Conjugation-Amenable Tetrasaccharide of the Side Chain of the Major Glycoprotein of the Bacillus anthracis Exosporium: A Large-Scale Preparation

Shujie Hou, Pavol Kováč

NIDDK, LBC, National Institutes of Health, Bethesda, MD 20892-0815, USA
Fax +1(301)4805703; E-mail: kpn@helix.nih.gov
Received 1 September 2008; revised 27 October 2008

Abstract: A new strategy towards the synthesis of the title tetrasaccharide is described. The novelty within the common (2+2) assembly lies in the use of a disaccharide glycosyl donor having the fully assembled anthrose as one of the constituent sugar residues. Also, the final deprotection and transformation of the spacer arm into an amine, to form a structure amenable to conjugation by different conjugation techniques, is a one-pot conversion. Compared to other synthetic approaches, the present synthesis involves fewer chemical manipulations with the assembled tetrasaccharide as well as fewer overall numbers of synthetic steps towards this important antigenic component of a potential conjugate vaccine for anthrax.

Key words: carbohydrates, oligosaccharides, glycosylations, conjugation, amines

The structure of the tetrasaccharide of the side chain of the major glycoprotein of the Bacillus anthracis exosporium, which was proposed by Daubenspeck et al. 1 to be the sequence β-Ant-(1→3)-α-L-Rha-(1→3)-α-L-Rha-(1→2)-α-L-Rha, was confirmed in this laboratory by chemical synthesis. We have previously described the syntheses of the α and β 5-methoxycarbonylpentyl glycosides of the tetrasaccharide, as well as of all structural fragments of the two glycosides. A shorter (2+2) and a (3+1) sequence have also been described. Preparation of the sequence α-L-Rha-(1→3)-α-L-Rha-(1→2)-α-L-Rha is not a very difficult task and, thus, the chemical synthesis of the tetrasaccharide depends largely on availability of a suitable glycosyl donor for anthrose or its precursor. We have recently reported 7 a synthesis of a glycosyl donor for latent anthrose that is more convenient than either our original preparation or the later-developed shorter approaches. The availability of an anthrose precursor from inexpensive, commercially available starting materials and the need for the tetrasaccharide in connection with extensive studies towards a conjugate vaccine for anthrax prompted us to develop a large-scale preparation of the sequence β-Ant-(1→3)-α-L-Rha-(1→3)-α-L-Rha-(1→2)-α-L-Rha amenable to conjugation. The new (2+2) construction involves a feature that has not been attempted before, namely the use of a disaccharide glycosyl donor containing fully assembled anthrose as one of its constituent sugars. This minimizes the number of chemical manipulations with the constructed tetrasaccharide and, together with the fewer synthetic steps involved, renders the overall synthesis more efficient.

The assembly from smaller oligosaccharide building blocks is often a method of choice to construct higher oligosaccharides. Such blockwise strategy (2+2) was also used in the previous synthesis of the tetrasaccharide sequence described here. There, the glycosyl donor for the upstream disaccharide terminus contained the 4-azido-3-O-benzyl-4,6-dideoxy-2-O-methyl-β-D-glucopyranosyl residue, a precursor of anthrose, which was made from D-fucose. Following the assembly of the tetrasaccharide, its upstream terminal monosaccharide residue was transformed into anthrose by a two-step conversion. Conjugation by reductive amination, required further chemical manipulation with the pentenyl glycoside of the tetrasaccharide. The synthesis presented here (Schemes 1–3) uses donor, which can be made from inexpensive methyl α- or β-galactopyranoside. It is more economical and makes tetrasaccharide more readily available, as it uses a building block containing the fully assembled anthrose. In addition, the final, one-pot deprotection effects deacetylation in the N-acetyl side chain and conversion of the ester group in the spacer into an amine, making the substance ready for attachment to carriers by many conjugation techniques.

