of 18 h, particulate matter was harvested from each microtiter well with use of an automated cell harvester (Skatron, Inc., Sterling, VA). Uptake of ^{3}H-hypoxanthine was measured by using a scintillation spectrophotometer (Model LS3801, Beckman Instruments, Irvine, CA). Concentration–response data were analyzed by nonlinear regression and the IC_{50} values (50% inhibitory concentrations) for each compound were calculated.

(b) In Vivo Antimalarial Studies. The suppressive blood schizonticidal and curative activities of these new compounds were measured in a test where mice were infected with 5.98×10^5 *P. berghei* parasitized cells intraperitoneally on day 0. Test compounds were dissolved in peanut oil and were administered subcutaneously once a day for three consecutive days commencing on day 3. The dose levels of compounds given were 640, 160, and 40 mg/kg per day. Blood films were taken on days 6, 13, and 20. Blood schizonticidal activity was determined by monitoring blood films for the appearance of parasites and for extended survival times compared to infected untreated controls. Mice surviving 60 days were considered cured. The infected untreated control mice (negative controls) died on either day 6 or 7. Compounds was considered active when the survival time of the treated mice was greater than twice the control mice, i.e., 12–14 days.

Acknowledgment. We thank Dr. Arba L. Ager, Jr., University of Miami, for performing the in vivo, and Dr. Wilbur Milhous of WRAIR for the in vitro antimalarial studies. This work was supported, in part, by the Office of Naval Research.

Registry No. **1**, 63968-64-9; **29**, 71939-50-9; **3**, 82596-30-3; **4**, 127971-91-9; **69**, 127971-92-0; **66**, 127997-42-6; **6c**, 127997-43-7; **6d**, 127971-93-1; **6e**, 127971-94-2; **6f**, 127971-95-3; **a**, 128050-94-2; 7b, 128052-60-8; 3-$FCoH_4NH_2$, 372-19-0; $C_6H_5NH_2$, 62-53-3; 2-aminothiazole, 96-50-4; 2-aminopyridine, 504-29-0; 2-aminopyrimidine, 109-12-0.

Supplementary Material Available: Tables of atomic coordinates, bond lengths, bond angles, anisotropic displacement coefficients, and H-atom coordinates for compound **6d** (5 pages). Ordering information is given on any current masthead page. Tables of atomic coordinates and bond lengths and angles have been deposited with the Crystallographic Data Centre, Cambridge University Chemical Laboratory, Cambridge CB2 1EW, England.

Synthesis and Antineoplastic Properties of Ether-Linked Thioglycolipids

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Ether-linked glycero-α- and β-D-glucopyranosides and glycero-1-thio-α- and β-D-glucopyranosides have been synthesized by modifications of the Königs–Knorr procedure, and their antitumor activities have been evaluated. The bioactivities of these compounds have been evaluated in five different cell lines (WEHI 3B, C653, X63/OMIL3, R6X-B15, and HL-60) and compared with the activities of 1-*O*-hexadecyl-2-*O*-methyl-*sn*-3-glycerophosphocholine (GPC) and its enantiomer, 3-*O*-hexadecyl-2-*O*-methyl-*sn*-1-GPC. The results indicate that a α-D-thioglucopyranoside [1-*O*-hexadecyl-2-*O*-methyl-3-*S*-(α-D-1′-thioglucopyranosyl-*sn*-glycerol)] is selective with respect to its action on target cells, with high activity for killing of WEHI 3B and C653 cells as determined by inhibition of [^{3}H]thymidine incorporation into DNA and HL-60 cell cytotoxicity, but unable to induce aggregation of rabbit platelets at 10^{-5} M. The corresponding β-linked thioglycolipid was ineffective with respect to cytotoxicity against each cell line tested, indicating the importance of configuration at the anomeric position; the β-thioglycoside was also ineffective with respect to inducing platelet aggregation. 1-*O*-Hexadecyl-2-*O*-methyl-*sn*-3-GPC and 3-*O*-hexadecyl-2-*O*-methyl-*sn*-1-GPC were potent inhibitors of growth of each cell line tested but also caused rabbit platelet aggregation at concentrations $\geq 10^{-7}$ M. Thus, 3-*S*-(α-thioglycopyranosyl)-*sn*-glycerols bearing a long-chain *O*-alkyl group at the *sn*-1 position and a methoxy group at the *sn*-2 position of glycerol appear to be a promising class of antineoplastic agents with lower risk of inducing thrombosis than the widely studied platelet activating factor analogue, 1-*O*-octadecyl-2-*O*-methyl-*rac*-3-GPC.

A synthetic glucosyl diglyceride containing a long alkyl chain at the *sn*-1 position of glycerol, a methoxy group at the *sn*-2 position, and a β-linked glucosyl moiety at the *sn*-3 position (1-*O*-hexadecyl-2-*O*-methyl-3-*O*-(β-D-glucopyranosyl)-*sn*-glycerol (**1**); see Figure 1) possesses cancerostatic activities.[1] "Alkyl lysophospholipids" that have a 16- or 18-carbon alkyl chain at the *sn*-1 position, a methoxy group at the *sn*-2 position, and a phosphocholine moiety at the *sn*-3 position (**4**) have been shown to specifically inhibit the growth of tumor cells, inhibit tumor cell invasion and metastasis, and enhance the tumoricidal capacity of macrophages.[2] Although the mechanisms responsible for the cytotoxic action of the alkyl lysophospholipids are not known, the possibility of a metabolite being the active component has been raised.[3] In Ehrlich ascites cells *rac*-1-*O*-[^{3}H]octadecyl-2-*O*-methylglycerophosphocholine (GPC) was converted to *rac*-1-*O*-[^{3}H]octadecyl-2-*O*-methylglycerol at a rate of 70 pmol/10^6 cells/h.[1a] The significance of this observation is not yet known, however, since it is not clear whether the viability

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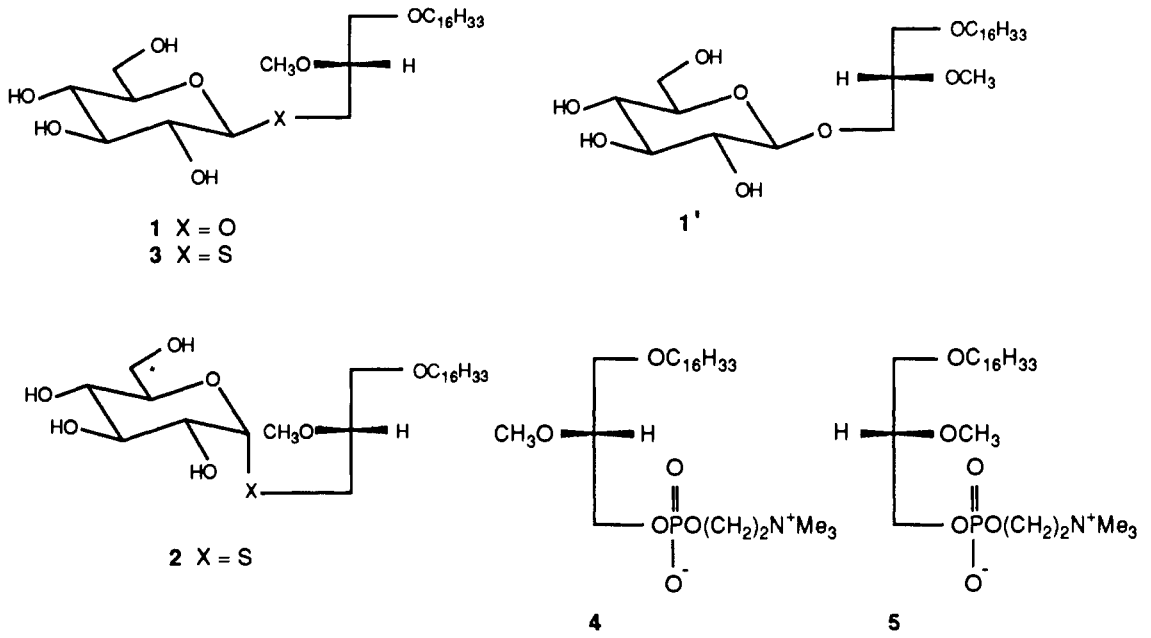

