Synthesis of novel 3′-*C*-branched 2′-deoxynucleosides. Incorporation of 3′-*C*-(3-hydroxypropyl)thymidine into oligodeoxynucleotides

Henrik M. Pfundheller,[a] Pia N. Jørgensen,†,[a] Ulrik S. Sørensen,‡,[a] Sunil K. Sharma,[b] Marie Grimstrup,†,[a] Claudia Ströch,[a] Poul Nielsen,[a] Garimella Viswanadham,[a] Carl Erik Olsen[c] and Jesper Wengel*,[b]

[a] *Department of Chemistry, Odense University, DK-5230 Odense M, Denmark*
[b] *Department of Chemistry, University of Copenhagen, Universitetsparken 5, DK-2100 Copenhagen, Denmark*
[c] *Department of Chemistry, Royal Veterinary and Agricultural University, DK-1871 Frederiksberg C, Denmark*

The methyl glycoside derivatives 4, 6, 10 and 32 have been used as precursors for the synthesis of novel 3′-*C*-alkyl-modified α- and β-2′-deoxynucleosides. Using an alternative linear strategy, 3′-*C*-methyl- and 3′-*C*-azidomethyl-modified thymidines 16 and 17 have been synthesized. Hybridization experiments with oligodeoxynucleotides containing 3′-*C*-(3-hydroxypropyl)thymidine monomers are reported.

Introduction

In recent years, much effort has been put into the design and synthesis of new nuclease-resistant oligonucleotides (ONs) retaining the ability of natural ONs to hybridize with complementary sequences.[1,2] A number of ONs containing sugar-modified pentofuranosyl nucleosides have been synthesized.[3] 2′-*O*-Alkyl- and 2′-deoxy-2′-fluoro-ONs have received considerable attention and have shown good hybridization properties towards complementary RNA.[4,5] ONs containing 5′-*C*-modified nucleosides[6] and 4′-*C*-branched nucleosides have also been synthesized.[7] Most of the above-mentioned sugar-modified ONs exhibit promising hybridization properties towards complementary DNA and/or RNA and enhanced nucleolytic stability relative to the corresponding unmodified ONs.

Previously, we have shown that incorporation of 3′-*C*-(hydroxymethyl)thymidine into oligodeoxynucleotides (ODNs) does not significantly change the hybridization properties but does increase the stability towards 3′-exonucleolytic degradation.[8] These results and the many potentially interesting applications of 3′-*C*-alkyl functionalities as attachment sites for, *e.g.*, intercalators or lipophilic groups in antisense molecules,[9] and our continued interest in 3′-*C*-modified ODNs, have prompted us to synthesize the novel 3′-branched nucleoside analogues described herein. Besides their potential as monomeric building blocks for ODN synthesis, these 3′-*C*-modified nucleosides are also interesting as potential biologically active compounds.[10] Herein, we report our results from different synthetic strategies towards 3′-*C*-branched 2′-deoxynucleosides.

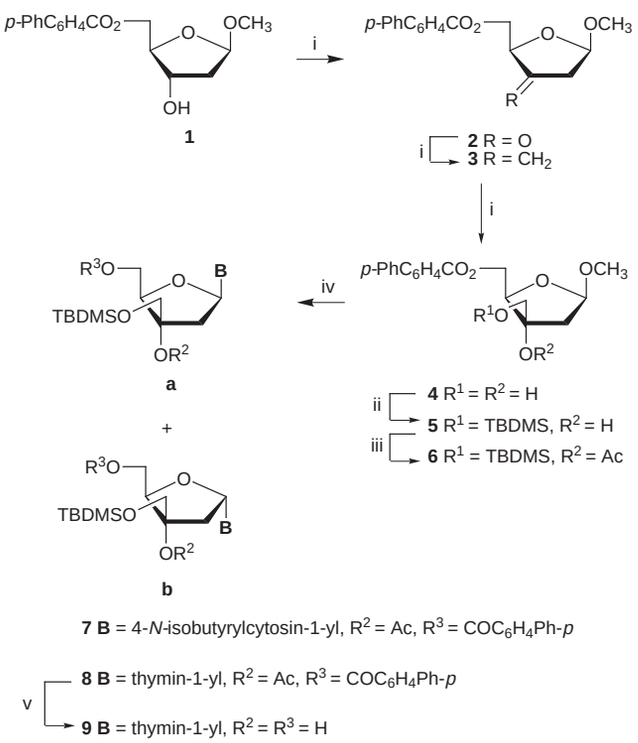
For synthesis of 3′-*C*-hydroxymethyl-modified 2′-deoxynucleosides containing other nucleobases than thymine,[8] we examined the possibility of using a convergent strategy. The goal was to synthesize a universal methyl 2-deoxy-3-*C*-hydroxymethyl-D-*erythro*-pentofuranoside precursor which would allow coupling of a variety of silylated nucleobases. Using a linear strategy, we have in addition investigated the synthesis of 3′-*C*-alkyl-modified nucleosides *via* a 3′-*C*-spiro epoxide. In continuation of our research on ODNs containing 3′-*C*-(hydroxymethyl)thymidines, we have also synthesized 3′-*C*-alkyl nucleosides containing more than one carbon atom in the branch. Thus, using a convergent strategy a 3′-*C*-(2-hydroxyethoxy)methyl-modified nucleoside was synthesized *via* a 3-*C*-spiro epoxide. For the synthesis of ODNs containing 3′-*C*-(3-hydroxypropyl)thymidine a convergent strategy involving deoxygenation at C-2′ was used *en route* to the 3′-*C*-alkyl monomeric building block.

Results and discussion

In our attempt to synthesize a universal 3-*C*-hydroxymethyl precursor for coupling with silylated nucleobases, stereoselective synthesis of the key intermediate, the methyl 2-deoxy-3-*C*-hydroxymethyl-β-D-*erythro*-pentofuranoside **4**, was performed in five steps from 2-deoxy-D-ribose essentially as described earlier.[11] However, instead of crystallization of methyl 2-deoxy-5-*O*-(4-phenylbenzoyl)-β-D-*erythro*-pentofuranoside **1** directly from the anomeric mixture, the two anomers were separated by column chromatography. Subsequent oxidation using pyridinium dichromate (PDC) in anhydrous CH_2Cl_2 in the presence of 3 Å molecular sieve powder[12] afforded 5-protected β-D-*glycero*-pentofuranosid-3-ulose **2** in 80% yield. This step was followed by Lombardo methylenation and stereoselective dihydroxylation as reported to give alkene **4** *via* ketone **3** (Scheme 1).[11]

Incorporation of 3′-*C*-hydroxymethyl nucleosides into ODNs requires protection of the 3′-*C*-hydroxymethyl group. Reaction of compound **4** with *tert*-butyldimethylsilyl chloride (TBDMSCl) in anhydrous DMF using imidazole as catalyst[13] gave the 3-*C*-(*tert*-butyldimethylsilyloxymethyl)pentofuranoside **5** in 99% yield (Scheme 1). Subsequent acetylation[14] of the sterically hindered tertiary hydroxy group using acetic anhydride in anhydrous CH_2Cl_2 in the presence of pyridine and 4-(*N*,*N*-dimethylamino)pyridine (DMAP) afforded methyl 3-*O*-acetyl-3-*C*-(*tert*-butyldimethylsilyloxymethyl)-2-deoxy-5-*O*-(4-phenylbenzoyl)-β-D-*erythro*-pentofuranoside **6** in 94% yield. Furanoside **6** was coupled with silylated 4-*N*-isobutyrylcytosine using trimethylsilyl trifluoromethanesulfonate (TMS triflate) as a Lewis acid following the methodology developed by Vorbrüggen *et al.*[15] Purification by column chromatography afforded the β-nucleoside **7a** as the more polar compound in 9% yield, the α-nucleoside **7b** in 10% yield, and a fraction contain-

† *Present address*: Acadia Pharmaceuticals A/S, Fabriksparken 58, DK-2600 Glostrup, Denmark.
‡ *Present address*: Department of Medicinal Chemistry, Royal Danish School of Pharmacy, Universitetsparken 2, DK-2100 Copenhagen, Denmark.

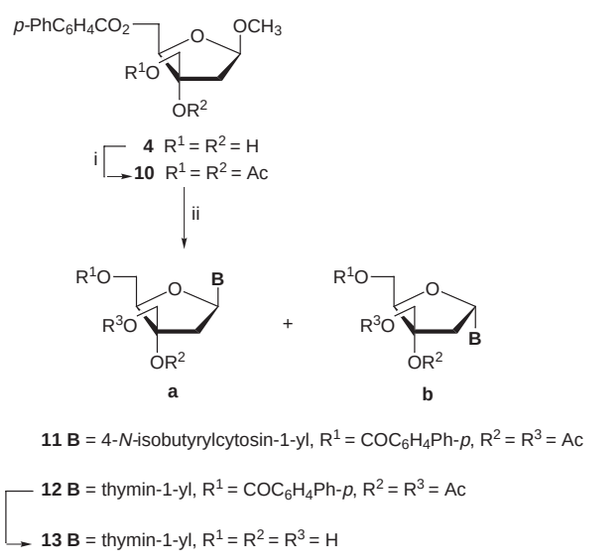


Scheme 1 *Reagents*: i, ref. 11; ii, TBDMSCl, imidazole, DMF; iii, Ac_2O, pyridine, DMAP, CH_2Cl_2; iv, A: 4-*N*-isobutyrylcytosine, HMDS, TMS triflate, CH_3CN; B: 4-*N*-isobutyrylcytosine or thymine, BSA, TMS triflate, CH_3CN; v, NH_3 in MeOH

ing an anomeric mixture (1 : 1; 9%). The structural assignment of the β- and α-nucleosides was done by nuclear Overhauser enhancement (NOE) experiments and one-dimensional ^{1}H NMR spectroscopy. As conclusive evidence, irradiation of H-4′ of the less polar compound gave a significant NOE effect in H-6 (and *vice versa*; not observed for the more polar compound) which confirms the less polar compound to be the α-anomer. The structural assignments of the anomers were further supported by the coupling constants of H-1′ and the relative chemical shifts of H^{a,b}-5′ and H-4′. The H-1′ signal of a β-anomer often appears as a pseudotriplet ($J_{1'\text{-}2'\alpha} \approx J_{1'\text{-}2'\beta}$), whereas the H-1′ signal of an α-anomer appears as a doublet of doublets ($J_{1'\text{-}2'\beta} > J_{1'\text{-}2'\alpha}$).[16] Generally, the H-4′ signal of the α-anomer is shifted downfield relative to the H-4′ signal of the β-anomer, causing the difference in the chemical shifts between the H^{a,b}-5′ and the H-4′ to be greater for the α-anomer than for the β-anomer.[16b,17] In the ^{1}H NMR spectrum of the more polar compound, the H^{a,b}-5′ and the H-4′ signals were coincident and the coupling constants for the H-1′ were found to be 5.8 and 8.0 Hz. The less polar compound showed clearly separated H^a-5′, H^b-5′ and H-4′ signals and a distinct doublet of doublets for the H-1′ signal ($J_{1'\text{-}2'\beta}$ 6.6 Hz > $J_{1'\text{-}2'\alpha}$ 2.2 Hz). Comparison of the ^{1}H NMR data and the NOE experiments confirmed the more polar compound to be the β-anomer and the less polar compound to be the α-anomer.

In an attempt to improve the yield of the nitrogen glycosylation between compound **6** and different nucleobases, other coupling methods and strategies were examined (Scheme 1). Direct nitrogen glycosylation[18,19] of compound **6** with 4-*N*-isobutyrylcytosine using *N*,*O*-bis(trimethylsilyl)acetamide (BSA) as silylating agent and TMS triflate in 1,2-dichloroethane was unsuccessful. However, exchange of 1,2-dichloroethane with the more polar and nucleophilic solvent CH_3CN afforded an anomeric mixture of nucleoside products **7a** and **7b** (2 : 1) in 64% yield after seven days at room temperature. When thymine was used as nucleobase, an inseparable anomeric mixture of the β- and α-nucleoside **8a** and **8b** was obtained in 59% yield. By comparison of the NMR data with those of the corresponding 4-*N*-isobutyrylcytosine nucleosides, the ratio between the β- and the α-anomer was determined to be 4 : 3. Deprotection of epimers **8a/8b** with methanolic ammonia afforded the anomeric mixture **9a/9b** in 56% yield. The silyl protecting group was retained as required for ODN synthesis.[8] Unfortunately, attempted separation of the thymine anomers by column chromatography, preparative TLC (PLC) as well as reversed-phase HPLC was unsuccessful in our hands. Analogously, according to analytical TLC and reversed-phased HPLC, the products obtained by treatment of the anomeric mixture of cytosine nucleosides **7a/7b** with methanolic ammonia were inseparable.

We likewise investigated whether an acetylated precursor (Scheme 2) was effective as glycosyl donor in coupling reactions. Quantitative yield of methyl 3-*C*-acetoxymethyl-3-*O*-acetyl-2-deoxy-5-*O*-(4-phenylbenzoyl)-β-D-*erythro*-pentofuranoside **10** was achieved by reaction of furanoside **4** with Ac_2O in anhydrous CH_2Cl_2 in the presence of pyridine and DMAP. Direct nitrogen glycosylation of compound **10** with 4-*N*-isobutyrylcytosine afforded an inseparable 1 : 2 anomeric mixture of the nucleosides **11a/11b** in 58% yield. Using thymine as the nucleobase, an inseparable (1 : 2) anomeric mixture of the nucleosides **12a/12b** in 69% yield was obtained. The anomeric configuration of the predominant anomers could not be assigned because of overlap of ^{1}H NMR signals. Deacetylation of compounds **12a/12b** gave, after column chromatographic purification, a 1 : 2 inseparable anomeric mixture **13a/13b** in 60% yield. Deacetylation of the anomeric mixture **11a/11b** analogously afforded an inseparable anomeric mixture according to analytical TLC and reversed-phase HPLC.



Scheme 2 *Reagents*: i, Ac_2O, pyridine, DMAP, CH_2Cl_2; ii, 4-*N*-isobutyrylcytosine or thymine, BSA, TMS triflate, CH_3CN; iii, NH_3 in MeOH

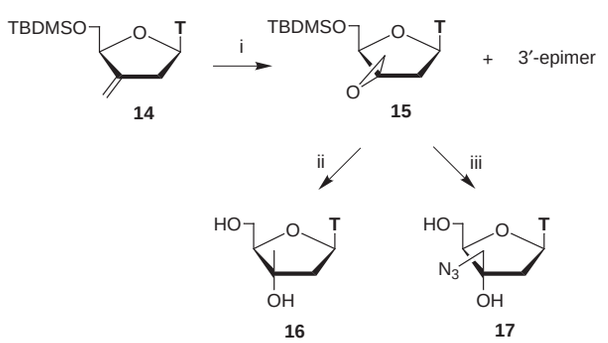
In order to reduce the number of synthetic steps, direct nitrogen glycosylation was carried out on methyl 2,3-dideoxy-3-*C*-methylene-5-*O*-(4-phenylbenzoyl)-β-D-*glycero*-pentofuranoside **3**[11] with 4-*N*-isobutyrylcytosine (data not shown). However, owing to a low yield and the possibility of dihydroxylation in the nucleobase[20] in the subsequent dihydroxylation step, this strategy was not further pursued.

Using the procedures described above, universal 3-*C*-hydroxymethyl precursors have been synthesized. Direct nitrogen glycosylation between silylated nucleobases and the precursors **4** and **6** were successful, affording anomeric mixtures of β- and α-nucleosides. Because of lack of stereoselectivity in the glycosylations and lack of success in separating the anomeric mixtures, this strategy has only limited utility for synthesis of stereochemically pure 3′-*C*-hydroxymethyl-modified ODNs. However, it provides a rapid method for obtaining anomeric

mixtures of 3′-*C*-hydroxymethyl-modified nucleosides for biological testing.

A linear synthetic route, starting from a nucleoside derivative, is an alternative strategy for synthesis of 3′-*C*-branched nucleosides. Although this approach limits straightforward access to nucleosides containing different nucleobases, time-consuming column chromatographic separation of anomers is avoided. In view of the potentially interesting biological properties, and our interest in incorporating 3′-*C*-alkyl-modified nucleosides with β-configuration into ODNs, we decided to synthesize the 3′-*C*-branched thymidine derivatives **16** and **17** (Scheme 3).

