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Note

Synthesis of the 6-deoxytalose-containing tetrasaccharide of the glycopeptidolipid from *Mycobacterium intracellare* serotype 7

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ABSTRACT

An efficient synthesis of 4-methoxyphenyl α-L-Rha*p*-(1→3)-α-L-Rha*p*-(1→3)-α-L-Rha*p*-(1→2)-6-deoxy-α-L-Tal*p*, the tetrasaccharide related to the GPLs of *Mycobacterium intracellare* serotype 7, was achieved with 4-methoxyphenyl 3,4-di-*O*-benzoyl-6-deoxy-α-L-talopyranoside (**6c**) as the key intermediate which was obtained through selective 3-O-benzoylation of 4-*O*-benzoyl-6-deoxy-α-L-taloside. Coupling of **6c** with 3-*O*-allyloxycarbonyl-2,4-di-*O*-benzoyl-α-L-rhamnopyranosyl trichloroacetimidate followed by removal of the allyloxycarbonyl protecting group afforded the disaccharide acceptor **11**. Condensation of **11** with 2,3,4-tri-*O*-benzoyl-α-L-rhamnopyranosyl-(1→3)-2,4-di-*O*-benzoyl-α-L-rhamnopyranosyl trichloroacetimidate and subsequent deprotection gave the target tetrasaccharide.

6-Deoxy-L-talose is a key building block of many biologically important glycopeptidolipids (GPLs)[1–4] and is an essential component of numerous antigenic bacterial lipopolysaccharides (LPSs)[5–9] Structural analysis revealed that most of these GPLs or LPSs possess the 6-deoxy-L-talose unit in which O-2 or O-3 is glycosylated with other sugar units. Recently, it was proposed that the oligosaccharide part of *Mycobacterium intracellare* serotype 7 GPLs contains a pentasaccharide, which is composed of one 6-deoxy-L-talose, three L-rhamnoses, and one terminal amido sugar unit, while the configuration of the terminal amido sugar remained undetermined (**I**, Chart 1).[10]

M. intracellulare is distributed ubiquitously in nature and is an important cause of respiratory and lymphatic disease in human and animals.[11] Since carbohydrates play an important role in bacterial physiology and pathogenesis, preparation of these GPL oligosaccharides is of considerable interest for structure–bioactivity studies of carbohydrates.[12–15] In this paper, we wish to report the efficient synthesis of the well-defined tetrasaccharide **II** (Chart 1) related to the GPLs of *M. intracellare* serotype 7.

For the synthesis of the tetrasaccharide, 4-methoxyphenyl 6-deoxy-α-L-talopyranoside acceptors (**6a–f**, **7a–d**) with the C-2 hydroxyl free were prepared through the regioselective acylation of 6-deoxy-α-L-taloside 2,3-diols (**4**, or **5**) with acyl chlorides. As outlined in Scheme 1, 4-methoxyphenyl 2,3-*O*-isopropylidene-α-L-rhamnopyranoside (**1**), obtained by a reported method from rhamnose,[16] was oxidized with PDC[17] to afford the corresponding glycos-4-ulose derivative **2** in 93% yield. Reduction of **2** with sodium borohydride gave 4-methoxyphenyl 6-deoxy-2,3-*O*-isopropylidene-α-L-talopyranoside (**3**) as crystals in 85% yield. The Tal-configuration of **3** was assigned from its ^{1}H NMR spectrum, showing characteristic signals at δ 3.63 ppm (dd, $J_{3,4} = J_{4,5}$ 5.1 Hz) for H-4.[14] Compared to the previously reported synthesis of methyl or benzyl deoxytalosides,[18–20] the main advantage for the synthesis of the *p*MP glycoside in the present work was that the *p*MP taloside could be purified by crystallization, avoiding the tedious column chromatography purification step. Acylation of **3** with benzoyl chloride or pivaloyl chloride in pyridine followed by de-isopropylidenation provided the 2,3-diol **4** (92%) or **5** (76%), respectively. Compounds **4** and **5** were employed for the selective acylation reactions. Because the equatorially oriented 3-OH is more reactive than the axial 2-OH, it was anticipated that the 3-OH could be selectively acylated. As a typical example, **4** was treated with acetyl chloride in dichloromethane at −10 °C in the presence of 4 equiv of pyridine and catalytic amounts of DMAP, resulting in the 3-*O*-acetyl derivative (**6a**) in excellent yield (93%). Other acylation reagents such as allyloxycarbonyl chloride, benzoyl chloride, chloroacetyl chloride, phenylacetyl chloride, and pivaloyl chloride were also investigated for the regioselective esterification, and in all cases the 3-OH acylated products **6b–f** and **7a–d** were obtained in high yield (80–95%). Low temperature and slow addition of the chlorides were necessary for optimal regioselectivity. The regioselectivity of the process was established by ^{1}H NMR spectroscopy, and the characteristic H-3 signal was found to move to downfield upon acylation ($\delta_{H\text{-}3}$ 4.33 ppm in **4** as compared to $\delta_{H\text{-}3}$ 5.49 ppm in **6a**). Further confirmation was supported by the