Figure 1. Structures of the antineoplastic compounds used.

of Ehrlich ascites cells is affected by alkylmethoxy–GPC; furthermore, the cytotoxic effect of alkylmethoxyglycerol on Ehrlich ascites cells has not been reported. It is also noteworthy that in undifferentiated HL-60 cells only about 1% of *rac*-1-*O*-[^{3}H]octadecyl-2-*O*-methyl-GPC was metabolized during a 24-h incubation.[4] Moreover, no cytotoxic activity was exhibited on the quantitative addition of 2.5 μM or 25 μM 1-*O*-octadecyl-2-*O*-methyl-*sn*-glycerol to the highly sensitive HL-60 cells.[5,6] It has been reported that both 1-*O*-octadecyl-2-*O*-methyl-*rac*-GPC[7] and 1-*O*-hexadecyl-2-*O*-methylglycerol[8] can inhibit protein kinase C. This phospholipid- and Ca^{2+}-dependent enzyme is a vital element of signal transduction and cell growth and differentiation, and therefore could be a relevant target.

In a search for new, more effective antineoplastic glycosylglycerolipids, we have synthesized 1-*O*-hexadecyl-2-*O*-methyl-3-*S*-(α-D-1′-thioglucopyranosyl)-*sn*-glycerol (**2**), since substitution of oxygen by sulfur in other lipids has been proposed to enhance lipophilicity.[9] In this communication, we report the syntheses of α- and β-thioglucopyranosyl derivatives **2** and **3**, respectively, and compare the activities of these compounds with those of the 3-*O*-β-D-glucopyranosyl lipid **1**, the widely studied 1-*O*-octadecyl-2-*O*-methyl-*rac*-GPC,[10] and 1-*O*-hexadecyl-2-*O*-methyl-*sn*-GPC (**4**) against leukemic cell lines. A disadvantage of compound **4** and its enantiomer **5** (or their 18-carbon analogues) with respect to use in intravenous administration as an antitumor drug is their proaggregatory behavior at concentrations exceeding 10^{-7} M.[11] We show here that α-thioglucopyranoside **2** does not aggregate rabbit platelets at 10^{-5} M, but is cytotoxic to a variety of cell lines in the concentration range 10–40 μM. The antineoplastic activity of **2**, together with its inability to induce platelet aggregation, suggests that α-linked thiosugar lipids merit further study in the treatment of leukemia.[12]

Results

I. Synthetic Chemistry. We report here a practical, short, stereoselective synthesis of the ether-linked α- and β-glycerothioglycosides **2** and **3** via reaction between 1-thio-β-D-glucose tetraacetate (**7**) and 1-*O*-hexadecyl-2-*O*-methyl-3-*O*-(*p*-tolylsulfonyl)-*sn*-glycerol (**6**). The latter was obtained from (*R*)-glycidyl tosylate in very high optical purity as described previously.[13] An advantage of the

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(10) 1-*O*-Octadecyl-2-*O*-methyl-*rac*-glycero-3-phosphocholine (ET18-OCH_3) is in a multi-institutional clinical phase II trial against non-small cell lung cancer in West Germany (Khanavkar, B.; Ulbrich, F.; Gatzemeier, U.; Meyer-Schwickerath, E.; Lorenz, J.; Schreml, W.; Brugger, R.; Schick, H. D.; von Pawel, J.; Nordström, R.; Drings, P. *Contrib. Oncol.* **1989**, *37*, 224–235). Clinical phase I studies were completed in Munich and Freiburg, FRG.[2a] 1-*O*-Octadecyl-2-*O*-methyl-*rac*-GPC has been used against a variety of human tumors[2] and has been used in purging bone marrow of leukemic cells prior to autologous transplantation (Glasser, L.; Somberg, L. B.; Vogler, W. R. *Blood* **1984**, *64*, 1288–1291).
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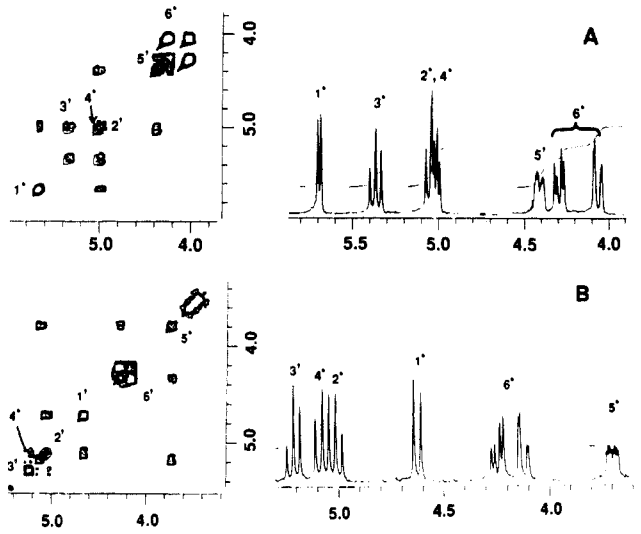

Figure 2. The 1- and 2-dimensional 300-MHz ^1H COSY nuclear magnetic resonance spectra for the glucosyl proton signals (H1′–H6′) in the α anomer **8** (A) and the β anomer **9** (B) in chloroform-*d*.

route outlined in Scheme I is that it makes possible the preparation of ^{35}S-labeled **2** and **3**, since **7** is available from the reaction of thiourea with tetra-*O*-acetyl-α-D-glucosyl bromide.[14] Glycosylation via the 1,8-diazabicyclo[5.4.0]-undec-7-ene (DBU) mediated displacement of the tosylate group by the thiol group of **7** gave a 3:1 mixture of the desired α and β anomers of the 1-thioglycopyranosides **8** and **9**. The formation of a mixture of α and β anomers probably occurs by opening of the pyranose ring to the free thioaldehyde form of **7**; attack by the hydroxy group at C-5 on the carbonyl carbon atom gives an anomeric mixture of **7**. A precedent for this reverse Königs–Knorr reaction in lipid chemistry is the displacement of iodide by reaction of **7** with 1-iodo-3-*O*-benzoylceramide.[15] The α and β anomers were readily separated by flash chromatography, and the tetra-*O*-acetates were hydrolyzed with use of methanolic barium oxide.

Scheme I also outlines the conversions of 1-*O*-hexadecyl-2-*O*-methyl-3-*O*-(*tert*-butyldiphenylsilyl)-*sn*-glycerol (**10**) and its enantiomer **10′** into the (*R*)-glycolipid **1** and the (*S*)-glycolipid **1′**, respectively. The Helferich modification of the Königs–Knorr reaction, i.e. use of mercury(II) cyanide instead of silver carbonate to promote coupling,[16] was used, followed by hydrolysis of the tetra-*O*-acetates with methanolic sodium hydroxide. The silyl ethers **10** and **10′** were prepared as described previously.[13] Briefly, the reaction sequence involves the BF_3 etherate catalyzed ring opening of (*R*)-(+)- and (*S*)-(−)-oxiranemethanol *tert*-butyldiphenylsilyl (TBDPS) ethers with 1-hexadecanol, giving the ring-opened intermediate in an enantiomeric excess of ≥94%, then methylation by using methyl iodide in benzene.[13]

The configuration at the anomeric carbon of the thioglycolipids was determined by ^1H NMR spectroscopy. The chemical shift of the glucosyl-H_1 proton and the $J_{1,2}$ coupling constant have been found to differ significantly in the α and β anomers of various glycosides and thioglycosides.[17] The ^1H NMR assignments of the glucosyl protons in the thioglycolipids were made from the through-space connectivities as deduced by 2D-NMR proton chemical-shift spectroscopy at 300 MHz (left-hand panels of Figure 2). It should be noted that earlier reports of the preparation of **1** did not include full assignments of these resonances.[1b,c] The assignments of the proton resonances of the glucosyl portion of **8** and **9** were assigned as shown in Figure 2. It is of interest to note the large difference in chemical shift of the glucosyl-$H_{5'}$ signal in the β and α anomers (3.69 and 4.41 ppm, respectively). The down-field shift of the glucosyl-$H_{5'}$ signal in the α anomer may arise from a deshielding influence of the axial sulfur on the axial proton at C_5.

