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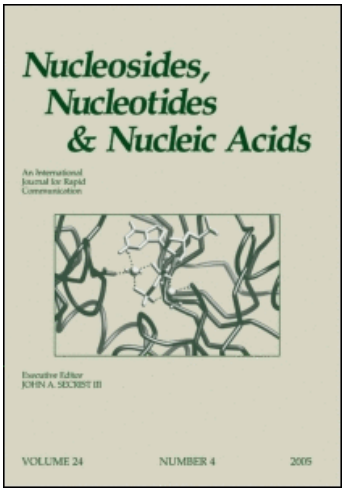

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E. Louise Hancox[a]; Richard T. Walker[a]
[a] School of Chemistry, The University of Birmingham, Birmingham, UK

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NUCLEOSIDES & NUCLEOTIDES, 15(1-3), 135-148 (1996)

SOME REACTIONS OF 4'-THIONUCLEOSIDES AND THEIR SULFONES

E. Louise Hancox and Richard T. Walker*†
School of Chemistry, The University of Birmingham, Edgbaston, Birmingham, B15 2TT, UK.

Abstract. We report interesting and novel reactions of 4'-thionucleosides and their sulfone derivatives when a good leaving group is present in the 5'-position. The results have important implications for the phosphorylation of these nucleoside analogues by standard chemical procedures. Possible mechanisms for these reactions are discussed.

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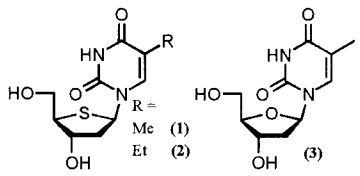
Nucleoside analogues have long been designed to act as potential antiviral agents and are now becoming increasingly important as building blocks in the synthesis of oligodeoxynucleotides for use in antisense strategy. One class of nucleoside analogue which has demonstrated a broad spectrum of antiviral activity is that of the 2'-deoxy-4'-thionucleosides.[1,2] The first synthetically useful strategies for the synthesis of these nucleoside analogues were devised independently by us and Secrist *et al.*[1,2] More recently, Uenishi *et al.*[1,2] have reported an elegant asymmetric synthesis from achiral precursors.[3] 1-(2-Deoxy-4'-thio-β-D-ribofuranosyl)thymine (4'-thiothymidine) (**1**) is an isoelectronic analogue of natural thymidine (**3**) in which the furanose ring oxygen is replaced by a sulfur atom. This nucleoside analogue is known to possess activity against herpes viruses including potent anti-cytomegalovirus (CMV) properties, but it is also extremely cytotoxic.[1]



4'-Thiothymidine has been incorporated into oligodeoxynucleotides (ODNs) and this appeared to have little effect upon the structure or stability of the duplex but was shown to have a drastic effect upon the interaction of the ODN with the enzymes of the *Eco*RV restriction-modification system.[4] X-Ray crystallographic data[5] for an ODN containing 4'-thiothymidine co-crystallised with *Eco*RV restriction endonuclease indicates that this drastic effect is due to steric clashes between the ODN and the protein, which arise as a result of the larger radius of the sulfur atom compared with that of the oxygen

† Dedicated to Dr. Yoshihisa Mizuno on the occasion of his 75th birthday

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atom present in the unmodified ODN. It remains unclear why 4'-thiothymidine should be toxic, whereas the 5-substituted pyrimidine-2'-deoxy-4'-thionucleosides are not, although it is likely to be linked to the fact that 4'-thiothymidine is a substrate for cellular and viral kinases. Information regarding why some nucleoside analogues are toxic and others are not, will be extremely useful in the design of such analogues in the future.

The initial synthetic target of this work was 4'-thiothymidine 5'-triphosphate, which was required to determine whether the modified nucleoside triphosphate could be incorporated into DNA by either cellular or viral DNA polymerases. However, it soon became clear that 4'-thiothymidine and other 4'-thionucleosides have the potential to demonstrate interesting and novel reactions when a good leaving group is present in the 5'-position.

Some of the reactions which are reported here were performed using either the α or β-anomers of 5-ethyl-4'-thio-2'-deoxyuridine (**2**) which were available and it is assumed that as far as the chemical reactions are concerned, the difference between a 5-methyl and a 5-ethyl substituent is negligible.

RESULTS AND DISCUSSION

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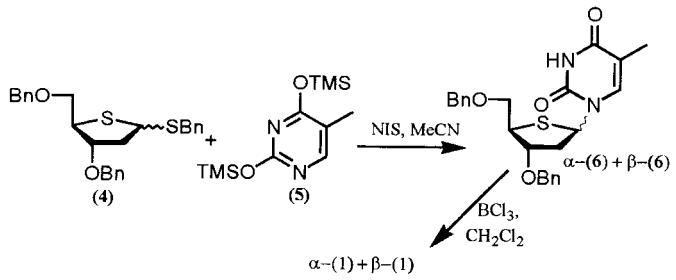
4'-Thiothymidine was prepared by adapting the methodology previously reported by Dyson *et al.*[1] The key intermediate in this synthesis was the thiosugar, benzyl 3,5-di-*O*-benzyl-1,4-dithio-D-*erythro*-pentoside (**4**) which was condensed with the appropriate heterocyclic base. Conditions used for the condensation of standard glycosides with heterocyclic bases were known to give poor yields when applied to the condensation of the thiosugar. An alternative method became available following the publication of a report by Sugimura *et al.*[6] in which *N*-bromosuccinimide (NBS) was used as a catalyst for the condensation of thioglycoside derivatives with 2,4-*bis*-*O*-trimethylsilyl-5-methyluracil (*bis*-TMS-thymine). These conditions have been successfully applied to the condensation of the thiosugar with silylated thymine (Scheme 1). The thiosugar, dissolved in acetonitrile, was added to the silylated base (**5**) followed - after ten minutes - by *N*-iodosuccinimide (NIS). By using NIS rather than NBS and performing the reaction in a high polarity solvent such as acetonitrile, 3',5'-di-*O*-benzyl-4'-thiothymidine (**6**) was synthesised as an anomeric mixture (α:β = 1:1.6) in a yield of 80%. Attempts to alter this anomeric ratio in favour of the β-anomer have failed to produce any improvement. Work done within this group has revealed that a similar ratio is obtained using a modification of the anomerisation procedure recently reported,[7] regardless of whether the anomerisation is performed on the pure β-anomer or the pure α-anomer. The β-anomer (**6**) was recrystallised from the anomeric mixture using methanol and the benzyl protecting groups were removed by the addition of four equiv. of fresh BCl_3 to a solution of the benzylated nucleoside in dichloromethane at -78 °C. It has proved crucial to maintain this low temperature throughout the reaction and during the quenching process. The debenzylation of the nucleoside α-(**6**) proceeded more slowly than for β-(**6**), presumably as a result of the steric interference from the base, which in the α-anomer is on the same face of the sugar moiety as the 3'-*O*-benzyl group.



