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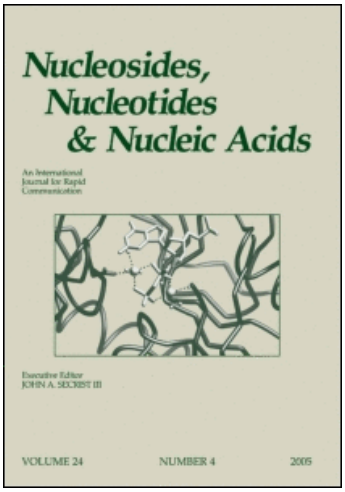

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THE SYNTHESIS OF SOME 5-ALKYL (CYCLOALKYL)-SUBSTITUTED 2'-DEOXY-4'-THIOURIDINES

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ABSTRACT: The silylated pyrimidine bases IIa-d were condensed with the benzyl 3,5-di-*O*-benzyl-2-deoxy-1,4-dithio-D-*erythro*-pentofuranoside III in acetonitrile under activation by *N*-iodosuccinimide, giving ca 1.5 : 1/α : β anomeric mixtures of the blocked nucleosides IVa-d and Va-d. in yields of 55-88%. After the separation on a silica column the pure anomers were deprotected by BCl_3 or $TiCl_4$, providing the free nucleosides VIa-d and VIIa,c,d in moderate to good overall yields. The β- or α-anomeric configuration, anti-glycosidic conformation and prevailing C2'-endo(S) thiosugar pucker in the synthesized compounds were established by the combined use of the ^1H, ^{13}C NMR and X-ray crystallography.

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INTRODUCTION

A recently synthesized series of 5-substituted and 5,6-disubstituted 2'-deoxyuridines,[1] did not yield compounds possessing significant antiviral activity, despite the fact that they all possessed the "natural" β-configuration, anti-glycosidic conformation and predominantly C2'-endo(S) sugar pucker in all 5-alkyl (cycloalkyl)-substituted members of this series. A possible explanation of this finding has been seen in their sensitivity towards pyrimidine phosphorylase, resulting in their degradation into the corresponding pyrimidine base and 2-deoxyribose-1-phosphate.[2] Many attempts have been made to increase nucleoside phosphorylase resistance via modification of either the base or the sugar moiety of the 2'-deoxynucleosides. Among them, replacement of *O*-4' with a methylene unit, which creates the carbocylic nucleosides, proved to be particularly effective.[3] Similarly, replacement of *O*-4' with sulfur in several 2'-deoxyuridines brought about interesting biological activities. The high antiviral activity of 4'-thiothymidine, particularly against HSV-1 and human cytomegalovirus is unfortunately also associated with severe cytotoxicity of this compound.[4,5] On the other hand, the 4'-thio analogue of (*E*)-5-(2-bromovinyl)-2'-deoxyuridine (4'-*S*-BVDU) was found to be equally as active against HSV-1 and VZV viruses as its highly active and selective predecessor BVDU. Moreover, in preliminary mouse bioavailability studies, it was non-toxic, with a significant half-life and

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

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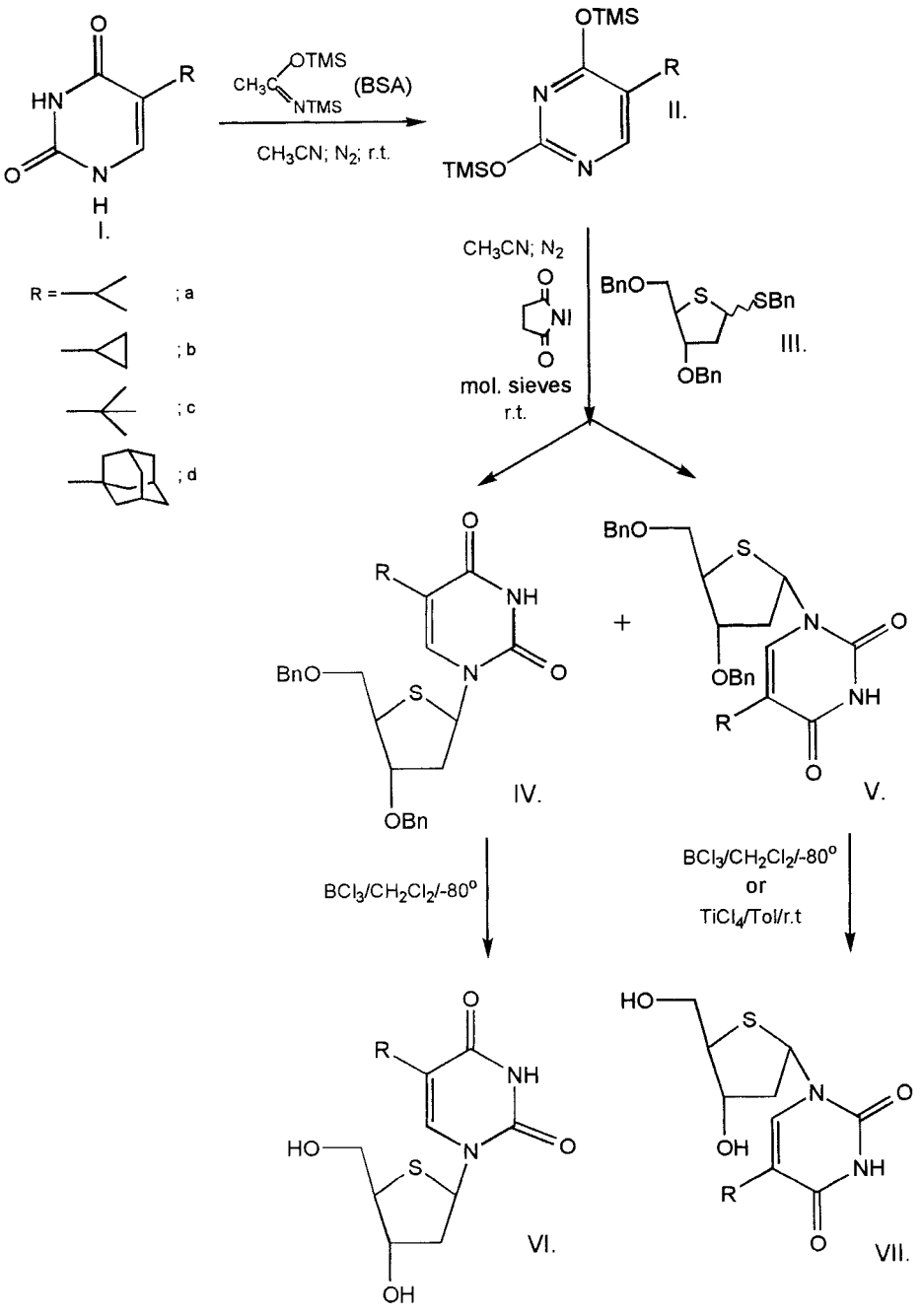

