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Separation of the enantiomeric intermediates of some platelet-activating factor analogues on a naphthylalanine-type Pirkle column

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SUMMARY

A chiral high-performance liquid-chromatographic separation method was developed for the analysis of some glycerol-based, ether-linked platelet-activating factor precursors. Using a D-naphthylalanine-type Pirkle-column and tetrahydrofuran–hexane eluents, the retention–structure relationships were determined for a large number of glycerol ether derivatives. The derivatives leading to the highest chiral selectivity factors in the analytical separations were surveyed for their applicability in large-scale isolation of chiral synthetic intermediates.

INTRODUCTION

Interest in ether-linked phospholipids has been steadily increasing over the last two decades[1], in part due to their well known platelet-activating, antihypertensive, antineoplastic and antiinflammatory properties[2,3]. The synthetic pathways reported to date for the preparation of racemic[4–6] or chiral ether lipids[7–9], however, are limited relative to the methodologies that have been developed for the synthesis of ester-linked phospholipids[10]. In the course of developing a new stereoselective synthesis of glycerol ether lipids from allylic alcohol precursors[11,12], a chiral analytical separation method was needed to monitor the stereospecificity of the reaction pathway. The method sought would also serve as the basis for the development of a preparative-scale isolation method to produce chiral materials from racemic mixtures of mono- and dialkylglycerolphosphate derivatives. If successful, this method would provide a facile, large-scale and low-cost route to these materials via nucleophilic ring-opening reactions of racemic glycidol esters[11] by aliphatic alcohols. An analogous route has been reported for the synthesis of chiral ether-linked phospholipids[12].

The 3,5-dinitrobenzoate (DNB) derivatives of several racemic mixtures of chiral alcohols were separated, though with low selectivity values, on naphthylalanine-type

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Pirkle phases[13]. This paper reports our results of a retention–structure relationship study aimed at establishing the best analytical chiral selectivity for a set of glycerol derivatives the substituents of which allow further manipulation of the ether lipid structure.

EXPERIMENTAL

Materials

Solvents were obtained from E. M. Science (Gibbstown, NJ, U.S.A.) and were reagent grade or better. Glycidol, 1-hexadecanol, benzoyl chloride, acetyl chloride and 4-nitrobenzoyl chloride were obtained from Aldrich (Milwaukee, WI, U.S.A.). Cyclohexylcarbonyl chloride and 3-nitrobenzenesulfonyl chloride were purchased from American Tokyo Kasei (Portland, OR, U.S.A.). 3,5-Dinitrobenzoyl chloride (99.5 plus %) was supplied by Fluka (Ronkonkoma, NY, U.S.A.). Trifluoromethanesulfonic acid was obtained from 3M (St. Paul, MN, U.S.A.).

The reaction pathway used to prepare the cmpounds studied is shown in Fig. 1. The solutes, in most cases, were synthesized by 1% trifluoromethanesulfonic acid-catalyzed ring opening of glycidyl sulfonate or carboxylate esters[11] with a threefold excess of 1-hexadecanol at 65–75°C for 5 h in the absence of solvent. Alkylated glycerol silyl ethers were prepared similarly using 2% tropylium tetrafluoroborate as catalyst. The hexadecyl-derivatized material was isolated from the reaction mixture by column chromatography [25 g silica per g of crude reaction mixture; 70–230 mesh; mobile phase: hexane–ethyl acetate (3:1)], recrystallized from light

