Synthesis and hydrolytic stability of the α and β anomers of 4′-thio-2′-deoxyuridine and their 5-substituted analogs. Competition between the acid-catalysed depyrimidination and isomerisation to a 5-thiopyranoside nucleoside

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The α and β anomers of 4′-thio-2′-deoxyuridine were readily synthesised as an anomeric mixture using adapted methodology and separated chromatographically as their 3′,5′-di-*O*-benzyl-*N*[3]-benzoyl derivatives. The 5-fluoro analogs were prepared in a similar manner, but the anomers could be separated simply as their 3′,5′-di-*O*-benzyl derivatives. The kinetics of acid-catalysed hydrolysis for the four compounds and their 5-alkylated analogs are reported. Under these conditions, cleavage of the *N*-glycosidic bond competes with the reversible isomerisation between the furano (4′-thio) and pyrano (5′-thio) ring systems. This was confirmed by isolation and NMR characterisation of the α and β pyranose intermediates of the parent compounds.

Introduction

The very first synthesis of a 2′-deoxy-4′-thioribonucleoside was described by Bobek *et al*. in 1976,[1] and consisted of a low yielding multistep synthesis of methyl 2-deoxy-4-thio-D-*erythro*-pentofuranoside which, following protection of the 5- and 3-hydroxy functions, was coupled with 5-fluorouracil. More successful syntheses of 2′-deoxy-4′-thioribonucleosides were based on the coupling of a suitably blocked 4′-thiosugar moiety with a bis-trimethylsilylated pyrimidine base.[1–3] Despite the difficult separation of the resulting anomeric mixture, this approach remains widely used.

At the same time, Dyson *et al*.[4] described a new 7-step synthesis of benzyl 3,5-di-*O*-benzyl-2-deoxy-1,4-dithio-D-*erythro*-pentofuranoside (**3**) from 2-deoxy-D-ribose with 11% overall yield. This method has subsequently been optimised and used on a multi-kilogram scale with an overall yield approaching 50% without any chromatographic separation.

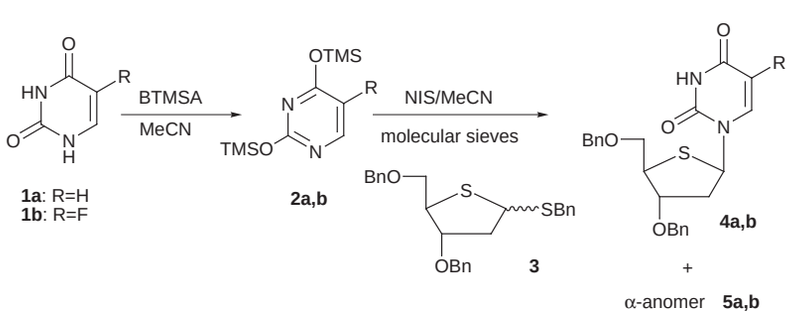
Exploiting these possibilities we were able to synthesise 4′-thiothymidine and (*E*)-5-(2-bromovinyl)-2′-deoxy-4′-thiouridine, as well as 3′-azido-2′-deoxy-4′-thiothymidine.[3] Subsequently an extensive programme was started for the synthesis of a range of different 5-substituted 2′-deoxy-4′-thiopyrimidine nucleosides. The programme so far has yielded several compounds with high anti HSV-1 or VZV activity.[5] The synthetic methodology has been based mainly on the condensation of the 1-*S*-benzyl thiosugar **3** with a bis-trimethylsilylated pyrimidine base promoted by *N*-bromo- (NBS) or *N*-iodosuccinimide (NIS). The methodology had originally been adapted for the synthesis of pentofuranosyl nucleosides by Sugimura *et al*.[6–9]

In the present study, we have applied the methodology to the syntheses of 2′-deoxy-4′-thiouridine and its 5-fluoro analog. The synthesis of 5-fluoro-4′-thio-2′-deoxyuridine has been reported previously by Bobek *et al*.,[1] but it was desirable to further investigate its potential use in anticancer chemotherapy. We have also studied the course of degradation of 2′-deoxy-4′-thiouridine and its 5-substituted derivatives in aqueous acid. The acid-catalysed cleavage of the *N*-glycosidic bond of the compounds was found to be in competition with isomerisation of the thionucleosides to their L-5-thiopyranoside derivatives. Accumulation of the latter, which was ascertained by NMR spectroscopic characterisation after isolation, indicates that hydrolysis takes a different course than the degradation of their native 4′-oxo-counterparts.

Results and discussion

Synthesis

Considering the ease with which 3′,5′-di-*O*-benzyl-4′-thiothymidine had been prepared[3] and the availability of the thiosugar **3**, it was decided to synthesise 4′-thio-2′-deoxyuridine using the same procedure (Scheme 1). 2,4-Bis(trimethylsilyl-



1a: R=H
1b: R=F

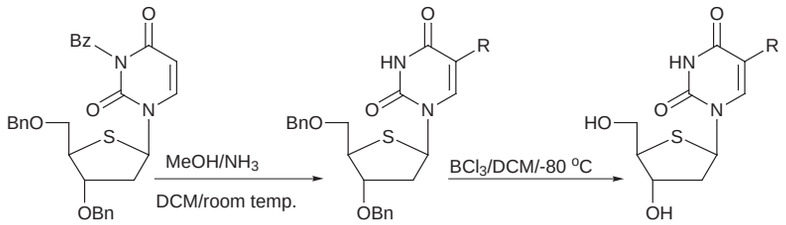
2a,b

3

4a,b

5a,b

Scheme 1



6 **4a**: R=H **4b**: R=F **8a**: R=H **8b**: R=F

Scheme 2

oxy)pyrimidine (**2a**) was prepared by silylating uracil (**1a**) with bis(trimethylsilyl)acetamide (BTMSA), which was then reacted *in situ* with thiosugar **3** using *N*-iodosuccinimide (NIS) to activate the thiobenzyl glycoside. An α/β anomeric mixture (3:2) of the 3′,5′-di-*O*-benzyl-4′-thio-2′-deoxyuridines (**4a**,**5a**) was obtained in good yield (74%). Attempted separation of the anomers by fractional crystallisation was unsuccessful, but they could be separated as earlier described[10] by column chromatography after benzoylation of the uracil N3 with benzoyl chloride and triethylamine in dichloromethane. Although the β-anomer, N^3-benzoyl-3′,5′-di-*O*-benzyl-4′-thio-2′-deoxyuridine (**6**) was isolated in 45% yield and the α-anomer (**7**) in 46% yield, it was, nevertheless, still a tedious process due to the close R_f values of the two anomers. Substitution at the N3-position with the sterically larger 1-adamantanoyl group did not improve the separation of the anomers.

Marquez and co-workers[10] reported that both the N^3-benzoyl group and the 3′,5′-di-*O*-benzyl groups could be simultaneously removed by treatment with boron tribromide in a yield of 60%. We have earlier used boron trichloride as a reagent for debenzylation and therefore a one-step removal of all protecting groups was attempted using boron trichloride. However, following work-up of the reaction mixture and subsequent flash column chromatography, uracil (**1a**) was identified as the major product (61%) by ^{1}H NMR, with the desired 4′-thio-2′-deoxyuridine (**8a**) present only as a minor product (24%). To circumvent the problem of the *N*-glycosidic cleavage, the benzoyl group was removed first by treatment with methanolic ammonia, after which the debenzylation was readily carried out by using boron trichloride (Scheme 2). The pure α-anomer of 3′,5′-di-*O*-benzyl-4′-thio-2′-deoxyuridine (**5a**) was prepared in a similar manner and was fully characterised.

