = 0 ppm, 25 °C): 15.41, 15.42, 15.59, 15.61 ($\text{PCH(OH)CH}_2\text{O-A,B}$); 23.47, 23.48, 23.52, 23.53 ($\text{PCH}_2\text{CH}_2\text{CO-A,B}$).

IR ν_{max} (KBr) 3129 (s, br), 2770 (m, vbr, sh), 2370 (w, vbr), 1691 (vs), 1633 (vs, br), 1605 (s, br, sh), 1571 (m, sh), 1534 (m), 1478 (m, sh), 1415 (w), 1171 (s), 1145 (m, sh), 1065 (s, br), 975 (w), 900 (m, br), 782 (w), 642 (w).

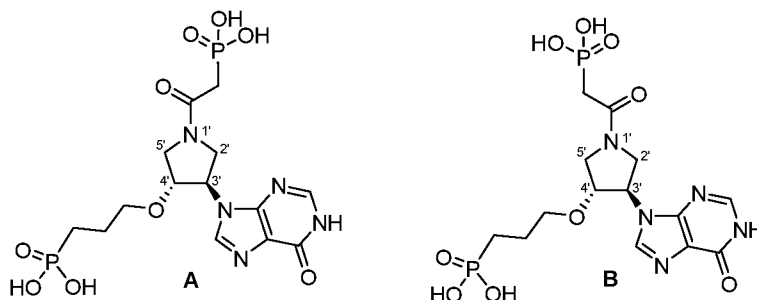
HRMS (ESI-) for $\text{C}_{14}\text{H}_{21}\text{N}_6\text{O}_{10}\text{P}_2$ (M-H) $^-$: calcd 495.07999, found 495.07928.

$[\alpha]^{20}_D = (c, \text{H}_2\text{O})$.

Example 20. [3*R*,4*R*]-4-(hypoxanthin-9-yl)-3-(phosphonoprop-3-yl)oxy-1-*N*-(phosphonoacetyl)pyrrolidine (10)



- 5 [3*S*,4*R*]-1-*N*-Boc-3-dimethoxytrityloxypyrrolidine (**iv**, 1.4 g, 2.77 mmol) was converted via the sequence of general methods G, H, F, C, and B to title compound **10** in 18% overall yield (105 mg, 190 μ mol) in the form of white amorphous lyophilizate as an ~ 1:1 mixture of rotamers A:B:



10

- ¹H NMR (500.0 MHz, D₂O, ref (dioxane) = 3.75 ppm, 25 °C): 1.43 – 1.60 (m, 4H, OCH₂CH₂CH₂P-A,B); 1.68-1.85 (m, 4H, (m, 4H, OCH₂CH₂CH₂P-A,B); 2.76-3.02 (m, 4H, OCCH₂P-A,B); 3.61-3.70 (m, 5H, H-5'b-A, OCH₂CH₂CH₂P-A,B); 3.82-3.89 (m, 2H, H-5'a-A, H-5'b-B); 4.03 (ddd, 1H, *J*_{gem} = 13.4, *J*_{2'b,3'} = 5.1, *J*_{H,P} = 1.4, H-2'b-B); 4.15 (ddd, 1H, *J*_{gem} = 13.4, *J*_{2'a,3'} = 7.6, *J*_{H,P} = 1.9, H-2'a-B); 4.25 (dd, 1H, *J*_{gem} = 11.9, *J*_{5'a,4'} = 5.9, H-5'a-B); 4.37 (dd, 1H, *J*_{gem} = 12.1, *J*_{2'b,3'} = 4.9, H-2'b-A); 4.42 (dd, 1H, *J*_{gem} = 12.1, *J*_{2'a,3'} = 7.1, H-2'a-A); 4.54 (dt, 1H, *J*_{4',5'} = 5.9, 4.1, *J*_{4',3'} = 4.1, H-4'-A); 4.60 (dt, 1H, *J*_{4',5'} = 5.9, 4.7, *J*_{4',3'} = 5.1, H-4'-B); 5.23 (dt, 1H, *J*_{3',2'} = 7.6, 5.1, *J*_{3',4'} = 5.1, H-3'-B); 5.29 (ddd, 1H, *J*_{3',2'} = 7.1, 4.9, *J*_{3',4'} = 4.1, H-3'-A); 8.19 (s, 1H, H-8-B); 8.20, 8.21 (2 × s, 2 × 1H, H-2-A,B); 8.29 (s, 1H, H-8-A).

- ¹³C NMR (125.7 MHz, D₂O, ref (dioxane) = 69.30 ppm, 25 °C): 26.22, 26.23 (2 × d, *J*_{C,P} = 3.8, OCH₂CH₂CH₂P-A,B); 27.00, 27.03 (2 × d, *J*_{C,P} = 134.6, OCH₂CH₂CH₂P-A,B); 39.60

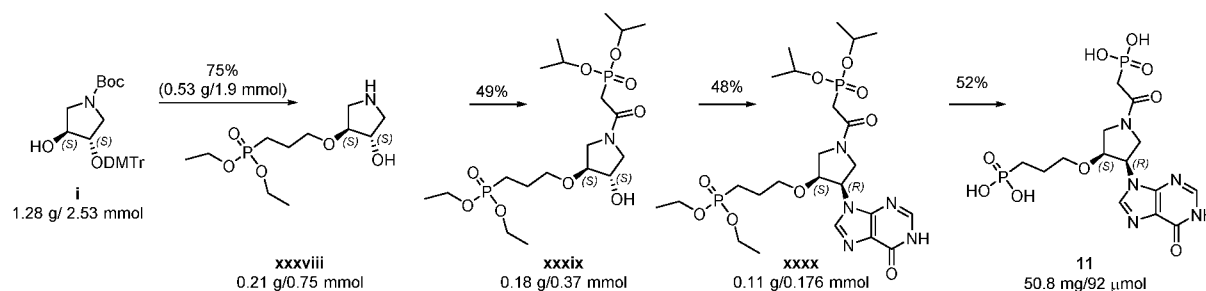
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(d, $J_{C,P}$ = 117.5, OCCH₂P-A or B); 39.67 (d, $J_{C,P}$ = 117.0, OCCH₂P-A or B); 50.80 (CH₂-2'-B); 52.05 (CH₂-2'-A); 52.12 (CH₂-5'-A); 53.65 (CH₂-5'-B); 59.83 (CH-3'-B); 60.94 (CH-3'-A); 73.15 (d, $J_{C,P}$ = 17.7, OCH₂CH₂CH₂P-A,B); 82.25 (CH-4'-A); 83.33 (CH-4'-B); 126.39, 126.44 (C-5-A,B); 142.65 (CH-8-B); 142.79 (CH-8-A); 148.54 (CH-2-A,B); 151.70 (C-4-A,B); 161.39 (C-6-A,B); 172.72 (d, $J_{C,P}$ = 6.0, NCO-A,B).

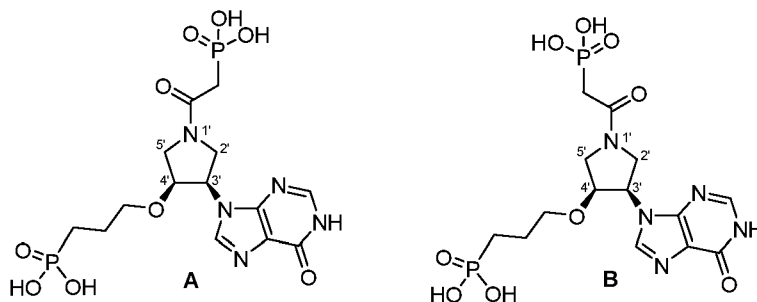
³¹P{¹H} NMR (202.3 MHz, D₂O, ref (external H₃PO₄) = 0 ppm, 25 °C): 13.56, 13.69 (PCH₂CO-A,B); 25.99 (PCH₂CH₂CH₂O-A,B).

HRMS (ESI-) for C₁₄H₂₀N₅O₉P₂ (M-H)⁻: calcd 464.07417, found 464.07432.

