Anal. Calcd. for $C_{13}H_{20}O_7S$: C, 48.73; H, 6.29; S, 10.02. Found: C, 48.77; H, 5.97; S, 10.32.

1,6-Anhydro-5,6-dideoxy-6-mercapto-β-D-*xylo*-hexofuranose (I).—5,6-Dideoxy-6-thioacetyl-D-*xylo*-hexofuranose, obtained from III by removal of the isopropylidene group as described above, was dissolved in 150 ml. of absolute methanol. After addition of 40 ml. of Amberlite IR-120 (H) resin, the mixture was heated with stirring at 65° for 20 hr. until the thiol activity of the solution disappeared as indicated by a negative sodium nitroprusside[13] or 2,3,5-triphenyl-2*H*-tetrazolium chloride[14] test. The resin was removed by filtration and was washed three times with methanol. Combined filtrate and washings were concentrated to 0.99 g. (72.5% yield) of sirup. This was purified by thin layer chromatography using irrigant A. Elution of the major component with acetone followed by concentration produced 0.55 g. (44.5% yield) of chromatographically pure sirup. This was further purified by distillation, b.p. 135–140° (0.01 mm.), $[\alpha]^{20}D$ −43.6° (*c* 2.04, water). The product was very hygroscopic. The freshly distilled material solidified to a white, waxy product but took up moisture very quickly on exposure to air and immediately liquefied. Its molecular weight was 145 (calcd., 165). On periodate oxidation it consumed 0.97 moles of periodate[15] in 5 hr. and did not liberate formic acid.[2]

1,6-Anhydro-5,6-dideoxy-2,3-di-*O*-(p-nitrobenzoyl)-6-mercapto-β-D-*xylo*-furanose was prepared by esterifying compound I in pyridine with *p*-nitrobenzoyl chloride. The ester was crystallized from methanol, m.p. 201–202°, $[\alpha]^{21}D$ +109° (*c* 1.0, chloroform).

Anal. Calcd. for $C_{20}H_{16}N_2O_9S$: C, 52.17; H, 3.50; S, 6.97. Found: C, 52.29; H, 3.64; S, 6.73.

1,6-Anhydro-5,6-dideoxy-2,3-di-*O*-acetyl-6-mercapto-β-D-*xylo*-furanose was prepared from compound I by acetylation with acetic anhydride in pyridine. The product was a sirup, b.p. 75–80° (0.01 mm.), $[\alpha]^{20}D$ −46.9° (*c* 1.28, chloroform).

Anal. Calcd. for $C_{10}H_{14}O_5S$: C, 48.77; H, 5.73; S, 13.02; mol. wt., 246. Found: C, 48.98; H, 5.85; S, 12.76; mol. wt., 242.

Formation of Compound I in Aqueous Medium.—5,6-Dideoxy-6-thioacetyl-D-*xylo*-hexofuranose obtained from compound III by the procedure described under the preparation of compound I was treated with IR-120 (H) in water at 65° for 20 hr. and was isolated as described above. It was characterized by preparing its *p*-nitrobenzoyl derivative in the usual manner, m.p. 201°. The mixture melting point with the authentic specimen described above remained unchanged. Treatment of 5-deoxy-1,2-isopropylidene-α-D-*xylo*-hexofuranose with IR-120 (H) in aqueous media under the same conditions, but for 48 hr., produced 5-deoxy-D-*xylo*-hexose which was identified as its osazone,[16] m.p. 153°.

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(14) W. E. Trevelyan, D. P. Procter, and J. S. Harrison, *Nature*, **166**, 444 (1950).

(15) R. D. Guthrie, *Methods Carbohydrate Chem.* **1**, 432 (1962).

(16) P. P. Regna, *J. Am. Chem. Soc.*, **69**, 246 (1947).

Pyrimidine Disaccharide Nucleosides. Synthesis of an Amino Sugar Disaccharide Nucleoside

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The 1-halo sugar derivatives of lactose, Ia and c, and cellobiose, Ib and d, were converted to their corresponding pyrimidine nucleoside derivatives, II, III, and IV, by two established procedures. The β-D configuration of the nucleoside bond was established by degradation of IIIa and b to the known nucleoside V. An amino sugar disaccharide nucleoside VIII was prepared from the appropriate chloro sugar VIIb by condensation with 2,4-diethoxypyrimidine. The β-D configuration of the nucleoside bond in VIII was established by its preparation *via* an alternate route involving formation of the disaccharide link between the nucleoside Xb and the halo sugar VI.

The antibiotic amicetin[1] has been shown to have a structure which includes in it a pyrimidine disaccharide nucleoside moiety. Specifically, the structural sequence in question has 4,6-dideoxy-4-dimethylamino-α-D-glucose[2] linked *via* an O-glycosidic bond to the 4-position of 2,3,6-trideoxy-β-D-*erythro*-hexopyranose.[3] The deoxy sugar, in turn, is linked *via* a β-glycosylamine bond to the 1-position of cytosine. The synthetic challenge involved in preparing a disaccharide nucleoside incorporating the unique amino sugar–deoxy sugar–pyrimidine sequence of amicetin has necessitated exploration into the general area of disaccharide nucleoside synthesis. We wish to report, herein, the first syntheses of pyrimidine disaccharide nucleosides and also the first synthesis of an amino sugar containing disaccharide nucleoside. Preparation of this latter compound was accomplished by two routes, the second of which comprises the first synthesis of a disaccharide nucleoside by the coupling of a *mono*saccharide nucleoside with a second monosaccharide.

The first synthetic disaccharide nucleoside, which contained the base purine and the disaccharide lactose, was prepared by Wolfrom[4] and his co-workers. Wolfrom subsequently prepared purine nucleosides of the disaccharides maltose and cellobiose.[5] Of the procedures available for the synthesis of pyrimidine nucleosides[6] the dialkoxypyrimidine method of Hilbert and Johnson[7] and the mercury salt procedure of Fox[8] appeared to be the methods of choice. For the initial

(1) (a) C. L. Stevens, K. Nagarajan, and T. H. Haskell, *J. Org. Chem.*, **27**, 2991 (1962); (b) S. Hanessian and T. H. Haskell, *Tetrahedron Letters*, **No. 36**, 2451 (1964), and references cited therein.

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(4) M. L. Wolfrom, P. McWain, F. Shafizadeh, and A. Thompson, *ibid.*, **81**, 6080 (1959).

