Research Article

Magnetic Resonance in Chemistry

Received: 2 September 2008 Revised: 11 October 2008 Accepted: 21 October 2008 Published online in Wiley Interscience: 15 December 2008

(www.interscience.com) DOI 10.1002/mrc.2374

A new method proposed for the determination of absolute configurations of α-amino acids†

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Enantiopure α-amino acids were converted to 4-substituted 2-aryl- and 2-alkyl-5(4*H*)-oxazolones under partial racemization. These nonracemic mixtures were dissolved in $CDCl_3$, an equimolar amount of the chiral dirhodium complex $Rh_2^{(II)}$[(*R*)−(+)− MTPA]$_4$ (MTPA-H = Mosher's acid) was added, and the ^{1}H NMR spectra of the resulting samples were recorded (*dirhodium method*). The relative intensities of ^{1}H signals dispersed by the formation of diastereomeric adducts allow to determine the absolute configuration (AC) of the starting α-amino acids. Binding atoms in the adducts were identified by comparing the ^{1}H and ^{13}C chemical shifts of the oxazolones in the absence and presence of $Rh_2^{(II)}$[(*R*)−(+)− MTPA]$_4$. Thereby, information about the scope and limits of this method can be extracted. A protocol how to use this method is presented.

Supporting information may be found in the online version of this article.

Keywords: ^{1}H and ^{13}C NMR; α-amino acids; 5(4*H*)-oxazolones; absolute configuration; chiral dirhodium complex

Introduction

Amino acids, peptides and proteins are among the most prominent compounds in nature, and the determination of their absolute configuration (AC) is of great importance. To achieve this, a variety of methods has been developed in the past, mostly using chromatographic or spectroscopic techniques but chemical correlation is still practiced where an amino acid of unknown AC is chemically converted into another compound of known AC.[1–5]

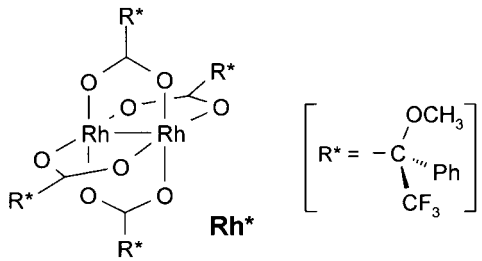
NMR spectroscopy is a method which is simple to use and ubiquitous in the chemical laboratories all over the world. Therefore, many NMR techniques for enantiodifferentiation and AC determination have been developed on the basis of the presence of chiral auxiliaries.[6] This is particularly true for alcohols and amines but for amino acids the choice of NMR methods is smaller and less elaborated. Among such auxiliaries are chiral shift reagents (CLSR)[6–8] and chiral derivatizing agents (CDA), such as Mosher's acid,[9] α-cyano-α-fluoro-*p*-tolylacetic acid (CFTA),[10] and some others.[6,11] Metal complexes of palladium[12] and platinum[13] have also been used for AC determination. The application of Courtieu's method, i.e. ^{2}H NMR of acetyl-d_3 derivatives in chiral solvents, should be mentioned as well.[14]

Experimental

Spectroscopy

Room-temperature ^{1}H (400.1 MHz) and ^{13}C (100.6 MHz) NMR measurements were performed on a Bruker Avance DPX-400 spectrometer. Samples were *ca* 0.01−0.025 mmolar in $CDCl_3$. Chemical shift standard was internal tetramethylsilane ($\delta = 0$).

Following parameters have been used for all one-dimensional NMR spectra: ^{1}H: acquisition time 4.0 s, relaxation delay 0.5 s, pulse duration 2.6 μs for a 30° flip angle, and spectral width 8224 Hz (20.6 ppm); 64 K points were used for data acquisition; 64 K points for Fourier transform (FT) transformation; digital resolution was 0.12 Hz/point. ^{13}C: acquisition time 2.6 s for a 30° flip angle, relaxation delay 0.5 s, pulse duration 2.3 μs, and spectral width 25.629 Hz (250 ppm); 128 K points were used for data acquisition; 128 K points for FT transformation; digital resolution was 0.19 Hz/point.



Scheme 1. Structure of the chiral dirhodium complex **Rh*** ($Rh_2^{(II)}$[(R)−(+)− MTPA]$_4$; Scheme 1) used as NMR auxiliary for chiral recognition.

Signal assignments were assisted by DEPT, COSY, HMQC and HMBC spectra (standard Bruker software and parameters).

