Novel Disaccharide Inhibitors of Human Glioma Cell Division

Begoña Aguilera,[†] Lorenzo Romero-Ramírez,[‡] José Abad-Rodríguez,[‡] Guillermo Corrales,[†] Manuel Nieto-Sampedro,*[,‡] and Alfonso Fernández-Mayoralas*[,†]

Instituto de Química Orgánica General, CSIC, Juan de la Cierva 3, 28006 Madrid, Spain, and Neural Plasticity Group, Instituto Cajal, CSIC, Avda. Doctor Arce 37, 28002 Madrid, Spain

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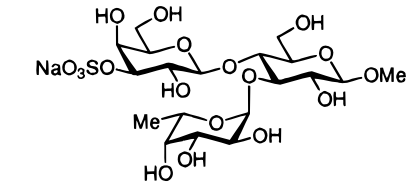
Several α-L-Fuc-(1→3)-α-D-GlcNAcOC$_8$H$_{17}$ disaccharide derivatives bearing different hydroxylated alkyl chains, with or without sulfate groups at C-4 and/or C-6 positions of the GlcNAc unit, have been synthesized and tested as inhibitors of human astrocytoma lines U-373 and U-118. The antimitotic activity was dependent on the structure and position of the hydroxylated chain linked to the disaccharide. The compounds with a pentaerythritol or L-glyceryl chain at the C-6 position showed the best inhibitory properties, with an ID$_{50}$ value of ca. 200 μM. On the contrary, sulfated disaccharide derivatives were inactive. The antimitotic activities of the compounds tested were essentially independent of the mitogen used to stimulate cell division.

Introduction

Astrocyte number is maintained constant in the mammalian central nervous system (CNS),[1] during adulthood and old age under normal circumstances, as a result of the balance of division promoters and division inhibitors. Mitogenic polypeptides are abundant in brain, and their structure has been known for many years. In contrast, evidence for the existence in mammalian brain of antimitotic molecules was presented only 10 years ago;[2] their purification has been achieved only recently,[3] and their precise structure remains unknown. The mitogen inhibitors are immunologically related to blood group oligosaccharides and to some glycidic epitopes of the epidermal growth factor receptor.[2,4] On the basis of these data, we synthesized a family of oligosaccharides with a common Lewis X-type structure and tested their activity as inhibitors of the proliferation of normal and transformed neural cells.[5−8]

The tetrasaccharide α-D-GalNac(1→3)-β-D-Gal-(1→4)-[α-L-Fuc-(1→3)]-β-D-GlcOMe inhibited the proliferation of astrocytes and astrocytoma in culture, showing ID$_{50}$ (50% inhibition) values in the micromolar range.[6] Astrocytes and neurons were viable at tetrasaccharide concentrations 10-fold higher than the ID$_{50}$, indicating that growth inhibition did not involve cytotoxicity. The effect of the tetrasaccharide may be related to the increased expression of connexin 43 observed in glioma cell cultures treated with the oligosaccharide.[7] However, the tetrasaccharide caused not only the inhibition but also the destruction of a malignant rat brain glioma after transplantation of C6 cells. This effect appeared to be caused indirectly, either by activation of natural killers cells, cytotoxic lymphocytes, or by inhibition of tumor vascularization.[7] From results obtained with the oligosaccharides tested in culture, some structure−activity relationships could be drawn: (i) an α-fucosyl residue linked to the C-3 position of a glucose moiety was important for the antimitotic activity and (ii) a negatively charged group at the nonreducing end of the oligosaccharide seemed beneficial for the inhibitory activity against tumoral cells. Thus, a sulfated trisaccharide (compound **1** in Chart 1) showed higher activity than the nonsulfated trisaccharide (ID$_{50}$ = 79 and 4300 μM, respectively).[8] A more flexible and straightforward synthesis of these molecules is required, both to get a deeper insight into their structure−activity relationship and to facilitate the large-scale preparation required for their possible clinical use.

Chart 1



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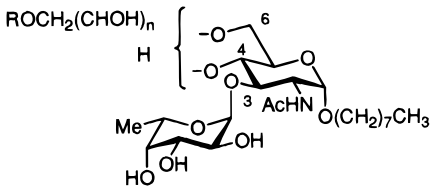

Structure I (R = H or NaO_3S)

The practical syntheses of a second generation of inhibitory compounds was carried out based on previous data. The compounds were tested as inhibitors of human astrocytoma grades 3 and 4. The new oligosaccharides have as common novel structural feature: a disaccharide fragment with the beneficial α-fucosyl residue linked to the C-3 position of the *N*-acetylglucosamine (structure I in Chart 1). The disaccharide was glycosylated with *n*-octanol giving the molecule an amphiphilic glycolipid character.[9] The different products originated from a variable hydroxylated alkyl chain, intended to mimic a glycoside unit linked to the C-4 and/or C-6 positions of the *N*-acetylglucosamine. Some derivatives with a sulfate group at the end of the hydroxylated chain will be useful for learning about the importance of a negatively charged group for antimitotic

* To whom correspondence should be addressed.
[†] Instituto de Química Orgánica General.
[‡] Instituto Cajal.

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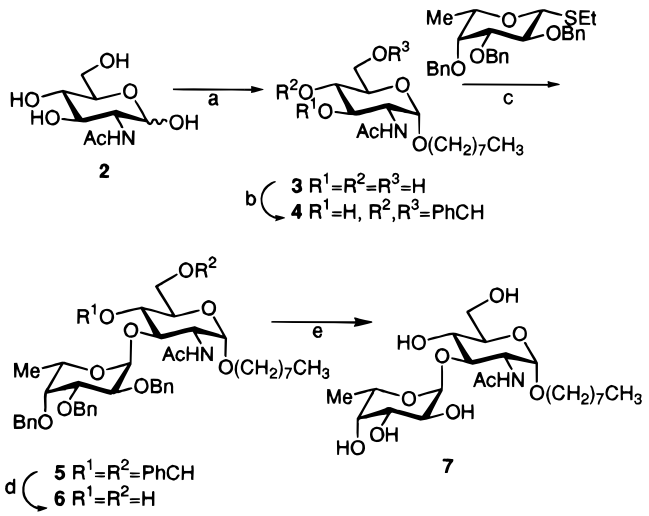
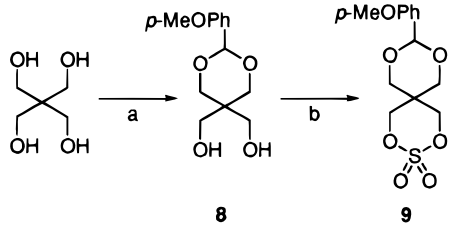
Scheme 1[a]



[a] Reagents and conditions: (a) octanol, $BF_3 \cdot Et_2O$, CH_3CN, reflux, 35%; (b) α,α′-dimethoxytoluene, *p*-TsOH, CH_3CN, rt, 73%; (c) 1. Br_2, CH_2Cl_2, 0 °C, 2. Bu_4NBr, DMF, rt, 87%; (d) camphorsulfonic acid, MeOH, 50 °C, 74%; (e) H_2, Pd/C, MeOH, rt, 97%.

Scheme 2[a]



[a] Reagents and conditions: (a) *p*-anisaldehyde, HCl, H_2O, rt, 84%; (b) NaH, 1,1′-sulfonyldiimidazole, THF, rt, 81%.

activity. In this paper we report the synthesis of the novel inhibitors and their antimitotic activity against human astrocytoma cells U-373 and U-118.

Results and Discussion

Chemistry. To minimize protecting group manipulations, the target compounds were obtained by carrying out partial alkylations on a common diol intermediate, **6**. The synthesis of the disaccharide derivative **6** started from inexpensive *N*-acetyl-D-glucosamine (**2**) (Scheme 1). Fischer-type glycosidation of **2** with *n*-octanol gave the α-glycoside **3**, which was subsequently benzylidenated to afford alcohol **4**. Glycosidation[10] of **4** with freshly prepared tri-*O*-benzyl-α-L-fucopyranosyl bromide gave protected disaccharide **5**. The α-configuration of the newly formed glycosidic bond was determined from the ^{1}H NMR spectrum of **5** by a doublet signal of J = 3.3 Hz at 5.23 ppm assigned to the H-1 of the α-fucosyl residue. Treatment of **5** with camphorsulfonic acid in MeOH gave the desired intermediate **6**. Hydrogenolysis of **6** afforded the unsubstituted disaccharide **7**.

