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Note

Efficient synthesis of a 6-deoxytalose tetrasaccharide related to the antigenic O-polysaccharide produced by *Aggregatibacter actinomycetemcomitans* serotype c

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ABSTRACT

Concise synthesis of a 6-deoxy-α-L-talose tetrasaccharide, 6-deoxy-α-L-Tal*p*-(1→3)-6-deoxy-α-L-Tal*p*-(1→2)-6-deoxy-α-L-Tal*p*-(1→3)-6-deoxy-α-L-Tal*p*, the dimer of the disaccharide repeating unit of the OPS from *Aggregatibacter actinomycetemcomitans* serotype **c**, has been accomplished through suitable protecting group manipulations and stereoselective glycosylation starting from commercially available L-rhamnose. The target oligosaccharide in the form of its *p*-methoxyphenyl glycoside is suitable for further glycoconjugate formation via selective cleavage of this group.

Aggregatibacter actinomycetemcomitans is a Gram-negative, non-motile, non-spore-forming, facultative anaerobic rod-shaped bacterium that has been implicated in the etiology of localized juvenile periodontitis,[1–3] adult periodontitis,[4] and severe non-oral human infections.[5] Normally, *A. actinomycetemcomitans* strains are divided into six serotypes: **a**, **b**, **c**, **d**, **e**, and **f**, according to their characteristic O-antigenic polysaccharides. These are forming the outer part of the LPS that is connected to the cell surfaces of the organism.[6–9] These molecules are involved in pathogenic processes and in mediating resistance to host defense mechanisms, and have been regarded as an important factor in the virulence of many animal and plant pathogens.[10] It was revealed that most of the polysaccharides in *A. actinomycetemcomitans* strains are made up of 6-deoxysugars, including 6-deoxy-D-talose, 6-deoxy-L-talose, D-rhamnose, and D-fucose.[6–9] Among them, serotype **c**-specific polysaccharide antigen is a 6-deoxy-L-talan composed of repeating disaccharide units: →3)-6-deoxy-L-Tal*p*-(1→2)-6-deoxy-α-D-Tal*p*-(1→, which are acetylated at the O-2 position of the 1,3-linked 6-deoxy-L-talose.[5,11] Although 6-deoxy-L-talose is a key building block of many biologically important glycopeptidolipids (GPLs)[12–15] and an essential component of numerous antigenic bacterial lipopolysaccharides (LPSs),[16–20] polysaccharides consisting of only 6-deoxytalose are rare. Synthetic studies on these antigenic O-polysaccharides are of considerable interests in view of developing novel vaccine candidates and studying structure–bioactivity relationship of carbohydrates.[21–24] In this paper, we wish to report the first total synthesis of the 6-deoxy-α-L-talose tetrasaccharide, the dimer of the disaccharide repeating unit of the antigenic O-polysaccharide produced by *A. actinomycetemcomitans* serotype **c**, in the form of its *p*-methoxyphenyl glycoside (Fig. 1). The *p*-methoxyphenyl group will, after cleavage, enable us to conjugate the oligosaccharide with a suitable aglycon, if necessary.

Since tetrasaccharide **1** consists of a single monosaccharide type, 6-deoxy-L-talose, our interest was firstly concentrated on the development of an efficient synthetic strategy for the large scale preparation of a 6-deoxy-L-talose building block, namely, *p*-methoxyphenyl 6-deoxy-α-L-talopyranoside **5**. As shown in Scheme 1, *p*-methoxyphenyl 2,3-*O*-isopropylidene-α-L-rhamnopyranoside **3** was prepared from L-rhamnose through a more convenient procedure than the previously reported one:[25] L-rhamnose

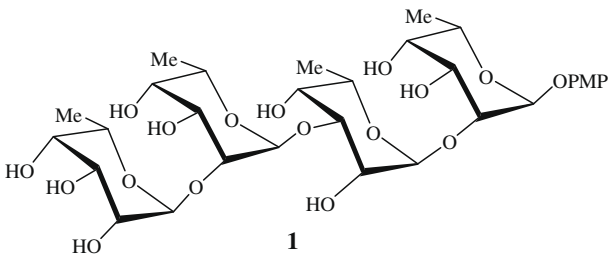

Figure 1. Structure of the target tetrasaccharide.

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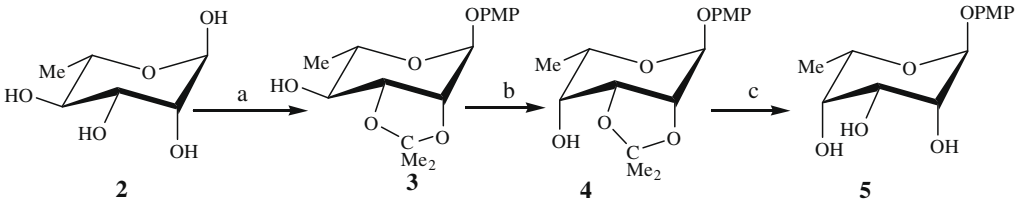
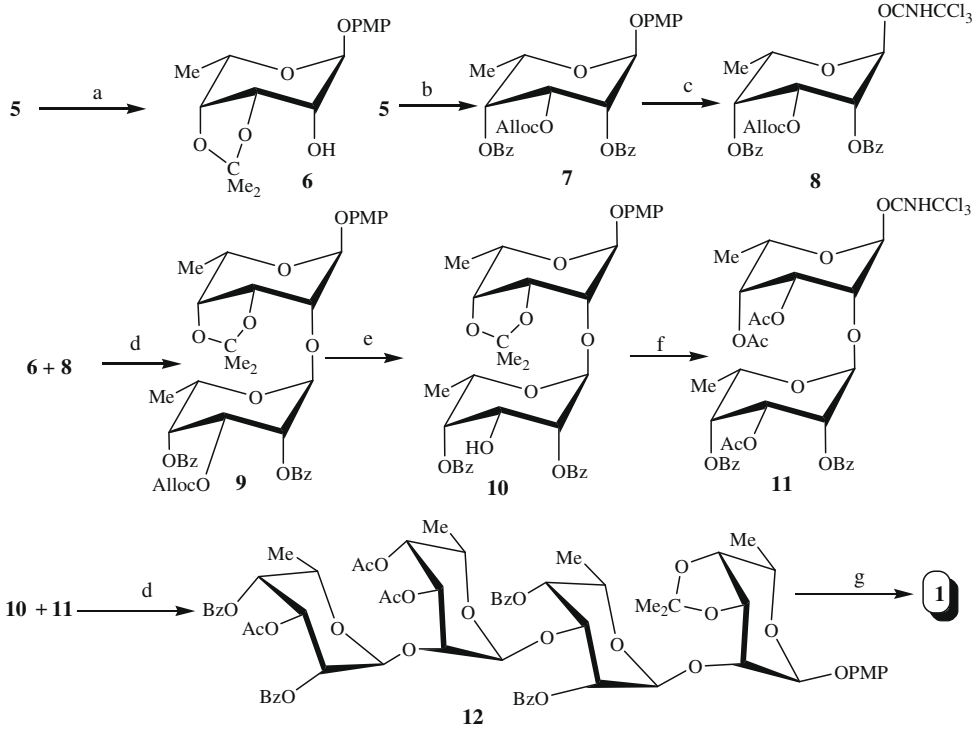


