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THE SYNTHESIS OF OLIGOSACCHARIDE–ASPARAGINE COMPOUNDS

PART V. *O*-α-D-MANNOPYRANOSYL-(1→6)-*O*-(2-ACETAMIDO-2-DEOXY-β-D-GLUCOPYRANOSYL)-(1→4)-2-ACETAMIDO-*N*-(L-ASPART-4-OYL)-2-DEOXY-β-D-GLUCOPYRANOSYLAMINE*†

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ABSTRACT

O-α-D-Mannopyranosyl-(1→6)-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→4)-2-acetamido-*N*-(L-aspart-4-oyl)-2-deoxy-β-D-glucopyranosylamine (**12**), used in the synthesis of glycopeptides and as a reference compound in the structure elucidation of glycoproteins, was synthesized *via* condensation of 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl bromide with 2-acetamido-4-*O*-(2-acetamido-3-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-3,6-di-*O*-acetyl-2-deoxy-β-D-glucopyranosyl azide (**5**) to give the intermediate, trisaccharide azide **7**. [Compound **5** was obtained from the known 2-acetamido-4-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-3,6-di-*O*-acetyl-2-deoxy-β-D-glucopyranosyl azide by de-*O*-acetylation, condensation with benzaldehyde, acetylation, and removal of the benzylidene group.] The trisaccharide azide **6** was then acetylated, and the acetate reduced in the presence of Adams' catalyst. The resulting amine was condensed with 1-benzyl *N*-(benzyloxycarbonyl)-L-aspartate, and the *O*-acetyl, *N*-(benzyloxycarbonyl), and benzyl protective groups were removed, to give the title compound.

INTRODUCTION

The title compound **12** was synthesized as a reference compound for the elucidation of the chemical structure of the inner core of glycoprotein carbohydrate chains.

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*Dedicated to Professor V. Deulofeu in honor of his 70th birthday.

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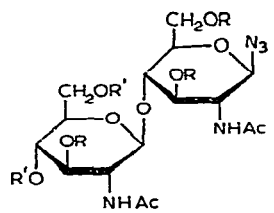
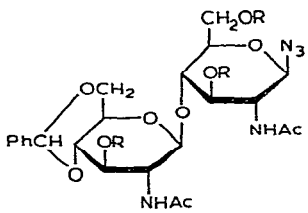
This compound is also of use for the determination of the nature of the antigenic determinants in the reaction between cancer- and virus-transformed cells with concanavalin A and wheat-germ agglutinin[1–4]. A mannosyl–di-*N*-acetylchitobiose–asparagine compound has been isolated from ribonuclease B, and the structure D-mannopyranosyl-(1→3)-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→4)-2-acetamido-*N*-(L-aspart-4-oyl)-2-deoxy-β-D-glucopyranosylamine was suggested for it[5]. Because of the known microheterogeneity of the carbohydrate structures of glycoproteins, other positions of the D-mannopyranosyl linkage may exist in the compound isolated from ribonuclease B, or in similar compounds which have been isolated from glycopeptides isolated from rat-liver microsomes[6], from ovalbumin, *Aspergillus alpha*-amylase, and pineapple bromelain[7], from λ-G myeloma glycoprotein[8], and from silk fibroin[9]. Compound **12** will be useful for comparison with the natural compounds, and for the study of the specificity of enzymes degrading these compounds.

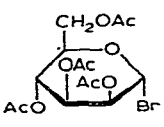
After this work had been completed, evidence was presented by Maley and co-workers[10] that the D-mannopyranosyl residue (1→3)- or (1→4)-linked in the



1 R = R′ = Ac
2 R = R′ = H
5 R = Ac R′ = H



3 R = H
4 R = Ac



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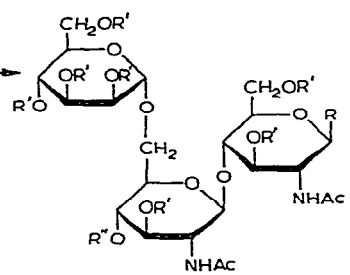

