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THE SYNTHESIS OF 5-AMINO-2,6-ANHYDRO-5-DEOXY-D-*glycero*-D-*gulo*-HEPTONIC ACID AND ITS POLYCONDENSATION TO OLIGOMERS*†

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ABSTRACT

5-Amino-2,6-anhydro-5-deoxy-D-*glycero*-D-*gulo*-heptonic acid has been synthesized by conventional introduction of an amino function *via* azide displacement, starting with a suitable derivative of 2,6-anhydro-D-*glycero*-L-*manno*-heptonic acid. The amino acid was converted into the methyl ester hydrochloride which, in methanolic sodium methoxide, gave oligomeric and polymeric amides, depending on the conditions applied. Four oligomeric esters, as well as the corresponding *N*-(2,4-dinitrophenyl) derivatives of the amino acids, could be separated by paper chromatography. The oligomers could be saponified under mild, basic conditions.

INTRODUCTION

Several amino acid derivatives having a pyranosyl ring as the typical structural feature of a hexose moiety were synthesized as potential monomers for the preparation of polysaccharide analogues[1,2]. Cellulose served as a model when 5-amino-2,6-anhydro-5-deoxy-D-*glycero*-D-*gulo*-heptonic acid (**12**) was conceived for the preparation of a polyamide having a carbohydrate appearance.

RESULTS AND DISCUSSION

2,6-Anhydro-5,7-*O*-benzylidene-D-*glycero*-L-*manno*-heptonic acid methyl ester[3] (**1**), prepared *via* 3,4,5,7-tetra-*O*-acetyl-2,6-anhydro-D-*glycero*-L-*manno*-heptononitrile[4], was the starting material in a multistep synthesis of 5-amino-2,6-anhydro-5-deoxy-D-*glycero*-D-*gulo* heptonic acid (**12**). On treatment with methanolic ammonia, **1** was converted into 2,6-anhydro-5,7-*O*-benzylidene-D-*glycero*-L-*manno*-heptonamide (**2**), acetylation of which yielded 3,4-di-*O*-acetyl-2,6-anhydro-5,7-*O*-benzylidene-D-*glycero*-L-*manno*-heptonamide (**3**). Removal of the benzylidene group from **3** by mild, acid treatment gave 3,4-di-*O*-acetyl-2,6-anhydro-D-*glycero*-L-*manno*-heptonamide (**4**). Trimethylsilylation of **4** gave **5**, which was treated with acetic acid and acetic anhydride

*Dedicated to the memory of Professor Edward J. Bourne.
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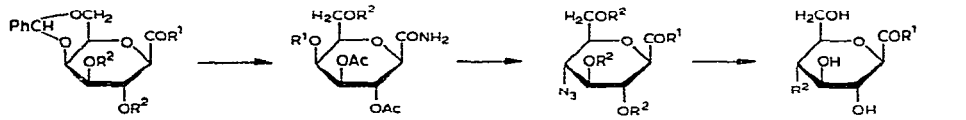
in pyridine[5]. This specific replacement reaction gave 3,4,7-tri-*O*-acetyl-2,6-anhydro-5-*O*-(trimethylsilyl)-D-*glycero*-L-*manno*-heptonamide (**6**). After removal of the trimethylsilyl group, 3,4,7-tri-*O*-acetyl-2,6-anhydro-D-*glycero*-L-*manno*-heptonamide (**7**) could be mesylated to give 3,4,7-tri-*O*-acetyl-5-*O*-mesyl-D-*glycero*-L-*manno*-heptonamide (**8**). Treatment of **8** with sodium azide[6] yielded 3,4,7-tri-*O*-acetyl-2,6-anhydro-5-azido-5-deoxy-D-*glycero*-D-*gulo*-heptonamide (**9**), deacetylation of which with methanolic ammonia afforded 2,6-anhydro-5-azido-5-deoxy-D-*glycero*-D-*gulo*-heptonamide (**10**). Saponification of **10** gave **11**, which was not isolated but hydrogenated to give the amino acid **12**, which was obtained in an over-all yield of 17% and could be converted into the methyl ester hydrochloride[7] **13**.