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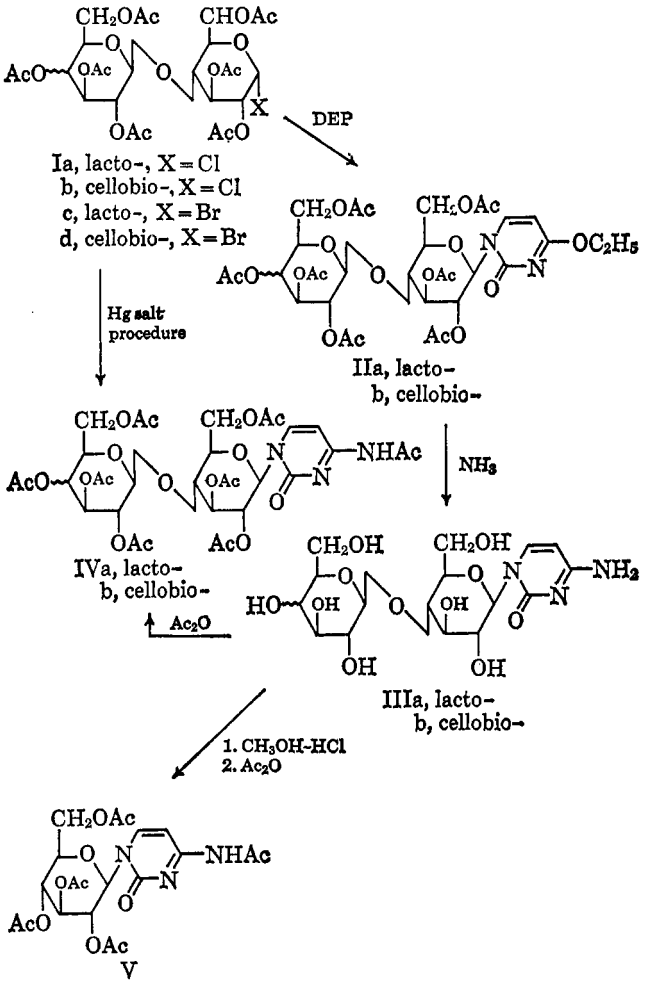
(6) J. J. Fox, *Record Chem. Prog.* (Kresge-Hooker Sci. Lib.), **19**, 173 (1958).

(7) G. E. Hilbert and T. B. Johnson, *J. Am. Chem. Soc.*, **52**, 4489 (1930).

(8) (a) J. J. Fox, N. Yung, J. Davoll, and G. Brown, *ibid.*, **78**, 2117 (1956); (b) J. J. Fox, N. Yung, I. Wempen, and I. L. Doerr, *ibid.*, **79**, 5060 (1957).

exploration, the readily available disaccharides lactose and cellobiose were chosen.

The reaction of hepta-O-acetyl-α-lactosyl chloride[9] (Ia) with excess 2,4-diethoxypyrimidine (DEP) at 125° for 6 days yielded the corresponding pyrimidine nucleoside, IIa, in 46% yield.[10] Heating the nucleoside IIa with ethanolic ammonia removed the acetyl protecting groups and converted it to 1-(β-lactosyl)-cytosine (IIIa) which was characterized as its hydrochloride salt. Acetylation of IIIa in pyridine gave the octaacetyl derivative IVa in 95% yield. The cytosine nucleoside IVa was then synthesized directly by the mercury salt procedure of Fox. Earlier work[11] had shown that the more reactive 1-bromo sugars were better suited for this procedure, and so hepta-O-acetyl-α-lactosyl bromide[12] (Ic) was used in this approach. The condensation of Ic with N-acetylcytosinemercury afforded IVa in 24% yield after alumina chromatography and reacetylation.[12]



A similar series of reactions was conducted with the disaccharide cellobiose. Thus, hepta-O-acetyl-α-cellobiosyl chloride[13] (Ib) yielded the nucleoside IIb in 55% yield upon condensation with diethoxypyrimidine. The ammonolysis reaction on IIb afforded 1-(β-cellobiosyl)cytosine (IIIb) in 75% yield. As before, the cytosine nucleoside was characterized as its hydrochloride salt. The octaacetyl derivative IVb was prepared in 66% yield by acetylation of IIIb, and in 46% yield by condensation of bromo sugar Id with acetylcytosine mercury. Again, use of the mercury salt condensation necessitated chromatographic purification of the reaction product. This time cellulose chromatography, with *prior* acetic anhydride treatment,[12] proved most suitable.

Recently, Fox has compiled conclusive evidence[14] that the dialkoxypyrimidine method of Hilbert and Johnson[7] can yield mixtures of α- and β-nucleosides of pentoses bearing a participating group in the 2-position. However, on the basis of the stereochemical configuration of the starting materials, these nucleosides prepared by Fox's mercury salt procedure, and, by identity, also those prepared from dialkoxy pyrimidine, would be expected to have the β-anomeric configuration.[15] Conclusive proof of this configuration was obtained by converting both IIIa and b to the known nucleoside V[8b] by a two-step sequence. Treatment of IIIa with methanolic hydrogen chloride selectively cleaved the O-glycosidic bond. Acetylation of that reaction product, without isolation, afforded V in 65% yield. The cellobiosyl nucleoside IIIb was converted to V in 46% yield by a similar procedure.

For the synthesis of an amino sugar disaccharide nucleoside, the disaccharide VIIa, described by Michael and Drescher,[16] was chosen since formation of a nucleoside of VIIa would give a model compound containing the amino sugar–neutral sugar–pyrimidine sequence found in amicetin.

Prior to their synthesis of VIIa, Michael and coworkers had shown that the bromo sugar VI was unstable, and, in ether solution, rapidly rearranged to the oxazoline XI.[17] The oxazoline, then, was the starting material that Michael and Drescher used in their synthesis of disaccharide VIIa. In our hands, the rearrangement of VI, as described, yielded very low yields of the oxazoline XI. Consequently, the bromo sugar VI was used as a starting material for the disaccharide synthesis. Even in a crude state, the bromo sugar VI gave the disaccharide VIIa in 33% yield on Koenigs–Knorr[18] coupling with 1,2,3,4-tetra-O-acetyl-β-D-glucopyranose.[19] The chloro sugar VIIb, prepared by hydrogen chloride treatment of VIIa, was an amorphous solid, but could be satisfactorily purified and characterized. Condensation of VIIb with diethoxypyrimidine yielded the amino sugar disaccharide nucleoside VIII in 21% yield.

