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Tetrahedron
Tetrahedron 64 (2008) 1257–1262
www.elsevier.com/locate/tet

Structural and thermodynamic investigations of an unusual enantiomeric separation: Lipodex E and compound B

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Received 26 July 2007; received in revised form 3 November 2007; accepted 22 November 2007
Available online 23 November 2007

Abstract

Compound B (1,1,3,3,3-pentafluoro-2-fluoromethoxy-1-methoxypropane) can be separated by gas chromatography with an extraordinary chiral separation factor on a column coated with Lipodex E (octakis(3-*O*-butanoyl-2,6-di-*O*-*n*-pentyl)-γ-cyclodextrin). The enantioselectivity is greatly reduced when employing the β-cyclodextrin analogue. To further investigate the background of this unusual separation, the complexation between 'compound B' and Lipodex E (octakis(3-*O*-butanoyl-2,6-di-*O*-*n*-pentyl)-γ-cyclodextrin) and heptakis(3-*O*-butanoyl-2,6-di-*O*-*n*-pentyl)-β-cyclodextrin were studied by NMR. Association constants of the interaction of the two enantiomers of compound B with Lipodex E and its β-cyclodextrin analogue were determined by NMR chemical shift titration and showed a large difference corroborating earlier GC results. Heteronuclear NOE measurements proved that inclusion complex formation is taking place with compound B situated inside the cavity of the cyclodextrin moiety. Differences between the inclusion complex structures and their connection to association constants are discussed.
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Keywords: Chiral recognition; Inclusion complex formation; Enantiomeric separation; Chromatography phases

1. Introduction

Cyclodextrins are cyclic oligosaccharides composed of α-(1→4)-linked α-D-glycosyl residues, of which α-, β- and γ-cyclodextrin, consisting of 6–8 glycosyl units, respectively, is the most studied.[1–3] These cyclodextrins can be described as toroidal, hollow, truncated cones with a hydrophilic exterior and a hydrophobic interior. This unique structure allows molecules (guests) with hydrophobic groups to at least partly enter the cavity and be bound by the cyclodextrin (host) by non-covalent forces only. The resulting entity is known as a guest–host complex or inclusion complex. This capability is exploited in many applications in the pharmaceutical, agrochemical, food, and chemical industries, e.g., as vehicle for drug delivery of poorly water-soluble drugs, for increasing stability of labile molecules, masking of unpleasant taste or odour and stabilisation of protein solutions against aggregation.[3–5] Moreover, cyclodextrins have gained a position as the most widely used eluent modifier for the separation of structurally similar molecules (e.g., enantiomers) by chromatography and electrophoresis. Numerous chemically modified cyclodextrin materials for chromatography purposes have been reported. Of these, Lipodex E (octakis(3-*O*-butanoyl-2,6-di-*O*-*n*-pentyl)-γ-cyclodextrin) has exhibited some of the largest separation factors between enantiomeric compounds.[6–12] The largest separation factor between enantiomeric compounds ever observed was with a degradation product of the inhalational anaesthetic sevoflurane (1,1,1,3,3,3-hexafluoro-2-(fluoromethoxy)-propane) called 'compound B' (1,1,3,3,3-pentafluoro-2-fluormethoxy-1-methoxypropane). The structures of sevoflurane and its degradation to compound B under basic conditions[13–16] are shown in Figure 1. A recent study[17] investigated the inclusion complex formation of compound B with another cyclodextrin derivative, (2,3-di-*O*-acetyl-6-*O*-*tert*-butyldimethylsilyl)-β-cyclodextrin, which does not show such outstanding separation factors. That study focused on

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doi:10.1016/j.tet.2007.11.069

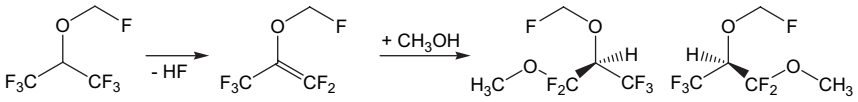

Figure 1. The chemical structure of sevoflurane and its degradation to compound B via compound A.

the interaction of compound B with the silyl groups attached to the cyclodextrin molecule and explained the moderate enantiodiscrimination with these interactions. No clear conclusions were presented regarding the interaction with the inside of the cyclodextrin cavity. In the light of these results, it seemed strange that Lipodex E, a compound lacking the silyl groups, is able to discriminate between the *S*- and *R*-enantiomer of compound B even stronger than the silyl modified cyclodextrin.