The spacer-equipped tetrasaccharide was built up from disaccharides and , which were synthesized from the common intermediate 1. Thus, coupling of thioglycoside with the linker-equipped rhannoside gave disaccharide, which was deacetylated to afford the disaccharide glycosyl acceptor (Scheme 1). To obtain glycosyl donor , alcohol was prepared from 1 as described and treated with the known imidate in the presence of trimethylsilyl trifluoromethanesulfonate (TMSOTf), to give disaccharide (89%) and a small amount of by-product (3%) (Figure 1), a product of glycone transfer.

SYNTHESIS 2009, No. 4, pp 0545–0550
Advanced online publication: 27.01.2009
DOI: 10.1055/s-0028-1083338; Art ID: M04708SS
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Figure 1 Molecular structure of the by-product

7A
Scheme 1  Synthesis of disaccharide 4. **Reagents and conditions**: (i) NIS, AgOTf, 4 Å MS, CH₂Cl₂; (ii) NaOMe, MeOH.

Scheme 2  Synthesis of glycosyl donor 12. **Reagents and conditions**: (i) 4 Å MS, TMSOTf, CH₂Cl₂, –78 °C; (ii) NaOMe, MeOH–CH₂Cl₂, 50 °C; (iii) KOH, MeI, DMSO; (iv) H₂S, H₂O–pyridine; (v) HATU, Hünig’s base, 3-hydroxy-3-methylbutanoic acid, CH₂Cl₂; (vi) Ac₂O, DMAP, CH₂Cl₂.

Scheme 3  Synthesis of tetrasaccharide 15. **Reagents and conditions**: (i) 4 Å MS, NIS, AgOTf, CH₂Cl₂, –50 °C; (ii) H₂, Pd/C, MeOH–EtOAc; (iii) H₂N(CH₂)₂NH₂, 50 °C overnight, then NaOMe, MeOH.
When the reaction was promoted by BF₃·OEt₂, a much higher proportion of 7A was formed (this conversion is not described in the experimental section). Compound 7 was functionalyzed, namely debenzoylated (Zemplén, → 8), methylaleted (9), treated successively with H₂S (→ 10) and 3-hydroxy-3-methylbutanoic acid in the presence of HATU. N-[4-(dimethylamino)-1H-1,2,3-triazolo[4,5-b]pyridin-1-ylmethylene]-N-methylthiaminium hexafluorophosphate N-oxide (→ 11). The product was acetylated to give the disaccharide glycosyl donor 12 having the fully assembled anthrone moiety at the upstream end (Scheme 2).

Couplings of the two building blocks 4 and 12 (Scheme 3) in the presence of N-iodosuccinimide (NIS) and AgOTf under various conditions (these reactions are not described in the Experimental) were accompanied by side reactions and afforded tetrasaccharide 13 in poor yields.

This situation improved considerably when the reaction was conducted at 50 °C using AgOTf as the promoter, to give 13 consistently in ~70% yield. It was interesting to note that 13 was obtained in much lower yield when the reaction was performed at -40 °C, -60 °C, -78 °C or at ambient temperature. Debenzylation of 13 was conducted at -50 °C using AgOTf as the promoter, to note that the reaction was complete. The mixture was filtered through a Celite pad into a separatory funnel containing 10% aq Na₂S₂O₃ (30 mL). The mixture was shaken and, after the organic phase had been drained, the aqueous solution was extracted with CH₂Cl₂ (30 mL). The combined organic phase was dried, concentrated, and the residue was chromatographed (hexane-acetone, 6:1) to afford 3; yield: 16.8 g (91%); colorless oil; [α]D₂⁺ 45.65 (c 1.7, CHCl₃).

