En route to a carbohydrate-based vaccine against *Burkholderia cepacia*

Régis Fauré, Tze Chieh Shiao, David Lagnoux, Denis Giguère and René Roy*

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We report a very high yielding first total synthesis of trisaccharide **5**, α-D-Rha*p*-(1→3)-α-D-Rha*p*-(1→4)-α-D-Gal*p*, corresponding to the repeating unit **1** of an *O*-polysaccharide present in the lipopolysaccharide of clinical isolate of *Burkholderia cepacia*. The approach included two successive glycosylations, based on D-rhamnosyl trichloroacetimidate donors **12** and **14**. The oligosaccharide **5** has been further functionalized by photochemical coupling or cross-metathesis with non-natural amino acid derivatives. Trisaccharidylamino acids **16** and **17** are now available, with the aim of preparing a novel synthetic carbohydrate-based vaccine.

Introduction

Over the past two decades, *Burkholderia cepacia* (*B. cepacia*), initially known to cause onion bulb rot, has emerged as an important life-threatening opportunistic pathogen, especially for patients afflicted with cystic fibrosis.[1] Pulmonary colonisation by these bacteria can lead to an accelerated decline in lung function and, in some cases, to a fatal necrotizing pneumonia, known as "cepacia syndrome". Despite such devastating effects on cystic fibrosis and immunocompromised patients, *B. cepacia* is also considered by the agricultural industry to be a potentially useful biopesticide. In addition, lipase from this organism has attracted considerable interest from the biotechnological industry for catalytic applications. Therefore, at present there is a clear conflict of interest between public health and industrial objectives.[2] To complicate matters, *B. cepacia*, a Gram-negative bacterium, displays multiple antibiotic resistance and is highly virulent and transmissible.[3] Taken together, these factors provide motivation for efforts aimed at the identification of an efficient therapeutic and preventative strategy.

Two types of polysaccharide, **1** and **2**, are present in the *O*-chain of the lipopolysaccharide of *B. cepacia* (Fig. 1).[4] These possess two and three unusual D-rhamnosyl residues respectively. These polysaccharides are thought to be interesting targets for vaccine development. Using the rhamnosyl-containing motifs as oligosaccharidic haptens coupled to an appropriate immunogenic peptide or protein carrier (T-cell epitope), it would be possible to induce a specific immune response against this pathogen. Indeed, the oligosaccharide–peptide conjugates will be capable of initiating a display of immunologic signals that result in both humoral and cell-mediated responses, notably through the major histocompatibility complex (MHC); thus resulting in an antibody secretion and an antigen-specific memory cell formation. Over the last decade, synthetic carbohydrate-based vaccines have emerged as powerful therapeutic agents.[5] Both our group and others have developed such glycoconjugate vaccines against *Haemophilus influenzae* type b,[6] and towards a variety of illnesses such as cancer,[7] malaria,[8] and even HIV.[9] However, one of the major problems associated with the elaboration of such carbohydrate-based vaccines is the poor synthetic yield of complex oligosaccharides. Consequently, it is essential to develop efficient synthesis strategies.

→3)-α-D-Rha*p*-(1→3)-α-D-Rha*p*-(1→4)-α-D-Gal*p*-(1→
1 (major)

→3)-α-D-Rha*p*-(1→3)-α-D-Rha*p*-(1→2)-α-D-Rha*p*-(1→
2 (minor)

Fig. 1 Linear trisaccharide repeating units of two polysaccharides present in the lipopolysaccharide of a clinical isolate of *B. cepacia*.

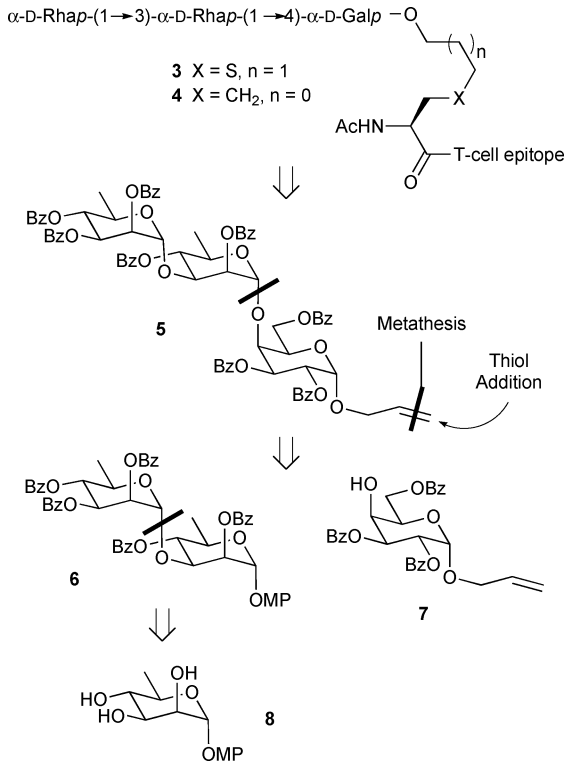
Herein, we report the first total synthesis, in very high yield, of the major trisaccharide repeating unit **1** of the *O*-polysaccharide of *B. cepacia*, as the first step toward a fully synthetic carbohydrate-based vaccine.