Again, by analogy with the nucleosides IIa and b and others,[14] the β configuration could be assigned to the nucleoside bond in VIII. However, conclusive proof of this was sought. In addition to this, the problems of stereochemical assignment and control at the anomeric carbon of a 2-deoxy sugar, such as exists in amicetin, was brought to mind. In condensa-

(9) E. Pacsu, *Ber.*, **B61**, 1508 (1928).

(10) Dimethoxypyrimidine yielded the corresponding methoxypyrimidone nucleoside on reaction with Ia. However, the reaction mixture was darker in color and the yield of nucleoside was lower than with the ethoxypyrimidine.

(11) T. S. Sulkowski, Doctoral Dissertation, Wayne State University, 1961.

(12) See Experimental section.

(13) D. H. Brauns, *J. Am. Chem. Soc.*, **48**, 2776 (1926).

(14) J. Farkas, L. Kaplan, and J. J. Fox, *J. Org. Chem.*, **29**, 1464 (1964).

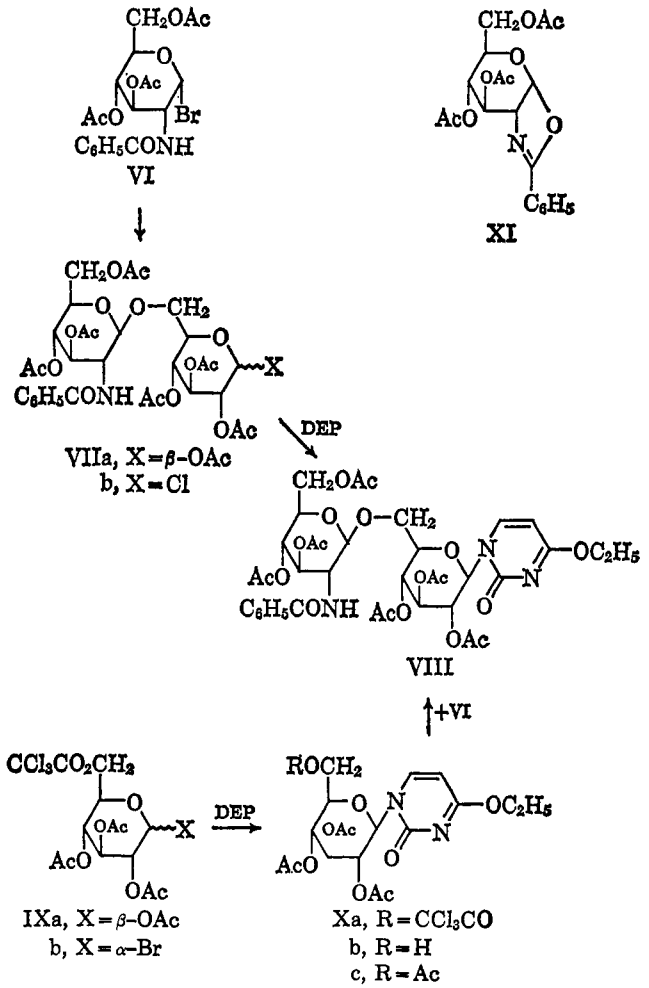
(15) J. J. Fox, J. F. Codington, N. C. Yung, L. Kaplan, and J. O. Lampen, *ibid.*, **80**, 5155 (1958), and references cited therein.

(16) F. Michael and E. Drescher, *Ber.*, **94**, 670 (1958).

(17) F. Michael, F. P. van de Kamp, and H. Peterson, *ibid.*, **90**, 521 (1957).

(18) W. Koenigs and E. Knorr, *ibid.*, **34**, 957 (1901).

(19) D. W. Reynolds and W. L. Evans, "Organic Syntheses," Coll. Vol. III, John Wiley and Sons, Inc., New York, N. Y., 1955, p. 434.



tions at the 1-carbon of furanosyl halides, loss of stereochemical control results when the 2-carbon bears a nonparticipating group or no hetero atom substituent at all. Thus, 2-deoxyfuranoses are known to yield α- and β-anomeric mixtures on nucleoside formation[20] and a *cis*-1,2 configurational arrangement has been shown to result on nucleoside formation when the C-2 oxygen-protecting group was the nonparticipating benzyl group.[21] Similar stereochemical results can be expected in the case of 2-deoxypyranoses. Currently, there exist no convenient, unequivocal physical methods applicable to the problem of configurational stereochemical assignment at the anomeric carbon in 2-deoxypyranose nucleosides. For these reasons, the synthesis of VIII *via* formation of the O-glycosidic bond between glucosamine and a nucleoside of known β configuration was attempted. Successful synthesis of VIII by this route would then conclusively establish the β configuration of the nucleoside bond and demonstrate the feasibility of this alternate route to disaccharide nucleosides.[22]

The desired nucleoside for the coupling reaction, Xb, was prepared in the following manner. The reaction of 1,2,3,4-tetra-O-acetyl-β-D-glucopyranose with trichloroacetyl chloride in pyridine afforded the 6-O-trichloroacetyl derivative IXa in 87% yield. The crystalline bromo sugar IXb was prepared and, on condensation with diethoxypyrimidine, was converted to the nucleoside Xa. Mild, anhydrous ammonolysis selectively cleaved the trichloroacetyl group of Xa, providing the desired Xb in 78% yield. Acetylation of Xb produced the known 1-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)-4-ethoxy-2(1H)-pyrimidone,[23] and Koenigs–Knorr condensation of Xb with bromo sugar VI produced the nucleoside VIII in 9% yield. Since only one base, silver carbonate, was tried in the coupling reaction, the low yield probably reflects nonoptimum reaction conditions rather than an inherent disadvantage in the synthetic route. Nevertheless, the feasibility of an alternate route to disaccharide nucleosides has been demonstrated.

Experimental

Lactose Octaacetate.—This material was prepared by acetylation of lactose with acetic anhydride in presence of pyridine as described by Hudson and Kunz.[24] The crude material obtained in this manner was crystallized once from ethanol, dried, and used without further purifications.

