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SYNTHESIS AND HYDROLYSIS OF *N,N'*-DIGLYCOPYRANOSYLETHYLENEDIAMINES

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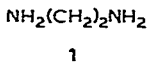
ABSTRACT

Condensation products from ethylenediamine and simple reducing sugars were prepared and their chemical behavior was studied in order to understand how ethylenediamine may react with the reducing ends of wood polysaccharides in ethylenediamine–soda pulping. The *N,N'*-diglycosyldiamines containing D-glucose, D-mannose, and cellobiose were hydrolyzed rapidly but not completely in water at moderate temperatures (35–65°). The reactions were at equilibrium; the greater the diamine concentration in solution, the less hydrolysis of the condensation products occurred.

INTRODUCTION

Despite its versatility, kraft pulping is increasingly burdened by its high capital costs and environmental pollutants. Other reagents, especially those containing no sulfur, are being sought that will provide satisfactory pulping of wood in alkaline media.

To replace the sodium sulfide in kraft pulping liquor, a wide variety of amines was studied by Kubes and Bolker[1], and ethylenediamine (**1**) proved to be particu-



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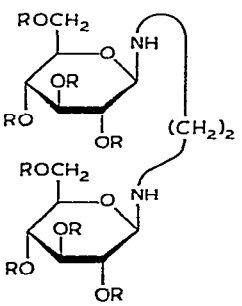
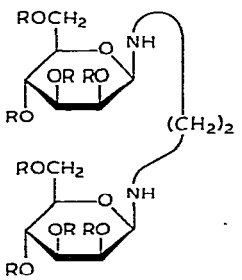
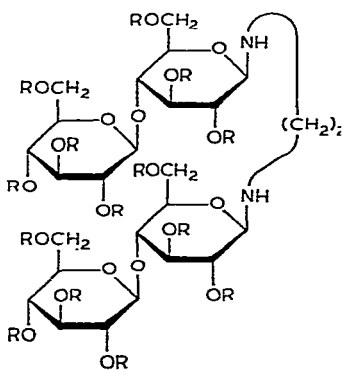


2 R = H
3 R = Ac
4 R = Bz



5 R = H
6 R = Ac



7 R = H
8 R = Ac

larly effective as an accelerator for soda pulping. Surprisingly, soda–ethylenediamine-unbleached and -bleached softwood pulps had unusually hightear-strengths, typically about 1.5 times greater than those for control kraft pulps. It was suggested[2] that **1** might interact chemically with the hemicelluloses in the wood, possibly maintaining their orientation in the fibers longer than would be expected in other pulping liquors, leading to the production of pulps having superior mechanical properties.

The reactions of **1** with some simple reducing sugars were studied in order to indicate how **1**, and by analogy, other simple amines, might react with the reducing-end residues of polysaccharides in wood. By use of this approach, fully-characterized ethylenediamine–carbohydrate compounds were prepared, and were then subjected to conditions representing the impregnation stage of two-stage soda–ethylenediamine pulping. Recent publications[3] have described some polymeric properties of cellulose in **1**, reactions of monoethanolamine with lignin model compounds[4], and hydrogen-bonded complexes[5] of reducing sugars with **1**.

Reducing sugars react rapidly with primary amines to form glycosylamines[6–8]. Reactions of some arylenediamines with aldoses are known[9–12]; in particular, reactions of *o*-phenylenediamine with reducing sugars to form benzimidazoles[13] have been extensively studied. Little investigation, however, has been devoted to the reactions of alkyldiamines with aldoses and ketoses, or to the chemical behavior of the products thus formed. In fact, the preparation of *N*,*N*′-di-D-glucopyranosylethylenediamine has been reported only twice[14–16], and its per-*O*-acetyl derivative once[17]. The condensation products of **1** with D-mannose and cellobiose are described here for the first time.

