and 212 mμ (ε 10,510), λ_{min}^{EtOH} 241 mμ (ε 7140); τ 8.2 (CH_3 singlet and 7.75 (CH_3 singlet) in d_6-DMSO.[28] *Anal.* Calcd. for $C_{10}H_{10}N_2O_3$: C, 58.24; H, 4.89; N, 13.59. Found: C, 58.42; H, 4.82; N, 13.48.

From 5′-O-Mesyl-2,3′-anhydrothymidine.[15]—A solution of 0.604 g. (2 mmoles) of the anhydronucleoside and 0.224 g. (2 mmoles) of *t*-BuOK in 15 ml. of DMSO was stirred at room temperature for 1 hr. Following the procedure outlined above, there was obtained 0.120 g. (29% yield) of XVIII, m.p. and m.m.p. 162–165°. The acetone-insoluble material, when examined spectrally (ultraviolet) clearly showed the presence of unreacted starting material.[29]

(28) The authors are grateful to Dr. Robert Scott, Parke, Davis and Company, Research Laboratories, Ann Arbor, Mich., for these measurements.

(29) The use of an abbreviated reaction period was deliberate in an (unsuccessful) attempt to trap the presumed intermediate, XVII.

Nucleosides. XXXI. 3′-Amino-3′-deoxyhexopyranosyl Nucleosides. IV. Nucleoside Conversions in the 3′-Aminohexose Series[1]

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1-(3′-Amino-3′-deoxy-β-D-mannopyranosyl)uracil (X) was prepared from its gluco isomer II in a seven-step synthesis proceeding *via* 1-(3′-acetamido-3′-deoxy-2′-*O*-mesyl-4′,6′-*O*-benzylidene-β-D-glucosyl)uracil (V). Compound V was converted to the 2,2′-anhydro derivative VI, the first of its kind in the hexopyranosyl nucleoside area. The structure of VI was established by its conversion to 1-(3′-acetamido-3′-deoxy-4′,6′-*O*-benzylidene-β-D-mannosyl)isocytosine (VII) with liquid ammonia and to 1-(3′-acetamido-3′-deoxy-4′,6′-*O*-benzylidene-β-D-mannosyl)uracil (VIII) with alkali, the latter of which, after removal of blocking groups, yielded X. An attempted conversion of the 3′-aminogluco nucleoside II to a 4′-amino-4′-deoxygulo nucleoside XVIII *via* the aziridine XIVb was carried out. Some indication of formation of XVIII was obtained along with the formation of the crystalline hydrochloride of 1-(3′-amino-3′-deoxy-β-D-galactopyranosyl)uracil (XVI). The latter nucleoside was also obtained directly from uridine by the periodate–nitromethane procedure.

Previous papers from this laboratory[2] described the facile synthesis of 3′-amino-3′-deoxyglucosyluracil (II) by treatment of uridinedialdehyde with nitromethane followed by reduction of the nitro group. It was indicated[2] that, although the 3′-nitrogluco derivative I was obtained as the predominant isomer, other isomers were present in the mother liquors. By analogy with the work of Baer, *et al.*,[3] with glycosides, it would be expected that a maximum of four isomers would form in the course of reaction of the dialdehyde with nitromethane (though eight isomers are theoretically possible). In addition to the gluco isomer already isolated,[2] the galacto, manno, and talo isomers should also be present. (In our recent studies[4] on the application of the periodate–nitromethane procedure to the purine nucleoside, adenosine, the glucosyl-, galactosyl-, and mannosyladenine derivatives were obtained.) In the present paper we report the isolation of some of these 3′-amino isomers from uridine as well as their unequivocal synthesis from II.

The five pyrimidine nucleoside antibiotics elaborated by *Streptomyces* (amicetin, bamicetin, plicacetin, blasticidin S, and gougerotin) all contain 4-amino-4-deoxyhexosyl moieties linked to cytosine.[5] None have been synthesized chemically, although obviously such syntheses would make possible the preparation of analogs of potential biochemical interest. In this report, we also describe some preliminary attempts in the conversion of 3′-aminohexosyl nucleosides to 4′-aminohexosyl nucleoside derivatives.

The 3′-aminoglucosyl nucleoside II served as a chemical precursor for the preparation of other epimeric nucleosides. For the synthesis of the mannosyl derivative X, the *N*-acetate[2] III was converted to the 4′,6′-*O*-benzylidene derivative IV by treatment of III with benzaldehyde and zinc chloride (see Scheme I). Compound IV was very insoluble in common organic solvents and exhibited a high melting point (306–308°). Treatment of a suspension of IV in anhydrous pyridine with methanesulfonyl chloride afforded the 2′-mesylate V.

Compound V could react with alkoxide in two possible ways. The neighboring *N*-acetate could conceivably participate[6] to form an oxazoline structure VIa. Alternatively, the 2-carbonyl group of the pyrimidine moiety could participate with the formation of a 2,2′-anhydromanno nucleoside VI. If the latter course prevails, it would be the first example of an anhydro nucleoside in the hexosyl nucleoside area.

Treatment of V with sodium ethoxide in a mixture of pyridine and ethanol[7] afforded colorless needles (VI) in ~60% yield. The ultraviolet absorption

(1) This investigation was supported in part by funds from the National Cancer Institute, National Institutes of Health, U. S. Public Health Service (Grant No. CA 03190-09).

(2) (a) K. A. Watanabe and J. J. Fox, *Chem. Pharm. Bull.* (Tokyo), **12**, 975 (1964), (b) K. A. Watanabe, J. Beránek, H. A. Friedman, and J. J. Fox, *J. Org. Chem.*, **30**, 2735 (1965).

(3) H. H. Baer, *J. Am. Chem. Soc.*, **84**, 83 (1962); H. H. Baer and F. Kienzle, *Can. J. Chem.*, **41**, 1606 (1963).

