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Chromatographic investigation of the slowly interconverting atropisomers of hindered naphthamides

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ABSTRACT

Chromatographic separation of the stereoisomers of a series of racemic N,N-disubstituted-2-methyl-1-naphthylenecarboxamide atropisomers using several recently developed chiral stationary phases is reported. Separation factors in excess of three are obtained in the best cases, allowing for convenient separation and investigation of the kinetics of stereoisomer interconversion. Examples of deracemization as well as an apparent cog wheel rotational process are also reported.

INTRODUCTION

The chromatographic separation of interconverting species has been a subject of considerable interest to chromatographers for quite some time. A theoretical treatment of the subject was given by Keller and Giddings in 1960 [1], with an expanded treatment provided by Horváth and co-workers in 1984 [2]. Just as nuclear magnetic resonance spectroscopy has proven useful in monitoring kinetic processes on the "NMR time scale", Mannschreck has illustrated the utility of HPLC in the study of kinetic processes occurring on the much slower "HPLC time scale" [3]. Mannschreck as well as others have reported on the liquid chromatographic separation of interconverting enantiomers, [4–9] while Schurig and co-workers have carried out similar studies using gas chromatography [10–12]. Recently, the liquid chromatographic separation of interconverting diastereomers containing proline residues has received considerable attention, [13–16] some of these compounds possessing significant activity as angiotensin converting enzyme (ACE) inhibitors.

The chromatographic separation of the enantiomers of compound **1a**, (Fig. 1) was described by Mannschreck and co-workers [4], who determined an energy barrier to enantiomer interconversion of 100.4 kJ/mol at 25.3°C in

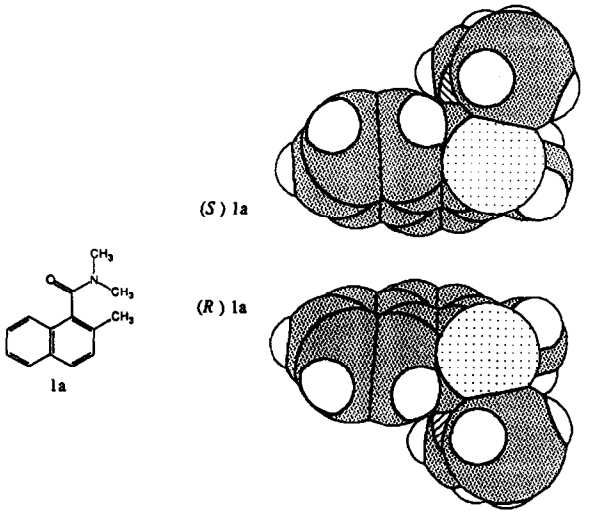

Fig. 1. Enantiomeric forms of compound **1a**.

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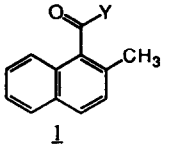
dioxane, which corresponds to an interconversion half-life of about 6 h. The two enantiomeric forms of **1a** are chiral owing to restricted rotation about the $C_{(carbonyl)}-C_{(aryl)}$ bond. These molecules adopt a conformation in which the plane of the carboxamide system is approximately perpendicular to the plane of the aromatic system, the β-methyl group serving as a steric impediment to enantiomer interconversion.

Our interest in these amides initially stemmed from considerations of their chromatographic behavior on a type of chiral stationary phase (CSPs 4–6, Fig. 3) developed recently in our laboratory. We have advanced a model to account for the separation of the enantiomers of naproxen and related 2-aryl-propionic acids on these CSPs [17,18]. This model suggests that the enantiomers of **1a** and similar analytes might also be separated using this type of CSP. As with naproxen, compound **1a** also possesses an electron-rich conjugated π system with a proximate hydrogen bond acceptor situated out of the plane of the π system.

To test this hypothesis, compounds **1a–j** (Fig. 2) were prepared and their chromatographic properties were studied using a series of CSPs



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compound	Y	compound	Y
1a		1f	
1b		1g	
1c		1h	
1d		1i	
1e		1j	

Fig. 2. N,N-2-methyl-1-naphthylenecarboxamide analytes.

