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(54) Title: METHODS OF PREPARING 2'-O-SUBSTITUTED PURINE NUCLEOSIDES

(57) Abstract: Provided herein are methods for preparing 2'-O-substituted purine nucleosides without protecting exocyclic amino groups on the base during alkylation. The methods are particularly useful in that the products are crystalline enabling purification without chromatography.

METHODS OF PREPARING 2'-O-SUBSTITUTED PURINE NUCLEOSIDES

FIELD OF THE INVENTION

Provided herein are methods for preparing 2'-O-substituted purine nucleosides without protecting exocyclic amino groups on the base during alkylation. The methods are particularly useful in that the products are crystalline enabling purification without chromatography.

BACKGROUND OF THE INVENTION

Oligonucleotides have been used in various biological and biochemical applications. They have been used as primers and probes for the polymerase chain reaction (PCR), as antisense agents used in target validation, drug discovery and development, as ribozymes, as aptamers, and as general stimulators of the immune system. This widespread use of oligonucleotides has led to an increasing demand for rapid, inexpensive and efficient methods for their synthesis.

Synthetic oligonucleotides are generally prepared through the repeated coupling reactions of nucleoside phosphoramidites to the 5'-hydroxyl group of a nucleoside monomer or the free 5'-hydroxyl group of a growing oligomer. The most commonly used method to perform oligomer synthesis is the phosphoramidite approach which is largely based on developments reported in the literature (see for example: Beaucage and Caruthers (1981) Tetrahedron Letters 22:1859-1862; McBride and Caruthers (1983) Tetrahedron Letters 24:245-248; Sinha et al. (1984) Nucleic Acids Res. 12:4539-4557 and Beaucage and Iyer (1992) Tetrahedron 48:2223-2311, each of which is incorporated herein by reference in its entirety).

Oligomer synthesis can be performed using solution or solid phase chemistries. Solid phase oligonucleotide synthesis (SPOS) is the preferred method. In SPOS, oligonucleotides are assembled in a cyclical manner, each cycle consisting of a series of three chemical reactions. The first reaction is a deblocking reaction, i.e. the removal of a hydroxyl protecting group from a nucleoside monomer or an oligomer bound to a support. Generally, this requires the removal of a dimethoxytrityl protecting group to provide a free hydroxyl group. The second reaction is the coupling reaction, normally performed in the presence of an activator, wherein the free hydroxyl group is reacted with a nucleoside phosphoramidite to provide a phosphite triester in the presence of an activator. The third reaction is the oxidation of the phosphite triester to a phosphate triester. Optionally, a capping step is included either directly before or after each oxidation reaction in order

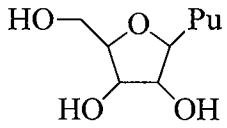
to block support bound nucleoside monomers or oligomers which failed to react in the coupling reaction and to prevent them from further chain elongation in subsequent coupling steps.

A major limiting factor for the cost efficient synthesis of oligonucleotides is the time and cost required to prepare nucleosides, including especially modified nucleosides, and their purification and conversion into phosphoramidites. The standard method used in the industry for purifying nucleoside phosphoramidites and the intermediates formed during their synthesis is column chromatography, especially flash column chromatography which primarily uses silica gel. Regardless of which particular type of column chromatography is used, purification by column chromatography requires large amounts of support material (silica gel for example) and large volumes of high purity solvents. The process also requires a lot of time as it is labor-intensive. Purine nucleosides tend to be more difficult to prepare and purify than pyrimidines. Due to the difficulties inherent in the methods currently being used, there exists an ongoing need in the art for more efficient methods for the synthesis and purification of nucleosides, especially modified nucleosides such as 2'-O-substituted purine nucleosides. Provided herein are methods that fulfill this need.

SUMMARY OF THE INVENTION

Provided herein are methods of preparing a 2'-O-substituted purine nucleoside comprising:

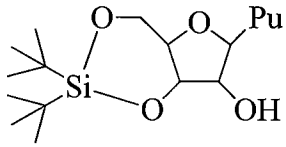
contacting a nucleoside having Formula I:



I

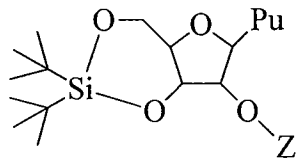
wherein Pu is an unprotected purine having at least one exocyclic amino group;

with a di-*t*-butyl silyl protecting reagent under conditions to provide a protected nucleoside having Formula II:



II;

contacting the protected nucleoside having Formula II with an alkylating agent under conditions to provide 2'-O-substituted purine nucleoside having Formula III:



III

wherein

Z is C_1-C_6 alkyl or substituted C_1-C_6 alkyl, C_2-C_6 alkenyl, substituted C_2-C_6 alkenyl, C_2-C_6 alkynyl or substituted C_2-C_6 alkynyl;

wherein each substituted group is, independently, mono or poly substituted with substituent groups independently selected from halogen, OJ_1, SJ_1, NJ_1J_2, N_3, CN, $C(=O)OJ_1$, $C(=O)NJ_1J_2$, $C(=O)J_1$, $O\text{-}C(=O)NJ_1J_2$, $N(H)C(=O)NJ_1J_2$ or $N(H)C(=S)NJ_1J_2$; and

each J_1 and J_2 is, independently, H, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 aminoalkyl or a protecting group.

In certain embodiments, the methods provided herein further comprise contacting the 2'-O-substituted purine nucleoside with a reagent capable of protecting any exocyclic amino groups located on the purine. In certain embodiments, the methods provided herein further comprise contacting the 2'-O-substituted purine nucleoside with a reagent capable of protecting any exocyclic amino groups located on the purine and or contacting the 2'-O-substituted purine nucleoside with a reagent capable of removing the di-*t*-butyl silyl protecting group.

In certain embodiments, the reagent used to protect exocyclic amino groups on the base is isobutyryl chloride. In certain embodiments, the reagent used to remove the di-*t*-butyl silyl protecting group is triethylamine trihydrofluoride.

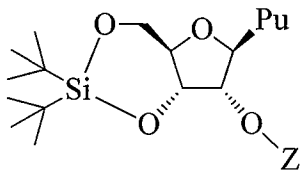
In certain embodiments, the unprotected purine is guanine, adenine or diaminopurine. In certain embodiments, the unprotected purine is guanine.

In certain embodiments, the di-*t*-butyl silyl protecting reagent is $t\text{Bu}_2\text{Si}(\text{OTf})_2$.

In certain embodiments, the alkylating agent comprises a C_1-C_6 alkyl or substituted C_1-C_6 alkyl and a leaving group. In certain embodiments, the alkylating agent comprises a substituted C_1-C_6 alky group and a leaving group. In certain embodiments, the alkylating agent comprises 1-iodo-2-methoxyethane.