7 R = N_3, R′ = Ac, R″ = H
8 R = N_3, R′ = R″ = Ac
9 R = NHCOCH$_2$CH(NHCbz)CO$_2$CH$_2$Ph, R′ = R″ = Ac (Cbz = CO$_2$CH$_2$Ph)
10 R = NHCOCH$_2$CH(NHCbz)CO$_2$Me, R′ = R″ = H
11 R = NHCOCH$_2$CH(NHCbz)CO$_2$H, R′ = R″ = H
12 R = NHCOCH$_2$CH(NH_2)CO$_2$H, R′ = R″ = H

oligosaccharide–asparagine compounds isolated from ribonuclease B and from ovalbumin was present in the β configuration. The synthesis described here, however, is useful as a model for the synthesis of trisaccharide–asparagine compounds, and, as more than one D-mannopyranosyl residue is linked to the *N*-diacetylchitobiosyl residue, there still exists the possibility that structure **12** is present in natural compounds.

DISCUSSION

The synthesis of *O*-α-D-mannopyranosyl-(1→6)-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→4)-2-acetamido-2-deoxy-D-glucose has been reported[11]. However, the synthesis of the azide derivative (**8**) of this trisaccharide, for subsequent reduction to the amine and coupling with a protected aspartic acid derivative, requires the preparation of an intermediate glycosyl halide, and this might cause extensive splitting of the glycosidic linkages. Consequently, in analogy to the preparation of 2-acetamido-*N*-(L-aspartyl-4-oyl)-2-deoxy-3-*O*-α-D-mannopyranosyl-β-D-glucopyranosylamine[3], the azide derivative (**1**) of di-*N*-acetylchitobiose was prepared first, because this disaccharide is readily available and possesses a rather resistant glycoside linkage[1]. After protecting groups had been introduced, the resulting azide (**5**) was condensed with a protected α-D-mannopyranosyl group, to give the trisaccharide azide **7**.

Previous work[11–13] has shown that the 4-hydroxyl group of a 2-acetamido-2-deoxy-D-glucopyranosyl group is not reactive in the Koenigs–Knorr condensation, and that, when both the 4- and 6-hydroxyl groups are free, this reaction occurs exclusively at O-6. Consequently, the peracetylated azide derivative[1] (**1**) of chitobiose was de-*O*-acetylated, and the resulting glycosyl azide (**2**) was condensed with benzaldehyde to give the benzylidene derivative **3**. Compound **3** was acetylated to give 2-acetamido-4-*O*-(2-acetamido-3-*O*-acetyl-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranosyl)-3,6-di-*O*-acetyl-2-deoxy-β-D-glucopyranosyl azide (**4**). The benzylidene group of **4** was removed by treatment with acetic acid, and the resulting disaccharide **5** was condensed with 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl bromide[14] (**6**), to give the trisaccharide azide **7**, obtained in crystalline form and in good yield (48%), with no indication that a second isomer had been formed. The peracetylated derivative (**8**) of **7** was reduced in the presence of Adams' catalyst, and the resulting amine was directly condensed with 1-benzyl *N*-(benzyloxycarbonyl)-L-aspartate[15] in the presence of *N,N'*-dicyclohexylcarbodiimide, to give the crystalline, fully protected asparagine–trisaccharide derivative **9** in 37% yield. Removal of the protective *O*-acetyl groups of **9** by the action of sodium methoxide gave simultaneously the methyl ester **10** (formed by transesterification of the benzyl ester group). Treatment of **9** with aqueous lithium hydroxide, however, gave the totally saponified compound **11**. Hydrogenolysis of the protective *N*-benzyloxycarbonyl group of this compound afforded *O*-α-D-mannopyranosyl-(1→6)-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→4)-2-acetamido-*N*-(L-aspart-4-oyl)-2-deoxy-β-D-glucopyranosylamine (**12**). All of the intermediate compounds were obtained in crystalline form.

As the condensation of the α-D-mannopyranosyl derivative **6** with the disaccharide azide **5** was performed under conditions similar to those used in the preparation of *O*-α-D-mannopyranosyl-(1 → 6)-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1 → 4)-2-acetamido-2-deoxy-D-glucose[11], the D-mannopyranosyl group was assumed to have the α configuration in compounds **7**–**12**. Similarly, the configuration of the 2-acetamido-2-deoxy-β-D-glucopyranosyl residue linked to asparagine was assumed to be β, on the basis of previously published work[1–4,16].

