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Synthesis of spacer-containing analogs of serogroup 6 pneumococcal oligosaccharides

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Abstract—An efficient convergent strategy for the synthesis of a range of spacer-containing pneumococcal oligosaccharides of serogroup 6 and derivatives thereof has been developed. The spacer-containing oligosaccharides were deprotected and are available for subsequent conjugation and immunological studies, which are underway in our laboratory.

Keywords: Carbohydrates; *Streptococcus pneumoniae*; Spacer; Synthetic vaccines; Glycosylation; Oligosaccharides

1. Introduction

Complex carbohydrates represent a unique family of multi-functional compounds involved in many biological phenomena.[1] Our current knowledge about the key roles of these fascinating natural compounds is not yet complete. However, thanks to the explosive growth of the field of glycobiology in the recent years, we already know that carbohydrates are involved in a broad range of life threatening processes such as bacterial and viral infections, development and growth of tumors, metastasis, tissue rejection are only a few to mention. The fact that many of these processes are directly associated with pathogenesis of many deadly diseases has already stimulated heated interest for the development of excellent carbohydrate-based therapeutics.[2]

Also, the pneumococcal bacterial cell is surrounded by a polysaccharide capsule, and preventive vaccination based on the related carbohydrates, which are commonly applied as sugar-protein conjugates, has been found a viable weapon against the bacterial invasion.[3] *Streptococcus pneumoniae* (SPn) have become one of the most frequent causes of invasive bacterial infections, particularly in patients with weakened or immature immune system.[4] Although the study of the pneumococcal disease over the last 120 years has led to important scientific and clinical insights,[5] it still accounts for nearly 20% of all childhood deaths under the age of five that is translated into two million deaths every year.[6]

Among 91 elucidated SPn serotypes,[7] serogroup 6 has been consistently ranked second–third most frequent cause of invasive pneumococcal disease worldwide.[8] This fact stimulated extensive structural[9] and synthetic studies[10,11] of SPn6 oligosaccharides and derivatives thereof. Because for the most part relatively small oligosaccharide fragments are non-immunogenic, many synthetic targets are equipped with a spacer moiety, suitable for conjugation to a carrier protein. These conjugates were proven to significantly increase the immune response and are immunogenic even in small children who are at the greatest risk to infection.[12] In continuation of our recent studies toward the stereoselective synthesis of pneumococcal oligosaccharides,[13] herein we report the synthesis of spacer-containing oligosaccharides (**1** and **2**) and derivatives thereof (**3**–**6**, Fig. 1) structurally related to the repeating unit of SPn6. The spacer moiety is essential for conjugation with a carrier protein and subsequent immunological studies.

2. Results and discussion

SPn6A and SPn6B repeating units consist of structurally related complex pseudo-tetrasaccharides, which in the

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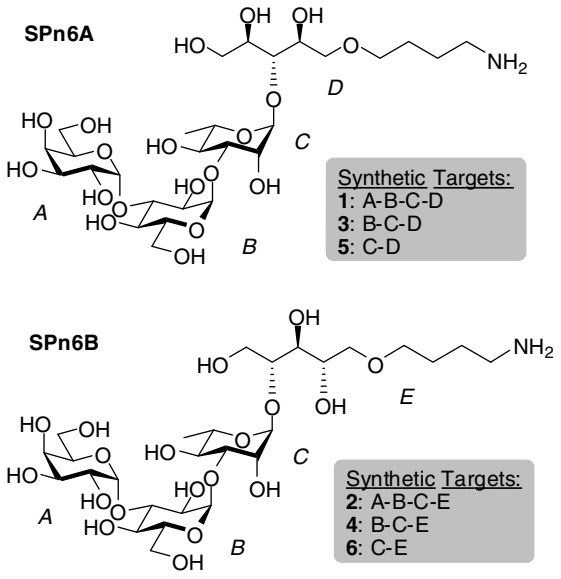


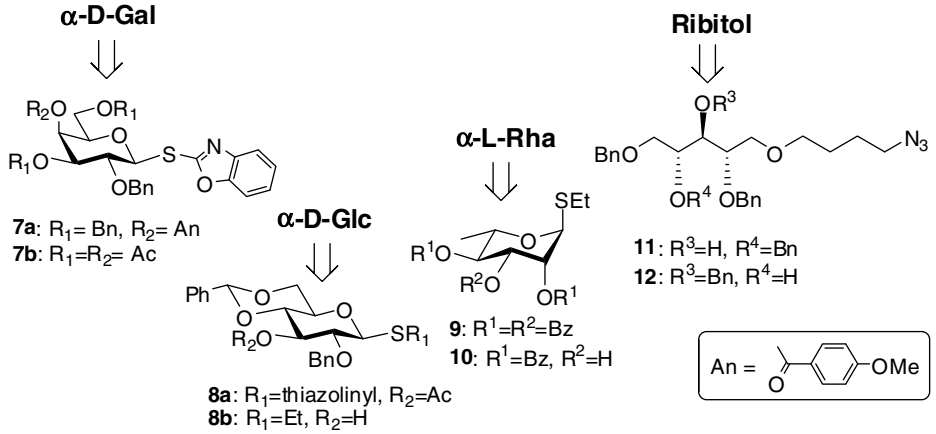
Figure 1.

natural polysaccharide are connected via a phosphate (5→2‴) linkage. In our previous work, we have already shown that the pseudo-disaccharides, -trisaccharides, and -tetrasaccharides of SPn6 series could be synthesized via selective activation strategy in the overall excellent yields and complete stereoselectivity.[13] Our current work reports the synthesis of spacer-containing oligosaccharides (**1**–**6**), using a similar approach. Nevertheless, some adjustments have been made to the scheme: while in our previous work, the assembly of ABCD tetrasaccharide was performed in (B + C) + A + D fashion, herein the assembly was carried out as follows: A + B + (C + D). These strategic adjustments required further optimization of the anomeric selectivity, and for this purpose two types of galactose building block have been investigated (**7a**,**b**). Our expectation was that anisoyl group at C-4 of galactose would help to achieve excellent 1,2-*cis* stereoselectivity.[14] The D-gluco and L-rhamno units will be introduced by using common building blocks **8**–**10**, whereas the synthesis of the spacer-containing ribitol units would require particular attention (Scheme 1). Efficient chemical synthesis of the spacer-containing oligosaccharides of SPn6 and derivatives thereof will help to quickly obtain substantial quantities of pure samples for conjugation and immunological studies.

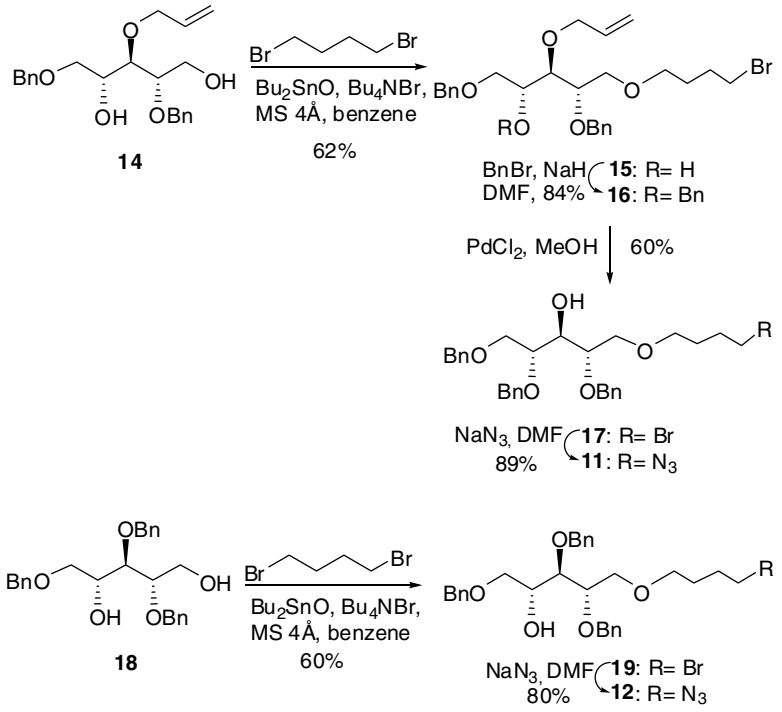
The synthesis of the SBox galactose building block (**7a**) was carried out from the known precursor ethyl 4-*O*-anisoyl-2,3,6-tri-*O*-benzyl-1-thio-β-D-galactopyranoside (**7**)[14] via conventional protocol involving bromination–thioglycosylation sequence.[15] 2-Benzyl-triacetate SBox galactoside **7b**, which provided excellent results in our previous work, was also generated for comparison studies.[15] Known derivatives **8**–**10** were projected for the introduction of units B and C and their synthesis was accomplished as described previously.[16]

Synthesis of the 3-OH ribitol building block **11** was carried out from the known precursor **14**[11] as shown in Scheme 2. First, diol **14** was subjected to dibutyltin oxide-mediated alkylation, which was required to ensure excellent regioselective protection of the primary hydroxyl.[17] The resulting compound **15** was obtained in 62% yield; it was then subjected to benzylation to afford **16**, and the latter was deallylated using $PdCl_2$ in MeOH to afford compound **17** in 60% yield. Bromine was then converted to the corresponding azide via conventional nucleophilic displacement to provide the requisite glycosyl acceptor **11** in 89% yield. For the synthesis of 4-OH ribitol building block **12**, the known precursor **18**[13] was alkylated and converted to the azido derivative using similar reaction conditions as described for the synthesis of acceptor **11**.

