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(54) Abstract Title
Hexitol Nucleosides

(57) The invention relates to nucleoside analogues with a 1,5-anhydrohexitol moiety as the sugar part, of which the hexitol ring is substituted with an alkoxy substituent at the 3-position and a nucleobase derived from Pyrimidine and Purine bases at the 2-position. It also involves methyl pyranose nucleosides with a nucleobase at the 2-position having a hydroxy or alkoxy group at the 3-position. This invention further relates to oligomers comprising or containing in part one or more of the afore mentioned nucleoside analogues which exhibit sequence-specific binding to complementary sequences of natural oligonucleotides. This invention further relates to the chemical synthesis of these oligomers and their use in antisense strategies which comprise diagnosis, hybridisation, isolation of nucleic acids, site-specific DNA modification, and therapeutics.

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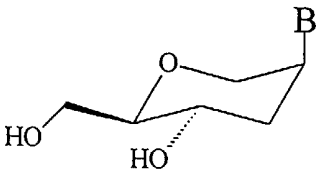
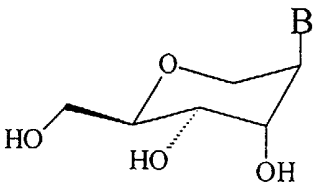
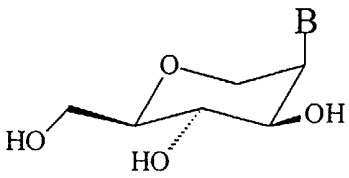


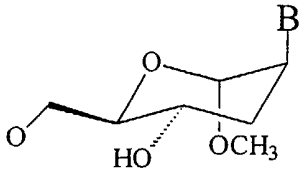
HNA monomers



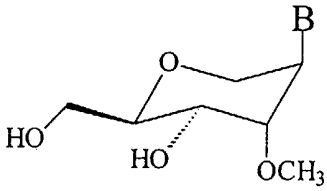
ANA monomers



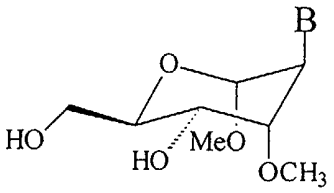
MNA monomers



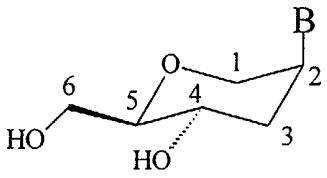
1-methoxy HNA analogues (26)



3-O-methyl ANA analogues (21)

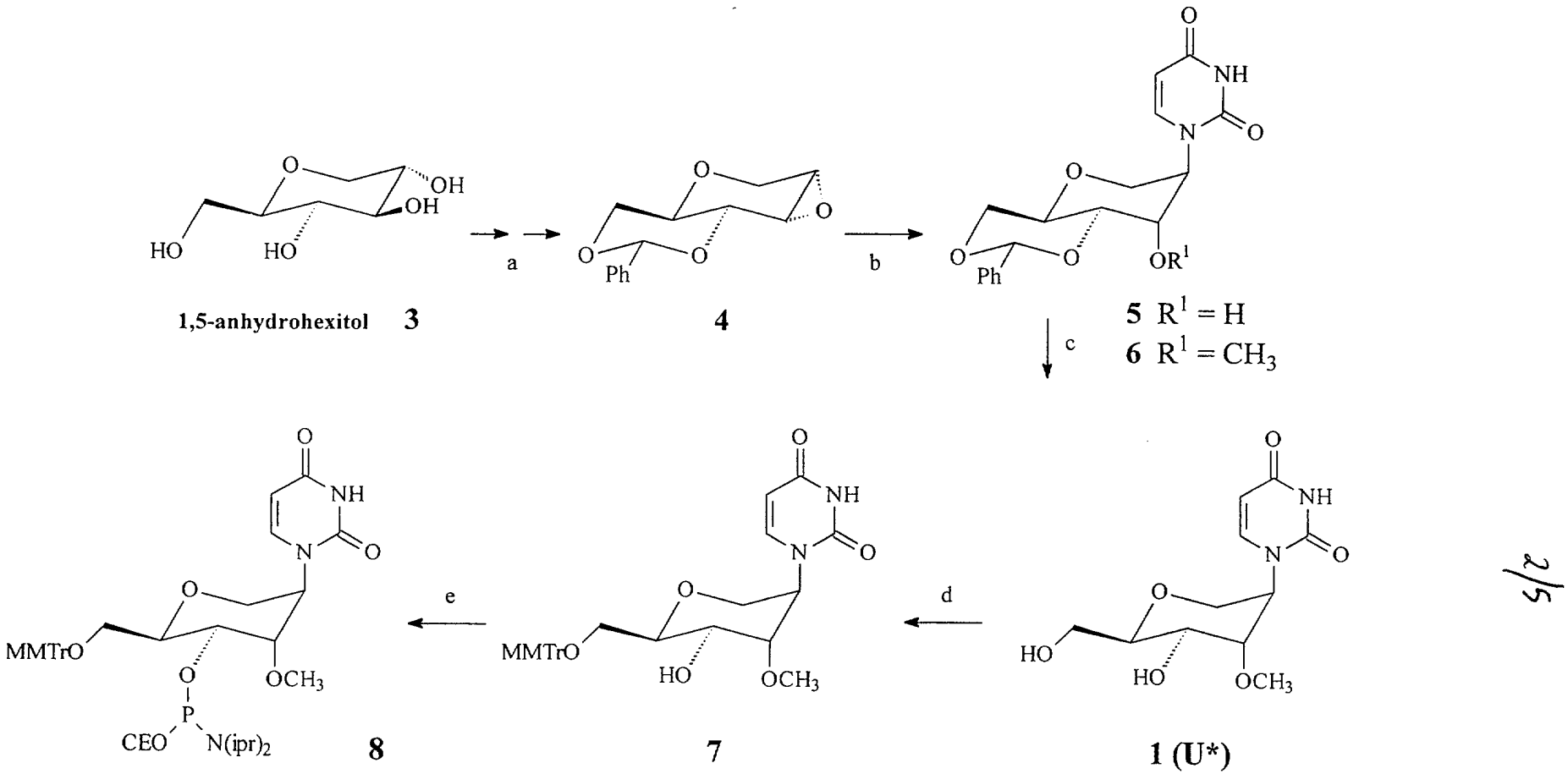


1-methoxy-3-O-methyl ANA analogues (28)



