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[16] ^1H NMR data for the $(CH_3)_2CH$ groups of the 2,6-iPr_2-bian derivative in $CDCl_3$: **B** (200 K): $\delta = 1.26$ (d) and 0.52 (d); **1**: (200 K): $\delta = 1.32$ and 0.54; **4**: (223 K): $\delta = 1.39$ (d), 1.27 (d), 1.09 (d) and 0.60 (d; no C_s plane perpendicular to the $Pd(X)CN_2$ plane for **4**).
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Conformation Design of a Fully Flexible βII-Hairpin Analogue**

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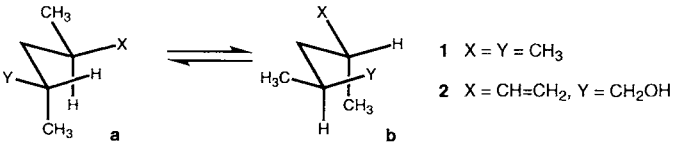
Isosteric, non-hydrolyzable analogues of secondary-structure elements of peptides are of high current interest in medicinal chemistry and serve as peptidomimetics. Such structural units yield important information on complex structure–activity relationships and are neccessary for a rational design of low molecular weight, non-peptide pharmaceutical agents.[1] Moreover, such analogues of peptide structures may be important for inducing α-helix or β-turn structures in adjacent peptide sequences.[2] Far less is known about the prerequisites for the induction of β-sheet structures, in which tertiary interactions play a decisive role. Gellman et al. pointed out that good model systems for studying the formation of β-hairpin structures are lacking.[3]

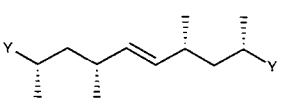
We are interested in a rational conformation design of open-chain hydrocarbon backbones that possess a strong conformational bias and yet maintain full flexibility.[4] We therefore tried to apply the principles of nature's conformation design, demonstrated in polyketide natural products, to designing new molecular backbones. We demonstrate here the value of such an approach with the design of a fully flexible β-hairpin analogue.

A β hairpin is the simplest form of an antiparallel β-sheet conformation, and is defined by a β-turn region flanked by two antiparallel peptide strands that are hydrogen bonded through the corresponding backbone CO and NH groups. Different structural types of β turns are characterized by the ϕ and ψ dihedral angles of the peptide backbone.[5] Figure 1 shows the structure of a βII-type hairpin with $\phi_1 = -60$, $\psi_1 = 120°$, $\phi_2 = 90°$, and $\psi_2 = 0°$. The requirements that a mimic must meet are 1) a reversal in the peptide-chain direction and 2) the promotion of intramolecular hydrogen-bond formation.[2] In addition, our approach allows preservation of conformational flexibilty similar to that of the natural prototype.

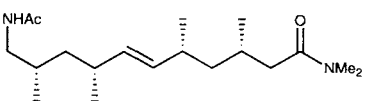
Our design is based on 2,4-dimethylpentane units such as the ones nature uses in its conformation design of polyketide natural products.[4] 2,4-Dimethylpentane (**1**) is biconformational, and equally populates, to greater than 90%, two enantiomorphous and, hence, isoenergetic low-energy conformations **1a** and **1b**.



The position of the conformer equilibrium could be biased to one side by varying the substituents X and Y. In **2a** X suffers an additional *gauche* interaction, which Y does not have, and is therefore in the sterically more encumbered position. When X in **2** is a less sterically demanding vinyl group and Y a hydroxymethyl group, conformation **2a** should be preferentially populated. In fact, an equilibrium ratio **a**:**b** of about 3.5:1 was found for **2** in $CDCl_3$ solution. Therefore, **2** represents a backbone segment with a conformational preference. It can be combined with itself or other building blocks to yield larger molecular frameworks. The combination of two segments of **2** results in structure **3**, which should have a U-shaped molecular backbone that is similar to β-turn und β-hairpin moieties of peptides.



3 Y = CH_2OH **4** Y = CH_3



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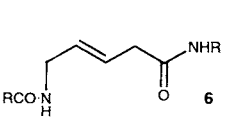

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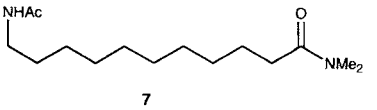

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Simple modeling (MM3*)[6] showed that the most stable conformation of the bis(amide) **5** derived from **3** matches the shape of a βII-hairpin **8** very well (Figure 1).