RESULTS AND DISCUSSION

Glycosylamines are prepared by heating the reducing sugar and amine in alcohol at reflux, sometimes in the presence of acids as catalysts. Unlike glycosides, glycosylamines can mutarotate, but the mechanism involved is not well understood[6,16]. More important in the context of this work is the observation that a glycosylamine in aqueous solution is hydrolyzed to give the amine and sugar components. The ease of hydrolysis of simple glycosylamines generally increases with the dissociation constant of the amine[14,18]. The reaction reaches an equilibrium, and the extent of hydrolysis depends on the initial glycosylamine concentration. In addition, the reaction temperature and the pH of the solution play a role[14], for example, in that the hydrolysis of L-arabinosylamine[19] is fastest at pH 5, but very slow above pH $\sim$ 7 or below $\sim$3. Mutarotation and hydrolysis of glycosylamines have been studied by polarimetry, but this technique provides no direct evidence of the disappearance of the starting material or the identity of the products formed. In this work, polarimetry has been used to supplement the quantitative analysis of products by g.l.c. and p.m.r. spectroscopy. By use of g.l.c.–m.s. and t.l.c., the extent of hydrolysis was also estimated and the products identified.

The diglycosyldiamines were readily obtained from treatment of the reducing

TABLE I

^{13}C CHEMICAL SHIFTS FOR COMPOUNDS **2**, **3**, **4**, AND **8**[a]

Compound	*C-1*	*Other ring C atoms*[b]				*C-6*	*-CH_2-*	*-$COCH_3$*
2	92.1	78.7	74.8	71.9		62.9	47.2	
3	85.9	80.4	74.7	73.2	68.6	62.0	41.7	20.4
4	92.2	73.8	72.3	70.1		62.9	43.9	
8	90.9	74.9	73.4	69.0		62.0	43.9	20.6

[a]All values in p.p.m. relative to the tetramethylsilane signal (0). Compounds **3**, **4**, and **8** were measured in chloroform-*d* solutions, compound **2** in dimethyl sulfoxide-d_6. [b]Positive assignments for C-2, C-3, C-4, and C-5 were not made.

sugars with **1** in methanol at reflux. The *N,N'*-di-D-glucopyranosyl- (**2**), *N,N'*-di-D-mannopyranosyl- (**5**), and *N,N'*-dicellobiosyl-ethylenediamine (**7**) were isolated as chromatographically pure (by t.l.c.) precipitates. In a previous report[14], **2** was stated to have been recrystallized from methanol–water (no proportions given); however, the presence of water may have caused partial hydrolysis, and the characterization provided no real evidence for its structure.

Compound **2** was analyzed by ^1H- and ^{13}C-n.m.r. spectroscopy. In addition, the per-*O*-acetyl (**3**) and per-*O*-benzoyl (**4**) derivatives were prepared, and their structures determined in a variety of ways. P.m.r. spectroscopy of **2** in dimethyl sulfoxide-d_6 showed broad resonance signals that were of limited help in elucidating its structure. The proton-decoupled ^{13}C-n.m.r. spectrum of **2** in the same solvent clearly showed the various resonance signals attributable to the carbon atoms of the D-glucose residues, plus the methylene carbons of the ethylenediamine residue (Table I). The ring-carbon signal farthest downfield (δ 92.1 p.p.m., Me_4Si = 0) was assigned to the two identical C-1 atoms. After 7 days, a small signal appeared at 88.5 p.p.m., and was assumed to be the C-1 resonance signal of the α anomer, the result of mutarotation in solution[6,16]. Thus, the initial C-1 signal corresponded to the C-1(β)–N linkages. The difference between the signals (3.6 p.p.m.) agreed with that found for *N*-phenyl-D-glucopyranosylamines[22] (3.9 p.p.m.).

The octa-*O*-acetyl derivative **3** of **2** was a crystalline compound showing no evidence of *N*-acetylation, and the anomeric signal (δ 5.92) was a doublet with $J_{1,2}$ 8.5 Hz, corresponding to the β configuration of the C-1–N linkages. Similarly, **4** had 8 *O*-benzoyl groups, and elemental analyses agreed with the molecular formulas assigned to **2**, **3**, and **4**. The per-*O*-acetyl derivative (**6**) of **5** did not crystallize, but the chromatographically pure material had a p.m.r. spectrum corresponding to that of an octaacetate. The p.m.r. spectrum of the crystalline peracetate **8** of **7** exhibited an *O*-acetyl multiplet incorporating 42 protons, as expected for 14 acetate groups.

