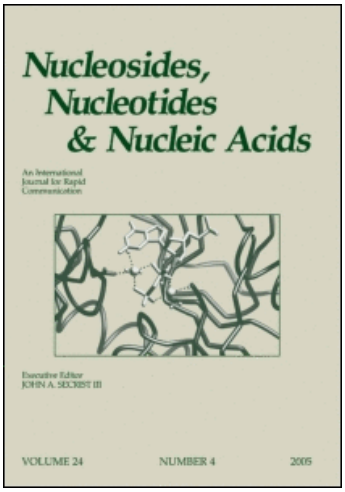

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Configurational Assignment in 2'-Deoxy-4'-Thionucleosides - A Review

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REVIEW

CONFIGURATIONAL ASSIGNMENT IN 2'-DEOXY-4'-THIONUCLEOSIDES — A REVIEW[§]

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ABSTRACT: Various NMR and physical data for about one hundred 2'-deoxy-4'-thio-nucleosides have been tabulated and examined as indicators of anomeric configuration. A minimal set of useful indicators is proposed.

Very many 2'-deoxynucleosides have been synthesised over the last thirty years, in many cases by direct glycosylation methods which generate a mixture of both anomers. The assignment of anomeric configuration to these nucleosides has relied mostly on characteristics of the NMR data and 'rules' have been established[1-5] on the basis of extensive data, supported by a large number of absolute structure determinations by X-ray diffraction analysis. There has been a huge upsurge in interest in nucleoside analogues as antitumour and antiviral agents in recent years, and that has stimulated interest in 2'-deoxy-4'-thio-nucleosides. These 4'-thionucleosides have been synthesised, almost entirely, by direct glycosylation methods and hence there is a need to address the problem of anomeric assignment in a class of compounds which is likely to have NMR characteristics different from those of conventional nucleosides (here designated as 4'-O-nucleosides).

Although the number of reported 2'-deoxy-4'-thionucleosides is small (about one hundred) we feel that there is a need to establish whether or not it is possible to find a set of criteria of wide applicability, which are based on normal compound characterisation data and which will allow for unequivocal assignment of the anomeric configuration of this class of nucleosides. It is particularly important that the criteria for configuration assignment of

[§]Dedicated to the memory of Dr. Yoshihisa Mizuno

2'-deoxy-4'-thionucleosides are reliable for routine application thus avoiding the need for further extensive work, such as is required for NOE difference spectroscopy and related NMR correlation techniques. We present here a survey of all available data for 2'-deoxy-4'-thionucleosides and a critical appraisal of the criteria for assignment of anomeric configuration.

We have chosen a set of readily available parameters which can be used for assignment of anomeric configuration and Table 1 presents a comprehensive collection of this data for all reported 2'-deoxy-4'-thionucleosides together with some prepublication data.[6] Notably ^{13}C NMR data are not included since it is our experience that these have little diagnostic value in this area. The individual parameters chosen for inclusion in Table 1 are rarely all available for any particular compound and one of the objectives of this survey will be to indicate which data should be regarded as essential. Before dealing with the data in Table 1 we shall discuss the 'absolute' methods of configuration assignment.

NMR correlation methods

The investigation of anomeric configuration using NOE difference spectroscopy (or less satisfactorily, using the NOESY method) can often provide an unequivocal result based on the presence of an inter-ring NOE contact between the base proton (H-6 in pyrimidines or H-8 in purines) and the protons on the *endo* face of the thiosugar ring in the β-anomer and protons on the *exo* face in the α-anomer. Thus assignments have been made on the basis of H-6 ↔ H-4' and H-6 ↔ H-3' contacts for several 2'-deoxy-4'-thionucleosides.[7,8] The existence of particular intra-ring contacts is also indicative of the anomeric configuration and contacts of the type H-1' ↔ H-3' in the α-anomer and H-1' ↔ H-4' in the β-anomer have been observed.[8,9] Similar results are found in the β-anomer of 2',3'-dideoxy-4'-thiocytidine.[10] Both intra- and inter-ring contacts can be observed with protons H-2'a and H-2'b but these cannot be used to determine configuration unless there is independent assignment of these two protons to the *exo* and *endo* positions. This is often established most convincingly by NOE contact, especially if the couplings do not fall in the normal ranges (see below).

