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Note

Synthesis of galactosyl and lactosyl derivatives as potential anti-metastasis compounds

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Abstract

Based on the known anti-metastasis activities of lactosides and galactosides, a galactosyl and a lactosyl trimannoside were prepared via the conventional Koenigs–Knorr and trichloroacetimidate methods, respectively. Through typical deblocking procedures, a tetrasaccharide α-D-Gal*p*-(1→2)-α-D-Man*p*-(1→2)-α-D-Man*p*-(1→6)-α-D-Man*p*OCH_3 and a pentasaccharide β-D-Gal*p*-(1→4)-β-D-Glc*p*-(1→2)-α-D-Man*p*-(1→2)-α-D-Man*p*-(1→6)-α-D-Man*p*OCH_3 were obtained. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: α-Galactosyl trimannoside; β-Lactosyl trimannoside; Glycosylation; Synthesis

1. Introduction

Raz and Lotan [1,2] discovered that lactose, D-galactose, D-glucosamine, and *N*-acetyl-D-galactosamine can inhibit the adhesion of some cancer cells, such as human melanoma A375, SH4, Hs294, Hs852, human adenoma Hela-S3, murine melanoma B16-F1, and murine fibrosarcoma UV-2237P. Among the aforementioned glycoses, lactose has the strongest inhibition activity, whereas other glycoses such as D-mannose, L-fucose, *N*-acetyl-D-glucosamine have none. The adhesion of cancer cells to other normal cells is the basis of cancer metastasis and proliferation. Oguchi et al. [3] reported that methyl β-lactoside can significantly inhibit murine melanoma B-16 cells from agglomerating in the lungs. Further study of the lactosides indicated that conjugates having a lactosyl group linked to a lysine–lysine peptide or other polylysine peptides through a carbon chain spacer arm have different activities in regard to inhibiting metastasis. Interestingly some of the glycoconjugates have strong anti-metastasis activity while others show no such activity or even enhance metastasis, a factor apparently related to the difference in length of the spacer arms [4]. In addition, such ramified mannans as hexamannoside Man6 and nonamannoside Man9 are also able to induce cancer cell adhesion, whereas trimannoside Man3 has not [5,6].

We therefore postulated that a combination of a galactosyl or lactosyl group and a trimannoside might lead to new anti-metastasis compounds. For this purpose, a tetrasaccharide α-D-Gal*p*-(1→2)-α-D-Man*p*-(1→2)

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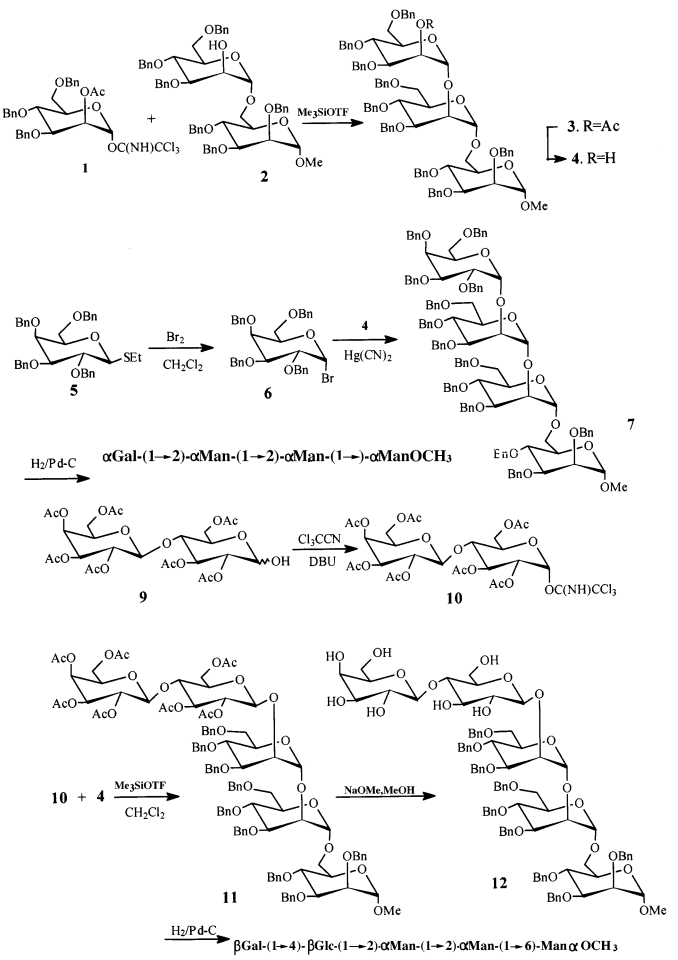
-α-D-Man*p*-(1→6)-α-D-Man*p*OCH$_3$ (**8**), and a pentasaccharide β-D-Gal*p*-(1→4)-β-D-Glc*p*-(1→2)-α-D-Man*p*-(1→2)-α-D-Man*p*-(1→6)-α-D-Man*p*OCH$_3$ (**13**) have been synthesized, using 1 + 3 and 2 + 3 coupling modes (see Scheme 1).