In the *standard dirhodium experiment*, **Rh*** {$Rh_2^{(II)}$[(*R*)−(+)− MTPA]$_4$; MTPA-H = methoxytrifluoromethylphenylacetic acid (Mosher's acid); shown in Scheme 1} and an equimolar amount of the ligands **1**–**11**, respectively (Scheme 3), were dissolved in 0.7 ml $CDCl_3$.[15,16] Quantities of *ca* 25 mg of **Rh*** corresponding to *ca* 0.025 mM concentration were employed. It may be noted that

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† *This work is dedicated to the memory of the late Prof. Dr. H.C. (H) Günther Snatzke (1928–1992) on the occasion of his 80th birthday.*

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$\Delta\nu$-values are B_0-dependent; in this work all dispersion values are given in Hz, as determined at $B_0 = 9.4$ Tesla corresponding to 400 MHz ^{1}H and 100.6 MHz ^{13}C.

Infrared (IR) spectra were recorded on a Bruker Vector 22 spectrometer. Electrospray-ionization mass spectrometry (ESI-MS) spectra were measured on a Micromass LCT instrument with lock-spray unit and EI-MS spectra on a Micromass LCT. Optical rotations $[\alpha]_D$ were measured on a Perkin-Elmer 341 at room temperature, 589 nm (Na_D-line).

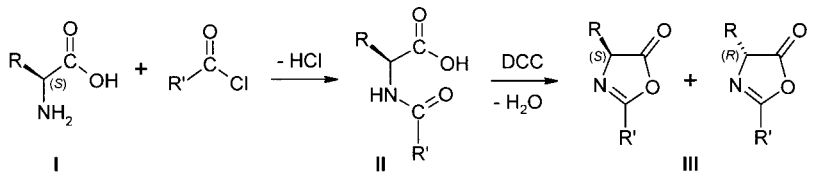
Thin layer chromatography (TLC) was performed to check reaction progress using aluminum foils coated with silica gel 60 F_{254} (Merck).

Acylation of α-amino acids

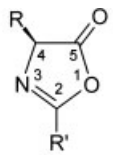
The respective acid chloride was added dropwise to a solution of the respective α-amino acid dissolved in 2N sodium hydroxide and kept at 0 °C. Then, the mixture was stirred for 20 min at room temperature. It was important to check during the addition that the pH of the solution stayed above 7. The solution was then cooled to 0 °C and concentrated hydrochloric acid was added slowly till neutral (pH = 7). The products precipitated were collected by filtration and washed with cool water. After drying, the acylated amino acids were obtained as white solids in 90–98% yields. Most of the acylated α-amino acids have been reported in the literature but spectroscopic data seem to be rare. So, complete data sets were collected in the Supporting Information material.

General procedure to convert acylated amino acids into 5(4H)-oxazolones (1–11)

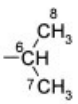
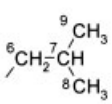
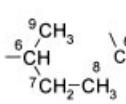
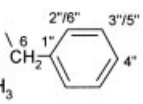
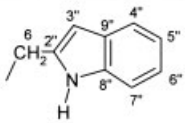
A solution of the *N*-acylated (*S*)-α-amino acid (0.6 mmol) in dichloromethane (20 ml) was added dropwise to a solution of 1,3-dicyclohexylcarbodiimide (DCC, 125 mg, 0.6 mmol) in dichloromethane (15 ml) at 0 °C. After stirring for 15–20 min, the suspension was filtered at 0 °C. *N*,*N*′-dicyclohexylurea (DCU), developed from DCC, was thereby removed and the organic layer was evaporated to dryness. The resulting optically active raw product was dissolved in 25 ml acetone, cooled to −78 °C under stirring and then filtered to remove residual DCU. Finally, the solvent was evaporated under reduced pressure giving the 5(4*H*)-oxazolones **1**–**11** as oil or crystals in high yields. They are stable for a few hours at room temperature and for few days at −20 °C. It may be noted that an extension of the reaction time from 15–20 to 60 min and more may result in a complete racemization after work-up.[17] Immediately after their synthesis,

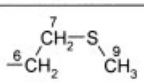
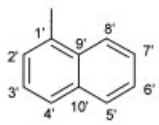


Scheme 2. Synthesis of 5(4*H*)-oxazolones from amino acids.



