New, and Accurate Method To Determine the Enantiomeric Purity of Amino Acids Based on Deuterium NMR in a Cholesteric Lyotropic Liquid Crystal

I. Canet, A. Meddour, and J. Courtieu*

Laboratoire de Chimie Structurale Organique
URA 1384, ICMO, Bât. 410
Université Paris-Sud, 91405 Orsay Cedex, France

J.L. Canet and J. Salaün*

Laboratoire des Carbocycles
URA 478, ICMO, Bât. 420
Université Paris-Sud, 91405 Orsay Cedex, France

Received October 13, 1993

The constant interest in enantioselective synthesis of amino acids has led to an increased demand for accurate, reliable, convenient methods of measuring enantiomeric purity. In the past this problem was addressed by using chiroptical methods, but this technique possesses severe limitations that have been described in the literature. Among them, nonideal behavior of the optical rotation with concentration and the effect of chiral impurities are well-known.[1] Although rapid progress has been made in the past five years in developing sensitive and accurate HPLC methods of analysis,[2] many practitioners of organic chemistry use NMR methods.[1] For amino acids, most of them require reaction with a chiral derivatizing agent—for instance, by means of the Mosher acid chloride[3] —in order to study the NMR spectra of the resulting diastereoisomers that are expected to be separated. Unhappily the spectral resolution is not always great enough to allow for an accurate measurement of the enantiomeric purity. An original method, using lyotropic amphiphilic liquid crystals, has been investigated by Radley *et al.* to study alanine.[4] We propose herein a new method to determine the enantiomeric excess of amino acids based on proton decoupled deuterium NMR in a cholesteric lyotropic liquid crystal solvent. The technique appears broadly applicable as, to date, we cannot report about any unsuccessful example.

Some synthetic polypeptides are known to form lyotropic cholesteric liquid crystals when dissolved in organic solvents.[5] Among them, we have recently reported that dichloromethane solutions of poly(γ-benzyl L-glutamate) (PBLG, 13.5% w/w[6]) can be used as a chiral deuterium NMR solvent to distinguish enantiomers.[7] The discrimination originates from the fact that, in such an anisotropic chiral medium, the averaged molecular ordering parameters are different for each enantiomer. We named this phenomenon the differential ordering effect of enantiomers. Among the NMR anisotropic interactions depending on the molecular ordering, the quadrupolar splitting, $\Delta\nu_Q$, is the most sensitive. Thus, deuterated enantiomers exhibit different $\Delta\nu_Q$ on their ^2H spectra, which allows for the measurement of their enantiomeric excesses (Figure 1).

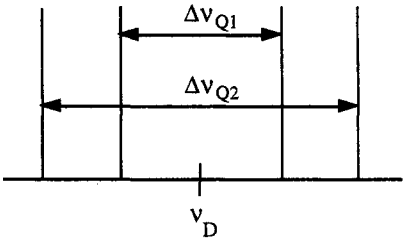


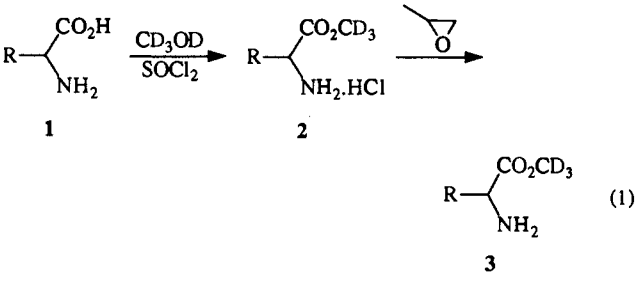
Figure 1. Theoretical ^2H NMR spectrum of a deuterated racemic molecule dissolved in the PBLG–CH_2Cl_2 lyotropic liquid crystal solvent. $\Delta\nu_{Q1}$ and $\Delta\nu_{Q2}$ are the quadrupolar splittings for each enantiomer.

Table 1. Observed Quadrupolar Splittings $\Delta\nu_Q$ and Determination of Enantiomeric Excesses of Amino Esters **4**[a]

amino ester[b]	R	$\Delta\nu_{Q1}$ (Hz)	$\Delta\nu_{Q2}$ (Hz)	enantiomeric excess obsd[c]	enantiomeric excess calcd[d]	absolute config
4a (Ala)	CH_3	18.4	5.7	39.5	40.0	*R*
4b (Val)	$(CH_3)_2CH$	37.1	25.7	39.1	40.0	*R*
4c (Leu)[e]	$(CH_3)_2CHCH_2$	54.3	41.3			
4d (Ile)	$CH_3CH_2(CH_3)CH$	62.1	48.7	60.8	60.0	*R*
4e (Phe)[e]	$C_6H_5CH_2$	38.0	10.9			
4f (Ser)[e,f]	$HOCH_2$	78.0	49.4			
4g (Met)	$CH_3SCH_2CH_2$	43.9	33.5	40.0	40.0	*S*

