Available online at www.sciencedirect.com
SCIENCE DIRECT®
Carbohydrate Research 339 (2004) 147–151
Carbohydrate RESEARCH

Note

A convenient synthesis of GDP D-*glycero*-α-D-*manno*-heptopyranose

Andrea Graziani, Alla Zamyatina and Paul Kosma*

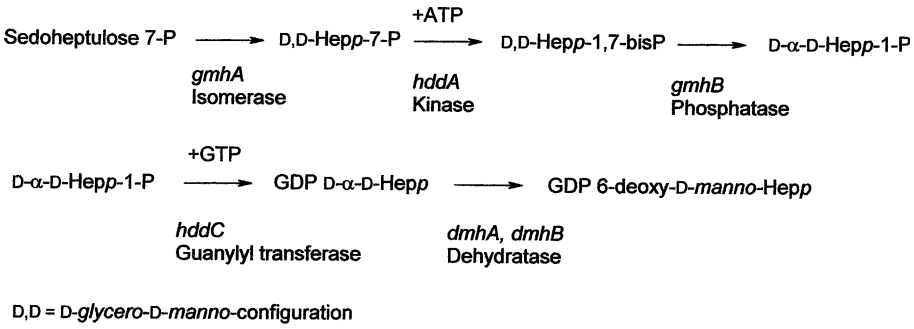
Institute of Chemistry, University of Natural Resources and Applied Life Sciences, Muthgasse 18, A-1190 Vienna, Austria

Received 6 August 2003; accepted 9 September 2003

Abstract—GDP D-*glycero*-α-D-*manno*-Heptopyranose has been prepared in good overall yield from 2,3,4,6,7-penta-*O*-acetyl-D-*glycero*-D-*manno*-heptopyranose by a short-step synthesis. Phosphitylation using the phosphoramidite procedure afforded the α-anomer in high selectivity. Subsequent oxidation and partial deprotection gave the acetylated phosphate derivative, which was subjected to the coupling reaction with GMP-morpholidate to furnish the acetylated heptose nucleoside diphosphate in good yield. De-*O*-acetylation and final purification afforded the target GDP D-*glycero*-α-D-*manno*-heptopyranose, which serves as the substrate of the heptosyl transferase in *Aneurinibacillus thermoaerophilus* DSM 10155 and occurs as an intermediate in the biosynthesis of GDP 6-deoxy-heptose in *Yersinia pseudotuberculosis*.

Keywords: Heptose; GDP-heptose; Sugar nucleotide; Lipopolysaccharide; Surface-layer glycoprotein

