TOPICS IN CHEMISTRY AND MATERIAL SCIENCE, Vol. 5 (2011) pp. 70–77
Current Issues in Organic Chemistry 2, edited by R.D. Nikolova, S. Simova,
P. Denkova, G.N. Vayssilov

Chemical Shift Anisotropy-Based Measurement of Enantiomeric Excess for Selected Tetrasubstituted Pyrrolidines

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Abstract
The measurement of enantiomeric excess is still a difficult analytical task in modern chemistry. One way of measuring this quantity is the integration of NMR spectra acquired in a chiral environment. Especially samples in chiral alignment media are known to be distinguishable by differential anisotropic NMR parameters. Here we show three racemic mixtures of relatively large tetrasubstituted pyrrolidines as examples for measuring enantiomeric excess via differences in residual chemical shift anisotropy induced by the well-known chiral alignment medium poly-γ-benzyl-L-glutamate (PBLG).

1 Introduction

NMR spectroscopy is one of the most important analytical tools in modern organic and inorganic chemistry as it is the only tool that allows the determination of molecular structures at atomic resolution in solution. It is usually used to identify the constitution, conformation and configuration of a given molecule, but there are also methods available for the measurement of enantiomeric excess. Most commonly used are chemical modifications with chiral substances of known chirality which leaves a diastereomeric pair in the end that can be distinguished with conventional NMR spectroscopy. The most popular example is the Mosher-ester and the many related compounds [1,2]. But the method relies on the existence of an appropriate functional group in the molecule of interest and especially when working with highly valuable compounds one doesn't necessarily want to irreversibly modify the substance.

Another possibility for the distinction of enantiomers is the orientation of the molecule of interest in a so-called chiral alignment medium [3-6]. In this case, the molecule is partially aligned and anisotropic NMR parameters can be measured. Such parameters can be residual quadrupolar couplings, residual dipolar couplings, and residual chemical shift anisotropy [5,6], where the

 ISSN 1314–0795

latter one is most easily acquired in a conventional gated decoupled ^{13}C 1D spectrum [7-9]. The distinction of enantiomers with chiral alignment media like PBLG [10,11], PCBLL (poly-ε-carbobenzyloxy-L-lysine) [12], PELG (poly-γ-ethyl-L-glytamate) [12,13], polyguanidines [14], or stretched gelatin [15-19], collagen [19], and polysaccharides [20] has so far been demonstrated for relatively simple molecules which fit into the grooves of the chiral polymers. This study deals with the enantiomeric distinction of three racemic tetrasubstituted pyrrolidines consisting of at least three ring systems with considerable potential flexibility, which can be considered a challenging task for differential alignment. As chiral orienting medium we used the well-established lyotropic mesophase of PBLG with $CDCl_3$ in all three cases.

2 Residual Chemical Shift Anisotropy and the Distinction of Enantiomers

While solid state spectra contain the full anisotropic NMR interactions, they are completely averaged out in isotropic samples. Partially aligned samples, on the other hand, have a complex averaging behavior, which is usually described by the Saupe matrix $\mathbf{S}$ [21] or the alignment tensor $\mathbf{A} = A_{\alpha\beta}$ with $\mathbf{A} = 2/3\mathbf{S}$. The alignment tensor describes the amount of alignment introduced by the so-called alignment medium. For a detailed introduction into this concept we refer the reader to [22].

Conventional, isotropic liquid samples have a vanishing alignment tensor $\mathbf{A} = \mathbf{0}$ and signals of such samples can be unambiguously characterized by the isotropic chemical shift. In general, however, the chemical shift is a tensor $\boldsymbol{\sigma} = \sigma_{\alpha\beta}$, which can be divided into an isotropic scalar σ^{iso} and the chemical shift anisotropy tensor $\sigma^{\text{aniso}}_{\alpha\beta}$. With the effective averaging the chemical shift value is given by [23]:

$$\delta = \sum_{\alpha,\beta=a,b,c} \sigma_{\alpha\beta} A_{\alpha\beta} = \sigma^{\text{iso}} + \sigma^{\text{aniso}}_{\alpha\beta} A_{\alpha\beta} \,.$$

The difference in chemical shift between isotropic and partially aligned sample $\sigma^{\text{aniso}}_{\alpha\beta} A_{\alpha\beta}$ is called residual chemical shift anisotropy (RCSA). It is apparently proportional to the chemical shift anisotropy $\sigma^{\text{aniso}}_{\alpha\beta}$ which varies strongly among different nuclei and chemical surroundings. Its size and therefore its discrimination power for enantiomers is only large enough in favourable cases, as e.g. for most ^{19}F and ^{31}P nuclei and aromatic, olefinic, or carbonyl carbons [6].

