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DIRECT SEPARATION OF NON-K-REGION MONO-OL AND DIOL ENANTIOMERS OF PHENANTHRENE, BENZ[*a*]ANTHRACENE, AND CHRYSENE BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY WITH CHIRAL STATIONARY PHASES*

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SUMMARY

The direct separation of 26 bay region and non-bay region mono-ol and diol enantiomers of phenanthrene, benz[*a*]anthracene, and chrysene was compared by high-performance liquid chromatography on commercially available columns, packed with γ-aminopropylsilanized silica to which either (*R*)-N-(3,5-dinitrobenzoyl)phenylglycine (*R*-DNBPG) or (*S*)-N-(3,5-dinitrobenzoyl)leucine (*S*-DNBL) was either ionically or covalently bonded. In general, enantiomers of bay region mono-ols and diols are more efficiently resolved than those of non-bay region derivatives. Elution orders of enantiomers on either chiral stationary phase are the same, regardless of whether the chiral stationary phase is ionically or covalently bonded. Except for the enantiomers of 4-hydroxy-4-methyl-1,2,3,4-tetrahydrobenz[*a*]anthracene, 1,2,3,4-tetrahydrobenz[*a*]anthracene *trans*-1,2-diol, and benz[*a*]anthracene *trans*-1,2-dihydrodiol, elution orders of resolved enantiomers on *R*-DNBPG are reversed on *S*-DNBL. The enantiomers are generally more efficiently resolved on *R*-DNBPG than on *S*-DNBL. With the exception of the elution order of the enantiomeric 4-hydroxy-1,2,3,4-tetrahydrochrysene, the results of this study are consistent with the chiral recognition mechanisms proposed by Pirkle and co-workers, who developed the chiral stationary phases used in this study.

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INTRODUCTION

Carcinogenic polycyclic aromatic hydrocarbons (PAHs) are inert by themselves and require metabolic activation by drug-metabolizing enzyme systems to exert their mutagenic and carcinogenic effects[1]. PAH metabolites such as epoxides, dihydrodiols, mono-ols, triols, tetrols, phenol-dihydrodiols, and dihydrodiol-epoxides are optically active[1,2]. Elucidation of the stereochemical pathways of metabolism requires resolution of the optical isomers and knowledge of their absolute configurations.

Previously, we reported a high-performance liquid chromatographic (HPLC) method for the direct resolution of some mono-ol, dihydrodiol, tetrahydrodiol, and epoxide enantiomers of various PAH on columns packed with γ-aminopropylsilanized silica to which either (*R*)-N-(3,5-dinitrobenzoyl)phenylglycine (*R*-DNBPG) or (*S*)-N-(3,5-dinitrobenzoyl)leucine (*S*-DNBL) is either ionically or covalently bonded[3–11]. This chiral stationary phase (CSP) HPLC method has been applied to determine the enantiomeric compositions of mono-ol, diol, and epoxide derivatives formed in the metabolism of some PAHs by hepatic microsomes[3,4,7–9,12–15].

Chiral recognition mechanisms have been proposed to predict the enantiomer of cyclic alcohols (mono-ols) that is more strongly retained by a CSP[16]. However, exact chiral recognition mechanisms are not clear, as enantiomers of some structually similar compounds cannot be resolved. Furthermore, different elution orders of enantiomers were observed among some structurally similar compounds[6,17].

It has been found that structural factors, such as conformation, presence of a methyl substituent, molecular size and shape, and ring saturation all contribute to chiral interactions between the CSP and the solute[6,10]. Detailed chiral recognition mechanisms could not be established, due to complex structural factors which influence enantiomeric resolutions and the lack of data on the absolute configurations of resolved enantiomers. Thus, it is necessary to know the absolute configuration of resolved enantiomers before the exact chiral recognition mechanisms can be established.

In this study, we examine the effect of the conformation of the hydroxyl groups of some mono-ols and diols of phenanthrene (PA), benz[*a*]anthracene (BA), and chrysene (CR) (Fig. 1) on the direct separation of enantiomers on CSP. BA and CR can be viewed as derivatives of PA with an additional benzo ring fused at the 6,7- or 7,8-position of PA.

The hydroxyl groups of mono-ols and diols adopt either quasi axial or quasi

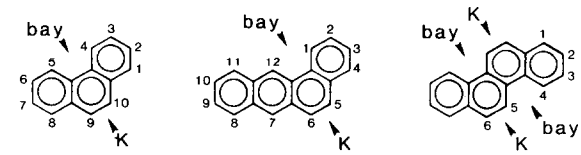

Fig. 1. K- and bay region designation and numbering system of phenanthrene (PA), benz[*a*]anthracene (BA), and chrysene (CR).

equatorial conformation. The absolute configurations of most of the compounds in this study have been established by the exciton chirality circular dichroism (CD) method[18]. With a knowledge of absolute configuration of resolved enantiomers it is possible to assess the role of quasi axial and quasi-equatorial hydroxyl groups in the CSP-solute interactions that contribute to enantiomeric separation.

EXPERIMENTAL

Materials

The following racemic cyclic alcohols were prepared by reduction of ketone precursors with $NaBH_4$: 1-hydroxy-1,2,3,4-tetrahydrophenanthrene (1-OH-H_4PA), 4-hydroxy-1,2,3,4-tetrahydrophenanthrene (4-OH-H_4PA), 1-hydroxy-1,2,3,4-tetrahydrobenz[*a*]anthracene (1-OH-H_4BA), 4-hydroxy-1,2,3,4-tetrahydrobenz[*a*]anthracene (4-OH-H_4BA), 1-hydroxy-1,2,3,4-tetrahydrochrysene (1-OH-H_4CR), 4-hydroxy-1,2,3,4-tetrahydrochrysene (4-OH-H_4CR).

4-Hydroxy-4-methyl-1,2,3,4-tetrahydrophenanthrene (4-OH-4-CH_3-H_4PA) was purchased from Aldrich (Milwaukee, WI, U.S.A.). Both 1-hydroxy-1-methyl-1,2,3,4-tetrahydrobenz[*a*]anthracene (1-OH-1-CH_3-H_4BA) and 4-hydroxy-4-methyl-1,2,3,4-tetrahydrobenz[*a*]anthracene (4-OH-4-CH_3BA) were prepared by methylation of the corresponding ketone precursors with methyl lithium.

