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# SELECTIVE RING-OPENING OF 3,4,5-TRI-*O*-ACETYL-2,6-ANHYDRO-D-*glycero*-L-*manno*-HEPTARIC ANHYDRIDE WITH AMMONIA: THE SYNTHESIS OF 7-AMINO-2,6-ANHYDRO-7-DEOXY-L-*glycero*-L-*galacto*-HEPTONIC ACID*†

ERNST-F. FUCHS AND JOCHEN LEHMANN
*Chemisches Laboratorium der Universität, D-78 Freiburg i.Br. (Deutschland)*
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## ABSTRACT

3,4,5-Tri-*O*-acetyl-2,6-anhydro-D-*glycero*-L-*manno*-heptaric anhydride (**3**) was synthesized from 2,6-anhydro-D-*glycero*-L-*manno*-heptaric acid (**1**) by treatment of the disodium salt with phosphoryl chloride. Due to steric hindrance, **3** reacts with ammonia selectively at position 1 to yield 2,6-anhydro-D-*glycero*-L-*manno*-heptaric acid 1-amide (**4**). 2,6-Anhydro-D-*glycero*-L-*manno*-heptaric acid 1-nitrile 7-methyl ester (**9**) was obtained by esterification, acetylation, and dehydration of **4**. Catalytic reduction and subsequent hydrolysis of **9** yielded 7-amino-2,6-anhydro-7-deoxy-L-*glycero*-L-*galacto*-heptonic acid (**13**).

## INTRODUCTION

The amino acids 7-amino-2,6-anhydro-7-deoxy-D-*glycero*-L-*manno*-heptonic acid and 7-amino-2,6-anhydro-7-deoxy-D-*glycero*-D-*gulo*-heptonic acid have been synthesized[1] as potential monomers for the preparation of oligo- and poly-saccharide analogues having amide links. Syntheses were carried out by conventional introduction of amino functions into the corresponding heptonic acids. We now report the synthesis of 3,4,5-tri-*O*-acetyl-2,6-anhydro-D-*glycero*-L-*manno*-heptaric anhydride (**3**), which reacts specifically with ammonia to form the 1-monoamide (**4**) of 2,6-anhydro-D-*glycero*-L-*manno*-heptaric acid (**1**). Compound **4** can be used for the preparation of 7-amino-7-deoxyheptonic acid derivatives.

## RESULTS AND DISCUSSION

The dicarboxylic acid **1** is obtained in reasonable yield from 2,6-anhydro-D-*glycero*-L-*manno*-heptonic acid[1] by oxidation with nitric acid[2]. Treatment of the disodium salt of 3,4,5-tri-*O*-acetyl-D-*glycero*-L-*manno*-heptaric acid (**2**) with phosphoryl chloride gave a compound, in good yield, which was not the expected acid chloride but the anhydride **3**. Compound **3** reacted smoothly with ammonia in

*Dedicated to the memory of Dr. Hewitt G. Fletcher, Jr.
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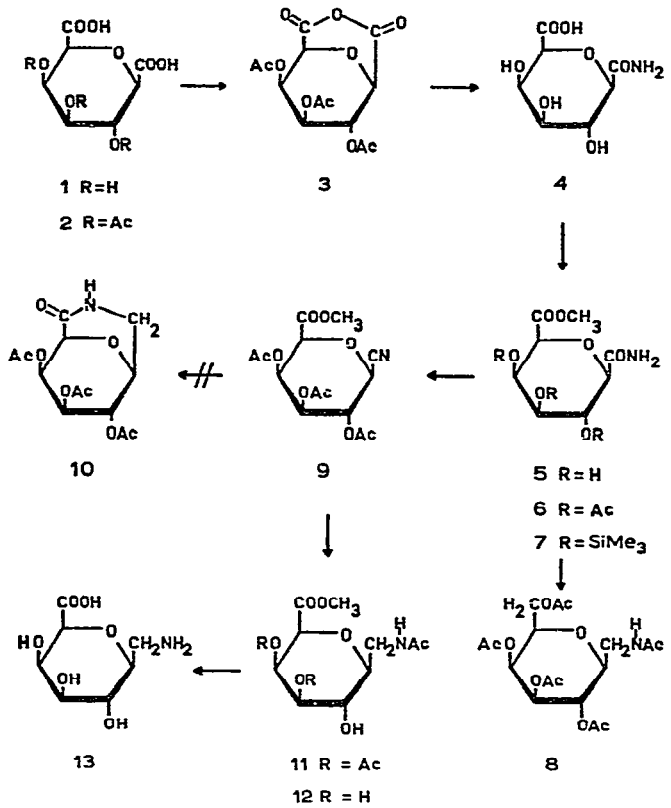


methanol to give 2,6-anhydro-D-*glycero*-L-*manno*-heptaric acid 1-amide (**4**) exclusively. This remarkable selectivity is probably due to steric hindrance of C-7 by the equatorial group at position 5. Such steric hindrance is operative only if attack of the nucleophile comes from an *exo* direction (Fig. 1); *endo* attack will be hindered by the pyranosyl ring oxygen.

