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Synthesis of a tetrasaccharide analog corresponding to the repeating unit of the *O*-polysaccharide of *Salmonella enterica* O59: unexpected stereo outcome in glycosylation

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ABSTRACT

Convergent synthesis of a tetrasaccharide analog corresponding to the repeating unit of the O-polysaccharide of *Salmonella enterica* O59 is presented. A thioglycoside disaccharide donor was prepared by the glycosylation of two thioglycosides by tuning their relative reactivity. An unexpected stereochemical outcome was observed in a glycosylation using an ethyl 2-*O*-acetyl-3-*O*-benzyl-4,6-*O*-bensylidene-thiogalactoside donor, where the alpha-galactoside was formed in spite of the presence of the 2-*O*-acetyl participating group.

1. Introduction

Salmonella is a Gram-negative facultative rod-shaped bacterium in the family of Enterobacteriaceae, commonly known as 'enteric' bacteria.[1] *Salmonella enterica* is responsible for the food borne gastrointestinal infections causing salmonellosis in animals and humans.[2] *Salmonella* live in the intestinal tracts of warm and cold blooded animals. In general, *Salmonella enterica* infects cattle and poultry, which act as the reservoir of infections to humans.[3] Approximately 1.5 million cases of salmonellosis (common symptoms are enteric fever and acute gastroenteritis) and a significant number of deaths caused due to this infection are noted annually in the developed and developing countries.[4] *Salmonella* species are divided into a large number of serotypes based on the structure of their cell wall lipopolysaccharides (*O*-antigen) and the flagellar structures (*H*-antigen).[5,6] However, only few of them are responsible for the infections in humans. Recently, Perepelov et al. reported the structure of the cell wall *O*-polysaccharide of *Salmonella enterica* O59,[7] which contains two D-glucosamine, one D-galactose and one L-rhamnose moieties (Fig. 1).

Several reports in the recent past elaborately demonstrated the useful role of *O*-antigenic polysaccharides in the development of carbohydrate based therapeutics.[8,9] In order to have better understanding of the pathogenic role of the *O*-antigenic polysaccharide of *Salmonella enterica* O59, several immunochemical experiments should be carried out requiring a large quantity of the tetrasaccharide. Since isolation of the polysaccharide fragments from the natural source can not meet the requirement, a concise chemical synthetic strategy for the synthesis of the tetrasaccharide and its close analogs is highly desirable. As a part of the ongoing program on the synthesis of complex oligosaccharides from the bacterial origin, synthesis of the tetrasaccharide repeating unit (Fig. 2, structure 1) corresponding to the *O*-antigenic lipopolysaccharide of *Salmonella enterica* O59 strain has been undertaken. However, during the course of the synthesis of the target tetrasaccharide **1**, an unusual stereochemical outcome of one of the glycoside formations was observed. Although using a thiogalactoside donor with a 2-*O*-participating group still the disaccharide formed was found to be alpha-linked. Continuing from this unexpected disaccharide derivative a tetrasaccharide analog **2** (Fig. 2) related to the *O*-antigenic lipopolysaccharide of *Salmonella enterica* O59 strain was synthesized.

→4)-α-L-Rha*p*-(1→3)-β-D-Glc*p*NAc-(1→2)-β-D-Gal*p*-(1→3)-α-D-Glc*p*NAc-(1→

Figure 1. Structure of the repeating unit of the *O*-polysaccharide of *Salmonella enterica* O59.

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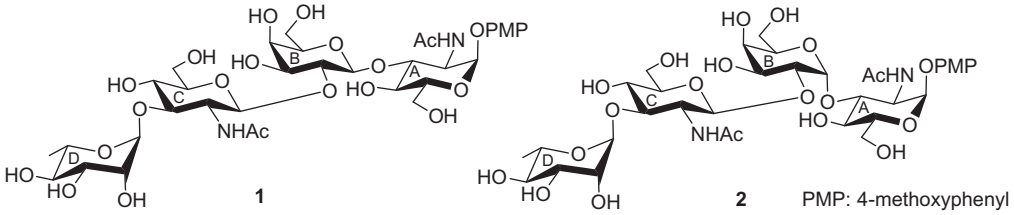


Figure 2. Chemical structure of the alpha –p-methoxybenzyl glycoside of the tetrasaccharide repeating unit of the *O*-polysaccharide of *Salmonella enterica* O59 (**1**) and structure of the synthesized tetrasaccharide **2** related to tetrasaccharide **1**.

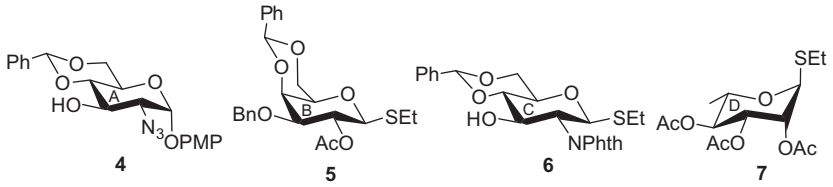
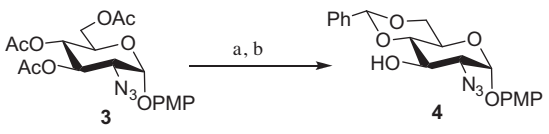


Figure 3. Monosaccharide intermediates used for the synthesis of compound **2**.

