Notes

Enantioselective Synthesis and Antiproliferative Properties of an Ilmofosine Analog, 2′-(Trimethylammonio)ethyl 3-(Hexadecyloxy)-2-(methoxymethyl)propyl Phosphate, on Epithelial Cancer Cell Growth

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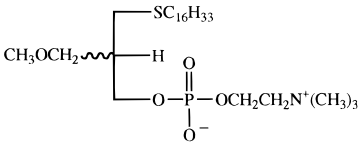
Received February 29, 1996⊗

An asymmetric synthesis of the 1-alkyloxy analog of the thioether phosphocholine ilmofosine (BM 41.440, *rac*-**1**), 2′-(trimethylammonio)ethyl 3-(hexadecyloxy)-2-(methoxymethyl)propyl phosphate (**2**), is described. Stereoselectivity was obtained in an asymmetric hydroboration−oxidation sequence carried out on a 2,2-disubstituted 1-alkene, 3-(hexadecyloxy)-2-(methoxymethyl)-1-propene (**9**), which was prepared by starting with either ethyl acrylate or ethyl α-(hydroxymethyl)acrylate (**3**). (*R*)- and (*S*)-**2** and *rac*-**1** were highly effective in inhibiting the proliferation of the breast adenocarcinoma cell line MCF-7 (IC_{50}, 2 μM), moderately effective against A549 (non-small-cell lung adenocarcinoma) (IC_{50}, 8−10 μM), and less effective against A427 (large cell lung carcinoma) (IC_{50}, ∼20 μM). The in vitro cytotoxicity against the three epithelial cancer cell lines was independent of the configuration about C-2 of the glycerol backbone of **2** and was also not altered by substitution of oxygen for sulfur in the *sn*-1 ether linkage of ilmofosine.

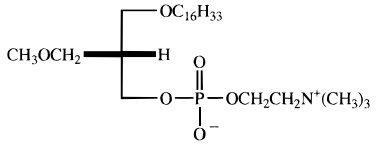
Introduction

Ilmofosine (*rac*-**1**, 2′-(trimethylammonio)ethyl 3-(hexadecylthio)-2-(methoxymethyl)propyl phosphate, BM 41.440) is an experimental anticancer drug with activity against a variety of malignant cell types of murine and human origin.[1−4] The antineoplastic action of the synthetic ether phospholipids is as yet not fully established, but it is membrane mediated. Interference in cell signaling pathways is clearly an important aspect of the mechanism of action.[5] Although *rac*-**1** inhibits protein kinase C,[6] its antineoplastic effects may arise from its ability to suppress the cyclin-dependent cdc2 kinase.[7] *rac*-**1** blocks the accumulation of cyclin B1/cdc2 complexes and arrests human lymphoma cells in the G2 phase of the cell cycle.[7] *rac*-**1** also possesses antileishmanial activity.[8]



***rac*-1** (ilmofosine, BM 41.440)

Thioether phospholipid *rac*-**1** was synthesized by a Boehringer-Mannheim group in a long route with low overall yield, starting with diethyl bis(hydroxymethyl)-malonate.[9] We recently communicated an improved synthesis of *rac*-**1** from ethyl α-(hydroxymethyl)acrylate (**3**) in good overall yield.[10] We report here an asymmetric synthesis of (*R*)- and (*S*)-**2**, the enantiomers of the oxygen analog of ilmofosine; the synthesis of *rac*-**2** from 2-methoxy-1,3-propanediol was reported previously.[11] There have been conflicting reports about the role of chirality in the in vitro antitumor activity of various ether lipids (e.g., ref 12), yet ilmofosine is used as the racemic form in clinical studies. The enantiomers of **2** have essentially the same in vitro cytotoxicity against the three epithelial cancer cell lines we tested, suggesting a lack of stereoselective interactions in vitro. (*R*)- and (*S*)-**2**, like the thioether phosphocholine *rac*-**1**, were highly effective in inhibiting the proliferation of MCF-7 cells.



