Sodium Salt Glycosylation in the Synthesis of Purine 2′-Deoxyribonucleosides: Studies of Isomer Distribution

Catherine Hildebrand† and George E. Wright*

Department of Pharmacology, University of Massachusetts Medical School, Worcester, Massachusetts 01655

Received October 31, 1991

A systematic study of 2-deoxyribonucleoside isomer distribution from the sodium salt glycosylation of substituted purines is reported. Reactions of 1-α-chloro-2-deoxy-3,5-di(p-toluyl)-*erythro*-pentofuranose with the sodium salts of purines in acetonitrile typically results in 9-β and 7-β regioisomers as major products in a ratio of about 4:1, results consistent with a S_N2 reaction of base anion with the 1-α chlorosugar. However, the reaction with 2,6-dibromopurine (**2**) gave 9-β and 9-α stereoisomers as major products in a 4:1 ratio. We have isolated and identified all nucleoside products from sodium salt glycosylations of several 2,6-disubstituted purines and 6-substituted purines. In addition to the major products, the 9-α and 7-α isomers were obtained in small yields in most cases. Rate studies showed that fastest glycosylations occurred with 2,6-bis(methylthio)purine (**3**). Glycosylations of 2,6-dichloropurine (**1**) and of **2** proceeded with nearly identical rates for the formation of the 9-β isomers and with comparable rates for the formation of 7-β and 9-α isomers, respectively. These observations indicate that the extent of sugar anomerization during glycosylation of **2** does not alone account for 9-α isomer formation, although, in a separate experiment, aging of chlorosugar solutions did increase the yield of 9-α product in the reaction. Studies of possible interconversion of isomers under the reaction conditions indicated that formation of the 9-α isomer from **2** was not the result of conversion of a kinetically favored (7-β) isomer, nor was the 7-β isomer from **1** derived from conversion of the 9-α isomer. We conclude that a combination of steric effect of the 6-bromo group and an as yet unidentified rate effect of the 2-bromo group is responsible for the significant yield of 9-α product from **2**. The ability of substituents to enhance the rate and regioselectivity in the sodium salt glycosylation was evaluated with 2-bromo-6-(methylthio)purine (**6**). This base afforded the highest total nucleoside yield (86%) and the highest 9-β isomer yield (68.3%) among all purines tested, suggesting a useful strategy to increase yield of intermediates that can be converted to biologically important purine 2′-deoxyribonucleosides.

The synthesis of biologically active (9-β) purine 2′-deoxyribonucleosides commonly involves direct glycosylation of the heterocycle with an activated 2-deoxyribose derivative.[1-4] Due to the somewhat severe conditions of classical reactions, glycosylations with purine bases have lacked both regioselectivity and stereoselectivity. N9 and N7, and in some cases, N1 and N3 regioisomers are produced with substituted purines.[3,4] In contrast to ribofuranose synthons which possess an acyloxy substituent at the 2 position that directs base attack on the β face of the sugar,[5] glycosylation with 2-deoxyribofuranoses may result in the formation of α and β anomeric products. Not only is the yield of desired product low in such reactions, but purification may be difficult due to similar mobility of isomeric products during chromatographic separations.

A recent technique with considerable improvement in the stereo- and regioselectivity of 2-deoxyribonucleoside synthesis involves reaction of the sodium salt of the base with a reactive 1-chlorosugar derivative in a polar aprotic solvent.[6] The success of this "sodium salt" method relies, in part, on the fact that the sugar, 1-chloro-2-deoxy-3,5-di(p-toluyl)-D-*erythro*-pentofuranose, can be prepared as a crystalline solid in the α anomeric configuration.[7,8] The reaction presumably occurs by S_N2 attack of base anion on the 1-α-chlorosugar, resulting in exclusive β nucleoside formation, and, with suitably substituted purines or related bicyclic bases, high yields (>50%) of N9 regioisomers.[6,9] Reactions of the 1-α-chlorosugar in acetonitrile with 6-chloro- and 2,6-dichloropurines,[6] 2-amino-6-chloropurines,[9,10] 2,6-dibromopurine,[11] and 2,6-bis(methylthio)purine[12] were reported to give the 9-β nucleosides as the major product in 50–60% yields. In agreement with the stereoselectivity expected for a S_N2 reaction, the second product in all cases, except that for 2,6-dibromopurine (see below), was the 7-β isomer isolated in 10–15% yields. Related reactions with alkali metal salts of adenine resulted in isolation of variable mixtures of α and β anomers of only the N9 regioisomer in total yields of up to 50%.[13]

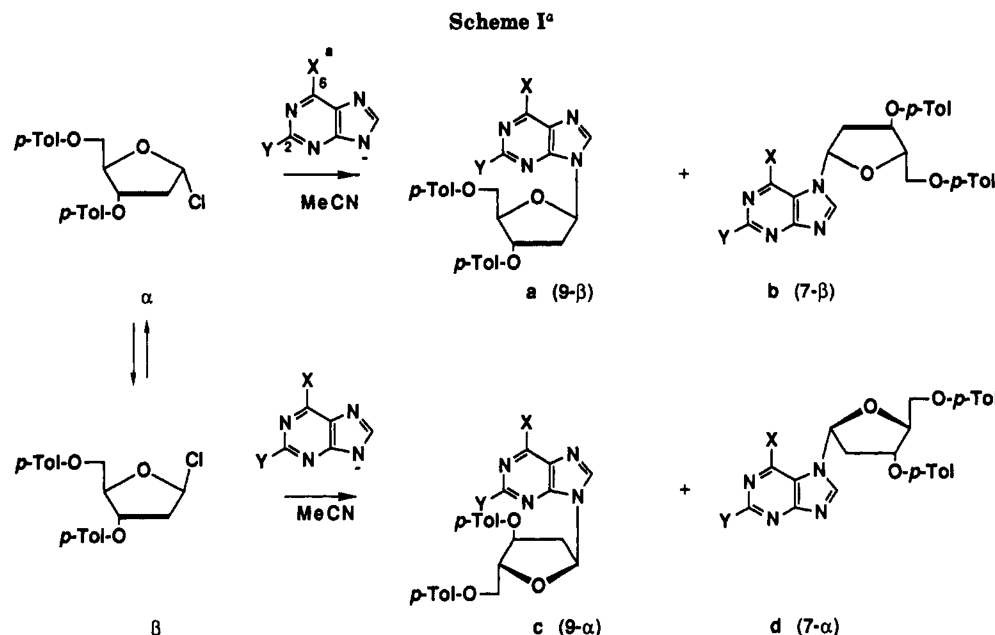
The finding that the second most abundant isomer from the sodium salt glycosylation of 2,6-dibromopurine was the 9-α nucleoside instead of the expected 7-β isomer[11] raised questions about the mechanism of this reaction. In particular, it was thought that an α nucleoside could only be formed if the sugar had anomerized to a mixture of α and β forms, a process reported to occur in solution,[14] or if the reaction did not follow the simple S_N2 mechanism. The possibility that a steric effect, inter alia, may be responsible for reduced N7 isomer formation for 2,6-dibromopurine was tested with 2,6-bis(methylthio)purine; glycosylation