2. Results and discussion

In the initial attempt to synthesize compound **1**, a number of suitably functionalized monosaccharide intermediates **3**,[10] **4**, **5**,[11] **6**[12], and **7**[12] (Fig. 3) were prepared from the commercially available reducing sugars using earlier reported reaction conditions. A convergent strategy involving [2+2] glycosylation has been adopted for the synthesis of compound **1**. Compound **4** was prepared in 77% yield from compound **3** using a two step sequence involving de-acetylation and iodine catalyzed 4,6-*O*-benzylidene acetal formation[13] (Scheme 1). Iodonium ion promoted stereoselective glycosylation[14] of compound **4** with thioglycoside **5** using *N*-iodosuccinimide (NIS) and trifluoromethane sulfonic acid (TfOH) furnished compound **8** in 77% yield having a newly formed 1,2-*cis* glycosyl linkage. Formation of 1,2-*cis* glycoside was confirmed from its spectral analysis [signals at δ 5.68 (d, J = 4.0 Hz, H-1_B), 5.52 (d, J = 3.5 Hz, H-1_A) in the ^{1}H NMR and δ 98.6 (C-1_A), 97.4 (C-1_B) in the ^{13}C NMR spectra]. Formation of compound **8** with 1,2-*cis* glycosyl linkage using 2-O-acetylated D-galactosyl thioethyl glycoside donor was unexpected due to the well known concept that the presence of an acyl protection group on 2-OH of a glycosyl donor commonly induces the exclusive formation of a 1,2-*trans* glycoside because of the neighboring group participation effect.[15,16] Using other 2-O-acetylated glycosyl donors (e.g., thiophenyl glycoside, trichloroacetimidate derivative) showed similar unexpected stereo outcome of the glycosylation. In a separate experiment, reaction of the same glycosyl acceptor with ethyl 2,3-di-*O*-acetyl-4,6-*O*-benzylidene-1-thio-β-D-galactopyranoside, exclusively furnished 1,2-*trans* glycosylated product (80%). In both galactosyl thioglycoside donors 4,6-*O*-benzylidene acetal is present, but the difference in 3-O-substituents (benzyl and acetyl) directs the stereochemical outcome of the glycosylation product. This observation indicated that the presence of 3-*O*-benzyl ether in the thioglycoside donor controls the stereo-outcome of the glycosylations. It is presumed that a five-member twisted boat transition state may form during the activation of the thioglycoside with NIS–TfOH, which forces the nucleophile to approach from the 1,2-*cis* orientation and thus furnishes 1,2-*cis* glycosylated product. In earlier reports by Satoh et al.,[17] Manabe et al.,[18] Oscarson et al.[19] and Crich et al.[20] it was considered that *endo*-cyclic post glycosylation anomerization plays the pivotal role in the formation of 1,2-*cis* glycosylation products. Recently, Oscarson and co-workers[21] reported the anomerization of disaccharides by kinetic measurements. However, in this particular case such possibilities may be excluded since the functional groups present in the glycosyl donor are different. Although the formation of compound **8** was unexpected and can not be used for the synthesis of the target tetrasaccharide **1**, we planned to use compound **8** in the preparation of an analogous tetrasaccharide **2** related to the target compound **1**. Analogous compounds are very important in evaluating their biological potential in comparison to the original compounds. Thus compound **8** was deacetylated using sodium methoxide to give disaccharide derivative **9** in quantitative yield.



Scheme 1. Reagents and conditions: (a) 0.1 M CH_3ONa, CH_3OH, room temperature, 2 h; (b) benzaldehyde dimethyl acetal, I_2, CH_3CN, 50 °C, 8 h, 77%.

