Functionalized Glycomers as Growth Inhibitors and Inducers of Apoptosis in Human Glioblastoma Cells

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Received December 27, 2002

The effects of functionalized aryl β-D-glycopyranosides (glycomers) on the proliferation, survival, and apoptosis of human glioblastoma cells in culture were evaluated as a way to control tumor progression. The results showed that inhibition of growth and/or induction of apoptosis can be achieved by these molecules in human glioblastoma cells. Inhibition of DNA synthesis precedes induction of apoptosis and growth inhibition. The substituents at C-1, C-2, C-3,C-4, and C-6 on the pyranosidic scaffold are important to modulate the action and the efficacy of these molecules. Human fibroblasts and brain-derived endothelial cells were less sensitive to glycomers than tumor cells. Thus, functionalized aryl β-D-glycopyranosides represent a new class of molecules potentially able to control the progression of brain tumors.

Introduction

Uncontrolled cell proliferation is involved in several human diseases, including cancers of any origin. The control of cancer growth by inhibition of cell division and induction of tumor cell death (apoptosis), in conjunction with other adjuvant anticancer treatments, could provide a therapeutic strategy with improved efficacy.

Glioblastomas are brain tumors with a high proliferative potential and a very poor prognosis, mainly due to two reasons. First, glioblastoma tumor cells as well as the cerebral vasculature forming the blood−brain barrier express multiple resistance pathways to chemotherapeutic agents,[1] precluding conventional treatments.[2] Second, while glioblastomas rarely metastasize outside of the brain, they have a very high migratory potential in the central nervous system,[3−5] precluding complete surgical resection. Thus, the development of drugs able to control the growth of glioblastoma cells without inducing drug resistance, would be of benefit for the treatment of brain cancer.[6] Disaccharide or tetrasaccharide derivatives able to control glioblastoma cell growth have been reported to be of potential therapeutic interest.[7,8] However, simpler functionalized monosaccharide derivatives could be advantageous in biological systems, due to their increased stability and potential to be transported across physiological barriers and cell membranes. Different binding capacities for saccharide epitopes in brain tumors have been described.[9] Thus, the control of glioblastoma tumor cell growth either by inhibition of cell division and/or induction of cell apoptosis by carbohydrate-based molecules may be possible.

We have previously shown that functionalized aryl β-D-glucopyranosides (glycomers) inhibit the growth of human epidermoid and colon carcinoma cells lines.[10] Here we report on the synthesis of a new series of glycomers by systematic functional group modifications of an aryl glycopyranosidic scaffold, and their effects on inhibition of proliferation and induction of apoptosis of human glioblastoma cells. The effect of these compounds toward human cerebral endothelial cells and normal primary human fibroblasts was also studied.

Synthesis

Glycomers in the aryl D-glucopyranoside series were synthesized from common precursors utilizing well-established methods starting with the known[11] 3-O-allyl-1,2,5,6-*O*-isopropylidene-D-glucofuranose **2** (Scheme 1). Acetolysis provided the tetraacetate **3**, which was converted to the β-iodophenyl glycoside **4** utilizing the enhanced nucleophilicity of tributylstannyloxy 4-iodobenzene toward a glucopyranosyl bromide.[12] Deacetylation and conversion to the 4,6-*O*-benzylidene derivative **6** allowed the preparation of a series of 2-esters **7a**−**d**. Cleavage of the acetal and selective sulfonylation with 3-trifluoromethylbenzenesulfonyl chloride afforded a set of glycomers **8a**−**e** with different esters at C-2 (Scheme 1). Glycomer **8e** was prepared from the corresponding 3-*O*-methyl ether as described for **8b**. The sulfonamides **10a** and **10b** were prepared by displacement of **8a** and **8c** with sodium azide to give the corresponding 6-azido derivatives **9a** and **9b**, which were reduced and *N*-sulfonylated (Scheme 2). Reductive cleavage of the 4,6-*O*-benzylidene ring in **7a** and **7c** with sodium cyanoborohydride in the presence of hydrogen chloride[13] afforded the 6-*O*-benzyl ethers **11a** and **11b** (Scheme 3).

The glycosidation reaction shown in Scheme 1 also afforded the *p*-iodophenyl α-D-glucopyranoside analogue **12** as a minor product (Scheme 4). This was transformed through a series of steps similar to the β-anomer affording glycomer **13**.

The preparation of analogues in the D-*galacto* series starting with β-D-galactopyranose pentaacetate was achieved as described for the D-*gluco* series in Schemes

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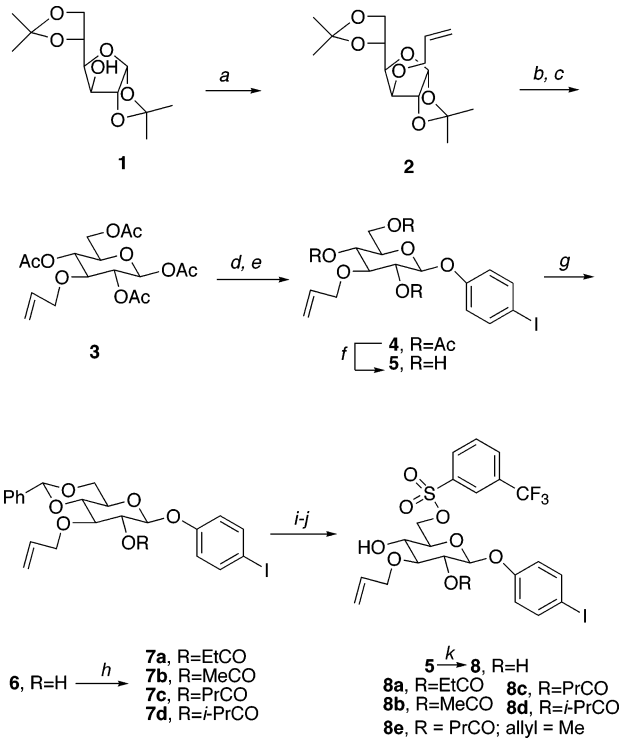
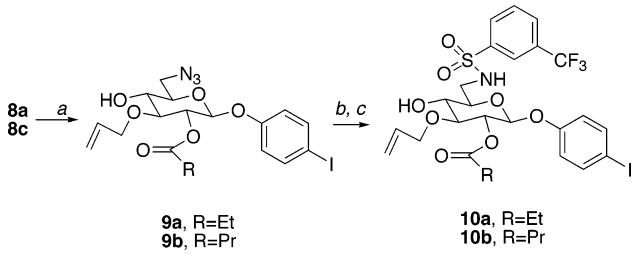
10.1021/jm0205853 CCC: $25.00
Published on Web 07/16/2003

Scheme 1[a]



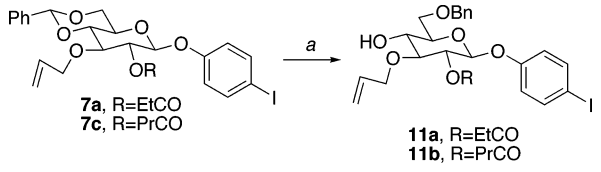
[a] NaH, allyl bromide; (b) ion-exchange resin IR-20 (H^+); (c) Ac_2O, pyridine; (d) HBr in AcOH, Ac_2O, CH_2Cl_2, 0 °C; (e) 4-iodotributylstannyl phenoxide, $SnCl_4$, benzene, reflux; (f) NaOMe,MeOH; (g) $PhCH(OCH_3)_2$, $HBF_4.Et_2O$, DMF; (h) $(RCO)_2O$, pyridine (for **7a**, 7**b**); RCOCl, Et_3N, CH_2Cl_2 (for **7c**, **7d**); (i) TFA, H_2O, THF; (j) 3-(trifluoromethyl)benzenesulfonyl chloride, Et_3N, CH_2Cl_2, (k) 3-(trifluoromethyl)benzenesulfonyl chloride, pyridine, 0 °C to rt.

Scheme 2[a]



[a] NaN_3, DMF; (b) PPh_3, H_2O, THF; (c) 3-(trifluoromethyl)benzenesulfonyl chloride, Et_3N, CH_2Cl_2.

Scheme 3[a]

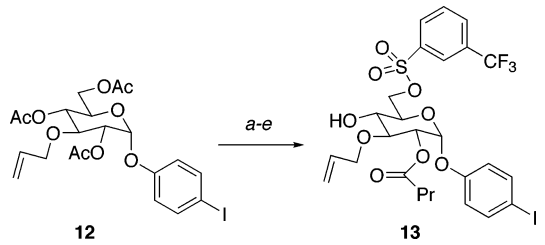


[a] $NaCNBH_3$, HCl, THF, 4A MS.

1−3. The 2-ester derivatives of the 6-sulfonates **16a**,**b**, the sulfonamides **17a**,**b**, and the 6-*O*-benzyl ethers **18a**,**b** were easily prepared as shown in Scheme 5 (Scheme 5).

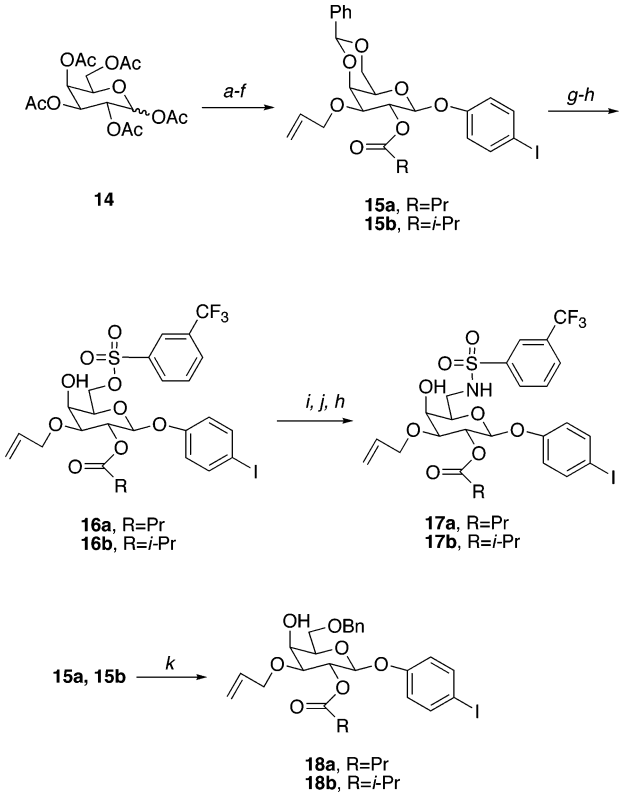
Evaluation of the Effects of Functionalized Glycomers in Human Glioblastoma Cells. First, we tested **8d** as a prototype for the inhibition of cell growth using the MTT (thiazole blue) assay, which determines the number of adherent metabolically active cells. For these experiments human glioblastoma LN18 and

Scheme 4[a]



[a] NaOMe,MeOH; (b) $PhCH(OCH_3)_2$, HBF_4·Et_2O, DMF; (c) RCOCl, Et_3N, CH_2Cl_2; (d) TFA, H_2O, THF; (e) 3-(trifluoromethyl)benzenesulfonyl chloride, Et_3N, CH_2Cl_2.

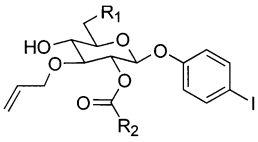
Scheme 5[a]



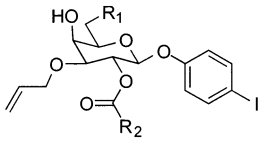
[a] HBr in AcOH, Ac_2O, CH_2Cl_2, 0 °C; (b) 4-iodotributylstannyl phenoxide, $SnCl_4$, benzene, reflux; (c) NaOMe, MeOH; (d) Bu_2SnO, allyl bromide, Bu_4NBr, benzene, reflux; (e) $PhCH(OCH_3)_2$, HBF_4·Et_2O, DMF; (f) RCOCl, Et_3N, CH_2Cl_2; (g) TFA, H_2O, THF; (h) 3-(trifluoromethyl)benzenesulfonyl chloride, Et_3N, CH_2Cl_2; (i) NaN_3, DMF; (j) PPh_3, H_2O, THF; (k) $NaCNBH_3$, HCl, THF, 4A MS.