Optical rotations were measured at ambient temperature with a Jasco automatic polarimeter, Model P-2000. All reactions were monitored by TLC on silica gel 60 gel coated glass slides. Column chromatography was performed by elution from columns of silica gel with CombiFlash Companion Chromatograph (Isco, Inc.). Unsymmetrical separation was performed by elution from columns of silica gel with 10% aq Na₂S₂O₃ (30 mL). The combined organic phase was dried, concentrated, and the residue was chromatographed (hexane-acetone, 6:1) to afford 3; yield: 16.8 g (91%); colorless oil; [α]D₂⁺ 45.65 (c 1.7, CHCl₃).


5-Methoxycarboxylpeptidyl 2-O-(3-O-Acetyl-2,4-di-O-benzyl-a-L-rhamnopyranosyl)-3,4-di-O-benzyl-a-L-rhamnopyranoside (4)

NaOMe in MeOH (1 M) was added to a solution of 7 (16.08 g, 19.1 mmol) in MeOH (500 mL) until the mixture became strongly basic (Scheme 3). After neutralization with Amberlite IR-120 (H⁺), the solution was filtered, the filtrate was concentrated, and the residue was chromatographed to give 4; yield: 14.5 g (94%); colorless oil; [α]D₂⁺ -8.76 (c 1.2, CHCl₃).


When the reaction was promoted by BF₃·OEt₂, a much higher proportion of 7A was formed (this conversion is not described in the experimental section). Compound 7 was functionalyzed, namely debenzoylated (Zemplén, → 8), methylaleted (9), treated successively with H₂S (→ 10) and 3-hydroxy-3-methylbutanoic acid in the presence of HATU. N-[4-(dimethylamino)-1H-1,2,3-triazolo[4,5-b]pyridin-1-ylmethylene]-N-methylthiaminium hexafluorophosphate N-oxide (→ 11). The product was acetylated to give the disaccharide glycosyl donor 12 having the fully assembled anthrone moiety at the upstream end (Scheme 2).

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Optical rotations were measured at ambient temperature with a Jasco automatic polarimeter, Model P-2000. All reactions were monitored by TLC on silica gel 60 gel coated glass slides. Column chromatography was performed by elution from columns of silica gel with CombiFlash Companion Chromatograph (Isco, Inc.). Unless stated otherwise, solvent mixtures less polar than those used for TLC were used at the onset of separations. NMR spectra were measured at 300 MHz (1H) and 75 MHz (13C) with a Varian Gemini or Varian Mercury spectrometers, or at 600 MHz (1H) and 150 MHz (13C) with a Bruker Avance 600 spectrometer. Assignments of NMR signals were made by homonuclear and heteronuclear 2-dimensional correlation spectroscopy, run with the software supplied with the spectrometers. When reporting assignment of NMR signals, sugar residues in oligosaccharides are serially numbered, beginning with the one bearing the linker, and individual nuclei are identified by a Roman numeral superscript. Nuclei associated with the N-butanimido side chain and the linker are identified by Arabic numerals, and those belonging to the aglycone linker arm are denoted with a prime. Attempts were made to obtain correct combustion analysis data for all new compounds. However, some compounds tenaciously retained traces of solvents, despite exhaustive drying, and analytical figures for carbon could not be obtained within ±0.4%. Structures of these compounds follow unequivocally from the mode of synthesis and NMR and MS data. Pd/C catalyst (5%, ESCAT 103) was a product of Engelhard Industries. HATU was purchased from Applied Biosystems. 3-Hydroxy-3-methylbutanoic acid was purchased from Alfa Aesar Chemical Company. Solutions in organic solvents were dried with anhyd Na₂SO₄, and concentrated at 40 °C/2 kPa.

