Synthesis and hybridization properties of β- and α-oligodeoxynucleotides containing β- and α-1-(3-*C*-allyl-2-deoxy-D-*erythro*-pentofuranosyl)thymine and α-1-[3-*C*-(3-aminopropyl)-2-deoxy-D-*erythro*-pentofuranosyl]thymine

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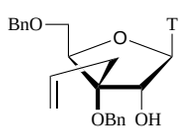
Convergent synthesis of β- and α-1-(3-*C*-allyl-2-deoxy-D-*erythro*-pentofuranosyl)thymine and their incorporation into β- and α-oligodeoxynucleotides (ODNs) is described. The thermal stabilities of duplexes formed between modified ODNs and complementary single-stranded DNA and RNA have been evaluated. In all cases stable duplexes are formed, but whereas β-ODNs containing β-3′-*C*-allylthymidine show moderately lowered thermal stability towards both DNA and RNA, α-ODNs containing α-3′-*C*-allylthymidine show significantly increased thermal stabilities compared with the corresponding β-ODN reference duplexes. Even more stable duplexes towards both DNA and RNA have been obtained using an α-ODN containing one α-1-[3-*C*-(3-aminopropyl)-2-deoxy-D-*erythro*-pentofuranosyl]thymine monomer.

Introduction

Synthesis of modified oligodeoxynucleotides (ODNs) for use in control of gene expression has been the subject of active research during the last few years.[1] In order to improve the binding affinity, enzyme stability, cell-uptake, and other pharmacokinetic properties, a variety of oligonucleotide analogues have been synthesized and evaluated.[2,3] In connection with a project directed towards the synthesis of new ODN analogues containing 3′-*C*-alkyl functionalities as attachment sites for *e.g.* intercalating agents or lipophilic carriers, we decided to synthesize 1-(3-*C*-allyl-2-deoxy-β-D-*erythro*-pentofuranosyl)-thymine **13** (β) (Scheme 1). The allyl group is suitable for diverse structural manipulations, and allows, *e.g.* easy access to both the 3′-*C*-(2-hydroxyethyl)- and the 3′-*C*-(3-hydroxypropyl)-modified nucleosides by either oxidative cleavage followed by reduction or hydroboration followed by *in situ* oxidation. Additionally, it would be interesting to evaluate 1-(3-*C*-allyl-2-deoxy-β-D-*erythro*-pentofuranosyl)thymine as a novel monomeric substitute in modified ODNs. Until recently, only a few examples of 3′-*C*-allyl-substituted nucleosides were known. 3′-*C*-Allyl-2′,3′-deoxynucleosides were obtained by addition of a 3′-centred radical to allyltributyltin which proceeded in a stereoselective way to afford only the *erythro*-isomer corresponding to addition from the less hindered α-face of the free-radical intermediate.[4] Recently, we have reported the synthesis of 3′-*C*-allyluridines by cerium-assisted Grignard additions of allylmagnesium bromide to 3′-ketouridines.[5] However, as in the majority of Grignard reactions on ketonucleosides,[6] the nucleophilic addition occurred preferentially from the α-face due to steric hindrance from the base moiety. Based on these results we decided to use a convergent synthetic strategy, hoping to achieve a favourable ratio between the desired *erythro*- and the *threo*-configurated products. In addition, the possibility of straightforward introduction of various nucleobases appealed to us. Recently, ODNs containing nucleoside monomers carrying aminoalkyl linkers tethered at the 1′-*C*, 2′-*O*, 3′-*O* or 4′-*C* positions of the carbohydrate moiety have been reported.[7] Here we introduce an ODN containing one 3′-*C*-aminopropyl-derivatized monomer synthesized from the parent 3′-*C*-allyl nucleoside.



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T = thymin-1-yl

Results and discussion

1-(3-*C*-Allyl-3,5-di-*O*-benzyl-β-D-ribofuranosyl)thymine[8] **1** was prepared in six steps from 5′-*O*-silyl-protected 1,2-*O*-isopropylidene-α-D-ribofuranosid-3-ulose in an overall yield of 47%. The reaction included a stereoselective Grignard addition of allylmagnesium bromide and a 2′-*O*-acyl-assisted nitrogen glycosylation to afford exclusively the β-nucleoside. Unfortunately, free-radical deoxygenation[9] of the 2′-hydroxy group of compound **1** *via* the corresponding pentafluorophenyl thionocarbonate by Bu_3SnH in the presence of azoisobutyronitrile (AIBN) in hot benzene was unsuccessful in our hands, probably due to uncontrolled intramolecular free-radical cyclizations. To overcome this problem we decided to use 2-deoxy-D-ribose as the starting compound for the synthesis of amidite **16a** (and **16b**). Conversion of 2-deoxy-D-ribose into an anomeric mixture of methyl 2-deoxy-D-*erythro*-pentofuranoside was done under kinetic control as previously described.[10] Reaction of methyl 2-deoxy-D-*erythro*-pentofuranoside with 1.03 mol equiv. of *tert*-butyldimethylsilyl chloride (TBDMSCl) and imidazole in dimethylformamide (DMF)[11] afforded, after column chromatography, pure 5-*O*-silylated α-anomer **2** (28% yield), the

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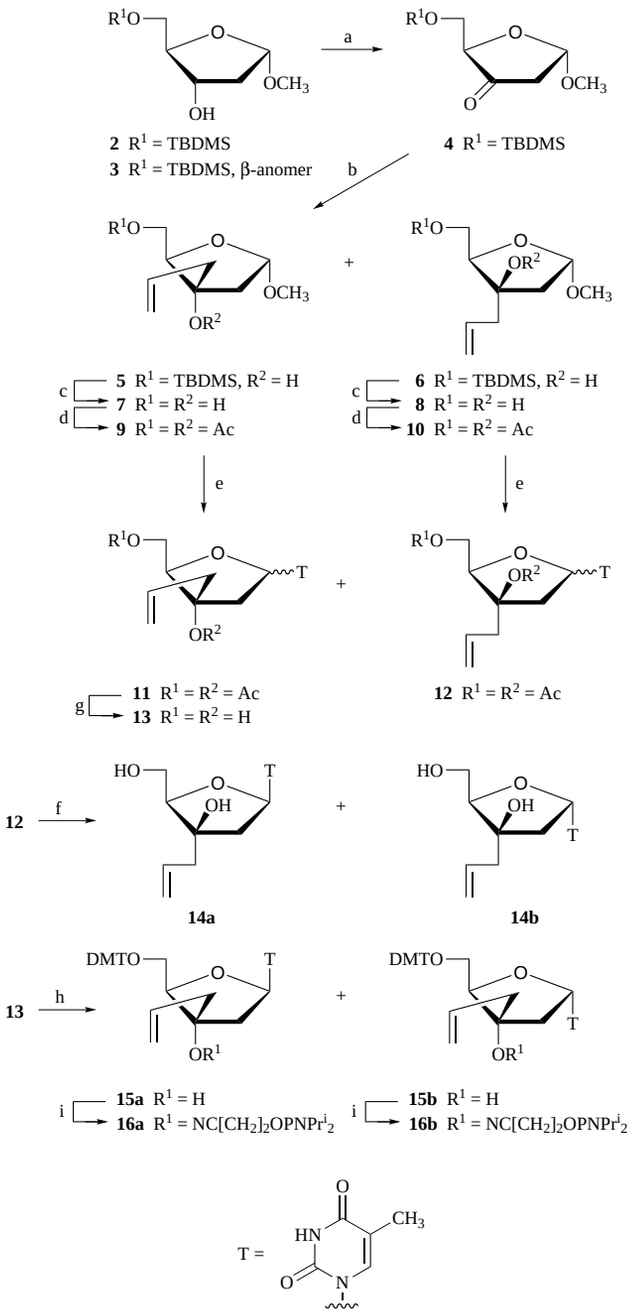
corresponding β-anomer **3** (19% yield) and a fraction containing an anomeric mixture of the disilylated glycosides (9% yield). Assignment of the anomeric configuration was done by ^{1}H–^{1}H 2-D chemical-shift-correlation spectroscopy (COSY) and nuclear Overhauser effect (NOE) difference experiments. In an attempt to control the stereoselectivity of the nucleophilic addition of allylmagnesium bromide to give the *erythro*-configurated product, the α-anomer **2** was selected. Oxidation of compound **2** with CrO_3–pyridine–acetic anhydride reagent[12] gave in 84% yield the corresponding 3-ulose **4**, which was used in the next step without further purification. Grignard addition of 1.0 mol equiv. of allylmagnesium bromide afforded the 3-*C*-allyl-α-D-*erythro* glycoside **5** in 16% yield, whereas the 3-*C*-allyl-α-D-*threo* glycoside **6** was obtained in 50% yield. The stereochemistry of the Grignard products was confirmed by ^{1}H–^{1}H COSY and NOE difference experiments. Thus, the α-*erythro* configuration of product **5** was confirmed by observing NOEs between H-1 and H-2β (~3%), 3-OH and H-4 (2%), and 3-OH and H-2α (3%). Mutual NOEs between 3-OH and H-2β and lack of an NOE between 3-OH and H-4 confirmed the α-*threo* configuration of product **6**. These results clearly show that the stereochemical outcome of the Grignard addition was determined by the bulky group at the 5-position and that no stereocontrolling effect was induced by the α-OMe substituent. In addition, our experiments indicated that no more than 1.0 mol equiv. of allylmagnesium bromide should be used in the Grignard reaction. Thus, reaction of 3-ulose **4** with an excess of allylmagnesium bromide afforded a mixture of acyclic derivatives as a result of additional nucleophilic attack at the anomeric carbon atom. Deprotection of the furanosides **5** and **6** using tetrabutylammonium fluoride (TBAF) in tetrahydrofuran (THF)[13] afforded the diols **7** and **8** in 91 and 82% yield, respectively. Subsequent acetylation of both the primary and the tertiary hydroxy groups using acetic anhydride in anhydrous pyridine in the presence of 4-(dimethylamino)pyridine (DMAP) afforded compounds **9** and **10** in 77 and 45% yield, respectively. Direct nitrogen glycosylation[14] of **9** and **10** with thymine using *N*,*O*-bis(trimethylsilyl)acetamide (BSA) as silylating agent and trimethylsilyl trifluoromethanesulfonate (TMS triflate) as a Lewis acid in anhydrous 1,2-dichloroethane afforded inseparable anomeric mixtures of nucleosides **11** (β : α ~ 1 : 2.3) and **12** (β : α ~ 1 : 1.1) in 52 and 64% yield, respectively. Exchange of 1,2-dichloroethane with the more polar solvent CH_3CN in the direct glycosylation reaction of compound **9** with thymine improved the yield to 74% and reversed the ratio between the β- and α-anomer (β : α ~ 1.5 : 1). Contrary to the alternative convergent strategy starting from 5′-*O*-silyl-protected 1,2-*O*-isopropylidene-α-D-ribofuranosid-3-ulose,[8] this strategy gives access to both anomers which enables evaluation of β- as well as α-ODN analogues. In this context it is notable that α-DNA is interesting as a potential antisense agent as it forms a more stable duplex with complementary RNA than does the corresponding β-DNA strand,[15] and is not cleaved by many nucleases.[16]