In spite of the obvious usefulness of assignments based on NOE contacts this cannot be regarded as a routine method since the required experimental procedures are not trivial.[8] Furthermore interpretation of the results of NOE measurements is complicated by the existence of *syn* and *anti* conformations for the base,[8,11] by the conformational flexibility of the thiosugar ring (it remains to be established what, in general, are the most prevalent twist

TABLE 1 Data for 2'-deoxy-4'-thionucleosides useful for the assignment of anomeric configuration.

Substituents[a]			NMR data[b]									
5'-sub	3'-sub	base	δH-1'	*J*(H-1')	ΔH-2'	ΔH-5'	δH-3'	δH-4'	$[\alpha]_D$ (S)[c]	mp[d]	Conf[e]	ref
OH	OH	U	5.99[f]	7.3,7.7	0.19	0.09	4.05	3.03	−30.7 (A)	182	β	8
OH	OH	U							+61.5 (B)		α[g]	17
OH	OH	U	6.26	7.5,7.5	0.04	0.05	4.35	3.28		188	β	3
OH	OH	U	6.14	3.3,8.3	0.46	0.10	4.32	3.55		192	α	3
OH	OH	T	6.30	7.7,7.7	0.0		4.36		−32.7 (A)	210	β	8
OH	OH	T	6.30	6.7,8.5	0.05		4.38	3.28		215	β	3
OH	OH	T	6.16	4.4,8.2	0.44		4.26			207	α	3
OH	OH	T	6.29	t			4.38			209	β	18
OH	OH	T	6.16	dd			4.26			196	α	18
OH[h]	OH	T	6.37[i]	1.5,4.0	0.15	0.28	4.42	3.98		167	(α)[h]	15
OH[h]	OH	T	6.14[i]	4.0.5.0	0.32	0.18	4.18	3.70		209	(β)[h]	15
OH	OH	CF_3-U	6.15	6.6,6.6	0.0	0.09	4.30	3.33	+11.2 (D)	208	β	8
OH	OH	CF_3-U	6.15	t	0	0.1	4.30	3.3		220	β	6
OH	OH	CF_3-U	6.15	d	0.3		4.42			195	α	6
OH	OH	Et-U	6.29	t	0	0	4.37	3.3		207	β	6
OH	OH	FEt-U	6.28	t	0	0.1	4.38	3.3		195	β	6
OH	OH	F_3Et-U	6.25	t	0.1		4.35	3.3		159	β	6
OH	OH	ClEt-U	6.28	t	0		4.39				β	6
OH	OH	ClEt-U	6.15	dd	0.4		4.28				α	6
OH	OH	MeOEt-U	6.27	7.5,7.5	0	0	4.37	3.24			β	6
OH	OH	Pr-U	6.30	t	0	0	4.38	3.3			β	6
OH	OH	2Pr-U	6.28	7.5,7.5	0	0	4.35	3.3		166	β	6
OH	OH	CyPr-U	6.24	7.5,7.5	0	0	4.38	3.3		184	β	6
OH	OH	*t*-Bu-U	6.31	7.5,7.5	0	0	4.35	3.3		142	β	6
OH	OH	2Bu-U	6.28	t	0		4.35				β	6
OH	OH	2Bu-U	6.20	dd	0.5		4.35				α	6
OH	OH	Ad-U	6.30	7.5,7.5	0	0	4.35	3.3		147	β	6
OH	OH	Vi-U	6.26	7.5,7.5			4.37	3.3		246	β	6

(continued)

TABLE 1. (continued)

