Synthesis of a New Turn Mimic Bearing a β-Lactam Moiety

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Dedicated to Prof. Dr. Dieter Seebach on the occasion of his 65th birthday

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A new turn mimic derived from PLG (prolyl-leucyl-glycine amide) containing a β-lactam in the turn area has been prepared. The β-lactam moiety was furnished by treating an Fmoc-protected leucine-derived diazo ketone **2** with a benzylidene-protected glycine ester in a photochemically induced Staudinger-type reaction. The *trans*-substituted β-lactams **3a/b** are formed in 70% yield (*dr* 70:30). Separation of the isomers, deprotection and attachment of Fmoc-proline using the pentafluorphenyl ester activation protocol yielded the protected peptidomimetic **4** in 93% yield. Deprotection and amidation resulted in formation of the target substrate **1** in 82% yield but with a low purity. Better results were obtained using Nsc ({2-[(4-nitrophenyl)sulfonyl]ethoxy}-carbonyl) as protection for proline. It could be cleaved yielding a spectroscopically pure product **1** whose structure was elucidated by X-ray crystallographic analysis. It shows an open turn conformation, i.e., the turn is not stabilized by a hydrogen bond between the termini of the turn region.
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Introduction

Peptides showing a turn conformation[1−4] are of major relevance since, in most cases, the turn regions are responsible for their biological activity. Well-known representatives showing a turn conformation are, inter alia, somatostatin[5−7] and oxytocin.[8] Substrates containing rigid frameworks, i.e., turn mimics, can be prepared by replacement of the turn region with other, mostly cyclic, templates.[9−11]

Johnson et al. have incorporated β-lactams into peptide chains (**A**) to mimic the β-turn-adopting tripeptide H−Pro−Leu−Gly−NH_2 (PLG, Figure 1)[12−15] and found that the products show a similar conformation and comparable biological activity through selectively enhancing the affinity of dopamine agonists to dopamine receptors. Further examples of peptidomimetics containing higher lactam moieties have been reported as well.[14,16−19]

Results and Discussion

Starting from α-amino acids, we have developed a β-lactam synthesis for use in the preparation of similar turn mimics **B** (Figure 1). Molecular modeling suggested that the additional alkylidene moiety between the four-membered lactam ring and the amide group should not have a negative influence on the formation of the turn conformation. Here we describe as a first example the construction of the PLG-derived turn mimic **1**.

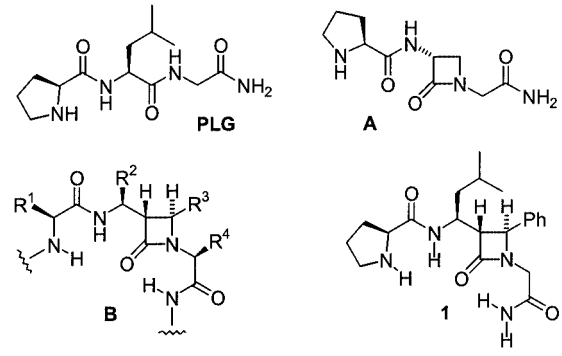

Figure 1. Targeted peptidomimetics

For the synthesis of the peptidomimetics, we started with suitably protected activated amino acids, which were allowed to react with diazomethane to yield the respective diazo ketones.[20−22] The latter were used in a photochemically induced Wolff reaction leading to ketenes which, in the presence of imines, furnished β-lactams (Scheme 1).[23−29] *N*-Peptidyl-substituted β-lactams are directly accessible when these imines have been prepared by condensation of an amino acid or a peptide derivative with an aldehyde.[26]

Although dipeptidic diazo ketones may be used in this protocol,[26] the yields are significantly higher when the tripeptide analogues were assembled by conventional peptide coupling. The Fmoc-leucine-derived diazo ketone **2** used