The reactions of D-xylose and D-galactose with **1** in methanol went to completion in 1 h at ~60°, with an initial monosaccharide-to-**1** molar ratio of 2:1. In neither

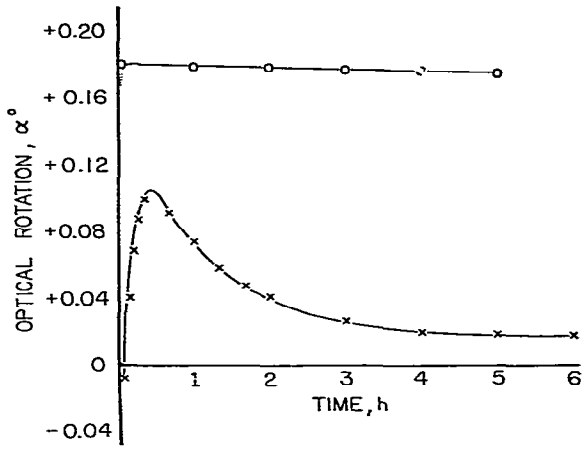


Fig. 1. Polarimetric curves (λ = 546 nm, Hg) for the hydrolyses of **2** and **7** in water at 65°; (O—O—O) **2** and (x—x—x) **7**.

TABLE II

CARBOHYDRATE PRODUCTS FROM THE HYDROLYSIS OF **2**, **5**, AND **7**

Reaction time	*Carbohydrate hydrolysis products found*[a]		
	From **2** (%)[b]	*From* **5** (%)[b]	*From* **7** (%)[c]
10 min	12.2	8.3 (30 min)	46.5
100 min	15.0	14.0 (60 min)	47.1
1 day	24.7	30.0 (120 min)	51.1
7 days	33.3	36.9	57.7
14 days	34.6	35.0	*d*
21 days	*d*	*d*	*d*

[a]Products are given as weight percentages of dried reaction samples. All reactions were performed in water at 65°; 5% diglycosyldiamine by weight. [b]Mixture of D-glucose, D-mannose, and D-fructose. [c]Mixture of cellobiose plus possible alkaline-isomerization products with retention times ±10% of those of the cellobiose anomers. [d]No change.

case, however, could the product be precipitated from the reaction solution. With D-fructose, the reaction reached completion only when a large molar excess of **1** (*e.g.*, 3 : 1) was used. Again, the product could not be precipitated. It has been shown previously[6] that ketoses generally form glycosylamines less readily than do aldoses. These reactions were not studied further.

The hydrolysis of **2**, **5**, and **7** was studied for 5% solutions in water, and the polarimetric curves for the hydrolysis of **2** and **7** at 65° are shown in Fig. 1. For both **2** and **7**, the initial change in optical rotation was extremely rapid. Allowing time for dissolution of the starting materials and loading of the measuring cell, it was impossible to record rotation values within the first 2 min of elapsed time. Each

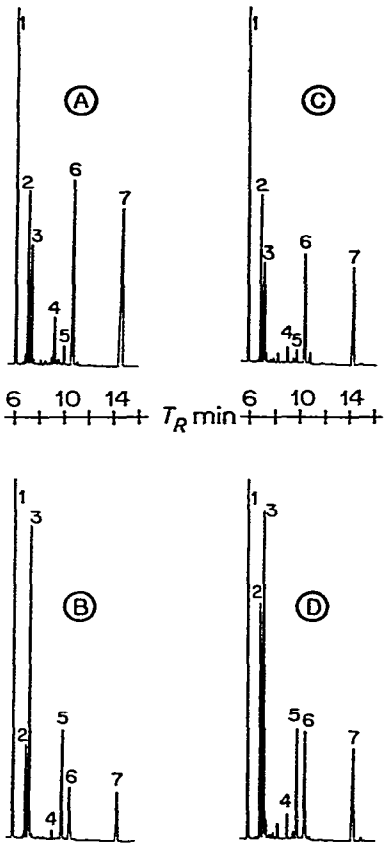
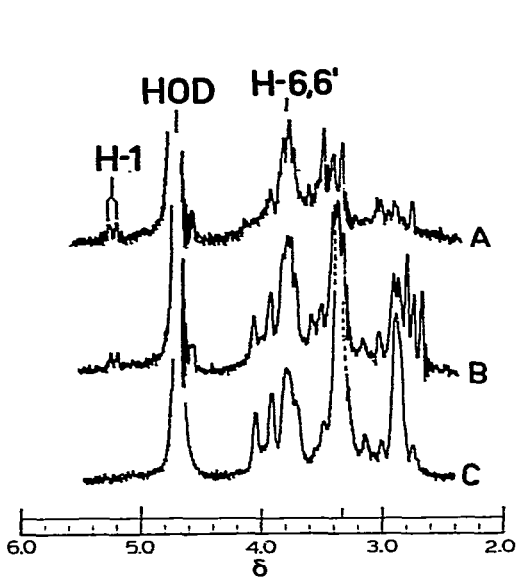