In this paper, we wanted to quantify the enantiodiscrimination between *R*- and *S*-compound by Lipodex E in terms of association constants and to find an explanation for the observed separation factors in these association constants as well as in structural data on the interaction. To this end, we investigated the complexation of enantiomerically pure *R*- and *S*-compound B with Lipodex E. In addition, we also investigated the β-cyclodextrin analogue of Lipodex E (heptakis(3-*O*-butanoyl-2,6-di-*O*-*n*-pentyl)-β-cyclodextrin). The α-cyclodextrin analogue of Lipodex E, hexakis(3-*O*-butanoyl-2,6-di-*O*-*n*-pentyl)-α-cyclodextrin, does not show any enantiodiscrimination.[7] It was of special interest to investigate, whether the observed differences can be explained by different geometries of the inclusion complex formation.

We used NMR spectroscopy for both parts of this study. For the measurement of association constants by NMR, several methods have been published. The standard method of choice is a titration, where the fraction of complexed guest molecule is determined from chemical shift changes induced by the complexation.[18] Another NMR-based method is to study NMR diffusion.[19,20] NMR-derived association constants have also been suggested as a mean to predict the extent of enantiodiscrimination.[18]

2. Results

2.1. Thermodynamic investigation of inclusion complex formation

Figure 2 shows some of the NMR spectra obtained for the titration of Lipodex E with *R*- and *S*-compound B, respectively. Figure 3 shows the data extracted from the spectra and the quality of the fit. Table 1 shows the obtained association constants and δ_{bound} values.

It is worthwhile to consider the uncertainty of the fit. This is not trivial, since we are fitting two parameters. The uncertainties obtained are correlated, in that certain combinations of deviations of the two parameters are more probable than others. We have attempted to visualise this by calculating the sum of square deviations of the experimental data points from the fitted function and plotting this against the two parameters. These plots are shown in Figure 3.

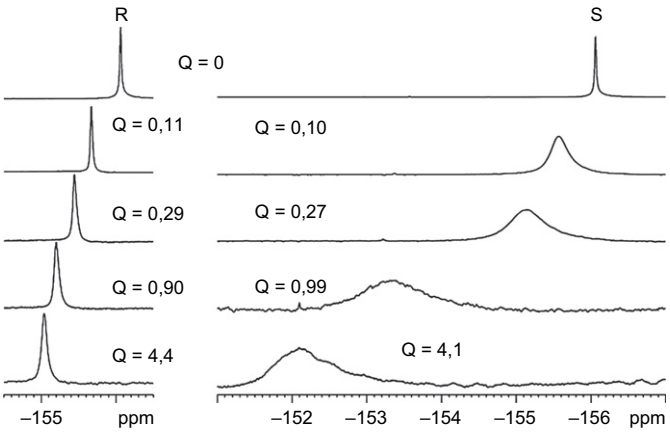

Figure 2. ^{1}H decoupled ^{19}F NMR signal of $-CH_2F$ of compound B in the presence of Lipodex E at different molar ratios *Q* (defined in Eq. 1) shown for *R*-compound B (left) and *S*-compound B (right).