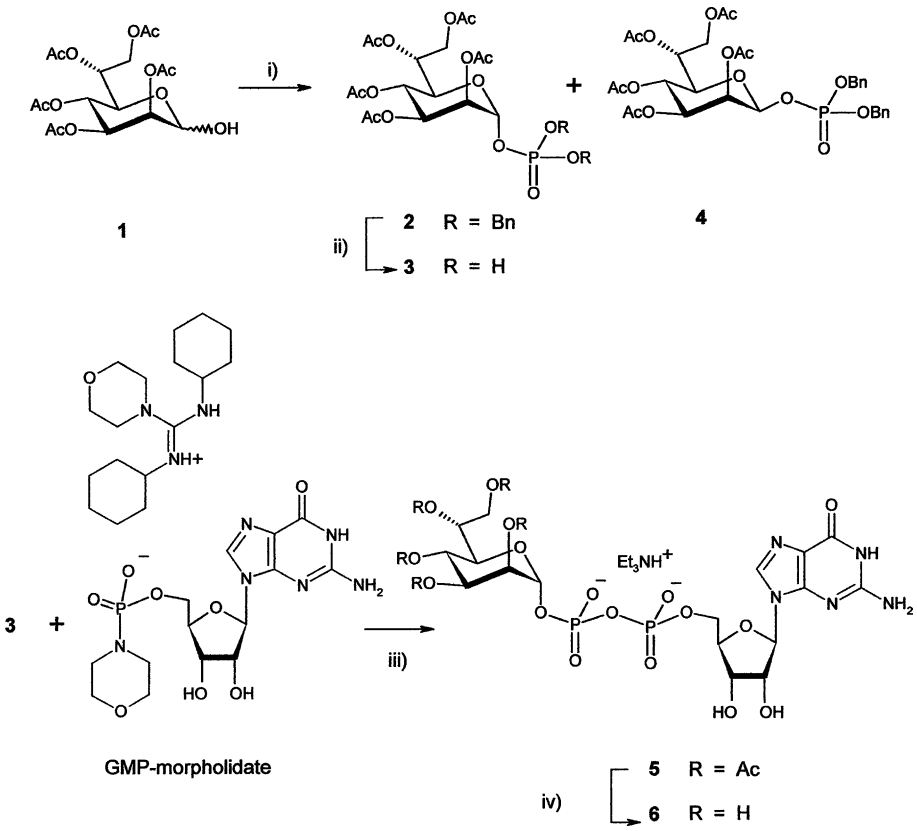
Heptoses of the D-*glycero*-D-*manno*- configuration are common constituents in the core region of numerous bacterial lipopolysaccharides and have also been detected in various O-antigenic chains.[1] For example, O-antigens from *Vibrio cholerae* serogroup O:3 and O:21 contain D-*glycero*-D-*manno*-heptose in the repeating units,[2,3] and β-linked D-*glycero*-D-*manno*-heptose and 6-deoxy-D-*manno*-heptose have been detected in the O-antigen from *Plesiomonas shigelloides* O54:H2.[4] Furthermore, β-heptopyranosyl residues are present in the surface-layer glycoprotein glycan in *Aneurinibacillus thermoaerophilus* DSM 10155.[5] The genes encoding the biosynthesis of the nucleotide-activated heptose in this Gram-positive thermophilic bacterium have been cloned, overexpressed in *E. coli* and the gene products have structurally and functionally been characterized.[6] Thus GDP D-*glycero*-α-D-*manno*-heptopyranose has been identified as the substrate for the bacterial glycosyltransferase involved in the assembly of the S-layer glycoprotein glycan in *A. thermoaerophilus* (Scheme 1).



Scheme 1. Biosynthetic steps and genes for GDP-activated heptoses in *A. thermoaerophilus* and *Y. pseudotuberculosis*.

* Corresponding author. Tel.: +43-1-36006-6055; fax: +43-1-36006-6059; e-mail: pkosma@edv2.boku.ac.at

0008-6215/$ - see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carres.2003.09.012



Scheme 2. Reagents and conditions: (i) Bis(benzyloxy)-*N*,*N*-diisopropylaminophosphine, 1*H*-tetrazole (3.5% in MeCN), 2 h, CH_2Cl_2, then *t*-BuOOH, 12 h, rt, 65% for **2**; (ii) 10% Pd/C, H_2, MeOH, Et_3N, 10 h, rt, 98% for **3**; (iii) 4′-morpholine-*N*,*N*′-dicyclohexylcarboxamidinium salt of GMP, pyridine, 3 days, rt, anion exchange on BioRad (Q) 5 mL cartridge (HCO_3^--form), elution 0.01 → 0.2 M TEAB, 65% for **5**; (iv) 7:3:1 MeOH–water–Et_3N, 3 h, rt, 98% for **6**.

Furthermore, a similar pathway leading to GDP 6-deoxy-D-*manno*-heptose has been identified in *Yersinia pseudotuberculosis* and the genes encoding the common steps of the biosynthesis of these nucleotide heptoses display a high degree of sequence homology.[7] Whereas ADP heptoses involved in the biosynthesis of the lipopolysaccharide core units of *E. coli* are present in the labile β-anomeric configuration, GDP heptose occurs in the significantly more stable α-anomeric linkage.[8,9]

In extension of previous work on the synthesis of both anomeric forms of ADP heptoses, we report herein on the chemical synthesis of GDP heptose to be used as a biochemical probe.[10]

The previously described D-*glycero*-D-*manno*-heptopyranose[11] **1** was subjected to phosphitylation using the phosphoramidite method.[12] Treatment of **1** with bis(benzyloxy)-*N*,*N*-diisopropylaminophosphine/1*H*-tetrazole and subsequent oxidation with *t*-BuOOH gave the α-phosphate **2** in 65% yield. The minor amount (5%) of β-anomer **4** was removed by chromatography.