The chemical shift anisotropy is identical for enantiomers as it does not change upon inversion. However, the alignment tensor $\mathbf{A}$ in a chiral alignment medium depends on the chirality of the solute molecule. The resulting alignment tensors for the R and S configuration are correspondingly $\mathbf{A}^R$ and $\mathbf{A}^S$. As the solute interacts with the chiral polymer, intermediate diastereomeric complexes are formed which, in principle, can be distinguished by NMR spectroscopy. The different alignment tensors $\mathbf{A}^R$ and $\mathbf{A}^S$ for the two enantiomers will lead to different observed averaged chemical shifts δ^R and δ^S, which can be used for enantiomeric distinction (see Figure 1 and [5]).

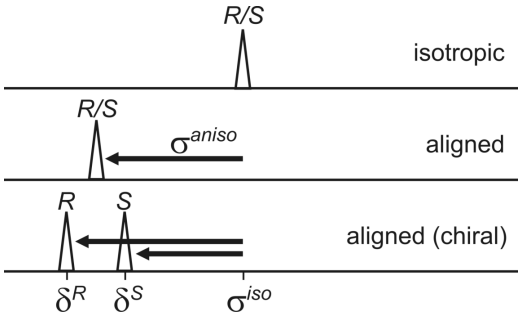

Figure 1. Schematic description of the effect of chiral alignment on chemical shifts. In the isotropic phase isotropic chemical shifts are observed that cannot distinguish enantiomers (top). When a sample is aligned in an achiral alignment medium, signals are shifted by RCSA, but R and S are still indistinguishable (middle). Alignment in a chiral alignment medium leads to two different RCSA values for the two enantiomers and eventually separates R from S (bottom).

Finally, the measurement of chemical shifts is trivial and can be done in any kind of spectrum with one or more chemical shift evolution periods. Probably the most effective way to measure enantiomeric excess is a proton-decoupled ^{13}C-1D spectrum. Due to the observed singlets with typically very narrow linewidths the discrimination power in this case is quite good and signal integration is directly related to corresponding populations from which enantiomeric excess can easily be calculated.

3 Tetrasubstituted Pyrrolidines

Three tetrasubstituted pyrrolidines as shown in Figure 2 were synthesized as described in detail in [24]. The compound **1** is the main reaction product of a 1,3-cycloaddition reaction between N-arylmethyleneaminomethyl-dimethylphosphineoxide and ethyl cinnamate and is identified as ethyl 2-(4-chlorophenyl)-5-(dimethylphosphoryl)-4-phenylpyrrolidine-3-carboxylate. Compounds **2** and **3** are products of the same reaction of N-arylmethyleneaminomethyldimethylphosphineoxide and benzylideneacetophenone to give 3-benzoyl-2-(4-chlorophenyl)-5-dimethylphosphinyl-4-phenyl-2,3,4,5-tetrahydropyrrole. The isotropic samples were prepared of: compound **1** – 17 mg sample dissolved in $CDCl_3$ to give 83.8 mM solution, compound **2** – 9.6 mg – 43.8 mM, compound **3** – 7.1 mg – 32 mM solution. The anisotropic samples contain 91.3 to 94.5 mg of commercial PBLG with degree of polymerisation of 782 and average molecular weight of 171300 obtained from Sigma. The compounds powder was inserted in the NMR tubes together with the polymer and then 0.3 to 0.5 ml $CDCl_3$ was added. The approximate concentrations in the aligned samples are as follows: 300 mM (compound **1**), 250 mM (compound‘**2**) and 230 mM (compound **3**). Corresponding quadrupolar splittings were measured as 495 Hz (sample **1**), 415 Hz (**2**) and 382 Hz (**3**). All samples were measured on 600 MHz spectrometers with

