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Synthesis of tetrasaccharides as possible metastatic inhibitors [1]

Xiao Xiang Zhu, Ping Yu Ding, Meng Shen Cai *

Department of Organic Chemistry, School of Pharmaceutical Sciences, Beijing Medical University, Beijing 100083, PR China

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Abstract

The synthesis is reported of the possible metastatic inhibitors — methyl β-D-galactopyranosyl-(1 → 4)-(2-acetamido-2-deoxy-α-D-glucopyranosyl)-(1 → 6)-β-D-galactopyranosyl-(1 → 4)-β-D-glucopyranoside (**11**) and methyl β-D-galactopyranosyl-(1 → 4)-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1 → 6)-β-D-galactopyranosyl-(1 → 4)-β-D-glucopyranoside (**14**) — by procedures for regio- and stereo-selective coupling, reduction of azido groups, *N*-acetylation, and *O*-deacetylation. © 1996 Elsevier Science Ltd.

Keywords: Tetrasaccharide; Chemoselective glycosylation; Metastatic inhibitor

1. Introduction

Metastasis is one of the major causes of mortality in cancer. The pathogenesis of metastases can be subdivided into a variety of sequential steps. During the sequential steps of metastasis, metastasizing tumor cells encounter various host cells (platelets, lymphocytes, or endothelial cells) and (or) extracellular matrix and basement-membrane components (laminin and fibronectin) [1,2]. As a result of adhesive interaction, this encounter may result in embolus formation which can subsequently enhance the survival, arrest, or invasiveness of tumor cells [3–5]. Specific incidents of tumor

* Corresponding author.

[1] Studies on carbohydrates XXIV.

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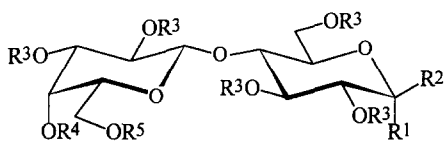
interaction with host cells or components are therefore fundamental events in the metastatic process. Consequently, both adhesion and detachment of cells are thought to be of prime importance in achieving control of the cellular function of diverse cell types, including highly metastatic cells.

Our research group observed that *N*-acetyllactosamine and lactose are capable of inhibiting the attachment of tumor cells (S180) to laminin, and that *N*-acetyllactosamine was more effective than lactose [6]. In addition, Raz and Lotan [7] demonstrated the presence of lactose-binding lectins at the tumor cell surface, and Oguchi and Toyokuni et al. [8] reported that methyl β-lactoside reduces the formation of lung colonies in mice injected with mouse B16 melanoma cells. We have, therefore, synthesized tetrasaccharides composed of lactose and *N*-acetyllactosamine to explore the possible prevention of metastatic spread by inhibiting the attachment of tumor cells to the macromolecules, and by blocking the cognitive interactions among tumor cells and between tumor and hosts with a competitive oligosaccharide.

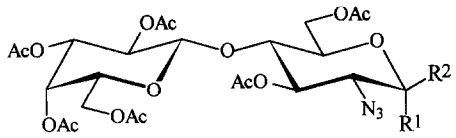
2. Results and discussion

Compound **2** was prepared by an improved procedure. Unlike the standard method [9], condensation of 2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-α-D-glucopyranosyl bromide (**1**) with MeOH in CH_2Cl_2 using silver zeolite as a promoter gave a single product **2** in high yield. Compound **2** was deacetylated with NaOMe in MeOH, benzylidenated with benzaldehyde and ethyl orthoformate in the presence of *p*-toluenesulfonic acid as catalyst, and *O*-acetylated to furnish **5** [9] in 66% overall yield. Treatment of **5** with 90% aqueous trifluoroacetic acid afforded debenzylidenated compound **6** [9] (86%). Both **7** and **8** were obtained by treatment of (2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1 → 4)-3,6-di-*O*-acetyl-2-azido-2-deoxy-D-glucopyranose with trichloroacetonitrile in the presence of K_2CO_3 [10].



