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<p>(21) International Application Number: PCT/US92/01020 (22) International Filing Date: 7 February 1992 (07.02.92) (30) Priority data: 652,978 8 February 1991 (08.02.91) US (71) Applicant: GILEAD SCIENCES, INC. [US/US]; 344 Lakeside Drive, Foster City, CA 94404 (US). (72) Inventors: BUHR, Chris ; 870 Campus Drive, #303, Daly City, CA 94015 (US). MATTEUCCI, Mark ; 1524 Co- lumbus Avenue, Burlingame, CA 94010 (US). BIS- CHOFBERGER, Norbert, W. ; 1116 Cedar Street, San Carlos, CA 94070 (US). FROEHLER, Brian ; 2310 Monserat Avenue, Belmont, CA 94002 (US).</p>		<p>(74) Agents: GRACEY, Nancy, Joyce et al.; Morrison & Foer- ster, 545 Middlefield Road, Suite 200, Menlo Park, CA 94025 (US). (81) Designated States: AT (European patent), AU, BE (Euro- pean patent), CA, CH (European patent), DE (Euro- pean patent), DK (European patent), ES (European pa- tent), FI, FR (European patent), GB (European patent), GR (European patent), IT (European patent), JP, KR, LU (European patent), MC (European patent), NL (Eu- ropean patent), NO, RU, SE (European patent). Published <i>With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i></p>
<p>(54) Title: METHYLENE PHOSPHONATE NUCLEOSIDE ANALOGS AND OLIGONUCLEOTIDE ANALOGS MADE THEREFROM</p> <p>(57) Abstract</p> <p>Oligonucleotide analogs and nucleoside analogs as well as methods for their synthesis are described. The nucleoside analogs are useful as antiviral agents and as agents to treat tumors or infectious agents. The oligonucleotides are useful in diagnostic and therapeutic applications.</p>		

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METHYLENE PHOSPHONATE NUCLEOSIDE ANALOGS
AND OLIGONUCLEOTIDE ANALOGS MADE THEREFROM

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15 Field of the Invention

This invention relates to methylene phosphonate nucleosides which exhibit antiviral and antitumor activity and oligonucleotides derived from methylene phosphonate nucleoside monomers that have enhanced
20 nuclease stability. The invention also relates to processes for preparing the compounds, their derivatives and oligonucleotides containing one or more 5' methylene phosphonate internucleotide linkages. The oligonucleotides are resistant to nuclease degradation
25 and are useful for diagnostic and therapeutic applications.

Background Art

Antisense and triple helix oligonucleotides are
30 synthetic oligonucleotides which bind complementary nucleic acids (i.e. sense strand RNA or duplex DNA sequences) via hydrogen bonding, thereby inhibiting expression of these sequences. Therapeutic intervention at the nucleic acid level using oligonucleotides offers a
35 number of advantages. Inhibition of gene expression is

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more efficient than inhibition of the protein encoded by the gene since transcription of a single DNA sequence gives rise to multiple copies of mRNA which, in turn, are translated into many protein molecules.

5 Oligonucleotides have been used to inhibit gene expression in a variety of systems. There are several reviews that discuss this topic (1-4). In addition, the use of oligonucleotides in sequence-specific recognition of double stranded DNA (5,6) as well as potential
10 chemotherapeutic agents (7) has been reviewed.

 An important feature of the antisense oligomeric probes is the design of the backbone of the administered oligomer. Specifically, the backbone should contain internucleoside linkages that are stable in vitro
15 and should be structured such that the oligomer is resistant to endogenous nucleases, such as nucleases that attack the phosphodiester linkage (8). At the same time, the oligomer must also retain its ability to hybridize to the target DNA or RNA. In order to ensure these
20 properties, a number of modified oligonucleotides have been constructed which contain alternate internucleoside linkages. Several of these oligonucleotides are described in Uhlmann, E. and Peyman, A., Chemical Reviews (1990) 90:543-584. Among these are methylphosphonates
25 (wherein one of the phosphorous-linked oxygens has been replaced by methyl); phosphorothioates (8,9) (wherein sulphur replaces one of these oxygens) and various amidates (wherein NH₂ or an organic amine derivative, such as morpholidates or piperazidates, replace an
30 oxygen). These substitutions confer enhanced stability, for the most part, but suffer from the drawback that they result in a chiral phosphorous in the linkage, thus leading to the formation of 2ⁿ diastereomers where n is the number of modified diester linkages in the oligomer.

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The presence of these multiple diastereomers considerably weakens the capability of the modified oligonucleotide to hybridize to target sequences. Some of these substitutions also retain the ability to support a negative charge and the presence of charged groups decreases the ability of the compounds to penetrate cell membranes. There are numerous other disadvantages associated with these modified linkages, depending on the precise nature of the linkage. Phosphorodithioate modified backbones have been made (9,10). These modified oligonucleotides are nuclease resistant and are diastereomerically pure. However, these modifications further reduce the affinity of the oligonucleotide for its intended target (10c). A variety of modified nonionic (11) oligonucleotides including methylphosphonate, phosphoroamidate, and phosphotriesters generally are either composed of a mixture of diastereomers, have a low affinity for intended targets, or both.

A deoxyoligonucleotide comprised from nucleotide monomers that contain a methylene (-CH₂-) group substituted for the 5'-oxygen can be resistant to nucleases, especially those that leave a 3'-phosphate moiety after cleavage of the internucleotidic bond. This results from the fact that the requisite P-C bond can not be cleaved under normal physiological conditions. Additionally, a single diastereomerically pure deoxyoligonucleotide could be prepared, as the internucleotide phosphorous linkages would be achiral. We refer to the nucleotides containing a methylene (-CH₂-) group substituted for the 5'-oxygen as 5'-methylene phosphonates.

The preparation of ribo (i.e. 2'-OH) 5'-methylene phosphonates is well documented in the

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literature (12). Uridine (13-15), adenosine (13-15), and guanosine (16) 5'-methylene phosphonates have been prepared. A number of analogues of adenosine 5'-methylene phosphonate have been prepared (17-23). In addition, ribavirin 5'-methylene phosphonate (24), as well as a ribo 5'-methylene phosphonate containing thiazole-4-carboxamide as the base, has been prepared (25). Ribo compounds having a 3'-methylene phosphonate have also been prepared (26-28).

10 There are very few reports of 2'-deoxy 5'-methylene phosphonates in the literature, and these are all related to thymidine. Only the syntheses of 5'-methylene phosphonates of thymidine (29), 3'-azidothymidine (AZT) (30,31), and 15 2'-deoxy-5-fluoro-uridine (32) have been reported. There have been no reports on the syntheses of 5'-methylene phosphonates derived from 2'-deoxyadenosine, 2'-deoxycytidine, or 2'-deoxyguanosine. There also have been no reports on the synthesis of 5' methylene 20 phosphonate nucleosides having 5-iodouracil, 2-aminopurine or 2,6-diaminopurine as the base. The 5-iodouridine 5' methylene phosphonate compound would be made in an analogous manner to that used to synthesize the 5' methylene phosphonate derived from thymidine as 25 described for compounds 33 and 37 below. The 2-aminopurine and 2,6-diaminopurine nucleoside 5' methylene phosphonates would be made in an analogous manner to that used to synthesize the 5' methylene phosphonate derived from deoxyadenosine as described for 30 compounds 36 and 40 below.

Several ribo 5'-methylene phosphonate dimers have been synthesized. These include UpCH₂U and UpCH₂A (33,34). Several ribo 3'-methylene phosphonate dimers (33), as well as a trimer (28) have been synthesized. 35 These ribo dimers and trimer were prepared using the

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diester method of oligonucleotide synthesis (35,36). This method suffers from low product yields, and difficulties in purification of the final product (35,36). The method is generally not useful in the preparation of longer oligonucleotides. Recently, a ribo oligonucleotide 10-mer consisting of 5'-methylene phosphonates, ApA(pCH₂A)₈, was prepared enzymatically using polynucleotide phosphorylase (37). This technique, however, cannot be used for the preparation of oligonucleotides having a defined sequence of mixed bases.

Only one 2'-deoxy dimer, TpCH₂T, and one 2'-deoxy trimer, TpCH₂TpCH₂T, have been reported in the literature (29). Only the 5'-methylene phosphonate derived from thymidine was used in the dimer and trimer. No mixed base 2'-deoxy 5'-methylene phosphonate dimers or longer mixed base, 2'-deoxy 5'-methylene phosphonate oligonucleotides have been reported. Additionally, no 2'-deoxy 5'-methylene phosphonate oligonucleotides longer than a 3-mer of any kind have been reported. However, recently the synthesis of oligodeoxynucleotides containing 5'-methylene phosphonates of 2'-deoxy-4'-carbocyclic nucleosides has been reported W. Frick and S.W. Schneller, Meetings Abstract, Conference on Nucleic Acid Therapeutics, January 13-17, 1991, Clearwater Beach, Florida, p 63).

Disclosure of the Invention

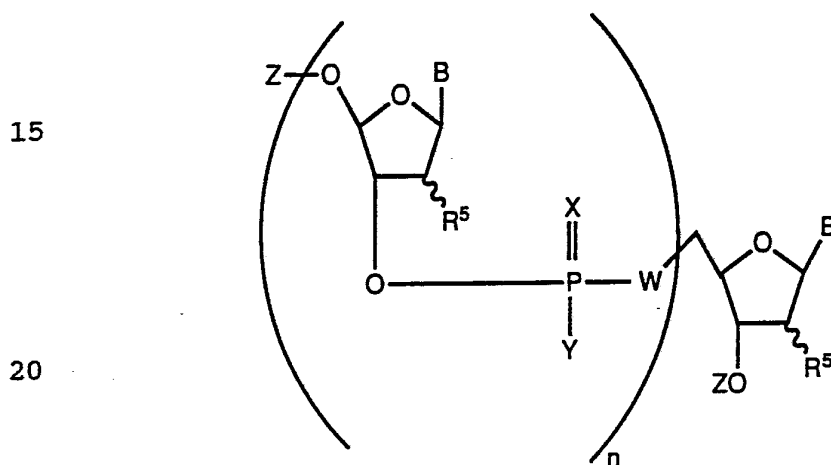
The present invention is directed to (i) monomeric 5'-methylene phosphonate nucleoside analogs, their tautomers, isomers and salts and (ii) modified oligonucleotides including dimers, trimers and tetramers wherein the modification comprises replacing at least one phosphodiester linkage, -OP(O)O⁻O-, of the

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oligonucleotide with a 5'-methylene phosphonate type linkage of the formula $-OPXYCH_2-$, wherein X is O or S and Y is OR^4 , $N(R^4)_2$ or SR^4 , wherein each R^4 is independently H, methyl, ethyl, propyl, isopropyl, butyl, phenyl, alkyl (4-18C) or substituted alkyl (1-18C). Other preferred alkyl and substituted alkyl groups are hexyl, nonyl, oleyl and methoxyethyl. Methods for synthesis of the modified oligonucleotides using the monomers are also disclosed.

The modified oligonucleotides may be represented as shown in general structural formula I:



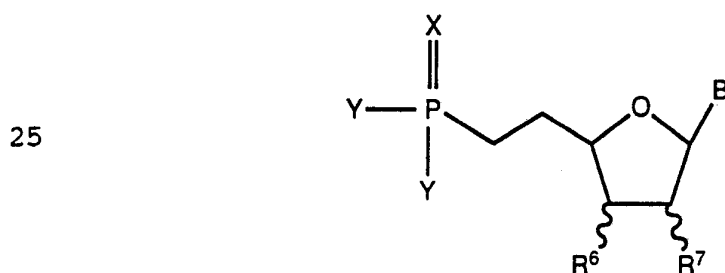
I

and include stereoisomers and salts thereof, wherein X and Y are as defined above, W is independently O or CH_2 , each B is independently a purine or pyrimidine base or modified form each Z is independently a noninterfering substituent, preferably hydrogen, PO_3^- or a protecting group; each R^5 is independently hydrogen, hydroxyl, O-allyl, S-allyl, O-methyl, S-methyl or fluorine; and n is an integer between 1 and 30 with the proviso that (i) at least one W is CH_2 , (ii) when B is thymine and n is 1 or

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2 then not all R^5 are H, (iii) when B is adenine and n is
 an integer from 1 to 9 then not all R^5 are OH, (iv) when
 B is uracil and n is 1 then not all R^5 are OH and (v)
 when n is 1 and the 5' B is uracil and the 3' B is
 5 adenine then not all R^5 are OH. Modified forms of bases
 as defined herein are base analogs such as 5-
 methylcytosine or derivatives of bases such as N^2 -
 isobutyrylguanine or N^4 -benzoylcytosine that contain
 10 standard blocking groups or other derivatives. Bases such
 as adenine, guanine, cytosine, thymine and uracil as well
 as modified forms (base analogs) such as
 5-methylcytosine, aziridinylcytosine,
 8-hydroxy- N^6 -methyladenine, pseudoisocytosine, 5-
 15 propynyluracil and 5-propynylcytosine are preferred.
 Preferred protecting groups are H-phosphonate,
 methylphosphonate, MMT, DMT, methylphosphoramidite and β -
 cyanoethylphosphoramidite.

The oligonucleotides can be synthesized from
 20 appropriate derivatives of monomers of formula (II):



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II

and their salts, zwitterions, solvates and tautomers,
 which can be present as diastereoisomers, wherein B, X
 and Y have the meanings defined above; R^6 is hydrogen,
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hydroxyl, fluorine, O-benzyl, O-t-butyldimethylsilyl, O-DMT and O-MMT; and R⁷ is hydrogen, O-allyl, S-allyl, O-methyl, S-methyl or fluorine or both R⁶ and R⁷, when taken together with the carbon atom to which each is attached, form a 2', 3' epoxide group or a carbon-carbon double bond which gives a 2' 3' dideoxydideohydro sugar analog. Also included are monomers and their isomers of compounds containing a double bond at the C5' methylene carbon as shown in Tables 2 and 5 below. Any hydroxyl group can be coupled to a standard protecting group.

Preferred bases include guanine, adenine, cytosine, thymine, uracil, inosine, xanthine, hypoxanthine, 5-iodouracil, 5-iodocytosine, 5-ethyluracil, 5-propynyluracil, 5-fluorouracil, 5-trifluoromethyluracil, 5-bromovinyluracil, 5-propynylcytosine, 5-methylcytosine, 8-hydroxy-N⁶-methyladenine, aziridinylcytosine, 2-aminopurine, 2,6-diaminopurine, 6-chloropurine, 3-deazaguanine, 7-deazaadenine, 8-bromoadenine, 7-deazaguanine, 3-deazaadenine, and 6-hydroxylaminopurine, 6-thiopurine or other base analogs or altered forms can be utilized. Unless specifically indicated, bases are linked to the sugar moiety at conventional positions such as N9 of adenine, guanine and other purines or the N1 of cytosine, thymine, uracil and other pyrimidines.

Stereoisomers of the monomers include α -anomers of the base residue. Dideoxy and dideoxyarafluoro monomers can be prepared essentially by a previously disclosed method using appropriate compounds disclosed herein (56).

2'-deoxy-5'-methylene phosphonate oligonucleotides of length 2-30 bases or more of mixed base composition can be synthesized according to the methods disclosed herein. These oligodeoxynucleotides are prepared using the phosphotriester method (38) from

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suitably protected 2'-deoxy 5'-methylene phosphonate nucleotide monomers. We prepared 5'-methylene phosphonates in both a protected form that was suitable for oligonucleotide synthesis, as well as in a completely deprotected form. Some of the 5'-methylene phosphonates that were prepared were derived from 2'-deoxycytidine and 2'-deoxyguanosine. The monomers described herein are suitable for solid phase oligonucleotide synthesis by triester chemistry. For example, compounds 87-90 in Table 6 which are derived from corresponding precursors 60, 62, 64 and 66 in Table 5 may be utilized in solid phase synthesis using described methods. Previous methods utilized diester chemistry which is more difficult and generates low yields of product.