Scheme 1. Reagents and conditions: (a) (i) Py–Ac_2O, 70 °C, 3 h; (ii) 4-methoxyphenol, $BF_3{\cdot}Et_2O$, CH_2Cl_2, 0–25 °C, 3 h; (iii) MeOH–MeONa; (iv) DMF, $CH_3C(OCH_3)_2CH_3$, cat. $TsOH{\cdot}H_2O$, 30 °C, 24 h. 61% for four steps; (b) (i) DMSO, Et_3N, $PhOPOCl_2$, CH_2Cl_2, −15 to −10 °C, 6–8 h; (ii) 50% $CH_3COOCH_2CH_3$, H_2O, $NaBH_4$. 86% for two steps; (c) 70%AcOH, 3 h, 70 °C, 93%.

was acetylated with pyridine–Ac_2O, after co-evaporated with toluene, the crude tetra-*O*-acetyl derivative was treated with *p*-methoxyphenol and boron trifluoride diethyl etherate affording crude *p*-methoxyphenyl 2,3,4-tri-*O*-acetyl-α-L-rhamnopyranoside. The crude product was deacetylated in MeOH–MeONa, providing the *p*-methoxyphenyl α-L-rhamnopyranoside as a white solid after precipitating from EtOAc–petroleum ether (2:1). Isopropylidenation of the solid in dry DMF with 2,2-dimethoxylpropane in the presence of catalytic amounts of $TsOH{\cdot}H_2O$ resulted in compound **3** that could be precipitated as a white solid when pouring the reaction mixture into crushed ice. These four steps were carried out in consecutive way without chromatographic separation, making the preparation of **3** in large scale possible with high yield (61% from **2**).

Inversion of the configuration at the C-4 atom of L-rhamnose derivative **3** was smoothly accomplished using the phenyl dichlorophosphate (PDCP)-mediated Pfitzner–Moffat oxidation[26] in methylene chloride followed by $NaBH_4$ reduction yielding *p*-methoxyphenyl 2,3-*O*-isopropylidene-6-deoxy-α-L-talopyranoside **4**[27] in 86% yield over two steps. In contrast to our previously reported PDC oxidation procedure[27] in which a chromatographic separation was unavoidable, the crude oxidation product could be isolated sufficiently pure to be used in the reduction after an aqueous workup. Subsequent deacetonation of **4** was accomplished by aqueous acetic acid to give *p*-methoxyphenyl 6-deoxy-α-L-talopyranoside **5** in 93% yield. By far, the 6-deoxy-α-L-talose derivative **5** was synthesized from L-rhamnose efficiently in an overall yield of 49%. The preparation could be easily scaled up to 20 g of starting material, giving ca. 16 g of compound **5**.

Subsequently, synthesis of the target tetrasaccharide **1** was accomplished through transformation of **5** into suitably protected building blocks **6** and **8**, followed by stepwise glycosylation and de-protection as depicted in Scheme 2. Kinetically controlled isopropylidenation[28] of *p*-methoxyphenyl 6-deoxy-α-L-taloside **5** with 2,2-dimethoxypropane resulted in the C-2–OH acceptor, *p*-methoxyphenyl 3,4-*O*-isopropylidene-6-deoxy-α-L-talopyranoside (**6**), exclusively in 85% yield. The regioselective 3,4-*O*-isopropylidenation product was confirmed by the ^1H NMR of the product, showing characteristic large coupling constant for the anomeric proton at δ 5.31 ppm ($J_{1,2}$ = 5.6 Hz).[23,28] An important change of conformation of the taloside takes place after formation of the 3,4-O-linked bicyclic acetal, and the molecule takes a boat or twist-boat conformation of the ring instead of the more common chair form of the precursors. The coupling constant between H-1 and H-2 indicated an almost diaxial orientation of these protons, and this phenomenon was also observed previously.[23,28] Meanwhile,