In certain embodiments, In certain embodiments, Z is CH_3, CH_2CH_3, $(CH_2)_2CH_3$, $(CH_2)_2\text{-}O\text{-}CH_3$, $CH_2\text{-}CH{=}CH_2$, $(CH_2)_3\text{-}N(R_1)(R_2)$, $(CH_2)_2\text{-}O\text{-}N(R_1)(R_2)$, $(CH_2)_2\text{-}O\text{-}(CH_2)_2\text{-}N(R_1)(R_2)$, $CH_2C(=O)\text{-}N(R_1)(R_2)$, $CH_2C(=O)\text{-}N(H)\text{-}(CH_2)_2\text{-}N(R_1)(R_2)$ or $CH_2\text{-}N(H)\text{-}C(=NR_1)[N(R_1)(R_2)]$ wherein R_1 and R_2 are each independently, H or C_1-C_2 alkyl.

In certain embodiments, the 2'-O-substituted purine nucleoside has the configuration of Formula IV:

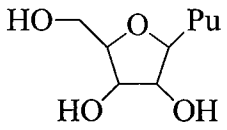


IV.

DETAILED DESCRIPTION OF THE INVENTION

Provided herein are methods for preparing 2'-O-substituted purine nucleosides without protecting exocyclic amino groups on the base during alkylation. The methods are particularly useful in that the products are crystalline enabling purification without chromatography.

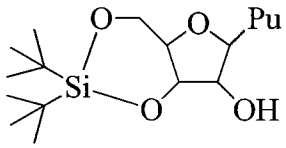
The methods include selecting a nucleoside having Formula I:



I

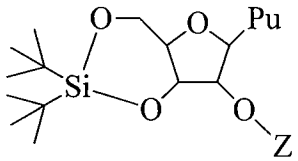
wherein Pu is an unprotected purine having at least one exocyclic amino group;

and contacting this selected nucleoside with a di-*t*-butyl silyl protecting reagent under conditions to provide a protected nucleoside having Formula II:



II.

The nucleoside having Formula II is further contacted with an alkylating agent under conditions to provide 2'-O-substituted purine nucleoside having Formula III:



III.

The alkylating agent can be selected and protected if needed to provide a desired Z group. Such Z groups include without limitation C_1-C_6 alkyl or substituted C_1-C_6 alkyl, C_2-C_6 alkenyl, substituted C_2-C_6 alkenyl, C_2-C_6 alkynyl or substituted C_2-C_6 alkynyl;

wherein each substituted group is, independently, mono or poly substituted with substituent groups independently selected from halogen, OJ_1, SJ_1, NJ_1J_2, N_3, CN, $C(=O)OJ_1$, $C(=O)NJ_1J_2$, $C(=O)J_1$, $O\text{-}C(=O)NJ_1J_2$, $N(H)C(=O)NJ_1J_2$ or $N(H)C(=S)NJ_1J_2$; and

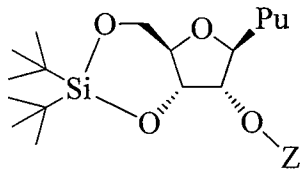
each J_1 and J_2 is, independently, H, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 aminoalkyl or a protecting group.

The methods provided herein are useful for the preparation of 2'-O-substituted purine nucleosides having at least one exocyclic amino group on the base. The methods provide for protection of the sugar 3' and 5' hydroxyl groups and subsequent alkylation of the 2' hydroxyl without having to protect base amino groups. Representative purine bases that have exocyclic amino groups include without limitation guanine, adenine and diaminopurine.

The selection of the alkylating agent will determine the 2'-O-substitutent (Z). Many alkylating agents can be purchased commercially or prepared from commercial materials. Such substituents (Z) include without limitation CH_3, CH_2CH_3, $(CH_2)_2CH_3$, $(CH_2)_2\text{-}O\text{-}CH_3$, $CH_2\text{-}CH{=}CH_2$, $(CH_2)_3\text{-}N(R_1)(R_2)$, $(CH_2)_2\text{-}O\text{-}N(R_1)(R_2)$, $(CH_2)_2\text{-}O\text{-}(CH_2)_2\text{-}N(R_1)(R_2)$, $CH_2C(=O)\text{-}N(R_1)(R_2)$, $CH_2C(=O)\text{-}N(H)\text{-}(CH_2)_2\text{-}N(R_1)(R_2)$ or $CH_2\text{-}N(H)\text{-}C(=NR_1)[N(R_1)(R_2)]$ wherein R_1 and R_2 are each independently, H or C_1-C_2 alkyl.

In certain embodiments, the 2'-O-substituted purine nucleoside prepared using the methods provided herein can be further contacted with a reagent capable of protecting any exocyclic amino groups located on the purine. In certain embodiments, the 2'-O-substituted purine nucleoside prepared using the methods provided herein can be further contacted with a reagent capable of protecting any exocyclic amino groups located on the purine and or contacted with a reagent capable of removing the di-*t*-butyl silyl protecting group.

In one embodiment, the 2'-O-substituted purine nucleosides prepared here have the configuration of Formula IV:



IV.

The terms "substituent" and "substituent group," as used herein, are meant to include groups that are typically added to other groups or parent compounds to enhance desired properties or provide other desired effects. Substituent groups can be protected or unprotected and can be added

to one available site or to many available sites in a parent compound. Substituent groups may also be further substituted with other substituent groups and may be attached directly or via a linking group such as an alkyl or hydrocarbyl group to a parent compound.

Substituent groups amenable herein include without limitation, halogen, hydroxyl, alkyl, alkenyl, alkynyl, acyl (-C(O)R_{aa}), carboxyl (-C(O)O-R_{aa}), aliphatic groups, alicyclic groups, alkoxy, substituted oxy (-O-R_{aa}), aryl, aralkyl, heterocyclic radical, heteroaryl, heteroarylalkyl, amino (-N(R_{bb})(R_{cc})), imino(=NR_{bb}), amido (-C(O)N(R_{bb})(R_{cc}) or -N(R_{bb})C(O)R_{aa}), azido (-N_3), nitro (-NO_2), cyano (-CN), carbamido (-OC(O)N(R_{bb})(R_{cc}) or -N(R_{bb})C(O)OR_{aa}), ureido (-N(R_{bb})C(O)-N(R_{bb})(R_{cc})), thioureido (-N(R_{bb})C(S)N(R_{bb})(R_{cc})), guanidinyl (-N(R_{bb})C(=NR_{bb})N(R_{bb})(R_{cc})), amidinyl (-C(=NR_{bb})N(R_{bb})(R_{cc}) or -N(R_{bb})C(=NR_{bb})(R_{aa})), thiol (-SR_{bb}), sulfinyl (-S(O)R_{bb}), sulfonyl (-$S(O)_2R_{bb}$) and sulfonamidyl (-$S(O)_2$N(R_{bb})(R_{cc}) or -N(R_{bb})$S(O)_2R_{bb}$). Wherein each R_{aa}, R_{bb} and R_{cc} is, independently, H, an optionally linked chemical functional group or a further substituent group with a preferred list including without limitation, H, alkyl, alkenyl, alkynyl, aliphatic, alkoxy, acyl, aryl, aralkyl, heteroaryl, alicyclic, heterocyclic and heteroarylalkyl. Selected substituents within the compounds described herein are present to a recursive degree.

