The 31P-NMR Spectroscopic Determination of the Enantiomeric Excess of Unprotected Amino Acids

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The ^{31}P-NMR Spectroscopic Determination of the Enantiomeric Excess of Unprotected Amino Acids**

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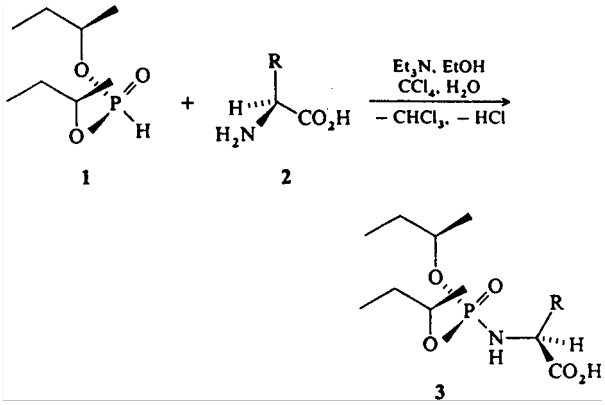
The increasing use of natural and synthetic amino acids and their derivatives for the modification of proteins and as chiral building blocks or chiral ligands in numerous asymmetric syntheses[1] demands new methodologies for the fast and accurate determination of the enantiomeric excess (*ee*) of these compounds. The enantiomeric purity of amino acids is routinely analyzed by gas or liquid chromatography through the use of chiral stationary phases and various (in situ) derivatizing techniques.[2]. A number of methods exist for the determination of the enantiomeric excess of amino acid derivatives by NMR spectroscopy, employing chiral[3] or achiral[4] derivatizing agents. Similar methods for *free amino acids* are scarce, mainly due to the low solubility in organic solvents[5] or to the lack of proper chiral derivatizing agents. We now report a simple and efficient ^{31}P-NMR method for the determination of the enantiomeric excess of unprotected amino acids which is based on the use of phosphonate **1** as new chiral derivatizing agent.

The reagent **1** is readily prepared from (*S*)-2-butanol and PCl_3.[6] All amino acids examined thus far react with **1** quantitatively in a few hours at room temperature providing phosphonic amides **3**. The formation of diastereomeric phosphonic amides **3** is most conveniently carried out using aqueous ethanolic solutions of amino acids **2** and reagent **1** dissolved in CCl_4 employing triethylamine as base.[7]



It should be emphasized that aqueous solutions of the amino acids can be analyzed following this methodology and, in particular, by using **1** that no purification of the adducts **3** is necessary, thus allowing rapid *ee* determination. Typical results of the ^{31}P-NMR analyses are summarized in Table 1.

Table 1. ^{31}P-NMR data of the phosphonic amides **3** prepared from amino acids and from chiral amines and alcohols and **1**, recorded in C_6D_6 [a].

Entry	Substrate	δ[b]	$\Delta\delta$[c]	Peak ratio
1	DL-Ala	6.51	0.099	49.5:50.5
		6.47	0.116	49.5:50.5
2	DL-Phe	5.93	0.025	50:50
		5.91	0.106	50:50
3	DL-Val	6.93	0.069	49.5:50.5
		6.92	0.127	49.5:50.5
4	DL-Try	6.03	0.038	49:51
		6.00	0.091	49:51
5	DL-Ser	6.11	0.079	49.5:50.5
		6.03	0.117	49.5:50.5
6	DL-phenylglycine	5.71	0.098	49.5:50.5
		5.69	0.172	49.5:50.5
7	DL-Tyr	5.65	0.093	49.5:50.5
	DL-Tyr [b]	5.59	0.191	49.5:50.5
8	DL-Cys	5.98	0.087	50:50
		5.98	0.156	50:50
9	DL-Pro	4.63	0.127	49:51
10	DL-α-Me-PG [d]	2.20	0.487	49.5:50.5
11	DL-α-Me-Phe	4.32	0.051	49.5:50.5
12	DL-α-Me-Phe-amide	5.85	0.035	49.5:50.5
13	DL-α-allyl-PG-amide [d]	5.50	0.185	49.5:50.5
14	DL-α-allyl-PG methyl ester [d]	5.48	0.037	49.5:50.5
15	DL-α-phenylethylamine	5.94	0.185	50:50
16	DL-α-phenylethyl alcohol	5.23	0.103	49.5:50.5
17	DL-menthol	5.63	0.127	50:50

[a] In each of the entries 1–8 the first row applies to the diastereomeric separations of **3** with half a molar equivalent of bound water, the second to the diastereomeric separations of **3** after removal of the bound water. [b] Mean of the δ-values of the two diastereomers. [c] Absolute value of the difference in the δ-values of the diastereomers. [d] PG = phenylglycine.

