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Synthesis of the Upstream Terminal Disaccharide of the O-Antigenic Polysaccharide of *Vibrio cholerae* O37

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The terminal disaccharide of the *O*-antigenic polysaccharide of *Vibrio cholerae* O37, 4-*O*-methyl-α-D-QuiNAc-(1→4)-α-D-QuiNAc, was synthesized as methyl glycoside involving glycosylation between glycosyl donor ethyl 2-azido-3-*O*-benzyl-2,6-dideoxy-4-*O*-methyl-6-iodo-1-thio-α-D-glucopyranoside and glycosyl acceptor methyl 2-azido-3-*O*-benzyl-2,6-dideoxy-6-iodo-α-D-glucopyranoside. Dehalogenation, global deprotection, and reduction of the azide to amine were effected in one step by catalytic hydrogenation. It was followed by selective *N*-acetylation to give the desired deprotected disaccharide.

Keywords *Vibrio cholerae* O37; *O*-Antigenic lipopolysaccharide; Synthesis; Upstream terminal disaccharide

INTRODUCTION

Vibrio cholerae serogroups O1 and O139 are considered the causative organisms of the disease cholera and are responsible for epidemic and pandemic outbreaks. More than 200 *V. cholerae* serogroups have been identified to date, but strains belonging to serogroups other than O1 and O139 are usually associated with sporadic outbreaks with cholera-like infection having much less severity and lacking pandemic potential.[1] The reason that other strains of *V. cholerae* do not have pandemic potential still remains unknown. However, the *V. cholerae* serogroup O37 caused major outbreaks of diarrheal disease in Sudan[2] in 1968 and later in Bangkok[3] during 1993–1995.

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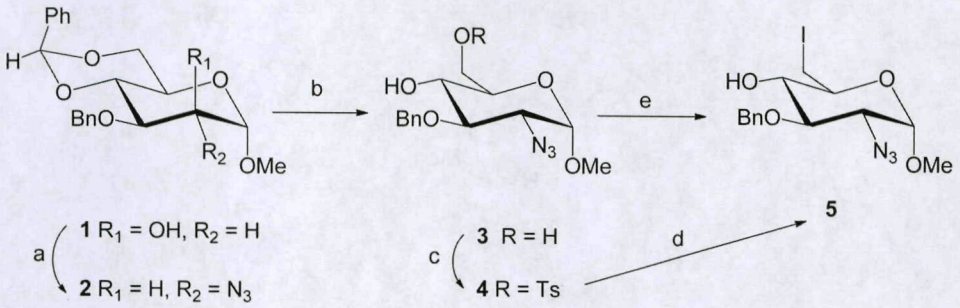
The bacterial cell surface lipopolysaccharides (LPSs) play an important role in antibacterial immunity and virulence. The structural architecture of the *O*-antigenic LPS of *V. cholerae* O37 had been established.[4] The upstream nonreducing terminal sugar was found to be α-D-quinovosamine, partially substituted with the 4-*O*-methyl group. It is interesting to mention that, in the case of *V. cholerae* O1, the *Ogawa* serotype contains terminal 2-*O*-methyl perosamine,[5] whereas the *Inaba* serotype contains only perosamine[6] as the terminal sugar, which determines the serotype specificity of *Inaba* and *Ogawa*.[7] Therefore, it is anticipated that the upstream nonreducing terminal sugar (i.e., partially substituted α-D-quinovosamine residue) may have a significant role in immunity and antigenicity as antigenic determinants in bacterial O-antigenic polysaccharides.[8] Synthesis of antigenic determinants of *V. cholerae* O1 and O139 was reported in the literature,[9] and some of these synthetic molecules have been used in immunochemical studies.[10] We initially tried to isolate the upstream terminal disaccharide of *V. cholerae* O37, that is, 2-acetamido-2,6-dideoxy-4-*O*-methyl-α-D-glucopyranosyl-(1→4)-2-acetamido-2,6-dideoxy-α-D-glucopyranose, by partial acid or enzymatic hydrolysis without success. Presently, no suitable endo-enzyme is available to produce the desired disaccharide. Therefore, we explored the chemical synthesis of the desired disaccharide as described herein.

RESULTS AND DISCUSSION

A convergent approach based on the glycosylation of suitably protected 4-*O*-methyl-α-D-quinovosamine as a glycosyl donor and α-D-quinovosamine as a glycosyl acceptor was applied for the preparation of the target disaccharide. Glycosyl acceptor **5** was synthesized from methyl 3-*O*-benzyl-4,6-*O*-benzylidene-α-D-mannopyran-oside **1** (Sch 1).[11] It was first converted to the corresponding triflate derivative followed by S_N2 displacement with azide to produce **2** in 60% yield. An earlier report showed that in the case of methyl α-D-mannopyranoside, inversion at C-2 using mesyl as leaving group is not feasible.[12] This was reasoned to the dipolar interaction between the approaching nucleophile and axially oriented C-1 methoxy group giving rise to a highly unstable transition stage originated due to the flattening of the pyranoside ring. The study also revealed that, in addition to the electrostatic interaction, there was some nonbonded steric interaction between the approaching nucleophile and conformationally positioned axial hydrogen atoms. Another report for the synthesis of **2** involves N-imidazolylsulfonyl derivatives as a leaving group, but the yield was again found to be poor (~23%).[13] In this synthesis a better leaving group "triflate" was used to achieve the inversion at rt with the azide as nucleophile, minimizing dipolar as well as 1,4-nonbonded steric interaction between the nucleophile and the C-5 hydrogen atom, making the reaction feasible.