SCHEME 1

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high plasma level and no (*E*)-5-(2-bromovinyl)uracil could be detected.[4] This clear higher stability towards phosphorylases with retained antiviral activity and non-toxicity, when compared with BVDU itself, proved the viability of the concept of *O*-4' replacement by sulfur, provided other structural conditions of the molecule are fulfilled. With the intention of investigating further similarities and differences between 5-substituted-2'-deoxyuridines and their 4'-thiocounterparts, we started the synthesis of a further series of 4'-thio-analogues, complementary to the normal nucleosides published earlier.[1]

CHEMISTRY

The first succesful syntheses of 2′-deoxy-4'-thioribonucleosides were based on the coupling of a suitably blocked 4'-thiosugar moiety with a bis-trimethylsilylated pyrimidine base,[4,5,8] and this strategy remains mostly used in spite of the complicated separation of the resulting anomeric mixtures of nucleosides.The very first synthesis of a 2′-deoxy-4'-thioribonucleoside was described by Bobek et al. in 1976,[8] and involved a low yielding and multistep (14) synthesis of methyl 2-deoxy-4-thio-D-*erythro*-pentofuranoside, which, following protection of the 5- and 3-hydroxyl functions as the di-*O*-p-toluoyl derivative was coupled with 5-fluorouracil.

Secrist et al.,[5] modified this procedure so that an approximately 1:1 anomeric mixture of 2,3-di-*O*-p-toluoyl-1-*O*-acetyl-2-deoxy-4-thioribose was obtained, which was directly coupled with uracil, thymine and cytosine under trimethylsilyl triflate catalysis. Anomeric mixtures of the blocked nucleosides (~1:1) were separated and deprotected with sodium methoxide, affording the corresponding pure β- and α-nucleosides.

At the same time, work in our laboratory described a new 7-step synthesis of benzyl 3,5-di-*O*-benzyl-2-deoxy-1,4-dithio-D-*erythro*-pentofuranoside (III) from 2-deoxy-D-ribose with 11 % overall yield (25 % when recycling unreacted intermediates). This preparation has subsequently been optimised and used on a multi-kilogram scale with an overall yield approaching 50 % without having to resort to chromatographic separations. This material can either be used directly for nucleoside production or can be deprotected and reprotected with ester functionality so that acid-labile nucleosides can be made. Exploiting these possibilities, we synthesized 4'-thiothymidine and (*E*)-5-(2-bromovinyl)-2′-deoxy-4'-thiouridine, as well as 3'-azido-2′-deoxy-4'-thiothymidine.[4] 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.[10] The synthetic methodology has been based mainly on the condensation of the 1-*S*-benzyl thiosugar (III) with a bis-trimethylsilylated pyrimidine base promoted by the thiophilic activators of glycosidation such as *N*-bromo- (NBS) or *N*-iodo-succinimide (NIS) in the presence of molecular sieves (type-4A).

This glycosidation methodology has been originally adopted for the synthesis of different pentofuranosyl nucleosides by Sugimura et al.[11-14] In a detailed study of the influence of different activators and solvents on the anomeric stereoselectivity,[11] they specified that NBS/CH_2Cl_2/Molecular sieves (type-4A) was the system providing nearly exclusively 2'-deoxy-β-D-*threo*-pentofuranosyl nucleosides from the corresponding 2-deoxy-D-*threo*-substrates. The authors also analysed the possible

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mechanism which could explain the observed high stereospecificity. They considered the preferential α-localization of the succinimide anion in its interaction with an intermediate oxonium cation, as well as the rapid anomerization creating equilibrium in favour of an α-bromosulfonium intermediate. Subsequent S_N2 atack of a silylated pyrimidine base preferentially from the β-face would then explain the final anomeric ratio of the product.

As is shown in SCHEME 2, we adapted the conditions described by Sugimura for use in the production of 5-substituted 1-[2′-deoxy-D-*erythro*-4′-thiopentofuranosyl] uracils and our standard conditions included the use of NIS/CH_3CN/Molecular sieves (type-4A). These conditions resulted from a previous optimization study using different activators and solvents.[15] This study also revealed that the anomeric ratio of the final blocked nucleoside does not depend on the anomeric ratio of the starting 1-*S*-benzyl thiosugar III, and hence starting from a 4-thiosugar, one has very little, if any, ability to control the stereochemical selectivity of the condensation reaction.

