Derivatives of 4-Amino-4-deoxy-D-glucose[1a]

ELMER J. REIST, ROLAND R. SPENCER,[1b] DIANNE F. CALKINS, B. R. BAKER,[1c] AND LEON GOODMAN

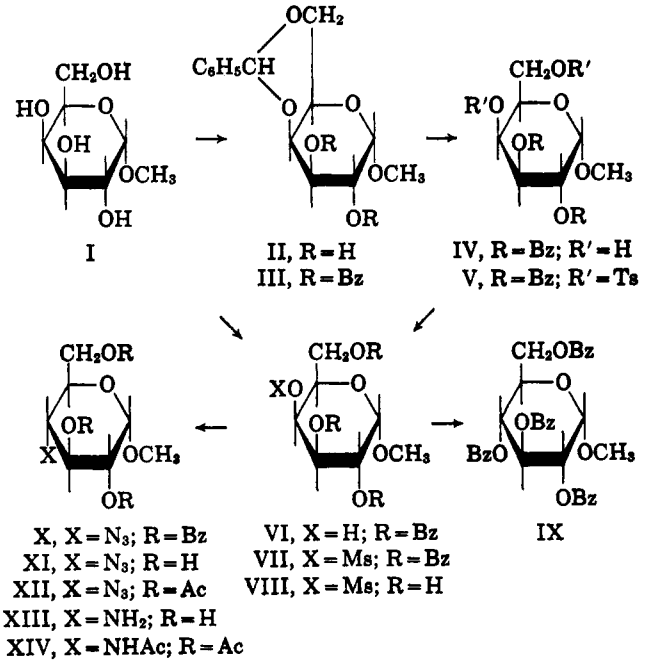
Life Sciences Research, Stanford Research Institute, Menlo Park, California

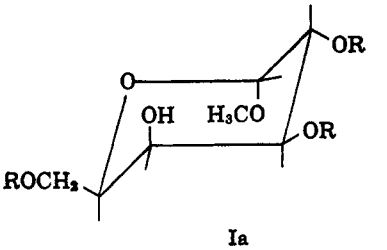
Received January 25, 1965

The displacement of the sulfonate of methyl 2,3,6-tri-O-benzoyl-4-O-methylsulfonyl-α-D-galactopyranoside (VII) by sodium azide or sodium benzoate in N,N-dimethylformamide to give substituted glucosides is described. From methyl 4-azido-2,3,6-tri-O-benzoyl-4-deoxy-α-D-glucopyranoside (X) a number of derivatives of 4-amino-4-deoxy-D-glucose (XVIII) were prepared, including methyl 4-amino-4-deoxy-α-D-glucopyranoside (XIII) and its nitrogen mustard (XXIV) hydrochloride. Efforts to prepare 4-amino-4-deoxy-D-glucose itself or its nitrogen mustard were unsuccessful. Also described is a one-step synthesis of methyl 2,3,6-tri-O-benzoyl-α-D-galactopyranoside (VI) from methyl-α-D-galactopyranoside (I) which involves the selective benzoylation of secondary equatorial hydroxyls in the presence of the secondary axial hydroxyl at C-4.

Over the last few years, the preparation of a number of "bis" and "one-armed" nitrogen mustards of carbohydrates was reported from these laboratories.[2] The biological activity of these mustards in the standard three-tumor screen[2b] was sufficiently encouraging to warrant an investigation of the nitrogen mustards of 4-amino-4-deoxy-D-glucose. At about this same time, Wheat and co-workers[3] reported on the isolation of 4-amino-4,6-dideoxy-D-glucose (viosamine) from *Chromobacterium violaceum* NCTC 7917; Strominger and coworkers[4] described the isolation of this same sugar from *Escherichia coli* B while Stevens, *et al.*,[5] isolated 4,6-dideoxy-4-dimethylamino-D-glucose (amosamine) from the antibiotic amicetin. The isolation and identification of these derivatives of 4-amino-4-deoxy-D-glucose together with the suggestion[5d] of a widespread natural occurrence of this type of amino sugar made it of interest to attempt the synthesis of the parent compound (XVIII).

A logical starting material for the synthesis of 4-substituted glucose derivatives was methyl α-D-galactopyranoside (I)[6] which was easily converted to methyl 4,6-O-benzylidene-α-D-galactopyranoside (II)[7] and then to the dibenzoate III.[8] Catalytic hydrogenation of dibenzoate III gave crude methyl 2,3-di-O-benzoyl-α-D-galactopyranoside (IV) as an amorphous foam which could not be crystallized. Crystalline methyl 2,3-di-O-benzoyl-4,6-di-O-(*p*-tolylsulfonyl)-α-D-galactopyranoside (V)[9a] was obtained when the amorphous dibenzoate IV was treated with *p*-tolylsulfonyl chloride in pyridine. When the ditosylate V was heated with sodium benzoate in N,N-dimethylformamide (DMF) at 140° for 24 hr., methyl 2,3,4,6-tetra-O-benzoyl-α-D-glucopyranoside (IX) was isolated.[9a] This reaction proved not only that the secondary ring sulfonate on C-4 of a sugar could be displaced with inversion by





Ia

this reagent, but that the tosylates of V were indeed on C-4 and C-6 so that there had been no significant amount of benzoate migration during the preparation of IV or during its subsequent tosylation to prepare V. Attempts to effect the selective displacement of the 6-tosylate of V by sodium benzoate in DMF in an effort to prepare methyl 2,3,6-tri-O-benzoyl-4-O-(*p*-tolylsulfonyl)-α-D-galactopyranoside were unsuccessful. The product isolated was apparently a mixture of tetra-

(1) (a) This work was carried out under the auspices of the Cancer Chemotherapy National Service Center, National Cancer Institute, National Institutes of Health, Public Health Service, Contract No. PH 43-64-500. The opinions expressed in this paper are those of the authors and not necessarily those of the Cancer Chemotherapy National Service Center. (b) U. S. Department of Agriculture, Western Regional Research Laboratory, Albany, Calif. (c) School of Pharmacy, University of Buffalo, Buffalo 14, New York.

(2) (a) E. J. Reist, R. R. Spencer, and B. R. Baker, *J. Am. Chem. Soc.*, **82**, 2025 (1960); (b) E. J. Reist, R. R. Spencer, M. E. Wain, I. G. Junga, L. Goodman, and B. R. Baker, *J. Org. Chem.*, **26**, 2821 (1961); (c) E. J. Reist, R. R. Spencer, L. Goodman, and B. R. Baker, *ibid*, **27**, 202 (1962).

(3) (a) R. W. Wheat, E. L. Rollins, and J. M. Leatherwood, *Biochem. Biophys. Res. Commun.*, **9**, 120 (1962); (b) E. J. Smith, J. M. Leatherwood, and R. W. Wheat, *J. Bacteriol.*, **84**, 1007 (1962).