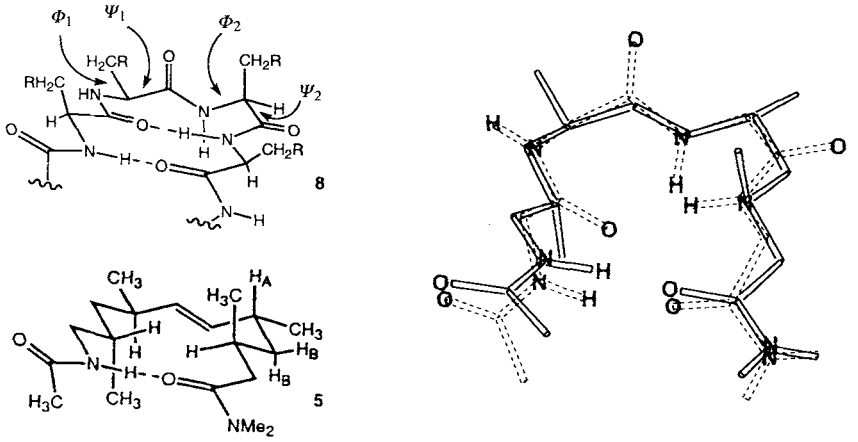
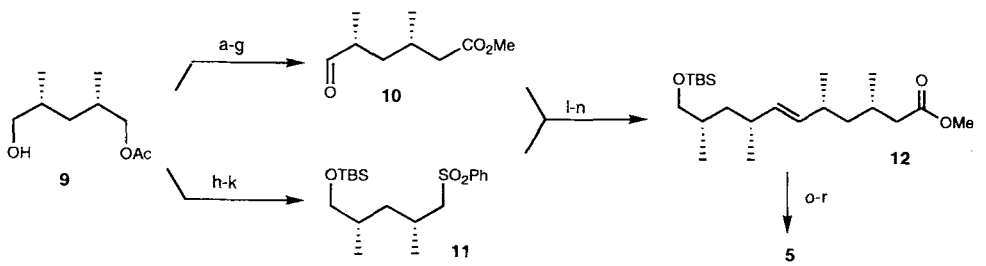


Figure 1. Superposition of the most stable conformation of **5** with a βII-hairpin **8**.

To find out whether **5** is a conformationally flexible βII-hairpin analogue, we synthesized **5** from optically active **9** (Scheme 1).[7] Compound **9** was transformed on the one hand in four conventional steps into sulfone **11**, and on the other hand in eight steps into aldehyde **10**. Julia olefination of the two components yielded alkene **12**, from which bis(amide) **5** could be obtained in four further steps.

IR and NMR spectroscopy was used to analyze the conformational properties of **5** in solution. The former is especially suited for studying intramolecular hydrogen bonds, because the transformation of hydrogen-bonded into non-hydrogen-bonded conformations is slow on the IR time scale. Both states can therefore be distinguished by separate N–H streching frequencies in nonpolar solvents. In contrast, this transformation is fast on the NMR time scale, so that the observed resonances are weighted averages of hydrogen-bonded and non-hydrogen-bonded states.



Scheme 1. a) Triisopropylsilyl chloride (TIPSCl), imidazole, 4-dimethylamino pyridine (DMAP), DMF, 50 °C, 96%; b) K_2CO_3, CH_3OH, 25 °C, 94%; c) CH_3SO_2Cl, NEt_3, CH_2Cl_2, −40 °C; LiBr, THF, 25 °C, 98%; d) NaCN, DMSO, 25 °C, 95%; e) NaOH, EtOH, 80 °C, 84%; f) CH_2N_2, Et_2O, 0 °C, 90%; g) pyridinium chlorochromate (PCC), CH_2Cl_2, SiO_2; h) I_2, PPh_3, imidazole, THF, 25 °C, 90%; i) $PhSO_2Na$, polyethylene glycol (PEG) 400, 130 °C, 91%; j) K_2CO_3, CH_3OH, 25 C, 91%; k) *tert*-butyldimethylsilyl chloride (TBSCl), imidazole, DMAP, DMF, 50 °C, 99%; l) sulfone **11**, *n*BuLi, THF, −78 °C, aldehyde **10**, 97%; m) Ac_2O, pyridine, 25 °C, 90%; n) 6% Na/Hg, CH_3OH, AcOEt, NaH_2PO_4, −30 °C, 88%; o) ClAl(Me)NMe_2, benzene, 80 °C, 95%; p) nBu_4NF, THF, 25 °C, 96%; q) CH_3SO_2Cl, NEt_3, CH_2Cl_2, −40 °C; NaN_3, DMF, 50 °C, 85%; r) PPh_3, THF, trace H_2O; Ac_2O, 25 °C, 96%.

