Stereolabile chiral compounds: analysis by dynamic chromatography and stopped-flow methods

Christian Wolf

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Enantiomerization and diastereomerization reactions of chiral compounds play a major role in all aspects of chemistry spanning a wide bridge from drug development to supramolecular chemistry. Traditionally, these reactions are studied by variable-temperature NMR spectroscopy and chiroptical methods such as polarimetry. However, powerful complimentary methods based on chromatography and electrophoresis have been developed and applied to a variety of stereolabile chiral compounds. This *tutorial review* explains the principles, applications, and limitations of dynamic chromatography and chromatographic and electrophoretic stopped-flow analysis for the investigation of isomerization reactions of chiral compounds.

Introduction

Dynamic stereochemistry deals with the three-dimensional structure of interconverting conformational or configurational isomers as a function of time. The determination of isomerization kinetics, *i.e.* rate constants and activation parameters ($\Delta G^{\neq}$, $\Delta H^{\neq}$, $\Delta S^{\neq}$), therefore plays a crucial role in the study of stereolabile compounds and is routinely performed using either spectroscopic, chiroptical or chromatographic techniques. As a rule of thumb, an activation barrier of 100 kJ mol^{-1} of an irreversible first-order reaction corresponds to a reaction rate of 1.89 $\times$ 10^{-5} s^{-1} at 25 °C. In general, $k = 2.084 \times 10^{10} \times T\,\mathrm{e}(-\Delta G^{\neq}/8.314 \times T)$, where T is the temperature in Kelvin and $\Delta G^{\neq}$ is the energy barrier in J mol^{-1}.

Christian Wolf

Christian Wolf graduated from the University of Hamburg, Germany, in 1993, where he received his Ph.D. in 1995. In 1997, after a post-doctoral appointment as a Feodor-Lynen Fellow at the University of Illinois in Urbana, IL, he took an R&D position at SmithKline Beecham Pharmaceuticals, King of Prussia, PA. Since 2000 he has held the position of Assistant Professor in the Chemistry Department at Georgetown University. He was a visiting scholar at the University of Reading, UK, in 1991 and at the University of Aix-Marseille, France, in 1995. He was awarded an Erasmus Fellowship (1991; European Union), a Ph.D. Fellowship (1993; Fund of the German Chemical Industry), a Feodor Lynen Fellowship (1996; Alexander von Humboldt Foundation), a Silver and a Gold Impact Award (1998, 2000; SmithKline Beecham Pharmaceuticals), and an NSF CAREER Award (2004; National Science Foundation). His current research interests are stereodynamics of chiral compounds, asymmetric synthesis, stereoselective sensing, palladium-catalyzed cross-coupling reactions, antimalarial drug development, and enantioselective chromatography.

Interconversion processes of conformationally or configurationally unstable chiral molecules can be treated macroscopically as a change toward a racemic (interconversion of enantiomers) or non-racemic (interconversion of diastereoisomers) mixture of stereoisomers at thermodynamic equilibrium or they can be analyzed microscopically, *i.e.* on the molecular level. The former approach discusses an irreversible process, whereas the latter can, in principle, provide individual rate constants for each interconversion reaction thus including reversibility (Fig. 1).[1] The interconversion of enantiomers can therefore macroscopically be considered an irreversible racemization process with a rate constant, k_{rac}, that is completed when 50% of an enantiopure compound has been converted to the other enantiomer. The corresponding half-life time is the time in which the enantiomeric excess of a chiral mixture has been reduced to half its initial value, for example from 100% to 50%. On a microscopic level, the process is perceived as a reversible enantiomerization reaction with a rate constant, $k_{enant} = 0.5\ k_{rac}$, that is completed after all molecules have been converted to the other enantiomer. The half-life time of

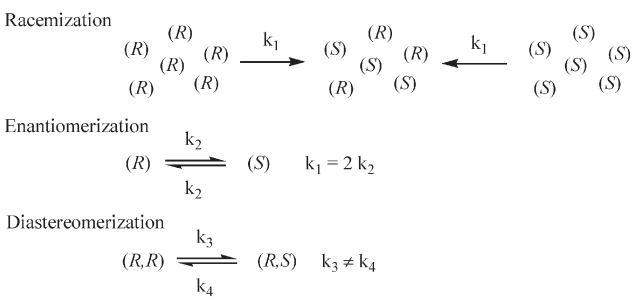

Fig. 1 Classification of isomerizations of chiral compounds that interconvert *via* one transition state.

enantiomerization is the time required for 50% interconversion, *i.e.* a change from 100% ee to 0% ee. Although enantiomerization reactions usually obey reversible first-order kinetics and therefore exclude complete enantioconversion such unidirectionality has been observed for (*R*)-profens that are enantioselectively and irreversibly converted to the (*S*)-enantiomer by coenzyme A conjugate.[2,3] Unidirectionality and deracemization can also be achieved if rapid enantioconversion is coupled with either stereoselective transformation of one of the enantiomers (asymmetric transformation of the first kind) or with separation such as selective crystallization of one stereoisomer (asymmetric transformation of the second kind). Monitoring the change in the optical rotation or circular dichroism of a chiral non-racemic mixture will provide the rate of racemization, while other methods such as dynamic chromatography or proton-deuterium substitution which can easily be monitored by ^{1}H-NMR spectroscopy afford enantiomerization rates. Although enantiomerization and racemization kinetics can be used to describe the same process it is important to distinguish between the two mathematical treatments and the corresponding different rate constants k_{enant} and k_{rac}.

The interconversion of diastereomers is a reversible microscopic process that is more complex than enantiomerization because the rate constant for the transformation of one diastereomer to another is usually different from the rate constant for the reverse reaction. An equimolar ratio is generally not observed at equilibrium due to the different thermodynamic stability of diastereomers. Epimerization is a special case of diastereomerization as it refers to the interconversion of epimers, which are defined as diastereoisomers that differ in only one configuration of two or more chiral elements. The macroscopic analog to epimerization is mutarotation, which refers to the irreversible change of the optical rotation of an epimeric mixture until equilibrium is reached.

In principle, fast dynamic molecular processes can directly be investigated by flash photolysis and various spectroscopic methods including microwave, infrared, electron spin resonance (ESR) or nuclear magnetic resonance (NMR) spectroscopy. The interconversion of chiral stereoisomers exhibiting activation barriers between 20 and 100 kJ mol^{-1} is often monitored by variable-temperature (dynamic) NMR spectroscopy, whereas chiroptical techniques (polarimetry, circular dichroism and optical rotary dispersion) and chromatographic methods are usually employed in isomerization studies of more stable compounds. The choice of a suitable technique for the study of a given enantio- or diastereoisomerization reaction mainly depends on the time scale, *i.e.* reaction rate, availability of isolated stereoisomers, and the ability of the above mentioned methods to differentiate between the interconverting stereoisomers. In contrast to chiroptical methods, the employment of variable-temperature NMR spectroscopy, dynamic chromatography, and chromatographic stopped-flow techniques in stereodynamic studies usually renders isolation of pure enantiomers or diastereoisomers unnecessary. The latter techniques also require smaller sample amounts and are much more convenient to use. In addition, stereoisomers exhibiting an interconversion barrier above 100 kJ mol^{-1} can often be isolated or enriched by chromatography or through crystallization at room temperature which greatly facilitates indirect studies of isomerization reactions. In these cases, preparative amounts of an isolated stereoisomer are heated and the isomerization processes are monitored externally by analyzing small aliquots of the reaction mixture with enantioselective chromatography, polarimetry or NMR spectroscopy.

This review will focus on the use of chromatographic and electrophoretic techniques for direct analysis of interconversion reactions of stereolabile chiral compounds. In the first section, principles of dynamic chromatography will be discussed. The following sections will examine the application spectrum and limitations of dynamic HPLC, GC, and related techniques. Finally, chromatographic and electrophoretic stopped-flow analysis will be introduced as a complimentary tool for the determination of isomerization kinetics of chiral compounds.

Dynamic chromatography

Chromatography (GC, HPLC, SFC, SubFC, CEC, and MEKC) and capillary zone electrophoresis (CZE) provide powerful means for the determination of stereoisomer composition, *i.e.* enantiomeric and diastereomeric excess. In particular, enantioselective gas chromatography (GC), high performance liquid chromatography (HPLC), and supercritical fluid chromatography (SFC) are widely used techniques for the separation of enantiomers for analytical and preparative purposes. In analogy to variable-temperature NMR spectroscopy with stereolabile compounds, dynamic processes resulting in peak coalescence can be observed by chromatography as a consequence of simultaneous resolution and on-column interconversion of stereoisomers. A successful chromatographic separation of stereoisomers affords two distinct peaks. However, stereoisomers may undergo interconversion during the chromatographic process at elevated temperatures. The competition between resolution and isomerization results in an elution profile showing a plateau between the peaks. The plateau formation is a consequence of on-column isomerization which increases with temperature. Finally, the interconversion process becomes much faster than the chromatographic separation at high temperatures and one observes peak coalescence (Fig. 2).

