Solid-Phase Synthesis of a β^3-Icosapeptide Containing the Homologs of the Twenty Common Proteinaceous Amino Acids

Preliminary Communication

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An icosapeptide, **1**, containing the β^3-amino acid residues with the 20 proteinogenic side chains has been assembled by manual solid-phase synthesis, according to the Fmoc strategy. The sequence was chosen in such a way that a possible 3_{14}-helical conformation (secondary structure) would be stabilized by salt bridges and have an amphipathic character (*Fig. 1,a*), and the N-terminal β^3hCys would lend itself to thioligations and disulfide formation (**2** and **3**, in *Figs. 1* and *2*). The products **1**–**3** were pure according to RP-HPLC, NMR, and MS analysis (*Fig. 1,b* and *c*, *Fig. 2,c* and *d*, and *Fig. 3*). With due caution, the CD spectra in aqueous solution (pH 7) and in MeOH (*Fig. 4*), with normalized *Cotton* effects $\theta = -14000$ to -16000 [deg · cm^2 · dmol^{-1}] between 209 and 210 nm, might be taken as an evidence for the presence of 3_{14}-helical conformations. An evaluation of the data from a 700-MHz 2D-NMR measurement of the disulfide **2** in CD_3OH is in progress.

The solution structures of longer β-peptides containing more than twelve residues [1] has, so far, been deduced only from CD spectra. These, in turn, have as yet been more confusing than illuminating [2–5][2]). We, therefore, consider the structure determination of β-peptides by NMR solution analysis as the only reliable method at this stage. Signal assigment in the NMR spectrum is facilitated by different β-amino acids in the sequence, and we considered it as a challenge to synthesize, on solid phase, a β^3-icosapeptide **1** with the 20 side chains of the common natural ribosomal amino acids[3])[4]).

Furthermore, the new oligomer will allow us to probe the stability of the 3_{14} helix as a function of chain length: a normal[5]) α-peptidic helix has a dipole moment that increases with increasing chain length, leading to destabilization of this secondary-

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1) Part of the projected Ph.D. thesis of *T. K.*, ETH-Zürich.

2) It is generally accepted that the CD spectra of 3_{14}-helical β-peptides built of homologated proteinaceous β^3-amino acids show a negative *Cotton* effect near 215 nm ($\theta \geq 15000$ deg · cm^2 · dmol^{-1}, normalized), a zero-crossing near 205, and a rise to large positive θ values at shorter wavelengths, as first reported by us in 1996 [6]. The positive, short-wavelength part is missing in the spectrum of *Gellman*'s 2-aminocyclohexane carboxylic acid oligomers, (see the Table in [2a] and refs. cit. therein). On the other hand, there are β-peptides that cannot possibly form a 3_{14} helix and still exhibit the characteristic trough near 215 nm in the CD spectrum [2a].

3) There are 22 of them by now [7].

4) The plan was actually emerged during a discussion with *Kurt Wüthrich*, who complained that there were too many β^3hPhe units in a β-peptide of which his group became involved in determining the NMR solution structure.

5) This is not true of the *12/10* β^2/β^3 helix [6c][8], which has no resulting dipole moment (macrodipole).

structural element[6]). In an α-peptidic 3.6_{13} helix, the macrodipole $\oplus \rightarrow \ominus$ points from the N- to the C-terminus, and there is additional destabilization by $\oplus$-pole$-{}^{\oplus}NH_3$ and $\ominus$-pole$-{}^{\ominus}O_2C$ charge repulsion. The β^3-peptidic 3_{14} helix has a macrodipole of opposite direction, pointing $\oplus \rightarrow \ominus$ from the C- to the N-terminus, and this leads to a charge–pole stabilizing effect: the helix stability in solution increases when the *termini* of the β^3-peptide are deprotected [3a][6b,c]. Thus, the question arises, up to what chain-length range can β^3-peptidic helices be observed in H_2O or MeOH solution, with or without salt-bridge or capping stabilization[2][6][7]).