* Author to whom correspondence should be addressed.
† Current address: Sandoz Research Institute, Vienna, Austria.

(1) (a) Hoffer, M.; Duschinsky, R.; Fox, J. J.; Yung, N. *J. Am. Chem. Soc.* **1959**, *81*, 4112–4113. (b) Ness, R. K.; Fletcher, H. G., Jr. *J. Am. Chem. Soc.* **1960**, *82*, 3434–3436. (c) Venner, H. *Chem. Ber.* **1960**, *93*, 140–149. (d) Robins, M. J.; Robins, R. K. *J. Am. Chem. Soc.* **1965**, *87*, 4934–4940.
(2) Christensen, L. F.; Broom, A. D.; Robins, M. J.; Bloch, A. *J. Med. Chem.* **1972**, *15*, 735–739.
(3) Vorbrüggen, H.; Krolikiewicz, K.; Bennua, B. *Chem. Ber.* **1981**, *114*, 1234–1255.
(4) Wright, G. E.; Dudycz, L. W. *J. Med. Chem.* **1984**, *27*, 175–181.
(5) Vorbrüggen, H.; Bennua, B. *Tetrahedron Lett.* **1978**, *15*, 1339–1342.
(6) Kazimierczuk, Z.; Cottam, H. B.; Revankar, G. R.; Robins, R. K. *J. Am. Chem. Soc.* **1984**, *106*, 6379–6382.
(7) Hoffer, M. *Chem. Ber.* **1960**, *93*, 2777–2781.
(8) Bhattacharya, A. K.; Ness, R. K.; Fletcher, H. E. *J. Org. Chem.* **1963**, *28*, 428–434.
(9) Hanna, N. B.; Ramasamy, K.; Robins, R. K.; Revankar, G. R. *J. Heterocycl. Chem.* **1988**, *25*, 1899–1903.
(10) (a) Wright, G. E.; Dudycz, L. W.; Kazimierczuk, Z.; Brown, N. C.; Khan, N. N. *J. Med. Chem.* **1987**, *30*, 109–116. (b) Focher, F.; Hildebrand, C.; Freese, F.; Ciarrocchi, G.; Noonan, T.; Sangalli, S.; Brown, N.; Spadari, S.; Wright, G. *J. Med. Chem.* **1988**, *31*, 1496–1500.
(11) Wright, G. E.; Hildebrand, C.; Freese, S.; Dudycz, L. W.; Kazimierczuk, Z. *J. Org. Chem.* **1987**, *52*, 4617–4618.
(12) Kazimierczuk, Z.; Vilpo, J.; Hildebrand, C.; Wright, G. *J. Med. Chem.* **1990**, *33*, 1683–1687.
(13) Kawakami, H.; Matsushita, H.; Naoi, Y.; Itoh, K.; Yoshikoshi, H. *Chem. Lett.* **1989**, 235–238.
(14) Hubbard, A. J.; Jones, A. S.; Walker, R. T. *Nucleic Acids Res.* **1984**, *12*, 6827–6837.

0022-3263/92/1957-1808$03.00/0 © 1992 American Chemical Society

Scheme I[a]



[a] For X, Y see Table I.

of this base, however, gave the 7-β nucleoside (19%) as the second most abundant product.[12]

The present paper contains results of a systematic study undertaken to explain the anomalous product distribution observed in the sodium salt glycosylation of 2,6-dibromopurine. It was thought that understanding of the reaction mechanism could lead to control of yield and isomer formation to produce biologically important deoxyribonucleosides and their intermediates. In addition, recent interest in α nucleosides in their own right, as enzyme inhibitors[15] and, in the form of α oligonucleotides, for regulation of gene expression,[16] makes the ability to tailor the product distribution in these reactions of considerable practical value.

Results and Discussion

Preparative Reactions. In order to evaluate the influence of base substituents on isomer distribution and to provide authentic nucleoside standards, preparative sodium salt glycosylation reactions were carried out with 6-substituted and 2,6-disubstituted purines (Scheme I). The reactions were performed by the addition of 1 equiv of freshly crystallized and dried 1-chloro-2-deoxy-3,5-di-(*p*-toluyl)-α-D-*erythro*-pentofuranose to a suspension of the purine anion, formed by treatment with 1.1 equiv of sodium hydride, in dry acetonitrile under nitrogen. After stirring for 1 h at 25 °C the mixtures were diluted with chloroform and filtered through Celite, and the filtrates were concentrated and passed through a silica gel column prior to isolation of products. All products were isolated by preparative HPLC on silica gel. The isolated yields of products are presented in Table I, and the properties of new compounds are summarized in Table II.

Isomer Identification. Products were identified by comparison with physical and spectral data from the literature (**1a–c**,[2,6] **2a–d**,[10] and **3a–d**[12]) or characterized by ^{1}H NMR (Table III) using standard criteria for assignment of regio- and stereoisomers. N9 and N7 purine 2'-deoxyribonucleosides are typically differentiated by ^{1}H NMR by the characteristic downfield chemical shifts for the anomeric 1'-H and the purine 8-H resonances of the N7 isomer relative to those of the N9 isomer.[6] These patterns were consistently observed for the regioisomers in all cases reported in Table III.

