(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) Publication number: **0 517 796 B1**

(12) # EUROPEAN PATENT SPECIFICATION

(45) Date of publication of patent specification: **19.04.95** (51) Int. Cl.[6]: **C07H 21/00**, A61K 31/70

(21) Application number: **91905593.9**

(22) Date of filing: **25.02.91**

(86) International application number:
PCT/US91/01066

(87) International publication number:
WO 91/15500 (17.10.91 91/24)

(54) **OLIGONUCLEOTIDE ANALOGS.**

(30) Priority: **10.04.90 US 507693**

(43) Date of publication of application:
16.12.92 Bulletin 92/51

(45) Publication of the grant of the patent:
19.04.95 Bulletin 95/16

(84) Designated Contracting States:
AT BE CH DE DK ES FR GB GR IT LI LU NL SE

(56) References cited:
WO-A-89/03683
WO-A-89/12060
WO-A-89/12060

The Journal of Antibiotics, vol. 37, n 6, June 1984, Japan Antibiotics Research

Association, (Tokyo, JP), K. Isono et al. : " Ascamycin and dealanylascamycin,nucleoside antibiotics from.Streptomyces sp" , pages 670-672 see the wholearticle cited in the application

(73) Proprietor: **THE DU PONT MERCK PHARMACEUTICAL COMPANY**
Barley Mill Plaza
Building 25
Wilmington,
Delaware 19898 (US)

(72) Inventor: **HUIE, Edward, McDonald**
1021 Rockwell Road
Wilmington, DE 19810 (US)
Inventor: **TRAINOR, George, Leonard**
9 Carillon Court
Wilmington, DE 19803 (US)

(74) Representative: **Jones, Alan John et al**
CARPMAELS & RANSFORD
43 Bloomsbury Square
London, WC1A 2RA (GB)

Note: Within nine months from the publication of the mention of the grant of the European patent, any person may give notice to the European Patent Office of opposition to the European patent granted. Notice of opposition shall be filed in a written reasoned statement. It shall not be deemed to have been filed until the opposition fee has been paid (Art. 99(1) European patent convention).

EP 0 517 796 B1

Tetrahedron Letters, vol. 27, n 33, 1986, Pergamon Journals Ltd, (GB), M.Ubukata et al.:" Total synthesis of nucleoside antibiotic, ascomycin", pages3907-3908 see the whole article cited in the application

The Journal of the American Chemical Society, vol. 91, n 12, 4 JUNE 1969, D.A.Shuman et al.: "The synthesis of adenine 5'-O-sulfamoyl nucleosides related tonucleocidin", pages 3391-3392 see the whole article cited in the application

Annual Reports in Medicinal Chemistry, vol. 23, 1988, Academic Press Inc., P.C.Miller et al.: "Oligonucleotide inhibitors of gene expression in living cells:New opportunities in drug design", pages 295-304 see the whole article cited inthe application

Pharmaceutical Research, vol.5, n 9, September 1988, Plenum Publishing corp.,G. Zon: "Oligonucleotide analogues as potential chemotherapeutic agents", pages539-549 see the whole article cited in the application

Description

FIELD OF THE INVENTION

This invention relates to novel compounds in which one or more of the internucleotide phosphodiester linkages in oligonucleotide analogs have been replaced by a sulfur based linkage.

BACKGROUND OF THE INVENTION

The repertoire of substances available for therapeutic purposes consists primarily of relatively low-molecular weight organic compounds. Recently, the repertoire has been expanded to include proteinaceous materials which have been engineered for efficacy, specificity and stability. Increasing attention is now being focused on the therapeutic potential of other classes of biomacromolecules, including nucleic acids.

Nucleic acids are linear phosphopentose polymers bearing pendant adenine (A), guanine (G), cytosine (C), and thymine (T) [or the related uracil (U)] base groups. The pentose may be ribose (RNA) or 2'deoxyribose (DNA). They are attractive candidates for therapeutics due to the high potential for selectivity. The basis for this high selectivity is the well-known ability of a nucleic acid to form an antiparallel, two-stranded, helical structure (or duplex) with its structural complement through the formation of hydrogen bonds between the bases on opposite strands (Watson-Crick base pairs). Complementarity is defined as the pairing of G with C and A with T [or U] on opposite strands. Duplexes with perfect complementarity are thermodynamically preferred. For short [<20 residues or nucleotides (nt)] oligonucleotides, a single improper pairing or mismatch can significantly destabilize the duplex. Thus one can, in principle, selectively address a single site in a 3×10^9 nt human genome (the genetic material of a human in its entirety) with an oligonucleotide of 16-20 nt. This is substantially greater selectivity than one can generally achieve with traditional, low-molecular weight agents. With this degree of potential selectivity, one can consider the approach of exerting a therapeutic effect at the level of gene expression. For viral agents which act through integration of their genetic material into the host system, one can envision blocking one of the many steps involved in integration and replication.

Most of the attempts to use nucleic acids as complementary addressed therapeutic agents have involved single-stranded targets. Such targets include messenger RNA (mRNA) and single-stranded viral genomes. In such cases, the reagent nucleic acids are complementary to the target and are referred to as "anti-sense reagents". The process of using such agents to exert a specific effect is referred to herein as "anti-sense targeting"[1-3,32].

More recently, a second, high-specificity mode of nucleic acid binding has been investigated. It has been found that certain sequences of duplex DNA will bind a third strand to form a triple helix or triplex[4]. Triplex formation involves the formation of base triples with the additional base forming hydrogen bonds in the so-called Hoogsteen mode. Reagents designed to bind in such a mode are referred to herein as "triplex reagents" and the process of using such reagents to exert a specific effect will be referred to as "triplex targeting". The advantage of the triplex targeting approach is that one can address double-stranded genomic DNA directly. The disadvantage is that, at least at this time, not all sequences can be addressed in this fashion.

The ease of synthesis and chemical stability of simple, unmodified oligodeoxynucleotides has led to widespread investigation of their use as anti-sense reagents. This approach has been used with varying degrees of success *in vitro* against human immunodeficiency virus (HIV), Rous sarcoma virus, and c-*myc* oncogene expression, among others.[28]

Simple oligodeoxynucleotide anti-sense reagents may exert their effects in one or both of two ways. They can simply bind their target through duplex formation thereby reducing the available concentration of functional single-stranded target. Alternatively, in the case where the target is a single-stranded RNA, the RNA/DNA hybrid may serve as a substrate for endogenous ribonuclease H (RNaseH). RNaseH is an enzyme which will cleave the RNA strand of an RNA/DNA hybrid through phosphodiester bond hydrolysis. The mediation of RNaseH can allow anti-sense reagents to operate at concentrations well below those required to drive all of the target to hybrid since the reagent itself is not cleaved and each molecule can direct the cleavage of many molecules of target.

A number of structural modification approaches to improve the function of oligodeoxynucleotides as anti-sense reagents have been investigated. One class of modification involves the attachment of chemical appendages to the reagent to stabilize the reagent/target duplex or cleave the target at the site of attachment. Acridine derivatives attached via flexible tethers have been shown to improve the thermodynamic stability of the duplex through intercalation[5]. Similarly, oligodeoxynucleotides bearing tethered

psoralens can be covalently cross-linked to target following irradiation of the duplex[6]. Cross-linking can also be accomplished through the use of tethered alkylating agents[7]. Cleavage of the target through the use of oligodeoxynucleotides bearing tethered ethylenediaminetetraacetic acid (EDTA)/iron[8] or 1,10-phenanthroline/copper[9] has been demonstrated *in vitro*. Numerous other attachments for these purposes have been described. Functionalization with poly (L-lysine) has been employed to improve transport.[31]

The use of oligodeoxynucleotides as anti-sense reagents *in vivo* is hampered, however, by two fundamental problems. The first problem is that small single-stranded oligodeoxynucleotides are rapidly digested by endogenous nucleases. As a result high *in vivo* concentrations are difficult to sustain. The second problem is that oligodeoxynucleotides are highly charged having roughly one full negative charge per nucleotide residue. This generally results in a reduced rate of transport across membranes which in some cases limits the access to the ultimate site of action. These two effects combine to afford relatively low bioavailability.

The attachment of chemical functionalities (as described above) to the terminii of oligodeoxynucleotides can provide enhanced nuclease-resistance in some cases. Alpha-Oligodeoxynucleotides, in which the attachment of the base to the ribofuranosyl ring has been changed from beta- to alpha-, form parallel stranded duplexes and show increased nuclease resistance[10].

In several approaches to solve the stability and transport problems, the central phosphorus atom in the linkage has been retained but attached atoms have been replaced or modified. O-Alkylphosphotriesters are uncharged but are more bulky than the natural linkage and show somewhat reduced chemical stability[11,12]. Phosphorothioate diesters are isostructural with the natural linkage and show increased resistance to nucleases but are still charged[13]. Methylphosphonates are uncharged and considerably more lipophilic but fully replaced hybrids are not substrates of RNaseH[14].

One fundamental difficulty with these analogues is the fact that all derive from a single replacement of one of the non-bridging oxygen atoms on the phosphodiester linkage. Since that phosphorus is prochiral within the linkage, non-specific replacement of one oxygen results in the formation of a chiral center and hence a pair of diastereomers. Each additional non-specific replacement doubles the number of diastereomers present. These diastereomers have differing physical properties complicating analysis and in some cases have been shown to have widely differing abilities to form hybrids[34]. Thus oligodeoxynucleotide analogues with multiple linkage replacements are generally complex mixtures of species which can have widely differing biological efficacy.

The diastereomer mixture problem can be circumvented by replacement of both bridging oxygens in the phosphodiester linkage with the same chemical group. Along these lines, phosphorodithioate diesters have been investigated[15]. However, like the phosphorothioate linkage, this moiety is still charged.

Approaches involving more extensive modifications have also been reported. Replacement of the phosphodiester linkage with a carbon centered, neutral carbamate have been investigated[16,17]. The planarity of the carbamate linkage ensures that its introduction does not generate diastereomers but it represents a departure from tetrahedral geometry of the phosphodiester with consequences that have not been fully explored. At least one derivative has been shown to be able to form duplex with complementary target. The carbamate's nuclease sensitivity and ability to activate RNaseH have not yet been reported on.

Several recent studies have focused on oligodeoxynucleotides with partial phosphodiester linkage replacement. The rationale here is that one may be able to engineer a balanced profile of desirable properties (e.g., nuclease resistance, hybrid stability, RNaseH activation, etc.). These studies while useful are unlikely to lead to a general solution to the problems listed above.

With some analogues, the situation is further complicated by the observation that biological effects are being exerted in a non-sequence-specific manner[18,19].The origins of these effects remain obscure.

The described list of modifications explored in attempts to enhance the function of oligodeoxynucleotides as anti-sense reagents is representative but is by no means exhaustive. Two recent reviews[1,2] and one monograph[32] dealing with this subject area are comprehensive.

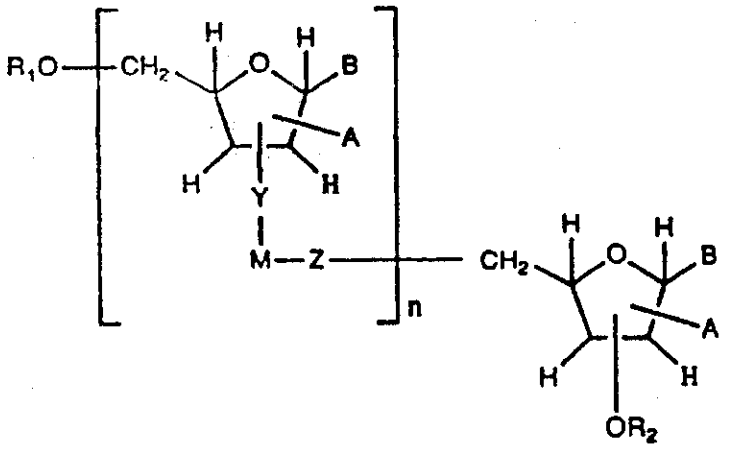
A series of naturally occurring sulfamoyl mononucleoside antibiotics and some synthetic analogs have been described[21–24]. In addition, analogs of DNA containing sulfides, sulfoxides and sulfones as linking groups between subunits capable of forming bonds with natural oligonucleotides have been described.[33] The β-decay of P^{32} oligonucleotides is expected to give a sulfate linkate oligonucleotide; however, such sulfate linked oligonucleotides have not been reported.

Applicants have developed a novel series of compounds in which one or more of the internucleotide phosphodiester linkages in oligonucleotide analogs have been replaced by a sulfur-based linkage. This linkage is isostructural and isoelectronic with the phosphodiester. Applicants have found the linkage to be synthetically accessible, chemically robust, nuclease resistant, and capable of supporting duplex formation. Compounds of this invention have excellent potential as anti-sense and triplex reagents for the therapeutic

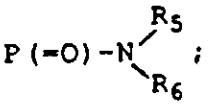
control of nucleic acid function. In addition, such compounds would have potential as antiviral agents and utility as hybridization probes.

SUMMARY OF THE INVENTION

There is provided by this invention a compound of the formula:



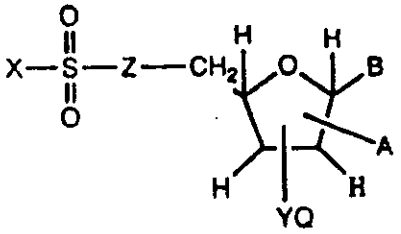
A is	H, OH, OR_8, OQ or halogen;
B is	a naturally occurring nucleic acid base or synthetic modified nucleic acid base;
Y is	RN or O;
Z is	R_7N or O;
M is	S(=O)=O, P(=O)-O$^-$, P(=O)-S$^-$, P(=S)-S$^-$, P(=O)-OR_3 , P(=O)-R_9, P(=O)-SR_4 or



provided at least one M is S(=O)=O and when M is S(=O)=O, only one of Y and Z are O;

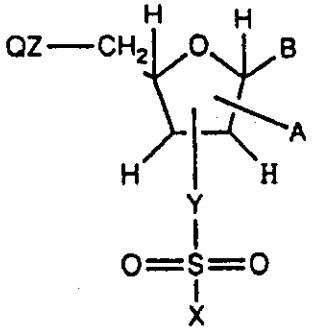
n is	1-200;
R is	H or C_{1-4} alkyl;
R_1 is	H_2PO_3, $H_3P_2O_5$, $H_4P_3O_7$ and their suitable salts, H or a protecting group;
R_2 is	H_2PO_3, $H_3P_2O_5$, $H_4P_3O_7$ and their suitable salts, H or a protecting group;
R_3 is	C_{1-4} alkyl or cyanoethyl;
R_4 is	H or C_{1-4} alkyl;
R_5 is	H or C_{1-4} alkyl;
R_6 is	H or C_{1-4} alkyl;
R_7 is	H or C_{1-4} alkyl;
R_8 is	C_{1-4} alkyl;
R_9 is	C_{1-4} alkyl or cyanoethyl; and
Q is	a protecting group.

In another aspect of this invention, intermediate compounds are provided:



wherein:

A is H, OH, OR_2, OQ or halogen;
B is a naturally occurring nucleic acid base or synthetic modified nucleic acid base;
Y is RN or O;
Z is R_1N or O;
X is a suitable leaving group;
Q is a protecting group;
R is H or C_{1-4} alkyl;
R_1 is H or C_{1-4} alkyl; and
R_2 is C_{1-4} alkyl or



Further provided by this invention is an anti-sense, triplex and antiviral reagent.

In another aspect of this invention, a hybridization probe is provided.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1. Demonstrates the melting profile of the modified oligodeoxynucleotides, 9a:9c, (circles) and the two oligodeoxynucleotides containing a single sulfamate linkage in each strand, 9b:9d, (closed squares). The buffer contained 200 mM sodium at pH 7.0.

Figure 2. Figure 2a shows an HPLC chromatogram of a standard mixture of dC, T, dG, and dA. Figure 2b shows an HPLC chromatogram of the product mixture resulting from prolonged digestion of oligodeoxynucleotide 9c. The HPLC conditions used were as follows: C18 Rainin analytical column (4.6mm x 25cm) with guard, flow of 1.0 ml/min, and a gradient of 10% water/methanol (solvent B) in 50mM KH_2PO_4/water (solvent A) of 0-20 min = 3.0%sol.B/sol.A), 20-40 min = 3-40% sol.B/sol.A (linear), 40-45 min = 40% sol.B/sol.A.

Figure 3. This figure is a photograph of an autoradiogram of a 20% denaturing polyacrylamide gel illustrating cleavage of sulfamate or phosphate linked oligonucleotides by *Nsi* I.

Figure 4. Demonstrates the meltings profile of d(AsA) vs. poly-uridine in a buffer of 10mM magnesium H at pH 7.5.

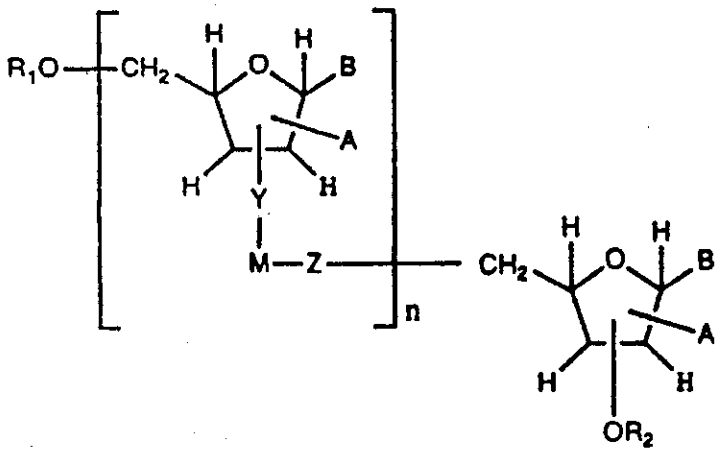
DETAILED DESCRIPTION OF THE INVENTION

All previous attempts to replace the phosphodiester linkage in oligodeoxynucleotide analogs for the purposes of enhancing function as anti-sense reagents represent partial solutions. No single phosphodiester

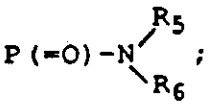
replacement has a completely satisfactory profile of properties. Applicants have generated a list of chemical, biochemical, and structural criteria which, if met by a compound, are likely to lead to superior functions such as anti-sense, anti-viral and triplex functions. The linkage should be: 1) isostructural (isoelectronic and isosterlc) with the phosphodiester; 2) nuclease resistant; 3) uncharged at physiological pH; 4) achiral; 5) chemically stable under physiological conditions; 6) synthetically accessible; and 7) amenable to analysis.

