Synthesis of 2′-*O*,3′-*C*-linked bicyclic nucleosides and bicyclic oligonucleotides

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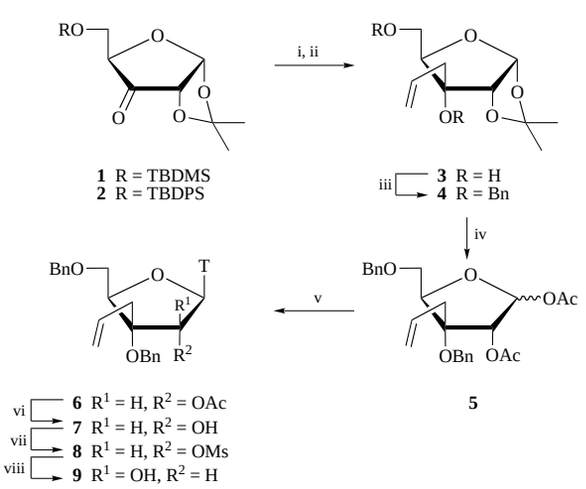
The 3′-*C*-allyl furanose 4 has been used as a precursor for synthesis of the novel 2′-O,3′-C-linked bicyclic thymine nucleosides 15, 16, 20 and 25. The three bicyclic β-nucleosides 15, 20 and 25 have been incorporated into oligodeoxynucleotides. One of these nucleosides, dioxabicyclo[3.3.0]octane derivative 25, induces increased thermal stability of duplexes towards complementary RNA.

Introduction

Modified oligonucleotides (ONs) able to hybridise effectively with single-stranded RNA (ssRNA) are currently being extensively evaluated as novel therapeutic agents (antisense or ribozyme approach).[1] In the present work, the possibility of enhancing duplex stability by incorporating conformationally preorganised monomeric nucleosides has been investigated.[2] A number of conformationally restricted monomers and the corresponding oligonucleotide analogues have been reported.[3] Thus, bicyclonucleosides with an additional 3′,5′-ethylene bridge,[4] bicyclic carbocyclic nucleosides with a 1′,6′- or a 4′,6′-methano bridge,[5] 2′,3′-riboacetal dimers,[6] 3′,6′-anhydrogluco-2′,5′-formacetal trimers[7] and 1,5-anhydro-2,3-dideoxy-D-arabinohexitol nucleosides[8] have been synthesised and incorporated into oligodeoxynucleotides (ODNs). In several cases,[4,5,8] increased thermal stabilities of duplexes towards complementary RNA were observed.

We decided to synthesise new bicyclic nucleosides containing an additional ring between the 2′- and the 3′-carbon atoms thereby hoping to introduce conformational preorganisation that could favourably influence the thermal stability of the corresponding duplexes. Based on molecular modelling studies and continuing our research on modified ODNs containing derivatised pentofuranose nucleosides linked through natural 5′-*O*-to-3′-*O*-phosphordiester linkages,[9] we chose nucleosides **15**, **20** and **25** as candidates. Retrosynthetic analysis revealed 3′-*C*-allyl-5-methyluridine to be an ideal precursor for the 3′-C,2′-*O*-linked bicyclic nucleosides. Synthesis of 3′-*C*-allyl-2′,3′-dideoxyuridine and 3′-*C*-allyl-3′-deoxythymidine has been accomplished using a free-radical method.[10] Synthesis of 2′-*O*-protected *xylo* and *ribo* configurated 3′-*C*-allyluridines has been reported as the first examples of allyl Grignard additions on 3′-ketonucleosides.[11] Other additions on either 2′-deoxy-3′-keto nucleosides or on 2′-*O*-protected 3′-ketonucleosides have been accomplished using ordinary or cerium-assisted Grignard, or organolithium reagents. Thus, methyl, ethyl, vinyl and ethynyl groups have been added to the 3′-position of nucleosides generally affording the *xylo* isomers as the major products.[12] Although 3′-*C*-allyluridine can be synthesised from a linear strategy,[11] the corresponding *xylo* isomer was obtained as well, and the radical method results in 3′-deoxy nucleosides.[10] We therefore preferred a convergent synthetic strategy for the present work.

Several 3′-*C*-alkyl nucleosides have been synthesised from 3-ketofuranoses and Grignard reagents followed by nucleobase coupling.[13–15] In one case starting from methyl 2-deoxy-3-ketofuranosides, *erythro* and *threo* isomers of both α- and β-nucleosides were obtained.[15] In other cases, 3-ketones of 5-protected 1,2-*O*-isopropylidene-α-D-xylofuranoses (compounds **1** and **2**) exhibited excellent stereoselectivities towards 3-*C*-alkyl ribofuranose products. As examples, 3′-*C*-methyl-[14,15] and 3′-*C*-ethynyl-β-D-*ribo*-nucleosides[13] were effectively synthesised using this approach. Based on these results, the syntheses of 3′-*C*-allyl-5-methyluridines described here (Scheme 1) were



Scheme 1 *Reagents and yields:* i, AllylMgBr, Et_2O, THF; ii, TBAF, THF (for two steps: 69% from **1**, 58% from **2**); iii, BnBr, NaH, DMF (86%); iv, (**1**) 80% aq. AcOH, (2) Ac_2O, pyridine (97%); v, thymine, BSA, CH_3CN, TMS triflate (86%); vi, CH_3ONa, CH_3OH (95%); vii, MsCl, pyridine (89%); viii, aq. NaOH, ethanol (74%). TBDMS = Bu^tMe_2Si, TBDPS = Bu^tPh_2Si. T = thymin-1-yl.

accomplished using a strategy comparable to the one used earlier by Schmit *et al.* for synthesis of 3′-*C*-methyluridines.[14]

The allyl group has the advantage of being a 'hidden protecting group' as the double bond *e.g.* can be oxidatively cleaved or hydrated and thus converted into a 2-hydroxyethyl or a 3-hydroxypropyl group. We envisaged that subsequent cyclisations with 2′-hydroxy groups, either before or after introduction of the nucleobase, should be a viable strategy for synthesis of the bicyclic building blocks **15**, **20** and **25**. Direct addition of a 3-hydroxypropyl group to a ketone using the Grignard reagent $MgCl(CH_2)_3OMgCl$[16,17] has been reported, but we preferred the allyl group which alleviated the need for an additional protecting group.

Results

Synthesis of 3′-*C*-allyl nucleosides 7 and 9

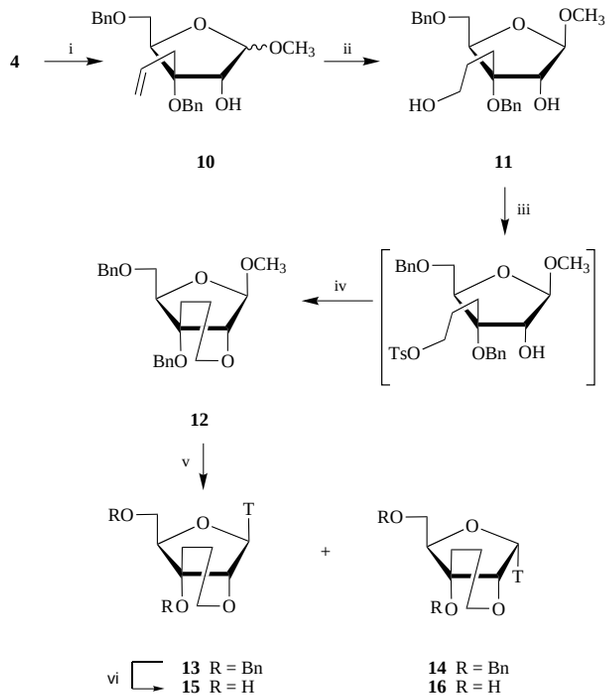
Starting from one of the well known uloses **1**[18] or **2**[19] (Scheme 1), Grignard addition of an allyl group using allylmagnesium bromide afforded 3-*C*-allyl-1,2-*O*-isopropylidene-α-D-ribofuranose **3** in good yield (69% from **1** and 58% from **2**) after removal of the silyl groups. For protection of the two hydroxy functionalities we needed a group showing high stability under both acidic and basic conditions, wherefore benzyl groups were chosen. Benzylation afforded the diprotected compound **4** in 86% yield. To prepare for nucleoside synthesis, the isopropylidene group was removed and the diacetylated compound **5** was synthesised in high yield (97% from **4**) using aq. acetic acid followed by acetylation. An attempt to use trifluoroacetic acid (TFA)[14] turned out to give a significantly lower yield of compound **5**. Taking advantage of anchimeric assistance from the 2′-*O*-acetyl group, standard Vorbrüggen coupling[20] with thymine as nucleobase afforded exclusively the β-nucleoside **6** in good yield (86%). Removal of the 2′-*O*-acetyl group afforded the key nucleoside **7** in a satisfactory overall yield (47%) from 3-ketofuranose **1**. The β-*ribo* configuration of compound **7** was verified by a nuclear Overhauser enhancement (NOE) experiment. Thus, mutual NOEs between H-1′ and H-4′ and between H-2′ and H-6 confirmed the anomeric configuration as β, and the positioning of the 3′-*C*-allyl group on the β-face of the furanose ring was verified by mutual NOEs between H-1″ and H-2′ and between H-1″ and H-5′.†

Synthesis of the *arabino*-isomer **9** was accomplished by inverting the configuration at the 2′-carbon atom of nucleoside **7** by using the 'anhydro approach'.[21] Thus, mesylation of *ribo*-alcohol **7** gave 2′-*O*-mesyl nucleoside **8** in good yield (89%) and subsequent treatment with aq. base afforded the 2,2′-anhydronucleoside as an intermediate, which was directly hydrolysed to give the nucleoside **9** in an acceptable yield (74%). The *arabino* configuration of product **9** was verified through an NOE experiment on nucleoside **20** (*vide infra*).

Synthesis of bicyclic nucleosides 15 and 16 from bicyclic furanoside 12

The isopropylidene-protected ribofuranose **4** was directly converted into the corresponding methyl furanoside **10** in good yield (82%) by using methanolic hydrochloric acid (Scheme 2). This anomeric mixture was boronated, then was subjected to alkaline hydrogen peroxide oxidation to give exclusively the anti-Markovnikov product. After chromatographic separation, the β-anomer **11** was isolated in a good yield (62%) along with a mixture of β-anomer **11** and its α-anomer (32%). Compound **11** was selectively tosylated at the primary hydroxy functionality and, without purification, was converted into the bicyclic methyl furanoside **12** in an acceptable yield (42%) by using potassium hydroxide. Attempts to synthesise this compound using Mitsunobu conditions failed. The structures of compounds **10**–**12** were verified using MS, ^{1}H NMR, ^{1}H–^{1}H chemical-shift correlation (COSY) and ^{13}C NMR experiments. The bicyclic structure of product **12** was indicated by mass spectrometry.

The bicyclic methyl furanoside **12** was coupled with thymine by using *N*,*O*-bis(trimethylsilyl)acetamide (BSA) and trimethylsilyl trifluoromethanesulfonate (TMS triflate) to give the two anomeric bicyclic nucleosides **13** and **14** in low yields of 19% and 31%, respectively. A first attempt using 1,2-dichloroethane as solvent instead of acetonitrile gave even lower yields combined with small amounts of acyclic derivatives as described earlier for other nucleoside couplings[22] [plasma desorption mass spectrometry (PDMS) and NMR indicated the presence of both the nucleobase and the 1′-*O*-methyl functionality]. Attempts to improve the yield of the nucleoside-coupling reaction by converting the methyl furanoside into a 1′-*O*-acetylated analogue using strong acid failed as the benzyl ethers were unstable under these conditions. The assigned structures of products **13** and **14** were verified using MS, ^{1}H NMR, ^{1}H–^{1}H COSY and NOE experiments. The mutual NOEs between H-1′ and H-4′, between H-2′ and H-5′, and between H-2′ and H-6 confirmed the β-*ribo* configuration of compound **13**. The bicyclic structure was verified by mutual NOEs between H-2′, H-1″ and H-3″ and between H-1″, H-2″ and 3′-OCH_2Ph. The mutual NOEs between H-1′ and H-2′ and between H-6 and H-4′ indicated an α-*ribo* configuration for compound **14**. The bicyclic structure was analogously verified by mutual NOEs between H-2′, H-1″ and H-3″.