The assignment of configuration at the anomeric carbon as α or β has been often based on the splitting pattern of 1'-H: a pseudotriplet is observed for β 2'-deoxyribonucleosides due to nearly equal coupling to the 2'-H and

Table I. Products of Sodium Salt Glycosylation of Purines[a]

compd	substituents	% yield a (9-β)	b (7-β)	c (9-α)	d (7-α)	% total nucleosides
1	2,6-diCl	50	15	1.5		66.5
2	2,6-diBr	50	6.6	12	3.8	72.4
3	2,6-diSMe	62	19	0.3	0.2	81.5
4	6-Cl	58	3	1.4	1.2	63.6
5	6-Br	45	13	2.6	1.4	62
6	2-Br-6-SMe	68.3	14.9	1.9	0.8	85.9

[a] Reactions were done as described in the Experimental Section, and all products were isolated by preparative HPLC.

Table II. Properties of (2-Deoxy-3,5-di(*p*-toluyl)-D-ribofuranosyl)purines

compd	mp (°C)	cryst solvent	UV (pH 7) λ_{max} nm (ϵ)	formula (anal.)
4c	85–88	MeOH	241.5 (35 400)	$C_{26}H_{23}N_4O_5Cl$ (C, H, N)
5a	127–128	MeOH	265.5 (12 100)	$C_{26}H_{23}N_4O_5Br$ (C, H, N, Br)
5b	137–138	MeOH	270.5 (10 200)	$C_{26}H_{23}N_4O_5Br$ (C, H, N, Br)
5c	101–103	MeOH	266 (12 000)	$C_{26}H_{23}N_4O_5Br$ (C, H, N, Br)
6a	140–142	MeOH	298.5 (21 500)	$C_{27}H_{25}N_4O_5SBr$ (C, H, N)
6b	138–140	MeOH	299 (13 800)	$C_{27}H_{25}N_4O_5SBr$ (C, H, N)
6c	80–82	MeOH/H_2O	299 (23 400)	$C_{27}H_{25}N_4O_5SBr$ (C, H, N)
6d	100 dec	nd[a]	nd	$C_{27}H_{25}N_4O_5SBr$ (C, H, N)

[a] nd, not done.

(15) Niedzwicki, J. G.; Abernethy, D. R. *Biochem. Pharmacol.* **1991**, *41*, 1615–1624.
(16) (a) Gautier, C.; Morvan, F.; Rayner, B.; Huynh-Dinh, T.; Igolen, J.; Imbach, J.-L.; Paoletti, C.; Paoletti, J. *Nucleic Acids Res.* **1987**, *15*, 6625–6641. (b) Thuong, N. T.; Asseline, U.; Roig, V.; Takasugi, M.; Hélène, C. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 5129–5133.

Table III. ^{1}H NMR Chemical Shifts of (2-Deoxy-3,5-di(p-toluyl)-D-ribofuranosyl)purines in Me_2SO-d_6[a]

compd	isomer	chemical shifts, δ (ppm) H-1′	H-2′	H-2″	H-3′	H-4′	H-5′,5″	H-2	H-8	CH_3Ph	2,6-H(Ph)	3,5-H(Ph)	CH_3S
1a	9-β	6.57 (t)	3.21	2.84	5.80	ca. 4.59	ca. 4.59		8.90	2.38, 2.34	7.93, 7.74	7.35, 7.24	
1b	7-β	6.83 (t)	3.19	2.99	5.73	4.68	4.58		9.18	2.40, 2.37	7.94, 7.74	7.37, 7.27	
1c	9-α	6.66 (q)	3.22	3.07	5.64	5.06	4.55		8.95	2.40, 2.37	7.93, 7.58	7.36, 7.25	
2a	9-β	6.57 (t)	3.24	2.84	5.81	4.63	4.65, 4.53		8.90	2.36, 2.40	7.95, 7.75	7.38, 7.26	
2b	7-β	6.91 (t)	3.18	2.96	5.72	4.68	4.58, 4.56		9.16	2.38, 2.40	7.93, 7.74	7.36, 7.26	
2c	9-α	6.65 (t)	3.05	3.05	5.63	5.07	4.53		8.92	2.36, 2.40	7.92, 7.56	7.35, 7.25	
2d	7-α	6.98 (bd d)	3.12	2.93	5.64	5.27	4.53		9.19	2.36, 2.40	7.93, 7.65	7.37, 7.26	
3a	9-β	6.55 (t)	3.35	2.79	5.8	ca. 4.62	ca. 4.62		8.26	2.43, 2.39	7.95, 7.80	7.29, 7.20	2.66, 2.63
3b	7-β	6.75 (q)	2.99	2.67	5.69	ca. 4.71	ca. 4.71		8.48	2.44, 2.39	7.97, 7.89	7.29, 7.23	2.73, 2.64
3c	9-α	6.57 (q)	3.26	3.0	5.67	4.91	4.61		8.39	2.49, 2.43	7.95, 7.54	7.27, 7.18	2.67, 2.59
3d	7-α	6.82 (bd d)	3.13	2.59	5.70	4.95	4.61		8.36	2.43, 2.38	7.97, 7.68	7.28, 7.21	2.72, 2.65
4a	9-β	6.59 (t)	3.34	2.81	5.82	ca. 4.58	ca. 4.58	8.74	8.64	2.36, 2.32	7.90, 7.71	7.32, 7.21	
4b	7-β	6.87 (t)	3.18	2.94	5.72	4.66	4.55	8.83	9.14	2.39, 2.35	7.93, 7.75	7.36, 7.26	
4c	9-α	6.70 (q)	3.10	3.10	5.64	5.02	4.54	8.72	8.86	2.38, 2.35	7.91, 7.54	7.34, 7.23	
5a	9-β	6.62 (t)	3.37	2.83	5.84	ca. 4.60	ca. 4.60	8.65	8.84	2.40, 2.36	7.94, 7.78	7.36, 7.26	
5b	7-β	6.98 (t)	3.16	2.95	5.72	4.65	4.56	8.78	9.13	2.37, 2.34	7.93, 7.77	7.36, 7.27	
5c	9-α	6.67 (q)	3.07	c	5.62	5.0	4.56	8.65	8.84	2.37, 2.34	7.90, 7.49	7.33, 7.22	
5d	7-α	7.0 (bd d)	3.16	2.85	5.64	5.13	4.52	8.72	9.05	2.36, 2.32	7.90, 7.59	7.33, 7.22	
6a	9-β	6.54 (t)	3.24	2.83	5.80	ca. 4.59	ca. 4.59		8.65	2.40, 2.36	7.95, 7.77	7.36, 7.26	2.66
6b	7-β	6.70 (t)	3.17	2.91	5.72	4.64	4.55		8.94	2.40, 2.37	7.93, 7.77	7.37, 7.28	2.67
6c	9-α	6.61 (t)	3.04	3.04	5.62	5.00	4.52		8.65	2.39, 2.36	7.92, 7.55	7.35, 7.24	2.65
6d	7-α	6.79 (bd d)	3.12	2.87	5.65	5.12	4.53		8.93	2.40, 2.36	7.93, 7.66	7.37, 7.28	2.67