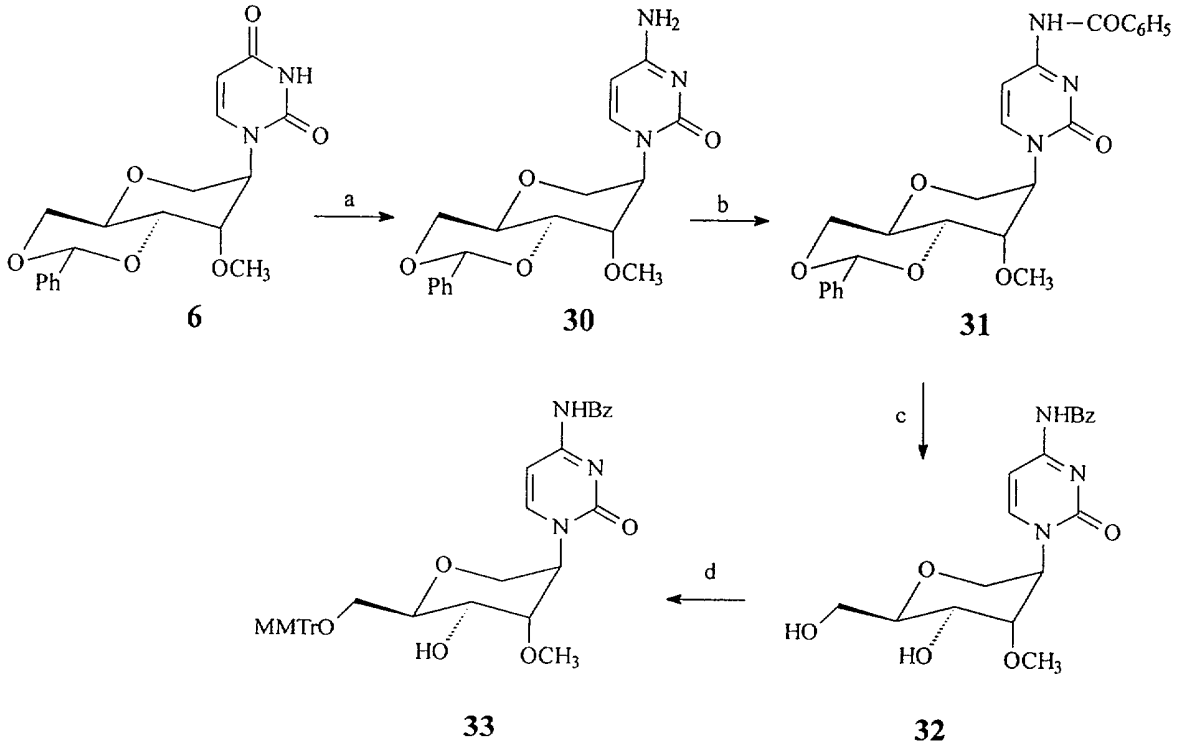
general numbering scheme

Figure 1



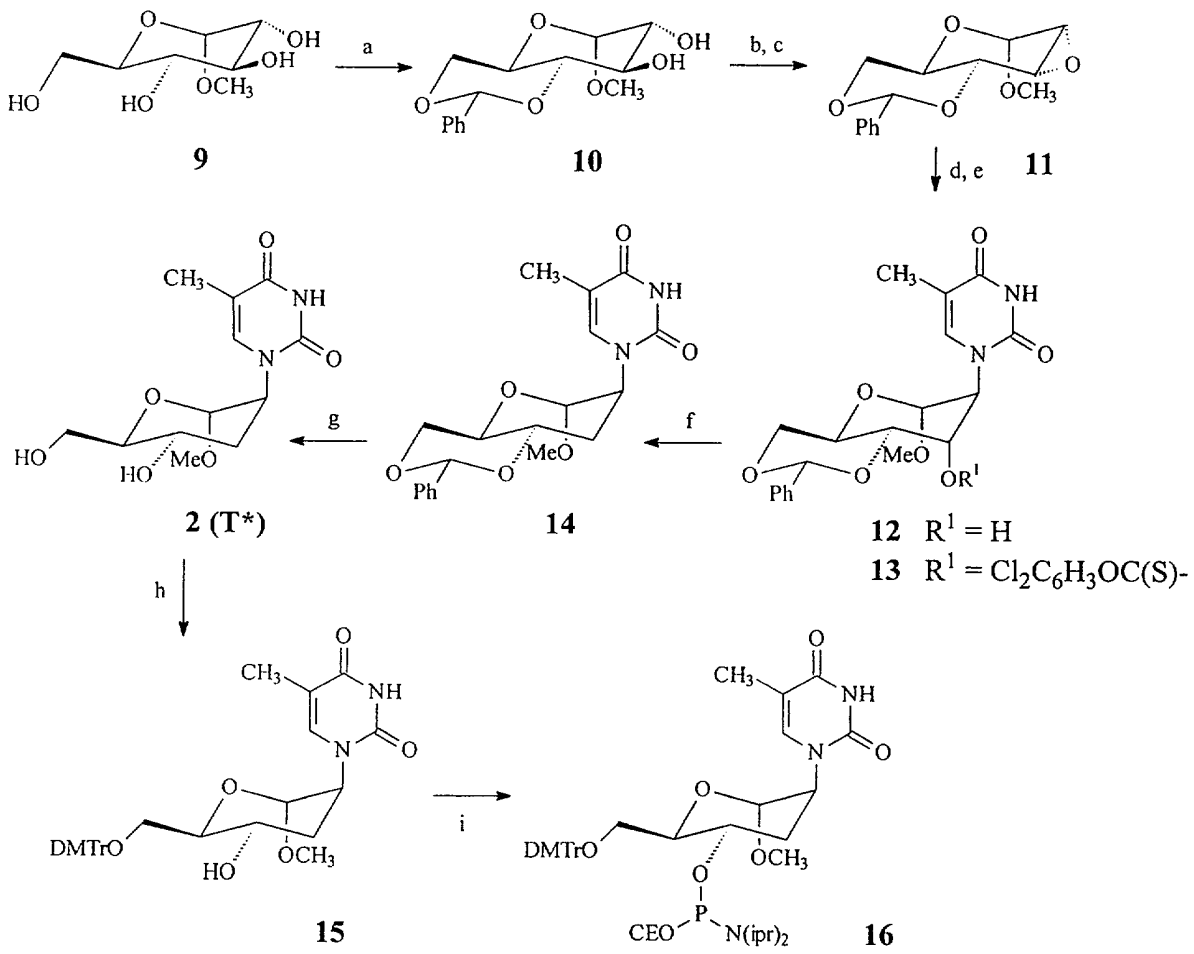
a) ref. 14; b) i) 3.2 eq. uracil, 3 eq. NaH, DMF 120°C 24h (86%); ii) 5 eq. NaH, 3 eq. CH_3I, THF, 7h 0°C (38%); c) 90% TFA (74%); d) MMTrCl, pyridine (89%); e) DIEA, CH_2Cl_2, $(iPr)_2N(CN)PCl$ (90%).

Scheme I



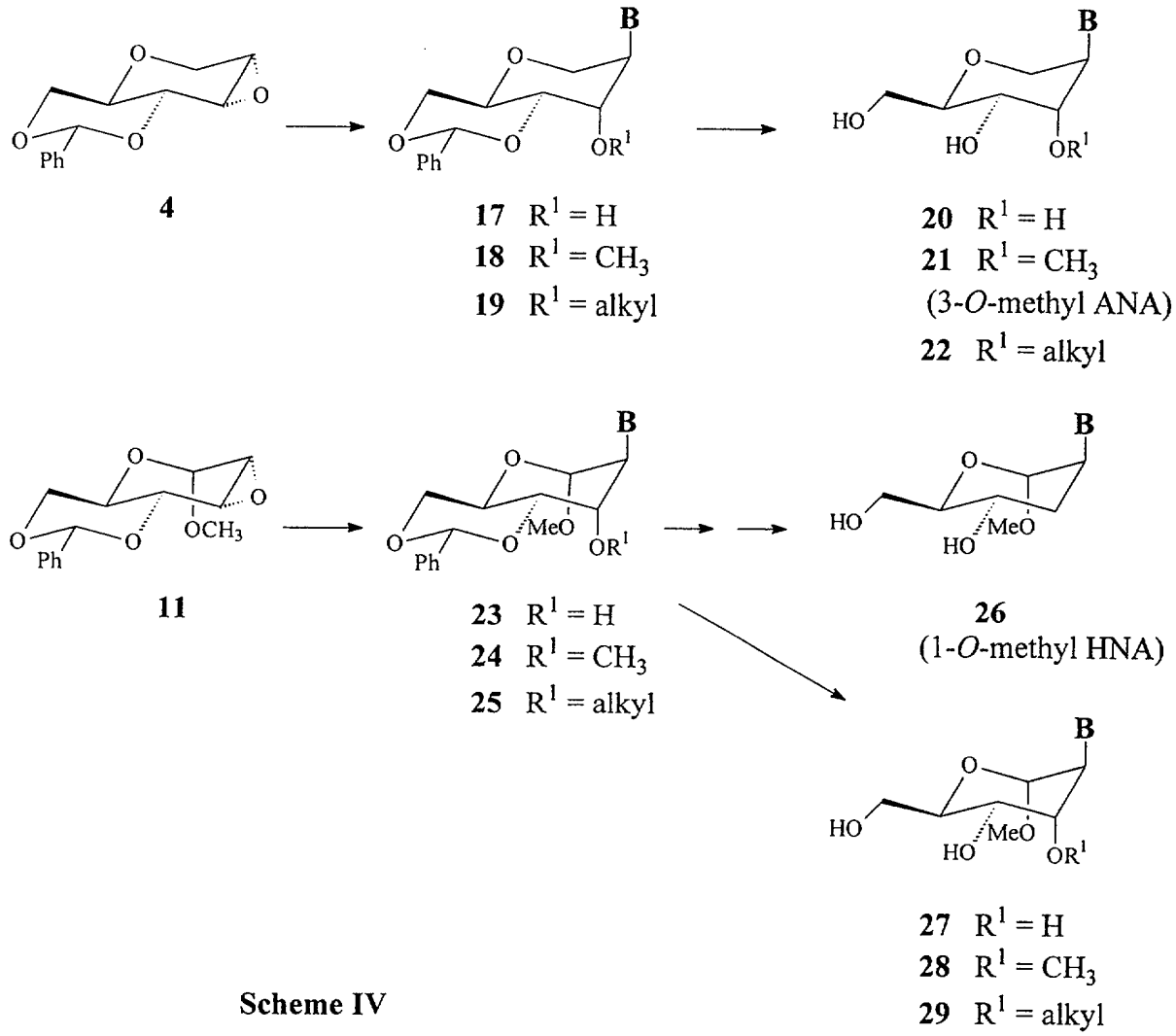
a) (1) $POCl_3$, 1,2,4-triazole, NEt_3, MeCN; (2) NH_4OH, dioxane (89%); b) (1) BzCl, pyridine; (2) NH_4OH, pyridine (75%); c) 90% aq. TFA (64%); d) MMTCl, pyridine (28%).

Scheme II synthesis of the 3'-O-methyl ANA cytosine congener



a) C_6H_5CHO, $ZnCl_2$, 72h (66%); b) 6 eq. $CH_3C_6H_5SO_2Cl$, pyridine, 72h, 60°C (78%); c) NaOMe, MeOH, CH_2Cl_2 (79%) (ref. 9 and 10); d) 3 eq. thymine, 2.8 eq. NaH, DMF, 96h, 120°C (71%); e) 2 eq. $CSCl_2$, 7 eq. DMAP, CH_2Cl_2 at -40°C followed by 4 eq. 2,4-$Cl_2C_6H_3OH$ at RT for 1h ; f) 1.5 eq. Bu_3SnH, AIBN, toluene 80°C (85% over 2 steps); g) 10% TFA-MeOH 3h (45%); alternatively H_2, Pd/C in MeOH-HOAc 98:2 for 18h (90%); h) DMTrCl, pyridine (85%); i) DIEA, CH_2Cl_2, $(iPr)_2N(CN)PCl$ (67%).

