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Incorporation of a S-glycosidic linkage into a glyconucleoside changes the conformational preference of both furanose sugars

Joanne Buckingham,[a] John A. Brazier,[a] Julie Fisher[b,*] and Richard Cosstick[a,*]

[a]*Department of Chemistry, University of Liverpool, Liverpool L69 7ZD, UK*
[b]*School of Chemistry, University of Leeds, Leeds LS2 9JT, UK*

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Abstract—A glyconucleoside containing a thioglycoside linkage, namely 1-(3-*S*-β-D-ribofuranosyl-2,3-dideoxy-3-thio-β-D-ribofuranosyl)-thymine, has been prepared through condensation of a suitably protected derivative of 3′-thiothymidine with an activated ribose sugar. NMR has been used to study the conformation of the S-disaccharide and the unmodified O-disaccharide. A full pseudorotational analysis showed that for the S-disaccharide, the ribose and deoxy ribose sugars have a preference for the south and north pucker, respectively; which is the reverse of what is seen for the O-disaccharide.

Keywords: Glyconucleosides; Thioglycoside; Conformational analysis; NMR; Modified nucleoside

1. Introduction

Glyconucleosides contain an additional carbohydrate residue that is linked to one of the nucleoside hydroxyl groups via an O-glycoside bond. These types of compounds show considerable structural diversity depending on the nature of the sugar, its position of attachment to the nucleoside and the configuration of the O-glycosidic linkage.[1] Of particular note are the 2′-*O*-ribofuranosyl nucleosides, which have been shown to occur with either an α- or β-glycosidic bond. 2′-*O*-β-D-Ribofuranosyl-(1″-2′)-adenosine-5″-*O*-phosphate (**1**) and its analogous guanosine derivative (**2**) have a β-glycoside linkage, and were identified as modified nucleosides at position 64 in initiator tRNAs of lower eukaryotes.[2] The 5″-phosphororibofuranosyl moiety is thought to be an important factor in determining whether the tRNA participates in initiation or elongation during protein synthesis.[3] Closely related is 2′-*O*-α-D-ribofuranosyl-(1″-2′)-adenosine (**3**), which has an α-glycosidic bond, and is a structural component in polyADP-ribose.[4] Glycosidic linkages to the 3′-position of the nucleoside have also been identified in several natural products including adenophostins[5] A and B (**4** and **5**) and HF-7[6] (**6**). The adenophostins and their analogues are potent agonists of *myo*-inisitol 1,4,5-triphosphate,[7] whilst HF-7 is a neuroactive compound found in the venom of the funnel-web spider.

Amongst the simplest of the glyconucleosides are the 3′-*O*-β-D-ribofuranosyl-2′-deoxynucleosides, which are conveniently prepared by condensation of the partially protected nucleoside with an activated ribofuranoside. This procedure has been applied to the synthesis of 3′-*O*-β-D-ribofuranosyl thymidine (**7**) and the 2′-deoxycytidine derivative.[8] We have become interested in preparing glyconucleosides in which the glycosidic oxygen atom is replaced by sulfur. These S-glycosides are of interest for two reasons: firstly, sulfur substitution can enhance the stability of the glycosidic linkage to enzymatic hydrolysis[9] and secondly, it can significantly alter the conformation of ribofuranose sugars. The conformational effect of replacing the 3′-O atom by sulfur is

* Corresponding authors. Tel.: +44 113 343 6577; fax: +44 113 343 6565 (J.F.); tel.: +44 151 794 3514; fax: +44 151 794 3588 (R.C.); e-mail addresses: j.fisher@chem.leeds.ac.uk; rcosstic@liv.ac.uk

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well established for 2′-deoxynucleotides, where it has been shown that the sugar to which the sulfur is attached shifts to the C-3′-*endo*/C-2′-*exo* or north conformation.

We now report the synthesis of a glyconucleoside containing a S-glycoside linkage (**8**) and show through NMR studies that in comparison to the O-glycoside (**7**), the conformational preferences of both the ribose and the deoxyribose sugars are significantly altered by the sulfur substitution (Fig. 1).

2. Results and discussion

2.1. Synthesis of 1-(3-*S*-β-D-ribofuranosyl-2,3-dideoxy-3-thio-β-D-ribofuranosyl)-thymine (8)

In what served as a model study for the synthesis of the S-glycoside (**8**), *O*-β-D-ribofuranosyl-(1″-3′)-thymidine (**7**) was prepared using the method previously described by Mikhailov.[8] In the key step, the fully protected O-glycoside (**11**) was prepared in 74% yield by condensation of 5′-*O*-*tert*-butyldiphenylsilyl (TBDPS) thymidine (**10**, Scheme 1) with 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose.

