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Synthesis of Tumor-associated Saccharides via *O*-Glycosyl Trichloroacetic

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Abstract: The trichloroacetic method was employed to synthesize di- and hexa-saccharides. *O*-glycosyl trichloroacetic, a stable and readily obtained intermediate, was activated to give a highly reactive glycosyl donor upon treatment with acid and coupled with the acceptor to afford complex glycosides with high stereoselectivity and in good yield. Two free hexa-saccharides will be used to explore the possible prevention of metastatic spread.

Introduction

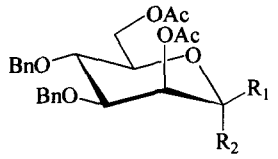
The complex series of events constituting tumor metastasis can be subdivided into a number of distinct steps, several of which involve the traversal of extracellular matrix barriers[1]. Extracellular matrices are composed of macromolecules that include laminin, fibronectin[2]. These molecules can promote cell adhesion and migration[3] and are believed to play a role in tumor cell invasion[4,5]. Our research group has observed that *N*-acetyllactosamine, lactose and Galβ(1→4)GlcNHAcβ(1→6)Manα(1→6)ManαOCH$_3$ (the core structure of the *N*-linked oligosaccharide of laminin) are capable of inhibiting the attachment of tumor cell (S180) to laminin and that Galβ(1→4)GlcNHAcβ(1→6)Manα(1→6)ManαOCH$_3$ is more effective than *N*-acetyllactosamine and lactose[6]. We have, therefore, synthesized their analogues **1** and **2** to explore the possible prevention of metastatic spread.

Results and Discussion

For the preparation of **1** and **2**, 1,2,6-tri-*O*-acetyl-3,4-di-*O*-benzyl-α-D-mannopyranose **3**[7], methyl 2,3,4-tri-*O*-benzyl-α-D-mannopyranoside **6**[8], 3,6-di-*O*-acetyl-2-deoxy-2-phthalimido-4-*O*-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-D-glucopyranose **9**[9] and 2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyrannosyl)-D-glucopyranose **14**[10] were chosen as starting materials.

3 was deacetylated at C-1 using hydrazine acetate and the product was treated with trichloroacetic anhydride in the presence of sodium trichloroacetic to yield **5** (overall yield 96%). Condensation of **5** with **6** in dichloromethane in the presence of trimethylsilyl triflate gave disaccharide derivative **7** (84%)[11]. Compound **7** was *O*-deacetylated with sodium methoxide in methanol to give **8** (92%).

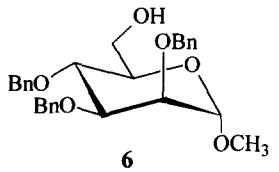
10 was readily obtained from **9**. Stereoselective coupling of **10** with **8** in dichloromethane using trimethylsilyl triflate as a promoter gave **11** (40.2%). Debenzylation of **11**, followed by dephthaloylation with hydrazine monohydrate, re-*N*,*O*-acetylation and de-*O*-acetylation gave the hexasaccharide **1** (overall yield 37.8%).