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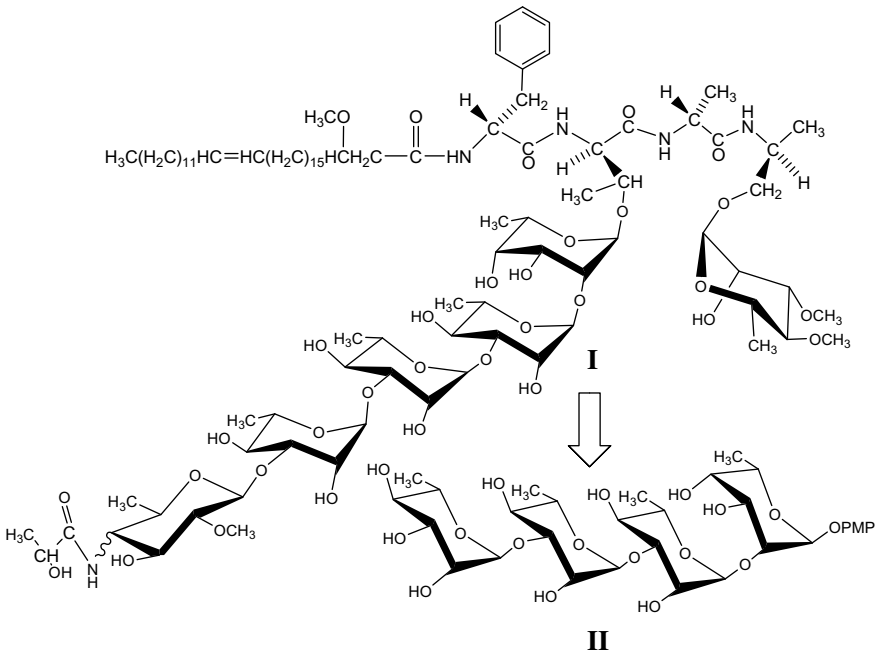
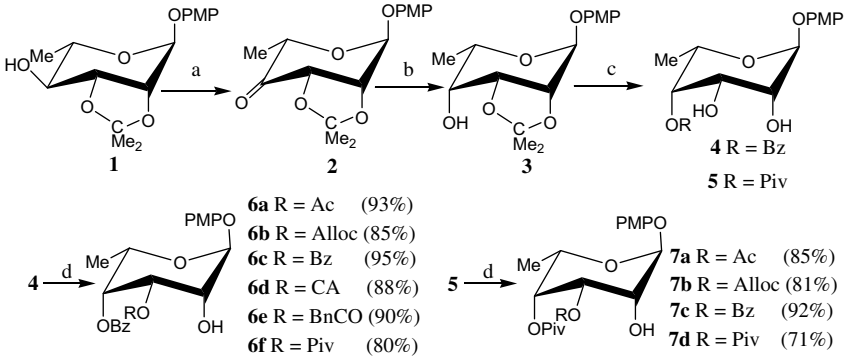


Chart 1. Proposed structure of the *M. intracellare* serotype 7 GPL **I** and the target tetrasaccharide **II**.



Scheme 1. Reagents and conditions: (a) PDC, CH_2Cl_2, reflux, 6–8 h, 93%; (b) EtOAc–water (7:3), $NaBH_4$, 85%; (c) (i) BzCl or PivCl, pyridine, cat DMAP, rt, 2 h; (ii) 70% AcOH, 75 °C, 2 h, 92% for **4**; 76% for **5**; (d) acyl chloride–pyridine, cat DMAP, CH_2Cl_2, rt.

single-crystal X-ray analysis of 4-methoxyphenyl 3-*O*-allyloxycarbonyl-2,4-di-*O*-benzoyl-6-deoxy-α-L-talopyranoside (**14**), obtained from benzoylation of compound **6b** (preparation of **14** and its X-ray data are included in the Supplementary data).

