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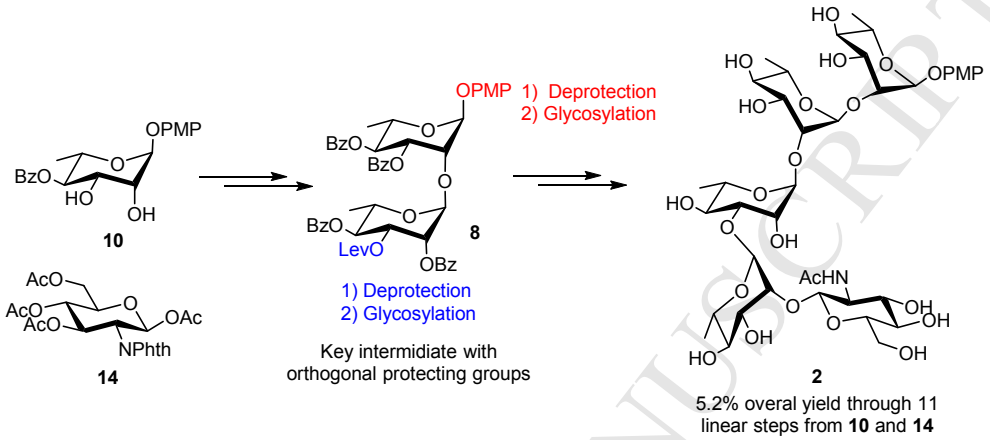

Synthesis of the repeating unit of O-specific polysaccharide isolated from the water-borne bacteria *Aeromonas bestiarum* 207

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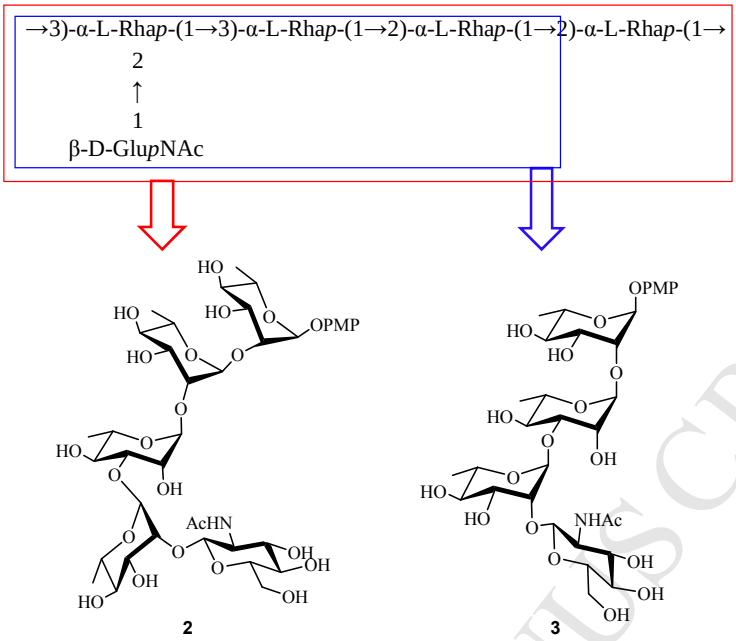
Abstract

Aeromonas bestiarum 207 is a bacterial pathogen with severe impact on aquaculture. In a recent study, the structure of OPS antigens from *Aeromonas* bestiarum was identified as pentasaccharide repeating units. Synthesis of the pentasaccharide repeating unit and its derivative are reported. Stereo- and regio-specific synthesis was achieved under Schmidt glycosylation conditions employing appropriately protected L-rhamopyranosyl and D-glucopyranosylamine building blocks. The pentasaccharide synthesis was achieved using a [3+2] strategy with an overall yield of 5.2% through 11 linear steps from the monosaccharide building blocks **10** and **14**.

Keywords: *Aeromonas bestiarum*; O-specific polysaccharide; pentasaccharide synthesis; aquaculture; carbohydrate pesticide.

1. Introduction

Pathogenic bacteria from genus *Aeromonas* are widespread in various aquatic environments and the intestinal tract of different animals.[1, 2] These bacteria are known as pathogens causing a wide spectrum of diseases among reptiles, frogs, fish and some mammals (including humans).[3] For example, *Aeromonas sobria and Aeromonas caviae* can cause wound infections, meningitis, endocarditis, and osteomyelitis in humans[4-7]. *Aeromonas punctata* can cause red leg disease in frogs[3]. *Aeromona salmonicida and Aeromonas bestiarum* can cause motile aeromonad infection/motile aeromonad septicemia (MAI/MAS)[8, 9] and haemorrhagic septicaemia[10] in fish. The putative virulence factors of genus *Aeromonas* include: exoproteins (cytotoxins, entertoxins, haemolysins), proteases, the lipopolysaccharides (LPS) and other cytoarchitecture on the cell surface layers. Among them, lipopolysaccharides (LPS) containing O-specific polysaccharide (OPS) antigens have strong immunogenicity. In addition, LPS are important in cell specific recognition[11, 12]. Identification of the virulent factor and molecular mechanism behind the host-pathogen interaction, will be helpful to prevent and control such outbreak of bacterial infections in aquaculture[1-7].The identification and synthesis of O-specific polysaccharide (OPS) and their derivatives can shed more light on host-pathogen interaction ways inhibit such interaction which can potentially lead to a safe carbohydrate based bio-pesticide for application in aquaculture.



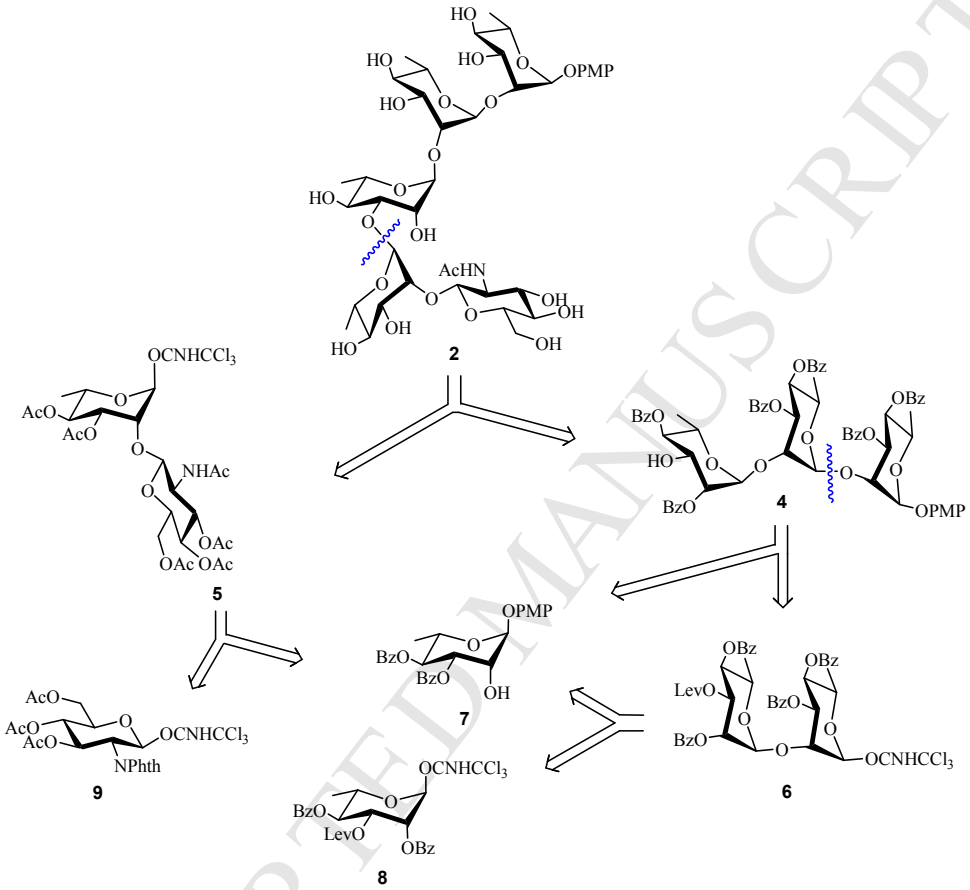
Scheme 1.The repeating unit of OPS from the water-borne bacteria *A. bestiarum* 207

Aeromonas bestiarum is a member of "*Aeromonas hydrophila"* complex, which belongs to mesophilic and motile aeromonads group bacterial species[13]. *Aeromonas bestiarum* 207 was isolated from diseased carp farmed in Poland and proved to be highly virulent for carp. High infection and/or death rates related to this bacterium are an increasing problem in commercial carp aquaculture[14].Recently, Anna Turska-Szewczuk and coworkers have isolated and characterized a OPS antigens (**scheme 1**) from *Aeromonas bestiarum* 207 as a pentasaccharide repeating unit[15],which consists of four α-linked L-Rha*p* in main chain with a terminal β-D-Glc*p*NAc unit. As a part of our ongoing research project to develop carbohydrate based pesticides from LPS O-antigens for controlling the genus *Aeromonas*, we studied the chemical synthesis of a pentasaccharide analogue **2** (Scheme 1) related to this repeating unit, and its tetrasaccharide derivative **3** with one L-Rha*p* missing in the reducing end of **2** (Scheme 1).