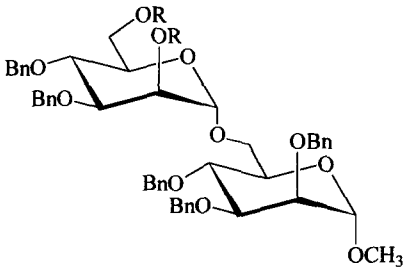
3 $R_1 = H, R_2 = OAc$

4 $R_1, R_2 = H, OH$

5 $R_1 = H, R_2 = OCOCCl_3$

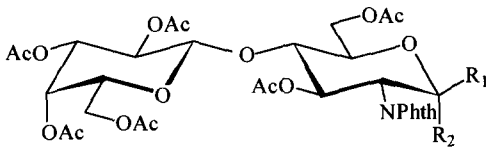


6



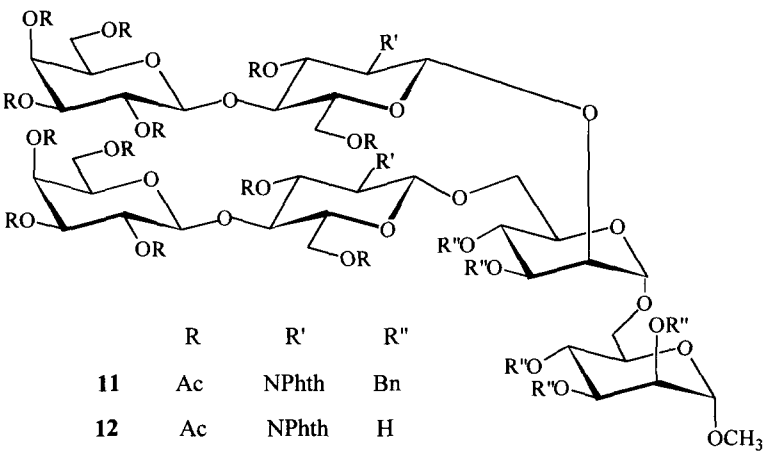
7 R = Ac

8 R = H



9 $R_1, R_2 = H, OH$

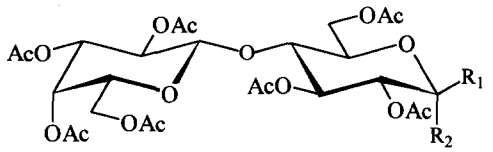
10 $R_1 = OCOCCl_3, R_2 = H$



	R	R'	R"
11	Ac	NPhth	Bn
12	Ac	NPhth	H
13	Ac	NHAc	Ac
1	H	NHAc	H

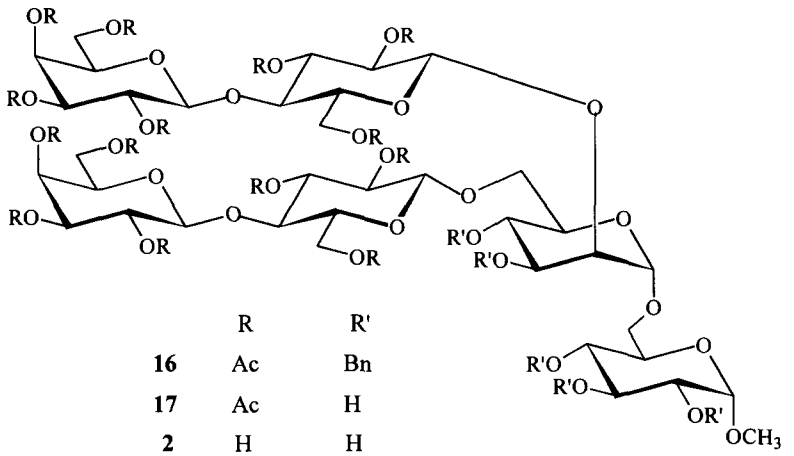
2,3,6-Tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-α/β-D-glucopyranosyl trichloroacetic **15** was obtained from 2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-D-glucopyranose **14** by treatment with trichloroacetic anhydride and sodium trichloroacetic in dichloromethane for 6 h. The ^{1}H NMR showed signals in the ratio 32:68 at δ 5.74 (d, J 7.5 Hz) and δ 6.24 (d, J 3.5 Hz), attributed to protons of β anomer and α anomer respectively. Coupling of **15** with **8** was performed in the presence of trimethylsilyl triflate and gave the hexasaccharide **16** (52%). Reductive debenzylation with

palladium hydroxide on carbon as a catalyst, followed by de-*O*-acetylation afforded free hexasaccharide **2** (overall yield 76%).



14 R_1 ,R_2 = H, OH

15 R_1, R_2 = H, $OCOCCl_3$



	R	R'
16	Ac	Bn
17	Ac	H
2	H	H

Experimental

General methods: See reference 12.