10 **Example 21.** [3*S*,4*R*]-4-(hypoxanthin-9-yl)-3-(phosphonoprop-3-yl)oxy-1-*N*-(phosphonoacetyl)pyrrolidine (**11**)



[3*S*,4*S*]-1-*N*-Boc-3-dimethoxytrityloxypyrrolidine (**i**, 1.28 g, 2.53 mmol) was converted via the sequence of general methods G, H, F, C, and B to title compound **11** in 9% overall yield (50.8 mg, 92 μmol) in the form of white amorphous lyophilizate as an ~ 6:7 mixture of rotamers A:B:



20 ¹H NMR (500.0 MHz, D₂O, ref (dioxane) = 3.75 ppm, 25 °C): 1.12 – 1.43 (m, 4H, OCH₂CH₂CH₂P-A,B); 1.47-1.65 (m, 4H, (m, 4H, OCH₂CH₂CH₂P-A,B); 2.78-3.05 (m, 4H, OCCH₂P-A,B); 3.30-3.38 (m, 2H, OCH_aH_bCH₂CH₂P-A,B); 3.54-3.64 (m, 2H, OCH_aH_bCH₂CH₂P-A,B); 3.67 (ddd, 1H, J_{gem} = 13.2, $J_{5'b,4'}$ = 3.9, $J_{H,P}$ = 2.0, H-5'b-A); 3.87

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(ddd, 1H, $J_{\text{gem}} = 13.2$, $J_{5'a,4'} = 5.6$, $J_{\text{H,P}} = 2.4$, H-5'a-A); 3.92 (dd, 1H, $J_{\text{gem}} = 11.9$, $J_{5'b,4'} = 4.4$, H-5'b-B); 4.07 (ddd, 1H, $J_{\text{gem}} = 12.8$, $J_{2'b,3'} = 6.8$, $J_{\text{H,P}} = 2.2$, H-2'b-B); 4.14 (ddd, 1H, $J_{\text{gem}} = 12.8$, $J_{2'a,3'} = 7.6$, $J_{\text{H,P}} = 2.1$, H-2'a-B); 4.17 (dd, 1H, $J_{\text{gem}} = 11.9$, $J_{5'a,4'} = 5.4$, H-5'a-B); 4.35 (dd, 1H, $J_{\text{gem}} = 11.3$, $J_{2'b,3'} = 7.2$, H-2'b-A); 4.42 (dd, 1H, $J_{\text{gem}} = 11.3$, $J_{2'a,3'} = 7.5$, H-2'a-A); 4.49 (ddd, 1H, $J_{4',5'} = 5.6$, 4.4, $J_{4',3'} = 4.9$, H-4'-A); 4.60 (ddd, 1H, $J_{4',5'} = 5.4$, 4.4, $J_{4',3'} = 4.9$, H-4'-B); 5.23 (dt, 1H, $J_{3',2'} = 7.6$, 6.8, $J_{3',4'} = 4.9$, H-3'-B); 5.29 (ddd, 1H, $J_{3',2'} = 7.5$, 7.2, $J_{3',4'} = 4.9$, H-3'-A); 8.22 (s, 1H, H-2-B); 8.23 (s, 1H, H-2-A); 8.27 (s, 1H, H-8-B); 8.29 (s, 1H, H-8-A).

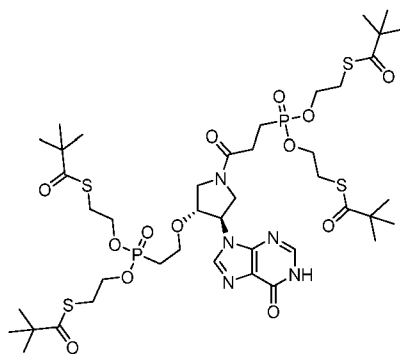
^{13}C NMR (125.7 MHz, D_2O , ref (dioxane) = 69.30 ppm, 25 °C): 26.00, 26.02 ($2 \times \text{d}$, $J_{\text{C,P}} = 3.7$, $\text{OCH}_2\text{CH}_2\text{CH}_2\text{P-A,B}$); 26.92 (d, $J_{\text{C,P}} = 134.8$, $\text{OCH}_2\text{CH}_2\text{CH}_2\text{P-B}$); 26.95 (d, $J_{\text{C,P}} = 134.8$, $\text{OCH}_2\text{CH}_2\text{CH}_2\text{P-A}$); 39.47 (d, $J_{\text{C,P}} = 117.9$, $\text{OCCH}_2\text{P-B}$); 39.56 (d, $J_{\text{C,P}} = 117.5$, $\text{OCCH}_2\text{P-A}$); 50.38 ($\text{CH}_2\text{-2'-B}$); 51.62 ($\text{CH}_2\text{-2'-A}$); 51.98 ($\text{CH}_2\text{-5'-A}$); 53.37 ($\text{CH}_2\text{-5'-B}$); 56.96 (CH-3'-B); 57.96 (CH-3'-A); 73.71 (d, $J_{\text{C,P}} = 18.7$, $\text{OCH}_2\text{CH}_2\text{CH}_2\text{P-A}$); 73.74 (d, $J_{\text{C,P}} = 18.6$, $\text{OCH}_2\text{CH}_2\text{CH}_2\text{P-B}$); 78.96 (CH-4'-A); 80.00 (CH-4'-B); 125.80 (C-5-A); 125.82 (C-5-B); 143.72 (CH-8-B); 143.81 (CH-8-A); 148.52 (CH-2-A,B); 152.07 (C-4-A); 152.12 (C-4-B); 161.40 (C-6-A,B); 172.59 (d, $J_{\text{C,P}} = 6.1$, NCO-A); 172.65 (d, $J_{\text{C,P}} = 6.1$, NCO-B).

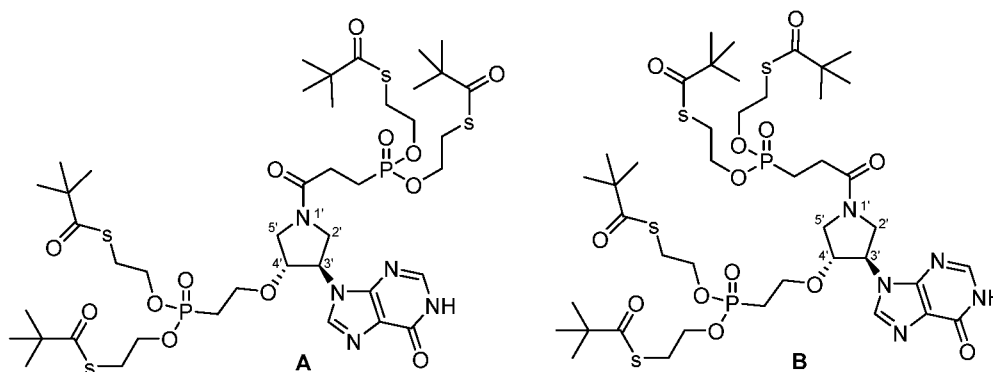
$^{31}\text{P}\{^1\text{H}\}$ NMR (202.3 MHz, D_2O , ref (external H_3PO_4) = 0 ppm, 25 °C): 13.57 (PCH₂CO-A); 13.80 (PCH₂CO-B); 25.94 (PCH₂CH₂CH₂O-A,B).

IR ν_{max} (KBr) 1685 (s), 1628 (vs), 1590 (m, sh), 1550 (w), 1517 (w), 1453 (w), 1414 (w), 1347 (w), 1222 (m), 892 (m, br), 793 (w), 647 (w).

HRMS (ESI-) for $\text{C}_{14}\text{H}_{20}\text{N}_5\text{O}_9\text{P}_2$ (M-H)⁻: calcd 464.07417, found 464.07330.