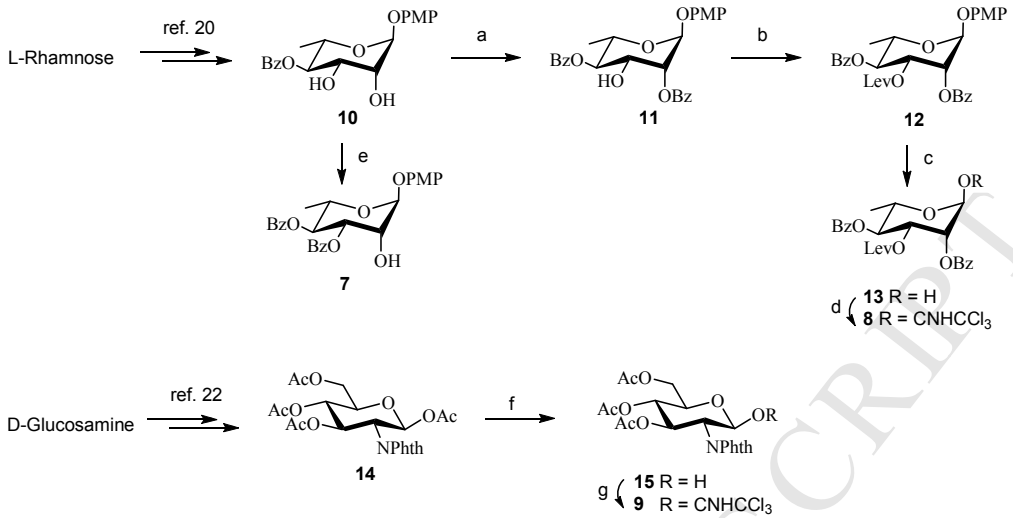
2. Results and Discussion

The retrosynthetic analysis of the target pentasaccharide **2** is as outlined in Scheme 2. Synthesis of the pentasaccharide **2** could be achieved through a convergent strategy involving [3+2] coupling of a trisaccharide acceptor **4** and a disaccharide donor **5**. The trisaccharide acceptor **4** could then be prepared by glycosylation of a α-(1→2)-linked disaccharide donor **6** and a rhamnosyl acceptor **7**[16-20]. Coupling of the rhamnosyl acceptor **7** with another

rhamnosyl donor **8** yield the disaccharide precursor for donor **6**. Rhamnosyl acceptor **7** could also be involved in the synthesis of the disaccharide donor **5** by coupling with a glucosyl donor **9**[21], followed by a two-step sequence involving selective de-1-O-aetylation and trichloroacetimidate formation.

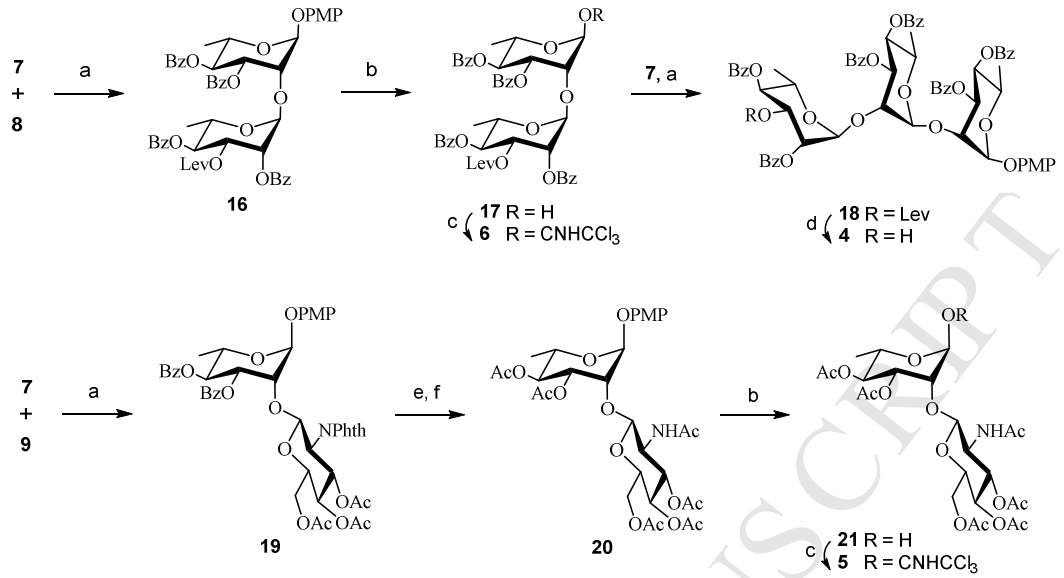


Scheme 2. Retrosynthetic analysis of the target pentasaccharide **2**.



Scheme 3. Synthesis of monosaccharide building blocks. Reagents and conditions: (a) triethyl orthobenzoate, *p*-TsOH, CH_2Cl_2, rt, 4 h; then 80% aq AcOH, 20 min, 74%; (b) levulinic acid, DCC, DMAP, CH_2Cl_2, rt, 4 h, 93%; (c) $Ce(NH_4)_2(NO_3)_6$, CH_3CN/H_2O (4:1 v/v), rt, 2 h, 76%; (d) CCl_3CN, DBU, CH_2Cl_2, 0 ^oC to rt, 2 h, 92%; (e) benzoyl chloride, DMAP, Py, CH_2Cl_2, −25 ^oC, 4 h, 69%; (f) ethylenediamine/CH_3COOH (1:1 v/v), THF, rt, 24 h, 72%; (g) CCl_3CN, DBU, CH_2Cl_2, 0 ^oC to rt, 2 h, 95%.

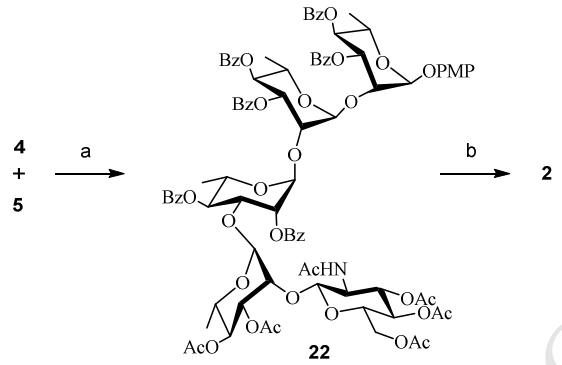
The synthesis commenced with preparation of L-rhamnosyl acceptor **7** and L-rhamnosyl donor **8**. These two L-rhamnosyl building blocks were obtained from the same intermediate **10**[22], which was synthesized through 6 steps from the commercially available L-rhamnose following the literature reported procedure. Treatment of **10** with triethyl orthobenzoate in the presence of *p*-TsOH formed an orthoester intermidiate, hydrolysis of which gave alcohol **11**[22].Staglich esterification of **11** with levulinic acid furnished compound **12**. Donor **8** was then synthesized in 67% yield from **12** using a two-step sequence involving selective de-1-O-aetylation followed by trichloroacetimidate formation. Starting from the commercially available D-glucosamine, glucosyl donor **9**[21] was synthesized over four steps including the ethylenediamine/CH_3COOH[23, 24] involved selective removal of anomeric acetate followed by reaction with trichloroacetonitrile and 1,8-diazobicyclo[5.4.0]undec-7-ene (DBU).



Scheme 4. Synthesis of trisaccharide acceptor **4** and disaccharide donor **5**. Reagents and conditions: (a) TMSOTf, CH_2Cl_2, 4 Å molecular sieves, –10 °C, 8 h, 66% for **16**, 75% for **18**, 65% for **19**; (b) $Ce(NH_4)_2(NO_3)_6$, CH_3CN/H_2O (4:1 v/v), rt, 2 h, 68% for **17**; 74% for **21**; (c) CCl_3CN, DBU, anhydrous CH_2Cl_2, 0 °C to rt, 2 h, 95% for **6**, 90% for **5**; (d) hydrazine acetate, DMF, rt, 4 h, 80%; (e) ethylenediamine, butanol, 85 °C, 8 h; (f) Ac_2O, Py, 24 h, 70% over two steps.

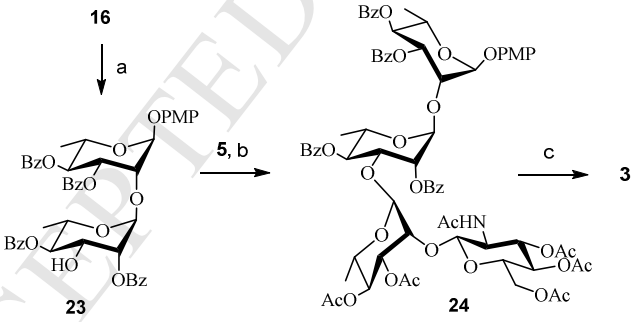
With the monosaccharide building blocks **7**, **8** and **9** in hand, we next focused on the construction of the trisaccharide acceptor **4** and disaccharide donor **5** (Scheme 4). Glycosylation of **7** with **8** promoted by TMSOTf afforded the corresponding α-(1→2)-linked disaccharide **16** in 65% yield. The cleavage of the 4-methoxyphenyl group in **16** with ceric ammonium nitrate (CAN), followed by reacting the resulting hemiacetal **17** with trichloroacetonitrile in the presence of DBU, afforded the disaccharide donor **6** in 67% yield over two steps. Further glycosylation of disaccharide donor **6** with the same L-rhamnosyl acceptor **7** in CH_2Cl_2 generated trisaccharide **18** smoothly in moderate yield (75%). Chemo-selective removal of Lev group on *C*-3″ by treating **18** with hydrazine acetate in DMF[25], gave trisaccharide acceptor **4** in 80% yield. Meanwhile, disaccharide donor **5** was synthesized following the route as shown in Scheme 4. The phthalimide (Phth) group at the C-2 position in glucosyl donor **9** lead the β-(1→2)-linkage to form disaccharide **19** in the glycosylation with L-rhamnosyl acceptor **7**. The 1,2-*trans* configuration was confirmed by ^{1}H NMR, in which the signal at 5.52 ppm showed a large coupling (d, 1H, *J* 8.4 Hz) for H-1-GluNPhth. The Phth group was then removed together with the other acetates when being treated with ethylenediamine in *n*-butanol [26]followed by per-acetylation gave disaccharide **20** in 70% yield over two steps. Converting **20** to the

corresponding disaccharide donor **5** was achieved in 63% yield over two steps following the same strategy to prepare disaccharide donor **6**.