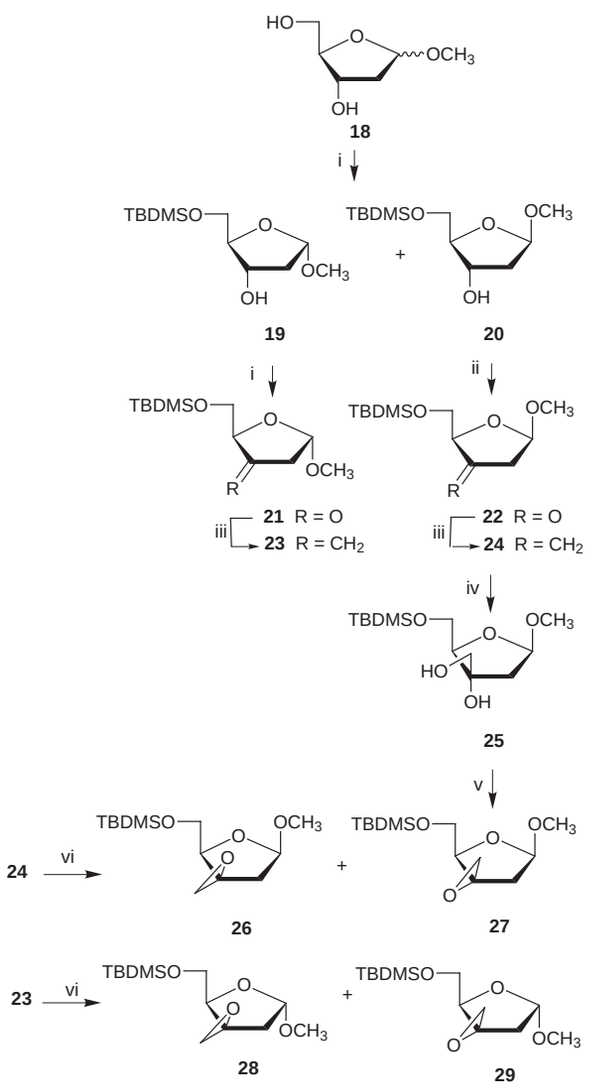
5′-*O*-(*tert*-Butyldimethylsilyl)-3′-deoxy-3′-*C*-methylene-thymidine **14** was synthesized from thymidine in three steps by our earlier published method.[21] Epoxidation of alkene **14** using 3-chloroperoxybenzoic acid (MCPBA) in CH_2Cl_2 gave the *erythro*-configurated epoxide **15** in 70% yield after column chromatographic separation from the corresponding 3′-epimer (data not shown). The configuration of compound **15** was assigned on the basis of NOE experiments; in particular the NOE effect between 3′-*C*-CH_2 and H-5′ confirmed the positioning of the 3′-*C* substituent at the β-face of the pentofuranose ring. For the synthesis of 3′-*C*-branched nucleosides with *erythro* configuration, epoxide **15** is an ideal key synthon. Reaction of compound **15** with lithium triethylborohydride in THF or with sodium azide in DMF followed by desilylation and column chromatographic purification gave the deprotected 3′-*C*-branched thymidine derivatives **16** and **17** in 83 and 74% yield, respectively. Compound **16** and some of its 5′-*O*-protected derivatives have been synthesized earlier by alternative or analogous strategies.[22]



Scheme 3 *Reagents*: i, MCPBA, CH_2Cl_2; ii, (1) lithium triethylborohydride in THF; (2) TBAF, THF; iii, (1) NaN_3, DMF, (2) TBAF, THF. **T** = thymin-1-yl.

Stimulated by the promising thermal stability of oligonucleotides containing 3′-*C*-(hydroxymethyl)thymidine when hybridized with complementary DNA and their enzymic stability,[8] we have investigated several strategies for obtaining modified nucleosides with a 3′-*C*-hydroxyalkyl branch containing more than one carbon atom. Furthermore, 3′-*C*-hydroxyalkyl substituents may prove useful for the attachment of, *e.g.*, intercalating agents or for the synthesis of branched ODNs.[23] In this context, it is noteworthy that synthesis of Y-shaped branched ONs employing the 3′-*O*-phosphoramidite of 5′-*O*-(4,4′-dimethoxytrityl)-3′-*O*-(4,4′-dimethoxytrityloxymethyl)thymidine failed in our hands.[24] We ascribe this to 3′-*O*-strand cleavage during detritylation due to attack from the liberated nucleophilic 3′-*C*-hydroxymethyl functionality. We envisage that, by extending the 3′-*C*-hydroxyalkyl group, this problem can be circumvented. We report the synthesis of 3′-*C*-(2-hydroxyethoxy)-methyl-modified nucleosides *via* a novel C-3 spiro carbohydrate epoxide which is generally useful for nucleophilic introduction of 3-*C*-alkyl substituents. In addition, we have synthesized 3′-*C*-(3-hydroxypropyl) nucleosides using another strategy involving addition of allylmagnesium bromide to a 3-ketopentofuranose derivative. ODNs containing 3′-*C*-(3-hydroxypropyl)-thymidine have been synthesized and evaluated for affinity towards complementary DNA and RNA.

As the first step in the synthesis of nucleosides with a 3′-*C*-(2-hydroxyethoxymethyl) modification (Scheme 4), selective silylation of an anomeric mixture of methyl pentofuranosides **18**[25] as previously described[26] afforded the pure anomers **19** and **20**. Oxidation of anomers **19** and **20** with a CrO_3–pyridine–Ac_2O complex[26,27] in CH_2Cl_2 afforded the 3-uloses **21**[26] and **22**, respectively, in high yields after purification by flash chromatography. Methylenation using the organometallic complex[28] of Zn–CH_2Br_2–$TiCl_4$ gave the 3-*C*-methylene compounds **23** and **24** in 40 and 51% yield, respectively. Wittig methylenation on ulose **22** afforded alkene **24** in only 3% yield, possibly due to β-elimination induced by this basic reagent.



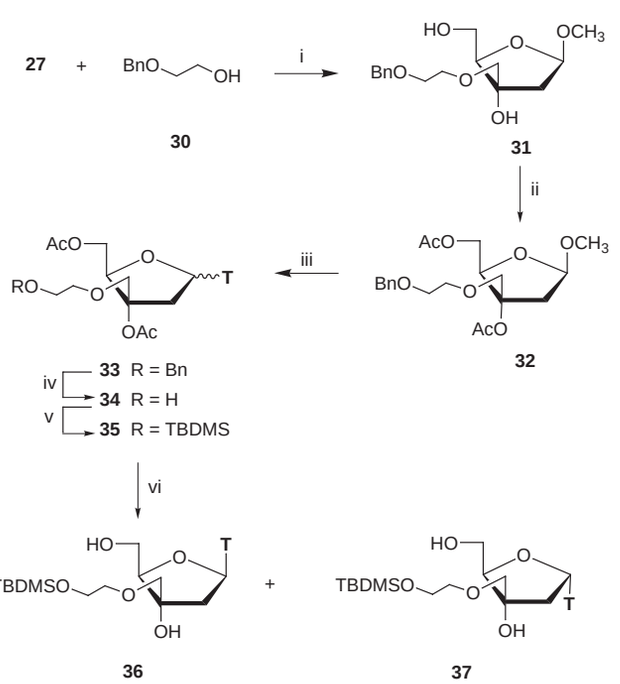
Scheme 4 *Reagents*: i, ref. 26; ii, CrO_3, pyridine, Ac_2O, CH_2Cl_2; iii, Zn, CH_2Br_2, $TiCl_4$, THF; iv, OsO_4, NMO, pyridine, water, Bu′OH; v, (1) TsCl, pyridine; (2) K_2CO_3, 18-crown-6, DMF; vi, $NaHCO_3$, MCPBA, CH_2Cl_2

Two different methods were used to obtain C-3 spiro epoxide **27**. Compound **24** was dihydroxylated by the method previously described[11] using osmium tetraoxide in basic aq. *tert*-butyl alcohol and *N*-methylmorpholine *N*-oxide[29] (NMO) as co-oxidant. This reaction was stereoselective, affording exclusively the *erythro* isomer **25** in 93% yield. The configuration at C-3 was determined using ^1H–^1H chemical-shift correlation spectroscopy (COSY) and NOE experiments. Irradiation of H^{β}-2 induced a significant NOE effect in CH_2OH, whereas no NOE effect was observed between these methylene protons and H^{α}-2. These configurational indications are in fine accord with previ-

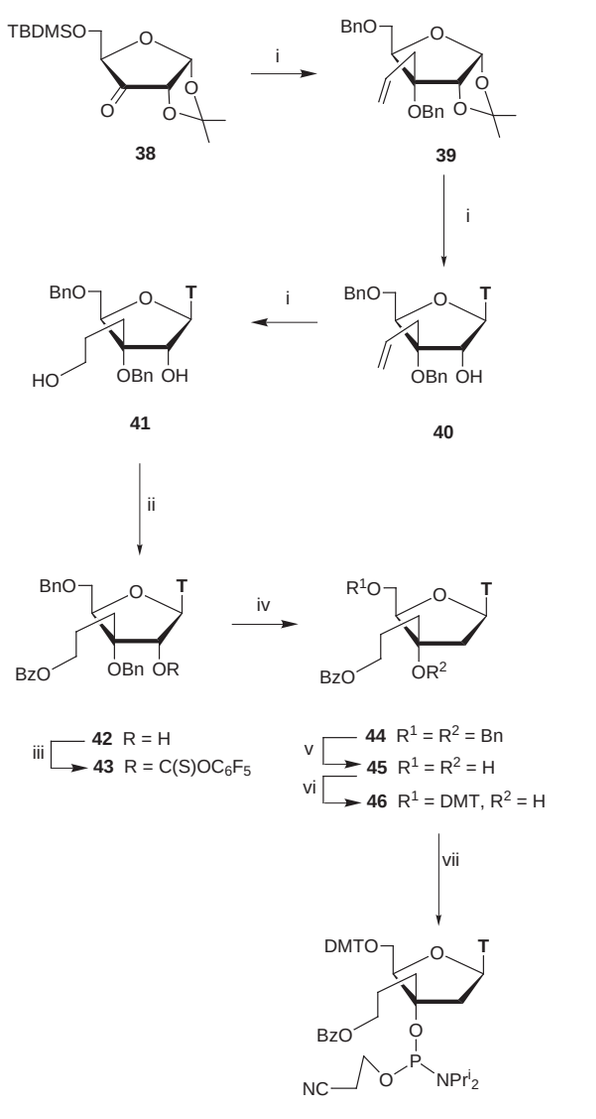
ous results on analogous β-pentofuranosides.[11] Tosylation of the primary hydroxy group in diol **25** with toluene-*p*-sulfonyl chloride followed by base-catalysed ring closure afforded the epoxide **27** in 39% yield. The alternative method leading to epoxides **26**–**29** involved direct epoxidation of the methylene group in alkene **23** or **24** using MCPBA. This gave for both anomers (**23**/**24**) a mixture of the two possible C-3 stereoisomers which, however, could easily be separated by silica gel column chromatography. By NOE experiments, the *threo* configuration of epoxides **26** and **28** was verified by the NOE effect between H-4 and the CH_2 protons of the epoxide ring. The *erythro* configuration of epoxides **27** and **29** was indicated by NOE effects between the methylene protons and H-5. In addition, these assignments were supported by the fact that compound **27** was also synthesized *via* diol **25**.

Compound **27** was ring opened by reaction with 2-(benzyloxy)ethanol[30] **30** and NaH in DMF (Scheme 5) to afford furanoside **31** in 57% yield resulting from nucleophilic attack on the less sterically hindered methylene carbon. The strongly basic conditions resulted in simultaneous desilylation. Acetylation of the primary and tertiary hydroxy functions using Ac_2O in pyridine and DMAP as catalyst gave compound **32** in 85% yield. Condensation of compound **32** with thymine in the presence of TMS triflate and BSA gave nucleoside **33** as an anomeric mixture (1:1.4 according to ^1H NMR analysis) in 75% yield. To obtain the free 2-hydroxyethoxymethyl substituent at C-3′, compound **33** was debenzylated using H_2 and 20% $Pd(OH)_2$–C as catalyst in absolute EtOH at 60 °C to afford nucleoside **34** in 89% yield. As our long-term aim is to synthesize novel ODN analogues the primary hydroxy group was protected by reaction with TBDMSCl as described earlier, giving the completely protected nucleosides **35** in 84% yield. The anomeric mixture **35** was subsequently deacetylated using saturated NH_3 in MeOH to afford the pure anomers **36** and **37** after preparative TLC in 24 and 65% yield, respectively. An NOE effect between H-1′ and H-4′ for compound **36** indicated a β-configuration whereas an NOE effect between H-4′ and H-6 indicated the α-configuration for anomer **37**. These anomeric assignments are also in accordance with the general rule that the α-configuration induces a downfield shift of H-4′ in the ^1H NMR spectrum due to shielding by the nucleobase.[16b,17b] Thus, H-4′ in α-nucleoside **37** has a chemical shift δ_H of 4.30 compared with δ_H 3.99 for H-4′ in epimer **36**. Novel α- and β-3′-*C*-branched nucleosides have been synthesized using this convergent strategy involving opening of novel C-3 spiro epoxides. This strategy should be generally useful for nucleophilic introduction of a wide variety of derivatized 3-*C*-alkoxymethyl substituents into 2-deoxypentofuranoses.

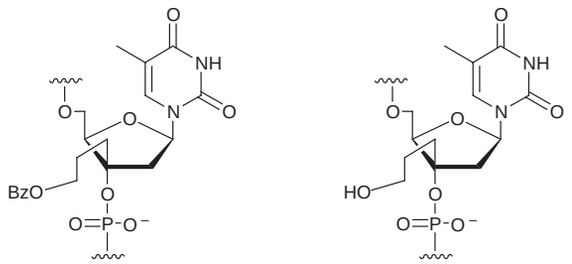
For the synthesis of ODNs containing 3′-*C*-(3-hydroxypropyl)thymidine the synthetic strategy depicted in Scheme 6 was used. Starting from 3-ketofuranoside **38**,[31] Grignard addition using allylmagnesium bromide followed by desilylation and dibenzylation afforded compound **39**, which was converted to the key intermediate **40** as described.[32] Nucleoside **4** was thereafter transformed into 3′,5′-di-*O*-benzyl-3′-*C*-(3-hydroxypropyl)thymidine **41**.[32] For synthesis of the monomeric phosphoramidite building block **47**, nucleoside **41** was treated with benzoyl chloride, using 2,6-lutidine (2,6-dimethylpyridine) as base, at −40 °C in CH_2Cl_2 to afford compound **42** in 62% yield after column chromatographic purification. Besides this main product, a fraction consisting of a mixture of compound **42** and a dibenzoylated derivative was obtained. A substantially higher yield of the dibenzoylated by-product was obtained when using pyridine as base and solvent at −20 °C. For the



Scheme 5 *Reagents*: i, NaH, DMF; ii, Ac_2O, pyridine, DMAP; iii, thymine, BSA, TMS triflate, 1,2-dichloroethane; iv, H_2, 20% $Pd(OH)_2$–C, EtOH; v, TBDMSCl, imidazole, DMF; vi, NH_3 in MeOH. **T** = thymin-1-yl.



Scheme 6 *Reagents*: i, ref. 32; ii, BzCl, 2,6-lutidine, CH_2Cl_2; iii, $C_6F_5OC(S)Cl$, DMAP, CH_2Cl_2–pyridine (1:1, v/v); iv, Bu_3SnH, AIBN, benzene; v, H_2, 20% $Pd(OH)_2$–C, EtOH; vi, DMTCl, pyridine; vii, 2-cyanoethyl *N*,*N*-diisopropylphosphoramidochloridite, DIPEA, CH_2Cl_2. **T** = thymin-1-yl.