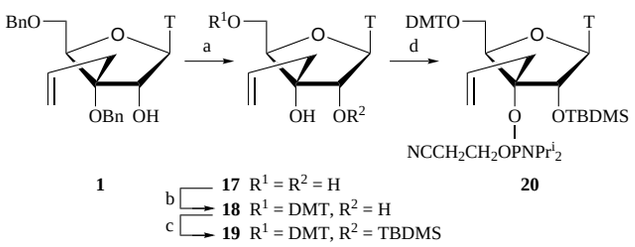
Deacetylation of the *threo*-anomers **12** using methanolic ammonia afforded the β-anomer **14a** in 39% yield and the α-anomer **14b** in 44% yield after column chromatographic separation. These two novel nucleosides are currently being evaluated for biological activity. Treatment of the *erythro* anomers **11** with CH_3ONa–CH_3OH afforded an inseparable anomeric mixture **13** in 81% yield. Dimethoxytritylation of mixture **13** in anhydrous THF–anhydrous pyridine, with $AgNO_3$ as catalyst,[17] afforded 1-[3-*C*-allyl-2-deoxy-5-*O*-(4,4′-dimethoxytrityl)-β-D-*erythro*-pentofuranosyl]thymine **15a** in 47% yield, and 1-[3-*C*-allyl-2-deoxy-5-*O*-(4,4′-dimethoxytrityl)-α-D-*erythro*-pentofuranosyl]thymine **15b** in 26% yield. The structural assignment of the β and α nucleosides was done by NOE difference experiments and one-dimensional ^{1}H NMR spectroscopy. The key mutual NOE between H-1′ and H-4′ observed for the β-anomer **15a** [irradiation of H-1′ gave an NOE-effect in H-4′ (3%), while irradiation of H-4′ gave an NOE-effect in H-1′ (7%)] was not seen for the α-anomer **15b** for which an NOE between H-4′ and H-6 (6%) was observed. These results were further supported by the relative chemical shifts of H-5′a,b and H-4′. The H-4′ signal of the α-anomer is shifted downfield relative to the signal of the β-anomer, causing the difference in the chemical shifts between the H-5′a,b and the H-4′ to be greater in the α-anomer than in the β-anomer.[18–19] In the ^{1}H NMR spectrum of the β-anomer **15a**, the H-5′a,b and the H-4′ signals appeared with a chemical-shift difference of 1.04 ppm which was extended to 1.29 ppm for the α-anomer **15b**, caused by a downfield shift of the H-4′. Phosphitylation[20] of compound **15a** and **15b** by reaction with 2-cyanoethyl *N*,*N*-diisopropylphosphoramido-



Scheme 1 (a) CrO_3, Pyridine, Ac_2O, CH_2Cl_2; (b) 1.0 mol equiv. allylMgBr, Et_2O; (c) TBAF, THF; (d) Ac_2O, DMAP, pyridine; (e) A: thymine, BSA, TMS triflate, CH_2ClCH_2Cl; B: thymine, BSA, TMS triflate, CH_3CN; (f) NH_3 in CH_3OH; (g) CH_3ONa in CH_3OH; (h) DMTCl, $AgNO_3$, pyridine, THF; (i) $NC(CH_2)_2OP(Cl)NPr^i_2$, DIPEA, CH_2Cl_2. T = thymin-1-yl

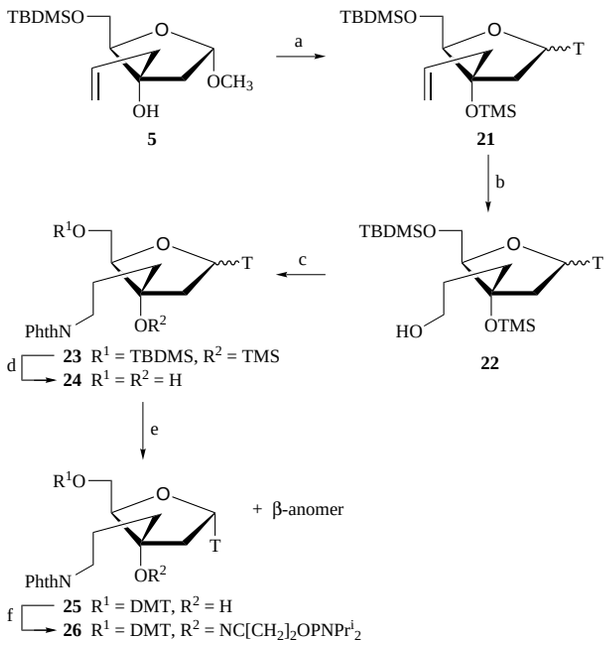
chloridite in the presence of *N*,*N*-diisopropylethylamine (DIPEA) in anhydrous CH_2Cl_2 afforded the nucleoside phosphoramidites **16a** in 75% yield and **16b** in 77% yield, respectively, after column chromatographic purification and precipitation from light petroleum.

With the aim of evaluating the properties of 3′-*C*-allyl β-D-*ribo* ODN-analogues, the phosphoramidite derivative **20** was synthesized (Scheme 2) from 1-(3-*C*-allyl-3,5-di-*O*-benzyl-β-D-ribofuranosyl)thymine **1**.[8] Debenzylation of compound **1** using BCl_3 in CH_2Cl_2 at −78 °C afforded nucleoside **17** in 73% yield. As expected, removal of the benzyl groups using H_2 and $Pd(OH)_2$–C as catalyst in absolute EtOH at room temperature caused concomitant reduction or the allylic double bond. Dimethoxytritylation of triol **17** using 1.2 mol equiv. of 4,4′-dimethoxytrityl chloride (DMTCl) in anhydrous pyridine afforded nucleoside **18** in 82% yield. This was followed by silylation of the free 2′-hydroxy group using TBDMSCl in anhydrous DMF and imidazole as catalyst to afford compound **19** in 82% yield. Subsequent phosphitylation, as described above for the preparation of compound **16a**, afforded the phosphoramidite **20** in 78% yield after column chromatographic purification and precipitation from light petroleum.



Scheme 2 (a) BCl_3, CH_2Cl_2, hexane; (b) DMTCl, pyridine; (c) TBDMSCl, imidazole, DMF; (d) $NC(CH_2)_2OP(Cl)NPr^i_2$, DIPEA, CH_2Cl_2. T = thymin-1-yl

The chemistry used to synthesize the phosphoramidite monomer **26**, which was used on an automated DNA synthesizer to introduce a 3′-*C*-(3-aminopropyl) linker into an oligonucleotide, is shown in Scheme 3. Direct nitrogen glycosylation[14] of furanoside **5** with thymine using BSA as silylating agent and TMS triflate as Lewis acid in CH_3CN afforded an inseparable anomeric mixture **21** of the α- and β-nucleoside (α : β ~ 3 : 1) in 83% yield. Hydroboration of this mixture with BH_3·1,4-oxathiane in THF followed by *in situ* oxidation gave 3′-*C*-(3-hydroxypropyl) nucleosides **22** in 63% yield. Mitsunobu[21] reaction of alcohol **22** with phthalimide afforded the phthaloyl-protected primary amines **23** in 86% yield. Treatment of the protected bis-ether **23** with TBAF in THF removed the silyl groups in 33% yield and the deprotected nucleosides **24** were allowed to react with DMTCl in anhydrous THF containing anhydrous pyridine, with $AgNO_3$ as catalyst, to protect the 5′-hydroxy groups. At this stage, the α- and β-nucleosides were easily separated by silica gel chromatography to give 1-[2-deoxy-5-*O*-(4,4′-dimethoxytrityl)-3-*C*-(phthalimidopropyl)-α-D-*erythro*-pentofuranosyl]-thymine **25** in 43% yield and the corresponding β-anomer in 15% yield. The structural assignment of the α-and β-nucleoside was based on one-dimensional ^{1}H NMR analysis. Thus, for the β-anomer, the H-5′a,b and the H-4′ signals appeared with a chemical-shift difference of 0.91 ppm which was extended to 1.36 ppm for the α-anomer **25**. The configuration of the α-anomer was further confirmed by a significant NOE of H-4′ (5%) by irradiation of H-6. The α-anomer **25** was treated with 2-cyanoethyl *N*,*N*-diisopropylphosphoramidochloridite in the presence of DIPEA in anhydrous CH_2Cl_2 to give the phosphoramidite **26** in 41% yield. Owing to the low yield obtained for the β-anomer corresponding to compound **25** incorporation of this anomer awaits completion of an alternative synthetic strategy.



Scheme 3 (a) Thymine, BSA, TMS triflate, CH_3CN; (b) BH_3·1,4-oxathiane, THF; NaOH, 30% H_2O_2; (c) pthalimide, Ph_3P, DEAD, THF; (d) TBAF, THF; (e) DMTCl, $AgNO_3$, pyridine, THF; (f) $NC(CH_2)_2OP(Cl)NPr^i_2$, DIPEA, CH_2Cl_2. T = thymin-1-yl. PhthN = phthalimido.

Synthesis of DMT-on ODNs **A**–**L** (Table 1) was performed by use of standard phosphoramidite methodology on an automated DNA-synthesizer using the appropriate building blocks [**16a**, **16b**, **26**, α-thymidine 3′-*O*-2-(cyanoethyl)phosphoramidite and commercial thymidine 3′-*O*-2-(cyanoethyl)phosphoramidite]. The coupling efficiency of the modified phosphoramidites **16a**, **16b**, and **26** were approximately 40% (two times 24 min coupling) compared with >99% for α-thymidine and standard phosphoramidites (2 min coupling). The coupling efficiency of the building block **20** was in all experiments below 5%. The low coupling yield obtained with compound **20** can be explained by the tertiary nature of the phosphitylated hydroxy group in connection with steric hindrance from the 2′-*O*-(*tert*-butyldimethylsilyl) group (compare with structures **16a**, **16b** and **26**). No ODNs containing compound **20** could therefore be obtained. The 5′-*O*-dimethoxytrityl-protected oligomers were removed from the solid support by treatment with conc. ammonia at room temperature for 72 h, which also removed the phosphate and nucleobase-protecting groups and the phthaloyl group in species **L**. Purification using disposable reversed-phase chromatography cartridges (which includes detritylation) afforded the unprotected oligomers **A**–**L**. The purity of the modified oligomers was confirmed by anion-exchange high-performance liquid chromatography (HPLC) analysis. The composition of the oligomers was verified by matrix-assisted laser desorption mass spectrometry (MALDI-MS), a powerful method for mass analysis of ligomers.[22,23] The observed relative molecular masses correspond within experimental error to those calculated (Table 2).

The hybridization properties of the modified oligomers towards their complementary DNA and RNA strands were checked by UV melting point (T_m) measurement as described.[24] The results are given in Table 1. Incorporation of **16a** (monomer **X**) once or twice in the middle of a 14-mer (ODNs **A**–**D**) induces a minor destabilization of the duplex formed with complementary DNA (ΔT_m ~ −1 °C modification). However, when RNA is used as the complementary strand, the resulting duplexes are destabilized by −2.8 to −4.0 °C per modification. These results support the assumption that the monomer **X** adopts a 2′-*endo* conformation, as reported earlier for a similar nucleoside,[25] which is favourable for DNA–DNA duplex form-

Table 1 Sequences and melting experiments of synthesized β- and α-ODNs.

