93. Conformational Studies on Peptides Containing Enantiomeric α-Methyl α-Amino Acids

Part I

Differential Conformational Properties of (*R*)- and (*S*)-2-Methylaspartic Acid

by **Karl-Heinz Altmann**[1])*, **Eva Altmann**[2]), and **Manfred Mutter**

Section de Chimie de l'Université de Lausanne, Rue de la Barre 2, CH–1005 Lausanne

(12.III.92)

The conformational properties of four model peptides of the general formula Ac-Tyr-Xaa-Yaa-Zaa-Ala-Lys-Glu-Ala-Ala-Glu-Lys-Ala-Zaa-Yaa-Xaa-Lys-NH_2 (Xaa-Yaa-Zaa = Ala-Ala-(*R*)-Asp(2-Me), **1**; Ala-Ala-(*S*)-Asp(2-Me), **2**; Ala-Aib-Asp, **3**; Ala-Ala-Asp, **4**; Asp(2-Me) = 2-methylaspartic acid; Aib = 2-aminoisobutyric acid) were studied by CD spectroscopy in solution, to evaluate the helix-inducing potential of enantiomerically pure 2-methylaspartic acid as a function of its chirality at C(2). At neutral pH and 1°, all peptides exhibit significant helix formation in aqueous solution, the degree of helicity increasing in the order **4** < **3** ≈ **2** < **1**. Lowering the pH to 2 results in a dramatic increase in helicity for peptide **1**, while the diastereoisomeric peptide **2** now exists in a predominantly unordered conformation. Helix induction by protonated (*R*)-Asp(2-Me) exceeds Aib-induced helix formation in peptide **3**, and the helix content of **1** in aqueous solution at pH 2 is comparable to the degree of helicity in the strongly helix-inducing solvent 2,2,2-trifluoroethanol.

Introduction. – The biological effects mediated by peptides and proteins are based on defined conformational features, *i.e.*, secondary and tertiary structures, either of these molecules free in solution or when bound to their corresponding receptors. However, small and medium-sized peptides often do not exhibit a defined secondary structure in aqueous solution to any appreciable extent [1], let alone the conformation required for receptor binding [2] [3]. The stabilization of particular conformational properties has thus been of major interest in the field of biologically active peptides (including protein fragments) [2] [3] and, more recently, the induction or stabilization of secondary structures in peptides has also become a major issue in conjunction with the *de novo* design of proteins [1] [3] [4]. Among the various approaches pursued for the stabilization of helical conformations, one of the major strategies is the incorporation of the helix-inducing amino acid 2-aminoisobutyric acid (= 2-methylalanine = 2-amino-2-methylpropanoic acid; Aib) [5] [6] (for other approaches to helix stabilization, *cf.* [7]). However, in contrast to the numerous studies conducted on the conformational properties of Aib-containing peptides [5] [6] [8], only little work has been devoted to the evaluation of the conformational characteristics of peptides containing α-methyl-substituted α-amino acids other than Aib[3]). This is rather surprising in view of the very interesting and useful properties of Aib and given the advanced synthetic methodology available for the stereospecific synthesis of these compounds [10] [11].

[1]) Present address: *Ciba-Geigy Ltd.*, Central Research Laboratories, R-1060.2.14, CH–4002 Basel.

[2]) Present address: *Ciba-Geigy Ltd.*, Pharmaceutical Research Division, K-136.4.92, CH–4002 Basel.

[3]) The conformations of homooligomers of symmetrically α,α-dialkyl-substituted α-amino acids have been studied in some detail (see, *e.g.*, [9]).

In a comparative study on the helical properties of peptides of the general formula Ac-Ala-Xaa-(Ala-)$_2$Xaa-(Ala-)$_2$Xaa-(Ala-)$_2$NH-PEGM^{5000}, with Xaa = Aib, (*S*)-2-ethylalanine (Ala(2-Et)), (*S*)-2-methylserine (Ser(2-Me)), and PEGM^{5000} = polyethylene glycol monomethyl ether (M_r = 5000), *Altmann et al.* [12] found that (*S*)-Ala(2-Et) has approximately the same helix-inducing potential as Aib, while incorporation of (*S*)-Ser(2-Me) does not result in any helix induction whatsoever. *Valle et al.* [13] reported the crystal structure of the pentapeptide Ac-Aib-Aib-(*R*)-Ala(2-Et)-Aib-Aib-OMe which exhibits a 3_{10}-helical conformation; however, in spite of the presence of the chiral Ala(2-Et) residue, the helix does not have a preferred screw sense. On the other hand, the tetrapeptides Boc-(*R*)-Ala(2-Et)-Hyp(Bzl)-Ala-Phol (Hyp = 4-hydroxy-L-proline, Phol = L-phenylalaninol) and Boc-(*R*)-Ala(2-Et)-Hyp(Bzl)-Aib-Phol were found to assume right-handed 3_{10}-helical conformations in the crystal structure [14], although the (*R*)-Ala(2-Et) residue can be formally derived from the (*R*)(or D)-enantiomer of 2-ethylglycine. Most recently, *Nebel et al.* [15] investigated the conformations of diastereoisomeric tri- and pentapeptides containing (*R*)- and (*S*)-Ala(2-Et) by X-ray diffraction as well as spectroscopic methods in $CDCl_3$ solution. The (β-turn) conformations observed for pairs of diastereoisomers were essentially independent of the chirality of the Ala(2-Et) residue.

To gain a better understanding of the conformational preferences of various α-methyl-substituted α-amino acids, we have now embarked on a program directed towards the elucidation of the potential helix-inducing effects of chiral α-methylated derivatives of naturally occurring α-amino acids, with special emphasis on the comparison of the behaviour of the corresponding (*R*)- and (*S*)-enantiomers. In this study, we want to report, as a first example, on the differential conformational behaviour of peptides containing either (*R*)- or (*S*)-2-methylaspartic acid ((*R*)- or (*S*)-Asp(2-Me))[4]). To address this question, the peptides **1**–**4** were synthesized and their conformational properties studied by circular dichroism (CD) spectroscopy in solution[5]).

$$\overset{1\ 2\ 3}{\text{Ac-Y-A-A-}}(R)\text{-Asp(2-Me)-}\overset{5\ 6\ 7\ 8\ 9\ 10\ 11\ 12}{\text{A-K-E-A-A-E-K-A-}}(R)\text{-Asp(2-Me)-}\overset{14\ 15\ 16}{\text{A-A-K-NH}_2} \quad \textbf{(1)}$$

$$\text{Ac-Y-A-A-}(S)\text{-Asp(2-Me)-A-K-E-A-A-E-K-A-}(S)\text{-Asp(2-Me)-A-A-K-NH}_2 \quad \textbf{(2)}$$

$$\text{Ac-Y-A-Aib-D-A-K-E-A-A-E-K-A-D-Aib-A-K-NH}_2 \quad \textbf{(3)}$$

$$\text{Ac-Y-A-A-D-A-K-E-A-A-E-K-A-D-A-A-K-NH}_2 \quad \textbf{(4)}$$

---

[4]) Although the choice of this particular amino acid as a starting point may be somewhat arbitrary, we strongly felt that we had to use the α-methylated derivative of a helix-destabilizing amino acid in order to realistically evaluate the conformational consequences of α-methylation (for the helical parameters of aspartic acid, *cf.* [16] [17]). For amino acids with an intrinsic preference for α-helical conformations, *e.g.*, Met, Leu, or Glu, the difference between the parent and the modified peptide seemed likely to be less pronounced. The preference of Asp over other helix-destabilizing amino acids, *e.g.*, Ser, was finally also determined by the ease of synthesis of the appropriately protected derivatives of the α-methylated amino acid [11].

