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β-Depsipeptides—the effect of a missing and a weakened hydrogen bond on the stability of the β-peptidic 3_{14}-helix†

Dieter Seebach,* Yogesh R. Mahajan‡, Ramanathan Senthilkumar, Magnus Rueping‡ and Bernhard Jaun*

Laboratorium für Organische Chemie der Eidgenössischen Technischen Hochschule, ETH-Hönggerberg, Wolfgang-Pauli-Strasse 10, CH-8093 Zürich, Switzerland. E-mail: seebach@org.chem.ethz.ch; Fax: +4116321144; Tel: +416322990

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The importance of hydrogen bonding in β-peptide 3_{14}-helices is demonstrated by an NMR analysis of three β-heptadepsipeptides containing a 3-hydroxybutanoic residue in position 2, 4 or 6.

Depsipeptides, *i.e.* peptides containing hydroxy acid residues, occur as natural products[1] and have also been synthesized to study the local folding propensities of peptides[2] and proteins.[3] We wondered what the effect of a missing NH-group in a β-peptide would be. We chose sequences of six β[3]-amino acids, with aliphatic side chains, which we know form a 3_{14}-helix in MeOH,[4] and inserted (*S*)-3-hydroxybutanoic acid (**HB**) residues, in the 2, 4 and 6 positions, see **1**–**3** (Fig. 1, left). The Boc-protected methyl ester of a β-heptadepsipeptide, with a central **HB** unit (*cf.* **2**) had been synthesized before and did not show the CD pattern characteristic of a 3_{14}-helix;[5] on the other hand, we know that fully deprotected β-peptides form more stable helices than the terminally protected derivatives;[6] we have also demonstrated that a β-hexadepside (consisting of six β-hydroxy acid residues) does not fold to a preferred conformation.[7]

The building blocks **4**–**19** for the construction of **1**–**3** are shown in Fig. 1, right. The depsipeptides were synthesized in solution using Boc-protection[4] and/or Z-protection. DCC/DMAP or EDC/DMAP coupling conditions were used for the ester bond formation and EDC/HOBt for the amide bonds. The fully protected β-heptadepsipeptide **1** was prepared from the β-tetradepsipeptide **18** (a coupling product of β-dipeptides **9** and **10**) and the β-tripeptide ester **15**, which was obtained from Boc-β-HAla-OH and **9**. Deprotection of the *C*-terminus by hydrogenolysis and of the *N*-terminus by treatment with trifluoroacetic acid gave the β-heptadepsipeptide **1**. The required building blocks **9** and **10** were prepared from Boc-β-HVal-OH and benzyl (*S*)-3-hydroxybutanoate **5**,[5,8] respectively. β-Tetradepsipeptide **17** was assembled from the Z-β-tripeptide **14** and *tert*-butyl (*S*)-3-hydroxybutanoate **4**.[9] Subsequent acidic deprotection of **17** and coupling with the β-tripeptide ester **16**, gave the fully protected β-heptadepsipeptide, which was deprotected at both ends by hydrogenolysis to give **2**. The required β-tripeptides **14** and **16** were prepared from *N*-Z protected dipeptide **6** and *N*-Boc protected dipeptide **8**. Finally, the protected β-heptadepsipeptide **3** was constructed from benzyl (*S*)-3-hydroxybutanoate **5**, Boc-β-HLeu-OH (→**11**), H-β-HVal-OBn (→**13**) and the tetrapeptide **19** (from **7** and **12**). Hydrogenolysis followed by treatment with trifluoroacetic acid gave **3**.

The β-heptadepsipeptides **1**–**3** were purified by reversed-phase HPLC and isolated as the trifluoroacetate salts, for which we obtained the correct high-resolution mass spectra. The CD spectra are shown in Fig. 2. All three β-depsipeptides display the characteristic CD pattern of a 3_{14}-helical structure in methanol.[6] Judging from the intensity of the negative *Cotton* effect near 215 nm, we conclude that the helix content in solution of **1** and **3**, with the **HB** unit incorporated at the 2 and 6 positions, is high, while the compound **2** with central **HB** unit shows only weak *Cotton* effects.