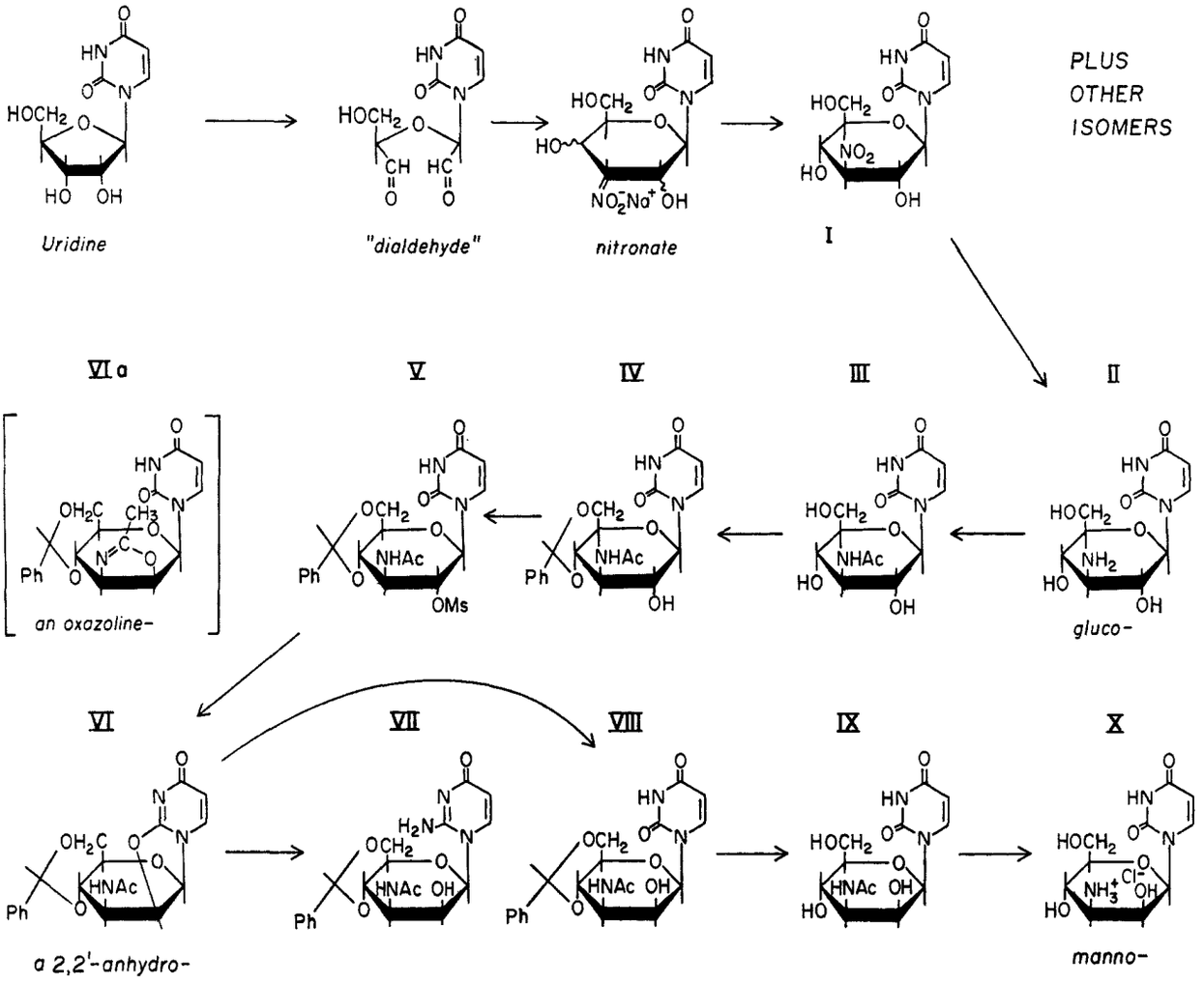
(4) J. Beránek, H. A. Friedman, K. A. Watanabe, and J. J. Fox, *J. Heterocyclic Chem.*, **2**, 188 (1965).

(5) J. J. Fox, Y. Kuwada, K. A. Watanabe, T. Ueda, and E. B. Whipple, *Antimicrobial Agents Chemotherapy*, 518 (1964), and leading references therein.

(6) B. R. Baker and T. Neilson, *J. Org. Chem.*, **29**, 1047 (1964), and leading references therein. The formation of a 2′,3′-aziridine ring by reaction of V with alkoxide is also conceivable. However, as demonstrated by Baker, *et al.*, the formation of an aziridine ring from a diequatorial arrangement of the 2′,3′ neighboring groups (as in V) in highly unlikely under these reaction conditions. The possibility of aziridine formation is discussed later (Scheme II, XIVb) with a related nucleoside.

(7) Compound V is highly insoluble in water and slightly soluble in ethanol. It is soluble in pyridine. The choice of solvent and alkoxide in this case is due to these solubility characteristics of V.

SCHEME I



spectrum of VI showed two maxima (240 and 225 mμ) which strongly suggests the 2,2′-anhydro nucleoside structure.[8] Furthermore, the infrared spectrum of VI differs from IV or V in the carbonyl region. Whereas IV and V give absorption bands at ~5.8 μ, compound VI shows no bands in this region.

Conclusive proof that VI is an anhydro nucleoside was obtained by treatment of VI with liquid ammonia[9,10] to yield the crystalline isocytosine nucleoside derivative VII. Compound VII exhibited an ultraviolet absorption spectrum similar to that for 1-β-D-arabinofuranosylisocytosine[10] derivatives and gave an analysis consistent with four nitrogen atoms. These data exclude structure VIa and firmly establish the anhydro nucleoside structure VI as depicted in Scheme I.

A study of molecular models shows that such an anhydro nucleoside could exist with the sugar in the C1 (chair) conformation as shown in Figure 1. Other conformations (two "boat" and two "twisted boat" forms) are also possible for VI. However, these conformations are considered less likely. The n.m.r. spectrum[11] of VI is shown in Figure 2. The anomeric proton resonance absorption is easily identified as a doublet[12] at δ 6.12 with a coupling constant, $J_{H_1',H_2'} \leqq$ 3.5 c.p.s.[13,14] The amide H at δ 8.5 is exchanged in deuterioacetic acid and a spin decoupling experiment shows that one of the four protons (H-3′) in the δ ~4 region is coupled to the NH proton. The assignments of H-4′ and H-5′ are not certain and may be reversed. Treatment of VI with dilute alkali opened the anhydro ring by attack at C-2 with the formation of the crystalline manno derivative VIII in good yield. The n.m.r. spectrum of VIII showed chemical shifts for the anomeric proton at δ 5.89 with a $J_{H_1',H_2'}$ of approximately zero, consistent with an axial–equatorial[12] (manno) orientation of the C-1′ and C-2′ protons.

1-(3′-Amino-3′-deoxy-β-D-mannopyranosyl)uracil (X) was obtained as the hydrochloride salt by refluxing VIII in aqueous acetic acid followed by removal

(8) D. M. Brown, A. R. Todd, and S. Varadarajan, *J. Chem. Soc.*, 2388 (1956); J. F. Codington, I. L. Doerr, and J. J. Fox, *J. Org. Chem.*, **30**, 476 (1965), and leading references therein. These references refer to anhydro nucleosides of 1-β-D-aldopentofuranosyluracils which also show twin maxima in approximately the same general region.

(9) The conversion of 2,2′-anhydroarabino nucleosides to their isocytosine analogs was first reported by Brown, *et al.*,[10] using alcoholic ammonia. A smoother reaction was innovated by I. L. Doerr (unpublished) of this laboratory using liquid ammonia on anhydro nucleosides. The authors are indebted to Miss Doerr for this method of proof of VI.

(10) D. M. Brown, A. R. Todd, and S. Varadarajan, *J. Chem. Soc.*, 868 (1957); D. M. Brown, D. B. Parihar, A. R. Todd, and S. Varadarajan, *ibid.*, 3028 (1958).