Benz[*a*]anthracene *trans*-1,2-dihydrodiol (BA *trans*-1,2-H_2diol), benz[*a*]anthracene *trans*-3,4-dihydrodiol (BA *trans*-3,4-H_2diol), chrysene *trans*-1,2-dihydrodiol (CR *trans*-1,2-H_2diol), and chrysene *trans*-3,4-dihydrodiol (CR *trans*-3,4-H_2diol) were obtained from the Chemical Repository of National Cancer Institute (Bethesda, MD, U.S.A.). Phenanthrene *trans*-1,2-dihydrodiol (PA *trans*-1,2-H_2diol) and phenanthrene *trans*-3,4-dihydrodiol (PA *trans*-3,4-H_2diol) were isolated by HPLC from a mixture of products formed in the incubation of phenanthrene by rat liver microsomes[19]. The biosynthetic diols are enriched in the *R,R* enantiomers. Repetitive chromatography enabled us to collect sufficient amounts of the *S,S* dihydrodiols for this study.

Tetrahydrodiols were prepared by catalytic hydrogenation (tetrahydrofuran, PtO_2, 1 atm, 30 min) of the dihydrodiols of phenanthrene, benz[*a*]anthracene, or chrysene, respectively. Benz[*a*]anthracene 1,2,3,4-tetrahydro-*cis*-1,2-diol (BA *cis*-1,2-H_4diol) was synthesized by reduction of 3,4-dihydrobenz[*a*]anthracene-1(2H)-one (Aldrich) with $NaBH_4$ to 1-OH-H_4BA, which was converted by acid-catalyzed dehydration to 3,4-H_2BA and subsequently oxidized with OsO_4 in pyridine for two days. Phenanthrene 1,2,3,4-tetrahydro-*cis*-1,2-diol (PA *cis*-1,2-H_4diol), phenanthrene 1,2,3,4-tetrahydro-*cis*-3,4-diol (PA *cis*-3,4-H_4diol), and chrysene 1,2,3,4-tetrahydro-*cis*-3,4-diol (CR *cis*-3,4-H_4diol) were similarly prepared from the respective ketone precursors.

Chromatography

Mono-ols and diols weere analyzed on HPLC columns (25 cm × 4.6 mm I.D.; Regis, Morton Grove, IL, U.S.A.) packed with (*R*)-N-(3,5-dinitrobenzoyl)-phenylglycine either ionically bonded (*R*-DNBPG-I) or covalently bonded (*R*-DNBPG-C) or packed with (*S*)-N-(3,5-dinitrobenzoyl)leucine, either ionically bonded (*S*-DNBL-I) or covalently bonded (*S*-DNBL-C) to spherical particles of 5 μm γ-ami-

nopropylsilanized silica[20,21]. HPLC was performed using a Waters Assoc. (Milford, MA, U.S.A.) Model 510 or 6000A solvent delivery system, a Model 440 absorbance detector (254 nm) or a Kratos (Ramsey, NJ) Model 757 variable-wavelength detector. An Autochrom (Milford, MA, U.S.A.) Model OPS solvent mixer was used for reversed-phase HPLC. Retention times and areas under the peaks were recorded with a Hewlett-Packard (Palo Alto, CA, U.S.A.) Model 3390A integrator. Samples were injected via a Valco (Houston, TX, U.S.A.) Model N60 loop injector. Separation of enantiomers was achieved isocratically at a flow-rate of 2 ml/min using premixed solvents of up to 15% (v/v) of solvent A (ethanol–acetonitrile, 2:1, v/v) in hexane at ambient temperature[3,6]. Optically pure enantiomers were obtained by repetitive chromatography. Solvent was removed from resolved enantiomers by either flash evaporation under reduced pressure or evaporation under nitrogen at ambient pressure. As previously described, any CSP which leached from ionically bonded CSP into resolved enantiomers was removed by reversed-phase HPLC with a DuPont (Wilmington, DE, U.S.A.) Zorbax ODS column prior to CD spectral measurement[3].

Absolute configurations of mono-ol and diol enantiomers

The absolute configurations of mono-ol and diol enantiomers were determined by the exciton chirality CD method[18]. Enantiomeric mono-ol or diol (0.1–0.3 mg) was dissolved in a test tube with 1 ml of ethyl acetate which had been dried with sodium hydride. Sodium hydride (*ca.* 1 mg) was then added, followed by the addition of *ca.* 5 mg of either *p*-N,N-dimethylaminobenzoyl chloride, *p*-nitrobenzoyl chloride, or benzoyl chloride. The test tube was placed on ice for about 5 min, 2 drops of *p*-N,N-dimethylaminopyridine (10 mg/ml in ethyl acetate) were added as a catalyst, and the solution was stirred for 16 h with a magnetic stirrer, allowing it to come to ambient temperature. Solid material was removed by centrifugation and the supernatant was dried, redissolved in tetrahydrofuran (THF)–methanol (1:1), and injected onto a DuPont Golden Series Zorbax ODS column (8 cm × 6.2 mm I.D.). The ODS column was eluted with a 15-min linear gradient of methanol–water (3:1, v/v) to methanol at a flow-rate of 1.5 ml/min. The bis-*p*-N,N-dimethylaminobenzoates derived from diols were eluted in 14–20 min. The *p*-nitrobenzoate derivatives of mono-ols were eluted in 14–18 min, while the benzoate derivatives of mono-ols were eluted in 8–12 min. The absolute configuration of an enantiomeric mono-ol or diol was assigned on the basis of the CD spectrum of either a benzoate or a dibenzoate derivative, according to the exciton chirality CD method[18].

Spectral analysis

UV–VIS absorption spectra of samples in methanol were determined using a 1-cm path length quartz cuvette with a Varian Model 118C spectrophotometer. Mass spectral analysis was performed on a Finnigan (San Jose, CA, U.S.A.) Model 4000 gas chromatograph–mass spectrometer–data system by electron impact with a solid probe at 70 eV and 250°C ionizer temperature. CD spectra of samples in methanol were measured in a quartz cell of 1-cm path length at room temperature, using a Jasco Model 500A spectropolarimeter, equipped with a Model DP-500 data processor. The concentration of the sample is indicated by A_λ/ml (number of absorbance unit at wavelength λ per ml of methanol). CD spectra are expressed by ellipticity (in millidegrees) for methanol solutions that have an absorbance of 1.0 unit at a specified wavelength[12].