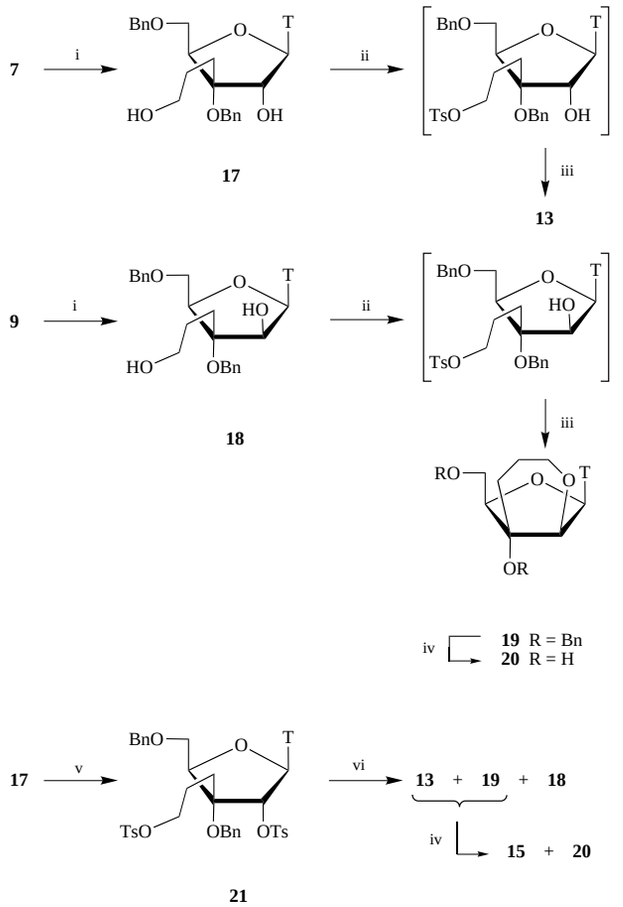
Scheme 2 *Reagents and yields:* i, 20% HCl in CH_3OH, water (82%); ii, (1) BH_3–1,4-oxathiane, THF, (2) aq. NaOH, H_2O_2 (62% isolated **11**); iii, TsCl, pyridine; iv, KOH, 18-crown-6, DMF (for two steps: 42%); v, thymine, BSA, CH_3CN, TMS triflate (19% isolated **13**, 31% isolated **14**); vi, H_2, 20% $Pd(OH)_2$/C, ethanol (73% isolated **15**, 70% isolated **16**). T = thymin-1-yl.

The nucleosides **13** and **14** were deprotected by using catalytic hydrogenation to give bicyclic nucleoside diols **15** and **16** in acceptable yields of 73% and 70%, respectively. The structures were verified from MS and NMR spectroscopy as described for precursors **13** and **14**. Summarising, the bicyclic β-nucleoside **15** was synthesised (through furanoside **12**) from furanose **4** in an overall yield of ~4%.

Linear synthesis of bicyclic nucleosides 13, 15, 20 and 25

Since the nucleoside **13** was synthesised in an unsatisfactory overall yield using the strategy described above, and since the 3′-*C*-allyluridine derivative **7** could be synthesised conveniently without separation of anomers, a linear synthesis of the bicyclic nucleoside **13** starting from *ribo*-nucleoside **7** was investigated (Scheme 3). Boronation of compound **7** by using borane–1,4-oxathiane, followed by oxidation with alkaline hydrogen peroxide, gave the anti-Markovnikov product **17** in 54% yield plus, surprisingly, a significant amount of a by-product tentatively assigned as the Markovnikov product (17% yield). The structure of the former product was verified from MS and NMR experiments showing the transformation of the allyl group into a 3″-hydroxypropyl group. The structure of the latter product was identified from NMR spectra showing two diastereoisomers and the 3′-*C*-alkyl group [intensive nuclei enhancement by polarisation transfer (INEPT)] as a 2″-hydroxypropyl.

† Double-prime locants refer to the numbering of the 3-*C*-allyl/propyl groups.



Scheme 3 *Reagents* (see text for yields): i, (1) BH_3–1,4-oxathiane, THF, (2) aq. NaOH, H_2O_2; ii, TsCl, pyridine; iii, NaH, DMF; iv, H_2, 20% $Pd(OH)_2/C$, ethanol; v, 3.0 mol equiv. TsCl, pyridine; vi, aq. NaOH, ethanol. T = thymin-1-yl.

Selective tosylation of the primary hydroxy functionality of compound **17** proved to be difficult. In all experiments (varying temperature, base and solvent), a significant amount of the ditosylated product **21** was obtained and column chromatographic separation was necessary. The monotosylated compound [verified from fast-atom bombardment mass spectrometry (FAB-MS)] was, without further purification, treated with strong base (sodium hydride gave better results than potassium hydroxide) and nucleoside **13** was obtained in a low yield (13% from **17**). Compound **21** was obtained in 58% yield starting from diol **17** using a large excess of toluene-*p*-sulfonyl chloride.

The *arabino*-configured bicyclic nucleoside **19** was likewise synthesised using a linear strategy (Scheme 3). Thus, hydroboration/oxidation of compound **9** gave nucleoside diol **18** in acceptable yield (58%) and only trace amount (according to analytical TLC) of the assumed Markovnikov product. Selective tosylation, chromatographic separation and cyclisation again proved difficult, and compound **19** was obtained in a disappointing yield (19%). The structure of this compound was only indicated from MS and 1D NMR spectroscopy, and by direct debenzylation to afford diol **20**.

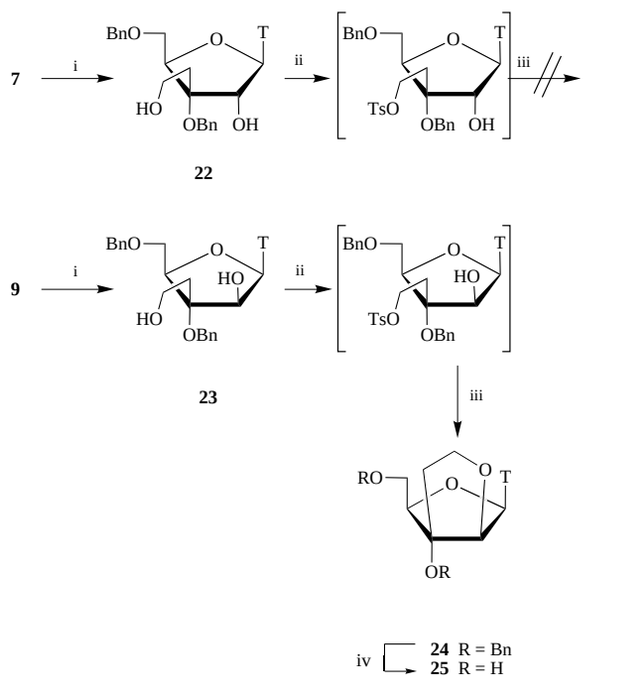
Attempts to generate the *arabino*-configured nucleoside **18** from the ditosylated *ribo*-configurated compound **21** by using aq. base and taking advantage of the 'anhydro' approach turned out to give not only hoped-for compound **18** in a small yield (19%) but also a mixture of isomers **13** and **19** in a surprisingly high yield of 50%. The ratio of isomers **13**:**19** as estimated from ^{1}H NMR spectroscopy was 3:7 and the mixture was inseparable on analytical TLC. After debenzylation of the mixture **13** + **19**, the isomeric bicyclic nucleosides **15** and **20** were separated and obtained in 15 and 38% yield, respectively.

The structure of compound **20** was verified by using MS, ^{1}H NMR, ^{1}H–^{1}H COSY and NOE experiments. The mutual NOEs between H-1′ and H-4′, between H-2′ and H-4′, and the absence of NOEs between H-5′, H-1′ and H-2′ indicated a β-*arabino* configuration.

As described above, the bicyclic nucleoside **15** was synthesised by the linear strategy in only ~3% overall yield from compound **4**. Compared with the strategy through furanoside **12** the overall yield was not improved, and both strategies include difficult separation procedures. The route *via* furanoside **12** has the advantage of involving fewer reaction steps.

Synthesis of the bicyclic nucleoside **20** was accomplished exclusively by applying a linear strategy in an overall yield of ~4% from starting material **4** through diol **18** (Scheme 3). The synthesis of diol **20** through fully protected nucleoside **21** was accomplished in an overall yield of ~5% from compound **4**. In this case, even though the yields were disappointing, a synthetic strategy based on nucleobase coupling on a precyclised furanose derivative was not investigated as the inversion of the configuration at C-2′ could be conveniently performed after nucleoside coupling.

Synthesis of bicyclic nucleosides with an additional 2′-*O*,3′-*C*-linked 5-membered ring was attempted using a linear strategy (Scheme 4). Starting from *ribo*-configured nucleoside **7**,



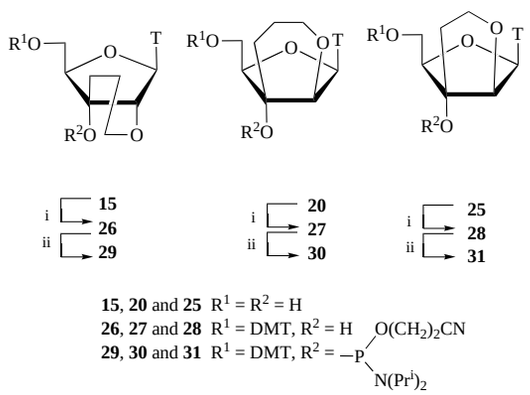
Scheme 4 *Reagents and yields:* i, (1) $NaIO_4$, cat. OsO_4, Bu^{t}OH, aq. THF, (2) $NaBH_4$, aq. THF (48% isolated **22**, 49% isolated **23**); ii, TsCl, pyridine, iii, NaH, DMF (for two steps: 83% isolated **24**); iv, H_2, 20% $Pd(OH)_2/C$, ethanol (82%). T = thymin-1-yl.

oxidative cleavage of the double bond by using osmium tetraoxide in catalytic amounts and sodium periodate as a co-oxidant followed by reduction using sodium borohydride, gave the 2″-hydroxyethyl nucleoside **22** in 48% yield. The structure was verified from MS and NMR spectroscopy showing only two methylene groups in the 3′-*C*-alkyl substituent. Selective tosylation of diol **22** was accomplished only with the same difficulties as described for the synthesis of compounds **13** and **19**, but no cyclised product could be obtained. The desired *ribo*-configured product probably involves unfavourable ring strain. However, the *arabino*-isomer **24** was easily synthesised. Cleavage of the double bond of nucleoside **9** afforded nucleoside diol **23** in 49% yield. Selective tosylation at the primary hydroxy group was uncomplicated and subsequent cyclisation gave compound **24** in high yield (83%). The structure was verified

from MS, 1D NMR, ^{1}H–^{1}H COSY and NOE experiments. The mutual NOEs between H-1′ and H-2′ and between H-1′ and H-4′ and the lack of an NOE between H-2′ and H-6 confirmed the β-*arabino* configuration. Nucleoside **24** was debenzylated using standard hydrogenation to give the bicyclic nucleoside **25** in 82% yield. This structure was analogously verified using 1D NMR, ^{1}H–^{1}H COSY and NOE experiments. The overall yield of compound **25** was a satisfactory 17% from furanose **4**. Compared with the syntheses of compounds **15** and **20**, the efficiency of the cyclisation was improved. The tosylation of diol **23** was selective, probably because of the proximity of the thymine base to the 2′-hydroxy group.

Synthesis of oligonucleotides

The bicyclic nucleosides were prepared for standard automated solid-phase oligonucleotide (ON) synthesis by the introduction of an acid-labile 5′-*O*-protecting group and a 3′-*O*-phosphoramidite functionality. Transformation of the bicyclic nucleosides **15**, **20** and **25** into the 5′-*O*-4,4′-dimethoxytrityl (DMT)-protected analogues **26**–**28** was accomplished in yields of 61–99% (Scheme 5). Subsequently, the phosphoramidites



15, **20** and **25** $R^1 = R^2 = H$
26, **27** and **28** R^1 = DMT, $R^2 = H$
29, **30** and **31** R^1 = DMT, $R^2 = -P(N(Pr^i)_2)O(CH_2)_2CN$

Scheme 5 *Reagents* (see text for yields): i, DMTCl, pyridine; ii, $NC(CH_2)_2OP(Cl)NPr^i_2$, $EtNPr^i_2$, CH_2Cl_2. T = thymin-1-yl.

