Pergamon

Tetrahedron Vol. 51, No. 45, pp. 12319-12336, 1995

0040-4020/95 $9.50+0.00

0040-4020(95)00777-6

Synthesis of 3'-Deoxy-3'-C-Hydroxymethyl-aldopentopyranosyl Nucleosides and their Incorporation in Oligonucleotides. Part II[1].

Bogdan Doboszewski, Hans De Winter, Arthur Van Aerschot and Piet Herdewijn*

Laboratory for Medicinal Research, Rega Institute for Medical Research, Katholieke Universiteit Leuven, Minderbroedersstraat 10, B-3000 Leuven (Belgium)

Abstract : The synthesis of 3'-deoxy-3'-C-hydroxymethyl-aldopentopyranosyl nucleosides using an intramolecular radical C-C bond formation reaction is described. This method gives good results for the synthesis of thymine and adenine nucleosides, but not for cytosine and guanine nucleosides. Dependent on the configuration (β-D-erythro or α-L-threo), the conformation of the adenine nucleosides is clearly different (axial base moiety for α-D-erythro and equatorial adenine base for α-L-threo nucleosides) which could be explained by the gauche effect. Oligonucleotides built up of 2',3'-dideoxy-3'-C-hydroxymethyl-α-L-threo-pentopyranosyl adenine are able to form duplexes with oligothymidylate although with less stability than natural dsDNA.

INTRODUCTION

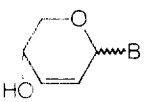
The application of synthetic oligonucleotides are numerous. One of the most exciting fields, however, is their use as antisense construct. These oligonucleotides have base sequences complementary to mRNA targets and by blocking the mRNA, they might be able to inhibit gene expression. This basic idea has stimulated several research groups to develop modified oligonucleotides. Indeed, major drawbacks of natural oligonucleotides are the susceptibility to nucleases and the poor cellular uptake. Another factor which might be important for an antisense oligonucleotide is the ability to activate RNase H, once a duplex with its target is formed. This enzyme cleaves the RNA target of a RNA-DNA duplex. The major goal of synthesizing unnatural oligonucleotides is to make the molecules more stable against enzymatic degradation and to discover oligomers which bind to their complement with a higher affinity than natural oligonucleotides. Such molecules might be less dependent on RNase H activity for their antisense effect.

One of the approaches we followed during the last years is to synthesize pyranose oligonucleotides based on the principle that conformationally constrained oligomers should have a free energy advantage over the flexible natural oligomers during hybridization[1]. This approach has also been followed by other research groups[2,3].

Here, we report on the synthesis and properties of oligonucleotides with 3'-deoxy-3'-hydroxymethyl-aldopentopyranosyl nucleosides as building blocks. The synthesis of the thymine and uracil nucleoside has been described before[4]. The rational for their synthesis is based on previously described modelling experiments[1,5]. Models of tetrameric double helices between 9-(3-deoxy-3-hydroxymethyl-aldopentopyranosyl)adenine (A_4^*)

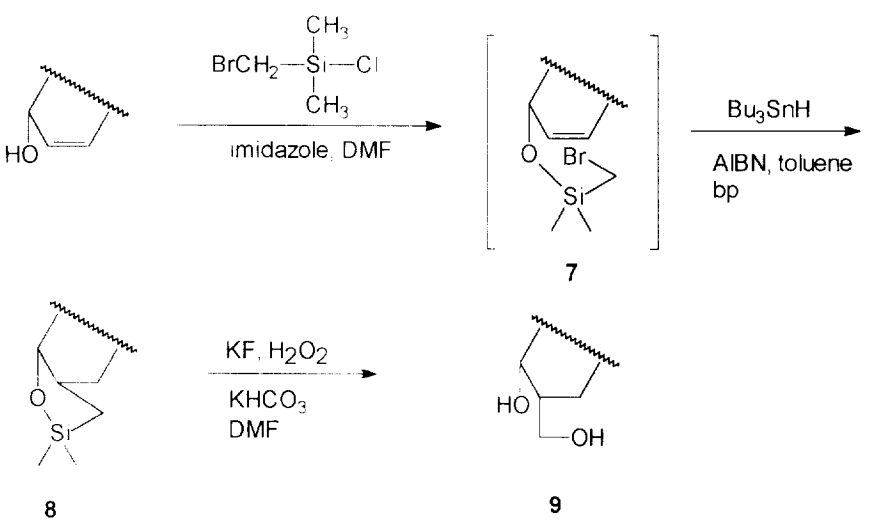
and thymidine [d(T)$_4$] were built forcing the adenine bases of the pyranosyl strand to base pair with the corresponding d(T)$_4$ and allowing the sugar and phosphate backbone to relax[1,5]. The high temperature molecular dynamics conformation search (200 ps at 1000 K) revealed that the energy needed for forcing the pyranose tetramer chain into a helix conformation is about 10 kcal/mol lower than the energy needed for the formation of the unmodified tetrameric double helix. These data were supplemented by molecular dynamics studies in an aqueous environment. Aqueous molecular dynamics simulations with the pyranose oligo as hexamers (supplied with 10 Na^+ counterions) in a water droplet of about 800 water molecules, revealed that the double helix remained intact and that the hydrogen bond motif was maintained over the course of the 200 ps simulation. These data stimulated us to start synthesis of the afore mentioned oligomers.

SYNTHESIS



B	A^{Bz}	C^{Bz}	G_{iBu}
β	**1**	**3**	**5**
α	**2**	**4**	**6**

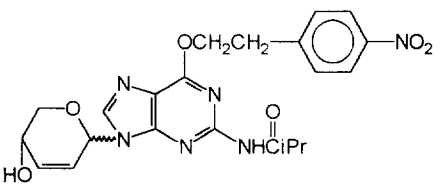
The starting 2',3'-unsaturated pentopyranosyl nucleosides **1-6** have been prepared as published before[6,7]. In order to introduce a hydroxymethyl substituent into the 3'-position, we have used a known three step procedure[8,9], which has already been used in nucleoside[4,10] and carbohydrate chemistry[11,12] (Scheme 1).



Scheme 1

Treatment of DMF solutions of the allylic alcohols **1-6** with a slight excess of bromomethyl chlorodimethylsilane in the presence of imidazole, followed by rapid aqueous work-up furnished unstable silyl ethers **7**, which, without characterization, have been subjected to a free-radical cyclization to yield species **8**. These compounds, without characterization, were subjected to oxidative desilylation to give branched products **9** having a hydroxymethyl group positioned *syn* with respect to the 4'-OH group. The products obtained are listed in Table 1.

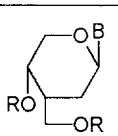
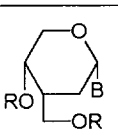
It should be mentioned, however, that no cyclization took place using O^6-p-nitrophenylethyl protected guanosine analogues **10** and **11**.



10 β

11 α

Table 1 : Yields, selected NMR parameters and conformational forms of the 2',3'-dideoxy-3'-C-hydroxy α and β D-erythro pentopyranosyl nucleosides.

						
	12	**14**	**16**	**13**	**15**	**17**
B	A	C^{Ac}	G_{Ac}	A	C^{Ac}	G_{Ac}
R	H	Ac	Ac	H	Ac	Ac
Yield %	76[b]	5[c]	13[c]	57[b]	28[c]	20[c]
$J_{1'2'}$[a]	4,0[d]	9.9[e,f]	5.5[e,f]	9.5[f,g]	10.5[e,f]	10.1[e,f]
$J_{1'2''}$[a]	4,9	3.3	< 1	< 1	2.2	2.7
$J_{4'5'}$[a]	5,5	9.6	ND[h]	ND	ND	ND
$J_{4'5''}$[a]	2,8	4.9	ND	ND	ND	ND
Conformation	$^1C_4$	$^4C_1$	$^4C_1 \leftrightarrow {}^1C_4$	$^1C_4$	$^1C_4$	$^1C_4$

a Values in Hz

b Cumulative yield of three consecutive steps

c Cumulative yield of four consecutive steps

d Recorded on a Varian Unity 500 spectrometer in CD_3OD solution

e in $CDCl_3$

f Recorded on a Varian Gemini 200 spectrometer

g in DMSO-d_6

h not determined

During the Tomao oxidation, N-deacylation took place in all cases. Only in the case of the adenosine analogue we were able to identify the N-benzoylated product **18** besides the main compound **12**. Upon addition of $KHCO_3$ and stirring for 5 h, the product **18** was converted into **12**. Yields of both β and α anomers of adenosine analogues were 76 % and 57 % respectively for three consecutive steps. High polarity of the branched N-deprotected cytidine and guanosine analogues prompted us to perform O,N-acetylation to facilitate their purification. Cytidine analogues could be O,N-acetylated using a conventional procedure (Ac_2O, Py, DMAP), to give **14** and **15**, whereas guanosine is known to furnish only O-acetylated products under these conditions[13,14]. However, using tetraalkylammonium hydroxides as bases[15,16] it was possible to get O,N-acetylated compounds **16** and **17**. The yields of the β-anomers **14** and **16**, however, turned out to be disappointingly low (Table 1). Surprisingly, the α anomer of cytidine **15** was isolated in about six times higher yield than the β anomer **14**, following the four step reaction sequence as outlined.