EXPERIMENTAL

General. — Melting points were determined with a Mettler FP-2 apparatus and correspond to "corrected melting points". Optical rotations were determined, in semimicrotubes, with a Perkin–Elmer Model 141 polarimeter. The chloroform used was analytical-reagent grade, and contained about 0.75% of ethanol. I.r. spectra were recorded, for potassium bromide discs, with a Perkin–Elmer Model 237 spectrophotometer. Column chromatography was performed on Silica Gel Merck (70–325 mesh; E. Merck, Darmstadt, Germany), used without pretreatment. The ratio of the weight of substance to the weight of silica gel was 1:60 to 1:100. The volume of the fractions collected was 3–4 ml per g of the substance to be chromatographed. The ratio of diameter of the column to its length was 1:13. Thin-layer chromatography (t.l.c.) was performed on precoated plates of Silica Gel G (layer thickness 0.25 mm; E. Merck, Darmstadt, Germany). The substances were revealed by spraying with 1:1:18 (v/v) anisaldehyde–concentrated sulfuric acid–ethanol, followed by heating on a hot plate for a few min. Each compound showed only one spot. Evaporations were conducted *in vacuo*, with the bath temperature below 45°. Solutions in volatile solvents of less than 5 ml were evaporated under a stream of nitrogen. Microanalyses were performed by Dr. W. Manser, Zurich, Switzerland.

2-Acetamido-4-O-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-2-deoxy-β-D-glucopyranosyl azide (**2**). — A solution of compound **1** (ref. 1; 1.1 g) in methanol (50 ml) was treated with M sodium methoxide in methanol (10 drops) for 4 h at room temperature. The solution was de-ionized by stirring it with Dowex 50 (H^+) ion-exchange resin, the suspension filtered, and the filtrate evaporated. The residue was crystallized from methanol, to give 730 mg (95%) of needles, m.p. 226–227° (dec.); $[\alpha]_D^{20}$ −31° (*c* 0.64, methanol); i.r. data: ν_{max}^{KBr} 1630 (CONH), 2140 (N_3), and 3400 cm^{-1} (broad, NH and OH); t.l.c. in 3:2 benzene–methanol: R_F 0.20; in 2:3 benzene–methanol: R_F 0.45.

Anal. Calc. for $C_{16}H_{27}N_5O_{10}$: C, 42.76; H, 6.06; N, 15.58; O, 35.60. Found: C, 42.80; H, 6.07; N, 15.66; O, 35.83.

2-Acetamido-4-O-(2-acetamido-3-O-acetyl-4,6-O-benzylidene-2-deoxy-β-D-glucopyranosyl)-3,6-di-O-acetyl-2-deoxy-β-D-glucopyranosyl azide (**4**). — (*a*) *From* **2**. Dry **2** (680 mg) was added to a solution of freshly fused zinc chloride (1 g) in benzaldehyde (25 ml), and the mixture was stirred under nitrogen for 24 h. The mixture was diluted with hexane (200 ml), and the benzaldehyde–hexane mixture was decanted from the

gummy residue. The residue was washed with hexane (3 × 100 ml), and then treated with pyridine (25 ml) and acetic anhydride (30 ml) for 16 h at room temperature. The mixture was diluted with chloroform (450 ml), and successively washed with ice-water, cold saturated sodium hydrogen carbonate solution, and water, evaporated without being dried, and the residue dried by azeotropic distillation with toluene. The residue was washed with hexane to remove the last traces of benzaldehyde, and then crystallized from 1:1 chloroform–methanol, to give 550 mg (54%) of needles, m.p. 222–224° (dec.); $[\alpha]_D^{20}$ −59° (*c* 0.6, pyridine); i.r. data: ν_{max}^{KBr} 1660 (CONH), 1735 (OAc), 2120 (N_3), and 3260 cm^{-1} (NH); t.l.c. in 9:1 chloroform–methanol: R_F 0.41.

Anal. Calc. for $C_{29}H_{37}N_5O_{13}$: C, 52.49; H, 5.62; N, 10.55; O, 31.34. Found: C, 52.48; H, 5.60; N, 10.52; O, 31.34.