Based on the rate of enantioconversion, *i.e.* the enantiomerization barrier, of a given chiral compound one can therefore expect three different chromatographic scenarios. If enantiomerization is slow compared to the chromatographic timescale and the chiral stationary phase (CSP) used can distinguish between the enantiomers, two baseline-separated peaks will be obtained. However, competition between chromatographic resolution and enantiomerization will result in the formation of a temperature-dependent plateau between the two peaks and only one peak is observed when the enantiomerization proceeds faster than the separation. This has been observed when 2,2′-di*iso*propyl-, 2-*tert*-butyl-2′-*iso*propyl-, and 2,2′-di-*tert*-butylbiphenyl were separated at 165 °C by GC using a selectively modified cyclodextrin CSP (Fig. 3).[4] Because of the high conformational stability of

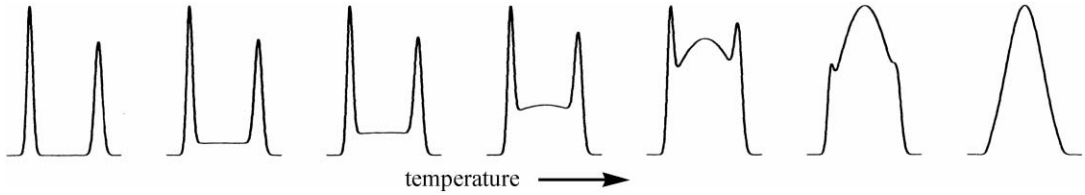

Fig. 2 Competition between chromatographic resolution and on-column stereointerconversion resulting in the formation of plateaus and peak coalescence.

2,2′-di-*tert*-butylbiphenyl both enantiomers are separated and show no sign of interconversion. Replacement of the sterically demanding *tert*-butyl moiety with the smaller *iso*propyl group decreases the rotational energy barrier and 2-*tert*-butyl-2′-*iso*propylbiphenyl undergoes significant isomerization in less than 10 minutes. Finally, 2,2′-di*iso*propylbiphenyl, which can be separated into enantiomers on cyclodextrin-derived CSPs at lower temperatures, is not stable to enantiomerization at 165 °C and only one racemic peak is observed.

The chromatographic separation process is often described as a discontinuous process using a theoretical plate model. Accordingly, the column is segmented into N theoretical plates exhibiting selective distribution of the analytes between the mobile and the stationary phase. Upon completion of the partitioning process in each plate, the mobile phase and its components are shifted to the next theoretical plate to undergo another separation step, and so on. In order to include isomerization competing with the chromatographic separation processes, each theoretical plate is considered a chemical reactor.[5] Schurig and co-workers were the first to systematically incorporate both resolution and enantiomerization of chiral compounds into a computer program based on the theoretical plate model (Fig. 4).[6] They were able to simulate the interconversion and partitioning equilibria of two enantiomers including achiral and chiral contributions to chromatographic retention and enantioconversion. The partition coefficient K_L denotes the distribution of the enantiomers between the mobile phase (MP) and the achiral stationary phase (SP), whereas the retention increment, R, describes the partitioning of the enantiomers between the achiral and the CSP. The enantiomers can undergo interconversion in the gas, achiral stationary, and in the chiral stationary phase exhibiting four different rate constants, k_{MP}, k_{SP}, k_{CSP}, and k'_{CSP}. The simulation of elution profiles requires the determination of the number of theoretical plates, N, void volume time, t_0, peak width at half height, $b_{0.5}$, retention times of the enantiomers, t_1 and t_2, and the enantioselectivity factor, α. Iterative optimization of the enantiomerization rate provides simulated elution profiles that are superimposable with the experimentally obtained chromatograms.

Wolf and coworkers observed plateau formation through peak analysis of the gas chromatograms obtained with 2,2′-bistrifluoromethylbiphenyl at varying temperature with octakis(6-*O*-methyl-2,3-di-*O*-pentyl)-γ-cyclodextrin as the CSP (Fig. 5).[7] Repetitive computer simulation of the experimentally obtained chromatograms with varying rates of enantiomerization gave identical elution profiles and allowed the determination of the rotational energy barrier as 109.7 (±0.2) kJ mol^{-1}. Noteworthy, they were also able to study the stereodynamics of enantioenriched 2,2′-bistrifluoromethylbiphenyl by polarimetry in ethanol in the same temperature range and obtained a barrier to enantiomerization of 109.6 (±0.1) kJ mol^{-1} showing that both methods are in excellent agreement. A comparison of computer simulations obtained by variation of the energy barrier to enantiomerization shows that energetic differences less than 0.2 kJ mol^{-1} can easily be visualized (Fig. 6). Providing additional tools for the investigation of stereodynamic processes with high accuracy and precision,

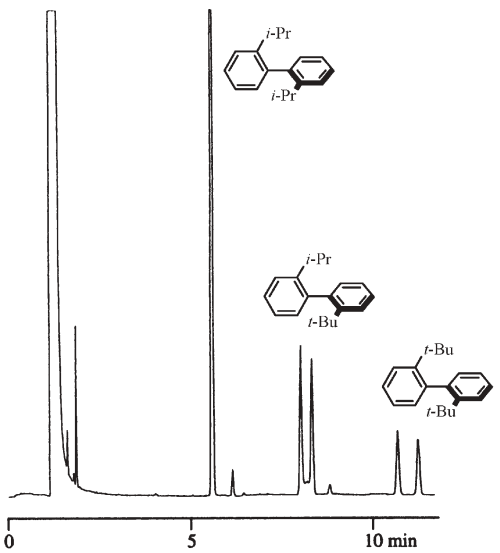

Fig. 3 Gas chromatographic resolution of 2,2′-disubstituted biphenyls at 165 °C using heptakis (6-*O*-*tert*-butyldimethylsilyl-2,3-di-*O*-methyl)-β-cyclodextrin.

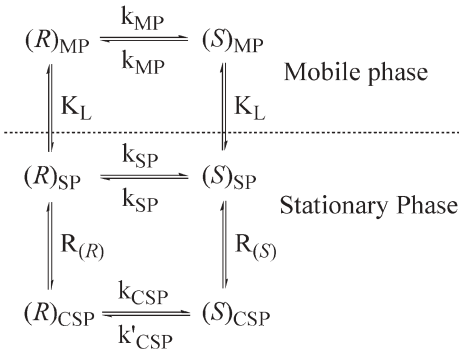

Fig. 4 Equilibria between two enantiomers of a chiral compound in a theoretical plate showing achiral and chiral contributions to chromatographic retention and enantioconversion.

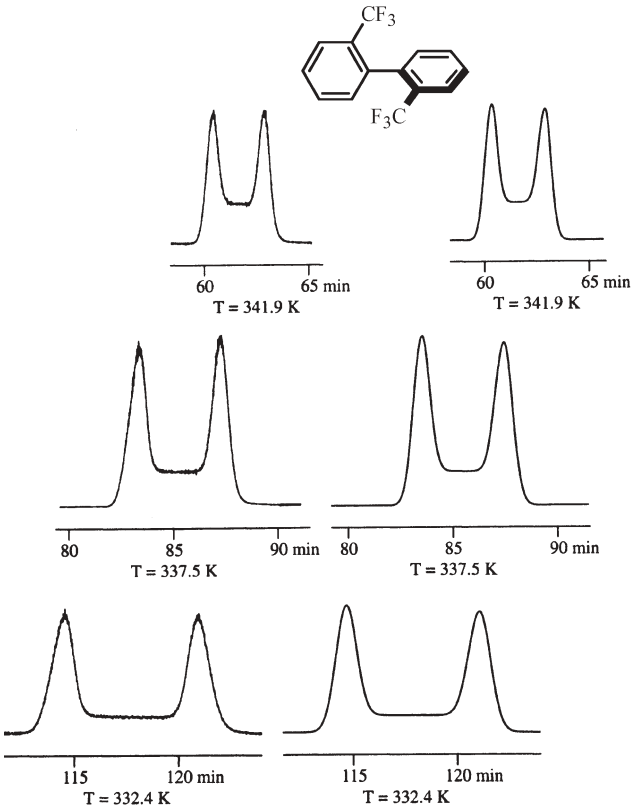

Fig. 5 Comparison of experimentally obtained (left) and simulated chromatograms (right) of 2,2′-bistrifluoromethylbiphenyl.

dynamic chromatography is a powerful supplement to NMR and chiroptical techniques.

Since all parameters required for the computer simulation are easily obtained from the chromatogram, enantioselective DGC and DHPLC have become widely used techniques for the determination of enantiomerization barriers of a broad variety of chiral compounds. Bürkle's computer program as well as computations based on a stochastic model have recently been further optimized to allow convenient and fast simulations of dynamic processes observed during chromatographic and electrophoretic separations.[8] Noteworthy, dynamic chromatography does not require preparative separation of enantiomers in contrast to chiroptical methods. Furthermore, only minute amounts of a racemic sample are used and chiral or achiral impurities do not interfere with the measurements as they are usually separated during the chromatographic process. On the other hand, a CSP capable of separating the enantiomers of interest at various temperatures remains an indispensable prerequisite and the enantiomerization proceeds at least partly in a chiral environment which may affect the enantiomerization process and thus the enantiomerization barriers measured. Because of the time-scales and different temperatures inherent to the chromatographic techniques mentioned above a wide range of isomerization barriers can be investigated with dynamic HPLC, GC, SFC, and MEKC.[9]

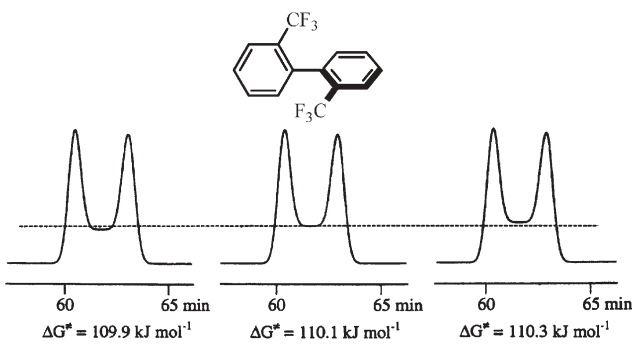

Fig. 6 Comparison of computed elution profiles showing the precision of dynamic chromatography.