(*S*)-**1** to (S)-**11**

	1 (Ala)	**2** (Val)	**3** (Leu)	**4** (Ile)	**5** (Phe)	**6** (Trp)
R						
R'	C_6H_5	C_6H_5	C_6H_5	C_6H_5	C_6H_5	C_6H_5

	7 (Met)	**8** (Ala)	**9** (Ala)	**10** (Ala)	**11** (Ala)
R					
R'	C_6H_5	CH_3	CH_2CH_3	$C(CH_3)_3$	

Scheme 3. Structures of the 5(4*H*)-oxazolones **1**–**11**, nonracemic mixture with the *S*-configurated enantiomer (see formula) as major constituent. ((Atoms of the phenyl group, R′ attached to C-2 in **1**–**7**, are numbered 1′, 2′/6′, 3′/5′ and 4′; those of the aliphatic CH-fragments at C-2 in **8**–**10** are 1′ and 2′; for **11** see structure scheme).

the 5(4*H*)-oxazolones **1**–**11** (Scheme 3) were subjected to the standard dirhodium experiment (given above). Melting points of the 5(4*H*)-oxazolones were not determined because the samples were nonracemic mixtures of varying compositions and decayed at elevated temperatures. Physical and spectroscopic data other than NMR are given in the Supporting Information material for all oxazolones **1**– **11**.

Results and Discussion

In two earlier reports, we have shown that the chiral NMR auxiliary **Rh*** {$Rh_2^{(II)}$[(*R*)− (+)− MTPA]$_4$; MTPA-H = methoxytrifluoromethylphenylacetic acid (Mosher's acid); shown in Scheme 1}, originally developed for the enantiodifferentiation of a great variety of functionalities (*dirhodium method*),[15,16] can be used for determination of AC if a series of structurally related compounds with limited conformational mobility are to be investigated.[18,19] Once the AC of one member of the series is known, those of the others can be derived by inspecting the signs of dispersion effects $\Delta\nu$; if they are equal, the other members of the series have the same AC and vice versa (correlation method).

5(4*H*)-Oxazolones (alias: Δ^2-oxazolin-5-ones, azlactones; Scheme 2) exist in the literature for many years.[17] Recently, they received increasing attention because of their pharmacological activities[20] and applications in polymer chemistry.[21] Therefore, a reliable determination of the enantiopurity and the AC assignment for those compounds as well as for the amino acids as their precursors are of vital importance. In our present communication, we follow the correlation approach by showing how an easy to perform two-step synthesis converts an enantiopure α-amino acid into a nonracemic mixture of a conformationally rigid derivative, the 5(4*H*)-oxazolones. As shown in Scheme 2, enantiopure α-amino acids (**I**) can be converted easily into their *N*-acyl derivatives (**II**) by reaction with carboxylic acid chlorides (Schotten-Baumann).[22] Finally, compounds **II** can be cyclized by DCC to form the 5(4*H*)-oxazolones (**III**).[17a,23] The target compounds **III** are not entirely stable; they racemize slowly during the cyclization reaction and in $CDCl_3$ solution,[17b] probably via tautomerization[24]; the prevailing enantiomer correlates stereochemically with the original α-amino acid whereas the minor one is produced by the racemization. If, alternatively, the starting amino acid already exists as a nonracemic mixture, this method works analogously as long as one enantiomer dominates clearly; otherwise, the signal intensities of the major and the minor enantiomers in the ^{1}H NMR spectrum may be too similar to achieve a reliable assignment to the respective enantiomers.

After preparing the optically active (*S*)-5(4*H*)-oxazolones **1**–**11** (Scheme 3), partial racemization was observed after a 20-min-

Table 1. ^{1}H and ^{13}C chemical shifts (δ, in ppm) of the 2-phenyl-5(4*H*)-oxazolones **1**–**7** and their complexations shifts ($\Delta\delta$, in ppm) due the addition of an equimolar amount of **Rh***