Having obtained the key monosaccharide building blocks, we turned our attention to the oligosaccharide assembly. For the synthesis of pseudo-disaccharides **5** and **6**, per-benzoylated rhamnose building block **9** was used as a glycosyl donor. Coupling of **9** with ribitol acceptors **11** and **12** in the presence of NIS–TfOH afforded glycosides **20** and **21** with complete α-selectivity in



Scheme 1. Retrosynthetic analysis of spacer-containing oligosaccharides of SPn6.

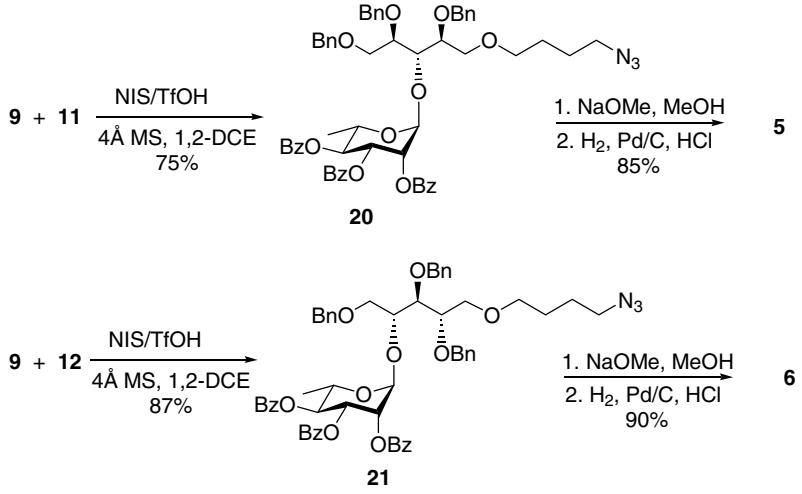


Scheme 2. Synthesis of ribitol acceptors **11** and **12**.

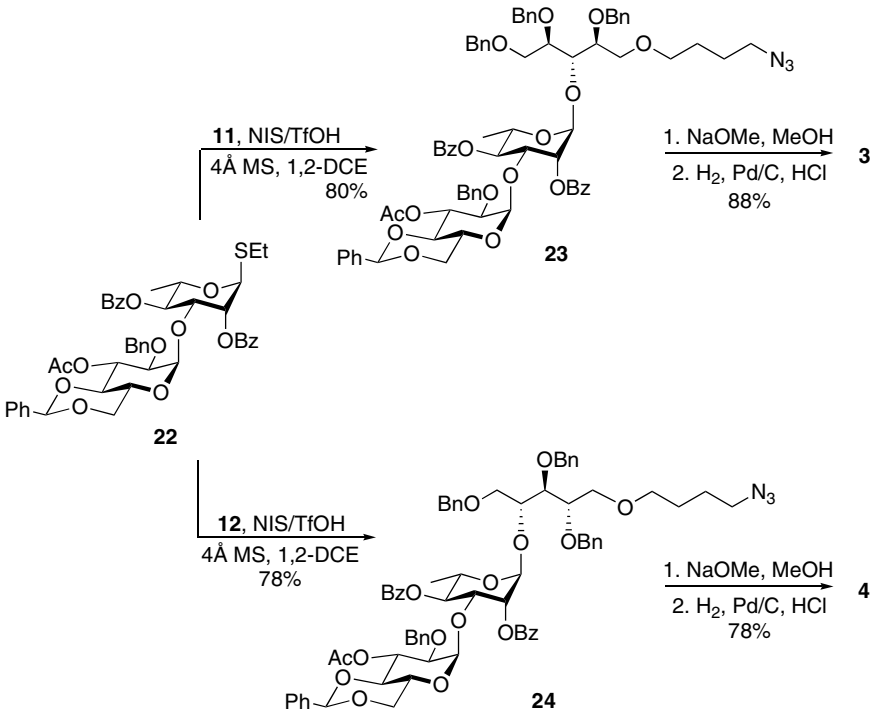
75% and 87% yields, respectively (Scheme 3). The compounds obtained were then subjected to a two-step sequential deprotection: deacylation (NaOMe in MeOH) and hydrogenation using palladium on charcoal in 5% HCl in EtOH to obtain pseudo-disaccharides **5** and **6** in 85% and 90% yields, respectively.

For the synthesis of pseudo-trisaccharides **3** and **4**, the *S*-ethyl anomeric moiety of disaccharide **22**, obtained from **8a** and **10** as described previously,[13] was activated with NIS–TfOH for the glycosylation of glycosyl acceptors **11** and **12** (Scheme 4). As a result, saccharides **23** and **24** were isolated in 80% and 78% yields, respectively. These rhamnosylations also proceeded with complete α-stereoselectivity. The protected pseudo-trisaccharides **23** and **24** were then subjected to the standard deprotection sequence, as described above for the synthesis of **5** and **6**, to afford compounds **3** and **4** in 88% and 78% yields, respectively.

Finally, we were aiming for the synthesis of pseudo-tetrasaccharides **1** and **2**, the ultimate targets of this work. For this purpose, galactose derivative **7b** was used as a glycosyl donor; unfortunately, this coupling resulted in a very poor stereoselectivity (α:β = 3:1). This was a rather unexpected result considering the success



Scheme 3. Synthesis of spacer-containing pseudo-disaccharides **5** and **6**.

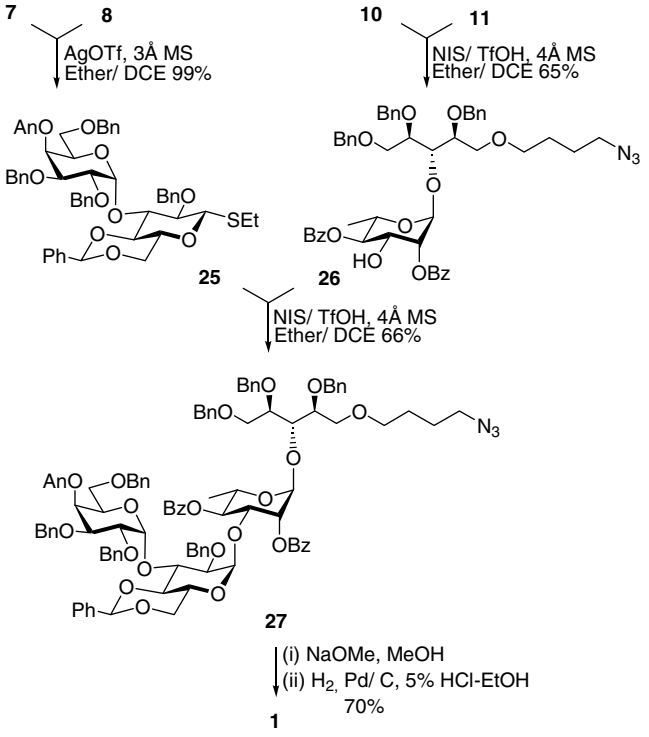


Scheme 4. Synthesis of spacer-containing pseudo-trisaccharides **3** and **4**.

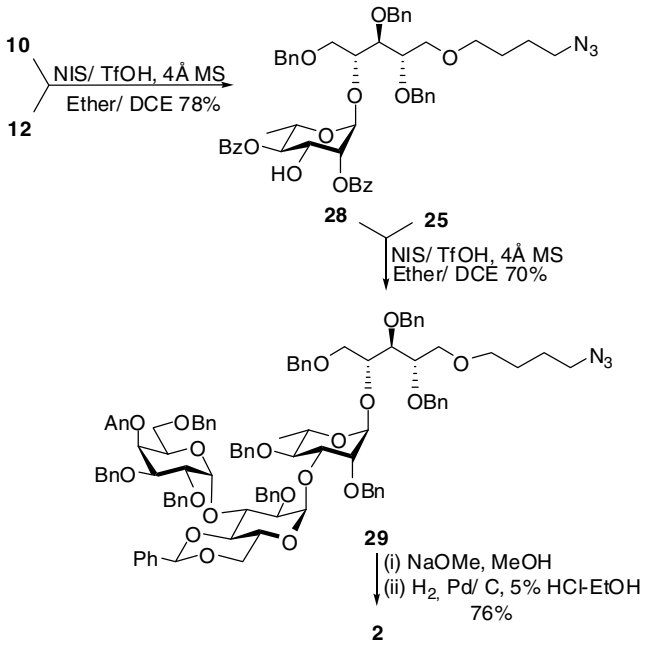
of our previous galactosylation using the A + BC strategy.[13] To improve this, we obtained galactose donor **7a**, containing an anisoyl group at the C-4 position, a known concept to improve the outcome of α-galactosylations. The SBox leaving group of **7a** was then selectively activated over the SEt moiety of glucosyl acceptor **8b**. This reaction was carried out in diethyl ether–1,2-dichloroethane (5:1) mixture of solvents to further enhance the stereoselectivity. This fine-tuning of reaction conditions using the previously elaborated methodologies allowed us to obtain disaccharide **25** in 99% yield and very good stereoselectivity (α:β = 14:1, Scheme 5).