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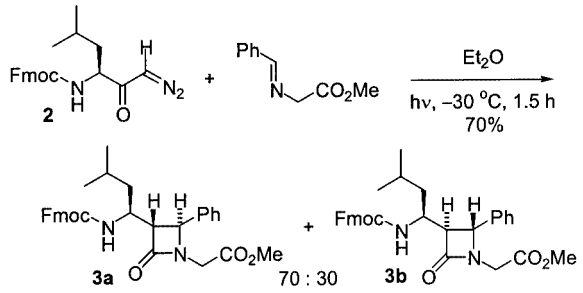
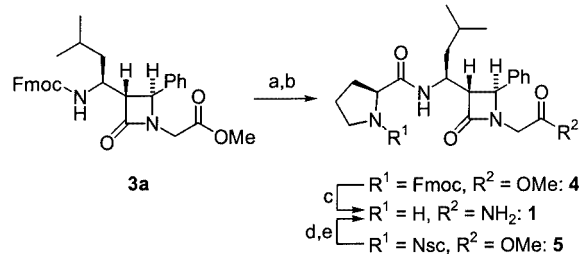


Scheme 1. Synthesis of the β-lactam moiety

here was prepared according to a procedure published by Seebach et al.;[22] the imine was prepared by condensation of glycine methyl ester with benzaldehyde.[30,31] In order to achieve high yields of about 70% in the photochemically induced β-lactam synthesis, it was necessary to use an imine containing no residual benzaldehyde and to perform the reaction at low temperature (−30 °C). The *trans*-substituted β-lactams **3a/b** (*dr* 70:30) formed in this reaction were separated by medium pressure chromatography (MPLC, ethyl acetate/light petroleum ether); both isomers were − separately − used for the preparation of turn mimics, although only the reaction of the major isomer **3a** is discussed here. On the basis of semi-empirical calculations (PM3), both isomers should lead to the formation of a stable turn conformation.

In the further course of the reaction sequence, the Fmoc group was cleaved with diethylamine, and peptide coupling with an Fmoc-protected proline pentafluorophenyl ester[32,33] led to the protected tripeptide analog **4** in 93% yield (two steps). Cleavage of the Fmoc group and amidation of the *C*-terminus were achieved with methanolic ammonia containing piperidine. The target turn mimic **1** was formed in 82% yield (crude material, determined by NMR spectroscopy), although purification by chromatographic methods was not feasible. Separation of the by-products formed during Fmoc cleavage [dibenzofulvene (DBF), polymerized DBF and a DBF amine adduct which is present in an equilibrium][34] was possible only with high losses during crystallization. When tris(2-aminoethyl)amine (TAEA)[35] was used as a base instead of piperidine, extraction with phosphate buffer was possible, although the gelatinous DBF polymer could not be removed by this method. Accordingly, we changed our strategy and used the {2-[(4-nitrophenyl)sulfonyl]ethoxy}carbonyl group (Nsc) for *N*-terminal protection, a protecting group that has been used only recently for solid-phase peptide synthesis even though it has been known for much longer.[34,36−38] The Nsc group is again cleaved by amine bases; the vinyl sulfone formed thereby is not prone to polymerization and the formation of the amine adduct is fast, irreversible and quantitative. The tripeptide analog **5** protected in this way was prepared from **3a** by attaching an Nsc-protected amino acid, which is easily prepared by published procedures or can be obtained from commercial sources.[34,36,39] The final cleavage of the protecting group was either achieved with TAEA (purification of the product by extraction with buffer) or with polymer-supported TAEA (purification by filtration). The latter reagent has been described before,[40] but has, to the best of our knowledge, not previously been used for a base-induced removal of protecting groups. Subsequent amidation with methanolic ammonia completed the reaction sequence.