Scheme 5. Synthesis of pentasaccharide **2**. Reagents and conditions: (a) TMSOTf, CH_2Cl_2, 4 Å molecular sieves, –10 °C, 8 h, 67%; (b) satd. NH_3–MeOH, 14 days, 80%.

Finally, the construction of the target pentasaccharide **2** was achieved as shown in Scheme 5. The Schmidt glycosylation of the trisaccharide acceptor **4** and the disaccharide donor **5** giving the fully protected pentasaccharide **22**. The successful glycosylation was supported by ^{13}C NMR, the signal at δ 97.6, 98.9, 99.3, 99.9 and 100.5 in which spectrum showed five anomeric carbons. Global deprotection of **22** in satd. NH_3–MeOH afforded the target pentasaccharide **2** in 80% yield. High resolution mass data confirmed the accomplishment of the target compound.



Scheme 6. Synthesis of tetrasaccharide **3**. Reagents and conditions: (a) hydrazine acetate, DMF, rt, 4 h, 79%; (b) TMSOTf, CH_2Cl_2, 4 Å molecular sieves, –10 °C, 8 h, 73%; (c) satd. $MeNH_2$–MeOH, 7 days, 80%.

During the synthesis of the target pentasaccharide **2**, we were able to construct the one-rhamnose missing analogue (tetrasaccharide **3**) at the reducing end of **2** using the key intermediate **16**. Chemo-selective removal of Lev protecting group in **16** following the same procedure to synthesis trisaccharide acceptor **4** giving disaccharide acceptor **23** in high yield. Schmidt glycosylation of acceptor **23** with the disaccharide donor 5 afforded fully protected

tetrasaccharide **24**. Final complete deprotection of **24** following the same strategy to obtain **2** giving the tetrasaccharide **3** in high yield.

3. Conclusion

In summary, we have reported a convergent total synthesis of a pentasaccharide analogue **2** and its tetrasccharide derivative **3** related to the repeating unit of O-specific polysaccharide isolated from water-borne bacteria *Aeromonasbestiarum* 207. The efficient synthesis features the preparation of a key disaccharide intermediate **16** containing an orthogonal protecting group (Lev), which could be easily converted to the disaccharide donor **6** or the disaccharide acceptor **23**. This study will enrich the O-specific polysaccharide library for our carbohydrate pesticide development project.

4. Experimental Procedures

4.1 Materials and methods

^{1}H and ^{13}C NMR spectra were recorded with Bruker AVANCE600 spectrometers (^{1}H NMR-300 MHz; ^{13}C NMR-75 MHz) for solutions in $CDCl_3$ or D_2O. Internal references: TMS (δ 0.000 ppm for 1H), $CDCl_3$ (δ 77.00 ppm for 13C). Data for ^{1}H NMR are reported as follows: chemical shift, integration, multiplicity (app = apparent, parobsc = partially obscure, ovrlp = overlapping, s = singlet, d = doublet, dd = doublet of doublet, t = triplet, q = quartet, m= multiplet) and coupling constants in Hertz. All ^{13}C NMR spectra were recorded with complete proton decoupling. High-resolution mass spectra (HRMS) were recorded with Bruker Daltonics Bio-TOF-Q III (ESIMS). Thin layer chromatography (TLC) was performed on silica gel HF254 plates, detected by charring using 15% (v/v) H_2SO_4 in MeOH or by means of a UV detector. All commercially available reagents were used without further purification, and purchased at Sinopharm, Shanghai, China. All reactions were monitored by TLC or by iodine fuming detection. Column chromatography was conducted on a silica gel plug (200-300 mesh) using a mixture of ethyl actetate (EtOAc) and petroleum ether (bp 60-90 °C) as the eluent.

4.2 Synthetic procedures

*4.2.1. p-Methoxyphenyl 3,4-di-O-benzoyl-α-L-rhamnopyranoside (**7**)*

To a solution of **10** (5.00 g, 13.35 mmol) in CH_2Cl_2 (200 mL) was added pyridine (20 ml) and DMAP (0.05 g, 0.41 mmol) at −25° C in N_2 atmosphere. The reaction mixture was stirred for 30 min, and BzCl (1.53 mL, 13.30 mmol) in CH_2Cl_2 (25 ml) was added dropwise. The resulting mixture was stirred for another 4 h, at which time TLC (petroleum ther-ethyl acetate 4:1) indicated the completion of reaction. The reaction was then quenched with cooled H_2O (200 mL), and extracted with CH_2Cl_2 (3 × 150 mL). The organic phases were combined, dried over Na_2SO_4, and concentrated. The residue was purified by flash column chromatography (petroleum ether/ethyl acetate 8:1) to give **7** (4.48 g, 69%) as colorless syrup. $[\alpha]_D^{22}$ −17.6° (*c* 1.0, $CHCl_3$).^{1}H NMR (300 MHz, $CDCl_3$): *δ* 7.99−7.94 (m, 4H, Ar*H*), 7.53−7.47 (m, 2H, Ar*H*), 7.39−7.34 (m, 4H, Ar*H*), 7.12−7.06 (m, 2H, Ar*H*), 6.89−6.85 (m, 2H, Ar*H*), 5.79 (dd, 1H, J_1= 3.5 Hz, J_2= 10.0 Hz, H-3), 5.66 (t, 1H, *J* = 9.8 Hz, H-4), 5.51 (d, *J* = 1.7 Hz, H-1), 4.85 (dd, 1H, J_1 = 2.0 Hz, J_2= 3.03 Hz, H-2), 4.28−4.18 (m, 1H, H-5), 3.79 (s, 3H, OCH_3), 1.29 (d, 3H, *J* = 6.3 Hz, H-6); ^{13}C NMR (75 MHz, $CDCl_3$) : *δ* 165.4 (*C*OPh), 165.3 (*C*OPh), 154.8, 149.9, 132.9, 132.8, 129.4, 129.3, 129.0, 128.9, 128.1, 128.0, 117.25, 114.4, 98.1 (C-1), 72.1, 71.1, 69.2, 66.9, 55.3 (O*C*H_3), 17.2 (C-6). ESI-HRMS $[M + Na]^+$calculated for $C_{27}H_{26}NaO_8$ 512.1525 found 512.1521.

*4.2.2. p-Methoxyphenyl 2,4-di-O-benzoyl-α-L-rhamnopyranoside (**11**)*

To a solution of **10** (5.00 g, 13.35 mmol) in CH_2Cl_2 (100 mL) was added triethylorthobenzoate (7.59 ml, 33.38 mmol) and *p*-TsOH (2.00 g, 0.12 mmol) at room temperature in N_2 atmosphere. The reaction mixture was stirred for 4 h, at which time TLC (petroleum ether-ethyl acetate 4:1) indicated the completion of reaction, the reaction mixture was concentrated and added 80% aq. AcOH 100 ml, the resulting mixture was stirred for another 1 h, atwhich time TLC (petroleum ether-ethyl acetate 5:1) indicated the completion of reaction. The reaction was then quenched withH_2O (200 mL), and extracted with CH_2Cl_2 (3 ×75 mL). Theorganic phases were washed with sated aq $NaHCO_3$ (250 ml) and brine (2 × 200 ml), dried over anhydrous sodium sulfate, filtered, and concentrated in vacuo. The residue was purified by flash column chromatography (petroleum ether/ethyl acetate 6:1) to give **11** (4.45 g, 74%) as colorless syrup. $[\alpha]_D^{22}$ −14.6° (*c* 1.0, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): *δ* 8.15−8.06 (m, 4H, Ar*H*), 7.65−7.57 (m, 2H, Ar*H*), 7.52−7.44 (m, 4H, Ar*H*), 7.08−7.02 (m, 2H, Ar*H*), 6.88−6.83 (m, 2H, Ar*H*), 5.58−5.56 (m, 2H, H-1, H-2), 5.34 (t, 1H, *J* = 9.9 Hz, H-4), 4.54−4.50 (m, 1H, H-3), 4.25−4.20 (m, 1H, H-5), 3.78 (s, 3H, OC*H*$_3$), 2.56 (d, 1H, *J* = 6.8 Hz, O*H*), 1.31 (d, 3H, *J* = 6.2 Hz, H-6); ^{13}C NMR (75 MHz, $CDCl_3$) : *δ* 166.7 (*C*OPh), 165.6 (*C*OPh), 154.9, 149.8, 133.2, 133.2, 129.6, 129.5, 129.1, 129.0, 128.3, 128.2, 117.4, 114.4, 96.2 (C-1), 75.2, 72.8, 68.6, 66.6, 55.3 (O*C*H_3), 17.3 (C-6). ESI-HRMS $[M + Na]^+$calculated for $C_{27}H_{26}NaO_8$ 512.1525 found 512.1520.