Atom		**1**[a]		**2**[b]		**3**[c]		**4**[d]		**5**[e]		**6**[f]		**7**[g]	
		δ	$\Delta\delta$	δ	$\Delta\delta$	δ	$\Delta\delta$	δ	$\Delta\delta$	δ	$\Delta\delta$	δ	$\Delta\delta$	δ	$\Delta\delta$
2	^{1}H	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	^{13}C	161.6	+7.0	161.7	+7.0	161.4	+7.0	161.6	+7.1	161.7	+6.6	161.7	+6.5	162.0	−1.7
4	^{1}H	4.46	+1.1	4.29	+0.8	4.42	+0.8	4.37	+0.8	4.69	+0.7	4.71	+0.6	4.61	+0.7
	^{13}C	61.1	+0.7	70.7	0	63.9	+1.6	69.8	+1.0	66.5	+0.4	66.6	+1.2	63.7	+1.1
5	^{1}H	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	^{13}C	179.0	−2.9	177.8	−3.9	179.0	−3.5	177.8	−3.7	177.6	−2.7	178.0	−2.3	178.4	−3.3
6	^{1}H	1.60	+0.3	2.39	+0.7	1.84 1.68	+0.4 +0.6	2.14	+0.7	3.37 3.19	+0.6 0	3.54 3.40	+0.5 0	2.32 2.16	+0.8 +0.6
	^{13}C	16.9	+0.8	31.3	−1.3	40.8	−1.9	37.7	−1.4	37.3	−0.8	27.2	−0.6	30.4	+0.7
7	^{1}H	–	–	1.02	0	2.07	−0.1	1.39 1.57	+0.1 +0.3	–	–	–	–	2.74	+0.5
	^{13}C	–	–	17.5	−2.2	25.2	−0.8	25.0	−1.2	–	–	–	–	30.0	−2.6
8	^{1}H	–	–	1.15	0	1.04	−0.1	0.97	−0.1	–	–	–	–	–	–
	^{13}C	–	–	18.8	−1.2	22.0	+0.8	11.7	+0.4	–	–	–	–	–	–
9	^{1}H	–	–	–	–	1.01	−0.1	1.07	0	–	–	–	–	2.12	+0.2
	^{13}C	–	–	–	–	22.7	+0.2	15.4	−1.8	–	–	–	–	15.1	+2.2

[a] Data of further nuclei, $\delta/\Delta\delta$: 8.0/+0.2 (H-2′/6′), 7.49/−0.2 (H-3′/5′), 7.58/−0.1 (H-4′), 125.9/−1.2 (C-1′), 127.9/+0.7 (C-2′/6′), 128.8/+1.2 (C-3′/5′), 132.8/+0.9 (C-4′).
[b] Data of further nuclei, $\delta/\Delta\delta$: 8.02/+0.2 (H-2′/6′), 7.49/−0.2 (H-3′/5′), 7.58/−0.2 (H-4′), 125.9/−1.2 (C-1′), 127.9/+0.7 (C-2′/6′), 128.8/+1.2 (C-3′/5′), 132.7/+0.9 (C-4′).
[c] Data of further nuclei, $\delta/\Delta\delta$: 8.0/+0.2 (H-2′/6′), 7.48/−0.2 (H-3′/5′), 7.57/−0.1 (H-4′), 126.0/−1.2 (C-1′), 127.9/+2.2 (C-2′/6′), 128.8/−0.2 (C-3′/5′), 132.6/+1.0 (C-4′).
[d] Data of further nuclei, $\delta/\Delta\delta$: 8.01/+0.2 (H-2′/6′), 7.49/−0.3 (H-3′/5′), 7.58/−0.2 (H-4′), 126.0/−1.3 (C-1′), 127.9/+2.1 (C-2′/6′), 128.8/−0.3 (C-3′/5′), 132.6/+0.9 (C-4′).
[e] Data of further nuclei, $\delta/\Delta\delta$: 7.92/+0.1 (H-2′/6′), 7.45/−0.1 (H-3′/5′), 7.55/−0.2 (H-4′), 125.8/−1.5 (C-1′), 127.9/+0.4 (C-2′/6′), 128.7/−0.4 (C-3′/5′), 132.7/+0.8 (C-4′), 7.26/0 (H-2″/6″), 7.22/0 (H-3″/5″), 7.22/0 (H-4″), 135.3/−1.7 (C-1″), 129.6/+0.9 (C-2″/6″), 128.4/+1.4 (C-3″/5″), 127.2/+0.3 (C-4″).
[f] Data of further nuclei, $\delta/\Delta\delta$: 7.88/+0.1 (H-2′/6′), 7.41/−0.3 (H-3′/5′), 7.52/−0.2 (H-4′), 125.9/−1.4 (C-1′), 127.9/+1.8 (C-2′/6′), 128.6/−0.5 (C-3′/5′), 132.6/+0.6 (C-4′), 8.02/−0.1 (H-1″), 7.13/−0.1 (H-3″), 7.2/+0.2 (H-4″), 7.11/−0.1 (H-5″), 7.15/−0.2 (H-6″), 7.29/−0.1 (H−7″), 109.7/−1.6 (C-2″), 123.3/+1.0 (C-3″), 119.2/+0.4 (C-4″), 119.6/+0.3 (C-5″), 122.1/−0.2 (C-6″), 110.9/−0.3 (C-7″), 135.9/−0.2 (C-8″), 127.4/+0.2 (C-9″).
[g] Data of further nuclei, $\delta/\Delta\delta$: 8.0/+0.2 (H-2′/6′), 7.49/−0.3 (H-3′/5′), 7.44/−0.1 (H-4′), 125.8/−1.2 (C-1′), 127.9/+1.8 (C-2′/6′), 128.8/−0.5 (C-3′/5′), 132.8/+0.6 (C-4′).