The term "alkyl," as used herein, refers to a saturated straight or branched hydrocarbon radical containing up to twenty four carbon atoms. Examples of alkyl groups include without limitation, methyl, ethyl, propyl, butyl, isopropyl, n-hexyl, octyl, decyl, dodecyl and the like. Alkyl groups typically include from 1 to about 24 carbon atoms, more typically from 1 to about 12 carbon atoms (C_1-C_{12} alkyl) with from 1 to about 6 carbon atoms being more preferred. The term "lower alkyl" as used herein includes from 1 to about 6 carbon atoms. Alkyl groups as used herein may optionally include one or more further substituent groups.

The term "alkenyl," as used herein, refers to a straight or branched hydrocarbon chain radical containing up to twenty four carbon atoms and having at least one carbon-carbon double bond. Examples of alkenyl groups include without limitation, ethenyl, propenyl, butenyl, 1-methyl-2-buten-1-yl, dienes such as 1,3-butadiene and the like. Alkenyl groups typically include from 2 to about 24 carbon atoms, more typically from 2 to about 12 carbon atoms with from 2 to about 6 carbon atoms being more preferred. Alkenyl groups as used herein may optionally include one or more further substituent groups.

The term "alkynyl," as used herein, refers to a straight or branched hydrocarbon radical containing up to twenty four carbon atoms and having at least one carbon-carbon triple bond. Examples of alkynyl groups include, without limitation, ethynyl, 1-propynyl, 1-butynyl, and the

like. Alkynyl groups typically include from 2 to about 24 carbon atoms, more typically from 2 to about 12 carbon atoms with from 2 to about 6 carbon atoms being more preferred. Alkynyl groups as used herein may optionally include one or more further substituent groups.

The term "acyl," as used herein, refers to a radical formed by removal of a hydroxyl group from an organic acid and has the general Formula -C(O)-X where X is typically aliphatic, alicyclic or aromatic. Examples include aliphatic carbonyls, aromatic carbonyls, aliphatic sulfonyls, aromatic sulfinyls, aliphatic sulfinyls, aromatic phosphates, aliphatic phosphates and the like. Acyl groups as used herein may optionally include further substituent groups.

The term "alicyclic" refers to a cyclic ring system wherein the ring is aliphatic. The ring system can comprise one or more rings wherein at least one ring is aliphatic. Preferred alicyclics include rings having from about 5 to about 9 carbon atoms in the ring. Alicyclic as used herein may optionally include further substituent groups.

The term "aliphatic," as used herein, refers to a straight or branched hydrocarbon radical containing up to twenty four carbon atoms wherein the saturation between any two carbon atoms is a single, double or triple bond. An aliphatic group preferably contains from 1 to about 24 carbon atoms, more typically from 1 to about 12 carbon atoms with from 1 to about 6 carbon atoms being more preferred. The straight or branched chain of an aliphatic group may be interrupted with one or more heteroatoms that include nitrogen, oxygen, sulfur and phosphorus. Such aliphatic groups interrupted by heteroatoms include without limitation, polyalkoxys, such as polyalkylene glycols, polyamines, and polyimines. Aliphatic groups as used herein may optionally include further substituent groups.

The term "alkoxy," as used herein, refers to a radical formed between an alkyl group and an oxygen atom wherein the oxygen atom is used to attach the alkoxy group to a parent molecule. Examples of alkoxy groups include without limitation, methoxy, ethoxy, propoxy, isopropoxy, *n*-butoxy, sec-butoxy, *tert*-butoxy, n-pentoxy, neopentoxy, n-hexoxy and the like. Alkoxy groups as used herein may optionally include further substituent groups.

The term "aminoalkyl" as used herein, refers to an amino substituted C_1-C_{12} alkyl radical. The alkyl portion of the radical forms a covalent bond with a parent molecule. The amino group can be located at any position and the aminoalkyl group can be substituted with a further substituent group at the alkyl and/or amino portions.

The terms "aralkyl" and "arylalkyl," as used herein, refer to an aromatic group that is covalently linked to a C_1-C_{12} alkyl radical. The alkyl radical portion of the resulting aralkyl (or

arylalkyl) group forms a covalent bond with a parent molecule. Examples include without limitation, benzyl, phenethyl and the like. Aralkyl groups as used herein may optionally include further substituent groups attached to the alkyl, the aryl or both groups that form the radical group.

The terms "aryl" and "aromatic," as used herein, refer to a mono- or polycyclic carbocyclic ring system radicals having one or more aromatic rings. Examples of aryl groups include without limitation, phenyl, naphthyl, tetrahydronaphthyl, indanyl, idenyl and the like. Preferred aryl ring systems have from about 5 to about 20 carbon atoms in one or more rings. Aryl groups as used herein may optionally include further substituent groups.

The terms "halo" and "halogen," as used herein, refer to an atom selected from fluorine, chlorine, bromine and iodine.

The terms "heteroaryl," and "heteroaromatic," as used herein, refer to a radical comprising a mono- or poly-cyclic aromatic ring, ring system or fused ring system wherein at least one of the rings is aromatic and includes one or more heteroatoms. Heteroaryl is also meant to include fused ring systems including systems where one or more of the fused rings contain no heteroatoms. Heteroaryl groups typically include one ring atom selected from sulfur, nitrogen or oxygen. Examples of heteroaryl groups include without limitation, pyridinyl, pyrazinyl, pyrimidinyl, pyrrolyl, pyrazolyl, imidazolyl, thiazolyl, oxazolyl, isooxazolyl, thiadiazolyl, oxadiazolyl, thiophenyl, furanyl, quinolinyl, isoquinolinyl, benzimidazolyl, benzooxazolyl, quinoxalinyl and the like. Heteroaryl radicals can be attached to a parent molecule directly or through a linking moiety such as an aliphatic group or hetero atom. Heteroaryl groups as used herein may optionally include further substituent groups.

The term "heteroarylalkyl," as used herein, refers to a heteroaryl group as previously defined that further includes a covalently attached C_1-C_{12} alkyl radical. The alkyl radical portion of the resulting heteroarylalkyl group is capable of forming a covalent bond with a parent molecule. Examples include without limitation, pyridinylmethyl, pyrimidinylethyl, napthyridinylpropyl and the like. Heteroarylalkyl groups as used herein may optionally include further substituent groups on one or both of the heteroaryl or alkyl portions.

The term "oxo" refers to the group (=O).

