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

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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.

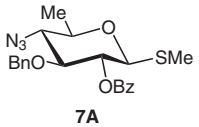
Key words: carbohydrates, oligosaccharides, glycosylations, conjugation, amines

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.[2] We have previously described the syntheses of the α and β 5-methoxycarbonylpentyl glycosides of the tetrasaccharide[2,3] as well as of all structural fragments[2] of the two glycosides. A shorter (2+2)[4] and a (3+1)[5] synthesis, as well as an asymmetric synthesis[6] of the tetrasaccharide 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[8] or the later-developed shorter approaches.[4–6,9] The availability of an anthrose precursor[7] 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[4] of the tetrasaccharide sequence described here. There,[4] the glycosyl donor for the upstream[10] 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,[11] required further chemical manipulation with the pentenyl glycoside of the tetrasaccharide. The synthesis presented here (Schemes 1– 3) uses donor **6**, which can be made[7] from inexpensive methyl α- or β-galactopyranoside. It is more economical and makes tetrasaccharide **15** more readily available, as it uses a building block **12** containing the fully assembled anthrose. In addition, the final, one-pot deprotection effects deacetylation in the *N*-acyl 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.[12–14]

The spacer-equipped tetrasaccharide **13** was built up from disaccharides **4** and **12**, which were synthesized from the common intermediate **1**.[15] Thus, coupling of thioglycoside **1** with the linker-equipped rhamnoside **2**[2] gave disaccharide **3**, which was deacetylated to afford the disaccharide glycosyl acceptor **4** (Scheme 1).

To obtain glycosyl donor **12**, alcohol **5** was prepared from **1** as described[15] and treated with the known[7] imidate **6** in the presence of trimethylsilyl trifluoromethanesulfonate (TMSOTf), to give disaccharide **7** (89%) and a small amount of by-product **7A** (3%) (Figure 1), a product of aglycone transfer.[16]



7A

Figure 1 Molecular structure of the by-product **7A**

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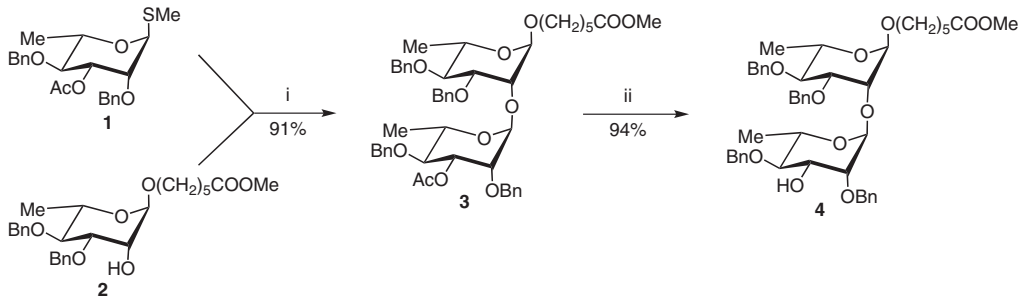
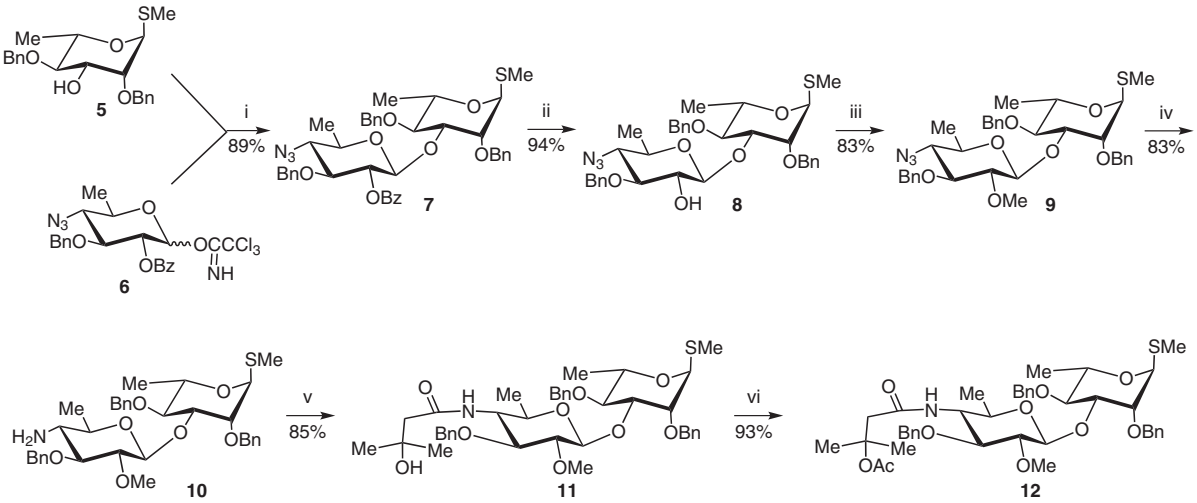
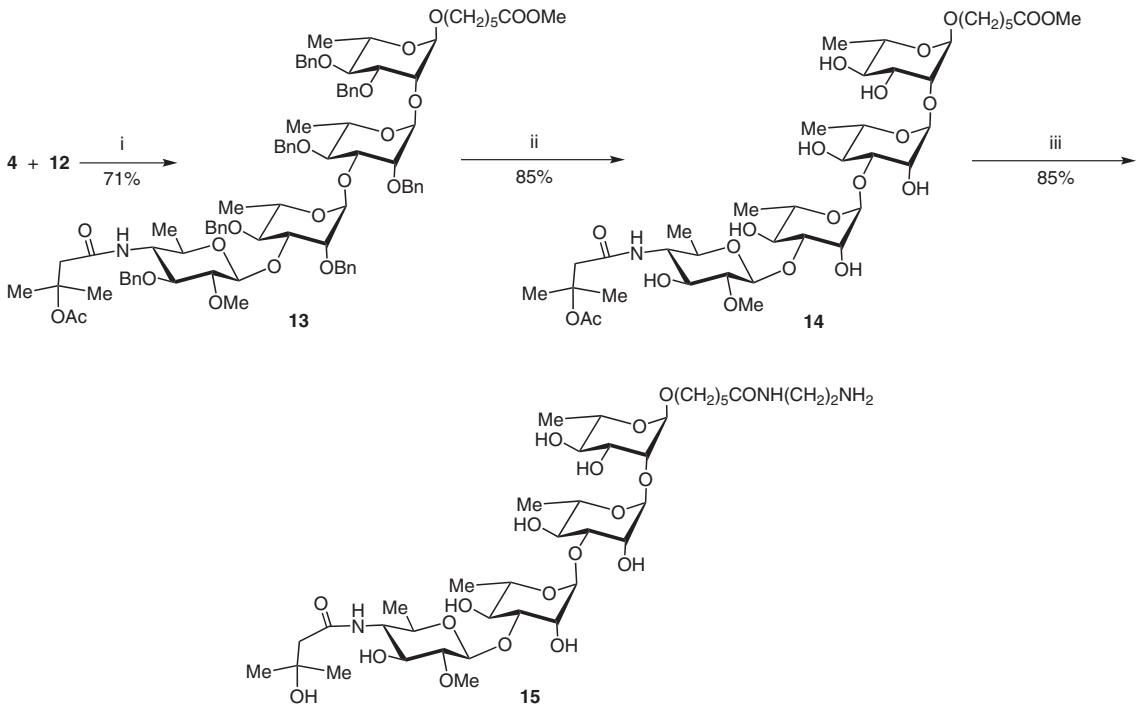