The fitted K_a value of the interaction of Lipodex E and *S*-compound B carries a large uncertainty. This is generally the case when trying to determine association constants of strong interactions. Nevertheless, it is evident that there is a large difference in K_a between *R*- and *S*-compound B, which corroborates earlier GC results.[7]

We have used cyclohexane as the solvent for NMR investigations. Earlier studies on similar compounds[21] have shown that only very little or no enantiodiscrimination can be observed in other apolar solvents like chloroform or benzene. This was also the case for our compounds (data not shown). The reason for this is unknown.

2.2. Structural investigation of inclusion complex formation

$\{^{19}F\}-^{1}H$ NOE difference experiments revealed only minor differences in nuclear Overhauser enhancements between the complexes of the two enantiomers of compound B. Figure 4 shows an example for NOE spectra obtained with irradiating different resonances. NOEs between fluorine atoms of compound B and hydrogen atoms of Lipodex E and its β-cyclodextrin based analogue were solely observed inside the cavity of cyclodextrin, i.e., to atoms H^{III}, H^{V} and H^{VI} of the cyclodextrin glucose moieties. No NOE enhancements could be detected for the aliphatic hydrogen atoms of the butanoyl and pentyl groups bound to the cyclodextrin skeleton. No, or only very weak, NOEs were observed to the hydrogen atoms H^{I}, H^{II} and H^{IV}, which are situated on the outside of the cyclodextrin molecule, whereas significant $\{^{19}F\}-^{1}H$ NOE signal enhancements were observed for those cyclodextrin atoms situated inside the cavity, H^{III}, H^{V} and H^{VI}. This is indicative of the interaction taking place mainly inside the cavity, which is