Table I. Time Course of Effect of **2** on Killing of WEHI 3B Cells

time of incubation of cells with **2** before addition of [^3H]thymidine[b]	% cell survival;[a] concn of **2**: 30 μM	40 μM
0 min	79 ± 7	49 ± 10
3 h	57 ± 4	49 ± 7
6 h	53 ± 6	27 ± 4
24 h	48 ± 7	20 ± 8
48 h	46 ± 11	0.5 ± 0
72 h	25 ± 8	0

[a] Mean ± standard deviation, $n = 8$. [b] After the cells were incubated with **2** for the indicated time period, [^3H]thymidine was added and incubation was continued for another 24 h at 37 °C. Cells were then harvested and [^3H]thymidine incorporated into DNA was counted as described in the Experimental Section.

Table II. Effects of **2** and **4** on WEHI-3B Cell Viability[a]

drug concn (μM)	% viable cells after 24 h: **4**	**2**	% viable cells after 48 h: **4**	**2**
0	100	100	100	100
1.25	44 ± 6	90 ± 5	48 ± 21	72 ± 8
2.5	39 ± 5	75 ± 6	18 ± 4	64 ± 7
5.0	8 ± 8	64 ± 8	1 ± 0.5	25 ± 4
10.0	0	53 ± 9	0	0.3 ± 0.1
30.0	0	48 ± 11	0	0

[a] Cell viability was assessed as the percentage of cells that took up trypan blue dye versus the controls.

Table III. Effects of Antineoplastic Agents on [^3H]Thymidine Incorporation into DNA of Various Cell Lines

compd (40 μM)	[^3H]thymidine incorporated (% of control)[a]: WEHI 3B	C653	X63/OMIL3	R6X-B15
2	7 ± 2 (15)	13 ± 1 (8)	71 ± 2 (8)	66 ± 17 (10)
3	80 ± 11 (15)	78 ± 4 (5)	84 ± 8 (5)	98 ± 10 (5)
1	26 ± 4 (5)	7 ± 2 (10)	42 ± 6 (8)	75 ± 7 (5)
1′	82 ± 9 (5)	70 ± 10 (13)	95 ± 5 (8)	89 ± 10 (5)
4	0.4 ± 0.3 (10)	4 ± 0.5 (8)	3 ± 0 (8)	55 ± 28 (10)
5	0.3 ± 0.2 (10)	5 ± 1 (8)	2.5 ± 1 (8)	45 ± 24 (10)

[a] Mean ± standard deviation; the number of individual experiments is given in parentheses. Cells were treated with the drug (40 μM) for 48 h prior to addition of [^3H]thymidine.

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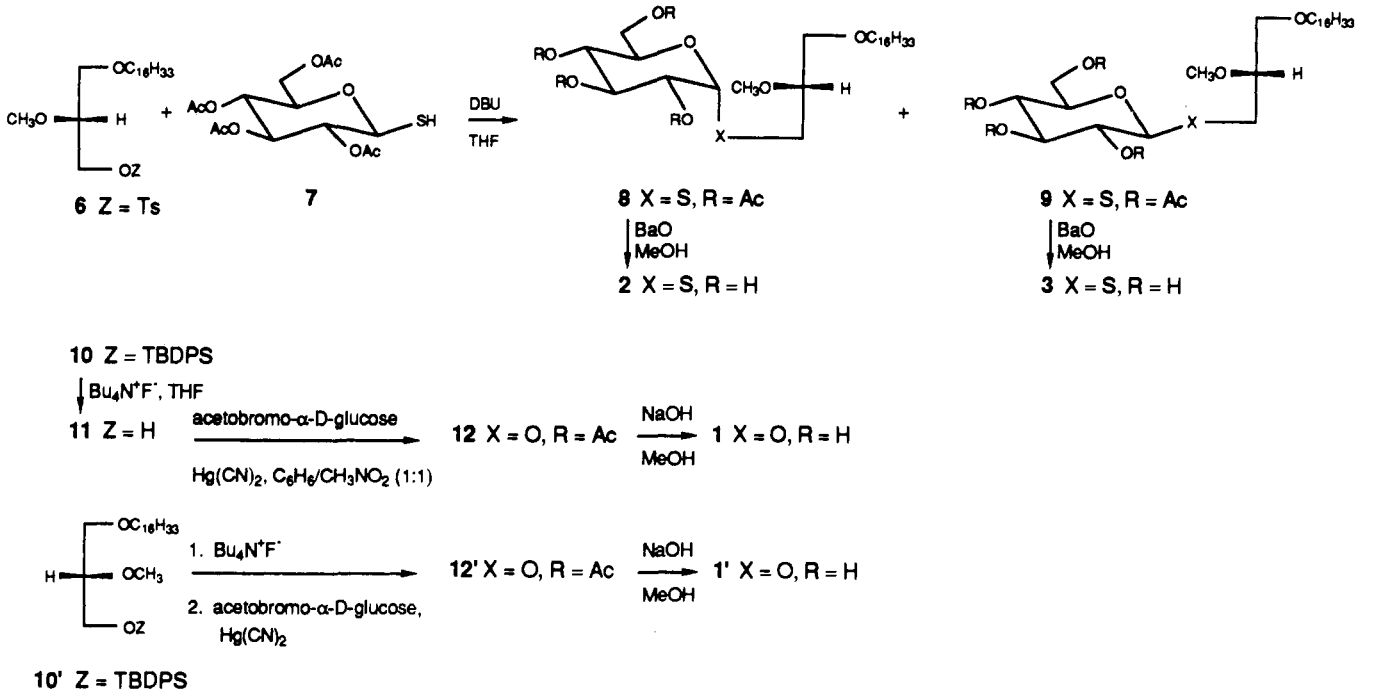
(16) For a review of glycolipid synthesis, see: Gigg, R. *Chem. Phys. Lipids* **1980**, *26*, 287–404.
(17) In the α anomer, the H-1 proton is shifted downfield and the $J_{1,2}$ value is smaller because of the axial–equatorial arrangement of H_1 and H_2. For example, in the α anomer of phenyl thioglycoside H-1 shows δ 5.58 ppm, d, $J = 5.0$ Hz (Nicolaou, K. C.; Dolle, R. E.; Papahatjis, D. P.; Randall, J. L. *J. Am. Chem. Soc.* **1984**, *106*, 4189–4192), whereas H-1 of the β anomer of phenyl or 3-phenylpropyl thioglycosides shows δ 4.5 ppm, d, J = 9–10 Hz (Nambiar, S.; Daeuble, J. F.; Doyle, R. J.; Taylor, K. G. *Tetrahedron Lett.* **1989**, *30*, 2179–2182. Pfäffli, P. J.; Hixson, S. H.; Anderson, L. *Carbohydr. Res.* **1972**, *23*, 195–206). Furthermore, the H-1 resonance of *n*-octyl 1-thio-β-D-glucopyranoside (Sigma) shows the following (200 MHz, $CDCl_3$): δ 4.45 ppm, d, $J = 9.30$ Hz.

