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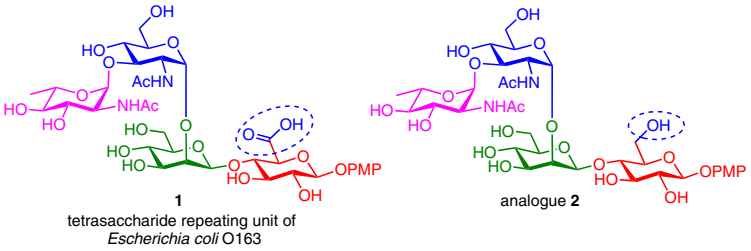
Expeditious Synthesis of a Tetrasaccharide Repeating Unit of the O-Antigen of *Escherichia coli* O163

Geeta Karki[a]
Harikesh Kumar[a]
Remya Rajan[a]
Pintu Kumar Mandal[*a,b]

[a] Medicinal and Process Chemistry Division, CSIR-Central Drug Research Institute, BS-10/1, Sector 10, Jankipuram extension, Sitapur Road, Lucknow, 226 031, India
pintuchem06@gmail.com
[b] Academy of Scientific and Innovative Research, New Delhi, 11000, India



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Abstract The synthesis of the tetrasaccharide repeating unit of the O-antigen of *Escherichia coli* O163 as its *p*-methoxyphenyl (PMP) glycoside was achieved followed by sequential glycosylation strategy through thioglycoside activation using sulfuric acid immobilized on silica (H_2SO_4–silica) in conjunction with *N*-iodosuccinimide as a Brønsted acid catalyst. The application of one-pot reaction conditions for two glycosylations and in situ PMB-group removal reduced the number of reaction steps significantly. The L-QuipNAc building block was obtained from known carbohydrate L-rhamnose precursors. The stereoselective outcomes of all glycosylation reactions were found to be very good. A late-stage TEMPO-mediated oxidation was performed for the formation of required uronic acid moiety. An analogue of the target tetrasaccharide was also prepared by using one-pot glycosylation approach. Such synthetic oligosaccharides could later be effectively conjugated with an appropriate protein to furnish glycoconjugate derivatives for their use in immunochemical studies.

Keywords polysaccharide, *Escherichia coli*, glycosylation, antigen, stereoselectivity

Escherichia coli (*E. coli*), immensely diversified gram-negative rod-shaped, anaerobic bacterial species facultative in nature resides in the lower intestine of humans and are generally harmless. They contribute a major part to the microflora within the gut and make themselves available for suppressing the growth and harmful effects of other microorganisms.[1,2] They are also found to be helpful in synthesizing an appreciable amount of vitamins within the body, for example, the synthesis of vitamin K_2 (menaquinone).[3] Although some of its bacterial strains are beneficial to the human hosts, a large diversity of its pathogenic forms are also found in nature.[4] The pathogenic forms are either food-borne or water-borne pathogens.[5]

There is an increasing awareness that out of the several pathogenic bacteria associated with sporadic cases of diseases, as well as outbreaks of food-borne illnesses, *E. coli* is the most predominant.[6]

People ingesting food contaminated with Shiga toxin-producing *E. coli* (STEC) may get infected with watery diarrhoea, abdominal pain, fever, vomiting, and in severe cases hemorrhagic colitis. It is also characterized by hemolytic-uremic syndrome, the symptoms of which include acute kidney failure, microangiopathic hemolytic anemia, and thrombocytopenia. These multidrug-resistant bacterial strains are a major threat to the mankind, especially children, and is an issue of serious concern.[4,7]

The O-antigen is an important component of the lipopolysaccharide unit of the bacteria. Till date, 174 types of O-antigenic strains of *E.coli* have been reported. The reason for this huge diversification is the genetic variation between *galF* and *gnd* genes on the chromosome of *E.coli* that codes for O-antigen.[8,9] Based on the types of pathogenic infections they cause, they are categorized as: a) enteropathogenic *E. coli* (EPEC), b) enterohemorrhagic *E. coli* (EHEC), c) enterotoxigenic *E. coli* (ETEC), d) enteroaggregative *E. coli* (EAEC), e) enteroinvasive *E. coli* (EIEC), etc.[10,11]