Fig. 2. Capillary gas–liquid chromatograms of the trimethylsilyl derivatives of the final hydrolysis products (in water, 65°) from **2** (A) and **5** (B); and final alkaline isomerization products (in 0.78% aq. **1**, 65°) from D-glucose (C) and D-mannose (D): 1, Internal standard; 2, β-D-fructopyranose; 3, α-D-mannopyranose; 4, β-D-fructofuranose; 5, β-D-mannopyranose; 6, α-D-glucopyranose; and 7, β-D-glucopyranose.

curve reached a maximum in less than 30 min, and then decreased. Color formation (yellow to brown) in the reaction of **2** was rapid, and prevented the measurement of optical rotation after about 6 h. In the hydrolysis of **7**, color formation (yellowing) was much less intense; the change in optical rotation after 15 min was very slow, and was not studied beyond 5 h.

The proportions and the identity of the carbohydrate products of hydrolysis from **2**, **5**, and **7** were determined by g.l.c. (see Table II). The hydrolysis of **2** at 65° reached equilibrium in ~14 days; the products were D-glucose and D-fructose, as determined by g.l.c. and g.l.c.–m.s., and D-mannose as determined by g.l.c. (see Fig. 2A). These hexoses represented ~35% (by weight) of the equilibrium mixture. A satisfactory g.l.c. analysis of the trimethylsilyl derivative of **2** could not be achieved. In the 10-min sample, α- and β-D-glucose were the major carbohydrate products of hydrolysis, as expected. As the reaction proceeded, it became obvious that the



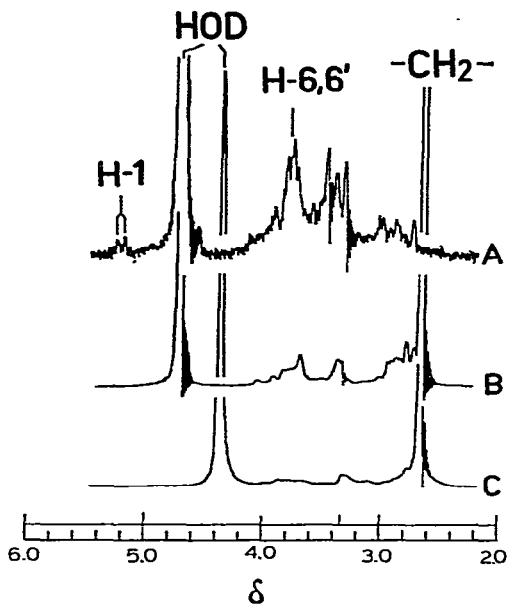


Fig. 3. (left). ^{1}H-N.m.r. spectra (60 MHz) of reaction mixtures from hydrolysis of **2** in deuterium oxide. Reaction times: A, 20 days; B, 4 days; C, 10 min.

Fig. 4. (right). ^{1}H-N.m.r. spectra (60 MHz) of equilibrium mixtures from reactions of **2** in deuterium oxide (A), and in 10% (B) and 40% (C) **1** in deuterium oxide. The methylene protons are part of **1**. Spectrum amplitudes: A, 20; B, 3.2; and C, 1.0.

increasing content of **1** in the reaction mixture was causing alkaline isomerization[20] of D-glucose to D-mannose and D-fructose. Thus, the isomers of all three monosaccharides were found in the 100-min and later samples (see Fig. 2A). Other amines are known to catalyze similar transformations[6,20].