1 R^1 = OMe; R^2 = H
2 R^1 = NH_2; R^2 = H
3 R^1 = NH_2; R^2 = Ac

4 R^1 = R^2 = H
5 R^1 = R^2 = $SiMe_3$
6 R^1 = $SiMe_3$; R^2 = Ac
7 R^1 = H; R^2 = Ac
8 R^1 = Ms; R^2 = Ac

9 R^1 = NH_2, R^2 = Ac
10 R^1 = NH_2; R^2 = H
11 R^1 = OH; R^2 = H

12 R^1 = OH, R^2 = NH_2
13 R^1 = OMe; R^2 = $NH_3^+Cl^-$
14 R^1 = ONa; R^2 = NH_2

When **13** was added to methanolic sodium methoxide at room temperature, condensation products could be detected (p.c.) almost immediately (Table I). After 5 min, the initially clear solution became turbid and a precipitate started to separate. The precipitate was water-soluble. Prolonged treatment (4 h) of the reaction mixture at 100° (autoclave) yielded a precipitate that was mainly water-insoluble. Starting material and oligomers had almost disappeared and, although the precipitate was not

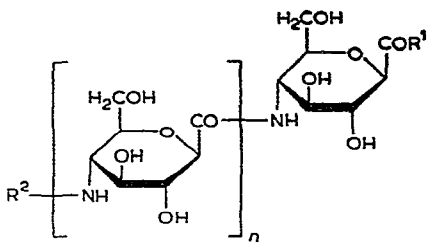
TABLE I

PRODUCTS OF POLYMERIZATION OF **13** AND THEIR DNP-DERIVATIVES

Compound	n	R_F[a]	R_F *of the N-DNP-derivatives*[b]	*Relative yields of condensation products* (%)[c]
2,4-Di-nitrophenol	—	—	0.75	—
13	0	0.72	0.47	45
Ia	1	0.67	0.27	23
IIa	2	0.59	0.13	19
IIIa	3	0.47	0.04	13
IVa	4	0.35	0.00	—

[a]Ethanol-M ammonium acetate (7:3), detection with periodate–Schiff's reagent. [b]*N*-DNP-derivatives in 1-Butanol–pyridine–water (6:4:3); detection by yellow colour of DNP derivatives and radiochromatogram-scanning of ^{14}C-*N*-DNP derivatives. [c]The figures represent the relative amounts of products in the reaction mixture after incubation of **13** under standard conditions, as calculated from the radioactivity of the ^{14}C-*N*-DNP derivatives. A small amount of ^{14}C-*N*-DNP-labelled material remaining at the origin was not considered.

further examined, it is reasonable to assume that polymeric products had been formed. Under standard conditions (1 h, room temperature), 4 oligomers and unchanged starting-material could be detected (p.c.), as could their *N*-(2,4-dinitrophenyl) (DNP) derivatives[8]. During the preparation of the DNP-derivatives, the *C*-terminal ester grouping was saponified. Although determination of the molecular weight of the oligomers was not carried out, it is reasonable to assume that compounds Ia–IVa as well as their *N*-DNP-derivatives are di-, tri-, tetra-, and penta-mers, respectively. Each of the oligomers was readily saponified in M sodium hydroxide. The ease of saponification is probably due to participation of HO-7. The oligomeric amino esters Ia–IVa gave only sodium 5-amino-2,6-anhydro-5-deoxy-D-*glycero*-D-*gulo*-heptonate (**14**). The *N*-DNP derivatives Ib–IVb gave **15** as well as sodium 2,6-anhydro-5-deoxy-5-(2,4-dinitrophenyl-amino)-D-*glycero*-D-*gulo*-heptonate (**14**). Compound **14** was identified by p.c.



