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A conformationally locked tricyclic nucleoside. Synthesis, crystal structure and incorporation into oligonucleotides

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A tricyclic nucleoside is synthesised from a bicyclic nucleoside precursor by applying a stereoselective dihydroxylation, a regioselective tosylation and an intramolecular ether formation. This tricyclic nucleoside is constructed as a conformationally locked thymidine analogue and has been analysed by X-ray crystallography. Thus, the furanose ring of this nucleoside adopts a perfect *S*-type conformation and the torsion angle γ, describing the C4′–C5′ bond is restricted in the $+ac$ range. The tricyclic nucleoside is incorporated into two nonameric oligonucleotide sequences displaying strongly decreased affinity towards complementary DNA and RNA when compared to the corresponding unmodified oligodeoxynucleotide sequences.

Introduction

The demand for oligonucleotides with efficient and selective recognition of complementary nucleic acid sequences for diagnostic or therapeutic purposes has recently motivated significant research activity.[1,2] Conformationally restricted nucleosides have proven to be a very successful approach for the construction of oligonucleotides preorganised for efficient nucleic acid recognition.[2,3] As a prime example, LNA (locked nucleic acid) has recently been introduced[4] as a nucleic acid analogue displaying unprecedented recognition of complementary DNA and RNA due to its nucleoside monomers being conformationally locked in an *N*-type (C3′-*endo*) conformation[5] by a bicyclic carbohydrate moiety.[4] Other bi- and tricyclic nucleoside analogues have been introduced in this context[3] including the pioneer example **1** by Leumann and co-workers (Fig. 1).[6] This nucleoside structure has been found to be restricted in an *S*-type (C1′-*exo*) conformation. However, the C4′–C5′ bond is also restricted and the torsion angle γ describing this bond[5] is found to prefer the $+ac/+ap$ range, which is known to be untypical in natural duplexes. Subsequently, oligonucleotides containing **1** have been shown to prefer the Hoogsteen base-pairing mode over the usual Watson–Crick base-pairing.[7] The binding affinities towards complementary nucleic acid sequences were slightly improved compared to unmodified oligodeoxynucleotides but were very dependent on the nucleotide sequences.[6,7] Improved binding affinities have been introduced with a 5′–6′ cyclopropanated tricyclic analogue of **1** in which γ is restricted to the less unfavourable $+ac$ range.[8]

Another bicyclic nucleoside with interesting properties is **2** which in a fully modified sequence demonstrated increased affinity for complementary RNA but not DNA.[9] This nucleoside has been shown by molecular modelling of the monomer,[10] as well as in NMR[11] and X-ray studies[12] of duplexes containing this modification, to adopt an *E*-type (O4′-*endo*) conformation.[5] The bicyclic nucleoside **3** has also been synthesised[13] and incorporated into oligonucleotides. However, very large decreases in duplex stability were observed.[14] This nucleoside might be suggested to be strongly restricted in a perfect *S*-type conformation but with γ in an unfavourable $+ap$ conformation due to a chair conformation of the six-membered ring.

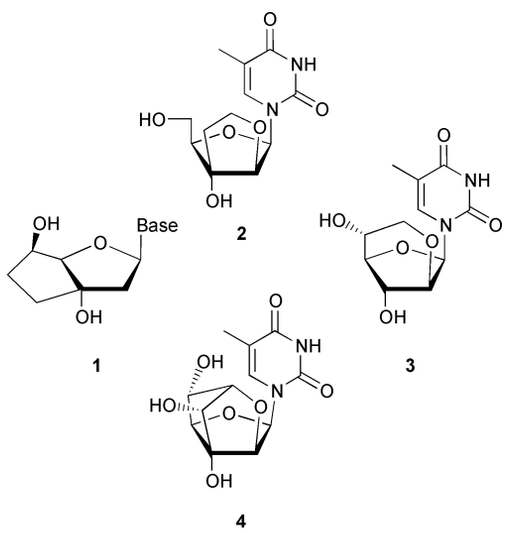

Fig. 1 Bicyclic and tricyclic nucleosides.

Furthermore, the 6′-carbon atom might sterically prevent the nucleobase from taking a proper *anti*-position[5] relative to the furanose ring.

Recently, we have introduced the syntheses of bicyclic *ribo*-nucleoside analogues by an RCM (ring-closing metathesis) method.[15] Thus, an olefinic moiety connecting the C3′ and the C5′ atoms provided the opportunity for the construction of analogues of **1** as well as *e.g.* tricyclic nucleosides.[15] Following up these results, we hereby introduce a nucleoside combining the ring-systems of the three bicyclic nucleosides **1**, **2**, and **3** in a tricyclic structure **4**. Thus, interesting properties concerning the recognition of complementary nucleic acids might be obtained. Thus, each of the three bicyclic ring systems in **1**, **2**, and **3** retain some degree of flexibility but are hereby strongly conformationally restricted, apparently towards more favourable conformations. Hence, simple modelling suggests that this tricyclic nucleoside **4** will be conformationally locked due to the very rigid [2.2.1]bicyclic system connected to the furanose ring. Furthermore, this furanose should adopt a perfect *S*-type conformation and γ should prefer a conformation that is less unfavourable than the ranges found for **1** and **3**.

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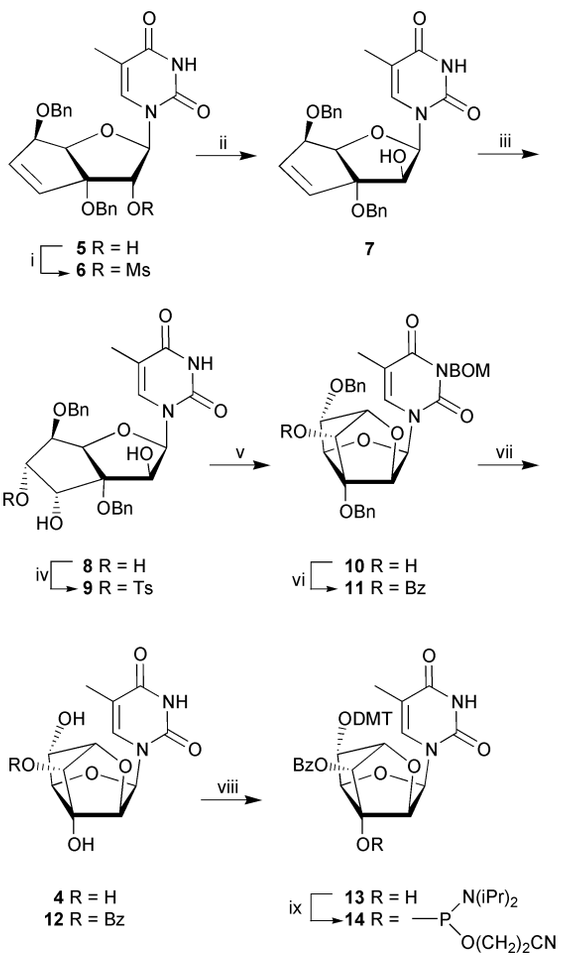
Results

Synthesis of the tricyclic nucleoside 4

The tricyclic nucleoside **4** and the corresponding phosphoramidite building block **14** were synthesised from the benzyl-protected bicyclic nucleoside **5** as outlined in Scheme 1. The