5'-sub	3'-sub	base	δH-1'	J(H-1')	ΔH-2'	ΔH-5'	δH-3'	δH-4'	$[\alpha]_{D\,(S)}$[c]	mp[d]	Conf[e]	ref
OH	OH	MeVi-U	6.29	t	0	0	4.35	3.3		170	β	6
OH	OH	ClVi-U	6.25	t	0	0	4.38	3.3		219	β	6
OH	OH	BrVi-U	6.25	t			4.36			191	β	18
OH	OH	BrVi-U	6.11	dd			4.27			187	α	18
OH	OH	ViMe-U	6.30	t	0	0	4.36	3.3			β	6
OH	OH	F_2Vi-U	6.30	t	0	0.0	4.34	3.3		169	β	6
OH	OH	Cl_2FVi-U	6.21	t	0	0	4.44	3.3			β	6
OH	OH	HC≡C-U	6.21	t	0	0	4.35	3.3		195	β	6
OH	OH	MeC≡C-U	6.22	t	0	0	4.32	3.3		265	β	6
OH	OH	Ac-U	6.20	t	0.1		4.29				β	6
OH	OH	CN-U	6.16	t	0	0	4.35	3.3		197	β	6
OH	OH	NO_2-U	6.10	t	0	0	4.30	3.3			β	6
OH	OH	NO_2-U	6.10	d	0.25		4.46				α	6
OH	OH	Cl-U	6.22	t	0.1	0	4.36	3.3		221	β	6
OH	OH	Br-U	6.20	t	0.1	0	4.35	3.3		224	β	6
OH	OH	Br-U	6.13	dd	0.4		4.40			195	α	6
OH	OH	I-U	6.19	7.5,7.5	0	0	4.32	3.3		226	β	6
OH	OH	C	6.34	6.4,8.4	0.09	0.1	4.33	3.26		132	β	3
OH	OH	C	6.20	4.1,8.1	0.46		4.26			S	α	3
OH	OH	C	6.36	6.6,8.4	0.08		4.34	3.27	−23.6 (A)	209	β	8
OH	OBn	T	6.21	dd			4.29	3.88		141	α	18
OTr	OH	T	6.44[i]	3.5,3.5[j]	0.45		4.48			L	β	19
OBn	OBn	T	6.30	t			4.30			142	β	18
OBn	OBn	T	6.23	d			4.25	4.08		L	α	18
OBn	OBn	BrVi-U	6.04	t			4.28			L	β	18
OBn	OBn	BrVi-U	6.19	d						L	α	18
OAc	OH	U	6.41[f]	7.0,7.0	0.40	0.06	4.40	3.61	−23.5 (B)	140	β	8
OAc	OH	U	6.31	3.1,8.1	0.35	0.07	4.39	3.80	+95.9 (B)	172	α	8
OAc	OH	T	6.33	6.4,9.1	0.11	0.11	4.37	3.45	−26.7 (B)	172	β	8
OAc	OH	CF_3-U	6.19	7.2,7.2	0.16			3.48	+10.2 (B)	173	β	8

TABLE 1. (continued)