[a] Spectra of compounds **3a–d** were obtained in $CDCl_3$.

Table IV. Coupling Constants (Hz) in ^{1}H NMR Spectra of 2,6-Dibromopurine Deoxyribonucleosides[a]

compd	isomer	$J_{1',2'}$	$J_{1',2''}$	$J_{2',3'}$	$J_{2'',3'}$	$J_{3',4'}$	$J_{4',5'}$	$J_{4',5''}$	$J_{2',2''}$	$J_{5',5''}$
2a	9-β	6.6	6.6	6.6	3.6	6.6	4.5	7.4	−14.4	−13.2
2b	7-β	6.4	6.4	6.3	3.9	5.4	3.9	3.9	−14.9	−12.9
2c	9-α	4.2	4.2	4.1	4.1	2.1	4.5	4.5	b	b
2d	7-α	6.3	0[c]	6.0	0[c]	0[c]	5.0	5.0	−15.3	b

[a] Spectra were obtained at 300 MHz in Me_2SO-d_6 at 25 °C. [b] Indeterminate. [c] <1 Hz.

2″-H ($J_{avg} \approx$ 6–7 Hz), and a doublet of doublets ("pseudoquartet") is seen for α nucleosides due to unequal coupling to the adjacent protons ($J_{avg} \approx$ 6–7, 1–3 Hz).[1d,17] Deviations from the typical pseudotriplet pattern for 1′-H in β-2′-deoxyribonucleosides have been observed, for example, in doublets of doublets for 8-substituted 2′-deoxyadenosines[18] and 5-(trifluoromethyl)-2′-deoxyuridine.[19] Similarly, we observed a doublet of doublets for 1′-H in the 9α isomer **2c** in deuteriochloroform solution,[11] but a pseudotriplet in deuteriodimethyl sulfoxide (Tables III and IV). Therefore, reliance on the appearance of the 1′-H resonance alone is insufficient to assign anomeric configuration. The data in Table III and in the literature[20] show that, for 3′,5′-diacyl derivatives, α anomers exhibit a large chemical shift difference between 4′-H and 5′,5″-H resonances (0.3–0.6 ppm) compared to their near coincidence in β anomers.

The strong overlap of 4′-H and 5′,5″-H in most β anomers (Table III) made coupling constant assignments difficult in these cases. Complete measurements presented in Table IV for the 2,6-dibromopurine nucleoside products, **2a–d**, illustrate the coupling constants characteristic of the four isomers. The pseudotriplet for 1′-H in the 9-α isomer (**2c**) was also observed in the same isomer of the 2-bromo-6-(methylthio)purine product (**6c**). The 7-α isomers gave deceptively simple spectra, as a consequence of near zero values of $J_{1',2''}$, $J_{2'',3'}$, and $J_{3',4'}$ (see Table IV for **2d**). Simple doublets were observed in the 7α isomers for 1′-H, 3′-H, and 5′,5″-H, the latter resulting from identical chemical shifts of the exocyclic protons.

Product Distributions. In all cases it was possible to isolate the four nucleoside isomers, 9-β, 7-β, 9-α, and 7-α, from sodium salt glycosylation of the respective purines. In addition, two dipurinyl nucleosides were found in the reaction with 6-chloropurine (**4**), and a minor product consistent with the 2′-deoxyinosine derivative was isolated from the reaction of 6-bromopurine (**5**) (data not shown). In several cases a disaccharide, 1,1′-(2-deoxy-D-ribofuranosyl-2′-deoxy-D-ribofuranoside) tetra-O-(p-toluate),[21] resulting from dimerization of starting sugar, was detected. In contrast to long reaction times reported for these and related reactions (18–24 h), we found that the reactions are essentially complete after about 10 min at ambient temperature and within 1 h at 0 °C (see below).

The percent yields represented in Table I show that the 9-β isomer predominated in glycosylations of **1–5** with yields varying from 45 to 62%. In all cases where a substantial amount (7–19%) of a second isomer was found, this was the 7-β isomer, except in the case of 2,6-dibromopurine (**2**) where the 9-α isomer was the second most abundant product.

Mechanism of Glycosylation. The sodium salt glycosylation of 6-bromopurine (**5**) did not result in a higher

(17) Srivastava, P. C.; Robins, R. K. *J. Heterocycl. Chem.* **1981**, *18*, 1659–1662.
(18) Long, R. A.; Robins, R. K.; Townsend, L. B. *J. Org. Chem* .**1967**, *32*, 2751–2756.
(19) Ryan, K. J.; Acton, E. M.; Goodman, L. *J. Org. Chem.* **1966**, *31*, 1181–1184.
(20) (a) Nuhn, P.; Zschunke, A.; Heller, D.; Wagner, G. *Tetrahedron* **1968**, *25*, 2139–2144. (b) Kazimierczuk, Z.; Binding, U.; Seela, F. *Helv. Chim. Acta* **1989**, *72*, 1527–1536.
(21) Keller, F.; Bunker, J. E.; Brown, L. H. *J. Org. Chem.* **1966**, *31*, 3840–3842.

yield of 9-α than 7-β nucleoside compared with 6-chloropurine (4), clearly eliminating a simple steric effect of the 6-bromo group in the formation of **2c** (Table I). Several experiments were undertaken in an attempt to explain the unexpected result of glycosylation of **2**.