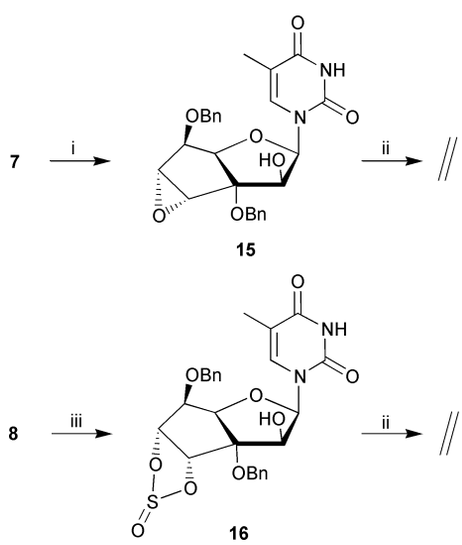
Scheme 1 *Reagents and conditions*: i, MsCl, pyridine; ii, aq. NaOH, EtOH; iii, OsO_4, NMO, aq. THF, *t*-BuOH; iv, TsCl, pyridine; v, BOM-Cl, DBU, CH_3CN; vi, BzCl, pyridine; vii, H_2, $Pd(OH)_2$–C, MeOH; viii, DMT-OTf, DMAP, pyridine; ix, $NC(CH_2)_2OP(Cl)N(i\text{-}Pr)_2$, $EtN(i\text{-}Pr)_2$, CH_2Cl_2.

synthesis of **5** in 13 steps and 36% overall yield from diacetone-α-D-glucose, taking advantage of an RCM strategy, has recently been described by us.[15] Inversion of configuration at C2′ was achieved using a 2,2′-anhydro approach.[9,16] Thus, conversion of the 2′-OH of **5** to the corresponding methanesulfonic ester to give **6** and subsequent treatment with aq. base in ethanol afforded the *arabino*-configured nucleoside **7** in 82% yield over the two steps. The alkene moiety of the bicyclic system was treated with a catalytic amount of osmium tetraoxide in the presence of *N*-methylmorpholine *N*-oxide (NMO) as a co-oxidant to give the dihydroxylated nucleoside **8** in 83% yield. The reaction proceeded with complete stereoselectivity giving only a single product. The stereochemistry of this product **8** was indicated by a large coupling constant $^3J_{H5'H6'} = 7.5$ Hz indicating the two protons to be in a *trans* position on the cyclopentane ring. This result was expected, since osmium tetraoxide should approach the double bond from the less hindered, convex side of the bicyclic system. Unfortunately, the reaction is relatively slow giving the product in 84% yield after stirring at 50 °C for at least 5 days. Raising the temperature above 50 °C did not speed up the reaction but led to a varying amount of by-products resulting from dihydroxylation of the thymine double bond.

In order to perform a ring-closing reaction between the 2′-OH group and C6′, the 6′-OH group was converted to an appropriate sulfonate as a potential leaving group. Taking advantage of the sterically demanding toluene-*p*-sulfonate group, the selective esterification between secondary alcohols has been observed before avoiding tosylation of the 2′-OH on a similar *arabino*-nucleoside substrate.[17] Thus, treatment of **8** with 7 equivalents of toluene-*p*-sulfonyl chloride in pyridine at room temperature resulted in a completely regioselective tosylation of 6′-OH to give the monotosylate **9** in 77% yield together with a small amount of unreacted starting material **8**. We suggest that the first sulfonic ester sterically prevents the formation of a second ester on the neighbouring 7′-OH group. Cyclisation of **9** by treatment with NaH in DMF and heating did not give the expected tricyclic compound. Instead, an analysis of the crude product indicated cyclisation between O2 of the thymine nucleobase and C6′ to give a 2,6′-anhydro compound. In order to avoid this unwanted reaction, we decided to replace the imide proton of the nucleobase with a benzyloxymethyl (BOM) group, thereby preventing a nucleophilic attack from the adjacent O2 carbonyl group. Surprisingly, this protection of N3 using benzyloxymethyl chloride and 1,8-diazabicyclo[5.4.0]-undec-7-ene (DBU) in CH_3CN did not afford the *N*-BOM-protected analogue of **9**, but instead a single product, which was identified as the cyclised compound **10**, was obtained in 76% yield. Thus, after BOM-protection DBU must have been able to promote the immediate cyclisation between 2′-OH and C6′. Finally, treatment of **10** with hydrogen in the presence of 20% $Pd(OH)_2$–C in methanol afforded the fully deprotected tricyclic nucleoside **4** in 65% yield. The structure of this novel nucleoside was confirmed by NMR, mass spectrometry and X-ray crystallographic data (*vide infra*).

Alternative approaches

In addition to the successful synthetic strategy shown (Scheme 1), two other strategies for the construction of **4** have been investigated (Scheme 2). Thus, conversion of the olefinic moiety



Scheme 2 *Reagents and conditions*: i, H_2O_2, K_2CO_3, PhCN, MeOH; ii, NaH, DMF; iii, $SOCl_2$, Et_3N, CH_2Cl_2.

of **7** to an epoxide was expected to proceed stereoselectively from the convex side of the bicyclic nucleoside affording a reasonable substrate for intramolecular ether formation. However, this potential was recognised for both the target structure **4** as well as for an alternative, but still interesting, tricyclic nucleoside containing a four-membered ring connecting 2′-O and C7′. After a standard epoxidation using MCPBA failed to give any product, the epoxide **15** was obtained as the only product in 68% yield from alkene **7** using the protocol of Payne *et al*. with

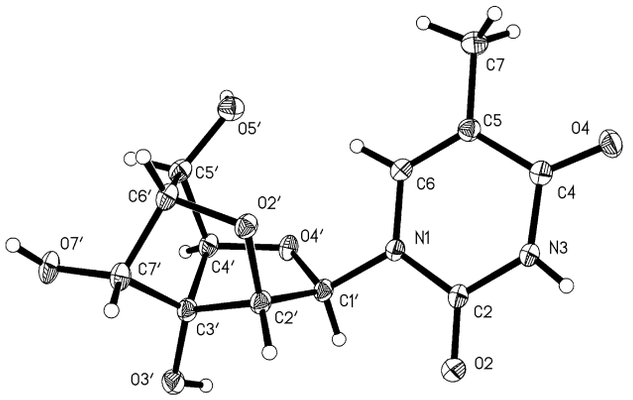


Fig. 2 Molecular structure (ORTEP-plot) of the tricyclic nucleoside **4**.

Table 1 Important torsion angles

Torsion	Definition[a]	Measured angle[b]/°
ν_0	C4′–O4′–C1′–C2′	−12.3
ν_1	O4′–C1′–C2′–C3′	35.9
ν_2	C1′–C2′–C3′–C4′	−42.8
ν_3	C2′–C3′–C4′–O4′	36.8
ν_4	C3′–C4′–O4′–C1′	−16.4
δ	O3′–C3′–C4′–C5′	152.5
γ	C3′–C4′–C5′–O5′	137.4
χ	O4′–C1′–N–C2	−151.2
	O3′–C3′–C7′–O7′	−61.6
P	Ref. 5	182.2
Φ_{max}	ν_2/cos P	42.8

[a] Torsion angles defined according to ref. 5. [b] Torsion angles measured from crystallographic data.