Applicants have developed compounds with at least one novel sulfur-centered linkages which meet all of these criteria. These linkages are sulfamate esters. Conceptually these derive from the sulfate diester which is isosteric and isoelectronic with the phosphodiester. Replacement of either bridging oxygen with a nitrogen affords a sulfamate ester. Applicants have found the internucleotide sulfamate linkage to be synthetically accessible, chemically robust, nuclease resistant, and capable of supporting duplex formation.

The novel compound of this invention is represented by the formula:



A is H, OH, OR_8, OQ or halogen;
B is a naturally occurring nucleic acid base or synthetic modified nucleic acid base;
Y is RN or O;
Z is R_7N or O;
M is $S(=O)=O$, $P(=O)\text{-}O^-$, $P(=O)\text{-}S^-$, $P(=S)\text{-}S^-$, $P(=O)\text{-}OR_3$, $P(=O)\text{-}R_9$, $P(=O)\text{-}SR_4$ or



provided at least one M is $S(=O)=O$ and when M is $S(=O)=O$, only one of Y and Z are O;
n is 1-200;
R is H or C_{1-4} alkyl;
R_1 is H_2PO_3, $H_3P_2O_5$, $H_4P_3O_7$ and their suitable salts, H or a protecting group;
R_2 is H_2PO_3, $H_3P_2O_5$, $H_4P_3O_7$ and their suitable salts, H or a protecting group;
R_3 is C_{1-4} alkyl or cyanoethyl;
R_4 is H or C_{1-4} alkyl;
R_5 is H or C_{1-4} alkyl;
R_6 is H or C_{1-4} alkyl;
R_7 is H or C_{1-4} alkyl;
R_8 is C_{1-4} alkyl;
R_9 is C_{1-4} alkyl or cyanoethyl; and
Q is a protecting group.

As far as the base (B) is concerned, no fundamental limitation is envisioned. One may include the naturally occurring nucleotide bases as well as synthetic modified nucleic acid bases such as inosine, deazaadenosine, etc. Preferably B is a residue of the naturally occurring nucleotide bases adenine, guanine, cytosine, thymine or uracil.[30,33]

With regard to the sugar, ribonucleotides, deoxyribonucleotides, and dideoxyribonucleotides are all possible. Sugars with halogen substitution (A is halogen) and altered sugar configuration (e.g., arabinosides and alpha-ribosides) may also be contemplated.[30]

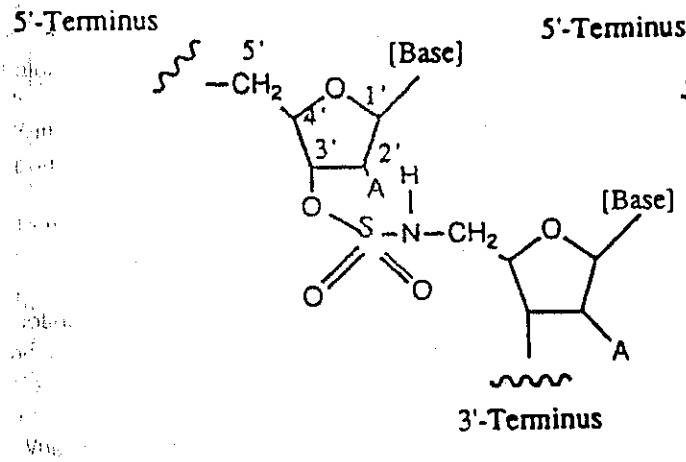
In the above compound, n is 1-200. An n of up to 200 is accessible by standard methods.

With regard to the internucleotide linkage (M), Applicants have demonstrated the preparation of compounds with only a single sulfur-based linkage as well as the preparation of fully replaced compounds. Compounds where at least one of the sulfur based linkages of this invention is substantially near at least one terminus of the compound, are expected to be particularly useful. Such compounds will be substantially protected from exonuclease activity. Compounds that are not fully replaced with the sulfur based linkages comprise linkages as defined by M, which are as found in DNA, RNA as well as synthetic modified nucleotides known to those skilled in the art. It is understood that materials with any degree of partial replacement of phosphorous based linkages should be accessible by the methods applicants have developed. Furthermore, compounds with mixed ribose structures (e.g., single strands containing both ribonucleotides and deoxyribonucleotides) may be prepared.

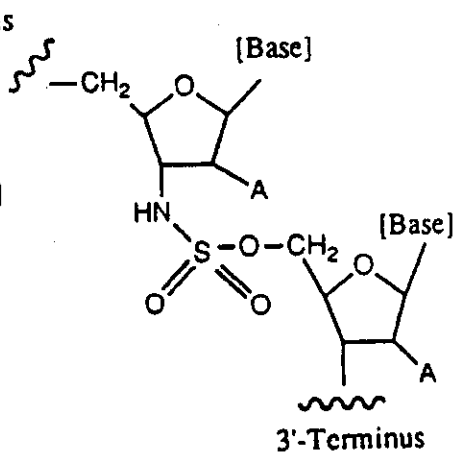
Where R substituents are alkyl, alkyls of less than 5 carbon atoms are expected to be useful.

Additionally, one may attach the chemical appendages to the compounds of this invention for the purposes of stabilizing a duplex, crosslinking, or cleaving a target sequence or for facilitating transport(*vide supra*).[5-9,31]

Unlike the phosphodiester linkage, the sulfamate has directionality. The linkage may be oriented in one of two ways with respect to the compound terminii. The two forms will be referred to as 3'-NSO or 5'-NSO depending on whether the nitrogen is on the 3' or 5' side of the attached sulfur.



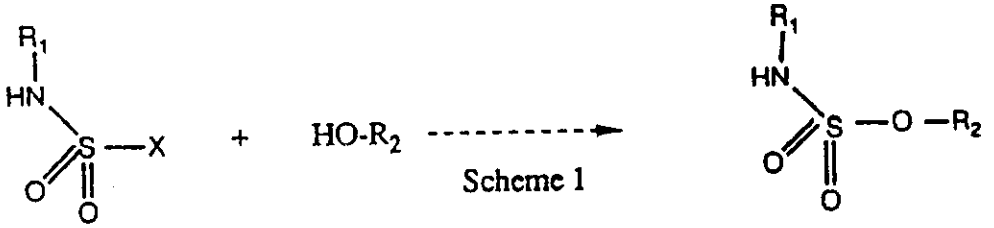
3'-NSO Sulfamate Linkage



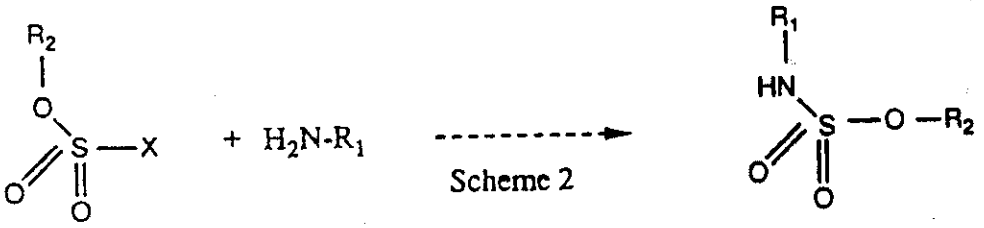
5'-NSO Sulfamate Linkage

Synthesis

Conceptually a disubstituted sulfamate can be prepared by the reaction of a hydroxy compound with a sulfamoyl-X [where X is a suitable leaving group] (Scheme 1) or by the reaction of an amino compound with alkoxysulfonyl-X (Scheme 2). Generally, the leaving group (X) is a group that is easily displaced (Examples include, halide, azide, sulfonates, etc.). Equivalent reactions with reduced sulfur reagents (e.g., sulfinyl groups) followed by oxidation may also be contemplated.

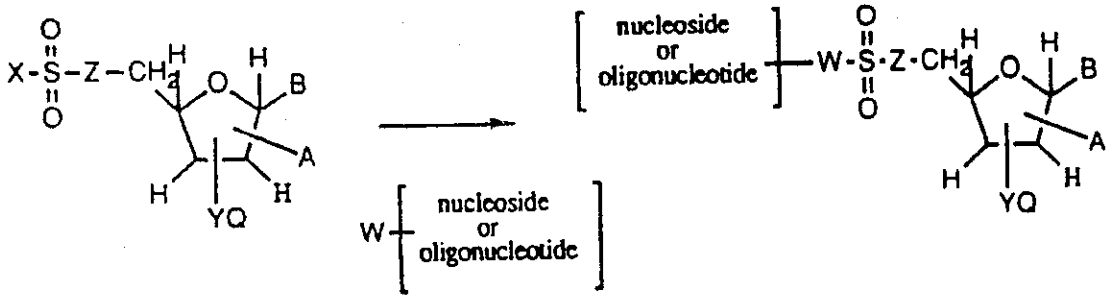


Scheme 1

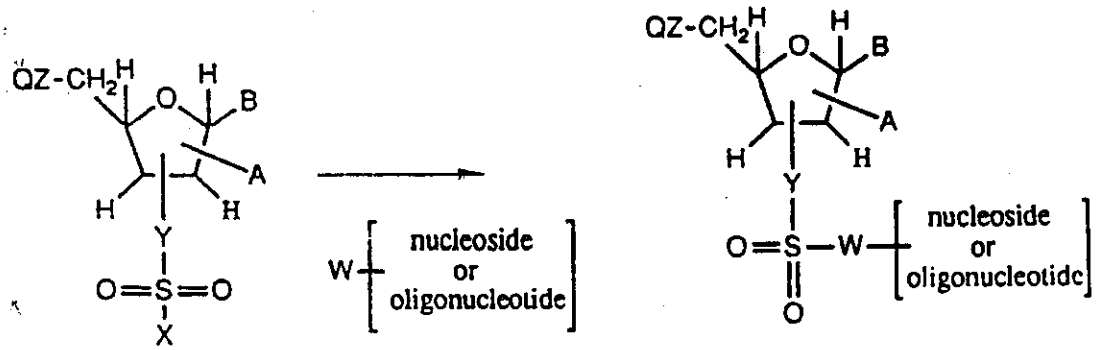


Scheme 2

Both of these routes are feasible for the preparation of the sulfamate-linked oligonucleotide derivatives that are the subject of this application. Applicants have found one specific embodiment of Scheme 1 to be particularly efficacious. In this process, a 5'-N-(X-sulfonyl)-5'-amino-5'-deoxynucleoside intermediate is reacted with a hydroxy-bearing nucleoside or hydroxy-bearing oligonucleotide to afford a 3'-NSO sulfamate linked derivative. In this process,



X is a suitable leaving group such as a halide, sulfonate, or azido group, with the azido group preferred. Similarly, 5'-NSO sulfamate linked derivatives may be prepared by reacting a 3'-N-(X-sulfonyl)-3'-amino-3'-deoxynucleoside with a hydroxy-bearing nucleoside or



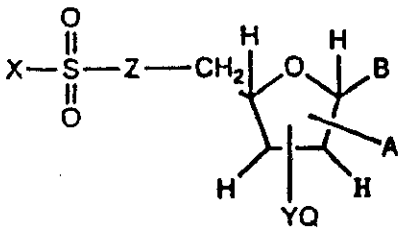
hydroxy-bearing oligonucleotide. These processes are generally carried out in the presence of a base but may also be facilitated by metal ions.

The key intermediates in these processes, the N-(X-sulfonyl)-amino-deoxynucleosides, may be conveniently prepared by the reaction of the appropriate amino-deoxynucleoside with a bifunctional sulfonating

agent X-SO_2-X'. For example, for X = azido, the N-(azidosulfonyl)-amino-deoxynucleoside is prepared by reacting the amino-deoxynucleoside with azidosulfonyl chloride.

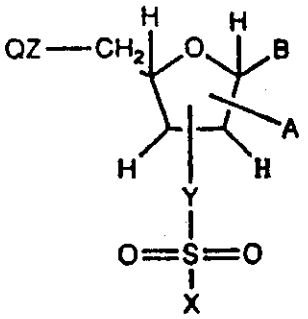
These N-(X-sulfonyl)-amino-deoxynucleoside intermediates may also be reacted with amino-bearing nucleosides or amino-bearing oligonucleotides, to afford sulfamide-linked oligonucleotide derivatives that are also the subject of this application.

The intermediates of this invention are compounds of the formula:



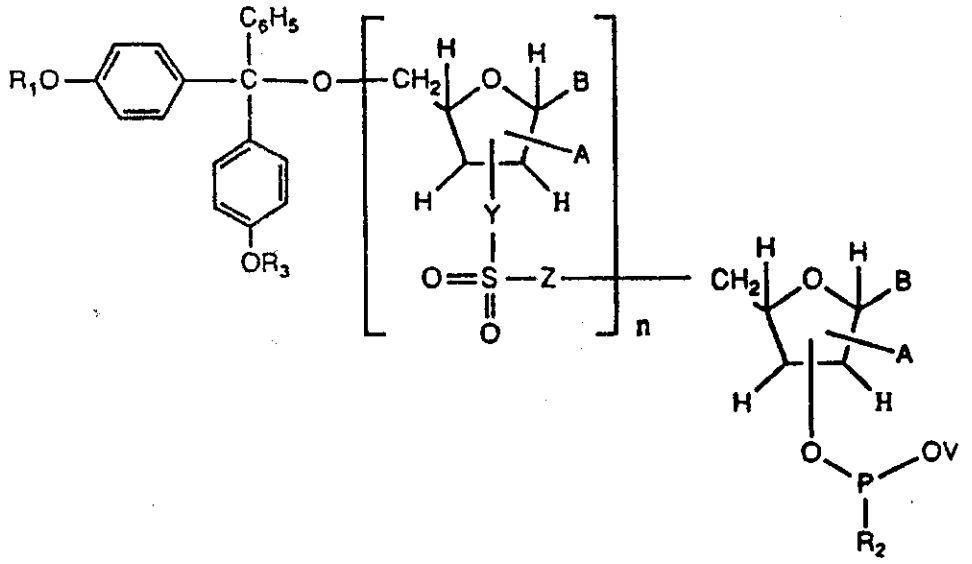
wherein:

A is	H, OH, OR_2, OQ or halogen;
B is	a naturally occurring nucleic acid base or synthetic modified nucleic acid base;
Y is	RN or O;
Z is	R_1N or O;
X is	a suitable leaving group;
Q is	a protecting group;
R is	H or C_{1-4} alkyl;
R_1 is	H or C_{1-4} alkyl; and
R_2 is	C_{1-4} alkyl or



The strategy for insertion of the sulfamate linkage into larger compounds of this invention depends on the degree of substitution desired. For introduction at a single site or just a few sites, a block-dimer approach is preferred. In this approach the standard chemistry of automated oligonucleotide synthesis based the stepwise addition of single nucleotides (3' to 5' growth) via nucleoside phosphoramidites is employed.[39] At a selected point in the synthesis, a block-dimer phosphoramadite of this invention, a 5'-dimethyoxytrityl-protected, sulfamate-linked dinucleoside 3'-phosphoramidite is used in place of the normal reagent. This results in the addition of two nucleotides, linked by a sulfamate, to the 5'-terminus of the growing chain. The block multi-mer of this invention would allow n consecutive sulfamate linkages to be introduced.

The block multi-mer phosphoramidite compound of this invention is of the formula:



A is H, OH, OR_4, OQ, or halogen;
B is a naturally occurring nucleic acid base or synthetic modified nucleic acid base;
n is 1-200;
R_1 is C_{1-4} alkyl;
R_2 is dialkylamino, morpholino, piperidino or pyrrolidino;
R_3 is C_{1-4} alkyl;
R_4 is C_{1-4} alkyl;
Y is RN or O;
Z is RN or O;
R is H or C_{1-4} alkyl;
Q is a protecting group; and
V is a protecting group.

R, R_1, R_3 and R_4 substituents with alkyls of less than 5 carbon atoms are expected to be useful.

V is a protecting group that can be cleaved under basic conditions, examples include cyanoethyl and methyl.

For all compounds of this invention, Q is alkyl or a protecting group possibly selected from acyl, trialkylsilyl, cyanoethyl, or a tetrahydropyran.

Where the A substituent contains an oxygen, three levels of protection can be imagined. In one case, the protecting group Q would be amenable to cleavage under the same conditions as for V. Another possibility is to make Q stable to the deprotection conditions used for removing V for stability reasons. The final case would be where Q is not to be removed at all, an alkyl group for example.

The issue of the stability of the sulfamate linkage is an important one in this approach. First, the linkage must be able to withstand repeated treatment under the cycling conditions: 1) removal of the 5'-trityl group with moderately strong acid; 2) coupling with a tetrazole activated phosphoramidite,; and 3) oxidation with iodine under basic conditions. Applicants have found the internucleotide linkage to be fully stable to repeated treatments under these conditions. Secondly, the linkage must be able to stand up to the conditions required to cleave the nascent oligonucleotide from the resin and remove the protecting groups from the nucleotide bases. The base protection generally involves blocking the exocyclic amino groups through acylation (for C, A: benzoylation; for G: isobutyrylation). Both cleavage from the resin and base-deprotection are generally carried out by treating with concentrated aqueous ammonia. The cleavage from the resin occurs rapidly at room temperature but complete base deprotection requires more prolonged treatment at elevated temperatures. The sulfamate linkage is fully resistant to this prolonged treatment with concentrated aqueous ammonia.

Using the block dimer approach, one may synthesize compounds, oligonucleotide analogs, bearing from one to multiple sulfamate linkages. The only limitations are that the first linkage (on the 3'-terminus) may not be a sulfamate and one may not introduce consecutive sulfamates. (These limitations may be

overcome, respectively, through the use of a resin linked sulfamate block dimer as a starter or through the use of block multimers.)