Overall yields and the α : β anomeric ratios of the blocked nucleosides IVa-d and Va-d, prepared according to SCHEME 1, are shown in TABLE 1. The average α : β ratio found in this series is about 1.5 : 1 in favour of the α–anomer, which is in good agreement with an α:β ~1.4:1 ratio found in coupling with thymine under the same conditions.[15] The differences in the yield and anomeric ratio in the case of the 5-(1-adamantyl)-derivatives (IVd+Vd) probably reflect substantially different steric/electronic properties of this group when compared with the rest of the.series. In an attempt to explain the observed more or less consistant predominance of the α-anomer, we adapted the model of Sugimura to the 4-thiosugar moiety as depicted in SCHEME 2. It seems reasonable to assume, that from the steric point of view, the benzyliodosulfonium cation or the succinimide anion in the intermediate ion is less hindered in the β-face location, due to the absence of bulky 3′-benzyloxo- group. Hence, regardless of which mechanism of nucleophilic substitution is dominant in the subsequent attack of the silylated pyrimidine base (S_N2 or S_N1), an observed excess of the α-anomer in the resulting blocked anomeric mixture becomes the logical expectation.

The results of deprotection of both β (IVa-d) and α (Va-d) anomers after their separation on a silica column with the optimal mixture of EtAc/n-Hexane as the eluent, are presented in TABLE 2. All the deprotected β-anomers VIa-d were obtained in good yields using BCl_3 in CH_2Cl_2.[4] Effective cooling, so that the temperature inside the reaction flask did not rise above -80 °C during the reaction and subsequent quenching of the reaction, was essential to prevent cleavage of the glycosidic bond and liberation of pyrimidine base. Deprotection of α-anomers with BCl_3 was not completed even after a long reaction time when a small amount of the liberated base appeared and partially deprotected intermediate(s) was still present. This resulted in a relatively lower yield of the deprotected nucleoside, as can be seen in the case of the 5-isopropyl-derivative VIIa. In the case of the 5-cyclopropyl-derivative VIIb, only liberated base was isolated after 22 hrs of the reaction, which indicates the slightly higher lability of the glycosidic bond in this nucleoside, apparently due to the "conjugation" effect of the cyclopropyl group.In an attempt to overcome the problems in the deprotection of α-anomers with BCl_3, we tried more severe conditions, using $TiCl_4$ in toluene at the room temperature.[16] These conditions were ideal for the deprotection of the 5-(1-adamantyl)-nucleoside Vd,

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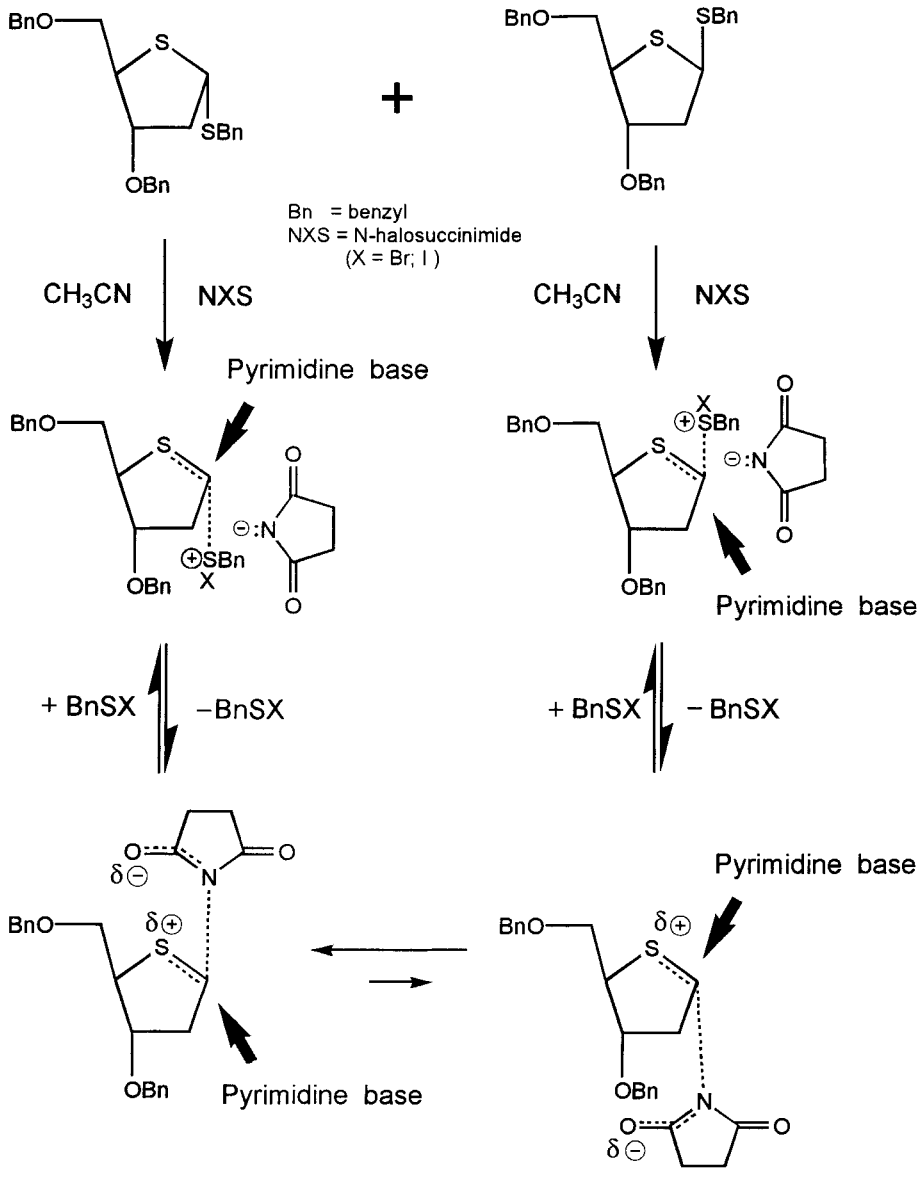

SCHEME 2

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TABLE 1 The overall yields and anomeric ratios in the condensation reaction

Base	Nucleoside	*Overall yield IV+V(%)	**Anomeric ratio α:β
Ia	IVa+Va	88	1.6:1
Ib	IVb+Vb	70	1.4:1
Ic	IVc+Vc	84	1.6:1
Id	IVd+Vd	55	1.3:1

* Overall yield after column chromatography

** Anomeric ratio in the crude reaction mixture, established from the ^{1}H NMR integrated signals of H-1′ and H-6

TABLE 2 Deprotection of 3′,5′-di-*O*-benzyl-nucleosides

Substrate	Product	[a]Method	Reaction time(hrs)	[b]Yield (%)
IVa	VIa	A	5	[c]65
IVb	VIb	A	5	[c]65
IVc	VIc	A	6	[c]60
IVd	VId	A	6	[c]79
Va	VIIa	A	94	[c]53
Vb	VIIb	[c]A(B)	22(8)	0
Vc	VIIc	B	3	[d]9
Vd	VIId	B	3	[c]76

[a] A: BCl_3/CH_2Cl_2/-80 °C

B: $TiCl_4$/Toluene/0 °C to room temp.