29–**31** were obtained in good yield (86–95%) using standard procedures.[9]

Compounds **29**–**31** as well as commercial 3′-*O*-phosphoramidites were used to synthesise ONs **32**–**45** (Scheme 6). The

Modified sequences

5′-T_7XT_6-3′	**32** (X = **15**)	**35** (X = **20**)	**38** (X = **25**)
5′-$T_6X_2T_6$-3′	**33** (X = **15**)	**36** (X = **20**)	**39** (X = **25**)
5′-T_6XTXT_6-3′	**34** (X = **15**)	**37** (X = **20**)	**40** (X = **25**)
5′-$T_5X_4T_5$-3′			**41** (X = **25**)
5′-$T_3(TX)_4T_3$-3′			**42** (X = **25**)
5′-$X_{13}T$-3′			**43** (X = **25**)

Unmodified and complementary sequences

5′-T_{14}-3′	**44**
5′-d(A_{14})-3′	**45**
5′-r(A_{14})-3′	**46**
5′-r(A_7GA_6)-3′	**47**

Scheme 6 *Synthesised and complementary sequences:* T = thymidine monomer; dA = 2′-deoxyadenosine monomer; rA = adenosine monomer; rG = guanosine monomer; X = bicyclic monomers derived from compound **15**, **20** or **25**

purity of the modified ONs **32**–**43** was verified by capillary gel electrophoresis. Only the electropherogram of compound **43** showed minor peaks (<5%) originating from shorter sequences ($X_{10}T$, $X_{11}T$ and $X_{12}T$). Analogously, in the matrix-assisted laser-desorption ionisation (MALDI) spectrum of compound **43** in addition to the expected peak for $X_{13}T$ (deviation of 1.3 Da), minor peaks assigned to $X_{10}T$, $X_{11}T$ and $X_{12}T$ were detected. MALDI spectra of ONs **32**–**42** were recorded and showed only the expected peaks with small deviations (0–2.8 Da) from the calculated masses. The complementary ONs **46** and **47** are commercially available.

Table 1 Melting experiments of modified ONs[a]

Modified sequence	Complementary sequence **45** T_m/°C	ΔT_m/°C	**46** T_m/°C	ΔT_m/°C	**47** T_m/°C
44	35		30		25
32	25	−10	24	−6	
33	16	−19	15	−15	
34	14	−21	14	−16	
35	26	−9	20	−10	
36	17	−18	18	−12	
37	18	−17	11	−19	
38	32	−3	27	−3	
39	31	−4	28	−2	
40	30	−5	23	−7	
41	23	−12	31	+1	
42	23	−12	16	−14	
43	<10		42	+12	37

[a] Measured at 260 nm in medium salt buffer: 1 mM EDTA, 10 mM Na_3PO_4, 140 mM NaCl, pH 7.2; concentration of each strand: 1.0 μM; T_m = melting temp. determined as the local maximum of the first derivative of the absorbance *vs.* temp. curve; ΔT_m = change in T_m compared with unmodified controls.

Melting properties of oligonucleotides

The modified bicyclic sequences **32**–**43** were hybridised with the complementary DNA-segment dA_{14} (**45**) and RNA-segment rA_{14} (**46**) and the melting behaviour of these duplexes was compared with results for the unmodified reference T_{14} (**44**) (Table 1). When the [4.3.0]bicyclic nucleoside **15** was incorporated once or twice in a T_{14}-strand, the mps of the duplexes towards DNA were ~10 °C lower per modification, and towards RNA 6–8 °C lower per modification, indicating that the double-helical structures are distorted from the natural ones. The mps of duplexes towards DNA for modified ONs containing the [4.3.0]bicyclic nucleoside **20** once or twice were approximately 9 °C lower per modification and the mps of the corresponding duplexes towards RNA 6–10 °C lower per modification. With nucleoside **20** incorporated twice in a row (**36**), a duplex towards RNA seems to be significantly less destabilised than with compound **20** incorporated twice alternating with unmodified thymidine (**37**).

When the [3.3.0]bicyclic nucleoside **25** was incorporated once, twice or four times, a decrease in the mps of duplexes towards DNA of ~2–3 °C per modification was induced, whereas the variation of the mps of duplexes towards RNA was more pronounced. Thus, when the modified nucleoside **25** was incorporated once or several times alternating with thymidine, the destabilisation induced was ~3 °C per modification, but when incorporated in a row, compound **25** stabilised the duplexes with ~1 °C per modification for the almost fully modified strand $X_{13}T$ (**43**). In all cases involving duplexes **38**–**44** sharp monophasic transitions with hyperchromicity values of ~1.2 were observed.

The selectivity in base-pairing of the ONs containing the nucleoside **25** was investigated by hybridising the unmodified and the fully modified strands (compounds **43** and **44**) with the non-matched sequence **47** containing one guanosine monomer. As the mps of both duplexes were 5 °C lower than those obtained towards the corresponding matched sequence **46**, the selectivity of the new analogue, based on this first experiment, seems to be comparable to natural nucleotides, but further experiments are needed on this subject.

Discussion

From preliminary molecular modelling the [4.3.0]bicyclic

nucleoside **15** was predicted to adopt a C-2′-*endo* (*S*-type) conformation. This conformation is known to be present in a B-type duplex (most DNA–DNA and some DNA–RNA duplexes).[3] Therefore, the more pronounced destabilising effect of nucleoside **15** on a duplex towards DNA must be due to steric effects of the additional bulky six-membered ring structure. As the duplexes towards RNA were also significantly destabilised it seems unlikely that ONs containing the *ribo*-configured bicyclic nucleoside **15** can be used in antisense research.

The conformational properties of the *arabino*-configured [4.3.0]bicyclic nucleoside **20** based on molecular modelling are more ambiguous as the furanose ring was predicted to be able to exist in several conformations. Consequently, the conformational restriction is expected to be less pronounced than for compound **15**. The nucleoside **20** destabilises duplexes towards DNA to a similar degree as does its analogue **15**, and duplexes towards RNA even more. Again, steric factors may play a role. Even though the destabilising effect towards RNA was less pronounced when the bicyclic nucleoside **20** was incorporated twice in a row, indicating that a fully modified sequence could form a stable duplex with complimentary RNA, its future applications will be hampered by its rather complicated synthetic route.

Contrary to the results obtained for compounds **15** and **20**, the *arabino*-configured [3.3.0]bicyclic nucleoside **25** forms duplex structures with complementary DNA which are only slightly destabilised compared with the unmodified duplex. Interestingly, duplexes towards complementary RNA were stabilised after consecutive incorporations. Preliminary molecular modelling predicts for the nucleoside monomer **25** a C-4′-*exo*-conformation (Puckering amplitude $P = 54°$),[5c] which is near the range of a *N*-type conformation as in *A*-type duplexes. A restriction of this conformation could explain the stabilising effect for the $X_{13}T:rA_{14}$ duplex. The destabilising effect observed when the bicyclic nucleoside **25** was incorporated once or several times alternating with unmodified thymidine monomers could be due to unfavourable structural irregularities in the duplex. This suggests that the $X_{13}T:rA_{14}$ (**43**:**45**) duplex has a helical structure which is nonidentical with the reference $T_{14}:rA_{14}$ (**44**:**45**) duplex. The conformational restriction of the bicyclic nucleoside **25** induced on C-2′ and C-3′ of the furanose ring indicates a restriction of the torsion angles ν_1, ν_2 and ν_3[5c] whereas other torsion angles should be less restricted. This could generate an advantage concerning duplex stabilities over the bicyclic DNA introduced by Leumann and co-workers, containing an additional 3′,5′-ethylene bridge restricting the torsion angle γ into a range different from natural duplexes.

Conclusions

Four different bicyclic nucleosides have been synthesised from the new 3′-*C*-allyl nucleoside **7**. Compared with natural nucleosides, they contain additional 5- or 6-membered rings linking 2′-C and 3′-C. The syntheses of the nucleosides with 6-membered rings (**15**, **16** and **20**) were complicated, whereas the synthesis of the nucleoside **25** with an additional tetrahydrofuran ring was more straightforward. The three new bicyclic β-nucleosides **15**, **20** and **25** were incorporated into ODNs. Whereas the nucleosides with additional 6-membered rings destabilise duplexes towards both DNA and RNA dramatically, the nucleoside with the additional 2′,3′-tetrahydrofuran ring stabilises duplexes towards RNA without compromising the ability of recognising a single guanosine mismatch. Based on these results, and the reported excellent 3′-exonucleolytic stability,[2] these 2′,3′-bicyclo-oligonucleotides (2′,3′-BcNAs) are attractive candidates for antisense molecules. Current experiments focus on biochemical studies and on synthesis of mixed sequences and chemically related structures.

Experimental

All reagents were obtained from commercial suppliers and were used without further purification. The silica gel (0.040–0.063 mm) used for column chromatography was purchased from Merck. Molecular modelling was performed using the HyperChem™ program (MM+ force field). 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 and at 202.33 MHz for ^{31}P NMR on a Varian Unity 500 spectrometer. δ-Values are in ppm relative to tetramethylsilane as internal standard (^{1}H and ^{13}C NMR) and relative to 85% H_3PO_4 as external standard (^{31}P NMR). Assignments of NMR peaks were made according to standard nucleoside nomenclature and numbering. EI mass spectra were recorded on a Varian Mat 311A spectrometer, FAB mass spectra on a Kratos MS50TC spectrometer, and Plasma Desorption mass spectra on an Applied Biosystems Biopolymer Mass Analyzer *BIO-ION 20R*. ONs were synthesised on a Gene Assembler Special® DNA-synthesiser (Pharmacia Biotech). Purification of 5′-*O*-DMT-on ONs was accomplished using disposable Oligopurification Cartridges (COP, Cruachem). MALDI mass spectra were obtained in positive mode on a Micromass TofSpec E mass spectrometer (matrix: diammonium hydrogen citrate and 2,6-dihydroxyacetophenone). Capillary gel electrophoresis was performed on a Beckman P/ACE System 5000. Light petroleum refers to the fraction with distillation range 40–60 °C.

3-*C*-Allyl-1,2-*O*-isopropylidene-α-D-ribofuranose 3

Method 1. A solution of furanose **1**[18] (17.8 g, 58.9 mmol) in anhydrous tetrahydrofuran (THF) (980 cm³) was stirred at 0 °C and 1 M allylmagnesium bromide in anhydrous diethyl ether (130 cm³, 130 mmol) was added dropwise. After stirring of the mixture for 2 h, saturated aq. ammonium chloride (800 cm³) was added and the mixture was extracted with dichloromethane (3 × 400 cm³). The organic phase was washed with brine (3 × 450 cm³) and dried (Na_2SO_4). The solvent was removed under reduced pressure and the residue was dissolved in anhydrous THF (700 cm³). A 1.1 M solution of tetrabutylammonium fluoride (TBAF) in THF (54.4 cm³, 59.8 mmol) was added and the mixture was stirred at room temperature for 1 h and evaporated to dryness. The residue was dissolved in dichloromethane (1700 cm³) and the solution was washed with saturated aq. sodium hydrogen carbonate (3 × 500 cm³) and dried (Na_2SO_4). The solvent was removed under reduced pressure and the residue was purified by silica gel column chromatography using dichloromethane–methanol (98:2, v/v) as eluent to give *title compound* **3** as a solid (9.42 g, 69%).

Method 2. Furanose **3** (2.99 g, 58%) was analogously synthesised from furanose **2**[19] (9.5 g, 22.2 mmol) using: anhydrous THF (425 cm³); 1 M allylmagnesium bromide in anhydrous diethyl ether (130 cm³, 130 mmol); saturated aq. ammonium chloride (490 cm³); extraction with diethyl ether (350 + 2 × 160 cm³); brine (2 × 160 cm³); 1.1 M TBAF in THF (22.3 cm³, 24.6 mmol); anhydrous THF (400 cm³); dichloromethane (1400 cm³); saturated aq. sodium hydrogen carbonate (3 × 500 cm³); brine (500 cm³) and (Na_2SO_4); $\delta_H(CD_3)_2SO$ 5.84 (1 H, m, 2′-H), 5.65 (1 H, d, *J* 3.8, 1-H), 5.12 (1 H, d, *J* 6.1, 3′-H^a), 5.06 (1 H, br s, 3′-H^b), 4.76 (1 H, s, 3-OH), 4.64 (1 H, t, *J* 5.4, 5-OH), 4.16 (1 H, d, *J* 3.8, 2-H), 3.84 (1 H, dd, *J* 2.2 and 8.1, 4-H), 3.56 (1 H, ddd, *J* 2.3, 5.6 and 11.8, 5-H^a), 3.42 (1 H, m, 5-H^b), 2.16 (1 H, dd, *J* 6.1 and 14.3, 1′-H^a), 1.98 (1 H, dd, *J* 8.2 and 14.3, 1′-H^b), 1.46 (3 H, s, CH_3) and 1.25 (3 H, s, CH_3); $\delta_C(CDCl_3)$ 133.5 (C-2′), 117.9 (C-3′), 110.8 [$C(CH_3)_2$], 102.9 (C-1), 82.6 and 81.0 (C-4 and -2), 77.7 (C-3), 59.4 (C-5), 36.4 (C-1′) and 26.4 and 26.3 (2 × CH_3) (Found: C, 57.4; H, 8.0. $C_{11}H_{18}O_5$ requires C, 57.4; H, 7.9%).