Scheme 1 Synthesis of disaccharide **4**. *Reagents and conditions*: (i) NIS, AgOTf, 4 Å MS, CH_2Cl_2; (ii) NaOMe, MeOH.



Scheme 2 Synthesis of glycosyl donor **12**. *Reagents and conditions*: (i) 4 Å MS, TMSOTf, CH_2Cl_2, –78 °C; (ii) NaOMe, MeOH–CH_2Cl_2, 50 °C; (iii) KOH, MeI, DMSO; (iv) H_2S, H_2O–pyridine; (v) HATU, Hünig's base, 3-hydroxy-3-methylbutanoic acid, CH_2Cl_2; (vi) Ac_2O, DMAP, CH_2Cl_2.



Scheme 3 Synthesis of tetrasaccharide **15**. *Reagents and conditions*: (i) 4 Å MS, NIS, AgOTf, CH_2Cl_2, –50 °C; (ii) H_2, Pd/C, MeOH–EtOAc; (iii) $H_2N(CH_2)_2NH_2$, 50 °C overnight, then NaOMe, MeOH.

When the reaction was promoted by $BF_3{\cdot}OEt_2$, a much higher proportion of **7A** was formed (this conversion is not described in the experimental section). Compound **7** was functionalized, namely debenzoylated (Zemplén, → **8**), methylated[17] (→ **9**), treated successively with H_2S (→ **10**) and 3-hydroxy-3-methylbutanoic acid in the presence of HATU {*N*-[(dimethylamino)-1*H*-1,2,3-triazolo-[4,5-*b*]pyridin-1-ylmethylene]-*N*-methylmethanaminium hexafluorophosphate *N*-oxide} (→ **11**). The product was acetylated to give the disaccharide glycosyl donor **12** having the fully assembled anthrose 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** by hydrogenolysis in the presence of Pd/C (→ **14**) was uneventful. Subsequent, overnight treatment with ethylenediamine at 50 °C effected complete conversion of the methyl ester group in **14** to give the corresponding amino amide, but the desired, concomitant deacetylation to form **15** was incomplete (TLC, NMR). The latter reaction could be readily completed under Zemplén[18,19] conditions. In this way, the two-step conversion **14** → **15** could be accomplished as a one-pot process. The isolated product **15**, obtained in 85% yield over two steps, was in all respects identical with previously described substance.[2]

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 (^{1}H) and 75 MHz (^{13}C) with a Varian Gemini or Varian Mercury spectrometers, or at 600 MHz (^{1}H) and 150 MHz (^{13}C) with a Bruker Avance 600 spectrometer. Assignments of NMR signals were made by hmonuclear 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*-butanamido 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 Na_2SO_4, and concentrated at 40 °C/2 kPa.

5-Methoxycarbonylpentyl 2-*O*-(3-*O*-Acetyl-2,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-3,4-di-*O*-benzyl-α-L-rhamnopyranoside (3)

A mixture of **1**[15] (10.81 g, 26.0 mmol), **2**[2] (10.10 g, 21.4 mmol), and 4 Å MS (4.20 g) in CH_2Cl_2 (210 mL) was stirred under N_2 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 $Na_2S_2O_3$ (30 mL), the mixture was shaken and, after the organic phase had been drained, the aqueous solution was extracted with CH_2Cl_2 (3 × 3 mL). The combined organic phase was dried, concentrated, and the residue was chromatographed (hexane–acetone, 6:1) to afford **3**; yield: 16.08 g (91%); colorless oil; $[\alpha]_D$ +5.65 (*c* 1.7, $CHCl_3$).

^{1}H NMR (600 MHz, $CDCl_3$): δ = 5.23 (dd, $J_{2,3}$ = 3.4 Hz, $J_{3,4}$ = 9.4 Hz, 1 H, H-3^{II}), 5.08 (d, $J_{1,2}$ = 2.0 Hz, 1 H, H-1^{II}), 4.88 (d, *J* = 10.8 Hz, 1 H, C*H*$_2$Ph), 4.70 (dd, *J* = 3.4, 11.2 Hz, 2 H, C*H*$_2$Ph), 4.66 (d, $J_{1,2}$ = 1.8 Hz, 1 H, H-1^{I}), 4.64–4.61 (m, 3 H, C*H*$_2$Ph), 4.43–4.21 (ABq, *J* = 12.3 Hz, 2 H, C*H*$_2$Ph), 4.02 (t, *J* = 2.0 Hz, 1 H, H-1^{I}), 3.93 (dd, $J_{2,3}$ = 3.4 Hz, $J_{1,2}$ = 2.1 Hz, 1 H, H-2^{II}), 3.87 (m, 1 H, H-5^{II}), 3.84 (dd, $J_{2,3}$ = 4.0 Hz, $J_{3,4}$ = 9.4 Hz, 1 H, H-3^{I}), 3.65 (s, 3 H, OCH_3), 3.64–3.57 (m, 3 H, H-5^{I}, H-4^{II}, H-1$_a$), 3.47 (t, *J* = 9.5 Hz, 1 H, H-4^{I}), 3.36–3.32 (m, 1 H, H-1$_b$), 2.31 (t, *J* = 7.5 Hz, 2 H, H-5), 1.93 (s, 3 H, $COCH_3$), 1.64 (m, 2 H, H-4), 1.54 (m, 2 H, H-2), 1.37 (m, 2 H, H-3), 1.35 (d, $J_{5,6}$ = 3.1 Hz, H-6^{II}), 1.34 (d, $J_{5,6}$ = 3.0 Hz, H-6^{I}).