Sequence		T_m (°C)[a]	ΔT_m (°C)[a]	T_m (°C)[b]	ΔT_m (°C)[b]
5′-TTTTTTTTTTTTTT-3′	**A**	35.5		29.0	
5′-TTTTTTT**X**TTTTTT-3′	**B**	34.5	−1.0	25.0	−4.0
5′-TTTTTT**XX**TTTTTT-3′	**C**	34.0	−0.8	23.5	−2.8
5′-TTTTT**X**TTT**X**TTTT-3′	**D**	33.0	−1.3	23.0	−3.0
5′-TTTTTTT**Y**TTTTTT-3′	**E**	18.5	−17.0		
5′-TTTTTT**YY**TTTTTT-3′	**F**	15.0	−10.3		
5′-TTTTT**Y**TTT**Y**TTTT-3′	**G**	14.5	−10.5		
5′-α-(TTTTTTTTTTTTTT)C-3′	**H**	38.0		43.5	
5′-α-(TTTTTTT**Y**TTTTTT)C-3′	**I**	37.0	−1.0	40.0	−3.5
5′-α-(TTTTTT**YY**TTTTTT)C-3′	**J**	37.0	−0.5	37.0	−3.3
5′-α-(TTTT**Y**TTT**Y**TTTT)C-3′	**K**	37.0	−0.5	36.0	−3.8
5′-α-(TTTTTT**Z**TTTTTT)C-3′	**L**	38.0	0.0	41.0	−2.5

T = thymidine monomer; C = 2′-deoxycytidine monomer; **X** = β-monomer derived from amidite **16a**; **Y** = α-monomer derived from amidite **16b**; **Z** = α-3-*C*-3-aminopropyl monomer derived from amidite **26**. T_m = melting temperature; ΔT_m = change in T_m per modification compared with the corresponding reference duplex. [a] Complexed with dA_{14}. [b] Complexed with rA_{14}.

Table 2 Mass analysis of synthesized ODNs.

ODN	Calc. $[M + H]^+$ (*m*/*z*)	Found $[M + H]^+$ (*m*/*z*)
A	4198	4200
B	4238	4241
C	4278	4280
D	4278	4280
E	4238	4240
F	4278	4280
G	4278	4280
H	4487	4487
I	4527	4529
J	4567	4565
K	4567	4566
L	4544	4545

ation whereas the conformation of the carbohydrate moiety in a DNA–RNA duplex is known to be 3′-*endo*.[26] As expected, incorporation of the α-monomer **Y** derived from compound **16b** into a β-ODN (**E**–**G**) results in a large destabilization of the duplex formed with complementary DNA. This further confirms the assigned configuration of the nucleosides. As mentioned, α-ODNs form thermally more stable duplexes with RNA than do β-ODNs. The melting experiments depicted in Table 1 show the same tendency as α-ODN values are on average 14.5 °C higher towards complementary RNA than are those for the corresponding β-ODN–RNA duplexes. However, incorporation of α-monomer **Y** once or twice in the middle of a α-T_{14} (ODNs **H**–**K**) induces a similar destabilizing effect ($\Delta T_m \sim -3.5$ °C/modification) on a duplex formed with complementary RNA as seen above for **X** when incorporated in a β-strand. When DNA is used as the complementary strand, incorporation of **Y** has only a minor effect on the hybridization properties of the modified α-ODNs. The hybridization obtained by modified β-ODNs with complementary DNA is similar to that obtained earlier for the corresponding 3′-*C*-(hydroxymethyl)thymidine.[27]

As depicted in Table 1, the hybridization properties of the ODN **L** containing one 3′-*C*-aminopropyl α-monomer **Z** in the middle were the most promising obtained towards both complementary DNA and RNA as no (towards DNA) or only minor (towards RNA) destabilizing effect was observed. These results could originate from the cationic nature of the amino group possibly leading to favourable partial neutralization of the phosphate backbone.

In summary, synthesis of the novel β- and α-anomers of 3′-*C*-allylthymidine has been accomplished and these nucleosides have been incorporated into β- and α-ODNs. Incorporation of the β-anomer into β-ODNs and the α-anomer into α-ODNs have a similar impact on duplex formation with complementary DNA and RNA. The increased thermal stabilities observed for all the modified α-ODNs, compared with the unmodified T_{14}, suggest them to be promising as bioactive molecules. The most likely application of 3′-*C*-allylnucleosides, however, will be as a precursor for the synthesis of 3′-*C*-(2-hydroxyethyl)- or 3′-*C*-(3-hydroxypropyl)nucleosides and their amine analogues. Interesting properties of the latter were demonstrated (ODN **L**) and further research in this direction seems justified.

Experimental

NMR spectra were recorded at 250 MHz for ^{1}H NMR and 62.9 MHz for ^{13}C NMR on a Bruker AC-250 spectrometer or at 500 MHz for ^{1}H NMR, 125 MHz for ^{13}C NMR and 202.3 MHz for ^{31}P NMR on a Varian Unity 500 spectrometer. Chemical shifts are in ppm relative to tetramethylsilane as internal standard (^{1}H NMR and ^{13}C NMR) and relative to 85% H_3PO_4 as external standard (^{31}P NMR). *J* Values are given in Hz. ^{1}H NMR peak assignments for compounds **2**, **3**, **5**, **6**, **14a**, **14b**, **15a**, **15b**, **17**–**19** and **21**–**25** were derived from ^{1}H–^{1}H COSY and/or NOE difference experiments. ^{13}C NMR peak assignments for compounds **3**, **5**, **6** and **17** were derived from intensive nuclei enhancement by polarization transfer (INEPT) and ^{1}H–^{13}C COSY experiments. Fast-atom bombardment (FAB) mass spectra were recorded on a Kratos MS 50 RF spectrometer. Microanalyses were performed at the Department of Chemistry, University of Copenhagen. The silica gel used for column chromatography (0.040–0.063 mm) was purchased from Merck. ODNs were synthesized on an Assembler Gene Special® DNA-Synthesizer (Pharmacia Biotech). Purification of 5′-*O*-DMT-on ODNs was accomplished using disposable Oligopurification Cartridges (COP, Cruachem). MALDI-MS was performed in positive mode on a Micromass TofSpec E mass spectrometer using a matrix of diammonium citrate and 2,6-dihydroxyacetophenone. Analytical anion-exchange HPLC (RESOURCE™ Q, 1 cm^3, Pharmacia Biotech) was performed on a Waters Delta Prep 3000 Preparative Chromatography System. Melting profiles of duplexes were obtained on a Perkin-Elmer UV/VIS spectrometer fitted with a PTP-6 Peltiér temperature-programming element. The reference oligonucleotide (rA_{14}) was purchased from DNA Technology ApS, Aarhus, Denmark. Light petroleum refers to the fraction with distillation range 60–80 °C.

Methyl 5-*O*-(*tert*-butyldimethylsilyl)-2-deoxy-α-D-*erythro*-pentofuranoside 2 and methyl 5-*O*-(*tert*-butyldimethylsilyl)-2-deoxy-β-D-*erythro*-pentofuranoside 3

An anomeric mixture of methyl 2-deoxy-D-*erythro*-pentofuranoside[10] (51.32 g, 0.35 mmol) and imidazole (57.06 g, 0.84 mol) was dissolved in anhydrous DMF (400 cm^3) under argon. TBDMSCl (58.02 g, 0.36 mol) as a solution in anhydrous DMF (100 cm^3) was added dropwise over a period of 1 h. After 5 h, the reaction mixture was concentrated under reduced pressure. Purification by silica gel column chromatography (0–25%

EtOAc in light petroleum) afforded α-anomer **2** (25.83 g, 28%) as the less polar compound, stereoisomer **3** (17.84 g, 19%) and a fraction of the diprotected glycoside (12.29 g, 9%).

Compound **2**: $\delta_C(CDCl_3)$ −5.64 and −5.42 [$Si(CH_3)_2$], 18.13 [$C(CH_3)_3$], 25.72 [$C(CH_3)_3$], 40.96 (C-2), 54.60 (OCH_3), 63.63 (C-5), 73.03 (C-3), 87.69 (C-4) and 105.50 (C-1); $\delta_H(CDCl_3)$ 0.02 [s, 6 H, $Si(CH_3)_2$], 0.85 [s, 9 H, $C(CH_3)_3$], 1.91–2.15 (m, 2 H, H_2-2), 2.84 (d, *J* 10.4, 1 H, OH), 3.34 (s, 3 H, OCH_3), 3.53 (dd, *J* 4.8 and 10.9, 1 H, H^a-5), 3.70 (dd, *J* 3.6 and 10.9, 1 H, H^b-5), 4.05–4.09 (m, 1 H, H-4), 4.11–4.18 (m, 1 H, H-3) and 5.04 (m, 1 H, H-1).

Compound **3**: $\delta_C(CDCl_3)$ −5.46 and −5.41 [$Si(CH_3)_2$], 18.27 [$C(CH_3)_3$], 25.88 [$C(CH_3)_3$], 40.93 (C-2), 54.98 (OCH_3), 65.01 (C-5), 73.63 (C-3), 85.66 (C-4) and 105.02 (C-1); $\delta_H(CDCl_3)$ 0.08 [s, 6 H, $Si(CH_3)_2$], 0.91 [s, 9 H, $C(CH_3)_3$], 1.99 (s, 1 H, OH), 2.06 (ddd, *J* 5.3, 6.6 and 13.3, 1 H, H^a-2), 2.22 (ddd, *J* 2.1, 6.8 and 13.3, 1 H, H^b-2), 3.32 (s, 3 H, OCH_3), 3.54 (dd, *J* 8.0 and 9.7, 1 H, H^a-5), 3.75–3.90 (m, 2 H, H^b-5 and H-4), 4.42–4.44 (m, 1 H, H-3) and 5.06 (dd, *J* 2.1 and 5.3, 1 H, H-1).

Anomeric mixture of compounds **2** and **3**: FAB-MS *m/z* 261 $[M - H]^-$ (Found: C, 54.74; H, 9.64. Calc. for $C_{12}H_{26}O_4Si$: C, 54.92; H, 9.99%).

Methyl 5-*O*-(*tert*-butyldimethylsilyl)-2-deoxy-α-D-*glycero*-pentofuranoside-3-ulose 4

To a suspension of CrO_3 (5.00 g, 50.0 mmol) in anhydrous CH_2Cl_2 (150 cm^3) were added anhydrous pyridine (8.1 cm^3, 0.10 mol) and methyl glycoside **2** (5.86 g, 22.3 mmol) followed by Ac_2O (4.7 cm^3, 49.7 mmol). The reaction mixture was stirred under argon for 4 h at room temp. EtOAc (500 cm^3) was added and the mixture was filtered through a silica gel column. Concentration of the organic phase under reduced pressure afforded title keto glycoside **4** as a pale yellow oil (4.77 g, 82%) which was used without further purification in the next step; $\delta_C(CDCl_3)$ −5.70 and 5.51 [$Si(CH_3)_2$], 18.17 [$C(CH_3)_3$], 25.70 [$C(CH_3)_3$], 44.18 (C-2), 54.82 (OCH_3), 62.25 (C-5), 78.81 (C-4), 104.80 (C-1) and 212.28 (C-3); $\delta_H(CDCl_3)$ 0.04 and 0.05 [2 × s, 6 H, $Si(CH_3)_2$], 0.86 [s, 9 H, $C(CH_3)_3$], 2.33–2.60 (m, 2 H, H_2-2), 3.44 (s, 3 H, OCH_3), 3.83–3.98 (m, 3 H, H_2-5 and H-4) and 5.36 (m, 1 H, H-1).

Methyl 3-*C*-allyl-5-*O*-(*tert*-butyldimethylsilyl)-2-deoxy-α-D-*erythro*-pentofuranoside 5 and methyl 3-*C*-allyl-5-*O*-(*tert*-butyldimethylsilyl)-2-deoxy-α-D-*threo*-pentofuranoside 6

Compound **4** (4.29 g, 16.46 mmol) was coevaporated with anhydrous toluene (2 × 25 cm^3) and dissolved under argon in anhydrous Et_2O (150 cm^3). The mixture was cooled to 0 °C, a 1 M solution of allylmagnesium bromide in anhydrous Et_2O (16.5 cm^3, 16.5 mmol) was added dropwise and the mixture was stirred for 16 h at room temp. The reaction was quenched by addition of saturated aq. NH_4Cl (400 cm^3) and the aqueous phase was extracted with Et_2O (3 × 250 cm^3). The organic phase was dried (Na_2SO_4) and evaporated. Purification by silica gel column chromatography (0–3% EtOAc in light petroleum, v/v) afforded *erythro* product **5** (789 mg, 16%) as the less polar compound and its *threo* stereoisomer **6** (2.51 g, 50%).