H_2O_2 and benzonitrile.[18] Nevertheless, subsequent treatment of **15** with NaH in DMF and heating did not give any traceable cyclisation, but rather a slow decomposition of the starting material. As an alternative, the same reaction conditions were applied to the cyclic sulfite **16** synthesised in 43% yield from the dihydroxylated nucleoside **8** by reaction with thionyl chloride, but again no cyclisation was observed. As no cyclisation involving the nucleobase was indicated with either **15** or **16**, the same BOM-protecting strategy as that successfully applied before with **9** was not attempted.

Crystal structure of 4

The molecular structure of the tricyclic nucleoside **4** was determined by a single-crystal X-ray diffraction study of the dihydrate. The molecules in the crystal are connected through a network of hydrogen bonds, especially O–H···O interactions involving two hydrate water molecules. A plot of the structure, as shown in Fig. 2, demonstrates the expected configuration of the nucleoside analogue and its constrained tricyclic nature. About the only flexibility of the molecule lies in the torsion around the anomeric N–C1′ bond. The remainder of the molecule is the expected rigid arrangement of interlocked rings leading to some strain as reflected in several of the bond angles around C1′ to C7′ that deviate considerably from the ideal tetrahedral values. The most notable deviation is found for C6′–C7′–C3′, which is 93.4°. The pyrimidine ring is planar as expected, having the attached methyl carbon 0.67 Å out of that plane. The most relevant torsional angles measured are displayed in Table 1. Thus, the torsions describing the furanose ring ν_0–ν_4 were measured, and from the general definition,[5] the pseudorotational angle P, as well as the puckering amplitude Φ_{max}, were calculated. Also, the torsions describing the backbone angles δ and γ, as well as the torsions describing the anomeric bond χ and the vicinal diol moiety of the C3′–C7′ bond, are shown in Table 1.

Synthesis of oligonucleotides

The tricyclic nucleoside **4** was incorporated into oligonucleotide sequences by using the standard automated solid-phase phosphoramidite methodology.[19] As a prerequisite, the 7′-hydroxy group should be appropriately protected with *e.g.* a base-labile protecting group, complying with the standard methods for oligonucleotide synthesis. Thus, the hydroxy group of **10** (Scheme 1) was esterified with benzoyl chloride in pyridine to give **11** in 83% yield, and subsequent debenzylation under standard hydrogenation conditions afforded the diol nucleoside **12** in a reasonable 57% yield, but surprisingly, also 20% yield of the fully deprotected nucleoside **4**. For the conversion of nucleoside **12** into an appropriate phosphoramidite, the 5′-hydroxy group was protected as its 4,4′-dimethoxytrityl (DMT) ether. The product **13** was successfully obtained in 64% yield using 4,4′-dimethoxytrityl triflate (DMT-OTf) in pyridine in the presence of dimethylaminopyridine (DMAP). This reagent has been developed by Leumann and co-workers[20] and used for the tritylation of secondary and tertiary alcohols of other bi- and tricyclic nucleosides.[20,21] Despite the predicted steric hindrance of the secondary 5′-hydroxy group of **12**, we expected the tritylation to be selective for this position. The structure of the only product **13** was verified by its ^{1}H NMR spectrum. Thus, in the ^{1}H NMR spectrum of **12** (recorded in DMSO-d_6) the 3′-OH signal occurs as a singlet at 6.72 ppm whereas the 5′-OH signal occurs as a doublet at 5.88 ppm. The fact that only a singlet at 6.79 ppm occurs in the corresponding spectrum of the DMT-protected analogue **13** strongly indicates the DMT-group to be situated at O5′. This compound was phosphorylated to give the phosphoramidite building block **14** as an impure material in quantitative yield. Thus, traces of hydrolysed phosphitylation reagent as well as a phosphonate were detected in ^{31}P NMR spectra. Nevertheless, this compound could be conveniently used in an automated solid-phase synthesis of oligodeoxynucleotides using the phosphoramidite approach.[19] Thus, two nonameric mixed sequences incorporating **4** (*via* phosphoramidite **14**) once and three times, respectively, were synthesised (Table 2). The use of standard 1*H*-tetrazole activation for the coupling reaction of **14** afforded only low coupling yields, whereas pyridinium hydrochloride improved the stepwise coupling yield of **14** to >85%. The modified oligonucleotides were synthesised by using the DMT-*ON* mode and purified by using disposable reversed-phase chromatography cartridges (Cruachem). The purities of the synthesised oligonucleotides were found to be >95% by capillary gel electrophoresis. The compositions of the sequences were verified by MALDI-MS spectra.

Melting properties of oligonucleotides

The modified sequences **18** and **19** were mixed with their complementary DNA and RNA sequences **20** and **21** and the thermal stabilities of the duplexes were determined (Table 2). When compared to the corresponding unmodified duplexes obtained by mixing the unmodified sequence **17** with the same complements, large decreases in thermal stability of 9 and 10 °C were observed for the sequence **18** with one tricyclic nucleoside incorporated. For the sequence **19** with three tricyclic nucleosides **4** incorporated, no stable duplexes with either complementary DNA or RNA could be observed.

Discussion

The target tricyclic nucleoside **4** has been obtained in 6 steps and 26% overall yield from **5** through an efficient synthetic strategy including a selective tosylation and subsequent ring-closing reaction. Including the procedures towards **5**,[15] **4** has been obtained in 19 steps and 9% overall yield from the cheap starting material diacetone-D-glucose taking advantage from a very efficient RCM-protocol.[15] The nucleoside has been

Table 2 Hybridisation data

Oligonucleotides		T_m/°C[a] DNA complement **20** 5′-GCATATCAG-3′	T_m/°C[a] RNA complement **21** 5′-GCAUAUCAG-3′
17	5′-CTGATATGC-3′	29	27
18	5′-CTGA**X**ATGC-3′	20	17
19	5′-C**X**GA**X**A**X**GC-3′	<8	<8

[a] Melting temperatures (T_m) obtained from the maxima of the first derivatives of the melting curve [A_{260} (absorbance at 260 nm) *vs*. temperature] recorded in a buffer containing 10 mM Na_2HPO_4, 100 mM NaCl, 0.1 mM EDTA, pH 7.0 using 1.5 μM concentrations of the two complementary sequences assuming identical absorption coefficients for all the thymine nucleotides. **X** corresponds to **4** incorporated *via* **14**.

efficiently incorporated into oligonucleotides through the phosphoramidite **14**. Thus, the problems with a phosphoramidite functionality positioned on a tertiary 3′-hydroxy functionality as described earlier[22] were not observed in this case. Neither was this expected, as the present tricyclic system should not be able to adopt the planar geometry of a potential 3′-cation and, therefore, no cleavage of the C3′–O bond occurred during oxidation.[22] On the other hand, rearrangement of the oligomers during deprotection in the form of a migration of the sequence in the 3′-end of the tricycle from 3′-OH to the free 7′-OH cannot be completely excluded from the MALDI-MS and electrophoretic data. However, we consider this migration to be very unlikely due to the relatively large distance (3.0 Å) between the two vicinal hydroxy functionalities. Thus, the constrained tricyclic system dictates an inflexible torsion angle O3′–C3′–C7′–O7′ of 62° (Table 1). Furthermore, no mixtures of different (including shorter) sequences have been observed, and the sequences **18** and **19** were obtained in yields as expected from the detected stepwise coupling yields.