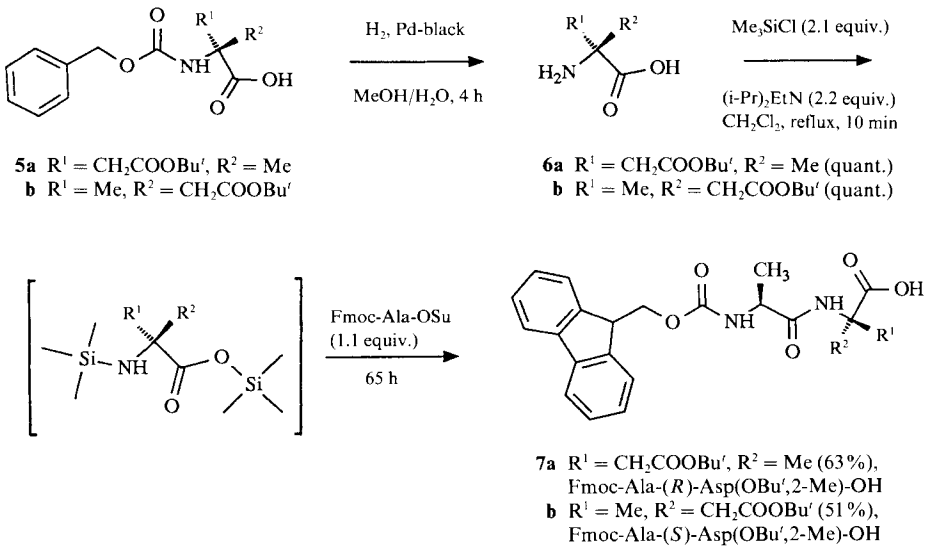
[5]) Proteinogenic amino acids are abbreviated according to the one-letter code [18] and are all of the L-configuration: A = Ala = alanine, K = Lys = lysine, E = Glu = glutamic acid, D = Asp = aspartic acid, Y = Tyr = tyrosine. The symbols suggested by *Marshall et al.* [14] for the designation of α,α-dialkyl-substituted α-amino acids and originally adopted by the authors for 2-methylaspartic acid (= α-MeAsp) were replaced by the editor by the less ambiguous symbols for side-chain-substituted amino acids [18], *i.e.* Asp(2-Me) instead of α-MeAsp.

Peptides **4** and **3** serve as reference points for the evaluation of the helix-inducing properties of α-methylated aspartic-acid derivatives as compared to L-Asp and especially Aib, respectively. The peptides were designed such that a helical conformation of the central part of the molecule should be stabilized by two attractive $i/(i+4)$ charge-charge interactions (at neutral pH) in all four cases [19], while the helical potential of the adjoining parts of the sequence should vary with their respective amino-acid composition, thus reflecting the helix-inducing potential of the different α-methyl-substituted α-amino acids.

Results and Discussion. – *Peptide Synthesis.* All peptides were synthesized by the semi-automatic solid-phase method [20] using a [(fluoren-9-yl)methoxy]carbonyl/*tert*-butyl (Fmoc/Bu^t) protection scheme [21] and diisopropylcarbodiimide/1*H*-benzotriazol-1-ol (DIC/HOBt) activation [22] [23] (for details of the protocol, *cf. Exper. Part*). Based on our previous experience [5], all α-methylated derivatives were incorporated by the coupling of appropriately protected dipeptides containing the α-methyl-substituted α-amino acid as the C-terminal residue. Thus difficulties in the coupling to the sterically crowded N-terminus of the α-methylated residues [24] as well as problems to monitor the coupling reaction by standard methods[6]) can be avoided.

The dipeptides Fmoc-Ala-(*R*)-Asp(OBu^t,2-Me)-OH (**7a**) and Fmoc-Ala-(*S*)-Asp(OBu^t,2-Me)-OH (**7b**) were synthesized according to a method originally described by *Kricheldorf* [25], involving the *in situ* silylation of the C-terminal Asp(OBu^t, 2-Me) (see **6**) and subsequent reaction with the *N*-hydroxysuccinimide ester of Fmoc-Ala (Fmoc-Ala-OSu) (see *Scheme*; for the synthesis of N^2-[(benzyloxy)carbonyl]-protected Asp(OBu^t,

Scheme



5a $R^1 = CH_2COOBu^t$, $R^2 = Me$
b $R^1 = Me$, $R^2 = CH_2COOBu^t$

6a $R^1 = CH_2COOBu^t$, $R^2 = Me$ (quant.)
b $R^1 = Me$, $R^2 = CH_2COOBu^t$ (quant.)

7a $R^1 = CH_2COOBu^t$, $R^2 = Me$ (63%), Fmoc-Ala-(*R*)-Asp(OBu^t,2-Me)-OH
b $R^1 = Me$, $R^2 = CH_2COOBu^t$ (51%), Fmoc-Ala-(*S*)-Asp(OBu^t,2-Me)-OH

[6]) In contrast to the usual (α-monoalkylated) α-amino acids, Asp(2-Me) as well as Aib give only very weak colour reactions with ninhydrin.

2-Me) **5**, *cf.* [11]). Fmoc-Asp(OBu^t)-Aib-OH (**8**) was obtained in the same way in 59% yield, except that the mixed anhydride of Fmoc-Asp(OBu^t)-OH with isobutyl hydrogencarbonate was used in the coupling reaction instead of the *N*-hydroxysuccinimide ester. Fmoc-Ala-Aib-OH (**9**) was obtained from HCl·H-Ala-Aib-OH and Fmoc-Cl [26]. The diastereoisomeric purity of dipeptides **7a** and **7b** was demonstrated by reversed-phase HPLC to be higher than 99%; the HPLC analysis required prior removal of the hydrophobic N-terminal Fmoc group, as no separation of diastereoisomers could be achieved at the fully protected stage or after removal of the Bu^t side-chain-protecting group. Although no detailed kinetic studies were conducted at this point, it is worth noting that the coupling kinetics of **7a** and **7b** were much slower than for **8** and **9**: while double couplings (*ca.* 4-fold excess of activated dipeptide) of 2 h each were sufficient to achieve > 99% coupling with **8** and **9**, double couplings of 8 h each (*ca.* 2-fold excess) were necessary to accomplish satisfactory incorporation (> 95%) of **7a** and **7b**.

Conformational Studies. As can be seen from the *Table*, the CD spectra of peptides **1**–**4** in 90% aq. MeOH are characterized by strong negative *Cotton* effects around 220 and 207 nm and a zero crossover at 200 nm, indicating a largely helical conformation in all four cases. The peptides display very similar overall conformational features in this solvent system, and it can be concluded that only minor differences exist in their helical stabilities under these helix-promoting conditions [27]. However, this situation changes when the helix-promoting capacity of the alcoholic solvent is modulated by dilution with H_2O, which is known to exert a helix-destabilizing effect [27]. In 10% aq. MeOH (*Table*), pronounced differences become apparent in the helix-forming potential of the individual peptides. Based on the location of the longer-wavelength component of the $\pi-\pi^*$ transition (205 nm), the wavelength of the zero crossover (197 nm), and in particular its *R* value (0.72), peptide **1** (containing two (*R*)-Asp(2-Me) residues) exhibits the highest degree of helicity under those experimental conditions. By the same criteria[7]), peptide **4** (containing only proteinogenic L-amino acids) clearly possesses the lowest helical potential, while **2** and **3** exhibit helical stabilities intermediate between those of **1** and **4**. Similar results were obtained in 90% and 10% aq. CF_3CH_2OH, respectively (data not shown). Under purely aqueous conditions at near neutral pH and 25°, the degree of helicity is even further reduced as compared to 10% aq. MeOH, which is manifested in a blue-shift of the wavelength λ_0 of the zero crossover and of the lower-wavelength negative *Cotton* effects as well as in lower *R* values (*Table*). Lowering the temperature from 25 to 1° results in a profound increase in helix stability in all four cases, the degree of helicity in H_2O at pH 6.6 being even higher than in 10% aq. MeOH at 25° (*Table*, *Fig. 1*). However, it is most important to note that all these data demonstrate a profound helix-inducing effect of the (*R*)-Asp(2-Me) residue in aq. solution at approximately neutral pH (*i.e.*, with ionized side chain); helix induction is also observed upon replacement of L-Asp in **4** by (*S*)-Asp(2-Me) (see **2**) (to about the same extent as upon substitution of Ala^3 and Ala^{14} in **4** by Aib (see **3**)).