2,6-Di-*O*-acetyl-3,4-di-*O*-benzyl-α-D-mannopyranosyl trichloroacetic 5. A solution of **3** (3 g, 6.17 mmol) and hydrazine acetate (0.6 g, 6.12 mmol) in dry *N*,*N*-dimethylformamide (120 ml) was stirred for 6 h at room temperature, and diluted with ethyl acetate (360 ml). The organic phase was washed with aqueous 5% sodium chloride and water, dried, filtered, and evaporated *in vacuo* to give **4** (2.7 g, 98.5%) as a syrup. R_F 0.23 (3:1 petroleum ether-acetone). A mixture of **4** (2.7 g, 6.08 mmol), trichloroacetic anhydride (5.6 ml, 30.7 mmol) and sodium trichloroacetic (5.7 g, 30.7 mmol) in dichloromethane (100 ml) was heated at reflux. After 1 h, the mixture was filtered and the solid was washed with dichloromethane (3×20 ml). The combined organic layer was washed with water, saturated aq. sodium hydrogencarbonate, and water, dried, and concentrated to yield **5** (3.5 g, 98%) as a syrup: $[\alpha]_D$ +21 (c 1, $CHCl_3$); R_F 0.41 (3:1 petroleum ether-acetone); ^1H NMR (300 MHz, $CDCl_3$): δppm 7.39-7.24 (m, 10 H, Ph), 5.86 (d, 1 H, J 2.2 Hz, H-1), 5.74 (q, 1 H, J 2.4 Hz, H-2), 2.21 and 2.20 (s, each 3 H, 2 Ac). Anal. Calcd. for $C_{26}H_{27}Cl_3O_9$: C, 52.94; H, 4.61. Found: C, 52.89; H, 4.65.

Methyl 2,3,4-Tri-*O*-benzyl-6-*O*-(2,6-di-*O*-acetyl-3,4-di-*O*-benzyl-α-D-mannopyranosyl)-α-D-mannopyranoside 7. A mixture of **5** (3 g, 5.09 mmol), **6** (2.4 g, 5.17 mmol) and powdered molecular sieves

(4Å, 2 g) in dry dichloromethane (50 ml) was stirred for 3 h at room temperature, and then cooled to -20°C. 2.5 ml of trimethylsilyl triflate in dry dichloromethane (1 M solution) was added dropwise. After 6 h, TLC (3:1 petroleum ether -acetone) indicated the formation of a main spot. To the mixture was added sodium hydrogencarbonate(1 g). The mixture was stirred for 30 minutes and filtered and filtrate was concentrated. Column chromatography (15:1 petroleum ether- acetone) of the residue on silica gel afforded **7** (3.8 g, 84%) as a colorless syrup: $[\alpha]_D$ +34 (c 0.5, $CHCl_3$); R_F 0.35 (15:1 petroleum ether-acetone); ^1H NMR (300 MHz, $CDCl_3$): δppm 5.51 (d, 1 H, J 2.1 Hz, H-1b), 4.95 (d, 1 H, J 2.0 Hz, H-1a), 3.28 (s, 3H, CH_3O), 2.18, 2.02 (s, each 3 H, 2 Ac). Anal. Calcd. for $C_{52}H_{58}O_{13}$: C, 70.10; H, 6.56. Found: C, 70.21; H, 6.50.

Methyl 2,3,4-Tri-*O*-benzyl-6-*O*-(3,4-di-*O*-benzyl-α-D-mannopyranosyl)-α-D-mannopyranoside 8. A catalytic amount of sodium was added to a solution of **7** (3 g) in methanol (200 ml). The solution was left at room temperature overnight, neutralized with 732 (H^+) cation-exchange resin, filtered and concentrated to dryness. Column chromatography (5:1 petroleum ether- acetone) of the residue on silica gel gave 8 (2.5 g, 92%) as a white solid: $[\alpha]_D$ +47 (c 2, $CHCl_3$); R_F 0.15 (petroleum ether-acetone); ^1H NMR (300 MHz, $CDCl_3$): δppm 5.45 (d, 1 H, J 2.0 Hz, H-1b), 4.93 (d, 1 H, J 2.0 Hz, H-1a), 3.28 (s, 3 H, OCH_3).