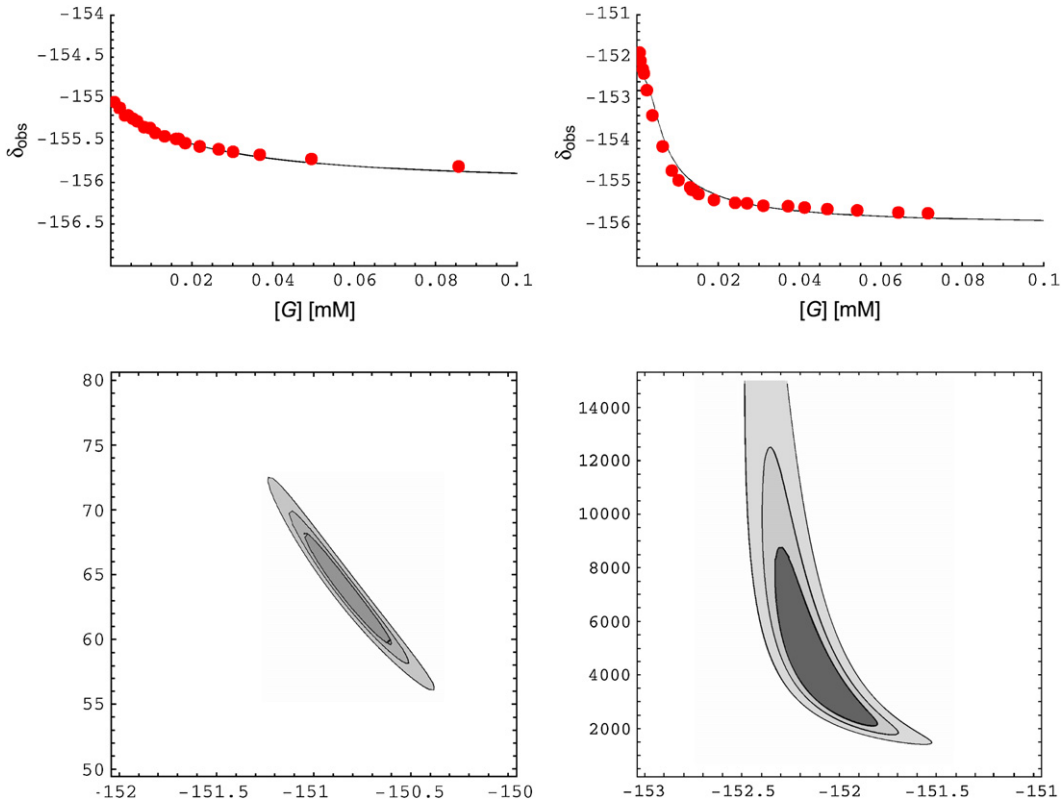

Figure 3. Results from titration of Lipodex E with *R*-compound B (upper left panel) and *S*-compound B (upper right panel). Red points are experimental data points and the solid line indicates the theoretical function with the fitted parameters (given in Table 1). The lower left (for *R*-compound B) and right (for *S*-compound B) panels show the uncertainty of the fit. The grey areas indicate combinations of K_a and δ_{bound} that increase the sum of square deviations of the experimental data points from the fitted curve by less than 5%, 10% and 20%, respectively.

somewhat surprising, given the hydrophobic nature of the solvent and the excellent solubility of the guest molecule, compound B, in the solvent cyclohexane.

In order to make the data independent of different complex concentrations (owing to different sample concentrations and different K_a values), and to simplify the interpretation of the data in terms of positioning of guest molecule atoms relative to the cyclodextrin atoms, the NOE enhancements have been normalised. It is thus possible to compare relative NOE enhancements. The comparison of the relative NOE intensities within the cavity can give clues about the average structure of the inclusion complexes. If the NOE from an atom belonging to the guest molecule to H^{III} is stronger than that to H^{V}, this means a placement of the atom near the wide rim of cyclodextrin, if an NOE is stronger to H^{V}, the atom is situated deeper inside the cavity, and strong NOEs to H^{VI} indicate a large depth of penetration. Figure 5 shows a graphical representation of the NOE enhancements obtained for the different complexes. The data are represented as

$$\lg\left(\frac{\eta_{F-H^{III}}}{\eta_{F-H^{x}}}\right)$$

where lg denotes the decadic logarithm, η denotes the heteronuclear NOE between F and H^{III}, or F and H^{x}, where x stands for V or VI. (NOE values to H^{VI} represent an average of the NOE values to the two H^{VI} atoms.) A value >0 corresponds to a position of the fluorine atom in question nearer to H^{III} than to H^{x}.

Comparing the relative NOE intensities between the inclusion complexes of *R*- and *S*-compound B with Lipodex E and its β-cyclodextrin analogue gives valuable information not only on the geometry of the inclusion, but also on the differences between the complexes.

In all cases, the NOEs from the CF_3 group to H^{V} and H^{VI} of the cyclodextrin moiety are stronger than NOEs from other fluorine atoms to H^{V} and H^{VI}, indicating that this part of compound B is situated deepest inside the cavity in all four inclusion complexes. For the inclusion complexes with the β-cyclodextrin analogue of Lipodex E, the NOE intensities of compound B atoms to H^{III} and H^{V} are comparable, whereas NOE enhancements of H^{VI} are weaker. In the case of

Table 1
Association constants of the complex formation of *R*- and *S*-compound B and Lipodex E and its β-cyclodextrin analogue as obtained by NMR titration

K_a and δ_{bound}	β-Cyclodextrin analogue of Lipodex E	Lipodex E
R-Compound B	K_a=4.6±1[a] [l mol^{-1}] δ_{bound}=−150.2±1[a] [ppm]	K_a=64±4[a] [l mol^{-1}] δ_{bound}=−150.9±0.3[a] [ppm]
S-Compound B	K_a=24±2[a] [l mol^{-1}] δ_{bound}=−154.0±0.2[a] [ppm]	K_a=4000±2000[a] [l mol^{-1}] δ_{bound}=−152.1±0.3[a] [ppm]

[a] The errors in the fitted values are correlated, i.e., only certain combinations of deviations are possible, e.g., a lower value of K_a means a higher value of δ_{bound}.

