

# High-Performance Liquid Chromatographic Separation of Enantiomeric Alkyl Glycerol Ethers<sup>1</sup>

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The resolution of a series of racemic 1-alkyl-3-trityl-*rac*-glycerols and 1-alkyl-2-benzyl-*rac*-glycerols into their enantiomers by high-performance liquid chromatography (HPLC) was investigated. Of the racemic glycerol derivatives studied, only three (1,2-*O*-isopropylidene-*rac*-glycerol, 1,2-*O*-isopropylidene-3-benzyl-*rac*-glycerol and 1-hexadecyl-2-benzyl-*rac*-glycerol) could be resolved into their enantiomers. The compounds were resolved on a silica (10  $\mu$ m) column coated with cellulose tribenzoate and eluted isocratically with hexane/isopropanol as mobile phase. The effects of mobile phase composition, column temperature and flow rate on the resolution of enantiomeric pairs were investigated. Also, the effect of substituents, *i.e.*, trityl, benzyl, hydroxyl or alkyl, on the resolution of the alkyl glycerol ethers by HPLC was evaluated. *Lipids* 26, 769-773 (1991).

enantiomers by HPLC, rather than to synthesize them by multistep stereospecific procedures. The enantiomeric resolution of diacylglycerols by HPLC has been reported previously (13) on other chiral liquid phases. However, such resolutions are accomplished after prior conversion of the diacylglycerols into urethane or naphthyl urea diastereomeric pairs.

In the present study, we investigated the use of HPLC for the chiral separation of glycerol ethers without derivatization. Several of the compounds are intermediates of PAF synthesis and of other biologically active ether lipids. Here we describe the enantiomer separation of some glycerol ethers by HPLC using a cellulose derivatized CSP column.

## MATERIALS AND METHODS

**Materials.** Racemic glycerol acetone 1 was obtained from Aldrich Chemical Co. (Milwaukee, WI). All other glycerol ether derivatives (compounds 2-12) (Fig. 1) were synthesized in our laboratory. 1-Bromohexadecane, benzyl chloride, trityl chloride, methyl iodide, and all catalysts used were obtained from Aldrich Chemical Co. n-Hexane and isopropanol used for HPLC separations were obtained from American Burdick & Jackson (Muskegon, MI).

**High-performance liquid chromatography.** The HPLC instrument used in this study was an HP 1090 Liquid Chromatograph (Hewlett-Packard, Avondale, PA) that included an ultraviolet detector. A Waters Differential Refractometer Model R 401 detector (Waters Associates, Milford, MA) was employed when necessary. The CSP HPLC column used was a 4.6 mm ID  $\times$  25 cm stainless steel type prepacked with 10  $\mu$ m silica coated with cellulose tribenzoate (Bakerbond Chiralcel OB, J.T. Baker, Phillipsburg, NJ). An analytical silica column (Zorbax SIL, 4.6 mm ID  $\times$  25 cm, 5-6  $\mu$ m, DuPont, Wilmington, DE) was used for determining purity of the glycerol ethers prepared. The integrator/recorder was a Chromatopac C-R3A System (Shimadzu, Columbia, MD). The samples were eluted isocratically with n-hexane/isopropanol varying in composition from 90:10 to 99:1 (v/v), at flow rates of 0.25 to 1 mL/min. Samples were dissolved in mobile phase (1% w/v; injection volumes for the chiral HPLC column were 20  $\mu$ L). Column temperature was 25  $\sim$  27°C, unless stated otherwise.

**Syntheses.** The glycerol ether derivatives used in this study were prepared using the procedures illustrated in Figure 1.

1-*O*-Hexadecyl-2,3-*O*-isopropylidene-*rac* glycerol 2 was synthesized by alkylation of 1 with 1-bromohexadecane in the presence of sodium hydride in dimethylformamide (4). 1-*O*-Hexadecyl-*rac*-glycerol 3 was obtained by hydrolysis of 2 with conc. HCl in methanol solution. Tritylation at the 3-position of 3 with trityl chloride in triethylamine gave 1-*O*-hexadecyl-3-*O*-trityl-*rac*-glycerol 4 (4). Benzoylation at the 2-position of 4 with benzyl chloride in the presence of sodium hydride gave 1-*O*-hexadecyl-2-*O*-benzyl-3-*O*-trityl-*rac*-glycerol 5 (5). 1-*O*-Hexadecyl-2-*O*-benzyl-*rac*-