Scheme III



Scheme IV

General scheme for synthesis of the different new nucleoside analogues

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Alkyl ether congeners of hexitol nucleoside analogues (HNA)

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Field of the invention

The present invention relates to nucleoside analogues with a 1,5-anhydrohexitol moiety as the sugar part, of which the hexitol ring is substituted with an alkoxy substituent at the 3-position and a nucleobase at the 2-position. It also involves methyl pyranose nucleosides with a nucleobase at the 2-position having a hydroxy or alkoxy group at the 3-position. This invention further relates to oligomers comprising or containing in part one or more of the afore mentioned nucleoside analogues which exhibit sequence-specific binding to complementary sequences of natural oligonucleotides. This invention further relates to the chemical synthesis of these oligomers and their use in antisense strategies which comprise diagnosis, hybridisation, isolation of nucleic acids, site-specific DNA modification, and therapeutics.

Technical background

Control of translation processes is a continuously growing research area and the use of antisense oligonucleotides reflects one of the possibilities enabling such control. This relies mostly on degradation of the mRNA target through assistance of RNase H, becoming activated upon recognition of the mixed DNA-RNA duplex. Oligonucleotides which do not activate RNase H after hybridizing with complementary RNA have to rely on a strong association with their nucleic acids target to obtain an antisense effect. If oligomers can be obtained which are able to induce strand displacement in double stranded RNA structures, targeting of RNA becomes independent of the secondary and tertiary structure of the mRNA and the number of possible RNA targets will increase considerably. One way to approach this problem is to synthesize carbohydrate modified oligonucleotides exemplified by hexitol nucleic acids,[1-4] 2'-O-(2-methoxy) ethyl oligonucleotides,[5] and bicyclic oligonucleotides,[6] with the compounds of the Wengel group[7] showing the strongest affinity for RNA. The strong hybridization characteristics with complementary RNA are generally attributed[8] to the formation of a preorganized conformation which fits the A-form of dsRNA, good stacking interactions between the bases which interact in a Watson-Crick type geometry with their complement and efficient hydration of the double stranded helix.

Hexitol nucleic acids (HNA) are composed of phosphorylated 2,3-dideoxy-D-arabino-hexitol units with a nucleobase situated in the 2-[S]-position. They hybridize sequence-

selectively with RNA in an antiparallel way. The observed increase in Tm per modification of a HNA:RNA duplex versus duplexes of natural nucleic acids is sequence- and length-dependent and varies from +0.9 °C/modification[3] to +5.8 °C/modification.[2] HNA is an efficient steric blocking agent as observed during investigations of HNA in cell-free translation experiments (giving IC_{50} values of 50 nM as inhibitors of Ha-ras mRNA translation).[9] Valuable results in cellular systems recently likewise have been reported (inhibition of Ha-ras and ICAM-1,[9] and antimalarial activity[10]).

An interesting observation made during hybridization experiments is that the HNA:RNA duplex is invariably more stable than the HNA:DNA duplex. Molecular dynamics simulation of HNA:RNA and HNA:DNA hybrids revealed that minor groove solvatation contributes to this difference in duplex stability.[11] In order to further increase minor groove hydration, in an effort to influence hybridization in a beneficial way, we synthesized D-altritol nucleic acids (ANA), consisting of a phosphorylated D-altritol backbone with nucleobases inserted in the 2'-position of the carbohydrate moiety[12] (Figure 1). They differ, structurally, from HNA[1-4] by the presence of a supplementary hydroxyl group in the 3'-α-position, meaning that carbon-3' of the hexitol moiety adopts the [S]-configuration. Inversion of configuration, giving the 3'-[R]-form leads to D-mannitol nucleic acids (MNA) which lack hybridization capabilities with natural nucleic acids.[13] This is due to conformational restriction of single stranded MNA in a partially unwound form by formation of intrastrand hydrogen bonds between the 3'-hydroxy and the 6'-O of the phosphate of the next nucleotide. This hydrogen bond, however, cannot be formed when using 2-deoxy-1,5-anhydro-D-altritol nucleosides as repeating unit in the backbone structure (ANA). The 3'-hydroxyl group of this nucleoside analogue is pointing into the minor groove of the ANA:RNA duplex and positively influences hybridization either by increasing hydration of the groove either by further stabilization of a preorganized single stranded structure.[12] The higher thermal stability for ANA:RNA was demonstrated when compared with HNA:RNA duplexes. Complexes formed between ANA and natural nucleic acids are, likewise, more stable than complexes between two natural nucleic acid strands. Moreover, as well the HNA complexes, as the ANA complexes retain their sequence-selectivity as well for polypurine sequences as for completely mixed sequences.

However, the technical difficulty of the latter monomers is the lenghty synthesis and the need of a supplementary protecting group for the 3'-hydroxyl position during oligomer

assembly.[12,14] The HNA monomers themselves, likewise obviate a long-routed synthesis in which twice a deoxygenation step is needed, when starting from ubiquitous glucose.[15-17] Therefore, constructs endowed with a further increase in affinity for an RNA taget, or constructs constituting an economically more viable alternative for the HNA or ANA monomers would be advantageous.

Reviews on the subject matter and background of antisense oligonucleotides are numerous[8, 18-20] and only a few references are given here as examples.

Description of the illustrative embodiments

The terminology used herein is for the purpose of describing particular embodiments only, and is not intended to limit the scope of the present invention. This invention is not limited to the particular methodology, protocols and reagents described, as these may vary.

The strong hybridizing potential of anhydrohexitol nucleic acids by virtue of its pre-organisation by now is well documented,[1-4] and some interesting biological antisense effects have been reported (inhibition of Ha-ras and ICAM-1,[9] and antimalarial activity[10]). To further augment the affinity for target RNA structures, two different roads can be explored by looking for analogues which either increase the conformational preorganisation of the monomeric structures, or which alternatively augment the hydrophobic interactions. In addition, cost and ease of synthesis need to be considered.

As an example therefore, as well 3'-O-methylated altrohexitol analogues, as 1'-O-methylglycosidic analogues were prepared and incorporated into HNA sequences. The former monomer was synthesized analogous to the preparation of the altrohexitol monomers,[14] with 3'-O-methylation of the pre-formed nucleoside analogue. For the latter monomers, the ubiquitous methyl glucopyranoside was used as starting material. Following traditional phosphoramidite chemistry, both monomers were incorporated with good yield into oligonucleotides within a stretch of HNA.
Thermal denaturation experiments indicated strong duplex stabilities for both series, as well for hybridisation with RNA targets, as for pairing with hexitol oligonucleotides (vide infra).

For reasons of clarity, a general figure has been inserted, depicting the different hexitol containing nucleoside analogues. These comprise the known 1,5-anhydrohexitol or

HNA monomers (**H**exitol **N**ucleic **A**cids), the 1,5-anhydroaltritol or ANA monomers (**A**ltritol **N**ucleic **A**cids) and the 1,5-anhydromannitol or MNA monomers (**M**annitol **N**ucleic **A**cids). In addition, the new structures are depicted as there are the 1'-methoxy HNA analogues **26**, the 3'-*O*-methyl ANA analogues **21**, and the 1'-methoxy-3'-*O*-methyl ANA analogues **28**. (**Figure 1**).

Synthesis of the **3'-O-methylated analogue 1 (U*)** is depicted in **scheme I**, and followed the route previously described for preparation of the altrohexitol monomers (ANA).[14] Ring opening of the 4,6-*O*-benzylidene protected allitol epoxide **4** with the uracil anion furnished the altrohexitol derivative **5**. Chemoselective methylation without temporary protection of the nucleobase gave the methylated nucleoside **6**. The methylation proceeded slowly and the yield was lower than reported for other derivatives.[21,22] The slow reaction compared to the previously described methylations is probably caused by the axial location of the hydroxyl group. Faster reactions are achieved with a primary alcohol[22] and a pseudoequatorial positioned secondary alcohol.[21] The selectivity of the methylation was confirmed by NMR, and only a small amount of the dimethylated compound was obtained. Further modification (monomethoxytritylation and phosphitylation) yielded the desired phosphitylated building block **8**, to be used for oligomer assembly.

The cytosine congener **33** was obtained in 5 steps from the uracil analogue **6** according to well-known procedures (**scheme II**). Reaction with $POCl_3$ and 1,2,4-triazole followed by treatment with aqueous NH_3 afforded the cytidine nucleoside **30**.[23] When using anhydrous pyridine as solvent for the reaction with 1,2,4-triazole followed by treatment with ammonia as previously described,[14] the cytosine nucleoside was obtained as a yellow substance in only 20% yield. However, by changing the solvent to anhydrous acetonitrile, the cytosine nucleoside could be obtained in 89% as a white substance. Benzoylation of the exocyclic aminogroup was followed by acidic hydrolysis to give the parent cytosine nucleoside derivative **32** which was subsequently tritylated to give **33** in 28% yield.

The **1'-*O*-methylglycosidic analogues (scheme III)** were obtained starting from ubiquitous methyl glucopyranoside. The chemistry starts by opening of the epoxide ring of methyl 2,3-anhydro-*allo*-hexopyranoside **11**,[24,25] followed by deoxygenation of the 3-position, affording the 3-deoxy analogue **14**. Removal of the benzylidene position with acid is possible, but less straightforward because of the glycosidic linkage, but can be accomplished alternatively via hydrogenation in almost quantitative yield, affording the

envisaged 1'-*O*-methylglycoside analogue **2 (T*)** of 1,5-anhydrohexitol nucleosides. Further modification yielded the desired phosphitylated building block **16**, to be used for oligomer assembly.

