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Note

Synthesis of oligosaccharide inhibitors of neural cell division

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The availability of inhibitors of astrocyte division has practical importance because most tumors of the central nervous system are gliomas and the so-called glial scar is the major obstacle to regeneration of the central axon[1]. A soluble mitogen inhibitor, immunologically related to the epidermal growth factor receptor (EGFR) and to blood groups A, H, or Le, occurs in brain extracts[2,3]. Based on the data available, the oligosaccharide glycosides methyl *O*-2-acetamido-2-deoxy-α-D-galactopyranosyl-(1 → 3)-*O*-β-D-galactopyranosyl-(1 → 4)-β-D-glucopyranoside (**1**) and methyl *O*-2-acetamido-2-deoxy-α-D-galactopyranosyl-(1 → 3)-*O*-β-D-galactopyranosyl-(1 → 4)-*O*-[α-L-fucopyranosyl-(1 → 3)]-β-D-glucopyranoside (**2**) have been designed, synthesised, and tested for the ability to inhibit the division of astrocytes, gliomas, and neuroblastomas. Compounds **1** and **2** are structurally related to the oligosaccharides of blood groups A and Le^x.

Partial benzylation[4,5] of methyl 3′-*O*-allyl-β-lactoside[6] (**3**) with benzyl chloride–potassium hydroxide gave the hexa-*O*-benzyl derivative **4** (41%) and the 2,6,2′,4′,6′-penta-*O*-benzyl derivative **5** (28%) with HO-3 unsubstituted. Deallylation of **4** gave **6** (55%), which was glycosylated with 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-α-D-galactopyranosyl bromide[7] (**7**), using mercuric salt promotion[8], to give the trisaccharide derivative **8** (87%) with α-stereospecificity (δ 5.17, d, $J_{1'',2''}$ 3.5 Hz, H-1″). Hydrogenolysis of the benzyl groups of **8**, reduction of the azide to amine, and *N*-acetylation gave **9**, *O*-deacetylation of which afforded the target glycoside **1**.

α-Fucosylation of **5** with 2,3,4-*O*-benzyl-α-L-fucopyranosyl bromide[9] in the presence of mercuric bromide gave the trisaccharide derivative **10** (84%; δ 5.61, $J_{1',2'}$ 3.3 Hz, H-1). Deallylation of **10** gave **11** (65%) with HO-3′ unsubstituted. Glyco-

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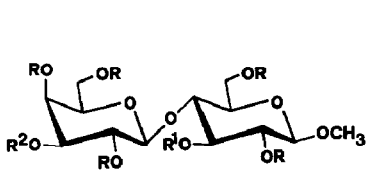
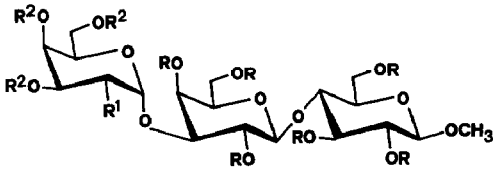


3 R = R¹ = H , R² = All

4 R = R¹ = Bn , R² = All

5 R = Bn , R¹ = H , R² = All

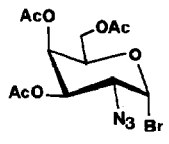
6 R = R¹ = Bn , R² = H



1 R = R² = H , R¹ = NHAc

8 R = Bn , R¹ = N₃ , R² = Ac

9 R = H , R¹ = NHAc , R² = Ac



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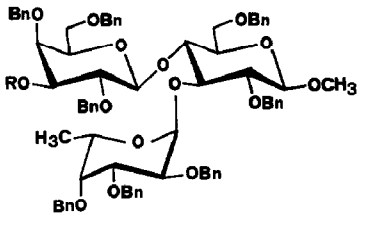
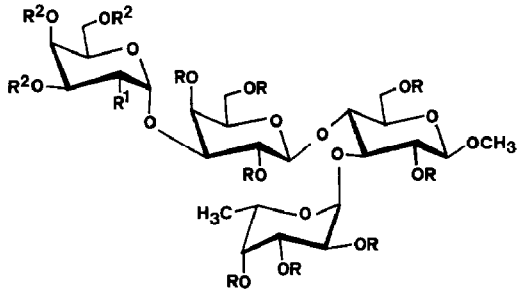