[a] NMR spectra were recorded on a Bruker AM 250 spectrometer (38.398 MHz) at 306 K using samples of PBLG–CH_2Cl_2 containing 50 mg of amino esters **4**. [b] Common abbreviations are given for the parent amino acids. [c] Optical purities (±1%) were calculated from the relative peak integrations. [d] Enriched mixtures (ee ± 1%) have been realized by weighing commercial *R* and *S* amino acids. [e] Spectra were recorded using racemic mixture. [f] Spectrum was recorded at 273 K.

To apply this new analytical tool to the determination of the enantiomeric purity of amino acids **1**, we first esterified the acid function of **1** in order to make the compound soluble in dichloromethane. Using perdeuterated methanol, in the presence of thionyl chloride (1.2 equiv),[8] allowed us to incorporate deuterium in the molecule in that same step. To regenerate the amino function from hydrochloride **2**, the esters were heated in propylene oxide to obtain quantitatively the amino esters **3**[9] (eq 1).

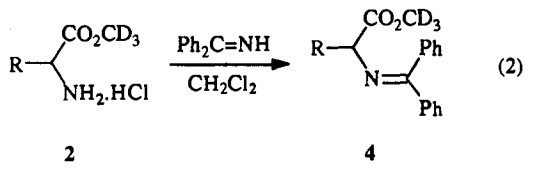


(1)

Meanwhile, amino esters such as **3** can undergo dimerization into solid diketopiperazines[10] within a few days or much more rapidly, with formation of deuterated methanol as a byproduct, which can disturb the measurement of the enantiomeric excess. To overcome this problem, we have protected the amino moiety of **2** by direct action of the benzophenone imine (1 equiv), which gave the (diphenylmethylene)amino ester **4** quantitatively[11] (eq 2).

(1) For a review, see: Parker, D. *Chem. Rev.* **1991**, *91*, 1441.
(2) Allenmark, S. G. *Chromatographic Enantioseparation: Methods and Applications*; Ellis Horwood: Chichester, 1988.
(3) Mosher, H. S.; Dale, J. A.; Dull, D. L. *J. Org. Chem.* **1969**, *34*, 2543.
(4) Radley, K.: Cattey, H. *Mol. Cryst. Liq. Cryst.* **1993**, *226*, 195. Radley, K.; Tracey, A. S. *Can. J. Chem.* **1985**, *63*, 95.
(5) Samulski, T. V.; Samulski, E. T. *J. Chem. Phys.* **1973**, *67*, 824. Toriumi, H.; Yahagi, K.; Uematsu, I.; Uematsu, Y. *Mol. Cryst. Liq. Cryst.* **1983**, *94*, 267. Abe, A.; Yamasaki, T. *Macromolecules* **1989**, *22*, 2138.
(6) PBLG is commercially available from Sigma (degree of polymerization (DP): 1078). The NMR sample was usually centrifuged back and forth several times until a homogeneous solution was obtained, introduced for some minutes in the magnetic field, and then centrifuged again. The operation was repeated until the spectral line width reached 1–2 Hz.
(7) Bayle, J. P.; Courtieu, J.; Gabetty, E.; Loewenstein, A.; Péchiné, J. M. *New J. Chem.* **1992**, *16*, 837. Canet, J. L.; Fadel, A.; Salaün, J.; Canet-Fresse, I.; Courtieu, J. *Tetrahedron: Asymmetry* **1993**, *4*, 31.
(8) Brenner, M.; Huber, W. *Helv. Chim. Acta* **1953**, *36*, 1109.
(9) Williams, R. M.; Fegley, G. J. *J. Am. Chem. Soc.* **1991**, *113*, 8796.
(10) Greenstein, J. P.; Winitz, M. *Chemistry of the Amino Acids*; John Wiley & Sons, Inc.: New York, NY, 1961; Vol. 2, p 716.
(11) O'Donnell, M. J.; Polt, R. L. *J. Org. Chem.* **1982**, *47*, 2663.