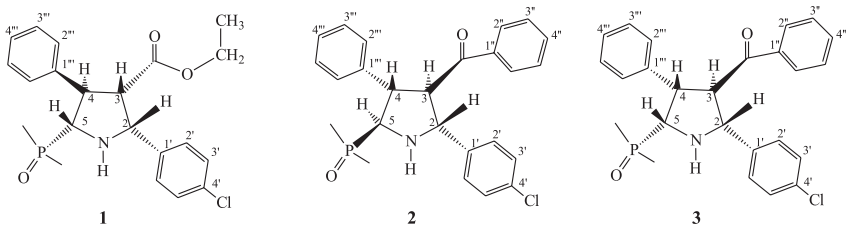

Figure 2. Three tetrasubstituted compounds 1, 2, and 3, for which the measurement of enantiomeric excess via gated decoupled ^{13}C NMR spectra in the chiral alignment medium PBLG has been attempted. Only one enantiomeric form is given for the racemic mixtures.

proton frequency of 600.13 MHz (Bruker DMX 600 for compound **1** in PBLG and Bruker AVII+ 600 for the others), and corresponding carbon and deuterium frequencies of 150.91 MHz and 92.12 MHz. Temperature was in all cases controlled with a BVT to 293 K. All samples with exception of compound **1** in PBLG were measured on a z-gradient PABBO BB-^{1}H probehead. The ^{13}C spectra were recorded with spectral width of 238.9 ppm, 32k data points and a digital resolution of 1.1 Hz with a relaxation delay of 1.5 s. The carbon spectrum in PBLG of sample **1** was recorded on a z-gradient TXI ^{1}H/^{13}C/^{15}N probehead. The spectral width is 16 ppm, with 8k data points and a digital resolution of 0.3 Hz with relaxation delay of 2 s. All spectra were zero filled to twice the amount of data points and apodized before Fourier-transform with an exponential function with a resulting increase in linewidth of 0.5 Hz. After baseline correction, integration of enantiomeric signals was achieved with the program Topspin 2.1.

4 Results

The three compounds **1**, **2**, and **3** were all synthesized as racemic mixtures which result in a single set of signals in conventional isotropic spectra with an achiral environment. The corresponding aromatic regions of gated decoupled ^{13}C 1D NMR spectra are shown in Figures 3-5A with the corresponding assignment. By adding the compounds to the chiral PBLG/$CDCl_3$ liquid crystalline phase as described above, the environment of the sample becomes chiral and different alignment properties of the enantiomers result in differences in RCSAs. Clearly most signals shift in the spectra shown in Figures 3-5B in the anisotropic phase, being a hint for partial alignment with the corresponding chemical shift change due to the anisotropic contribution. In addition, as can be nicely seen in Figure 3B, for example, two sets of carbon signals are visible, representing the R and S configuration of molecule **1**. Integration of the separated peaks for C3' results in a relative ratio of the two enantiomers of 1.00:1.02 and an enantiomeric excess of less than 1%. In contrast, the equivalent spectrum of compound **2**, shown in Figure 4B, exhibits still a single set of resonances with significantly broadened lines. Apparently, most signals are again shifted, but the alignment

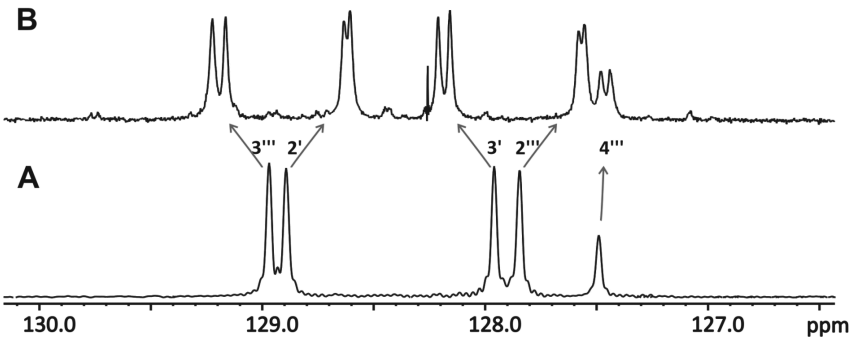

Figure 3. Gated decoupled ^{13}C 1D NMR spectra of the racemic mixture of compound 1 in isotropic $CDCl_3$ solution (A) and in PBLG/$CDCl_3$ (B). Assignment of the carbons according to the nomenclature defined in Figure 2 and corresponding chemical shift changes upon alignment are annotated. The intensities of signals separated in the anisotropic spectrum measured by integration of signals at 128.25 and 128.19 ppm is identical within the error of the experiment.

properties for the two enantiomers do not differ sufficiently for enantiomeric discrimination. This is an interesting result when comparing the spectra with the ones for compound **3**, which only differs with compound **2** by the inverted stereogenic center at carbon 5. In this case, again a clean separation of enantiomers is possible as with compound **1** and the enantiomeric excess can be determined to be less than 2%.