Out of the various strains so far identified, which are associated with each clinical symptom, *E. coli* 0163 strains had been found to cause results that shows a high rate of acute renal failure in children and renal and nonrenal sequelae in survivors. These strains are also identified as STEC strain and cause diarrhoea, gastrointestinal infections. *E. coli* O163 isolated from children suffering from hemolytic-uremic syndrome has been found to be transmitted through sheep and swine feces.[12]

The structure of the O-antigenic polysaccharide present in the cell wall of *E. coli* 0163 strain has been reported by A. V. Perepelov et al.[13] which is composed of D-glucuronic acid (GlcA), 2-acetamido-2,6-dideoxy-L-glucose (QuiNAc), D-mannose (Man), D-glucasamine (GlcNAc, Figure 1).

→3)-α-L-Qui*p*NAc-(1→3)-α-D-GlcpNAc-(1→2)-β-D-Man*p*-(1→4)-β-D-Glc*p*A-(1→

Figure 1 Structure of the repeating unit of the *O*-antigen of *Escherichia coli* O163

Due to the emerging resistance of the bacterial cells towards the various antibacterial agents presently available in market, the synthesis of potent vaccines is the only hope left for the treatment of these bacterial infections which when left untreated may even be fatal. Vaccines are biological preparations that provide active acquired immunity to a particular disease. Since cell wall O-antigen contributes to the virulence factor of the bacterial cells, its biological studies is of prime importance.[14,15] Since the antigen plays an important role in the initial stages of infection, vaccines with O-antigens linked to a conjugate (protein, lipid) seems to be pertinent. In the recent past, a number of reports appeared in the literature for the synthesis and evaluation of glycoconjugate vaccines against bacterial infections.[16–19] However, due to the difficulty in isolation of these lipopolysaccharides from bacterial cell walls in sufficient amount and purity followed by problems related with handling of live bacterial strains and tedious isolation procedures, development of appropriate synthetic strategies for the synthesis of these lipopolysaccharides has become a prime requisite. This will help in synthesizing the antigens in appreciable amounts both for the biological studies and vaccine synthesis.

In addition, we therefore decided to synthesize the target tetrasaccharide **1** and its analogue **2** moiety corresponding to the repeating unit of the cell wall O-specific polysaccharide of *E. coli* O163 strain using a one-pot synthetic strategy.

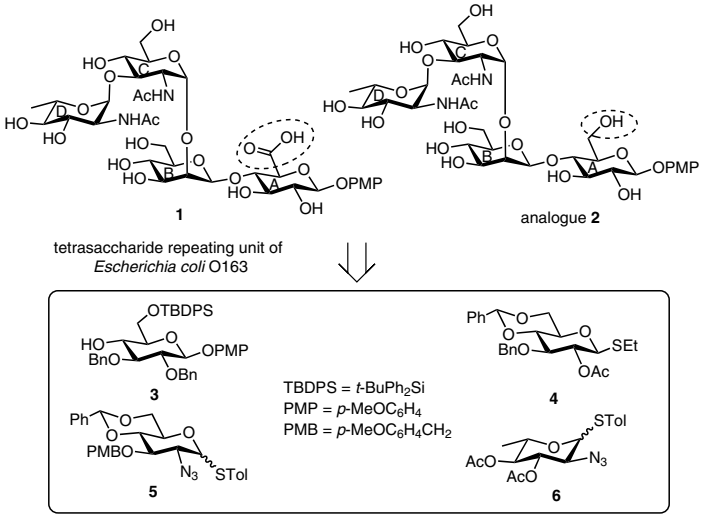
The strategy for the synthesis of the tetrasaccharide as its *p*-methoxyphenyl (PMP) glycoside **1** and its analogue **2** involves sequential glycosylations of suitably functionalized monosaccharide intermediates (Scheme 1). The selection of the *p*-methoxyphenyl (PMP) glycoside as the final reducing-end glycoside could provide the option handle for further glycoconjugate formation after oxidative removal of the PMP group[16] through trichloroacetimidate chemistry.

As per the requirement, a set of suitably functionalized monosaccharide intermediates **3**, **4**,[20] **5**,[21] and **6** were prepared in good yield from the commercially available reducing sugars using the reaction conditions available in the literature (Scheme 2).