OMe

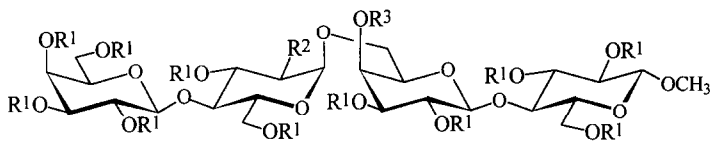
	R1	R2	R3	R4	R5
1	Br	H	Ac	Ac	Ac
2	H	OMe	Ac	Ac	Ac
3	H	OMe	H	H	H
4	H	OMe	H	PhCH	
5	H	OMe	Ac	PhCH	
6	H	OMe	Ac	H	H



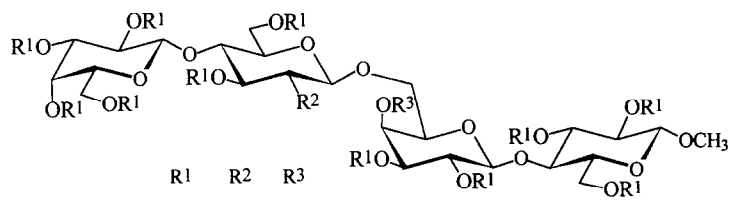
7 R1 = OC(NH)CCl3, R2 = H

8 R1 = H, R2 = OC(NH)CCl3

Regio- and stereo-selective coupling of **8** with **6** in CH_2Cl_2 using boron trifluoride diethyl etherate as a promoter gave tetrasaccharide derivative **9** [11] (57%). Similarly, regio- and stereo-selective coupling of **7** with **6** in CH_2Cl_2 in the presence of trimethylsilyl triflate afforded tetrasaccharide derivative **12** (53%) [12]. Compounds **9** and **12** were hydrogenated using 10% Pd–C as a catalyst and then *N*-acetylated to give **10** and **13** in 89 and 81% yields, respectively. Treatment of **10** and **13** with NaOMe in MeOH led to the desired tetrasaccharides **11** and **14**, respectively. The free tetrasaccharides will be tested for their activity in the prevention of tumor metastasis in vitro and in vivo.



	R1	R2	R3
9	Ac	N_3	H
10	Ac	NHAc	Ac
11	H	NHAc	H



	R1	R2	R3
12	Ac	N_3	H
13	Ac	NHAc	Ac
14	H	NHAc	H

3. Experimental

General methods.—Optical rotations were determined with a Perkin–Elmer Model 241 MC polarimeter at 15 °C at the sodium D-line. Column chromatography was

performed on Silica Gel H (Qingdao) and fractions were monitored by TLC on Silica Gel GF_{254} (Qingdao). Detection was effected by examination under UV light and by charring with 5% phosphomolybdic acid hydrate in EtOH. The elemental analysis data were determined on a PE 240C spectrometer. ^{1}H NMR spectra were recorded at 300 MHz with a Varian VXR 300 and at 500 MHz with a Bruker AM 500 apparatus at 25 °C. ^{13}C NMR spectra were recorded at 75 MHz with a Varian VXR 300 and at 125 MHz with a Bruker AM 500 apparatus at 25 °C. The values of δ_H and δ_C are expressed in ppm downward from the signal for internal Me_4Si for solutions in $CDCl_3$ and for external sodium 4,4-dimethyl-4-silapentane-1-sulfonate for solution in D_2O. The letters a, b, c, d are used to designate the glycosyl residue in which a cited H and C atom is located. The bp of petroleum ether is 60–90 °C.