Scheme 1

4'-Thiothymidine was subjected to the phosphorylation conditions reported in the literature[8] but analysis by both TLC and HPLC indicated that no reaction had occurred. Work done within this group has shown that dimethoxytritylation of 4'-thiothymidine proceeds more slowly than for thymidine,[4] therefore the phosphorylation reaction was repeated allowing 3 days for conversion to the phosphodichloridate. The failure of 4'-thiothymidine to react under these conditions indicated that the problem may be the result of an electronic rather than a steric effect. The van der Waals radius of a sulfur atom is larger than that of an oxygen atom (1.85 Å and 1.40 Å, respectively). The lone pair of electrons associated with the sulfur atom are held much more loosely than the corresponding electrons associated with an oxygen atom, with the result that the sulfur electrons are more available for participation in reactions. Using this reasoning, the formation of a bicyclic episulfonium intermediate (7) *via* displacement of the leaving group at the 5'-position was postulated. The intermediate could then be converted back to starting material during the aqueous work-up (Scheme 2). Uenishi *et al.* have also recently suggested such and intermediate in their proposed mechanism for the formation of the thiosugar from the γ,γ-diethoxy episulfide,[3] and similar reactions in six-membered ring systems have been reported by Hughes *et al.*[9] where more than one product was identified.

It was considered that if this were the case, the situation might be improved by reducing the reaction time for the initial phosphorylation rather than by increasing it. However, reducing the reaction time with phosphoryl chloride to 1 min., failed to produce any reaction. At this point it was decided to attempt to isolate the 5'-monophosphate by quenching the reaction with buffer, prior to the addition of the inorganic pyrophosphate. Again, monitoring by TLC indicated that no reaction occurred over a period of 3 days. Huang and Hui have recently reported the chemical synthesis of 4'-thiothymidine 5'-triphosphate in which they claimed to have isolated the monophosphate in 55% yield after reacting with phosphoryl chloride for 3 days.[10] However it is clear that their starting material was incorrectly characterized and the further characterization of the triphosphate is unconvincing.[11,12]

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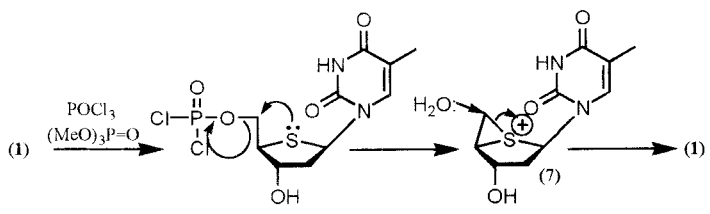

Scheme 2

At this stage some definitive chemical evidence was needed to support the bicyclic episulfonium intermediate theory. The tosyl group is a very effective leaving group and the formation of 5'-tosylthymidine is known to proceed smoothly in good yields. If it could be demonstrated that the 5'-hydroxyl group of 4'-thiothymidine could not be tosylated, then this in itself would provide good evidence for rapid displacement of a reactive 5'-substituent, possibly proceeding *via* a bicyclic episulfonium ion intermediate.

The model reaction of thymidine with tosyl chloride in dry pyridine was complete within a few hours and the expected product was isolated in 70% yield and was fully characterised showing that the tosylation was selective for the 5'-hydroxyl group. Under identical conditions, 4'-thiothymidine remained largely unreacted (less than 20% conversion after 3 days) and the NMR of the isolated product confirmed that it was a complex mixture of nucleosides. The ability to tosylate both 5'-*O*-DMT-thymidine and 5'-*O*-DMT-4'-thiothymidine at the 3'-hydroxyl position using tosyl chloride illustrates that it is the relative positions of the sulfur atom and leaving group that result in these unusual reactions of 4'-thionucleosides.

To simplify the reaction, the tosylation was performed on 3'-*O*-acetyl-4'-thiothymidine. The best strategy for the synthesis of 2'-deoxy-4'-thionucleosides in which the 3'-hydroxyl group is masked was found to involve the use of the monomethoxytrityl (MMT) protecting group.[13] This group was introduced in excellent yield, was easy to work with and, after subsequent acetylation, was removed quantitatively using 80% acetic acid to give the required nucleoside. Scheme 3 illustrates this strategy applied to the synthesis of 3'-*O*-acetyl-5-ethyl-4'-thio-2'-deoxyuridine. As far as chemical reactions are concerned we believe there to be little difference between the 5-ethyl and 5-methyl derivatives of 4'-thio-2'-deoxynucleosides. Thus this scheme should be equally effective for the preparation of 3'-*O*-acetyl-4'-thiothymidine although we had previously used a less efficient route for the preparation of this particular compound (data not shown).