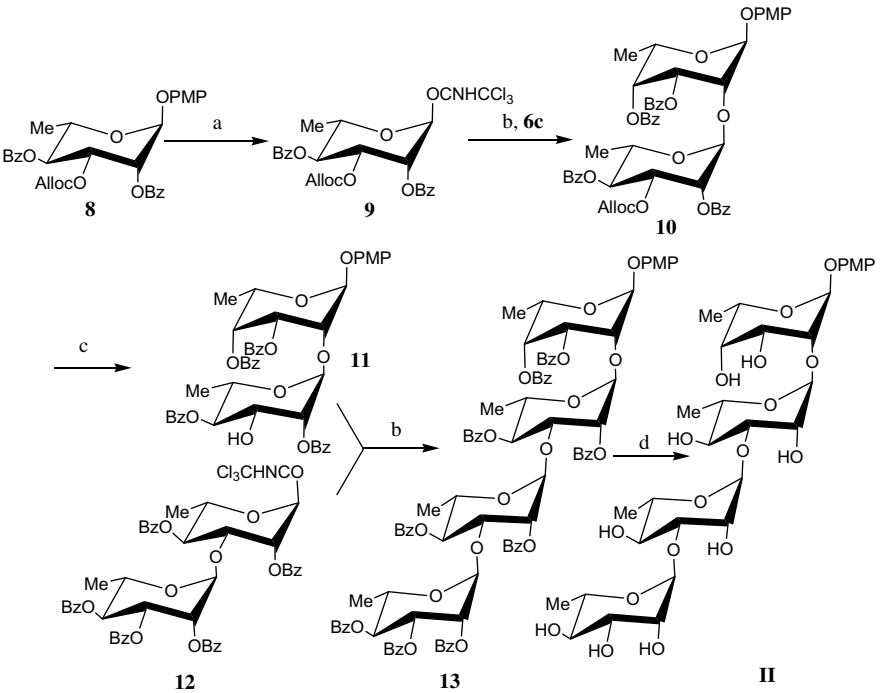
With the 6-deoxytaloside acceptors possessing a free 2-OH at hand, synthesis of the target tetrasaccharide was readily achieved as shown in Scheme 2. In the synthesis, 4-methoxyphenyl 3-*O*-allyloxycarbonyl-2,4-di-*O*-benzoyl-α-L-rhamnopyranoside (**8**) and the disaccharide donor 2,3,4-tri-*O*-benzoyl-α-L-rhamnopyranosyl-(1→3)-2,4-di-*O*-benzoyl-α-L-rhamnopyranosyl trichloroacetimidate (**12**) were prepared from L-rhamnose according to the previously reported procedures.[21,22] Cleavage of the 4-methoxyphenyl group of **8** with ceric ammonium nitrate (CAN), followed by trichloroacetimidation, provided **9**. Then, condensation of the donor **9** with 4-methoxyphenyl 3,4-di-*O*-benzoyl-6-deoxy-α-L-talopyranoside (**6c**) in the presence of TMSOTf[23] afforded the disaccharide **10**, and subsequent de-allyloxycarbonylation of **10** with palladium catalyst ($Pd[P(C_6H_5)_3]_4$, 0.05 equiv), $P(C_6H_5)_3$ (0.3 equiv), and Et_3N (2 equiv) in THF[24] gave the disaccharide acceptor **11** in satisfactory yield. TMSOTf-catalyzed coupling reaction of the acceptor **11** with the donor **12** provided the tetrasaccharide **13** in 76% yield. The ^1H NMR spectrum of **13** showed a doublet at δ 5.75 ppm (J 1.2 Hz) for H-1 of α-Tal along with three doublets at δ 5.29 ppm (J 1.8 Hz), δ 4.84 ppm (J 1.2 Hz), and δ 4.69 ppm (J 0.6 Hz) for the three α-Rha anomeric protons, respectively. The ^{13}C NMR spectrum showed peaks at δ 99.8, 99.0, 99.0, and 98.6 for the four anomeric carbons. Finally deacylation of **13** in ammonium-saturated methanol gave the target tetrasaccharide **II** (Chart 1).

In conclusion, we have explored the highly selective acylation at O-3 of 6-deoxy-α-L-taloside 2,3-diols with acyl chloride and pyridine as mild esterification reagents, and a 6-deoxy-α-L-talopyranose-containing tetrasaccharide from the GPLs of *M. intracellare* serotype 7 was efficiently synthesized.