Table 2. ^{1}H and ^{13}C chemical shifts (δ, in ppm) of the 2-substituted 5(4*H*)-oxazolones **8**–**11** and their complexations shifts ($\Delta\delta$, in ppm) due the addition of an equimolar amount of **Rh***

		8[a]		**9**[b]		**10**[c]		**11**[d]	
Atom		δ	$\Delta\delta$	δ	$\Delta\delta$	δ	$\Delta\delta$	δ	$\Delta\delta$
2	^{1}H	–	–	–	–	–	–	–	–
	^{13}C	170.3	+0.4	166.6	+7.6	171.7	+5.9	161.3	+8.8
4	^{1}H	4.20	+0.7	4.19	+0.8	4.21	+0.6	4.62	+0.6
	^{13}C	60.4	+0.2	60.4	+0.1	60.5	+1.2	61.6	− 0.2
5	^{1}H	–	–	–	–	–	–	–	–
	^{13}C	179.1	− 3.4	179.4	− 3.6	179.7	− 1.5	178.7	− 2.8
6	^{1}H	1.47	+0.3	1.48	+0.3	1.47	0	1.69	+0.2
	^{13}C	16.5	− 0.3	16.7	− 0.2	16.8	+1.3	17.1	+0.6

[a] Data of further nuclei, $\delta/\Delta\delta$: 2.22/+0.4 (H-1′), 15.2/−0.9 (C-1′).
[b] Data of further nuclei, $\delta/\Delta\delta$: 2.51/+0.5 (H-1′), 1.26/0 (H-2′), 22.5/−0.5 (C-1′), 9.1/+0.7 (C-2′).
[c] Data of further nuclei, $\delta/\Delta\delta$: 1.29/+0.1 (H-2′), 34.1/+1.5 (C-1′), 26.7/+0.4 (C-2′).
[d] Data of further nuclei, $\delta/\Delta\delta$: 8.17/0 (H-2′), 7.56/−0.5 (H-3′), 8.06/−0.2 (H-4′), 7.93/−0.1 (H-5′), 7.58/−0.1 (H-6′), 7.66/−0.2 (H-7′), 9.26/−1.2 (H-8′), 133.8/−0.8 (C-1′), 130.2/+1.3 (C-2′), 124.6/+0.3 (C-3′), 133.6/−0.3 (C-4′), 128.8/−0.3 (C-5′), 126.6/−0.2 (C-6′), 128.2/−0.6 (C-7′), 125.9/−1.1 (C-8′), 130.8/−0.2 (C-9′), 121.7/+0.7 (C-10′).

reaction time, for the dehydration of enantiopure acyl *S*-amino acids with DCC and work-up; consequently, the major component of **1**–**11** is *S*-configurated. Their preparation was immediately followed by recording the ^{1}H and ^{13}C NMR spectra. Subsequently, an equimolar amount of the chiral auxiliary **Rh*** was added, and immediately thereafter, new NMR spectra were recorded. Details of the reaction are given in the Experimental Part and in the Supporting Information Material.

In $CDCl_3$ solution, a rapid-exchange equilibrium between the free components (**Rh*** and the ligands **L** = **1**–**11**, respectively) and their adducts [**Rh*** ←**L**] is formed.[16] There are two diastereomeric adducts which differ in the chemical shifts of all ligand nuclei, ^{1}H and ^{13}C (chiral recognition; *dirhodium method*).[16] The difference between the ν-values of corresponding nuclei (ν is the chemical shift in Hz) is called dispersion $\Delta\nu$ (recorded at 400 MHz here) and can be positive or negative according to the following definition:

$$\Delta\nu = \nu(S) - \nu(R). \quad (1)$$

The results of the dirhodium experiments with the oxazolones **1**–**11** are listed in the Tables 1–3. NMR signals can be shifted moderately by the presence of **Rh*** because of adduct formation.[16] These complexations shifts $\Delta\delta$ are positive if the binding site is close in terms of intervening covalent bonds; ^{13}C provides stronger effects than ^{1}H. So, complexations shifts offer an easy way to identify the ligand atom bound to rhodium. Nuclei further apart hardly experience any shift unless they are positioned inside the anisotropy cones of the **Rh*** phenyl groups (ring-current effect); generally, such shifts are negative.