The reaction of 3'-*O*-acetylthymidine with tosyl chloride was extremely slow (TLC indicated that starting material was still present after 5 days). The expected product, 5'-*O*-tosyl-3'-*O*-acetylthymidine, was isolated in a yield of 20% and the remaining 80% was shown to be unreacted starting material. The reason why this reaction is so much slower than with underivatised thymidine is unclear, although it is possible that the presence of the acetyl group in the 3'-position alters the pucker of the furanose ring,

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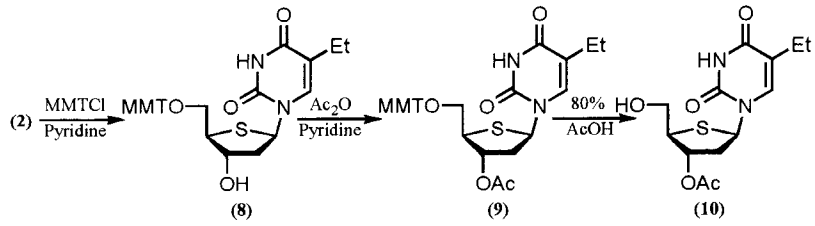

Scheme 3

making the 5'-hydroxyl group less accessible. When 3'-*O*-acetyl-4'-thiothymidine (**11**) was reacted under identical conditions, the extent of reaction was similarly poor with only 15% conversion of the starting material. Unreacted starting material was recovered and the more lipophilic product was isolated by column chromatography. The NMR spectrum of this product revealed that it contained at least 3 nucleosides (2 major products in equal amounts and approximately 10% of a minor product). The essential feature of the NMR spectra of the separated products and that of the mixture was that none of the products contained a tosyl function. The minor product appeared to have all of the protons required for a nucleoside except for the H-5' and H-4' multiplets and in addition gave 2 sharp doublets at 6.8 ppm. The spectrum of this minor product is consistent with the vinylic nucleoside **12**. The other two products have been identified as 3'-*O*-acetyl-5'-chloro-4'-thiothymidine (**13**) and 3'-*O*-acetyl-2,5'-anhydro-4'-thiothymidine (**14**). Although these products initially appeared to be consistent with the proposed bicyclic episulfonium ion intermediate, a more likely explanation is that the 5'-*O*-tosyl is formed (albeit extremely slowly) and is highly reactive. Thus **14** can be formed by direct displacement of the reactive tosylate by a chloride ion. The backside attack of O-2 on C-5' which would be necessary to open the episulfide ring giving rise to **14** is impossible on steric grounds. Anhydronucleoside formation is usually brought about by the action of a strong base but, given the high reactivity of the 5'-tosylate, the action of a weaker base such as pyridine (pK_a = 5.21) could explain the presence of compound **14**. These alternative mechanisms are summarised in Scheme 4.

Further experiments to demonstrate the difference between oxygen and sulfur at the 4'-position were performed with the sulfone. Again, the secondary hydroxyl group of the 4'-thionucleoside was protected to restrict the tosylation reaction to the 5'-hydroxyl position.

The required sulfone **17** was prepared by oxidising the fully protected nucleoside **15** with *m*-CPBA and removing the DMT group using benzenesulfonic acid (BSA). The reaction of this sulfone with an excess of tosyl chloride produced the diacetyl sulfone **18** in a yield of 20% (Scheme 5).

There are several chemical consequences of oxidising the sulfide to the sulfone which arise from the electron-withdrawing effect of the sulfone. Firstly, the proton which is in the position α to the sulfone (the H-4' proton) is considerably more acidic than in 4'-

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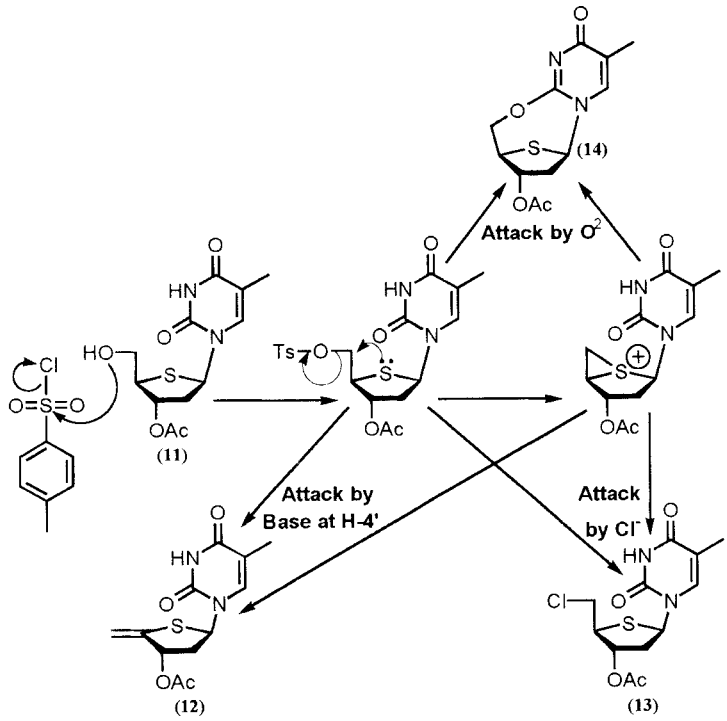
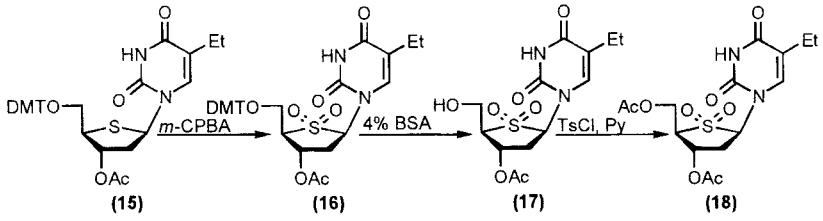


Scheme 4



Scheme 5

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thiothymidine. Secondly, the tosylation itself is likely to be more difficult since the nucleophilicity of the 5'-hydroxyl group is reduced and thus, the C-5' will be prone to nucleophilic attack. The formation of the diacetate could be rationalised mechanistically by invoking the initial formation of the 5'-*O*-tosyl sulfone **19**. As we have discussed with regard to 4'-thiothymidine, this 5'-tosylate appears to be highly reactive. Thus two mechanistic possibilities exist; in the presence of pyridine the H-4' proton could be abstracted (*via* an E2 mechanism) causing displacement of the tosyl group (which is a better leaving group than SO_2^-) to produce the vinyl sulfone **20** in which the double bond is conjugated with the sulfone group. Alternatively, species **21**, in which the anion is stabilised by the adjacent sulfone group, may be produced. These two species would presumably be in equilibrium with each other as shown in Scheme 6. The other possibility is that the 5'-*O*-tosyl sulfone **19** is the key intermediate.