LNZ308 cell lines[14] or human brain-derived endothelial cells (HCEC),[15] in cell cultures with and without exposure to serum (fetal calf serum, FCS) were used. The results showed that in the presence of FCS only low inhibition of growth was observed. Removal of FCS immediately prior to the addition of the glycomers restored growth inhibition, and a preincubation period of 24 h in the absence of FCS further increased the growth inhibitory response. Glioblastoma cells were found to be more sensitive to **8d** than HCEC brain-derived endothelial cells. The effect of cell density on growth inhibition showed that subconfluent cells were more sensitive to growth inhibition than confluent cells under these experimental conditions. Thus, for screening and evaluation of glycomer efficacy, cells were routinely split and left to adhere for 24 h in the presence of FCS, then deprived of FCS for another 24 h period,

Table 1. Glycomer Activity against Human Glioblastoma LN18 and LNZ308 Cells

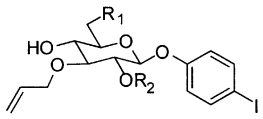


8a, b, d; 10a, b; 11a, b

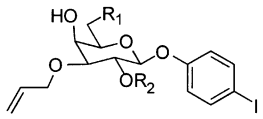


16b, 17b

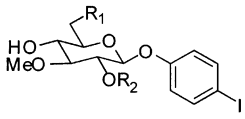
compound	R_1	R_2	LN18[a]	LNZ308
	A. Glycomers with IC_{50} < 20 μM			
8a	OSO_2m-TMB[b]	Et	7.0 ± 1.5	11.5 ± 3.0
8b	OSO_2m-TMB	Me	12.5 ± 2.0	13.0 ± 2.0
8d	OSO_2m-TMB	i-Pr	12.5 ± 2.0	8.5 ± 2.0
10a	$NHSO_2$m-TMB	Et	12.5 ± 1.5	18.5 ± 2.5
10b	$NHSO_2$m-TMB	Pr	6.5 ± 1.0	17.5 ± 2.0
11a	OCH_2Ph	Et	11.5 ± 2.5	17.5 ± 4.5
11b	OCH_2Ph	Pr	7.5 ± 1.5	11.5 ± 2.5
16b	OSO_2m-TMB	i-Pr	18.5 ± 2.0	18.0 ± 2.0
17b	$NHSO_2$m-TMB	i-Pr	12.5 ± 2.0	7.5 ± 1.0



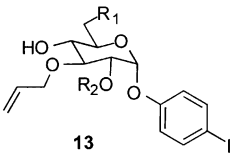
8, 8c



16a, 17a, 18a



8e



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	B. Glycomers with IC_{50} 20−45 μM			
8	OSO_2m-TFMB[b]	COMe	21 ± 2.5	19 ± 2.5
8c	OSO_2m-TFMB	COPr	43 ± 8.5	41 ± 7.5
8e	OSO_2m-TFMB	COPr	26 ± 3.5	22 ± 2.0
13	OSO_2m-TFMB	COPr	25 ± 5.0	42 ± 8.0
16a	OSO_2m-TFMB	COPr	40 ± 4.0	29 ± 3.5
17a[c]	$NHSO_2$m-TFMB	COPr	25 ± 3.0	26 ± 3.5
17d	$NHSO_2$m-TFMB	H	16 ± 2.5	27 ± 4.5
18a	OCH_2Ph	COPr	20 ± 2.5	37 ± 5.5

[a] Cells were grown for 24 h with FCS, then deprived of FCS for 24 h. Glycomers were added to cells for 24 h and MTT assay was performed. The % growth inhibition was calculated as the ratio of MTT reduction (absorbance at 540 nm) of treated to control cells and IC_{50} was calculated from dose−response curves as the concentration inducing 50% of growth inhibition. Experiments were done in triplicate and means ± standard deviations were calculated. [b] TMB = trifluoromethyl benzenesulfonyl. [c] Phenylglycoside analogue (I = H).

before exposure to glycomers and evaluation of growth by the MTT assay. Cell density seeding was adjusted to have confluent cell layers in the control wells at the end of the experiment period.

Using the experimental conditions described above, a systematic evaluation of glycomers with chemical modifications in C-1, C-2, C-3, C-4, and C-6 positions was performed (Table 1A,B) (Figure 1A−E). The results showed that the glycomers could be grouped in three classes: high efficacy with IC_{50} < 20 μM (Table 1A); low efficacy with IC_{50} 20−50 μM, Table 1B), and glycomers with no efficacy (IC_{50} > 50 μM, Chart 1). Examination of the functional features in these classes of aryl glycopyranosides led to the identification of critical groups involved in the observed inhibitory effects. Most interesting was the nature of the *p*-phenyl substituent at the anomeric position. The *p*-iodo group (Tables 1A,B)

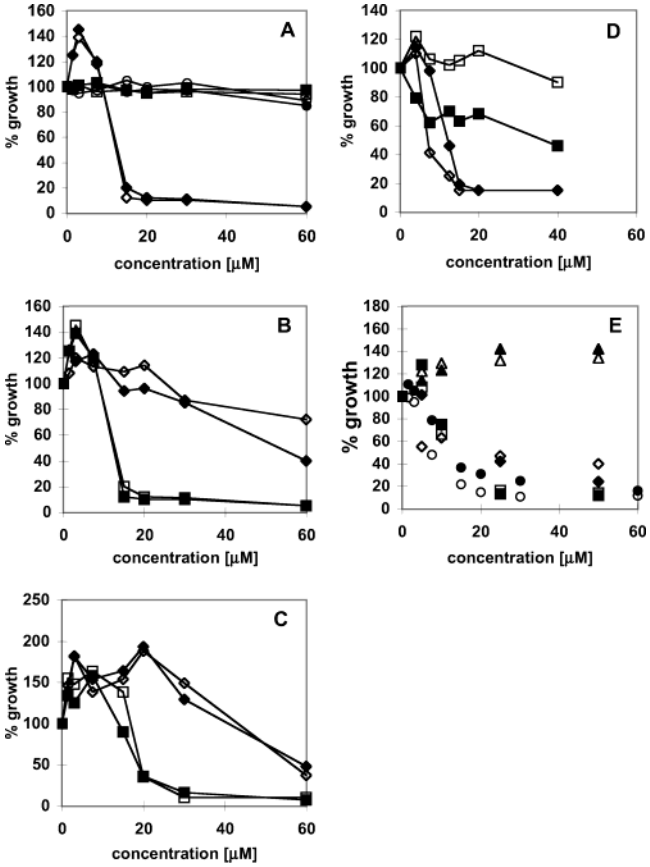


Figure 1. Growth inhibition of glioblastoma cells as a function of systematic chemical modifications of the glucopyranoside backbone. Cells were left to adhere for 24 h in the presence of FCS and incubated for another 24 h in the absence of FCS. Medium was changed and glycomers were added to cells at increasing concentrations in the absence of FCS for 24 h. MTT assay was performed during the last 2 h of incubation. Percent of growth was calculated as the ratio of the MMT reduction (absorbance at 540 nm) of treated versus control cells. Open symbols: LN18 cells; closed symbols: LNZ308 cells A: modification at position C-1; □ ■: **29**; ○ ●: **30**; ◆ ◇: **8b** B: modification at position C-2: □ ■: **8b**; ◆ ◇: **27** C: modification at position C-3: □ ■: **8**; ◆ ◇: **28** D: modification at position C-4: □ ■: **8a**; ◆ ◇: **32** E: modification at position C-6: ◆ ◇: **8d**; □ ■: **11b**; ▲ △: **31**; ○ ●: **17b**.

was preferred to hydrogen, bromo, and methoxy analogues (Chart 1). No significant difference in efficacy was observed between *gluco*- and *galacto*-isomers (Table 1A, **10a**, **17b**; **8a**, **16b**) or between the α/β-configuration of the glycomers (Table 1B, **8c**, **13**).

In position C-2, an aliphatic short-chain ester substituent improves efficacy, the propionate **8a** or acetate **8b** derivatives being the most favorable (compare **8b** and **27**, Figure 1B), possibly representing the preference of cellular esterases for short-chain alcohols, as we observed using esters of 5-aminolevulinic acid.[16] In position C-3, an allyl ether is more favorable than an methyl ether substitution (compare **8** and **28**, Figure 1C). The C-4 position must be unsubstituted (compare **8a** and **32**, Figure 1D). In position C-6, substitution of a sulfonate derivative with sulfonamide or ether derivatives does not modify the effect, while an ester substitution is detrimental, which we interpreted as a result of hydrolysis due to the presence of cellular esterases (compare **8d**, **11b**, and **17b** with **31**, Figure 1E). Thus, the structure of the most efficient molecule, as defined

Chart 1. Glycomers with $IC_{50} > 50\ \mu M$ against Human Glioblastoma LN18 and LNZ308 Cell Lines

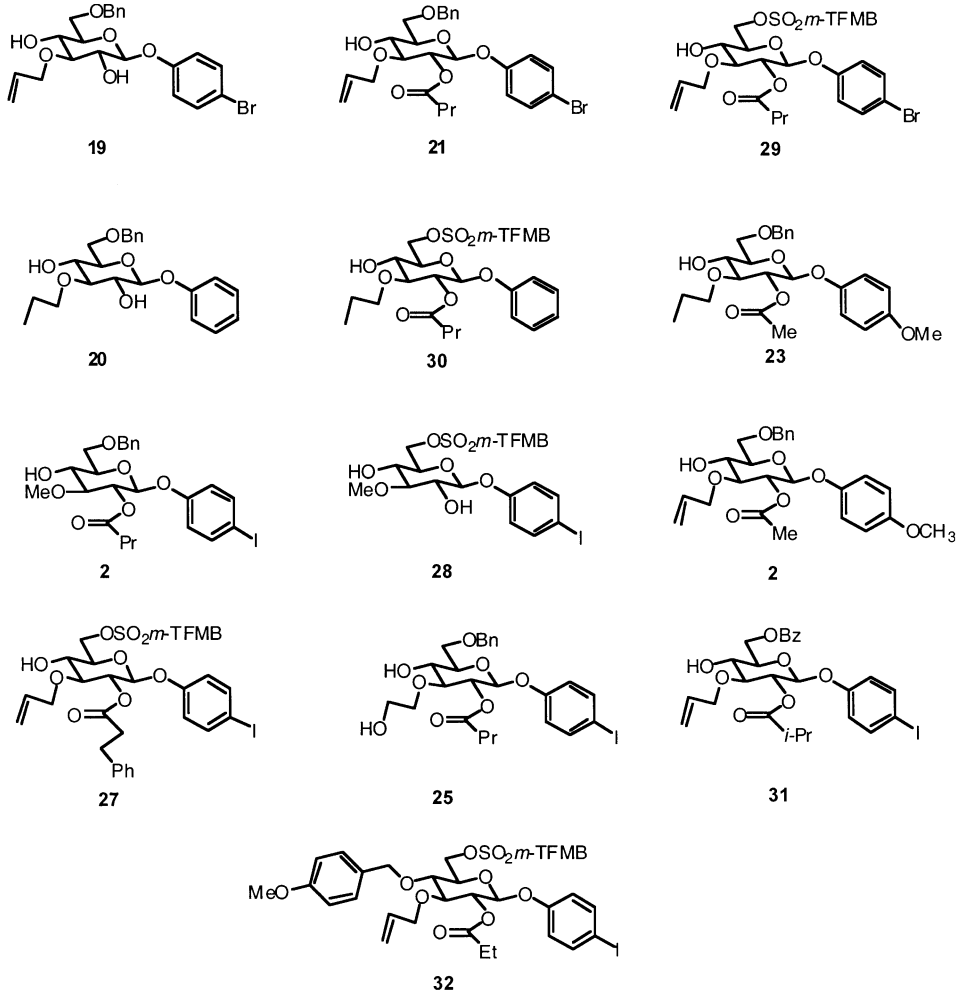
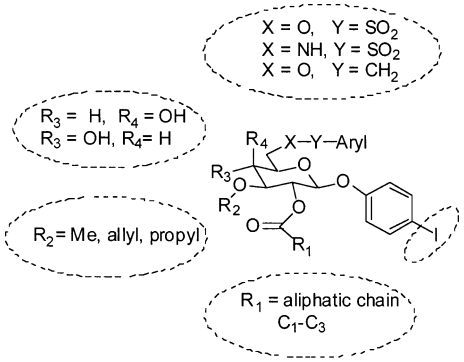


Chart 2 The Active Glycomers



by MTT assay, has a *p*-iodophenyl substitution in position C-1, a short-chain aliphatic ester in position C-2, an allyl group in position C-3, no substituent at position C-4, and an aromatic sulfonate, sulfonamide, or ether, but not ester, substitution at position C-6 (Chart 2).

