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Organic Stereochemistry and Conformational Analysis from Enantioselective Chromatography and Dynamic Nuclear Magnetic Resonance Measurements

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Important progress in organic chemistry has often been linked to the availability of new separation and spectroscopic techniques. Furthermore, the need for accurate, precise, and reliable methods for determining enantiomeric purity is ubiquitous in organic synthesis and in related areas dealing with optically active compounds. Separation sciences, especially enantioselective liquid chromatography (LC), gas chromatography (GC), supercritical fluid chromatography (SFC), and capillary electrophoresis (CE) have greatly affected stereochemical practice. The results obtained through a separation on a chiral phase[1] can provide valuable information not only about the analyte under investigation (stereochemical composition and stereodynamics) but also about its selective interaction with the chiral component of the chromatographic system (enantioselective molecular recognition).

The potential of modern enantioselective chromatographic techniques as a well-established, powerful tool within the field of stereochemistry is evident from the large number of reviews, books, and data bases on this topic that have appeared in the last years.[2-5] Direct chromatographic techniques based on chiral stationary phases (CSPs) are the most appropriate for enantiomer separations in analytical investigations or on a preparative scale; moreover, the use of chiroptical detectors greatly extends the usefulness of these phases in solving stereochemical problems.[6-9]

In the present Account, stereodynamic processes mainly will be highlighted and, in particular, dynamic enantioselective chromatography (on CSP) and dynamic NMR (DNMR) will be considered together. In fact, the complementary use of these techniques allows the complete elucidation of complex dynamic phenomena in a simple and precise way.

Starting from optically active *trans*-1,2-diaminocyclohexane, a family of brush-type chiral stationary phases has been designed and synthesized in our

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(1) The following symbols are commonly used in enantioselective chromatography: $k'_{R(S)}$, the retention factor of R (or S) enantiomers, defined as the ratio of the adjusted retention time and the holdup time; $\alpha_{R,S}$, the enantioseparation factor, defined as the ratio of the retention factors of R and S; these two thermodynamically controlled parameters determine the extent of the enantioseparation; the efficiency of the column (expressed by the number of theoretical plates, N) influences band shapes and contributes to the overall separation process.

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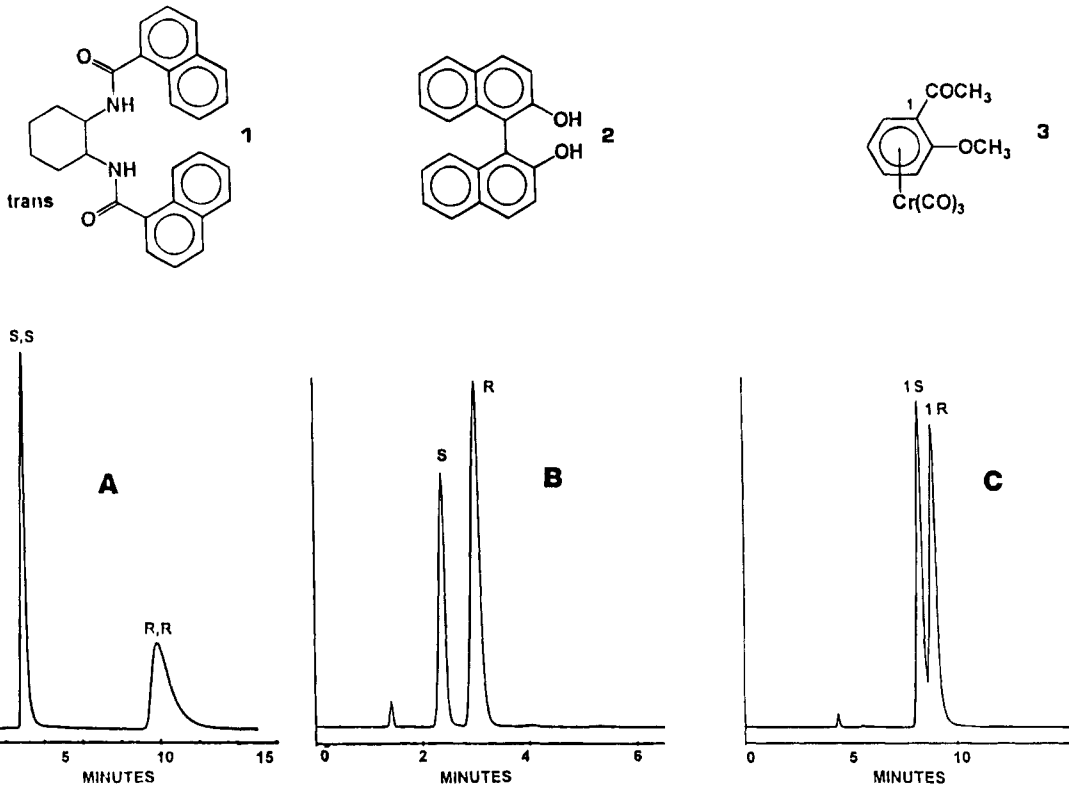
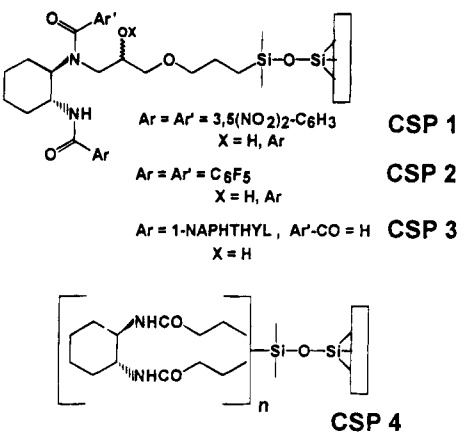