Example 22. Compound 12, Tetra-(pivaloylthioethyl) prodrug of Compound 1





¹H NMR (500.0 MHz, CDCl₃, 25 °C): 1.202, 1.206, 1.215, 1.22 (4 × s, 72H, (CH₃)₃C-A,B); 2.07 - 2.30 (m, 8H, OCH₂CH₂P-A,B, COCH₂CH₂P-A,B); 2.57 - 2.74 (m, 4H, COCH₂CH₂P-A,B); 3.05 - 3.19 (m, 16H, SCH₂CH₂O-A,B); 3.58 - 3.99 (m, 8H, H-5'-A,B, OCH₂CH₂P-A,B); 4.02 - 4.25 (m, 20H, H-2'-A,B, SCH₂CH₂O-A,B); 4.39 (m, 1H, H-4'-A); 4.46 (m, 1H, H-4'-B); 5.07 (dt, 1H, *J*_{3',2'} = 6.9, 4.0, *J*_{3',4'} = 4.0, H-3'-B); 5.45 (dt, 1H, *J*_{3',2'} = 7.0, 4.7, *J*_{3',4'} = 4.7, H-3'-A); 7.79 (s, 1H, H-8-B); 7.86 (s, 1H, H-8-A); 8.10 (s, 2H, H-2-A,B).

¹³C NMR (125.7 MHz, CDCl₃, 25 °C): 20.41 (d, *J*_{C,P} = 143.7, COCH₂CH₂P-B); 20.43 (d, *J*_{C,P} = 143.9, COCH₂CH₂P-A); 27.80, 26.83 (2 × d, *J*_{C,P} = 141.2, OCH₂CH₂P-A,B); 27.25 - 27.32 ((CH₃)₃C-A,B, COCH₂CH₂P-A,B); 28.67 - 28.87 (SCH₂CH₂O-A,B); 46.50 ((CH₃)₃C-A,B); 47.87 (CH₂-2'-B); 48.55 (CH₂-2'-A); 49.42 (CH₂-5'-A); 50.11 (CH₂-5'-B); 57.25 (CH-3'-B); 58.91 (CH-3'-A); 63.98 - 64.38 (OCH₂CH₂P-A,B, OCH₂CH₂S-A,B); 79.73 (CH-4'-A); 81.51 (CH-4'-B); 125.11 (C-5-A); 125.15 (C-5-B); 138.00 (CH-8-B);

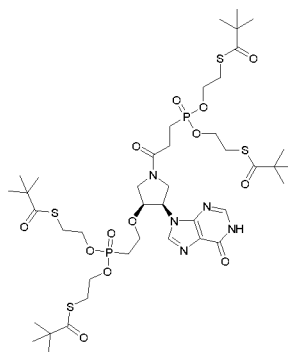
- 67 -

138.30 (CH-8-A); 145.01 (CH-2-A,B); 148.68 (C-4-A); 148.72 (C-4-B); 158.53 (C-6-A); 158.55 (C-6-B); 169.62 (d, $J_{C,P}$ = 17.0, NCO-A); 169.76 (d, $J_{C,P}$ = 17.0, NCO-B); 205.67 (SCO-A,B).

$^{31}\text{P}\{^1\text{H}\}$ NMR (202.3 MHz, CDCl_3 , 25 °C): 28.97 (PCH₂CH₂O-A); 29.03 (PCH₂CH₂O-B);
 5 32.67, 32.69 (PCH₂CH₂CO-A,B).

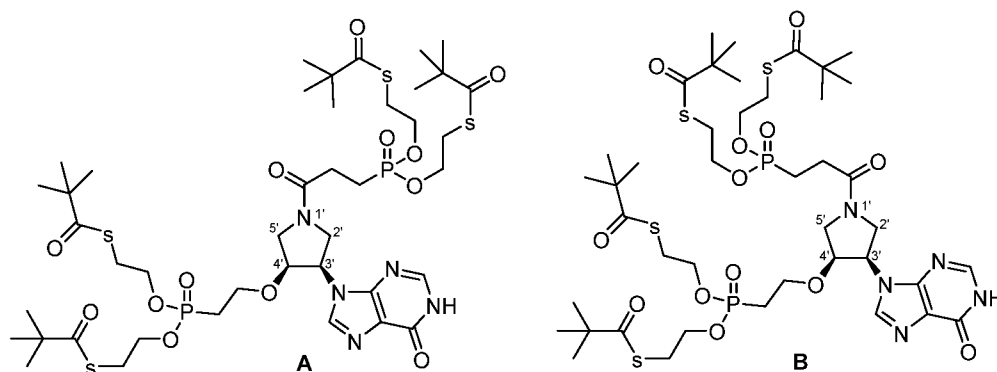
HRMS (ESI+) for C₄₂H₇₀N₅O₁₃P₂³²S₄ (M+H)⁺: calcd 1042.33227, found 1042.33104.

Example 23. Compound 13, Tetra-(pivaloylthioethyl) prodrug of Compound 2



10 A mixture of compound **2** (0.28 g, 0.32 mmol; triethylammonium salt), trioctylamine (0.56 ml, 1.2 mmol) and methylimidazole (0.18 ml, 2.24 mmol) was co-evaporated with pyridine (2x 15 ml) and dissolved in a mixture of pyridine (5 ml) and DMF (5 ml). TPSCl (0.68 g, 2.24 mmol) was added and the reaction mixture was stirred for 30 min. at rt. 2-pivaloylthioethanol (0.31 g, 1.92 mmol) was added and the reaction mixture was stirred for
 15 5 days. 2M aq. TEAB (1 ml) was added and the reaction mixture was concentrated *in vacuo*. The residue was dissolved in chloroform (40 ml) and washed with sat. soln. NaHCO₃ (2x30 ml) and 3% citric acid (2x30 ml). The organic phase was dried over sodium sulfate, and concentrated *in vacuo*. The title compound was obtained by chromatography on silica gel using linear gradient of ethanol in chloroform followed by
 20 preparative HPLC using a linear gradient of methanol in water in 21% yield (69.7 mg, 67 μmol) as an ~ 1:1 mixture of rotamers A:B:

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^1H NMR (500.0 MHz, CD_3OD , 25 °C): 1.209, 1.210, 1.220, 1.222, 1.24 ($5 \times \text{s}$, 72H, $(\text{CH}_3)_3\text{C-A,B}$); 1.98 - 2.17 (m, 4H, $\text{OCH}_2\text{CH}_2\text{P-A,B}$); 2.17 - 2.32 (m, 4H, $\text{COCH}_2\text{CH}_2\text{P-A,B}$); 2.61 - 2.82 (m, 4H, $\text{COCH}_2\text{CH}_2\text{P-A,B}$); 3.08 - 3.13, 3.15 - 3.21 ($2 \times \text{m}$, $2 \times 8\text{H}$, $\text{SCH}_2\text{CH}_2\text{O-A,B}$); 3.50 - 3.66 (m, 2H, $\text{OCH}_a\text{H}_b\text{CH}_2\text{P-A,B}$); 3.72 (dd, 1H, $J_{\text{gem}} = 13.2$, $J_{5'b,4'} = 4.4$, H-5'b-A); 3.75 - 3.85 (m, 2H, $\text{OCH}_a\text{H}_b\text{CH}_2\text{P-A,B}$); 3.89 (dd, 1H, $J_{\text{gem}} = 13.2$, $J_{5'a,4'} = 2.0$, H-5'a-A); 3.92 - 3.97 (m, 2H, H-5'-B); 3.97 - 4.08 (m, 9H, H-2'b-B, $\text{SCH}_2\text{CH}_2\text{O-A,B}$); 4.08 - 4.20 (m, 10H, H-2'a-B, H-2'b-A, $\text{SCH}_2\text{CH}_2\text{O-A,B}$); 4.23 (dd, 1H, $J_{\text{gem}} = 10.1$, $J_{2'a,3'} = 8.0$, H-2'a-A); 4.39 (td, 1H, $J_{4',5'} = 4.4$, 2.0, $J_{4',3'} = 4.4$, H-4'-A); 4.48 (td, 1H, $J_{4',5'} = 4.5$, 3.4, $J_{4',3'} = 4.5$, H-4'-B); 5.38 (td, 1H, $J_{3',2'} = 8.1$, $J_{3',4'} = 4.5$, H-3'-B); 5.45 (ddd, 1H, $J_{3',2'} = 9.4$, 8.0, $J_{3',4'} = 4.4$, H-3'-A); 8.08, 8.09 ($2 \times \text{s}$, $2 \times 1\text{H}$, H-2-A,B); 8.26 (s, 1H, H-8-B); 8.33 (s, 1H, H-8-A).