[b] Isolated yield, [c] Crystallization, [d] Column chromatography

[c] Additional $TiCl_4$ added after 4 hrs

when the product precipitated directly in the reaction mixture and was, therefore, protected against excessive treatment with $TiCl_4$. As a result a high yield (76 %) of the deprotected nucleoside (VIId) was obtained. On the contrary, 5-tert-butyl nucleoside VIIc remained dissolved in the reaction mixture, being exposed to the severe conditions resulting from the excess of $TiCl_4$. As a result, only 9 % of the product VIIc was isolated. Not surprisingly, 5-cyclopropyl nucleoside VIIb did not survive the hard conditions of the $TiCl_4$ and was not prepared at all. In general, the success of deprotection with $TiCl_4$ very much depends on the solubility of the product and does

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TABLE 3. ^{1}H NMR spectra of the synthesized 3',5'-di-*O*-benzyl-2'-deoxy-4'-thiouridines ($CDCl_3$; ppm)

Compd	H - 1'	H - 2'a,b	H - 3'	H - 4'	H - 5'a,b	-CH_2-(Bn)	C_6H_5 (Bn)	H - 6	H - R_5
IVa	6.48 t, 1	2.49; 2.15 2m, 2	4.24 m, 1	3.58 m, 1	3.70 m, 2	4.58; 4.53 2m, 4	7.39 - 7.25 m, 10	7.66 d, 1	2.80 (m, 1, CH); 1.10 and 1.07 (2d, 6, $2CH_3$, J = 7 Hz)
IVb	6.44 t, 1	2.49; 2.17 2m, 2	4.22 m, 1	3.58 m, 1	3.70 m, 2	4.26; 4.53 2m, 4	7.38 - 7.27 m, 10	7.68 d, 1	1.57 (m, 1, CH); 0.74 and 0.47 (2m, 4, $2CH_2$)
IVc	6.49 dd, 1	2.49; 2.11 2m, 2	4.23 m, 1	3.57 m, 1	3.71 m, 2	4.56; 4.53 2m, 4	7.39 - 7.24 m, 10	7.63 s, 1	1.22 (s, 9, $3CH_3$)
IVd	6.49 t, 1	2.49; 2.11 2m, 2	4.22 m, 1	3.58 m, 1	3.72 m, 2	4.57; 4.53 2m, 4	7.39 - 7.25 m, 10	7.51 s, 1	1.80 (m, 3); 1.71 (m, 6); 0.60 (m, 6)
Va	6.36 dd, 1	2.49; 2.27 2m, 2	4.30 m, 1	3.98 m, 1	3.50; 3.34 2m, 2	4.70 m, 4	7.39 - 7.24 m, 10	7.93 d, 1	2.73 (m, 1, CH); 1.01 and 0.93 (2d, 6, $2CH_3$, J = 7 Hz)
Vb	6.35 dd, 1	2.48; 2.25 2m, 2	4.31 m, 1	3.98 m, 1	3.50; 3.34 2m, 2	4.70 m, 4	7.38 - 7.25 m, 10	7.84 d, 1	1.53 (m, 1, CH); 0.63 and 0.31 (2m, 4, $2CH_2$)
Vc	6.36 dd, 1	2.47; 2.27 2m, 2	4.29 m, 1	3.98 m, 1	3.49; 3.34 2m, 2	4.53 m, 4	7.39 - 7.24 m, 10	8.00 s, 1	1.14 (s, 9, $3CH_3$)
Vd	6.35 dd, 1	2.49; 2.29 2m, 2	4.30 m, 1	3.99 m, 1	3.50; 3.36 2m, 2	4.54 m, 4	7.39 - 7.25 m, 10	7.88 s, 1	1.90 (m, 3); 1.80 (m, 6); 1.63 (m, 6)

TABLE 4 ^{1}H NMR spectra of the synthesized 2'-deoxy-4'-thiouridines (DMSO-d_6; ppm).