Linking groups or bifunctional linking moieties such as those known in the art are useful for attachment of chemical functional groups, conjugate groups, reporter groups and other groups to selective sites in a parent compound such as for example an oligomeric compound. In general, a bifunctional linking moiety comprises a hydrocarbyl moiety having two functional groups. One of

the functional groups is selected to bind to a parent molecule or compound of interest and the other is selected to bind to essentially any selected group such as a chemical functional group or a conjugate group. In some embodiments, the linker comprises a chain structure or a polymer of repeating units such as ethylene glycols or amino acid units. Examples of functional groups that are routinely used in bifunctional linking moieties include without limitation, electrophiles for reacting with nucleophilic groups and nucleophiles for reacting with electrophilic groups. In some embodiments, bifunctional linking moieties include amino, hydroxyl, carboxylic acid, thiol, unsaturations (e.g., double or triple bonds), and the like. Some nonlimiting examples of bifunctional linking moieties include 8-amino-3,6-dioxaoctanoic acid (ADO), succinimidyl 4-(N-maleimidomethyl) cyclohexane-1-carboxylate (SMCC) and 6-aminohexanoic acid (AHEX or AHA). Other linking groups include without limitation, substituted C_1-C_{10} alkyl, substituted or unsubstituted C_2-C_{10} alkenyl or substituted or unsubstituted C_2-C_{10} alkynyl, wherein a nonlimiting list of preferred substituent groups includes hydroxyl, amino, alkoxy, carboxy, benzyl, phenyl, nitro, thiol, thioalkoxy, halogen, alkyl, aryl, alkenyl and alkynyl.

The term "protecting group," as used herein, refers to a labile chemical moiety which is known in the art to protect reactive groups including without limitation, hydroxyl, amino and thiol groups, against undesired reactions during synthetic procedures. Protecting groups are typically used selectively and/or orthogonally to protect sites during reactions at other reactive sites and can then be removed to leave the unprotected group as is or available for further reactions. Protecting groups as known in the art are described generally in Greene's Protective Groups in Organic Synthesis, 4th edition, John Wiley & Sons, New York, 2007.

Groups can be selectively incorporated into oligomeric compounds as provided herein as precursors. For example an amino group can be placed into a compound as provided herein as an azido group that can be chemically converted to the amino group at a desired point in the synthesis. Generally, groups are protected or present as precursors that will be inert to reactions that modify other areas of the parent molecule for conversion into their final groups at an appropriate time. Further representative protecting or precursor groups are discussed in Agrawal *et al.*, *Protocols for Oligonucleotide Conjugates*, Humana Press; New Jersey, 1994, 26, 1-72.

The term "orthogonally protected" refers to functional groups which are protected with different classes of protecting groups, wherein each class of protecting group can be removed in any order and in the presence of all other classes (see, Barany *et al., J. Am. Chem. Soc.*, 1977, *99*, 7363-7365; Barany *et al., J. Am. Chem. Soc.*, 1980, 102, 3084-3095). Orthogonal protection is widely

used in for example automated oligonucleotide synthesis. A functional group is deblocked in the presence of one or more other protected functional groups which is not affected by the deblocking procedure. This deblocked functional group is reacted in some manner and at some point a further orthogonal protecting group is removed under a different set of reaction conditions. This allows for selective chemistry to arrive at a desired compound or oligomeric compound.

Examples of hydroxyl protecting groups include without limitation, acetyl, t-butyl, t-butoxymethyl, methoxymethyl, tetrahydropyranyl, 1-ethoxyethyl, 1-(2-chloroethoxy)ethyl, p-chlorophenyl, 2,4-dinitrophenyl, benzyl, 2,6-dichlorobenzyl, diphenylmethyl, p-nitrobenzyl, bis(2-acetoxyethoxy)methyl (ACE), 2-trimethylsilylethyl, trimethylsilyl, triethylsilyl, t-butyldimethylsilyl, t-butyldiphenylsilyl, triphenylsilyl, [(triisopropylsilyl)oxy]methyl (TOM), benzoylformate, chloroacetyl, trichloroacetyl, trifluoroacetyl, pivaloyl, benzoyl, p-phenylbenzoyl, 9-fluorenylmethyl carbonate, mesylate, tosylate, triphenylmethyl (trityl), monomethoxytrityl, dimethoxytrityl (DMT), trimethoxytrityl, 1(2-fluorophenyl)-4-methoxypiperidin-4-yl (FPMP), 9-phenylxanthine-9-yl (Pixyl) and 9-(p-methoxyphenyl)xanthine-9-yl (MOX). Wherein more commonly used hydroxyl protecting groups include without limitation, benzyl, 2,6-dichlorobenzyl, t-butyldimethylsilyl, t-butyl-diphenylsilyl, benzoyl, mesylate, tosylate, dimethoxytrityl (DMT), 9-phenylxanthine-9-yl (Pixyl) and 9-(p-methoxyphenyl)xanthine-9-yl (MOX).

Examples of amino protecting groups include without limitation, carbamate-protecting groups, such as 2-trimethylsilylethoxycarbonyl (Teoc), 1-methyl-1-(4-biphenylyl)ethoxycarbonyl (Bpoc), t-butoxycarbonyl (BOC), allyloxycarbonyl (Alloc), 9-fluorenylmethyloxycarbonyl (Fmoc), and benzyloxycarbonyl (Cbz); amide-protecting groups, such as formyl, acetyl, trihaloacetyl, benzoyl, and nitrophenylacetyl; sulfonamide-protecting groups, such as 2-nitrobenzenesulfonyl; and imine- and cyclic imide-protecting groups, such as phthalimido and dithiasuccinoyl.

Examples of thiol protecting groups include without limitation, triphenylmethyl (trityl), benzyl (Bn), and the like.

As is known in the art, a nucleoside is a base-sugar combination. The base portion of the nucleoside is normally a heterocyclic base moiety. The two most common classes of such heterocyclic bases are purines and pyrimidines. Nucleotides are nucleosides that further include a phosphate group covalently linked to the sugar portion of the nucleoside. For those nucleosides that include a pentofuranosyl sugar, the phosphate group can be linked to either the 2', 3' or 5' hydroxyl moiety of the sugar. In forming oligonucleotides, the phosphate groups covalently link adjacent nucleosides to one another to form a linear polymeric compound. The respective ends of this linear

polymeric structure can be joined to form a circular structure by hybridization or by formation of a covalent bond. However, open linear structures are generally desired. Within the oligonucleotide structure, the phosphate groups are commonly referred to as forming the internucleoside linkages of the oligonucleotide. The normal internucleoside linkage of RNA and DNA is a 3' to 5' phospho-diester linkage.

As used herein the term "modified nucleoside" is meant to include all manner of modified nucleosides that can be incorporated into an oligomeric compound using oligomer synthesis. The term is intended to include modifications made to a nucleoside such as modified stereochemical configurations, one or more substitutions, and deletion of groups as opposed to the use of surrogate groups which are described elsewhere herein. The term includes nucleosides having a furanose sugar (or 4'-S analog) portion and can include a heterocyclic base or can include an abasic site. One group of representative modified nucleosides includes without limitation, substituted nucleosides (such as 2', 5', and/or 4' substituted nucleosides) 4'-S-modified nucleosides, (such as 4'-S-ribonucleosides, 4'-S-2'-deoxyribonucleosides and 4'-S-2'-substituted ribonucleosides), bicyclic modified nucleosides (such as for example, bicyclic nucleosides wherein the sugar group has a 2'-O-CHR_a-4' bridging group, wherein R_a is H, alkyl or substituted alkyl) and base modified nucleosides. The sugar can be modified with more than one of these modifications listed such as for example a bicyclic modified nucleoside further including a 5'-substitution or a 5' or 4' substituted nucleoside further including a 2' substituent. The term modified nucleoside also includes combinations of these modifications such as a base and sugar modified nucleosides. These modifications are meant to be illustrative and not exhaustive as other modifications are known in the art and are also envisioned as possible modifications for the modified nucleosides described herein.