Scheme I. Conversion of Ether-Linked Glycerols 6, 10, and 10′ into Glyceroglycolipids 1, 1′, 2, and 3



II. Biological Properties. Table I presents the antiproliferative and cytotoxic effects of **2** on WEHI 3B cells. At a drug concentration of 30 μM, approximately 50% of the cells were killed after a 3-h incubation period on the basis of inhibition of [^{3}H]thymidine incorporation. Incubation with 40 μM of **2** for 6 h resulted in even greater inhibition of [^{3}H]thymidine uptake, with ~75% of the cells killed. Inhibition of the incorporation of [^{3}H]thymidine was correlated with cell cytotoxicity, since trypan blue exclusion revealed that ~50% of the cells were killed after 3 h of incubation with 30 μM of **2** (Table II). Similar experiments with 30 μM of **4** resulted in killing of essentially all of the cells within 6 h, as indicated by the inability of the cells to incorporate [^{3}H]thymidine into DNA, as well as their ability to take up trypan blue.

Figure 3 shows the inhibition of [^{3}H]thymidine uptake into WEHI 3B cells by various lipids containing a *sn*-2 methoxy group and a *O*-hexadecyl chain at the 1-position. The most potent agent for cell killing is **4**, which essentially blocked [^{3}H]thymidine uptake at concentrations ≥10 μM (EC_{50} ~2.5 μM) after a 24-h incubation period. α-Thioglycolipid **2** is somewhat more cytotoxic than the oxygen-containing glycolipid **1**, which has the same configuration at the 2-position of the glycerol backbone (see Table III). The fact that β-thioglycolipid **3** is significantly less cytotoxic than α-thioglycoside **2** demonstrates the importance of configuration at the anomeric position.

Figure 3 also shows that (*S*)-glycolipid **1′** (which has the *S* configuration at C-2) is less potent against this cell line than is (*R*)-glycolipid **1** at high concentrations. However, at concentrations of ≤20 μM, **1** and **1′** exert similar cytotoxic effects on both WEHI 3B (Figure 3) and HL-60 cells (see Figure 4), indicating that the configuration at C-2 of the glycerol backbone plays a minor role in determining the cytotoxic properties at low concentrations of these agents.

Cytotoxic properties of compounds **1–5** at 40 μM against four cell lines are summarized in Table III. The R6X-B15 cell line was less responsive to these agents than were the others. The cytotoxicity of **2** was dependent on the cell line used, with activity in the relative order of WEHI 3B > C653 ≫ R6X-B15 = X63/OMIL3. Cytotoxicity of

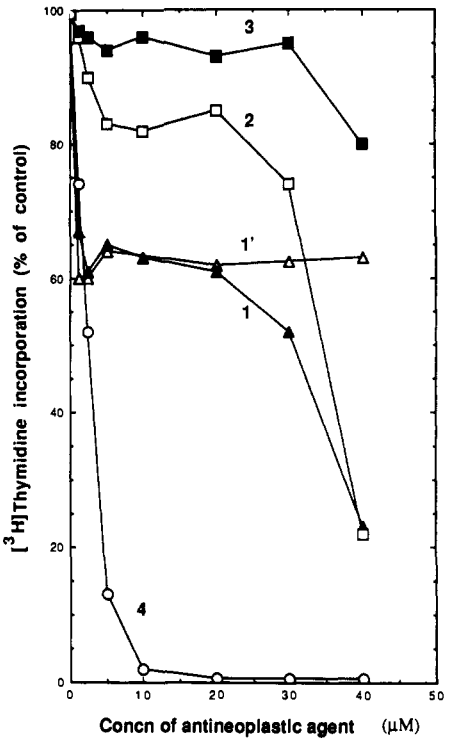


Figure 3. Effects of antineoplastic agents on the incorporation of [^{3}H]thymidine into WEHI 3B cells: **3** (■); **1′** (△); **2** (□); **1** (▲); **4** (○). Cells were treated with the drug 24 h prior to the addition of [^{3}H]thymidine. Data represent the mean of at least eight experiments. The approximate EC_{50} values are as follows: **4**, 2; **2**, 35; **1**, 31; **1′** and **3**, >40 μM.

(*R*)-glycolipid **1** followed the order of C653 > WEHI 3B > X63/OMIL3 > R6X-B15. (*S*)-Glycolipid **1′** is much less potent than **1** at 40 μM, whereas there is little effect of configuration at the 2-position of the glycerol backbone on the cytotoxicity of the GPC derivatives **4** and **5**.

The effects of the α- and β-thioglycosides and of the (*R*)- and (*S*)-glycolipids on the growth of the human promyelocytic leukemic cell line HL-60 are shown in Figure 4. The β-thioglycoside **3** has markedly lower cytotoxic activity

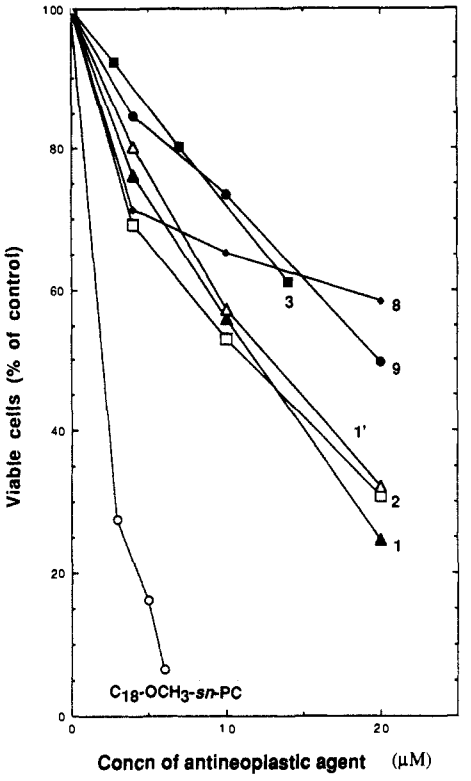


Figure 4. Cytotoxicity of antineoplastic agents toward HL-60 cells as a function of lipid concentration: 1-*O*-octadecyl-2-*O*-methyl-*sn*-3-GPC (○); 1 (▲); 2 (□); 1′ (△); 3 (■); 9 (●); 8 (◇). Each value corresponds to the average of duplicate determinations and is representative of three separate experiments.

than the α anomer **2**. The O-acetylated forms of the α and β anomers (**8** and **9**) have poor cytotoxic activities, indicating a requirement for free hydroxyl groups in the D-glucosides for cytotoxicity. Although the oxygen- and sulfur-containing lipids **1** and **2** have similar effects on HL-60 cell cytotoxicity, their potency is significantly lower than that of 1-*O*-alkyl-2-*O*-methyl-GPC. The activity of the latter compound is shown in Figure 4 by the use of 1-*O*-octadecyl-2-*O*-methyl-*sn*-3-GPC.

We evaluated the relative platelet aggregation activity of **1–5** using washed rabbit platelets. As shown in Figure 5A, platelet activating factor (PAF) induced platelet aggregation at concentrations exceeding 10^{-13} M, whereas much higher concentrations ($>10^{-7}$ M) of the methoxy analogue of PAF (**4**) were required to induce significant aggregation of rabbit platelets. [(*R*)-2-*O*-Methyl-GPC (**4**) was about 50 times more effective than its enantiomer (**5**); unpublished results.] The glycosides **1**, **1′**, and **3** did not cause platelet aggregation up to 10^{-5} M. Furthermore, **2** did not show antagonistic properties toward PAF receptors, since platelets that were pretreated with 10^{-5} M **2** for 2 min were still able to respond to 10^{-10} M PAF (Figure 5B).

