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Synthesis of a Novel β-Turn Mimetic and its Incorporation in Leu-Enkephalin

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Abstract: A conformationally restricted β-turn mimetic, in which an ethylene bridge replaces the intramolecular hydrogen bond between residues *i* and *i+3* of β-turns, has been prepared and incorporated in Leu-enkephalin. Amino acids and α-bromoacids were used as building blocks in the synthesis of the mimetic. © 1999 Elsevier Science Ltd. All rights reserved.

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In a β-turn the polypeptide chain reverses its direction over four residues so that a hydrogen bond is often formed between the C=O of residue *i* and the NH of residue *i+3* (Figure 1).[1] β-Turns make up a large part of proteins and are predominantly located at the protein surface, where they may serve as sites of recognition. In addition, several small, biologically active peptides such as the hormones LHRH and somatostatin have been found to adopt β-turn conformations. Substantial interest has been directed towards synthesis of mimetics of β-turns to be used in *i)* studies of their capacity to nucleate β-sheet formation in small peptides or *ii)* in efforts to investigate and improve the biomedicinal activity of peptides through conformational restriction.[2-7] Introduction of a mimetic may also facilitate absorption and provide stability towards enzymatic degradation. The second of the two above objectives is inherently more demanding since the conformation of the mimetic should then accurately resemble that of the peptide backbone, while still allowing introduction of side chains at desired positions for interaction with a receptor. In fact, with a few exceptions,[4] most β-turn mimetics described to date serve only as nucleators of β-sheet formation.[7]

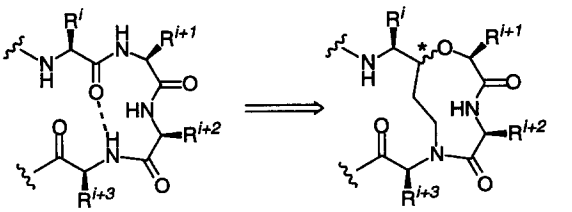

Figure 1. A β-turn and a conformationally restricted mimetic, in which the intramolecular hydrogen bond is replaced by an ethylene bridge.

We now describe an approach to a novel type of β-turn mimetic with potential for retaining the biological activity of the parent peptide (Figure 1). As an initial example of the approach the first four residues of Leu-enkephalin (**Tyr-Gly-Gly-Phe**-Leu), which may adopt a β-turn both in the crystal[8] and in solution,[9] have been replaced by such a mimetic (cf. **19**, Scheme 2). Synthesis of the turn mimetic started from commercial Z-L-Tyr(tBu)-OMe (**1**, Scheme 1). Compound **1** was converted to aldehyde **2** by sodium borohydride reduction followed by oxidation of the resulting alcohol. Attempts to perform the oxidation under Swern conditions[10] led to racemic **2**, but use of Dess-Martin periodinane[11] gave the aldehyde in enantiomerically