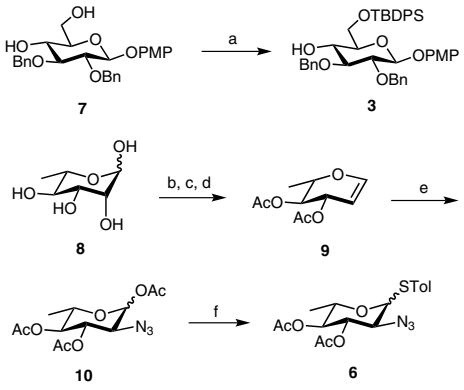
To start with, known diol glucoside **7**[22] (prepared from commercial D-glucose[23]) was allowed to react with TBDPSCl and imidazole in dry DMF to give 4-O-unprotected glucoside **3** in 88% yield. The synthesis of the building block **6** was carried out with efficient approaches towards starting from commercially available L-rhamnose monohydrate.

The commercially available reducing sugars L-rhamnose (**8**) was transformed into the rhamnal **9** in 72% yield in three steps in the following sequence of reactions: peracetylation with acetic anhydride in the presence of perchloric acid; bromination using HBr/AcOH; and finally reductive elimination using NaH_2PO_4 and zinc dust.[24,25]

Azidonitration[26,27] of **9** with sodium azide in the presence of ceric ammonium nitrate (CAN) in MeCN at –15 °C followed by acetolysis[25] with acetic acid, Ac_2O, and NaOAc



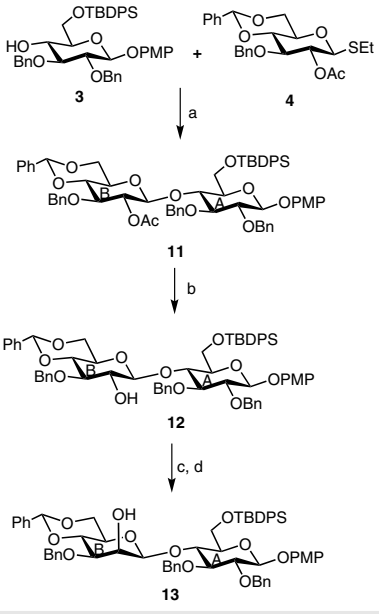
Scheme 1 Structure of the synthesized tetrasaccharide **1** and its analogue **2** as their *p*-methoxyphenyl (PMP) glycosides and their synthetic intermediates



Scheme 2 *Reagents and conditions*: (a) TBDPSCl, imidazole, DMF, r.t., 6 h, 88%; (b) Ac_2O, $HClO_4$ (cat.), 0 °C, 10 min; (c) HBr/AcOH, CH_2Cl_2, 4 h; (d) Zn, NaH_2PO_4, EtOAc–H_2O, 30 min, (**9**: 72%, from L-rhamnose); (e) NaN_3, CAN, MeCN, –15 °C, 3 h, then NaOAc, AcOH, Ac_2O (cat.), 100 °C, 1 h, 77%; (f) *p*-thiocresol, $BF_3{\cdot}OEt_2$, CH_2Cl_2, r.t., 4 h, 85%.

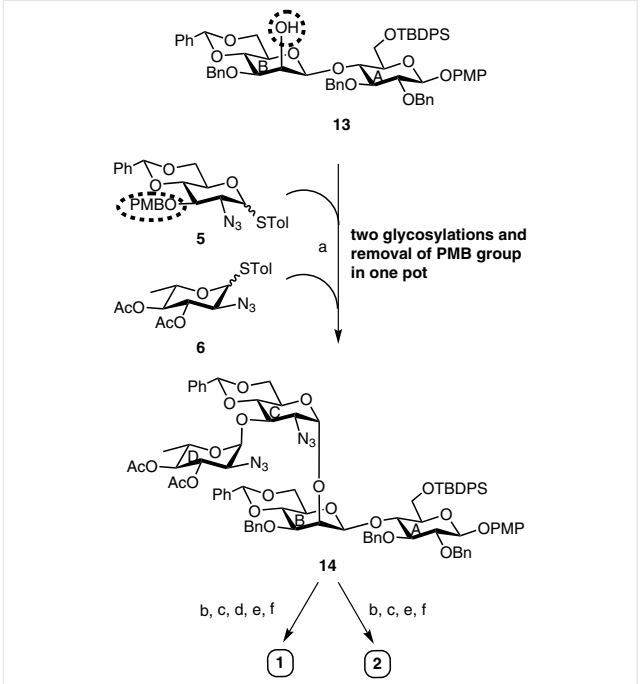
at reflux for one hour gave the reducing deoxy glucoside **10** in 77% yield (Scheme 2). Compound **10** was further subjected to treatment with *p*-thiocresol in the presence boron trifluoridediethyl etherate, and the glycosylating donor **6** was synthesized in 85% yield.

