Central Neural Tumor Destruction by Controlled Release of a Synthetic Glycoside Dispersed in a Biodegradable Polymeric Matrix

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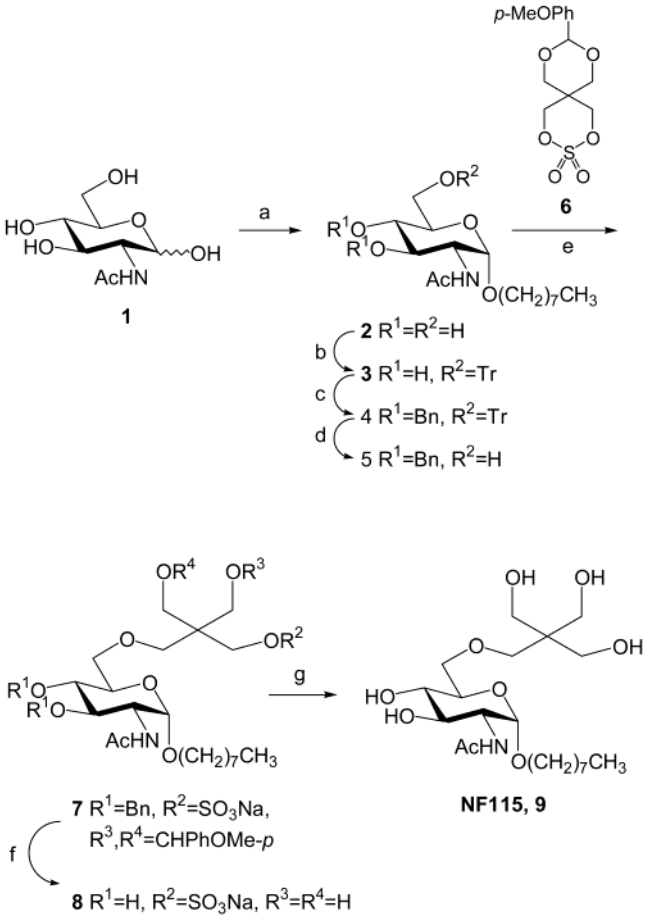
Abstract: An octyl *N*-acetylglucosaminide derivative with a pentaerythritol chain at position 6 has been synthesized and evaluated as an inhibitor of neural tumor growth. The glycoside inhibited the growth of a neuroectodermic tumor implanted in rats and, when loaded on a slow-delivery polymer disk, caused the destruction of cultured human astroblastoma obtained after surgical biopsy.

Treatment of central nervous system tumors is always problematic. Despite important advances in therapeutic oncology, the treatment of glioma has yielded only meager increases in survival time. Moreover, drug delivery further restricts brain tumor treatment with chemotherapy. The investigation of new antitumoral substances and delivery systems is, therefore, important.

The presence in brain extracts of inhibitors of astroblast and astrocytoma division was first described by one of us.[1] The inhibitor had glycidic epitopes immunologically related to those of the epidermal growth factor receptor and of blood groups A, H, or Le.[2] On the basis of these observations, we synthesized a family of oligosaccharides with a common Lewis X-type structure and tested their activities as inhibitors of division of normal and transformed neural cells.[3] The tetrasaccharide α-D-GalNAc(1,3)-*β*-D-Gal(1,4)[α-L-Fuc(1,3)]-D-GlcNAc inhibited the division of astrocytes and astrocytoma cells in culture and caused the destruction of a malignant glioma formed in the rat brain after transplantation of the C6 glioma line.[4] The practical synthesis of a second generation of α-L-Fuc(1,3)-D-GlcNAc disaccharide derivatives was carried out, and the compounds were tested as inhibitors of human glioma growth.[5] Disaccharides with a pentaerythritol or L-glyceryl chain at the C-6 position of a GlcNAc unit showed the best inhibitory properties. We show now that octyl 2-acetamido-2-deoxy-6-*O*-[2,2-bis(hydroxymethyl)-3-hydroxypropyl]-α-D-glucopyranoside (NF115, **9**, Scheme 1), containing the pentaerythritol chain at position 6 and a hydrophobic octyl chain at the anomeric position, inhibits the growth of cultured cells of a biopsy sample of human astroblastoma and causes a progressive decrease of volume, eventually leading to its total disappearance, of a primitive neuroectodermal tumor implanted in rats.

Scheme 1[a]



[a] Reagents and conditions: (a) *n*-octanol, $BF_3{\cdot}Et_2O$, CH_3NO_2, 100 °C, 42%; (b) TrCl, 4-DMP, pyridine, 100 °C, 84%; (c) BnBr, NaH, THF, 80 °C, 89%; (d) *p*-TsOH, CH_2Cl_2-MeOH, room temp, 95%; (e) **6**, NaH, 1:9 DMF−THF, 100 °C, 98%; (f) H_2, 10% Pd−C, MeOH, room temp, quantitative; (g) 1 M H_2SO_4, 3:1 dioxane−MeOH, room temp, 99%.

Compound **9** was efficiently synthesized starting from readily available *N*-acetyl-D-glucosamine (**1**, Scheme 1). Reaction of **1** with *n*-octanol, promoted by $BF_3{\cdot}Et_2O$, gave α-glycoside **2**, which was selectively tritylated at the HO-6 hydroxy group with high yield (84%). Benzylation on **3** followed by detritylation afforded alcohol **5** through **4**. Alkylation of **5** with cyclic sulfate **6** gave sulfate derivative **7**, which was submitted to hydrogenolysis of benzyl and benzylidene groups to give **8**. Finally, acid hydrolysis of the sulfate group in **8** furnished the target **9**. This synthetic scheme allowed the preparation of **9** on a multigram scale.