1. Experimental

1.1. General methods

Optical rotations were determined with a Perkin–Elmer model 241-MC automatic polarimeter for solns in a 1-dm jacketed cell. ^1H and ^{13}C NMR spectra were recorded with Bruker DPX300 and Bruker AVANCE600 spectrometers in $CDCl_3$ or D_2O solns. Internal references: TMS (δ 0.000 ppm for ^1H), $CDCl_3$ (δ 77.00 ppm for ^{13}C), and HOD (δ 4.700 for ^1H). ^1H NMR signals of some compounds



Scheme 2. Reagents and conditions: (a) (i) 80% MeCN, CAN, 35 °C, 0.5 h, 78%; (ii) CCl_3CN, DBU, CH_2Cl_2, rt, 0.5 h, 89%; (b) TMSOTf, CH_2Cl_2, −10 °C to rt, 2 h, 88% for **10**; 76% for **13**; (c) $Pd[P(C_6H_5)_3]_4$, $P(C_6H_5)_3$, Et_3N, THF, rt, 81%; (d) satd NH_3–MeOH, rt, 96 h, 91%.

were assigned with the aid of COSY. Elemental analysis was performed on a Yanaco CHN Corder MF-3 automatic elemental analyzer. MALDI and ESI mass spectra were performed by the Institute of Chemistry of the Chinese Academy of Sciences. Thin-layer chromatography (TLC) was performed on Silica Gel HF with detection by charring with 30% (v/v) H_2SO_4 in MeOH or by UV detection. Column chromatography was conducted by elution of a column of silica gel (200–300 mesh) with EtOAc–petroleum ether (bp 60–90 °C) as the eluent. Solns were concentrated at a temperature <60 °C under diminished pressure.

1.2. 4-Methoxyphenyl 6-deoxy-2,3-*O*-isopropylidene-α-L-*lyxo*-hexopyranosid-4-ulose (2)

A mixture of 4-methoxyphenyl 2,3-*O*-isopropylidene-α-L-rhamnopyranoside (**1**)[16] (31.0 g, 0.1 mol), PDC (23.0 g, 0.06 mol), and acetic anhydride (28.4 mL, 0.3 mol) in CH_2Cl_2 (200 mL) was stirred at reflux for 8 h, at the end of which time TLC (4:1 petroleum ether–EtOAc) indicated that the reaction was complete. After direct concentration of the reaction mixture, the dark brown residue was diluted with EtOAc (60 mL) and the soln was passed through a short (5–10 cm) silica-gel column. The column was eluted with EtOAc and the eluents were concentrated and coevaporated with toluene. The residue was subjected to silica-gel column chromatography again (4:1 petroleum ether–EtOAc) to give **2** (28.6 g, 93%) as a syrup; $[\alpha]_D^{25}$ −90.1 (*c* 0.5 $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$): δ 1.38 (d, $J_{5,6}$ 6.8 Hz, 3H, H-6), 1.41, 1.54 (2s, 6H, C*Me*$_2$), 3.78 (s, 3H, O*Me*), 4.30 (q, $J_{5,6}$ 6.8 Hz, 1H, H-5), 4.55–4.65 (m, 2H, H-2, H-3), 5.59 (s, 1H, H-1), 6.84 (m, 2H, Ar-*H*), 7.00 (m, 2H, Ar-*H*). Anal. Calcd for $C_{16}H_{20}O_6$: C, 62.33; H, 6.54. Found: C, 62.09; H, 6.86.

1.3. 4-Methoxyphenyl 6-deoxy-2,3-*O*-isopropylidene-α-L-talopyranoside (3)

To a soln of **2** (15.4 g, 0.05 mol) in 7:3 EtOAc–water (150 mL) at 0 °C was added $NaBH_4$ (2.1 g, 0.055 mol). The mixture was stirred at 0 °C for 15 min, and TLC (4:1 petroleum ether–EtOAc) indicated that the reaction was complete. The aq soln was extracted with EtOAc (3 × 200 mL), the extract was washed with M HCl and satd aq $NaHCO_3$, dried (Na_2SO_4), and concentrated to give crude **3** as a syrup. Purification of the crude product by crystallization (4:1 petroleum ether–EtOAc) provided **3** (13.2 g, 85%) as white crystals (mp 93–94 °C); $[\alpha]_D^{25}$ −75.0 (*c* 1.0 $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$): δ 1.29 (d, $J_{5,6}$ 6.6 Hz, 3H, C*H*$_3$), 1.42, 1.61 (2s, 6H, C*Me*$_2$), 2.32 (d, 1H, O*H*, exchangeable with D_2O), 3.63 (dd, $J_{3,4} = J_{4,5} = 5.1$ Hz, 1H, H-4), 3.76 (s, 3H, O*Me*), 4.00 (m, 1H, H-5), 4.27 (dd, $J_{1,2}$ 0.8 Hz, $J_{2,3}$ 6.4 Hz, 1H, H-2), 4.36 (d, $J_{2,3}$ 6.4 Hz, $J_{3,4}$ 5.1 Hz, 1H, H-3), 5.66 (s, 1H, H-1), 6.84 (m, 2H, Ar-*H*), 7.00 (m, 2H, Ar-H). Anal. Calcd for $C_{16}H_{22}O_6$: C, 61.92; H, 7.15. Found: C, 62.11; H, 7.02.