The ^{1}H-decoupled ^{31}P-NMR spectra of **3**, derived from racemic amino acids, show, in all the cases, two well separated singlets corresponding to the two diastereomers. On the

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other hand, a singlet is observed in each case for the enantiomerically pure compounds **3**. Figure 1 shows the ^{31}P-NMR spectra of an amino acid at different *ee* values.

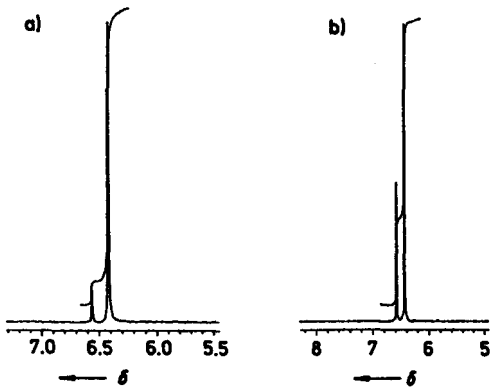

Fig. 1. Noise-decoupled ^{31}P-NMR spectrum of **3** (R = Me) in C_6D_6. a) 82% *ee*. b) 40% *ee* for the (*S*)-enantiomer of **2**.

Though diastereomeric peak separations have also been observed in the ^{1}H-NMR spectra the phosphorus–proton long range coupling gives rise to rather complex spectra.

Phosphonic amides **3** form stable complexes with water, alcohols, and ethyl acetate. After normal workup, 0.5 equivalents of H_2O are complexed which can be removed by heating at 60 °C under high vacuum. Removal of the complexed water results in nearly doubling of the chemical shift differences of the diastereomers of **3** (Table 1).

Some typical results of the *ee* determinations performed on partially enriched compounds are listed in Table 2 together with the *ee*'s determined by the known α-chloropropionyl chloride method[5] and by rotation measurements. These results clearly show that the enantiomeric purities obtained via the different methods are in excellent agreement and that no racemization or kinetic resolution occurs during phosphonic amide formation.

Table 2. Comparison of the *ee* values, those for samples with known enantiomeric composition determined ^{31}P-NMR spectroscopically by rotation [8], and by the α-chloropropionyl chloride method [5] [a].

Entry [b]	Ratio of enantiomers		
	by polarimetry	^{1}H-NMR	^{31}P-NMR
1	76.40:23.60	76.35:23.65	76.40:23.60
2	73.45:26.55	74.10:25.90	74.10:25.90
15	70.80:29.20	70.10:29.90	70.50:29.50

[a] Estimated errors: ^{31}P-NMR 2%, polarimetry 3%, ^{1}H-NMR 2%. [b] Numbering of entries as in Table 1.

1 is not only suitable as derivatizing reagent for the determination of the enantiomeric excess of unprotected amino acids but can also be used in the case of α-alkylated amino acids (Table 1, entries 10–14), amino acid esters and amides, chiral amines, and alcohols. Typical examples are given in Table 1.

The new method has some unique features and several advantages over existing methods. To our knowledge this is the first ^{31}P-NMR method to be reported for the determination of the *ee* of free amino acids using facile in situ derivatization and broad solvent tolerance including aqueous solutions; the chiral derivatizing agent **1** is readily prepared, and excellent results are obtained with α-alkylated amino acids and their derivatives. Furthermore, the method compares favorably with currently available NMR techniques for amino acids in view of the large shift differences and the possibility of using phosphorus nuclei besides other nuclei.

Experimental

(*S*,*S*)-*O*,*O*-Di-*s*-butylphosphonate **1** was synthesized according to known literature procedures [6].

Synthesis of phosphonic amide **3**: A suspension of amino acid (1.0 mmol) in Et_3N (0.4 mL), H_2O (0.2 mL) and ethanol (0.5 mL) was cooled to 0 °C and treated dropwise with a solution of the phosphonate **1** (1.15 mmol) in CCl_4 (0.5 mL) and the mixture stirred at 20 °C for 2 h. The reaction was quenched by acidifying the mixture to pH 2 with 10% HCl solution. After extraction of the mixture with EtOAc (3 × 5 mL) the combined EtOAc phases were washed with water (5 mL) and dried (Na_2SO_4). The solvent was then removed by evaporation and the oily residue used as such for the *ee* determination. The phosphonic amides were purified by crystallization from EtOAc/petroleum ether or column chromatography on silica gel, providing white solids or oils.

With other substrates such as amino acid esters, the use of water as cosolvent may be omitted. When alcohols are used, addition of a catalytic amount of *N*,*N*-dimethylaminopyridine is recommended or alternatively the potassium alkoxides can be used.

The ^{31}P-{^{1}H}-NMR spectra were recorded on a Varian VXR 300 spectrometer operating at 121.42 MHz; the chemical shifts are given relative to those of $(NPCl_2)_3$ (δ = 19.91).

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[8] Materials used for entries 1, 2, and 15 were obtained from Janssen Chimica.

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