Compd	H - 1'	H - 2'a,b	H - 3'	H - 4'	H - 5'a,b	OH-3'	OH-5'	NH - 3	H - 6	H - R_5
VIa	6.28, t, 1	2.27 -2.12 m, 2	4.35 m, 1	3.30 m, 1	3.68 - 3.54 m, 2	5.26 d, 1	5.22 t, 1	11.30 s, 1	7.77 s, 1	2,74 (sept, 1, CH); 1.09 (d, 6, 2 CH_3, J = 7 Hz)
VIb	6.25 t, 1	2.26 - 2.07 m, 2	4.33 m, 1	3.28 m, 1	3.68 - 3.55 m, 2	5,26 brs, 1	5.26 brs, 1	11.32 brs, 1	7.67 s, 1	1.65 - 1.54 (m, 1, CH); 0.75 - 0.68, 0.63 - 0.47 (2 m, 4, 2 CH_2)
VIc	6.29 t, 1	2.26 - 2.10 m, 2	4.35 m,1	3.30 m, 1	3.67 - 3.50 m, 2	5.27 d, 1	5.20 t, 1	11.22 s, 1	7.72 s, 1	1.22 (s, 9, 3 CH_3)
VId	6.30 t, 1	2.25 - 2.10 m, 2	4.34 m, 1	3.31 m, 1	3.67 - 3.52 m, 2	5.27 d, 1	5.20 t, 1	11.17 s, 1	7.59 s, 1	1.98 (m, 3); 1.89 (m, 6); 1.68 (m, 6)
VIIa	6.22 dd, 1	2.03; 2.50 2 m, 2	4.37 m, 1	3.60 - 3.52 (m, 1) 3.45 - 3.30 (m, 2)		5.55 d, 1	5.04 t, 1	11.21 s, 1	8,17 s, 1	2.73 (sept, 1, CH); 1.07 (d, 6, 2 CH_3, J = 7 Hz)
VIIc	6.23 dd, 1	2.02; 2.50 2 m, 2	4.40 m, 1	3.57 - 3.50 (m, 1) 3.43 - 3.28 (m, 2)		5.56 d, 1	5.05 t, 1	11.12 s, 1	8.23 s, 1	1.21 (s, 9, 3 CH_3)
VIId	6.23 dd, 1	2.03; 2.50 2 m, 2	4.39 m, 1	3.58 - 3.50 (m, 1) 3.41 - 3.29 (m, 2)		5.54 d, 1	5.03 t, 1	11.08 s, 1	8.09 s, 1	2.03 (m, 3); 1.97 (m, 6); 1.89 (m, 6)
* 4'STβ	6.30 dd, 1	2.19 - 2.14 m, 2	4.38 brs, 1	3.28 m, 1	3.67 - 3.52 m, 2	5.24 d, 1	5.16 brs, 1	11.32 brs, 1	7.81 s, 1	1.80 (d, 3, CH_3, $J_{6,5\text{-Me}}$ = 1.1 Hz)
** 4'STα	6.16 dd, 1	2.05; 2.49 dt,1; ddd,1	4.26 m, 1	3.60 - 3.48 (m, 2) 3.38 - 3.33 (m, 1)		5.48 brs, 1	4.99 brs, 1	11.24 brs, 1	8.11 s, 1	1.80 (s, 3, CH_3)

* 4'-Thiothymidine,[5]

** α-4'-Thiothymidine [1-(2-Deoxy-4'-thio-α-D-*erythro*-pentofuranosyl) thymine],[5]

not provide any advantage, compared to the more or less standard, if not ideal, BCl_3 procedure.

^{1}H NMR spectra of the synthesized blocked nucleosides IVa-d and Va-d are shown in TABLE 3. Within each anomeric series, the chemical shift values of the similar protons are very close to each other, if not identical, indicating the same anomeric configuration and the same or very similar conformation around the glycosidic bond. The pucker of the sugar ring seems to be also very similar for all compounds in each anomeric series. In the β-anomers, the value for the characteristic triplet of the anomeric proton H-1' varies from 6.44 to 6.49 ppm and similarly, in the α-anomers, the value for characteristic doublet of doublet is 6.35 - 6.36 ppm.

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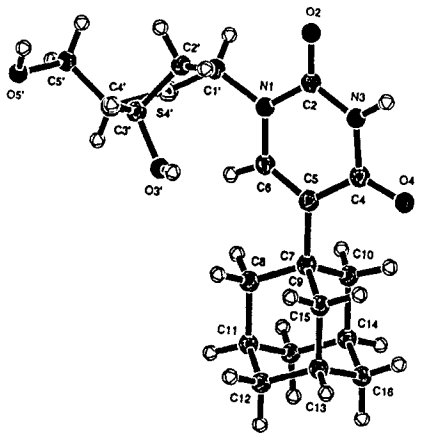
The conformation of 4'-thiothymidine has previously been studied in detail by 500-MHz ^{1}H NMR (in D_2O) and by X-ray crystallography.[17] Because of the limited solubility of some of our nucleosides in water, we measured the NMR spectra in DMSO-d_6 and compared the obtained values with the detailed data of 4'-thiothymidine (both β– and α–anomers) obtained also in DMSO-d_6 and published earlier.[5] ^{1}H NMR data of the new 2'-deoxy-4'-thiouridines VIa-d and VIIa,c,d (excluding the α-5-cyclopropyl-2'-deoxy-4'-thiouridine, which was not prepared) are in the TABLE 4. Again, as with the blocked nucleosides, the striking similarity of data of the compounds within one anomeric series and their overall similarity with the data of the corresponding anomer of the 4'-thiothymidine,[5] unequivocally point to the glycosidic anti-conformation in both anomeric series. and a $C_{2'}$-endo(S) thiosugar ring pucker,[5,17] seems to be characteristic for the whole β–anomeric series. The conclusive information about the prevailing thiosugar ring pucker in α-4'-thiothymidine is not available from the literature. This fact increases the importance of the X-ray crystallographic structure of the α-5-(1-adamantyl)-2'-deoxy-4'-thiouridine (VIId) presented in the FIGURE 1.



The structure brings about further confirmation of the anti-conformation and reveals the $C_{2'}$-endo(S) thiosugar ring pucker even in the case of the bulky 1-adamantyl derivative. It seems reasonable to suppose that the same conformational situation will exist throughout the whole α-anomeric series (VIIa,c,d), because the $J_{1',2'a}$ and $J_{1',2'b}$ coupling constants are very close within this series and are rather similar to those of the α–4'-thiothymidine (TABLE 5).