The sequence of the 20 β^3-amino acids in the peptide **1** (*Fig. 1*) was chosen such that the 3_{14}-helical conformation would be amphipathic, with one streak of side chains being hydrophobic, and two streaks containing polar side chains and a salt bridge each; furthermore, we chose an N-terminal β^3hCys for possible coupling with other peptides by thioligation [3d] (see formula in *Fig. 1* and helical-wheel-type presentation in *Fig. 1,a*).

The Fmoc-β^3-amino acids with *t*-Bu (Asp, Glu, Ser, Thr, Tyr), Boc (Lys, Trp), Trt (Asn, Gln, His), or Pmc (Arg) protection of the side-chain functionalities were used as building blocks. Of these, all but Fmoc-β^3hTrp(Boc)-OH [12], Boc-β^3hCys(Trt)-OH, and Fmoc-β^3hHis(Trt)-OH are commercial[8][9]). The β^3hCys derivative was obtained from the previously described Boc-β^3hCys(Bn)-OH [13] by change of the side-chain protecting group (*Birch* reduction followed by tritylation). The histidine derivative was prepared by an eight-step synthesis (starting from Boc-His(Tos)-OH), which will be published elsewhere[10]).

For the construction of the icosapeptide **1**, we used manual solid-phase synthesis on *Wang* resin (300 mg), with HATU as coupling reagent (two times with 3 equiv. of Fmoc-β^3-amino acid, 'double couple', for at least 1 h each) and piperidine/DBU for Fmoc deprotection (four times for 10 min each)[11]). Removal from the resin with deprotection of the side chains and of the Boc-protected N-terminus was carried out with CF_3CO_2H/i-Pr_3SiH/$(HSCH_2)_2$/H_2O to give the crude product **1**, which was purified by multiple preparative RP-HPLC (a total of 25 mg of **1** was isolated)[12]). The coupling procedures required 110 h altogether, and the purification two weeks! To avoid inadvertent disulfide formation on contact with air, we prepared the mixed disulfide **2** (*cf. Fig. 1*) by treatment with methyl methanethiosulfonate (MMTS). We also

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[6]) The macrodipole of an α-peptidic 3.6_{13} helix is given by an increment in dipole moment of 3.2–3.4 D per amino acid [9a]. Natural α-peptide helices are often 'capped' by having negatively charged side chains near the positive end of the dipole and *vice versa* [9b].

[7]) Helices of β^3-heptapeptides with Orn-Glu or Arg-Glu salt-bridge stabilization, as determined by NMR analysis [10], and of a tetradecamer with salt bridges and terminal capping, as deduced from CD spectra [11], have been described.

[8]) Suppliers are, for instance, *Fluka AG* (CH-Buchs) and *PeptTech Corp.* (Cambridge, USA).

[9]) We gratefully acknowledge discount prices by *Fluka AG.*

[10]) *G. Lelais* and *T. Kimmerlin*, hitherto unpublished results, ETH-Zürich, 2001–2003.

[11]) For generally accepted abbreviations in peptide and protein chemistry, see [14].

[12]) The procedures for coupling, for following the progress of each coupling step, for the Fmoc deprotection, and for the removal of the peptide from the resin were taken from the arsenal of the α-peptide synthesis [15].