Scheme 2. Reagents and conditions: (a) DMF, $CH_3C(OCH_3)_2CH_3$, cat. $TsOH{\cdot}H_2O$, 30 °C, 24 h, 90%; (b) AllocCl, Py, DMAP, CH_2Cl_2; −15 to 0 °C, 3 h; then BzCl, 0–25 °C, 12 h; 73%; (c) (i) 80% CH_3CN, CAN, 35 °C, 0.5 h; (ii) CCl_3CN, DBU, CH_2Cl_2, rt, 0.5 h, 81% for two steps; (d) TMSOTf, CH_2Cl_2, −10 °C to rt, 2 h, 88% for **9**; 71% for **12**; (e) MeOH–THF = 1:1, $NaBH_4$, $Pd[P(C_6H_5)_3]_4$, CH_3COONH_4, 90%; (f) (i) 70% AcOH, 3 h, 70 °C, 90%; (ii) Ac_2O, Py; (iii) 80% CH_3CN, CAN, 35 °C, 0.5 h; (iv) CCl_3CN, DBU, CH_2Cl_2, rt, 0.5 h, 67% for four steps; (g) (i) 70% AcOH, 3 h, 70 °C, (ii) satd NH_3–MeOH, rt, 96 h, 75% for two steps.

treatment of **5** with allyloxycarbonyl chloride in dichloromethane at −10 °C in the presence of 10 equiv of pyridine and catalytic amounts of DMAP, the C-3 hydroxyl group was selectively acylated to give *p*-methoxyphenyl 3-*O*-allyloxycarbonyl-6-deoxy-α-L-talopyranoside, which was benzoylated with 3 equiv of benzoyl chloride to provide *p*-methoxyphenyl 3-*O*-allyloxycarbonyl-2,4-di-*O*-benzoyl-6-deoxy-α-L-talopyranoside (**7**) in 73% yield. The ^{1}H NMR of compound **7** was in accordance with our previously reported data.[27,29] Since the equatorially oriented C-3–OH is more reactive than the axial C-2–OH or C-4–OH, the regioselectivity was not unexpected. Cleavage of the *p*-methoxyphenyl group of **6** with ceric ammonium nitrate (CAN) in CH_3CN–H_2O followed by trichloroacetimidation with trichloroacetonitrile in the presence of DBU[30] provided 3-*O*-allyloxycarbonyl-2,4-di-*O*-benzoyl-α-L-talopyranosyl trichloroacetimidate (**8**) in 81% yield. Glycosylation of acceptor **6** with donor **8** was accomplished within 40 min using TMSOTf as the catalyst at −5 °C, giving the α-linked disaccharide **9** in satisfactory yield (88%) after column chromatography, and the configuration of the glycosyl bond formed in the product was deduced from the corresponding coupling constants (δ 5.39 ppm, $J_{1,2}$ = 1.1 Hz).

With disaccharide **9** in hand, assembly of the tetrasaccharide **12** was achieved through three steps: (1) the allyloxycarbonyl group of **9** was successfully removed in MeOH–THF[31] in the presence of CH_3COONH_4, $Pd[P(C_6H_5)_3]_4$, and $NaBH_4$, within 5 min without affecting any of the other protecting groups, giving the desired acceptor **10** in 90% yield; (2) preparation of disaccharide glycosyl donor **11** as follows: firstly, removal of the isopropylidene ring of **10** with 70% HOAc at 70 °C for 3 h provided the triol intermediate, then, acetylation of the intermediate with py-Ac_2O provided *p*-methoxyphenyl 3-*O*-acetyl-2,4-di-*O*-benzoyl-6-deoxy-α-L-talopyranosyl-(1→2)-3,4-di-*O*-acetyl-6-deoxy-α-L-talopyranoside, which was the corresponding disaccharide donor **11** through C-1 deprotection and trichloroacetimidation. Since the C-1 deprotection was carried out in MeCN–H_2O with the presence of CAN, transformation of the acidic liable isopropylidene-protecting group to relatively stable acetyl groups was necessary; (3) TMSOTf-catalyzed coupling reaction of disaccharide acceptor **10** with donor **11** took place smoothly at 0 °C, giving fully protected tetrasaccharide **12** in 71% yield. The structure of **12** was confirmed by its ^{1}H NMR and ^{13}C NMR spectra, showing the characteristic signals such as δ 5.44, δ 5.35, 5.34, and 5.32 ppm for H-1, δ 98.8, 98.7, 96.9, and 96.2 ppm for C-1, and δ 16.4, 16.3, 16.1, and 15.3 ppm for C-6. The configuration of the glycosyl bond formed in the product was concluded from the corresponding coupling constants (δ 5.35 ppm, $J_{1'',2''}$ 1.1 Hz). Finally, deisopropylidenation of **12** in 70% HOAc followed by deacylation in ammonium-saturated methanol afforded the target tetrasaccharide **1** in 79% yield and its bioassay is in progress and will be reported in due course.

1. Experimental

1.1. General methods

Optical rotations were determined with a Perkin–Elmer model 241-MC automatic polarimeter for solution in a 1-dm, jacketed cell. ^{1}H and ^{13}C NMR spectra were recorded with Bruker DPX300 and Bruker AVANCE600 spectrometers in $CDCl_3$ or D_2O solutions. Internal references: TMS (δ 0.000 ppm for ^{1}H), $CDCl_3$ (δ 77.00 ppm for ^{13}C), HOD (δ 4.700 for ^{1}H). ^{1}H NMR signals of some compounds 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. Solutions were concentrated at a temperature <60 °C under diminished pressure.

1.2. *p*-Methoxyphenyl 2,3-*O*-isopropylidene-α-L-rhamnopyranoside (3)