5'-sub	3'-sub	base	δH-1'	J(H-1')	ΔH-2'	ΔH-5'	δH-3'	δH-4'	$[\alpha]_D$ (S)[c]	mp[d]	Conf[e]	ref
OAc	OTbs	U	6.45[i]	6.2,8.1	0.39	0.11	4.42	3.53	−30.1 (B)	146	β[f]	8
OAc	OTbs	U	6.34[i]	1.8,7.7	0.38	0.19	4.49	3.74	+40.9 (B)	51	α[f]	8
OAc	OTbs	T	6.49[i]	6.2,8.4	0.33	0.14	4.41	3.51	+2.1 (C)	153	β	8
OAc	OTbs	T	6.23[i]	2.4,7.9	0.39	0.16	4.47	3.75	+38.4 (C)	92	α	8
OAc	OTbs	CF_3-U	6.39[i]	6.9,6.9	0.38	0.15	4.40	3.55	+18.6 (C)	168	β	8
OAc	OTbs	CF_3-U	6.22[i]	0,6.5	0.23		4.51	3.8	+61.5 (C)	113	α	8
OAc	OAc	U	6.52[i]	6.6,8.2	0.38	0.14	5.38	3.71	−20.4 (C)	L	β	8
OAc	OAc	TP	6.58[i]	7.0,7.0	0.53	0.14	5.37	3.78	−30.1 (B)	146	β	8
OTl	OTl	BrVi-U	6.43	t			5.83	3.96		184	β	18
OTl	OTl	BrVi-U	6.28	d			5.68			172	α	18
OTl	OTl	U	6.61[i]	6.6,8.2	0.35	0	5.75	4.00		184	β	3
OTl	OTl	U	6.43[i]	1.2,7.0	0.33	0.08		4.24		120	α	3
OTl	OTl	T	6.66[i]	6.5,9.1	0.32	0	5.76	4.00		182	β	3
OTl	OTl	T	6.46[i]	2.3,7.7	0.35	0.08	5.71	4.25		148	α	3
OTl	OTl	C	6.69[i]	6.4,7.2	0.38	0	5.71	3.95		L	β	3
OTl	OTl	C	6.45[i]	2.1,7.1	0.24	0.07	5.76	4.18		L	α	3
OTl[h]	OTl	T	6.62[i]	t	0.57		5.95	4.25		L	(α)[h]	8
OTl[h]	OTl	T	6.37[i]	dd	0.17		6.62	4.23		L	(β)[h]	8
OH	N_3	U	6.15	6.8,7.1	0.07	0.0	4.46	3.41	−75.0 (A)	130	β	7
OH	N_3	U	6.12	6.3,6.9	0.46	0.08	4.25	3.70	+43.0 (A)	L	α	7
OH	N_3	T	6.18[i]	3.5,3.5[j]			4.51	3.38		121	β	19
OH	N_3	T	6.17	7.0,7.5	0.14	0	4.51	3.39	−62.4 (A)	123	β	7
OH	N_3	T	6.15	7.2,7.2	0.44	0.15	4.15	3.74	+100 (A)	L	α	7
OH	N_3	F-U	6.10	6.0,7.0	0.15	0.0	4.48	3.39	−17.5 (A)	S	β	7
OH	N_3	F-U	6.12	6.5,7.0	0.40	0.13	4.18	3.77	+19.2 (A)	L	α	7
OH	N_3	Cl-U	6.10	6.6,6.6	0.19	0.0	4.46	3.43	−43.0 (A)	167	β	7
OH	N_3	Cl-U	6.11	6.8,6.8	0.35	0.11	4.20	3.78	+7.2 (A)	L	α	7
OH	N_3	Br-U	6.07	6.2,6.2	0.19	0.0	4.46	3.40	−52.0 (A)	169	β	7
OH	N_3	Br-U	6.09	6.8,6.8	0.34	0.11	4.21	3.79	+7.2 (A)	L	α	7
OH	N_3	Me-C	6.18	6.8,8.5	0.09	0	4.48	3.36	+60 (A)	88	β	7

(continued)

TABLE 1. (continued)

5'-sub	3'-sub	base	δH-1'	J(H-1')	ΔH-2'	ΔH-5'	δH-3'	δH-4'	$[\alpha]_{D\,(S)}$[c]	mp[d]	Conf[e]	ref
OH	N_3	Me-C	6.14	7.2,7.2	0.48	0.12	4.16	3.67	+6.0 (A)	L	α	7
OTr	N_3	T	6.37[i]	3.5,3.5[j]	0.43		4.29	3.26		L	β	19
OH	CH_2OH	T	6.05	5.5,5.8		0.12	2.38	3.35	−34.9 (E)		β	9
OH	CH_2OH	T	6.18	6.5,9.5	0.39	0.37	2.13	3.67	+81.6 (E)		α	9
OH	CH_2OH	C	6.08	4.3,6.0		0.15		3.44	−53.6 (E)		β	9
OH	CH_2OH	C	6.25	6.5,9.6	0.48	0.35	2.10	3.62	+99.6 (E)		α	9
OH	CH_2OH	A	6.10	3.2,5.8		0.19			−24.2 (A)		β	9
OH	CH_2OH	A	6.19	6.8,8.8					+69.9 (A)		α	9
OH	OH	2-ClA	6.17	6.0,6.0	0.25	0.18	4.50	3.30			β	20
OBz	OBz	6-ClP	6.47[i]	6.7,7.4	0.23	0.10	5.91	4.08			β	21
OBz	OBz	6-ClP	6.49[i]	2.5,6.4	0.06	0.04	5.89	4.41			α	21