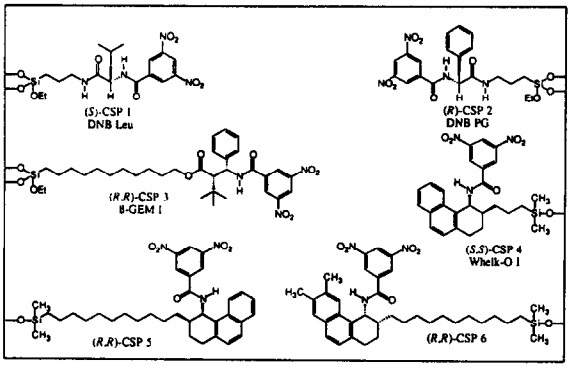


Fig. 3. π-Acidic CSPs used in the study.

(Fig. 3) developed in our laboratory. The commercial versions of CSPs 1–3 were used. The data reported for CSP 4 were collected using the prototype which, generally speaking, shows less enantioselectivity and efficiency than the newly developed "Whelk-O 1" commercial version (Regis).

MATERIALS AND METHODS

Apparatus

Chromatographic analysis was performed using a Rainin Rabbit HP solvent delivery system, a Rainin pressure monitor, a Rheodyne Model 7125 injector with a 20-μl sample loop, a Milton Roy UV monitor (254 nm) and an Altex C-R1A integrating recorder. Signs of optical rotation were determined using an Autopol III Automatic Polarimeter as an inline chromatography detector. ^{1}H NMR spectra were recorded on a Varian XL 200 FT NMR spectrometer.

Materials

All chemicals used were of reagent grade quality and were used without further purification. Chromatography solvents were EM Science HPLC grade. CSPs 1, 2, and 3 were obtained from Regis Chemical Company, Morton Grove, IL (USA). CSPs 4 and 5 were described previously [17,18]. CSP 6 will be described in a forthcoming publication.

Methods

All chromatographic experiments were conducted at a flow-rate of 2 ml/min at ambient

temperature. Column void time was determined by injection of tri-*tert.*-butyl benzene [19]. ^{1}H NMR chemical shifts are reported in ppm (δ) relative to tetramethylsilane.

Synthesis of analytes **1a–j** involved initial preparation of 2-methyl-1-naphthoyl chloride (method of Adams and Binder [20]), followed by reaction with the appropriate amine in a two-phase system consisting of dichloromethane and 1 *M* NaOH. Following an extractive workup and purification by flash chromatography on a silica support, compounds **1a–j** were all satisfactorily characterized by ^{1}H NMR.

RESULTS AND DISCUSSION

Data pertinent to the chromatographic separation of the enantiomers of analytes **1a–i** on CSPs 1–6 are shown in Table I. All of the π-acidic CSPs examined in the study provide some degree of separation for the enantiomers of at least some of analytes **1a–i**. The greatest separation factors (α's) are provided by CSPs 4 and 6, which also show the greatest enantioselectivity in the separation of naproxen enantiomers. The amino acid-derived CSPs 1 and 2 display but moderate enantioselectivities for these analytes, with the β-amino acid-derived CSP 3 [21] performing somewhat better. Retention, indicated by the capacity factor (k_1') for the first-eluted enantiomer, is greatest on CSPs containing aromatic substituents at the stereogenic center. This suggests that face-to-edge $\pi-\pi$ interaction, invoked to account for separation of the enantiomers of naproxen and related analytes on these CSPs, [17,18] may be important in the case of analytes **1a–i** as well.

The molecular interactions responsible for chiral recognition of analytes **1a–i** on the π-acidic CSPs 1–6 are believed to be similar to those proposed for the separation of naproxen enantiomers on these CSPs [17,18]. Fig. 4 depicts a schematic representation of what is believed to be the more stable diastereomeric adsorbate formed between the more strongly retained enantiomer of **1a** and each of the CSPs 1–4

For the DNB Leu CSP (CSP 1), access of analytes to the two faces of the planar π-acidic dinitrobenzamide ring system is presumed to be governed by the steric bulk of the isobutyl side chain. Approaching from the more sterically accessible side, the (*S*) enantiomer of compound **1a** can undergo simultaneous $\pi-\pi$ interaction and hydrogen bonding interactions with the CSP to a greater extent than can the (*R*) enantiomer (Fig. 4a).

A rationale based on steric approach might

TABLE I

CHROMATOGRAPHIC SEPARATION OF THE ENANTIOMERS OF ANALYTES **1a–i** USING CSPs 1–6

Conditions: mobile phase, 20% 2-propanol in hexane; flow-rate, 2.00 ml/min; temperature, ambient; void time determined by injection of tri-*tert.*-butylbenzene; k_1' = capacity factor for initially eluted enantiomer; α = separation factor.

