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Studies on Carbohydrates XX. Synthesis of Hexasaccharide Containing Lactosamine Unit Using Glycosyl Trichloroacetates as Glycosyl Donors

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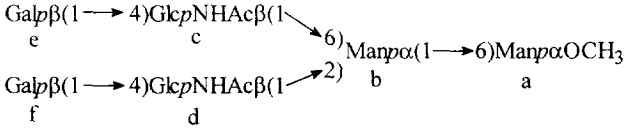
Key Words: disaccharide; hexasaccharide; oligosaccharide; O-glycosyl trichloroacetate; tumor metastasis

Abstract: O-Glycosyl trichloroacetate, a stable and readily obtained intermediate, was activated as a highly reactive glycosyl donor upon treatment with acid and coupled with the acceptor to afford complex glycosides with high stereoselectivity. Copyright © 1996 Elsevier Science Ltd

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].

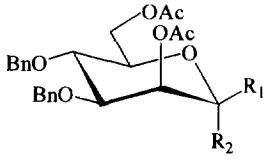
Although some oligosaccharides containing *N*-acetyllactosamine have been synthesized, their inhibition against tumor metastasis has not been studied[6,7,8]. Our research group observed that acetyllactosamine is capable of inhibiting the attachment of the tumor cell (S180) to laminin substrate and that a synthesized tetrasaccharide consisting of *N*-acetyllactosamine and mannose is more effective than *N*-acetyllactosamine in inhibiting tumor metastasis in *vitro* and in *vivo*[9]. We have, therefore, synthesized their analogues 1 to explore the possible prevention of metastatic spread. In the classical and frequently used Koenigs-Knorr method for the synthesis of oligosaccharides, relatively harsh conditions are needed for the generation of the glycosyl halide; the glycosyl halides exhibit low thermal stability and are highly sensitive to hydrolysis; expensive or toxic heavy-metal salts are used as a catalyst. Due to disadvantages of the Koenigs-Knorr method, the trichloroacetate method was employed to synthesize di- and hexa-saccharide[10,11,12]. *O*-Glycosyl trichloroacetate, a stable and readily obtained intermediate, coupled with the acceptor in the presence of trimethysilyl triflate in good yield and with high stereoselectivity.



1

1 was prepared from 1,2,6-tri-*O*-acetyl-3,4-di-*O*-benzyl-α-D-mannopyranose(**3**)[13], methyl 2,3,4-tri-*O*-benzyl-α-D-mannopyranoside(**6**)[14], 3,6-di-*O*-acetyl-2-deoxy-2-phthalimido-4-*O*-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-D-glucopyranose(**9**)[15].

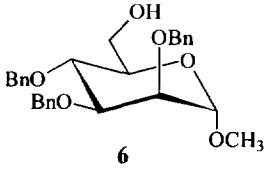
3 was deacetylated at C-1 using hydrazine acetate to afford **4**. A mixture of **4** (2.7 g, 6.08 mmol), trichloroacetic anhydride (5.6 ml, 30.7 mmol) and sodium trichloroacetate (5.7 g, 30.7 mmol) in dichloromethane (100 ml) was heated at reflux. After 1 h, the mixture was filtered and the solid was 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** (overall yield 96%). A mixture of **5** (3g, 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. A solution of trimethysilyl triflate in dry dichloromethane (2.5 ml, 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, then filtered, and the 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. Compound **7** was *O*-deacetylated with sodium methoxide in methanol to give **8** (92%).