Results and discussion

Lipopolysaccharides are the major components of the outer surface of Gram-negative bacteria. These are of interest in medicine for their immunogenic properties. Given the fact that D-rhamnose is only encountered in microorganisms, notably in the O-antigens of *B. cepacia*,[4] and not in humans, animals or plants, rhamnose-containing conjugates are promising therapeutic targets. Potential vaccines **3** or **4** could be obtained from a common *O*-allyl intermediate **5** (Scheme 1). Thiol addition using protected cysteine or cross-metathesis with protected allyl glycine on **5** gave the desired intermediates *en route* to the vaccine, the thioether **3** or the *C*-analogue **4**, respectively. Trisaccharide **5** was synthesized from dirhamnoside **6** and tribenzoylated *O*-allylgalactoside **7**. The key intermediate **6** was assembled from the glycosylation of acceptor **9** and donor **12**, both derived from D-rhamnoside **8**.

In order to synthesize dirhamnoside **6** (Scheme 2), readily available rhamnoside **8** (prepared in 87% yield over five steps from D-mannose, using sequential glycosidation, iodination and reduction) was first converted into 3-*O*-glycosyl acceptor **9** in 76% over four steps, through selective benzylation.[10] Hemiacetal **11**, obtained by benzoylation of unprotected rhamnoside **8** followed by oxidative deprotection of the *p*-methoxyphenyl

Équipe PharmaQÀM, Département de Chimie, Université du Québec à Montréal, P. O. Box 8888, Succ. Centre-Ville, H3C 3P8, Montréal, Québec, Canada. E-mail: roy.rene@uqam.ca; Fax: +1 514 987 4054; Tel: +1 514 987 3000#2546



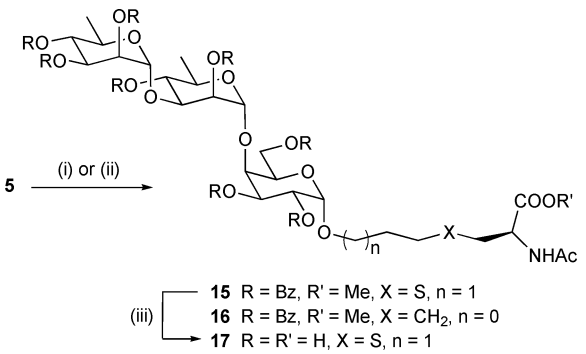
Scheme 1 Retrosynthetic analysis of targeted synthetic carbohydrate-based vaccines **3** and **4**.

group of **10** using cerium(IV) in nearly quantitative yield,[10] was then activated with trichloroacetonitrile in basic media to afford glycosyl trichloroacetimidate donor **12** in 96% yield. Coupling 3-*O*-unprotected rhamnoside acceptor **9** with rhamnosyl trichloroacetimidate **12** using trimethylsilyl triflate (TMSOTf) as activator afforded dirhamnoside **6** in almost quantitative yield.

The same strategy was applied toward the synthesis of dirhamnosyl donor **14**, *i.e.* removal of the *p*-methoxyphenyl group on **6** and then trichloroacetimidate synthesis from hemiacetal **10** performed in 89% yield over two steps. Galactoside **7** was obtained by selective tribenzoylation of commercial allyl α-D-galactopyranoside in 88% yield.[11] Despite the lack of reactivity of the axial 4-OH in the galactoside acceptor **7**, subsequent coupling with dirhamnosyl trichloroacetimidate **14** was achieved using TMSOTf to give key trisaccharide **5** in 66% yield.

Synthesis of trisaccharide **5** was thus performed in 16 linear steps, from D-mannose, with an overall yield of 36%. The α-stereochemistry of the *O*-glycosidic linkages was ascertained by comparison of experimental $^{1}J_{C\text{-}1,H\text{-}1}$ coupling constants (171.4, 173.4 and 172.3 Hz), determined by a coupled HSQC experiment, with known data for α-glycosides (*ca.* 170 Hz).[12]

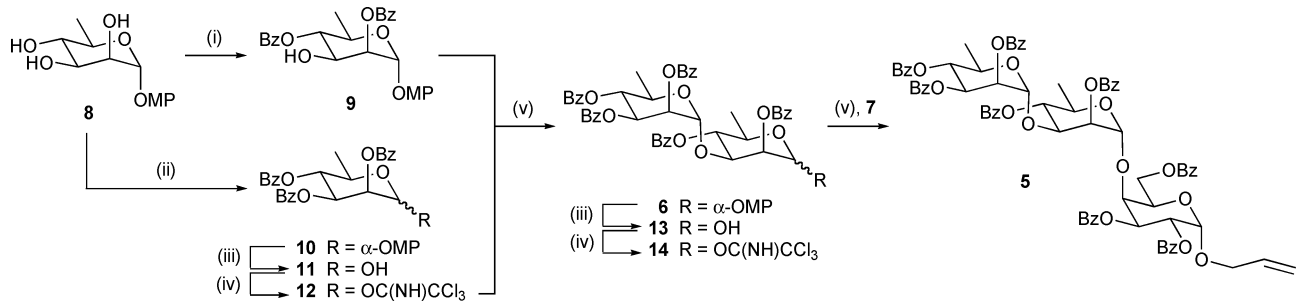
Two different approaches were used for final functionalisation of allyl trisaccharide **5** (Scheme 3). The first one consisted of a radical addition with *N*-acetyl-L-cysteine methyl ester, leading to trisaccharide **15** in 79% yield.[13] The second conjugation consisted of a cross-metathesis between **5** and commercially available *N*-acetyl-L-allylglycine methyl ester using Grubbs' first-generation catalyst. Hydrogenolysis of the nascent double bond in the resulting mixture of *E*- and *Z*-stereoisomers using 10% palladium-on-carbon gave **16** in 60% yield over two steps.[14] Global deprotection was achieved on thioether **15** using LiOH to afford the ultimate intermediate **17** in quantitative yield, ready for solid-phase peptidic coupling.