X Y

Table 1 Sequences synthesized and hybridization data towards complementary DNA[a] and RNA[b]

	Sequences	T_m (°C)[a]	ΔT_m (°C)[a]	T_m (°C)[b]	ΔT_m (°C)[b]
I	5′-TTTTTTTTTTTTTT-3′	36.0		29.0	
II	5′-TTTTTTT**X**TTTTTT-3′	n.d.		n.d.	
III	5′-TTTTTTT**Y**TTTTTT-3′	n.d.		n.d.	
IV	5′-TTTTTT**YY**TTTTTT-3′	34.0	−1.0	23.0	−3.0
V	5′-TTTTTTTTTTTT**YT**-3′	33.0	−3.0	26.0	−3.0
VI	5′-TTTTTTTTTTT**YYT**-3′	31.0	−2.5	25.0	−2.0

T = thymidine monomer; T_m = melting temperature (1 mM EDTA, 10 mM Na_2HPO_4, 140 mM NaCl, pH 7.2); ΔT_m = change in T_m/modification. n.d. = not determined. See text for further explanation.

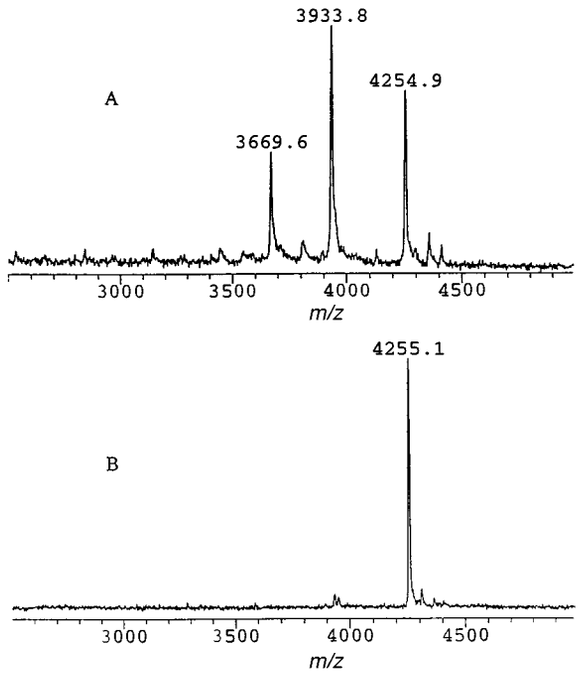


Fig. 1 MALDI-MS of ODN **V** in positive-ion mode (A) and in negative-ion mode (B). See text for explanation.

radical deoxygenation of secondary alcohols it is known that a phenoxythiocarbonyl group containing electron-withdrawing substituents increases the radicophilicity and thus the rate of the desired fragmentation.[33] Deoxygenations at C-2′ in 1-(3-*O*-benzyl-6-*O*-trityl-β-D-allopyranosyl)thymine[18] and at C-2′ in 3′,5′-di-*O*-benzyl-3′-*C*-methylthymidine[22] *via* the phenoxythiocarbonyl derivatives were accomplished only with difficulties. However, the desired deoxygenated products were prepared *via* the 2,4-dichlorophenoxythiocarbonyl derivative[18] or the pentafluorophenoxythiocarbonyl derivative.[22] Based on these results, pentafluorophenoxythiocarbonyl nucleoside derivative **43** was synthesized in 52% yield by using pentafluorophenylthiocarbonyl chloride in pyridine–CH_2Cl_2 (1:1) as solvent and catalytic amounts of DMAP. This reaction was followed by Barton deoxygenation using Bu_3SnH in refluxing benzene and 2,2′-azo(2-methylpropionitrile) (AIBN) as initiator to afford the reductively cleaved 2′-deoxy product **44** in 52% yield. The primary and tertiary benzyl protecting groups were subsequently removed using H_2 and 20% $Pd(OH)_2$–C in absolute EtOH at room temperature affording diol **45** in 65% yield. Initially, we used TBDMS as protecting group at the 3′-*C*-hydroxypropyl functionality instead of the benzoyl group. Unexpectedly, debenzylation conditions as described above caused contemporary removal of the silyl group. Removal of primary and secondary benzyl groups in the presence of TBDMS-protected primary and secondary alcohols under the same reaction conditions has been reported.[33] However, we had to use significantly larger amounts of the palladium catalyst and longer reaction times to remove the tertiary benzyl group, which could be an explanation for the silyl-group cleavage. The debenzylated nucleoside **45** was subsequently prepared for ODN synthesis by reaction first with 4,4′-dimethoxytrityl chloride (DMTCl) in anhydrous pyridine to afford the 5′-*O*-protected nucleoside **46** in 90% yield. The desired phosphoramidite **47** was finally synthesized in 71% yield, after column chromatographic purification followed by precipitation in light petroleum, by reaction of tertiary alcohol **46** with 2-cyanoethyl *N*,*N*-diisopropylphosphoramidochloridite [$NCCH_2CH_2OP(Cl)NPr^i_2$] in the presence of *N*,*N*-diisopropylethylamine (DIPEA).

ODNs **I**–**VI** (Table 1) were synthesized on an automated DNA synthesizer using phosphoramidite **47** and commercial nucleoside phosphoramidites. The coupling efficiency of compound **47** (3 × 12 min coupling, 2 × 24 min coupling or 3 × 24 min coupling) using tetrazole as activator was ≈20% compared with 99% (2 min coupling) for commercial deoxynucleoside phosphoramidites as evaluated by monitoring of the release of the dimethoxytrityl cation after each coupling step. The low coupling efficiency seems to be a general problem for tertiary monocyclic amidites, and steric problems could be contributing to this effect.[8,26] ODN **II** (Table 1) was removed from the solid support by treatment with 32% aq. ammonia at room temperature for three days, which also removed the phosphate protecting groups, and was purified on a disposable reversed-phase chromatography cartridge (COP™ columns, Cruachem) which includes detritylation. However, to our surprise, analysis of ODN **II** by matrix-assisted laser desorption/ionization mass spectrometry (MALDI MS)[34] revealed that the major part of the resulting ODN product still contained the benzoyl protecting group on the carbohydrate moiety [measured mass 4361.4 Da (M + H). Calc. 4360.9 Da (M + H) for ODN **II** containing one monomer **X** with one benzoyl group]. By treatment of ODNs **II** and **IV**–**VI** with 32% aq. ammonia at 55 °C overnight followed by purification on COP™ columns for ODNs **IV**–**VI**, and desalting on an NAP-10 column for ODN **II** giving ODN **III**, removal of the benzoyl protecting groups was accomplished efficiently as verified by MALDI MS as described below.

The composition of ODNs **III**–**VI** was analysed by MALDI MS using a linear time-of-flight mass analyser. When these experiments were performed in the positive-ion mode, peaks in addition to the signals for the expected ODNs were seen. For example, for ODN **V** the molecular mass of 4254.9 Da (M + H) (Calc. 4255.9 Da, M + H) was confirmed, but also present in the spectrum were peaks at *m/z* 3669.6 and 3933.8 Da (Fig. 1A). These peaks can be explained by fragmentation on either the 5′- or the 3′-side of the modified nucleoside **Y** (producing 5′-T_{12}-3′ with phosphate attached to the 3′ end or 5′-T_{12}**Y**-3′ without 3′-end phosphate) leading to fragments with calculated molecular masses of 3670.4 Da and 3934.7 Da (M + H), respectively. When the same sample was analysed in the negative-ion mode, only the peak corresponding to 5′-T_{12}-**Y**-3′ (measured 3932.3 Da, low intensity) was detected in addition to the expected signal from ODN **V** (Fig. 1B). It therefore appears that the analytes undergo noticeable fragmentation only in the positive-ion mode.

ODN **III** containing one modification gave a molecular mass of 4257.1 Da (M + H) (Calc. 4255.9 Da, M + H), and an add-

itional non-assigned peak (30% intensity) with a molecular mass of 4229.2 Da (M + H) (this peak was seen in both the negative- and positive-ion mode). ODNs **IV** and **VI** containing two modifications gave molecular masses of 4312.8 Da and 4312.0 Da (M − H), respectively (Calc. 4311.9 Da, M − H). ODNs **III**–**VI** were additionally analysed by capillary gel electrophoresis and showed high purity of ODNs **IV**–**VI** and confirmed the presence of an impurity in the ODN **III** sample.

The hybridization properties of the modified ODNs were measured as previously described.[8] The melting temperature (T_m) and the differences between modified and unmodified oligomers as the change in melting temperature per modification (ΔT_m) are listed in Table 1 for ODNs containing the 3′-*C*-(3-hydroxypropyl)thymidine monomer (**Y**). We have refrained from evaluating hybridization properties of ODNs **II** and **III** because of the presence of ON-impurities which could not be removed preparatively (low coupling efficiency of amidite **47** and some material consumed). Promising results were obtained when species **Y** was incorporated twice in the middle of a 14-mer (ODN **IV**). Thus, towards complementary DNA a minor decrease in ΔT_m of only −1 °C was observed which indicates a less destabilizing effect of incorporating 3′-*C*-branched monomers consecutively. However, ODN **IV** exhibited a significant decrease in ΔT_m (−3 °C) when hybridized towards complementary RNA. The presence of monomer **Y** once (ODN **V**) or twice (ODN **VI**) in the 3′ end of a 14-mer caused comparable decreases in ΔT_m (between −3 and −2 °C) towards complementary DNA and RNA. Similar hybridization properties towards complementary DNA and RNA were obtained for ODNs containing 3′-*C*-hydroxymethylthymidine.[8a,35] The preferential DNA recognition observed for ODN **IV** can be explained by conformational considerations. In a DNA : RNA A-type duplex, the sugar conformation of the monomer is of the *N*-type (3′-*endo*) contrary to a DNA : DNA duplex (*S*-type sugar conformation, 2′-*endo*). As the additional 3′-*C*-branch of monomer **Y** shifts the conformational equilibrium towards the *S*-type,[26] ODN **IV** is structurally preorganized for DNA binding.

It has been shown that among 5′- and 3′-exonucleases and endonucleases, 3′-exonucleases play a predominant role in the *in vivo* degradation of natural oligonucleotides.[36] We therefore decided to investigate the stability of ODNs **V** and **VI** towards snake venom phosphordiesterase (SVPDE, 3′-exonuclease) using a qualitative procedure previously described.[37] The unmodified control (T_{14}) was completely degraded within 10 min. However, ODN **V** containing one modification in the 3′-end showed an increased resistance towards degradation, and no degradation of ODN **VI** containing two modifications in the 3′-end was observed during the period monitored (30 min).

In this report, coupling of several methyl 3-*C*-hydroxymethyl pentofuranoside derivatives with cytosine and thymine nucleobases was achieved. Anomeric mixtures of the corresponding nucleosides were obtained. Using a linear strategy, 3′-*C*-methyl- and 3′-*C*-azidomethyl-modified thymidines **16** and **17** were synthesized *via* a 3′-*C*-spiro nucleoside epoxide in good yields. The spiro methyl glycoside **27** proved useful for synthesis of 3′-*C*-functionalized α- and β-nucleosides (**36** and **37**). ODNs containing 3′-*C*-(3-hydroxypropyl)thymidine exhibited moderate decreases in melting temperature of −1 to −3 °C per modification in duplexes towards complementary DNA but slightly larger decreases towards RNA. Generally, the convergent synthetic strategies described should be useful for generation of anomeric mixtures of 3′-*C*-branched 2′-deoxynucleosides for biological testing. The low yields and difficult anomeric separations make these routes non-ideal if, *e.g.*, stereochemically pure β-nucleosides are needed. If so, the linear route *via* a 3′-spiro-2′-deoxynucleoside epoxide is convenient. The results described herein and earlier[8,26] on hybridization properties of ODNs containing 3′-*C*-branched 2′-deoxynucleosides suggest that these monomers should find applicability as discrete attachment sites in otherwise unmodified ODNs.

Experimental

An inert atmosphere of nitrogen or argon was applied for reactions performed in anhydrous solvents. The silica gel (0.040–0.063 mm) used for column chromatography was purchased from Merck. NMR spectra were obtained on a Bruker AC250 spectrometer or a Varian Gemini 2000 spectrometer. δ-Values are reported in ppm relative to $SiMe_4$ as internal standard for ^1H (250 MHz or 300 MHz) and ^{13}C NMR (62.9 MHz) and relative to 85% H_3PO_4 as external standard for ^{31}P NMR (202.3 MHz). Couplings constants (J) are in Hz. ^1H–^1H COSY spectra were obtained for compounds **5**–**13**, **24**–**29**, **32**, **44** and **45**. A double quantum-filtered (DQF) COSY spectrum was obtained for compound **24**. ^1H NOE spectra were obtained for compounds **7a**, **7b**, **15**, **25**, **26**–**29**, **36** and **37**. Non-decoupled ^{13}C NMR spectra were recorded for compounds **5**–**13**, **26** and **27** and intensive nuclei enhancement by polarization transfer (INEPT) spectra were recorded for compounds **24**, **27**, **32** and **44**. Fast-atom bombardment (FAB) mass spectra were recorded on a Kratos MS 50 RF spectrometer. Exact mass determination for compound **45** was performed on a JEOL JMS-AX505W. MALDI MS was performed using a Micromass TofSpec E mass spectrometer. Capillary gel electrophoresis was performed on a Beckman P/ACE System 5000. Microanalyses were performed at the University of Copenhagen. Oligodeoxynucleotides were synthesized on a Pharmacia Gene Assembler® Special DNA-Synthesizer. Purification of 5′-*O*-DMT-ON oligodeoxynucleotides was accomplished using disposable Oligopurification Cartridges (COP, Cruachem) following manufacturer's protocol. The unmodified sequences dT_{14} and dA_{14} were desalted using NAP-10 columns (Pharmacia). The complementary oligonucleotide rA_{14} was purchased from DNA technology ApS, Aarhus, Denmark. Determination of T_ms[8] and evaluation of 3′-exonucleolytic stabilities[37] was performed as described earlier. Light petroleum refers to the fraction with distillation range 60–80 °C.

Methyl 3-*C*-(*tert*-butyldimethylsilyloxymethyl)-2-deoxy-5-*O*-(4-phenylbenzoyl)-β-D-*erythro*-pentofuranoside 5

To a solution of compound **4**[11] (630 mg, 1.75 mmol) in anhydrous DMF (5 cm^3) were added imidazole (386 mg, 5.67 mmol) and TBDMSCl (494 mg, 3.28 mmol). The reaction mixture was stirred under argon at room temperature for 3.5 h. The solvent was removed under reduced pressure, the crude product was redissolved in CH_2Cl_2 (50 cm^3) and the solution was washed successively with saturated aq. $NaHCO_3$ (2 × 25 cm^3) and water (25 cm^3). The organic phase was dried (Na_2SO_4), and evaporated under reduced pressure. Purification using silica gel column chromatography (CH_2Cl_2) afforded furanoside **5** (822 mg, 99%) as a clear oil which was used in the next step without further purification; $\delta_H(CDCl_3)$ 0.11 [6 H, s, $Si(CH_3)_2$], 0.92 [9 H, s, $C(CH_3)_3$], 2.01 (1 H, dd, J_1 3.1, J_2 13.9, H^a-2), 2.32 (1 H, dd, J_1 5.8, J_2 13.9, H^b-2), 3.37 (3 H, s, OCH_3), 3.77 (1 H, s, J 10.0, $CH_2{}'^a$), 3.88 (1 H, d, J 10.0, $CH_2{}'^b$), 4.31 (1 H, dd, J_1 3.3, J_2 10.3, H^a-5), 4.33 (1 H, m, H-4), 4.60 (1 H, dd, J_1 3.4, J_2 11.0, H^b-5), 5.18 (1 H, dd, J_1 3.1, J_2 5.8, H-1), 7.38–7.49 (3 H, m, ArH), 7.64 (4 H, m, ArH) and 8.15 (2 H, m, ArH); $\delta_C(CDCl_3)$ −5.61 and −5.49 [$Si(CH_3)_2$], 18.16 [$C(CH_3)_3$], 25.79 [$C(CH_3)_3$], 43.15 (C-2), 55.36 (OCH_3), 64.34 (C-5), 65.73 (CH_2'), 80.67 (C-3), 84.33 (C-4), 104.93 (C-1), 127.00, 127.23, 128.10, 128.87, 130.19, 139.98 and 145.69 (C-Ar) and 166.19 (O*C*OC-Ar).