Ia-IVa R^1 = OMe; R^2 = H

EXPERIMENTAL

General. — T.l.c. was performed on silica gel F_{254} (Merck) with 4:1 benzene–methanol for compounds having free hydroxyl groups, and 4:1 ether–light petroleum (b.p. 60–70°) for fully protected compounds. Detection was effected by charring with conc. sulphuric acid at 150°. Paper chromatography (p.c.) was performed on Whatman No. 1 paper with either 6:4:3 1-butanol–pyridine–water or 7:3 ethanol–M ammonium acetate. Compounds containing vicinal hydroxyl groups were detected with periodate–Schiff's reagent[9]. ^{14}C-Labelled compounds were detected by using a radiochromatogram scanner (Packard, Model 7200). G.l.c. was carried out using glass columns containing Chromosorb G coated with 3% of SE-52, with nitrogen as carrier gas and flame-ionization detection. For pertrimethylsilylation, samples (10 mg) were treated with a 2:1:10 mixture (1.3 ml) of hexamethyldisilazane, chlorotrimethylsilane, and pyridine[10]. I.r. and n.m.r. data (methyl sulphoxide-d_6, internal Me_4Si) were obtained with Perkin–Elmer Infracord Model 137 and Varian A-60 spectrometers, respectively.

2,6-Anhydro-5,7-O-benzylidene-D-glycero-L-manno-*heptonamide* (**2**). — A solution of 2,6-anhydro-5,7-*O*-benzylidene-D-*glycero*-L-*manno*-heptonic acid methyl

ester[3] (**1**; 9.3 g, 30 mmol) in methanolic ammonia (200 ml, saturated at 0°) was kept at room temperature for 3 h, and then concentrated under reduced pressure. The syrupy residue crystallized on addition of benzene, and recrystallization from benzene–methanol yielded **2** (8.5 g, 96%), m.p. 138° (with preliminary sintering), $[\alpha]_{578}^{22}$ +58° (*c* 1, methanol); ν_{max}^{KBr} 3500–3300 (OH and NH) and 1670 cm^{-1} ($CONH_2$).

Anal. Calc. for $C_{14}H_{17}NO_6$: C, 56.94; H, 5.80; N, 4.74. Found: C, 57.04; H, 5.91; N, 4.97.

*3,4-Di-*O*-acetyl-2,6-anhydro-5,7-*O*-benzylidene-*D-glycero-L-manno-*heptonamide* (**3**). — Conventional treatment of **2** (29.5 g, 0.1 mol) with pyridine (170 ml) and acetic anhydride (150 ml), with recrystallization of the product by adding 1:1 ether–light petroleum (b.p. 60–70°) to a hot, methanolic solution, gave **3** (32.3 g, 88%), m.p. 192°, $[\alpha]_{578}^{22}$ +119° (*c* 1, chloroform); ν_{max}^{KBr} 1730 (OAc) and 1680 cm^{-1} ($CONH_2$). N.m.r. data ($CDCl_3$): δ 2.07 (s, 6H, 2Ac), 5.50 (s, 1H, benzyl), 7.2–7.5 (m, 5H, phenyl).

Anal. Calc. for $C_{18}H_{21}NO_8$: C, 56.99; H, 5.58; N, 3.69. Found: C, 56.78; H, 5.69; N, 3.73.

*3,4-Di-*O*-acetyl-2,6-anhydro-*D-glycero-L-manno-*heptonamide* (**4**). — Treatment of **3** (37.9 g, 0.1 mol) with boiling, 50% acetic acid (500 ml) for 10–15 min was followed by concentration of the mixture under reduced pressure with addition of water (3 × 300 ml). Recrystallization of the residue from methanol (300 ml) and ether (500 ml) gave **4** (19.2 g, 66%), m.p. 186–190°, $[\alpha]_{578}^{22}$ +48° (*c* 1, methanol); ν_{max}^{KBr} 3600–3250 (OH and NH), 1755 (OAc), and 1680 cm^{-1} ($CONH_2$). N.m.r. data: δ 1.96 and 2.02 (2s, 6H, 2Ac).