L-Rhamnose (**2**) (20.0 g, 0.11 mol) was dissolved in a mixture of pyridine (100 mL) and Ac_2O (80 mL), and the solution was stirred at rt overnight. The solvents were evaporated in vacuo, and then co-evaporated with toluene, providing the tetra-*O*-acetyl derivative as a syrup. Then to a cold (0 °C) solution of the syrup and *p*-methoxyphenol (20.7 g, 0.17 mol) in dry CH_2Cl_2 (50 mL) was added boron trifluoride diethyl etherate (70 mL, 0.55 mol) for 30 min. Then, the mixture was agitated for 6 h at rt until TLC (petroleum ether–EtOAc; 2:1) indicated that the reaction was complete. The reaction mixture was poured into crushed ice, and the excess boron trifluoride diethyl etherate was neutralized by the careful addition of $NaHCO_3$. The organic layer was washed with water, dried (Na_2SO_4), and concentrated in vacuo to provide a white solid. The solid was dried under high vacuum for 2 h and then dissolved in 150 mL absolute MeOH, 200 mg MeONa was added to the reaction mixture and stirred at rt for 3 h. Neutralization of the reaction mixture was with acidic ion exchange resin (Amberlite IR-120 (H^+), Alfa Aesar) and the organic phase was concentrated to a volume of 50 mL. Then 300 mL of EtOAc–petroleum ether (2:1) was added to the mixture with vigorous stirring, and the target compound was precipitated from the mixture as a white solid. Drying under high vacuum for 2 h, the above-obtained product was dissolved in dry DMF under nitrogen atmosphere, TsOH–H_2O (0.32 g, 1.7 mmol) and 2,2-dimethoxylpropane (21 mL, 0.17 mol) were added, and the mixture was stirred for 6 h at rt until completion (petroleum ether–EtOAc; 2:1). The reaction mixture was poured into 5%$NaHCO_3$, and a white solid was obtained after standing for 12 h at rt, after filtrating and drying, *p*-methoxyphenyl 2,3-*O*-isopropylidene-α-L-rhamnoside (**3**) was fulfilled (23.2 g, 61% over four steps). The ^{1}H NMR data were in accordance with the literature.[25]

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

A solution of $PhOPOCl_2$ (9.2 mL, 62 mmol) in *i*-PrOAc (18 mL) was added dropwise over 1 h to a solution of *p*-methoxyphenyl 2,3-*O*-isopropylidene-α-L-rhamnoside (**3**) (11.4 g, 36.7 mmol), DMSO (10.4 mL, 146 mmol), and TEA (25.6 mL, 184 mmol) in *i*-PrOAc (192 mL) at −15 °C. The mixture was stirred for 1 h at −15 °C and then poured into 1% aqueous H_3P0_4. The aqueous phase was extracted with *i*-PrOAc (3 × 50 mL), and the combined organic phases were washed with saturated aqueous $NaHCO_3$ (50 mL) and evaporated in vacuo to give *p*-methoxyphenyl 6-deoxy-2,3-*O*-isopropylidene-α-L-lyxohexopyranosid-4-ulose as a yellowish syrup. Then to a cold (0 °C) solution of the syrup in 50% aq EtOAc was added $NaBH_4$ (1.5 g, 42 mmol). The mixture was stirred at 0 °C for 15 min, and TLC (petroleum ether–EtOAc; 4:1) indicated that the reaction was complete. The aqueous solution was extracted with EtOAc (3 × 150 mL), the extract was washed with M HCl and satd aq $NaHCO_3$, dried (Na_2SO_4), and concentrated to give crude **4** as a syrup. Purification of the crude product by crystallization (petroleum ether–EtOAc; 4:1) provided **4** (9.8 g, 86% over two steps) as white crystals. The ^{1}H NMR data were in accordance with the literature.[27]

1.4. *p*-Methoxyphenyl 6-deoxy-α-L-talopyranoside (5)

Compound **4** (6.2 g, 20 mmol) was dissolved in 70% aq AcOH (50 mL) and the solution was stirred at 70 °C for 3 h. The solvents were evaporated in vacuo and then co-evaporated with toluene,

which afforded the triol **5** (5.0 g, 93%) as a white solid after precipitating from EtOAc–petroleum ether (2:1). $[\alpha]_D^{25}$ −60 (*c* 1.0, $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$) δ: 7.01–6.95 (m, 2H, Ar-*H*), 6.84–6.77 (m, 2H, Ar-*H*), 5.50 (s, 1H, H-1), 4.46 (s, 2H, 2 × O*H*), 4.11–4.00 (m, 4H), 3.77 (m, 1H), 3.76 (s, 3H, OCH_3), 1.27 (d, 3H, $J_{5,6}$ 6.5 Hz, C-CH_3). HRMS calcd for $C_{13}H_{18}O_6Na$ $(M+Na)^+$: 293.1001, found: 293.1030.

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

To a solution of compound **5** (4.6 g, 17 mmol) with $TsOH\cdot H_2O$ (0.048 g, 0.26 mmol) in dry DMF under an N_2 atmosphere was added 2,2-dimethoxypropane (4.1 mL) dropwise. The mixture was allowed to stir at room temperature for 2 h when TLC (petroleum ether–EtOAc; 1:1) indicated that the reaction was complete. White precipitate was formed when pouring the reaction mixture into crushed ice. Filtration and then vacuum-drying on the filter for about 1 h gave acceptor **6** (4.7 g, 90%) as a white solid. $[\alpha]_D^{25}$ −20 (*c* 1.0, $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$) δ: 7.00–7.05 (m, 2H, Ar-*H*), 6.80–6.85 (m, 2H, Ar-*H*), 5.32 (d, 1H, $J_{1,2}$ 5.6 Hz, H-1), 4.61 (dd, 1H, $J_{3,4}$ 7.5 Hz, $J_{4,5}$ 3.4 Hz, H-4), 4.18 (dd, 1H, $J_{2,3}$ 2.0 Hz, $J_{3,4}$ 7.5 Hz, H-3), 4.00–3.92 (m, 2H, H-2, H-5), 3.77 (s, 3H, OCH_3), 2.43 (s, 1H, O*H*), 1.54, 1.39 (2s, 6H, C(CH_3)$_2$), 1.26 (d, $J_{5,6}$ 6.5 Hz, 3H, C-CH_3). Anal. Calcd for $C_{16}H_{22}O_6$: C, 61.92; H, 7.15. Found: C, 61.80; H, 7.28.