1-*O*-Alkyl-2-acetyl-*sn*-glycero-3-phosphocholine (commonly referred to as platelet activating factor, PAF) and its analogs have been attracting considerable attention because of their role in initiating or mediating various biological processes (1). It also has been shown that there are distinct differences in the biological activity between the enantiomers of PAF and of its analogs (2,3). In order to prepare pure PAF enantiomers, several total synthetic methods, employing optically active starting materials, have been developed (3-6). However, such stereospecific syntheses usually require numerous steps, and overall yields are relatively low. Moreover, in some cases synthetic intermediates are labile and racemize quite readily. Thus, convenient methods for the preparation of pure enantiomeric ether lipids are urgently needed.

High-performance liquid chromatography (HPLC) technology for the chiral separation of enantiomers has undergone rapid development in recent years. Several types of chiral stationary phases (CSP) for HPLC separation are now available (7). Some of the more useful CSP are the cellulose derivatives on which various compounds have been separated successfully into pure enantiomers without prior derivatization (8-12). Moreover, even large scale chiral separations by HPLC appear feasible, since cellulose derivatized CSP columns are reported to have good stability and durability. Therefore, we considered that in preparing enantiomeric ether lipids, it may be easier to separate selected racemic intermediates into their

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Abbreviations:  $\alpha$ , Selectivity; CSP, chiral stationary phase; HPLC, high-performance liquid chromatography; IR, infrared;  $\kappa'$ , capacity factor; PAF, platelet activating factor; Rs, resolution.

## METHODS

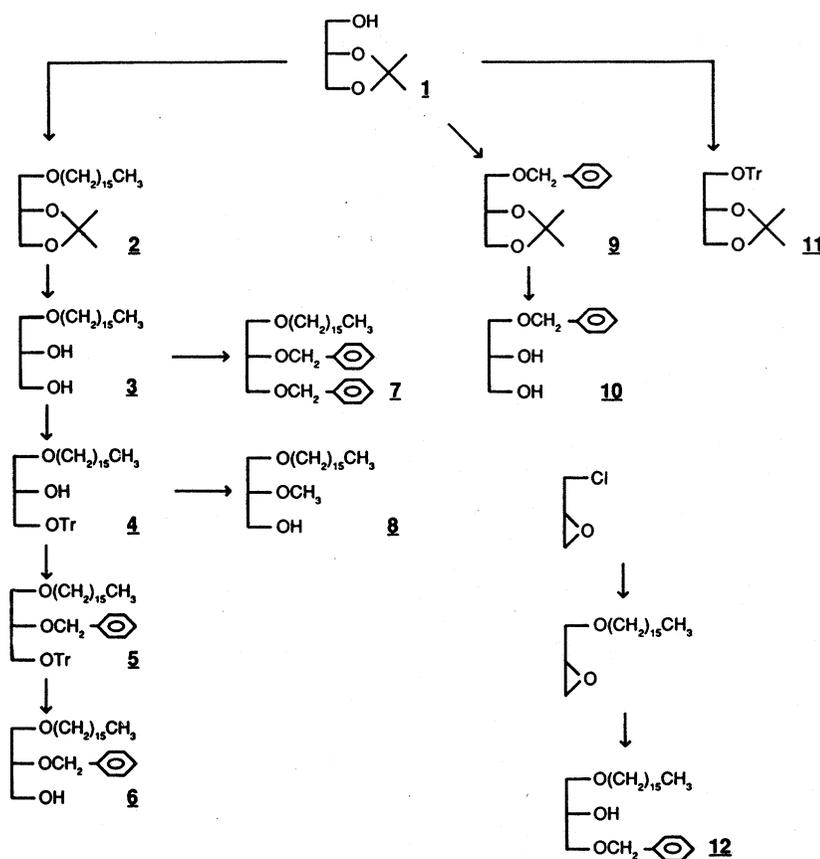


FIG. 1. Preparation of glycerol ethers.

glycerol 6 was prepared by detritylation of 5 with *p*-toluenesulfonic acid in methanol (6).

1-*O*-Hexadecyl-2-*O*-methyl-*rac*-glycerol 8 was obtained from 4 by methylation with methyl iodide in the presence of sodium hydride in dimethylformamide followed by detritylation (5). Benzoylation of 3 gave 1-*O*-hexadecyl-2,3-*O*-dibenzyl-*rac*-glycerol 7 (6).