In order to obtain the diacetate as product, the mechanism must account for a source of acetate anions. As shown in Scheme 7, there are two potential sources of acetate and, while both require the loss of an acetate anion rather than a tosylate anion, they both lead to the most highly substituted (and therefore most favourable) α,β-unsaturated sulfone **22** (Scheme 7). The yield of the diacetate is in fact 40% rather than 20% since the maximum possible yield is only 50%. The final step in the proposed mechanism involves the attack of the acetate anion, either at the 5'-position in the 5'-*O*-tosyl compound **19** or at the β carbon atom in the vinyl sulfone **20** (Scheme 8). Since there was no evidence for two diastereoisomers of 5'-*O*-acetyl which would result from acetylation of **20**, we favour the intermediacy of the 5'-tosylate and a direct displacement of the tosyl group in compound **19** by an acetate anion. As the tosylation of compound **17** proceeds rather slowly, it is also possible that an acetate anion is formed *via* a direct elimination from this compound.

Given this proposed mechanism, it was of interest to repeat the reaction using a derivative which had a better leaving group than acetyl at the 3'-hydroxyl position. The 4'-thionucleoside **23** in which the secondary alcohol was protected as a carbamate, was provided by the Wellcome Foundation. Oxidation using *m*-CPBA gave the required substrate **24** in excellent yield. Upon reaction of this substrate with tosyl chloride in pyridine the novel 5'-pyridinium nucleoside **25** was isolated in a yield of 84% (Scheme 9).

Once again there are two possible pathways to this unusual nucleoside. One alternative assumes the initial formation of the 5'-*O*-tosyl derivative which, by an E2 elimination produces the vinyl sulfone **26** as described above. This α,β-unsaturated sulfone is then attacked by pyridine in an S_N2' mechanism, the carbamate is displaced and decomposes to provide the reaction with a large driving force (Scheme 10). Alternatively, the carbamate may leave first generating an allylic alcohol which, upon tosylation, would produce a highly reactive allylic tosylate which would be readily displaced by pyridine to yield nucleoside **25**.

In summary, we have described the first application of a novel condensation method applied to the coupling of 2'-deoxythiosugars with heterocyclic bases using NIS as a catalyst. Furthermore we have shown that the phosphorylation of 4'-thionucleosides by standard chemical methods is not possible and that future attempts should focus on the use of enzymatic methods of phosphorylation. We have extended our study and have

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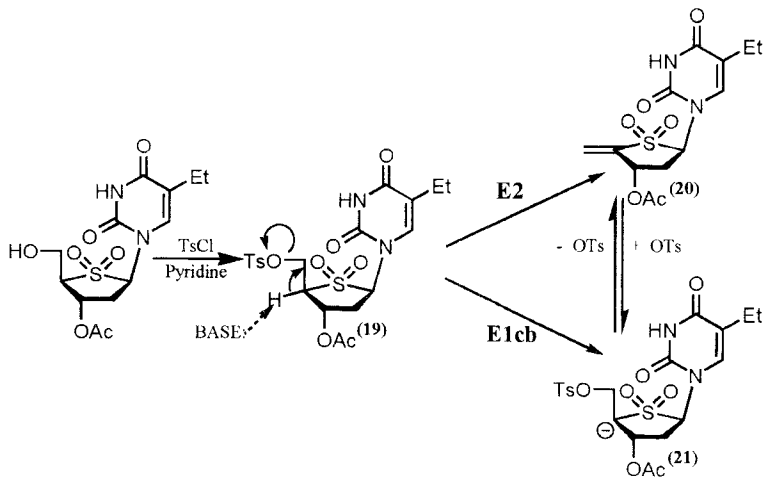

Scheme 6

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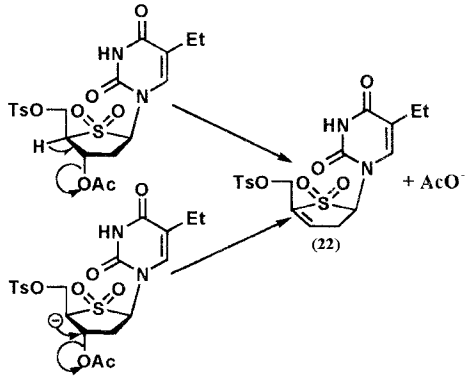
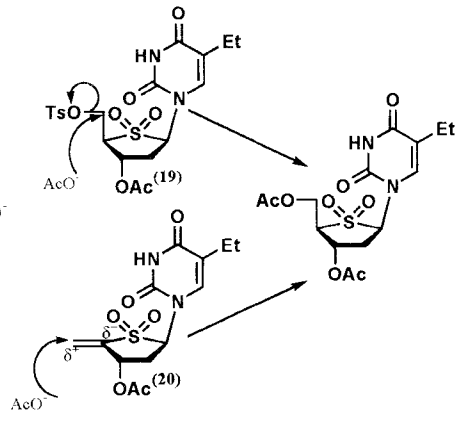
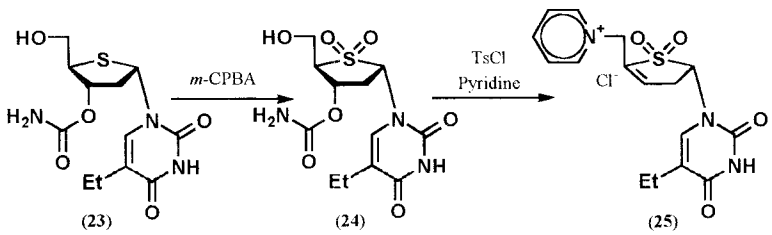