For the synthesis of the disaccharide acceptor **26**, rhamnose building block **10** was glycosidated with ribitol acceptor **11** to afford the requisite disaccharide in 65% yield. No self-condensation of compound **10**, bearing both leaving and hydroxyl moieties, was detected. To complete the assembly, the disaccharide donor **25** was coupled with the disaccharide acceptor **26** in the presence of NIS and catalytic TfOH. As a result, pseudo-tetrasaccharide derivative **27** was obtained in 66% yield. Finally, the deprotection of **27** was carried out using conventional deacylation–hydrogenation sequence to afford pseudo-tetrasaccharide **1** in 70%.

For the synthesis of pseudo-tetrasaccharide **2**, corresponding to the carbohydrate part of the repeating unit of SPn6B, disaccharide acceptor **28** was obtained from building blocks **10** and **12** in 78% yield. Glycosylation of **28** with the disaccharide donor **25** led to the fully protected pseudo-tetrasaccharide **29** in 70% yield with complete α-stereoselectivity. The conventional deprotection of **29** afforded the target compound **2** in 76% yield (Scheme 6).



Scheme 5. Synthesis of spacer-containing pseudo-tetrasaccharide of SPn6A **1**.



Scheme 6. Synthesis of spacer-containing pseudo-tetrasaccharide of SPn6B **2**.

In conclusion, we have developed an efficient convergent strategy for the synthesis of a range of spacer-containing pneumococcal oligosaccharides, serogroup 6 (SPn6). Further studies related to the synthesis of other spacer-containing oligosaccharides and their conjugation to the carrier protein and subsequent immunological studies are underway in our laboratory.

3. Experimental

3.1. General

Column chromatography was performed on silica gel 60 (EM Science, 70–230 mesh), reactions were monitored by TLC on Kieselgel 60 F_{254} (EM Science). The compounds were detected by examination under UV light and by charring with 10% sulfuric acid in methanol. Solvents were removed under reduced pressure at <40 °C. $ClCH_2CH_2Cl$ was distilled from CaH_2 directly prior to application. Methanol was dried by refluxing with magnesium methoxide, distilled and stored under argon. Pyridine was dried by refluxing with CaH_2 and then distilled and stored over molecular sieves (3 Å). Anhydrous DMF (EM Science) and ether were used as is. Molecular sieves (3 Å or 4 Å), used for the reactions, were crushed and activated in vacuo at 390 °C for 8 h in the first instance and then for 2–3 h at 390 °C directly prior to application. AgOTf (Acros) was first dried by co-evaporation with toluene (3 × 10 mL) and then placed in vacuo for 2–3 h directly prior to application. Optical rotations were measured at 'Jasco P-1020' polarimeter. Unless noted otherwise, ^1H NMR spectra were recorded in $CDCl_3$ at 300 MHz (Bruker Avance), ^{13}C NMR spectra and two-dimensional experiments were recorded in $CDCl_3$ at 75 MHz (Bruker Avance) or at 125 MHz (Bruker ARX-500). HRMS determinations were made with the use of JEOL MStation (JMS-700) Mass Spectrometer.

3.2. Benzoxazolyl 4-*O*-anisoyl-2,3,6-tri-*O*-benzyl-1-thio-β-D-galactopyranoside (7a)

The solution of ethyl 4-*O*-anisoyl-2,3,6-tri-*O*-benzyl-1-thio-β-D-glucopyranoside[14] (3.98 g, 6.34 mmol) and activated molecular sieves (3 Å, 3.17 g) in CH_2Cl_2 (95 mL) was stirred under argon for 1 h. Freshly prepared solution of Br_2 in CH_2Cl_2 (60 mL, 1:165) was then added and the reaction mixture was kept for 5 min at rt. After this, the solid was filtered off and the filtrate was concentrated in vacuo at rt. Crude residue was then treated with KSBox (7.6 mmol) and 18-crown-6 (0.76 mmol) in dry acetone (12 mL) under argon for 16 h at rt. Upon completion, the mixture was diluted with CH_2Cl_2, the solid was filtered off and the residue was washed with CH_2Cl_2. The combined filtrate (200 mL) was washed with 1% aq NaOH (50 mL) and water (3 × 50 mL). The organic layer was separated, dried with $MgSO_4$, and concentrated in vacuo. The residue was purified by silica gel column chromatography (EtOAc–toluene gradient elution) to afford **7a** as a syrup (2.2 g, 50%). Analytical data for **7a**: $R_f = 0.46$ (EtOAc–hexanes 3:7); $[\alpha]_D^{29}$ +10.8 (*c* 1.0, $CHCl_3$); ^1H NMR: δ 3.40–3.53 (m, 2H, H-6a, 6b), 3.69 (s, 3H, –OCH_3), 3.74 (dd, 1H, $J_{2,3} = 2.8$ Hz, H-3), 3.89–3.97 (m, 2H, H-2, 5), 4.18–4.82 (m, 6H, 4 × CH_2Ph), 5.36 (dd, 1H, $J_{3,4} = 2.3$ Hz, H-4), 6.76–7.98 (m, 25H, aromatic); ^{13}C NMR: δ 56.2, 67.5, 68.6, 72.4, 74.3, 76.4, 81.9, 85.7, 110.7, 114.4, 119.7, 122.7, 124.9, 125.1, 128.3, 128.4, 128.5 (×2), 128.8 (×4), 128.9, 129.1, 132.7, 138.1, 138.2, 138.3, 142.4, 161.9, 164.2, 165.9. FABMS *m/z* $[M+H]^+$ calcd for $C_{42}H_{40}NO_8S$: 718.2475. Found: 718.2452.

3.3. 3-*O*-Allyl-2,5-di-*O*-benzyl-1-*O*-(4-bromobutyl)-D-ribitol (15)

To a solution of 3-*O*-allyl-2,5-di-*O*-benzyl-D-ribitol **14**[11] (871 mg, 2.34 mmol) in benzene (18 mL), freshly activated molecular sieves (4 Å, 4 g) were added and the mixture was stirred under argon for 1 h. Bu_2SnO (786 mg, 3.2 mmol) and Bu_4NBr (784 mg, 2.4 mmol) were then added followed by the addition of 1,4-dibromobutane (1.3 mL, 14.04 mmol). The reaction mixture was refluxed for 20 h under argon, then cooled to rt. Molecular sieves were filtered off through a pad of Celite, rinsed with toluene (2 × 5 mL), and the combined filtrate was then concentrated in vacuo. The residue was purified by column chromatography on silica gel

(EtOAc–hexanes gradient elution) to afford **15** as a colorless liquid (730 mg, 62% yield). Analytical data for **15**: $R_f = 0.50$ (EtOAc–hexanes 2:3); $[\alpha]_D^{28}$ +70.1 (*c* 1.0, $CHCl_3$); ^1H NMR: δ 1.63–1.68 (m, 2H, CH_2sp), 1.84–1.89 (m, 2H, CH_2sp), 2.81 (d, 1H, –OH), 3.33–3.43 (m, 4H, 2 × CH_2sp), 3.54–3.63 (m, 3H, H-3,5), 3.64–3.68 (m, 2H, H-1), 3.77–3.79 (m, 1H, H-2), 3.87–3.91 (m, 1H, H-2), 3.87–3.91 (m, 1H, H-4), 4.00–4.11 (m, 2H, OCH_2), 4.44–4.66 (m, 4H, 2 × *CH*$_2$Ph), 5.08–5.18 (m, 2H, CH_2=), 5.72–5.87 (m, 1H, =CH–), 7.24–7.28 (m, 10H, aromatic); ^{13}C NMR: δ 28.8, 30.2, 34.3, 70.8, 70.9, 71.4, 71.8, 73.0, 73.3, 73.9, 79.3, 79.6, 117.4, 128.1, 128.2, 128.3 (×2), 128.4 (×2), 128.9 (×2), 129.0 (×2), 135.5, 138.6, 138.9. FABMS *m*/*z* $[M+Na]^+$ calcd for $C_{26}H_{35}BrO_5Na$: 529.1566. Found: 529.1542.