FIGURE 1
X-Ray structure of the nucleoside VIId

^{13}C NMR data of the new 2'-deoxy-4'-thiouridines are in TABLE 6, including the data of both β- and α-anomers of 4'-thiothymidine,[5] for comparison. The literature data of 5-substituted uracils,[18] also helped to assign unequivocally all the chemical shift values of individual carbon atoms. Once again, the chemical shift values of the same type of carbon atom are very close for all compounds within each anomeric series. The presence of the small, but a distinctive difference between the chemical shifts of both the methyl-groups in the β-5-isopropyl derivative VIa (21.45 and 21.37 ppm) is experimental proof of their diastereotopic character. In the corresponding α-anomer VIIa the same diastereotopic methyl-groups are not resolved (single chemical shift value of 21.67 ppm). The presence of two very distinctive signals at 5.79 and 5.17 ppm of two

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TABLE 5 ^{1}H NMR coupling constants of the synthesized 2′-deoxy-4′-thiouridines (anomeric and hydroxylic protons, DMSO-d_6, Hz)

Compd	$J_{1',2'a}$	$J_{1',2'b}$	$J_{3',OH}$	$J_{5'(a,b),OH}$
VIa	7.3		3.5	5.0
VIb	7.3		-	-
VIc	7.5		4.0	5.0
VId	7.5		4.0	5.0
VIIa	2.5	8.5	3.0	5.5
VIIc	2.0	9.0	3.0	5.5
VIId	2.0	9.0	3.0	5.5
*4′STβ	6.7	8.5	3.7	-
**4′STα	4.4	8.2	-	-

* 4′-Thiothymidine,[5]

** α-4′-Thiothymidine :
[1-(2-Deoxy-4-thio-α-D-*erythro*-pentofuranosyl)thymine],[5]

TABLE 6 ^{13}C NMR spectra of the synthesized 2′-deoxy-4′-thiouridines (DMSO-d_6; ppm)

Compound	C-2	C-4	C-5	C-6	C-1′	C-2′	C-3′	C-4′	C-5′	C-R$_5$
VIa.	150.44	162.79	119.96	135.37	60.56	41.99	74.02	58.97	63.16	25.77(CH); 21.45, 21.37(2CH$_3$)
VIb.	150.31	163.30	115.55	135.24	60.40	41.80	73.84	58.81	62.85	8.22(CH); 5.79,5.17(2CH$_2$)
VIc.	150.38	162.24	121.45	135.39	60.60	41.95	74.19	59.01	63.29	32.85(C); 28.54(CH$_3$)
VId.	150.02	161.93	121.64	135.60	60.36	41.78	73.99	58.86	63.17	36.24(6CH$_2$); 34.66(C); 27.80(3CH)
*4′STβ	150.60	163.37	109.82	136.70	59.93	40.98	73.40	59.01	63.51	12.14(CH$_3$)
VIIa.	150.67	163.09	119.31	137.68	61.33	42.39	74.61	60.59	64.02	25.76(CH); 21.67(2CH$_3$)
VIIc.	150.61	162.51	120.62	138.08	61.58	42.27	74.57	60.76	63.94	32.98(C); 28.73 (3CH+3)
VIId.	150.37	162.32	121.04	138.38	61.39	42.27	74.52	60.63	63.92	36.49(6CH$_2$); 34.92(C); 28.04(3CH)
**4′STα	150.62	163.47	108.94	138.28	59.77	42.03	74.05	59.53	63.57	12.26(CH$_3$)

* 4′-Thiothymidine,[5]

** 1-(2-Deoxy-4-thio-α-D-*erythro*-pentofuranosyl)thymine,[5]

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methylenes of the cyclopropyl group in the β-5-cyclopropyl nucleoside VIb is even stronger experimental evidence of their diastereotopic character. Similarly,the two distinctive signals at 5.74 and 5.58 ppm of the two methylene groups were found in 5-cyclopropyl-2'-deoxyuridine.[1] Theoretical calculations by the PCILO method for four different temperatures (223 K, 273 K, 298 K and 323 K) revealed,[19] that in this molecule, the *gauche*-conformation of the cyclopropyl group towards the pyrimidine ring plane (dihedral angel H-C_α-$C_{(5)}$-$C_{(6)}$ φ = 120°) represents the most populated energetical minimum in all the above mentioned temperatures. Another energetical minimum corresponding to the *trans*-conformation (φ = 0°) is sharp and slightly less populated.

The UV-spectra values of the synthesized 2'-deoxy-4'-thiouridines VIa-d and VIIa,c,d are presented with each compound in the EXPERIMENTAL. As expected, λ_{max} values of corresponding pairs of the anomers, as well as within each anomeric series, are very close and in the range of 268-270 nm. The only exception is the cyclopropyl-derivative VIb with λ_{max}=276 nm. The observed bathochromic shift of 7nm well corresponds to the similar effect of the cyclopropyl group observed in the series of the 5-substituted uracils earlier,[21] and further confirms the "conjugation ability" of the cyclopropyl group in this type of molecules.[20]

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ANTIVIRAL ACTIVITY

The nucleosides synthesized in this program (SCHEME 1) have substituents in position-5 of the pyrimidine ring varying from those with some similarity to the methyl group in thymidine, such as isopropyl (VIa, VIIa) or cyclopropyl (VIb) to those bulky lipophilic substituents, such as tert-butyl (VIc, VIIc) and 1-adamantyl (VId, VIId). Both 5-isopropyl- and 5-cyclopropyl-2'-deoxyuridine had previously been claimed to have antiviral activity,[6,7] but we could not confirm this.[1] 5-tert-Butyl- and 5-(1-adamantyl)-2'-deoxyuridines have very similar stereochemistry to thymidine[1] in as much as the sugar pucker and glycosyl torsion angles are concerned. Due to the steric bulk of the 5-substituent, it is not surprising that these analogues have no activity, as they are unlikely to be kinase substrates.