For the synthesis of the corresponding S-glycoside (**8**), we adopted an analogous procedure based on 5′-*O*-TBDPS-3′-deoxy-3′-thiothymidine (**14**), as outlined in Scheme 1. Thus, the anhydronucleoside (**12**) was prepared in 85% yield from 5′-*O*-TBDPS-thymidine (**10**) by a Mitsunobu reaction. Opening of the anhydronucleoside was achieved by treatment with excess sodium thiobenzoate in DMF at 110 °C, to give the product thioester (**13**) in 72%, following chromatography. Hydrolysis of the 5′-*O*-TBDPS protected thioester (**13**) was performed using sodium hydroxide in aqueous ethanol, as previously described[10] for the corresponding dimethoxytrityl-protected nucleoside and gave 5′-*O*-TBDPS-3′-deoxy-3′-thiothymidine (**14**) in 78% yield. Whilst thionucleosides are susceptible to oxidation to the symmetrical disulfides, **14** was clearly established as the thiol from the ^{1}H NMR spectrum, which revealed a single compound with a characteristic doublet attributable to the mercapto proton (1.53 ppm). Condensation of the thionucleoside (**14**) with 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose using tin tetrachloride in dichloromethane gave **15** in 67% yield, comparable to the 74% yield obtained for the corresponding O-disaccharide (**11**). Deprotection was accomplished in two steps, initial treatment with tetrabutylammonium fluoride removed the TBDPS group (60% yield, following chromatography) and subsequent reaction with methanolic ammonia gave the fully deprotected thioglycoside (**8**) as an analytically pure amorphous solid after extraction with dichloromethane. The beta configuration

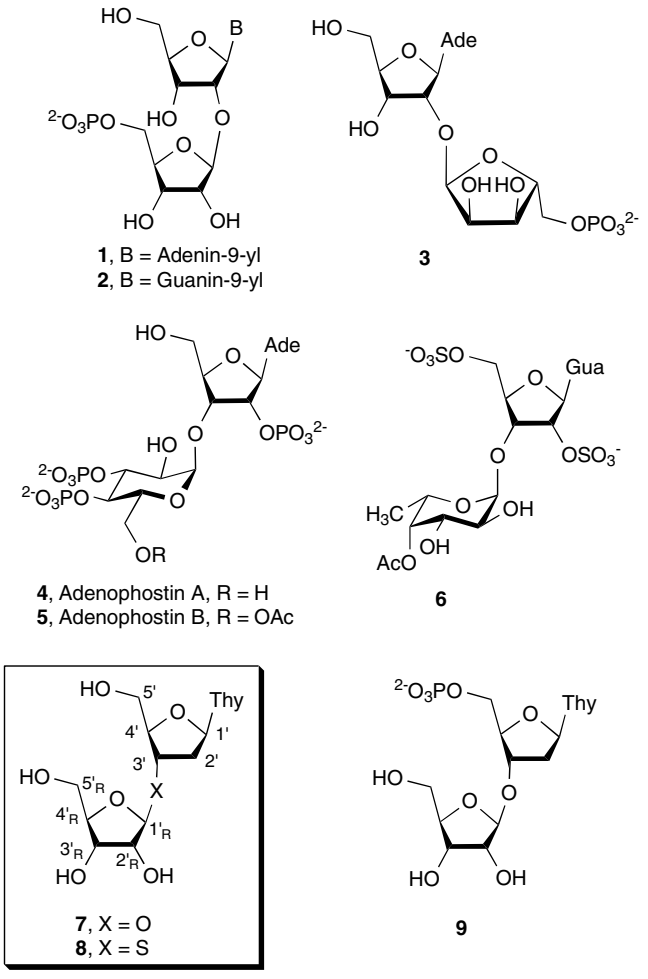
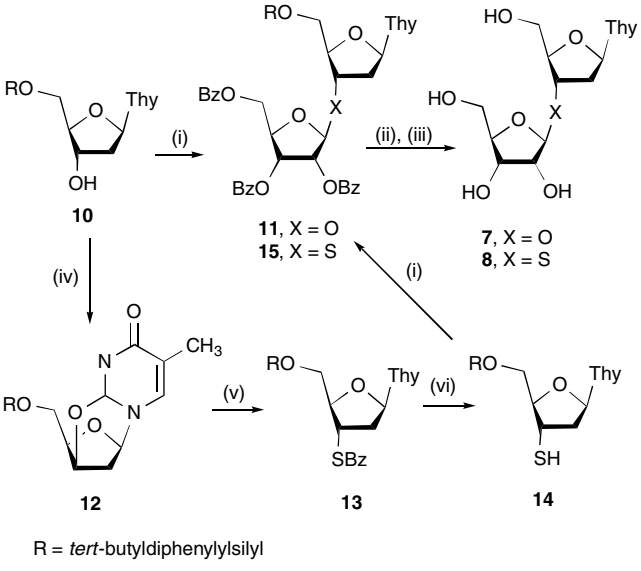


Figure 1. Glyconucleoside structures. Subscript R indicates a ribose sugar.



Scheme 1. Reagents and conditions: (i) 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose, $SnCl_4$, CH_2Cl_2, 0 °C; (ii) TBAF, THF; (iii) NH_3 saturated MeOH; (iv) DIAD, PPh_3, ethyl acetate; (v) sodium thiobenzoate, DMF, 110 °C; (vi) NaOH (0.4 M) H_2O, EtOH.

of the ribose glycosidic bond of the S-disaccharide (**8**) was established through NOE measurements (see below and Supplementary data).