10 R = All

11 R = H



2 R = R² = H , R¹ = NHAc

12 R = Bn , R¹ = N₃ , R² = Ac

13 R = H , R¹ = NHAc , R² = Ac

sylation of **11** with **7**[7], as for **6**, gave the tetrasaccharide derivative **12**, which could not be isolated pure, but was debenzylated and then reduced in the presence of acetic anhydride to give **13** (74% from **11**). The ^{1}H-NMR data for **13** (δ 5.40, d, $J_{1'',2''}$ 3.7 Hz, H-1″) demonstrated the new glycosidic bond to be α. *O*-Deacetylation of **13** provided the target tetrasaccharide glycoside **2**.

Tests in cultures of neonatal astrocytes, A7 astrocytoma, neuro-2a (N2a) neuroblastoma, and RN22 schwannoma (Table I) showed that **2** was a better mitosis inhibitor of each cell line than **1**, suggesting that the fucose moiety in **2** is important, although the effect of this residue varied with the cell type. Neither **1** nor **2** was cytotoxic. More detailed information of this biological activity will be given elsewhere[10].

EXPERIMENTAL

General.—Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. TLC was performed on Silica Gel GF_{254} (Merck) with detection by charring with H_2SO_4. Column chromatography was performed on silica gel (Merck 70–230). NMR spectra were recorded with a Varian XL-300 (^1H, 300 MHz), Bruker AM-200 (^1H, 200 MHz; ^{13}C, 50 MHz), or Bruker WP-80 spectrometer (^{13}C, 20 MHz). Optical rotations were determined with a Perkin–Elmer 141 polarimeter.

Partial benzylation of methyl 3'-O-allyl-β-lactoside (**3**).—A mixture of **3**[4] (5 g, 14 mmol), benzyl chloride (30 mL), and KOH (9 g) was stirred for 30 min at 100°, then cooled, and diluted with $CHCl_3$ (150 mL). The organic solution was washed with water, M H_2SO_4, and water, dried (Na_2SO_4), and concentrated. Column chromatography (hexane–EtOAc, 1:0 → 4:1) of the residue gave, first, methyl 3'-*O*-allyl-2,3,6,2',4',6'-hexa-*O*-benzyl-β-lactoside[11] (**4**; 4.90 g, 41%) and then methyl 3'-*O*-allyl-2,6,2',4',6'-penta-*O*-benzyl-β-lactoside (**5**; 2.92 g, 28%), $[\alpha]_D$ −10° (*c* 0.6, $CHCl_3$).

Anal. Calcd for $C_{51}H_{58}O_{11}$: C, 72.32; H, 6.90. Found: C, 72.60; H, 7.09.

Methyl O-(3,4,6-tri-O-acetyl-2-azido-2-deoxy-α-D-galactopyranosyl)-(1 → 3)-O-(2,4,6-tri-O-benzyl-β-D-galactopyranosyl)-(1 → 4)-2,3,6-tri-O-benzyl-β-D-glucopyranoside (**8**).—Compound **4** (5 g, 5.34 mmol) was stirred with EtOAc–EtOH–acetic acid–water (2:2:1:1, 60 mL) and 10% Pd–C (200 mg) for 10 h at 80–90°, then filtered. The insoluble material was washed with $CHCl_3$, and the combined filtrate and washings were washed with satd aq $NaHCO_3$ and water, and concentrated. Column chromatography (hexane–EtOAc, 5:1 → 3:1) of the residue gave **6** (2.61 g, 55%). A mixture of **6** (1.5 g, 1.67 mmol), mercuric cyanide (2.41 g, 9.53 mmol), mercuric bromide (3.48 g, 9.65 mmol), molecular sieves 4A (7 g), and CH_2Cl_2 (85 mL) was stirred for 1 h at room temperature under Ar. A solution of the glycosyl bromide **7**[7] (0.77 g, 1.95 mmol) in CH_2Cl_2 (5 mL) was added slowly. After 13 and 22 h, more **7** (0.85 g per addition) was added, and the reaction was continued for 60 h. The mixture was filtered, washed with aq 10% NaI, satd aq $NaHCO_3$, and water, dried (Na_2SO_4), and concentrated. Column chromatography (hexane–EtOAc, 3:1 → 7:4) of the residue gave **8**, isolated as a syrup (1.7 g, 87%), $[\alpha]_D$