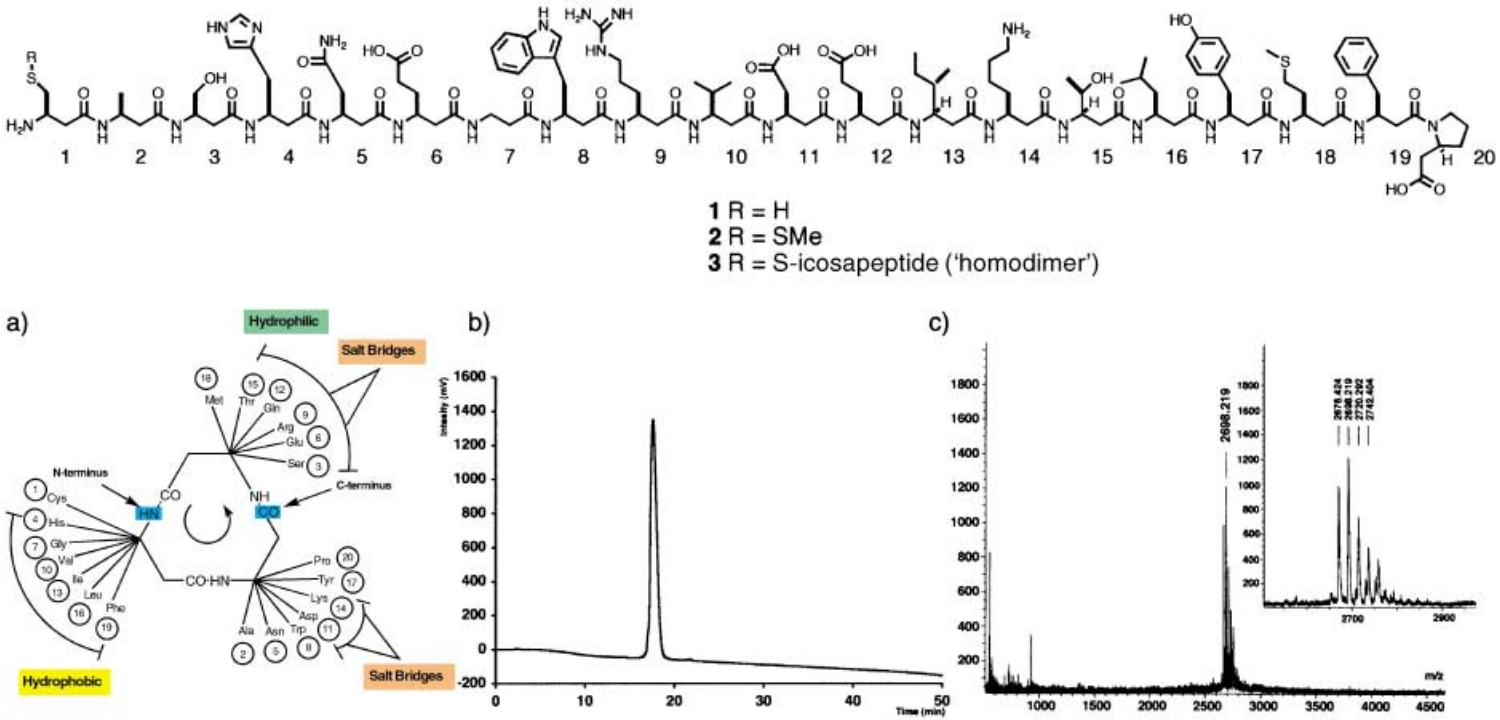

Fig. 1. *Formula, schematic view of a hypothetical 3_{14}-helical conformation, RP-HPLC traces, and mass spectrum of the β^3-icosapeptide* **1**. The molecular formulae of β^3-icosapeptide **1**, the mixed disulfide **2**, and the 'homodimer' **3** are shown. *a*) Schematic presentation along the axis of an idealized 3_{14}-helical secondary structure of **1**. *b*) Analytical-HPLC profile of purified **1** (RP-HPLC C_8 column, linear gradient of 0.1% TFA in H_2O and MeCN). *c*) MALDI-TOF Mass spectrum of **1** with multiply charged Na^+ ion peaks; molecular weight of **1**: 2675.4 Da.