2.2. Conformational studies

The O-disaccharide (**7**) has previously been prepared[8] and its ^{1}H NMR spectrum recorded at 200 MHz, in D_2O solution, has been analyzed. Use of a 500 MHz instrument in this current study has now resulted in the previously reported overlapped-multiplets being resolved and a full assignment of the protons is now described (Table 1). The 500 MHz ^{1}H NMR spectrum of S-disaccharide (**8**) in D_2O solution was assigned using DQF-COSY and TOCSY spectra. The relative assignments of the 2′/2″ protons for **7** were made as described previously.[11] For **8**, the 2′/2″ assignments were confirmed by analysis of 1D NOE data; H-6-2′ (2%), and comparison with literature data[12] for a related compound. Nuclear Overhauser data (Supplementary data) recorded for **8** also permitted confirmation of the presence of the β-anomer for each sugar ring. In the case of the ribose sugar of **8**, the characteristic NOEs[13] established that H-4′ and H-1′ are on the same face as each other and no NOE connection was observed between H-1′ and H-3′. Care is needed when interpreting the absence of an NOE; however, as the stereochemistry at C-4′ is established at the outset, these data taken together are consistent with the glycosidic bond being beta.

To determine accurate coupling constants necessary for conformational analysis (see below), ^{1}H NMR spectra recorded for **7** and **8** were computer simulated following our previous methodology.[12] The chemical shifts and coupling constants from each experimental spectrum were entered into a spectral simulation program, gNMR[14] and new spectra calculated. To extract the coupling constants, peaks in the calculated spectrum were assigned to observed transitions in the corresponding experimental spectrum, and then an automated iterative approach to improving agreement between the calculated and the observed spectrum was adopted. Each sugar system was taken separately and the results of these simulations are shown in Table 2. From the error analysis, all singular values are large and comparable in magnitude to within a factor of 100, indicating well determined data. From the variance–covariance matrix, the estimated standard deviations of all parameters were at most of the order of 10^{-3}. This procedure was repeated for spectra recorded at 21, 26 and 31 °C.

The conformation of five-membered rings can be described by the concept of pseudorotation.[15] Using this approach the phase angle, P, and puckering amplitude, Φ, are sufficient to uniquely define the ring shape.[15,16] Furanose rings of nucleotides generally exist in an equilibrium between two extreme conformers (Fig. 2) notated north (N) and south (S); these labels encompass the conformations C-2′-*exo*/C-3′-*endo* and C-2′-*endo*/C-3′-*exo*, respectively.[16] Consequently, five quantities are actually required for a complete description: the pseudorotational values for the north and south conformers (P_N, Φ_N, P_S, Φ_S) and the mole fraction of one of the conformers (X_N or X_S). These may be calculated from vicinal ^{1}H–^{1}H coupling constants, involving sugar ring protons, using a generalized form of the Karplus equation[17] embedded in the computer program PSEUROT.[18] In using the latest version of this program, the methodology previously adopted was followed here.[12] A summary of the results of the pseudorotational analysis of **7** and **8** is provided in Table 3.

An initial interpretation of the H-1′ to H-2′/H-2″ coupling constant data[11] for the deoxyribose ring suggested that in the O-disaccharide (**7**), the sugar pucker would be south and that in S-disaccharide (**8**) would be north (Table 2). These preliminary conclusions were confirmed following the pseudorotation analysis. Thus, in the deoxysugar of the O-disaccharide (**7**), the south (or

Table 1. ^{1}H NMR chemical shifts (ppm) for **7** and **8** in D_2O solution at 21 °C from gNMR simulations

Proton	Chemical shifts of sugar protons			
	S-Disaccharide (**8**)		O-Disaccharide (**7**)	
	Deoxy	Ribo	Deoxy	Ribo
1′	6.164	5.125	6.232	5.091
2′	2.635	4.055	2.359	4.067
2″	2.577	—	2.532	—
3′	3.587	4.169	4.426	4.195
4′	4.009	4.006	4.106	4.006
5′	3.954	3.746	3.799	3.806
5″	3.842	3.671	3.740	3.640

Table 2. Vicinal coupling constants (Hz) for sugar ring protons in each of the disaccharides

	Coupling constants at 21 °C				
	1′-2′	1′-2″	2′-3′	2″-3′	3′-4′
S-Disaccharide					
Deoxy	3.48	7.62	8.51	9.13	8.86
Ribo	5.76	—	5.08	4.71	3.63
O-Disaccharide					
Deoxy	6.99	6.58	6.96	3.42	3.75
Ribo	1.23	—	4.55	—	6.98

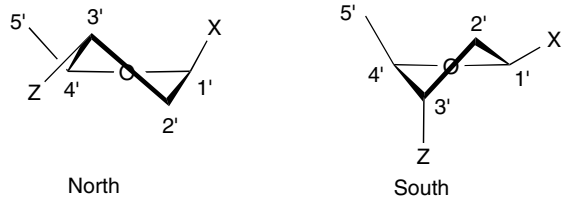


Figure 2. Representations of the two extreme sugar conformations: X = N, S or O, Z = O or S.

