Deprotonation of the peptide NH groups and diastereoselective hydrogenation of *N*-acetyl-α,β-dehydrodipeptides

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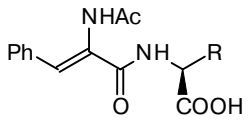
The peptide protons in *N*-acetyl-α,β-dehydrodipeptides (DHDP) dissociate in aqueous methanol in the presence of magnesium salts upon the addition of alkali, which favors the diastereoselectivities of their hydrogenation over Pd/C. By contrast, the N—H bonds of the *N*-acetyl groups in DHDP seem not to dissociate under these conditions. The dissociation of the peptide N—H bonds in strongly alkaline solutions was studied by ^{19}F NMR spectroscopy for model compounds containing the 4-FC_6H_4 fragment.

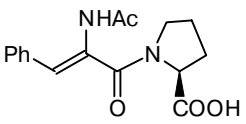
Key words: *N*-acetyl-α,β-dehydrodipeptides, deprotonation of the peptide NH groups, diastereoselective hydrogenation, ^{19}F NMR spectroscopy.

Dissociation of the peptide NH protons is a decisive factor in complexation of peptides with transition metals. These protons dissociate in aqueous media in the presence of a metal cation even at pH ~5, but they cannot be titrated without the metal cation at pH < 12.[1]

In the present work, it was shown that the deprotonation of the peptide NH groups of *N*-acetyl-α,β-dehydrodipeptide (DHDP) in alcoholic media at pH > 12 in the presence of Mg salts (non-transition metal) favors complexation. According to a general rule, the formation of cyclic complexes strongly limits conformational mobility and hence increases asymmetric induction in chemical reactions. We studied the diastereoselective hydrogenation of complexes of *N*-acetyl-α,β-dehydrophenylalanyl-(*S*)-valine (**1**), *N*-acetyl-α,β-dehydrophenylalanyl-(*S*)-leucine (**2**), and *N*-acetyl-α,β-dehydrophenylalanyl-(*S*)-proline (**3**) with Mg^{2+} over Pd/C in alkaline media to the corresponding saturated *N*-acetyldipeptides.



1, 2



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1: R = $CHMe_2$

2: R = CH_2CHMe_2

Peptides **1** and **2** contain three protons that can dissociate, namely, a COOH proton, a peptide proton, and an amide proton. Peptide **3** possesses no peptide proton.

Previously,[2] we have shown that DHDP reacts with Ca or Mg salts in 95% MeOH, the acidity of the carboxy groups increasing by two orders of magnitude.

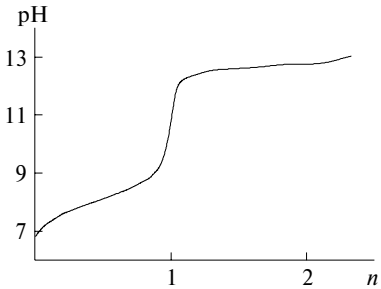

Fig. 1. Potentiometric titration of complex **1** · $MgCl_2$ in 95% MeOH (*n* is the number of equivalents of NaOH).

The potentiometric titration of **1** in the presence of $MgCl_2$ in 95% MeOH shows that only the COOH protons dissociate, while the peptide protons are not involved in complexation at pH < 12 (Fig. 1). Above pH 12, these cannot be seen in the titration curve since the range of dissociation is beyond the scope of the method. Nevertheless, it turned out that the amount of NaOH used to prepare DHDP complexes with Mg^{2+} markedly influences the diastereomeric excess in their hydrogenation, as can be seen in Table 1.

We attributed this effect to the formation of more rigid Mg chelates upon deprotonation of the peptide NH groups. To prove this hypothesis, we examined methanolic solutions of *N*-acetyl-α,β-dehydro-*p*-fluorophenylalanine (**4**), *N*-acetyl-α,β-dehydro-*p*-fluorophenylalanyl-(*S*)-valine (**5**), and *p*-FC_6H_4COOH all containing an indicator F atom by ^{19}F NMR spectroscopy in the presence of different amounts of alkali. Earlier,[3,4] we showed that this method provides valuable information on the state of electron density in relevant complexes.