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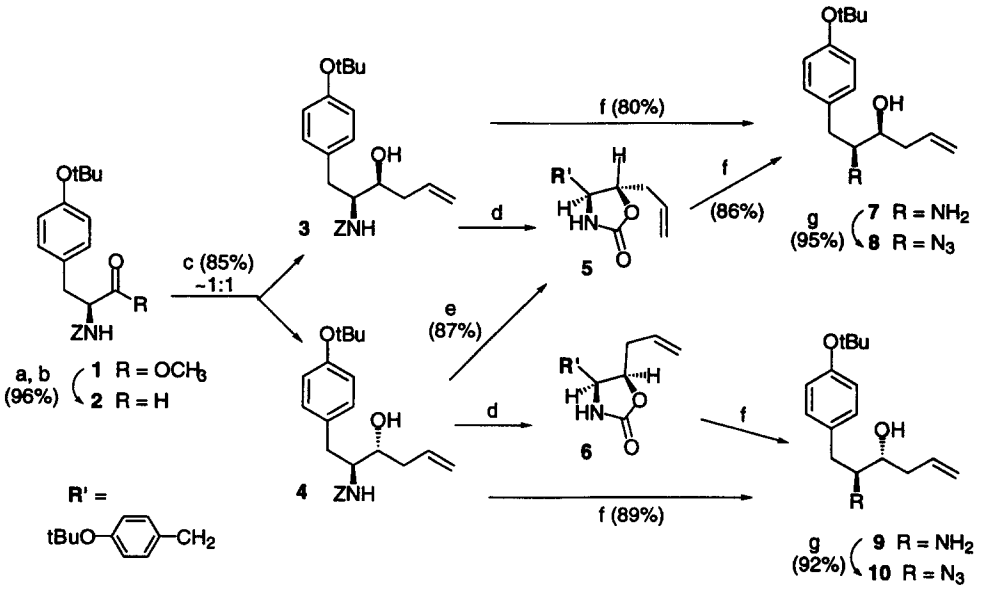
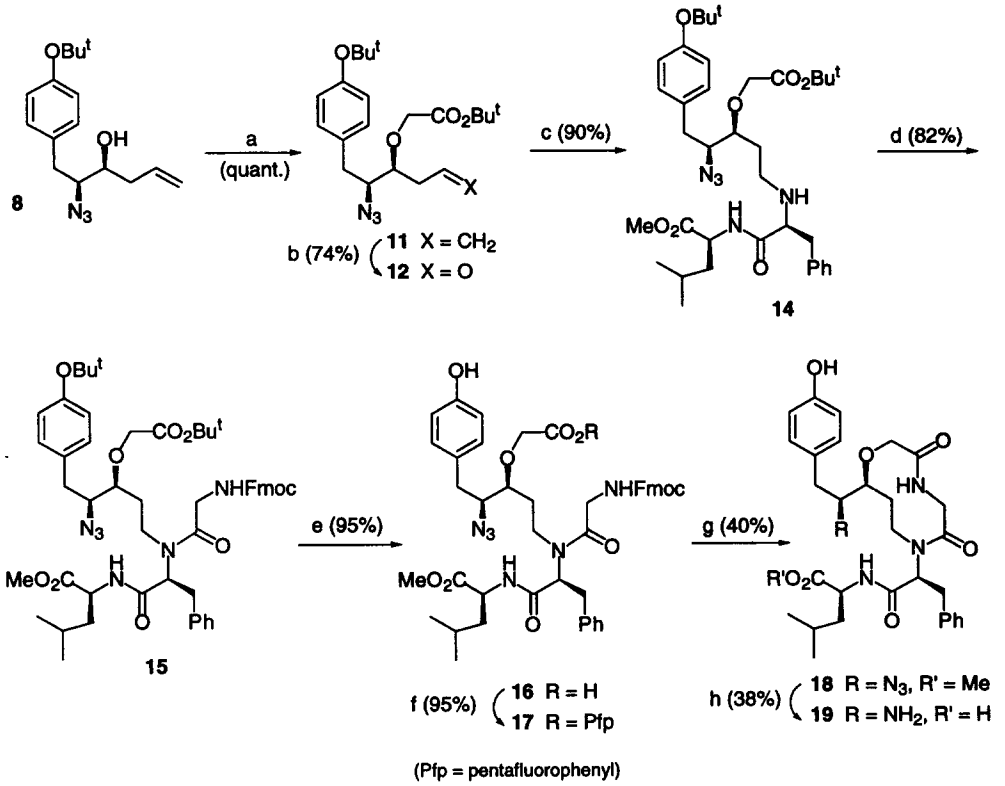
pure form. Allylation of **2** was best performed with allyl bromide and zinc under aqueous conditions[12] to give a ~1:1 mixture of the *syn*- and *anti*-2-amino alcohols **3** and **4**, which could be separated by careful flash column chromatography. Compounds **3** and **4** are key building blocks that contain both residue *i* and the ethylene bridge of the β-turn mimetic, and their relative stereochemistry was elucidated by base-catalyzed conversion into oxazolidinones **5** and **6**, respectively. For **5** irradiation of H-4 gave a 5% nOe for H-5, whereas a 14% enhancement was found for H-5 on saturation of H-4 in **6**.



Scheme 1. a) $NaBH_4$, THF/MeOH (20:1), -10 °C → room temp., 16 h; b) Dess-Martin periodinane, CH_2Cl_2, room temp., 1 h; c) Allyl bromide, Zn, THF/aq. NH_4Cl (1:5), 0 °C, 1.5 h; d) aq. 7.5 M KOH/MeOH/THF (1:2:4), room temp., 5 h; e) MsCl, NEt_3, CH_2Cl_2, 0 °C, 2 h then 1,2-dichloroethane, reflux, 24 h; f) aq. 1 M KOH/EtOH (1:1), reflux, 5 h; g) TfN_3, DMAP, $CuSO_4$ (cat.), CH_2Cl_2, room temp., 2 h.