The crystallographic data of **4** prove the nucleoside to be restricted in a typical *S* conformation with a pseudorotational angle $P = 182°$ and a puckering amplitude $\Phi_{max} = 43°$ (Table 1). Hence, this nucleoside can be considered as a perfect mimic of nucleosides in *S*-type conformations. The relatively large puckering amplitude further verifies the conformational rigidity of the tricyclic system. The C4′–C5′ bond is very strongly restricted or locked in $\gamma = 137°$, *i.e.* in the $+ac$ range. However, this is not typically found in nucleic acid duplexes. Nevertheless, the deviation from the typical $+sc$ range (around 60°) is smaller than that observed for another tricyclic nucleoside recently described by us (178°)[10] or the bicyclic nucleosides **1** (140–160°),[6,7] but is larger compared to the more successful tricyclic nucleosides constructed by Steffens and Leumann (110–130°).[8] The hybridisation data (Table 2), however, demonstrate the tricyclic nucleoside structure **4** to be very unfavourable for duplex formation in contrary to those of the bicyclic nucleoside **1**[6,7] and its 5′–6′ cyclopropanated tricyclic analogue.[8] At least, higher affinity for complementary DNA than for RNA might have been expected as the *S*-type conformation dictates a B-type duplex as typically found in DNA–DNA duplexes. One reason for the strong general destabilisation of duplexes might be found in the highly locked structure of **4** in combination with an imperfect angle γ. Hence, no small adjustments during hybridisation are allowed. On the other hand, both X-ray data and simple modelling also suggest the anomeric bond described by the torsion angle χ to be less flexible than expected and, perhaps, unfavourably restricted. Apparently, the nucleobase is prevented from adopting a typical *anti* position relative to the furanose ring due to steric hindrance from the 2′-O atom and the 5′-O atom both irreversibly locked in their positions by the tricyclic ring system. Thus, the torsion angle found, −151° (Table 1), deviates slightly from the values of around −130° typically found for nucleosides in C2′-*endo* (*S*-type) conformations.[5] With the free rotation around the anomeric bond prohibited, the correct position of the nucleobase for favourable base-pairing and duplex formation cannot be found. Finally, steric destabilisation or crucial disturbance of the duplex hydration pattern by the additional rings and the secondary alcohol moiety (7′-OH) cannot be excluded.

The present results suggest that even though strong conformational restriction in locked nucleoside structures can be extremely useful as with LNA,[4] the same strategy can also be extremely unfavourable for duplex formation, if an imperfect conformation is favoured. In the present case, the effect of adding a few additional atoms to the natural deoxynucleoside structure, thereby restricting its conformational freedom, is at the same level of destabilisation as formerly demonstrated by increasing the same conformational freedom through the introduction of acyclic nucleoside moieties.[23] Nevertheless, the possibility of obtaining high-affinity nucleic acid recognition by using nucleosides that are locked in *S*-type conformations is still an obvious opportunity. However, the C4′–C5′ bond should be either flexible or, perhaps even better, restricted in a more favourable $+sc$ conformational range. Likewise, a proper *anti*-positioning of the nucleobase should be allowed.

Conclusions

A tricyclic nucleoside has been efficiently synthesised through a bicyclic precursor which has been conveniently obtained *via* an RCM strategy. From X-ray crystallography, the tricyclic nucleoside has been demonstrated to adopt a perfect *S*-type conformation but with both the C4′–C5′ bond and the anomeric bond restricted in partly unfavourable conformations. Subsequently, the tricyclic nucleoside was found to strongly decrease the affinity of oligodeoxynucleotides for complementary DNA and RNA sequences. Nevertheless, the results give new insight into the process of duplex formation and describe the limits for the design of future conformationally restricted nucleosides. Alternatively, the present tricyclic nucleoside structure might be a significant tool in the study of conformational preferences of nucleoside/nucleotide converting enzymes or receptors. Further work on the design of conformationally restricted bi- and tricyclic nucleosides for the general study of the scopes and limitations of high-affinity nucleic acid recognition is in progress in our laboratory.

Experimental

All commercial reagents were used as supplied except for toluene-*p*-sulfonyl chloride, which was recrystallised from chloroform, and thionyl chloride, which was distilled before use. All reactions were performed under an atmosphere of nitrogen. Column chromatography was carried out on glass columns using Silica gel 60 (0.040–0.063 mm). NMR spectra were obtained on a Bruker AC250, a Varian Gemini 2000 or a Varian Unity 500 spectrometer. ^{1}H NMR spectra were recorded at 250, 300 or 500 MHz and ^{13}C NMR spectra were recorded at 62.5 or 75 MHz. Values for δ are in ppm relative to tetramethylsilane as internal standard. A ^{31}P NMR spectrum was recorded at 121.5 MHz with 85% H_3PO_4 as external standard. ^{1}H–^{1}H-COSY spectra were recorded for compounds **4**, **8**, **9**, **10**, **11**, **12**,

15 and **16** and a ^{1}H–^{13}C-COSY spectrum was recorded for compound **10**. Assignments of NMR signals follow standard carbohydrate and nucleoside style. However, the compound names are given according to von Baeyer nomenclature. FAB mass spectra were recorded in positive ion mode on a Kratos MS50TC spectrometer, and EI mass spectra were recorded on a SSQ710 Finnigan MAT spectrometer. Microanalyses were performed at the Microanalytical Laboratory, Department of Chemistry, University of Copenhagen.

(1*R*,3*R*,4*R*,5*R*,8*R*)-5,8-Bis(benzyloxy)-4-methylsulfonyloxy-3-(thymin-1-yl)-2-oxabicyclo[3.3.0]oct-6-ene 6

Methanesulfonyl chloride (0.8 cm^3, 10.3 mmol) was added dropwise to a stirred solution of **5**[15] (1.21 g, 2.62 mmol) in anhydrous pyridine (15 cm^3) at 0 °C. The reaction mixture was stirred for 3 h at room temperature, quenched with ice-cold water (30 cm^3), and extracted with CH_2Cl_2 (3 × 40 cm^3). The combined extracts were washed with saturated aq. $NaHCO_3$ (3 × 20 cm^3) and then dried ($MgSO_4$). The solvent was removed by distillation under reduced pressure and the residue was co-evaporated with toluene (2 × 50 cm^3) and then purified by column chromatography [0–2% (v/v) MeOH in CH_2Cl_2] to give the product **6** (1.36 g, 96%) as a white solid material (Found: C, 60.54; H, 5.58; N, 5.28. $C_{27}H_{28}N_2O_8S$ requires C, 59.99; H, 5.22; N, 5.18%); δ_H ($CDCl_3$) 1.66 (3H, d, *J* 1.2, CH_3), 3.22 (3H, s, SO_2CH_3), 4.38 (1H, d, *J* 11.4, C*H*$_2$Ph), 4.58 (1H, d, *J* 11.5, C*H*$_2$Ph), 4.63 (1H, d, *J* 11.5, C*H*$_2$Ph), 4.72 (1H, m, 5′-H), 4.76 (1H, d, *J* 11.4, C*H*$_2$Ph), 4.81 (1H, d, *J* 4.8, 4′-H), 5.18 (1H, d, *J* 3.5, 2′-H), 6.03 (1H, d, *J* 5.9, 7′-H), 6.17 (1H, d, *J* 5.9, 6′-H), 6.17 (1H, d, *J* 3.5, 1′-H), 7.26–7.36 (10H, m, Ph), 7.74 (1H, d, *J* 1.2, 6-H), 9.26 (1H, br s, NH); δ_C ($CDCl_3$) 12.2, 39.2, 67.6, 72.4, 81.6, 82.9, 84.4, 91.3, 92.8, 110.9, 127.6, 127.8, 127.9, 128.2, 128.4, 128.6, 131.2, 135.7, 137.1, 137.3, 138.9, 150.8, 163.6; FAB-MS *m*/*z* 541 [M + H$^+$].