With all the required building blocks in hand, 4-O-unprotected glucoside **3** was glycosylated with thioglycoside derivative **4** in the presence of *N*-iodosuccinimide (NIS) in conjunction with sulfuric acid immobilized on silica (H_2SO_4–silica)[28–33] to afford the disaccharide **11** in 87% yield. The disaccharide **11** was then deacetylated under Zemplén conditions[34] to furnish the disaccharide alcohol **12** in 93% yield. Oxidation of the C-2 hydroxyl group of compound **12** using Dess–Martin periodinane[35] followed by stereoselective reduction of the resulted ketone using sodium borohydride[36] furnished disaccharide acceptor **13** in a yield of 74% (Scheme 3). Formation of the β-D-mannoside containing disaccharide moiety **13** with exact stereochemistry was prepared exclusively from the β-D-glucoside containing disaccharide derivative **12** by epimerization at C-2, and it was unambiguously confirmed by NMR spectroscopy.



Scheme 3 *Reagents and conditions*: (a) NIS, H_2SO_4–silica, CH_2Cl_2, MS 4 Å, –20 °C, 30 min, 87%; (b) NaOMe, MeOH, r.t., 1 h, 93%; (c) Dess–Martin periodinane, CH_2Cl_2, r.t., 3 h; (d) $NaBH_4$, MeOH, r.t., 10 h, 74% over two steps.

In order to synthesize the target tetrasaccharide derivative **14**, a three-step, one-pot-sequence starting from disaccharide acceptor **13** was employed (Scheme 4). Thus, acceptor **13** and 3-*O*-PMB-protected 2-azido-glucoside donor **5** was made to react in the presence of NIS and H_2SO_4–silica at a low temperature, providing the expected trisaccharide intermediate. After complete consumption of the 3-*O*-PMB-protected 2-azido-glucoside donor **5**, which was indicated by TLC, by raising the reaction temperature of the reaction vessel, the 3-*O*-PMB group was removed in the same pot[37] and produced the desired trisaccharide acceptor. The reaction mixture was once again cooled, and a mixture of 2-azido-2,6-dideoxy-L-glucopyranoside donor **6** and another



Scheme 4 *Reagents and conditions*: (a) **5**, NIS, H_2SO_4–silica, CH_2Cl_2–Et_2O (1:2), MS 4 Å, –20 °C, 30 min; then 10 °C, 30 min; then **6**, NIS, –20 °C, 30 min, 61%; (b) MeCOSH, pyridine, r.t., 10 h; (c) Bu_4NF–THF, AcOH, 0 °C to r.t., 6 h; (d) TEMPO, NaBr, NaOCl, TBAB, $NaClO_2$, 2-methylbut-2-ene, NaH_2PO_4, $NaHCO_3$, CH_2Cl_2, 0 °C to r.t., 5 h; (e) H_2, 10% $Pd(OH)_2$/C, MeOH, r.t., 24 h; (f) 0.1 M NaOMe, MeOH, r.t., 1 h (overall yield for **1** 54% and for **2** 62%).

fresh portion of NIS were added to the reaction mixture. The tetrasaccharide derivative **14**[38] was obtained in 61% overall yield using a three-step, one-pot-sequence strategy and the formation of compound **14**[38] was unambiguously confirmed by a combination of 2D-NMR techniques including $^{1}H–^{1}H$ and $^{1}H–^{13}C$ experiments.

Generation of a minor amount of other isomeric glycosylation products was also observed, which were removed from compound **14** using flash column chromatography. Carrying out three reactions in a one-pot setup significantly reduced the number of steps in this process which would improve ease in synthesis of the target compound. A one-pot multistep strategy required less bench time and afforded superior yields, avoiding the workup and isolation procedures required in the intermediate steps.