All oligos were assembled on a propanediol containing universal support, obviating the need of modified supports.[26] The new analogues were used either for homopolymers, for incorporation within HNA stretches, or for incorporation within stretches of RNA. Hereto, a 0.11 M amidite concentration was used as was done for the HNA building blocks, with a coupling time of 3 minutes. Coupling yields were consistently over 95% and higher. Oligos were purified as usual[26] on a Mono Q ® (Pharmacia) column with a NaCl gradient at pH 12 to disrupt possible secondary structures. MS of the isolated oligos were run following gel filtration, RP-HPLC with a 0.025M TEAB containing acetonitrile gradient and occasionally the addition of extra ion exchange beads under $TEAH^+$ form to reduce all sodium adducts. Electrospray ionization mass spectrometry (ESI-MS) in negative mode was performed on a quadruple / orthogonal-acceleration time-of-flight (Q/oaTOF) tandem mass spectrometer (qTof 2, Micromass, Manchester, UK) equipped with a standard electrospray ionisation interface. Samples were infused in an acetonitrile : water (1:1) mixture at 3 μL/min. Monoisotopic masses were consistently within 0.5 Da of the calculated masses.

As an example a study with pyrimidine hexamers was done as depicted in Table 1. When hybridised with complementary HNA, the introduction of the 3'-*O*-methylated uridine nucleoside **(1)** into a HNA strand results in an increased thermal stability of the duplex compared to the unmodified HNA:HNA duplex (ΔT_m = +0.6°C/modification), entry **A** and **B**, Table 1.

However, this increase is less pronounced than the increase in thermal stability obtained by modifying the nucleobase with a methyl substituent in the 5-position (ΔT_m = +1.1°C/ modification), (compare entry **A** and **D**). Hybridisation of the modified ON with complementary ANA results in a duplex with decreased thermal stability compared to the parent ANA:ANA duplex (however, entry **C** represents fully modified ANA, thus with 6 modifications versus 3 for entry **A**!). Hybridising the modified ONs with complementary RNA corroborates this pattern of thermal stabilisation of the duplexes. The duplex between the 3'-*O*-methylated ON and complementary RNA is slightly more stable than the corresponding HNA duplex (ΔT_m = +0.2°C/modification), entry **A** and **B**, but considerably

less stable as compared to the corresponding ANA:RNA duplex (ΔT_m = -1.1°C/modification, cave, fully modified ANA) and the duplex between RNA and the base modified HNA oligo (thymine replacing for uracil) (ΔT_m = -2.6°C/modification), entry **C** and **D**. As expected, none of the hexitol based ONs hybridised with complementary DNA. Clearly, for this series the results indicate that a 5-methyl is more important than a 3'-*O*-methyl and that methylation of the 3'-hydroxyl group in ANA is destabilising when pairing to an RNA sequence is envisaged.

Table 1. Hybridisation data for hexameric hexitol sequences (6'→4') with incorporation of 3 methylated building blocks 1 or 2.

	Sequence	HNA complement	ANA complement	RNA complement	DNA complement
A	U*CU* CCU* (HNA)	52.4 (64)	58.8 (71)	31 (42)	no T_m
B	UCU CCU (HNA)	50.7 (61.2)	55#	30.5 (40)	no T_m
C	UCU CCU (ANA)	54	61.8 (71.2)	38.4 (47.6)	no T_m
D	TCT CCT (HNA)	54	60.6	39 (48)	no T_m
E	T*CT* CCT* (HNA)	56.7	62.7	39.9 (49.5)	no T_m

T_m's obtained in a buffer consisting of 0.1 M NaCl and 20mM phosphate, pH 7.4 with a duplex concentration of 4μM. Numbers in brackets are T_m's in a high salt buffer (1.0 M NaCl). U* denotes a 3'-*O*-methylated ANA monomer **(1)**, T* denotes a 1'-*O*-methylated HNA monomer **(2)**
lit data incorrect (in ref. 12, the duplex was HNA-HNA, instead of HNA-ANA.

For the pyranosylated analogue **2** comparison is more straightforward, and the thermal stabilisation is of the same order as for **1** when compared to HNA, as well in its pairing with hexitol oligonucleotides ($\Delta T_m \approx$ +0.8°C/modification) as with RNA sequences ($\Delta T_m \approx$ +0.3°C/modification). Therefore, introduction of **2** seems to be slightly more favorable over addition of a HNA monomer.

Table 2. Thermal stability of octameric sequences with a single incorporation of a methylated analogue 1 or 2 versus RNA

Entry	Sequence	T_m /°C [a]	ΔT_m /°C [b]
A	GCG UA GCG (HNA)	52	ref.
B	GCG U*A GCG (HNA)	52.4	+0.4
C	GCG TA GCG (HNA)	54	+2.0
D	GCG T*A GCG (HNA)	55.6	+3.6
E	GCG UA GCG (full ANA)	59.6	+1.0

[a] T_m's towards complementary RNA, obtained in a buffer consisting of 0.1 M NaCl and 20mM phosphate, pH 7.4 with a duplex concentration of 4μM; [b] ΔT_m/modification

The results in Table 2 emphasise the results of Table 1. Thus, incorporation of a 3'-*O*-methyl-ANA nucleoside into a HNA sequence results in a duplex with complementary RNA being thermally more stable than the duplex between the parent HNA and complementary RNA (entry **B** vs. **A**). The effect is however less than for the duplexes containing ANA or those containing the 5-methyl modification (entry **E** with a ΔT_m/modification = 1°C and **C** with ΔT_m/modification = 2°C, respectively). However, the modification **2** with the pyranose moiety gave a solid increase of 1.6°C compared with the hexitol T reference (entries **C** and **D**), and therefore introduction of monomers like **2** remains worthwhile to evaluate in more detail.

However, thermal unwinding of a self-complementary duplex gave another pattern as shown in Table 3. In contrast to the results depicted in Tables 1 and 2, incorporation of 3'-*O*-methyl-ANA nucleosides **1** into a self complementary sequence results in considerable stabilisation of the duplex (ΔT_m = +3°C/modification) exceeding the stabilisation obtained for the substitution of uracil for thymine (ΔT_m = +2.4°C/modification). This change in stabilisation effect might be an effect of the studied sequence or could be explained by a more continuous run of hydrophobic methyl groups. On the other hand, the effect of the 1'-*O*-methyl glycoside is less in this case, but this could be explained by the high melting temperature of the reference duplex (entry **C**) which becomes difficult to surmount.

Table 3. Thermal stability of self complementary HNA sequences containing 3'- or 1'-*O*-methyl modifications (1 or 2).

Sequence	T_m /°C	ΔT_m /°C [a]
GUGU ACAC	65.0	ref.
GU*GU* ACAC	76.7	+3
GTGT ACAC	74.5	+2.4 / ref.
GT*GT* ACAC	76.9	+0.6

T_m's obtained in 0.1 M NaCl, 20mM phosphate, pH 7.4 with an oligo concentration of 8μM (4μM of duplex). [a] ΔT_m/modification.

Generally, the ONs containing the 3'-*O*-methyl derivative **(1)** showed a small increase in thermal stability towards complementary sequences as compared to HNA, except in the case of a self-complementary sequence for which an increase in thermal stability of 3°C per modification was observed. Compared to ANA, however, the 3'-*O*-methylation caused a decrease in thermal stability of duplexes between a modified ON and a complementary target, especially when targeting RNA. The introduction of a hydrophobic moiety at the rim of the

minor groove does not seem to have a large destabilising effect and the slightly decreased affinity of the methylated analogue as compared to parent ANA towards complementary sequences is probably due to the reduced ability to form hydrogen bonds, *i.e.* lost ability to act as a proton donor. These results suggest that it is possible to derivatise the 3'-hydroxyl group in ANA without significantly affecting the thermal stability of the duplexes with complementary sequences leaving room for alkylation using different alkyl moieties. In addition, for the glycosidic analogues **2**, the potential seems to be really there to obtain antisense compounds with higher affinity for RNA in comparison with well-known HNA, while at the same time having economically more favorable monomers, which are more easy to prepare.

In addition, incorporation of a single modification of either **1 (U*)** or **hU** (the HNA monomer with a uracil base) into RNA monomers was done within different sequence context, and the obtained modified oligos were evaluated versus RNA complementary sequences. The modified RNA oligos thus comprised two successive steps of change in overall geometry from a ribofuranosyl ring to either a 3'-*O*-methylated hexitol ring or a 3'-deoxygenated hexitol ring, and back again. Nevertheless, strong hybridizing complexes were obtained and these have been compared with incorporation of 2'-*O*-methylated uridine monomers at exactly the same position. As expected from the literature, the 2'-O-methyluridine containing oligos displayed increased affinity for the RNA complement over the non-methylated reference oligos. However, likewise a systematic increase in affinity was noticed of both the methylated and non-methylated hexitol modification containing oligos for their respective complementary sequences. The oligos with the plain hexitol modification (hU) surpassed the reference RNA oligos in affinity for their target, while the oligos containing the 3'-*O*-methylated altrohexitol modification **1 (U*)** proved to be endowed with an even better affinity (surpassing the Tm values for the reference oligos mostly by 3 to 4°C).

As pointed out before, we have to keep the change in conformation in mind, which takes place by incorporation of the modification. Therefore multiple incorporation of these modified building blocks could result in even higher stabilities of the formed complexes and thus higher affinities of the backbone modified oligos for RNA. Clearly, it is possible to incorporate the new modified monomers into RNA oligonucleotides, without comprising the affinity for their respective RNA target. In contrast, overall a clear increase in affinity is noticed.