Oligonucleotides containing 2'-deoxy-2'-fluoro-ribonucleotides are of interest because the conformation of the sugar closely resembles that of RNA and consequently these oligonucleotides have a higher affinity to DNA than normal oligodeoxyribonucleotides (M. Ikehara, Heterocycles 1984, 21, 75). The oligonucleotide analogs may be used as conventional probes or primers for various diagnostic assays.

Synthesis of oligonucleotides containing a covalent crosslinking aziridinylcytosine residue is disclosed in commonly assigned co-pending U.S. application serial no. 640,654, synthesis of oligonucleotides containing a region of inverted polarity is disclosed in commonly assigned co-pending PCT application serial no. PCT/US90/06128, synthesis of oligonucleotides containing improved base analogs, such as 8-hydroxy-N⁶-methyladenine and pseudoisocytosine, for triple helix formation are disclosed in commonly assigned co-pending U.S. application serial no. 643,382 and synthesis of oligonucleotides containing noninterfering

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substituents at either the 5' or 3' ends of oligonucleotides is disclosed in commonly assigned co-pending U.S. application serial no. 482,943. All documents cited herein are incorporated herein by reference. 5' methylene phosphonate nucleoside having N⁴-etheno-5-methylcytosine (aziridinylcytosine) as the base can be prepared from the thymidine 5' methylene phosphonate (compound 37 below) by transient silyl protection, formation of the appropriate triazolide, removal of silyl protecting groups and then reaction with aziridine in DMF using the procedures described in pending PCT application serial no. PCT/US90/03680.

The free 5'-methylene phosphonate nucleosides present enzymatically nonhydrolysable isosteres of mononucleotides. As such they can be converted intracellularly by cellular kinases to the corresponding nucleoside phosphono triphosphates, incorporated into DNA by polymerases and thus interfere with cellular metabolism. The inhibitory effects of nucleoside and nucleotide analogs often exert their effects through interaction with DNA or RNA polymerase enzymes. Nucleoside phosphonates can exhibit activity against viruses, tumors, parasites such as malaria parasites, trypanosomes and yeasts or other infectious agents. For example, several acyclic methylene phosphonates such as the methylene phosphonates derived from ganciclovir, and acyclovir are potent antivirals (39-42). The analogs disclosed herein are isosteric with the methylene phosphonates disclosed herein. A similar class of methylene phosphonate compounds has been shown to have both antiviral activity and activity against the DNA polymerase enzyme of the malaria parasite Plasmodium falciparum (54). Other nucleoside phosphonates have been described in PCT publication no. WO 84/04748. The compounds of the present invention can thus be used to

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treat cancers or tumors, especially those caused by viruses in addition to their use as agents to treat various pathogenic agents. Nucleoside analogs have also been used to treat tumors or malignant cells (55).

5 Physiologically acceptable salts, zwitterions and solvates of the compounds of this invention are prepared by methods known in the art. The salts include amine or ammonium salts and salts of physiologically acceptable metals, particularly Li^+ , K^+ , Na^+ , Ca^{++} and ,
10 Mg^{++} , and are novel compounds and comprise a further aspect of the invention. Metal salts can be prepared by reacting a metal hydroxide with a compound of the invention. Examples of metal salts which can be prepared in this way are salts containing Li^+ , Na^+ , and K^+ . A
15 less soluble metal salt can be precipitated from a solution of a more soluble salt by addition of a suitable metal compound. Acid salts can be prepared by reacting a compound of the invention with an acid such as HCl , HBr , H_2SO_4 , or an organic sulphonic acid.

20 Nucleoside monomer compounds possess antiviral activity and can be used in the control or prevention of viral infections, e.g. of herpes simplex viral or HCMV infections. The in vitro activity of the compounds of formula I and their tautomers in inhibiting herpes
25 simplex virus type 2 (HSV-2) can be demonstrated by means of plaque reduction or cytopathic effects assays. Host VERO or human fibroblast cells are infected with virus stock containing a known number of infectious virions in the presence of various concentrations of compound.
30 Plaques in the cell monolayer are then counted and compared to untreated controls and to acyclovir treated controls. The degree of cytopathic effects inhibition or titer reduction at each concentration of compound is expressed as a percentage of the control titer (100%).
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The EC50 value, namely the concentration of compound which inhibits viral activity or titer by 50%, is then calculated. The results that are obtained with representative compounds show that virus titer reductions occur.

Compound numbers 38 and 39 as described in Table 3 were tested for antiviral activity against the human herpesviruses HSV-1 (strain 377), HSV-2 (strain MS) and HCMV (strain AD169) in vitro. Efficacy was assayed by measuring reduction of cytopathic effects in primary human foreskin fibroblasts. Compound 38 was administered to cells in tissue culture as the monosodium, hydrogentriethylammonium salt and compound 39 was used as the disodium salt. Both compounds were administered to cells immediately prior to infection of cells on microtiter wells. CPE reduction was determined at 72 h post infection for HSV or at 14 d post infection for HCMV. Compound 38 was found to have an EC₅₀ of 28.3 µg/mL for HCMV, >100 µg/mL for HSV-1 and HSV-2 and compound 39 was found to have an EC₅₀ of >100 µg/mL for HCMV, HSV-1 and HSV-2.

The compounds disclosed herein can be used as medicaments in the form of pharmaceutical preparations which contain them in association with a compatible pharmaceutical carrier material. This can be an organic or inorganic carrier suitable for enteral, e.g. oral, or parenteral administration. Examples of such carriers are water, gelatin, gum arabic, lactose, starch, magnesium stearate, talc, vegetable oils, polyalkylene glycols and petroleum jelly. The pharmaceutical preparations can be made up in a solid form, e.g. as tablets, dragees, suppositories or capsules, or in a liquid form, e.g. as solutions, suspensions or emulsions; they can be subjected to standard pharmaceutical operations, e.g.

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sterilization and/or may contain adjuvants, e.g. preserving, stabilizing, wetting or emulsifying agents, salts for varying the osmotic pressure or buffers. The compounds may also be formulated in a manner suitable for
5 administration as an aerosol. They may also contain other therapeutically valuable substances.

The compounds disclosed herein and their tautomers can be administered for the control or prevention of viral infection, such as herpes simplex
10 viral infections, to warmblooded animals in need of such treatment. The disclosed compounds and their tautomers can be administered to adult humans in a daily dosage of from about 1 to 1000 mg, preferably about 5 to 500 mg. The daily dosage can be administered as a single dose or
15 in divided doses. The above dosage range is given by way of example only and can be varied upwards or downwards depending on factors such as the particular compound being administered, the route of administration, the severity of the indication being treated and the
20 condition of the patient.

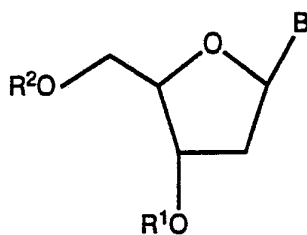
Experimental Section

General. Flash chromatography refers to the procedure of
25 Still et. al (43). Drying refers to drying over Na_2SO_4 filtration, and concentration. All reactions requiring dry solvents were run under a dry argon atmosphere. The following six tables show structures for compounds 1
30 through 120.

35

Table 1

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10

Compound	B	R ¹	R ₂
1	G ^{Ib}	H	H
15	3	C ^{Bz}	H
5	A ^{Bz}	H	H
2	G ^{Ib}	H	DMT
4	C ^{Bz}	H	DMT
6	A ^{Bz}	H	DMT
20	7	T	DMT
8	G ^{Ib}	TBS	H
9	C ^{Bz}	TBS	H
10	A ^{Bz}	TBS	H
25	11	T	H
12	T ^{Bn}	Bn	H

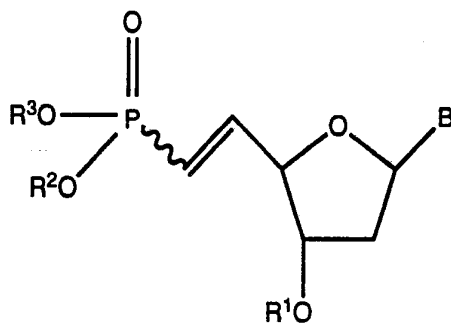
For tables 1-6; G = guanine; C = cytosine; A = adenine;
 T = thymine; G^{Ib} = N²-isobutyrylguanine; C^{Bz} =
 30 N⁴-benzoylcytosine; A^{Bz} = N⁶-benzoyladenine; T^{Bn} =
 N³-benzylthymine; Bn = benzyl; DMT =
 4,4'-dimethoxytrityl; TBS = t-butyldimethylsilyl;
 +HTEA = hydrogentriethylammonium; A^{DMT} = N⁶-
 dimethoxytrityladenine, U = uracil and Me = methyl.

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Table 2

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Compound	B	R ¹
13	G ^{Ib}	TBS
15	C ^{Bz}	TBS
17	A ^{Bz}	TBS
19	T	TBS
21	T ^{Bn}	Bn

For definition of abbreviations, see Table 1.

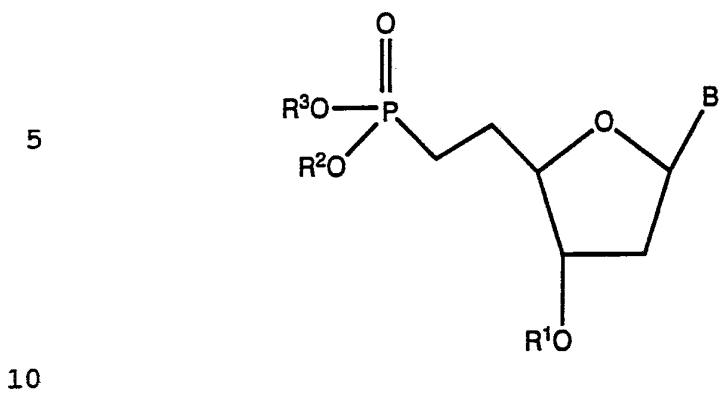
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Table 3



Compound	B	R ¹	R ²	R ³
14	G ^{Ib}	TBS	Ph	Ph
16	C ^{Bz}	TBS	Ph	Ph
15 18	A ^{Bz}	TBS	Ph	Ph
20	T	TBS	Ph	Ph
22	T ^{Bn}	Bn	Ph	Ph
23	T ^{Bn}	Bn	Me	Me
20 24	T ^{Bn}	H	Me	Me
25	T ^{Bn}	Bn	Bn	Bn
26	G ^{Ib}	H	Ph	Ph
27	C ^{Bz}	H	Ph	Ph
28	A ^{Bz}	H	Ph	Ph
25 29	T	H	Ph	Ph
30	G ^{Ib}	H	Me	Me
31	C ^{Bz}	H	Me	Me
32	A ^{Bz}	H	Me	Me
33	T	H	Me	Me
30 34	G ^{Ib}	H	H	H
35	C ^{Bz}	H	H	H
36	A ^{Bz}	H	H	H
37	T	H	H	H
38	G	H	H	H

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	39	C	H	H	H
	40	A	H	H	H
	41	G ^{Ib}	DMT	Ph	Ph
	42	C ^{Bz}	DMT	Ph	Ph
5	43	A ^{Bz}	DMT	Ph	Ph
	44	T	DMT	Ph	Ph
	45	G ^{Ib}	DMT	Ph	+HTEA
	46	C ^{Bz}	DMT	Ph	+HTEA
	47	A ^{Bz}	DMT	Ph	+HTEA
10	48	T	DMT	Ph	+HTEA
	91	G ^{IB}	TBS	Me	Me
	92	G	H	Me	Na ⁺
	93	G	H	Na ⁺	Na ⁺
15	94	C ^{Bt}	TBS	Me	Me
	95	C	H	Me	Na ⁺
	96	C	H	Na ⁺	Na ⁺
	119	G	H	Na ⁺	+HTEA

For definition of abbreviations, see Table 1.

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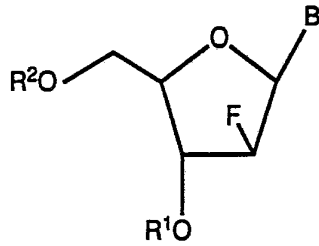
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Table 4

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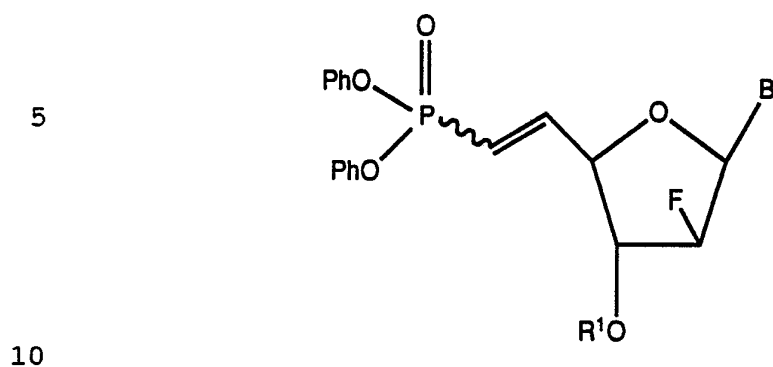


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Compound	B	R ¹	R ²
49	G ^{Ib}	H	H
51	C ^{Bz}	H	H
53	A ^{Bz}	H	H
50	G ^{Ib}	H	DMT
52	C ^{Bz}	H	DMT
54	A ^{Bz}	H	DMT
55	T	H	DMT
56	G ^{Ib}	TBS	H
57	C ^{Bz}	TBS	H
58	A ^{Bz}	TBS	H
59	T	TBS	H
100	U	H	DMT
101	U	TBS	H
110	A ^{DMT}	H	DMT
111	A	TBS	H

For definition of abbreviations, see Table 1.

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Table 5

Compound	B	R ¹
60	G ^{Ib}	TBS
15 62	C ^{Bz}	TBS
64	A ^{Bz}	TBS
66	T	TBS
102	U	TBS
112	A	TBS

20 For definition of abbreviations, see Table 1.

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Table 6

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Compound	B	R ¹	R ²	R ³
61	G ^{Ib}	TBS	Ph	Ph
63	C ^{Bz}	TBS	Ph	Ph
15 65	A ^{Bz}	TBS	Ph	Ph
67	T	TBS	Ph	Ph
68	G ^{Ib}	H	Ph	Ph
69	C ^{Bz}	H	Ph	Ph
70	A ^{Bz}	H	Ph	Ph
20 71	T	H	Ph	Ph
72	G ^{Ib}	H	Me	Me
73	C ^{Bz}	H	Me	Me
74	ABz	H	Me	Me
25 75	T	H	Me	Me
76	G ^{Ib}	H	H	H
77	C ^{Bz}	H	H	H
78	A ^{Bz}	H	H	H
79	T	H	H	H
30 80	G	H	H	H
81	C	H	H	H
82	A	H	H	H
83	G ^{Ib}	DMT	Ph	Ph
84	C ^{Bz}	DMT	Ph	Ph
35 85	A ^{Bz}	DMT	Ph	Ph

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	86	T	DMT	Ph	Ph
	87	G ^{Ib}	DMT	Ph	+HTEA
	88	C ^{Bz}	DMT	Ph	+HTEA
	89	A ^{Bz}	DMT	Ph	+HTEA
5	90	T	DMT	Ph	+HTEA
	97	T	TBS	Me	Me
	98	T	H	Me	Na ⁺
	99	T	H	Na ⁺	Na ⁺
10	103	U	TBS	Ph	Ph
	104	U	TBS	Me	Me
	105	U	H	Me	Na ⁺
	106	U	H	Na ⁺	Na ⁺
	107	C	TBS	Ph	Ph
15	108	C	H	Ph	Na ⁺
	109	C	H	Na ⁺	Na ⁺
	113	A	TBS	Ph	Ph
	114	A	H	Ph	Na ⁺
	115	A	H	Na ⁺	Na ⁺
20	116	G ^{Ib}	TBS	Me	Me
	117	G	H	Me	Na ⁺
	118	G	H	Na ⁺	Na ⁺
	120	C	H	Ph	HTEA ⁺

For definition of abbreviations, see Table 1.