1.4. 4-Methoxyphenyl 4-*O*-benzoyl-6-deoxy-α-L-talopyranoside (4)

To a soln of **3** (12.4 g, 0.04 mol) in pyridine (60 mL) was added benzoyl chloride (5.6 mL, 0.048 mol) dropwise. After stirring for 8 h at rt, TLC (3:1 petroleum ether–EtOAc) indicated that the reaction was complete. MeOH (1 mL) was added to quench the reaction and then water (100 mL) was added to the reaction mixture. The aq soln was extracted with EtOAc (3 × 200 mL), the extract was washed with M HCl and satd aq sodium hydrogencarbonate, dried (Na_2SO_4), and concentrated. The residue was dissolved in 70% AcOH (200 mL) and stirred for 3 h at 75 °C, at the end of which time TLC (2:1 petroleum ether–EtOAc) indicated completion of the reaction. The mixture was concentrated under diminished pressure and then coevaporated with toluene (2 × 40 mL). The residue was passed through a short silica-gel column with 5:2 petroleum ether–EtOAc as the eluent to give **4** (13.8 g, 92% for two steps) as a foamy solid; $[\alpha]_D^{25}$ +48.0 (*c* 0.5 $CHCl_3$); ^1H NMR (600 MHz, $CDCl_3$): δ 1.22 (d, $J_{5,6}$ 6.6 Hz, 3H, C*H*$_3$), 2.65 (br s, 2H, 2O*H*), 3.82 (s, 3H, O*Me*), 4.03 (dd, $J_{1,2}$ 1.8 Hz, $J_{2,3}$ 3.6 Hz, 1H, H-2), 4.30 (q, $J_{5,6}$ 6.6 Hz, 1H, H-5), 4.33 (dd, $J_{2,3} = J_{3,4} = 3.6$ Hz, 1H, H-3), 5.53 (d, $J_{3,4}$ 3.6 Hz, 1H, H-4), 5.61 (d, $J_{1,2}$ 1.8 Hz, 1H, H-1), 6.87 (m, 2H, Ar-*H*),

7.04 (m, 2H, Ar-*H*), 7.47–8.08 (m, 5H, Bz-*H*). Anal. Calcd for $C_{20}H_{22}O_7$: C, 64.16; H, 5.92. Found: C, 63.90; H, 6.20.

1.5. 4-Methoxyphenyl 6-deoxy-4-*O*-pivaloyl-α-L-talopyranoside (5)

To a soln of **3** (6.2 g, 20 mmol) with DMAP (1.0 g, 8.0 mmol) in pyridine (30 mL) was added pivaloyl chloride (4.8 mL, 40 mol) dropwise. After stirring for 2 d at rt, TLC (3:1 petroleum ether–EtOAc) indicated that the reaction was complete. MeOH (0.5 mL) was added to the reaction mixture followed by stirring for 10 min. Water (100 mL) was added and the aq soln was extracted with CH_2Cl_2 (3 × 50 mL), the extract was washed with M HCl and satd aq sodium hydrogencarbonate, dried (Na_2SO_4), and concentrated. The residue was dissolved in 70% AcOH (200 mL) and stirred for 3 h at 70 °C, at the end of which time TLC (2:1 petroleum ether–EtOAc) indicated that the reaction was complete. The mixture was concentrated and coevaporated with toluene (2 × 40 mL). The residue was passed through a short silica-gel column with 5:2 petroleum ether–EtOAc as the eluent to give **5** (5.4 g, 76% for two steps) as a foamy solid; $[\alpha]_D^{25}$ +44.5 (*c* 0.5 $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.13 (d, $J_{5,6}$ 5.0 Hz, 3H, C*H*$_3$), 1.30 (s, 9H, (C*H*$_3$)$_3$CCO), 2.56 (d, *J* 11.3 Hz, 1H, O*H*), 2.69 (d, *J* 7.8 Hz, 1H, O*H*), 3.78 (s, 3H, OC*H*$_3$), 3.95 (m, 1H, H-2), 4.15–4.21 (m, 2H, H-3, H-5), 5.25 (d, $J_{3,4}$ 3.7 Hz, 1H, H-4), 5.58 (d, $J_{1,2}$ 1.5 Hz, 1H, H-1), 6.82–6.86 (m, 2H, Ar-*H*), 6.96–7.02 (m, 2H, Ar-*H*). Anal. Calcd for $C_{18}H_{26}O_7$: C, 61.00; H, 7.39. Found: C, 60.86; H, 7.22.