^{13}C NMR (150 MHz, $CDCl_3$): δ = 174.0 (C=O), 170.0 (C=O), 99.1 (C-1^{II}, $J_{C\text{-}H}$ = 171 Hz), 98.8 (C-1^{I}, $J_{C\text{-}H}$ = 169 Hz), 80.4 (C-4^{I}), 80.2 (C-3^{I}), 79.1 (C-4^{II}), 75.8 (C-2^{II}), 75.4 (*C*H$_2$Ph), 74.9 (C-2^{I}), 74.8 (*C*H$_2$Ph), 73.3 (C-3^{II}), 72.7 (*C*H$_2$Ph), 72.3 (*C*H$_2$Ph), 68.0 (2 × C, C-5^{I}, C-5^{II}), 67.1 (C-1), 51.4 (OCH_3), 33.9 (C-5), 29.1 (C-2), 25.7 (C-3), 24.7 (C-4), 21.0 (CO*C*H$_3$), 18.0 (C-6^{I}), 17.8 (C-6^{II}).

MS (TOF): *m*/*z* = 858.4 $[M + NH_4]^+$, 863.3 $[M + Na]^+$.

HRMS (TOF): *m*/*z* $[M + Na]^+$ calcd for $C_{49}H_{60}O_{12}$ + Na: 863.3982; found: 863.3996.

Anal. Calcd for $C_{49}H_{60}O_{12}$: C, 69.98; H, 7.19. Found: C, 69.85; H, 7.22.

5-Methoxycarbonylpentyl 2-*O*-(2,4-Di-*O*-benzyl-α-L-rhamnopyranosyl)-3,4-di-*O*-benzyl-α-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; $[\alpha]_D$ –8.76 (*c* 1.2, $CHCl_3$).

^{1}H NMR (600 MHz, $CDCl_3$): δ = 5.15 (br s, 1 H, H-1^{II}), 4.89 (dd, *J* = 2.9, 10.7 Hz, 2 H, C*H*$_2$Ph), 4.72–4.61 (m, 5 H, C*H*$_2$Ph, H-1^{I}), 4.46–4.23 (AB$_q$, *J* = 12.0 Hz, 2 H, C*H*$_2$Ph), 4.02 (br s, 1 H, H-2^{II}), 3.98 (ddd, *J* = 3.8, 9.3 Hz, H-3^{II}), 3.84 (dd, *J* = 2.9, 9.4 Hz, 1 H, H-3^{I}), 3.78–3.74 (m, 2 H, H-2^{II} and H-5^{II}), 3.65 (s, 3 H, OCH_3), 3.63–3.58 (m, 2 H, H-5^{I} and H-1$_a$), 3.40 (t, *J* = 9.4 Hz, 1 H, H-4^{I}), 3.34 (m, 1 H, H-1$_b$), 3.28 (t, *J* = 9.3 Hz, 1 H, H-4^{II}), 2.31 (t, *J* = 7.5 Hz, 2 H, H-5), 2.26 (d, *J* = 9.4 Hz, OH-3^{II}), 1,63 (m, 2 H, H-4), 1.54 (m, 2 H, H-2), 1.37 (m, 2 H, H-3), 1.32 (d, $J_{5,6}$ = 6.2 Hz, 3 H, H-6^{II}), 1.25 (d, $J_{5,6}$ = 6.3 Hz, 3 H, H-6^{I}).

^{13}C NMR (150 MHz, $CDCl_3$): δ = 174.0 (C=O), 98.8 (C-1^{I}), 98.2 (C-1^{II}), 82.2 (C-4^{II}), 80.5 (C-4^{I}), 80.1 (C-3^{I}), 78.1 (C-2^{II}), 75.4

(*C*H$_2$Ph), 74.9 (*C*H$_2$Ph), 74.4 (C-2^I), 72.6 (*C*H$_2$Ph), 72.2 (*C*H$_2$Ph), 71.2 (C-3^{II}), 67.8 (C-5^I), 67.5 (C-5^{II}), 67.1 (C-1), 51.4 (OCH$_3$), 33.9 (C-5), 29.1 (C-2), 25.7 (C-3), 24.7 (C-4), 18.0 (C-6^I), 17.9 (C-6^{II}).

MS (TOF): *m*/*z* = 816.4 [M + NH$_4$]$^+$, 821.3 [M + Na]$^+$.

HRMS (TOF): *m*/*z* [M + Na]$^+$ calcd for $C_{47}H_{58}O_{11}$ + Na: 821.3877; found: 821.3860.

Anal. Calcd for $C_{47}H_{58}O_{11}$: C, 70.65; H, 7.32. Found: C, 70.45; H, 7.25.

Methyl 3-*O*-(4-Azido-2-*O*-benzoyl-3-*O*-benzyl-4,6-dideoxy-β-D-glucopyranosyl)-2,4-di-*O*-benzyl-1-thio-α-L-rhamnopyranoside (7) and Methyl 4-Azido-2-*O*-benzoyl-3-*O*-benzyl-4,6-dideoxy-1-thio-β-D-glucopyranoside (7A)

A mixture of methyl 2,4-di-*O*-benzyl-1-thio-α-L-rhamnopyranoside (**5**;[15] 12.83 g, 34.3 mmol) and 4 Å MS (12.3 g) in CH_2Cl_2 (420 mL) was stirred under N_2 at r.t. for 15 min. The mixture was cooled to –78 °C and a solution of TMSOTf (283 μL, 1.56 mmol) in CH_2Cl_2 (3 mL) was added, followed by a solution of 4-azido-2-*O*-benzoyl-3-*O*-benzyl-4,6-dideoxy-α-D-glucopyranose 1-*O*-trichloroacetimidate (**6**;[7] 16.38 g, 31.08 mmol) in CH_2Cl_2 (10 mL). The stirring at the same temperature was continued for 1 h, when TLC (7:1 hexane–EtOAc) showed that the imidate was consumed. Et_3N (1.0 mL) was added to quench the reaction, the mixture was filtered through a Celite pad, the filtrate was concentrated, and chromatography gave aglycone transfer **7A** in the first fraction; yield: 400 mg (3%); colorless oil; [α]$_D$ +115.6 (*c* 1.1, $CHCl_3$).

^{1}H NMR (600 MHz, $CDCl_3$): δ = 5.31 (dd, $J_{1,2}$ = 9.9 Hz, $J_{2,3}$ = 9.4 Hz, 1 H, H-2), 4.70 (AB$_q$, *J* = 10.8 Hz, 2 H, C*H*$_2$Ph), 4.40 (d, $J_{1,2}$ = 9.9 Hz, 1 H, H-1), 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, SCH_3), 1.41 (d, $J_{5,6}$ = 6.0 Hz, 3 H, H-6).