Dynamic high performance liquid chromatography

In 1982, Horváth and co-workers observed *cis*/*trans*-isomerization of small peptides during reversed-phase HPLC. They found that proline-derived peptides undergo rotation about the amide bond containing the imide nitrogen of proline residues under neutral and acidic conditions at ambient temperature during the chromatographic separation. Investigating the effects of peptide isomerization within the chromatographic time scale, Horváth was thus first to employ HPLC in kinetic studies of interconverting stereoisomers. Developing the experimental and theoretical foundation for dynamic chromatography, they were able to determine the two corresponding rate constants for the reversible first-order interconversion between the *cis*- and *trans*-diastereoisomers of (*S*)-alanine-(*S*)-proline and (*S*)-valine-(*S*)-proline dipeptides.[10–12] Mannschreck *et al.* employed dynamic HPLC in enantiomerization studies using various CSPs in conjunction with both photometric and polarimetric detection. They observed that chiral 2,2′-spirobichromenes undergo on-column enantiomerization in methanol *via* reversible thermal ring opening at 35 to 45 °C.[13] For example, computer simulation of the elution profiles obtained with tribenzoylcellulose as the CSP yielded an energy barrier for the ring-opening step of 2,2′-spirobichromene of 100.0 kJ mol^{-1} at 45.0 °C (Fig. 7). This was confirmed by polarimetric racemization studies with enantiomerically enriched samples of 2,2′-spirobichromene under the same conditions. Mannschreck's group also studied the enantiomerization of axially chiral 1-dimethylamino-8-dimethylcarbamoylnaphthalene and helical 1,3,7-trimethylbenzo[*c*]phenanthrene using triacetylcellulose as the CSP.[14,15] Veciana and Crespo utilized DHPLC to determine the energy barrier for the enantiomerization of tris(2,4,6-trichlorophenyl)methane as 80.4 kJ mol^{-1} at −5.0 °C using a (+)-poly(triphenylmethylmethacrylate) column (Fig. 7).[16] They also investigated the isomerization of diastereomeric biradicals of perchloro-1,3-bisdiphenylmethylbenzene using an achiral C_{18} reversed phase column and obtained an interconversion barrier of 96.0 kJ mol^{-1} at 44.0 °C (Fig. 7). Cabrera *et al.* introduced DHPLC to enantiomerization studies of chiral pharmaceutical drugs such as chlorthalidone, oxazepam, phenylthiohydantoin-phenylalanine, and prominal using ChiraDex as the CSP. They reported that the stereochemical stability strongly depends on the solvent and pH used, which may have important implications with respect to the pharmacokinetic integrity of these chiral drugs.[17,18] Li

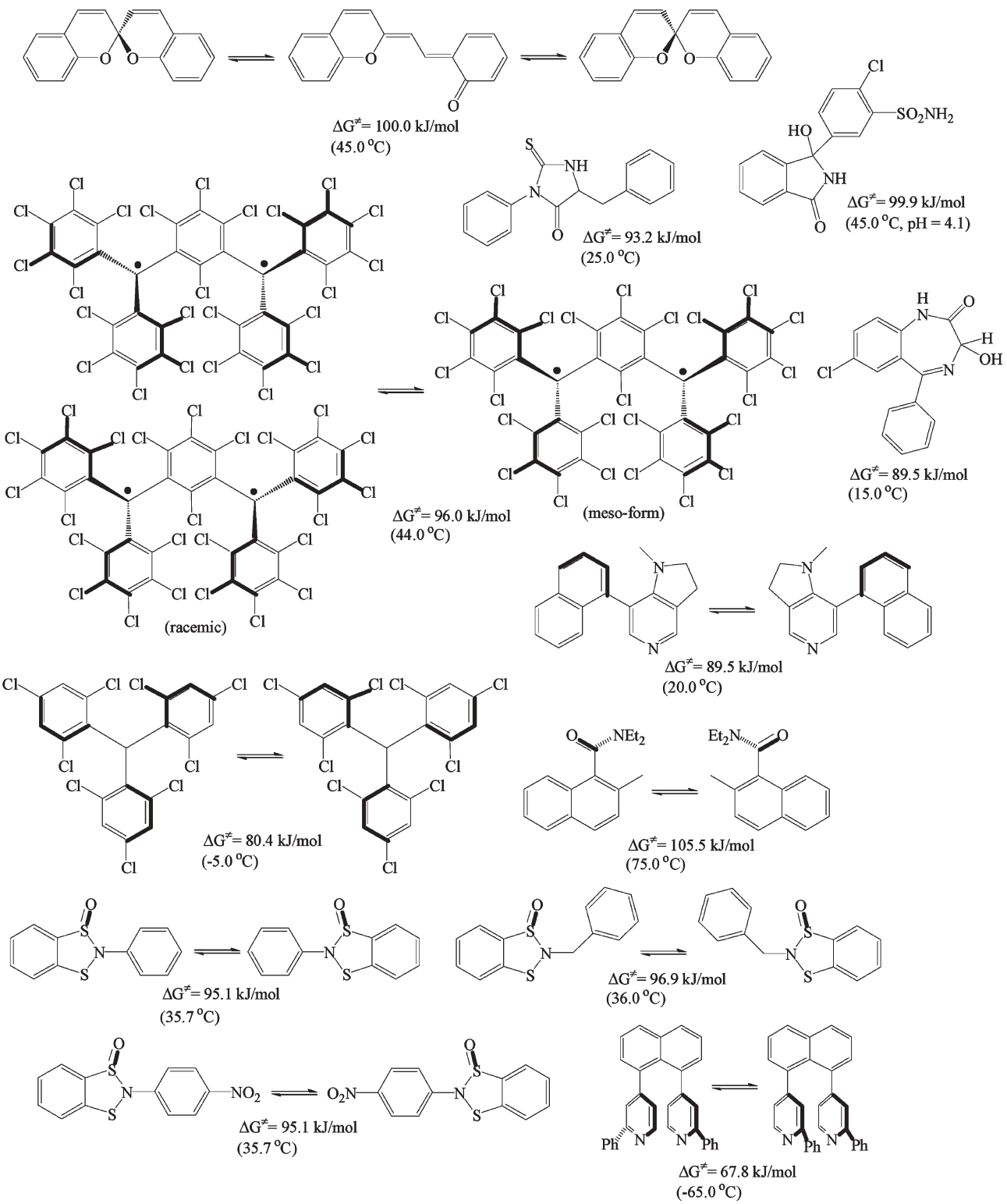

Fig. 7 Isomerization reactions of some chiral compounds studied by DHPLC.

and co-workers were able to determine the diastereomerization barrier of stereolabile trityloxymethyl butyrolactol using an achiral C_{18}-column under reversed phase conditions.[19] The atropisomerization of a series of axially chiral biaryl derivatives of 4-(dimethylamino)pyridine, DMAP, has been investigated by Spivey *et al.* using Chiralcel OB, OD, OJ or Chiralpak AD as the CSP in order to identify conformationally stable, DMAP-derived catalyst candidates for effective kinetic resolution of secondary alcohols.[20]

Wolf and Tumambac investigated the isomerization of 1,8-bis(2′-phenyl-4′-pyridyl)naphthalene over a temperature range of more than 100 °C using dynamic HPLC and computer

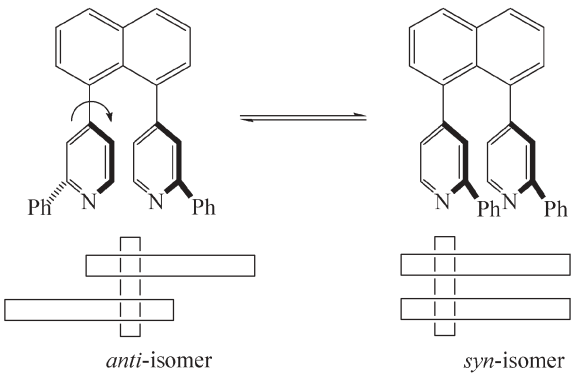

Fig. 8 Isomerization of the conformational diastereoisomers of 1,8-bis(2′-phenyl-4′-pyridyl)naphthalene having either parallel (*syn*) or antiparallel (*anti*) 2-phenylpyridyl rings.

simulation as well as variable-temperature NMR spectroscopy. Rotation of either pyridyl ring of 1,8-bis(2′-phenyl-4′-pyridyl)naphthalene about the pyridyl-naphthalene bond results in interconversion between the meso *syn*- and the two axially chiral *anti*-isomers (Fig. 8).[21]

Variable-temperature NMR studies revealed that the diastereomerization between the almost equienergetic rotamers of 1,8-bis(2′-phenyl-4′-pyridyl)naphthalene is fast at room temperature and the energy barrier to rotation about the chiral naphthyl-pyridyl axis was determined as 73 kJ mol^{-1} at 40.3 °C. Chromatographic separation of the *syn*- and *anti*-isomers of this stereolabile atropisomer was found to require cryogenic conditions. Accordingly, the isomers were fully separated using a cyano HPLC column at −70.0 °C while plateau formation was observed when the column temperature was increased to 65.0 °C. Because of rapid diastereomerization relative to the HPLC time scale, peak coalescence was observed at −39.0 °C (Fig. 9). Computer simulation of the experimentally obtained chromatograms then allowed determination of the isomerization rate constants under these cryogenic conditions.

