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Selector enantioselectivity derived from chromatographic and NMR data

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Abstract—Selectivity factors describing the chiral discrimination exerted by an enantiopure bicyclic diamide selector (DEABA) towards lorazepam enantiomers have been obtained by liquid chromatography (immobilized selector, $\alpha = k_2/k_1$) and by NMR (free selector, $\alpha = K_2/K_1$). Although the NMR results are in agreement with the elution order found in the chromatographic experiments, the α-values obtained by NMR were lower than expected. A possible cause of this discrepancy could be a self-association of the selector in solution, as indicated by a concentration-dependent chemical shift change found.

1. Introduction

The enantioselectivity exerted by a chiral selector can often be significantly changed after binding to a solid support. It may therefore be of interest to compare selectivity factors (α) obtained from chromatography with those obtained with the free selector in solution. While an α-value is readily determined from the chromatographic retention factors, its evaluation from studies of the free selector involves determination of the equilibrium constants for the respective enantiomers of the analyte. Of the spectroscopic methods useful for this purpose, NMR spectroscopy has attracted the most interest with various procedures for the extraction of thermodynamic parameters from spectroscopic data being described.[1–5] Our earlier studies in this field have focused on the possible estimation of the contribution of nonselective interactions in the chromatographic situation.[6,7] Herein, we report a study of the enantioselectivity shown by a recently synthesized chiral selector, DEABA,[8] against a benzodiazepinone, lorazepam, as evaluated from chromatographic and NMR data, respectively.

2. Results and discussion

2.1. Determination of equilibrium constants from NMR data

On the NMR time scale, the equilibrium between the selector and the analyte is fast enough to yield a single peak, with a position determined by $\delta^{obs} = X_A\delta_A + X_{AS}\delta_{AS}$, where X_A and X_{AS} denote the mole fractions of free and selector-bound analyte, A, respectively. If X_{AS} is expressed as a function of the equilibrium constant K, one obtains the expression given in Eq. 1.[1,4,6,7] Here, Δ equals $\delta^{obs} - \delta_A$, Δ_0 is the difference $\delta_{AS} - \delta_A$, $m = S/A$ (the selector to analyte total concentration ratio) and K is the equilibrium constant to be calculated. A plot of Δ against m gives a curve starting in origin of coordinates ($\Delta = 0$ for $m = 0$) and yielding $\Delta = \Delta_0$ as $m \to \infty$.

$$\Delta = \tfrac{1}{2}[1 + m + 1/KA - \sqrt{(1 + m + 1/KA)^2 - 4m}]\Delta_0. \quad (1)$$

Eq. 1 can be simplified if certain approximations are made.[6] In the first case, we assume that $m \gg 1$, which means that $S \gg A$ and the expression for K reduces to $K = x/[S(A - x)]$, where x denotes the concentration of bound A (i.e., AS). It can be shown that this leads to Eq. 2.

$$\Delta = \frac{KS}{KS + 1} \cdot \Delta_0. \quad (2)$$

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Two limiting cases can be distinguished: (1) If $KS \gg 1$ Eq. 2 reduces to $\Delta = \Delta_0$, which represents the totally bound analyte. (2) If $KS \ll 1$ Eq. 2 yields $\Delta = mKA\Delta_0$, that is a linear dependence of Δ on m.

In the second approximation we assume that $m \ll 1$, which means that $S \ll A$ and A can be assumed to be constant. Then $K = x/[A(S - x)]$, which leads to Eq. 3, where Δ is linearly dependent on m.

$$\Delta = m \cdot \frac{KA}{KA + 1} \cdot \Delta_0. \quad (3)$$

Again, two limiting cases can be found: (3) If $KA \gg 1$, Eq. 3 reduces to $\Delta = m\Delta_0$ and (4) if $KA \ll 1$ we again get $\Delta = mKA\Delta_0$.

Experimentally, m was varied at constant A by increasing the selector concentration S and measuring Δ at each m-value. This was done for each enantiomer, either in separate runs or in single experiments using the analyte in racemic form.

