Relative Reactivities of Hydroxyl Groups in Carbohydrate Derivatives. Specific Nuclear Magnetic Resonance Spectral Assignments of Acetyl Groups in Methyl α-D-Glucopyranoside Tetraacetate and Related Derivatives[1,2]

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Methyl α-D-glucopyranoside tetraacetates (**1**) having a trideuterioacetyl group at C-2 (**1a**), C-3 (**1b**), C-4 (**1c**), and C-6 (**1d**) were synthesized by the unambiguous routes shown in Scheme I to permit assignment of each individual acetoxy-group signal in the nmr spectrum of **1**. The 6-acetoxy group resonates at lower field than the other acetoxy groups in chloroform-*d*, pyridine-d_5, and benzene-d_6 (where its signal coincides with that of the 3-acetoxy group). In pyridine-d_5 and benzene-d_6 the highest field signal is that of the 2-acetoxy group, but in chloroform-*d* it is that of the 3-acetoxy group. The acetoxy-group signals in methyl 2,3-di-*O*-acetyl-4,6-*O*-benzylidene-α-D-glucopyranoside (**6**) were assigned similarly by synthesis of the 2-trideuterioacetyl (**6a**) and 3-trideuterioacetyl (**6b**) analogs. It was shown that acetyl groups in **1** do not exchange with the reagent under the normal conditions for acetylation with acetic anhydride–pyridine. The relative extents of acetylation of the various hydroxyl positions, when methyl α-D-glucopyranoside and its 4,6-benzylidene acetal were treated with a limited proportion of acetic anhydride in pyridine, were determined.

Studies on relative reactivities of the various hydroxyl groups in carbohydrates and their derivatives[3] are of theoretical interest and also have significant practical implications in connection with industrially important *O*-substituted derivatives of cellulose[4] and starch.[5] It is well known that primary hydroxyl groups in carbohydrates are tritylated much more readily than secondary hydroxyl groups,[6] and there is a considerable amount of evidence based on product isolation to suggest that the 2-hydroxyl group in methyl glucopyranosides, starch, and cellulose is more reactive than the other secondary hydroxyl groups.[7] Unless all products of a reaction are determined quantitatively, no firm conclusions on extent of reaction at each position can be made, and inferences drawn on the basis of crystalline products isolated in relatively low yield may be expecially misleading. Degradative methods to determine total distribution of substituents have been applied with partially acetylated cellulose,[8] partially phosphorylated starch,[5] partially methylated amylose,[9] and partially 2-(diethylamino)ethylated cellulose,[10] and various simple analogs.[9,10] All of these methods require conversion of the carbohydrate into a partially substituted derivative under various conditions, followed by separation and quantitative determination of the products. This method can be made reliable when stable substituents such as aliphatic ethers are used, but the lability of ester substituents makes determination of their distribution much more difficult because of possible migration or deacylation reactions during the analytical procedure.

Determination of Extent of Substitution at Each Position by Nmr Spectroscopy.—The present approach was designed as a method, not involving separations or degradations, for determining the quantitative extent of substitution at each position in a partially substituted carbohydrate. The method is potentially applicable with various types of substituents, but will be described with reference to acetylated derivatives in particular.

In the nmr spectra of acetylated sugars, individual signals for acetyl groups at various positions in the molecule can be observed. It is possible, in principle, to determine the extent of acetylation at each position if signals for acetyl groups at each position are resolved sufficiently to permit quantitative determination by integration, and if the individual signals can be assigned unambiguously to specific positions of substitution.

The separation of each acetoxy-group signal can frequently be achieved if multiple-solvent techniques[11] and the excellent resolving power of spectrometers operating at high field strengths (≡100 and 220 MHz for protons) are employed. The positional assignment of individual signals is more difficult. Numerous such assignments of individual acetoxy-group resonances have been recorded in the literature, based on rationalizations of comparative data for related compounds. Some of these assignments appear to have statistical validity, especially for fully substituted compounds that are related very closely.[12] However, the chemical shifts of acetyl-group resonances may be affected profoundly by the nature of the solvent[11] and by the presence of substituents, expecially aryl groups, within the molecule.[13] Because assignments of this type, made on single, pure compounds, are open to question, no reliance whatsoever can be placed on assignments, based on comparative data, made for acetoxy-group signals in partially acetylated carbohydrates, where the situation is made even more complex by the simultaneous presence of several different molecular species in the product.

(1) A preliminary report of this work has been given: D. Horton, J. H. Lauterbach, and K. D. Philips, Abstracts, 154th National Meeting of the American Chemical Society, Chicago, Ill., Sept 1967, E028.

(2) Supported in part by a Grant-in-Aid from the Ohio State University Development Fund, Grant No. 170200.

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In the present method, the problems created by the presence of several molecular species in the partially acetylated product are overcome by acetylating the product to completion with deuterated acetylating reagent. This gives a single peracetylated product this is heterogeneous only to the extent of replacement of CH_3 by CD_3 at various positions in the molecules. The nmr spectrum of this acetylated product will be identical with that of the all-protiated material, since isotopic substitution is too small a change to affect signal positions of the remaining hydrogen atoms; the only difference will be that the signals for the acetate methyl protons will be diminished in intensity to the extent of the labeling by deuterium. By comparing the acetoxy-group signal intensities with those of the fully protiated peracetate the fractional degree of substitution by deuterium at each position can be determined.

For the procedure to be effective as a method for determining degree of substitution at each position in the partially labeled, peracetylated derivative it is necessary (a) to find a system in which signals of acetoxy groups at each position can be observed individually, (b) to assign each acetoxy-group signal unequivocally to a specific position in the molecule, and (c) to establish that acetyl groups and trideuterioacetyl groups do not exchange under conditions of acylation. For the data thereby obtained to be valid for the original, partially acetylated product it is further necessary to establish that no significant migration of acetyl groups takes place during treatment with deuterated acetylating reagents.

The present paper records the synthesis of specifically deuterated derivatives of methyl α-D-glucopyranoside tetraacetate (**1**) for assignment of the acetoxy resonances together with a study of the solvent and concentration dependence of these resonances. It is shown that no exchange of acetyl groups for trideuterioacetyl groups occurs with **1** in acetic anhydride-d_6–pyridine. Some experiments are described on the distribution of acetyl groups in partially acetylated methyl α-D-glucopyranoside and its 4,6-benzylidene acetal.

Synthesis of Specifically Deuterated Derivatives.—The first assignment of all acetyl-group signals in the nmr spectrum of a carbohydrate derivative, by synthesis of specifically deuterated derivatives, was that reported[14] for 2-acetamido-1,3,4,6-tetra-*O*-acetyl-2-deoxy-α-D-glucopyranose. Some of the procedures used in that investigation were adapted in the present work.