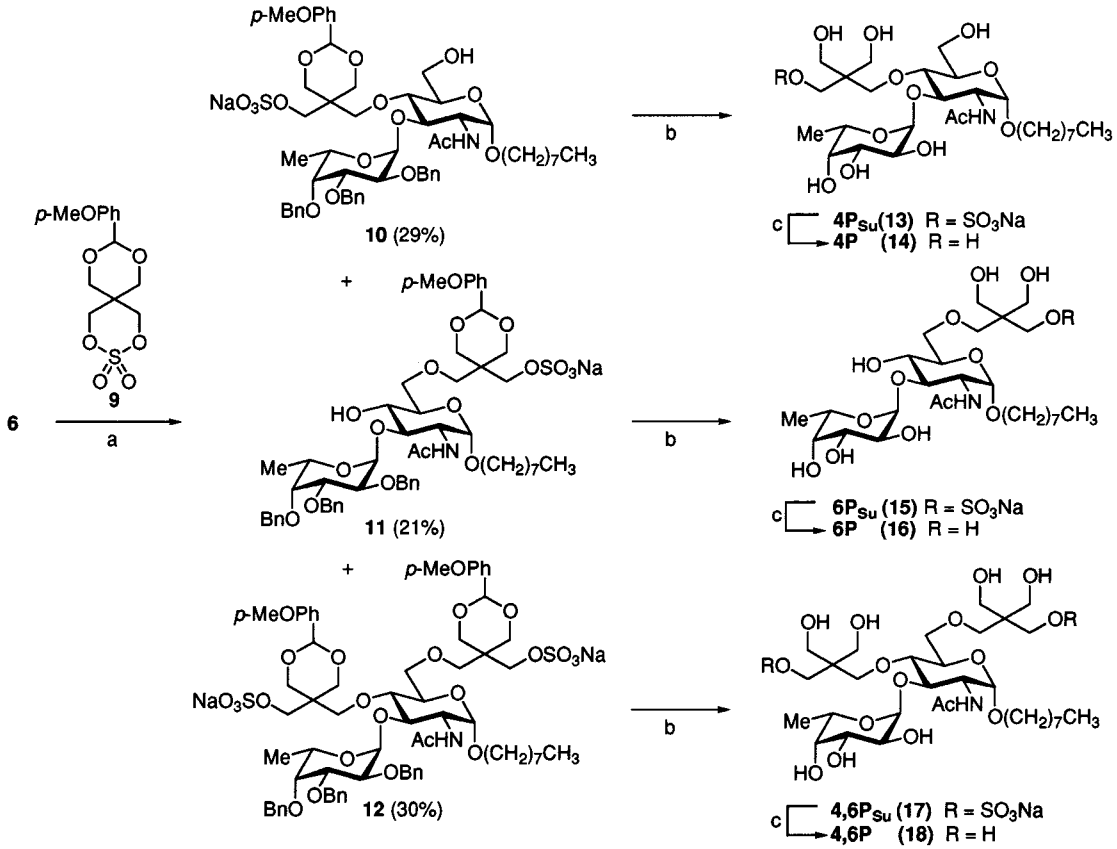
From intermediate **6** we first prepared a series of compounds containing a sulfated pentaerythritol chain (P series). A straightforward synthesis of sulfates $4P_{Su}$ (**13**), $6P_{Su}$ (**15**), and $4,6P_{Su}$ (**17**) (Scheme 3) was performed by using the cyclic sulfate derivative **9** as alkylating agent, easily prepared from pentaerythritol (Scheme 2). The nucleophilic ring opening of **9** allowed to introduce the pentaerythritol chain generating, at the same time, the negatively charged sulfate group (Scheme 3). Thus, treatment of diol **6** with NaH followed by reaction with 1.5 equiv of cyclic sulfate **9** at 60 °C gave a mixture of mono- and dialkylated compounds, **10**−**12**. After column chromatography **10**, **11**, and **12** were isolated in 30%, 21%, and 30% yields, respectively. Separated hydrogenolysis of **10**−**12** provided target sulfates **13**, **15**, and **17**. The nonsulfated compounds 4P (**14**), 6P (**16**), and 4,6P (**18**) were obtained by treatment of **13**, **15**, and **17**, respectively, with sulfuric acid in dioxane.

The structures of **13**−**18** were unequivocally determined by their NMR spectra. Thus, the number of protons and carbons in the ^{1}H and ^{13}C NMR spectra of **13**−**18** was in agreement with the presence of one or two pentaerythritol chains. In the case of monoalkylated derivatives **13**−**16**, the position of the chain was determined by analyzing the ^{13}C NMR spectra on the basis of the downfield α-effect caused by alkylation.[11] The assignment of the signals was previously carried out using DEPT, HMQC, and HMBC experiments. The C-6 carbon is 7.0−8.0 ppm more deshielded in **15** and **16** than in **13** and **14**, indicating that the former compounds are bearing the hydroxylated alkyl chain at the C-6 position. Finally, the presence of the sulfate groups was characterized by a sharp band at 1200 cm^{-1} in the IR spectra of compounds **13**, **15**, and **17**.

We next synthesized another series of compounds containing a linear hydroxylated chain derived from glycerol (G series). Partial alkylations on diol **6** were carried out using commercially available tosylates **19** and **20** derived from D- and L-glycerol (Scheme 4). The reaction of **6** with 1.5 equiv of tosylate **19** in the presence of NaH gave a mixture of **21**, **23**, and **25** in 52%, 22%, and 16% yields. Under similar conditions the L-glyceryl tosylate **20** afforded a mixture of **22**, **24**, and **26** in 56%, 24%, and 14% yields. Hydrogenation of **21**−**26** separately in the presence of trifluoroacetic acid led to the cleavage of benzyl and isopropylidene groups providing target products **27**−**32**. The structure of **27**−**32** was again determined from their ^{1}H and ^{13}C NMR spectra. It is interesting to note that in both cases a selective alkylation on the secondary hydroxyl HO-4 over the primary HO-6 took place. A similar preference, although to a lesser extent, was also observed in the above-described alkylation using the cyclic sulfate. Schmidt has reported[12] a selective allylation on the secondary C-3 position in the presence of two primary hydroxyls of an azidolactose derivative using allyl bromide and NaH. The surprising result was explained by intramolecular complexation of the sodium ion from the O-3 alkoxide. Similarly, in diol **6** a preferential formation of O-4 alkoxide could take place due to Na^+ chelation with O-3 and the ring oxygen atom of the fucose moiety (Chart 2). As evidence to support this hypothesis, the alkylation of diol lacking the fucose residue (**33**) with tosylate **20** led to a complete reverse of the regioselectivity, with a predominant formation of the 6-O-alkylated derivative.

For comparative purposes the 6-*O*-galactosyl trisaccharide derivative **36** was prepared (Scheme 5). From intermediate **6** a highly regio- and stereoselective galactosylation was achieved using the trichloroacetimidate **34** in the presence of TMSOTf as promoter. The 6-O-β-glycosylated product **35** was obtained in 82%

Scheme 3[a]



[a] Reagents and conditions: (a) NaH, **9**, THF−DMF, 60 °C; (b) H_2, Pd/C, rt, **13** (96%), **15** (96%), **17** (quant); (c) H_2SO_4 (1 M), dioxane−MeOH, rt, **14** (64%), **16** (71%), **18** (38%).

yield, which was transformed, by removal of the benzoyl and benzyl groups, into the desired product **36**.