2.2. Selector–analyte system investigated

The chiral selector DEABA (*trans*-9,10-dihydro-9,10-ethanoanthracene-11,12-dicarboxylic acid bis-allylamide) was chosen since it has shown a high enantioselectivity towards a series of benzodiazepinone racemates.[8] As the analyte, lorazepam (yielding a selectivity factor of 2.1 in 5% 2-propanol in hexane by chromatography on a DEABA-based sorbent) was selected (Fig. 1). A complicating factor was found in the relatively fast racemization of the lorazepam enantiomers taking place in protic solvents, which excludes 2-propanol-containing solvents for studies of each enantiomer separately. A further complication was the low solubility of the selector in both cyclohexane and 2-propanol. Due to the low solubility in nonpolar solvents, a chloroform-*d*/cyclohexane-d_{12} solvent system with a maximum concentration of 20% cyclohexane-d_{12} had to be used for the NMR studies. The chemical shift of the NH proton in the analyte (δ^{obs}) was measured as a function of added selector ($m = S/A$). The proton at the stereogenic centre could not be used due to overlapping signals from the vinylic protons of the terminal groups of the selector, giving a multiplet at ca. 5.1 ppm.

The change in the chemical shift for the NH proton of both enantiomers of the analyte as a function of

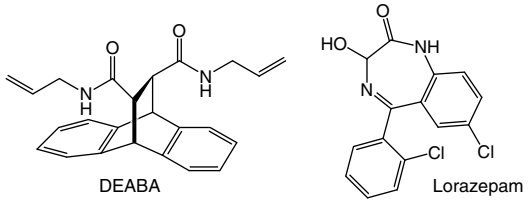

Figure 1. Structures of the selector [(+)-(*S*,*S*)-DEABA] and analyte (lorazepam) used.

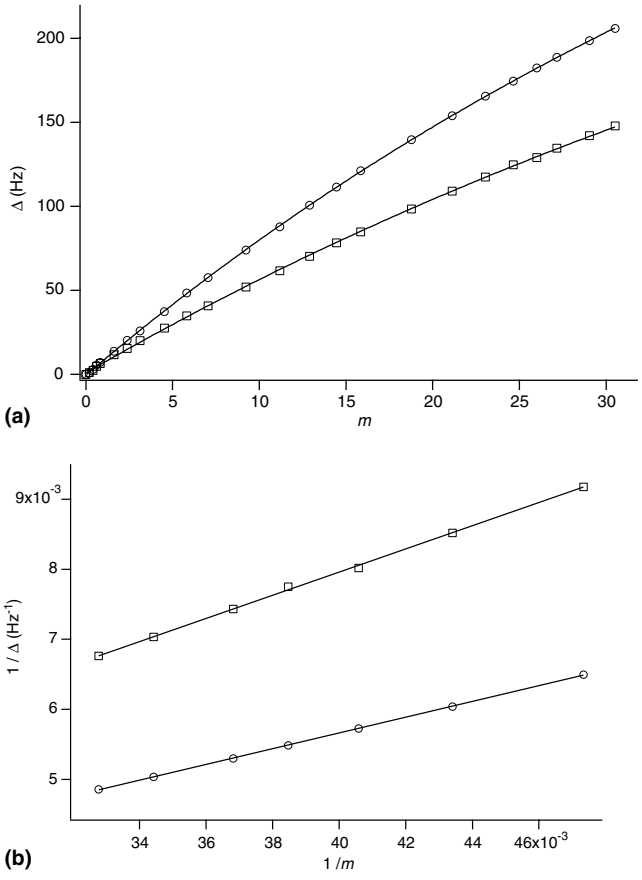

Figure 2. (a) Illustration of the chemical shift difference generated between the two enantiomers of lorazepam with increasing selector concentration and (b) double reciprocal plots yielding the respective equilibrium constrants.