In another experiment, stereoselective glycosylation of thioglycoside **6** and thioglycoside **7** in the presence of a combination of NIS–TfOH[14] furnished disaccharide derivative **10** in an 81% yield. Appearance of signature peaks in the NMR spectra [signals at δ 5.37 (d, J = 10.5 Hz, H-1_C), 4.51 (br s, H-1_D) in the ^{1}H NMR and δ 97.3 (C-1_D), 81.9 (C-1_C) in the ^{13}C NMR spectra] confirmed the formation of compound **10**. Although both compounds **6** and **7** are thioethyl glycosides and can be activated by the combination of NIS–TfOH, the selective activation of compound **7** was achieved because of the fact that deoxy sugars are more highly reactive than the amino sugars, which has been established in several earlier reports by Wong and co-workers using relative reactivity values.[22,23] Presence of a deactivating *N*-phthalimido group at C-2 position of compound **6** induces disarming effect to act as glycosyl acceptor in the presence of a comparatively activated 6-deoxy-L-sugar derived thioglycoside **7**. This kind of selective activation has also been documented by Demchenko and co-workers[24] and Bols and co-workers[25] considering super armed/disarmed glycosylation approach. Iodonium ion promoted stereoselective glycosylation of disaccharide **9** with disaccharide **10** using NIS–TfOH combination furnished tetrasaccharide derivative **11** in a 74% yield. Formation of compound **11** was supported by it's spectral analysis [signals at δ 5.55 (d, J = 4.0 Hz, H-1_B), 5.48 (d, J = 3.5 Hz, H-1_A), 5.39 (d, J = 8.0 Hz, H-1_C), 4.45 (d, J = 1.5 Hz, H-1_D) in the ^{1}H NMR and δ

99.6 (C-1_B), 98.6 (C-1_A), 98.0 (C-1_C), 97.1 (C-1_D) in the ^{13}C NMR spectra]. Compound **11** was subjected to a series of reactions involving (a) removal of *N*-phthalimido group followed by N-acetylation,[26] (b) hydrogenolysis[27] followed by N-acetylation and (c) saponification to furnish compound **2** in a 56% over all yield. Presence of signals in the NMR spectra confirmed its formation [signals at δ 5.62 (d, J = 4.0 Hz, H-1_B), 5.42 (d, J = 8.5 Hz, H-1_C), 5.31 (d, J = 3.5 Hz, H-1_A), 4.56 (d, J = 1.5 Hz, H-1_D) in the ^1H NMR and δ 102.9 (C-1_D), 101.7 (C-1_C), 100.3 (C-1_B), 99.1 (C-1_A) in the ^{13}C NMR spectra] (Scheme 2). A comparison of the chemical shifts of the anomeric centers of the synthesized tetrasaccharide as its 4-methoxyphenyl glycoside (**2**) with the tetrasaccharide fragment corresponding to the *O*-antigenic lipopolysaccharide of *Salmonella enterica* O59 is presented in Table 1. A slight deviation of the chemical shifts in the synthesized compound **2** may be due to the presence of a 4-methoxyphenyl group at the reducing end whereas the isolated tetrasaccharide was a hemiacetal. From the chemical shift values it is clear that D-galactose moiety is 1,2-*cis* linked in compound **2**.

Table 1
Comparison of the NMR chemical shifts (δ, ppm) and coupling constants (J in Hz) of the anomeric centers of compound **2** and the tetrasaccharide repeating unit isolated from the natural source[7]

Sugar residue	H-1 (δ, ppm)		C-1 (δ, ppm)	
	Compd **2**	Isolated[7]	Compd **2**	Isolated[7]
→3)-α-D-GlcpNAc (A)	5.31 (J = 3.5 Hz)	4.99	99.1	99.2
→2)-α/β-D-Galp (B)	5.62 (J = 4.0 Hz)(α)	4.56 (β)	100.3	102.2
→3)-β-D-GlcpNAc (C)	5.42 (J = 8.5 Hz)	4.80	101.7	102.5
α-L-Rhap (D)	4.56 (J = 1.5 Hz)	4.87	102.9	102.2

3. Conclusion

In conclusion, it was observed that the stereo chemical outcome of the glycosylation is strongly dependent on the functionalities present in the donor and acceptor and often deviates from the generally accepted explanation of neighboring group participating effect for the 1,2-*trans* glycoside formation. A tetrasaccharide analog related to the repeating unit of the *O*-polysaccharide of *Salmonella enterica* O59 has been synthesized using a [2+2] block glycosylation strategy. An intermediate disaccharide donor was prepared by glycosylation of two thioglycosides by tuning their relative reactivity. All intermediates were obtained in a high yield.

