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Development of 2,3-diazabicyclo[2.2.1]heptane as a constrained azapeptide template and its uses in peptidomimetic studies

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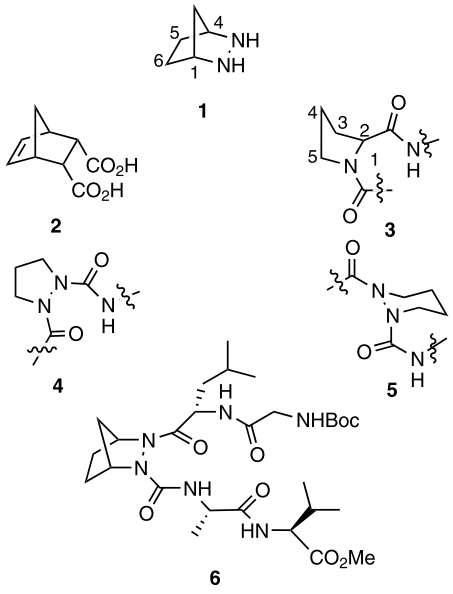
Abstract—2,3-Diazabicyclo[2.2.1]heptane **1** is used for the first time as a peptidomimetic scaffold in the synthesis of a conformationally constrained analogue **6** of azaproline (or azapipecolic acid) containing peptides.

Modification of the peptide backbone by inserting structurally constrained nonpeptide scaffolds is a common method routinely used by chemists to induce desirable conformational biases in small peptides.[1] Azapeptides with a nitrogen atom at the α-carbon site in an amino acid residue have emerged as an important class of such modified peptides.[2] In the present work, we used 2,3-diazabicyclo[2.2.1]heptane **1** as a novel template for the synthesis of azapeptide-based peptidomimetics. While a similar carbon-based scaffold, norbornene-2,3-dicarboxylic acid **2**, has already been utilized in peptidomimetic studies,[3] this is the first report, to the best of our knowledge, on the development of an aza analogue of **2**.



The built-in assembly of a proline-type moiety in **1**, similar to a C4-flapped envelope conformation of a proline ring **3** with a φ value of ca. 0°, appears particularly attractive, especially as a constrained mimic of an azaproline (AzPro, **4**)[2i,j] or azapipecolic acid (AzPip, **5**).[2a] The steric strain between the two carbonyls in an envelope conformation of the pyrazolidine ring in AzPro-containing peptides forces its nitrogens to adopt partial sp^3 character with nonplanarity of the bonds on each one of them, resulting in an unusual φ value of ca. ±110°, in contrast to that of ca. −60° in L-Pro-peptides. While proline in the i+1 position is known to nucleate the classical β-turn in peptides involving an intramolecular i+3→i hydrogen bond, the AzPro (or, AzPip) in the i+2 position induces a βVI type reverse turn with its preceding amino acid which forms a *cis* amide bond with the AzPro.[2a,i,j] In fact, opening of the pyrazolidine ring is shown not to alter significantly the molecular conformation in other aza amino acid containing peptides. Bridging the Cβ and Cδ residues of AzPro with a dimethylene linker as in **1** would lock the pyrazolidine ring into an envelope conformation and lead to an interesting structural variation. Incorporation of **1** into the peptidomimetic molecule **6** and its conformational studies by NMR are reported in this communication.

The synthesis of compound **6** is outlined in Scheme 1. A Diels–Alder reaction between cyclopentadiene and diethyl azodicarboxylate and subsequent reduction of the double bond by hydrogenation using Pd/C as catalyst, following reported procedures,[4] provided the start-

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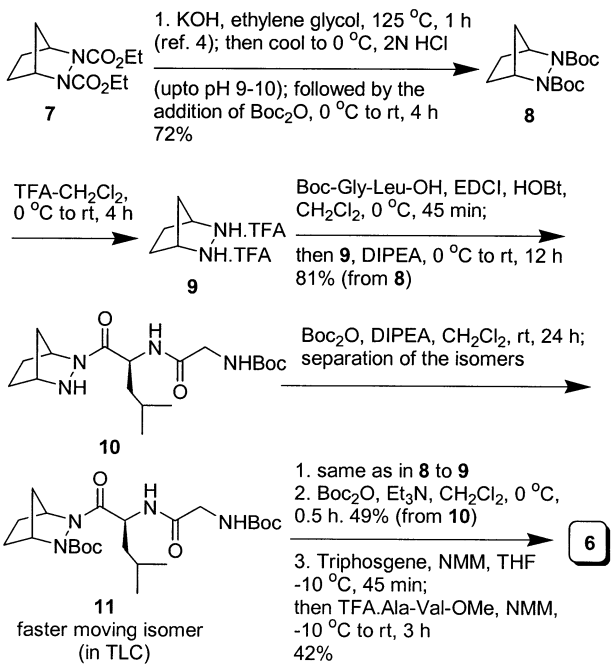

Scheme 1. Synthesis of the peptide **6**.