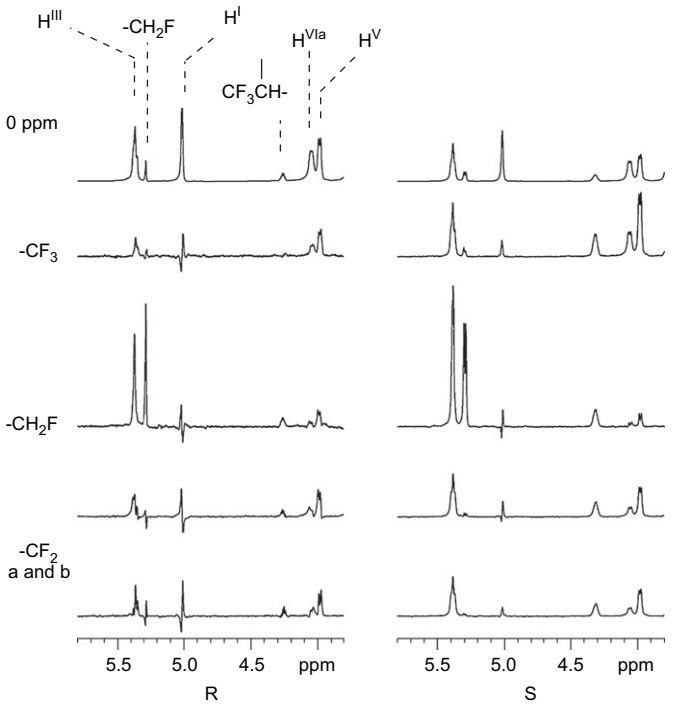

Figure 4. {^{19}F}–^{1}H NMR spectra of mixtures of the β-cyclodextrin analogue of Lipodex E with *R*- and *S*-compound B, respectively. The uppermost line shows the spectra with irradiation at 0 ppm, i.e., far away from any ^{19}F NMR signal and the other lines show the NOE difference spectra obtained after irradiation of the ^{19}F NMR resonances of the atoms noted at the left margin.

S-compound B, the fluorine atom of the CH_2F group is, however, situated much nearer the wider rim of the cyclodextrin cavity. This is not the case for *R*-compound B. This difference suggests a different orientation of the two guest molecules in the cyclodextrin cavity.

Comparing the NOE pattern for the inclusion of *R*-compound B into Lipodex E, and its β-cyclodextrin analogue, the overall orientation of the guest molecule inside the cavity is very similar, with slightly lower ratios of NOEs to H^{III} than to H^{V} and H^{VI}, indicating a slightly deeper penetration of the guest molecule into the (wider cavity) of the γ-cyclodextrin moiety. This is not surprising and one is tempted to speculate that the larger association constants obtained for the Lipodex E are probably a consequence of this difference.

The difference in NOE pattern between *R*- and *S*-compound B interacting with Lipodex E is less distinct than in the case of the β-cyclodextrin analogue. In fact, the differences between the NOEs from the different fluorine atoms to the cyclodextrin atoms seem to be smaller. The small differences in NOE intensities from the fluorine atoms to H^{III} and especially H^{V} and H^{VI} point at a structure, where the distances of the fluorine atoms to all atoms within the cavity of the cyclodextrin moiety are similar. This is difficult to picture. The explanation for this observation is rather a dynamic complex with a multitude of structures interchanging at a fast time scale.

Overall, the NOE data seem not to offer an unambiguous explanation of the exorbitantly different association constants of the complexes of Lipodex E with the two enantiomers of compound B.