The synthesis of α- and β-5-fluoro-4′-thio-2′-deoxyuridine **8b** and **9b** is analogous to that of 4′-thio-2′-deoxyuridine **8a** and its α-anomer **9a** (Schemes 1 and 2). Condensation of the silylated 5-fluorouracil **1b** with thiosugar **3**, activated with NIS, yielded the α- and β-anomers in a 1.7:1 molar ratio, as shown by ^{1}H NMR. In contrast to **4a** and **5a**, the dibenzylated compounds **4b** and **5b** could be separated without further derivatisation. Despite the unfavourable ratio, it was possible to directly crystallise out a 16% yield of the β-anomer **4b**. Separation by column chromatography of the mother liquor residue provided an extended yield of the pure β- and α-anomers. The total yield of nucleoside recovered from the reaction was 96%.

In an attempt to avoid the use of BCl_3, which requires extended reaction time at low temperatures and can risk glycosidic bond cleavage, $TiCl_4$ was investigated as an alternative reagent for debenzylation. β-Nucleoside anomer **4b** was successfully debenzylated in 85% yield with $TiCl_4$ in toluene, to give an overall yield of 5-fluoro-4′-thio-2′-deoxy-β-uridine **8b** of 27%. However, the yield for the deprotection of the α-anomer **5b** was only 60%, to give an overall yield of 5-fluoro-4′-thio-2′-deoxy-α-uridine **9b** of 14%.

$TiCl_4$ has a considerable advantage over BCl_3 as a reagent for debenzylation as the reaction can be carried out rapidly at room temperature. However, difficulty in isolating the product may result in variable yields. The stability of nucleoside **4b** to BCl_3 deprotection conditions was also confirmed, affording compound **8b** in 88% yield after purification.

Acid-catalysed hydrolysis of 4-oxopyrimidine 2′-deoxy-4′-thionucleosides

In a previous paper[11] we have reported the kinetics of the acid-catalysed hydrolysis of purine and cytosine 2′-deoxy-4′-thionucleosides. The 4′-thio analogs were shown to be from 7 to 70 times more stable than their native 4′-oxo counterparts (pH 1–4). However, on the basis of structural kinetic effects it was suggested[11] that cleavage of the *N*-glycosidic linkage of those 4′-thionucleosides follows the same kind of mechanism as that followed by the native purine and cytosine nucleosides. The rate-limiting stage of this mechanism involves a unimolecular departure of a protonated base moiety, to leave a cyclic oxocarbenium ion formed from the sugar.[12] Accordingly, it was suggested that the higher stability of the 4′-thio analogs derives from the lower stability of the cyclic thiocarbenium ion compared to the corresponding oxocarbenium ion.

It is well documented that the hydrolytic behavior of 4-oxopyrimidine 2′-deoxynucleosides considerably differs from that of their purine and cytosine analogs.[12–14] The main evidence for this is that thymidine and 2′-deoxyuridine are known[13] to be both anomerised to their α-counterparts, and isomerised to α- and β-pyranosides during the course of acid-catalysed hydrolysis. This is consistent with a mechanism involving protonation of the 4′-oxygen and opening of the sugar ring. Hydrolysis of the Schiff-base intermediate thus formed is in competition with recyclisation, which may lead to accumulation of the isomers of the original nucleoside.

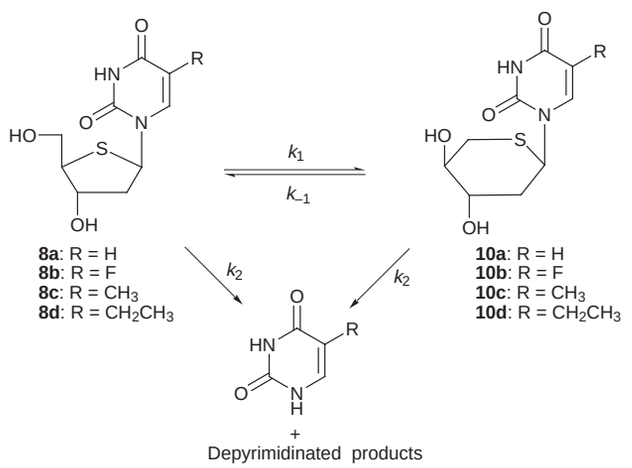
According to this mechanism, one could propose that substitution of 4′-oxygen by sulfur may have a more marked effect on the rate and product distribution of acidic hydrolysis of 4-oxopyrimidine nucleosides than that observed[11] with the purine and cytosine 2′-deoxy-4′-thionucleosides. Even some earlier results support this proposal. Namely, it has been shown[15] that the 5′-hydroxy group of a 4′-thionucleoside may not be phosphorylated with phosphoryl oxychloride by a method conventionally employed with native nucleosides. Even trials[15] to tosylate the 5′-O were unsuccessful and yielded a complicated product mixture. It was suggested[15] that a good leaving-group at the 5′-position could be readily replaced by attack of the 4′-sulfur on the 5′-carbon, leading to formation of an episulfonium intermediate. Episulfonium formation has previously been documented[16] even with several non-nucleosidic 5-thiopyranoside derivatives, where displacement of a sulfonate ester was found to occur by attack of sulfur sited at the β-position with respect to the leaving group.

When the hydrolysis of 4′-thio-2′-deoxyuridine (**8a**) in 1 M aqueous hydrogen chloride was followed by HPLC, release of uracil was found to be accompanied by the accumulation (25% of total peak area at maximum) of an intermediate having a slightly longer retention time than the starting nucleoside on a reversed phase (RP) HPLC column. The intermediate was isolated and purified by HPLC. By NMR analysis (see below) the intermediate was shown to be the

Table 1 The first-order rate constants for the hydrolytic reactions of 5-substituted uracil 4′-thio-2′-deoxynucleosides in aqueous hydrogen chloride solutions at 363.2 K[a]

Base	$c(H^+)$/M	$k_1/10^{-5}$ s^{-1}	$k_{-1}/10^{-5}$ s^{-1}	$k_2/10^{-5}$ s^{-1}
Uracil	4.0	5.2	5.1	3.7
	1.0	0.31	0.27	0.30
Thymine	4.0	6.9	3.5	8.3
	1.0	0.44	0.24	0.39
5-Ethyluracil	4.0	6.5	4.1	6.1
	2.0	0.76	0.30	0.95
	1.0	0.36	0.14	0.32
	0.1	0.02	0.03	0.04
Uracil,	4.0	1.8	Not detected	6.2
α-anomer	1.0	0.13		0.3
Thymine,	4.0	2.5		6.6
α-anomer	1.0	0.19		0.39

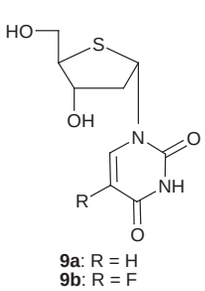
[a] The rate constants are defined in Scheme 3.