Employing the DHPLC and DNMR results in the Eyring equation, $k = k_B T/h[e(\Delta S^{\neq}/R)e(-\Delta H^{\neq}/RT)]$, the activation enthalpy, $\Delta H^{\neq}$, and activation entropy, $\Delta S^{\neq}$, of the isomerization of 1,8-bis(2′-phenyl-4′-pyridyl)naphthalene were determined as 57.5 kJ mol^{-1} and −43.4 J $K^{-1}mol^{-1}$, respectively. The corresponding rotational energy barrier was calculated as 67.8 kJ mol^{-1} at −65.0 °C and observed to slightly increase to 68.7 kJ mol^{-1} at −39.0 °C due to the negative activation entropy. The Eyring plot also shows that DHPLC and DNMR are complementary methods that allow kinetic investigations of stereolabile compounds over a wide temperature range, *i.e.* more than 100 °C (Fig. 10).

Trapp and co-workers investigated the reversible enantiomerization of a chiral tetrabenzoxazine resorcarene showing plateau formation during HPLC on Chiralpak AD at room temperature. This macrocycle exhibits a labile hemiaminal group that is readily cleaved under slight acidic conditions. Consecutive ring opening of each oxazine ring *via* an iminium intermediate followed by ring closure with the opposite hydroxyl group of the resorcinol unit thus results in

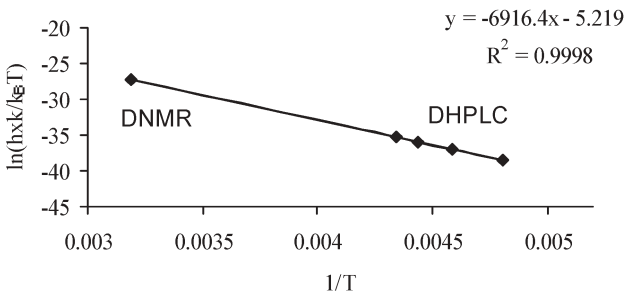

Fig. 10 Eyring equation and plot for the diastereomerization of 1,8-bis(2′-phenyl-4′-pyridyl)naphthalene.

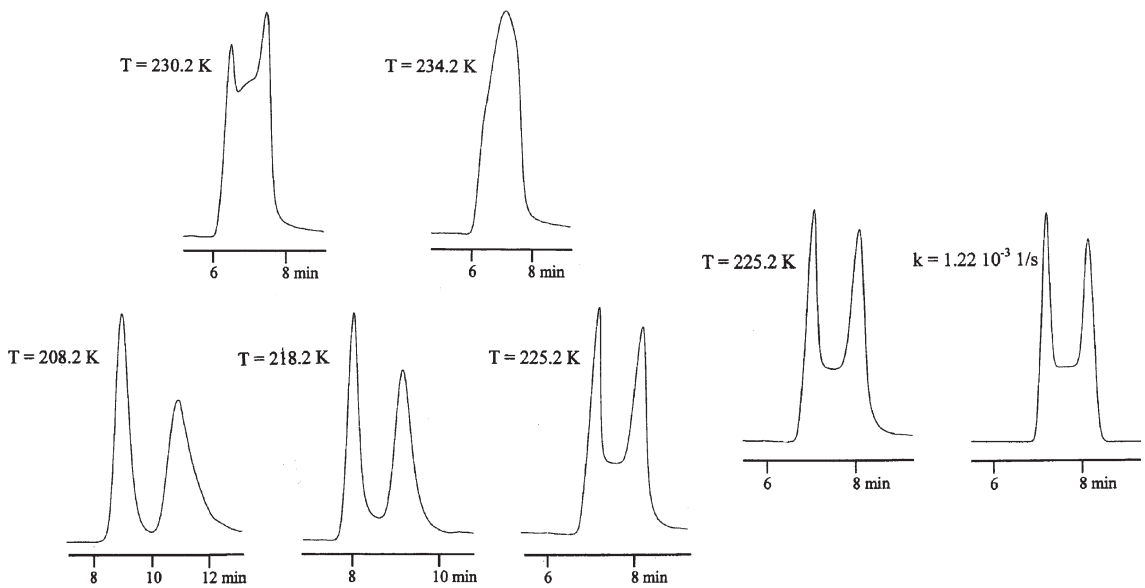

Fig. 9 Cryogenic HPLC separations of the *syn*- and *anti*-isomers of 1,8-bis(2′-phenyl-4′-pyridyl)naphthalene and plateau formation at various temperatures (left). Comparison of experimentally obtained and simulated elution profile (right).

enantiomerization at low temperatures. DHPLC studies at various temperatures gave an enantiomerization barrier, $\Delta G^{\neq}$, of 92 kJ mol^{-1} and the activation parameters $\Delta H^{\neq}$ = 53 kJ mol^{-1} and $\Delta S^{\neq} = -131$ J K^{-1}mol^{-1}. The low activation enthalpy and the highly negative activation entropy were attributed to the dissociative enantiomerization mechanism (Fig. 11).[22]

Villani and co-workers investigated the stereodynamics of numerous axially chiral 2-methyl and 2-ethoxy-1-naphthylcarboxamides, sulfones and sulfoxides by DHPLC using a (3*R*,4*S*)-Whelk-O1 CSP (Fig. 7).[23,24] Comparison of the rotational energy barriers determined by computer simulation of elution profiles obtained by DHPLC with results from off-column racemization experiments revealed that the CSP increases the energy barrier of these atropisomers by 1–4 kJ mol^{-1}. Similarly, Oxelbark and Allenmark determined the configurational stability of *N*-aryl and *N*-benzyl-1,3,2-benzodithiazole-1-oxides by both DHPLC and off-column racemization experiments using CD spectroscopy (Fig. 7).[25,26] They were able to differentiate between interconversion in the CSP and in the mobile phase and found that the (3*R*,4*S*)-Whelk-O1 CSP increases the barrier to enantiomerization by approximately 2 kJ mol^{-1}. For example, the enantiomerization barrier of 2-benzyl-1,3,2-benzodithiazole-1-oxide complexed by the CSP was determined as 96.9 ($\pm$0.1) kJ mol^{-1} at 36.0 °C by DHPLC, whereas racemization experiments in free solution, *i.e.* the mobile phase, gave an energy barrier of 94.7 kJ mol^{-1}. In general, the rate of enantiomerization of stereodynamic compounds has been found to slightly decrease in the presence of (3*R*,4*S*)-Whelk-O1 CSP, *i.e.* the energy barrier is usually enhanced by 1–4 kJ mol^{-1}, which is commonly attributed to stabilization of the ground state of the interconverting stereoisomers during complexation by the chiral selector. However, other chiral stationary phases such as poly(triphenylmethylmethacrylate) and triacetylcellulose have been reported to exhibit small destabilizing effects on the conformational or configurational stability of chiral compounds and comparison of isomerization barriers obtained by dynamic chromatography and variable-temperature NMR spectroscopy or polarimetric studies usually shows excellent agreement between the methods.[27] To date, DHPLC and computer simulation has been applied in kinetic studies of a variety of enantiomerization and diastereomerization reactions of chiral compounds exhibiting Gibbs activation energies, $\Delta G^{\neq}$, between 60 and 120 kJ mol^{-1} (Fig. 7).

Dynamic gas chromatography

At the same time and independently from the DHPLC studies reported by Horváth in 1982, Schurig and co-workers observed plateau formation due to on-column enantiomerization of 1-chloro-2,2-dimethylaziridine and 1,6-dioxaspiro[4.4]-nonane during complexation gas chromatography using nickel(II)-bis[(1*R*)-3-heptafluorobutyryl)camphorate] dissolved in squalane, as the chiral stationary phase.[28] They were first to quantitatively calculate the kinetics of interconverting enantiomers and have since then been most prominent in establishing dynamic chromatography and DGC in particular. Introducing the theoretical background for dynamic chromatography and the necessary tools for peak shape analysis based on computer simulation they determined the enantiomerization barrier 1-chloro-2,2-dimethylaziridine as 104.9 kJ mol^{-1} at 60.0 °C.[29] Studying the enantiomerization of homofuran between 60 and 130 °C by DGC and polarimetry they found