The following nucleosides from our series (SCHEME 1) were found to have significant antiviral activity {Figures given are IC_{50} values [μM] and no toxicity was seen at values up to 500 μM}: 5-isopropyl-2'-deoxy-4'-thiouridine(VIa): HSV-1, 2.2; VZV, 4.1. 5-cyclopropyl-2'-deoxy-4'-thiouridine (VIb): HSV-1, 1.6; VZV, 0.5. These results show that the relationship between the substituent at C-5 and antiviral activity is largely steric in nature, presumably caused by the inability of viral kinases to recognize analogues with bulky substituents. However, we also see that the compounds VIa and VIb show significant activity where we have previously shown, that the corresponding "normal" deoxyribonucleosides have no activity. This almost certainly has nothing to do with any difference in these molecules in their ability to be recognised by the viral kinase,[22] and it is unlikely that the lack of activity of the latter compounds is due to degradation by phosphorylases in this in vitro experiment. This observation is the subject of further intensive investigation.

EXPERIMENTAL

The ^{1}H and ^{13}C NMR spectra were recorded on the BRUCKER AC300 and AMX400 spectrometers and the chemical shift values are in ppm. Chemical ionisation (CI) mass spectra were recorded on the VG PROSPEC, fast atom bombartment (FAB) and accurate mass measurements were recorded on the VG ZABSPEC mass spectrometer. Ultraviolet spectra were recorded on a PERKIN-ELMER Lambda 2 UV/VIS spectrometer, in ethanol. Precoated Merck silica gel 60 F_{254} plates were used for TLC and the spots were detected under UV light (254 nm). Column chromatography was performed using Kieselgel 60, 230-400 mesh ASTM, type 9385. Glass columns were slurry-packed under gravity. Solvent systems used for TLC and column chromatography were S_1 = n-hexane/ethyl acetate and S_2 = chloroform/methanol in the ratios, which are specified in each experiment. Acetonitrile was dried by heating under reflux over calcium hydride, distilled and stored over type-4A molecular sieves. Dichloromethane was dried by heating under reflux over phosphorus pentoxide, distilled and stored over type-4A molecular sieves.Methanol was dried by heating under reflux over magnesium methoxide, distilled and stored over type-4A molecular sieves. Low temperature experiments (-80 °C) were done using HAAKE EK101 cryostat. When dry, oxygen-free reaction conditions were used, solutions of the reactants were added by syringe via Suba-seal stoppers in the reaction flask. Melting points are not corrected.

3',5'-Di-*O*-benzyl-2'-deoxy-4'-thiouridines IVa-d,Va-d (general method). To 3.5 mmol of 5-alkyl(cycloalkyl)uracil (**Ia-d**),[1] in a two necked 100 ml round bottomed flask with a magnetic stirrer and under the stream of dry nitrogen, 1.4 g (7 mmol) of bis-trimethylsilylacetamide (ALDRICH) in 20 ml of dry acetonitrile was added and the reaction mixture was stirred intensively at room temperature. When all the solid pyrimidine base had dissolved and the silylation was completed (different pyrimidine bases needed different time to complete the silylation, e.g. **Ia** - *ca.* 2 hrs, **Id** - overnight at the room temperature), 2 g of dry molecular sieves (type 4) were added, then 1.75 g (4 mmol) of thiosugar III in 20 ml of dry acetronitrile and finally 900 mg (4 mmol) of *N*-iodosuccinimide (ALDRICH) in 10 ml of dry acetonitrile. The reaction mixture immediately turned to dark red-brown and was further stirred at the room temperature, until the pyrimidine base disappeared (TLC, S_1). The reaction time necessary to complete the condensation was 3-6 hrs. Molecular sieves were filtered off and washed with ethyl acetate. The combined filtrates were evaporated to dryness (rotavap) and coevaporated with ethyl acetate (2-3 times). The resulting black, honey-like residue, comprising the anomeric mixture of the blocked nucleosides **(IVa-d) + (Va-d)** was purified and separated on a column using the solvent system S_1. The pure anomers **IVa-d** and **Va-d**, obtained as a colourless glassy residues upon the evaporation of the eluate from the column, were characterized (^{1}H NMR; MS) and used directly in the deprotection experiments.

2'-Deoxy-4'-thiouridines VIa-d, VIIa (general method). To 10 ml (10 mmol) of the 1.0 M BCl_3 (CH_2Cl_2; ALDRICH) in a three necked round bottomed flask with a thermometer and magnetic stirrer, cooled to -80 °C in a cryostat, was added a solution of 1 mmol of the blocked nucleoside IVa-d or Va in 25 ml of dry CH_2Cl_2 under a slight stream of dry nitrogen and intensive stirring. It was crucial to perform the addition dropwise, so that the temperature in the reaction mixture was kept below -75 °C all the time. The reaction mixture was further stirred (-80 °C) for 4 hrs (94 hrs in the case of

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Va), then quenched with 40 ml of the 1:1 mixture of dry CH_2Cl_2 and dry CH_3OH. The quenching solution was added dropwise, so that the temperature in the reaction mixture was all the time below -75 °C. Then the reaction mixture was allowed to warm up to room temperature, evaporated (rotavap, 40 °C) and the product was purified on a column using the solvent system S_2. The fractions, containing the product only, were combined and evaporated to a glassy residue which was sufficiently pure for characterization. Some compounds were further purified by crystallization, as specified in each case.

2'-Deoxy-4'-thiouridines VIIc,d (general method). To the stirred reaction mixture of 1mmol of the blocked nucleoside Vc or Vd in10 ml of toluene, was dropwise added at 0 °C (ice-bath) a solution of 580 mg (3 mmol) of $TiCl_4$ in 1.5 ml of toluene. The reaction mixture was stirred at room temperature for 3 hrs, then cooled to 0 °C (ice-bath). Methyl ethyl ketone (4 ml) was added followed by a solution of 580 mg (9 mmol) of citric acid in 4 ml of water and the reaction mixture was stirred for 20 min at 0 °C, before being worked-up as specified in each case.

5-Isopropyl-3',5'-di-*O*-benzyl-2'-deoxy-4'-thiouridine [IVa(β) and Va(α)]. Solvent system for chromatography S_1 = 1:1, $R_f(\beta)$ = 0.47, $R_f(\alpha)$ = 0.34; MS(CI): 467$[M+1]^+$, 155$[M+1\text{-sugar}]^+$.