Methyl 2,3,4,6-tetra-O-*acetyl*-β-D-*galactopyranosyl-(1 → 4)-2,3,6-tri*-O-*acetyl*-β-D-*glucopyranoside* (**2**).—A mixture of 2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-α-D-glucopyranosyl bromide (**1**) (8 g, 11.4 mmol), dry MeOH (25 mL), and silver zeolite (2 g) in dry CH_2Cl_2 (60 mL) was vigorously stirred overnight at rt. The mixture was filtered through a bed of Celite and the organic phase was evaporated affording **2** (7.2 g, 97%) as a colourless syrup without further purification; R_f 0.24 (2:1 petroleum ether–acetone); ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.32–169.06 (C=O), 101.45 (C-1b), 101.11 (C-1a), 76.34 (C-4a), 72.97 (C-3a), 72.76 (C-5a), 71.79 (C-2a), 71.09 (C-3b), 70.62 (C-5b), 69.30 (C-2b), 66.76 (C-4b), 62.12 (C-6a), 60.92 (C-6b), 56.92 (OCH_3), 20.81–20.61 (Ac).

Methyl 2,3-di-O-*acetyl-4,6*-O-*benzylidene*-β-D-*galactopyranosyl-(1 → 4)-2,3,6-tri*-O-*acetyl*-β-D-*glucopyranoside* (**5**).—A solution of **2** (3.4 g, 5.2 mmol) in dry MeOH (30 mL) was treated with NaOMe (pH 9). After 12 h, the solution was neutralized with 732 (H^+) cation-exchange resin, filtered through cotton, and concentrated in vacuo affording **3** (1.8 g, 97%) as a white solid. A mixture of **3** (1.8 g, 5.06 mmol), benzaldehyde (5 mL), ethyl orthoformate (1.7 mL), *N*,*N*-dimethylformamide (15 mL), and *p*-toluenesulfonic acid monohydrate (0.1 g) in dry acetonitrile (40 mL) was stirred for 6 h at 50 °C. After neutralization with Et_3N (0.2 mL), the mixture was evaporated in vacuo to give **4** (1.73 g, 77%); R_f 0.34 (4:1 $CHCl_3$–EtOH). The crude **4** was dissolved in pyridine (20 mL) and Ac_2O (20 mL) and then stirred at rt for 24 h. Work-up in the usual manner gave **5**, which was purified by column chromatography with 4:1 petroleum ether–acetone as eluent (2.27 g, 89%); [α] +42° (*c* 1, $CHCl_3$); R_f 0.32 (2:1 petroleum ether–acetone); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.45–7.35 (m, 5 H, Ph), 5.45 (s, 1 H, CH–Ph), 4.46 (d, 1 H, *J* 7.8 Hz, H-1a), 4.38 (d, 1 H, *J* 8.7 Hz, H-1b), 3.46 (s, 3 H, OCH_3), 2.15, 2.10, 2.08, 2.05, 2.02 (s, each 3 H, 5 Ac).

Methyl 2,3-di-O-*acetyl*-β-D-*galactopyranosyl-(1 → 4)-2,3,6-tri*-O-*acetyl*-β-D-*glucopyranoside* (**6**).—Aqueous trifluoroacetic acid (90%, 5 mL) was added to a cold solution of **5** (2 g, 3 mmol) in CH_2Cl_2 (50 mL), and the mixture was stirred for 1 h at 0 °C. The mixture was then washed with water, aq satd $NaHCO_3$, and finally with water, dried, filtered, and concentrated. Column chromatography (12:5 petroleum ether–acetone) of the residue gave **6** (1.49 g, 86%) as a white solid; [α] −12.5° (*c* 0.5, $CHCl_3$); R_f 0.12 (2:1 petroleum ether–acetone); ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.48 (d, 1 H, *J* 7.5 Hz, H-1a), 4.39 (d, 1 H, *J* 7.8 Hz, H-1b), 3.46 (s, 3 H, OCH_3), 2.10, 2.09, 2.08, 2.06, 2.03 (s, each 3 H, 5 Ac); ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.53–169.4

(C=O), 101.23 (C-1b), 101.05 (C-1a), 76.36, 74.42, 73.54, 73.25, 72.57, 71.58, 69.68, 67.64, 62.23, 61.95 (sugar ring C), 56.97 (OCH_3).