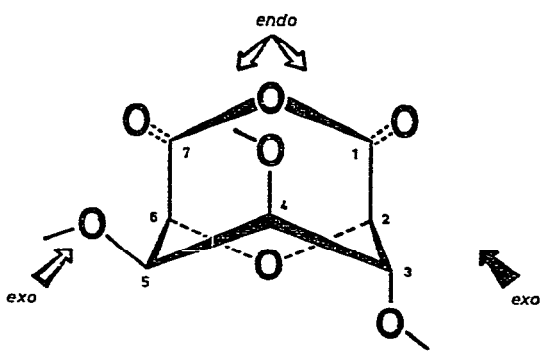


Fig. 1. Directions for nucleophilic attack of anhydride **3**.

The structure of **4** was proved by converting the 7-ester-1-amide **5** *via* its tris-(trimethylsilyl) ether (**7**) into 1-acetamido-3,4,5,7-tetra-*O*-acetyl-2,6-anhydro-1-deoxy-D-*glycero*-L-*manno*-heptitol (**8**), which was identical with an authentic sample[3] obtained by reduction and acetylation of 3,4,5,7-tetra-*O*-acetyl-2,6-anhydro-D-*glycero*-L-*manno*-heptononitrile[4]. Compound **5**, obtained by treatment of **4** with diazomethane, was acetylated and converted into the nitrile **9** by heating[5] with tosyl chloride in pyridine. Catalytic reduction of **9** in acetic acid gave a syrupy acetate which was not isolated but heated in benzene to give 3,4,5-tri-*O*-acetyl-7-amino-2,6-anhydro-7-deoxy-L-*glycero*-L-*galacto*-heptonic acid lactam (**10**), as described[6] for the preparation of ethyl 5-phenyl-6-ketonipecotate. However, the main product, obtained in crystalline form, was shown by n.m.r. spectroscopy to be 7-acetamido-3,4-di-*O*-acetyl-2,6-anhydro-7-deoxy-L-*glycero*-L-*galacto*-heptonic acid methyl ester (**11**). Catalytic *O*-deacetylation of **11** with sodium methoxide in methanol gave 7-acetamido-2,6-anhydro-7-deoxy-L-*glycero*-L-*galacto*-heptonic acid methyl ester (**12**), hydrolysis of which with sodium hydroxide yielded crystalline 7-amino-2,6-anhydro-7-deoxy-L-*glycero*-L-*galacto*-heptonic acid (**13**). Compound **13** shows the same chromatographic properties as the recently described amino acids[1], and also reacts with ninhydrin.

## EXPERIMENTAL

*General methods.* — 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°. G.l.c. was carried out in glass columns containing Chromosorb G coated with silicon rubber (SE 52/3%), using 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[7]. I.r. and n.m.r. data (internal $Me_4Si$) were obtained with Perkin–Elmer Infracord Model 137 and Varian A-60 spectrometers, respectively.

*2,6-Anhydro*-D-glycero-L-manno-*heptaric acid* (**1**). — A solution of sodium nitrite (20 mg) in 1:1 conc. nitric acid–fuming nitric acid (26 ml) was heated to 55–60°. During 30 min, 2,6-anhydro-D-*glycero*-L-*manno*-heptonic acid[1] (20.8 g, 0.1 mol) was added to the stirred solution, which was then kept at 55–60° for 1 h. The reaction mixture was diluted with water (200 ml), neutralized to pH 7–8 with 2M sodium hydroxide, and concentrated under reduced pressure. A solution of the residue in water (50 ml) was stirred into ice-cold methanol (500 ml). The amorphous precipitate was collected, and dissolved in water (200 ml), and the solution was passed through Amberlite IR-120($H^+$) resin (300 ml). The acidic eluate was treated with charcoal and concentrated, and the crystalline residue was washed with cold acetone to give **1** (13.7 g, 62%), m.p. 205–208° (dec.), $[\alpha]_{578}^{25}$ +33° (*c* 1, water); $\nu_{max}^{KBr}$ 3600–2300 (OH) and 1740 $cm^{-1}$ (COOH). A trimethylsilylated sample of **1** was homogeneous in g.l.c., and 66.5 mg of **1** (0.3 mmol) consumed 5.94 ml of 0.1M NaOH.

