6-Mercapto and Other Substituted Derivatives of 9-D-Arabino- and 9-D-Xylopyranosylpurine.[1,2] Synthesis and Spectral Properties

ABELARDO P. MARTINEZ AND WILLIAM W. LEE

Life Sciences Research, Stanford Research Institute, Menlo Park, California 94025

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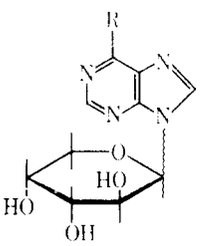
The 6-mercaptopurine nucleosides of α- and β-D-arabinopyranose and β-D-xylopyranose (**3a**, **3b**, and **10b**, respectively) and 9-(β-D-xylopyranosyl)thioguanine (**18**), together with some intermediates and derivatives, have been synthesized and their properties have been studied. The nmr evidence support the *1C* conformation for all nucleosides of α-arabinopyranose and the *C1* conformation for those of β-xylopyranose. A pronounced shielding effect upon the nmr signals of the acetyl protons is noted in those purine pentopyranosides where both the C-2′ acetoxy group and the C-1′ purine ring are equatorial. This effect is absent from the corresponding anomers where the purine ring is axial. Some of these pentopyranosyl purines disobeyed Hudson's isorotation rules.

Interest in the nucleosides of 6-mercaptopurine (6-MP) and thioguanine (TG) stems from their antitumor activity and other biological activity. Among these nucleosides are the ribosides of 6-MP[3] and TG,[4] the arabinofuranoside of 6-MP,[5] and the α- and β-deoxyribofuranosides of TG.[6] We have recently prepared other nucleosides[7] of these bases and now report the synthesis and properties of the β-D-xylopyranosides of 6-MP and TG (**10b** and **18**, respectively) and the α- and β-D-arabinopyranosides of 6-MP (**3a** and **3b**). The spectral properties of these compounds and their derivatives are of considerable interest. The present results extend our recent observations on the conformations and optical rotations of the anomeric pairs of D-arabinopyranosyl- and D-xylopyranosyladenines.[2a]

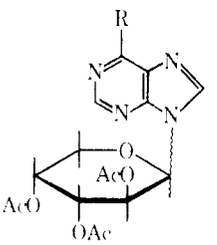
The 6-MP nucleosides were readily prepared in good yields by the usual reactions.[7] Nitrous acid converted 9-(α-D-arabinopyranosyl)adenine[2a] (**1a**) into the corresponding hypoxanthine nucleoside **2a**. Acetylation (to **6a**), thiation with phosphorus pentasulfide (to **7a**), and deacetylation afforded the desired **3a**. The β anomer **3b** and the β-D-xyloside **10b** were similarly prepared from **1b**[2a] and 9-(β-D-xylopyranosyl)adenine,[2a,8] respectively.

The acetylated arabinosides **4a** and **4b** were needed for an examination of their physical properties. Acetylation of **1b** in pyridine with acetic anhydride at room temperature afforded a mixture of tri- and tetraacetates, **4b** and **5b**, according to the nmr spectrum. However, the triacetate **4b** could be isolated and purified by crystallization. The same acetylation conditions afforded an even more highly acetylated mixture from **1a**. Heating this mixture in refluxing 80% acetic acid afforded the homogeneous tetraacetate **5a**. Pure **4a** was obtained by acetylation of **1a** in a limited amount of acetic anhydride.



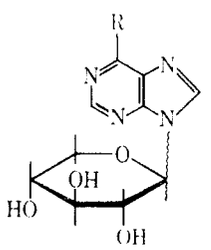
1, R = NH_2
2, R = OH[9]
3, R = SH[9]

a series = α anomer
b series = β anomer



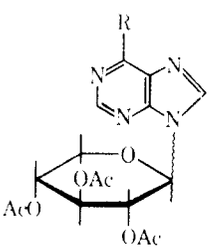
4, = NH_2
5, R = NHAc
6, R = OH
7, R = SH

a series = α anomer
b series = β anomer

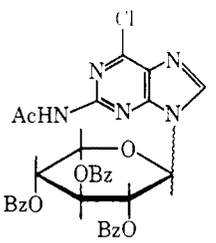


8, R = NH_2
9, R = OH[9]
10, R = SH[9]

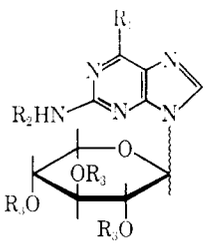
a series = α anomer
b series = β anomer



11, R = OH (α)
12, R = OH (β)
13, R = SH (β)



14



15, R_1 = OH;[9] R_2 = R_3 = H
16, R_1 = OH; R_2 = H; R_3 = Ac
17, R_1 = OH; R_2 = R_3 = Ac
18, R_1 = SH;[9] R_2 = R_3 = H
19, R_1 = SH; R_2 = H; R_3 = Ac

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(2) (a) This is paper II on pentopyranosyl nucleosides. For paper I, see A. P. Martinez, W. W. Lee, and L. Goodman, *J. Org. Chem.*, **34**, 92 (1969). (b) A portion of this material was presented at the 155th National Meeting of the American Chemical Society, San Francisco, Calif., April 1968.

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(4) F. R. White, *ibid.*, **11**, 202 (1961).

(5) (a) See A. P. Kimball, G. A. LePage, and B. Bowman, *Can. J. Biochem.*, **42**, 1753 (1964), for antitumor activity; (b) see A. P. Kimball, G. A. LePage, B. Bowman, and S. J. Herriot, *Proc. Soc. Exptl. Biol. Med.*, **119**, 248 (1965), for suppression of homograft response.