Methyl 3-*O*-acetyl-3-*C*-(*tert*-butyldimethylsilyloxymethyl)-2-deoxy-5-*O*-(4-phenylbenzoyl)-β-D-*erythro*-pentofuranoside 6

To a solution of compound **5** (457 mg, 0.96 mmol) in anhydrous CH_2Cl_2 (7 cm^3) were added DMAP (118 mg, 0.97 mmol), anhydrous pyridine (0.6 cm^3, 6.8 mmol) and Ac_2O (0.4

cm^3, 4.2 mmol). The reaction mixture was stirred under argon at room temperature for 24 h before being diluted with CH_2Cl_2 (25 cm^3) and the reaction was quenched with 1 M HCl (25 cm^3). After successive washings with saturated aq. $NaHCO_3$ (2 × 25 cm^3) and water (25 cm^3), the organic phase was dried (Na_2SO_4), and evaporated to afford an oil under reduced pressure. Purification using silica gel column chromatography (CH_2Cl_2) afforded furanoside **6** (698 mg, 94%) as a clear oil, $\delta_H(CDCl_3)$ 0.05 and 0.06 [2 × 3 H, 2 s, $Si(CH_3)_2$], 0.90 [9 H, s, $C(CH_3)_3$], 2.05 (3 H, s, $OCOCH_3$), 2.32 (1 H, dd, J_1 2.9, J_2 14.7, H^a-2), 2.61 (1 H, dd, J_1 5.9, J_2 14.7, H^b-2), 3.36 (3 H, s, OCH_3), 4.11 (1 H, d, J 11.1, $CH_2{}'^a$), 4.27 (1 H, d, J 11.1, $CH_2{}'^b$), 4.46 (1 H, dd, J_1 7.7, J_2 11.3, H^a-5), 4.61 (1 H, dd, J_1 3.4, J_2 7.7, H-4), 4.75 (1 H, dd, J_1 3.4, J_2 11.3, H^b-5), 5.13 (1 H, dd, J_1 2.9, J_2 5.8, H-1), 7.34–7.59 (3 H, m, ArH), 7.64 (4 H, m, ArH) and 8.16 (2 H, m, ArH); $\delta_C(CDCl_3)$ −5.67 and −5.62 [$Si(CH_3)_2$], 18.05 [$C(CH_3)_3$], 21.78 ($OCOCH_3$), 25.67 [$C(CH_3)_3$], 41.81 (C-2), 55.37 (OCH_3), 61.73 (CH_2'), 64.69 (C-5), 83.00 (C-4), 89.48 (C-3), 104.75 (C-1), 126.98, 127.23, 128.07, 128.86, 128.94, 130.19, 140.02 and 145.63 (C-Ar), 166.15 (O*C*OC-Ar) and 170.30 (O*C*OCH_3) (Found: C, 65.3; H, 7.4. Calc. for $C_{28}H_{38}O_7Si$: C, 65.3; H, 7.4%).

3′-*O*-Acetyl-3′-*C*-(*tert*-butyldimethylsilyloxymethyl)-2′-deoxy-4-*N*-isobutyryl-5′-*O*-(4-phenylbenzoyl)cytidine 7a and 1-[3-*O*-acetyl-3-*C*-(*tert*-butyldimethylsilyloxymethyl)-2-deoxy-5-*O*-(4-phenylbenzoyl)-α-D-*erythro*-pentofuranosyl]-4-*N*-isobutyryl-cytosine 7b

Method A. 4-*N*-Isobutyrylcytosine[38] (515 mg, 2.83 mmol) was suspended in hexamethyldisilazane (HMDS, 10 cm^3). $(NH_4)_2SO_4$ (10 mg) was added and the mixture was refluxed under argon. After 2 h a clear solution was obtained, which was cooled to 35 °C before evaporation off of the excess of HMDS under reduced pressure to give silylated 4-*N*-isobutyrylcytosine as an oil. The methyl glycoside **6** (732 mg, 1.42 mmol) was dissolved in anhydrous CH_3CN (20 cm^3) and the solution was added to the oil under argon. The mixture was cooled to −30 °C and TMS triflate (0.52 cm^3, 2.8 mmol) was added dropwise. After 30 min at −30 °C, the reaction mixture was allowed to warm to room temperature and was stirred for six days. The mixture was diluted with CH_2Cl_2 (50 cm^3) and washed successively with saturated aq. $NaHCO_3$ (2 × 25 cm^3) and water (25 cm^3). The organic phase was dried (Na_2SO_4), and evaporated under reduced pressure. The crude product was purified by silica gel column chromatography (CH_2Cl_2–MeOH 97 : 3, v/v) to give as solid materials the β-anomer **7a** (83 mg, 9%) as the more polar isomer, α-anomer **7b** (97 mg, 10%) as the less polar isomer, and an anomeric mixture (1 : 1, 87 mg, 9%).

Method B. General method for coupling reactions. To a stirred suspension of the methyl glycoside **6** (499 mg, 0.97 mmol) and 4-*N*-isobutyrylcytosine (557 mg, 3.08 mmol) in anhydrous CH_3CN (15 cm^3) was dropwise added BSA (1.5 cm^3, 6.07 mmol) under argon at room temperature. The mixture was stirred for 1 h until clear. The reaction mixture was cooled to −30 °C, and TMS triflate (0.7 cm^3, 3.87 mmol) was added dropwise. After being stirred for seven days at room temperature, the mixture was diluted with CH_2Cl_2 (50 cm^3) and washed 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 evaporated under reduced pressure. Purification using silica gel column chromatography (CH_2Cl_2–MeOH 99 : 1, v/v) afforded an anomeric mixture of the nucleosides **7a** and **7b** (2 : 1) (415 mg, 64%).

Isomer **7a**: $\delta_H(CDCl_3)$ 0.06 [6 H, s, $Si(CH_3)_2$], 0.89 [9 H, s, $C(CH_3)_3$], 1.16 and 1.18 [2 × 3 H, 2 d, J 6.9, $CH(CH_3)_2$], 2.10 (3 H, s, $OCOCH_3$), 2.11 (1 H, m, H^{β}-2′), 2.59 [1 H, septet, J 6.9, $CH(CH_3)_2$], 3.26 (1 H, dd, J_1 5.8, J_2 14.5, H^{α}-2′), 4.00 (1 H, d, J 10.9, $CH_2{}''^a$), 4.26 (1 H, d, J 10.9, $CH_2{}''^b$), 4.61–4.84 (3 H, m, H-5′ and H-4′), 6.20 (1 H, dd, J_1 5.8, J_2 8.0, H-1′), 7.37–7.50 (3 H, m, ArH), 7.64 (4 H, m, ArH), 7.65 (1 H, d, J 8.6, H-5), 8.01–8.07 (3 H, m, H-6, ArH) and 8.76 (1 H, s, NH); $\delta_C(CDCl_3)$ −5.67 [$Si(CH_3)_2$], 18.03 [$C(CH_3)_3$], 18.84 and 19.00 [$CH(CH_3)_2$], 21.02 ($OCOCH_3$), 25.63 [$C(CH_3)_3$], 36.51 [$CH(CH_3)_2$], 41.78 (C-2′), 61.71 (CH_2''), 63.52 (C-5′), 83.52 (C-4′), 86.32 (C-1′), 88.68 (C-3′), 96.43 (C-5), 127.13, 127.21, 128.08, 128.22, 128.87, 130.02, 139.70 and 143.63 (C-Ar), 146.05 (C-6), 154.89 (C-2), 162.47 (C-4), 165.93 (O*C*OC-Ar), 170.24 (O*C*OCH_3) and 176.93 (CONH).

Isomer **7b**: $\delta_H(CDCl_3)$ 0.00 [6 H, s, $Si(CH_3)_2$], 0.89 [9 H, s, $C(CH_3)_3$], 1.21 and 1.22 [2 × 3 H, 2 d, J 6.9, $CH(CH_3)_2$], 1.93 (3 H, s, $OCOCH_3$), 2.73 [1 H, septet, J 6.9, $CH(CH_3)_2$], 2.81 (1 H, dd, J_1 2.2, J_2 15.0, H^{α}-2′), 2.92 (1 H, dd, J_1 6.6, J_2 15.0, H^{β}-2′), 4.05 (1 H, d, J 11.1, $CH_2{}''^a$), 4.35 (1 H, d, J 11.1, $CH_2{}''^b$), 4.53 (1 H, dd, J_1 5.6, J_2 12.2, H^a-5′), 4.71 (1 H, dd, J_1 3.6, J_2 12.2, H^b-5′), 5.06 (1 H, dd, J_1 3.6, J_2 5.6, H-4′), 6.23 (1 H, dd, J_1 2.2, J_2 6.6, H-1′), 7.37–7.51 (3 H, m, ArH), 7.61–7.67 (4 H, m, ArH), 7.70 (1 H, d, J 8.5, H-5), 8.01 (2 H, m, ArH), 8.11 (1 H, d, J 8.5, H-6) and 9.16 (1 H, s, NH); $\delta_C(CDCl_3)$ −5.75 [$Si(CH_3)_2$], 17.97 [$C(CH_3)_3$], 18.88 and 19.08 [$CH(CH_3)_2$], 21.80 ($OCOCH_3$), 25.60 [$C(CH_3)_3$], 36.40 [$CH(CH_3)_2$], 40.68 (C-2′), 61.23 (CH_2''), 63.45 (C-5′), 85.29 (C-4′), 88.21 (C-1′), 89.10 (C-3′), 95.53 (C-5), 127.22, 128.05, 128.19, 128.88, 130.05, 139.73 and 143.63 (C-Ar), 146.11 (C-6), 154.96 (C-2), 162.72 (C-4), 165.86 (O*C*OC-Ar), 169.82 (O*C*OCH_3) and 177.38 (CONH) (Found: C, 62.7; H, 6.6; N, 6.0. Calc. for $C_{35}H_{45}N_3O_8Si{\cdot}0.25H_2O$: C, 62.9; H, 6.9; N, 6.3%).

3′-*O*-Acetyl-3′-*C*-(*tert*-butyldimethylsilyloxymethyl)-5′-*O*-(4-phenylbenzoyl)thymidine 8a and 1-[3-*O*-acetyl-3-*C*-(*tert*-butyldimethylsilyloxymethyl)-2-deoxy-5-*O*-(4-phenylbenzoyl)-α-D-*erythro*-pentofuranosyl]thymine 8b

Same procedure as for isomers **7a** and **7b** (Method B); used amounts: methyl glycoside **6** (390 mg, 0.76 mmol), thymine (294 mg, 2.33 mmol), anhydrous CH_3CN (14 cm^3), BSA (1.7 cm^3, 6.88 mmol) and TMS triflate (0.55 cm^3, 3.03 mmol). Flash chromatographic purification on a silica gel column (CH_2Cl_2–MeOH 99 : 1, v/v) afforded an inseparable anomeric mixture of nucleosides **8a** and **8b** (4 : 3) (275 mg, 59%) as a solid material, $\delta_H(CDCl_3)$ 0.08 and 0.09 [2 × s, $Si(CH_3)_2$*, $Si(CH_3)_2$], 0.91 and 0.92 [2 × s, $C(CH_3)_3$*, $C(CH_3)_3$], 1.72 (CH_3), 1.96* (CH_3), 2.01* (s, $OCOCH_3$), 2.12 (s, $OCOCH_3$), 2.19 (dd, J_1 9.1, J_2 14.2, H^a-2′), 2.71* (m, H^a-2′), 2.85* (dd, J_1 7.1, J_2 15.2, H^b-2′), 2.90 (dd, J_1 5.5, J_2 14.2, H^b-2′), 3.99* (d, J 11.0, $CH_2{}''^a$), 4.09 (d, J 10.8, $CH_2{}''^a$), 4.29 (d, J 10.8, $CH_2{}''^b$), 4.33* (d, J 10.8, $CH_2{}''^b$), 4.53* (dd, J_1 6.0, J_2 12.1, H^a-5′), 4.62–4.70 (m, H-4′, H^a-5′), 4.77–4.85 (m, H^b-5′*, H^b-5′), 4.97* (dd, J_1 3.7, J_2 5.8, H-4′), 6.26* (dd, J_1 3.2, J_2 7.1, H-1′), 6.31 (dd, J_1 5.4, J_2 9.1, H-1′), 7.26–7.72 (m, ArH*, ArH), 8.08–8.13 (m, H-6′*, H-6, ArH*, ArH), 9.32 (s, NH) and 9.40* (s, NH); $\delta_C(CDCl_3)$ −5.68 and −5.61 [$Si(CH_3)_2$*, $Si(CH_3)_2$], 12.16 (CH_3), 12.61* (CH_3), 18.02 [$C(CH_3)_3$], 18.06* [$C(CH_3)_3$], 21.68 ($OCOCH_3$), 21.76* ($OCOCH_3$), 25.67 [$C(CH_3)_3$], 25.96* [$C(CH_3)_3$], 40.34 (C-2′), 40.56* (C-2′), 61.34* (CH_2''), 61.71 (CH_2''), 63.52 (C-5′*, C-5′), 82.58 (C-4′), 83.53 (C-1′), 84.49* (C-4′), 86.19* (C-1′), 88.75 (C-3′), 88.84* (C-3′), 110.00* (C-5), 111.51 (C-5), 127.07, 127.21, 128.14, 128.26, 128.91, 130.01, 130.09, 139.67, 139.76, 146.08 and 146.23 (C-Ar*, C-Ar), 134.50 (C-6), 135.15* (C-6), 150.22* (C-2), 150.36 (C-2), 163.60 (C-4), 163.96* (C-4), 165.89 (O*C*OC-Ar*, O*C*OC-Ar), 169.81* (O*C*OCH_3) and 170.37 (O*C*OCH_3); FAB-MS *m*/*z* 609 $[M + H]^+$ (Found: C, 62.9; H, 6.6; N, 4.5. Calc. for $C_{32}H_{40}N_2O_8Si$: C, 63.1; H, 6.6; N, 4.6%).

* Minor isomer: **8b**.

3′-*C*-(*tert*-Butyldimethylsilyloxymethyl)thymidine 9a and 1-[3-*C*-(*tert*-butyldimethylsilyloxymethyl)-2-deoxy-α-D-*erythro*-pentofuranosyl]thymine 9b

An anomeric mixture of nucleosides **8a/8b** (114 mg, 0.19 mmol) was dissolved in a saturated solution of NH_3 in MeOH (10

cm^3). After 22 h at room temperature, the solvent was removed under reduced pressure and the crude product was purified by silica gel column chromatography (CH_2Cl_2–MeOH 99:1, v/v) to give an anomeric mixture (2:1) of the two nucleosides **9a** and **9b** (41 mg, 56%) as a solid material, $\delta_H(CDCl_3)$ 0.12 [s, $Si(CH_3)_2$*, $Si(CH_3)_2$], 0.92 [s, $C(CH_3)_3$*, $C(CH_3)_3$], 1.91 (CH_3*, CH_3), 2.06* (dd, J_1 2.3, J_2 14.5, H^a-2′), 2.16–2.34 (m, H^a-2′, H^b-2′), 2.56* (dd, J_1 8.1, J_2 14.5, H^b-2′), 3.68–3.97 (m, H_2-5′*, H_2-5′, CH_2″* and CH_2″), 4.00 (m, H-4′), 4.28* (m, H-4′), 6.18 (dd, J_1 5.6, J_2 9.2, H-1′), 6.37* (dd, J_1 2.3, J_2 8.0, H-1′), 7.58 (s, H-6), 7.77* (s, H-6) and 9.12 (br s, NH*, NH); $\delta_C(CDCl_3)$ −5.58 [$Si(CH_3)_2$*, $Si(CH_3)_2$], 12.39 (CH_3), 12.50* (CH_3), 18.14 [$C(CH_3)_3$], 18.73* [$C(CH_3)_3$], 25.75 [C(CH_3)$_3$*, C(CH_3)$_3$], 40.78 (C-2′), 42.05* (C-2′), 61.68 (CH_2″), 61.95* (CH_2″), 65.29 (C-5′), 65.37* (C-5′), 80.65* (C-3′), 81.06 (C-3′), 86.05* (C-1′), 86.31 (C-1′), 87.73 (C-4′), 89.86* (C-4′), 110.23* (C-5′), 111.04 (C-5′), 137.36 (C-6), 137.43* (C-6), 150.81 (C-2), 151.01* (C-2), 164.18 (C-4) and 164.42* (C-4); FAB-MS *m/z* 387 $(M + H)^+$ (Found: C, 52.4; H, 7.7; N, 7.0. Calc. for $C_{17}H_{30}N_2O_6Si$: C, 52.8; H, 7.8; N, 7.3%).