Hepta-O-acetyl-α-lactosyl Chloride (Ia).—A solution of 5 g. (7.36 mmoles) of lactose octaacetate in 52 ml. of absolute chloroform was treated with a solution of 1.4 g. (7.36 mmoles) of titanium tetrachloride in 20 ml. of absolute chloroform. The reaction flask was fitted with a reflux condenser containing a calcium chloride drying tube and the reaction mixture was heated to gentle reflux on the steam bath for 3 hr. The reaction mixture was cooled and poured into 200 ml. of water. The chloroform layer was separated, washed once with water, and dried over anhydrous sodium sulfate; the solution was concentrated *in vacuo* to a small volume. The concentrate was warmed slightly on the steam bath and warm anhydrous ethyl ether was added until cloudy. Crystallization was induced by scratching the walls of the flask with a glass rod. The product was filtered and recrystallized by dissolution of the material in a small volume of warm chloroform, then addition of ethyl ether until turbid. The recrystallized product, 3.14 g. (65%), melted at 118–120° (reported[9] 91% yield, m.p. 122°).

Hepta-O-acetyl-α-lactosyl Bromide (Ib).—Lactose octaacetate (10 g., 15 mmoles) was dissolved in 14 ml. of hot acetic anhydride. After cooling, 25 ml. of a saturated solution of anhydrous hydrogen bromide in glacial acetic acid was added and the reaction mixture was allowed to stand at room temperature for 2 hr. The solution was poured on ice–water and the resultant mixture was extracted twice with chloroform. The chloroform extracts were combined, washed with ice–water, sodium bicarbonate solution, and ice–water, and then dried over anhydrous sodium sulfate. The chloroform solution was concentrated *in vacuo* to about 30 ml., 50 ml. of anhydrous ethyl ether was added, followed by petroleum ether (b.p. 30–60°) until turbidity persisted. When the solution was warmed slightly until the turbidity disappeared, crystal formation proceeded rapidly. After 1 hr. at room temperature, the product was filtered, washed with a small volume of ethyl ether, and dried to yield 7.0 g. (68%) of product, m.p. 140–142° (lit.[25] 80–85% yield, m.p. 141–142°).

1-(Hepta-O-acetyl-β-D-lactosyl)-4-methoxy-2(1H)-pyrimidone.[10]—A mixture of 3.30 g. (5 mmoles) of hepta-O-acetyl-α-D-lactosyl-chloride (Ia) and 4.5 ml. of 2,4-dimethoxypyrimidine was heated (oil bath) at 125–130° (bath temperature) for 60 hr. A small sample was withdrawn from the reaction mixture; treatment with alcoholic silver nitrate showed a negative halide test. The reaction mixture was cooled and triturated three times with hot diisopropyl ether. The residue was dissolved in hot methanol and a slight excess of diisopropyl ether was added. A crystalline solid was obtained. The product was recrystallized from absolute ethanol. An additional recrystallization from methanol gave 1.56 g. (42%) of pure material, m.p. 170–175° (foaming),

(20) J. J. Fox, N. C. Yung, I. Wempen, and M. Hoffer, *J. Am. Chem. Soc.*, **83**, 4066 (1961).

(21) C. Glaudemans and H. G. Fletcher, *J. Org. Chem.*, **28**, 3004 (1963).

(22) Regarding the synthesis of analogs closer to amicetin, the importance of this route has been increased with the recent successful syntheses of 2,3,6-trideoxypyranose nucleosides with established anomeric configurations: C. L. Stevens, N. A. Nielsen, P. Blumbergs, and K. G. Taylor, *J. Am. Chem. Soc.*, **86**, 5695 (1964).

(23) G. E. Hilbert and E. F. Jansen, *ibid.*, **58**, 60 (1936).

(24) C. S. Hudson and A. Kunz, *ibid.*, **47**, 2052 (1925).

(25) E. Fisher and H. Fisher, *Ber.*, **43**, 2521 (1910).

λ_{max}^{EtOH} 274 mμ (ε 5470), $[\alpha]^{25}D$ +18.0 (*c* 1, chloroform). The analytical sample was dried immediately before analysis for 1 min. at 180°.

Anal. Calcd. for $C_{31}H_{40}N_2O_{19}$: C, 50.00; H, 5.41; N, 3.76. Found: C, 50.14; H, 5.57; N, 3.66.

1-(Hepta-O-acetyl-β-lactosyl)-4-ethoxy-2-(1H)pyrimidone (IIa).—A mixture of 2.85 g. (4.35 mmoles) of chloro sugar Ia and 6 ml. of 2,4-diethoxypyrimidine was heated at 120–125° for 6 days. To the cooled reaction mixture was added 2 ml. of diisopropyl ether and 6 ml. of ethyl ether, and the solution was allowed to stand at room temperature overnight. The solid that had formed was filtered and recrystallized from methanol to yield 0.78 g. of material, m.p. 146–148° (foaming). The mother liquors were concentrated to a small volume, then rediluted with 20 ml. of diisopropyl ether. After 2 days at room temperature, additional material had separated. The solid was filtered and recrystallized twice from methanol to yield 0.75 g. of product, m.p. 147–148.5° (foaming). The total yield of product was 1.53 g. (46%). After two additional recrystallizations from methanol, an analytical sample, m.p. 147.5–150°, λ_{max}^{EtOH} 275 mμ (ε 6450), $[\alpha]^{24}D$ +20.1 (*c* 1, chloroform), was obtained. Before analysis, the sample was dried at 150° for 1 min.

Anal. Calcd. for $C_{32}H_{42}N_2O_{19}$: C, 50.66; H, 5.58; N, 3.69. Found: C, 50.63; H, 5.75; N, 3.72.

1-(β-Lactosyl)cytosine (IIIa) by Ammonolysis of IIa.—A mixture of 1.5 g. (2.01 mmoles) of 1-hepta-O-acetyl-β-lactosyl)-4-ethoxy-2(1H)-pyrimidone (IIa) and 25 ml. of ethanol, saturated with anhydrous ammonia at 0°, was heated in an autoclave at 100° for 15 hr. The resultant solution was concentrated *in vacuo* to a gum. The gum was dissolved in the minimum amount of hot water and an excess of hot absolute ethanol was added. A gum separated. The mixture was cooled to 0° for 1 hr. and the supernatant liquid was decanted. The residual gum was dissolved in a few drops of hot water. Five drops of concentrated hydrochloric acid was added, followed by hot absolute ethanol until the solution was turbid. After cooling, the product was filtered and dried. The filtrate was concentrated *in vacuo* and rediluted with hot absolute ethanol to give an additional crop of product. The total yield of product, m.p. 207° dec., $\lambda_{max}^{50\% MeOH}$ 277 mμ (ε 13,210), $[\alpha]D$ +23.1 (*c* 1, water), was 0.53 g. (57%). Recrystallization of the material did not raise the melting point.