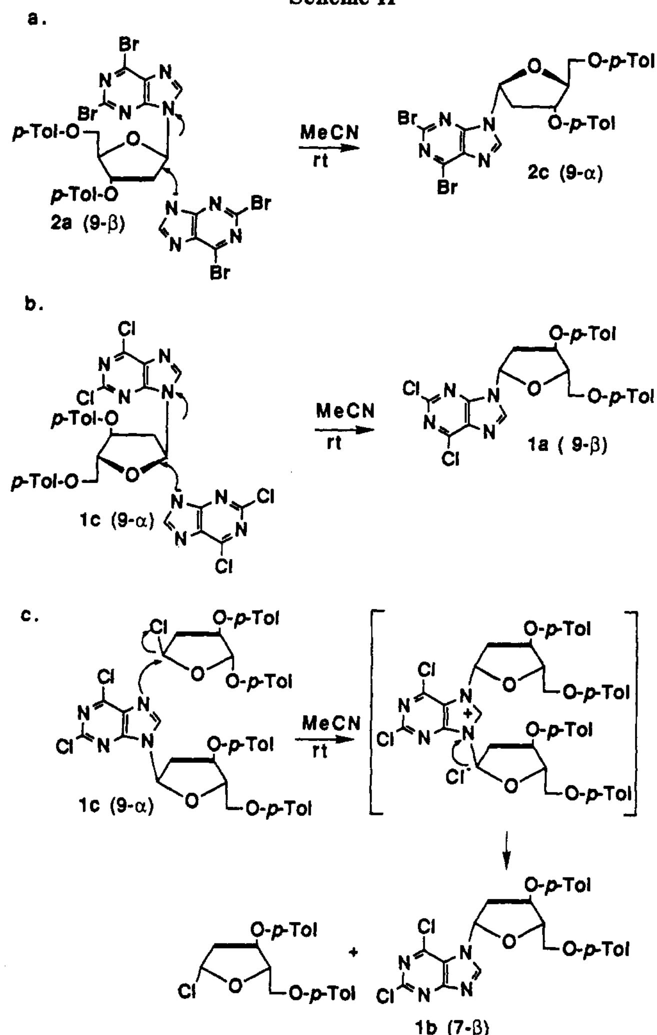
Sugar Anomerization. The isolation of α anomers in all of the sodium salt glycosylations in Table I indicate that this reaction is not completely stereoselective as previously reported,[6,9] and it seemed likely that the formation of α nucleosides resulted from anomerization of the chlorosugar in solution.[14] Therefore, assuming that the reaction proceeded by a S_N2 mechanism, we predicted that the α/β product ratio would increase after aging of the chlorosugar in acetonitrile solution prior to the reaction. The standard reaction was done for 15 min with the sodium salt of 2,6-dibromopurine (**2**) in acetonitrile (see Experimental Section) with solutions of chlorosugar in acetonitrile either freshly prepared or allowed to stand for 10 or 30 min at room temperature. Analytical HPLC on a 5-μm silica gel column and authentic nucleoside standards were used to identify and quantitate the products of the reactions. The ratios of the yields of 9-α and 9-β products, **2c**/**2a**, were 0.47, 0.76, and 0.78 after aging of chlorosugar for 0, 10, and 30 min, respectively. In addition, after aging of chlorosugar for 30 min the overall yield of nucleoside products decreased by 40%. The increase in the relative amount of 9-α product (**2c**) after aging of chlorosugar, if the result of sugar anomerization, is consistent with a S_N2 mechanism for the reaction.

Nucleoside Interconversion. The lack of a straightforward theory to explain the product distribution in glycosylation of **2** led us to hypothesize that it may result from equilibration of initially formed products to more thermodynamically stable products. This phenomenon was observed in syntheses of purine ribonucleosides,[22] and several experiments were designed to test this possibility. Selected product isomers were incubated in anhydrous acetonitrile at room temperature in the presence of either excess purine anion or added chlorosugar. Aliquots were taken during 30 min, and the possible appearance of products was monitored by analytical HPLC as described above.

First we tested the possibility that the 9-β nucleoside **2a** was converted, in the presence of the anion of **2**, to the 9-α isomer **2c** as a result of S_N2 attack, resulting in inversion of configuration (Scheme IIa). The results indicated no change in the concentration of **2a**, nor did a peak corresponding to **2c** appear in the HPLC chromatogram. Similarly, we tested the postulate that the 9-α 2,6-dichloropurine nucleoside (**1c**) may be formed during glycoylation of **1**, but that it is converted to the 9-β isomer **1a** by reaction with the anion of **1** (Scheme IIb). (Such a postulate would explain the lower yield of 9-α anomers in all cases *except* that of **2**.) Analysis of the appropriate reaction mixture, however, showed no change in the concentration of **1c** and no formation of **1a**. Finally, as illustrated in Scheme IIc, we tested the possibility that **1c** could react with the chlorosugar to give a 7,9-bis(2-deoxyribofuranosyl) intermediate, followed by conversion to the (more stable) 7-β isomer **1b**, as observed in reactions of purines with acylated ribofuranoses.[22] In the incubation mixture, however, no change in concentration of **1c** and no formation of **1b** were observed.

The results of these experiments indicate that the nucleoside products were stable in the presence of excess base

Scheme II



anion or chlorosugar. Thus, we conclude that the product distributions observed in the sodium salt glycosylation of purines (Table I) are not likely the result of equilibration to more stable isomers.

Rate Studies. Analytical HPLC analysis of the sodium salt glycosylation reactions indicated that, at 25 °C, the reactions were substantially complete after 10 min. The rates of these reactions are clearly faster than the reported rate of anomerization of the chlorosugar in acetonitrile.[14] We, therefore, tested the possibility that the formation of **2c** resulted from differences in the rates of attack of the anion of **2**, relative to the other bases, on the starting 1-α-chlorosugar. That is, 2,6-dibromopurine anion reacted more slowly with 1-α-chlorosugar, allowing a significant amount of the more reactive[14] 1-β-chlorosugar to be formed, resulting in increased formation of 9-α nucleoside (**2c**).