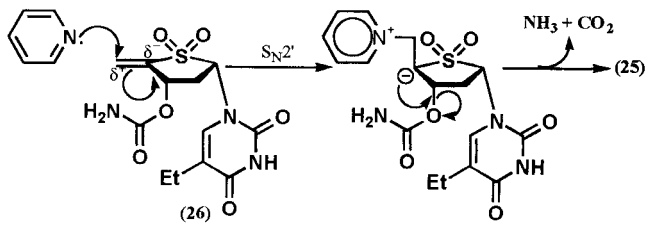
Scheme 7



Scheme 8



Scheme 9



Scheme 10

revealed that attempts to tosylate the 5'-position of 4'-thionucleosides give unexpected products whose formation we have attempted to rationalise. Although we initially favoured the intermediacy of a bicyclic episulfonium ion, our results at both the sulfide and sulfone level indicate that a reactive group in the 5'-position is highly susceptible to direct displacement. The reactions of the sulfone derivatives have been demonstrated to be largely influenced by the acidity of the H-4' proton and the protecting group present at the 3'-position, but are consistent with rapid displacement of reactive substituents in the 5'-position.

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EXPERIMENTAL

Thin Layer Chromatography. Precoated, aluminium-backed silica gel plates were supplied by E. Merck A. G., Darmstadt, Germany (silica gel 60 F_{254}, thickness 0.2 mm). Development was by the ascending method. Detection was by quenching of fluorescence at 254 nm or by spraying with 10% sulfuric acid in ethanol followed by charring. ***Column Chromatography.*** Glass columns were slurry-packed in the chosen eluent under gravity with silica gel (Kieselgel 60, 70-250 mesh ASTM, type 7734 supplied by E. Merck A. G.). Samples were applied as a concentrated solution in the same eluent or adsorbed onto silica gel. ***NMR Spectroscopy.*** ^{1}H NMR spectra were recorded on Jeol GX-270 (270 MHz) and Bruker AC-300 (300 MHz) spectrometers. All spectra were recorded relative to an internal trimethylsilane standard. ***Mass Spectroscopy.*** Spectra were recorded on a Kratos MS-80 mass spectrometer with a DS-55 data system with automatic digital readout or a Kratos MS-580RF mass spectrometer. Chemical ionisation (CI) methods used NH_3 as carrier gas and fast atom bombardment (FAB) methods used 3-nitrobenzyl alcohol or glycerol as matrix, with sodium or potassium ion doping when necessary. ***Elemental analysis.*** Analyses were obtained using a Perkin-Elmer 240 elemental analyser. ***Solvents and reagents.*** All solvents were dried and distilled according to standard procedures. Solid reagents were dried under high vacuum either over phosphorus pentoxide or self-indicating silica gel if acid sensitive.

PROCEDURE A: Oxidation Using *m*-CPBA To a solution of the nucleoside in dichloromethane-methanol 9:1 at -10 °C (ice-salt bath) was added *m*-CPBA dissolved in

dichloromethane, dropwise with stirring. Triethylamine (2% v/v) was added and the solution was left at 4 °C overnight. The crude product was washed successively with a saturated aqueous solution of sodium bicarbonate, saturated aqueous sodium chloride solution and water, dried ($MgSO_4$) and concentrated *in vacuo*. The compound was purified by column chromatography to yield the product.

PROCEDURE B: Reaction with Acetic Anhydride To a solution of the nucleoside in dry pyridine at room temperature was added freshly distilled acetic anhydride dropwise with stirring. After stirring for 18 hours at room temperature, the pyridine was removed by co-evaporation with toluene and then methanol and the product purified by eluting from a silica (7734) column with the appropriate solvent.

PROCEDURE C: Reaction with *p*-Toluenesulfonyl Chloride To a solution of the nucleoside stirring at 0 °C in pyridine was added *p*-toluenesulfonyl chloride portionwise. The solution was allowed to warm to room temperature and was stirred until TLC indicated no further change. The reaction mixture was then diluted with ethyl acetate and acidified with 1M hydrochloric acid. The organic layer was washed successively with a saturated aqueous solution of sodium bicarbonate and a saturated aqueous solution of sodium chloride, dried ($MgSO_4$) and evaporated *in vacuo*.

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2,4-*Bis*-*O*-trimethylsilyl-5-methyluracil (*bis*-TMS-thymine) (5) Thymine (0.87 g, 6.9 mmol) was suspended in hexamethyldisilazane (12 ml). To this was added chlorotrimethylsilane (2 ml). The mixture was heated at 130 °C under a stream of dry nitrogen until complete dissolution had occurred. The solvent was removed by reduced pressure distillation and the silylated base was dissolved in acetonitrile *in situ*.

3',5'-Di-*O*-benzyl-4'-thiothymidine and its α-anomer (6) A solution of benzyl 3,5-di-*O*-benzyl-2-deoxy-1,4-dithio-D-*erythro*-pentoside (1.50 g, 3.44 mmol), *bis*-TMS-thymine (1.86 g, 6.88 mmol) and crushed 4 Å molecular sieves (1.20 g) in acetonitrile (20 ml) were stirred under an atmosphere of nitrogen for 10 minutes. *N*-Iodosuccinimide (0.85 g, 3.79 mmol) dissolved in acetonitrile (10 ml) was added and the mixture was stirred for 4 hours. Following concentration *in vacuo*, the mixture was dissolved in dichloromethane, extracted with saturated aqueous sodium thiosulfate, dried ($MgSO_4$) and evaporated *in vacuo* to reveal a brown oil. The product was obtained as a white solid (1.20 g, 80%) following elution from silica (7734) column using hexane-ethyl acetate 1:1. ^{1}H-NMR indicated the ratio of α- to β-anomers to be 1:1.6. δ (^{1}H NMR; 300 MHz, DMSO-d_6) 11.4-11.35(1H, bs, NH), 7.95-7.69(1H, s, H-6), 7.38-7.30(10H, m, aromatics), 6.33-6.20(1H, m, H-1'), 4.62-4.46(4H, m, PhC$\underline{H}_2O$), 4.37-4.24(1H, m, H-3'), 4.10(1H, m, H-4'), 3.75-3.40(2H, m, H-5'), 2.39-2.32(2H, m, H-2'), 1.67-1.54(3H, s, CH_3).