5-Methoxycarboxylpeptidyl 2-O-(3-O-Acetyl-2,4-di-O-benzyl-a-L-rhamnopyranosyl)-3,4-di-O-benzyl-a-L-rhamnopyranoside (3)

A mixture of 11 (10.81 g, 26.0 mmol), 2 (10.10 g, 21.4 mmol), and 4 Å MS (4.20 g) in CH₂Cl₂ (210 mL) was stirred under N₂ for 15 min at r.t. After cooling to 0 °C, NIS (6.73 g, 29.9 mmol) was added, followed by solid AgOTf (2.75 g, 10.8 mmol). Red color developed within 5 min and, after 30 min, TLC (3:1 hexane-EtOAc) showed that the reaction was complete. The mixture was filtered through a Celite pad into a separatory funnel containing 10% aq Na₂S₂O₃, allowing solution of 11 (1500 mL). The mixture was shaken and, after the organic phase had been drained, the aqueous solution was extracted with CH₂Cl₂ (3 × 30 mL). The combined organic phase was dried, concentrated, and the residue was chromatographed (hexane-acetone, 6:1) to afford 3; yield: 16.8 g (91%); colorless oil; [α]D₂⁺ 45.65 (c 1.7, CHCl₃).


5-Methoxycarboxylpeptidyl 2-O-(2,4-Di-O-benzyl-a-L-rhamnopyranosyl)-3,4-di-O-benzyl-a-L-rhamnopyranoside (4)

NaOMe in MeOH (1 M) was added to a solution of 3 (16.08 g, 19.1 mmol) in MeOH (500 mL) until the mixture became strongly basic to litmus. The mixture was stirred overnight at r.t., when TLC (3:1 hexane-EtOAc) showed that all starting material was converted into a more polar product. After neutralization with Amberlite IR-120 (H⁺), the solution was filtered, the filtrate was concentrated, and the residue was chromatographed to give 4; yield: 14.5 g (94%); colorless oil; [α]D₂⁺ -8.76 (c 1.2, CHCl₃).


Yield: 20.46 g (89%); colorless oil; 

\[ \delta (J=3.2, CHCl_3) \]

3H NMR (600 MHz, CDCl3): \( \delta = 5.40 \) (dd, \( J = 9.9 \) Hz, \( J = 9.6 \) Hz, 1 H, H-2), 4.70 (ABq, \( J = 10.8 \) Hz, 2 H, CH2Ph), 4.40 (d, \( J = 9.9 \) Hz, 1 H, H-3), 3.71 (t, \( J = 9.1 \) Hz, 1 H, H-3), 3.40–3.25 (m, 2 H, H-4 and H-5), 2.16 (s, 3 H, SCH3), 1.41 (d, \( J = 6.0 \) Hz, 3 H, H-6).

13C NMR (150 MHz, CDCl3): \( \delta = 82.9 \) (C-1), 82.6 (C-3), 75.4 (CH2Ph), 75.3 (C-5), 71.9 (C-2), 67.9 (C-4), 18.8 (C-6) (SCH3).


HRMS (TOF): \( m/z = [M + Na]^+ \) calculated for C35H43N3O7S + Na: 436.1307; found: 436.1299.


Compound 7 was eluted out in the next fraction.

Yield: 20.46 g (89%); colorless oil; \( [\alpha]_D^{25} = -11.95 \) (c 1.2, CHCl3).

1H NMR (300 MHz, CDCl3): \( \delta = 5.40 \) (dd, \( J = 9.9 \) Hz, \( J = 9.6 \) Hz, 1 H, H-1), 5.05 (d, \( J = 1.1 \) Hz, 1 H, H-1), 4.84 (d, \( J = 8.3 \) Hz, 1 H, H-1), 4.79–4.43 (m, 6 H, CH2Ph), 4.01 (dd, \( J = 9.3 \) Hz, \( J = 3.0 \) Hz, 1 H, H-4), 3.94 (dd, \( J = 3.0 \) Hz, 1 H, H-2), 3.90 (m, 1 H, H-5), 3.64 (t, \( J = 9.3 \) Hz, 1 H, H-3), 3.48 (t, \( J = 9.6 \) Hz, 1 H, H-3), 3.30 (m, 1 H, H-5), 3.25 (t, \( J = 9.8 \) Hz, 1 H, H-4), 1.32 (d, \( J = 5.7 \) Hz, 3 H, H-6), 1.16 (t, \( J = 4.3 \) Hz, 3 H, H-6).