We then evaluated whether the inhibitory effects were dependent on increased cell death or decreased proliferation or both. Examination under the microscope of cells exposed to glycomers in the presence of MTT, an indicator of mitochondrial function, did not indicate mitochondrial toxicity, since adherent and rounded cells displayed similar violet coloration of mitochondria (not shown). We first investigated growth inhibition by evaluating the incorporation of tritiated thymidine to quantitate DNA synthesis. The effects of **8c**, a weak inhibitor, and **11b**, a potent inhibitor, on inhibition of DNA synthesis were first determined in the two glioblastoma cell lines after 8 or 27 h of exposure (results not shown). While **11b** completely inhibited DNA synthesis already after 8 h, **8c** was much less efficient and only partial inhibition was observed after 27 h exposure. These results demonstrated that inhibition of DNA synthesis is not due to a nonspecific effect of glycomers in glioblastoma cells, but is related to the potency of the drug. Exposure to **8a** induced cell rounding and detachment (as observed under the microscope), starting after 6−8 h. Inhibition of DNA synthesis was already observed 2 h after addition of **8a** and increased only slightly with longer exposure time (Figure 2A). To observe a decrease in the number of metabolically active cells, using the MTT assay as a marker and the quantification of protein content to ascertain cell number (Figure 2B) in the culture wells, it was necessary to expose to **8a** for periods longer than 8 h. A maximal effect was observed after 24 h, which did not further increase by incubating the cells for 48 h. Continuous exposure to **8a** was not necessary to observe an effect on DNA synthesis, since diminution of DNA synthesis was seen already after 5 min, reaching a maximum after 60 min exposure of glioblastoma cells (Figure 3A). Thus the primary effect of these glycomers appears to be a

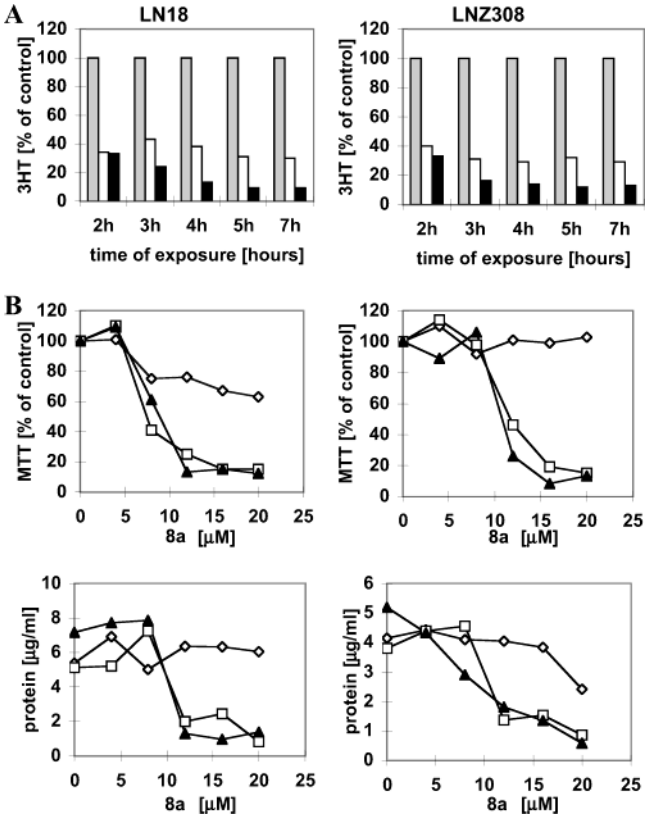


Figure 2. Inhibition of DNA synthesis precedes inhibition of cell growth and loss of adherence. Cells were left to adhere for 24 h in the presence of FCS, then incubated for another 24 h in the absence of FCS. Medium was changed, and **8a** was added to cells at increasing concentrations in the absence of FCS for 2−24 h. Thymidine incorporation and MTT assay were performed for the two last hours of incubation, and protein content was determined at the end of the incubation period. **(A)**: Thymidine incorporation. Grey bars: no **8a** ; white bars: 2 μM **8a** ; black bars: 4 μM **8a**. **(B):** MTT assay and protein content. ◇: 8 h exposure; □: 24 h exposure; ▲: 48 h exposure.

rapid initial inhibition (<5 min) of DNA synthesis in glioblastoma cells, followed by death of cells (>8 h) which is maximal at 24 h.

Endothelial cells derived from human brain (HCEC) were less sensitive to inhibition of DNA synthesis in response to short-time exposure to **8a** than glioblastoma cells (Figure 3B). The growth of normal primary human fibroblasts, as determined by an MTT assay, was inhibited at slightly higher concentrations of **8a** (IC_{50} = 20−25 μM) than glioblastoma tumor cells (Figure 4A). Inhibition of growth (MTT) and DNA synthesis (3HT incorporation) (Figure 4B) in fibroblasts (Figure 4B, upper panels) by another glycomer, **10a**, was also induced at higher glycomer concentration in fibroblasts than in LN18 and LNZ308 glioblastoma cells (Figure 4B, lower panels). Thus, some concentration-dependent selectivity toward tumor cells versus stromal cells can also been obtained by some glycomers.

We then studied whether growth inhibition of glioblastoma cells by the glycomers can also be achieved through induction of apoptosis. To this end, we determined chromatin condensation in the nuclei of adherent cells in culture using DAPI, a fluorescent dye labeling double-stranded DNA (Figure 5A), or histological nuclear and cytoplasmic staining (hematoxylin-eosin and Giemsa histological stains) (Figure 5B), or quantitated

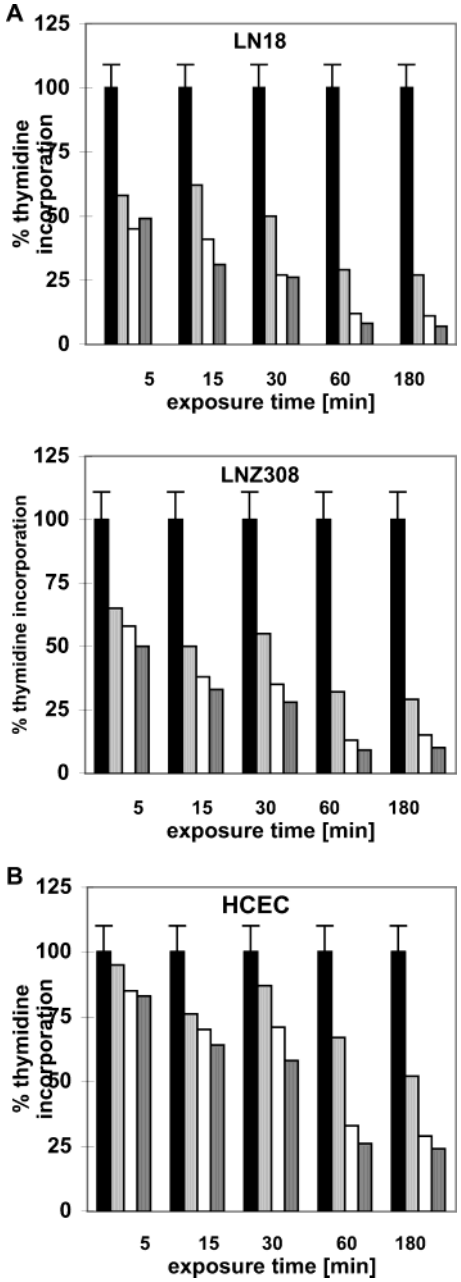


Figure 3. Continuous exposure to glycomers is not necessary for effect in human glioblastoma cells and endothelial cells. Endothelial cells are less sensitive than glioblastoma cells to short time exposure to glycomers. Cells were left to adhere for 24 h in the presence of FCS, then incubated for another 24 h in the absence of FCS. Medium was changed and **8a** was added to cells at 0, 4, 12, and 20 μM concentrations in the absence of FCS for 5, 15 30, 60, or 180 min. Following exposure, **8a** was removed from the culture wells, medium without FCS and without glycomer was added and incubation was continued to reach 60 min, then thymidine incorporation was performed for the two next hours. A: glioblastoma cells; B: endothelial cells. Black bars: no **8a**; light gray bars: 4 μM **8a**; white bars: 12 μM **8a**; dark gray bars: 20 μM **8a**.

in cell extracts histone-associated DNA fragments (Table 2), which are end-point markers of apoptotic cell death. Using quantification of nucleosome fragments, the apoptosis index in the absence of glycomer was defined as 1.00. The results are shown in Table 2. Compounds **19** and **20** (Chart 1) did not induce apoptosis, based on the observation that growth inhibition and loss of adhesivity was not observed, while in the presence of the most

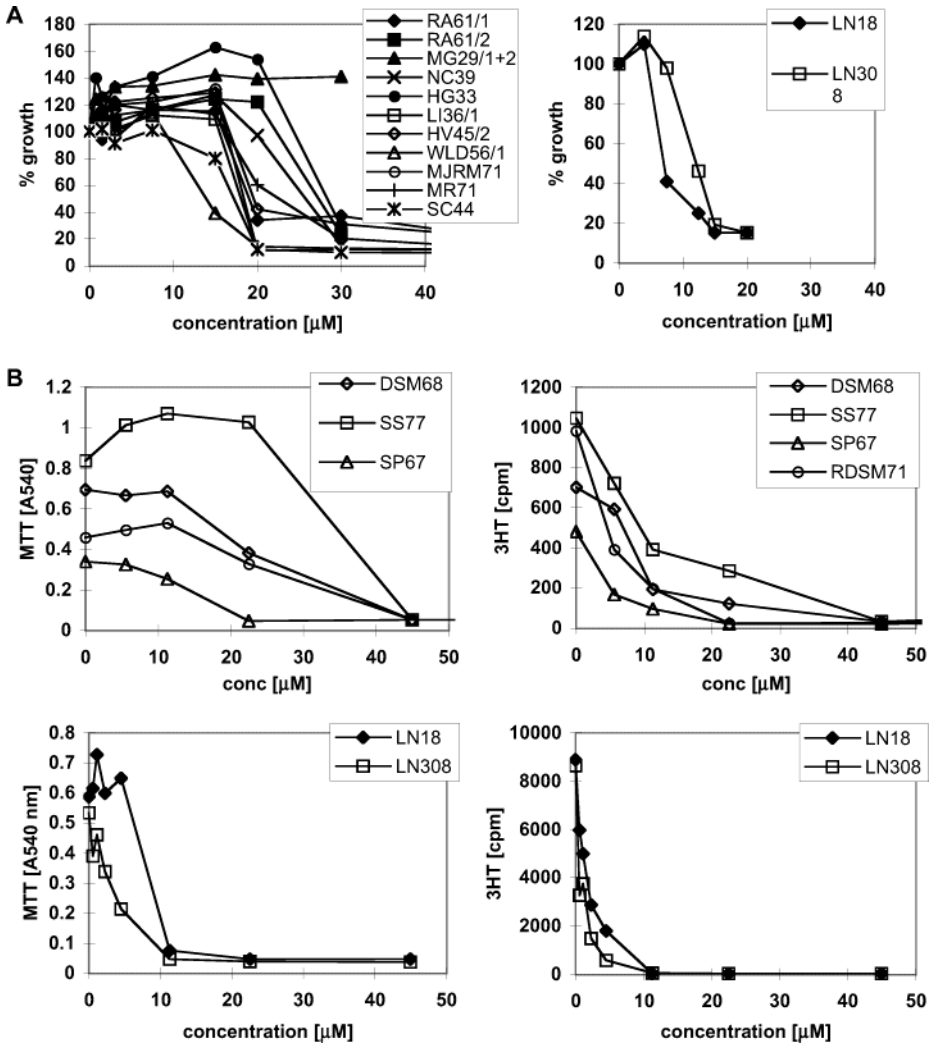


Figure 4. Growth and DNA synthesis inhibition of primary human fibroblasts by **8a** and **10a**. Comparison with human glioblastoma cells. Cells were grown in the presence of FCS, then incubated for 24 h in the absence of FCS. Medium was changed and **8a** or **10a** was added to cells at increasing concentrations in the absence of FCS for 24 h. MTT assay or thymidine incorporation was performed during the last 2 h of incubation. A: **8a** inhibits growth in cultures of human fibroblasts from 11 different surgical specimens and two glioblastoma cell lines. B: dose-dependent inhibition of growth (MTT, left) and DNA synthesis (^{3}HT incorporation, right) by **10a** in four different cultures of human fibroblasts (upper panels) or in 2 glioblastoma cell lines (lower panels).