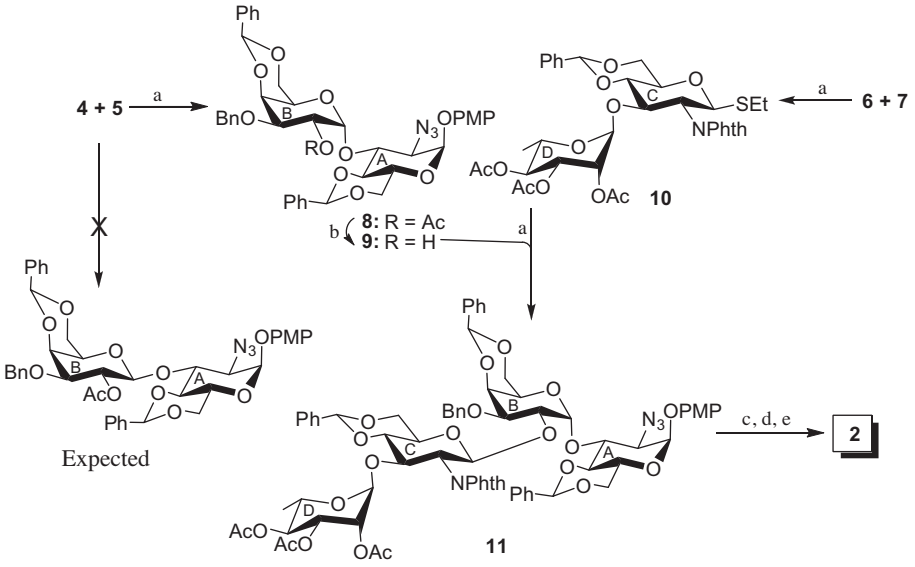
4. Experimental

4.1. General methods

All reactions were monitored by thin layer chromatography over silica gel-coated TLC plates. The spots on TLC were visualized by warming ceric sulfate (2% $Ce(SO_4)_2$ in 2 N H_2SO_4)-sprayed plates on a hot plate. Silica gel 230–400 mesh was used for column chromatography. ^1H and ^{13}C NMR, DEPT 135, 2D COSY, and HSQC NMR spectra were recorded on Brucker Avance DRX 500 MHz spectrometers using $CDCl_3$ and CD_3OD as solvents and TMS as internal reference unless stated otherwise. Chemical shift values are expressed in δ ppm. ESI-MS were recorded on a Micromass mass spectrometer. Elementary analysis was carried out on Carlo Erba analyzer. Optical rotations were measured at 25 °C on a Jasco P-2000 polarimeter. Commercially available grades of organic solvents of adequate purity are used in all reactions.

4.2. 4-Methoxyphenyl 2-azido-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranoside (4)

A solution of compound **3** (4.0 g, 9.15 mmol) in 0.1 M CH_3ONa in CH_3OH (30 mL) was allowed to stir at room temperature for 2 h, neutralized with Dowex 50W X8 (H^+) resin and the solvents were removed under reduced pressure. To a solution of the product in anhydrous CH_3CN (25 mL) were added benzaldehyde dimethylacetal (2.7 mL, 18.0 mmol) and iodine (500 mg, 1.97 mmol) and the reaction mixture was allowed to stir at 50 °C for 8 h. To the reaction mixture was added Et_3N (2 mL) and it was evaporated to dryness under reduced pressure. The crude mass was dissolved in CH_2Cl_2 (150 mL) and successively washed with 5% $Na_2S_2O_3$, satd $NaHCO_3$, and water. The organic layer was dried (Na_2SO_4) and concentrated under reduced pressure to give the crude product, which was purified over SiO_2 using hexane–EtOAc (4:1) as eluant to give



Scheme 2. Reagents and conditions: (a) *N*-iodosuccinimide, TfOH, CH_2Cl_2, MS 4 Å, −30 °C, 45 min, 77% for compound **8**, 81% for compound **10,** and 74% for compound **11**; (b) 0.1 M CH_3ONa, CH_3OH, room temperature, 2 h, quantitative; (c) (i) $NH_2NH_2{\cdot}H_2O$, EtOH, 80 °C, 8 h; (ii) acetic anhydride, pyridine, room temperature, 1 h; (d) (i) H_2, 20% $Pd(OH)_2$-C, CH_3OH, 24 h, room temperature; (ii) acetic anhydride, pyridine, room temperature, 1 h; (e) 0.1 M CH_3ONa, CH_3OH, room temperature, 2 h, 56% over all.

pure compound **4** (2.8 g, 77%). Yellow oil; $[\alpha]_D^{25}$ +120 (*c* 1.2, $CHCl_3$); IR (neat): 2963, 2944, 2116, 1752, 151, 1230, 1044, 1035, 819, 786, 543 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz): δ 7.49–7.36 (m, 5H, Ar-H), 7.01 (d, *J* = 9.0 Hz, 2H, Ar-H), 6.85 (d, *J* = 9.0 Hz, 2H, Ar-H), 5.53 (s, 1H, PhC*H*), 5.39 (d, *J* = 3.3 Hz, 1H, H-1), 4.38–4.32 (m, 1H, H-6_a), 4.25–4.20 (m, 1H, H-6_b), 4.06–3.97 (m, 1H, H-5), 3.76 (s, 3H, OCH_3), 3.71 (t, *J* = 10.2 Hz each, 1H, H-4), 3.55 (t, *J* = 9.6 Hz each, 1H, H-3), 3.36 (dd, *J* = 9.9, 3.6 Hz, 1H, H-2); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 155.6–114.7 (Ar-C), 102.1 (PhCH), 98.3 (C-1), 81.7, 68.8, 68.7, 66.3, 63.1, 55.7; ESI-MS: 422.1 $[M+Na]^+$; Anal. Calcd for $C_{20}H_{21}N_3O_6$ (399.14): C, 60.14; H, 5.30. Found: C, 60.30; H, 5.50.