3-*C*-Allyl-3,5-di-*O*-benzyl-1,2-*O*-isopropylidene-α-D-ribofuranose 4

A 60% suspension of sodium hydride (4.9 g, 123 mmol) in

anhydrous dimethylformamide (DMF) (100 cm^3) was stirred at 0 °C and a solution of furanose **3** (9.42 g, 40.9 mmol) in anhydrous DMF (65 cm^3) was added dropwise over a period of 45 min. The solution was stirred for 1 h at 50 °C and cooled to 0 °C. A mixture of benzyl bromide (14.5 cm^3, 121 mmol) and anhydrous DMF (14.5 cm^3) was added dropwise and the mixture was stirred at room temp. for 18 h. The reaction mixture was evaporated to dryness and a solution of the residue in dichloromethane (700 cm^3) was washed with saturated aq. sodium hydrogen carbonate (2 × 450 cm^3) and dried (Na_2SO_4). The solvent was removed under reduced pressure and the residue was purified by silica gel column chromatography using light petroleum–ethyl acetate (9:1, v/v) as eluent to give *title compound* **4** as an oil (14.5 g, 86%), $\delta_H(CDCl_3)$ 7.39–7.21 (10 H, m, Ph), 5.92 (1 H, m, 2′-H), 5.71 (1 H, d, *J* 3.8, 1-H), 5.17–5.09 (2 H, m, 2 × 3′-H), 4.67 (2 H, m, CH_2Ph), 4.60 (1 H, d, *J* 12.2, C*H*HPh), 4.52 (1 H, d, *J* 12.1, CH*H*Ph), 4.43 (1 H, m, 4-H), 4.42 (1 H, d, *J* 3.8, 2-H), 3.73 (1 H, dd, *J* 3.2 and 10.8, 5-H^a), 3.66 (1 H, dd, *J* 7.4 and 10.8, 5-H^b), 2.50 (1 H, dd, *J* 7.7 and 14.9, 1′-H^a), 2.39 (1 H, dd, *J* 6.5 and 14.9, 1′-H^b), 1.60 (3 H, s, CH_3) and 1.34 (3 H, s, CH_3); $\delta_C(CDCl_3)$ 138.6 and 138.1 (C-aryl), 132.6 (C-2′), 128.3, 128.2, 127.7, 127.5, 127.4 and 127.4 (C-aryl), 118.5 (C-3′), 112.6 [*C*$(CH_3)_2$], 104.1 (C-1), 86.5 (C-3), 82.1 and 80.4 (C-4 and -2), 73.4 and 68.6 (*C*H_2Ph), 67.0 (C-5), 35.8 (C-1′) and 26.8 and 26.6 (2 × CH_3); FAB-MS *m/z* 433 $[M + Na]^+$ (Found: C, 73.4; H, 7.4. $C_{25}H_{30}O_5$ requires C, 73.2; H, 7.4%).

3-*C*-Allyl-1,2-di-*O*-acetyl-3,5-di-*O*-benzyl-D-ribofuranose 5

A solution of furanose **4** (12.42 g, 30.3 mmol) in 80% aq. acetic acid (150 cm^3) was stirred at 90 °C for 3 h. The solvent was removed under reduced pressure and the residue was co-evaporated successively with ethanol (3 × 75 cm^3), toluene (3 × 75 cm^3) and anhydrous pyridine (2 × 75 cm^3) and re-dissolved in anhydrous pyridine (60 cm^3). Acetic anhydride (46 cm^3) was added and the solution was stirred at room temp. for 48 h. A mixture of ice–water (300 cm^3) was added and the resulting mixture was extracted with dichloromethane (2 × 300 cm^3). The organic phase was washed with saturated aq. sodium hydrogen carbonate (3 × 200 cm^3) and dried (Na_2SO_4). The solvent was evaporated off and the residue was purified using silica gel column chromatography with light petroleum–ethyl acetate (4:1, v/v) as eluent to give the *anomeric mixture* **5** (β:α ~ 2:1) as an oil (13.3 g, 97%), $\delta_C(CDCl_3)$ 169.7 and 169.6 (2 × C=O), 138.7, 138.4, 137.7 and 137.6 (C-aryl), 132.4 and 132.2 (2 × C-2′), 128.4, 128.4, 128.2, 128.2, 127.8, 127.7, 127.7, 127.6, 127.3, 127.3, 126.9 and 126.8 (C-aryl), 118.5 (C-3′), 99.4 and 93.5 (2 × C-1), 84.8, 83.7, 83.2, 82.0, 79.1 and 75.5 (2 × C-2, 2 × C-3 and 2 × C-4), 73.7, 73.5, 69.3 and 68.7 (*C*H_2Ph), 66.1 (C-5), 35.5 and 34.9 (2 × C-1′) and 21.1, 21.0, 20.7 and 20.6 (CH_3) (Found: C, 68.7; H, 6.7. $C_{26}H_{30}O_7$ requires C, 68.8; H, 6.6%).

1-(2-*O*-Acetyl-3-*C*-allyl-3,5-di-*O*-benzyl-β-D-ribofuranosyl)-thymine 6

To a stirred solution of the anomeric mixture **5** (β:α ~ 2:1; 11.8 g, 26.0 mmol) and thymine (6.55 g, 52.0 mmol) in anhydrous acetonitrile (250 cm^3) was added BSA (44.9 cm^3, 182 mmol). The reaction mixture was stirred at reflux for 1 h and cooled to 0 °C. TMS triflate (8.00 cm^3, 44.0 mmol) was added dropwise and the solution was stirred at room temperature for 12 h. Ice-cold saturated aq. sodium hydrogen carbonate (270 cm^3) was added and the mixture was extracted with dichloromethane (3 × 125 cm^3). The organic phase was washed successively with saturated aq. sodium hydrogen carbonate (2 × 125 cm^3) and brine (2 × 125 cm^3) and dried (Na_2SO_4). The solvent was removed under reduced pressure and the residue was purified by silica gel column chromatography using dichloromethane–methanol (98:2, v/v) as eluent to give nucleoside **6** as a solid (11.6 g, 86%), $\delta_H(CDCl_3)$ 8.34 (1 H, br s, NH), 7.75 (1 H, d, *J* 0.9, 6-H), 7.41–7.25 (10 H, m, Ph), 6.43 (1 H, d, *J* 8.2, 1′-H), 5.88 (1 H, m, 2″-H), 5.66 (1 H, d, *J* 8.2, 2′-H), 5.12 (1 H, s, 3″-H^a), 5.07 (1 H, dd, *J* 1.5 and 8.5, 3″-H^b), 4.85 (1 H, d, *J* 11.2, C*H*HPh), 4.64 (2 H, s, CH_2Ph), 4.63 (1 H, d, *J* 11.2, CH*H*Ph), 4.33 (1 H, br s, 4′-H), 3.81 (1 H, dd, *J* 2.7 and 11.1, 5′-H^a), 3.65 (1 H, m, 5′-H^b), 2.81–2.65 (2 H, m, 1″-H_2), 2.08 (3 H, s, Ac) and 1.52 (3 H, d, *J* 0.8, CH_3); $\delta_C(CDCl_3)$ 170.1 (C=O), 163.6 (C-4), 150.9 (C-2), 138.1 and 136.6 (C-aryl), 136.0 (C-6), 131.6 (C-2″), 128.8, 128.4, 128.3, 127.6, 127.5 and 127.1 (C-aryl), 118.5 (C-3″), 111.1 (C-5), 84.2, 83.4, 83.1 and 77.4 (C-1′, -2′, -3′ and -4′), 73.6 and 69.2 (*C*H_2Ph), 65.6 (C-5′), 33.7 (C-1″), 20.8 (CO*C*H_3) and 11.9 (CH_3) (Found: C, 66.8; H, 6.3; N, 5.1. $C_{29}H_{32}N_2O_7$ requires C, 66.9; H, 6.2; N, 5.4%).

1-(3-*C*-Allyl-3,5-di-*O*-benzyl-β-D-ribofuranosyl)thymine 7

To a stirred solution of nucleoside **6** (11.6 g, 22.3 mmol) in methanol (110 cm^3) was added sodium methoxide (3.03 g, 55.5 mmol). The reaction mixture was stirred at room temp. for 16 h and neutralised (pH 7) with dil. hydrochloric acid. The solvent was partly evaporated off and the residue was dissolved in dichloromethane (400 cm^3). The organic phase was washed with saturated aq. sodium hydrogen carbonate (3 × 250 cm^3) and dried (Na_2SO_4). The solvent was removed under reduced pressure to give *title compound* **7** as a solid (10.1 g, 95%), $\delta_H(CDCl_3)$ 8.77 (1 H, br s, NH), 7.58 (1 H, d, *J* 1.2, 6-H), 7.41–7.25 (10 H, m, Ph), 6.14 (1 H, m, 2″-H), 6.12 (1 H, d, *J* 7.8, 1′-H), 5.23 (1 H, m, 3″-H^a), 5.17 (1 H, br s, 3″-H^b), 4.68 (1 H, d, *J* 10.8, C*H*HPh), 4.59 (2 H, s, CH_2Ph), 4.55 (1 H, d, *J* 10.9, CH*H*Ph), 4.39 (1 H, br s, 4′-H), 4.26 (1 H, dd, *J* 7.8 and 10.7, 2′-H), 3.84 (1 H, dd, *J* 3.1 and 11.0, 5′-H^a), 3.58 (1 H, dd, *J* 1.4 and 11.0, 5′-H^b), 3.04 (1 H, d, *J* 10.8, 2′-OH), 2.82–2.78 (2 H, m, 1″-H_2) and 1.51 (3 H, d, *J* 1.0, CH_3); $\delta_C(CDCl_3)$ 163.5 (C-4), 151.1 (C-2), 137.3 and 136.7 (C-aryl), 136.0 (C-6), 132.2 (C-2″), 128.8, 128.5, 128.3, 127.9 and 127.6 (C-aryl), 118.5 (C-3″), 111.2 (C-5), 87.4, 82.6, 81.1 and 79.3 (C-1′, -2′, -3′ and -4′), 73.8 and 69.8 (*C*H_2Ph), 64.7 (C-5′), 35.1 (C-1″) and 11.9 (CH_3) (Found: C, 67.8; H, 6.1; N, 5.5. $C_{27}H_{30}N_2O_6$ requires C, 67.8; H, 6.3; N, 5.9%).

1-(3-*C*-Allyl-3,5-di-*O*-benzyl-2-*O*-methylsulfonyl-β-D-ribofuranosyl)thymine 8

To a stirred solution of nucleoside **7** (3.50 g, 7.31 mmol) in anhydrous pyridine (23 cm^3) at 0 °C was added methanesulfonyl chloride (1.69 cm^3, 21.89 mmol). The reaction mixture was stirred for 1 h at room temp., water (100 cm^3) was added, and extraction was performed using dichloromethane (3 × 150 cm^3). The organic phase was washed with saturated aq. sodium hydrogen carbonate (3 × 200 cm^3) and dried (Na_2SO_4). The solvent was removed under reduced pressure and the residue was purified by silica gel column chromatography using dichloromethane–methanol (99:1) as eluent to give the *methanesulfonate* **8** as a solid (3.64 g, 89%), $\delta_H(CDCl_3)$ 8.95 (1 H, br s, NH), 7.71 (1 H, d, *J* 1.1, 6-H), 7.39–7.25 (10 H, m, Ph), 6.52 (1 H, d, *J* 8.0, 1′-H), 5.90 (1 H, m, 2″-H), 5.34 (1 H, d, *J* 7.9, 2′-H), 5.20–5.09 (2 H, m, 3″-H_2), 4.91 (1 H, d, *J* 11.2, C*H*HPh), 4.68 (1 H, d, *J* 11.3, CH*H*Ph), 4.64 (2 H, s, CH_2Ph), 4.33 (1 H, br s, 4′-H), 3.81 (1 H, dd, *J* 2.5 and 11.1, 5′-H^a), 3.73 (1 H, dd, *J* 1.1 and 11.1, 5′-H^b), 3.08 (1 H, dd, *J* 5.5 and 5.7, 1″-H^a), 2.99 (3 H, s, CH_3), 2.68 (1 H, m, 1″-H^b) and 1.51 (3 H, d, *J* 0.8, CH_3); $\delta_C(CDCl_3)$ 163.4 (C-4), 150.8 (C-2), 137.9 and 136.3 (C-aryl), 135.5 (C-6), 131.0 (C-2″), 128.8, 128.3, 127.5 and 127.2 (C-aryl), 119.3 (C-3″), 111.6 (C-5), 84.1, 83.6, 82.4 and 82.2 (C-1′, -2′, -3′ and -4′), 73.6 and 68.8 (*C*H_2Ph), 66.1 (C-5′), 38.7 (CH_3), 33.0 (C-1″) and 11.9 (CH_3) (Found: C, 60.5; H, 5.8; N, 4.9. $C_{28}H_{32}N_2O_8S$ requires C, 60.4; H, 5.8; N, 5.0%).