*4.2.3. p-Methoxyphenyl 2,4-di-O-benzoyl-3-O-levulinoyl-α-L-rhamnopyranoside (**12**)*

To a solution of **11** (4.00 g, 8.36 mmol) in CH_2Cl_2 (100 mL) was added DCC (3.45 g, 16.72 mmol) and DMAP (0.05 g, 0.41 mmol) at 0° C in N_2 atmosphere. The reaction mixture was stirred for 10 min, and levulinic acid (1.46 g 12.54 mmol) in CH_2Cl_2 (20 ml) was added dropwise. The resulting mixture was stirred for another 3 h, at which time TLC (petroleum ether-ethyl acetate 1:1) indicated the completion of reaction. The reaction was then filtered through a pad of celite, and the filtrate was concentrated. The residue was purified by flash column

chromatography (petroleum ether/ethyl acetate 1:1) to give **3** (4.44 g, 93%) as colorless syrup. $[\alpha]_D^{22}$ 37.1° (*c* 1.0, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.15−8.01 (m, 4H, Ar*H*), 7.65−7.43 (m, 6H, Ar*H*), 7.09−7.04 (m, 2H, Ar*H*), 6.87−6.82 (m, 2H, Ar*H*), 5.81 (dd, 1H, J_1 = 3.4 Hz, J_2 = 10.1 Hz, H-3), 5.71 (dd, 1H, J_1 = 1.9 Hz, J_2 = 3.4 Hz, H-2), 5.58− 5.51 (m, 2H, H-1, H-4), 4.29−4.20 (m, 1H, H-5), 3.77 (s, 3H, OCH_3), 2.64−2.34 (m, 4H, COCH_2CH_2COCH_3), 1.93 (s, 3H, $COCH_2CH_2COCH_3$), 1.31 (d, *J* = 6.2 Hz, H-6); ^{13}C NMR (75 MHz, $CDCl_3$) :δ 205.4 (CH_2COCH_3), 171.3 (*C*(O)OCH_2CH_2), 165.4 (*C*OPh), 165.2 (*C*OPh), 154.9, 149.7, 133.2, 133.0, 129.6, 129.5, 128.9, 128.3, 128.1, 117.3, 114.3, 96.1 (C-1), 71.3, 70.1, 69.0, 67.0, 55.3 (O*C*H_3), 37.5, 29.0, 27.6, 17.3 (C-6). ESI-HRMS $[M + Na]^+$ calculated for $C_{32}H_{32}NaO_{10}$ 599.1898 found 599.1895.

*4.2.4. 2,4-di-O-benzoyl-3-O-levulinoyl-α-L-trichloroacetimidate (**8**)*

To a solution of **12** (4.00 g, 6.93 mmol) in CH_3CN:H_2O (4:1 v/v) 75 ml was added $Ce(NH_4)_2(NO_3)_6$ (15.20 g, 27.75 mmol) at room temperature. The resulting mixture was stirred for another 2 h, at which time TLC (petroleum ether-ethyl acetate 4:1) indicated the completion of reaction. The reaction was then quenched with cooled H_2O (200 mL), and extracted with CH_2Cl_2 (3 × 150 mL). The organic phases were combined, dried over Na_2SO_4, and concentrated. The residue was purified by flash column chromatography (petroleum ether/ethyl acetate 3:1) to give **13** (2.30 g, 76%). Subsequently dissolving **13** in CH_2Cl_2 (50 ml) and added trichloroacetonitrile (1.23 ml, 12.2 mmol) and DBU (0.05mL) in N_2 atmosphere. The reaction mixture was stirred for 2 h under this condition, and then concentrated. The residue was subjected to flash column chromatography (petroleum ether/ethyl acetate 4:1) to give **8** (2.88 g 92%) as colorless syrup. $[\alpha]_D^{22}$ 16.6° (*c* 1.0, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.80 (s, 1H, OCN*H*CCl_3), 7.98−7.88 (m, 4H, Ar*H*), 7.53−7.46 (m, 2H, Ar*H*), 7.40−7.32 (m, 4H, Ar*H*), 6.32 (d, 1H, *J* = 1.7 Hz, H-1), 5.76 (dd, 1H, J_1 = 3.4 Hz, J_2 = 10.2 Hz, H-3), 5.68−5.59 (m, 2H, H-2, H-4), 4.37−4.28 (m, 1H, H-5), 2.73 (s, 4H, OCOCH_2CH_2COCH_3), 2.14 (s, 3H, $OCOCH_2CH_2COCH_3$), 1.37 (d, 3H, *J* = 6.2 Hz, H-6); ^{13}C NMR (75 MHz, $CDCl_3$) :δ 205.7 (*C*OCH_3), 171.4*C*(O)OCH_2CH_2, 165.6*C*OPh, 165.4*C*OPh, 159.9 (O*C*NHCH_3), 133.4, 133.2, 129.7, 129.7, 129.0, 129.0, 128.4, 128.3, 94.7(C-1), 90.7, 70.8, 69.6, 69.5, 68.6, 37.7, 29.6, 27.8, 17.6 (C-6). ESI-HRMS $[M + Na]^+$ calculated for $C_{27}H_{26}NNaO_9$ 636.0576 found 636.0572.

*4.2.5. 3,4,6-tri-O-acetyl-2-deoxy-2-phthalimido-D-glucopyranoside (**15**)*

To a solution of ethylene diamine : CH_3COOH (0.85 ml : 0.85 ml v/v) in THF (250 ml) at 0^{o}C and compound **14** (5.00 g, 10.47 mmol) in THF (50 ml) was added dropwise. The resulting mixture was stirred for 24 h at room temperature, at which time TLC (petroleum ether-ethyl acetate 3:1) indicated the completion of reaction. The reaction was then quenched with 1M HCl (200 mL), and extracted with CH_2Cl_2 (3 × 100 mL). The organic phases were washed with sated aq. $NaHCO_3$ (200 ml), and brine (2 × 200 ml), dried over anhydrous sodium sulfate, filtered, and concentrated in vacuo. The residue was purified by flash column chromatography (petroleum ether/ethyl acetate 4:1) to give **15** (3.43 g, 72%) as colorless syrup. $[\alpha]_D^{22}$ 16.7° (*c* 1.0, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.90−7.82 (m, 2H, Ar*H*), 7.78−7.71 (m, 2H, Ar*H*), 5.84 (dd, 1H, J_1 = 9.1 Hz, J_2 = 10.7 Hz, H-3), 5.63 (t, 1H, *J* = 7.2 Hz, H-4), 5.17 (dd, 1H, J_1 = 9.2 Hz, J_2 = 10.1 Hz, H-2), 4.32−4.16 (m, 3H, H-1, H-6), 3.95−3.90 (m, 1H, H-5), 3.48 (d, 1H, *J* = 6.4 Hz, O*H*),

1.86, 2.03, 2.10 (3s, 9H, OCOC*H*$_3$); ^{13}C NMR (75 MHz, $CDCl_3$) : δ 170.4 (*C*OCH$_3$), 169.7 (*C*OCH$_3$), 169.1 (*C*OCH$_3$), 134.0, 131.0, 123.3, 92.3 (C-1), 71.7, 70.1, 68.6, 61.7, 55.7 (C-6), 20.4 (CO*C*H$_3$), 20.2 (CO*C*H$_3$), 20.0 (CO*C*H$_3$). ESI-HRMS [M + Na]$^+$ calculated for $C_{20}H_{21}NNaO_{10}$ 458.1063 found 458.1058.

*4.2.6. 3,4,6-tri-O-acetyl-2-deoxy-2-phthalimido-D-trichloroacetimidate (**9**)*

To a solution of **15** (3.00 g, 6.89 mmol) in CH_2Cl_2 (50 mL) at 0^{o}C with in N_2 atmosphere was added trichloroacetonitrile (1.72 ml, 17.2mmol) and DBU (0.05 mL). The reaction mixture was stirred for 2 h under this condition, and then concentrated. The residue was subjected to flash column chromatography (petroleum ether/ethyl acetate 4:1) to give **15** (3.75 g 95%) which was immediately used in the synthesis of 1**9.**

*4.2.7. p-Methoxyphenyl 2,4-di-O-benzoyl-3-O-levulinoyl-α-L-rhamnopyranosyl-(1→2)-3,4-di-O-benzoyl-α-L-rhamnopyranoside (**16**)*