Table 4. Thermal stability of RNA sequences (5'→3') containing in the middle a single incorporation of a modified building block hybridized to complementary RNA nonamers.

Entry	Sequence	T_m /°C [a]	ΔT_m /°C [b]
A	GCG U U U GCG	51.4 (59.3)	reference
B	GCG U $\mathbf{U_{OMe}}$ U GCG	53.0 (60.8)	1.6 (1.5)
C	GCG U **hU** U GCG	54.4 (61.6)	3.0 (2.3)
D	GCG U **1** U GCG	55.4 (62.4)	4.0 (3.1)
E	GCU G U G UCG	55.9 (62.8)	reference
F	GCU G $\mathbf{U_{OMe}}$ G UCG	57.3 (64.6)	1.4 (1.8)
G	GCU G **hU** G UCG	57.1 (64.7)	1.2 (1.9)
H	GCU G **1** G UCG	59.3 (66.5)	3.4 (3.7)
I	GCA C U C ACG	56.9 (63.8)	reference
J	GCA C $\mathbf{U_{OMe}}$ C ACG	58.0 (65.1)	1.1 (1.3)
K	GCA C **hU** C ACG	60.0 (66.9)	3.1 (3.1)
L	GCA C **1** C ACG	60.8 (67.7)	3.9 (3.9)
M	GCC A U A CCG	57.1 (64.4)	reference
N	GCC A $\mathbf{U_{OMe}}$ A CCG	58.8 (66.4)	1.7 (2.0)
O	GCC A **hU** A CCG	57.2 (64.9)	0.1 (0.5)
P	GCC A **1** A CCG	58.9 (66.2)	1.8 (1.8)

[a] T_m's towards complementary RNA, obtained in a buffer consisting of 0.1 M NaCl (respectively 1 M NaCl) and 20mM phosphate, pH 7.4 with a duplex concentration of 4μM; [b] ΔT_m/modification; $\mathbf{U_{OMe}}$ characterizes 2'-*O*-methyluridine, **hU** characterizes a 1,5-anhydrohexitol uracil monomer, and **1** characterizes a 3'-*O*-methyl-1,5-anhydroaltritol uracil monomer.

Taking together, the present invention eliminates the problem of the supplementary protecting group as necessary in altritol nucleic acids (ANA) by alkylation of the [S]-hydroxyl group which is liberated upon opening of the allitol epoxide by introduction of the heterocyclic base moiety.[14] Such alkylation reaction paves the way for a series of new nucleoside analogues, the 3'-*O*-methyl altritol nucleoside analogues (**21**), or more generally 3'-*O*-alkyl altritol nucleoside analogues (**22**), useful for incorporation into oligonucleotides **(Scheme IV)**.

In addition, the present invention details the use of ubiquitous methylglucoside as starting material for synthesis of 3'-deoxy-1'-*O*-methylglycosidic analogues (**26**) of 1,5-

anhydrohexitol nucleosides, eliminating the need for reductive deoxygenation of the C1-position.

Both new types of nucleoside analogues have been functionalized to allow incorporation into oligonucleotides, and the newly constructed oligomers showed strong pairing potential for RNA oligonucleotides.

Finally, it is clear for the specialist in the field that both afore mentioned modifications can be combined in one synthesis, leading to the novel 1'-*O*-methylglycosidic nucleoside analogues (**28 and 29**), having a 3'-*O*-alkyl moiety with the [S]-configuration.

Experimental procedures

1,5-anhydro-4,6-*O*-benzylidene-3-*O*-methyl-2-(uracil-1-yl)-2-deoxy-D-*altro*-hexitol (6).

1,5-anhydro-4,6-*O*-benzylidene-2-(uracil-1-yl)-2-deoxy-D-*altro*-hexitol (**5**)[14] (1.59 g, 4.6 mmol) was coevaporated with anhydrous acetonitrile (3×13 mL) and dissolved in anhydrous THF (38 mL). NaH (552 mg, 13.8 mmol) was added, and the reaction was left to stir 30 min at 0°C, whereupon CH_3I (1.35 mL, 23 mmol) was added. After 5 hours stirring at 0°C an additional amount of CH_3I (1 mL, 17 mmol) was added, and the reaction was left to stir another 2 hours at 0°C. The reaction was quenched with water (20 mL), diluted with EtOAc (200 mL) and washed with $NaHCO_3$ (2×50 mL). The combined aqueous phase was extracted with dichloromethane (50 mL), whereupon the combined organic phase was dried (Na_2SO_4), filtered and evaporated to dryness. Purification by silica column chromatography (0-2% MeOH/dichloromethane) afforded the methylated nucleoside (829 mg, 2.28 mmol, 50% (69% based on recovered starting material)) as a white foam. R_f: 0.3 (5% MeOH/dichloromethane).

δ ^{1}H-NMR ($CDCl_3$): 9.69 (s, 1h, NH), 8.04 (d, J=8.06 Hz, 1H, 6-H), 7.34-7.49 (m, 5H, Ph), 5.80 (d, J=8.06 Hz, 1H, 5-H), 5.30 (s, 1H, PhC<u>H</u>), 4.53 (t, J= 2.93 Hz, 1H, 2'-H), 4.37 (dd, J= 5.49Hz, 9.89 Hz, 1H, 6'-H_e), 4.32 (dd, J= 3.29, 13.18 Hz, 1H, 1'H_e), 4.08 (dt, J= 5.12, 9.89 Hz, 1H, H-5'), 4.03 (d, J= 13.92 Hz, 1H, 1'H), 3.86 (br t, 1H, 3'-H), 3.81 (d, j= 10.26 Hz, 1H, 6'-H_a), 3.64 (dd, J= 2.56, 9.53 Hz, 1H, 4'-H), 3.63 (s, 3H, OCH_3). δ ^{13}C-NMR ($CDCl_3$): 163.30 (C-4), 150.79 (C-2), 142.05 (C-6), 137.01, 129.03, 128.15, 126.00 (Ph), 102.60 (C-5), 102.23 (Ph<u>C</u>H), 76.22 (C-4'), 74.58 (C-3'), 68.70 (C-6'), 66.45 (C-5'), 64.11 (C-1'), 59.41 (OCH_3), 54.64 (C-2'). HRMS (thgly) calc. for $C_{18}H_{20}N_2NaO_6$ $(M+Na)^+$: 383.1219, found 383.1229.

1,5-anhydro-3-*O*-methyl-2-(uracil-1-yl)-2-deoxy-D-*altro*-hexitol (1).

1,5-anhydro-4,6-*O*-benzylidene-3-*O*-methyl-2-(uracil-1-yl)-2-deoxy-D-*altro*-hexitol (**6**) (390 mg, 1.08 mmol) was dissolved in 90% aq. trifluoroacetic acid (6 mL) and stirred at room temperature for 1 hour. Upon completion, the mixture was evaporated to dryness and coevaporated with toluene (2×10 mL). Purification by silica column chromatography (5-10% MeOH in dichloromethane) afforded the deprotected nucleoside **1** as a white foam (210 mg, 0.77 mmol, 71%). R_f: 0.28 (10% MeOH/dichloromethane).

δ ^{1}H-NMR (DMSO-*d*6): 11.32 (s, 1H, NH), 7.98 (d, J=8.06 Hz, 1H, 6-H), 5.57 (dd, J= 2.2, 8.06 Hz, 1H, 5-H), 4.85 (d, J= 6.23 Hz, 1H, 4'-OH), 4.60 (t, J= 5.86 Hz, 1H, 6'-OH), 4.46 (AB, J= 3.66, 1H, 2'-H), 3.86 (d, J= 3.66 Hz, 2H, 1'-H), 3.51-3.68 (m, 5H, 3'-H, 4'-H, 5'-H, 6'-H), 3.39 (s, 3H, OCH_3). δ ^{13}C-NMR (DMSO-*d*6): 163.42 (C-4), 151.31 (C-2), 143.27 (C-6), 101.35 (C-5), 78.01, 77.23 (C-3' and C-4'), 63.60 (C-5'), 63.08 (C-1'), 60.14 (C-6'), 57.62 (OCH_3), 52.77 (C-2'). HRMS (thgly) calc. for $C_{11}H_{15}N_2Na_2O_6$ $(M-H+2Na)^+$: 317.07255, found 317.07232.

1,5-anhydro-3-*O*-methyl-5-*O*-monomethoxytrityl-2-(uracil-1-yl)-2-deoxy-D-*altro*-hexitol (7).

1,5-anhydro-3-*O*-methyl-2-(uracil-1-yl)-2-deoxy-D-*altro*-hexitol (**1**) (460 mg, 1.69 mmol) was coevaporated with anhydrous pyridine (2×5 mL) and redissolved in anhydrous pyridine (10 mL). Monomethoxytritylchloride (532 mg, 1.73 mg) was added, and the reaction was left to stir for 20 hours. After completion, the reaction was quenched with methanol (2 mL) and evaporated to dryness. The last residues of pyridine were removed by coevaporation with toluene. Purification by silica column chromatography (1-5 % MeOH/dichloromethane) afforded the tritylated compound as a white foam (816 mg, 1.50 mmol, 89 %). R_f: 0.79 (5% MeOH/dichloromethane).