[a] Tbs = *t*-butyldimethylsilyl, Tr = triphenylmethyl, Tl = p-toluoyl. The abbreviations U, T, C and A are used for the bases uracil, thymine, cytosine and adenine respectively. The uracil 5-substituent is indicated, with FEt = 2-fluoroethyl, ClEt = 2-chloroethyl, F_3Et = 2,2,2-trifluoroethyl, MeOEt = 2-methoxyethyl, 2Pr = 2-propyl, CyPr = cyclopropyl, 2Bu = 2-butyl, Ad = adamantyl, ViMe = 1-methylvinyl, MeVi = 2-methylvinyl, BrVi = 2-bromovinyl, F_2Vi = 2,2-difluorovinyl, Cl_2FVi = 2,2-dichloro-1-fluorovinyl. TP is the base 4-(1,2,4-triazol-1-yl)-pyrimidin-2(1H)-one and Me-C is 4-*N*-methoxycytosine. 6-ClP is 6-chloropurine. [b] Missing entries were either unreported or indeterminable. ΔH is the chemical shift separation for methylene protons, given as 0 for cases where a very tightly coupled pair was not analysed fully, *J*(H-1') is the pair of couplings (Hz) to H-1' or the reported multiplicity. NMR solvent is $(CD_3)_2SO$ unless otherwise indicated. [c] Rotation solvents: A = MeOH, B = EtOH, C = $CHCl_3$, D = Me_2CO, E = H_2O. [d] No value for amorphous solids (S) and gums and syrups (L). [e] Anomeric configuration as assigned by original workers [f] NMR solvent CD_3OD - $CDCl_3$, 1:3. [g] These configurations were reversed in an earlier report[17]. [h] This pair of anomers have the L-configuration at C-4 and hence the meaning of the anomeric labels is reversed. [i] NMR solvent $CDCl_3$ [j] These couplings should probably be doubled in value.

or envelope forms for a 4-thiofuranose ring) and can be frustrated by coincident chemical shifts and relayed or negative NOE contributions.

Crystal structures

Although there are many hundreds of crystal structures reported for conventional 2'-deoxy-nucleosides only three have been published for 2'-deoxy-4'-thionucleosides. The archetypal compound with a β-configuration, 4'-thiothymidine,[11,12] has an *anti* conformation for the base ($\chi = -144.2°$) which places H-6 approximately above the centre of the ring, a South conformation ($^2T_3$ form) for the thiosugar ring ($P = 178°$) with a relatively high

degree of ring pucker (ν_{max} = 47.9°) and an *ap* conformation for the 5'-hydroxyl group (γ = 179°). The crystal structure of 5-(2-bromovinyl)-2'-deoxy-4'-thiouridine[11] indicates that the conformational parameters for this compound are virtually identical to those for 4'-thiothymidine. Only one structure for a compound with an α-configuration has been reported.[13] 1-(5-*O*-Acetyl-2-deoxy-4-thio-α-D-*erythro*-pentofuranosyl)uracil also has an *anti* conformation for the base and a South conformation for the thiosugar ring. The crystal structure of 4'-thio-5-fluorouridine has also been determined,[14] revealing that this ribonucleoside has the base in an *anti* conformation (χ = −111° †), the thiosugar ring has a South conformation (C-2' endo) and the 5'-OH group is in an *ap* conformation.

Although there is clearly very limited data, the crystal structures of these 2'-deoxy-4'-thiopyrimidine nucleosides show that, when compared with analogous 4'-O-nucleosides, there is conformational similarity (preference for a South conformation) and conformational dissimilarity (preference for an C-4'—C-5' *ap* conformation). When the greater length of a C—S bond compared to a C—O bond is also taken into account it is likely that the NMR characteristics of the two classes of nucleoside may be different.

NMR parameters δH-1' and *J*(H-1')

The chemical shift δH-1' and the splitting pattern *J*(H-1') of the anomeric proton H-1' were probably the first NMR diagnostics used for assignment of anomeric configuration in 4'-O-nucleosides.[1] Generally this multiplet was observed as a triplet (or pseudo triplet) in β-anomers and doublet or double doublet in α-anomers, with H-1' to lower frequency (upfield) in the β-anomer relative to the α-anomer. Using modern instrumentation the quality of the spectra is such that both anomers often show a resolved double doublet splitting for H-1' but with a clear difference in the magnitude of the coupling $J_{1,2exo}$ which is small (ca 2 Hz) in the α-anomer and large (*ca* 7 Hz) in the β-anomer, in keeping with the change in relative orientation of the coupled protons.