1.6. *p*-Methoxyphenyl 3-*O*-allyloxycarbonyl-2,4-di-*O*-benzoyl-6-deoxy-α-L-talopyranoside (7)

To a cold (−10 °C) solution of compound **5** (2.3 g, 8.5 mmol) in dry CH_2Cl_2 (20 mL) containing dry pyridine (5.6 mL, 68 mmol) was added slowly allyl chloroformate (0.85 mL, 8.9 mmol) in anhyd CH_2Cl_2 (5 mL) under an N_2 atmosphere. After complete addition, the mixture was allowed to stir at rt for 1 h when TLC (petroleum ether–EtOAc; 1:1) showed complete conversion of the starting material to a faster moving spot. Then benzoyl chloride (2.2 mL, 19 mmol) in pyridine (5 mL) was added dropwise to the solution over 30 min at −0 °C. The reaction mixture was slowly raised to rt and stirred overnight, at the end of which time TLC (petroleum ether–EtOAc; 2:1) indicated that the reaction was complete. The reaction mixture was diluted with CH_2Cl_2 (50 mL), washed with ice-water, M HCl, and dried (Na_2SO_4). The solution was concentrated, and purification of the residue by column chromatography on silica gel (petroleum ether–EtOAc; 4:1) gave compound **7** (3.5 g, 73%) as a white solid.[27,29] $[\alpha]_D^{25}$ −88 (*c* 1.0, $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$) δ: 8.17–7.17 (m, 10H, Bz-*H*), 7.08–7.05 (m, 2H, Ar-*H*), 6.87–6.84 (m, 2H, Ar-*H*), 5.93 (m, 1H, CH_2=C*H*CH_2OCO), 5.71 (m, 1H, H-2), 5.67 (m, 1H, H-4), 5.59–5.56 (m, 2H, H-1, H-3), 5.38–5.22 (m, 2H, CH_2=HCH_2OCO), 4.69–4.65 (m, 2H, CH_2=HCH_2OCO), 4.46 (m, 1H, H-5), 3.78 (s, 3H, CH_3O), 1.29 (d, 3H, $J_{5,6}$ 6.5 Hz, H-6). Anal. Calcd for $C_{31}H_{30}O_{10}$: C, 66.18; H, 5.38. Found: C, 66.40; H, 5.52.

1.7. 3-*O*-Allyloxycarbonyl-2,4-di-*O*-benzoyl-6-deoxy-α-L-talopyranosyl trichloroacetimidate (8)

To a solution of compound **7** (3.0 g, 5.3 mmol) in acetonitrile (80 mL) and water (20 mL) was added CAN (11.6 g, 21.2 mmol). The mixture was stirred for 20 min at 30 °C, at the end of which time TLC (petroleum ether–EtOAc; 3:1) indicated that the reaction was complete. The solvent was evaporated under diminished pressure at 50 °C to give a residue, and then the residue was dissolved in CH_2Cl_2 and washed with water. The organic phase was dried (Na_2SO_4) and concentrated. Purification by silica gel chromatography with 4:1 petroleum ether–EtOAc as the eluent afforded 3-*O*-allyloxycarbonyl-2,4-di-*O*-benzoyl-α-L-6-deoxy-talopyranoside as a foamy solid. A mixture of this compound, trichloroacetonitrile (2.0 ml, 20 mmol), and 1,8-diazabicyclo[5.4.0] undecene (DBU) (0.20 ml, 20 mmol) in dry CH_2Cl_2 (70 ml) was stirred for 0.5 h and then concentrated. The residue was purified by chromatography with 5:1 petroleum ether–EtOAc as the eluent to give **8** (2.6 g, 81%). $[\alpha]_D^{25}$ −51 (*c* 1.0, $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$) δ: 8.81 (s, 1H, CN*H*CCl_3), 8.15–7.20 (m, 10H, Bz-*H*), 6.56 (d, 1H, $J_{1,2}$ 1.5 Hz, H-1), 5.92 (m, 1H, CH_2=*H*CH_2OCO), 5.70 (m, 1H, H-2), 5.63 (m, 1H, H-4), 5.45 (dd, 1H, $J_{2,3}$, $J_{3,4}$ 3.7 Hz, H-3), 5.36–5.22 (m, 2H, CH_2=HCH_2OCO), 4.68–4.64 (m, 2H, CH_2=HCH_2OCO), 4.53 (m, 1H, H-5), 1.35 (d, 3H, $J_{5,6}$ 6.5 Hz, H-6). Anal. Calcd for $C_{26}H_{24}Cl_3NO_9$: C, 51.97; H, 4.03; N, 2.33. Found: C, 51.74; H, 4.21; N, 2.78.

1.8. *p*-Methoxyphenyl 3-*O*-allyloxycarbonyl-2,4-di-*O*-benzoyl-6-deoxy-α-L-talopyranosyl-(1→2)-3,4-*O*-isopropylidene-6-deoxy-α-L-talopyranoside (9)