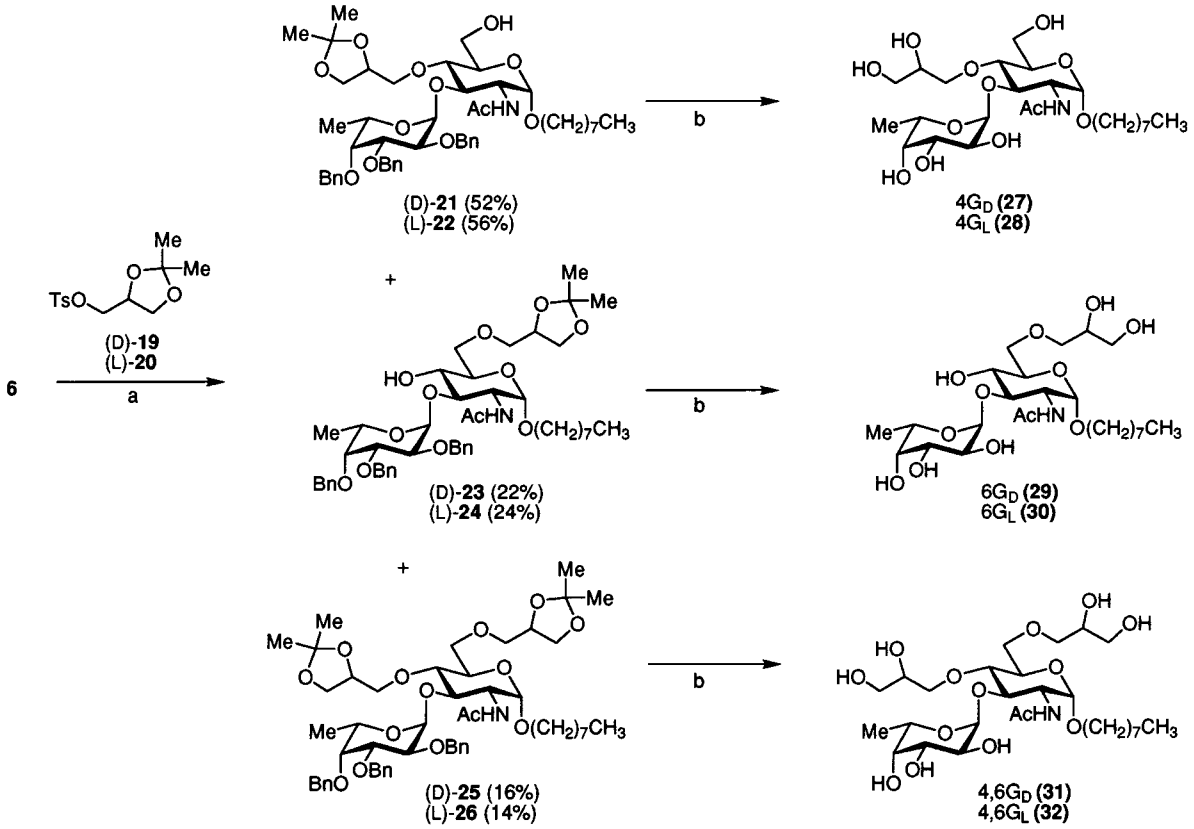
Biological Activity. The antimitotic activity of compounds containing a pentaerythritol chain (**13**−**18**, P series) as well as that of the nonsubstituted disaccharide **7** was tested on rat (C6) and human glioma (U-373 and U-118) cell cultures, stimulated with different mitogens. The results are summarized in Table 1. In contrast to our previous results using the sulfated trisaccharide **1** (Chart 1) on rat C6 glioma,[8] sulfated compounds $4P_{Su}$ (**13**), $6P_{Su}$ (**15**), and $4,6P_{Su}$ (**17**) were inactive in all the cell types. However, the nonsulfated derivatives 4P (**14**), 6P (**16**), and 4,6P (**18**) showed moderate inhibitory activity on thymidine incorporation promoted by EGF, the ID_{50} value of which depended on the position of the pentaerythritol chain. The substitution at the C-4 position (compound 4P, **14**) led to a slight increase in activity compared with the nonsubstituted disaccharide **7**, while 6P (**16**), containing the pentaerythritol chain at C-6, showed the best antimitotic activity of this family.

All the compounds of the G series, substituted with a glyceryl chain, showed ID_{50} values below 1 mM (Table 2) improving the inhibitory activity with respect to the P series. Some appreciable differences were observed depending on both the position and the configuration of the glyceryl chain. The 6-L-glyceryl derivative $6G_L$ (**30**) was the best inhibitor with ID_{50} values ranging between 105 and 234 μM. Its 4-L-glyceryl regioisomer $4G_L$ (**28**) showed a 2−4-fold higher ID_{50} value depending on the mitogen used. A similar, though smaller, loss of activity was observed in $6G_D$ (**29**), substituted at C-6 with a D-glyceryl chain, with respect to its diastereoisomer $6G_L$(**30**). As in the pentaerythritol series, wherea second alkyl chain at C-4 of 6P (**16**) led to the less inhibitory compound 4,6P (**18**), the disubstituted derivative 4,6GL (**32**) showed a decrease of activity as compared to the monosubstituted disaccharide $6G_L$ (**30**).

Taken together, the results indicate that a hydroxylated alkyl chain at the C-6 position of the glucosamine enhanced the inhibitory activity, although the effect of the substitution was dependent on the relative orientation of the hydroxyl groups. Thus, a small modification such as the chirality of the glyceryl chain in $6G_L$ (**30**) or $6G_D$ (**29**) produced a change in the activity. Steric factors could also influence the inhibitory properties of these compounds since a second alkyl chain at C-4 gave less active inhibitors. Besides, the trisaccharide derivative **36** with a bulky galactosyl residue at C-6 was inactive against U-373 glioma cells (Table 2).

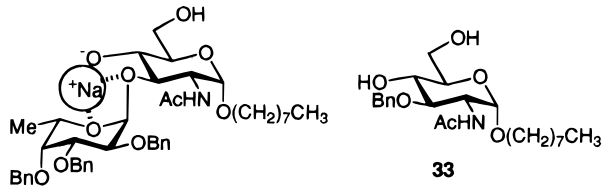
Human glioma U-373 cells produced molecules which would allow them to proliferate, when cultured in the absence of mitogens (DMEM medium). Several authors have reported the expression of growth factor[13] and cytokine[13,14] receptors by U-373 cells, although the astrocytoma proliferated much better in the presence of exogenous growth factors, such as EGF and bFGF. $6G_L$ (**30**) and $6G_D$ (**29**) were the most active molecules of the two series studied here, with small differences depending on whether division was driven by EGF, bFGF, or autocrine (DMEM only). Therefore, antimitotic activity was substantially independent of the

Scheme 4[a]

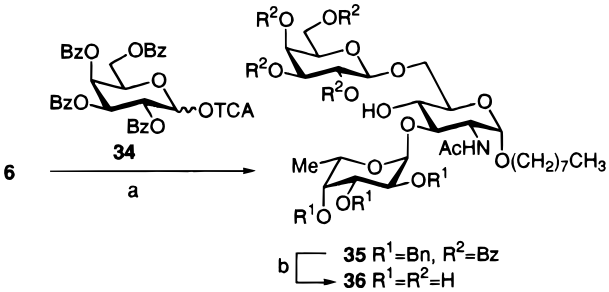


[a] Reagents and conditions: (a) NaH, **19** or **20**, DMF, rt; (b) H_2, Pd/C, TFA, rt, **27** (quant), **28** (quant), **29** (97%), **30** (96%), **31** (quant), **32** (98%).

Chart 2



Scheme 5[a]



[a] Reagents and conditions: (a) TMSOTf, CH_2Cl_2, rt, 82%; (b) 1. NaOMe, MeOH, rt, 2. H_2, Pd/C, rt, 93%.

mitogen, and the inhibitor must be acting at a common point downstream from the different kinases induced by the various mitogens. This is advantageous for the possible therapeutic use of these molecules because, under physiological conditions, tumor growth is promoted by many different mitogens. New compounds, based on the structures and synthetic strategies presented here, will provide valuable information on structure−function relationships and permit the production of compounds with very low ID_{50} values.