(4) T. Okazaki, R. Okazaki, J. L. Strominger, and S. Suzuki, *Biochem. Biophys. Res. Commun.*, **7**, 300 (1962).

(5) (a) C. L. Stevens, P. Blumbergs, and F. A. Daniher, *J. Am. Chem. Soc.*, **85**, 1552 (1963); (b) C. L. Stevens, K. Nagarajan, and T. H. Haskell, *J. Org. Chem.*, **27**, 2991 (1962); (c) C. L. Stevens, R. J. Gasser, T. K. Mukherjee, and T. H. Haskell, *J. Am. Chem. Soc.*, **78**, 6212 (1956); (d) C. L. Stevens, P. Blumbergs, F. A. Daniher, R. W. Wheat, A. Kujomoto, and E. L. Rollins, *ibid.*, **85**, 3061 (1963).

(6) J. K. Dale and C. S. Hudson, *ibid.*, **52**, 2534 (1930).

(7) A. Müller, M. Móricz, and G. Verner, *Ber.*, **72B**, 745 (1939).

(8) M. Gyr and T. Reichstein, *Helv. Chim. Acta*, **28**, 226 (1945).

(9) Certain portions of this work have been described in preliminary communications: see (a) E. J. Reist, R. R. Spencer, and B. R. Baker, *J. Org. Chem.*, **24**, 1618 (1559); (b) E. J. Reist, R. R. Spencer, B. R. Baker, and L. Goodman, *Chem. Ind.* (London), 1794 (1962).

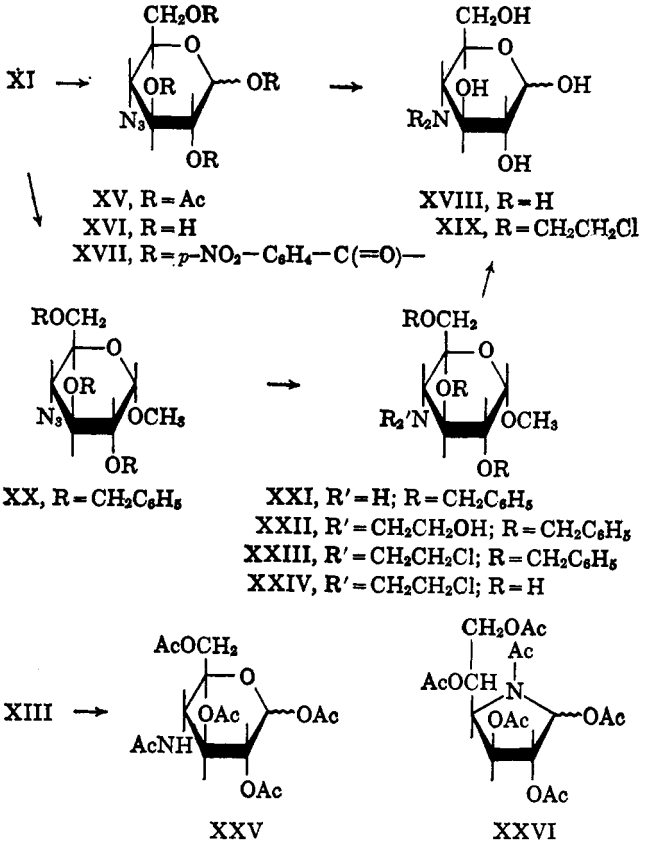
benzoate IX and starting material (V). It is interesting to note that a selective displacement of the 6-tosylate of V by iodide was reported by Stevens, *et al.*,[5a] to give methyl 2,3-di-O-benzoyl-6-deoxy-6-iodo-4-O-(*p*-tolylsulfonyl)galactopyranoside, although chromatography on alumina was required to separate it from the diiodo by-product and starting material.

In view of the unsuccessful efforts to effect a selective displacement by benzoate on the ditosylate for the preparation of a suitable 4-sulfonylated galactoside, the selective benzoylation of the amorphous dibenzoate IV was investigated. Treatment of IV with benzoyl chloride in pyridine at 0° gave a 32% yield of crystalline methyl 2,3,6-tri-O-benzoyl-α-D-galactopyranoside (VI)[9b] which could be easily mesylated to crystalline methyl 2,3,6-tri-O-benzoyl-4-O-methylsulfonyl-α-D-galactopyranoside (VII). That the methylsulfonyl function of VII was on position 4 was shown by the treatment of VII with methanolic sodium methoxide. A crystalline mesylate, presumably methyl 4-O-methylsulfonyl-α-D-galactopyranoside (VIII), was obtained rather than an epoxide or anhydro derivative which would be expected from a mesylate on the 2-, 3-, or 6-hydroxyls of methyl α-D-galactopyranoside under these conditions. Furthermore, the mesylate VII could be displaced with inversion by sodium benzoate in DMF to give methyl 2,3,4,6-tetra-O-benzoyl-α-D-glucopyranoside (IX).

Thus, the key starting material for the preparation of 4-aminoglucose derivatives was available by the five-step sequence outlined. However, on scale-up of the reactions to obtain the necessary quantities of VII, the reactions were unwieldy—particularly the hydrogenation of the benzylidene group of III—and variable yields (0–50%) of the tribenzoate VI were obtained. Examination of a molecular model of methyl α-D-galactopyranoside (I), see Ia, suggests that, in the favored conformation, the C-4 hydroxyl is unique in that it occupies an axial configuration, whereas the remaining secondary hydroxyls at C-2 and C-3 are equatorial. The lower reactivity of axial hydroxyls as compared with equatorial hydroxyls is well known in steroid and alicylic chemistry. That this difference in reactivity applies equally well to carbohydrates was demonstrated by Turvey and Williams[10] in the sulfonation of methyl 2,3-O-benzyl-6-O-trityl-β-D-galactopyranoside with sulfur trioxide in pyridine. They reported a reaction time of 21 hr. to complete the sulfonation, whereas a period of 5 hr. is normally required. They attributed this lower reactivity of the C-4 hydroxyl to its axial orientation in the favored conformation.

It seemed reasonable to expect that the axial C-4 hydroxyl of I would be sufficiently more difficult to benzoylate than the C-2 and C-3 equatorial hydroxyls to permit a selective benzoylation at C-2, C-3, and C-6. Thus, the 2,3,6-tribenzoate VI would be obtained in one step from I. This expectation was realized and the selective benzoylation of I gave a 57% yield of the tribenzoate VI, identical in all respects with VI[9b] obtained by the sequence described above. Yields of at least 55% have been obtained consistently, starting with as much as 130 g. of the glycoside I.