2. Results and discussion

The trimannoside **3** was prepared using 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl trichloroacetimidate (**1**) as glycosyl donor and the dimannoside **2** [7] as acceptor. The condensation was carried out in a solution of dichloromethane with trimethylsilyl triflate (Me_3SiOTf) as promoter and the yield was good (72.7%). Zemplén deacetylation gave the trisaccharide acceptor **4** in 83.7% yield. 2,3,4,6-Tetra-*O*-benzyl-α-D-galactopyranosyl bromide (**6**), obtained readily by in situ conversion of the corresponding ethyl 1-thioglycoside **5** [8] reacting with bromine in dichloromethane [9], was coupled to **4** by the conventional Koenigs–Knorr method with $Hg(CN)_2$ as promoter to give in 54% yield of an exclusive α-galacosylated tetrasaccharide **7**, which was then debenzylated by catalytic hydrogenolysis to afford the deblocked tetrasaccharide **8**. Compound **5** could be coupled directly to glycosyl acceptor **4** using methyl triflate as promoter to afford tetrasaccharide **7**, but the yield was relatively low (20–30%). The configuration of **8** was confirmed by the galactosyl anomeric $^3J_{H\text{-}1,2}$, value of 3.68 Hz.

To prepare pentasaccharide **11**, hepta-*O*-acetyllactosyl trichloroacetimidate **10** [9], obtained by reaction of lactoseheptaacetate **9** and trichloroacetonitrile in the presence of DBU as catalyst, was chosen as glycosyl donor to couple with **4** in the presence of Me_3SiOTf as promoter. The resultant pentasaccharide **11** was obtained in moderate yield (45%). Deacetylation of **11** in NaOMe–Me_3OH gave compound **12** in 81.7% yield. Subsequent catalytic hydrogenolysis gave the deblocked pentasaccharide **13** in 82.6% yield. ^1H NMR spectroscopy indicated that the non-reducing terminal galactosyl and glucosyl anomeric $^3J_{H\text{-}1,2}$ values are 7.97 and 7.99 Hz, respectively, and thus the β configuration of the lactosylation was thus confirmed.

Compounds **8** and **13**, along with several other related oligosaccharides synthesized in our laboratory are being tested for their anti-metastasis activity, and the results will be reported later.



Scheme 1.