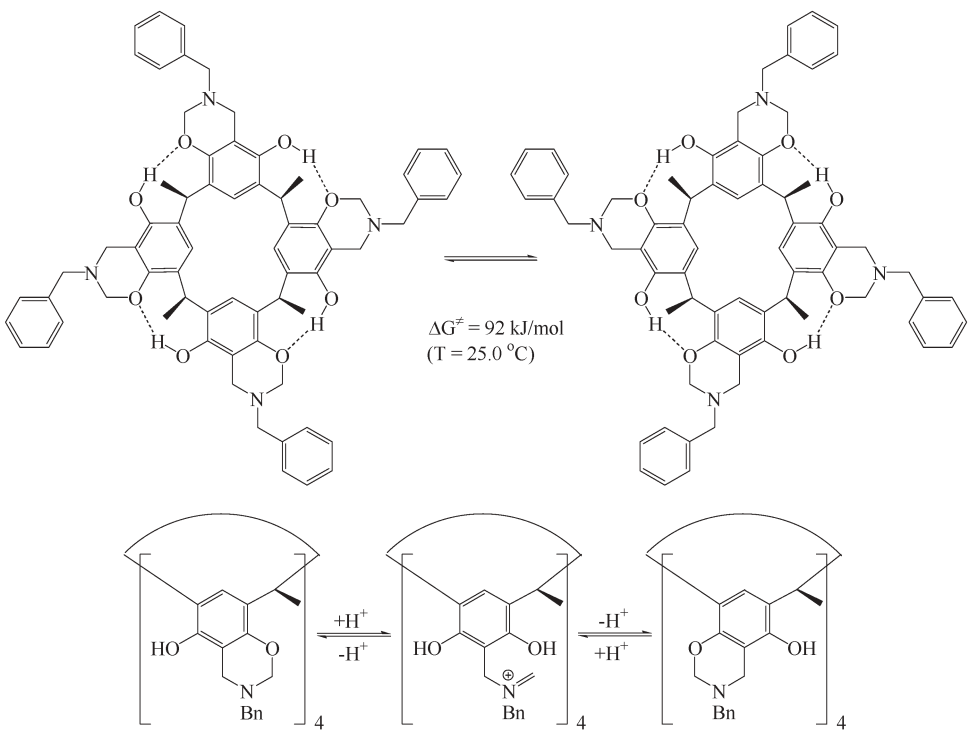

Fig. 11 Enantiomerization of a chiral tetrabenzoxazine resorcarene derivative and reversible oxazine ring opening.

that energy barriers obtained by both methods are in excellent agreement.[30] Noteworthy, Klärner and Schröer determined the enantiomerization barrier of homofuran as 113.8 kJ mol^{-1} at 90.0 °C and found that the reaction proceeds *via* a carbonyl-ylide-like intermediate formed by an orbital symmetry allowed disrotatory electrocyclic ring-opening obeying Woodward–Hoffman rules, (Fig. 14).[31] Marriott and Lai utilized DGC to investigate *syn*/*anti*-isomerizations of axially chiral phenanthrenes and anthracenes.[32] For instance, peak shape analysis of the plateau formed as a result of on-column interconversion of the diastereomeric *anti*-isomers and the meso *syn*-form of 9,10-bis(2-methylphenyl)phenanthrene at 240.0 °C gave an energy barrier of 132 kJ mol^{-1}. Schurig and co-workers have since then employed Chirasil-Dex, *i.e.* permethylated β-cyclodextrin immobilized on dimethylpolysiloxane, and Chirasil-Nickel, *i.e.* polysiloxane-anchored nickel(II)-bis[(1*R*)-3-heptafluorobutyryl)camphorate] as CSPs in dynamic gas chromatography to determine the enantiomerization barrier of various diaziridines and allenes.[33–35] Computer simulation of the interconversion elution profiles revealed an enantiomerization barrier for 1,2,3,4-tetramethyldiaziridine which interconverts *via* two consecutive nitrogen inversions of 115.0 kJ mol^{-1} at 80.0 °C. Interestingly, Chirasil-Dex was found to accelerate the rate of enantiomerization and reduces the measured energy barrier by 1–3 kJ mol^{-1}. A more pronounced effect of the CSP used in DGC studies of interconverting enantiomers was found with axially chiral dimethyl-2,3-pentadienedioate. An interconversion barrier of 128 kJ mol^{-1} at 120 °C was obtained using stopped-flow multidimensional gas chromatography which allows kinetic studies in the absence of a CSP (*vide infra*), whereas DGC measurements using Chirasil-Nickel and Chirasil-Dex provided energy barriers of 117.5 and 114.9 kJ mol^{-1}, respectively, at the same temperature (Fig. 14). Investigating the isomerization of spiroketal chalcogran, which is an important beetle aggregation pheromone, Trapp and Schurig extended DGC to the study of epimerization reactions. Because of the inherently different ground state energies of epimers two rate constants for the forward and backward reactions have to be considered which further complicates peak shape analysis. Using the powerful computer simulation program ChromWin and DGC studies between 70 and 120 °C they were able to calculate the individual Gibbs free activation energies for the interconversion of (2*R*,5*R*)- and (2*R*,5*S*)-2-ethyl-1,6-dioxaspiro[4.4]nonane as 108.0 and 108.5 kJ mol^{-1} at 25 °C.[36] The corresponding low activation enthalpies and the highly negative activation entropies were attributed to a dissociative, heterolytic mechanism proceeding *via* a zwitterionic/enol ether intermediate (Fig. 12).

Wolf and co-workers studied the rotational energy barrier of a broad variety of axially chiral biphenyls by DGC using selectively modified cyclodextrins as CSPs.[37] The significance of steric and CH/π-interactions of *ortho*-substituents on the conformational stability of 2,2′-disubstituted biphenyls was investigated (Fig. 13). They were first to systematically investigate and quantify electronic and buttressing effects of *para*- and *meta*-substituents on the rotational energy barrier of biphenyls using DGC and polarimetric techniques. Noteworthy, energy barriers determined by DGC were in excellent agreement with results obtained by polarimetric studies of enantioenriched samples albeit the CSPs used were

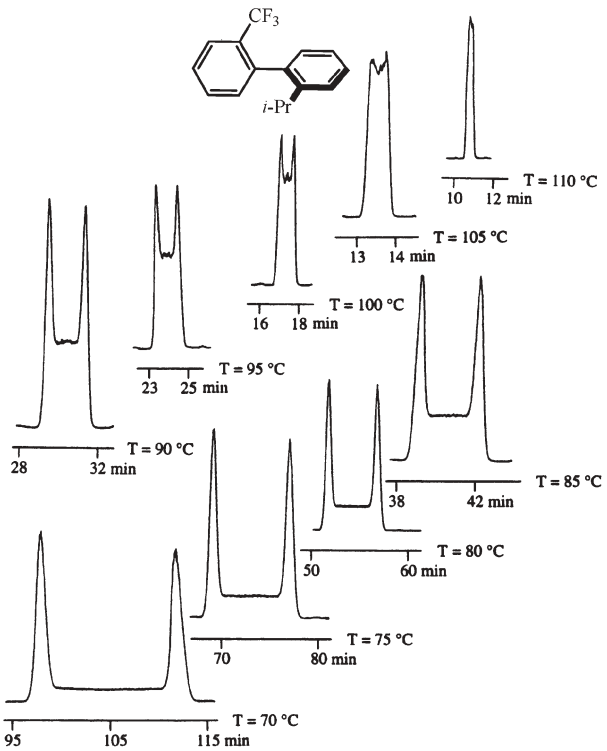

Fig. 13 DGC elution profiles of 2-isopropyl-2′-trifluoromethylbiphenyl using octakis(6-*O*-methyl-2,3-di-*O*-pentyl)-γ-cyclodextrin as the CSP.

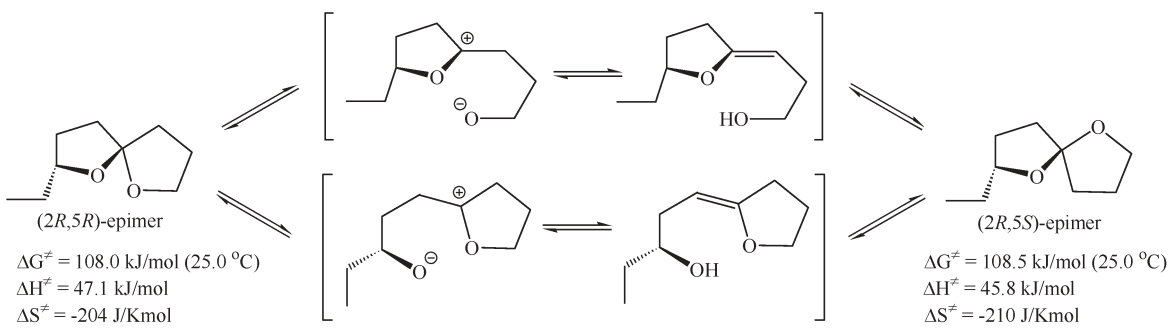

Fig. 12 Dissociative epimerization mechanism of chalcogran.

found to exhibit small effects on the rotational energy barriers of the biphenyls studied. For example, simulation of the experimentally obtained elution profiles of 2,2′-diisopropylbiphenyl obtained by DGC using heptakis(6-*O*-*tert*-butyldimethylsilyl-2,3-di-*O*-pentyl)-β-cyclodextrin as the CSP gave an energy barrier of 112.4 (±0.2) kJ mol^{-1} (78.5–83.5 °C) whereas a value of 114.6 (±0.2) kJ mol^{-1} (78.4–88.1 °C) was obtained when octakis(6-*O*-methyl-2,3-di-*O*-pentyl)-γ-cyclodextrin was employed as the CSP. Polarimetric studies of enantiomerically enriched 2,2′-diisopropylbiphenyl obtained by HPLC on microcrystalline triacetylcellulose were conducted in the same temperature range as the DGC measurements and yielded an energy barrier of 112.8 (±0.1) kJ mol^{-1}. Since additional polarimetric studies in various solvents including ethanol and hexanes showed negligible solvent effects on the conformational stability of 2,2′-diisopropylbiphenyl, the difference in energy barriers obtained by DGC with selectively substituted cyclodextrins was attributed to small but unpredictable stabilization or destabilization effects of the CSPs used.