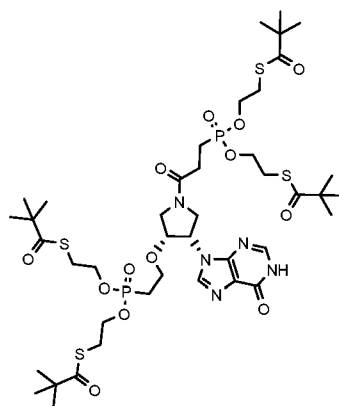
^{13}C NMR (125.7 MHz, CD_3OD , 25 °C): 21.14, 21.19 ($2 \times \text{d}$, $J_{\text{C,P}} = 143.8$, $\text{COCH}_2\text{CH}_2\text{P-A,B}$); 27.12, 27.20 ($2 \times \text{d}$, $J_{\text{C,P}} = 141.0$, $\text{OCH}_2\text{CH}_2\text{P-A,B}$); 27.69, 27.71, 27.72 ($(\text{CH}_3)_3\text{C-A,B}$); 27.90, 28.16 ($2 \times \text{d}$, $J_{\text{C,P}} = 3.3$, $\text{COCH}_2\text{CH}_2\text{P-A,B}$); 29.64 - 29.84 ($\text{SCH}_2\text{CH}_2\text{O-A,B}$); 47.52, 47.53, 47.56 ($(\text{CH}_3)_3\text{C-A,B}$); 48.76 ($\text{CH}_2\text{-2'-B}$); 49.07 ($\text{CH}_2\text{-2'-A}$); 50.53 ($\text{CH}_2\text{-5'-A}$); 50.94 ($\text{CH}_2\text{-5'-B}$); 55.37 (CH-3'-B); 56.41 (CH-3'-A); 64.69, 64.96 (d, $J_{\text{C,P}} = 3.5$, $\text{OCH}_2\text{CH}_2\text{P-A,B}$); 65.58, 65.63, 65.65, 65.79, 65.81 ($5 \times \text{d}$, $J_{\text{C,P}} = 6.5$, $\text{OCH}_2\text{CH}_2\text{S-A,B}$); 77.87 (CH-4'-A); 79.20 (CH-4'-B); 124.83, 124.89 (C-5-A,B); 141.42, 141.65 (CH-8-A,B); 146.75, 146.81 (CH-2-A,B); 150.56, 150.66 (C-4-A,B); 158.93, 158.94 (C-6-A,B); 172.00 (d, $J_{\text{C,P}} = 15.6$, NCO-A); 172.11 (d, $J_{\text{C,P}} = 15.1$, NCO-B); 206.98, 206.99, 207.00, 207.02, 207.04 (SCO-A,B).

$^{31}\text{P}\{^1\text{H}\}$ NMR (202.3 MHz, CD_3OD 25 °C): 30.95, 31.13 ($\text{PCH}_2\text{CH}_2\text{O-A,B}$); 33.91, 34.03 ($\text{PCH}_2\text{CH}_2\text{CO-A,B}$).

HRMS (ESI+) for $\text{C}_{42}\text{H}_{70}\text{N}_5\text{O}_{13}\text{P}_2^{32}\text{S}_4$ ($\text{M}+\text{H}$) $^+$: calcd 1042.33227, found 1042.33287.

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Example 24. Compound 14, Tetra-(pivaloylthioethyl) prodrug of [3R,4S]-4-hypoxanthin-9-yl-3-phosphonoethoxy-1-N-(phosphonopropionyl)pyrrolidine



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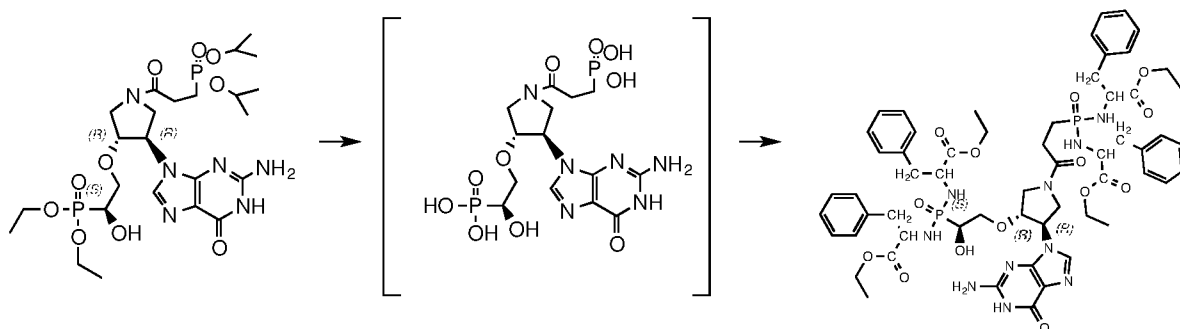
A mixture of [3R,4S]-4-hypoxanthin-9-yl-3-phosphonoethoxy-1-N-(phosphonopropionyl)pyrrolidine (0.23 g, 0.26 mmol; triethylammonium salt) and 4-methoxypyridine-1-N-oxide (66 mg, 0.53 mmol) was co-evaporated with pyridine (2x 15 ml) and dissolved in pyridine (4 ml). 2-Chloro-5,5-dimethyl-1,3,2-dioxaphosphorinane 2-oxide (0.33 g, 1.82 mmol) was added and the reaction mixture was stirred for 30 min. at rt. 2-pivaloylthioethanol (0.21 g, 1.3 mmol) was added and the reaction mixture was stirred for 5 days. Water (0.5 ml) was added and the reaction mixture was concentrated *in vacuo*. The residue was dissolved in chloroform (40 ml) and washed with sat. soln. NaHCO₃ (2x30 ml) and 3% citric acid (2x30 ml). The organic phase was dried over sodium sulfate and concentrated *in vacuo*. The title compound was obtained by chromatography on silica gel using a linear gradient of ethanol in chloroform in 88% yield (0.238 g, 0.23 mmol).

¹H NMR (600.1 MHz, D₂O, 25 °C), ¹³C NMR (150.9 MHz, D₂O, 25 °C), and ³¹P{¹H} NMR (202.3 MHz, D₂O, 25 °C) spectra were identical to those of enantiomeric Compound 13.

20

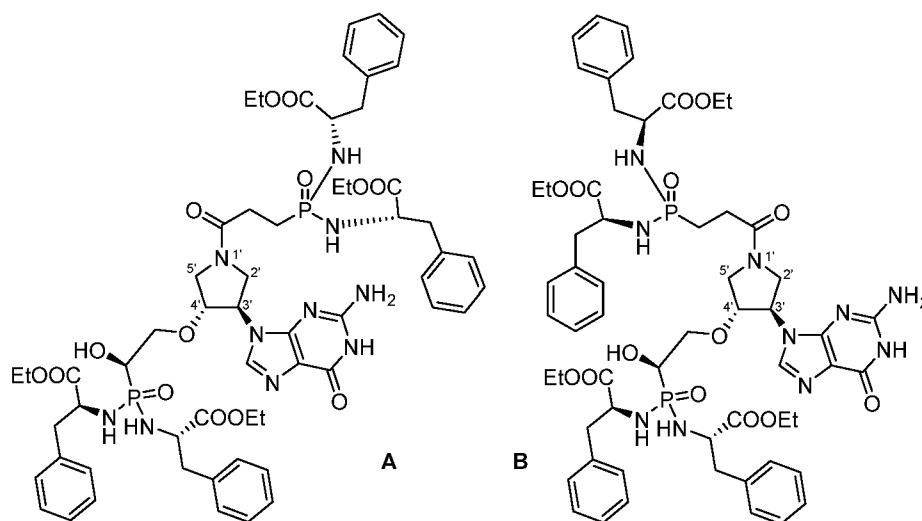
HRMS (ESI-) for C₄₂H₇₀N₅O₁₃P₂³²S₄ (M-H)⁻ : calcd 1042.33227, found 1042.33175.