Compound **5**: $\delta_C(CDCl_3)$ −5.65 and −5.49 [$Si(CH_3)_2$], 18.10 [$C(CH_3)_3$], 25.81 [$C(CH_3)_3$], 38.67 (C-2), 44.71 (CH_2), 54.90 (OCH_3), 62.57 (C-5), 79.88 (C-3), 89.20 (C-4), 104.93 (C-1), 117.30 (=CH_2) and 134.49 (=CH); $\delta_H(CDCl_3)$ 0.06 and 0.07 [2 × s, 6 H, $Si(CH_3)_2$], 0.89 [s, 9 H, $C(CH_3)_3$], 1.88–1.93 (m, 1 H, H^a-2), 2.14 (dd, *J* 5.2 and 13.2, 1 H, H^b-2), 2.36–2.55 (m, 2 H, CH_2), 3.39 (s, 3 H, OCH_3), 3.59 (s, 1 H, OH), 3.66–3.69 (m, 2 H, H_2-5), 4.05–4.08 (m, 1 H, H-4), 5.05–5.08 (m, 2 H, H-1 and =CH_2^a), 5.12–5.15 (m, 1 H, =CH_2^b) and 5.93–6.10 (m, 1 H, =CH) (Found: C, 59.71; H, 9.88. Calc. for $C_{15}H_{30}O_4Si$: C, 59.56; H, 10.00%).

Compound **6**: $\delta_C(CDCl_3)$ −5.61 and 5.49 [$Si(CH_3)_2$], 18.12 [$C(CH_3)_3$], 25.75 [$C(CH_3)_3$], 44.27 (CH_2), 47.10 (C-2), 55.09 (OCH_3), 62.42 (C-5), 80.64 (C-3), 81.69 (C-4), 103.93 (C-1), 118.24 (=CH_2) and 133.80 (=CH); $\delta_H(CDCl_3)$ 0.11 [s, 6 H, $Si(CH_3)_2$], 0.91 [s, 9 H, $C(CH_3)_3$], 2.01 (dd, *J* 2.9 and 13.8, 1 H, H^a-2), 2.17 (dd, *J* 5.6 and 13.8, 1 H, H^b-2), 2.37–2.50 (m, 2 H, CH_2), 3.35 (s, 3 H, OCH_3), 3.66 (s, 1 H, OH), 3.78–3.81 (m, 1 H, H-4), 3.93–3.95 (m, 2 H, H_2-5), 5.07–5.11 (m, 2 H, H-1 and =CH_2^a), 5.15–5.16 (m, 1 H, =CH_2^b) and 5.85–5.99 (m, 1 H, =CH); FAB-MS *m/z* 303 $[M + H]^+$ (Found: C, 59.37; H, 9.60%).

Methyl 3-*C*-allyl-2-deoxy-α-D-*erythro*-pentofuranoside 7

Compound **5** (1.08 g, 3.57 mmol) was dissolved in anhydrous THF (15 cm^3)under argon. 1.1 M TBAF in THF (3.2 cm^3, 3.57 mmol) was added and the mixture was stirred for 15 min. EtOAc (70 cm^3) was added and the organic phase was washed with saturated aq. $NaHCO_3$ (3 × 30 cm^3). The water phase was extracted with EtOAc (60 cm^3), and the organic phases were combined, dried (Na_2SO_4) and concentrated under reduced pressure. Purification using silica gel column chromatography (20% EtOAc in light petroleum) afforded title diol **7** as an oil (612 mg, 91%); $\delta_C(CDCl_3)$ 38.76 (C-2), 44.95 (CH_2), 54.92 (OCH_3), 61.99 (C-5), 79.41 (C-3), 89.20 (C-4), 104.53 (C-1), 117.79 (=CH_2) and 133.72 (=CH); $\delta_H(CDCl_3)$ 2.00–2.03 (m, 2 H, H_2-2), 2.27–2.47 (m, 2 H, CH_2), 2.65 (br s, 1 H, OH), 3.40 (s, 3 H, OCH_3), 3.52–3.73 (m, 2 H, H_2-5), 4.14 (dd, *J* 3.7 and 4.9, 1 H, H-4), 5.09–5.17 (m, 3 H, H-1 and =CH_2) and 5.89–6.06 (m, 1 H, =CH) (Found: C, 57.60; H, 8.47. Calc. for $C_9H_{16}O_4$: C, 57.43; H, 8.57%).

Methyl 3-*C*-allyl-2-deoxy-α-D-*threo*-pentofuranoside 8

Compound **6** (739 mg, 2.44 mmol) was dissolved in anhydrous THF (10 cm^3) under argon. 1.1 M TBAF in THF (2.2 cm^3, 2.4 mmol) was added and the mixture was stirred for 15 min. EtOAc (40 cm^3) was added and the organic phase was washed with saturated aq. $NaHCO_3$ (3 × 20 cm^3). The water phase was extracted with EtOAc (40 cm^3), and the organic phases were combined, dried (Na_2SO_4) and concentrated under reduced pressure. Purification using silica gel column chromatography (20% EtOAc in light petroleum) afforded title diol **8** as an oil (377 mg, 82%); $\delta_C(CDCl_3)$ 43.77 (CH_2), 47.49 (C-2), 55.23 (OCH_3), 61.23 (C-5), 80.82 (C-3), 81.87 (C-4), 103.81 (C-1), 119.18 (=CH_2) and 133.11 (=CH); $\delta_H(CDCl_3)$ 2.04 (dd, *J* 2.9 and 14.0, 1 H, H^a-2), 2.20 (dd, *J* 5.64 and 14.0, 1 H, H^b-2), 2.43–2.47 (m, 2 H, CH_2), 2.70 (br s, 1 H, OH), 3.37 (s, 3 H, OCH_3), 3.80–3.82 (m, 1 H, H-4), 3.93–3.94 (m, 2 H, H_2-5), 5.13–5.17 (m, 2 H, H-1 and =CH_2^a), 5.20–5.21 (m, 1 H, =CH_2^b) and 5.83–6.00 (m, 1 H, =CH); FAB-MS *m/z* 187 $[M - H]^-$.

Methyl 3,5-di-*O*-acetyl-3-*C*-allyl-2-deoxy-α-D-*erythro*-pentofuranoside 9

To a solution of compound **7** (1.71 g, 9.09 mmol) in anhydrous pyridine (30 cm^3) under argon were added DMAP (1.11 g, 9.10 mmol) and Ac_2O (6.01 cm^3, 54.54 mmol). After stirring of the mixture for 48 h at room temperature, the solvent was evaporated off and saturated aq. $NaHCO_3$ (80 cm^3) was added. The aqueous phase was extracted with CH_2Cl_2 (3 × 125 cm^3), and the organic phases were combined, dried (Na_2SO_4) and concentrated under reduced pressure. Silica gel column chromatography (10–30% EtOAc in light petroleum) gave title diacetate **9** (1.90 g, 77%); $\delta_C(CDCl_3)$ 20.75 and 21.53 (2 × CH_3), 37.23 (CH_2), 44.66 (C-2), 54.98 (OCH_3), 63.22 (C-5), 81.69 (C-4), 87.25 (C-3), 103.35 (C-1), 118.87 (=CH_2), 131.90 (=CH) and 170.24 and 170.49 (2 × $COCH_3$); $\delta_H(CDCl_3)$ 2.02 and 2.09 (2 s, 6 H, 2 × CH_3), 2.25–2.45 (m, 3 H, H_2-2 and CH_2^a), 2.99 (dd, *J* 7.4 and 14.5, 1 H, CH_2^b), 3.37 (s, 3 H, OCH_3), 4.20 (dd, *J* 7.0 and 11.6, 1 H, H^a-5), 4.44 (dd, *J* 2.9 and 7.0, 1 H, H-4), 4.57 (dd, *J* 2.9 and 11.6, 1 H, H^b-5), 5.03 (dd, *J* 2.3 and 4.9, 1 H, H-1), 5.07–5.10 (m, 1 H, =CH_2^a), 5.14–5.15 (m, 1 H, =CH_2^b) and 5.64–5.80 (m, 1 H, =CH); FAB-MS *m/z* 272 $[M + H]^+$.

Methyl 3,5-di-*O*-acetyl-3-*C*-allyl-2-deoxy-α-D-*threo*-pentofuranoside 10

To a solution of compound **8** (2.92 g, 1.55 mmol) in anhydrous pyridine (5 cm^3) under argon were added DMAP (195 mg, 1.6 mmol) and Ac_2O (0.68 cm^3, 6.20 mmol). After stirring of the mixture for 48 h at room temperature, additional Ac_2O (0.68 cm^3, 6.20 mmol) was added. The reaction mixture was stirred for another 72 h, the solvent was evaporated off, and saturated aq. $NaHCO_3$ (15 cm^3) was added. The aqueous phase was extracted with CH_2Cl_2 (3 × 25 cm^3), and the organic phases were combined, dried (Na_2SO_4) and concentrated under reduced pressure. Silica gel column chromatography (30% EtOAc in light petroleum, v/v) gave title diacetate **10** (191 mg, 45%); $\delta_C(CDCl_3)$ 20.81 and 21.58 (2 × CH_3), 40.05 and 44.74 (CH_2 and C-2), 54.93 (OCH_3), 63.61 (C-5), 81.06 (C-4), 87.82 (C-3), 103.59 (C-1), 119.34 (=CH_2), 131.75 (=CH) and 169.65 and 170.72 (2 × *C*OCH_3); $\delta_H(CDCl_3)$ 2.00 and 2.10 (2 s, 6 H, 2 × CH_3), 2.33 (dd, *J* 1.7 and 14.9, 1 H, H^a-2), 2.65 (dd, *J* 5.7 and 15.0, 1 H, H^b-2), 2.65–2.73 (m, 1 H, $CH_2{}^a$), 3.05–3.13 (m, 1 H, $CH_2{}^b$), 3.36 (s, 3 H, OCH_3), 4.12–4.21 (m, 2 H, H_2-5), 4.38–4.47 (m, 1 H, H-4), 5.05–5.17 (m, 3 H, H-1 and =CH_2) and 5.67–5.81 (m, 1 H, =CH).

1-(3,5-Di-*O*-acetyl-3-*C*-allyl-2-deoxy-α,β-D-*erythro*-pentofuranosyl)thymine 11

Method A. Methyl glycoside **9** (1.79 g, 6.57 mmol) and thymine (1.66 g, 13.14 mmol) were dissolved in anhydrous CH_2ClCH_2Cl (100 cm^3) under argon and BSA (9.75 cm^3, 39.45 mmol) was added. The mixture was refluxed for 15 min at 78 °C. The resulting clear mixture was allowed to cool to room temperature and TMS triflate (1.83 cm^3, 9.20 mmol) was added dropwise over a period of 10 min. After being stirred for 48 h at room temperature, the mixture was diluted with CH_2Cl_2 (100 cm^3) and poured into ice–water (50 cm^3) saturated with $NaHCO_3$. The aqueous phase was extracted with CH_2Cl_2 (3 × 150 cm^3), and the organic phase was combined, dried (Na_2SO_4) and concentrated under reduced pressure. Purification using silica gel column chromatography (10–30% EtOAc in light petroleum) afforded an anomeric mixture of the β- and α-nucleoside **11** (β : α; 1 : 2.3) (1.25 g, 52%).