^{13}C NMR (150 MHz, $CDCl_3$): δ = 82.9 (C-1), 82.6 (C-3), 75.4 (*C*H$_2$Ph), 75.3 (C-5), 71.9 (C-2), 67.9 (C-4), 18.8 (C-6), 11.4 (SCH_3).

MS (TOF): *m*/*z* = 431.1 [M + NH$_4$]$^+$, 436.1 [M + Na]$^+$.

HRMS (TOF): *m*/*z* [M + Na]$^+$ calcd for $C_{21}H_{23}N_3O_4S$ + Na: 436.1307; found: 436.1299.

Anal. Calcd for $C_{21}H_{23}N_3O_4S$: C, 61.00; H, 5.61. Found: C, 61.17; H, 5.63.

Compound **7** was eluted out in the next fraction.

Yield: 20.46 g (89%); colorless oil; [α]$_D$ –11.95 (*c* 1.2, $CHCl_3$).

^{1}H NMR (300 MHz, $CDCl_3$): δ = 5.40 (dd, $J_{1,2}$ = 7.9 Hz, $J_{2,3}$ = 9.6 Hz, 1 H, H-2^{II}), 5.05 (d, $J_{1,2}$ = 1.1 Hz, 1 H, H-1^I), 4.84 (d, *J* = 8.3 Hz, 1 H, H-1^{II}), 4.79–4.23 (m, 6 H, C*H*$_2$Ph), 4.01 (dd, $J_{3,4}$ = 9.3 Hz, $J_{4,5}$ = 3.0 Hz, 1 H, H-4^I), 3.94 (dd, $J_{2,3}$ = 3.0 Hz, 1 H, H-2^I), 3.90 (m, 1 H, H-5^I), 3.64 (t, *J* = 9.3 Hz, 1 H, H-3^{II}), 3.48 (t, *J* = 9.6 Hz, 1 H, H-3^I), 3.30 (m, 1 H, H-5^{II}), 3.25 (t, *J* = 9.8 Hz, 1 H, H-4^{II}), 1.32 (d, $J_{5,6}$ = 5.7 Hz, 3 H, H-6^{II}), 1.16 (d, $J_{5,6}$ = 4.3 Hz, 3 H, H-6^I).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 101.7 (C-1^{II}), 84.4 (C-1^I), 81.5 (C-3^{II}), 80.5 (C-3^I), 80.0 (C-4^I), 79.7 (C-2^I), 75.3 (*C*H$_2$Ph), 74.9 (*C*H$_2$Ph), 74.2 (C-2^{II}), 73.3 (*C*H$_2$Ph), 70.9 (C-5^{II}), 68.6 (C-5^I), 68.0 (C-4^{II}), 18.5 (C-6^{II}), 17.9 (C-6^I), 13.7 (SCH_3).

MS (TOF): *m*/*z* = 757.3 [M + NH$_4$]$^+$, 762.2 [M + Na]$^+$.

HRMS (TOF): *m*/*z* [M + Na]$^+$ calcd for $C_{41}H_{45}N_3O_8S$ + Na: 762.2825; found: 762.2841.

Anal. Calcd for $C_{41}H_{45}N_3O_8S$: C, 66.56; H, 6.13. Found: C, 66.39; H, 6.18.

Methyl 3-*O*-(4-Azido-3-*O*-benzyl-4,6-dideoxy-β-D-glucopyranosyl)-2,4-di-*O*-benzyl-1-thio-α-L-rhamnopyranoside (8)

Methanolic 1 M NaOMe was added to a solution of **7** (18 g, 24.35 mmol) in MeOH (400 mL) and CH_2Cl_2 (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.

^{1}H NMR (300 MHz, $CDCl_3$): δ = 5.03 (d, $J_{1,2}$ = 1.3 Hz, 1 H, H-1^I), 4.92–4.60 (m, 6 H, C*H*$_2$Ph), 4.43 (d, $J_{1,2}$ = 7.7 Hz, 1 H, H-1^{II}), 4.01–3.95 (m, 2 H, H-5^I and H-3^I), 3.92 (dd, $J_{1,2}$ = 1.3 Hz, $J_{2,3}$ = 3.0 Hz, 1 H, H-2^I), 3.65 (t, *J* = 9.4 Hz, 1 H, H-4^I), 3.55 (ddd, $J_{2,OH}$ = 2.2 Hz, 1 H, H-2^{II}), 3.38 (t, *J* = 9.0 Hz, 1 H, H-3^{II}), 3.21 (m, 1 H, H-5^{II}), 3.10 (t, *J* = 9.4 Hz, 1 H, H-4^{II}), 2.54 (d, *J* = 2.2 Hz, 1 H, 2^{II}-OH), 2.05 (s, 3 H, SCH_3), 1.35 (d, $J_{5,6}$ = 6.3 Hz, 3 H, H-6^I), 1.29 (d, $J_{5,6}$ = 6.1 Hz, 3 H, H-6^{II}).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 104.0 (C-1^{II}), 84.0 (C-1^I), 82.5 (C-3^{II}), 80.9 (C-4^I), 80.6 (C-3^I), 79.5 (C-2^I), 75.7 (C-2^{II}), 75.6 (*C*H$_2$Ph), 75.1 (*C*H$_2$Ph), 73.1 (*C*H$_2$Ph), 70.9 (C-5^{II}), 68.7 (C-5^I), 67.4 (C-4^{II}), 18.7 (C-6^{II}), 18.2 (C-6^I), 13.9 (SCH_3).

MS (TOF): *m*/*z* = 653.3 [M + NH$_4$]$^+$, 658.2 [M + Na]$^+$.

HRMS (TOF): *m*/*z* [M + Na]$^+$ calcd for $C_{34}H_{41}N_3O_7S$ + Na: 658.2563; found: 658.2559.

Methyl 3-*O*-(4-Azido-3-*O*-benzyl-4,6-dideoxy-2-*O*-methyl-β-D-glucopyranosyl)-2,4-di-*O*-benzyl-1-thio-β-L-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 MeI (0.8 mL, 12.5 mmol). The mixture was stirred at r.t. under N_2 for 1 h, when TLC (6:1 hexane–EtOAc) showed that the reaction was complete. EtOAc (100 mL) was added and, after washing with brine (3 × 30 mL), the organic phase was dried (Na_2SO_4) and concentrated. The residue was chromatographed to give **9**; yield: 1.36 g (83%); mp 67–69 °C (EtOH); [α]$_D$ –37.64 (*c* 1.4, $CHCl_3$).