To test the kinetic hypothesis, the sodium salt glycosylation was done with 2,6-dichloro-, 2,6-dibromo-, and 2,6-bis(methylthio)purines (**1**, **2**, and **3**). Reactions were run at 0 °C in order to permit convenient sampling times. The rates of appearance of products were determined, after HPLC analysis of aliquots of the reaction mixtures (see Experimental Section), using standard curves derived from authentic standards. Plots of % yields of 9-β nucleoside products as a function of reaction time (Figure 1a) show that the initial rate of formation of **2a** was similar to that of **1a**, but that the initial rate of formation of **3a** was greater than for the dihalo compounds. Rate constants for formation of **1a**, **2a**, and **3a** were 1.83, 2.38, and 5.47

(22) (a) Dudycz, L. W.; Wright, G. E. *Nucleosides Nucleotides* 1984, *3*, 33–44. (b) Garner, P.; Ramakanth, S. *J. Org. Chem.* 1988, *53*, 1294–1298.

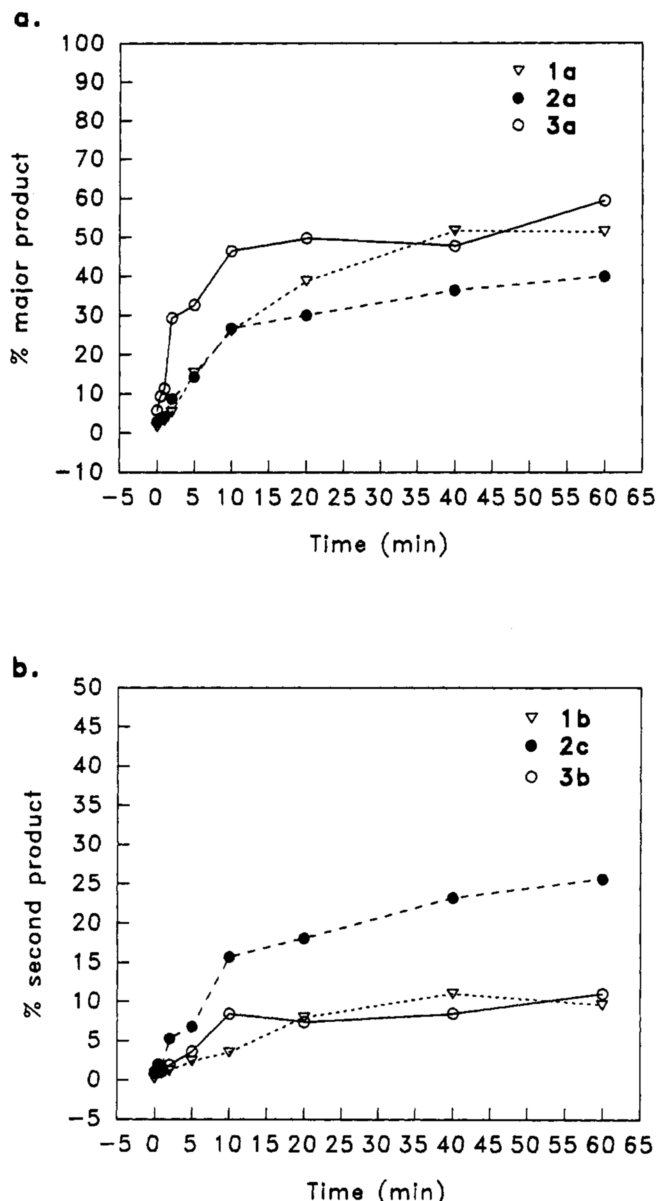


Figure 1. Sodium salt glycosylation of 2,6-disubstituted purines at 0 °C. Reactions were performed as described in the Experimental Section. Aliquots were removed, and the concentrations of products were determined by analytical HPLC at the times indicated. a. Percent yields of the major product of each reaction as a function of reaction time. b. Percent yields of the second product of each reaction as a function of reaction time.

($\times 10^{-3}$ M^{-1} s^{-1}), respectively, calculated from the initial slopes of the curves of Figure 1a, assuming a second-order reaction with equal concentrations of reactants. The higher rate of formation of **3a** is consistent with the higher yield of that product, relative to **1a** and **2a**, in the preparative reactions (Table I) and the expected greater nucleophilicity of the anion of **3** compared with those of **1** and **2** (see below).

Relative rates of formation of the second most abundant product in each reaction, illustrated in the plots in Figure 1b, show that the 9-α 2,6-dibromo nucleoside, **2c**, was formed faster than the 7-β 2,6-dichloro- and 2,6-bis(methylthio) nucleosides, **1b** and **3b**. In fact, the overall "yield" of **2c**, ca. 25%, was greater than that found in the preparative reaction (Table I).

The greater rate of formation of **3a** than of **1a** and **2a** suggests that the electron-releasing methylthio groups of **3** increase the nucleophilicity of N9 of the corresponding anion.[23] However, the influence of substituents on the rates of formation of the minor products is less clear. Although high nucleophilicity expected for N7 of the anion of **3** would explain the high yield of the 7-β isomer, **3b**, no such difference would be expected for N7 of the dihalopurines.[23] A steric effect of the 6-SMe group of **3** may limit the rate of formation of 7-isomers during glycosylation of **3**. Consequently, a combination of reduced charge at N7 in the anion of **2** (relative to **3**) and steric repulsion by the 6-halo group of **2** (relative to **1**) may retard formation of 7-isomers during glycosylation of **2**. Formation of the 9-α isomer from **2** could then occur as a consequence of rapid reaction of the base at N9 with anomerized 1-β-chlorosugar in competition with direct reaction with 1-α-chlorosugar.