An experiment was performed to establish that acetyl groups, once bonded to the carbohydrate, do not exchange with the acetylating medium. Methyl 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranoside (**1**) was treated with a 7 molar excess of acetic anhydride-d_6 in dry pyridine for 7 days at room temperature. In the nmr spectrum of the mixture the ratio of the integrals for the methoxyl proton signal and the acetoxy proton signals was 1.0:4.0 at the outset and also at the end of the experiment, indicating that acetyl groups in **1** did not undergo detectable exchange with the reagent under the conditions of the experiment. This experiment employed a reaction time much longer than that normally used for acetylation of sugars.

Methyl 2,3,4-tri-*O*-acetyl-6-*O*-trideuterioacetyl-α-D-glucopyranoside (**1d**) was prepared by detritylation[14] of methyl 2,3,4-tri-*O*-acetyl-6-*O*-trityl-α-D-glucopyranoside (**2**) to give the known[15] methyl 2,3,4-tri-*O*-acetyl-α-D-glucopyranoside (**3**), followed by acetylation of **3** with acetic anhydride-d_6 and pyridine to give **1d**. In order to exclude any possibility of 4 → 6 acetyl migration during the last step, the triacetate **3** was first dissolved in dry benzene, the acetic anhydride-d_6 was then added, and after the reactants had been mixed thoroughly the pyridine was added. Since compound **3** can be retritylated[15] in pyridine to give **2** without 4 → 6 acetyl migration, and since tritylation is a slower reaction than acetylation, the possibility can be excluded that **3** undergoes acetyl migration on acetylation under the conditions used (Scheme I).

The triacetate, mono(trideuterioacetate) **1d**, gave an nmr spectrum identical with that of **1** except that one of the acetoxy-group signals was absent, and this missing signal could thus be assigned to the 6-acetoxy group (Figure 1 and Table I).

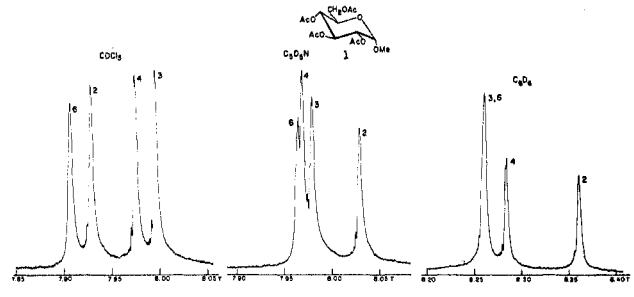


Figure 1.—The acetoxy-group signals in the nmr spectra of methyl 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranoside (**1**) at 100 MHz in chloroform-*d*, pyridine-d_5, and benzene-d_6. Numbers correspond to the individual acetoxy groups in compound 1.

TABLE I

CHEMICAL SHIFTS OF METHOXYL GROUPS AND ACETOXYL GROUPS AS ASSIGNED BY SYNTHESIS OF SPECIFICALLY DEUTERATED DERIVATIVES[a]

Compd	Solvent	-OMe	2-OAc	3-OAc	4-OAc	6-OAc
1	$CDCl_3$	6.58	7.929	7.995	7.975	7.907
	C_5D_5N	6.65	8.029	7.979	7.968	7.965
	C_6D_6	7.00	8.359	8.257	8.281	8.257
6	$CDCl_3$	6.56	7.92	7.96		
	C_5D_5N	6.68	8.008	8.005		
	C_6D_6	7.05	8.31	8.28		
8	$CDCl_3$	6.52		7.90		

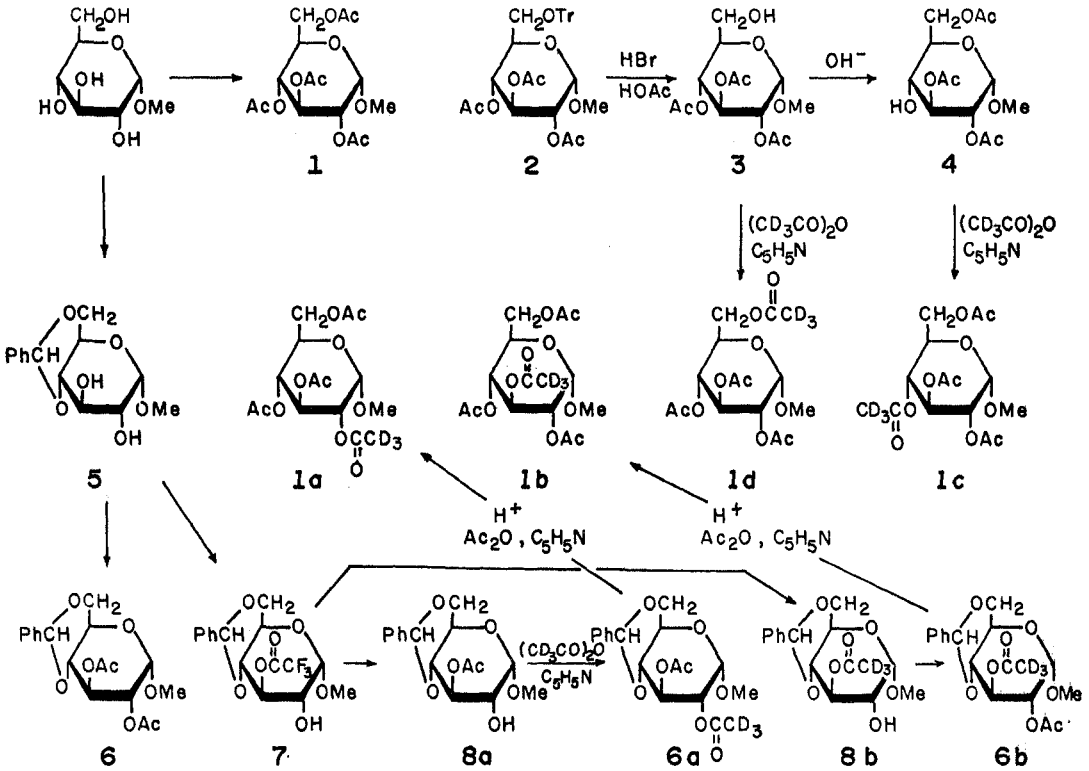
[a] Chemical shifts are on the τ scale, for 10% solutions.