The displacement of the mesylate VII by sodium azide in DMF gave a good yield of methyl 4-azido-2,3,6-tri-O-benzoyl-4-deoxy-α-D-glucopyranoside (X) as a sirup.[9b] A similar reaction in which diethylene glycol dimethyl ether was substituted for DMF gave no product and starting material was recovered quantitatively. Debenzoylation of X with methanolic sodium methoxide afforded crystalline methyl 4-azido-4-deoxy-α-D-glucopyranoside (XI), which, on hydrogenation over 5% palladium on carbon, gave methyl 4-amino-4-deoxy-α-D-glucopyranoside (XIII). Treatment of the glycoside XIII or its tetraacetate XIV with 6 *N* hydrochloric acid in order to obtain 4-amino-4-deoxy-D-glucose (XVIII) itself was unsuccessful. The amorphous product obtained was not homogeneous on paper chromatography and analyzed poorly for XVIII hydrochloride. It seemed reasonable to suspect that the 4-amino group was involved in ring formation to give, eventually, a pyrrole[9b] which would not be stable to the rigors of an acid hydrolysis. Therefore, alternate routes not requiring strong acid treatment were considered for the synthesis of XVIII. Acetolysis of the azide XI gave sirupy 1,2,3,6-tetra-O-acetyl-4-azido-4-deoxy-D-glucose (XV) which had fair analytical values. Deacetylation of XV, however, gave 4-azido-4-deoxy-D-glucose (XVI) as a hygroscopic amorphous foam which was not homogeneous on paper chromatography and which resisted all efforts of purification. Characterization of the crude azide XVI was accomplished



through the crystalline *p*-nitrobenzoate XVII. Regeneration of XVI from the recrystallized *p*-nitrobenzoate XVII again gave amorphous material with exceedingly poor analytical values and which was again nonhomogeneous on paper chromatography. Hydrogenation of the resulting crude azide XVI over palladium on carbon was unsuccessful, and the resulting sirup gave no reducing test with Benedicts reagent or

(10) J. R. Turvey and T. P. Williams, *J. Chem. Soc.*, 2119 (1962).

aniline citrate and appeared to be a hydroxylated pyrrolidine on the basis of an n.m.r. analysis of the sirup and its acetate.[11]

Another route to the preparation of 4-amino-4-deoxy-D-glucose XVIII was suggested by a recent report by Foster and co-workers[12] in which they utilized boron trichloride in dichloromethane to cleave methyl ethers of 2-acetamido-2-deoxy-D-glucose. In the present work boron trichloride was used to cleave both the methyl glycoside function and benzyl ethers employed as blocking groups. The condensation of methyl 4-azido-4-deoxy-α-D-glucopyranoside (XI) with α-chlorotoluene gave crude sirupy methyl 4-azido-2,3,6-tri-O-benzyl-4-deoxy-α-D-glucopyranoside (XX) which was treated directly with lithium aluminum hydride to give methyl 4-amino-2,3,6-tri-O-benzyl-4-deoxy-α-D-glucopyranoside (XXI) isolated as the crystalline *p*-toluenesulfonic acid salt. The free base XXI was regenerated by means of aqueous sodium bicarbonate and was treated with boron trichloride in dichloromethane for 18 hr. in the manner described by Foster, *et al.*[12] Normal work-up conditions in which methanol was used to remove all traces of boric acid resulted in the partial regeneration of a methyl glycoside of XVIII. When methanol was avoided and an ion-exchange resin was used to remove the boric acid, the product isolated analyzed for a diborate complex of XVIII. Retreatment of this product with resin failed to remove any additional inorganic borate.

A third route which could conceivably yield 4-amino-4-deoxy-D-glucose (XVIII) or its N-acetate began with the acetolysis of either methyl 4-amino-4-deoxy-α-D-glucopyranoside (XIII) or its tetraacetate XIV. The product isolated was crystalline 4-acetamido-1,2,3,6-tetra-O-acetyl-4-deoxy-D-glucopyranose (XXV) rather than the furanose XXVI as shown by the presence of 5-acetyl methyl groups according to n.m.r. data and an NHAc group according to the infrared spectrum and also by analytical data. It is interesting to note[13] that the acetolysis of methyl 4-acetamido-4-deoxy-α-D-ribopyranoside gave the ribofuranose analog of XXVI rather than the ribopyranose analog corresponding to XXV. Treatment of XXV with methanolic sodium methoxide effected the deacetylation; however, the resulting product was quite impure according to paper chromatography, and a characterizable product could not be isolated.

Finally, an attempt was made to prepare the bis nitrogen mustard XIX of 4-amino-4-deoxy-D-glucose. The reaction of methyl 4-amino-2,3,6-tri-O-benzyl-4-deoxy-α-D-glucopyranoside (XXI) with ethylene oxide gave an 81% yield of methyl 2,3,6-tri-O-benzyl-4-deoxy-4-[bis(2-hydroxyethyl)amino]-α-D-glucopyranoside (XXII) as a crude sirup which was treated directly with thionyl chloride to give an 87% yield of methyl 2,3,6-tri-O-benzyl-4-[bis(2-chloroethyl)amino]-4-deoxy-α-D-glucopyranoside (XXIII) as a sirup which had fair analytical values. Hydrogenation of the blocked mustard XXIII with 5% palladium on carbon afforded a 75% yield of methyl 4-[bis(2-chloroethyl)amino]-4-deoxy-α-D-glucopyranoside (XXIV) hydrochloride as a hygroscopic sirup. All efforts to prepare the free sugar mustard XIX from XXIV by acid hydrolysis or from XXIII by boron trichloride scission of the benzyl ethers and methyl glycoside were unsuccessful. A product, presumably XIX, could be obtained, but it was always contaminated by various decomposition products and purification was unsuccessful.

A recent publication by Jeanloz and Rapin[14] described the preparation of a number of derivatives of 4-amino-4-deoxy-D-glucose; however, they also failed to prepare the parent compound. The failure to obtain XVIII is surprising in view of the apparent stability of 4-amino-4,6-dideoxy-D-glucose and the ease with which it can be isolated.[3]

Experimental[15]

Methyl 2,3-Di-O-benzoyl-α-D-galactopyranoside (IV).—A solution of 2.0 g. (4.1 mmoles) of methyl 2,3,-di-O-benzoyl-4,6-O-benzylidene-α-D-galactopyranoside (III)[8] in 30 ml. of absolute ethanol containing 200 mg. of palladium black was hydrogenated at a temperature of 60° and at atmospheric pressure for 2.5 hr., by which time hydrogen uptake was complete. The reaction mixture was filtered and the filtrate was evaporated to dryness *in vacuo* at 50°. The last traces of ethanol were removed by the addition and removal *in vacuo* of two 10-ml. portions of benzene to give 1.62 g. (98%) of product as a light green foam, λ_{max}^{Nujol} 2.90 (OH) and 5.80 (C=O) μ.

Anal. Calcd. for $C_{21}H_{22}O_8$: C, 62.7; H, 5.48. Found: C 62.4; H, 5.60.