A different strategy is employed for the synthesis of compounds where all of the internucleotide linkages are to be replaced by the sulfamate. Here a stepwise synthesis is preferred. Synthesis is carried out in a 5'-3' direction adding a single nucleotide unit at a time. The 3'-protected-5'-azidosulfonamido monomer is activated *in situ* and added to the 3'-hydroxyl of the growing oligonucleotide.

This direction of chain-synthesis is the reverse of that employed in normal automated oligodeoxynucleotide synthesis. Thus the two processes are not trivially compatible. However, it is possible to conceive of cycles where either the sulfamate or the phosphodiester could be added as desired. For example, it should be possible using a 3'-azidosulfonamido-5'-protected monomer to incorporate sulfamate linkages at any point desired. This is a consequence of the extension direction now being 3' to 5', the same direction used in phosphoramidite extension.

In general an activated monomer approach is superior to a block dimer approach in that: a) all possible sequences can be addressed; and b) only four, as opposed to sixteen reagents are required.

Utility

The compounds of this invention should find application as anti-sense reagents (i.e., in therapeutics) as these compounds have substantially demonstrated those physical properties which have been shown to correlate with anti-sense function in other systems. Similarly, Applicants expect that these reagents should be able to function in triplex targeting applications.

There are other possible therapeutic applications. For example, sulfamate analogues of 2',5'-oligoadenosines might be expected to show anti-viral properties.[26]

One might expect sulfamate replaced oligodeoxynucleotides to show some of the same non-sequence-specific anti-viral efficacy observed with other analogues.[18,19]

There are many potential applications in research areas as well. The sulfamate-linked systems may be useful as hybridization probes, as reagents for mutagenesis, and as structural analogues for the study of protein/nucleic acid interactions. Sulfamate-linkage replacement could confer useful properties when incorporated in catalytic RNA systems[35].

Similar utility is expected for the oligonucleotide analog compound where M is S(=O)=O and both Y and Z are 0.

Duplex Formation:

To function as an anti-sense reagent, the compound of this invention must retain the ability to recognize (hybridize to, anneal to, form duplex with) a complementary sequence. For short oligonucleotides, this may be conveniently assayed by observing the changes in UV absorption which accompany duplex formation. Generally, the absorption at 260 nm is measured as a function of temperature.

To study the effect of replacing a single phosphodiester linkage with the sulfamate on duplex formation, a pair of modified complementary 18-mers, each having a single sulfamate linkage, were compared to the corresponding pair of unmodified 18-mers. The two pairs showed virtually identical melting profiles (smooth S-curves) with the curve for the modified pair displaced to a slightly lower temperature. That the magnitude of the absorption change is identical and the curves are nearly identical in shape suggest that the duplex is perturbed very little by the sulfamate linkage and that it undergoes single-domain melting. The slightly lower transition temperature indicates that the modified duplex is slightly less stable than its unmodified counterpart under these conditions.

Attempts to demonstrate annealing of a T-homopolymer bearing all sulfamate linkages to a short dA homopolymer (with phosphodiesters) have been unsuccessful. This is somewhat perplexing but a similar phenomenon has been observed with carbamates and methylphosphonates: oligoTs (and Us) anneal poorly or not at all to polyA, yet sequences with other bases anneal well[17,20]. This may be a peculiarity of oligoTs when the linkage is uncharged.

The annealing of a sulfamate-linked adenosine dimer to polyU has been demonstrated. Here the interaction was very strong with a transition temperature some 10 degrees higher than the corresponding unmodified adenosine dimer.

Nuclease Resistance:

For stability *in vivo*, it is desirable that an anti-sense reagent be resistant to exonucleases, which digest from either terminus in a stepwise fashion, and endonucleases, which cleave in the middle of the chain.

To test for exonuclease resistance, several compounds of this invention having a single sulfamate linkage were subjected to prolonged treatment with a mixture of an exonuclease, snake venom phosphodiesterase, and alkaline phosphatase (to facilitate analysis). In each case, the sulfamate link was recovered intact (as the dimer) with the rest of the compounds fully digested to its constituent nucleosides.

Endonuclease resistance was tested by examining the cleavage of a short double stranded oligomer containing the *Nsi I* restriction endonuclease recognition sequence. This endonuclease normally cuts both strands within its recognition sequence in a staggered fashion. Replacement of the phosphodiester bond at the cleavage site in either or both strands conferred resistance to cleavage in the altered strand(s).

EXAMPLES

Example 1

Preparation of N6-benzoyl-3'-O-t-butyldimethylsilyl-5'-sulfamoylazido-2',5'-dideoxyadenosine

A mixture of 8.9g (24 mmol) of 5'-Azido-N6-benzoyl-2',5'-dideoxyadenosine (which can be prepared as in reference 36), 5.4g (35 mmol) of t-butyldimethylsilyl chloride, and 3.3g (48 mmol) of imidazole in 100ml of anhydrous dimethylformamide (DMF) was stirred at room temperature (RT) for 20 hr. The solvent was removed under vacuum, and the residue was partitioned between one half saturated brine and EtOAc (2x200ml). The combined organic layers were washed with brine, dried with sodium sulfate (dried), and evaporated (evap.). Purification by chromatography on silica (silica gel (240-400 mesh) from EM Science) using 20 to 40% EtOAc/DCM gave 11.1g of 5'-Azido-N6-benzoyl-3'-O-t-butyldimethylsilyl-2',5'-dideoxyadenosine as a white foam: NMR (300MHz,d6-DMSO) δ 11.25 (br,1H), 8.78 (s,1H), 8.71 (s,1H), 8.05 (m,2H), 7.5-7.7 (m,3H), 6.52 (t,1H,J=7Hz), 4.72 (m,1H), 4.02 (m,1H), 3.67 (dd,1H,J=13,7Hz), 3.56 (dd,1H,J=13,5Hz), 3.09 (m,1H), 2.43 (m,1H), 0.92 (s,9H), 0.14 (s,6H).

A mixture of 5.08g (10.4 mmol) of 5'-Azido-N6-benzoyl-3'-O-t-butyldimethylsilyl-2',5'-dideoxyadenosine and 2.1g of 10% Pd/C in 69ml of ethanol and 3.0ml of acetic acid was stirred under 1 atm. of hydrogen for 4hr. The slurry was filtered through celite washing with methanol. p-Toluenesulfonic acid monohydrate (1.97g, 10.4 mmol) was added and the mixture was agitated until dissolution. The solution was evap., and the resulting solid was chromatographed on silica (1 to 12% methanol/DCM) giving 4.63g of 5'-Amino-N6-benzoyl-3'-O-t-butyldimethylsilyl-2',5'-dideoxyadenosine, p-toluenesulfonate salt: NMR (300MHz,d6-DMSO) δ 8.76 (s,1H), 8.74 (s,1H), 8.05 (d,2H,J=8Hz), 7.67 (t,1H,J=8Hz), 7.57 (t,2H,J=8Hz), 7.48 (d,2H,J=8Hz), 7.11 (d,2H,J=8Hz), 6.53 (t,1H,J=7Hz), 4.68 (m,1H), 4.01 (m,1H), 3.06 (m,2H), 2.56 (m,1H), 2.39 (m,1H), 2.29 (s,3H), 0.93 (s,9H), 0.15 (s,6H).

A mixture of 4.24g (6.68 mmol) of 5'-amino-N6-benzoyl-3'-O-t-butyldimethylsilyl-2',5'-dideoxyadenosine and 4Å molecular sieves (~5g) in 10ml of anhydrous acetonitrile and 20ml of anhydrous DCM was stirred under nitrogen for 2 hr. After adding 0.93ml (6.68 mmol) of triethylamine, the mixture was stirred for 5 min, and 0.42ml of chlorosulfonylazide (1M in acetonitrile) were added. After stirring the reaction for 1 hr., the slurry was filtered and the filtrate was partitioned between water and EtOAc (2x100ml). The combined organic layers were washed with brine, dried, and evap. Purification using chromatography on silica (1 to 3% MeOH/DCM) gave 0.89g of N6-benzoyl-3'-O-t-butyldimethylsilyl-5'-sulfamoylazido-2',5'-dideoxyadenosine as a white foam: NMR (300MHz,d6-DMSO) δ 11.22 (br,1H), 9.28 (br,1H), 8.75 (s,1H), 8.71 (s,1H), 8.05 (d,2H,J=8Hz), 7.66 (t,1H,J=8Hz), 7.56 (t,2H,J=8Hz), 6.52 (t,1H,J=8Hz), 4.67 (m,1H), 4.00 (m,1H), 3.07 (m,1H), 2.34 (m,1H), 0.93 (s,9H), 0.15 (s,6H). IR (KBr) 3400, 3080 2950, 2930, 2880, 2860, 2130, 1700, 1610, 1585, 1510, 1370, 1170 cm-1.

Example 2

Preparation of N6-benzoyl-3'-O-t-butyldimethylsilyl-5'-sulfamoylazido-2',5'-dideoxycytidine

A mixture of 12.09g (35.1 mmol) of 5'-Azido-N6-benzoyl-2',5'-dideoxycytidine (which can be prepared as in reference 36), 7.94g (52.7 mmol) of t-butyldimethylsilyl chloride, and 4.78g (70.3 mmol) of imidazole were stirred in 70ml of anhydrous DMF under nitrogen at RT for 20hr. The DMF was removed under vacuum, and the resulting mixture was dissolved in DCM and loaded directly on a column of silica. A 25%

EtOAc/DCM solution was used to elute the product from the column. Evap. of the fractions containing the product gave 13.35g of 5'-Azido-N6-benzoyl-3'-O-t-butyldimethylsilyl-2',5'-dideoxycytidine as a white foam: NMR (300MHz,d6-DMSO) δ 11.31 (br,1H), 8.18 (d,1H,J = 8Hz), 8.01 (m,2H), 7.63 (m,1H), 7.56 (t,2H,J = 7Hz), 7.39 (d,1H,J = 8Hz), 6.20 (t,1H,J = 7Hz), 4.38 (m,1H), 3.96 (m,1H), 3.68 (dd,1H,J = 13,6Hz), 3.61 (dd,1H,J = 13,5Hz), 2.30 (m,1H), 0.88 (s,9H), 0.10 (s,6H).

A mixture of 6.88g (15 mmol) of 5'-Azido-N6-benzoyl-3'-O-t-butyldimethylsilyl-2',5'-dideoxycytidine and 3.0g of 10% Pd/C in 130ml of ethanol and 5ml of acetic acid was stirred under 1 atm. of hydrogen for 4hr. The slurry was filtered through celite washing with methanol. p-Toluenesulfonic acid monohydrate (2.85g, 15 mmol) was added and the mixture was agitated until dissolution. The solution was evap., and the resulting solid was chromatographed on silica (1 to 12% methanol/DCM) giving 6.32g of pure 5'-amino-N6-benzoyl-3'-O-t-butyldimethylsilyl-2',5'-dideoxycytidine p-toluenesulfonate salt as a tan solid: NMR (300MHz,d6-DMSO) δ 8.31 (d,1H,J = 8Hz), 8.02 (d,2H,J = 8Hz), 7.64 (m,1H), 7.52 (m,4H), 7.38 (d,1H,J = 8Hz), 7.12 (d,2H,J = 8Hz), 6.19 (t,1H,J = 6.5Hz), 4.43 (m,1H), 3.97 (m,1H), 3.02 (m,2H), 2.30 (m,5H), 0.89 (s,9H), 0.11 (s,6H).

A mixture of 3.40g (5.63 mmol) of 5'-amino-N6-benzoyl-3'-O-t-butyldimethylsilyl-2',5'-dideoxycytidine p-toluenesulfonate salt and 4Å molecular sieves (~5g) in 30ml of 50% anhydrous DCM/acetonitrile was stirred under nitrogen for 1hr. After cooling to 0°C, 0.78ml (5.63 mmol) of triethylamine was added and stirring was continued for 10 min. Finally 3.9 ml (3.9 mmol) of chlorosulfonylazide (1M in acetonitrile) was added, the cold bath was removed, and the reaction was stirred for 2.5 hr. The slurry was filtered, and the filtrate was partitioned between one half saturated brine and 300 ml of EtOAc. The organic layer was washed with brine, dried, and evap. Purification by chromatography on silica (1 to 4% methanol/DCM) gave 0.99g of product that contained two impurities. Furthur chromatography afforded pure N6-benzoyl-3'-O-t-butyldimethylsilyl-5'-sulfamoylazido-2',5'-dideoxycytidine as a white foam: NMR (300MHz,d6-DMSO) δ 11.27 (br,1H), 9.30 (br,1H), 8.21 (d,1H,J = 8Hz), 8.01 (m,2H), 7.62 (m,1H), 7.50 (t,2H,J = 8Hz), 7.37 (d,1H,J = 8Hz), 6.17 (t,1H,J = 6.5Hz), 4.39 (m,1H), 3.95 (m,1H), 3.35 (m,2H), 2.27 (m,2H), 0.89 (s,9H), 0.10 (s,6H).

Example 3

Preparationof3'-O-t-butyldimethylsilyl-N4-i-butyryl-O6-(4-nitrophenethyl)-5'-sulfamoylazido-2',5'-dideoxyguanosine

Using the procedure in reference 37, 5.00g (17.5 mmol) of 2'-deoxyguanosine (Sigma, St. Louis, MO), 63ml of anhydrous DCM, 13ml of anhydrous pyridine, and 9.3ml (88 mmol) of isobutyryl chloride gave 10.34g of crude N4,(3',5'-O)-tri-i-butyryl-2'-deoxyguanosine.

After dissolving 10.34g (~17 mmol) of crude N4,(3',5'-O)-tri-i-butyryl-2'-deoxyguanosine in 70ml of anhydrous DCM under nitrogen, 8.91g (34 mmol) of triphenylphosphine and 5.68g (34 mmol) of 4-nitrophenethyl alcohol were added and dissolved with stirring. After cooling to 0°C, 5.3ml (34 mmol) of diethyldiazodicarboxylate were added over 5 minutes (min). The cold bath was removed, and the reaction was stirred for 18hr. A TLC of the reaction mixture (10% methanol/DCM) indicated some of the starting material was still present. Triphenylphosphine (4.45g), 4-nitrophenethyl alcohol (5.30g), and diethyldiazodicarboxylate (2.65ml) were added sequentially, and the mixture was stirred another one hr. The solvent was evaporated, and the residue was used directly in the next reaction. A sample was purified by chromatography on silica (1 to 5% methanol/DCM) to give N4,(3',5'-O)-tri-i-butyryl-O6-(4-nitrophenethyl)-2'-deoxyguanosine as a white foam: NMR (300MHz,CdCl3) δ 8.17 (d,2H,J = 8Hz), 8.07 (br,1H), 7.97 (s,1H), 7.53 (d,2H,J = 8Hz), 6.36 (dd,1H,J = 7,6Hz), 5.43 (m,1H), 4.84 (t,2H,J = 7Hz), 4.54 (dd,1H,J = 12,5Hz), 4.39 (m,2H), 3.33 (t,2H,J = 7Hz), 2.5-3.1 (m,5H), 1.39 (d,6H,J = 7Hz), 1.32 (d,6H,J = 7Hz), 1.26 (d,3H,J = 7Hz), 1.24 (d,3H,J = 7Hz);

Crude N4,(3',5'-O)-tri-i-butyryl-O6-(4-nitrophenethyl)-2'-deoxyguanosine (~17 mmol) was dissolved in 400ml of methanol and 400ml of 27% ammonium hydroxide were added. The mixture was cloudy so enough methanol was added (100ml) to make the reaction homogeneous. After stirring for 20 hr at RT, the solvents were evap. and the residue was taken up in 100ml of DCM. Enough hexane was added to make the solution cloudy. After standing overnight, the light yellow crystals were collected to give 5.83g of N4-i-butyryl-O6-(4-nitrophenethyl)-2'-deoxyguanosine. A second crystallization gave an additional material for a total of 8.60g of N4-i-butyryl-O6-(4-nitrophenethyl)-2'-deoxyguanosine:NMR (300MHz,20% d4-methanol/CDCl3) δ 8.18 (d,2H,J = 9Hz), 8.17 (s,1H), 7.57 (d,2H,J = 9Hz), 6.38 (t,1H,J = 7Hz), 4.84 (t,2H,J = 7Hz), 4.72 (m,1H), 4.07 (q,1H,J = 4Hz), 3.83 (ddd,2H,J = 33,12,4Hz), 3.33 (t,2H,J = 7Hz), 2.75-2.9 (m,2H), 2.44 (ddd,1H,J = 14,7,4Hz), 1.37 (d,6H,J = 8Hz).

A solution of 7.28g (14.9 mmol) of N4-i-butyryl-O6-(4-nitrophenethyl)-2'-deoxyguanosine in 75ml of anhydrous pyridine was cooled to 0 °C under nitrogen. After adding 2.84g (14.9 mmol) of p-toluenesulfonyl chloride, the reaction was stirred at 0 °C for 2hr and then at 5 °C for 18hr. The pyridine was evap., and the residue was partitioned between water and EtOAc. The organic layer was washed with brine, dried, and evap. to give 6.22g. Purification by chromatography on silica (1 to 5% methanol/DCM) gave 4.08g of N4-i-butyryl-O6-(4-nitrophenethyl)-5'-O-p-toluenesulfonyl-2'-deoxyguanosine as a white foam: (300MHz,20% d6-DMSO) δ 10.34 (br,1H), 8.24 (s,1H), 8.19 (d,2H,J = 8Hz), 7.67 (d,2H,J = 8Hz), 7.61 (d,2H,J = 8Hz), 7.22 (d,2H,J = 8Hz), 6.38 (t,1H,J = 7Hz), 5.46 (d,1H,J = 5Hz), 4.82 (t,2H,J = 7Hz), 4.64 (m,1H), 4.33 (ddd,2H,J = 26,12,8Hz), 3.97 (m,1H), 3.35 (t,2H,J = 7Hz), 2.82 (m,2H), 2.27 (s,3H), 1.12 (d,3H,J = 7Hz), 1.10 (d,3H,J = 7Hz).