A mixture of **8** (2.50 g, 4.18 mmol), **7** (2.00 g, 4.18 mmol) and 4 Å MS (0.5 g) in anhydrous CH_2Cl_2 (70 mL) was stirred at rt for 30 min in N_2 atmosphere, before it was cooled to 0^o C, then TMSOTf (10 uL) was added. The mixture was stirred at same temperature for 8 h, and then neutralized with Et_3N. The mixture was diluted with CH_2Cl_2 (10 mL), filtered through a pad of celite, and the filtrate was concentrated. The residue was purified by flash column chromatography (petroleum ether/ethyl acetate 4:1) to give **16** (2.53 g, 66%) as colorless syrup. $[\alpha]_D^{22}$ 11.9° (*c* 1.0, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.08–7.95 (m, 8H, Ar*H*), 7.62− 7.56 (m, 2H, Ar*H*), 7.52−7.29 (m, 10H, Ar*H*), 7.12−7.08 (m, 2H, Ar*H*), 6.89−6.86 (m, 2H, Ar*H*), 5.96 (dd, 1H, J_1 = 3.5 Hz, J_2 = 10.0 Hz, H-3), 5.76−5.73 (m, 2H, H-2, H-3), 5.68 (t, 1H, *J* = 9.8 Hz, H-4), 5.55 (d, 1H, *J* = 1.6 Hz, H-1), 5.48 (t, 1H, *J* = 9.9 Hz, H-4), 5.14 (s, 1H, H-1), 4.47 (m, 1H, H-2), 4.28−4.22 (m, 2H, H-5, H-5), 3.79 (s, 3H, OC*H*$_3$), 2.59−2.38 (m, 4H, OCOC*H*$_2$C*H*$_2$COCH$_3$), 1.94 (s, 3H, OCOCH$_2$CH$_2$COC*H*$_3$), 1.36 (d, 3H, *J* = 6.3 Hz, H-6), 1.30 (d, 3H, *J* = 6.3 Hz, H-6); ^{13}C NMR (75 MHz, $CDCl_3$) : δ 205.4 (*C*OCH$_3$), 171.0 (*C*(O)OCH$_2$CH$_2$), 165.5 (*C*OPh), 165.4 (*C*OPh), 165.1 (*C*OPh), 164.7 (*C*OPh), 154.9, 149.8, 133.0, 132.8, 132.7, 129.8, 129.6, 129.5, 129.4, 129.1, 128.9, 128.6, 128.1, 128.1, 127.9, 117.3, 114.3, 99.1 (C-1), 97.4 (C-1), 75.9, 71.5, 71.3, 70.6, 69.0, 68.8, 67.3, 67.2, 55.3 (O*C*H$_3$), 37.5, 29.0, 27.7, 17.3 (C-6), 17.2 (C-6). ESI-HRMS [M+NH$_4$]$^+$ calculated for $C_{52}H_{54}NO_{16}$ 948.3437, found 948.3431.

*4.2.8. 2,4-di-O-benzoyl-3-O-levulinoyl-α-L-rhamnopyranosyl-(1→2)-3,4-di-O-benzoyl-α-L-trichloroacetimidate (**6**)*

To a solution of **16** (1.50 g, 1.61 mmol) in CH_3CN : H_2O (4:1 v/v) 25 ml was added $Ce(NH_4)_2(NO_3)_6$ (3.70 g, 6.45 mmol) at room temperature. The resulting mixture was stirred for another 2 h, at which time TLC (petroleum ether-ethyl acetate 5:1) indicated the completion of reaction. The reaction was then quenched with cooled H_2O (50 mL), and extracted with CH_2Cl_2 (3 ×25 mL). The organic phases were combined, dried over Na_2SO_4, and concentrated. The residue was purified by flash column chromatography (petroleum ether/ethyl acetate 5:1) to give **17** (0.93 g 68%). Subsequently dissolving **17** in CH_2Cl_2 20 ml and added trichloroacetonitrile (0.28 mL, 2.82 mmol) and DBU (0.02 mL). The reaction mixture was stirred for 2 h under this condition, and then concentrated. The residue was subjected to flash column chromatography

(petroleum ether/ethyl acetate 7:1) to give ***6*** (980 mg 95%) which was immediately used in the synthesis of **18.**

*4.2.9. p-Methoxyphenyl 2,4-di-O-benzoyl-3-O-levulinoyl-α-L-rhamnopyranosyl-(1→2)-2,4-di-O-benzoyl-α-L-rhamnopyranosyl-(1→2)-3,4-di-O-benzoyl-α-L-rhamnopyranoside (**18**)*

A mixture of **7** (572 mg, 1.20 mmol), **6** (980 mg, 1.01 mmol) and 4 Å MS (0.4 g) in anhydrous CH_2Cl_2 (30 mL) was stirred at room temperature for 30 min in N_2 atmosphere, before it was cooled to -10^o C, then TMSOTf (10 uL) was added. The mixture was stirred at same temperature for 8 h, and then neutralized with Et_3N. The mixture was diluted with CH_2Cl_2 (20 mL), filtered through a pad of celite, and the filtrate was concentrated. The residue was purified by flash column chromatography (petroleum ether/ethyl acetate 4:1) to give **18** (890 mg, 75%) as colorless syrup. $[\alpha]_D^{22}$ 37.9° (*c* 1.0, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.02−7.94 (m, 12H, Ar*H*), 7.63−7.53 (m, 2H, Ar*H*), 7.51−7.30 (m, 14H, Ar*H*), 7.24−7.17 (m, 2H, Ar*H*), 7.17−7.10 (m, 2H, Ar*H*), 6.92−6.82 (m, 2H, Ar*H*), 5.90−5.80 (m, 2H, H-3, H-3), 5.71−5.57 (m, 4H, H-2, H-3, H-4, H-4), 5.60 (s, 1H, H-1), 5.32 (t, 1H, *J* = 9.7 Hz, H-4), 5.17 (d, 1H, *J* = 1.4 Hz, H-1), 4.79 (s, 1H, H-1), 4.63−4.51 (m, 1H, H-2), 4.40−4.33 (m, 1H, H-2), 4.32−4.23 (m, 2H, H-5, H-5), 4.01−3.99 (m, 1H, H-5), 3.79 (s, 3H, OCH_3), 2.60−2.34 (2m, 4H, OCOCH_2CH_2CH$_3$), 1.92 (s, 3H, COCH_3), 1.38 (d, 3H, *J* = 6.3 Hz, H-6), 1.34 (d, 3H, *J* = 6.3 Hz, H-6), 0.97 (d, 3H, *J* = 6.26 Hz, H-6); ^{13}C NMR (75 MHz, $CDCl_3$) : δ 205.4 (*C*OCH$_3$), 170.9 (*C*(O)OCH$_2$CH$_2$), 165.6 (*C*OPh), 165.4 (*C*OPh), 165.1 (*C*OPh), 165.1 (*C*OPh), 164.6 (*C*OPh), 154.9, 149.9, 133.0, 133.0, 132.9, 132.8, 132.7, 129.6, 129.5, 129.4, 129.3, 129.1, 129.0, 128.9, 128.8, 128.7, 128.1, 128.1, 128.0, 128.0, 117.3, 114.4, 100.3 (C-1), 99.0(C-1), 97.7 (C-1), 71.4, 71.2, 70.2, 69.8, 68.8, 67.4, 67.1, 55.3 (O*C*H$_3$), 37.4, 29.0, 27.6, 17.3 (C-6), 17.3 (C-6), 16.8 (C-6). ESI-HRMS $[M+NH_4]^+$ calculated for $C_{71}H_{69}NO_{22}$ 1302.4540 found 1302.4531.

*4.3.0. p-Methoxyphenyl 2,4-di-O-benzoyl-α-L-rhamnopyranosyl-(1→2)-2,4-di-O-benzoyl-α-L-rhamnopyranosyl-(1→2)-3,4-di-O-benzoyl-α-L-rhamnopyranoside (**4**)*

To a solution of 1**6** (800 mg, 0.62 mmol) in DMF (25 mL) was added hydrazine acetate (243 mg, 3.1 mmol) at room temperature in N_2 atmosphere. The reaction mixture was stirred for 4 h, at which time TLC (petroleum ether/ethyl acetate 2:1) indicated the completion of reaction. The reaction was then quenched with sat. aq. NH_4Cl (50 mL) and then extracted with dichloromethane (100 mL). The organic phase was washed with sat. aq. $NaHCO_3$ (2 x 50 mL) and water (50 mL). After the solution was dried over Na_2SO_4, the solvent was removed under reduced pressure and the residue was purified by flash chromatography (petroleum ether/ethyl acetate 3:1) to give **4** (589 mg, 80%) as colorless syrup. $[\alpha]_D^{22}$ 38.4° (*c* 1.0, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ8.09−7.93 (m, 12H, Ar*H*), 7.62−7.52 (m, 2H, Ar*H*), 7.62−7.32 (m, 14H, Ar*H*), 7.21−7.04 (m, 4H, Ar*H*), 6.91−6.86 (m, 2H, Ar*H*), 5.89−5.79 (m, 2H, H-3, H-3), 5.68 (t, 1H, *J* = 9.7 Hz, H-4), 5.56 (s, 1H, H-1), 5.59−5.53 (t, 1H, *J* = 9.8 Hz, H-4), 5.43 (dd, 1H, J_1 = 1.6 Hz, J_2 = 3.3 Hz, H-2), 5.16 (s, 1H, H-1), 5.16−5.09 (t, 1H, *J* = 11.7 Hz, H-4), 4.78 (d, 1H, *J* = 1.3 Hz, H-1), 4.56 (dd, 1H, J_1 = 2.1 Hz, J_2 = 3.0 Hz, H-2), 4.36−4.32 (m, 2H, H-2, H-3), 4.30−4.22 (m, 2H, H-5, H-5), 4.06−3.96 (m, 1H, H-5), 3.80 (s, 3H, OCH_3), 2.34 (d, 1H, *J* = 7.3 Hz, O*H*), 1.37 (d, 3H, *J* = 6.2 Hz, H-6), 1.32 (d, 3H, *J* = 6.2 Hz, H-6), 0.97 (d, 3H, *J* = 6.2 Hz, H-6); ^{13}C NMR (75 MHz, $CDCl_3$) : δ 166.6 (*C*OPh), 165.6 (*C*OPh), 165.4 (*C*OPh), 165.2 (*C*OPh), 165.1 (*C*OPh), 165.0 (*C*OPh), 154.9, 149.9, 133.0, 132.9, 132.8, 132.8, 132.7, 129.5, 129.5, 129.3,

129.1, 129.0, 128.9, 128.7, 128.1, 128.0, 128.0, 117.3, 114.4, 100.5 (C-1), 98.9 (C-1), 97.7 (C-1), 76.5, 74.7, 72.4, 71.4, 71.3, 71.2, 70.1, 68.3, 67.4, 67.1, 66.7, 55.3 (O*C*H$_3$), 17.4 (C-6), 17.3 (C-6), 16.9 (C-6). ESI-HRMS [M+NH$_4$]$^+$ calculated for $C_{67}H_{66}NO_{20}$ 1204.4178 found 1204.4174.