(*R*)-2

Results and Discussion

Chemistry. (*R*)- and (*S*)-**2** were prepared by using two routes. One route used 2-methylene-1,3-propanediol (**5**) as the key intermediate (Scheme 1). As shown in Scheme 1, **5** was made from ethyl acrylate by a coupling reaction with formaldehyde catalyzed by DABCO in THF, giving ethyl α-(hydroxymethyl)acrylate (**3**).[10] Reduction of **3** with 1 equiv of AlH_3, which was generated *in situ* by the reaction of $LiAlH_4$ with *n*-butyl bromide,[13] is assumed to give aldehyde **4**. The latter was reduced without isolation with $NaBH_4$ to give 2-methylene-1,3-propanediol (**5**) in 57% overall yield from **3**. Mono-*O*-alkylation of diol **5** via a 1,3-cyclic stannoxane intermediate[14] in chloroform/methanol (10:

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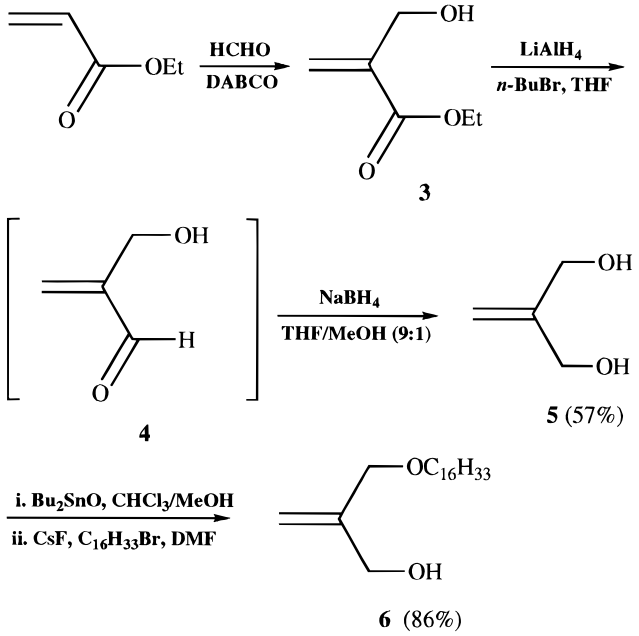
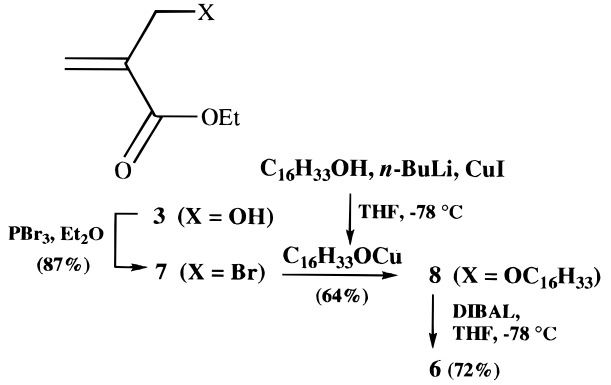
⊗ Abstract published in *Advance ACS Abstracts,* April 1, 1997.

S0022-2623(96)00165-3 CCC: $14.00

Scheme 1. Synthesis of **6** from Ethyl Acrylate via 2-Methylene-1,3-propanediol (**5**)



Scheme 2. Synthesis of **6** from Ethyl α-(Hydroxymethyl)acrylate (**3**) via Ethyl α-(Bromomethyl)acrylate (**7**)



1), followed by treatment with cesium fluoride and 1-bromohexadecane in DMF, gave 3-(hexadecyloxy)-2-(hydroxymethyl)-1-propene (**6**) in 86% overall yield.

In the other route, ethyl α-(hydroxymethyl)acrylate (**3**) was used as the starting material. Scheme 2 outlines the conversion of **3** into **6**. Ethyl α-(bromomethyl)acrylate (**7**) was prepared from **3** as described previously.[15] The reaction of bromide **7** with hexadecyloxycopper(I), generated by the reaction of the lithium salt of 1-hexadecanol with cuprous iodide in THF at −23 °C, provided ethyl 2-[(hexadecyloxy)methyl]propenoate (**8**) in 64% yield. Reduction of ester **8** with DIBAL in THF at −78 °C gave alcohol **6** in 72% yield.