Discussion

The DBU-mediated synthesis of 1,2-di-*O*-alkyl-3-(α- and β-D-1′-thioglucopyranosyl)-*sn*-glycerol (**2** and **3**) proceeds under mild conditions and in high yield (92%, 3:1 ratio of α and β anomers). The anomers were separated by flash chromatography, affording the tetra-*O*-acetates **8** and **9**. Mechanistically, the reaction is considered to take place by the intermediate formation of the free thioaldehyde form of **7**. Tosylate **6** was selected as the glycosyl acceptor, affording the 1-*O*-alkyl-2-methoxythioglycolipids with the desired *R* configuration at C-2 of the glycerol backbone. For the synthesis of the oxygen-linked glycolipid **1**, the TBDPS ether **10** was used instead of the corresponding tosylate **6**. The stereoselective reaction with acetobromo-α-D-glucose by the Königs–Knorr method with anhydrous mercuric cyanide as catalyst in benzene–nitromethane (1:1) gave β-D-glucopyranoside **12** in high yield.

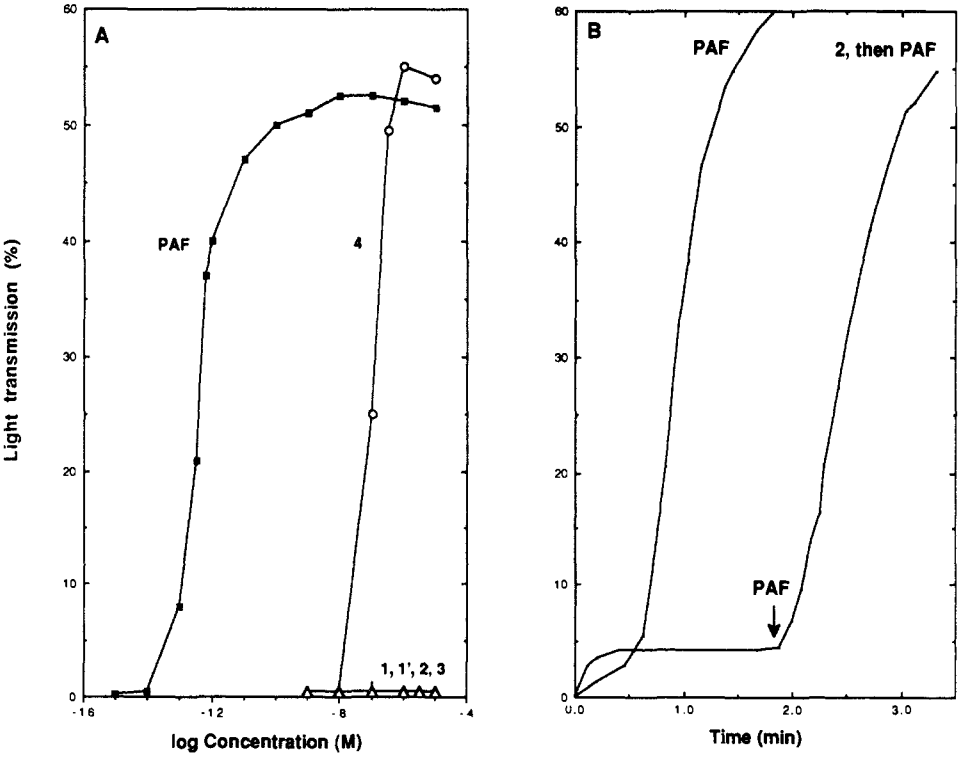


Figure 5. Effects of ether lipids on the aggregation of washed rabbit platelets (200×10^6/mL) as a function of (A) lipid concentration and (B) pretreatment with compound **2**. A: PAF (■); **4** (○); **3**, **2**, **1**, **1′** (△). Platelets were treated with 10^{-5} M **2** for 2 min, then 10^{-10} M PAF was added. For comparison, aggregation induced directly by addition of 10^{-10} M PAF is also shown. The results, which are the mean of three separate experiments, are expressed as percent light transmitted during platelet aggregation.

To prepare derivative 12′, which has the *S* configuration at C-2 of the glycerol backbone, the TBDPS ether 10′ was used to give the glycosyl acceptor 11′.

Thioglycolipid **2** is an effective inhibitor of cancer cell growth. Its activity against WEHI 3B cells is higher than that of the oxygen-containing glycolipid **1**, but lower against two other leukemic cell lines (C653 and X63/OMIL3). This observation suggests that **2** is more toxic against neoplastic cells of monocytic/granuloma origin (WEHI 3B) than of lymphocytic origin (C653 and X63/OMIL3). The activities of **1** and **2** against WEHI 3B and C653 cells approach that exhibited by the alkylmethoxy-GPC **4**. This observation indicates that the moiety at the *sn*-3 position need not be phosphocholine in order for antineoplastic activities to be expressed against these two cell lines; nevertheless, cell cytotoxicity is observed with lower concentrations of **4** (EC_{50} ~2.5 μM against WEHI 3B cells and ~2.0 μM against HL-60 cells) compared with **2** (EC_{50} ~20 μM against WEHI 3B cells and ~11.5 μM against HL-60 cells). The β-linked thioglycolipid **3** lacked significant cytotoxic properties against each of the cell lines used in this study, indicating that the configuration at the anomeric position is critical for recognition or uptake by the cells.

We have also evaluated the importance of the stereochemistry at the 2-position of the glycerol backbone on the cytotoxic properties of **1** and **4**. Table III shows that at 40 μM the (*R*)-glycolipid **1** is more potent than its *S* diastereomer 1′ against WEHI 3B, C653, and X63/OMIL3 cells after a 48-h incubation period. However, the action of GPCs **4** and **5**, which have the *R* and *S* configuration at C-2, respectively, was the same on the four cell lines listed in Table III. It is thus unclear whether specific binding sites are involved. Further work is required to establish whether binding of **1** to the surfaces of WEHI 3B, C653, and X63/OMIL3 cells differs from that of 1′ at low drug concentrations.

The potencies of the ether lipids vary with the cell type. Although the reasons for the observed cell specificities are not clear, possible explanations of the differential cytotoxicity are of interest. Less sensitive cells may contain the tetrahydropteridine-dependent 1-*O*-alkyl cleavage enzyme that degrades the alkyl bond in these lipids.[4,18] Thus, it has been proposed that the poorly metabolizable, methoxy-containing lipids may accumulate in neoplastic cells deficient in this enzyme and interfere with membrane functions. (Two recent studies, however, reported that the susceptibility of human leukemic cell lines to *rac*-1-*O*-octadecyl-2-*O*-methyl-GPC is not controlled by the activity of the *O*-alkyl cleavage enzyme.[3a,4]) In addition, the methoxy-GPC analogue **4** inhibits the activities of acyl-CoA transferase and other enzymes involved in the metabolism of phospholipids;[19] this finding, however, may not account for the different cell sensitivities.

Other mechanisms that may contribute to the biological effects of the methoxy analogue of PAF include inhibition of protein kinase C[7] and activation of certain subtypes of PAF receptors.[20] Since WEHI 3B cells are known to possess PAF receptors[21] and are highly sensitive to the antineoplastic agents we used, it is possible that the cell specificity represents variation in the numbers or structural specificity of PAF receptors on the cell surfaces. On the other hand, our observation that **2** did not activate PAF receptors in rabbit platelets and did not act as an antagonist of PAF (Figure 5) casts doubt on the involvement of PAF receptors in the mechanism of action of **2** in leukemic cells, assuming that PAF receptors behave the same in these two systems. There is, however, the possibility that several types of PAF receptors exist. In addition, it appears unlikely that the action of alkylmethoxy-GPC involves PAF receptors in other cell lines,[22] in contrast to the report of Bazill and Dexter.[20] Thus, although selective antineoplastic activities of some PAF analogues have been attributed, at least in part, to interactions with PAF receptors[20,23] further studies are required to establish whether PAF receptors play a significant role in mediating the action of antineoplastic lipids in leukemic cells.