Catalytic hydrogenation in the presence of 10% Pd/C and treatment with triethylamine produced the known[11] triethylammonium salt of penta-*O*-acetyl-heptosyl phosphate **3**. The partially protected derivative **3** is fairly soluble in pyridine (Scheme 2) and thereby increases the efficiency of the nucleoside diphosphate formation.[13] The coupling of **3** with GMP-morpholidate was carried out in pyridine under strictly anhydrous conditions for 3 days.[14] The acetylated GDP D-*glycero*-α-D-*manno*-heptose **5** was isolated in 65% yield after anion-exchange chromatography. Removal of the acetyl groups with triethylamine in MeOH–water produced GDP heptose **6** as the triethylammonium salt in 98% yield. The presence of the diphosphate in compound **6** was confirmed by the chemical shift of the ^{31}P NMR signals at −11.2 (phosphate attached to ribose) and −13.6 ppm (phosphate linked to heptose unit), respectively. The ^{1}H and ^{13}C NMR data of **6** (Fig. 1) were in full agreement with those found for the native nucleotide-activated sugar in *A. thermoaerophilus*, thereby confirming the structure of the bacterial GDP heptose.[6] In addition, the ^{13}C NMR signals of the heptopyranosyl unit are nearly identical (within 0.05 ppm) to those of the related ADP D-*glycero*-α-D-*manno*-heptose, thus fully confirming the structural assignments.[11]

In conclusion, the method reported herein together with the previously described ADP heptose synthesis constitutes a flexible and efficient strategy for the synthesis of purine-nucleotide heptoses.