Method B. To a stirred suspension of the methyl glycoside **9** (901 mg, 3.32 mmol) and thymine (843 mg, 6.68 mmol) in anhydrous CH_3CN (30 cm^3) under argon at room temperature was dropwise added BSA (5 cm^3, 20.23 mmol). The mixture was stirred for 1 h until clearness was attained. The reaction mixture was cooled to −30 °C, and TMS triflate (0.95 cm^3, 5.25 mmol) was added dropwise. The reaction mixture was stirred for 72 h at room temperature, then was diluted with CH_2Cl_2 (50 cm^3), and the reaction was quenched with saturated aq. $NaHCO_3$ (2 × 50 cm^3). After being washed with water (2 × 50 cm^3), the organic phase was dried (Na_2SO_4) and concentrated under reduced pressure. Purification using silica gel column chromatography (0–1% MeOH in CH_2Cl_2) afforded an anomeric mixture of the β- and α-nucleoside **11** (β : α; 1.5 : 1) (894 mg, 74%); $\delta_C(CDCl_3)$ 12.41 and 12.45 (2 × CH_3), 20.70, 21.52 and 21.59 (4 × CO*C*H_3), 35.75 and 36.05 (2 × C-2′), 42.85 and 43.62 (2 × $CH_2{}'$), 62.79 and 63.05 (2 × C-5′), 82.24, 83.92, 84.04, 85.76, 88.49 and 88.68 (2 × C-1′, -3′ and -4′), 109.55 and 111.04 (2 × C-5), 119.41 and 119.53 (2 × =$CH_2{}'$), 131.03 and 131.16 (2 × =CH′), 134.48 and 135.17 (2 × C-6), 150.39 and 150.48 (2 × C-2), 163.81 and 164.12 (2 × C-4) and 169.71, 169.92 and 170.10 (4 × *C*OCH_3); $\delta_H(CDCl_3)$ 1.94, 1.96, 2.07 and 2.13 (4 s, 2 × CH_3, 4 × $COCH_3$), 2.36–2.85 (m, 2 × H_2-2 and 2 × $CH_2{}'^a$), 3.08–3.29 (m, 2 × $CH_2{}'^b$), 4.11–4.19 (m, 2 × H-4′), 4.33–4.41 and 4.69–4.72 (2 m, 2 × H_2-5′), 5.00–5.17 (m, 2 × =$CH_2{}'$), 5.63–5.75 (m, 2 × =CH′), 6.18–6.27 (m, 2 × H-1′), 7.36 and 7.39 (2 s, 2 × H-6) and 9.99 and 10.03 (2 s, 2 × NH); EI-MS *m*/*z* 366 [M^+, 25%] (Found: C, 55.54; H, 6.17; N, 7.31. Calc. for $C_{17}H_{22}N_2O_7$: C, 55.73; H, 6.05; N, 7.65%).

1-(3,5-Di-*O*-acetyl-3-*C*-allyl-2-deoxy-α,β-D-*threo*-pentofuranosyl)thymine 12

Method A. Used amounts. Methyl glycoside **10** (129 mg, 0.47 mmol), thymine (120 mg, 0.95 mmol), anhydrous CH_2ClCH_2Cl (7 cm^3), BSA (0.70 cm^3, 2.84 mmol) and TMS triflate (0.13 cm^3, 0.65 mmol). Purification using preparative TLC (PLC) (2% CH_3OH in CH_2Cl_2, double run) afforded an anomeric mixture of the β- and α-nucleoside **12** (112 mg, 64%); $\delta_C(CDCl_3)$ 12.33 and 12.44 (2 × CH_3), 20.66, 20.68, 21.46 and 21.66 (4 × CO*C*H_3), 37.99, 38.03, 40.52 and 41.65 (2 × C-2′ and $CH_2{}'$), 62.64 and 63.37 (2 × C-5′), 83.13, 83.59, 84.08, 86.38, 86.81 and 88.39 (2 × C-1′, -3′ and -4′), 110.46 and 110.95 (2 × C-5), 120.31 and 120.67 (2 × =$CH_2{}'$), 130.55 and 131.01 (2 × =CH′), 134.63 and 136.00 (2 × C-6), 150.31 and 150.34 (2 × C-2), 163.84 and 164.01 (2 × C-4) and 169.23, 169.74, 170.43 and 170.60 (4 × *C*OCH_3); $\delta_H(CDCl_3)$ 1.86, 1.87, 1.93, 2.00, 2.04 and 2.05 (6 s, 2 × CH_3, 4 × $COCH_3$), 2.35 (dd, *J* 7.6 and 15.0, $CH_2{}'^a$), 2.62–2.95 (m, 2 × H_2-2′ and $CH_2{}'^b$), 3.16 (dd, *J* 6.6 and 15.0, $CH_2{}'^b$), 4.07–4.15 (m, H_2-4′), 4.38–4.43 (m, H_2-5′), 5.10–5.25 (m, 2 × =$CH_2{}'$), 5.61–5.77 (m, 2 × =CH′), 5.99–6.06 (m, 2 × H-1′), 7.04 and 7.35 (2 s, 2 × H-6) and 9.89 and 9.93 (2 s, 2 × NH); FAB-MS *m*/*z* 367 $[M + H]^+$ (Found: C, 54.99; H, 6.00; N, 7.53. Calc. for $C_{17}H_{22}N_2O_7 \cdot 0.25H_2O$: C, 55.06; H, 6.12; N, 7.55%).

1-(3-*C*-Allyl-2-deoxy-α,β-D-*erythro*-pentofuranosyl)thymine 13

The anomeric mixture of nucleosides **11** (894 mg, 2.41 mmol) was dissolved in anhydrous CH_3OH (25 cm^3) under argon and CH_3ONa (660 mg, 12.2 mmol) was added. After stirring of the mixture for 12 h at room temp., water (25 cm^3) was added and the mixture was neutralized with 4 M HCl. The aqueous phase was extracted with 3-methylbutan-1-ol (3 × 100 cm^3) and the organic phase was concentrated under reduced pressure. Purification using silica gel column chromatography (0–5% CH_3OH in CH_2Cl_2) afforded an anomeric mixture of β- and α-nucleoside **13** (β : α; 1.5 : 1) (569 mg, 81%); $\delta_C(CD_3OD)$ 12.50 and 12.57 (2 × CH_3), 40.94, 41.13, 44.32 and 45.76 (2 × C-2′, 2 × $CH_2{}'$), 62.20 and 62.37 (2 × C-5′), 80.48, 81.51, 85.97, 87.64, 90.00 and 91.87 (2 × C-1′, -3′, -4′), 110.02 and 111.34 (2 × C-5), 118.67 and 118.85 (2 × =$CH_2{}'$), 134.64 and 134.72 (2 × =CH′), 138.61 and 139.26 (2 × C-6), 152.32 and 152.44 (2 × C-2) and 166.36 and 166.61 (2 × C-4); $\delta_H(CD_3OD)$ 2.02 and 2.08 (2 s, 2 × CH_3), 2.14–2.38 (m, 2 × H^a-2′ and H^b-2′), 2.57–2.73 (m, 2 × $CH_2{}'$ and H^b-2′), 3.73–3.98 (m, 2 × H-4′ and -5′), 4.05 (m, OH), 4.34 (m, OH), 5.25–5.33 (m, 2 × =$CH_2{}'$), 6.00–6.16 (m, 2 × =CH′), 6.33 (dd, *J* 2.1 and 7.7, H-1′), 6.46 (dd, *J* 5.4 and 9.3, H-1′), 8.21 and 8.22 (2 s, 2 × H-6) and 9.97 and 10.03 (2 s, 2 × NH); EI-MS *m*/*z* 282 (M^+, 9%); HR-MS (Found: M^+, 282.1210. Calc. for $C_{13}H_{18}N_2O_5$: M, 282.1216) (Found: C, 53.20; H, 6.31; N, 9.94. Calc. for $C_{13}H_{18}N_2O_5 \cdot 0.25H_2O$: C, 53.60; H, 6.57; N, 9.62%).

1-(3-*C*-Allyl-2-deoxy-β-D-*threo*-pentofuranosyl)thymine 14a and 1-(3-*C*-allyl-2-deoxy-α-D-*threo*-pentofuranosyl)thymine 14b

An anomeric mixture of nucleosides **12** (79 mg, 0.22 mmol) was dissolved in a saturated solution of NH_3 in methanol (10 cm^3). After stirring of the mixture for 15 h at room temp., the solvent was removed under reduced pressure and the crude product was purified by silica gel column chromatography (3% CH_3OH in CH_2Cl_2) to give anomers **14a** (23 mg, 39%) and **14b** (26 mg, 44%).

β-Isomer **14a**: $\delta_C(CDCl_3)$ 12.36 (CH_3), 42.56 and 44.63 (C-2′, $CH_2{}'$), 60.87 (C-5′), 79.61 (C-4′), 84.48, 85.11 (C-1′ and -3′), 109.47 (C-5), 119.43 (=$CH_2{}'$), 132.57 (=CH′), 138.08 (C-6), 150.83 (C-2) and 164.50 (C-4); $\delta_H(CDCl_3)$ 1.81 (s, 3 H, CH_3), 2.32–2.58 (m, 4 H, H_2-2′, $CH_2{}'$), 3.74 (m, 1 H, H-4′), 3.84 (br s, 1 H, OH), 4.08–4.16 (m, 2 H, H_2-5′), 4.78 (s, 1 H, OH), 5.15–5.22 (m, 2 H, =$CH_2{}'$), 5.84–6.00 (m, 1 H, =CH′), 6.08 (dd, *J* 2.9 and 7.5, 1 H, H-1′), 7.99 (s, 1 H, H-6) and 9.93 (s, 1 H, NH).

α-Isomer **14b**: $\delta_C(CDCl_3)$ 12.46 (CH_3), 42.05 and 45.01 (C-2′,

CH_2'), 60.87 (C-5′), 81.01 (C-4′), 85.28 and 86.09 (C-1′ and -3′), 111.14 (C-5), 119.28 (=CH_2'), 132.69 (=CH′), 135.74 (C-6), 150.74 (C-2) and 164.39 (C-4); $\delta_H(CDCl_3)$ 2.00 (s, 3 H, CH_3), 2.36–2.45 (m, 4 H, H-2′, CH_2'), 3.46 (s, 1 H, OH), 3.96–4.02 (m, 2 H, H_2-5′), 4.10 (m, 1 H, H-4′), 4.60 (br s, 1 H, OH), 5.13–5.18 (m, 2 H, =CH_2'), 5.82–5.98 (m, 1 H, =CH′), 6.28 (dd, *J* 6.1 and 8.1, 1 H, H-1′), 7.19 (s, 1 H, H-6) and 10.05 (br s, 1 H, NH).

1-[3-*C*-Allyl-2-deoxy-5-*O*-(4,4′-dimethoxytrityl)-β-D-*erythro*-pentofuranosyl]thymine 15a and 1-[3-*C*-allyl-2-deoxy-5-*O*-(4,4′-dimethoxytrityl)-α-D-*erythro*-pentofuranosyl]thymine 15b

An anomeric mixture of nucleoside **13** (569 mg, 2.11 mmol), $AgNO_3$ (358 mg, 2.11 mmol) and DMTCl (1.785 g, 5.27 mmol) were dissolved in anhydrous THF (50 cm^3), and anhydrous pyridine (0.85 cm^3, 10.5 mmol) was added. The mixture was stirred at room temp. for 72 h. The mixture was filtered into 5% aq. sodium hydrogen carbonate (100 cm^3). The product was extracted into CH_2Cl_2 (4 × 100 cm^3), and the combined extract was dried (Na_2SO_4), and concentrated under reduced pressure. Purification by PLC (1% pyridine, 3% CH_3OH in CH_2Cl_2, v/v/v, 3 runs) gave the α-anomer **15b** (304 mg, 26%) as the less polar isomer and the β-anomer **15a** (545 mg, 47%).