Anal. Calc. for $C_{11}H_{17}NO_8$: C, 45.36; H, 5.88; N, 4.81. Found: C, 45.04; H, 5.82; N, 4.76.

*3,4,7-Tri-*O*-acetyl-2,6-anhydro-5-*O*-(trimethylsilyl)-*D-glycero-L-manno-*hepton-amide* (**6**). — Compound **4** (29.1 g, 0.1 mol) was treated[10] with hexamethyldisilazane (40 g) and chlorotrimethylsilane (25 g) in pyridine (450 ml) to yield crude 3,4-di-*O*-acetyl-2,6-anhydro-5,7-di-*O*-(trimethylsilyl)-D-*glycero*-L-*manno*-heptonamide (**5**, 43.5 g). Compound **5** was dissolved in a mixture of pyridine (200 ml) and acetic anhydride (150 ml), and the selective replacement of the 7-TMS group by an *O*-acetyl group was started by adding glacial acetic acid (12 g). After 70 h at room temperature, the reaction mixture was concentrated *in vacuo* and a solution of the syrupy residue in chloroform (400 ml) was washed with water (2 × 200 ml), treated with activated charcoal, dried ($CaSO_4$), and concentrated *in vacuo* to give crystalline **6** (38.5 g, 95%). A pure sample, obtained by recrystallization from ethanol, had m.p. 102°, $[\alpha]_{578}^{22}$ +34° (*c* 1, chloroform); ν_{max}^{KBr} 1750 (OAc) and 1690 cm^{-1} ($CONH_2$). N.m.r. data: δ 0.10 (s, 9H, Me_3Si), 1.95 and 2.06 (2s, 9H, 3Ac).

Anal. Calc. for $C_{16}H_{27}NO_9Si$: C, 47.39; H, 6.71. Found: C, 47.33; H, 6.83.

*3,4,7-Tri-*O*-acetyl-2,6-anhydro-*D-glycero-L-manno-*heptonamide* (**7**). — Crude **6** (20.3 g, 0.05 mol) was desilylated with methanol (130 ml) and 30% aqueous acetic acid (150 ml) for 4 h at room temperature. The organic layer was discarded and the aqueous solution was concentrated *in vacuo*, with the addition of water (2 × 200 ml),

to give crystalline **7** (16.5 g). A pure sample, obtained by recrystallization from ethanol, had m.p. 184–189°, $[\alpha]_{578}^{22}$ +42° (*c* 1, methanol); ν_{max}^{KBr} 3400 (OH), 1740 (OAc), and 1680 cm^{-1} ($CONH_2$). N.m.r. data: δ 1.97 and 2.04 (2s, 9H, 3Ac).

Anal. Calc. for $C_{13}H_{19}NO_9$: C, 46.85; H, 5.76; N, 4.20. Found: C, 46.95; H, 5.81; N, 4.19.

*3,4,7-Tri-*O*-acetyl-2,6-anhydro-5-*O*-methanesulphonyl-*D*-glycero-*L*-manno-heptonamide* (**8**). — A solution of crude **7** (16.6 g, 0.05 mol) in pyridine (250 ml) was treated with methanesulphonyl chloride (19 g) in the cold during 2 h. After a further 3 h, the mixture was stirred into ice–water (3 l). The precipitate was collected, and recrystallized from methanol to give **8** (13.0 g, 63%), m.p. 234°, $[\alpha]_{578}^{22}$ +9° (*c* 1 methyl sulphoxide); ν_{max}^{KBr} 1740 (OAc) and 1680 cm^{-1} ($CONH_2$). N.m.r. data: δ 1.98 and 2.06 (2s, 9H, 3Ac), 3.32 (s, 3H, Ms).

Anal. Calc. for $C_{14}H_{21}NO_{11}S$: C, 40.88; H, 5.15; N, 3.41; S, 7.79. Found: C, 40.61; H, 4.94; N, 3.46; S, 7.70.