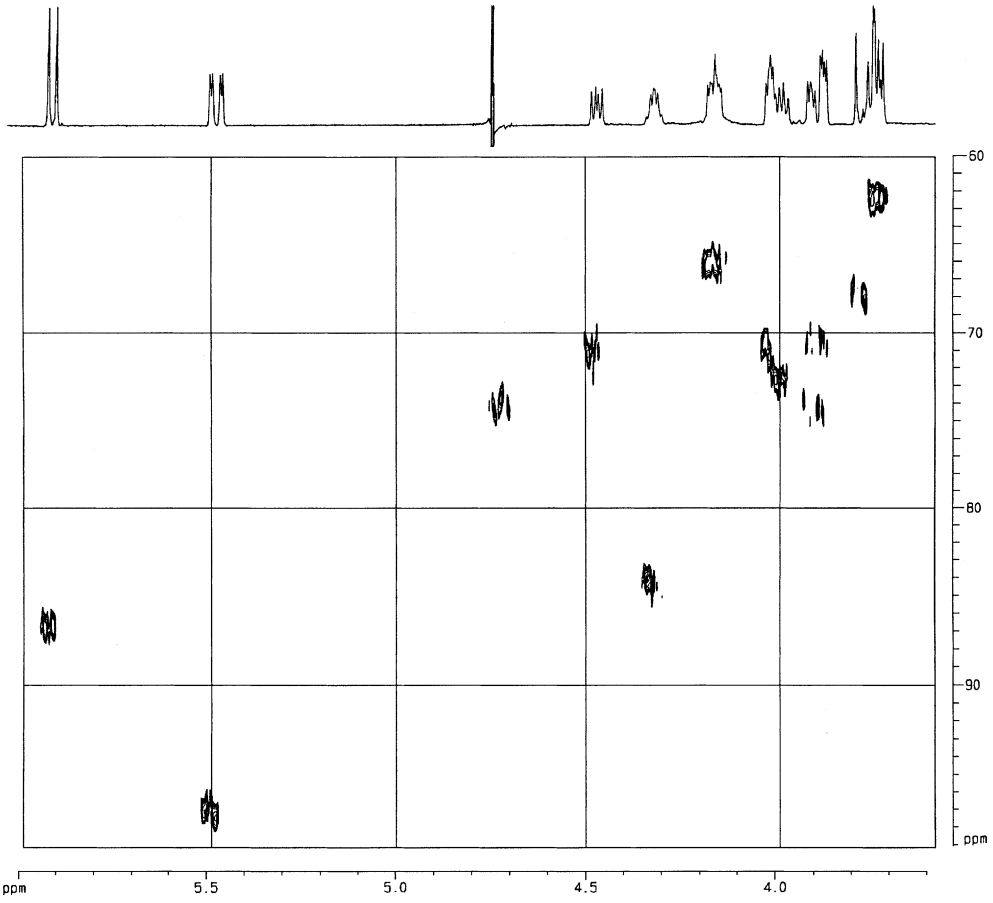


Figure 1. Contour plot of the HMQC spectrum of GDP heptose **6** with 300 MHz ^{1}H NMR projection. The spectrum was recorded at 300 K for a sample at pD 6.3.

1. Experimental

1.1. General

All solvents were purified and dried by standard procedures. Column chromatography was performed on Silica Gel 60 (230–400 mesh, E. Merck). Anion-exchange chromatography was performed on a strong anion-exchange resin (BioRad, 5 mL cartridge, HCO_3^- form). Size-exclusion chromatography was performed on Sephadex G-25 M. The anion-exchange column was connected to the FPLC system (Pharmacia) and the eluates were monitored at 280 nm. Analytical TLC was performed using silica gel 60 F_{254} HPTLC plates with 2.5 cm concentration zone (E. Merck). Spots were detected by treatment with anisaldehyde–H_2SO_4; guanosine-containing compounds were also detected by UV light examination. Triethylammonium bicarbonate (TEAB) buffer was prepared by passing a stream of CO_2-gas through a cooled solution of triethylamine (2 M) in de-ionized water until a near neutral solution (pH 7.5) was obtained. Optical rotations were measured with a Perkin–Elmer 243 B polarimeter. NMR spectra were recorded at 297 K in D_2O and $CDCl_3$ with a Bruker DPX 300 spectrometer (^{1}H at 300.13 MHz, ^{13}C at 75.47 MHz and ^{31}P at 121.50 MHz) using standard Bruker NMR software. ^{1}H NMR spectra were referenced to tetramethylsilane or 2,2-dimethyl-2-silapentane-5-sulfonic acid. ^{13}C NMR spectra were referenced to chloroform for solutions in $CDCl_3$ (δ 77.00) or dioxane (δ 67.40) for solutions in D_2O. ^{31}P NMR spectra were referenced externally to 85% aq H_3PO_4 (δ 0.0). MALDI-TOFMS spectra were recorded on a Dynamo (Thermo BioAnalysis) instrument using water and 2,5-dihydroxybenzoic acid as matrix. TOF-ESMS spectra were recorded on a Waters QTOF-ES-MS instrument.

1.2. Dibenzyl (2,3,4,6,7-penta-*O*-acetyl-D-*glycero*-α-D-*manno*-heptopyranosyl) phosphate (2) and dibenzyl (2,3,4,6,7-penta-*O*-acetyl-D-*glycero*-β-D-*manno*-heptopyranosyl) phosphate (4)

Heptose pentaacetate **1** (60 mg, 0.14 mmol) and bis-(benzyloxy)-*N*,*N*-diisopropylaminophosphine (117 μL, 0.35 mmol) were dried by repeated evaporations with dry toluene (3 × 10 mL) and then under diminished pressure for 10 h. Then the flask was charged with CH_2Cl_2 (3 mL), a soln of 1*H*-tetrazole (30 mg,