Table 1. Inhibition of Human (U-373 and U-118) and Rat (C6) Glioma Cell Cultures by Compounds of the Pentaerythritol (P) Series

	ID_{50} (mM)			
compd	U-373 1% FCS[a]	U-373 EGF[a]	U-118 1% FCS[a]	C6 10% FCS[a]
7	>1000	>1000	993	756
$4P_{Su}$ (**13**)	>1000	>1000	>1000	nd[b]
$6P_{Su}$ (**15**)	>1000	>1000	>1000	nd[b]
$4,6P_{Su}$ (**17**)	>1000	>1000	>1000	nd[b]
4P (**14**)	>1000	710	785	>1000
6P (**16**)	202	210	425	nd[b]
4,6P (**18**)	>1000	433	>1000	>1000

[a] Cell cultures were stimulated with different mitogens: FCS, fetal bovine serum; EFG, epidermal growth factor. For details of the assay, see the Experimental Section. [b] nd, not detectable.

Experimental Section

Chemistry. General Methods. Melting points are not corrected. TLC was performed using TLC plates GF_{254} with detection by charring with 5% H_2SO_4 in EtOH. Column chromatography was performed on silica gel (230−400 mesh). The eluent used is indicated, and solvent ratios refer to volume. Solvents were distilled over drying agents: dimethylformamide, BaO; dichloromethane, CaH_2; tetrahydrofuran, sodium/benzophenone ketyl; acetonitrile, CaH_2; and pyridine, BaO. ^1H NMR spectra were registered at 500, 400, 300, or 200 MHz. ^{13}C NMR spectra were obtained at 125, 75, or 50 MHz. Elemental analyses were determined in a Perkin-Elmer 240 analyzer.

Octyl 2-Acetamido-2-deoxy-α-D-glucopyranoside (3). To a suspension of *N*-acetyl-D-glucosamine (4.42 g, 20 mmol) and octanol (9.45 mL, 60 mmol) in dry acetonitrile (160 mL) was added under Ar boron trifluoride diethyl etherate (406 μL, 3.3 mmol), and the reaction mixture was stirred under reflux for 18 h. After cooling, the unreacted starting material

Table 2. Inhibition of Human U-373 Glioma Cell Cultures by Compounds of the Glycerol (G) Series

compd	ID_{50} (mM), U-373		
	DMEM[a]	EGF[a]	bFGF[a]
$4G_D$ (**27**)	>1000	788	604
$4G_L$ (**28**)	430	490	710
$6G_D$ (**29**)	195	291	443
$6G_L$ (**30**)	105	205	234
$4,6G_D$ (**31**)	900	500	529
$4,6G_L$ (**32**)	363	500	363
36	>1000	>1000	>1000

[a] Human U-373 glioma cell cultures were stimulated with the appropriate mitogen and treated with the different molecules at various concentrations as indicated in the Experimental Section: DMEM, Dulbecco's modified Eagle's medium; EGF, epidermal growth factor; bFGF, basic fibroblast growth factor.

was filtered off, and the filtrate was evaporated to dryness. The residue was purified by column chromatography (CH_2Cl_2−MeOH 20:1→6:1) to give **3** as a solid (2.34 g, 35%). R_f: 0.54 (CH_2Cl_2−MeOH, 10:1). Mp: 157−159 °C. $[\alpha]_D$: +129.5° (*c* 1, MeOH). Anal. ($C_{16}H_{31}NO_6$) C, H, N.

Octyl 2-Acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranoside (4). To a suspension of **3** (2.34 g, 7.02 mmol) in dry acetonitrile (50 mL) under Ar were added *p*-toluenesulfonic acid (66 mg, 0.34 mmol) and then α,α′-dimethoxytoluene (5.2 mL, 35.09 mmol), and the reaction mixture was stirred at room temperature for 3 h. After this time, the mixture was diluted with dichloromethane, neutralized with triethylamine and concentrated. Purification of the residue by column chromatography (CH_2Cl_2−MeOH, 40:1→20:1) gave **4** as a solid (2.17 g, 73%). R_f: 0.61 (CH_2Cl_2−MeOH, 10:1). Mp: 187−189 °C. $[\alpha]_D$: +49.7° (*c* 1, $CHCl_3$). Anal. ($C_{23}H_{35}NO_6$) C, H, N.

Octyl *O*-(2,3,4-Tri-*O*-benzyl-α-L-fucopyranosyl)-(1→3)-2-acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranoside (5). Ethyl 2,3,4-tri-*O*-benzyl-1-thio-β-L-fucopyranoside[18] (2.82 g, 5.89 mmol) was dissolved in dry dichloromethane (45 mL) under argon. At 0 °C, bromine (0.31 mL, 6 mmol) was added, and the mixture was stirred for 30 min. The mixture was concentrated in vacuo, and residual bromine was removed by evaporation with toluene (3 × 20 mL). The crude bromide was used without purification in the next step.

Acceptor **4** (1.64 g, 3.91 mmol) was dissolved in dry DMF (36 mL), and 4 Å molecular sieves (5 g) and Bu_4NBr (1.28 g, 3.95 mmol) were added. The mixture was kept under argon atmosphere, and the crude bromide, obtained as shown before (ca 5.89 mmol), dissolved in dichloromethane (16 mL) was added. After stirring for 18 h, the reaction was quenched with ethanol. After 30 min, the mixture was diluted with dichloromethane (300 mL) and filtered through a pad of Celite. The filtrate was washed with saturated aqueous $NaHCO_3$ (200 mL) and brine (200 mL), dried (Na_2SO_4), and concentrated in vacuo. The residue was purified by column chromatography (hexane−acetate, 4:1) to give **5** as a solid (2.86 g, 87%). R_f: 0.34 (hexane−acetone, 2:1). Mp: 93−96 °C. $[\alpha]_D$: −28.7° (*c* 1, $CHCl_3$). Anal. ($C_{50}H_{63}NO_{10}$) C, H, N.

Octyl *O*-(2,3,4-Tri-*O*-benzyl-α-L-fucopyranosyl)-(1→3)-2-acetamido-2-deoxy-α-D-glucopyranoside (6). A mixture of **5** (2.61 g, 3.11 mmol) and camphorsulfonic acid (180 mg, 0.77 mmol) in methanol (60 mL) was stirred for 90 min at 50 °C. After cooling, the mixture was neutralized with triethylamine and concentrated. The residue was purified by column chromatography(hexane−AcOEt (1:1)→AcOEt) to give **6** as a solid (1.73 g, 74%). R_f: 0.11 (hexane−acetone, 2:1). Mp: 156−159 °C. $[\alpha]_D$: +9.4° (*c* 1, $CHCl_3$). Anal. ($C_{43}H_{59}NO_{10}$) C, H, N.

Octyl *O*-(α-L-Fucopyranosyl)-(1→3)-2-acetamido-2-deoxy-α-D-glucopyranoside (7). To a solution of **6** (150.9 mg, 0.2 mmol) in methanol (15 mL) was added Pd/C 10% (100 mg), and the mixture was stirred under H_2 atmosphere for 5 h. After this time, the mixture was filtered through Celite and evaporated to dryness to give **7** as a solid (93.6 mg, 97.4%). R_f: 0.65 (CH_2Cl_2−MeOH, 3:1). Mp: 214−217 °C. $[\alpha]_D$: +4.4° (*c* 0.6, MeOH). ^{1}H NMR (300 MHz, CD_3OD): δ 5.16 (d, 1H, *J* = 2.4 Hz), 4.97 (d, 1H, *J* = 3.6 Hz), 4.45 (m, 1H), 4.24 (dd, 1H, *J* = 3.6 Hz, *J* = 10.5 Hz), 4.03−3.60 (m, 10H), 2.16 (s, 3H), 1.81 (m, 2H), 1.53 (m, 10H), 1.38 (d, 1H, *J* = 6.6 Hz), 1.09 (m, 3H). ^{13}C NMR (50 MHz, CD_3OD): δ 174.03, 101.69, 98.54, 81.57, 73.82, 73.61, 71.64, 70.82, 70.12, 69.01, 68.45, 62.60, 54.56, 32.98, 30.51, 30.39, 27.27, 23.67, 22.77, 16.56, 14.37. Anal. ($C_{22}H_{41}NO_{10}$) C, H, N.