Compound	CSP 1 DNB Leu		CSP 2 DNB PG		CSP 3 β-GEM I		CSP 4		CSP 5		CSP 6	
	k_1'	α	k_1'	α	k_1'	α	k_1'	α	k_1'	α	k_1'	α
1a	2.82	1.17	7.96	1.07	6.93	1.62	4.91	2.21	3.93	1.74	3.67	2.24
1b	1.80	1.11	4.47	1.08	4.57	1.68	3.29	2.46	2.53	1.91	2.42	2.34
1c	1.46	1.10	3.56	1.08	3.70	1.63	3.23	2.66	–	–	2.29	2.53
1d	0.98	1.00	1.90	1.09	2.82	1.93	2.05	3.00	1.40	2.31	1.46	2.86
1e	0.80	1.00	1.57	1.09	2.42	1.92	1.71	3.09	1.21	2.41	1.27	2.91
1f	3.35	1.00	7.56	1.10	7.18	1.68	5.11	2.04	3.78	1.61	3.94	2.01
1g	4.51	1.14	10.3	1.10	8.29	1.59	6.24	2.63	4.76	2.02	4.41	2.63
1h	3.46	1.09	6.34	1.00	6.12	1.47	4.46	2.08	3.36	1.65	3.30	2.08
1i	6.37	1.18	13.8	1.05	7.59	1.61	7.95	2.43	5.50	1.92	5.79	2.45