Finally, the tetraaccharide derivative **14** was subjected to a series of functional-group transformations, which comprised of: a) treatment with thioacetic acid[39] to convert azido group into acetamido group, b) fluoride ion mediated desilylation[40], c) TEMPO-mediated oxidation of the primary hydroxyl group,[41] d) removal of benzyl ethers and benzylideneacetal under catalytic transfer hydrogenation conditions using hydrogen and 10% Pd/C,[42] and finally, e) saponfication using sodium methoxide[34] to furnish the desired tetrasaccharide *p*-methoxyphenyl (PMP) glycoside **1**[43] in 54% overall yield. The NMR spectrum of compound **1**[43] unambiguously supported its structure. The analogue **2**[44] of the desired tetrasaccharide *p*-methoxyphenyl (PMP) glycoside **1** was also synthesized from compound **14** with the same experimental protocol that was used for compound **1** skipping the TEMPO-mediated oxidation of the primary hydroxyl group (Scheme 4).

In summary, oligosaccharide unit corresponding to the O-antigen of *E. coli* O163 as its sodium salt and *p*-methoxyphenyl glycoside was synthesized, applying three-step, one-pot sequence reaction conditions for two stereoselective glycosylation reactions and removal of the PMB group in situ. TEMPO-mediated phase-transfer oxidation was performed efficiently at a late stage of the total synthesis. An analogue of the concerned tetrasaccharide was also synthesized in very good yield using the same glycosylation approach. During the synthetic process, H_2SO_4 immobilized on silica was used as a thiophilic glycosylation activator in all stereoselective glycosylation reactions. The choice of the *p*-methoxyphenyl group as reducing end glycoside in all cases offers further glycoconjugate formation for future biological evaluation of the synthesized oligosaccharides.

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(38) ***p*-Methoxyphenyl (3,4-di-*O*-Acetyl 2-azido-2,6-dideoxy-α-L-glucopyranosyl)-(1→3)-(2-azido-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranosyl)-(1→2)-(3-*O*-benzyl-4,6-*O*-benzylidene-β-D-mannopyranosyl)-(1→4)-6-*O*-*tert*-butyldiphenylsilyl-2,3-di-*O*-benzyl-β-D-glucopyranoside (14)**