Anal. Calcd. for $C_{16}H_{26}ClN_3O_{11}$: C, 40.73; H, 5.55; N, 8.91. Found: C, 40.47; H, 5.87; N, 8.84.

1-(Hepta-O-acetyl-β-lactosyl)-4-acetamido-2(1H)-pyrimidone (IVa). A. By the Reaction of Ic with N-Acetylcytosinemercury.—To an azeotropically dried mixture of 1 g. (2.85 mmoles) of N-acetylcytosinemercury in 100 ml. of benzene was added 4 g. (5.71 mmoles) of the bromo sugar Ic and the vigorously stirred mixture was refluxed for 14 hr. To the cooled reaction mixture was added 200 ml. of petroleum ether, the product was filtered, and the filtrate was discarded. The solid was dissolved in chloroform; the chloroform solution was washed with 30% potassium iodide solution and then water, and dried over anhydrous sodium sulfate. The solvent was removed under reduced pressure to leave a gum. The gum was chromatographed over neutral alumina (activity grade Brockman II). The column was eluted with 150 ml. of ethyl acetate–benzene (1:4), 150 ml. of ethyl acetate–benzene (2:3), and 100 ml. of ethyl acetate to remove impurities. The column was then washed with 300 ml. of methanol, the solution was concentrated to dryness, and the residue was treated with excess acetic anhydride and pyridine. The excess reagents were removed under reduced pressure, the residue was dissolved in the minimum amount of chloroform, diluted with excess ethyl acetate, concentrated again, and rediluted with hot ethanol. A crystalline product was obtained. The material was recrystallized by dissolving in the minimum amount of hot chloroform and adding hot ethanol. In this manner 0.39 g. of material, m.p. 279–280° dec., was obtained. Concentration of the mother liquors gave additional product. The total yield of pure product, m.p. 279–280° dec., λ_{max}^{MeOH} 249 mμ (ε 18,270) and 298 mμ (ε 6680), $[\alpha]^{25}D$ +23.1 (*c* 1, chloroform), was 0.53 g. (24%). An additional recrystallization did not raise the melting point. Prior to analysis the compound was dried *in vacuo* at 140° for 12 hr.

Anal. Calcd. for $C_{32}H_{41}N_3O_{19}$: C, 49.81; H, 5.36; N, 5.45. Found: C, 50.08; H, 5.57; N, 5.40.

B. By Acetylation of IIIa.—1-(β-Lactosyl)cytosine hydrochloride (IIIa), 0.14 g. (0.30 mmole), was dissolved in 15 ml. of acetic anhydride and 5 ml. of pyridine and allowed to stand at room temperature for 3 days. The solution was then concentrated *in vacuo* to dryness; the residue was dissolved in chloroform, washed with sodium bicarbonate solution and water, and then dried over anhydrous sodium sulfate. The solvent was removed under reduced pressure and hot ethanol was added to the residue. A crystalline solid was obtained. Recrystallization from chloroform–ethanol gave 0.22 g. (95%) of the desired product, m.p. 278–279°, $[\alpha]^{25}D$ +24.6 (*c* 1.2, chloroform). The infrared absorption spectra of this material was identical in every respect with the spectra of the same compound prepared by the mercury salt procedure.

Hepta-O-acetyl-α-cellobiosyl Chloride (Ib).—To a solution of 5 g. (7.36 mmoles) of cellobiose octaacetate in 50 ml. of absolute chloroform was added 1.4 g. (7.36 mmoles) of titanium tetrachloride and the mixture was refluxed for 24 hr. The cooled reaction mixture was shaken with dilute hydrochloric acid, the chloroform layer was separated, and the aqueous portion was extracted twice with chloroform. The combined chloroform fractions were washed with water, dried over anhydrous sodium sulfate, and concentrated *in vacuo* to dryness. The residual product was crystallized from ethyl acetate–ethanol to yield 3.75 g. (78%) of material: m.p. 191–194°, $[\alpha]^{25}D$ +68.2 (*c* 1.1, chloroform); lit.[13] m.p. 200–201°, $[\alpha]^{20}D$ +71.7. This material was used without further purification.

Hepta-O-acetyl-α-cellobiosyl Bromide (Id).—This material was prepared by a modified procedure[26] of Fisher and Zemplen.[27] From 10 g. of starting cellobiose octaacetate, 6.70 g. (65%) of product, m.p. 189° dec., was obtained (lit.[27] 62% yield, m.p. 180°).

1-(Hepta-O-acetyl-β-cellobiosyl)-4-ethoxy-2(1H)-pyrimidone (IIb).—A mixture of 1.5 g. (2.44 mmoles) of the chloro sugar Ib and 3.5 ml. of 2,4-diethoxypyrimidine was heated at 130–135° for 10 days, then at 135–140° for 12 hr. To the cooled reaction mixture was added 50 ml. of diisopropyl ether, the mixture was filtered, and the solid was recrystallized twice from ethanol. The product obtained weighed 0.96 g. (55%) and melted at 197–198.5°. Two additional recrystallizations from ethanol gave pure material, m.p. 199–200°, λ_{max}^{EtOH} 275 mμ (ε 6030), $[\alpha]^{25}D$ +7.0 (*c* 0.8, chloroform).

Anal. Calcd. for $C_{32}H_{42}N_2O_{19}$: C, 50.66; H, 5.58; N, 3.69. Found: C, 50.53; H, 5.62; N, 3.75.

1-(β-Cellobiosyl)cytosine (IIIb) Hydrochloride.—1-(Hepta-O-acetyl-β-cellobiosyl)-4-ethoxy-2(1H)-pyrimidone (IIb), 1 g. (1.32 mmoles), was partially dissolved in 25 ml. of ethanol; the mixture was saturated at 0° with anhydrous ammonia and heated in an autoclave at 100° for 14 hr. The resultant solution was concentrated *in vacuo* to yield a gum. The gum was dissolved in the minimum amount of water and hot absolute ethanol was added to the solution. On cooling, an amorphous solid was obtained. The product was filtered and dissolved in 5 ml. of water, 5 drops of concentrated hydrochloric acid was added, and the solution was diluted with 30 ml. of hot absolute ethanol containing 3–4 ml. of benzene. The crystalline product was filtered and recrystallized to yield 0.47 g. (75%) of material, m.p. 238–239° dec. Two additional recrystallizations gave an analytically pure product, m.p. 242–243° dec., $\lambda_{max}^{0.1\ N\ HCl}$ 275 mμ (ε 13,510), $[\alpha]^{25}D$ +9.66 (*c* 1, water).