(11) The authors are indebted to Dr. E. B. Whipple of the Union Carbide Research Institute, Tarrytown Site, N. Y., for the n.m.r. data.

(12) R. U. Lemieux, R. K. Kullnig, H. J. Bernstein, and W. G. Schneider, *J. Am. Chem. Soc.*, **80**, 6098 (1958).

(13) This coupling constant is consistent with the C1 conformation in which the projected dihedral angle between H-1′ and H-2′ is ~45°.[14]

(14) See L. D. Hall, *Advan. Carbohydrate Chem.*, **19**, 51 (1964), for a general discussion of angular dependence of coupling constants.

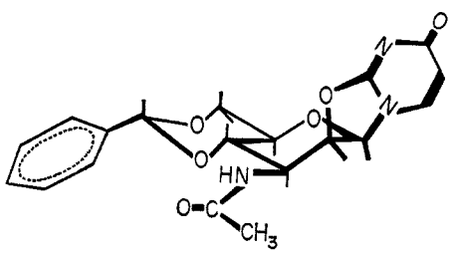


Figure 1.

galacto derivative (XVI) would arise if the reaction proceeded *via* a 2,4′-anhydro nucleoside intermediate (XVa). In a recent report, zu Reckendorf[15] showed that treatment of the glucoside derivative XIX (in which the juxtaposition of the 3-mesylate and 2-benzamido groups is diequatorial) with sodium methylate gave almost exclusively the oxazoline XXI as expected. However, when XIX was treated with potassium cyanide in N,N-dimethylformamide (DMF) at 100°, a 17% yield of the crystalline aziridine XX was ob-

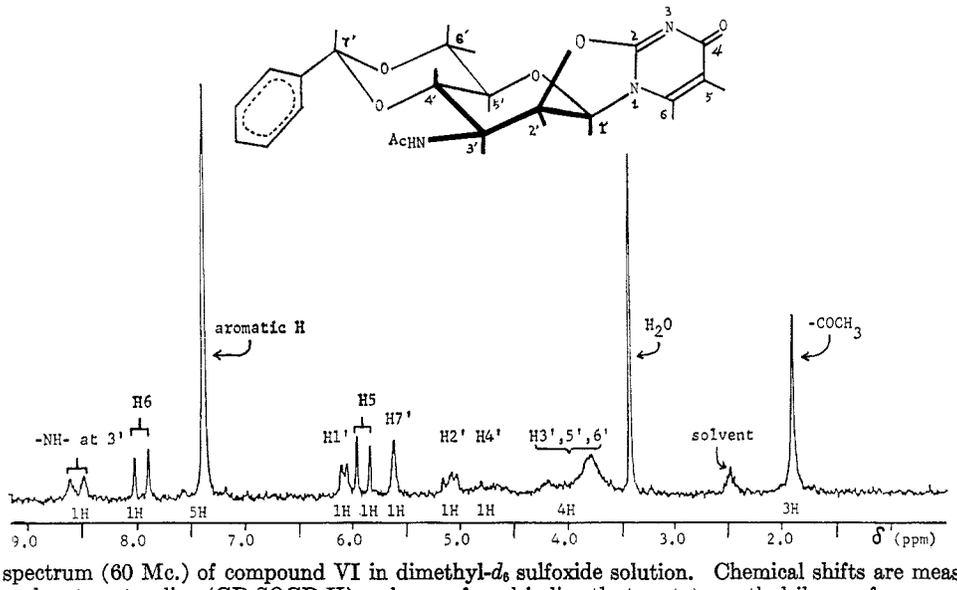


Figure 2.—N.m.r. spectrum (60 Mc.) of compound VI in dimethyl-d_6 sulfoxide solution. Chemical shifts are measured from a weak solvent proton line (CD_3SOCD_2H) and are referred indirectly to a tetramethylsilane reference.

of the N-acetyl group by refluxing IX with Dowex 50 (H^+) in water and elution of product with dilute hydrochloric acid. The ultraviolet absorption of X was similar to that for the gluco isomer II. Compound X differed from the hydrochloride of II as expected with respect to optical rotation (X, 51°; II, 33°) and mobility in paper electrophoresis (borate buffer, pH 9.2).

Compound IV also served as a chemical precursor for the synthesis of the 3′-aminogalacto nucleoside XVI and might be useful for the synthesis of 4′-amino-4′-deoxyhexosyl nucleosides. (See Scheme II.) Acetylation of IV with acetic anhydride in pyridine suspension gave a high yield of diacetate XI which, like IV, was insoluble in common organic solvents and exhibited a high melting point (316–318°). The 4′,6′-*O*-benzylidine group was removed by refluxing XI in 80% aqueous acetic acid to yield crystalline XII which was tritylated to XIII. Mesylation of XIII with methanesulfonyl chloride in pyridine afforded the mesylate XIV in good yield. Attempts to obtain crystalline XIV were unsuccessful. However, compound XIV exhibited a strong absorption band at ∽8.5 μ for the sulfonyl group and two strong phenyl absorption bands for the trityl group at 13.1 and 14.2 μ and was of sufficient purity for subsequent conversions.