Mono-*p*-methoxybenzilidene-pentaerythritol (8). In a flask were placed pentaerythritol (10 g, 73.4 mmol) and water (72 mL). The mixture in the flask was heated with stirring until all the solid was dissolved and then was allowed to cool undisturbed. When the solution had cooled to room temperature, stirring was started and concentrated hydrochloric acid (370 μL) was added, followed by *p*-anisaldehyde (1 mL, 8.2 mmol). When the precipitate of mono-*p*-methoxybenzilidene-pentaerythritol started forming, dropwise addition of *p*-anisaldehide (8.32 mL, 68.4 mmol) was begun. After the addition of *p*-anisaldehide was complete (ca 2 h), the mixture was stirred for an additional 3 h. The precipitate was collected on a Büchner funnel and washed with ice-cold water which had been made slightly alkaline by addition of sodium carbonate. The solid was dried over phosphorus pentoxide to give **8** as a white solid (15.7 g, 84%). R_f: 0.47 (CH_2Cl_2−MeOH, 10:1). Mp: 163−166 °C. Anal. ($C_{13}H_{18}O_5$) C, H.

3,3-Dioxo-9-(*p*-methoxyphenyl)-2,4,8,10-tetraoxo-3-thiaspiro[5.5]decane (9). To a suspension of **8** (2 g, 7.84 mmol) in dry tetrahydrofuran (40 mL) was added sodium hydride (423 mg, 17.6 mmol) and 1,1′-sulfonyldiimidazole (2.3 g, 11.7 mmol) dissolved in dry tetrahydrofuran (30 mL). The reaction mixture was stirred at room temperature for 2 h. Then, methanol (1 mL) was added, and the mixture was evaporated to dryness. The residue was purified by column chromatography (hexane−CH_2Cl_2, 5:1) to give **9** as a solid (2 g, 81%). R_f: 0.77 (CH_2Cl_2−MeOH 10:1). Mp: 181−184 °C. IR (KBr): 1410, 1210 cm^{-1}. Anal. ($C_{13}H_{16}O_7S$) C, H, S.

Alkylation Reaction of Diol 6 with Cyclic Sulfate 9. To a solution of **6** (1 g, 1.33 mmol) in a mixture of dry tetrahydrofuran−dimethylformamide (7:1, 48 mL) were added sodium hydride (128 mg, 5.33 mmol) and then **9** (634 mg, 2 mmol), and the reaction mixture was heated at 60 °C for 1.5 h. After cooling, methanol (5 mL) was added and the mixture was evaporated to dryness. The residue was purified by column chromatography (CH_2Cl_2−MeOH, 20:1) to give **10** (386 mg, 30%), **11** (283 mg, 21%), and **12** (570 mg, 30%). **10**: R_f 0.28 (CH_2Cl_2−MeOH, 8:1). **11**: R_f 0.57 (CH_2Cl_2−MeOH, 8:1). **12**: R_f 0.16 (CH_2Cl_2−MeOH, 8:1).

Octyl *O*-(α-L-Fucopyranosyl)-(1→3)-2-acetamido-2-deoxy-4-*O*-[2,2-bis(hydroxymethyl)-3-hydroxy-3-*O*-(oxosulfonyl)propyl]-α-D-glucopyranoside ($4P_{Su}$ (13)). To a solution of **10** (200 mg, 0.184 mmol) in methanol (15 mL) was added 10% Pd/C (100 mg), and the reaction mixture was stirred under H_2 atmosphere for 5 h. After this time, the mixture was filtered through Celite and evaporated to dryness to give **13** as a solid (124 mg, 96%). R_f: 0.10 (CH_2Cl_2−MeOH, 3:1). Mp: 149−153 °C. $[\alpha]_D$: +12.4° (*c* 0.7, MeOH). ^{1}H NMR (300 MHz, CD_3OD): δ 5.34 (d, 1H, *J* = 2.7 Hz), 5.16 (d, 1H, *J* = 3.4 Hz), 4.37 (m, 1H), 4.27−3.59 (m, 19H), 2.18 (s, 3H), 1.75 (m, 2H), 1.50 (m, 10H), 1.44 (d, 3H, *J* = 6.5 Hz), 1.09 (m, 3H). ^{13}C NMR (50 MHz, CD_3OD): δ 173.47 (CO), 101.87 (C-1 Fuc), 97.76 (C-1 GlcNAc), 80.04, 78.61, 73.49, 73.29, 72.17 (C-1′), 71.06, 70.86, 69.25, 69.01 (CH_2 (octyl)), 67.88 (C-3′), 62.39 (CH_2-OH), 62.27 (CH_2OH), 61.90 (C-6), 55.77 (C-2 GlcNAc), 46.98 (C-2′), 32.98 (CH_2), 30.43 (CH_2), 27.23 (CH_2), 23.68 (CH_2), 22.79 (NHAc), 16.70, 14.41. IR (KBr): 1240, 1080 cm^{-1}.

Octyl *O*-(α-L-Fucopyranosyl)-(1→3)-2-acetamido-2-deoxy-6-*O*-[2,2-bis(hydroxymethyl)-3-hydroxy-3-*O*-(oxosulfonyl)propyl]-α-D-glucopyranoside ($6P_{Su}$ (15)). **10** (200 mg, 0.184 mmol) was reacted by the procedure used to synthesize compound **13** and afforded the title compound as a solid (124 mg, 96%). R_f: 0.10 (CH_2Cl_2−MeOH, 3:1). Mp: 121−124 °C. $[\alpha]^{435}_{Hg}$: +3.0° (*c* 0.5, MeOH). ^{1}H NMR (300 MHz, CD_3OD): δ 5.16 (s, 1H), 4.93 (d, 1H, *J* = 3.6 Hz), 4.50 (m, 1H), 4.30−3.54 (m, 19H), 2.17 (s, 3H), 1.81 (m, 2H), 1.58

(m, 10H), 1.38 (d, 3H, $J = 6.6$ Hz), 1.09 (m, 3H). ^{13}C NMR (50 MHz, CD_3OD): δ 173.97 (CO), 101.42 (C-1 Fuc), 98.69 (C-1 GlcNAc), 81.07, 73.67, 72.97, 72.14 (C-1′), 71.73, 71.68 (C-6), 70.73, 70.17, 69.23 (OCH_2 (octyl)), 68.35, 68.21 (C-3′), 62.99 (CH_2OH), 62.92 (CH_2OH), 54.65 (C-2), 46.66 (C-2′), 32.92 (CH_2), 30.50 (CH_2), 30.47 (CH_2), 30.32 (CH_2), 27.27 (CH_2), 23.61 (CH_2), 22.82 (NHAc), 16.51, 14.30. IR (KBr): 1250, 1060 cm^{-1}.

Octyl *O*-(α-L-Fucopyranosyl)-(1→3)-2-acetamido-2-deoxy-4,6-bis-*O*-[2,2-bis(hydroxymethyl)-3-hydroxy-3-*O*-(oxosulfonyl)propyl]-α-D-glucopyranoside (4,6P$_{Su}$ (17)). **12** (206 mg, 0.144 mmol) was reacted by the procedure used to synthesize compound **13** and afforded the title compound as a solid (133 mg, quantitative). R_f: 0.25 (CH_2Cl_2−MeOH, 3:2). Mp: 204−208 °C. $[\alpha]_D$: +12.3° (*c* 0.5, MeOH). ^{1}H NMR (300 MHz, CD_3OD): δ 5.15 (d, 1H, $J_{1,2} = 2.2$ Hz), 4.93 (d, 1H, $J = 3.4$ Hz), 4.69−3.55 (m, 28H), 1.99 (s, 3H), 1.52 (m, 10H), 1.56 (m, 2H), 1.25 (d, 3H, $J = 6.6$ Hz), 0.89 (m, 3H). ^{13}C NMR (50 MHz, CD_3OD): δ 173.56 (CO), 101.65 (C-1 Fuc), 97.90 (C-1 GlcNAc), 79.77, 78.39, 73.50, 72.37 (C-1′), 72.31, 71.59 (C-1′), 71.11, 70.89, 70.59 (C-6), 69.25, 69.19 (CH_2 (octyl)), 68.45 (C-3′), 68.15 (C-3′), 62.87 (CH_2OH), 62.40 (CH_2OH), 55.75 (C-2 GlcNAc), 46.86 (C-2′), 46.51 (C-2′), 33.02 (CH_2), 30.46 (CH_2), 27.27 (CH_2), 23.72 (CH_2), 22.83 (NHAc), 16.73, 14.45. IR (KBr): 1250, 1045 cm^{-1}.