The antimitotic activity of **9** was first tested on the human glioma line U-373 cell, measuring the incorporation of [^{3}H]thymidine in cultures of the cells. Compound **9** inhibited the division of U-373 cells with an IC_{50} of

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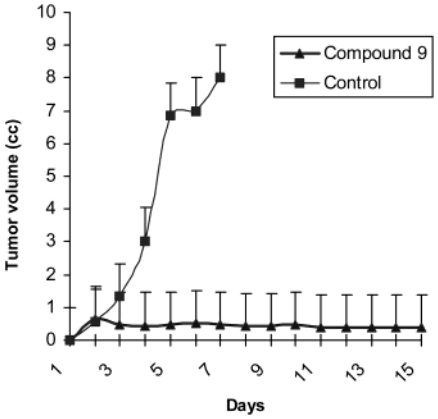

Figure 1. Time course of tumoral growth in rats treated with (▲) **9** and (■) saline.

43 ± 14 μM. We next studied the effect of **9** on the growth of a tumor in vivo. We used 45 male or female Wistar rats (1 month old and weighting 125−150 g) bearing an ENU-induced primitive neuroectodermal,[6] growing in the epicranial subcutaneous space, with a mean volume of 0.032 cm^3. Animals were treated by intratumor administration of compound **9** (4 mg/day in 0.1 cm^3 of saline; experimental group, $n = 25$) or 0.1 cm^3 of saline (control group, $n = 20$) during the following 15 days. The tumor grew very large in controls after implantation (Figure 1), and the rats had to be sacrificed after 8 days. Administration of **9** slowed tumor growth and caused a progressive reduction of its volume until it totally disappeared. A group of five rats treated with **9** was kept without further treatment for an additional 30 days. After this time, no relapse of the tumor was observed in any case. In addition, no clinically significant adverse reactions were attributable to compound **9**.

The clinical treatment of brain tumors with the glycoside requires controlled targeted delivery of the drug. The association with synthetic polymers has emerged as a promising method for local drug delivery.[7,8] We have recently described[9] the synthesis of a polymeric matrix capable of steady release of the glycoside for several days in a brain homogenate. The polymer matrix is a graft copolymer of poly(ϵ-caprolactone) on poly(methyl methacrylate) (copolymer composition of 52:48), and it shows a low degradation rate, adequate for long-term delivery. We have now tested the effect of polymer disks loaded with compound **9** on cultures of cells obtained by biopsy of a tumor in a patient suffering repeated relapses after surgical resection of a brain tumor. The tumor was typified as a malignant astroblastoma. Cells were incubated in the presence of drug-loaded disks weighing 360 mg (compound **9** was 17% of the weight) and unloaded control disks weighing 300 mg. Four days later, a monolayer of viable tumor cells was observed in controls, whereas cultures treated with drug-loaded disks showed floating aggregates of rounded nonviable cells (Figure 2). Therefore, glycoside **9** was released from the disk and efficiently destroyed the astroblastoma cells.

We conclude that glycoside **9** in a polymer disk may be a promising therapy for central nervous system tumors. Intracerebral implantation in pigs of drug-loaded disks showed lack of acute clinical toxicity.[9]

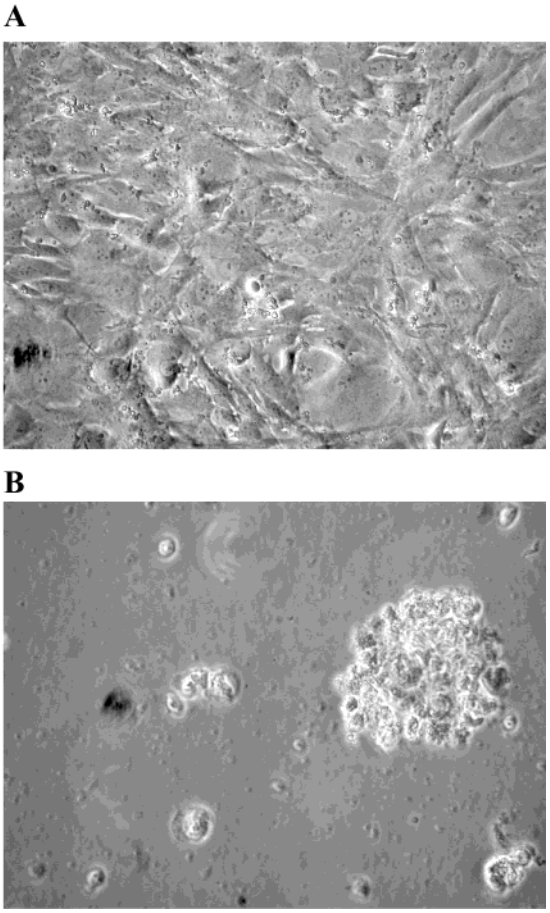

Figure 2. Tumor cell biopsy cultures in the presence of (A) unloaded and (B) **9**-loaded polymer disks.

These preliminary studies indicate tolerance of cerebral tissue to drug-loaded disks and encourages future clinical trials of the compound. Further work toward clinical evaluation is in progress.

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Supporting Information Available: Experimental section containing preparation of **9** and description of the in vitro and in vivo assays. This material is available free of charge via the Internet at http://pubs.acs.org.

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