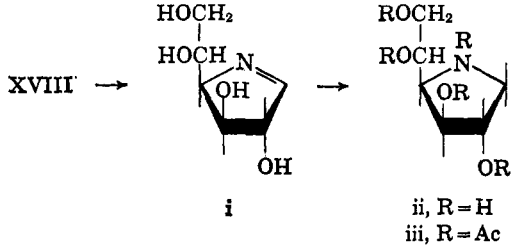
Methyl 2,3-Di-O-benzoyl-4,6-di-O-(*p*-tolylsulfonyl)-α-D-galactopyranoside (V).[9a]—To a solution of 0.86 g. (2.1 mmoles) of methyl 2,3-di-O-benzoyl-α-D-galactopyranoside (IV) in 5 ml. of anhydrous pyridine was added 1.0 g. (5.27 mmoles) of *p*-toluenesulfonyl chloride with stirring. The reaction was heated at 50° for 48 hr. while protected from moisture, then was cooled to room temperature, and poured with stirring into 25 ml. of saturated aqueous sodium bicarbonate. The resulting suspension was extracted with three 10-ml. portions of chloroform. The chloroform extracts were washed with water, dried, and evaporated to dryness *in vacuo* to give 1.15 g. of a tan-colored sirup. Crystallization from 5 ml. of methanol–ether (1:1) gave 0.92 g. (60%) of product as white crystals, m.p. 128–129°, $[\alpha]^{29}D$ +150° (*c* 1, chloroform), λ_{max}^{Nujol} 5.80 (C=O) and 8.50 (—SO_2—) μ.

Anal. Calcd. for $C_{35}H_{34}O_{12}S_2$: C, 59.1; H, 4.80; S, 9.00. Found: C, 58.8; H, 4.91; S, 9.11.

Methyl 2,3,6-Tri-O-benzoyl-α-D-galactopyranoside (VI).[9b]

A. From Methyl 2,3-Di-O-benzoyl-α-D-galactopyranoside (IV).—A solution of 7.5 g. (18.6 mmoles) of dibenzoate IV in 25 ml.

(11) The loss of reducing character during the hydrogenolysis of the azide XI might be rationalized if one assumes a spontaneous cyclic Schiff base formation of 4-amino-4-deoxy-D-glucose to give the 1-pyrroline i. This compound would be rapidly hydrogenated to the pyrrolidine ii, a compound which will give a negative test to the reagents used for detecting reducing sugars. Acetylation of the crude hydrogenation product (presumably ii) gave a material which had infrared and n.m.r. spectra compatible with iii. Thus the infrared showed an amide I band at 6.0 μ but no amide II at 6.5 μ—indicating a tertiary amide. The n.m.r. spectrum showed the presence of 15 acetate methyl protons at τ 7.88–7.98.



i ii, R = H iii, R = Ac

(12) A. B. Foster, D. Horton, N. Salim, M. Stacey, and J. M. Webber, *J. Chem. Soc.*, 2587 (1960).

(13) E. J. Reist, D. E. Gueffroy, and L. Goodman, *J. Am. Chem. Soc.*, **87**, 677 (1965).

(14) R. W. Jeanloz and A. M. C. Rapin, *J. Org. Chem.*, **28**, 2978 (1963).

(15) Melting points were determined with a Fisher-Johns apparatus. Paper chromatograms were run by the descending technique on Whatman No. 1 paper using butanol–acetic acid–water (4:1:5) as the developing solvent. Aniline citrate was used to develop the reducing sugar spots. Rotations were determined with a Rudolph photoelectric polarimeter. Magnesium sulfate was used to dry organic solutions.

of dry pyridine was cooled to 10°, then 1.71 ml. (18.8 mmoles) of benzoyl chloride was added dropwise with stirring. The reaction mixture was left at room temperature for 18 hr. while protected from moisture, then was added dropwise with stirring to 200 ml. of saturated aqueous sodium bicarbonate. The aqueous mixture was extracted with three 20-ml. portions of chloroform. The chloroform extracts were washed with 10 ml. of water, dried, and evaporated to dryness *in vacuo* to give 6.78 g. (74%) of crude product as a colorless sirup. Crystallization from 95% ethanol gave 2.9 g. (32%) of crystalline VI, m.p. 121–125°. Recrystallization from 95% ethanol gave the analytical sample, m.p. 135.5–137.0°, $[\alpha]^{30}D$ +120.0° (*c* 1, chloroform), λ_{max}^{Nujol} 2.87 (OH) and 5.79 (C=O) μ.

Anal. Calcd. for $C_{28}H_{26}O_9$: C, 66.3; H, 5.18. Found: C, 66.0; H, 5.50.

B. From Methyl α-D-Galactopyranoside (I).—A solution of 6.81 g. (35.1 mmoles) of methyl α-D-galactopyranoside (I) in 70 ml. of dry pyridine was cooled to 0°, then 14 ml. (121 mmoles) of benzoyl chloride was added dropwise with stirring. The reaction mixture was left at room temperature for 3 days, then the excess benzoyl chloride was decomposed by the dropwise addition of the reaction mixture to 200 ml. of ice-cold saturated aqueous sodium bicarbonate with stirring. The aqueous mixture was extracted with 250 ml. of chloroform. The chloroform layer was washed with water, dried, then evaporated to dryness *in vacuo* to give 18.4 g. of crude product as a white solid. Recrystallization from methanol gave 10.0 g. (57%) of tribenzoate VI, m.p. 132–136°, which was identical in all respects with tribenzoate prepared by route A and which gave no depression on mixture melting point with tribenzoate prepared by route A.

Yields up to 65% were obtained in the scale-up of this reaction.

Methyl 2,3,6-Tri-O-benzoyl-4-O-methylsulfonyl-α-D-galactopyranoside (VII).[9b]—To a solution of 5.0 g. (10 mmoles) of methyl 2,3,6-tri-O-benzoyl-α-D-galactopyranoside (VI) in 20 ml. of dry pyridine was added 2.5 ml. (32 mmoles) of methylsulfonyl chloride. The reaction was stirred at room temperature for 18 hr. while protected from moisture; then the excess methylsulfonyl chloride was decomposed by the addition of 0.5 ml. of water. The resulting reaction mixture was dissolved in 60 ml. of chloroform and washed with two 50-ml. portions of saturated aqueous sodium bicarbonate and 30 ml. of water, then dried and evaporated to dryness *in vacuo* to give 5.97 g. of a dark sirup. Crystallization from 95% ethanol gave 4.72 g. (82%) of product, m.p. 142–143°. The analytical sample had m.p. 141–142°; λ_{max}^{Nujol} 5.80 (C=O), 7.75, 7.82 (benzoate C–O–C), and 8.50 (OSO_2) μ.