Figure 1. Enantiomeric separations of compounds **1–3** with different stereogenic elements. (A) Column: 150 × 4 mm i.d. packed with (*R,R*)-CSP 2. Eluent: *n*-hexane/2-propanol (90:10, v/v). Flow rate: 2 mL/min. *T*: 25 °C. Detector, UV at 230 nm. $K'_{S,S}$ = 2.89; α = 4.21. (B) Column: 150 × 4 mm i.d. packed with (*R,R*)-CSP 4. Eluent: CH_2Cl_2/2-propanol (90:10, v/v). Flow rate: 1 mL/min. *T*: 25 °C. Detector: UV at 280 nm. K'_S = 0.68; α = 1.69. (C) Column: 250 × 4 mm i.d. packed with (*R,R*)- CSP 1. Eluent: *n*-hexane/$CHCl_3$ (70:30, v/v). Flow rate: 2 mL/min. *T*: 25 °C. Detector: UV at 480 nm. K'_{1S} = 5.50; α = 1.10.

laboratories (Scheme 1, CSPs 1–4).[10–15] These CSPs, based on low molecular weight synthetic selectors, show good levels of enantioselectivity, high chemical and thermal inertness, and "tunable" selectivity toward specific compounds, obtained by functional group variations on the basic cyclic diamine structure (Figure 1).

Scheme 1



Dynamic Enantioselective Chromatography and Dynamic NMR Spectroscopy: Two Powerful Tools in the Study of Conformational Chirality

The difficulties often encountered in dealing with conformational chirality[16] arise partly because conformational enantiomers and diastereoisomers cannot

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(6) Lloyd, D. K.; Goodall, D. M. *Chirality* **1989**, *1*, 251.

be separated at room temperature and spectroscopic or chromatographic techniques are often unable to differentiate the rapidly interconverting species.

Conformational chirality is, however, of considerable importance in the discussion of reaction mechanism in asymmetric synthesis or drug–receptor interactions and, in general, in the elucidation of the enantiomer discrimination mechanism. Often, unfortunately, no clear distinction is made between the averaged structures observed at room temperature and the individual isomers which are important in understanding reaction mechanisms or recognition processes.

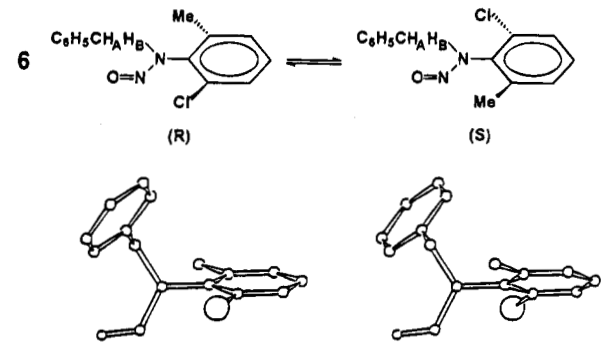
In the field of high-resolution enantioselective chromatography, techniques like high-performance liquid chromatography (HPLC), SFC, and high-resolution gas chromatography (HRGC), based on the utilization of chiral stationary phases, are extremely useful for the study of conformational chirality. The high speed of analysis and the broad range of the operating temperatures (−90 to 250 °C) permit a study of dynamic phenomena in solution in an effective way. Furthermore, the simultaneous use of DNMR is of fundamental importance because it allows the complete elucidation and assignment of very complicated patterns.

Dynamic NMR

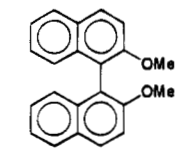
The NMR spectra of two enantiomers in nonchiral solvents are identical and, thus, indistinguishable. The same is true for a racemic mixture, which in fact yields the same spectrum as that of each of the enantiomers.[17] This is not necessarily true in the solid state where the NMR spectrum of a racemic mixture (racemate) differs, in principle, from the spectrum of the pure *S* or *R* enantiomer.[19] This is because in the solid state the NMR shifts of the racemate are affected by the intermolecular *R,S* interactions whereas in the pure enantiomer (e.g., *R*) the shifts are affected by the *R,R* interactions.