efficient growth inhibitors **8a**, **11a**, and **11b** (Table 1A), nucleosome fragmentation was observed (Tables 2 and 3). However, **31** (Chart 1), which did not inhibit growth and cell adherence after 24 h treatment, could induce apoptosis. Glycomer **10a**, which is a potent inhibitor of DNA synthesis and cell growth at low concentration, was a poorer inducer of apoptosis at these concentrations. Thus, the concentration able to inhibit cell growth or induce apoptosis could be dissociated for some functionalized glycomers. When studied for a shorter time-course (4−8 h of exposure), before cell detachment was observed but at the time of cell rounding, glycomers **8a** and **11a** were able to induce apoptosis as determined by chromatin condensation and revealed by DAPI staining (Figure 5A) or nuclear staining by hematoxylin-eosin or Giemsa histological stains (Figure 5B). These results show that functionalized *p*-iodophenyl glycosides are able to inhibit DNA synthesis and induce apoptosis in human glioblastoma cells. Therefore they represent a new class of molecules with a potential to control brain tumor progression.

To exclude the possible alkylating effect of sulfonated glycomers such as **8a**, the efficacy of inhibition of DNA synthesis or induction of apoptosis in glioblastoma cells was directly compared with the sulfonamide **10a** and the ether **11a** analogues (Table 3). We calculated the efficacy of these glycomers by reporting the ratio of thymidine incorporation or of apoptosis index to the level of MTT reduction. These experiments indicate that the efficacy indexes, at concentrations above the IC50 are similar for **8a**, **10a**, and **11a**, and that an alkylating of sulfonate glycomers is not necessary for effects.

Discussion

Glioblastoma tumors express binding sites for saccharide epitopes.[9] A few studies reporting the evaluation of carbohydrate-based antiproliferative agents for glioblastoma tumor cells have been published.[7,8,17] We show here that substituted aryl glycopyranosides (glycomers) cause selective inhibition of DNA synthesis, thus controlling the replicative/proliferative potential of glioblastoma cells, as well as induction of apoptosis. These

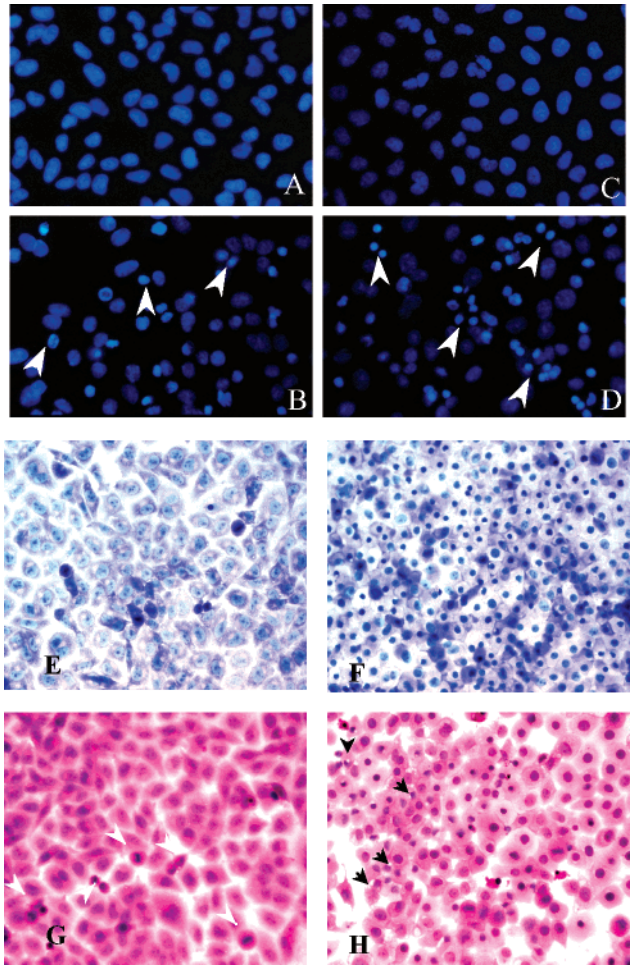


Figure 5. **8a** and **11a** glycomers block mitosis and induce nuclear condensation and apoptosis in human glioblastoma cells. Cells were left to adhere for 24 h on histological slides in the presence of FCS, then incubated for another 24 h in the absence of FCS. Medium was changed, and **8a** (A) or **11a** (B) was added for 7 h to cells in the absence of FCS. Then cells were fixed, exposed to the DNA labeling agent DAPI (A–D), and photographed under a fluorescent microscope. Alternatively following treatment, cells were fixed and stained using either Giemsa (E,F) or hematoxylin-eosin (G,H) histological stainings. (Part A) DAPI staining: LN18 (A,B) or LNZ308 (C,D) cells were exposed to control medium (A,C) or 20 μM **8a** (B,D), then fixed in Carnoy and exposed to the DNA labeling agent DAPI and photographed under a fluorescence microscope (white arrowheads: apoptotic cells). (Part B) hematoxylin-eosin and Giemsa stainings: LNZ308 cells were exposed to control medium (E,G) or to 22.5 μM **11a** (F,H), then fixed in 4% buffered paraformaldehyde and stained using Giemsa staining (E,F), which mainly stains nuclei or hematoxylin-eosin staining (G,H), which stains both cytoplasm and nucleus (G: white arrowheads: mitotic cells; H: black arrowheads: preapoptotic nucleus displaying nuclear blebbing). Identical information was obtained using LN18 cells (not shown).

combined effects would result in the control of glioblastoma growth.

The most active glycomers where *p*-iodophenyl β-D-glucopyranosides or β-D-galactopyranosides with short ester groups at C-2, an allyl ether at C-3, a free hydroxyl at C-4, and sulfonate, sulfonamide or benzyl ether at C-6. In the original series[10] the active glycomers contained a C-6 sulfonate, which is a potential alkylating molecule for proteins or nucleic acids. We therefore explored the chemically and biologically more stable sulfonamide or benzyl ether derivatives which were equally active as antiproliferative agents. Thus, these compounds are not active by virtue of a covalent alkylation mechanism. In view of the fact that glioblastomas are polyclonal and heterogeneous tumors, both cell lines displayed slightly different responses to apoptosis induction. Using bosentan and Fas Ligand[18] as an alternative approach to aptoptosis, we also observed a graded response of these cells. Interestingly, apoptosis was induced in a shorter time and at lower concentration by **8a** compare to other glycomers, for both cell lines. It appears that subtle changes in the nature of the ester group have significant effects on the induction of apoptosis (compare **8a**, **8c**, **8d**) (Table 2). This trend was also observed in the 6-*O*-benzyl series (compare **11a** to **11b**). We anticipate that this may be possibly due to the efficiency of cleavage by esterases.

Table 2. Induction of Apoptosis by Selected Glycomers.

compd	concn [μM]	total exposure,[a] time [h]	apoptosis index LN18	apoptosis index LNZ308
8a	16	10	3.82	8.97
8c	25	24	0.44	0.41
8d	25	24	1.10	1.09
10a	11	15	0.98	1.45
11a	11	15	3.67	0.91
11b[b]	25	24	1.60	2.27
19	25	24	1.19	ND
20	25	24	1.29	ND

[a] Cells were grown for 24 h with FCS, deprived of FCS for 24 h, then glycomers were added to cells. Quantification of nucleosome fragments was performed in the still adherent cells at the end of the incubation. Apoptosis index was defined as 1.00 for untreated cell culture wells under identical culture conditions than the treated cells. [b] Initial concentration 25 μM, precipitation of the compound in the culture medium starting after 15 min of incubation. [c] ND: not determined.

Glioblastomas present both compact tumor areas and brain tissue diffusely infiltrated by tumor cells. Depending on tumor cell density, the volume of tumor mass, the presence of tumor-derived factors, and dysfunction of the blood–brain barrier, extravasation of serum in some tumor areas may be facilitated. The inhibitory role of serum and the increased sensitivity to glycomers in nonconfluent cell cultures indicate that glycomers may be more effective on scattered tumor cells at the invasion front in brain areas where there is an intact blood–brain barrier compared to the primary tumor site, where tumor cell density and serum leakage are high. The passage of molecules through the cerebral vascular system forming the blood–brain is a difficult task to achieve. However, the fact that endothelial cells in nonangiogenic areas are not actively dividing, thus would be less sensitive to inhibition of DNA synthesis, and that brain-derived endothelial cells are more resistant to apoptosis induction than tumor cells, augurs well for molecules that have a selective effect. The preliminary results presented here confirm that selectivity between glioblastoma and brain-derived endothelial cells or primary human fibroblasts used as a model for normal untransformed cells may be achieved with these functionalized glycomers.

Conclusion

While no efficient treatment exists for high-grade glioblastoma, some drugs belonging to diverse classes, including microtubule destabilizing agents, topoisomerase

Table 3. Comparison of the Efficacy Indexes on Inhibition of DNA Synthesis and Induction of Apoptosis of Sulfonate (**8a**), Sulfonamide (**10a**), and Ether (**11a**) Substitution in Position C-6 of Glycomers[a]

	8a		10a		11a	
concn [μM]	^{3}H-T /MTT	apoptosis/MTT	^{3}H-T/MTT	apoptosis/MTT	^{3}H-T/MTT	apoptosis/MTT
LN18						
0	16.25	0.93	16.25	0.93	16.25	0.93
4.5	4.87	1.19	3.11	0.88	6.23	1.92
11.3	0.23	3.51	0.07	1.63	0.09	4.85
22.5	0.30	9.60	0.47	8.43	0.58	8.30
LN308						
0	9.79	0.95	9.79	0.95	9.79	0.95
4.5	5.00	0.99	6.01	0.72	5.65	0.62
11.3	1.23	2.03	0.64	1.81	2.04	0.70
22.5	0.24	5.09	0.35	6.18	0.34	2.32

[a] Cells were grown for 24 h with FCS, then deprived of FCS for 10 h. Glycomers were added to cells for 12 h, and MTT reduction (absorbance at 540 nm) and incorporation of tritiated thymidine (cpm) or apoptosis (apoptosis index: amount of nucleosme fragments in treated versus control cells) were determined in adherent cells. Efficacy indexes were obtained by calculating the ratio of either thymidine incorporation or apoptosis index to MTT reduction.

inhibitors, inhibitors of damage-repair enzymes, and alkylating and intercalating agents, are under experimental or clinical (re)evaluation.[2,5,6,19]

We have evaluated a series of new substituted aryl β-D-glycopyranosides that exhibit specific effects on growth inhibition and apoptosis induction in human glioblastoma cells in culture. Studies of systematic substitution at selected positions of the glycopyranosidic backbone indicate that the most important groups for biological activity are the *p*-iodophenyl in position C-1, no substitution in position C-4, while substitutions at positions C-2, C-3, and C-6 exert fine-tuning of the effects (Chart 2). The principal event induced by exposure of glioblastoma cells to functionalized glycomers is an inhibition of DNA synthesis, ultimately resulting in loss of adhesive properties and induction of apoptosis. Thus, these molecules represent interesting new potential agents to control the progression of glioblastomas.