Table 3. Pseudorotational parameters for the north and south conformers of the sugar rings in each of the disaccharides

	S-Disaccharide		O-Disaccharide	
	Deoxy	Ribo	Deoxy	Ribo
Puckers				
Φ_N	31.2°	40.1°	35.0°	36.8°
Φ_S	34.5°	38.4°	28.1°	35.0°
P_N	42.8°	39.5°	9.5°	−16.3°
P_S	162.6°	149.2°	143.8°	162.6°
RMS error/Hz	0.540	0.053	0.160	0.120
% South				
21 °C	3	58	70	5
26 °C	2	57	72	3
31 °C	3	57	70	4

C-2′-*endo*/C-3′-*exo*) conformer is dominant (70% mole fraction), which is typical of deoxyribonucleosides.[16] The preference for this conformation arises from a combination of electronic and steric factors covered by the anomeric and *gauche* effects;[19] the anomeric 'contribution' or generalized anomeric effect is now considered as a special case of a general preference for gauche conformations.[20] The anomeric effect of the nucleobase (or any other substituent with electron lone pairs attached to the C-1′ (anomeric) carbon) is thought to originate from the tendency of one of the lone pairs on O-4′ to adopt an antiperiplanar orientation relative to the glycosidic bond. The *gauche* effect is the stabilization of the *gauche* versus the *trans* orientation in the X–C–C–Y fragment, where X and Y are electronegative groups, O or S in this instance. Thus, both factors are determined to some extent by the electronegativity of substituents attached to the sugar ring.[20,21] If the anomeric effect were to dominate then the sugar puckers would be north. In a normal deoxyribose ring, that is, with a 3′-O, the *gauche* effect dominates and the sugar pucker is south. When the 3′-O is substituted for a sulfur atom (with much lower electronegativity), the *gauche* effect is dramatically reduced and the anomeric effect wins. This is clearly seen for the deoxyribose ring in the S-disaccharide (**8**) where the N conformer is seen almost exclusively (97–98% mole fraction). This result is consistent with our previous studies on 3′-S-modified dinucleotides, which revealed the same conformational preference for this sugar.[12]

In ribose rings, additional *gauche* interactions arise and the balance is such that the north pucker is generally preferred and this is seen for the ribose ring of the O-disaccharide (**7**) where this conformer dominates to the extent of about 95% mole fraction. However, this balance is disturbed when the electronegativity of ribose ring substituents is changed.[21] Thus, when the O-1′ atom is substituted by a sulfur atom, as in the case of the ribose sugar of S-disaccharide (**8**), the anomeric effect is reduced, as is the *gauche* interaction between O-2′ and the C-1′ sulfur substituent and the result is a more equal population of the conformers with the south pucker being slightly favoured (57–58% S).

In relation to our studies on the O-disaccharide (**7**), comparison can be drawn to previous work on its phosphorylated derivative (**9**). Compound **9** was found to have ribose and deoxyribose sugars with pseudorotation parameters[22] (mole fraction south 74% and 11% and P_S 160°, and 162°, for the deoxyribose and ribose rings, respectively) very similar to those calculated here for **7** (see Table 3). Slight differences in the pseudorotational parameters between **9** and **7** are likely to arise from the presence of the bulky 5′-phosphate group in **9** and the slight temperature difference between the two sets of measurements; 19 °C for **9**,[22] whilst data for **7** were collected over three different temperatures, which is generally accepted to give more reliable solutions to the pseudorotation analysis.[18] Alternatively, as coupling constants used for the analysis of **9** were taken directly from spectra with the assumption of first order behaviour,[22] this may be another cause of the difference.

2.3. Conclusion

In summary, the first example of a glyconucleoside containing a S-glycosidic linkage has been prepared. The S-glycosidic linkage was introduced by condensation of a 3′-thiothymidine derivative with 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose and the yield (67%) for this condensation reaction was slightly lower than that used to generate the O-glycoside (74% yield). The beta configuration of the S-glycosidic linkage was established by NOE experiments. Conformational studies, including a full pseudorotational analysis, showed that for the S-disaccharide the ribose and deoxy ribose sugars have a preference for the south and north pucker, respectively. In the south, arrangement, the C-2′ atom is above the plane formed by the bonds between C-1′, O-4′, C-4′ and C-3′ below this plane. In the north arrangement, C-3′ is above the plane described by C-4′, O-4′, C-1′ and C-2′ below. These observations for the S-disaccharide (**8**) are the reverse of what is seen for the O-disaccharide (**7**). The switch in the conformational preferences of the sugars, which is induced by the sulfur substitution, results through changing the interplay of the different anomeric and gauche factors. The altered conformational features of the S-disaccharide may prove useful in exploring the relationship between sugar pucker and biological activity in glyconucleosides.

3. Experimental

3.1. General methods

1-*O*-Acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose was purchased from Chemgenes, Wilmington, MA, USA.