(1*R*,3*R*,4*S*,5*R*,8*R*)-5,8-Bis(benzyloxy)-4-hydroxy-3-(thymin-1-yl)-2-oxabicyclo[3.3.0]oct-6-ene 7

To a solution of **6** (1.05 g, 1.94 mmol) in EtOH (25 cm^3) and H_2O (25 cm^3) was added 2.0 M aq. NaOH (4 cm^3, 8 mmol) and the reaction mixture was stirred under reflux for 16 h. After cooling to room temperature, the mixture was neutralised with 1 M aq. HCl and the solvent was partly removed by distillation under reduced pressure. The residue was saturated with NaCl and then extracted with CH_2Cl_2 (3 × 40 cm^3). The combined extracts were washed with saturated aq. $NaHCO_3$ (2 × 50 cm^3) and then dried ($MgSO_4$). The solvent was removed by distillation under reduced pressure and the residue purified by column chromatography [0–2% (v/v) MeOH in CH_2Cl_2] to give the product **7** (761 mg, 85%) as a white solid material (Found: C, 67.32; H, 5.77; N, 6.11. $C_{26}H_{26}N_2O_6$ requires C, 67.52; H, 5.67; N, 6.06%); δ_H ($CDCl_3$) 1.52 (3H, s, C*H*$_3$), 4.00 (1H, d, *J* 7.7, OH), 4.43 (1H, d, *J* 11.4, C*H*$_2$Ph), 4.54 (1H, d, *J* 5.6, 5′-H), 4.60 (1H, d, *J* 11.4, C*H*$_2$Ph), 4.61 (1H, m, 2′-H), 4.62 (1H, d, *J* 11.4, C*H*$_2$Ph), 4.68 (1H, d, *J* 5.6, 4′-H), 4.79 (1H, d, *J* 11.4, C*H*$_2$Ph), 6.21 (2H, br s, 6′-H and 7′-H), 6.35 (1H, d, *J* 3.9, 1′-H), 7.25–7.34 (10H, m, Ph), 7.57 (1H, s, 6-H), 9.58 (1H, br s, NH); δ_C ($CDCl_3$) 12.1, 67.2, 72.4, 74.8, 80.4, 82.5, 89.2, 99.6, 108.7, 127.4, 127.7, 127.8, 128.1, 128.4, 128.6, 131.9, 136.8, 137.2, 137.8, 138.5, 150.6, 163.5.

(1*R*,3*R*,4*S*,5*R*,6*R*,7*R*,8*R*)-5,8-Bis(benzyloxy)-4,6,7-trihydroxy-3-(thymin-1-yl)-2-oxabicyclo[3.3.0]octane 8

To a solution of **7** (840 mg, 1.82 mmol) in THF (20 cm^3) and H_2O (20 cm^3) were added *N*-methylmorpholine *N*-oxide (640 mg, 5.45 mmol) and a 2.5% w/w solution of OsO_4 in *tert*-butyl alcohol (1.0 cm^3, 0.077 mmol), and the reaction mixture was stirred at 50 °C for 6 days. After cooling to room temperature the reaction was quenched with 5% aq. $Na_2S_2O_5$ (10 cm^3) and the solvent was partly removed by distillation under reduced pressure. The residue was extracted with EtOAc (4 × 50 cm^3) and the combined organic extracts were dried ($MgSO_4$). The solvent was removed by distillation under reduced pressure and the residue purified by column chromatography [3–8% (v/v) MeOH in CH_2Cl_2] to give the product **8** (761 mg, 83%) as a white solid material (Found: C, 61.51; H, 5.62; N, 5.58. $C_{26}H_{28}N_2O_8{\cdot}\frac{1}{2}H_2O$ requires C, 61.77; H, 5.78; N, 5.54%); δ_H (DMSO-d_6) 1.61 (3H, s, CH_3), 3.99 (1H, dd, *J* 7.5 and 6.6, 5′-H), 4.07–4.20 (2H, m, 6′-H and 7′-H), 4.27–4.33 (2H, m, 2′-H, 4′-H), 4.53 (1H, d, *J* 11.6, C*H*$_2$Ph), 4.55 (1H, d, *J* 11.5, C*H*$_2$Ph), 4.66 (1H, d, *J* 11.6, C*H*$_2$Ph), 4.82 (1H, d, *J* 11.5, C*H*$_2$Ph), 4.91 (1H, d, *J* 4.3, 6′-OH), 5.05 (1H, d, *J* 6.8, 7′-OH), 5.95–6.00 (2H, m, 1′-H and 2′-OH), 7.26–7.44 (10H, m, Ph), 7.58 (1H, s, 6-H), 11.34 (1H, s, NH); δ_C (DMSO-d_6) 12.4, 66.3, 69.1, 71.1, 72.0, 75.8, 81.0, 82.2, 86.2, 92.1, 106.9, 127.8, 128.0, 128.2, 128.4, 128.5, 128.6, 137.8, 138.5, 138.7, 150.1, 163.8; FAB-MS *m*/*z* 497 [M + H$^+$].

(1*R*,3*R*,4*S*,5*R*,6*R*,7*R*,8*R*)-5,8-Bis(benzyloxy)-4,6-dihydroxy-3-(thymin-1-yl)-7-(4-tolylsulfonyloxy)-2-oxabicyclo[3.3.0]octane 9

To a solution of **8** (750 mg, 1.51 mmol) in anhydrous pyridine (50 cm^3) was added toluene-*p*-sulfonyl chloride (2.0 g, 10.5 mmol) and the mixture was stirred at room temperature for 48 h. The reaction was quenched with H_2O (50 cm^3) and extracted with CH_2Cl_2 (3 × 100 cm^3). The combined extracts were washed with saturated aq. $NaHCO_3$ (2 × 150 cm^3) and then dried ($MgSO_4$). The solvent was removed by distillation under reduced pressure and the residue was co-evaporated with toluene (2 × 50 cm^3) and then purified by column chromatography [1–3% (v/v) MeOH in CH_2Cl_2] to give unreacted starting material **8** (53 mg, 7%) and the product **9** (759 mg, 77%) as white solid materials (Found: C, 60.99; H, 5.24; N, 4.29. $C_{33}H_{34}N_2O_{10}S$ requires C, 60.91; H, 5.27; N, 4.31%); δ_H ($CDCl_3$) 1.48 (3H, s, CH_3), 2.43 (3H, s, $PhCH_3$), 4.24–4.56 (7H, m, 4′-H, 5′-H, 7′-H, C*H*$_2$Ph), 4.88 (1H, m, 2′-H), 5.29 (1H, dd, *J* 8.2 and 3.6, 6′-H), 6.15 (1H, d, *J* 2.8, 1′-H), 7.35–7.20 (12H, m, Bn, Ts), 7.68 (1H, s, 6-H), 7.80 (2H, d, *J* 8.1, Ts), 11.32 (1H, br s, NH); δ_C ($CDCl_3$) 11.9, 21.6, 67.5, 68.5, 71.5, 72.4, 79.0, 82.4, 85.6, 88.8, 93.3, 107.6, 127.8, 127.8, 127.9, 128.0, 128.1, 128.3, 128.6, 128.6, 129.8, 133.6, 137.2, 137.3, 145.1, 150.5, 166.6; FAB-MS *m*/*z* 651 [M + H$^+$].