Scheme 3 *Reagents and conditions*: (i) *N*-acetyl-L-cysteine methyl ester, AIBN, THF, 254 nm, rt, 24 h, 79%; (ii) *N*-acetyl-L-allylglycine methyl ester, Grubbs' first-generation catalyst, CH_2Cl_2; then H_2, 10% Pd/C, MeOH, 60%; (iii) LiOH, THF–H_2O, quantitative yield.

Conclusions

In conclusion, we have developed a very high-yielding synthesis towards a new potential carbohydrate-based vaccine against *B. cepacia*. Coupling of trisaccharide **16** or unprotected trisaccharide **17** with a T-cell epitope is currently under investigation. Finally, dirhamnosyl donor **14** is also a key intermediate for the synthesis of the minor trisaccharidic repeating unit **2**, which is also under study.



Scheme 2 *Reagents and conditions*: (i) see ref. 10: 76% (over four steps); (ii) BzCl, pyridine, rt, 12 h, 99%; (iii) CAN, toluene–acetonitrile–water, rt, 12 h, **11**: 99%, **13**: 95%; (iv) CCl_3CN, DBU, CH_2Cl_2, rt, 1 h, **12**: 96%, **14**: 94%; (v) TMSOTf, 4 Å MS, CH_2Cl_2, −50 °C, 30 min, **6**: 99%, **5**: 66%.

Experimental

General methods

All reactions in organic media were carried out under nitrogen atmosphere using freshly distilled solvents.[15] After work-up, organic phases were dried over anhydrous Na_2SO_4. Evolution of reactions was monitored by analytical thin-layer chromatography using silica gel 60 F_{254} precoated plates (E. Merck). Purifications by column chromatography were performed using silica gel Si 60 (40–63 μm) with the indicated eluent. Optical rotations were measured with a JASCO P-1010 polarimeter. Melting points were measured on a Fisher Jones apparatus. Roman numerals in ascending order are given to the residues from the reducing end. NMR spectra were recorded on Varian Gemini 300 and Gemini 500 spectrometers. Proton and carbon chemical shifts (δ) are reported in ppm downfield from TMS and/or with internal reference of residual solvents.[16] Coupling constants (J) are reported in Hertz (Hz), and the following abbreviations are used: singlet (s), doublet (d), doublet of doublets (dd), triplet (t), multiplet (m), broad (b). Analysis and assignments were made using COSY, DEPT, and HSQC experiments. Low-resolution (MS) and high-resolution mass spectra (HRMS) were carried out by Dr Alexandra Furtos and Karine Venne (Mass Spectrometry Laboratory, Université de Montréal, Quebec, Canada).

4-Methoxyphenyl 2,3,4-tri-*O*-benzoyl-α-D-rhamnopyranoside (10). To a cooled (0 °C) solution of rhamnoside **8**[10] (0.540 g, 2.0 mmol) in pyridine (20 mL) was added dropwise benzoyl chloride (3.4 mL, 7.2 mmol, 3.6 equiv.). After stirring for 12 h at rt, the reaction was quenched by addition of MeOH at 0 °C, concentrated and co-evaporated with toluene. The residue was dissolved in CH_2Cl_2, then successively washed with saturated aqueous potassium hydrogensulfate, sodium hydrogencarbonate, water and brine, dried and concentrated. Flash chromatography (petroleum ether–EtOAc, 3 : 1 v/v) gave the benzoylated **10** (1.160 g, 99%), which crystallized in CH_2Cl_2–petroleum ether. mp 63–65 °C; $[\alpha]_D^{25} = 60$ (c 1.0 in $CHCl_3$); δ_H ($CDCl_3$, 300 MHz): 8.14–7.25 (15H, m, CO*Ph*), 7.14–7.11, 6.89–6.86 (4H, m, $C_6H_4OCH_3$), 6.05 (1H, dd, $J_{3,4} = 10.0$ Hz, H-3), 5.85 (1H, dd, $J_{2,3} = 3.2$ Hz, H-2), 5.76 (1H, t, $J_{4,5} = 10.0$ Hz, H-4), 5.63 (1H, d, $J_{1,2} = 1.6$ Hz, H-1), 4.40–4.31 (1H, m, H-5), 3.79 (3H, s, $C_6H_4OCH_3$), 1.36 (3H, d, $J_{5,6} = 6.2$ Hz, H-6); δ_C ($CDCl_3$, 75 MHz): 165.7, 165.6, 165.5 (*C*OPh), 155.2, 150.1 (C-q of $C_6H_4OCH_3$), 133.5–128.3 (CO*Ph*), 117.7, 114.7 (others $C_6H_4OCH_3$), 96.6 (C-1), 71.7, 70.7, 69.8, 67.3 (C-2 to C-5), 55.6 ($C_6H_4OCH_3$), 17.7 (C-6); ES-MS: $m/z = 605.2$ $(M + Na)^+$.