Treatment of the triacetate **3** with base by the method of Helferich and Bredereck[15] caused 4 → 6 acetyl migration to give methyl 2,3,6-tri-*O*-acetyl-α-D-glucopyranoside (**4**). This product appeared to contain ~10% of **3** in equilibrium with **4**. Acetylation of this crude **4** with acetic anhydride-d_6–pyridine gave methyl 2,3,6-tri-*O*-acetyl-4-*O*-trideuterioacetyl-α-D-glucopyranoside (**1c**). The nmr spectrum of **1c** was identical with that of **1** except that one of the four signals had only ~10% of the intensity of the others, and this signal was thus assigned to the 4-acetoxy group (Figure

(14) D. Horton, Abstracts, 150th National Meeting of the American Chemical Society, Atlantic City, N. J., Sept 1965, p 5D; D. Horton, W. E. Mast, and K. D. Philips, *J. Org. Chem.*, **32**, 1471 (1967).

(15) B. Helferich and H. Bredereck, *Ber.*, **64**, 2411 (1931).

SCHEME I



1 and Table I). The signal for the 6-acetoxy group was ~90% of its normal intensity, indicating that the product contained ~90% of **1c** and ~10% of **1d**.

Synthesis of the tetraacetates **1** deuterated specifically in the 2-acetoxy group (**1a**) and 3-acetoxy group (**1b**) was accomplished by way of methyl 4,6-*O*-benzylidene-3-*O*-trifluoroacetyl-α-D-glucopyranoside[16] (**7**). When **7** is treated with acetic anhydride in dry pyridine the trifluoroacetyl group migrates[16] to C-2 to give methyl 3-*O*-acetyl-4,6-*O*-benzylidene-2-*O*-trifluoroacetyl-α-D-glucopyranoside, which on treatment with warm ethanol loses the C-2 substituent to give methyl 3-*O*-acetyl-4,6-*O*-benzylidene-α-D-glucopyranoside (**8a**). The structure of **8a** has been established[16] by methylation followed by graded acid hydrolysis to the known[17] methyl 2-*O*-methyl-α-D-glucopyranoside. The nmr spectrum of **8a** (Tables I–IV) provided independent evidence for the location of the acetoxy group at C-3 by the fact that the ring-methine signal at lowest field (τ 4.68 in chloroform-*d*, AcOCH) was a wide triplet characteristic of an axial proton flanked by vicinal, axial protons (H-3). Had the acetoxy group been at C-2, the signal of the lowest-field ring proton (H-2, AcOCH) would have been a doublet of narrow doublets, probably perturbed by the proximity of the H-2 signal to that of H-1. Treatment of the trifluoroacetate **7** with acetic anhydride-d_6 in dry pyridine, followed by treatment of the product with warm ethanol, gave the 3-trideuterioacetate analog (**8b**) of **8a**.

Acetylation of **8a** with acetic anhydride-d_6 in pyridine gave methyl 3-*O*-acetyl-4,6-*O*-benzylidene-2-*O*-trideuterioacetyl-α-D-glucopyranoside (**6a**), and acetylation of compound **8b** with acetic anhydride in pyridine gave methyl 2-*O*-acetyl-4,6-*O*-benzylidene-3-*O*-trideuterioacetyl-α-D-glucopyranoside (**6b**). Both **6a** and **6b** had nmr spectra identical with that of methyl 2,3-di-*O*-acetyl-4,6-*O*-benzylidene-α-D-glucopyranoside (**6**) except that, in each instance, only one of the two acetoxy-group signals present in **6** was observed. The missing signals could thus be assigned to the individual acetyl groups in **6** (Table I).

TABLE II

CHEMICAL SHIFTS OF BENZYLIDENE, METHOXYL, HYDROXYL, AND ACETOXYL GROUPS[a]

Compd	Solvent	Benzylidene		Methoxyl	Hydroxyl	Acetyl
		Aromatic	Side chain			
2	$CDCl_3$	2.5–2.9		6.54		7.94, 8.00, 8.26
3	$CDCl_3$			6.58	7.54	7.92, 7.94, 7.98
	C_6D_6			6.89	7.45	8.21, 8.28, 8.31
5	Me_2SO-d_6	3.08	4.08	6.32	4.49, 4.68	
6	$CDCl_3$	2.60	4.47	6.56		*b*
	C_5D_5N	2.70	4.26	6.68		*b*
	C_6D_6	2.84	4.70	7.05		*b*
7	$CDCl_3$[c]	2.83	4.49	6.52	7.90	
8	$CDCl_3$	2.60	4.52	6.56	7.52	*b*

[a] Chemical shifts are on the τ scale, for 10% solutions. [b] See Table I. [c] Saturated solution.

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(17) W. N. Haworth, E. L. Hirst, and E. G. Teece, *ibid.*, 2858 (1931).

TABLE III

CHEMICAL SHIFTS OF RING PROTONS AND SIDE CHAIN METHYLENE PROTONS[a]

Compd	Solvent	H-1	H-2	H-3	H-4	H-5	H-6	H-6′
1	$CDCl_3$	5.04	5.11	4.51	4.93	6.08	5.71	5.91
	C_5D_5N	4.83	4.74	4.10	4.58	5.87	5.57	5.70
	C_6D_6	5.11	4.96	4.20	4.73	6.17	5.71	5.94
2	$CDCl_3$	4.97	5.07	4.54	4.92	6.08	6.8–6.9	6.8–6.9
3	C_6D_6	5.03	4.93	4.14	4.72	6.27	6.30	6.48
5	Me_2SO-d_6	5.01	6.25	*b*	*b*	5.47	*b*	*b*
6	$CDCl_3$	5.04	5.07	4.33	*b*	*b*	5.67	*b*
	C_5D_5N	4.77	4.75	3.98	*b*	*b*	5.63	*b*
	C_6D_6	5.09	4.89	3.98	6.52	6.05	6.00	6.53
7	$CDCl_3$[c]	5.16	*b*	4.61	*b*	*b*	5.18	*b*
8	$CDCl_3$	5.22	*b*	4.68	*b*	*b*	5.72	*b*

[a] Chemical shifts are on the τ scale, for 10% solutions. [b] Chemical shifts not measured because of second-order effects. [c] Saturated solution.