Hochmuth and König used variable temperature gas chromatography to study the enantiomerization of substituted [10]-, [11]-, and [12]paracyclophanes exhibiting a chiral plane.[38,39] They reported baseline separations of the enantiomers of various [10]paracyclophanes but did not observe any sign of on-column interconversion even at 170 °C which was attributed to the high conformational stability of these atropisomers. By contrast, [12]paracyclophanes were found to undergo fast interconversion compared to the GC time scale and enantiomers could not be resolved above 40 °C. As was expected, the enantiomerization barriers of substituted dioxa- and carbocyclic [11]paracyclophanes are suitable for DGC studies and showed formation of a plateau during GC separation on cyclodextrin-derived CSPs. A broad range of [11]paracyclophanes exhibiting enantiomerization barriers between 115 and 135 kJ mol^{-1} were thus analyzed by DGC and computer simulation.

Since its introduction by Schurig in 1982, DGC has been applied to kinetic studies of a variety of enantiomerization and diastereomerization reactions of chiral compounds exhibiting Gibbs free activation energies, $\Delta G^{\neq}$, between 100 and 150 kJ mol^{-1} (Fig. 14).

Dynamic supercritical fluid chromatography and dynamic electrokinetic chromatography

The principles of dynamic chromatography and computer simulation discussed above have also been applied to other separation techniques. Although competition between isomerization and separation of chiral compounds has mainly been observed through HPLC and GC, other pressure-driven methods such as super- or subcritical fluid chromatography and electroosmotically-driven separations such as electrophoresis with chiral additives have been found to further extend the application spectrum of this approach. The range of energy barriers of dynamic processes that can be studied by DHPLC and DGC is limited by the chromatographic time scale, solubility and thermal stability of the eluent, and the temperature range inherent to these techniques. Because of practical difficulties due to low analyte solubility and increasing viscosity of the mobile phase causing high back pressures at low temperature, HPLC is commonly performed between 0 °C and 120 °C albeit the use of efficient brush-type CSPs has allowed operating temperatures to be extended down to −50 °C in some cases. By contrast, GC requires sufficient volatility and thermal stability of the analyte and temperatures between 40 °C and 180 °C are usually employed in chiral separations. A further extension of this temperature range is

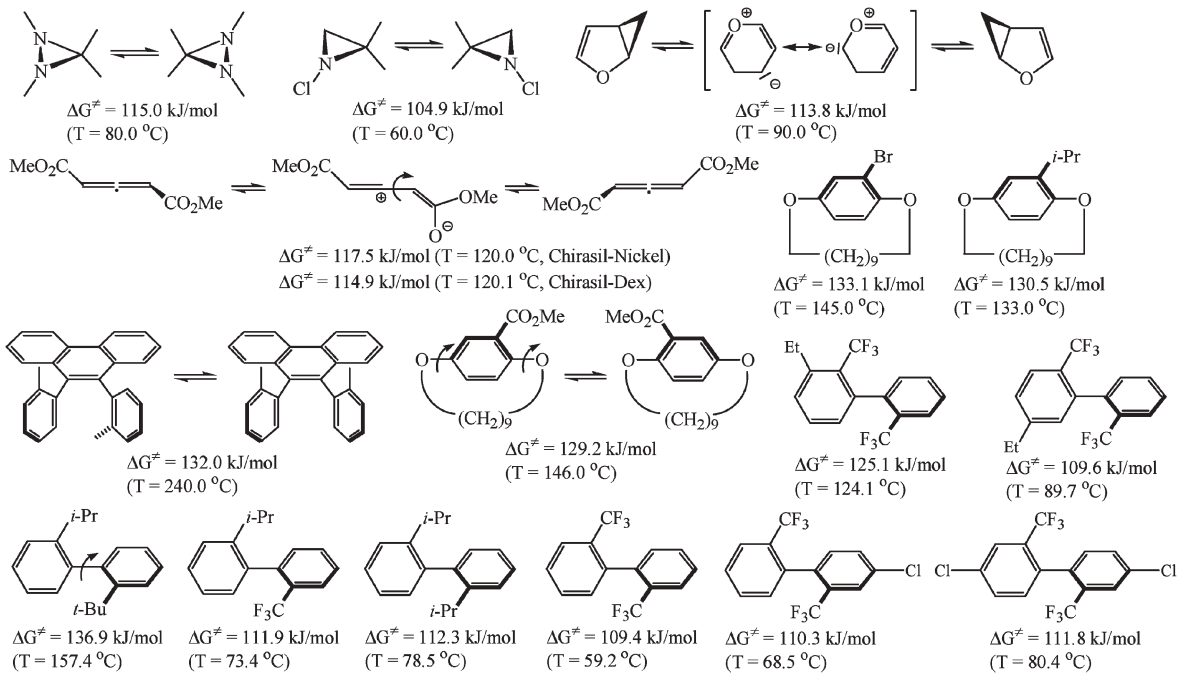

Fig. 14 Isomerization reactions of selected chiral compounds studied by DGC.

not possible because of the general thermal instability of CSPs above 180 °C. The inherently higher diffusivity and lower viscosity of subcritical fluids provide a significant advantage of subcritical fluid chromatography (SubFC) over HPLC when cryogenic conditions are required. Wolf and co-workers introduced dynamic subcritical fluid chromatography (DSubFC) using a brush-type CSP and subcritical carbon dioxide modified with small amounts of methanol or acetonitrile as the mobile phase as a highly useful tool for the study of rapidly interconverting stereoisomers.[40] They observed that the enantioseparation of axially chiral arylnaphthalene lignans on a polyWhelk-O CSP can be achieved under cryogenic conditions, *i.e.* between −50 °C and 0 °C, while on-column enantiomerization occurred as the temperature was progressively increased. Computer simulation of the temperature-dependent elution profiles showing plateau formation due to enantiomerization gave rotational energy barriers between 75.0 and 92.0 (±0.2) kJ mol^{-1} thus significantly extending the application spectrum of DGC and DHPLC (Fig. 15). A change in the mobile phase composition did not show any influence on the rotational energy barriers obtained by simulation. Importantly, subcritical and supercritical fluid chromatography cover the combined temperature range of HPLC and GC and therefore allow the study of isomerization processes exhibiting energy barriers between 60 and 150 kJ mol^{-1}.

Schurig *et al.* developed dynamic micellar electrokinetic chromatography (DMEKC) for the determination of the enantiomerization kinetics of chiral benzodiazepine drugs, *i.e.* oxazepam, temazepam, and lorazepam, using aqueous buffers as the mobile phase and sodium cholate as a chiral surfactant.[41] This technique allows kinetic studies of stereolabile drugs exhibiting energy barriers to interconversion between 80 and 120 kJ mol^{-1} under biologically relevant conditions and at ambient temperatures (Fig. 15).

Chromatographic and electrophoretic stopped-flow analysis

Chiral chromatography is very useful for the analytical or preparative separation of enantiomers and for the investigation of the interconversion of stereolabile compounds based on the principles of dynamic chromatography and computer simulation of the temperature-dependent elution profiles. The development of stopped-flow chromatography by Weseloh, Wolf and König has provided another useful tool for kinetic studies of the interconversion of stereoisomers and enantiomers in particular.[42] They observed that 4,4′-diammonium-2,2′-diisopropylbiphenyl can be separated into enantiomers with high resolution, *i.e.* selectivity and efficiency, by capillary zone electrophoresis using heptakis(2,3,6-tri-*O*-methyl)-β-cyclodextrin as the resolving chiral additive in the electrophoretic buffer at pH = 3 and 20 kV. Combining highly enantioselective electrophoresis with selectively modified cyclodextrins and the known stereodynamic properties of axially chiral biphenyls they were able to develop a chromatographic method that allows one to monitor isomerization or enantiomerization reactions through on-column interconversion experiments (Fig. 16). A racemic sample of 4,4′-diammonium-2,2′-diisopropylbiphenyl was injected electrokinetically and separated at low temperature into the enantiomers using heptakis(2,3,6-tri-*O*-methyl)-β-cyclodextrin. Then, the voltage was switched off exactly in the middle of the electrophoretic separation process and the temperature was increased to 82.6 °C to allow interconversion of the atropisomers. At this point the enantiomers were already well-separated residing at different locations and within the same distance before or after the middle of the capillary. After a certain time, the on-column interconversion was stopped and the buffer was cooled to the original temperature. The electrophoretic process was resumed by switching on the applied voltage of 20 kV and the separation continued in the second part of the capillary.