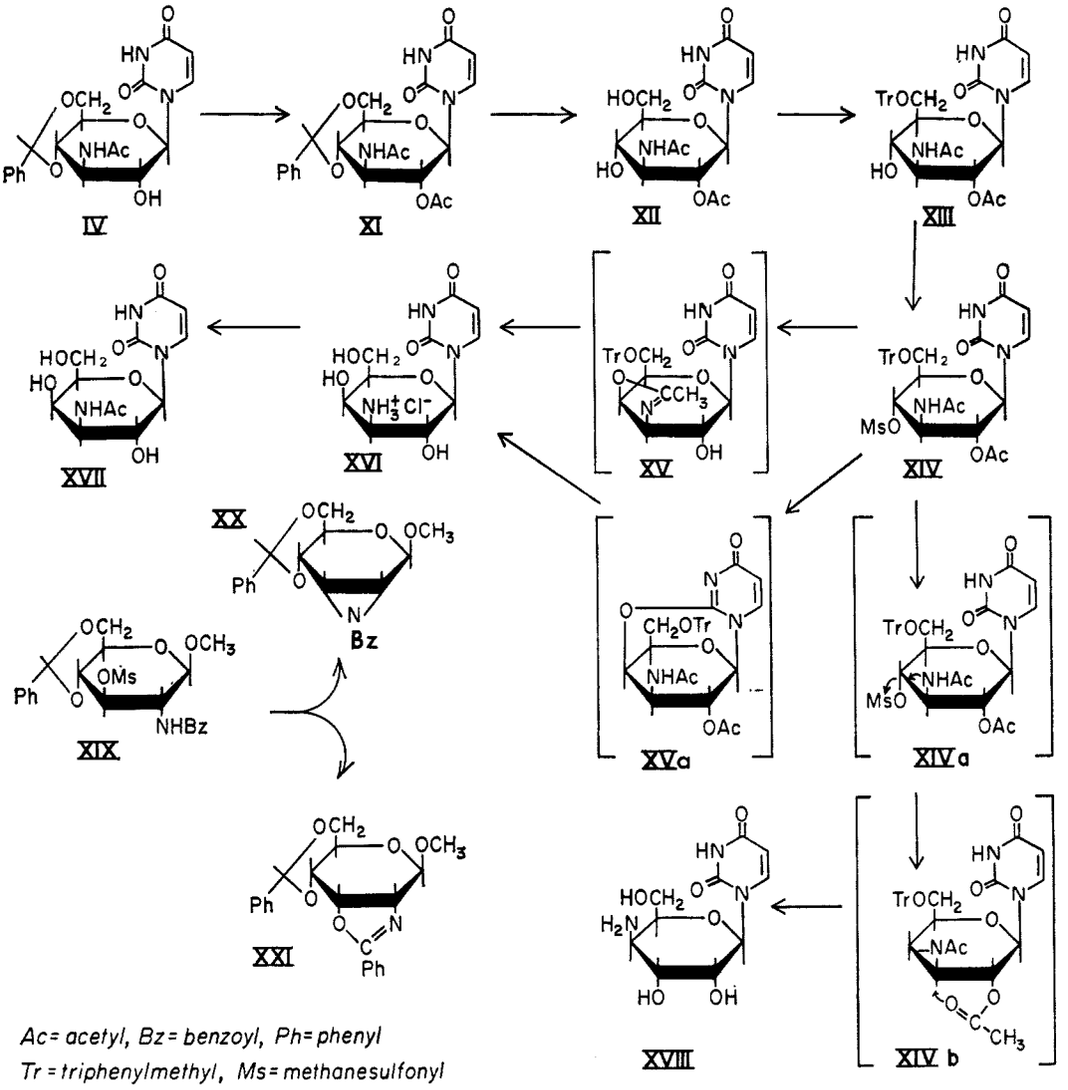
The arrangement of the 3′,4′ substituents in XIV is diequatorial (as in V of Scheme I). Nucleophilic displacement of the mesyloxy function could proceed in several ways. Treatment of XIV with alkoxide could give rise to an oxazoline intermediate (XV) which upon subsequent hydrolyses would yield the 3′-aminogalacto nucleoside XVI. Alternatively, the same tained together with a 63% yield of the oxazoline XXI. It is conceivable, then, that XIV might also lead to some aziridine formation (*e.g.*, XIVb) when reacted with potassium cyanide in DMF. This aziridine should then undergo a second displacement on C-3′ by the 2′-acetoxy group to form, after hydrolyses, the 4′-aminogulo nucleoside XVIII. Thus, in the reaction of XIV with KCN in DMF, at least three routes are possible, proceeding *via* XV, XVa, and/or XIVa,b.

Compound XIV was treated with potassium cyanide in DMF at 100° for 5 hr. according to zu Reckendorf's procedure. The reaction mixture became very dark and no crystalline organic compound could be isolated. This tarry mixture, which gave a positive ninhydrin test, was detritylated with 80% acetic acid and deacetylated with methanolic ammonia. A syrup was obtained which by paper electrophoresis showed at least four components. The residue was then refluxed with Dowex 50 (H^+) to remove any remaining N-acetyl groups. The resin containing mixed amino nucleosides was packed in a column and eluted with 0.04 *N* HCl to give one fraction (A) which was electrophoretically pure. A second fraction (B) was obtained by elution with 0.06 *N* HCl. Fraction B was not pure and showed three components in paper electrophoresis, none of which was similar to the component of fraction A.

From fraction A a crystalline compound (XVI) was obtained which gave elemental analyses and an ultraviolet spectrum at several pH values consistent with a hydrochloride salt of an aminohexosyl nucleoside.

(15) W. M. zu Reckendorf, *Chem. Ber.*, **97**, 325 (1964).

SCHEME II



It differed in optical rotation and in migration in paper electrophoresis from the parent gluco nucleoside II or the manno isomer X. *N*-Acetylation of XVI gave XVII which did not consume metaperiodate over a 5-day period. It is thus established that compound XVI is a 3′-amino-3′-deoxyhexosyl nucleoside. Since XVI is not the gluco (II) [or manno (X)] nucleoside, it must be 1-(3′-amino-3′-deoxy-β-D-galactopyranosyl)-uracil. It is not determined whether XV or XVa were intermediates in the synthesis of XVI from XIV. This aspect will be pursued in this laboratory.

Fraction B [from the Dowex 50 (H^+) column], in which three components were present, was concentrated to a syrup and *N*-acetylated[16,17] to a yellow glass. Attempts to obtain crystalline *N*-acetate derivatives were unsuccessful.

The yellow glass was subjected to periodate oxidation. A slow uptake of 0.4 mole of oxidant/mole[18,19] was observed over a 5-day period. The consumption of oxidant is best explained by the presence of 4′-acetamido-4′-deoxyhexosyluracil in the yellow glass obtained from fraction B and supports the conclusion that the aziridine pathway (XIVa,b) occurred to a significant extent in the reaction of XIV with potassium cyanide in refluxing DMF. The *slow* uptake of oxidant is also noteworthy. We have previously observed in our studies[5] on the nucleoside antibiotic, gougerotin, that the *N*-acetyl derivative of 1-(4′-amino-4′-deoxy-β-D-galactopyranosyl)cytosine[20] also consumes periodate slowly and required several days for completion.

Although we were unable to obtain crystalline material from the *N*-acetylation of fraction B, the data obtained thus far indicate that the synthetic approach to convert a 3′-aminoglucosyl derivative (*e.g.*, XIV) to a 4′-aminohexosyluracil has merit and is worthy of further study.

As mentioned previously,[2b] the reaction of the dialdehyde obtained from uridine (Scheme I) with nitromethane should yield, in addition to the nitro-

(16) *N*-Acetylation of this syrup and of XVI was done by the procedure of Roseman and Ludowieg[17] using acetic anhydride in water with Dowex 1 (CO_3^{2-}). To avoid possible contamination of product(s) with any *O*-acetylated by-products, the resulting syrup was treated with dilute alkali and then with Dowex 50 (H^+) to remove any free amino nucleoside. (See Experimental Section.)

(17) S. Roseman and J. Ludowieg, *J. Am. Chem. Soc.*, **76**, 301 (1954).

(18) Concentration of substrate in these periodate oxidation studies was determined by ultraviolet extinction coefficients with the assumption that the ϵ_{max} of *N*-acetylated 4′-(or 3′-) aminohexopyranosyluracils will be essentially similar to that for hexopyranosyluracils.[19]

(19) J. J. Fox and D. Shugar, *Biochim. Biophys. Acta*, **9**, 369 (1952).