The β-anomer was obtained as fine white crystals (m.p. 142 °C) and the α-anomer was isolated as a colourless oil upon recrystallisation of the mixture from methanol. δ (^{1}H NMR; 300 MHz, DMSO-d_6) (β-anomer): 11.37(1H, bs, NH), 7.69(1H, s, H-6), 7.38-7.28 (10H, m, aromatics), 6.34-6.27(1H, m, H-1'), 4.62-4.52(4H, m, PhC$\underline{H}_2O$), 4.30(1H, m, H-4'), 3.75-3.60(3H, m, H-3',H-5'), 2.40-2.34(2H, m, H-2'), 1.66(3H, s, CH_3); *m/z*: 439.5$(M+H)^+$, 331.3$(M\text{-}O\text{Bn})^+$.

β-4'-Thiothymidine (1) To a 1M solution of boron trichloride in dichloromethane (10 ml, 10 mmol) at -78 °C was added a solution of β-3'5'-di-*O*-benzyl-4'-thiothymidine (1.00 g, 2.28 mmol) in dichloromethane, dropwise with stirring. Stirring was continued at this temperature for 4 hours, after which time a 1:1 solution of methanol-dichloromethane (100 ml) was added over a period of 1 hour. The reaction mixture was allowed to warm to room temperature and the solvent was removed *in vacuo* and co-evaporation with methanol revealed the title compound as a white solid (0.70 g, 88%). This was recrystallised from methanol to give white crystals (m.p. 209 °C) δ (^{1}H NMR; 300 MHz, DMSO-d_6) 11.45 (1H, s, NH), 7.81(1H, s, H-6), 6.32-6.25(1H, m, H-1'), 5.34(1H, d, OH-3'), 5.24 (1H, t, OH-5'), 4.40-4.34(1H, m, H-3'), 3.73-3.55(2H, m, H-5'), 3.34-3.28(1H, m, H-4'), 2.34-2.14 (2H, m, H-2'), 1.87(3H, s, CH_3); *m/z*: 259$(M+H)^+$. Found: C, 46.40; H, 5.40; N, 10.80: $C_{10}H_{14}N_2O_4S$ requires C, 46.50; H, 5.46; N, 10.85.

5'-*O*-(Monomethoxytriphenylmethyl)-5-ethyl-4'-thio-2'-deoxyuridine (8) To a solution of 5-ethyl-4'-thio-2'-deoxyuridine (1.00 g, 3.67 mmol) in pyridine (40 ml) was added monomethoxytriphenylmethyl chloride (1.60 g, 5.14 mmol) portionwise and the resultant yellow solution was stirred at room temperature for 18 hours. The solvent was removed *in vacuo* by co-evaporation with toluene and then ethanol and the residue was dissolved in dichloromethane, extracted twice with saturated aqueous sodium bicarbonate, once with saturated aqueous sodium chloride, dried ($MgSO_4$), filtered and concentrated *in vacuo*. The cream foam was purified by eluting from a silica (7734) column with ethyl acetate-hexane 3:1 to give the title compound as a white foam (1.78 g, 90%). δ (^{1}H NMR; 300 MHz, $CDCl_3$) 8.20(1H, s, NH), 7.46-6.84(15H, m, aromatic, H-6), 6.39(1H, dd, H-1'), 4.47(1H, m, H-3'), 3.80(3H, s, OCH_3), 3.60-3.20(3H, m, H-4', H-5'), 2.48-2.01 (5H, m, H-2', $C\underline{H}_2CH_3$, OH-3'), 0.96(3H, t, $CH_2C\underline{H}_3$); *m/z*: 567$(M+Na)^+$, 545$(M)^+$. HRMS (EI): Calculated for $C_{31}H_{32}N_2O_5S$ (544.20320); Found M^+: 544.20383.

5'-*O*-(4-Monomethoxytriphenylmethyl)-3'-*O*-acetyl-5-ethyl-4'-thio-2'-deoxyuridine (9) 5'-*O*-(4-Monomethoxytriphenylmethyl)-5-ethyl-4'-thio-2'-deoxyuridine (1.46 g, 2.68 mmol) was treated with acetic anhydride (1.26 ml, 13 mmol) according to procedure B. The title compound was obtained as a white foam by eluting from a silica (7734) column with ethyl acetate-hexane 3:2 (1.36 g, 87%). δ (^{1}H NMR; 300 MHz, $CDCl_3$) 8.34(1H, s, NH), 7.48-6.83(15H, m, aromatic, H-6), 6.53(1H, dd, H-1'), 5.52(1H, m, H-3'), 3.80(3H, s, OCH_3), 3.66(1H, m, H-4'), 3.50(1H, m, H-5'), 3.23(1H, m, H-5') 2.46(1H, m, H-2'), 2.17-1.95 (3H, m, H-2', $C\underline{H}_2CH_3$), 2.11(3H, s, OCH_3), 0.92(3H, t, $CH_2C\underline{H}_3$); *m/z*: 586$(M)^+$, 273$(MMT)^+$. Found: C, 67.17; H, 5.74; N, 4.69: $C_{33}H_{34}N_2O_6S$ requires C, 67.57; H, 5.84; N, 4.78.