13C NMR (75 MHz, CDCl3): \( \delta = 101.7 \) (C-1), 84.4 (C-1), 81.5 (C-3), 80.5 (C-5), 80.0 (C-4), 75.7 (C-2), 75.3 (CH2Ph), 74.9 (CH2Ph), 74.2 (C-2), 73.3 (CH2Ph), 70.9 (C-5), 68.6 (C-5), 68.0 (C-4), 18.5 (C-6), 17.9 (C-6), 13.7 (SCH3).

MS (TOF): \( m/z = 757.3 \) [M + Na]+. 762.2 [M + Na]+.


Methyl 3-O-(4-Azido-3-oxymethyl-4,6-dideoxy-ß-D-glucopyranosyl)-2,4-di-O-benzyl-1-thio-ß-D-rhamnopyranoside (8)

Methanolic 1 M NaOMe was added to a solution of 7 (18 g, 24.35 mmol) in MeOH (400 mL) and CH2Cl2 (10 mL) until the solution became strongly basic to litmus. The mixture was stirred overnight at 50 °C, when TLC (4:1 hexane–EtOAc) showed that the reaction was complete. After neutralization with Amberlite IR-120 (H+), filtration, and concentration of the filtrate, chromatography gave 8; yield: 14.5 g (94%); colorless oil.

HRMS (TOF): \( m/z = 757.3 \) [M + Na]+. 762.2 [M + Na]+.

HRMS (TOF): \( m/z = [M + Na]^+ \) calculated for C41H45N3O8S + Na: 762.2563; found: 762.2559.

Methyl 3-O-(4-Azido-3-oxymethyl-4,6-dideoxy-ß-D-glucopyranosyl)-2,4-di-O-benzyl-1-thio-ß-D-rhamnopyranoside (9)

Powdered KOH (750 mg, 12.5 mmol) was added with stirring to a solution of 8 (1.6 g, 2.52 mmol) in DMSO (10 mL), followed by Mel (0.8 mL, 12.5 mmol). The mixture was stirred at r.t. under N2 for 1 h, when TLC (61:hexane–EtOAc) showed that the reaction was complete. EtOAc (100 mL) was added and, after washing with brine (3 x 30 mL), the organic phase was dried (Na2SO4) and concentrated. The residue was chromatographed to give 9; yield: 1.36 g (83%); mp 67–69 °C (EtOH); \( [\alpha]_D^{25} = -37.64 \) (c 1.4, CHCl3).

HRMS (TOF): \( m/z = 673.2 \) [M + Na]+. 672.2 [M + Na]+.

HRMS (TOF): \( m/z = [M + Na]^+ \) calculated for C35H43N3O7S + Na: 672.2710; found: 672.2693.

Anal. Calcd for C35H43N3O7S: C, 64.69; H, 6.67. Found: C, 64.33; H, 6.75.

Methyl 3-O-(4-Amino-3-O-benzyl-4,6-dideoxy-2-ß-methyl-ß-D-glucopyranosyl)-2,4-di-O-benzyl-1-thio-ß-D-rhamnopyranoside (10)

H2O (30 mL) was added to a solution of 9 (6.8 g, 10.48 mmol) in pyridine (50 mL) until slight turbidity, followed by a few drops of pyridine until a clear solution was formed. A slow stream of H2S gas was passed through the solution for 1.5 h, and the mixture was stirred overnight at r.t., when TLC (2.1:0.1 hexane–EtOAc–25% NH4OH) showed that the conversion was complete. After concentration, the residue was chromatographed to afford 10; yield: 5.4 g (83%); colorless oil.

HRMS (TOF): \( m/z = 103.1 \) [C11H9O5N + Na]+. 102.1 [C11H9O5N]+.