Anal. Calcd. for $C_{16}H_{26}ClN_3O_{11}$: C, 40.73; H, 5.55; Cl, 7.52; N, 8.91. Found: C, 40.90; H, 5.58; Cl, 7.67; N, 9.01.

1-(Hepta-O-acetyl-β-cellobiosyl)-4-acetamido-2(1H)-pyrimidone (IVb). A. By the Reaction of Id with N-Acetylcytosinemercury.—The reaction of 0.50 g. (1.42 mmoles) of N-acetylcytosinemercury with 2.0 g. (2.86 mmoles) of hepta-O-acetyl-α-cellobiosyl bromide (Id) in 50 ml. of benzene as solvent was conducted in a manner similar to that described for the preparation of the nucleoside IVa. The crude reaction product was treated with excess acetic anhydride and pyridine for 12 hr. The excess reagents were removed under reduced pressure and the residue was dissolved in chloroform. The chloroform was washed with aqueous saturated sodium bicarbonate solution and water and dried over anhydrous sodium sulfate. Removal of the solvent *in vacuo* left a heavy gum. The gum was redissolved in a small volume of chloroform and the solution was divided into three equal portions for chromatography. Each portion was carefully pipetted on top of a tightly packed cellulose column (100 g. of cellulose for 1.0 g. of compound) and washed onto the column with 50 ml. of petroleum ether. Elution with 300 ml. of a petro-

(26) G. Zemplen, *Ber.*, **53**, 996 (1920).

(27) E. Fisher and G. Zemplen, *ibid.*, **43**, 2536 (1910).

leum ether–chloroform mixture (3:1) removed undesirable side products which were discarded. Elution with 200 ml. of chloroform removed IVb in slightly impure form. One additional chromatography, followed by crystallization of the material from a chloroform–ethyl acetate mixture, gave a total of 0.50 g. (46%) of pure product, m.p. 261.5–262.5°. Two additional recrystallizations gave an analytically pure sample, m.p. 262.5–263°, λ_{max}^{EtOH} 251 mμ (ε 6600), $[\alpha]^{25}D$ +12.1 (*c* 1, chloroform).

Anal. Calcd. for $C_{32}H_{41}N_3O_{19}$: C, 49.81; H, 5.36; N, 39.39; O, 5.45. Found: C, 49.75; H, 5.47; N, 39.43; O, 5.42.

B. By Acetylation of IIIb.—A solution of 0.15 g. (0.32 mmole) of 1-(β-cellobiosyl)cytosine (IIIb) hydrochloride in 5 ml. of acetic anhydride and 5 ml. of pyridine was heated on the steam bath for 1 hr., then stored at room temperature for 20 hr. The solution was poured in ice–water and the mixture was extracted with chloroform. The chloroform extracts were washed with saturated sodium bicarbonate solution and water and dried over anhydrous sodium sulfate. Evaporation of the solvent under reduced pressure gave a solid residue which, after recrystallization from absolute ethanol, amounted to 0.17 g. (67%), m.p. 259–261°, $[\alpha]^{25}D$ +11.7 (*c* 1, chloroform). A mixture of this compound with the product obtained by method A showed no depression in melting point.

1-(Tetra-O-acetyl-β-D-glucopyranosyl)-4-acetamido-2(1H)-pyrimidone (V). A. By the Cleavage of IIIa.—A mixture of 0.1 g. (0.21 mmole) of 1-(β-lactosyl)cytosine (IIIa) hydrochloride in 25 ml. of methanol was saturated at 0° with anhydrous hydrogen chloride and allowed to stand at room temperature for 20 hr. The resultant solution was concentrated *in vacuo* to a small volume, then chromatographed over ion-exchange resin (Dowex 50 W-X2, acid form). The product was eluted with a 3% solution of ammonia in methanol. The residue obtained after concentration of the eluates was treated with acetic anhydride and pyridine at room temperature for 1 day. Removal of the excess reagents *in vacuo* left a solid residue. Two recrystallizations from ethanol gave 66 mg. (65%) of product, m.p. 225–226°, $[\alpha]^{25}D$ +40.0 (*c* 1, chloroform).

A sample of the authentic V,[8b] recrystallized from ethanol, had m.p. 225–226° and $[\alpha]D$ +40.8 (*c* 1.2, chloroform). The melting point of a mixture of this compound and the lactose cleavage product was undepressed, and the infrared absorption curves of the two samples were identical in every respect.

B. By the Cleavage of IIIb.—1-(β-Cellobiosyl)cytosine (IIIb) hydrochloride (0.2 g., 0.42 mmole) was treated at room temperature with 100 ml. of a saturated solution of hydrogen chloride in methanol for 36 hr. Work-up of the reaction mixture as described above yielded 95 mg. (47%) of the desired product, m.p. 225–226°, $[\alpha]^{25}D$ +40.4 (*c* 1.1, chloroform). The melting point of this material was not depressed by admixture with authentic V and the infrared absorption curve was identical in every respect to that of the authentic nucleoside.

2-Deoxy-2-benzamido-3,4,6-tri-O-acetyl-α-D-glucopyranosyl Bromide (VI).—A mixture of 5 g. (0.011 mole) of 1,3,4,6-tetraO-acetyl-2-deoxy-2-benzamido-β-D-glucopyranose,[28] 5 ml. of acetic anhydride, and 15 ml. of glacial acetic acid was cooled to 0° and saturated with anhydrous hydrogen bromide. The resultant solution was stored at 0° for 4 hr., diluted with ice-cold chloroform, and poured on ice. The chloroform layer was separated and the water was extracted rapidly with chloroform. The combined chloroform extracts were washed with ice–water and ice-cold saturated sodium bicarbonate solution and immediately dried over anhydrous sodium sulfate. Evaporation of the solvent under reduced pressure left a gum which was dissolved in a small volume of anhydrous ethyl ether and stored in the refrigerator. A crystalline product could not be obtained and the crude product was used as such in the next step.