1.6. General procedure for the preparation of compounds 6a–e and 7a–d

Compound **4** or **5** (2 mmol), in a 100 mL round-bottomed flask, was dried under high vacuum for 4 h, and then anhyd CH_2Cl_2 (20 mL), pyridine (4.0 mL, 50 mmol) and, in the case of **6f** and **7d**, catalytic amounts of DMAP (50 mg, 0.4 mmol) were successively added to the flask under N_2 atmosphere. The flask was cooled to −15 °C in an ice-salt-acetone bath and a soln of acyl chloride (2.2 mmol) in dry CH_2Cl_2 (10 mL) was added dropwise within 40 min. The reaction mixture was slowly warmed to rt and stirred for a further 2 h, at the end of which time TLC (3:1 petroleum ether–EtOAc) indicated the completion of the reaction. Water (20 mL) was added to the reaction mixture. The aq soln was extracted with CH_2Cl_2 (3 × 20 mL), the extract was washed with M HCl and satd aq sodium hydrogencarbonate, dried (Na_2SO_4), and concentrated. The residue was subjected to silica-gel column chromatography (3:1–6:1 petroleum ether–EtOAc) to give the desired products **6a–f** and **7a–d** in high yields.

Analytical data for compounds **6a–f** and **7a–d** are enclosed in Supplementary data.

1.7. 3-*O*-Allyloxycarbonyl-2,4-di-*O*-benzoyl-α-L-rhamnopyranosyl trichloroacetimidate (9)

To a soln of 4-methoxyphenyl 3-*O*-allyloxycarbonyl-2,4-di-*O*-benzoyl-α-L-rhamnopyranoside (**8**)[21] (8.43 g, 0.015 mol) in 80% MeCN (400 mL) at 35 °C was added CAN (34.1 g, 0.06 mol). The mixture was stirred at 35 °C for 0.5 h while TLC (4:1 petroleum ether–EtOAc) indicated the completion of the reaction. The solvent was evaporated under diminished pressure at 50 °C to give a residue, which was dissolved in CH_2Cl_2, and washed with water. The organic phase was dried (Na_2SO_4), concentrated, and then purified by silica-gel column chromatography with 3:1 petroleum ether–EtOAc as the eluent to give 3-*O*-allyloxycarbonyl-2,4-di-*O*-benzoyl-α-L-rhamnopyranose (5.34 g, 78%) as a yellow foam. A mixture of this compound (5.34 g, 11.7 mmol), trichloroacetonitrile (4.0 mL, 40 mmol), and 1,8-diazabicyclo[5.4.0] undecene (DBU) (0.30 mL, 30 mmol) in dry CH_2Cl_2 (40 mL) was stirred until completion of the reaction (TLC, 3:1 petroleum ether–EtOAc). Concentration and purification of the residue by flash chromatography (6:1 petroleum ether–EtOAc) gave **9** (6.26 g, 89%) as a white foamy solid; $[\alpha]_D^{25}$ +60.0 (*c* 1.0 $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.39 (d, $J_{5,6}$ 6.2 Hz, 3H, C*H*$_3$), 4.30 (m, 1H, H-5), 4.49–4.51 (m, 2H, OC*H*$_2$CH=CH$_2$), 5.02–5.18 (m, 2H, OCH$_2$CH=C*H*$_2$), 5.52–5.72 (m, 3H, OCH$_2$C*H*=CH$_2$, H-3, H-4), 5.82 (dd, $J_{1,2}$ 2.0 Hz, $J_{2,3}$ 3.2 Hz, 1H, H-2), 6.44 (d, $J_{1,2}$ 2.0 Hz, H-1), 7.44–7.62 (m, 6H, Ar-*H*), 8.04–8.14 (m, 4H, Ar-*H*), 8.81 (s, 1H, CN*H*CCl$_3$). Anal. Calcd for $C_{26}H_{24}Cl_3NO_9$: C, 51.97; H, 4.03; N, 2.33. Found: C, 51.82; H, 4.34; N, 2.72.

1.8. 4-Methoxyphenyl 3-*O*-allyloxycarbonyl-2,4-di-*O*-benzoyl-α-L-rhamnopyranosyl-(1→2)-3,4-di-*O*-benzoyl-6-deoxy–L-talopyranoside (10)