2,3,4-Tri-*O*-benzoyl-D-rhamnopyranose (11). A mixture of rhamnoside **10** (0.890 g, 1.53 mmol) and ammonium cerium nitrate (CAN, 8.370 g, 15.3 mmol, 10 equiv.) in toluene–acetonitrile–water (1 : 1.4 : 1 v/v/v, 27 mL) was stirred at rt for 12 h. The reaction mixture was diluted with CH_2Cl_2 then successively washed with brine, saturated aqueous sodium hydrogencarbonate and water, dried, concentrated and purified by flash chromatography (hexane–EtOAc, 3 : 1 v/v) to afford the hemiacetal **11** (0.720 g, 99%), which crystallized in petroleum ether–EtOAc. mp 204–206 °C; δ_H ($CDCl_3$, 300 MHz): 8.12–7.23 (15H, m, CO*Ph*), 5.94 (1H, dd, $J_{2,3} = 3.4$ and $J_{3,4} = 10.2$ Hz, H-3), 5.73–5.67 (2H, m, H-2 and H-4), 5.47 (1H, dd, $J_{1,2} = 1.6$ and $J_{1,OH} = 4.0$ Hz, H-1), 4.52–4.42 (1H, m, H-5), 3.71 (1H, d, OH-1), 1.37 (3H, d, $J_{5,6} = 6.3$ Hz, H-6); δ_C ($CDCl_3$, 75 MHz): 165.8, 165.7, 165.6 (*C*OPh), 133.5–128.2 (CO*Ph*), 92.2 (C-1), 71.9, 71.3, 69.7, 66.7 (C-2 to C-5), 17.7 (C-6); ES-MS: $m/z = 499.1$ $(M + Na)^+$.

2,3,4-Tri-*O*-benzoyl-D-rhamnopyranosyl trichloroacetimidate (12). To a solution of the hydroxy rhamnose **11** (318 mg, 0.66 mmol) in CH_2Cl_2 (10 mL) was added trichloroacetonitrile (0.34 mL, 3.34 mmol, 5 equiv.) and DBU (0.04 mL, 0.26 mmol, 0.4 equiv.). The solution was stirred for 1 h at rt, then concentrated and purified by flash chromatography (hexane–EtOAc, 5 : 1 v/v) using silica gel neutralized with triethylamine to give the trichloroacetimidate **12** (396 mg, 96%). δ_H ($CDCl_3$, 300 MHz): 8.83 (1H, s, NH), 8.13–7.25 (15H, m, CO*Ph*), 6.50 (1H, d, $J_{1,2} = 1.4$ Hz, H-1) 5.93–5.88 (2H, m, H-2 and H-3), 5.79 (1H, t, $J_{3,4} = J_{4,5} = 9.9$ Hz, H-4), 4.46–4.37 (1H, m, H-5), 1.43 (3H, d, $J_{5,6} = 6.3$ Hz, H-6); δ_C ($CDCl_3$, 75 MHz): 165.6, 165.4, 165.2 (*C*OPh), 160.0 (CNH), 133.6–128.3 (CO*Ph*), 94.7 (C-1), 71.0, 69.6, 69.6, 69.0 (C-2 to C-5), 17.7 (C-6); ES-MS: $m/z = 642.1$ $(M + Na)^+$.