The coupling constants of the H1' protons in compounds **12-17** (Table 1) enabled evaluation of their preferred conformations in solution. The α anomer of adenine **13**, both anomeric cytidines **14**, **15** and α anomer of guanosine **17** adopt a conformation which assures an equatorial position of the aglycons. Large diaxial coupling constants observed for the anomeric protons (9,5-10,5 Hz) accompanied by much smaller axial-equatorial couplings indicate conformational preferences as shown in Figure 1.

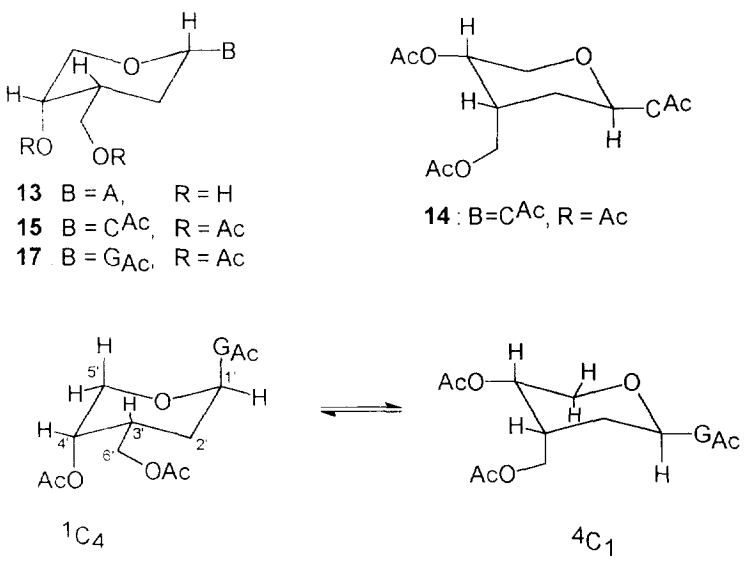
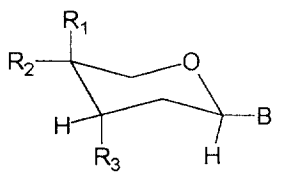


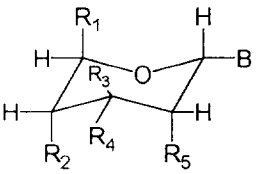
Figure 1

Minimizing steric repulsion could explain this conformational preference. This preference is in accordance with earlier observations that nucleobases in pentopyranosyl nucleosides are often positioned equatorially even at the expense of axial orientation of one, two or three other substituents as shown in Figure 2. The same pattern has been observed for 2'-deoxy-α-D-ribohexopyranosyl nucleosides[17], 2',4'-dideoxy-α-D-erythrohexopyranosyl

nucleosides[18] and also 2',3'-dideoxy-α-D-erythrohexopyranosyl nucleosides[19]. Interestingly, when a purine/pyrimidine moiety is located on a C2' position of a carbohydrate skeleton as in 1,5-anhydrohexitols **19** and **20**, the bases are oriented axially[20].

Figure 2 : The conformation preferences of pyranosyl nucleosides as described in literature.

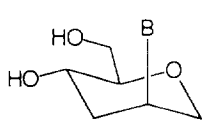




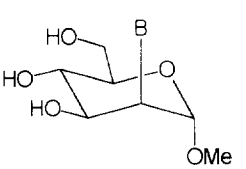
R_1	R_2	R_3	B
H	OH	H	U/A[7]
H	OH	CH_2OH	T/U[4]
OH	H	CH_2OH	T/U[4]

R_1	R_2	R_3	R_4	R_5	B
H	OAc	OAc	H	OAc	U[4]
H	OBz	OH	H	OH	U[4]
H	OH	H	H	H	U/A[7]
CH_2OH	H	H	OH	H	A/G/C/T[18]
CH_2OH	OH	H	OH	H	U,2-chloro-6-amino-purine[17]
CH_2ONBz	ONBz	H	ONBz	H	U,2,6-dichloropurine[17]
CH_2OH	OH	H	H	H	A/G/C/T[19]

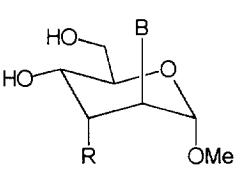
The same is true for compounds like **21** and **22**[21] However, in the latter cases the strong anomeric effect is clearly a conformation driving factor. The reason for the conformational preference of **19** and **20** is less clear.



19 B : 5-iodouracil-1-yl
20 B : hypoxanthin-9-yl



21

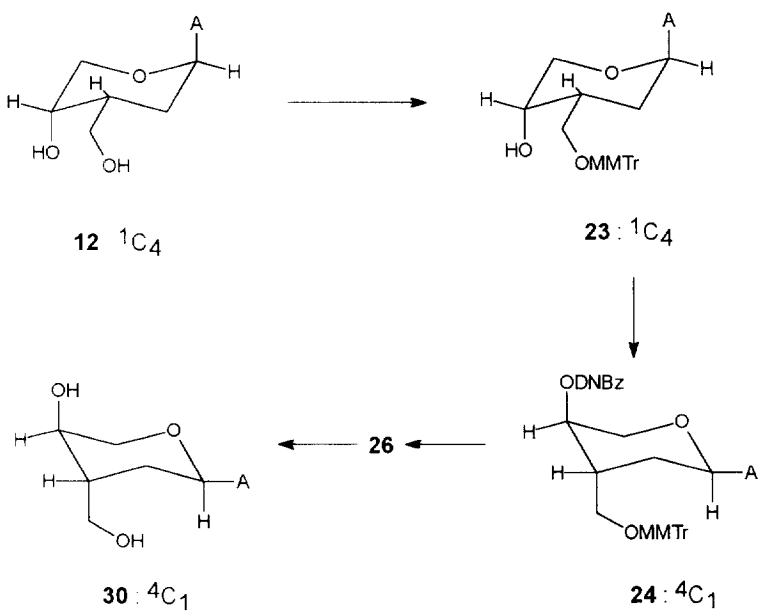


22 : R = H, OH

The β-configured guanosine analogue **16** is present in an equilibrium between $^1C_4$ and $^4C_1$ forms (Figure 1). The steric effect of a purine base moiety is somewhat less than of a pyrimidine base. For an analogue like **16**, interactions in the $^1C_4$ form between the axially oriented base and axial protons H3' and H5' are evidently comparable in magnitude to the interactions between a 3'-hydroxymethyl appendix and protons H1' and H5' in a $^4C_1$ form. Interplay of these interactions apparently results in a mixture of both conformers with evident excess of a $^4C_1$ form, albeit the $^1C_4$ conformation is supplementary stabilized by a gauche effect which is not so for the

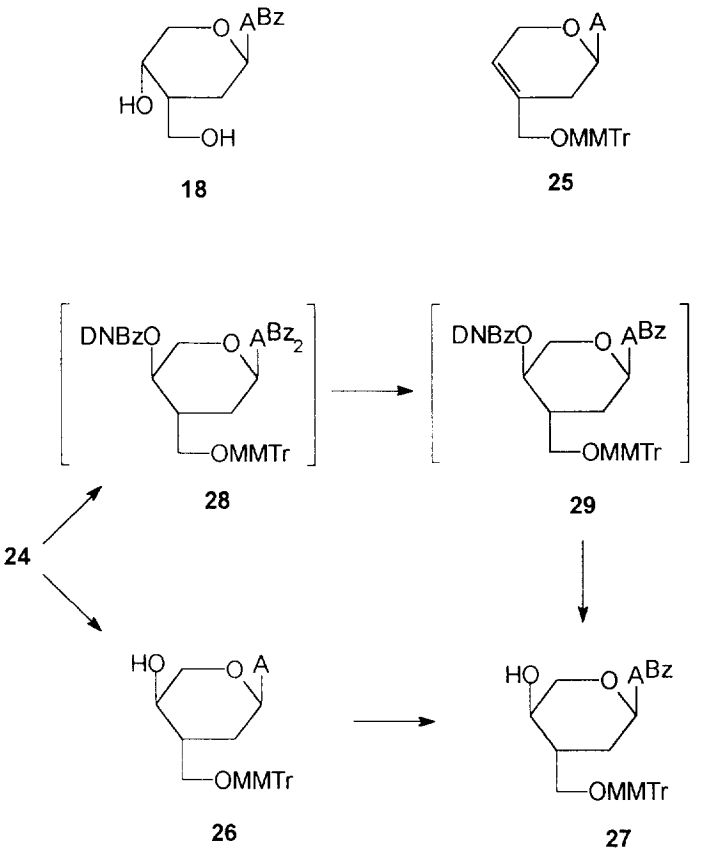
$^4C_1$ conformation. Surprisingly, the β anomer of adenosine **12** adopts exclusively a $^1C_4$ conformation (Scheme 3) with an axial adenine. This form is evident as judged from a small coupling between a H4' and H5" protons, both of which are oriented equatorially. This conformation is in contrast with preferences of the other members of this series (see further).