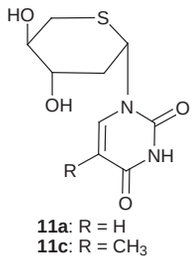
Scheme 3

β-L-thiopyranoside isomer, **10a** (see Scheme 3), of the original nucleoside. The isomerisation was found to be reversible, since **8a** accumulated when **10a** was hydrolysed under the same conditions.

Analogously, isomerisation competed with depyrimidination also for the earlier[3] synthesised 5-methyl- and 5-ethyl-2′-deoxy-4′-thiouridines (**10c**,**d**). Even 5-fluoro-2′-deoxy-4′-thiouridine (**8b**) seems to behave analogously, since an intermediate accumulated (*ca.* 10% of total peak area at maximum) during hydrolysis of **8b** in 1 M aqueous hydrogen chloride. However, no attempts were made to isolate and characterise the intermediate in this case. The substituent at the base moiety had little effect on the rate of isomerisation (Table 1). In each case, isomerisation is strictly stereospecific in both directions, since only one nucleosidic isomer was found to accumulate in each run. Moreover, when an α-nucleoside (**9a** or **9c**) was used as

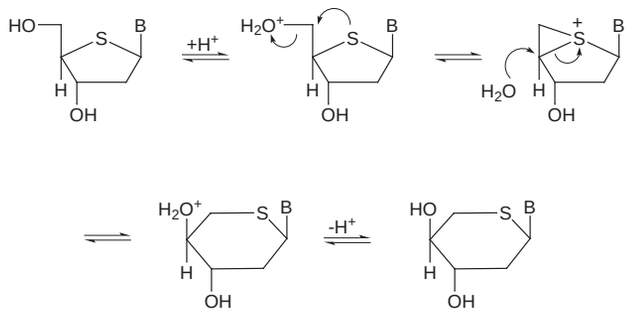


starting material, the corresponding α-thiopyranoside accumulated. In contrast with the β-anomers, however, no evidence for reversibility of the reaction was obtained with the α-anomers: accumulation of uracil α-D-thiofuranoside (**9a**) was not detected, when hydrolysis of the α-L-thiopyranoside (**11a**) was



followed. Both reactions, isomerisation and depyrimidination, are acid-catalysed, showing first-order dependence on the acidity of the solution over the pH-range studied (0.1 M to 4 M hydrogen chloride). The pyrimidine base was released at about an equal rate when either thiofuranoside or thiopyranoside was employed as starting material.

Taking into account both the findings described above and previous studies,[15,16] the mechanism shown in Scheme 4 may be



Scheme 4

proposed to explain the formation of the pyranose intermediates. Protonation of the C5 hydroxy and subsequent displacement (as a water molecule) by the strategically positioned sulfur results in formation of the relatively stable cyclic episulfonium ion intermediate. Subsequent attack by a water molecule at C4′ leads to thiopyranoside formation after deprotonation. A consequence of this mechanism is inversion at C4′, consistent with the NMR data. Attack at C5′ results in regeneration of the starting nucleoside. Attack of a water molecule at C1′, on the other hand, could result in cleavage of the *N*-glycosidic bond. However, it has to be noted that the present data as such do not reveal if the depyrimidination takes place from a common intermediate with isomerisation, or if there are competing mechanisms working. Nevertheless, the base moiety appears to be released about as fast from the thiofuranoside and thiopyranoside derivatives. The substituent at the C5 of the pyrimidine base does not markedly affect the rate of either isomerisation or depyrimidination of the compounds. Furthermore, since the α-4′-thionucleosides are isomerised analogously, and only slightly slower than their β-counterparts, we may exclude reaction mechanisms involving either attack of 5′OH at the C6 of the base moiety, or attack of the O2 of the base moiety on the episulfonium ion. Furthermore, ring-opening of the sugar moiety is unlikely, since anomerisation was not evident in any of the samples. With normal, oxygen only based, sugar moieties ring-opening (and hence anomerisation) is observed.[13]

According to the proposed mechanism, the different behaviour of the 4′-thio- and 4′-oxynucleosides could be explained, tentatively, by the lower basicity of 4′-sulfur compared with 4′-oxygen and the higher nucleophilicity of sulfur than oxygen towards carbon. With the 4′-thionucleosides protonation of 5′OH may successfully compete with protonation of the 4′S.

The episulfonium intermediate can also be formed from the β-thiopyranoside isomer, as shown by accumulation of **8a** during hydrolysis of **10a**. However, as mentioned above, the α-thiofuranoside nucleoside was not detected during hydrolysis of the α-L-thiopyranoside derivative. One possible explanation for this could be unfavourable conformational equilibria of the α-thiopyranoside ring for episulfonium formation (4′OH is axial in the preferred β-thiopyranoside conformer and equatorial in the preferred α-thiopyranoside conformer).

The *N*-glycosidic linkages of 4′-thiothymidine and 4′-thio-2′-deoxyuridine are more than one order of magnitude more stable towards acidic hydrolysis than are those of their native counterparts. The first-order rate constants determined for hydrolysis of thymidine and 2′-deoxyuridine in 1.0 M hydrogen chloride at 363.2 K were 8.91 and 5.11×10^{-5} s^{-1}, respectively. The stability difference is comparable to that observed with purine nucleosides.[11] With the latter, the stability of the thionucleosides was explained[11] by the destabilising effect of sulfur on the key intermediate of hydrolysis, *viz.* cyclic thiocarbenium ion compared to the corresponding oxocarbenium ion. In contrast, with uracil and thymine 2′-deoxynucleosides the 4′-thiosubstitution appears to completely change the mechanism of hydrolysis.

Characterization of the pyranose intermediates

The isolated thiopyranoside derivatives, **10a** and **11a**, and their respective starting materials, **8a** and **9a**, were examined in detail by NMR. Both compounds **8a** and **9a** gave proton and carbon spectra consistent with their expected structures. Starting from assignment of the anomeric protons at 6.25 ppm (**8a**) and 6.14 ppm (**9a**), respectively, the concerted use of COSY and CH correlation spectra readily yielded the carbon and attached proton assignments of the sugar ring. The base moiety assignments, proton and carbon, were readily made by comparison to the assignments made for an authentic sample of uridine and confirmed by 2D spectra (HMQC, HMBC). NOE difference spectra readily indicated the H2 proton assignments and, importantly, confirmed the stereochemistry given for the compounds. Distinction between the H5′ and H5″ was not made in either compound. All chemical shifts and coupling constants were unexceptional and in accord with expectations.