4-Methoxyphenyl 2,3,4-tri-*O*-benzoyl-α-D-rhamnopyranosyl-(1→3)-2,4-di-*O*-benzoyl-α-D-rhamnopyranoside (6). A suspension of trichloroacetimidate **12** (316 mg, 0.51 mmol, 1.3 equiv.), acceptor **9**[10] (188 mg, 0.39 mmol, 1 equiv.) and freshly powdered 4 Å molecular sieves in CH_2Cl_2 (10 mL) was stirred for 1 h at rt under nitrogen atmosphere, cooled to −50 °C, and followed by the addition of a solution of TMSOTf (36 μL, 0.20 mmol, 0.5 equiv.) in CH_2Cl_2 (1 mL). After stirring for 30 min at −50 °C, the mixture was raised to rt, neutralized by adding triethylamine, filtered through Celite, and concentrated. Flash chromatography (hexane–EtOAc, 7 : 1 v/v) afforded the disaccharide **6** (366 mg, 99%). $[\alpha]_D^{25} = 78$ (c 1.0 in $CHCl_3$); δ_H ($CDCl_3$, 300 MHz): 8.31–7.19 (25H, m, CO*Ph*), 7.13–7.08, 6.91–6.86 (4H, m, $C_6H_4OCH_3$), 5.75–5.63 (4H, m), 5.55 (1H, t, $J_{3,4} = J_{4,5} = 9.8$ Hz, H-4^{II}), 5.36–5.34 (2H, m), 4.72 (1H, dd, $J_{2,3} = 3.3$ and $J_{3,4} = 9.6$ Hz, H-3^{I}), 4.29-4.16 (2H, m, H-5$^{I\text{ and }II}$), 3.80 (3H, s, $C_6H_4OCH_3$), 1.38 (3H, d, $J_{5,6} = 6.3$ Hz, H-6^{I}), 1.21 (3H, d, $J_{5,6} = 6.3$ Hz, H-6^{II}); δ_C ($CDCl_3$, 75 MHz): 166.1, 165.8, 165.5, 165.0, 164.7 (*C*OPh), 155.2, 150.0 (C-q of $C_6H_4OCH_3$), 133.6–128.1 (CO*Ph*), 117.7, 114.7 (others $C_6H_4OCH_3$), 99.4, 96.4 (C-1$^{I\text{ and }II}$, $^1J_{C\text{-}1,H\text{-}1} = 171.2$ and 173.8 Hz), 76.3, 72.9, 72.2, 71.5, 70.7, 69.3, 67.5, 67.4 (C-2 to C-5$^{I\text{ and }II}$), 55.6 ($C_6H_4OCH_3$), 17.8, 17.4 (C-6$^{I\text{ and }II}$); ES-MS: $m/z = 959.3$ $(M + Na)^+$; ES-HRMS: m/z $(M + Na)^+$, $C_{54}H_{48}O_{15}Na$ requires 959.2885; found: 959.2877.

2,3,4-Tri-*O*-benzoyl-α-D-rhamnopyranosyl-(1→3)-2,4-di-*O*-benzoyl-D-rhamnopyranose (13). A mixture of disaccharide **6** (93 mg, 99 μmol) and ammonium cerium nitrate (CAN, 548 mg, 1.0 mmol, 10 equiv.) in toluene–acetonitrile–water (1 : 1.4 : 1 v/v/v, 3.4 mL) was stirred at rt for 12 h. The reaction mixture was diluted with CH_2Cl_2 then successively washed with brine, saturated aqueous sodium hydrogencarbonate and water, dried, concentrated and purified by flash chromatography (hexane–EtOAc, 3 : 1 v/v) to afford the expected hemiacetal **13** (78 mg, 95%). δ_H ($CDCl_3$, 300 MHz): 8.27–7.18 (25H, m, CO*Ph*), 5.66–5.47 (5H, m, H-1^{I}, H-2^{I}, H-4^{I}, H-3^{II} and H-4^{II}), 5.31 (1H, dd, $J_{2,3} = 3.3$ Hz, H-2^{II}), 5.25 (1H, d, $J_{1,2} = 1.7$ Hz, H-1^{II}), 4.59 (1H, dd, $J_{2,3} = 3.4$ and $J_{3,4} = 9.6$ Hz, H-3^{I}), 4.39–4.29 (1H, m, H-5^{I}), 4.20–4.11 (1H, m, H-5^{II}), 3.08 (1H, bs, OH-1), 1.37 (3H, d, $J_{5,6} = 6.3$ Hz, H-6^{I}), 1.18 (3H, d, $J_{5,6} = 6.2$ Hz, H-6^{II}); δ_C ($CDCl_3$, 75 MHz): 166.2,

165.8, 165.6, 165.0, 164.7 (*C*OPh), 133.5–128.1 (CO*Ph*), 99.2, 92.0 (C-1$^{I\text{ and }II}$), 75.8, 73.1, 72.6, 71.5, 70.7, 69.3, 67.4, 66.9 (C-2 to C-5$^{I\text{ and }II}$), 17.8, 17.4 (C-6$^{I\text{ and }II}$); ES-MS: m/z = 853.3 (M + Na)$^+$.