A solution of 4.08g (6.35 mmol) of N4-i-butyryl-O6-(4-nitrophenethyl)-5'-O-p-toluenesulfonyl-2'-deoxyguanosine and 467mg (9.52 mmol) of lithium azide (Kodak, Rochester, NY) in 21ml of anhydrous DMF was heated to 100 °C for 3hr. The solvent was removed under vacuum. The residue was partitioned between water and EtOAc. The organic layer was washed with brine, dried, and evap. to give 3.95g of crude product. Purification by chromatography on silica (1 to 10% methanol/DCM) gave 1.47g of the less polar material and 419mg of the more polar material. The less polar material was a white foam and was found to be 5'-azido-N4-i-butyryl-O6-(4-nitrophenethyl)-2',5'-dideoxyguanosine: NMR (300MHz, d6-DMSO) δ 10.40 (br,1H), 8.41 (s,1H), 8.18 (d,2H,J = 8Hz), 7.64 (d,2H,J = 8Hz), 6.37 (t,1H,J = 7Hz), 5.46 (d,1H,J = 4Hz), 4.79 (t,2H,J = 7Hz), 4.63 (m,1H), 3.97 (m,1H), 3.86 (dd,1H,J = 13,8Hz), 3.53 (dd,1H,J = 13.5Hz), 3.36 (t,2H,J = 7Hz), 3.05 (m,1H), 2.84 (m,1H), 2.28 (ddd,1H,J = 14,6,3Hz), 1.12 (d,6H,J = 8Hz).

In the same manner as for 5'-Azido-N6-benzoyl-3'-O-t-butyldimethylsilyl-2',5'-dideoxycytidine, 1.47g (2.87 mmol) of 5'-azido-N4-i-butyryl-O6-(4-nitrophenethyl)-2',5'-dideoxyguanosine, 0.65g (4.3mmol) of t-butyldimethylsilyl chloride, 0.39g (5.7 mmol) of imidazole, and 10ml of anhydrous DMF was used to obtain after chromatography on silica (10% EtOAc/DCM) 1.49g of 5'-azido-3'-O-t-butyldimethylsilyl-N4-i-butyryl-O6-(4-nitrophenethyl)-2',5'-dideoxyguanosine as a white foam: NMR (300MHz, d6-DMSO) δ 10.40 (br,1H), 8.40 (s,1H), 8.18 (d,2H,J = 9Hz), 7.65 (d,2H,J = 9Hz), 6.35 (t,1H,J = 7Hz), 4.78 (t,2H,J = 7Hz), 4.73 (m,1H), 3.94 (m,1H), 3.84 (dd,1H,J = 13,8Hz), 3.56 (dd,1H,J = 13,5Hz), 3.31 (t,2H,J = 7Hz), 3.10 (m,1H), 2.82 (m,1H), 2.29 (m,1H), 1.10 (d,6H,J = 7Hz), 0.89 (s,9H), 0.14 (s,3H), 0.12 (s,3H).

A mixture of 100mg (.159 mmol) of 5'-azido-3'-O-t-butyldimethylsilyl-N4-i-butyryl-O6-(4-nitrophenethyl)-2',5'-dideoxyguanosine, 0.064ml (.64 mmol) of 1,3-propanedithiol, 0.089ml (.64 mmol) of triethylamine, and 1ml of methanol was stirred under nitrogen at RT for 4 days. After adding 0.5ml of acetic acid, the reaction was concentrated under vacuum. Purification by chromatography on silica (1 to 15% methanol/DCM) gave 121mg of the acetic acid salt of the product. This material was partitioned between saturated potassium carbonate and EtOAc. The organic layer was dried and evap to give 91mg of 5'-amino-3'-O-t-butyldimethylsilyl-N4-i-butyryl-O6-(4-nitrophenethyl)-2',5'-dideoxyguanosine: NMR (300MHz, CDCl3) δ 8.17 (d,2H,J = 8Hz), 7.98 (s,1H), 7.90 (br,1H), 7.52 (d,2H,J = 8Hz), 6.28 (t,1H,J = 7Hz), 4.80 (t,2H,J = 7Hz), 4.71 (m,1H), 3.93 (q,1H,J = 4Hz), 3.32 (t,2H,J = 7Hz), 3.04 (dd,1H,J = 14,4Hz), 2.93 (dd,1H,J = 14,6Hz), 2.84 (m,1H), 2.39 (m,1H), 1.28 (d,6H,J = 7Hz), 0.93 (s,9H), 0.13 (s,3H), 0.11 (s,3H).

A solution of 90mg (.15 mmol) of 5'-amino-3'-O-t-butyldimethylsilyl-N4-i-butyryl-O6-(4-nitrophenethyl)-2',5'-dideoxyguanosine in 2ml of anhydrous DCM was stirred at RT under nitrogen while 0.11ml of chlorosulfonylazide (1M in acetonitrile) was added. After stirring for 1 hr., the solution was partitioned between one half saturated brine and EtOAc. The organic layer was washed with brine, dried, and evap. The residue was purified by chromatography on silica (1 to 3% methanol/DCM) to give 18mg of 3'-O-t-butyldimethylsilyl-N4-i-butyryl-O6-(4-nitrophenethyl)-5'-sulfamoylazido-2',5'-dideoxyguanosine: NMR (300MHz, CDCl3) δ 8.67 (br,1H), 8.46 (t,1H,J = 6Hz), 8.16 (d,2H,J = 8Hz), 7.81 (s,1H), 7.54 (d,2H,J = 8Hz), 6.17 (dd,1H,J = 8,6Hz), 4.87 (m,3H), 4.17 (m,1H), 3.57 (m,1H), 3.34 (t,2H,J = 7Hz), 2.98 (m,1H), 2.65 (m,1H), 2.25 (m,1H), 1.27 (d,3H,J = 7Hz), 1.26 (d,3H,J = 7Hz), 0.93 (s,9H), 0.16 (s,3H), 0.14 (s,3H).

Example 4

Preparation of 3'-O-t-butyldimethylsilyl-5'-sulfamoylazido-5'-deoxythymidine

A solution of 25.0g (93.6 mmol) of 5'-azido-5'-deoxythymidine (which can be prepared as in reference 36), 21.1g (140 mmol) of t-butyldimethylsilyl chloride, and 12.7g (187 mmol) imidazole in 190ml of anhydrous DMF was stirred at RT under nitrogen for 18hr. The DMF was removed under vacuum. The oil was partitioned between ether (500ml) and 1N NaOH (2x300ml). The aqueous layers were cooled with ice, and 650ml of 1N HCl were added. The product was extracted with EtOAc (2x750ml), and the organic layer was washed with brine, dried, and evap. After remaining under high vacuum for 24hr, the oil crystallized to

give 27.9g of 5'-azido-3'-O-t-butyldimethylsilyl-5'-deoxythymidine as a white solid: NMR (300MHz,d6-DMSO) δ 11.34 (br,1H), 7.50 (s,1H), 6.16 (t,1H,J=7Hz), 4.36 (m,1H), 3.84 (m,1H), 3.55 (m,2H), 2.34 (m,1H), 2.05 (m,1H), 1.79 (s,3H), 0.86 (s,9H), 0.08 (s,6H).

A mixture of 15.0g (39.3 mmol) of 5'-azido-3'-O-t-butyldimethylsilyl-5'-deoxythymidine and 4.0g of 10% Pd/C in 200ml of EtOAc was stirred vigorously under 1 atm. of hydrogen for 4.5 hr. The slurry was filtered through celite, rinsing with methanol. The filtrate was evap. to give 15.5g of a thick oil (contains some solvent). A sample was purified by chromatography on silica (2-10% methanol/DCM) to give pure 5'-amino-3'-O-t-butyldimethylsilyl-5'-deoxythymidine as a white solid: NMR (300MHz,d6-DMSO) δ 7.64 (s,1H), 6.13 (dd,1H,J=8,6Hz), 4.37 (m,1H), 3.65 (m,1H), 2.78 (m,2H), 2.21 (m,1H), 2.01 (ddd,1H,J=12,6,4Hz), 1.79 (s,3H), 0.88 (s,9H), 0.08 (s,6H).

A mixture of 5.52g of 5'-amino-3'-O-t-butyldimethylsilyl-5'-deoxythymidine and 168mg of ammonium sulfate in 150ml of hexamethyldixilazane (HMDS) was heated to dissolution under nitrogen. After stirring at RT for 18hr, the slurry was heated to 80 °C for 2hr. The HMDS was removed under vacuum to give a thick oil which was dissolved in 45ml of anhydrous acetonitrile under nitrogen. This solution was added to an addition funnel and dripped into 10ml of chlorosulfonylazide (1M in acetonitrile) precooled to 0 °C over 25 minutes. After the addition, the cold bath was removed, and the mixture was stirred for 1hr. The reaction was poured onto 200ml of half saturated brine and extracted with EtOAc (2x200ml). The combined organic layers were washed with brine, dried, and evap. to give 5.29g of a solid. Purification by chromatography on silica (1 to 5% methanol/DCM) gave 3.70g of reasonably pure 3'-O-t-butyldimethylsilyl-5'-sulfamoylazido-5'-deoxythymidine as a tan foam: NMR (300MHz,CDCl3) δ 8.57 (br,1H), 7.02 (s,1H), 6.88 (br,1H), 5.75 (t,1H,J=8Hz), 4.49 (m,1H), 4.04 (m,1H), 3.48 (m,2H), 2.72 (m,1H), 2.18 (m,1H), 1.92 (s,3H), .89 (s,9H), .11 (m,6H).

Example 5

Preparation of 3'-O-acetyl-5'-sulfamoylazido-5'-deoxythymidine

A mixture of 1.00g (3.53 mmol) of 5'-amino-3'-O-acetyl-5'-deoxythymidine (which can be prepared as in reference 38) and 94mg of ammonium sulfate was stirred in 20ml of HMDS under nitrogen at RT for 18hr. The slurry was gently heated (60 °C) for 24hr. The HMDS was removed under vacuum into a trap at -78 °C. Acetonitrile (40ml) was added under nitrogen giving a homogenous solution. After adding 5.0ml of chlorosulfonylazide (1.0M in acetonitrile) to the reaction, the mixture was stirred for 1.5hr. Water (50ml) was added and the product was extracted with EtOAc (2x150ml). The organic layers were washed with brine, dried, and evap. giving 0.89g of a solid. Purification by chromatography on silica (1 to 5% methanol/DCM) gave 0.64g of pure 3'-O-acetyl-5'-sulfamoylazido-5'-deoxythymidine as a foam: NMR (300MHz,d6-DMSO) δ 11.40 (Br,1H), 9.31 (Br,1H), 7.59 (s,1H), 6.17 (dd,1H,J=7.5,6.5Hz), 5.14 (m,1H), 4.03 (m,1H), 3.44 (m,2H), 2.47 (m,1H), 2.25 (m,1H), 2.07 (s,3H), 1.80 (s,3H); IR (film) 3200, 2920, 2840, 2135, 1730, 1685, 1365, 1240, 1170 cm-1; Mass Spectrum (FAB) meas 388.98 m/z, calcd for C12H16N6O7S 389.08 (M+H);

Example 6

Preparation of 5'-dimethoxytrityl-3'-sulfamoylazido-3'-deoxythymidine

A slurry of 2.17g of 3'-azido-3'-deoxythymidine (Aldrich, Milwaukee, WI) and .91g of 10% Pd/C in 25ml of ethanol were vigorously stirred under 1 atm. of hydrogen for 4 hr. The mixture was filtered through celite, washed with methanol, and the filtrate was evap. Purification by chromatography on silica (2 to 40% methanol/DCM) gave 1.72g of 3'-amino-3'-deoxythymidine as a light yellow solid: NMR (300MHz,d6-DMSO) δ 7.76 (s,1H), 6.07 (t,1H,J=6Hz), 4.97 (m,1H), 3.5-3.7 (m,3H), 3.39 (q,1H,J=7Hz), 3.33 (br,2H), 1.9-2.15 (m,2H), 1.77 (s,3H).

A solution of 1.04g of 3'-amino-3'-deoxythymidine, 0.90ml of triethylamine, and 1.5ml of ethyl trifluoroacetate in 10ml of methanol was stirred under nitrogen at RT for 20 hr. Evaporation gave a product that was used without purification. A sample was subjected to column chromatography on silica (1 to 20% methanol/DCM) affording pure 3'-trifluoroaceamido-3'-deoxythymidine: NMR (300MHz,d6-DMSO) δ 11.33 (br,1H), 9.84 (br,1H), 7.76 (s,1H), 6.26 (t,1H,J=7Hz), 5.15 (t,1H,J=5Hz), 4.47 (m,1H), 3.89 (m,1H), 3.6 (m,2H), 2.27 (m,2H), 1.78 (s,3H).

To a solution of crude 3'-trifluoroaceamido-3'-deoxythymidine in pyridine was added 1.2ml of triethylamine, 0.10g of N,N-dimethylaminopyridine, and 2.2g of dimethoxytrityl chloride under nitrogen. After stirring for 18 hr., the solvent was evap. The dark oil was partitioned between one half saturated bicarbonate

and EtOAc. The organic layer was washed with brine, dried, and evap. Purification by chromatography on silica (1 to 7% methanol/DCM) gave 2.18g of pure 5'-dimethoxytrityl-3'-trifluoroacetamido-3'-deoxythymidine as a yellow solid: NMR (300MHz,CDCl3) δ 8.89 (br,1H), 8.31 (d,1H,J=7Hz), 7.65 (s,1H), 7.2-7.45 (m,9H), 6.84 (d,4H,J=9Hz), 6.51 (t,1H,J=7Hz), 4.72 (m,1H), 4.09 (m,1H), 3.78 (s,6H), 3.53 (m,2H), 2.45 (m,2H), 1.64 (s,3H).

A solution of 1.54g of 5'-dimethoxytrityl-3'-trifluoroacetamido-3'-deoxythymidine in methanol was saturated with ammonia gas at RT. The reaction vessel was sealed for 4 days. After the solvent was evap., the residue was purified using chromatography on silica (1 to 10% methanol/DCM) giving 1.17g of 3'-amino-5'-O-dimethoxytrityl-3'-deoxythymidine: NMR (300MHz,d6-DMSO) δ 7.51 (s,1H), 7.2-7.45 (m,9H), 6.88 (d,4H,J=8Hz), 6.14 (t,1H,J=6Hz), 3.74 (s,6H), 3.68 (m,1H), 3.50 (q,1H,J=7Hz), 3.33 (br,2H), 3.21 (m,2H), 2.21 (m,1H), 2.03 (m,1H), 1.47 (s,3H).

In the same manner as with example 4, 605mg of 3'-amino-5'-O-dimethoxytrityl-3'-deoxythymidine, 20ml of HMDS, 0.78ml of chlorosulfonylazide (1M in acetonitrile), and 6ml of anhydrous acetonitrile were used to obtain (after purification on silica using 1 to 5% methanol/DCM) 217mg of 5'-dimethoxytrityl-3'-sulfamoylazido-3'-deoxythymidine as a light orange solid: NMR (300MHz,CDCl3) δ 7.58 (s,1H), 7.2-7.4 (m,9H), 6.85 (d,4H,J=9Hz), 6.55 (t,1H,J=7Hz), 4.31 (m,1H), 4.23 (m,1H), 3.78 (s,6H), 3.50 (dd,1H,J=11,3Hz), 3.39 (dd,1H,J=11,3Hz), 2.46 (m,2H), 1.47 (s,3H).

Example 7

Preparation of the sulfamate linked dimer d(AsT)

Stirred a mixture of 480mg of the compound from Example 4 and 493mg of N6-benzoyl-5'-dimethoxytrityl-2'-deoxyadenosine (Aldrich, Milwaukee, WI) in 7ml of dry acetonitrile with some 4Å mol. sieves for 2hr. Added 0.17ml of triethylamine to the reaction and stirred 18hr. The mixture was filtered and evap. Purification by chromatography on silica (1-7% methanol/DCM) gave 621mg of (DMTO)A^{Bz}sT(OTBDMS) as a white solid: NMR (300MHz,d6-DMSO) δ 11.33 (s,1H), 11.23 (br,1H), 8.58 (s,1H), 8.57 (s,1H), 8.51 (br,1H), 8.05 (m,2H), 7.5-7.7 (m,4H), 7.34 (m,2H), 7.21 (m,7H), 6.80 (dd,4H,J=8,5Hz), 6.52 (t,1H,J=7Hz), 6.14 (t,1H,J=7Hz), 5.28 (m,1H), 4.37 (m,2H), 3.81 (m,1H), 3.71 (s,6H), 3.29 (m,4H), 3.14 (dd,1H,J=17,5Hz), 2.78 (m,1H), 2.26 (m,1H), 2.04 (m,1H), 1.77 (s,3H), 0.87 (s,9H), 0.09 (s,6H). mass spectrum (FAB) m/z 1075.47 (assigned M+H), calcd for C54H62N8O12SSi + H 1075.40. IR (CH2Cl2) 3380, 3180, 3060, 2960, 2930, 2860, 1695, 1610, 1585, 1510, 1360, 1180, 1250 CM-1.

The fully protected dimer, (DMTO)A^{Bz}sT(OTBDMS), was treated with a total of 6ml of 1M tetra-n-butylammonium fluoride/THF (Aldrich) in four equal aliquots in one hr. intervals (small s designates sulfamate linkage). After the final additon, the reaction was stirred for 1hr. The solution was partioned between 1M sodium dihydrogen phosphate and ethyl acetate (EtOAc). The organic layer was washed with brine, dried, and evap. The crude product was chrom. on silica (1-7% methanol/DCM) gaving 420mg of (DMTO)A^{Bz}sT(OH) as a solid: NMR (300MHz,d6-DMSO) x 11.31 (Br,1H), 11.22 (br,1H), 8.58 (s,2H), 8.47 (br,1H), 8.03 (m,2H), 7.45-7.7 (m,4H), 7.34 (m,2H), 7.20 (m,7H), 6.81 (dd,4H,J=8,5Hz), 6.52 (t,1H,J=7Hz), 6.14 (t,1H,J=7Hz), 5.39 (d,1H,J=5Hz), 5.29 (m,1H), 4.38 (m,2H), 4.17 (m,1H), 3.80 (m,1H), 3.72 (s,6H), 3.3 (m,4H), 3.14 (m,1H), 2.79 (m,1H), 2.4 (m,1H), 2.15 (m,1H), 1.76 (s,3H). IR (KBR) 3400, 3180, 3055, 2955, 2930, 2830, 1690, 1610, 1580, 1510, 1360, 1250, 1180 cm-1. mass spectrum (FAB) 961.66 (M+H), calcd for C48H48N8O12S + H 961.07.