Similarly for the isolated material **10a**, starting with the assignment of the anomeric proton at 5.84 ppm, the concerted use of COSY and CH correlation experiments readily provided the carbon sequence and attached proton assignments of the sugar ring without ambiguity. The base moiety assignments were clear by comparison, but were also confirmed by 2D spectra (HMQC, HMBC) and appeared to be essentially unaffected. Notable, though, was that consideration of the carbon chemical shifts implied that C3 and C4 were O-bound (confirmed also by the exchangeable protons which were coupled to H3 and H4, respectively), whilst C1 and C5 (still a methylene carbon) were S-bound. Consequently a thiopyranoside structure was proposed, and this was further confirmed from a HMBC spectrum showing a correlation between H5′β and C1—the most likely correlation given the final structure and conformation whereby H5′β is equatorial resulting in a 180° dihedral angle. Consistent with a change from a furanoside to a pyranoside ring were the multiplicities of the two hydroxy protons, from a doublet and a triplet to a pair of doublets, respectively. Also consistent with the change were the chemical shift differences (0.6 and 0.7 ppm for the H2's and H5's, respectively) and couplings of both sets of methylene protons (most significantly, the large axial–axial coupling between H1′ and H2′β). Further evidence in support of this structure came from NOE difference spectra, primarily the interaction between H1′ and H5′α.

A chair conformation with the base moiety in an equatorial position is the only expected preferred conformation, and uniquely yields expected coupling constants in accordance with those observed. The large axial–axial coupling of H2′β also indicated its stereochemical assignment, confirmed by NOE difference spectra—which also facilitated the rest of the stereochemical assignments. The main implication of the determined stereochemistry, based on coupling constants and NOEs, is that inversion at C4′ has occurred.

For isolated compound **11a**, again the concerted use of COSY and CH correlation experiments readily provided the carbon sequence and attached proton assignments of the sugar ring without ambiguity starting with the assignment of the anomeric proton at 5.60 ppm. The base moiety assignments, carbon and proton, were again also clearly evident by comparison, but were also supported by HMQC, and appeared to be essentially unaffected. From comparison to **10a**, it was readily apparent that a similar transformation had occurred as the carbon chemical shifts were grossly similar. The proton spectrum also showed a clear transformation from a five-membered ring to a six-membered ring on examination of the couplings of each of the four methylene protons; although the clear chemical shift differences seen between H2′β and H2′α (0.6 ppm) and H5′β and H5′α (0.7 ppm) for **10a** were not exhibited here (0.15, 0.06 ppm for the H2′ and H5′ methylene pairs, respectively). The hydroxy protons were only evident as one broad singlet and so did not provide any evidence, based on multiplicities, for the furanoside to pyranoside transformation.

A chair conformation with the base moiety in an equatorial position again uniquely yields expected coupling constants in accordance with those observed. The large axial–axial couplings of H2′α and H5′β clearly indicated their stereochemical assignment, the former also confirmed by NOE difference spectra. Furthermore, based on coupling constants, inversion at C4′ is again also evident.

With the 5-ethyl analog **8d**, the pyranoside intermediate **10d** was identified on the basis of ^{13}C NMR spectra taken on aliquots from various stages of the reaction. A set of peaks associated with the pyranoside intermediate **10d** (*i.e.* with the appropriate chemical shift changes from **8d**) from amongst sets of peaks for the starting material **8d**, free base, *etc.*, was clearly evident.

Experimental

Spectra were acquired primarily using a JEOL Alpha 500 NMR spectrometer equipped with either a 5 mm normal configuration probe or a 5 mm inverse field-gradient probe and a NM-AFG field gradient unit (20 A) operating at 500.16 MHz for ^{1}H and 125.78 MHz for ^{13}C, or, alternatively, a JEOL Lambda 400 NMR spectrometer equipped with a 5 mm inverse probe operating at 399.78 MHz for ^{1}H and 100.54 MHz for ^{13}C. Spectra were recorded of samples in d_6-DMSO at 30 °C initially but, where appropriate, were also run at higher temperatures (75, 90 °C) in order to minimise the overlap between sample signals and the residual water peak. The spectra were referenced internally to TMS, assigned as 0 ppm for both proton and carbon.

1D proton spectra were acquired with single-pulse excitation, 45° flip angle, spectral widths of 8 kHz (digital resolution 0.11 Hz per point) and with presaturation of the residual water signal. NOE difference spectra were acquired on samples flushed with dry, nitrogen gas and using irradiation times of 6–8 s with 8 k data points and with 1 Hz exponential weighting applied prior to Fourier transformation. 2D homonuclear experiments included both phase-sensitive DQF COSY and absolute-value SERF (programmed as presented in Kalinowski *et al.*,[17] except that all three selective pulses used were Gaussian) and were acquired with spectral widths appropriately optimised from the 1D spectra (100 Hz was used for the f1 dimension of SERF).

1D carbon spectra were acquired with single-pulse excitation,

45° flip angle, spectral widths of 34 kHz (digital resolution 0.52 Hz per point), and with 1 Hz exponential weighting applied prior to Fourier transformation. DEPT spectra (90° and 135°) were acquired under similar conditions. 2D heteronuclear experiments included HMQC, phase-sensitive HMQC with BIRD filter, field gradient HMQC, and field gradient HMBC, and were acquired with spectral widths appropriately optimised from the 1D spectra. The delay used for the BIRD filter was optimised by minimisation of the incoming FID (*ca.* 0.5 s).

3′,5′-Di-*O*-benzyl-4′-thio-2′-deoxyuridine (4a) and its α-anomer 5a

To a stirred suspension of uracil **1a** (7.93 g, 70.8 mmol) in acetonitrile (150 ml) was added bis(trimethylsilyl)acetamide (28.88 g, 36.5 ml, 141.6 mmol). The reaction was stirred at room temperature for 30 minutes, during which time a clear solution formed, and was then placed under a nitrogen atmosphere. The addition of crushed 4 Å molecular sieves (*ca.* 5 g) and a solution of benzyl 3,5-di-*O*-benzyl-2-deoxy-1,4-dithio-D-*erythro*-pentofuranoside **3** (25.76 g, 59.0 mmol) in acetonitrile (100 ml) followed. After 10 minutes, a solution of *N*-iodosuccinimide (14.03 g, 64.9 mmol) in acetonitrile (150 ml) was added, upon which the reaction mixture turned dark brown. After 14 hours, the reaction was quenched by the addition of saturated aqueous sodium thiosulfate (200 ml) followed by filtration through a layer of Celite® 521 (10 mm), which was washed with dichloromethane. The organic layer was separated, washed with saturated aqueous $NaHCO_3$ (200 ml) and NaCl, dried with $MgSO_4$ and then filtered. The solvent was removed *in vacuo* to yield a beige syrup with an α/β-anomeric ratio of 3:2 (determined by ^{1}H NMR). The crude product was purified by column chromatography, (silica, EtOAc–hexane, 1:1) yielding a beige viscous syrup containing an anomeric mixture of product (18.43 g, 73.7%). A 1:1 α/β mixture of the product (8.83 g, 35%) was obtained as a white solid after recrystallisation from methanol. R_f(EtOAc–hexane, 4:1) 0.48. δ_H(300 MHz, $CDCl_3$) 8.97 (br d, HN3β), 8.90 (br d, HN3α), 8.16 (d, ^3J 8.2, H6 α), 8.00 (d, ^3J 8.3, H6β), 7.41–7.22 (20H, m, 4 × *Ph*CH_2 α/β), 6.43 (dd, ^3J 7.0, H1′β), 6.32 (dd, ^3J 1.8, 7.6, H1′α), 5.55 (dd, ^3J 8.3, ^4J 2.2, H5α), 5.31 (dd, ^3J 8.3, ^4J 2.3, H5β), 4.60–4.42 (8H, m, 4 × PhCH_2 α/β), 4.31 (m, H3′α), 4.23 (m, H3′β), 4.00–3.30 (6H, m, 2 × H5′ and H5″ and 2 × H4′), 2.55–2.12 (4H, m, 2 × H2′α and 2 × H2′β). *m*/*z* (FAB) 425 ([M + H]$^+$). (Found: C, 64.98; H, 5.55; N, 6.50. $C_{23}H_{24}O_4N_2S$ requires: C, 65.08; H, 5.70; N, 6.60%).