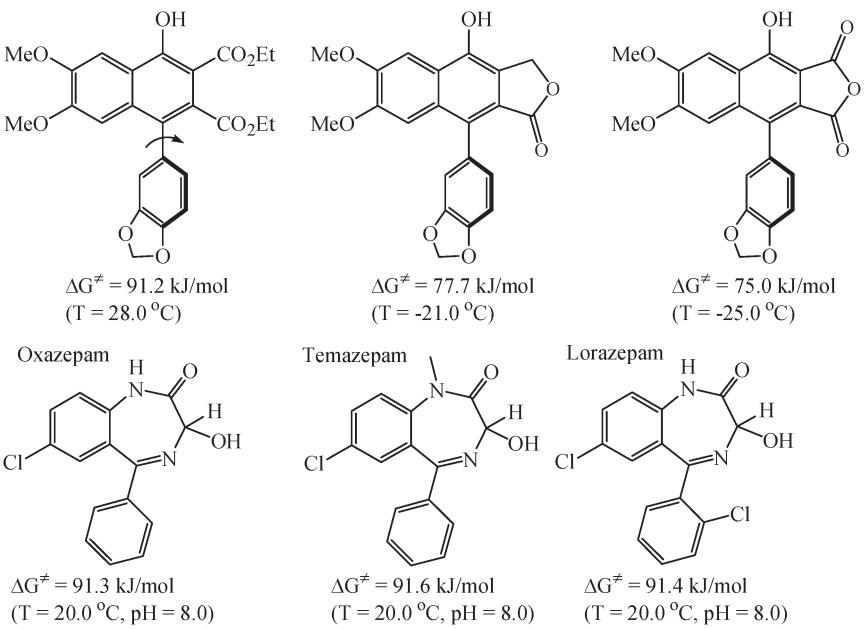

Fig. 15 Enantiomerization of selected chiral compounds studied by DSubFC (top) and DMEKC (bottom).

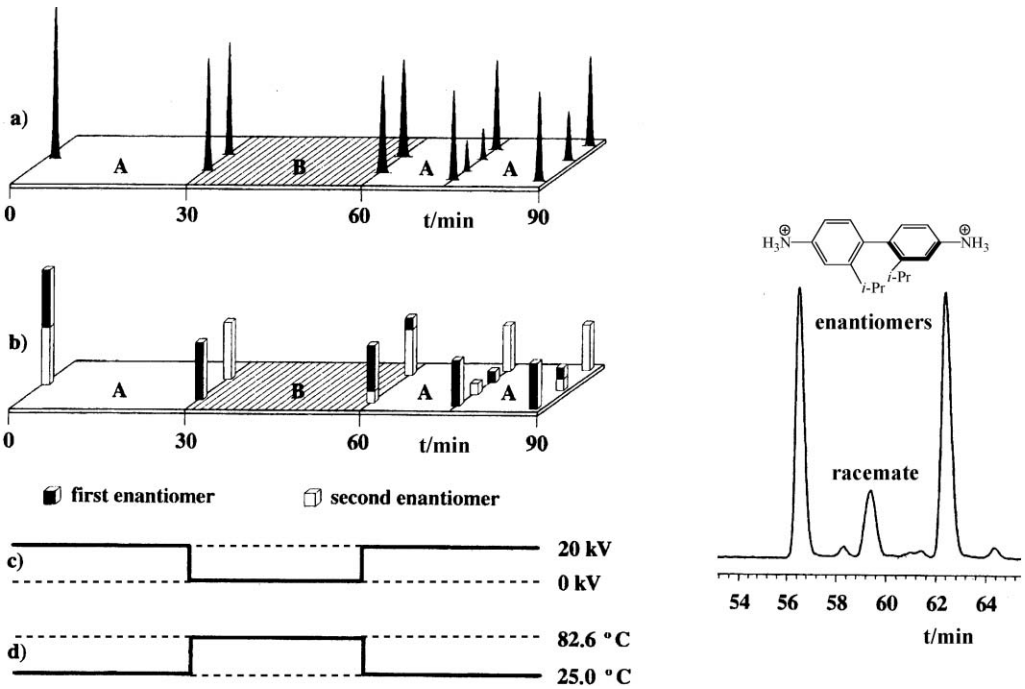

Fig. 16 Illustration of the electrophoretic separation of the enantiomers of 4,4′-diammonium-2,2′-diisopropylbiphenyl and the on-column enantiomerization experiment (a) and the enantiomeric composition of each peak (b). Enantioseparation proceeds in sections A while enantiomerization takes place in section B. The applied voltage and temperature are shown in (c) and (d). A typical electropherogram is shown on the right.

This stopped-flow electrophoresis experiment resulted in the elution of three peaks (Fig. 17). The electropherogram showed a new racemic peak in between the signals of the enantiomers that did not undergo on-column enantiomerization during the allotted reaction time. The rotational energy barrier, $\Delta G^{\neq}$, of 4,4′-diammonium-2,2′-diisopropylbiphenyl was then calculated from the integrated peak areas, the applied temperature, and enantiomerization time as 115.2 kJ mol^{-1}. Similar to dynamic chromatography, this method requires only minute amounts of racemate but eliminates the need for computer simulation. Since electrophoresis is usually conducted with aqueous buffers, isomerization reactions can be investigated at temperatures between 25 and 95 °C. Noteworthy, energy barriers to isomerization ranging from 100 to 130 kJ mol^{-1} can be determined with high accuracy ($\pm$0.2 kJ mol^{-1}) and reproducibility.

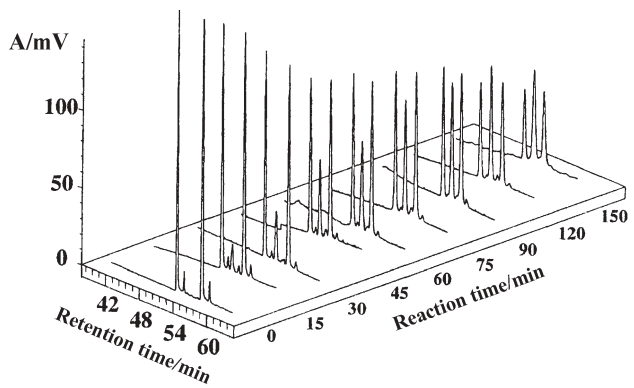

Fig. 17 Electropherograms obtained through variation of the on-column enantiomerization time. The peak in the middle corresponds to the amount of racemate formed.

However, the presence of a chiral selector can increase or decrease the enantiomerization barrier of the sample, *e.g.* either by stabilizing the ground state or by catalyzing the interconversion. The stopped-flow method was therefore further refined to allow kinetic studies in the absence of the chiral additive in order to exclude enantioselective effects on the rotational energy barrier of the biphenyl atropisomers as a result of stabilizing or destabilizing interactions with the chiral cyclodextrin host.[43] Through careful rinsing procedures and control of the electro-osmotic flow, the capillary was segmented into three different buffer zones. Following the stopped-flow method described above, the enantiomers of 4,4′-diammonium-2,2′-bistrifluoromethylbiphenyl were separated in the first and third buffer zone containing the cyclodextrin selector, whereas the enantiomerization experiment was conducted in the middle section which did not contain any chiral additives. Noteworthy, the electrophoretic separation must not be stopped exactly in the middle of the capillary because only one part of the capillary is heated to selectively employ one atropisomer to enantiomerization at elevated temperature. Thus, segmentation of the capillary into different buffer and heating zones allows one to selectively determine the rotational energy barrier of either enantiomer in the presence or in the absence of the chiral additive (Fig. 18).

Investigation of the enantiomerizaton of 4,4′-diammonium-2,2′-bistrifluoromethylbiphenyl using a 30 mM phosphate buffer at pH = 2.4 gave a rotational energy barrier of 106.9 kJ mol^{-1} at 71.0 °C in the absence of a cyclodextrin additive which is in excellent agreement with polarimetric studies of an

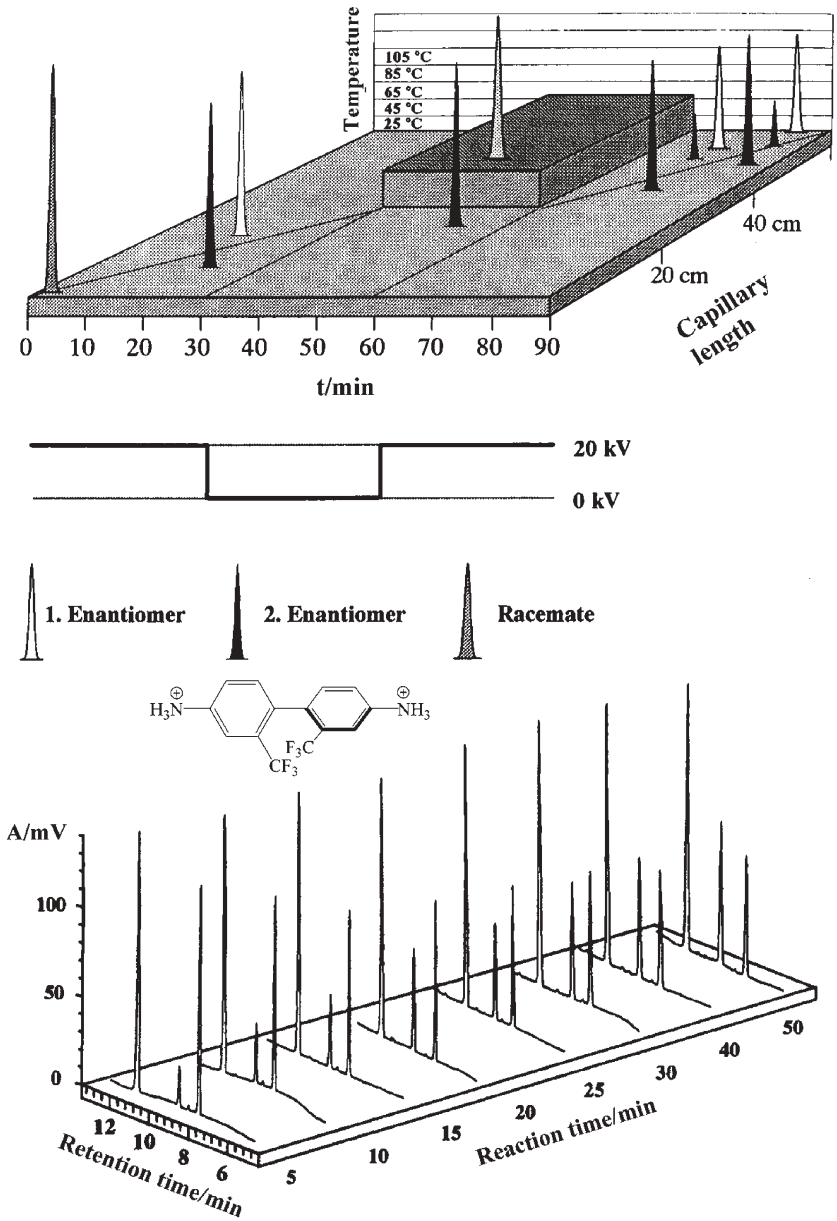

Fig. 18 Illustration of the CZE enantioseparation and on-column enantiomerization of one enantiomer of 4,4′-diammonium-2,2′-bistrifluoromethylbiphenyl (top). Electropherograms obtained after different times of enantiomerization (bottom). The emerging peak in the middle corresponds to the amount of enantiomer formed during selective on-column heating of the less retained enantiomer.

