(*S*)-(−)-**4**[30b] in 57% yield and 48% ee.[31]

The absolute configurations[31,37] in Scheme I show that the helix **7** winds so as to place the silyloxyl derived from **5** on the outer face.

Acknowledgment. We are grateful to the National Science Foundation for support under Grant DMR-82-13794.

Supplementary Material Available: ^{1}H NMR spectra of **2**, **8**, their double-bond isomer, and **9**, the ^{13}C NMR spectrum of **9**, and CD and UV spectra of **3**, **8**, and **9** (8 pages). Ordering information is given on any current masthead page.

(36) Sudhakar, A. Ph.D. Dissertation, Columbia University, New York, 1985.

(37) The absolute configuration of **8** was assigned assuming that, like other helicenes, the *M* enantiomer is levorotatory at 578 nm and exhibits negative Cotton effects in methanol for the p bands (λ = 371 nm, $[\theta] = -2.45 \times 10^5$ deg cm^2 mol^{-1}; 354 nm, $[\theta] = -2.46 \times 10^5$ deg cm^2 mol^{-1}) and the β band (λ = 329 nm, $[\theta] = -5.50 \times 10^5$ deg cm^2 mol^{-1}).[38]

(38) (a) Laarhoven, W. H.; Prinsen, W. J. C. *Top. Curr. Chem.* **1984**, *125*, 63 and references cited therein (on page 91). (b) Groen, M. B.; Wynberg, H. *J. Am. Chem. Soc.* **1971**, *93*, 2970. (c) Martin, R. H.; Marchant, M. J. *Tetrahedron* **1974**, *30*, 343. (d) Weigang, O. E., Jr.; Trouard Dodson, P. A. *J. Chem. Phys.* **1968**, *49*, 4248.

2,8-Dimethyl-4-(carboxymethyl)-6-(aminomethyl)phenoxathiin *S*-Dioxide: An Organic Substitute for the β-Turn in Peptides?

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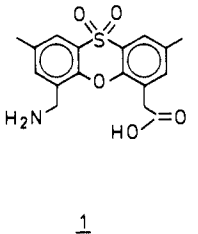
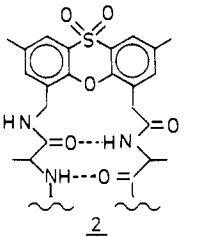
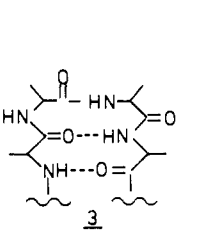
Cyclic peptides are known to adopt several conformations in solution; single rigid conformations are found only for small rings with a specific combination of amino acids. Attempts to stabilize specific peptide conformations incorporating nonpeptide residues are rare.[1] We propose the use of the spacer **1** to force hydrogen



1



2

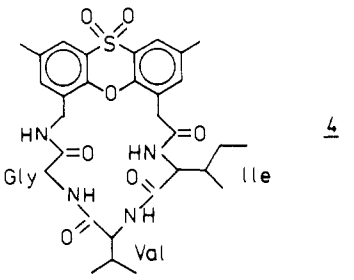


3

bridging between antiparallel peptide strains (**2**) in a similar manner as a β-turn (**3**).

Here we report the synthesis and conformational investigation of compound **4**, a cyclic peptide consisting of **1** and the amino acid sequence Ile-Val-Gly. Two low-energy conformations of **4** were found with the MM2 force field. One- and two-dimensional ^{1}H NMR experiments support **4A** as the prominent conformation in Me_2SO. **4A** contains a β-type hydrogen bridge and possibly a γ-loop, a situation that is found in several cyclic pentapeptides. Therefore, **1** may be used as an organic substitute simulating a pair of amino acids preferring the $i + 1$ and $i + 2$ positions of the β-reverse turn in peptides.

(1) (a) Kellogg, R. M. *Angew. Chem.* **1984**, *96*, 769–781; *Angew. Chem., Int. Ed. Engl.* **1984**, *23*, 782. (b) Mosberg, H. I.; Omnaas, J. R. *J. Am. Chem. Soc.* **1985**, *107*, 2986–2987. (c) Ravi, A.; Balaram, P. *Tetrahedron* **1984**, *40*, 2577–2583. (d) Natural examples are some peptide antibiotics, e.g., ristocetin[11] or bouvardin: Jolad, S. D.; Hoffmann, J. J.; Torrance, S. J.; Wiedhopf, R. M.; Cole, J. R.; Arora, J. K.; Bates, R. B.; Gargiulo, R. L.; Kriek, G. R. *J. Am. Chem. Soc.* **1977**, *99*, 8040–8044.