* Minor isomer: **9b**.

Methyl 3-*C*-acetoxymethyl-3-*O*-acetyl-2-deoxy-5-*O*-(4-phenylbenzoyl)-β-D-*erythro*-pentofuranoside 10

To a solution of furanoside **4**[11] (1.015 g, 2.82 mmol) in anhydrous CH_2Cl_2 (20 cm^3) were added DMAP (349 mg, 2.86 mmol), anhydrous pyridine (2 cm^3, 22.5 mmol) and Ac_2O (2.1 cm^3, 22.5 mmol). The reaction mixture was stirred under argon at room temperature for 4 h before being diluted with CH_2Cl_2 (50 cm^3) and the reaction was quenched with 1 M HCl (50 cm^3). After being washed successively with saturated aq. $NaHCO_3$ (2 × 50 cm^3) and water (50 cm^3) the organic phase was dried (Na_2SO_4), and evaporated to afford an oil under reduced pressure. Purification using silica gel column chromatography (CH_2Cl_2) afforded title compound **10** (1.24 g, 100%) as a clear oil, $\delta_H(CDCl_3)$ 2.04 and 2.05 (2 × 3 H, 2 s, CH_3), 2.39 (1 H, dd, J_1 2.8, J_2 14.6, H^a-2), 2.65 (1 H, dd, J_1 5.7, J_2 14.6, H^b-2), 3.36 (3 H, s, OCH_3), 4.48 (1 H, m, H^a-5), 4.66–4.80 (4 H, m, H^b-5, CH_2′ and H-4), 5.13 (1 H, dd, J_1 2.8, J_2 5.7, H-1), 7.38–7.49 (3 H, m, ArH), 7.60–7.68 (4 H, m, ArH) and 8.12–8.17 (2 H, m, ArH); $\delta_C(CDCl_3)$ 20.60 and 21.64 ($CO\mathit{C}H_3$), 42.10 (C-2), 55.41 (OCH_3), 62.79 (CH_2′), 63.80 (C-5), 82.54 (C-4), 87.33 (C-3), 104.39 (C-1), 127.04, 127.21, 128.10, 128.60, 128.86, 130.17, 139.91 and 145.77 (C-Ar), 165.98 ($O\mathit{C}OC_{arom}$) and 170.16 and 170.22 ($\mathit{C}OCH_3$) (Found: C, 65.0; H, 5.9. Calc. for $C_{24}H_{26}O_8$: C, 65.2; H, 5.9%).

3′-*C*-Acetoxymethyl-3′-*O*-acetyl-2′-deoxy-4-*N*-isobutyryl-5′-*O*-(4-phenylbenzoyl)cytidine 11a and 1-[3-*C*-acetoxymethyl-3-*O*-acetyl-2-deoxy-5-*O*-(4-phenylbenzoyl)-α-D-*erythro*-pentofuranosyl]-4-*N*-isobutyrylcytosine 11b

Same procedure as for compounds **7a** and **7b** (Method B); used amounts: methyl glycoside **10** (371 mg, 0.84 mmol), 4-*N*-isobutyrylcytosine (455 mg, 2.51 mmol), anhydrous CH_3CN (15 cm^3), BSA (1.9 cm^3, 7.69 mmol) and TMS triflate (0.61 cm^3, 3.37 mmol). Purification using silica gel column chromatography (CH_2Cl_2–MeOH 99:1, v/v) afforded nucleosides **11a** and **11b** as an inseparable anomeric mixture (1:2) (294 mg, 58%) as a solid, $\delta_H(CDCl_3)$ 1.14* [d, *J* 7.0, $CH(C\mathit{H}_3)_2$], 1.21 [d, *J* 6.8, $CH(C\mathit{H}_3)_2$], 1.92 and 2.05 (2 s, 2 × $OCOCH_3$), 2.07* and 2.12* (2 s, 2 × $OCOCH_3$), 2.19* (dd, J_1 8.3, J_2 14.4, H^a-2′), 2.60* [septet, *J* 7.0, $C\mathit{H}(CH_3)_2$], 2.74 [septet, *J* 6.8, $C\mathit{H}(CH_3)_2$], 2.92 (m, H_2-2′), 3.30* (dd, J_1 5.5, J_2 14.4, H^b-2′), 4.45 (d, *J* 4.2, CH_2″^{a}), 4.50 (d, *J* 4.2, CH_2″^{b}), 4.53* (d, *J* 4.1, CH_2″^{a}), 4.58* (d, *J* 4.1, CH_2″^{b}), 4.68–4.83 (m, H_2-5′* and H_2-5′), 4.90* (m, H-4′), 5.21 (m, H-4′), 6.17–6.25 (m, H-1′*, H-1′), 7.28–7.72 (m, H-5′*, H-5, ArH*, ArH), 8.00–8.11 (m, H-6*, H-6, ArH*, ArH), 8.85* (s, NH) and 9.16 (s, NH); $\delta_C(CDCl_3)$ 18.77, 18.85, 18.89 and 19.04 [$CH(\mathit{C}H_3)_2$*, $CH(\mathit{C}H_3)_2$], 20.44, 20.49, 21.61 and 21.68 ($OCO\mathit{C}H_3$*, $OCO\mathit{C}H_3$), 36.35 [$\mathit{C}H(CH_3)_2$*, $\mathit{C}H(CH_3)_2$], 41.57 (C-2′), 41.65* (C-2′), 62.02* (CH_2″), 62.33 (CH_2″), 62.63* (C-5′), 62.71 (C-5′), 82.67* (C-4′), 84.42 (C-4′), 86.38* (C-1′), 87.12 (C-3′), 87.20* (C-3′), 87.97 (C-1′), 95.58 (C-5), 96.50* (C-5), 127.16, 127.26, 127.66, 127.70, 128.20, 128.86, 129.93, 130.04, 130.23, 139.53, 139.61, 143.32 and 143.54 (C-Ar*, C-Ar), 146.16* (C-6), 146.22 (C-6), 154.86* (C-2), 154.95 (C-2), 162.53* (C-4), 162.76 (C-4), 165.93 ($O\mathit{C}OC$-Ar*, $O\mathit{C}OC$-Ar), 169.94 ($O\mathit{C}OCH_3$), 170.05* ($O\mathit{C}OCH_3$), 176.93* (CONH) and 177.38 (CONH) (Found: C, 61.8; H, 5.7; N, 6.9. Calc. for $C_{31}H_{33}N_3O_9\cdot0.5H_2O$: C, 62.0; H, 5.7; N, 7.0%).

* Minor isomer.

3′-*C*-Acetoxymethyl-3′-*O*-acetyl-5-*O*-(4-phenylbenzoyl)-thymidine 12a and 1-[3-*C*-acetoxymethyl-3-*O*-acetyl-2-deoxy-5-*O*-(4-phenylbenzoyl)-α-D-*erythro*-pentofuranosyl]thymine 12b

Same procedure as for compounds **7a** and **7b** (Method B); used amounts: methyl glycoside **10** (539 mg, 1.22 mmol), thymine (461 mg, 2.55 mmol), anhydrous CH_3CN (20 cm^3), BSA (2.71 cm^3, 10.96 mmol) and TMS triflate (0.88 cm^3, 4.86 mmol). Purification using silica gel column chromatography (CH_2Cl_2–MeOH 99:1, v/v) afforded nucleosides **12a** and **12b** as an inseparable anomeric mixture (1:2) (454 mg, 69%) as a white solid, $\delta_H(CDCl_3)$ 1.56 (s, CH_3), 1.72* (s, CH_3), 1.95* and 2.01* (2 s, 2 × $OCOCH_3$), 2.11 and 2.14 (2 s, 2 × $OCOCH_3$), 2.15 (m, H^a-2′), 2.82* (m, H^a-2′, H^b-2′), 2.89 (dd, J_1 5.5, J_2 14.2, H^b-2′), 4.47* (m, H^a-5′), 4.51 (m, H^a-5′), 4.66* (dd, J_1 3.8, J_2 12.5, H^b-5′), 4.75–4.93 (m, CH_2″*, CH_2″, H-4′ and H^b-5′), 5.10* (m, H-4′), 6.24–6.33 (m, H-1′* and H-1′), 7.24–7.72 (m, H-6*, H-6, ArH*, ArH), 8.08–8.12 (m, ArH*, ArH), 9.26 (s, NH) and 9.39* (s, NH); $\delta_C(CDCl_3)$ 11.97 (CH_3), 12.60* (CH_3), 20.53, 20.57, 21.60 and 21.71 (2 × $OCO\mathit{C}H_3$*, 2 × $OCO\mathit{C}H_3$), 41.40* (C-2′), 41.52 (C-2′), 62.08, 62.51 and 62.80 (CH_2″*, CH_2″, C-5′*, C-5′), 81.84 (C-4′), 83.58 (C-1′), 83.73* (C-4′), 86.07* (C-1′), 86.98* (C-3′), 87.33 (C-3′), 110.06* (C-5), 111.61 (C-5), 127.21, 127.30, 127.43, 127.73, 127.78, 128.25, 128.36, 128.91, 128.94, 130.01, 130.10, 139.54, 139.68, 146.29 and 146.54 (C-Ar*, C-Ar), 134.20 (C-6), 134.95* (C-6), 150.22* (C-2), 150.32 (C-2), 163.44 (C-4), 163.86* (C-4), 165.66 ($O\mathit{C}OC$-Ar), 165.76* ($O\mathit{C}OC$-Ar), 169.88*, 170.01* (2 × $O\mathit{C}OCH_3$), 170.23 and 170.55 (2 × $O\mathit{C}OCH_3$); FAB-MS *m/z* 537 $(M + H)^+$ (Found: C, 62.0; H, 5.2; N, 5.2. Calc. for $C_{28}H_{28}N_2O_9\cdot0.25H_2O$: C, 62.2; H, 5.3; N, 5.2%).

* Minor isomer.

3′-*C*-Hydroxymethylthymidine[8a] 13a and 1-(2-deoxy-3-*C*-hydroxymethyl-α-D-*erythro*-pentofuranosyl)thymine 13b

An anomeric mixture of nucleosides **12a/12b** (493 mg, 0.92 mmol) was dissolved in a saturated solution of NH_3 in MeOH (25 cm^3). After 24 h at room temperature, the solvent was removed under reduced pressure and the crude product was purified by silica gel column chromatography (CH_2Cl_2–MeOH 19:1, v/v; one teaspoon of silica gel was added to the reaction mixture, and was then placed on top of the column after evaporation to dryness), to give an anomeric mixture (1:2) of the two nucleosides **13a** and **13b** as a white solid (154 mg, 60%), $\delta_H[(CD_3)_2SO]$ 1.78 (s, CH_3*, CH_3), 1.85* (dd, J_1 2.9, J_2 14.2, H^a-2′), 1.99 (m, H^a-2′, H^b-2′), 2.44* (m, H^b-2′), 3.44–3.68 (m, CH_2″*, CH_2″, H_2-5′* and H_2-5′), 3.80 (m, H-4′), 4.12* (m, H-4′), 4.82* (m, OH), 5.03 (m, OH), 6.16–6.21 (m, H-1′*, H-1′), 7.86* (s, H-6), 7.91 (s, H-6) and 11.21 (s, NH*, NH); $\delta_C[(CD_3)_2SO]$ 12.29 (CH_3), 12.37* (CH_3), 40.80 (C-2′), 41.99* (C-2′), 60.50* (C-5′), 60.61 (C-5′), 63.88* (CH_2″), 63.99 (CH_2″), 80.25* (C-3′), 80.71 (C-3′), 83.33 (C-1′), 84.73* (C-1′), 87.52 (C-4′), 89.01* (C-4′), 108.35* (C-5), 109.28 (C-5), 136.36 (C-6), 137.37* (C-6), 150.46* (C-2), 150.54 (C-2), 163.74 (C-4) and 163.91* (C-4); FAB-MS *m/z* 273 $(M + H)^+$ (Found: C, 48.1; H, 5.9; N, 10.0. Calc. for $C_{11}H_{16}N_2O_6\cdot0.25H_2O$: C, 47.7; H, 6.0; N, 10.1%).

* Minor isomer.

(3*R*,4*R*,6*R*)-4-(*tert*-Butyldimethylsilyloxymethyl)-6-(thymin-1-yl)-1,5-dioxaspiro[2.4]heptane 15

To a solution of 5′-*O*-(*tert*-butyldimethylsilyl)-3′-deoxy-3′-*C*-methylenethymidine[8c] **14** (500 mg, 1.42 mmol) in CH_2Cl_2 (25 cm^3) was added MCPBA (60%; 500 mg, 1.74 mmol) and the solution was stirred at room temperature for 8 h and then was washed successively with saturated aq. Na_2CO_3 (4 × 20 cm^3) and water (2 × 20 cm^3). The organic phase was dried (Na_2SO_4) and the solvent was evaporated off under reduced pressure. The crude product was purified by silica gel column chromatography (light petroleum–EtOAc 17 : 3, v/v) to afford nucleoside **15** as a solid (352 mg, 70%) which was used without further purification in the next step, $\delta_H(CDCl_3)$ 0.11 and 0.12 [6 H, 2 s, $Si(CH_3)_2$], 0.93 [9 H, s, $C(CH_3)_3$], 1.93 (3 H, d, *J* 1.0, CH_3), 2.18 (1 H, dd, J_1 5.6, J_2 13.8, H^{β}-2′), 2.40 (1 H, dd, J_1 9.2, J_2 13.7, H^{α}-2′), 2.96 (1 H, d, *J* 4.0, 3′-CH_2^a), 3.05 (1 H, d, *J* 4.0, 3′-CH_2^b), 3.64 (1 H, dd, J_1 2.5, J_2 12.0, H^a-5′), 3.92–3.98 (2 H, m, H-4′, H^b-5′), 6.43 (1 H, dd, J_1 5.6, J_2 9.0, H-1′), 7.59 (1 H, d, *J* 1.0, H-6) and 8.39 (1 H, s, N-H); $\delta_C(CDCl_3)$ −5.53, −5.41, 12.46, 18.21, 25.83, 38.56, 47.46, 63.17, 63.51, 82.36, 83.53, 111.10, 135.33, 152.34 and 164.20.

3′-*C*-Methylthymidine 16[22]

A solution of nucleoside **15** (80 mg, 0.23 mmol) in a 1.0 M solution of lithium triethylborohydride in THF (0.5 cm^3, 0.5 mmol) was stirred under nitrogen for 20 min. Ethyl acetate (4 cm^3) was added and the solution was washed with saturated aqueous NH_4Cl (2 × 10 cm^3) and saturated brine (2 × 10 cm^3). The organic phase was dried (Na_2SO_4), and evaporated under reduced pressure. The residue was dissolved in anhydrous THF (1 cm^3) and this was followed by addition of TBAF (1.0 cm^3 of a 1.0 M solution in THF, 1.0 mmol). The reaction mixture was stirred at room temperature for 20 min before being evaporated and the residue was dissolved in CH_2Cl_2 (5 cm^3) and the solution was washed with brine (3 × 5 cm^3). The organic phase was dried (Na_2SO_4), and evaporated under reduced pressure. The residue was purified by silica gel column chromatography (CH_2Cl_2–MeOH 19 : 1, v/v) to give nucleoside **16** (48 mg, 83%) as a solid, $\delta_H(CD_3OD)$ 1.63 (3 H, d, *J* 1.0), 2.07 (3 H, s), 2.29 (1H, dd, J_1 9.2, J_2 12.7), 2.41 (1 H, dd, J_1 5.5, J_2 12.7), 3.84 (1 H, dd, J_1 3.5, J_2 12.0), 3.98 (1 H, dd, J_1 3.1, J_2 12.0), 4.06 (1 H, t, *J* 3.2), 6.52 (1 H, dd, J_1 5.5, J_2 9.3) and 8.26 (1 H, d, *J* 1.0); $\delta_C(CD_3OD)$ 12.51, 22.51, 46.15, 62.74, 79.66, 85.84, 90.32, 111.43, 138.69, 152.59 and 166.52.