Experimental Section

Solvents were distilled under positive pressure of dry nitrogen before use and dried by standard methods; THF and ether, from K/benzophenone; CH_2Cl_2 and toluene from $CaCl_2$. All commercially available reagents were used without further purification. All reactions were performed under nitrogen atmosphere. NMR (^{1}H, ^{13}C) spectra were recorded on AMX-300 and ARX-400 spectrometers in $CDCl_3$ or CD_3OD with tetramethylsilane as the internal standard. In some cases carbon resonances were coincident. Low- and high-resolution mass spectra were recorded on VG Micromass, AEI-MS 902, or Kratos MS-50 spectrometers using fast atom bombardment (FAB) or electrospray techniques. Optical rotations were recorded on a Perkin-Elmer 241 polarimeter in a 1 dm cell at ambient temperature. Analytical thin-layer chromatography was performed on Merck $60F_{254}$ precoated silica gel plates. Visualization was performed by UV or by development using $KMnO_4$ or $FeCl_3$ solutions. Flash column chromatography were performed using (40−60 μm) silica gel at increased pressure. Melting points recorded were uncorrected.

Biology. Cells. The human LN18 and LNZ308 glioblastoma cell lines were developed from surgical specimens of human glioblastoma[14] (a kind gift of AC. Diserens and N. de Tribolet, Neurosurgery Division, CHUV, Lausanne). Human brain-derived endothelial cells[15] (HCEC cells) were a kind gift of Dr. D. Stanimirovic, Ottawa, Canada. Human primary urogenital fibroblasts were cultured from surgical biopsies performed during delivery or reconstructive surgery in women, according to a protocol approved by the Ethics Committee of the CHUV in Lausanne, using the explant technique. All cells were grown in DMEM medium (Gibco-BRL, Basel, Switzerland) containing 4.5 g/L glucose, 10% fetal calf serum (FCS), and antibiotics.

Cell Culture and Treatments. The cell culture reagents were from Life Technologies or Gibco (both in Basel, Switzerland). The human cell lines were routinely maintained in DMEM supplemented with 10% FCS and detached from the plate using trypsin-EDTA. Cells were seeded in 48-well plates (Costar) as monolayers for 24 h in DMEM-10% FCS to half confluence and, unless otherwise stated, incubated 24 h in DMEM without FCS, then exposed for the time indicated to the glycomers under investigation. Then either thymidine incorporation to evaluate DNA synthesis, or protein content and MTT reduction to evaluate cell viability, or determination of nucleosome−DNA fragments to quantitate apotosis was performed. Experiments were done in triplicate and means and standard deviations (SD) were calculated.

Thymidine Incorporation. Thymidine incorporation was used to ascertain DNA synthesis. Following treatment, cells were exposed to 0.8 μCi/mL [^{3}H]-thymidine (Amersham Pharmacia, Dübendorf, Switzerland) for 2−3 h, and radioactivity was quantitated in a beta-counter (LKB) after precipitation with 10% trichloracetic acid and solubilization in 0.1% SDS−0.1 N NaOH and 5 mL Optiphase scintillation cocktail (Wallac, Fisher Chemicals, England).

Evaluation of Cell Proliferation by MTT. MTT ((3,4,5-dimethylthiazol-yl)-2,5-diphenyl tetrazolium); Sigma, Buchs, Switzerland) was used to quantify the number of metabolically active cells. Following glycomer treatment, cells were exposed to 0.2 mg/mL MTT in DMEM medium for 2 h, supernatant was aspirated, and the precipitated formazan was dissolved in 0.1 N HCl in 2-propanol and quantified at 540 nm in a multiwell plate reader (iEMS Reader MF, Labsystems, Bioconcepts, Switzerland).

Apoptosis Determination and Quantification. Apoptosis was determined in cells in culture by evaluation of nuclear chromatin condensation using the fluorescent nuclear marker 4′,6′-diamidino-2-phenylindolylhydrochloride, (DAPI, Boehringer Roche). Cells were grown on histological sides and exposed to glycomers according to the same protocols than cells grown in culture wells. Following fixation in methanol/acetic acid (3:1), slides were exposed to 1 μg/mL DAPI for 30 min at 37 °C, then mounted in 20% glycerol in PBS and examined and photographed under a fluorescent microscope (360/500 nm, excitation/emission wavelength, respectively). Alternatively, following buffered paraformaldehyde fixation, Giemsa and hematoxylin-eosin staining to visualize nuclei were performed according to standard procedures.

Apoptosis was quantified in the adherent cell population at the end of the incubation using the Cell Death Detection ELISA^{PLUS} (Roche, Rotkreuz, Switzerland), a photometric enzyme-linked immunoassay for quantitative in vitro determination of cytoplasmic histone-associated DNA fragments (mono- and oligo-nucleosomes), a marker of DNA breakdown and laddering, which is considered as a landmark of apoptosis. Determinations were performed according to the supplier's instructions. Using this method, the increase in absorbance

at 405 nm is proportional to apoptosis. The enrichment in apoptotic cell proportion (apoptosis index) was calculated as the ratio of absorbance of treated cells/absorbance of untreated cells as previously described.[20]

Protein Concentration. Protein content was determined with the BCA protein assay kit (Pierce, Socochim, Switzerland) and bovine serum albumin as standard.

Chemistry. *p*-Iodophenyl 3-*O*-Allyl-β-D-glucopyranoside (5). To a solution of **3**[11] (2.38 g, 6.13 mmol) in CH_2Cl_2 (20 mL) at 0 °C was added dropwise HBr (30 wt % in acetic acid, 14 mL). After being stirred for 20 min at 0 °C, the reaction mixture was diluted with CH_2Cl_2, washed with cold water twice, cold saturated aqueous $NaHCO_3$ solution, and cold water again, dried over Na_2SO_4, and concentrated. The syrupy residue was dissolved in dry CH_2Cl_2 (20 mL). A mixture of 4-iodophenol (1.35 g, 6.13 mmol) and bis[tri-*n*-butyltin] oxide (1.56 mL) was refluxed (100 °C) in benzene (250 mL) with a Dean−Stark apparatus. After 1 h, the solvent was removed in vacuo, and the crude tributylstannyl 4-iodophenoxide was added to the glycosyl bromide in CH_2Cl_2 (30 mL), after which $SnCl_4$ (6.7 mL, 1 M in CH_2Cl_2) was added dropwise at 0 °C. After being stirred 30 min, the reaction mixture was allowed to warm to room temperature, stirred for 4 h, and quenched with dilute $NaHCO_3$ and ether, and the organic layer was processed as usual. Purification by flash column chromatography (hexanes−ethyl acetate 2/1) gave **4** (2.84 g, 75%) as a white foam; $[\alpha]_D$ −22.0 (*c* 1.065, CH_3Cl); IR (KBr) 1758, 1727 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.59−7.55 (m, 2H), 6.77−6.74 (m, 2H), 5.82−5.75 (m 1H, vinyl-H), 5.26−5.10 (m, 4H, 2-H, 4-H and vinyl-Hs), 4.94 (d, 1H, $J_{1,2}$ = 7.8 Hz, 1-H), 4.22-(dd, $J_{5,6}$ = 5.8 Hz and $J_{6',6}$ = 12.3 Hz, 6-H), 4.14 (dd, 1H, $J_{5,6'}$ = 2.7 Hz, 6′-H), 4.11−4.09 (fm, 2H, allyl-Hs), 3.77−3.73 (m, 1H, 5-H), 3.68(t, $J_{3,4}$ = $J_{2,3}$ = 9.3 Hz, 3-H), 2.10 (s, 6H), 2.07 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.99(0), 169.68(0), 169.46(0), 157.23(0), 138.77, 134.46, 119.55, 117.63(−), 99.49, 86.29(0), 79.90, 73.36(−), 72.74, 72.52, 69.77, 62.65(−), 21.46, 21.26, 21.13; MS (FAB) *m*/*z* 548.0 $[M]^+$.

To a solution of **4** (548 mg, 1.0 mmol) in dried MeOH (7 mL) was added a catalytic amount of NaOMe (pH ∼ 9). After 2 h, the solution was neutralized with Amberlite IR-200 (H^+) and filtered, and the filtrate was evaporated to a residue that was used in the next step without further purification. ^1H NMR (400 MHz, $CDCl_3$) δ 7.59 (dt, 2H), 6.91 (dt, 2H), 6.08−5.98 (m 1H, vinyl-H), 5.30 (ddt, 1H, vinyl-H), 5.14 (ddt, 1H, vinyl-H), 4.88 (d, 1H, $J_{1,2}$ = 7.8 Hz, 1-H), 4.44−4.34 (m, 2H, ally-Hs), 3.88 (dd, $J_{5,6}$ = 2.0 Hz and $J_{6',6}$ = 12.1 Hz, 6-H), 3.69 (dd, 1H, $J_{5,6'}$ = 5.3 Hz, 6′-H), 3.52 (dd, $J_{2,3}$ = 9.1 Hz, 2-H), 3.47−3.44 (m, 2H), 3.36−3.31 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 159.210), 139.57, 137.11, 120.31, 116.91(−), 102.29, 85.84, 85.51(0), 78.20, 75.33(−), 75.02, 71.13, 62.53(−).

***p*-Iodophenyl 3-*O*-Allyl-4,6-*O*-benzylidene-β-D-glucopyranoside (6).** To a solution of **5** (∼1.0 mmol) in DMF (18 mL) were added benzaldehyde dimethyl acetal (225 μL, 1.5 mmol) and tetrafluoroboric acid (54% in ether, 2.1 mL, 1.5 mmol). The reaction mixture was stirred overnight at room temperature, the solvent removed in vacuo, and the residue dissolved in $CHCl_3$, filtered through a short layer of silica gel, concentrated, and purified by chromatography (hexanes−ethyl acetate 5/1) to yield **6** (434 mg, 85%, over two steps) as a white foam; $[\alpha]_D$ −11.7 (*c* 0.75, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 7.60 (dd, 2H), 7.50−7.48 (m, 2H), 7.40−7.26 (m, 3H), 6.83 (dd, 2H), 6.02−5.92 (m, 1H, vinyl-H), 5.57 (s, 1H, *CH*Ph), 5.32 (dq, 1H, vinyl-H), 5.22 (dq, 1H, vinyl-H), 5.00 (d, 1H, $J_{1,2}$ = 7.7 Hz, 1-H), 4.48 (dq, 1H, allyl-H), 4.36 (dd, 1H, $J_{5,6}$ = 5.0 Hz, $J_{6,6'}$ = 10.5 Hz, 6-H), 4.29 (dq, 1H, allyl-H), 3.84−3.64 (m, 4H, 2-H, 3-H, 4-H and 6′-H), 3.59−3.55 (m, 1H, 5-H), 2.68 (d, 1H, 2-OH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 157.10(0), 138.88, 137.44(0), 135.03, 129.52, 128.73, 126.39, 119.70, 118.13(−), 101.72, 101.43, 86.40(0), 81.46, 80.32, 74.16(−), 74.10, 68.95-(−), 67.00; HRMS calcd for $C_{22}H_{24}O_6I$: 511.06177; found: 511.05980.