Low yields of the branched products for β-cytidine **14** and β guanosine **16** excluded the application of these derivatives as substrates for inversion of configuration at the C4' position to allow further incorporation into oligos for antisense studies. This, however, was feasible with the β-adenosine analogue **12** (Scheme 2, 3). Conventional selective monomethoxytritylation gave **23** (Scheme 2) which has been subjected to inversion of configuration at the 4' position using Mitsunobu conditions[22-24]. 2,4-Dinitrobenzoic acid-triphenylphosphine-diethyl azodicarboxylate system has been used to furnish 46 % of the dinitrobenzoate **24** (Scheme 2) accompanied by an elimination product **25** (Scheme 3) formed in 30 % yield. Evidently, a dinitrobenzoyl anion is still sufficiently basic to promote elimination[25]. Inversion of configuration at the 4' position induces a conformational change on going from **12** *via* **23** to **24**. Like **12**, the compound **23** is present in a $^1C_4$ form as judged from the coupling constants at the anomeric proton : $J_{1'2'}$ = 3.6 Hz, $J_{1'2"}$ = 4.8 Hz (in DMSO). However, the values $J_{1'2'}$ = 2.1 Hz and $J_{1'2"}$ = 8.3 Hz (in $CDCl_3$) recorded for **24** (Table 2), suggest a predominant (if not exclusive) conformation $^4C_1$ with equatorial adenine and both functionalities at the atoms C3',4' oriented axially.



Scheme 2

Compound **24** has been de-esterified using Zemplen conditions (cat. NaOMe-MeOH) to furnish **26**. This was transformed into its N-benzoate analogue **27** using a transient protection methodology[26,27].



Scheme 3

Alternatively, **24** was N,N-bis-benzoylated to afford **28** which was treated with 2N NH_4OH in a one flask procedure. This procedure hydrolyzed only one N-benzoyl group to furnish **29**, which without characterization was transformed into **27** by NaOMe/MeOH treatment. Compound **27** was further phosphitylated for incorporation in oligonucleotides. Fully deprotected compound **30** was obtained from **26** upon hydrolysis with 80 % HOAc. Selected NMR data for the compounds **23**, **24**, **26**, **27** and **30** and their preferential conformations are listed in Table 2. The product **24** adopts a $^4C_1$ conformation as mentioned above. The 4'-O-deprotected derivatives **26** and **27** are present in a roughly equally populated $^4C_1 \leftrightarrow {}^1C_4$ equilibrium or they adopt a $^1C_4$ form as judged from the comparison of the $J_{1'2'}$ and $J_{1'2''}$ values of **26** with those of **12**. In the latter case determination of conformation was possible as the values $J_{4'5'}$ and $J_{4'5''}$ could be gathered from a spectrum. This was not the case for neither **26** nor **27**. Deprotected target compound **30** in turn adopts a $^4C_1$ form, in accordance with the data for the thymine and uracil counterparts[17] (Scheme 3). Thus, removal of the di-nitrobenzoyl moiety in **24** (**24** → **26**) must be a factor which destabilizes a $^4C_1$ form for the compound **26**, whereas de-methoxytritylation of **26** (**26** → **30**) re-establishes a preference towards a $^4C_1$ form. N-Benzoylation of **26** (**26** → **27**) doesn't change the conformational preference

The difference between the conformational preferences of **12** and **30** ($^4C_1$) is of interest. Upon epimerization at the 4'-position the conformation changes from a $^1C_4$ to a $^4C_1$ form. This can be explained by the gauche effect which is present in the $^1C_4$ form of **12** (O-CH_2-CH(4')-O) which disappears upon 4'-epimerization. Changing the conformation of **30** to the $^4C_1$ form, reestablishes this gauche effect. Similar gauche effects could stabilize the conformation of **13**, **15**, **16** (partially), **17** and several of the structures depicted in Figure 2.

Table 2. Selected NMR parameters and conformational features of the product **23** and the 2',3'-dideoxy-3'-C-hydroxymethyl-α-L-threopentopyranosyl adenines (coupling constants in Hz).

	23	**24**[a]	**26**[a]	**27**[a]	**30**[b]
$J_{1'2'}$	3.6	2.1	4.2	4.7	3.7
$J_{1'2''}$	4.8	8.3	4.8	4.7	7.9
$J_{4'5'}$	ND[c]	1.8	ND	ND	2.9
$J_{4'5''}$	ND	2.8	ND	ND	ca 5
preferred conformation	$^1C_4$	$^4C_1$	$^4C_1 \leftrightarrow {}^1C_4$ or $^1C_4$	$^4C_1 \leftrightarrow {}^1C_4$ or $^1C_4$	$^4C_1$

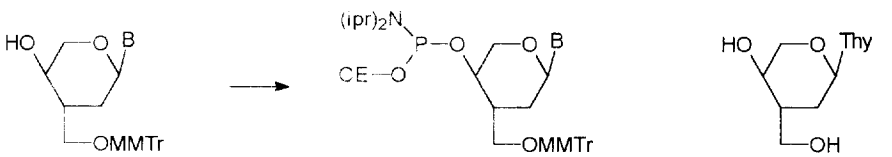
a. Recorded on a Varian Gemini 200 spectrometer in $CDCl_3$

b. Recorded on a Varian Unity 500 spectrometer, in D_2O

c. Not determined

OLIGONUCLEOTIDES

The monomethoxytritylated deoxyadenosine analogue **27** was phosphitylated under standard conditions, to yield the phosphoramidite **31** in 70% yield. Likewise, the tritylated thymidine analogue **33** [4] was converted to **34** with the same yield. To avoid synthesis of the respective succinates, fully modified sequences have been assembled on a support functionalized with a propanediol moiety. The extra 3'-propanediolphosphodiester does not influence the melting behaviour of the control duplexes as shown before.[28]



27 B = N-benzoyl-adenin-9-yl **31** B = N-benzoyl-adenin-9-yl **32**

33 B = Thymin-1-yl **34** B = Thymin-1-yl

Incorporation of the branched analogues in the middle of a sequence, as expected seriously disrupts the duplex (Table 3) : the functionalization of the nucleoside is in the opposite direction compared to normal oligonucleotide synthesis. Substitution of the middle thymidine of a oligodT 13-mer with the branched pyranosylated thymidine analogue **32**, destabilizes its duplex with $(dA)_{13}$ by approximately 15°C (Table 3). On the other hand, incorporating the branched adenosine analogue **30** only caused 9°C destabilization of the tridecaoligonucleotide duplex. Incorporation of the analogues versus the end is less destabilizing. Main purpose of the project however, was the synthesis of full length modified oligonucleotides. The branched trideca- as well as the heptadecathymidine analogues were not able to hybridize with a complementary homo dA strand, neither at low nor at high salt concentrations. The latter should promote duplex formation because of increased shielding of the repulsive phosphate interactions.

In contrast however, the trideca oligonucleotide made up of the branched analogue **30**, clearly favoured duplexation with tridecathymidine (as predicted by modelling experiments), albeit the duplex was less strong compared to the natural complement. As well under fysiological conditions (0.1 M), as under high salt conditions (1 M NaCl) a drop in Tm of approximately 20°C was noticed. Duplex formation of oligonucleotides having a completely modified backbone with a natural complement have not been reported many times. The duplexation seen here, proves modeling[1,5] of such new complicated structures is a valuable although not yet fully optimised tool for predicting the hybridisation possibilities of so far unknown structures.

Table 3 : Melting temperatures as determined at 4 μM of each strand in 0.1 M NaCl (1 M NaCl), 0.1 μM EDTA, 20 μM KH_2PO_4 pH 7.5. A* and T* denominate the branched nucleoside analogues **30** and **32**.