0.43 mmol) in dry MeCN (1.5 mL) was added and the mixture was stirred at room temperature for 2 h under N_2. Monitoring of the reaction by TLC showed the formation of intermediate phosphite triesters (2.5:7.5, *n*-hexane–diethyl ether). The reaction mixture containing phosphite triesters was cooled to 0 °C and a soln of *t*-BuOOH (35 μL of an 80% soln in di-*tert*-butyl peroxide) in CH_2Cl_2 (1.5 mL) was gradually added (~20 min). The reaction mixture was warmed to room temperature and stirred for 12 h. The solvent was evaporated using a stream of N_2. The residue was redissolved in diethyl ether and washed sequentially with saturated aq $NaHCO_3$–water and brine. The organic phase was dried (Na_2SO_4) and concentrated. The α- and β-phosphates were separated by chromatography on silica gel (1:9, *n*-hexane–diethyl ether) to give **2** (62 mg, 65%) as a syrup. R_f 0.40; analytical data were similar to the previously described compound.[11] ^{1}H NMR ($CDCl_3$): δ 7.37–7.33 (m, 10H, 2Ph), 5.57 (dd, 1H, $^3J_{1,2}$ 1.7, $^3J_{1,P}$ 6.5 Hz, H-1), 5.30 (t, 1H, $^3J_{4,3} = {}^3J_{4,5}$ 9.8 Hz, H-4), 5.28 (d, 1H, $^3J_{3,2}$ 3.3 Hz, H-3), 5.21–5.14 (m, 2H, H-2, H-6), 5.09 (d, 2H, $^3J_{H,P}$ 8.5 Hz, CH_2Ph), 5.08 (d, 2H, $^3J_{H,P}$ 8.5 Hz, CH_2Ph), 4.33 (dd, 1H, $^3J_{7a,6}$ 4.0, $^2J_{7a,7b}$ 12.0 Hz, H-7a), 4.19 (dd, 1H, $^3J_{7b,6}$ 7.7 Hz, H-7b), 4.15 (m, 1H, H-5), 2.13, 2.09, 2.03, 1.99 and 1.93 (5s, 15H, 5Ac).

Further elution gave **4** (4.8 mg, 5%) as a syrup. R_f 0.24; ^{1}H NMR ($CDCl_3$): δ 7.37–7.29 (m, 10H, 2Ph), 5.47 (dd, 1H, $^3J_{1,2}$ 1.5, $^3J_{1,P}$ 7.8 Hz, H-1), 5.42 (dd, 1H, $^3J_{2,1}$ 1.5, $^3J_{2,3}$ 3.3 Hz, H-2), 5.26 (t, 1H, $^3J_{4,3} = {}^3J_{4,5}$ 9.3 Hz, H-4), 5.25 (m, 1H, H-6), 5.10 (d, 2H, $^3J_{H,P}$ 8.0 Hz, CH_2Ph), 5.05 (d, 2H, $^3J_{H,P}$ 8.0 Hz, CH_2Ph), 5.04 (dd, 1H, $^3J_{3,4}$ 9.3, $^3J_{3,2}$ 3.3 Hz, H-3), 4.41 (dd, 1H, $^3J_{7a,6}$ 3.6, $^2J_{7a,7b}$ 12.0 Hz, H-7a), 4.25 (dd, 1H, $^3J_{7b,6}$ 7.0 Hz, H-7b), 4.15 (dd, 1H, $^3J_{5,6}$ 3.7 Hz, H-5), 2.13, 2.10, 2.04, 2.01 and 1.99 (5s, 15H, 5Ac).

1.3. 2,3,4,6,7-Penta-*O*-acetyl-D-*glycero*-α-D-*manno*-heptopyranosyl phosphate (triethylammonium salt) (3)