3,6-Di-*O*-acetyl-2-deoxy-2-phthalimido-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-β-D-glucopyranosyl trichloroacetic 10. To a solution of **9** (1.1 g, 1.52 mmol) in dry dichloromethane was added trichloroacetic anhydride (1.1 ml) and sodium trichloroacetic (1.2 g). The mixture was boiled under reflux until the formation of a single product. Work-up in the usual manner afforded **10** (1.29 g, 98%) as a syrup: $[\alpha]_D$ +21 (c 1, $CHCl_3$); R_F 0.3 (3:2 petroleum ether- acetone); ^1H NMR (300 MHz, $CDCl_3$) δppm 7.83-7.72 (m, 4 H, Phth), 6.51 (d, 1 H, J 8.7 Hz, H-1a), 5.82 (dd, 1 H, J 8.4 and 10.8 Hz, H-3a), 5.32 (d, J 3 Hz, H-4b), 5.10 (dd, 1 H, J 7.1 and 10 Hz H-2b), 4.94 (dd, 1 H, J 3.3 and 10.1 Hz, H-3b), 2.13, 2.11, 2.03, 2.01, 1.94 and 1.90 (6s, each 3 H, 6 OAc). Anal. Calcd. for $C_{34}H_{36}Cl_3NO_{19}$: C, 46.99; H, 4.18, N, 1.61. Found: C, 46.91; H, 4.23; N, 1.68.

Methyl *O*-(2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-*O*-(3,6-di-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→2)-*O*-[(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-*O*-(3,6-di-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→6)]-*O*-(3,4-di-*O*-benzyl-α-D-mannopyranosyl)-(1→6)-2,3,4-tri-*O*-benzyl-α-D-mannopyranoside 11. A mixture of **10** (1.22 g, 1.4 mmol), **8** (370 mg, 0.46 mmol) and powdered molecular sieves (4Å, 1.5 g) in dry dichloromethane (20 ml) was stirred for 3 h at room temperature and cooled to -20°C. Then trimethylsilyl triflate (0.7 ml of 1 M solution in CH_2Cl_2) was added dropwise. After 12 h, sodium hydrogencarbonate (0.6 g) was added, and the mixture was stirred for 30 minutes. The mixture was filtered through a bed of silica gel and the solid was washed with dichloromethane (3 ×10 ml). The combined organic layer was concentrated *in vacuo*. Column chromatography (3:2 petroleum ether- acetone) of the residue on silica gel gave **11** (0.41g, 40.2%) as a white solid. $[\alpha]_D$ +15 (c 1, $CHCl_3$); R_F 0.14 (3:2 petroleum ether- acetone); FD-MS 2239 $[M + Na]^+$; 2217 $[M + 1]^+$; ^1H NMR (300 MHz, $CDCl_3$): δppm 7.78-7.09 (m, 33 H, 5 Ph and 2 Phth), 5.80 (dd, 1H, J 8.5 and 10.0 Hz, H-3c), 5.49 (dd, 1H, J8.2 and 10.5 Hz, H-3d), 5.41 (d, 1H, J 10 Hz, H-1c), 3.33 (s, 3 H, OCH_3), 2.17, 2.16, 2.14, 2.12, 2.10, 2.08, 2.07, 2.02, 1.94, 1.92, 1.90 and 1.86 (s, each 3 H, 12 Ac); ^{13}C NMR (75 MHz, $CDCl_3$): δppm 170.3-169.0 (C=O), 138.5-137.6 and 128.3-127.6 (Ph), 134.1-123.2 (Phth), 101.2 (2 C) (C-1e, C-1f), 99.0 and

98.6 (2 C) (C-1c, C-1d), 97.4 (C-1b), 96.9 (C-1a), 62.6 and 62.4 (C-6c, C-6d), 53.9 (OCH_3), 20.8 (CH_3). Anal. Calcd. for $C_{112}H_{124}N_2O_{45}$: C, 60.65; H, 5.63; N, 1.26. Found: C, 60.51; H, 5.76; N, 1.32.