HRMS (TOF): \( m/z = [M + Na]^+ \) calculated for C35H43N3O7S + Na: 102.1307; found: 102.1299.

Anal. Calcd for C35H43N3O7S: C, 64.69; H, 6.67. Found: C, 64.33; H, 6.75.
H, H-5I), 3.98 (m, 1 H, H-2 I), 3.96 (m, 1 H, H-2 I), 3.39 (dd, 2,3 = 9.0 Hz, 1 H, H-2II), 2.60 (ABq, 2,3 = 9.1 Hz, 1 H, H-2II), 2.20 (ABq, 2,3 = 9.0 Hz, 1 H, H-2II), 2.06 (s, 3 H, SCH2), 1.36 (d, J = 6.3 Hz, 3 H, H-6I), 1.23 (s, 3 H, CH3), 1.20 (s, 3 H, CH3), 1.16 (d, J = 5.6 Hz, 3 H, H-6II).

13C NMR (150 MHz, CDCl3): δ = 171.0 (C=O), 169.3 (C=O), 103.8 (C-1'), 84.5 (C-2'), 84.3 (C-1'), 80.7 (C-4'), 80.7 (C-3'), 80.4 (C-3'), 80.0 (C-2'), 79.2 (C-3'), 74.8 (CH2Ph), 73.4 (CH2Ph), 73.2 (CH2Ph), 71.0 (C-5'), 68.3 (C-5'), 60.6 (OCH3), 55.7 (C-4'), 47.2 (C-2'), 26.6 and 26.5 ([C(CH3)]2, 22.4 (CH2CO), 18.0 (C-6'), 17.9 (C-6'), 13.5 (SCH3).

MS (TOF): m/z = 783.3 [M + Na]+, 788.3 [M + Na]+.

HRMS (TOF): m/z [M + Na]+ calculated for C24H30N2O5S: 783.3890; found: 783.3878.

5-Methoxybenzylpropyl 4-(3-O-Acetyl-3-methylbutanamidido)-3-0-benzyl-4,6-dideoxy-2-0-methyl-β-D-glucopyranosyl-(1→2)-3,4-di-O-benzyl-a-L-rhamnopyranosyl-(1→3)-4,3-di-o-benzyl-a-L-rhamnopyranoside (13)

A mixture of 4 (4.41 g, 5.53 mmol), 12 (4.23 g, 5.53 mmol), and 4 Å MS (3.0 g) in CH2Cl2 (200 mL) was stirred under N2 at r.t. for 30 min, cooled to −50 °C, and NIS (1.86 g, 8.30 mmol) followed by AgOTf (991 mg, 3.87 mmol) was added. The stirring was continued at the same temperature for 6 h, and then overnight at r.t. When TLC (8:1 toluene–acetone) showed that the reaction was complete, Et2N (2.5 mL) was added to quench the reaction and, after filtration through a Celite pad, the filtrate was washed successively with 10% Na2SO4 (2 × 50 mL) and brine (150 mL). Concentration of the organic phase and chromatography of the residue gave 13; yield: 5.96 g (71%); colorless oil; [α]D = −21.60 (c 1.19, CHCl3).

1H NMR (600 MHz, CDCl3): δ = 5.15 (d, J = 9.1 Hz, 1 H, NH), 5.09 (br s, 1 H, H-1I), 5.07 (d, J = 1.8 Hz, 1 H, H-1I), 5.00–4.42 (m, 16 A 16 H, CH2 and CH3), 4.12 (t, J = 3.0 Hz, 1 H, H-3I), 3.95 (d, J = 2.0 Hz, 1 H, H-2I), 3.90 (dd, J = 1.8 Hz, J = 3.0 Hz, 1 H, H-1I), 3.82–3.75 (m, 4 H, H-2 II, H-3 II, H-5 II, H-5 III), 3.66–3.58 (m, 10 H, H-1 a, H-5 I, H-4 III, H-4 IV, H-5 IV), 2.55 (ABp, J = 13.7 Hz, 2 H, H-2I), 2.30 (t, J = 7.5 Hz, 1 H, H-5), 1.86 (s, 3 H, CH3CO), 1.63 (m, 2 H, H-1I), 1.54 (m, 2 H, H-2I), 1.49 and 1.48 [2 s (CH2)], 1.34 (m, 2 H, H-3I), 1.28 (d, J = 5.4 Hz, 3 H, 2 × 3 H, H-6I), 1.26 (d, J = 6.2 Hz, 3 H, H-6I), 1.23 (d, J = 6.3 Hz, 3 H, H-6II), 1.02 (d, J = 6.2 Hz, 3 H, H-6II).