δ ^{1}H-NMR (DMSO-*d*6): 11.40 (s, 1H, NH), 8.06 (d, J= 8.06 Hz, 6-H), 6.88-7.43 (m, 14H, MMTr), 5.58 (d, J= 8.06 Hz, 1H 5-H), 4.80 (d, J= 6.59 Hz, 1H, 4'-OH), 4.45 (m, 1H, 2'-H), 3.95 (m, 2H, 1'-H), 3.60-3.86 (m, 5H, MMTr-OCH_3, 4'-H, 5'-H), 3.54 (pt, J= 3.67 Hz, 1H, 3'-H), 3.41 (s, 3H, OCH_3), 3.21 (d, J= 2.57 Hz, 2H, 6'-H). δ ^{13}C-NMR (DMSO-*d*6): 163.41 (C-4), 158.41 (MMTr), 151.21 (C-2), 144.75 (MMTr), 143.08 (C-6), 135.28 (MMTr), 127.02-130.36 (MMTr), 113.33 (MMTr), 101.41 (C-5), 85.56 (MMTr), 77.37, 75.91 (C-3' and C-4'), 63.80 (C-5'), 63.25 (C-1'), 62.25 (C-6'), 58.00 (OCH_3), 55.15 (MMTr), 52.78 (C-2'). HRMS (thgly) calc. for $C_{31}H_{32}N_2NaO_7$ $(M+Na)^+$: 567.2107, found 567.1817.

1,5-anhydro-3-*O*-methyl-4-*O*-(*P*-β-cyanoethyl-*N*,*N*-diisopropylaminophosphinyl)-6-*O*-monomethoxytrityl-2-(uracil-1-yl)-2-deoxy-D-*altro*-hexitol (8).

The monomethoxytritylated derivative **7** (495 mg, 0.90 mmol) was dissolved in 6 mL dichloromethane under argon and diisopropylethylamine (470 μL, 2.70 mmol) and 2-cyanoethyl N,N-diisopropylchlorophosphoramidite (305 μL, 1.35 mmol) were added and the solution was stirred for 2 hours. An additional amount of 1.35 mmol DIPEA and 0.65 mmol of the amidite were added and the mixture was stirred for another 2 hours TLC indicated complete reaction. Water (3 mL) was added, the solution was stirred for 10 min. and partitioned between CH_2Cl_2 (50 mL) and aqueous $NaHCO_3$ (30 mL). The organic phase was washed with aqueous sodium chloride (2x30 mL) and the aqueous phases were back extracted with CH_2Cl_2 (30 mL). Evaporation of the organics left an oil which was flash purified twice on 40 g of silica gel (hexane: acetone: TEA, 49:49:2) to afford the product as a foam after coevaporation with dichloromethane. Dissolution in 2 mL of dichloromethane and precipitation in 60 mL cold (-70°C) hexane afforded 605 mg (0.81 mmol, 90%) of the title product **8** as a white powder. Rf (hexane: acetone: TEA 49:49:2): 0.32.

ESI-MS pos. calcd. for $C_{40}H_{50}N_4O_8P_1$ 745.33660 found 745.3429 $[M+H]^+$;
^{31}P-NMR δ (ppm, external ref. = H_3PO_4 capil.) 148.11, 150.40.

1,5-anhydro-4,6-*O*-benzylidene-3-*O*-methyl-2-(cytosin-1-yl)-2-deoxy-D-*altro*-hexitol (30)

To a solution of 1,5-anhydro-4,6-*O*-benzylidene-3-*O*-methyl-2-(uracil-1-yl)-2-deoxy-D-*altro*-hexitol (**6**) (602 mg, 1.67 mmol) in anhydrous acetonitrile (21 mL) was added 1,2,4-triazole (1.08 g, 15.7 mmol) and $POCl_3$ (0.31 mL, 3.33 mmol). The reaction mixture was cooled to 0°C and anhydrous triethylamine (2.1 mL, 15.1 mmol) was added and the reaction was left to stir for 18 hours at room temperature. The reaction was quenched with triethylamine (1.38 mL) and water (0.4 mL) and stirring was continued for another 10 minutes, before the mixture was evaporated to dryness. The residue was dissolved in ethylacetate (100 mL) and washed with aq. $NaHCO_3$ (2×10 mL) and water (10 mL). The aqueous phase was extracted with dichloromethane (50 mL) and the combined organic extract was dried (Na_2SO_4), filtered and evaporated to dryness. The residue was dissolved in dioxane (10 mL) and concentrated ammoniumhydroxide (2 mL) was added, and the reaction was left to stir for 3 days, whereupon it was evaporated to dryness. Silica gel column chromatography (3, 5, 10%

MeOH/dichloromethane) afforded the cytosine congener **30** as a white foam (540 mg, 1.49 mmol, 89%). R_f: 0.21 (7% MeOH/dichloromethane).

δ ^{1}H-NMR (DMSO-*d*6): 7.90 (d, J= 7.69 Hz, 1H, 6-H), 7.33-7.42 (m, 5H, Ph), 7.19 (br. S, 2H, NH_2), 5.79 (d, J= 7.33 Hz, 1H, 5-H), 5.64 (s, 1H, PhC<u>H</u>), 4.47 (m, 1H, 2'-H), 4.22 (dd, J= 4.39, 9.52 Hz, 1H, 6'-H_e), 4.09 (s, 2H, 1'-H), 3.81 (m, 1H, 5'-H), 3.63-3.73 (m, 3 H, 3'H, 4'-H and 6'-H_a), 3.49 (s, 3H, OCH_3). δ ^{13}C-NMR (DMSO-*d*6): 165.86 (C-4), 155.54 (C-2), 143.37 (C-6), 137.94 (Ph), 129.05, 128.26 and 126.37 (Ph), 101.27 (Ph<u>C</u>H), 94.29 (C-5), 76.34 (C-4'), 74.81 (C-3'), 68.25(C-6'), 66.28(C-5'), 64.25(C-1'), 58.45 (OCH_3), 54.08 (C-2'). HRMS (thgly) calc. for $C_{18}H_{22}N_3O_5$ $(M+H)^+$: 360.1559, found 360.1572.

1,5-anhydro-4,6-*O*-benzylidene-3-*O*-methyl-2-(N^4-benzoylcytosin-1-yl)-2-deoxy-D-*altro*-hexitol (31).

To a solution of **30** (499 mg, 1.39 mmol) in anhydrous pyridine (8 mL) was added benzoylchloride (0.8 mL, 6.9 mmol) at 0°C, and stirring was continued at room temperature for 3 hours. The reaction mixture was cooled to 0°C and water (1.6 mL) was added, and after 5 min. concentrated ammoniumhydroxide (3.2 mL) was added. Stirring was continued for 30 min., whereupon the reaction mixture was evaporated to dryness. Purification by silica gel column chromatography (0-5% MeOH/dichloromethane) afforded the benzoylated nucleoside as a white foam (480 mg, 1.04 mmol, 75%). R_f: 0.74 (7% MeOH/dichloromethane).

δ ^{1}H-NMR (DMSO-*d*6): 8.41 (d, J= 7.69 Hz, 1H, 6-H), 8.03 (d, J= 6.96 Hz, 2H, Bz), 7.34-7.68 (m, 11 H, Bz, Ph, 5-H), 4.61 (br s, 1H, 2'-H), 4.10-4.28 (m, 3H, 1'-H, 6'-H_e), 3.70-3.91 (m, 4H, 3'-H, 4'-H, 6'-H_a), 3.53 (s, 3H, OCH_3). δ ^{13}C-NMR (DMSO-*d*6): 168.18, 167.75 (CO), 163.20 (C-4), 155.04 (C-2), 147.93 (C-6),126.41-137.93 (2Bz + Ph), 101.31 (Ph<u>C</u>H), 96.97 (C-5), 75.91 (C-4'), 74.27 (C-3'), 68.20 (C-6'), 66.44 (C-5'), 64.07 (C-1'), 58.58 (OCH_3), 55.12 (C-2'). HRMS (thgly) calc. for $C_{25}H_{26}N_3O_6$ $(M+H)^+$: 464.1821, found 464.1890.

1,5-anhydro-3-*O*-methyl-2-(N^4-benzoylcytosin-1-yl)-2-deoxy-D-*altro*-hexitol (32).

1,5-anhydro-4,6-*O*-benzylidene-3-*O*-methyl-2-(N^4-benzoylcytosin-1-yl)-2-deoxy-D-*altro*-hexitol (**31**) (480 mg, 1.04 mmol) was dissolved in 90% aq. TFA (20 mL) and left to stir at room temperature for 3 hours. Upon completion, the mixture was evaporated to dryness, and silica gel column chromatography (5, 10% MeOH/dichloromethane) afforded the deprotected nucleoside as a pale yellow foam (250 mg, 0.67 mmol, 64%)., R_f: 0.20 (5% MeOH/dichloromethane).

δ ^{1}H-NMR (DMSO-*d*6): 4.49 (d, J= 7.5 Hz, 1H, 6-H), 8.01 (d, J= 8.5 Hz, 2H, Ph_o), 7.63 (t, J= 7 Hz, 1H, Ph_p), 7.53 (t, J= 8 Hz, 2H, Ph_m), 7.32 (d, J= 7 Hz, 1H, 5-H), 4.63 (d, J= 4 Hz, 1H, 2'-H), 4.01 (dAB, J= 2.5, 12.5 Hz, 2H, 1'-H), 3.65 (m, 3H, 4'-H, 5'-H, 6'-H_A), 3.60 (m, 2H, 3'-H, 6'-H_B), 3.46 (s, 3H, OCH_3). δ ^{13}C-NMR (DMSO-*d*6): 167.69 (CO), 162.94 (C-4), 155.18 (C-2), 148.32 (C-6), 133.39, 132.94, 128.66 (Ph), 96.40 (C-5), 77.64 (C-5'), 76.82 (C-3'), 63.24 (C-4'), 63.13 (C-1'), 60.23 (C-6'), 57.81 (OCH_3), 54.44 (C-2').
HRMS (thgly) calc. for $C_{18}H_{22}N_3O_6$ $(M+H)^+$: 376.1509, found 376.1499.