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N²-Isobutyryl-2'-deoxyguanosine (1). The acylation by transient protection method of R. A. Jones (44) was used. To a stirred mixture of 4.28 g (15.0 mmol) of 2'-deoxyguanosine monohydrate (that was first concentrated from dry pyridine) in 150 mL of dry pyridine that was cooled on an ice water bath was added 9.75 mL (76.8 mmol, 5.12 equiv) of chlorotrimethylsilane dropwise, over several minutes. After 30 min., 12.8 mL (76.9 mmol, 5.13 equiv.) of isobutyric anhydride was added dropwise, over several minutes. The ice bath was

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removed and stirring was continued for 2 h. The reaction mixture was then cooled on an ice water bath, and 30 mL of cold H₂O was added to the reaction. After 15 min., 30 mL of concentrated aqueous ammonia was added. The reaction was stirred for 30 min., and then concentrated. The residue was taken up in 100 mL of H₂O and extracted with Et₂O. The title compound was either crystallized from the aqueous layer, or was isolated by flash column chromatography.

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N²-Isobutyryl-5'-O-(4,4'-dimethoxytrityl)-2'-deoxyguanosine (2). The tritylation procedure of Jones (45) was modified such that no DMAP was used. To 3.37 g (10.0 mmol) of N²-isobutyryl-2'-deoxyguanosine (that was first concentrated from dry pyridine) in 50 mL of dry pyridine, was added 4.06 g (12.0 mmol, 1.20 equiv.) of 4,4'-dimethoxytrityl chloride. The reaction was stirred for 15 h, and then concentrated. The residue was partitioned between CH₂Cl₂ and 0.5% aq. NaHCO₃, shaken, and separated. The organic layer was washed with 0.5% aq. NaHCO₃ and dried. The crude product was purified by flash chromatography.

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N⁴-Benzoyl-2'-deoxycytidine (3). This compound was prepared from 2'-deoxycytidine monohydrate by the same procedure used for the preparation of N²-isobutyryl-2'-deoxyguanosine except that 9.0 mL (77.5 mmol, 5.17 equiv.) of benzoyl chloride was used instead of isobutyric anhydride.

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N⁴-Benzoyl-5'-O-(4,4'-dimethoxytrityl)-2'-deoxycytidine (4). This compound was prepared from N⁴-benzoyl-2'-deoxycytidine by the same procedure used for the

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preparation of N²-isobutyryl-5'-O-(4,4'-dimethoxytrityl)-2'-deoxyguanosine.

5 N⁶-Benzoyl-2'-deoxyadenosine (5). This compound was prepared from 2'-deoxyadenosine monohydrate by the same procedure used for the preparation of N⁴-benzoyl-2'-deoxycytidine.

10 N⁶-Benzoyl-5'-O-(4,4'-dimethoxytrityl)-2'-deoxyadenosine (6). This compound was prepared from N⁶-benzoyl-2'-deoxyadenosine by the same procedure used for the preparation of N²-isobutyryl-5'-O-(4,4'-dimethoxytrityl)-2'-deoxyguanosine.

15 5'-O-(4,4'-Dimethoxytrityl)-thymidine (7). This compound was prepared from thymidine by the same procedure used for the preparation of N²-isobutyryl-5'-O-(4,4'-dimethoxytrityl)-2'-deoxyguanosine.

20 3'-O'-t-Butyldimethylsilyl-N²-isobutyryl-2'-deoxyguanosine (8). To a stirred solution of 2.00 g (3.13 mmol) of N²-isobutyryl-5'-O-(4,4'-dimethoxytrityl)-2'-deoxyguanosine and 1.54 g (22.6 mmol, 7.22 equiv.) of imidazole in 12.5 mL of dry DMF was added
25 1.16 g (7.70 mmol, 2.46 equiv.) of t-butyldimethylsilyl chloride. The reaction was stirred at room temperature for 3.5 h and then concentrated. The residue was partitioned between CH₂Cl₂ and H₂O, shaken, and
30 separated. The organics were washed with H₂O and concentrated (not dried). The crude residue was then stirred in 100 mL of 80% aq. HOAc for 1.5 h and then concentrated. The residue was partitioned between CH₂Cl₂ and H₂O, shaken, and separated. The organics were washed
35 with sat. aq. NaHCO₃, H₂O, and dried. The crude product

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was purified by flash chromatography on a 40 mm column using one column volume of 2% TEA in CH₂Cl₂, then one column volume of 2% TEA and 2% MeOH in CH₂Cl₂, and then 2% TEA and 4% MeOH in CH₂Cl₂. The product was
5 concentrated from toluene affording 1.18 g (83.7% yield).

3'-O-t-Butyldimethylsilyl-N⁴-benzoyl-2'-deoxycytidine (9). This compound was prepared from 29.09g (45.90 mmol) of
10 N⁴-benzoyl-5'-O-(4,4'-dimethoxytrityl)-2'-deoxycytidine by the same procedure used for the preparation of 3'-O-t-butyl-
dimethylsilyl-N²-isobutyryl-2'-deoxyguanosine. Column chromatography of the crude material afforded
15 15.29 g (74.8% yield) of product.

3'-O-t-Butyldimethylsilyl-N⁶-benzoyl-2'-deoxyadenosine (10). This compound was prepared from N⁶-benzoyl-
5'-O-(4,4'-dimethoxytrityl)-2'-deoxyadenosine by the same
20 procedure used for the preparation of 3'-O-t-butyl-
dimethylsilyl-N²-isobutyryl-2'-deoxyguanosine.

3'-O-t-Butyldimethylsilylthymidine (11). This compound was prepared from 5'-O-(4,4'-dimethoxytrityl)-thymidine
25 by the same procedure used for the preparation of 3'-O-t-butyl-
dimethylsilyl-N²-isobutyryl-2'-deoxyguanosine.

3'-O,N³-Dibenzylthymidine (12). To a stirred solution of 2.18 g of 5'-O-(4,4'-dimethoxytrityl)-thymidine (4.00
30 mmol) in 52 mL of dry DMF was carefully added 2.00 g of a 60% oil dispersion of NaH. The reaction was stirred at room temperature for 5 min. To the mixture was added 4.77 mL (40.1 mmol, 10.0 equiv.) of benzyl bromide dropwise, over several minutes. After 1 h, the reaction
35 was cooled on an ice-water bath. Then, 12 mL of sat. aq.

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NaHCO₃ was carefully added (vigorous hydrogen gas evolution) dropwise, over several minutes. The mixture was stirred for 10 min, and then concentrated. The residue was then stirred in 100 mL of 80% aq. HOAc at room temperature for 1.5 h, and then concentrated. The crude residue was partitioned between CH₂Cl₂ and H₂O, shaken, and separated. The organic layer was washed with sat. aq. NaHCO₃, H₂O, and then dried. The crude product was purified by flash chromatography on a 50 mm column using two column volumes of CH₂Cl₂, two column volumes of 1% MeOH in CH₂Cl₂, and then 2.5% MeOH in CH₂Cl₂ as eluents. This afforded 1.49 g of product (88.2% yield) as a colorless solid.

Diphenyl [9-(3-O-t-Butyldimethylsilyl-2,5,6-trideoxy-β-D-ribo-hex-5-enofuranosyl)-N²-isobutyrylguanaine]-6'-phosphonate (13). Literature methods (39) were adapted for the preparation of the title compound. To a solution of 106 mg of 3'-O-t-butyldimethylsilyl-N²-isobutyryl-2'-deoxyguanosine (0.236 mmol) and 294 mg of dicyclohexylcarbodiimide DCC (1.42 mmol, 6.02 equiv.) in 1.3 mL of dry DMSO was added 11.3 mg of methylphosphonic acid (0.118 mmol, 0.50 equiv.). The reaction was stirred at room temperature. After 18 h, dry pyridine (0.080 mL) and then 120 mg (0.236 mmol, 1.00 equiv.) of diphenyl [(triphenylphosphoranylidene)methyl]phosphonate (46) were added. Another 0.80 mL of dry DMSO was added. The reaction was stirred at room temperature. After 27 h, the reaction was diluted with CH₂Cl₂, washed with 2 x H₂O, and dried. The crude material was flashed on a 25 mm column using one column volume of CH₂Cl₂, then one column volume of 3% MeOH in CH₂Cl₂, and then 6% MeOH in CH₂Cl₂ as eluents. The product containing fractions were combined and concentrated. The product was purified

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again purified by flash chromatography on a 25 mm column using one column volume of 12.5% EtOAc in CH₂Cl₂, then one column volume of 25% EtOAc in CH₂Cl₂, and then 50% EtOAc in CH₂Cl₂ as eluents. This procedure afforded 9.4 mg (6.0% yield) of product.

Diphenyl [9-3-O-t-Butyldimethylsilyl-2,5,6-trideoxy-β-D-ribohexofuranosyl)-N²-isobutyrylguanidine]-6'-phosphonate (14). To a solution of 9.4 mg (0.0138 mmol) of diphenyl [9-(3-O-t-butyldimethylsilyl-2,5,6-trideoxy-β-D-ribo-hex-5-enofuranosyl)-N²-isobutyrylguanidine]-6'-phosphonate in 20 mL of MeOH was added a catalytic amount of 10% Pd on carbon. The mixture was hydrogenated at 260 psi of H₂ (in a Parr reaction vessel) for 3 h. The mixture was filtered through Celite and concentrated. The crude product was purified by flash chromatography on a 15 mm column using one column volume of CH₂Cl₂, then one column volume of 12.5% EtOAc in CH₂Cl₂, then one column volume of 25% EtOAc in CH₂Cl₂, and then 50% EtOAc in CH₂Cl₂ as eluents. This procedure afforded 2.0 mg (21.3% yield) of product.

Diphenyl [1-(3-O-t-Butyldimethylsilyl-2,5,6-trideoxy-β-D-ribo-hex-5-enofuranosyl)-N⁴-benzoylcytosine]-6'-phosphonate (15). This compound was prepared from 9.87 g (22.15 mmol) of 3'-O-t-butyldimethylsilyl-N⁴-benzoyl-2'-deoxycytidine by the same procedure used for the preparation of diphenyl [9-(3-O-t-butyldimethylsilyl-2,5,6-trideoxy-β-D-ribo-hex-5-enofuranosyl)-N²-isobutyrylguanosine]-6'-phosphonate. Chromatography of the residue on silica gel afforded 9.42 g (63.2% yield) of product.

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Diphenyl [1-(3-O-t-Butyldimethylsilyl-2,5,6-trideoxy-β-D-ribohexofuranosyl)-N⁴-benzoylcytosine]-6'-phosphonate (16). This compound was prepared from 9.00 g (13.36 mmol) of diphenyl

5 [1-(3-O-t-butyldimethylsilyl-2,5,6-trideoxy-β-D-ribo-hex-5-enofuranosyl)-N⁴-benzoylcytosine]-6'-phosphonate by the same procedure used for the preparation of diphenyl [9-(3-O-t-butyldimethylsilyl-2,5,6-trideoxy-β-D-ribohexo
10 furanosyl)-N²-isobutyrylguanosine]-6'-phosphonate. Chromatography of the residue on silica gel afforded 4.08 g (55.4% yield) of product.

Diphenyl [9-(3-O-t-Butyldimethylsilyl-2,5,6-trideoxy-β-D-ribo-hex-5-enofuranosyl)-N⁶-benzoyladenine]-6'-
15 phosphonate (17). This compound is prepared from 3'-O-t-butyldimethylsilyl-N⁶-benzoyl-2'-deoxyadenosine by the same procedure used for the preparation of diphenyl [9-(3-O-t-butyldimethylsilyl-2,5,6-trideoxy-β-D-ribo-hex-
20 5-enofuranosyl)-N²-isobutyrylguanosine]-6'-phosphonate.

Diphenyl [9-(3-O-t-Butyldimethylsilyl-2,5,6-trideoxy-β-D-ribohexofuranosyl)-N⁶-benzoyladenine]-6'-phosphonate (18). This compound is prepared from diphenyl [9-(3-
25 O-t-butyldimethylsilyl-2,5,6-trideoxy-β-D-ribo-hex-5-enofuranosyl)-N⁶-benzoyladenine]-6'-phosphonate by the same procedure used for the preparation of diphenyl [9-(3-O-t-butyldimethylsilyl-2,5,6-trideoxy-β-D-ribo-hexofuranosyl)-N²-isobutyrylguanosine]-6'-phosphonate.

30 Diphenyl [1-(3-O-t-Butyldimethylsilyl-2,5,6-trideoxy-β-D-ribo-hex-5-enofuranosyl)-thymine]-6'-phosphonate (19). This compound is prepared from 3'-O-t-butyldimethylsilyl-thymidine by the same procedure used for the preparation

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of diphenyl [9-(3-O-t-butyldimethylsilyl-2,5,6-trideoxy- β -D-ribo-hex-5-enofuranosyl)-N²-isobutyrylguanosine]-6'-phosphonate.

5 Diphenyl [1-(3-O-t-Butyldimethylsilyl-2,5,6-trideoxy- β -D-ribohexofuranosyl)-thymine]-6'-phosphonate (20). This compound is prepared from diphenyl [1-(3-O-t-butyldimethylsilyl-2,5,6-trideoxy- β -D-ribo-hex-5-enofuranosyl)-thymine]-6'-phosphonate by the same
10 procedure used for the preparation of diphenyl [9-(3-O-t-butyldimethylsilyl-2,5,6-trideoxy- β -D-ribohexofuranosyl)-N²-isobutyrylguanosine]-6'-phosphonate.

15 Diphenyl [1-(3-O-benzyl-2,5,6-trideoxy- β -D-ribo-hex-5-enofuranosyl)-N³-benzylthymine]-6'-phosphonate (21). The title compound was prepared by modification of related known procedures (13,19). To a stirred solution of 300 mg of 3'-O,N³-dibenzylthymidine (0.710 mmol) and
20 874 mg of (4.24 mmol, 5.97 equiv.) of dicyclohexylcarbodiimide (DCC), in 2.37 mL of DMSO was added 0.356 mL of a 1.0 M solution (0.356 mmol, 0.50 equiv.) of orthophosphoric acid (Aldrich) in DMSO. The reaction was stirred at room temperature. After 19 h,
25 0.237 mL of dry pyridine was added, followed by 412 mg (0.710 mmol, 1.0 equiv.) of diphenyl [(triphenylphosphoranylidene)methyl]phosphonate. The reaction was stirred for 31 h. The reaction mixture was partitioned between CH₂Cl₂ and H₂O, shaken and separated.
30 The organic layer was washed with H₂O and dried. The residue was purified by flash chromatography on a 25 mm column using one column volume of CH₂Cl₂, one column volume of 5% EtOAc in CH₂Cl₂, and then 10% EtOAc in
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CH₂Cl₂ as eluents. This afforded 334 mg (80.5% yield) of product.

5 Diphenyl [1-(3-O-benzyl-2,5,6-trideoxy-β-D-ribohexofuranosyl)-N³-benzylthymine]-6'-phosphonate (22).
To a stirred solution of 334 mg (0.513 mmol) of diphenyl [1-(3-O-benzyl-2,5,6-trideoxy-β-D-ribohexofuranosyl)-N³-benzylthymine]-6'-phosphonate in 7.7 mL
10 of dry Et₂O was added 307 mg (1.03 mmol, 2.01 equiv.) of 2,4,6-tri-isopropylbenzenesulphonyl hydrazide (47), followed by 0.143 mL of dry TEA. The reaction was refluxed for 14 h. The mixture was partitioned between Et₂O and sat. aq. NaHCO₃, shaken, and separated. The
15 organic layer was washed with H₂O and dried. The residue was purified by flash chromatography on a 25 mm column using one column volume of CH₂Cl₂, one column volume of 5% EtOAc in CH₂Cl₂, and then 10% EtOAc in CH₂Cl₂ as eluents. This afforded 244 mg (72.8% yield) of product.