In order to distinguish the NMR solution spectra of a pair of enantiomers it is necessary to obtain the spectrum in a chiral environment. Thus if an optically pure enantiomer (henceforward called chiral solvating agent, CSA) is added for this purpose to a solution containing a racemic mixture of a certain compound, intermolecular diastereomeric interaction will occur

Scheme 2



between the CSA and the *R* and *S* enantiomers, respectively.[19,20] As an example, the introduction of Pirkle's alcohol (−)-ArCH(OH)CF_3, where Ar = 9-anthryl, **4**-(*l*)[21] to a $CDCl_3$ solution of the racemic derivative **5** yields an NMR spectrum for (*R*)-**5** different from that of (*S*)-**5**.[22] This method is widely used



to measure the enantiomeric excess of configurationally stable enantiomers. Often, however, the stereomutation between two enantiomers can be fast at room temperature where we are dealing with conformational rather than configurational enantiomers. If such a circumstance occurs, even in the presence of a CSA the NMR spectra of the two (rapidly interconverting) enantiomers will be indistinguishable. However, if these spectra are recorded at a temperature low enough to make the interconversion rate slow on the NMR time scale, the different spectra for the *R* and *S* conformational enantiomers will be again detected in a chiral environment.

One of the first examples reported was that of (*E*)-(2-chloro-6-methylphenyl)benzylnitrososamine, **6**, which in the presence of a CSA such as (+)-(*S*)-2,2,2-trifluoro-1-phenylethanol yields distinct NMR spectra for the *R* and *S* enantiomers at a temperature equal to or lower than 10 °C (Scheme 2). Here, the methyl group in position 2 on the phenyl ring displays two lines separated by 0.02 ppm.[23] An even larger separation is obtained[24] when the paramagnetic derivative $Eu(hfc)_3$, i.e., (+)-tris[3-[(heptafluoropropyl)hydroxymethylene]-*d*-camphorato]europium(III), is used as the CSA.

When the temperature is raised above 10 °C, the rotation becomes faster and the *R* enantiomer interconverts into the *S* (in other words, the molecule acquires a dynamic plane of symmetry, thus losing its chirality). As the temperature is raised, the spectral lines broaden and eventually coalesce much in the same way observed for the usual exchange process (DNMR).[25]

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(8) Mannschreck, A. *Chirality* **1992**, *4*, 163.
(9) Salvadori, P.; Rosini, C.; Bertucci, C. *J. Org. Chem.* **1984**, *49*, 5050.
(10) Gasparrini, F.; Misiti, D.; Villani, C. *Chirality* **1992**, *4*, 447.
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(13) Gasparrini, F.; Misiti, D.; Villani, C.; La Torre, F.; Sinibaldi, M. *J. Chromatogr.* **1988**, *457*, 235.
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(15) Gasparrini, F.; Maggio, F.; Misiti, D.; Villani, C.; Andreolini, F.; Mapelli, G. P. *J. High Resolut. Chromatogr.* **1994**, *17*, 43.
(16) Dodziuk, H. *Tetrahedron: Asymmetry* **1992**, *3*, 43.
(17) In a few cases the occurrence of strong diastereomeric solute–solute interactions can cause nonracemic mixtures of enantiomers to yield distinct NMR solution spectra,[18] much in the same way as usually observed in the solid state spectra.[19]
(18) (a) Williams, T.; Pitcher, R. G.; Bommer, P.; Gutzwiller, J.; Uskokowic, M. *J. Am. Chem. Soc.* **1969**, *91*, 1871. (b) Harger, M. J. P. *J. Chem. Soc., Perkin Trans. 2* **1977**, 1882. (c) Dobashi, A.; Saito, N.; Motoyama, Y.; Hara, S. *J. Am. Chem. Soc.* **1986**, *108*, 307. (d) Luchinat, C.; Roelens, S. *J. Am. Chem. Soc.* **1986**, *108*, 4873 and references quoted therein.
(19) (a) Hill, H. C.; Zens, A. P.; Jacobus, J. *J. Am. Chem. Soc.* **1979**, *101*, 7090. (b) Andersen, K. V.; Bildsfe, H.; Jacobsen, H. J. *Magn. Reson. Chem.* **1990**, *20*, 347. (c) Parker, D. *Chem. Rev.* **1991**, *91*, 1441.
(20) Rinaldi, P. L. *Prog. Nucl. Magn. Reson. Spectrosc.* **1983**, *15*, 291.
(21) Pirkle, W. H. *J. Am. Chem. Soc.* **1966**, *88*, 1837.
(22) Gottarelli, G.; Spada, G. P. *J. Org. Chem.* **1992**, *56*, 2096.
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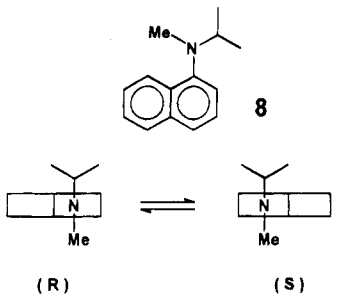
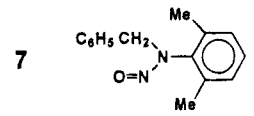
(25) Sandstroem, J. *Dynamic NMR Spectroscopy*; Academic Press: London, 1982.