Example 25. Compound 15, Tetra-(ethyl L-phenylalanine) tetraamide prodrug of Compound 5



- A mixture of tetraester (0.34 g, 0.53 mmol), dry pyridine (8 ml) and BrSiMe_3 (1 ml) was stirred overnight at rt under argon atmosphere. After evaporation and co-distillation with pyridine under argon atmosphere, the residue was dissolved in pyridine (8 ml) and ethyl (L)-phenylalanine hydrochloride (1.5 g, 10 mmol) and triethylamine (2.5 ml) were added. The mixture was heated to 70 °C under argon atmosphere and then solution of Aldrithiol (1.85 g, 8 mmol) and triphenylphosphine (2.1 g, 8 mmol) in pyridine (8 ml) was added.
- The reaction mixture was stirred at 70 °C under argon atmosphere for three days. The solvent was evaporated and the residue was purified by chromatography on silica gel using linear gradient of ethanol in chloroform followed by preparative HPLC on reversed phase purification using linear gradient of methanol in water. The phosphoramidate prodrug was obtained as a foam in 25% yield (161 mg, 134 μmol) as an ~ 1:1 mixture of rotamers A:B:

15



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¹H NMR (500.0 MHz, CD₃OD, 25 °C): 1.17 – 1.26 (m, 24H, CH₃CH₂O-A,B); 1.59 – 1.78 (m, 4H, COCH₂CH₂P-A,B); 2.09 – 2.33 (m, 4H, COCH₂CH₂P-A,B); 2.65 – 3.17 (m, 16H, CH₂-Phe-A,B); 3.26 (bm, 1H, H-5'b-B); 3.40 (bdd, 1H, $J_{\text{gem}} = 13.1$, $J_{5'b,4'} = 3.7$, H-5'b-A); 3.61 - 3.82 (m, 6H, H-5'a-A,B, OCH₂CH(OH)P-A,B); 3.83 – 3.99 (m, 10H, H-2'-A,B, CH-Phe-A,B, OCH₂CH(OH)P-A,B) 4.07 – 4.28 (m, 21H, H-4'-A, CH-Phe-A,B, CH₃CH₂O-A,B); 4.30 (m, 1H, H-4'-B); 4.78 (dt, 1H, $J_{3',2'} = 7.0$, 3.8, $J_{3',4'} = 3.8$, H-3'-B); 4.85 (m, 1H, H-3'-A, overlapped with water signal); 7.08 – 7.34 (m, 40H, H-*o,m,p*-Ph); 7.60, 7.70 (2 × s, 2 × 1H, H-8-A,B).

¹³C NMR (125.7 MHz, CD₃OD, 25 °C): 14.43, 14.45, 14.51, 14.52 (CH₃CH₂O-A,B); 24.57, 24.65 (2 × d, $J_{\text{C,P}} = 118.2$, COCH₂CH₂P-A,B); 28.40, 28.46 (2 × d, $J_{\text{C,P}} = 3.0$, COCH₂CH₂P-A,B); 41.13 – 41.59 (CH₂-Phe-A,B); 48.75 (CH₂-2'-B); 49.42 (CH₂-2'-A); 50.80 (CH₂-5'-A); 51.45 (CH₂-5'-B); 55.24, 55.41, 55.52, 55.69, 55.72, 55.87, 55.96, 56.03 (CH-Phe); 58.24 (CH-3'-B); 59.87 (CH-3'-A); 62.31, 62.36, 62.46, 62.48, 62.50, 62.53 (CH₃CH₂O-A,B); 69.93 (d, $J_{\text{C,P}} = 133.5$, OCH₂CH(OH)P-AorB); 69.95 (d, $J_{\text{C,P}} = 132.8$, OCH₂CH(OH)P-AorB); 71.57, 71.62 (2 × d, $J_{\text{C,P}} = 11.1$, OCH₂CH(OH)P-A,B); 81.40 (CH-4'-A); 82.93 (CH-4'-B); 118.09 (C-5-A,B); 127.86, 127.91, 127.92, 127.96, 127.97, 128.01, 128.03 (CH-*p*-Ph-A,B); 129.38, 129.49, 129.55 (CH-*m*-Ph-A,B); 130.62, 130.63, 130.72, 130.75, 130.86, 130.88, 130.89 (CH-*o*-Ph-A,B); 137.41, 137.86 (CH-8-A,B); 137.88, 138.00, 138.43, 138.50, 138.52, 138.58, 138.60, 138.63 (C-*i*-Ph-A,B); 153.01, 153.03 (C-4-A,B); 155.31 (CH-2-A,B); 159.50, 159.52 (C-6-A,B); 172.77 (d, $J_{\text{C,P}} = 12.4$, NCO-AorB); 172.81 (d, $J_{\text{C,P}} = 12.4$, NCO-AorB); 169.76 (d, $J_{\text{C,P}} = 13.4$, NCO-AorB); 174.35 (d, $J_{\text{C,P}} = 7.1$, COOEt-AorB); 174.48 (d, $J_{\text{C,P}} = 5.9$, COOEt-AorB); 174.67 (d, $J_{\text{C,P}} = 4.0$, COOEt-AorB); 174.74 (d, $J_{\text{C,P}} = 3.4$, COOEt-AorB); 174.84, 174.86, 175.11, 175.20 (4 × d, $J_{\text{C,P}} = 1.9$, COOEt-AorB).

³¹P{¹H} NMR (202.3 MHz, CD₃OD, 25 °C): 26.62 (PCH₂CH₂O-A); 26.65 (PCH₂CH₂O-B); 32.16 (PCH₂CH₂CO-A,B).

HRMS (ESI+) for C₅₈H₇₄N₁₀O₁₄P₂Na (M+Na)⁺: calcd 1219.47534, found 1219.47617.

Example 26. Anti-malarial Activity of the Compounds of the Invention

The anti-malarial activity of a number of the compounds of the invention was assessed using the following assay methodology.

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Determination of K_i values

Human HGPRT was stored in 0.1 M Tris-HCl, 0.01 M $MgCl_2$, pH 7.4, 200 μM PRib-PP, 1 mM dithiothreitol (DTT), $-80^\circ C$. PfHGXPRT was stored in 0.01 M phosphate, 60 μM hypoxanthine, 200 μM PRib-PP, pH 7.2, 1 mM DTT as previously described (Keough, D. T., *et al.*, *Mol. Biochem. Parasitol.* 1999, 98 (1), 29-41). This difference is because the Pf enzyme is completely inactive under the conditions used to store the human enzyme. For enzyme assays, the buffer was 0.1 M Tris-HCl, 0.01 M $MgCl_2$, pH 7.4. The K_i values were calculated by Hanes' plots at a fixed concentration of guanine (60 μM) and at variable concentrations of PRib-PP (14 - 1000 μM) depending on the $K_{m(app)}$ in the presence of the inhibitor.

Antimalarial cell based data

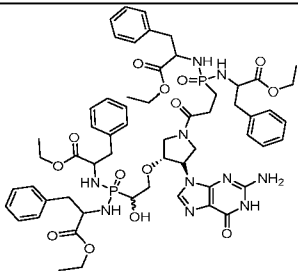
Pf D6 (Sierra-Leone) laboratory line, sensitive to most antimalarial drugs and W2 (Indochina) line, resistant to chloroquine and pyrimethamine, were maintained in RPMI-1640-LPLF complete medium, containing 10% human plasma, at 4% hematocrit and 1% to 8% parasitemia as previously described (Trager, W.; Jensen, J. B., *Science* 1976, 193 (4254), 673-675). Cultures were routinely synchronised using D-sorbitol. To evaluate the anti-malarial activity of the compounds, the previously described [3H]-hypoxanthine growth inhibition assay was utilized (Desjardins, R. E., *et al. Antimicrob. Agents and Chemother.* 1979, 16 (6), 710-718), where the uptake of [3H]-hypoxanthine by malaria parasites is used as a surrogate marker for parasite growth. For these assays, stock solutions of ANPs were made to concentrations of 20-40 mM in DMSO or water and subsequently diluted in hypoxanthine-free complete media prior to assay. The assays (in 96-well plate format) were initiated when the majority of parasites (>90%) were at early trophozoite (ring) stage. Parasite cultures (100 μL per well) at 0.5% initial parasitemia and 2% hematocrit in hypoxanthine-free RPMI1640-LPLF medium were exposed to ten 2-fold serial dilutions of the ANPs and chloroquine (CQ) (reference drug) for 96 hours, with [3H]-hypoxanthine (0.2 μCi /well) added ~48 hours after beginning of the experiment.