2,3,4-Tri-*O*-benzoyl-α-D-rhamnopyranosyl-(1→3)-2,4-di-*O*-benzoyl-D-rhamnopyranosyl trichloroacetimidate (14). To a solution of the hydroxy compound **13** (47 mg, 57 μmol) in CH_2Cl_2 (5 mL) was added trichloroacetonitrile (29 μL, 283 μmol, 5 equiv.) and DBU (4 μL, 26 μmol, 0.5 equiv.). The solution was stirred for 1 h at rt, then concentrated and purified by flash chromatography (hexane–EtOAc, 3 : 1 v/v) using silica gel neutralized with triethylamine to give the trichloroacetimidate **14** (52 mg, 94%). δ_H ($CDCl_3$, 300 MHz): 8.83 (1H, s, NH), 8.29–7.18 (25H, m, CO*Ph*), 6.51 (1H, d, $J_{1,2}$ = 1.6 Hz, H-1^I), 5.75–5.69 (2H, m, H-2$^{I\text{ or }II}$ and H-4$^{I\text{ or }II}$), 5.61 (1H, dd, $J_{3,4}$ = 10.0 Hz, H-3^{II}), 5.52 (1H, t, $J_{4,5}$ = 10.0 Hz, H-4$^{I\text{ or }II}$), 5.34 (1H, dd, $J_{2,3}$ = 3.3 Hz, H-2$^{I\text{ or }II}$); 5.26 (1H, bs, H-1^{II}), 4.60 (1H, dd, $J_{2,3}$ = 3.3 and $J_{3,4}$ = 9.9 Hz, H-3^I), 4.33–4.18 (2H, m, H-5$^{I\text{ and }II}$), 1.41 (3H, d, $J_{5,6}$ = 6.3 Hz, H-6$^{I\text{ or }II}$), 1.20 (3H, d, $J_{5,6}$ = 6.0 Hz, H-6$^{I\text{ or }II}$); δ_C ($CDCl_3$, 75 MHz): 165.8, 165.6, 165.5, 164.9, 164.7 (*C*OPh), 159.9 (CNH), 133.7–128.1 (CO*Ph*), 99.3, 94.6 (C-1$^{I\text{ and }II}$), 75.1, 72.5, 71.4, 70.7, 70.4, 69.8, 69.2, 67.6 (C-2 to C-5$^{I\text{ and }II}$), 17.8, 17.4 (C-6$^{I\text{ and }II}$); ES-MS: m/z = 996.2 (M + Na)$^+$.

Allyl 2,3,4-tri-*O*-benzoyl-α-D-rhamnopyranosyl-(1→3)-2,4-di-*O*-benzoyl-α-D-rhamnopyranosyl-(1→4)-2,3,6-tri-*O*-benzoyl-α-D-galactopyranoside (5). A suspension of trichloroacetimidate **14** (149 mg, 150 μmol, 1 equiv.), acceptor **7** (160 mg, 300 μmol, 2 equiv.) and freshly powdered 4 Å molecular sieves in CH_2Cl_2 (5 mL) was stirred for 1 h at rt under nitrogen atmosphere, cooled to −50 °C, and followed by the addition of a solution of TMSOTf (14 μL, 76 μmol, 0.5 equiv.) in CH_2Cl_2 (1 mL). After stirring for 30 min at −50 °C, the mixture was raised to rt, neutralized by adding triethylamine, filtrated through Celite, and concentrated. Flash chromatography (hexane–EtOAc, 6 : 1 v/v) afforded the trisaccharide **5** (132 mg, 66%). $[\alpha]_D^{25}$ = −7 (*c* 1.0 in $CHCl_3$); δ_H ($CDCl_3$, 300 MHz): 8.16–7.17 (40H, m, CO*Ph*), 5.92–5.73 (3H, m, including OCH$_2$C*H*CH$_2$ and H-3^I), 5.64–5.60 (2H, m, including H-2^{II}), 5.56–5.47 (2H, m, H-4$^{II\text{ and }III}$), 5.43 (1H, d, J = 2.7 Hz), 5.38–5.34 (2H, m), 5.24 (1H, d, J = 1.4 Hz), 5.30–5.11 (2H, m, OCH$_2$CHC*H*$_2$), 4.88 (1H, dd, $J_{5,6a}$ = 5.5 and $J_{6a,6b}$ = 9.9 Hz, H-6a^I), 4.66 (1H, bd, J = 1.9 Hz, H-4^I), 4.61–4.89 (3H, m, H-3^{II}, H-5^I and H-6b^I), 4.29–4.23 (2H, m, H-5^{II} and OC*H*$_2$CHCH$_2$), 4.18–4.06 (2H, m, H-5^{III} and OC*H*$_2$CHCH$_2$), 1.16 (3H, d, $J_{5,6}$ = 6.0 Hz, H-6^{III}), 0.66 (3H, d, $J_{5,6}$ = 6.0 Hz, H-6^{II}); δ_C ($CDCl_3$, 75 MHz): 166.1, 166.1, 166.0, 165.9, 165.7, 165.5, 164.9, 164.7 (*C*OPh), 133.4–128.1 (CO*Ph* and OCH$_2$*C*HCH$_2$), 117.8 (OCH$_2$CH*C*H$_2$), 99.5, 98.7, 95.6 (C-1$^{I,\,II\text{ and }III}$, $^1J_{C\text{-}1,H\text{-}1}$ = 171.4, 173.4 and 172.3 Hz), 76.2, 75.9, 72.6, 72.5, 71.5, 70.5, 70.1, 69.5, 68.8, 68.8, 67.8, 67.7, 67.5 (C-2 to C-5$^{I,\,II\text{ and }III}$ and O*C*H$_2$CHCH$_2$), 62.0 (C-6^I), 17.3, 17.2 (C-6$^{I\text{ and }II}$); ES-MS: m/z = 1367.4 (M + Na)$^+$; ES-HRMS: m/z (M + Na)$^+$, $C_{77}H_{68}O_{22}Na$ requires 1367.4094; found: 1367.4062. Anal. calcd for $C_{77}H_{68}O_{22}$: C 68.74, H 5.09; found: C 68.85; H, 5.71.