3′-*C*-(Azidomethyl)thymidine 17

To a solution of nucleoside **15** (100 mg, 0.28 mmol) in anhydrous DMF (4 cm^3) was added sodium azide (90 mg, 1.38 mmol) and the reaction mixture was stirred under nitrogen at 100 °C for 6 h. The solution was cooled, diethyl ether (10 cm^3) was added, and the mixture was washed with brine (3 × 20 cm^3). The organic phase was dried (Na_2SO_4), and evaporated under reduced pressure to give a product which was desilylated and purified as described for analogue **16** to afford title azide **17** (62 mg, 74%) as a solid, $\delta_H(CD_3OD)$ 2.06 (3 H, d, *J* 1.0), 2.30 (1 H, dd, J_1 9.4, J_2 12.8), 2.45 (1 H, dd, J_1 5.5, J_2 12.7), 3.80 (2 H, s) 3.92 (1 H, dd, J_1 3.0, J_2 12.0), 4.0 (1 H, dd, J_1 3.0, J_2 12.0), 4.17 (1 H, t, *J* 3.0), 6.46 (1 H, dd, J_1 5.4, J_2 9.3) and 8.26 (1 H, d, *J* 1.0); $\delta_C(CD_3OD)$ 12.53, 43.29, 56.88, 60.00, 82.46, 85.86, 88.92, 111.58, 138.53, 152.55 and 166.45.

Methyl 5-*O*-(*tert*-butyldimethylsilyl)-2-deoxy-β-D-*glycero*-pentofuranosid-3-ulose 22

CrO_3 (3.3 g, 33.0 mmol) was dissolved in anhydrous CH_2Cl_2 (150 cm^3). To this stirred solution were slowly added anhydrous pyridine (7.0 cm^3, 86 mmol), compound **20**[26] (4.28 g, 16.31 mmol) and Ac_2O (5.0 cm^3, 52.9 mmol). After 4 h, EtOAc (500 cm^3) was added and the residue obtained after evaporation was purified by silica gel column chromatography (EtOAc) to yield ketone **22** (3.26 g, 77%) as a clear oil, which was used in the next step without further purification; $\delta_H(CDCl_3)$ 0.08 (6 H, s), 0.90 (9 H, s), 2.36–2.44 (1 H, m), 2.73 (1 H, dd, J_1 5.7, J_2 18.2), 3.46 (3 H, s), 3.77 (1 H, dd, J_1 6.3, J_2 11.0), 3.86 (1 H, dd, J_1 3.5, J_2 11.0) and 5.33 (1 H, dd, J_1 1.8, J_2 5.7); $\delta_C(CDCl_3)$ −5.54, −5.44, 18.29, 25.78, 43.62, 54.92, 64.43, 81.37, 102.16 and 211.89.

Methyl 5-*O*-(*tert*-butyldimethylsilyl)-2,3-dideoxy-3-*C*-methylene-α-D-*glycero*-pentofuranoside 23

A mixture of zinc dust (12.44 g, 0.19 mol) and CH_2Br_2 (4.3 cm^3, 61.3 mmol) in anhydrous THF (100 cm^3) was cooled to −40 °C under argon. To this stirred mixture was carefully added $TiCl_4$ (5.0 cm^3, 45.5 mmol). After stirring of the mixture for 4 days at 5 °C, compound **21**[26] (2.25 g, 8.62 mmol) was added dropwise to the solution. After 1 h the reaction mixture was poured onto an ice-cooled mixture of water (150 cm^3), saturated aq. $NaHCO_3$ (150 cm^3) and CH_2Cl_2 (150 cm^3). The mixture was extracted with CH_2Cl_2 (3 × 150 cm^3) and the combined organic phase was dried (Na_2SO_4) and evaporated. Purification by silica gel column chromatography (CH_2Cl_2) gave furanoside **23** (898 mg, 40%) as a clear oil, $\delta_H(CDCl_3)$ 0.06 (6 H, s), 0.90 (9 H, s), 2.46–2.52 (1 H, m), 2.65–2.76 (1 H, m), 3.36 (3 H, s), 3.70 (2 H, m), 4.45–4.49 (1 H, m) and 5.05–5.09 (3 H, m); $\delta_C(CDCl_3)$ −5.35, −5.29, 18.35, 25.89, 39.90, 54.45, 66.13, 80.30, 103.90, 106.33 and 146.45.

Methyl 5-*O*-(*tert*-butyldimethylsilyl)-2,3-dideoxy-3-*C*-methylene-β-D-*glycero*-pentofuranoside 24

Same procedure as for anomer **23**; used amounts: zinc dust (13.80 g, 0.21 mol), CH_2Br_2 (5.0 cm^3, 71.8 mmol), anhydrous THF (100 cm^3), $TiCl_4$ (5.7 cm^3, 51.9 mmol) and ketone **22** (8.71 g, 33.4 mmol). Work-up as described for anomer **23** afforded furanoside **24** (4.36 g, 51%) as a clear oil, $\delta_H(CDCl_3)$ 0.08 and 0.09 [6 H, 2 s, $Si(CH_3)_2$], 0.92 [9 H, s, $C(CH_3)_3$], 2.51–2.59 (1 H, m, H^a-2), 2.75–2.83 (1 H, m, H^b-2), 3.36 (3 H, s, OCH_3), 3.61–3.74 (2 H, m, H_2-5), 4.47–4.53 (1 H, m, H-4) and 5.02–5.07 (3 H, m, H-1, $=CH_2$); $\delta_C(CDCl_3)$ −5.35 [$Si(CH_3)_2$], 18.32 [$C(CH_3)_3$], 25.89 [$C(CH_3)_3$], 40.07 (C-2), 54.53 (OCH_3), 67.73 (C-5), 81.50 (C-4), 104.21 (C-1), 106.60 ($=CH_2$) and 146.52 (C-3).

Methyl 5-*O*-(*tert*-butyldimethylsilyl)-2-deoxy-3-*C*-(hydroxymethyl)-β-D-*erythro*-pentofuranoside 25

Compound **24** (309 mg, 1.19 mmol) was dissolved in *tert*-butyl alcohol (15 cm^3). To this solution were added NMO (1.0 g, 8.54 mmol), pyridine (0.6 cm^3, 7.43 mmol), water (0.70 cm^3) and OsO_4 (55 mm^3 of a 2.5% solution in Bu^{t}OH, 0.18 μmol). After refluxing of the mixture for 3 h at 76 °C the reaction was quenched by addition of 20% aq. $Na_2S_2O_5$ (20 cm^3) and the mixture was evaporated under reduced pressure. The residue was dissolved in EtOAc (100 cm^3) and the solution was washed with brine (50 cm^3). The water phase was extracted with EtOAc (2 × 100 cm^3) and the combined organic phase was dried ($MgSO_4$), and evaporated under reduced pressure. Purification by silica gel column chromatography (EtOAc) afforded furanoside **25** (324 mg, 93%) as a clear oil, $\delta_H(CDCl_3)$ 0.13 [6 H, s, $Si(CH_3)_2$], 0.92 [9 H, s, $C(CH_3)_3$], 1.78 (1 H, dd, J_1 3.9, J_2 14.2, H^a-2), 2.00 (1 H, br s, OH), 2.24 (1 H, dd, J_1 5.8, J_2 14.1, H^b-2), 3.35 (3 H, s, OCH_3), 3.36–3.66 (2 H, m, H_2-5), 3.68 (1 H, s, OH), 3.71 (1 H, d, *J* 2.4, CH_2^a), 3.88–3.98 (2 H, m, H-4, CH_2^b) and 5.14 (1 H, dd, J_1 3.9, J_2 5.9, H-1); $\delta_C(CDCl_3)$ −5.68, −5.61, 18.13, 25.72, 41.69, 55.58, 63.50, 65.22, 82.09, 87.28 and 105.13; EI-MS 291 $[M - H]^-$ (Found: C, 53.4; H, 9.6. Calc. for $C_{13}H_{28}O_5Si$: C, 53.4; H, 9.7%).

(3*S*,4*R*,6*R*)-4-(*tert*-Butyldimethylsilyloxymethyl)-6-methoxy-1,5-dioxaspiro[2.4]heptane 26 and (3*R*,4*R*,6*R*)-4-(*tert*-butyldimethylsilyloxymethyl)-6-methoxy-1,5-dioxaspiro[2.4]heptane 27

Method A: cyclization. To a stirred solution of furanoside **25** (324 mg, 1.11 mmol) in pyridine (4.0 cm^3) was added TsCl

(508 mg, 2.66 mmol). After 17 h the solvent was evaporated off and the residue was dissolved in EtOAc (8 cm^3) followed by washing successively with brine (2 × 5 cm^3) and saturated aq. $NaHCO_3$ (2 × 5 cm^3). The organic phase was dried (Na_2SO_4), and evaporated under reduced pressure. The residue was coevaporated with anhydrous toluene (2 × 15 cm^3) and dissolved in anhydrous DMF (5 cm^3). To this mixture were added K_2CO_3 (110 mg, 0.80 mmol) and 18-crown-6 (211 mg, 0.80 mmol). After stirring of the mixture for 3 h at 65 °C the solvent was evaporated off under reduced pressure and the crude product was dissolved in EtOAc (15 cm^3) and washed with saturated aq. $NaHCO_3$ (2 × 8 cm^3). After drying (Na_2SO_4) and evaporation of the organic phase under reduced pressure, purification by silica gel column chromatography (hexane–EtOAc 9 : 1, v/v) afforded epoxide **27** (118 mg, 39%) as a clear oil.

Method B: oxidation. Compound **24** (742 mg, 2.87 mmol) was dissolved in CH_2Cl_2 (15 cm^3). After cooling of this reagent to 0 °C, $NaHCO_3$ (130 mg, 10.92 mmol) and MCPBA (1.32 g, 4.36 mmol; 57–86%) were added. After stirring of the mixture for 24 h, saturated aq. Na_2CO_3 (40 cm^3) was added and the mixture was extracted with CH_2Cl_2 (3 × 75 cm^3). The combined organic phase was dried (Na_2SO_4), and evaporated under reduced pressure. The two stereoisomers **26** and **27** were separated by silica gel column chromatography (hexane–EtOAc 9 : 1, v/v) to afford as clear oils epoxide **26** [234 mg, 30%; R_f = 0.4 (hexane–EtOAc 7 : 3, v/v)] and epoxide **27** [350 mg, 44%; R_f 0.5 (hexane–EtOAc 7 : 3, v/v)].

Compound **26**: $\delta_H(CDCl_3)$ 0.06 and 0.07 [6 H, 2 s, $Si(CH_3)_2$], 0.89 [9 H, s, $C(CH_3)_3$], 1.71–1.87 (1 H, m, H^a-2), 2.52 [1 H, dd, J_1 5.1, J_2 14.1, H^b-2], 2.85 (1 H, d, J 4.8, $CH_2'^a$), 2.91 (1 H, d, J 4.8, $CH_2'^b$), 3.40 (3 H, s, OCH_3), 3.58 (1 H, dd, J_1 5.9, J_2 10.4, H^a-5), 3.68 (1 H, dd, J_1 6.3, J_2 10.4, H^b-5), 4.21 (1 H, m, H-4) and 5.07 (1 H, m, H-1); $\delta_C(CDCl_3)$ −5.46 [$Si(CH_3)_2$], 18.29 [$C(CH_3)_3$], 25.87 [$C(CH_3)_3$], 39.53 (C-2), 46.93 (CH_2'), 54.69 (OCH_3), 62.32 (C-5), 63.52 (C-3), 78.68 (C-4) and 103.49 (C-1) (Found: C, 56.8; H, 9.4. Calc. for $C_{13}H_{26}O_4Si$: C, 56.9; H, 9.6%).

Compound **27**: $\delta_H(CDCl_3)$ 0.06 (6 H, s), 0.89 (9 H, s), 2.23–2.26 (2 H, m), 2.92 (1 H, d, J 4.8), 3.23 (1 H, d, J 4.8), 3.38 (3 H, s), 3.65–3.69 (2 H, m), 3.87 (1 H, dd, J_1 5.6, J_2 7.0) and 5.18 (1 H, dd, J_1 3.8, J_2 5.0); $\delta_C(CDCl_3)$ −5.50 [$Si(CH_3)_2$], 18.17 [$C(CH_3)_3$], 25.81 [$C(CH_3)_3$], 38.49 (C-2), 48.28 (CH_2'), 55.18 (OCH_3), 64.19 (C-5), 64.27 (C-3), 81.25 (C-4) and 104.68 (C-1) (Found: C, 56.7; H, 9.5%).

(3*S*,4*R*,6*S*)-4-(*tert*-Butyldimethylsilyloxymethyl)-6-methoxy-1,5-dioxaspiro[2.4]heptane 28 and (3*R*,4*R*,6*S*)-4-(*tert*-butyldimethylsilyloxymethyl)-6-methoxy-1,5-dioxaspiro[2.4]heptane 29

Same procedure as for compounds **26** and **27** (Method B); used amounts: compound **23** (338 mg, 1.31 mmol), CH_2Cl_2 (7 cm^3), $NaHCO_3$ (55 mg, 4.62 mmol) and MCPBA (499 mg, 1.65 mmol; 57–86%). After 15 h, saturated aq. solution of Na_2CO_3 (20 cm^3) was added, and work-up was performed as described for compounds **26** and **27**. Separation by silica gel column chromatography (hexane–EtOAc 19 : 1, v/v) afforded epoxide **28** [142 mg, 40%; R_f 0.5 (hexane–EtOAc 7 : 3, v/v)] and epoxide **29** [78 mg, 21%; R_f 0.4 (hexane–EtOAc 7 : 3, v/v)] as clear oils.

Compound **28**: $\delta_H(CDCl_3)$ 0.05 [6 H, s, $Si(CH_3)_2$], 0.88 [9 H, s, $C(CH_3)_3$], 1.62–1.82 (1 H, m, H^a-2), 2.47 (1 H, dd, J_1 5.1, J_2 14.0, H^b-2), 2.92 (1 H, d, J 4.4, $CH_2'^a$), 3.05 (1 H, d, J 4.4, $CH_2'^b$), 3.42 (3 H, s, OCH_3), 3.59 (1 H, dd, J_1 3.2, J_2 11.2, H^a-5), 3.72 (1 H, dd, J_1 3.9, J_2 11.2, H^b-5), 3.94 (1 H, m, H-4) and 5.13 (1 H, m, H-1); $\delta_C(CDCl_3)$ −5.57, −5.39, 18.15, 25.80, 39.42, 47.22, 54.67, 63.26, 63.40, 80.35 and 104.02; FAB-MS *m*/*z* 273 $[M - H]^-$ (Found: C, 56.7; H, 9.8. Calc. for $C_{13}H_{26}O_4Si$: C, 56.9; H, 9.6%).

Compound **29**: $\delta_H(CDCl_3)$ 0.06 and 0.07 [6 H, 2 s, $Si(CH_3)_2$], 0.89 [9 H, s, $C(CH_3)_3$], 2.15 (1 H, dd, J_1 1.6, J_2 14.4, H^a-2), 2.31 (1 H, dd, J_1 5.5, J_2 14.4, H^b-2), 2.86 (1 H, d, J 4.9, $CH_2'^a$), 2.95 (1 H, d, J 4.9, $CH_2'^b$), 3.39 (3 H, s, OCH_3), 3.59 (1 H, dd, J_1 5.0, J_2 10.9, H^a-5), 3.68 (1 H, dd, J_1 5.2, J_2 10.9, H^b-5), 4.13 (1 H, m, H-4) and 5.15 (1 H, dd, J_1 1.6, J_2 5.5, H-1); $\delta_C(CDCl_3)$ −5.44, −5.41, 18.28, 25.86, 38.97, 49.86, 54.82, 61.91, 62.88, 76.56 and 103.00 (Found: C, 56.7; H, 9.6%).