3. Conclusion

We present here association constants and NOE data of the complexes of both enantiomeric forms of compound B with Lipodex E and the β-cyclodextrin analogue of Lipodex E. The association constants obtained show a large difference, possibly the largest so far reported for pairs of enantiomers. However, NOE data fail to explain these differences by the structure of the inclusion complexes. From our data, it was found, that the interaction between the host and the guest molecule took place exclusively inside the cyclodextrin cavity. This is somewhat surprising in the light of the findings reported on the interaction of compound B with 2,3-di-*O*-acetyl-6-*tert*-butyldimethylsilyl)-β-cyclodextrin.[17] There, a significant part of the interaction is attributed to the interaction with the silyl groups used to modify the cyclodextrin; no relevant dipolar interactions between compound B (hydrogen) atoms and H^{V} are reported and it is concluded that the CH_3O group is the only part of compound B entering the cavity, though not very deep. The results are very different from ours, yet the experimental conditions (temperature, solvent) used for both NMR studies were identical. That study used homonuclear ROEs instead of heteronuclear NOEs, but also in their case, only minor differences in ROE patterns could be observed. This other study also investigated the influence of the presence of the guest molecule on the host geometry and found some differences there.

Overall, it seems that complex geometries at the level of precision that they can be obtained today are in some cases not suitable as the sole models to explain stability constants of inclusion complexes and their differences. Complex formation is a process on a multidimensional energy landscape; the magnitude of the association constant is dependent on the number of minima on this landscape and their depths—while the investigation of complex geometries cannot account for the depths of the minima. Clear-cut NMR data on the complex geometry are only available if there is a single minimum on the complexation energy landscape, which is energetically much more favourable than the other minima; a multitude of minima with similar energies will result in a multitude of possible complex geometries, thus blurring the picture that solution-state NMR can provide. However, from the thermodynamic viewpoint, a multitude of energy minima does not necessarily mean that the interaction is weak; it is the sum of possible conformations and their complexation enthalpies that decides the strength of the interaction.

A possibility to improve the interpretation of solution-state NMR data in cases like this would be the development of structural calculation techniques that take into account the ensemble nature of NMR data, as has been demonstrated for protein structural determination.[22]

Another point that needs more consideration is the solvent and its behaviour. As outlined above, cyclohexane has been found to mimic the conditions under a GC experiment best,