N^3-Benzoyl-3′,5′-di-*O*-benzyl-4′-thio-2′-deoxyuridine 6 and its α-anomer 7

To a stirred solution at 0 °C of an α/β-mixture (1:1) of 3′,5′-di-*O*-benzyl-4′-thio-2′-deoxyuridine (**4a**/**5a**, 4.10 g, 9.67 mmol) in dichloromethane (50 ml) was added triethylamine (5.86 g, 8.10 ml, 58.02 mmol), followed by benzoyl chloride (6.79 g, 5.61 ml, 48.35 mmol). The reaction mixture was allowed to warm to room temperature. After 16 hours, the reaction was quenched by the addition of 5% $NaHCO_3$ (100 ml). The organic layer was then separated, washed with 5% $NaHCO_3$ (50 ml) and water (75 ml), dried with $MgSO_4$ and filtered. Removal of the solvent *in vacuo* yielded a brown oil that was purified by flash column chromatography (hexane–EtOAc, 3:1), resulting in isolation of the β-anomer (2.28 g, 45%) and the α-anomer (2.34 g, 46%). Both anomers gave fluffy, white crystals when recrystallised from hot ethanol. β-**Anomer 6**: R_f(EtOAc–hexane, 1:1) 0.56, (EtOAc–hexane, 1:2) 0.27, (EtOAc–hexane, 1:3) 0.18. Mp 116–119 °C. δ_H(300 MHz, $CDCl_3$) 8.14 (d, ^3J 8.2, H6), 7.90 (2H, m, *m*-benzoyl), 7.64 (1H, m, *p*-benzoyl), 7.49 (2H, m, *o*-benzoyl), 7.41–7.28 (10H, m, 2 × *Ph*CH_2), 6.40 (dd, ^3J 7.0, H1′), 5.38 (d, ^3J 8.2, H5), 4.53 (4H, m, 2 × PhCH_2), 4.25 (m, H3′), 3.83 (m, H4′), 3.74–3.65 (2H, m, H5′), 2.58–2.49 and 2.27–2.18 (2H, m, H2′α and H2′β). δ_C(75 MHz, $CDCl_3$) 41.4 (C2′), 54.0 (C4′), 62.3 (C1′), 71.5, 71.6 (2 × Ph*C*H_2), 74.0 (C5′), 82.5 (C3′), 102.5 (C5), 127.8, 128.1, 128.3, 128.5, 128.7, 128.8 (benzyl aromatic), 129.3, 130.6 (benzoyl aromatic), 131.7 (quaternary benzoyl aromatic), 135.2 (*p*-benzoyl aromatic), 137.5, 137.7 (quaternary benzyl aromatic), 141.5 (C6), 149.8 (C2), 162.1 (C4), 169.0 (benzoyl carbonyl). *m*/*z* (FAB) 529 ([M + H]$^+$). λ_{max}(10% aqueous ethanol) 253.2 nm, ε = 20100 M^{-1} cm^{-1}. (Found: C, 68.42; H, 5.22; N, 5.09; S, 6.31. $C_{30}H_{28}O_5N_2S$ requires: C, 68.16; H, 5.34; N, 5.30; S, 6.07%). α-**Anomer 7**: R_f(EtOAc–hexane 1:1) 0.62, (EtOAc–hexane, 1:2) 0.32, (EtOAc–hexane, 1:3) 0.25. Mp 129–132 °C. δ_H(300 MHz, $CDCl_3$) 8.28 (d, ^3J 8.2, H6), 7.93 (2H, m, *m*-benzoyl), 7.68–7.30 (13H, m; 1H, *p*-benzoyl; 2H, *o*-benzoyl; 10H, 2 × *Ph*CH_2), 6.30 (dd, ^3J 7.0, 2.2, H1′), 5.66 (d, ^3J 8.2, H5), 4.54 (4H, m, 2 × PhCH_2), 4.35 (m, H3′), 4.02 (m, H4′), 3.54–3.49 and 3.39–3.32 (2H, m, H5′α and H5′β), 2.52–2.35 (2H, m, H2′α and H2′β). δ_C(75 MHz, $CDCl_3$) 41.6 (C2′), 55.0 (C4′), 64.0 (C1′), 71.3, 71.9 (2 × Ph*C*H_2), 73.4 (C5′), 83.2 (C3′), 101.2 (C5), 127.7, 127.9, 128.0, 128.2, 128.6, 128.7 (benzyl aromatic), 129.2, 130.5 (benzoyl aromatic), 131.5 (quaternary benzoyl aromatic), 135.1 (*p*-benzoyl aromatic), 137.0, 137.6 (quaternary benzyl aromatic), 143.2 (C6), 150.0 (C2), 162.3 (C4), 169.0 (benzoyl carbonyl). *m*/*z* (FAB) 529 ([M + H]$^+$), 551 ([M + Na]$^+$). λ_{max}(10% aqueous ethanol) 252.9 nm, ε = 19800 M^{-1} cm^{-1}. (Found: C, 68.02; H, 5.28; N, 5.41; S, 5.96. $C_{30}H_{28}O_5N_2S$ requires: C, 68.16; H, 5.34; N, 5.30; S, 6.07%).

3′,5′-Di-*O*-benzyl-4′-thio-2′-deoxyuridine 4a

To a stirred solution of N^3-benzoyl-3′,5′-di-*O*-benzyl-4′-thio-2′-deoxyuridine (6.58 g, 12.46 mmol) in dichloromethane (25 ml) at room temperature was added a solution (2.0 M) of ammonia in methanol (63 ml, 126 mmol). After 15 hours the solvent was removed *in vacuo* and the resultant brown viscous syrup was purified by column chromatography (silica, EtOAc–hexane, 2:1), yielding a white, solid product (4.84 g, 92%). Fine, colourless crystals of the pure product were obtained by recrystallization from ethanol. R_f(EtOAc–hexane, 4:1) 0.48. Mp 127–129 °C. δ_H(300 MHz, $CDCl_3$) 8.99 (s, H-N3), 8.00 (d, ^3J 8.3, H6), 7.41–7.27 (10H, m, 2 × *Ph*CH_2), 6.43 (dd, ^3J 7.0, H1′), 5.32 (dd, ^3J 8.0, ^4J 1.8, H5), 4.60–4.46 (4H, m, 2 × PhCH_2), 4.23 (m, H3′), 3.83–3.76 (m, H4′), 3.74–3.61 (2H, m, H5′α and H5′β), 2.56–2.43 and 2.24–2.11 (2H, m, H2′α and H2′β). δ_C(75 MHz, $CDCl_3$) 41.2 (C2′), 53.7 (C4′), 61.8 (C1′), 71.4 (2 × Ph*C*H_2), 73.8 (C5′), 82.3 (C3′), 102.6 (C5), 128.0, 128.1, 128.3, 128.6, 128.7 (2 × *Ph*CH_2), 137.3, 137.6 (2 × quaternary benzyl aromatic), 141.6 (C6), 150.9 (C2), 163.6 (C4). *m*/*z* (FAB) 425 ([M + H]$^+$), 447 ([M + Na]$^+$). λ_{max}(10% aqueous ethanol) 264.3 nm, ε = 9200 M^{-1} cm^{-1}. (Found: C, 65.04; H, 5.52; N, 6.71; S, 7.34. $C_{23}H_{24}O_4N_2S$ requires: C, 65.08; H, 5.70; N, 6.60; S, 7.55%).