increasing selector concentration and a double reciprocal plot of the Δ versus m data, are given in Figure 2.[9] It is clear that even at relatively high m-values, the equilibrium had not reached the plateau region (Fig. 2a). This is a consequence of the comparatively low K-values in the solvent system used.[7] All attempts to decrease the polarity, and thereby increase K, by addition of more cyclohexane-d_{12} failed, however, due to solubility problems. The m-values used for the plot in Figure 2b are all >20, which justifies the use of the approximation given by Eq. 2. In the double reciprocal plot, the determination of the equilibrium constants from the slope $1/(KA\Delta_0)$ was dependent on the Δ_0-value obtained from the intercept $1/\Delta_0$. An error in the extrapolation to the intercept would have been reflected in the K-value. An alternative method, where K is determined without the need of extrapolation, is the Foster–Fyfe procedure.[5] By this method (used at high m-values), where Δ/S is plotted against Δ, the equilibrium constant given by the slope and Δ_0 is obtained from the intercept corresponding to $K\Delta_0$.

2.3. Comparison of chromatographic and NMR data

The results from the NMR studies of the individual enantiomers of lorazepam were compared to the results

Table 1. NMR data from Foster–Fyfe plots and calculations for the racemate and the separate enantiomers of lorazepam in chloroform-*d*/cyclohexane-d_{12} 80:20

	K_1 (M^{-1})	K_2 (M^{-1})	$\Delta_{0,1}$ (Hz)	$\Delta_{0,2}$ (Hz)	α
Data from Foster–Fyfe plot					
Separate enantiomers	30.8	37.9	741	881	1.23
Racemate	37.3	45.9	638	779	1.23
Calculated data					
Separate enantiomers	30.9	39.2	744	862	1.27
Racemate	32.6	41.4	721	852	1.27

obtained from the studies of racemic lorazepam. Since the results obtained from the Foster–Fyfe plots and from the calculations are similar for the racemate and the separate enantiomers (Table 1), it can be assumed that the competition between the enantiomers for the selector can be neglected when the *m*-values are high and equilibrium constants are relatively low. The only deviating values are the Δ_0-values for the racemate determined by the Foster–Fyfe plot. By utilizing the computational *K*-values in the calculation of $\Delta_{0,1}$ and $\Delta_{0,2}$ the more appropriate values of 731 and 864 Hz, respectively, were obtained.

In Figure 3 the plots of Δ versus *m* and the Foster–Fyfe plots for the NH proton of racemic lorazepam in 20% cyclohexane-d_{12} in chloroform-*d* are shown. Results