Test of Substituent Effects. The greater regioselectivity of the sodium salt glycosylation of **2** than of **1** and **3** (Table I) suggested that a combination of bromo and methylthio groups in a purine base could result in a higher yield of 9-β product in this reaction. In 2-bromo-6-(methylthio)purine (**6**), for example, the methylthio group may both increase overall reactivity of the anion and provide steric hindrance to reaction at N7, and the bromo group may further decrease nucleophilicity of N7. Sodium salt glycosylation of this base (Table I) gave the highest yield of 9-β product, **6a**, among all glycosylation reactions of purines reported in this work and by others.[6,9,10] The improvement in stereoselectivity in glycosylation of **6** relative to that of **2**, i.e., $[9\text{-}\alpha]/[9\text{-}\beta] = 0.03$ and 0.24, respectively, is consistent with increased rate of the reaction as a result of the methylthio group. Regioselectivity in the glycosylation of **6**, i.e., $[9\text{-}\beta]/[7\text{-}\beta]$, improved by a factor of 1.4 relative to reactions of **1** and **3**, perhaps due to a combination of reduced nucleophilicity of N7 and a steric effect of the 6-methylthio group. The results illustrate that yields and distribution of products of the sodium salt glycosylation can be controlled by manipulation of substituents on the base.

Conclusions

Based on the studies of Hubbard et al.[14] showing that 1-chloro-2-deoxy-3,5-(di-*p*-toluyl)-α-D-*erythro*-pentofuranose anomerized in solution in organic solvents, exclusive formation of β nucleoside products could only occur if the rate of glycosylation of the base was much faster than that of anomerization of the sugar or if the 1-β chlorosugar were unreactive. The results presented above indicate that sodium salt glycosylation reactions of purines at 25 °C are complete after 10 min. Furthermore, Hubbard et al. concluded that the 1-β chlorosugar was even more reactive toward pyrimidine bases than the 1-α chlorosugar.[14] We, therefore, propose that steric or electronic repulsion by the 6-bromo group, and an as yet unidentified effect of the 2-bromo group, prevent attack by N7 of the 2,6-dibromopurine anion,[24] allowing sufficient anomerization of sugar to occur and resulting in the formation of 9-α nucleoside product.

(23) MNDO calculations using the MOPAC program, v. 5 (Stewart, J. J. P. *Quant. Chem. Exch.* **1987**, Program 455) were performed for the anions of 1, 2, and 3 in order to estimate the net electronic charges on heteroatoms in the ions. For example, for 1, N7 = −0.282, N9 = −0.297; for 2, N7 = −0.287, N9 = −0.299; for 3, N7 = −0.322, N9 = −0.313. The results indicate that there is no difference in net charges on N7 and N9 between the dihalopurine anions but that those in the di(methylthio)-purine anion are greater. The charge on N7 of 3 is, in fact, greater than that on N9. (We thank Dr. Frank Mari for these results.)

(24) Alkylation of a series of 2-amino-6-substituted purines gave N9- and N7-alkylated products in ratios (N9/N7) which were correlated positively with both the resonance parameter, $\mathcal{R}$, and the hydrophobic parameter, π, of the 6-substituents (Geen, G. R.; Grinter, T. J.; Kincey, P. M.; Jarvest, R. L. *Tetrahedron* **1990**, *46*, 6903–6914). For example, products from 6-bromo- and 6-chloro-2-aminopurines were obtained in N9/N7 ratios of 7.3 and 5.5, respectively.

This and related work[10–12] have described methods for efficient synthesis of purine 2′-deoxyribonucleosides, and, via deblocking and selective amination and/or hydrolysis of 2 and 6 substituents, strategies to prepare biologically important 9-β 2′-deoxyribonucleosides in high yields. The finding that glycosylation of the anion of 2,6-dibromopurine with aged solutions of 1-α-chloro-2-deoxy-3,5-di-(p-toluyl)-*erythro*-pentofuranose results in a high yield of the 9-α 2′-deoxyribonucleoside, **2c**, suggests a similar strategy to prepare 9-α isomers of the natural nucleosides.[25] For example, amination of **2c** followed by hydrogenolysis gave α-2′-deoxyadenosine in 40% overall yield,[12] and hydrolysis followed by amination gave α-2′-deoxyguanosine.[26]

Experimental Section

Melting points were determined on a Mel-temp apparatus and are uncorrected. UV spectra were obtained with a Gilford Response spectrophotometer. ^{1}H NMR spectra were recorded at 200 MHz with a Bruker AC200 spectrometer or at 300 MHz with a Varian Unity 300 spectrometer. Chemical shifts are reported in ppm (δ) relative to internal tetramethylsilane.

Preparative HPLC was done with a Waters Model 6000 pump and Model 401 differential refractometer detector. Analytical HPLC employed a Rainin Rabbit-HP pump with analytical (5-mL) pump heads, a Knauer variable wavelength UV detector, and a Hewlett-Packard Integrating recorder. Elemental analyses were performed by the Microanalysis Laboratory, University of Massachusetts, Amherst; analytical results were within ±0.4% of calculated values. The following compounds were prepared as described previously: 2,6-bis(methylthio)purine;[27] 6-(methylthio)purine;[28] 6-bromopurine and 2,6-dibromopurine;[29] and 1-chloro-2-deoxy-3,5-di(p-toluyl)-α-D-*erythro*-pentofuranose.[7] 6-Chloropurine and 2,6-dichloropurine were purchased from Aldrich Chemical Co. Anhydrous acetonitrile was prepared by distillation over phosphorous pentoxide prior to use.

2-Bromo-6-(methylthio)purine. Sodium hydrosulfide hydrate (2.6 g) was added to a solution of 2,6-dibromopurine (2.65 g, 9.5 mmol) in ethanol (100 mL). After being heated at 60 °C for 40 min, the mixture was diluted with water, and the solution was brought to pH 3 with concd HCl. The precipitate was filtered, and the filtrate was concentrated to half volume. After chilling, the precipitated product was collected by filtration to give 1.76 g (76%) of 2-bromo-6-thiopurine. A suspension of this product (1.59 g, 6.5 mmol) in ethanol (200 mL) was treated with triethylamine (0.66 g, 6.5 mmol) and iodomethane (0.91 g, 6.5 mmol). After being stirred at ambient temperature for 1 h, the solution was evaporated to dryness, and the residue was dissolved in 0.1 N NaOH. After filtration, 594 mg of the product was precipitated with glacial acetic acid. The filtrate was evaporated to dryness, and the residue was purified by chromatography on silica gel (60 g, 70–230 mesh). Additional product (300 mg; total yield 59%) was eluted with a gradient of chloroform to 3% methanol in chloroform (2 L). Crystallization from methanol gave an analytical sample, mp 229 °C dec. ^{1}H NMR (Me_2SO-d_6) δ 8.44 (s, 1 H, C8-H), 2.65 (s, 3 H, SCH_3). Anal. ($C_6H_5N_4SBr$): C, H, N.