β-Isomer **15a**. $\delta_C(CDCl_3)$ 11.22 (CH_3), 39.89 (C-2′), 44.16 (CH_2'), 55.22 (OCH_3), 62.46 (C-5′), 80.13 (C-3′), 83.97 (C-1′), 87.34 (*C*Ph_3, C-4′), 111.21 (C-5), 113.18, 127.92, 128.01, 128.44, 129.92, 130.25, 130.28, 134.71, 134.91, 143.64 and 158.82 (C_{arom}), 119.84 (=CH_2'), 132.50 (=CH′), 136.12 (C-6), 150.59 (C-2) and 163.87 (C-4); $\delta_H(CDCl_3)$ 1.22 (s, 3 H, CH_3), 2.12–2.41 (m, 4 H, CH_2', H_2-2′), 3.02 (dd, *J* 2.4 and 10.8, 1 H, H^a-5′), 3.67 (dd, *J* 3.5 and 10.8, 1 H, H^b-5′), 3.79 (s, 6 H, 2 × OCH_3), 4.06 (m, 1 H, H-4′), 4.90 (dd, *J* 1.4 and 17.1, 1 H, =$CH_2'^a$), 5.09 (dd, *J* 1.4 and 10.2, 1 H, =$CH_2'^b$), 5.68–5.82 (m, 1 H, =CH′), 6.51 (dd, *J* 5.1 and 9.4, 1 H, H-1′), 6.84 (m, 4 H, ArH), 7.21–7.45 (m, 9 H, ArH), 7.86 (s, 1 H, H-6) and 9.20 (s, 1 H, NH).

α-Isomer **15b**: $\delta_C(CDCl_3)$ 12.50 (CH_3), 39.93 (C-2′), 45.43 (CH_2'), 55.19 (OCH_3), 63.17 (C-5′), 79.57 (C-3′), 86.96 (C-4′), 87.20 (*C*Ph_3), 89.32 (C-1′), 109.15 (C-5), 113.27, 126.91, 127.96, 128.04, 129.91, 129.93, 135.43, 135.72, 144.37 and 158.57 (C_{arom}), 120.02 (=CH_2'), 132.63 (=CH′), 137.58 (C-6), 150.52 (C-2) and 164.21 (C-4); $\delta_H(CDCl_3)$ 1.90 (s, 3 H, CH_3), 2.17–2.34 (m, 3 H, CH_2', H^{α}-2′), 2.79 (dd, *J* 7.7 and 14.3, 1 H, H^{β}-2′), 3.02 (dd, *J* 2.6 and 10.8, 1 H, H^a-5′), 3.44 (dd, *J* 3.7 and 10.8, 1 H, H^b-5′), 3.78 (s, 6 H, 2 × OCH_3), 4.31 (m, 1 H, H-4′), 4.98 (dd, *J* 1.3 and 17.0, 1 H, =$CH_2'^a$), 5.10 (dd, *J* 1.3 and 10.1, 1 H, =$CH_2'^b$), 5.69–5.80 (m, 1 H, =CH′), 6.41 (m, 1 H, H-1′), 6.85 (m, 4 H, ArH), 7.16–7.45 (m, 9 H, ArH), 7.71 (s, 1 H, H-6) and 9.13 (s, 1 H, NH); FAB-MS *m*/*z* 584 $[M]^+$ (Found: C, 69.28; H, 6.20; N, 5.19. Calc. for $C_{34}H_{36}N_2O_7 \cdot 0.1H_2O$: C, 69.63; H, 6.22; N, 4.78%).

1-{3-*C*-Allyl-3-*O*-[cyanoethoxy(diisopropylamino)phosphino]-2-deoxy-5-*O*-(4,4′-dimethoxytrityl)-β-D-*erythro*-pentofuranosyl}-thymine 16a

Method C. General method for phosphitylation. Nucleoside **15a** (545 mg, 0.93 mmol) was coevaporated with anhydrous CH_3CN (3 × 2 cm^3) and was then dissolved under argon in anhydrous CH_2Cl_2 (3.7 cm^3). DIPEA (1.02 cm^3, 5.97 mmol) was added followed by dropwise addition of 2-cyanoethyl *N*,*N*-diisopropylphosphoramidochloridite (0.44 cm^3, 1.86 mmol). After 5 h, CH_3OH (1 cm^3) was added and the reaction mixture was diluted with EtOAc (20 cm^3) containing triethylamine (0.2 cm^3), washed successively with saturated aq. $NaHCO_3$ (3 × 30 cm^3) and saturated aq. NaCl (2 × 30 cm^3), dried (Na_2SO_4) and evaporated under reduced pressure. Purification using silica gel column chromatography (EtOAc–CH_2Cl_2–Et_3N–light petroleum, 15:30:5:50, v/v/v/v) followed by precipitation in light petroleum (200 cm^3) at −20 °C [after re-dissolution in anhydrous toluene (2 cm^3)] afforded compound **16a** as a solid (534 mg, 75%); $\delta_P(CDCl_3)$ 138.88 and 138.28.

1-{3-*C*-Allyl-3-*O*-[cyanoethoxy(diisopropylamino)phosphino]-2-deoxy-5-*O*-(4,4′-dimethoxytrityl)-α-D-*erythro*-pentofuranosyl}-thymine 16b

Method C. Used amounts. Nucleoside **15b** (304 mg, 0.52 mmol), anhydrous CH_2Cl_2 (2.1 cm^3), DIPEA (0.57 cm^3, 3.33 mmol) and 2-cyanoethyl *N*,*N*-diisopropylphosphoramidochloridite (0.25 cm^3, 1.06 mmol) as above. Purification using silica gel column chromatography (EtOAc–CH_2Cl_2–Et_3N–light petroleum, 15:30:5:50, v/v/v/v) followed by precipitation in light petroleum (150 cm^3) at −20 °C [after re-dissolution in anhydrous toluene (1.5 cm^3)] afforded compound **16b** as a solid (306 mg, 77%); $\delta_P(CDCl_3)$ 143.94.

1-(3-*C*-Allyl-β-D-ribofuranosyl)thymine 17

To a solution of compound **1**[8] (2.16 g, 4.51 mmol) in anhydrous CH_2Cl_2 (70 cm^3) under argon at −78 °C was added dropwise BCl_3 (1 M solution in hexane; 18.1 cm^3, 18.1 mmol). The mixture was stirred for 3.5 h at −78 °C, additional BCl_3 was added (1 M hexane solution; 4.0 cm^3, 4.0 mmol), and the mixture was stirred for a further 2 h. MeOH (50 cm^3) was added to the mixture, which was then stirred overnight at room temp. After concentration under reduced pressure and coevaporation with MeOH (3 × 5 cm^3), the residue was purified using silica gel column chromatography (3–6% MeOH in CH_2Cl_2) to give title compound **17** (977 mg, 73%), which was used in the next step without further purification. An analytical sample was obtained by recrystallization from MeOH; $\delta_C(CD_3OD)$ 12.68 (CH_3), 39.65 (CH_2'), 62.22 (C-5′), 78.62 (C-2′), 79.91 (C-3′), 88.78 (C-4′), 89.05 (C-1′), 112.14 (C-5), 118.91 (=CH_2'), 135.11 (=CH′), 139.63 (C-6), 153.68 (C-2) and 166.90 (C-4); $\delta_H(CD_3OD)$ 1.88 (s, 3 H, CH_3), 2.49–2.56 (dd, *J* 8.3 and 14.8, 1 H, $CH_2'^a$), 2.56–2.63 (dd, *J* 6.2 and 14.8, 1 H, $CH_2'^b$), 3.72–3.77 (dd, *J* 2.6 and 12.1, 1 H, H^a-5′), 3.80–3.84 (dd, *J* 2.3 and 12.2, 1 H, H^a-5′), 3.94–3.96 (t, *J* 2.6, 1 H, H-4′), 4.16 (d, *J* 7.9, 1 H, H-2′), 5.12–5.21 (m, 2 H, =CH_2'), 5.99–6.05 (m, 1 H, =CH′), 6.00 (d, *J* 7.9, 1 H, H-1′) and 8.05 (s, 1 H, H-6); FAB-MS *m*/*z* 299 $[M + H]^+$ (Found: C, 52.39; H, 5.84; N, 9.26. Calc. for $C_{13}H_{18}N_2O_6$: C, 52.34; H, 6.08; N, 9.39%).

1-[3-*C*-Allyl-5-*O*-(4,4′-dimethoxytrityl)-β-D-ribofuranosyl]-thymine 18

Nucleoside **17** (977 mg, 3.28 mmol) was coevaporated with anhydrous pyridine (3 × 10 cm^3) and re-dissolved in anhydrous pyridine (8 cm^3). DMTCl (1.33 g, 3.93 mmol) was added and the mixture was stirred for 16 h under argon at room temp. The solution was evaporated under reduced pressure, the residue was re-dissolved in CH_2Cl_2 (40 cm^3), and the solution was washed with saturated aq. NaCl (3 × 30 cm^3). The water phase was extracted with CH_2Cl_2 (2 × 10 cm^3). The combined organic phases were dried (Na_2SO_4), and concentrated under reduced pressure. Purification using silica gel column chromatography (20–60% EtOAc in light petroleum, 0.5% pyridine, v/v/v) gave title compound **18** (1.66 g, 82%); $\delta_C(CDCl_3)$, 11.19 (CH_3), 38.25 (CH_2'), 55.18 (OCH_3), 62.04 (C-5′), 77.83 and 78.31 (C-2′ and -3′), 86.00, 87.39 and 87.58 (C-1′, -4′ and *C*Ph_3), 111.28 (C-5), 113.26, 113.30, 127.38, 128.07, 128.61, 132.50, 134.80, 134.90, 136.70, 143.80 and 158.96 (=CH′, C-6 and C_{arom}), 118.75 (=CH_2'), 152.01 (C-2) and 164.37 (C-4); $\delta_H(CDCl_3)$ 1.13 (s, 3 H, CH_3), 2.21–2.29 (dd, *J* 8.3 and 14.5, 1 H, $CH_2'^a$), 2.46–2.53 (dd, *J* 5.6 and 14.5, 1 H, $CH_2'^b$), 3.24–3.29 (dd, *J* 2.2 and 10.7, 1 H, H^a-5′), 3.64–3.68 (dd, *J* 2.9 and 10.9, 1 H, H^b-5′), 3.76 (s, 6 H, 2 × OCH_3), 4.17 (m, 1 H, H-4′), 4.28 (d, 1 H, *J* 7.2, H-2′), 4.49 (d, 1 H, *J* 17.1, =$CH_2'^a$), 4.90 (d, 1 H, *J* 10.3, =$CH_2'^b$), 5.75–5.83 (m, 1 H, =CH′), 6.15 (d, 1 H, *J* 7.2, H-1′), 6.82–6.86 (m, 4 H, ArH), 7.23–7.38 (m, 9 H, ArH) and 7.79 (s, 1 H, H-6); FAB-MS *m*/*z* 600 $[M]^+$ (Found: C, 67.88; H, 5.86; N, 5.13. Calc. for $C_{34}H_{36}N_2O_8 \cdot 0.2C_5H_5N$: C, 68.19; H, 6.05; N, 5.00%).