Anhydrous *N*,*N*-dimethylformamide (Sure-seal™) was purchased from Aldrich. Dichloromethane was distilled from calcium hydride. Analytical thin layer chromatography was performed on Merck TLC aluminium sheets coated with silica gel 60 F_{254}. The developed chromatographs were visualized with a UV lamp (254 nm). Carbohydrates were developed by treatment with *p*-anisaldehyde stain [*p*-anisaldehyde (6 cm^3) was mixed with sulfuric acid (8.0 cm^3), acetic acid (2.4 cm^3) and ethanol (218 cm^3)], turning blue upon charring with a heat gun. Flash chromatography was performed on Merck silica (particle size 40–63 μM, supplied by BDH), prepared as a homogeneous slurry in the column eluent and applied to the column over a base layer of sand. The material to be purified was then introduced on to the column in a minimal volume of the eluent and ran with the appropriate eluents. All NMR spectra were recorded on a Bruker 400 MHz, a Bruker 500 MHz or a Varian Unity Inova 500 MHz NMR spectrometer. For ^1H and ^{13}C NMR spectra ran in deuterated dimethylsulfoxide ($(CD)_3SO$), all chemical shifts are reported in parts per million relative to $(CD)_3SO$ (^1H ppm: 2.50, ^{13}C ppm: 39.52 ± 0.06). For the disaccharide derivatives, resonances referring to the ribose sugars are denoted with a subscript 'R'. All mass spectra were recorded on a Micromass LCT mass spectrometer using electrospray ionization (EI) and direct infusion syringe pump sampling. All samples were diluted with MeOH. For the preparation of sodium thiobenzoate, thiobenzoic acid (10.11 g, 73.2 mmol) was added dropwise to a stirred solution of 3 M sodium hydroxide (24.13 cm^3, 72.4 mmol) over a 30 min period. The precipitate formed was removed by filtration and the filtrate concentrated in vacuo to yield sodium thiobenzoate as a pale yellow solid (10.99 g, 68.4 mmol, 94%).

3.2. 5′-*O*-(*tert*-Butyldiphenylsilyl)-2,3′-anhydrothymidine (12)

Diisopropylazodicarboxylate (1.23 cm^3, 6.24 mmol) dissolved in ethyl acetate (2 cm^3) was added dropwise to a solution of triphenyl phosphine (1.64 g, 6.24 mmol) and 5′-*O*-(*tert*-butyldiphenylsilyl)thymidine (2.00 g, 4.16 mmol) dissolved in ethyl acetate (11 cm^3) and the reaction mixture left to stir overnight at rt. The solvents were subsequently removed under reduced pressure to afford a crude yellow foam. Purification by flash silica chromatography, eluting with 5:1 ethyl acetate–*n*-hexane followed by 5:1:2 ethyl acetate–*n*-hexane–MeOH, afforded the title compound as a white amorphous solid (1.64 g, 3.55 mmol, 85%). ^1H NMR (400 MHz; DMSO-d_6): δ 0.97 (9H, s, $CH_3{}^tBu$(TBDPS)), 1.76 (3H, d, 5-CH_3, *J* 1.11 Hz), 2.46–2.61 (2H, m, $H_{2'}$, $H_{2''}$), 3.61 (1H, dd, $H_{5'}$, *J* 7.48, 10.8 Hz), 3.84 (1H, dd, $H_{5''}$, *J* 5.56, 10.64 Hz), 4.43–4.46 (1H, m, $H_{4'}$), 5.33 (1H, m, $H_{3'}$), 5.87 (1H, d, $H_{1'}$, *J* 3.66 Hz), 7.35–7.65 (11H, m, $10 \times CH$(Ph, TBDPS), H_6). ^{13}C NMR (100 MHz; DMSO-d_6): δ 13.4 (5-CH_3), 19.1 (SiC(Me)$_3$), 26.8 ($CH_3{}^tBu$(TBDPS)), 39.8 ($C_{2'}$), 63.2 ($C_{5'}$), 77.3 ($C_{3'}$), 85.1 ($C_{4'}$), 116.4 (C_5), 128.3 (C_6), 130.3, 132.6, 132.7, 137.1 (CH(TBDPS), SiC(Ph)(TBDPS)), 153.7 (C_2), 171.1 (C_4). *m*/*z* (ES+): 463.2 $[M+H]^+$, 485.2 $[M+Na]^+$, 501.2 $[M+K]^+$. Acc mass: $[M+H]^+$ 463.2040, $C_{26}H_{31}N_2O_4Si$ requires 463.2053; $[M+Na]^+$ 485.1859, $C_{26}H_{30}N_2O_4SiNa$ requires 485.1873; $[M+K]^+$ 501.1616, $C_{26}H_{30}N_2O_4SiK$ requires 501.1612.