RESULTS

Enantiomeric separations of bay region and non-bay region mono-ols and diols on *R*-DNBPG and *S*-DNBL, either ionically bonded or covalently bonded to γ-aminopropylsilanized silica, are shown in Tables I and II. For the purpose of comparison, the absolute configurations of the enantiomers of bay region and non-bay region mono-ols and diols more strongly retained by *R*-DNBPG and *R*-DNBL are shown in Fig. 2, although the *S*-DNBL column was actually used in this study. Elution orders of enantiomers were the same on a CSP regardless of whether the CSP is ionically or covalently bonded. In general, enantiomers of bay region mono-ols and diols were more efficiently separated than those of non-bay region derivatives. For reason(s) unknown, the elution orders of enantiomers of 4-OH-4-CH_3-H_4BA (Table II), BA *trans*-1,2-H_2diol (Table I), and BA *trans*-1,2-H_4diol (Table I) are the same on both *R*-DNBPG and *S*-DNBL. *R*-DNBPG generally gave better resolution of enantiomers than *S*-DNBL, although exceptions do exist.

Bay region mono-ols and diols

Due to steric crowding in the bay region, the benzylic hydroxyl group of bay region mono-ols and diols preferentially adopts a quasi axial conformation. Enan-

TABLE I

CSP-HPLC RESOLUTION OF BAY REGION MONO-OL AND DIOL ENANTIOMERS OF PHENANTHRENE (PA), BENZ[*a*]ANTHRACENE (BA) AND CHRYSENE (CR)

H_2 = dihydro, H_4 = 1,2,3,4-tetrahydro; abbreviations of CSPs are described in the Experimental section.

Compound	*CSP*	*A(%)**	*Retention time***		*RV****
			Peak No. 1	*Peak No. 2*	
4-OH-H_4PA	*R*-DNBPG-I	2.5	11.7(*R*)	13.3(*S*)	2.5
	R-DNBPG-C	2.5	11.0(*R*)	12.8(*S*)	2.2
	S-DNBL-I	2.5	7.5(*S*)	7.6(*R*)	0.2
	S-DNBL-C	2.5	7.1(*S*)	7.9(*R*)	0.3
4-OH-4-CH_3-H_4PA	*R*-DNBPG-I	2.5	5.6(*R*)	6.7(*S*)	2.8
	R-DNBPG-C	2.5	5.7(*R*)	7.1(*S*)	3.7
	S-DNBL-I	2.5	4.6(*S*)	5.1(*R*)	1.2
	S-DNBL-C	2.5	4.5(*S*)	4.9(*R*)	1.9
PA *trans*-3,4-H_2diol	*R*-DNBPG-I	10	25.5(3*S*,4*S*)	26.3(3*R*,4*R*)	0.8
	R-DNBPG-C	10	21.2	21.2	0
	S-DNBL-I	10	21.0(3*R*,4*R*)	22.4(3*S*,4*S*)	1.3
	S-DNBL-C	10	15.7(3*R*,4*R*)	16.1(3*S*,4*S*)	0.1
PA *trans*-3,4-H_4diol	*R*-DNBPG-I	10	16.9(3*S*,4*S*)	18.8(3*R*,4*R*)	2.2
	R-DNBPG-C	10	14.5(3*S*,4*S*)	15.4(3*R*,4*R*)	1.4
	S-DNBL-I	10	14.0(3*R*,5*R*)	15.2(3*S*,4*S*)	1.5
	S-DNBL-C	10	11.3(3*R*,4*R*)	11.8(3*S*,4*S*)	0.6
PA *cis*-3,4-H_4diol	*R*-DNBPG-I	10	15.9(3*R*,4*S*)	20.1(3*S*,4*R*)	4.6
	R-DNBPG-C	10	10.7(3*R*,4*S*)	12.7(3*S*,4*R*)	3.0
	S-DNBL-I	10	10.2(3*S*,4*R*)	10.5(3*R*,4*S*)	0.7
	S-DNBL-C	10	7.9(3*S*,4*R*)	8.3(3*R*,4*S*)	0.9

(*Continued on p. 216*)

TABLE I (*continued*)

Compound	*CSP*	*A(%)**	*Retention time***		*RV****
			Peak No. 1	*Peak No. 2*	
1-OH-H_4BA	*R*-DNBPG-I	2.5	19.4(*R*)	21.6(*S*)	2.6
	R-DNBPG-C	2.5	17.3(*R*)	20.0(*S*)	3.2
	S-DNBL-I	2.5	10.7	10.7	0
	S-DNBL-C	2.5	11.2	11.2	0
1-OH-1-CH_3-H_4BA	*R*-DNBPG-I	2.5	9.7(*R*)	12.6(*S*)	5.7
	R-DNBPG-C	2.5	9.7(*R*)	12.5(*S*)	4.1
	S-DNBL-I	2.5	6.5(*S*)	7.2(*R*)	2.2
	S-DNBL-C	2.5	6.6(*S*)	7.2(*R*)	1.8
BA *trans*-1,2-H_2diol§	*R*-DNBPG-I	15	21.8(1*S*,2*S*)	22.2(1*R*,2*R*)	0.1
	R-DNBPG-C	15	17.2	17.2	0
	S-DNBL-I	15	17.8	17.8	0
		10	49.0(1*S*,2*S*)	49.5(1*R*,2*R*)	0.1
	S-DNBL-C	15	11.7	11.7	0
BA *trans*-1,2-H_4diol§	*R*-DNBPG-I	15	14.4(1*S*,2*S*)	15.7(1*R*,2*R*)	2.4
	R-DNBPG-C	15	11.8(1*S*,2*S*)	12.5(1*R*,2*R*)	1.4
	S-DNBL-I	15	12.3	12.3	0
		10	20.8	20.8	0
	S-DNBL-C	15	9.0	9.0	0
		10	14.2(1*S*,2*S*)	14.4(1*R*,2*R*)	0.1
BA *cis*-1,2-H_4diol§	*R*-DNBPG-I	15	16.8(1*S*,2*R*)	24.6(1*R*,2*S*)	8.9
	R-DNBPG-C	15	10.5(1*S*,2*R*)	13.7(1*R*,2*S*)	5.5
	S-DNBL-I	10	19.2(1*R*,2*S*)	19.7(1*S*,2*R*)	0.6
	S-DNBL-C	10	10.7(1*R*,2*S*)	11.2(1*S*,2*R*)	0.8
4-CH_3-H_4CR	*R*-DNBPG-I	0	8.4	8.6	0.3
	R-DNBPG-C	0	7.8	7.9	0.1
	S-DNBL-I	0	3.5	3.5	0
	S-DNBL-C	0	5.2	5.2	0
4-OH-H_4CR	*R*-DNBPG-I	2.5	32.1(*S*)	32.6(*R*)	0.2
	R-DNBPG-C	2.5	29.3(*S*)	29.4(*R*)	0.1
	S-DNBL-I	2.5	23.6(*R*)	24.5(*S*)	0.9
	S-DNBL-C	2.5	14.2(*R*)	15.2(*S*)	1.2
CR *trans*-3,4-H_2diol§	*R*-DNBPG-I	10	38.1	38.1	0
	R-DNBPG-C	10	35.2	35.2	0
	S-DNBL-I	10	40.6(3*R*,4*R*)	42.0(3*S*,4*S*)	0.7
	S-DNBL-C	10	21.8	21.8	0
CR *trans*-3,4-H_4diol§	*R*-DNBPG-I	10	32.1(3*S*,4*S*)	37.0(3*R*,4*R*)	3.3
	R-DNBPG-C	10	22.4(3*S*,4*S*)	25.1(3*R*,4*R*)	2.6
	S-DNBL-I	10	25.4(3*R*,4*R*)	27.3(3*S*,4*S*)	1.6
	S-DNBL-C	10	15.5(3*R*,4*R*)	16.0(3*S*,4*S*)	0.6
CR *cis*-3,4-H_4diol	*R*-DNBPG-I	10	34.5(3*R*,4*S*)	45.9(3*S*,4*R*)	5.2
	R-DNBPG-C	10	19.6(3*R*,4*S*)	25.6(3*S*,4*R*)	5.0
	S-DNBL-I	10	22.6(3*S*,4*R*)	24.3(3*R*,4*S*)	1.4
	S-DNBL-C	10	13.2(3*S*,4*R*)	14.5(3*R*,4*S*)	1.3