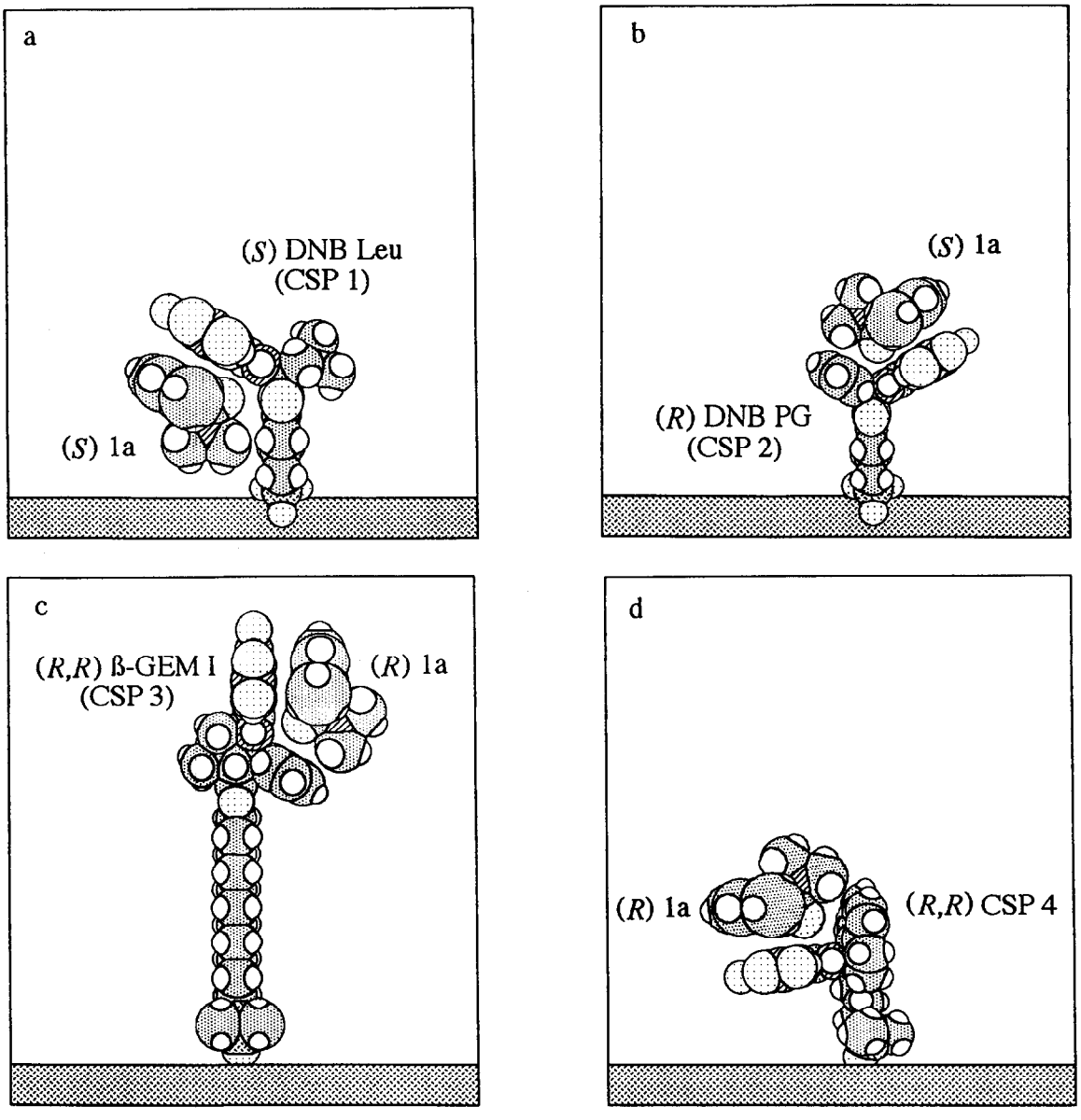


Fig. 4. Schematic representation of chiral recognition rationale for separation of the enantiomers of **1a** on several π-acidic CSPs.

lead one to predict preferential retention of the (*R*) enantiomer of **1a** by the (*R*)-DNB PG CSP (CSP 2), however, recent studies suggest that the phenyl ring at the stereogenic center of this CSP may be involved in beneficial face-to-edge $\pi-\pi$ interaction with analytes which approach the dinitrobenzamide system from the side of the phenyl substituent. To the extent that such face-to-edge $\pi-\pi$ complexation is important, retention of the (*S*) enantiomer of **1a** on the (*R*)-DNB PG CSP is expected (Fig. 4b).

The β-GEM I CSP (CSP 3) is similar to the DNB PG CSP in that it also bears an aromatic substituent at the stereogenic center. Furthermore, this β-amino acid-derived CSP 3 contains a second stereogenic center bearing a *tert.*-butyl substituent which may be useful in restricting access to the face of the dinitrobenzamide system

opposite the phenyl substituent. The (*R*)-β-GEM I CSP is therefore expected to selectively retain the (*R*) enantiomer of **1a**, which can more readily approach from the side of the phenyl substituent (Fig 4c). It should be pointed out that while the "shapes" of CSPs 2 and 3 appear to be effectively "enantiomeric" in Fig. 4, a priority inversion in the Cahn, Ingold, Prelog stereochemistry nomenclature system [22] assigns an (*R*) absolute configuration to CSP 2 and an (*R*,*R*) absolute configuration to CSP 3.

CSP 4 and the closely related CSPs 5 and 6 (Fig. 3) were designed specifically to better utilize the face-to-edge $\pi-\pi$ interactions which are believed to be important in the DNB PG and β GEM I CSPs. These CSPs contain an aromatic substituent at the stereogenic center which is held in a conformation favorable for simultaneous face-to-face and face-to-edge $\pi-\pi$ interactions with the analyte. In addition, the tether connecting the selector to the silica support may serve to restrict access to the "undesired" face of the dinitrobenzamide system. (*S*,*S*) CSP 4 is thus expected to selectively retain the (*S*) enantiomer of **1a** (Fig. 4d).

The sign of the optical rotation at 589 nm was determined for each enantiomer using a polarimetric detector. Table II shows the sign of rotation for the more retained enantiomer of analyte **1a** on each of the CSPs used in the study as well as the absolute configuration assigned to the more retained enantiomer using the model illustrated in Fig. 4. All of the models proposed to account for enantioselective recognition of **1a** by the various CSPs assign the (*S*) absolute configuration to the levorotatory enantiomer and the (*R*) absolute configuration to the dextrorotatory enantiomer. Moreover, the circular dichroism spectra of the enantiomers of **1a** are similar to those of the configurationally related enantiomers of several alkyl 2-methyl-naphthyl ketones [23].

TABLE II

ELUTION ORDERS FOR COMPOUND **1a** ON CSPs 1–6

Column	Rotation [a]	Assigned [b]
(*S*)-CSP 1	(−)	(*S*)
(*R*)-CSP 2	(−)	(*S*)
(*R*,*R*)-CSP 3	(+)	(*R*)
(*S*,*S*)-CSP 4	(−)	(*S*)
(*R*,*R*)-CSP 5	(+)	(*R*)
(*R*,*R*)-CSP 6	(+)	(*R*)

[a] Sign of rotation of the more retained enantiomer of analyte **1a**.

[b] Absolute configuration of the more retained enantiomer of analyte **1a** based upon the model illustrated in Fig. 4.