To a solution of 102mg of (DMTO)A^{Bz}sT(OH) in 5ml of acetonitrile was added 0.22ml of dichloroacetic acid. The orange solution was stirred at RT for 15 min. After pouring the reaction into one half saturated bicarbonate, the product was extracted with EtOAc (2x40ml). The combined organic layers were washed with brine, dried, and evap. Purification by chromatography on silica (1 to 10% methanol/DCM) gave 68mg of (HO)A^{Bz}sT(OH): NMR (300MHz,d6-DMSO) δ 11.33 (br,1H), 11.26 (br,1H), 8.77 (s,1H), 8.70 (s,1H), 8.47 (br,1H), 8.06 (d,2H,J=8Hz), 7.66 (t,1H,J=8Hz), 7.59 (s,1H), 7.55 (d,2H,J=8Hz), 6.52 (t,1H,J=7Hz), 6.18 (t,1H,J=7Hz), 5.41 (d,1H,J=5Hz), 5.32 (t,1H,J=5Hz), 5.23 (m,1H), 4.25 (m,1H), 4.19 (m,1H), 3.82 (m,1H), 3.65 (m,2H), 3.0-3.3 (m,3H), 2.78 (m,1H), 2.0-2.3 (m,2H), 1.79 (s,3H). IR (KBr) 3420, 1690, 1620, 1585, 1360, 1175 cm-1. mass spectrum (FAB) meas. 659.23 (M+H), calcd for C27H30N8O10S + H: 659.19.

A slurry of 31mg of (HO)A^{Bz}sT(OH) in 3ml of methanol was saturated at RT with ammonia gas. The reaction was sealed and heated to 55°C for 4 hr. The solvent was evap. Purification by chromatography on silica (methanol/DCM gradient) gave 19mg of d(AsT): NMR (300MHz,d6-DMSO) δ [partial spectrum] 8.34 (s,1H), 8.14 (s,1H), 7.35 (s,1H), 7.41 (br,2H), 6.37 (dd,1H,J=9,6Hz), 6.17 (t,1H,J=7Hz), 5.67 (br,1H), 5.39 (d,1H,J=5Hz), 5.18 (m,1H), 4.2 (m,2H), 3.81 (m,1H), 3,63 (m,2H), 3.19 (m,1H), 3.01 (m,1H), 2.66 (m,1H), 2.18 (m,1H), 1.78 (s,3H).

Example 8

Preparation of a Sulfamate Ester linked dA-Nucleoside Dimer d(AsA)

A mixture of 57mg (.10 mmol) of the compound from Example 1, 65mg (.10 mmol) and N6-benzoyl-5'-dimethoxytrityl-2'-deoxyadenosine (Aldrich) in 0.5ml of acetonitrile was stirred at RT under nitrogen. Triethylamine .028ml (.2 mmol) was added and the reaction was stirred for 20 hr. After evaporation of the mixture and purification on silica (1-3% methanol/DCM) 87mg of the fully protected dimer, (DMTO)$A^{Bz}sA^{Bz}$-(OTBDMS), were obtained: NMR [partial spectrum] (300MHz,d6-DMSO) δ 11.23 (br,2H), 8.66 (s,1H), 8.65 (s,1H), 8.58 (s,1H), 8.56 (s,1H), 8.56 (br,1H), 8.04 (d,4H,J=8Hz), 7.5-7.7 (m,6H), 7.15-7.35 (m,9H), 6.77 (dd,4H,J=8,6Hz), 6.53 (t,1H,J=7Hz), 6.47 (t,1H,J=7Hz), 5.27 (m,1H), 4.64 (m,1H), 4.34 (m,1H), 4.01 (m,1H), 3.68 (s,6H), 3.02 (m,1H), 2.78 (m,1H), 2.36 (m,1H), 0.91 (s,9H), 0.15 (s,6H).

51mg of (DMTO)$A^{Bz}sA^{Bz}$(OTBDMS) were treated under nitrogen with a total of 1.5ml of 1M tetra-n-butylammonium fluoride in THF added in three aliquots over 1hr intervals. The reaction was stirred at RT for 1 hr. after the final addition of the fluoride reagent. The reaction was partitioned between 1M sodium dihydrogen phosphate and EtOAc. The organic layer was washed with brine, dried, and evap. Purification by column chromatography on silica (1-6% methanol/DCM) gave 36mg of pure (DMTO)d($A^{Bz}sA^{Bz}$)(OH): NMR (300MHz,d6-DMSO) δ 11.22 (br,2H), 8.67 (s,1H), 8.65 (s,1H), 8.58 (br,2H), 8.54 (br,1H), 8.05 (d,4H,J=7Hz), 7.5-7.7 (m,6H), 7.33 (m,2H), 7.2 (m,7H), 6.79 (dd,4H,J=7Hz), 6.52 (t,1H,J=7Hz), 6.47 (t,1H,J=7Hz), 5.53 (d,1H,J=5Hz), 5.37 (m,1H), 4.45 (m,1H), 4.34 (m,1H), 4.01 (m,1H), 3.69 (s,6H), 3.1-3.5 (m,5H(est.)), 2.7-2.95 (m,2H), 2.38 (m,1H).

Treated 21 mg of (DMTO)($A^{Bz}sA^{Bz}$)(OH) with 3ml of saturated NH3/MeOH in a sealed vial for 3 days at RT. The solvent was evaporated and the residue was stirred with 1ml of 80% HOAc/water at RT for 45min. The solvent was removed using a vacuum pump/rotovap and a water bath at RT. Pure d(AsA) was obtained by HPLC: C8 Rainin 10mm x 25cm column (with guard), gradient = 10-60% MeOH/water (linear), flow = 3.5ml/min, sample dissolved in 20% MeOH/water, 6c eluted at 21.2min. NMR [partial spectrum] (300MHz,d4-MeOH,all chemical shifts relative to MeOH @ 3.30) δ 8.23 (s,1H), 8.20 (s,1H), 8.19 (s,1H), 8.11 (s,1H), 6.37 (m,2H), 5.22 (d,1H,J=6Hz), 4.59 (m,1H), 4.33 (m,1H), 4.16 (dd,1H,J=7,4Hz), 3.74 (dq,2H,J=13,3Hz), 3.47 (m,2H), 2.85-3.0 (m,2H), 2.69 (ddd,1H,J=13,6,1.5Hz), 2.37 (ddd,1H,J=13,6,3Hz).

Example 9

Preparation of an Oligonucleotide with a Sulfamate Linked to the 2' or 3' position of a ribonucleoside [((Ph)-3CO)[rU]sT(OTBDMS)].

To a solution of 200mg of 5'-trityluridine (Sigma, St. Louis, MO) and 189mg of 3'-O-t-butyldimethylsilyl-5'-sulfamoylazido-5'-deoxythymidine (from Example 4) in 2.5ml of anhydrous acetonitrile under nitrogen was added 0.11ml of triethylamine. After stirring at RT for 20 hr., the solvent was evaporated, and the residue was purified by chromatography on silica (1 to 7% methanol/DCM) giving 81mg of the major product as one spot on TLC. Analytical HPLC shows a 50:50 mixture of products. Prep HPLC (C8 10x250mm column with guard (Rainin), gradient: 80-90% over 25 min. of methanol/0.1M triethylammonium bicarbonate, flow=4ml/min, sample: 10-15mg/injection) afforded pure ((Ph)3CO)[rU]sT(OTBDMS) (2' linked) and pure (-(Ph)3CO)[rU]sT(OTBDMS) (3' linked). 2' linked: retention time=16.61 min.; NMR (300MHz,d6-DMSO) δ 11.47 (br,1H), 11.34 (br,1H), 8.39 (br,1H), 7.69 (d,1H,J=8Hz), 7.25-7.4 (m,15H), 6.15 (dd,1H,J=7,6Hz), 5.93 (d,1H,J=4Hz), 5.71 (m,1H), 5.49 (d,1H,J=8Hz), 5.01 (m,1H), 4.42 (m,1H), 4.36 (m,1H), 3.99 (m,1H), 3.78 (m,1H), 3.23 (m,2H), 2.25 (m,1H), 2.03 (m,1H), 1.77 (s,3H), 0.87 (s,9H), 0.09 (s,6H). 3' linked: ret. time=18.15 min.; NMR (300MHz,d6-DMSO) δ 11.46 (br,1H), 11.33 (br,1H), 8.37 (br,1H), 7.65 (d,1H,J=9Hz), 7.35-7.5 (m,16H), 6.13 (dd,1H,J=8,6Hz), 6.07 (br,1H), 5.79 (d,1H,J=6Hz), 4,87 (m,1H), 4.47 (m,1H), 4.34 (m,1H), 4.29 (m,1H), 3.80 (m,1H), 3.1-3.4 (m,7H), 2.24 (m,1H), 2.03 (m,1H), 1.76 (s,1H), 0.87 (s,9H), 0.08 (s,6H).

Example 10

Preparation of Sulfamate Ester Linked Block Dimer Phosphoramidite [(DMTO)A^{Bz}sT(OP(OCH_2CH_2CN)N-(iPr_2))]

After dissolving 294mg of (DMTO)A^{Bz}sT(OH) in 3ml of dry DCM, 116ul of 2-cyanoethyl N,N,N',N',-tetraisopropylphosphorodiamidite (Aldrich, Milwaukee, WI) and 31mg of diisopropylammonium tetrazolide

salt (prepared by mixing equal amounts of tetrazole and diisopropylamine) were added and the mixture was stirred for 1hr. The reaction not complete by TLC (10% methanol/DCM). Another 60ul of 4d and 15mg of the tetrazole salt were added and stirring was continued for another 1hr. The reaction was partitioned between half saturated bicarbonate and EtOAc. The organic layer was washed with brine, dried, and evap. The product was chrom. on silica (1-5% methanol/DCM with 0.5% pyridine) giving 452mg of semi-pure (DMTO)A^{Bz}sT(OP(OCH_2CH_2CN)N(iPr_2)). This material was dissolved in 3ml of DCM and added this to 150ml of pentane at -78 • C with stirring. The precipitate was filtered and collected. Repeated this procedure once. The white solid was evaporated from dry pyridine (2x) followed by dry toluene. Obtained (DMTO)-A^{Bz}sT(OP(OCH_2CH_2CN)N(iPr_2)) as white solid (used without furthur purification): ^{31}P NMR (300MHz,d6-DMSO) ∂ 151.133 (s), 150.596 (s) (minor impurities at 15-21 ppm); ^{1}H NMR (300MHz,d6-DMSO) ∂ 11.33 (br,1H), 11.21 (br,1H), 8.58 (s,2H), 8.52 (br,1H), 8.03 (m,2H), 7.1-7.7 (m), 6.80 (m,4H), 6.52 (t,1H,J = 7Hz), 6.15 (t,1H,J = 7Hz), 5.16 (m,1H), 4.46 (m,1H), 4.37 (m,1H), 3.9-4.1 (m), 2.88 (t,2H,J = 6Hz), 2.77 (q,2H,J = 6Hz), 2.2 (m,1H), 1.76 (s,3H), 1.17 (m). mass spectrum (FAB) meas. 1161.76 (M + H), calcd. for C57H65N10O13PS + H 1161.43.

Example 11

Preparation of Oligodeoxyribonucleotides with a Single Sulfamate Linkage

The oligodeoxynucleotide $^{5'}$d(**GCGTGCATGC-[AsT]CGTACG**)$^{3'}$ (**9c**) was synthesized on the CODER automated DNA synthesizer on a 1 micromole scale using standard protocols with the block dimer phosphoramidite ((DMTO)A^{Bz}sT(OP(OCH_2CH_2CN)N(iPr_2))) (from Example 10) in a 0.1M acetonitrile solution placed on the X port. The oligomer was cleaved from the solid support using 27% ammonium hydroxide with slow elution over 1hr. This solution was put in a sealed vial and heated to 55 • C for 4hr. After evaporation on a rotary evaporator, the crude product was purified by HPLC (C8 column, 10mm x 25cm) using a linear gradient of 5-15% acetonitrile/0.1M triethylammonium acetate (TEAA). The oily product was evaporated from 50% ethanol/water until a white powder was obtained. The purified product had a retention time of 9.00 min. (C8 analytical 10cm columm, 1.0ml/min, 5-20% CH3CN/0.1M TEAA over 15 min.). A stock solution containing 5.0 ODs 9c/ml water (sterile) was prepared and stored in the freezer.

Example 12

Annealing of a Pair of Oligodeoxyribonucleotides with Single Sulfamate Linkages

The buffer used was pH7.00, 10 mM aqueous sodium phosphate, containing 200mM sodium chloride and 0.1 mM EDTA. The reference cells contained this buffer. Complementary strands (1.5 μM each) were allowed to anneal 15 • C and then the UV absorption at 260 nm was measured as the termperature was raised to 75 • C (0.4 • C/min). The resulting melting curves are shown in *Figure 1*. Solid diamonds show data from the oligonucleotide pair **9a**:9c (estimated T_m = 68 • C) and the open squares show data from the oligonucleotide pair **9b**:**9d** (estimatedT_m = 72 • C). [See below for oligonucleotide sequences **9a**, **9b**, and **9d**.]

Example 13

Demonstration of the Exonuclease Resistance of an Oligodeoxyribonucleotide with a Single Sulfamate Linkage

The enzyme stock solutions used were 1.8 Units/ml of SVP, (phosphodiesterase I, Sigma, St. Louis, MO) and 147 Units/ml of AP (alkaline phosphatase, Sigma, St. Louis, MO). The buffer solutions used were tris (100mM, pH 9) and $MgCl_2$ (50mM). The digestion was carried out in a 1.5ml eppendorf tubes by incubating 0.1 Abs. units of the compound from example 14 with 25.6ul of tris buffer, 24ul of $MgCl_2$ buffer, 10ul of SVP stock solution, and 13.6ul of AP stock solution at 37 • for 18hr. Next 10ul of 2.5M sodium acetate was added followed by 250ul of ethanol. After chilling to -78 • for 30min and centrifuging (12K rpm, 10min), the supernatant was diluted to 1ml with ethanol and respun.The supernatant was collected and evaporated on a speed-vac. The residue was redissolved in 1.0ml of sterile water, and was stored at 4 • until analyzed. *Figure 2a* shows an HPLC chromatogram of a standard mixture of dC, T, dG, and dA. *Figure 2b* shows an HPLC chromatogram of the product mixture resulting from prolonged digestion of oligodeoxynucleotide 9c. It can be seen that when the ODN containing the sulfamate ester linkage is

digested for an extended length of time, the block dimer emerges intact. An HPLC chromatogram of a sample of the totally deprotected block dimer, d(AsT), which was independently synthesized (see example 19), confirmed that the peak labled as [AsT] had the same retention time as the synthetic material. This demonstrates that the sulfamate ester linkage is extremely resistant to exonucleases.

Example 14

Demonstration of the Endonuclease Resistance of Duplexes from Oligodeoxyribonucleotides with Single Sulfamate Linkages

The oligonucleotide 9a was synthesized as described in Example 11 for **9c**. Oligonucleotides 9b and 9d were synthesized by standard methods well known to those skilled in the art. The additional sequences are:

9a $^{5'}$d(**CGTACGATGC[AsT]GCACGC**)$^{3'}$

9b $^{5'}$d(**CGTACGATGCATGCACGC**)$^{3'}$

9d $^{5'}$d(**GCGTGCATGCATCGTACG**)$^{3'}$

Each of the four oligomer strands **9a-9d** (10-100 pmol ends) were 5'-end-labelled using T4 polynucleotide kinase (10 units) with γ-^{32}P ATP(3000 Ci/mmol). Strands were purified by phenol:chloroform extraction, chloroform extraction, ether extraction and two successive ethanol precipitations. Excess ATP was removed using a NACS PrePac column following the standard protocol (BRL, Bethesda, MD).

The 5'-endlabelled single-stranded oligomers (12,000 cpm , ~1 pmol) were annealed to their unlabelled complements (10 pmol) in 11 µl of buffer containing 50 mM tris-HCl (pH 8.0), 10 mM $MgCl_2$ and 100 mM NaCl. The mixture was heated to 80 °C for 15 min and then cooled slowly to room temperature for 60 min. Restriction endonuclease *Nsi* I (10 units in 1 µl) and 7 µl of enzyme reaction buffer was added to each of the annealed samples. The duplex DNA and *Nsi* I mixtures were placed at 37 °C for 60 min. The samples were ethanol precipitated with the addition of 10 µl of 7.5 M ammonium acetate, 1 µl of tRNA and 90 µl of 100 % ethanol. Samples were centrifuged 15 min at 14,000 rpm at room temperature, supernatent liquid was removed and the DNA pellets were dried *in vacuo*. Samples were resuspended in 3 µl of formamide dye containing 80% deionized formamide, 0.1% xylene cyanol and 0.1% bromophenol blue, and were loaded onto a 20% denaturing polyacrylamide gel. Samples were electrophoresed for two hours at 2000 V. Gel was exposed to Kodak XAR film for 8-24 hours at -70 °C. The results are shown in *Figure 3*. Lanes 1 and 2 contain strand 9a annealed to strands 9c and 9d respectively; lanes 3 and 4 contain strand 9b annealed to strands 9c and 9d respectively; lanes 5 and 6 contain strand 9c annealed to strands 9b and 9a respectively; lanes 7 and 8 contain strand 9d annealed to strands 9b and 9a respectively. -S-indicates the sulfamate linkage and -P- indicates the phosphodiester linkage.

Example 15

Annealing of a Sulfamate Linked A-Nucleoside Dimer with PolyU

The buffer used was 10mM in magnesium chloride and Tris (base), and was adjusted to pH 7.5 with 0.1M HCl. The reference cell contained this buffer. The sample cell contained .660 OD units of poly U (Sigma, St. Louis, MO) and .330 OD units of d-AsA in 1.00 ml of buffer. The cells were equilibrated at 0.6 °C for 1 hour, and the temperature was ramped at 1 °C/min. The melting profile is shown in *Figure 4*. The Tm is estimated to be 15-18 °C with a hypochromic effect of 35%. The reported literature value for the corresponding natural phosphodiester dimer, d-ApA, is 7.6 °C[25]. This example demonstrates that oligodeoxynucleotides containing only sulfamate linkage(s) can hybridize to their complementary unmodified counterparts and that the hybrid can have greater stability.