***p*-Iodophenyl 3-*O*-Allyl-4,6-*O*-benzylidene-2-*O*-propionyl-β-D-glucopyranoside (7a).** A solution of **6** (83 mg, 0.16 mmol) and propionic anhydride (0.07 mL) in pyridine (2 mL) was stirred overnight at room temperature, MeOH (0.2 mL) was added, and the mixture was stirred for 1 h, then concentrated. The residue was dissolved in CH_2Cl_2 and processed in the usual way. Chromatography (hexanes−ethyl acetate, 3/1) gave **7a** (87 mg, 96%) as a white foam; $[\alpha]_D$ −8.7(*c* 0.67, $CHCl_3$); IR (KBr) 1746 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.55 (d, 2H), 7.47−7.44 (m, 2H), 7.38−7.34 (m, 3H), 6.74 (d, 2H), 5.87−5.74 (m, 1H, vinyl-H), 5.55 (s, 1H, CHPh), 5.27−5.23 (m, 2H, 2-H and vinyl-H), 5.12 (dd, 1H, vinyl-H), 5.02 (d, 1H, $J_{1,2}$ = 7.8 Hz, 1-H), 4.38−4.32 (m, 2H, 6-H and allyl-H), 4.17−4.08 (dd, 1H, allyl-H), 3.84−3.68 (m, 3H, 6-H, 4-H and 3-H), 3.59−3.51 (m, 1H, 5-H), 2.33 (q, 2H), 1.14 (t, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 173.22(0), 157.26(0), 138.92, 137.42(0), 134.96, 129.54, 128.74, 126.42, 119.59, 117.40(−), 101.73, 100.17, 86.38(0), 81.44, 78.86, 73.74(−), 72.74, 68.97(−), 66.95, 28.04(−), 9.69; MS (FAB,) *m*/*z* 566.1 $[M]^+$.

***p*-Iodophenyl 2-*O*-Acetyl-3-*O*-allyl-4,6-*O*-benzylidene-β-D-glucopyranoside (7b).** Prepared as described above to give **7b** (120 mg, 98%) as a white foam; $[\alpha]_D$ -10.0 (*c* 0.51, $CHCl_3$); IR 1747 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 2.08 (s, 3H); MS (FAB), *m*/*z* 553.1 $[MH^+]$.

***p*-Iodophenyl 3-*O*-Allyl-4,6-*O*-benzylidene-2-*O*-butyryl-β-D-glucopyranoside (7c).** To a solution of **6** (50 mg, 0.1 mmol) in CH_2Cl_2 were added Et_3N (15 μL, 0.11 mmol) and butyryl chloride (11 μL, 0.11 mmol), and the mixture was stirred at room temperature. After 2 h, another portion of Et_3N (15 μL, 0.11 mmol) and butyryl chloride (11 μL, 0.11 mmol) was added. After TLC indicated the completion of the reaction, MeOH (50 μL) and Et_3N (20μL) were added, and the solution was stirred for 1 h. The solvent was removed in vacuo and the residue dissolved in CH_2Cl_2, processed as usual, and purified by chromatography (hexanes−ethyl acetate, 3/1) to give **7c** (56 mg, 99%) as a white foam; $[\alpha]_D$ −11.6 (*c* 0.58, $CHCl_3$); IR (KBr) 1746 cm^{-1}; HRMS calcd for $C_{26}H_{30}O_7I$: 581.10364; found: 581.10140.

***p*-Iodophenyl 3-*O*-Allyl-4,6-*O*-benzylidene-2-*O*-isobutyryl-β-D-glucopyranoside (7d).** Prepared as described above to give **7d** (60 mg, 94%) as a white foam; $[\alpha]_D$ −11.4 (*c* 0.175, $CHCl_3$); MS (FAB) *m*/*z* 581.0 $[MH]^+$.

***p*-Iodophenyl 3-*O*-Allyl-6-*O*-(m-trifluoromethyl)benzenesulfonyl-β-D-glucopyranoside (8).** To a solution of **5** (42 mg, 0.1 mmol) in dry pyridine (1.5 mL) was added 3-(trifluoromethyl)benzenesulfonyl chloride (19 mL, 0.12 mmol) dropwise at 0 °C. The reaction mixture was stirred for 1 h at 0 °C and then allowed to warm to room temperature. After TLC indicated completion of the reaction, the solvent was removed in vacuo and the residue dissolved in CH_2Cl_2, processed as usual, and purified by chromatography (hexanes−ethyl acetate, 5/3) to yield **8** (53 mg, 85%) as a white foam; $[\alpha]_D$ −33.3 (c1.5, CH_3Cl); IR (neat/NaCl) 1485, 1327, 1239, 1187 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.14 (s, 1H), 8.01 (d, 1H), 7.81 (d, 1H), 7.57−7.52 (m, 3H), 6.73−6.69 (m, 2H), 6.00−5.91 (m, 1H, vinyl-H), 5.32 (dd, 1H, vinyl-H), 5.23 (dd, 1H, vinyl-H), 4.77 (d, 1H, $J_{1,2}$ = 7.7 Hz, 1-H), 4.49−4.25 (m, 4H, 6-H, 6′-H and allyl-Hs), 3.67−3.62 (m, 2H, 2-H and 5-H), 3.54 (dt, 1H, $J_{3,4}$ = $J_{4,5}$ = 8.9 Hz, $J_{4,\ OH}$ = 2.8 Hz, 4-H), 3.78(t, 1H, $J_{2,3}$ = 8.9 Hz, 3-H), 2.65 (d, 1H, 2-OH), 2.52 (d, 1H, 4-OH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 156.46(0), 138.27, 136.76(0), 134.50, 130.96, 130.36-(0), 130.33, 124.88(0), 124.84, 118.79, 117.68(−), 100.36, 85.67-(0), 82.84, 73.81(−), 73.64, 73.16, 69.00(−), 68.72; HRMS calcd for $C_{22}H_{22}F_3IO_8S$: 630.00322; found: 630.00387.

***p*-Iodophenyl 3-*O*-Allyl-2-*O*-propionyl-6-*O*-(*m*-trifluoromethyl)benzenesulfonyl-β-D-glucopyranoside (8a).** The reaction mixture of **7a** (140 mg, 0.24 mmol) and TFA (2.4 mL) in water/THF (5/20 mL) was refluxed at 85 °C for 2 h, and the reaction was monitored by TLC (hexanes−ethyl acetate 1/1). The crude was transferred to a separatory funnel, diluted with CH_2Cl_2, and washed with saturated $NaHCO_3$ solution, and the organic phase was processed as usual. The residue was purified by chromatography (hexanes−ethyl acetate 2/1) to yield 4-iodophenyl 3-*O*-allyl-2-*O*-propionyl-β-D-glucopyranoside (100 mg, 98%) as a white foam. To a solution of this compound (67 mg, 0.14 mmol) in pyridine (2.0 mL) containing a catalytic amount of DMAP was added 3-(trifluoromethyl)benzenesulfo-

nyl chloride (27 μL, 1.2 equiv) at 0 °C and the solution stirred for 30 min, then allowed to warm to room temperature. The solvent was removed in vacuo, the residue dissolved in CH_2Cl_2 and washed with water, 1 N HCl, and water, and the organic layer separated and concentrated. The residue was purified by chromatography (hexanes−ethyl acetate 2/1) to yield **8a** (91 mg, 95%) as a white foam; $[\alpha]_D$ −24.9 (*c* 0.515, $CHCl_3$); IR (film) 1745, 1611 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.14 (s, 1H), 8.02 (d, 1H), 7.81 (d, 1H), 7.57−7.52 (m, 3H), 6.64 (d, 2H), 5.91−5.81 (m, 1H, vinyl-H), 5.30−5.18 (m, 2H, vinyls-Hs), 5.10 (dd, 1H, $J_{2,3}$ = 9.0 Hz, 2-H), 4.87 (d, 1H, $J_{1,2}$ = 7.9 Hz, 1-H), 4.48 (dd, 1H, $J_{5,6}$ = 1.2 Hz, $J_{6,6'}$ = 11.3 Hz, 6-H), 4.36 (dd, 1H, $J_{5,6'}$ = 4.9 Hz, 6′-H), 4.22−4.17 (m, 2H, allyl-Hs), 3.74−3.64 (m, 2H, 4-H and 5-H), 3.49 (t, *J* = 9.3 Hz, 3-H), 2.99 (s, 1H), 2.34 (q, 2H), 1.15(t, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.27(0), 157.15(0), 138.84 137.20(0), 134.66, 131.59, 131.04, 131.0, 130.54, 125.44, 125.39, 119.26, 118.23(−), 99.37, 86.18(0), 82.25, 74.05(−), 73.84, 72.54, 69.57(−), 69.52, 28.09(−), 9.61; HRMS calcd for $C_{25}H_{26}F_3IO_9S$: 686.02944; found 686.02488.

***p*-Iodophenyl 3-*O*-Allyl-2-*O*-acetyl-6-*O*-(*m*-trifluoromethyl)benzenesulfonyl-β-D-glucopyranoside (8b).** Prepared as described above to yield **8b** (90%) as a white foam; $[\alpha]_D$ −20.4 (*c* 0.69, $CHCl_3$); MS (FAB), *m/z*, 672.0; HRMS: calcd for $C_{24}H_{24}F_3IO_9S$: 672.01379; found 672.01332.

***p*-Iodophenyl 3-*O*-Allyl-2-*O*-butyryl-6-*O*-(*m*-trifluoromethyl)benzenesulfonyl-β-D-glucopyranoside (8c).** Prepared as described above to yield **8c** (94%) as a white foam; $[\alpha]_D$ −28.8 (*c* 0.7, $CHCl_3$); HRMS calcd for $C_{26}H_{29}O_9ISF_3$: 701.05292; found: 701.05570.

***p*-Iodophenyl 2-*O*-Isobutyryl-3-*O*-allyl-6-*O*-(*m*-trifluoromethyl)benzenesulfonyl-β-D-glucopyranoside (8d).** Prepared as described for **8a** to yield **8d** (90%) as a white foam; $[\alpha]_D$ −17.4 (*c* 0.5, $CHCl_3$); HRMS calcd for $C_{26}H_{29}O_9ISF_3$: 701.05292; found: 701.05765.

***p*-Iodophenyl 3-*O*-allyl-6-azido-6-deoxy-2-*O*-propionyl-β-D-glucopyranoside (9a).** A mixture of **8c** (600 mg, 0.9 mmol) and NaN_3 (340 mg, 5.4 mmol) in DMF (9 mL) was stirred 1 h at 60−65 °C, and the solvent was removed in vacuo. The residue was partitioned between CH_2Cl_2 and water, the aqueous phase was extracted with excess of CH_2Cl_2, and the combined organic layer was dried over Na_2SO_4 and concentrated. The crude product was purified by flash column chromatography (hexanes−ethyl acetate 5/2) to yield **9a** (375 mg, 86%) as a white foam; $[\alpha]_D$ −52.3 (*c* 0.325, $CHCl_3$); IR (KBr) 2102, 1744 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.61−7.58 (fm, 2H), 6.79−6.77 (fm, 2H), 5.94−5.84 (m, 1H, vinyl-H), 5.32−5.21 (m, 3H, 2-H, and vinyl-Hs), 4.94 (d, 1H, $J_{1,2}$ = 7.8 Hz, 1-H), 4.27−4.13 (m, 2H, allyl-Hs), 3.70−3.62 (m, 3H, 4-H, 6-H and 6′-H), 3.53−3.49 (m, 2H, 3-H and 5-H), 2.65 (d, 1H, OH), 2.36 (q, 2H), 1.19 (t, 3H); ^{13}C NMR (75 MHz, CDCl3) δ 173.24(0), 157.31(0), 138.90, 134.65, 119.72, 118.31(−), 99.91, 86.47(0), 82.52, 75.49, 73.91(−), 72.83, 70.78, 51.91(−), 28.14(−), 9.65; HRMS: calcd for $C_{18}H_{22}O_6IN_3$: 503.05534; found: 503.05765.