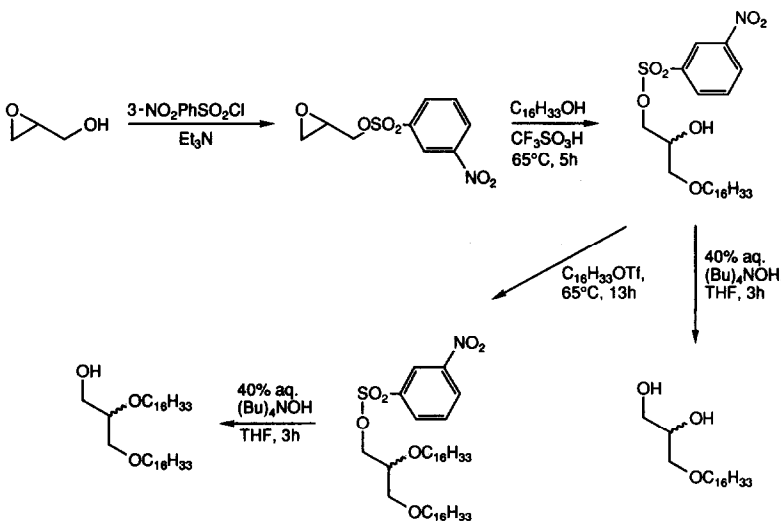

Fig. 1. Synthetic pathway for the preparation of mono- and dialkyl glycerol ethers from glycidyl 3-nitrobenzenesulfonate. Carboxylate esters were prepared in a similar fashion using the corresponding glycidyl carboxylate ester as starting material. DNB esters were prepared from the alcohol or diol precursor and DNB chloride. Ph = Phenyl; Et = ethyl; Bu = butyl; THF = tetrahydrofuran; aq. = aqueous; Tf = trifluoromethyl sulfonate.

petroleum (b.p. 37.2–57.8°C) ether, and characterized by NMR, Fourier transform (FT)-IR and mass spectrometry. Sulfonate ester cleavage was effected with a stoichiometric quantity of 40% aqueous tetrabutylammonium hydroxide in tetrahydrofuran to produce the free alcohols. DNB derivatives were prepared from these precursors in the usual manner prior to HPLC analysis[13].

HPLC system

Separations were carried out with a custom-built liquid chromatograph assembled from a Model 2020 pump, Model 2050 variable-wavelength UV detector and Model RI 3 refractive index detector (all from Varian, Walnut Creek, CA, U.S.A.), a pneumatically controlled Model 7010 injection valve equipped with a 10-μl sample loop (Rheodyne, Cotati, CA, U.S.A.), and a Maxima chromatographic work station (Millipore, Bedford, MA, U.S.A.).

Rexchrom 250 mm × 4.6 mm I.D. columns, packed with 5-μm D-naphthylalanine silica stationary phase (Regis, Morton Grove, IL, U.S.A.) and thermostated at 30°C [for the capacity factor (k') studies] by a circulating water bath (Science-Electronics, Dayton, OH, U.S.A.) were used to effect the chiral separations. Eluents were prepared from HPLC grade *n*-hexane and tetrahydrofuran (THF) (Fisher, Fair Lawn, NJ, U.S.A.) according to the gravimetric method[14]. The THF–hexane (10:90, v/v) eluent was found to give $0.5 < k' < 30$ values for all the solutes and was used throughout the experiments.

RESULTS AND DISCUSSION

The synthetic objectives of this study require that the intermediates contain a protecting group (X) that can be readily cleaved with retention of configuration at the asymmetric center while retaining favorable chiral selectivity for preparative scale separations. Chiral selectivity factors, peak resolution factors (R_s), and the k' values of the more strongly retained enantiomers (with *n*-heptane as the dead volume marker) for the materials studied are listed in Table I.

Compounds 1–9 in Table I represent leaving groups of differing overall polarity. It can be seen that solute retention increases significantly with the polarity of X (compounds 1–6). When the X groups have comparable polarities, secondary effects, such as size (compounds 2 *vs.* 3) may slightly alter the retention order. Comparison of the k' values of compounds 4 and 7 shows that when the two polar functional groups are in the 1,3 positions, rather than in the 1,2 positions, larger retention occurs. This agrees with a recent observation by Pirkle[15], who noted that the retention of di(2,4-dinitrophenyl)-α,ω-alkyldiamines varied significantly as the length of the alkyl chain was varied, with maximum solute retention occurring when the chains contained four methylene units. They postulated that the two distant polar sites bind to two different strands of the chiral stationary phase, rather than to two sites on the same strand. This effect is believed to be operational here as well.

It also can be seen that the presence of a very polar, π-acidic functional group and the concomitant large retention alone is not sufficient to effect chiral recognition: the k' values of compounds 5 and 6 are almost identical, yet only the DNB functional group, believed to interact selectively with the naphthyl group of the stationary phase[13], will lead to noticeable chiral recognition. Since the α value is only 1.02, even

TABLE I

CAPACITY FACTOR (k'), CHIRAL SELECTIVITY (α) AND PEAK RESOLUTION (R_s) DATA FOR THE 1-X-2-Y-3-Z-GLYCEROL DERIVATIVES

Stationary phase: D-naphthylalanine silica; eluent: THF–hexane (10:90, v/v); temperature: 30°C.