The alkaline isomerization of D-glucose to D-mannose and D-fructose in aqueous **1** was confirmed (see Fig. 2C), as well as that of D-mannose (see Fig. 2D). In addition, evidence (t.l.c.) of condensation products with **1** demonstrated that reducing sugars could react with aqueous **1** even at very low concentrations of **1** (*i.e.*, 0.78%).

The hydrolysis of **5** in water at 65° appeared to be comparable in every way to that of **2** (see Fig. 2-B). Hydrolysis of **7** at 65° reached equilibrium in less than 7 days, cellobiose being the dominant carbohydrate hydrolysis product, in admixture with compounds assumed to be produced by alkaline isomerization of cellobiose; the disaccharides accounted for ~58% of the equilibrium mixture, some starting material remaining intact. As shown in Table II, it is clear that **7** is hydrolyzed faster and to a greater extent than are **2** and **5**. The polarimetric curves (Fig. 1) may be explained by the g.l.c. results. Both **2** and **7** apparently undergo very rapid mutarotation plus hydrolysis initially, resulting in a fast increase of the optical rotation values as the primary carbohydrate product (D-glucose or cellobiose) is formed. As the reaction time increases, alkaline isomerization of these primary hydrolysis products leads to a gradual decrease of the optical rotation, even as hydrolysis continues.

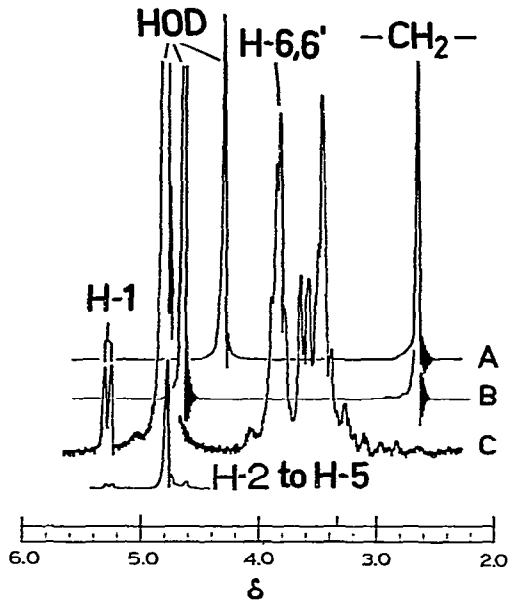


Fig. 5. ^{1}H-N.m.r. reference spectra (60 MHz) of D-glucose (C) and of 10% (B) and 40% (A) solutions of **1** in deuterium oxide. Proton assignments for D-glucose are taken from Perlin and Casu[21].

The aqueous hydrolysis of **2**, **5**, and **7** was investigated qualitatively by ^{1}H-n.m.r. spectroscopy (60 MHz) and t.l.c. on solutions in deuterium oxide. All three compounds underwent rapid initial hydrolysis. The p.m.r. spectrum of **2** at 10 min (see Fig. 3) exhibited strong signals associated with D-glucose (δ 3.80) and with the starting material (δ 3.40, 3.92, and 4.04). After 4 days, the spectrum contained a small doublet (δ 5.23) corresponding to the anomeric proton of α-D-glucose. At 20 days, very few signals associated with the starting material remained. A similar investigation of the behavior of **2** at a concentration of 20% in 10% and 40% solutions of **1** in deuterium oxide at ~35° (see Fig. 4) showed virtually no evidence of formation of D-glucose after two weeks. For comparison, the ^{1}H-n.m.r. spectra of D-glucose and 10% and 40% solutions of **1** in D_2O are shown in Fig. 5. These results are in accord with the hypothesis that the greater the concentration of **1** in the solution, the more the equilibrium is forced toward the preservation of the diglycosyldiamine. On solutions of 5% of **2** in 10% and 40% aqueous solutions of **1** at 65°, t.l.c. analysis after 24 h showed only a trace of D-glucose in the 40% aqueous solution of **1**, and slightly more in the 10% solution. In water only, the hydrolysis of **2** was substantial, as judged qualitatively by the amount of D-glucose observed. Analogous results were obtained when **5** and **7** were hydrolyzed in 10% and 40% aqueous solutions of **1**.