* Percent of solvent A (ethanol–acetonitrile, 2:1) in hexane at 2 ml/min.

** See text for the assignments of absolute configurations of resolved enantiomers.

*** RV = resolution value = $2(V_2 - V_1)/(W_2 + W_1)$, where V is the retention volume and W is the width at the peak base. The void time was 1.2 min.

§ Enantiomeric resolutions of these compounds were reported earlier[9,10] and are included for comparison.

TABLE II

CSP-HPLC RESOLUTION OF NON-BAY REGION MONO-OL AND DIOL ENANTIOMERS OF PHENANTHRENE (PA), BENZ[*a*]ANTHRACENE (BA), AND CHRYSENE (CR)

For abbreviations see Table I.

Compound	*CSP*	*A(%)*	*Retention time*		*RV*
			Peak No. 1	*Peak No. 2*	
1-OH-H_4PA	*R*-DNBPG-I	2.5	13.9(*R*)	14.1(*S*)	0.2
	R-DNBPG-C	2.5	14.1	14.1	0
	S-DNBL-I	2.5	10.1(*S*)	10.4(*R*)	0.6
	S-DNBL-C	2.5	9.4(*S*)	9.9(*R*)	1.0
PA *trans*-1,2-H_2diol	*R*-DNBPG-I	7.5	24.0(1*S*,2*S*)	24.8(1*R*,2*R*)	0.7
	R-DNBPG-C	7.5	15.1(1*S*,2*S*)	15.4(1*R*,2*R*)	0.1
	S-DNBL-I	7.5	19.7	19.7	0
	S-DNBL-C	7.5	12.4	12.4	0
PA *trans*-1,2-H_4diol	*R*-DNBPG-I	7.5	26.2(1*S*,2*S*)	27.9(1*R*,2*R*)	1.6
	R-DNBPG-C	7.5	17.4(1*S*,2*S*)	17.9(1*R*,2*R*)	0.2
	S-DNBL-I	7.5	21.4	21.4	0
	S-DNBL-C	7.5	13.6	13.6	0
PA *cis*-1,2-H_4diol*	*R*-DNBPG-I	10	17.3(1*S*,2*R*)	17.6(1*R*,2*S*)	0.2
	R-DNBPG-C	10	11.7(1*S*,2*R*)	11.9(1*R*,2*S*)	0.1
	S-DNBL-I	10	11.5	11.5	0.1
	S-DNBL-C	10	8.7	8.7	0
4-OH-H_4BA	*R*-DNBPG-I	2.5	26.2(*R*)	26.8(*S*)	0.5
	R-DNBPG-C	2.5	22.1	22.1	0
	S-DNBL-I	2.5	14.0(*S*)	14.5(*R*)	0.7
	S-DNBL-C	2.5	13.3(*S*)	14.0(*R*)	1.1
4-OH-4-CH_3-H_4BA**	*R*-DNBPG-I	2.5	16.7(*R*)	17.2(*S*)	0.7
	R-DNBPG-C	2.5	16.8(*R*)	17.0(*S*)	0.1
	S-DNBL-I	2.5	10.5	10.5	0
	S-DNBL-C	2.5	10.3(*R*)	10.8(*S*)	1.0
BA *trans*-3,4-H_2diol	*R*-DNBPG-I	10	31.6(3*S*,4*S*)	32.1((3*R*,4*R*)	0.2
	R-DNBPG-C	10	19.6	19.6	0
	S-DNBL-I	10	20.6(3*R*,4*R*)	21.0(3*S*,4*S*)	0.2
	S-DNBL-C	10	14.0	14.0	0
BA *trans*-3,4-H_4diol	*R*-DNBPG-I	10	32.9(3*S*,4*S*)	34.8(3*R*,4*R*)	1.3
	R-DNBPG-C	10	21.3(3*S*,4*S*)	21.7(3*R*,4*R*)	0.3
	S-DNBL-I	10	22.2	22.2	0
	S-DNBL-C	10	14.9	15.1	0.1
1-OH-H_4CR	*R*-DNBPG-I	2.5	21.7(*R*)	24.2(*S*)	3.1
	R-DNBPG-C	2.5	19.6(*R*)	22.7(*S*)	3.1
	S-DNBL-I	2.5	16.3(*S*)	16.7(*R*)	0.3
	S-DNBL-C	2.5	11.7(*S*)	11.8(*R*)	0.1
CR *trans*-1,2-H_2diol	*R*-DNBPG-I	10	38.9(1*S*,2*S*)	41.0(1*R*,2*R*)	1.1
	R-DNBPG-C	10	18.6	18.6	0
	S-DNBL-I	10	25.6	25.6	0
	S-DNBL-C	10	15.9	15.9	0
CR *trans*-1,2-H_4diol	*R*-DNBPG-I	10	42.5(1*S*,2*S*)	47.4(1*R*,2*R*)	2.3
	R-DNBPG-C	10	22.0(1*S*,2*S*)	22.4(1*R*,2*R*)	0.3
	S-DNBL-I	10	27.3(1*R*,2*R*)	27.5(1*S*,2*S*)	0.1
	S-DNBL-C	10	15.0	15.0	0

* Absolute configurations of resolved enantiomers are tentatively assigned (see text for discussion).