The inability of **2** at 10^{-5} M to produce platelet aggregation suggests that this compound is promising with respect to intravenous usage, since the risk of inducing thrombosis is reduced. In contrast, extensive aggregation of rabbit platelets was noted on addition of **4** at 10^{-7} M. The effect of **4** shown in Figure 5 is consistent with a previous report that *rac*-**4**,**5** is 600 times less effective than PAF with respect to aggregation of rabbit platelets.[11a,b] In addition, *rac*-1-*O*-octadecyl-2-*O*-methyl-GPC had markedly lower hypotensive activity than PAF and was not effective in releasing 5-HT from rabbit platelets.[11a] 1-*O*-Octadecyl-2-*O*-methyl-*rac*-GPC was also found to be ineffective in stimulating aggregation and serotonin release in human platelets.[24]

Experimental Section

Materials and General Procedures. The solvents were dried as follows: THF, distilled from sodium benzophenone ketyl and collected over sodium; nitromethane, dried over calcium chloride and distilled prior to use; benzene, washed with concentrated sulfuric acid, then aqueous 10% sodium bicarbonate and water, then dried over calcium chloride, and distilled over sodium; methanol, dried over magnesium sulfate. 1-*O*-Hexadecyl-2-*O*-methyl-3-*O*-(*p*-tolylsulfonyl)-*sn*-glycerol (**6**), 1-*O*-hexadecyl-2-*O*-methyl-3-*O*-(*tert*-butyldiphenylsilyl)-*sn*-glycerol (**10**), 3-*O*-hexadecyl-2-*O*-methyl-1-*O*-(*tert*-butyldiphenylsilyl)-*sn*-glycerol (10′), and 1-*O*-hexadecyl-2-*O*-methyl-*sn*-glycerol (**11**) were synthesized as described previously.[13] Acetobromo-α-D-glucose (2,3,4,6-tetra-*O*-acetyl-α-bromo-D-glucose) was obtained from Sigma Chemical Co. 1-Thio-β-D-glucose 2,3,4,6-tetra-*O*-acetate (**7**), mercuric cyanide, barium oxide, ethyl formate, and DBU were purchased from Aldrich Chemical Co. The cytotoxic ether-linked phospholipids **4** and **5** were prepared in very high optical purity from derivatives of (*R*)- or (*S*)-glycidol as described previously.[13] Dowex 50W-X8 was from J. T. Baker. Reactions were monitored on 0.25-mm thick silica gel GF TLC plates (Analtech, Newark, DE). Compounds were detected by spraying with 10% sulfuric acid in ethanol. Flash chromatography was carried out with silica gel

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60 (230–400 ASTM mesh) from E. Merck, purchased from Aldrich. ^{1}H NMR spectra were recorded on a GE Model QE spectrometer (300.5 MHz) and on a IBM-Bruker (200 MHz) spectrometer. Chemical shifts are given in parts per million from tetramethylsilane as internal standard. Infrared spectra were recorded on a Perkin-Elmer 1600 spectrophotometer. Optical rotations were measured at room temperature in a 1-dm cell on a JASCO DIP-140 digital polarimeter. Melting points are uncorrected. Elemental analyses were performed by Desert Analytics (Tucson, AZ).

Cell Culture. The following cell lines were kindly provided by Dr. John Schrader, Biomedical Research Centre, University of British Columbia: WEHI 3B, a myelomonocytic leukemic cell line; R6X-B15, a variant of a mast cell megacaryocyte mouse-derived cell line; C653, a B-cell myeloma mouse-derived cell line; and X63/OMIL3, a T-cell hybridoma mouse-derived cell line.[25] The cells were maintained at 37 °C in RPMI 1640 medium supplemented with 10% fetal bovine serum (heat inactivated, GIBCO Labs, Grand Island, NY), penicillin (50 units/mL), streptomycin (50 μg/mL), and mercaptoethanol (50 μM) in an atmosphere of 5% CO_2/95% O_2. The cell lines were passaged weekly by serial dilutions of 1/10 to 1/10^4. HL-60 cells were maintained in culture in RPMI 1640 medium supplemented with 10% fetal bovine serum (heat inactivated), penicillin (100 units/mL), streptomycin (100 μg/mL), and L-glutamate (4 mM).

[^{3}H]Thymidine Incorporation. Cells (2 × 10^4 cells/well) were grown in 200 μL of medium and incubated with [^{3}H]thymidine (0.1 μCi/well) for 24 h. [^{3}H]Thymidine (specific activity 70–85 Ci/mmol) was purchased from Amersham (Arlington Heights, IL). Stock solutions of the drugs (0.1 M) were prepared in ethanol. The desired drug concentrations were purchased by diluting the stock solutions with RPMI 1640 medium supplemented with 10% fetal bovine serum. Five μL of the drug or of RPMI 1640 (initially containing ethanol for control cultures) were added, and the 96 well plates were incubated in a CO_2 incubator for various times, as indicated in the text and tables. The cells were then harvested in a Brandel Model M-12 Cell Harvester (Gaithersburg, MD). Cells were collected on glass microfiber filters (Whatman 934-AH, 1.0 × 14 in.). The cells were washed with 2 mL of water to remove free thymidine and lysed cells. The filters were air-dried and counted in a Beckman LS 5000CE liquid scintillation counter with use of 4 mL of Eculom cocktail (Beckman, Palo Alto, CA).

Platelet Aggregation Assay. Washed rabbit platelets (200 × 10^6/mL) were suspended in Tyrode's solution,[26] pH 7.2, containing 1.3 mM calcium chloride. Aliquots of 0.5 mL were assayed by using a Bio/Data aggregometer (Hatboro, PA). Bioactivity was determined as the percent increase in light transmission registered by the aggregometer. Platelet activating factor at 10^{-10} M (Sigma Chemical Co., catalog number P4904) was used as a control (see Figure 5B).

Cytotoxicity Assay. WEHI-3B cells were diluted 1:10 in a solution of 2.5% trypan blue dye (Sigma), and 1 mm^3 was counted under a light microscope. Cells that took up the dye were considered dead. Cytotoxicity was determined as the percentage of dead cells in drug-treated cells compared with control (nontreated) cells. The cytotoxicity assay system used for undifferentiated HL-60 cells was described previously.[4,27] 1-*O*-Octadecyl-2-*O*-methyl-GPC (Figure 4) was obtained from Sigma. The PAF analogues were incubated with the HL-60 cells for 24 h.

Chemical Syntheses. 1-*O*-Hexadecyl-2-*O*-methyl-*sn*-glycero-3-*S*-D-1′-thioglucopyranoside 2,3,4,6-Tetra-*O*-acetate (Ca. 3:1 Mixture of α [8] and β [9] Anomers) (X = S, R = Ac). To a mixture of 220 mg (0.46 mmol) of 1-*O*-hexadecyl-2-*O*-methyl-*sn*-glycerol 3-*p*-toluenesulfonate (**6**) and 182 mg (0.5 mmol) of 2,3,4,6-tetra-*O*-acetyl-1-mercapto-β-D-glucopyranose (**7**) in 4 mL of dry tetrahydrofuran was added 75 μL (0.5 mmol) of DBU. The mixture was stirred under nitrogen at room temperature for 18 h; TLC (hexane–ethyl acetate 4:1) indicated that all of the starting tosylate had been consumed. The solvent was evaporated under reduced pressure, giving a brown residue that was purified by flash chromatography (hexane–ethyl acetate 4:1). There was obtained 312 mg (92%) of a mixture of the α and β anomers, which were separated by flash chromatography (hexane–ethyl acetate 4:1) to give 234 mg (69%) of the α tetraacetate (**8**, R_f 0.38) and 75 mg (22%) of the β tetraacetate (**9**, R_f 0.27) as pale yellow oils. IR (neat): 2919, 2849, 1749, 1461, 1367, 1224, 1113, 1090, 1037, 912 cm^{-1}. Anal. Calcd for $C_{34}H_{60}O_{11}S$: C, 60.33; H, 8.93; S, 4.74. Found: (α anomer **8**) C, 60.48; H, 8.86; S, 4.69; (β anomer **9**) C, 60.37; H, 8.91; S, 4.71.