4-Iodophenyl 3-*O*-Allyl-6-azido-6-deoxy-2-*O*-butyryl-β-D-glucopyranoside (9b). Prepared as described above to yield **9b** (125 mg, 83%) as a white foam; $[\alpha]_D$ −81.8 (*c* 0.5, $CHCl_3$); IR (KBr) 2102, 1745 cm^{-1}; MS (FAB) *m/z* 518.1$[MH]^+$, 540.2 $[MNa]^+$; HRMS calcd for $C_{19}H_{25}O_6IN_3$: 518.07880; found: 518.07960.

***p*-Iodophenyl 3-*O*-Allyl-2-*O*-propionyl-6-deoxy-6-*N*-(*m*-trifluoromethyl)benzenesulfonyl-β-D-glucopyranoside (10a).** A mixture of **9a** (352 mg, 0.7 mmol) and triphenylphosphine (220 mg, 0.84 mmol) in THF (3.5 mL) containing water (15 μL) was stirred at room temperature for 20 h, then concentrated in vacuo. The residue was purified by flash column chromatography (CH_2Cl_2−MeOH−NH_4OH 9:1:0.25, lower layer used as eluant) to yield 4-iodophenyl 3-*O*-allyl-6-amino-2-*O*-butyryl-6-deoxy-β-D-glucopyranoside (242 mg, 96%) as a white foam. To a solution of the amine (48 mg, 0.1 mmol) and Et_3N (15 μL, 0.11 mmol) in CH_2Cl_2 (2.0 mL) was added 3-(trifluoromethyl)benzenesulfonyl chloride (18 μL 0.11 mmol) at room temperature. After the mixture was stirred overnight, MeOH (50 μL) and Et_3N (9 μL) were added, and the mixture was stirred for 1 h, diluted with CH_2Cl_2, and processed as usual. The residue was chromatographed (hexanes−ethyl acetate 2/1) to yield **10a** (96%) as a white foam; $[\alpha]_D$ −33.0 (*c* 0.385, $CHCl_3$); IR (KBr) 1743, 1484, 1327, 1236, 1164 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.10 (s, 1H), 8.01 (d, 1H), 7.80 (d, 1H), 7.61 (t, 1H), 7.52 (dd, 2H), 6.66 (dd, 2H), 5.90−5.81-(m, 1H, vinyl-H), 5.54 (t, 1H, NH), 5.28−5.09 (m, 3H, vinyls-Hs and 2-H), 4.92 (d, 1H, $J_{1,2}$ = 8.0 Hz, 1-H), 4.26−4.16 (m, 2H, allyl-Hs), 3.72 (dt, 1H, 4-H), 3.60−3.47 (m, 3H, 3-H, 5-H, and OH), 3.38−3.35 (m, 2H, 6-H and 6′-H), 2.33 (q, 2H), 1.13 (t, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.57(0), 157.16(0), 141.53(0), 138.94, 134.91, 130.65, 130.53, 129.90, 129.86, 127.91, 119.25, 117.92(−), 99.51, 86.24(0), 81.86, 74.72, 74.09-(−), 72.67, 70.65, 44.06(−), 28.10(−), 9.63; MS (FAB) *m/z* 708.0 $[MNa]^+$; HRMS calcd for $C_{25}H_{26}F_3INO_8SNa$: 708.03519; found 708.03877.

***p*-Iodophenyl 3-*O*-Allyl-2-*O*-butyryl-6-deoxy-6-*N*-(*m*-trifluoromethyl)benzenesulfonyl)-β-D-glucopyranoside (10b).** Prepared as described above to yield **10b** (25 mg, 83%) as a white foam; $[\alpha]_D$ −21.8 (*c* 1.30, $CHCl_3$); MS (FAB) *m/z* 722.0 $[MNa]^+$; HRMS calcd for $C_{26}H_{29}O_8NSF_3INa$: 722.05084; found: 722.04890.

***p*-Iodophenyl 3-*O*-Allyl-6-*O*-benzyl-2-*O*-propionyl-β-D-glucopyranoside (11a).** 1N HCl was added at room temperature to a solution of **7a** (28 mg, 0.05 mmol) and sodium cyanoborohydride (39 mg, 0.6 mmol) in THF (10 mL) containing molecular sieves until the evolution of gas ceased. The mixture was diluted with CH_2Cl_2 and water and filtered over Celite, the filtrate was extracted with CH_2Cl_2 and processed as usual, and the product was purified with flash column chromatography (hexanes−ethyl acetate, 2/1) to give **11a** (25 mg, 89%) as a white foam; $[\alpha]_D$ −11.1 (*c* 0.54, $CHCl_3$); IR (neat/NaCl) 1745 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.48 (d, 2H), 7.29−7.21 (m, 5H), 6.70 (d, 2H), 5.87−5.78 (m, 1H, vinyl-H), 5.24−5.11 (m, 3H, 2-H and vinyl-Hs), 4.86 (d, 1H, $J_{1,2}$ = 7.9 Hz, 1-H), 4.51 (AB, 2H, *J* = 11.9 Hz and *J* = 1 6.4 Hz, CH_2Ph), 4.17−4.15 (fm, 2H, allyl-Hs), 3.80−3.66 (m, 3H, 6, 6′-H and 4-H), 3.60−3.54 (m, 1H, 5-H), 3.45 (t, 1H, *J* = 9.2 Hz, 3-H), 2.76 (d, 1H, 4-OH), 2.30 (q, 2H), 1.12 (t, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.27(0), 157.44(0), 138.78, 138.02-(0), 134.93, 128.90, 128.30, 128.14, 119.52, 117.79(−), 99.66, 85.98(0), 82.50, 74.96, 74.16(−), 73.83(−),72.69, 71.83, 70.44(−), 53.86, 28.11(−), 9.64; HRMS: calcd for $C_{25}H_{29}IO_7$: 568.09580,: found 568.10654.

***p*-Iodophenyl 3-*O*-Allyl-6-*O*-benzyl-2-*O*-butyryl-β-D-glucopyranoside (11b).** Prepared as described above to yield **11b** (95%) as a white foam; $[\alpha]_D$ = −26.2 (*c* 0.45, $CHCl_3$); IR (KBr) 1744 cm^{-1}; HRMS calcd for $C_{25}H_{29}IO_7$: 582.11145: found 582.11234.

***p*-Iodophenyl 2,4,6-Tri-*O*-acetyl-3-*O*-allyl-α-D-glucopyranoside (12).** Obtained as a byproduct of **4**, in <10%, white foam; $[\alpha]_D$ +119 (*c* 0.45, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 7.59−7.57(m, 2H), 6.87−6.84(m, 2H), 5.85−5.81 (m 1H, vinyl-H), 5.68(d, 1H, $J_{1,2}$ = 3.7 Hz, 1-H), 530−5.08(m, 3H, 4-H and vinyl-Hs), 4.94(dd, 1-H, $J_{2,3}$ = 10.0 Hz, 2-H), 4.27−4.11 (m, 4H, 6-H, 6′-H and allyl-Hs), 4.03 (t, 1H, 3-H), 3.98−3.94 (m, 1H, 5-H), 2.10(s, 6H), 2.07(s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.04(0), 170.48(0), 169.79(0), 156.42(0), 138.88, 134.80, 119.37, 117.25(−), 94.93, 86.06(0), 77.09, 74.28(−), 73.05, 69.82, 68.98, 62.25(−), 21.24, 21.09; MS (FAB), *m/z* 452.2 $[M]^+$.

***p*-Iodophenyl 3-*O*-Allyl-2-*O*-butyryl-6-*O*-(*m*-trifluoromethyl)benzenesulfonyl-β-D-glucopyranoside (13).** Prepared as described for **8a** to yield **13** (50% overall) as a colorless syrup; $[\alpha]_D$ +68.3 (*c* 0.12, $CDCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 8.18(s, 1H), 8.07 (d, 1H), 7.93 (d, 1H), 7.72 (t, 1H), 7.54 (dd, 2H), 6.73 (dd, 2H), 5.98−5.87 (m, 1H, vinyl-H), 5.52(d, 1H, $J_{1,2}$ = 3.6 Hz, 1-Hα), 5.31 (dd, 1H, vinyl-H), 5.22 (dd, 1H, vinyl-H), 4.75 (dd, 1H, $J_{2,3}$ = 9.9 Hz, 2-H), 4.42 (dd, $J_{5,6}$ = 3.3 Hz, $J_{5,6'}$ = 11.2 Hz, 6-H), 4.37−4.30 (m, 2H), 4.23 (dd, 1H, *J* = 5.9 Hz, *J* = 12.7 Hz), 3.91−3.83 (m, 2H), 3.65 (t, 1H, *J* = 9.1 Hz, 3-H), 2.70−2.59 (broad, 1H, OH), 2.32 (t, 2H), 1.62 (tq, 2H), 0.92 (t, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.17, 156.51, 138.91, 138.00, 134.87, 131.54, 130.96, 130.49, 125.43, 119.23,

117.89, 95.25, 85.06, 79.13, 74.51, 72.95, 70.25, 69.43, 69.25, 36.48, 18.73, 13.97; HRMS calcd for $C_{26}H_{28}F_3IO_9S$: 700.04509; found: 700.05943.

***p*-Iodophenyl 3-*O*-Allyl-4,6-*O*-benzylidene-2-*O*-butyryl-β-D-galactopyranoside (15a).** Prepared as described for **7a** to give **15a** (76%) as a white foam; $[\alpha]_D$ −10.4 (*c* 1.0, $CHCl_3$); IR (neat/NaCl) 1747, 1485 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.56−7.53 (m, 4H), 7.37−7.34 (m, 3H), 6.78 (dd, 2H) 5.94−5.83(m, 1H, vinyl-H), 5.63−5.57 (m, 2H, 2-H and CHPh), 5.32−5.19 (m, 2H, vinyl-Hs), 4.99 (d, 1H, $J_{1,2}$ = 8.0 Hz 1-H), 4.36−4.34 (m, 2H, 4-H and 6-H), 4.22−4.10 (m, 3H, $H_{6'}$ and allyl-Hs), 3.67 (dd, 1H, $J_{2,3}$ =10.1 Hz and $J_{3,4}$ = 3.4 Hz, 3-H), 3.57−3.55 (m, 1H, 5-H), 2.31 (t, 2H), 1.69−1.63 (m, 2H), 0.94 (t, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.36(0), 157.54(0), 138.65, 137.80(0), 134.97, 129.46, 128.57, 126.87, 119.79, 117.92(−), 101.62, 100.10, 85.90(0), 77.37, 73.54, 70.99(−), 69.85, 69.38(−), 67.26, 36.63(−), 18.96(−), 14.04; MS (FAB), *m*/*z* 581.0 $[MH]^+$.

***p*-Iodophenyl 3-*O*-Allyl-4,6-*O*-benzylidene-2-*O*-isobutyryl-β-D-galactopyranoside (15b).** Prepared as described **7a** to give **15b** (95%) as a white foam; $[\alpha]_D$ −9.1 (*c* 0.515, $CHCl_3$); IR (neat/NaCl) 1746, 1485 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 2.60−2.56 (m,1H), 1.21−1.17(m, 6H); MS(FAB), *m*/*z* 579.1 $[M - H]^+$, 603.1 $[MNa]^+$. See also Supporting Information.

***p*-Iodophenyl 3-*O*-Allyl-2-*O*-butyryl-6-*O*-(*m*-trifluoromethyl)benzenesulfonyl-β-D-galactopyranoside (16a).** Prepared as described for **8a** to give **16a** (82%) as a white foam; $[\alpha]_D$ −33.3 (*c* 1.0, $CHCl_3$); IR (neat/NaCl) 1744, 1485, 1369, 1327, 1185, 1075 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.15 (s, 1H), 8.04 (d, 1H, *J* = 7.9 Hz), 7.88 (d, 1H, *J* = 7.9 Hz), 7.61(t, 1H, *J* = 7.9 Hz), 7.54 (dd, 2H, *J* = 2.1 Hz and 6.9 Hz), 6.70 (dd, 2H, *J* = 2.1 Hz and 6.9 Hz), 5.87−5.80 (m, 1H, vinyl-H), 5.35 (dd, 1H, $J_{1,2}$ = 8.1 Hz and $J_{2,3}$ = 9.8 Hz, 2-H), 5.31−5.22 (m, 2H, vinyl-H), 4.88(d, 1H, 1-H), 4.42−4.32 (m, 2H, 6-H and 6′-H), 4.19−4.01 (m, 3H, allyl-H and 4-H), 3.95−3.92 (m, 1H, 5-H), 3.57 (dd, 1H, $J_{3,4}$ = 3.4 Hz, 3-H), 2.52 (s, 1H, OH), 2.30 (t, 2H, *J* = 7.3), 1.68−1.61 (m, 2H), 0.93 (t, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.93(0), 156.57(0), 138.23, 136.56(0), 133.37, 130.98, 130.56, 130.03, 124.84, 118.74, 118.33(−), 99.78, 85.53(0), 77.72, 72.15, 71.02(−), 69.25, 68.86(−), 65.53, 35.99(−), 18.33(−), 13.43; HRMS calcd for $C_{26}H_{29}O_9ISF_3$: 701.05292; found: 701.05889.