(1*S*,3*S*,4*R*,6*R*,7*R*,8*R*,9*R*)-7,9-Bis(benzyloxy)-8-hydroxy-4-(3-*N*-benzyloxymethylthymin-1-yl)-2,5-dioxatricyclo[4.2.1.0^{3,7}]nonane 10

To a solution of **9** (592 mg, 0.91 mmol) in anhydrous CH_3CN (10 cm^3) were added benzyl chloromethyl ether (0.2 cm^3, 1.5 mmol) and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) (0.3 cm^3, 2.0 mmol) and the mixture was stirred at room temperature for 16 h. After adding additional benzyl chloromethyl ether (0.1 cm^3, 0.73 mmol) and DBU (0.15 cm^3, 1.0 mmol) the mixture was stirred for a further 6 h. The mixture was concentrated by distillation under reduced pressure and the residue was purified by column chromatography [0–2% (v/v) MeOH in CH_2Cl_2] to give the product **10** (415 mg, 76%) as a white solid material (Found: C, 67.52; H, 5.79; N, 4.68. $C_{34}H_{34}N_2O_8{\cdot}\frac{1}{3}H_2O$ requires C, 67.54; H, 5.78; N, 4.63%); δ_H ($CDCl_3$) 1.34 (3H, s, CH_3), 2.92 (1H, br s, OH), 4.07 (1H, m, 6′-H), 4.38 (1H, dd, *J* 6.6 and 1.1, 5′-H), 4.43 (1H, d, *J* 1.7, 2′-H), 4.48 (1H, br s, 7′-H), 4.55 (1H, d, *J* 10.7, C*H*$_2$Ph), 4.66 (2H, s, C*H*$_2$Ph), 4.70 (1H, d, *J* 10.7, C*H*$_2$Ph), 4.72 (1H, d, *J* 11.7, C*H*$_2$Ph), 4.72 (1H, d, *J* 6.6, 4′-H), 4.78 (1H, d, *J* 11.7, C*H*$_2$Ph), 5.44 (2H, AB, *J* 9.9, NC*H*$_2$O), 6.30 (1H, d, *J* 1.7, 1′-H), 7.21–7.42 (15H, m, Ph), 8.36 (1H, s, 6-H); δ_C ($CDCl_3$) 12.3 (CH_3), 68.1 (*C*H$_2$Ph), 70.4 (NC*H*$_2$O), 71.9, 72.1, 72.3 (4′-C, 2 × *C*H$_2$Ph), 76.7 (5′-C), 76.7 (2′-C), 79.2 (7′-C), 79.3 (6′-C), 86.5 (1′-C), 94.7 (3′-C), 108.3 (5-C), 127.3, 127.6, 127.7, 128.2, 128.2, 128.3, 128.4, 128.6, 128.7 (3 × Ph), 136.9, 137.5, 137.8 (3 × Ph), 138.3 (6-C), 151.1 (2-C), 163.8 (4-C); FAB-MS *m*/*z* 599 [M + H$^+$].

(1*S*,3*S*,4*R*,6*R*,7*R*,8*R*,9*R*)-7,8,9-Trihydroxy-4-(thymin-1-yl)-2,5-dioxatricyclo[4.2.1.0^{3,7}]nonane 4

A solution of **10** (31 mg, 0.022 mmol) in methanol (1.0 cm^3) was added 20% $Pd(OH)_2$–C (20 mg) and the mixture was degassed with argon and flushed with H_2 for 5 min. After stirring under an atmosphere of H_2 for 16 h the mixture was filtered through a pad of Celite and the solvent was removed by distillation under reduced pressure to give the product **4** (10 mg, 65%) as a white solid; single crystals were grown by slow evaporation of a solution of **4** in methanol; δ_H (CD_3OD) 1.84 (3H, d, *J* 1.1, CH_3), 3.87 (1H, dd, *J* 2.9 and 1.3, 6′-H), 4.12 (1H, d, *J* 1.8, 2′-H), 4.19 (1H, d, *J* 2.9, 7′-H), 4.28 (1H, d, *J* 7.0, 4′-H), 4.51 (1H, dd, *J* 7.0 and 1.3, 5′-H), 6.13 (1H, d, *J* 1.8, 1′-H), 8.82 (1H, d, *J* 1.1, 6-H); δ_C (CD_3OD) 12.5, 73.4, 74.8, 80.0, 80.7, 84.0, 87.5, 91.0, 109.9, 142.1, 152.4, 166.7; FAB-MS *m*/*z* 299 [M + H$^+$].

(1*S*,3*S*,4*R*,6*R*,7*R*,8*R*,9*R*)-8-Benzoyloxy-7,9-bis(benzyloxy)-4-(3-benzyloxymethylthymin-1-yl)-2,5-dioxatricyclo[4.2.1.0^{3,7}]-nonane 11

To a solution of **10** (308 mg, 0.515 mmol) in anhydrous pyridine (10 cm^3) was added benzoyl chloride (0.2 cm^3, 1.7 mmol), and the mixture was stirred for 1 h at room temperature. The reaction was quenched with H_2O (10 cm^3) and extracted with CH_2Cl_2 (3 × 25 cm^3). The combined extracts were washed with saturated aq. $NaHCO_3$ (2 × 50 cm^3) and then dried ($MgSO_4$). The solvent was removed by distillation under reduced pressure and the residue was co-evaporated with toluene (2 × 30 cm^3) and then purified by column chromatography [0–1% (v/v) MeOH in CH_2Cl_2] to give the product **11** (303 mg, 83%) as a white solid material (Found: C, 69.47; H, 5.28; N, 4.16. $C_{41}H_{38}N_2O_9\cdot{}^1/_4H_2O$ requires C, 69.63; H, 5.49; N, 3.96%); δ_H ($CDCl_3$) 1.38 (3H, s, CH_3), 4.25 (1H, dd, *J* 6.6 and 1.1, 5′-H), 4.42 (1H, dd, *J* 3.0 and 1.1, 6′-H), 4.61 (1H, d, *J* 10.4, C*H*$_2$Ph), 4.63 (1H, d, *J* 1.8, 2′-H), 4.69 (2H, s, C*H*$_2$Ph), 4.72 (1H, d, *J* 11.5, C*H*$_2$Ph), 4.74 (1H, d, *J* 10.4, C*H*$_2$Ph), 4.80 (1H, d, *J* 11.5, C*H*$_2$Ph), 4.81 (1H, d, *J* 6.6, 4′-H), 5.48 (2H, AB, *J* 9.8, NC*H*$_2$O), 5.70 (1H, d, *J* 3.0, 7′-H), 6.39 (1H, d, *J* 1.8, 1′-H), 7.22–7.40 (15H, m, Ph), 7.57 (2H, m, Bz), 7.71 (1H, m, Bz), 7.95 (2H, m, Bz), 8.30 (1H, d, *J* 1.1, 6-H); δ_C ($CDCl_3$) 12.4, 68.6, 70.4, 71.9, 72.1, 72.5, 74.8, 75.8, 78.9, 79.7, 86.4, 93.7, 108.7, 127.6, 127.7, 127.7, 128.3, 128.3, 128.5, 128.5, 128.6, 128.6, 128.7, 128.7, 129.7, 134.0, 136.4, 136.5, 137.8, 138.0, 151.3, 163.6, 164.9; FAB-MS *m*/*z* 703 [M + H$^+$].