1,2,3,4-Tetra-O-acetyl-6-(2-deoxy-2-benzamido-3,4,6-tri-O-acetyl-β-D-glucopyranosyl)-β-D-glucopyranose (VIIa).—In a 125-ml. erlenmeyer flask fitted with a drying tube were placed 30 ml. of azeotropically dried benzene, 5 g. of Drierite (dried at 200° for 12 hr.), the crude bromo sugar VI (prepared from 5 g. of the 1-acetate), 4.5 g. (0.013 mole) of 1,2,3,4-tetra-O-acetyl-β-D-glucopyranose,[19] and 1.3 g. of silver carbonate. The reaction mixture, protected from light, was agitated at room temperature with a magnetic stirrer for 1 hr. The mixture gelled and was diluted with 50 ml. of chloroform. After stirring for 2 days, the mixture was filtered, the residue was washed thoroughly with chloroform, the filtrate and washings were combined, and the solvent was removed *in vacuo*. The partially solid product was crystallized once from methanol, yielding 2.74 g. (33% for the two steps) of pure product: m.p. 252–253°, $[\alpha]^{27}D$ −2.1 (*c* 1, chloroform); lit.[16] m.p. 251–253°, $[\alpha]^{22}D$ −2.3.

2,3,4-Tetra-O-acetyl-6-(2-deoxy-2-benzamido-3,4,6-tri-O-acetyl-β-D-glucopyranosyl)-D-glucopyranosyl Chloride (VIIb).—A mixture containing 2 g. (2.70 mmoles) of VIIa and 20 ml. of acetic anhydride was saturated at 0° with anhydrous hydrogen chloride, then stored at 15° for 3 hr. At the end of this period the reaction mixture was diluted with 30 ml. of ice-cold chloroform and poured on ice. The chloroform layer was separated and the water layer was extracted with chloroform. The chloroform extracts were combined, washed with ice–water, ice-cold saturated sodium bicarbonate solution, and ice–water, and dried over anhydrous sodium sulfate. The solution was concentrated *in vacuo* to a small volume and diluted with anhydrous ether. A gelatinous solid formed. The solution was suction filtered; the solid was washed with ether and dried *in vacuo* to yield 0.81 g. (42%) of product, m.p. 150–151° dec. For the preparation of an analytical sample the compound was dissolved in a small volume of chloroform and precipitated by the addition of ether. Two reprecipitations gave a pure product, m.p. 152–153° dec., $[\alpha]^{25}D$ +21.5 (*c* 1, chloroform).

Anal. Calcd. for $C_{31}H_{38}ClNO_{16}$: C, 52.00; H, 5.35; Cl, 4.95. Found: C, 52.19; H, 5.51; Cl, 4.75.

1,2,3,4-Tetra-O-acetyl-6-O-trichloroacetyl-β-D-glucopyranose (IXa).—To a solution of 6 g. (0.017 mole) of 1,2,3,4-tetra-O-β-D-glucopyranose[19] in 60 ml. of benzene was added 6 ml. of pyridine followed by a solution of 4.3 g. (0.024 mole) of trichloroacetyl chloride in 10 ml. of benzene. The reaction mixture was stored for 6 hr. at room temperature, then poured into 100 ml. of water. The benzene layer was separated, washed with water, dilute acetic acid, and water, and then taken to dryness *in vacuo*. The solid was washed with 15 ml. of ethanol and dried to yield 7.22 g. of product, m.p. 104–105°. Concentration of the washings gave additional material. The total yield was 7.41 g. (87%). Recrystallization from a ligroin–diisopropyl ether mixture did not raise the melting point.

Anal. Calcd. for $C_{16}H_{19}Cl_3O_{11}$: C, 38.93; H, 3.88; Cl, 21.55. Found: C, 38.94; H, 4.00; Cl, 21.47.

2,3,4-Tri-O-acetyl-6-O-trichloroacetyl-α-D-glucopyranosyl Bromide (IXb).—A solution of 4 g. (8.1 mmoles) of IXa in 20 ml. of glacial acetic acid and 4 ml. of acetic anhydride was saturated at 0° with anhydrous hydrogen bromide. The solution was kept at 0° for 4.5 hr. and then poured on ice, and the mixture was extracted three times with chloroform. The chloroform extracts were combined, washed with ice–water, ice-cold saturated sodium bicarbonate solution, and ice–water, and dried immediately over anhydrous sodium sulfate. The chloroform was removed under reduced pressure and the crystalline residue was triturated with 10 ml. of diisopropyl ether. The product was separated by suction filtration, washed with 10 ml. of diisopropyl ether, and dried to yield 3.80 g. (91%) of material, m.p. 167–168.5°. For purification, the compound was dissolved in the minimum volume of warm chloroform and the solution was diluted with a fourfold excess of diisopropyl ether. Two crystallizations gave analytically pure product, m.p. 168.5–169°, $[\alpha]^{24}D$ +161 (*c* 1.3, chloroform).

Anal. Calcd. for $C_{14}H_{16}BrCl_3O_9$: C, 32.68; H, 3.13; O, 27.98. Found: C, 32.89; H, 3.38; O, 28.24.

1-(2,3,4-Tri-O-acetyl-6-O-trichloroacetyl-β-D-glucopyranosyl)-4-ethoxy-2(1H)-pyrimidone (Xa).—A mixture of 2.75 g. (5.34 mmoles) of IXb and 5.5 ml. of 2,4-diethoxypyrimidine was heated at 80–85° for 20 hr., then at 90–95° for 24 hr. The reaction mixture was diluted with 40 ml. of ether–diisopropyl ether (1:3) and filtered and the residual solid was crystallized from absolute ethanol to yield 1.15 g. of material, m.p. 221–222.5°. Additional product was obtained from the concentrated mother liquors. The total yield was 1.25 g. (41%). Two recrystallizations from ethanol gave analytically pure product, m.p. 221.5–223°, λ_{max}^{EtOH} 276 mμ (ε 6410), $[\alpha]^{25}D$ +43.8 (*c* 1.1, chloroform).

Anal. Calcd. for $C_{20}H_{23}Cl_3N_2O_{11}$: C, 41.87; H, 4.04; N, 4.88. Found: C, 42.09; H, 4.04; N, 4.84.

1-(2,3,4-Tri-O-acetyl-β-D-glucopyranosyl)-4-ethoxy-2(1H)-pyrimidone (Xb).—To a solution of 1 g. (1.74 mmole) of Xa in 50 ml. of benzene was added 25 ml. of toluene previously saturated at 0° with anhydrous ammonia. The reaction mixture was placed in an ice-bath for 2 hr. and then concentrated to dryness *in vacuo*. The solid residue was washed with 5 ml. of ether and recrystallized from an ethanol–diisopropyl ether mixture to yield

(28) M. Bergmann and L. Zervas, *Ber.*, **B64**, 975 (1931).