Anal. Calcd. for $C_{29}H_{28}O_{11}S$: C, 59.6; H, 4.80; S, 5.48. Found: C, 59.3; H, 4.80; S, 5.30.

Methyl 4-O-Methylsulfonyl-α-D-galactopyranoside (VIII).—A suspension of 10.0 g. (17.1 mmoles) of methyl 2,3,6-tri-O-benzoyl-4-O-methylsulfonyl-α-D-galactopyranoside (VII) in 60 ml. of methanol was cooled to 0° and a solution of 0.50 g. of sodium methoxide in 5 ml. of methanol was added. The reaction was kept at 5–10° for 18 hr., then neutralized with glacial acetic acid. The resulting solution was evaporated to dryness *in vacuo* and the residue was partitioned between 30 ml. each of chloroform and water. The water layer was treated with Norit and then evaporated to dryness *in vacuo* to give a semisolid residue. Recrystallization from 95% ethanol gave 2.72 g. (58%) of product, m.p. 159–161°.

The analytical sample had m.p. 159–161°; λ_{max}^{Nujol} 2.92, 3.00, 3.03 (OH), and 8.44 (OSO_2) μ.

Anal. Calcd. for $C_8H_{16}O_8S$: C, 35.3; H, 5.88; S, 11.8. Found: C, 35.6; H, 6.11; S, 11.8.

Methyl 2,3,4,6-Tetra-O-benzoyl-α-D-glucopyranoside (IX).

A. From Methyl 2,3-Di-O-benzoyl-4,6-di-O-(*p*-tolylsulfonyl)-α-D-galactopyranoside (V).—A suspension of 0.50 g. (0.7 mmole) of the ditosylate V and 0.70 g. (4.3 mmoles) of sodium benzoate in 15 ml. of DMF was heated at 140° with stirring for 24 hr. The reaction was cooled, diluted with 80 ml. of water, and extracted with three 10-ml. portions of chloroform. The chloroform extracts were washed with 10 ml. of water, dried, and evaporated to dryness *in vacuo* to give 430 mg. of crude product as a dark sirup. Crystallization from absolute ethanol gave 210 mg. (49%) of material, m.p. 101–104°, $[\alpha]^{31}D$ +78° (*c* 0.5, chloroform). A mixture melting point with authentic methyl 2,3,4,6-tetra-O-benzoyl-α-D-glucopyranoside gave no depression, and the infrared spectra were identical.

Ness, *et al.*,[16] reported m.p. 105–108° and $[\alpha]D$ +84° (*c* 0.95, chloroform) for IX.

B. From Methyl 2,3,6-Tri-O-benzoyl-4-O-methylsulfonyl-α-D-galactopyranoside (VII).—Treatment of 1.0 g. of the mesylate VII with 0.53 g. of sodium benzoate in 15 ml. of DMF in the manner described starting with the ditosylate V gave a 23% yield of crystalline tetrabenzoate IX which was identical in all respects with authentic methyl 2,3,4,6-tetra-O-benzoyl-α-D-glucopyranoside.[16]

Methyl 4-Azido-4-deoxy-α-D-glucopyranoside (XI).—A suspension of 20.2 g. (36.4 mmoles) of mesylate VII and 8.0 g. (123 mmoles) of sodium azide in 150 ml. of DMF was heated at reflux with stirring for 4 hr. The reaction mixture was cooled and then partitioned between 150 ml. each of ether and water. The aqueous phase was extracted with two additional 50-ml. portions of ether. The ether extracts were washed with water, dried, and evaporated to dryness *in vacuo* to give 15.0 g. (79%) of crude azide tribenzoate X as a yellow sirup: λ_{max}^{film} 4.75 (N_3), 5.80 (C=O), and 7.85 (benzoate C–O–C) μ.

A solution of 14.9 g. of the above blocked azide (X) in 50 ml. of methanol was treated with 10 ml. of 1 *N* methanolic sodium methoxide at reflux temperature for 1.5 hr. The reaction was cooled to room temperature, then neutralized to pH 7 with IRC 50 (H)[17] (previously washed with methanol). The neutralized solution was evaporated to dryness *in vacuo* and the residue was partitioned between 20 ml. each of water and ether. The ether layer was washed with 5 ml. of water, and then the combined aqueous fractions were evaporated to dryness *in vacuo* to give 5.22 g. of a tan-colored sirup.

Crystallization of the sirup from acetonitrile gave 2.8 g. (37% from VII) of methyl 4-azido-4-deoxy-α-D-glucopyranoside (XI), m.p. 84–88°. Recrystallization from acetonitrile gave the analytical sample, m.p. 108–109°, $[\alpha]^{28}D$ +231° (*c* 0.5, water), λ_{max}^{Nujol} 3.0 (OH) and 4.72 (N_3) μ.

Anal. Calcd. for $C_7H_{13}N_3O_5$: C, 38.4; H, 5.98; N, 19.2. Found: C, 38.2; H, 6.39; N, 19.1.

Methyl 2,3,6-Tri-O-acetyl-4-azido-4-deoxy-α-D-glucopyranoside (XII).—To a cold (10°) solution of 2.61 g. (11.9 mmoles) of azide XI in 20 ml. of dry pyridine was added 20 ml. of acetic anhydride with stirring. The reaction was left at room temperature for 18 hr. while protected from moisture, and then was decomposed with 10 ml. of methanol. The mixture was added to 200 ml. of saturated aqueous sodium bicarbonate and extracted with three 20-ml. portions of chloroform. The chloroform layers were dried and evaporated to dryness *in vacuo* to give 3.20 g. (78%) of crude product which showed no hydroxyl absorption at 2.9 μ in the infrared spectrum.

Evaporative distillation of 1.0 g. of the crude product at 100° and 0.1 mm. gave 0.66 g. of product as a colorless oil: λ_{max}^{film} 4.73 (N_3), 5.70 (acetate C=O), 7.28 (CH_3), and 8.10 (acetate C–O–C) μ.

Anal. Calcd. for $C_{13}H_{19}N_3O_8$: C, 45.2; H, 5.55; N, 12.2. Found: C, 45.2; H, 5.48; N, 12.3.

Methyl 4-Amino-4-deoxy-α-D-glucopyranoside (XIII).—To a solution of 0.54 g. (2.46 mmoles) of methyl 4-azido-4-deoxy-α-D-glucopyranoside (XI) in 8 ml. of water was added 200 mg. of 5% palladium on carbon and the mixture was hydrogenated at room temperature and atmospheric pressure for 2.75 hr. The catalyst was removed by filtration, and the filtrate was evaporated to dryness *in vacuo* to give 0.44 g. of crude product as a colorless sirup that showed no azide absorption at 4.7 μ in the infrared spectrum. Crystallization from methanol–acetonitrile gave 0.32 g. (67%) of white crystals, m.p. 158–159°.