1-*O*-Benzyl-2,3-*O*-isopropylidene-*rac*-glycerol 9 was prepared from 1 by benzoylation; hydrolysis of 9 with conc. HCl in methanol at 0°C gave 1-*O*-benzyl-*rac* glycerol 10 (5). 1-*O*-Trityl-2,3-*O*-isopropylidene-*rac*-glycerol 11 was obtained by tritylation of 1 (5).

1-*O*-Hexadecyl-3-*O*-benzyl-*rac*-glycerol 12 was prepared from epichlorohydrin and 1-hexadecanol in the presence of sodium hydride in dimethylformamide, followed by selective reaction at the 3-position with benzyl alcohol in the presence of sodium hydride in dimethylformamide (6).

All glycerol ether derivatives were identified by infrared (IR) and <sup>13</sup>C nuclear magnetic resonance (NMR) spectroscopy, and purity was confirmed by HPLC with the analytical silica column (Table 1).

## RESULTS AND DISCUSSION

**Enantiomer separation by CSP column.** Various chiral compounds have been reported to be separated into their enantiomers on the cellulose tribenzoate CSP column (Chiralcel OB). However, only three of the glycerol

derivatives (1, 6 and 9) which we prepared were separated into their enantiomers on the column used (Fig. 2).

Table 2 gives the chromatographic data for the enantiomeric separation of compounds 1, 6 and 9. Although elution times with lower polarity mobile phases were longer, better enantiomer separations were obtained. Racemic glycerol acetonide 1 was separated into its enantiomers, as previously reported (14). Good resolution (*R<sub>s</sub>* 1.24) was obtained with *n*-hexane/isopropanol (99:1, v/v). However, 1 may not be a good intermediate for enantiomer separation since the enantiomers tend to racemize in the process. 1-*O*-Benzyl-2,3-*O*-isopropylidene-*rac*-glycerol 9 was separated into its enantiomers. However, resolution (*R<sub>s</sub>*) was less than 0.8 under optimal conditions. 1-*O*-Hexadecyl-2-*O*-benzyl-*rac*-glycerol 6 was separable into its enantiomers although it has a long alkyl chain at the 1-position of the glycerol backbone. A desirable baseline resolution (*R<sub>s</sub>* 1.47) was obtained with *n*-hexane/isopropanol (99:1, v/v) as mobile phase. The observed order of elution, *R* enantiomers before *S* enantiomers, shown in Figure 2, is in agreement with work previously reported for compound 1 (14). Moreover, the order of elution for compounds 1 and 9 was confirmed by the direct preparation of the *R* enantiomer of 1 and the *S* enantiomer of 9.

We also checked the effect of mobile phase flow rate as well as column temperature on the enantiomer separation of 6 (Table 3). Column selectivities ( $\alpha$ ) were similar at all flow rates studied. However, enantiomer resolution (*R<sub>s</sub>*)

TABLE 1

## Characterization of Glycerol Derivatives 1-12

Glycerol derivative	IR (cm <sup>-1</sup> )	Purity (%) <sup>c</sup> HPLC	Retention time (min)
1	3580, 3460 (OH), 1380, 1370 (gem. dimethyl) 1210, 1160, 1050 (C-O-C)	100 <sup>d</sup>	11.0 <sup>g</sup>
2	1380, 1370 (gem. dimethyl) <sup>a</sup> 1110, 1050 (C-O-C)	100 <sup>e</sup>	8.6 <sup>f</sup>
3	3580, 3460 (OH), 1120 (C-O-C) <sup>b</sup>	100 <sup>d</sup>	10.8 <sup>g</sup>
4	3580 (OH) 3100 ~ 3000, 1495 (arom) <sup>a</sup> 1220, 1120, 1095 (C-O-C)	99 <sup>e</sup>	12.0 <sup>f</sup>
5	3100 ~ 3000, 1495 (arom) <sup>a</sup> 1220, 1120, 1095 (C-O-C)	95 <sup>e</sup>	8.3 <sup>f</sup>
6	3580 (OH) 3100 ~ 3000, 1495 (arom) <sup>a</sup> 1220, 1120, 1095 (C-O-C)	100 <sup>a</sup>	13.2 <sup>f</sup>
7	3100 ~ 3000, 1495 (arom) <sup>a</sup> 1180, 1100 (C-O-C)	83 <sup>a</sup>	8.5 <sup>f</sup>
8	3580 (OH) 1120, 1040 (C-O-C) <sup>a</sup>	100 <sup>d</sup>	7.9 <sup>g</sup>
9	3100 ~ 3000, 1495 (arom) <sup>a</sup> 1380, 1370 (gem. dimethyl) 1220, 1160, 1090 (C-O-C)	94 <sup>d</sup>	9.1 <sup>f</sup>
10	3400 (OH) 3200 ~ 3000, 1495 (arom) <sup>a</sup>	100 <sup>d</sup>	18.1 <sup>g</sup>
11	3100 ~ 3000, 1600, 1495 (arom) 1380, 1370 (gem. dimethyl) 1220, 1160, 1080 (C-O-C)	100 <sup>d</sup>	8.3 <sup>f</sup>
12	3580 (OH), 3200 ~ 3000, 1495 (arom) <sup>a</sup> 1120, 1095 (C-O-C)	94 <sup>e</sup>	8.4 <sup>g</sup>