^{1}H NMR (300 MHz, $CDCl_3$): δ = 5.07 (d, $J_{1,2}$ = 1.1 Hz, 1 H, H-1^I), 4.98–4.70 (m, 5 H, C*H*$_2$Ph), 4.57 (m, 2 H, including d, $J_{1,2}$ = 8.0 Hz, H-1^{II} and C*H*$_2$Ph), 4.03–3.96 (m, 2 H, H-3^I and H-5^I), 3.92 (dd, $J_{2,3}$ = 3.3 Hz, 1 H, H-2^I), 3.68–3.6 (m, 4 H, including s, 3 H, OCH_3 and t, *J* = 7.7 Hz, H-4^I), 3.38 (t, *J* = 9.0 Hz, H-4^{II}), 3.20–3.04 (m, 3 H, H-2^{II}, H-3^{II}, and H-5^{II}), 2.06 (s, 3 H, SCH_3), 1.35 (d, $J_{5,6}$ = 6.4 Hz, 3 H, H-6^{II}), 1.25 (d, $J_{5,6}$ = 5.8 Hz, 3 H, H-6^I).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 103.1 (C-1^{II}), 84.9 (C-1^I), 84.3 (C-3^{II}), 82.9 (C-4^{II}), 81.0 (C-4^I), 80.3 (C-2^I), 79.3 (C-3^I), 75.6 (*C*H$_2$Ph), 75.1 (*C*H$_2$Ph), 73.3 (*C*H$_2$Ph), 70.1 (C-5^{II}), 68.6 (C-5^I), 67.8 (C-2^{II}), 60.9 (OCH_3), 18.6 (C-6^I), 18.1(C-6^{II}), 13.8 (SCH_3).

MS (TOF): *m*/*z* = 667.3 [M + NH$_4$]$^+$, 672.2 [M + Na]$^+$.

HRMS (TOF): *m*/*z* [M + Na]$^+$ calcd for $C_{35}H_{43}N_3O_7S$ + Na: 672.2710; found: 672.2693.

Anal. Calcd for $C_{35}H_{43}N_3O_7S$: 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-*O*-methyl-β-D-glucopyranosyl)-2,4-di-*O*-benzyl-1-thio-α-L-rhamnopyranoside (10)

H_2O (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 H_2S 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% NH_4OH) showed that the conversion was complete. After concentration, the residue was chromatographed to afford **10**; yield: 5.4 g (83%); colorless oil.

^{1}H NMR (600 MHz, $CDCl_3$): δ = 5.07 (d, $J_{1,2}$ = 1.6 Hz, 1 H, H-1^I), 5.01–4.58 (m, 7 H, including d, $J_{1,2}$ = 7.5 Hz at 4.64, H-1^{II} and C*H*$_2$Ph), 4.06 (dd, $J_{2,3}$ = 3.2 Hz, $J_{3,4}$ = 9.6 Hz, 1 H, H-3^I), 4.00 (m, 1

H, H-5^{I}), 3.96 (dd, $J_{1,2}$ = 1.6 Hz, $J_{2,3}$ = 3.2 Hz, 1 H, H-2^{I}), 3.66 (m, 4 H, OCH_3 and H-4^{I}), 3.18–3.12 (m, 3 H, H-2^{II}, H-3^{II}, and H-5^{II}), 2.50 (t, J = 9.3 Hz, 1 H, H-4^{II}), 2.06 (s, 3 H, SCH_3), 1.35(d, $J_{5,6}$ = 6.4 Hz, 3 H, H-6^{I}), 1.12 (d, $J_{5,6}$ = 5.8 Hz, 3 H, H-6^{II}).

^{13}C NMR (150 MHz, $CDCl_3$): δ = 103.9 (C-1^{II}), 85.2 (C-5^{II}), 84.6 (C-1^{I}), 84.7 (C-3^{II}), 80.8 (C-4^{I}), 80.1 (C-2^{I}), 78.7 (C-3^{I}), 75.0 (*C*H$_2$Ph), 74.8 (*C*H$_2$Ph), 73.0 (*C*H$_2$Ph), 72.5 (C-2^{II}), 68.3 (C-5^{I}), 60.4 (OCH_3), 58.1 (C-4^{II}), 18.0 (C-6^{II}), 17.9 (C-6^{I}), 13.5 (SCH_3).

MS (TOF): m/z = 624.3 [M + H]$^+$, 646.2 [M + Na]$^+$.

HRMS (TOF): m/z [M + H]$^+$ calcd for $C_{35}H_{46}NO_7S$: 624.2995; found: 624.3011.

Methyl 2,4-Di-*O*-benzyl-3-*O*-[3-*O*-benzyl-4,6-dideoxy-4-(3-hydroxy-3-methylbutanamido)-2-*O*-methyl-β-D-glucopyranosyl]-1-thio-α-L-rhamnopyranoside (11)

HATU (4.96 g, 13.1 mmol) followed by Hünig's base (1.68 g, 13.1 mmol) was added to a solution of **10** (5.4 g, 8.7 mmol) and 3-hydroxy-3-methylbutanoic acid (1.36 g, 13.1 mmol) in CH_2Cl_2 (100 mL). The mixture was stirred at r.t. for 1.5 h when TLC (1:1 hexane–EtOAc) showed that compound **10** was completely consumed. After partitioning between CH_2Cl_2 (200 mL) and a mixture of aq $NaHCO_3$ (50 mL) and brine (150 mL), the organic phase was dried, concentrated, and the residue was chromatographed to give **11**; yield: 5.28 g (85%); colorless oil; $[\alpha]_D$ –84.32 (*c* 2.3, $CHCl_3$).

^{1}H NMR (600 MHz, $CDCl_3$): δ = 5.42 (d, J = 9.0 Hz, 1 H, NH), 5.05 (d, $J_{1,2}$ = 1.2 Hz, 1 H, H-1^{I}), 5.00–4.58 (m, 7 H, including d, $J_{1,2}$ = 7.8 Hz at 4.60 H-1^{II} and C*H*$_2$Ph), 4.00 (dd, $J_{2,3}$ = 3.3 Hz, $J_{3,4}$ = 9.6 Hz, 1 H, H-3^{I}), 3.98 (m, 1 H, H-5^{I}), 3.96 (m, 1 H, H-2^{I}), 3.72–3.64 (m, 5 H, H-4^{I}, H-4^{II} and OCH_3), 3.36 (dd, $J_{2,3}$ = 9.1 Hz, $J_{3,4}$ = 10.2 Hz, 1 H, H-3^{II}), 3.30 (m, 1 H, H-5^{II}), 3.20 (dd, $J_{1,2}$ = 7.8 Hz, $J_{2,3}$ = 9.1 Hz, 1 H, H-2^{II}), 2.20 (AB_q, J = 15 Hz, 2 H, H-2′), 2.06 (s, 3 H, SCH_3), 1.36 (d, $J_{5,6}$ = 6.3 Hz, 3 H, H-6^{I}), 1.23 (s, 3 H, CH_3), 1.20 (s, 3 H, CH_3), 1.16 (d, $J_{5,6}$ = 5.6 Hz, 3 H, H-6^{II}).