To a solution of compound **13** (1.5 g, 1.50 mmol) and compound **5** (730 mg, 1.65 mmol) in anhydrous CH_2Cl_2–Et_2O (1:2 v/v, 30 mL) MS 4 Å (3 g) was added and cooled to –20 °C under argon. To the cooled reaction mixture, NIS (410 mg, 1.82 mmol) and H_2SO_4–SiO_2 (100 mg) were added, and it was allowed to stir at the same temperature for 30 min. After consumption of the starting materials (TLC; hexane–EtOAc, 3:1), the temperature of the reaction mixture was raised to 10 °C and stirred for 30 min. After the formation of a new spot which was confirmed by TLC (hexane–EtOAc, 3:1), again the reaction mixture was cooled to –20 °C. To the cooled reaction mixture, a solution of compound **6** (689 mg, 1.81 mmol) in CH_2Cl_2–Et_2O (1:2 v/v, 5 mL) and NIS (448 mg, 1.99 mmol) were added, and the reaction mixture was then again stirred at –20 °C for another 30 min. The reaction mixture was filtered through a Celite bed, and the filtering bed was washed with CH_2Cl_2 (100 mL). The combined organic layer was successively washed with 5% $Na_2S_2O_3$, sat. $NaHCO_3$ and water, dried (Na_2SO_4), and concentrated. The crude product was purified over SiO_2 using hexane–EtOAc (4:1) as the eluent to give pure compound **14** (1.4 g, 61%); colorless oil; $[\alpha]_D^{25}$ +18 (*c* 1.0, $CHCl_3$). IR (neat): 3089, 2866, 1849, 1732, 1435, 1236, 1022, 988, 698 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 7.66–7.64 (m, 4 H, ArH), 7.52–7.50 (m, 2 H, ArH), 7.40–7.20 (m, 26 H, ArH), 7.15–7.11 (m, 1 H, ArH), 7.01–6.97 (m, 2 H, ArH), 6.96 (d, *J* = 9.0 Hz, 2 H, ArH), 6.80 (d, *J* = 9.0 Hz, 2 H, ArH), 5.62 (s, 1 H, PhCH), 5.55 (s, 1 H, PhCH), 5.32 (dd, *J* = 3.8, 9.8 Hz, 1 H, H-3_D), 5.25 (br s, 1 H, H-1_C), 5.10 (d, *J* = 10.4 Hz, 1 H, $PhCH_2$), 5.02 (d, *J* = 11.0 Hz, 1 H, $PhCH_2$), 4.89 (d, *J* = 7.8 Hz, 1 H, H-1_A), 4.87 (br s, 1 H, H-1_B), 4.86–4.83 (m, 2 H, H-4_D, $PhCH_2$),4.82 (d, *J* = 11.7 Hz, 1 H, $PhCH_2$), 4.77 (d, *J* = 10.8 Hz, 1 H, $PhCH_2$), 4.65 (d, *J* =11.8 Hz, 1 H, $PhCH_2$), 4.64–4.59 (m, 1 H, H-2_C), 4.56 (br s, 1 H, H-1_D), 4.27–4.22 (m, 3 H, H-3A, H-3_C, H-6_{aB}), 4.15–4.13 (m, 1 H, H-2_D), 4.09–4.05 (m, 3 H, H-2_B, H-3_B, H-6_{bB}), 4.01–3.96 (m, 2 H, H-6_{aA}, H-4_C), 3.94–3.88 (m, 2 H, H-5_C, H-5_D), 3.83–3.76 (m, 3 H, H-2_A, H-6_{bA}, H-6_{aC}), 3.78 (s, 3 H, OCH_3), 3.72–3.67 (m, 2 H, H-4_B, H-6_{bC}), 3.52–3.49 (m, 1 H, H-5_A), 3.41–3.78 (m, 1 H, H-4_A), 3.28–3.22 (m, 1 H, H-5_B), 2.04 (s, 3 H, $COCH_3$), 1.92 (s, 3 H, $COCH_3$), 1.06 [s, 9 H, $SiC(CH_3)_3$], 0.35 (d, *J* = 6.1 Hz, 3 H, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 169.9 (2 C, $COCH_3$), 155.4–114.6 (ArC), 103.2 (C-1_A), 102.2 (PhCH), 101.5 (PhCH), 99.7 (2 C, C-1_B, C-1_C), 95.7 (C-1_D), 83.5 (C-2_A), 82.0 (C-4_B), 79.1 (C-3_B), 78.7 (C-5_A), 77.4 (C-4_C), 76.8 (C-3_A), 76.4 ($PhCH_2$), 75.7 (C-4_A), 75.1 ($PhCH_2$), 74.6 (C-2_B), 73.8 (C-3_C), 73.6 ($PhCH_2$), 70.9 (2 C, C-3_D, C-4_D), 68.7 (C-6_B), 68.5 (C-6_C), 67.2 (C-5_B), 66.3 (C-5_C), 63.8 (C-2_C), 62.2 (C-6_A), 61.6 (C-2_D), 61.4 (C-5_D), 55.7 (OCH_3), 26.8 [$SiC(CH_3)_3$], 20.7 ($COCH_3$), 20.6 ($COCH_3$), 19.4 [$SiC(CH_3)_3$], 16.1 (CH_3). HRMS (ESI-TOF): *m/z* calcd for $C_{86}H_{94}N_6O_{21}Si$ [M + Na]: 1597.6241; found: 1597.6259.

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(43) ***p*-Methoxyphenyl (2-Acetamido-2,6-dideoxy-α-L-glucopyranosyl)-(1→3)-(2-acetamido-2-deoxy-α-D-glucopyranosyl)-(1→2)-(β-D-mannopyranosyl)-(1→4)-β-D-glucopyranosyluronic Acid (1)**

