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SYNTHESIS AND ANALYSIS OF LEU-ENKEPHALIN ANALOGUES CONTAINING REVERSE TURN PEPTIDOMIMETICS§

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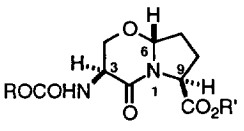
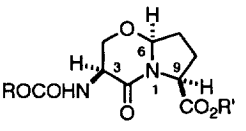
Abstract: the synthesis and ^{1}H n.m.r. analysis of peptidomimetics of Leu-enkephalin containing bicyclic lactams is described.

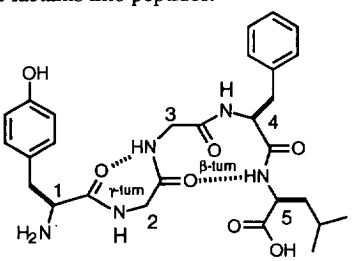
Interest in peptide mimetics including analogues of reverse turns particularly β-turns is currently high.[1] Examples of peptidomimetics which have been synthesised in order to induce reverse turns include several lactams[2], spirocyclic compounds[3] and benzodiazopines[4]. N-Methylated[5], α,α-dialkyl[6] and dihydroamino acids[7] have also been used in order to induce reverse turns. We have recently reported[8] the synthesis of 6,5-bicyclic lactams (**1** and **2**) intended for evaluation as β or reverse turn dipeptide mimetics. Herein we demonstrate the that it is possible to incorporate the (3*S*,6*R*,9*S*) (**1**) and (3*S*,6*S*,9*S*) (**2**) bicyclic lactams into peptides.



(**1**)-(3*S*,6*R*,9*S*)
(**3**) R = $PhCH_2$, R' = CH_2Ph
(**5**) R = ^{t}Bu, R' = CH_2Ph



(**2**)-(3*S*,6*S*,9*S*)
(**4**) R = $PhCH_2$, R' = CH_2Ph
(**6**) R = ^{t}Bu, R' = CH_2Ph



Leu-enkephalin

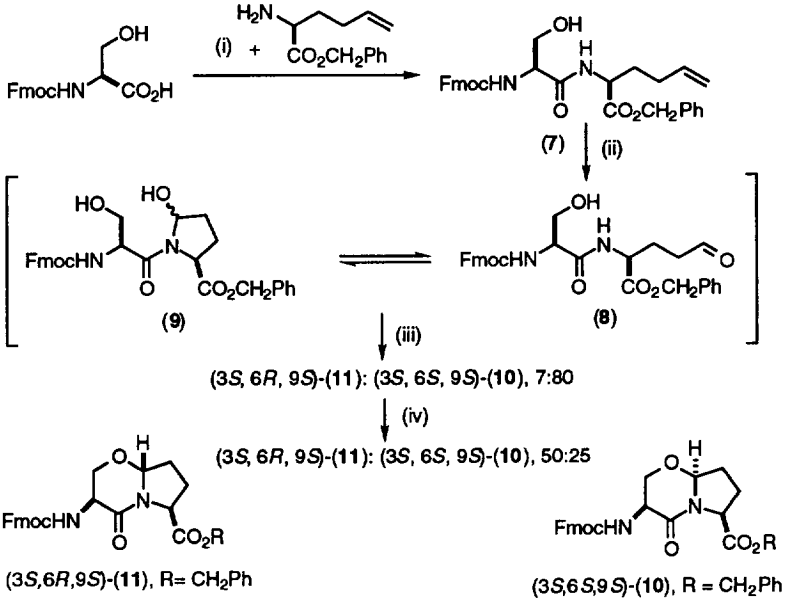
Figure 1

Previous studies on Leu-enkephalin (Tyr-Gly-Gly-Phe-Leu)[9, 10] have shown it to adopt, predominantly, a β-turn centred on Gly3-Phe4 with a γ-turn centred on Gly2 present simultaneously[10] (Figure 1). We were interested in the incorporation of the bicyclic lactams (**1**) and (**2**) into Leu-enkephalin analogues in order to probe the bioactive conformation of Leu-enkephalin.