4.3.1. p- Methoxyphenyl 3,4,6-tri-O-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→2)

*-3,4-di-O-benzoyl-α-L-rhamnopyranoside (**19**)*

A mixture of **10** (1.80 g, 3.47 mmol), **9** (2.00 g, 3.45 mmol) and 4 Å MS (0.5 g) in anhydrous CH_2Cl_2 (40 mL) was stirred at rt for 30 min in N_2 atmosphere, before it was cooled to −10^o C, then TMSOTf (10 uL) was added. The mixture was stirred at same temperature for 8 h, and then neutralized with Et_3N. The mixture was diluted with CH_2Cl_2 (10 mL), filtered through a pad of celite, and the filtrate was concentrated. The residue was purified by flash column chromatography (petroleum ether/ethyl acetate 4:1) to give **19** (2.00 g 65%) as colorless syrup. $[\alpha]_D^{22}$ −15.5° (*c* 1.0, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.80−7.77 (m, 2H, Ar*H*), 7.54−7.51 (m, 4H, Ar*H*), 7.45−7.28 (m, 6H, Ar*H*), 7.11−7.04 (m, 4H, Ar*H*), 6.87−6.82 (m, 2H, Ar*H*), 5.71 (s, 1H, H-1-Rham*p*), 5.70 (t, 1H, *J* = 10.5 Hz, H-3-Glc*p*), 5.52 (d, 1H, *J* = 8.4 Hz, H-1-Glc*p*), 5.49 (dd, 1H, J_1 = 3.3 Hz, J_2 = 10.2 Hz, H-3-Rham*p*), 5.33 (t, 1H, *J* = 9.9 Hz, H-4-Rham*p*), 5.08 (t, 1H, *J* = 10.1 Hz, H-4-Glc*p*), 4.61 (m, 1H, H-2-Rham*p*), 4.48 (dd, 1H, J_1 = 8.4 Hz, J_2 = 10.5 Hz, H-2-Glc*p*), 4.19 (dd, 1H, J_1 = 6.1 Hz, J_2 = 12.2 Hz, H-6-Glc*p*), 4.11−4.06 (m, 2H, H-5, H-6), 3.83−3.78 (m, 1H, H-5), 3.77 (s, 3H, OC*H*$_3$), 1.80, 1.84, 1.98 (3s, 9H, OCOC*H*$_3$), 1.20 (d, 3H, *J* = 6.2 Hz, H-6); ^{13}C NMR (75 MHz, $CDCl_3$) :δ 170.2 (*C*OCH$_3$), 169.6 (*C*OCH$_3$), 169.0 (*C*OCH$_3$), 167.0 (Phth), 165.9 (*C*OPh), 164.7 (*C*OPh), 154.7, 149.9, 133.5, 132.6, 132.4, 130.8, 129.1, 129.1, 128.7, 127.9, 123.1, 117.1, 114.3, 98.9 (C-1), 97.6 (C-1), 71.9, 70.6, 70.1, 68.7, 66.8, 61.8, 55.3, 54.2 (O*C*H$_3$), 20.2 (CO*C*H$_3$), 20.0 (CO*C*H$_3$), 17.2 (C-6). ESI-HRMS [M+NH$_4$]$^+$ calculated for $C_{47}H_{49}N_2O_{17}$ 913.3026 found 913.3024.

4.3.2. p-Methoxyphenyl 3,4,6-tri-O-acetyl-2-deoxy-2-acetamido-β-D-glucopyranosyl-(1→2)-

*3,4-di-O-acetyl-α-L-rhamnopyranose (**20**)*

Ethylene diamine (1.49 ml, 22.3 mmol) was added to a suspension of compound **19** (2.00 g, 2.23 mmol) in n-butyl alcohol (100 mL). The reaction mixture was stirred for 8 h at 85^o C. The solvent was removed under reduced pressure and repeatedly co-distilled with toluene (3 x 150 mL) and ethanol (2 x 100 mL). The resulting oil was dissolved in pyridine (50 mL), treated with acetic anhydride (10 mL) and stirred for 24 h at room temperature. The reaction was poured into ice-water (200 mL) and then extracted with dichloromethane (200 mL). The organic phase was washed with 0.1 M HCl (150 mL), sat. aq. $NaHCO_3$ (2 x 100 mL) and water (100 mL). After the solution was dried over Na_2SO_4, the solvent was removed under reduced pressure and the residue was purified by flash chromatography (petroleum ether/ethyl acetate 1:2) to give **20** (1.60 g 70%) as colorless syrup. $[\alpha]_D^{22}$ −7.7° (*c* 1.0, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.98−6.95 (m, 2H, Ar*H*), 6.82−6.74 (m, 2H, Ar*H*), 5.87 (d, 1H, *J* = 7.6 Hz, H-1-Glc*p*), 5.65 (dd, 1H, J_1 = 9.3 Hz, J_2 = 10.5 Hz, H-3-Glc*p*), 5.43 (d, 1H, J = 1.7 Hz, H-1-Rham*p*), 5.39 (dd, 1H, J_1 = 3.1 Hz, J_2 = 10.2 Hz, H-3-Rham*p*), 5.10−5.03 (m, 2H, H-2-Glc*p*, H-4), 4.95 (t, 1h, *J* = 9.9 Hz, H-4), 4.22−4.16 (m, 2H, H-2-Rham*p*, H-6-Glc*p*), 4.03−3.98 (dd, 1H, J_1 = 2.1 Hz, J_2 = 12.1 Hz, H-6-Glc*p*), 3.99−3.89 (m, 1H, H-5), 3.73 (s, 3H, OC*H*$_3$), 3.50−3.41 (m, 1H, H-5), 1.88, 1.97, 1.98, 2.00, 2.09 (5s, 18H, OCOC*H*$_3$), 1.14 (d, 3H, *J* = 6.2 Hz, H-6); ^{13}C NMR (75 MHz, $CDCl_3$) :δ 170.5 (*C*OCH$_3$), 170.2 (*C*OCH$_3$), 170.1 (*C*OCH$_3$), 170.0 (*C*OCH$_3$), 169.5 (*C*OCH$_3$), 169.2(*C*OCH$_3$), 154.6, 149.6, 133.8,

131.5, 123.0, 117.0, 114.3, 100.1 (C-1), 97.4 (C-1), 75.7, 71.3, 70.8, 70.5, 68.8, 66.6, 61.8, 55.7, 55.2, 22.9 (CO*C*H_3), 22.3 (CO*C*H_3), 20.4 (CO*C*H_3), 20.3 (CO*C*H_3), 20.2 (CO*C*H_3), 20.1 (CO*C*H_3), 17.0 (C-6). ESI-HRMS $[M+NH_4]^+$ calculated for $C_{31}H_{45}N_2O_{16}$ 701.2764 found 701.2758.