(*b*) *From* **3**. A solution of compound **3** (500 mg) in pyridine (20 ml) was treated with acetic anhydride (10 ml) for 16 h at room temperature. The mixture was evaporated, and the residue was dried by repeated addition and distillation of toluene. It was crystallized from 1:1 chloroform–methanol, to give 500 mg (89%) of needles having m.p., and mixed m.p. with the compound just described, 223–224° (dec.).

*2-Acetamido-4-*O*-(2-acetamido-4,6-*O*-benzylidene-2-deoxy-β-*D*-glucopyranosyl)-2-deoxy-β-*D*-glucopyranosyl azide* (**3**). — (*a*) *From* **4**. A solution of **4** (100 mg) in methanol (50 ml) was treated with 0.09M sodium methoxide in methanol for 16 h at 4°. The cold solution was rapidly passed through a bed of Dowex 50 (H^+) cation-exchange resin, and the effluent evaporated. The residue was crystallized from water–methanol, to give 75 mg (92%) of fine needles, m.p. 229–230° (dec.); $[\alpha]_D^{20}$ −74° (*c* 1.04, pyridine); i.r. data: ν_{max}^{KBr} 1650 (CONH), 2140 (N_3), and 3400 cm^{-1} (broad, NH and OH); t.l.c. in 1:1 benzene–methanol: R_F 0.53.

Anal. Calc. for $C_{23}H_{31}N_5O_{10}$: C, 51.39; H, 5.81; N, 13.03; O, 29.76. Found: C, 51.59; H, 5.80; N, 12.96; O, 29.36.

(*b*) *From* **2**. Dry **2** (900 mg) was added to a solution of fused zinc chloride (1.5 g) in benzaldehyde (30 ml). The mixture was stirred for 24 h under an atmosphere of nitrogen, and was then diluted with hexane (300 ml). The benzaldehyde–hexane mixture was decanted, and the gummy residue was washed three additional times with hexane, and then triturated with ice–water. The resulting crystalline substance was rapidly filtered off, washed with a small volume of cold water, and recrystallized from water–methanol to give 655 mg (61%) of fine needles, m.p. 230–231°, that did not depress the melting point of the compound described under (*a*).

*2-Acetamido-4-*O*-(2-acetamido-3-*O*-acetyl-2-deoxy-β-*D*-glucopyranosyl)-3,6-di-O-acetyl-2-deoxy-β-*D*-glucopyranosyl azide* (**5**). — A suspension of **4** (350 mg) in 50% acetic acid (10 ml) was heated for 45 min at 100°. The resulting clear solution was diluted with water (150 ml), and then freeze-dried. [Evaporation at room temperature of the acetic acid solution gave an additional minor product, which moved faster in t.l.c. than **5**, and is the 3,6,3′,6′-tetra-*O*-acetyl derivative (see refs. 17 and 18).] The freeze-dried residue was crystallized from methanol–ether, to give 275 mg (90%) of needles, m.p. 204–205° (dec.); $[\alpha]_D^{20}$ −42° (*c* 1.1, methanol); t.l.c. in 3:2 benzene–

methanol: R_F 0.45; i.r. data: ν_{max}^{KBr} 1640 (CONH), 1720, 1740 (OAc), 2140 (N_3), and 3400 cm^{-1} (broad, NH and OH).

Anal. Calc. for $C_{22}H_{33}N_5O_{13}$: C, 45.92; H, 5.78; N, 12.17; O, 36.14. Found: C, 45.88; H, 5.71; N, 12.08; O, 36.14.