*3,4,7-Tri-*O*-acetyl-2,6-anhydro-5-azido-5-deoxy-*D*-glycero-*D*-gulo-heptonamide* (**9**). — Compound **8** (20.5 g, 0.05 mol) was stirred with sodium azide (23.5 g) in methyl sulphoxide (420 ml) at 80° for 100 h. Acetone (4 l) was added to the cooled mixture which was then filtered, and concentrated under reduced pressure to remove the acetone. The residual solution was mixed with water (500 ml) and continuously extracted with ether. The extract was dried ($CaSO_4$) and concentrated, and the residue was recrystallized from ethanol–light petroleum (b.p. 60–70°) to give **9** (11.5 g, 64%), m.p. 145°, $[\alpha]_{578}^{22}$ +56° (*c* 1, chloroform); ν_{max}^{KBr} 2120 (N_3), 1750 (OAc), and 1680 cm^{-1} ($CONH_2$). N.m.r. data ($CDCl_3$): δ 2.08, 2.12, and 2.16 (3s, 9H, 3Ac).

Anal. Calc. for $C_{13}H_{18}N_4O_8$: C, 43.58; H, 5.06; N, 15.64. Found: C, 43.50; H, 5.12; N, 15.51.

*2,6-Anhydro-5-azido-5-deoxy-*D*-glycero-*D*-gulo-heptonamide* (**10**). — Treatment of **9** (7.2 g, 0.02 mol) with methanolic ammonia (150 ml, saturated at 0°) for 15 h was followed by concentration and removal of acetamide in high vacuum by sublimation at 60° (bath), recrystallization of the residue from methanol–ether gave **10** (4.3 g, 93%), m.p. 198–202° (dec.), $[\alpha]_{578}^{22}$ +100° (*c* 1, water); ν_{max}^{KBr} 2120 (N_3) and 1680 cm^{-1} ($CONH_2$).

Anal. Calc. for $C_7H_{12}N_4O_5$: C, 36.21; H, 5.21; N, 24.13. Found: C, 36.34; H, 5.31; N, 24.43.

*5-Amino-2,6-anhydro-5-deoxy-*D*-glycero-*D*-gulo-heptonic acid* (**12**). — Compound **10** (2.2 g, 10 mmol) was saponified at room temperature with 10% aqueous sodium hydroxide (8 ml). After 16 h, water (15 ml) was added and the solution was passed through a column of Amberlite IR-120 (H^+) resin (50 ml). The eluate was concentrated to 20 ml. The 5-azido-2,6-anhydro-5-deoxy-D-*glycero*-D-*gulo*-heptonic acid (**11**) was not isolated, but hydrogenated (Adams' catalyst) overnight at room temperature and atmospheric pressure. The mixture was then filtered, and concentrated *in vacuo*. Compound **12** crystallized on adding ethanol, and recrystallization from water–ethanol gave the hydrate (1.74 g, 84%), m.p. 205–290° (sintering with

dec.), $[\alpha]_{578}^{22}$ −3° (*c* 1, water), R_F 0.47 (Whatman No. 1 paper; ethanol–M ammonium acetate, 7:3; detection with periodate–Schiff's reagent).

Anal. Calc. for $C_7H_{13}NO_6 \cdot H_2O$: C, 37.33; H, 6.71; N, 6.22. Found: C, 37.70; H, 6.50; N, 5.95.

Dehydration of the foregoing product was effected by dissolution in water, freeze-drying, and drying overnight *in vacuo* at 100°.

Anal. Calc. for $C_7H_{13}NO_6$: C, 40.58; H, 6.32; N, 6.76. Found: C, 40.46; H, 6.09; N, 6.72.