2,3,4,6-Tetra-O-*acetyl*-β-D-*galactopyranosyl*-(*1 → 4*)-*3,6-di*-O-*acetyl*-*2-azido*-*2-deoxy*-α(*and* β)-D-*glucopyranosyl trichloroacetimidate* **7**) and **8**).—A mixture of *O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1 → 4)-3,6-di-*O*-acetyl-2-azido-2-deoxy-D-glucopyranose (2.0 g, 3.23 mmol), trichloroacetonitrile (2 mL), and anhyd K_2CO_3 (2 g) in dry CH_2Cl_2 (50 mL) was vigorously stirred at rt. After 3 h, more anhyd K_2CO_3 (0.9 g) was added and the mixture was stirred for another 3 h. The mixture was filtered and the filtrate was concentrated in vacuo. Column chromatography (7:2 petroleum ether–acetone) of the residue gave α anomer **7** (0.82 g, 33%) and β anomer **8** (1.23 g, 50%).

Compound **7** had [α] +112° (*c* 1, $CHCl_3$); R_f 0.45 (2:1 petroleum ether–acetone); The chemical shifts for ^1H NMR (300 MHz, $CDCl_3$) agreed with those reported [13]: δ 8.81 (s, 1 H, NH), 6.44 (d, 1 H, *J* 4.2 Hz, H-1a), 4.51 (d, 1 H, *J* 7.8 Hz, H-1b).

Compound **8** had [α] −2° (*c* 1, $CHCl_3$); R_f 0.50 (2:1 petroleum ether–acetone); The chemical shifts for ^1H NMR (300 MHz, $CDCl_3$) agreed with those reported [13]: δ 8.79 (s, 1 H, NH), 5.67 (d, 1 H, *J* 7.8 Hz, H-1a), 4.46 (d, 1 H, *J* 7.5 Hz, H-1b).

Methyl 2,3,4,6-tetra-O-*acetyl*-β-D-*galactopyranosyl*-(*1 → 4*)-(*3,6-di*-O-*acetyl*-*2-azido*-*2-deoxy*-α-D-*glucopyranosyl*)-(*1 → 6*)-(*2,3-di*-O-*acetyl*-β-D-*galactopyranosyl*)-(*1 → 4*)-*2,3,6-tri*-O-*acetyl*-β-D-*glucopyranoside* (**9**).—A mixture of **8** (0.26 g, 0.34 mmol), **6** (0.17 g, 0.30 mmol), and molecular sieves (4 Å) in CH_2Cl_2 (10 mL) was stirred for 3 h at rt and cooled to −20 °C. To the mixture was added dropwise trimethylsilyl triflate (0.3 mL of 0.5 M solution in CH_2Cl_2). After 4 h, TLC indicated the formation of a new spot. The mixture was neutralized with $NaHCO_3$ (100 mg), filtered, and concentrated in vacuo. Column chromatography of the residue afforded **9** as a white powder (200 mg, 57%); [α] +26° (*c* 1, $CHCl_3$); R_f 0.12 (3:2 petroleum ether–acetone); IR (KBr, cm^{-1}): ν 3504 (O–H), 2111 (N_3), 1749 (C=O); ^1H NMR (300 MHz, $CDCl_3$): δ 4.65 (d, 1 H, *J* 3.0 Hz, H-1c), 4.50–4.47 (2 H, H-1b, H-1d), 4.43 (d, 1 H, *J* 8.0 Hz, H-1a), 3.38 (s, 3 H, OMe); ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.24–166.89 (C=O), 101.33, 101.23, 101.03, 100.62 (C-1a, C-1b, C-1c, C-1d), 20.80 (Ac). Anal. Calcd for $C_{47}H_{65}N_3O_{31}$: C, 48.33; H, 5.61; N, 3.60. Found: C, 48.17; H, 5.63; N, 3.58.