As used herein the terms, "unmodified nucleobase" and "naturally occurring nucleobase" include the purine bases adenine (A) and guanine (G), and the pyrimidine bases thymine (T), cytosine (C) and uracil (U). Modified nucleobases include other synthetic and natural nucleobases such as 5-methylcytosine (5-me-C), 5-hydroxymethyl cytosine, xanthine, hypoxanthine, 2-aminoadenine, 6-methyl and other alkyl derivatives of adenine and guanine, 2-propyl and other alkyl derivatives of adenine and guanine, 2-thiouracil, 2-thiothymine and 2-thiocytosine, 5-halouracil and cytosine, 5-propynyl (-C≡C-CH_3) uracil and cytosine and other alkynyl derivatives of pyrimidine bases, 6-azo uracil, cytosine and thymine, 5-uracil (pseudouracil), 4-thiouracil, 8-halo, 8-amino, 8-thiol, 8-thioalkyl, 8-hydroxyl and other 8-substituted adenines and guanines, 5-halo particularly 5-bromo, 5-trifluoromethyl and other 5-substituted uracils and cytosines, 7-methylguanine and 7-

methyladenine, 2-F-adenine, 2-amino-adenine, 8-azaguanine and 8-azaadenine, 7-deazaguanine and 7-deazaadenine, 3-deazaguanine and 3-deazaadenine, universal bases, hydrophobic bases, promiscuous bases, size-expanded bases, and fluorinated bases as defined herein. Further modified nucleobases include tricyclic pyrimidines such as phenoxazine cytidine([5,4-b][1,4]benzoxazin-2(3H)-one), phenothiazine cytidine (1H-pyrimido[5,4-b][1,4]benzothiazin-2(3H)-one), G-clamps such as a substituted phenoxazine cytidine (e.g. 9-(2-aminoethoxy)-H-pyrimido[5,4-b][1,4]benzoxazin-2(3H)-one), carbazole cytidine (2H-pyrimido[4,5-b]indol-2-one), pyridoindole cytidine (H-pyrido[3',2':4,5]pyrrolo[2,3-d]pyrimidin-2-one). Modified nucleobases may also include those in which the purine or pyrimidine base is replaced with other heterocycles, for example 7-deaza-adenine, 7-deazaguanosine, 2-aminopyridine and 2-pyridone. Further nucleobases include those disclosed in United States Patent No. 3,687,808, those disclosed in *The Concise Encyclopedia Of Polymer Science And Engineering*, Kroschwitz, J.I., Ed., John Wiley & Sons, 1990, 858-859; those disclosed by Englisch *et al.*, *Angewandte Chemie*, International Edition, 1991, *30*, 613; and those disclosed by Sanghvi, Y.S., Chapter 15, *Antisense Research and Applications*, Crooke, S.T. and Lebleu, B., Eds., CRC Press, 1993, 273-288.

The heterocyclic base moiety of each of the 2'-O-substituted purine nucleosides can be modified with one or more substituent groups to enhance one or more properties such as affinity for a target strand or affect some other property in an advantageous manner. Modified nucleobases include without limitation, universal bases, hydrophobic bases, promiscuous bases, size-expanded bases, and fluorinated bases as defined herein. Certain of these nucleobases are particularly useful for increasing the binding affinity of the oligomeric compounds as provided herein. These include 5-substituted pyrimidines, 6-azapyrimidines and N-2, N-6 and O-6 substituted purines, including 2-aminopropyladenine, 5-propynyluracil and 5-propynylcytosine. 5-methylcytosine substitutions have been shown to increase nucleic acid duplex stability by 0.6-1.2 °C (*Antisense Research and Applications*, Sanghvi, Y.S., Crooke, S.T. and Lebleu, B., Eds., CRC Press, Boca Raton, 1993, 276-278).

Representative United States patents that teach the preparation of certain of the above noted modified nucleobases as well as other modified nucleobases include without limitation, U.S. 3,687,808; 4,845,205; 5,130,302; 5,134,066; 5,175,273; 5,367,066; 5,432,272; 5,457,187; 5,459,255; 5,484,908; 5,502,177; 5,525,711; 5,552,540; 5,587,469; 5,594,121; 5,596,091; 5,614,617; 5,645,985; 5,681,941; 5,750,692; 5,763,588; 5,830,653 and 6,005,096, certain of which are commonly owned with the instant application, and each of which is herein incorporated by

reference in its entirety.

In certain embodiments, examples of substituent groups useful for modifying furanose sugar moieties (e.g., sugar substituent groups used for nucleosides), include without limitation 2'-F, 2'-allyl, 2'-amino, 2'-azido, 2'-thio, 2'-O-allyl, 2'-OCF_3, 2'-O-C_1-C_{10} alkyl, 2'-O-CH_3, OCF_3, 2'-O-CH_2CH_3, 2'-O-$(CH_2)_2CH_3$, 2'-O-$(CH_2)_2$-O-CH_3 (MOE), 2'-O$(CH_2)_2SCH_3$, 2'-O-CH_2-CH=CH_2, 2'-O-$(CH_2)_3$-N(R_m)(R_n), 2'-O-$(CH_2)_2$-O-N(R_m)(R_n), 2'-O-$(CH_2)_2$-O-$(CH_2)_2$-N(R_m)(R_n), 2'-O-CH_2C(=O)-N(R_m)(R_n), 2'-O-CH_2C(=O)-N(H)-$(CH_2)_2$-N(R_m)(R_n) and 2'-O-CH_2-N(H)-C(=NR_m)[N(R_m)(R_n)], 5'-vinyl, 5'-methyl (R or S) and 4'-S wherein each R_m and R_n is, independently, H, substituted or unsubstituted C_1-C_{10} alkyl or a protecting group. Further examples of modified sugar moieties include without limitation bicyclic sugars (e.g. bicyclic nucleic acids or bicyclic nucleosides discussed below).

Combinations of these modifications are also provided for herein without limitation, such as 2'-F-5'-methyl substituted nucleosides (see PCT International Application WO 2008/101157 Published on 8/21/08 for other disclosed 5', 2'-bis substituted nucleosides) and replacement of the ribosyl ring oxygen atom with S and further substitution at the 2'-position (see published U.S. Patent Application US2005-0130923, published on June 16, 2005) or alternatively 5'-substitution of a bicyclic nucleic acid (see PCT International Application WO 2007/134181, published on 11/22/07 wherein a 4'-CH_2-O-2' bicyclic nucleoside is further substituted at the 5' position with a 5'-methyl or a 5'-vinyl group).