5-Cyclopropyl-3',5'-di-*O*-benzyl-2'-deoxy-4'-thiouridine [IVb(β) and Vb(α)]. Solvent system for chromatography S_1 = 1:1, $R_f(\beta)$ = 0.33, $R_f(\alpha)$ = 0.26; MS(CI): 465$[M+1]^+$, 153$[M+1\text{-sugar}]^+$.

5-tert-Butyl-3',5'-di-*O*-benzyl-2'-deoxy-4'-thiouridine [IVc(β) and Vc(α)]. Solvent system for chromatography S_1 = 1.5:1, $R_f(\beta)$ = 0.62, $R_f(\alpha)$ = 0.5; MS(CI): 481$[M+1]^+$, 169$[M+1\text{-sugar}]^+$.

5-(1-Adamantyl)-3',5'-di-*O*-benzyl-2'-deoxy-4'-thiouridine [IVd(β) and Vd(α)]. Solvent system for chromatography S_1 = 1.5:1, $R_f(\beta)$ = 0.41, $R_f(\alpha)$ = 0.29; MS(CI): 560$[M+1]^+$, 247$[M+1\text{-sugar}]^+$.

5-Isopropyl-2'-deoxy-4'-thio-β-uridine [VIa]. Solvent system for chromatography S_2 = 6:1, R_f = 0.5; UV: λ_{max} 270 nm (ε 14900), λ_{min} 237 nm (ε 3900); MS(CI): 287$[M+1]^+$, 239$[M-47]^+$, 217$[M-69]^+$, 181$[M-105]^+$, 168$[M-118]^+$, 155$[M+1\text{-sugar}]^+$; EA ($C_{12}H_{18}N_2O_4S$) calculated: C = 50.35, H = 6.29, N = 9.79, found: C = 50.1, H = 6.4, N = 9.7; Accurate mass: for calc. mass $[M+1]^+$ = 287.1065, found: 287.1071; M.p. 164-6 °C (ether/methanol).

5-Isopropyl-[1-(2-deoxy-4-thio-α-D-*erythro*-pentofuranosyl)uracil] [VIIa]. Solvent system for chromatography S_2 = 6:1, R_f = 0.5; UV: λ_{max} 270 nm (ε 11530), λ_{min} 237 nm (ε 3740); MS(FAB): 287$[M+1]^+$, 155$[M+1\text{-sugar}]^+$; Accurate mass: for calc. mass $[M+1]^+$ = 287.1065, found: 287.1060; M.p. 168-70 °C (ether/methanol).

5-Cyclopropyl-2'-deoxy-4'-thio-β-uridine [VIb]. Solvent system for chromatography S_2 = 6:1, R_f = 0.3, UV: λ_{max} 276 nm (ε 11440), λ_{min} 242 nm (ε 3310); MS(CI): 285$[M+1]^+$, 269$[M-15]^+$, 251$[M-33]^+$, 170$[M-114]^+$, 153$[M+1\text{-sugar}]^+$; EA ($C_{12}H_{16}N_2O_4S$) calculated: C = 50.69, H = 5.67, N = 9.85, found: C = 49.9, H = 5.7, N = 9.3; M.p. 183-4 °C (ether).

5-tert-Butyl-2'-deoxy-4'-thio-β-uridine [VIc]. Solvent system for chromatography S_2 = 6:1, R_f = 0.4; UV: λ_{max} 268 nm (ε 11670), λ_{min} 236 nm (ε 3360); MS(FAB):

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301[M+1]$^+$, 251[M-49]$^+$, 195[M-105]$^+$, 169[M+1-sugar]$^+$; EA ($C_{13}H_{20}N_2O_4S$ x 0.4 H_2O) calculated: C = 50.78, H = 6.77, N = 9.11, found: C = 50.7, H = 6.8, N = 8.9; Accurate mass: for calc. mass [M+1]$^+$ = 301.1222, found: 301.1231; M.p. 140-2 °C (ether).

5-tert-Butyl-[1-(2-deoxy-4-thio-α-D-*erythro*-pentofuranosyl)uracil] [VIIc]. Solvent system for chromatography S_2 = 6:1, R_f = 0.5; UV: λ_{max} 268 nm (ε 11050), λ_{min} 236 nm (ε 3140); MS(FAB): 301[M+1]$^+$, 169[M+1-sugar]$^+$; Accurate mass: for calc. mass [M+1]$^+$ = 301.1222, found 301.1229.

5-(1-Adamantyl)-2'-deoxy-4'-thio-β-uridine [VId]. Solvent system for chromatography S_2 = 6:1, R_f = 0.4; UV: λ_{max} 269 nm (ε 10980), λ_{min} 237 (ε 2890); MS(CI): 378[M]$^+$, 363[M-15]$^+$, 331[M-47]$^+$, 264[M-114]$^+$, 247[M+1-sugar]$^+$; EA ($C_{19}H_{26}N_2O_4S$): calculated: C = 60.31, H = 6.88, N = 7.41, found: C = 60.5, H = 7.4, N = 6.6; Accurate mass: for calc. mass [M+1]$^+$ = 379.1691, found: 379.1698; M.p. 196-7 °C (ether).

5-(1-Adamantyl)-[1-(2-deoxy-4-thio-α-D-*erythro*-pentofuranosyl)uracil] [VIId]. Solvent system for chromatography S_2 = 9:1, R_f = 0.3; UV: λ_{max} 269 nm (ε 11450), λ_{min} = 237 (ε 3160); MS(FAB): 379[M+1]$^+$, 247[M+1-sugar]$^+$; Accurate mass: for calc. mass [M+1]$^+$ = 379.1691, found 379.1700; M.p. 129-131 °C (ether/methanol).

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