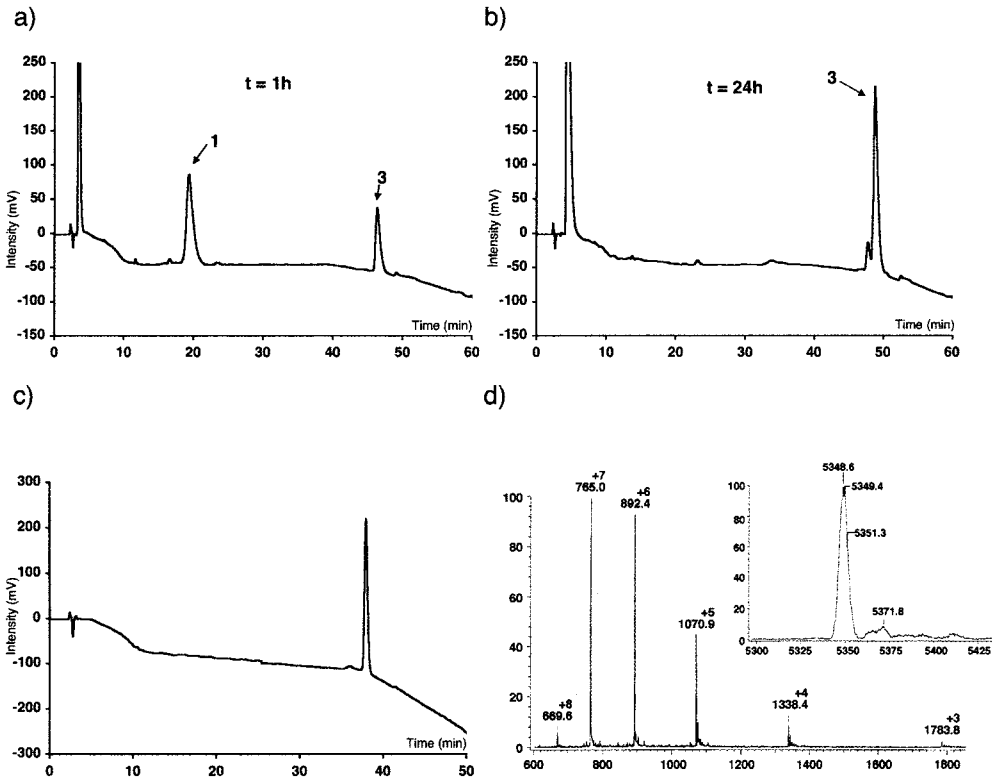

Fig. 2. *Disulfide formation*. Analytical RP-HPLC chromatogram of the oxidation reaction at *a*) $t = 1$ h, *b*) $t =$ 24 h. *c*) Analytical RP-HPLC chromatogram of the purified 'homodimer' **3** (RP-HPLC C_8 column, linear gradient of 0.1% TFA in H_2O and MeCN). *d*) Electrospray mass spectrum of **3** with multiply (positively) charged ion peaks and with the molecular-ion peak; molecular weight of **3**: 5348.8 Da.

dimerized the icosamer **1** to the disulfide **3** by stirring it in aqueous solution containing 10% DMSO (*Fig. 2*).

All three compounds were purified by RP-HPLC and identified by mass spectrometry (MALDI-TOF or ESI, see *Figs. 1* and *2*). The NMR spectrum of the icosapeptide derivative **2** is well-resolved; the chemical shifts of the backbone CONH H-atoms are spread over a range of *ca.* 2 ppm (*Fig. 3*), a promising feature for the ongoing interpretation of a 700-MHz 2D-NMR spectrum.

The CD spectra of the disulfides **2** and **3** in MeOH and in aqueous buffer (pH 7) solution are shown in *Fig. 4*; all of them exhibit the negative *Cotton* effect ($\theta = -10000$ to -16000 [deg · cm^2 · dmol^{-1}] per amino acid, *i.e.*, normalized) between 210 and 215 nm, which is considered characteristic of a 3_{14}-helical conformation[2]). On the other hand, only with the aqueous solution of the monomeric mixed disulfide **2** do we observe the likewise 3_{14}-helix-typical steep rise to large positive values near 200 nm in the CD spectrum (*Fig. 4,b*, red curve). As with other large β^3-peptides of as yet unknown structure prepared by us previously [3d,e], there is hardly any zero crossing in the CD spectra of the dimer **3** measured in MeOH and in H_2O solutions (black curves in