(20) This substance is listed as compound IV in ref. 5. As pointed out in this reference, the hexosyl moiety of IV (previously reported by others as a 3′-amino-3′-deoxyallosyl sugar derivative) is actually of the 4′-amino-4′-deoxygalactosyl configuration.[5]

gluco nucleoside I, the galacto, manno, and talo isomers. It was pointed out further that water played a critical role in the production of isomers during the neutralization of the *aci*-nitro nucleoside to I. If the neutralization of the nitronate is carried out in aqueous alcohol, a high yield of the crystalline nitroglucosyl derivative I is obtained. If, however, nonaqueous conditions are employed, no crystalline nucleoside is obtained. Baer[3] had first observed the mutarotation of nitronates of glycosides. We have also observed the mutarotation of the nucleoside nitronate of I in dilute aqueous alkali. From these data it is clear that the formation of nitrohexose isomers is highly dependent upon neutralization conditions and suggests that a higher proportion of isomers other than the gluco derivative I might be obtained by neutralization of the *aci*-nitro mixture under relatively anhydrous conditions.

A suspension of the nitronate mixture derived from uridinedialdehyde was neutralized in absolute alcohol with "anhydrous" Dowex 50 (H^+) which was prewashed with anhydrous alcohol. Crystalline nitrogluco nucleoside I could not be isolated from the reaction mixture. The mixture was hydrogenated with Raney nickel to give a mixture of 3′-aminohexosyl nucleosides which was subjected to ion-exchange chromatography [Dowex 50 (H^+)]. Elution with 0.02 *N* hydrochloric acid yielded two main fractions. The first of these fractions contained only one isomer and, after removal of solvent, yielded the crystalline galacto isomer XVI identical with that obtained by the synthetic route (IV → XVI, see Scheme II). The second fraction yielded crystalline material which was a mixture of the gluco (II) and galacto (XVI) nucleoside isomers. It is highly probable that other isomers (manno and talo) are present in the original reaction mixture.

Experimental Section[21]

1-(3′-Acetamido-3′-deoxy-4′,6′-*O*-benzylidene-β-D-glucosyl)uracil (IV).—The *N*-acetyl derivative III[2] (2.31 g., 0.0073 mole) was shaken with a mixture of freshly distilled benzaldehyde (23 ml.) and freshly fused and pulverized zinc chloride (2.3 g.) at room temperature overnight. The clear mixture was diluted with 200 ml. of ether. The precipitate was collected by decantation and washed with 100 ml. of 5% ethylenediaminetetraacetic acid (EDTA) disodium salt solution. The solid was then washed with 50 ml. of water and 50 ml. of acetone. Finally the white solid was suspended in 30 ml. of acetone and refluxed gently for 30 min. The white amorphous material changed to fine colorless needles, 2.56 g. (85%), m.p. 303–305° (to a brown liquid). For analysis a small amount of the compound was recrystallized from a large amount of acetone. Fine needles were obtained, m.p. 306–308°. Compound IV is insoluble in common organic solvents.

Anal. Calcd. for $C_{19}H_{21}N_3O_7$: C, 56.57; H, 5.26; N, 10.41. Found: C, 56.26; H, 5.22; N, 10.18.

1-(3′-Acetamido-3′-deoxy-2′-*O*-mesyl-4′,6′-*O*-benzylidene-β-D-glucosyl)uracil (V).—Compound IV (2.6 g., 0.0065 mole) was suspended in 50 ml. of pyridine. To the ice-cold suspension was added 0.7 ml. of methanesulfonyl chloride with stirring. The stirring was continued overnight at room temperature. A small amount of insoluble material was removed by filtration. The filtrate was evaporated to near dryness at room temperature under reduced pressure, and treated with 25 g. of ice–water. Precipitation of a white solid was induced by scratching the wall of the flask. The crude product, 2.75 g. (89%), was recrystallized from methanol and afforded 1.9 g. of colorless needles, m.p. 198–200°. From the mother liquor, an additional 0.6 g. of the same material was obtained.

Anal. Calcd. for $C_{20}H_{23}N_3O_9S$: N, 8.73; S, 6.65. Found: N, 8.95; S, 6.78.

2,2′-Anhydro-1-(3′-acetamido-3′-deoxy-4′,6′-*O*-benzylidene-β-D-mannosyl)uracil (VI).—The methanesulfonyl derivative V (1.60 g., 0.0033 mole) was treated with 110 ml. of ethanol, 16 ml. of pyridine, and 34 ml. of 0.1 *N* sodium ethoxide in ethanol. The mixture was refluxed for 30 min., after which the solvent was evaporated to dryness under reduced pressure at room temperature. The syrupy residue was triturated with 20 ml. of water. Colorless needles of anhydro nucleoside VI, 802 mg. (63%), m.p. 221–225°, were obtained. Recrystallization of this material from ethanol–water afforded analytically pure material (615 mg., 48%): sintered at 209° and melted at 226°; λ_{max}^{EtOH} 245, 225 mμ (ε 8800, 10,300).

Anal. Calcd. for $C_{19}H_{19}N_3O_6$: C, 59.27; H, 4.94; N, 10.91. Found: C, 58.95; H, 5.20; N, 10.61.

1-(3′-Acetamido-3′-deoxy-4′,6′-*O*-benzylidene-β-D-mannosyl)isocytosine (VII).—The anhydro nucleoside VI (0.178 g., 0.46 mmole) was mixed with *ca.* 25 ml. of liquid ammonia in a glass-lined steel bomb. After 4 days at room temperature, a homogeneous reaction mixture was obtained. Most of the ammonia was removed by evaporation at room temperature, and the residual white solid was placed under vacuum to remove traces of ammonia. The residue was crystallized from ethanol. The yield of colorless rods was 161 mg. (86%): m.p. 266–269° dec., $\lambda_{max}^{90\%\ EtOH}$ 252 mμ (ε 6400), $\lambda_{max}^{10\%\ 1\ N\ HCl\ in\ EtOH}$ 255 mμ (ε 8600). No distinct maximum was observed in alkaline media.

Anal. Calcd. for $C_{19}H_{21}N_4O_6 \cdot 1.5H_2O$: C, 53.27; H, 5.61; N, 13.08. Found: C, 53.24; H, 5.88; N, 12.67.

1-(3′-Acetamido-3′-deoxy-4′,6′-*O*-benzylidene-β-D-mannosyl)uracil (VIII).—To an ethanolic solution (30 ml.) of anhydro nucleoside VII (0.577 g., 1.5 mmoles) was added 15 ml. of 0.1 *N* sodium hydroxide. The ultraviolet absorption spectrum of the mixture immediately changed to a uridine-like curve (maximum at ∽260 mμ with loss of maxima at 225 and 245 mμ). The reaction mixture was neutralized by addition of 2 ml. of Dowex 50 (H^+) and stirred for 3 min. The resin was filtered and washed with 20 ml. of methanol. The combined filtrate and washings were evaporated to dryness *in vacuo*. A colorless crystalline residue was obtained which, upon recrystallization from methanol–water, gave colorless needles, 492 mg. (81%), m.p. 297–299°.