<sup>a</sup>In CCl<sub>4</sub>.<sup>b</sup>In CHCl<sub>3</sub>.<sup>c</sup>Peak area (%) of HPLC with analytical silica column. Column: Zorbax Sil 4.6 mm ID × 25 cm, 5–6 μm (DuPont).<sup>d</sup>Mobile phase, n-hexane/isopropanol (90:10, v/v).<sup>e</sup>Mobile phase, n-hexane/isopropanol (90:2, v/v).<sup>f</sup>Flow rate, 0.5 mL/min.<sup>g</sup>Flow rate, 1.0 mL/min.

TABLE 2

## Enantiomer Separation of Glycerol Derivatives 1, 6 and 9

Glycerol derivative		Mobile phase (n-hexane/isopropanol)			Enantiomer elution order
		99:1	99:2	95:5	
1	$\alpha$ <sup>c</sup>	4.00	2.33	1.21	R,S
	$\alpha$ <sup>d</sup>	1.21	1.21	1.15	
	Rs <sup>e</sup>	1.24	1.04	0.57	
6 <sup>a</sup>	$\alpha$	1.00	0.52	0.30	R,S
	$\alpha$	1.46	1.34	1.21	
	Rs	1.47	0.95	0.54	
9 <sup>b</sup>	$\alpha$	3.37	2.77	2.26	R,S
	$\alpha$	1.21	1.14	1.08	
	Rs	0.75	0.48	0.42	

<sup>a</sup>Flow rate of mobile phase, 0.25 mL/min; column temperature, 25°C.<sup>b</sup>Flow rate of mobile phase, 0.5 mL/min; column temperature, 25°C.<sup>c</sup>Capacity factor of the first eluted enantiomer.<sup>d</sup>Column selectivity:  $\alpha = \alpha_1'/\alpha_1'$ , the ratio of the capacity factors.<sup>e</sup>Resolution:  $RS = 2(t_2 - t_1)/(wt_1 + wt_2)$ ; t, retention time; wt, peak width.