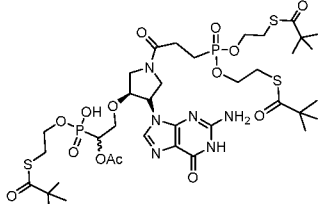
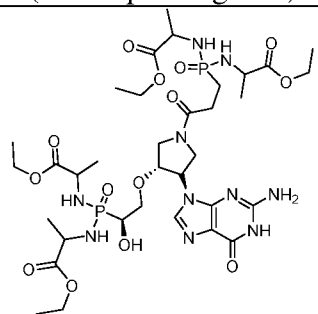
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The [^3H]-hypoxanthine incorporation data were analysed and sigmoidal growth inhibition curves were produced by non-linear regression analysis of the [^3H]-hypoxanthine incorporation data versus log-transformed concentrations of the compounds using Graphpad Prism V5.0 software (GraphPad Software Inc. USA). The inhibitory concentration (IC_{50}) that results in 50% inhibition of parasite growth was determined. The IC_{50} values were based on three independent experiments with mean $\pm$ SD calculated.

The results of the study are shown in Table 1 below. The K_i values provided in the Table correspond to the value obtained for the parent compound of the prodrugs listed in the Table. To obtain the K_i values, the parent compounds were either synthesized and tested or the prodrug compounds were converted to the parent compound by hydrolysis of the prodrug attachments. As is evident for Compound **5** and its prodrug counterparts, the prodrug attachments greatly enhance transport of the parent compound across the cellular membrane to act against the D6 and W2 malaria strains in the cell based assay. As can be seen, the compounds of the invention are effective inhibitors against both strains of malaria and some of the compounds display selectivity for parasitological enzyme HG(X)PRT over human enzyme HGPRT.

Table 1 Anti-malarial activity for the compounds of the invention against the *Pf* D6 and W2 strains and against human HGPRT (hu) and *Plasmodium falciparum* HG(X)PRT (*Pf*).

Compound	K_i (hu) (μM)	K_i (<i>Pf</i>) (μM)	IC_{50} (D6) (μM)	IC_{50} (W2) (μM)
5	0.003	0.007	>250	>1000
 (Compound 15 , prodrug of 5)	*	*	12	13

 (SATE prodrug of 5)	*	*	97	226
 (alanine ethyl ester prodrug of 5)	*	*	15	14
12**	0.02	0.014	4.6	7.8
13**	0.2	0.01	26	45
14**	0.7	1.2	5.1	8.4

* K_i value for the parent compound shown for compound **5**, the prodrug compound *per se* does not inhibit enzyme; ** K_i values are shown for the parent compounds and the cell based data are for the prodrugs analogs.

5 Example 27. Anti-Tuberculosis Activity of the Compounds of the Invention

The ability of the compounds of the invention to inhibit *Mycobacterium tuberculosis* was assessed using the following assay methodology.

Inhibition Studies

- 10 The K_i values for *Mycobacterium tuberculosis* HGPRT were determined in 0.1 M Tris-HCl, 12 mM MgCl₂, pH 7.4 at 25°C. The concentration of guanine was fixed at 67.5 μ M and the concentration of the variable substrate, PRib-PP, was in the range 20-1500 μ M, depending on the value for $K_{m(app)}$ at that concentration of inhibitor. The concentration of inhibitor in the assay ranged from 1.4 μ M (for the best inhibitor) to 56 μ M (for the weakest
- 15 inhibitor). The reaction was initiated by the addition of enzyme to give a final concentration of 200 nM. The reaction was followed at 257.5 nm for 60 s with a total change in absorbance of 0.05 units. The $K_{m(app)}$, V_{max} and K_i values were calculated using Prism6 (GraphPad Software, Inc., La Jolla, CA).

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Cell based assays under normal growth conditions

M. tuberculosis H37Rv/a (ATCC 25177) was grown in Middlebrook 7H9 broth medium supplemented with OADC (oleic acid dextrose catalase), 0.5% glycerol and 0.02% tyloxapol. Freshly seeded cultures were grown at 37°C, for approximately 14 days, to mid-exponential phase (OD₆₀₀ 0.4-0.8) for use in inhibition assays. The potency of the inhibitors was measured by a resazurin reduction microplate assay, as previously described (Taneja, N. K. and Tyagi, J. S., *J. Antimicrob. Chemother.*, 2007, 60 (2), 288-293; West, N. P., *et al.*, *Chem. Commun.*, 2011, 47(18), 5166-5168), with some alterations. *M. tuberculosis*, grown to mid-exponential phase (OD₆₀₀ 0.4-0.8), was diluted to OD₆₀₀ 0.001 in 7H9S media (Middlebrook 7H9 with OADC, 0.5% glycerol, 0.75% tween-80, 1% tryptone) containing 0.5% DMSO. 96-well microtitre plates were set up with 100 µl of inhibitors, serially diluted into 7H9S media. 100 µl of diluted *M. tuberculosis*, representing ~2x10⁴ CFU/mL was added to each well. Plates were incubated for five days at 37°C in a humidified incubator prior to the addition of 30 µl of a 0.02% resazurin solution and 12.5 µl of 20% Tween 80 to each well. Sample fluorescence was measured 30h later on a FluoroStar Omega fluorescent plate reader (BMG) with an excitation wavelength of 530 nm and emission read at 580 nm. Changes in fluorescence relative to positive control wells (H37Rv with no inhibitor) minus negative control wells (no H37Rv) were plotted for determination of MIC₅₀.

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Cell based assays under normoxic conditions

The hypoxic assay is identical to the normoxic REMA with the following modifications:

1. Incubation takes place inside the hypoxic chamber with an O₂ concentration of 0.1%.
2. Following the addition of resazurin, fluorescence is read at 48hrs as opposed to 24hrs in the normoxic REMA.

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M. tuberculosis macrophage intracellular MIC drug assay

PMA (phorbol 12-myristate 13-acetate) was added to THP-1 cells (5x10⁵ cells/ml) at a concentration of 50ng/ml in RPMI media. PMA is capable of differentiating the monocytic THP-1 cells into macrophages. 200 µL of induced THP-1 cells were added to a 96-well plate and incubated for 3 days at 37°C/5% CO₂. This facilitated differentiation

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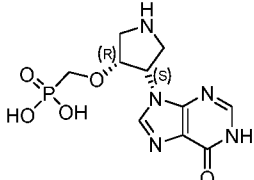
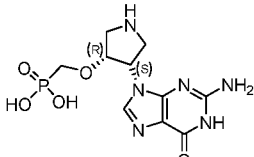
- into macrophages. Media was removed from the macrophages and replaced with *M. tuberculosis* (pmCherry) in RPMI at an MOI=1. Infection proceeded for 2hrs at 37°C/5% CO₂. Macrophages were washed 2x with fresh RPMI media. Candidate compounds were added to the infected macrophages and incubated for 4 days at 37°C/5% CO₂.
- 5 Fluorescence was then recorded. *Media for THP-1 cells* RPMI, L-glutamine (2mM final), 10% FBS, β-mercaptoethanol (0.05mM final). Made fresh and kept for 1-2 weeks at 4°C

- The results of the study are shown in Table 2 below. The K_i values are representative of the parent compound. Once again, addition of the prodrug attachments greatly enhances
- 10 transport of the compound across the cellular membrane in the cell based assay. As is evident, the compounds of the invention effectively inhibit *M. tuberculosis*.