^{1}H NMR (300 MHz, $CDCl_3$) α anomer **8**: δ 5.69 (d, J = 5.77 Hz, 1 H, glucosyl-$H_{1'}$), 5.36 (dd (apparent t), J = 9.98 Hz, J = 9.53 Hz, 1 H, glucosyl-$H_{3'}$), 5.03 (m, 2 H, glucosyl-$H_{2'}$ and $H_{4'}$), 4.41 (m, 1 H, glucosyl-$H_{5'}$), 4.29 (dd, $J_{H6'a-H5'}$ = 4.55 Hz, $J_{H6'a-H6'b}$ = 12.33 Hz, glucosyl-$H_{6'a}$), 4.07 (dd, $J_{H6'b-H5'}$ = 1.85 Hz, $J_{H6'a-H6'b}$ = 12.33 Hz, glucosyl-$H_{6'b}$), 3.38–3.57 (m, 8 H, with singlet at δ 3.41 (3 H), $CH_2OCH_2C_{15}H_{31}$, CH_3OCH), 2.60–2.87 (distorted AB quartet, 2 H, CH_2S), 2.09 (s, 3 H, OAc), 2.05 (s, 3 H, OAc), 2.03 (s, 3 H, OAc), 2.01 (s, 3 H, OAc), 1.55 (br t, 2 H, $OCH_2CH_2C_{14}H_{29}$), 1.26 (br s, 26 H, $(CH_2)_{13}$), 0.89 (br t, 3 H, ω-CH_3).

^{1}H NMR (300 MHz, $CDCl_3$) β anomer **9**: δ 5.21 (dd (apparent t), J = 9.32 Hz, J = 9.27 Hz, 1 H, glucosyl-$H_{3'}$), 5.08 (dd (apparent t), J = 9.82 Hz, J = 9.20 Hz, 1 H, glucosyl-$H_{4'}$), 5.01 (dd (apparent t), J = 9.61 Hz, J = 9.37 Hz, glucosyl-$H_{2'}$), 4.63 (d, J = 10.08 Hz, 1 H, glucosyl-$H_{1'}$), 4.25 (dd, $J_{H6'a-H5'}$ = 4.84 Hz, $J_{H6'a-H6'b}$ = 12.04 Hz, glucosyl-$H_{6'a}$), 4.12 (dd, $J_{H6'b-H5'}$ = 2.06 Hz, $J_{H6'a-H6'b}$ = 12.04 Hz, glucosyl-$H_{6'b}$), 3.69 (m, 1 H, glucosyl-$H_{5'}$), 3.36–3.55 (m, 8 H, with singlet at δ 3.42 (3 H), $CH_2OCH_2C_{15}H_{31}$, CH_3OCH), 2.75–2.92 (distorted AB quartet, 2 H, CH_2S), 2.09 (s, 3 H, OAc), 2.05 (s, 3 H, OAc), 2.03 (s, 3 H, OAc), 2.01 (s, 3 H, OAc), 1.56 (br t, 2 H, $OCH_2CH_2C_{14}H_{29}$), 1.26 (br s, 26 H, $(CH_2)_{13}$), 0.89 (br t, 3 H, ω-CH_3).

1-*O*-Hexadecyl-2-*O*-methyl-3-*S*-(α-D-1′-thioglucopyranosyl)-*sn*-glycerol (2, X = S, R = H). The tetra-*O*-acetate of the α anomer **8** was hydrolyzed by adding 4.5 mg (0.03 mmol) of barium oxide to a solution of 102 mg (0.15 mmol) of the tetraacetate in 4 mL of dry methanol. After the mixture had stirred for 24 h at room temperature under nitrogen, Dowex 50W-X8 (H^+ form) was added, and stirring was continued for an additional 2 h (during which period the solution became clear). The mixture was filtered to remove the ion-exchange resin, which was washed with 20 mL of THF. The filtrate was dried over sodium bicarbonate to remove traces of acid. After filtration, the solvents were removed under reduced pressure to give 75 mg (100%) of the product as white crystals: mp ~210 °C dec; R_f 0.45 (chloroform–methanol 85:15); $[\alpha]^{25}_D$ 122.4° (*c* 1.06, THF), 119.8° (*c* 0.45, chloroform–methanol 1:1); IR (KBr) 3359, 2919, 2849, 1407, 1365, 1223, 1112, 1090, 1037, 913 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.40 (d, J = 5.31 Hz, 1 H, glucosyl-$H_{1'}$), 3.41–4.0 (m, 14 H, with a singlet (OCH_3) at δ 3.44), 2.74 (m, 2 H, CH_2S-glucose), 1.53 (br t, 2 H, $OCH_2CH_2C_{14}H_{29}$), 1.26 (br s, 26 H), 0.89 (br t, 3 H). Anal. Calcd for $C_{26}H_{52}O_7S$: C, 61.38; H, 10.30. Found: C, 61.17; H, 10.21.

1-*O*-Hexadecyl-2-*O*-methyl-3-*S*-(β-D-1′-thioglucopyranosyl)-*sn*-glycerol (3, X = S, R = H). The tetra-*O*-acetate of the β anomer **9** was hydrolyzed as described above for the α anomer: R_f 0.30 (chloroform–methanol 85:15); $[\alpha]^{25}_D$ 14.4° (*c* 0.125, THF), 45.52° (*c* 0.125, chloroform–methanol, 1:1); IR (KBr) identical with that of **2**; ^{1}H NMR (200 MHz, $CDCl_3$) δ 4.45 (d, J = 10.16 Hz, 1 H, glucosyl-$H_{1'}$), 3.33–3.92 (m, 14 H, with a singlet (OCH_3) at δ 3.46), 2.76 (m, 2 H, CH_2S-glucose), 1.58 (br t, 2 H, OCH_2CH_2), 1.26 (br s, 26 H), 0.89 (br t, 3 H).