1-[3-*C*-Allyl-2-*O*-(*tert*-butyldimethylsilyl)-5-*O*-(4,4′-dimethoxytrityl)-β-D-ribofuranosyl]thymine 19

Nucleoside **18** (1.61 g, 2.68 mmol) was coevaporated with

anhydrous CH_3CN (3 × 10 cm^3) and re-dissolved in anhydrous DMF (10 cm^3). Imidazole (1.46 g, 21.4 mmol) was added followed by the addition of TBDMSCl (1.61 g, 10.7 mmol). The mixture was stirred at room temp. under argon for 72 h. MeOH (3 cm^3) was added and the solution was concentrated under reduced pressure. The residue was re-dissolved in CH_2Cl_2 (30 cm^3) and this solution was washed with water (3 × 20 cm^3). The water phase was extracted with CH_2Cl_2 (20 cm^3) and the combined organic phases were dried (Na_2SO_4), and concentrated under reduced pressure. Purification using silica gel column chromatography (20–30% EtOAc in light petroleum, 0.5% pyridine, v/v/v) gave title compound **19** (1.54 g, 82%); $\delta_C(CDCl_3)$ −4.62 and −4.56 (2 × $SiCH_3$), 10.43 (CH_3), 17.70 [$C(CH_3)_3$], 25.52 [$C(CH_3)_3$], 38.40 (CH_2'), 55.19 and 55.28 (2 × OCH_3), 61.68 (C-5′), 78.00 and 78.46 (C-2′ and -3′), 84.57, 85.93 and 87.66 (C-1′, -4′ and CPh_3), 111.83 (C-5), 113.26, 113.30, 127.68, 128.04, 128.93, 130.66, 132.34, 134.41, 134.44, 136.44, 143.29 and 159.15 (=CH′, C-6 and C_{arom}), 118.37 (=CH_2), 150.94 (C-2) and 163.77 (C-4); $\delta_H(CDCl_3)$ 0.00 and 0.19 (2 s, 6 H, 2 × $SiCH_3$), 0.92 [s, 9 H, $C(CH_3)_3$], 0.95 (s, 3 H, CH_3), 2.32 (d, *J* 6.8, 2 H, CH_2'), 3.42 (d, *J* 10.6, 1 H, H^a-5′), 3.72–3.76 (dd, *J* 3.1 and 10.1, 1 H, H^b-5′), 3.79 (s, 6 H, 2 × OCH_3), 4.03–4.10 (m, 2 H, =$CH_2'^a$, H-4′), 4.38 (d, *J* 7.5, 1 H, H-2′), 4.78 (d, *J* 10.7, 1 H, =$CH_2'^b$), 5.71–5.77 (m, 1 H, =CH′), 6.22 (d, *J* 7.4, 1 H, H-1′), 6.84 (m, 4 H, ArH), 7.20–7.34 (m, 9 H, ArH), 7.83 (s, 1 H, H-6) and 8.76 (s, 1 H, NH); FAB-MS *m/z* 714 $[M]^+$(Found: C, 66.78; H, 6.90; N, 3.99. Calc. for $C_{40}H_{50}N_2O_8Si·0.25\ H_2O$: C, 66.78; H, 7.08; N, 3.89%).

1-{3-*C*-Allyl-2-*O*-(*tert*-butyldimethylsilyl)-3-*O*-[2-cyanoethoxy-(diisopropylamino)phosphino]-5-*O*-(4,4′-dimethoxytrityl)-β-D-ribofuranosyl]thymine 20

Method C. Used amounts. Nucleoside **19** (252 mg, 0.35 mmol), anhydrous CH_2Cl_2 (3 cm^3), DIPEA (2.63 mmol, 0.45 cm^3), 2-cyanoethyl *N*,*N*-diisopropylphosphoramidochloridite (1.4 mmol, 0.33 cm^3) as above. After 24 h, the reaction was quenched by addition of CH_3OH (1 cm^3). Purification using silica gel column chromatography (0.5–1% Et_3N in CH_2Cl_2, v/v) followed by precipitation in light petroleum (250 cm^3) at −40 °C after re-dissolution in anhydrous toluene (2 cm^3) afforded title compound **20** (249 mg, 78%); $\delta_P(CDCl_3)$ 139.50 and 140.75.

1-[3-*C*-Allyl-5-*O*-(*tert*-butyldimethylsilyl)-2-deoxy-3-*O*-trimethylsilyl-α,β-D-*erythro*-pentofuranosyl]thymine 21

To a stirred suspension of the methyl glycoside **5** (720 mg, 2.38 mmol) and thymine (604 mg, 4.79 mmol) in anhydrous CH_3CN (25 cm^3) under argon at room temp. was added dropwise BSA (4.7 cm^3, 19.01 mmol). The mixture was stirred for 1 h until clearness. The reaction mixture was then cooled to −30 °C, and TMS triflate (0.86 cm^3, 4.75 mmol) was dropwise added. The reaction mixture was stirred for 7 days at room temp. before being diluted with CH_2Cl_2 (50 cm^3) and the reaction quenched with saturated aq. $NaHCO_3$ (2 × 50 cm^3). After being washed with water (2 × 50 cm^3) the organic phase was dried (Na_2SO_4), and concentrated under reduced pressure. Purification using silica gel column chromatography (0–1% MeOH in CH_2Cl_2, v/v) afforded a (1:2) anomeric mixture of nucleosides **21** (926 mg, 83%); $\delta_C(CDCl_3)$ −5.92, −5.81, −5.76 and −5.61 [2 × $Si(CH_3)_2$], 1.92, 2.03 and 2.17 [2 × $Si(CH_3)_3$], 12.39 and 12.45‡ (2 × CH_3), 17.92 and 18.01‡ [2 × $C(CH_3)_3$], 25.65 and 25.78 [2 × $C(CH_3)_3$], 39.41‡ and 39.84 (2 × C-2′), 44.74‡ and 46.16 (2 × 62.56‡ and 62.93 (2 × C-5′) 83.85 and 83.92‡ (2 × C-3′), 85.44‡ and 85.92 (2 × C-1′), 88.62‡ and 89.99 (2 × C-4′), 109.66 and 109.99‡ (2 × C-5), 117.83‡ and 118.18 (2 × =CH_2''), 133.63 (2 × =CH″), 135.76‡ and 137.12 (2 × C-6), 150.47‡ and 150.65 (2 × C-2) and 164.28‡ and 164.38 (2 × C-4); $\delta_H(CDCl_3)$ 0.01–0.20 [2 × $Si(CH_3)_2$ and 2 × $Si(CH_3)_3$], 0.92 [s, 2 × $C(CH_3)_3$], 1.93 (s, 2 × CH_3), 1.98–2.11 (m, 2 × H^a-2′), 2.40–2.65 (m, 2 × H^b-2′, 2 × CH_2'), 3.73–3.98 (m, 2 × H_2-5′), 4.04‡ (m, H-4′), 4.22 (m, H-4′), 4.94–5.17 (m, 2 × =CH_2'), 5.74–5.99 (m, 2 × =CH′), 6.15‡ (dd, *J* 4.9 and 9.2, H-1′), 6.27 (dd, *J* 2.5 and 8.1 H-1′), 7.61‡ and 7.64 (2 s, 2 × H-6) and 9.22 and 9.34‡ (2 s, 2 × NH); FAB-MS *m/z* 469 $[M + H]^+$ (Found: C, 56.47; H, 8.20; N, 5.78. Calc. for $C_{22}H_{40}N_2O_5Si_2$: C, 56.37; H, 8.60; N, 5.98%).

‡ Minor anomer.

1-[5-*O*-(*tert*-Butyldimethylsilyl)-2-deoxy-3-*C*-(3-hydroxypropyl)-3-*O*-trimethylsilyl-α,β-D-*erythro*-pentofuranosyl]thymine 22

To a stirred solution of allyl compound **21** (918 mg, 1.96 mmol) in anhydrous THF (4 cm^3) under argon at room temp. was added BH_3·1,4-oxathiane (0.27 cm^3 of a 7.8 M solution in 1,4-oxathiane, 2.11 mmol). The mixture was cooled to 0 °C and 2 M aq. NaOH (1.1 cm^3) was slowly added followed by the dropwise addition of 30% aq. H_2O_2 (0.28 cm^3); stirring was continued for 60 min at room temp. The reaction mixture was poured into ice–water (50 cm^3) and extracted with Et_2O. The organic phase was washed successively with saturated aq. $NaHCO_3$ (2 × 50 cm^3) and water (50 cm^3), dried (Na_2SO_4), and evaporated under reduced pressure. The crude product was purified by silica gel column chromatography (0–40% EtOAc in light petroleum, v/v) to give title compound **22** as a solid (602 mg, 63%); $\delta_C(CDCl_3)$ −5.75, −5.61 and −5.44 [2 × $Si(CH_3)_2$], 2.00 and 2.27 [2 × $Si(CH_3)_3$], 12.50 and 12.56‡ (2 × CH_3), 18.07 and 18.17‡ [2 × $C(CH_3)_3$], 25.78 and 25.90‡ [2 × $C(CH_3)_3$], 27.71‡ and 27.82 (2 × C-2″), 31.49‡ and 31.81 (2 × C-1″), 44.67‡ and 46.01 (2 × C-2′), 62.79, 62.94 and 63.19 (2 × C-5′ and 2 × C-3″), 84.38 and 84.48‡ (2 × C-3′), 85.79 (2 × C-1′), 88.61‡ and 89.93 (2 × C-4′), 109.69 and 109.97‡ (2 × C-5), 135.88‡ and 137.30 (2 × C-6), 150.44‡ and 150.65 (2 × C-2) and 164.15‡ and 164.26 (2 × C-4); $\delta_H(CDCl_3)$ 0.01–0.24 [2 × $Si(CH_3)_2$ and 2 × $Si(CH_3)_3$], 0.91 [s, 2 × $C(CH_3)_3$], 1.59–2.04 (m, 2 × CH_3, H^a-2′‡, H_2-1″), 2.10 (dd, *J* 1.7 and 14.1, H^a-2′), 2.48‡ (m, H^b-2′), 2.53 (dd, *J* 8.3 and 14.1, H^b-2′), 3.67–3.90 (m, 2 × H_2-5′ and H_2-3″), 4.07‡ (m, H-4′), 4.23 (m, H-4′), 6.18‡ (dd, *J* 4.9 and 9.2, H-1′), 6.27 (dd, *J* 2.5 and 8.2, H-1′), 7.63‡ and 7.69 (2 s, 2 × H-6) and 9.00 (br s, 2 × NH); FAB-MS *m/z* 487 $[M + H]^+$ (Found: C, 54.34; H, 8.44; N, 5.59. Calc. for $C_{22}H_{42}N_2O_6Si_2$: C, 54.29; H, 8.70; N, 5.76%).