4.3. 4-Methoxyphenyl (2-*O*-acetyl-3-*O*-benzyl-4,6-*O*-benzylidene-α-D-galactopyranosyl)-(1→3)-2-azido-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranoside (8)

To a solution of compound **4** (1.0 g, 2.5 mmol) and compound **5** (1.3 g, 2.9 mmol) in anhydrous CH_2Cl_2 (10 mL) was added MS 4 Å (1.0 g) and the reaction mixture was stirred under argon at room temperature for 30 min. The reaction mixture was cooled to −30 °C and *N*-iodosuccinimide (NIS; 780 mg, 3.46 mmol) and trifluoromethane sulfonic acid (TfOH; 15 μL) were added to it. After stirring at the same temperature for 45 min the reaction mixture was filtered through a Celite® bed and washed with CH_2Cl_2 (100 mL). The organic layer was successively washed with 5% $Na_2S_2O_3$, satd. $NaHCO_3$, and water, dried (Na_2SO_4) and concentrated under reduced pressure. The crude product was purified over SiO_2 using hexane–EtOAc (6:1) as eluant to give pure compound **8** (1.5 g, 77%). Yellow oil; $[\alpha]_D^{25}$ +109 (*c* 1.2, $CHCl_3$); IR (neat): 2920, 2864, 2106, 1743, 1508, 1235, 1093, 1029, 744, 698 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz): δ 7.33–7.26 (m, 15H, Ar-H), 7.04 (d, 9.0 Hz, 2H, Ar-H), 6.86 (d, *J* = 9.0 Hz, 2H, Ar-H), 5.68 (d, *J* = 4.0 Hz, 1H, H-1_B), 5.52 (d, *J* = 3.5 Hz, 1H, H-1_A), 5.50 (s, 1H, PhC*H*), 5.47 (s, 1H, PhC*H*), 5.44 (dd, *J* = 10.5, 3.5 Hz, 1H, H-2_B), 4.73–4.67 (AB_q, *J* = 12.0 Hz each, 2H, PhCH_2), 4.54 (t, *J* = 9.5 Hz each, 1H, H-3_A), 4.34–4.31 (m, 1H, H-6_{aA}), 4.26 (d, *J* = 3.5 Hz, 1H, H-4_B), 4.25–4.22 (m, 1H, H-6_{bA}), 4.12–4.07 (m, 1H, H-5_A), 4.05 (dd, *J* = 10.5, 3.0 Hz, 1H, H-3_B), 4.03–4.02 (m, 1H, H-5_B), 4.00–3.98 (m, 1H, H-6_{aB}), 3.78 (s, 3H, OCH_3), 3.77–3.73 (m, 2H, H-4_A, H-6_{bB}), 3.29 (dd, *J* = 10.5, 3.5 Hz, 1H, H-2_A), 1.74 (s, 3H, COCH_3); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 169.4 (*C*OCH$_3$), 155.6–114.8 (Ar-C), 101.9 (PhCH), 101.0 (PhCH), 98.6 (C-1_A), 97.4 (C-1_B), 82.1 (C-4_A), 74.2 (C-4_B), 73.4 (C-3_B), 72.6 (C-3_A), 71.7 (PhCH_2), 69.6 (C-6_A), 69.1 (C-2_B), 68.7 (C-6_B), 63.4 (C-5_B), 63.0 (C-5_A), 61.4 (C-2_A), 55.6 (OCH_3), 20.9 (CO*C*H$_3$); ESI-MS: 804.2 $[M+Na]^+$; Anal. Calcd for $C_{42}H_{43}N_3O_{12}$ (781.28): C, 64.52; H, 5.54. Found: C, 64.30; H, 5.75.

4.4. 4-Methoxyphenyl (3-*O*-benzyl-4,6-*O*-benzylidene-α-D-galactopyranosyl)-(1→3)-2-azido-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranoside (9)