3.3. 3′-Deoxy-3′-thio-3′-*S*-benzoyl-2′-deoxy 5′-*O*-(*tert*-butyldiphenylsilyl)-thymidine (13)

Sodium thiobenzoate (0.443 g, 2.77 mmol) was added to a stirred solution of compound **12** (0.512 g, 1.11 mmol) dissolved in anhydrous dimethylformamide (6 cm^3) under N_2 at 110 °C. Additional portions of sodium thiobenzoate (0.443 g, 2.77 mmol) were then added every 30 min for the next 2 1/2 h. Following the addition of the final portion of sodium thiobenzoate, the reaction mixture was left to stir for a further 30 min after which the solvent was removed under reduced pressure. The deep red residue that remained was taken up in ethyl acetate (100 cm^3) and washed with satd aq $NaHCO_3$ (2×100 cm^3). The organic layer was then dried over anhydrous sodium sulfate, filtered and concentrated in vacuo to afford the impure product as a pale brown foam. Purification by flash silica chromatography, eluting with CH_2Cl_2 followed by a gradient of 0–1% MeOH in CH_2Cl_2, yielded the title compound as a pale brown amorphous solid (0.478 g, 0.795 mmol, 72%). ^1H NMR (400 MHz; DMSO-d_6): δ 1.02 (9H, s, ^tBu(TBDPS)), 1.59 (3H, s, 5-CH_3), 2.46–2.72 (2H, m, $H_{2'}$, $H_{2''}$), 3.91 (1H, dd, $H_{5'}$, *J* 3.97, 7.79 Hz), 3.98 (1H, dd, $H_{5''}$, *J* 2.54, 6.99 Hz), 4.12–4.16 (1H, m, $H_{4'}$), 4.40 (1H, q, $H_{3'}$, *J* 7.92 Hz), 6.23 (1H, dd, $H_{1'}$, *J* 5.40, 6.27 Hz), 7.34–7.93 (16H, m, $10 \times CH$(Ph, TBDPS), $5 \times CH$(Bz), H_6), 11.36 (1H, br s, N_3H). ^{13}C NMR (100 MHz; DMSO-d_6): δ 11.8 (5-CH_3 thy), 18.8 (SiC(Me)$_3$), 26.6 (CH_3 ^tBu(TBDPS)), 37.4 ($C_{2'}$), 39.8 ($C_{3'}$) 63.3 ($C_{5'}$), 83.7 ($C_{1'}$), 109.7 (C_5), 127.3, 128.2, 129.6, 130.3, 134.6, 135.3, 135.4, 136.0 ((CH(Ph, TBDPS)), SiC(Ph)(TBDPS), CH(Bz), SCOC(Bz)), 136.3 (C_6) 150.3 (C_2), 163.6 (C_4), 190.0 (SCOBz). *m*/*z* (ES+): 623.2 $[M+Na]^+$. Acc mass: $[M+Na]^+$ 623.1995, $C_{33}H_{36}N_2O_5SSiNa$ requires 623.2012.

3.4. 3′-Deoxy-3′-thio-5′-*O*-(*tert*-butyldiphenylsilyl)-thymidine (14)

N_2 was bubbled through a stirred solution of 0.4 M sodium hydroxide in ethanol (52 cm^3) for 30 min. 3′-Deoxy-3′-thio-3′-*S*-benzoyl-5′-*O*-(*tert*-butyldiphenylsilyl)-thymidine (0.478 g, 0.80 mmol) was then added and

the reaction mixture stirred under N_2 at rt for a further 2 h. A 1 M solution of potassium dihydrogen phosphate (93 cm^3, 93.70 mmol) was then added dropwise over a 15 min period. The cream precipitate that formed was filtered, washed with distilled water (3×50 cm^3) and dried over phosphorus pentoxide to yield the title compound as a cream solid (0.308 g, 0.619 mmol, 78%). ^{1}H NMR (400 MHz; DMSO-d_6): δ 1.01 (9H, s, ^{t}Bu(TBDPS)), 1.53 (1H, d, S*H*, *J* 5.70 Hz), 1.56 (3H, d, 5-CH_3, *J* 0.80 Hz), 2.29–2.37 (1H, m, $H_{2'}$), 2.50–2.57 (1H, m, $H_{2''}$), 3.52 (1H, q, $H_{3'}$, *J* 8.59 Hz), 3.80–3.98 (3H, m, $H_{5'}$, $H_{5''}$, $H_{4'}$), 6.14 (1H, dd, $H_{1'}$, *J* 3.66, 5.73 Hz), 7.39–7.68 (11H, m, $H_{6'}$, $10 \times$ C*H*(Ph, TBDPS)), 11.29 (1H, br s, N_3H). ^{13}C NMR (100 MHz; DMSO-d_6): δ 12.2 (5-*C*H_3), 19.2 (Si*C*(Me)$_3$), 27.0 (CH_3^{t}Bu(TBDPS)), 34.7 ($C_{3'}$), 41.4 ($C_{2'}$), 62.8 ($C_{5'}$), 83.4 ($C_{1'}$), 87.9 ($C_{4'}$), 109.9 (C_5), 128.2, 130.3, 135.4, 136.0 (*C*H(TBDPS), Si*C*(Ph)(TBDPS)), 136.2 (C_6) 150.8 (C_4), 164.0 (C_2). *m*/*z* (ES+): 519.2 $[M+Na]^+$. Acc mass: $[M+Na]^+$ 519.1749, $C_{26}H_{32}N_2O_4$SSiNa requires 519.1750.