(6) See G. A. LePage and I. G. Junga, *Mol. Pharmacol.*, **3**, 37 (1967), for utilization by murine tumor cells.

(7) (a) G. L. Tong, K. J. Ryan, W. W. Lee, E. M. Acton, and L. Goodman, *J. Org. Chem.*, **32**, 859 (1967); (b) G. L. Tong, W. W. Lee, and L. Goodman, *ibid.*, **32**, 1984 (1967).

(8) G. W. Kenner, B. Lythgoe, and A. R. Todd, *J. Chem. Soc.*, 652 (1944).

(9) The purine rings are all written in the completely aromatic form for convenience, although the 6-hydroxy and 6-thiol derivatives exist as the *keto* tautomers.

The reaction of bromo-2,3,4-tri-*O*-benzoyl-D-xylose[10] with the mercury derivative[11] of 2-acetamido-6-chloropurine afforded the nucleoside **14**, which could be isolated and characterized although the reaction was not complete under any of the conditions studied. Reaction of crude **14** with sodium hydroxyethylmercaptide[7] afforded the guanine nucleoside **15**. Because of its tendency to gel, **15** was best isolated as its crystalline sodium salt. Subsequent acidification gave **15** as a more tractable solid.

Compound **15** was very insoluble in any acetylation medium. Consequently, after 80 hr in acetic anhydride and pyridine, a trace of insoluble **15** remained along with some triacetate **16** and tetracetate **17**. However, the crude mixture was thiated satisfactorily with phosphorus pentasulfide in pyridine. Deacylation in methanolic sodium methoxide afforded the desired thioguanine nucleoside **18**. Attempts to prepare **18** by the reaction of sodium hydrogen sulfide with either crude or crystalline chloropurine nucleoside **14** afforded mixtures of products from which crystalline **18** could not be isolated. The properties of these compounds are given in Tables I–VI.

We previously reported[2a] that the anomers of D-arabinopyranosyladenine, **1a** and **1b**, disobeyed Hudson's isorotation rules[12] while the corresponding D-xylopyranosides, **8a** and **8b**, obeyed the rules. Compounds **1a** and **1b** were the first exceptions[2a] to the generalization that purine nucleosides[13a] obey Hudson's rules while pyrimidine nucleosides[13b] disobeyed them. Since the generalizations were based on furanosyl nucleosides, it is of interest to obtain more information on pentopyranosyl nucleosides. Table I records the results obtained in this study. It is apparent that the arabinopyranosides of hypoxanthine (**2a** and **2b**) and 6-MP (**3a** and **3b**) also disobey Hudson's rules in the solvents used. When these nucleosides are acetylated, to afford **4**, **6**, and **7**, a variety of optical rotatory behavior resulted. Thus, depending on the solvent, these anomeric pairs may or may not obey Hudson's rules. This was true also of the one available pair of xylopyranosides, **11** and **12**.

It is well known[14] that the solvent influences both the magnitude *and* sign of the optical rotations of the solute in various ways. These include (1) the degree of solvation with the solute[14] and (2) the change in population of various dissymmetric conformations.[14] The second includes changes in conformation of the sugar ring, in rotamer distribution of the sugar substituents, and in freedom of rotation of the base about the glycosyl bond. Whether one or more of these factors are responsible for the variety of behavior recorded in Table I is not obvious. However, the nmr data in Table II suggest little or no conformational change in the sugar moiety of the arabino- and the xylopyranosyl nucleosides upon acetylation.

TABLE I

OPTICAL ROTATIONS ([α]D, DEGREE)[a] OF SOME PENTOPYRANOSYL NUCLEOSIDES

	Solvent			
	H_2O[b]		DMF[c]	
Compd	α	β	α	β
1[d]	−35	+35	−48	+50
2	−45	+32	−64	+71
3	*e*	*e*	−35	0
4	0	*e*	−18	−5
6	+10[f]	−5[f]	−28	+8
7	+39[f,g]	−37[f,g]	+27[f]	−16[f]
11, 12	−11[f]	−29[f]	−36	−33
8[d]	−7[f]	−30[f]	−30[f]	−46[f]
9b		−23		
10b				−31
15				+9
18				−28
13				−83
16				−11
17				−25
19				−58

[a] Optical rotations were measured at ambient temperature (21–24°) with a Perkin-Elmer Model 141 automatic polarimeter. [b] Concentrations were 0.15–0.18%. [c] Concentrations in *N*,*N*-dimethylformamide were 0.5% except **2** and **16** at 0.25% and **6**, **11**, and **12** at 0.35%. [d] From ref 2a. [e] Not determined because of low solubility. [f] Obeys Hudson's isorotation rules. [g] Run in methanol at 0.25% concentration.

The signals of the H-1′ protons of all of the α-arabinosides (**2a**, **3a**, **4a**, **5a**, **6a**, and **7a**) and all of the β-xylosides (**9b**, **10b**, **12**, **13**, **15**, **16**, **17**, **18**, and **19**), whether acetylated or not, have the large coupling constants that show that H-1′ and H-2′ are *trans* diaxial.[15a] This is only possible for the α-arabinosides in the *1C* conformation and the β-xylosides in the *C1* conformation.