3.4. 3-*O*-Allyl-2,4,5-tri-*O*-benzyl-1-*O*-(4-bromobutyl)-D-ribitol (16)

Benzyl bromide (0.3 mL, 2.1 mmol) was added to a stirring solution of **15** (0.73 g, 1.44 mmol) in dry DMF (10 mL) at 0 °C. Sodium hydride (60% suspension in mineral oil, 0.12 g, 2.8 mmol) was added slowly until the evolution of hydrogen gas has seized. The reaction mixture was stirred for 5 h at rt until complete disappearance of the starting material as indicated by TLC. The reaction mixture was poured in crushed ice (~50 mL), stirred for 15 min, extracted with EtOAc–ether (1:1, 3 × 30 mL). The combined organic extract was dried with anhydrous $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (EtOAc–hexanes gradient elution) to afford compound **16** as a colorless liquid (0.72 g, 84% yield). Analytical data for **16**: $R_f = 0.57$ (EtOAc–hexanes 2:3); $[\alpha]_D^{28}$ −2.2 (*c* 1.0, $CHCl_3$); ^1H NMR: δ 1.87–2.00 (m, 2H, CH_2sp), 2.13–2.27 (m, 2H, CH_2sp), 3.64–3.68 (m, 4H, 2 × CH_2sp), 3.85–4.10 (m, 7H, H-1, 2, 3, 4, 5), 4.38–3.41 (m, 2H, OCH_2), 4.75–5.00 (m, 6H, 3 × *CH*$_2$Ph), 5.36–5.50 (m, 2H, CH_2=), 6.08–6.13 (m, 1H, =CH–), 7.54–7.65 (m, 15H, aromatic); ^{13}C NMR: δ 26.6, 28.5, 29.9, 70.3, 70.5, 70.9, 72.3, 72.5, 72.9, 73.5, 78.6, 78.7, 116.5, 127.6, 127.7, 127.8 (×2), 127.9 (×4), 128.4 (×4), 128.5 (×2), 128.6, 135.2, 135.4, 138.4, 138.6, 138.8. FABMS *m*/*z* $[M+H]^+$ calcd for $C_{33}H_{42}BrO_5$: 597.2216. Found: 597.2198.

3.5. 2,4,5-Tri-*O*-benzyl-1-*O*-(4-bromobutyl)-D-ribitol (17)

To a stirring solution of **16** (0.7 g, 1.2 mmol) in MeOH (7 mL), $PdCl_2$ (0.25 g) was added and the reaction was stirred at rt for 3 h. The solid was then filtered off through Celite, rinsed with EtOAc (3 × 10 mL), and the combined filtrate was concentrated in vacuo. The residue was purified by column chromatography on silica gel (EtOAc–hexanes gradient elution) to afford compound **17** as a yellow syrup (0.42 g, 60% yield). Analytical data for **17**: $R_f = 0.45$ (EtOAc–hexanes 3:7); $[\alpha]_D^{25}$ −3.8 (*c* 1.0, $CHCl_3$); ^1H NMR: δ 1.69–1.76 (m, 2H, CH_2sp), 1.80–1.98 (m, 2H, CH_2sp), 2.97 (d, 1H, –OH), 3.40–3.47 (m, 4H, 2 × CH_2sp), 3.53–3.63 (m, 3H, H-4, 5), 3.68–3.80 (m, 3H, H-1,2), 4.03–4.06 (m, 1H, H-3), 4.49–4.75 (m, 6H, 3 × *CH*$_2$Ph), 7.26–7.33 (m, 15H, aromatic); ^{13}C NMR: δ 27.2, 28.4, 29.6, 33.9, 70.4, 70.7, 70.9, 71.2 (×2), 71.8, 72.2 (×3), 73.7 (×2), 77.6, 78.1, 127.8, 127.9 (×2), 128.1 (×2), 128.5 (×4), 128.6 (×2), 138.3, 138.6. FABMS *m*/*z* $[M+H]^+$ calcd for $C_{30}H_{38}BrO_5$: 559.1887. Found: 559.1885.

3.6. 1-*O*-(4-Azidobutyl)-2,4,5-tri-*O*-benzyl-D-ribitol (11)

Sodium azide (86 mg, 1.75 mmol) was added to a stirring solution of **17** (0.42 g, 0.75 mmol) in dry DMF (6 mL). The reaction was heated at 60 °C for 5 h, then poured in crushed ice (~30 mL), stirred for 15 min, and extracted with EtOAc (3 × 20 mL). The combined organic extract was dried with anhydrous $MgSO_4$ and concentrated in vacuo. The residue was purified by column chromatography on silica gel (EtOAc–hexanes gradient elution) to afford compound **11** as a colorless syrup (0.35 g, 89% yield). Analytical data for **11**: $R_f = 0.48$ (EtOAc–hexanes 2:3); $[\alpha]_D^{29}$ −4.8 (*c* 1.0, $CHCl_3$); ^1H NMR: δ 1.56 (br s, 4H, 2 × CH_2sp), 2.95 (br s, 1H, –OH), 3.17–3.24 (m, 2H, CH_2sp), 3.33–3.35 (m, 2H, CH_2sp), 3.52–3.60 (m, 2H, H-2, 4), 3.62–3.73 (m, 4H, H-1, 5), 3.95 (br s, 1H, H-3), 4.40–4.67 (m, 6H, 3 × *CH*$_2$Ph), 7.15–7.25 (m, 15H, aromatic); ^{13}C NMR: δ 25.8, 26.9, 51.3, 70.6, 71.3, 71.4, 72.1, 72.4, 73.9, 78.3, 78.4, 127.6 (×2), 127.7, 127.8 (×2), 127.9 (×4), 128.3 (×5), 128.4 (×4), 138.2, 138.5. FABMS *m*/*z* $[M+H]^+$ calcd for $C_{30}H_{38}N_3O_5$: 520.2811. Found: 520.2827.

3.7. 2,3,5-Tri-*O*-benzyl-1-*O*-(4-bromobutyl)-D-ribitol (19)

The title compound was obtained from **18**[13] as described for the synthesis of compound **15** in 60% yield. Analytical data for **19**: $R_f = 0.56$ (EtOAc–hexanes 2:3); $[\alpha]_D^{29}$ +16.3 (*c* 1.0, $CHCl_3$); ^1H NMR: δ 1.60–1.65 (m, 2H, CH_2sp), 1.81–1.88 (m, 2H, CH_2sp), 2.77 (br s, 1H, –OH), 3.30–3.39 (m, 4H, 2 × CH_2sp), 3.53–3.61 (m, 3H, H-3, 5), 3.66–3.71 (m, 2H, H-1), 3.84–3.96 (m, 1H, H-2), 4.10–4.12 (m, 1H, H-4), 4.42–4.63 (m, 6H, 3 × *CH*$_2$Ph), 7.21–7.29 (m, 15H, aromatic); ^{13}C NMR: δ 28.4, 29.8, 33.9, 70.4, 70.6, 71.0, 71.3, 72.6, 73.5 (×2), 73.8, 79.0, 79.3, 127.7, 127.8 (×2), 127.9 (×2), 128.0, 128.2 (×2), 128.5 (×4), 128.6, 138.1, 138.5, 138.6. FABMS *m*/*z* $[M+H]^+$ calcd for $C_{30}H_{38}BrO_5$: 559.1887. Found: 559.1885.

3.8. 1-*O*-(4-Azidobutyl)-2,3,5-tri-*O*-benzyl-D-ribitol (12)

The title compound was obtained from **19** as described for the preparation of compound **11** in 80% yield. Analytical

data for **12**: $R_f = 0.40$ (EtOAc–hexanes 3:7); $[\alpha]_D^{29}$ +29.0 (*c* 1.0, $CHCl_3$); ^1H NMR: δ 1.61 (br s, 4H, 2 × CH_2sp), 2.83 (d, 1H, –OH), 3.19–3.23 (m, 2H, CH_2sp), 3.38–3.40 (m, 2H, CH_2sp), 3.54–3.58 (m, 3H, H-3, 5), 3.66–3.72 (m, 2H, H-1), 3.82–3.87 (m, 1H, H-2), 3.93 (br s, 1H, H-4), 4.42–4.69 (m, 6H, 3 × CH_2Ph), 7.18–7.29 (m, 15H, aromatic); ^{13}C NMR: δ 25.9, 27.0, 51.4, 70.4, 70.9, 71.0, 71.3, 72.6, 73.5, 73.9, 79.0, 79.3, 127.7, 127.8 (×2), 127.9 (×4), 128.0 (×2), 128.1 (×2), 128.5 (×4), 128.6 (×2), 138.2, 138.6. FABMS *m/z* $[M+H]^+$ calcd for $C_{30}H_{38}N_3O_5$: 520.2811. Found: 520.2827.