3.5. 1-[5-*O*-*tert*-Butyldiphenylsilyl-2,3-dideoxy-3-thio-3-*S*-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-β-D-ribofuranosyl]-thymine (15)

A 1.0 M solution of tin tetrachloride in CH_2Cl_2 (1.07 cm^3, 1.073 mmol) was added to a cold solution (0 °C) of 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose (0.489 g, 0.936 mmol) in dry CH_2Cl_2 (4 cm^3) under N_2 and the resulting reaction mixture stirred at 0 °C for 10 min. A solution of 3′-deoxy-5′-*O*-(*tert*-butyldiphenylsilyl)-3′-thiothymidine (0.395 g, 0.795 mmol) in dry CH_2Cl_2 (2 cm^3) was then added and the reaction mixture stirred under the same conditions for a further 1 h. A 10% aqueous solution of sodium bicarbonate (10 cm^3) was then added and the reaction mixture left to stir for a further 20 min. The white suspension that was observed to form was removed by filtration through Celite and the filtrate was washed with water (30 cm^3) and reduced in vacuo to afford a crude cream foam. Purification by flash silica chromatography, eluting with 2:1 *n*-hexane–ethyl acetate followed by 1:1 *n*-hexane–ethyl acetate, afforded the title compound as a white amorphous solid (0.501 g, 0.532 mmol, 67%). ^{1}H NMR (400 MHz; DMSO-d_6): δ 0.95 (9H, s, ^{t}Bu(TBDPS)), 1.49 (3H, d, 5-*C*H_3, *J* 0.64 Hz), 2.42–2.56 (2H, m, $H_{2'}$, $H_{2''}$), 3.82–3.98 (4H, m, $H_{3'}$, $H_{4'}$, $H_{5'}$, $H_{5''}$), 4.58 (1H, dd, $H_{5'R}$, *J* 4.61, 12.16 Hz), 4.69 (1H, dd, $H_{5''R}$, *J* 3.66, 12.08 Hz), 4.78 (1H, q, $H_{4'R}$, *J* 4.45 Hz), 5.70 (1H, t, $H_{1'R}$, *J* 4.77 Hz), 5.76 (1H, d, $H_{2'R}$, *J* 4.45 Hz), 5.83 (1H, t, $H_{3'R}$, *J* 5.25 Hz), 6.13 (1H, dd, $H_{1'}$ *J* 4.77, 7.31 Hz), 7.32–8.05 (26H, m, $10 \times$ C*H*(Ph, TBDPS), $15 \times$ C*H*(Bz), H_6), 11.31 (1H, br s, N_3H). ^{13}C NMR (100 MHz; DMSO-d_6): δ 12.2 (5-*C*H_3), 19.2 (Si*C*(Me)$_3$), 27.0 (*C*H_3 ^{t}Bu(TBDPS)), 39.0 ($C_{2'}$), 40.8 ($C_{3'}$), 63.2 ($C_{5'}$), 64.3 ($C_{5'R}$), 72.4 ($C_{3'R}$), 75.4 ($C_{1'R}$), 80.3 ($C_{4'R}$), 83.6 ($C_{1'R}$), 84.8 ($C_{4'}$), 85.3 ($C_{2'R}$), 109.9 (C_5), 128.1, 128.2, 128.8, 129.0, 129.1, 129.1, 129.6, 129.7, 129.7, 130.2, 130.2, 133.1, 133.9, 135.3, 135.4 (*C*H(TBDPS), Si*C*(Ph)(TBDPS)/*C*H(Bz), OCO*C*(Bz)), 135.9 (C_6), 150.7 (C_2), 164.0 (C_4), 164.9. 165.1, 165.8 (*C*OBz). *m*/*z* (ES+): 963.3$[M+Na]^+$. Acc mass: $[M+Na]^+$ 963.2999, $C_{52}H_{52}N_2O_{11}$SSiNa requires 963.2959.

3.6. 1-[2,3-Dideoxy-3-thio-3-*S*-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-β-D-ribofuranosyl]thymine

A 0.5 M solution of tetrabutyl ammonium fluoride trihydrate in tetrahydrofuran (0.71 cm^3, 0.7085 mmol) was added to a solution of **15** (0.480 g, 0.510 mmol) and the reaction mixture stirred for 2 h at rt. Co-evaporation of the solvent with chloroform afforded the impure product as a cream foam. Purification by flash silica chromatography, eluting with CH_2Cl_2 followed by a gradient of 0–2% MeOH in CH_2Cl_2, yielded the title compound as a white amorphous solid (0.214 g, 0.304 mmol, 60%). ^{1}H NMR (400 MHz; DMSO-d_6): δ 1.76 (3H, d, 5-CH_3, *J* 0.95 Hz) 2.39–2.48 (2H, m, $H_{2'}$, $H_{2''}$), 3.59–3.75 (3H, m, $H_{3'}$, $H_{5'}$, $H_{5''}$), 3.84 (1H, dt, $H_{4'}$, *J* 3.02, 8.10 Hz), 4.56 (1H, dd, $H_{5'R}$, *J* 4.6, 12.0 Hz), 4.68 (1H, dd, $H_{5'R}$, *J* 3.81, 12.0 Hz), 4.75 (1H, q, $H_{4'R}$, *J* 4.29 Hz), 5.18 (1H, br t, 5′-O*H*, *J* 4.93 Hz), 5.66–5.69 (1H, m, $H_{1'R}$), 5.77–5.81 (2H, m, $H_{2'R}$, $H_{3'R}$), 6.04 (1H, dd, $H_{1'}$, *J* 4.29, 6.76 Hz), 7.44–8.04 (16H, m, H_6, $15 \times$ C*H*(Bz)), 11.24 (1H, br s, N_3H). ^{13}C NMR (100 MHz; DMSO-d_6): δ 12.6 (5-*C*H_3), 40.1 ($C_{2'}$), 40.6 ($C_{3'}$), 60.2 ($C_{5'}$), 64.3 ($C_{5'R}$), 72.5 ($C_{3'R}$), 75.5 ($C_{1'R}$), 80.1 ($C_{4'R}$), 83.9 ($C_{1'}$), 85.5 ($C_{2'R}$), 85.8 ($C_{4'}$), 109.3 (C_5), 128.9, 129.0, 129.1, 129.2, 129.6, 129.7, 133.9, 134.2, 134.3 (*C*H(Bz), OCO*C*(Bz)), 136.5 (C_6), 150.7 (C_2), 164.1 (C_4), 164.9, 165.1, 165.8 (*C*OBz). *m*/*z* (ES+): 725.2 $[M+Na]^+$. Acc mass: $[M+Na]^+$ 725.1760, $C_{36}H_{34}N_2O_{11}$SNa requires 725.1781.