3. Experimental

General methods.—Spectra were recorded with the following instruments: ^1H and ^{13}C NMR, Bruker ARX-400; the ^1H NMR spectra were recorded with Me_4Si as the internal standard and ^{13}C NMR with $CDCl_3$ as solvent and internal standard; Mass spectra, VG ZAB-Hs; IR, Perkin–Elmer 983. Elemental analyses were performed on a Perkin–Elmer 240C instrument. Optical rotations were measured at 25 °C with a Optical Activity AA-10R polarimeter. The progress of reactions

was monitored by thin-layer chromatography (TLC) on Silica Gel GF_{254} (Hai Yang Chemical Factory, Qingdao, Shandong, PR China). Detection was effected by examination under UV light and by charring with 5% phosphomolybdic acid hydrate in EtOH or 20% concd H_2SO_4 in EtOH and heating. PTLC was performed on Silica Gel GF_{254} and column chromatography on silica gel H (Hai Yang Chemical Factory, Qingdao, Shandong, PR China). The solvent systems indicated are volume volume ratios, and the petroleum ether used in the experiment has the boiling range of 60–90 °C.

Methyl 2,3,4-tri-O-*benzyl-6*-O-*[3,4,6-tri*-O-*benzyl-2*-O-*(2*-O-*acetyl-3,4,6-tri*-O-*benzyl*-α-D-*mannopyranosyl)*-α-D-*mannopyranosyl]*-α-D-*mannopyranoside* (**3**).—2-*O*-Acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl trichloroacetimidate (**1**) (1.04 g, 1.63 mmol) and methyl 2,3,4-tri-*O*-benzyl-6-*O*-(3,4,6-tri-*O*-benzyl-2-*O*-α-D-mannopyranosyl)-α-D-mannopyranoside (**2**) (1.32 g, 1.44 mmol) were dissolved in dry CH_2Cl_2 (25 ml). To the solution was added powdered 4Å molecular sieve (1.0 g) and the mixture was stirred at room temperature (rt) for 1 h and then cooled to −10 °C for another 20 min. Trimethylsilyl triflate (ten drops) was added and the mixture was stirred for 5 h, during which time the mixture attained to rt. The mixture was filtered through a layer of Celite and rinsed with CH_2Cl_2. The combined filtrate was washed with water, satd aq $NaHCO_3$, and water, and then dried ($MgSO_4$). The residue obtained by evaporation of the solvent was subjected to silica gel column chromatography eluting with 6:1 petroleum—acetone to give compound **3** (yield 1.47 g, 72.7%) as a colorless syrup. R_f 0.50 (4:1 cyclohexane–acetone); $[\alpha]_D$ +10.5° (*c* 0.39, $CHCl_3$). Anal. Calcd. for $C_{84}H_{90}O_{17}$: C, 73.58; H, 6.57. Found: C, 73.37; H, 6.28. IR: 1732 cm^{-1} (C=O).

Methyl 2,3,4-tri-O-*benzyl-6*-O-*[3,4,6-tri*-O-*benzyl-2*-O-*(3,4,6-tri*-O-*benzyl*-α-D-*mannopyranosyl)*-α-D-*mannopyranosyl]*-α-D-*mannopyranoside* (**4**).—To a solution of compound **3** (1.33 g, 0.95 mmol) in abs MeOH (30 ml), metal sodium (30 mg) was added. The solution was stirred at rt overnight, decationized and evaporated. Column chromatography of the residue on silica gel, eluting with 4:1 cyclohexane–acetone gave compound **4** (yield 1.08 g, 83.7%) as a colorless syrup. R_f 0.37 (4:1 cyclohexane–acetone); $[\alpha]_D$ +19.6° (*c*, 0.12, $CHCl_3$); IR: 3460 cm^{-1} (OH); ^{1}H NMR: δ 2.60 (br, 1 H, O*H*), 3.22 (s, 3 H, OCH_3), 3.67–4.70 (m, 49 H, H-1, 2, 3, 4, 5, 6, H-1′, 2′, 3′, 4′, 5′, 6′, H-1″, 2″, 3″, 4″, 5″, 6″, 9 × PhCH_2), 7.20–7.32 (m, 45 H, aromatic H); ^{13}CNMR: δ 54.61 (O*C*H$_3$), 65.27–77.36 (C-2, 3, 4, 5, 6, C-2′, 3′, 4′, 5′, 6′, C-1″, 2″, 3″, 4″, 5″, 6″, 9 × Ph*C*H$_2$), 98.82, 99.10, 101.13 (C-1, C-1′, C-1″), 127.27–128.41 (aromatic carbon), 138.09, 138.12, 138.25, 138.29, 138.47, 138.55 (2 C), 138.68, 138.72 (aromatic quaternary carbon).