1-(3-*C*-Allyl-3,5-di-*O*-benzyl-β-D-arabinofuranosyl)thymine 9

A solution of nucleoside **8** (3.59 g, 6.45 mmol) in ethanol (72 cm^3), water (72 cm^3) and 1 M aq. sodium hydroxide (20.6 cm^3)

was stirred under reflux for 18 h. After neutralisation (pH 7) with dil. hydrochloric acid, the solvent was removed under reduced pressure and the residue was dissolved in dichloromethane (3 × 150 cm^3). The organic phase was washed with saturated aq. sodium hydrogen carbonate (3 × 200 cm^3) and dried (Na_2SO_4). The solvent was removed under reduced pressure and the residue was purified by silica gel column chromatography using dichloromethane–methanol (99 : 1, v/v) as eluent to give *title compound* **9** as a solid (2.32 g, 74%), $\delta_H(CDCl_3)$ 7.60 (1 H, d, *J* 1.2, 6-H), 7.50–7.23 (10 H, m, Ph), 6.22 (1 H, d, *J* 2.9, 1′-H), 5.80 (1 H, m, 2″-H), 5.15–5.08 (2 H, m, 3″-H_2), 4.86–4.33 (6 H, m, 2 × CH_2Ph, 2′- and 4′-H), 3.82–3.71 (2 H, m, 5′-H_2), 2.72 (1 H, m, 1″-H^a), 2.52 (1 H, dd, *J* 7.6 and 16.1, 1″-H^b) and 1.70 (3 H, d, *J* 0.9, CH_3); $\delta_C(CDCl_3)$ 165.1 (C-4), 150.4 (C-2), 138.4 and 136.8 (C-aryl), 137.7 (C-6), 132.3 (C-2″), 128.7, 128.4, 128.3, 128.0, 127.9 and 127.6 (C-aryl), 118.5 (C-3″), 107.8 (C-5), 88.0, 87.8 and 83.7 (C-1′, -3′ and -4′), 73.7, 72.9 and 69.4 (CH_2Ph and C-2′), 64.7 (C-5′), 31.1 (C-1″) and 12.4 (CH_3) (Found: C, 67.5, H, 6.3; N, 5.3. $C_{27}H_{30}N_2O_6$·0.25H_2O requires C, 67.1; H, 6.4; N, 5.8%).

Methyl 3-*C*-allyl-3,5-di-*O*-benzyl-D-ribofuranoside 10

A solution of furanose **4** (3.08 g, 7.50 mmol) in a mixture of hydrochloric acid in methanol (20%, w/w; 100 cm^3) and water (14 cm^3) was stirred at room temperature for 18 h. After neutralisation (pH 7) with sodium hydrogen carbonate, the solvents were removed under reduced pressure and the residue was dissolved in dichloromethane (2 × 200 cm^3). The organic phase was washed with water (2 × 100 cm^3) and dried (Na_2SO_4). The solvent was removed under reduced pressure and the residue was purified by silica gel column chromatography using dichloromethane–methanol (99 : 1, v/v) as eluent to give the *anomeric mixture* **10** (β : α ~ 2 : 1) as an oil (2.37 g, 82%), $\delta_C(CDCl_3)$ 137.8, 137.8 and 137.7 (C-aryl), 132.8 and 132.7 (2 × C-2′), 128.4, 128.2, 127.7, 127.7, 127.7, 127.6, 127.3 and 127.0 (C-aryl), 118.5 and 117.9 (2 × C-3′), 109.4 (C-1), 101.9 (C-1), 83.3, 82.1, 81.2, 80.4, 80.0 and 77.2 (2 × C-2, 2 × C-3 and 2 × C-4), 73.6, 73.6, 70.2 and 69.4 (CH_2Ph), 65.4 and 65.1 (2 × C-5), 55.8 and 55.3 (2 × OCH_3) and 36.3 and 34.4 (2 × C-1′); FAB-MS *m*/*z* 407 $[M + Na]^+$.

Methyl 3,5-di-*O*-benzyl-3-*C*-(3-hydroxypropyl)-β-D-ribofuranoside 11

To a stirred solution of furanosides **10** (β : α ~ 2 : 1; 1.36 g, 3.54 mmol) in anhydrous THF (12 cm^3) was added dropwise 7.8 M BH_3 in 1,4-oxathiane (0.45 cm^3, 3.54 mmol). The solution was stirred at room temp. for 45 min. After cooling of the solution to 0 °C, a mixture of 2 M aq. sodium hydroxide (2.03 cm^3) and 35% hydrogen peroxide (0.43 cm^3) was added dropwise. The mixture was stirred at room temp. for 1 h and was extracted with diethyl ether (3 × 75 cm^3). The organic phase was washed successively with water (3 × 50 cm^3) and saturated aq. sodium hydrogen carbonate (3 × 50 cm^3) and dried (Na_2SO_4). The solvent was removed under reduced pressure and the residue was purified by silica gel column chromatography using dichloromethane–methanol (99 : 1, v/v) as eluent to give the β-*anomer* **11** as an oil (898 mg, 62%) as the only anomer isolated pure, $\delta_C(CDCl_3)$ 137.9, 137.8, 128.5, 128.4, 127.9, 127.8, 127.8 and 127.5 (C-aryl), 109.7 (C-1), 83.6, 82.1 and 80.5 (C-2, -3 and -4), 73.6 and 70.3 (CH_2Ph), 65.1 (C-5), 62.8 (C-3′), 56.0 (OCH_3) and 26.5 and 26.2 (C-1′ and -2′) (Found: C, 67.9; H, 7.4. $C_{23}H_{30}O_6$·0.25H_2O requires C, 67.9; H, 7.6%).

(1*R*,6*R*,7*R*,9*R*)-6-Benzyloxy-7-benzyloxymethyl-9-methoxy-2,8-dioxabicyclo[4.3.0]nonane 12

A solution of furanoside **11** (683 mg, 1.70 mmol) in anhydrous pyridine (3.7 cm^3) was stirred at 0 °C and toluene-*p*-sulfonyl chloride (386 mg, 2.04 mmol) was added. After stirring of the mixture for 1.5 h, water (1 cm^3) was added and extraction was performed with dichloromethane (2 × 50 cm^3). The organic phase was washed with saturated aq. sodium hydrogen carbonate (3 × 30 cm^3) and dried (Na_2SO_4). The solvent was removed under reduced pressure and the residue was co-evaporated with anhydrous toluene (3 × 10 cm^3) and dissolved in anhydrous DMF (9 cm^3). The solution was stirred at room temperature for 4 h after addition of a mixture of 18-crown-6 (416 mg, 1.58 mmol) and pulverised potassium hydroxide (448 mg, 7.93 mmol). After evaporation under reduced pressure, the residue was dissolved in dichloromethane (60 cm^3) and washing was performed successively with saturated aq. sodium hydrogen carbonate (3 × 30 cm^3) and brine (2 × 30 cm^3) and the organic phase was dried (Na_2SO_4). The solvent was removed under reduced pressure and the residue was purified by silica gel column chromatography using light petroleum–ethyl acetate (4 : 1, v/v) as eluent to give *title bicycle* **12** as an oil (275 mg, 42%), $\delta_C(CDCl_3)$ 138.6 and 137.9 (C-aryl), 128.4, 128.3, 127.7, 127.3 and 127.1 (C-aryl), 104.8 (C-1), 83.4, 79.4 and 79.3 (C-2, -3 and -4), 73.6 and 70.5 (CH_2Ph), 68.7 (C-3′), 64.2 (C-5), 57.2 (OCH_3), 25.4 (C-1′) and 20.6 (C-2′); FAB-MS *m*/*z* 385 $[M + H]^+$ and 407 $[M + Na]^+$ (Found: C, 71.1; H, 7.1. $C_{23}H_{28}O_5$·0.25H_2O requires C, 71.0; H, 7.4%).

(1*R*,6*R*,7*R*,9*R*)-6-Benzyloxy-7-benzyloxymethyl-9-(thymin-1-yl)-2,8-dioxabicyclo[4.3.0]nonane 13 and (1*R*,6*R*,7*R*,9*S*)-6-benzyloxy-7-benzyloxymethyl-9-(thymin-1-yl)-2,8-dioxabicyclo[4.3.0]nonane 14

To a stirred solution of compound **12** (475 mg, 1.24 mmol) in anhydrous acetonitrile (19 cm^3) were added thymine (313 mg, 2.49 mmol) and BSA (1.85 cm^3, 7.38 mmol). The mixture was heated under reflux for 15 min and subsequently cooled to 0 °C and TMS triflate (0.313 cm^3, 1.59 mmol) was added dropwise. After being stirred first at room temp. for 18 h and then at 50 °C for 4 h, the mixture was evaporated under reduced pressure. The residue was dissolved in dichloromethane (150 cm^3) and the solution was washed with saturated aq. sodium hydrogen carbonate (3 × 100 cm^3) and dried (Na_2SO_4). The solvent was removed under reduced pressure and the residue was purified by silica gel column chromatography using light petroleum–ethyl acetate (1 : 1, v/v) as eluent to give compounds **13** (113 mg, 19%) and **14** (182 mg, 31%) as solids.

For *compound* **13**: $\delta_H(CDCl_3)$ 8.37 (1 H, br s, NH), 7.54 (1 H, d, *J* 0.8, 6-H), 7.36–7.24 (10 H, m, Ph), 6.39 (1 H, d, *J* 8.7, 1′-H), 4.68 (2 H, s, CH_2Ph), 4.56 (1 H, d, *J* 11.9, CHHPh), 4.45 (1 H, d, *J* 11.9, CHHPh), 4.33 (1 H, br s, 4′-H), 4.13 (1 H, m, 3″-H^a), 3.96 (1 H, d, *J* 8.6, 2′-H), 3.88 (1 H, dd, *J* 3.6 and 10.9, 5′-H^a), 3.56–3.45 (2 H, m, 5′-H^b, 3″-H^b), 2.35 (1 H, d, *J* 13.2, 1″-H^a), 2.00 (1 H, m, 2″-H^a), 1.84 (1 H, m, 1″-H^b), 1.58 (3 H, s, CH_3) and 1.51 (1 H, m, 2″-H^b); $\delta_C(CDCl_3)$ 163.5 (C-4), 150.5 (C-2), 138.0 and 137.0 (C-aryl), 136.0 (C-6), 128.7, 128.4, 128.2, 127.5 and 126.9 (C-aryl), 111.0 (C-5), 83.8, 81.7, 79.9 and 79.1 (C-1′, -2′, -3′ and -4′), 73.8 and 69.9 (CH_2Ph), 68.6 (C-3″), 64.0 (C-5′), 25.0 (C-1″), 20.5 (C-2″) and 12.1 (CH_3); plasma desorption mass spectrometry (PD-MS) *m*/*z* 479.1 $[M + H]^+$ and 501.9 $[M + Na]^+$ (Found: C, 66.6; H, 6.3; N, 5.5. $C_{27}H_{30}N_2O_6$·0.5H_2O requires C, 66.5; H, 6.4; N, 5.8%); for *compound* **14**: $\delta_H(CDCl_3)$ 8.19 (1 H, br s, NH), 7.46 (1 H, d, *J* 1.1, 6-H), 7.42–7.25 (10 H, m, C-aryl), 6.32 (1 H, d, *J* 7.3, 1′-H), 4.66 (1 H, m, 4′-H), 4.58 (2 H, m, CH_2Ph), 4.52 (1 H, d, *J* 10.6, CHHPh), 4.35 (1 H, d, *J* 10.6, CHHPh), 4.14 (1 H, d, *J* 7.3, 2′-H), 4.13 (1 H, m, 3″-H^a), 3.64 (1 H, dd, *J* 4.6 and 10.6, 5′-H^a), 3.51 (1 H, dd, *J* 3.5 and 10.7, 5′-H^b), 3.51 (1 H, m, 3″-H^b), 2.41 (1 H, m, 1″-H^a), 1.93 (1 H, m, 2″-H^a), 1.71 (1 H, m, 1″-H^b), 1.49 (1 H, m, 2″-H^b) and 1.21 (3 H, d, *J* 1.0, CH_3); $\delta_C(CDCl_3)$ 163.7 (C-4), 150.9 (C-2), 138.8 (C-6), 137.3 and 136.8 (C-aryl), 128.7, 128.6, 128.1, 128.0, 127.9 and 127.7 (C-aryl), 108.2 (C-5), 82.0, 81.9, 81.5 and 78.8 (C-1′, -2′, -3′, -4′), 73.8, 69.6 and 69.5 (Bn, C-3″), 64.6 (C-5′), 25.3 (C-1″), 20.5 (C-2″) and 11.7 (CH_3). PD-MS *m*/*z* 479.4 $[M + H]^+$ and 501.9 $[M + Na]^+$.