***N*-Acetyl-(*S*)-{3-[2,3,4-tri-*O*-benzoyl-α-D-rhamnopyranosyl-(1→3)-2,4-di-*O*-benzoyl-α-D-rhamnopyranosyl-(1→4)-2,3,6-tri-*O*-benzoyl-α-D-galactopyranosyloxy]propyl}-L-cysteine methyl ester (15).** To a solution of trisaccharide **5** (33.2 mg, 25 μmol) in deoxygenated THF (3 mL) was added *N*-acetyl-L-cysteine methyl ester (22.4 mg, 126 μmol, 5 equiv) and a catalytic amount of AIBN. The reaction was irradiated at 254 nm under nitrogen atmosphere for 24 h, and then the solvent was evaporated and taken up in CH_2Cl_2 (4 mL). Resin Trt-Cl (350 mg, 0.9 mmol g^{-1}) and DIPEA (20 μL, 121 μmol, 5 equiv.) were added and stirred for 2 h. The solution was then filtrated and concentrated. Flash chromatography (toluene–AcOEt, 7 : 3 v/v) afforded derivative **15** (29.7 mg, 79%). $[\alpha]_D^{25}$ = +10 (*c* 1.0 in $CHCl_3$); δ_H ($CDCl_3$, 300 MHz): 8.16–7.20 (40H, m, CO*Ph*), 6.47 (1H, d, J = 7.8 Hz, NH), 5.78–5.67 (2H, m), 5.64–5.59 (2H, m), 5.56–5.47 (2H, m, H-4$^{II\text{ and }III}$), 5.38–5.34 (3H, m), 5.24 (1H, d, J = 1.5 Hz), 4.87 (1H, dd, J = 6.2 and 10.7 Hz), 4.80–4.74 (1H, m, SCH$_2$C*H*), 4.63–4.46 (4H, m), 4.29–4.23 (1H, m, H-5^{II}), 4.15–4.10 (1H, m, H-5^{III}), 3.89–3.82 (1H, m, OC*H*$_2$CH$_2$CH$_2$S), 3.73 (3H, s, CO$_2$C*H*$_3$), 3.58 (1H, m, OC*H*$_2$CH$_2$CH$_2$S), 2.93–2.80 (2H, m, SC*H*$_2$CH), 2.58–2.53 (2H, m, OCH$_2$CH$_2$C*H*$_2$S), 2.03 (3H, s, NHCOC*H*$_3$), 1.88–1.78 (2H, m, OCH$_2$C*H*$_2$CH$_2$S), 1.15 (3H, d, $J_{5,6}$ = 6.3 Hz, H-6^{III}), 0.66 (3H, d, $J_{5,6}$ = 6.0 Hz, H-6^{II}); δ_C ($CDCl_3$, 75 MHz): 171.2, 169.8 (NH*C*OCH$_3$ and *C*O$_2$CH$_3$), 166.1, 166.1, 166.1, 166.0, 165.7, 165.5, 164.9, 164.7 (*C*OPh), 133.5–128.1 (CO*Ph*), 99.5, 98.7, 96.5 (C-1$^{I,\,II\text{ and }III}$), 76.2, 75.8, 72.6, 72.5, 71.5, 70.5, 70.1, 69.5, 69.0, 67.7, 67.6, 67.5, 66.5 (C-2 to C-5$^{I,\,II\text{ and }III}$ and O*C*H$_2$(CH$_2$)$_2$S), 62.0 (C-6^I), 52.6, 51.8 (SCH$_2$*C*H and CO$_2$*C*H$_3$), 34.3, 29.5, 29.0, 23.0 (OCH$_2$(*C*H$_2$)$_2$S*C*H$_2$CH and NHCO*C*H$_3$), 17.3, 17.2 (C-6$^{I\text{ and }II}$); ES-MS: m/z = 1522.0 (M + H)$^+$; ES-HRMS: m/z (M + H)$^+$, $C_{83}H_{80}NO_{25}S$ requires 1522.4735; found: 1522.4724.