To a cooled soln (−10 °C) of **9** (3.99 g, 6.6 mmol) and **6c** (2.65 g, 5.5 mmol) in anhyd CH_2Cl_2 (45 mL) was added TMSOTf (25 μL, 0.14 mmol). The mixture was stirred at this temperature for 2 h, and then quenched with Et_3N (2 drops). The solvents were evaporated under diminished pressure to give a residue, which was purified by silica-gel column chromatography (5:1 petroleum ether–EtOAc) to give disaccharide **10** (4.43 g, 88%) as a foamy solid; $[\alpha]_D^{25}$ −28.0 (*c* 1.0 $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.26 (d, *J* 5.6 Hz, 3H, C*H*$_3$), 1.33 (d, *J* 5.9 Hz, 3H, C*H*$_3$), 3.80 (s, 3H, OC*H*$_3$), 4.23–4.32 (m, 2H), 4.47–4.49 (m, 3H, H-2, OC*H*$_2$CH=CH$_2$), 5.03–5.19 (m, 2H, OCH$_2$CH=C*H*$_2$), 5.26 (s, 1H), 5.50–5.56 (m, 2H), 5.64–5.76 (m, 4H), 5.87 (dd, $J_{2,3}$ = $J_{3,4}$ = 3.6 Hz, 1H, H-3), 6.88–6.91 (m, 2H, Ar-*H*), 7.09–7.12 (m, 2H, Ar-*H*), 7.31–7.64 (m, 12H, Bz-*H*), 7.96–8.20 (m, 8H, Bz-*H*); ^{13}C NMR (300 MHz, $CDCl_3$) δ 16.3, 17.6, 55.7, 65.9, 67.6, 67.9, 68.7, 69.2, 70.1, 71.9, 72.4, 72.7, 98.7 (C-1), 99.1 (C-1), 114.8, 117.5, 118.8, 128.4, 128.4, 128.5, 128.6, 129.2, 129.2, 129.3, 129.8, 129.9, 130.1, 131.2, 133.0, 1332, 133.3, 133.5, 150.1, 153.9, 155.2, 164.8, 165.5, 165.6, 166.4. Anal. Calcd for $C_{51}H_{48}O_{16}$: C, 66.80; H, 5.28. Found: C, 66.67; H, 5.39.

1.9. 4-Methoxyphenyl 2,4-di-*O*-benzoyl–L-rhamnopyranosyl-(1→2)-3,4-di-*O*-benzoyl-6-deoxy–L-talopyranoside (11)

To a soln of **10** (1.28 g, 1.4 mmol) and Et$_3$ N (0.38 mL, 2.8 mmol) in THF (15 mL) was added PPh_3 (110 mg, 0.42 mmol) and Pd[P(C$_6$H$_5$)$_3$]$_4$ (81 mg, 0.07 mmol), and the mixture was stirred at 25 °C until TLC (3:1 petroleum ether–EtOAc) indicated completion of the reaction. The reaction mixture was concentrated under diminished pressure, and the residue was purified by flash chromatography on a silica-gel column (4:1 petroleum ether–EtOAc) to give **11** (0.94 g, 81%) as a white solid; $[\alpha]_D^{25}$ −32.0 (*c* 0.5 $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$); δ 1.31 (d, *J* 4.3 Hz, 3H, C*H*$_3$), 1.33 (d, *J* 4.6 Hz, 3H, C*H*$_3$), 2.07 (d, *J* 6.6 Hz, 1H, O*H*, exchangeable with D_2O), 3.80 (s, 3H, OC*H*$_3$), 4.28–4.33 (m, 2H), 4.49–4.51 (m, 2H), 5.19 (s, 1H, H-1), 5.26–5.35 (m, 2H), 5.69 (s, 1H, H-1), 5.70 (m, 1H), 5.82 (dd, $J_{2,3}$ = $J_{3,4}$ = 3.6 Hz, 1H, H-3), 6.88–6.91 (m, 2H, Ar-*H*), 7.08–7.11 (m, 2H, Ar-*H*), 7.35–7.64 (m, 12 H, Bz-*H*), 7.98–8.20 (m, 8H, Bz-*H*). Anal. Calcd for $C_{47}H_{44}O_{14}$: C, 67.78; H, 5.33. Found: C, 67.70; H, 5.60.

1.10. 4-Methoxyphenyl 2,3,4-tri-*O*-benzoyl–L-rhamnopyranosyl-(1→3)-2,4-di-*O*-benzoyl–L-rhamnopyranosyl-(1→3)-2,4-di-*O*-benzoyl–L-rhamnopyranosyl-(1→2)-3,4-di-*O*-benzoyl-6-deoxy–L-talopyranoside (13)