enantioenriched sample employed in racemization studies under the same conditions. Interestingly, it was found that cyclodextrin hosts can either catalyze the or impede the atropisomerization process. Stopped-flow CZE studies of 4,4′-diammonium-2,2′-bistrifluoromethylbiphenyl conducted in the presence of permethylated β-cyclodextrin at 71 °C afforded a reduced rotational energy barrier of 105.2 kJ mol^{-1} while hydroxypropyl-β-cyclodextrin (degree of substitution = 0.9) increased the conformational stability to 108.3 kJ mol^{-1}. The stopped-flow technique developed by Weseloh, Wolf and König thus allows the investigation of dynamic processes including enantiomerization reactions and also provides new means to determine stereoselective interactions between stereodynamic compounds and cyclodextrin hosts or other chiral selectors.

The interconversion of the enantiomers of a variety of planar chiral 1,11-diaza[11]paracyclophanes at 95.5 °C has been studied by König and coworkers using the stopped-flow electrophoresis technique described above (Fig. 19).[44] They obtained pH-dependent energy barriers ranging from 113 to 126 kJ mol^{-1} that were dramatically reduced by 6–8 kJ mol^{-1} in the presence of cyclodextrin derivatives. Schurig and coworkers applied the principles of stopped-flow chromatography to gas chromatography albeit with significantly lower accuracy when the measurements required very high temperatures. They were able to determine the rotational barrier of

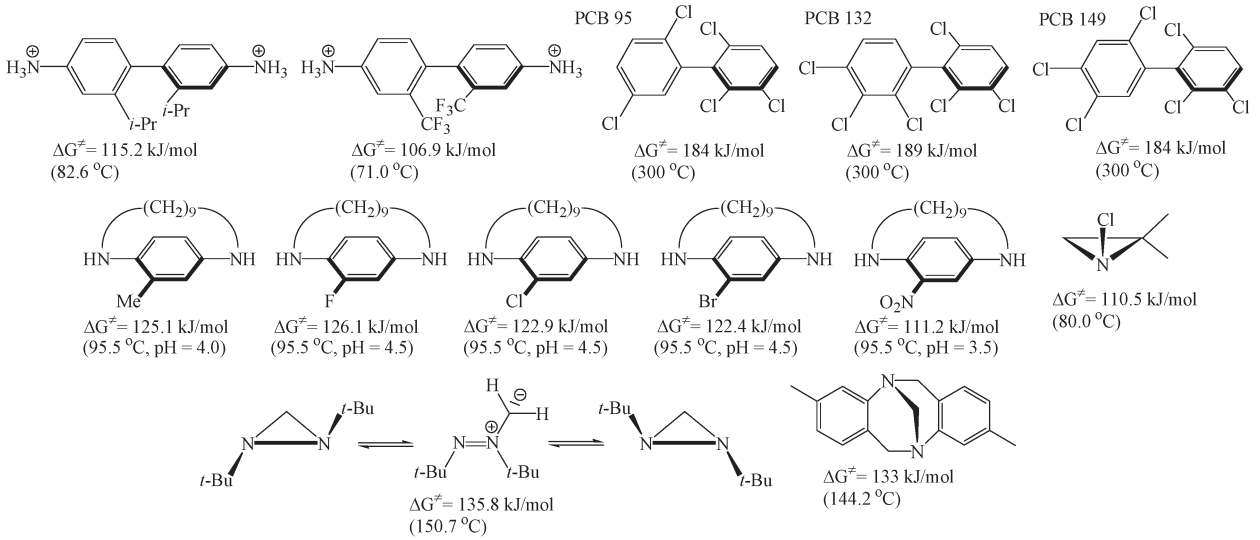

Fig. 19 Enantiomerization of chiral compounds studied by chromatographic and electrophoretic stopped-flow analysis.

hexachlorobiphenyl PCB 132 in the presence of a cyclodextrin-derived chiral stationary phase as approximately 184 ($\pm$2) kJ mol^{-1}.[45] Moreover, the development of stopped-flow multi-dimensional gas chromatography provided a method for the investigation of isomerization barriers ranging between 70 and 200 kJ mol^{-1} in the presence or absence of a chiral stationary phase.[46,47] For example, multi-dimensional GC has been used for the separation of the enantiomers of 1-chloro-2,2-dimethylaziridine on a cyclodextrin column and subsequent selective introduction of one enantiomer to a second achiral column. The enantiomer was thus subjected to racemization in an achiral environment at high temperature. The sample was then separated into enantiomers on a third column at lower temperature and the peaks were integrated to determine the amount of gas phase enantiomerization in order to calculate the enantiomerization barrier based on the retention time, *i.e.* the time of enantiomerization, and temperature of the second reactor column. The studies revealed that enantiomerization of 1-chloro-2,2-dimethylaziridine proceeds at 80 °C with a nitrogen inversion barrier of 110.5 ($\pm$0.5) kJ mol^{-1}. Similarly, the enantiomerization barrier of 1,2-di-*tert*-butyldiaziridine was determined as 135.8 ($\pm$0.2) kJ mol^{-1} at 150.7 °C.[48] The nitrogen inversion was attributed to a reversible thermally favored conrotatory electrocyclic ring opening mechanism (Fig. 19). Stopped-flow multi-dimensional gas chromatography has also been used to determine the rotational energy barrier, $\Delta G^{\neq}$, of PCBs 95, 132, and 136 at 300 °C as 184, 189, and 184 kJ mol^{-1}, respectively. The increased rotational energy barrier of PCB 132 was attributed to the buttressing effect of the additional *meta*-substituent. Tröger's base is a widely used chiral solvating agent exhibiting two asymmetric bridgehead nitrogen atoms that do not show pyramidal inversion and thus enantiomerization under normal conditions, *i.e.* without breaking a bond. However, Trapp and Schurig found that Tröger's base undergoes enantioconversion at high temperatures in the gas phase either through retro-hetero-Diels Alder ring opening or *via* a zwitterionic intermediate and determined the enantiomerization barrier by stopped-flow multi-dimensional gas chromatography as 133 ($\pm$1.5) kJ mol at 144.2 °C.[49]

Summary

Dynamic chromatography-computer simulation as well as chromatographic and electrophoretic stopped-flow techniques have become powerful tools for the investigation of stereodynamic processes of chiral compounds and compliment variable-temperature NMR spectroscopy and chiroptical techniques. In contrast to chiroptical methods, dynamic chromatography, and stopped-flow techniques render isolation of pure enantiomers or diastereoisomers unnecessary. Furthermore, only minute amounts ($\sim$ng) of a racemic sample are required and chiral or achiral impurities do not interfere with the measurements as they are usually separated during the chromatographic process. On the other hand, a chiral stationary phase capable of separating the enantiomers of interest at various temperatures remains an indispensable prerequisite and the chiral selector used may slightly affect the enantiomerization barrier measured. The range of energy barriers of dynamic processes that can be studied by dynamic chromatography is determined by the chromatographic time scale, solubility and thermal stability of the eluent, and the temperature range inherent to these techniques. However, a wide range of isomerization barriers can be investigated with DHPLC (60–120 kJ mol^{-1}), DGC (100–150 kJ mol^{-1}), DSFC or DSubFC (60–150 kJ mol^{-1}), and DMEKC (80–120 kJ mol^{-1}).

Similarly, electrophoretic stopped-flow analysis requires only minute racemic amounts of the analyte but eliminates the need for computer simulation. Since electrophoresis is usually conducted with aqueous buffers, isomerization reactions can be investigated at temperatures between 25 and 95 °C and in aqueous solutions. Noteworthy, energy barriers to isomerization ranging from 100 to 130 kJ mol^{-1} can be determined with high accuracy and precision while the affect of

chiral additives can conveniently be excluded or even systematically studied. The extension of this technique to stopped-flow multi-dimensional gas chromatography has provided additional means for the investigation of isomerization reactions with interconversion barriers ranging between 70 and 200 kJ mol^{-1}.

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Christian Wolf
Department of Chemistry, Georgetown University, Washington DC 20057
E-mail: cw27@georgetown.edu

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