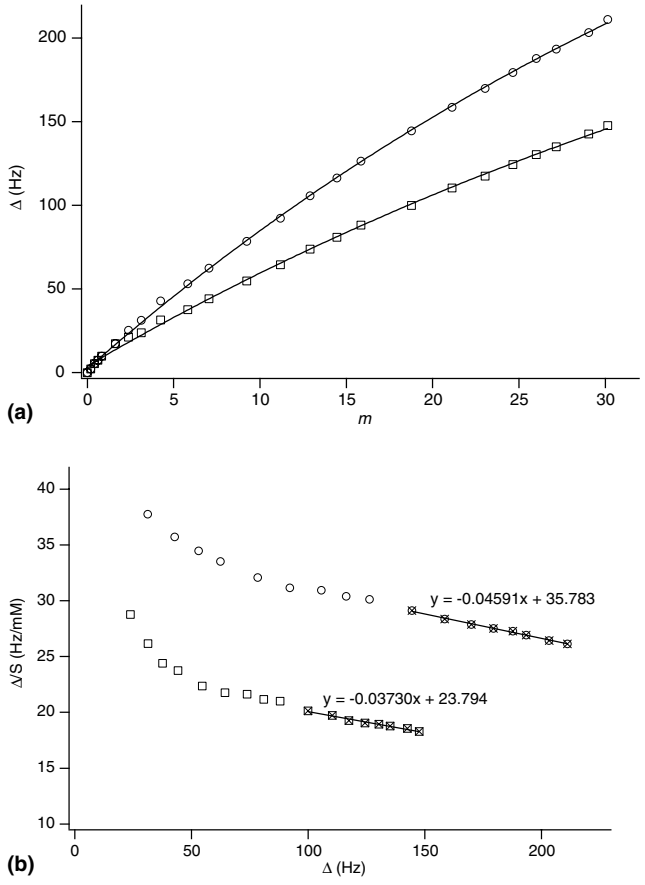

Figure 3. (a) The Δ-values plotted against *m*-values and (b) Foster–Fyfe plots in 20% cyclohexane-d_{12} in chloroform-*d*, where squares represent the chromatographically first eluted enantiomer and circles the second eluted.

obtained from chromatographic resolution of lorazepam on a column containing immobilized DEABA, as compared to NMR data produced by the free selector in three solvent systems with 0%, 10% or 20% cyclohexane-d_{12} in chloroform-*d*, are given in Table 2. Although the α-values obtained by NMR correctly predict the chromatographic elution order, they are lower than expected for a system where fewer nonselective interactions should take place. The reason for this unexpectedly low selectivity is still not quite clear, but is most likely connected to self-association phenomena[10] in the solvents used. This is supported by our findings that the chemical shift of the protons on the bridging carbons at positions 11 and 12 of DEABA is slightly concentration dependent. The chemical shift declined exponentially with increasing selector concentration, indicating a large contribution from self-association at concentrations above 5 mM (Fig. 4), at which the NMR data for lorazepam are obtained. Self-association of the selector in solution should impair stereoselective binding of the analyte and lead to reduced α-values as compared to those obtained in the chromatographic situation where this possibility does not exist. A self-association, taking place via intermolecular dual hydrogen bonding of the amide groups, is strongly supported by molecular docking experiments using semiempirical (AM1) calculations. Moreover, we have previously found that >CO···HN< dual hydrogen bonding contributes significantly to the retention of lorazepam on immobilized DEABA.[8]

One could argue, that in order to avoid self-association during the NMR experiments, it would be possible to use the opposite concentration situation, that is, A ≫ S. However, apart from certain experimental difficulties, we preferred to use conditions less far from those present during chromatography. The column used had a DEABA selector density of 0.16 mmol/g sorbent,[11] whereas the amount of analyte applied was 0.016 μmol, so consequently linear sorption isotherm conditions (corresponding to S ≫ A) were present during chromatography.

It is noticeable that the small amount of ethanol (≈0.8%) used as stabilizer of chloroform had a large effect on the chromatographic retention and resolution of lorazepam. With ethanol-free chloroform as the mobile phase, retention significantly increased (k_1 from 0.46 to 0.95), while the separation factor decreased (α reduced from 2.01 to 1.78). It seems most likely that the ethanol favourably competed for nonselective sites on the CSP, thereby increasing selectivity.

Table 2. Chromatographic and NMR data representing the selector–analyte interactions in three different solvent media

Solvent	Chromatographic data			NMR data		
% *c*-C_6H_{12} in $CHCl_3$[a]	k_1	k_2	α	K_1 (M^{-1})	K_2 (M^{-1})	α
0	0.95	1.69	1.78	25.8	27.3	1.06
10	1.23	2.20	1.78	32.2	36.4	1.13
20	1.55	2.84	1.83	37.3	45.9	1.23

[a] Deuterated solvents used for NMR.