A solution of compound **14** (400 mg, 0.26 mmol) and thioacetic acid (0.2 mL) in pyridine (15 mL) was stirred at room temperature for 10 h. The solvent was removed under reduced pressure, and the crude product was passed through a short pad of SiO_2. To the solution of the N-acetylated product in THF (10 mL) was added Bu_4NF in THF (5 mL), and the reaction mixture was stirred at room temperature for 6 h. The solvents were removed, and the crude mass was dissolved in CH_2Cl_2 (40 mL). The organic layer was washed with sat. $NaHCO_3$ and water, dried, and concentrated. To a solution of the crude product in CH_2Cl_2 (20 mL) and H_2O (3.5 mL) were added aqueous solution of NaBr (1 mL, 1 M), aqueous solution of TBAB (2 mL; 1 M), TEMPO (80 mg, 0.4 mmol), sat. aqueous solution of $NaHCO_3$ (8 mL), and 4% NaOCl aq (10 mL) in succession, and the reaction mixture was stirred at 0–5 °C for 2 h. The reaction mixture was neutralized with 1 N HCl aq solution followed by addition of *t*-BuOH (25 mL), 2-methyl-but-2-ene (30 mL, 2 M solution in THF), aqueous solution of $NaClO_2$ (1 g in 5 mL), and aqueous solution of NaH_2PO_4 (1 g in 5 mL). The resultant mixture was allowed to stir at room temperature for 5 h and then diluted with sat. aqueous solution of NaH_2PO_4 and extracted with CH_2Cl_2 (3 × 50 mL). The combined organic layer was washed with water, dried (Na_2SO_4), and concentrated to dryness to give the oxidized product. To a solution of the oxidized product in MeOH (10 mL) was added 10% Pd/C (100 mg) and it was allowed to stir at room temperature for 24 h under a positive pressure of hydrogen. The reaction mixture was filtered through a Celite® bed and the filtering bed was washed with MeOH (50 mL). The combined solution was concentrated under reduced pressure to give the crude product, which was dissolved in 0.1 M NaOMe in MeOH (20 mL), and the solution was stirred at room temperature for 1 h. The reaction mixture was neutralized with Dowex 50W-X8 (H^+) resin, filtered, and concentrated to give compound **1**, which was passed through a column of Sephadex LH-20 (25% MeOH–H_2O) to furnish pure compound **1** (121 mg, 54%) as white powder; $[\alpha]_D^{25}$ –28 (*c* 1.0, H_2O). IR (neat): 3419, 2910, 1598, 1327, 1142, 988, 679 cm^{-1}. ^1H NMR (400 MHz, D_2O): δ = 7.06 (d, *J* = 8.1 Hz, 2 H, ArH), 6.92 (d, *J* = 8.1 Hz, 2 H, ArH), 5.18 (br s, 1 H, H-1_C), 5.04 (d, *J* = 7.6 Hz, 1 H, H-1_A), 4.83 (br s, 1 H, H-1_D), 4.69 (br s, 1 H, H-1_B), 4.59–4.57 (m, 1 H, H-2_D), 4.22–4.20 (m, 1 H, H-2_C), 4.11–4.09 (m, 3 H, H-5_A, H-2_B, H-5_C), 3.95–3.90 (m, 4 H, H-4_B, H-5_D, H-6_{aB}, H-6_{aC}), 3.88–3.82 (m, 3 H, H-4_A, H-6_{bB}, H-3_C), 3.76 (s, 3 H, OCH_3), 3.72–3.61 (m, 6 H, H-2_A, H-3_A, H-3_B, H-4_C, H-6_{bC}, H-3_D), 3.42–3.33 (m, 2 H, H-5_B, H-4_D), 2.02 (s, 6 H, $NHCOCH_3$), 1.25 (d, *J* = 6.0 Hz, 3 H, CH_3). ^{13}C NMR (100 MHz, D_2O, CD_3OD internal standard at δ = 49.5 ppm): δ = 175.5(2 C, C-6_A, $NHCOCH_3$), 174.9 ($NHCOCH_3$), 155.5–115.6 (ArC), 101.6 (C-1_A), 100.7 (C-1_B), 100.4 (C-1_C), 95.9 (C-1_D), 81.1 (C-4_A), 77.5 (C-5_B), 76.3 (C-2_B), 74.3 (2 C, C-5_A, C 3_C), 74.1 (C-4_D), 73.3 (C-3_B), 72.9 (2 C, C-5_C, C-3_D), 70.0 (C-3_A), 69.5 (C-5_D), 69.2 (C-4_B), 67.2 (C-4_C), 65.2 (C-2_A), 61.4 (C-6_C),

60.7 (C-6_B), 56.4 (OCH_3), 53.3 (C-2_C), 49.0 (C-2_D), 22.6 (NHCO-CH_3), 22.3 ($NHCOCH_3$), 17.2 (CH_3). HRMS (ESI-TOF): *m/z* calcd for $C_{35}H_{51}N_2O_{22}$ [M + Na]: 874.2831; found: 874.2843.