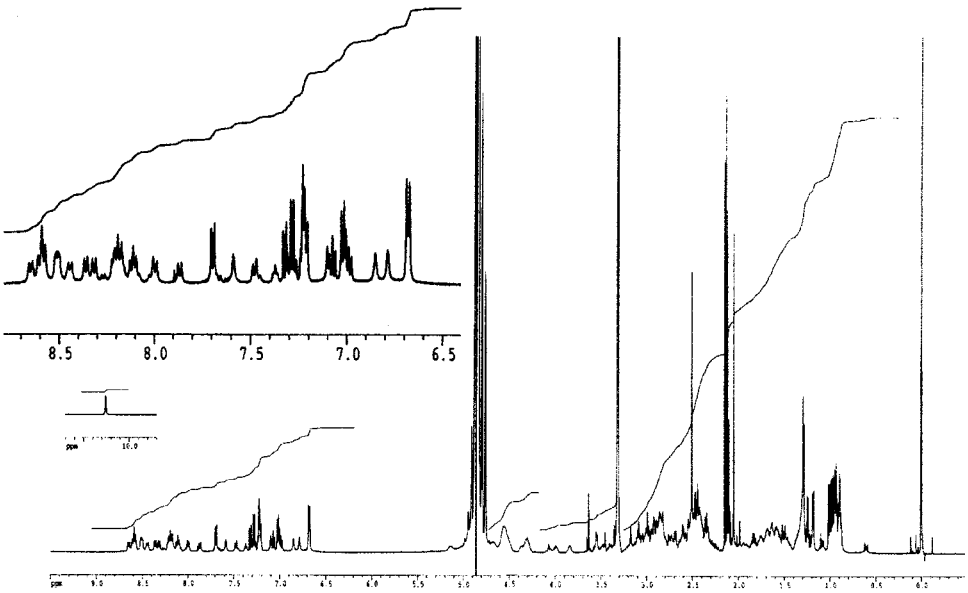

Fig. 3. *1D ^{1}H-NMR* (500 MHz) *spectrum of the β^3-icosapeptide derivative* **2**. Low-field region of the ^{1}H-NMR spectrum of **2** in CD_3OH, showing the NH and the aromatic H-atoms.

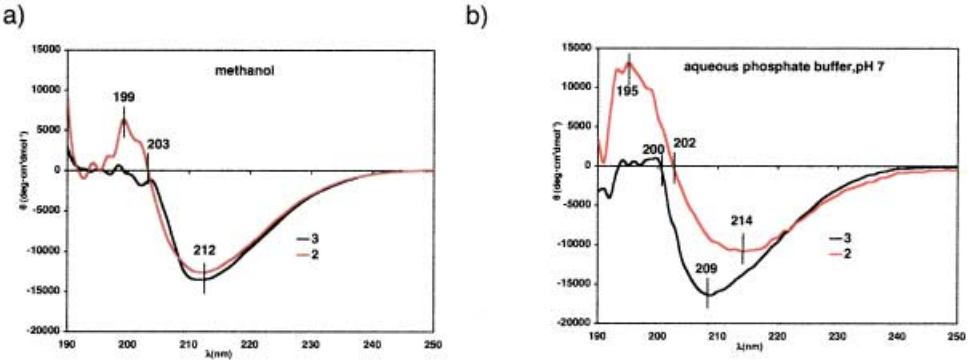

Fig. 4. *Normalized CD spectra* a) *in MeOH and* b) *in buffer of the β^3-icosapeptide derivative* **2** *and of the 'homodimer'* **3**. The spectra were recorded at 20° at a concentration of 0.2 mM. The peptides were used as the TFA salts as obtained after lyophilization.

Fig. 4,a and *b*). Only a full NMR investigation will enable us to assign structures to the new compounds described herein.

The synthesis of the β^3-icosapeptide **1** constitutes a demonstration that any β^3-peptide with the proteinaceous side chains will be accessible, and the NMR solution-structure elucidation of **2** will hopefully shed light on how some of the *miraculous* [3c] spectra of longer β^3-peptides ought to be interpreted [4].

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