TABLE IV

FIRST-ORDER COUPLING CONSTANTS FOR THE RING PROTONS[a]

Compd	Solvent	$J_{1,2}$	$J_{2,3}$	$J_{3,4}$	$J_{4,5}$	$J_{5,6}$	$J_{5,6'}$	$J_{6,6'}$
1	$CDCl_3$	3.5	9.5	9.5	10.0	4.6	2.5	12.2
	C_5D_5N	4.0	9.5	9.5	10.0	5.0	2.5	12.5
	C_6D_6	3.5	9.5	9.5	10.0	4.5	2.5	12.2
2	$CDCl_3$	3.5	9.5	9.5	10.0	4.5	3.0	*b*
3	C_6D_6	3.5	9.5	9.5	9.5	3.0	5.0	12.5
5	Me_2SO-d_6	3.8	*b*	*b*	*b*	*b*	*b*	*b*
6	$CDCl_3$	4.0	*b*	*b*	*b*	3	*b*	*b*
	C_5D_5N	4.0	*b*	*b*	*b*	3	*b*	*b*
	C_6D_6	4.0	10.0	10.0	10.0	5	10	11.2
7	$CDCl_3$	4.0	10	10	*b*	*b*	*b*	*b*
8	$CDCl_3$	4	9	9	*b*	*b*	*b*	*b*

[a] Coupling constants are given in hertz. [b] Coupling constant not measured because of second-order effects.

Attempts to remove the *O*-benzylidene group from **6** (or **6a** or **6b**) by hydrogenation over palladium black[18] in ethyl acetate[18] or in 2-methoxyethanol[19] did not lead to satisfactory results, but treatment of **6** with refluxing 0.0025 *M* hydrogen chloride in acetone[20] smoothly cleaved the *O*-benzylidene group to give syrupy methyl 2,3-di-*O*-acetyl-α-D-glucopyranoside. The nmr spectrum of the latter (in pyridine-d_5) showed only two signals for acetoxy groups, at τ 7.98 and 8.00. Compounds **6a** and **6b** were hydrolyzed by the same procedure[20] and the resultant mono(trideuterioacetylated) diacetates were acetylated to completion with acetic anhydride in pyridine to give methyl 3,4,6-tri-*O*-acetyl-2-*O*-trideuterioacetyl-α-D-glucopyranoside (**1a**) and methyl 2,4,6-tri-*O*-acetyl-3-*O*-trideuterioacetyl-α-D-glucopyranoside (**1b**), respectively. The nmr spectrum of **1a** was identical with that of **1** except that one signal was only ~10% of the intensity of the others, and the latter was thus assigned to the 2-acetoxy group (Figure 1 and Table I). Similarly, in the spectrum of **1b**, one acetoxy-group signal was only ~10% of the intensity of the corresponding signal for **1** and this signal was assigned, therefore, to the 3-acetoxy group (Figure 1 and Table I).

The fact that the preparations of **1a** and **1b** showed small peaks for 2- and 3-acetoxy groups, respectively, indicated that a small amount of acetyl migration had occurred during one of the steps leading to **1a** and **1b**. However, since the assignment of 2- and 3-acetoxy groups had been approached from two separate routes (**7** → **8a** → **6a** → **1a** and **7** → **8b** → **6b** → **1b**), with mutually consistent results, the possibility of extensive acetyl migration (~90%) in these sequences can be ruled out.

Solvent and Concentration Dependence of Acetate-Methyl Group Signals.—The appearance of the acetoxy-group signals of methyl α-D-glucopyranoside tetraacetate (**1**) at 100 MHz in chloroform-*d*, pyridine-d_5, and benzene-d_6 is shown in Figure 1. The signal positions were found to be unaffected (within ±0.004 ppm) by changes of concentration between 1 and 20%, indicating that the chemical shifts were reproducible without the need for specifying any fixed concentration within these limits.

Signals of two of the acetyl groups overlapped in the spectrum measured in benzene-d_6. All signals were separated in the chloroform-*d* and pyridine-d_5 spectra, but chloroform-*d* is regarded as the better of the two solvents for quantitative work because the signals are separated more widely. Solvents such as acetone-d_6 and methyl sulfoxide-d_6 were not included in the study because the protiated forms of these solvents, always present in small concentration in the commercial deuterated solvents, would interfere with determination of acetyl-proton signal intensities by integration.

It is well known that aromatic solvents cause an upfield shift of acetate signals in nmr spectra;[11,21] the signals are observed ~0.4 ppm to higher field than their positions in nonaromatic solvents. With assignment of all signals in **1** it is possible to observe the effect of solvent on each individual acetoxy group. It is especially noteworthy (Figure 1 and Table I) that the signal of the 2-acetoxy group, which appears at second to lowest field of the four signals when chloroform-*d* is the solvent, is specifically shifted to highest field when the spectrum is measured in benzene-d_6 or pyridine-d_5. It has been noted in earlier work from this laboratory[13] that aryl substituents in a peracetylated carbohydrate derivative cause specific shielding or deshielding of individual acetyl groups and ring protons, because of the spatial relationship between the aryl groups and various protons in the molecule. Similar effects are also involved when the aromatic molecules constitute the solvent.[11,21] Specifically deuterated derivatives such as those used in the present study may be useful for gaining insight into the favored orientation of aromatic substituents in organic molecules and the orientation of the solvent sheath with solutions in aromatic solvents.

The coupling constants recorded in Table IV for **1** and other derivatives used in this study are unexceptional. The chemical shifts of ring protons in these derivatives (Table III), established where necessary with the aid of spin decoupling, again illustrate the great utility of multiple solvent techniques[11] for spectral analyses; the large dispersion of the methine-proton signals in the spectrum of **1** in benzene-d_6 particularly facilitates interpretations.

Partial Acetylation Experiments.—Treatment of methyl α-D-glucopyranoside with 1 equiv of acetic anhydride in pyridine for 30 min at room temperature, followed by removal of the reagents *in vacuo* at room temperature, and peracetylation of the product with

(18) H. B. Wood, Jr., H. W. Diehl, and H. G. Fletcher, Jr., *J. Amer. Chem. Soc.*, **79**, 1986 (1957).

(19) R. J. Ferrier and L. R. Hatton, *Carbohyd. Res.*, **5**, 135 (1967).

(20) P. A. Levene and A. L. Raymond, *J. Biol. Chem.*, **97**, 772 (1932).

(21) L. D. Hall, *Advan. Carbohyd. Chem.*, **19**, 66 (1964); M. H. Freemantle and W. G. Overend, *Chem. Commun.*, 503 (1968).

excess acetic anhydride-d_6–pyridine gave the tetraacetate 1 containing a total of 1 mol/mol of protioacetate groups, indicating that all of the original acetic anhydride had reacted to give a total degree of substitution (DS) of 1.0 for protioacetate. The distribution of the acetyl groups, as revealed by nmr spectroscopy, showed that the product had DS for protioacetate of 0.4 at C-6, 0.2 at C-4, 0.2 at C-3, and 0.2 at C-2.