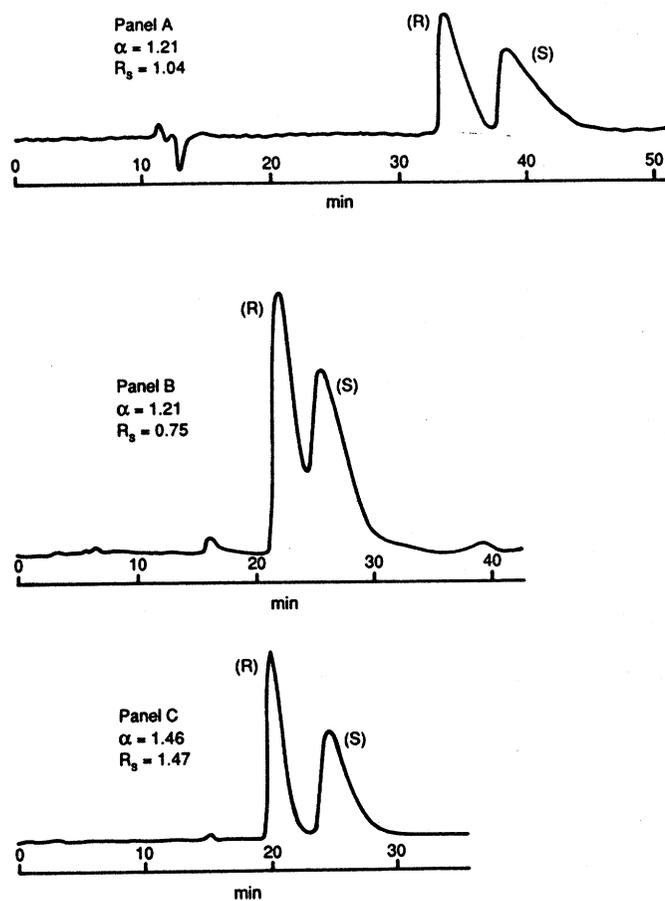


FIG. 2. Enantiomer separation of glycerol derivatives 1, 6 and 9 on Chiralcel OB column (4.6 × 250 mm). Panel A: compound 1, mobile phase, n-hexane/isopropanol (98:2, v/v); flow rate, 0.25 mL/min; RI detector. Panel B: compound 9; mobile phase n-hexane/isopropanol (99:1, v/v); flow rate, 0.5 mL/min; UV detector (254 nm). Panel C: compound 6; mobile phase, n-hexane/isopropanol (99:1, v/v) flow rate, 0.25 mL/min; UV detector (254 nm).

decreased at higher flow rates, presumably because of decreased column efficiency. No appreciable difference in  $R_s$  was observed between 25°C and 32°C. However, resolution decreased at 40°C. This may be attributed to a decrease in column efficiency at the higher column temperature. As a result of the above experiments, we concluded that in order to obtain good enantiomer separation, this particular CSP column should be used with a lower polarity solvent, at relatively low flow rate, and at, or near, room temperature (20–30°C).

**Structure of glycerol ethers and separation of enantiomers.** In the present study only three of the glycerol derivatives, 1, 6 and 9 were resolved into their enantiomers. Judging from the structure of compounds 1, 6 and 9, the combination of a hydroxy group, a benzyl group and an isopropylidene group seems to provide chiral recognition by CSP. The structures probably interact with CSP through hydrogen bonding and  $\pi$ - $\pi$  interaction that are also governed by steric effects. Furthermore, more than two of the three groups appear to be required for successful enantiomer separation. This seems plausible because multiple site interactions between the racemates

TABLE 3

Dependence of Enantiomer Separation of (6) on Flow Rate and Column Temperature

		$\alpha$	$R_s$
Flow rate <sup>a</sup>	0.25 mL/min	1.42	1.32
	0.5 mL/min	1.41	1.08
	1 mL/min	1.41	0.86
Column temperature <sup>b</sup>	25°C	1.46	1.48
	32°C	1.39	1.51
	40°C	1.32	1.17

<sup>a</sup>Mobile phase, n-hexane/isopropanol (99:1, v/v); column temperature, 25°C.

<sup>b</sup>Mobile phase, n-hexane/isopropanol (99:1, v/v); flow rate of mobile phase, 0.25 mL/min.

and CSP would be expected to be required for good chiral recognition (14). If two of the functional groups occur in the structure, they are not necessarily effective as recognition sites, as compound 10 and 12 were not separated into enantiomers. These results support the idea that for chiral recognition to be effective, factors other than the mere presence of chemical recognition sites (11) of the analyte play a role. It appears that the morphology of cellulose tribenzoate on the silica gel, for example, is also important. Moreover, it has been reported that electron-donating substituents on the aromatic ring of a racemate (15) or the cellulose tribenzoate CSP (11) tend to improve enantiomer separations. Such substituents seem to work by changing the electron density of the aromatic ring which relates to the  $\pi$ - $\pi$  interaction of the analyte and CSP. Accordingly, the enantiomer separation of 6 and 9 might be improved by attaching an electron-donating unit to the benzyl group.

In conclusion, of the glycerol derivatives studied, compound 6 has the best potential for preparative enantiomer separation by HPLC because of the good column selectivity ( $\alpha$ ) and resolution ( $R_s$ ) obtained with this compound (Table 2). The S-isomer of 6 would be the intermediate of choice for the stereoselective synthesis of PAF.

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