A solution of compound **8** (1.3 g, 1.66 mmol) in 0.1 M CH_3ONa in CH_3OH (15 mL) was allowed to be stirred at room temperature and neutralized with Dowex 50W X8 (H^+) resin. The reaction mixture was filtered, concentrated, and passed through a short pad of SiO_2 to give pure compound **9** (1.2 g, 98%). Yellow oil; $[\alpha]_D^{25}$ +89 (*c* 1.2, $CHCl_3$); IR (neat): 3529, 2924, 2104, 1508, 1370, 1210, 1027, 743, 697 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz): 7.52–7.25 (m, 15H, Ar-H), 7.04 (d, *J* = 7.5 Hz, 2H, Ar-H), 6.85 (d, *J* = 7.5 Hz, 2H, Ar-H), 5.62 (br s, 1H, H-1_B), 5.59 (s, 1H, PhC*H*), 5.50 (br s, 1H, H-1_A), 5.45 (s, 1H, PhC*H*), 4.73 (br s, 2H, PhCH_2), 4.54 (t, *J* = 8.0 Hz, 1H, H-3_A), 4.35–4.34 (m, 1H, H-6_{aA}), 4.27–4.26 (m, 3H, H-3_B, H-4_B, H-$6b_A$), 4.07–4.03 (m, 2H, H-3_B, H-5_A), 3.39 (br s, 1H, H-5_B), 3.88–3.72 (m, 3H, H-4_A, H-6_{abB}), 3.74 (s, 3H, OCH_3), 3.33 (dd, *J* = 9.5, 3.0 Hz, 1H, H-2_A); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 155.7–114.8 (Ar-C), 101.6 (PhCH), 100.8 (PhCH), 100.3 (C-1_A), 98.5 (C-1_B), 82.2 (C-4_A), 76.4 (C-4_B), 73.6 (C-3_B), 73.0 (C-3_A), 71.4 (PhCH_2), 69.8 (C-6_A), 68.6 (C-6_B), 67.9 (C-2_B), 63.7 (C-5_B), 63.1 (C-5_A), 61.9 (C-2_A), 55.7 (OCH_3); ESI-MS: 762.2 $[M+Na]^+$; Anal. Calcd for $C_{40}H_{41}N_3O_{11}$ (739.27): C, 64.94; H, 5.59. Found: C, 64.72; H, 5.85.

4.5. Ethyl (2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl)-(1→3)-4,6-*O*-benzylidene-2-deoxy-2-*N*-phthalimido-1-thio-β-D-glucopyranoside (10)

To a solution of compound **6** (1.0 g, 2.26 mmol) and compound **7** (840 mg, 2.51 mmol) in anhydrous CH_2Cl_2 (5 mL) was added MS 4 Å (1.0 g) and the reaction mixture was stirred under argon at room temperature for 30 min. The reaction mixture was cooled to −30 °C and NIS (550.0 mg, 2.44 mmol) and TfOH (10 μL) were added to it. After stirring at same temperature for 45 min the reaction mixture was filtered through a Celite® bed and washed with CH_2Cl_2 (50 mL). The organic layer was successively washed with 5% $Na_2S_2O_3$, satd. $NaHCO_3$ and water, dried (Na_2SO_4), and concentrated under reduced pressure. The crude product was purified over SiO_2 using hexane–EtOAc (5:1) as eluant to give pure compound **10** (1.3 g, 81%). Yellow oil; $[\alpha]_D^{25}$ −26 (*c* 1.2, $CHCl_3$); IR (neat): 2983, 1751, 1716, 1383, 1224, 1096, 1045, 721, 545 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz): δ 7.73–7.31 (m, 9H, Ar-H), 5.57 (s, 1H, PhC*H*), 5.37 (d, *J* = 10.5 Hz, 1H, H-1_C), 5.20 (dd, *J* = 10.0, 3.0 Hz, 1H, H-3_D), 4.80 (t, *J* = 10.0 Hz each 1H, H-4_D), 4.71–4.70 (m, 1H, H-2_D), 4.66 (t, *J* = 9.5 Hz each, 1H, H-3_C), 4.51 (br s, 1H, H-1_D), 4.42–4.39 (m, 1H, H-6_{ac}), 4.38 (t, *J* = 10.5 Hz each,1H, H-2_C), 4.10–3.97 (m, 1H, H-5_D), 3.83–3.79 (m, 1H, H-6_{bc}), 3.77–3.71 (m, 2H, H-4_C, H-5_C), 2.68–2.60 (m, 2H, SCH_2CH$_3$), 1.93, 1.90, 1.76 (3 s, 9H, 3 COCH_3), 1.71 (t, *J* = 7.5 Hz each, 3H, SCH$_2$CH_3), 0.57 (d, *J* = 6.5 Hz, 3H, CCH_3); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 169.8, 169.6, 169.9, 169.3 (*C*OCH$_3$), 137.0–123.2 (Ar-C), 102.0 (PhCH), 97.3 (C-1_D), 81.9 (C-1_C), 80.2 (C-5_C), 75.3 (C-3_C), 71.1 (C-4_D), 70.8 (C-4_C), 70.2 (C-2_D), 68.6 (C-6_C), 68.2 (C-3_D), 66.4 (C-5_D), 55.1 (C-2_C), 24.1 (SCH_2CH$_3$), 20.7, 20.6, 20.4 (3 COCH$_3$), 16.3 (CCH$_3$), 14.8 (SCH$_2$CH$_3$); ESI-MS: 736.2 $[M+Na]^+$; Anal. Calcd for $C_{35}H_{39}NO_{13}S$ (713.21): C, 58.90; H, 5.51. Found: C, 58.73; H, 5.72.