$T_{13} \cdot dA_{13}$	33.2 (47.6)		
$T^*_{13} \cdot dA_{13}$	-	$T_{13} \cdot A^*_{13}$	11.7 (29.5)
$T^*_{17} \cdot dA_{17}$	-	$T^*_{13} \cdot A^*_{13}$	-
$T_6T^*T_6 \cdot dA_{13}$	17.8	$T_{13} \cdot dA_6A^*dA_6$	24.0
$TT^*T_9T^*T \cdot dA_{13}$	22.8	$T_{13} \cdot A^*dA_{12}$	32.1

EXPERIMENTAL

General

Glassware has been dried at 130° and cooled under dry nitrogen. Reactions involving water-sensitive reagents have been performed under dry nitrogen. After evaporations, vacuum has been broken with dry nitrogen using a baloon. NMR spectra have been recorded on a Varian Gemini 200 spectrometer or Varian Unity 500 spectrometer using tetramethylsilane as internal standard. Coupling constants through an even number of bonds are assumed to be negative. Exact mass measurements have been performed on a Kratos Concept 1H Mass Spectrometer using electron impact (EI), chemical ionisation (CI) or liquid secondary ion mass spectra (LSIMS) (with Cs^+ as primary ion beam) modes with matrices shown.

2',3'-Dideoxy-3'-C-hydroxymethyl-β-D-erythro-pentopyranosyl adenine 12 and α anomer 13

Compound **1** (1.17 g, 3.47 mmol) and imidazole (0.71 g, 10.4 mmol), were dissolved in DMF (16 mL), and cooled on ice-bath. To this was added bromomethyldimethylsilyl chloride (0.70 ml, 5.3 mmol). Stirring was continued for 1 h at room temperature. The solution was diluted with 10 mL of $CHCl_3$ and transferred to a separatory funnel charged with chloroform and ice-water. Rapid extraction was performed, and the organic layer was dried ($MgSO_4$) and filtered. All these operations should not exceed ca 15 min to minimize hydrolysis of the unstable bromomethyl dimethylsilyl ether. After evaporation and drying on oil pump, 100 mL of toluene was added and the flask was immersed in an oil bath. When the solution reached bp, tributyltin hydride (2.3 ml, 8.7 mmol) and AIBN (0.14 g, 0.87 mmol) in 50 mL of toluene were added dropwise over a 1 h period. Heating was continued over a total of 4 h. When the flask reached room temperature, toluene was evaporated and final drying was accomplished on an oil pump. The residue was transferred to a smaller flask (50 mL) using 35 mL of DMF. To this solution, cooled in an ice-bath, was added 1.5 g of KF, 1.3 g of $KHCO_3$ and 6 mL of 35 % H_2O_2. Stirring was maintained overnight. TLC shows a spot corresponding to the product **12**, R_f 0.40 in CH_2Cl_2-MeOH 4:1, and a less polar compound identified as 9-[2,3-dideoxy-β-D-pent-2-enopyranosyl]-adenine. Inorganic material was removed by filtration through a sintered glass funnel, and volatiles were removed by evaporation using an oil pump. Flash chromatography furnished 0.58 g of the branched product **12**. Repurification of the forerunning fractions yielded 0.14 g of the unreacted N-debenzoylated starting compound. Yield of **12** is 76 % [for three consecutive reactions] based on recovered allylic alcohol substrate or 63 % counting on the total substrate taken for the reaction.

When $KHCO_3$ was omitted in the last stage, two more compounds could be identified by TLC: the starting substrate **1** and **18** (the benzoylated analogue of **12**, not isolated). Both of them disappeared after addition of $KHCO_3$ and stirring during 5 h.

12: mp (cryst. from MeOH) partial melting 205-210°, solidification ca 220°, no mp up to 300°, dec.
^1H (500 MHz, CD_3OD) 8.407, 8.288 H2,8, 6.230 (1H, t, $J_{1'2'}$ = 4.0 Hz, $J_{1'2''}$ = 4.9 Hz, H1'); 4.057 (1H, quintette, $J_{4'5'}$ = 5.3 Hz, $J_{4'3'}$ = $J_{4'5'}$ = 2.7 Hz, H4'); 3.928 (1H, dd, $J_{6'3'}$ = 5.8 Hz, $J_{6'6''}$ = -11.3 Hz, H6'); 3.854 (1H, dd, $J_{5'4'}$ = 5.8 Hz, $J_{5'5''}$ = -12.2 Hz, H5'); 3.822 (1H, dd, $J_{6''3'}$ = 6.7 Hz, $J_{6''6'}$ = -11.0 Hz, H6''); 3.732 (1H, dd, $J_{5''4'}$ = 2.8 Hz, $J_{5''5'}$ = -11.9 Hz, H5''); 2.511 (1H, ddd, $J_{2'ax3'}$ = 8.9 Hz, $J_{2'ax1'}$ = 5.2 Hz, $J_{2'ax2'eq}$ = -18.5 Hz, H2'ax); 2.42-2.35 (2H, unresolved, H2'eq, 3'-
^{13}C (D_2O): 155.77 C6, 152.05 C2, 149.28 C4, 141.40 C8, 118.93 C5, 79.12 C1', 66.10, 62.34 C5',6', 64.23 C4', 36.58 C3', 24.87 C2'. UV (MeOH) λ_{max} 260 nm (ε 13100). Combustion analysis: calc. for $C_{11}H_{15}N_5O_3$: C, 49.81, H, 5.70, N, 26.40; found: C, 49.63, H, 5.75, N, 26.71. Exact mass (thioglycerol) calc. for $C_{11}H_{15}N_5O_3$+H 266.1253; found 266.1244.

Using the same protocol and proportional amounts of reagents, 0.69 g of the α anomer **2** furnished 0.31 g, 57 % of **13** counted on total substrate used for the reaction.
13: mp 209-211° (cryst. MeOH).
^1H (D_2O) 8.02, 7.84 (two s, H2,8), 5.45 (1H, [illegible] $J_{1'2''}$ = 4.5 Hz, H1'), 4.02 (1H, d, $J_{5'5''}$ = -11.8 Hz, H5'); 3.88 (1H, s, half-width = 4 Hz, H4'), 3.85 (1H, [illegible] $J_{5''5'}$ = -12.5 Hz, H5''), 3.96 (1H, dd, $J_{6'3'}$ = 6.9 Hz, $J_{6'6''}$ = -10.9 Hz, H6'), 3.53 (1H, dd, $J_{5''4'}$ = 5.5 Hz, $J_{6''}$ [illegible] Hz, H6''), 2.20-1.76 (3H, AB_2 system, 2xH2', H3').

^{13}C (D_2O) 155.36 C6, 152.71 C2, 148.13 C4, 139.63 C8, 118.31 C5, 81.49 C1', 72.81, 62.89 C5',6', 63.77 C4', 40.89 C3', 27.49 C2'. UV (MeOH) λ_{max} 260 nm, ε = 13300. Combustion analysis : calc. for $C_{11}H_{15}N_5O_3$: C, 49.81; H, 5.70; N, 26.40; found : C, 50.01; H, 5.77. Exact mass (thioglycerol) calc. for $C_{11}H_{15}N_5O_3$+H 266.1253, found 266.1251.

4',6'-Di-O-acetyl-2',3'-dideoxy-3'-C-hydroxymethyl-β-D-erythro-pentopyranosyl(N^4-acetyl)cytosine 14 and α anomer 15

Compound **3** (1.0 g, 3.2 mmol) was converted into the 4'-O-bromomethyl dimethylsilyl ether exactly as described for the adenosine analogue, using 0.57 mL (4.2 mmol) of bromomethyl dimethylsilyl chloride and 0.62 g (9.1 mmol) of imidazole in 20 mL of DMF. Cyclization step : substrate in 300 mL of toluene; Bu_3SnH 2.1 mL (8 mmol) and AIBN 0.13 g (0.8 mmol) in 50 mL of toluene; after 4.5 h at bp more Bu_3SnH 0.5 mL and AIBN 0.050 g were added directly to the reaction flask to bring cyclization to the end. Total cyclization time was 5.5 h. Oxidation step : KF 1.4 g, H_2O_2 5.2 mL, DMF, 20 mL overnight. Complete N-deprotection took place without addition of $KHCO_3$. After filtration of the inorganic material and evaporation of DMF, the residue was passed through a short column of silica gel in CH_2Cl_2-MeOH conc. NH_4OH 20:5:0.6 → 20:5:1, to afford 0.52 g of a crude product. For purification purpose, acetylation was performed in which 0.60 g of the crude product was treated with 2:1 mixture of pyridine and acetic anhydride for two days. The deeply brown-red solution was evaporated. TLC demonstrated a mixture of at least six compounds. The product **14** was localized by comparison with tri-acetate **15** (see below). Flash chromatography (CH_2Cl_2-MeOH 20:0.7) furnished 0.061 g (5 % over the four steps) of **14**.