Methyl 2,3,4-tri-O-*benzyl-6*-O-*{3,4,6-tri*-O-*benzyl-2*-O-*[3,4,6-tri*-O-*benzyl-2*-O-*(2,3,4,6-tetra*-O-*benzyl*-α-D-*galactopyranosyl)*-α-D-*mannopyranosyl]*-α-D-*mannopyranosyl}*-α-D-*mannopyranoside* (**7**).—To a solution of compound **4** (550 mg, 0.40 mmol) in 25 mL of dry CH_2Cl_2, $Hg(CN)_2$ (0.33 g) and powdered 4Å molecular sieve (1.0 g) were added and the mixture was stirred for 1 h at rt. Meanwhile, to a solution of compound **5** (780 mg, 1.33 mmol) in 15 mL of dry CH_2Cl_2 was added liquid bromine dropwise (0.25 mL) with stirring for 45 min at rt. The solvent was evaporated in vacuo and toluene (2 × 25 mL) was co-distilled from the residue. The resulting galactosyl bromide **6** was dissolved in dry CH_2Cl_2 (10 mL) and the solution added dropwise to the aforementioned mixture. After stirring for 24 h at rt, the mixture was filtered and the filtrate was washed successively with satd brine, satd $NaHCO_3$ solution, and water, respectively, and dried ($MgSO_4$). Evaporation of the solvent in vacuo gave a yellowish residue, which was subjected to column chromatography on silica gel eluting with 5:1 petroleum ether–EtOAc to give tetrasaccharide **7** (yield 420 mg, 54.0%) as a colorless syrup; R_f 0.33 (5:1 petroleum ether–EtOAc); $[\alpha]_D$ +37.1° (*c* 0.35, $CHCl_3$). ^{1}H NMR: δ 3.17 (s, 3 H, OCH_3), 3.49–5.20 (54 H, sugar ring H and 13 × PhCH_2), 7.17–7.45 (65 H, aromatic H). ^{13}C NMR: δ 53.48 (O*C*H$_3$), 60.42–80.50 (C-2, 3, 4, 5, 6, C-2′, 3′, 4′, 5′, 6″, C-2″, 3″, 4″, 5″, 6″, C-2‴, 3‴, 4‴, 5‴, 6‴, 13 × Ph*C*H$_2$), 97.96, 98.91, 99.05, 101.12 (C-1, C-1′, C-1″, C-1‴),

127.00–128.53 (aromatic carbon), 138.01, 138.25, 138.36, 138.53, 138.62, 138.70, 138.73, 138.79, 138.85, 138.90, 138.95, 139.17, 139.21 (^{13}C, aromatic quaternary carbon).