Scheme 2. Assembly of peptidomimetic **1**; a) $HNEt_2$, THF, room temp., 4.5 h; b) R^1−Pro−OPfp (R^1 = Fmoc, Nsc), THF, room temp., 2 h, 93−96% (two steps); c) piperidine, MeOH/NH_3, 0 °C to room temp., 16 h, 82%; d) TAEA or polymer-supported TAEA, CH_2Cl_2, 0.5 h, room temp., then extraction with phosphate buffer or filtration; e) MeOH/NH_3, 0 °C to room temp., 16 h, 90% (two steps)

The thus accessible tripeptide analog **1** was spectroscopically pure without further purification, and a single recrystallization (from *i*PrOH) gave crystals suitable for an X-ray crystallographic analysis (Figure 2).[41] Surprisingly, the substrate has a turn conformation even though no stabilizing central hydrogen bond is present. This so-called open turn conformation[42] is in fact stabilized by a hydrogen bond between the β-lactam carbonyl group (O1 in Figure 2) and the neighboring NH moiety (N2). The resulting six-membered twist-boat-like ring is not only present in the solid state but also in solution in $CDCl_3$, as has been determined by NMR spectroscopy (by inspection of the respective coupling constants). This stabilization is only possible as a result of the additional alkylidene group between the nitrogen atom and the β-lactam ring. The angle between incoming and leaving peptide strands (i.e. the vectors at

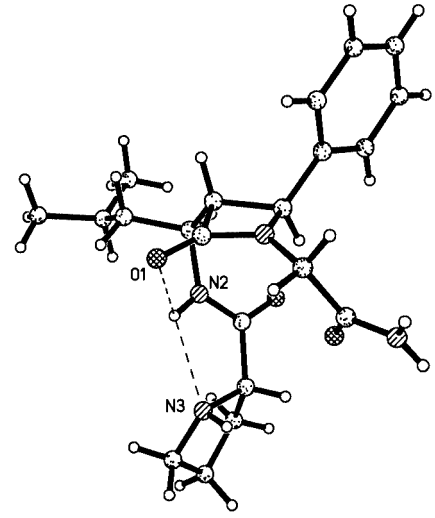


Figure 2. X-ray crystal structure of the turn mimic **1** (labels are due to the X-ray crystallographic processing, they do not indicate the usual turn numbering)

which the peptide chain enters and leaves the turn mimetic) is 185.3°, demonstrating the almost perfect turn conformation of the substrate.[43]

Conclusion

Most published turn mimetics do not allow for a flexible positioning of side chains in the turn area. The substrates **B** presented here carry up to three residues which perfectly mimic a peptide in the turn region. This provides for further flexibility in the construction and optimization of possibly biologically active compounds.[44,45] The fact that no central hydrogen bond is needed to stabilize the turn conformation gives further possibilities for variation. It is feasible that the peptide backbone, which usually supplies the hydrogen bonds, is not even essential in the termini of the turn region.

Investigations to elucidate the scope of the accessible turn mimics and to incorporate this and similar substrates in longer peptide chains are now in progress.

Experimental Section

Synthesis of Turn Mimic 1. Method A: The protected peptide analog **5** (30 mg, 45 μmol) in CH_2Cl_2 (5 mL) was added to polymer-bound TAEA (1.00 g, 700 μmol) and stirred for about 72 h at room temp. (the reaction was monitored by TLC). The mixture was then filtered through a sintered-glass frit and the solvent was removed in a rotary evaporator. The resulting clear oil was chilled to 0 °C, cold methanolic NH_3 (2 mL, ca. 7.5 M) was added and the mixture was stirred for 16 h whilst warming to room temp. The volatile material was removed in a rotary evaporator yielding a clear oil which slowly crystallized. Yield: 16 mg, 92%. **Method B:** The protected peptide analog **5** (30 mg, 0.045 mmol) and TAEA (135 μL, 900 μmol) in CH_2Cl_2 (1 mL) were stirred for about 30 min at room temp.; CH_2Cl_2 was then added and the solution was extracted with brine (three times), buffer (once; 13.1 g Na_2HPO_4, 40.7 g $NaH_2PO_4 \cdot 2H_2O$ in 200 mL H_2O) and brine (once), and then dried with $MgSO_4$. The solvent was removed in a rotary evaporator giving a clear oil which was chilled to 0 °C. Cold methanolic NH_3 (2 mL, ca. 7.5 M) was added and the mixture was stirred for 16 h whilst warming to room temp. The volatile material was removed in a rotary evaporator yielding a clear oil which slowly crystallized. Yield: 14 mg, 81%.