A soln of **2** (23 mg, 0.034 mmol) in dry MeOH (7 mL) was hydrogenated in the presence of 10% Pd/C (7 mg) for 10 h at atmospheric pressure. After completion of the reaction, the catalyst was removed by filtration through a pad of Celite and washed with MeOH. The combined filtrates were neutralized by addition of Et_3N (0.1 mL) and concentrated. The residue was lyophilized from water to give the triethylammonium salt of **3** (20 mg, 98%) as a white fluffy solid which was used in the next step without further purification. R_f 0.34 (35:20:2:2, $CHCl_3$–MeOH–25% aq NH_4OH–water); ^{1}H NMR (D_2O): δ 5.42 (dd, 1H, $^3J_{1,P}$ 7.5, $^3J_{1,2}$ 2.0 Hz, H-1), 5.38 (dd, 1H, $^3J_{2,3}$ 2.9, $^3J_{3,4}$ 9.8 Hz, H-3), 5.30 (t, 1H, $^3J_{4,5}$ 9.8 Hz, H-4), 5.26 (br s, 1H, H-2), 5.25 (ddd, 1H, H-6), 4.43 (dd, 1H, $^3J_{7a,6}$ 3.4, $^2J_{7a,7b}$ 12.0 Hz, H-7a), 4.40 (dd, 1H, $^3J_{5,6}$ 2.5 Hz, H-5), 4.26 (dd, 1H, $^3J_{7b,6}$ 7.6 Hz, H-7b), 3.16 (q, 6H, NCH_2), 2.15, 2.11, 2.09, 2.04, 1.99 (5s, 15H, 5Ac), 1.24 (t, 9H, CH_3, Et_3N).

1.4. Guanosine 5′-(D-*glycero*-α-D-*manno*-heptopyranosyl) diphosphate (triethylammonium salt) (6)

Pentaacetyl heptosyl phosphate **3** (20 mg, 0.0334 mmol) was made anhyd by repeated dissolution in dry pyridine and evaporation (4 × 10 mL). After each evaporation step, dry N_2 was flushed into the rotary evaporator. Guanosine-5′-monophosphate morpholidate (4′-morpholine-*N*,*N*′-dicyclohexylcarboxamidinium salt) (36 mg, 0.0496 mmol) was dissolved in dry pyridine and evaporated to dryness and the process was repeated three times with exclusion of moisture under N_2. Both components were finally dissolved in pyridine and combined in a one-neck round-bottom flask under N_2-atmosphere. The reaction mixture was repeatedly evaporated from pyridine (3 × 10 mL) and flushed with dry N_2. A final amount of pyridine (10 mL) was added to give a clear solution. About 70% of pyridine was removed by concentration and the reaction vessel was sealed under N_2. The solution was vigorously stirred and the progress of the reaction was monitored by TLC-analysis (35:20:2:2, $CHCl_3$–MeOH–25% aq NH_4OH–H_2O). The reaction was kept for 3 days and progress was monitored by the appearance of a major UV-positive spot of GDP-Hep. The reaction was stopped by evaporation of pyridine. The diphosphate **5** was isolated using anion-exchange chromatography. The crude reaction products were dissolved in 10 mL water and the soln was allowed to slowly adsorb on a resin bed of anion-exchange column (BioRad, 5 mL cartridge, HCO_3^--form) connected to an FPLC-system. The column was operated at 0.7 mL/min, fractions (1 mL) were collected. The column was washed first with water and was then developed with a linear gradient of TEAB buffer. The eluate was monitored at 280 nm, **5** was eluted at a concentration of ~0.15 M TEAB. The fractions containing GDP-Hep were pooled, concentrated to 10 mL vol, the soln was cooled to 0 °C, and the pH was adjusted to 4.5 with Dowex 50 (H^+) resin. The resin was removed by filtration, the total eluate was made neutral by addition of Et_3N, concentrated to 5 mL vol at 25 °C and lyophilized to give guanosine 5′-(2,3,4,6,7-penta-*O*-acetyl-D-*glycero*-α-D-*manno*-heptopyranosyl) diphosphate (triethylammonium salt) (**5**) as a solid. Yield: 20.7 mg (65%); R_f 0.37 (35:20:2:2, $CHCl_3$–MeOH–25% aq NH_4OH–water); ^{31}P NMR: δ −11.0 (d, $^2J_{P,P}$ 19 Hz; P_{Rib}), −14.2 (d, P_{Hep}); ^{1}H NMR (D_2O): δ 8.11 (br s, 1H, H-8_{Gua}), 5.91 (d, 1H, $^3J_{1,2}$ 5.8 Hz, H-1_{Rib}), 5.58 (dd, 1H, $^3J_{1,2}$ 1.8, $^3J_{1,P}$ 7.5 Hz, H-1_{Hep}), 5.36 (m, 1H, H-2_{Hep}), 5.35–5.28 (m, 2H, H-3_{Hep}, H-4_{Hep}), 5.22 (m, 1H, H-6_{Hep}), ~4.73 (H-2_{Rib}, underneath the water signal), 4.49 (dd, 1H, $^3J_{3,4}$ 5.1, $^3J_{3,2}$