The α anomer **4** was converted to **15** using the same procedure and the following quantities of reagents : substrate **4**, 2.27 g (7.25 mmol).
Silylation step : bromomethyl dimethylsilyl chloride 1.28 mL (9.4 mmol), imidazole 1.28 g (18.8 mmol) and DMF 16 mL. Cyclization step : Bu_3SnH 4.9 mL (18 mmol), AIBN 0.30 g (1.8 mmol) in toluene 100 mL, substrate in 250 ml of toluene. More Bu_3SnH 2 mL and AIBN 0.1 g were added after 5 h and boiling was continued overnight. Oxidation step : KF 3.7 g, 35 % H_2O_2, 13.8 mL, substrate in 100 mL of DMF, overnight. Flash chromatography as stated for the β anomer furnished 0.76 g, 44 % of crude product which was only slightly contaminated. Acetylation of 0.117 g of this material in 16 mL of pyridine and 8 mL of Ac_2O overnight resulted in a clean reaction. TLC indicated a single product R_f 0.37 in CH_2Cl_2-MeOH 20:0.7. After evaporation of volatiles and flash chromatography, 0.055 g of **15** was obtained. Yield of **15** is 64 % (acetylation step only) or 28 % from **4**.

14 : glassy compound
^1H ($CDCl_3$) 9.26 (1H, s, NH), 7.88 (1H, d, J_{56} = 7.6 Hz, H6; 7.48 (1H, d, J_{65} = 7.5 Hz, H5); 6.00 (1H, dd, $J_{1'2'eq}$ = 3.3 Hz, $J_{1'2'ax}$ = 9.9 Hz, H1'); 5.16 (1H, dt, $J_{4'5'ax}$ = 9.6 Hz, $J_{4'5'eq}$ and $J_{4'3'}$ = 4.9 and 5.0 Hz, H4'); 4.41 (1H, dd, $J_{6'3'}$ = 6.2 Hz, $J_{6'6''}$ = -11.7 Hz, H6'); 4.31 (1H, dd, $J_{6''3'}$ = 7.4 Hz, $J_{6''6'}$ = -11.5 Hz, H6''); 4.10 (1H, dd, $J_{5'eq4'}$ = 4.8 Hz, $J_{5'eq5'ax}$ = -11.2 Hz, H5'eq); 3.85 (1H, dd, $J_{5'ax4'}$ = 9.5 Hz, $J_{5'ax5'eq}$ = -11.6 Hz, H5'ax); 2.74-2.59 (1H, unresolved, H3'); 2.40 (1H, dt, $J_{2'eq1'}$ = $J_{2'eq3'}$ = 3.7 Hz, $J_{2'eq2'ax}$ = -14.0 Hz, H2'eq); 2.29 (3H) and 2.13 (6H), acetyl; 1.81 (1H, ddd, $J_{2'ax3'}$ = 5.2 Hz, $J_{2'ax1'}$ = 9.2 Hz, $J_{2'ax2'eq}$ = -15.1 Hz, H2'ax).

^{13}C ($CDCl_3$) 170.73, 170.45, 169.97, $COCH_3$, C62.40, 154.43, 143.89, 96.82 C5, 80.08 C1', 67.09 C4', 65.47, 61.59 C5',6', 33.57 C3', 31.33 C2', 24.92, 20.85 $COCH_3$. Exact mass (thioglycerol) calc. for $C_{16}H_{21}N_3O_7$+H 368.14566, found 368.1461.

15 : mp 192-195° (cryst. CH_2Cl_2-MeOH)

^1H ($CDCl_3$) 9.57 (1H, bs, NH), 7.91 and 7.52 (1H each, d, J_{56} = 7.5 Hz, H5,6; 5.81 (1H, dd, $J_{1'2'eq}$ = 2.2 Hz, $J_{1'2'ax}$ = 10.5 Hz, H1'), 4.98 (1H, bs, half-width = 6.8 Hz, H4'); 4.31 (1H, dd, $J_{5'eq5'ax}$ = -13.1 Hz, $J_{5'eq4'}$ = 1.4 Hz, H5'eq), 4.13 (1H, dd, $J_{6'3'}$ = 8.2 Hz, $J_{6'6"}$ = -11.0 Hz, H6'); 3.93 (1H, dd, $J_{6"3'}$ = 6.6 Hz, $J_{6"6'}$ = -10.9 Hz, H6"); 3.83 (1H, dd, $J_{5'ax5'eq}$ = -13.1 Hz, $J_{5"ax4'}$ = 1.0 Hz, H5'ax); 2.54-2.35 (1H, unresolved, H3'); 2.31, 2.18, 2.07 (3H each, $COCH_3$); 2.18-2.07 (H2'eq superimposed on acetyl signals); 1.55 (1H, q, $J_{2'ax1'} = J_{2'ax3'} = |J_{2'ax2'eq}|$ = 12.0 Hz, H2'ax). ^{13}C ($CDCl_3$) 170.66, 170.25, $\underline{C}OCH_3$, 162.61 C4, 154.34 C2, 144.03 C6, 96.99 C5, 82.65 C1', 69.76, 63.45 C5',6', 65.35 C4', 36.74 C3', 29.35 C2', 24.91, 20.97, 20.67 $CO\underline{C}H_3$. Exact mass (thioglycerol) calc. for $C_{16}H_{21}N_3O_7$+H 368.14566, found 368.1454.

4',6'-Di-O-acetyl-2',3'-dideoxy-3'-C-hydroxymethyl-β-D-erythro-pentopyranosyl(N^2-acetyl)guanine 16 and α anomer 17

Compounds **16** and **17** were prepared exactly as described for cytidine analogues **14** and **15**. Chromatography system to get crude unprotected compound was CH_2Cl_2-MeOH-conc. NH_4OH 15:6:2. O,N-Acetylation was performed using Bu_4NOH solubilization[15,16] and co-evaporation with pyridine (five times) followed by Ac_2O-Py-DMAP treatment during 3 days and flash chromatography in CH_2Cl_2-MeOH 20:1. Compound **16** required additional HPLC purification to remove a marginally less polar impurity. Yields were for **16** : 45 % and **17** : 8.3 % (over four steps).

16 : mp 172-175° (cryst. CH_2Cl_2-Et_2O)

^1H ($CDCl_3$) 11.94 and 9.43 (two bs, NH), 7.85, H8, 6.01 (1H, d, $J_{1'2'}$ = 5.5 Hz, H1'); 4.97 (1H, bs, half-width = 6.5 Hz, H4'); 4.39 (1H, dd, $J_{6'3'}$ = 5.0 Hz, $J_{6'6"}$ = -11.0 Hz, H6'); 3.95 (1H, t, $J_{6"3'}$ = 9.9 Hz, $J_{6"6'}$ = -11.0 Hz, H6"), 3.94 (1H, dd, $J_{5'4'}$ = 2.0 Hz, $J_{5'5"}$ = -13.1 Hz, H5'); 3.45 (1H, dd, $J_{5"4'}$ = 1.3 Hz, $J_{5"5'}$ = -13.1 Hz, H5"), 3.34-3.17 (1H, m, H3'), 2.55 (1H, dd, $J_{2'eq3'}$ = 4.0 Hz, $J_{2'eq2'ax}$ = -13.8 Hz, H2'eq), 2.34 Ac; 2.26-2.11 (H2'ax superimposed on the acetyl groups signals), 2.21, 2.15, Ac. ^{13}C ($CDCl_3$) 172.10, 170.34 $\underline{C}OCH_3$, 155.70, 148.16, 147.07, 139.66, 121.11 guanine, 80.33 C1', 65.73 C4', 63.61, 63.04, C5',6', 33.16 C3', 24.14 C2', 21.13, 20.93 $COCH_3$. UV (MeOH) : λ_{max} 257 nm, ε = 16300; pH 14 : λ_{max} 268 nm, ε = 14200. Exact mass (thioglycerol) calc. for $C_{17}H_{21}N_5O_7$+H 408.1519; found 408.1509.

17 : mp 200-202° (CH_2Cl_2-MeOH)

^1H ($CDCl_3$) 7.90 (1H, s H8), 5.58 (1H, dd, $J_{1'2'eq}$ = 2.7 Hz, $J_{1'2'ax}$ = 10.1 Hz, H1'); 4.96 (1H, bs, half-width = 6 Hz, H4'); 4.22 (1H, dd, $J_{5'5"}$ = -12.5 Hz, H5'), 4.14 (1H, dd, $J_{6'3'}$ = 8.1 Hz, $J_{6'6"}$ = -11.0 Hz, H6'); 3.99 (1H, dd, $J_{6"3'}$ = 6.2 Hz, $J_{6"6'}$ = -10.8 Hz, H6"), 3.79 (1H, d, $J_{5"5'}$ = -13.2 Hz, H5"); 2.52-2.38 (1H, unresolved, H3'(2'); 2.35, 2.17, 2.07, Ac, 2.19-1.97 (the signals of H2",2'(3') superimposed on the acetyl signals). ^{13}C ($CDCl_3$) 172.69, 170.75, 170.40 $\underline{C}OCH_3$, 155.88, 148.01, 147.79, 136.45, 120.61, 80.51 C1', 69.46, 63.54

C5',6', 65.26 C4', 37.02 C3', 29.19 C2', 24.28, 20.95, 20.68 $CO\underline{C}H_3$. Exact mass (glycerol) calc. for $C_{17}H_{21}N_5O_7$+H 408.1519, found 408.1509.