---

[7]) CD Spectra are evaluated on a purely qualitative basis using mainly the location of the negative *Cotton* effect related to the $\pi-\pi^*$ transition ($\lambda_{\pi-\pi^*,\min}$), the wavelength λ_0 of the zero crossover, and the *R* value ($= [\theta]_{n-\pi^*}/[\theta]_{\pi-\pi^*,\min}$), as the criteria to establish a relative scale of helicities [5] [28]. The corresponding values for the ideal α-helix were reported as 208 nm, 202 nm, and *ca.* 1, respectively [29]. Any decrease in the degree of helicity results in a blue-shift of $\lambda_{\pi-\pi^*,\min}$ and λ_0 as well as in a decreased *R* value [29].

Table. *CD-Spectral Characteristics of Peptides* **1**–**4** *under Different Experimental Conditions*

	Solvent	T	$[\theta]_{n-\pi^*}$[a])	$[\theta]_{\pi-\pi^*}$[b])		λ_o[c])	R[d])
1	90% aq. MeOH	25°	−28.1 (220)	−37.0 (207)	n.d.[e])	200	0.76
2	90% aq. MeOH	25°	−31.8 (220)	−39.3 (207)	n.d.[e])	200	0.79
3	90% aq. MeOH	25°	−30.9 (220)	−40.4 (207)	n.d.[e])	200	0.76
4	90% aq. MeOH	25°	−32.3 (220)	−44.1 (207)	n.d.[e])	200	0.74
1	10% aq. MeOH	25°	−13.8 (221)	−19.2 (205)	23.3 (190)	197	0.72
2	10% aq. MeOH	25°	−13.2 (220)	−21.9 (204)	n.o.[f])	195	0.60
3	10% aq. MeOH	25°	−12.0 (220)	−20.3 (204)	n.o.[f])	195	0.59
4	10% aq. MeOH	25°	−11.1 (220)	−25.6 (202)	n.o.[f])	192	0.43
1	pH 6.6[g])	25°	−8.85 (220)	−15.9 (204)	9.42 (189)	194	0.56
2	pH 6.6[g])	25°	−10.1 (220)	−21.0 (203)	n.o.[f])	191	0.48
3	pH 6.6[g])	25°	−9.40 (219)	−20.8 (202)	n.o.[f])	191	0.45
4	pH 6.6[g])	25°	−9.03 (219)	−28.1 (200)	n.o.[f])	190	0.32
1	pH 6.6[g])	1°	−20.0 (221)	−21.8 (206)	35.8 (189)	198	0.92
2	pH 6.6[g])	1°	−18.8 (220)	−25.4 (205)	24.2 (188)	196	0.74
3	pH 6.6[g])	1°	−15.8 (220)	−22.1 (204)	20.9 (190)	197	0.72
4	pH 6.6[g])	1°	−17.9 (220)	−27.9 (203)	23.5 (188)	194	0.64
1	pH 2.0[h])	25°	−25.0 (220)	−27.8 (206)	51.8 (191)	199	0.90
2	pH 2.0[h])	25°	−4.45 (220)	−19.5 (198)	n.o.[f])	n.o.	0.23
3	pH 2.0[h])	25°	−11.9 (221)	−20.4 (204)	n.o.[f])	194	0.58
4	pH 2.0[h])	25°	−11.2 (219)	−25.4 (201)	n.o.[f])	192	0.44
1	pH 2.0[h])	1°	−34.6 (221)	−33.5 (206)	74.5 (191)	200	1.03
2	pH 2.0[h])	1°	−10.0 (222)	−22.8 (202)	n.o.[f])	n.o.	0.44
3	pH 2.0[h])	1°	−19.7 (220)	−24.5 (206)	31.7 (190)	198	0.80
4	pH 2.0[h])	1°	−21.4 (220)	−30.0 (204)	n.d.[e])	195	0.71

[a]) Total molar ellipticity $[\theta]$ (in $°\cdot cm^2\cdot dmol^{-1}\cdot 10^{-4}$) and wavelength (in nm; in parentheses) of the negative *Cotton* effect related to the n–π^* transition; for cases where no distinct negative *Cotton* effect could be observed (*e.g.*, at pH 6.6 and 25°), the wavelength assignment may be somewhat arbitrary.

[b]) Total molar ellipticities $[\theta]$ (in $°\cdot cm^2\cdot dmol^{-1}\cdot 10^{-4}$) and wavelengths (in nm; in parentheses) of the *Cotton* effects related to the π–π^* transition; lefthand column, negative *Cotton* effect, and righthand column, positive *Cotton* effect of this transition.

[c]) Wavelength (in nm) of the zero crossover.

[d]) $R = [\theta]_{n-\pi^*}/[\theta]_{\pi-\pi^*\text{(higher-wavelength component)}}$.

[e]) Not determined; a positive *Cotton* effect was clearly present around 190 nm; however, because of very poor signal-to-noise ratios, the ellipticity values were not determined.

[f]) Not observed above 188 nm.

[g]) 1 mM Phosphate buffer, pH 6.6.

[h]) 0.010M HCl.

The difference in the helix-inducing potential between (*R*)- and (*S*)-Asp(2-Me) observed at near neutral pH widens to a dramatic gap when lowering the pH from 6.6 to 2.0. As indicated by a drastic increase in ellipticities (*Fig. 2* (at 25°) and *3* (at 1°)) and also by a significant increase in the *R* value, the degree of helicity of peptide **1** under acidic conditions is considerably higher than at near neutral pH. In contrast, the spectra of peptide **2** at pH 2 (*Figs. 2* and *3*) are dominated by a negative *Cotton* effect around 200 nm, and no zero crossover can be detected above 190 nm. These spectral features are indicative of a largely unordered conformation of peptide **2** under acidic conditions, and

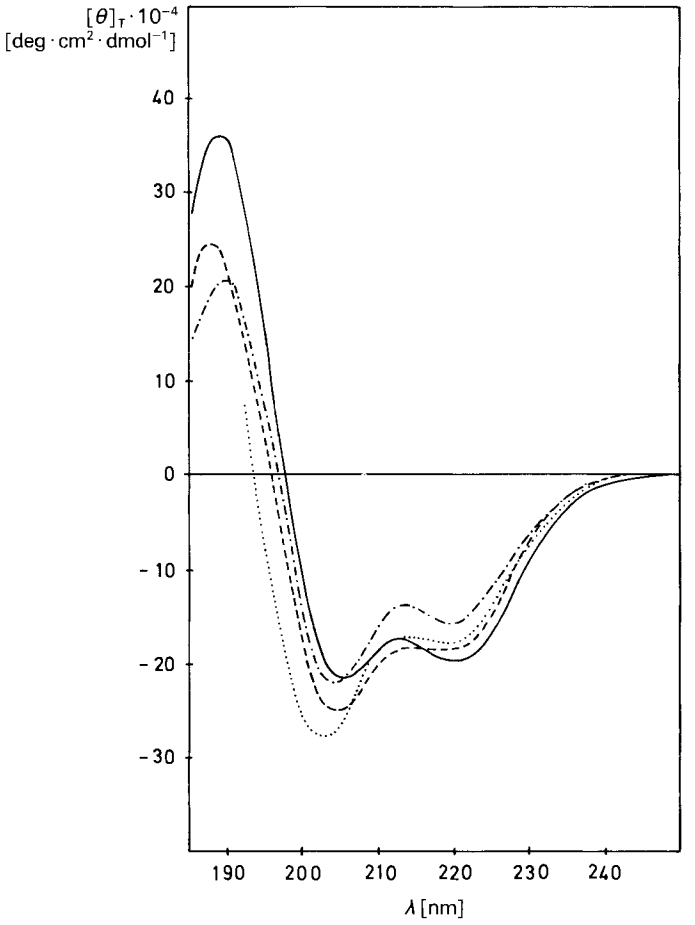


Fig. 1. *CD Spectra of peptides* **1**–**4** *at 1° in 1 mM phosphate buffer.* pH 6.6, $c = 80$ μM. **1** (—), **2** (- - -), **3** (- · - · -), **4** (· · · ·).

especially at 25° (*Fig. 2*) the degree of helicity is probably negligible. The spectra obtained for peptides **1** and **2** at pH 2 are independent of concentration (identical spectra were obtained at 80 μM and 8 μM concentration, in both cases), which rules out peptide aggregation as the source of the difference in the conformational properties of **1** and **2** under acidic conditions.