If the step of removal of all reagents after partial acetylation was omitted, the final product obtained under the conditions of the experiment had a DS of 1.3 for protioacetate. The incorporation of more than 1 mol of CH_3CO per mole may be attributed to rapid interaction of 1 mol of $CH_3CO_2^-$, present in the solution at the end of the acetylation, with acetic anhydride-d_6, to give the mixed anhydride $CH_3COOCOCD_3$, which then reacts with the sugar to cause incorporation of more CH_3CO groups.

Methyl 4,6-*O*-benzylidene-α-D-glucopyranoside (**5**), which contains two equatorial, secondary hydroxyl groups, was treated with 1 equiv of acetic anhydride in pyridine for 30 min at room temperature. Removal of the reagents and addition of an excess of acetic anhydride-d_6 in pyridine gave the peracetate **6**, which showed a DS for protioacetate groups of 0.67, indicating that acetylation had gone only two-thirds to completion under the condtions. The nmr spectrum showed that the DS for protioacetate at C-3 was 0.37 and that at C-2 was 0.30.

Further reports in this series will present details on the extent of acylation of various compounds under different conditions of reaction, and will also consider problems of acyl migration and deacylation.

Experimental Section

General Methods.—Unless otherwise noted, solutions were concentrated below 50° under diminished pressure. Melting points were determined with a Thomas–Hoover "Unimelt" apparatus (Arthur H. Thomas Co., Philadelphia, Pa.) and are corrected. Infrared spectra were measured with a Perkin-Elmer "Infracord" Model 137 infrared spectrophotometer. Nmr spectra were measured with a Varian HA-100 nmr spectrometer operating at 100 MHz in the field-sweep mode. Spin-decoupling experiments were performed with the HA-100 instrument operating in the frequency-sweep mode. Unless otherwise specified, specta were measured at a concentration of 10% (w/v). Solutions also contained 5% (w/v) of tetramethylsilane (τ = 10.000) as an internal standard and to provide a lock signal. Chemical shifts are on the τ scale and were taken from the chart recording and/or were measured electronically by using the "Diff 1" postion on a Varian V-4354A internal reference nmr stabilized controller (Publication No. 87-107-402, Varian Associates, Palo Alto, Calif., p 5) in conjunction with a Varian V-4315 frequency counter. The temperature in the probe was approximately 23°. The recorded first-order coupling constants are the measured peak spacings. X-Ray powder diffraction data give interplanar spacings (ångströms) for Cu Kα radiation. The camera diameter was 114.59 mm. Relative intensities were estimated visually: m, moderate; s, strong; v, very; w, weak. The strongest lines are numbered (1, strongest); double numbers indicate approximately equal intensities.

Preparation of Methyl 2,3,4,6-Tetra-*O*-acetyl-α-D-glucopyranoside (1).—Methyl α-D-glucopyranoside (5.0 g, 26 mmol) in 30 ml of dry pyridine was treated with 20 ml (0.20 mol) of acetic anhydride. The mixture was shaken until all of the solids had dissolved; and the mixture was kept for 18 hr at room temperature. The mixture was poured over ice and stirred well. The product was extracted into methylene chloride and the solution was washed with saturated aqueous sodium hydrogen carbonate and water. After drying over magnesium sulfate, the extracts were evaporated and coevaporated with toluene and then with carbon tetrachloride. The residue was crystallized from 95% ethanol and recrystallized from ethanol–petroleum ether (bp 60–110°) to give **1**: yield 8.01 g (89%); mp 101–101.5° (lit.[22] mp 100–101°); λ_{max}^{KBr} 5.71 (OAc), 7.28, 8.12, 9.15 μm; nmr data, see Tables I, III, and IV; X-ray powder diffraction data, 10.91 vw, 7.09 s (1), 6.37 w, 5.50 m (3), 4.66 vw, 4.26 m (2,2), 4.03 m (2,2), 3.36 w.

Methyl 2,3,4-Tri-*O*-acetyl-6-*O*-trityl-α-D-glucopyranoside (2).—A modification[14] of the procedure of Helferich, *et al.*,[23] was used. A mixture of methyl α-D-glucopyranoside (10 g, 50 mmol), dry pyridine (80 ml), and chlorotriphenylmethane (14 g, 50 mmol) was shaken at room temperature until the solution became clear (3 days). Acetic anhydride (37 ml, 0.39 mol) was added and after 18 hr at room temperature the solution was poured into 200 ml of ice–water and stirred well. The amorphous solid that precipitated was filtered off, washed with water, and recrystallized from ethanol–water: yield 20 g (69%); mp 132–135° (lit.[6] mp 136°); λ_{max}^{KBr} 5.70 (OAc), 6.90, 7.30, 8.15, 9.32, 9.57, 12.92, 13.15, 13.42, 14.02, 14.22, 14.45 μm; nmr data are listed in Tables II, III, and IV.

Methyl 2,3,4-Tri-*O*-acetyl-α-D-glucopyranoside (3).—The procedure used was a modification of the one used[14] for 2-acetamido-1,3,4-tri-*O*-acetyl-2-deoxy-α-D-glucopyranose. A solution of methyl 2,3,4-tri-*O*-acetyl-6-*O*-trityl-α-D-glucopyranoside (**2**, 10 g, 20 mmol) in acetic acid (32 ml) was cooled to 20°, and a solution of hydrogen bromide in acetic acid that had been saturated at 0° (3.2 ml) was added. The mixture was shaken for 60 sec and then filtered, the filtrate passing directly into 200 ml of a mixture of ice and water. The bromotriphenylmethane that remained on the filter was washed with cold water, and the combined filtrate and washings were extracted with three 200-ml portions of chloroform. The combined extracts were evaporated without washing, and the product was freed from acetic acid by coevaporation with toluene. The residue was dissolved in chloroform, and the solution was dried over magnesium sulfate and evaporated. The residue was crystallized from ether–petroleum ether (bp 30–60°) and recrystallized from the same solvent system: yield 5.1 g (85%); mp 108–110° (lit.[15] mp 111°); λ_{max}^{KBr} 5.72 (OAc), 7.31, 8.17, 9.61 μm; nmr data, see Tables II, III, and IV.

Methyl 2,3,4-Tri-*O*-acetyl-6-*O*-trideuterioacetyl-α-D-glucopyranoside (1d).—Methyl 2,3,4-tri-*O*-acetyl-α-D-glucopyranoside (**3**, 0.476 g, 1.48 mmol) was dissolved in dry benzene (3 ml) and acetic anhydride-d_6 (0.15 ml) was added. After the acetic anhydride-d_6 had mixed in well, dry pyridine (2 ml) was added. The reaction mixture was kept for 24 hr at room temperature and was then evaporated. The residue was coevaporated with toluene and carbon tetrachloride. The product was crystallized from ether–petroleum ether (bp 30–60°): yield 0.439 g (80%); mp 100–101°. The infrared spectrum (KBr) was indistinguishable from that of **1**. The nmr spectra of **1d** in chloroform-*d*, pyridine-d_5, and benzene-d_6 were identical with those of **1** except for the lack of 3-proton singlets at τ 7.907, 7.965, and 8.257, respectively. The X-ray powder diffraction pattern was indistinguishable from that of **1**.