Methyl 2-(*S*)-acetamido-6-[2,3,4-tri-*O*-benzoyl-α-D-rhamnopyranosyl-(1→3)-2,4-di-*O*-benzoyl-α-D-rhamnopyranosyl-(1→4)-2,3,6-tri-*O*-benzoyl-α-D-galactopyranosyloxy]hexanoate (16). To a solution of **5** (20.6 mg, 15.3 μmol) and *N*-acetyl-L-allylglycine methyl ester (13.1 mg, 76.5 μmol, 5 equiv.) in CH_2Cl_2 (1 mL) was added Grubbs' first-generation catalyst (1.2 mg, 1.5 μmol, 0.1 equiv) and the reaction was refluxed. After 8 h, the solution was concentrated and purified by flash chromatography (toluene–EtOAc, 1 : 1 v/v). The resulting mixture of isomers was dissolved in MeOH (1 mL), and Pd/C (1 mg) was added. Hydrogen was bubbled into the solution until disappearance of the starting material. After this time, the reaction mixture was filtered over Celite, concentrated and purified by flash chromatography (toluene–EtOAc, 1 : 1 v/v) to give **16** (13.6 mg, 60%). $[\alpha]_D^{25}$ = +3 (*c* 0.3 in $CHCl_3$); δ_H ($CDCl_3$, 500 MHz): 8.16–7.05 (40H, m, CO*Ph*), 6.20 (1H, d, J = 8.1 Hz, NH), 5.74–5.68 (2H, m, H-3^I and H-2^{II}), 5.64–5.60 (2H, m), 5.55–5.48 (2H, m, H-4$^{II\text{ and }III}$), 5.36–5.33 (3H, m), 5.29–5.25 (1H, m), 4.90–4.87 (1H, m, H-6a^I), 4.67–4.57 (2H, m, O(CH$_2$)$_4$C*H* and H-4^I), 4.55–4.45 (3H, m, H-3^{II}, H-5^I and H-6b^I), 4.31–4.22 (1H, m, H-5^{II}), 4.17–4.06 (1H, m, H-5^{III}), 3.78–3.75 (1H, m, OC*H*$_2$(CH$_2$)$_3$CH), 3.69 (3H, s, CO$_2$C*H*$_3$), 3.54–3.47 (1H, m, OC*H*$_2$(CH$_2$)$_3$CH), 2.01 (1H, s, NHCOC*H*$_3$), 1.66–1.58 (2H, m, O(CH$_2$)$_3$C*H*$_2$CH), 1.36–1.26 (4H, m, OCH$_2$C*H*$_2$C*H*$_2$CH$_2$CH), 1.15 (3H, d, $J_{5,6}$ = 6.3 Hz, H-6^{III}), 0.65 (3H, d, $J_{5,6}$ = 6.1 Hz, H-6^{II}); δ_C ($CDCl_3$, 125 MHz): 170.1, 169.6 (NH*C*OCH$_3$ and *C*O$_2$CH$_3$), 166.5, 166.3, 166.2, 166.1, 166.1, 166.0, 165.8, 165.5 (*C*OPh), 133.3–128.1 (CO*Ph*), 99.5, 98.7, 96.3 (C-1$^{I,\,II\text{ and }III}$), 77.9, 76.5, 72.6, 72.6, 71.4, 70.5, 69.4, 68.9, 68.8, 68.1, 67.6, 67.5, 67.3 (C-2 to C-5$^{I,\,II\text{ and }III}$ and O*C*H$_2$(CH$_2$)$_3$CH), 61.8 (C-6^{III}), 52.3, 51.9 (O(CH$_2$)$_4$*C*H and CO$_2$*C*H$_3$), 32.1, 29.7, 28.8, 21.9 (OCH$_2$(*C*H$_2$)$_3$CH and NHCO*C*H$_3$), 17.3, 17.2 (C-6$^{I\text{ and }II}$);

ES-HRMS: m/z $(M + H)^+$, $C_{83}H_{80}NO_{25}$ requires 1490.5030; found: 1490.5013.

***N*-Acetyl-(*S*)-{3-[α-D-rhamnopyranosyl-(1→3)-α-D-rhamnopyranosyl-(1→4)-α-D-galactopyranosyloxy]propyl}-L-cysteine methyl ester (17).** To a solution of **15** (19.8 mg, 13 μmol) in THF (0.2 mL) was added a solution of LiOH (9.0 mg, 210 μmol, 16 equiv.) in H_2O (0.5 mL). The reaction was stirred overnight at rt and then acidified using Amberlite IR-120 (H^+) resin. After filtration, the filtrate was concentrated and lyophilized, to afford the trisaccharide **17** (8,7 mg, quantitative yield). $[\alpha]_D^{25} = +86$ (c 0.1 in MeOH); δ_H (D_2O, 300 MHz): 5.05 (1H, d, $J = 1.2$ Hz), 4.95–4.76 (3H, m), 4.36 (1H, q, $J = 4.5$ and 8.1 Hz), 4.18–3.78 (11H, m), 3.72–3.70 (2H, m), 3.63–3.44 (3H, m), 3.05 (1H, dd, $J = 4.1$ and 13.8 Hz), 2.91–2.83 (1H, m), 2.70 (2H, t, $J = 6.7$ Hz), 2.05 (3H, s), 2.00–1.89 (2H, m), 1.30 (3H, d, $J_{5,6} = 6.3$ Hz, H-$6^{II\,or\,III}$), 1.26 (3H, d, $J_{5,6} = 6.2$ Hz, H-$6^{II\,or\,III}$); δ_C (D_2O, 75 MHz): 180.8, 180.8 ($NHCOCH_3$ and CO_2H), 103.0, 102.2, 99.0 (C-$1^{I,\,II\,and\,III}$), 79.1, 78.5, 72.6, 71.9, 71.8, 70.8, 70.8, 70.7, 70.0, 69.8, 69.5, 69.1, 67.3 (C-2 to C-$5^{I,\,II\,and\,III}$ and $OCH_2(CH_2)_2S$), 61.3 (C-6^{I}), 55.1 (SCH_2CH), 34.4, 29.2, 29.0, 23.1 ($OCH_2(CH_2)_2SCH_2CH$ and $NHCOCH_3$), 17.3, 17.2 (C-$6^{I\,and\,II}$); ES-HRMS: m/z $(M + Na)^+$, $C_{26}H_{45}NO_{17}SNa$ requires 698.2300; found: 698.2279.

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