Scheme 3



In the case quoted above the $\Delta G^{\ddagger}$ value was found to be 17.1 or 16.5 kcal/mol, depending on the chiral auxiliary employed.[24,26] In **6** a prochiral probe is also present, i.e., the methylene group of the benzyl moiety.[27,28] Below 10 °C derivative **6** does not display any element of symmetry; thus the two CH_2 hydrogens yield a pair of signals *even* in a nonchiral environment. Thus the enantiomerization rate, in the present case, can also be monitored in the absence of CSA and the $\Delta G^{\ddagger}$ compared with that obtained in a chiral solvent. The value obtained for **6** in CCl_4 (16.5 kcal/mol) matches well[26] that previously obtained in the chiral auxiliary Eu(hfc)$_3$, using the NMR signals of the Me group. When the chlorine atom of **6** is replaced by a methyl group, derivative **7** will maintain a plane of symmetry even when the Ar–N rotation is slow: i.e., the two enantiomers now will become enantiotopomers.[29]



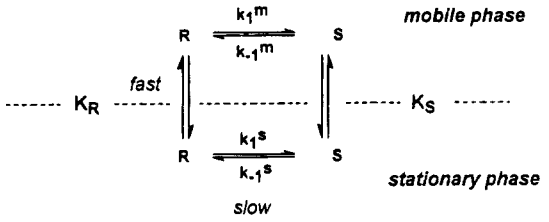
As a consequence, in an achiral environment the NCH_2 hydrogens will be isochronous even at low temperature and, consequently, the corresponding signal can no longer be used to monitor the internal motion. In a chiral environment, on the contrary, two different signals for the two methyl groups will be visible at an appropriate low temperature. These methyl groups are in fact diastereotopic with respect to the stereogenic center of the CSA when the Ar–N rotation is slow. Although in **7** we are no longer dealing with a pair of enantiomers, we can still monitor the Ar–N rotational process and obtain the corresponding enantiotopomerization barrier.[24]

With the progress in NMR techniques, this type of investigation has been extended into a much lower temperature range, allowing the NMR detection of much shorter living conformational enantiomers.

For instance, *N*-methyl-*N*-isopropyl-1-naphthylamine (**8**) exists as a pair of conformational enantiomers since the dynamic plane containing the rapidly inverting N atom and the plane of the naphthalene ring do not coincide, as shown in Scheme 3.

At a temperature of −80 °C this situation becomes NMR visible in an achiral solvent where the two isopropyl methyl groups yield anisochronous signals. If the spectrum is obtained at the same temperature

Scheme 4



in the presence of a CSA such as Pirkle's alcohol **4**-(*l*), two groups of signals are expected for the *R* and *S* conformational enantiomers. Variable temperature studies revealed an enantiomerization barrier for **8** as low as 10.7 kcal/mol.[30] In derivative **9** (*N*-methyl-*N*-*tert*-butyl-1-naphthylamine) the presence of a CSA is essential for the NMR detection of conformational chirality. Only under these conditions can one observe, for instance, two signals for the *N*-methyl group (below 19 °C) which coalesce at 52 °C, allowing the determination of a much higher enantiomerization barrier than for **8** (i.e., 19.1 kcal/mol). Also in the case of *N*,*N*-dimethyl-1-naphthylamine (**10**), the presence of the auxiliary chiral Pirkle's alcohol allows the detection of the diastereotopicity of the *N*,*N*-dimethyl group at −120 °C.[31] On warming above −93 °C the signals coalesce and a rotational barrier ($\Delta G^{\ddagger}$ = 8.3 kcal/mol) can be determined.

Introduction of CSAs into a solution to create diastereotopic relationships between otherwise enantiotopically related groups has been found effective also in cases where inversion, rather than rotation processes, is involved. For instance, in dimethylethylamine (Me_2NEt, **11**) the inversion at the N atom cannot be monitored by NMR in achiral environments because the plane bisecting the CH_2 moiety of the prochiral ethyl group is also a molecular plane of symmetry even for a pyramidal nitrogen. In the presence of a chiral solvating agent, however, the two methyl groups will become diastereotopic at a temperature of −90 °C, where the lifetime of the pyramidal conformation is long enough with respect to the NMR time scale; the N inversion, which interconverts one enantiotopomer into the other, can be monitored under these conditions and a $\Delta G^{\ddagger}$ value of 10.8 kcal/mol determined.[31]

Dynamic Enantioselective Chromatography

Chromatography of chiral compounds, undergoing reversible interconversion on an optically active sorbent, produces typical peak shape deformations resembling those encountered in dynamic NMR spectroscopy. In the simplest case of two interconverting enantiomers *R* and *S* analyzed on a chiral stationary phase, two sets of equilibria occur during the passage of the analytes through the column, as illustrated in Scheme 4.[32,33]