20 Dimethyl[1-(3-O-benzyl-2,5,6-trideoxy-β-D-ribohexofuranosyl)-N³-benzylthymine]-6'-phosphonate (23).
Commercially available CsF (100 mg) was flame dried while under vacuum, and allowed to cool to room temperature.
25 To the dried solid was added 3.00 mL of dry MeOH, followed by 143 mg (0.219 mmol) of diphenyl [1-(3-O-benzyl-2,5,6-trideoxy-β-D-ribohexofuranosyl)-N³-benzylthymine]-6'-phosphonate. The reaction was stirred for 20 h, and then concentrated. The residue was
30 partitioned between CH₂Cl₂ and H₂O, shaken, and separated. The organics were washed with H₂O and dried. The residue was purified on a 25 mm column using one column volume of CH₂Cl₂, one column volume of 2.5% MeOH in CH₂Cl₂, and then 5% MeOH in CH₂Cl₂ as eluents. This
35 procedure afforded 85.9 mg (74.0% yield) of product.

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Dimethyl [1-(2,5,6-trideoxy- β -D-ribohexofuranosyl)-
N³-benzylthymine]-6'-phosphonate (24). Known literature
methods (48) were adapted to remove the benzyl protecting
group from the 3'-oxygen. Dimethyl [1-(3-O-benzyl-
5 2,5,6-trideoxy- β -D-ribohexofuranosyl)-N³-benzylthymine]-
6'-phosphonate (3.0 mg, 0.00567 mmol) was added to a 4.4%
solution of HCO₂H in MeOH (prepared from 96% HCO₂H)
followed by a catalytic amount of 10% Pd on carbon. The
reaction was stirred at room temperature for 19 h. The
10 reaction was then filtered through Celite and
concentrated. This procedure afforded 2.0 mg (80.6%
yield) of product as a colorless solid.

Dibenzyl [1-(3-O-benzyl-2,5,6-trideoxy- β -D-
15 ribohexofuranosyl)-N³-benzylthymine]-6'-phosphonate (25).
This procedure was based on a related procedure (25). To
a solution of 416 mg (0.638 mmol) of diphenyl
[1-(3-O-benzyl-2,5,6-trideoxy- β -D-ribohexofuranosyl)-N³-
20 benzylthymine]-6'-phosphonate in 3.0 mL of benzyl
alcohol, was added 2.0 mL of a solution prepared by the
addition of 200 mg of NaH to 16.7 mL of benzyl alcohol.
After 1 h, the reaction mixture was diluted with 50 mL of
Et₂O. Excess gaseous CO₂ was bubbled into the mixture.
A gel like mixture formed which was dissolved in EtOAc.
25 This solution was concentrated onto silica gel. The
silica gel was loaded onto a previously equilibrated 25
mm column and eluted with one column volume of CH₂Cl₂,
then one column volume of 10% EtOAc in CH₂Cl₂, and then
30 20% EtOAc in CH₂Cl₂ as eluents. This afforded 127 mg
(29.3% yield) of product.

Diphenyl [9-(2,5,6-trideoxy- β -D-ribohexofuranoxyl)-
N²-isobutyrylguanine]-6'-phosphonate (26).

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This reaction is based on a similar procedure by Barton et al (30). To 5.00 mmol of diphenyl [9-(3-O-t-butyldimethylsilyl-2,5,6-trideoxy- β -D-ribohexofuranosyl)-N²-isobutyrylguanine]-6'-phosphonate in 100 mL of dry THF is added 5.5 mL (5.5 mmol, 1.1 equiv.) of a 1.00 M solution of tetrabutylammonium fluoride (TBAF) in THF. The reaction is stirred at room temperature for 1 h. Then 20 mL of MeOH is added. The reaction is stirred for 5 min., and then concentrated. The residue is purified by flash chromatography.

Diphenyl [1-(2,5,6-trideoxy- β -D-ribohexofuranosyl)-N⁴-benzoylcytosine]-6'-phosphonate (27). This compound is prepared from diphenyl [1-(3-O-t-butyldimethylsilyl-2,5,6-trideoxy- β -D-ribohexofuranosyl)-N⁴-benzoylcytosine]-6'-phosphonate by the same procedure used for the preparation of diphenyl [9-(2,5,6-trideoxy- β -D-ribohexofuranosyl)-N²-isobutyrylguanine]-6'-phosphonate.

Diphenyl [9-(2,5,6-trideoxy- β -D-ribohexofuranosyl)-N⁶-benzoyladenine]-6'-phosphonate (28). This compound is prepared from diphenyl [9-(3-O-t-butyldimethylsilyl-2,5,6-trideoxy- β -D-ribohexofuranosyl)-N⁶-benzoyladenine]-6'-phosphonate by the same procedure used for the preparation of diphenyl [9-(2,5,6-trideoxy- β -D-ribohexofuranosyl)-N²-isobutyrylguanine]-6'-phosphonate.

Diphenyl [1-(2,5,6-trideoxy- β -D-ribohexofuranosyl)-thymine]-6'-phosphonate (29). This compound is prepared from diphenyl [1-(3-O-t-butyldimethylsilyl-2,5,6-trideoxy- β -D-ribohexofuranosyl)-thymine]-6'-phosphonate by the same procedure used for the preparation of diphenyl [9-(2,5,6-trideoxy- β -D-ribohexofuranosyl)-N²-isobutyrylguanine]-6'-phosphonate.

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Dimethyl [9-(2,5,6-trideoxy- β -D-ribohexofuranosyl)- N^2 -isobutyrylguanaine]-6'-phosphonate (30). This compound is prepared from diphenyl [9-(2,5,6-trideoxy- β -D-ribohexofuranosyl)- N^2 -isobutyrylguanaine]-6'-phosphonate and CsF in MeOH by the same procedure used for the preparation of dimethyl [1-(3-O-benzyl-2,5,6-trideoxy- β -D-ribo-hexofuranosyl)- N^3 -benzylthymine]-6'-phosphonate. After the aqueous extraction and drying, the crude product is purified by flash chromatography.

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Dimethyl [1-(2,5,6-trideoxy- β -D-ribohexofuranosyl)- N^4 -benzoylcytosine]-6'-phosphonate (31). This compound is prepared from diphenyl [1-(2,5,6-trideoxy- β -D-ribohexofuranosyl)- N^4 -benzoylcytosine]-6'-phosphonate by the same procedure used for the preparation of dimethyl [9-(2,5,6-trideoxy- β -D-ribohexofuranosyl)- N^2 -isobutyrylguanaine]-6'-phosphonate.

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Dimethyl [9-(2,5,6-trideoxy- β -D-ribohexofuranosyl)- N^6 -benzoyladenine]-6'-phosphonate (32). This compound is prepared from diphenyl [9-(2,5,6-trideoxy- β -D-ribohexofuranosyl)- N^6 -benzoyladenine]-6'-phosphonate by the same procedure used for the preparation of dimethyl [9-(2,5,6-trideoxy- β -D-ribohexofuranosyl)- N^2 -isobutyrylguanaine]-6'-phosphonate.

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Dimethyl [1-(2,5,6-trideoxy- β -D-ribohexofuranosyl)-thymine]-6'-phosphonate (33). This compound is prepared from diphenyl [1-(2,5,6-trideoxy- β -D-ribohexofuranosyl)-thymine]-6'-phosphonate by the same procedure used for the preparation of dimethyl [9-(2,5,6-trideoxy- β -D-ribohexofuranosyl)- N^2 -isobutyrylguanaine]-6'-phosphonate.

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[9-(2,5,6-trideoxy- β -D-ribohexofuranosyl)-N²-isobutyrylguanine]-6'-phosphonic acid (34). This reaction is based on a similar procedure by Barton et al (30). To a stirred, ice-cooled mixture of dimethyl

5 [9-(2,5,6-trideoxy- β -D-ribohexofuranosyl)-N²-isobutyrylguanine]-6'-phosphonate in 150 mL of CH₂Cl₂ is added 1.98 mL (15.0 mmol, 3.0 equiv.) of bromotrimethylsilane dropwise, over several minutes. The reaction is stirred for 30 min., and then the ice bath is removed.

10 After stirring for an additional 10 h, 20 mL of MeOH is added. The reaction is stirred for 5 min., and then concentrated. The product is used without further purification.

15 [1-(2,5,6-Trideoxy- β -ribohexofuranosyl)-N⁴-benzoyl cytosine]-6'-phosphonic acid (35). This compound is prepared from dimethyl [1-(2,5,6-trideoxy- β -D-ribohexofuranosyl)-N⁴-benzoylcytosine]-6'-phosphonate by the same procedure used for the preparation of [9-(2,5,6-

20 trideoxy- β -D-ribohexofuranosyl)-N²-isobutyrylguanine]-6'-phosphonic acid.

[9-(2,5,6-Trideoxy- β -D-ribohexofuranosyl)-N⁶-benzoyladenine]-6'-phosphonic acid (36). This compound

25 is prepared from dimethyl [9-(2,5,6-trideoxy- β -D-ribohexofuranosyl)-N⁶-benzoyladenine]-6'-phosphonate by the same procedure used for the preparation of [9-(2,5,6-trideoxy- β -D-ribohexofuranosyl)-N²-isobutyrylguanine]-6'-phosphonic acid.

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[1-(2,5,6-Trideoxy- β -D-ribohexofuranosyl)-thymine]-6'-phosphonic acid (37). This compound was prepared from dimethyl [1-(2,5,6-trideoxy- β -D-ribohexofuranosyl)-

35 thymine]-6'-phosphonate by the same procedure used for

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the preparation of [9-(2,5,6-trideoxy- β -D-ribohexofuranosyl)-N²-isobutyrylguanine]-6'-phosphonic acid.

5 [9-(2,5,6-Trideoxy- β -D-ribohexofuranosyl)-guanine]-6'-phosphonic acid (38). The entire crude [9-(2,5,6-trideoxy- β -D-ribohexofuranosyl)-N²-isobutyrylguanine]-6'-phosphonic acid, from above, is heated in 150 mL of concentrated aqueous ammonia at 55°C for 18 h, and then
10 concentrated.

[1-(2,5,6-Trideoxy- β -D-ribohexofuranosyl)-cytosine]-6'-phosphonic acid (39). This compound is prepared from
15 [1-(2,5,6-trideoxy- β -D-ribohexofuranosyl)-N⁴-benzoylcytosine]-6'-phosphonic acid by the same procedure used for the preparation of [9-(2,5,6-trideoxy- β -D-ribohexofuranosyl)-guanine]-6'-phosphonic acid.

20 [9-(2,5,6-Trideoxy- β -D-ribohexofuranosyl)-adenine]-6'-phosphonic acid (40). This compound is prepared from [9-(2,5,6-trideoxy- β -D-ribohexofuranosyl)-N⁶-benzoyladenine]-6'-phosphonic acid by the same procedure used for the preparation of [9-(2,5,6-trideoxy- β -D-ribohexofuranosyl)-guanine]-6'-phosphonic acid.
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Diphenyl [9-(3-O-[4,4'-Dimethoxytrityl]-2,5,6-trideoxy- β -D-ribohexofuranosyl)-N²-isobutyrylguanine]-6'-phosphonate (41). To 5.00 mmol of diphenyl
30 [9-(2,5,6-trideoxy- β -D-ribohexofuranosyl)-N²-isobutyrylguanine]-6'-phosphonate (that is first concentrated from dry pyridine) in 30 mL of dry pyridine, is added 2.03 g (6.0 mmol, 1.20 equiv.) of 4,4'-dimethoxytrityl chloride. The reaction is stirred for 15 h, and then concentrated. The residue is
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partitioned between CH_2Cl_2 and 0.5% aq. NaHCO_3 , shaken, and separated. The organic layer is washed with 0.5% aq. NaHCO_3 and dried. The crude product is purified by flash chromatography.

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Diphenyl [1-(3-O-[4,4'-Dimethoxytrityl]-2,5,6-trideoxy- β -D-ribohexofuranosyl)- N^4 -benzoylcytosine]-6'-phosphonate (42). This compound is prepared from diphenyl [1-(2,5,6-trideoxy- β -D-ribohexofuranosyl)- N^4 -benzoylcytosine]-6'-phosphonate by the same procedure used for the preparation of diphenyl [9-(3-O-[4,4'-dimethoxytrityl]-2,5,6-trideoxy- β -D-ribohexofuranosyl)- N^2 -isobutyrylguanine]-6'-phosphonate.

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Diphenyl [9-(3-O-[4,4'-Dimethoxytrityl]-2,5,6-trideoxy- β -D-ribohexofuranosyl)- N^6 -benzoyladenine]-6'-phosphonate (43). This compound is prepared from diphenyl [9-(2,5,6-trideoxy- β -D-ribohexofuranosyl)- N^6 -benzoyladenine]-6'-phosphonate by the same procedure used for the preparation of diphenyl [9-(3-O-[4,4'-dimethoxytrityl]-2,5,6-trideoxy- β -D-ribohexofuranosyl)- N^2 -isobutyrylguanine]-6'-phosphonate.

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Diphenyl [1-(3-O-[4,4'-Dimethoxytrityl]-2,5,6-trideoxy- β -D-ribohexofuranosyl)-thymine]-6'-phosphonate (44). This compound is prepared from diphenyl [1-(2,5,6-trideoxy- β -D-ribohexofuranosyl)-thymine]-6'-phosphonate by the same procedure used for the preparation of diphenyl [9-(3-O-[4,4'-dimethoxytrityl]-2,5,6-trideoxy- β -D-ribohexofuranosyl)- N^2 -isobutyrylguanine]-6'-phosphonate.

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Monophenyl [9-(3-O-[4,4'-Dimethoxytrityl]-2,5,6-trideoxy- β -D-ribohexofuranosyl)- N^2 -isobutyrylguanine]-6'-phosphonate hydrogentriethylammonium salt (45). A

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mixture of 3.00 mmol of diphenyl [9-(3-O-[4,4'-dimethoxytrityl]-2,5,6-trideoxy-β-D-ribohexofuranosyl)-N²-isobutyrylguanaine]-6'-phosphonate is stirred in 100 mL of concentrated aqueous ammonia at room temperature.

5 The reaction is monitored by TLC. After ca. 1 h, the mixture is concentrated. The product is purified by flash chromatography.

10 Monophenyl [1-(3-O-[4,4'-Dimethoxytrityl]-2,5,6-trideoxy-β-D-ribohexofuranosyl)-N⁴-benzoylcytosine]-6'-phosphonate hydrogentriethylammonium salt (46). This compound is prepared from diphenyl [1-(3-O-[4,4'-dimethoxytrityl]-2,5,6-trideoxy-β-D-ribohexofuranosyl)-N⁴-benzoylcytosine]-6'-phosphonate by the same procedure
15 used for the preparation of monophenyl [9-(3-O-[4,4'-dimethoxytrityl]-2,5,6-trideoxy-β-D-ribohexofuranosyl)-N²-isobutyrylguanaine]-6'-phosphonate hydrogentriethylammonium salt.

20 Monophenyl [9-(3-O-[4,4'-Dimethoxytrityl]-2,5,6-trideoxy-β-D-ribohexofuranosyl)-N⁶-benzoyladenine]-6'-phosphonate hydrogentriethylammonium salt (47). This compound is prepared from diphenyl [9-(3-O-[4,4'-dimethoxytrityl]-2,5,6-trideoxy-β-D-ribohexofuranosyl)-N⁶-
25 -benzoyladenine]-6'-phosphonate by the same procedure used for the preparation of monophenyl [9-(3-O-[4,4'-dimethoxytrityl]-2,5,6-trideoxy-β-D-ribohexofuranosyl)-N²-isobutyrylguanaine]-6'-phosphonate hydrogentriethyl-
30 ammonium salt.

Monophenyl [1-(3-O-[4,4'-Dimethoxytrityl]-2,5,6-trideoxy-β-D-ribohexofuranosyl)-thymine]-6'-phosphonate hydrogentriethylammonium salt (48). This compound is prepared from diphenyl [1-(3-O-[4,4'-dimethoxytrityl]-
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2,5,6- trideoxy- β -D-ribohexofuranosyl)-thymine]-
6'-phosphonate by the same procedure used for the
preparation of monophenyl [9-(3-O-[4,4'-dimethoxytrityl]-
2,5,6-trideoxy- β -D-ribohexofuranosyl)-N²-
5 isobutyrylguanaine]-6'-phosphonate hydrogen-
triethylammonium salt.