** Note that the elution orders of enantiomers are the same on *R*-DNBPG-I, *R*-DNBPG-C, and *S*-DNBL-C.

tiomers of these compounds are resolved with varying degrees of efficiency (RV = 0–8.9) by at least one CSP (Table I). The enantiomers of BA *trans*-1,2-H_2diol, 4-CH_3-H_4CR, and CR *trans*-3,4-H_2diol were less efficiently resolved (RV $\leqslant$ 0.7). Enantiomeric resolution of all diols was improved when a dihydrodiol is converted to a tetrahydrodiol. The resolution values (Table I) for bay region mono-ols with and

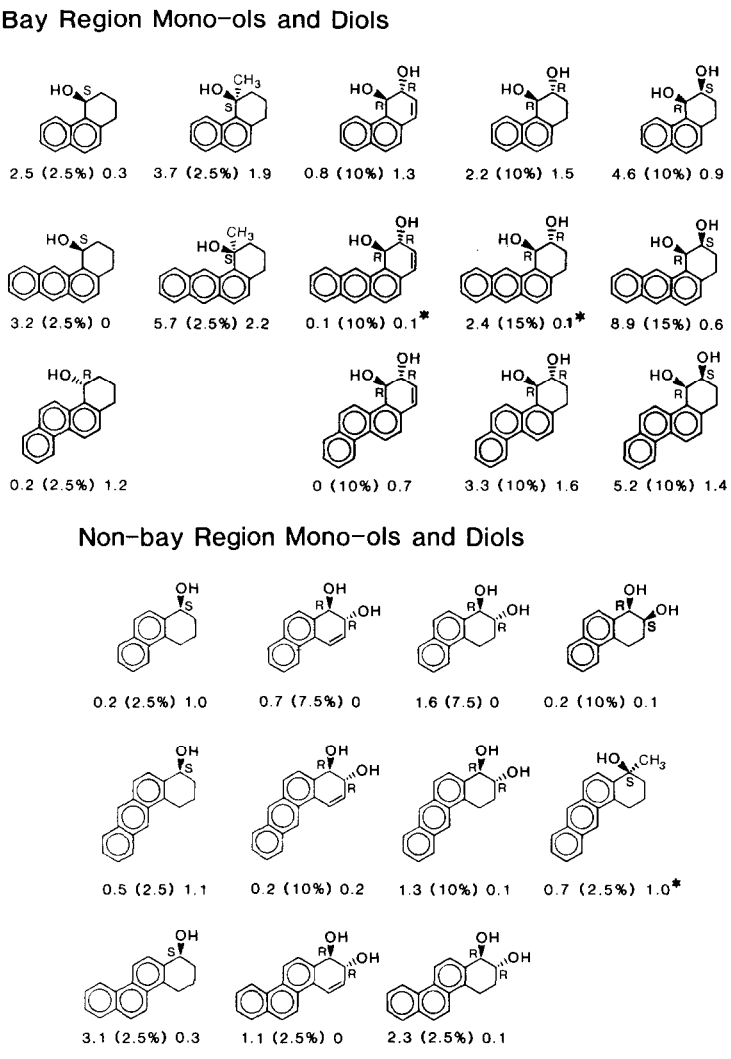

Fig. 2. Structures of bay region and non-bay region mono-ol and diol enantiomers that are more strongly retained by either *R*-DNBPG or *R*-DNBL. Structures are drawn in a manner analogous to the chiral recognition model proposed by Pirkle *et al.*[16]. The percentage of solvent A (ethanol–acetonitrile, 2:1) in hexane used in chromatography is shown in parenthesis. Resolution values on *R*-DNBPG and *R*-DNBL are shown at the left and right sides of the parenthesis, respectively. The larger of two resolution values on the ionically and covalently bonded *R*-DNBPG or *R*-DNBL is shown. The asterisks indicate the elution orders of enantiomers that are the same on *R*-DNBPG and *S*-DNBL (Tables I and II).

without a geminal methyl group indicated that the presence of a geminal methyl group substantially enhanced the enantiomeric separation. The enantiomers of most mono-ols and diols were better resolved on *R*-DNBPG than on *S*-DNBL. However, 4-OH-H_4CR and CR *trans*-3,4-H_2diol were more efficiently resolved on *S*-DNBL than on *R*-DNBPG (Table I). Enantiomers were separated better on ionically bonded columns than on covalently bonded columns, except for 4-OH-4-CH_3-H_4PA, 1-OH-H_4BA, and 4-OH-H_4CR.

Non-bay region mono-ols and diols

Due to the absence of steric constraint, the benzylic hydroxyl group of non-bay region mono-ols and *trans*-diols preferentially adopts a quasi equatorial conformation. The benzylic hydroxyl group of non-bay region *cis*-diols (*e.g.*, PA *cis*-1,2-H_4diol, Fig. 2) adopts either a quasi equatorial or a quasi axial conformation. Enantiomers of this class of compounds were resolved with various efficiency (RV = 0–3.1) by at least one CSP (Table II). The enantiomers of BA *trans*-3,4-H_2diol were not efficiently resolved on any of the four CSP tested. Enantiomeric resolution of this and other dihydrodiols was also improved by conversion to tetrahydrodiols (Tables I and II). The enantiomers of most mono-ols and diols were more efficiently resolved on *R*-DNBPG than on *S*-DNBL. The enantiomers of 1-OH-H_4PA and 4-OH-H_4BA were better resolved on *S*-DNBL-C than on the other CSP. Resolution values of 4-OH-H_4BA and 4-OH-4-CH_3-H_4BA (Table II) indicate that the geminal methyl group does not significantly change the efficiency of enantiomeric separation. Except for 4-OH-4-CH_3-H_4BA, elution orders of non-bay region mono-ol and diol enantiomers on *R*-DNBPG were reversed on *S*-DNBL (Table II).