TABLE I

Inhibition of cell proliferation by **1** and **2**

Compound	ID_{50} (μg/mL) [a]			
	Astrocytes	A7	N2a	RN22
1	131	203	181	137
2	54	34	17	27

[a] ID_{50} is the dose that inhibited 50% of the incorporation of [^3H]thymidine into cells, promoted by 10% foetal calf serum, and was obtained from dose–response curves. The incorporation of [^3H]thymidine was measured as described[2].

+61° (*c* 0.7, $CHCl_3$). ^{1}H-NMR data ($CDCl_3$): δ 7.4–7.1 (m, 30 H, 6 Ph), 5.33 (dd, 1 H, $J_{2'',3''}$ 11.0, $J_{3'',4''}$ 3.3 Hz, H-3''), 5.17 (d, 1 H, $J_{1'',2''}$ 3.5 Hz, H-1''), 5.04 (dd, 1 H, $J_{4'',5''}$ 1.0 Hz, H-4''), 4.46 (d, 1 H, $J_{1,2}$ 7.5 Hz, H-1), 3.50 (s, 3 H, OMe), 2.05, 2.01, and 1.80 (3 s, 3 H each, 3 Ac).

Anal. Calcd for $C_{67}H_{75}N_3O_{18}$: C, 66.49; H, 6.25; N, 3.47. Found: C, 66.70; H, 6.10; N, 3.35.

Methyl O-*(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-*D*-galactopyranosyl)-(1→3)-*O*-β-*D*-galactopyranosyl-(1→4)-β-*D*-glucopyranoside* (**9**).—A solution of **8** (1.75 g, 1.45 mmol) in EtOH (100 mL) and acetic anhydride (2 mL) was shaken under H_2 for 4 days at room temperature in the presence of 10% Pd–C, then filtered through Celite, and concentrated. Column chromatography ($CHCl_3$–MeOH, 8:1) of the residue gave **9** (0.75 g, 75%), mp 149–151°, $[\alpha]_D$ +94° (*c* 0.6, MeOH). ^{1}H-NMR data (CD_3OD): δ 5.40 (dd, 1 H, $J_{3'',4''}$ 3.3, $J_{4'',5''}$ 1.2 Hz, H-4''), 5.23 (dd, 1 H, $J_{2'',3''}$ 11.5 Hz, H-3''), 5.05 (d, 1 H, $J_{1'',2''}$ 3.7 Hz, H-1''), 4.50 (dd, 1 H, H-2''), 3.51 (s, 3 H, OMe), 2.12, 2.00 (2 s, 3 H each, 2 Ac), and 1.93 (s, 6 H, 2 Ac).

Anal. Calcd for $C_{27}H_{43}NO_{19}$: C, 47.30; H, 6.32; N, 2.04. Found: C, 46.95; H, 6.44; N, 2.34.