Example 16

Demonstration of the stability of the Sulfamate when Linked to the 2' or 3' position of a ribonucleotide

A solution of 1mg of 3' linked ((Ph)3CO)[rU]sT(OTBDMS) (from Example 9) in 3ml of 27% ammonium hydroxide was incubated in a sealed vial at RT for 24 hr. The solvent was evap. When the residue was analysized by HPLC (C18 Rainin analytical column (4.6mm x 25cm) with guard, flow of 1.5 ml/min, and a linear gradient of 80-90% methanol/water over 15 min.) none of the peak at 12.375 min. had converted to the 2'-linked ((Ph)3CO)[rU]sT(OTBDMS) (ret. time 11.110 min.). When a 1mg sample of the 2' linked ((Ph)-3CO)[rU]sT(OTBDMS) was treated in a similar manner, no equilibration was seen either. This demonstrates

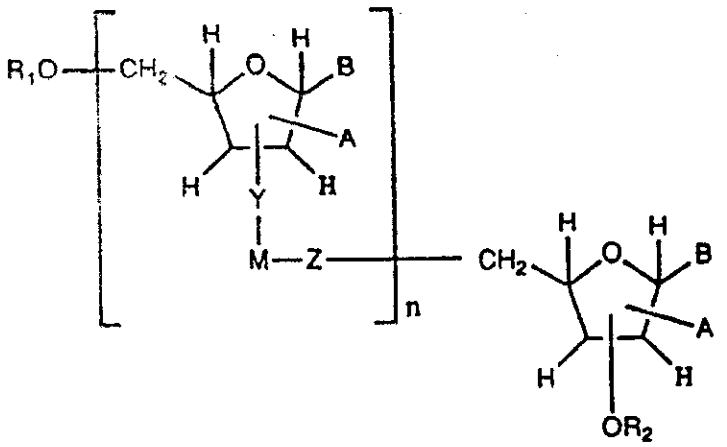
that sulfamate linked oligoribonucleotide derivatives are base stable with respect to cleavage and migration.

1. G. Zon, *Pharmaceut. Res.*, 5, 539-549 (1988).
2. P. S. Miller and P.O.P.Ts'O. in *Ann. Rep. Med. Chem., No. 23*, Vinick, ed., p. 295-304, Academic Press, New York (1988).
3. "Antisense RNA and DNA", *Current Communications in Molecular Biology series*, Cold Spring Harbor Press, Cold Spring Harbor (1988).
4. H. E. Moser and P. B. Dervan, *Science*, **238**, 645-650 (1987).
5. C. Cazenave, N. Loreau, N. T. Thuong, J.-J. Toulme, and C. Helene, *Nucl. Acids Res.*, **15**, 4717-4736 (1987).
6. U. Pieles and U. Englisch, *Nucl. Acids. Res.*, **17**, 285-299 (1989).
7. V. V. Vlassov, V. F. Zarytova, I. V. Kitiavin, S. V. Mamaev, and M. A. Podyminogin, *Nucl. Acids. Res.*, **14**, 4065-4076 (1986).
8. B. C. F. Chu and L. E. Orgel, *Proc. Nat. Acad. Sci. USA*, **82**, 963-967 (1985).
9. C. B. Chen and D. S. Sigman, *J. Amer. Chem. Soc.*, **110**, 6570-6572 (1988).
10. F. Morvan, B. Rayner, J.-L. Imbach, S. Thenet, J. -R. Bertrand, J. Paoletti, C. Malvy, and C. Paoletti, *Nucl. Acids Res.*, **15**, 3421-3437 (1987).
11. M. Weinfeld and D. C. Livingston, *Biochem.*, **25**, 5083-5091 (1986).
12. H. M. Moody, M. H. P. van Genederen, L. H. Koole, H. J. M. Kocken, E. M. Meijer, and H. M. Buck, *Nucl. Acids Res.*, **17**, 4769-4782 (1989).
13. M. Matsukura, K. Shinozuka, G. Zon, H. Mitsuya, M. Reitz, J. S. Cohen, and S. Broder, *Proc. Nat. Acad. Sci. USA*, **84**, 7706-7710 (1987).
14. P. J. Furdon, Z. Dominski, and R. Kole, *Nucl. Acids. Res.*, **17**, 9193-9204 (1989).
15. W. K.-D. Brill, J. -Y. Tang, Y. -X. Ma, and M. H. Caruthers, *J. Amer. Chem. Soc.*, **111**, 2321-2322 (1989).
16. J. M. Coull, D. V. Carlson, and H. L. Weith, *Tet. Lett.*, **28**, 745-748 (1987).
17. E. P. Stirchak, J. E. Summerton, and D. D. Weller, *J. Org. Chem.*, **52**, 4202-4206 (1987).
18. S. Agrawal, J. Goodchild, M. P. Civeira, A. H. Thronton, P. S. Sarin, and P. C. Zamecnik, *Proc. Nat. Acad. Sci. USA*, **85**, 7079-7083 (1988).
19. M. H. Caruthers, W. K -D Brill, Y. -X. Ma, W. S. Marshall, J. Nielsen, H. Sasmor, and J. -Y. Tang, J. Cell. Biochem., *Supp. 13D*, 17 (1989).
20. Poster presented by C. Bionski at Conference on "Recognition Studies in Nucleic Acids", Sheffield, England, April 16-21, 1989.
21. D. A. Shuman, R. K. Robins, and M. J. Robins, *J. Amer. Chem. Soc.*, **91**, 3391-3392 (1969).
22. G. R. Gough, D. N. Nobbs, J. C. Middleton, F. Penglis-Caredes, and M. H. Maguire, *J. Med. Chem.*, **21**, 520-525 (1978).
23. K. Isono, M. Uramoto, H. Kusakabe, N. Miyata, T. Koyama, M. Ubukata, S. K. Sethi, and J. A. McCloskey, *J. Antibiot.*, **37**, 670-672 (1984).
24. M. Ubukata and K. Isono, *Tet. Lett.*, **27**, 3907-3908 (1986).
25. P. S Miller, K. N. Fang, N. S. Kondo, and P. O. P. Ts'o, *J. Amer. Chem. Soc.*, **93**, 6657-6665 (1971).
26. Charachon, G., Sobol, R. W. Bisbal, C., Salehzada, T., Silhol, M., Charubula, R., Pfleiderer, W., Lebleu, B. & Suhadolnik, *Biochemistry*, **29**, 2550-2555 (1990).
27. Zamecnik, P. R., Goodchild. J., Taguchi, Y. and Sarin, P.S., *Proc. Natl. Acad. Sci., USA*, **83**, (1986) pp. 1028-1032.
28. Harel-Bellan, A. Ferris, D. K., Vinocour, M., Holt, J. T., and Farrar, W. L., *J. Immunol.*, **140**, (1988) 2431-2435.
29. Walder, R. Y., and Walder, J. A., (1988) *Proc. Natl. Acad. Sci. USA*, **85**, (1988), 5011-5015.
30. Principles of Nucleic Acid Structures, (1984) Springer-Verlag, New York, pp. 51-104, 159-200.
31. Lamaitre, M., Bayard, B. and Leblew, B. (1987) *Proc. Natl. Acad. Sci. USA*, **84**, 648-652.
32. "Oligodeoxynucleotides: Antisense Inhibitors of Gene Experssion", *Topics in Molecular and Structural Biology series*, CRC Press Inc., Boca Raton, Florida (1989).
33. S. Benner, WO 89/12060.
34. P. S. Miller, J. Yano, E. Yano, C. Carroll, K. Joyaraman, and P. Tso, *Biochem.*, **18**, 5134-5143 (1979).
35. R. B. Waring, *Nucl. Acids Res.*, 17, 10281-10293 (1989).
36. I. Yamamoto, M. Sekine, and T. Hata, *J. Chem. Soc., Perkin Trans. 1*, 306-310 (1980).
37. Charubala, Uhlmann, Beter, and Pfleiderer, *Synthesis*, 965 (1984).
38. T.-S. Lin, *J. Pharm. Sci.*, 73, 1568-1570 (1984).
39. *Oligonucleotide Synthesis: A Practical Approach*, Ed. by M. J. Gait, IRL Press Ltd., Washington, DC (1984).

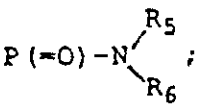
All references are incorporated herein.

Claims

1. A compound of the formula:



A is H, OH, OR_8, OQ or halogen;
B is a naturally occurring nucleic acid base or synthetic modified nucleic acid base;
Y is RN or O;
Z is R_7N or O;
M is $S(=O)=O$, $P(=O)-O^-$, $P(=O)-S^-$, $P(=S)-S^-$, $P(=O)-OR_3$, $P(=O)-R_9$, $P(=O)-SR_4$ or



provided at least one M is $S(=O)=O$ and when M is $S(=O)=O$, only one of Y and Z are O;
n is 1-200;
R is H or C_{1-4} alkyl;
R_1 is H_2PO_3, $H_3P_2O_5$, $H_4P_3O_7$ and their suitable salts, H or a protecting group;
R_2 is H_2PO_3, $H_3P_2O_5$, $H_4P_3O_7$ and their suitable salts, H or a protecting group;
R_3 is C_{1-4} alkyl or cyanoethyl;
R_4 is H or C_{1-4} alkyl;
R_5 is H or C_{1-4} alkyl;
R_6 is H or C_{1-4} alkyl;
R_7 is H or C_{1-4} alkyl;
R_8 is C_{1-4} alkyl;
R_9 is C_{1-4} alkyl or cyanoethyl; and
Q is a protecting group.

2. The compound of Claim 1 wherein when M is $S(=O)=O$, one of Y or Z is RN or R_7N and R or R_7 is H.

3. The compound of Claim 1 wherein R is an alkyl of less than 5 carbon atoms.

4. The compound of Claim 1 wherein R_3 is an alkyl of less than 5 carbon atoms.

5. The compound of Claim 1 wherein R_4 is an alkyl of less than 5 carbon atoms.

6. The compound of Claim 1 wherein R_5 is an alkyl of less than 5 carbon atoms.

7. The compound of Claim 1 wherein R_6 is an alkyl of less than 5 carbon atoms.

8. The compound of Claim 1 wherein R_7 is an alkyl of less than 5 carbon atoms.

9. The compound of Claim 1 wherein R_8 is an alkyl of less than 5 carbon atoms.

10. The compound of Claim 1 wherein R_9 is an alkyl of less than 5 carbon atoms.

11. The compound of Claim 1 wherein the A substituent is in the 2'-position.

12. The compound of Claim 1 wherein A is selected from the group consisting of H and OH.

13. The compound of Claim 1 wherein B is adenine, guanine, cytosine, thymine or uracil.

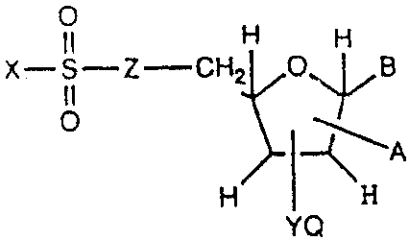
14. The compound of Claim 1 wherein at least one M is S(=O)=O and is substantially near at least one terminus of the compound.

15. The compound of Claim 1 wherein M is S(=O)=O.

16. The compound of Claim 1 wherein M is S(=O)=O or P(=O)-O$^-$.

17. The compound of Claim 16 wherein one of Y or z are RN or R_7N and R or R_7 is H.

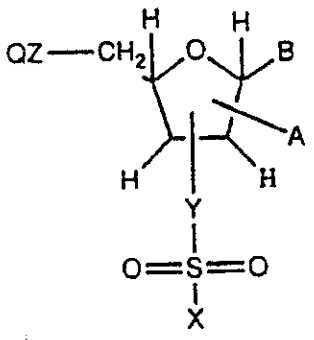
18. A compound of the formula:



wherein:

A is	H, OH, OR_2, OQ or halogen;
B is	a naturally occurring nucleic acid base or synthetic modified nucleic acid base;
Y is	RN or O;
Z is	R_1N or O;
X is	a suitable leaving group;
Q is	a protecting group;
R is	H or C_{1-4} alkyl;
R_1 is	H or C_{1-4} alkyl; and
R_2 is	C_{1-4} alkyl.

19. A compound of the formula:



wherein:

A is	H, OH, OR_2, OQ or halogen;
B is	a naturally occurring nucleic acid base or synthetic modified nucleic acid base;
Y is	RN or O;
Z is	R_1N or O;
X is	a suitable leaving group;
Q is	a protecting group;
R is	H or C_{1-4} alkyl;
R_1 is	H or C_{1-4} alkyl; and
R_2 is	C_{1-4} alkyl.

20. The compound of Claim 18 or 19 wherein X is selected from the group consisting of halides, azide and sulfonates.

21. The compound of Claim 18 or 19 wherein R is an alkyl of less than 5 carbon atoms.

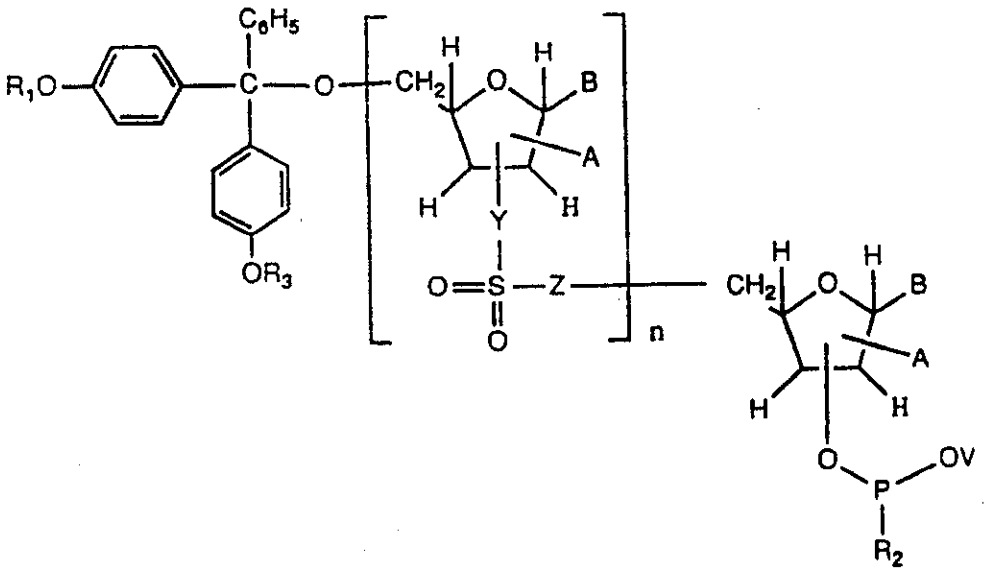
22. The compound of Claim 18 or 19 wherein R_1 is an alkyl of less than 5 carbon atoms.

23. The compound of Claim 18 or 19 wherein R_2 is an alkyl of less than 5 carbon atoms.

24. A process to prepare the compound of Claim 1 wherein M is S(=O)=O comprising contacting the compound of Claim 18 or 19 with a hydroxy or amino bearing nucleoside, nucleotide or oligonucleotide.

25. The process of Claim 24 wherein a metal catalyst is used.

26. A compound of the formula:



A is		H, OH, OR_4, OQ, or halogen;
B is		a naturally occurring nucleic acid base or synthetic modified nucleic acid base;
n is		1-200;
R_1 is		C_{1-4} alkyl;
R_2 is		dialkylamino, morpholino, piperidino or pyrrolidono;
R_3 is		C_{1-4} alkyl;
R_4 is		C_{1-4} alkyl;
Y is		RN or O;
Z is		RN or O;
R is		H or C_{1-4} alkyl;
Q is		a protecting group; and
V is		a protecting group.

27. The compound of Claim 26 wherein V is cyanoethyl or methyl.

28. The compound of Claim 1, 18, 19 or 26 wherein Q is selected from the group consisting of C_1-C_5 trialkylsilyl, C_1-C_5 alkyl, acyl, cyanoethyl or tetrahydropyran.

29. The compound of Claim 26 wherein R is an alkyl of less than 5 carbon atoms.

30. The compound of Claim 26 wherein R_1 is an alkyl of less than 5 carbon atoms.

31. The compound of Claim 26 wherein R_3 is an alkyl of less than 5 carbon atoms.

32. The compound of Claim 26 wherein R_4 is an alkyl or less than 5 carbon atoms.

33. An anti-sense reagent comprising the compound of Claim 1.

34. An antiviral reagent comprising the compound of Claim 1.

35. A hybridization probe comprising the compound of Claim 1.

36. A triplex reagent comprising the compound of Claim 1.

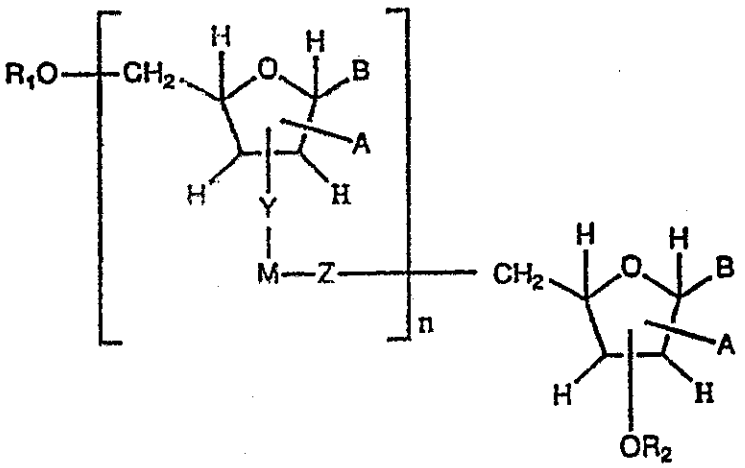
37. An antisense reagent comprising the compound of Claim 1 wherein when M is S(=O)=O both Y and Z are 0.