The enantioselective synthesis of **2** was completed as shown in Scheme 3. After the hydroxy group of **6** was methylated by using sodium hydride in THF in the presence of a phase transfer catalyst to give 3-(hexadecyloxy)-2-(methoxymethyl)-1-propene (**9**), asymmetric hydroboration was carried out with (+)-diisopinylcampheylborane ((+)-Ipc_2BH)[16,17] followed by oxidation of the intermediate borane with alkaline hydrogen peroxide to give (*R*)-**10** in 94% yield. Analysis of the (*R*)-(+)-α-methoxy-α-(trifluoromethyl)phenylacetic acid (MTPA) ester derivative of (*R*)-**10** by chiral HPLC analysis

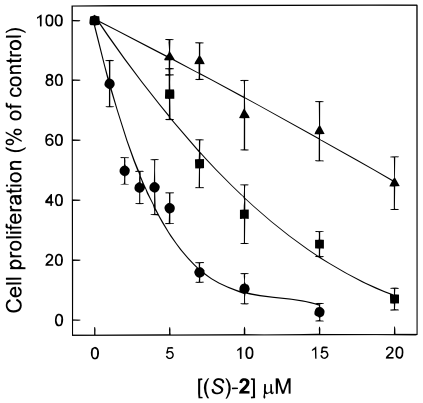
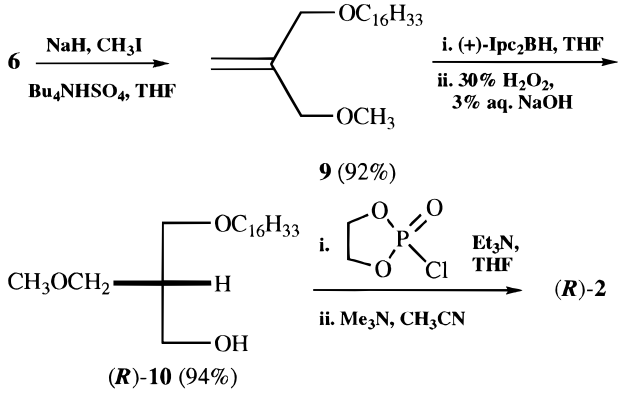


Figure 1. Effect of (*S*)-**2** on the proliferation of MCF-7 (●), A549 (■), and A427 (▲) cells. Cells were treated with the drug for 48 h. The increase in cell number after 48 h was determined and expressed as a percent of controls. The results are the means ± SD of eight different determinations.

Scheme 3. Synthesis of (*R*)-**2** via Asymmetric Hydroboration−Oxidation



indicated that the enantiomeric excess (ee) of **10** was 84%. Although asymmetric hydroboration of prochiral 2-substituted 1-alkenes generally proceeds in low ee,[17,18] there is a precedent for a large difference in size,[19] such as that between the hexadecyloxy and methoxy groups of the 2,2-disubstituted 1-alkene **9**, giving rise to a high level of asymmetric induction in this reaction. The insertion of the phosphocholine group into alcohol **10** was accomplished by using 2-chloro-1,3,2-dioxaphospholane 2-oxide, followed by ring opening of the intermediate cyclic phospholane with trimethylamine in a pressure tube, as described elsewhere,[20] to give the desired product (*R*)-**2**. (*S*)-**2** was synthesized in analogous fashion, using (−)-Ipc_2BH, which was prepared from (+)-α-pinene.

Antiproliferative Properties of (*R*)-2 and (*S*)-2 and Comparison with *rac*-1 Activity. The stereochemical requirements about C-2 of the glycerol backbone of various ether lipids for optimal antitumor or antiviral activity has been probed in previous in vitro studies. There are conflicting reports about the role of chirality of ether phospholipids in antineoplastic activity.[21,22] In several closely related antitumor ether lipids, no marked dependence on phospholipid chirality was noted in vitro.[12,23−26] However, it was recently reported that the *S* enantiomer of SRI 62-834 was somewhat more active than the *R* enantiomer against various murine tumor cell lines.[21] Further, stereoselectivity was detected in the antiviral activity of ether−lipid−3′-azido-3′-deoxythymidine conjugates in HIV-infected human