Methyl 2,3,6-Tri-*O*-acetyl-α-D-glucopyranoside (4).—The procedure of Helferich and Bredereck[15] was used, as modified by not including vacuum distillation of the product. To 55 ml of ethanol was added 1.35 ml of 0.1 *N* aqueous sodium hydroxide and methyl 2,3,4-tri-*O*-acetyl-α-D-glucopyranoside (**3**, 4.5 g, 71 mmol). The solution was kept for 6 hr at room temperature and was then neutralized with acetic acid. The solution was evaporated, and the residue was taken up in water and extracted four times with 50-ml portions of chloroform. The extract was dried (magnesium sulfate) and evaporated to give **4** as a syrup, yield 2.8 g (62.5%). The nmr spectrum of **4** in chloroform was different from that of **3**; the H-3 signal in **4** was observed at τ 4.67, whereas this signal is observed at τ 4.5 in **3** (chloroform-*d*).

Methyl 2,3,6-Tri-*O*-acetyl-4-*O*-trideuterioacetyl-α-D-glucopyranoside (1c).—Methyl 2,3,6-tri-*O*-acetyl-α-D-glucopyranoside (**4**, 0.476 g, 1.48 mmol) was dissolved in dry pyridine and was evaporated to ensure dryness of the starting material. To the syrupy residue, acetic anhydride-d_6 (0.15 ml, 1.94 mmol) and dry pyridine (2 ml) were added. The mixture was kept overnight at room temperature and was then evaporated. The residue was coevaporated with toluene and carbon tetrachloride. The product was crystallized from ether–petroleum ether (bp 30–60°) and recrystallized from the same solvent: yield 0.270 g (50%);

(22) C. S. Hudson and J. K. Dale, *J. Amer. Chem. Soc.*, **37**, 1264 (1915).
(23) B. Helferich, W. Klein, and W. Schafer, *Ber.*, **59**, 81 (1926).

mp 100–101°. The infrared spectrum (KBr) was indistinguishable from that of **1**. The nmr spectra of **1c** in chloroform-*d*, pyridine-d_5, and benzene-d_6 were identical with those of **1** except that the 3-proton singlets at τ 7.975, 7.968, and 8.281, respectively, were only about 10% of their normal intensity. The X-ray powder diffraction pattern was indistinguishable from that of **1**.

Methyl 4,6-*O*-Benzylidene-α-D-glucopyranoside (5).—Prepared by the method of Richtmyer,[24] this compound was obtained in 57% yield: mp 165–166° (lit.[24] mp 161–163°); λ_{max}^{KBr} 2.90 (OH), 7.30, 8.23, 8.56, 8.77, 8.95, 9.35, 9.60, 9.72, 10.02, 10.28, 13.25, 14.05 μm; nmr data, see Tables II, III, and IV.

Preparation of Methyl 2,3-Di-*O*-acetyl-4,6-*O*-benzylidene-α-D-glucopyranoside (6).—Acetylation of **5** with acetic anhydride–pyridine and recrystallization of the product from ethanol–water gave **6**: yield 84.5%; mp 107–108.5° (lit.[25] mp 108–109°); λ_{max}^{KBr} 5.72 (OAc), 6.85, 7.25, 7.31, 8.10, 8.33, 8.72, 8.88, 9.15, 9.42, 9.60, 9.97, 10.05, 10.38, 10.78, 12.89, 13.45, 14.21 μm; nmr data, see Tables I, III, IV; X-ray powder diffraction data, 8.94 vs (1), 7.09 vw, 6.66 vw, 5.19 vs (1, 1), 4.88 s (3), 4.55 w, 4.17 s (2, 2), 3.99 s (2, 2), 3.44 m.

Methyl 4,6-*O*-Benzylidene-3-*O*-trifluoroacetyl-α-D-glucopyranoside (7).—The method of Bourne, *et al.*,[16] was used to convert **5** into **7**, and the product was recrystallized from carbon tetrachloride: yield 58%; mp 208–210° (lit.[16] mp 211°); λ_{max}^{KBr} 2.92 (OH), 5.58 ($COCF_3$), 7.30, 8.20, 8.45, 8.65, 8.77, 9.28, 9.45, 10.05, 13.32 μm; nmr data see Tables II, III, and IV.

Methyl 3-*O*-Acetyl-4,6-*O*-benzylidene-α-D-glucopyranoside (8a).—The method of Bourne, *et al.*,[16] was used to convert **7** into **8a** and the product was recrystallized from petroleum ether (bp 60–110°): yield 47.5%; mp 174–175.5° (lit.[16] mp 174°); λ_{max}^{KBr} 5.72 (OAc), 7.30, 7.92, 8.00, 8.08, 9.10, 9.15, 9.45, 10.03, 13.35 μm; nmr data, see Tales I, III, and IV.

Methyl 3-*O*-Acetyl-4,6-*O*-benzylidene-2-*O*-trideuterioacetyl-α-D-glucopyranoside (6a).—Methyl 3-*O*-acetyl-4,6-*O*-benzylidene-α-D-glucopyranoside (**8a**, 4.0 g, 12.3 mmol) was dissolved in dry pyridine (25 ml) and acetic anhydride-d_6 (1.44 ml, 18.8 mmol) was added. The mixture was kept for 18 hr at room temperature, and then ice–water was added until precipitation was complete. The product was filtered off and recrystallized from ethanol–water: yield 3.7 g (82%); mp 107–108°. The infrared spectrum (KBr) was indistinguishable from that of **6**. The nmr spectra of **6a** in chloroform-*d*, pyridine-d_5, and benzene-d_6 were identical with those of **6** except that the 3-proton singlets at τ 7.92, 8.008, and 8.31, respectively, were absent. The X-ray powder diffraction pattern was indistinguishable from that of **6**.