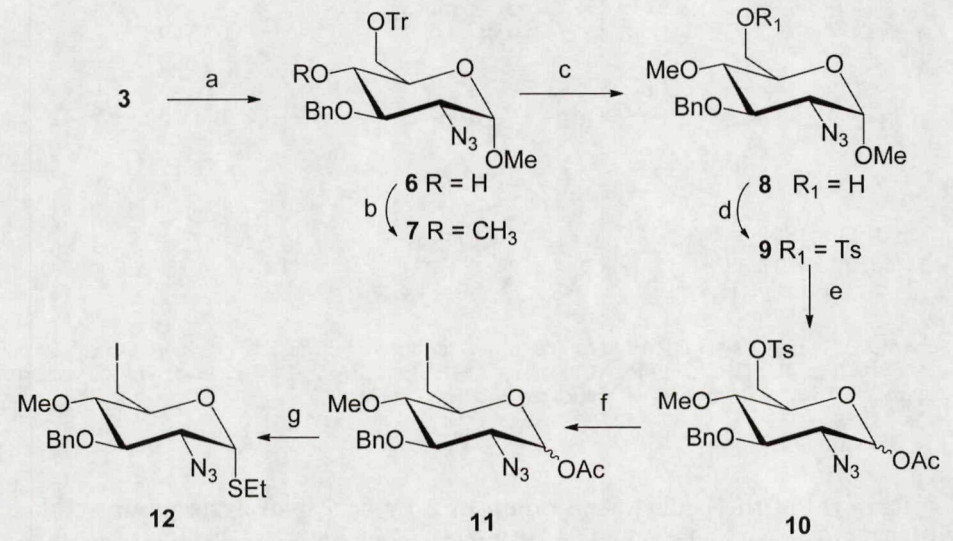


Scheme 1: Reagents and conditions: (a) (i) Tf_2O, pyridine, −15°C, 2 h; (ii) NaN_3, DMF, rt, 24 h; (b) 80% HOAc, 100°C, 2 h; (c) Tosyl chloride, DMAP, Et_3N, CH_2Cl_2-DMF (2:1), rt, 4 h; (d) sodium iodide, DMF, 120°C, 2 h; (e) Ph_3P, imidazole, iodine, 30 min.

Removal of the benzylidene group in **2** by acid hydrolysis produced diol **3**.[14] The primary hydroxyl group of **3** was then tosylated selectively, with tosyl chloride and 4-dimethylamino pyridine[15] in CH_2Cl_2-DMF [2:1 (v/v)] at rt in high yield. The ^1H NMR spectrum of **4** showed a characteristic downfield shift to 4.32 ppm from 3.33 ppm for H_b-6, confirming that the reaction was regioselective for O-6. A downfield shift of the C-6 signal from 62.4 ppm to 68.9 ppm was also observed in the ^{13}C NMR spectrum. Moreover, the chemical shift of *H*-4 at 3.71 ppm with $J_{4,5} = 10.0$ Hz remained unaltered. Compound **4** was then allowed to react with sodium iodide[16] in DMF, producing the desired compound **5** in high yield. Alternatively, diol **3** could be easily converted to iodo derivative **5** in a one-step procedure using triphenylphosphine and iodine under mild basic conditions.[17] Imidazole was found to act more prominently than other bases such as triethyl amine or DBU. The reaction was more effective than the two-step conversion proceeded via 6-*O*-tosylated derivative **4** and the reaction could be completed within 30 min.

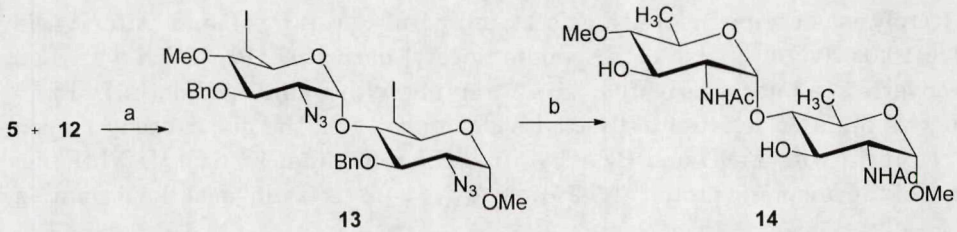
The glycosyl donor **12** was also prepared from diol intermediate **3** (Sch 2). Diol **3** was converted to 6-*O*-trityl derivative by treating with trityl chloride in pyridine[18] at rt followed by methylation[19] at *O*-4 of **6** to produce **7**. The trityl group was then removed[20] using 80% HOAc at 100°C to give **8**, which on treatment with tosyl chloride and pyridine afforded 6-*O*-tosyl derivative **9**. Acetolysis of compound **9** using acetic anhydride, acetic acid, and sulfuric acid [25:10:0.5 (v/v)][21] gave corresponding acetyl derivative **10**, which was then converted to 6-iodo derivative **11** by treating with sodium iodide in DMF.[22] It was finally converted to thioglycoside donor **12** as the major product upon treatment with EtSH and BF_3-Et_2O in dichloromethane.[23] The ^1H NMR signal of the anomeric proton at 5.25 ppm ($J_{1,2} = 4.2$ Hz) confirmed the formation of α-glycoside.