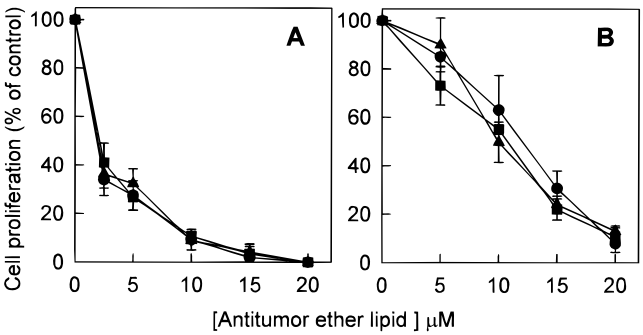


Figure 2. Effects of *rac*-**1** (▲), (*S*)-**2** (●), and (*R*)-**2** (■) on (A) MCF-7 cell proliferation and (B) A549 cell proliferation. See caption to Figure 1 for experimental details.

T-lymphocyte cell cultures.[27] In an extension of these studies, we probed the cytotoxicity of (*R*)- and (*S*)-**2** (see the Experimental Section). Figure 1 shows the antiproliferative effects of (*S*)-**2** on various epithelial cell lines after a 48-h treatment. The relative order of selectivity against these cell lines was MCF-7 > A549 > A427 (IC_{50} values of 2.0, 7.5, and 20 μM, respectively). The cytotoxicity profile of (*R*)-**2** was very similar (not shown) to that of (*S*)-**2**, with IC_{50} values of 2.0, 10, and $\geq$20 μM, respectively, against MCF-7, A549, and A427 cells. The enantiomers of ilmofosine analog **2** have the same cytotoxicity as *rac*-**1** against MCF-7 and A549 cells (Figure 2).

At the concentrations of drugs we examined, the viability of A549 and A427 cells, as assessed by trypan blue exclusion, did not differ significantly in the drug-treated vs untreated cells. With MCF-7 cells, drug concentrations > 15 μM resulted in significantly lower viability relative to untreated controls.

Replacement of an oxygen in the glycerol backbone by sulfur is one of the many chemical modifications that have been introduced in ether phospholipid analogs in efforts to improve the therapeutic efficiency. Substitution of a 1-alkylthio chain, as in **1**, for the 1-alkyloxy chain found in ET18-OCH_3 or in the anti-HIV nucleoside ether phospholipid conjugates has been reported in many cases to either increase[28] or not change[26] the cytotoxicity of the ether lipid. We found that replacing the sulfur of **1** by oxygen did not alter the cytotoxicity against the three epithelial cancer cell lines.

Conclusion

(*R*)- and (*S*)-**2** are enantiomers of an *O*-alkyl analog of thioether **1**. They were prepared in a short synthetic route, starting with either ethyl acrylate or ethyl α-(hydroxymethyl)acrylate (**3**), in which the key step is asymmetric hydroboration of the prochiral alkene **9** with Ipc_2BH followed by oxidation. No marked dependence on chirality was found in inhibition of proliferation of three epithelial cancer cell lines. (*R*)-**2**, (*S*)-**2**, and *rac*-**1** have high cytotoxicity in vitro against the breast cancer cell line MCF-7 (IC_{50}, 2 μM). The three compounds had a similar potency with respect to inhibition of A549 (non-small-cell lung adenocarcinoma) proliferation (IC_{50} values of 8−10 μM) and were less effective against A427 cells (large cell lung carcinoma) (IC_{50} values of ~20 μM).