The results obtained are presented in Table 2.

It can be seen from the data for compound **5** that successive addition of one and two equivalents of NaOH shifts the signal for the ^{19}F atom upfield by 0.34 and

† Deceased.

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Table 1. Effect of the amount of NaOH on the diastereoselectivity of the hydrogenation of DHDP complexes with Mg^{2+}

Complex	NaOH (equiv.)	*de* (*SS*)* (%)
1 · $MgSO_4$	—	10
	1	35 (22**)
	2	60 (33**)
	3	52
2 · $MgCl_2$	—	7
	1	19
	2	34
3 · $MgCl_2$	—	30
	1	58
	2	56

* *de* (*RS*) for complex **3** · $MgCl_2$.
** In the absence of $MgSO_4$.

Table 2. Effect of the amount of NaOH on the ^{19}F chemical shifts in complexes with **4**, **5**, and *p*-FC_6H_4COOH

Compound	NaOH (equiv.)	$-\delta_F$
4	—	2.73
	1	0.14
	2	0.01
5	—	1.97
	1	1.63
	2	0.97
	3	1.01
p-FC_6H_4COOH	—	6.51
	1	1.77
	2	1.78
	3	1.80

* The ^{19}F shift was determined with respect to PhF according to Taft. More negative values indicate that the ^{19}F signal is shifted downfield.

1.0 ppm, respectively, compared to the signal for the nonionized molecule. This suggests that the F atom is shielded by negative charges appearing in succession in the molecule of **5** upon dissociation of both the COOH proton of the substrate (the first equivalent) and the peptide or amide NH proton (the second equivalent). The addition of a third equivalent of alkali does not virtually change the position of the signal for the ^{19}F atom, indicating the ionization of only one NH proton. The ^{19}F NMR spectrum of *p*-FC_6H_4COOH remains unchanged upon the addition of the second and third equivalents of alkali, *i.e.*, the magnetic environment of the dipole in alkaline medium does not affect the ^{19}F chemical shift. In the case of compound **4**, the addition of one equivalent of alkali gives the carboxylate anion, and a signal for the F atom is strongly shifted upfield (by 2.59 ppm). The second equivalent of alkali only slightly changes its position (an additional shift of 0.13 ppm), and therefore the amide proton seems to remain nonionized.

The ionization of only COOH and peptide NH protons was indirectly confirmed by the hydrogenation of complexes **3** with $MgCl_2$, which were formed in the presence of one or two equivalents of NaOH (see Table 1). As this peptide does not contain the peptide proton, only the COOH group is ionized, the second equivalent of alkali producing no effect on the stereoselectivity of the reaction.

In conclusion, it should be emphasized that ^{19}F NMR spectroscopy makes it possible to reveal the deprotonation of the peptide NH groups in strongly alkaline media and thus supplement the data from potentiostatic titration for the range where the latter fails.

Experimental

^1H NMR spectra were recorded on a Bruker WP-200SY spectrometer in CD_3OD. ^{19}F NMR spectra were recorded as described in Ref. 3. Potentiostatic titration was carried out under the conditions reported in Ref. 5.

All DHDP were prepared by the azlactone method according to the known procedure.[4,5]

***N*-Acetyl-α,β-dehydro-*p*-fluorophenylalanine (4)** was synthesized by alkaline hydrolysis of 2-methyl-4-(*p*-fluorobenzylidene)oxazol-5-one, m.p. 217—218 °C (from EtOH) (*cf.* Ref. 6: 214—216 °C).

The procedures described in Ref. 3 were used to hydrogenate the complexes and determine the diastereomeric ratios of the dipeptides.

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