The IR spectrum of **5** (10^{-3} M in CCl_4) shows only one sharp band, the wavenumber of which (3360 cm^{-1}) is characteristic for the N–H streching vibration of hydrogen-bonded amide protons.[8] That the preorganization of the amide groups in **5** by the hydrocarbon backbone is the origin of the predominance of the hydrogen-bonded state can be seen by a comparison with the IR spectrum of the simple ω-acetamidocarboxamide **7**. The latter shows, in addition to two bands for hydrogen-bonded NH-groups, an intensive absorption due to non-hydrogen-bonded amide protons (Figure 2). The concentration dependence of the amide proton ^1H NMR chemical shift of **5** was then determined to exclude aggregation as the cause of hydrogen bonding. From the constant value in solutions of varying concentration (10^{-1}–10^{-4} M, CCl_4) it can be unequivocally deduced that **5** is intra- rather than intermolecularly hydrogen bonded (Figure 3). In contrast, **7** exhibits a pronounced concentration dependence of the amide-proton chemical shift, which corresponds to the behavior of *N*-methylacetamide. The data clearly show that no aggregation occurs in a 10^{-3} M solution, the concentration at which the IR spectra were obtained.

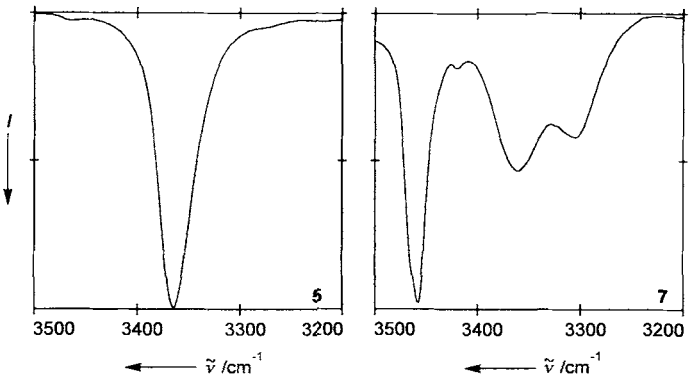


Figure 2. IR spectra (transmittance) of the bis(amide)s **5** and **7** (1 mM in CCl_4, d = 3.5 cm, NaCl): **5**, maximum at 3365 cm^{-1}; **7**, maxima at 3458, 3361 and 3305 cm^{-1}.

The conformational preference of the hydrocarbon backbone of **5** can be deduced from the vicinial ^1H–^1H NMR coupling constants. The divergence of the diagnostic[9] ^1H NMR coupling constants between H_A (Figure 1) and the two protons H_B (2.9 Hz and 10.5 Hz, $CDCl_3$) proves a pronounced conformational preference of the hydrocarbon backbone of **5**. This preference, however, is not a consequence of conformational constraints induced by the hydrogen bond: MM3* calculations show that the most stable conformation of **4** is virtually identical to that of the bis(amide) **5** (Figure 4). Therefore, the backbone conformation of **4** is already ideally suited to preorient the amide groups of **5** for formation of an intramolecular hydrogen bond. Clearly, conformation design of **5** is the origin of the spontaneous formation of the 14-membered cyclic conformation, as multiconformational **7** is converted into an essentially monoconformational