3′,5′-Di-*O*-benzyl-4′-thio-2′-deoxy-α-uridine 5a

Compound **7** (0.90 g, 1.70 mmol) was debenzoylated in a similar manner to **6** to provide α-anomer **5a** (0.68 g, 94%) as a colourless syrup. R_f(EtOAc–hexane, 4:1) 0.48. δ_H(300 MHz, $CDCl_3$) 9.40 (br d, H-N3), 8.16 (d, ^3J 8.2, H6), 7.40–7.22 (10H, m, 2 × *Ph*CH_2), 6.32 (dd, ^3J 7.5, 1.5, H1′), 5.55 (dd, ^3J 8.2, ^4J 1.8 Hz, H5), 4.58–4.44 (4H, m, 2 × PhCH_2), 4.31 (m, H3′), 3.98 (m, H4′), 3.54–3.29 (2H, m, H5′ and H5″), 2.51–2.27 (2H, m, H2′ and H2″). δ_C(75 MHz, $CDCl_3$) 41.4 (C2′), 54.9 (C4′), 63.3 (C1′), 71.2, 71.9 (2 × Ph*C*H_2), 73.3 (C5′), 83.1 (C3′), 101.5 (C5), 127.7, 127.9, 128.0, 128.1, 128.6 (2 × *Ph*CH_2), 137.0, 137.6 (2 × quaternary benzyl aromatic), 143.3 (C6), 151.1 (C2), 163.5 (C4). *m*/*z* (FAB) 425 ([M + H]$^+$), 447 ([M + Na]$^+$). λ_{max}(10% aqueous ethanol) 264.3 nm, ε = 9700 M^{-1} cm^{-1}. (Found: C, 64.96; H, 5.85; N, 6.47; S, 7.49. $C_{23}H_{24}O_4N_2S$ requires: C, 65.08; H, 5.70; N, 6.60; S, 7.55%).

4′-Thio-2′-deoxyuridine 8a

To a stirred solution of boron trichloride in dichloromethane (1.0 M) (51.4 ml, 51.4 mmol) at −80 °C under a nitrogen atmosphere was added dropwise a solution of 3′,5′-di-*O*-benzyl-4′-thio-2′-deoxyuridine **4a** (4.36 g, 10.28 mmol) in dichloromethane (50 ml). Stirring was continued at this temperature for a further 16 hours. The reaction was then quenched, at −80 °C, by the dropwise addition of methanol–dichloromethane (1:1) (50 ml). Upon warming the reaction mixture to room temperature, the solvent was removed *in vacuo* followed by co-evaporation with methanol. The resultant white solid was purified by flash column chromatography (dichloromethane–methanol, 9:1). The appropriate fractions were combined to give the desired product (2.67 g, 97%). Colourless crystals of the pure product were obtained by recrystallisation from ethanol. R_f(DCM–MeOH, 4:1) 0.52; (DCM–MeOH, 9:1) 0.14. Mp 184–187 °C. δ_H(500 MHz, d_6-DMSO) 7.938 (d, $^3J_{H5}$ 8.1, H6), 6.249 (dd, $^3J_{H2'\beta}$ 7.9, $^3J_{H2'\alpha}$ 6.8, H1′), 5.631 (d, $^3J_{H6}$ 8.1, H5), 4.349 (~qt, $^3J_{H2'\alpha}$~$^3J_{H2'\beta}$~$^3J_{H4'}$ 3.7, $^3J_{H4'}$ (from SERF) 3.4, H3′), 3.618 (d(AB)d, $^3J_{H5''}$ 11.3, $^3J_{H4'}$ 6.6, H5′), 3.576 (d(AB)d, $^3J_{H5'}$ 11.3, $^3J_{H4'}$ 5.5, H5″), 3.306 (~td, $^3J_{H5'}$~$^3J_{H5''}$ 6.0, $^3J_{H3'}$ 2.9, H4′), 2.202 (d(AB)dd, $^2J_{H2'\beta}$ 13.3, $^3J_{H'1}$ 6.7, $^3J_{H'3}$ 4.1, H2′α), 2.144 (d(AB)dd, $^2J_{H2'\alpha}$ 13.2, $^3J_{H'1}$ 8.2, $^3J_{H'3}$ 4.3, H2′β). The exchangeable protons (OHs, NH) were not observed in this sample but were observed in a different sample at 300 MHz: 11.35 (br s, HN3), 5.30 (d, 3′OH), 5.16 (t, 5′OH). δ_C(125 MHz, d_6-DMSO) 41.29 (C2′), 59.02 (C4′), 60.17 (C1′), 63.52 (C5′), 73.49 (C3′), 102.22 (C5), 141.41 (C6), 150.82 (C2), 163.02 (C4). *m/z* (FAB) 245 ([M + H]$^+$), 267 ([M + Na]$^+$). λ_{max}(10% aqueous ethanol) 265.0 nm, $\varepsilon = 9300$ M^{-1} cm^{-1}. (Found: C, 44.38; H, 4.72; N, 11.36; S, 13.42. $C_{10}H_{14}O_4N_2S$ requires: C, 44.25; H, 4.95; N, 11.47; S, 13.13%).

4′-Thio-2′-deoxy-α-uridine 9a

Compound **5a** (0.90 g, 1.70 mmol) was debenzylated in a similar manner to **4a,** to provide α-anomer **9a** (0.68 g, 94%) in a similar yield. R_f(DCM–MeOH, 4:1) 0.52, (DCM–MeOH, 9:1) 0.14. Mp 184–187 °C. δ_H (500 MHz, d_6-DMSO) 8.261 (d, $^3J_{H5}$ 8.2, H6), 6.137 (dd, $^3J_{H2'\beta}$ 8.2, $^3J_{H2'\alpha}$ 3.3, H1′), 5.647 (d, $^3J_{H6}$ 8.1, H5), 5.487 (d, $^3J_{H3'}$ 2.7, 3′OH), 5.041 (t, $^3J_{H5'} = {}^3J_{H5''}$ 4.8, 5′OH), 4.320 (m, H3′), 3.554 (td, $^3J_{H5'} = {}^3J_{H5''}$ 6.8, $^3J_{H3'}$ 2.9, unres. $^4J_{H2'\alpha}$ ~ 0.4, H4′), ~3.44 and 3.35 (2H, m, H5′ and H5″), 2.487 (1H, ddd (part. overlapped with DMSO), $^2J_{H2'\alpha}$ 14.3, $^3J_{H1'}$ 8.3, $^3J_{H3'}$ 4.7, H2′β), 2.048 (dt, $^2J_{H2'\beta}$ 14.3, $^3J_{H1'} = {}^3J_{H3'}$ 3.4, unres. $^4J_{H4'}$ ~ 0.6, H2′α), δ_C (125 MHz, d_6-DMSO) 42.14 (C2′), 59.98 (C4′), 60.97 (C1′), 63.55 (C5′), 74.15 (C3′), 101.18 (C5), 143.00 (C6), 150.72 (C2), 163.04 (C4). *m/z* (FAB) 245 ([M + H]$^+$), 267 ([M + Na]$^+$).