No.	*X*	*Y*	*Z*	k'_2[a]	α	R_s
1	H	H	$C_{16}H_{33}$	0.35	1	0
2	COC_6H_5	H	$C_{16}H_{33}$	0.52	1	0
3	$COCH_3$	H	$C_{16}H_{33}$	0.55	1	0
4	COC_6H_4-4-(NO_2)	H	$C_{16}H_{33}$	1.0	1	0
5	COC_6H_3-3,5-(NO_2)$_2$	H	$C_{16}H_{33}$	2.08	1.02	0.5
6	$SO_2C_6H_4$-3-(NO_2)	H	$C_{16}H_{33}$	2.11	1	0
7	COC_6H_4-4-(NO_2)	$C_{16}H_{33}$	H	1.15	1	0
8	$SO_2C_6H_4$-3-(NO_2)	$C_{16}H_{33}$	$C_{16}H_{33}$	0.46	1	0
9	COC_6H_3-3,5-(NO_2)$_2$	$C_{16}H_{33}$	$C_{16}H_{33}$	0.93	1.01	0.3
10	COC_6H_{11}	COC_6H_3-3,5-(NO_2)$_2$	$C_{16}H_{33}$	1.84	1.02	0.4
11	$COCH_3$	COC_6H_3-3,5-(NO_2)$_2$	$C_{16}H_{33}$	2.61	1.02	0.4
12	COC_6H_5	COC_6H_3-3,5-(NO_2)$_2$	$C_{16}H_{33}$	2.81	1.02	0.4
13	COC_6H_4-4-(NO_2)	COC_6H_3-3,5-(NO_2)$_2$	$C_{16}H_{33}$	6.85	1.05	0.9
14	$SO_2C_6H_4$-3-(NO_2)	COC_6H_3-3,5-(NO_2)$_2$	$C_{16}H_{33}$	16.59	1.02	0.7
15	COC_6H_3-3,5-(NO_2)$_2$	COC_6H_3-3,5-(NO_2)$_2$	$C_{16}H_{33}$	31.38	1.06	1.3
16	COC_6H_{11}	$C_{16}H_{33}$	COC_6H_3-3,5-(NO_2)$_2$	2.36	1.01	0.3
17	$COCH_3$	$C_{16}H_{33}$	COC_6H_3-3,5-(NO_2)$_2$	3.23	1.02	0.4
18	COC_6H_4-4-(NO_2)	$C_{16}H_{33}$	COC_6H_3-3,5-(NO_2)$_2$	8.76	1.01	0.3

[a] k'_2 = Capacity factor of the more retained enantiomer.

with the DNB group, an analytically useful separation requires the use of columns with comparatively large plate numbers (two 250-mm columns in series, operated at a flow-rate of 0.5 ml/min and temperature of 15°C). The chromatogram, which can be completed in less than 1 h represents a possible, though certainly not optimum solution of the separation problem.

Compounds 8 and 9, the dialkyl ethers, are the most desirable compounds for further synthetic work. The presence of a second hexadecyl ether group in position 2, however, greatly reduces the retention of the solutes regardless of whether the leaving group is the 3-nitrobenzenesulfonate (NBS) or the DNB group. The second hexadecyl ether group in position 2 also greatly diminishes the extent of chiral recognition offered by the DNB group. Therefore, though synthetically attractive, these solutes cannot be used to achieve the final goals of this study.

An alternative approach requires the attachment of a chiral selectivity-enhancing leaving group first, followed by epoxide ring opening (Fig. 1) and derivatization of the resulting hydroxy group in position 2 with DNB to effect chiral resolution. Chiral selectivity might be sufficiently increased by judicious choice of intermolecular interactions and synthetic utility of the leaving group. Compounds 10–15 were synthesized to test this possibility.

It can be seen in Table I that with a DNB group in position 2, retention again increases rapidly as the polarity of the leaving group is increased. Just as with the hydroxy group in position 2, the change of X from benzoate to 4-nitrobenzoate (NB) results in an almost twofold increase in retention, as does the change from NB to NBS.