^{13}C NMR (150 MHz, $CDCl_3$): δ = 172.3 (C=O), 103.8 (C-1^{II}), 84.8 (C-2^{II}), 84.2 (C-1^{I}), 80.7 (C-4^{I}), 80.0 (C-3^{II}), 79.9 (C-2^{I}), 79.2 (C-3^{I}), 74.8 (*C*H$_2$Ph), 73.7 (*C*H$_2$Ph), 73.2 (*C*H$_2$Ph), 70.9 (C-5^{II}), 69.4 (C-3′), 68.3 (C-5^{I}), 60.5 (OCH_3), 55.6 (C-4^{II}), 47.7 (C-2′), 29.3 [2 × C, C(*C*H$_3$)$_2$], 18.0 (C-6^{II}), 17.9 (C-6^{I}), 13.5 (SCH_3).

MS (TOF): m/z = 741.3 [M + NH_4]$^+$, 746.3 [M + Na]$^+$.

HRMS (TOF): m/z [M + NH_4]$^+$ calcd for $C_{40}H_{57}N_2O_9S$: 741.3785; found: 741.3781.

Anal. Calcd for $C_{40}H_{53}NO_9S$: C, 66.37; H, 7.38. Found: C, 66.20; H, 7.39.

Methyl 2,4-Di-*O*-benzyl-3-*O*-[3-*O*-benzyl-4,6-dideoxy-4-(3-*O*-acetyl-3-methylbutanamido)-2-*O*-methyl-β-D-glucopyranosyl]-1-thio-α-L-rhamnopyranoside (12)

Ac_2O (10 mL, 109 mmol) was added at 0 °C to a mixture of **11** (5.26 g, 7.27 mmol) and 4-dimethylaminopyridine (887 mg, 7.27 mmol) in anhyd CH_2Cl_2 (50 mL). The cooling bath was removed and the mixture was stirred overnight, when the reaction was complete (TLC, 3:2 hexane–EtOAc). MeOH (10 mL) was added to quench the reaction, the mixture was concentrated, and the residue was chromatographed to give **12**; yield: 5.38 g (93%); colorless oil.

^{1}H NMR (600 MHz, $CDCl_3$): δ = 5.23 (d, J = 9.0 Hz, 1 H, NH), 5.05 (d, $J_{1,2}$ = 1.4 Hz, 1 H, H-1^{I}), 4.97–4.58 (m, 7 H, H-1^{II} and C*H*$_2$Ph), 4.01 (dd, $J_{2,3}$ = 3.2 Hz, $J_{3,4}$ = 9.5 Hz, 1 H, H-3^{I}), 3.98 (m, 1 H, H-5^{I}), 3.95 (dd, $J_{1,2}$ = 1.5 Hz, $J_{2,3}$ = 3.2 Hz, 1 H, H-2^{I}), 3.70–3.63 (m, 5 H, H-4^{I}, H-4^{II} and OCH_3), 3.39 (dd, $J_{2,3}$ = 9.0 Hz, $J_{3,4}$ = 10.2 Hz, 1 H, H-3^{II}), 3.30 (m, 1 H, H-5^{II}), 3.19 (dd, $J_{1,2}$ = 7.8 Hz, $J_{2,3}$ = 9.0 Hz, 1 H, H-2^{II}), 2.60 (AB_q, J = 13.8 Hz, 2 H, H-2′), 2.05 (s, 3 H, SCH_3), 1.91 (s, 3 H, CH_3CO), 1.51 (s, 3 H, CH_3), 1.50 (s, 3 H, CH_3), 1.36 (d, $J_{5,6}$ = 6.3 Hz, 3 H, H-6^{I}), 1.16 (d, $J_{5,6}$ = 5.6 Hz, 3 H, H-6^{II}).

^{13}C NMR (150 MHz, $CDCl_3$): δ = 171.0 (C=O), 169.3 (C=O), 103.8 (C-1^{II}), 84.5 (C-2^{II}), 84.3 (C-1^{I}), 80.7 (C-4^{I}), 80.7 (C-3′), 80.4 (C-3^{II}), 80.0 (C-2^{I}), 79.2 (C-3^{I}), 74.8 (*C*H$_2$Ph), 73.4 (*C*H$_2$Ph), 73.2 (*C*H$_2$Ph), 71.0 (C-5^{II}), 68.3 (C-5^{I}), 60.6 (OCH_3), 55.7 (C-4^{II}), 47.2 (C-2′), 26.6 and 26.5 [C(*C*H$_3$)$_2$], 22.4 (CH_3CO), 18.0 (C-6^{I}), 17.9 (C-6^{II}), 13.5 (SCH_3).

MS (TOF): m/z = 783.3 [M + NH_4]$^+$, 788.3 [M + Na]$^+$.

HRMS (TOF): m/z [M + NH_4]$^+$ calcd for $C_{42}H_{59}N_2O_{10}S$: 783.3890; found: 783.3878.

5-Methoxycarbonylpentyl 4-(3-*O*-Acetyl-3-methylbutanamido)-3-*O*-benzyl-4,6-dideoxy-2-*O*-methyl-β-D-glucopyranosyl-(1→3)-2,4-di-*O*-benzyl-α-L-rhamnopyranosyl-(1→3)-2,4-di-*O*-benzyl-α-L-rhamnopyranosyl-(1→2)-3,4-di-*O*-benzyl-α-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 CH_2Cl_2 (200 mL) was stirred under N_2 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. Et_3N (2.5 mL) was added to quench the reaction and, after filtration through a Celite pad, the filtrate was washed successively with 10% aq $Na_2S_2O_3$ (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; $[\alpha]_D$ –21.60 (*c* 1.19, $CHCl_3$).