Table 2. Anti-tuberculosis activity of the compounds of the invention

Compound	K _i value (μM)	Prodrug substituent (attached to the phosphate groups)	Cell based assays for <i>M. tuberculosis</i>		
			REMA assay normal growth	REMA assay in hypoxic (Latent)	Macrophage
3	0.041	-	-	-	-
4	0.064	-	-	-	-
5	0.064	 (Compound 15)	22*	-	-
6	0.2	-	-	-	-
7	0.127	-	-	-	-
9	0.3	 (Compound 15)	14** 20*	29**	85**

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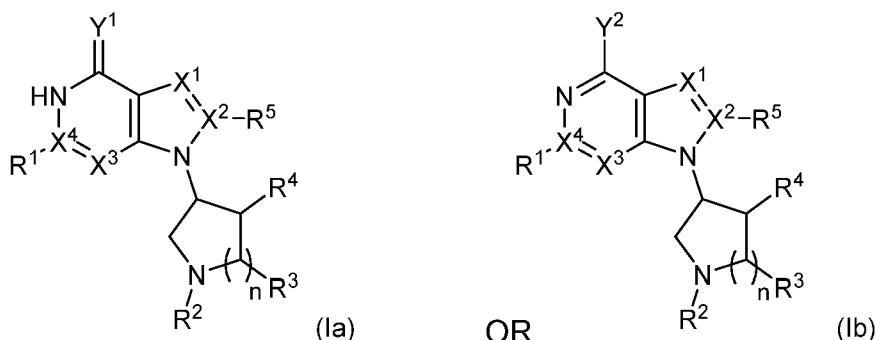
	88	-	-	-	-
	2.8	-	-	-	-

* Non-virulent H37Ra *Mycobacterium tuberculosis* strain. Value is MIC₉₀ (μM).

** Virulent H37Rv *Mycobacterium tuberculosis* strain. Value is MIC₉₀ (μM).

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. A compound of formula (Ia) or (Ib):



5 wherein:

R^1 is alkyl, aryl, heteroaryl, alkenyl, alkynyl, arylalkyl, heteroarylalkyl, NR^6_2 , halogen, OR^6 , H or NH_2 ;

R^2 is H, $-C(O)L^1P(O)R^7R^8$ or $-C(O)L^2P(O)R^7R^8$;

10 the or each R^3 is independently selected from H, $-L^1OH$, $-L^1OR^6$, $-CH_2OC(O)L^1P(O)R^7R^8$, $-CH_2C(O)L^1P(O)R^7R^8$, $-OC(O)L^1P(O)R^7R^8$, and $-OL^1P(O)R^7R^8$;

R^4 is $-OC(O)L^2P(O)R^7R^8$, $-OL^1P(O)R^7R^8$ or $-NHC(O)L^1P(O)R^7R^8$; or

R^4 is $-C(O)L^1P(O)R^7R^8$ when R^2 is $-C(O)L^2P(O)R^7R^8$; or

R^4 is H when R^3 is other than H;

15 R^5 is H, alkyl, aryl, heteroaryl, NR^6_2 , halogen or OR^6 ;

R^7 and R^8 are independently selected from OH, OR^6 , SR^6 , NHR^6 , NR^6_2 , and R^p ;

wherein R^p is a prodrug substituent and wherein the or each R^6 , where present, is independently selected from alkyl, alkenyl, alkynyl, aryl, acyl and arylalkyl optionally containing one or more heteroatoms;

20 L^1 C_{1-4} alkylene, C_{2-4} alkenylene or C_{2-4} alkynylene;

L^2 is C_{2-4} alkylene, C_{2-4} alkenylene or C_{2-4} alkynylene;

wherein L^1 and L^2 are optionally substituted with one or more OH, C_{1-4} alkyl, C_{2-4} alkenyl or C_{2-4} alkynyl;

X^1 is selected from N or CH;

25 X^2 is C or X^2-R^5 forms N;

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X^3 is selected from N or CH;

X^4 is C or X^4-R^1 forms N;

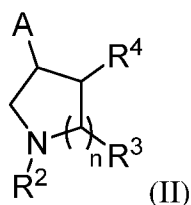
Y^1 where present is selected from S and O,

Y^2 where present is halogen; and

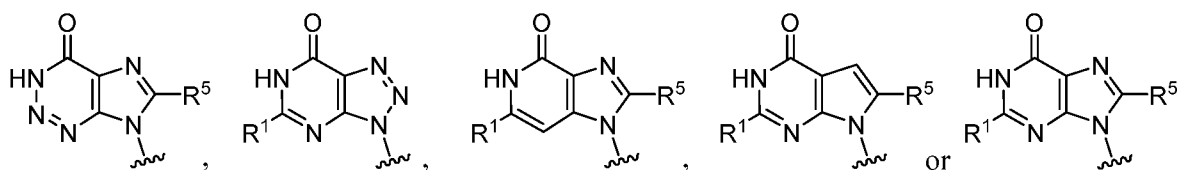
5 n is 1 or 2;

or a pharmaceutically acceptable salt thereof.

2. A compound according to claim 1 of formula (II):



10 wherein A is selected from one of the following:



and wherein:

15 R^1 is alkyl, aryl, heteroaryl, alkenyl, alkynyl, arylalkyl, heteroarylalkyl, NR^6_2 , halogen, OR^6 , H or NH_2 ;

R^2 is H, $-C(O)L^1P(O)R^7R^8$ or $-C(O)L^2P(O)R^7R^8$;

the or each R^3 is independently selected from H, $-L^1OH$, $-L^1OR^6$, $-CH_2OC(O)L^1P(O)R^7R^8$, $-CH_2C(O)L^1P(O)R^7R^8$, $-OC(O)L^1P(O)R^7R^8$, and $-OL^1P(O)R^7R^8$;

20 R^4 is $-OC(O)L^2P(O)R^7R^8$, $-OL^1P(O)R^7R^8$ or $-NHC(O)L^1P(O)R^7R^8$; or

R^4 is $-C(O)L^1P(O)R^7R^8$ when R^2 is $-C(O)L^2P(O)R^7R^8$; or

R^4 is H when R^3 is other than H;

R^5 is H, alkyl, aryl, heteroaryl, NR^6_2 , halogen or OR^6 ;

R^7 and R^8 are independently selected from OH, OR^6 , SR^6 , NHR^6 , NR^6_2 , and R^p ;

25 wherein R^p is a prodrug substituent and wherein the or each R^6 , where present, is independently selected from alkyl, alkenyl, alkynyl, aryl, acyl and arylalkyl

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optionally containing one or more heteroatoms;

L^1 is C_{1-4} alkylene, C_{2-4} alkenylene or C_{2-4} alkynylene;

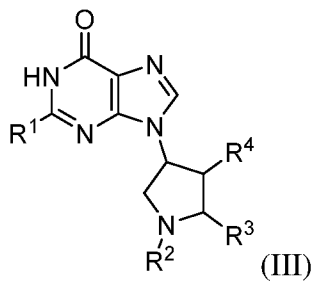
L^2 is C_{2-4} alkylene, C_{2-4} alkenylene or C_{2-4} alkynylene;

wherein L^1 and L^2 are optionally substituted with one or more OH, C_{1-4} alkyl, C_{2-4} alkenyl or C_{2-4} alkynyl; and

n is 1 or 2;

or a pharmaceutically acceptable salt thereof.

3. A compound according to claim 1 or claim 2 of formula (III):



wherein:

R^1 is H or NH_2 ;

R^2 is H, $-C(O)L^1P(O)R^7R^8$ or $-C(O)L^2P(O)R^7R^8$;

R^3 is H;

R^4 is $-OC(O)L^2P(O)R^7R^8$, $-OL^1P(O)R^7R^8$ or $-NHC(O)L^1P(O)R^7R^8$; or

R^4 is $-C(O)L^1P(O)R^7R^8$ when R^2 is $-C(O)L^2P(O)R^7R^8$;

R^7 and R^8 are independently selected from OH, OR^6 , SR^6 , NHR^6 , NR^6_2 , and R^P ;

R^P is a prodrug substituent;

the or each R^6 , where present, is independently selected from alkyl, alkenyl, alkynyl, aryl, acyl and arylalkyl optionally containing one or more heteroatoms;

L^1 is C_{1-4} alkylene; and

L^2 is C_{2-4} alkylene; or

or a pharmaceutically acceptable salt thereof.