*Anal.* Calc. for $C_7H_{10}O_8$: C, 37.85; H, 4.54. Found: C, 37.54; H, 4.58.

*3,4,5-Tri-*O*-acetyl-2,6-anhydro-*D-glycero-L-manno-*heptaric acid* (**2**). — A mixture of **1** (8.9 g, 40 mmol) and acetic anhydride (50 ml) containing conc. sulphuric acid (0.2 ml) was heated to 110°. After 30 min, when dissolution was complete, the mixture was cooled and stirred into ice–water (500 ml), and after storage at room temperature for 1 h the solution was concentrated *in vacuo* to ~50 ml and cooled to give **2**. Recrystallisation from water gave the dihydrate of **2** (12.3 g, 80%), m.p. 72–74°, $[\alpha]_{578}^{25}$ +26° (*c* 1, acetone); $\nu_{max}^{KBr}$ 1740 $cm^{-1}$ (COOH and OAc). N.m.r. data (acetone-$d_6$): $\delta$ 1.9–2.3 (3 s, 9 H, 3 AcO).

*Anal.* Calc. for $C_{13}H_{16}O_{11}\cdot 2H_2O$: C, 40.63; H, 5.26. Found: C, 40.39; H, 5.54.

*3,4,5-Tri-*O*-acetyl-2,6-anhydro-*D-glycero-L-manno-*heptaric anhydride* (**3**). — A solution of **2** (11.5 g, 30 mmol) in water (200 ml) was neutralized with M sodium hydroxide and freeze-dried. The powdered, amorphous disodium salt was added portionwise with stirring to phosphoryl chloride (30 ml) at 50°. There was an immediate, vigorous reaction. The mixture was then boiled under reflux for 15 min to complete the reaction. Dry benzene (80 ml) was added, and the hot mixture was filtered through Celite. Addition of light petroleum (b.p. 60–70°) to the cooled filtrate gave **3**. After recrystallisation from benzene–light petroleum (b.p. 60–70°), the product (7.8 g, 79%) had m.p. 127°, $[\alpha]_{578}^{25}$ +67° (*c* 1, chloroform); $\nu_{max}^{KBr}$ 1830 and 1770 $cm^{-1}$ (–CO–O–CO–). N.m.r. data ($CDCl_3$): $\delta$ 1.9–2.2 (3 s, 9 H, 3 AcO).

*Anal.* Calc. for $C_{13}H_{14}O_{10}$: C, 47.28; H, 4.27. Found: C, 47.52; H, 4.49.

*2,6-Anhydro-*D-glycero-L-manno-*heptaric acid 1-amide* (**4**). — A solution of **3** (9.9 g, 30 mmol) in methanolic ammonia (150 ml, saturated at 0°) began to deposit the ammonium salt of **4** after ~1 h. The mixture was stirred for an additional 5 h and then cooled to 0°, the amorphous precipitate was collected, and a solution in water (50 ml) was passed down a column of Amberlite IR-120($H^+$) resin (50 ml). The acidic eluate was concentrated *in vacuo* to give **4** which was recrystallised from 9:1 ethanol–water by the addition of ether. The product (5.1 g, 77%) had m.p. 224° (dec.), $[\alpha]_{578}^{25}$ +51° (*c* 1, water); $\nu_{max}^{KBr}$ 3400–2300 (OH and NH), 1730 (COOH), and 1670 $cm^{-1}$ ($CONH_2$). The trimethylsilylated product of **4** was homogeneous in g.l.c.

*Anal.* Calc. for $C_7H_{11}NO_7$: C, 38.01; H, 5.01; N, 6.33. Found: C, 38.28; H, 5.04; N, 6.21.