Octyl *O*-(α-L-Fucopyranosyl)-(1→3)-2-acetamido-2-deoxy-4-*O*-[2,2-bis(hydroxymethyl)-3-hydroxypropyl]-α-D-glucopyranoside (4P (14)). To a solution of **13** (190 mg, 0.27 mmol) in dioxane (7.8 mL) and methanol (2.5 mL) was added 1 M sulfuric acid (0.012 mL, 0.012 mmol), and the reaction mixture was stirred at room temperature for 24 h. After this time, the mixture was neutralized with saturated $NaHCO_3$ solution and concentrated in vacuo. The residue was purified by column chromatography (EtOAc−MeOH, 4:1) to give **14** as a solid (104 mg, 64%). R_f: 0.44 (CH_2Cl_2−MeOH, 3:1). Mp: 61−65 °C. $[\alpha]_D$: +11.0° (*c* 1, MeOH). ^{1}H NMR (300 MHz, CD_3OD): δ 5.31 (d, 1H, $J = 3.2$ Hz), 5.22 (d, 1H, $J = 3.3$ Hz), 4.39 (m, 1H), 4.21−3.51 (m, 19H), 2.18 (s, 3H), 1.75 (m, 2H), 1.64 (m, 10H), 1.43 (d, 3H, $J = 6.6$ Hz), 1.09 (m, 3H). ^{13}C NMR (50 MHz, CD_3OD): δ 173.52 (CO), 102.75 (C-1 Fuc), 95.66 (C-1 GlcNAc), 80.38, 79.39, 73.43, 72.49 (C-1′), 71.08, 70.97, 69.60, 68.99 (CH_2 (octyl)), 62.95 (CH_2OH), 61.93 (C-6), 55.89 (C-2 GlcNAc), 40.52 (C-2′), 33.02 (CH_2), 30.46 (CH_2), 27.28 (CH_2), 23.72 (CH_2), 22.79 (NHAc), 16.72 (Me-Fuc), 14.45 (CH_3). Anal. ($C_{27}H_{51}NO_{13}\cdot H_2O$) C, H, N.

Octyl *O*-(α-L-Fucopyranosyl)-(1→3)-2-acetamido-2-deoxy-6-*O*-[2,2-bis(hydroxymethyl)-3-hydroxypropyl]-α-D-glucopyranoside (6P (16)). Compound **15** (180 mg, 0.26 mmol) was reacted by the procedure used to synthesize compound **14** and afforded the title compound as a solid (110 mg, 71%). R_f: 0.36 (CH_2Cl_2−MeOH, 3:1). Mp: 55−59 °C. $[\alpha]_D$: +4.8° (*c* 1, MeOH). ^{1}H NMR (300 MHz, CD_3OD): δ 5.16 (s, 1H), 4.94 (d, 1H, $J = 3.5$ Hz), 4.48 (m, 1H), 4.25 (dd, 1H, $J = 3.5$ Hz, $J = 10.6$ Hz), 3.96−3.50 (m, 18H), 2.16 (s, 3H), 1.82 (m, 2H), 1.61−1.43 (m, 10H), 1.38 (d, 3H, $J = 6.6$ Hz), 1.1 (m, 3H). ^{13}C NMR (50 MHz, CD_3OD): δ 174.05 (CO), 101.66 (C-1 Fuc), 98.67 (C-1 GlcNAc), 80.94, 73.66, 72.73, 72.47 (C-1′), 71.89 (C-6), 71.66, 70.95, 70.09, 69.15 (CH_2 (octyl)), 68.29, 63.20 (CH_2OH), 54.62 (C-2 GlcNAc), 46.96 (C-2′), 33.04 (CH_2), 30.57 (CH_2), 30.48 (CH_2), 27.35 (CH_2), 23.74 (CH_2), 22.75 (NHAc), 16.60 (Me-Fuc), 14.45 (CH_3). Anal. ($C_{27}H_{51}NO_{13}$) C, H, N.

Octyl *O*-(α-L-Fucopyranosyl)-(1→3)-2-acetamido-2-deoxy-4,6-bis-*O*-[2,2-bis(hydroxymethyl)-3-hydroxypropyl]-α-D-glucopyranoside (4,6P (18)). Compound **17** (38.7 mg, 0.04 mmol) was reacted by the procedure used to synthesize compound **14**. The yield after column chromatography was 38% (11.5 mg). R_f: 0.32 (CH_2Cl_2−MeOH, 3:1); $[\alpha]_D$: +24.3° (*c* 1, MeOH). ^{1}H NMR (300 MHz, CD_3OD): δ 5.30 (d, 1H, $J = 3.2$ Hz), 5.22 (d, 1H, $J = 3.4$ Hz), 4.38 (m, 1H), 4.18−3.51 (m, 27H), 2.18 (s, 3H), 1.77 (m, 2H), 1.49 (m, 10H), 1.43 (d, 3H, $J = 6.6$ Hz), 1.09 (m, 3H). ^{13}C NMR (50 MHz, CD_3OD): δ 173.52 (CO), 102.79 (C-1 Fuc), 97.68 (C-1 GlcNAc), 80.24, 79.28, 73.36, 72.40 (C-1′), 72.21, 71.75 (C-1′), 71.03, 70.89, 70.56 (C-6), 69.61, 69.11 (CH_2 (octyl)), 63.10 (CH_2OH), 62.79 (CH_2OH), 55.83 (C-2 GlcNAc), 32.97 (CH_2), 30.44 (CH_2), 30.41 (CH_2), 27.24 (CH_2), 23.69 (CH_2), 22.74 (NHAc), 16.70 (CH_3-Fuc), 14.43 (CH_3). Anal. ($C_{32}H_{61}NO_{16}\cdot H_2O$) C, H, N.

Alkylation Reaction of Diol 6 with Tosylate 19. To a solution of **6** (330 mg, 0.44 mmol) in dry dimethylformamide (4.8 mL) at room temperature under Ar was added sodium hydride (27 mg, 1.1 mmol), and the mixture was stirred for 5 min. Then, **19** (188 mg, 0.66 mmol) dissolved in dimethylformamide (2 mL) was added dropwise, and the reaction mixture was heated at 40 °C for 8 h. After cooling, the reaction was quenched with methanol (1 mL) and the solvent was evaporated under reduced pressure. The residue was purified by column chromatography (hexane−acetone, 5:1→1:1) to give **21** (194 mg, 52%), **23** (82 mg, 22%), and **25** (66 mg, 16%). **21**: R_f 0.34 (hexane−acetone, 1:1). **23**: R_f 0.51 (hexane−acetone, 1:1). **25**: R_f 0.54 (hexane−acetone, 1:1).