***p*-Iodophenyl 3-*O*-Allyl-2-*O*-isobutyryl-6-*O*-(*m*-trifluoromethyl)benzenesulfonyl-β-D-galactopyranoside (16b).** Prepared as described for **8a** to yield **16b** (95%) as a white foam; $[\alpha]_D$ −31.5 (*c* 0.355, $CHCl_3$); IR (neat/NaCl) 1743, 1485, 1328, 1186, 1074 cm^{-1}; HRMS calcd for $C_{26}H_{28}F_3SIO_9Na$: 723.03485; found: 723.03310.

***p*-Iodophenyl 3-*O*-Allyl-2-*O*-butyryl-6-deoxy-6-*N*-(*m*-trifluoromethyl)benzene sulfonyl-β-D-galactopyranoside (17a).** Prepared as described for **10a** to yield **17a** (82%) as a white foam; $[\alpha]_D$ −19.0 (*c* 1.225, $CHCl_3$); IR (neat/NaCl) 1742, 1485, 1327, 1165, 1072 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.12 (s, 1H), 8.02 (d, 1H,), 7.80 (d, 1H), 7.63−7.57 (m, 3H), 6.71 (d, 2H), 5.90−5.80 (m, 1H, vinyl-H), 5.36 (dd, 1H, $J_{2,3}$ = 9.7 Hz, 2-H), 5.31−5.18 (m, 3H, vinyl-Hs and 6-NH), 4.92 (d, 1H, $J_{1,2}$ = 8.1 Hz 1-H), 4.20−4.04 (m, 3H, allyl-Hs and 4-H), 3.84−3.82 (m, 1H, 5-H), 3.57 (dd, 1H, $J_{3,4}$ = 3.4 Hz, 3-H), 3.40−3.37 (m, 2H, 6-H and 6′-H), 2.68 (s, 1H, 4-OH), 2.30 (t, 2H), 1.69−1.62 (m, 2H), 0.94 (t, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.64(0), 157.04(0), 141.44(0) 138.91, 133.97, 132.41(0), 131.96, 130.55, 130.42, 129.84, 124.45, 124.40, 118.99, 118.86(−), 99.17, 85.96(0), 78.42, 73.68, 71.52(−), 69.87, 66.84, 43.89(−) 36.56(−), 18.89(−), 13.98; MS (FAB) *m*/*z* 722.0 $[MNa]^+$; HRMS calcd for $C_{26}H_{29}F_3SIO_8NNa$: 722.05084; found: 722.04820. See also Supporting Information.

***p*-Iodophenyl 3-*O*-Allyl-6-deoxy-2-*O*-isobutyryl-6-*N*-(*m*-trifluoromethyl)benzenesulfonyl-β-D-galactopyranoside (17b).** Prepared as described for **10a** to yield **17b** (95%) as a white foam; $[\alpha]_D$ −22.6 (*c* 0.97, $CHCl_3$); IR (neat/NaCl) 1741, 1484, 1072 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 2.55−2.43(m, 1H), 1.12−1.08 (m, 6H); MS (FAB) *m*/*z* 697.9 $[M]^+$, 721.9 $[MNa]^+$; HRMS calcd for $C_{26}H_{29}F_3SIO_8NaN$: 722.05084; found: 722.03310.

***p*-Iodophenyl 3-*O*-Allyl-6-*O*-benzyl-2-*O*-butyryl-β-D-galactopyranoside (18a).** Prepared as described for **11a** to yield **18a** (91%) as a white foam; $[\alpha]_D$ +5.0 (*c* 1.105, CH_3Cl); ^1H NMR (400 MHz, $CDCl_3$) δ 7.53 (d, 2H),7.36−7.31 (m, 5H), 6.79 (d, 2H), 5.93−5.80 (m, 1H, vinyl-H), 5.44 (dd, 1H, $J_{2,3}$ = 9.7 Hz, 2-H), 5.32−5.20 (m, 2H, vinyl-Hs), 4.90 (d, 1H, $J_{1,2}$ = 8.0 Hz, 1-H), 4.58 (s, 2H, CH_2PH), 4.21−4.15 (m, 1H, allyl-H), 4.11 (d, 1H, $J_{4,5}$ = 0, 4-H), 4.07−4.01 (m, 1H, allyl-H), 3.89−3.74 (m, 3H, 5-H, 6-H and 6′-H), 3.55 (dd, 1H, $J_{3,4}$ = 3.4 Hz, 3-H), 2.32 (t, 2H), 1.70−1.63 (m, 2H), 0.95 (t, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.54(0), 157.43(0), 138.71, 138.24, 134.25, 128.87, 128.25, 128.20, 119.56, 118.45(−), 99.70, 85.81(0), 78.93, 74.45, 74.23(−), 71.25, 70.32, 69.45(−), 66.60, 36.62(−), 18.92(−), 14.01; HRMS calcd for $C_{26}H_{31}IO_7$: 582.11145; found 582.11432.

***p*-Iodophenyl 3-*O*-Allyl-6-*O*-benzyl-2-*O*-isobutyryl-β-D-galactopyranoside (18b).** Prepared as described for **11a** to yield **18b** (95%) as a white foam; $[\alpha]_D$ +1.1 (*c* 1.135, CH_3Cl); HRMS calcd for $C_{26}H_{31}IO_7$: 582.11145; found 582.12876.

Acknowledgment. The authors thank the Swiss League and Research against Cancer (grant KFS 947-09-1999), the Swiss National Science Foundation for Scientific Research (grant 3152-059219.99), and the Swiss Society for Multiple Sclerosis for financial support. We are very grateful to Drs. Sylvain Meyer, Lausanne, for providing the human surgical specimens for fibroblast cultures and R. C. Janzer for critical reading of the manuscript. We also thank NSERC for generous financial assistance through the Medicinal Chemistry Chair Program (S.H., L.Z.).

Supporting Information Available: Copies of LC/MS data for selected compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Bredel, M. Anticancer drug resistance in primary human brain tumors. *Brain Res. Rev*. **2001**, *35*, 161−204.
(2) Brandes, A. A.; Basso, U.; Pasetto, L. M.; Ermani, M. New strategy development in brain tumor therapy. *Curr. Pharm. Des*. **2001**, *7*, 1553−1580.
(3) Berens, M. E.; Giese, A. Biology and oncology of invasive glioma cells. *Neoplasia* **1999**, *1*, 208−219.
(4) Prados, M. D.; Levin, V. Biology and treatment of malignant glioma. *Sem. Oncol*. **2000**, *27*, 1−10.
(5) Hofer, S.; Herrmann, R. Chemotherapy for malignant brain tumors of astrocytic and oligodendroglial lineage. *J. Cancer Res. Clin. Oncol.* **2001**, *127*, 91−95.
(6) Newton, H. B. Novel chemotherapeutic agents for the treatment of brain cancer. *Exp. Opinion Invest. Drugs* **2000**, *9*, 2815−2829.
(7) Nieto-Sampedro, M.; Bailon, C.; Fernandez-Mayoralas, A.; Martin-Lomas, M.; Mellström, B.; Naranjo, J. R. Experimental brain glioma : growth arrest and destruction by a blood-group-related tetrasaccharide. *J. Neuropath. Exp. Neurol.* **1996**, *55*, 169−177.
(8) Aguilera, B.; Romera-Ramirez, L.; Abad-Rodriguez, J.; Corrales, G.; Nieto-Sampiedro, M. Novel disaccharide inhibitors of human glioblastoma cell division. *J. Med. Chem*. **1998**, *41*, 4599−4606.
(9) Camby, I.; Decaestecker, C.; Gordower, L.; DeDecker, R.; Kacem, Y.; Lemmers, A.; Siebert, H. C.; Bovin, N. V.; Wesseling, P.; Danguy, A.; Salmon, I.; Gabius, H. J.; Kiss, R. Distinct differences in binding capacity to saccharide epitopes in supratentorial pilocytic astrocytomas, astrocytomas, anaplastic astrocytomas and glioblastomas. *J. Neuropath. Exp. Neurol.* **2001**, *60*, 75−84.
(10) Hanessian, S.; Saavedra, O. M.; Xie, F.; Amboldi, N.; Battistini, C. Design and synthesis of functionalized glycomers as nonpeptidic ligands for SH2 binding and as inhibitors of A-431 human epidermoid and HT-29 colon carcinoma cell lines. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 439−442.
(11) Bessel, E. M.; Westwood, J. M. (*R*,*S*)-2,3-Ethoxypropyl ethers and glycosides of D-glucopyranose. *Carbohydr. Res.* **1992**, *25*, 11−21.
(12) Ogawa, T.; Matsui, M. An approach to the synthesis of glycosides: enhancement of nucleophilicity of hydroxyl gorups by trialkylstannylation. *Carbohydr. Res*. **1976**, *51*, C13−C18.
(13) Garegg, P.; Hultberg, H.; Wallin, S. A novel reductive ring opening of carbohydrate benzylidene acetals. *Carbohdr. Res.* **1982**, *108*, 97−101.

(14) Diserens, A. C.; de Tribolet, N.; Martin-Achard, G.; Gaide, A. C.; Schnegg J. F.; Carrel, S. Characterization of an established human malignant glioma cell line : LN18. *Acta Neuropathol*. **1981**, *53*, 21−28.
(15) Muruganandan A.; Herz, L. M.; Monette, R.; Durkin, J. P.; Stanimirovic, D. Development of immortalized human cerebrovascular endothelial cell line as an in vitro model of the human blood-brain barrier. *FASEB J*. **1997**, *11*, 1187−1197.
(16) Uehlinger, P.; Zellweger, M.; Wagnières, G.; Juillerat-Jeanneret, L.; van den Bergh, H.; Lange, N. 5-aminolevulinic acid and its derivatives : physical chemical properties and protoporphyrin IX formation in cultured cells. *J. Photochem. Photobiol. B: Biol*. **2000**, *54*, 72−80.
(17) Agbaria, R.; Kelley, J. A.; Jackman, J.; Viola, J. J.; Ram, Z.; Oldfield, E.; Johns, D. G. Antiproliferative effects of cyclopentenyl cytosine (NSC 375575) in human glioblastoma cells. *Oncol. Res*. **1997**, *11*−*118*, 1997.
(18) Egidy, G.; Peduto Eberl, L.; Valdenaire, O.; Irmler, M.; Majdi, R.; Diserens, A. C.; Fontana, A.; Janzer, R. C.; Pinet, F.; Juillerat-Jeanneret, L. The endothelin system in human glioblastoma. *Lab. Invest*. **2000**, *80*, 1681−1689.
(19) Uckun, F. M. Rationally designed anti-mitotic agents with promitotic activity. *Curr. Pharm. Des*. **2001**, *7*, 1627−1639.
(20) Peduto Eberl, L.; Valdenaire, O.; Saintgiorgio, V.; Jeannin, J. F.; Juillerat-Jeanneret, L. Endothelin receptor blockade potentiates FasL-induced apoptosis in rat colon carcinoma cells. *Int. J. Cancer* **2000**, *86*, 182−187.

JM0205853