To a cooled soln (−10 °C) of **11** (400 mg, 0.48 mmol) and **12**[22] (500 mg, 0.53 mmol) in anhyd CH_2Cl_2 (25 mL) was added TMSOTf (25 μL, 0.14 mmol). The mixture was stirred at this temperature for 2 h, and then quenched with Et_3N (2 drops). The solvent was

evaporated under diminished pressure to give a residue, which was purified by silica-gel column chromatography (3:1 petroleum ether–EtOAc) to give the tetrasaccharide **13** (600 mg, 76%) as a foamy solid; $[\alpha]_D^{25}$ +78 (*c* 1.0 $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): δ 0.79 (d, *J* 6.6 Hz, 3H, C*H*$_3$), 0.80 (d, *J* 6.6 Hz, 3H, C*H*$_3$), 1.31 (d, *J* 6.6 Hz, 3H, C*H*$_3$), 1.34 (d, *J* 6.3 Hz, 3H, C*H*$_3$), 3.74–3.76 (m, 1H, H-5′), 3.79 (s, 3H, OC*H*$_3$), 3.81 (m, 1H, H-5″), 4.18 (dd, $J_{2,3}$ 3.0 Hz, $J_{3,4}$ 9.6 Hz, 1H, H-3′), 4.29 (dd, $J_{1,2}$ 1.2 Hz, $J_{2,3}$ 3.6 Hz, 1H, H-2), 4.30–4.33 (m, 1H, H-5‴), 4.38 (dd, $J_{2,3}$ 3.0 Hz, $J_{3,4}$ 9.6 Hz, 1H, H-3″), 4.48 (q, 1H, H-5), 4.69 (d, $J_{1,2}$ 0.6 Hz, 1H, H-1′), 4.84 (d, $J_{1,2}$ 1.2 Hz, 1H, H-1‴), 5.13 (dd, $J_{1,2}$ 0.6, $J_{2,3}$ 3.0 Hz, 1H, H-2′), 5.17 (dd, $J_{1,2}$ 1.2 Hz, $J_{2,3}$ 3.0 Hz, 1H, H-2‴), 5.29 (d, $J_{1,2}$ 1.8 Hz, 1H, H-1″), 5.30 (dd, $J_{3,4}$ = $J_{4,5}$ = 9.6 Hz, 1H, H-4′), 5.35 (dd, $J_{3,4}$ = $J_{4,5}$ = 9.6 Hz, 1H, H-4‴), 5.46 (dd, $J_{2,3}$ 3.0 Hz, $J_{3,4}$ 9.6 Hz, 1H, H-3‴), 5.50 (dd, $J_{1,2}$ 1.8 Hz, $J_{2,3}$ 3.0 Hz, 1H, H-2″), 5.55 (dd, $J_{3,4}$ = $J_{4,5}$ = 9.6 Hz, 1H, H-4″), 5.66 (d, $J_{3,4}$ 3.6 Hz, 1H, H-4), 5.75 (d, $J_{1,2}$ 1.2 Hz, 1H, H-1), 5.83 (dd, $J_{2,3}$ = $J_{3,4}$ = 3.6 Hz, 1H, H-3), 6.86–7.24 (m, 7H), 7.27–7.75 (m, 32H), 8.10–8.14 (m, 10H); ^{13}C NMR (300 MHz, $CDCl_3$); δ 16.4, 16.9, 17.3, 17.8, 98.6 (C-1), 99.0 (C-1), 99.0 (C-1), 99.8 (C-1), 164.7, 165.4, 165.5, 165.5, 165.5, 165.7, 166.1, 166.3, 167.1 (9 Bz*C*O). Anal. Calcd for $C_{94}H_{84}O_{27}$: C, 68.61; H, 5.14. Found: C, 68.89; H, 5.30.

1.11. 4-Methoxyphenyl L-rhamnopyranosyl-(1→3)–L-rhamnopyranosyl-(1→3)–L-rhamnopyranosyl-(1→2)-6-deoxy–L-talopyranoside (II)

Tetrasaccharide **13** (200 mg, 0.12 mmol) was dissolved in satd NH_3–MeOH (30 mL). After 96 h at rt, the reaction mixture was concentrated, and the residue was purified by chromatography on Sephadex LH-20 (MeOH) to afford **II** (77 mg, 91%) as a foamy solid; $[\alpha]_D^{25}$ +55.8 (*c* 0.5 H_2O); ^{1}H NMR (300 MHz, D_2O): δ 1.09–1.40 (m, 12H), 3.23–3.49 (m, 4H), 3.66–3.81 (m, 12H), 3.96–4.09 (m, 7H), 4.93 (s, 3H, 3 H-1), 5.41 (s, 1H, H-1), 6.84–6.97 (m, 4H, Ar-*H*); ^{13}C NMR (300 MHz, D_2O): δ 15.4, 16.6, 16.7, 16.7 (4 *Me*), 25.0 (O*Me*), 98.9, 102.2, 102.5, 102.6 (4 C-1); MALDI-TOFMS: *m/z* 731.5 [M+Na^+]; HRESIMS: calcd for [$C_{31}H_{48}O_{18}$]Na^+: 731.2735, found: *m/z* 731.2733.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.carres.2008.09.010.

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