To a cooled (−10 °C) solution of **6** (1.1 g, 3.6 mmol) and **8** (2.4 g, 4.0 mmol) in anhydrous, redistilled CH_2Cl_2 (40 mL) was added 4 Å molecular sieves (2.0 g) and the mixture was stirred under an N_2 atmosphere for 30 min. Then TMSOTf (36 μL, 0.2 mmol) was added to the mixture. The reaction mixture was stirred at −10 °C for 0.5 h, during which time the mixture was allowed to gradually warm to ambient temperature. TLC (petroleum ether–EtOAc; 4:1) indicated that the reaction was complete. Then the reaction mixture was neutralized with Et_3N (two drops) and filtrated. The filtrate was evaporated in vacuo to give a residue, which was purified by silica gel column chromatography (petroleum ether–EtOAc; 6:1) to give disaccharide **9** (1.5 g, 88%). $[\alpha]_D^{25}$ −73 (*c* 1.0, $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$) δ: 8.14–7.17(m, 10H, Bz-*H*), 7.07–6.98 (m, 2H, Ar-*H*), 6.87–6.84 (m, 2H, Ar-*H*), 5.90 (m, 1H, CH_2=*H*CH_2OCO), 5.59 (m, 1H, H-2′), 5.53 (m, 1H, H-3′), 5.45 (d, 1H, $J_{1,2}$ 6.4 Hz, H-1), 5.39 (d, 1H, $J_{1,2}$ 1.1 Hz, H-1), 5.38 (m, 1H, H-4), 5.34–5.20 (m, 2H, CH_2=HCH_2OCO), 4.72 (dd, 1H, $J_{2,3}$ 2.5 Hz, $J_{3,4}$ 7.6 Hz, H-3), 4.63–4.59 (m, 3H, H-5′, CH_2=HCH_2OCO), 4.17 (dd, 1H, $J_{3,4}$ 7.6 Hz, $J_{4,5}$ 1.7 Hz, H-4), 4.15 (dd, 1H, $J_{1,2}$ 6.4 Hz, $J_{2,3}$ 2.5 Hz, H-2), 3.94 (m, 1H, H-5), 3.78 (s, 3H, CH_3O), 1.56, 1.42 (2s, 6H, C(CH_3)$_2$), 1.31, 1.23 (2d, 6H, *J* 6.5 Hz, H-6, H-6′). ^{13}C NMR (300 MHz, $CDCl_3$) δ: 166.4, 166.1 (2 × *C*OPh), 155.1, 153.9 (OCOO), 150.8, 118.9, 118.8, 118.5, 118.4(2), 114.7(2), 110.7 (*C*(CH$_3$)$_2$), 98.7, 96.2 (2 × C-1), 55.7 (*C*H$_3$O), 26.2, 25.5 (C(*C*H$_3$)$_2$), 16.2, 15.3 (2 × C-6). Anal. Calcd for $C_{40}H_{44}O_{14}$: C, 64.16; H, 5.92. Found: C, 64.37; H, 5.98.

1.9. *p*-Methoxyphenyl 2,4-di-*O*-benzoyl-6-deoxy-α-L-talopyranosyl-(1→2)-3,4-*O*-isopropylidene-6-deoxy-α-L-talopyranoside (10)

To a cooled (−5 °C) solution of compound **9** (1.2 g, 2.5 mmol) in 1:1 MeOH–THF (40 mL) was added CH_3COONH_4 (1.9 g, 25 mmol). With vigorous stirring, $NaBH_4$ (0.027 g, 0.75 mmol), $Pd[P(C_6H_5)_3]_4$ (0.12 g, 0.10 mmol), and $NaBH_4$ (0.11 g, 3.0 mmol) were added in three portions immediately one after another. One minute after the addition of the second portion of $NaBH_4$, TLC (petroleum ether–EtOAc; 3:1) indicated that the reaction was complete. The reaction mixture was concentrated under vacuum, and the residue was dissolved in CH_2Cl_2 (20 mL) and washed with brine (10 mL), then the organic phase was dried over Na_2SO_4. Evaporation and purification by flash column chromatography (petroleum ether–EtOAc; 5:1) afforded compound **10** as a white solid (1.5 g, 90%). $[\alpha]_D^{25}$ −62 (*c* 1.0, $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$) δ: 8.12–7.22 (m, 10H, Bz-*H*), 7.00–6.97 (m, 2H, Ar-*H*), 6.87–6.84 (m, 2H, Ar-*H*), 5.44–5.39 (m, 3H, H-2′, H-4′, H-1), 5.38 (s, 1H, H-1′), 4.74 (dd, 1H, $J_{2,3}$ 2.3 Hz, $J_{3,4}$ 7.6 Hz, H-3), 4.54 (m, 1H, H-5′), 4.45 (t, 1H, $J_{3',4'}$, $J_{4',5'}$ 3.8 Hz, H-3′), 4.18 (dd, 1H, $J_{3,4}$ 7.6 Hz, $J_{4,5}$ 1.4 Hz, H-4),

4.15 (dd, 1H, $J_{2,3}$ 2.3 Hz, $J_{1,2}$ 6.4 Hz, H-2), 3.95 (m, 1H, H-5), 3.79 (s, 3H, CH_3O), 1.56, 1.42 (2s, 6H, C(CH_3)$_2$), 1.32, 1.23 (2d, 6H, J 6.5 Hz, H-6, H-6′). Anal. Calcd for $C_{36}H_{40}O_{12}$: C, 65.05; H, 6.07. Found: C, 64.82; H, 6.31.

1.10. *p*-Methoxyphenyl 3-*O*-acetyl-2,4-di-*O*-benzoyl-6-deoxy-α-L-talopyranosyl-(1→2)-3,4-di-*O*-acetyl-6-deoxy-α-L-talopyranosyl trichloroacetimidate (11)

Deisopropylidenation of compound **10** (1.3 g, 2.0 mmol) was carried out in 70% aq AcOH (30 mL) at 70 °C for 3 h, at the end of which time TLC (petroleum ether–EtOAc; 2:1) indicated completion of the reaction. The mixture was concentrated under diminished pressure and then co-evaporated with toluene (2 × 20 mL) to give the crude product. The crude product was then acetylated with Ac_2O (5 mL) in pyridine (20 mL) without purification to obtain its acetylated derivative. The disaccharide trichloroacetimidate **11** was prepared by following the same procedure as described above for the preparation of compound **8**. After purification by column chromatography on silica gel (petroleum ether–EtOAc; 4:1), **11** (0.97 g, 66% over four steps) was obtained as a white foam. $[\alpha]_D^{25}$ −43 (*c* 1.0, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.68 (s, 1H, CN*H*CCl$_3$), 8.18–7.15 (m, 10H, Bz-*H*), 6.47 (d, 1H, $J_{1,2}$ 1.4 Hz, H-1), 5.62 (t, 1H, $J_{2',3'}$, $J_{3',4'}$ 3.8 Hz, H-3′), 5.55 (m, 1H, H-2′), 5.37 (m, 1H, H-4), 5.32–5.30 (m, 2H, H-1′, H-4′), 5.30 (t, 1H, $J_{2,3}$, $J_{3,4}$ 3.6 Hz, H-3), 4.55 (m, 1H, H-5), 4.39 (m, 1H, H-5′), 4.12 (m, 1H, H-2), 2.28, 2.10, 2.00 (3s, 9H, 3 × CH_3CO), 1.34, 1.27 (2d, 6H, J 6.2 Hz, J 6.5 Hz, 2 × C-CH$_3$). Anal. Calcd for $C_{34}H_{36}Cl_3NO_{14}$: C, 51.76; H, 4.60; N, 1.78. Found: C, 51.53; H, 4.78; N, 1.92.