ing material **7**. Alkaline hydrolysis of the carboxylate groups of **7** furnished **1**,[4] which was protected in situ using Boc_2O, for easier purification purposes, to give **8** in 72% yield. Treatment of **8** with trifluoroacetic acid (TFA) in CH_2Cl_2 provided the di-TFA salt **9** which was coupled with an amino-terminally protected peptide residue Boc-Gly-Leu-OH under standard solution phase methods using 1-ethyl-3-(3-(dimethylamino)-propyl)carbodiimide hydrochloride (EDCI) and 1-hydroxybenzotriazole (HOBt) as coupling agents and dry CH_2Cl_2 as solvent. Only one of the amino groups of **9** underwent coupling, even when an excess of the peptide component was used, giving the product **10** as a mixture of diastereomers in 81% yield (from **8**). Next, a three-step protocol was followed to obtain an isomerically pure component of **10**. Reaction of **10** with Boc_2O in the presence of *N*,*N*-diisopropylethylamine (DIPEA) for an extended period of time gave di-Boc-protected intermediate **11**. A pure diastereomer could be separated at this stage following standard silica gel column chromatography. Deprotection of this purified diastereomer of **11** (higher running in TLC) and selective protection of its terminal Gly-NH_2 furnished a diastereomerically pure version of **10** in 49% overall yield in three steps. Treatment of this pure isomer, whose identity has not yet been determined, with triphosgene in the presence of *N*-methylmorpholine (NMM), according to the reported procedure,[5] was followed by coupling with Ala-Val-OMe to provide the target molecule **6**[6,7] in 42% yield.

Detailed conformational analysis of peptide **6** was performed using various NMR techniques. The assignments were carried out with the help of two-dimensional total correlation spectroscopy (TOCSY)[8] and were further confirmed by rotating-frame nuclear Overhauser effect spectroscopy (ROESY) experiments,[8] which, in addition, provided the information on the proximity of the protons.

Although the NMR spectrum of compound **6** in DMSO-d_6 was very well resolved, it did not reveal any significant secondary structure. The temperature coefficients ($\Delta\delta/\Delta T$) of the amide protons of **6**, obtained from variable temperature experiments (between 30 and 70°C), had large magnitudes, except that of AlaNH with a moderately low value of −4.0 ppb/K.

In a noncompetitive solvent such as $CDCl_3$, intramolecularly hydrogen bonded amide protons resonate downfield.[9] Compound **6** had only one amide proton, AlaNH with a chemical shift >7 ppm in $CDCl_3$. Solvent titration studies revealed that the addition of increasing amounts of DMSO-d_6, a hydrogen bonding solvent, to a $CDCl_3$ solution (1 mM) of **6** caused a very small change in the AlaNH chemical shift ($\Delta\delta = 0.12$ ppm on addition of 40% v/v of DMSO-d_6) as shown in Fig. 1. These observations indicate that the AlaNH is probably involved in intramolecular hydrogen bonding(s).

Some of the important ROE cross-peaks obtained in $CDCl_3$ are shown schematically in Fig. 2. The charac-

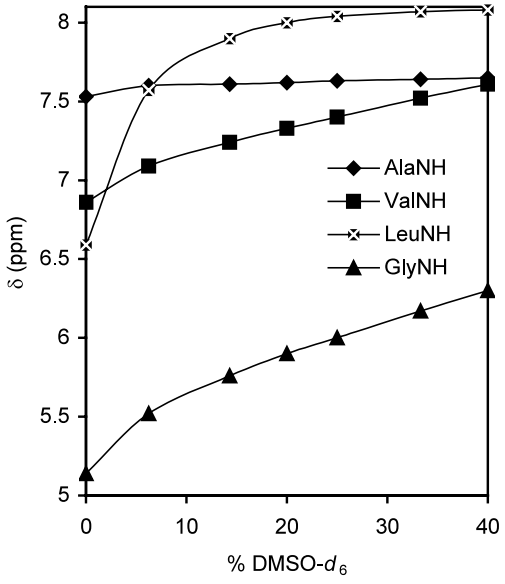

Figure 1. Solvent titration plots for the amide protons of **6**.

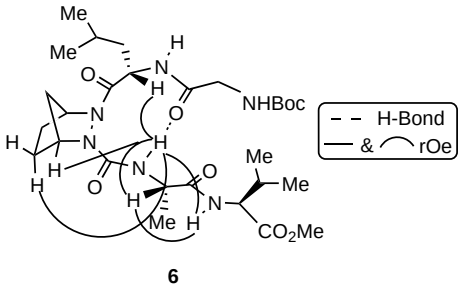

Figure 2. Schematic representation of the proposed structure of **6** with the long-range ROEs seen in the ROESY spectrum in $CDCl_3$.