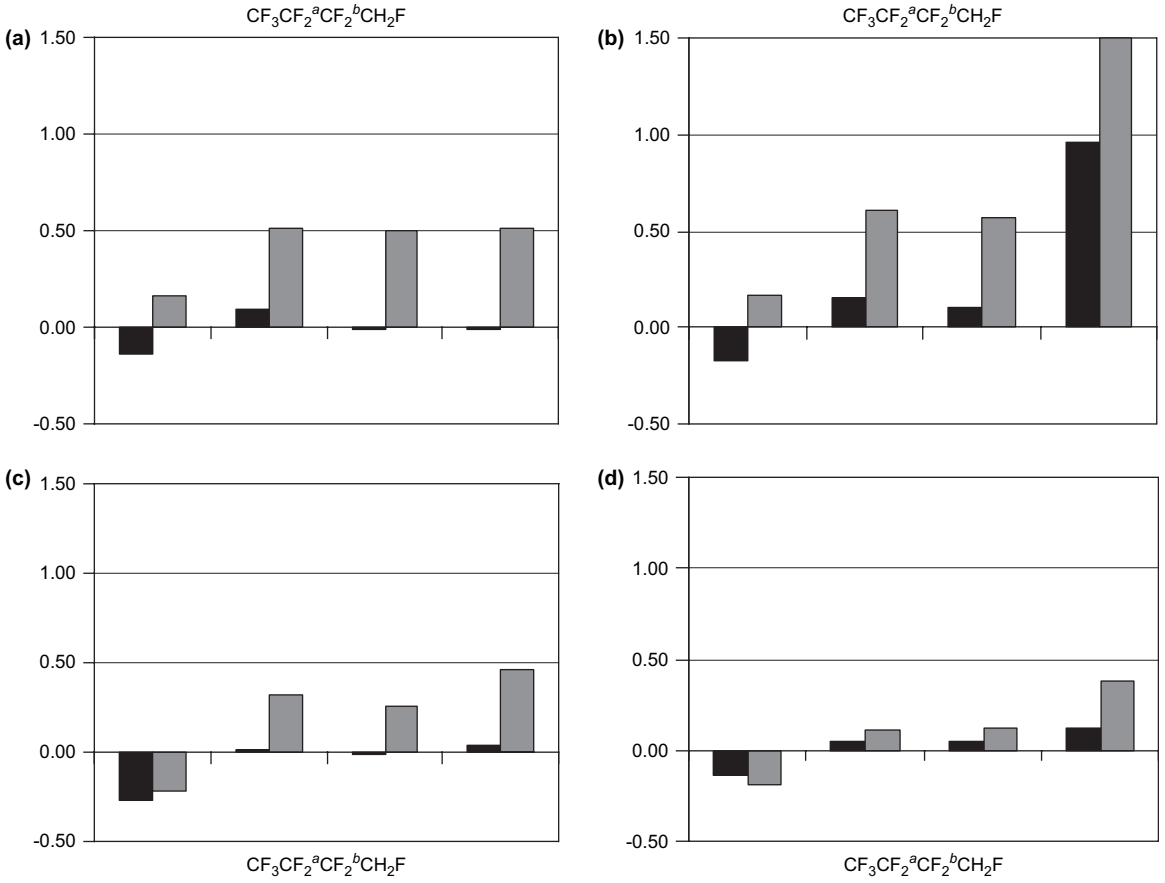

Figure 5. NOE enhancements obtained on ^{1}H atoms of Lipodex E upon irradiation of ^{19}F resonances. NOE enhancements (η) are represented as $\lg(\eta(F-H^{III})/\eta(F-H^{V}))$ (dark grey) and $\lg(\eta(F-H^{III})/\eta(F-H^{VI}))$ (light grey). A positive value means that the NOE from the denoted fluorine atom to H^{III} is higher than that to H^{V} (or H^{VI}), and thus the average distance to H^{III} is shorter than that to H^{V} or H^{VI}. (a) β-Analogue+*R*-compound B, (b) β-analogue+*S*-compound B, (c) Lipodex E+*R*-compound B, (d) Lipodex E+*S*-compound B.

while other solvents are not able to reproduce the enantiodiscrimination or do not even yield complex formation. The reason for this is yet unknown. However, it stands clear that the solvent plays an important role in the complex formation. Future investigations should focus on the influence of the solvent on complex formation and enantiodiscrimination.

4. Experimental

4.1. General

The enantiomers of compound B (ee >99.9%) were prepared as described previously.[7] Cyclohexane-d_{12} was obtained from Deutero AG, Germany.

Butanoylated/pentylated cyclodextrins **1** and **2** were synthesised according to the procedure of König and Krebber[6] and slightly modified.[23,9]

In order to minimise the competitive effect of the solvent in the association process both dichloromethane and benzene were tested but no enantiodiscrimination was observed. In the apolar solvent cyclohexane, C_6D_{12}, a significant differentiation of the enantiomers of compound B was detected in the presence of Lipodex E.

4.2. NMR

All NMR experiments were carried out at 293.1 K on a BRUKER DRX600 NMR spectrometer operating at a field strength of 14.1 T. ^{1}H NMR measurements were obtained using a TXI (H/C/N) probe and ^{19}F measurements were obtained using a QXI (H/N/P/F) probe.

4.2.1. Determination of association constants

The determination of association constants follows the method described by Grosenick et al.[21] Small aliquots of compound B were added to solutions of the β-cyclodextrin based analogue of Lipodex E (4.2 mM) or Lipodex E (3.8 mM) in cyclohexane-d_{12}. Both ^{1}H and ^{19}F spectra were obtained for each titration point. The chemical shifts of the signals of compound B were plotted against Q, defined in Eq. 1:

$$Q = \frac{[H]_0}{[G]_0} \tag{1}$$

where $[H]_0$ is the total concentration of host molecule (cyclodextrin derivative), free or bound, and $[G]_0$ is the total concentration of guest molecule (compound B), free or bound.