*4.3.3. 3,4,6-tri-O-acetyl-2-deoxy-2-acetamido-β-D-glucopyranosyl-(1→2)-3,4-di-O-acetyl-L-trichloroacetimidate (**5**)*

To a solution of **20** (1.50 g, 2.18 mmol) in CH_3CN : H_2O (4:1 v/v) 25 ml was added $Ce(NH_4)_2(NO_3)_6$ (4.90 g, 8.75 mmol) at room temperature. The resulting mixture was stirred for another 2 h, at which time TLC (petroleum ether-ethyl acetate 4:1) indicated the completion of reaction. The reaction was then quenched with cooled H_2O (50 mL), and extracted with CH_2Cl_2 (3 ×25 mL). The organic phases were combined, dried over Na_2SO_4, and concentrated. The residue was purified by flash column chromatography (petroleum ether/ethyl acetate 1:3) to give **21** (0.90 g, 70%). Subsequently dissolving **21** in CH_2Cl_2 30 ml and added DBU (0.1 ml) with N_2 protection. The reaction mixture was stirred for 12 h under this condition, and then filtered through a pad of celite, and the filtrate was concentrated. The residue was subjected to flash column chromatography (petroleum ether/ethyl acetate 1:4) to give **5** (2.85 g 90%) as colorless syrup. $[\alpha]_D^{22}$ −14.3° (*c* 1.0, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.66 (s, 1H, OCN*H*CCl_3), 6.27 (d, 1H, *J* = 2.0 Hz, H-1-Rham*p*), 5.83 (d, 1H, *J* = 7.4 Hz, H-1-Glc*p*), 5.67 (dd, 1H, J_1 = 9.2 Hz, J_2 = 10.5 Hz, H-3-Glc*p*), 5.25 (dd, 1H, J_1 = 3.0 Hz, J_2 = 10.3 Hz, H-3-Rham*p*), 5.16−5.09 (m, 2H, H-4-Rham*p*, H-4-Glc*p*), 5.00 (t, 1H, *J* = 9.8 Hz, H-2-Glc*p*), 4.23 (m, 2H, H-2-Rham*p*, H-6-Glc*p*), 4.15-4.05 (m, 1H, H-6-Glc*p*), 3.74−3.68 (m, 1H, H-5), 3.46−3.37 (m, 1H, H-5), 2.08, 2.03, 2.03, 2.02, 1.99, 1.98 (6s, 18H, OCOC*H*$_3$), 1.22 (d, 3H, *J* = 6.2 Hz, H-6-Rham*p*); ^{13}C NMR (75 MHz, $CDCl_3$) :δ 170.4 (*C*OCH_3), 170.3 (*C*OCH_3), 170.0 (*C*OCH_3), 169.9 (*C*OCH_3), 169.5 (*C*OCH_3), 169.1 (*C*OCH_3), 159.7 (O*C*NHCH$_3$), 99.9 (C-1), 96.0 (C-1), 90.5 (OCNH*C*Cl_3), 74.2, 71.3, 70.8, 70.2, 70.1, 68.9, 68.7, 61.7, 56.0 (C-2), 22.9 (CO*C*H_3), 20.3 (CO*C*H_3), 20.3 (CO*C*H_3), 20.2 (CO*C*H_3), 17.0 (C-6). ESI-HRMS $[M+NH_4]^+$ calculated for $C_{26}H_{39}N_3O_{15}$ 738.1449 found 738.1442.

*4.3.4. p-Methoxyphenyl 3,4,6-tri-O-acetyl-2-deoxy-2-acetamido-β-D-glucopyranosyl-(1→2)-3,4-di-O-acetyl-α-L-rhamnopyranosyl-(1→3)-2,4-di-O-benzoyl-α-L-rhamnopyranosyl-(1→2)-3,4-di-O-benzoyl-α-L-rhamnopyranosyl-(1→2)-3,4-di-O-benzoyl-α-L- rhamnopyranose (**22**)*

A mixture of **5** (304 mg, 0.43 mmol), **4** (500 mg, 0.42 mmol) and 4 Å MS (0.3 g) in anhydrous CH_2Cl_2 (30 mL) was stirred at rt for 30 min in N_2 atmosphere, before it was cooled to -10^oC, then TMSOTf (10 uL) was added.The mixture wasstirred at same temperature for 8 h, and then neutralized with Et_3N. The mixture was diluted with CH_2Cl_2 (10 mL), filtered through a pad of celite, and the filtrate was concentrated. The residue was purified by flash column chromatography (petroleum ether/ethyl acetate 1:4) to give **22** (447 mg 67%) as colorless syrup. $[\alpha]_D^{22}$ 28.4° (*c* 1.0, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.07−7.91 (m, 12H, Ar*H*), 7.62−7.32 (m, 15H, Ar*H*),7.20−7.06 (m, 5H, Ar*H*), 6.89−6.86 (m, 2H, Ar*H*), 5.88−5.84 (dd, 1H, J_1 = 3.0 Hz, J_2 =10.2 Hz, H-3), 5.82−5.78 (dd, 1H, J_1 = 3.0 Hz, J_2 =9.8 Hz, H-3), 5.70−5.64 (m, 2H, H-3, H-4), 5.60−5.43 (m, 3H, H-4, H-4, H-4), 5.57 (s, 1H, H-1-Rham*p*),5.29 (t, 1H, *J* = 9.8 Hz, H-4), 5.14 (s, 1H, H-1-Rham*p*), 5.08−5.04 (dd, 1H, J_1 = 2.1 Hz, J_2 = 9.5 Hz, H-2), 5.01 (s, 1H, H-1-Rham*p*), 4.89 (t, 1H, J = 9.7 Hz, H-3), 4.80 (s, 1H, H-1-Rham*p*), 4.77−4.71 (m, 1H, H-2), 4.72

(d, 1H, *J* = 8.0 Hz, H-1-Glc*p*), 4.55 (m, 1H, H-2), 4.48−4.43 (dd, 1H, J_1 = 2.9 Hz, J_2 =9.7 Hz, H-3), 4.55 (m, 1H, H-2), 4.31−4.22 (m, 2H, H-6), 3.95−3.79 (m, 2H, H-5), 3.79 (s, 3H, OCH_3), 3.59 (m, 2H, H-2, H-5), 3.38−3.30 (m, 2H, H-5),1.89, 1.93, 1,95, 1.96, 1.98, 2.00 (6s, 18H, OCOCH_3), 1.37 (d, 3H, *J* = 6.2 Hz, H-6), 1.31 (d, 3H, *J* = 6.2 Hz, H-6), 1.03 (d, 3H, *J* = 6.2 Hz, H-6), 0.89 (d, 3H, *J* = 6.2 Hz, H-6); ^{13}C NMR (75 MHz, $CDCl_3$) : *δ* 170.3, 170.2, 169.9, 169.7, 169.2, 165.7, 165.2, 164.9, 154.9, 132.93, 132.8, 129.6, 129.5, 129.4, 129.3, 129.2, 129.2, 129.1, 129.0, 128.7, 128.2, 128.1, 128.0, 128.0, 117.4, 114.7, 100.5 (C-1-Rham*p*), 99.9 (C-1-Rham*p*), 99.3 (C-1-Glc*p*), 98.9 (C-1-Rham*p*), 97.6 (C-1-Rham*p*), 76.5 (C-2, C-2), 74.8 (C-3, C-4), 73.4 (C-3, C-4, C-4), 72.8 (C-4, C-4, C-3), 71.5 (C-3), 71.2 (C-5), 70.9 (C-5), 70.4 (C-5), 70.2 (C-5), 68.6 (C-2), 67.4 (C-2), 67.1(C-3), 67.0 (C-6), 61.4 (C-5), 55.5 (C-2), 55.3 (OCH_3), 22.8, 20.4, 20.3, 20.2, 17.3 (C-6, C-6), 16.9 (C-6), 16.7 (C-6). ESI-HRMS $[M+NH_4]^+$ calculated for $C_{91}H_{99}N_2O_{34}$ 1763.6079 found 1763.6075.

*4.3.5. p-Methoxyphenyl 2-deoxy-2-acetamido-β-D-glucopyranosyl-(1→2)-α-L-rhamnopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-rhamnopyranosyl-(1→2)-α-L-rhamnopyranose (**2**)*

To a solution of **22** (400 mg, 0.23 mmol) in MeOH (20 mL) was added satd. MeOH-NH_3 (MeOH solution) 40 ml. The resulting mixture was stirred for 14 days and the solvent was removed under reduced pressure to give **2** (168 mg 80%) as yellow syrup. $[\alpha]_D^{22}$ 26.3°(*c* 1.0, MeOH). ^{1}H NMR (300 MHz, D_2O): *δ* 6.96−6.93 (m, 2H, Ar*H*), 6.84−6.81 (m, 2H, Ar*H*), 5.34 (s, 1H, H-1-Rham*p*), 5.15 (s, 1H, H-1-Rham*p*), 5.06 (s, 1H, H-1-Rham*p*), 4.86 (s, 1H, H-1-Rham*p*), 4.57 (d, 1H, *J* = 8.4 Hz, H-1-Glc*p*), 4.04−3.99 (m, 5H), 3.83−3.57 (m, 15H, OCH_3), 3.48−3.20 (m, 10H), 1.94 (s, 3H, ONHCOCH_3), 1.19−1.13 (m, 12H, H-6); ^{13}C NMR (75 MHz, D_2O) : *δ* 174.5, 154.5, 149.3, 118.6, 114.9, 102.5 (C-1-Glc*p*), 101.6 (C-1-Rham*p*), 100.7 (C-1-Rham*p*), 100.6 (C-1-Rham*p*), 98.0 (C-1-Rham*p*), 78.9, 78.1, 77.9, 75.5 , 73.3, 72.0, 71.8, 69.7, 69.6, 69.5, 69.3, 69.1, 68.9, 60.3, 55.6, 55.5, 22.0, 16.5 (C-6, C-6), 16.4 (C-6). ESI-HRMS $[M + H]^+$calculated for $C_{39}H_{62}NO_{23}$ 912.3707 found 912.3700.