4.6. 4-Methoxyphenyl (2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl)-(1→3)-(4,6-*O*-benzylidene-2-deoxy-2-*N*-phthalimido-β-D-glucopyranosyl)-(1→2)-(3-*O*-benzyl-4,6-*O*-benzylidene-α-D-galactopyranosyl)-(1→3)-2-azido-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranoside (11)

To a solution of compound **9** (1.0 g, 1.35 mmol) and compound **10** (1.0 g, 1.40 mmol) in anhydrous CH_2Cl_2 (5 mL) was added MS 4 Å (1.0 g) and the reaction mixture was stirred under argon at room temperature for 30 min. The reaction mixture was cooled to −30 °C and NIS (350.0 mg, 1.55 mmol) and TfOH (5 μL) were added to it. After stirring at the same temperature for 45 min the reaction mixture was filtered through a Celite® bed and washed with CH_2Cl_2 (50 mL). The organic layer was successively washed with 5% $Na_2S_2O_3$, satd $NaHCO_3$ and water, dried (Na_2SO_4), and concentrated under reduced pressure. The crude product was purified over SiO_2 using hexane–EtOAc (4:1) as eluant to give pure compound **11** (1.4 g, 74%). Yellow oil; $[\alpha]_D^{25}$ +86 (*c* 1.2, $CHCl_3$); IR (neat): 3475, 2932, 2107, 1751, 1717, 1508, 1387, 1223, 1045, 698 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz): δ 7.71–7.18 (m, 24H, Ar-H), 6.12 (s, 1H, PhC*H*), 5.55 (d, *J* = 4.0 Hz, 1H, H-1_B), 5.48 (d, *J* = 3.5 Hz, 1H, H-1_A), 5.39 (d, *J* = 8.0 Hz, 1H, H-1_C), 5.30 (dd, *J* = 10.0, 3.5 Hz, 1H, H-3_D), 5.24 (s, 1H, PhC*H*), 5.22 (s, 1H, PhC*H*), 4.84 (t, *J* = 10.0 Hz each, 1H, H-4_D), 4.75–4.74 (m, 1H, H-2_D), 4.53–4.46 (m, 2H, H-3_A, H-4_C), 4.45 (d, *J* = 1.5 Hz, 1H, H-1_D), 4.41 (d, *J* = 12.8 Hz, 1H, PhCH_2), 4.30–4.27 (m, 2H, H-2_B, H-6_{aB}), 4.25–4.22 (m, 1H, H-6_{aA}), 4.11–4.09 (m, 1H, H-6_{bA}), 4.06–4.00 (m, 3H, H-3_C, H-6_{aC}, PhCH_2), 3.98–3.91 (m, 4H, H-2_C, H-5_C, H-5_D, H-6_{bB}), 3.88–3.87 (d, *J* = 3.5 Hz, 1H, H-4_B),

3.81 (br s, 1H, H-5$_B$), 3.78 (s, 3H, OC*H*$_3$), 3.65 (dd, *J* = 10.5, Hz, 1H, H-3$_B$), 3.43–3.37 (m, 1H, H-5$_A$), 3.20 (dd, *J* = 10.0, 3.5 Hz, 1H, H-2$_A$), 3.05 (t, *J* = 10.0 Hz each, 1H, H-6$_{bC}$), 2.53 (t, *J* = 10.0 Hz each, 1H, H-4$_A$), 1.99, 1.95, 1.79 (3 s, 9H, 3 COC*H*$_3$), 0.57 (d, *J* = 6.5 Hz, 1H, CC*H*$_3$); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 169.9, 169.8, 169.4 (3 *C*OCH$_3$), 167.9, 167.8 (PhthCO), 155.6–114.7 (Ar-C), 101.4 (PhCH), 101.2 (PhCH), 101.0 (PhCH), 99.6 (C-1$_B$), 98.6 (C-1$_A$), 98.0 (C-1$_C$), 97.1 (C-1$_D$), 82.0 (C-5$_C$), 79.3 (C-4$_A$), 75.9 (C-3$_B$), 74.3 (C-4$_B$), 74.0 (C-4$_C$), 72.4 (C-3$_A$), 71.8 (PhCH$_2$), 71.1 (C-2$_B$), 71.0 (C-4$_D$), 70.3 (C-2$_D$), 69.7 (C-6$_B$), 68.7 (C-6$_A$), 68.5 (C-3$_D$), 68.0 (C-6c), 66.3 (C-5$_D$), 65.4 (C-5$_A$), 63.2 (C-3$_C$), 63.0 (C-5$_B$), 61.7 (C-2$_A$), 57.0 (C-2$_C$), 55.6 (OCH$_3$), 20.7, 20.6, 20.5 (3 COCH$_3$), 16.4 (CCH$_3$); ESI-MS: 1413.4 [M+Na]$^+$; Anal. Calcd for $C_{73}H_{74}N_4O_{24}$ (1390.47): C, 63.02; H, 5.36. Found: C, 63.21; H, 5.60.