Methyl *O*-(2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-*O*-(2-acetamido-3,6-di-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→2)-*O*-[(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-*O*-(2-acetamido-3,6-di-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→6)]-*O*-(3,4-di-*O*-acetyl-α-D-mannopyranosyl)-(1→6)-2,3,4-tri-*O*-acetyl-α-D-mannopyranoside 13. A solution of **11** (380 mg, 0.17 mmol) in 5:3 ethanol-ethyl acetate (160 ml) was hydrogenolysed using palladium hydroxide on carbon (130 mg) as a catalyst for 12 h at room temperature. TLC (R_F 0.53, 4:1 chloroform-methanol) showed the debenzylation to be complete. The mixture was filtered through Celite, and the filtrate was concentrated affording **12** (302 mg, quantitative). To a solution of **12** (302 mg, 0.17 mmol) in ethanol (30 ml) was added hydrazine monohydrate (1.5 ml). After 2 h at reflux the mixture was concentrated *in vacuo* and co-concentrated with toluene (3×30 ml). The residue was dissolved in pyridine (20 ml) and acetic anhydride (10 ml), and a catalytic amount of *N,N*-dimethylaminopyridine was added. After stirring for two days, TLC indicated the formation of a new spot , and the solution was concentrated, and co-concentrated with toluene (3 ×30 ml). Column chromatography (1:1 petroleum ether-acetone) of residue on silica gel afforded **13** (121 mg, 39%) as a white powder. $[\alpha]_D$ +7 (c 1, $CHCl_3$); R_F 0.1 (1:1 petroleum ether-acetone); ^{13}C NMR (75 MHz, $CDCl_3$): δppm 101.2, 100.8, 100.6 and 99.7 (4C, C-1c, C-1d, C-1e, C-1f), 98.9 (C-1b), 97.8 (C-1a).

Methyl *O*-β-D-Galactopyranosyl-(1→4)-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→2)-*O*-[β-D-galactopyranosyl)-(1→4)-*O*-(acetamido-2-deoxy-β-D-glucopyranosyl)-(1→6)]-*O*-(α-D-mannopyranosyl)-(1→6)-α-D-mannopyranoside 1. A catalytic amount of sodium was added to a solution of **13** (91 mg) in methanol (20 ml). The mixture was stirred for 24 h, neutralized with 732 (H^+) cation-exchange resin, filtered and concentrated *in vacuo*. After de-salting and freeze-drying, **1** was obtained as an amorphous powder (53 mg, 97%). $[\alpha]_D$ +3 (c 0.5, H_2O); ^{13}C NMR (75 MHz, D_2O): δppm 176.2 and 175.8 (2C, C=O), 104.3 (2C, C-1e, C-1f), 103.6 and 102.8 (2C, C-1c, C-1d), 101.5 (C-1b), 100.7 (C-1a), 22.4 (2C, NHAc). Anal. Calcd. for $C_{41}H_{70}N_2O_{31}$: C, 45.30; H, 6.49; N, 2.58. Found: C, 45.18; H, 6.41; N, 2.65.

2,3,4-Tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-α/β-D-glucopyranosyl trichloroacetic 15. A mixture of **14** (1.4 g, 2.2 mmol), trichloroacetic anhydride (2 ml), sodium trichloroacetic (2.04) in dry dichloromethane (20 ml) was boiled under reflux for 6 h. Work-up in usual manner afforded **15** (1.70 g, 99%) as a colorless syrup. $[\alpha]_D$ +15 (c 1, $CHCl_3$); R_F 0.25 (2:1 petroleum ether-acetone); ^1HNMR (300 MHz, $CDCl_3$): δppm 6.24 (d, J 3.5 Hz, H-1a, α anomer), 5.74 (d, J 7.5 Hz, H-1a, β anomer), 5.24 (d, 1 H, J 4.5 Hz, H-4b), 5.18 (t, 1 H, J 7.8 Hz, H-3a), 2.05, 2.02, 1.97, 1.95, 1.94, 1.92, 1.86 (s, each 3 H, 7 Ac). Anal. Calcd. for $C_{28}H_{35}Cl_3O_{19}$: C, 43.01; H, 4.51. Found: C, 43.14; H, 4.61.