Care must be taken in extrapolating the results just discussed to the far more complex case of impregnation of wood chips at ~80° with **1**. The following conclusions seem to be warranted: (a) By use of 40% of **1** on oven-dry wood and a liquor-to-wood ratio[1] of 4:1, **1** should react readily with the accessible reducing ends of the polysaccharide molecules. For a molecule of **1** to condense with two

reducing end groups (*N,N'*-substitution), the C-1 atoms of the end groups would have to be located within 0.55 nm of each other. In either case, alkaline "peeling" of the polysaccharide should be inhibited in a subsequent "soda cook". Indeed, at comparable yields, α-cellulose contents were found to be[1]: soda–ethylenediamine pulp, 44.6%; soda pulp, 41.0% (based on oven-dry wood). (b) The chemically heterogeneous situation in wood-chip impregnation is probably not a hindrance to the condensation reaction (see preparation of **7**). (c) Because the condensation reaction of **1** with a reducing sugar results in an equilibrium, the reaction will be more complete the greater the concentration of **1**. Conversely, the higher the proportion of water, the greater will be the hydrolysis of the glycosylamine back to **1** and carbohydrate constituents. (d) Even at very low concentrations of **1** in water, some degree of condensation probably occurs. (e) If **1** condenses with the reducing ends of wood polysaccharides during impregnation at ~80°, some proportion of the condensation products may remain stable at least until enough water is introduced (*e.g.*, final pulp washing) to complete the hydrolysis. It is known that some glycosylamines hydrolyze quickly under moderately acidic conditions (pH 4–6), but hardly at all in strongly acidic or alkaline solutions[6,16]. Thus, the alkaline pulping stage that follows impregnation would probably tend to preserve glycosylamine products, providing that the pH of the liquor remains above ~7. High pulping temperatures (160–170°) alone may not cause the decomposition of the condensation products, but would accelerate condensation and hydrolysis rates.

EXPERIMENTAL

General methods. — Melting points were determined on a calibrated Gallenkamp apparatus. T.l.c. was performed on microscope slides or 20 × 20-cm glass plates coated with Silica Gel G (Merck); developing solvents were: (*A*) 1:1 (v/v) and (*B*) 2:1 (v/v) methanol–isopropyl ether; (*C*) 2:1 (v/v) ethyl acetate–chloroform. Optical rotations were recorded with a Perkin–Elmer 141 MC (589 nm, Na) or a Bendix NPL-143 polarimeter (546 nm, Hg). ^{1}H-N.m.r. spectra were recorded with Varian T-60 and HA-100 spectrometers, tetramethylsilane being the internal standard for chloroform-*d* and dimethyl sulfoxide-d_6 solutions, and sodium 2,2-dimethyl-2-silapentane-5-sulfonate for deuterium oxide solutions. Proton-decoupled ^{13}C-n.m.r. spectra were recorded with a Bruker WH-90 spectrometer operating at 22.6 MHz. G.l.c. was performed with Hewlett–Packard 5751B and 5830 chromatographs equipped with flame-ionization detectors, and helium was the carrier gas. Conditions for packed column work were: 3% OV-17 on 100–120 mesh Chromosorb W (AW-DMCS), 1.5 m × 0.32 cm o.d., stainless steel; He, 65 mL/min; column temp. from 120→220° increased by 4°/min, injector at 250°, and detector at 265°. For capillary column work: WCOT OV-17 glass column, 19 m × 0.25 mm i.d., at a split ratio of 1:140; column temp. at 185° isothermal, injector at 220°, and detector at 230°. G.l.c.–m.s. analyses were performed with a Hewlett–Packard 5992 instrument having

a microprocessor input–output–control system; the g.l.c. component of the instrument was operated under the conditions for packed column just described.