Interconverting enantiomers offer some interesting possibilities in chiral recognition and separation studies. In one study, the enantiomers of analyte **1a** were preparatively resolved using an analytical size (25 cm × 4.6 mm I.D.) β-GEM I column (CSP 3). The reversion of a single enantiomer of **1a** to the racemate was monitored, and the rate of enantiomerization was determined to be in 0.023/h ($t_{1/2}$, 15.1 h; solvent, 20% 2-propanol in hexane; temperature, 25°C), a finding which is close to the value of 0.058/h determined for this compound by Mannschreck at 25.3°C in dioxane [4].

When one enantiomer of a racemic solution is preferentially adsorbed by a CSP, an excess of the opposite enantiomer exists in solution. When a pathway for enantiomer interconversion is introduced, this solution excess will become "racemized" leading to a net enrichment in the more retained enantiomer. In a study of the deracemization of **1a**, a racemic solution was pumped onto a β-GEM I column using 20% 2-propanol in hexane as a mobile phase. The column was then removed from the HPLC system, column end plugs were inserted, and the column was allowed to stand at room temperature of a period of one week. The column was then eluted with 5% methanol in dichloromethane, the effluent showing an enrichment in the more strongly retained enantiomer. Analogous solution studies using the soluble β-GEM I selector result in enrichment of the more strongly complexed enantiomer. The extent of enrichment increases when larger ratios of chiral selector to analyte are used, the limiting enantiomeric ratio (1.68:1) approximating the separation factor obtained when the racemate is chromatographed on the β-GEM I CSP ($\alpha = 1.62$). This kind of experiment finds a close analogy in earlier studies of the deracemization of a π-

acidic analyte in the presence of a chiral π-basic selector [24].

When amide **1j** is chromatographed on CSP 4, four stereoisomers are found to be present. This indicates that rotation about the $C_{(carbonyl)}$–$N_{(amide)}$ bond as well as the $C_{(aryl)}$–$C_{(carbonyl)}$ bond is slow on the HPLC time scale (Fig. 5).

The *E* and *Z* amide rotamers of **1j** were separated by chromatography on silica gel. Fig. 6 shows ^{1}H NMR spectra of the mixture of diastereomers (i), and the high (ii) and low (iii) R_F bands obtained from separation by preparative

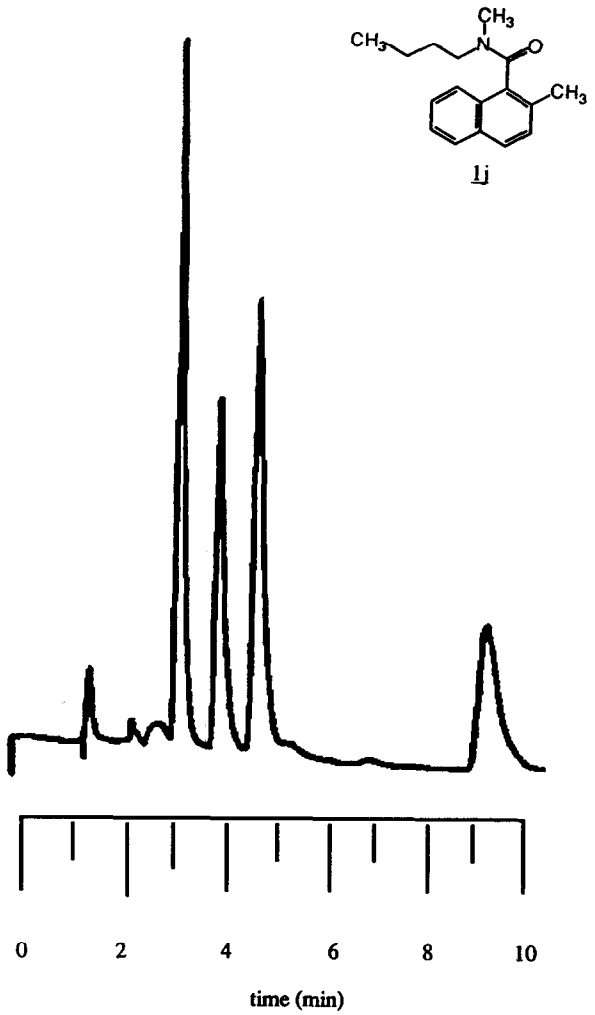
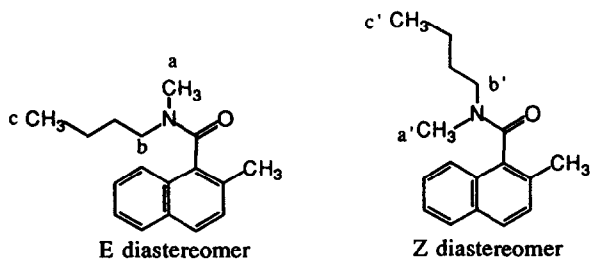