4

2,8-Dimethylphenoxathiin,[2] lithiated α to the oxygen, reacts with bromoacetic acid to form 2,8-dimethyl-4-(carboxymethyl)-phenoxathiin.[3] This was converted by H_2O_2 in acetic acid to the *S*-dioxide. Subsequent treatment with (hydroxymethyl)phthalimid in concentrated H_2SO_4 gave 2,8-dimethyl-4-(carboxymethyl)-6-(phthalimidomethyl)-phenoxathiin *S*-dioxide (**5**), the N-protected derivative of **1**. The tripeptide Ile-Val-Gly-OMe was coupled with **5** by propanephosphonic anhydride in CH_2Cl_2[4] (33%). Deprotection with hydrazine and cyclization by the Medzihradszky method yields **4** in 35% yield.[5]

The ^{1}H NMR spectrum of **4** in Me_2SO-d_6 was completely assigned with the aid of two-dimensional scalar correlated spectroscopy. The weak temperature coefficient of the chemical shift of the Ile-NH proton (Ile-NH, 0.5×10^{-3}; Val-NH, 3.3×10^{-3}; Gly-NH, 3.9×10^{-3}; **1**-NH, 4.7×10^{-3} ppm/deg) indicates that the proton is shielded from the solvent. This can be attributed to various types of intramolecular interactions—the most probably one is a hydrogen bridge to the Gly-CO (see below). Force-field calculations[6] revealed two basic low-energy conformations **4A** and **4B**, both possessing trans peptide bonds (Figure 1). Whereas **4A** has the expected "β-loop" with a hydrogen bridge from the Ile-NH to the Gly-CO (and in addition a γ-loop), conformation **4B** contains two γ-loops. It is possible to "invert" the phenoxathiin part in **4A** without significant change in the energy or distortion of the peptide moiety (see Figure 1). The NH–α-CH dihedral angles, derived from NMR coupling constants, support both conformations **4A**,**B** if fast inversion of the phenoxathiin part is assumed.[8] More definitive conclusions, however, can be drawn from the intramolecular distances measured by the nuclear Overhauser experiments.

We observed cross peaks due to chemical exchange in the 2D NOE spectra at 78 °C. Inspection of the 1D spectrum proves the presence of small amounts (4%) of a second conformation in slow exchange with the dominant form. To slow the exchange rate, in order to determine the NOE connectivity pattern of the main component, the experiments were run in a Me_2SO/CCl_4 solvent mixture at −3 °C. Here, the NOEs are negative and of medium size (2–18% in 1D experiments with 2.8-s presaturation).

(2) Tomita, M. *J. Pharm. Soc. Jpn.* **1938**, *58*, 510. See also: Suter, C. M.; McKenzie, J. P.; Maxwell, C. E. *J. Am. Chem. Soc.* **1936**, *58*, 717–720.

(3) Addition at −100 °C, in THF; chromatography on Silica gel (CH_2Cl_2, 1% MeOH); yield 31%. For analogous reaction conditions, see: Neidlein, R.; Kramer, W. *Helv. Chim. Acta* **1981**, *64*, 939–942.

(4) BOC protection during peptide synthesis; coupling conditions: Wissmann, H.; Kleiner, H.-J. *Angew. Chem.* **1980**, *92*, 129–130; *Angew. Chem., Int. Ed. Engl.* **1980**, *19*, 133–134.

(5) Azide cyclization in DMF at 6×10^{-2} mol/L; 4 °C; 6 days; after workup, 2 times recrystallization from MeOH; purity 98% by HPLC; Anal. ($C_{30}H_{38}N_4O_7S$) C, H, N. The monomeric structure of 4 is proved by its mass spectrum: EI 598 (M^+), most intense peak above mass 90; no peaks were detected at masses higher than 598.

(6) An undated version of the MM2 program of Allinger[7a]—obtained by courtesy of Molecular Design Ltd., Hayward, CA—was parametrized for amide functions giving reasonable energies and geometries for small *N*-alkyl amides as peptide models. H bonds are formed by the attraction of the NH and CO dipoles; the van der Waals repulsion of the NH proton was reduced in an interaction with a carbonyl oxygen.[7b] Six preconceived backbone conformations of 4 were used as starting points in the energy minimization; no attempts were made to explore the total conformational energy surface.