It should be noted that also peptides **3** and **4** exhibit a definitive (though far less dramatic) increase in helix content at acidic pH (*Fig. 3*, *Table*), which seems rather surprising as two attractive $i/(i + 4)$ charge-charge interactions are lost upon protonation of acidic side chains [19]. The reasons for this unexpected behaviour are not known, and it is not the objective of this paper to address general questions of the relationship between charge effects and helical stability. However, this finding indicates that a minor part of the increase in helix stability of peptide **1** at pH 2 might have to be attributed to factors unrelated to the presence of (*R*)-Asp(2-Me). Nevertheless, the very different helix

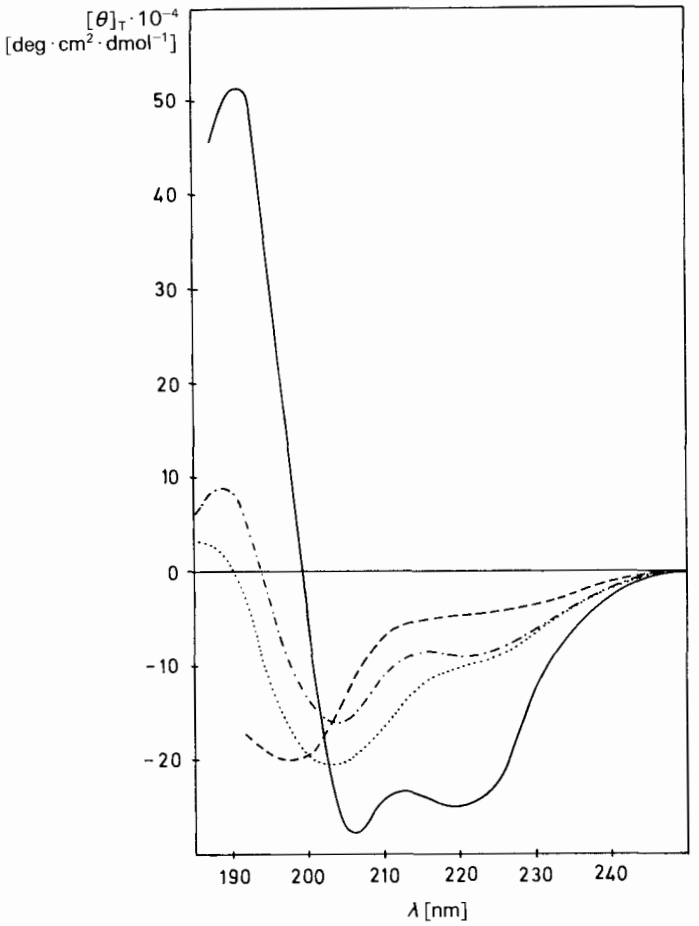


Fig. 2. *CD Spectra of peptides* **1** and **2** at 25° (c = 80 μM). **1** in 10 mM HCl, pH 2 (——); **2** in 10 mM HCl, pH 2 (- - -); **1** in 1 mM phosphate, pH 6.6 (- · - · -); **2** in 1 mM phosphate, pH 6.6 (· · · ·).

capacity of peptides **1** and **2** at pH 2 should mainly reflect a profoundly different *intrinsic* helical potential of the protonated forms of (*R*)- and (*S*)-Asp(2-Me), the former being a much superior helix former than the latter. This is further underscored by the observation that the degree of helicity of **1** in aqueous solution at pH 2 and 1° is in fact comparable to its helix content in the strongly helix-promoting solvent CF_3CH_2OH (*Fig. 3*), spectral data obtained in CF_3CH_2OH being often postulated to be characteristic for complete (100%) helix formation [19] [30–32].

Regarding the divergent pH dependence of the conformational properties of peptides **1** and **2**, this rather intriguing phenomenon deserves some special comment. Based on the orientation of the CH_2COOH side chain of (*R*)-Asp(2-Me) in a right handed helical structure, interactions with residues in position $i + 3$ (but not $i - 3$!) may be significantly more important than for (*S*)-α-amino acids. However, in peptide **1** at neutral pH, this would simply result in one more relevant attractive (*R*)-Asp(2-Me)^{13}/Lys^{16} and one more

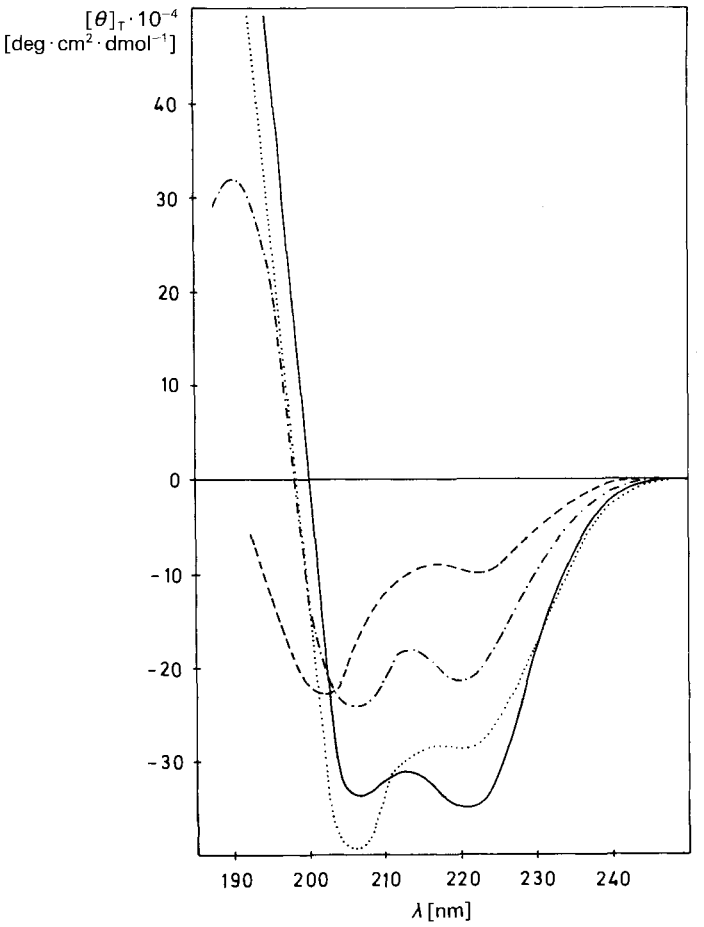


Fig. 3. *CD Spectra of peptide* **1** ($c = 40$ μM) *at 1° in* CF_3CH_2OH/H_2O *20:1* (· · · ·) *and of peptides* **1**–**3** ($c = 80$ μM) *at 1° in 10 mM HCl, pH 2.* **1** (——), **2** (- - -), **3** (- · - · -); for reasons of clarity, the spectrum of **4** at 1° and pH 2 is not shown (*cf. Table*).

relevant repulsive (*R*)-Asp(2-Me)[4]/Glu[7] charge-charge interaction (as compared to **2**); both interactions would be lost upon protonation of acidic side chains. It seems, therefore, unlikely that any unique charge effects that would be related to the orientation of the CH_2COOH side chain of (*R*)-Asp(2-Me) should be of major importance for the observed pH effect[8]). On the other hand, no significant difference should exist with respect to any charge-charge interactions between peptide **2** and peptides **3** and **4**, respectively (the orientation of the CH_2COOH side chain of (*S*)-Asp(2-Me) in a right-handed helical