Standard Sodium Salt Glycosylation Reaction. A mixture of the purine base and sodium hydride (60% suspension in mineral oil, 1.1 equiv) was stirred in anhyd acetonitrile under a dry nitrogen atmosphere at rt (ca. 25 °C) for 20 min. Freshly prepared, crystalline 1-chloro-2-deoxy-3,5-di(p-toluyl)-α-D-*erythro*-pentofuranose (1 equiv) was added in one portion, and stirring was continued for 1 h. After addition of an equal volume of chloroform the mixture was filtered through Celite, and the Celite was washed with chloroform to remove all UV-absorbing material. The combined filtrates were concentrated and layered on a column of silica gel (20 × theoretical yield, 230–400 mesh). The combined products were eluted with chloroform, and the solvent was evaporated to give a syrup. The syrup was purified by preparative HPLC (silica gel, 50 cm × 22.5 mm) using isocratic elution with the appropriate acetone:toluene mixtures at a flow rate of 15.75 mL min^{-1}. In cases where the major product was insoluble in toluene, the syrupy residue was mixed with toluene, the precipitate of 9-β product was filtered, and the soluble products were separated by preparative HPLC as described above. Yields of all products and physical data for all new compounds are presented in Tables I and II, respectively.

Analytical HPLC. For monitoring the outcome of sodium salt glycosylation reactions on a small scale, analytical HPLC employed a 5-μm Microsorb silica gel column and UV detector set at 277 nm for 2,6-dibromo- and 2,6-dichloropurines and 260 nm for 2,6-bis(methylthio)purine. Elution of products was done with 20% chloroform in cyclohexane containing 1% 2-propanol, 0.1% acetic acid, and 0.1% ethyl acetate at a flow rate of 1 mL min^{-1}. The integrating recorder, calibrated with authentic product standards, was used to determine concentrations of the products.

Sugar Aging Study. Glycosylation of 2,6-dibromopurine (25 mg, 0.09 mmol), as the anion in anhyd acetonitrile (4 mL), was done with 1 equiv of 1-α-chlorosugar as described for the standard sodium salt glycosylation (above). Two additional reactions were carried out by the same procedure except that the samples of 1-α-chlorosugar were dissolved in anhyd acetonitrile (2 mL each) and allowed to stand for 10 and 30 min at rt before adding to the suspension of base anion. An aliquot (5 μL) was removed from each reaction mixture after 15 min and mixed with chloroform (0.5 mL) to quench the reaction. The aliquot solutions were evaporated to dryness, and the residues were dissolved in 20% chloroform in cyclohexane (0.5 mL). After filtration through a 0.45-μm filter, 10-μL aliquots were analyzed by HPLC, and the products were quantitated as described above.

Rate Studies. A suspension of the purine base (25 mg) in anhyd acetonitrile was stirred with sodium hydride (60% suspension in mineral oil, 1.1 equiv) for 20 min at rt and then cooled to 0 °C. Fresh 1-chloro-2-deoxy-3,5-di(p-toluyl)-α-D-*erythro*-pentofuranose (1 equiv) was added to the cooled suspension in one portion. Aliquots of 5 μL each were removed immediately and at various times after the addition and added to excess chloroform to quench the reaction. Samples were processed by analytical HPLC as described above. Rate constants for the formation of the major and second products were estimated from plots of the integrated rate equation based on the assumption of a second order reaction with equal concentrations of reactants.

Isomer Interconversion Studies. a. 2a plus the Sodium Salt of 2,6-Dibromopurine. A suspension of the sodium salt of 2,6-dibromopurine in anhyd acetonitrile, prepared from 25 mg (0.09 mmol) of base and 1.1 equiv of sodium hydride, was added to a solution of **2a** (3 mg, 0.005 mmol) in anhyd acetonitrile (2 mL) at rt. Aliquots of 50 μL were removed immediately and at various times after the addition and quenched and processed by analytical HPLC as described above.

b. 1c plus the Sodium Salt of 2,6-Dichloropurine. A suspension of the sodium salt of 2,6-dichloropurine in anhyd acetonitrile, prepared from 25 mg (0.13 mmol) of base and 1.1 equiv of sodium hydride, was added to a solution of **1c** (2 mg, 0.004 mmol) in anhyd acetonitrile (2 mL) at rt. Removal and analysis of aliquots were done as described above.

c. 1c plus 1-Chloro-2-deoxy-3,5-di(p-toluyl)-α-D-*erythro*-pentofuranose. The 1-α chlorosugar (3.1 mg, 0.008 mmol) was added to a solution of **1c** (7.8 mg, 0.016 mmol) in anhyd acetonitrile (2 mL) at rt. Removal and analysis of aliquots were done as described above.

Acknowledgment. The authors thank Dr. Lech Dudycz for helpful advice and Joseph Gambino for graphics. This work was supported by National Institutes of Health grant No. GM21747. The 300-MHz NMR was purchased with a Shared Instrumentation Grant, no. RR04659, from the National Institutes of Health.

(25) A recent method for 9-α 2′-deoxyribonucleoside synthesis relies on the TMS-triflate-catalyzed trans-2-deoxyribosylation of acetylated 2′-deoxycytidine (Yamaguchi, T.; Saneyoshi, M. *Chem. Pharm. Bull.* **1984**, *32*, 1441–1450). With this method α-2′-deoxyadenosine was obtained in 28% yield; however, 6-methylthiopurine gave 9-α and 9-β isomers in 36% and 27% yields, respectively.
(26) Unpublished results.
(27) Montgomery, J. A.; Holum, L. B.; Johnson, T. P. *J. Am. Chem. Soc.* **1959**, *81*, 3963–3967.
(28) Beaman, A. G.; Gerster, J. F.; Robins, R. K. *J. Org. Chem.* **1962**, *27*, 986–990.
(29) Elion, G. B.; Hitchings, G. H. *J. Am. Chem. Soc.* **1956**, *78*, 3508–3510.