Boldface arrows indicate adsorption equilibria of *R* and *S* between the mobile and stationary phase (primary equilibria). These are the processes that

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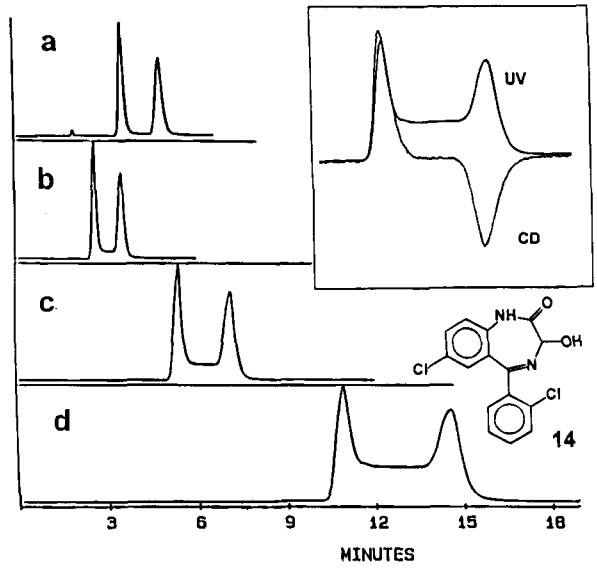

Figure 2. Variable temperature (a, b) and variable flow (b–d) HPLC of racemic stereolabile 3-hydroxybenzodiazepin-2-one (**14**) on (*R,R*)-CSP 4 (150 × 4.0 mm i.d.). Eluent: CH_2Cl_2/2-propanol (90:10, v/v). Flow rate (mL/min): (a, b) 1.0, (c) 0.5, (d) 0.25. *T* (°C): (a) 25, (b–d) 65. Detector: UV at 265 nm. Inset: UV and CD detections at 265 nm; other conditions as in part d ($\Delta G^{\ddagger}_{298}$ = 22.7 ± 0.1 kcal/mol).

govern both retention and enantioselectivity, and if enantiomer separation occurs, K_R is different from K_S, i.e., $\alpha \neq 1$.

The second set of equilibria is related to the reversible exchange processes between *R* and *S* occurring in the mobile and stationary phases (secondary equilibria). If the mobile phase is achiral, as in chiral-GC and in most of the chiral-HPLC systems, the two rate constants k_1^m and k_{-1}^m are equal; on the other hand, the two rate constants, k_1^s and k_{-1}^s are different if *R* and *S* have different retention times. The chiral component of the stationary phase not only discriminates between *R* and *S* but also selectively influences the rates of the forward and backward enantiomerization in the stationary phase.

Moreover, secondary equilibria may take place at different rates in the mobile phase and on the stationary phase, i.e., $k^m \neq k^s$.[34] Variation of the original enantiomeric ratio of the enantiomers after passage through a column is unlikely to occur under usual chromatographic conditions. However, in a recently described[35] on-column deracemization experiment, enantiomeric enrichment of an atropisomeric naphthylamide (*N,N*-dimethyl-2-methyl-1-naphthalenecarboxamide, **12**) was observed after a 5 day contact time period of the racemate with the β-amino acid derived CSP [(*R,R*)-undecyl 3-[*N*-(3,5-dinitrobenzoyl)-amino]-3-phenyl-2-(1,1-dimethylethyl)propanoate, **13**]. If the exchange and separation processes have comparable characteristic times, a typical chromatographic profile, with an intermediate plateau between the resolved peaks, will be observed (Figure 2c, d). The overlapping zone between the extreme peaks arises from the detection of molecules of *R* and *S* which have undergone at least one inversion during their flow through the column and has a racemic composition which cannot be identified by chiroptical detectors.

As a rule of the thumb, peak deformations due to secondary equilibria can be observed if the half-life of the exchanging species is of the same order of magnitude as their average retention times ($\tilde{t}_{RS}$). For a standard HPLC separation with $\tilde{t}_{RS}$ = 20 min at 25 °C, chromatographic pattern deformations will appear if the energy barrier for the exchange reaction is around 22 kcal/mol, provided the column shows sufficient enantioselectivity and efficiency.

At lower temperatures the separation process becomes faster than the isomerization and two peaks, without an intermediate plateau, will be observed. In