Anal. Calcd. for $C_{19}H_{21}N_3O_7$: C, 56.58; H, 5.21; N, 10.42. Found: C, 56.29; H, 5.45; N, 10.20.

1-(3′-Amino-3′-deoxy-β-D-mannopyranosyl)uracil Hydrochloride (X).—The benzylidene mannoside VIII (398 mg., 0.99 mmole) was refluxed with 20 ml. of 80% aqueous acetic acid for 1 hr. The solvent was evaporated to dryness under reduced pressure. In order to remove benzaldehyde, 30 ml. of water was added to the residue and the mixture was evaporated to dryness under reduced pressure at ∽70°. This procedure was repeated four times, and finally the residue was dissolved in 30 ml. of water. A very small amount of insoluble amorphous material was filtered and the filtrate was evaporated to dryness under reduced pressure. The colorless syrup obtained was dried azeotropically with 30 ml. of toluene under reduced pressure. A pale yellow glass of IX, 310 mg. (quantitative yield), was obtained. Attempts to crystallize the glass from several solvents or solvent systems were unsuccessful. Descending paper chromatography in the solvent system, 1-butanol–acetic acid–water (5:2:3), showed a single spot with an R_f of 0.47.

The colorless glass IX (158 mg.) was dissolved in 20 ml. of water. About 5 ml. of Dowex 50 (H^+) was added to the solution and the mixture was refluxed for 4 hr. with stirring. The resin was then packed in a small column and washed with 300 ml. of water. The column was then treated with 1 *N* hydrochloric acid (300 ml.). The eluates were evaporated under reduced pressure. The residual syrup was crystallized from methanol and ethanol. Fine colorless needles of X were obtained, 52 mg., m.p. 212–218° dec., $[\alpha]^{23}D$ +51° (*c* 0.79, water), R_f 0.30 (BuOH–AcOH–H_2O, 5:2:3, descending paper chromatography). The electrophoretic migration of X was +5.1 cm. (pH 9.2 borate buffer, 900 v., 8 hr.).

Anal. Calcd. for $C_{10}H_{15}N_3O_6 \cdot HCl \cdot H_2O$: C, 36.64; H, 5.49; N, 12.82. Found: C, 36.54; H, 5.44; N, 12.70.

(21) Analyses were performed by Galbraith Laboratories, Inc., Knoxville, Tenn., and by Spang Microanalytical Laboratory, Ann Arbor, Mich. All melting points are corrected. Electrophoretic data were obtained using Whatman 3 MM paper.

1-(3′-Acetamido-3′-deoxy-2′-*O*-acetyl-4′,6′-*O*-benzylidene-β-D-glucosyl)uracil (XI).—A mixture of IV (2.40 g., 0.006 mole) and acetic anhydride (10 ml.) in 50 ml. of pyridine was shaken overnight at room temperature. A clear reaction mixture was obtained. The solvent was removed under reduced pressure at about 50°. The semisolid residue was dissolved in 50 ml. of hot pyridine and treated with charcoal. To the filtrate was added an equal volume of petroleum ether (b.p. 30–60°). A gel-like material precipitated, which, on standing overnight at room temperature, changed to colorless needles, 2.02 g. (76%), m.p. 316–318°. Crystalline XI is sparingly soluble in ether, chloroform, methanol, ethanol, acetone, and pyridine.

Anal. Calcd. for $C_{21}H_{23}N_3O_8$: C, 56.63; H, 5.17; N, 9.44. Found: C, 56.57; H, 5.31; N, 9.18.

1-(3′-Acetamido-3′-deoxy-2′-*O*-acetyl-β-D-glucopyranosyl)uracil (XII).—Crystalline XI (1.10 g., 2.4 mmoles) was refluxed in 30 ml. of 80% aqueous acetic acid for 30 min. The solvent was evaporated *in vacuo*. The residue, which had a strong benzaldehyde odor, was treated with 30 ml. of water and the benzaldehyde was removed by steam distillation under reduced pressure at *ca.* 70°. Finally, the residue was dried by azeotropic distillation with 30 ml. of toluene at *ca.* 70° *in vacuo*. The residue was crystallized from a large amount of methanol to yield XII, 0.34 g. (39%), m.p. 253–256° dec. From the mother liquor, ~500 mg. of a glass was recovered, but attempts to crystallize this glass were unsuccessful.

Anal. Calcd. for $C_{14}H_{19}N_3O_8$: C, 47.06; H, 5.32; N, 11.76. Found: C, 47.18; H, 5.46; N, 11.56.

1-(3′-Acetamido-3′-deoxy-2′-*O*-acetyl-6′-*O*-trityl-β-D-glucopyranosyl)uracil (XIII).—Crystalline XII (432 mg., 0.0012 mole) was dissolved in 20 ml. of anhydrous pyridine. To the solution was added 298 mg. (0.0011 mole) of trityl chloride and the reaction was shaken for several minutes. The homogeneous mixture was allowed to remain overnight at ~60°. The reaction mixture was poured into a mixture of ice–water and stirred vigorously for 5 min. The white solid which precipitated was filtered and washed with 50 ml. of cold water. The solid was suspended in 50 ml. of a 1:1 mixture of ether and petroleum ether. The suspension was stirred vigorously for 5 min. and filtered. The white powder was dried *in vacuo* at room temperature for 4 hr., then at 40° for 2 hr., and finally at 60° overnight. A fluffy white powder, 596 mg. (82%), was obtained. Crystallization of the powder from several solvents or solvent systems was unsuccessful. The powder liquified gradually at about 202–213° to a brown oil.

Anal. Calcd. for $C_{33}H_{33}N_3O_8$: C, 66.09; H, 5.51; N, 7.01. Found: C, 65.60; H, 5.50; N, 6.94.

1-(3′-Acetamido-3′-deoxy-2′-*O*-acetyl-4′-*O*-mesyl-6′-*O*-trityl-β-D-glucosyl)uracil (XIV).—To the stirred ice-cooled solution of XIII (421 mg., 0.7 mmole) in 20 ml. of anhydrous pyridine was added methanesulfonyl chloride (0.7 ml., 0.9 mmole). Cooling and stirring were continued for 2 hr. The reaction was then allowed to remain overnight at room temperature. The mixture was poured onto 100 ml. of ice–water and shaken vigorously for 5 min. The white precipitate was filtered and washed with 50 ml. of cold water and 50 ml. of a 1:1 mixture of ethanol and petroleum ether. The powder was dried under reduced pressure at ~40° for 2 hr. A fluffy white powder of XIV, 402 mg. (85%), was obtained. Attempts to crystallize the powder from several solvents or solvent systems were unsuccessful. Purification was accomplished by dissolving the material in a minimum amount of ethanol followed by addition of a large amount of petroleum ether to effect precipitation: λ_{max}^{KBr} 3.0 (–NH–), 5.7 (*O*-acetyl), 5.9 (*N*-acetyl), 8.5 (sulfonic ester), 9.2, 9.4, 9.7 (–C–O–C–), 13.1, and 14.2 μ (phenyl).