3'-*O*-Acetyl-5-ethyl-4'-thio-2'-deoxyuridine (10) 5'-*O*-(Monomethoxytriphenylmethyl)-3'-*O*-acetyl-5-ethyl-4'-thio-2'-deoxyuridine (0.30 g, 0.51 mmol) was stirred at room temperature in 80% acetic acid (10 ml) for 8 hours. The reaction mixture was neutralised by the careful addition of a saturated aqueous solution of sodium bicarbonate. The mixture was diluted with dichloromethane and extracted with saturated aqueous sodium bicarbonate, saturated aqueous sodium chloride, dried ($MgSO_4$), filtered and

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concentrated *in vacuo*. The product was obtained following elution from a silica (7734) column with ethyl acetate-hexane 2:1 (0.16 g, 100%). δ (^{1}H NMR; 300 MHz, $CDCl_3$) 9.47(1H, s, NH), 7.80(1H, s, H-6), 6.46(1H, dd, H-1'), 5.43(1H, m, H-3'), 4.01-3.71(2H, m, H-5'), 3.56(1H, m, H-4'), 2.90(1H, bt, OH-5'), 2.50-2.28(4H, m, H-2',C$\underline{H}_2$CH$_3$), 2.08(3H, s, CH_3), 1.09(3H, t, $CH_2C\underline{H}_3$); *m/z*: 315(M)$^+$, 273(M-*O*Ac)$^+$. Found: C, 49.58; H, 5.76; N, 8.57: $C_{13}H_{18}N_2O_5S$ requires C, 49.67; H, 5.78; N, 8.91.

5'-Chloro-3'-*O*-acetyl-4'-thiothymidine (13) and 3'-*O*-Acetyl-2,5'-anhydro-4'-thiothymidine (14) 3'-*O*-Acetyl-4'-thiothymidine (0.11 g, 0.37 mmol) and *p*-toluenesulfonyl chloride (0.21 g, 1.08 mmol) were reacted for 4 days according to procedure C. Following elution from a silica (7734) column with chloroform-ethanol 19:1, a mixture of products was isolated, followed by unreacted starting material (60 mg, 55%). The product mixture was separated by elution from a silica (7734) column with chloroform-ethyl acetate 7:3. The first product to be eluted was the title compound (**13**) (10 mg, 8.6%). δ (^{1}H NMR; 300 MHz, $CDCl_3$) 8.31(1H, s, NH), 7.55(1H, s, H-6), 6.51(1H, dd, H-1'), 5.48(1H, m, H-3'), 3.89(1H, m, H-5'), 3.78(1H, m, H-4'), 3.69(1H, m, H-5'), 2.53(1H, m, H-2'), 2.31(1H, m, H-2'), 2.13(3H, s, *O*CH$_3$), 1.97(3H, s, CH_3); Found: C,45.60; H, 4.62; N, 8.53: $C_{12}H_{15}N_2ClO_4S$ requires C, 45.20; H, 4.74; N, 8.78. This was followed by the title compound (**14**) (10 mg, 11.0%). δ (^{1}H NMR; 300 MHz, $CDCl_3$) 8.59(1H, s, NH), 7.27(1H, s, H-6), 6.32(1H, dd, H-1'), 5.21(1H, m, H-3'), 4.35(2H, m, H-5'), 4.25(1H, m, H-4'), 2.53(1H, m, H-2'), 2.31(1H, m, H-2'), 2.11(3H, s, OCH_3), 1.96(3H, s, CH_3). Found: C, 51.43; H, 5.01; N, 9.58: $C_{12}H_{14}N_2O_4S$ requires C, 51.05; H, 5.00; N, 9.92.

5'-*O*-(4,4'-Dimethoxytriphenylmethyl)-3'-*O*-acetyl-5-ethyl-4'-thio-2'-deoxyuridine (15) 5'-*O*-(4,4'-Dimethoxytriphenylmethyl)-5-ethyl-4'-thio-2'-deoxyuridine (1.00 g, 1.74 mmol) was treated with acetic anhydride (0.83 ml, 8.70 mmol) according to procedure B. The title compound was obtained as a white foam by eluting from a silica (7734) column with dichloromethane-acetone 19:1 containing 2% (v/v) triethylamine (0.80 g, 75%). δ (^{1}H NMR; 300 MHz, DMSO-d_6) 11.40(1H, s, NH), 8.33(1H, s, H-6), 7.43-6.89(13H, m, aromatic), 6.25 (1H, dd, H-1'), 5.43(1H, m, H-3'), 3.74(6H, s, *O*CH$_3$), 3.54(1H, m, H-4'), 3.36-3.23(2H, m, H-5'), 2.40(2H, m, H-2'), 2.05(3H, s, *O*CH$_3$), 2.04(2H, q, C$\underline{H}_2$CH$_3$), 0.89(3H, t, $CH_2C\underline{H}_3$); *m/z*: 639(M+Na)$^+$, 616(M)$^+$, 303(DMT)$^+$. Found: C, 66.00; H, 5.70; N, 4.60: $C_{34}H_{36}N_2O_7S$ requires C, 66.22; H, 5.88; N, 4.54.

Sulfone of 5'-*O*-(4,4'-dimethoxytriphenylmethyl)-3'-*O*-acetyl-5-ethyl-4'-thio-2'-deoxyuridine (16) Solutions of 5'-*O*-(4,4'-dimethoxytriphenylmethyl)-3'-*O*-acetyl-4'-thio-5-ethyl-2'-deoxyuridine (0.60 g) in dichloromethane-methanol (30 ml) and *m*-CPBA (0.74 g) in dichloromethane (7 ml) were reacted according to procedure A. The title compound was obtained as a pale yellow foam (0.61 g, 96%) which did not require purification by column chromatography. δ (^{1}H NMR; 300 MHz, DMSO-d_6) 11.68(1H, s, NH), 7.43-6.88 (14H, m, aromatic, H6), 5.97(1H, dd, H-1'), 5.25(1H, m, H-3'), 3.74(6H, s, *O*CH$_3$), 3.57-3.48 (3H, m, H-4',H-5'), 2.90-2.58(2H, m, H-2'), 2.20(2H, q, C$\underline{H}_2$CH$_3$), 2.30(3H, s, OCH_3), 0.99(3H, t, $CH_2C\underline{H}_3$); *m/z*: 664(M+NH_3)$^+$, 648(M)$^+$, 303(M-DMT)$^+$.