Glycosylation of acceptor **5** with donor **12** (Sch 3) was carried out in the presence of NIS/AgOTf[24] to produce the desired disaccharide **13** in 73% yield.



Scheme 2: Reagents and conditions: (a) Trityl chloride, pyridine, rt, 72 h; (b) DMSO/NaOH, MeI, rt; (c) 80% HOAc, 100°C, 2 h; (d) tosyl chloride, pyridine, rt, 24 h; (e) Ac_2O, HOAc, H_2SO_4 (25:10:0.5; (v/v)), rt, 2 h; (f) NaI, DMF, 120°C, 2 h; (g) EtSH, BF_3-Et_2O, CH_2Cl_2, rt, 24 h.

The ^{1}H NMR spectrum of **13** confirmed the formation of the desired disaccharide. The two anomeric signals appear at 5.20 ppm ($J_{1,2} = 3.6$ Hz) and 4.97 ppm ($J_{1,2} = 3.3$ Hz), confirming the α-linkages of the protected disaccharide. Hydrogenation followed by selective *N*-acetylation of compound **13** afforded the target disaccharide **14**, which was purified by gel permeation chromatography (Bio-Gel P-2, fine) using water as eluent. In the ^{1}H NMR spectrum of disaccharide **14**, signals at 5.05 ppm with $J_{1,2} = 3.8$ Hz and 4.84 ppm with $J_{1,2} = 3.5$ Hz indicated the presence of two β-anomeric protons, again confirming the α-glycosidic linkages of the disaccharide. The spectrum also showed signals at 3.78 ppm (dd, $J_{3,4} = 10.2$ Hz) for H-3^{I} and 4.20 ppm (t, $J_{3,4} = 10.6$ Hz) for H-3^{II}, confirming that these positions were not acetylated, so the *N*-acetylation was selective in the presence of free hydroxyl groups. The ^{13}C NMR spectrum



Scheme 3: Reagents and conditions: (a) NIS/AgOTf, −15°C, CH_2Cl_2, 1 h; (b) Pd-C (10%)/MeOH, Ac_2O, rt, 30 h.

exhibited the presence of 18 carbon signals, which further supported the assignment of the desired disaccharide.

In conclusion, a highly convergent and efficient method was developed for the synthesis of the upstream terminal disaccharide of the *O*-antigenic polysaccharide of *V. cholerae* O37.

EXPERIMENTAL

Materials and Methods

All reactions were monitored with TLC on silica gel 60 F_{254} coated on aluminum plates. Column chromatography was performed on 100–200 mesh silica gel. The spots were visualized using UV light or by charring with aqueous 10% sulfuric acid. All solvents were distilled and dried before use. Evaporations were conducted in a rotary evaporator at a temperature below 40°C unless stated otherwise. Optical rotations were measured with a Perkin Elmer model 241 MC polarimeter for solutions in a 1-dm cell. Nuclear magnetic resonance (NMR) data were extracted from spectra measured in $CDCl_3$ or D_2O at 25°C on a Bruker DPX spectrometer recorded at 300 MHz (^{1}H NMR) and 75 MHz (^{13}C NMR). Chemical shifts were measured from the resonance signals of Me_4Si and HDO ($\delta_{HDO} = 4.78$) in $CDCl_3$ and D_2O, respectively. For ^{13}C NMR spectra in D_2O, acetone ($\delta_C = 29.84$) was used as internal standard unless otherwise mentioned. Electron spray ionization mass spectrometry (ESI-MS) was performed with an ESI-Q-TOF Micro Mass spectrometer.

Methyl 2-azido-3-O-benzyl-4,6-O-benzylidene-2-deoxy-α-D-gluco-pyran-oside (2)

To a solution of **1** (2 g, 5.4 mmol) in dry CH_2Cl_2 (15 mL), triflic anhydride (1.4 mL, 8.1 mmol) and dry pyridine (1.6 mL) were added at −15°C and stirred for 1 h. TLC analysis (10:1, toluene-EtOAc) indicated completion of the reaction. The reaction mixture was then successively washed with saturated $NaHCO_3$ and cold water and dried over Na_2SO_4. The solvent was evaporated under reduced pressure keeping the bath temperature below 25°C and dried under vacuum for 1 h. The crude material was immediately dissolved in dry DMF (15 mL), and then NaN_3 (0.72 g, 10.9 mmol) was added and stirred at rt. After 16 h, TLC {hexane-EtOAc [5:1 (v/v)]} indicated two spots; the major one was identified as the desired product **2** (1.3 g, 60%): $[\alpha]_D^{29} = +12$ ($c = 1.1$; $CHCl_3$); IR (KBr): 2103 cm^{-1} (C-N); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 7.51$–7.23 (m, 10H, aromatic protons), 5.57 (s, 1H, C*H*-Ph), 4.94 (d, 1H, $J = 10.96$ Hz, CH_2Ph), 4.79 (d, 1H, $J = 10.9$ Hz, CH_2Ph), 4.77 (d, 1H, $J_{1,2} = 3.6$ Hz, *H*-1), 4.28 (dd, 1H, $J_{5,6b} = 4.4$ Hz, *H*-6-b), 4.06 (t, 1H, $J_{3,4} = 9.3$ Hz, *H*-3), 3.87 (t, 1H, $J_{4,5} = 9.6$ Hz, *H*-4), 3.75 (t, 1H, $J_{5,6a} = 10.0$ Hz, *H*-6-a), 3.70 (m, 1H, *H*-5), 3.44