9-(2-Deoxy-2-fluoro- β -D-arabinofuranosyl)-N²-
isobutyrylguanaine (49). This compound is prepared from
10 9-(2-deoxy-2-fluoro- β -D-arabinofuranosyl)-guanine (49,
57) by the same procedure used for the preparation of
N²-isobutyryl-2'-deoxyguanosine.

9-[2-Deoxy-5-O-(4,4'-dimethoxytrityl)-2-fluoro- β -D-arabin
15 ofuranosyl]-N²-isobutyrylguanaine (50). This compound is
prepared from 9-(2-deoxy-2-fluoro- β -D-arabinofuranosyl)-
-N²-isobutyrylguanaine by the same procedure used for the
preparation of N₂-isobutyryl-5'-O-(4,4'-dimethoxytrityl)-
2'-deoxyguanosine.
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1-(2-Deoxy-2-fluoro- β -D-arabinofuranosyl)-N⁴-
benzoylcytosine (51). This compound is prepared from
1-(2-deoxy-2-fluoro- β -D-arabinofuranosyl)-cytosine (50)
25 by the same procedure used for the preparation of
N⁴-benzoyl-2'-deoxycytidine.

1-[2-Deoxy-5-O-(4,4'-dimethoxytrityl)-2-fluoro- β -D-
30 arabinofuranosyl]-N⁴-benzoylcytosine (52). This compound
is prepared from 1-(2-deoxy-2-fluoro- β -D-
arabinofuranosyl)-N⁴-benzoylcytosine by the same
procedure used for the preparation of N⁴-benzoyl-5'-
O-(4,4'-dimethoxytrityl)-2'-deoxycytidine.

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9-(2-Deoxy-2-fluoro- β -D-arabinofuranosyl)-N⁶-benzoyladenine (53). This compound is prepared from 9-(2-deoxy-2-fluoro- β -D-arabinofuranosyl)-adenine (49) by the same procedure used for the preparation of N⁶-benzoyl-2'-deoxyadenosine.

9-[2-Deoxy-5-O-(4,4'-dimethoxytrityl)-2-fluoro- β -D-arabinofuranosyl]-N⁶-benzoyladenine (54). This compound is prepared from 9-(2-deoxy-2-fluoro- β -D-arabinofuranosyl)-N⁶-benzoyladenine by the same procedure used for the preparation of N⁶-benzoyl-5'-O-(4,4'-dimethoxytrityl)-2'-deoxyadenosine.

1-[2-Deoxy-5-O-(4,4'-dimethoxytrityl)-2-fluoro- β -D-arabinofuranosyl]-thymine (55). This compound was prepared from 2.90 g (11.1 mmol) of 1-(2-deoxy-2-fluoro- β -D-arabinofuranosyl)-thymine (51) by the same procedure used for the preparation of 5'-O-(4,4'-dimethoxytrityl)-thymidine. Chromatography of the residue on silica gel afforded 6.98 g (89.4% yield) of product.

9-(3-O-t-Butyldimethylsilyl-2-deoxy-2-fluoro- β -D-arabinofuranosyl)-N²-isobutyrylguanine (56). This compound is prepared from 9-[2-deoxy-5-O-(4,4'-dimethoxytrityl)-2-fluoro- β -D-arabinofuranosyl]-N²-isobutyrylguanine by the same procedure used for the preparation of 3'-O-t-butyl dimethylsilyl-N²-isobutyryl-2'-deoxyguanosine.

1-(3-O-t-Butyldimethylsilyl-2-deoxy-2-fluoro- β -D-arabinofuranosyl)-N⁴-benzoylcytosine (57). This compound is prepared from 1-[2-deoxy-5-O-(4,4'-dimethoxytrityl)-2-fluoro- β -D-arabinofuranosyl]-N⁴-benzoylcytosine by the

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same procedure used for the preparation of
3'-O-t-butyldimethylsilyl-N⁴-benzoyl-2'-deoxycytidine.

5 9-(3-O-t-Butyldimethylsilyl-2-deoxy-2-fluoro-β-D-
arabinofuranosyl)-N⁶-benzoyladenine (58). This compound
is prepared from 9-[2-deoxy-5-O-(4,4'-dimethoxytrityl)-
2-fluoro-β-D-arabinofuranosyl]-N⁶-benzoyladenine by the
same procedure used for the preparation of
10 3'-O-t-butyldimethylsilyl-N⁶-benzoyl-2'-deoxyadenosine.

1-(3-O-t-Butyldimethylsilyl-2-deoxy-2-fluoro-β-D-
arabinofuranosyl)-thymine (59). This compound was
prepared from 6.28 g (11.16 mmol) of 1-[2-deoxy-5-O-
15 (4,4'-dimethoxytrityl)-2-fluoro-β-D-
arabinofuranosyl]-thymine by the same procedure used for
the preparation of 3'-O-t-butyldimethylsilylthymidine.
Column chromatography of the crude residue afforded 3.59
g (85.9% yield) of product.

20 Diphenyl [9-(3-O-t-Butyldimethylsilyl-2,5,6-trideoxy-
2-fluoro-β-D-arabino-hex-5-enofuranosyl)-N²-
isobutyrylguanidine]-6'-phosphonate (60). This compound is
prepared from 9-(3-O-t-butyldimethylsilyl-2-deoxy-
25 -2-fluoro-β-D-arabinofuranosyl)-N²-isobutyrylguanidine by
the same procedure used for the preparation of diphenyl
[9-(3-O-t-butyldimethylsilyl-2,5,6-trideoxy-β-D-ribo-hex-
5-enofuranosyl)-N²-isobutyrylguanosine]-6'-phosphonate.

30 Diphenyl [9-(3-O-t-Butyldimethylsilyl-2,5,6-trideoxy-2-
fluoro-β-D-arabino-hexofuranosyl)-N²-isobutyrylguanidine]-6'
-phosphonate (61). This compound is prepared from
diphenyl [9-(3-O-t-butyldimethylsilyl-2,5,6-trideoxy-
2-fluoro-β-D-arabino-hex-5-enofuranosyl)-N²-

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isobutyrylguanine]-6'-phosphonate by the same procedure used for the preparation of diphenyl [9-(3-O-t-butyl dimethylsilyl-2,5,6-trideoxy- β -D-ribohexofuranosyl)-N²-isobutyrylguanine]-6'-phosphonate.

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Diphenyl [1-(3-O-t-Butyldimethylsilyl-2,5,6-trideoxy-2-fluoro- β -D-arabino-hex-5-enofuranosyl)-N⁴-benzoylcytosine]-6'-phosphonate (62). This compound is prepared from 1-(3-O-t-butyl dimethylsilyl-2-deoxy-2-fluoro- β -D-arabinofuranosyl)-N⁴-benzoylcytosine by the same procedure used for the preparation of diphenyl [1-(3-O-t-butyl dimethylsilyl-2,5,6-trideoxy- β -D-ribo-hex-5-enofuranosyl)-N⁴-benzoylcytosine]-6'-phosphonate.

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Diphenyl [1-(3-O-t-Butyldimethylsilyl-2,5,6-trideoxy-2-fluoro- β -D-arabino-hex-5-enofuranosyl)-N⁴-benzoylcytosine]-6'-phosphonate (63). This compound is prepared from diphenyl [1-(3-O-t-butyl dimethylsilyl-2,5,6-trideoxy-2-fluoro- β -D-arabino-hex-5-enofuranosyl)-N⁴-benzoylcytosine]-6'-phosphonate by the same procedure used for the preparation of diphenyl [1-(3-O-t-butyl dimethylsilyl-2,5,6-trideoxy- β -D-ribohexofuranosyl)-N⁴-benzoylcytosine]-6'-phosphonate.

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Diphenyl [9-(3-O-t-Butyldimethylsilyl-2,5,6-trideoxy-2-fluoro- β -D-arabino-hex-5-enofuranosyl)-N⁶-benzoyladenine]-6'-phosphonate (64). This compound is prepared from 9-(3-O-t-butyl dimethylsilyl-2-deoxy-2-fluoro- β -D-arabinofuranosyl)-N⁶-benzoyladenine by the same procedure used for the preparation of diphenyl [9-(3-O-t-butyl dimethylsilyl-2,5,6-trideoxy- β -D-ribo-hex-5-enofuranosyl)-N⁶-benzoyladenine]-6'-phosphonate.

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Diphenyl [9-(3-O-t-Butyldimethylsilyl-2,5,6-trideoxy-2-fluoro- β -D-arabinohexofuranosyl)-N⁶-benzoyladenine]-6'-phosphonate (65). This compound is prepared from diphenyl [9-(3-O-t-butyldimethylsilyl-2,5,6-trideoxy-2-fluoro- β -D-arabino-hex-5-enofuranosyl)-N⁶-benzoyladenine]-6'-phosphonate by the same procedure used for the preparation of diphenyl [9-(3-O-t-butyldimethylsilyl-2,5,6-trideoxy- β -D-ribohexofuranosyl)-N⁶-benzoyladenine]-6'-phosphonate.

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Diphenyl [1-(3-O-t-Butyldimethylsilyl-2,5,6-trideoxy-2-fluoro- β -D-arabino-hex-5-enofuranosyl)-thymine]-6'-phosphonate (66). This compound was prepared from 3.12 g (8.33 mmol) of 1-(3-O-t-butyldimethylsilyl-2-deoxy-2-fluoro- β -D-arabinofuranosyl)-thymine by the same procedure used for the preparation of diphenyl [1-(3-O-t-butyldimethylsilyl-2,5,6-trideoxy- β -D-ribo-hex-5-enofuranosyl)-thymine]-6'-phosphonate. Column chromatography of the crude residue afforded 1.19 g (70.4% yield) of product.

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Diphenyl [1-(3-O-t-Butyldimethylsilyl-2,5,6-trideoxy-2-fluoro- β -D-arabinohexofuranosyl)-thymine]-6'-phosphonate (67). This compound was prepared from 1.69 g (2.80 mmol) of diphenyl [1-(3-O-t-butyldimethylsilyl-2,5,6-trideoxy-2-fluoro- β -D-arabino-hex-5-enofuranosyl)-thymine]-6'-phosphonate by the same procedure used for the preparation of diphenyl [1-(3-O-t-butyldimethylsilyl-2,5,6-trideoxy- β -D-ribohexofuranosyl)-thymine]-6'-phosphonate. Column chromatography of the crude residue afforded 249 mg (27.6% yield) of product.

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Diphenyl [9-(2,5,6-trideoxy-2-fluoro-β-D-arabinohexofuranosyl)-N²-isobutyrylguanine]-6'-phosphonate (68). This compound is prepared from diphenyl [9-(3-O-t-butyldimethylsilyl-2,5,6-trideoxy-2-fluoro-β-D-arabinohexofuranosyl)-N²-isobutyrylguanine] 5 -6'-phosphonate by the same procedure used for the preparation of diphenyl [9-(2,5,6-trideoxy-β-D-ribohexofuranosyl)-N²-isobutyrylguanine]-6'-phosphonate.

10 Diphenyl [1-(2,5,6-trideoxy-2-fluoro-β-D-arabinohexofuranosyl)-N⁴-benzoylcytosine]-6'-phosphonate (69). This compound is prepared from diphenyl [1-(3-O-t-butyldimethylsilyl-2,5,6-trideoxy-2-fluoro-β-D-arabinohexofuranosyl)-N⁴-benzoylcytosine]-6'-phosphonate 15 by the same procedure used for the preparation of diphenyl [1-(2,5,6-trideoxy-β-D-ribohexofuranosyl)-N⁴-benzoylcytosine]-6'-phosphonate.

20 Diphenyl [9-(2,5,6-trideoxy-2-fluoro-β-D-arabinohexofuranosyl)-N⁶-benzoyladenine]-6'-phosphonate (70). This compound is prepared from diphenyl [9-(3-O-t-butyldimethylsilyl-2,5,6-trideoxy-2-fluoro-β-D-arabinohexofuranosyl)-N⁶-benzoyladenine]-6'-phosphonate 25 by the same procedure used for the preparation of diphenyl [9-(2,5,6-trideoxy-β-D-ribohexofuranosyl)-N⁶-benzoyladenine]-6'-phosphonate.

Diphenyl [1-(2,5,6-trideoxy-2-fluoro-β-D-arabinohexofuranosyl)-thymine]-6'-phosphonate (71). This 30 compound is prepared from diphenyl [1-(3-O-t-butyldimethylsilyl-2,5,6-trideoxy-2-fluoro-β-D-arabinohexofuranosyl)-thymine]-6'-phosphonate by the same procedure used for the preparation of diphenyl 35 [1-(2,5,6-trideoxy-β-D-ribohexofuranosyl)-thymine]-6'-

phosphonate.

Dimethyl [9-(2,5,6-trideoxy-2-fluoro- β -D-arabinohexofuranosyl)-N²-isobutyrylguanine]-6'-phosphonate (72). This compound is prepared from diphenyl [9-(2,5,6-trideoxy-2-fluoro- β -D-arabinohexofuranosyl)-N²-isobutyrylguanine]-6'-phosphonate by the same procedure used for the preparation of dimethyl [9-(2,5,6-trideoxy- β -D-ribohexofuranosyl)-N²-isobutyrylguanine]-6'-phosphonate.

Dimethyl [1-(2,5,6-trideoxy-2-fluoro- β -D-arabinohexofuranosyl)-N⁴-benzoylcytosine]-6'-phosphonate (73). This compound is prepared from diphenyl [1-(2,5,6-trideoxy-2-fluoro- β -D-arabinohexofuranosyl)-N⁴-benzoylcytosine]-6'-phosphonate by the same procedure used for the preparation of dimethyl [1-(2,5,6-trideoxy- β -D-ribohexofuranosyl)-N⁴-benzoylcytosine]-6'-phosphonate.

Dimethyl [9-(2,5,6-trideoxy-2-fluoro- β -D-arabinohexofuranosyl)-N⁶-benzoyladenine]-6'-phosphonate (74). This compound is prepared from diphenyl [9-(2,5,6-trideoxy-2-fluoro- β -D-arabinohexofuranosyl)-N⁶-benzoyladenine]-6'-phosphonate by the same procedure used for the preparation of dimethyl [9-(2,5,6-trideoxy- β -D-ribohexofuranosyl)-N⁶-benzoyladenine]-6'-phosphonate.

Dimethyl [1-(2,5,6-trideoxy-2-fluoro- β -D-arabinohexofuranosyl)-thymine]-6'-phosphonate (75). This compound is prepared from diphenyl [1-(2,5,6-trideoxy-2-fluoro- β -D-arabinohexofuranosyl)-thymine]-6'-

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phosphonate by the same procedure used for the preparation of dimethyl [1-(2,5,6-trideoxy- β -D-ribohexofuranosyl)-thymine]-6'-phosphonate.

5 [9-(2,5,6-trideoxy-2-fluoro- β -D-arabinohexofuranosyl)-N²-isobutyrylguanine]-6'-phosphonic acid (76). This compound is prepared from dimethyl [9-(2,5,6-trideoxy-2-fluoro- β -D-arabinohexofuranosyl)-N²-isobutyrylguanine]-6'-phosphonate by the same procedure used for the
10 preparation of [9-(2,5,6-trideoxy- β -D-ribohexofuranosyl)-N²-isobutyrylguanine]-6'-phosphonic acid.

[1-(2,5,6-trideoxy-2-fluoro- β -D-arabinohexofuranosyl)-N⁴-benzoylcytosine]-6'-phosphonic acid (77). This compound
15 is prepared from dimethyl [1-(2,5,6-trideoxy-2-fluoro- β -D-arabinohexofuranosyl)-N⁴-benzoylcytosine]-6'-phosphonate by the same procedure used for the preparation of [1-(2,5,6-trideoxy- β -D-ribohexofuranosyl)-N⁴-benzoylcytosine]-6'-phosphonic
20 acid.