3′,5′-Di-*O*-benzyl-5-fluoro-4′-thio-2′-deoxyuridine 4b and its α-anomer 5b

To 5-fluorouracil (5 g, 38.43 mmol) in dry acetonitrile (200 ml) was added bistrimethylsilylacetamide (19 ml, 76.86 mmol), and the mixture was stirred at room temperature for 2 hours. The reaction was put under an atmosphere of dry argon followed by the addition of molecular sieves (19.75 g, type 4 Å). The reaction was stirred for 5 minutes before the addition of 3,5-di-*O*-benzyl 2-deoxy-1,4-dithio-D-*erythro*-pentofuranoside **3** (19.25 g, 44.09 mmol) in dry acetonitrile (125 ml). Finally, a solution of *N*-iodosuccinimide (9.93 g, 44.08 mmol) in dry acetonitrile (125 ml) was added. The reaction mixture immediately turned dark red and was left to stir for 16 hours at which time TLC (EtOAc–hexane, 1:1) confirmed the reaction to be complete. The molecular sieves were removed by filtration and the solution concentrated *in vacuo* and further co-evaporated with EtOAc (2 × 30 ml). ^{1}H NMR indicated an α:β anomeric ratio of 1.7:1. The crude material was partially purified on a column of fine silica (110 × 100 mm, EtOAc–hexane, 1:1). The isolated α/β mixture of nucleoside was dissolved in EtOAc and kept at *ca.* 5 °C, resulting in the crystallisation of some β-anomer (2.73 g, 16%). The mother liquor was taken to dryness *in vacuo* and purified on a larger column (230 × 90 mm, EtOAc–hexane, EtOAc increased incrementally gradually from 0–50%). The β-anomer eluted first and was isolated as a light coloured syrup (2.65 g, 16%). δ_H(300 MHz, d_6-DMSO) 11.85 (m, H-N3), 8.19 (d, ^3J 7.5, H6), 7.26–7.42 (10H, m, *Ph*CH$_2$), 6.25 (m, H1′), 4.48–4.62 (4H, m, PhC*H*$_2$), 4.25 (m, H3′), 3.74 (m, H4′), 3.38–3.73 (2H, m, H5′, H5″), 2.51–2.68 (2H, m, H2′, H2″); δ_C(75 MHz, d_6-DMSO) 156.3 (C4), 148.5 (C2), 141.0 (C5), 138.3, 138.2 (quaternary benzyl aromatic), 128.4, 127.6 (benzyl aromatic), 125.3 (C6), 81.5 (C3′), 72.3, 71.8 (Ph*C*H$_2$), 70.1 (C5′), 61.2 (C1′), 52.9 (C4′), 40.5 (C2′). *m/z* (FAB) 443 ([M + H]$^+$).

Middle fractions were shown by TLC to contain an α/β mixture of nucleosides, they were combined to yield a syrup (6.88 g, 40%). Finally, fractions containing pure α-anomer were eluted to yield a syrup (4.06 g, 24%). δ_H(300 MHz, d_6-DMSO) 11.85 (m, H3), 8.36 (d, ^3J 7.5, H6), 7.26–7.42 (10H, m, *Ph*CH$_2$), 6.21 (m, H1′), 4.48–4.62 (4H, m, PhC*H*$_2$), 4.25 (m, H3′), 4.08 (m, H4′), 3.38–3.73 (2H, m, H5′, H5″), 2.51–2.68 (2H, m, H2′, H2″); δ_C(75 MHz, d_6-DMSO) 156.2 (C4), 149.3 (C2), 140.8 (C5), 138.1, 137.6 (quaternary benzyl aromatic), 128.3, 127.7, 127.5, 127.2 (benzyl aromatic), 126.7 (C6), 82.7 (C3′), 72.0, 71.7 (Ph*C*H$_2$), 70.4 (C5′), 62.1 (C1′), 53.8 (C4′), 40.4 (C2′). *m/z* (FAB) 443 ([M + H]$^+$). (Found: C, 62.67; H, 5.41; N, 6.18; S, 7.08. $C_{23}H_{23}N_2O_4FS$ requires: C, 62.42; H, 5.24; N, 6.33; S, 7.24%). Overall yield 16.3 g (96%).

5-Fluoro-4′-thio-2′-deoxyuridine 8b

To a solution of 3′,5′-di-*O*-benzyl-5-fluoro-4′-thio-2′-deoxyuridine **4b** (1.02 g, 2.26 mmol) in freshly dried toluene (8 ml) was added $TiCl_4$ (0.72 ml, 6.77 mmol) in dry toluene (3 ml). Monitoring by TLC (DCM–methanol, 9:1) indicated all of the starting material to have been consumed after 90 minutes and the reaction was thence cooled in an ice-bath prior to the addition of methyl ethyl ketone (MEK) (8 ml) and citric acid (1.42 g) in water (10 ml), after which the reaction was warmed to room temperature. The organic and aqueous phases were separated and the toluene layer extracted with water (3 × 10 ml). The combined aqueous extracts were cooled to 0 °C and neutralised with aqueous ammonia (density 0.88), after which the aqueous phase was exhaustively extracted with MEK (10 × 25 ml). The combined MEK extracts were dried ($MgSO_4$), filtered and taken to dryness *in vacuo*. The white solid residue was purified on a column of fine silica (80 × 50 mm, DCM–methanol, 9:1), yielding a white solid (0.52 g, 85%). δ_H(300 MHz, d_6-DMSO) 11.83 (m, H-N3), 8.37 (d, ^3J 7.5, H6), 6.26 (m, H1′), 4.38 (m, H3′), 3.55–3.66 (3H, m, H4′, H5′, H5″), 3.25–3.47 (2H, m, 3′ and 5OH′), 2.05–2.23 (2H, m, H2′, H2″); δ_C(75 MHz, d_6-DMSO) 169.8 (C4), 149.6 (C2) 138.2 (C5), 127.9 (C6), 74.4 (C3′), 63.9 (C5′), 62.3 (C1′), 60.6 (C4′), 42.2 (C2′). *m/z* (CI) 263 ([M + H]$^+$). λ_{max}(0.1 M HCl in 10% aq. ethanol) 275 nm ($\varepsilon = 9100$ M^{-1} cm^{-1}); λ_{max}(pH 7) 273 nm ($\varepsilon = 11000$ M^{-1} cm^{-1}); λ_{max}(0.1 M NaOH) 274 nm ($\varepsilon = 8500$ M^{-1} cm^{-1}). (Found: C, 41.03; H, 4.52; N, 10.43; S, 12.02. $C_9H_{11}N_2O_4FS$ requires: C, 41.22; H, 4.23; N, 10.68; S, 12.22%).