[8]) It should be noted that any unique destabilizing charge effects involving the ionized side chain of (*R*)-Asp(2-Me) would lead to an *overestimation* of the difference between the helix-inducing potential of this amino acid in its protonated and its ionized state (and would thus offer a potential explanation for the dramatic increase in helicity at pH 2); however, at the same time, this would be equivalent to an *underestimation* of the *intrinsic* helical potential of (*R*)-Asp(2-Me) at neutral pH.

conformation of peptide **2** should resemble that of the L-Asp side chain in **3** and **4**). It, therefore, has to be concluded that the lower helicity of peptide **2** at acidic pH truly reflects a significant decrease in the *intrinsic* helical potential of the (*S*)-Asp(2-Me) residue upon side-chain protonation. As a result, protonated (*S*)-Asp(2-Me) exhibits an even lower helical potential than the protonated form of the parent amino acid L-Asp, which is contrary to the contention that α-methylation will generally result in an enhanced helical potential as compared to the corresponding parent amino acid [3]. The observed pH dependence of the helix-forming ability of (*S*)-Asp(2-Me) is also in strong contrast to the behaviour of the proteinogenic amino acids L-Glu [35] and L-Asp [16], whose intrinsic helical parameters significantly increase upon side-chain protonation (as observed for (*R*)-Asp(2-Me)).

About the reasons for the superior helix-inducing properties of the 'unnatural' (*R*)-isomer of Asp(2-Me) one can only speculate at this stage; however, it should be pointed out that (*R*)-Asp(2-Me) may be formally derived either from the unnatural D-isomer of aspartic acid or from the natural L-isomer of alanine (and *vice versa* for (*S*)-Asp(2-Me)). As was demonstrated in a number of recent studies [17] [31–33], L-Ala may be the best helix former among the naturally occurring amino acids, and L-Ala-based medium-sized peptides (15–20 amino-acid residues) generally exhibit an unusually high degree of helicity [30] [34], although the origin of this helix-stabilizing effect is not understood[9]). It is thus conceivable that α-alkylated derivatives of L-Ala (*i.e.*, the (*R*)-isomers of α-methylated derivatives of naturally occurring amino acids (except for the case of L-cysteine)) may generally be more helix-stabilizing than their corresponding enantiomers. In addition to the general restriction of the accessible conformational space due to α,α-bis-alkylation, the 'correct' orientation of the methyl side chain could be a crucial determinant for the helix-inducing potential of α-methyl-substituted α-amino acids. This notion is in agreement with conformational-energy calculations on Ac-(*R*)-Ala(2-Et)-NHMe [37] that suggest a right-handed helical conformation of this derivative to be *ca.* 4 kcal/mol more favourable than a left-handed one.

Obviously, the above hypothesis can be tested experimentally by investigating other enantiomeric pairs of α-methylated α-amino acids and other peptide sequences; studies along these lines are currently in progress in our laboratory.

Conclusions. – We have demonstrated for the first time that the conformational consequences caused by the incorporation of asymmetrically α,α-dialkyl-substituted α-amino acids in peptide sequences may strongly depend on the chirality of these monomers. In the present case, the (*R*)-enantiomer derived from the *nonnatural* D-enantiomer of aspartic acid by α-methylation displays a much higher helix-inducing potential than the (*S*)-enantiomer, which is derived from the *naturally* occurring L-aspartic acid. For the peptide sequences investigated in this study, the helix-inducing potential of (*R*)-2-methylaspartic acid clearly exceeds even that of 2-aminoisobutyric acid. In contrast, the helical potential of protonated (*S*)-2-methylaspartic acid is even *lower* than that of L-aspartic acid itself. We are presently investigating whether the findings described

[9]) No data are available on the intrinsic (right-handed!) helical potential of D-Ala in the background of a peptide sequence otherwise composed of L-amino acids. However, it was explicitly demonstrated for aspartic and glutamic acid that the D-isomers exhibit a significantly lower intrinsic (right-handed) helical potential than the natural L-isomers [16] [36].

in this report are representative for the conformational behaviour of enantiomeric 2-methylaspartic acids in general, and whether similar effects are displayed by pairs of enantiomers of other α-methyl-substituted α-amino acids.

Experimental Part

1. *General.* If not noted otherwise, all amino acids are of the (*S*)-configuration; protected derivatives of proteinogenic amino acids were purchased from *Novabiochem*, Läufelfingen, Switzerland, and *Bachem*, Bubendorf, Switzerland, and were generally used without further purification. Aminomethyl-[poly(styrene)/1% divinylbenzene] (NH_2CH_2-PS) and 2-{4-{(2′,4′-dimethoxyphenyl){{[(fluoren-9-yl)methoxy]carbonyl}amino}methyl} phenoxy}acetic acid ($(MeO)_2C_6H_4CH(FmocNH)$-$C_6H_4OCH_2COOH$) were from *Bachem*. The capacity of NH_2CH_2-PS was specified by the manufacturer to be 0.7 mmol/g and was not specifically verified. The 1*H*-benzotriazol-1-ol containing 12–17% of H_2O (HOBt · H_2O) was obtained from *Fluka* and used as such. Diisopropylcarbodiimide (DIC) was also from *Fluka*. Fmoc-Ala-Aib-OH (**9**) was synthesized by Dr. *R. Hersperger* [26]; Fmoc-Ala-OSu was prepared by standard procedures [38] and twice recrystallized from i-PrOH. O^4-(*tert*-Butyl) hydrogen (*R*)- and (*S*)-N^2-[(benzyloxy)carbonyl]-2-methylaspartate ((*R*)- and (*S*)-Z-Asp(OBu^t, 2Me)-OH; **5a** and **5b**, resp.) were synthesized according to [11]. Melting points (m.p.) are uncorrected. Flash chromatography (FC): silica gel *60* (230–400 mesh, 0.04–0.063 mm, *Merck*). Anal. HPLC: *Waters* HPLC system; gradient controller *600*, two pumps *510*, variable-wavelength UV detector *486*, data module *748*; 25 × 0.4 cm C_{18} (4 μm; reversed-phase) stainless-steel column from *Vydac*. Prep. HPLC: *Waters Prep LC 3000* system; UV detector *486* from *Waters*, one-pen chart recorder from *Kipp & Zonen*; 25 × 2.5 cm C_{18} (7 μm; reversed-phase) stainless-steel column from *Vydac*; solvent systems, 0.09% aq. CF_3COOH (*A*) and 0.09% CF_3COOH in MeCN/H_2O 9:1 (*B*); flow rates, 1 ml/min (anal.) and 18 ml/min (prep.); if not noted otherwise, detection at 210 (anal.) or 214 nm (prep.). ^{1}H-NMR Spectra: *Bruker-250-FT* (250 MHz) instrument. CI-MS: *Nermag-R-10-10C* mass spectrometer, University of Lausanne. High-resolution (HR) FAB-MS were obtained through the mass-spectral facility of the Ecole polytechnique fédérale at Lausanne (EPFL), and FAB-MS of peptides **1**–**4** were recorded at *Ciba-Geigy Ltd.*, Basel, Switzerland.

2. O^4-(tert-*Butyl) Hydrogen (*R*)-2-Methylaspartate ((*R*)-Asp(OBu^t, 2-Me)*; **6a**). To a soln. of **5a** (1.80 g, 5.37 mmol) in 55% aq. MeOH (110 ml), Pd-black (80 mg) was added and a slow stream of H_2 passed through the mixture for 3 h. Precipitated material was then redissolved by heating in a hot water-bath, the catalyst removed by filtration, and the filtrate evaporated. The solid white residue was twice reevaporated with MeOH and then suspended in Et_2O. The product was collected by filtration and dried under high vacuum over P_2O_5: **6a** (quant.). $[\alpha]_D^{25} = -28.4$ ($c = 0.65$, H_2O). ^{1}H-NMR (D_2O): 2.95 (*d*, 1 H, CH_2(3)); 2.73 (*d*, 1 H, CH_2(3)); 1.47 (*s*, Me–C(2)); 1.42 (*s*, *t*-Bu). CI-MS ($C_9H_{17}NO_4$ (203.238)): 204 (53, $[M + 1]^+$).