(7) (a) Allinger, N. L. *J. Am. Chem. Soc.* **1977**, *99*, 8127–8134. (b) Hagler, A. T.; Huler, E.; Lifson, S. *J. Am. Chem. Soc.* **1974**, *96*, 5319–5327.

(8) Coupling constants $^3J_{NH,\alpha H}$, derived dihedral angles (Karplus), MM2 angles in **4A**,**B**: Ile 7.1 Hz, 25° or 130°, 139.5° and 133.6°; Val 7.6 Hz, 20° or 140°, 138.6° and 153.4°; Gly-α_1 8.1 Hz, 10° or 150°, 156.8° (*Pro-S*) and 24.4° (Pro-R); Gly-α_2 5.3 Hz, 130° or 30°, 38.3° (*Pro-R*) and 142.9° (*Pro-S*); **1**-α_1 and **1**-α_2 both 6 Hz, not compatible with Karplus equation.

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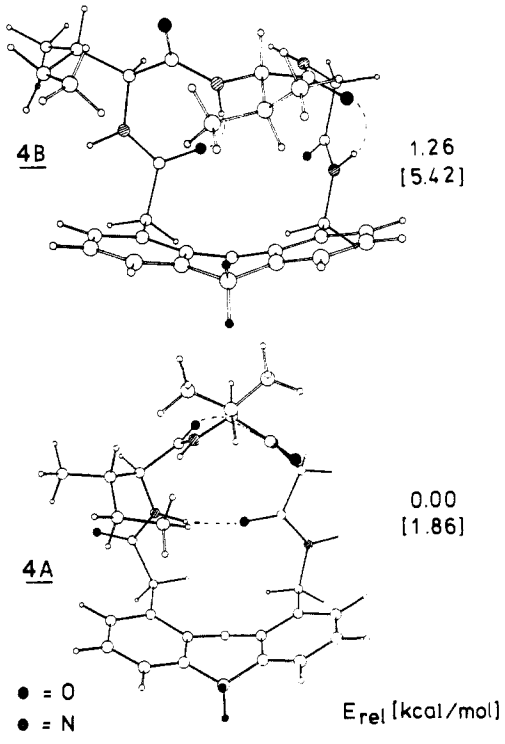

Figure 1. Geometries and relative steric energies of the conformations **4A,B** derived from force-field calculations. The numbers in brackets refer to alternative conformations (not shown) in which the phenoxathiin part is bent away from the view point. Aromatic methyl groups are replaced by hydrogens.

Table I. Comparison of Proton Distances r_{ij} Derived from NOE Experiments with the Minimum-Energy Geometries **4A,B**

connected protons	exptl r_{ij}[a]		force field	
	2D NMR	1D NMR	**4A**	**4B**
I, Ile-NH-**1**-CH_2CO[b]	2.44	2.24	2.43	2.53
II, Val-NH-Ile-βH	2.35	2.26	2.36	4.52
III, Gly-NH-Val-αH	2.37	2.30	2.43	2.92
IV, **1**-NH-**1**-CH_2N[c]	2.32	2.25	2.26	2.64
V, **1**-CH_2CO[c]-**1**-CH_2N[d]	2.52	*e*	2.26	2.93

[a] Values in Å, error limits ca ±0.2. [b] *Pro-S* proton in **4A**; *Pro-R* proton in **4B**. [c] *Pro-S* proton. [d] *Pro-R* proton. [e] A selective irradiation in the 1D experiment is impossible due to the small chemical shift difference.

A representative 2D NOE spectrum is shown in Figure 2.

Distances between protons were calculated in two ways—from the buildup rate of the 2D NMR cross-peak intensities with increasing mixing time[9] and from time-dependent 1D NOE experiments.[11,12] The results of both techniques are compared with the minimum-energy geometries in Table I. The experimental evidence suggests that **4A** is the observed low-energy conformation. **4B** can be eliminated for it has two r_{ij} which are to long to explain the NOE buildup rates. The γ-loop involving the Val residue in **4A** is in concordance with the II and III NOE effects (Table I). However, the temperature coefficient of the presumably hydrogen-bonded Gly-NH (see above) indicates exposure to the solvent; so the local conformation at the Val residue may be still flexible.

In summary, three independent NMR experiments—NH temperature shifts, coupling constants, and NOE connectivities—support structure **4A** which contains the anticipated

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(10) Macura, S.; Huang, Y.; Suter, D.; Ernst, R. R. *J. Magn. Reson.* **1981**, *43*, 259-281.