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Sulfone of 3'-*O*-acetyl-5-ethyl-4'-thio-2'-deoxyuridine (17) 5'-*O*-(4,4'-Dimethoxytriphenylmethyl)-3'-*O*-acetyl-5-ethyl-4'-thio-2'-deoxyuridine (0.50 g, 0.77 mmol) was dissolved in chloroform and the solution cooled to 0 °C. To this was added a 4% (w/v) solution of benzenesulfonic acid in chloroform (30 ml). The colour changed instantly from colourless to deep maroon and stirring at 0 °C was continued for 90 minutes. The mixture was extracted using a saturated aqueous solution of sodium chloride until the final solution was pale yellow in colour. The organic layer was dried ($MgSO_4$), filtered and following elution from a silica (7734) column with chloroform-ethanol 19:1, the title compound was isolated as a pale yellow powder (0.16 g, 62%). δ (^{1}H NMR; 300 MHz, DMSO-d_6) 11.66(1H, s, NH), 7.51(1H, s, H6), 5.96(1H, dd, H-1'), 5.44(1H, t, OH-5'), 5.39(1H, m, H-3'), 3.88-3.45(3H, m, H-4', H-5'), 2.96-2.60(2H, m, H-2'), 2.23(2H, q, $C\underline{H}_2CH_3$), 2.11(3H, s, OCH_3), 1.03(3H, t, $CH_2C\underline{H}_3$); *m/z*: 369(M+Na)$^+$, 347(M+H)$^+$, 205(M-5-ethyluracil)$^+$.

Sulfone of 3',5'-di-*O*-acetyl-5-ethyl-4'-thio-2'-deoxyuridine (18) The sulfone of 3'-*O*-acetyl-5-ethyl-4'-thio-2'-deoxyuridine (0.10 g, 0.29 mmol) and *p*-toluenesulfonyl chloride (0.25 g, 1.28 mmol) were reacted for 36 hours according to procedure C. Following elution from a silica (7734) column with chloroform-ethanol 19:1, the title compound was isolated as a white foam (22 mg, 20%). δ (^{1}H NMR; 300 MHz, DMSO-d_6) 11.7(1H, s, NH), 7.52(1H, s, H-6), 6.0(1H, dd, H-1'), 5.42(1H, m, H-3'), 4.48-4.36(2H, m, H-5'), 3.70(1H, m, H-4'), 3.03-2.61(2H, m, H-2'), 2.25(2H, q, $C\underline{H}_2CH_3$), 2.11(3H, s, OCH_3), 2.05 (3H, s, OCH_3), 1.04(3H, t, $CH_2C\underline{H}_3$); *m/z*: 389(M+H)$^+$, 346(M-acetyl)$^+$, 141(Ethyluracil+H)$^+$. Found: C, 46.38; H, 5.41; N, 6.91: $C_{15}H_{20}N_2O_8S$ requires C, 46.39; H, 5.19; N, 7.21.

Sulfone of 3'-*O*-carbamoyl-5-ethyl-4'-thio-2'-deoxyuridine (24) Solutions of 3'-*O*-carbamoyl-5-ethyl-4'-thio-2'-deoxyuridine (0.70 g) in dichloromethane-methanol (30 ml) and *m*-CPBA (1.73 g) in dichloromethane (10 ml) were reacted according to procedure A. The product precipitated from solution after standing at 4 °C overnight and following concentration *in vacuo*, was isolated as a white solid by trituration with methanol (0.62 g, 80%). δ (^{1}H NMR; 300 MHz, DMSO-d_6) 11.60(1H, s, NH), 7.63(1H, s, H6), 6.83(2H, bs, NH_2), 5.81(1H, m, H-3'), 5.36(1H, bs, OH-5'), 5.05(1H, dd, H-1'), 3.90-3.77 (2H, m, H-5'), 3.55-3.48(1H, m, H-4'), 2.91-2.82(1H, m, H-2'), 2.58-2.47 (1H, m, H-2'), 2.26(2H, q, $C\underline{H}_2CH_3$), 1.05(3H, t, $CH_2C\underline{H}_3$); *m/z*: 386(M+K)$^+$, 370(M+Na)$^+$, 348(M+H)$^+$, 332(M-NH_3)$^+$. Found: C, 41.38; H, 4.92; N, 12.06: $C_{12}H_{17}N_3O_7S$ requires C, 41.49; H, 4.93; N, 12.10.

Sulfone of 5'-pyridinium-3',4'-didehydro-5-ethyl-4'-thio-2'-deoxyuridine (25) The sulfone of 3'-*O*-carbamoyl-5-ethyl-4'-thio-2'-deoxyuridine (0.18 g, 0.52 mmol) and *p*-toluenesulfonyl chloride (0.30 g, 1.55 mmol) were reacted for 12 hours according to procedure C. Following co-evaporation with toluene and then methanol, the title compound was isolated as a white solid upon trituration with methanol (0.15 g, 84%). δ (^{1}H NMR; 300 MHz, DMSO-d_6) 11.63(1H, s, NH), 9.25(2H, d, aromatic), 8.69(1H, dd, aromatic), 8.22(2H, dd, aromatic), 7.59(1H, m, H-3'), 7.36(1H, s, H-6), 6.98(1H, dd, H-1'), 5.92(2H, s, H-5'), 3.60-3.18(2H, m, H-2'), 2.21(2H, q, $C\underline{H}_2CH_3$), 0.98(3H, t,

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$CH_2C\underline{H}_3$); *m/z*: 348(M)$^+$, 269(M-Py)+. Found: C, 49.70; H, 4.90; N, 10.80: $C_{16}H_{18}N_3ClO_4S$ requires C, 50.07; H, 4.73; N, 10.95.

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