1-[5-*O*-(*tert*-Butyldimethylsilyl)-2-deoxy-3-*C*-(3-phthalimidopropyl)-3-*O*-trimethylsilyl-α,β-D-*erythro*-pentofuranosyl]thymine 23

Compound **22** (602 mg, 1.24 mmol), phthalimide (237 mg, 1.61 mmol) and triphenylphosphine (444 mg, 1.69 mmol) were dissolved in anhydrous THF (1.3 cm^3) under argon. The mixture was cooled to 0 °C and a solution of diethyl azodicarboxylate (DEAD) in anhydrous THF (0.62 cm^3 THF; 1.59 mmol) was added dropwise. After 24 h at room temperature, the solvent was evaporated off under reduced pressure. The crude product was dissolved in CH_2Cl_2 (50 cm^3) and washed successively with saturated aq. $NaHCO_3$ (2 × 40 cm^3) and water (2 × 40 cm^3). The organic phase was dried (Na_2SO_4), and concentrated under reduced pressure. Silica gel column chromatography (CH_2Cl_2) afforded title compound **23** as a solid (656 mg, 86%); $\delta_C(CDCl_3)$ −6.07 and −5.86 [2 × $Si(CH_3)_2$], 1.81 and 2.05 [2 × $Si(CH_3)_3$], 12.42 and 14.28‡ (2 × CH_3), 17.83 and 17.95‡ [2 × $C(CH_3)_3$], 25.57 and 25.72‡ [2 × $C(CH_3)_3$], 23.63‡ and 23.90 (2 × C-2″), 32.40‡ and 32.81 (2 × C-1″), 37.88 and 37.97‡ (2 × C-3″), 45.94 (2 × C-2′), 61.98 and 63.05‡ (2 × C-5′), 84.18‡ and 84.20 (2 × C-3′), 85.61‡ and 85.77 (2 × C-1′), 88.45‡ and 89.76 (2 × C-4′), 109.71 and 110.00‡ (2 × C-5), 123.30‡, 123.33, 132.10, 132.15‡, 134.06‡ and 134.09 (2 × C_{arom}), 135.82‡ and 137.30 (2 × C-6), 150.28‡ and 150.50 (2 × C-2), 164.05 (2 × C-4) and 168.40‡ and 168.43 (2 × CON); $\delta_H(CDCl_3)$ 0.01–0.18 [2 × $Si(CH_3)_2$ and 2 × $Si(CH_3)_3$], 0.81

[s, 2 × $C(CH_3)_3$], 1.71–1.96 (m, 2 × CH_3, H^a-2′‡, H_2-1″ and H-2″), 2.05 (dd, *J* 14.3, H^a-2′), 2.40‡ (dd, *J* 4.8 and 12.5, H^b-2′), 2.45 (dd, *J* 8.2 and 14.2, H^b-2′), 3.60–3.71 (m, H^a-5′‡, H-5′a and H_2-3″), 3.81‡ (m, H^b-5′), 4.01‡ (m, H-4′), 4.16–4.23 (m, H-4′ and H^b-5′), 6.15‡ (dd, *J* 4.8 and 9.2, H-1′), 6.24 (dd, *J* 2.0 and 8.2, H-1′), 7.58‡ and 7.65 (2 s, H_2-6), 7.66–7.81 (2 × ArH) and 8.58 and 8.69‡ (2 s, 2 × NH); FAB-MS *m*/*z* 616 $[M + H]^+$.

1-[2-Deoxy-3-*C*-(3-phthalimidopropyl)-α,β-D-*erythro*-pentofuranosyl]thymine 24

To a solution of nucleoside **23** (668 mg, 1.09 mmol) in anhydrous THF (27 cm^3) was added 1.1 M TBAF in THF (2.2 cm^3, 2.42 mmol). The reaction mixture was stirred under argon for 20 min and then was concentrated to dryness. Purification using silica gel column chromatography (20% EtOAc in light petroleum, v/v) afforded title compound **24** as solid (154 mg, 33%); $\delta_C(CDCl_3)$ 12.12 and 12.22‡ (2 × CH_3), 23.37‡ and 23.57 (2 × C-2″), 32.21‡ and 32.80 (2 × C-1″). 38.09‡ and 38.24 (2 × C-3″), 42.60‡ and 44.56 (2 × C-2′), 58.17 and 62.02‡ (2 × C-5′), 79.60, 80.78‡, 85.78‡, 87.32, 88.93‡ and 91.51 (C-1′, -3′ and -4′), 108.43 and 110.74‡ (2 × C-5), 123.29, 131.96 and 134.06 (2 × C_{arom}), 137.29‡ and 137.93 (2 × C-6), 151.06‡ and 151.32 (2 × C-2), 164.34‡ and 164.72 (2 × C-4) and 168.72‡ and 168.83 (CON); $\delta_H(CDCl_3)$ 1.75–2.62 (m, 2 × CH_3, H_2-2′, H_2-1″ and H_2-2″), 3.67–4.55 (m, 2 × H-4′, H_2-5′ and H_2-3″), 6.17 (d, *J* 5.0, H-1′), 6.27‡ (dd, *J* 5.2 and 9.2, H-1′), 7.66–7.81 (m, 2 × H-6 and ArH) and 10.90 (br s, 2 × NH); FAB-MS *m*/*z* 430 $[M + H]^+$.

1-[2-Deoxy-5-*O*-(4,4′-dimethoxytrityl)-3-*C*-(3-phthalimidopropyl)-α-D-*erythro*-pentofuranosyl]thymine 25

An anomeric mixture of nucleoside **24** (368 mg, 0.86 mmol), $AgNO_3$ (174 mg, 1.02 mmol) and DMTCl (1.45 g, 4.28 mmol) were dissolved in anhydrous THF (35 cm^3), and pyridine (0.35 cm^3, 4.33 mmol) was added. The mixture was stirred at room temp. for 72 h with exclusion of light. The mixture was filtered into 5% aq. $NaHCO_3$ (50 cm^3). The product was extracted into CH_2Cl_2 (4 × 100 cm^3), and the organic phase was dried (Na_2SO_4), and concentrated under reduced pressure. Purification by PLC (2% pyridine, 5% CH_3OH in CH_2Cl_2, v/v/v, two runs) gave the α-anomer **25** (267 mg, 43%) as the less polar isomer and the corresponding β-anomer (94 mg, 15%).

β-Anomer **25**: $\delta_C(CDCl_3)$ 11.18 (CH_3), 23.36 (C-2″), 31.81 (C-1″), 37.92 (C-3″), 43.99 (C-2′), 55.12 (OCH_3), 62.69 (C-5′), 80.98 (C-3′), 84.15 (C-1′), 87.32 (C-4′), 87.84 (*C*Ph_3), 111.33 (C-5), 113.24, 127.35, 128.01, 130.28, 130.34, 134.78, 134.99, 143.75 and 158.88 (DMT), 123.31, 131.99 and 134.06 (Phth), 136.58 (C-6), 150.76 (C-2), 163.96 (C-4) and 168.62 (CON); $\delta_H(CDCl_3)$ 1.22 (s, 3 H, CH_3), 1.45–1.76 (m, 4 H, H_2-1″ and -2″), 2.09 (dd, *J* 9.5 and 12.4, 2 H, H^a-2′), 2.40 (dd, *J* 4.8 and 12.4, 1 H, H^b-2′), 3.15 (dd, *J* 2.2 and 10.8, 1 H, H^a-5′), 3.53 (m, 2 H, H_2-3″), 3.62 (dd, *J* 3.3, 10.8, 1 H, H^b-5′), 3.79 (s, 6 H, 2 × OCH_3), 4.06 (m, 1 H, H-4′), 6.49 (dd, *J* 4.8 and 9.5, 1 H, H-1′), 6.84 (m, 4 H, DMT), 7.23–7.38 (m, 9 H, DMT), 7.65–7.85 (m, 5 H, Phth and H-6) and 9.18 (s, 1 H, NH).

α-Anomer **25**: $\delta_C(CDCl_3)$ 12.26 (CH_3), 23.56 (C-2″), 32.05 (C-1″), 37.82 (C-3″), 44.94 (C-2′), 54.88 (OCH_3), 63.28 (C-5′), 80.08 (C-3′), 86.74 (C-4′), 87.36 (*C*Ph_3), 89.86 (C-1′), 108.64 (C-5), 113.19, 126.79, 127.91, 129.81, 129.88, 135.46, 135.79, 144.46 and 158.47 (DMT), 123.75, 131.85 and 133.87 (Phth), 137.92 (C-6), 150.66 (C-2), 164.71 (C-4) and 168.37 (CON); $\delta_H(CDCl_3)$ 1.24–1.79 (m, 4 H, H_2-1″ and -2″), 1.84 (s, 3 H, CH_3), 2.32 (d, *J* 14.0, 1 H, H^α-2′), 2.72 (dd, *J* 7.6 and 14.4, 1 H, H^β-2′), 2.99 (dd, *J* 2.0 and 10.7, 1 H, H^a-5′), 3.41 (dd, *J* 3.6 and 10.7, 1 H, H^b-5′), 3.59 (m, 2 H, H_2-3″), 3.78 (s, 6 H, 2 × OCH_3), 4.35 (m, 1 H, H-4′), 6.36 (d, *J* 7.0, 1 H, H-1′), 6.84 (m, 4 H, DMT), 7.17–7.43 (m, 9 H, DMT), 7.64–7.77 (m, 5 H, Phth, H-6) and 10.03 (s, 1 H, NH); FAB-MS *m*/*z* 731 $[M]^+$.

1-{3-*O*-[2-Cyanoethoxy(diisopropylamino)phosphino]-2-deoxy-5-*O*-(4,4′-dimethoxytrityl)-3-*C*-phthalimidopropyl-α-D-*erythro*-pentofuranosyl}thymine 26

Nucleoside **25** (267 mg, 0.37 mmol) was coevaporated with anhydrous CH_3CN (3 × 2 cm^3) and dissolved under argon in anhydrous CH_2Cl_2 (2 cm^3). DIPEA (0.4 cm^3, 2.34 mmol) was added followed by dropwise addition of 2-cyanoethyl *N*,*N*-diisopropylphosphoramidochloridite (0.17 cm^3, 0.72 mmol). After stirring of the mixture for 5 h, CH_3OH (0.5 cm^3) was added and the reaction mixture was diluted with EtOAc (20 cm^3) containing triethylamine (0.2 cm^3), washed successively with saturated aq. $NaHCO_3$ (3 × 30 cm^3) and saturated aq. NaCl (2 × 30 cm^3), dried (Na_2SO_4), and evaporated under reduced pressure. Purification using silica gel column chromatography (EtOAc–CH_2Cl_2–Et_3N–petroleum ether, 15:30:5:50, v/v/v/v) followed by precipitation in light petroleum (30 cm^3) at −20 °C [after re-dissolution in anhydrous toluene (1 cm^3)] afforded compound **26** as a solid (141 mg, 41%); $\delta_P(CDCl_3)$ 140.78 and 140.90.

Synthesis of oligonucleotides

The synthesis of ODNs was carried out on a 0.2 μmol scale (5 μmol amidite per cycle, Pharmacia Primer Support™) using compounds **16a**, **16b**, **26**, αT-cyanoethylphosphoramidite and commercial βT-cyanoethylphosphoramidite. The synthesis followed the regular protocol for the DNA synthesizer. For the modified phosphoramidites the coupling time was increased from 2 to 24 min and the cycle was repeated twice. The ODNs were removed from the support and deblocked by treatment with conc. ammonia at room temp. for 72 h. Purification and detritylation was achieved on Cruachem oligonucleotide purification cartridges using the standard procedure. The purity of the ODNs was confirmed by analytical anion-exchange HPLC. The solvent systems consisting of 10 mM NaOH (A) and 10 mM NaOH + 1.8 M NaCl (B) were used in the following order: 10 min linear gradient of 25–30% B in A, 40 min linear gradient of 30–45% B in A, 1 min 45–100% B in A, 1 min 100% B, 1 min linear gradient of 100–25% B in A. Flow rate 1.67 cm^3 min^{-1}. The purified ODNs eluted as one peak after approximately 14 min for products **H**–**L** and after approximately 20 min for products **A**–**G**.

Melting experiments

The melting experiments were carried out in medium salt buffer, 1 mM ethylenediaminetetra-acetic acid (EDTA), 10 mM Na_2HPO_4, 140 mM NaCl, pH 7.2 at a concentration of 2 nmol for each strand. The increase in absorbance at 260 nm as a function of time was recorded while the temperature was raised from 10 to 60 °C at a rate of 1 °C per min. The melting temperature was determined as the maximum of the first-derivative plot of the 260 nm transition.

Acknowledgements

The Danish Natural Science Research Council is thanked for financial support.

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Paper 7/03173D
Received 8*th May* 1997
Accepted 25*th June* 1997