Inspecting the 5(4*H*)-oxazolones data (Tables 1 and 2), it is obvious from the positive C-2 and C-4 signal shifts that the major binding site is N-3, the endocyclic nitrogen. As a consequence of this N-3 complexation, most aromatic carbons (1′–6′) show significant shifts with alternating signs indicating resonance effects within the phenylimino moiety. Similar effects on nitrogen-containing aromatic compounds have been reported.[25]

Only compound **7** displays a different $\Delta\delta$-pattern because the sulfur atom in the side chain is a soft-base ligand and strong donor dominating in binding to **Rh***.[16,18,19] This is revealed by the positive $\Delta\delta$-value of C-9 and the absence of a significant value at C-2. So, any amino acid bearing soft Lewis acid atom (sulfur, phosphorus etc.) has to be excluded from the method described here.

As noted above, dispersion effects $\Delta\nu$ are a consequence of the existence of two diastereomeric **Rh*** ←**L** adducts. Since we are dealing with nonracemic mixtures of enantiomers–the (*S*)-oxazolones are always prevailing–it is easy to assign the two data sets by their difference in signal intensities. Anisotropy (ring-current) effects from aromatic groups of **Rh*** shield ^{1}H nuclei significantly if those atoms are situated above or below the ring; ^{13}C nuclei with their much larger chemical shift range are not as sensitive to anisotropy influences.

As can be seen from Table 3, many pronounced $\Delta\nu$-values exist. It is striking to note that in the series of 2-aryl derivatives (**1**–**6**) all H-4 and H-6 values are large and positive indicating that those protons have the larger chemical shift in the *S*-enantiomers. This remarkable uniformity in the $\Delta\nu(^{1}H)$-values–many of them show enormous magnitudes up to +129 Hz corresponding to *ca* 0.5 ppm–suggests that all compounds within the series have a similar complexation behavior and adopt similar adduct conformation equilibria. Thus, the H-4 and H-6 atoms are spatially exposed to the MTPA-phenyl groups in a similar way. Surprisingly, compound **7** with the sulfur atom is not an exception although this ligand has different binding properties as compared to the others (see above). However, since equal signs of the dispersion values should be originated in similar adduct geometries, the observation for seven is probably coincidental.

As a result of the large magnitudes of the $\Delta\nu$-values, signal overlap is not a serious danger, and it is generally easy to analyze which is the major and which the minor constituent in a nonracemic mixture; Fig. 1 shows an example.

Comparing the alanyl derivative **1** (2-phenyl) with members of the 2-alkyl series **8**–**10** and with the 2-(α-naphthyl) analogue **11**, one can find that there is a significant drop in the H-4 value from +12 Hz (**1**) even to +1 Hz (**11**) and to negative values for

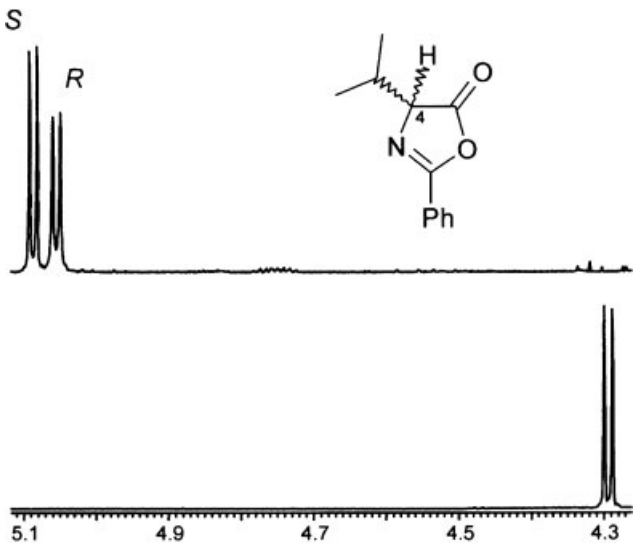


Figure 1. Signal dispersion of H-4 ($\Delta\nu$ = +13Hz) of **2**; bottom: free ligand; top: in the presence of an equimolar amount of **Rh***.

