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PEPTIDE BACKBONE-TO-BACKBONE CYCLISATION AS AN AVENUE TO β-TURN MIMICS

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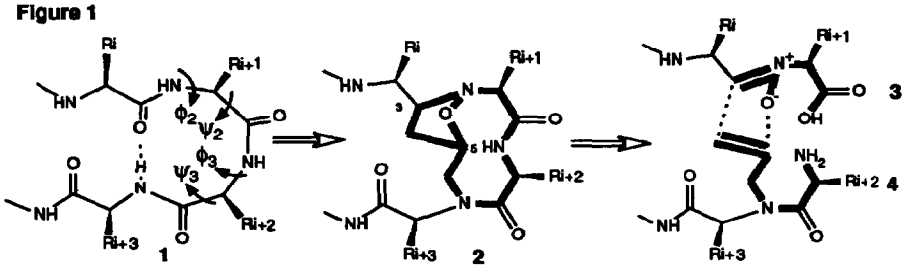
Abstract: 1,3-Dipolar cycloaddition of the nitrone functionality of **13** and the alkene functionality of **8** yields the backbone-to-backbone cyclised peptides **14-16**. The conformation of these structures is such that they are β-turn mimics. They differ in their C3/C5 stereochemistry with discrete conformational differences.

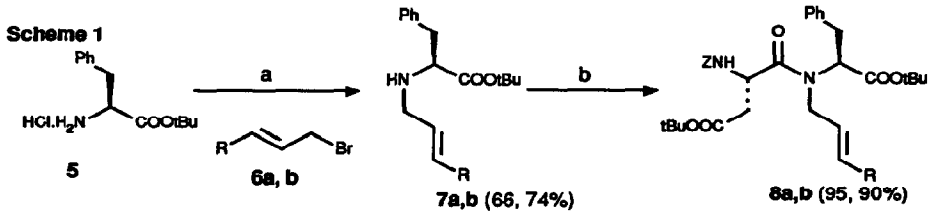
An increasing interest in peptide secondary structure mimics (*i.e.* α-helix, γ- and β-turns) built-up from scaffold-like molecules is notable.[1] Among these secondary structure mimics, considerable attention has been paid to those mimicking β-turns. Thirteen types of β-turns can be distinguished that vary in their dihedral angles ϕ_2, ψ_2, ϕ_3, and ψ_3 (Figure 1).

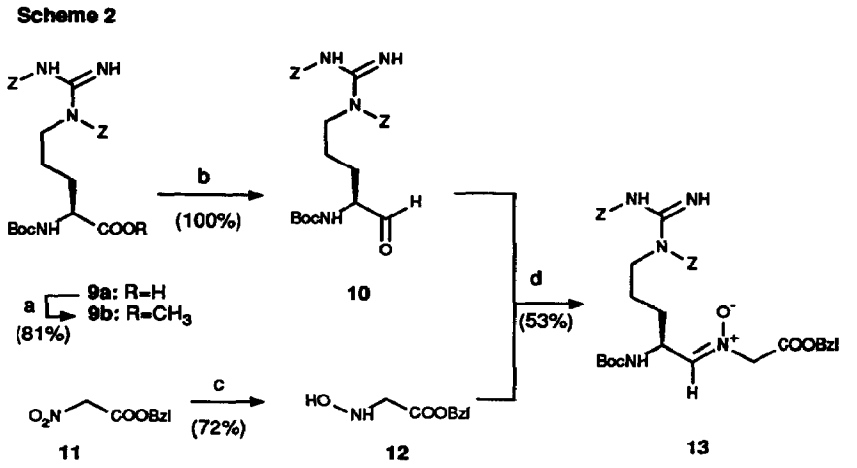
Figure 1



A mimic of a β-turn should fulfil the following criteria.[1] It should: *(i)* reproduce the spacial area of a β-turn *(ii)* contain the side chains of the amino acid residues i+1 and i+2 in the correct stereochemistry *(iii)* minimize steric interactions beyond the peptide backbone[1f] *(iv)* still contain the N- and C-terminal ends. A backbone-to-backbone cyclisation in a peptide sequence would fulfil these criteria.[2] This cyclisation might be achieved by replacement of the N-H---C=O H-bridge in **1** by a covalent bond.[3] For the formation of this covalent bond we selected the 1,3-dipolar nitrone-alkene cycloaddition reaction. The reaction partners **3** and **4** are accessible starting from amino acids and the adduct is the bicyclic, rigid scaffold **2**. Part of this structure is a isoxazolidine moiety having two new chiral centres (C(3) and C(5)). Thus the cycloaddition reaction will give a mixture of stereoisomers representing several types of the β-turn.[4]

As a touchstone of this approach, we set out to prepare the RGDF β-turn mimics[5] **14-16**, all sharing the scaffold **2**. Alkylation of **5** with the allylbromides **6** was performed under *phase transfer conditions* (PTC) resulting in the desired mono-alkylated products **7** as main product (Scheme 1). As side-product, some bis-alkylated amine was isolated (5-10%). Several of the established methods for peptide coupling failed to give **8** on reaction of **7** with Z-Asp(OtBu)-OH.[6] The method of choice was found to be the addition of two