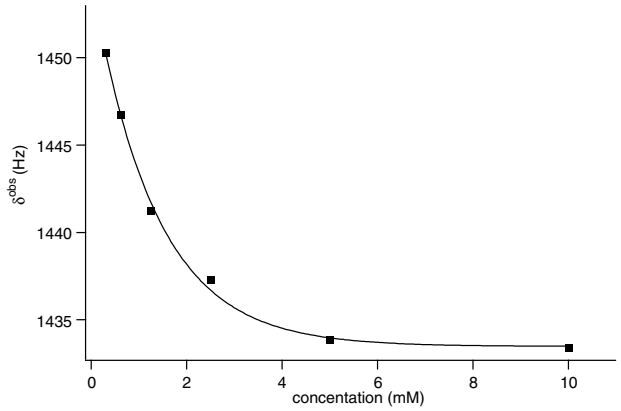

Figure 4. The change of chemical shift for the protons on C11 and C12 of DEABA with varying selector concentration.

3. Experimental

3.1. General

Analytical liquid chromatography was performed on a system of a Varian 9012Q solvent delivery pump and a Varian 9050 variable wavelength UV detector. Samples were injected via a Rheodyne injector (5 μL loop). The preparative liquid chromatographic system consisted of a Shimadzu LC-8A solvent delivery pump, a Shimadzu SPD-10A UV–vis detector and a Rheodyne injector (1000 μL loop). All NMR experiments were recorded at 500 MHz with a Varian Unity 500 NMR spectrometer at the probe temperature 25 °C.

The solvents used for chromatography were of HPLC grade. Ethanol-free chloroform was obtained from Sigma–Aldrich. The solvents used in the NMR experiments were $CDCl_3$ of 99.8% isotopic purity from Dr. Glaser AG Basel and cyclohexane-d_{12} of 99.7% purity from Larodan Fine Chemicals AB. The selector and the column with immobilized DEABA phase were prepared according to Ref. 11. Lorazepam was a gift from AstraZeneca R&D (Mölndal, Sweden).

3.2. Chromatography

The analytical chromatographic separations of lorazepam were obtained on a column (250×3.2 mm i.d.) with an immobilized DEABA phase. The mobile phases were 0%, 10% and 20% cyclohexane in chloroform while the flow was 0.75 mL/min. Samples were dissolved in the mobile phase to a concentration 1 mg/mL with a 5 μL volume injected. Detection was made at $\lambda = 225$ nm. Retention factors and selectivity factors are shown in Table 2.

Preparative liquid chromatographic separation was obtained on a Kromasil CHI-DMB column of size 250×20 mm i.d. Due to racemization of lorazepam in solvents containing 2-propanol, the mobile phase used was 10% methyl *tert*-butyl ether in hexane. The flow was 20 mL/min and the injection volume 1000 μL. Samples were dissolved in 2-propanol to a concentration 5 mg/mL. Detection was made at $\lambda = 225$ nm. $k'_1 = 3.43$ and $\alpha = 1.46$.

3.3. NMR

In the NMR spectra of solutions of (+)-, (−)- or (±)-lorazepam (0.17 mg, 0.53 μmol) in 0%, 10% or 20% cyclohexane-d_{12} in chloroform (2 mL), the chemical shift for the NH proton of lorazepam in absence of the selector was determined. The selector (5.83 mg, 15.6 μmol) was dissolved in a solution of lorazepam (1.4 mL) to keep the analyte concentration constant during the addition of the selector. To an NMR tube with 500 μL of the 0.26 mM solution of lorazepam, the 11.18 mM solution of the selector was added in volumes of 4×2.5, 3×10, 3×20, 5×40, 6×100 and 2×200 μL with syringes equipped with chaney adapters. After every addition, an NMR spectrum was recorded and the new observed chemical shift for the NH proton determined for each current selector concentration.

3.4. Computation

Evaluation of *K*-values by double reciprocal and other plots based on Eq. 2 was made by the use of Igor Pro (ver. 2.04) and Excel programs. Refinement of the calculations was made by means of the MATLAB (ver. 5.3.1) program (The MathWorks, Inc.) by minimization of the function:

$F(K) = \sum_i (\delta_i^{\mathrm{obs}} - x_{\mathrm{A}_i}\delta_{\mathrm{A}} - x_{\mathrm{AS}_i}\delta_{\mathrm{AS}})^2$ as described previously.[2,4,7]

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