Methyl 3-*C*-[2-(benzyloxy)ethoxymethyl]-2-deoxy-β-D-*erythro*-pentofuranoside 31

Compound **30**[30] (812 mg, 5.34 mmol) was dissolved in anhydrous DMF (40 cm^3). After addition of NaH (214 mg of a 60% dispersion in mineral oil, 5.35 mmol) the reaction mixture was stirred for 30 min followed by the addition of compound **27** (905 mg, 3.30 mmol). After being stirred for 3 days at room temperature, the reaction mixture was poured into ice-cooled, saturated aq. $NaHCO_3$ (40 cm^3) and extracted with CH_2Cl_2 (3 × 75 cm^3). The combined organic phase was dried (Na_2SO_4) and evaporated under reduced pressure. Purification by silica gel column chromatography (hexane–EtOAc 9 : 1, v/v) afforded furanoside **31** (589 mg, 57%) as a clear oil, $\delta_H(CDCl_3)$ 1.89 (1 H, dd, J_1 3.8, J_2 14.2), 2.28 (1 H, dd, J_1 6.1, J_2 14.2), 3.18 (1 H, br s), 3.41 (3 H, s), 3.58–3.73 (8 H, m), 3.86 (1 H, d, J 9.6), 4.06 (1 H, t, J 3.7), 4.54 (2 H, s), 5.19 (1 H, dd, J_1 3.8, J_2 6.0) and 7.25–7.38 (5 H, m); $\delta_C(CDCl_3)$ 43.60, 55.59, 62.22, 68.84, 70.84, 73.01, 73.59, 80.76, 88.26, 105.11, 127.57, 128.25 and 137.64 (Found: C, 61.3; H, 7.9. Calc. for $C_{16}H_{24}O_6$: C, 61.5; H, 7.7%).

Methyl 3,5-di-*O*-acetyl-3-*C*-[2-(benzyloxy)ethoxymethyl]-2-deoxy-β-D-*erythro*-pentofuranoside 32

Compound **31** (472 mg, 1.51 mmol) was dissolved in anhydrous pyridine (15 cm^3). DMAP (10 mg, 0.08 mmol) was added followed by dropwise addition of Ac_2O (1.13 cm^3, 12.09 mmol). The mixture was stirred at room temperature for 2 days, evaporated under reduced pressure, and the residue was dissolved in saturated aq. $NaHCO_3$ (50 cm^3) and extracted with CH_2Cl_2 (3 × 75 cm^3). The combined organic phase was dried (Na_2SO_4), and evaporated under reduced pressure. Purification by silica gel column chromatography (hexane–EtOAc 9 : 1, v/v) afforded furanoside **32** (509 mg, 85%) as a clear oil, $\delta_H(CDCl_3)$ 2.02 and 2.08 (6 H, 2 s, 2 × CH_3), 2.28 (1 H, dd, J_1 3.0, J_2 14.7, H^a-2), 2.60 (1 H, dd, J_1 5.8, J_2 14.7, H^b-2), 3.34 (3 H, s, OCH_3), 3.57–3.66 (4 H, m, H_2-2′ and H_2-3′), 3.96 (1 H, d, J 3.5, H^a-1′), 4.08 (1 H, d, J 3.4, H^b-1′), 4.13–4.51 (3 H, m, H-4, H_2-5), 4.54 (2 H, s, Bn), 5.09 (1 H, dd, J_1 3.0, J_2 5.8, H-1) and 7.24–7.34 (5 H, m, Bn); $\delta_C(CDCl_3)$ 20.81 and 21.66 (2 × CH_3), 41.90 (C-2), 55.27 (OCH_3), 63.90 (C-5), 69.13, 69.24, 70.72 and 73.00 (C-1′, -2′, -3′ and Bn), 82.97 (C-4), 88.35 (C-3), 104.67 (C-1), 127.42, 127.47, 128.20 and 138.16 (Bn) and 170.38 and 170.52 (2 × C=O).

1-{3,5-Di-*O*-acetyl-3-*C*-[2-(benzyloxy)ethoxymethyl]-2-deoxy-α,β-D-*erythro*-pentofuranosyl}thymine 33

Compound **32** (335 mg, 0.85 mmol) was coevaporated with anhydrous toluene (2 × 20 cm^3) and dissolved in anhydrous 1,2-dichloroethane (15 cm^3). Thymine (213 mg, 1.69 mmol) and BSA (1.24 cm^3, 5.07 mmol) were added and the mixture was stirred under reflux for 10 min at 78 °C. The clear solution was cooled to room temperature and TMS triflate (0.21 cm^3, 1.18 mmol) was added dropwise over a period of 10 min. After 15 h, 0.10 cm^3 (0.55 mmol) of TMS triflate was added and the reaction mixture was stirred for 2 days at room temperature. After dilution with CH_2Cl_2 (40 cm^3) the mixture was poured into ice-cooled, saturated aq. $NaHCO_3$. The organic phase was isolated and the aqueous phase was extracted with CH_2Cl_2 (3 × 40 cm^3). The combined organic phase was dried (Na_2SO_4), and evaporated under reduced pressure. PLC (CH_2Cl_2–MeOH 19 : 1, v/v) afforded an anomeric mixture of nucleosides **33** (1 : 1.4; 307 mg, 75%) as a solid, $\delta_H(CDCl_3)$ 1.93, 1.93, 1.97, 2.07, 2.08 and 2.08 (6 s), 2.24 (dd, J_1 5.5, J_2 14.3), 2.70–2.74 (m), 2.83 (dd, J_1 5.9, J_2 14.4), 3.61–3.69 (m), 3.83–4.49 (m), 4.54 (s), 4.84 (dd, J_1 3.5, J_2 5.5), 6.19 (dd, J_1 3.7, J_2 6.5), 6.29 (dd, J_1 5.9, J_2 8.7), 7.25–7.36 (m) and 9.81 and 9.86 (2 s); $\delta_C(CDCl_3)$ 12.36, 12.44, 20.67,

21.53, 40.14, 40.57, 62.85, 62.91, 68.74, 68.96, 69.11, 69.37, 70.62, 70.77, 82.26, 83.08, 84.34, 85.99, 86.70, 87.71, 109.79, 111.15, 127.42, 127.45, 127.51, 128.19, 134.89, 135.20, 137.82, 137.94, 150.25, 150.42, 163.75, 164.01, 169.78 and 170.15; MS-FAB *m*/*z* 491 $[M + H]^+$.

1-[3,5-Di-*O*-acetyl-2-deoxy-3-*C*-(2-hydroxyethoxymethyl)-α,β-D-*erythro*-pentofuranosyl]thymine 34

Compound **33** (191 mg, 0.19 mmol) was dissolved in absolute EtOH (2.5 cm^3) and 20% $Pd(OH)_2$–C (190 mg) was added. After degassing with H_2 the reaction mixture was heated to 60 °C under H_2 and was stirred for 3 days. Filtration through Celite, evaporation under reduced pressure, and PLC (CH_2Cl_2–MeOH; 19 : 1, v/v) of the residue afforded nucleoside **34** (69 mg, 89%) as a solid which was used in the next step without further purification; $\delta_H(CDCl_3)$ 1.94, 2.01, 2.10, 2.12 and 2.12 (18 H, 5 s), 2.62–2.87 (4 H, m), 3.46 (2 H, s), 3.58–3.73 (8 H, m), 3.84–4.46 (9 H, m), 4.83–4.84 (1 H, m), 6.18–6.24 (2 H, m), 7.30–7.39 (2 H, m), 9.66 and 9.70 (2 H, 2 br s); $\delta_C(CDCl_3)$ 12.39, 12.50, 20.77, 21.62, 40.19, 40.69, 61.41, 62.97, 68.83, 69.44, 72.96, 82.24, 83.53, 84.20, 85.92, 86.95, 87.66, 110.04, 111.22, 135.14, 150.32, 150.41, 163.99, 169.97, 170.37 and 170.51.

1-{3,5-Di-*O*-acetyl-3-*C*-[2-(*tert*-butyldimethylsilyloxy)ethoxymethyl]-2-deoxy-α,β-D-*erythro*-pentofuranosyl}thymine 35

Compound **34** (55 mg, 0.14 mmol) was dissolved in anhydrous DMF (0.5 cm^3) and imidazole (23 mg, 0.34 mmol) and TBDMSCl (30 mg, 0.20 mmol) were added. After being stirred for 2 h at room temperature, the reaction mixture was evaporated under reduced pressure and saturated aq. $NaHCO_3$ (5 cm^3) was added followed by extraction with CH_2Cl_2 (4 × 8 cm^3). The combined organic phase was dried ($MgSO_4$), and evaporated under reduced pressure. PLC (CH_2Cl_2–MeOH, 49 : 1, v/v) afforded nucleosides **35** (59 mg, 84%) as a solid, $\delta_H(CDCl_3)$ 0.06 (s), 0.88 and 0.89 (2 s), 1.94, 2.00, 2.11 and 2.12 (4 s), 2.15–2.25 (m), 2.70–2.85 (m), 3.50–3.77 (m), 3.80–4.51 (m), 4.83 (dd, J_1 3.4, J_2 5.6), 6.20 (dd, J_1 3.7, J_2 6.4), 6.28 (dd, J_1 5.9, J_2 8.8), 7.35–7.37 (m), 9.50 and 9.53 (2 br s); $\delta_C(CDCl_3)$ −5.44, 12.52, 12.54, 18.17, 20.80, 21.65, 21.67, 25.75, 40.24, 40.62, 62.32, 62.42, 62.92, 62.97, 68.98, 69.59, 73.04, 73.13, 82.41, 83.27, 84.55, 86.06, 86.93, 87.93, 109.92, 111.27, 134.91, 135.21, 150.24, 150.40, 163.70, 163.94, 169.79 and 170.20.

3′-*C*-[2-(*tert*-Butyldimethylsilyloxy)ethoxymethyl]thymidine 36 and 1-{3-*C*-[2-(*tert*-butyldimethylsilyloxy)ethoxymethyl]-2-deoxy-α-D-*erythro*-pentofuranosyl}thymine 37

Compound **35** (50 mg, 0.10 mmol) was dissolved in a saturated solution of NH_3 in MeOH (8 cm^3) in a sealed flask and the mixture was stirred at room temperature for 2 days. After evaporation under reduced pressure, PLC (EtOAc) afforded anomer **36** [10 mg, 24%; R_f 0.4 (TLC run twice in hexane–EtOAc 1 : 1, v/v)] and anomer **37** (27 mg, 65%; R_f 0.3 (TLC run twice in hexane–EtOAc 1 : 1, v/v)] as solids.

Compound **36**: $\delta_H(CDCl_3)$ 0.08 (6 H, s), 0.91 (9 H, s), 1.91 (3 H, s), 2.18–2.30 (2 H, m), 3.33 and 3.46 (2 H, 2 br s), 3.65–3.83 (8 H, m), 3.99 (1 H, t, J 2.8), 6.20 (1 H, dd, J_1 5.9, J_2 8.7), 7.66 (1 H, m) and 8.73 (1 H, br s); $\delta_C(CDCl_3)$ −5.38, 12.49, 18.33, 25.87, 40.89, 61.70, 62.47, 73.26, 73.40, 80.41, 86.54, 88.07, 110.95, 137.42, 150.50 and 163.67.

Compound **37**: $\delta_H(CDCl_3)$ 0.09 (6 H, s), 0.91 (9 H, s), 1.90 (3 H, s), 2.09 (1 H, dd, J_1 1.8, J_2 14.4), 2.55 (1 H, dd, J_1 8.1, J_2 14.6), 3.00 (1 H, br s), 3.59–3.82 (9 H, m), 4.30 (1 H, t, J 3.7), 6.34 (1 H, dd, J_1 2.2, J_2 8.0), 7.79–7.80 (1 H, m) and 9.18 (1 H, br s); $\delta_C(CDCl_3)$ −5.39, 12.51, 18.29, 25.85, 42.12, 62.06, 62.64, 73.56, 73.71, 80.24, 85.99, 90.05, 110.13, 137.46, 150.81 and 164.17.

1-[3-*C*-(3-Benzoyloxypropyl)-3,5-di-*O*-benzyl-β-D-ribofuranosyl]thymine 42

Nucleoside **41**[32] (2.63 g, 5.30 mmol) was coevaporated with anhydrous CH_3CN (3 × 15 cm^3) and redissolved in anhydrous CH_2Cl_2 (30 cm^3). 2,6-Lutidine (1.85 cm^3, 15.9 mmol) was added and the mixture was cooled to −40 °C. To the stirred solution was added dropwise benzoyl chloride (0.65 cm^3, 5.57 mmol) and the mixture was allowed to warm to room temperature. After 2 h, the same amounts of benzoyl chloride and 2,6-lutidine were again added at −40 °C whereupon the mixture was allowed to warm to room temperature. After additional stirring for 1 h, the reaction was quenched with ice-cold water (20 cm^3) and the mixture was extracted with CH_2Cl_2 (3 × 30 cm^3). The combined extract was washed successively with ice-cold, saturated aq. NaCl acidified with HCl to pH ≈ 2 (2 × 30 cm^3) and with saturated aq. $NaHCO_3$ (2 × 20 cm^3). The organic phase was dried (Na_2SO_4), and evaporated under reduced pressure. Purification by silica gel column chromatography (CH_2Cl_2–MeOH 97 : 3, v/v) afforded nucleoside **42** (1.98 g, 62%) as a solid, $\delta_H(CDCl_3)$ 1.47 (3 H, d, J 0.6), 1.95–2.15 (3 H, m), 2.20–2.38 (1 H, m), 3.53 (1 H, d, J 10.3), 3.81–3.87 (1 H, dd, J_1 2.9, J_2 11.0), 4.26 (1 H, d, J 7.8), 4.34–4.40 (3 H, m), 4.52–4.57 (4 H, m), 6.18 (1 H, d, J 7.8), 7.25–7.56 (12 H, m), 7.62 (1 H, d, J 1.0), 8.00–8.10 (3 H, m) and 9.31 (1 H, br s); $\delta_C(CDCl_3)$ 11.7, 22.4, 26.7, 64.3, 64.9, 69.7, 73.5, 79.2, 81.0, 82.5, 87.3, 111.2, 127.3, 127.9, 128.2, 128.3, 128.5, 128.6, 129.3, 129.9, 130.0, 132.9, 136.0, 136.5, 137.0, 151.2, 163.8 and 166.5; FAB-MS *m*/*z* 601 $[M + H]^+$ (Found: C, 67.5; H, 6.0; N, 4.2. Calc. for $C_{34}H_{36}N_2O_8 \cdot 0.25H_2O$: C, 67.5; H, 6.0; N, 4.6%).

1-[3-*C*-(3-Benzoyloxypropyl)-3,5-di-*O*-benzyl-2-*O*-(pentafluorophenoxythiocarbonyl)-β-D-ribofuranosyl]thymine 43

Nucleoside **42** (2.85 g, 4.7 mmol) was coevaporated in anhydrous pyridine (3 × 10 cm^3) and redissolved in a mixture of anhydrous pyridine (20 cm^3) and anhydrous CH_2Cl_2 (20 cm^3). DMAP (15 mg, 0.12 mmol) was added and the solution was cooled to −12 °C. $C_6F_5OC(S)Cl$ (1.53 cm^3, 9.5 mmol) was added slowly with vigorous stirring and the mixture was allowed to warm to room temperature and was stirred overnight. The mixture was poured into ice-cold water (50 cm^3), filtered, and extracted with CH_2Cl_2 (3 × 30 cm^3). The combined extract was washed with saturated aq. $NaHCO_3$ (2 × 30 cm^3), dried (Na_2SO_4), and evaporated under reduced pressure. Purification by silica gel column chromatography (CH_2Cl_2–MeOH 199 : 1, v/v) afforded nucleoside **43** (2.02 g, 52%) as a light brown solid which was used in the next step without further purification, $\delta_H(CDCl_3)$ 1.40 (3 H, d, J 1.0), 1.90–2.19 (4 H, m), 3.65 (1 H, d, J 10.9), 3.92–3.97 (1 H, dd, J_1 2.4, J_2 11.0), 4.31–4.37 (2 H, m), 4.48 (1 H, br s), 4.55–4.59 (2 H, m), 4.64 (1 H, d, J 10.5), 4.75 (1 H, d, J 11.5), 6.26 (1 H, d, J 8.0), 6.65 (1 H, d, J 8.0) and 7.25–8.61 (1 H, m); FAB-MS *m*/*z* 827 $[M + H^+]$.

