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Synthesis and Growth Inhibitory Properties of Glucosamine-Derived Glycerolipids

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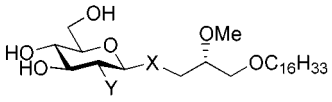
ABSTRACT



1a X = O, Y = NH_2 antiproliferative
1b X = CH_2, Y = NH_2 an active *C*-analog

2-Amino *C*-glycerolipid 1b was synthesized by using the Ramberg–Bäcklund rearrangement as the key step. *β*-*C*-Glycerolipid 1b exhibits in vitro antiproliferative effects strikingly similar to those of *O*-glycoside analogue 1a.

The study of *C*-glycoside analogues of bioactive *O*- and *N*-glycosides is a mature field.[1] Since the publication of the two books and several monograph chapters cited in ref 1, hundreds of articles have appeared. A great deal of work has focused on the structural and conformational properties of *C*-glycosides as probes of the anomeric and exo-anomeric effects. *C*-Glycosides are essentially inert to degradation by glycosidases because the anomeric carbon has been transformed from a hydrolytically labile *O*- or *N*-acetal linkage to an ether linkage. The underlying assumption for the use of *C*-glycoside analogues in glycobiology is that the conformational differences between the *O*- (or *N*-) linked natural material and the *C*-linked analogue will be minimal. The corollary to the minimal difference hypothesis is that the recognition and binding of the *C*-analogue will be similar to that of the natural material.

Until now, in contrast to the large number of *C*-glycosides that have been synthesized, few direct comparisons of O vs C biological activity have been made.[2] The most thorough comparison has been done for the *C*-lactose/*O*-lactose pair reported in significant papers in 1995, 1996, and 1998 by

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(2) We are focusing on comparisons between *O*-glycosides and other identical materials with the simple replacement of glycoside O by CH_2. We use the word "exact" to characterize the analogue. It is not possible to review the many bioactive *C*-glycoside materials that do not have an exact *O*-analogue for comparison. To cite a few notable cases: (a) Schmidt, R. R.; Dietrich, H. *Angew. Chem., Int. Ed. Engl.* **1991**, *30*, 1328−1329. (b) Michael, K.; Wittman, V.; Konig, W.; Sandow, J.; Kessler, H. *Int. J. Peptide Protein Res.* **1996**, *48*, 59−70. (c) Nagy, J. O.; Wang, P.; Gilbert, J. H.; Schefer, M. E.; Hill, T. G.; Callstrom, M. R.; Bednarski, M. D. *J. Med. Chem.* **1992**, *35*, 4501−4502. (d) Vyplel, H.; Scholz, D.; Macher, I.; Schindlmaier, K.; Schutze, E. *J. Med. Chem.* **1991**, *34*, 2759−2767. Refs 1a and 1b should be consulted for in-depth surveys of bioactive *C*-glycosides.

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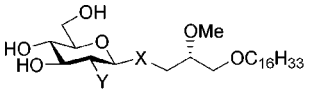
the Kishi and Schmidt and Jiminez-Barbero groups, which focused on NOE data and modeling results. There is partial but not complete agreement with regard to the similarities and differences in the conformation of ground-state and of binding conformations.[3−5] Nonetheless, the K_i values for *O*- and *C*-lactose for the competitive inhibition of β-galactosidase-catalyzed cleavage of *p*-nitrophenyl galactose are 1 and 3 μM, respectively, which suggest a close similarity if not perfect identity of the two materials in their binding to the enzyme. Three other close comparisons include binding of a blood group trisaccharide to a leguminous lectin,[6] oligo-β-1,6-galactosides to three monoclonal immunoglobulins,[7] and a trimannose analogue containing one *C*-linkage to concanavalin A.[8] In the first two of these comparisons, the affinities of the *O*-glycosides and their exact *C*-analogues were essentially identical. In the last report, the binding decreased by 66-fold (from 3 μM for the *O*-trimannose to 198 μM for the mono-*C*-analog). An early comparison is that between the antitumor activity of daunomycin and its nonexact *C*-analogue, with ED_{50} values vs L1210 cells of 0.013 and 4 μM, respectively.[9] Recently we had reported a comparison between an antiproliferative 2-deoxyglucosyl glycerolipid and its exact *C*-analogue in which the *C*-glycoside showed a severalfold weaker activity.[10] We now wish to describe an example in which the *O*- and *C*-glycerolipids of glucosamine display very similar micromolar antiproliferative activity against nine tumor cell lines.