Octyl *O*-(α-L-Fucopyranosyl)-(1→3)-2-acetamido-2-deoxy-4-*O*-(2(*R*),3-dihydroxypropyl)-α-D-glucopyranoside (4G$_D$ (27)). To a solution of **21** (100 mg, 0.116 mmol) in methanol (9 mL) were added trifluoroacetic acid (2.6 μL, 0.035 mmol) and then Pd/C (132 mg), and the mixture was stirred under H_2 atmosphere for 4 h. After this time, the reaction mixture was filtered through Celite and evaporated to give **27** as a solid (65 mg, quantitative). R_f: 0.24 (CH_2Cl_2−MeOH, 5:1). Mp: 96−99 °C. $[\alpha]_D$: +16.7° (*c* 1, MeOH). ^{1}H NMR (300 MHz, CD_3OD): δ 5.31 (d, 1H, $J = 2.2$ Hz), 5.11 (d, 1H, $J = 3.0$ Hz), 4.38 (m, 1H), 4.18−3.54 (m, 16H), 2.17 (s, 3H), 1.75 (m, 2H), 1.41 (m, 10H), 1.09 (m, 3H). ^{13}C NMR (50 MHz, CD_3OD): δ 101.77 (C-1 Fuc), 98.10 (C-1 GlcNAc), 79.97, 78.18, 75.59 (C-1′), 73.50 (C-1′), 73.23, 72.73, 71.25, 70.68, 69.00 (CH_2 (octyl)), 68.75, 64.29 (C-3′), 61.87 (C-6), 55.69, 32.99 (CH_2), 30.47 (CH_2), 30.43 (CH_2), 27.27 (CH_2), 23.69 (CH_2), 22.76 (NHAc), 16.79 (Me-Fuc), 14.41 (CH_3). Anal. ($C_{25}H_{47}NO_{12}$) C, H, N.

Octyl *O*-(α-L-Fucopyranosyl)-(1→3)-2-acetamido-2-deoxy-6-*O*-(2(*R*),3-dihydroxypropyl)-α-D-glucopyranoside (4G$_D$ (29)). **23** (64 mg, 0.074 mmol) was reacted by the procedure used to synthesize compound **27** and afforded the title compound (40 mg, 97%). R_f: 0.24 (CH_2Cl_2−MeOH, 5:1); $[\alpha]_D$: +6.2° (*c* 0.8, MeOH). ^{1}H NMR (300 MHz, CD_3OD): δ 5.16 (s, 1H), 4.94 (d, 1H, $J = 3.5$ Hz), 4.50 (m, 1H), 4.23 (dd, 1H, $J = 3.5$ Hz, $J = 10.6$ Hz), 4.00−3.55 (m, 15H), 2.16 (s, 3H), 1.81 (m, 2H), 1.50 (m, 10H), 1.38 (d, 3H, $J = 6.5$ Hz), 1.11 (m, 3H). ^{13}C NMR (50 MHz, CD_3OD): δ 101.61 (C-1 Fuc), 98.66(C-1 GlcNAc), 80.94, 74.14 (C-1′), 73.64, 72.85, 72.22, 71.66, 71.63 (C-6), 70.69, 70.09, 69.15 (CH_2 (octyl)), 68.27, 64.42 (C-3′), 54.60, 33.00 (CH_2), 30.53 (CH_2), 30.45 (CH_2), 30.43 (CH_2), 27.29 (CH_2), 23.71 (CH_2), 22.74 (NHAc), 16.57 (Me-Fuc), 14.41 (CH_3). Anal. ($C_{25}H_{47}NO_{12}$) C, H, N.

Octyl *O*-(α-L-Fucopyranosyl)-(1→3)-2-acetamido-2-deoxy-4,6-bis-*O*-(2(*R*),3-dihydroxypropyl)-α-D-glucopyranoside (4,6G$_D$ (31)). **25** (46 mg, 0.047 mmol) was reacted by the procedure used to synthesize compound **27** and afforded the title compound (30 mg, quantitative). R_f: 0.17 (CH_2Cl_2−MeOH, 3:1); $[\alpha]_D$: +15.2° (*c* 0.3, MeOH). ^{1}H NMR (300 MHz, CD_3OD): δ 5.31 (d, 1H, $J = 2.7$ Hz), 5.01 (d, 1H, $J = 3.3$ Hz), 4.38 (m, 1H), 4.20−3.51 (m, 21H), 2.17 (s, 3H), 1.75 (m, 2H), 1.49 (m, 10H), 1.41 (d, 3H, $J = 6.5$ Hz), 1.09 (m, 3H). ^{13}C NMR (50 MHz, CD_3OD): δ 101.79 (C-1 Fuc), 98.19 (C-1 GlcNAc), 79.80, 78.12, 75.58 (C-1′), 73.93 (C-1′), 73.50, 72.60, 72.34, 72.25, 71.26, 70.76 (C-6), 70.67, 69.17 (CH_2 (octyl)), 68.74, 64.41 (C-3′), 64.28 (C-3′), 55.65, 32.99 (CH_2), 30.47 (CH_2), 30.43 (CH_2), 27.27 (CH_2), 23.67 (CH_2), 22.74 (NHAc), 16.79 (Me-Fuc), 14.41 (CH_3). Anal. ($C_{28}H_{53}NO_{14}$) C, H, N.

Alkylation Reaction of Diol 6 with Tosylate 20. **6** (425 mg, 0.56 mmol) was treated with **20** (243 mg, 0.85 mmol), following the procedure shown above, to give after column chromatography (hexane−acetone, 5:1→1:1) **22** (278 mg, 56%), **24** (114 mg, 24%), and **26** (75 mg,14%). **22** R_f: 0.33 (hexane−acetone, 1:1). **24**: R_f 0.51 (hexane−acetone, 1:1). **26**: R_f 0.54 (hexane−acetone, 1:1).

Octyl *O*-(α-L-Fucopyranosyl)-(1→3)-2-acetamido-2-deoxy-4-*O*-(2(*S*),3-dihydroxypropyl)-α-D-glucopyranoside (4G$_L$ (28)). **22** (145 mg, 0.168 mmol) was reacted by the procedure used to synthesize compound **27** and afforded the

title compound (93 mg, quantitative). R_f: 0.34 (CH_2Cl_2−MeOH, 3:1). Mp: 96−99 °C. $[\alpha]_D$: +19.0° (*c* 1, MeOH). ^{1}H NMR (300 MHz, CD_3OD): δ 5.32 (d, 1H, J = 2.8 Hz), 5.11 (d, 1H, J = 3.3 Hz), 4.40 (m, 1H), 4.18 (dd, 1H, J = 8.7 Hz, J = 10.7 Hz), 4.05−3.52 (m, 14H), 2.17 (s, 3H), 1.75 (m, 2H), 1.49 (m, 10H), 1.41 (d, 3H, J = 6.5 Hz), 1.09 (m, 3H). ^{13}C NMR (50 MHz, CD_3OD): δ 102.84 (C-1 Fuc), 98.94(C-1 GlcNAc), 81.00, 79.33, 75.84 (C-1′), 74.42, 74.11, 73.36, 72.15, 71.64, 69.92 (CH_2 (octyl)), 69.79, 65.35 (C-3), 62.86 (C-6), 56.62, 33.87 (CH_2), 31.36 (CH_2), 28.16 (CH_2), 24.59 (CH_2), 23.70 (NHAc), 17.69, 15.33. Anal. ($C_{25}H_{47}NO_{12}$) C, H, N.

Octyl *O*-(α-L-Fucopyranosyl)-(1→3)-2-acetamido-2-deoxy-6-*O*-(2(*S*),3-dihydroxypropyl)-α-D-glucopyranoside (6G$_L$ (30)). **24** (65 mg, 0.075 mmol) was reacted by the procedure used to synthesize compound **27** and afforded the title compound (40 mg, 96%). R_f: 0.34 (CH_2Cl_2−MeOH, 3:1); $[\alpha]_D$: +9.9° (*c* 0.9, MeOH). ^{1}H NMR (300 MHz, CD_3OD): δ 5.16 (s, 1H), 4.95 (d, 1H, J = 3.2 Hz), 4.48 (m, 1H), 4.23 (dd, 1H, J = 3.5 Hz, J = 10.6 Hz), 4.00−3.55 (m, 15H), 2.16 (s, 3H), 1.83 (m, 2H), 1.50 (m, 10H), 1.38 (d, 3H, J = 6.5 Hz), 1.11 (m, 3H). ^{13}C NMR (50 MHz, CD_3OD): δ 101.59 (C-1 Fuc), 98.66 (C-1 GlcNAc), 80.88, 74.05 (C-1′), 73.64, 72.87, 72.12, 71.64, 71.55 (C-6), 70.66, 70.07, 69.14 (CH_2 (octyl)), 68.24, 64.40 (C-3′), 54.59, 33.01 (CH_2), 30.56 (CH_2), 30.54 (CH_2), 30.44 (CH_2), 27.29 (CH_2), 23.71 (CH_2), 22.73 (NHAc), 16.57, 14.42. Anal. ($C_{25}H_{47}NO_{12}$) C, H, N.