[9-(2,5,6-trideoxy-2-fluoro- β -D-arabinohexofuranosyl)-N⁶-benzoyladenine]-6'-phosphonic acid (78). This compound
25 is prepared from dimethyl [9-(2,5,6-trideoxy-2-fluoro- β -D-arabinohexofuranosyl)-N⁶-benzoyladenine]-6'-phosphonate by the same procedure used for the preparation of [9-(2,5,6-trideoxy- β -D-ribohexofuranosyl)-N⁶-benzoyladenine]-6'-phosphonic acid.

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[1-(2,5,6-trideoxy-2-fluoro- β -D-arabinohexofuranosyl)-thymine]-6'-phosphonic acid (79). This compound is prepared from dimethyl [1-(2,5,6-trideoxy-2-fluoro- β -D-arabinohexofuranosyl)-thymine]-6'-phosphonate by the
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same procedure used for the preparation of
[1-(2,5,6-trideoxy- β -D-ribohexofuranosyl)-thymine]-
6'-phosphonic acid.

5 [9-(2,5,6-trideoxy-2-fluoro- β -D-arabinohexofuranosyl)-
guanine]-6'-phosphonic acid (80). This compound is
prepared from [9-(2,5,6-trideoxy-2-fluoro- β -D-
arabinohexofuranosyl)-N²-isobutyrylguanine]-6'-phosphonic
acid by the same procedure used for the preparation of
10 [9-(2,5,6-trideoxy- β -D-ribohexofuranosyl)-guanine]-6'-
phosphonic acid.

[1-(2,5,6-trideoxy-2-fluoro- β -D-arabinohexofuranosyl)-
cytosine]-6'-phosphonic acid (81). This compound is
15 prepared from [1-(2,5,6-trideoxy-2-fluoro- β -D-
arabinohexofuranosyl)-N⁴-benzoylcytosine]-6'-phosphonic
acid by the same procedure used for the preparation of
[1-(2,5,6-trideoxy- β -D-ribohexofuranosyl)-cytosine]-6'-
phosphonic acid.

20 [9-(2,5,6-trideoxy-2-fluoro- β -D-arabinohexofuranosyl)-
adenine]-6'-phosphonic acid (82). This compound is
prepared from [9-(2,5,6-trideoxy-2-fluoro- β -D-
arabinohexofuranosyl)-N⁶-benzoyladenine]-6'-phosphonic
25 acid by the same procedure used for the preparation of
[9-(2,5,6-trideoxy- β -D-ribohexofuranosyl)-adenine]-6'-
phosphonic acid.

Diphenyl [9-(3-O-[4,4'-Dimethoxytrityl]-2,5,6-trideoxy
30 -2-fluoro- β -D-arabinohexofuranosyl)-N²-isobutyrylguanine]
-6'-phosphonate (83). This compound is prepared from
diphenyl [9-(2,5,6-trideoxy-2-fluoro- β -D-
-arabinohexofuranosyl)-N²-isobutyrylguanine]-6'-

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phosphonate by the same procedure used for the preparation of diphenyl [9-(3-O-[4,4'-dimethoxytrityl]-2,5,6-trideoxy- β -D-ribohexofuranosyl)-N²-isobutyrylguanine]-6'-phosphonate.

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Diphenyl [1-(3-O-[4,4'-Dimethoxytrityl]-2,5,6-trideoxy-2-fluoro- β -D-arabinohexofuranosyl)-N⁴-benzoylcytosine]-6'-phosphonate (84). This compound is prepared from diphenyl [1-(2,5,6-trideoxy-2-fluoro- β -D-arabinohexofuranosyl)-N⁴-benzoylcytosine]-6'-phosphonate by the same procedure used for the preparation of diphenyl [1-(3-O-[4,4'-dimethoxytrityl]-2,5,6-trideoxy- β -D-ribohexofuranosyl)-N⁴-benzoylcytosine]-6'-phosphonate.

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Diphenyl [9-(3-O-[4,4'-Dimethoxytrityl]-2,5,6-trideoxy-2-fluoro- β -D-arabinohexofuranosyl)-N⁶-benzoyladenine]-6'-phosphonate (85). This compound is prepared from diphenyl [9-(2,5,6-trideoxy-2-fluoro- β -D-arabinohexofuranosyl)-N⁶-benzoyladenine]-6'-phosphonate by the same procedure used for the preparation of diphenyl [9-(3-O-[4,4'-dimethoxytrityl]-2,5,6-trideoxy- β -D-ribohexofuranosyl)-N⁶-benzoyladenine]-6'-phosphonate.

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Diphenyl [1-(3-O-[4,4'-Dimethoxytrityl]-2,5,6-trideoxy-2-fluoro- β -D-arabinohexofuranosyl)-thymine]-6'-phosphonate (86). This compound is prepared from diphenyl [1-(2,5,6-trideoxy-2-fluoro- β -D-arabinohexofuranosyl)-thymine]-6'-phosphonate by the same procedure used for the preparation of diphenyl [1-(3-O-[4,4'-dimethoxytrityl]-2,5,6-trideoxy- β -D-ribohexofuranosyl)-thymine]-6'-phosphonate.

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Monophenyl [9-(3-O-[4,4'-Dimethoxytrityl]-2,5,6-trideoxy-2-fluoro- β -D-arabinohexofuranosyl)-N²-isobutyrylguanine]-

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6'-phosphonate hydrogentriethylammonium salt (87). This compound is prepared from diphenyl [9-(3-O-[4,4'-dimethoxytrityl]-

2,5,6-trideoxy-2-fluoro-β-D-arabinohexofuranosyl)-N²-isobutyrylguanine]-6'-phosphonate by the same procedure used for the preparation of monophenyl [9-(3-O-[4,4'-dimethoxytrityl]-2,5,6-trideoxy-β-D-ribohexofuranosyl)-N²-isobutyrylguanine]-6'-phosphonate hydrogentriethylammonium salt.

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Monophenyl [1-(3-O-[4,4'-Dimethoxytrityl]-2,5,6-trideoxy-2-fluoro-β-D-arabinohexofuranosyl)-N⁴-benzoylcytosine]-6'-phosphonate hydrogentriethylammonium salt (88). This compound is prepared from diphenyl [1-(3-O-[4,4'-

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dimethoxytrityl]-2,5,6-trideoxy-2-fluoro-β-D-arabinohexofuranosyl)-N⁴-benzoylcytosine]-6'-phosphonate by the same procedure used for the preparation of monophenyl [1-(3-O-[4,4'-dimethoxytrityl]-2,5,6-trideoxy-β-D-ribohexofuranosyl)-N⁴-benzoylcytosine]-6'-phosphonate hydrogentriethylammonium salt.

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Monophenyl [9-(3-O-[4,4'-Dimethoxytrityl]-2,5,6-trideoxy-2-fluoro-β-D-arabinohexofuranosyl)-N⁶-benzoyladenine]-6'-phosphonate hydrogentriethylammonium salt (89). This compound is prepared from diphenyl [9-(3-O-[4,4'-dimethoxytrityl]-

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2,5,6-trideoxy-2-fluoro-β-D-arabinohexofuranosyl)-N⁶-benzoyladenine]-6'-phosphonate by the same procedure used for the preparation of monophenyl [9-(3-O-[4,4'-dimethoxytrityl]-2,5,6-trideoxy-β-D-ribohexofuranosyl)-N⁶-benzoyladenine]-6'-phosphonate hydrogentriethylammonium salt.

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Monophenyl [1-(3-O-[4,4'-Dimethoxytrityl]-2,5,6-trideoxy-2-fluoro- β -D-arabinohexofuranosyl)-thymine]-6'-phosphonate hydrogentriethylammonium salt (90). This compound is prepared from diphenyl [1-(3-O-[4,4'-
5 dimethoxytrityl]-2,5,6-trideoxy-2-fluoro- β -D-arabinohexofuranosyl)-thymine]-6'-phosphonate by the same procedure used for the preparation of monophenyl [1-(3-O-[4,4'-
10 dimethoxytrityl]-2,5,6-trideoxy- β -D-ribohexofuranosyl)-thymine]-6'-phosphonate hydrogentriethylammonium salt.

Synthesis of oligonucleotides. Oligonucleotides are synthesized from the 5'-end to the 3'-end. The phosphotriester method of oligonucleotide synthesis
15 described by Sproat and Gait is used (38). Appropriately protected 3'-O-(4,4'-dimethoxytrityl)-nucleosides having a free 5'-hydroxyl group are required for the solid phase synthesis (52). These nucleosides are affixed to a long chain alkylamine controlled pore glass (LCAA/CPG) via a
20 succinate linker using standard methods (38). The 3'-O-DMT group on the support bound nucleoside is cleaved with 3% (v/v) dichloroacetic acid in 1,2-dichloroethane (DCE). After washing with DCE, and then pyridine, coupling of the appropriate monophenyl
25 nucleoside-6'-phosphonate as its hydrogentriethylammonium salt is effected with the coupling agent 1-mesitylenesulphonyl-3-nitro-1,2,4-triazole (MSNT) and 1-methylimidazole (NMI) in pyridine. This coupling is allowed to occur from 15-45 minutes. The support is then
30 washed with pyridine. The oligo containing support is then treated with an Ac_2O /lutidine/DMAP capping solution. The capping agent and its use is described by Atkinson and Smith (53). After capping, the support is washed with first DCE, pyridine, and then DCE again. Then the
35 cycle is repeated (ie. deprotection, coupling, capping).

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After the last coupling step, the fully protected oligonucleotide is cleaved from the support and fully deprotected using a mixture of pyridine-2-carbaldoxime and tetramethylguanidine in dioxane/water (38). This
5 deprotection is allowed to occur at 37°C for 20 h. After drying in vacuo, the oligonucleotide is purified by either HPLC or polyacrylamide gel electrophoresis (PAGE).

Diphenyl [9-(3-O-t-Butyldimethylsilyl-2,5,6-trideoxy
10 - β -D-ribohexofuranosyl)-N²-isobutyrylguanine]-6'-phosphonate (14). Another procedure for the preparation of the title compound follows. A solution of 2.70 g (3.97 mmol) of diphenyl [9-(3-O-t-butyldimethylsilyl-2,5,6-trideoxy- β -D-ribo-hex-5-enofuranosyl)-N²-
15 isobutyrylguanine]-6'-phosphonate was reduced with 2,4,6-tri-isopropylbenzenesulphonyl hydrazide and triethylamine as in the preparation of compound 22. After extractive workup, the crude product was used in the next step without further purification.

20 Dimethyl [9-(3-O-t-Butyldimethylsilyl-2,5,6-trideoxy - β -D-ribohexofuranosyl)-N²-isobutyrylguanine]-6'-phosphonate (91). The entire crude compound 14 from above was treated with CsF and MeOH as in the preparation
25 of compound 23. After workup, the product was purified by flash chromatography and afforded 476 mg (21.5% yield) of product.

30 Monomethyl [9-(2,5,6-Trideoxy- β -D-ribohexofuranosyl) -guanine]-6'-phosphonate sodium salt (92). A solution of 288 mg (0.516 mmol) of compound 91 in 8.5 mL of 0.5 N NaOH solution of MeOH/H₂O 4:1 was heated at 80°C for 1.5 h. The volatiles were rotovapped off. To the mixture
35 was added 5.70 mL of H₂O, and this mixture was

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neutralized to pH = 7.0 with 1 N aqueous HCl. The aqueous mixture was extracted with Et₂O several times. The aqueous layer was concentrated. The residue was purified by reverse phase HPLC on a C18 column using
5 CH₃CN/H₂O as eluents. This afforded 148 mg (75.2% yield) of product.

[9-(2,5,6-Trideoxy-β-D-ribohexofuranosyl)-guanine]-6'-phosphonate disodium salt (93). To 60.0 mg (0.157 mmol)
10 of compound 92 in 25 mM tris and 1 mM MgCl₂ was added 1 unit of phosphodiesterase (from *Crotalus duriss*, that was purchased from Boehringer Mannheim as a 50% w/v solution in glycerol). The pH of the solution was kept between
15 8.5 and 9.5 during the course of the reaction by adding solid tris to the reaction. After 17 h, the phosphodiesterase was removed on a size exclusion column (Centriprep-10 column, purchased from Amicon). The product was purified on a reverse phase C18 column using
20 a combination of 100 mM triethylammonium acetate (TEAA) and CH₃CN as eluents. The product was azeotroped from EtOH/H₂O several times to remove excess TEAA. The triethylammonium counter ion was exchanged for Na⁺ on a Bio-Rad Poly-Prep cation exchange column (AG 50W-x8
25 resin, Na⁺ form). The product was desalted by reverse phase HPLC on a C18 column using CH₃CN/H₂O. This afforded 38.2 mg (63.9% yield) of product.

[9-(2,5,6-Trideoxy-β-D-ribohexofuranosyl)-guanine]-6'-phosphonate monosodium monohydrogentriethylammonium salt
30 (119). The procedure for the preparation of compound 93 was used except that the cation exchange column was not treated with enough aqueous NaOH before the phosphonate was loaded onto the column. The resulting acidic resin caused some depurination, and only partial exchange of
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Na⁺ for hydrogentriethylammonium cation. The resulting mixture was separated on a reverse phase C18 column using CH₃CN/H₂O as eluents. This afforded 14.1 mg (19.2% yield) of product as the monohydrogentriethylammonium salt.

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Dimethyl [1-(3-O-t-Butyldimethylsilyl-2,5,6-trideoxy-β-D-ribohexofuranosyl)-N⁴-benzoylcytosine]-6'-phosphonate (94). This compound was prepared from 3.88 g (5.74 mmol) of compound 16 by the same procedure used for the preparation of compound 91. Purification of the product by silica gel chromatography afforded 700 mg (22.1 % yield) of product.

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Monomethyl [1-(2,5,6-Trideoxy-β-D-ribohexofuranosyl)-cytosine]-6'-phosphonate sodium salt (95). This compound was prepared from 500 mg (0.906 mmol) of compound 94 by the same procedure used for the preparation of compound 92. Purification by HPLC on a C18 column using CH₃CN/H₂O afforded 336 mg (> 100% yield) that contained only the product and less than one equivalent of benzoic acid by ¹H NMR.

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[1-(2,5,6-Trideoxy-β-D-ribohexofuranosyl)-cytosine]-6'-phosphonate disodium salt (96). This compound was prepared from 100 mg (0.293 mmol) of compound 95 by the same procedure used for the preparation of compound 93. This procedure afforded 61.3 mg (59.9% yield) of product.

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Dimethyl [1-(3-O-t-Butyldimethylsilyl-2,5,6-trideoxy-2-fluoro-β-D-arabinohexofuranosyl)-thymine]-6'-phosphonate (97). This compound was prepared from 1.14 g (1.88 mmol) of compound 67 by the same procedure used for the preparation of compound 91. Column chromatography of the

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crude residue on silica gel afforded 249 mg (27.6% yield) of product.

5 **Monomethyl [1-(2,5,6-Trideoxy-2-fluoro-β-D-arabinohexofuranosyl)-thymine]-6'-phosphonate sodium salt (98).** This compound was prepared from 220 mg (0.458 mmol) of compound 97 by the same procedure used for the preparation of compound 92. HPLC purification afforded 90.0 mg (40.9% yield) of product.

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[1-(2,5,6-Trideoxy-2-fluoro-β-D-arabinohexofuranosyl)-thymine]-6'-phosphonate disodium salt (99). This compound was prepared from 80.0 mg (0.214 mmol) of compound 98 by the same procedure used for the preparation of compound 93. This procedure afforded 18.1 mg (22.1% yield) of product.

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1-[2-Deoxy-5-O-(4,4'-dimethoxytrityl)-2-fluoro-β-D-arabinofuranosyl]-uracil (100). This compound was prepared from 4.00 g (17.2 mmol) of 1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-uracil (51b) by the same procedure used for the preparation of compound 55. Column chromatography of the crude residue afforded 6.60 g (69.9% yield) of product.

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1-(3-O-t-Butyldimethylsilyl-2-deoxy-2-fluoro-β-D-arabinofuranosyl)-uracil (101). This compound was prepared from 5.60 g (10.2 mmol) of compound 100 by the same procedure used for the preparation of compound 59. Column chromatography of the crude residue afforded 2.70 g (73.4% yield) of product.