^{1}H NMR (600 MHz, $CDCl_3$): δ = 5.15 (d, J = 9.1 Hz, 1 H, NH), 5.09 (br s, 1 H, H-1^{III}), 5.07 (d, $J_{1,2}$ = 1.8 Hz, 1 H, H-1^{II}), 5.00–4.42 (m, 16 H, 7 × C*H*$_2$Ph, H-1^{I}, H-1^{IV}), 4.12 (t, J = 3.0 Hz, 1 H, H-3^{II}), 4.10 (t, J = 3.0 Hz, 1 H, H-3^{III}), 3.99 (t, J = 2.0 Hz, 1 H, H-2^{I}), 3.90 (dd, $J_{1,2}$ = 1.8 Hz, $J_{2,3}$ = 3.0 Hz, 1 H, H-2^{III}), 3.82–3.75 (m, 4 H, H-3^{I}, H-2^{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}, CO_2CH_3 and OCH_3), 3.53 (m, 1 H, H-4^{II}), 3.39 (t, J = 9.4 Hz, 1 H, H-4^{I}), 3.35–3.31 (m, 2 H, H-1$_b$ and H-3^{IV}), 3.18–3.15 (m, 2 H, H-2^{IV}, H-5^{IV}), 2.55 (AB_q, J = 13.7 Hz, 2 H, H-2′), 2.30 (t, J = 7.5 Hz, H-5), 1.86 (s, 3 H, CH_3CO), 1.63 (m, 2 H, H-4), 1.54 (m, 2 H, H-2), 1.49 and 1.48 [2 s, C(CH_3)$_2$], 1.34 (m, 2 H, H-3), 1.28 (d, $J_{5,6}$ = 5.4 Hz, 3 H, H-6^{III}), 1.26 (d, $J_{5,6}$ = 6.2 Hz, 3 H, H-6^{I}), 1.23 (d, $J_{5,6}$ = 6.3 Hz, 3 H, H-6^{II}), 1.02 (d, $J_{5,6}$ = 6.2 Hz, 3 H, H-6^{IV}).

^{13}C NMR (150 MHz, $CDCl_3$): δ = 103.7 (C-1^{IV}), 100.4 (C-1^{III}), 98.9 (C-1^{II}), 98.8 (C-1^{I}), 84.5 (C-2^{IV}), 80.8 (C-4^{III}), 80.6 (C-4^{I}), 80.5 (C-4^{II}), 80.4 (C-3^{I}), 80.3 (C-3′), 80.0 (C-3^{IV}), 79.2 (C-2^{III}), 78.7 (C-3^{III}), 78.0 (2 × C, C-2^{II} and C-3^{II}), 75.4 (*C*H$_2$Ph), 74.8 (2 × C, C-2^{I} and *C*H$_2$Ph), 74.5 (*C*H$_2$Ph), 73.4 (*C*H$_2$Ph), 73.3 (*C*H$_2$Ph), 72.3 (*C*H$_2$Ph), 72.0 (*C*H$_2$Ph), 70.9 (C-5^{IV}), 68.5 (C-5^{II}), 68.3 (C-5^{III}), 67.8 (C-5^{I}), 67.1(C-1), 60.5 (OCH_3), 55.6 (C-4^{IV}), 51.4 (CO_2CH_3), 47.2 (C-2′), 33.9 (C-5), 29.1 (C-2), 26.6 and 26.5 [C(*C*H$_3$)$_2$], 25.7 (C-3), 24.7 (C-4), 22.4 (*C*H$_3$CO), 17.9 (4 × C, C-6^{I}, C-6^{II}, C-6^{III}, C-6^{IV}).

MS (TOF): m/z = 1533.6 [M + NH_4]$^+$, 1538.6 [M + Na]$^+$.

Anal. Calcd for $C_{88}H_{109}NO_{21}$: C, 69.68; H, 7.24; N, 0.92. Found: C, 69.42; H, 7.38; N, 1.07.

5-Methoxycarbonylpentyl 4-(3-*O*-Acetyl-3-methylbutanamido)-4,6-dideoxy-2-*O*-methyl-β-D-glucopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-rhamnopyranoside (14)

A mixture of compound **13** (7.38 g, 4.91 mmol) and 5% Pd/C catalyst (4.0 g) in 5:1 MeOH–EtOAc (300 mL) was stirred in a H_2 atmosphere at r.t. for 24 h. TLC (6:4:1 CH_2Cl_2–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.

^{1}H NMR (600 MHz, CD_3OD): δ = 5.06 (d, $J_{1,2}$ = 1.6 Hz, 1 H, H-1^{III}), 4.91 (d, $J_{1,2}$ = 1.7 Hz, 1 H, H-1^{II}), 4.78 (d, $J_{1,2}$ = 1.5 Hz, 1 H, H-1^{I}), 4.62 (d, $J_{1,2}$ = 7.8 Hz, 1 H, H-1^{IV}), 4.18 (dd, $J_{1,2}$ = 1.8 Hz,

$J_{2,3}$ = 3.2 Hz, 1 H, H-2^{III}), 4.06 (dd, $J_{1,2}$ = 2.0 Hz, $J_{2,3}$ = 3.1 Hz, 1 H, H-2^{II}), 3.90 (dd, $J_{2,3}$ = 3.3 Hz, $J_{3,4}$ = 9.5 Hz, 1 H, H-3^{III}), 3.83 (ddd, J = 4.8, 7.9, 12.5 Hz, 1 H, H-5^{III}), 3.80–3.77 (m, 2 H, H-3^{II}, H-2^{I}), 3.74 (m, 2 H, H-5^{II}, H-3^{I}), 3.69–3.64 (m, 7 H, H-1$_a$, 2 × OCH_3), 3.61–3.48 (m, 4 H, H-4^{IV}, H-4^{III}, H-4^{II}, H-5^{I}), 3.44–3.34 (m, 4 H, H-3^{IV}, H-5^{IV}, H-4^{I}, and H-1$_b$), 3.01 (dd, $J_{1,2}$ = 8.0 Hz, $J_{2,3}$ = 8.9 Hz, 1 H, H-2^{IV}), 2.72 (AB_q, J = 13.5 Hz, 2 H, H-2′), 2.34 (t, J = 7.4 Hz, 2 H, H-5), 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 × CH_3), 1.46–1.37 (m, 2 H, H-3), 1.29 (d, J = 6.2 Hz, 3 H, H-6^{III}), 1.25 (dd, J = 5.2 Hz, 6.0 Hz, 6 H, H-6^{II}, H-6^{I}), 1.20 (d, $J_{5,6}$ = 6.2 Hz, 3 H, H-6^{IV}).