*4.3.6. p-Methoxyphenyl 2,4-di-O-benzoyl -α-L-rhamnopyranosyl-(1→2)-3,4-di-O-benzoyl-α-L-rhamnopyranose (**23**)*

To a solution of **16** (2.00 g, 2.15 mmol) in DMF (100 mL) was added hydrazine acetate (0.80 g, 10.75 mmol) and at room temperature in N_2 atmosphere. The reaction mixture was stirred for 4 h, at which time TLC (petroleum ether/ethyl acetate 2:1) indicated the completion of reaction. The reaction was then quenched with sat. aq. NH_4Cl (200 mL) and then extracted with dichloromethane (200 mL). The organic phase was washed with sat. aq. $NaHCO_3$ (2 x 100 mL) and water (100 mL). After the solution was dried over Na_2SO_4, the solvent was removed under reduced pressure and the residue was purified by flash chromatography (petroleum ether/ethyl acetate 3:1) to give **23** (1.38 g 79%) as colorless syrup. $[\alpha]_D^{22}$ 18.8° (*c* 1.0, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): *δ* 8.13−7.97 (m, 8H, Ar*H*), 7.64−7.33 (m, 12H, Ar*H*), 7.13−7.09 (m, 2H, Ar*H*), 6.91−6.87 (m, 2H, Ar*H*), 5.98 (dd, 1H, J_1 = 3.2 Hz, J_2 = 10.0 Hz, H-2), 5.70−5.64 (m, 2H, H-3, H-4), 5.58 (d, 1H, *J* = 1.7 Hz, H-1), 5.32 (t, 1H, *J* = 9.8 Hz, H-4), 5.19 (s, 1H, H-1), 4.53−4.46 (m, 2H, H-2, H-3), 4.31−4.24 (m, 2H, H-5, H-5), 3.79 (s, 3H, OCH_3), 2.52 (d, 1H, *J* = 7.4 Hz, O*H*), 1.36 (d, 3H, *J* = 6.3 Hz, H-6), 1.31 (d, 3H, *J* = 6.3 Hz, H-6); ^{13}C NMR (75 MHz,

$CDCl_3$) : δ 166.6 (*C*OCH_3), 165.5 (*C*OCH_3), 165.3 (*C*OCH_3), 165.2 (*C*OCH_3), 153.9, 149.9, 133.1, 133.0, 132.9, 132.8, 129.6, 129.5, 129.5, 129.5, 129.4, 129.1, 129.0, 129.0, 128.2, 128.2, 128.1, 128.0, 117.3, 114.4, 99.2 (C-1), 97.5 (C-1), 76.5, 74.8, 72.5, 71.5, 70.5, 68.5, 67.2, 67.0, 55.3 (O*C*H_3), 17.4 (C-6), 17.3 (C-6). ESI-HRMS $[M+NH_4]^+$ calculated for $C_{47}H_{48}NO_{14}$ 850.3069 found 850.3064.

*4.3.7. p-Methoxyphenyl 3,4,6-tri-O-acetyl-2-deoxy-2-acetamido-β-D-glucopyranosyl-(1→2)-3,4-di-O-acetyl-α-L-rhamnopyranosyl-(1→3)-2,4-di-O-benzoyl-α-L-rhamnopyranosyl-(1→2)-3,4-di-O-benzoyl-α-L-rhamnopyranose (**24**)*

After a mixture of **5** (430 mg, 0.60 mmol), **23** (500 mg, 0.60 mmol) and 4 Å MS (0.2 g) in anhydrous CH_2Cl_2 (20 mL) was stirred at rt for 30 min in N_2 atmosphere, before it was cooled to 0^oC, then TMSOTf (10 uL) was added. The mixture was stirred at same temperature for 8 h, and then neutralized with Et_3N. The mixture was diluted with CH_2Cl_2 (10 mL), filtered through a pad of celite, and the filtrate was concentrated. The residue was purified by flash column chromatography (petroleum ether/ethyl acetate 1:2) to give **24** (586 mg 73%) as colorless syrup. $[\alpha]_D^{22}$ 29.7° (*c* 1.0, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.09−8.05 (m, 4H, Ar*H*), 8.00−7.94 (m, 4H, Ar*H*), 7.63−7.30 (m, 12H, Ar*H*), 7.10−7.15 (m, 2H, Ar*H*), 6.89−6.84 (m, 2H, Ar*H*), 5.97-5.93 (dd, 1H, J_1 = 3.2 Hz, J_2 = 10.0 Hz, H-3), 5.67-5.60 (m, 3H, H-4, H-4, H-4), 5.55 (d, 1H, *J* = 1.7 Hz, H-1), 5.51−5.43 (m, 2H, H-2, H-3), 5.18 (d, 1H, *J* = 1.7 Hz, H-1), 5.12−5.08 (dd, 1H, J_1 = 2.9 Hz, J_2 = 10.0 Hz, H-2), 5.07 (d, 1H, *J* = 1.7 Hz, H-1), 4.92 (t, 1H, *J* = 9.8 Hz, H-3), 4.76−4.73 (d, 1H, *J* = 8.7 Hz, H-1), 4.79−4.73 (t, 1H, *J* = 10.1 Hz, H-4), 4.59−4.55 (dd, 1H, J_1 = 3.4 Hz, J_2 = 9.7 Hz, H-3), 4.47−4.45 (dd, J_1 = 2.0 Hz, J_2 = 3.1 Hz, H-2), 4.26−4.14 (m, 2H, H-6), 3.94−3.89 (m, 1H, H-5), 3.84−3.82 (dd, 1H, J_1 = 2.0 Hz, J_2 = 3.1 Hz, H-2), 3.78 (s, 3H, OCH_3), 3.65−3.61 (m, 1H, H-5), 3.40−3.31 (m, 2H, H-5), 2.04, 1.99, 1.97, 1.96, 1.94, 1.91 (6s, 18H, OCOCH_3), 1.33 (d, 3H, *J* = 6.3 Hz, H-6), 1.25 (d, 3H, *J* = 6.3 Hz, H-6), 1.08 (d, 3H, *J* = 6.3 Hz, H-6); ^{13}C NMR (75 MHz, $CDCl_3$) : δ 170.3 (*C*OCH_3), 170.2 (*C*OCH_3), 170.0 (*C*OCH_3), 169.7 (*C*OCH_3), 165.4 (*C*OCH_3), 165.1 (*C*OCH_3), 154.9, 149.9, 133.1, 132.9, 132.8, 129.6, 129.5, 129.5, 129.4, 129.1, 128.7, 128.2, 128.1, 128.0, 117.3, 114.3, 100.0 (C-1-Rham*p*), 99.3 (C-1-Glc*p*), 99.0 (C-1-Rham*p*), 97.5 (C-1-Rham*p*), 76.8 (C-2), 74.9 (C-3), 73.4 (C-4, C-5), 72.9 (C-4), 71.5 (C-3), 71.2 (C-3), 70.9 (C-2), 70.6 (C-2), 70.4 (C-3), 68.6 (C-4), 67.4 (C-4), 67.2 (C-6), 67.1 (C-5), 61.4 (C-5), 55.6 (C-2, C-5), 55.3 (O*C*H_3), 22.9, 20.5, 20.4, 20.3, 17.4 (C-6, C-6), 16.7 (C-6). ESI-HRMS $[M+NH_4]^+$ calculated for $C_{71}H_{81}N_2O_{28}$ 1409.4970 found 1409.4972.

*4.3.8. p-Methoxyphenyl 2-deoxy-2-acetamido-β-D-glucopyranosyl-(1→2)-α-L-rhamnopyranosyl -(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-rhamnopyranose (**3**)*

To a solution of **24** (500 mg, 0.36 mmol) in MeOH (20 mL) was added 30% $MeNH_2$ (MeOH solution) 30 ml. The resulting mixture was stirred for 7 days and the solvent was removed under reduced pressure to give **3** (220 mg, 80%) as yellow syrup. $[\alpha]_D^{22}$ 29.7° (*c* 1.0, MeOH).^{1}H NMR (300 MHz, D_2O): δ 6.97−6.83 (2m, 4H, Ar*H*), 5.39 (s,1H, H-1-Rham*p*), 5.18 (s,1H, H-1-Rham*p*), 4.91 (s,1H, H-1-Rham*p*), 4.59 (d, 1H, *J* = 8.1 Hz, H-1-Glc*p*), 4.10−3.96 (m, 5H), 3.85−3.57 (m,

9H), 3.69 (s, 3H, OCH_3), 3.49−3.24 (m, 8H) , 1.97 (s, 3H, OCOCH_3), 1.15−1.12 (m, 9H, H-6). ^{13}C NMR (75 MHz, D_2O) : δ 174.6, 154.6, 149.3, 118.7, 114.9, 102.5 (C-1-Glc*p*), 101.8 (C-1-Rham*p*), 100.7 (C-1-Rham*p*), 98.0 (C-1-Rham*p*), 78.9, 78.3, 77.8, 75.5, 73.3, 72.0, 71.9, 71.1, 69.6, 69.5, 69.4, 69.4, 69.1, 68.9, 60.3, 55.6, 55.5, 24.3, 22.1, 16.5, 16.4. ESI-HRMS [M + H]$^+$calculated for $C_{33}H_{52}NO_{19}$ 766.3128 found 766.3121.

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Supplementary Data

Supplementary data associated with this article can be found, in the online version, at http://~~.

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Highlights:

1. we have reported a convergent total synthesis of a pentasaccharide analogue and its tetrasccharide derivative
2. Stereo- and regio-specific synthesis was achieved in Schmidt glycosylation employing appropriately protected L-rhamopyranosyl and D-glucopyranosylamine building blocks.
3. The efficient synthesis features the preparation of a key disaccharide intermediate containing an orthogonal protecting group (Lev), which could be easily converted to the disaccharide donor or the disaccharide acceptor