*1-Amido-2,6-anhydro-*D-glycero-L-manno-*heptaric acid 7-methyl ester* (**5**). — To a solution of **4** (3.3 g, 15 mmol) in water (15 ml) and methanol (150 ml), excess 1–2% ethereal diazomethane was added until the yellow colour persisted. The product crystallised from the reaction mixture, and precipitation was completed by the addition of ether and cooling to 0°. Recrystallisation from 9:1 methanol–water by the addition of ether gave **5** (2.9 g, 82%), m.p. 200–230° (dec.), $[\alpha]_{578}^{25}$ +40° (*c* 1, water); $\nu_{max}^{KBr}$ 3450–3200 (OH and NH), 1730 (COOMe), and 1660 $cm^{-1}$ ($CONH_2$).

*Anal.* Calc. for $C_8H_{13}NO_7$: C, 40.85; H, 5.57; N, 5.96. Found: C, 40.76; H, 5.57; N, 5.72.

*3,4,5-Tri-*O*-acetyl-1-amido-2,6-anhydro-*D-glycero-L-manno-*heptaric acid 7-methyl ester* (**6**). — Conventional acetylation of **5** (4.7 g, 20 mmol) with pyridine

(30 ml) and acetic anhydride (25 ml) and crystallization of the product from water gave the hydrate of **6** (6.4 g, 84%), m.p. 76°, $[\alpha]_{578}^{25}$ +48° (*c* 1, acetone); $\nu_{max}^{KBr}$ 1750 (COOMe and OAc) and 1690 $cm^{-1}$ ($CONH_2$). N.m.r. data (acetone-$d_6$): $\delta$ 1.9–2.2 (3 s, 9 H, 3 AcO), 3.75 (s, 3 H, COOMe).

*Anal.* Calc. for $C_{14}H_9NO_{10}\cdot H_2O$: C, 44.33; H, 5.58; N, 3.69. Found: C, 44.10; H, 5.51; N, 3.85.

*1-Amido-2,6-anhydro-3,4,5-tri*-O-(*trimethylsilyl*)-D-glycero-L-manno-*heptaric acid 7-methyl ester* (**7**). — Compound **5** (2.35 g, 10 mmol) was treated[7] with hexamethyldisilazane (8 g) and chlorotrimethylsilane (5 g) in pyridine (50 ml). The product **7** (4.1 g, 91%) had b.p. 195°/0.8 mmHg, m.p. 111° [from light petroleum (b.p. 60–70°)], $[\alpha]_{578}^{25}$ +48° (*c* 1, chloroform); $\nu_{max}^{KBr}$ 1760 (COOMe) and 1680 $cm^{-1}$ ($CONH_2$).

*Anal.* Calc. for $C_{17}H_{37}NO_7Si_3$: C, 45.20; H, 8.26. Found: C, 45.48; H, 8.47.

*1-Acetamido-3,4,5,7-tetra*-O-*acetyl-2,6-anhydro-1-deoxy*-D-glycero-L-manno-*heptitol* (**8**). — A solution of **7** (0.64 g, 1.4 mmol) in ether (3 ml) was added dropwise to a stirred suspension of lithium aluminium hydride (0.2 g) in ether (3 ml). The mixture was then boiled under reflux for 2 h. Excess reductant was decomposed with water (2 ml), and the mixture was concentrated. The solid residue was stirred with methanol (10 ml) and acetic acid (15 ml, 30%) for 4 h, and the clear solution was then diluted with water (100 ml) and extracted with chloroform (2 × 20 ml). The aqueous layer was concentrated to dryness and the residue was stirred for 5 h with pyridine (15 ml) and acetic anhydride (10 ml). The mixture was concentrated to dryness and the residue was extracted with chloroform (2 × 50 ml). The combined organic layers were washed with water (2 × 20 ml), dried ($Na_2SO_4$), and concentrated. The syrupy residue crystallised on the addition of ether and was recrystallised from ethanol–ether to give **8** (0.28 g, 49%), m.p. 153°, $[\alpha]_{578}^{25}$ +1° (*c* 1, chloroform); $\nu_{max}^{KBr}$ 3200 and 1570 (NH), 1745 (OAc), and 1640 $cm^{-1}$ (NAc); lit.[3] m.p. 153–154°, $[\alpha]_D^{20}$ +1.3° (*c* 1, chloroform). The product was identical (g.l.c., i.r. spectrum, and mixture m.p.) with an authentic sample[3].