The data in Table 1 include thirty anomeric pairs of pyrimidine nucleosides where a comparison of δH-1' values is possible and in twentyone cases H-1'β appears at higher frequency. The main exceptions are the 3'-azido and 3'-hydroxymethyl compounds where the difference between the two H-1' chemical shifts is small and often reversed. In the subset

†Recalculated from the given χCN value.[14]

consisting of uracil compounds without hydroxy protecting groups not all anomer pairs are reported but δH-1'β falls in the range 6.20 - 6.36 (twentynine values) and δH-1'α in the range 6.10 - 6.20 (eleven values). The only deviating compounds are the 5-CN-, 5-NO_2- and 5-CF_3-uracil derivatives. The presence of O-protecting groups at either or both 3' and 5' sites increases the range of both shifts. Clearly the value of δH-1' cannot be an ideal indicator of configuration especially if only one anomer is available.

Examination of the data in Table 1 for coupling to the anomeric proton in 2'-deoxy-4'-thionucleosides shows that, where the 3'-substituent is hydroxy, alkoxy or acyloxy, the splitting pattern or measured couplings for H-1' in fortynine β-anomers and twentyone α-anomers accords with the established trends noted above for 4'-O-nucleosides. There are no exceptions. In the α-anomers $J_{1,2exo}$ is in the range 0 - 4.1 Hz and in the range 6.0 - 7.5 Hz in the β-anomer. This trend has been noted by other workers[8] and described as a greater peak width for H-1' in the β-anomers. However, although the 5'-substituent does not seem to have any effect, this rule for *J*(H-1') breaks down when the 3'-substituent is azido or hydroxymethyl. These substituents must induce significant conformational change in the α-anomers such that the couplings become similar in the two anomers or even show a reversed trend.

Thus the H-1' splitting pattern is not a reliable configurational indicator in every case but can be taken as such for at least those 4'-thionucleosides with a 3'-OH or 3'-OR substituent.

NMR parameter ΔH-2'

This parameter is defined as δH-2'b – δH-2'a and has been widely cited[2,3,5] as an indicator of configuration in 4'-O-nucleosides. The general observation when both α and β forms have been characterised, is that ΔH-2' is small for β-anomers (0 - 0.2) and large for α-anomers (0.3 - 0.5). In many cases the H-2' multiplet is too tightly coupled to be fully analysed and the value of ΔH-2' is not evaluated accurately. On the basis of about thirtyeight β-anomers and fourteen α-anomers in Table 1, the value of ΔH-2' for 2'-deoxy-4'-thionucleosides falls in the ranges noted above. The rule holds for any group in the 3'-position (OH, N_3, OBn, CH_2OH), but does not hold for 2'-deoxy-4'-thionucleosides which are 5'-*O*-substituted. For this group of compounds there is no consistent difference in the range of values for the two anomers and there are cases where the β-anomer has the larger value.[8,9]

This is consistent with a further deshielding of the *endo*-H-2' proton which increases the separation of the H-2' protons in the β-anomer. It is notable that ΔH-2' for the anomers of the L-*threo*furanose analogue[15] of 4'-thiothymidine also fall into the appropriate ranges for ΔH-2' (noting that these stereochemical labels have their meaning reversed relative to H-3' which is still (R). See footnote *h* in Table 1).

The evidence of Table 1 shows that ΔH-2' is not a universal indicator of configuration but within certain structural constraints may be very reliable.

NMR parameter ΔH-5'

This parameter is defined in the same way as ΔH-2'. In our work on the 3'-azido nucleosides[7] we found that the value of ΔH-5' had a parallel behaviour to that of ΔH-2'. For the β-anomer this parameter is about zero and for the α-anomer in the range 0.08 - 0.15. Other data confirm that the relative magnitudes are always in this order for this parameter but the ranges overlap significantly (0.1 - 0.37 for the α-anomers and 0 - 0.2 for the β-anomers). The general trend in the value of ΔH-5' is consistent with one H-5' proton being shielded by lying above the sugar ring (*ap* conformation). This results in a larger separation in H-5' protons in the α-anomers and a smaller separation in the β-anomers since this effect is offset by the deshielding effect of the base in the latter case. At best this parameter should be regarded as a suppporting diagnostic and only then if both anomers are available.