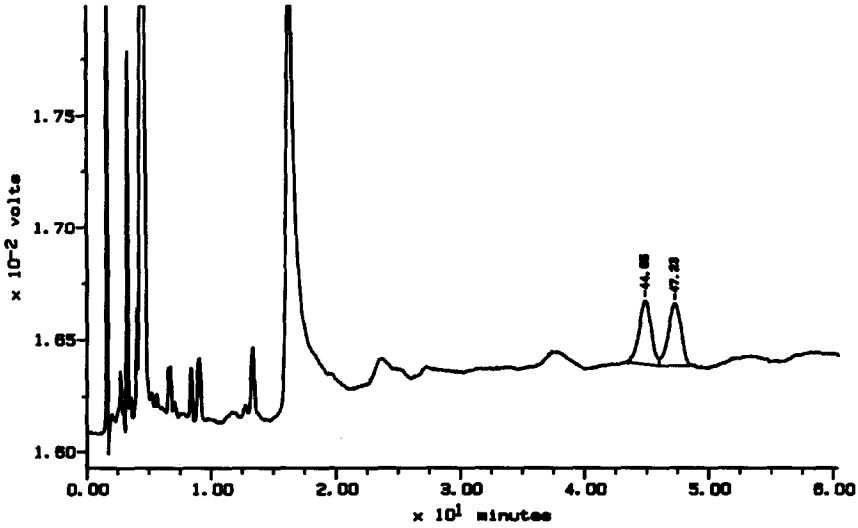

Fig. 2. Chromatogram of compound 15. Conditions: 250 mm × 4.6 mm I.D. Rexchrom D-naphthylalanine column; THF–hexane (10:90, v/v) eluent; flow-rate: 2.0 ml/min; temperature: 30°C; detection (UV): 205 nm. Peaks at 44.85 and 47.23 min: enantiomers of compound 15.

However, unlike in the case of compounds 5 and 6, where NBS and DNB substitution lead to identical increases in retention, the addition of DNB to position 1 results in an almost twofold increase in k' (compare compounds 14 *vs.* 15).

The chiral selectivity values for the cyclohexylcarboxylate, acetate and benzoate derivatives (compounds 10, 11 and 12) are as low (1.02) as that of the mono-DNB derivative, compound 5, indicating that the lower polarity of these auxiliaries brings no improvement in the separation. The situation is much more favorable, however, with both the NB and the DNB functional groups: chiral selectivity increases to 1.05 and 1.06 for compounds 13 and 15, respectively. These selectivities are sufficiently large to afford baseline–baseline resolution of the enantiomers of compound 15 on a single 250-mm column, even with an eluent flow-rate as high as 2 ml/min (Fig. 2). The separation of compound 15, which can be completed in less than 1 h, promises to be slightly better for preparative work than compound 13; however, the different protecting groups of compound 13 provide greater regiochemical control of the reaction products.

When the DNB substituent is moved to position 3 and the hexadecyl ether group to position 2 (compounds 16–18), solute retention increases, presumably due to multistrand binding, as in the case of compounds 4 and 7. However, it can also be seen that multistrand binding decreases the chances of chiral resolution (which is based on sterically localized interactions), and equal or lower chiral selectivity values are obtained for each pair (compounds 10 *vs.* 16, 11 *vs.* 17 and 13 *vs.* 18).

CONCLUSIONS

Based on the structure–retention relationships of the glycerol ether derivatives reported here, it can be concluded that:

(i) The presence of a 3,5-dinitrobenzoate functional group is mandatory for chiral resolution.

(ii) Solute retention increases with the polarity of the second polar group in the order: hexadecyl ether < alcohol < cyclohexylcarboxylate < acetate < benzoate < 4-nitrobenzoate < 3,5-dinitrobenzoate < 3-nitrobenzenesulfonate.

(iii) Chiral selectivity is highest when the second functional group is either the 3,5-dinitrobenzoate or the 4-nitrobenzoate group.

(iv) Chiral selectivities are identical for the 1-(3,5-dinitrobenzoate)-2-X and the 1-X-2-(3,5-dinitrobenzoate) derivatives.

(v) Solute retention is larger and chiral selectivity is lower with the 3,5-dinitrobenzoate group and the second polar functional group in the 1,3 positions *versus* the 1,2 positions.

(vi) Both solute retention and chiral selectivity are greatly diminished for the dialkyl ether derivatives, even with the 3,5-dinitrobenzoate group in position 1.

Work is under way in our laboratories using compounds 5, 13 and 15 to determine their adsorption isotherms and develop viable preparative separation methods to further their synthetic utility.

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