The signals of the H-1′ protons of all of the β-arabinosides (**2b**, **3b**, **4b**, **6b**, and **7b**) and the one α-xyloside (**11**), whether acetylated or not, have small values for $J_{1',2'}$ and are downfield from the H-1′ protons of their corresponding anomers. On conformational grounds it had been previously stated that the α-xyloside **8a**, should, like the β anomer **8b** be in the *C1* conformation.[2a] Since the H-1′ equatorial proton of **8a** was downfield from the axial H-1′ proton of **8b**, it appeared to obey the generalization that equatorial protons signals are downfield from those of axial protons in the same chemical and steric environment.[2a,15] If this generalization is valid for other nucleoside pairs, then the H-1′ protons of the α-xyloside **11** and the β-arabinosides are equatorial, so that the conformation of **11** is *C1* and that of the β-arabinosides (**2b**, **3b**, **4b**, **6b**, and **7b**) is *1C*. Since there are exceptions to the above generalization,[15] other evidence for the assignment of conformation was examined.

A complete assignment of the nmr signals to the pyranose protons would definitely establish the conformations of the nucleosides. This was not possible

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(15) (a) L. D. Hall, *Advan. Carbohyd. Chem.*, **19**, 51 (1964). (b) R. U. Lemieux and J. D. Stevens, *Can. J. Chem.*, **43**, 2059 (1965). (c) The latter authors point out that axial acetoxy signals are generally at lower field than equatorial ones, but there are exceptions. Assigning conformations on the basis of acetoxy signals alone is not always unequivocal. In the present work the acetoxy signal results and the H-1′ signal results agree and mutually support the conformational assignments.

TABLE II

NMR DATA FOR PENTOPYRANOSYL NUCLEOSIDES[a]

Compd	H-1′,[b] δ	$J_{1',2'}$, cps	Acetyl H,[b] δ: Shielded	Equational	Axial	Purine H[b] δ		Δδ	Solvent
1a[c]	5.48	9				8.29	8.43	0.16	A + C
1b[c]	6.13	1.5				8.28	8.41	0.13	A + C
2a	5.30	9				8.04	8.19	0.15	A
2b	6.03	<1				8.11	8.19	0.08	A
3a	5.25	9				8.18	8.36	0.18	A
3b	5.97	<1				8.20	8.29	0.09	A
2a	5.51	9				8.18	8.33	0.15	C
2b	6.13	1				8.19	8.35	0.16	C
8a[c]	6.11	2				8.27	8.47	0.20	A + C
8b[c]	5.43	9				8.23	8.34	0.11	A + C
9b	5.26	9				7.98	8.16	0.18	A
10b	5.36	9				8.10	8.33	0.23	A
15	5.10	9				7.82			A + C
18	5.01	9				7.92			A
4a	5.90	8	1.61 (3)	1.88 (3)	2.09 (3)	8.10	8.16	0.06	A
4b	6.28	2		2.03 (6)	2.18 (3)	8.19 (2)		0	A
5a	5.87	8	1.61 (3)	1.87 (6)[e]	2.10 (3)	8.11	8.18	0.07	A
6a	5.83	8	1.68 (3)	1.87 (3)	2.09 (3)	8.00	8.11	0.11	A
6b	6.15	2.5		1.92, 1.95 (6)	2.11 (3)	8.02 (2)		0	A
7a	5.86	8	1.69 (3)	1.88 (3)	2.10 (3)	8.14	8.33	0.19	A
7b	6.16	2		1.93, 1.96 (6)	2.10 (3)	8.15	8.22	0.06	A
4a	5.91	9	1.81 (3)	2.07 (3)	2.26 (3)	8.12	8.40	0.28	B
4b	6.34	2		2.05 (6)	2.33 (3)	8.05	8.38	0.35	B
5a	5.85	~8	1.78 (3)	2.04 (6)[e]	2.23 (3)	8.15	8.35	0.20	B
6b	6.28	<1		2.06 (6)	2.23 (3)	8.08	8.38	0.30	B
11	6.09	2		1.96, 2.05, 2.10 (9)		8.02 (2)		0	A
12	*d*	*d*	1.74 (3)	2.00, 2.02 (6)		8.08	8.32	0.24	A
13	*d*	*d*	1.74 (3)	1.99, 2.01 (6)		8.21	8.51	0.30	A
16	*d*	*d*	1.72 (3)	2.04, 2.07 (6)		7.93		...	A + C
17	*d*	*d*	1.79 (3)	2.01, 2.04 (6)	2.23 (3)[e]	8.19		...	A + C
19	*d*	*d*	1.70 (3)	1.90, 1.93 (6)		8.00		...	A

[a] Nmr data were obtained on Varian A-60 with TMS external reference for DMSO-d_6 (A) and A + C (C is D_2O); TMS was internal reference for $DCCl_3$ (B). [b] All H-1′ signals are doublets and purine H signals are singlets. All acetyl H's are singlets. Where necessary the number of protons are shown in parenthesis. All signal intensities indicated the proper number of protons. [c] From ref 2a. [d] The H-1′ signals are hidden in a group of protons between δ 5.5 and 6.0. Only one of these, **12**, has been run at higher resolution (see Tables III and IV) and shows the expected large $J_{1',2'}$. [e] Protons of N-Ac here.

TABLE III

CHEMICAL SHIFTS OF PYRANOSE PROTONS IN SOME NUCLEOSIDES[a]

Compd	H-1′	H-2′	H-3′	H-4′	H-5′
	δ				
7a	5.96 d[b]	5.69 t	5.44 q	5.28 m	4.01 d
					4.22 d
7b	6.22 d	5.10 q	5.46 q	5.2 m	4.06 m
12	5.97 d	5.72 t	5.49 t	5.11 s	4.10 q, 3.81 t

[a] Chemical shift values were obtained on Varian HA-100 with TMS internal reference. [b] Symbols are d, doublet; t, triplet; q, quartet; m, multiplet; s, sextet.