13C NMR (150 MHz, CDCl3): δ = 103.7 (C-1'), 100.4 (C-1'), 98.9 (C-1'), 98.8 (C-1'), 84.5 (C-2'), 80.8 (C-4'), 80.6 (C-4'), 80.5 (C-4'), 80.4 (C-3'), 80.3 (C-4'), 80.0 (C-3'), 79.2 (C-2'), 78.7 (C-3'), 78.0 (2 × C-2, C-2'), 75.4 (CH2Ph), 74.8 (2 × C-2, C-2' and CH2Ph), 74.5 (CH2Ph), 73.4 (CH2Ph), 73.2 (CH2Ph), 72.0 (CH2Ph), 71.9 (OCH3), 68.5 (C-2), 67.5 (C-3, OCH3), 67.4 (C-2), 67.1 (C-1), 60.5 (OCH3), 55.6 (C-4'), 51.4 (CO2CH3), 47.2 (C-2'), 33.9 (C-5), 29.1 (C-2'), 26.6 and 26.5 ([CD(3)], 25.7 (C-3), 24.7 (C-4), 22.4 (CH2CO), 17.9 (4 × C, C-6-'), C-6', C-6''-).

MS (TOF): m/z = 1533.6 [M + Na]+*, 1538.6 [M + Na]+.

Anal. Calcd for C58H100O28S: C, 69.68; H, 7.24; N, 0.92. Found: C, 69.42; H, 7.38; N, 1.07.

5-Methoxybenzylpropyl 4-(3-O-Acetyl-3-methylbutanamidido)-4,6-dideoxy-2-0-methyl-β-D-glucopyranosyl-(1→3)-3,4-di-O-benzyl-a-L-rhamnopyranosyl-(1→2)-a-L-rhamnopyranoside (14)

A mixture of compound 13 (7.38 g, 4.91 mmol) and 5% Pd/C catalyst (4.0 g) in 5 MeOH–EtOAc (300 mL) was stirred in a H2 atmosphere at r.t. for 24 h. TLC (6:4:1 CH2Cl2–acetone–MeOH) showed that the reaction was complete. After filtration through a Celite pad and concentration of the filtrate, the residue was chromatographed to give 14; yield: 3.62 g (85%); colorless oil.