0002-7863/94/1516-2155$04.50/0



(2)

2 4

The results obtained for different amino esters **4** are listed in Table 1. For all the amino esters studied, it was possible to differentiate the enantiomers on the NMR spectra with very good resolution. Some examples of spectra are shown in Figure 2. For the compounds **4a**, **4b**, **4d**, and **4g**, the measured enantiomeric excesses were in agreement with the calculated ones. In the case of the amino ester **4f**, the spectrum has been recorded at a lower temperature to increase the resolution. Indeed, a temperature variation can increase the difference between the quadrupolar splittings of each enantiomer.[7] Finally, to prove the sensitivity of our method, we have studied the compound (*R*)-**4a**, with a calculated enantiomeric excess of 98%; as shown in Figure 2, it was possible to detect the presence of traces of the *S* amino ester.

In conclusion, from deuterated methylene amino esters readily and quantitatively synthesized from the corresponding amino acids, using deuterium NMR in cholesteric lyotropic liquid crystal solvent, it is possible to differentiate remarkably the enantiomers of these compounds, and to measure with high precision their enantiomeric purity. The simplicity of the ^{2}H NMR spectra and the high sensitivity of the ee measurement make this new method general and much more discriminating than previously reported NMR methods.

Furthermore, for the compounds **4a**, **4b**, **4d**, and **4g**, we made an attempt to correlate the values of the quadrupolar splittings to the absolute configuration of the enantiomers. The molecular orientation of a small molecule dissolved in liquid crystals essentially depends on geometrical factors. This remark can be illustrated by the evolution of the ratio $\Delta\nu_{Q1}/\Delta\nu_{Q2}$ with the volume of the alkyl substituent, R. Consequently, we defined the absolute configurations following the Cahn–Ingold–Prelog rules using a hierarchy of the substituents based on the volume defined by the van der Waals radii[12] instead of the atomic numbers. We used then the designations R_v and S_v following the definition given by Krabbe *et al.*[13] On the spectra of the four enriched amino esters **4**, the outside doublet always corresponds to the S_v enantiomer and the inside doublet to the R_v enantiomer. At this stage of our work it is too ambitious to claim that this is a general rule, so complementary studies are underway in order to know if this quadrupolar splitting–configuration correlation ($\Delta\nu_Q(R_v) < \Delta\nu_Q(S_v)$) can be applied to other methylene amino esters, which then could allow a direct assignment of their absolute configuration based on ^{2}H NMR spectra.

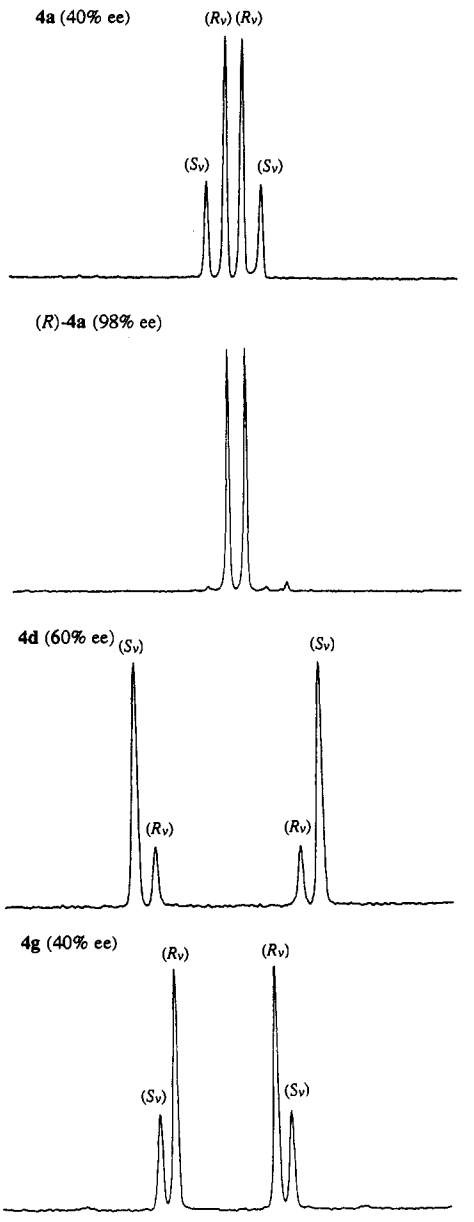


Figure 2. Spectra of amino esters **4** in the PBLG–CH_2Cl_2 solvent.

(12) This comparison has been made using the relative van der Waals volumes (see: Bondi, A. *J. Phys. Chem.* **1964**, *68*, 441) and also using molecular modeling calculation (MAD version 2.0, available from Oxford Molecular, Ecole Polytechnique, Palaiseau, France).

(13) Krabbe, H. J.; Heggemeier, H.; Schrader, B.; Korte, E. H. *J. Chem. Res. (Synop.)* **1978**, 238.