TABLE IV

PYRANOSE PROTON COUPLING CONSTANTS OF SOME NUCLEOSIDES[a]

Compd	1′,2′	2′,3′	3′,4′	4′,5′a	4′,5′e	5′a,5′e
	J, cps					
7a	9.0[b]	9.5	3.5	<1	1.5	13.0
7b	2.0	5.0	3.0	*c*	*c*	*c*
12	8.5	9.0	9.5	10.5	5.5	11.0

[a] Coupling constants were obtained on Varian HA-100 with TMS internal reference. [b] Values are given to the nearest 0.5 cps. [c] Not determined.

for the unblocked nucleosides because all of the pyranose proton signals fell too close together at 100 Mc as well as 60 Mc. However, the protons of the acetylated nucleosides showed improved resolution in the 100-Mc spectra. Three acetylated nucleosides were examined and the results are given in Tables III and IV. Spin decoupling experiments were used to confirm the proton assignments. The 60-Mc spectra of these three are like those of the other corresponding nucleoside anomers where only the base is changed. Hence the 100-Mc spectra of these three are representative of the other nucleosides also.

The β-xyloside **12** showed H-1′ as a doublet, H-2′ and H-3′ as triplets, H-4′ as a sextet, H-5′e as two pairs of doublets at δ 4.10, and H 5′a as a triplet (because $J_{4',5'a}$ and $J_{5'a,5'e}$ are nearly equal) at δ 3.81. All of the signals are well separated and clearly resolved. The large coupling constants for all the neighboring protons (except H-5′e) clearly established their *trans*-diaxial relationships and the conformation of **12** as *C1*.

The α-arabinoside **7a** showed H-1′ as a doublet, H-2′ as a triplet, and H-3′ as two pairs of doublets. The large coupling constants between these protons showed that H-1′, H-2′, and H-3′ have a *trans*-diaxial relationship to each other as required by the *1C* conformation. The signals of H-4′ appeared as a narrow multiplet; those of H-5′e and H-5′a appear as two pairs of doublets; the doublets of one of these pairs were barely resolved. The *trans*-diaxial relationships of H-1′, H-2′, and H-3′ establish the conformation of **7a** as *1C*.

The β-arabinoside **7b** showed H-1′, a doublet, at lowest field and H-3′, two pairs of doublets, at next lowest field. The signals of H-2′ are shifted considerably upfield (compared with those of **12** and **7a**) and overlapped those of H-4′. Spin decoupling was used to establish H-2′ as a pair of doublets with $J_{2',3'}$ of 5 cps. This value was smaller than those observed for the *trans*-diaxial protons in **7a** and **12**. The signals for H-4′ and those for the H-5′ protons were not sufficiently resolved for interpretation. The H-5′ protons signals of **7b** were definitely not like those of **12**, but were more similar to those of **7a**. These H-5′ signals of **7b** should be (1) like those of **12** (in which H-4′ and H-5′a were *trans* diaxial) if **7b** were in the *C1* conformation or (2) like those of **7a** if **7b** were in the *1C* conformation. The data for H-2′ and H-5′ suggest that **7b** may not be in the ideal *1C* chair form or that it may exist in conformational equilibrium with the *1C* form being favored.[16] This same conformational situation may hold for the other β-D-arabinopyranosyl nucleosides, whether acetylated or not.

The location of the acetoxy signals of the arabinosides (see Table II) also support the *1C* conformations which require one axial acetoxy group and two equatorial ones, with the signals of the former being downfield[15a,b] from the latter two. This is the case with **4b**, **6b**, and **7b**. For the α anomers, **4a**, **5a**, **6a**, and **7a**, the axial group signal is located at the same place as for the β anomers (δ 2.09–2.18). However, one of the two equatorial group signals is shifted upfield (δ 1.61–1.69). This shift suggests that for these α anomers the purine moiety is spatially located so that ring current anisotropy[17] can effectively shield the C-2′ acetoxy protons. Examination of Dreiding models show that statistically there is greater opportunity for the C-2′ acetoxy protons to be above the plane of the equatorial purine ring in the α anomers than when the purine ring is axial as in the β anomers.

If shielding can occur when both C-1′ purine and C-2′ acetoxy are equatorial, then the same effect should be seen for the β anomers of the D-xylopyranosyl nucleosides, but not the α anomers. This is indeed the case for the β anomers **12**, **13**, **16**, **17**, and **19** where the shielded equatorial acetoxy signals fall between δ 1.70 and 1.79 and are well separated from the other two equatorial acetoxy signals (1.90–2.07). See Table II. The single acetylated α anomer in the xylose series, **11**, appears to retain the *C1* conformation. Its H-1′ proton is at δ 6.09 like the equatorial H-1′ proton of **8a** (6.11). None of the signals of its acetoxy groups is shifted by ring-current shielding to δ 1.70–1.79. However, the three equatorial acetoxy group signals are spread apart more than those in any other example in Table II, suggesting some differences in magnetic environment for these three groups.