Methyl *O*-(2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-*O*-(2,3,6-di-*O*-acetyl-β-D-glucopyranosyl)-(1→2)-*O*-[(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-*O*-(2,3,6-di-*O*-acetyl-β-D-glucopyranosyl)-(1→6)]-*O*-(3,4-di-*O*-benzyl-α-D-mannopyranosyl)-(1→6)-2,3,4-tri-*O*-benzyl-α-D-mannopyranoside 16. A mixture of **15** (1.64 g, 2.1 mmol), **8** (560 mg, 0.69 mmol) and powdered molecular sieves (4Å, 1.5 g) in dry dichloromethane (20 ml) was stirred for 3 h at room temperature and cooled to -20°C.

Then 0.5 ml of trimethylsilyl triflate in dry dichloromethane (1 M solution) was added dropwise. After 12 h, TLC (3:2 petroleum ether-acetone) showed the formation of a new spot. Sodium hydrogencarbonate (0.8 g) was added, and the mixture was stirred for 30 minutes. The mixture was filtered through a bed of silica gel and the solid was washed with dichloromethane (3×10 ml). The combined organic layer was concentrated *in vacuo*. Column chromatography (3:2 petroleum ether-acetone) of the residue on silica gel afforded **16** (0.73 g, 52%) as a white powder. $[\alpha]_D$ +35 (c 1, $CHCl_3$); R_F 0.17 (3:2 petroleum ether-acetone); FD-MS: 2045 [M + 1]$^+$, 2044 (M^+), 2001, 1954, 1442; ^{13}C NMR (300 MHz, $CDCl_3$): δppm 170.2-168.9 (C=O), 138.3-136.8, 128.2-127.6 (Ph), 101.4, 101.3, 101.1 and 100.8 (4C, C-1c, C-1d, C-1e, C-1f), 98.9 (C-1b), 98.7 (C-1a), 54.6 (OCH_3), 21.3 (CH_3). Anal. Calcd. for $C_{100}H_{122}O_{45}$: C, 58.76; H, 6.02. Found: C, 58.58; H, 6.11.

Methyl *O*-β-D-Galactopyranosyl-(1→4)-*O*-β-D-glucopyranosyl-(1→2)-*O*-(β-D-galactopyranosyl-(1→4)-*O*-β-D-glucopyranosyl-(1→6))-*O*-α-D-mannopyranosyl-(1→6)-α-D-mannopyranoside 2. A solution of **16** (0.7 g, 0.34 mmol) in 5:3 ethanol-ethyl acetate (300 ml) was hydrogenolysed using palladium hydroxide on carbon (230 mg) as a catalyst at room temperature. After 12 h, TLC (4:1 chloroform-methanol) showed the debenzylation to be complete and the mixture was filtered through Celite, concentrated affording **17** (0.54 g, quantitative). A catalytic amount of sodium was added to a solution of **17** (0.54 g, 0.34 mmol) in methanol (40 ml). The mixture was stirred for 24 h, and neutralized with 732 (H^+) cation-exchange resin. The resin was filtered off and washed with methanol, and the combined filtrate and washings were evaporated *in vacuo*. After freeze-drying, **2** was obtained as an amorphous powder (0.26 g, 76%). $[\alpha]_D$ +7 (c 2, H_2O); ^1H NMR (500 MHz, D_2O): δppm 4.48 (d, 2 H, J 7.9 Hz, H-1e, H-1f), 4.38 (d, 2 H, J 7.8 Hz, H-1c, H-1d), 3.33 (OCH_3); ^{13}C NMR (125 MHz, D_2O): δppm 105.4 (2C), 105.0 (1C), 103.5 (2C) and 102.1 (1C) (C-1a, C-1b, C-1c, C-1d, C-1e and C-1f), 57.3 (OCH_3). Anal. Calcd. for $C_{37}H_{64}O_{31}$: C, 44.22; H, 6.42. Found: C, 44.35; H, 6.48.

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