Fig. 5. Chromatographic separation of the stereoisomers of **1j**. Conditions: CSP, (*R*,*R*)-CSP 4; mobile phase, 20% 2-propanol in hexane: flow-rate, 2.0 ml/min; ambient temperature.



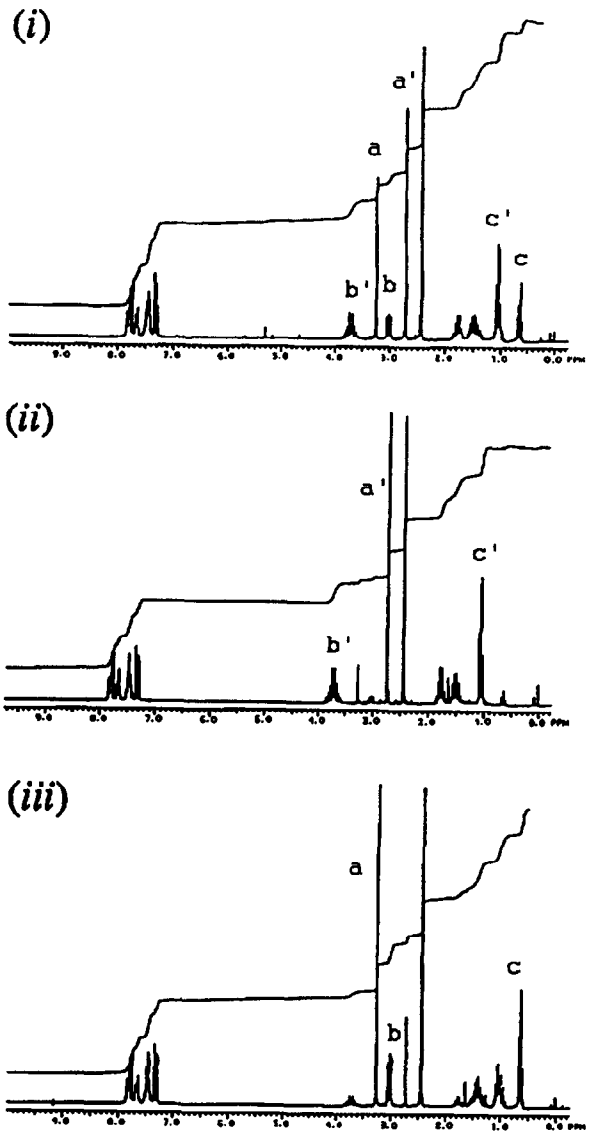


Fig. 6. 200 MHz ^{1}H NMR spectra (C^2HCl_3) of: (i) mixture of *E* and *Z* diastereomers before chromatographic fractionation; (ii) high R_F band from preparative TLC separation, assigned as *Z* rotamer; (iii) low R_F band from preparative TLC separation, assigned as *E* rotamer.

TLC (2% methanol in dichloromethane). Analysis of molecular models suggests that it is the *Z* amide rotamer which should have the signal for the amide methyl group (protons a′) in the furthest upfield position, this group being strongly shielded by the naphthyl ring. The corresponding methyl group of the *E* rotamer (protons a) is positioned close to the lone pair of the carbonyl oxygen and would accordingly be expected to occur at a relatively downfield position. Similarly the signal from the α-methylene protons of the *E* rotamer (protons b) are found upfield of the corresponding signals in the *Z* rotamer (protons b′).

Based on these arguments, the high R_F band from the preparative TLC separation contains the *Z* rotamer, while the low R_F band contains the *E* rotamer. Interestingly, even the butyl C-methyls (protons c, c′) show chemical shift differences which are consistent with this assignment, the *E* rotamer methyl resonance (proton c) occurring at the more upfield position.