3.8 Hz, H-3_{Rib}), 4.37–4.15 (m, 6H, H-4_{Rib}, H-5_{Hep}, H-5a,b_{Rib}, H-7a,b_{Hep}), 3.18 (q, 12H, CH_2, Et_3N), 2.16, 2.11, 2.05, 1.98, 1.90 (5s, 15H, 5Ac) and 1.27 (t, 18H, CH_3, Et_3N).

A soln of **5** (20.7 mg, 0.022 mmol) in 7:3:1 MeOH–water–Et_3N (2 mL) was stirred at ambient temperature for 3 h at pH 12. The reaction mixture was diluted with water and purified on a size-exclusion column (Sephadex G-25 M, PD-10 column, Pharmacia). The eluate was concentrated and lyophilized to give **6** (17 mg, 98%) as a white solid. R_f 0.60 (5:10:2:2, $CHCl_3$–MeOH–25% aq NH_4OH–water); $[\alpha]_D^{20}$ +4.6 (*c* 0.2, water); ^{31}P NMR: δ −11.2 (d, $^2J_{P,P}$ 20.9 Hz, P_{Rib}), −13.6 (d, P_{Hep}); ^1H NMR (D_2O): δ 8.04 (br s, 1H, H-8_{Gua}), 5.91 (d, 1H, $^3J_{1,2}$ 6.2 Hz, H-1_{Rib}), 5.48 (dd, 1H, $^3J_{1,2}$ 2.0, $^3J_{1,P}$ 7.8 Hz, H-1_{Hep}), 4.73 (H-2_{Rib}, underneath the HOD signal), 4.48 (dd, 1H, $^3J_{3,4}$ 3.1, $^3J_{3,2}$ 5.2 Hz, H-3_{Rib}), 4.33 (m, 1H, H-4_{Rib}), 4.18 (m, 1H, H-5_{Rib}), 4.02 (dd, 1H, $^3J_{2,3}$ 3.4 Hz, H-2_{Hep}), 4.00 (ddd, 1H,$^3J_{6,5}$ 3.3, $^3J_{6,7a}$ 4.1, $^3J_{6,7b}$ 6.5 Hz, H-6_{Hep}), 3.91 (dd, 1H, $^3J_{5,4}$ 9.3 Hz, H-5_{Hep}), 3.87 (dd, 1H, $^3J_{3,4}$ 9.3 Hz, H-3_{Hep}), 3.76 (t, 1H, H-4_{Hep}), 3.76 (dd, 1H, $^3J_{7a,7b}$ 12.1 Hz, H-$7a_{Hep}$), 3.72 (dd, 1H, H-$7b_{Hep}$), 3.19 (q, ~9H, CH_2, Et_3N), 1.27 (t, ~13.5H, CH_3, Et_3N); ^{13}C NMR (D_2O): δ 160.78 (C-6_{Gua}), 152.44 (C-4_{Gua}), 136.8 (C-8_{Gua}), 118.01 (C-5_{Gua}), 97.05 (C-1_{Hep}, $^2J_{1,P}$ 5.8 Hz), 86.71 (C-1_{Rib}), 84.23 (C-4_{Rib}, $^3J_{4,P}$ 9.0 Hz), 74.32 (C-2_{Rib}, C-5_{Hep}), 72.86 (C-6_{Hep}), 71.18 (C-3_{Rib}), 70.72 (C-2_{Hep}, $^3J_{2,P}$ ~9.0 Hz), 70.71 (C-3), 67.88 (C-4_{Hep}), 66.04 (C-5_{Rib}, $^2J_{5,P}$ 5.8 Hz), 62.61 (C-7_{Hep}), 47.07 (CH_2, Et_3N), 9.16 (CH_3, Et_3N); QTOF-ES-MS: $m/z = 636.029$ $[M+H]^+$.