Methyl O-*(2-acetamido-2-deoxy-α-*D*-galactopyranosyl)-(1→3)-*O*-β-*D*-galactopyranosyl-(1→4)-β-*D*-glucopyranoside* (**1**).—A solution of **9** (0.75 g, 1.12 mmol) in methanolic 0.1 M sodium methoxide (4 mL) was kept for 30 min at room temperature, then neutrallised with Amberlite IR-120 (H^+) resin, and filtered. The solvent was evaporated to give **1** (0.54 g, 95%), mp 293–298°, $[\alpha]_D$ +124° (*c* 0.5, H_2O). ^{1}H-NMR data (D_2O): δ 4.96 (d, 1 H, $J_{1'',2''}$ 3.8 Hz, H-1''), 4.38 and 4.28 (2 d, each 1 H, H-1,1'), 3.45 (s, 3 H, OMe), and 1.92 (s, 3 H, Ac); ^{13}C, δ 104.4, 104.1 (C-1,1'), 95.3 (C-1''), 80.0, 78.1, 76.5, 76.0, 75.8, 74.1, 72.2, 70.9, 69.7, 68.9, 66.1, 62.3 (2 C), 61.5, 58.5, 51.0 (OCH_3), and 23.3 (CH_3CO).

Anal. Calcd for $C_{21}H_{37}NO_{16}$: C, 45.08; H, 6.66; N, 2.50. Found: C, 44.69; H, 6.75; N, 2.46.

Methyl O-*(3-*O*-allyl-2,4,6-tri-*O*-benzyl-β-*D*-galactopyranosyl)-(1→4)-*O*-[2,3,4-tri-*O*-benzyl-α-*L*-fucopyranosyl-(1→3)]-β-*D*-glucopyranoside* (**10**).—A mixture of **5** (2.94 g, 3.48 mmol), mercuric bromide (0.4 g, 1.11 mmol), molecular sieves 4A (5.7 g), and CH_2Cl_2 (40 mL) was stirred for 1 h at room temperature. A solution of 2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl bromide[9] (2.4 g, 4.83 mmol) in CH_2Cl_2 (40 mL) was then added during 5 h, stirring was continued for 1 h, and the mixture was filtered through Celite, washed with aq 10% NaI, satd aq $NaHCO_3$, and water, dried (Na_2SO_4), and concentrated. Column chromatography (hexane–EtOAc, 5:1) of the residue gave **10** (3.94 g, 84%), $[\alpha]_D$ −40° (*c* 0.7, $CHCl_3$). ^{1}H-NMR data ($CDCl_3$): δ 7.4–7.2 (m, 40 H, 8 Ph), 6.0–5.4 (m, 1 H, CH_2=C*H*CH_2), 5.61 (d, 1 H, $J_{1',2'}$ 3.3 Hz, H-1'), 3.46 (s, 3 H, OMe), and 1.09 (d, 3 H, $J_{5',6'}$ 6.5 Hz, H-6').

Anal. Calcd for $C_{78}H_{86}O_{15}$: C, 74.14; H, 6.86. Found: C, 73.99; H, 7.01.

Methyl O-*(2,3,4-tri-*O*-benzyl-β-*D*-galactopyranosyl)-(1→4)-*O*-[2,3,4-tri-*O*-benzyl-α-*L*-fucopyranosyl (1→3)]-β-*D*-glucopyranoside* (**11**).—A solution of **10** (3.57 g, 2.83 mmol) in EtOAc (28 mL) and 95% EtOH (140 mL) was treated with toluene-*p*-

sulfonic acid (0.28 g) in the presence of 10% Pd–C (0.52 g) at 80° for 1.5 h, then filtered through Celite, treated with triethylamine (0.5 mL), and concentrated. Column chromatography (hexane–EtOAc, 4:1) of the residue gave **11** (2.24 g, 65%), $[\alpha]_D$ −41° (*c* 0.56, $CHCl_3$). ^{1}H-NMR data ($CDCl_3$): δ 7.4–7.1 (m, 40 H, 8 Ph), 5.61 (d, 1 H, $J_{1',2'}$ 3.2 Hz, H-1'), 3.44 (s, 3 H, OMe), 2.20 (d, 1 H, OH), and 1.09 (d, 3 H, $J_{5',6'}$ 6.5 Hz, H-6').

Anal. Calcd for $C_{75}H_{82}O_{15}$: C, 73.63; H, 6.75. Found: C, 73.71; H, 7.01.