NMR parameters δH-3' and δH-4'

Several workers[5,4] have noted for 4'-O-nucleosides that the sugar ring protons which are *syn* to the base, are shifted to higher frequency (due to the deshielding effect of the heteroaromatic ring) and hence H-3' in the β-anomer and H-4' in the α-anomer should show characteristic deshielding shifts relative to the corresponding shifts in the other respective anomers. Although these two chemical shifts are not always recorded due to spectral complexity, the data in Table 1 show that for 2'-deoxy-4'-thionucleosides the shift of H-4' does fit this 'rule' without exception (eighteen examples), showing a shift in the range 0.18 - 0.4. However for proton H-3' only twentyone out of twentyeight examples show the expected shift. The exceptional behaviour can be traced to the presence of a bulky or electronegative group at the 5-position of the uracil base or a bulky group (*t*-butyldimethylsilyl) on the 3'-O atom.

It is clear that, in general, the deshielding effect of the heterocyclic base will shift the ring protons on the same side of the sugar ring to higher frequency in 2'-deoxy-4'-thionucleosides and this effect probably also accounts for the relative changes observed in the H-2' proton chemical shifts.

Optical Rotation

Generally the β-anomers of all 2'-deoxy-4'-thionucleosides with the 5'-hydroxy group unprotected have a negative rotation irrespective of the nature of the base or the 3'-substituent and the corresponding α-anomers have positive rotations. Values of $[\alpha]_D$ between $-20°$ and $-75°$ are found for β-anomers and between $+7°$ and $+100°$ for α-anomers. Thus the β-anomers are generally of the order of 100° more negative. Coincidentally this trend is in agreement with Hudson's rules for conventional glycosides. The same sign difference is found for the $[\alpha]_D$ values of 4'-thioribonucleosides[14,16] but for the anomers of 2',3'-dideoxy-4'-thiocytidine[10] the $[\alpha]_D^{20}$ values are $-43.8°$ (β-anomer) and $-14.3°$ (α-anomer).

The only exception to this rule for nucleosides of regular heterocyclic bases is a derivative of thymine with the 5'-hydroxy group acetylated and a large *t*-butyldimethysilyl protecting group on the 3'-hydroxy group, where the β-anomer has a rotation of $+2°$ compared to $+38°$ for the α-anomer. Derivatives of 5-trifluoromethyluracil also have small positive values for the β-anomers, in the range $+10°$ to $+19°$ but the corresponding α-anomer is still 40° more positive in the one case where a comparison is possible. The only complete contravention of the trend for the rotation of the β-anomer to be much more negative is found in the anomeric pair of 3'-azidonucleosides containing the base, 4-*N*-methoxycytosine.[7] These values, or the assignment, must be suspect.

Melting Point

This parameter is not always accessible since many nucleosides have a tendency to form amorphous solids or gums. However for ten out of twelve cases where a direct comparison is possible the β-anomer has the higher melting point. This may be a useful supportive diagnostic when other criteria are less then unequivocal.

Conclusion

This survey of routine anomeric configuration indicators for 2'-deoxy-4'-thio-nucleosides shows that there is *not a single criterion which gives an unequivocal assignment*

across all the structural variations so far examined (nearly one hundred nucleosides). However we suggest that, for new compounds of this type particularly those with different 3'-substituents, if a set of criteria are examined and each criterion is considered in the light of the limitations detailed above then the anomeric configuration should be determinable with confidence, *even if only one anomer is available.*

The minimum set of parameters which should be examined consists of the following:

The chemical shift and the coupling constants (or splitting pattern) of proton H-1'

The chemical shift separation of protons H-2'

The chemical shift of proton H-3'

The optical rotation.

These criteria can usefully be supported by the other NMR and physical data as indicated by our survey.