0.58 g. (78%) of product, m.p. 218–219°, λ_{max}^{EtOH} 276 mμ (ε 6430), $[\alpha]^{26}D$ +46.5 (*c* 1, chloroform).

Anal. Calcd. for $C_{18}H_{24}N_2O_{10}$: C, 50.54; H, 5.65; N, 6.54. Found: C, 50.77; H, 5.78; N, 6.32.

Acetylation of Xb.—Xb (70 mg., 0.16 mmole) was treated with excess acetic anhydride and pyridine at room temperature for 12 hr. The solution was poured on an ice–potassium carbonate mixture and extracted with chloroform. The chloroform extract was washed with water and dried over anhydrous sodium sulfate and the solvent was removed *in vacuo*. The solid product, Xc, was recrystallized from 3 ml. of ethanol to yield 65 mg. (86%) of material, m.p. 203.5–204.5°, $[\alpha]^{26}D$ +34.9 (*c* 1, chloroform).

An authentic sample of this material was prepared in 46% yield as described by Hilbert and Jansen.[29] After two recrystallizations from ethanol, a pure product, m.p. 203.5–204.5°, $[\alpha]^{26}D$ +33.9 (*c* 1, chloroform), was obtained (lit.[29] m.p. 206°, $[\alpha]D$ +36.1). Admixture of this materal with the product obtained by the acetylation of Xb did not depress the melting point and the infrared absorption curves of both samples were identical in every respect.

1-[2,3,4-Tri-O-acetyl-6-(2-deoxy-2-benzamido-3,4,6-tri-O-acetyl-β-D-glucopyranosyl)-β-D-glucopyranosyl]-4-ethoxy-2(1H)-pyrimidone (VIII). A. By the Reaction of VIIc with Diethoxypyrimidine.—A mixture of 0.80 g. (1.1 mmoles) of the chloro sugar VIIc and 2 ml. of 2,4-diethoxypyrimidine was heated at 85° for 40 hr. and then at 130° for 6 hr. The cooled reaction mixture was diluted with 40 ml. of diisopropyl ether; the solid was separated by filtration and crystallized from methanol to yield 80 mg. of product, m.p. 285–287°. The mother liquors were concentrated and two additional crops of product, m.p. 285–286°, were obtained. The total yield was 0.20 g. (21%). Two additional recrystallizations from methanol gave an analytically pure compound, m.p. 287–288°, λ_{max}^{EtOH} 274 mμ (ε 4970), $[\alpha]^{25}D$ −6.0 (*c* 1, chloroform).

Anal. Calcd. for $C_{37}H_{45}N_3O_{18}$: C, 54.21; H, 5.53; N, 5.13. Found: C, 54.23; H, 5.43; N, 5.14.

B. By the Reaction of IXb with 1-(2,3,4-Tri-O-acetyl-β-D-glucopyranosyl)-4-ethoxy-2(1H)-pyrimidone.—A vigorously stirred mixture of 0.47 g. (1.1 mmoles) of the nucleoside Xb, 0.80 g. of silver carbonate, 3 g. of Drierite (predried at 200° for 12 hr.), the bromo sugar IXb (prepared from 1.5 g. of IXa), and 10 ml. of azeotropically dried benzene was refluxed for 3 hr. in the dark, then stirred at room temperature for additional 10 hr. The mixture was then diluted with chloroform and filtered, the residue was washed thoroughly with chloroform, the filtrate and washing were combined, and the solvent was evaporated under reduced pressure. The residual gum, when triturated with ether, solidified. The solid was dissolved in methanol and, after a treatment with charcoal, the solution was concentrated to a small volume. The crystalline product obtained was recrystallized once more from methanol to yield 90 mg. (9% based on Xb) of analytically pure product, m.p 287–288°, $[\alpha]^{26}D$ −6.5 (*c* 1, chloroform). Admixture with the compound obtained by procedure A did not depress the melting point and the infrared absorption curve was identical in every respect with the infrared absorption curve of the nucleoside obtained by procedure A.

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Amino Derivatives of Starches. 2,6-Diamino-2,6-dideoxy-D-mannose Dihydrochloride[1]

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2,6-Diamino-2,6-dideoxy-β-D-mannose dihydrochloride (VI), a reference compound required in studies on aminated starch derivatives, was synthesized from 2-acetamido-2-deoxy-D-mannose. Phenyl 2-acetamido-2-deoxy-α-D-mannopyranoside (III), prepared from 2-acetamido-1,3,4,6-tetra-*O*-acetyl-2-deoxy-β-D-mannopyranose (I), was *p*-toluenesulfonated at the 6-position, and the 6-*p*-tolylsulfonyloxy group was replaced by the azido group. Reduction of the azido group and removal of the protecting groups gave the diamino sugar VI by a stereochemically unambiguous route.

Our laboratory has been concerned with the preparation of aminated starch and starch derivatives, and with methods for structural characterization of the products. Selective *p*-toluenesulfonation of a slightly derivatized amylose has been shown[2] to yield a product, degree of substitution (D.S.) 1.7, which was considered to be preponderantly the 2,6-di-*p*-toluenesulfonate ester. Hydrazinolysis of this product followed by reduction and selective *N* acetylation, gave an *N*-acetylated, aminated amylose of D.S. 1.4. This derivative is undoubtedly a complex hetero polymer, since several possible reactions between hydrazine and the *p*-toluenesulfonated units of the amylose derivative can be envisaged. We have conducted a series of studies on simple sugar derivatives to provide model systems for the reaction leading to aminated amylose and to provide reference compounds for comparison with possible fragmentation products obtained from aminated amylose in structural studies by degradative methods.

In the amination by a nitrogen nucleophile of a 2,6-di-*O*-*p*-tolylsulfonyl-α-D-glucopyranose monomeric unit in the (1→4)-linked amylose chain, at least three reasonable and possible mechanistic pathways may be postulated, as shown in Scheme I. The products are shown as having been converted into the free amino derivatives. If direct replacement of both *p*-tolylsulfonyloxy groups takes place (pathway a), inversion will occur at C-2, and a 2,6-diamino-2,6-dideoxy-D-mannose residue will be formed. If the reagent functions as a strong base, the initial reaction may involve intramolecular attack by the anion of the C-3 hydroxyl group to displace the 6-*p*-tolylsulfonyloxy group (pathway c) and give a 3,6-anhydro-2-*O*-

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