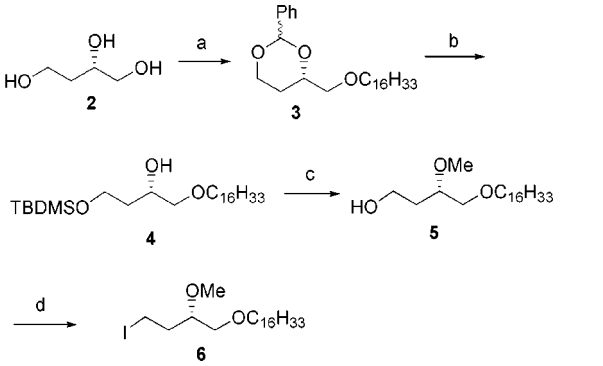


1a X = O, Y = NH_2 ; **1b** X = CH_2, Y = NH_2; **1c** X = CH_2, Y = H

Our plan was to compare glucosamine derivatives **1a** and **1b** since we had shown earlier that *O*-glycoside **1a** had micromolar antiproliferative activity in assays against several tumor cell lines.[11] This lead compound had been prepared via a zinc chloride catalyzed version of the Koenigs−Knorr reaction of 1-chlorotetra-*O*-acetylglucosamine (the intermediate in the conversion of **7** to **8**) and the appropriate modified glycerol. For the preparation of **1b**, we chose to test the Ramberg-Bäcklund (RB)[12] method for the synthesis of *C*-glycosides, under development by both our group and Taylor's.[13] After the rather guarded outlook for the synthesis of *C*-glycosides of 2-amino sugars that was expressed in 1996,[14] several useful approaches have been reported.[15] However, we believed that our method offers both simplicity and certain β-anomeric selectivity.

Previously, we had synthesized 2-deoxy *C*-glycoside **1c** by introducing a methyl ether into its thioglycoside precursor via *O*-methylation of the side chain hydroxyl immediately prior to the RB rearrangement. The corresponding *O*-methylation step is not clean in the 2-acetaminoglucose series because *N*-methylation also takes place. Therefore, the sequence was modified by installing the *O*-methyl group before the thioglycoside was prepared. The synthesis of the lipid (*S*)-4-*O*-hexadecyl-3-*O*-methyl-1-iodobutane (**6**) was easily accomplished starting from (*S*)-(−)-1,2,4-butanetriol **2** (Scheme 1). This procedure is based on selective protection

Scheme 1[a]



[a] Reagents and conditions: (a) ref 10; (b) (1) 80% AcOH, reflux, 81%, (2) TBDMSCl, CH_2Cl_2, imidazole, 87%; (c) (1) NaH, MeI, THF, 92%, (2) Bu_4NF, THF, 83%; (d) Ph_3P, I_2, imidazole, toluene, reflux, 70%.

of **2** followed by *O*-alkylation.[10] Deprotection of **3** using 80% acetic acid at reflux, followed by selective silylation of the primary alcohol afforded silyl ether **4**. *O*-Methylation followed by deprotection of silyl group gave primary alcohol **5**. 4-*O*-Hexadecyl-3-*O*-methyl-1-iodobutane (**6**) was prepared from **5** and I_2/Ph_3P at reflux in toluene.

N-Acetyl-3,4,6-tri-*O*-acetyl-1-glucosamine thioacetate (**8**) was synthesized from commercial *N*-acetyl-D-glucosamine

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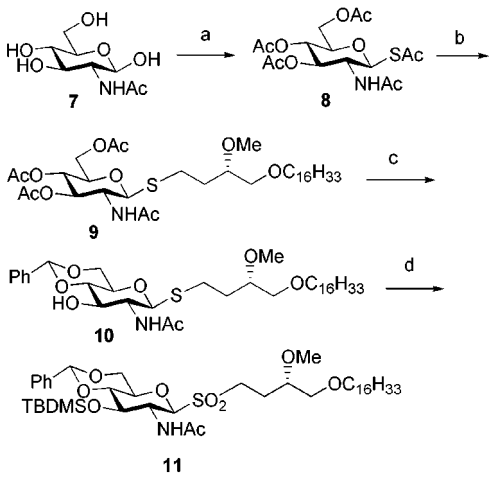
Scheme 2[a]



[a] Reagents and conditions: (a) (1) AcCl, overnight, (2) KSAc, acetone, 70%, two steps; (b) NH_2NH_2·HOAc, DMF, **6**, Et_3N, 85%; (c) (1) guanidine, EtOH/CH_2Cl_2, (2) $PhCH(OMe)_2$, *p*-TsOH, DMF, 72%, two steps; (d) (1) TBDMSCl, imidazole, DMF, 93%, (2) MMPP, 95%.