2',3'-Dideoxy-3'-C-(methoxytrityl)-oxymethyl-β-D-erythro-pentopyranosyl adenine 23

Compound **12** (1.3 g, 4.9 mmol) in 30 mL of pyridine and cat. amount of DMAP was reacted with 1.8 g (5.9 mmol) of methoxytrityl chloride at 75°. After 5 h more of the chloride was added (0.38 g) and the reaction mixture was left at room temperature overnight. Extractive work-up ($CHCl_3$-aq.sat. $NaHCO_3$), evaporation of the volatiles followed by co-evaporation with p-xylene and flash chromatography (in CH_2Cl_2-MeOH 20:1.5) furnished 1.5 g (57 %) of the title compound as a glassy solid.

23

^1H ($CDCl_3$) 8.38, 8.01 (2H, two s, H2,8); 7.52-7.23, 6.94-6.84 (14H, two groups of signals, H aromatic); 6.05 (3H, irregular signal, H1', NH_2); 4.05 (1H, bs, half-width = 8.6 Hz, H4'); 3.83 (1H, dd, $J_{5'4'}$ = 4.2 Hz, $J_{5'5''}$ = -12.3 Hz, H5'); 3.83 (3H, s, OMe); 3.69 (1H, dd, $J_{5''4'}$ = 2.0 Hz, $J_{5''5'}$ = -12.1 Hz, H5''); 3.51 (1H, dd, $J_{6'3'}$ = 6.1 Hz, $J_{6'6''}$ = -9.7 Hz, H6'); 3.41 (1H, dd, $J_{6''3'}$ = 4.7 Hz, $J_{6''6'}$ = -9.7 Hz, H6''); protons H2',2'',3' form an unresolved AB_2 pattern, 2.86-2.70 (1H) and 2.45-2 28 (2H). ^{13}C (DMSO-d_6) 158.03, 144.64, 144.51, 135.29, 129.97, 127.94, 127.77, 126.68, 113.10, -OMMT, 156.01 C6, 152.48 C2, 149.33 C4, 139.10 C8, 118.86 C5, 85.58 -$C(Ph)_2$PhOMe, 78.26 C1', 67.03, 61.90 C5',6', 63.87 C4', 54.93 OMe, 35.72 C3', 27.16 C2'. Exact mass (glycerol, NaOAc) calc. for $C_{31}H_{31}N_5O_4$+Na 560.22739, found 560.2279.

4'-O-(2,4-Dinitrobenzoyl)-2',3'-dideoxy-3'-C-(methoxytrityl)oxymethyl-α-L-threo-pentopyranosyl adenine 24 and elimination product 25

To a cooled (ice bath) solution of the compound **23** (1.50 g, 2.8 mmol), triphenylphosphine (14.0 mmol, 3.7 g) and 2,4-dinitrobenzoic acid (14 mmol, 3.0 g) in dioxane 80 mL, was injected diethyl azodicarboxylate (14.0 mmol, 2.2 mL). After 4 h at room temperature the substrate had reacted nearly completely. The reaction mixture was evaporated to near dryness and passed through a column of silica gel (in CH_2Cl_2-MeOH 20:0.75) to absorb the excess of dinitrobenzoic acid. Franctions containing Ph_3P, Ph_3PO and both reaction products (**24** : R_f 0.40 and **25** : R_f 0.30 in CH_2Cl_2-MeOH 20:0.75) were subjected to flash chromatography to furnish 0.95 g (46 %) of **23** and 0.44 g (30 %) of **25** (both amorphous, glassy compounds).

24

^1H ($CDCl_3$) 8.78 (1H, d, $|J_{H_aH_b}|$ = 2.1 Hz, H_a*); 8 54 (1H, dd, $|J_{H_bH_a}|$ = 2.1 Hz, $J_{H_bH_c}$ = 8.4 Hz, H_b*); 8.35, 8.09 (1H each, s, H2,8); 7.98 (1H, d, $J_{H_bH_c}$ = 8.4 Hz, H_c*); 7.54-7.28, 6.93-6.85 (14H, -OMMT); 5.91 (1H, dd, $J_{1'2'eq}$ = 2.1 Hz, $J_{1'2'ax}$ = 8.3 Hz, H1'); 5.81 (-NH_2, partly superimposed on the H1' signal [+ D_2O : 5.84, dd, $J_{1'2'eq}$ = 2.8 Hz, $J_{1'2'ax}$ = 9.4 Hz, H1']; 5.29 (1H, apparent d, $J_{4'5'ax}$ = 2.5 Hz, H4'); 3.97 (2H, AB,

* H_a, H_b, H_c refer to the protons of the 2,4-di-nitrobenzoyl group

$J_{5'eq4'}$ = 1.8 Hz, $J_{5'ax4'}$ = 2.8 Hz, J_{AB} = 13.3 and 19.1 Hz, H5'eq,5'ax); 3.82 (3H, s, -OMe); 3.54-3.43 (2H, unresolved, 2xH6'); 2.78-2.57 (1H) and 2.18-2.07 (2H), AB_2 system, H2'eq,2'ax,3'. ^{13}C (DMSO-d_6) 162.69 C carbonyl, 158.33, 156.16, 152.85, 149.32, 149.22, 147.91, 144.32, 144.22, 138.63, 134.93, 132.04, 130.84, 130.19, 128.10, 127.07, 119.86, 118.77, 133.42 -OMMT, adenine, 86.26 -O<u>C</u>(Ph)$_2$PhOMe, 77.29 C1', 70.21 C4', 64.73, 62.02 C5',6', 55.13 OMe, 36.38 C3', 27.75 C2'. Exact mass (thioglycerol-NaOAc) calc. for $C_{38}H_{33}N_7O_9$+Na 754.22376, found 754.2247

25

^1H ($CDCl_3$) 8.40, 8.06 (1H each, s, H2,8), 7.51-7.26 and 6.90-6.86 (14H, MMT); 6.12 (1H, dd, $J_{1'2'}$ = 4.5 Hz, $J_{1'2''}$ = 7.0 Hz, H1'), 5.98 (1H, bs, half-width = 6.9 Hz, H4'); 5.87 (2H, s, NH2); 4.50 (1H, dd, $J_{5'4'}$ = 2.1 Hz, $J_{5'5''}$ = -16.5 Hz, H5'); 4.29 (1H, d, $J_{5''5'}$ = -16.6 Hz, H5''); 3.83 (3H, s, OMe); 3.68 (2H, s, 2x6'); 2.75 (1H, dd, $J_{1'2'}$ = 6.0 Hz, $J_{2'2''}$ = -16.0 Hz, H2'); 2.57 (1H, two bs, half-width = 9 Hz, $J_{2''2'}$ = -14.9 Hz, H2''). ^{13}C ($CDCl_3$) 158.60, 144.25, 135.43, 130.20, 128.23, 127.85, 127.00, 113.14 MMT, 155.44 C6, 153.23 C2, 149.69 C4, 138.48 C8, 119.53 C5, 131.48 C3', 120.59 C4', 86.62 -O<u>C</u>(Ph)$_2$PhOMe, 77.79 C1', 66.06, 64.47 C5',6', 55.17 OMe, 36.70 C2'. Exact mass (thioglycerol-NaOAc) calc. for $C_{38}H_{35}N_5O_5$+Na 664.2536, found 664.2545

2',3'-Dideoxy-3'-C-(methoxytrityl)oxymethyl-α-L-threo-pentopyranosyl(N^6-benzoyl)adenine 27

A. From **24**

To a cooled (ice-bath) solution of compound **24** (0.31 g, 0.42 mmol) in 20 mL of pyridine was added benzoyl chloride (0.15 ml, 1.7 mmol). After 2 h at room temperature the flask was cooled again, and 4 mL of water, followed by 4 mL of conc NH_4OH, were added dropwise. After 0.5 h at ice-bath temperature and 0.5 h at room temperature, TLC showed the product **28** having R_f 0.48 (in CH_2Cl_2-MeOH 20:0.3) and traces of the unreacted substrate **24** near startpoint. After extractive work-up (CH_2Cl_2-aq $NaHCO_3$), evaporation, and co-evaporation with p-xylene, the residue was purified by chromatography (CH_2Cl_2-MeOH 100:25) to yield 0.26 g (73 %) of **28** [exact mass calc. for $C_{45}H_{38}N_7O_{10}$+Na 858.24997, found (thioglycerol-NaOAc matrix) 858.2499]. This material was dissolved in dioxane 10 mL. Methanol 20 mL was added, followed by 1.9 mL of a solution of sodium methoxide in methanol (prepared from 0.015 g, 0.65 mmol of sodium and 50 mL of MeOH corresponding to 0.026 mmol of NaOMe). After 5 h the reaction mixture was neutralized with solid CO_2 and evaporated. Flash chromatography (CH_2Cl_2-MeOH 10:1) gave 0.144 g (73 %) of **27** as an amorphous compound.