(44) ***p*-Methoxyphenyl (2-Acetamido-2,6-dideoxy-α-L-glucopyranosyl)-(1→3)-(2-acetamido-2-deoxy-α-D-glucopyranosyl)-(1→2)-(β-D-mannopyranosyl)-(1→4)-β-D-glucopyranoside (2)**

A solution of compound **14** (600 mg, 0.39 mmol) and thioacetic acid (0.2 mL) in pyridine (20 mL) was stirred at room temperature for 10 h. The solvent was removed under reduced pressure, and the crude product was passed through a short pad of SiO_2. To the solution of the N-acetylated product in THF (10 mL) was added Bu_4NF in THF (5 mL), and the reaction mixture was stirred at room temperature for 6 h. The solvents were removed, and the crude product was passed through a short pad of SiO_2. To a solution of the desilylated product in MeOH (10 mL) was added 10% Pd/C (150 mg), and it was allowed to stir at room temperature for 24 h under a positive pressure of hydrogen. The reaction mixture was filtered through a Celite® bed, and the filtering bed was washed with MeOH (60 mL). The combined solution was concentrated under reduced pressure to give the crude product, which was dissolved in 0.1 M MeONa in MeOH (30 mL), and the solution was stirred at room temperature for 1 h. The reaction mixture was neutralized with Dowex 50W-X8 (H^+) resin, filtered, and concentrated to give compound **1**, which was passed through a column of Sephadex LH-20 (25% MeOH–H_2O) to furnish pure compound **1** (142 mg, 62%) as white powder;$[\alpha]_D^{25}$ –12 (*c* 1.0, H_2O). IR (neat): 3445, 2936, 1546, 1377, 1030, 988, 669 cm^{-1}. ^1H (400 MHz, D_2O): δ = 7.12 (d, *J* = 8.1 Hz, 2 H, ArH), 6.98 (d, *J* = 8.1 Hz, 2 H, ArH), 5.20 (br s, 1 H, H-1_C), 5.03 (d, *J* = 7.4 Hz, 1 H, H-1_A), 4.87 (br s, 1 H, H-1_D), 4.81 (br s, 1 H, H-1_B), 4.63–4.62 (m, 1 H, H-2_D), 4.26–4.25 (m, 1 H, H-2_C), 4.19 (br s, 1 H, H-2_B), 4.15–4.13 (m, 2 H, H-5_A, H-5_C), 3.99–3.90 (m, 6 H, H-4_B, H-5_D, H-6_{abA}, H-6_{aB}, H-6_{aC}), 3.82 (s, 3 H, OCH_3), 3.79–3.71 (m, 7 H, H-2_A, H-4_A, H-3_B, H-3_C, H-4_C, H-6_{bB}, H-6_{bC}), 3.70–3.58 (m, 2 H, H-3_A, H-3_D), 3.51–3.48 (m, 1 H, H-5_B), 3.38–3.36 (m, 1 H, H-4_D), 2.07 (s, 3 H, $NHCOCH_3$), 2.06 (s, 3 H, $NHCOCH_3$), 1.31 (d, *J* = 6.0 Hz, 3 H, CH_3). ^{13}C NMR (100 MHz, D_2O, CH_3OD internal standard at δ = 49.5 ppm): δ = 175.2 ($NHCOCH_3$), 174.6 (NHCO-CH_3), 155.3–115.6 (ArC), 101.6 (C-1_A), 100.6 (2 C, C-1_B, C-1_C), 95.9 (C-1_D), 79.9 (C-4_A), 77.5 (C-2_B), 76.8 (C-5_B), 75.4 (C-3_D), 74.7 (C-3_C), 74.1 (C-3_B), 73.4 (C-5_C), 73.0 (C-5_A), 72.6 (C-3_A), 72.3 (C-4_D), 69.5 (C-5_D), 69.2 (C-4_B), 67.3 (C-4_C), 65.2 (C-2_A), 61.5 (C-6_C), 60.8 (C-6_B), 60.6 (C-6_A), 56.4 (OCH_3), 53.3 (C-2_C), 49.1 (C-2_D), 22.6 ($NHCOCH_3$), 22.3 ($NHCOCH_3$), 17.2 (CH_3). HRMS (ESI-TOF): *m/z* calcd for $C_{35}H_{54}N_2O_{21}$ [M + Na]: 861.3219; found: 861.3158.