1,5-anhydro-3-*O*-methyl-5-*O*-monomethoxytrityl-2-(N^4-benzoylcytosin-1-yl)-2-deoxy-D-*altro*-hexitol (33).

1,5-anhydro-3-*O*-methyl-2-(N^4-benzoylcytosin-1-yl)-2-deoxy-D-*altro*-hexitol (**32**) (200mg, 0.53 mmol) was co-evaporated with anhydrous pyridine (10 mL) and dissolved in anhydrous pyridine (5 mL). Monomethoxytritylchloride (250 mg, 0.81 mmol) was added, and the reaction was left to stir overnight at room temperature, whereupon additional monomethoxytritylchloride (100 mg, 0.32 mmol) and triethylamine (0.5 mL) was added. After stirring for an additional 2 days, the reaction was quenched with MeOH (2 mL) and evaporated to dryness. Purification by silica column chromatography (0-2% MeOH/dichloromethane) afforded the tritylated nucleoside as a pale yellow foam (100 mg, 0.15 mmol, 28 %)

δ ^{1}H-NMR (DMSO-*d*6): 11.34 (s, 1H, N^4-H), 8.67 (d, J= 7.32 Hz, 1H, 6-H), 8.04 (d, J= 6.96 Hz, 2H, Bz), 7.20-7.68 (m, 16H, Bz, MMTr, 5-H), 6.92 (d, J= 8.79 Hz, 2H, MMTr), 4.78 (d, J= 6.59 Hz, 1H, 4'-OH), 4.64 (m, 1H, 2'-H), 3.84 (m, 1H, 4'-H), 4.18 (d, J= 13.55 Hz, 1H, 1'-H_A), 4.02 (dd, J= 3.29, 13.55 Hz, 1H, 1'-H_B), 3.74 (s, 3H, MMTr), 3.67 (m, 2H, 3'-H, 5'-H), 3.48 (s, 3H, OCH_3), 3.21 (m, 2H, 6'-H). δ ^{13}C-NMR ($CDCl_3$): 166.53 (CO), 162.25 (C-4), 158.64 (MMTr), 155.58 (C-2), 148.11 (C-6), 144.35 (MMTr), 135.79 (MMTr), 133.21 (Cx), 127.02-130.23 (MMTr), 113.15 (MMTr), 97.00 (C-5), 86.32 (MMTr), 76.67 (C-5'), 76.09 (C-3'), 63.86 and 63.46 (C-1' and C-4'), 62.31 (C-6'), 58.61 (OCH3), 55.15 (MMTr), 53.45 (C-2').
HRMS (thgly) calc. for $C_{38}H_{38}N_3O_7$ $(M+H)^+$: 648.2721, found 648.2710.

Methyl 4,6-*O*-benzylidene-2-(thymin-1-yl)-2-deoxy-D-*altro*-hexopyranoside (12).

Thymine (3.78 g, 30 mmol) was suspended in 250 ml of anhydrous DMF to which was added 1.13 g of a 60% oil dispersion of sodium hydride (28 mmol) and the mixture was heated on an oil bath for 1 hour at 90°C. The allitol epoxide[22] **11** (2.64 g, 10 mmol) was added and the mixture was heated for 4 days at 120°C, after which the reaction was cooled, quenched with sodium bicarbonate and concentrated. The residue was partitioned between 200 ml of ethyl acetate and 200 ml of 5% aqueous sodium bicarbonate, and the organics were washed twice

with brine. Purification of the organic residue on silica gel (0-2% MeOH/dichloromethane) afforded 2.77 g (7.1 mmol, 71%) of the title compound as a foam.

UV (MeOH) λmax 269 nm; FABMS 391 (M+H);
^{1}H-NMR (DMSO-*d*6): δ : 1.81 (s, 3H, 5-CH_3), 3.30 (s, 3H, 1'-OCH_3), 3.75-3.95 (m, 3H), 4.10-4.32 (m, 2H), 4.52 (d, 1H, J=1.7Hz, 2'-H), 4.88 (s, 1H, 1'-H), 5.38 (d, 1H, J=4.6Hz), 5.72 (s, 1H, PhC<u>H</u>), 7.30-7.50 (m, 5H, arom-H), 7.55 (d, J= 1.1 Hz, 6-H), 11.40 (s, 1H, NH);
^{13}C-NMR ($CDCl_3$) δ : 163.81 (C-4), 150.85 (C-2), 137.55 (C-6), 137.88, 128.95, 128.10, 126.50 (Ph), 109.34 (C-5), 101.00 (Ph<u>C</u>H), 98.59 (C-1'), 75.20 (C-4'), 68.25 (C-6'), 66.38 (C-5'), 58.34 (C-3'), 57.85 (C-2'), 54.91 (OCH_3), 12.66 (5-CH_3); ESI-MS pos.: HRMS calcd. for $C_{33}H_{36}N_2O_8Na$ 611.2369; found 611.2364 $[M+Na]^+$.

Methyl 4,6-*O*-benzylidene-3-*O*-(2,4-dichlorophenoxythiocarbonyl)-2-(thymin-1-yl)-2-deoxy-D-*altro*-hexopyranoside (13).

The methyl 4,6-*O*-benzylidene-2-(thymin-1-yl)-2-deoxy-D-*altro*-hexopyranoside (**12**) (390 mg, 1 mmol) obtained in the previous preparation, and 856 mg (7 mmol) of dimethylaminopyridine were dissolved in 15 mL of dry dichloromethane. The reaction mixture was cooled to –40°C, and 0.158 mL (2 mmol) of thiophosgene was added with vigorous stirring. The mixture was brought to room temperature, and after stirring for 1 hour, 656 mg (4 mmol) of 2,4-dichlorophenol was added and stirring was continued for 2 hours more. The mixture was poured in 20 mL of a 1 M solution of KH_2PO_4 and extracted twice with dichloromethane. The organic layers were dried, and after evaporation the residue was purified by flash chromatography (0-2% MeOH/dichloromethane). The product was immediately used in the next step.
FABMS 391 (M+H).

Methyl 4,6-*O*-benzylidene-2-(thymin-1-yl)-2,3-dideoxy-D-*arabino*-hexopyranoside (14).

The obtained thiocarbonyl compound was dissolved in 15 mL of anhydrous toluene. After nitogen gas was bubbled through the solution for 10 min., 0.41 mL (1.5 mmol) of tributyltin hydride and 20 mg of 2,2'-azobis(2-methylpropionitrile) were added, and the mixture was heated at 80°C overnight, when TLC indicated complete reaction. The mixture was evaporated and purified on silica gel (0-2% MeOH/dichloromethane) affording 320 mg (0.85 mmol, 85%) of the title compound.

UV (MeOH) λmax 269 nm; FABMS 375 (M+H);
^{1}H-NMR ($CDCl_3$) δ : 1.98 (s, 3H, 5-CH_3), 2.12-2.40 (m, 2H, 3'-H), 3.45 (s, 3H, 1'-OCH_3), 3.65-3.80 (m, 1H, 5'-H), 3.80 (d, J= 10 Hz, 1H, 6'-H_a), 3.90-4.04 (dd, J= 4.3, 9.5 Hz, 1H,

4'-H), 4.34 (dd, J= 4.3 Hz, 9.9 Hz, 1H, 6'-H_e), 4.78 (s, 1H, 1'-H), 4.81 (t, 1H, J=2.4 Hz, 2'-H), 5.58 (s, 1H, PhC<u>H</u>), 7.25-7.50 (m, 5H, arom-H), 7.72 (s, 1H, 6-H), 8.92 (s, 1H, NH); ^{13}C-NMR ($CDCl_3$) δ : 163.39 (C-4), 150.64 (C-2), 137.27 (C-6), 137.04, 129.18, 128.31, 126.07 (Ph), 110.68 (C-5), 102.15 (Ph<u>C</u>H), 98.39 (C-1'), 73.20 (C-4'), 69.11 (C-6'), 65.08 (C-5'), 55.07 (OCH_3), 53.59 (C-2'), 29.60 (C-3'), 12.84 (5-CH_3).

Methyl 2-(thymin-1-yl)-2,3-dideoxy-D-*arabino*-hexopyranoside (2).

Method A

An amount of 500 mg (1.33 mmol) of the benzylidene protected compound **14** was dissolved in 25 mL of methanol and 2.5 mL of trifluoroacetic acid was added. The solution was stirred for 3 hours, evaporated to dryness and coevaporated twice with dioxane. The residue was dissolved in methanol, adsorbed on silica gel by evaporation, and purified by flash chromatography on silica gel (0-15% MeOH/dichloromethane) to afford the title compound in 45% yield (172 mg, 0.6 mmol)

Method B

An amount of 1.08 g (2.89 mmol) of the benzylidene protected compound **14** was dissolved in 40 mL of methanol in a Parr container, and 0.5 mL of acetic acid was added. The solution was degassed by bubbling nitrogen for 10 min. after which 450 mg of 10% Pd on carbon was added and the mixture was hydrogenated overnight on a Parr apparatus at 45 psi. The mixture was filtered, the filter was washed with hot ethanol, the volatiles were removed in vacuo and the residue was coevaporated twice with dioxane. Crystallization from hexane afforded the title compound in 90% yield (743 mg, 2.60 mmol).