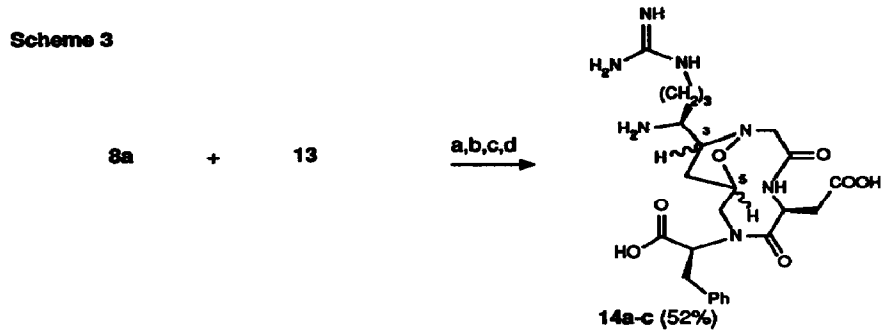


equivalents of DCC as well as Z-Asp(OtBu)-OH to **7a** (**7b**).

Boc-Arg(Z)$_2$-OH (**9a**)[7] was transformed into the corresponding argininal derivative **10** *via* the methyl ester **9b** (Scheme 2). The aldehyde was not isolated as such,[8-10] but immediately treated with N-hydroxyglycine (**12**), obtained from compound **11**.[11] We assume that the nitrone moiety of the dipeptide **13** has a Z-configuration.[12] 1,3-Dipolar cycloaddition of the nitrone functionality of **13** and the alkene moiety of **8a** under high pressure conditions[13] gave the backbone-to-backbone linkage (Scheme 3). Subsequently, compound **14** was prepared by the following three steps: reductive removal of the protective groups Z and Bzl, amide bond formation and finally removal of the protective groups Boc and tBu. Preparative HPLC separation gave three compounds **14** in a ratio of **14a/14b/14c** = 1/1/3. These compounds differ in the stereochemistry at the atom C(3) and C(5); The NMR spectra were too complex to allow an assignment of the relative stereochemistry.[14]

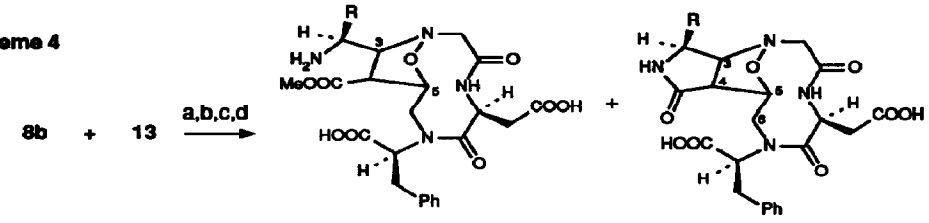
In an analogous fashion, the alkene **8b** was subjected to the nitrone cycloaddition reaction. Surprisingly, a mixture of the two diastereomers **15a** and **15b** (ratio 1:1) and the two tricyclic diastereomers **16a** and **16b** (ratio 2:1) was isolated in an overall yield of 49% (Scheme 4).[15] Apparently, an additional cyclisation had occurred in those diastereomers of **15** of which the protons of C(3) and C(4) of the isoxazolidine moiety have a *cis*-relationship, resulting in the tricyclic compounds **16a,b**. NOE measurements on the protons

Scheme 3



a) toluene, 15 kbar, 50°C, 2 days b) MeOH, Pd/C, H_2 c) DMF (0.0001M), TBTU (1 equiv.) pH 8.0, RT d) TFA/phenol/H_2O/HSi(i-C_3H_7)$_3$ (88/5/5/2)

Scheme 4



a) toluene, 15 kbar, 50°C, 2 days b) MeOH, Pd/C, H_2 c) DMF (0.0001M), TBTU (1 equiv.), pH 8.0, RT d) TFA/phenol/H_2O/HSi(i-C_3H_7)$_3$ (88/5/5/2)

Hα(Arg), H3, H4 and H5 could discriminate between compounds **16a** and **16b**. For both compounds, cross-peaks (NOE) were observed between the protons H3/H4; in the NOESY spectra no cross-peak was observed between H3/H5. For **16a**, an additional cross-peak between the protons H3/Hα(Arg) was present. For the bicyclic compounds **15a,b**, NOE experiments failed to give conclusive evidence regarding the configuration of the carbon atoms C(3) and C(5).

None of the compounds **14-16** showed inhibitory activity in the ADP-induced human platelet aggregation assay (GP IIb/IIIa receptor).[16] This finding suggests that the bioactive conformation of RGD required for the GP IIb/IIIa receptor is not mimicked by the β-turns **14-16**.[17]

However, we demonstrated that the 1,3-dipolar cycloaddition as method for the backbone-to-backbone cyclisation provides several stereoisomers of a relatively rigid, scaffold-based molecule, allowing the study of the bioactive conformation of a given peptide. In the present study, we focussed on the β-turn motif; however, we expect that the 1,3-dipolar cycloaddition as a method for backbone-to-backbone cyclisation might also be applicable to generate mimics featuring a γ-turn or an α-helical scaffold.