As used herein the terms "bicyclic nucleic acid" and "bicyclic nucleoside" refer to nucleosides wherein the sugar portion of the nucleoside is bicyclic (e.g. bicyclic sugar). In certain embodiments, a bicyclic nucleic acid comprises a nucleoside wherein the furanose ring comprises a bridge between two non-geminal ring carbon atoms. Examples of bicyclic nucleosides include without limitation nucleosides comprising a bridge between the 4' and the 2' ribosyl ring atoms. In certain embodiments, oligomeric compounds provided herein include one or more bicyclic nucleosides wherein the bridge comprises one of the formulae: 4'-(CH_2)-O-2' (LNA); 4'-(CH_2)-S-2'; 4'-$(CH_2)_2$-O-2' (ENA); 4'-CH(CH_3)-O-2' and 4'-CH(CH_2OCH_3)-O-2' (and analogs thereof see U.S. Patent 7,399,845, issued on July 15, 2008); 4'-C(CH_3)(CH_3)-O-2' (and analogs thereof see published International Application WO/2009/006478, published January 8, 2009); 4'-CH_2-N(OCH_3)-2' (and analogs thereof see published International Application WO/2008/150729, published December 11, 2008); 4'-CH_2-O-N(CH_3)-2' (see published U.S. Patent Application US2004-0171570, published September 2, 2004); 4'-CH_2-N(R)-O-2', wherein R is H, C_1-C_{12} alkyl, or a protecting group (see

U.S. Patent 7,427,672, issued on September 23, 2008); 4'-CH_2-C(H)(CH_3)-2' (see Chattopadhyaya, *et al., J. Org. Chem.*,2009, *74*, 118-134); and 4'-CH_2-C(=CH_2)-2' (and analogs thereof see published International Application WO 2008/154401, published on December 8, 2008). Each of the foregoing bicyclic nucleosides can be prepared having one or more stereochemical sugar configurations including for example α-L-ribofuranose and β-D-ribofuranose (see PCT international application PCT/DK98/00393, published on March 25, 1999 as WO 99/14226).

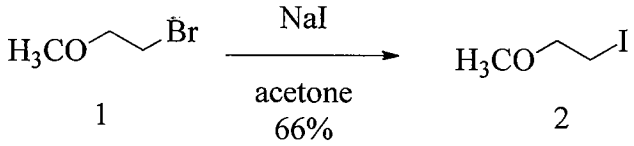
While in certain embodiments, methods of preparing 2'-O-substituted purine nucleosides have been described with specificity, the following examples serve only to illustrate and are not intended to be limiting.

Examples (General)

^{1}H NMR spectra were recorded on a 300 MHz Bruker spectrometer and purity was determined by HPLC/LCMS, respectively.

Example 1

Preparation of 1-Iodo-2-methoxy-ethane, Compound 2

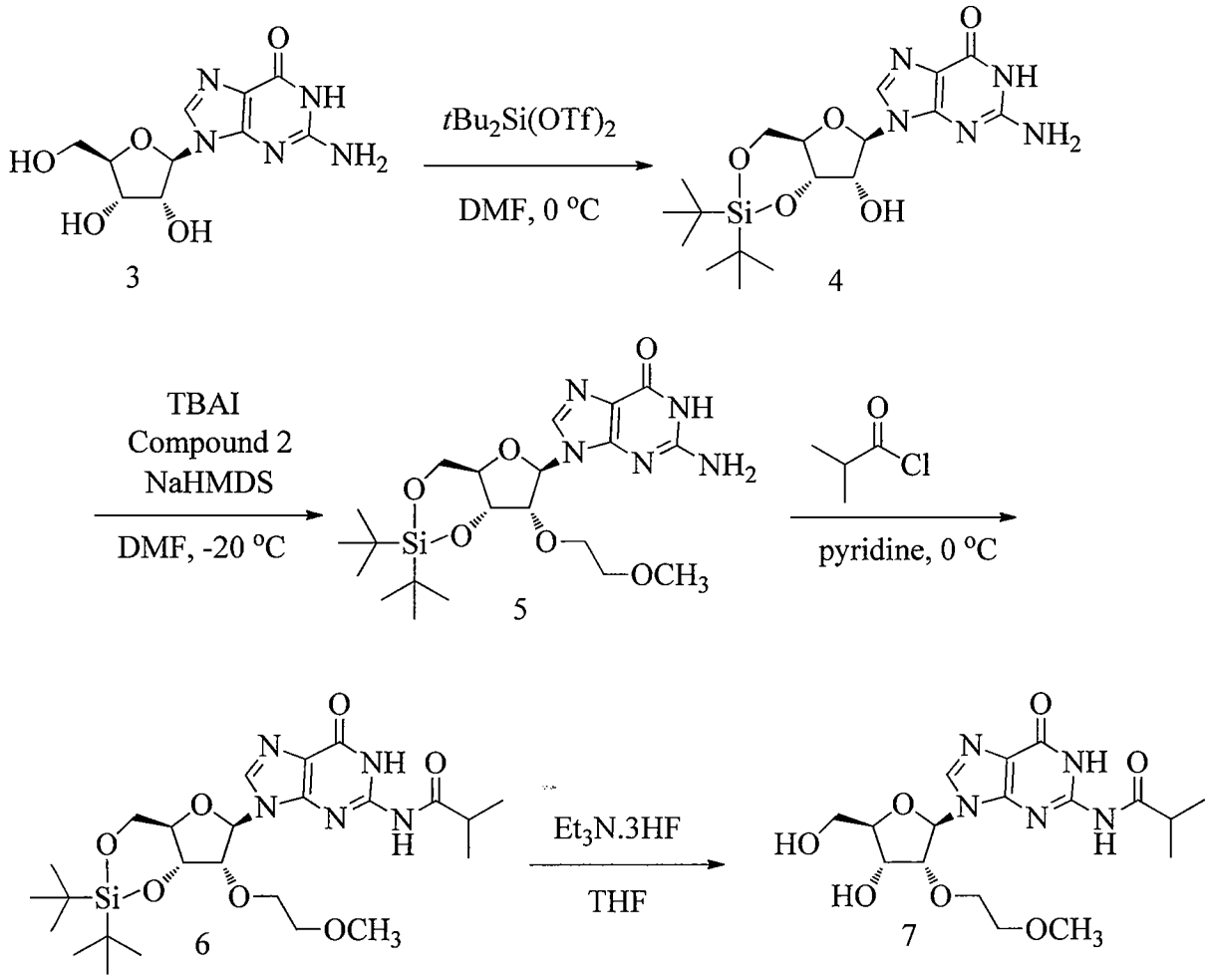


1 2

Compound 2, was prepared according to the procedures illustrated in WO 2003/091227. Compound 1 (15 g, 0.11 mol, 1.0 eq, commercially available) was added to a solution of sodium iodide (22.5 g, 0.15 mol, 1.36 eq) in acetone (50 mL) and stirred overnight under reflux. The reaction mixture initially turned brown, then slowly changed to dark orange. The byproduct, sodium bromide, was observed as it crashed out as a white precipitate. After stirring for 12 h, the reaction mixture was poured into ice water (200 mL) and extracted with diethyl ether (3 x 100 mL). The combined organic layers were sequentially washed with saturated sodium thiosulfate (3 x 50 mL), water and brine then dried over $MgSO_4$. The solution was filtered through a thin pad of Celite and silica gel and concentrated *in vacuo* to furnish 1-iodo-2-methoxy-ethane, Compound 2 (13.31 g, 66%). To maximize shelf life, Compound 2 was stored in a freezer at -20 °C under nitrogen. Compound 2 can also be further purified by vacuum distillation.