The analytical sample was recrystallized from acetonitrile and had m.p. 166.0–166.5°, λ_{max}^{Nujol} 3.00 (OH, NH_2) and 6.25 (NH_2) μ.

Anal. Calcd. for $C_7H_{15}NO_5$: C, 43.5; H, 7.78; N, 7.25. Found: C, 43.4; H, 7.34; N, 7.38.

Methyl 4-Acetamido-2,3,4-tri-O-acetyl-4-deoxy-α-D-glucopyranoside (XIV).—A solution of 0.319 g. (1.65 mmoles) of methyl 4-amino-4-deoxy-α-D-glucopyranoside (XIII) in 4 ml. of dry pyridine was acetylated with 2.4 ml. of acetic anhydride in the normal fashion to yield 0.58 g. of crude crystalline product. Recrystallization from methanol–ether gave 0.269 g. (45%) of

(16) R. K. Ness, H. G. Fletcher, Jr., and C. S. Hudson, *J. Am. Chem. Soc.*, **72**, 2200 (1950).

(17) A weak acid cation-exchange resin manufactured by the Rohm and Haas Co., Philadelphia, Pa. When used for the neutralization of methanolic solutions of sodium methoxide during the course of this work, it was washed initially with methanol until the methanol washes were colorless.

product, m.p. 138.0–138.5°. The analytical sample had m.p. 140–141°; $[\alpha]^{25}D$ +145° (*c* 0.98, chloroform); λ_{max}^{Nujol} 3.04 (NH), 5.76 (acetate C=O), 6.02 (amide C=O), and 6.50 (amide II) μ.

Anal. Calcd. for $C_{15}H_{23}NO_9$: C, 49.9; H, 6.37; N, 3.88. Found: C, 50.2; H, 6.54; N, 4.00.

4-Azido-4-deoxy-1,2,3,6-tetra-O-(*p*-nitrobenzoyl)-D-glucofuranose (XVII).—To a solution of 8.71 g. (39.7 mmoles) of methyl 4-azido-4-deoxy-α-D-glucopyranoside (XI) in 150 ml. each of glacial acetic acid and acetic anhydride was added 15 ml. of concentrated sulfuric acid dropwise with stirring. The solution was left at room temperature for 3 days, 27.6 g. of sodium acetate was added to neutralize the sulfuric acid, and the mixture was evaporated to dryness *in vacuo*. The residue was partitioned between 200 ml. each of chloroform and water. The chloroform layer was washed with water, dried, and evaporated to dryness *in vacuo*. The last traces of acetic acid were removed by the addition and evaporation *in vacuo* of 25 ml. of toluene to yield 14.0 g. (95%) of 1,2,3,6-tetra-O-acetyl-4-azido-4-deoxy-D-glucopyranose (XV) as a brown oil: λ_{max}^{film} 4.74 (N_3), 5.70 (C=O), 8.10, and 8.21 (acetate C—O—C) μ.

Anal. Calcd. for $C_{14}H_{19}N_3O_9$: C, 45.1; H, 5.12; N, 11.2. Found: C, 45.5; H, 4.66; N, 10.9.

A solution of 9.0 g. (24.1 mmoles) of the tetraacetate XV in 100 ml. of methanol was stirred with 5 ml. of 1 *N* methanolic sodium methoxide at room temperature for 4 hr., then neutralized with IRC 50 (H)[17] and evaporated to dryness *in vacuo*. The residue was partitioned between 50 ml. each of chloroform and water. The water layer was treated with Norit and then evaporated to dryness *in vacuo* to give 5.3 g. of crude 4-azido-4-deoxy-D-glucose (XVI) as a hygroscopic yellow foam which was free of acetate absorption at 5.7 μ according to infrared spectroscopy.

A solution of 2.0 g. (*ca.* 9.75 mmoles) of crude XVI in dry pyridine was treated with 10.0 g. (54 mmoles) of *p*-nitrobenzoyl chloride under a nitrogen atmosphere at room temperature for 18 hr. and was poured into *ca.* 100 ml. of ice–water. The aqueous mixture was extracted with 100 ml. of chloroform. The chloroform layer was washed with three 50-ml. portions of saturated aqueous sodium bicarbonate and 50 ml. of water, then was dried and evaporated to dryness *in vacuo* to yield 6.3 g. of crude tetranitrobenzoate XVII as a brown solid. Trituration with isopropyl alcohol gave 5.27 g. of a tan solid, m.p. 185–195°.

The analytical sample from a previous run was obtained by recrystallization from ethyl acetate–methanol and had m.p. 205–210°; λ_{max}^{Nujol} 4.70 (N_3), 5.72 (C=O), 6.50, 7.38 (NO_2), and 7.88 (*p*-nitrobenzoate C—O—C) μ.

Anal. Calcd. for $C_{34}H_{23}N_7O_{17}$: C, 50.9; H, 2.89; N, 12.2. Found: C, 51.0; H, 3.04; N, 12.2.

4-Acetamido-1,2,3,6-tetra-O-acetyl-4-deoxy-D-glucopyranose (XXV).—To a cold (0°) solution of 1.32 g. (6.83 mmoles) of methyl 4-amino-4-deoxy-D-glucopyranoside (XIII) in 25 ml. each of acetic acid and acetic anhydride was added 1.44 ml. of concentrated sulfuric acid dropwise with stirring and continued cooling. After the addition was complete, the reaction was left at room temperature for 72 hr. and was decomposed by being added slowly to 700 ml. of vigorously stirred saturated aqueous sodium bicarbonate solution. The aqueous mixture was extracted with three 50-ml. portions of chloroform. The chloroform extracts were washed with water, dried, and evaporated to dryness *in vacuo* to give 2.67 g. of product as a pale yellow sirup. This sirup was dissolved in ethyl acetate, hexane was added to the cloud point, and the solution was stored at 0° for 48 hr. The gummy solid which separated weighed 2.07 g. (78%) and had m.p. 55–57.5°; λ_{max}^{Nujol} 3.0 (NH), 5.70 (acetate C=O), 5.90 (amide C=O), and 6.45 (amide II) μ.

The n.m.r. spectrum showed 15 protons at τ 7.8 to 8.1 indicative of the 5-acetyl groups of the pyranose (XXV) rather than 6-acetyl groups which would be expected for the furanose (XXVI).

Anal. Calcd. for $C_{16}H_{23}N_{10}\cdot 0.5H_2O$: C, 48.3; H, 6.04; N, 3.52. Found: C, 48.5; H, 6.01; N, 3.57.