We have previously described the synthesis of the (3*S*,6*R*,9*S*) and (3*S*,6*S*,9*S*) N-benzyloxycarbonyl [(**3**) and (**4**), respectively] and N-*tert* butyloxycarbonyl [(**5**) and (**6**), respectively] protected bicyclic lactams.[8] However, it was not possible to effect efficient selective deprotection of one protecting group in the case of the N-Z protected compounds. Attempted alkaline hydrolysis of the benzyl ester led to partial epimerisation of the C-3 stereochemistry[8]. The corresponding N-Boc compounds were also not ideal for further elaboration since the acid labile nature of the amino protecting group made efficient epimerisation of the (3*S*,6*S*,9*S*) lactam (**6**) to the

(3*S*,6*R*,9*S*) lactam (**5**) difficult. Thus, in order to make the attachment of the other amino acid residues onto the lactams possible, the Boc group was replaced by a fluorenylmethoxycarbonyl (Fmoc) protecting group. It was envisaged that the amino protected 6,5-bicylic lactams would be useful for incorporation into peptides (and combinatorial libraries) via the 'Fmoc strategy' for solid phase peptide synthesis[11].

Thus, N-Fmoc-*S*-serine[12] was coupled with *S*-but-3-enyl glycine benzyl ester[13] to give dipeptide (**7**). Reaction of ozone with (**7**) followed treatment with PPh_3 gave the aldehyde (**8**) which was in equilibrium with the epimeric hemi-aminals (**9**) [by ^1H n.m.r. (500MHz) analysis]. The ozonolysis procedure was found to be superior to the previously reported oxidation procedure using $NaIO_4$ and cat. OsO_4[8]. The crude aldehyde/hemiaminal mixture was then converted by treatment with cat. CF_3CO_2H (CH_2Cl_2, reflux, 1 hour) to a *ca.* 9:1 mixture of the (3*S*,6*S*,9*S*) (**10**) to (3*S*,6*R*,9*S*) (**11**) mixture of bicyclic lactams (by ^1H n.m.r. analysis of the crude reaction mixture). The (3*S*,6*S*,9*S*) lactam (**10**) [isolated yield 80%] was easily purified from (3*S*,6*R*,9*S*) (**11**) [isolated yield 7%] by flash chromatography (diethyl ether).

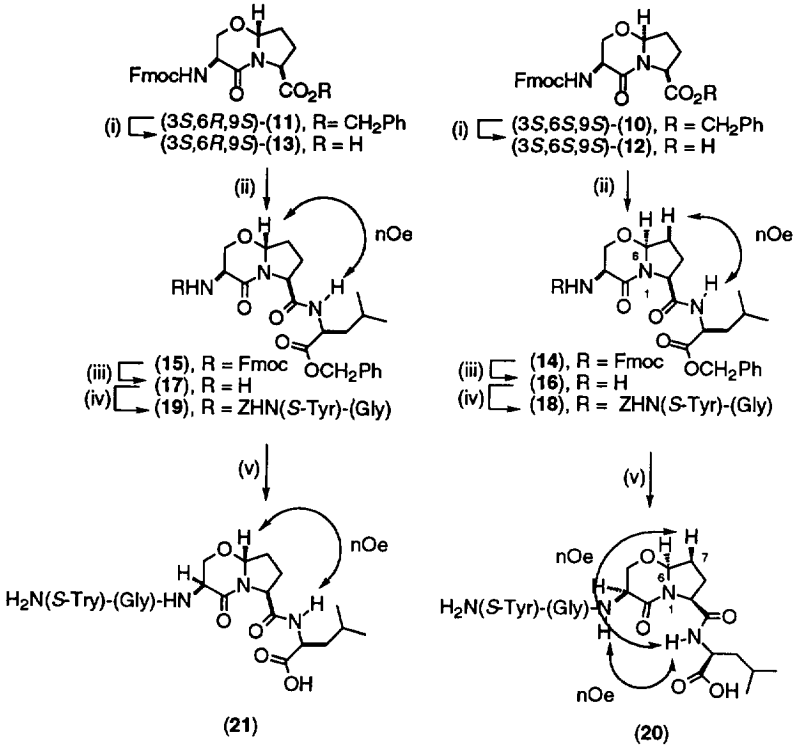


(i) EDCI/HOBT (76%); (ii) O_3, CH_2Cl_2, -78 °C, then Ph_3P; (iii) cat. CF_3CO_2H, CH_2Cl_2, reflux 1 hour [(3*S*,6*R*,9*S*)-(**11**) (7%), (3*S*,6*S*,9*S*)-(**10**) (80%), 2 steps]; (iv) CF_3CO_2H, 3 days [(3*S*,6*R*,9*S*)-(**11**) (50%), (3*S*,6*S*,9*S*)-(**10**) (25%). All yields refer to isolated compounds. Satisfactory microanalytical and/or spectroscopic data were obtained for all isolated compounds.