Reaction of XIV with Potassium Cyanide in DMF. A. Isolation of 1-(3′-Amino-3′-deoxy-β-D-galactopyranosyl)uracil Hydrochloride (XVI).—A stirred mixture of XIV (502 mg., 0.74 mmole), potassium cyanide (482 mg., 7.4 mmoles), and 50 ml. of DMF was heated to 95–105° for 5 hr. Within 5 min. the reaction became homogeneous. The solution gradually darkened during the reaction period. The reaction mixture was poured onto 70 g. of stirred crushed ice and stored in the refrigerator overnight. The mixture became slightly turbid. The mixture was evaporated to dryness under reduced pressure at ~40°. Chloroform (30 ml.) was added to the residue and the insoluble material (506 mg.) was filtered and washed with 30 ml. of chloroform. The filtrate and washings were combined and dried over anhydrous magnesium sulfate. The dark chloroform solution was concentrated to dryness under reduced pressure to a dark syrup, 380 mg., which was dissolved in 30 ml. of 80% aqueous acetic acid and refluxed for 45 min. The solvent was evaporated to dryness under reduced pressure and the residue was dried further azeotropically with 30 ml. of toluene. The residue was extracted with ether (three 30-ml. portions). (From the ether extracts crystalline triphenylcarbinol was obtained.) The residue was dissolved in 30 ml. of methanol. The solution was cooled to 0° and then saturated with ammonia. A dark-colored amorphous substance precipitated which was insoluble in water and, since it showed no selective absorption in the ultraviolet, was filtered and discarded. The orange filtrate was evaporated to dryness under reduced pressure. The residue (163 mg.) was dissolved in 30 ml. of water and refluxed with 3 ml. of Dowex 50 (H^+) (200–400 mesh) for 4 hr. The resin was put onto the Dowex 50 (H^+) (200–400 mesh) column (1.2 × 19 cm.). The column was developed as follows: 500 ml. of water, 1000 ml. of 0.04 *N* HCl, and finally 0.06 *N* HCl (1000 ml.). The fraction eluted by 0.04 *N* HCl (fraction A) contained 2200 total optical density (T.O.D.) units. Evaporation of the solvent gave 72 mg. of a syrup XVI which crystallized from a small volume of methanol to colorless needles, m.p. 234° eff., $[\alpha]^{23}D$ +67° (*c* 0.76, water). Electrophoretic migration in borate buffer (pH 9.2, 900 v., 8 hr.) was +12.1 cm. (Under similar conditions, X migrated +5.1 cm. and II migrated +6.0 cm.)

Anal. Calcd. for $C_{10}H_{15}N_3O_6 \cdot HCl \cdot H_2O$: C, 36.64; H, 5.49; N, 12.82. Found: C, 36.90; H, 5.30; N, 12.96.

The crystalline amino nucleoside XVI (33.8 mg.) was dissolved in a mixture of 1.0 ml. of water and 0.1 ml. of methanol. To the solution was added 1.2 ml. of Dowex 1 (CO_3^{2-}) and 0.025 ml. of acetic anhydride. The mixture was stirred overnight below 10°. The resin was filtered and washed with 2 ml. of cold water. The combined filtrate and washings were evaporated to dryness under reduced pressure. A colorless solid was obtained, which contained a small amount of impurity which migrated at higher R_f [paper chromatography BuOH–H_2O–AcOH (5:3:2), R_f 0.48 (main spot), R_f 0.69 (impurity)]. The colorless solid was dissolved in 2 ml. of 0.5 *N* NaOH, which, on standing at room temperature for 1 hr., gave a single spot on paper chromatography. Dowex 50 (H^+), 0.5 ml., was added and the mixture stirred for 3 min. at room temperature and filtered. The resin was washed with cold water (2 ml.). The washings and filtrate were combined and evaporated to dryness under reduced pressure. A colorless solid was obtained (32 mg.), which did not consume any metaperiodate over a 5-day period.

B. Evidence for the Presence of 4′-Amino Nucleoside XVIII.—The fraction of 0.06 *N* HCl (fraction B, T.O.D. = 1500 units) was concentrated to dryness to afford 49 mg. of a syrup. The syrup contained at least three components which were detected by paper electrophoresis (migrations in borate buffer, pH 9.2, 900 v., 8 hr.: +14.5, 7.3, and 4.7 cm.]. The syrup was dissolved in 1.3 ml. of water and treated with 0.13 ml. of methanol, 1.3 ml. of Dowex 1 (CO_3^{2-}), and 0.03 ml. of acetic anhydride. The mixture was stirred overnight in the cold. The resin was filtered and washed with 2 ml. of cold water. To the combined filtrate and washings was added 3 ml. of 1 *N* NaOH and the mixture was kept standing for 1 hr. at room temperature. Dowex 50 (H^+) (0.7 ml.) was added to the alkaline mixture and stirred for 5 min. The resin was filtered and washed with 2 ml. of water. The combined filtrate and washings were evaporated to dryness. The residue (30.2 mg.) gave a negative ninhydrin test on paper consistent with an *N*-acetate structure. The syrup was subjected to metaperiodate titration. A slow uptake of oxidant was observed which was completed at 96 hr. at which time a total of 0.39 mole of periodate/mole was consumed.

Direct Preparation of 1-(3′-Amino-3′-deoxy-β-D-galactopyranosyl)uracil Hydrochloride (XVI). From Uridine.—Uridine (9.75 g., 0.04 mole) was dissolved in 40 ml. of water and treated while stirring with sodium metaperiodate (9.56 g., 5% excess) in water (40 ml.). After 5 hr. the reaction was poured into 500 ml. of vigorously stirred ethanol. Precipitated inorganic salts were removed and washed with 100 ml. of ethanol. The combined filtrate and washings were evaporated to dryness under reduced pressure at about 40°. The glassy "dialdehyde" was dried by azeotropic distillation with 100 ml. of benzene and then dissolved in a mixture of 80 ml. of anhydrous ethanol and 2.2 ml. of nitromethane. To the stirred mixture was added 27.5 ml. of 3% methanolic solution of sodium methoxide. The sodium salts of *aci*-nitro compounds (nitronates) precipitated. The stirring was continued for 30 min. after methoxide addition was com-

pleted. Anhydrous Dowex 50 (H^+) (washed with anhydrous methanol and dried) (40 ml.) was added to the reaction mixture and stirred vigorously until complete disappearance of the nitronate precipitates was observed (2 hr.). The resin was filtered and washed with 80 ml. of anhydrous ethanol. The filtrate and washings were combined and hydrogenated with 40 g. (wet wt.) of activated alcoholic Raney nickel at an initial pressure of 3 atm. The uptake of hydrogen ceased within 2 hr. The catalyst was filtered and the filtrate was diluted to 2 l. with deionized water. The solution (T.O.D. of 330,000 units) was passed through a column of Dowex 50 × 8 (H^+) (50–100 mesh) (2.2 × 28 cm.). The column was washed with 500 ml. of water; 99,000 T.O.D. units were unabsorbed on the column and were eluted with water. The column was then eluted with 0.02 *N* HCl. The first 3.6 l. was discarded. The next 5.3 l. of acid eluted fraction 1 which contained 88,000 T.O.D. units and the last 2 l. (fraction 2) contained 89,000 T.O.D. units. The over-all recovery of T.O.D. units from the column was 90%.