Methyl 2,3,4,6-tetra-O-*acetyl*-β-D-*galactopyranosyl*-(*1 → 4*)-(*2-acetamido*-*3,6-di*-O-*acetyl*-*2-deoxy*-α-D-*glucopyranosyl*)-(*1 → 6*)-(*2,3,4-tri*-O-*acetyl*-β-D-*galactopyranosyl*)-(*1 → 4*)-*2,3,6-tri*-O-*acetyl*-β-D-*glucopyranoside* (**10**).—A solution of **9** (150 mg, 0.129 mmol) in MeOH (50 mL) was hydrogenated at 0.3 MPa over 10% Pd–C (80 mg). After 12 h, the mixture was filtered, concentrated, and treated with pyridine (5 mL) and Ac_2O (5 mL). The mixture was concentrated to dryness, and column chromatography of the residue gave **10** (140 mg, 89%) as a white powder; [α] +5° (*c* 1, $CHCl_3$); R_f 0.15 (1:1 petroleum ether–acetone); IR (KBr, cm^{-1}): ν 3301 (N–H), 1747 (C=O), 1673 (amide I), 1521 (amide II); ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.81–168.86 (C=O), 101.27, 101.21, 101.04, 97.7 (C-1a, C-1b, C-1c, C-1d), 22.81 (NHAc), 20.64 (OAc). Anal. Calcd for $C_{51}H_{71}NO_{33}$: C, 49.96; H, 5.84; N, 1.14. Found: C, 49.75; H, 5.82; N, 1.09.

Methyl β-D-*galactopyranosyl*-(*1 → 4*)-(*2-acetamido*-*2-deoxy*-α-D-*glucopyranosyl*)-(*1 → 6*)-β-D-*galactopyranosyl*-(*1 → 4*)-β-D-*glucopyranoside* (**11**).—A solution of **10**

(140 mg, 0.114 mmol) in dry MeOH (30 mL) was treated with a catalytic amount of NaOMe (pH 9). After 24 h, the solution was neutralized with 732 (H^+) cation-exchange resin, filtered, and concentrated. The product **11** (81 mg, 98%) was dissolved in water and lyophilized; [α] +1° (*c* 1, H_2O); ^{1}H NMR (500 MHz, D_2O): δ 4.73 (d, 1 H, *J* 3.5 Hz, H-1c), 4.54 (d, 1 H, *J* 7.6 Hz, H-1b), 4.49 (d, 1 H, *J* 7.8 Hz, H-1a), 4.45 (d, 1 H, *J* 8.4 Hz, H-1d), 3.36 (s, 3 H, OMe), 1.84 (s, 3 H, NHAc); ^{13}C NMR (125 MHz, D_2O): δ 176.94 (C=O), 105.25, 105.18, 103.61, 102.64 (C-1a, C-1b, C-1c, C-1d), 24.58 (NHAc). Anal. Calcd for $C_{27}H_{47}NO_{21}$: C, 44.94; H, 6.56; N, 1.94. Found: C, 44.75; H, 6.48; N, 2.01.