3′-*C*-(3-Benzoyloxypropyl)-3′,5′-di-*O*-benzylthymidine 44

A stirred solution of nucleoside **43** (2.03 g, 2.46 mmol) and AIBN (0.202 g, 1.23 mmol) in anhydrous benzene (10 cm^3) was degassed by bubbling argon through the solution for 30 min. Bu_3SnH (1.96 cm^3, 7.4 mmol) was added at 90 °C and the mixture was refluxed for 1 h. The mixture was then allowed to cool to room temperature and the solvent was removed under reduced pressure. Purification by silica gel column chromatography [(1) light petroleum, (2) light petroleum–EtOAc 9 : 1, v/v, (3) CH_2Cl_2–MeOH 99 : 1, v/v] afforded 2′-deoxynucleoside **44** (764 mg, 52%) as a solid, $\delta_H(CDCl_3)$ 1.55 (3 H, d, J 0.5, CH_3), 1.89–2.09 (5 H, m, H^a-2′, H_2-2″, H_2-1″), 2.57–2.64 (1 H, dd, J_1 5.2, J_2 12.8, H^b-2′), 3.57–3.62 (1 H, dd, J_1 1.3, J_2 11.0, H^a-5′), 3.81–3.87 (1 H, dd, J_1 2.9, J_2 10.9, H^b-5′), 4.32–4.42 (3 H, m, 4′-H, H_2-3″), 4.47 (2 H, d, J 2.8, Bn), 4.52 (2 H, s, Bn), 6.40–6.46 (1 H, dd, J_1 5.1, J_2 9.4, H-1′), 7.23–7.55 (13 H, m, Bn, Bz), 7.75 (1 H, d, J 1.0, H-6), 7.99–8.03 (2 H, m, Bn) and 8.87 (1 H, br s, NH); $\delta_C(CDCl_3)$ 11.9 (CH_3), 23.6 and 26.8 (C-1″, -2″), 41.8 (C-2′), 63.9 and 64.6 (C-3″ and -5′), 70.1 and 73.5 (Bn),

82.9, 84.4 and 86.3 (C-1′, -3′, -4′), 110.5 (C-5), 127.0, 127.3, 127.5, 128.1, 128.3, 128.4, 128.5, 129.3, 130.0, 132.9, 136.1, 136.7 and 137.7 (C-6, Bn, Bz), 150.3 (C-2), 163.7 (C-4) and 166.4 (C=O); FAB-MS *m/z* 585 $[M + H]^+$ (Found: C, 68.4; H, 6.1; N, 4.8. Calc. for $C_{34}H_{36}N_2O_7 \cdot 0.75H_2O$: C, 68.3; H, 6.3; N, 4.7%).

3′-*C*-(3-Benzoyloxypropyl)thymidine 45

To a stirred solution of nucleoside **44** (694 mg, 1.19 mmol) in absolute EtOH (8 cm^3) was added 20% $Pd(OH)_2$–C (570 mg). After degassing several times with H_2, the reaction mixture was stirred overnight under H_2 at room temperature. The mixture was filtered through a Celite pad saturated with 10% MeOH in CH_2Cl_2. The pad was washed thoroughly with 10% MeOH in CH_2Cl_2 and the filtrate was evaporated under reduced pressure. Purification by silica gel column chromatography (CH_2Cl_2–MeOH 24:1, v/v) afforded nucleoside diol **45** (314 mg, 65%) as a solid which was used in the next step without further purification, $\delta_H(CD_3OD)$ 1.96 (3 H, d, *J* 0.7, CH_3), 1.97–2.12 (4 H, m, H_2-1″, H_2-2″), 2.13–2.22 (1 H, dd, J_1 9.5, J_2 12.7, H^a-2′), 2.32–2.39 (1 H, dd, J_1 5.4, J_2 12.6, H^b-2′), 3.78–3.84 (1 H, dd, J_1 3.4, J_2 12.0, H^a-5′), 3.87–3.93 (1 H, dd, J_1 3.1, J_2 12.0, H^b-5′), 4.02 (1 H, t, *J* 3.1, H-4′), 4.43–4.48 (2 H, m, H_2-3″), 6.42–6.48 (1 H, dd, J_1 5.3, J_2 9.4, H-1′), 7.53–7.69 (3 H, m, Bz), 8.10–8.13 (2 H, m, Bz) and 8.19 (1 H, d, *J* 0.9, H-6); $\delta_C(CD_3OD)$ 12.8, 25.3, 33.1, 44.4, 62.8, 66.7, 82.2, 86.4, 90.7, 111.6, 129.9, 130.8, 131.8, 134.5, 139.0, 152.8, 166.8 and 168.4 (Found: FAB-MS $[M + H]^+$, 405.1653. Calc. for $C_{20}H_{25}N_2O_7$: *m/z*, 405.1662).

3′-*C*-(3-Benzoyloxypropyl)-5′-*O*-(4,4′-dimethoxytrityl)-thymidine 46

Nucleoside **45** (282 mg, 0.70 mmol) was coevaporated with anhydrous pyridine (4 × 5 cm^3) and redissolved in anhydrous pyridine (5 cm^3). DMTCl (473 mg, 1.39 mmol) was added and the mixture was stirred overnight at room temperature. The reaction was quenched with MeOH (1 cm^3), and the mixture was evaporated under reduced pressure. The residue was dissolved in CH_2Cl_2 (30 cm^3) and the solution was washed with brine (3 × 20 cm^3). The water phase was extracted with CH_2Cl_2 (3 × 10 cm^3) and the combined organic phase was dried (Na_2SO_4), and evaporated under reduced pressure. Purification by silica gel column chromatography (CH_2Cl_2–MeOH–pyridine 98:1:1, v/v/v) afforded nucleoside **46** (444 mg, 90%) as a solid, $\delta_H(CD_3OD)$ 1.24 (3 H, d, *J* 0.6), 1.94–2.08 (4 H, m), 2.36 (1 H, dd, J_1 9.8, J_2 12.6), 2.51 (1 H, dd, J_1 5.2, J_2 12.6), 3.33 (1 H, dd, J_1 2.0, J_2 10.9), 3.80 (1 H, dd, J_1 3.4, J_2 11.1), 3.85 (3 H, s), 3.86 (3 H, s), 4.14 (1 H, br s), 4.25–4.29 (2 H, m), 6.62 (1 H, dd, J_1 5.1, J_2 9.8), 6.89–6.95 (4 H, m), 7.34–7.72 (12 H, m) and 8.08–8.14 (3 H, m); $\delta_C(CD_3OD)$ 11.5, 24.7, 33.3, 44.3, 55.7, 63.8, 66.5, 81.7, 85.5, 88.7, 89.7, 111.8, 114.1, 128.4, 128.9, 129.6, 129.8, 130.5, 131.5, 131.7, 134.2, 135.7, 136.2, 138.2, 145.1, 152.5, 160.4, 160.5, 166.3 and 168.0; FAB-MS *m/z* 706 $[M + H]^+$ (Found: C, 69.7; H, 6.0; N, 4.2. Calc. for $C_{41}H_{42}N_2O_9$: C, 69.7; H, 6.0; N, 4.0%).

3′-*C*-(3-Benzoyloxypropyl)-3′-*O*-[2-cyanoethoxy(diisopropylamino)phosphino]-5′-*O*-(4,4′-dimethoxytrityl)thymidine 47

Nucleoside **46** (390 mg, 0.55 mmol) was coevaporated with anhydrous CH_3CN (3 × 2 cm^3) and dissolved in CH_2Cl_2 (2 cm^3). DIPEA (0.55 cm^3) was added followed by dropwise addition of 2-cyanoethyl *N*,*N*-diisopropylphosphoramidochloridite (0.26 cm^3, 1.1 mmol) at room temperature. After 4 h, the reaction was quenched with MeOH (0.2 cm^3) and the mixture was diluted with EtOAc (5 cm^3). The solution was washed successively with saturated aq. $NaHCO_3$ (2 × 10 cm^3) and brine (2 × 10 cm^3). The organic phase was dried (Na_2SO_4), and evaporated under reduced pressure. The residue was purified by silica gel column chromatography (CH_2Cl_2–NEt_3 49:1, v/v) and after evaporation the product was redissolved in anhydrous toluene (3 cm^3) and precipitated from light petroleum (250 cm^3) at −65 °C. The product was collected by filtration and was dried (Na_2SO_4) to afford derivative **47** (357 mg, 71%) as a solid, $\delta_P(CDCl_3)$ 139.97 and 140.33 [besides which a minor (<25% intensity) non-identified peak at δ_P 136.27 was observed]; FAB-MS *m/z* 929 $[M + Na]^+$.

Acknowledgements

The Danish Natural Science Research Council and The Danish International Development Agency are thanked for generous financial support.

References

1 E. Uhlmann and A. Peyman, *Chem. Rev.*, 1990, **90**, 543.
2 S. L. Beaucage and R. P. Iyer, *Tetrahedron*, 1993, **49**, 6123.
3 Y. S. Sanghvi and P. D. Cook, *Carbohydrate Modifications in Antisense Research*, ACS Symposium Series No. 580, American Chemical Society, Washington, DC, 1994.
4 E. A. Lesnik, C. J. Guinosso, A. M. Kawasaki, H. Sasmor, M. Zounes, L. L. Cummins, D. J. Ecker, P. D. Cook and S. M. Freier, *Biochemistry*, 1993, **32**, 7832.
5 A. M. Kawasaki, M. D. Casper, S. M. Freier, E. A. Lesnik, M. C. Zounes, L. L. Cummins, C. Gonzales and P. D. Cook, *J. Med. Chem.*, 1993, **36**, 831.
6 (*a*) A. K. Saha, T. J. Caulfield, C. Hobbs, D. A. Upson, C. Waychunas and A. M. Yawman, *J. Org. Chem.*, 1995, **60**, 788; (*b*) G. Wang and P. J. Middleton, *Tetrahedron Lett.*, 1996, **37**, 2739; (*c*) J. Fensholdt and J. Wengel, *Acta Chem. Scand.*, 1996, **50**, 1157.
7 (*a*) H. Thrane, J. Fensholdt, M. Regner and J. Wengel, *Tetrahedron*, 1995, **51**, 10 389; (*b*) G. Wang and W. E. Seifert, *Tetrahedron Lett.*, 1996, **37**, 6515.
8 (*a*) P. N. Jørgensen, P. C. Stein and J. Wengel, *J. Am. Chem. Soc.*, 1994, **116**, 2231; (*b*) P. N. Jørgensen, M. L. Svendsen, C. Scheuer-Larsen and J. Wengel, *Tetrahedron*, 1995, **51**, 2155; (*c*) P. N. Jørgensen, M. L. Svendsen, C. Nielsen and J. Wengel, *Nucleosides, Nucleotides*, 1995, **14**, 912.
9 R. W. Wagner, *Nature*, 1994, **372**, 333.
10 D. M. Huryn and M. Okabe, *Chem. Rev.*, 1992, **92**, 1745.
11 C. Scheuer-Larsen, H. M. Pfundheller and J. Wengel, *J. Org. Chem.*, 1995, **60**, 7298.
12 M. L. Froehlich, D. J. Swartling, R. E. Lind, A. W. Mott and D. E. Bergstrom, *Nucleosides, Nucleotides*, 1989, **8**, 1529.
13 K. K. Ogilvie, *Can. J. Chem.*, 1973, **51**, 3799.
14 V. G. Höfle, W. Steglich and H. Vorbrüggen, *Angew. Chem.*, 1978, **90**, 602.
15 H. Vorbrüggen, K. Krolikiewicz and B. Bennua, *Chem. Ber.*, 1981, **114**, 1234.
16 (*a*) R. U. Lemieux and M. Hoffer, *Can. J. Chem.*, 1961, **39**, 110; (*b*) U. Séquin and Ch. Tamm, *Helv. Chim. Acta*, 1972, **55**, 1196; (*c*) R. A. Long, R. K. Robins and L. B. Townsend, *J. Org. Chem.*, 1967, **32**, 2751.
17 (*a*) M. Okabe, R.-C. Sun, S. Y.-K. Tam, L. J. Todaro and D. L. Coffen, *J. Org. Chem.*, 1988, **53**, 4780; (*b*) K. C. Schneider and S. A. Benner, *Tetrahedron Lett.*, 1990, **31**, 335.
18 K. Augustyns, J. Rozenski, A. Van Aerschot, G. Janssen and P. Herdewijn, *J. Org. Chem.*, 1993, **58**, 2977.
19 M. Koga, A. Wilk, M. F. Moore, C. L. Scremin, L. Zhou and S. L. Beaucage, *J. Org. Chem.*, 1995, **60**, 1520.
20 M. R. Barvian and M. M. Greenberg, *J. Org. Chem.*, 1993, **58**, 6151.
21 J. Wengel, M. L. Svendsen, P. N. Jørgensen and C. Nielsen, *Nucleosides, Nucleotides*, 1995, **14**, 1465.
22 (*a*) C. Schmit, M.-O. Bévierre, A. De Mesmaeker and K.-H. Altmann, *Bioorg. Med. Chem. Lett.*, 1994, **4**, 1969; (*b*) I. I. Fedorov, E. M. Kazmina, N. A. Novicov, G. V. Gurskaya, A. V. Bochkarev, M. V. Jasco, L. S. Victorova, M. K. Kukhanova, J. Balzarini, E. De Clerq and A. A. Krayevski, *J. Med. Chem.*, 1992, **35**, 4567; (*c*) G. Wang, P. J. Middleton, L. He, V. Stoisavljevic and W. E. Seifert, *Nucleosides, Nucleotides*, 1997, **16**, 445.
23 M. von Büren, G. V. Petersen, K. Rasmussen, G. Brandenburg, J. Wengel and F. Kirpekar, *Tetrahedron*, 1995, **51**, 8491.
24 G. Viswanadham and J. Wengel, unpublished results.
25 M. S. Motawia and E. B. Pedersen, *Liebigs Ann. Chem.*, 1990, 599.
26 P. N. Jørgensen, U. S. Sørensen, H. M. Pfundheller, C. E. Olsen and J. Wengel, *J. Chem. Soc., Perkin Trans. 1*, 1997, 3275.
27 P. J. Garegg and B. Samuelsson, *Carbohydr. Res.*, 1978, **67**, 267.

28 (*a*) L. Lombardo, *Tetrahedron Lett.*, 1982, **23**, 4293; (*b*) M. Sharma and M. Bobek, *Tetrahedron Lett.*, 1990, **31**, 5839.
29 V. VanRheenen, R. C. Kelly and D. Y. Cha, *Tetrahedron Lett.*, 1976, 1973.
30 G. M. Bennett, *J. Chem. Soc.*, 1925, **127**, 1277.
31 Y. Yoshimura, T. Sano, A. Matsuda and T. Ueda, *Chem. Pharm. Bull.*, 1988, **36**, 162.
32 P. Nielsen, H. M. Pfundheller and J. Wengel, (*a*) *Chem. Commun.*, 1997, 825; (*b*) P. Nielsen, H. M. Pfundheller, C. E. Olsen and J. Wengel, *J. Chem. Soc.*, *Perkin Trans. 1*, 1997, 3423.
33 K. C. Nicolaou, R. A. Daines, J. Uenishi, W. S. Li, D. P. Papahatjis and T. K. Chakraborty, *J. Am. Chem. Soc.*, 1988, **110**, 4672.
34 U. Pieles, W. Zürcher, M. Schär and H. E. Moser, *Nucleic Acids Res.*, 1993, **21**, 3191.
35 S. K. Sharma and J. Wengel, unpublished results.
36 (*a*) D. M. Tidd and H. M. Warenius, *Br. J. Cancer*, 1989, **60**, 343; (*b*) J. P. Shaw, K. Kent, J. Bird, J. Fishback and B. Froehler, *Nucleic Acids Res.*, 1991, **19**, 747.
37 M. L. Svendsen, J. Wengel, O. Dahl, F. Kirpekar and P. Roepstorff, *Tetrahedron*, 1993, **49**, 11 341.
38 Y. Sugiura, S. Furuya and Y. Furukuwa, *Chem. Pharm. Bull.*, 1988, **36**, 3253.

Paper 7/08801I
Received 8*th December* 1997
Accepted 10*th February* 1998