(dd, 1H, $J_{2,3}$ = 9.3 Hz, *H*-2), 3.35 (s, 3H, O-CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 138.28 (C), 137.67 (C), 130.40–126.71 (aromatic carbons), 101.88 (*C*H-Ph), 99.83 (*C*-1), 88.19, 76.77, 63.66, 63.01 (*C*-4, *C*-3, *C*-2, *C*-5), 75.42, 71.59 (CH_2-Ph, *C*-6), 55.84 (O-CH_3); HRESI-MS calcd for $C_{21}H_{23}N_3O_5Na$ 420.1534, found 420.1541.

Methyl 2-azido-3-O-benzyl-2-deoxy-6-O-tosyl-α-D-glucopyranoside (4)

Compound **3** was synthesized from **2** according to the procedure described earlier.[13] Compound **3** (0.1 g, 0.32 mmol) was first dissolved in CH_2Cl_2-DMF-pyridine [2:1:1 (v/v), 10 mL] and cooled to 0°C. *p*-Toluenesulphonyl chloride (69 mg, 0.36 mmol) and 4-dimethylamino pyridine (5 mg) were then added and the reaction mixture was allowed to stand at rt. After 4 h, TLC {toluene-EtOAc [2:1 (v/v)]} showed completion of the reaction. The crude reaction mixture was condensed under reduced pressure and the residue was extracted with CH_2Cl_2. The organic layer was washed with saturated solution of $NaHCO_3$ and water and dried over Na_2SO_4. The product was purified by column chromatography to give **4** (0.12 g, 80%): $[\alpha]_D^{29}$ = +58 (*c* = 1.6; $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.80–7.26 (m, 9H, aromatic protons), 4.94–4.71 (2d, 2H, *J* = 11.2 Hz, CH_2Ph), 4.72 (d, 1H, $J_{1,2}$ = 3.7 Hz, *H*-1), 4.32 (dd, 1H, $J_{5,6b}$ = 2.6 Hz, *H*-6-b), 4.23 (m, 1H, *H*-5), 3.76 (dd, 1H, $J_{3,4}$ = 9.8 Hz, *H*-3), 3.71 (dd, 1H, $J_{4,5}$ = 10.0 Hz, *H*-4), 3.65 (t, 1H, $J_{5,6a}$ = 11.0 Hz, *H*-6-a), 3.57 (dd, 1H, $J_{2,3}$ = 8.9 Hz, *H*-2), 3.35 (s, 3H, OCH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 145.40, 138.28, 133.27 (tertiary carbons), 130.26–128.38 (aromatic carbons), 99.20 (*C*-1), 80.23, 70.40, 69.87, 63.40 (*C*-4, *C*-3, *C*-5, *C*-2), 75.59, 68.90 (CH_2-Ph, C-6), 55.86 (O-CH_3 at C-1), 22.05 (CH_3 group of tosyl); HRESI-MS calcd for $C_{21}H_{25}N_3O_7SNa$ 486.1311, found 486.1319.

Methyl 2-azido-3-O-benzyl-2,6-dideoxy-6-iodo-α-D-glucopyranoside (5)

To a solution of **4** (0.12 g, 0.26 mmol) in dry DMF (5 mL), NaI (59 mg, 0.39 mmol) was added. The reaction mixture was heated to 120°C for 1 h when TLC {toluene-EtOAc [10:1 (v/v)]} indicated completion of the reaction. DMF was then removed under reduced pressure and the reaction mixture was extracted with CH_2Cl_2. The organic layer was washed with $Na_2S_2O_7$ and distilled water and finally evaporated to dryness. The residue was purified by column chromatography to produce **5** (76 mg, 70%). In an alternative approach compound **5** was synthesized directly from diol derivative **3**. To a solution of **3** (50 mg, 0.16 mmol) in dry toluene (10 mL) were added triphenylphosphine (83 mg, 0.32 mmol), imidazole (83 mg, 0.8 mmol), and iodine (213 mg, 0.56 mmol). The mixture was heated under reflux for 15 min, when the violet

color of iodine disappeared. A saturated solution of sodium bicarbonate in water was then added, and stirring was continued for 5 min. Iodine was further added in the reaction mixture until the color of the solution remained purple. Aqueous solution of sodium thiosulphate (10%) was then added to consume the excess iodine. The mixture was diluted with ethyl acetate and washed with water, concentrated under reduced pressure. Column chromatography afforded pure **5** (48 mg, 72%): $[\alpha]_D^{29} = +58$ ($c = 1.6$; $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 7.38$–7.33 (m, 5H, aromatic Hs), 4.99–4.82 (2d, 2H, $J = 11.4$ Hz, CH_2Ph), 4.82 (d, 1H, $J_{1,2} = 3.6$ Hz, *H*-1), 3.79 (t, 1H, $J_{3,4} = 10.2$ Hz, *H*-3), 3.53 (m, 1H, H-5), 3.41 (dd, 1H, $J_{4,5} = 10.2$ Hz, *H*-4), 3.48 (dd, 1H, $J_{2,3} = 10.2$ Hz, *H*-2), 3.38 (s, 3H, OCH_3), 3.31–3.28 (m, 2H, *H*-6); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 137.77$ (tertiary carbon), 98.79 (*C*-1), 128.87–128.20 (aromatic carbons), 79.83, 74.21, 70.05, 63.34 (*C*-4, *C*-3, *C*-5, *C*-2), 75.15 (CH_2-Ph), 55.61 (O-CH_3), 6.72 (CH_2-I); HRESI-MS calcd for $C_{14}H_{18}N_3IO_4Na$ 442.0240, found 442.0266.