Acknowledgements

Authors are grateful to Andreas Hofinger for providing NMR spectra and for financial support by FWF (grant P14978-MOB).

References

1. (a) Holst, O. In *Endotoxin in Health and Disease*; Brade, H., Opal, S. M., Vogel, S. N., Morrison, D. C., Eds.; Marcel Dekker: New York, Basel, USA, 1999; pp 115–154, and references cited therein; (b) Wilkinson, S. G. *Prog. Lipid Res.* **1996**, *35*, 283–343.
2. Chowdhury, T. A.; Jansson, P.-E.; Lindberg, B.; Lindberg, J.; Gustafsson, B.; Holme, T. *Carbohydr. Res.* **1991**, *215*, 303–314.
3. Ansari, A. A.; Kenne, L.; Lindberg, B.; Gustafsson, B.; Holme, T. *Carbohydr. Res.* **1986**, *150*, 213–219.
4. (a) Niedziela, T.; Lukasiewicz, J.; Jachymek, W.; Dzieciatkowska, M.; Lugowski, C.; Kenne, L. *J. Biol. Chem.* **2002**, *277*, 11653–11663; (b) Czaja, J.; Jachymek, W.; Niedziela, T.; Lugowski, C.; Aldova, E.; Kenne, L. *Eur. J. Biochem.* **2000**, *267*, 1672–1679.
5. Kosma, P.; Wugeditsch, T.; Christian, R.; Zayni, S.; Messner, P. *Glycobiology* **1995**, *5*, 791–796.
6. Kneidinger, B.; Graninger, M.; Puchberger, M.; Kosma, P.; Messner, P. *J. Biol. Chem.* **2001**, *276*, 20935–20944.
7. Pacinelli, E.; Wang, L.; Reeves, P. R. *Infect. Immun.* **2002**, *70*, 3271–3276.
8. (a) Kneidinger, B.; Marolda, C. L.; Graninger, M.; Zamyatina, A.; McArthur, F.; Kosma, P.; Valvano, M. A.; Messner, P. *J. Biol. Chem.* **2002**, *184*, 363–369; (b) Gronow, S.; Oertelt, C.; Ervelä, E.; Zamyatina, A.; Kosma, P.; Skurnik, M.; Holst, O. *J. Endotoxin Res.* **2001**, *7*, 263–270.
9. Valvano, M. A.; Messner, P.; Kosma, P. *Microbiology* **2002**, *148*, 1979–1989.
10. Zamyatina, A.; Gronow, S.; Oertelt, C.; Puchberger, M.; Brade, H.; Kosma, P. *Angew. Chem., Int. Ed.* **2000**, *39*, 4150–4153.
11. Zamyatina, A.; Gronow, S.; Puchberger, M.; Graziani, A.; Hofinger, A.; Kosma, P. *Carbohydr. Res.* **2003**, *338*, 2571–2589.
12. Bannwarth, W.; Trzeciak, A. *Helv. Chim. Acta* **1987**, *70*, 175–186.
13. (a) Warren, C. D.; Jeanloz, R. W. *Biochemistry* **1972**, *11*, 2565–2572; (b) Baisch, G.; Öhrlein, R. *Bioorg. Med. Chem.* **1997**, *5*, 383–391; (c) Liemann, S.; Klaffke, W. *Liebigs Ann. Chem.* **1995**, 1779–1787; (d) Naundorf, A.; Natsch, S.; Klaffke, W. *Tetrahedron Lett.* **2000**, *41*, 189–192.
14. Roseman, S.; Distler, J. J.; Moffatt, J. G.; Khorana, H. G. *J. Am. Chem. Soc.* **1961**, *83*, 659–663.