1.11. *p*-Methoxyphenyl 3-*O*-acetyl-2,4-di-*O*-benzoyl-6-deoxy-α-L-talopyranosyl-(1→2)-3,4-di-*O*-acetyl-6-deoxy-α-L-talopyranosyl-(1→3)-2,4-di-*O*-benzoyl-6-deoxy-α-L-talopyranosyl-(1→2)-3,4-*O*-isopropylidene-6-deoxy-α-L-talopyranoside (12)

Glycosylation between disaccharide acceptor **10** (0.40 g, 0.60 mmol) and donor **11** (0.77 g, 0.96 mmol) was accomplished by following the same procedure as described above for the preparation of disaccharide **9**. After purification, tetrasaccharide **12** (0.94 g, 74%) was afforded as a white foamy solid. $[\alpha]_D^{25}$ −10 (*c* 1.0, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.17–7.16 (m, 20H, Bz-*H*), 7.02–6.98 (m, 2H, Ar-*H*), 6.88–6.85 (m, 2H, Ar-*H*), 5.61 (t, 1H, $J_{2''',3'''}$, $J_{3''',4'''}$ 3.7 Hz, H-3‴), 5.54–5.45 (m, 3H, H-4′, H-4‴, H-2‴), 5.44 (d, 1H, $J_{1,2}$ 6.4 Hz, H-1), 5.35 (d, 1H, $J_{1'',2''}$ 1.1 Hz, H-1″), δ 5.34 (d, 1H, $J_{1',2'}$ 0.8 Hz, H-1′), 5.31 (m, 1H, H-2′), 5.32 (d, 1H, $J_{1''',2'''}$ 1.0 Hz, H-1‴), 5.19 (m, 1H, H-4″), 4.95 (m, 1H, $J_{2'',3''}$, $J_{3'',4''}$, 3.6 Hz, H-3″), 4.73 (dd, 1H, $J_{2,3}$ 2.4 Hz, $J_{3,4}$ 7.5 Hz, H-3), 4.56–4.51 (m, 2H, 2 × H-5), 4.45 (t, 1H, $J_{2',3'}$, $J_{3',4'}$, 3.9 Hz, H-3′), 4.28 (m, 1H, H-5), 4.18 (dd, 1H, $J_{3,4}$ 7.5 Hz, $J_{4,5}$ 1.7 Hz, H-4), 4.15 (dd, 1H, $J_{1,2}$ 6.4 Hz, $J_{2,3}$ 2.4 Hz, H-2), 3.98 (m, 1H, H-5), 3.79 (s, 3H, CH_3O), 3.66 (m, 1H, H-2″), 2.23, 1.97, 1.92 (3s, 9H, 3 × CH_3CO), 1.58, 1.42 (2s, 6H, C(CH_3)$_2$), 1.38, 1.34, 1.24, 1.20 (4d, 12H, J 6.5 Hz, 4 × C-CH_3). ^{13}C NMR (300 MHz, $CDCl_3$) δ: 171.2, 169.8, 169.2 (3 × CH_3CO), 166.5, 166.4, 166.2, 165.9 (4 × *C*OPh), 110.6 (*C*(CH$_3$)$_2$), 98.8, 98.7, 96.9, 96.2 (4 × C-1), 55.6 ($C_6H_4OCH_3$), 26.2, 25.5 (C(CH_3)$_2$), 20.7 (2), 20.6 (3 × *C*H$_3$CO), 16.4, 16.3, 16.1, 15.3 (4 × C-CH_3). Anal. Calcd for $C_{68}H_{74}O_{25}$: C, 63.25; H, 5.78. Found: C, 63.02; H, 5.71.

1.12. *p*-Methoxyphenyl 6-deoxy-α-L-talopyranosyl-(1→2)-6-deoxy-α-L-talopyranosyl-(1→3)-6-deoxy-α-L-talopyranosyl-(1→2)-6-deoxy-α-L-talopyranoside (1)

Compound **12** (0.51 g, 0.39 mmol) was dissolved in 70% aq AcOH (25 mL) and the solution was stirred at 70 °C for 3 h. The solvents were evaporated in vacuo and then co-evaporated with toluene. The crude mixture thus obtained was purified by flash chromatography using petroleum ether–EtOAc (3:1) to get a white foam. Then the white foam was dissolved in satd NH_3–MeOH (40 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 **1** (0.21 g, 75% over two steps) as a foamy solid. $[\alpha]_D^{25}$ −70 (*c* 1.0, H_2O). ^{1}H NMR (300 MHz, D_2O) δ: 6.99–6.86 (2d, 4H, J 9.0 Hz, $C_6H_4OCH_3$), 5.40, 5.13, 5.06, 5.02 (4s, 4H, 4 × H-1), 4.11–3.87 (m, 13H), 3.71 (s, 3H, CH_3O), 3.69–3.66 (m, 3H), 1.20, 1.18, 1.16, 1.13 (4d, 12H, J 6.5 Hz, 4 × C-CH_3). ^{13}C NMR (300 MHz, D_2O) δ: 157.2, 152.1, 121.0(2), 117.3(2) (Ar-*C*), 105.9, 105.7, 101.2, 99.4 (4 × C-1), 57.9 ($C_6H_4OCH_3$), 18.0(2), 17.9, 17.8 (4 × C-*C*H$_3$). HRMS calcd for $C_{31}H_{48}O_{18}Na$ $(M+Na)^+$: 731.2738, found: 731.2716.