References and Notes

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12. NMR spectroscopy did not reveal any E-Z isomerisation. In analogy with analogous nitrones a Z-configuration was asumed, see Grundke, G.; Keese, W.; Rimpler, M. *Liebigs Ann.Chem.* **1987**, 73.
13. Cycloaddition at elevated temperature (60-80°C) failed as a result of the thermal instability of **13**.
14. The purity of **14a-c** (HPLC) was between 90 and 95%; FABMS 534 (M+H), 532 (M-H)
15. **15a-b**: HPLC purities were between 80 and 90%; FABMS 591 (M+H), 589 (M-H); The NMR spectra were to complex to allow an assignment of the relative stereochemistry.
16a: HPLC 96%; FABMS (%) 560 ([M+H]$^+$, 22), 558 ([M-H]$^+$, 11); δ(D_2O) 7.42-7.27 (m, 5H, C_6H_5), 4.90 (dd, 1H, J=5.6 Hz and J=9.0 Hz, $C_\alpha H$(Phe)), 4.65 (d, 1H, $C_\alpha H$(Asp), J=11.0 Hz), 4.24 (dd, 1H, H(3), $J_{3,4}$=6.2Hz,$J_{3,\alpha H(Arg)}$=4.0Hz), 3.99 (dd, 1H,, H(5), $J_{4,5}$<0.3 Hz, $J_{5,6}$=4.4 Hz, $J_{5,6'}$=11.0 Hz), 3.81 (m, 1H, $C_\alpha H$(Arg)), 3.82 (d, 1H, CH_A(Gly), ^{2}J=14.0 Hz), 3.36 (m, 1H, H(6')), 3.26 (m, 1H, H(6)), 3.25 (d, 1H, CH_B(Gly), ^{2}J=14.0 Hz), 3.25-3.19 (m, 2H, $C_\delta H_2$(Arg)), 3.16 (d, 1H, H(4), $J_{4,3}$=6.2 Hz), 2.82 (dd, 1H, $C_\beta H_A$(Asp), J=11.0 Hz, ^{2}J=16.5 Hz), 2.78 (dd, 1H, $C_\beta H_A$(Phe), J=9.0 Hz, ^{2}J=15.0 Hz), 2.41 (dd, 1H, $C_\beta H_B$(Phe), J=5.6 Hz, ^{2}J=15.0 Hz), 2.01 (br d, 1H, $C_\beta H_B$(Asp), ^{2}J=16.5 Hz), 1.74-1.59 (m, 4H, $C_\beta H_2 C_\gamma H_2$ (Arg))
16b: HPLC 97.3%; FABMS 560 (M+H), 558 (M-H); δ(D_2O) 7.45-7.32 (m, 5H, C_6H_5), 5.47 (d, 1H, J=1.0 Hz), 5.13 (t, 1H, H(3), $^3J_{3,4}$=8.0 Hz), 4.32 (dt, 1H, $C_\alpha H$(Asp), J=1.0 Hz, J=4.0 Hz), 4.27 (dt, 1H, H(5), $J_{5,4}$=11.5 Hz, $J_{5,6}$=2.0 Hz, $J_{5,6'}$=9.5 Hz), 4.05 (m, 1H, $C_\alpha H$(Arg)), 3.99 (t, 1H, $C_\alpha H$(Phe), J=7.0 Hz), 3.49 (dd, 1H, H(6), $J_{6,5}$=2.0 Hz, $^2J_{6,6'}$=13.0 Hz), 3.34 (dd, 1H, H(6'), $J_{6',5}$=9.5 Hz, $^2J_{6',6}$=13.0 Hz), 3.26 (d, 2H, $C_\beta H_2$(Phe)), J=7.0 Hz), 3.27-3.15 (m, 2H, $C_\delta H_2$(Arg)), 2.91 (dd, 1H, $C_\beta H_A$(Asp), J=4.0 Hz, ^{2}J=16.5 Hz), 2.65 (dd, 1H, H(4), $J_{4,3}$=8.0 Hz), $J_{4,5}$=11.5 Hz), 2.62 (dd, 1H, $C_\beta H_B$(Asp), J, 4.0 Hz, ^{2}J=16.5 Hz), 1.85-1.50 (m, 4H, $C_\beta H_2 C_\gamma H_2$ (Arg)).
16. Activation of platelets by ADP is mediated by the GP IIb/IIIa receptor.
17. Upon completion of these studies, this suggestion was corroborated by the finding that the bioactive conformation for this receptor has a turn at Arg, extended φ, ψ angles at Gly and a γ-turn at Asp, see; Ku, T.W.; Ali, F.E.; Barton, L.S.; Bean, J.W. *et.al. J.Am.Chem.Soc.* **1993**, *115*, 8861 and references 13-15 therein. In retrospect, our choice to test the RGD-turn mimics reported here on the GP IIb/IIIa receptor was unfortunate.

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