3.9. Typical glycosylation procedures: preparation of pseudo-oligosaccharides

3.9.1. Method A. AgOTf-promoted activation of the SBox glycosyl donors. A mixture of the glycosyl donor (0.11 mmol), glycosyl acceptor (0.10 mmol), and freshly activated molecular sieves (3 Å, 200 mg) in $ClCH_2CH_2Cl$ (2 mL) was stirred under argon for 1.5 h. Freshly conditioned AgOTf (0.22 mmol) was added and the reaction mixture was stirred for 15 min at rt, then diluted with CH_2Cl_2, the solid was filtered off and the residue was washed with CH_2Cl_2. The combined filtrate (30 mL) was washed with 20% aq $NaHCO_3$ (15 mL) and water (3 × 10 mL), the organic phase was separated, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (EtOAc–hexanes gradient elution) to afford a pseudo-oligosaccharide derivative.

3.9.2. Method B. NIS–TfOH-promoted activation of *S*-ethyl glycosyl donors. A mixture of the glycosyl donor (0.13 mmol), glycosyl acceptor (0.10 mmol), and freshly activated molecular sieves (4 Å, 200 mg) in $ClCH_2CH_2Cl$ (2 mL) or ether–DCE was stirred for 1 h under argon. NIS (0.25 mmol) and TfOH (0.025 mmol) were added at −30 °C to 0 °C and the reaction mixture was stirred for 10 min. Upon completion, the solid was filtered off and the residue was washed with CH_2Cl_2. The combined filtrate (30 mL) was washed with 20% aq $Na_2S_2O_3$ (15 mL) and water (3 × 10 mL). The organic phase was separated, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (EtOAc–hexanes gradient elution) or Sephadex LH-20 (methanol–CH_2Cl_2, 1:1) to afford a pseudo-oligosaccharide derivative.

3.10. 1-*O*-(4-Azidobutyl)-3-*O*-(2,3,4-tri-*O*-benzoyl-α-L-rhamnopyranosyl)-2,4,5-tri-*O*-benzyl-D-ribitol (20)

The title compound was obtained by Method B from **9** and **11** in 75% yield. Analytical data for **20**: $R_f = 0.50$ (EtOAc–hexanes 3:7); $[\alpha]_D^{29}$ +37.8 (*c* 1, $CHCl_3$); ^1H NMR: δ 1.11 (d, 3H, H-6′), 1.58 (br s, 4H, 2 × CH_2sp), 3.18 (s, 2H, CH_2sp), 3.36 (d, 2H CH_2sp), 3.66–3.70 (d, 4H, H-1, 5), 3.72–3.75 (m, 1H, H-2), 3.82–3.86 (m, 1H, H-4), 4.20–4.32 (m, 2H, H-3, 5′), 4.40–4.60 (m, 6H, 3 × CH_2Ph), 5.21 (s, 1H, H-1′), 5.52–5.59 (m, 2H, H-2′, 4′), 5.67 (dd, 1H, $J_{2',3'} = 3.1$ Hz, H-3′), 7.17–8.06 (m, 30H, aromatic); ^{13}C NMR: δ 18.2, 26.4, 27.5, 51.9, 67.8, 69.0, 70.7, 71.2, 71.3, 71.5, 72.4, 72.6, 72.7, 73.8, 78.3, 98.3, 128.2 (×3), 128.4 (×3), 128.5 (×6), 128.9 (×6), 129.0 (×3), 129.2 (×3), 129.6, 129.8, 130.1, 130.3 (×4), 130.5 (×2), 133.7, 133.9, 134.0, 138.8, 139.0, 166.1, 166.2, 166.4. FABMS *m/z* $[M+Na]^+$ calcd for $C_{57}H_{59}N_3O_{12}Na$: 1000.3996. Found: 1000.3963.

3.11. 1-*O*-(4-Aminobutyl)-3-*O*-(α-L-rhamnopyranosyl)-D-ribitol (5)

To a solution of **20** (60 mg, 0.06 mmol) in dry methanol (1.0 mL) was added 1M NaOMe to pH 9 (~0.1 mL). The reaction mixture was stirred for 15 h at rt, then neutralized with Dowex (H^+), filtered, and concentrated in vacuo. The crude residue was dissolved in a mixture of ethanol–HCl (12:0.03) and 10% Pd–C (70 mg) was added. The reaction mixture was stirred under an atmosphere of H_2 for 8 h. When TLC showed the formation of ninhydrin positive spot on the baseline, the catalyst was filtered off and the filtrate was neutralized with Dowex (OH^-) resin and concentrated under reduced pressure. The residue was co-evaporated with water (2 × 2 mL) and then purified by column chromatography on Sephadex G-15 (water elution) to afford compound **5** as a syrup (19 mg, 85% yield). Analytical data for **5**: $[\alpha]_D^{26}$ −24.4 (*c* 0.5, H_2O); ^1H NMR (D_2O): δ 1.32 (d, 3H, H-6′), 1.72–1.74 (m, 4H, 2 × CH_2sp), 2.99–3.02 (m, 2H, CH_2sp), 3.51 (dd, 1H, $J_{3',4'} = 9.6$ Hz, H-4′), 3.62–3.65 (m, 2H, CH_2sp), 3.69–3.71 (m, 2H, H-1b, 5b), 3.75–3.90 (m, 5H, H-1a, 5a, 3, 3′, 5′), 3.99–4.03 (m, 2H, H-2, 4), 4.08–4.10 (m, 1H, H-2′), 4.99 (d, 1H, $J_{1',2'} = 1.7$ Hz, H-1′) ppm; ^{13}C NMR (D_2O): δ 16.9, 24.8, 26.1, 39.8, 62.7, 69.7, 70.3, 70.5, 70.7, 70.8, 71.1, 71.8, 72.2, 80.4, 100.9. FABMS *m/z* $[M+H]^+$ calcd for $C_{15}H_{32}O_9N$: 370.2077. Found: 370.2071.

3.12. 1-*O*-(4-Azidobutyl)-4-*O*-(2,3,4-tri-*O*-benzoyl-α-L-rhamnopyranosyl)-2,3,5-tri-*O*-benzyl-D-ribitol (21)

The title compound was obtained by Method B from **9** and **12** in 87% yield. Analytical data for **21**: $R_f = 0.50$ (EtOAc–hexanes 3:7); $[\alpha]_D^{25}$ +61.2 (*c* 1, $CHCl_3$); ^1H NMR: δ 1.07 (d, 3H, H-6′), 1.59 (s, 4H, 2 × CH_2sp), 3.13 (d, 2H, CH_2sp), 3.41–3.50 (m, 2H, CH_2sp), 3.61–3.67 (m, 3H, H-3, 5), 3.71 (d, 1H, H-2), 3.74–3.80 (m, 2H, H-1), 4.18–4.23 (m, 1H, H-5′), 4.28–4.33 (m, 1H, H-4), 4.37–4.70 (m, 6H, 3 × CH_2Ph), 5.36 (dd, 1H, $J_{1',2'} = 7.4$ Hz, H-1′), 5.55 (dd, 1H, $J_{3',4'} = 9.9$ Hz, H-3′), 5.66–5.77 (m, 2H, H-2′, 4′), 7.11–8.02 (m, 30H, aromatic); ^{13}C NMR: δ 18.2, 26.5, 27.6, 51.9, 67.7, 70.7, 71.3, 71.5, 71.6, 72.5, 73.4, 74.0, 74.1, 76.4, 78.8, 79.6,

97.4, 128.2, 128.3 (×2), 128.4 (×3), 128.5 (×2), 128.7 (×2), 128.9 (×2), 129.0 (×6), 129.2 (×2), 129.9, 130.1, 130.2, 130.3 (×4), 130.4 (×2), 130.6 (×2), 133.7, 133.9, 134.0, 138.7, 138.8, 139.1, 166.1, 166.2, 166.4. FABMS *m/z* $[M+Na]^+$ calcd for $C_{57}H_{59}N_3O_{12}Na$: 1000.3996. Found: 1000.3963.