Methyl 2-azido-3-O-benzyl-2-deoxy-6-O-trityl-α-D-glucopyranoside (6)

To a solution of **3** (0.3 g, 0.97 mmol) in dry pyridine (10 mL), trityl chloride (0.41 g, 1.5 mmol) was added and the reaction mixture was stirred at rt for 48 h. The solvents were co-evaporated with toluene and the product was purified by column chromatography using toluene as eluent to give **6** (0.37 g, 70%): $[\alpha]_D^{29} = +24$ ($c = 1.9$; $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 7.46$–7.24 (m, 20H, aromatic protons), 4.95–4.74 (2H, 2d, $J = 10.9$ Hz, CH_2Ph), 4.80 (d, 1H, $J_{1,2} = 3.3$ Hz, *H*-1), 3.80 (dd, 1H, $J_{5,6b} = 3.8$ Hz, *H*-6-b), 3.75 (m, 1H, *H*-5), 3.66 (t, 1H, $J_{3,4} = 8.7$ Hz, *H*-3), 3.60 (t, 1H, $J_{4,5} = 9.9$ Hz, *H*-4), 3.50 (dd, 1H, $J_{2,3} = 8.9$ Hz, *H*-2), 3.38 (t, 1H, $J_{5,6a} = 8.2$ Hz, *H*-6-a), 3.35 (s, 3H, O-CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 144.08$, 138.50 (tertiary carbons), 130.42–127.66 (aromatic carbons), 99.06 (*C*-1), 87.50 (*C*-Ph_3), 80.48, 73.10, 70.05, 63.54 (*C*-4, *C*-3, *C*-5, *C*-2), 75.66, 64.26 (CH_2-Ph, *C*-6), 55.59 (O-CH_3); HRESI-MS calcd for $C_{33}H_{33}N_3O_5Na$ 574.2318, found 574.2332.

Methyl 2-azido-3-O-benzyl-2-deoxy-4-O-methyl-6-O-trityl-α-D-gluco-pyranoside (7)

To a solution of **6** (0.34 g, 0.62 mmol) in dry DMSO (5 mL), powdered NaOH (40 mg, 0.93 mmol) was added under N_2 atmosphere. The reaction mixture was stirred at rt for 15 min. To the reaction mixture, MeI (58 μL, 0.93 mmol) was added and the mixture was stirred for 30 min at rt when TLC (toluene) showed completion of the reaction. The reaction mixture was neutralized with 25% AcOH at 0°C. It was then diluted with CH_2Cl_2 and the organic layer was washed with distilled water twice. The combined organic layer was evaporated to dryness. Column chromatography (toluene) afforded pure **7** (0.33 g, 95%):

$[\alpha]_D^{29}$ +61 (c = 1.7, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$): δ = 7.50–7.22 (m, 20H, aromatic Hs), 4.89 (d, 1H, $J_{1,2}$ = 3.5 Hz, H-1), 4.83 (s, 2H, CH_2Ph), 3.83 (dd, 1H, $J_{5,6b}$ = 3.8 Hz, H-6-b), 3.67 (m, 1H, H-5), 3.52 (dd, 1H, $J_{3,4}$ = 10.2 Hz, H-3), 3.49 (dd, 1H, $J_{4,5}$ = 0.2 Hz, H-4), 3.48 (dd, 1H, $J_{2,3}$ = 9.9 Hz, H-2), 3.31 (t, 1H, $J_{5,6a}$ = 10.1 Hz, H-6-a), 3.44 (s, 3H, OCH_3), 3.26 (s, 3H, OCH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 144.35 (C), 138.32 (C), 129.18–127.41 (aromatic carbons), 98.96 (C-1), 86.78 (C-Ph_3), 81.08, 80.92, 71.12, 64.03 (C-4, C-3, C-5, C-2), 76.01, 62.57 (CH_2-Ph, C-6), 61.01, 55.46 (O-CH_3 at C-1 and C-4); HRESI-MS calcd for $C_{34}H_{35}N_3O_5Na$: 588.2474, found 588.2477.