1: Colorless crystals, m.p. 199−201 °C. $[\alpha]_D^{20} = -36.5$ ($c = 1$, $CHCl_3$). IR (KBr): $\tilde{\nu}$ = 3418, 3253, 2940, 1755, 1670, 1635, 1520 cm^{-1}. ^1H NMR ($CDCl_3$, 500 MHz): δ = 0.86 (d, J = 6.7 Hz, 3 H, H-δ_{Leu}), 0.91 (d, J = 6.6 Hz, 3 H, H-δ_{Leu}), 1.32 (ddd, J_{gem} = 13.9, J = 9.2, J = 4.7 Hz, 1 H, H-β_{Leu}), 1.56 (dseptd, J = 9.2, J = 6.6, J = 5.1 Hz, 1 H, H-γ_{Leu}), 1.65−1.76 (m, 2 H, H-γ_{Pro}), 1.78 (ddd, J_{gem} = 13.9, J = 10.7, J = 5.1 Hz, 1 H, H-β_{Leu}), 1.88 (dddd, J_{gem} = 12.9, J = 7.5, J = 5.8, J = 5.7 Hz, 1 H, H-β_{Pro}), 2.15 (dddd, J_{gem} = 12.9, J = 9.2, J = 7.5, J = 7.5 Hz, 1 H, H-β_{Pro}), 2.23 (br. s, 1 H, $NHPr_o$), 2.90 (ddd, J_{gem} = 10.0, J = 6.4, J = 5.9 Hz, 1 H, H-δ_{Pro}), 3.00 (ddd, J_{gem} = 10.0, J = 7.1, J = 7.0 Hz, 1 H, H-δ_{Pro}), 3.09 (dd, J = 4.1, J = 2.2 Hz, 1 H, H-3), 3.26 (d, J_{gem} = 17.1 Hz, 1 H, H-α_{Gly}), 3.82 (dd, J = 9.2, J = 5.5 Hz, 1 H, H-α_{Pro}), 4.27 (d, J_{gem} = 17.1 Hz, 1 H, H-α_{Gly}), 4.45 (dddd, J = 10.7, J = 9.8, J = 4.7, J = 4.1 Hz, 1 H, H-α_{Leu}), 4.60 (d, J = 2.2 Hz, 1 H, H-4), 5.68, 6.47 (br. s, 2 H, CONH_2), 7.28−7.31 (m, 2 H, aryl-CH), 7.32−7.40 (m, 3 H, aryl-CH), 7.86 (d, J = 9.8 Hz, 1 H, NH_{Leu}) ppm. ^{13}C NMR ($CDCl_3$, 125 MHz): δ = 21.5, 23.2 (C-δ_{Leu}), 25.0 (C-γ_{Leu}), 26.2 (C-γ_{Pro}), 30.6 (C-β_{Pro}), 42.4 (C-β_{Leu}), 43.5 (C-α_{Gly}), 45.1 (C-α_{Leu}), 47.1 (C-δ_{Pro}), 59.5 (C-4), 60.6 (C-α_{Pro}), 65.6 (C-3), 126.5, 128.7, 129.1 (aryl-C), 136.8 (aryl-C_{ipso}), 168.5, 169.5, 176.5 (C=O) ppm. MS (EI, 70 eV): m/z (%) = 386 [M^+], 70 [$C_4H_7NH^+$]. HRMS (EI, 70 eV): $^{12}C_{21}{}^1H_{30}{}^{14}N_4{}^{16}O_3$: calcd. 386.2317; found 386.2317. $C_{21}H_{30}N_4O_3$ (386.5): calcd. C 65.26, H 7.82, N 14.50; found C 64.95, H 7.78, N 14.33.

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