3.13. 1-*O*-(4-Azidobutyl)-4-*O*-(α-L-rhamnopyranosyl)-D-ribitol (6)

The title compound was obtained from **21** using the same reaction conditions as described for the synthesis of compound **5** in 90% yield. Analytical data for **6**: $[\alpha]_D^{26}$ −81.5 (*c* 1.0, H_2O); ^1H NMR (D_2O): δ 1.34 (d, 3H, H-6′), 1.71–1.82 (m, 4H, 2 × CH_2sp), 3.06–3.09 (m, 2H, CH_2sp), 3.52 (dd, 1H, $J_{3',4'} = 9.7$ Hz, H-4′), 3.60–3.69 (m, 3H, H-1b, CH_2sp), 3.75–3.85 (m, 4H, H-2, 3, 3′, 5′), 3.89–3.95 (m, 4H, H-1a, 4, 5), 4.07 (dd, 1H, $J_{2',3'} = 1.7$ Hz, H-2′), 5.06 (dd, 1H, $J_{1',2'} = 1.5$ Hz, H-1′); ^{13}C NMR (D_2O): δ 17.1, 24.1, 26.1, 39.7, 59.6, 69.6, 70.6, 70.7, 70.9, 71.7, 72.3, 72.4, 78.6, 100.3; HR-FAB HR-FAB MS $[M+H]^+$ calcd for $C_{15}H_{32}O_9N$: 370.2077. Found: 370.2071.

3.14. *O*-(3-*O*-Acetyl-2-*O*-benzyl-4,6-*O*-benzylidene-α-D-glucopyranosyl)-(1→3)-*O*-(2,4-di-*O*-benzoyl-α-L-rhamnopyranosyl)-(1→3)-1-*O*-(4-azidobutyl)-2,4,5-tri-*O*-benzyl-D-ribitol (23)

The title compound was obtained by Method B from **22** and **11** in 80% yield. Analytical data for **23**: $R_f = 0.38$ (EtOAc–hexanes 3:7); $[\alpha]_D^{22}$ +50.3 (*c* 0.8, $CHCl_3$); ^1H NMR: δ 1.07 (d, 3H, H-6′), 1.55 (br s, 4H, 2 × CH_2sp), 1.73 (s, 3H, $COCH_3$), 3.13–3.19 (m, 2H, CH_2sp), 3.22 (dd, 1H, $J_{1a,1b} = 9.6$ Hz, H-1a), 3.32–3.40 (m, 3H, H-2′, CH_2sp), 3.59–3.73 (m, 5H, H-1b, 2, 4, 5), 3.79–3.83 (m, 3H, H-3, 3′, 4′), 4.14–4.19 (m, 3H, H-5′, 5″, 6b″), 4.24–4.30 (m, 1H, H-6a″), 4.39–4.69 (m, 8H, 4 × *CH*$_2$Ph), 4.99 (dd, 1H, $J_{1',2'} = 3.2$ Hz, H-1′), 5.10–5.17 (m, 3H, H-1″, 3″, C*H*Ph), 5.45–5.51 (m, 2H, H-2″, 4″), 6.94–7.99 (m, 35H, aromatic); ^{13}C NMR: δ 17.8, 20.9, 25.9, 27.1, 51.4, 62.9, 67.6, 68.7, 68.9, 69.0, 70.5, 70.6, 70.8, 70.9, 72.1, 72.2, 72.4, 72.6, 73.4, 79.4, 95.1, 97.8, 101.3, 126.5 (×2), 127.7 (×4), 127.8 (×4), 127.9 (×4), 128.0 (×6), 128.3 (×4), 128.5 (×6), 128.7 (×4), 129.6, 129.8, 130.2, 133.3, 133.4, 137.2, 137.9, 138.4, 138.5, 138.6, 165.7, 166.4, 169.3. FABMS *m/z* $[M+Na]^+$ calcd for $C_{72}H_{77}N_3O_{17}Na$: 1278.5151. Found: 1278.5138.

3.15. *O*-(α-D-Glucopyranosyl)-(1→3)-*O*-(α-L-rhamnopyranosyl)-(1→3)-1-*O*-(4-aminobutyl)-D-ribitol (3)

The title compound was obtained from **23** as described for the synthesis of compound **5** in 88% yield. Analytical data for **3**: $[\alpha]_D^{25}$ +33.2 (*c* 1.0, H_2O); ^1H NMR (D_2O): δ 1.35 (d, 3H, H-6′), 1.74–1.79 (m, 4H, 2 × CH_2sp), 3.06–3.08 (m, 2H, CH_2sp), 3.51 (dd, 1H, $J_{4'',5''} = 9.2$ Hz, H-4″), 3.58–3.65 (m, 4H, H-4′, 2″, CH_2sp), 3.68–3.72 (m, 2H, H-1b, 5b), 3.77–3.93 (m, 8H, H-1a, 3, 5a, 3′, 5′, 3″, 6a″, 6b″), 3.99–4.03 (m, 2H, H-2, 4), 4.09–4.11 (m, 1H, H-5″), 4.23 (dd, 1H, $J_{2',3'} = 2.6$ Hz, H-2′), 5.05 (d, 1H, $J_{1',2'} = 1.7$ Hz, H-1′), 5.15 (d, 1H, $J_{1'',2''} = 3.5$ Hz, H-1″); ^{13}C NMR (D_2O): δ, 17.2, 24.1, 26.1, 39.7, 60.7, 62.7, 62.9, 67.3, 69.7, 69.8, 70.5, 70.7, 71.1, 71.7, 72.0, 72.5, 73.3, 75.7, 80.4, 95.8, 100.5. FABMS *m/z* $[M+H]^+$ calcd for $C_{21}H_{42}NO_{14}$: 532.2605. Found: 532.2606.

3.16. *O*-(3-*O*-Acetyl-2-*O*-benzyl-4,6-*O*-benzylidene-α-D-glucopyranosyl)-(1→3)-*O*-(2,4-di-*O*-benzoyl-α-L-rhamnopyranosyl)-(1→4)-1-*O*-(4-azidobutyl)-2,3,5-tri-*O*-benzyl-D-ribitol (24)

The title compound was obtained by Method B from **22** and **12** in 78% yield. Analytical data for **24**: $R_f = 0.41$ (EtOAc–hexanes 3:7); $[\alpha]_D^{22}$ +58.7 (*c* 1, $CHCl_3$); ^1H NMR: δ 1.09 (d, 3H, H-6′), 1.59 (bs, 4H, 2 × CH_2sp), 1.76 (s, 3H, $COCH_3$), 3.15 (br s, 2H, CH_2sp), 3.26 (dd, 1H, $J_{3'',4''} = 9.3$ Hz, H-4″), 3.38–3.45 (m, 4H, H-3, 2″, CH_2sp), 3.60–3.74 (m, 5H, H-2, 5, 5″, 6b″), 3.78–3.86 (m, 3H, H-1, 6a″), 4.12–4.15 (m, 1H, H-5′), 4.22–4.28 (m, 1H, H-4), 4.33 (s, 1H, H-3′), 4.38–4.70 (m, 8H, 4 × *CH*$_2$Ph), 5.12–5.18 (m, 3H, H-1″, 3″, C*H*Ph), 5.36 (dd, 1H, $J_{1',2'} = 1.5$ Hz, H-1′), 5.54 (dd, 1H, $J_{3',4'} = 9.8$ Hz, H-4′), 5.66 (d, 1H, $J_{2',3'} = 2.7$ Hz, H-2′), 6.95–8.11 (m, 35H, aromatic); ^{13}C NMR: δ 17.9, 20.9, 25.9, 27.1, 51.4, 62.8, 67.6, 68.7, 70.1, 70.4, 71.0, 71.1, 71.7, 72.1, 72.6, 72.9, 73.5, 75.7, 78.6, 79.2, 79.4, 94.4, 96.8, 101.2, 126.5 (×2), 127.6, 127.7 (×3), 127.8 (×2), 127.9 (×3), 128.0 (×6), 128.1 (×3), 128.3 (×3), 128.5 (×6), 128.6 (×3), 128.8, 129.6, 129.7, 129.8, 130.2, 133.2, 133.4, 137.2, 137.9, 138.2, 138.3, 138.5, 165.7, 166.3, 169.3. FABMS *m/z* $[M+Na]^+$ calcd for $C_{72}H_{77}N_3O_{17}Na$: 1278.5151. Found: 1278.5138.

3.17. *O*-(α-D-Glucopyranosyl)-(1→3)-*O*-(α-L-rhamnopyranosyl)-(1→4)-1-*O*-(4-aminobutyl)-D-ribitol (4)

The title compound was obtained from **24** as described for the synthesis of compound **5** in 78% yield. Analytical data for **4**: $[\alpha]_D^{27}$ +25.4 (*c* 0.4, H_2O); ^1H NMR (D_2O): δ 1.35 (d, 3H, H-6′), 1.70–1.72 (m, 4H, 2 × CH_2sp), 2.82–2.84 (m, 2H, CH_2sp), 3.51 (dd, 1H, $J_{4'',5''} = 9.5$ Hz, H-4″), 3.61–3.65 (m, 4H, H-4′, 2″, CH_2sp), 3.75–3.87 (m, 7H, H-1, 2, 3, 5′, 3″, 6a″), 3.91–3.95 (m, 5H, H-5, 3′, 5″, 6b″), 4.01–4.04 (m, 1H, H-4), 4.27 (dd, 1H, $J_{2',3'} = 2.5$ Hz, H-2′), 5.12 (d, 1H, $J_{1',2'} = 1.3$ Hz, H-1′), 5.18 (d, 1H, $J_{1'',2''} = 3.2$ Hz, H-1″); ^{13}C NMR (D_2O): δ 17.1, 24.5, 26.2, 40.0, 59.6, 60.6, 67.4, 69.7 (×2), 70.5 (×2), 71.2, 71.6, 71.7, 72.0, 72.4, 73.2, 75.9, 78.8, 95.9, 99.9. FABMS *m/z* $[M+H]^+$ calcd for $C_{21}H_{42}NO_{14}$: 532.2605. Found: 532.2606.