Methyl 2-azido-3-O-benzyl-2-deoxy-4-O-methyl-α-D-glucopyranoside (8)

Compound **7** (0.3 g, 0.53 mmol) was dissolved in 80% HOAc (10 mL) and was heated at 100°C for 1 h. TLC (2:1, EtOAc-toluene) showed the complete conversion of the starting material. HOAc was then removed under reduced pressure by co-distillation with toluene and the product was purified by column chromatography {toluene-EtOAc [1:2 (v/v)]} to give **8** (0.16 g, 90%): $[\alpha]_D^{29}$ = +120 (c = 1.4; $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$): δ = 7.43–7.26 (m, 5H, aromatic Hs), 4.86 (s, 2H, CH_2Ph), 4.76 (d, 1H, $J_{1,2}$ = 3.4 Hz, H-1), 3.88 (dd, 1H, $J_{3,4}$ = 8.8 Hz, H-3), 3.82 (m, 2H, H-6), 3.63 (m, 1H, H-5), 3.57 (dd, 1H, $J_{4,5}$ = 10.1 Hz, H-4), 3.46 (dd, 1H, $J_{2,3}$ = 8.8 Hz, H-2), 3.40 (3H, s, OCH_3), 3.28 (3H, s, OCH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 138.25 (C), 128.89–128.35 (aromatic carbons), 99.07 (C-1), 80.57, 80.50, 63.86 (C-4, C-3, C-5, C-2), 75.85, 61.99 (CH_2-Ph, C-6), 61.31, 55.86 (O-CH_3 at C-1 and C-4); HRESI-MS calcd for $C_{15}H_{21}N_3O_5Na$ 346.1379, found 346.1355.

Methyl 2-azido-3-O-benzyl-2-deoxy-4-O-methyl-6-O-tosyl-α-D-gluco-pyranoside (9)

To the solution of **8** (0.15 g, 0.46 mmol) in dry pyridine (5 mL), tosyl chloride (0.14 g, 0.70 mmol) was added under N_2 atmosphere. The reaction mixture was stirred at r.t. for 24 h when TLC {toluene-EtOAc [10:1 (v/v)]} indicated the completion of reaction. Pyridine was removed under reduced pressure and the compound was extracted with CH_2Cl_2. The organic layer was washed with saturated solution of $NaHCO_3$, distilled water and dried over Na_2SO_4. The organic layer was evaporated under reduced pressure and the crude product was purified by column chromatography to afford **9** (0.19 g, 85%): $[\alpha]_D^{29}$ = +104 (c = 0.5; $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$): δ = 7.82–7.19 (9H, aromatic Hs), 4.86–4.78 (2d, 2H, J = 10.6 Hz, CH_2Ph), 4.67 (d, 1H, $J_{1,2}$ = 3.5 Hz, H-1), 4.29 (dd, 1H, $J_{5,6b}$ = 4.1 Hz, H-6-b), 4.20 (m, 1H, H-5), 3.82 (m, 2H, H-3 and H-4), 3.71 (t, 1H, $J_{5,6a}$ = 10.6 Hz, H-6-a), 3.48 (s, 3H, O-CH_3), 3.35 (s, 3H, O-CH_3), 3.40 (dd, 1H, $J_{2,3}$ = 8.9 Hz, H-2), 2.37 (3H, s, CH_3); ^{13}C NMR

(75 MHz, $CDCl_3$): δ = 145.38, 138.06, 133.33 (tertiary carbons), 130.24–128.41 (aromatic carbons), 98.92 (*C*-1), 80.51, 79.96, 69.42, 63.58 (*C*-4, *C*-3, *C*-5, *C*-2), 75.80, 68.58 (CH_2-Ph, *C*-6), 61.31, 55.85 (O-CH_3 at *C*-1 and *C*-4), 22.08 (CH_3); HRESI-MS calcd for $C_{22}H_{27}N_3O_7SNa$ 500.1467; found 500.1361.

1-O-Acetyl-2-azido-3-O-benzyl-2-deoxy-4-O-methyl-6-O-tosyl-α-D-gluco-pyranose (10)

To **9** (0.15 g, 0.31 mmol), a mixture of acetic anhydride, acetic acid, and sulfuric acid [25:10:0.5 (v/v), 5 mL] was added at 0°C and the reaction mixture was allowed to stand at rt for 2 h when TLC {toluene-EtOAc [2:1 (v/v)]} indicated the completion of reaction. The reaction mixture was cooled (10°C), neutralized with saturated solution of $NaHCO_3$, and extracted twice with CH_2Cl_2. The organic layer was washed with distilled water and dried over Na_2SO_4. Column chromatography {toluene-EtOAc [2:1 (v/v)]} afforded **10** with the α-anomer as the major product (α:β = 6:1) (0.13 g, 80%): $[\alpha]_D^{29}$ = +49 (*c* = 1.0; $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.8–7.30 (m, 9H, aromatic protons), 6.08 (d, 1H, $J_{1,2}$ = 3.6 Hz, *H*-1), 4.85 (s, 2H, CH_2Ph), 4.32 (dd, 1H, $J_{5,6b}$ = 3.3 Hz, *H*-6-b), 4.14 (m, 1H, *H*-5), 3.79 (dd, 1H, $J_{3,4}$ = 9.3 Hz, *H*-3), 3.76 (t, 1H, $J_{5,6a}$ = 10.8 Hz, *H*-6-a), 3.65 (dd, 1H, $J_{4,5}$ = 8.9 Hz, *H*-4), 3.46 (dd, 1H, $J_{2,3}$ = 9.8 Hz, *H*-2), 3.33 (s, 3H, O-CH_3), 2.45 (3H, s, CH_3), 2.12 (3H, s, $COCH_3$); ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 169.15 ($COCH_3$), 145.46, 137.88, 133.13, 130.25–127.45 (aromatic carbons), 90.56 (*C*-1), 80.53, 79.23, 71.72, 62.67 (*C*-4, *C*-3, *C*-5, *C*-2), 75.89, 68.01 (CH_2-Ph, *C*-6), 61.52 (O-CH_3), 22.05, 21.29 (CH_3, $COCH_3$); HRESI-MS calcd for $C_{23}H_{27}N_3O_8SNa$ 528.1417, found 528.1411.