O-(2,3,4,6-Tetra-O-acetyl-α-D-mannopyranosyl)-(1→6)-O-(2-acetamido-3-O-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→4)-2-acetamido-3,6-di-O-acetyl-2-deoxy-β-D-glucopyranosyl azide (**7**). — A solution of **5** (700 mg) and mercuric cyanide (1 g) in 1:1 benzene–nitromethane (200 ml) was concentrated at atmospheric pressure to 150 ml. The mixture was cooled to room temperature, and then treated with a solution of 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl bromide[14] (**6**, 1.5 g) in benzene (7.5 ml), and stirred for 2 days at room temperature. Additional amounts of bromide (0.5 g) and mercuric cyanide (0.5 g) were added, and the mixture was stirred for a further 2 days. The mixture was filtered, and the filtrate was diluted with chloroform (150 ml), successively washed with water, cold saturated potassium hydrogen carbonate solution, and water, dried (sodium sulfate), and evaporated; the residue was chromatographed on a column of silica gel, first with elution with 1:1 ethyl acetate–ether, and then with 9:1 chloroform–ethanol. Fractions obtained with the latter solvent mixture gave, on evaporation, a residue which was rechromatographed with the same solvent mixtures to give pure **7** (t.l.c. in 9:1 chloroform–ethanol: R_F 0.40). Crystallization from chloroform–ether gave 540 mg (48%) of microcrystals, m.p. 133–134°; $[\alpha]_D^{20}$ −12.3° (*c* 0.5, chloroform); i.r. data: ν_{max}^{KBr} 1670 (CONH), 1740 (OAc), 2130 (N_3), and 3350 cm^{-1} (NH and OH).

Anal. Calc. for $C_{36}H_{51}N_5O_{22}$: C, 47.47; H, 5.68; N, 7.73; O, 38.86. Found: C, 47.85; H, 5.71; N, 7.45; O, 38.80.

O-(2,3,4,6-Tetra-O-acetyl-α-D-mannopyranosyl)-(1→6)-O-(2-acetamido-3,4-di-O-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→4)-2-acetamido-3,6-di-O-acetyl-2-deoxy-β-D-glucopyranosyl azide (**8**). — A solution of **7** (400 mg) in pyridine (5 ml) was treated with acetic anhydride (5 ml) for 16 h at room temperature. The solvent was evaporated, and the residue was dried by repeated addition and distillation of toluene. Crystallization of the residue from chloroform–ether gave 380 mg (91%) of microcrystals, m.p. 130–131°; $[\alpha]_D^{20}$ −21° (*c* 0.3, chloroform); i.r. data: ν_{max}^{KBr} 1670 (CONH), 1740 (OAc), 2130 (N_3), and 3350 cm^{-1} (NH); t.l.c. in 9:1 chloroform–ethanol: R_F 0.46.

Anal. Calc. for $C_{38}H_{53}N_5O_{23}$: C, 48.67; H, 5.70; N, 7.47; O, 38.18. Found: C, 48.33; H, 5.69; N, 7.01; O, 38.64.

O-(2,3,4,6-Tetra-O-acetyl-α-D-mannopyranosyl)-(1→6)-O-(2-acetamido-3,4-di-O-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→4)-2-acetamido-3,4-di-O-acetyl-N-[1-benzyl N-(benzyloxycarbonyl)-L-aspart-4-oyl]-2-deoxy-β-D-glucopyranosylamine (**9**). — A solution of **8** (250 mg) in ethanol (30 ml) was hydrogenated for 3 h at room temperature and atmospheric pressure in the presence of Adams' catalyst (60 mg). The catalyst was filtered off, and the filtrate, which gave a positive ninhydrin reaction, was evaporated; the last traces of ethanol were removed by repeated addition and distillation of dichloromethane. The residue was dissolved in dichloromethane (15 ml), and 1-ben-

zyl *N*-(benzyloxycarbonyl)-L-aspartate (112 mg) and *N,N'*-dicyclohexylcarbodiimide (100 mg) were added. The mixture was stirred for 24 h at room temperature. Acetic acid (5 drops) was then added, and the mixture was stirred for 10 min. The *N,N'*-dicyclohexylurea that had crystallized was filtered off, the filtrate was evaporated to dryness, and the residue was chromatographed on a column of silica gel. Elution with chloroform removed the noncarbohydrate compounds. Fractions eluted with 19:1 chloroform–ethanol gave, after evaporation, 125 mg (37%)of pure **9**. It was crystallized from chloroform–ether–pentane, to give microcrystals, m.p. 150–152°; $[\alpha]_D^{20}$ +13.5° (*c* 0.4, chloroform); i.r. data: ν_{max}^{KRr} 1670 (CONH), 1745 (C=O ester), and 3350 cm^{-1} (NH); t.l.c. in 9:1 chloroform–ethanol: R_F 0.59.