Methyl 4-Amino-2,3,6-tri-O-benzyl-4-deoxy-α-D-glucopyranoside (XXI) Toluenesulfonate Salt.—A solution of 19.4 g. (0.088 mole) of methyl 4-azido-4-deoxy-α-D-glucopyranoside (XI) in 100 ml. of α-chlorotoluene containing 15.0 g. (0.375 mole) of powdered sodium hydroxide was stirred at 100° for 7 hr. The reaction mixture was evaporated to dryness *in vacuo* to remove the excess α-chlorotoluene. The residue was partitioned between 50 ml. each of chloroform and water. The water layer was extracted with an additional 20 ml. of chloroform. The combined chloroform layers were dried and evaporated to dryness *in vacuo* to give 43.6 g. of the blocked azide XX as a dark sirup which was essentially free of hydroxyl absorption at 2.9 μ in the infrared and which was of satisfactory quality for the reduction to the amine.

A solution of the crude azide XX in 125 ml. of anhydrous diethyl ether was added to a stirred mixture of 9.0 g. (0.23 mole) of lithium aluminum hydride in 125 ml. of ether at a rate such that the temperature remained below 30° throughout the addition. The reaction mixture was stirred at room temperature for 24 hr. and then was cooled to 0° in an ice bath and the excess hydride was decomposed by the cautious addition of 15 ml. of absolute ethanol followed by 15 ml. of 10% aqueous sodium hydroxide and 30 ml. of water. The ether layer was separated from the white sludge and washed with 20 ml. of water. The sludge was extracted with three 50-ml. portions of ether. The combined ether extracts were dried and evaporated to dryness to give 39.6 g. of crude product (XXI) as the free base.

A solution of crude XXI in 200 ml. of dry ether was cooled to 0° and 500 ml. of ether which had been previously saturated with *p*-toluenesulfonic acid was added dropwise with stirring and continued cooling. After cooling for several hours, the white crystalline sulfonic acid salt of XXI was filtered and washed with ether to give 30.5 g. (54% based on 4-azide XI), m.p. 184–186°.

The analytical sample was recrystallized from methanol–ether and had m.p. 204.5–205.0°; λ_{max}^{Nujol} 3.6–3.9, 6.20 (NH_3^+), 9.65, and 9.88 (OTs^-) μ.

Anal. Calcd. for $C_{28}H_{33}NO_5\cdot C_7H_8O_3S$: C, 66.2; H, 6.46; N, 2.22; S, 5.04. Found: C, 65.9; H, 6.75; N, 2.76; S, 5.13.

The free base XXI was regenerated by partitioning the toluenesulfonic acid salt between 50 ml. each of chloroform and saturated aqueous sodium bicarbonate. The chloroform layer was washed with water, dried, and evaporated to dryness *in vacuo* to give 21.25 g. of XXI free base as a pale yellow sirup: λ_{max}^{film} 2.98, 6.22, 6.28 (NH_2), 13.08, and 14.35 ($C_6H_5CH_2$) μ.

Anal. Calcd. for $C_{28}H_{33}NO_5\cdot H_2O$: C, 69.9; H, 7.28; N, 2.91. Found: C, 70.5; H, 7.26; N, 2.78.

Methyl 2,3,6-Tri-O-benzyl-4-[bis(2-chloroethyl)amino]-4-deoxy-α-D-glucopyranoside (XXIII).—To a solution of 15.5 g. (33.5 mmoles) of amine XXI, which had been regenerated from its toluenesulfonic acid salt, in 40 ml. of benzene was added 20 ml. of freshly distilled ethylene oxide. The reaction mixture was heated in a Parr bomb at 180° for 18 hr., cooled, washed with 40 ml. of water, dried, and evaporated to dryness *in vacuo* to give 15.6 g. of methyl 2,3,6-tri-O-benzyl-4-deoxy-4-[bis-(2-hydroxyethyl)amino]-α-D-glucopyranoside (XXII) as an amber sirup, λ_{max}^{film} 2.90 (OH) and 6.20 (weak, NH_2) μ.

Acetylation of an aliquot of XXII with acetic anhydride in pyridine gave a sirup which contained 10–15% of N-acetate absorption at 6.0 μ in the infrared,[2c] indicative of incomplete hydroxyethylation. Repetition of the hydroxyethylation reaction gave 15.0 g. (81%) of material which was completely hydroxyethylated according to the acetylation technique and was of satisfactory purity for the subsequent chlorination.

A solution of 5.4 g. (9.8 mmoles) of the bishydroxyethylamine XXII in 25 ml. of dry dichloromethane was cooled to 0° and 6.0 ml. (82 mmoles) of thionyl chloride was added dropwise with stirring. After the addition was complete, the reaction was heated at reflux for 1.5 hr., cooled, and diluted with 25 ml. of dry dichloromethane. The diluted solution was added dropwise with stirring to 200 ml. of saturated aqueous sodium bicarbonate. The organic layer was separated, dried, and evaporated to dryness *in vacuo* to give 5.1 g. (87%) of blocked mustard XXIII as a yellow sirup which showed no hydroxyl absorption at 2.9 μ in the infrared spectrum.

Anal. Calcd. for $C_{32}H_{39}Cl_2NO_5$: C, 65.3; H, 6.64; Cl, 12.0; N, 2.38. Found: C, 64.8; H, 6.71; Cl, 11.7; N, 2.31.

Methyl 4-[Bis(2-chloroethyl)amino]-4-deoxy-D-glucopyranoside (XXIV) Hydrochloride.—A solution of 2.90 g. (4.66 mmoles) of blocked mustard XXIII in 20 ml. of methanol was saturated with hydrogen chloride gas and then evaporated to dryness *in vacuo*. The resulting hydrochloride was redissolved in 20 ml. of methanol and added to a suspension of 0.5 g. of 5% palladium on carbon in 20 ml. of acetic acid. The reaction was hydrogenated in a Parr shaker at 40 p.s.i. of hydrogen at room temperature for 20 hr. and then was filtered; the filtrate was evaporated to dryness *in vacuo* to give 2.53 g. of a dark sirup which still contained some benzyl ether as shown by its infrared absorption at 14.3 μ. The hydrogenation was repeated using the same quan-

tities of catalyst as before, to give on work-up 1.31 g. (75%) of mustard XXIV hydrochloride as a yellow sirup: λ_{max}^{film} 3.0 (OH), 9.18, 9.32, and 9.55 (C–O–C) μ. There was no absorption at 14.3 μ due to $C_6H_5CH_2$.

Anal. Calcd. for $C_{11}H_{21}Cl_2NO_5 \cdot HCl \cdot 0.25H_2O$: C, 36.8; H, 6.28; Cl, 29.6; N, 3.90. Found: C, 36.4; H, 6.36; Cl, 29.8; N, 3.70.

Acknowledgment.—The authors thank Dr. Peter Lim and his staff for the infrared, ultraviolet, and n.m.r. spectra, paper chromatography, and optical rotations. The authors are also indebted to Mr. O. P. Crews, Jr., and his staff for large-scale preparations of some intermediates.