(1*S*,3*S*,4*R*,6*R*,7*R*,8*R*,9*R*)-8-Benzoyloxy-7,9-dihydroxy-4-(thymin-1-yl)-2,5-dioxatricyclo[4.2.1.0^{3,7}]nonane 12

To a solution of **11** (271 mg, 0.386 mmol) in methanol (5 cm^3) was added 20% $Pd(OH)_2$–C (100 mg), and the mixture was degassed with argon and flushed with H_2 for 5 min. After stirring under an atmosphere of H_2 for 16 h, additional catalyst (30 mg) was added and the mixture was stirred for a further 48 h. The mixture was filtered through a pad of Celite and the solvent was removed by distillation under reduced pressure and the residue was purified by column chromatography [1–10% (v/v) MeOH in CH_2Cl_2] to give **4** (23 mg, 20%) and the product **12** (89 mg, 57%) as a white solid material; δ_H (DMSO-d_6) 1.76 (3H, d, *J* 1.0, CH_3), 4.15 (1H, m, 6′-H), 4.23 (1H, d, *J* 1.8, 2′-H), 4.34 (1H, dd, *J* 6.8 and 3.9, 5′-H), 4.40 (1H, d, *J* 6.8, 4′-H), 5.32 (1H, d, *J* 2.8, 7′-H), 5.88 (1H, d, *J* 3.9, 5′-OH), 6.12 (1H, d, *J* 1.8, 1′-H), 6.72 (1H, s, 3′-OH), 7.57 (2H, m, Bz), 7.71 (1H, m, Bz), 8.02 (2H, m, Bz), 8.76 (1H, d, *J* 1.0, 6-H), 11.33 (1H, s, NH); δ_C (DMSO-d_6) 13.0, 71.7, 75.0, 76.4, 78.8, 82.5, 85.5, 88.6, 107.4, 129.1, 129.4, 130.1, 134.4, 140.0, 151.0, 164.3, 165.4; FAB-MS *m*/*z* 403 [M + H$^+$].

(1*S*,3*S*,4*R*,6*R*,7*R*,8*R*,9*R*)-8-Benzoyloxy-9-(4,4′-dimethoxytrityloxy)-7-hydroxy-4-(thymin-1-yl)-2,5-dioxatricyclo[4.2.1.0^{3,7}]-nonane 13

To a solution of **12** (40 mg, 0.10 mmol) in anhydrous pyridine (0.5 cm^3) were added dimethylaminopyridine (DMAP) (18 mg, 0.15 mmol) and 4,4′-dimethoxytrityl triflate (DMT-OTf) (135 mg, 0.30 mmol), and the mixture was stirred for 3 h at 60 °C. Additional DMT-OTf (45 mg, 0.10 mmol) was added and the mixture was stirred for a further 16 h. The reaction was quenched by the addition of saturated aq. $NaHCO_3$ (3 cm^3) and extracted with CH_2Cl_2 (3 × 8 cm^3). The combined extracts were dried ($MgSO_4$) and the solvent was removed by distillation under reduced pressure and the residue purified by column chromatography [0–4% (v/v) MeOH and 0.25% pyridine (v/v) in CH_2Cl_2] to give the product **13** (45 mg, 64%) as a white solid material; δ_H (DMSO-d_6) 1.34 (3H, s), 3.00 (1H, br s), 3.70 (3H, s), 3.71 (3H, s), 4.10–4.23 (3H, m), 5.04 (1H, d, *J* 2.5), 6.22 (1H, br s), 6.79 (1H, s, 3′-OH), 6.80–6.88 (2H, m), 7.16–7.38 (8H, m), 7.38–7.52 (4H, m), 7.64–7.72 (2H, m), 8.46 (1H, s), 11.35 (1H, s); δ_C (DMSO-d_6) 12.0, 55.1, 74.2, 74.6, 75.0, 78.4, 81.8, 84.3, 87.1, 88.3, 107.8, 113.5, 127.0, 127.4, 128.1, 128.2, 128.5, 128.8, 128.9, 129.0, 129.5, 129.5, 134.0, 135.1, 135.5, 138.4, 144.6, 150.5, 158.4, 163.7, 164.4; FAB-MS *m*/*z* 705 [M + H$^+$].

(1*S*,3*S*,4*R*,6*R*,7*R*,8*R*,9*R*)-8-Benzoyloxy-7-[2-cyanoethoxy-(diisopropylamino)phosphanyloxy]-9-(4,4′-dimethoxytrityloxy)-4-(thymin-1-yl)-2,5-dioxatricyclo[4.2.1.0^{3,7}]nonane 14

2-Cyanoethyl *N*,*N*-diisopropylphosphoramidochloridite (0.02 cm^3, 0.09 mmol) was added dropwise to a solution of **13** (45 mg, 0.064 mmol) in anhydrous CH_2Cl_2 (0.5 cm^3) and diisopropylethylamine (0.05 cm^3). After stirring at room temperature for 3 h, EtOAc (4 cm^3) was added to the mixture, which was washed with saturated aq. $NaHCO_3$ (2 × 3 cm^3) and brine (2 × 3 cm^3) and then dried ($MgSO_4$). The solvent was removed by distillation under reduced pressure and the residue purified by column chromatography [0.5% (v/v) triethylamine in CH_2Cl_2] to give a yellow oil. This oil was subsequently dissolved in a minimum of toluene and added dropwise to petroleum ether (20 cm^3, −40 °C). The yellow precipitate was filtered off and re-dissolved in CH_3CN and the solvent was removed by distillation under reduced pressure to give the product **14** (64 mg) as an impure yellow oil which was used without further purification for automated oligonucleotide synthesis; δ_P ($CDCl_3$) 146.4.