1-*O*-Hexadecyl-2-*O*-methyl-*sn*-glycero-3-*O*-β-D-glucopyranoside 2,3,4,6-Tetraacetate (12, X = O, R = Ac). A mixture of 330 mg (1.0 mmol) of 1-*O*-hexadecyl-2-*O*-methyl-*sn*-glycerol (**11**), 495 mg (1.2 mmol) of acetobromoglucose, and 308 mg (1.2 mmol) of mercuric cyanide in 10 mL of dry benzene–nitromethane (1:1) was stirred under nitrogen at 80 °C for 8 h. Additional amounts of mercuric cyanide (156 mg, 0.6 mmol) and acetobromoglucose (248 mg, 0.6 mmol) were added, and the mixture was stirred for an additionl 16 h. After cooling to room temperature, the mixture was diluted with ether (50 mL) to precipitate the mercury salts and then filtered through a Celite pad, which was washed with 100 mL of ether. The filtrate was washed twice with 10% aqueous sodium bicarbonate solution (75 mL), brine (75 mL), and water (75 mL). The ether layer was dried over Na_2SO_4, and the solvent was removed under reduced pressure. The residue was purified by flash chromatography (elution with

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hexane-ethyl acetate 2:1) to give 560 mg (85%) of the product as white crystals: mp 61-62 °C (lit.[1b] mp 61-62 °C); R_f 0.41 (hexane-ethyl acetate 2:1); $[\alpha]^{25}_D$ -13.56° (c 1.0, chloroform-methanol 1:1); IR (CCl_4) 2919, 2849, 1749, 1367, 1224, 1118, 1090, 1038, 911 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 5.24 (dd (apparent t), J = 9.42 Hz, J = 9.39 Hz, 1 H, glucosyl-$H_{3'}$), 5.12 (dd (apparent t), J = 9.76 Hz, J = 9.43 Hz, 1 H, glucosyl-$H_{4'}$), 5.01 (dd, $J_{H2'-H3'}$ = 9.42 Hz, $J_{H1'-H2'}$ = 7.98 Hz, 1 H, glucosyl-$H_{2'}$), 4.59 (d, J_{H1-H2} = 7.91 Hz, 1 H, glucosyl-$H_{1'}$), 4.31 (dd, $J_{H6'-H6'b}$ = 12.6 Hz, $J_{H6'a-H5'}$ = 4.75 Hz, 1 H, glucosyl-$H_{6'a}$), 4.17 (dd, $J_{H6'a-H6'b}$ = 12.6 Hz, $J_{H6'b-H5'}$ = 2.30 Hz, 1 H, glucosyl-$H_{6'b}$), 3.93 (dd, $J_{H1'-H3a}$ = 3.62 Hz, $J_{H3a-H3b}$ = 9.65 Hz, 1 H, C_3-H_a), 3.74 (m, 1 H, glucosyl-$H_{5'}$), 3.68 (dd, $J_{H1'-H3b}$ = 4.41 Hz, $J_{H3a-H3b}$ = ~9.6 Hz, 1 H, C_3-H_b), 3.41-3.58 (m, 8 H, with a singlet at δ 3.46, $CH_2OCH_2C_{15}H_{31}$, CH_3OCH), 2.09 (s, 3 H, OAc), 2.05 (s, 3 H, OAc), 2.03 (s, 3 H, OAc), 2.01 (s, 3 H, OAc), 1.56 (br t, 2 H, $OCH_2CH_2C_{14}H_{29}$), 1.26 (br s, 26 H, $(CH_2)_{13}$), 0.88 (br t, J = 7.8 Hz, 3 H).

3-*O*-Hexadecyl-2-*O*-methyl-*sn*-glycero-1-*O*-β-D-glucopyranoside 2,3,4,6-Tetraacetate (12′, X = O, R = Ac). This compound was prepared in 87% yield by the same procedure as described above: mp 56-58 °C [lit.[1b] mp of racemate at C-2 52-54 °C]; R_f 0.41 (hexane-ethyl acetate 2:1); $[\alpha]^{25}_D$ -6.33° (c 1.0, chloroform-methanol 1:1); ^1H NMR (300 MHz, $CDCl_3$) same as its C-2 enantiomer in the region δ 4.17-5.24; different at δ 3.98 (dd, $J_{H1'-H1b}$ = 3.65 Hz, $J_{H1a-H1b}$ = 9.71 Hz, 1 H, C_1-H_a), 3.74 (m, 1 H, glucosyl-$H_{5'}$), 3.61 (dd, $J_{H1'-H1b}$ = 4.45 Hz, J_{H1a-Hb} = 9.7 Hz, C_1-H_b).

1-*O*-Hexadecyl-2-*O*-methyl-3-*O*-(β-D-glucopyranosyl)-*sn*-glycerol (1, X = O, R = H). The hydrolysis procedure of Weber and Benning[1b] was used without modification: yield, 100%; mp 200 °C dec; $[\alpha]^{25}_D$ -11.9° (c 1.0, chloroform-methanol 1:1); lit.[1b] $[\alpha]^{20}_D$ -11° (c 1.0, chloroform-methanol 1:1); IR (KBr) 3359, 2919, 2849, 1112, 1090, 1037, 913 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 5.40 (d, 1 H, J = 5.31 Hz), 3.41-3.8 (m with a singlet at δ 3.44, 17 H), 1.55 (br t, 2 H), 1.26 (br s, 26 H, $(CH_2)_{14}CH_3$), 0.89 (br t, 3 H, ω-CH_3).

3-*O*-Hexadecyl-2-*O*-methyl-1-*O*-(β-D-glucopyranosyl)-*sn*-glycerol (1′, X = O, R = H). This compound was prepared as described above: $[\alpha]^{25}_D$ -7.30° (c 1.0, chloroform-methanol 1:1); ^1H NMR (200 MHz, $CDCl_3$) essentially identical with that of 1, except singlet at δ 3.46.

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Synthesis and in Vitro LTD_4 Antagonist Activity of Bicyclic and Monocyclic Cyclopentylurethane and Cyclopentylacetamide *N*-Arylsulfonyl Amides

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The dissociation constants (K_B) at the LTD_4 receptor on guinea pig trachea of a series of monocyclic and bicyclic cyclopentylurethane and cyclopentylacetamide *N*-arylsulfonyl amides have been measured. The K_B was found to be remarkably tolerant of changes in the electronic constitution and lipophilicity of the bicyclic ring system (template). Thus, *N*-[4[[6-[[(cyclopentyloxy)carbonyl]amino]benzimidazol-1-yl]methyl]-3-methoxybenzoyl]benzenesulfonamide (**11a**) and *N*-[4-[[5-[[(cyclopentyloxy)carbonyl]amino]benzo[*b*]thien-3-yl]methyl]-3-methoxybenzoyl]benzenesulfonamide (**25a**) had closely similar affinities (pK_B, 9.20 and 9.31, respectively; LTE_4 as agonist). It has been shown that the hetero-ring of the template need not be aromatic in order to achieve high affinity, since indoline **31** and 2,3-dihydrobenz-1,4-oxazines **37a-c** had pK_Bs > 9. Further, it has been shown that an *o*-aminophenone (see **42** and Figure 3) can function as a template; the template in **42** [see iii] is bicyclic by virtue of the presence of an intramolecular hydrogen bond. In contrast, when the template is a phenyl ring (**48**), receptor affinity is markedly reduced. These findings support the notion that central bicyclic ring system in this family of peptidoleukotriene antagonists is a molecular feature which helps to preorganize the acylamino and acidic chains and thereby facilitate the molecular recognition event.

Previous papers from these laboratories have described the discovery and some aspects of structure/activity relationships of a novel family of leukotriene (LT) D_4 antagonists.[1] Two series of indoles and two series of indazoles were highlighted in those reports (Figure 1): cyclopentylurethane and cyclopentylacetamide indole arylsulfonyl amides (**1**[1a,b] and **3**[1c]), and the corresponding indazole arylsulfonyl amides (**2**[1a,b] and **4**[1c]), where the group R in **3** and **4** was frequently a methyl group.

The series of compounds **1-4** were shown to contain selective LTD_4 antagonists of unusually high affinity, dissociation constants for the most part being in the range 10^{-9}-10^{-11} M against LTE_4 on isolated guinea pig trachea. In addition, many of the compounds were found to be orally effective in blocking leukotriene-induced dyspnea in guinea pigs. *N*-Methylindole ICI 204,219[1c,2] (a compound belonging to series **3**) was a product of those investigations, and is currently under clinical evaluation for asthma.

The previous studies demonstrated that the bicyclic ring system (the template) in this family of molecules could be modified in specific ways which were compatible with effective receptor recognition. In a considerable extension of that work, the present paper describes modification of

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