UV (MeOH) λmax 269 nm; FABMS 287 (M+H).

6-*O*-Dimethoxytrityl-2-(thymin-1-yl)-2,3-dideoxy-D-methylglucopyranoside (15).

Following coevaporation with anhydrous pyridine an amount of 910 mg (3.18 mmol) of the thymine glucopyranoside **1** was dissolved in 25 mL of pyridine and dimethoxytrityl chloride (1.19 g, 3.5 mmol) was added. The mixture was stirred for 16 h at ambient temperature, quenched with 3 mL of methanol and neutralized with some aqueous sodium bicarbonate. The mixture was concentrated and partitioned twice between dichloromethane and aqueous sodium bicarbonate. The organic layer was purified on 40 g of silica gel with a methanol step gradient (0 to 1%) in dichloromethane containing 0.5% of pyridine, affording 1600 mg (2.72 mmol, 85%) of the title compound **2** as a foam.

^{1}H-NMR 500 MHz ($CDCl_3$) δ : 1.82 (s, 3H, 5-CH_3), 2.00-2.07 (ddd, 1H, 3'-H), 2.12-2.18 (ddd, 1H, 3"-H), 2.28 (d, 1H, J=3.5 Hz, xx), 3.38 (s, 3H, 1'-OCH_3), 3.45 (d, J=3.6Hz, 2H, 6'-H), 3.69 (dt, 1H, J = 9 and 8.5 Hz, 5'-H), 3.78 (s, 6H, 2xOCH_3), 3.95 (m, 1H, 4'-H), 4.70 (t, 1H, J=6.5Hz, 2'-H), 4.75 (s, 1H, 1'-H), 6.84 (2d, 4H, J=9Hz, arom-H), 7.20-7.47 (m, 9H, arom-H), 7.74 (d, J= 1.1 Hz, 6-H), 9.05 (s, 1H, NH); ^{13}C-NMR ($CDCl_3$) δ : 163.59 (C-4), 150.88 (C-2), 137.98 (C-6), 110.51 (C-5), 98.14 (C-1'), 86.69 (Ph_3C), 72.33 (C-5'), 63.54 (C-4'), 63.05 (C-6'), 55.20 (2xCH_3O), 54.91 (1'-OCH_3), 53.50 (C-2'), 31.95 (C-3'), 12.65 (5-CH_3) + aromatic signals; ESI-MS pos.: HRMS calcd. for $C_{33}H_{36}N_2O_8Na$ 611.2369; found 611.2364 $[M+Na]^+$.

6-*O*-Dimethoxytrityl-2-(thymin-1-yl)-4-*O*-(*P*-β-cyanoethyl-*N*,*N*-diisopropylamino-phosphinyl)-2,3-dideoxy-β-D-methylglucopyranoside (16).

The dimethoxytritylated derivative **2** (800 mg, 1.36 mmol) was dissolved in 10 mL dichloromethane under argon and diisopropylethylamine (710 µL, 4.08 mmol) and 2-cyanoethyl N,N-diisopropylchlorophosphoramidite (455 µL, 2.05 mmol) were added and the solution was stirred for 15 minutes when TLC indicated complete reaction. Water (4 mL) was added, the solution was stirred for 10 min. and partitioned between CH_2Cl_2 (50 mL) and aqueous $NaHCO_3$ (30 mL). The organic phase was washed with aqueous sodium chloride (2x30 mL) and the aqueous phases were back extracted with CH_2Cl_2 (30 mL). Evaporation of the organics left an oil which was flash purified twice on 40 g of silica gel (hexane: acetone: TEA, 68:30:2) to afford the product as a foam after coevaporation with dichloromethane. Dissolution in 2 mL of dichloromethane and precipitation in 80 mL cold (-70°C) hexane afforded 718 mg (0.91 mmol, 67%) of the title product **3** as a white powder.

Rf (hexane: acetone: TEA 49:49:2): 0.37; ESI-MS pos.: 789.5 $[M+H]^+$, 811.4 $[M+Na]^+$; HRMS calcd. for $C_{42}H_{54}N_4O_9P_1$ $[M+H]^+$: 789.36281, found: 789.3640; ^{31}P-NMR δ (ppm, external ref. = H_3PO_4 capil.) 148.55, 149.01; ^{13}C-NMR ($CDCl_3$) δ : 163.43 (C-4), 150.78 (C-2), 137.98 (C-6), 117.2 (CN), 110.68 (C-5), 98.22 (C-1'), 86.13 (Ph_3C), 72.57 (C-5'), 63.42 (d, J=17.5Hz, C-4'), 62.08 (C-6'), 58.10 and 57.65 (2xd, J=18.6Hz, PO$\underline{CH_2}$), 55.20 (2xCH_3O), 54.88 (1'-OCH_3), 53.60 (C-2'), 43.11 (2xPN$\underline{CH}$), 31.90 (C-3'), 24.60 and 24.20 (4xCH$\underline{CH_3}$), 20.20 ($\underline{CH_2}$CN), 12.49 (5-CH_3) + aromatic signals.

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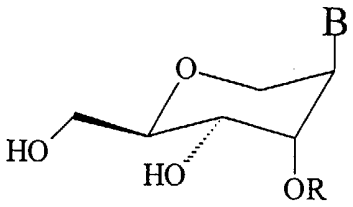
WHAT WE CLAIM IS :

1. 2-deoxy-3-*O*-alkyl-1,5 –anhydro-*altro*-hexitol nucleoside analogues, represented by general formula I



I

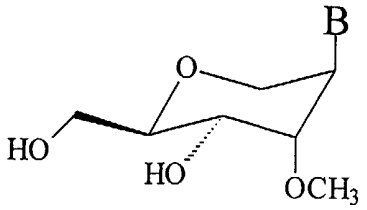
wherein:

B is a heterocyclic ring derived from the group consisting of pyrimidine and purine bases, and

R is an alkyl group, with:

alkyl being a straight or branched chain, saturated or unsaturated, substituted or unsubstituted hydrocarbon radical having from 1 to 6 carbon atoms.

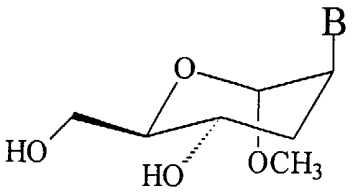
2. 2-deoxy-3-*O*-methyl-1,5 –anhydro-*altro*-hexitol nucleoside analogues, represented by general formula II



II

wherein B is a heterocyclic ring derived from the group consisting of pyrimidine and purine bases.

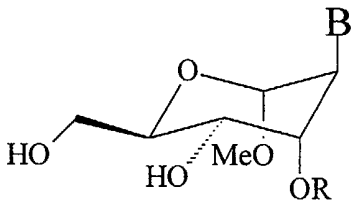
3. methyl 2,3-dideoxy-β-D-hexopyranoside nucleoside analogues, represented by general formula III



III

wherein B is a heterocyclic ring derived from the group consisting of pyrimidine and purine bases.

4. methyl 2-deoxy-3-*O*-alkyl-β-D-hexopyranoside nucleoside analogues, represented by general formula IV



IV

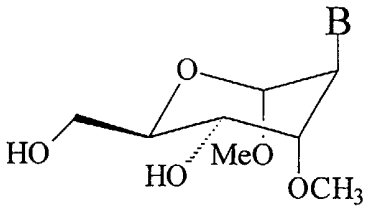
wherein:

B is a heterocyclic ring derived from the group consisting of pyrimidine and purine bases, and

R is an alkyl group, with:

alkyl being a straight or branched chain, saturated or unsaturated, substituted or unsubstituted hydrocarbon radical having from 1 to 6 carbon atoms.

5. methyl 2-deoxy-3-*O*-methyl-β-D-hexopyranoside nucleoside analogues, represented by general formula V



V

wherein B is a heterocyclic ring derived from the group consisting of pyrimidine and purine bases.

6. use of one or more of the nucleoside analogues as claimed in either claim 1, or in claim 2, or in claim 3, or in claim 4, or in claim 5, for incorporation into oligonucleotides.

7. use of the oligonucleotides as claimed in claim 6, in antisense strategies which comprise diagnosis, hybridisation, isolation of nucleic acids, site-specific DNA modification, and therapeutics.

Application No:	GB 0021221.7	**Examiner:**	S.I. AHMAD
Claims searched:	1,2, 4-7	**Date of search:**	31 January 2001

Patents Act 1977
Search Report under Section 17

Databases searched:

UK Patent Office collections, including GB, EP, WO & US patent specifications, in:

UK Cl (Ed.S): In view of CAS results, the C2C files were not searched

Int Cl (Ed.7): C07D-405/04

Other: DATA-BASE: CAS-ON-LINE

Documents considered to be relevant:

Category	Identity of document and relevant passage	Relevant to claims
	NO RELEVANT DOCUMENT	

X	Document indicating lack of novelty or inventive step	A	Document indicating technological background and/or state of the art.
Y	Document indicating lack of inventive step if combined with one or more other documents of same category.	P	Document published on or after the declared priority date but before the filing date of this invention.
&	Member of the same patent family	E	Patent document published on or after, but with priority date earlier than, the filing date of this application.