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Diphenyl [1-(3-O-t-Butyldimethylsilyl-2,5,6-trideoxy-2-fluoro-β-D-arabino-hex-5-enofuranosyl)-uracil]-6'-

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phosphonate (102). This compound was prepared from 3.50 g (9.71 mmol) of compound 101 by the same procedure used for the preparation of compound 66. Column chromatography of the crude residue afforded 4.02 g (70.3% yield) of product.

Diphenyl [1-(3-O-t-Butyldimethylsilyl-2,5,6-trideoxy-2-fluoro-β-D-arabinohexofuranosyl)-uracil]-6'-phosphonate (103). This compound was prepared from 3.80 g (6.46 mmol) of compound 102 by the same procedure used for the preparation of compound 67. Column chromatography of the crude residue afforded 1.20 g (31.4% yield) of product.

Monomethyl [1-(2,5,6-Trideoxy-2-fluoro-β-D-arabinohexofuranosyl)-uracil]-6'-phosphonate sodium salt (105). A solution of 350 mg (0.592 mmol) of compound 103 in 0.5 N NaOH in MeOH/H₂O 4:1 was heated at 80°C for 1.5 h. The volatiles were removed on a rotovap. The mixture was diluted with H₂O, cooled on ice, neutralized with 1 N aqueous HCl, and then extracted with Et₂O. The aqueous layer was concentrated and the product purified by reverse phase HPLC on a C18 column using CH₃CN/H₂O as eluents. This afforded 97.6 mg (45.8% yield) of product.

[1-(2,5,6-Trideoxy-2-fluoro-β-D-arabinohexofuranosyl)-uracil]-6'-phosphonate disodium salt (106). This compound was prepared from 89.6 mg (0.249 mmol) of compound 105 by the same procedure used for the preparation of compound 99. This procedure afforded 28.9 mg (31.5% yield) of product.

Diphenyl [1-(3-O-t-Butyldimethylsilyl-2,5,6-trideoxy-2-fluoro-β-D-arabinohexofuranosyl)-cytosine]-6'-phosphonate (107). To an ice-cooled mixture of 758 mg (1.28 mmol) of

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compound 103, 5.20 mL of TEA, and 523 mg of
1,2,4-triazole in 17.3 mL of dry CH₃CN was added 230
microliters of POCl₃ dropwise over five minutes. After
15 minutes, the ice bath was removed, and stirring was
5 continued for an additional hour. The reaction was
concentrated, partitioned between CH₂Cl₂ and H₂O, shaken,
and separated. The organic layer was dried (Na₂SO₄),
filtered, and concentrated. The residue was concentrated
10 once from dry CH₃CN, and then taken up in 38 mL of dry
CH₃CN. The reaction was saturated with anhydrous NH₃,
and the flask was tightly stoppered. After 65 h, the
reaction was concentrated. The residue was taken up in
CH₂Cl₂, washed with H₂O, dried (Na₂SO₄), filtered, and
15 concentrated. Column chromatography of the crude product
on silica gel afforded 410 mg (54.5% yield) of product.

**Monophenyl [1-(2,5,6-Trideoxy-2-fluoro-β-D-
arabinohexofuranosyl)-cytosine]-6'-phosphonate sodium
salt (108).** A solution of 350 mg (0.596 mmol) of
20 compound 107 in 20 mL of 0.5 N NaOH in dioxane/H₂O 1:1
was heated at 80°C for 1.5 h. The volatiles were removed
on a rotovap. The mixture was diluted with H₂O, cooled
on ice, neutralized with 1 N aqueous HCl, and extracted
25 with Et₂O. The aqueous layer was concentrated to give
crude product. The monophenyl phosphonate was purified
as the hydrogentriethylammonium salt in the next step.

**Monophenyl[1-(2,5,6-Trideoxy-2-fluoro-β-D-arabinohexo
furanosyl)-cytosine]-6'-phosphonate
30 hydrogentriethylammonium salt (120).** The entire crude
compound 108 from above was purified by reverse phase
HPLC on a C18 column using 100 mM aqueous
triethylammonium acetate (pH = 6.5) and CH₃CN as eluents.
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This afforded 227 mg (76.2% yield from compound 107) of product.

5 [1-(2,5,6-Trideoxy-2-fluoro- β -D-arabinohexofuranosyl)-
cytosine]-6'-phosphonate disodium salt (109). This
compound was prepared from 180 mg (0.360 mmol) of
compound 120 by the same procedure used for the
preparation of compound 106. This procedure afforded
10 81.8 mg (61.9% yield) of product.

9-[2-Deoxy-5-O-(4,4'-dimethoxytrityl)-2-fluoro- β -D-
arabinofuranosyl]-N⁶-(4,4'-dimethoxytrityl)-adenine
(110). To 2.70 g (10.03 mmol) of 9-(2-deoxy-2-
15 fluoro- β -D-arabinofuranosyl)-adenine⁴⁹ in 65 mL of dry
pyridine was added 8.98 g (26.6 mmol) of
4,4'-dimethoxytrityl chloride. The reaction was stirred
at room temperature for 63 h and then concentrated. The
residue was taken up in CH₂Cl₂, washed with 0.5% aqueous
20 NaHCO₃, dried (Na₂SO₄), filtered, and concentrated.
Column chromatography of the crude product on silica gel
afforded 5.71 g (63.4% yield) of product.

25 9-(3-O-t-Butyldimethylsilyl-2-deoxy-2-fluoro- β -D-arabino
furanosyl)-adenine (111). This compound was prepared
from 5.46 g (6.25 mmol) of compound 110 by the same
procedure used for the preparation of compound 101. The
product was not purified by chromatography, but was
precipitated from CH₂Cl₂ with hexanes affording 1.87 g
30 (77.9% yield) of product.

Diphenyl [9-(3-O-t-Butyldimethylsilyl-2,5,6-trideoxy-2-
-fluoro- β -D-arabino-hex-5-enofuranosyl)-adenine]-6'-

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phosphonate (112). This compound was prepared from 1.67 g (4.35 mmol) of compound 111 by the same procedure used for the preparation of compound 102. Column chromatography of the crude product on silica gel
5 afforded 1.49 g (56.0% yield) of product.

Diphenyl [9-(3-O-t-Butyldimethylsilyl-2,5,6-trideoxy-2-fluoro-β-D-arabinohexofuranosyl)-adenine]-6'-phosphonate (113). This compound was prepared from 1.11
10 g (1.82 mmol) of compound 112 by the same procedure used for the preparation of compound 103. Column chromatography of the crude product on silica gel afforded 133 mg (11.9% yield) of product.

15 Monophenyl [9-(2,5,6-Trideoxy-2-fluoro-β-D-arabinohexofuranosyl)-adenine]-6'-phosphonate sodium salt (114). This compound is prepared from compound 113 by the same procedure used for the preparation of compound 108.

20 [9-(2,5,6-Trideoxy-2-fluoro-β-D-arabinohexofuranosyl)-adenine]-6'-phosphonate disodium salt (115). This compound is prepared from compound 114 by the same procedure used for the preparation of compound 109.

25 Dimethyl [9-(3-O-t-Butyldimethylsilyl-2,5,6-trideoxy-2-fluoro-β-D-arabinohexofuranosyl)-N²-isobutyrylguanine]-6'-phosphonate (116). This compound is prepared from compound 61 by the same procedure used for the
30 preparation of compound 91.

Monomethyl [9-(2,5,6-Trideoxy-2-fluoro-β-D-arabinohexofuranosyl)-guanine]-6'-phosphonate sodium salt (117). This compound is prepared from compound 116 by
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the same procedure used for the preparation of compound 92.

5 [9-(2,5,6-Trideoxy-2-fluoro- β -D-arabinohexofuranosyl)-
guanine]-6'-phosphonate disodium salt (118). This
compound is prepared from compound 117 by the same
procedure used for the preparation of compound 93.

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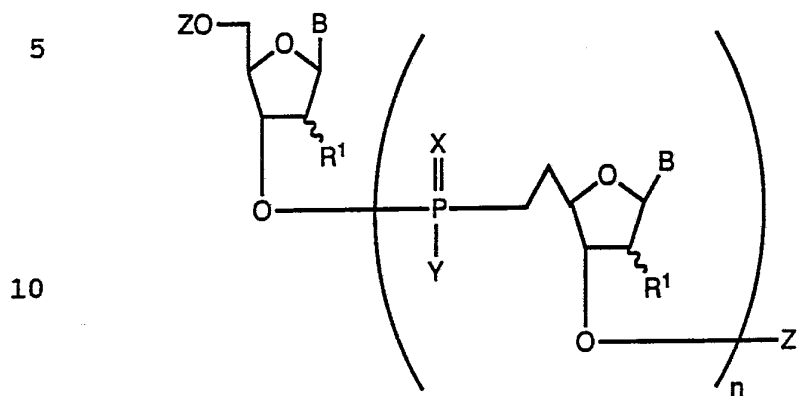
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What is claimed is:

1. A modified oligonucleotide of formula (I):



15

I

and stereoisomers and salts thereof, wherein:

each B is independently a purine or pyrimidine base or modified form;

20 each Z is independently a noninterfering substituent, hydrogen or a protecting group;

each R⁵ is independently hydrogen, hydroxyl, fluorine, O-methyl, O-allyl, S-methyl or S-allyl;

25 each Y is independently OR⁴, N(R⁴)₂ or SR⁴ wherein,

each R⁴ is independently hydrogen, methyl, ethyl, propyl, isopropyl, butyl, phenyl, alkyl (4 - 18C) or substituted alkyl (1-18C);

30 X is selected from oxygen and sulfur; and

n is an integer from 1 to 30, with the proviso that (i) at least one W is CH₂, (ii) when B is thymine and n is 1 or 2 then not all R⁵ are H, (iii) when B is adenine and n is an integer from 1 to 9 then not all R⁵ are OH, (iv) when B is uracil and n is 1 then not all R⁵

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are OH and (v) when n is 1 and the 5' B is uracil and the 3' B is adenine then not all R⁵ are OH.

5 2. The modified oligonucleotide of claim 1 wherein Y is O⁻, OH or OR⁴.

 3. The modified oligonucleotide of claim 2 wherein X is oxygen.

10 4. The modified oligonucleotide of claim 3 which is a dimer, trimer or tetramer.

 5. The modified oligonucleotide of claim 3 comprising
15 a first nucleotide sequence containing at least three nucleotide residues, said sequence having either 3' to 5' or 5' to 3' polarity, and, coupled thereto,
 a second nucleotide sequence containing at least one nucleotide residue, said second sequence having
20 polarity inverted from that of the first sequence.

 6. The oligonucleotide of claim 3 which is capable of forming a triplex with a target duplex DNA.

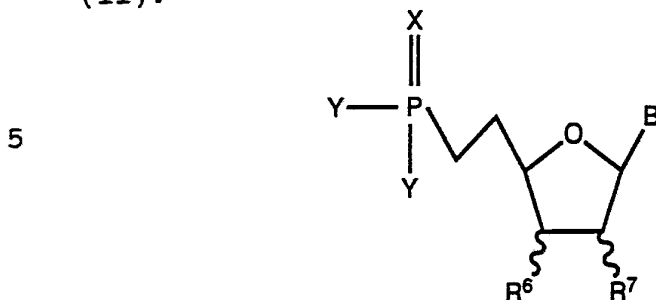
25 7. The oligonucleotide of claim 3 which is capable of forming a covalent crosslink with a target duplex DNA.

30 8. The modified oligonucleotide of claim 3 wherein R⁵ is hydrogen, hydroxyl or fluorine.

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9. A compound having the following formula
(II):



II

and stereoisomers and tautomers thereof, wherein:

B is a purine or pyrimidine base or modified form;

15 R^6 is hydrogen, hydroxyl, fluorine, O-benzyl, O-t-butyldimethylsilyl, O-DMT and O-MMT; and R^7 is hydrogen, O-allyl, S-allyl, O-methyl, S-methyl or fluorine or both R^6 and R^7 , when taken together with the carbon atom to which each is attached, form a 2', 3' epoxide group or a carbon-carbon double bond;

20 each Y is independently OR^4 , $N(R^4)_2$ or SR^4 wherein,

25 each R^4 is independently hydrogen, methyl, ethyl, propyl, isopropyl, butyl, phenyl, alkyl (4 - 18C) or substituted alkyl (1-18C);

X is selected from oxygen and sulfur;
and the corresponding salts, zwitterions, and solvates.

30 10. The compound of claim 9 wherein, R^6 is hydroxyl, O-DMT or O-t-butyldimethylsilyl;

R^7 is hydrogen;

X is oxygen; and

35 each Y is independently hydroxyl, O-methyl, O-ethyl, O-propyl, O-isopropyl, O-butyl or O-phenyl, with

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the proviso that R^6 has the α epimeric configuration corresponding to the 3' hydroxyl of ribose.

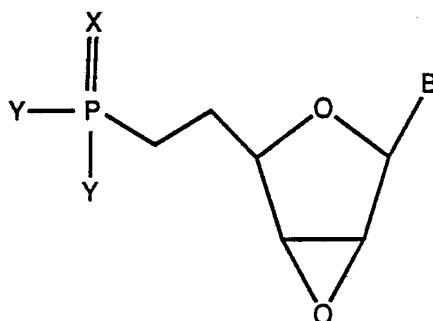
- 5
11. The compound of claim 10 wherein B is guanine.
12. The compound of claim 10 wherein B is adenine.
13. The compound of claim 10 wherein B is cytosine.
- 10
14. The compound of claim 10 wherein B is inosine, uracil, xanthine, hypoxanthine, 5-iodouracil, 5-iodocytosine, 5-ethyluracil, 5-propynyluracil, 5-fluorouracil, 5-trifluoromethyluracil, 5-bromovinyluracil, 5-propynylcytosine, 5-methylcytosine,
- 15
- 8-hydroxy- N^6 -methyladenine, aziridinylcytosine, 2-aminopurine, 2,6-diaminopurine, 3-deazaguanine, 6-chloropurine, 7-deazaadenine, 8-bromoadenine, 7-deazaguanine, 3-deazaadenine, 6-hydroxylaminopurine and 6-thiopurine.
- 20
15. The compound of claim 9 wherein,
 R^6 and R^7 are both hydrogen;
X is oxygen; and
each Y is independently hydroxyl, O-methyl, O-
- 25
- ethyl, O-propyl, O-isopropyl, O-butyl or O-phenyl, with the proviso that R^6 has the α epimeric configuration corresponding to the 3' hydroxyl of ribose.
- 30
16. The compound of claim 15 wherein B is thymine.
17. The compound of claim 15 wherein B is adenine.
18. The compound of claim 15 wherein B is cytosine.
- 35

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19. The compound of claim 15 wherein B is inosine.

20. The compound of claim 15 wherein B is guanine, uracil, xanthine, hypoxanthine, 5-iodouracil, 5-iodocytosine, 5-ethyluracil, 5-propynyluracil, 5-fluorouracil, 5-trifluoromethyluracil, 5-bromovinyluracil, 5-propynylcytosine, 5-methylcytosine, 8-hydroxy-N⁶-methyladenine, aziridinylcytosine, 2-aminopurine, 2,6-diaminopurine, 3-deazaguanine, 6-chloropurine, 7-deazaadenine, 8-bromoadenine, 7-deazaguanine, 3-deazaadenine, 6-hydroxylaminopurine or 6-thiopurine.

21. The compound of claim 9 having the formula (III):

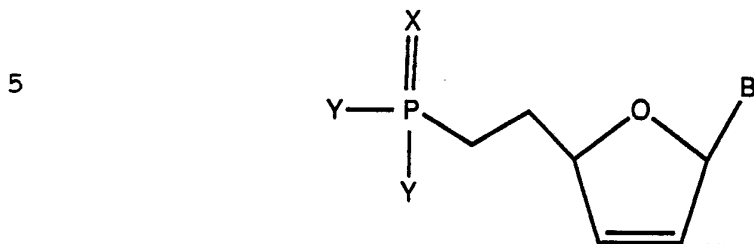


III

and stereoisomers thereof, wherein B, Y and X have any of the meanings given in claim 9 and the corresponding salts and solvates thereof.