Fig. 7 depicts chromatograms obtained when the two fractions from the preparative TLC separation were evaluated on (*R*,*R*) CSP 4. The stereoisomers giving rise to the first and third peaks are assigned as the enantiomers of the *Z* diastereomer, whereas those giving rise to the second and fourth peaks are assigned as arising from the *E* diastereomer. Based upon the model depicted in Fig. 4d, the four stereoisomers resolved upon (*R*,*R*) CSP 4 are assigned, in the order of their elution, as: *Z*-(*R*); *E*-(*R*); *Z*-(*S*); *E*-(*S*).

Preparative separation of a sample of **1j** on (*R*,*R*) CSP 4 afforded a sample which was highly enriched in the *Z*-(*R*) isomer. The equilibration of this enriched sample to the racemic mixture of diastereomers was monitored to determine interconversion rates for the C–N and C–C bond rotations. A schematic diagram of the possibilities for interconversion of the four species is shown in Fig. 8. Conversion of the *Z*-(*R*) to the *E*-(*R*) isomer is an amide bond rotation process which requires a single $C_{(carbonyl)}$–$N_{(amide)}$ bond rotation. Conversion of the *Z*-(*R*) to the *Z*-(*S*) isomer is an enantiomer interconversion process and requires a single $C_{(aryl)}$–$C_{(carbonyl)}$ bond rotation. Conversion of the *Z*-(*R*) to the *E*-(*S*)

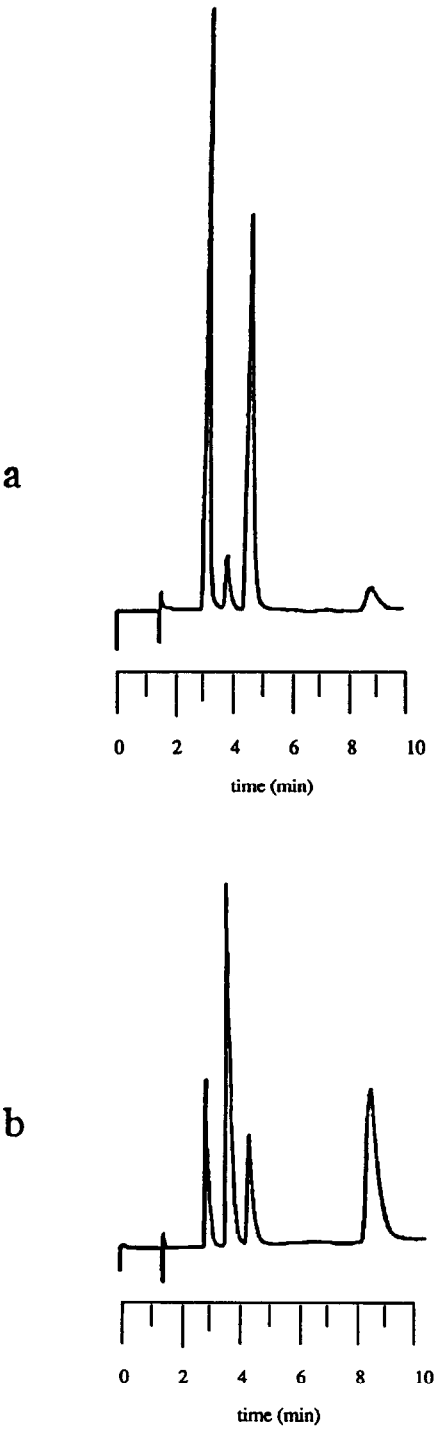


Fig. 7. Chromatographic separation of the enantiomers of the preparatively separated diastereomers of **1j**. (a) High R_F fraction; (b) low R_F fraction. Conditions: CSP, (*R*,*R*)-CSP 4; mobile phase, 20% 2-propanol in hexane; flow-rate, 2.0 ml/min; ambient temperature.

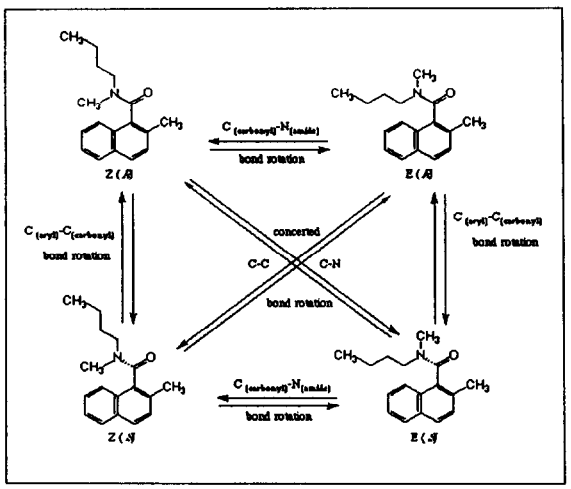


Fig. 8. Schematic representation of the interconversion of the four stereoisomers of analyte **1j**.