It should be emphasised that these criteria are based on data for 2'-deoxy-4'-thio-pyrimidine nucleosides. There is very little data available for 2'-deoxy-4'-thiopurine nucleosides. We have include in Table 1, as the final entries, data from Secrist[20] for a 2-chloro-2'-deoxy-4'-thioadenosine and our data[21] for an anomeric pair of 6-chloro-2'-deoxy-4'-thiopurine nucleosides. It not possible to draw any definitive conclusions on the basis of such meagre data but some similarities with the pyrimidine thionucleosides can be identified.

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References

1. Robins, M. J.; Robins, R. K.; *J. Org. Chem.*, **1969**, *34*, 2160-2163.
2. David, S.; Fischer, J. S.; *Carbohydr. Res.*, **1971**, *18*, 39.
3. Srivastava, P.C.; Robins, R.K.; Takusagawa, F.; Berman, H.M.; *J. Heterocycl. Chem.*, **1981**, *18*, 1659-1662.
4. Okabe, M.;. Sun, R.-C.; Tam, S. Y.-K.; Todaro, L. J.; Coffen, D. L.; *J. Org. Chem.*, **1988**, *53*, 4780-4786.
5. Svansson, L.; Kvarnström, I.; Classon, B.; Samuelsson, B.; *J. Org. Chem.*, **1991**, *56*, 2993-2997.
6. Rahim, S. G.; Walker, R. T.; unpublished work.

7. Tber, B.; Fahmi, N.; Ronco, G.; Villa, P.; Ewing, D. F.; Mackenzie, G.; *Carbohydr.Res.*, **1995**, *267*, 203-215.
8. Secrist III, J. A.; Tiwari, K. N.; Riordan, J. M.; Montgomery, J. A.; *J. Med. Chem.*, **1991**, *34*, 2361-2366.
9. Brånalt, J.; Kvarnström, I.; Niklasson, G.; Svensson, S. G. T.; Classon, B.; Samuelsson, B.; *J. Org. Chem.*, **1994**, *59*, 1783-1788.
10. Rassu, G.; Spanu, P.; Pinna, L.; Zanardi, F.; Casiraghi, G.; *Tetrahedron Lett.*, **1995**, *36*, 1941-1944.
11. Koole, L. H.; Plavec, J.; Liu, H.; Vincent, B. R.; Dyson, M. R.; Coe, P. L.; Walker, R. T.; Hardy, G. W.; Rahim, S. G.; Chattopadhyaya, J.; *J. Am. Chem. Soc.*,**1992**, *114*, 9936-9943.
12. Uenishi, J.; Takahashi, K.; Motoyama, M.; Akashi, H.; *Chem. Lett.*, **1993**, 255-256.
13. Uenishi, J.; Takahashi, K.; Motoyama, M.; Akashi, H.; Sasaki, T.; *Nucleosides Nucleotides*, **1994**, *13*, 1347-1361.
14. Bobek, M.; Bloch, A.; Parthasarathy, R.; Whistler, R.L.; *J.Med. Chem.*, **1975**, *18*, 784-787.
15. Tiwari, K. N.; Montgomery, J. A.; Secrist III, J. A.; *Nucleosides Nucleotides*, **1993**, *12*, 841-846.
16. Bellon, L.; Barascut, J.-L; Imbach, J.-L.; *Nucleosides Nucleotides*, **1992**, *11*, 1467-1479
17. Uenishi, J.; Motoyama, M.; Nishiyama, Y.; Wakabayashi, S.; *J. Chem. Soc., Chem. Commun.*, **1991**, 1421-1422.
18. Dyson, M. R.; Coe, P. L.; Walker, R. T.; *J. Med. Chem.*, **1991**, *34*, 2782-2786.
19. Secrist III, J. A.; Riggs, R. M.; Tiwari, K. N.; Montgomery, J. A.; *J. Med. Chem.*, **1992**, *35*, 533-538.
20. Tiwari, K. N.; Secrist III, J. A.; Montgomery, J.A.; *Nucleosides Nucleotides*, **1994**, *13*, 1819-1828.
21. Ewing, D. F.; Mackenzie, G.; Ronco, G.; Villa, P; unpublished observations.