*3,4,5-Tri*-O-*acetyl-2,6-anhydro*-D-glycero-L-manno-*heptaric acid 7-methyl ester 1-nitrile* (**9**). — The water of crystallization was removed from **6** (3.8 g, 10 mmol) by freeze-drying an aqueous solution. After the addition of pyridine (80 ml) and toluene-*p*-sulphonyl chloride (8 g) to the anhydrous compound, the mixture was heated to 80–85°. Dehydration was complete after 4 h (g.l.c.). The dark reaction mixture was then stirred into ice–water (300 ml), and, after 2 h, the aqueous solution was decolourised with charcoal and extracted with chloroform (6 × 150 ml). The combined organic layers were dried ($MgSO_4$) and concentrated. The residue crystallised on addition of ethanol, and recrystallisation from water gave **9** (2.5 g, 74%), $[\alpha]_{578}^{25}$ +61° (*c* 1, chloroform); $\nu_{max}^{KBr}$ 1750 $cm^{-1}$ (COOMe and OAc); the CN group being quenched does not show the typical i.r. absorption (2000–2300 $cm^{-1}$)[8].

*Anal.* Calc. for $C_{14}H_{17}NO_9$: C, 48.98; H, 4.99; N, 4.08. Found: C, 48.73; H, 5.04; N, 4.21.

*7-Acetamido-3,4-di*-O-*acetyl-2,6-anhydro-7-deoxy*-L-glycero-L-galacto-*heptonic*

*acid methyl ester* (**11**). — A solution of **9** (1.71 g, 5 mmol) in acetic acid (10 ml) was hydrogenated over platinum from 0.2 g of $PtO_2$. Hydrogen uptake stopped after ~4 h. The mixture was then filtered and concentrated to dryness, and a solution of the syrupy residue in dry benzene (50 ml) was boiled for 6 h under reflux. T.l.c. then showed that **11** had been formed in 80–85% yield. The solution was concentrated and the residue was eluted from a column (4 × 60 cm) of Kieselgel 60 (Merck, 0.063–0.2 mm), using 4:1 benzene–methanol. Fractions which contained **11** were combined and concentrated. Crystallisation of the product from ethyl acetate and recrystallisation from ethyl acetate–ether gave **11** (1.15 g, 66%), m.p. 157°, $[\alpha]_{578}^{25}$ +177° (*c* 1, chloroform); $\nu_{max}^{KBr}$ 3400–3100 (OH and NH), 1750 (COOMe and OAc), 1630 (NAc), and 1560 $cm^{-1}$ (NH). N.m.r. data ($CDCl_3$): $\delta$ 1.9–2.2 (2 s, 9 H, OAc and NAc), 3.75 (s, 3 H, COOMe). The exceptionally high optical rotation may be due to ring formation by the addition of HO-3 to the acetamido group.

*Anal.* Calc. for $C_{14}H_{21}NO_9$: C, 48.41; H, 6.09; N, 4.03. Found: C, 48.24; H, 6.04; N, 4.22.

*7-Acetamido-2,6-anhydro-7-deoxy*-L-glycero-L-galacto-*heptonic acid methyl ester* (**12**). — A solution of **11** (0.69 g, 2 mmol) in 0.02M methanolic sodium methoxide (3.5 ml) was stored for 30 min and then concentrated. The residue was recrystallized from methanol–ether to give **12** (0.43 g, 82%), m.p. 180°, $[\alpha]_{578}^{25}$ −7° (*c* 1, water); $\nu_{max}^{KBr}$ 3350–3200 (OH and NH), 1750 (COOMe), 1650 (NAc), and 1550 $cm^{-1}$ (NH).

*Anal.* Calc. for $C_{10}H_{17}NO_7$: C, 45.62; H, 6.51; N, 5.32. Found: C, 45.36; H, 6.73; N, 5.49.

*7-Amino-2,6-anhydro-7-deoxy*-L-glycero-L-galacto-*heptonic acid* (**13**). — Compound **12** (130 mg, 0.5 mmol) was saponified for 20 h at 100° with 2M sodium hydroxide (1 ml). After dilution with water (3 ml), the mixture was percolated through a column of Amberlite IRA-400($HO^-$) resin (15 ml). The column was washed with water until the eluate was neutral, and **13** was then eluted with 2M acetic acid (200 ml). Concentration of the acid solution, followed by evaporation of water twice from the syrupy residue, and recrystallisation from aqueous ethanol gave **13** (84 mg, 81%), m.p. 275–315° (dec.), $[\alpha]_{578}^{25}$ −23° (*c* 0.5, water), $R_F$ 0.24 (Whatman No. 1 paper; ethanol–M ammonium acetate, 7:3; detection with ninhydrin).

*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.

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