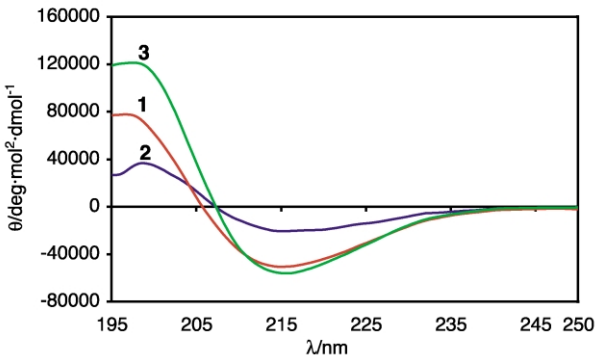
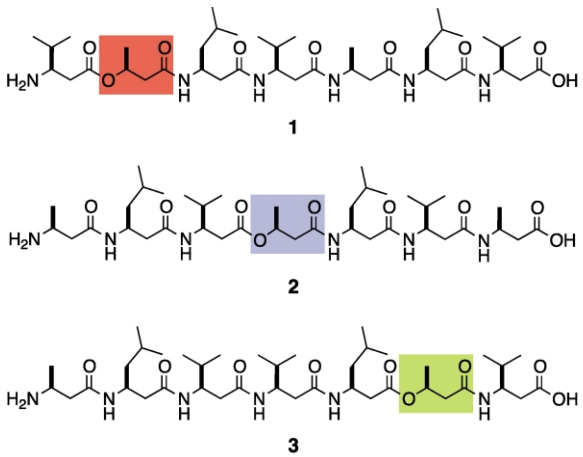


Fig. 2 CD-Spectra of **1**–**3** in methanol solution (all measurements were carried out with 0.2 mM solutions). The minimum near 215 nm is considered to be characteristic of an (*M*) 3_{14}-helical structure.

† Electronic supplementary information (ESI) available: NMR and NOE data. See http://www.rsc.org/suppdata/cc/b2/b204187c/



4 H-HB-O^{t}Bu
5 H-HB-OBn
6 Z-β-HAla-β-HLeu-OH
7 Boc-β-HAla-β-HLeu-OH
8 Boc-β-HLeu-β-HVal-OH
9 H-β-HLeu-β-HVal-OBn
10 Boc-β-HVal-HB-OH
11 Boc-β-HLeu-HB-OH
12 H-β-HVal-β-HVal-OMe
13 H-β-HLeu-HB-β-HVal-OBn
14 Z-β-HAla-β-HLeu-β-HVal-OH
15 H-β-HAla-β-HLeu-β-HVal-OBn
16 H-β-HLeu-β-HVal-β-HAla-OBn
17 Z-β-HAla-β-HLeu-β-HVal-HB-OH
18 Boc-β-HVal-HB-β-HLeu-β-HVal-OH
19 Boc-β-HAla-β-HLeu-β-HVal-β-HVal-OH

Fig. 1 Molecular formulae of the β-heptadepsipeptides **1**–**3** and the building blocks used for their synthesis.