N,N'-*Di-β*-D-*glucopyranosylethylenediamine* (**2**). — Ethylenediamine (**1**, 3.5 g, 0.06 mol, analytical-reagent grade) was dissolved in methanol (70 mL, anhydrous), and D-glucose (21.6 g, 0.12 mol) was added. At 50°, under continuous magnetic stirring, the D-glucose dissolved in ~15 min; after ~45 min, a white precipitate formed. After ~1 h, t.l.c. (*A*) showed no remaining D-glucose (R_F 0.51) and one product (R_F 0.05). The reaction mixture was stored for 24 h at ~5°, and the precipitate was filtered off, washed with cold, absolute methanol, and dried *in vacuo* for 48 h at 40° (yield 16.4 g, 71%), m.p. 135–137° (dec.), $[\alpha]_{589}^{20}$ −18.2° (*c* 1.37, dimethyl sulfoxide), $[\alpha]_{546}^{22}$ −25.1° (*c* 1.75, dimethyl sulfoxide); lit.[14] m.p. 152–154° (dec.), $[\alpha]_{589}^{25}$ −17° → +14.5° (*c* 2, 50% aq. ethanol); ^{1}H-n.m.r. (dimethyl sulfoxide-d_6): δ 3.07 (s, 4 H, methylene); ^{13}C-n.m.r.: see Table I.

Anal. Calc. for $C_{14}H_{28}N_2O_{10}$: C, 43.8; H, 7.29; N, 7.29. Found: C, 43.7; H, 7.54; N, 7.09.

N,N'-*Di-(2,3,4,6-tetra*-O-*acetyl-β*-D-*glucopyranosyl)ethylenediamine* (**3**). — Compound **2** was acetylated[23] with acetic anhydride in anhydrous pyridine, and the product crystallized from absolute ethanol, m.p. 152–154°, $[\alpha]_{589}^{20}$ +8.0° (*c* 1.13, chloroform); t.l.c. (*A*): R_F 0.82; ^{1}H-n.m.r. (60 MHz, chloroform-*d*): δ 2.00–2.08 (m, 24 H, OAc), 3.32 (d, 4 H, -CH_2-CH_2-), and 3.80–5.50 (incompletely resolved, 16 H); (100 MHz): δ 5.92 (d, *J* 8.5 Hz, 2 identical anomeric H-α); ^{13}C-n.m.r.: see Table I.

Anal. Calc. for $C_{30}H_{44}N_2O_{18}$: C, 50.0; H, 6.12; N, 3.30. Found: C, 50.0; H, 6.11; N, 3.89.

N,N'-*Di-(2,3,4,6-tetra*-O-*benzoyl-β*-D-*glucopyranosyl)ethylenediamine* (**4**). — Compound **2** was benzoylated[24] with benzoyl chloride in anhydrous pyridine. The product, isolated as a semicrystalline solid that could not be recrystallized, was chromatographically pure [t.l.c. (*B*): R_F 0.92]; m.p. 122–124°, $[\alpha]_{589}^{20}$ +54.7 (*c* 1.28, chloroform); ^{1}H-n.m.r. (chloroform-*d*): δ 3.58 [s (?), 4 H, -CH_2-CH_2-], 3.70–6.40 (incompletely resolved, 16 H), 7.10–7.60 (m, 24 H, *m*- and *p*- Ar-H), and 7.70–8.20 (m, 16 H, *o* Ar-H); ^{13}C-n.m.r.: see Table I.

Anal. Calc. for $C_{70}H_{60}N_2O_{18}$: C, 69.0; H, 5.04; N, 2.18. Found: C, 69.1; H, 4.93; N, 2.30.

N,N'-*Di-β*-D-*mannopyranosylethylenediamine* (**5**). — Compound **5** was prepared by the same procedure described for **2**, giving a white precipitate (yield 1.30 g, 58%, from 2.1 g of D-mannose), m.p. 125–127° (dec.): t.l.c. (*A*), R_F 0.04, and no remaining mannose (R_F 0.50).

A portion of **5** was acetylated with acetic anhydride in anhydrous pyridine to give **6** as a colorless, glassy solid, pure by t.l.c. (*A*, R_F 0.78); ^{1}H-n.m.r. (60 MHz, chloroform-*d*): δ 2.00–2.25 (m, 24 H, OAc).