In the same way, **6b** was obtained. $[\alpha]_D^{25} = +29.5$ ($c = 0.61$, H_2O). All other anal. data are identical with those of **6a**.

3. {N^2-{*[(Fluoren-9-yl)methoxy]carbonyl*}L-*alanyl*}-[O^4-(tert-*Butyl) Hydrogen (*R*)-2-Methylaspartate] (Fmoc-Ala-(*R*)-Asp(OBu^t,2-Me)-OH*; **7a**). To a suspension of **6a** (0.902 g, 4.44 mmol) in CH_2Cl_2 (5 ml) under Ar, 1.21 ml (9.55 mmol) of Me_3SiCl were added. After 10 min heating to reflux, the resulting clear soln. was cooled in a cold water-bath, 1.60 ml (9.32 mmol) of (i-Pr)$_2$EtN were added, and the mixture was briefly brought to reflux (*ca.* 2 min). After recooling in a cold water-bath, 2.04 g (5.00 mmol) of Fmoc-Ala-OSu in CH_2Cl_2 (10 ml) were added, and the mixture was stirred under a gentle stream of Ar at r.t. for 65 h. The solvent was then evaporated, the residue distributed between Et_2O and sat. aq. $NaHCO_3$ soln. (pH *ca.* 8.5; 150 ml), the aq. layer washed twice with 50-ml portions of Et_2O, the combined Et_2O extract once reextracted with 50 ml of sat. aq. $NaHCO_3$ soln., and the combined aq. extract brought to pH 2.5 by addition of 1N HCl. The product was extracted into AcOEt (3 × 150 ml) and the combined org. extract washed with 5% $KHSO_4$ soln. (3 × 100 ml) and H_2O (3 × 100 ml) and dried (Na_2SO_4). Evaporation gave crude **7a** as a white solid that was purified by FC (CH_2Cl_2/AcOH 10:1) and then crystallized from AcOEt/pentane: 1.40 g (63%) of pure **7a**. TLC (toluene /AcOH 7:3): no Fmoc-Ala-OH present. HPLC (45% *B* → 65% *B* in 30 min, detection at 265 nm): no Fmoc-Ala-OH at 10.0 min (< 0.1%), t_R (**7a**) 16.8 min. M.p. 161–163°. $[\alpha]_D^{25} = -25.56$ ($c = 0.575$, MeOH). ^{1}H-NMR ((D$_6$)DMSO): 8.0–7.3 (*m*, 8 arom. H, 2 NH); 4.25 (*m*, H–C(9), CH_2–C(9) (Fmoc)); 4.0 (*m*, H–C(2) (Ala)); 2.85 (br. *s*, CH_2(3) (Asp(2-Me))); 1.50 (*s*, Me–C(2) (Asp(2-Me))); 1.40 (*s*, *t*-Bu); 1.20 (*d*, Me(3) (Ala)). HR-FAB-MS ($C_{27}H_{32}N_2O_7$): 497.2301 ($[M + H]^+$, calc. 497.2287).

By the same procedure, **7b** was obtained from **6b** in 51% yield. The preparation was completely free of Fmoc-Ala-OH (< 0.1%). M.p. 160–162°. $[\alpha]_D^{25} = -7.30$ ($c = 0.575$, MeOH). ^{1}H-NMR: identical with those of **7a**. HR-FAB-MS ($C_{27}H_{32}N_2O_7$): 497.2303 ([M + H]$^+$, calc 497.2287).

4. *Diastereoisomeric Purity of* **7a** *and* **7b**. Samples (*ca.* 1 mg) of **7a** and **7b** were dissolved in 200 µl of piperidine/DMF 1:1. After 30 min, the solvent was evaporated at 40° and the residue treated with Et_2O. After decantation of Et_2O and air drying, the dipeptides were redissolved in 1 ml of 0.09% aq. CF_3COOH, and 50 µl of these solns. were analyzed by anal. HPLC (10% *B* to 25% *B* in 25 min, detection at at 265 nm): t_R(H-Ala-(*R*)-Asp(OBu^t, 2-Me)-OH) 13.50 min, t_R(H-Ala-(*S*)-Asp(OBu^t, 2-Me)-OH) 14.85 min. The diastereoisomeric purity of each dipeptide was thus > 99%.

5. *{O^4-(*tert*-Butyl) N^2-{[(Fluoren-9-yl)methoxy]carbonyl}-L-aspartyl}-(2-aminoisobutyric Acid) (Fmoc-Asp(OBu^t)-Aib-OH*; **8**). A soln. of bis-silylated Aib in CH_2Cl_2 was prepared as described for **6a** in the synthesis of **7a** from 1.03 g (10 mmol) of Aib, 2.80 ml (22 mmol) of Me_3SiCl, and 2.20 ml (20 mmol) of *N*-methylmorpholine (NMM). This suspension (precipitate of NMM·HCl) was cooled to −20° and then added to a soln. of the preformed mixed anhydride of Fmoc-Asp(OBu^t)-OH and isobutyl hydrogencarbonate in THF (prepared from 2.06 g (5 mmol) of Fmoc-Asp(OBu^t)-OH and 0.69 ml of isobutyl chloroformate (5 mmol) in the presence of 0.56 ml (5 mmol) of NMM in 7 ml of THF at −20°, 5 min) at −25 to −15°. After 1 h at −20 to −15° and 1 h at −10 to −5°, the mixture was allowed to warm up to r.t., the solvent evaporated, and the residue distributed between AcOEt (100 ml) and 5% $KHSO_4$ soln. (100 ml). The org. layer was washed 3 times with 50-ml portions of 5% $KHSO_4$ soln. and then with H_2O, dried (Na_2SO_4), and evaporated. The residue was purified by FC (CH_2Cl_2/AcOH 16:1) and subsequently crystallized from Et_2O/pentane at −20°: 2.49 g (59%). Anal. HPLC (50% *B* → 60% *B* in 20 min, detection at 265 nm): t_R (**8**) 13.4 min, no Fmoc-Asp(OBu^t)-OH (< 0.5%) at 14.4 min. M.p. 81–83°. $[\alpha]_D^{25} = -12.02$ (*c* = 1.04, MeOH). ^{1}H-NMR ((D$_6$)DMSO): 8.05–7.30 (*m*, 8 arom. H, 2 NH); 4.35 (*m*, H–C(2) (Asp)); 4.20 (*m*, H–C(9), CH_2–C(9) (Fmoc)); 2.50 (*m*, overlapping with solvent signal, CH_2(3) (Asp)); 1.35 (*s*, *t*-Bu); 1.30, 1.32 (2*s*, 2 Me (Aib)). HR-FAB-MS ($C_{27}H_{32}N_2O_7$): 497.2293 ([*M* + H]$^+$, calc. 497.2287).

6. *{{2-{4-{(2′,4′-Dimethoxyphenyl){{[(fluoren-9-yl)methoxy]carbonyl}amino}methyl}phenoxy}acetamido}methyl}-[poly(styrene) 1% divinylbenzene]* (**10**). To 1.9 g of NH_2CH_2-PS (0.7 mmol/g, 1.33 mmol) was added a freshly prepared soln. of 1.51 g (2.8 mmol) of $(MeO)_2C_6H_4CH$(FmocNH)-$C_6H_4OCH_2COOH$, 0.473 g (2.88 mmol) of HOBt·H_2O, and 0.477 ml (3.08 mmol) of DIC in 20 ml of DMF/CH_2Cl_2 1:1. After 4.5 at r.t., the mixture was filtered and the resin washed with 30-ml portions of DMF (5 × 1 min) and then CH_2Cl_2 (2 × 1 min). After treatment with CH_2Cl_2/Ac_2O/pyridine 10:1:1 (24 ml) for 30 min at r.t., the resin was washed as before and dried: 2.54 g of **10** (97%).