A few nmr spectra were run in deuteriochloroform ($DCCl_3$). The results for **4a**, **4b**, **5a**, and **6b** show the same relationship between acetoxy groups that are axial, equatorial, and equatorially shielded as in deuterated dimethyl sulfoxide (DMSO-d_6). All of the proton signals are shifted downfield relative to those in DMSO-d_6.[18,19] Of interest is the fact that the difference in chemical shifts between the two purine protons H-2 and H-8, Δδ, is considerably greater in $DCCl_3$ than in DMSO-d_6.[20] In the latter solvent, the value of Δδ becomes 0 for three acetylated nucleosides (**4b**, **6b**, and **11**) where the purine is axial but not for the corresponding unacetylated nucleosides (**1b** and **2b**) nor for the acetylated anomers (**4a**, **6a**, and **12**) where the purine is equatorial.

Two nmr spectra were obtained in D_2O. These also show that the H-1′ and H-2′ of the α-arabinopyranoside **2a** are *trans* diaxial and that H-1′ is upfield from H-1′ of the β-arabinopyranoside **2b** which is equatorial. Thus, changing the solvent from D_2O to DMSO-d_6 to $DCCl_3$ does not result in any sugar conformation change.

In the previous communication,[2a] the shielding effect of the benzene ring of a C-2′ benzyloxy group upon one of the purine protons was noted when both groups were equatorial. Reported here is the ring-current shielding effect of the purine upon a C-2′ group when both are equatorial. An earlier example of this is 9-(3-acetamido-tri-*O*-acetyl-3-deoxy-β-D-glucopyranosyl)hypoxanthine.[19] The upfield shift of its equatorial C-2′ acetoxy protons (to δ 1.76) relative to the other acetoxy signals is ascribed to the difference in shielding caused by an adjacent bulky substituent at C-1′ [19] (which is equatorial). No shielding effect of the phenyl ring upon the C-2′ acetoxy protons is observed for benzyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyranoside[21] and 2,3,4,6-tetra-*O*-acetyl-1-*S*-benzoyl-1-thio-β-D-glucopyranose[21] where the C-1 and C-2 groups are both equatorial. This may be due to the extra two atoms of S and C moving the phenyl ring to a less favorable position away from the C-2 acetoxy group. For the acetylated pyrimidine nucleo-

TABLE V

ULTRAVIOLET SPECTRA OF SOME PYRANOSYLPURINES

Compd	Maxima, λ, mμ (ε × 10^{-3}) pH 1[a]	pH 7[a]	pH 13[a]
2a	247 (11.0)	247 (11.3)	253 (11.9)
2b	248 (13.0)	248 (12.7)	253 (13.7)
3a	322 (24.2)	317 (23.4)	310 (22.7)
3b	321 (23.2)	317 (23.7)	309 (23.2)
4a	255 (15.1)	258 (15.1)	258 (15.1)
6a	247 (12.5)	247 (12.7)	252 (14.0)
7a	322 (24.8)	317 (21.9)	312 (22.8)
7b	322 (24.6)	318 (21.8)	310 (22.8)
9	247 (11.8)	247 (12.3)	253 (12.8)
10	322 (25.0)	318 (23.9)	310 (26.1)
12	243 sh[b]	243 sh	253 (14.7)
	247 (14.2)	247 (14.2)	
13	322 (25.0)	318 (21.5)	311 (22.8)
15	256 (11.9)	252 (13.1)	262 (10.8)
16	255 (13.3)	252 (14.7)	252–265 (12.2)
	275 sh		
17	262 (8.7)	265 sh	251 (14.8)
	343 (22.9)	342 (24.2)	272 (7.1)

[a] Solvents were 0.1 *N* HCl for pH 1, Beckman 3581 buffer for pH 7, and 0.1 *N* NaOH for pH 13. [b] sh, shoulder.

(16) As a comparison, β-L-arabinopyranose tetraacetate is reported to exist in conformational equilibrium containing about 80% of the favored conformation (*C1* for the L series or *1C* for the D series). See ref 15b.

(17) A. D. Broom, M. P. Schweizer, and P. O. P. Ts'o [*J. Amer. Chem. Soc.*, **89**, 3612 (1967)] have observed that ring-current diamagnetic anisotropy of one purine ring can effectively shield the protons of another purine ring in nucleosides that stack together in solution.

(18) See Table I in ref 17a.

(19) F. W. Lichtenthaler and H. P. Albrecht, *Chem. Ber.*, **99**, 575 (1966).

(20) One referee has suggested that this is due to the hydrogen bonding between the DMSO solvent and the "acidic" H-8 proton, first detected by L. Katz and S. Penman, *J. Mol. Biol.*, **15**, 220 (1966).

(21) C. V. Holland, D. Horton, and M. J. Miller, *J. Org. Chem.*, **32**, 3077 (1967).