(1*R*,6*R*,7*R*,9*R*)-6-Hydroxy-7-hydroxymethyl-9-thymin-1-yl-2,8-dioxabicyclo[4.3.0]nonane 15

A solution of nucleoside **13** (110 mg, 0.226 mmol) in ethanol (1.6 cm^3) was stirred at room temperature and 20% palladium hydroxide over carbon (50 mg) was added. The mixture was degassed with argon and placed in a hydrogen atmosphere. After being stirred for 2 h the mixture was directly purified by silica gel column chromatography using dichloromethane–methanol (97 : 3, v/v) as eluent to give compound **15** as a solid (52 mg, 73%), $\delta_H(CD_3)_2SO$ 11.3 (1 H, br s, NH), 7.78 (1 H, s, 6-H), 6.02 (1 H, d, *J* 8.6, 1′-H), 5.19 (1 H, m, 5′-OH), 5.05 (1 H, s, 3′-OH), 3.91–3.85 (2 H, m, 4′-H, 3″-H^a), 3.72 (1 H, d, *J* 8.7, 2′-H), 3.65 (1 H, m, 5′-H^a), 3.51 (1 H, m, 5′-H^b), 3.31 (1 H, s, 3″-H^b), 1.97 (1 H, m, 2″-H^a), 1.79 (3 H, s, CH_3) and 1.92–1.35 (3 H, m, 1″-H_2 and 2″-H^b); $\delta_C(CDCl_3)$ 163.5 (C-4), 150.9 (C-2), 135.8 (C-6), 109.7 (C-5), 86.2, 81.9 and 80.3 (C-1′, -2′ and -4′), 72.7 (C-3′), 67.7 (C-3″), 60.3 (C-5′), 28.9 (C-1″), 19.7 (C-2″) and 12.2 (CH_3); EI-MS *m*/*z* 298 [M^+, 19%] (Found: C, 49.9; H, 6.1; N, 8.5. $C_{13}H_{18}N_2O_6 \cdot 0.9H_2O$ requires C, 49.7; H, 6.4; N, 8.9%).

(1*R*,6*R*,7*R*,9*S*)-6-Hydroxy-7-hydroxymethyl-9-(thymin-1-yl)-2,8-dioxabicyclo[4.3.0]nonane 16

The same procedure as for preparation of isomer **15** was used with compound **14** (112 mg, 0.234 mmol), ethanol (1.8 cm^3) and 20% palladium hydroxide over carbon (55 mg) to give compound **16** as a solid (49 mg, 70%), $\delta_H(CD_3OD)$ 7.89 (1 H, d, *J* 1.1, 6-H), 6.30 (1 H, d, *J* 6.6, 1′-H), 4.47 (1 H, t, *J* 4.0, 4′-H), 4.16 (1 H, d, *J* 6.6, 2′-H), 4.15 (1 H, m, 3″-H^a), 3.88 (1 H, dd, *J* 4.1 and 12.4, 5′-H^a), 3.77–3.66 (2 H, m, 5′-H^b and 3″-H^b), 2.23–1.98 (3 H, m, 1″-H_2 and 2″-H^a), 2.01 (3 H, d, *J* 1.1, CH_3) and 1.59 (1 H, m, 2″-H^b); $\delta_C(CD_3OD)$ 166.8 (C-4), 152.6 (C-2), 140.3 (C-6), 108.6 (C-5), 90.3, 84.3 and 82.9 (C-1′, -2′, -4′), 74.7 (C-3′), 70.4 (C-3″), 62.0 (C-5′), 30.8 (C-1″), 21.2 (C-2″) and 12.6 (CH_3).

1-[3,5-Di-*O*-benzyl-3-*C*-(3-hydroxypropyl)-β-D-ribofuranosyl]-thymine 17

To a stirred solution of nucleoside **7** (3.42 g, 7.15 mmol) in anhydrous THF (20 cm^3) was added dropwise 7.8 M BH_3 in 1,4-oxathiane (1.00 cm^3, 7.80 mmol). The solution was stirred at room temp. for 45 min. After cooling of the solution to 0 °C, a mixture of 2 M NaOH (4.00 cm^3, 8.0 mmol) and 35% hydrogen peroxide (1.00 cm^3, 14.5 mmol) was added dropwise. After stirring of the mixture at room temp. for 1 h and addition of a mixture of ice and water (100 cm^3), extraction with dichloromethane (3 × 50 cm^3) was performed. The organic phase was washed successively with water (3 × 75 cm^3) and saturated aq. sodium hydrogen carbonate (3 × 75 cm^3) and dried (Na_2SO_4). The solvent was removed under reduced pressure and the residue was purified by silica gel column chromatography using dichloromethane–methanol (98 : 2, v/v) as eluent to give *title compound* **17** as a solid (1.92 g, 54%), $\delta_H(CDCl_3)$ 9.03 (1 H, br s, NH), 7.60 (1 H, d, *J* 1.1, 6-H), 7.34–7.23 (10 H, m, Ph), 6.13 (1 H, d, *J* 7.8, 1′-H), 4.56 (2 H, m, CH_2Ph), 4.52 (2 H, s, CH_2Ph), 4.34 (1 H, br s, 4′-H), 4.18 (1 H, m, 2′-H), 3.84–3.30 (4 H, m, 5′- and 3″-H_2), 2.15–1.71 (4 H, m, 1″- and 2″-H_2) and 1.49 (3 H, d, *J* 0.6, CH_3); $\delta_C(CDCl_3)$ 163.7 (C-4), 151.3 (C-2), 137.5 and 136.8 (C-aryl), 136.1 (C-6), 128.7, 128.5, 128.4, 128.2, 127.8, 127.7 and 127.4 (C-aryl), 111.2 (C-5), 87.4, 82.8, 81.4 and 79.2 (C-1′, -2′, -3′ and -4′), 73.7 and 69.8 (*C*H_2Ph), 64.3 (C-5′), 62.7 (C-3″), 26.4 and 25.8 (C-1″ and -2″) and 11.9 (CH_3); FAB-MS *m*/*z* 497 $[M + H]^+$ and 519 $[M + Na]^+$ (Found: C, 64.6; H, 6.5; N, 5.4. $C_{27}H_{32}N_2O_7 \cdot 0.25H_2O$ requires C, 64.7; H, 6.5; N, 5.6%).

Alternative method for preparation of compound 13

A solution of nucleoside **17** (295 mg, 0.594 mmol) in anhydrous pyridine (1.5 cm^3) was stirred at 0 °C and toluene-*p*-sulfonyl chloride (168 mg, 0.891 mmol) was added. After stirring of the mixture for 18 h, a mixture of water and ice (10 cm^3) was added and the mixture was extracted with dichloromethane (2 × 20 cm^3). The organic phase was washed with saturated aq. sodium hydrogen carbonate (3 × 20 cm^3) and dried (Na_2SO_4). The solvent was removed under reduced pressure and the residue was purified by silica gel column chromatography using dichloromethane–methanol (99 : 1–96 : 4, v/v) as eluent to afford three fractions: nucleoside **21** (44 mg, 9%, *vide infra*), starting material **17** (105 mg, 36% recovery) and a residue (135 mg), which was dissolved in anhydrous DMF (0.25 cm^3). This solution was added to a stirred suspension of 60% sodium hydride (16 mg, 0.23 mmol) in anhydrous DMF (0.25 cm^3) at 0 °C. The mixture was stirred for 18 h, diluted with dichloromethane (5 cm^3), and washed with saturated aq. sodium hydrogen carbonate (3 × 5 cm^3). The organic phase was dried ($NaSO_4$) and evaporated to dryness under reduced pressure and the residue was purified by silica gel column chromatography using light petroleum–ethyl acetate (4 : 1, v/v) as eluent to give compound **13** as an oil (36 mg, 13%).

1-[3,5-Di-*O*-benzyl-3-*C*-(3-hydroxypropyl)-β-D-arabinofuranosyl]thymine 18

The same procedure as for preparation of *ribo*-isomer **17** was used with compound **9** (194 mg, 0.402 mmol), anhydrous THF (1.7 cm^3), 7.8 M BH_3 in oxathiane (0.052 cm^3, 0.405 mmol), 2 M NaOH (0.231 cm^3, 0.46 mmol) and 35% hydrogen peroxide (0.049 cm^3, 0.71 mmol) to give *title compound* **18** as a solid (116 mg, 58%), $\delta_H(CDCl_3)$ 9.58 (1 H, br s, NH), 7.50 (1 H, d, *J* 0.5, 6-H), 7.40–7.23 (10 H, m, Ph), 6.21 (1 H, d, *J* 3.2, 1′-H), 4.82 (1 H, d, *J* 8.6, C*H*HPh), 4.62–4.28 (5-H, m, 4′- and 2′-H and CH_2Ph), 3.79–3.59 (4 H, m, 5′- and 3″-H_2), 2.16 (1 H, br s, 1″-H^a), 1.89–1.65 (3 H, m, 1″-H^b and 2″-H_2) and 1.73 (3 H, d, *J* 0.4, CH_3); $\delta_C(CDCl_3)$ 164.6 (C-4), 150.4 (C-2), 137.9 and 136.6 (C-aryl), 137.8 (C-6), 128.7, 128.4, 128.4, 128.0, 127.6 and 127.3 (C-aryl), 108.3 (C-5), 88.8, 88.2 and 82.2 (C-1′, -3′ and -4′), 73.9 and 73.8 (*C*H_2Ph, C-2′), 69.6 (*C*H_2Ph), 64.3 (C-5′), 62.4 (C-3″), 26.2 and 22.7 (C-1″ and -2″) and 12.4 (CH_3).

(1*S*,6*R*,7*R*,9*R*)-6-Benzyloxy-7-benzyloxymethyl-9-(thymin-1-yl)-2,8-dioxabicyclo[4.3.0]nonane 19

The same procedure as the alternative method for preparation of compound **13** was used with diol **18** (116 mg, 0.234 mmol), anhydrous pyridine (0.6 cm^3), toluene-*p*-sulfonyl chloride (66 mg, 0.350 mmol), 60% sodium hydride (12 mg, 0.29 mmol) and anhydrous DMF (2 × 0.25 cm^3) to give *title compound* **19** as a solid (21 mg, 19%), $\delta_H(CDCl_3)$ 8.58 (1 H, br s, NH), 7.48–7.25 (11 H, m, 6-H and Ph), 6.33 (1 H, d, *J* 3.5, 1′-H), 4.65–4.50 (5 H, m, 2 × CH_2Ph, 4′-H), 3.92–3.78 (4 H, m, 3″-H^a, 2′-H and 5′-H_2), 3.36 (1 H, m, 3″-H^b), 2.38 (1 H, m, 1″-H^a), 1.91 (1 H, m, 1″-H^b), 1.87 (3 H, d, *J* 0.9, CH_3) and 1.68–1.59 (2 H, m, 2″-H_2); $\delta_C(CDCl_3)$ 163.8 (C-4), 150.1 (C-2), 137.9, 137.6 and 137.5 (C-aryl, C-6), 128.5, 128.3, 127.9, 127.5 and 127.0 (C-aryl), 108.5 (C-5), 86.1, 82.4, 81.6 and 80.4 (C-1′, -2′, -3′ and -4′), 73.5 and 70.4 (*C*H_2Ph), 65.9 (C-3″), 64.2 (C-5′), 25.5 (C-1″), 22.1 (C-2″) and 12.5 (CH_3); PD-MS *m*/*z* 478.7 $[M + H]^+$ and 501.7 $[M + Na]^+$.