Absolute configurations of enantiomeric bay region diols

The absolute configurations of enantiomeric PA *trans*-3,4-H_2diol[19], BA *trans*-1,2-H_2diol[22], BA *trans*-1,2-H_4diol[22], BA *cis*-1,2-H_4diol[10], and CR *trans*-3,4-H_2diol[9,19] have been established. The absolute configurations of enantiomeric PA *trans*-3,4-H_4diol and CR *trans*-3,4-H_4diol were established by catalytic hydrogenation of the corresponding dihydrodiol of known absolute stereochemistry.

The CD spectrum of a bis-*p*-N,N-dimethylaminobenzoate, derived from the more strongly retained enantiomer of PA *cis*-3,4-H_4diol (M^+ at *m/z* 508) on *R*-DNBPG-C showed a pair of strong and symmetrical Cotton effects; positive at 302 nm and negative at 325 nm, passing through zero at 314 nm (Fig. 3). This negative chirality resulting from electric dipole–dipole interactions between the benzoate groups indicates that the diol under consideration has a 3*S*,4*R* absolute stereochemistry[18]. Thus, the elution order–absolute configuration relationship of enantiomeric PA *cis*-3,4-H_4diol is established in this study. Previously, the absolute configuration of a (−)PA *cis*-3,4-H_4diol enantiomer was established to have a 3*S*,4*R* absolute stereochemistry by the CD spectrum of an enantiomeric 4-benzoyloxy derivative[23]. Based on the results of this and earlier studies[23], it can be concluded that (−)PA *cis*-3*S*,4*R*-H_4diol is the more strongly retained enantiomer on both *R*-DNBPG and *R*-DNBL (Table I).

The CD spectrum (not shown) of a bis-*p*-N,N-dimethylaminobenzoate, derived from the less strongly retained enantiomer of CR *cis*-3,4-H_4diol on *S*-DNBL-C, showed a pair of strong and symmetrical Cotton effects; positive at 304 nm and

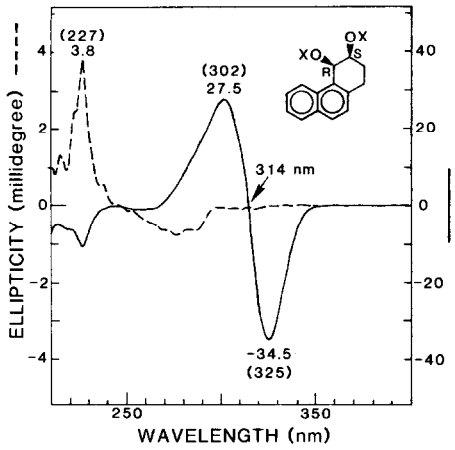

Fig. 3. CD spectra of PA *cis*-3,4-tetrahydrodiol enantiomer (– – –, 1.0 A_{227}/ml), more strongly retained by covalently bonded *R*-DNBPG, and its bis-*p*-N,N-dimethylaminobenzoate derivative (———, X = *p*-N,N-dimethylaminobenzoyl; 1.0 A_{312}/ml).

negative at 325 nm, passing through zero at 317 nm. According to the dibenzoate rule[18], the negative chirality resulting from electric dipole–dipole interactions between the benzoate groups indicates that the diol under consideration has a 3*S*,4*R* absolute stereochemistry.

Absolute configurations of enantiomeric non-bay region diols

The absolute configurations of enantiomeric PA *trans*-1,2-H_2diol[19], BA *trans*-3,4-H_2diol[24,25], and CR *trans*-1,2-H_2diol[9,19] have been established. The absolute configurations of enantiomeric PA *trans*-1,2-H_4diol, BA *trans*-3,4-H_4diol, and CR 1,2-H_4diol were established by catalytic hydrogenation of the corresponding dihydrodiol of known absolute stereochemistry.

Due to dual conformational possibilities (3-quasiaxial–4-quasiequatorial and 4-quasiaxial–3-quasiequatorial), the absolute configuration of an enantiomeric PA *cis*-1,2-H_4diol could not be established by the dibenzoate method[11]. The absolute configuration of an enantiomeric PA *cis*-1,2-H_4diol has been established by the CD spectrum of an enantiomeric 2-benzoyloxy derivative[23]. Unfortunately, the information on the absolute configuration reported earlier[23] could not be directly related to the results of this study. When a sample of PA *cis*-1,2-H_2diol (enriched in the 1*R*,2*S* enantiomer), formed in the metabolism of PA by soil bacteria[23], becomes available for our study, the elution order–absolute configuration relationship of enantiomeric PA *cis*-1,2-H_4diol can be readily established. By comparing the structures of enantiomeric mono-ols and diols more strongly retained by the CSP (Tables I and II), the PA *cis*-1,2-H_4diol enantiomer more strongly retained by the *R*-DNBPG is tentatively assigned to have a 1*R*,2*S* absolute stereochemistry.

Absolute configurations of enantiomeric mono-ols

The absolute configuration of the 4-OH-H_4PA enantiomer less strongly retained by *R*-DNBPG-I was determined by the exciton chirality CD method by con-