Experimental Section

3-(Hexadecyloxy)-2-(hydroxymethyl)-1-propene (6). This compound was prepared by two different procedures. **Procedure A.** A mixture of **5** (4.0 g, 45 mmol) and di-*n*-butyltin oxide (13.96 g, 56 mmol) in 60 mL of $CHCl_3$/MeOH (10:1) was refluxed until the solids were dissolved. The solvents were evaporated under vacuum, and 14.71 g (97 mmol) of CsF was added to the residue. After the mixture was further dried at room temperature under vacuum for 2 h, 50 mL of dry DMF was added. The mixture was stirred at 40 °C for 30 min and then cooled to room temperature. 1-Bromohexadecane (17.13 g, 56 mmol) was added over a 10-min period. After the mixture was stirred for 36 h, water (2 mL) and ether (100 mL) were added, with stirring for 30 min. The solids were filtered through a pad of Celite, which was washed with Et_2O (100 mL). The combined filtrate was washed with brine (20 mL × 2); the ether layer was dried (Na_2SO_4), concentrated, and purified by silica gel chromatography (elution with hexane:EtOAc, 9:1) to give 12.2 g (86%) of the monoalkylation product **6** as a low-melting solid: R_f 0.70 (hexane:EtOAc, 3:1).

Procedure B. To a stirred solution of 1.92 g (5.4 mmol) of **8** in 40 mL of dry toluene under nitrogen was added 8.0 mL (12 mmol) of DIBAL (a 1.5 M solution in toluene) slowly via cannula so as to keep the temperature of the reaction mixture below −70 °C. The reaction mixture was stirred for an additional 5 h at −78 °C; then the reaction was quenched by the slow addition of MeOH (10 mL). The resulting emulsion was poured slowly into 30 mL of 1 N HCl over 15 min, and the product was extracted with EtOAc (50 mL × 3). The combined organic layers were washed with brine, dried (Na_2SO_4), filtered, and concentrated under reduced pressure to give the crude product as a semisolid. Purification by silica gel chromatography (elution with hexane:EtOAc, 9:1) gave 1.25 g (73%) of **6**: mp 45−46 °C; same R_f, IR, and ^1H NMR as **6** prepared as above.

3-(Hexadecyloxy)-2-(methoxymethyl)-1-propene (9). To a suspension of 0.29 g (10 mmol, 80% in white oil, washed with dry hexane twice) of NaH in 30 mL of dry THF was added a solution of 1.00 g (3.2 mmol) of **6** in 2 mL of THF at room temperature under nitrogen. After the evolution of hydrogen had stopped, 0.70 mL (11.2 mmol) of MeI and 53.4 mg (0.16 mmol) of *n*-Bu_4NHSO_4 were added, and the reaction mixture was stirred for 10 h at room temperature. Ethanol (1 mL) and water (1 mL) were added, the solvents were removed under vacuum, and the residue was extracted with hexane (50 mL). The organic layer was washed with brine (20 mL), dried (Na_2SO_4), and concentrated. The residue was purified by silica gel chromatography (elution with hexane), yielding 960 mg (92%) of **9** as a colorless oil: R_f 0.60 (hexane:EtOAc, 9:1).

3-(Hexadecyloxy)-2(*R*)-2-(methoxymethyl)-1-propanol ((*R*)-10). To a stirred solution of (−)-α-pinene (1.44 g, 10.6 mmol) in 0.70 mL of THF was added 0.50 mL (5.6 mmol) of borane−methyl sulfide complex in THF dropwise at −10 °C over a 10-min period under nitrogen. After the mixture was stirred for 1.5 h at 0 °C, (+)-Ipc_2BH started to crystallize. The resulting slurry was stored without stirring under nitrogen at −4 °C for 3 days and then washed with cold dry hexane (10 mL × 2) under nitrogen. The white solid of (+)-Ipc_2BH was suspended in 10 mL of THF and cooled to −25 °C, 0.82 g (2.51 mmol) of **9** was added, and the mixture was stirred for 6 h at −25 °C and then warmed to room temperature. Methanol (20 mL) was added to the suspension followed by 5 mL of 3 N NaOH and 8 mL of 30% H_2O_2. After the reaction mixture was stirred at room temperature for 5 h, the product was extracted with ether (30 mL × 3). The combined ether extract was washed with brine (10 mL × 2), dried (Na_2SO_4), and concentrated. The residue was purified by silica gel chromatography (elution with hexane:EtOAc, 9:1) to give 0.82 g (94%) of (*R*)-**10** as a white solid: mp 46−47 °C; R_f 0.47 (hexane:EtOAc, 3:1); 84% ee. The % ee was estimated by chiral HPLC (Pirkle type IA column, 4.6 × 250 mm, J. T. Baker) of the crude (*R*)-(+)-MTPA ester derived from (*R*)-**10**: t_R 39.6, 41.2 min; elution with hexane/2-PrOH, 99:1; flow rate 0.3 mL/min. The MTPA ester was prepared as described previously.[29]