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References

1. Asikainen, S.; Lai, C. H.; Alaluusua, S.; Slots, J. *Oral Microbiol. Immunol.* **1991**, *6*, 115–118.
2. Slots, J.; Reynolds, H. S.; Genco, R. J. *Infect. Immun.* **1980**, *29*, 1013–1020.
3. Zambon, J. J. *J. Clin. Periodontol.* **1985**, *12*, 1–20.
4. Slots, J.; Bragd, L.; Wikstrom, M.; Dahlen, G. *J. Clin. Periodontol.* **1986**, *13*, 570–577.
5. Perry, M. B.; MacLean, L. M.; Brisson, J. R.; Wilson, M. E. *Eur. J. Biochem.* **1996**, *242*, 682–688.
6. Gmur, R.; McNabb, H.; van Steenbergen, T. J.; Baehni, P.; Mombelli, A.; van Winkelhoff, A. J.; Guggenheim, B. *Oral Microbiol. Immunol.* **1993**, *8*, 116–120.
7. Kaplan, J. B.; Perry, M. B.; MacLean, L. L.; Furgang, D.; Wilson, M. E.; Fine, D. H. *Infect. Immun.* **2001**, *69*, 5375–5384.
8. Saarela, M.; Asikainen, S.; Alaluusua, S.; Pyhuala, L.; Lai, C. H.; Jousimies-Somer, H. *Oral Microbiol. Immunol.* **1992**, *7*, 277–279.
9. Zambon, J. J.; Slots, J.; Genco, R. J. *Infect. Immun.* **1983**, *41*, 19–27.
10. Roberts, I. S. *Annu. Rev. Microbiol.* **1996**, *50*, 285–315.
11. Shibuya, N.; Amano, K.; Azuma, J.; Nishihara, T.; Noguchi, T.; Koga, T. *J. Biol. Chem.* **1991**, *266*, 16318–16323.
12. Chatterjee, D.; Bozic, C.; Aspinall, G. O.; Brennan, P. J. *J. Biol. Chem.* **1988**, *263*, 4092–4097.
13. Aspinall, G. O.; Crane, A. M.; Gammon, D. W.; Ibrahim, I. H.; Khare, N. K.; Chatterjee, D.; Rivoire, B.; Brennan, P. J. *Carbohydr. Res.* **1991**, *216*, 337–355.
14. Aspinall, G. O.; Khare, N. K.; Sood, R. K.; Chatterjee, D.; Rivoire, B.; Brennan, P. J. *Carbohydr. Res.* **1991**, *216*, 357–373.
15. Daubenspeck, J. M.; Zeng, H.; Chen, P.; Dong, S.; Steichen, C. T.; Krishna, N. R.; Pritchard, D. G.; Turnbough, C. L. *J. Biol. Chem.* **2004**, *279*, 30945–30953.
16. Zahringer, U.; Rettenmaier, H.; Moll, H.; Senchenkova, S. N.; Knirel, Y. A. *Carbohydr. Res.* **1997**, *300*, 143–151.
17. Forsberg, L. S.; Bhat, U. R.; Carlson, R. W. *J. Biol. Chem.* **2000**, *275*, 18851–18863.
18. Zatonsky, G. V.; Kocharova, N. A.; Veremeychenko, S. P.; Zdorovenko, E. L.; Shashkov, A. S.; Zdorovenko, G. M.; Knirel, Y. A. *Carbohydr. Res.* **2002**, *337*, 2365–2370.
19. Castro, C. D.; Gargiulo, V.; Lanzetta, R.; Parrilli, M. *Biomacromolecules* **2007**, *8*, 1047–1051.
20. Turska-Szewczuk, A.; Palusinska-Szysz, M.; Russa, R. *Carbohydr. Res.* **2008**, *343*, 477–482.
21. Liptak, A.; Kerekgyarto, J.; Popsavin, V.; Kajtar-Peredy, M.; Radies, L. *J. Carbohydr. Chem.* **1988**, *7*, 337–357.
22. Zuurmond, H. M.; Veeneman, G. H.; Mare, G. A.; Boom, J. H. *Carbohydr. Res.* **1993**, *241*, 153–164.
23. Heidelberg, T.; Martin, O. R. *J. Org. Chem.* **2004**, *69*, 2290–2301.
24. Fekete, A.; Gyergyoi, K.; Kover, K. E.; Bajza, I.; Liptak, A. *Carbohydr. Res.* **2006**, *341*, 1312–1321.
25. Sarkar, K.; Mukherjee, I.; Roy, N. *J. Carbohydr. Chem.* **2003**, *22*, 95–107.
26. Liu, H. J.; Nyangulu, J. M. *Tetrahedron Lett.* **1988**, *29*, 3167–3170.
27. Yan, S.; Liang, X.; Diao, P.; Yang, Y.; Zhang, J.; Wang, D.; Kong, F. *Carbohydr. Res.* **2008**, *343*, 3107–3111.
28. Janos, K.; Zoltan, S.; Andras, L. *Carbohydr. Res.* **1993**, *245*, 65–80.
29. Yan, S. Q.; Wu, X. M.; Liang, X. M.; Zhang, J. J.; Wang, D. Q. *Chin. Chem. Lett.* **2009**, *20*, 582–585.
30. Schmidt, R. R. *Angew. Chem., Int. Ed. Engl.* **1986**, *25*, 212–235.
31. Zong, G. H.; Yan, S. Q.; Liang, X. M.; Zhang, J. J.; Wang, D. Q.; Kong, F. Z. *Chin. Chem. Lett.* **2009**, *20*, 127–130.