4. The compound according to any one of claims 1 to 3, wherein L^1 is methylene or ethylene and L^2 is ethylene.

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5. The compound according to any one of claims 1 to 4, wherein R^7 and R^8 are independently selected from OH, OR^6 and R^p , wherein R^6 is C_{1-3} alkyl.
6. The compound according to any one of claims 1 to 5, wherein R^7 and R^8 are OH.
- 5 7. The compound according to any one of claims 1 to 5, wherein R^p is an amino acid residue, or derivative or ester thereof, and an optionally substituted alkoxy group.
8. The compound according to claim 7 wherein R^p is selected from a C_{1-3} alkyl ester
10 derivative of phenylalanine and $-OCH_2CH_2SC(O)R$ (SATE) wherein R is C_{1-4} alkyl.
9. A pharmaceutical composition comprising a therapeutically effective amount of a compound according to any one of claims 1 to 8, or a pharmaceutically acceptable salt thereof, together with at least one pharmaceutically acceptable carrier or diluent.
- 15 10. A combination comprising a therapeutically effective amount of a compound according to any one of claims 1 to 8, or a pharmaceutically acceptable salt thereof, together with at least one other therapeutic agent.
- 20 11. Use of a compound according to any one of claims 1 to 8, or a pharmaceutically acceptable salt thereof, in the inhibition of a 6-oxopurine phosphoribosyltransferase enzyme.
12. Use according to claim 11, wherein the 6-oxopurine phosphoribosyltransferase
25 enzyme is hypoxanthine-guanine-(xanthine) phosphoribosyltransferase (HG(X)PRT).
13. Use of a compound according to any one of claims 1 to 8, or a pharmaceutically acceptable salt thereof, in the manufacture of a medicament for the prevention or treatment of a microbial infection.
- 30 14. Use according to claim 13, wherein the microbial infection is caused by one or

more of *Plasmodia* spp. (especially *Plasmodium falciparum* and *Plasmodium vivax*), *Giardia* spp. (especially *Giardia lamblia*), *Helicobacter* spp. (such as *Helicobacter pylori*), *Mycobacterium tuberculosis*, a uropathogenic *Escherichia coli*, *Schistosoma* spp. or *Trypanosoma* spp.

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15. A method of preventing or treating a microbial infection comprising administering a therapeutically effective amount of a compound according to any one of claims 1 to 8, or a pharmaceutically acceptable salt thereof, to a subject in need thereof.

10 16. The method according to claim 15, wherein the microbial infection is caused by one or more of *Plasmodia* spp. (especially *Plasmodium falciparum* and *Plasmodium vivax*), *Giardia* spp. (especially *Giardia lamblia*), *Helicobacter* spp. (such as *Helicobacter pylori*), *Mycobacterium tuberculosis*, a uropathogenic *Escherichia coli*, *Schistosoma* spp. or *Trypanosoma* spp.

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17. A compound according to any one of claims 1 to 8, or a pharmaceutically acceptable salt thereof, for use in the prevention or treatment of a microbial infection.

18. The compound for use according to claim 17, wherein the microbial infection is
20 caused by one or more of *Plasmodia* spp. (especially *Plasmodium falciparum* and *Plasmodium vivax*), *Giardia* spp. (especially *Giardia lamblia*), *Helicobacter* spp. (such as *Helicobacter pylori*), *Mycobacterium tuberculosis*, a uropathogenic *Escherichia coli*, *Schistosoma* spp. or *Trypanosoma* spp.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU2016/051238

A. CLASSIFICATION OF SUBJECT MATTER

C07F 9/6561 (2006.01) A61K 31/675 (2006.01) A61K 31/683 (2006.01) A61P 33/06 (2006.01) A61P 31/06 (2006.01)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

STN (REGISTRY AND CAPLUS). Structure search based on the compounds of formula (Ia) and (Ib). Broader structure search based on the compounds of formula (Ia) and (Ib) with control terms "PLASMODIUM FALCIPARUM" or "ANTIMALARIALS" or "MYCOBACTERIUM TUBERCULOSIS", with keywords "?TRANSFERASE?" or "HGPRT OR HG!PRT OR HG!!!PRT", or "?BACTERIA? OR ?PROTOZ? OR ?MICROB?" or "?FUNG? OR ?ALGAE? OR ?ALGI?" or "?VIRAL? OR ?VIRUS?"; **Google Scholar and Espacenet.** Inventor search. "De Jersey John" or "Guddat Luke" or "Hockova Dana" or "Keough Dianne" or "Pohl Radek" or "Rejman Dominik"; **Espacenet.** Applicant and IPC search. "University of Queensland and C07D" or "INSTITUTE OF ORGANIC CHEMISTRY AND BIOCHEMISTRY and C07D" or "Acad of Science Czech Republic and C07D"

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	Documents are listed in the continuation of Box C	



Further documents are listed in the continuation of Box C



See patent family annex

* Special categories of cited documents:	
"A" document defining the general state of the art which is not considered to be of particular relevance	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"E" earlier application or patent but published on or after the international filing date	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O" document referring to an oral disclosure, use, exhibition or other means	"&" document member of the same patent family
"P" document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search
2 March 2017

Date of mailing of the international search report
02 March 2017

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INTERNATIONAL SEARCH REPORT		International application No.
C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		PCT/AU2016/051238
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Pohl, R., et al., "Synthesis, conformational studies, and biological properties of phosphonomethoxyethyl derivatives of nucleobases with a locked conformation via a pyrrolidine ring" Organic & Biomolecular Chemistry, 2015, Vol. 13, No. 16, pages 4693-4705. Abstract, page 4694, Fig. 2, compounds 15b-c and 16b-c, page 4700-4702, page 4698, 3rd paragraph and Table 1	1-6 and 9-18
Y	Abstract, page 4694, Fig. 2, compounds 15b-c and 16b-c, page 4700-4702, page 4698, 3rd paragraph and Table 1	7-8
X	Peterson, M.L., et al., "Synthesis and biological evaluation of 4-purinylypyrrolidine nucleosides" Journal of Medicinal Chemistry, 1991, Vol. 34, No. 9, pages 2787-2797. Abstract, page 2791, Scheme IV, compounds 27 and 29; page 2796, second column 1st paragraph, compound 29.2HCl; and Scheme V, compound 36, page 2791, Scheme V, compound 35	1-2, 4, 17 and 18
X	Kaspersen, F.M., et al., "Unconventional nucleotide analogues. Part XIII. (2S,4S)-2-Hydroxymethyl- and 2-Carboxy-4-(purin-9-yl)pyrrolidines" Journal of the Chemical Society, Perkin Transactions 1: Organic and Bio-Organic Chemistry (1972-1999), 1975, Vol. 16, pages 1617-1622. Page 1617, Scheme 1, compound 1h; and page 1618, Scheme 3, compound 14b	1-2, 4, 17 and 18
X	Li, Y., et al. "Synthesis of 9-(1-amino-4-fluoromethyl-3-pyrrolidinyl)-2,6-dichloropurine from trans-4-hydroxy-D-proline" Chinese Journal of Applied Chemistry, 2008, Vol. 25, No. 3, pages 345-349. Page 346, Scheme 1, compound 10	1, 4, 17 and 18
Y	Keough, D.T., et al., "Inhibition of the Escherichia coli 6-Oxopurine Phosphoribosyltransferases by Nucleoside Phosphonates: Potential for New Antibacterial Agents", Journal of Medicinal Chemistry, 2013, Vol. 56, No. 17, pages 6967-6984. Page 6976, column 2, 3rd paragraph, in particular compound 2, and the prodrug form thereof disclosed on page 6977, Figure 8, compound 2(b), page 6969, Table 1; and pages 6970-6972, Table 2, see in particular compounds 2 and 10 on page 6971	7-8
Form PCT/ISA/210 (fifth sheet) (July 2009)		