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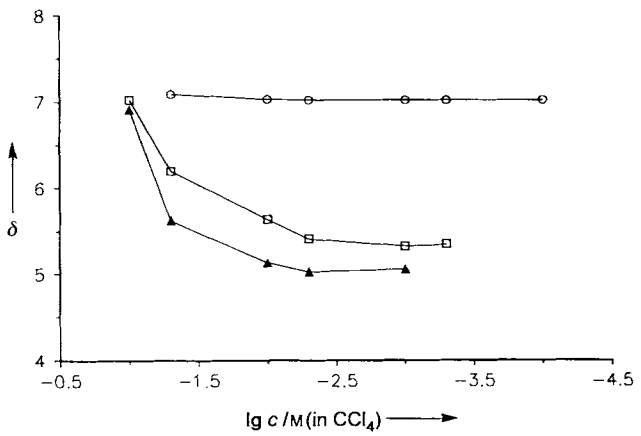

Figure 3. NMR chemical shift of the amide protons in CCl_4 at 300 K as a function of the logarithm of concentration: **5** (○), **7** (□), *N*-methylacetamide (▲).

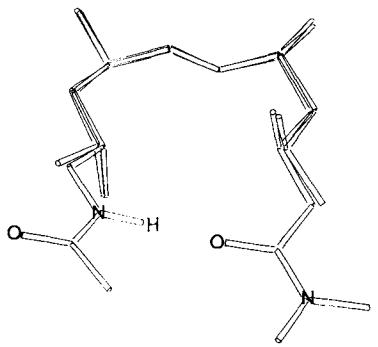

Figure 4. Superposition of the most stable conformation of **5** with that of **4**.

unit **5** by rational introduction of a *trans* double bond *and* four methyl groups. Simple introduction of a double bond into an ω-amido-alkyl-carboxamide does not suffice to induce a β-turn conformation, as Gellman et al.[10] showed with **6**.[11] Conclusion: **5** is not only isostructural with a βII hairpin, but also retains the conformational flexibility that is typical for peptides.

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[11] With two methyl substituents at the double bond of **6**, a conformation corresponding to a β turn can be induced due to 1,3-allylic strain [10].

NADH-Induced Changes of the Nickel Coordination within the Active Site of the Soluble Hydrogenase from *Alcaligenes eutrophus*: XAFS Investigations on Three States Distinguishable by EPR Spectroscopy**

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Dedicated to Professor Günter Schmid on the occasion of his 60th birthday

Hydrogenases are enzymes that catalyse the reversible activation of molecular hydrogen in numerous aerobic and anaerobic microorganisms.[1] This capability has attracted increasing interest especially in view of possible applications of the catalytic principle in industrial processes or as source for "biological hydrogen" (hydrogen technology).[2]

Most of the hydrogenases known today are metalloenzymes that contain nickel and iron as essential constituents (NiFe hydrogenases) in distinction to the less widespread "iron-only" species.[1] The nickel binding site of these enzymes shows characteristic EPR signals in specific stages of the catalytic cycle indicating an uncommon redox chemistry. Thus, the nickel center is considered the site of hydrogen activation. The NiFe hydrogenase from *Desulfovibrio gigas* has recently been the focus of special attention since the crystal structure of this enzyme revealed the presence of a binuclear Ni/Fe center with cysteine sulfur bridges.[3]

The soluble NAD^+-reducing hydrogenase from the aerobic H_2-oxidizing bacterium *Alcaligenes eutrophus* H16 (E. C. 1.12.1.2) is a heterotetrameric enzyme. Composed of two heterodimeric proteins of different function ($\beta\delta$ and $\alpha\gamma$), this enzyme is of higher complexity than the "typical" heterodimeric hydrogenases.[4] In addition to the nickel center, the O_2-insensitive holoenzyme contains different iron–sulfur clusters (2Fe-2S, 3Fe-4S, 4Fe-4S) and a flavine residue (FMN) as redox-active prosthetic groups.

In this context, we were interested to learn whether and how the coordination of nickel changes upon reductive activation of the enzyme and, at the same time to tackle the question of possible structural relationships between the nickel centers of the hydrogenases from *A. eutrophus* and from *D. gigas*. To this end, high-resolution X-ray absorption spectroscopy (XAFS) was chosen as the method of choice. This technique was developed to determine the structure in the vicinity of excited atoms, and, in contrast to diffraction methods, can also be used to investigate noncrystalline systems. Thus, we characterized the soluble hydrogenase from *A. eutrophus* by XAFS analysis in three different states distuingishable by EPR spectroscopy.[5] Herein we report on the evaluation of the X-ray absorption near-edge structure (XANES) and extended X-ray absorption

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