Scheme 1

As previously described in the case of the N-benzyloxycarbonyl series, in order to optimise production of the (3*S*,6*R*,9*S*) lactam (**11**), either the 9:1 mixture of (3*S*,6*S*,9*S*) (**10**): (3*S*,6*R*,9*S*) (**11**) or pure (3*S*,6*S*,9*S*) (**10**) was treated with neat CF_3CO_2H for 3 days to give a ca. 50% yield of (3*S*,6*R*,9*S*) (**11**) and ca. 25 % of recovered (3*S*,6*S*,9*S*,) (**10**) after chromatographic purification (Scheme 1). Hydrogenolysis (H_2/Pd/C) of benzyl esters (**10**)

and (**11**) gave acids (**12**) and (**13**), which were coupled with (*S*)-leucine benzyl ester to give (**14**) and (**15**) respectively. Cleavage of the Fmoc groups of (**14**) and (**15**) was effected using KF in the presence of 18-crown-6[14] to give (**16**) and (**17**). No epimerisation (by ^{1}H n.m.r. analysis) was observed using this procedure which gave better yields then the use of piperidine for Fmoc deprotection. Coupling with N-benzyoxycarbonyl-(*S*)-tyrosinyl-glycine gave the desired Leu-enkephalin analogues (**18**) and (**19**) in protected form which were deprotected by hydrogenolysis to give (**20**) and (**21**) respectively (Scheme 2). At no stage during the synthesis of the analogues (**20**) and (**21**) was any evidence for epimerisation of the (3*S*,6*S*,9*S*) to (3*S*,6*R*,9*S*) diastereomer or vice versa observed by ^{1}H n.m.r. or t.l.c. analysis.



(**21**) (**20**)

(i) H_2/Pd/C, EtOH [97% for (3*S*,6*S*,9*S*)-(**12**), 96% for (3*S*,6*R*,9*S*)-(**13**)]; (ii) (*S*)-leucine benzyl ester, EDCI/HOBT [86% for (3*S*,6*S*,9*S*)-(**14**) and 71% for (3*S*,6*R*,9*S*)-(**15**); (iii) KF, cat. 18-crown-6, HCONMe2 [96% for (3*S*,6*S*,9*S*)-(**16**) and 79% for (3*S*,6*R*,9*S*)-(**17**); (iv) EDCI/HOBT/Z-(S)-tyr-gly-OH [69% for (3*S*,6*S*,9*S*)-(**18**) and 73% for (3*S*,6*R*,9*S*)-(**19**); (v) H_2/Pd/C, H_2O, THF [quantative for (3*S*,6*S*,9*S*)-(**20**) and 93% for (3*S*,6*R*,9*S*)-(**21**). For the synthesis of Z-(*S*)-Tyr-Gly-OH: Z-(*S*)-Tyr-OH was coupled (EDCI,HOBT,Et_3N) with the HCl salt of Gly-O^{t}Bu to give Z-(*S*)-Tyr-Gly-O^{t}Bu (86%) followed by removal of the tert-butyl ester (CF_3CO_2H,PhOMe) (96%). All yields refer to isolated compounds. Satisfactory microanalytical and/or spectroscopic data were obtained for all isolated compounds.

Scheme 2

The two protected analogues (**18**) and (**19**), (**20**) and (**21**) and Leu-enkephalin itself were analysed by variable temperature studies, 2D rotating frame Overhauser effect spectroscopy (ROESY)[15]and in the case of ambiguity in the 2D spectra by 1D nOe difference spectroscopy.

Residue	Leu-enkephalin	Protected (*S, R, S*) (**19**)	Protected (*S, S, S*) (**18**)	Unprotected (*S, R, S*) (**21**)	Unprotected (*S, S, S*) (**20**)
Tyr1	*	-9.6	-9.8	*	*
Gly2	-13.0¶	-8.1	-8.0	-15.5	-10.1
Res3	-7.6	-6.4	-7.4	-9.6	-9.0
Res4	-8.9	/	/	/	/
Leu5	-3.9	-6.4	-7.0	-5.3	+2.8