(11) Williams, D. H.; Williamson, M. P.; Butcher, D. W.; Hammond, S. J. *J. Am. Chem. Soc.* **1983**, *105*, 1332-1339 and references cited therein.

(12) Known distances were used for calibration, e.g., the geminal protons of **1** or of Gly in 4.

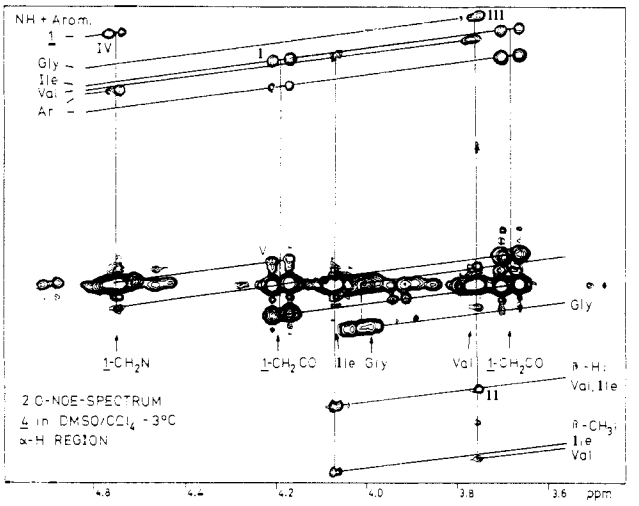

Figure 2. αH region of the 400-MHz 2D NOE spectrum of **4** in Me_2SO-d_6/CCl_4 at -3 °C. The echo pulse sequence (90°-t_1/2-90°-t_m-90°-t_1/2-FID) was used. A small random variation (±5 ms) of the mixing time t_m (375 ms) was applied to cancel unwanted signals due to J coupling.[10] The spectrum is recorded in the N-type mode which gives the somewhat unusual direction of the lines of connectivity. The numbers I to V refer to Table I.

hydrogen bridge of a β-loop type. The structure resembles cyclic pentapeptides—compounds that contain normally at least one D-amino acid or glycine. Similar cycles with different amino acid compositions are being synthesized in order to show whether the rules derived for the conformation of cyclic pentapeptides[13] are still applicable when **1** substitutes two of the amino acids.

Acknowledgment. This work was supported by the Deutsche Forschungsgemeinschaft.

(13) Kessler, H.; Hehlein, W.; Schuck, R. *J. Am. Chem. Soc.* **1982**, *104*, 4534-4540. (b) Gierasch, L. M.; Karle, I. L.; Rockwell, A. L.; Yenal, K. *J. Am. Chem. Soc.* **1985**, *107*, 3321-3327.

Productive Conformation in the Bound State and Hydrolytic Behavior of Thiopeptide Analogues of Angiotensin-Converting Enzyme Substrates

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We wish to report the unexpected behavior of angiotensin-converting enzyme (ACE; dipeptidyl carboxypeptidase EC 3.4.15.1) toward the thiopeptide analogues *N*-(furylacryloyl)-L-thiophenylalanylglycyl-L-proline [FA-Phe-Ψ-(CSNH)-Gly-Pro, **1**] and *N*-(furylacryloyl)-L-thiophenylalanyl-L-alanyl-L-proline [FA-Phe-Ψ-(CSNH)-Ala-Pro, **3**][1] of the well-known tripeptide substrates **2** and **4** (Table I). These thioamide analogues appeared attractive as potential ligands of the active-site zinc ion of the enzyme, the thiocarbonyl function being susceptible in principle to effective coordination by the metal. We found that thiopeptide **1** suffers ready hydrolysis by ACE at a rate comparable to that of **2** whereas analogue **3**, in contrast to the choice parent substrate **4**, is not hydrolyzed even over extended periods of time. This good substrate property of **1** was unexpected on the basis of the reported behavior of thioamide analogues of peptide substrates toward carboxypeptidase A (CPA),[3,4] an enzyme whose catalytic mech-

(1) For IUPAC-IUB nomenclature, see: *Eur. J. Biochem.* **1984**, *138*, 9.

(2) Holmquist, B.; Bunning, P.; Riordan, J. F. *Anal. Biochem.* **1979**, *95*, 540.

(3) Campbell, P.; Nashed, N. T. *J. Am. Chem. Soc.* **1982**, *104*, 5221.

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