38. An antiviral reagent comprising the compound of Claim 37.

39. A triplex reagent comprising the compound of Claim 37.

Patentansprüche

1. Verbindung der Formel



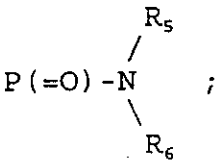
A ist H, OH, OR_8, OQ oder Halogen;

B ist eine natürlich vorkommende Nucleinsäurebase oder eine synthetische, modifizierte Nucleinsäurebase;

Y ist RN oder O;

Z ist R_7N oder O;

M ist S(=O)=O, P(=O)-O^-, P(=O)-S^-, P(=S)-S^-, P(=O)-OR_3, P(=O)-R_9, P(=O)-SR_4 oder



;

vorausgesetzt, daß wenigstens ein M S(=O)=O ist und wenn M S(=O)=O ist, nur eines von Y und Z O ist;

n 1 - 200 ist;

R H oder C_{1-4}-Alkyl ist;

R_1 H_2PO_3, $H_3P_2O_5$, $H_4P_3O_7$ und ihre geeigneten Salze, H oder eine Schutzgruppe ist;

R_2 H_2PO_3, $H_3P_2O_5$, $H_4P_3O_7$ und ihre geeigneten Salze, H oder eine Schutzgruppe ist;

R_3 C_{1-4}-Alkyl oder Cyanethyl ist;

R_4 H oder C_{1-4}-Alkyl ist;

R_5 H oder C_{1-4}-Alkyl ist;

R_6 H oder C_{1-4}-Alkyl ist;

R_7 H oder C_{1-4}-Alkyl ist;

R_8 C_{1-4}-Alkyl ist;

R_9 C_{1-4}-Alkyl oder Cyanethyl ist und

Q eine Schutzgruppe ist.

2. Verbindung des Anspruchs 1, worin wenn M S(=O)=O ist, eines von Y oder Z RN oder R_7N ist und R oder R_7 H ist.

3. Verbindung des Anspruchs 1, worin R Alkyl mit weniger als 5 Kohlenstoffatomen ist.

4. Verbindung des Anspruchs 1, worin R_3 Alkyl mit weniger als 5 Kohlenstoffatomen ist.

5. Verbindung des Anspruchs 1, worin R_4 Alkyl mit weniger als 5 Kohlenstoffatomen ist.

6. Verbindung des Anspruchs 1, worin R_5 Alkyl mit weniger als 5 Kohlenstoffatomen ist.

7. Verbindung des Anspruchs 1, worin R_6 Alkyl mit weniger als 5 Kohlenstoffatomen ist.

8. Verbindung des Anspruchs 1, worin R_7 Alkyl mit weniger als 5 Kohlenstoffatomen ist.

9. Verbindung des Anspruchs 1, worin R_8 Alkyl mit weniger als 5 Kohlenstoffatomen ist.

10. Verbindung des Anspruchs 1, worin R_9 Alkyl mit weniger als 5 Kohlenstoffatomen ist.

11. Verbindung des Anspruchs 1, worin der Substituent A in 2'-Stellung ist.

12. Verbindung des Anspruchs 1, worin A aus der aus H und OH bestehenden Gruppe ausgewählt ist.

13. Verbindung des Anspruchs 1, worin B Adenin, Guanin, Cytosin, Thymin oder Uracil ist.

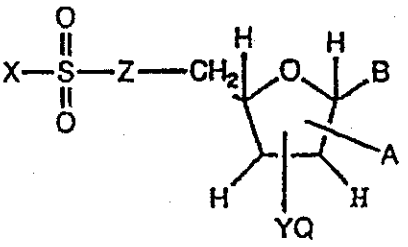
14. Verbindung des Anspruchs 1, worin wenigstens ein M S(=O)=O ist und sich im wesentlichen in der Nähe eines Terminus der Verbindung befindet.

15. Verbindung des Anspruchs 1, worin M S(=O)=O ist.

16. Verbindung des Anspruchs 1, worin M S(=O)=O oder P(=O)-O$^-$ ist.

17. Verbindung des Anspruchs 16, worin eines von Y oder Z RN oder R_7N ist und R oder R_7 H ist.

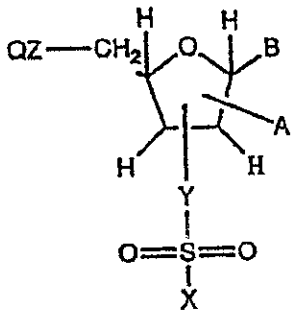
18. Verbindung der Formel



worin:

A	H, OH, OR_2, OQ oder Halogen ist;
B	eine natürlich vorkommende Nucleinsäurebase oder synthetische, modifizierte Nucleinsäurebase ist;
Y	RN oder O ist;
Z	R_1N oder O ist;
X	eine geeignete Abgangsgruppe ist;
Q	eine Schutzgruppe ist;
R	H oder C_{1-4}-Alkyl ist;
R_1	H oder C_{1-4}-Alkyl ist und
R_2	C_{1-4}-Alkyl ist.

19. Verbindung der Formel:



worin

A H, OH, OR_2, OQ oder Halogen ist;

B eine natürlich vorkommende Nucleinsäurebase oder synthetische, modifizierte Nucleinsäurebase ist;

Y RN oder O ist;

Z R_1N oder O ist;

X eine geeignete Abgangsgruppe ist;

Q eine Schutzgruppe ist;

R H oder C_{1-4}-Alkyl ist;

R_1 H oder C_{1-4}-Alkyl ist und

R_2 C_{1-4}-Alkyl ist.

20. Verbindung des Anspruchs 18 oder 19, worin X aus der aus Halogeniden, Azid und Sulfonaten bestehenden Gruppe ausgewählt ist.

21. Verbindung des Anspruchs 18 oder 19, worin R Alkyl mit weniger als 5 Kohlenstoffatomen ist.

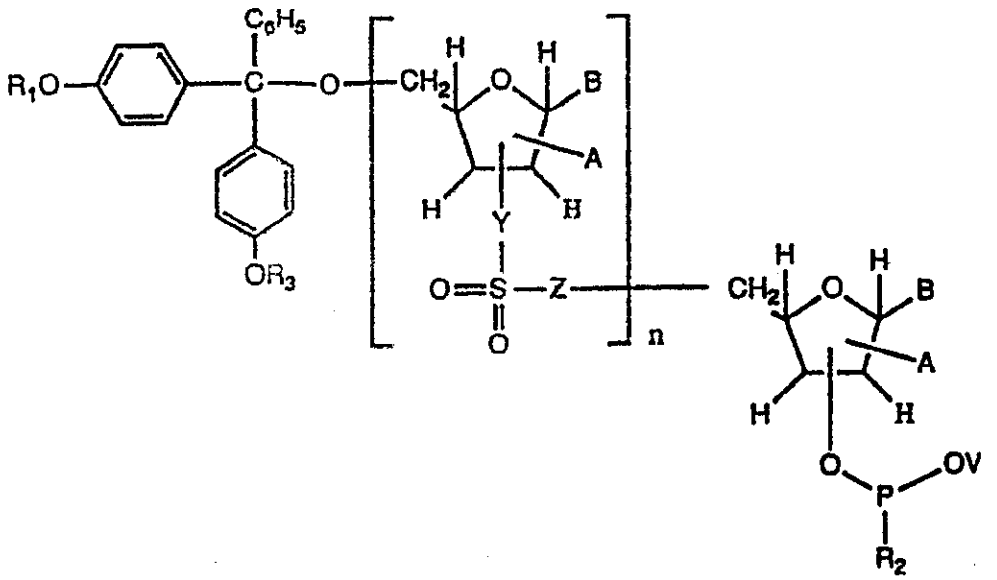
22. Verbindung des Anspruchs 18 oder 19, worin R_1 Alkyl mit weniger als 5 Kohlenstoffatomen ist.

23. Verbindung des Anspruchs 18 oder 19, worin R_2 Alkyl mit weniger als 5 Kohlenstoffatomen ist.

24. Verfahren zum Herstellen der Verbindung des Anspruchs 1, worin M S(=O)=O ist, welches das Zusammenbringen der Verbindung des Anspruchs 18 oder 19 mit einem Hydroxy oder Amino tragenden Nucleosid, Nucleotid oder Oligonucleotid umfaßt.

25. Verfahren des Anspruchs 24, wobei ein Metallkatalysator verwendet wird.

26. Verbindung der Formel:



worin

A ist H, OH, OR_4, OQ oder Halogen;
B ist eine natürlich vorkommende Nucleinsäurebase oder synthetische, modifizierte Nucleinsäurebase;
n ist 1 - 200;
R_1 ist C_{1-4}-Alkyl;
R_2 ist Dialkylamino, Morpholino, Piperidino oder Pyrrolidino;
R_3 ist C_{1-4}-Alkyl;
R_4 ist C_{1-4}-Alkyl;
Y ist RN oder O;
Z ist RN oder O;
R ist H oder C_{1-4}-Alkyl;
Q ist eine Schutzgruppe und
V ist eine Schutzgruppe.

27. Verbindung des Anspruchs 26, worin V Cyanethyl oder Methyl ist.

28. Verbindung des Anspruchs 1, 18, 19 oder 26, worin Q aus der aus C_1-C_5-Trialkylsilyl, C_1-C_5-Alkyl, Acyl, Cyanethyl oder Tetrahydropyran bestehenden Gruppe ausgewählt ist.

29. Verbindung des Anspruchs 26, worin R Alkyl mit weniger als 5 Kohlenstoffatomen ist.

30. Verbindung des Anspruchs 26, worin R_1 Alkyl mit weniger als 5 Kohlenstoffatomen ist.

31. Verbindung des Anspruchs 26, worin R_3 Alkyl mit weniger als 5 Kohlenstoffatomen ist.

32. Verbindung des Anspruchs 26, worin R_4 Alkyl mit weniger als 5 Kohlenstoffatomen ist.

33. anti-Sinnreagenz, das die Verbindung des Anspruchs 1 umfaßt.

34. Antivirales Reagenz, das die Verbindung des Anspruchs 1 umfaßt.

35. Hybridisierungssonde, welche die Verbindung des Anspruchs 1 umfaßt.

36. Triplexreagenz, das die Verbindung des Anspruchs 1 umfaßt.

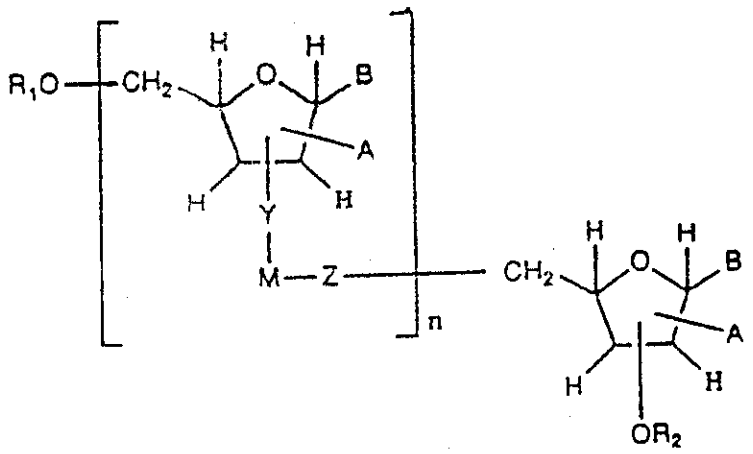
37. anti-Sinnreagenz, das die Verbindung des Anspruchs 1 umfaßt, worin M S(=O)=O ist und Y und Z beide O sind.

38. Antivirales Reagenz, das die Verbindung des Anspruchs 37 umfaßt.

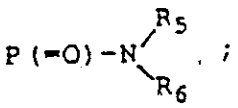
39. Triplexreagenz, das die Verbindung des Anspruchs 37 umfaßt.

Revendications

1. Un composé de la formule :



A est H, OH, OR_8, OQ ou un halogène ;
B est une base d'acide nucléique naturelle ou une base d'acide nucléique modifiée synthétique ;
Y est RN ou O ;
Z est R_7N ou O ;
M est S(=O)=O, P(=O)-O^-, P(=O)-S^-, P(=S)-S^-, P(=O)-OR_3, P(=O)-R_9, P(=O)-SR_4 ou



à condition qu'au moins un M soit S(=O)=O et que lorsque M est S(=O)=O, un seul de Y et Z soit O ;
n est 1 à 200 ;
R est H ou un groupe alkyle en C_1-C_4 ;
R_1 est H_2PO_3, $H_3P_2O_5$, $H_4P_3O_7$ et leurs sels appropriés, H ou un groupe protecteur ;
R_2 est H_2PO_3, $H_3P_2O_5$, $H_4P_3O_7$ et leurs sels appropriés, H ou un groupe protecteur ;
R_3 est un groupe alkyle en C_1-C_4 ou cyanoéthyle ;
R_4 est H ou un groupe alkyle en C_1-C_4 ;
R_5 est H ou un groupe alkyle en C_1-C_4 ;
R_6 est H ou un groupe alkyle en C_1-C_4 ;
R_7 est H ou un groupe alkyle en C_1-C_4 ;
R_8 est un groupe alkyle en C_1-C_4 ;
R_9 est un groupe alkyle en C_1-C_4 ou cyanoéthyle ; et
Q est un groupe protecteur.

2. Le composé de la revendication 1, dans lequel, lorsque M est S(=O)=O, l'un de Y et Z est RN ou R_7N et R ou R_7 est H.

3. Le composé de la revendication 1, dans lequel R est un groupe alkyle ayant moins de 5 atomes de carbone.

4. Le composé de la revendication 1, dans lequel R_3 est un groupe alkyle ayant moins de 5 atomes de carbone.

5. Le composé de la revendication 1, dans lequel R_4 est un groupe alkyle ayant moins de 5 atomes de carbone.

6. Le composé de la revendication 1, dans lequel R_5 est un groupe alkyle ayant moins de 5 atomes de carbone.

7. Le composé de la revendication 1, dans lequel R_6 est un groupe alkyle ayant moins de 5 atomes de carbone.

8. Le composé de la revendication 1, dans lequel R_7 est un groupe alkyle ayant moins de 5 atomes de carbone.

9. Le composé de la revendication 1, dans lequel R_8 est un groupe alkyle ayant moins de 5 atomes de carbone.

10. Le composé de la revendication 1, dans lequel R_9 est un groupe alkyle ayant moins de 5 atomes de carbone.

11. Le composé de la revendication 1, dans lequel le substituant A se trouve à la position 2'.

12. Le composé de la revendication 1, dans lequel A est choisi dans la classe formée par H et OH.

13. Le composé de la revendication 1, dans lequel B est l'adénine, la guanine, la cytosine, la thymine ou l'uracile.

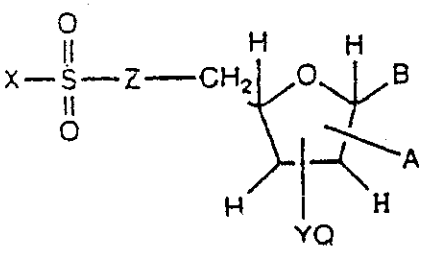
14. Le composé de la revendication 1, dans lequel au moins un M est S(=O)=O et se trouve près d'au moins une extrémité du composé.

15. Le composé de la revendication 1, dans lequel M est S(=O)=O.

16. Le composé de la revendication 1, dans lequel M est S(=O)=O ou P(=O)-O^-.

17. Le composé de la revendication 16, dans lequel l'un de Y et Z est RN ou R_7N et R ou R_7 est H.

18. Un composé de la formule :



dans laquelle :

A est H, OH, OR_2, OQ ou un halogène ;

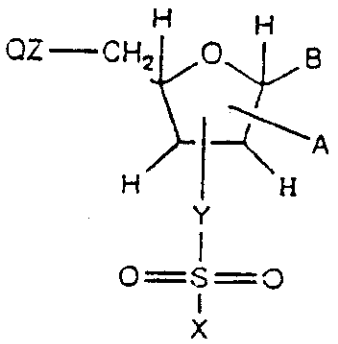
B est une base d'acide nucléique naturelle ou une base d'acide nucléique modifiée synthétique ;

Y est RN ou O ;

Z est R_1N ou O ;

X est un groupe labile approprié ;
Q est un groupe protecteur ;
R est H ou un groupe alkyle en C_1-C_4 ;
R_1 est H ou un groupe alkyle en C_1-C_4 ; et
R_2 est un groupe alkyle en C_1-C_4.

19. Un composé de la formule :



dans laquelle :
A est H, OH, OR_2, OQ ou un halogène ;
B est une base d'acide nucléique naturelle ou une base d'acide nucléique modifiée synthétique ;
Y est RN ou O ;
Z est R_1N ou O ;
X est un groupe labile approprié ;
Q est un groupe protecteur ;
R est H ou un groupe alkyle en C_1-C_4 ;
R_1 est H ou un groupe alkyle en C_1-C_4 ; et
R_2 est un groupe alkyle en C_1-C_4.

20. Le composé de la revendication 18 ou 19, dans lequel X est choisi dans la classe formée par les radicaux halogénures, azide et sulfonates.

21. Le composé de la revendication 18 ou 19, dans lequel R est un groupe alkyle ayant moins de 5 atomes de carbone.

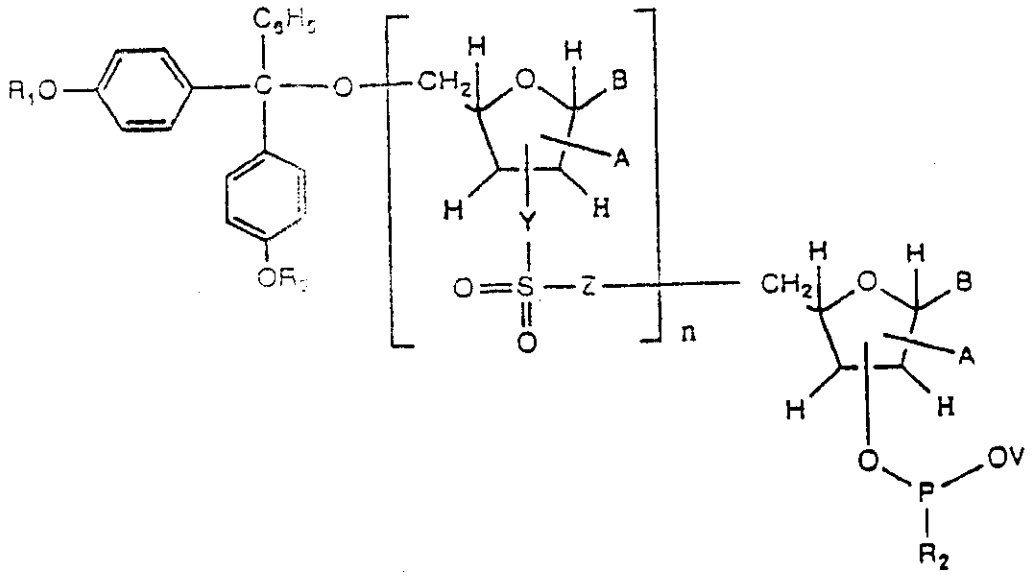
22. Le composé de la revendication 18 ou 19 dans lequel R_1 est un groupe alkyle ayant moins de 5 atomes de carbone.