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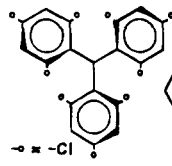
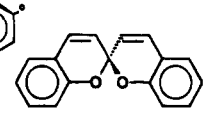
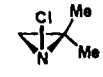
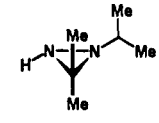
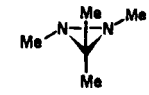
Table 1. Peak Shape Analysis in Enantioselective Chromatography

compound	 **15**	 **16**	 **17**	 **18**	 **19**	 **20**
$\Delta G^{\#}_{sim}$ (kcal/mol)	19.2	23.9	25.1	26.1	27.5	27.0
temperature (°K)	268	318	333	353	353	363
technique	HPLC	HPLC	complexation GC	inclusion GC	inclusion GC	complexation GC
eluent	methanol	methanol	/	/	/	/
method	A	A	B	B	B	B
reference	32	42	34	33	33	43
$\Delta G^{\#}_{exper}$ (kcal/mol)	19.6	23.7	27.6	~26	~28	27.2
temperature (°K)	268	318	338	363	363	363
technique	DNMR	polarimetry	chiral GC	DNMR	DNMR	polarimetry
solvent	C_4Cl_6/$^2H_6$-DMSO (1:1)	methanol	gas phase	$^2H_8$-toluene	$^2H_8$-toluene	dioxane
reference	32	42	44	45	45	46

A: Stochastic model. B: Discontinuous plate model

contrast a single peak will be observed upon complete coalescence at higher temperatures.

Variable temperature and variable flow HPLC are illustrated in Figure 2 for the resolution of a racemic, stereolabile 3-hydroxybenzodiazepin-2-one (**14**).[36] The effect of changing temperature or flow rate (or both) on the elution profile is diagnostic of on-column interconversion. The amount of substance detected in the reaction zone between the *R* and *S* peaks is indicative of the relative rates of separation and isomerization, for each combination of temperature and eluent flow rate.

Recently, kinetic data for the exchange processes occurring during chromatography have been obtained by peak form analysis using either a continuous flow model[37] or a stochastic model[38,39] (A) or a discontinuous plate model[40,41] (B). The entire elution profile can be simulated this way, yielding the apparent rate constants for the overall process in both phases. If the rate constant in one phase (usually the mobile phase) is known from independent experiments, the simulation procedure gives the two separate rate constants k^m and k^s as well as the rate constants for the forward and backward isomerization occurring on the stationary phase. Successful applications of this procedure to the study of interconverting chiral compounds are listed in Table 1.[32-34,42-46]

Inspection of the data reveals that the peak form analysis gives values for the energy barriers very close to those determined by other, well-established techniques (DNMR, racemization of optically enriched samples followed by polarimetry or enantioselective chromatography). Attractive features of this new technique are the low amounts of (racemic) samples required (10^{-8} g) and the typical working temperatures (from −80 to 120 °C for HPLC up to 200 °C for GC) which enable the study of isomerization processes with energy barriers in the range 17−30 kcal/mol for HPLC and 22−36 kcal/mol for GC. Extension of this temperature range is limited (at high *T*) by the thermal stability of chiral GC phases. In the low-*T* range, practical difficulties are encountered in HPLC as a consequence of low sample solubility and increased eluent viscosity; in this context, subcritical fluid chromatography (sub-FC) has a particular advantage over HPLC, because very high linear flow rates and/or low column temperatures can be used without degrading the efficiency of the column.[47]

Additional examples of on-column isomerization reactions of chiral compounds during HPLC have been reported for 1-(dimethylamino)-8-(dimethylcarbamoyl)naphthalene,[48] various *N,N*-dimethylthiobenzamides,[49] and 1,3,7-trimethylbenzo[*c*]phenanthrene.[50] For the latter compound, which shows flow and temperature dependent peak deformations upon enantioselective chromatography on CSP, a deconvolution procedure was employed to obtain two separate elution profiles for each of the enantiomers. An additional application of this technique, based on a double polarimetric/photometric detection followed by computer deconvolution, is shown in the determination of enantiomeric purity in the presence of overlapped peaks.[51]

In comparison with other techniques, GC and HPLC offer the advantage that nearly pure enantiomers can be isolated for further nonchromatographic investigations. For example, CD spectra recording and thermal racemization can be carried out conveniently after chromatography on a CSP, either using on-line chiroptical detectors equipped with a temperature control facility[7] or collecting the individual isomers at the appropriate temperature.

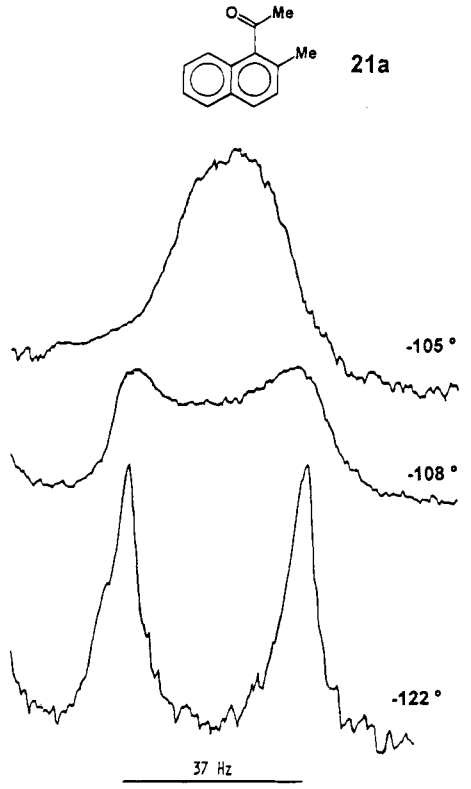


Figure 3. The 200 MHz spectrum of the Me group of **21a** in a chiral medium displays two lines at −122 °C for the two conformational enantiomers (atropisomers). The signals coalesce at −108 °C, having $\Delta G^{\ddagger} \approx 8$ kcal/mol for the enantiomerization process.