4.7. 4-Methoxyphenyl (α-L-rhamnopyranosyl)-(1→3)-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→2)-(α-D-galactopyranosyl)-(1→3)-2-acetamido-2-deoxy-α-D-glucopyranoside (2)

To a solution of compound **11** (1.0 g, 0.72 mmol) in EtOH (20 mL) was added hydrazine monohydrate (0.2 mL, 4.12 mmol) and the reaction mixture was allowed to stir at 80 °C for 8 h. After removal of the solvents the crude mass was dissolved in acetic anhydride-pyridine (5 mL; 1:1 v/v) and kept at room temperature for 1 h. The solvents were removed under reduced pressure to give the crude acetylated product. To a solution of the crude mass in CH_3OH (10 mL) was added 20% $Pd(OH)_2$-C (200 mg) and the reaction mixture was stirred at room temperature for 24 h. The reaction mixture was filtered through a Celite® bed and evaporated to dryness. A solution of the crude product in acetic anhydride-pyridine (3 mL; 1:1 v/v) was kept at room temperature for 1 h and concentrated under reduced pressure. A solution of the crude product in 0.1 M CH_3ONa in CH_3OH (10 mL) was allowed to stir at room temperature for 2 h and neutralized with Dowex 50W X8 (H^+) resin. The reaction mixture was filtered and concentrated to dryness to give compound **2**, which was passed through a Sephadex LH-20 column using CH_3OH–H_2O (4:1) as eluant to give pure compound **2** (340 mg, 56%). White powder; $[\alpha]_D^{25}$ +78 (*c* 1.3, CH_3OH); IR (KBr): 3423, 2928, 1767, 1655, 1578, 1211, 967 cm^{-1}; ^{1}H NMR (CD_3OD, 500 MHz): δ 7.07 (d, *J* = 9.0 Hz, 2H, Ar-H), 6.84 (d, *J* = 9.0 Hz, 2H, Ar-H), 5.62 (d, *J* = 4.0 Hz, 1H, H-1$_B$), 5.42 (d, *J* = 8.5 Hz, 1H, H-1$_C$), 5.31 (d, *J* = 3.5 Hz, 1H, H-1$_A$), 4.56 (d, *J* = 1.5 Hz, 1H, H-1$_D$), 4.41 (dd, *J* = 8.0 Hz each, 1H, H-3$_C$), 4.19 (dd, *J* = 8.5 Hz each, 1H, H-2$_C$), 4.15 (dd, *J* = 10.5, 3.5 Hz, 1H, H-2$_A$), 4.13–4.08 (m, 1H, H-5$_A$), 4.03–3.98 (m, 3H, H-2$_B$, H-4$_B$, H-6$_{aA}$), 3.87–3.81 (m, 1H, H-6$_{bA}$), 3.80–3.74 (m, 6H, H-3$_A$, H-3$_B$, H-3$_D$, H-5$_C$, H-6$_{abB}$), 3.73 (s, 3H, OC*H*$_3$), 3.72–3.65 (m, 2H, H-4$_D$, H-6$_{ac}$), 3.58–3.49 (m, 4H, H-4$_C$, H-5$_B$, H-5$_D$, H-6$_{bc}$), 3.39–3.37 (m, 1H, H-2$_D$), 3.28 (t, *J* = 9.5 Hz each, 1H, H-4$_A$), 2.14, 1.94 (2 s, 6H, 2 COC*H*$_3$), 1.21 (d, *J* = 6.0 Hz, 3H, CC*H*$_3$); ^{13}C NMR (CD_3OD, 125 MHz): δ 173.7 (2 C, 2 *C*OCH$_3$), 156.8–115.6 (Ar-C), 102.9 (C-1$_D$), 101.7 (C-1$_C$), 100.3 (C-1$_B$), 99.1 (C-1$_A$), 81.1 (C-3$_C$), 79.8 (C-5$_A$), 79.6 (C-2$_B$), 78.3 (C-5$_B$), 76.5 (C-5$_D$), 74.1 (C-3$_A$), 73.5 (C-4$_A$), 72.5 (C-2$_D$), 72.2 (C-4$_B$), 71.9 (C-4$_B$), 71.8 (C-5$_C$), 71.1 (C-4$_C$), 70.7 (C-4$_D$), 68.3 (C-3$_D$), 62.6 (2 C, C-6$_A$, C-6$_B$), 62.4 (C-6$_C$), 57.3 (C-2$_A$), 56.0 (OCH$_3$), 53.6 (C-2$_C$), 22.8 (2 C, 2 COCH$_3$), 17.8 (CCH$_3$); ESI-MS: 861.3 [M+Na]$^+$; Anal. Calcd for $C_{35}H_{54}N_2O_{21}$ (838.32): C, 50.12; H, 6.49. Found: C, 49.94; H, 6.72.

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Supplementary data

Supplementary data associated (copies of the 1D and 2D NMR spectra of compounds **8**–**11** and **2**) with this article can be found, in the online version, at doi:10.1016/j.carres.2012.01.026.

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