Methyl 3,4,6-Tri-*O*-acetyl-2-*O*-trideuterioacetyl-α-D-glucopyranoside (1a).—Methyl 3-*O*-acetyl-4,6-*O*-benzylidene-2-*O*-trideuterioacetyl-α-D-glucopyranoside (**6a**, 1.00 g, 2.72 mmol) was hydrolyzed essentially according to the procedure used by Levene and Raymond[20] for the β-D anomer of **6**. Compound **6a** was dissolved in an acetone–hydrochloric acid mixture (10 ml) made by diluting 2.5 ml of 0.1 *N* hydrochloric acid with 97.5 ml of acetone. The mixture was refluxed for 5 hr, cooled, and neutralized with silver oxide. The solution was filtered through Celite to remove silver chloride, and the filtrate was evaporated. The residue was taken up in water and extracted three times with an equal volume of petroleum ether (bp 30–60°). The aqueous layer was evaporated to a syrup and coevaporated with pyridine to remove final traces of water. The residue was dissolved in dry pyridine (2 ml) and acetic anhydride (0.77 ml, 8.2 mmol) was added. The reaction mixture was kept overnight at room temperature and was then evaporated. The residue was crystallized from ether–petroleum ether (bp 30–60°), and was recrystallized from the same solvent system: yield 0.690 g (71%); mp 100–101°. The infrared spectrum (KBr) was indistinguishable from that of **1**. The nmr spectra of **1a** in chloroform-*d*, pyridine-d_5, and benzene-d_6 were identical with those of **1** except that the 3-proton singlets at τ 7.929, 8.029, and 8.359, respectively, were only about 10% of their normal intensity. The X-ray powder diffraction pattern was indistinguishable from that of **1**.

Methyl 4,6-*O*-benzylidene-3-*O*-trideuterioacetyl-α-D-glucopyranoside (8b) was prepared by the same method as that used for **8a**. From methyl 4,6-*O*-benzylidene-3-*O*-trifluoroacetyl-α-D-glucopyranoside (**7**, 2.0 g, 5.29 mmol), acetic anhydride-d_6 (1.40 ml, 1.8 mmol), and dry pyridine (4.5 ml), 1.40 g of product (82%) was obtained, mp 174–176°. The infrared spectrum (KBr) was indistinguishable from that of **8a**. The nmr spectrum of **8b** in chloroform-*d* was identical with that of **8a** except for lack of any acetoxy absorptions. The X-ray powder diffraction pattern was indistinguishable from that of **8a**.

Methyl 2-*O*-acetyl-4,6-*O*-benzylidene-3-*O*-trideuterioacetyl-α-D-glucopyranoside (6b) was prepared by the route used for **6a** except that the starting material was **8b** instead of **8a**. A mixture of methyl 4,6-*O*-benzylidene-3-*O*-trideuterioacetyl-α-D-glucopyranoside (**8b**, 1.00 g, 3.06 mmol), acetic anhydride (0.43 ml, 4.5 mmol), and dry pyridine (7 ml) yielded 0.77 g of product (68%), mp 107–108°. The infrared spectrum (KBr) was indistinguishable from that of **6a**. The nmr spectra of **6b** in chloroform-*d*, pyridine-d_5, and benzene-d_6 were identical with those of **6a** except that the 3-proton singlets at τ 7.96, 8.005, and 8.28, respectively, were absent. The X-ray powder diffraction pattern was indistinguishable from that of **6**.

Methyl 2,4,6-Tri-*O*-acetyl-3-*O*-trideuterioacetyl-α-D-glucopyranoside (1b).—The procedure used was the same as that for **1a**. The hydrolysis was effected on methyl 2-*O*-acetyl-4,6-*O*-benzylidene-3-*O*-trideuterioacetyl-α-D-glucopyranoside (**6b**, 0.50 g, 1.36 mmol) by using 5.00 ml of the acidified acetone solution. The purified syrup was treated with acetic anhydride (0.40 ml, 4.3 mmol) in dry pyridine (3 ml) and was processed in the same way as **1a**: yield 0.228 g (48%); mp 100–101°. The infrared spectrum (KBr) was indistinguishable from that of **1**. The nmr spectra of **1b** in chloroform-*d* and pyridine-d_5 were identical with those of **1** except that the 3-proton singlets at τ 7.995 and 7.979, respectively, were only about 10% of their normal intensity; and in benzene-d_6, the peak at τ 8.257 was 50% of normal intensity. The X-ray powder diffraction pattern was indistinguishable from that of **1**.

Studies on Relative Distribution of Product on Acetylation of Methyl α-D-Glucopyranoside.—Methyl α-D-glucopyranoside (0.914 g, 1.00 mmol) was dissolved in dry pyridine (1.00 ml) and 1.00 ml of a 1.00 *M* solution of acetic anhydride in pyridine was added. The solution was shaken for 10 min and kept at room temperature for another 20 min. The solution was then evaporated under high vacuum at 30°, with subsequent coevaporation with toluene and carbon tetrachloride. The dry solid was then dissolved in dry pyridine (2 ml) and acetic anhydride-d_6 (0.40 ml, 5.15 mmol). The solution was kept at room temperature for 18 hr and then evaporated, and the product was crystallized from ether–petroleum ether (bp 30–60°): yield 0.225 g (63%); mp 100–101°. Nmr integration (chloroform-*d*) showed that the product contained a total of 1 mol of protioacetate for every mole of methoxyl group. The degree of substitution (DS) of protioacetate groups at individual positions, measured by nmr integration, showed 6-acetoxy, 0.4; 2-, 3-, and 4-acetoxy, 0.2 each. Except for intensity of the acetate peaks the rest of the nmr spectrum was identical with that of **1**.

When the experiment was repeated, but with omission of the evaporation step, the product had a DS for protioacetate groups of 1.3 mol/mol, with similar proportional distribution between the four positions.

Relative Distribution of Product on Acetylation of Methyl 4,6-*O*-Benzylidene-α-D-glucopyranoside.—Methyl 4,6-*O*-benzylidene-α-D-glucopyranoside (**5**, 0.282 g, 1.00 mmol) was dissolved in dry pyridine (1.00 ml) and 1.00 ml of a 1.00 *M* solution of acetic anhydride in dry pyridine was added. The mixture was shaken for 10 min and kept for another 20 min at room temperature. The solution was then evaporated at 30° under high vacuum, and the residue was coevaporated with toluene and carbon tetrachloride. The dry solid was dissolved in dry pyridine (2 ml) and acetic anhydride-d_6 (0.40 ml, 5.15 mmol) was added. The mixture was kept for 18 hr at room temperature. The product was precipitated by the addition of water, filtered off, and recrystallized from ethanol–water: yield 0.272 g (74%); mp 107–108°. Nmr integration (chloroform-*d*) showed a total DS of 0.67 mol of protioacetate for every mole of methoxyl group. The DS of protioacetate groups at the two positions, measured by nmr integration, was 0.37 at C-3 and 0.30 at C-2. Except for the intensity of the acetate peaks, the spectrum was identical with that of **6**.