Methyl 2,3,4,6-tetra-O-*acetyl*-β-D-*galactopyranosyl-(1 → 4)-(3,6-di*-O-*acetyl-2-azido-2-deoxy*-β-D-*glucopyranosyl)-(1 → 6)-(2,3-di*-O-*acetyl*-β-D-*galactopyranosyl)-(1 → 4)-2,3,6-tri*-O-*acetyl*-β-D-*glucopyranoside* (**12**).—A mixture of **6** (0.56 g, 0.99 mmol) and **7** (0.56 g, 0.73 mmol) in CH_2Cl_2–n-C_6H_{14} (1:1, 4 mL) was stirred with powdered molecular sieves (4 Å) for 3 h. To the solution at −20 °C was added dropwise diethyl ether–boron trifluoride (0.7 mL of a 0.5 M solution in CH_2Cl_2. After 6 h, the mixture was neutralized with $NaHCO_3$ (200 mg), filtered, and concentrated in vacuo. Column chromatography (2:1 petroleum ether–acetone) of the residue afforded **12** (0.45 g, 53%) as a white powder; [α] −12° (*c* 1, $CHCl_3$); R_f 0.1 (3:2 petroleum ether–acetone); IR (KBr, cm^{-1}): ν 3484 (O–H), 2112 (N_3), 1750 (C=O); ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.54 (d, 1 H, *J* 7.8 Hz, H-1b), 4.50 (d, 1 H, *J* 8.4 Hz, H-1c), 3.50 (s, 3 H, OMe); ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.34–168.67 (C=O), 101.77, 101.38, 101.29, 101.10 (C-1a, C-1b, C-1c, C-1d), 20.72 (Ac). Anal. Calcd. for $C_{47}H_{65}N_3O_{31}$: C, 48.33; H, 5.61; N, 3.60. Found: C, 48.26; H, 5.51; N, 3.63.

Methyl 2,3,4,6-tetra-O-*acetyl*-β-D-*galactopyranosyl-(1 → 4)-(2-acetamido-3,6-di*-O-*acetyl-2-deoxy*-β-D-*glucopyranosyl)-(1 → 6)-(2,3,4-tri*-O-*acetyl*-β-D-*galactopyranosyl)-(1 → 4)-2,3,6-tri*-O-*acetyl*-β-D-*glucopyranoside* (**13**).—Hydrogenation of **12** (200 mg, 0.17 mmol) and *N*-acetylation was carried out by the procedures as described for the preparation of compound **10**, affording **13** (0.17, 81%) as a white powder; [α] −6° (*c* 1, $CHCl_3$); R_f 0.12 (1:1 petroleum ether–acetone); IR (KBr, cm^{-1}): ν 3481 (N–H), 1751 (C=O), 1668 (amide I), 1529 (amide II); ^{13}C NMR (125 MHz, $CDCl_3$): δ 101.65, 101.44, 101.26, 100.80 (C-1a, C-1b, C-1c, C-1d), 22.69 (NHAc), 20.71–20.57 (OAc). Anal. Calcd. for $C_{51}H_{71}NO_{33}$: C, 49.96; H, 5.84; N, 1.14. Found: C, 49.99; H, 5.77; N, 1.13.

Methyl β-D-*galactopyranosyl-(1 → 4)-(2-acetamido-2-deoxy*-β-D-*glucopyranosyl)-(1 → 6)-*β-D-*galactopyranosyl-(1 → 4)-*β-D-*glucopyranoside* (**14**).—A solution of **13** (170 mg, 0.139 mmol) in dry MeOH (30 mL) was treated with a catalytic amount of NaOMe (pH 9). After 24 h, the solution was neutralized with 732 (H^+) cation-exchange resin, filtered, and concentrated in vacuo. After lyophilizing, the product **14** (91 mg, 91%) was obtained as an amorphous powder; [α] −4.5° (*c* 1, H_2O); ^{1}H NMR (500 MHz, D_2O): δ 4.62 (d, 1 H, *J* 7.7 Hz, H-1c), 4.29 (d, 1 H, *J* 8.8 Hz, H-1a), 4.25 (d, 1 H, *J* 7.8 Hz, H-1d), 4.20 (d, 1 H, *J* 7.8 Hz, H-1b), 3.21 (s, 3 H, OMe), 2.05 (s, 3 H, NHAc); ^{13}C NMR (125 MHz, D_2O): δ 176.75 (C=O), 105.40, 105.27, 105.11, 103.20 (C-1a, C-1b, C-1c, C-1d), 22.54. Anal. Calcd for $C_{27}H_{47}NO_{21}$: C, 44.94; H, 6.56; N, 1.94. Found: C, 44.88; H, 6.60; N, 1.89.

Acknowledgement

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