A large number of interesting chiral compounds have been separated and thoroughly investigated, taking advantage of the broad selectivity offered by modern chiral phases. Absolute configuration or stereochemical stability or both have been obtained for molecular propellers,[52] triarylboranes,[53] decakis(dichloromethyl)biphenyl,[54] overcrowded ethylenes,[55,56,57] substituted phenanthrenes,[58] *N*-aryl-4-pyridones,[59] polarized alkenes,[60] benzo[2.2]metacyclophane,[61] and substituted diarylbenzocycloheptene[62] with energy barriers to interconversion ranging from 16.1 to 31.6 kcal/mol.

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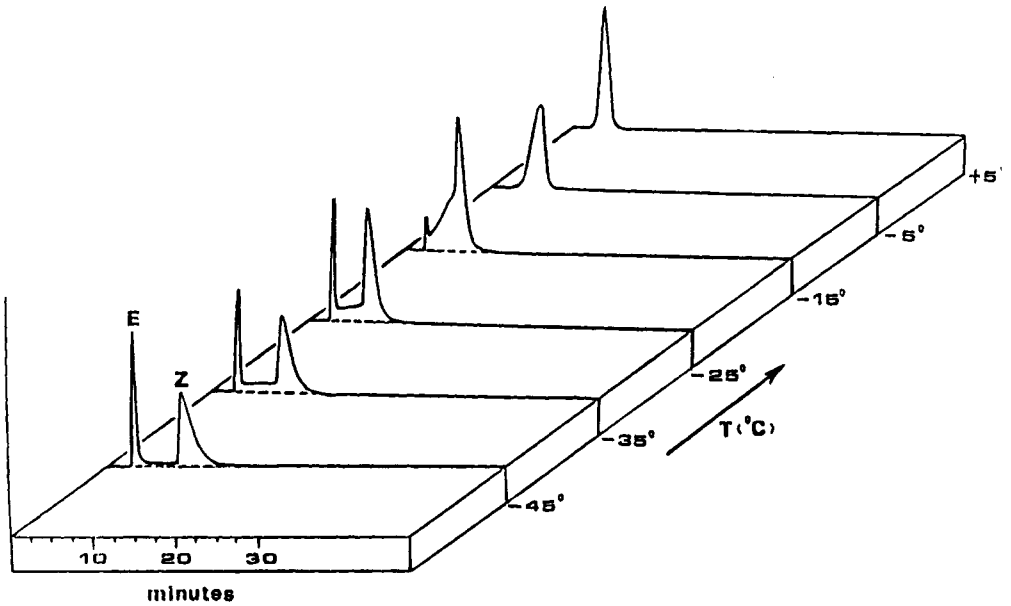
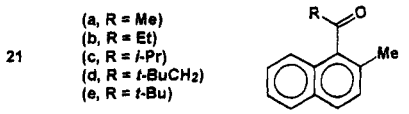


Figure 4. Dynamic HPLC of racemic **25d** on Rac-CSP1 LiChrosorb Si100 5 μ (racemic version of CSP 1) (150 × 4.0 mm i.d.). Eluent: *n*-hexane/2-propanol/methanol (80:20:0.5, v/v/v). Flow rate: 2.0 mL/min. Detector: UV at 300 nm.

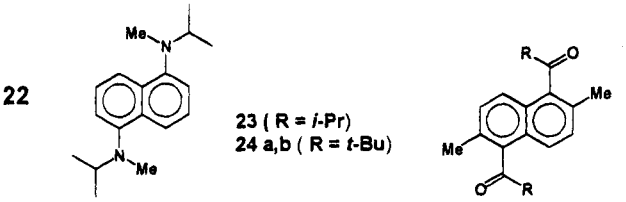
Dynamic Chromatography and Dynamic NMR: Two, in Tandem, Powerful Tools in the Study of Complex Conformational Chirality Problems

Hindered ketones such as **21** adopt a twisted conformation (i.e., the RCO plane is not coplanar with the naphthalene ring) and may thus exist as a pair of conformational enantiomers. When R is not a prochiral



probe (for instance, when R = Me, **21a**, or *t*-Bu, **21e**), the process will be detectable only in the presence of a CSA. Consider Figure 3, where the H-1 signal of the 2-Me group of **21a** in the presence of Pirkle's alcohol, **7**, is shown at various temperatures in a CHF_2Cl solution. The signal, which is a sharp singlet down to −80 °C, broadens at −100 °C, decoalesces at −110 °C and eventually yields *two* sharp singlets (intensity 1:1) below −120 °C. Line shape analysis yields an enantiomerization barrier of about 8 kcal/mol.[63]