Initial attempts to *O*-alkylate amino alcohols **3** and **4** with esters of α-bromo acetic acid failed and gave only oxazolidinones **5** and **6** which were alkylated on the ring nitrogen. The benzyloxycarbonyl group was therefore removed from **3** and **4** by basic hydrolysis to give amino alcohols **7** and **9**, most likely with **5** and **6** as intermediates. Amines **7** and **9** were converted[13] into azides **8**[14] and **10** by treatment with triflic azide in the presence of a catalytic amount of $CuSO_4$. This transformation served the dual purpose of introducing an inert *N*-protective group as well as reducing the steric bulk in the vicinity of the hydroxyl group. Compounds **8** and **10** could then be alkylated with *tert*-butyl bromoacetate using phase-transfer catalysis.[15] In this manner **8** was converted in quantitative yield to **11** (Scheme 2), whereas **10** gave a somewhat less pure product. The *O*-alkylation products were unstable during flash chromatography and it was decided to proceed directly with crude **11** towards a β-turn mimetic. The overall yield of the *syn*-amino alcohol **7**, and thereby *O*-alkylated **11**, could be improved by diastereoconversion[16,17] of **4**. Thus, mesylation of the hydroxyl group in **4** followed by intramolecular ring-closure, gave oxazolidinone **5** which was hydrolyzed to **7** (75 % from **4**).



Scheme 2. a) $BrCH_2CO_2tBu$, $Bu_4N^+HSO_4^-$, aq. 50% NaOH/C_6H_6 (1:1), room temp., 1 h. b) OsO_4 (cat.), NMO, THF/acetone/H_2O (1:1:1), room temp., 2 h, then $Pb(OAc)_4$, Na_2CO_3, benzene, 0 °C → room temp., 3 h; c) H-Phe-Leu-OMe (**15**), $NaBH(OAc)_3$, NEt_3, 1,2-dichloroethane, room temp., 30 min; d) Fmoc-Gly-OH, DIC, CH_2Cl_2, 0 °C → room temp., 16 h; e) HCO_2H, room temp., 16 h; f) PfpOH, DCC, EtOAc, 0 °C, 2 h; g) DBU, dioxane, 100 °C, 4.5 h; h) aq. 1 M LiOH, dioxane, 10 °C → room temp., 2 h then H_2, Pd/C, MeOH, 48 h.

Ozonolytic cleavage of the alkene moiety of **11** failed and aldehyde **12** was instead obtained by osmium tetroxide catalysed dihydroxylation followed by cleavage of the resulting diol with lead tetraacetate (Scheme 2). Reductive amination[18] of the dipeptide H-Phe-Leu-OMe (**13**) with **12** using sodium triacetoxyborohydride as reducing agent gave **14** and thus introduced the phenylalanine residue at the *i+3* position of the turn mimetic. The last unit of the mimetic was then introduced at the *i+2* position by coupling of Fmoc-Gly-OH to **14** using diisopropylcarbodiimide as coupling reagent. Treatment of the resulting **15** with formic acid removed the *tert*-butyl ester, as well as the phenolic *tert*-butyl ether, to give acid **16**. Attempts to convert **16** into **18** via Fmoc-cleavage followed by amide bond formation promoted by coupling reagents such as HATU[19] all failed. Instead **16** was converted into pentafluorophenyl ester **17** which was added to DBU in hot dioxane under high dilution conditions. Thus **18**[14] was obtained in a one-pot procedure[20] involving Fmoc-deprotection followed by ring closure of the 10-membered ring. Finally, deprotection of **18** to give Leu-enkephalin mimetic **19** was performed on a small scale by hydrolysis of the methyl ester followed by

hydrogenation of the azido group. Most likely the yields in these two final deprotection steps can be improved.

In summary, the design and synthesis of a novel type of β-turn mimetic assembled from amino acids and α-bromoacids as building blocks has been described. Future studies will deal with insertion of building blocks with more complex side-chains at the *i+1* and *i+2* positions of the turn mimetic. The conformation induced by incorporation of the mimetics in peptide hormones, as well as the biological activity of the resulting peptidomimetics, will also be investigated.

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