3.18. Ethyl *O*-(4-*O*-anisoyl-2,3,6-tri-*O*-benzyl-α-D-galactopyranosyl)-(1→3)-2-*O*-benzyl-4,6-*O*-benzylidene-1-thio-β-D-glucopyranoside (25)

The title compound was obtained by Method A from **7** and **8** in 99% yield. Analytical data for **25**: $R_f = 0.50$ (EtOAc–hexanes 3:7); $[\alpha]_D^{29}$ +42.7 (*c* 1, $CHCl_3$); ^1H NMR: δ 1.15 (t, 3H, CH_3), 2.77–2.81 (m, 2H, CH_2), 3.16 (dd, 1H, $J_{5',6a'} = 5.2$ Hz, $J_{6a',6b'} = 4.9$ Hz, H-6a′), 3.34 (dd, 1H, $J_{5',6b'} = 6.9$ Hz, H-6b′), 3.46–3.51 (m, 1H, H-4), 3.57 (dd, 1H, $J_{3,4} = 4.9$ Hz, H-3), 3.81 (dd, 1H, $J_{6a,6b} = 10.3$ Hz, H-6b), 3.85–3.91 (m, 4H, H-6a, 2′, –OCH_3), 4.05 (dd, 1H, $J_{3',4'} = 3.2$ Hz, $J_{2',3'} = 6.9$ Hz, H-3′), 4.12–4.19 (m, 4H, 2 × *CH*$_2$Ph), 4.33–4.37 (m, 2H, H-5, 5′), 4.52 (dd, 1H, $J_{2,3} = 3.2$ Hz, H-2), 4.57–4.83 (m, 4H, 2 × *CH*$_2$Ph), 5.05 (dd, 1H, $J_{1,2} = 7.1$ Hz, H-1), 5.45 (dd, 1H, $J_{4',5'} = 2.6$ Hz, H-4′), 5.50 (s, 1H, C*H*Ph), 5.71 (dd, 1H, $J_{1',2'} = 3.5$ Hz, H-1′), 6.85–7.95 (m, 30H, aromatic); ^{13}C NMR: δ 15.3, 25.3, 53.6, 67.5, 68.9 (×2), 70.1, 72.0, 72.3, 72.9, 74.7, 75.4, 75.8, 76.4, 80.3, 82.6, 85.2, 96.8, 102.1, 113.7 (×2), 127.4, 127.6 (×2), 127.7, 127.8 (×2), 128.1 (×2), 128.2 (×5), 128.4 (×5), 128.5 (×3), 128.6 (×3), 128.7 (×3), 129.5, 132.1 (×2), 137.3, 137.7, 138.3, 138.6. FABMS *m*/*z* $[M+H]^+$ calcd for $C_{57}H_{61}O_{12}S$: 969.3883. Found: 969.3884.

3.19. 1-*O*-(4-Azidobutyl)-3-*O*-(2,4-di-*O*-benzoyl-α-L-rhamnopyranosyl)-2,4,5-tri-*O*-benzyl-D-ribitol (26)

The title compound was obtained by Method B from **10** and **11** in 65% yield. Analytical data for **26**: $R_f = 0.57$ (EtOAc–hexanes 2:3); $[\alpha]_D^{28}$ −14.9 (*c* 1, $CHCl_3$); ^1H NMR: δ 1.11 (d, 3H, H-6′), 1.59 (br s, 4H, 2 × CH_2sp), 3.18–3.22 (m, 2H, CH_2sp), 3.31–3.35 (m, 2H, CH_2sp), 3.62–3.73 (d, 4H, H-1, 5), 3.78–3.86 (m, 2H, H-2, 4), 4.15–4.24 (m, 3H, H-3, 3′, 5′), 4.42–4.63 (m, 6H, 3 × *CH*$_2$Ph), 5.13–5.21 (m, 2H, H-1′, 4′), 5.32–5.34 (m, 1H, H-2′), 7.19–8.11 (m, 25H, aromatic); ^{13}C NMR: δ 17.7, 25.9, 27.2, 51.5, 67.0, 68.6, 69.3, 70.7, 71.0, 72.1, 72.3, 73.3, 73.5, 75.8, 77.9, 97.5, 127.7 (×2), 127.9 (×3), 128.0 (×6), 128.4 (×6), 128.7 (×4), 129.6, 129.7, 130.1 (×4), 133.7 (×2), 138.4 (×2), 138.6, 166.1, 167.4. FABMS *m*/*z* $[M+Na]^+$ calcd for $C_{50}H_{55}N_3O_{11}Na$: 896.3734. Found: 896.3732.

3.20. *O*-(4-*O*-Anisoyl-2,3,6-tri-*O*-benzyl-α-D-galactopyranosyl)-(1→3)-*O*-(2-*O*-benzyl-4,6-*O*-benzylidene-α-D-glucopyranosyl)-(1→3)-*O*-(2,4-di-*O*-benzoyl-α-L-rhamnopyranosyl)-(1→3)-1-*O*-(4-azidobutyl)-2,4,5-tri-*O*-benzyl-D-ribitol (27)

The title compound was obtained by Method B from **25** and **26** in 66% yield. Analytical data for **27**: $R_f = 0.60$ (EtOAc–hexanes 2:3); $[\alpha]_D^{28}$ +55.6 (*c* 1.0, $CHCl_3$); ^1H NMR: δ 1.19 (d, 3H, H-6′), 1.60–1.64 (m, 4H, 2 × CH_2sp), 3.24 (d, 4H, H-6a‴, 6b‴, CH_2sp), 3.38–3.42 (m, 2H, CH_2sp), 3.45–3.53 (m, 2H, H-4″, 6a″), 3.59 (dd, 1H, H-2″), 3.67–3.70 (m, 5H, H-1, 5, 2‴), 3.76–3.80 (m, 3H, H-5″, 6b″, 3‴), 3.85 (s, 3H, –OCH_3), 3.88–3.93 (m, 2H, H-2, 4), 3.97–4.05 (m, 1H, H-3), 4.07 (dd, 1H, $J_{3'',4''} = 4.8$ Hz, H-3″), 4.17–4.28 (m, 4H, H-5′, 5‴, C*H*$_2$Ph), 4.36–4.40 (m, 3H, H-3′, C*H*$_2$Ph), 4.45–4.77 (m, 10H, 5 × C*H*$_2$Ph), 5.08 (d, 1H, $J_{1'',2''} = 3.5$ Hz, H-1″), 5.28 (s, 1H, H-1‴), 5.30 (s, 2H, H-1′, C*H*Ph), 5.52 (dd, 1H, $J_{3''',4'''} = 3.3$ Hz, H-4‴), 5.62–5.66 (m, 2H, H-2′, 4′), 6.81–8.09 (m, 55H, aromatic); ^{13}C NMR: δ 17.9, 25.9, 27.1, 36.8, 51.4, 55.6, 62.6, 67.1, 67.8, 68.0, 68.4, 68.8, 68.9, 69.4, 70.8, 70.9, 71.7, 71.9, 72.1, 72.2, 72.3, 72.4, 72.8, 72.9, 73.2, 73.4, 74.7, 75.9, 77.7, 77.8, 77.9, 82.9, 94.9, 96.8, 97.7, 101.9, 113.6, 123.1, 126.8 (× 2), 127.3, 127.4, 127.6 (×2), 127.7 (×2), 127.9 (×5), 127.96 (×3), 128.0 (×4), 128.1 (×6), 128.2 (×2), 128.3 (×4), 128.5 (×6), 128.6 (×3), 128.7, 128.8, 129.2, 129.7, 129.8, 129.9, 130.2, 132.0 (×4), 133.3, 133.5, 137.4, 137.5, 138.4, 138.5 (×2), 138.6 (×2), 138.8, 163.3, 165.6, 165.8, 166.2. FABMS *m*/*z* $[M+Na]^+$ calcd for $C_{105}H_{109}N_3O_{23}Na$: 1802.7344. Found: 1802.7366.