*Methyl 6-*O*-[2-*O*-(2-*O*-α-*D*-galactopyranosyl-α-*D*-mannopyranosyl)-α-*D*-mannopyranosyl]-α-*D*-mannopyranoside* (**8**).—Compound **7** (320 mg, 0.17 mmol) was dissolved in 4:1 MeOH–EtOAc (65 mL) and the solution was hydrogenlyzed with palladium on charcoal (200 mg) in a hydrogen stream under a pressure of 330 KPa for 24 h. The mixture was filtered and the resulting solution was evaporated to give compound **8** a colorless syrup, which was then dissolved in distilled water and lyophilized to a colorless foam (98 mg, 83.3%), FABMS: (*m*/*z*) 681 $[M+1]^+$; $[\alpha]_D$ +54.0° (*c* 0.63, water). ^{1}H NMR (400 MHz, D_2O): δ 3.43 (s, 3 H, OCH_3), 3.37–4.25 (m, 37 H, sugar ring H, 13 × OH), 4.77 (d, 1 H, $J_{1,2}$ 1.48 Hz, mannosyl H-1), 5.17, 5.42 (2 s, 2 H, mannosyl H-1), 5.18 (d, 1 H, $J_{1,2}$ 3.68 Hz, galactosyl H-1); ^{13}C NMR: δ 57.33 (OCH_3), 63.46, 63.60, 63.82 (mannosyl C-6), 68.35 (galactosyl C-6), 69.05, 69.47, 69.68, 71.37, 71.82 (2 C), 72.41, 72.74, 72.88, 73.80 (2 C), 73.93, 75.31, 75.66, 81.48, 82.09 (C-2, 3, 4, 5, C-2′, 3′, 4′, 5′, C-2″, 3″, 4″, 5″, C-2‴, 3‴, 4‴, 5‴), 100.51 (galactosyl C-1), 103.20, 103.57, 103.74 (mannosyl C-1). Anal. Calcd. $C_{25}H_{44}O_{21}$: C, 44.05; H, 6.47. Found: C, 44.36; H, 6.61.

*2,3,6-Tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-*D*-galactopyranosyl)-*D*-glucopyranosyl trichloroacetimidate* (**10**).—To a solution of 2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-D-glucopyranose [9] (**9**, 1.13 g, 1.78 mmol) in dry CH_2Cl_2 (20 mL), trichloroacetonitrile (1 mL) was added and the mixture was stirred in an ice-water bath for 10 min. DBU (ten drops) was then added and the mixture was stirred for 2 h during which time the bath reached rt. Evaporation of the solvent in vacuo gave a dark-brown oil, which was purified by column chromatography eluting with 1:1 petroleum ether–EtOAc to give compound **10** (1.1 g, 79.7%), as a yellow syrup. R_f 0.31 (1:1 petroleum ether–EtOAc).

*Methyl (2,3,4,6-tetra-*O*-acetyl-β-*D*-galactopyranosyl)-(1→4)-(2,3,6-tri-*O*-acetyl-β-*D*-glucopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-α-*D*-mannopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-α-*D*-mannopyranosyl)-(1→6)-2,3,4-tri-*O*-benzyl-α-*D*-mannopyranoside* (**11**).—A mixture of compound **10** (270 mg, 0.35 mmol), compound **4** (290 mg, 0.22 mmol) and powered 4 Å molecular sieve (1 g) in dry CH_2Cl_2 (20 mL) was stirred for 2 h at rt and then cooled to −15 °C. Trimethylsilyl triflate (10 drops) was added and the mixture was stirred for 5 h. The mixture was filtered through a layer of Celite, rinsed with CH_2Cl_2, and then dried ($MgSO_4$). The residue resulting from evaporation of the solvent was subjected to silica gel column chromatography eluting with 3:1 cyclohexane–acetone to give compound **11** (yield 190 mg, 45%) as a colorless syrup; $[\alpha]_D$ +11.5° (*c* 0.17, $CHCl_3$); IR: 1748 cm^{-1} (C=O). ^{1}H NMR: δ 1.97, 2.04, 2.06, 2.08, 2.10, 2.15, 2.17 (7 s, 21 H, 7 × CH_3CO), 3.23 (s, 3 H, OCH_3), 3.68–5.22 (m, 53 H, sugar ring H, 9 × $PhCH_2$), 7.20–7.30 (45 H, aromatic H). ^{13}C NMR: δ 20.49, 20.62 (2 C), 20.71, 20.76, 20.80, 20.85 (7 × CH_3CO), 54.65 (OCH_3), 60.95–77.40 (39 C, sugar ring carbon, 9 × $PhCH_2$), 98.90, 99.0 (2 C) (C-1, C-1′, C-1″), 99.35, 101.14 (C-1‴, C-1⁗), 127.41–128.88 (aromatic carbon), 138.04, 138.30, 138.33, 138.37(2 C), 138.49(2 C), 138.53, 138.62 (aromatic quaternary carbon), 168.99, 169.46, 169.85, 170.03, 170.15, 170.25, 170.36 (7 C, CH_3CO). Anal. Calcd. for $C_{108}H_{122}O_{17}$: C, 66.60; H, 6.27. Found: C, 67.03; H, 6.20.