Table 1: Temperature coefficients (ppb/K). Solutions were prepared in ($^2H_7$)-DMF and 1D spectra recorded at 5° intervals between 303 and 328 K; * Not possible to estimate as the resonance was excessively broadened by exchange with residual H_2O; ¶ This proton was undergoing exchange with residual H_2O. This probably contributes to the high coefficient and cause the observed slight curvature of the shift vs. temperature plot. The value reported is the best fit linear value. All data were acquired on a Bruker AMX500 instrument. ROESY experiments were performed with the phase alternating spin-lock sequence (5.9 kHz field) to suppress coherent magnetisation transfer, as suggested by Shaka.[15] Amino acid residues are numbered sequenencially from the N-terminus, with the bicyclic lactam as residues 3 and 4. The available $^3J_{NH\alpha}$ values for the mimetics [(**18**), (**19**), (**20**) and (**21**)] were unremarkable in the 6-8 Hz range for non glycyl residues.

Temperature coefficients for the amide protons of Leu5 and Gly3 in Leu-enkephalin[9] are consistent with the shielding of these protons, suggestive of the requisite intramolecular H-bonds in the proposed β and γ reverse turns. Furthermore, the presence of the Phe4 NH-Leu5 NH nOe observed in our n.m.r. experiments on Leu-enkephalin is in accord with the presence of the β-turn.[16] Due to the lack of an amide proton at the equivalent 4 position of the peptidomimetics (**18**), (**19**), (**20**) and (**21**), it is not possible to observe the analogous NH-NH nOe. However, in the protected (3*S*, 6*R*, 9*S*) mimetic (**19**) an nOe observed between the Leu NH and the 6-H (Scheme 2), suggests the presence of a turn in the backbone.[16] Similarly, in the (3*S*, 6*S*, 9*S*) analogue (**18**), in which the 6-H is on the opposite face to the Leu residue, a Leu5 NH to *pro-S* 7-H nOe was apparent (Scheme 2), which again suggested a population of conformers containing a reverse turn. The temperature data for the protected mimetics (**18**) and (**19**) are less indicative of the intramolecular H-bonds than those of Leu-enkephalin. Whilst some shielding was again observed for residue 3 and Leu5, those of the latter are significantly less than those of Leu-enkephalin, and suggest only a small population of conformers with internal H-bonding. However, the temperature coefficients of the unprotected (3*S*, 6*R*, 9*S*) mimetic (**21**) suggests the Leu5 amide proton to be significantly shielded, and once again the nOe between this proton and 6-H was apparent. These data suggest a significant population of conformers containing a β-turn. For this peptide (**21**) and Leu-enkephalin, the coupling between the amide and α protons of Gly2 was lost by exchange decoupling such that the α proton became a sharp AB spin system as the temperature was increased. These data are consistent with the high temperature coefficients observed for the Gly2 amide protons and indicate them to be solvent exposed and free to undergo rapid exchange. Gly2 of the unprotected (3*S*, 6*S*, 9*S*) peptide (**20**) displayed similar behaviour. Curiously, the temperature coefficient for the (3*S*, 6*S*, 9*S*) mimetic (**20**) Leu5 amide is small but positive, indicating a downfield shift with increasing temperature. This suggests the peptide (**20**) may adopt a conformation that enhances the solvent shielding of the amide proton at higher temperature (<325K in the present study). Once again, a Leu5

NH to pro-S 7-H nOe was observed, in addition to an nOe between the Leu5 NH and Gly2 NH (Scheme 2), which is not anticipated for the conventional β-turn types[16], and it seems that the turn in the (3*S*, 6*S*, 9*S*) peptide (**20**) is somewhat distorted from the geometry of an ideal β-turn.[17, 18] It was also noted that at lower temperatures (300K) the ^{1}H spectrum of the (3*S*, 6*R*, 9*S*) peptide (**21**) displayed slight broadening of all resonances indicating the onset of slower conformational exchange in the NMR timescale.

In summary, this work demonstrates that the 6,5 lactam peptidomimetics [(**1**) and (**2**)] can be incorporated into peptides and that they can induce reverse turns. Detailed studies on the conformation of the induced turns and their relationship to the different categories of β-turn[17] are the subject of ongoing investigations.

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18) Modelling studies suggest that a close approach of the Gly2 CO and Leu5 NH of (**20**) occurs if an ideal β-turn is formed, hence distortion would be anticipated to relieve this conflict. For extensive modelling studies on reverse turn analogues including species containing bicyclic lactams [including (**1**) and (**2**)] see Marshall *et al.*, ref. 5.

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