Table 3. Diastereomeric dispersion effects $\Delta\nu = \nu(S) - \nu(R)$ at the ^{1}H and ^{13}C signals of the 5(4*H*)-oxazolones **1**–**11**; in Hz, recorded at 400 MHz

	Atom	**1**[a]	**2**[b]	**3**[c]	**4**[d]	**5**[e]	**6**[f]	**7**[g]	**8**[h]	**9**[i]	**10**[j]	**11**[k]
2	^{1}H	–	–	–	–	–	–	–	–	–	–	–
	^{13}C	0	+16	8	+24	0	n.d.[l]	0	−8	−7	−1	0
4	^{1}H	+12	+13	+32	+69	+50	+62	+42	−29	−23	−31	+1
	^{13}C	−1	−4	+13	−90	0	−18	0	+6	+7	+7	−10
5	^{1}H	–	–	–	–	–	–	–	–	–	–	–
	^{13}C	0	0	+7	−19	0	0	0	0	0	0	0
6	^{1}H	+39	n.d.[l]	+129[m] +52	+18	+41[m] +35	+94[m] +86	n.d.[l] +48	+12	+12	+44	+19
	^{13}C	+10	+11	0	0	+3	0	+12	0	−1	+10	−24
7	^{1}H	–	+24	0	−38	–	–	−48	–	–	–	–
	^{13}C	–	0	+14	0	–	–	+7	–	–	–	–
8	^{1}H	–	+16	+64	−20	–	–	–	–	–	–	–
	^{13}C	–	+6	+76	−34	–	–	–	–	–	–	–
9	^{1}H	–	–	−11	−13	–	–	+9	–	–	–	–
	^{13}C	–	–	−38	−205	–	–	0	–	–	–	–

[a] Data of further nuclei, $\Delta\nu$: n.d. (H-2′/6′), n.d. (H-3′/5′), +9 (H-4′), +4 (C-1′), −1 (C-2′/6′), +12 (C-3′/5′), +2 (C-4′).
[b] Data of further nuclei, $\Delta\nu$: +11 (H-2′/6′), n.d. (H-3′/5′), +9 (H-4′), −7 (C-1′), +2 (C-2′/6′), +13 (C-3′/5′), +6 (C-4′).
[c] Data of further nuclei, $\Delta\nu$: −3 (H-2′/6′), n.d. (H-3′/5′), +6 (H-4′), +6 (C-1′), +17 (C-2′/6′), +4 (C-3′/5′), 0 (C-4′).
[d] Data of further nuclei, $\Delta\nu$: −12 (H-2′/6′), n.d. (H-3′/5′), +15 (H-4′), +20 (C-1′), +10 (C-2′/6′), −2 (C-3′/5′), −18 (C-4′).
[e] Data of further nuclei, $\Delta\nu$: +12 (H-2′/6′), n.d. (H-3′/5′), n.d. (H-4′), n.d. (H-2′/6′), n.d. (H-3′/5′) n.d. (H-4′), n.d. (C-1′), +3 (C-2′/6′), +8 (C-3′/5′), +8 (C-4′), +10 (C-1′), −3 (C-2′/6′), +20 (C-3′/5′), n.d. (C-4′).
[f] Data of further nuclei, $\Delta\nu$: +26 (H-2′/6′), n.d. (H-3′/5′), 0 (H-4′), +9 (C-1′), +29 (C-2′/6′), +21 (C-3′/5′), 0 (C-4′), 0 (H-1′), +9 (H-3′), +18 (H-4′), +25 (H-5′), 0 (H-6′), n.d. (H-7′), −3 (C-2′), −7 (C-3′), −5 (C-4′), 0 (C-5′), −3 (C-6′), 0 (C-7′), 0 (C-8′), +7 (C-9′).
[g] Data of further nuclei, $\Delta\nu$: −36 (H-2′/6′), n.d. (H-3′/5′), +80 (H-4′), +4 (C-1′), +6 (C-2′/6′), −6 (C-3′/5′), −21 (C-4′).
[h] Data of further nuclei, $\Delta\nu$: +4 (H-1′), +5 (C-1′).
[i] Data of further nuclei, $\Delta\nu$: n.d. (H-1′), −8 (H-2′), +2 (C-1′), +2 (C-2′).
[j] Data of further nuclei, $\Delta\nu$: +8 (H-2′), −3 (C-1′), −3 (C-2′).
[k] Data of further nuclei, $\Delta\nu$: 4 (H-2′), +10 (H-3′), −31 (H-4′), −31 (H-5′), +37 (H-6′), +27 (H-7′), −75 (H-8′), −24 (C-1′), −9 (C-2′), −13 (C-3′), −18 (C-4′), −11 (C-5′), −9 (C-6′), +5 (C-7′), −24 (C-8′), −9 (C-9′), −15 (C-10′).
[l] 'n.d.' means 'not detectable due to signal overlap'.
[m] Diastereotopic hydrogens; no stereochemical assignment.