7. *Solid-Phase Synthesis of Peptides* **1**–**4**. All syntheses were performed starting from *ca.* 500 mg of resin **10** (0.51 mmol Fmoc/g) and employing a Fmoc/*tert*-butyl protection scheme. Fmoc removal was achieved by double treatment (10 and 3 min) with 20% piperidine/DMF. Preformed HOBt esters of single amino-acid derivatives or protected dipeptides were used in the coupling reactions. For simple Fmoc-amino acids, the preactivation procedure involved the dissolution of 1 mmol of Fmoc-amino acid in 2.5 ml of 0.4M HOBt·H_2O in DMF (1.35 g of commercial HOBt·H_2O in 20 ml of DMF; this soln. was freshly prepared as soon as it became coloured), followed by the addition of 1 ml of 1.1M DIC in CH_2Cl_2 at r.t. After 10 min at r.t., this mixture was directly added to the deprotected resin pre-swollen in *ca.* 1.0–1.5 ml of DMF (if, as sometimes happened, a precipitate was formed in the preactivation mixture, it was filtered through cotton before addition to the resin). The standard coupling time was 1 h; if the semi-quantitative *Kaiser* test [39] indicated the presence of unreacted free amino groups after this period, the resin was either directly acetylated or a second 1 h coupling (with freshly prepared HOBt ester) was performed followed by acetylation. Acetylations were carried out with 5 ml of either CH_2Cl_2/Ac_2O/pyridine 8:1:1 (for peptide chain lengths < 10 amino-acid residues) or DMF/Ac_2O/pyridine 8:1:1 (chain lengths ≥ 10 residues) for 20 min. For **8** and **9**, preactivation was conducted at 40° for 25 min [40]. Double couplings (2 h, 1 h) were performed in both cases (only 0.75 mmol of HOBt ester were used in each coupling in the case of **8**). For couplings involving Asp(2-Me) dipeptides **7a** and **7b**, only 0.5 mmol of dipeptide were used, and preactivation was routinely carried out at 40° for 45 min. Double couplings and extended coupling times (*ca.* 8 h for each coupling) were necessary to achieve > 95% (estimated) incorporation.

Removal of peptides from the resin (with concurrent deprotection of side-chain functionalities) was achieved by treatment of 280–290 mg of protected peptide resin with a soln. of 800 mg of phenol in 15 ml of CF_3COOH for 2 h at r.t. under Ar. The red resin beads were then removed by filtration and washed with CF_3COOH (13 ml), the filtrate was evaporated at 30° and the oily residue treated with 60 ml of Et_2O. The precipitate thus obtained was spun down in a centrifuge and the Et_2O was removed by decantation. The peptide was washed with fresh Et_2O (3 × 60 ml) and then dried *in vacuo*. Yields of crude peptides: 118 mg (81%, based on loading of starting resin) of **1**; 115 mg (80%) of **2**; 113 mg (80%) of **3**; 115 mg (77%) of **4**. All peptides were purified by prep. HPLC; typically, 15–16 mg of material dissolved in *ca.* 1 ml of starting buffer were injected in a single run (**1**–**3**: 5% *B* → 35% *B* in 60 min; **4**: 5% *B* → 25% *B* in 40 min). Recoveries (on a weight basis) were between 70% (**4**) and 90% (**3**).

Anal. HPLC (**1**–**3**: 12% *B* → 27% *B* in 30 min; **4**: 10% *B* → 25% *B* in 30 min): All peptides were > 99.5% pure. FAB-MS: **1** ($C_{73}H_{118}H_{20}O_{26}$(1691.7)): 1692.4 ($[M + H]^+$); **2** ($C_{73}H_{118}N_{20}O_{26}$(1691.7)): 1692.4 ($[M + H]^+$); **3** ($C_{73}H_{118}N_{20}O_{26}$(1691.7)): 1692.1 ($[M + H]^+$); **4** ($C_{71}H_{114}N_{20}O_{26}$(1663.7)): 1664.2 ($[M + H]^+$).

CD Spectroscopy. CD Spectra: *Jobin-Yvon Mark VI* circular dichrometer, IsoDichro 1.1 software; calibration with an aq. (+)-camphorsulfonic acid soln. [41]; quartz cells of 0.05, 0.1, 1.0, or 2.0 cm pathlength in the wavelength range 250–185 nm; peptide concentrations, *ca.* 80 μM. Sample solns. were prepared by dilution of a stock soln. of the corresponding peptide in HPLC-grade H_2O (*ca.* 2 mg/ml) into the appropriate buffer. The peptide concentration of the stock solns. was accurately determined using tyrosine absorbance at 280 nm (ε 1280 $cm \cdot l \cdot mol^{-1}$ [41]). Where checked (all spectra at pH 6.6 and 25°, spectra of **2** at pH 2 and 25°. spectra of **1** at pH 2 and 1°), no differences were observed between spectra acquired at 8 μM and 80 μM peptide concentration. The spectra obtained for individual sample solns. were averaged over 3 runs; typically, the data presented in the *Table* and *Figs. 1–3* are averaged over 2 or 3 independent samples (*i.e.*, obtained by dilution of independently prepared stock solns.).

This work was supported by a grant of the *Swiss National Science Foundation* to *M.M.* We thank *F. Raschdorf, Ciba-Geigy Ltd.*, Basel, and Dr. *D. Stahl*, EPFL Lausanne, for effective and expeditious help in obtaining mass-spectral data.