^{13}C NMR (150 MHz, CD_3OD): δ = 175.8 (C=O), 172.4 (2 × C, 2 × C=O), 105.4 (C-1^{IV}), 103.9 (C-1^{II}), 103.5 (C-1^{III}), 100.3 (C-1^{I}), 85.7 (C-2^{IV}), 81.7 (C-3^{II}), 81.5 (C-3′), 80.1 (C-2^{II}), 79.0 (C-3^{II}), 74.8 (C-3^{IV}), 74.3 (C-4^{I}), 73.2 (C-4^{II}), 73.0 (C-4^{III}), 72.2 (C-5^{IV}), 72.1 (C-3^{I}), 71.8 (C-2^{I}), 71.7 (C-2^{III}), 70.5 (C-5^{II}), 70.1 (C-5^{III}), 69.9 (C-5^{I}), 68.3 (C-1), 61.2 (OCH_3), 58.0 (C-4^{IV}), 52.0 (CO_2CH_3), 47.5 (C-2′), 34.7 (C-5), 30.2 (C-2), 27.0 (C-3), 26.8 [2 × C, (C′$_3$)(*C*H$_3$)$_2$], 25.7 (C-4), 22.4 (*C*H$_3$CO), 18.4 (C-6^{IV}), 18.1 and 17.9 (3 × C, C-6^{I}, C-6^{II}, C-6^{III}).

MS (TOF): *m*/*z* = 886.0 $[M + H]^+$, 903.4 $[M + NH_4]^+$, 908.3 $[M + Na]^+$.

HRMS (TOF): *m*/*z* $[M + H]^+$ calcd for $C_{39}H_{68}NO_{21}$: 886.4284; found: 886.4236.

(2-Aminoethylamido)carbonylpentyl 4,6-Dideoxy-4-(3-hydroxy-3-methylbutyramido)-2-*O*-methyl-β-D-glucopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-rhamnopyranoside (15)

A solution of **14** (3.60 g, 4.07 mmol) in $NH_2(CH_2)_2NH_2$ (10 mL) was stirred overnight at 50 °C, when TLC (CH_2Cl_2–MeOH–25% NH_4OH, 10:9:1) showed that all starting material was consumed and that two poorly resolved products (one major) were present. ^{1}H NMR showed absence of the signal for the CO_2CH_3 group and presence of a disproportionately small signal for the $COCH_3$ group. The mixture was concentrated and the residue was dissolved in MeOH (100 mL). Methanolic 1 M NaOMe was added to the solution until the solution became strongly basic to litmus, and the mixture was kept at r.t. overnight. TLC (1:1:0.1 CH_2Cl_2–MeOH–25% NH_4OH) showed that only one substance, more polar than the starting material **14** was present. Concentration and chromatography of the residue gave **15**, whose aqueous solution was freeze-dried; yield: 3.0 g (85%); colorless amorphous solid.

^{1}H NMR (600 MHz, D_2O): δ = 5.03 (d, $J_{1,2}$ = 1.7 Hz, 1 H, H-1^{III}), 4.92 (d, $J_{1,2}$ = 1.7 Hz, 1 H, H-1^{II}), 4.87 (d, $J_{1,2}$ = 1.5 Hz, 1 H, H-1^{I}), 4.73 (d, $J_{1,2}$ = 8.0 Hz, 1 H, H-1^{IV}), 4.27 (dd, $J_{1,2}$ = 1.8 Hz, $J_{2,3}$ = 3.3 Hz, 1 H, H-2^{III}), 4.14 (dd, $J_{1,2}$ = 2.0 Hz, $J_{2,3}$ = 3.1 Hz, 1 H, H-2^{II}), 3.98 (dd, $J_{2,3}$ = 3.3 Hz, $J_{3,4}$ = 9.7 Hz, 1 H, H-3^{III}), 3.90 (dd, $J_{1,2}$ = 1.7 Hz, $J_{2,3}$ = 3.3 Hz, 1 H, H-2^{I}), 3.87–3.74 (m, 4 H, H-3^{I}, H-3^{II}, H-5^{III}, H-5^{II}), 3.72–3.65 (m, 2 H, H-1$_a$, H-5^{I}), 3.63–3.58 (m, 5 H, H-4^{III}, H-4^{IV}, OCH_3), 3.57–3.44 (m, 5 H, H-1$_b$, H-4^{I}, H-4^{II}, H-3^{IV}, H-5^{IV}), 3.28–3.21 (m, 2 H, H-6), 3.12 (dt, J = 5.1, 5.9 Hz, 1 H, H-2^{IV}), 2.75 (dd, J = 3.5, 5.6 Hz, 2 H, H-7), 2.50–2.40 (m, 2 H, H-2′), 2.25 (q, J = 7.1 Hz, 2 H, H-5), 1.68–1.53 (m, 4 H, H-2 and H-4), 1.45–1.32 (m, 2 H, H-3), 1.30–1.24 [m, 15 H, H-6^{I}, H-6^{II}, H-6^{III}, C(CH$_3$)$_2$], 1.21 (d, $J_{5,6}$ = 6.1 Hz, 3 H, H-6^{IV}).

^{13}C NMR (150 MHz, D_2O): δ = 180.0 (C=O), 176.8 (C=O), 106.4 (C-1^{IV}), 104.9 (C-1^{II}), 104.6 (C-1^{III}), 101.0 (C-1^{I}), 86.0 (C-2^{IV}), 82.4 (C-3^{III}), 81.5 (C-2^{I}), 80.7 (C-3^{I}), 75.6 (C-5^{IV}), 74.9 (C-3^{IV}), 74.0 (C-4^{I}), 73.9 (C-4^{III}), 73.5 (C-4^{II}), 73.0 (C-3^{II}), 72.8 (C-3′), 72.6 (2 × C, C-2^{II} and C-2^{III}), 72.14 (2 × C, C-5^{II} and C-5^{III}), 71.6 (C-5^{I}), 70.6 (C-1), 62.8 (OCH_3), 59.3 (C-4^{IV}), 51.7 (C-2′), 43.8 (C-6), 42.5 (C-7), 38.5 (C-5), 31.0 (CH_3), 30.9 (2 × C, C-4 and CH_3), 27.8 and 27.7 (C-2 and C-3), 19.85 (C-6^{IV}), 19.4 (3 × C, C-6^{I}, C-6^{II}, C-6^{III}).

MS (TOF): *m*/*z* = 872.3 $[M + H]^+$, 894.3 $[M + Na]^+$.

HRMS (TOF): *m*/*z* $[M + H]^+$ calcd for $C_{38}H_{70}N_3O_{19}$: 872.4604; found: 872.4606.

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

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