When two stereogenic axes are present in the same molecule, they may give rise to a pair of conformational diastereoisomers. An example is offered by the 1,5-disubstituted derivatives of naphthalene **22** or **23** that exist as meso (*trans*) or racemic (*cis*) conformers. It is, however, difficult to assign the structure to the corresponding low-temperature NMR spectrum as the observed chemical shifts are not diagnostic for this purpose.[64,65]



Moreover, solid state NMR can occasionally help to distinguish the two species[64] as it is sensitive to the molecular symmetry. An unambiguous method requires the use of a chromatographic separation on an enantioselective column (enantioselective chromatography) cooled at convenient low temperatures (the appropriate temperature can be deduced by a knowledge of the conformer lifetimes as determined by DNMR experiments).

In these columns the racemic diastereoisomer (**24b**) will be further separated into a pair of conformational

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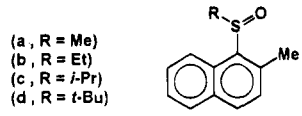
enantiomers whereas the meso conformer (**24a**) will not. Combination of the basic dynamic chromatography experiment (in the form of variable temperature analysis) with chiral/achiral column switching and simultaneous UV–CD detections resulted in a complete conformational description of 2,6-dimethyl-1,5-bis(2,2-dimethylpropanoyl)naphthalene (**24**).[63]

The dynamic chromatographic pattern shows fast and slow exchange at two extremes of the temperature range and an intermediate range (0–35 °C) where extensive peak broadening was observed as a consequence of comparable rates for the exchange and separation processes. The total number of conformers and their relative populations were easily obtained by chromatography at low temperature on (*R,R*)-CSP 1 [**24a** (meso) = 76%; **24b** (chiral) = 24%]. The enantiomeric/diastereomeric relationships between the observed peaks are evident from the coalescence of the last two eluted ones (when **24** is analyzed) on the racemic version of CSP 1 and from the bisignate CD trace observed for the same peaks only on the chiral phase.

The difference in the populations of the meso (**24a**) and racemic conformers (**24b**) observed in the chromatographic separation corresponds to the difference in the intensities of the NMR signals, thus allowing the assignment of the corresponding chemical shifts. On-line CD spectral acquisition at low temperature completed the conformational/configurational description of **24** ($\Delta G^{\ddagger}$ = 19.8 kcal/mol; *R,R* absolute configuration assigned to the most retained enantiomer of **24b**).[63]

A further consequence of conformational enantiomerism also can be anticipated if a molecule possesses a conformational stereogenic axis and, in addition, a configurational stereogenic center. In this case, in fact, a pair of conformational diastereoisomers should be detectable. An example of such a situation occurs in the naphthyl sulfoxides **25**. These molecules adopt



two distinct *E,Z* conformations, due to restricted rotation about the Ar–SO bond. Furthermore, the sulfur atom provides a configurational stereogenic center. As a consequence, sulfoxides **25** are expected to exist as a pair of *E,Z* diastereomeric conformers of different stability, and these were indeed detected by NMR at appropriate low temperatures.[66] In the case of **25d**, nuclear Overhauser experiments (NOE) and lanthanide-induced shift (LIS) experiments allowed us to ascertain the structure of the most stable species. The *Z* structure (which is the only form present in the solid state) actually corresponds to that of the conformer found to be the more stable in solution. HPLC on a CSP at room temperature only allows the separation of the two configurational enantiomers due to the sulfur atom. On lowering of the temperature to −35 °C, four stereoisomers can be separated: *ES*, *ER*, *ZS*, and *ZR*. At intermediate temperatures a single, broad peak containing all the interconverting species was detected. Dynamic enantioselective chromatography carried out on the racemic version of the CSP (Figure 4) showed a simplified pattern with the number of peaks being reduced as only the two diastereomeric conformers were resolved.

At −45 °C the extent of on-column isomerization was negligible, as judged by the absence of peak deformations. The relative populations of the *E* and *Z* conformers, obtained by peak integration, were found to be in good agreement with those determined by NMR.[66]

To assign each eluted peak to the appropriate structure it was necessary only to know the absolute configuration at the sulfur atom, since the *E,Z* conformation, due to the restricted Ar–SO rotation, had been established previously by NOE and LIS experiments in solution. An asymmetric synthesis allowed the assignment of the absolute configuration to the enantiomers due to the sulfur atom. When this result was combined with the NMR determinations, it was established that the elution order, at −35 °C, corresponds to the sequence *ES*, *ER*, *ZS*, *ZR* on the (*S,S*) chiral phase.

Final Comment

Parallel application of dynamic NMR and dynamic chromatography greatly enhances the quality and quantity of stereochemical information obtainable by each of the individual techniques alone; NMR provides direct structural information and has a characteristic time considerably lower than HPLC or GC; on the other hand, chromatography allows a facile physical separation of the stereoisomers of interest, thus enabling further structural investigations to be carried out in a straightforward manner.

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AR930079V

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