(1*R*,2*R*,4*R*,5*R*,6*R*,8*R*,9*S*)-1,5-Bis(benzyloxy)-9-hydroxy-8-(thymin-1-yl)-3,7-dioxatricyclo[4.3.0.0^{2,4}]nonane 15

To a solution of **7** (33 mg, 0.070 mmol) in MeOH (3 cm^3) were added benzonitrile (85 mg, 0.83 mmol), a 35% aq. solution of H_2O_2 (80 mg, 0.82 mmol) and K_2CO_3 (10 mg, 0.0725 mmol) and the mixture was stirred at room temperature for 3 h. The mixture was diluted with Et_2O (6 cm^3) and H_2O (3 cm^3) and extracted with CH_2Cl_2 (3 × 10 cm^3). The combined extracts were washed with brine (10 cm^3) and then dried ($MgSO_4$). The solvent was removed by distillation under reduced pressure and the residue purified by column chromatography [1–2% (v/v) MeOH in CH_2Cl_2] to give the product **15** (23 mg, 68%) as a white solid material. δ_H ($CDCl_3$) 1.85 (3H, s, CH_3), 3.87 and 3.68 (2H, 2 × br s, 6′-H and 7′-H), 4.15 (1H, d, *J* 6.0, 5′-H), 4.28 (1H, d, *J* 6.0, 4′-H), 4.60 (1H, d, *J* 11.9, C*H*$_2$Ph), 4.76 (1H, d, *J* 11.2, C*H*$_2$Ph), 4.79 (1H, d, *J* 11.9, C*H*$_2$Ph), 4.86 (1H, d, *J* 11.2, C*H*$_2$Ph), 4.95 (1H, d, *J* 3.8, OH), 5.18 (1H, dd, *J* 5.6 and 3.8, 2′-H), 6.27 (1H, d, *J* 5.6, 1′-H), 7.25–7.40 (10H, m, Ph), 8.05 (1H, s, 6-H), 10.71 (1H, br s, NH); δ_C ($CDCl_3$) 12.4, 53.5, 57.4, 68.1, 72.4, 75.7, 77.9, 79.7, 86.1, 91.7, 108.2, 127.6, 128.0, 128.1, 128.5, 128.5, 128.6, 137.4, 137.6, 141.5, 151.0, 165.8; FAB-MS *m*/*z* 479 [M + H$^+$].

(4-*R*$_S$/*S*$_S$-1*R*,2*R*,6*R*,7*R*,8*R*,10*R*,11*S*)-1,7-Bis(benzyloxy)-11-hydroxy-4-oxo-10-(thymin-1-yl)-3,5,9-trioxa-4-thiatricyclo-[6.3.0.0^{2,6}]undecane 16

A stirred solution of **8** (34 mg, 0.069 mmol) in CH_2Cl_2 (2 cm^3) and triethylamine (0.1 cm^3) was cooled to 0 °C, and a 0.41 M

solution of thionyl chloride in CH_2Cl_2 (0.2 cm^3, 0.081 mmol) was added dropwise. The reaction mixture was stirred at 0 °C for 30 min and then quenched with H_2O (4 cm^3). The phases were separated and the aqueous phase was extracted with CH_2Cl_2 (3 × 10 cm^3). The combined extracts were washed with saturated aq. NaCl (2 × 15 cm^3) and then dried ($MgSO_4$). The solvent was removed by distillation under reduced pressure and the residue purified by column chromatography [1–2% (v/v) MeOH in CH_2Cl_2] to give the product **16** (16 mg, 43%) as a mixture of isomers (1 : 1). The product is a white solid material; δ_H ($CDCl_3$) 1.17 (s, CH_3), 1.21 (s, CH_3), 4.00 (dd, *J* 8.6 and 4.2, 5′-H), 4.27 (m, 2′-H), 4.42 (m, 2′-H), 4.55 (d, *J* 8.6, 4′-H), 4.63 (s, C*H*$_2$Ph), 4.66 (s, C*H*$_2$Ph), 4.67 (dd, *J* 8.7 and 3.6, 5′-H), 4.80 (d, *J* 8.7, 4′-H), 5.09 (d, *J* 5.8, 7′-H), 5.28 (dd, *J* 5.8 and 3.6, 6′-H), 5.37 (t, *J* 4.2, 6′-H), 5.48 (br s, 7′-H), 5.68 (br s, 2′-OH), 5.83 (br s, 2′-OH), 6.10 (m, 1′-H), 7.16–7.45 (m, Ph), 7.79 (s, 6-H), 7.90 (s, 6-H), 11.48 (br s, NH); FAB-MS *m*/*z* 543 [M + H$^+$].

Crystallographic data of 4†

$C_{12}H_{14}N_2O_7 \cdot 2H_2O$, $M = 334.28$, monoclinic, $a = 24.555(3)$, $b = 8.5576(11)$, $c = 6.7140(8)$ Å, $\beta = 98.416(10)$, $V = 1395.6(3)$ Å^3, space group *C*2 (no. 5), $Z = 4$, $D_x = 1.591$ g cm^{-3}, $F(000) = 704$, graphite monochromated Mo-Kα radiation, $\lambda = 0.71073$ Å, $\mu = 0.138$ mm^{-1}, $T = 120$ K. Crystal size 0.45 × 0.43 × 0.19 mm, colourless plates. The intensities of 8619 reflections were measured on a Siemens/Bruker SMART 1K CCD diffractometer to $\theta_{max} = 29.79°$ and were merged to 3464 unique reflections ($R_{int} = 0.0145$). Data collection, integration of frame data and conversion to intensities were performed using the programs SMART,[24] SAINT[24] and SADABS.[25] Structure solution, refinement and analysis of the structure, and production of crystallographic illustrations were carried out using the programs SHELXTL[26] and PLATON.[27] The refinement using 280 parameters converged at $R_1 = 0.0262$ [for $F_o > 4\sigma(F_o)$].

Synthesis of oligodeoxynucleotides

Synthesis of oligonucleotides **17**–**20** was performed on a 0.2 μmol scale using commercially available 2-cyanoethyl phosphoramidites and compound **14**. The synthesis followed the regular protocol for the DNA-synthesiser. However, for compound **14** a prolonged coupling time of 10 min was used and the coupling efficiency was ~30% using 1*H*-tetrazole and ~85% using pyridinium hydrochloride. Coupling yields for unmodified 2-cyanoethyl phosphoramidites were >99%. The 5′-*O*-DMT-*ON* oligonucleotides were removed from the solid support by treatment with conc. ammonia at 50 °C for 16 h, which also removes the protecting groups. Subsequent purification using disposable reversed-phase cartridges, including 5′-*O* detritylation, afforded the pure oligonucleotides **18** and **19**. MALDI-MS [M + H$^+$] gave the following results: **18**, 2772.7 (calc. 2768.8) and **19** 2881.6 (calc. 2880.9).

Melting experiments

Melting experiments were carried out in a medium salt buffer containing 10 mM Na_2HPO_4, 100 mM NaCl, 0.1 mM EDTA, pH 7.0 using 1.5 μM concentrations of the two complementary sequences. The absorption coefficient was calculated to be 77.7 OD μmol^{-1} assuming the absorption coefficient to be identical for all the thymine nucleotides. The increase in absorbance at 260 nm as a function of time was recorded while the temperature was raised linearly from 8 to 60 °C at a rate of 0.5 °C min^{-1}. The melting temperature was determined as the local maximum of the first derivatives of the absorbance *vs*. temperature curve.

† CCDC reference number 164511. See http://www.rsc.org/suppdata/p1/b1/b104438a/ for crystallographic files in .cif or other electronic format.

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