5-Amino-2,6-anhydro-5-deoxy-D-glycero-D-gulo-*heptonic acid methyl ester hydrochloride* (**13**). — A solution of **12** (2.25 g, 10 mmol) in water (20 ml) was freeze-dried. The resulting, amorphous powder was suspended in dry methanol (30 ml), and dry hydrogen chloride was bubbled through until a clear solution was obtained. Saturation was completed at 0°. The reaction mixture was concentrated with repeated addition of dry methanol. The amorphous, hygroscopic residue was dried *in vacuo* at 60° to give **13** (2.55 g), $[\alpha]_{578}^{22}$ −23° (*c* 1, methanol); ν_{max}^{KBr} 1730 cm^{-1} (COOMe).

Anal. Calc. for $C_8H_{16}ClNO_6$: C, 37.29; H, 6.26; N, 5.44. Found: C, 36.39; H, 6.63; N, 5.43.

Polycondensation of **13**. — To a solution of **13** (5.15 mg, 0.02 mmol) in methanol (20 µl), M methanolic sodium methoxide (20 µl) was added. After 5 min at 24°, precipitation began. The mixture was left at room temperature for 1 h (standard conditions). Addition of water (40 µl) then gave again a clear solution, which was chromatographed on Whatman No. 1 paper using ethanol–M ammonium acetate (7:3) and detection with the periodate-Schiff's reagent. The R_F values of the mobile products are listed in Table I. If, instead of water, M sodium hydroxide (40 µl) was added, then, after 10 h at room temperature, the sole, detectable product (p.c.) was **14**, as shown by comparison with an authentic sample obtained by saponification of **13**.

Formation of N-(*2,4-dinitrophenyl*) *derivatives*. — The foregoing, standard, polycondensation mixture was shaken vigorously for 16 h at room temperature with 5% aqueous sodium hydrogen carbonate (200 µl) and 1-fluoro-2,4-dinitrobenzene (FDNB, 10 mg) in ethanol (400 µl); the ester groups are labile under these conditions. The mixture was concentrated to dryness, and the residue was dissolved in water (500 µl) and extracted with ether (4 × 100 µl). P.c. of the aqueous solution using 1-butanol–pyridine–water showed, in addition to a little immobile material, five yellow spots, the R_F values of which are given in Table I. The spot of highest R_F value was 2,4-dinitrophenol as could be shown by comparison with an authentic sample.

In order to obtain the relative yield of each polycondensation product formed under standard conditions, the ^{14}C-*N*-DNP derivatives were prepared. The standard polycondensation mixture (10 µl) was treated with aqueous sodium hydrogen carbonate (60 µl) and ^{14}C-FDNB (Amersham Buchler, 50 µCi, 17 mCi/mmol) in ethanol (150 µl) as described above. Radiochromatogram-scanning of the labelled products gave the relative amounts of condensation products, as listed in Table I.

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REFERENCES

1 E.-F. FUCHS AND J. LEHMANN, *Carbohyd. Res.*, 45 (1975) 135–141.
2 E.-F. FUCHS AND J. LEHMANN, *Chem. Ber.*, 108 (1975) 2254–2260.
3 M. BROCKHAUS AND J. LEHMANN, unpublished results.
4 B. HELFERICH AND K. L. BETTIN, *Chem. Ber.*, 94 (1961) 1159–1160.
5 E.-F. FUCHS AND J. LEHMANN, *Chem. Ber.*, 107 (1974) 721–724.
6 S. HANESSIAN AND T. H. HASKELL, *J. Org. Chem.*, 28 (1963) 2604–2610.
7 J. P. GREENSTEIN AND M. WINITZ, *Chemistry of the Amino Acids*, Vol. 2, Wiley, 1961, pp. 925–928.
8 F. SANGER, *Biochem. J.*, 39 (1945) 507–515.
9 J. G. BUCHANAN, C. A. DEKKER, AND A. G. LONG, *J. Chem. Soc.*, (1950) 3162–3167.
10 C. C. SWEELEY, R. BENTLEY, M. MAKITA, AND W. W. WELLS, *J. Amer. Chem. Soc.*, 85 (1963) 2497–2507.