REFERENCES

[1] M. Mutter, *Angew. Chem. Int. Ed.* **1985,** *24,* 639.
[2] a) E. T. Kaiser, F. J. Kézdy, *Science* **1984,** *223,* 249; b) J. W. Taylor, G. Ösapay, *Acc. Chem. Res.* **1990,** *23,* 338.
[3] W. F. DeGrado, *Adv. Protein Chem.* **1988,** *39,* 59.
[4] a) M. Mutter, S. Vuilleumier, *Angew. Chem. Int. Ed.* **1989,** *28,* 535; b) W. F. DeGrado, Z. A. Wasserman, J. D. Lear, *Science* **1989,** *243,* 622; c) I. L. Karle, J. L. Flippen-Anderson, K. Uma, M. Sukumar, P. Balaram, *J. Am. Chem. Soc.* **1990,** *112,* 9350; d) K.-H. Altmann, M. Mutter, *Int. J. Biochem.* **1990,** *22,* 947.
[5] M. Mutter, K.-H. Altmann, A. Flörsheimer, J. Herbert, *Helv. Chim. Acta* **1986,** *69,* 786.
[6] a) F. R. Carbone, B. S. Fox, R. H. Schwartz, Y. Paterson, *J. Immunol.* **1987,** *138,* 1838; b) I. L. Karle, J. L. Flippen-Anderson, K. Uma, P. Balaram, *Biochemistry* **1989,** *28,* 6696; c) W. F. DeGrado, J. D. Lear, *Biopolymers* **1990,** *29,* 205.
[7] a) D. S. Kemp, T. P. Curran, *Tetrahedron Lett.* **1988,** *29,* 4931; b) D. S. Kemp, T. P. Curran, *ibid.* **1988,** *29,* 4935; c) A. M. Felix, E. P. Heimer, C.-T. Wang, T. J. Lambros, A. Fournier, T. F. Mowles, S. Maines, R. M. Campbell, B. B. Wegrzynski, V. Toome, D. Fry, V. S. Madison, *Int. J. Pept. Protein Res.* **1988,** *32,* 441; d) F. Ruan, Y. Chen, P. B. Hopkins, *J. Am. Chem. Soc.* **1990,** *112,* 9403; e) M. R. Ghadiri, C. Choi, *ibid.* **1990,** *112,* 1630; f) M. Lieberman, T. Sasaki, *ibid.* **1991,** *113,* 1470; g) D. Y. Jackson, D. S. Kling, J. Chmielewski, S. Singh, P. G. Schultz, *ibid.* **1991,** *113,* 9391.
[8] a) G. Jung, N. Dubischar, D. Leibfritz, *Eur. J. Biochem.* **1975,** *54,* 395; b) Y. Paterson, S. M. Rumsey, E. Benedetti, G. Némethy, H. A. Scheraga, *J. Am. Chem. Soc.* **1981,** *103,* 2947; c) B. V. Venkataram Prasad, P. Balaram, *CRC Crit. Rev. Biochem.* **1984,** *16,* 307; d) R. Bosch, G. Jung, H. Schmitt, W. Winter, *Biopolymers* **1985,** *24,* 961; e) V. Pavone, B. Di Blasio, A. Santini, E. Benedetti, C. Pedone, C. Toniolo, M. Crisma, *J. Mol. Biol.* **1990,** *214,* 633; f) G. R. Marshall, E. E. Hodgkin, D. A. Langs, G. D. Smith, J. Zabrocki, M. T. Leplawy, *Proc. Natl. Acad. Sci. U.S.A.* **1990,** *87,*487; g) E. E. Hodgkin, J. D. Clark, K. R. Miller, G. R. Marshall, *Biopolymers* **1990,** *30,* 533.
[9] C. Toniolo, G. M. Bonora, A. Bavoso, E. Benedetti, B. Di Blasio, V. Pavone, C. Pedone, V. Barone, F. Lelj, M. T. Leplawy, V. Kaczmarek, A. Redlinski, *Biopolymers* **1988,** *27,* 373, and ref. cit. therein.
[10] *Cf., e.g.*, a) U. Schöllkopf, *Topics Curr. Chem.* **1983,** *109,* 65; b) K. Weinges, H. Brachmann, P. Stahnecker, H. Rodewald, M. Nixdorf, H. Irngartinger, *Liebigs Ann. Chem.* **1985,** 566; c) D. Seebach, R. Imwinkelried, T. Weber, in 'Modern Synthetic Methods', Ed. R. Scheffold, Springer Verlag, Heidelberg, 1986, Vol. 4, pp. 125–259; d) K. Nebel, M. Mutter, *Tetrahedron* **1988,** *44,* 4793; e) R. M. Williams, in 'Synthesis of Optically Active Amino Acids', Eds. J. E. Baldwin and P. D. Magnus, Pergamon Press, Oxford, 1989; f) D. Seebach, E. Dziadulewicz, L. Behrendt, S. Cantoreggi, R. Fitzi, *Liebigs Ann. Chem.* **1989,** 1215.
[11] E. Altmann, K. Nebel, M. Mutter, *Helv. Chem. Acta* **1991,** *74,* 800.
[12] E. Altmann, K.-H. Altmann, K. Nebel, M. Mutter, *Int. J. Pept. Protein Res.* **1988,** *32,* 344.

[13] G. Valle, M. Crisma, C. Toniolo, R. Beisswenger, A. Rieker, G. Jung, *J. Am. Chem. Soc.* **1989,** *111,* 6828.
[14] G. R. Marshall, J. D. Clark, J. B. Dunbar, Jr., G. D. Smith, J. Zabrocki, A. S. Redlinski, M. T. Leplawy, *Int. J. Pept. Protein Res.* **1988,** *32,* 544.
[15] K. Nebel, E. Altmann, M. Mutter, R. Bardi, A. M. Piazesi, M. Crisma, G. M. Bonora, C. Toniolo, *Biopolymers* **1991,** *31,* 1135.
[16] Y. Kobayashi, F. Cardinaux, B. O. Zweifel, H. A. Scheraga, *Macromolecules* **1977,** *10,* 1271.
[17] K. T. O'Neil, W. F. DeGrado, *Science* **1990,** *250,* 646.
[18] IUPAC-IUB, 'Nomenclature and Symbolism for Amino Acids and Peptides (Recommendations 1983)', *Pure Appl. Chem.* **1984,** *56,* 595.
[19] a) S. Marqusee, R. L. Baldwin, *Proc. Natl. Acad. Sci. U.S.A.* **1987,** *84,* 8898; b) G. Merutka, E. Stellwagen, *Biochemistry* **1991,** *30,* 1591.
[20] G. Barany, R. B. Merrifield, in 'Peptides – Analysis, Synthesis, Biology', Eds. E. Gross and J. Meienhofer, Academic Press, New York, 1980, Vol. 2, pp. 1–284.
[21] G. B. Fields, R. L. Noble, *Int. J. Pept. Protein Res.* **1990,** *35,* 161.
[22] W. König, R. Geiger, *Chem. Ber.* **1970,** *103,* 788.
[23] D. Hudson, *J. Org. Chem.* **1988,** *53,* 617.
[24] M. T. Leplawy, D. S. Jones, G. W. Kenner, R. C. Sheppard, *Tetrahedron* **1960,** *11,* 39.
[25] H. R. Kricheldorf, *Liebigs Ann. Chem.* **1972,** *763,* 17.
[26] R. Hersperger, Ph. D. Thesis, University of Basel, 1989.
[27] G. Ebert, *Topics Curr. Chem.* **1985,** *128,* 1.
[28] a) T. S. Sudha, E. K. S. Vijayakumar, P. Balaram, *Int. J. Pept. Protein Res.* **1983,** *22,* 464; b) E. K. S. Vijayakumar, T. S. Sudha, P. Balaram, *Biopolymers* **1984,** *23,* 877.
[29] N. Greenfield, G. D. Fasman, *Biochemistry* **1969,** *8,* 4108.
[30] a) S. Marqusee, V. H. Robbins, R. L. Baldwin, *Proc. Natl. Acad. Sci. U.S.A.* **1989,** *86,* 5286; b) S. Padmanabhan, S. Marqusee, T. Ridgeway, T. M. Laue, R. L. Baldwin, *Nature (London)* **1990,** *344,* 268.
[31] P. C. Lyu, M. I. Liff, L. A. Marky, N. R. Kallenbach, *Science* **1990,** *250,* 669.
[32] G. Merutka, W. Lipton, W. Shalongo, S.-H. Park, E. Stellwagen, *Biochemistry* **1990,** *29,* 7511.
[33] J. M. Scholtz, S. Marqusee, R. L. Baldwin, E. J. York, J. M. Stewart, M. Santoro, D. W. Bolen, *Proc. Natl. Acad. Sci. U.S.A.* **1991,** *88,* 2854.
[34] J. M. Scholtz, E. J. York, J. M. Stewart, R. L. Baldwin, *J. Am. Chem. Soc.* **1991,** *113,* 5102.
[35] F. R. Maxfield, J. E. Alter, G. T. Taylor, H. A. Scheraga, *Macromolecules* **1975,** *8,* 479.
[36] K.-H. Altmann, J. Wójcik, M. Vásquez, H. A. Scheraga, *Biopolymers* **1990,** *30,* 107.
[37] E. Benedetti, C. Toniolo, P. Hardy, V. Barone, A. Bavoso, B. Di Blasio, P. Grimaldi, F. Lelj, V. Pavone, C. Pedone, G. M. Bonora, I. Lingham, *J. Am. Chem. Soc.* **1984,** *106,* 8146.
[38] G. W. Anderson, J. E. Zimmerman, F. M. Callahan, *J. Am. Chem. Soc.* **1964,** *86,* 1839.
[39] E. Kaiser, R. L. Colescott, C. D. Bossinger, P. I. Cook, *Anal. Biochem.* **1970,** *34,* 595.
[40] H. Schmitt, G. Jung, *Liebigs Ann. Chem.* **1985,** 321.
[41] W. C. Johnson, *Proteins* **1990,** *7,* 205.