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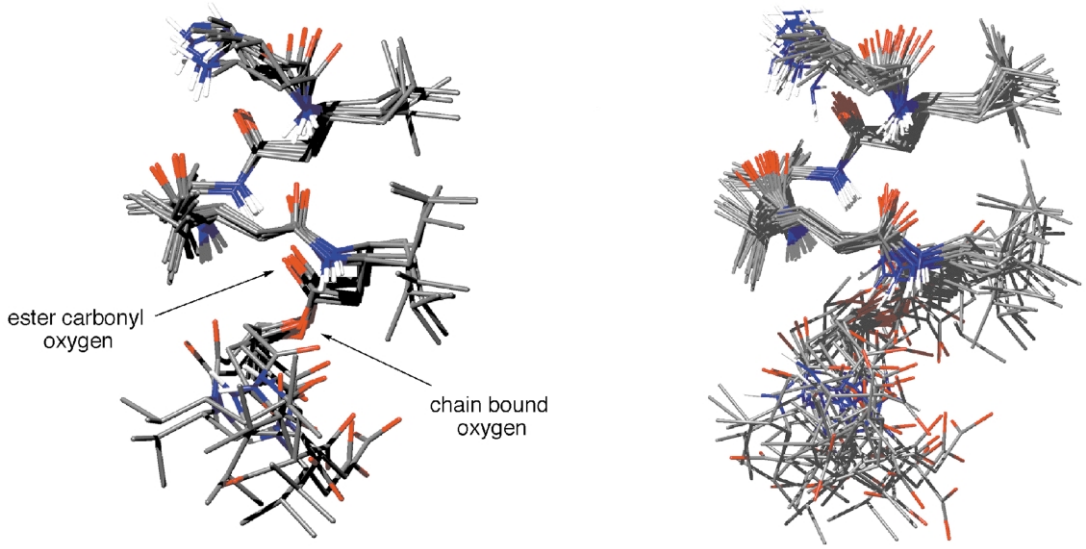

Fig. 3 Solution structure of the β-heptadepsipeptide **3** in methanol, represented as a bundle of 5 and 25 lowest-energy structures obtained by simulated annealing, using NMR-derived dihedral angles and NOE-distance restraints.

In order to ascertain whether the observations from the CD measurements are in agreement with the NMR solution structures, β-depsipeptides **1**–**3** have been examined by means of high-resolution NMR techniques. 2D-NMR Studies were carried out in MeOH solutions. DQF-COSY and TOCSY techniques were used to assign all ^{1}H resonances, and HSQC and HMBC experiments led to the assignment of the sequences. From the large ^3J(NH, C(β)-H) coupling constants it can be concluded that the NH and the C(β)-H protons are in an antiperiplanar arrangement. The diastereotopic CH_2(α) protons were assigned by assuming that in a 3_{14}-helix, the axial protons exhibit a large and the lateral a small coupling with H-C(β), as evident from the cross peak volume in the COSY spectra. This is in agreement with stronger NOEs from H-C(β) to the lateral H-C(α) protons than to the axial H-C(α) protons, and with stronger NOEs from NH_{i+1} to the axial H-C(α)$_i$ protons.[10] ROESY Spectra were acquired at different mixing times (150, 300 ms) for all three peptides. Qualitative analysis revealed that NOEs typical for a 3_{14}-helix are present in the ROESY spectra of β-depsipeptides **1**–**3**. However, these NOEs are only observed for residues 3–7 for **1**, residues 5–6 for **2** and residues 1–5 for **3**. Moreover, for β-depsipeptides **1** and **2** a second set of weak NOEs from NH_i to H-C(β)$_{i-1}$ (i = 3–7 for **1**, i = 5–6 for **2**) is present, that is not compatible with a 3_{14}-helix. A short distance between NH_i and H-C(β)$_{i-1}$ is only possible, if the dihedral backbone angle around the H_2C(α)/C=O bond is in the synclinal range as opposed to the anticlinal conformation in the 3_{14}-helix. This type of NOE has been observed in β-peptides before,[10] and it indicates that no single conformer of **1** and **2** is consistent with all observed NOEs. Hence, other conformations besides the regular 3_{14}-helix must be populated. The incorporation of the β-hydroxy acid residue leads to a loss of a hydrogen bond and weakening of another and thereby destabilizes the secondary structure. This effect is less relevant for β-depsipeptide **3**, since the ester bond is placed near the *C*-terminus where it is not involved in further hydrogen-bonding, and it suggests that **3** forms the most stable helix of the three depsipeptides. Indeed, the simulated annealing calculation using the NOE data and coupling-constant-derived distance and torsion angle constraints provided a 3_{14}-helical structure. This calculation yielded a set of 25 structures of which bundles of 5 and 25 lowest energy confomers are displayed in Fig. 3. The structures show a left-handed 3_{14}-helix which is well defined for residues 1–5, but less defined at the *C*-termini. This might be due to the decreased hydrogen-acceptor ability and lower rotational barrier (~10–13 kcal mol^{-1}) around the ester C(O)–O bond, compared to the amide bond (~18–22 kcal mol^{-1}).