B. From **26**

Compound **26** (0.58 g, 1.1 mmol) in pyridine, 20 mL, cooled in ice-bath was treated with trimethylsilyl chloride (0.4 mL, 3.2 mmol). After 40 min at room temperature, benzoyl chloride was added (0.3 ml, 3.2 mmol). After 2.5 h the flask was cooled again in ice-bath, and 4 mL of water was added dropwise followed by 4 ml of conc NH_4OH. Half an hour later TLC showed two compounds of which the lower proved identical with **27**. The upper compound was probably the 4'-O-benzoate and intensity of this spot didn't change with time. Probably 4'-OH group of the substrate **26** did not react completely with trimethylsilyl

chloride, and was subsequently benzoylated. The reaction mixture was extractively worked-up (CH_2Cl_2-aq. $NaHCO_3$), the organic layer was dried ($MgSO_4$), evaporated, and co-evaporated twice with xylene. The residue was dissolved in methanol and treated with cat. NaOMe as above. With progress of time the upper compound transformed into the lower compound **27**. After ca 5 h the mixture was treated with solid CO_2, evaporated and purified by chromatography to furnish 0.47 g (68 %) of **27**.

27 : amorphous

^1H ($CDCl_3$) 9.20 (1H, s, NH); 8.83, 8.18 (2H, 2s, H2,8); 8.18-8.02, 7.61-7.24, 6.87-6.83 (19H, three groups of signals, H aromatic); 5.93 (1H, t, $J_{1'2'ax} = J_{1'2'eq} = 4.7$ Hz, H1'); 3.89-3.77 (5H, OMe, H5',5"); 3.54-3.43 (2H, unresolved, H4', H6'); 3.30 (1H, t, J = 8.1 Hz, J = 9.6 Hz, H6"), 2.97 (1H, dt, $J_{2'1'} = 5.0$ Hz, $J_{2'3'} = 5.0$ Hz, $J_{2'2"} = -14.2$ Hz, H2'); 2.36-2.18 (1H, unresolved, H3'); 1.93 (1H, ddd, $J_{2"1'} = 4.0$ Hz, $J_{2"3'} = 8.2$ Hz, $J_{2"2'} = -13.5$ Hz, H2"). ^{13}C ($CDCl_3$) 164.59 C carbonyl, 158.72, 152.77, 151.60, 149.61, 144.03, 143.80, 141.59, 135.03, 133.65, 132.77, 130.25, 128.84, 128.21, 128.01, 127.85, 127.42, 127.15, 123.12, 113.27, 112.28 C aromatic, adenine, 87.28 $\underline{C}(Ph)_2PhOMe$, 80.13 C1', 67.67 C4', 66.52, 65.44 C5',6', 55.24 OMe, 38.79 C3', 28.20 C2'. Exact mass (thioglycerol, NaOAc) calc. for $C_{38}H_{35}N_5O_5$+Na 664.2536, found 664.2545.

2',3'-Dideoxy-3'-C(methoxytrityl)oxymethyl-α-L-threo-pentopyranosyl adenine 26

Compound **24** (0.95 g, 1.30 mmol) in 15 mL of dioxane and 30 mL of methanol was 4'-O-deesterified with cat. NaOMe. After neutralization, evaporation and chromatography (in CH_2Cl_2-MeOH 10:1) 0.58 g (83 %) of amorphous **26** was been obtained

26 : amorphous

^1H ($CDCl_3$) 8.38, 7.99 (1H each, s, H2,8); 7.52-7.17 and 6.94-6.82 (14H, MMT); 5.88 (3H, s, H1', NH_2) [+ D_2O : t, $J_{1'2'} = 4.2$ Hz, $J_{1'2"} = 4.8$ Hz, H1']; 3.92-3.75 (5H, 2x5', OMe); 3.59-3.41 (H4' overlapped with the H6' signal) [after irradiation of 2x5' signal : 3.52, d, $J_{4'3'} = 6.4$ Hz, H4']; 3.47 (1H, dd, $J_{6'3'} = 6.2$ Hz, $J_{6'6"} = -9.5$ Hz, H6'); 3.34 (1H, t, $J_{6"3'} = 8.1$ Hz, $J_{6"6'} = -9.3$ Hz, H6"), 2.91 (1H, dt, $J_{2'3'} = J_{2'1'} = 5.1$ Hz, $J_{2'2"} = -13.8$ Hz, H2'); 2.40-2.23 (1H, unresolved, H3'); 1.93 (1H, ddd, $J_{2"1'} = 3.9$ Hz, $J_{2"3'} = 7.9$ Hz, $J_{2"2'} = -14.0$ Hz, H2"). ^{13}C ($CDCl_3$) 158.65, 144.05, 143.89, 135.10, 130.24, 130.01, 128.22, 127.95, 127.07, 113.23, OMMT, 155.44 C6, 153.09 C2, 149.75 C4, 139.01 C8, 119.76 C5, 87.14 $C(Ph)_2PhOMe$, 79.51 C1', 67.49 C4', 66.50, 65.26 C5',6', 55.18 OMe, 38.91 C3', 28.19 C2'. Exact mass (glycerol, NaOAc) calc. for $C_{31}H_{31}N_5O_4$+Na 560.22739, found 560.2279.

2',3'-Dideoxy-3'-C-hydromethyl-α-L-threo-pentopyranosyl adenine 30

Conventional de-methoxytritylation of **26** using 80 % acetic acid during 1.5 h and flash chromatography (CH_2Cl_2-MeOH 4:1) furnished **30** in 80 % yield. This analogue **30** proved slighly more polar on TLC than the 4' "down" epimer **12**

30 : no mp up to 300° (cryst. MeOH)

^{1}H (500 MHz, CD_3OD) 8.45, 8.29 (s, H2,8), 6.01 (1H, dd, $J_{1'2'eq}$ = 3.7 Hz, $J_{1'2'ax}$ = 7.9 Hz, H1'); 3.95 (1H, dd, $J_{5'eq4'}$ = 2.9 Hz, $J_{5'eq5'ax}$ = -12.2 Hz, H5'eq); 3.94 (1H, dd, $J_{6'3'}$ = 6.3 Hz, $J_{6'6''}$ = -11.1 Hz, H6'); 3.77 (1H, dd, $J_{6''3'}$ = 6.7 Hz, $J_{6''6'}$ = -11.1 Hz, H6''); 3.61 (1H, ddd, J = 0.8 Hz, $J_{5'ax4'}$ = 4.9 Hz, $J_{5'ax5'eq}$ = -12.2 Hz, H5'ax); 3.70 (1H, dt, $J_{4'5'eq}$ = 2.9 Hz, $J_{4'5'ax}$ = 5.2 Hz, $J_{4''3'}$ = 5.2 Hz Hz, H4'); 2.72 (1H, ddd, $J_{2'ax3'}$ = 5.2 Hz, $J_{2'ax1'}$ = 7.9 Hz, $J_{2'ax2'eq}$ = -14.0 Hz, H2'ax); 2.18 (1H, sextette, J = 5.9 Hz, H3'); 2.03 (1H, ddd, $J_{2'eq2'}$ = 3.8 Hz, $J_{2'eq3'}$ = 6.1 Hz, $J_{2'eq2'ax}$ = -14.2 Hz, H2'eq). ^{13}C (50 MHz, CD_3OD) 156.48 C6, 152.92 C2, 149.33 C4, 139.81 C8, 119.16 C5, 79.05 C1', 67.77, 62.02 C5',6', 65.22 C4', 41.76 C3', 28.33 C2'. UV (MeOH) λ_{max} 260 nm, ε = 12900. Exact mass (glycerol) calc. for $C_{11}H_{15}N_5O_3$+H 266.12530, found 266.1267. Combustion analysis calc. for C 49.81, H 5.70, N 26.40; found C 49.74, H 5.69, N 26.15.