3.21. *O*-(α-D-Galactopyranosyl)-(1→3)-*O*-(α-D-glucopyranosyl)-(1→3)-*O*-(α-L-rhamnopyranosyl)-(1→3)-1-*O*-(4-aminobutyl)-D-ribitol (1)

The title compound was obtained from **27** in 70% yield similarly to that described for the synthesis of compound **5**, the only difference being pH 12 to ensure the rapid removal of the anisoyl moiety. Analytical data for **1**: $[\alpha]_D^{25}$ +80.8 (*c* 1.0, H_2O); ^1H NMR (D_2O): δ 1.21 (d, 3H, H-6′), 1.71–1.76 (m, 4H, 2 × CH_2sp), 3.04–3.07 (m, 2H, CH_2sp), 3.55–3.69 (m, 16H), 3.71–3.88 (m, 14H), 3.92–4.07 (m, 8H), 4.22–4.27 (m, 2H, H-2′, 5‴), 5.02 (d, 1H, $J_{1',2'} = 1.8$ Hz, H-1′), 5.13 (d, 1H, $J_{1'',2''} = 6.3$ Hz, H-1″), 5.40 (d, 1H, $J_{1''',2'''} = 3.7$ Hz, H-1‴); ^{13}C NMR. (D_2O): δ 17.1, 24.1, 26.1, 39.7, 60.5, 61.2, 62.7 (×2), 67.1, 68.9, 69.5, 69.7, 69.8, 70.1, 70.2, 70.4, 70.7, 71.1, 71.8, 72.4 (×2), 75.5, 79.9, 80.4, 90.0, 95.7, 99.7, 100.5. FABMS *m*/*z* $[M+H]^+$ calcd for $C_{27}H_{52}NO_{19}$: 694.3134. Found 694.3141.

3.22. 1-*O*-(4-Azidobutyl)-4-*O*-(2,4-di-*O*-benzoyl-α-L-rhamnopyranosyl)-2,3,5-tri-*O*-benzyl-D-ribitol (28)

The title compound was obtained by Method B from **10** and **12** in 78% yield. Analytical data for **28**: $R_f = 0.49$ (EtOAc–hexanes 3:7); $[\alpha]_D^{29}$ +3.2 (*c* 0.9, $CHCl_3$); ^1H NMR: δ 1.07 (d, 3H, H-6′), 1.56 (s, 4H, 2 × CH_2sp), 2.40 (d, 1H, –OH), 3.06–3.09 (m, 2H, CH_2sp), 3.38–3.40 (d, 2H, CH_2sp), 3.57–3.65 (m, 3H, H-2, 3, 5), 3.70–3.73 (m, 2H, H-1), 4.00–4.14 (m, 2H, H-3′, 5′), 4.22 (bs, 1H, H-4), 4.36–4.86 (m, 6H, 3 × *CH*$_2$Ph),

5.14 (dd, 1H, $J_{1',2'} = 9.8$ Hz, H-1′), 5.31–5.37 (m, 2H, H-2′, 4′), 7.14–8.05 (m, 25H, aromatic); ^{13}C NMR: δ 18.2, 26.4, 27.6, 51.8, 67.3, 67.7, 70.9, 71.3, 71.5, 73.1, 74.0, 74.1 (×2), 76.0, 76.2, 78.5, 79.7, 97.0, 128.2, 128.3 (×2), 128.4 (×3), 128.5 (×2), 128.6 (×2), 128.9 (×6), 129.1 (×2), 129.2 (×2), 130.2, 130.5 (×2), 130.6 (×2), 134.1 (×2), 138.7, 138.9 (×2), 166.6, 167.8. FABMS m/z $[M+Na]^+$ calcd for $C_{50}H_{55}N_3O_{11}Na$: 896.3734. Found: 896.3732.

3.23. *O*-(4-*O*-Anisoyl-2,3,6-tri-*O*-benzyl-α-D-galactopyranosyl)-(1→3)-*O*-(2-*O*-benzyl-4,6-*O*-benzylidene-α-D-glucopyranosyl)-(1→3)-*O*-(2,4-di-*O*-benzoyl-α-L-rhamnopyranosyl)-(1→4)-1-*O*-(4-azidobutyl)-2,3,5-tri-*O*-benzyl-D-ribitol (29)

The title compound was obtained by Method B from **25** and **28** in 70% yield. Analytical data for **29**: $R_f = 0.56$ (EtOAc–hexanes 2:3); $[\alpha]_D^{29}$ +54.8 (c 1.0, $CHCl_3$); ^{1}H NMR: δ 1.15 (d, 3H, H-6′), 1.64 (br s, 4H, 2 × CH_2sp), 3.21–3.23 (m, 4H, CH_2sp, H-6a‴, 6b‴), 3.40–3.43 (m, 3H, CH_2sp, H-6b″), 3.44–3.47 (m, 1H, H-6a″), 3.49–3.51 (dd, 1H, H-2″), 3.57–3.61 (m, 2H, H-2‴, H-4″), 3.64–3.70 (m, 5H, H-2, 3, 5, 3‴), 3.74–3.80 (m, 6H, H-1, 5″, –OCH_3), 3.86–3.90 (m, 1H, H-3), 3.96 (dd, 1H, $J_{3'',4''} = 9.5$ Hz, H-3″), 4.17–4.21 (m, 3H, H-5′, CH_2Ph), 4.24–4.26 (m, 1H, H-5‴), 4.33–4.35 (m, 1H, H-4), 4.40–4.54 (m, 9H, H-3′, 4 × CH_2Ph), 4.59–4.75 (m, 4H, 4 × CH_2Ph), 5.16 (d, 1H, $J_{1'',2''} = 3.3$ Hz, H-1″), 5.28 (s, 2H, H-1‴, C*H*Ph), 5.45 (s, 1H, H-1′), 5.49 (br s, 1H, H-4‴), 5.66 (dd, 1H, $J_{4',5'} = 9.8$ Hz, H-4′), 5.74 (br s, 1H, H-2′), 6.80–8.09 (m, 55H, aromatic); ^{13}C NMR: δ 17.9, 26.0, 27.1, 51.4, 55.6, 62.5, 67.0, 67.7, 68.1, 68.4, 68.9, 70.2, 71.1, 71.6, 71.7, 71.8, 72.0, 72.7, 72.9, 73.0, 73.2, 73.6, 73.8, 74.7, 75.9, 76.0, 77.4 (×3), 78.7, 79.3, 82.8, 94.2, 96.7, 96.8, 101.8, 113.5 (×2), 123.1, 126.7 (×2), 127.3 (×2), 127.4, 127.6 (×2), 127.8 (×2), 127.9 (×6), 128.0 (×3), 128.1 (×3), 128.15 (×5), 128.19 (×3), 128.25 (×3), 128.3 (×3), 128.4 (×2), 128.6 (×6), 128.7 (×2), 128.8 (×2), 129.2, 129.7, 129.9, 130.2, 131.9, 133.3, 133.5, 137.3, 137.4, 138.3 (×2), 138.4, 138.5 (×2), 138.8, 163.3, 165.6, 165.7, 166.1. FABMS m/z $[M+Na]^+$ calcd for $C_{105}H_{109}N_3O_{23}Na$: 1802.7344. Found: 1802.7366.

3.24. *O*-(α-D-Galactopyranosyl)-(1→3)-*O*-(α-D-glucopyranosyl)-(1→3)-*O*-(α-L-rhamnopyranosyl)-(1→4)-1-(4-aminobutyl)-D-ribitol (2)

The title compound was obtained from **29** as described for the synthesis of compound **1** in 76% yield. Analytical data for **1**: $[\alpha]_D^{25}$ +73.8 (c 0.8, H_2O); ^{1}H NMR (D_2O): δ 1.23 (d, 3H, H-6′), 1.72–1.74 (m, 4H, 2 × CH_2sp), 2.98–3.01 (m, 2H, CH_2sp), 3.57–3.63 (m, 6H), 3.63–3.70 (m, 7H), 3.71–3.84 (m, 12H), 3.87–3.99 (m, 9H), 4.01–4.05 (m, 2H), 4.28–4.31 (m, 2H, H-2′, 5‴), 5.11 (d, 1H, $J_{1',2'} = 1.8$ Hz, H-1′), 5.16 (d, 1H, $J_{1'',2''} = 3.3$ Hz, H-1″), 5.45 (d, 1H, $J_{1''',2'''} = 3.7$ Hz, H-1‴); ^{13}C NMR (D_2O): δ 17.5, 24.4, 26.1, 39.8, 60.7, 61.4, 62.1, 63.9, 67.0, 68.2, 69.9, 70.4, 70.6 (×2), 71.1, 71.7, 72.1, 72.8, 72.9, 73.3, 76.5, 79.8, 81.1, 96.8, 100.6, 101.5. FABMS m/z $[M+H]^+$ calcd for $C_{27}H_{52}NO_{19}$: 694.3134. Found: 694.3141.

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