teristic ROE cross-peaks, especially the interstrand ROE cross-peak between AlaNH and LeuCαH and the participation of the AlaNH in intramolecular hydrogen bonding as shown by solvent titration studies are consistent with the presence of a turn in the solution conformation of **6** and support the proposed structure displayed in Fig. 2. This was further supported by constrained molecular dynamics (MD) simulation studies.[10] The cross-peak intensities in the ROESY spectrum of **6** were used for obtaining the restraints in the MD calculations. The two-spin approximation was used to obtain the intermolecular distances. The upper and lower bounds of these distance restraints were fixed at ±15% of the derived distances. Several long-range (more than four bonds) distance constraints from the ROEs shown in Fig. 2 were used in the energy calculations and MD studies. The superimposed display of the 20 structures (Fig. 3), sampled at regular intervals of 5 ps during a 100 ps MD run, subsequently energy-minimized and superimposed aligning the hydrogen bonded parts, clearly reveals a β-turn-like structure of the molecule. In this structure, the AlaNH is strongly hydrogen bonded to GlyC=O intramolecularly (i+3→i) leading to a ten-membered ring structure. The diazabicycloheptane (AzBch) unit is at the i+2 position in this turn structure and behaves similar to the aza amino acid residues in AzPro- and AzPip-peptides. The similarity of the structure induced by AzBch to those found in AzPro and AzPip containing peptides implies its participation in a type-VI β-turn-like structure. However, the configuration of the Leu-AzBch (diazabicycloheptane) amide bond that is supposed to be *cis* for this type of turn structure could not be ascertained unambiguously. Further work on the conformational studies of this and related molecules are currently in progress.

In conclusion, the rigid scaffold of the 2,3-diazabicyclo[2.2.1]heptane **1** molecule can serve as a conformationally constrained template as shown here, and is expected to find applications in developing novel peptidomimetics with interesting structures and useful properties.

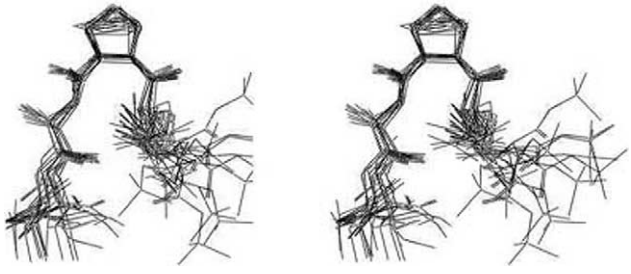

Figure 3. Stereoview of the 20 superimposed structures sampled at 5 ps intervals during 100 ps MD simulations of **6**, subsequently energy-minimized and superimposed aligning the hydrogen-bonded regions.

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6. Compound **6**: ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.53 (d, $J=7.3$ Hz, 1H, AlaN*H*), 6.86 (d, $J=8.7$ Hz, 1H, ValN*H*), 6.57 (br s, 1H, LeuN*H*), 5.11 (t, $J=5.3$ Hz, 1H, GlyN*H*), 4.95 (br d, $J=3.4$ Hz, 1H, C4-*H*), 4.61 (br s, 1H, C1-*H*), 4.51 (dd, $J=8.7$, 5.3 Hz, 1H, ValαH), 4.50 (m, 1H, LeuαH), 4.30 (p, $J=7.3$ Hz, 1H, AlaαH), (two dd, $J=16.7$, 5.3 Hz, 2H, GlyαH and $\alpha H'$), 3.68 (s, 3H, OMe), 2.10 (dp, $J=6.6$ and 5.3 Hz, 1H, ValβH), 1.89 (m, 2H, C6-*H*), 1.73 (m, 1H, LeuγH), 1.64 (m, 2H, C5-*H*), 1.54 (m, 2H, C7-H_2), 1.48 (d, $J=7.3$ Hz, 3H, AlaβH), 1.46 (s, 9H, Boc), 1.45 (m, 1H, LeuβH), 1.37 (m, 1H, Leu$\beta H'$), 0.95 (d, $J=6.7$ Hz, 3H, LeuδH), 0.90 (d, $J=6.6$ Hz, 3H, ValγH), 0.895 (d, $J=6.7$ Hz, 3H, Leu$\delta H'$), 0.887 (d, $J=6.6$ Hz, 3H, Val$\gamma H'$); MS (ESI): m/z (%): 620 (94) [M^++H+Na], 519 (100) [M^++Na−$C_5H_8O_2$].
7. Although compound **6** is diastereomerically pure, the distinction between the two possible structures shown below could not be ascertained. However, the secondary structure of the final assembly as established in this paper is applicable to either of them as interchanging the strands does not alter their relative spatial arrangements.
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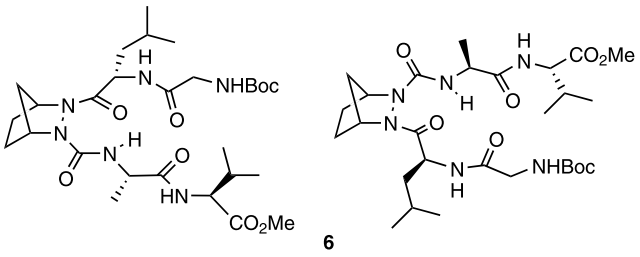

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