2',3'-Dideoxy-3'-C(p-methoxyphenyldiphenylmethyloxy-methyl)-4'-*O*-(*P*-β-cyanoethyl-*N*,*N*-diisopropylaminophosphinyl)-α-L-threo-pentopyranosyl-(N^6-benzoyl)adenine 31

The monomethoxytritylated derivative **27** (311 mg, 0.48 mmol) was dissoved in 5 mL dichloromethane under argon and diisopropylethylamine (0.26 mL, 1.45 mmol) and 2-cyanoethyl N,N-diisopropylchlorophosphoramidite (0.165 mL, 0.73 mmol) were added and the solution was stirred for a half hour. Ethanol (2 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 (3 x 30 mL) and evaporation of the organics left an oil which was flash purified on 35 g of silica gel (hexane : acetone : TEA, 60:38:2) to afford the product as a foam after coevaporation with dichloromethane. Dissolution in 1 mL of dichloromethane and precipitation in 50 mL cold (-70°C) hexane afforded 287 mg (0.34 mmol, 70%) of the title product **31** as a white powder

R_f (hexane : acetone : TEA 49:49:2) : 0.46 LSIMS (NBA) m/z 842 (MH^+, 2), 273 (MMTr, 100). ^{13}C NMR ($CDCl_3$) : δ (ppm) 164.6 (CO), 152.6 (C2), 151.7 (C6), 149.5 (C4), 141.4 (C8), 123.2 (C5), 117.4 (CN), 79.2 (C1'), 67.7, 67.4 (2d, J = 15 Hz, C4'), 66.4 (d, J = 9 Hz, C5'), 62.9 (C6'), 58.2 (d, J = 18.5 Hz, $POCH_2$), 43.1 (d, J = 12 Hz, NCH), 39.0 (d, J = 3 Hz, C3'), 29.5 (d, J = 7.3 Hz, C2'), 24.5 ($NCCH_3$), 20.3, 20.2 (2d, J = 6.4 Hz, CH_2CN), 12.4 (5-CH_3).

2',3'-Dideoxy-3'-C(p-methoxyphenyldiphenylmethyloxy-methyl)-4'-*O*-(*P*-β-cyanoethyl-*N*,*N*-diisopropylaminophosphinyl)-α-L-threo-pentopyranosylthymine 34

The monomethoxytritylated derivative **33** (320 mg, 0.6 mmol) was dissoved in 5 mL dichloromethane under argon and diisopropylethylamine (0.32 mL, 1.8 mmol) and 2-cyanoethyl N,N-diisopropylchlorophosphoramidite (0.2 mL, 0.9 mmol) were added and the solution was stirred for a half hour. Work-up was as described for the previous preparation, provided column purification was done using hexane:EtOAc:TEA, 40:58:2. Precipitation in 50 mL cold (-70°C) hexane afforded 305mg (0.42 mmol, 70%) of the title product **34** as a white powder.

R_f (hexane:EtOAc:TEA 40:58:2) 0.38. LSIMS (NBA-NaOAc) m/z 773 (M+Na, 10), 751 (MH^+, 10), 273 (MMTr, 100). ^{13}C NMR ($CDCl_3$) : δ (ppm) 163.3 (C4), 149.6 (C2), 135.1 (C6), 117.3 (CN), 110.8 (C5), 77.9

(C1'), 67.9 (C5'), 66.9, 66.6 (2d, J = 18 Hz, C4'), 62.4 (C6'), 58.2 (d, J = 18.5 Hz, $POCH_2$), 43.2 (d, J = 12.8 Hz, NCH), 39.0 (d, J = 14.0 Hz, C3'), 28.1 (C2'), 24.5 ($NCCH_3$), 20.5 (CH_2CN), 12.4 (5-CH_3).

ACKNOWLEDGEMENT

Dr. Peter Sandor, Varian GmbH, Darmstadt, Germany, is acknowledged for 500 MHz NMR measurement, Dr. Jef Rozenski for exact mass measurements, and Mieke Vandekinderen for editorial work.

REFERENCES

1. Herdewijn, P.; De Winter, H.; Doboszewski, B.; Verheggen, I.; Augustyns, K.; Hendrix, C.; Saison-Behmoaras, T.; De Ranter, C.; Van Aerschot, A. Hexopyranosyl-like oligonucleotide. *ACS Symposium Series 580. Carbohydrate Modifications in Antisense Research* **1994**, Eds. Y.S. Sanghvi, P.D. Cook, pp. 80-99.
2. Eschenmoser, A. *Pure & Appl. Chem.* **1993**, *65*, 1179-1188.
3. Tari, L.W.; Sadana, K.L.; Secco, A.S. *Nucleosides & Nucleotides* **1995**, *14*, 175-183.
4. Doboszewski, B.; Blaton, N.; Rozenski, J.; De Bruyn, A.; Herdewijn, P. *Tetrahedron* **1995**, *51*, 5381-5396.
5. De Winter, H.; De Ranter, C.; Van Aerschot, A.; Herdewijn, P. *J. Comp. Chem.* (submitted).
6. Doboszewski, B.; Blaton, N.; Herdewijn, P. *Tetrahedron Lett.* **1995**, *36*, 1321-1324.
7. Doboszewski, B.; Blaton, N.; Herdewijn, P. *J. Org. Chem.*, submitted.
8. Nashiyama, H.; Kitoyima, T.; Matsumato, M.; Itoh, K. *J. Org. Chem.* **1984**, *49*, 2298-2300.
9. Stork, G.; Khan, M. *J. Am. Chem. Soc.* **1985**, *107*, 500-501.
10. Augustyns, K.; Rozenski, J.; Van Aerschot, A.; Busson, R.; Claes, P.; Herdewijn, P. *Tetrahedron* **1994**, *50*, 1189-1198.
11. Bonner, R.V.; Davis, M.J.; Howarth, J.; Jenskins, P.R.; Lawrance, N.J. *J. Chem. Soc. Perkin I* **1992**, 27-29.
12. Pedretti, Y.; Mallet, J.-M., Sinaÿ, P.S. *Carbohydr. Res.* **1993**, *244*, 247-257.
13. Hayes, D.H.; Michelson, A.M.; Todd, A.R. *J. Chem. Soc.* **1955**, 808-815.
14. Nair, V.; Turner, G.A.; Chamberlain, S.D. *J. Am. Chem. Soc.* **1987**, *109*, 7223-7224.
15. Schaller, H.; Weimann, G.; Lerch, B.; Khorana, H.G. *J. Am.Chem. Soc.* **1963**, *85*, 3821-3827.
16. Büchi, H.; Khorana, H.G. *J. Mol. Biol.* **1972**, *72*, 251-288.
17. Nord, L.D.; Dalley, N.K.; McKernan, P.A.; Robins, R.K. *J. Med. Chem.* **1987**, *30*, 1044-1054.
18. Augustyns, K.; Rozenski, J.; Van Aerschot, A.; Janssen, G.; Herdewijn, P. *J. Org. Chem.* **1993**, *58*, 2977-2982.
19. Böhringer, M.; Roth, H.-J.; Hunziker, J.; Göbel, M.; Krishnan, R.; Giger, A.; Schweitzer, B.; Schreiber, J.; Leumann, C.; Eschenmoser, A. *Helv. Chim. Acta* **1992**, *75*, 1416-1477.
20. Verheggen, I.; Van Aerschot, A.; Van Meervelt, L.; Rozenski, J.; Wiebe, L.; Snoeck, R.; Andrei, G.; Balzarini, J.; Claes, P.; De Clercq, E.; Herdewijn, P. *J. Med. Chem.* **1995**, *38*, 826-835.

21. Ohrui, H., Waga, T., Meguro, H. *Biosci. Biotech. Biochem.* **1993**, *57*, 1040-1041.
22. Mitsunobu, O. *Synthesis* **1981**, 1-28
23. Castro, B.R. *Org. React.* **1983**, *29*, 1-162
24. Hughes, D.L. *Org. React.* **1992**, *42*, 335-656
25. Dodge, J.A., Trujillo, J.I., Presnell, M. *J. Org. Chem.* **1994**, *59*, 234-236.
26. Ti, G.S.; Gaffney, B.L.; Jones, R.A. *J. Am. Chem. Soc.* **1982**, *104*, 1316-1319.
27. Jones, R.A., *In : Oligonucleotide synthesis. A practical appraoch.* M.J. Gait, ed., JRL Press, **1984**, Chapter 2.
28 Van Aerschot, A., Saison-Behmoaras, T., Rozenski, J., Hendrix, C.; Schepers, G.; Verhoeven, G.; Herdewijn, P. *Bull. Soc. Chim. Belg.*, submitted

(*Received in UK* 27 *July* 1995; *revised* 14 *September* 1995; *accepted* 15 *September* 1995)