Alternative method for preparation of compounds 13 and 19

A solution of nucleoside **17** (778 mg, 1.57 mmol) in anhydrous pyridine (4.0 cm^3) was stirred at 0 °C and toluene-*p*-sulfonyl chloride (886 mg, 4.71 mmol) was added. After stirring of the mixture for 2 h, a mixture of water–ice (100 cm^3) was added and extraction was performed with dichloromethane (2 × 100 cm^3). The organic phase was washed with aq. sodium hydrogen carbonate (3 × 75 cm^3) and dried (Na_2SO_4). The solvent was removed under reduced pressure and the residue was purified by silica gel column chromatography using dichloromethane–methanol (99 : 1, v/v) as eluent to give a solid (729 mg, 58%) tentatively assigned as 1-{3,5-*di*-O-*benzyl*-2-O-(p-*tolylsulfonyl*)-3-C-[3-(p-*tolylsulfonyloxy*)*propyl*]-β-D-*ribofuranosyl*}*thymine* **21**, FAB-MS *m*/*z* 805 $[M + H]^+$ and 827 $[M + Na]^+$ (Found: C,

61.2; H, 5.7; N, 3.9. $C_{41}H_{49}N_2O_{11}S_2$ requires C, 61.2; H, 5.5; N, 3.5%).

To a stirred solution of this nucleoside intermediate (728 mg, 0.904 mmol) in ethanol (10 cm^3)–water (11.5 cm^3) was added 2 M sodium hydroxide (2.9 cm^3). The reaction mixture was stirred under reflux for 18 h, neutralised (pH 7) with hydrochloric acid and extracted with dichloromethane (3 × 30 cm^3). The organic phase was washed with aq. sodium hydrogen carbonate (3 × 30 cm^3) and dried (Na_2SO_4). The solvent was removed under reduced pressure and the residue was purified by silica gel column chromatography using dichloromethane–methanol (99:1–96:4, v/v) as eluent to give a mixture of diastereoisomers **13** and **19** as a solid (215 mg, 50% from **21**) and diol **18** (84 mg, 19% from **21**).

(1*S*,6*R*,7*R*,9*R*)-6-Hydroxy-7-hydroxymethyl-9-(thymin-1-yl)-2,8-dioxabicyclo[4.3.0]nonane 20 and an alternative method for preparation of compound 15

The same procedure as for preparation of compound **15** was used with the mixture of diastereoisomers **13** and **19** (212 mg, 0.443 mmol), ethanol (3.0 cm^3) and 20% palladium hydroxide over carbon (100 mg) to give diastereoisomers **15** (20 mg, 15%) and **20** (51 mg, 38%) as solids after silica gel column chromatographic separation using dichloromethane–methanol (97:3, v/v) as eluent, $\delta_H[(CD_3)_2SO]$ 11.3 (1 H, br s, NH), 7.43 (1 H, d, *J* 1.1, 6-H), 6.10 (1 H, d, *J* 3.5, 1′-H), 5.32 (1 H, s, OH), 4.92 (1 H, m, 4′-H), 3.95–3.82 (2 H, m, 5′-H^a, 3″-H^a), 3.66 (1 H, m, 5′-H^b), 3.57 (1 H, d, *J* 3.5, 2′-H), 3.32 (1 H, s, OH), 3.22 (1 H, m, 3″-H^b), 2.15 (1 H, d, *J* 7.9, 2″-H^a), 1.78 (3 H, d, *J* 1.0, CH_3), 1.88–1.52 (3 H, m, 1″-H_2 and 2″-H^b); $\delta_C(CDCl_3)$ 163.7 (C-4), 150.1 (C-2), 137.4 (C-6), 107.0 (C-5), 89.6, 85.4 and 81.0 (C-1′, -2′ and -4′), 75.0 (C-3′), 65.4 (C-3″), 61.8 (C-5′), 29.3 (C-1″), 22.0 (C-2″) and 12.3 (CH_3); EI-MS *m*/*z* 298 [M^+, 22%].

Alternatively, the same procedure was used with compound **19** (21 mg, 0.044 mol), ethanol (0.5 cm^3), 20% palladium hydroxide over carbon (10 mg) to give compound **20** (yield not determined).

1-[3,5-Di-*O*-benzyl-3-*C*-(2-hydroxyethyl)-β-D-ribofuranosyl]-thymine 22

To a stirred solution of nucleoside **7** (1.00 g, 2.09 mmol) in THF (5.4 cm^3)–water (5.4 cm^3) was added sodium periodate (1.34 g, 6.27 mmol) and a 2.5% (w/w) solution of osmium tetraoxide in *tert*-butyl alcohol (0.265 cm^3, 19 μmol). The solution was stirred at room temp. for 45 min. Water (25 cm^3) was added and the solution was extracted with dichloromethane (2 × 50 cm^3). The organic phase was washed with saturated aq. sodium hydrogen carbonate (3 × 30 cm^3) and dried (Na_2SO_4). The solvent was removed under reduced pressure and the residue was re-dissolved in THF (5.4 cm^3)–water (5.4 cm^3). The mixture was stirred at room temp. and sodium borohydride (79 mg, 2.08 mmol) was added. After stirring of the mixture for 1.5 h, water (25 cm^3) was added and the solution was extracted with dichloromethane (2 × 50 cm^3). The organic phase was washed with saturated aq. sodium hydrogen carbonate (3 × 30 cm^3) and dried (Na_2SO_4). The solvent was removed under reduced pressure and the residue was purified by silica gel column chromatography using dichloromethane–methanol (98:2, v/v) as eluent to give *title compound* **22** as a solid (488 mg, 48%), $\delta_H(CDCl_3)$ 9.14 (1 H, br s, NH), 7.60 (1 H, d, *J* 1.1, 6-H), 7.40–7.22 (10 H, m, Ph), 6.25 (1 H, d, *J* 7.7, 1′-H), 4.59 (1 H, d, *J* 7.1, C*H*HPh), 4.49 (1 H, d, *J* 7.1, CH*H*Ph), 4.39–3.30 (8 H, m, 4′- and 2′-H, CH_2Ph and 5′- and 2″-H_2), 2.23–2.00 (2 H, m, 1″-H_2) and 1.49 (3 H, d, *J* 0.7, CH_3); $\delta_C(CDCl_3)$ 163.5 (C-4), 151.2 (C-2), 137.1 and 136.5 (C-aryl), 135.7 (C-6), 128.7, 128.5, 128.2, 127.8, 127.6 and 127.2 (C-aryl), 111.3 (C-5), 87.0, 82.7, 81.1 and 78.3 (C-1′, -2′, -3′ and -4′), 73.7 and 69.6 (*C*H_2Ph), 64.4 (C-5′), 57.0 (C-2″), 32.4 (C-1″) and 11.8 (CH_3) (Found: C, 63.9; H, 6.3; N, 5.4. $C_{26}H_{30}N_2O_7$·0.25H_2O requires C, 64.1; H, 6.3; N, 5.8%).

1-[3,5-Di-*O*-benzyl-3-*C*-(2-hydroxyethyl)-β-D-arabinofuranosyl]thymine 23

The same procedure as for *ribo*-isomer **22** was used with nucleoside **9** (2.26 g, 4.68 mmol), THF (12 cm^3), water (12 cm^3), sodium periodate (3.04 g, 14.2 mmol) and a 2.5% (w/w) solution of osmium tetraoxide in *tert*-butyl alcohol (0.603 cm^3, 40 μmol); then treatment with THF (12 cm^3), water (12 cm^3) and sodium borohydride (182 mg, 4.71 mmol) to give *title compound* **23** as a solid (1.13 g, 49%), $\delta_H(CDCl_3)$ 9.29 (1 H, br s, NH), 7.47 (1 H, d, *J* 1.1, 6-H), 7.38–7.25 (10 H, m, Ph), 6.22 (1 H, d, *J* 3.4, 1′-H), 4.62 (2 H, s, CH_2Ph), 4.60 (1 H, m, 4′-H), 4.46 (2 H, s, CH_2Ph), 4.35 (1 H, dd, *J* 3.4 and 7.5, 2′-H), 3.83–3.73 (4 H, m, 5′- and 2″-H_2), 2.67 (1 H, br s, OH), 2.07–2.01 (2 H, m, 1″-H_2) and 1.77 (3 H, d, *J* 0.5, CH_3); $\delta_C(CDCl_3)$ 164.3 (C-4), 150.3 (C-2), 137.6 and 137.4 (C-aryl and C-6), 136.7, 128.6, 128.4, 128.2, 127.8, 127.6, 127.3 and 127.1 (C-aryl), 108.4 (C-5), 88.0, 87.7, 81.6 and 74.7 (C-1′, -2′, -3′ and -4′), 73.7 and 69.6 (*C*H_2Ph), 64.6 (C-5′), 57.7 (C-2″), 28.6 (C-1″) and 12.4 (CH_3); FAB-MS *m*/*z* 483 $[M + H]^+$ and 505 $[M + Na]^+$ (Found: C, 63.6; H, 6.2; N, 5.4. $C_{26}H_{30}N_2O_7$·0.5H_2O requires C, 63.5; H, 6.4; N, 5.7%).

(1*S*,5*R*,6*R*,8*R*)-5-Benzyloxy-6-benzyloxymethyl-8-(thymin-1-yl)-2,7-dioxabicyclo[3.3.0]octane 24

A solution of nucleoside **23** (1.08 g, 2.20 mmol) in anhydrous pyridine (5.0 cm^3) was stirred at 0 °C and a solution of toluene-*p*-sulfonyl chloride (462 mg, 2.47 mmol) in anhydrous pyridine (2.0 cm^3) was added dropwise. After stirring of the mixture at room temp. for 20 h and addition of a mixture of water–ice (70 cm^3), extraction was performed with dichloromethane (2 × 75 cm^3). The organic phase was washed with saturated aq. sodium hydrogen carbonate (3 × 50 cm^3) and dried (Na_2SO_4). The solvent was removed under reduced pressure and the residue was purified by silica gel column chromatography using dichloromethane–methanol (99:1, v/v) as eluent to give an intermediate which, after evaporation, was dissolved in anhydrous DMF (4.0 cm^3). The solution was added dropwise to a stirred suspension of 60% sodium hydride (203 mg, 4.94 mmol) in anhydrous DMF (4.0 cm^3) at 0 °C. The mixture was stirred for 18 h and water (20 cm^3) was added. After neutralisation (pH 7) with hydrochloric acid, dichloromethane (75 cm^3) was added. The organic phase was washed with saturated aq. sodium hydrogen carbonate (3 × 50 cm^3) and dried (Na_2SO_4). The solvent was removed under reduced pressure and the residue was purified by silica gel column chromatography using dichloromethane–methanol (98:2, v/v) as eluent to give *title compound* **24** as a solid (858 mg, 83%), $\delta_H(CDCl_3)$ 8.88 (1 H, br s, NH), 7.37–7.24 (11 H, m, 6-H and 2 × Ph), 6.02 (1 H, d, *J* 3.7, 1′-H), 4.67–4.49 (5 H, m, 2′-H and 2 × CH_2Ph), 4.29 (1 H, m, 4′-H), 3.99–3.96 (2 H, m, 2″-H_2), 3.84–3.79 (2 H, m, 5′-H_2), 2.36–2.12 (2 H, m, 1″-H_2) and 1.91 (3 H, d, *J* 1.0, CH_3); $\delta_C(CDCl_3)$ 163.5 (C-4), 150.0 (C-2), 137.5, 137.3 and 137.1 (C-aryl and C-6), 128.4, 128.4, 127.9, 127.7 and 127.0 (C-aryl), 109.1 (C-5), 93.7, 84.9, 83.7 and 80.7 (C-1′, -2′, -3′ and -4′), 73.5 and 70.2 (*C*H_2Ph), 68.4 and 66.4 (C-2″ and -5′), 32.4 (C-1″) and 12.5 (CH_3); PD-MS *m*/*z* 465.2 $[M + H]^+$ and 487.2 $[M + Na]^+$ (Found: C, 66.9; H, 6.2; N, 5.8. $C_{26}H_{28}N_2O_6$·0.25H_2O requires C, 66.6; H, 6.1; N, 6.0%).