Example 3

Preparation of Compound 7



a) Preparation of Compound 4

Compound 3 (purchased from a commercial source as a dihydrate) was dried by refluxing in toluene with a Dean-Stark trap and placed in a vacuum oven over P_2O_5 for storage. The anhydrous Compound 3 (20 g, 0.071 mol, 1.0 eq) was dissolved in anhydrous DMF (550 mL) and the volume was reduced to 500 mL *in vacuo* at 50 °C. The reaction mixture was cooled to 0 °C while stirring under nitrogen and tBu$_2$Si(OTf)$_2$ (25 mL, 0.077mol, 1.0 eq) was added dropwise *via* a syringe. After stirring for 30 min at 0 °C, the reaction was complete as determined by LCMS. The reaction mixture was carefully poured into a basic solution consisting of $NaHCO_3$ (20 g), H_2O (1.5 L) and crushed ice (500 g). It is noteworthy to mention that triflic acid byproduct tends to cause depurination if the crude is over-exposed to acidic medium. The resulting precipitate was stirred for 30 min and was collected by filtration. The crude solid was re-suspended in H_2O and the stirring

was continued for another 30 min. The solid was collected by filtration, dried overnight under vacuum at 45 °C and further dried by refluxing in toluene with a Dean-Stark trap to provide the crude Compound 4 (27.6 g, 93% with 94.175% purity).

b) Preparation of Compound 5

The conditions used in preparing Compound 5 was in part carried out using similar procedures to those reported in the literature (Sanghvi, Y. S.; Theodorakis, E. A. et al *J. Org. Chem.* 2002, *67*, 7887-7889 and *Nucleosides, Nucleotides and Nucleic Acids* 2003, *22,* 583-587). Compound 2 was prepared per the procedures illustrated in Example 1. Compound 4 (10 g, 0.024 mol, 1.0 eq) and tetrabutyl ammonium iodide or TBAI (2.66 g, 0.0072 mol, 0.3 eq) were dissolved in anhydrous DMF (600 mL) and the volume was reduced to 500 mL *in vacuo* at 50 °C. The reaction mixture was cooled to -20 °C with stirring under nitrogen and a solution of NaHMDS (70 mL, 1M NaHMDS in THF, 0.07 mol, 3 eq) was added dropwise at 2 mL/min. The stirring was continued for 10 min at -20 °C followed by the addition of 1-iodo-2-methoxy-ethane (13.17 g, 0.071 mol, 3 eq). The progress of the reaction was monitored by LCMS and additional NaHMDS and alkylating reagent were added until Compound 4 was completely consumed. After standing for 64 h at -20 °C, the reaction was quenched with cold MeOH (10 mL) and was poured into a solution of crushed ice (200 g) and water (1 L). The reaction mixture was diluted with EtOAc (500 mL) followed by the addition of saturated brine solution (500 mL). The organic layer was collected after partitioning and the aqueous layer was extracted twice with EtOAc (2 x 250 mL). The combined organic layers were sequentially washed with water and brine then dried ($MgSO_4$), concentrated *in vacuo* and dried under high vacuum to furnish the crude, Compound 5 as an orange oil, which was used without any further purification.

c) Preparation of Compound 6

Isobutyryl chloride (3.5 mL, 0.033 mol, 1.5 eq) was added to a solution of Compound 5 at 0 °C which was prepared by co-evaporating Compound 5 with anhydrous pyridine (2 x 100 mL) and dissolving the coevaporated Compound 5 (10.75 g, 0.022 mol, 1.0 eq) in anhydrous pyridine (250 mL). Additional isobutyryl chloride is added until the starting material is completely consumed. After stirring under nitrogen for 2 h at 0 °C, the reaction was complete as indicated by LCMS analysis. MeOH (10 mL) was added to the reaction mixture to quench any unreacted acid chloride and the solvent was evaporated under reduced pressure. The resulting residue was further co-

evaporated with CH_3CN (2 x 100 mL). The crude solid was suspended in boiling MeOH (400 mL) followed by the addition of dichloromethane (50 mL) until a solution resulted. After standing for 5 h, crystals began for form with additional crystals observed after storage overnight at 4 °C. The crystals were collected by filtration and dried over P_2O_5 in a vacuum oven at 45 °C to furnish pure Compound 6 (6.12 g, 46 % from Compound 4 with greater than 99% purity).

d) Preparation of Compound 7

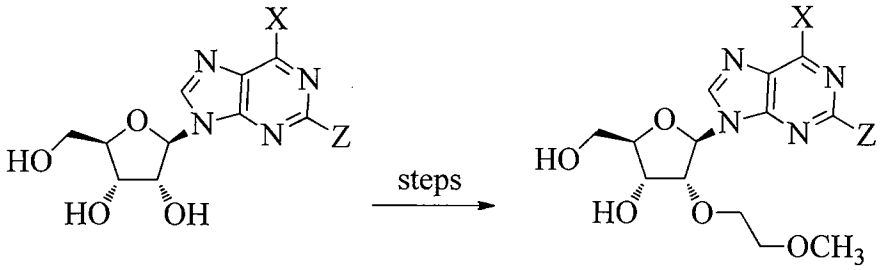
Triethylamine trihydrofluoride (1.2 mL, 0.0073 mol, 0.66 eq) was added to a solution of Compound 6 (6.1 g, 0.011 mol, 1.0 eq) suspended in anhydrous THF (100 mL). A minimum amount of triethylamine trihydrofluoride was added until all the starting material was consumed. The reaction was stirred at ambient temperature for 30 min after TLC analysis (10% MeOH in CH_2Cl_2) indicated that Compound 6 was consumed. The reaction mixture was concentrated under reduced pressure and the residue was diluted with diethyl ether (25 mL). The resulting white precipitate was collected by filtration and dried briefly under high vacuum. The crude solid was then recrystallized in acetone/H_20 (10:1 v/v, 100 mL) to provide pure Compound 7 (4.9 g, 94% yield with 99.67% purity) as crystals.

Structural analysis for Compounds 2-7 was confirmed by ^1H NMR and the purity for Compounds 4, 6 and 7 were determined by HPLC/LCMS.