Interestingly, the CD measurements initially indicated that β-depsipeptides **2** and **3** adopt an equally stable 3_{14}-helix. This in contrast to the results of the NMR investigations, which illustrate that β-depsipeptide **3** forms a complete 3_{14}-helix, while **1** and **2** are only partially folded. This observation confirms again that CD spectroscopy is not a conclusive tool for determining β-peptidic secondary structures and is certainly not able to give information about the stability and population of a helix.

In conclusion an (*S*)-3-hydroxybutanoate residue incorporated in positions 2, 4 or 6 of a β-heptapeptide (consisting of L-β^3-amino acids with the side chains of Val, Ala, and Leu) destabilizes the 3_{14}-helical structure. NMR Analysis reveals that only the β-heptadepsipeptide **3** with the ester bond next to the *C*-terminus exhibits two turns of a 3_{14}-helix in MeOH solution, demonstrating that hydrogen bonding is more important in stabilizing β-peptidic helices than the β-amino acid residue's backbone.[11]§

Notes and references

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1 M. M. Schemjakin, *Angew. Chem.*, 1960, **72**, 342.
2 G. Wouters, R. Katakai, W. J. Becktel and M. Goodman, *Macromolecules*, 1982, **15**, 31 and references cited therein; I. L. Karle, C. Das and P. Balaram, *Biopolymers*, 2001, **59**, 276; T. Ohyama, H. Oku, M. Yoshida and R. Katakai, *Biopolymers*, 2001, **58**, 636 and references cited therein.
3 J. T. Koh, V. W. Cornish and P. G. Schultz, *Biochemistry*, 1997, **36**, 11314; W-Y. Lu, M. A. Starovasnik, J. J. Dwyer, A. K. Kossiakoff, S. B. H. Kent and W. Lu, *Biochemistry*, 2000, **39**, 3575; G. S. Beligere and P. E. Dawson, *J. Am. Chem. Soc.*, 2000, **122**, 12079.
4 D. Seebach, M. Overhand, F. N. M. Künhle, B. Martinoni, L. Oberer, U. Hommel and H. Widmer, *Helv. Chim. Acta*, 1996, **79**, 913.
5 D. Seebach, P. E. Ciceri, M. Overhand, B. Jaun, D. Rigo, L. Oberer, U. Hommel, R. Amstutz and H. Widmer, *Helv. Chim. Acta*, 1996, **79**, 2043.
6 D. Seebach, J. V. Schreiber, S. Abele, X. Daura and W. F. van Gunsteren, *Helv. Chim. Acta*, 2000, **83**, 34.
7 M. Albert, D. Seebach, E. Duckhardt and H. Schwalbe, *Helv. Chim. Acta*, 2002, **85**, 633.
8 U. D. Lengweiler, M. G. Fritz and D. Seebach, *Helv. Chim. Acta*, 1996, **79**, 670.
9 B. M. Bachmann and D. Seebach, *Helv. Chim. Acta*, 1998, **81**, 2430.
10 D. Seebach, A. Jacobi., M. Rueping, K. Gademann, M. Ernst and B. Jaun, *Helv. Chim. Acta*, 2000, **83**, 2115.
11 Y-D. Wu and D-P. Wang, *J. Am. Chem. Soc.*, 1999, **121**, 9352–9362.