5-Fluoro-4′-thio-2′-deoxy-α-uridine 9b

3′,5′-Di-*O*-benzyl-5-fluoro-4′-thio-2′-deoxy-α-uridine (4.06 g, 9.17 mmol) (**5b**) was debenzylated in a similar manner to **4b** to provide **9b** as a white solid (1.42 g, 60%). δ_H(300 MHz, d_6-DMSO) 11.83 (m, H-N3), 8.60 (d, ^3J 7.5, H6), 6.17 (m, H1′), 4.38 (m, H3′), 3.55–3.66 (3H, m, H4′, H5′, H5″), 3.25–3.47 (2H, m, 3′- and 5′-OH), 2.05–2.23 (2H, m, H2′, H2″); δ_C(75 MHz, d_6-DMSO) 169.8 (C4), 149.6 (C2) 138.2 (C5), 127.9 (C6), 74.4 (C3′), 63.9 (C5′), 62.3 (C1′), 60.6 (C4′), 42.2 (C2′). *m/z* (CI) 263 ([M + H]$^+$). λ_{max} in ethanol–water 1:9; λ_{max}(0.1 M HCl) 274 nm ($\varepsilon = 11000$ M^{-1} cm^{-1}); λ_{max}(pH 7) 273 nm

($\varepsilon = 12000$ M^{-1} cm^{-1}); λ_{max}(0.1 M NaOH) 274 nm ($\varepsilon = 11000$ M^{-1} cm^{-1}). (Found: C, 41.19; H, 4.47; N, 10.82; S, 12.00. $C_9H_{11}N_2O_4FS$ requires: C, 41.22; H, 4.23; N, 10.68; S, 12.22%).

Characterisation of the hydrolysis products

Intermediates **10a** and **11a** were isolated from the hydrolysis mixtures of **8a** and **9a**, respectively, by HPLC using similar conditions to those used for kinetic analysis (see below). Full NMR characterisation and assignment were performed using techniques described above.

Uracil-1-yl 2-deoxy-5-thio-β-L-ribopyranoside 10a

δ_H(500 MHz, d_6-DMSO, 75 °C) 7.699 (d, $^3J_{H5}$ 8.1, H6), 5.838 (dd, $^3J_{H2'\beta}$ 11.5, $^3J_{H2'\alpha}$ 3.1, H1′), 5.634 (d, $^3J_{H6}$ 8.0, H5), 5.002 (d, $^3J_{H3'}$ 3.7, 3′OH), 4.798 (d, $^3J_{H4'}$ 5.3, 4′OH), ~3.83 (1.2 H, m, part. overlapped with impurity, $^3J_{H4'}$ (from SERF) 4.4, H3′), ~3.60 (1.2 H, m, part. overlapped with impurity, $^3J_{H3'}$ (from SERF) 4.2, H4′), 3.294 (dd, $^3J_{H5'\beta}$ 13.7, $^3J_{H4'}$ 2.1, H5′α), 2.562 (dd, $^3J_{H5'\alpha}$ 13.7, $^3J_{H4'}$ 4.3, H5′β), 2.434 (~ddd, $^2J_{H2'\alpha}$ 13.5, $^3J_{H1'}$ 11.5, $^3J_{H3'}$ 2.1, H2′β), 1.823 (ddd, $^2J_{H2'\beta}$ 13.1, $^3J_{H3'}$ 4.8, $^3J_{H1'}$ 3.3, H2′α). δ_C(125 MHz, d_6-DMSO) 31.22 (C5′), 33.80 (C2′), 48.80 (C1′), 64.14 (C4′), 68.59 (C3′), 102.25 (C5), 141.50 (C6), 150.04 (C2), 162.74 (C4).

Uracil-1-yl 2-deoxy-5-thio-α-L-ribopyranoside 11a

δ_H(500 MHz, d_6-DMSO, 75 °C) 7.675 (d, $^3J_{H5}$ 8.0, H6), 5.606 (d, $^3J_{H6}$ 8.0, H5), 5.604 (dd, $^3J_{H2'\alpha}$ 12.0, $^3J_{H2'\beta}$ 2.9, H1′), 4.904 and 4.796 (2H, br s, 3′OH and 4′OH), ~3.39 (2H, m, part. overlapped with H3′, H4′), ~3.35 (2H, m, part. overlapped with H4′, H3′), 2.739 (d(AB)d, $^3J_{H5'\beta}$ 13.5, $^3J_{H4'}$ 4.2, H5′α), 2.677 (d(AB)d, $^3J_{H5'\beta}$ 13.5, $^3J_{H4'}$ 9.7, H5′β), 2.225 (d(AB)~t, $^2J_{H2'\alpha}$ 12.5, $^3J_{H3'}$ 3.7, $^3J_{H1'}$ 3.1, H2′β), 2.084 (1H, t(AB)d, part. overlapped with impurity, $^2J_{H2'\beta} = {}^3J_{H1'} = 12.1$, $^3J_{H3'}$ 10.4, H2′α). δ_C(125 MHz, d_6-DMSO) 31.72 (C5′), 40.73 (C2′), 52.60 (C1′), 71.66 (C4′), 72.71 (C3′), 102.26 (C5), 141.22 (C6), 149.88 (C2), 162.70 (C4).

Kinetic measurements

The hydrolytic reactions were followed by an HPLC method described previously.[14] The chromatographic separations were carried out on a Hypersil ODS5 column (4 × 250 mm, 5 μm). With the **8a** and **10a**, and **9a** and **11a**, an acetic acid–sodium acetate buffer at pH 4.2, containing 0.1 M ammonium chloride and 2% (*v*/*v*) of acetonitrile, was used as eluent. The 5-alkylated nucleosides (**8c**,**d**) were separated from their thiopyranoside analogs by eluting with a formic acid–sodium formate buffer (pH 3.2), containing 0.1 M tetramethyl ammonium chloride and 4 to 6% (*v*/*v*) ethanol. The integrated peak areas of the isomers were assumed to be proportional with concentrations, due to the unchanged base moieties.

The rate constants for depyrimidination were calculated from the diminution of the sum of the peak areas of the thiofuranoside and thiopyranoside compounds. No deviation from first-order kinetics was observed, and the rate constants obtained were almost equal, when the reaction was started from either isomer. Based on this observation, the first-order rate constants for isomerization were calculated by the equations of reversible first-order reactions (eqn. (1) and (2)), assuming that both isomers are degraded to secondary products at equal rates.

$$(k_1 + k_{-1})t = \ln\frac{1 - x_e}{x - x_e} \quad (1)$$

$$k_1/k_{-1} = (1 - x_e)/x_e \quad (2)$$

In the equations, x stands for the mole fraction of the thiofuranoside in the isomeric mixture at moment t and x_e is the corresponding value at equilibrium. Two-parameter least-squares fitting was applied to obtain both $(k_1 + k_{-1})$ and x_e. The uncertainty of the rate constants given in Table 1 is in some cases as large as ±20 to 30%. However, in spite of the large error limits, the data should allow the deductions made above.

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