TABLE VI

PURINE NUCLEOSIDES OF D-ARABINOPYRANOSE AND D-XYLOPYRANOSE

Compd	Yield,[a] %	Mp, °C	Solvent[b]	Chromatography[c] Solvent	R_f	Formula	Calcd, % C	H	N	S	Found, % C	H	N	S
2a	95[d]	219–220	M	ME-20	0.07	$C_{10}H_{12}N_4O_5 \cdot CH_3OH$	44.0	5.37	18.7		43.9	5.21	18.9	
2b	63	260–261	W	ME-30	0.30	$C_{10}H_{12}N_4O_5$	44.8	4.51	20.9		44.7	4.60	21.1	
3a	98	260–262	M			$C_{10}H_{12}N_4O_4S$	42.2	4.22		11.25	42.3	4.29		11.22
3b	73	212.5–215.5	M			$C_{10}H_{12}N_4O_4S \cdot {}^1/_8CH_3OH$	42.1	4.56		10.88	42.1	4.73		10.90
4a	59	228–229	D[e]	ME-0	0.18	$C_{16}H_{19}N_5O_7$	48.9	4.87	17.8		48.5	4.87	17.6	
5a		105–110	D–H	ME-4	0.29	$C_{18}H_{21}N_5O_8 \cdot {}^1/_4HOAc$	49.3	4.92	15.6		49.0	5.17	15.4	
6a	65	268–269	F[e]	ME-5	0.19	$C_{16}H_{18}N_4O_8$	48.7	4.60	14.2		48.8	4.82	14.2	
6b	(76)	216–217	D–F	ME-30	0.67	$C_{16}H_{18}N_4O_8 \cdot {}^1/_4H_2O$	48.1	4.67	14.0		48.1	4.53	14.2	
7a	(100)	282–283	M	ME-5	0.67	$C_{16}H_{18}N_4O_7S$	46.8	4.43	13.7	7.80	46.7	4.63	13.8	7.92
7b	(59)	243–245[f]	M	E	0.69	$C_{16}H_{18}N_4O_7S$	46.8	4.43	13.7		46.5	4.66	13.7	
9b	(71)	221–222[g]	W	ME-20	0.10	$C_{10}H_{12}N_4O_5 \cdot H_2O$	42.0	4.93	19.6		42.2	5.03	19.2	
10b	(76)	218–226	W			$C_{10}H_{12}N_4O_4S \cdot {}^1/_2H_2O$	40.9	4.46	19.1	10.9	40.9	4.15	19.1	10.5
11	89	262–263	F			$C_{16}H_{18}N_4O_8 \cdot H_2O$	46.6	4.89	13.6		46.7	4.71	13.7	
12	69	283–284	F	ME-20	0.53	$C_{16}H_{18}N_4O_8$	48.7	4.60	14.2		48.9	4.72	14.2	
13	100	190–200	C–M			$C_{16}H_{18}N_4O_7S$	46.8	4.42		7.80	46.7	4.63		7.86
14	(7)	184.5–185.5	B–D	EC-20	0.29	$C_{33}H_{26}ClN_5O_8$	60.5	4.00	10.7	5.40[h]	60.4	3.96	10.7	5.35[h]
15	28	287–288	D[e]	ME-30	0.21	$C_{10}H_{12}N_5O_5 \cdot H_2O$	39.9	5.02	23.3		40.3	5.27	23.6	
16	(65)	291–292	A	H	1.37	$C_{16}H_{19}N_5O_8$	46.9	4.67	17.1		46.7	4.63	16.7	
17	13	294.5–295.0	C–T	EC[i]	0.20	$C_{18}H_{21}N_5O_9 \cdot 0.9CCl_4$	38.5	3.59	11.87	21.7	38.6	3.72	11.95	21.7
18	(73)	235–237[j]	M	ME-30	0.54	$C_{10}H_{13}N_5O_4S \cdot CH_3OH$	39.8	5.17	21.1	9.67	39.8	5.15	20.8	9.56
19	(67)	305–306	E[e]	H	1.32	$C_{16}H_{19}N_5O_7S$	45.1	4.50		7.52	44.8	4.58		7.67
15 Na salt	24	...	M[e]			$C_{10}H_{12}N_5O_5Na \cdot CH_3OH$	39.2	4.79	20.8		39.6	4.81	20.4	

[a] Yields are for analytical sample except values in parenthesis which are for homogeneous product suitable for use in the next reaction. [b] Solvents for crystallization are A, acetone; B, benzene; D, diethyl ether; C, chloroform; E, ethanol; F, ethyl acetate; H, *n*-hexane; M, methanol; T, carbon tetrachloride; W, water. [c] Solvents for tlc on silica gel HF plates are denoted by ME-20, etc., where M = methanol, E = ethyl acetate, and 20 = per cent of first solvent; likewise, C = chloroform. The spots are located relative to the front with R_f = 1.00. For paper chromatography, the solvent systems are E, 5% aqueous Na_2HPO_4, pH 8.9; H, *n*-butanol–acetic acid–water (5:2:3). Spots are referred to adenine with R_{Ad} = 1.00. [d] From **4a**. [e] Triturated with this solvent. All attempts to recrystallize **19** resulted in darkening and decomposition. [f] Tan color. [g] G. W. Kenner, B. Lythgoe, and A. R. Todd [*J. Chem. Soc.*, 652 (1944)] reported mp 220° from methanol. [h] Chlorine analysis. [i] Developed twice. [j] Decomposition temperature.

sides, some shielding effects have been noted. Cushley, *et al.*,[22] observed an anisotropic effect of the pyrimidine 5,6 double bond on the acetoxy groups which resulted in shielding or deshielding of these proton signals, depending on their spatial relationship to the 5,6 double bond.

Acetylation may affect the sugar–purine conformations even though the pyranose ring conformations are unchanged. The pronounced shielding effect upon the C-2′ acetoxy group that is found in one but not the other anomer suggest differences in restriction of rotation about the glycosidic bond between these anomers. These differences may change when no acetyl groups are on the hydroxyl groups, and hydrogen bonding is possible. In addition, the spatial relationship of base to sugar is changed as the base changes from an equatorial to an axial position. All of these differences may contribute to the factors that determine if these nucleosides obey Hudson's isorotation rules.

Table V lists the ultraviolet (uv) spectra of some pyranosylpurines.