The association constant (K_a) of the process

$$H + G \rightleftharpoons HG$$

is described by Eq. 2:

$$K_a = \frac{[HG]}{[H][G]} = \frac{[HG]}{([H]_0 - [HG])([G]_0 - [HG])} \quad (2)$$

where [H] is the concentration of free host molecules, [G] is the concentration of free guest molecules and [HG] is the concentration of complex.

The fraction of bound guest molecules can be determined from the chemical shift of their signals. Guest molecules have different chemical shifts in the bound (δ_{bound}) and the free (δ_{free}) forms. In case of an equilibrium between a free form and a bound form and an exchange rate between these two states that is faster than the difference in resonance frequency, only one signal will be observed with an observed chemical shift (δ_{obs}) that is a weighted average between δ_{bound} and δ_{free}. For the following, we define the observed change in chemical shift ($\Delta\delta_{obs}$) and the change in chemical shift upon binding ($\Delta\delta_{bound}$):

$$\Delta\delta_{obs} = \delta_{obs} - \delta_{free} \quad (3)$$

$$\Delta\delta_{bound} = \delta_{bound} - \delta_{free} \quad (4)$$

The ratio between $\Delta\delta_{obs}$ and $\Delta\delta_{bound}$ is thus equivalent to the mole fraction of bound guest molecules:

$$\frac{[\Delta\delta_{obs}]}{[\Delta\delta_{bound}]} = \frac{[HG]}{[G]_0} \quad (5)$$

[HG] can be obtained from Eq. 5 and the result was substituted into Eq. 2. Thus, Eq. 6 is obtained, yielding the association constant as a function of $\Delta\delta_{obs}$ and $\Delta\delta_{bound}$.

$$K_a = \frac{\Delta\delta_{obs}/\Delta\delta_{bound}}{\{1 - (\Delta\delta_{obs}/\Delta\delta_{bound})\}\{[G]_0 - [H]_0(\Delta\delta_{obs}/\Delta\delta_{bound})\}} \quad (6)$$

Eq. 6 can be solved for $\Delta\delta_{obs}$ to yield Eq. 7, the function of the titration curve $\Delta\delta_{obs}=f([G]_0)$ with the two unknown parameters $\Delta\delta_{bound}$ and K_a:

$$\Delta\delta_{obs} = (\Delta\delta_{bound}/2[H]_0)\left[\{[G]_0 + [H]_0 + K_a^{-1}\} - \{([G]_0 - [H]_0)^2 + (2[H]_0)^2 - (2[H]_0/K_a) + (2[G]_0/K_a) + K_a^{-2}\}^{1/2}\right] \quad (7)$$

$\Delta\delta_{bound}$ and K_a were determined by fitting Eq. 7 to the experimental data with the nonlinear regression module of Mathematica. Only ^{19}F chemical shifts were used here because of problems with overlapping ^1H resonances of compound B and the cyclodextrin derivatives.

4.2.2. NOE difference measurements

$\{^{19}F\}-^1H$ NOE difference measurements were obtained by recording ^1H NMR spectra after 2.5 s irradiation of each single resonance in the ^{19}F spectrum and at 0 ppm in an interleaved manner and subtracting the spectra obtained with irradiation at 0 ppm from the spectra obtained with irradiation. For irradiation, a continuous wave spin lock of 312 Hz was used.

Attempts to record 2D-heteronuclear NOESY experiments did not yield any crosspeaks, probably due to fast relaxation of ^{19}F signals.

Acknowledgements

A.B. thanks the Graduate College 'Chemistry in Interphases' of the Deutsche Forschungsgemeinschaft for financial support. The NMR laboratory at Aalborg University is supported by the Obel Foundation.

We wish to thank BRUKER BioSpin, Karlsruhe, for loan of a suitable probehead for ^{19}F measurements and especially Dr. Wolfgang Bermel from BRUKER for technical assistance. We wish to acknowledge the invaluable contribution of Professor V. Schurig to this publication.

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