3.7. 1-(3-*S*-β-D-Ribofuranosyl-2,3-dideoxy-3-thio-β-D-ribofuranosyl)-thymine (8)

A solution of 1-[2,3-dideoxy-3-thio-3-*S*-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-β-D-ribofuranosyl]-thymine (0.206 g, 0.293 mmol) dissolved in 7 M methanolic ammonia (3.52 cm^3) was stirred at rt under N_2 for 5 days. The solvents were then removed under reduced pressure and the residue that remained was taken up in distilled water (20 cm^3) and washed with CH_2Cl_2 (2×20 cm^3). The aqueous layer was subsequently co-evaporated to dryness with acetone to yield the title compound as a white amorphous solid (0.113 g, 0.289 mmol, 99%). ^{1}H NMR (500 MHz; DMSO-d_6): δ 1.76 (3H, s, 5-CH_3), 2.32–2.45 (2H, m, $H_{2'}$, $H_{2''}$),

3.38–3.55 (3H, m, $H_{3'}$, $H_{5'R}$, $H_{5''R}$), 3.58–3.63 (1H, m, $H_{5'}$), 3.71–3.80 (4H, m, $H_{5''}$, $H_{4'}$, $H_{4'R}$, $H_{2'R}$), 3.84–3.87 (1H, m, $H_{3'R}$), 4.70 (1H, t, $5'_R$-O*H*, *J* 5.77 Hz), 4.94 (1H, d, $3'_R$-O*H*, *J* 5.56 Hz), 4.96 (1H, d, $H_{1'R}$, *J* 5.21 Hz), 5.18 (1H, t, 5′-O*H*, *J* 5.34 Hz), 5.23 (1H, d, $2'_R$-O*H*, *J* 5.98 Hz), 6.03 (1H, dd, $H_{1'}$, *J* 7.05, 4.27 Hz), 7.76 (1H, s, H_6), 11.24 (1H, br s, N_3H). ^{13}C NMR (125 MHz; DMSO-d_6): δ 12.7 (5-*C*H$_3$), 38.9 ($C_{3'}$), 39.5 ($C_{2'}$) 60.5 ($C_{5'}$), 62.8 ($C_{5'R}$), 71.6 ($C_{3'R}$), 75.6 ($C_{2'R}$), 84.2 (H'_1), 85.7 ($C_{4'}$), 86.3 ($C_{4'R}$), 87.7 ($C_{1'R}$), 109.8 (C_5), 136.9 (C_6), 151.0 (C_2), 164.7 (C_4). *m*/*z* (ES+): 413.1 $[M+Na]^+$. Acc mass: $[M+Na]^+$ 413.0987, $C_{15}H_{22}N_2O_8S$ requires 413.0995.

3.8. Conformational analysis

1D ^{1}H NMR spectra for **7** and **8** in D_2O solution were recorded on a Varian Unity Inova 500, at 21, 26 and 31 °C, in 16k of complex data points over a spectral width of 6000 Hz. A relaxation delay of 1 s was used followed by 1.5 s presaturation time. Prior to Fourier transformation a, −1 Hz line broadening and a 0.6 Gaussian factor were applied for resolution enhancement. 1D NOE spectra were recorded using the 'cyclenoe' routine and a total NOE buildup time of 7 s. DQF-COSY and TOCSY spectra were recorded with hypercomplex phase cycling, in 2k of data points and 256 pairs of t_1 increments. An 80 ms spinlock period was used in the TOCSY experiment. Prior to Fourier transformation a Gaussian window function using LB = −1 Hz and GB = 0.06 was applied and the fid zero filled to 2k in F1. All spectral processing was performed using vnmr. 1D ^{1}H NMR spectra were simulated using gNMR[14] version 4.1.2 (Adept Scientific, Oxford). Computation of the conformational analysis was performed on a PC using the program PSEUROT version 6.3.[18]

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.carres.2006.11.007.

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