Anal. Calc. for $C_{57}H_{72}N_4O_{28}$: C, 54.30; H, 5.75; N, 4.44; O, 35.53. Found: C, 54.04; H, 5.88; N, 4.05; O, 35.59.

O-α-D-*Mannopyranosyl*-(*1→6*)-O-(*2-acetamido-2-deoxy-β*-D-*glucopyranosyl*)-(*1→4*)-*2-acetamido-2-deoxy*-N-[*1-methyl* N-(*benzyloxycarbonyl*)-L-*aspart-4-oyl*]-*β*-D-*glucopyranosylamine* (**10**). — A solution of **9** (450 mg) in dry methanol (20 ml) was treated with 0.1M sodium methoxide in methanol (3 ml) for 16 h at 4°. The solution was de-ionized by stirring with Dowex 50 (H^+) cation-exchange resin, and then evaporated. The residue was crystallized from methanol–ether, to give 265 mg (98%) of microcrystals, m.p. 182–184°; $[\alpha]_D^{20}$ +37.0° (*c* 0.4, methanol); i.r. data: ν_{max}^{KBr} 1650 (CONH); 1725 (COOMe), and 3350 cm^{-1} (broad, NH and OH).

Anal. Calc. for $C_{35}H_{52}N_4O_{20}$: C, 49.52; H, 6.18; N, 6.60; O, 37.70. Found: C, 49.55; H, 6.32; N, 6.32; O, 37.66.

O-α-D-*Mannopyranosyl*-(*1→6*)-O-(*2-acetamido-2-deoxy-β*-D-*glucopyranosyl*)-(*1→4*)-*2-acetamido*-N-[N-(*benzyloxycarbonyl*)-L-*aspart-4-oyl*]-*2-deoxy-β*-D-*glucopyranosylamine* (**11**). — Compound *9* (43 mg) was stirred with a solution of lithium hydroxide (30 mg) in water (10 ml) for 2 h at room temperature. The solution was de-ionized by passage through a bed of Dowex 50 (H^+) cation-exchange resin, and then evaporated. Crystallization of the residue from methanol–ether gave 24 mg (86%) of **11**, decomposing at 212° (effervescence); $[\alpha]_D^{20}$ +30° (*c* 0.4, methanol); i.r. data: ν_{max}^{KBr} 1650 (CONH), 1700 (COOH), and 3375 cm^{-1} (broad, NH and OH).

Anal. Calc. for $C_{34}H_{50}N_4O_{20}$: C, 48.93; H, 6.04; N, 6.71. Found: C, 48.73; H, 6.12; N, 6.50.

O-α-D-*Mannopyranosyl*-(*1→6*)-O-(*2-acetamido-2-deoxy-β*-D-*glucopyranosyl*)-(*1→4*)-*2-acetamido*-N-(L-*aspart-4-oyl*)-*2-deoxy-β*-D-*glucopyranosylamine* (**12**). — A solution of **11** (100 mg) in methanol (30 ml) was hydrogenolyzed for 5 h at atmospheric pressure and room temperature in the presence of palladium-on-charcoal (50 mg). The catalyst was filtered off, and the filtrate was evaporated. The residue, which gave a positive ninhydrin reaction, was crystallized from methanol–ether to give 64 mg (73%) of microcrystals that started to decompose, without melting, at 160°; $[\alpha]_D^{20}$ +39° (*c* 0.4, methanol); i.r. data: ν_{max}^{KBr} 1650 (CONH), 1725 (COOH), and 3400 cm^{-1} (broad, NH and OH). The compound contained solvent of crystallization, as shown by loss of weight on being dried. Extensive drying caused decomposition; consequently, the analytical sample was dried at room temperature *in vacuo* in the

presence of phosphorus pentaoxide. It contained 2 molecules of water of crystallization; correct results could not be obtained for nitrogen.

Anal. Calc. for $C_{26}H_{44}N_4O_{18}\cdot 2\,H_2O$: C, 42.39; H, 6.57; N, 7.61 Found: C, 42.39; H, 6.39; N, 6.92.

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