1-O-Acetyl-2-azido-3-O-benzyl-2,6-dideoxy-6-iodo-4-O-methyl-α-D-gluco-pyranose (11)

To a solution of **10** (0.13 g, 0.26 mmol) in dry DMF (5 mL), NaI (59 mg, 0.39 mmol) was added. The reaction mixture was heated to 120°C for 1 h when TLC {toluene-EtOAc [10:1 (v/v)]} indicated completion of the reaction. DMF was then removed under reduced pressure and the reaction mixture was extracted with CH_2Cl_2. The organic layer was washed with $Na_2S_2O_7$ and water and concentrated. Column chromatography {toluene-EtOAc [10:1 (v/v)]} afforded **11** (α:β = 15:1) (0.11 g, 92%): $[\alpha]_D^{29}$ = +58 (*c* = 2.0; $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.49–7.20 (m, 5H, aromatic protons), 6.21 (d, 1H, $J_{1,2}$ = 3.6 Hz, *H*-1), 4.85 (s, 2H, CH_2Ph), 3.87 (dd, 1H, $J_{3,4}$ = 8.8 Hz, *H*-3), 3.66 (dd, 1H, $J_{4,5}$ = 9.7 Hz, *H*-4), 3.57 (m, 1H, *H*-5), 3.46 (dd, 1H, $J_{3,4}$ = 9.4 Hz, *H*-2), 3.30 (s, 3H, O-CH_3), 3.23 (m, 2H, *H*-6), 2.18 (s, 3H, $COCH_3$); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 169.30 ($COCH_3$), 137.89, 128.95–128.51 (aromatic carbons), 90.84 (*C*-1), 83.80, 80.53, 71.90, 62.91 (*C*-4, *C*-3, *C*-5, *C*-2), 76.04 (CH_2-Ph), 61.66 (O-CH_3), 21.39 ($COCH_3$), 7.21 (CH_2-I); HRESI-MS calcd for $C_{16}H_{20}N_3O_5INa$ 484.0345, found 484.0398.

Ethyl 2-azido-3-O-benzyl-2,6-dideoxy-6-iodo-4-O-methyl-1-thio-α-D-glucopyranoside (12)

BF_3-OEt_2 (50 μL, 0.39 mmol) was added dropwise to a solution of **11** (0.1 g, 0.21 mmol) and EtSH (20 μL, 0.27 mmol) in CH_2Cl_2 (5 mL) at 0°C in N_2 atmosphere, and the reaction mixture was stirred at rt. After 4 h, the reaction mixture was diluted with CH_2Cl_2 and washed with saturated solution of $NaHCO_3$ and water. Column chromatography {toluene-EtOAc [10:1 (v/v)]} of the crude product afforded pure **12** (70 mg, 70%): $[\alpha]_D^{29} = +51$ (c = 1.1, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 5.25 (d, 1H, $J_{1,2}$ = 4.2 Hz, H-1), 4.88–4.79 (2H, 2d, J = 10.3 Hz, CH_2Ph), 4.33 (m, 1H, H-5), 4.22 (dd, 1H, $J_{3,4}$ = 10.3 Hz, H-3), 3.83 (dd, 1H, $J_{4,5}$ = 9.1 Hz, H-4), 3.75 (dd, 1H, $J_{2,3}$ = 9.1 Hz, H-2), 3.42 (s, 3H, O-CH_3), 3.34 (m, 2H, H-6), 2.84 (m, 2H, S-CH_2-CH_3), 1.20 (t, 3H, J = 7.4 Hz, S-CH_2-CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 135.6 (C), 124.10–128.84 (aromatic carbons), 102.80 (C-1), 82.22, 79.89, 73.00, 61.70 (C-4, C-3, C-5, C-2), 60.5 (O-CH_3), 76.04 (CH_2-Ph), 24.8 (S-CH_2-CH_3), 15.54 (S-CH_2-CH_3), 7.42 (CH_2-I); HRESI-MS calcd for $C_{16}H_{22}N_3O_3ISNa$ 486.0324, found 486.1319.