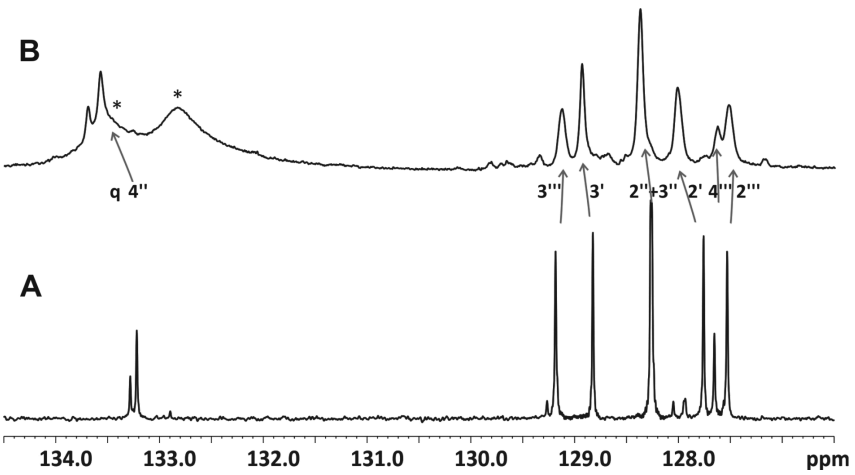

Figure 4. Gated decoupled ^{13}C 1D NMR spectra of the racemic mixture of compound 2 in isotropic $CDCl_3$ solution (A) and in PBLG/$CDCl_3$ (B). Assignment of the carbons according to the nomenclature defined in Figure 2 and corresponding chemical shift changes upon alignment are annotated. The difference in alignment tensors $\mathbf{A}^R$ and $\mathbf{A}^S$ apparently is not sufficient in this case to separate the two enantiomers.