(1*S*,5*R*,6*R*,8*R*)-5-Hydroxy-6-hydroxymethyl-8-(thymin-1-yl)-2,7-dioxabicyclo[3.3.0]octane 25

The same procedure as for preparation of compound **15** was used with precursor **24** (846 mg, 1.80 mmol), ethanol (10.0 cm^3) and 20% palladium hydroxide over carbon (400 mg) to give *title compound* **25** as a solid (444 mg, 82%), $\delta_H[(CD_3)_2SO]$ 11.3 (1 H, br s, NH), 7.36 (1 H, d, *J* 1.1, 6-H), 5.80 (1 H, d, *J* 4.3, 1′-H), 5.61 (1 H, s, OH), 4.86 (1 H, m, 4′-H), 3.89 (1 H, d, *J* 4.2, 2′-H), 3.85 (1 H, m, 2″-H^a), 3.83–3.64 (3 H, m, 5′-H_2 and 2″-H^b), 2.14 (1 H, m, 1″-H^a), 1.81 (1 H, m, 1″-H^b) and 1.78 (3 H, d, *J* 1.0, CH_3); $\delta_C(CD_3OD)$ 166.7 (C-4), 152.2 (C-2), 139.7 (C-6), 110.1 (C-5), 89.4, 89.1, 85.5 and 85.2 (C-1′, -2′, -3′ and

-4′), 71.4 (C-2″), 61.6 (C-5′), 37.0 (C-1″) and 12.7 (CH_3) (Found: C, 47.4; H, 5.7; N, 9.0. $C_{12}H_{16}N_2O_6 \cdot H_2O$ requires C, 47.7; H, 6.0; N, 9.3%).

(1*R*,6*R*,7*R*,9*R*)-7-(4,4′-Dimethoxytrityloxymethyl)-6-hydroxy-9-(thymin-1-yl)-2,8-dioxabicyclo[4.3.0]nonane 26

A solution of nucleoside **15** (70 mg, 0.235 mmol) in anhydrous pyridine (0.5 cm^3) was stirred at room temp. and DMTCl (114 mg, 0.353 mmol) was added. After stirring of the mixture for 3 h, additional DMTCl (100 mg, 0.310 mmol) was added. After further stirring of the mixture for 2 h, methanol (0.5 cm^3) was added and the mixture was evaporated. The residue was dissolved in dichloromethane (5 cm^3) and the solution was washed with saturated aq. sodium hydrogen carbonate (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 with dichloromethane–methanol (99 : 1, v/v) as eluent to give *title compound* **26** as a solid (140 mg, 99%), $\delta_H(CDCl_3)$ 8.53 (1 H, br s, NH), 7.75 (1 H, s, 6-H), 7.40–7.15 (9 H, m, ArH), 6.87–6.84 (4 H, m, ArH), 6.31 (1 H, d, *J* 8.4, 1′-H), 4.21–4.13 (2 H, m, 4′-H and 3″-H^a), 3.98 (1 H, d, *J* 8.4, 2′-H), 3.80 (6 H, s, 2 × OCH_3), 3.76 (1 H, m, 5′-H^a), 3.56 (1 H, m, 5′-H^b), 3.12 (1 H, m, 3″-H^b), 1.93–1.51 (4 H, m, 2″- and 1″-H_2) and 1.26 (3 H, s, CH_3); $\delta_C(CDCl_3)$ 163.4 (C-4), 158.9 (C-aryl), 150.7 (C-2), 143.7 (C-aryl), 135.7 (C-6), 134.9, 134.6, 130.3, 130.2, 129.0, 128.4, 128.0, 127.4 and 113.3 (C-aryl), 111.6 (C-5), 87.8, 84.3, 82.9 and 82.4 (Ar_3C, C-1′, -2′ and -4′), 74.1 (C-3′), 69.2 (C-3″), 62.3 (C-5′), 55.2 (OCH_3), 28.8 (C-1″), 20.3 (C-2″) and 11.3 (CH_3).

(1*S*,6*R*,7*R*,9*R*)-7-(4,4′-Dimethoxytrityloxymethyl)-6-hydroxy-9-(thymin-1-yl)-2,8-dioxabicyclo[4.3.0]nonane 27

The same procedure as for preparation of diastereoisomer **26** was used with precursor diol **20** (38 mg, 0.128 mmol), anhydrous pyridine (0.3 cm^3) and DMTCl (116 mg, 0.360 mmol) to give *title compound* **27** as a solid (47 mg, 61%), $\delta_H(CDCl_3)$ 7.71–7.22 (10 H, m, 6-H and ArH), 6.91–6.82 (4 H, m, ArH), 6.39 (1 H, d, *J* 3.5, 1′-H), 4.33 (1 H, m, 4′-H), 4.12–3.14 (4 H, m, 3″- and 5′-H_2), 3.78 (6 H, s, 2 × OCH_3), 3.74 (1 H, d, *J* 3.5, 2′-H), 2.19–1.27 (4 H, m, 2″- and 1″-H_2) and 1.77 (3 H, s, CH_3); $\delta_C(CDCl_3)$ 164.1 (C-4), 158.6 (C-aryl), 150.6 (C-2), 144.6 (C-aryl), 137.9 (C-6), 135.9, 135.8, 129.9, 129.9, 128.1, 127.8, 126.9 and 113.1 (C-aryl), 108.7 (C-5), 87.6, 86.6, 85.8 and 81.4 (Ar_3C, C-1′, -2′ and -4′), 76.6 (C-3′), 65.6 (C-3″), 64.3 (C-5′), 55.2 (OCH_3), 29.9 (C-1″), 22.0 (C-2″) and 12.5 (CH_3).

(1*S*,5*R*,6*R*,8*R*)-6-(4,4′-Dimethoxytrityloxymethyl)-5-hydroxy-8-(thymin-1-yl)-2,7-dioxabicyclo[3.3.0]octane 28

The same procedure as for the preparation of analogue **26** was used with precursor diol **25** (310 mg, 1.09 mmol), anhydrous pyridine (2.5 cm^3) and DMTCl (593 mg, 1.83 mmol) to give *title compound* **28** as a solid (618 mg, 97%), $\delta_H(CDCl_3)$ 9.04 (1 H, br s, NH), 7.47–7.16 (10 H, m, 6-H, ArH), 6.86–6.82 (4 H, m, ArH), 6.06 (1 H, d, *J* 4.1, 1′-H), 4.35 (1 H, d, *J* 4.1, 2′-H), 4.03 (1 H, m, 4′-H), 3.89 (1 H, m, 2″-H^a), 3.79 (6 H, s, 2 × OCH_3), 3.61 (1 H, m, 5′-H^a), 3.32–3.26 (2 H, m, 5′- and 2″-H^b), 1.94–1.69 (2 H, m, 1″-H_2) and 1.89 (3 H, s, CH_3); $\delta_C(CDCl_3)$ 163.4 (C-4), 158.6 (C-aryl), 150.1 (C-2), 144.3 (C-aryl), 137.2 (C-6), 135.6, 135.3, 129.9, 129.9, 128.9, 128.1, 127.9, 126.9, 125.2 and 113.2 (C-aryl), 109.3 (C-5), 88.7, 87.3, 86.9, 83.5 and 81.0 (Ar_3C, C-1′, -2′, -3′ and -4′), 69.7 (C-2″), 62.1 (C-5′), 55.1 (OCH_3), 36.5 (C-1″) and 12.5 (CH_3).

(1*R*,6*R*,7*R*,9*R*)-6-[2-Cyanoethoxy(diisopropylamino)phosphinoxy]-7-(4,4′-dimethoxytrityloxymethyl)-9-(thymin-1-yl)-2,8-dioxabicyclo[4.3.0]nonane 29

A solution of nucleoside **26** (108 mg, 0.180 mmol) in anhydrous dichloromethane (0.5 cm^3) and diisopropylethylamine (0.157 cm^3) was stirred at room temp. and 2-cyanoethyl *N*,*N*-diisopropylphosphoramidochloridite (0.081 cm^3, 0.360 mmol) was added. After stirring of the mixture for 1.5 h, methanol (0.4 cm^3) and ethyl acetate (5 cm^3) were added and the mixture was washed successively with saturated aq. sodium hydrogen carbonate (3 × 5 cm^3) and brine (3 × 5 cm^3). The organic phase was dried (Na_2SO_4), and evaporated under reduced pressure. The residue was dissolved in toluene (1 cm^3) and precipitated from hexane at −30 °C to give title compound **29** as a solid (124 mg, 86%), $\delta_P(CDCl_3)$ 142.5 and 140.4.

(1*S*,6*R*,7*R*,9*R*)-6-[2-Cyanoethoxy(diisopropylamino)phosphinoxy]-7-(4,4′-dimethoxytrityloxymethyl)-9-(thymin-1-yl)-2,8-dioxabicyclo[4.3.0]nonane 30

The same procedure as for the preparation of diastereoisomer **29** was used with precursor **27** (41 mg, 0.068 mmol), anhydrous dichloromethane (0.2 cm^3), diisopropylethylamine (0.060 cm^3) and 2-cyanoethyl *N*,*N*-diisopropylphosphoramidochloridite (0.031 cm^3, 0.137 mmol) to give title compound **30** as a solid (52 mg, 95%), $\delta_P(CDCl_3)$ 141.6 and 140.4.

(1*S*,5*R*,6*R*,8*R*)-5-[2-Cyanoethoxy(diisopropylamino)phosphinoxy]-6-(4,4′-dimethoxytrityloxymethyl)-8-(thymin-1-yl)-2,7-dioxabicyclo[3.3.0]octane 31

The same procedure as for preparation of analogue **29** was used with precursor **28** (436 mg, 0.743 mmol), anhydrous dichloromethane (2.2 cm^3), diisopropylethylamine (0.62 cm^3) and 2-cyanoethyl *N*,*N*-diisopropylphosphoramidochloridite (0.33 cm^3, 1.457 mmol) to give title compound **31** as a solid (517 mg, 88%) after silica gel column chromatography using dichloromethane–triethylamine (97 : 3, v/v) as eluent, evaporation, dissolution in toluene (1 cm^3) and precipitation from hexane at −30 °C, $\delta_P(CDCl_3)$ 142.0 and 141.9.

Synthesis of oligodeoxynucleotides

Synthesis of oligonucleotides **32**–**45** was performed on 0.2 μmol scale using commercial 2-cyanoethyl phosphoramidites and compounds **29**–**31**. The syntheses followed the regular protocol for the DNA-synthesiser for commercial 2-cyanoethyl phosphoramidites. However, for compounds **29**–**31** we used two 12-min couplings for each step, and the coupling efficiencies were lower (~30% for **29**, 50% for **30** and 95% for **31**) than those obtained for the unmodified amidites (~99%). The 5′-*O*-DMT-on ONs were removed from the solid support by treatment with conc. ammonia at room temp. for 72 h which also removed the protecting groups. Subsequent purification using disposable reversed-phase chromatography cartridges (including 5′-*O*-detritylation) afforded the pure ONs **32**–**45**. MALDI-MS $[M + H]^+$ gave the following results: 4252.6 (**32**, Calc. 4252.9), 4307.4 (**33**, Calc. 4308.9), 4307.4 (**34**, Calc. 4308.9), 4251.2 (**35**, Calc. 4252.9), 4307.0 (**36**, Calc. 4308.9), 4307.5 (**37**, Calc. 4308.9), 4241.0 (**38**, Calc. 4238.8), 4283.2 (**39**, Calc. 4280.9), 4284.7 (**40**, Calc. 4280.9), 4364.1 (**41**, Calc. 4364.9), 4363.7 (**42**, Calc. 4364.9) and 4743.0 (**43**, Calc. 4743.3).

Melting experiments

Melting experiments were carried out in medium salt buffer, 1 mM EDTA, 10 mM Na_3PO_4, 140 mM NaCl, pH 7.2.[23] The increase in absorbance at 260 nm as a function of time was recorded while the temp. was raised linearly from 10 to 60 °C at a rate of 0.5 °C min^{-1}. The melting temp. was determined as the local maximum of the first derivative of the absorbance *vs.* temp. curve.

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