Methyl 2-azido-3-O-benzyl-2,6-dideoxy-6-iodo-4-O-methyl-α-D-gluco-pyranosyl-(1→4)-2-azido-3-O-benzyl-2,6-dideoxy-6-iodo-α-D-glucopyranoside (13)

To a stirred mixture of **5** (58 mg, 0.13 mmol) and **12** (60 mg, 0.13 mmol) in dry CH_2Cl_2 (5 mL) containing 4Å molecular sieves, NIS (44 mg, 0.2 mmol) was added under N_2 atmosphere. The reaction mixture was stirred at −20°C for 15 min, and a solution of AgOTf (34 mg, 0.13 mmol) in toluene (0.5 mL) was added. The mixture was stirred for 15 min at −20°C and was then allowed to reach rt. After 15 min, TLC {toluene-EtOAc [10:1 (v/v)]} indicated completion of the reaction. The reaction was then quenched with Et_3N, diluted with CH_2Cl_2, and filtered through Celite. The filtrate was washed with 10% aqueous $Na_2S_2O_7$ and water and concentrated. Column chromatography afforded pure **13** (79 mg, 73%): $[\alpha]_D^{29} = +86$ (c = 1.1, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 5.20 (d, 1H, $J_{1,2}$ = 3.6 Hz, H-1^{II}), 4.97 (d, 1H, $J_{1,2}$ = 3.3 Hz, H-1^{I}), 4.81–4.50 (4d, 4H, J = 11.0 Hz, 2 × CH_2-Ph), 4.22 (dd, 1H, $J_{4,5}$ = 8.8 Hz, H-4^{I}), 4.05 (m, 1H, H-5^{II}), 3.98 (m, 1H, H-5^{I}), 3.95 (dd, 1H, $J_{4,5}$ = 10.1Hz, H-4^{II}), 3.93 (dd, 1H, $J_{3,4}$ = 9.2 Hz, H-3^{I}), 3.75 (dd, 1H, $J_{3,4}$ = 8.2 Hz, H-3^{II}), 3.52 (dd, 1H, $J_{2,3}$ = 9.7 Hz, H-2^{I}), 3.49 (dd, 1H, $J_{2,3}$ = 10.6 Hz, H-2^{II}), 3.27 (m, 2H, H-6^{II}), 3.24 (m, 2H, H-6^{I}), 3.35 (3H, s, OCH_3), 3.27 (3H, s, OCH_3); ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 138.84 (C), 137.90 (C), 130.44–127.84 (aromatic carbons), 100.05, 98.20 (C-1^{II}, C-1^{I}), 84.32, 82.03, 74.80, 76.05, 72.08, 70.01, 63.96, 62.57 (C-4^{I}, C-4^{II}, C-3^{I}, C-3^{II}, C-5^{I}, C-5^{II}, C-2^{I}, C-2^{II}), 76.12, 75.06 (2×CH_2-Ph), 61.94, 55.40 (O-CH_3 at C-1 and C-4), 7.51, 7.32 (C-6^{I}, C-6^{II}); HRESI-MS calcd for $C_{28}H_{34}N_6O_7I_2Na$ 843.0476, found 843.0442.

Methyl 2-acetamido-2,6-dideoxy-4-O-methyl-α-D-glucopyranosyl-(1→4)-2-acetamido-2,6-dideoxy-α-D-glucopyranoside (14)

A solution of the disaccharide **13** (50 mg, 0.061 mmol) and Ac_2O (0.5 mL) in MeOH (5 mL) was stirred with 10% Pd-C under H_2 at rt for 30 h. The reaction mixture was filtered through a Celite pad, and then the solvent was removed under reduced pressure. The residue was dissolved in water and purified on a Bio-Gel P-2 column (1 × 30 cm) with water as eluent to produce pure **14** (15 mg, 62%): $[\alpha]_D^{29} = +108$ (c = 0.6, H_2O); ^1H NMR (300 MHz, D_2O): δ = 5.05 (1H, d, $J_{1,2}$ = 3.8 Hz, H-1^{II}), 4.84 (1H, d, $J_{1,2}$ = 3.5 Hz, H-1^{I}), 4.29 (dd, 1H, $J_{4,5}$ = 9.9 Hz, H-4^{II}), 4.20 (t, 1H, $J_{3,4}$ = 10.6 Hz, H-3^{II}), 3.82 (dd, 1H, $J_{4,5}$ = 8.9 Hz, H-4^{I}), 3.78 (dd, 1H, $J_{3,4}$ = 10.2 Hz, H-3^{I}), 3.73–3.62 (m, 2H, H-5^{I}, H-5^{II}), 3.30 (dd, 1H, $J_{2,3}$ = 10.0 Hz, H-2^{I}), 3.21 (dd, 1H, $J_{2,3}$ = 8.8 Hz, H-2^{II}), 3.57 (s, 3H, OCH_3), 3.40 (s, 3H, OCH_3), 2.23 (s, 3H, $NHCOCH_3$), 2.05 (s, 3H, $NHCOCH_3$), 1.35 (d, 3H, $J_{5,6}$ = 5.6 Hz, H-6^{II}), 1.1 (d, 3H, $J_{5,6}$ = 6.6 Hz, H-6^{I}); ^{13}C NMR (75 MHz, D_2O): δ = 175.05 ($NHCOCH_3$), 174.5 ($NHCOCH_3$), 101.31 (C-1^{II}), 99.07 (C-1^{I}), 80.50, 75.86, 71.63, 68.25, 63.86, 61.99 (C-3^{I}, C-3^{II}, C-4^{I}, C-4^{II}, C-5^{I}, C-5^{II}), 61.31, 55.61 (2 × OCH_3), 51.84, 51.44 (C-2^{I} and C-2^{II}), 23.1 ($NHCOCH_3$), 22.66 ($NHCOCH_3$), 16.56, 16.05 (C-6^{I} & C-6^{II}); HRESI-MS calcd for $C_{18}H_{32}N_2O_9H$ 421.2186, found 421.2181.

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