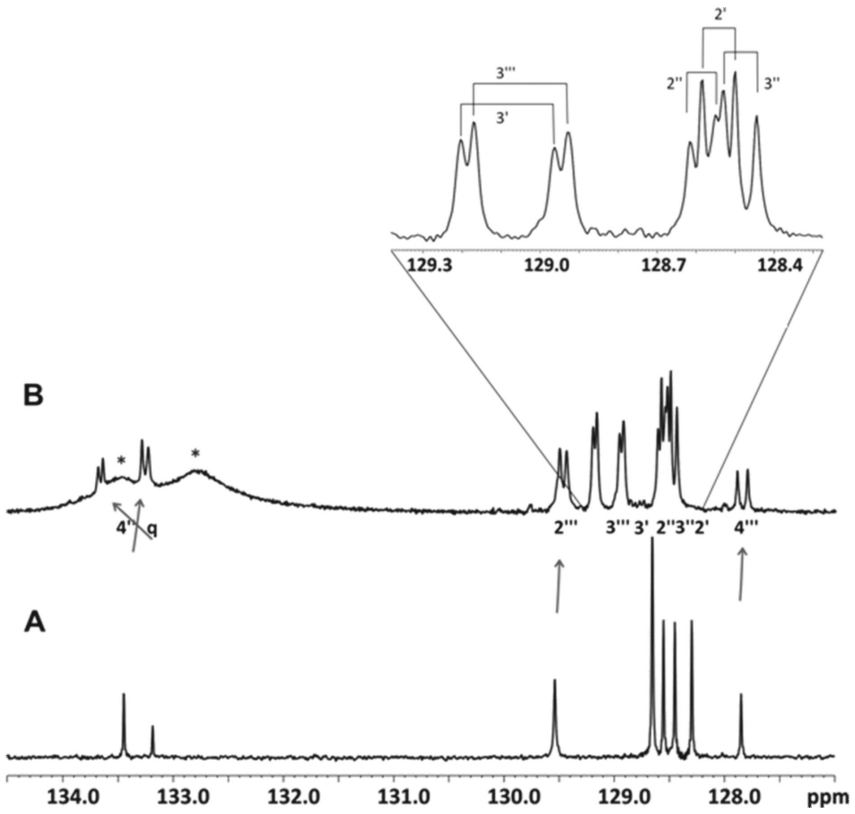

Figure 5. Gated decoupled ^{13}C 1D NMR spectra of the racemic mixture of compound 3 in isotropic $CDCl_3$ solution (A) and in PBLG/$CDCl_3$ (B). Assignment of the carbons according to the nomenclature defined in Figure 2 and corresponding chemical shift changes upon alignment are annotated. The ratio of the intensities of signals separated in the anisotropic spectrum measured by integration of signals at 127.90 and 127.80 ppm is again identical within the experimental error.

5 Discussion

The examples of the three tetrasubstituted pyrrolidines shown in Figure 2 demonstrate yet again that the distinction of enantiomers and the measurement of enantiomeric excess in chiral alignment media using ^{13}C 1D NMR spectroscopy is possible. The three compounds should be among the largest compounds tested so far. The results shown here are therefore evidence that the method is even applicable in cases where the molecule does not fully fit into the groove of the chiral polymer used. This is relatively surprising as the chiral nature of the interaction between the polymer and the solute molecules leading to the differential alignment tensor for the enantiomers must be considered to take place in this groove [5,6].

However, the three molecules behave differently: While compounds **1** and **3**

result in easily distinguishable and separated sets of signals, compound **2** only provides considerable linebroadening of a seemingly single set of resonances. In all cases we see chemical shift changes upon alignment in the range of +0.6 to -0.2 ppm. These chemical shift changes can be caused by the alignment medium itself [25,26] and by residual chemical shift anisotropy [5,6]. The measurement of a large quadrupolar coupling of the deuterated solvent is evidence that all samples have been aligned and because of the clear distinction of enantiomers for compounds **1** and **3** we conclude that the RCSA contribution is significant and most likely dominating the chemical shift changes.

Why does compound **2** not result in two distinguishable sets of resonances? The lines in the ^{13}C 1D spectrum of the aligned sample shown in Figure 4 are broad, which can have two reasons: Either the differential effect on the alignment tensors $\mathbf{A}^S$ and $\mathbf{A}^R$ are not sufficient for separation or the T_2 relaxation times are significantly shortened by a relatively tight interaction with the polymer. As we do not observe extensive linebroadening on the aliphatic carbons, we can exclude the T_2 relaxation as the major effect. Three acceptable explanations for the lack of discrimination for compound **2** are feasible – either a specific interaction with the PBLG as in the other compounds do not occur, compound **2** is more flexible than **1** and **3**, or the configuration of C5 in **2** does prevent the compound from fitting into the groove of PBLG.

Since compound **2** is a diastereomer of compound **3**, we suppose that the interaction of the tetrasubstituted pyrrolidines with PBLG takes place close to carbon 5, mediated by the phosphinyl and the NH groups. We assume therefore that the distinction of enantiomers in PBLG is only possible as long as this interacting side can access the groove of the α-helix of PBLG. Without this specific interaction the PBLG polymer could be fully approximated by a cylindrical polymer as done in the prediction program PALES, which, however, would not allow the distinction of enantiomers.

6 Conclusion

We demonstrate that partial alignment of samples in chiral alignment media can be a powerful tool to distinguish enantiomers and to measure enantiomeric excess. This is even the case for relatively large synthetic compounds, although the capabilities of the method have to be evaluated on an individual basis.

Acknowledgements

B.L. thanks the Deutsche Forschungsgemeinschaft (Heisenberg-Fellowship LU835/3,4,7 and Forschergruppe FOR934), the Biointerface program from the Helmholtz foundation, and the Fonds der Chemischen Industrie for financial support. The financial support of the National Science Fund for the purchase of Bruker AVII+ 600 NMR spectrometer in the framework of the Program “Promotion of the Research Potential through Unique Scientific Equipment” – Project UNA-17/2005 is gratefully acknowledged. We thank T. Cholakova, Y. Zagraniarsky, S. Varbanov and A. Dobrev for the supply of the sample materials.

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