Experimental Section[23]

Hypoxanthine Nucleosides.—By the usual procedure[7b] the reaction of 2.0 g (7.5 mmol) of **1b** with 3.8 g (55 mmol) of sodium nitrite in 100 ml of water and 12.5 ml of acetic acid at room temperature for 3 days, during which a second portion of 0.5 g of sodium nitrite was added after 24 hr, afforded the hypoxanthine nucleoside **2b**. The completeness of reaction was monitored by tlc with solvent ME-30. With some of the other adenine nucleosides, a reaction time of 20 hr was sufficient.

All of the acetylations (*e.g.*, **2b** → **6b**) were performed at room temperature, generally overnight, using acetic anhydride and pyridine.[7b] Completeness of reaction was monitored by tlc, with ME-30 or other solvent systems.

6-Mercaptopurine Nucleosides.—The hypoxanthine nucleoside, *e.g.*, **6a**, was refluxed with about 6 mol ratios of phosphorus pentasulfide in pyridine under nitrogen[7b] for about 4 hr and worked up to afford **7a**, or the appropriate mercaptopurine nucleosides. In the case of **7a** the initial crude product (100%) yield), mp 232–234°, was reddish rather than white, was homogeneous by tlc, and analyzed correctly for sulfur. One recrystallization with a charcoal treatment removed the color.

The deacetylation of **7a** and the other acetylated derivatives was carried out in hot methanolic sodium methoxide.[7b]

2-Acetamido-6-chloro-9-(2,3,4-tri-*O*-benzoyl-β-D-xylopyranosyl)-9*H*-purine (14).—A mixture containing 5.20 g (8.18 mmol) of 2-acetamido-6-chloropurine mercuric oxide complex[11] on 30% Celite and 200 ml of xylene was heated at reflux temperature with 4.2 g (8.6 mmol) of 2,3,4-tri-*O*-benzoyl-α-D-xylopyranosyl bromide[10] for 3.5 hr by the usual procedure[7a] to afford 5.00 g (93%) of a solid foam which contained mainly **14** (R_f 0.20 in EC-20) and about three other components (R_f 0, 0.7, and 1.0 in EC-20). This crude product was suitable for the next step.

For the analytical sample, a 6.64-g portion of crude product of the above purity was chromatographed on a column 20 cm long containing 40 g of alumina (neutral, Bio Rad AG 7, 100–200 mesh), eluting successively with benzene (400 ml) and benzene–ethyl acetate (6:4), 800 ml. Evaporation of the last 500 ml of benzene–ethyl acetate afforded a residue which was crystallized from toluene–ether (2:25) to afford 0.58 g (7.2%) of **14**, mp 178.5–180.0°. Another crystallization from benzene–ether (1:10) afforded the analytical sample of **14**.

9-(β-D-Xylopyranosyl)guanine (15).—Crude **14** (from 65.4 mmol of 2-acetamido-6-chloropurine–mercuric oxide complex), 20 ml (0.28 mol) of 2-mercaptoethanol, 175 ml of methanol, and 250 ml of 1 *N* methanolic sodium methoxide was heated at reflux under a nitrogen atmosphere for 4 hr. The hot reaction mixture was treated with 2.0 g (0.37 mol) of sodium methoxide and refrigerated for 18 hr at 0°. The crystalline sodium salt of **15** was collected, washed with 100 ml of cold methanol, then 200 ml of anhydrous ether, and dried to afford 5.31 g (24%). See Table VI for analysis. Treatment of an aqueous solution of the sodium salt with acetic acid afforded **15**, still gellike, but

(22) R. J. Cushley, K. A. Watanabe, and J. J. Fox, *J. Amer. Chem. Soc.*, **89**, 394 (1967).

(23) Melting points were determined on a Fisher-Johns apparatus and are uncorrected. Paper chromatograms were run by the descending technique on Whatman No. 1 paper. Tlc was run on silica gel HF (E. Merck AG Darmstadt) in the appropriate solvent. All spots on chromatograms were detected by uv light. Solutions were dried with magnesium sulfate, anhydrous. All solutions were concentrated *in vacuo* with a bath temperature of less than 50° unless noted otherwise. *N*,*N*-Dimethylformamide is designated as DMF. Skellysolve B is a petroleum fraction, essentially *n*-hexane, bp 62–70°. Celite is a diatomaceous earth product of Johns-Manville.

easily filterable. This crystallized when stirred in acetone; see Table VI.

9-(β-D-Xylopyranosyl)thioguanine (18).—A mixture of 2.00 g (6.64 mmol) of **15**, 70 ml of pyridine, and 13 ml of acetic anhydride was stirred at room temperature for 88 hr, then worked up to afford a tan foam (M), R_f 0.06 (major spot) and 0.20 (**16** and **17**, respectively) in solvent EC-100, when developed thrice. This foam was suitable for thiation. For analysis, the tan foam was stirred for several hours in ether, collected, and dried to afford 1.78 g (65%) of the triacetate **16**. This was recrystallized for analysis. The pure tetraacetate **17** was obtained from M which was partially freed of **16** by fractional precipitation from methylene chloride. The methylene chloride soluble material was then recrystallized from chloroform–carbon tetrachloride (1:1) to give **17**.

A 2.40-g portion (*ca.* 5.86 mmol) of the mixture of **16** and **17**, of purity equivalent to M, was heated with 10.0 g (45 mol) of phosphorus pentasulfide in 200 ml of pyridine[7] at reflux for 4.5 hr under a nitrogen atmosphere and worked up to afford 1.65 g (67%) of white, crystalline **19**. For analysis, see Table VI.