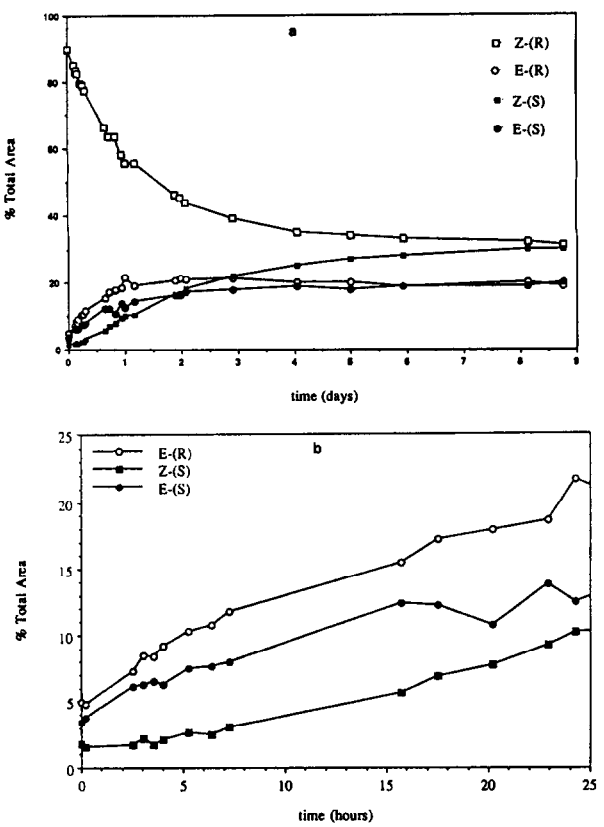


Fig. 9. (a) Time course for the equilibration at room temperature in 20% 2-propanol in hexane of the preparatively enriched *Z*-(*R*) isomer with the other stereoisomers of **1j** to afford a racemic mixture of diastereomers. (b) Expanded view of initial data showing the unexpectedly rapid formation of the *E*-(*S*) isomer.

isomer requires both a $C_{(carbonyl)}$–$N_{(amide)}$ bond rotation and a $C_{(aryl)}$–$C_{(carbonyl)}$ bond rotation. We therefore expected to see the formation of the *E*-(*S*) isomer only after significant amounts of the *E*-(*R*) and *Z*-(*S*) isomers had been formed.

Fig. 9 shows the results of this study in which the equilibration of the *Z*-(*R*) isomer to afford a racemic mixture of diastereomers was monitored by HPLC. Interestingly, the rate of $C_{(carbonyl)}$–$N_{(amide)}$ rotation is seen to be quite slow, on an order with the $C_{(aryl)}$–$C_{(carbonyl)}$ bond rotation rate, as evidenced by the rate of appearance of the *E*-(*R*) isomer. Quite unexpectedly, the rate of formation of the *E*-(*S*) isomer is even greater than the rate of formation of the *Z*-(*S*) isomer. This result can only be explained by the contribution of a concerted $C_{(carbonyl)}$–$N_{(amide)}$ and $C_{(aryl)}$–$C_{(carbonyl)}$ bond rotation, represented by the diagonals in Fig. 8. This situation, reminiscent of "dynamic gearing" described by Mislow and others [25], will be the subject of future studies.

CONCLUSION

The enantiomers of a number of axially chiral aromatic carboxamide analytes are conveniently separated using several π-acidic chiral stationary phases, the recently described CSP IV affording the better separations. Rationales accounting for the observed enantiomer separations on all of the CSPs used in the study have been advanced. Signs of optical rotation for the retained enantiomers on all of these CSPs are consistent with the proposed models, and absolute configuration of the enantiomers of these analytes have been tentatively assigned on this basis. The use of these CSPs to monitor rates of stereoisomer interconversion and to afford deracemization have been demonstrated. Amide **1j** has been shown to exist as a mixture of four slowly interconverting stereoisomers, all separable by HPLC. The rate at which the *Z*-(*R*) form equilibrates with other rotameric forms was followed, the data suggesting that concerted $C_{(aryl)}$–$C_{(carbonyl)}$ and $C_{(carbonyl)}$–$N_{(amide)}$ bond rotations may be important in the interconversion of the rotamers of hindered naphthamides.

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