7 in two steps[16] (Scheme 2). After the *S*-acetate was selectively cleaved (NH_2NH_2·HOAc, DMF), alkylation with iodide **6** in Et_3N gave thioglycoside **9** in good yield.[17] Selective deprotection of the *O*-acetyl groups using guanidine,[18] followed by benzylidene acetal protection of the 4,6-diol, afforded thioglycoside **10**. Treatment of **10** with TBDMSCl followed by oxidation using MMPP provided sulfone **11**.[19] The RB rearrangement of sulfone **11** using 25% KOH on alumina in $CBrF_2CBrF_2$ at reflux gave alkene **12** (*Z* isomer only, which was confirmed by a NOE experiment) in 78% yield (Scheme 3).[20]

RB product 2-deoxy-2-*N*-acetyl glycal **12** is much more stable than the corresponding RB product in the 2-deoxyglucose series. Exo glycal **12** can be stored at 0 °C for more than 1 month without decomposition. Simultaneous benzylidene deprotection and reduction of alkene **12** (H_2, 10% Pd/C) afforded β-*C*-glycoside **13** in 85% yield.[21] Of the several methods attempted for cleavage of the silyl group, Bu_4NF, formic acid, acidic ionic exchange resin (Dowex

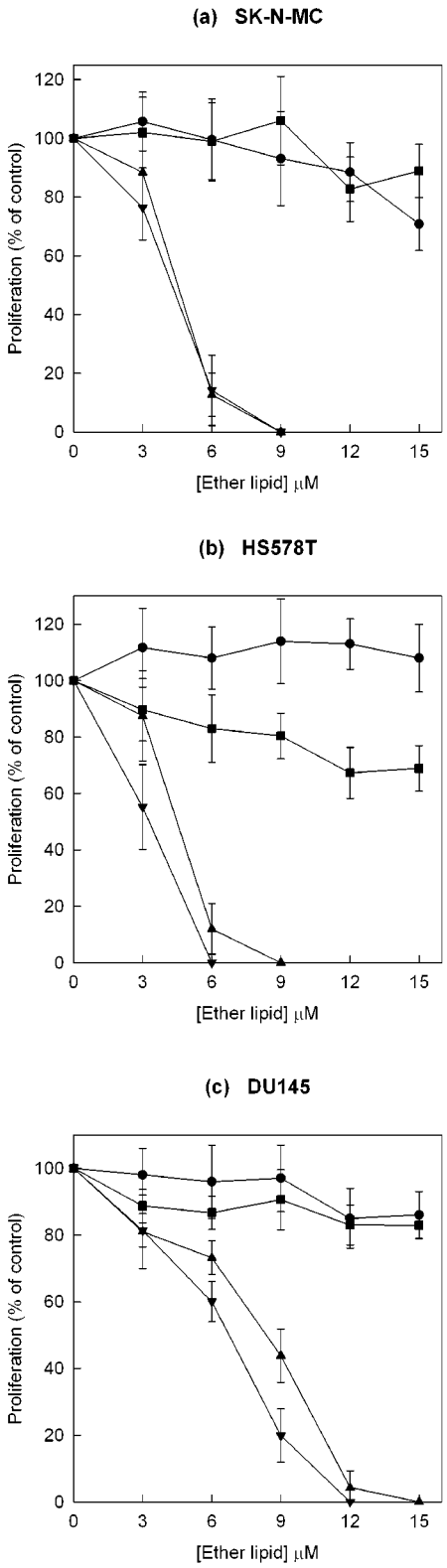


Figure 1. Effects of antitumor ether lipids **1a** (▼), **1b** (▲), **1c** (●), and **14** (■) on the proliferation of (a) SK-N-MC, (b) HS578T, and (c) DU145 cells. Cells were treated with each compound for 48 h.

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(19) This protection scheme was implemented (i) to replace KOH-sensitive acetates and (ii) to avoid alkylations with benzyl bromide, which would react with the amide function.