We have developed and successfully demonstrated the synthesis of Compound 7 in high purity on a multigram scale with 44% overall yield employing a four-step synthetic method described herein. This method provided crystalline compounds without requiring protection of the purine base during either the protection with di-*t*-butyl silyl or the alkylation step. The intermediates and the final base protected Compound 7 are purified by crystallization without the need of chromatography. Although the method is illustrated for preparing a 2'-$O(CH_2)_2OCH_3$ substituted guanosine nucleoside, the method is also expected to be useful for the preparation of additional 2'-O-substituted nucleosides. Such other 2'-O-substituted nucleosides include for example adenosine and diaminopurine.

Example 4

Preparation of Compounds 10 and 11



8 X = NH_2, Z = H
9 X = NH_2, Z = NH_2

10 X = NH_2, Z = H
11 X = NH_2, Z = NH_2

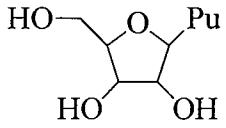
Compounds 10 and 11 are prepared as per the procedures illustrated in Example 3 using Compounds 8 and 9 as starting materials (Compounds 8 and 9 are commercially available).

All publications, patents, and patent applications referenced herein are incorporated herein by reference. While in the foregoing specification this invention has been described in relation to certain embodiments thereof, and many details have been set forth for purposes of illustration, it will be apparent to those skilled in the art that the invention is susceptible to additional embodiments and that certain of the details described herein may be varied considerably without departing from the basic principles of the invention.

What is Claimed is:

1. A method of preparing a 2'-O-substituted purine nucleoside comprising:

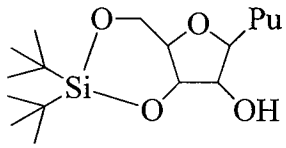
contacting a nucleoside having Formula I:



I

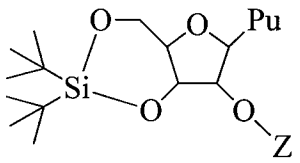
wherein Pu is an unprotected purine having at least one exocyclic amino group;

with a di-*t*-butyl silyl protecting reagent under conditions to provide a protected nucleoside having Formula II:



II

contacting the protected nucleoside having Formula II with an alkylating agent under conditions to provide 2'-O-substituted purine nucleoside having Formula III:



III

wherein:

Z is C_1-C_6 alkyl or substituted C_1-C_6 alkyl, C_2-C_6 alkenyl, substituted C_2-C_6 alkenyl, C_2-C_6 alkynyl or substituted C_2-C_6 alkynyl;

wherein each substituted group is, independently, mono or poly substituted with substituent groups independently selected from halogen, OJ_1, SJ_1, NJ_1J_2, N_3, CN, $C(=O)OJ_1$, $C(=O)NJ_1J_2$, $C(=O)J_1$, $O\text{-}C(=O)NJ_1J_2$, $N(H)C(=O)NJ_1J_2$ or $N(H)C(=S)NJ_1J_2$; and

each J_1 and J_2 is, independently, H, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 aminoalkyl or a protecting group.

2. The method of claim 1 further comprising contacting the 2'-O-substituted purine nucleoside with a reagent capable of protecting any exocyclic amino groups located on the purine.

3. The method of claim 1 further comprising contacting the 2'-O-substituted purine nucleoside with a reagent capable of protecting any exocyclic amino groups located on the purine and or contacting the 2'-O-substituted purine nucleoside with a reagent capable of removing the di-*t*-butyl silyl protecting group.

4. The method of claim 3 wherein the reagent capable of protecting exocyclic amino groups is isobutyryl chloride.

5. The method of claim 3 wherein the reagent capable of removing the di-*t*-butyl silyl protecting group is triethylamine trihydrofluoride.

6. The method of claim 1 wherein the unprotected purine is guanine, adenine or diaminopurine.

7. The method of claim 4 wherein the unprotected purine is guanine.

8. The method of claim 1 wherein the di-*t*-butyl silyl protecting reagent is $t\mathrm{Bu}_2\mathrm{Si(OTf)}_2$.

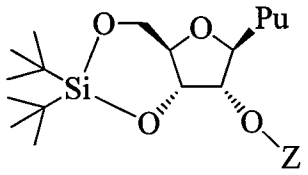
9. The method of claim 1 wherein the alkylating agent comprises a C_1-C_6 alkyl or substituted C_1-C_6 alkyl and a leaving group.

10. The method of claim 1 wherein the alkylating agent comprises a substituted C_1-C_6 alky group and a leaving group.

11. The method of claim 1 wherein the alkylating agent comprises 1-iodo-2-methoxyethane.

12. The method of claim 1 wherein Z is CH_3, CH_2CH_3, $(CH_2)_2CH_3$, $(CH_2)_2$-O-CH_3, CH_2-$CH{=}CH_2$, $(CH_2)_3$-$N(R_1)(R_2)$, $(CH_2)_2$-O-$N(R_1)(R_2)$, $(CH_2)_2$-O-$(CH_2)_2$-$N(R_1)(R_2)$, $CH_2C(=O)$-$N(R_1)(R_2)$, $CH_2C(=O)$-N(H)-$(CH_2)_2$-$N(R_1)(R_2)$ or CH_2-N(H)-$C(=NR_1)[N(R_1)(R_2)]$ wherein R_1 and R_2 are each independently, H or C_1-C_2 alkyl.

13. The method of claim 1 wherein the 2'-O-substituted purine nucleoside has the configuration of Formula IV:



IV.

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2011/027953

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07H23/00 C07H19/067
ADD.

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)
C07H

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 practical, search terms used)
EPO-Internal, BEILSTEIN Data, CHEM ABS Data, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	ZLATEV ET AL: "Convenient synthesis of N<2>-isobutyryl-2'-O-methyl guanosine by efficient alkylation of O<6>-trimethylsilylethyl-3',5'-di-tert-butyls ilanediyl guanosine", TETRAHEDRON, ELSEVIER SCIENCE PUBLISHERS, AMSTERDAM, NL, vol. 63, no. 45, 27 September 2007 (2007-09-27), pages 11174-11178, XP022274994, ISSN: 0040-4020, DOI: DOI:10.1016/J.TET.2007.08.006	1-4, 6-10,12, 13
Y	page 11175; figure 1	11
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[X] Further documents are listed in the continuation of Box C.

[X] 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

"E" earlier document but published on or after the international filing date

"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)

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Date of the actual completion of the international search	Date of mailing of the international search report
26 July 2011	02/08/2011

Name and mailing address of the ISA/	Authorized officer
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INTERNATIONAL SEARCH REPORT

International application No
PCT/US2011/027953

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	V. SEREBRYANY ET AL: "Synthesis of 2'-O-Substituted Ribonucleosides", NUCLEOSIDES, NUCLEOTIDES & NUCLEIC ACIDS, vol. 22, no. 5-8, 1 January 2003 (2003-01-01), pages 1007-1009, XP55001977, ISSN: 1525-7770, DOI: 10.1081/NCN-120022724	1-3,6,9, 10,12,13
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