N,N'-*Di-β-cellobiosylethylenediamine* (**7**). — A large molar excess of **1** was required to bring the reaction to completion, as an initial ratio of 2 mol of cellobiose per mol of **1** resulted in very little product formation. Thus, **1** (960 mg, 16 mmol)

was dissolved in methanol (15 mL, anhydrous), and cellobiose (1.37 g, 4 mmol) was added. At 50° under continuous magnetic stirring, t.l.c. (*B*) showed, after 1 h, that the suspended material was product 7 (R_F 0.05) and that no cellobiose (R_F 0.73) remained. Throughout the 1-h period, the reaction mixture was heterogeneous. After being kept for 24 h at 5°, the product was filtered off, washed with cold, absolute methanol, and dried *in vacuo* for 24 h at 40° (yield, 940 mg, 66%), m.p. 236–238° (dec.), $[\alpha]_{589}^{20}$ +25.7° (*c* 1.48, dimethyl sulfoxide), $[\alpha]_{546}^{22}$ +13.7° (*c* 1.83, dimethyl sulfoxide).

N,N'-*Di*-(*2,3,6,2',3',4',6'-hepta*-O-*acetyl*-β-*cellobiosyl*)*ethylenediamine* (**8**). — Compound **7** was acetylated with acetic anhydride in anhydrous pyridine. The semi-crystalline product (**8**) was chromatographically pure (t.l.c., *A*, R_F 0.76), m.p. 155–157°; ^{1}H-n.m.r. (chloroform-*d*): δ 2.00–2.08 (m, 42 H, OAc); ^{13}C-n.m.r.: see Table I.

Anal. Calc. for $C_{54}H_{76}N_2O_{34}$: C, 50.0; H, 5.86; N, 2.16. Found: C, 50.1; H, 6.04; N, 2.18.

Hydrolysis studies. — *G.l.c.* Unless otherwise noted, the initial concentration of diglycosyldiamine in water was 5% by weight. The required amount of redistilled, de-ionized water, preconditioned to the desired temperature, was rapidly added to the starting material in a screw-capped glass flask, and sealed with a Teflon-faced septum. The flask was placed in a constant-temperature water bath (65° ± 0.1°). Samples (1.0 mL) were taken by syringe, introduced into glass vials (4 mL), and frozen by partial immersion in liquid nitrogen; freezing was complete within 15 s. The frozen samples were freeze-dried at −50°, maintaining the vials at −10° or colder to prevent any melting. After being freeze-dried, the samples were placed in a vacuum oven for 24 h at 50°. For g.l.c. analysis, the samples were treated with silylation-grade dimethyl sulfoxide (0.3–0.4 mL) and Tri-Sil Concentrate (0.1–0.2 mL) (both reagents from Pierce Chemical Co., Rockford, IL 61105); after being warmed and mechanically shaken (~2 h), the g.l.c. sample was taken from the upper layer of the resulting two-phase system and injected into the chromatograph.

Polarimetry. Hydrolysis reactions were started as for g.l.c. analysis. As quickly as possible, some of the reaction solution was transferred to a measuring cell maintained at a constant temperature. The change in optical rotation at 546 nm (Hg) was continuously recorded. Measurement of optical rotation was terminated at 6 h; color development after this time rendered further measurements impossible.

^{1}H-N.m.r. spectroscopy. A 20% (w/v) solution of the diglycosyldiamine in deuterium oxide was prepared in a 5-mm n.m.r. tube; the solvent was preconditioned to normal probe temperature (35°). ^{1}H-N.m.r. spectra were recorded at various times over a span of several weeks. Between measurements, the sample tubes were maintained at 35°.

T.l.c. Samples from the hydrolysis reactions at 65° were analyzed by t.l.c. to monitor the consumption of starting material and the formation of products. The silica gel plates (20 × 20 cm) were developed in solvent *A*; the compounds were detected by spraying with an anisaldehyde–acetic acid–sulfuric acid solution[25] and heating for 20–30 min at ~100°.

The reactions of D-glucose and D-mannose with aqueous **1** were conducted as follows: the aldose (280 mg, 1.56 mmol) and **1** (46.8 mg, 0.78 mmol) were allowed to react in water (6.0 mL) at 65°. Samples were taken by syringe, frozen in liquid nitrogen, freeze-dried, trimethylsilylated, and analyzed by g.l.c. as just described. Methyl β-D-xylopyranoside and methyl α-D-glucopyranoside, the internal standards used for g.l.c. analysis, were unchanged after 28 days in 10% aqueous **1** at 65°.

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