*Methyl (β-*D*-galactopyranosyl)-(1→4)-(β-D-glucopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-α-*D*-mannopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-α-*D*-mannopyranosyl)-(1→6)-(2,3,4-tri-*O*-benzyl-α-*D*-mannopyranoside)* (**12**).—To a solution of compound **11** (180 mg, 0.09 mmol) in abs MeOH (20 mL) CH_3ONa (10 mg) was added and the mixture was stirred for 2 h at rt. TLC showed that the reaction was complete. The solution was decationized, evaporated, and was purified by PTLC (3:1 $CHCl_3$–MeOH) to afford compound **12** (yield 125 mg, 81.7%) as a colorless syrup. R_f 0.33 (3:1 $CHCl_3$–MeOH).

*Methyl (β-*D*-galactopyranosyl)-(1→4)-(β-D-glucopyranosyl)-(1→2)-(α-D-mannopyranosyl)-(1→2)-(α-D-mannopyranosyl)-(1→6)-(α-D-mannopyranoside)* (**13**).—To a solution of compound **12** (114 mg, 0.07 mmol) in

MeOH (50 mL) was added Pd–C (10%) and the mixture was hydrogenolyzed in a hydrogen stream under a pressure of 33 KPa for 24 h. The mixture was filtered and the catalyst was rinsed with MeOH and a small amount of distilled water. Evaporation and lyophilization of the filtrate gave compound **13** (yield 48 mg, 82.6%) as a white amorphous solid; FABMS: (m/z) 843 $[M+1]^+$, 865 $[M+Na]^+$; $[\alpha]_D$ +35.6° (c 0.51, water). ^{1}H NMR (400 MHz, D_2O): δ 3.44 (s, 3 H, OCH_3), 3.35–3.93 (m, sugar ring H), 4.05 (dd, 1 H, mannosyl H-2), 4.27 (dd, 1 H, mannosyl H-2), 4.48 (d, 1 H, $J_{1,2}$ 7.97 Hz, glucosyl H-1), 4.59 (d, 1 H, $J_{1,2}$ 7.99 Hz, galactosyl H-1), 4.76 (d, 1 H, $J_{1,2}$ 1.48 Hz, mannosyl H-1), 5.14 (d, 1 H, $J_{1,2}$ 1.44 Hz, mannosyl H-1), 5.16 (d, 1 H, $J_{1,2}$ 1.28 Hz, mannosyl H-1). ^{13}C NMR: δ 57.37 (OCH_3), 62.58, 63.10, 63.48, 63.52, 68.29 (C-6, C-6′, C-6″, C-6‴, C-6⁗), 69.09, 69.46, 71.13 (2 C), 72.08, 72.45, 72.75, 73.21, 73.32, 73.53, 74.98, 75.09, 75.36, 75.73, 76.66, 77.34, 77.95 (2 C), 79. 86, 80.82, 81.38 (C-2, 3, 4, 5, C-2′, 3′, 4′, 5′, C-2″, 3″, 4″, 5″, C-2‴, 3‴, 4‴, 5‴, C-2⁗, 3⁗, 4⁗, 5⁗), 100.50, 102.87, 103.62, 104.01, 105.54 (C-1, C-1′, C-1″, C-1‴, C-1⁗). Anal. Calcd. for $C_{31}H_{56}O_{26}$: C, 44.18; H, 6.65. Found: C, 43.96; H, 6.88.

Acknowledgements

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