Methyl O-(2-acetamido-2-deoxy-α-D-galactopyranosyl)-(1 → 3)-O-β-D-galactopyranosyl-(1→4)-O-[α-L-fucopyranosyl-(1→3)]-β-D-glucopyranoside (**2**).—A mixture of **11** (2.12 g, 1.73 mmol), $Hg(CN)_2$ (2.1 g, 8.31 mmol), $HgBr_2$ (3.04 g, 8.43 mmol), molecular sieves 4A (11.4 g), and CH_2Cl_2 (76 mL) was stirred for 1 h at room temperature and then a solution of **7** (2 g, 5.07 mmol) in CH_2Cl_2 (40 mL) was added. After 20, 30, 52, and 76 h, more **7** (0.4, 0.43, 0.23, and 0.2 g, respectively) was added, and, after 92 h, more $Hg(CN)_2$ (1.05 g) and $HgBr_2$ (1.52 g). After 120 h, the mixture was filtered through Celite, washed with aq. 10% NaI, satd aq $NaHCO_3$, and water, dried (Na_2SO_4), and concentrated. Column chromatography (hexane–EtOAc, 3:1 → 7:4) of the residue gave a fraction (2.2 g) containing **12** and **7**, a solution of which in EtOH (100 mL) and acetic anhydride (1.5 mL) was treated with H_2 in the presence of 10% Pd–C (0.5 g) for 60 h at room temperature, then filtered through Celite, and concentrated. Column chromatography ($CHCl_3$–MeOH, 6:1) of the residue gave methyl *O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-galactopyranosyl)-(1→3)-*O*-β-D-galactopyranosyl-(1→4)-*O*-[α-L-fucopyranosyl-(1→3)]-β-D-glucopyranoside (**13**; 0.86 g, 74%), mp 165–175°, $[\alpha]_D$ +28° (*c* 0.9, MeOH). ^{1}H-NMR data ($CDCl_3$): δ 5.44 (d, 1 H, $J_{1',2'}$ 3.9 Hz, H-1'), 5.40 (dd, 1 H, $J_{3''',4'''}$ 3.2, $J_{4''',5'''}$ 0.9 Hz, H-4'''), 5.24 (dd, 1 H, $J_{2''',3'''}$ 11.5, H-3'''), 5.04 (d, 1 H, $J_{1''',2'''}$ 3.7 Hz, H-1'''), 4.51 (dd, 1 H, H-2'''), 4.45 (t, 1 H, $J_{3'',4''} = J_{4'',5''}$ 3.7 Hz, H-4''), 4.22 (dd, 1 H, $J_{5''',6'''a}$ 6.7, $J_{6'''a,6'''b}$ 11.1 Hz, H-6'''a), 4.18 (d, 1 H, $J_{1,2}$ 7.9 Hz, H-1), 2.12, 2.01 (2 s, 3 H each, 2 Ac), 1.90 (s, 6 H, 2 Ac), and 1.20 (d, 1 H, $J_{5',6'}$ 6.6 Hz, H-6').

A solution of **13** (0.81 g, 0.97 mmol) in methanolic 0.1 M sodium methoxide (150 mL) was kept at room temperature for 30 min, then neutrallised with Amberlite IR-120 (H^+) resin, filtered, and concentrated to give **2** (0.69 g, 100%), mp 165–167°, $[\alpha]_D$ +34° (*c* 0.4, H_2O). ^{1}H-NMR data (D_2O): δ 5.27 (d, 1 H, $J_{1',2'}$ 4.0 Hz, H-1'), 4.87 (d, 1 H, $J_{1''',2'''}$ 3.7 Hz, H-1'''), 4.28 and 4.20 (2 d, 1 H each, H-1,1''), 1.84 (s, 3 H, Ac), and 1.01 (d, 1 H, $J_{5',6'}$ 6.7 Hz, H-6').

Anal. Calcd for $C_{27}H_{47}NO_{20} \cdot 2H_2O$: C, 43.71; H, 6.93; N, 1.89. Found: C, 43.53; H, 6.66; N, 2.13.

ACKNOWLEDGMENTS

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