Octyl *O*-(α-L-Fucopyranosyl)-(1→3)-2-acetamido-2-deoxy-4,6-bis-*O*-(2(*S*),3-dihydroxypropyl)-α-D-glucopyranoside (4,6G$_L$ (32)). **26** (52 mg, 0.053 mmol) was reacted by the procedure used to synthesize compound **27** and afforded the title compound (33 mg, 98%). R_f: 0.16 (CH_2Cl_2−MeOH, 3:1); $[\alpha]_D$: +18.0° (*c* 0.5, MeOH). ^{1}H NMR (300 MHz, CD_3OD): δ 5.32 (d, 1H, J = 2.5 Hz), 5.12 (d, 1H, J = 3.3 Hz), 4.40 (m, 1H), 4.16 (dd, 1H, J = 8.9 Hz, J = 11.8 Hz), 4.06−3.50 (m, 20H), 2.17 (s, 3H), 1.77 (m, 2H), 1.49 (m, 10H), 1.41 (d, 3H, J = 6.5 Hz), 1.10 (m, 3H). ^{13}C NMR (50 MHz, CD_3OD): δ 102.03 (C-1 Fuc), 98.11 (C-1 GlcNAc), 79.93, 78.39, 74.98 (C-1′), 73.51, 72.45, 72.33, 72.26, 72.14, 71.22 (C-6), 69.15 (CH_2 (octyl)), 68.93, 64.42 (C-3′), 64.37 (C-6), 55.70, 32.99 (CH_2), 30.44 (CH_2), 27.26 (CH_2), 23.69 (CH_2), 22.73 (NHAc), 16.76 (Me-Fuc), 14.41 (CH_3). Anal. ($C_{28}H_{53}NO_{14}$) C, H, N.

Octyl *O*-(2,3,4-Tri-*O*-benzyl-α-L-fucopyranosyl)-(1→3)-*O*-[(2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl-(1→6)]-2-acetamido-2-deoxy-α-D-glucopyranoside (35). A mixture of 2,3,4,6-tetra-*O*-benzoyl-α/β-D-galactopyranosyl trichloroacetimidate[19] (178 mg, 0.24 mmol), **6** (150 mg, 0.2 mmol), and molecular sieves (4 Å) (ca 60 mg) in dry dichloromethane (2 mL) was stirred at room temperature under Ar for 30 min. Then, a solution of TMSOTf in dichloromethane (0.1 M, 0.4 mL) was added dropwise, and the reaction mixture was stirred at room temperature under Ar for 3 h. After this time, the mixture was neutralized with triethylamine, filtered through Celite, and evaporated to dryness. The residue was purified by column chromatography (hexane−acetone, 4:1) to give **35** (217 mg, 82%). R_f: 0.44 (hexane−AcOEt, 1:1); $[\alpha]_D$: +45.5°. Anal. ($C_{77}H_{85}NO_{19}$) C, H, N.

Octyl *O*-(α-L-Fucopyranosyl)-(1→3)-*O*-[(β-D-galactopyranosyl-(1→6)]-2-acetamido-2-deoxy-α-D-glucopyranoside (36). **35** (86 mg, 0.065 mmol) was treated with a solution of sodium methoxide in methanol (0.1 M, 2.6 mL, 0.26 mmol) for 2 h at room temperature. After this time, the reaction mixture was neutralized with Amberlite IR-120 (H^+), filtered, and concentrated. The residue was dissolved in methanol (5 mL), Pd/C (74 mg) was added, and the reaction mixture was stirred under H_2 atmosphere for 3 h. Then, the mixture was filtered through Celite and evaporated to dryness to give **36** (38 mg, 93%). R_f: 0.22 (CH_2Cl_2−MeOH, 2:1). Mp: 223−226 °C. $[\alpha]_D$: +2.0° (*c* 0.7, MeOH). ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.16 (d, 1H, J = 1.7 Hz), 4.93 (d, 1H, J = 3.6 Hz), 4.48 (d, 1H, J = 7.7 Hz), 4.48 (m, 1H), 4.33 (dd, 1H, J = 1.7 Hz, J = 10.8 Hz), 4.25 (dd, 1H, J = 10.7 Hz, J = 3.5 Hz), 4.03−3.51 (m, 15H), 2.16 (s, 3H), 1.81 (m, 2H), 1.59 (m, 10H), 1.38 (d, 1H, J = 6.5 Hz), 1.09 (m, 3H). ^{13}C NMR (50 MHz, CD_3OD): δ 105.45, 101.64, 99.66, 80.95, 76.77, 74.99, 73.70, 72.89, 72.62, 71,72, 70.57, 70.34, 70.18, 69.16, 68.32, 62.54, 54.61, 32.79, 30.60, 30.54, 30.47, 27.30, 23.73, 22.75, 16.61, 14.42. Anal. ($C_{28}H_{51}NO_{15}\cdot 2H_2O$) C, H, N.

Cell Culture. C6 rat glioma cells[15] and U-373 MG and U-118 MG human glioma cells[16] were seeded on plastic flasks and maintained in DMEM culture medium, supplemented with 10% fetal bovine serum (FCS), 2 mM glutamine, 50 IU/mL penicillin, and 50 μg/mL streptomycin (DM-10S) and maintained in a humidified atmosphere of 5% CO_2, at 37 °C. All the plastic material was obtained from Costar (Cambrigde, MA). Culture media, supplements, and antibiotics were from Sigma Spain (Madrid, Spain).

Inhibition of Cell Proliferation. Proliferation assays were performed on 96-well plates, using a modification of the method described by Nieto-Sampedro,[2,17] as follows. All cells lines were seeded in DMEM supplemented with 10% FCS, at 5×10^3 cells/well, and allowed to attach for 6 h. For human glioma culture, after the cells were attached to the substrate, the medium was changed to serum-free DMEM and the cells were incubated for 48 h. By comparison, C6 cells were incubated in DMEM supplemented with 0.5% FCS, overnight. After either 48 h (human lines) or 24 h (rat glioma C6), the medium was replaced by DMEM medium containing the test inhibitors (from 1 mM to 62 μM, in triplicate) and mitogen. The mitogens were 10% FCS for C6 cells and 1% FCS for U-118. Astrocytoma U-373 proliferation was driven by 1% FCS, by human EGF (10 ng/mL), or by human bFGF (100 ng/mL), as indicated for each experiment. The cultures were further incubated for 48 h and washed with serum-free DMEM medium, and [^{3}H]thymidine incorporation was measured following a 6-h pulse (0.5 μCi/well). Nonincorporated radioactivity was washed, and the cells were collected on glass fiber filters, using a cell harvester(Skatron Inc., Norway). Radioactivity was measured on a Pharmacia-LKB liquid scintillation counter. Inhibition (%) was calculated as follows:

$$\% \text{ inhibition} = 100 - 100[(X - B)/(A - B)]$$

where A is the [^{3}H]thymidine dpm incorporated by cells maintained in 10% FCS, 1% FCS, or growth factors (high mitosis control), B is the dpm incorporated by cells in mitogen-free medium (low mitosis control), and X represents the dpm incorporated by cells treated with test inhibitors. Dose−response plots of percent inhibition versus concentration were adjusted to sigmoidal curves from which ID_{50} values were calculated.

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Supporting Information Available: NMR spectral data for compounds **3**−**6**, **8**−**12**, **21**−**26**, and **35** (4 pages). Ordering information is given on any current masthead page.

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