Determination of the Amount of Acetyl Exchange between *O*-Acetyl Groups on Methyl 2,3,4,6-Tetra-*O*-acetyl-α-D-glucopyranoside and Acetic Anhydride-d_6.—This experiment was performed at 60 MHz with a Varian A-60A spectrometer. Methyl 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranoside (**1**, 0.100 g, 0.284 mmol), acetic anhydride-d_6, (0.15 ml, 2.00 mmol), and dry pyridine (1 ml) were mixed together and an aliquot of this mix-

(24) N. K. Richtmyer, *Methods Carbohyd. Chem.*, **1**, 108 (1962).
(25) D. S. Mathers and G. J. Robertson, *J. Chem. Soc.*, 698 (1933).

ture was kept in an nmr tube. The spectrum was observed at various time intervals during a 7-day period; at all times the ratios of the methoxyl-group integral to the acetyl-group integral remained constant at 1.0 to 4.0. No deuterium incorporation could be detected in 1 recovered after 7 days.

Change in Nmr Peak Positions with Concentration.—Spectra of methyl 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranoside (1) were measured at concentrations of 1, 5, 10, and 20% (w/v) in chloroform-*d*, which also contained 5% (w/v) tetramethylsilane. The acetoxy-group signals did not vary in field position by more than 0.004 ppm.

Registry No.—**1**, 604-70-6; **1a**, 18031-45-3; **1b**, 18031-46-4; **1c**, 18031-47-5; **1d**, 18031-48-6; **2**, 18031-49-7; **3**, 7432-72-6; **4**, 18031-51-1; **5**, 3162-96-7; **6**, 4141-45-1; **6a**, 18031-54-4; **6b**, 18031-55-5; **7**, 18031-56-6; **8a**, 18031-57-7; **8b**, 18031-58-8.

The Structure and Properties of Some D-Arabino- and D-Xylopyranosyladenines[1]

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The anomeric pairs of both 9-D-arabino- and 9-D-xylopyranosyladenine have been prepared (**10a**, **10b** and **11a**, **11b**, respectively), together with the 7-α-D-arabino- and 7-β-D-xylopyranosyladenines. Evidence was obtained to show that all the arabinosides appeared to be in the 1*C* conformation; and all the xylosides, the *C*1 conformation. The arabinosides **10a** and **10b** violated Hudson's isorotation rules while the xylosides **11a** and **11b** obeyed them. The β-arabinoside **10b** was more conveniently prepared from the D-arabinopyranosyl halide blocked with *p*-nitrobenzoyl groups rather than with nonparticipating benzyl groups. The effect of other acyl blocking groups on anomer distribution of the nucleoside products was also examined.

The demonstrated anticancer activity of the furanose forms of 9-β-D-arabinosyl-[2a] and 9-β-D-xylosyladenine[2b] suggested that the pyranose forms be prepared in sufficient quantity for comparative studies. This manuscript describes the synthesis of the unknown α and β anomers of 9-D-arabinopyranosyladenine, the known 9-β-D-xylopyranosyladenine,[3] and its unknown α anomer. In addition, interesting observations bearing on the conformations of these compounds on Hudson's isorotation rules[4] and the *trans* rule[5] are recorded.

The known 9-β-D-xylopyranosyladenine[3] (**11b**) was prepared in good yield by condensation of chloromercuri-6-benzamidopurine with 2,3,4-tri-*O*-benzoyl-D-xylopyranosyl bromide[6] in refluxing xylene followed by deacylation with methanolic sodium methoxide (Scheme I). The properties of **11b** agreed well with those reported previously.[3] Because of the arabinose work discussed below, the α anomer, **11a**, was sought and found by chromatography of the crude product through a Dowex 1 (OH) column.[7] There was also isolated a minute amount of another xylopyranosyladenine (**Y**) which, on the evidence discussed later, was shown to be the 7-substituted nucleoside.

Preparation of 9-β-D-arabinopyranosyladenine **10b** from a halo sugar required that the arabinose be blocked by nonparticipating groups in order to maximize the yield of β anomer. The benzyl-blocked haloarabinose **4** was chosen because the corresponding furanose isomer was known to react with 6-benzamidopurine to afford, after deblocking, mainly 9-β-D-arabinofuranosyladenine.[8]

The halo sugar **4** was obtained from methyl β-D-arabinoside.[9] This was converted into **1** with sodium hydride and benzyl chloride in hot N,N-dimethylformamide (DMF).[10] This procedure required a smaller excess of reagents, a less laborious work-up and gave purer product in our hands than the literature procedure for the L isomer[11] of **1**. Hydrolysis of **1** afforded **2** which was treated with *p*-nitrobenzoyl chloride to afford **3** as a syrup that could be induced to crystallize partially. However, the entire syrup was generally converted into the halo sugar **4**. This was used immediately for the nucleoside condensation.

6-Benzamidopurine did not react with **4** under the mild conditions that were suitable for the furanose isomer.[8] However, chloromercuri-6-benzamidopurine reacted readily with **4** in refluxing xylene to give an anomeric mixture of **8** that was treated with sodium methoxide to give **9**. Some of the anomeric mixture of **9** was separated by preparative thin layer chromatography. The crystalline anomer of **9**, later found to be the α anomer, was readily debenzylated by sodium in liquid ammonia.[12] However, debenzylation of the gummy anomeric mixture of **9** was difficult and was successful only when the gummy **9** was suspended on coarse Celite.[13] The anomeric mixture was most conveniently separated at **10** using a Dowex-1 (OH) column.[7] An equal mixture of α and β anomers of **10** was obtained together with a minor, third component (**Z**) of mp 268–

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(10) For other alkylations of carbohydrates employing sodium hydride and an aprotic solvent, see (a) U. E. Diner, F. Sweet, and R. K. Brown, *Can. J. Chem.*, **44**, 1591 (1966); (b) D. M. W. Anderson and G. M. Cree, *Carbohyd. Res.*, **2**, 162 (1966), and (c) J. S. Brimacombe, B. D. Jones, M. Stacey, and J. J. Willard, *ibid.* **2**, 167 (1966).

(11) S. Tejima and H. G. Fletcher, Jr. [*J. Org. Chem.*, **28**, 2999 (1963)] have prepared the L isomer by benzylation with potassium hydroxide.

(12) E. J. Reist, V. J. Bartuska, and L. Goodman, *ibid.*, **29**, 3725 (1964).

(13) A diatomaceous earth product of Johns-Manville; Celite Grade 560 was suitable.