2′-(Trimethylammonio)ethyl 3-(Hexadecyloxy)-2(*R*)-2-(methoxymethyl)propyl Phosphate ((*R*)-2). To a solution of 55 mg (0.16 mmol) of (*R*)-**10** and 51 mg (0.50 mmol) of Et_3N in 20 mL of THF was added slowly a solution of 2-chloro-1,3,2-dioxaphospholane 2-oxide (44 μL, 0.49 mmol) in THF at 0 °C.

The reaction mixture was stirred at 0 °C for 30 min and then at room temperature for 5 h. The cyclic phosphate was opened with anhydrous Me_3N (1 mL) in dry CH_3CN (10 mL) in a pressure bottle as described previously,[20,29] giving 46 mg (56%) of (*R*)-**2**: R_f 0.38 ($CHCl_3$:MeOH:H_2O, 65:25:4).

For the synthesis of 2′-(trimethylammonio)ethyl 3-(hexadecyloxy)-2(*S*)-2-(methoxymethyl)propyl phosphate ((*S*)-**2**), the enantiomer of Ipc_2BH (prepared from (+)-α-pinene) was used for the asymmetric hydroboration−oxidation of **9**. The % ee was 84, as estimated by chiral HPLC of the crude (*R*)-(+)-MTPA ester derived from (*S*)-**10**: t_R 40.6, 42.5 min; elution with hexane/2-PrOH, 99:1; flow rate 0.3 mL/min.

Ethyl 2-[(Hexadecyloxy)methyl]propenoate (8). To a solution of 5.02 g (20.7 mmol) of 1-hexadecanol in 120 mL of dry THF was added 8.2 mL (20.5 mmol) of *n*-butyllithium (a 2.5 M solution in hexane). After the mixture was stirred for 45 min at −23 °C under nitrogen, CuI (3.90 g, 20.5 mmol) was added, and the reaction mixture was stirred for 1 h. A solution of 2.0 g (10.4 mmol) of **7** in 10 mL of THF was added, and the reaction mixture was stirred at −23 °C for 4 h and at 0 °C for 20 h. After addition of water (20 mL), THF was evaporated under vacuum, and the product was extracted with CH_2Cl_2 (50 mL × 2). The organic layer was washed with 20% aqueous NH_4OH solution until the blue-green color persisted, washed with water (25 mL × 2), dried (Na_2SO_4), and concentrated. The residue was purified by silica gel chromatography (elution with petroleum ether) to yield 2.35 g (64%) of **8** as a white solid: mp 35−37 °C; R_f 0.78 (hexane:EtOAc, 9:1).

Antiproliferative Studies. The human epithelial cancer cell lines were obtained from ATCC and grown as described previously.[30] The cell number was monitored with a Coulter ZM counter. Log-phase cells were treated with medium containing (*R*)- or (*S*)-**2** or *rac*-**1** at the desired concentration for 48 h, and the cell number was compared with control cells grown in media that did not contain the drug. Stock solutions of the drugs (30 mM) were prepared in ethanol. Media containing the drug at 30 μM were prepared and serially diluted to give the required concentrations. The final concentration of ethanol in all wells was 0.1% (v/v). Cell viability was assessed by trypan blue dye exclusion.[31]

Acknowledgment. We are grateful to Dr. Dieter B. J. Herrmann, Boehringer-Mannheim GmbH, Mannheim, Germany, for supplying *rac*-**1**. This work was supported in part by a grant from the National Cancer Institute of Canada with funds from the Cancer Research Society to G.A. FAB-MS were recorded at the Michigan State University Mass Spectrometer Facility.

Supporting Information Available: Spectral data for compounds **2**, **6**, and **8**−**10** (3 pages). See any current masthead page for ordering information.

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JM960165B