(20) We found that the yield of the RB reaction using freshly prepared KOH/Al_2O_3 is much higher than using the material that has been stored for 1 month in the desiccator.

(21) Both Taylor's group and ours have observed clean β-*C*-glycoside formation upon hydrogenation deoxyglycals, almost certainly due to a chairlike transition state when the hydrogen is transferred to the α-face. Conversely, β-face approach of hydrogen requires a twist-boat like TS during H-transfer.

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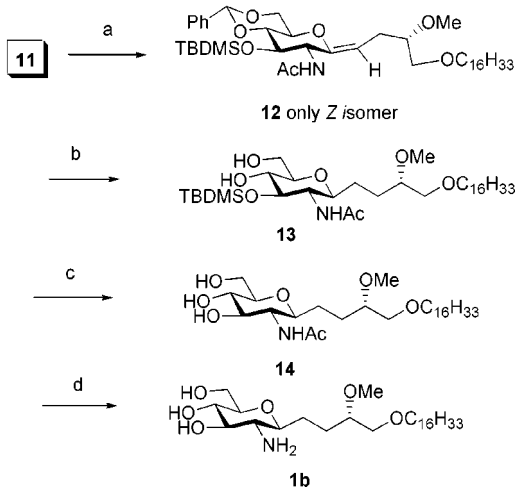
Scheme 3[a]



[a] Reagents and conditions: (a) $CBrF_2CBrF_2$, *t*-BuOH, 25% KOH/Al_2O_3, reflux, 70%; (b) H_2, 10%Pd/C, EtOAc, 80%; (c) $BF_3{\cdot}Et_2O$, CH_3CN, 0 °C, 93%; (d) 2 N KOH/EtOH, 120 °C, 75%.

50W), and $BF_3{\cdot}Et_2O$, only $BF_3{\cdot}Et_2O$ in CH_3CN[22] gave a clean reaction. The *N*-acetyl group was cleaved by using 2 N KOH in EtOH at 120 °C to afford final product **1b**.

In summary, our synthesis proved to be quite facile. Worthy of note is the very high stereoselectivity observed in each synthetic step, particularly in the RB sequence to afford exo glycal **12** and its reduction to afford *C*-glycoside **13**. It is of interest to note that the most rigorous conditions in the sequence involved the deacylation of **14** to afford **1b**. In addition to stereoselectivity, the combination of simplicity and convergence makes our approach an attractive and novel method for *C*-glycolipid synthesis.

Table 1 summarizes the comparative test results for **1a** and **1b**; also included are the data for **1c**, the 2-deoxy analogue of **1b**, which was described previously.[10] Figure 1 shows the data for the assay against SK-N-MC, HS578T, and DU145 cells. In all nine examples, the IC_{50} values[23] (drug concentrations required to inhibit growth by 50%) indicate that *C*-glycoside analogue **1b** shows antiproliferative activity remarkably parallel to that of the parent *O*-glycoside **1a**. It is interesting that a similar level of activity of ether glycerophospholipids bearing a deoxyinositol headgroup was reported recently by Kozikowski et al.[24] We are developing additional *C*-glycolipids in the aminosugar family, which will be described in the future.

Table 1. Growth Inhibitory Properties of **1a**, **1b**, and **1c**: IC_{50} Values for Inhibition of Cell Proliferation[a]

	IC_{50} (μM)		
cell line	**1a**	**1b**	**1c**
MCF-7, breast	8.0	8.1	25.6
MDA-MB-468, breast	7.0	9.0	34.4
MDA-MB-231, breast	7.1	9.1	40.0
HS578T, breast	3.1	5.1	21.0
BT549, breast	6.5	8.9	28.5
A498, kidney	6.9	8.5	ND
SK-N-SH, neuronal	3.8	4.1	ND
SK-N-MC, neuronal	4.1	4.1	ND
DU145, prostate	6.5	7.9	ND

[a] The IC_{50} values for **1a**, **1b**, and **1c** were determined as described in ref 23. Briefly, exponentially growing cells were incubated with the drugs (0−15 μM), and the increase in cell numbers after 48 h was determined and expressed as a percentage of the controls, which had no drug. ND means not determined but > 15 μM.

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Supporting Information Available: Experimental procedures and spectroscopic data for compounds **3**−**14**, and **1b**. This material is available free of charge via the Internet at http://pubs.acs.org.

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