methyl, −29 Hz (**8**), −23 Hz (**9**) and −31 Hz (**10**). The respective $\Delta\nu$ -values of H-6 remain positive like in the 2-phenyl cases but their absolute values appear to be somewhat smaller. Only the 2-*tert.*-butyl analogue **10** has an H-6 value with a magnitude comparable to those of derivatives with phenyl residues (**1**–**7**). Apparently, large groups next to the binding site are important; possibly, they are required to reduce the mobility of the ligand when bound in the adduct. This leads to the recommendation that the AC determination method introduced here should be executed with 2-phenyl-5(4*H*)-oxazolones where H-4 as well as H-6 signals of the *S*-configurated enantiomer have the larger chemical shift (left-hand signal). If one decides for a 2-*tert.*-butyl-analogue, the different sign behavior of the H-4 dispersions, negative instead of positive, has to be kept in mind.

It should be noted that the ^{13}C NMR spectra are not required for AC determination; they were needed here only for identifying the binding sites. Following the protocol below, only one single ^{1}H NMR spectrum in the presence of **Rh*** is sufficient for AC determination.

The described results allow to propose a protocol for the AC determination of amino acids by the *dirhodium method*:

1. Prepare a nonracemic mixture of 2-phenyl-5(4*H*)-oxazolones by reaction of an enantiopure α-amino acid of unknown AC with benzoyl chloride and condense the benzamide with DCC; restrict the time of the latter reaction to a maximum of 20 min including work-up. Nonracemic mixtures of the α-amino acid can be treated analogously as long as one enantiomer is clearly prevailing.
2. Record a ^{1}H NMR spectrum of the prepared oxazolone in the presence of an equimolar amount of **Rh*** and inspect the splitting of the H-4 (between $\delta = 5$ and 6 ppm) and H-6 signals (the H-6 chemical shift depends on the amino acid side chain). The left-hand signal is from the *S*- and the right-hand one from the *R*-configurated oxazolone.

With $\Delta\nu' = \nu$(major enantiomer) $- \nu$(minor enantiomer),
if $\Delta\nu'$(H-4) and $\Delta\nu'$(H-6) $> 0 \Rightarrow$ the amino acid is *S*-configured;
$\Delta\nu'$(H-4) and $\Delta\nu'$(H-6) $< 0 \Rightarrow$ the amino acid is *R*-configured.

Conclusion

The conversion of α-amino acids into 5(4*H*)-oxazolones by cyclization of their respective *N*-benzoyl or *N*-pivaloyl derivatives offers a new way to determine the AC of α-amino acids. The advantages of this method are the following:

1. The derivatization affords a nonracemic mixture in which the AC of the original amino acid–originally being either enantiopure or the major constituent of a nonracemic mixture–always prevails. Thus, the method allows a direct comparison of both enantiomers under identical conditions.

2. Most of the conformational mobility, which generally exists in substituted amino acids, is removed by the ring formation; an important principle for reliable AC determination by NMR correlation methods.
3. In applying the *dirhodium method* (addition of one equivalent of **Rh*** in $CDCl_3$), the ^1H signal sets of the two enantiomers can be identified and assigned easily to the major and the minor enantiomer. In the case of the 2-phenyl-5(4*H*)-oxazolones (like **1**–**6**), positive H-4 and H-6 dispersion effects $\Delta\nu'$ [$= \nu$(major constituent)$-\nu'$(minor constituent)] indicate that the original α-amino acid, or the major enantiomer in a nonracemic mixture, respectively, was *S*-configurated, and vice versa. In the case of (*S*)-2-*tert*.-butyl derivatives of amino acids, the H-4 dispersions are negative and those of H-6 positive for an *S*-amino acid, and vice versa.
4. ^1H NMR measurements of the pure oxazolone derivatives prior to the dirhodium experiment are not necessary for AC determination because only the sign of $\Delta\nu'$ (as mentioned in step 3) is required, not the absolute ν-values themselves (for example the top spectral trace in Fig.1). Thereby, experimental input and spectrometer time are greatly reduced.
5. Amino acids bearing a soft Lewis acid atom (sulphur, phosphorus etc.) have to be excluded from the method described here because they have a different complexation behavior.

Supporting information

Supporting information may be found in the online version of this article.

Acknowledgement

This work was supported by the German Research Foundation (Deutsche Forschungsgemeinschaft, grant DU 98/30-1).

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