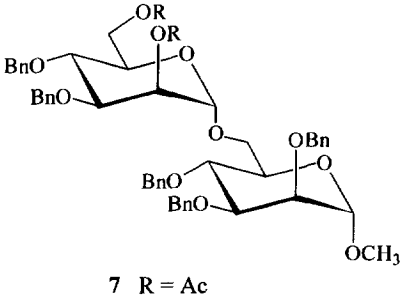
3 R_1 = H, R_2 = OAc

4 R_1, R_2 = H, OH

5 R_1 = H, R_2 = $OCOCCl_3$



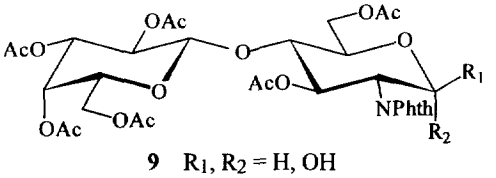
6



7 R = Ac

8 R = H

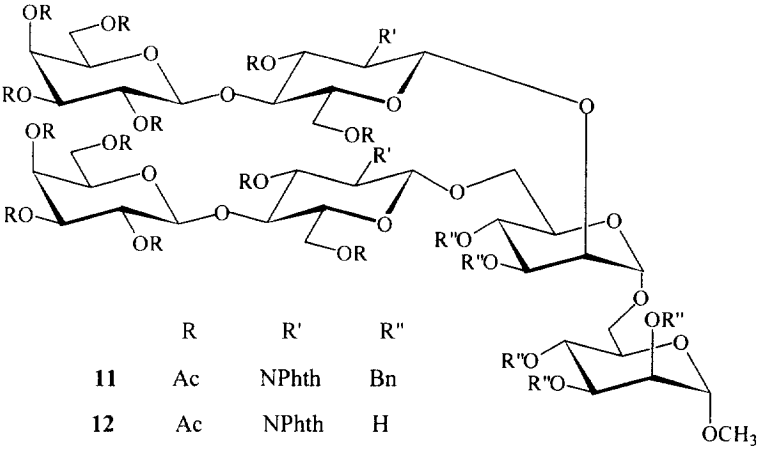
To a solution of **9** (1.1 g, 1.52 mmol) in dry dichloromethane was added trichloroacetic anhydride (1.1 ml) and sodium trichloroacetate (1.2 g). The mixture was boiled under reflux until the formation of a single product. Work-up in the usual manner afforded **10** (1.29 g, 98%) as a syrup.



9 R_1, R_2 = H, OH

10 R_1 = $OCOCCl_3$, R_2 = H

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, The mixture was neutralized with sodium hydrogencarbonate (0.6 g), then 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** (0.41, 40.2%) as a white solid. 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%). The free hexasaccharide will be used to explore the possible prevention of metastatic spread.



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

All compounds gave satisfactory data (The letters a, b, c, d, e, f are used to designate the glycosyl residue in which a cited H and C atom is located):

5: [α] +21° (C 1, $CHCl_3$); ^1H NMR (300 MHz, CDCl3): δppm 7.39-7.24 (m, 10 H, Ph), 5.86 (d, 1 H, J 2.2 Hz, H-1), 5.74 (dd, 1 H, J 2.4 Hz and J 3.2 Hz, H-2), 2.21 and 2.20 (2 s, each 3 H, 2 Ac).

7: [α] +34° (c 0.5, $CHCl_3$); ^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 (2 s, each 3 H, 2 Ac).

8: [α] +47° (c 2, $CHCl_3$); ^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).

10: [α] +21° (c 1, $CHCl_3$); ^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).

11: [α] +15° (c 1, $CHCl_3$); 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), 2.17-1.86 (12 Ac); ^{13}C NMR (75 MHz, $CDCl_3$):

δppm 170.3-169.0 (C=O), 138.5-123.2 (aromatic), 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 (2 C, C-6c, C-6d), 53.9 (OCH_3), 20.8 (Ac).

13: [α] +7° (c 1, $CHCl_3$); ^{13}C NMR (75 MHz, $CDCl_3$): δppm 101.2, 100.8, 100.6 and 99.7 (4 C, C-1c, C-1d, C-1e, C-1f), 98.9 (C-1b), 97.8 (C-1a).

1: [α] +3° (c 0.5, H_2O); ^{13}C NMR (75 MHz, D_2O): δppm 176.2 and 175.8 (2 C, C=O), 104.3 (2 C, C-1e, C-1f), 103.6 and 102.8 (2 C, C-1c, C-1d), 101.5 (C-1b), 100.7 (C-1a), 22.4 (Ac).

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