Fraction 1 was evaporated under reduced pressure at about 40° to a syrup. The syrup was dissolved in a minimum amount of hot methanol. To the solution was added an equal volume of ethanol. On cooling to room temperature, precipitation occurred. The semisolid was collected by decantation and again dissolved in a minimum amount of hot methanol and a few drops of ethanol. The solution was kept standing for 2 days at 0–5°. The precipitated solid was filtered and crystallized from methanol to yield 302 mg. of colorless needles, m.p. 234–235° eff., $[\alpha]^{23}D$ +67° (*c* 0.68, water).

Anal. Calcd. for $C_{10}H_{15}N_3O_6 \cdot HCl \cdot H_2O$: C, 36.64; H, 5.49; N, 12.82. Found: C, 36.84; H, 5.50; N, 13.05.

From the mother liquor of crystallization an additional 624 mg. of the same compound was obtained.

Electrophoretic migration (+12.1 cm., borate buffer, pH 9.2, 900 v., 8 hr.) and the optical rotation of this material were identical with those of the galacto derivative XVI.

Evaporation of fraction 2 afforded a syrup which was dissolved in a small amount of methanol. The solution was treated with a twofold volume of ethanol. The precipitate was gathered by decantation and was again dissolved in a small amount of hot methanol and treated with ethanol. This procedure was repeated once more and the mixture was kept at ~0° for several hours. Colorless needles crystallized, 276 mg., m.p. 212–214°, $[\alpha]^{23}D$ +52° (*c* 0.59, water). From the mother liquor an additional 775 mg. of crystalline material was obtained.

Anal. Calcd. for $C_{10}H_{15}N_3O_6 \cdot HCl \cdot 0.5\ H_2O$: C, 37.68; H, 5.37; N, 13.19. Found: C, 37.52; H, 5.60; N, 13.25.

Paper electrophoresis showed that the crystalline material from fraction 2 was a mixture of the gluco II and the galacto XVI derivatives. No mannosyl derivative X was detected electrophoretically in this crystalline material.

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Synthetic Nucleosides. LXIX.[1,2] Synthesis of Some New Types of Branched-Chain Amino Sugars

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Reaction of methyl 3-benzamido-4,6-*O*-benzylidene-3-deoxy-α-D-*arabino*-hexopyranosid-2-ulose (III) with methylmagnesium iodide proceeded by a stereospecific axial attack to give methyl 3-benzamido-4,6-*O*-benzylidene-3-deoxy-2-*C*-methyl-α-D-glucopyranoside (IV) in 82% yield; since III is readily obtained from methyl α-D-glucopyranoside *via* oxidation of methyl 3-benzamido-4,6-*O*-benzylidene-3-deoxy-α-D-altropyranoside (II) with the Pfitzner–Moffatt reagent, this sequence readily leads to amino sugar derivatives with a branch on a carbon not bearing the amino group. A similar Grignard reaction with methyl 2-benzamido-4,6-*O*-benzylidene-2-deoxy-α-D-*ribo*-hexopyranosid-3-ulose (VIII) proceeded by equatorial attack with formation of methyl 2-benzamido-4,6-*O*-benzylidene-2-deoxy-3-*C*-methyl-α-D-allopyranoside (X) in 65% yield; thus compounds of type X are also fairly accessable from methyl α-D-glucopyranoside *via* oxidation of methyl 2-benzamido-4,6-*O*-benzylidene-2-deoxy-α-D-altropyranoside (IX) to VIII.

As yet, no branched-chain amino sugars have been found in nature; since many unusual amino sugars[3] and many branched-chain sugars[4] are found in antibiotics or bacterial cell walls, it does not seem unreasonable that branched-chain amino sugars will ultimately be found in nature. Even though the first structure determinations will be made with the aid of n.m.r. spectroscopy and mass spectrometry, new synthetic methods for such branched-chain amino sugars are worthy of exploration; such branched-chain amino sugars could also be converted to unusual nucleosides which might have interesting biological properties.

Two groups of investigators[5,6] have independently developed a route to sugars containing the amino and branched moieties on the same carbon by a variation of the now classical sugar dialdehyde–nitromethane route[7]; by its synthetic nature, this route is limited to branching on the carbon bearing the amino group. A new route to branched-chain amino sugars has now been developed which is complementary to the nitroalkane route[5–7] in that branching can presumably be introduced on a carbon of the sugar not bearing the amino group; this new route is the subject of this paper.

In a recent publication from this laboratory,[8] the oxidation of either methyl 3-benzamido-4,6-*O*-benzylidene-3-deoxy-α-D-glucopyranoside (I) or methyl 3-benzamido-4,6-*O*-benzylidene-3-deoxy-α-D-altropyranoside (II) with phosphoric acid and dicyclohexylcarbodiimide in dimethyl sulfoxide—the Pfitzner–

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(2) Paper LXVIII: B. R. Baker and T. L. Hullar, *J. Org. Chem.*, **30**, 4053 (1965).

(3) R. W. Jeanloz and E. A. Balasz, Ed., "The Amino Sugars," Academic Press Inc., New York, N.Y., 1964.

(4) J. Staněk, M. Černý, J. Kocourek, and J. Pacak, "The Monosaccharides," Academic Press Inc., New York, N. Y., 1963, p. 83.

(5) S. W. Gunner, W. G. Overend, and N. R. Williams, *Chem. Ind.* (London), 1523 (1964).

(6) (a) H. H. Baer and G. V. Rao, *Ann.*, **686**, 210 (1964); (b) H. H. Baer, *Tetrahedron*, **20**, Suppl. 1, 263 (1964).

(7) H. H. Baer and H. O. L. Fischer, *J. Am. Chem. Soc.*, **82**, 3709 (1960); (b) F. W. Lichtenthaler, *Angew. Chem. Intern. Ed. Engl.*, **3**, 211 (1964).

(8) (a) B. R. Baker and D. H. Buss, *J. Org. Chem.*, **30**, 2308 (1965), paper LXIV of this series; (b) *ibid.*, **30**, 2304 (1965), paper LXV of this series.