1H NMR (600 MHz, CD3OD): δ = 5.06 (d, J = 1.6 Hz, 1 H, H-1I), 4.91 (d, J = 1.7 Hz, 1 H, H-1I), 4.78 (d, J = 1.5 Hz, 1 H, H-1'), 4.62 (d, J = 1.8 Hz, 1 H, H-1'), 4.58 (d, J = 1.7 Hz, 1 H, H-1'), 4.51 (d, J = 1.6 Hz, 1 H, H-1'), 4.48 (d, J = 1.6 Hz, 1 H, H-1').
$J_{1,2} = 3.2$ Hz, $H - 2\text{II}$), 4.06 (dd, $J_{1,2} = 2.0$ Hz, $J_{1,2} = 3.1$ Hz, 1 H, $H - 2\text{III}$), 3.90 (dd, $J_{1,2} = 3.3$ Hz, $J_{1,2} = 3.8$ Hz, 1 H, $H - 3\text{II}$), 3.83 (ddd, $J = 4.8$, 7.9, 12.5 Hz, 1 H, $H - 3\text{II}$), 3.80–3.77 (m, 2 H, $H - 3\text{III}$, $H - 2\text{IV}$), 3.74 (m, 2 H, $H - 5\text{III}$, $H - 3\text{IV}$), 3.69–3.64 (m, 7 H, $H - 1\text{a}$, 2 $\times$ OCH$_3$), 3.61–3.48 (m, 4 H, $H - 4\text{II}$, $H - 4\text{III}$, $H - 4\text{IV}$), 3.44–3.34 (m, 4 H, $H - 3\text{IV}$, $H - 5\text{I}$, $H - 4$), and 1 H), 3.01 (dd, $J_{1,2} = 8.0$ Hz, $J_{1,2} = 8.9$ Hz, 1 H, $H - 2\text{III}$), 2.72 (AB$_{p} q J = 13.5$ Hz, 2 H, $H - 2\text{II}$), 2.34 (t, $J = 7.4$ Hz, 2 H, $H - 5\text{I}$), 1.97 (s, 3 H, COCH$_3$), 1.70–1.56 (m, 4 H, $H - 2$ and $H - 4$), 1.55 (s, 6 H, 2 $\times$ CH$_3$), 1.46–1.37 (m, 2 H, $H - 3$), 1.29 (d, $J = 6.2$ Hz, 3 H, $H - 6\text{II}$), 1.25 (dd, $J = 5.2$ Hz, 6.0 Hz, 6 H, $H - 6\text{III}$, $H - 6\text{IV}$), 1.20 (d, $J_{3,4} = 6.2$ Hz, 3 H, $H - 6\text{V}$).

$^{13}$C NMR (150 MHz, CD$_2$OD): $\delta = 175.8$ (C-1), 172.4 (2 $\times$ C-2, 2 $\times$ C-3), 105.4 (C-1$\text{I}$), 103.9 (C-1$\text{II}$), 103.5 (C-1$\text{III}$), 100.3 (C-1$\text{IV}$), 85.7 (C-2$\text{III}$), 81.7 (C-3$\text{III}$), 81.5 (C-3$\text{IV}$), 80.1 (C-2$\text{IV}$), 79.0 (C-2$\text{III}$), 74.8 (C-3$\text{IV}$), 74.3 (C-4), 73.2 (C-4$\text{II}$), 73.0 (C-4$\text{III}$), 72.2 (C-5$\text{III}$), 72.1 (C-3$\text{II}$), 71.8 (C-2$\text{II}$), 71.7 (C-2$\text{IV}$), 70.5 (C-5$\text{IV}$), 70.1 (C-5$\text{III}$), 69.9 (C-5$\text{II}$), 68.3 (C-1), 61.2 (OCH$_3$), 58.0 (C-4$\text{II}$), 52.0 (CO$_2$CH$_3$), 47.5 (C-2$\text{I}$), 34.7 (C-5), 30.2 (C-2), 27.0 (C-3), 26.8 ($2 \times C$, (C$_3$)$_2$CH$_2$), 25.7 (C-4), 22.4 (CH$_2$CO), 18.4 (C-6$\text{IV}$), 18.1 and 17.9 ($3 \times C$, C-6$\text{II}$, C-6$\text{II}$, C-6$\text{IV}$).

MS (TOF): $m/z$ = 886.0 [M + H]$^+$, 903.4 [M + NH$_4$$]^+$, 908.3 [M + Na$]^+$. HRMS (TOF): $m/z$ [M + H]$^+$ calc for C$_{38}$H$_{70}$N$_3$O$_{19}$: 872.4604; found: 872.4606.

Supporting Information for this article is available online at http://www.thieme-connect.com/ejournals/toc/synthesis.

Acknowledgment

This research was supported by the Intramural Research Program of the NIH, NIDDK.

References