23. Le composé de la revendicacion 18 ou 19 dans lequel R_2 est un groupe alkyle ayant moins de 5 atomes de carbone.

24. Un procédé pour préparer le composé de la revendication 1 dans lequel M est S(=O)=O, comprenant la mise en contact du composé de la revendication 18 ou 19 avec un nucléoside, nucléotide ou oligonucléotide portant la fonction hydroxyle ou amino.

25. Le procédé de la revendication 24, dans lequel on utilise un catalyseur métallique.

26. Un composé de la formule :



A est	H, OH, OR_4, OQ ou un halogène ;
B est	une base d'acide nucléique naturelle ou une base d'acide nucléique modifiée synthétique ;
n est	1 à 200 ;
R_1 est	un groupe alkyle en C_1-C_4 ;
R_2 est	un groupe dialkylamino, morpholino, pipéridino ou pyrrolidono ;
R_3 est	un groupe alkyle en C_1-C_4 ;
R_4 est	un groupe alkyle en C_1-C_4 ;
Y est	RN ou O ;
Z est	RN ou O ;
R est	H ou un groupe alkyle en C_1-C_4 ;
Q est	un groupe protecteur ; et
V est	un groupe protecteur.

27. Le composé de la revendication 26, dans lequel V est un groupe cyanoéthyle ou méthyle.

28. Le composé de la revendication 1, 18, 19 ou 26, dans lequel Q est choisi dans la classe formée par un groupe trialkylsilyle en C_1-C_5, alkyle en C_1-C_5, acyle, cyanoéthyle ou tétrahydropyranne.

29. Le composé de la revendication 26, dans lequel R est un groupe alkyle ayant moins de 5 atomes de carbone.

30. Le composé de la revendication 26, dans lequel R_1 est un groupe alkyle ayant moins de 5 atomes de carbone.

31. Le composé de la revendication 26, dans lequel R_3 est un groupe alkyle ayant moins de 5 atomes de carbone.

32. Le composé de la revendication 26, dans lequel R_4 est un groupe alkyle ayant moins de 5 atomes de carbone.

33. Un réactif antisens comprenant le composé de la revendication 1.

34. Un réactif antiviral comprenant le composé de la revendication 1.

35. Une sonde d'hybridation comprenant le composé de la revendication 1.

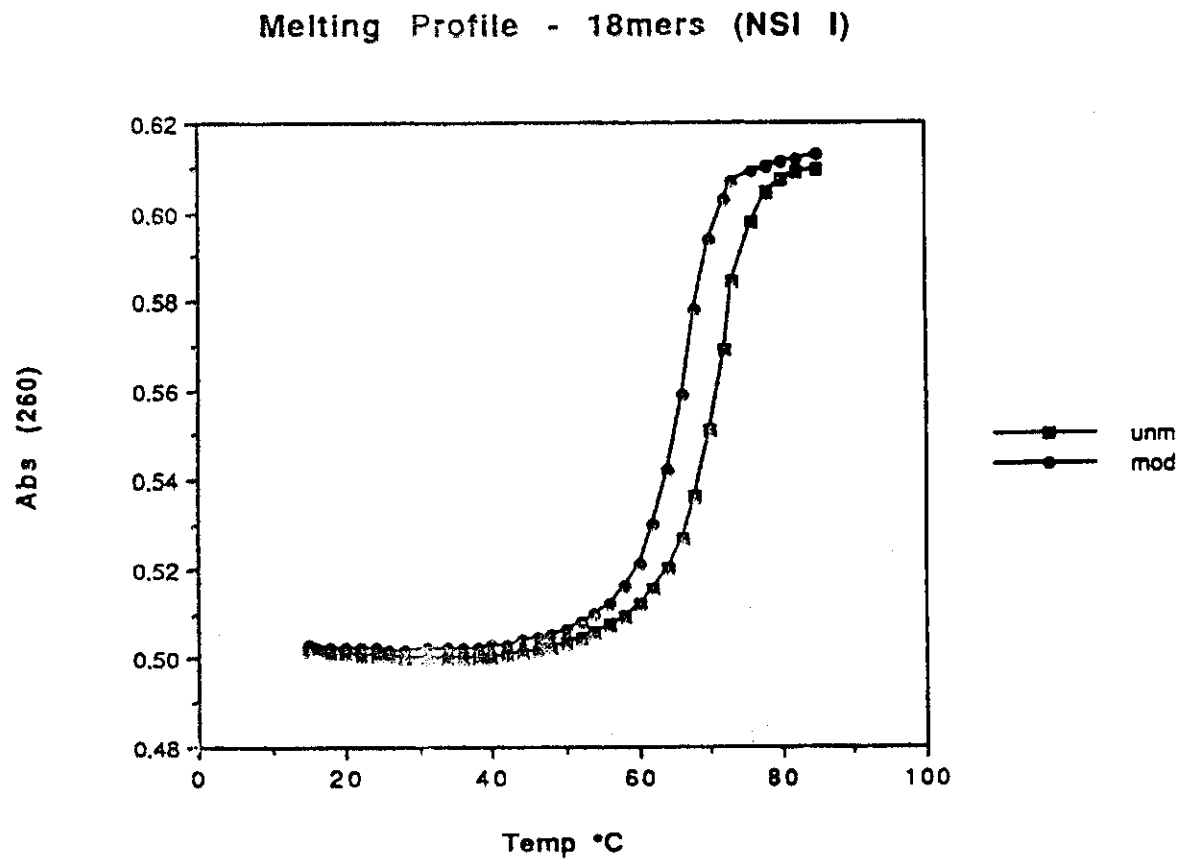
36. Un réactif formateur de triplex comprenant le composé de la revendication 1.

37. Un réactif antisens comprenant le composé de la revendication 1 dans lequel, lorsque M est S(=O)-=O, Y et Z sont tous deux O.

38. Un réactif antiviral comprenant le composé de la revendication 37.

39. Un réactif formateur de triplex comprenant le composé de la revendication 37.

FIG. 1


Melting Profile - 18mers (NSI I)
Abs (260)
0.62
0.60
0.58
0.56
0.54
0.52
0.50
0.48
0
20
40
60
80
100
Temp °C
unm
mod

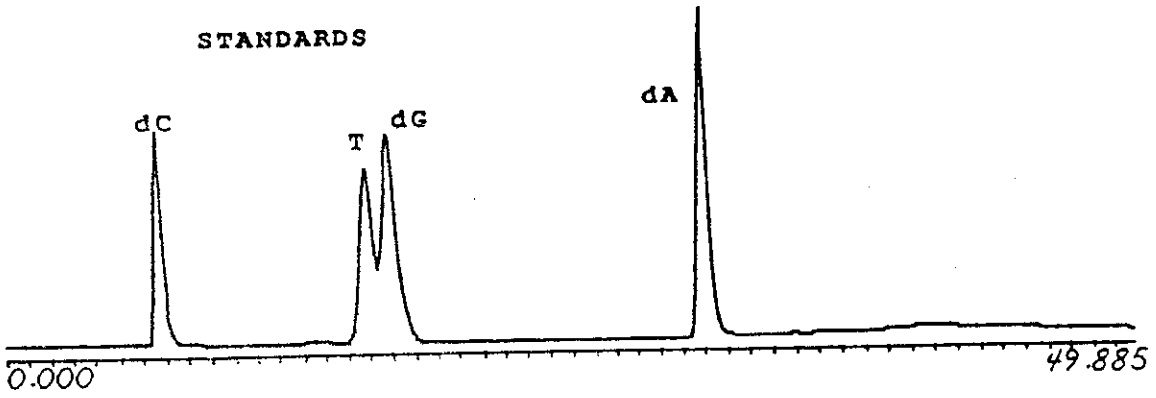


FIG. 2a

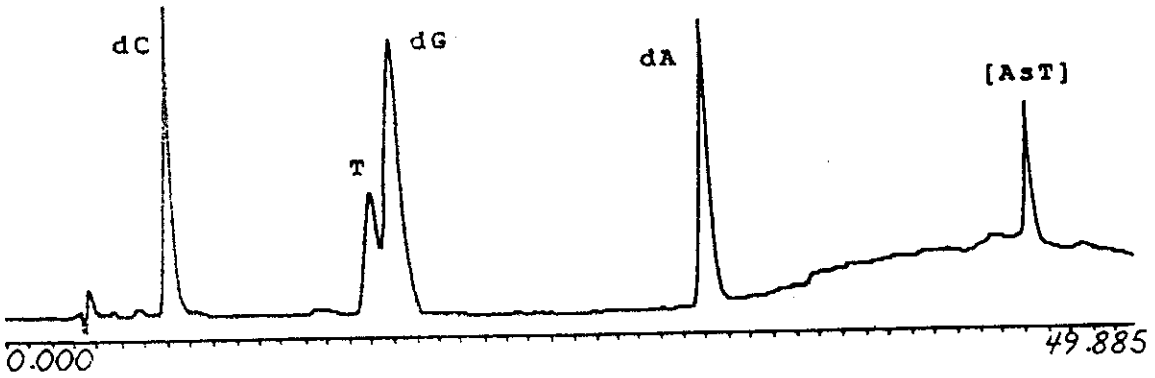


FIG. 2b

FIG. 3

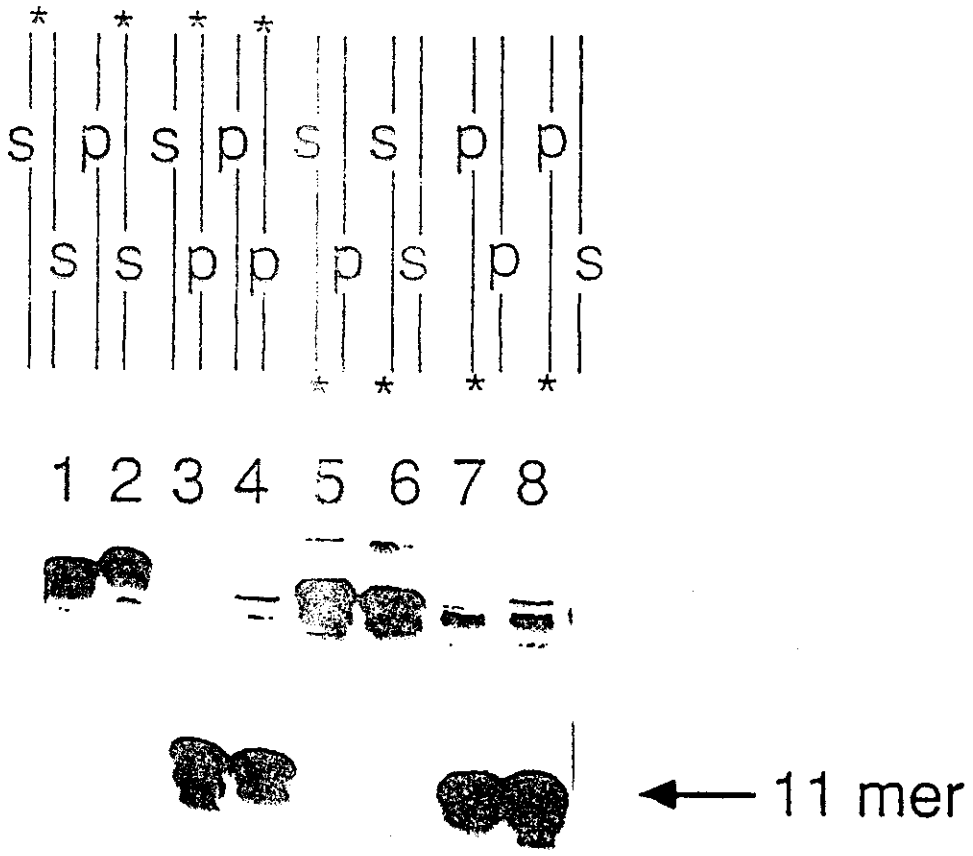
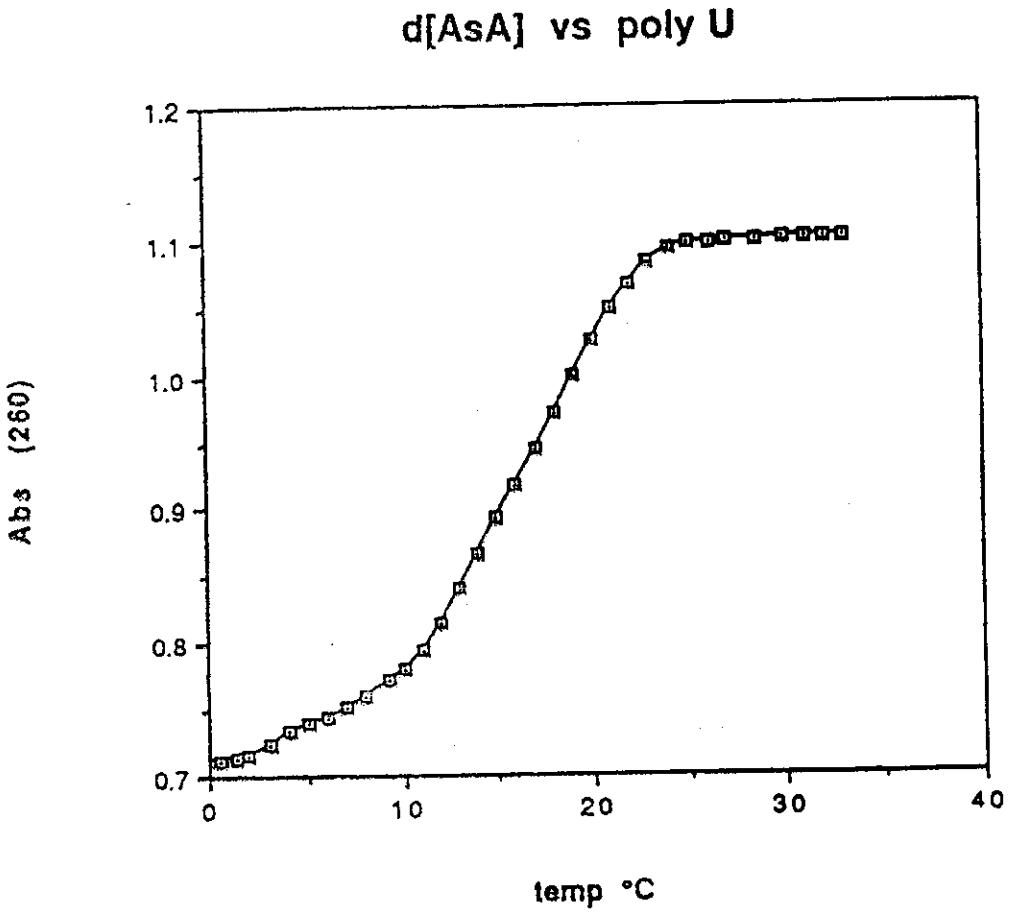


FIG. 4



REGISTER ENTRY FOR EP0517796

European Application No EP91905593.9 filing date 25.02.1991

Priority claimed:
10.04.1990 in United States of America - doc: 507693

PCT EUROPEAN PHASE
PCT Application PCT/US91/01066 Publication No WO91/15500 on 17.10.1991

Designated States BE CH DE DK ES FR GB GR IT LI LU NL SE AT

Title OLIGONUCLEOTIDE ANALOGS.

Applicant/Proprietor
E.I. DU PONT DE NEMOURS AND COMPANY, 1007 Market Street, Wilmington Delaware 19898, United States of America [ADP No. 50576693002]

Inventors
MCDONALD EDWARD HUIE, 1021 Rockwell Road, Wilmington, DE 19810, United States of America [ADP No. 60385994001]

LEONARD GEORGE TRAINOR, 9 Carillon Court, Wilmington, DE 19803, United States of America [ADP No. 60386000001]

Classified to
C07H A61K

Address for Service
CARPMAELS & RANSFORD, 43 Bloomsbury Square, LONDON, WC1A 2RA, United Kingdom [ADP No. 00000083001]

EPO Representative
ALAN JOHN JONES, CARPMAELS & RANSFORD 43 Bloomsbury Square, London, WC1A 2RA, United Kingdom [ADP No. 50873736001]

Publication No EP0517796 dated 16.12.1992
Publication in English

Examination requested 02.10.1992

Patent Granted with effect from 19.04.1995 (Section 25(1)) with title OLIGONUCLEOTIDE ANALOGS.

14.06.1993 Notification from EPO of change of Applicant/Proprietor details
from
E.I. DU PONT DE NEMOURS AND COMPANY, 1007 Market Street, Wilmington Delaware 19898, United States of America [ADP No. 50576693002]
to
THE DU PONT MERCK PHARMACEUTICAL COMPANY, Barley Mill Plaza Building 25, Wilmington, Delaware 19898, United States of America [ADP No. 58976820006]
Entry Type 25.14 Staff ID. RD06 Auth ID. EPT

**** END OF REGISTER ENTRY ****

RENEWAL DETAILS

PUBLICATION NUMBER EP0517796

PROPRIETOR(S)

THE DU PONT MERCK PHARMACEUTICAL COMPANY, Barley Mill Plaza
Building 25, Wilmington, Delaware 19898, United States of America

DATE FILED 25.02.1991

DATE GRANTED 19.04.1995

DATE NEXT RENEWAL DUE 25.02.1996

DATE NOT IN FORCE

DATE OF LAST RENEWAL

YEAR OF LAST RENEWAL 00

STATUS PATENT IN FORCE

**** END OF REPORT ****