Syntheses with Partially Benzylated Sugars. IV.[1] A Route to Some 1-O-Acyl-2-acylamido-2-deoxy-D-glucopyranoses and -D-galactopyranoses

ROGER HARRISON[2] AND HEWITT G. FLETCHER, JR.

National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Public Health Service, U. S. Department of Health, Education, and Welfare, Bethesda, Maryland 20014

Received November 24, 1964

2-Acylamido-2-deoxyhexoses of the D-glucose and D-galactose series can be benzylated to give benzyl 2-acylamido-3,4,6-tri-O-benzyl-2-deoxyhexopyranosides. When such glycosides are successively hydrolyzed by acid, acylated, and O-deacylated, they afford 2-acylamido-3,4,6-tri-O-benzyl-2-deoxyhexopyranoses. Through a sequence, involving acylation and debenzylation, these latter substances serve as precursors of the 1-O-acyl-2-acylamido-2-deoxyhexopyranoses.

It has been suggested that some of the 2-acetamido-2-deoxy-D-galactosyl moieties in ovine submaxillary gland mucoprotein (OSM) are bound by a C-1 ester linkage to the nonpeptide-bonded carboxyl groups of aspartic and glutamic acids.[3] For this reason, the properties of 1-O-acyl derivatives of the 2-acetamido-2-deoxyhexoses become of interest and we wish to report here a study of the synthesis of some representatives of this class of substance.

Aldoses, fully etherified with benzyl groups except at C-1 and C-4 (aldofuranoses) or C-5 (aldopyranoses), can be obtained through the hydrolysis of fully benzylated aldopyranosides[4–6] or aldofuranosides.[6,7] By esterification of such ethers at C-1 and subsequent removal of the benzyl groups by catalytic hydrogenolysis, C-1 esters of the aldoses can be prepared; by this route Schmidt and Schmadel[8] were able to synthesize the two anomeric 1-O-galloyl-D-glucopyranoses while Tejima and Fletcher[6] similarly prepared the anomeric 1-O-benzoyl-L-arabinopyranoses as well as the anomeric 1-O-benzoyl-L-arabinofuranoses. The application of this type of process to the synthesis of 1-O-acyl-2-acylamido-2-deoxyaldopyranoses requires 2-acylamido-2-deoxyaldopyranoses etherified with benzyl groups except at C-1 and C-5. For the synthesis of such an intermediate, attention was first turned to *t*-butyl 3,4,6-tri-O-acetyl-2-benzamido-2-deoxy-β-D-glucopyranoside (I), a substance reported by Micheel and Köchling[9]; the glycosidic linkage in such a compound should be relatively labile to acid while the benzamido group should be more stable than the acetamido group. Treatment of I with benzyl chloride and potassium hydroxide in boiling tetrahydrofuran caused replacement of the acetyl groups by benzyl groups, giving the tribenzyl ether II (Scheme I); hydrolysis of II in a mixture of tetrahydrofuran and hydrochloric acid afforded 2-benzamido-3,4,6-tri-O-benzyl-2-deoxy-D-glucopyranose (III) in 61% yield.[10] It is evident, therefore, that the *t*-butyl group may be removed from C-1 without substantial loss of the benzoyl group from the nitrogen. However, the synthesis of I is both laborious and time consuming and a more direct route to III was sought.

Kuhn and Trischmann[11] have shown that cautious methylation of 2-acetamido-2-deoxy-D-glucopyranose in N,N-dimethylformamide with methyl iodide, barium oxide, and barium hydroxide octahydrate affords a very high yield of methyl 2-acetamido-2-deoxy-3,4,6-tri-O-methyl-β-D-glucopyranoside. This procedure was adapted to the benzylation of 2-benzamido-2-deoxy-D-glucopyranose (IV), using benzyl bromide, and a crystalline benzyl 2-benzamido-3,4,6-tri-O-benzyl-2-deoxy-D-glucopyranoside (V) was obtained. By analogy with the findings of Kuhn and Trischmann,[11] one might expect this glycoside to have the β configuration; the n.m.r. spectrum of the substance confirmed this expectation. Hydrolysis of this glycoside V afforded 2-benzamido-3,4,6-tri-O-benzyl-2-deoxy-D-glucopyranose (III) in 62% yield, demonstrating that a benzyl glycoside is as suitable as a *t*-butyl glycoside for the preparation of this substance.

Since the 2-amino-2-deoxyaldoses most frequently occur in nature as *N*-acetyl derivatives, we studied next the synthesis of 2-acetamido-3,4,6-tri-O-benzyl-2-deoxy-D-glucopyranose (VIII). Direct benzylation of 2-acetamido-2-deoxy-D-glucopyranose (VI) readily gave benzyl 2-acetamido-3,4,6-tri-O-benzyl-2-deoxy-β-D-glucopyranoside (VII) in 76% yield. However, in contrast to the conversion of V to III, the hydrolysis of benzyl 2-acetamido-3,4,6-tri-O-benzyl-2-deoxy-β-D-

(1) Paper III of this series: C. P. J. Glaudemans and H. G. Fletcher, Jr., *J. Org. Chem.*, **28**, 3004 (1963).

(2) Fellow in the Visiting Progam, 1963–1964.

(3) E. R. B. Graham, W. H. Murphy, and A. Gottschalk, *Biochim. Biophys. Acta*, **74**, 222 (1963); K. Tanaka, M. Bertolini, and W. Pigman, *Biochem. Biophys. Res. Commun.*, **16**, 404 (1964); S. Harbon, G. Herman, B. Rossignol, P. Jollès, and H. Clauser, *ibid.*, **17**, 57 (1964); V. P. Bhavanandan, E. Buddecke, R. Carubelli, and A. Gottschalk, *ibid.*, **16**, 353 (1964) *cf.* also B. Anderson, N. Seno, P. Sampson, J. Riley, P. Hoffman, and K. Meyer, *J. Biol. Chem.*, **239**, 2716 (1964).

(4) O. T. Schmidt, T. Auer, and H. Schmadel, *Ber.*, **93**, 556 (1960).

(5) M. E. Tate and C. T. Bishop, *Can. J. Chem.*, **41**, 1801 (1963).

(6) S. Tejima and H. G. Fletcher, Jr., *J. Org. Chem.*, **28**, 2999 (1963).

(7) R. Barker and H. G. Fletcher, Jr., *ibid.*, **26**, 4605 (1961).

(8) O. T. Schmidt and H. Schmadel, *Ann.*, **649**, 149 (1961).

(9) F. Micheel and H. Köchling, *Ber.*, **91**, 673 (1958).

(10) While this substance is dextrorotatory, $[\alpha]^{20}D$ +89° ($CHCl_3$) and $[\alpha]^{20}D$ +89° (pyridine), it was not observed to mutarotate; assignment of an anomeric configuration to it cannot be made with assurance at this time.

(11) R. Kuhn and H. Trischmann, *Ber.*, **96**, 284 (1963).