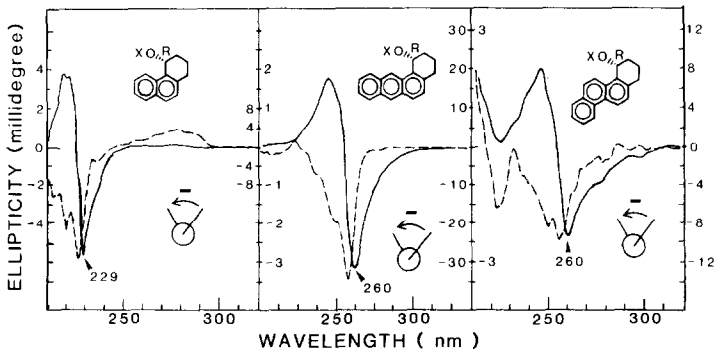

Fig. 4. Left panel: CD spectra of a 4-OH-H_4PA enantiomer, less strongly retained by the ionically bonded *R*-DNBPG (– – –, X = H; 1.0 A_{227}/ml), and its benzoate derivative (———, X = benzoyl; 1.0 A_{224}/ml). Middle panel: CD spectra of a 1-OH-H_4BA enantiomer, less strongly retained by the ionically bonded *R*-DNBPG (– – –, X = H; 1.0 A_{256}/ml), and its *p*-nitrobenzoate derivative (———, X = *p*-nitrobenzoyl; 1.0 A_{256}/ml). The CD spectra of 1-OH-1-CH_3-H_4BA (Φ_{256}/A_{256} = −0.8 millidegree) and 1-OH-H_4BA enantiomers, less strongly retained by *R*-DNBPG, are similar. Right panel: CD spectra of a 4-OH-H_4CR enantiomer, less strongly retained by the ionically bonded *S*-DNBL (– – –, X = H; 1.0 A_{257}/ml), and its *p*-nitrobenzoate derivative (———, X = *p*-nitrobenzoyl; 1.0 A_{257}/ml). The scale of ellipticity on the left of each panel is for the mono-ol and on the right is for the benzoate derivative. The chirality (positive or negative) between the electric transition dipoles of benzoate and aromatic nucleus is shown in each panel.

verting to a benzoate by reaction with benzoyl chloride. Mass spectral analysis of the benzoate derivative indicated molecular ions at *m/z* 302 with a fragment ion at *m/z* 180 (loss of C_6H_5COOH). The CD spectrum exhibited a pair of strong and symmetrical Cotton effects; positive at 218 nm and negative at 229 nm, passing through zero at 226 nm (Fig. 4, left panel). This negative chirality indicates that the less strongly retained enantiomer by *R*-DNBPG-I has a 4*R* absolute stereochemistry[18]. This assignment is consistent with those made in earlier reports[16,17]. The CD spectrum of the 4-OH-4-CH_3-H_4PA enantiomer less strongly retained by *R*-DNBPG is similar to that of the 4*R*-OH-H_4PA enantiomer (Fig. 4, left panel) which is also less strongly retained by *R*-DNBPG, and the enantiomer is therefore assigned to have a 4*R* absolute stereochemistry. This assignment is consistent with the elution order of enantiomers predicted by Pirkle *et al.*[16].

The absolute configuration of the 1-OH-H_4BA enantiomer less strongly retained by *R*-DNBPG was also established by the exciton chirality CD method by converting the mone-ol to a *p*-nitrobenzoate derivative (M^+ at *m/z* 397 and a fragment ion at *m/z* 230 (loss of $NO_2C_6H_4COOH$)). The CD spectrum of this *p*-nitrobenzoate showed a pair of strong and symmetrical Cotton effects; positive at 246 nm and negative at 260 nm, passing through zero at 255 nm (Fig. 4, middle panel). The negative chirality resulting from the electric transition dipole–dipole interactions between the *p*-nitrobenzoate and the anthracenic ring indicated that the mono-ol under consideration has a 1*R* absolute stereochemistry. This assignment is consistent with the elution order of enantiomers, predicted by the chiral recognition mechanism proposed by Pirkle *et al.*[16]. The enantiomers of 1-OH-1-CH_3-H_4BA were resolved by all four CSP columns. The CD spectrum of the more strongly retained enantiomer

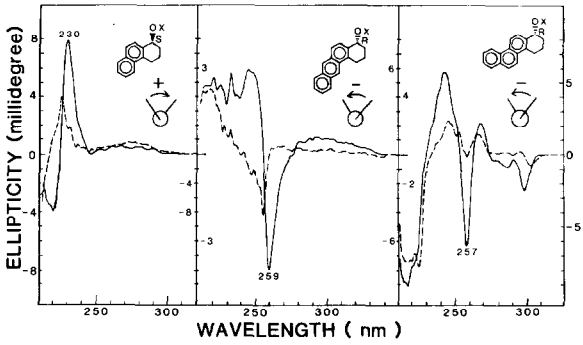

Fig. 5. Left panel: CD spectra of a 1-OH-H_4PA enantiomer, less strongly retained by the covalently bonded *S*-DNBL (– – –, X = H; 1.0 A_{225}/ml), and its benzoate derivative (———, X = benzoyl; 1.0 A_{219}/ml). Middle panel: CD spectra of a 4-OH-H_4BA enantiomer, more strongly retained by the covalently bonded *S*-DNBL (– – –, X = H; 1.0 A_{256}/ml), and its *p*-nitrobenzoate derivative (———, X = *p*-nitrobenzoyl; 1.0 A_{257}/ml). The CD spectra of 4-OH-1-CH_3-H_4BA (Φ_{256}/A_{255} = −0.6 millidegree) and 4-OH-H_4BA enantiomers, more strongly retained by covalently bonded *S*-DNBL, are similar. Right panel: CD spectra of a 1-OH-H_4CR enantiomer, less strongly retained by ionically bonded *R*-DNBPG (– – –, X = H; 1.0 A_{255}/ml), and its *p*-nitrobenzoate derivative (———, X = *p*-nitrobenzoyl; 1.0 A_{255}/ml). The scale of ellipticity on the left of each panel is for the mono-ol and on the right is for the benzoate derivative. The chirality (positive or negative) between the dipole moments of benzoate and aromatic nucleus is shown in each panel.

on *R*-DNBPG is similar to that of 1*S*-OH-H_4BA (Fig. 4, middle panel). On the basis of its CD spectrum, the 1-OH-1-CH_3-H_4BA enantiomer more strongly retained by *R*-DNBPG is assigned to have the 1*S* absolute stereochemistry.

The 4-OH-H_4BA enantiomer more strongly retained by *S*-DNBL-C was also determined by the CD spectrum of a *p*-nitrobenzoate derivative (Fig. 5, middle panel) to have the 4*R* absolute configuration. The more strongly retained enantiomer of 4-OH-4-CH_3-H_4BA by *R*-DNBL-C was assigned to be the 4*R* enantiomer, based on its CD spectrum, which is similar to that of 4*R*-OH-H_4BA.