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22. The compound of claim 9 having the formula (IV):



10

IV

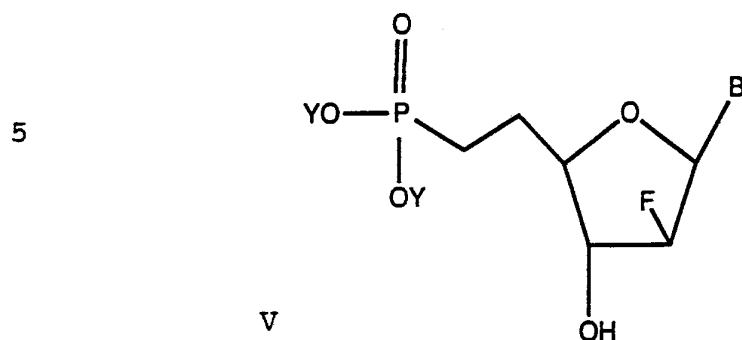
and stereoisomers thereof wherein B, Y and X have any of the meanings given in claim 9 and the corresponding salts and solvates thereof.

23. The compound of claim 22 wherein X is oxygen.
24. The compound of claim 23 wherein B is adenine.
- 20 25. The compound of claim 23 wherein B is cytosine.
26. The compound of claim 23 wherein B is thymine.
- 25 26. The compound of claim 23 wherein B is guanine, uracil, inosine, xanthine, hypoxanthine, 5-iodouracil, 5-iodocytosine, 5-ethyluracil, 5-propynyluracil, 5-fluorouracil, 5-trifluoromethyluracil, 5-bromovinyluracil, 5-propynylcytosine, 5-methylcytosine, 30 8-hydroxy-N⁶-methyladenine, aziridinylcytosine, 2-aminopurine, 2,6-diaminopurine, 3-deazaguanine, 6-chloropurine, 7-deazaadenine, 8-bromoadenine, 7-deazaguanine, 3-deazaadenine, 6-hydroxylaminopurine or 6-thiopurine.

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27. The compound of claim 9 having the formula (V):



wherein Y has the meaning defined in claim 9; and

B is selected from the group consisting of
 guanine, adenine, cytosine, thymine, uracil, inosine,
 xanthine, hypoxanthine, 5-iodouracil, 5-iodocytosine, 5-
 15 ethyluracil, 5-propynyluracil, 5-fluorouracil, 5-
 trifluoromethyluracil, 5-bromovinyluracil, 5-
 propynylcytosine, 5-methylcytosine,
 8-hydroxy-N⁶-methyladenine, aziridinylcytosine,
 2-aminopurine, 2,6-diaminopurine, 3-deazaguanine, 6-
 20 chloropurine, 7-deazaadenine, 8-bromoadenine, 7-
 deazaguanine, 3-deazaadenine, 6-hydroxylaminopurine and
 6-thiopurine.

28. The compound of claim 27 wherein B is guanine.

25

29. The compound of claim 27 wherein B is adenine.

30. The compound of claim 27 wherein B is cytosine.

30

31. The compound of claim 27 wherein B is thymine.

32. The compound of claim 27 wherein B is uracil.

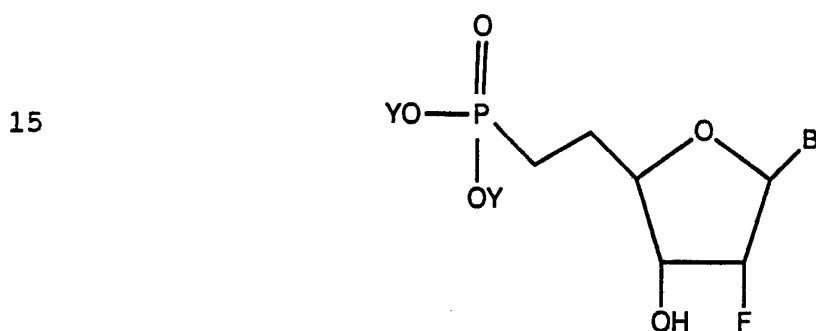
33. The compound of claim 27 wherein B is inosine.

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34. The compound of claim 27 wherein B is xanthine, hypoxanthine, 5-iodouracil, 5-iodocytosine, 5-ethyluracil, 5-propynyluracil, 5-fluorouracil, 5-trifluoromethyluracil, 5-bromovinyluracil, 5-propynylcytosine, 5-methylcytosine, 2-aminopurine, 2,6-diaminopurine, 3-deazaguanine, 6-chloropurine, 7-deazaadenine, 8-bromoadenine, 7-deazaguanine, 3-deazaadenine, 6-hydroxylaminopurine or 6-thiopurine.

10 35. The compound of claim 9 having the formula (VI):



VI

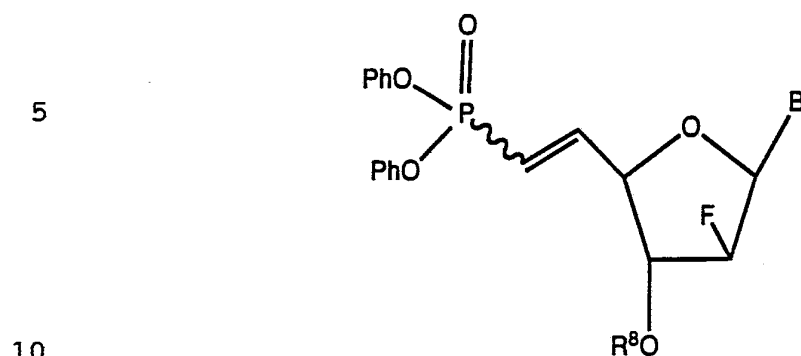
wherein Y has the meaning defined in claim 9; and

B is selected from the group consisting of guanine, adenine, cytosine, thymine, uracil, xanthine, hypoxanthine, 5-iodouracil, 5-iodocytosine, 5-ethyluracil, 5-propynyluracil, 5-fluorouracil, 5-trifluoromethyluracil, 5-bromovinyluracil, 5-propynylcytosine, 5-methylcytosine, 8-hydroxy-N⁶-methyladenine, aziridinylcytosine, 2-aminopurine, 2,6-diaminopurine, 3-deazaguanine, 6-chloropurine, 7-deazaadenine, 8-bromoadenine, 7-deazaguanine, 3-deazaadenine, 6-hydroxylaminopurine and 6-thiopurine.

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36. A compound having the formula (VII):



VII

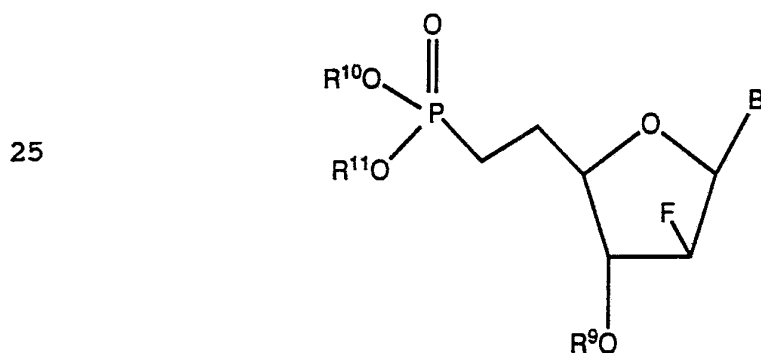
and stereoisomers thereof wherein B is a purine or pyrimidine base or a modified form; and

- 15 R^8 is t-butyldimethylsilyl.

37. The compound of claim 36 wherein B is N^2 -isobutyrylguanine, N^4 -benzoylcytosine, N^6 -benzoyladenine, thymine, uracil or adenine.

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38. A compound having the formula (VIII):



VIII

wherein,

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B is a purine or pyrimidine base or a modified form;

R⁹ is hydrogen, DMT or TBS;

R¹⁰ is hydrogen, methyl, phenyl, TBS, alkyl (2-
5 18C) or substituted alkyl (1-18C); and

R¹¹ is hydrogen, phenyl, +HTEA, Na⁺, methyl, alkyl (2-18C) or substituted alkyl (1-18C) and the corresponding salts, zwitterions and solvates.

10 39. The compound of claim 38 wherein B is guanine, adenine, cytosine, thymine, uracil, N²-isobutyrylguanine, N⁴-benzoylcytosine or N⁶-benzoyladenine.

15 40. A pharmaceutical composition useful for treatment of a viral infection or malignant condition which comprises an effective amount of a compound of claim 1 in combination with a pharmaceutically acceptable carrier.

20 41. A pharmaceutical composition useful for treatment of a viral infection or which comprises an effective amount of a compound of claim 9 in combination with a pharmaceutically acceptable carrier.

25 42. The composition of claim 9 in unit dosage form.

43. The composition of claim 27 in unit dosage form.

30 44. A method for treatment of parasitic infection or malignant condition which comprises administering to an individual in need of such treatment an effective amount of a compound of claim 9.

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45. A method for treatment of viral infection which comprises administering to an individual in need of such treatment an effective amount of a compound of claim 9.

5 46. The oligonucleotide of claim 1 which is exonuclease stable by having 2 or more 5' methylene phosphonate linkages at both 5' and 3' terminal internucleotide residues.

10 47. The modified oligonucleotide of claim 1 wherein each B is independently selected from adenine, guanine, cytosine, 5-methylcytosine, aziridinylcytosine, 8-hydroxy-N⁶-methyladenine, thymine, uracil, pseudoisocytosine and inosine.

15

48. A pharmaceutical composition useful for treatment of a tumor or parasitic infection or which comprises an effective amount of a compound of claim 9 in combination with a pharmaceutically acceptable carrier.

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49. A pharmaceutical composition useful for treatment of a tumor or parasitic infection which comprises an effective amount of a compound of claim 27 in combination with a pharmaceutically acceptable carrier.

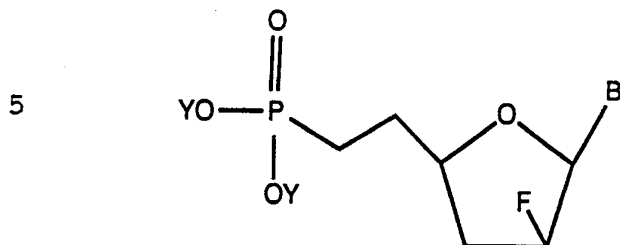
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50. The compound of claim 9 having the formula
(IX):



IX

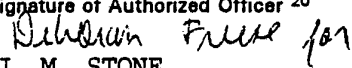
wherein Y has the meaning defined in claim 9; and

B is selected from the group consisting of
 guanine, adenine, cytosine, thymine, uracil, inosine,
 15 xanthine, hypoxanthine, 5-iodouracil, 5-iodocytosine, 5-
 ethyluracil, 5-propynyluracil, 5-fluorouracil, 5-
 trifluoromethyluracil, 5-bromovinyluracil, 5-
 propynylcytosine, 5-methylcytosine,
 8-hydroxy-N⁶-methyladenine, aziridinylcytosine,
 20 2-aminopurine, 2,6-diaminopurine, 3-deazaguanine, 6-
 chloropurine, 7-deazaadenine, 8-bromoadenine, 7-
 deazaguanine, 3-deazaadenine, 6-hydroxylaminopurine and
 6-thiopurine.

- 25 51. The compound of claim 50 wherein B is guanine.
52. The compound of claim 50 wherein B is adenine.
53. The compound of claim 50 wherein B is cytosine.
- 30 54. The compound of claim 50 wherein B is thymine.
55. The compound of claim 50 wherein B is uracil.
- 35 56. The compound of claim 50 wherein B is inosine.

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US92/01020

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)³		
According to International Patent Classification (IPC) or to both National Classification and IPC		
IPC (5): Please See Attached Sheet. US CL : Please See Attached Sheet.		
II. FIELDS SEARCHED		
Minimum Documentation Searched ⁴		
Classification System	Classification Symbols	
U.S.	514/44, 45, 46, 47, 48, 49, 50, 51; 536/27; 544/243, 244	
Documentation Searched other than Minimum Documentation to the extent that such Documents are included in the Fields Searched ⁵		
CAS ONLINE, MEDLINE, APS, BIOSIS		
III. DOCUMENTS CONSIDERED TO BE RELEVANT¹⁴		
Category*	Citation of Document, ¹⁶ with indication, where appropriate, of the relevant passages ¹⁷	Relevant to Claim No. ¹⁸
X,Y	Tetrahedron Letters, Vol. 28, No. 31, issued 1987, Padyukova et al, "A New Scheme for the Synthesis of 5'-Nucleotide Phosphonate Analogs", pages 3623-3626, see the entire document.	9, 10, 12, 14, 15, 17, 20, 41, 42, 48/1-8, 11, 13, 16, 18, 19, 40, 46, 47
X/Y	Nucleic Acids Research, Vol. 18, No. 10, issued 1990, Breaker et al, "Polynucleotide phosphorylase forms polymers from an ADP analog in which the 5' oxygen is replaced by a methylene group", pages 3085-3086, see the entire document.	1-8, 40, 46, 47/9-39, 41-45, 48-56
X/Y	Journal of Medicinal Chemistry, Volume 29, No. 6, issued 1986, Kappler et al, "Isozyme-Specific Enzyme Inhibitors. 11. L-Homocysteine-ATP S-C5' Covalent Adducts as Inhibitors of Rat Methionine Adenosyltransferases", pages 1030-1038, see the entire document.	9, 10, 12, 15, 17, 41, 42, 48/11, 13, 14, 16, 18-20
Y	US, A, 4, 757,055 (Miller et al), 12 July 1988, see the entire document.	1-56
<p>* Special categories of cited documents:¹⁵</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"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)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> <p>"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</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"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</p> <p>"&" document member of the same patent family</p>		
IV. CERTIFICATION		
Date of the Actual Completion of the International Search ²		Date of Mailing of this International Search Report ²
26 May 1992		10 JUN 1992
International Searching Authority ¹		Signature of Authorized Officer ²⁰
ISA/US		 J. M. STONE

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET		
X/Y	Abstracts, International Union Of Biochemistry, Conference on Nucleic Acid Therapeutics, 13-17 January 1991, Frick et al, "Carbocyclic Nucleoside 5'-Phosphonates", page 63, see the entire abstract.	1-14, 40-42, 46-49/15-39, 43-45, 50-56
Y	Chemical Reviews, Volume 90, No. 4, issued June 1990, Uhlmann et al, "Antisense Oligonucleotides: A New Therapeutic Principle", pages 543-584, see page 546-553, 565-567, 578-579.	1-56
V. <input type="checkbox"/> OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE¹		
<p>1. <input type="checkbox"/> Claim numbers _ because they relate to subject matter (1) not required to be searched by this Authority, namely:</p> <p>2. <input type="checkbox"/> Claim numbers _ because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out (1), specifically:</p> <p>3. <input type="checkbox"/> Claim numbers _ because they are dependent claims not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).</p>		
VI. <input type="checkbox"/> OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING²		
<p>This International Searching Authority found multiple inventions in this international application as follows:</p> <p>1. <input type="checkbox"/> As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.</p> <p>2. <input type="checkbox"/> As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:</p> <p>3. <input type="checkbox"/> No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:</p> <p>4. <input type="checkbox"/> As all searchable claims could be searched without effort justifying an additional fee, the International Search Authority did not invite payment of any additional fee.</p> <p>Remark on protest</p> <p><input type="checkbox"/> The additional search fees were accompanied by applicant's protest.</p> <p><input type="checkbox"/> No protest accompanied the payment of additional search fees.</p>		

FURTHER INFORMATION CONTINUED FROM PREVIOUS SHEETS

I. CLASSIFICATION OF SUBJECT MATTER:

IPC (5) :

C07H 19/20; C07H 19/10; A61K 48/00, 31/675

I. CLASSIFICATION OF SUBJECT MATTER:

US CL :

514/44, 45, 46, 47, 48, 49, 50, 51; 536/27; 544/243, 244