A solution of 1.20 g of **19**, 2.0 ml of 2-mercaptoethanol, and 0.20 g of sodium methoxide in 250 ml of methanol was heated at reflux for 3 hr under a nitrogen atmosphere. The solution was cooled to about 40°, treated with 20 g of IRC-50 (H^+) resin (prewashed with methanol), and stirred until pH 5–6 was attained (about 20 min). The mixture was filtered, treated with charcoal, filtered again, and evaporated. The residue was triturated with methanol–acetone (1:19) and the solid was collected to afford 0.68 g (73%) of **18**, homgeneous in solvent ME-30 with R_f 0.50. This was recrystallized once for analysis; see Table VI.

Registry No.—**2a**, 18520-77-9; **2b**, 18520-78-0; **3a**, 18520-79-1; **3b**, 18520-80-4; **4a**, 18520-81-5; **4b**, 18520-82-6; **5a**, 18520-83-7; **6a**, 18520-85-9; **6b**, 18520-84-8; **7a**, 18520-86-0; **7b**, 18520-87-1; **9b**, 18520-88-2; **10b**, 18520-89-3; **11**, 18520-90-6; **12**, 18520-91-7; **13**, 18520-92-8; **14**, 18520-93-9; **15**, 18520-94-0; **15** Na salt, 18520-95-1; **16**, 18520-96-2; **17**, 18598-35-1; **18**, 18598-36-2; **19**, 18530-32-0.

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Oligonucleotide Syntheses on Insoluble Polymer Supports. II. Pentathymidine Tetraphosphate

L. RUSSELL MELBY AND DONALD R. STROBACH

Contribution No. 1427 from the Central Research Department, Experimental Station, E. I. du Pont de Nemours and Company, Wilmington, Delaware 19898

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The linear oligonucleotide pentathymidine tetraphosphate (TpTpTpTpT) has been synthesized stepwise on an insoluble polymer support containing thymidine bound *via* 5′ ether linkage to a styrene–divinylbenzene copolymer containing methoxytrityl functional groups. Condensation of the bound thymidine with 3′-O-acetylthymidine 5′-phosphate activated by 2,4,6-triisopropylbenzenesulfonyl chloride or picryl chloride followed by 3′-O-deacetylation gave polymer-supported thymidylyl-(3′→5′)-thymidine in 70–80% conversions based on thymidine, corresponding to about 350–380 μmol of dinucleoside phosphate per gram of polymer. Repetition of the condensation and deacetylation steps gave the higher oligomers each in approximately 35–80% conversion based on the next lowest member. The over-all conversion into pentamer was about 10% based on initial polymer-bound thymidine.

The procedural advantages which accrue from stepwise synthesis of complex oligomeric substances on inert polymer supports have been discussed by Merrifield, particularly with respect to polypeptide synthesis;[1] more recently the application of this concept to oligonucleotides has been investigated in several other laboratories.[2–6] We have continued our work on oligonucleotides using an insoluble polymer bearing methoxytrityl chloride functional groups to which the nucleoside is attached by 5′-ether formation. The oligonucleotide chain is then extended by condensation of appropriately protected 5′-nucleotides with the free 3′-hydroxyl group of the polymer-bound nucleoside. Our system thus incorporates the characteristics of insolubility found in the polymers of Letsinger, *et al.*,[2] and the functionality of the soluble polymers described by Hayatsu and Khorana[3] and Cramer, *et al.*[4] The Letsinger system utilizes polymer-bound carbonyl chloride functional groups as nucleoside attachment sites through amide[2a,b] or ester[2c,d] formation. In other recent work Blackburn and coworkers have explored a system in which the polymer-bound oligonucleotide terminus is a nucleotide attached to an insoluble polymer by a phosphoramidate linkage.[6]

This paper will describe the synthesis of thymidine homooligonucleotides up to the pentanucleoside tetraphosphate stage. Several features different from our previous procedure[5] have led to increased per cent conversions. The effect of altering reaction variables will be discussed for each step of the synthesis outlined in Scheme I.[7]

(1) R. B. Merrifield, *Science*, **150**, 178 (1965).

(2) (a) R. L. Letsinger and V. Mahadevan, *J. Amer. Chem. Soc.*, **87**, 3526 (1965); (b) *ibid.*, **88**, 5319 (1966); (c) R. L. Letsinger, M. H. Caruthers, and D. M. Jerina, *Biochemistry*, **6**, 1379 (1967); (d) R. L. Letsinger, M. H. Carothers, P. S. Miller, and K. K. Ogilvie, *J. Amer. Chem. Soc.*, **89**, 7146 (1967).

(3) (a) H. Hayatsu and H. G. Khorana, *ibid.*, **88**, 3182 (1966); (b) *ibid.*, **89**, 3880 (1967).

(4) F. Cramer, R. Helbig, H. Hettler, K. H. Scheit, and H. Seliger, *Angew. Chem. Intern. Ed. Engl.*, **5**, 601 (1966).

(5) L. R. Melby and D. R. Strobach, *J. Amer. Chem. Soc.*, **89**, 450 (1967).

(6) G. M. Blackburn, M. J. Brown, and M. R. Harris, *J. Chem. Soc., C*, 2438 (1967).

(7) The symbol —Ⓟc denotes the cross-linked polystyrene backbone and (CH_3O)TrT the pendant methoxytrityl group with thymidine (T) attached *via* 5′-ether linkage; TpT and TpTOAc, respectively, refer to thymidylyl-(3′→5′)-thymidine and its 3′-O-acetate. The higher oligomers are abbreviated in the conventional way.