Using the same technique, the 4-OH-H_4CR enantiomer less strongly retained by *S*-DNBL-C was determined to have a 4*R* absolute configuration (Fig. 4, right panel). Likewise, the 1-OH-H_4CR enantiomer less strongly retained by *R*-DNBPG-I was determined to have a 1*R* absolute stereochemistry (Fig. 5, right panel). The 4-OH-H_4BA enantiomer more strongly retained by *S*-DNBL-C was determined by the CD spectrum of a *p*-nitrobenzoate derivative (Fig. 5, middle panel) to have a 4*R* absolute configuration. The more strongly retained enantiomer of 4-OH-4-CH_3-H_4BA by *R*-DNBL-C was assigned to be the 4*R* enantiomer, based on its CD spectrum, which is similar to that of 4*R*-OH-H_4BA. The 1-OH-H_4PA enantiomer less strongly retained by *S*-DNBL-C are more strongly retained by *R*-DNBPG-I was similarly determined to have a 1*S* absolute stereochemistry (Fig. 5, left panel).

DISCUSSION

Bay region mono-ols and diols have a common structural feature. Due to steric crowding in the bay region, the benzylic hydroxyl preferentially adopts a quasiaxial conformation. On the other hand, the benzylic hydroxyl of non-bay region mono-

ols and diols preferentially adopts a quasiequatorial conformation. The importance of quasiaxial benzylic hydroxyls in mono-ols and diols may be seen in the improved resolution of most enantiomers with quasiaxial benzylic hydroxyls when compared with enantiomers having quasiequatorial benzylic hydroxyl groups (Table I *vs.* Table II). The quasiaxial conformation of benzylic hydroxyl apparently allows a stronger CSP–solute interaction, thus accounting for the increased resolution of enantiomers (Table I *vs.* Table II). However, the enantiomers of some bay region dihydrodiols (*e.g.*, BA *trans*-1,2-H_2diol and CR *trans*-3,4-H_2diol) are not efficiently resolved. Furthermore, efficiencies of enantiomeric separation vary, depending on the type of CSP used (Tables I and II).

The results of this study indicate that all but one of the more strongly retained enantiomers have the same absolute configuration in their benzylic hydroxyl groups on *R*-DNBPG (Fig. 2). An exception in the elution order of enantiomers is seen with the bay region mono-ol 4-OH-H_4CR (Table I and Fig. 2). Other exceptions to the elution orders of enantiomers, proposed by Pirkle *et al.*[16], have been noted earlier[26]. The enantiomers of two bay region diols (BA *trans*-1,2-H_2diol and BA *trans*-1,2-H_4diol) and one non-bay region mono-ol (4-OH-4-CH_3-H_4BA) have the same elution orders on both *R*-DNBPG and *S*-DNBL (Tables I and II).

Substantial improvement in enantiomeric separation (as reflected by the resolution value) is observed for the following series of bay region diols on *R*-DNBPG: PA *trans*-3,4-H_2diol < PA *trans*-3,4-H_4diol < PA *cis*-3,4-H_4diol; BA *trans*-1,2-H_2diol < BA *trans*-1,2-H_4diol < BA *cis*-1,2-H_4diol; CR *trans*-3,4-H_2diol < CR *trans*-3,4-H_4diol < CR *cis*-3,4-H_4diol (Table I and Fig. 2). Improvement in enantiomeric separation on *R*-DNBPG is also observed for the non-bay region *trans*-diols: PA *trans*-1,2-H_2diol < PA *trans*-1,2-H_4diol; BA *trans*-3,4-H_2diol < BA *trans*-3,4-H_4diol; CR *trans*-1,2-H_2diol < CR *trans*-1,2-H_4diol. Unlike bay-region diols, the enantiomers of the non-bay region PA *cis*-1,2-H_4diol were less efficiently resolved than those of PA *trans*-1,2-H_4diol on *R*-DNBPG. On the *S*-DNBL column, the improvement in enantiomeric separation varies from compound to compound, and the enantiomers are generally less efficiently resolved than on *R*-DNBPG. The results of this study provide additional evidence that the allylic hydroxyl group of bay region *cis*-tetrahydrodiols may be involved in chiral interaction with the non-amide carbonyl oxygen of *R*-DNBPG[10].

Enantiomeric resolution of most dihydrodiols used in this and earlier studies[3,5,6,10] is substantially improved on *R*-DNBPG when a dihydrodiol is converted to a tetrahydrodiol (Tables I and II). It is apparent that the flexibility of the tetrahydro benzo-ring enhanced the interaction between the CSP and the diol.

The retention time of a bay region *trans*-tetrahydrodiol is shorter than that of the same bay region *trans*-dihydrodiol. In contrast, a non-bay region *trans*-tetrahydrodiol is eluted with a longer retention time than the same non-bay region *trans*-dihydrodiol. However, the non-bay region *cis*-tetrahydrodiol is eluted with a shorter retention time than the same non-bay region *cis*-dihydrodiol[6]. Saturation of vicinal double bond of a bay region *trans*-dihydrodiol results in a more flexible tetrahydro benzo ring, which partially relieves the steric strain, allowing the hydroxyl group to become slightly less quasiaxial and, hence, less bulky and less polar. On the other hand, when a quasiequatorial *trans*-dihydrodiol is converted to a *trans*-tetrahydrodiol, the conformation of the hydroxyl groups are changed slightly toward a quasi-

diaxial conformation due to the relatively flexible tetrahydro benzo ring. In general, quasidiaxial diols are bulkier and, hence, more polar than quasidiequatorial diols.

It is interesting to note that the enantiomers of 4-CH_3-H_4CR are partially resolved by *R*-DNBPG (Table I). Enantiomers of 10-methyl-7,8,9,10-tetrahydrobenzo[*a*]pyrene are also resolved by *R*-DNBPG[6]. Thus, a methyl group may participate in the interaction with *R*-DNBPG to effect the separation of enantiomers. Comparison of the resolution values for the enantiomeric separation of 4-OH-H_4CR and 4-CH_3-H_4CR indicates that the methyl–CSP interaction is considerably weaker than the hydroxy–CSP interaction.

In summary, the understanding of elution order and CSP–solute interaction requires not only the knowledge of absolute configuration but also the conformation of the mono-ols and diols of interest. By studying the direct enantiomeric resolution of some bay region and non-bay region mono-ol and diol derivatives of some PAH with known conformation and absolute configuration of enantiomers, greater insight is gained into the chiral recognition mechanisms that effect enantiomeric separation. With the exception of the elution order of the enantiomeric 4-OH-H_4CR, the results of this study are consistent with the chiral recognition mechanisms proposed by Pirkle *et al.*[16] for the *R*-DNBPG column.

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