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Synthesis of an external β-turn based on the GLDV motif of cell adhesion proteins

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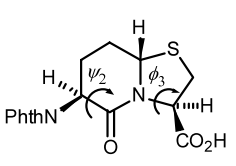
Abstract—The (3*S*,6*S*,10*S*)-7/5 bicyclic lactam **4**, designed as an external turn constraint, was synthesised by a new stereoselective route involving Eschenmoser condensation. Calculated preferred conformations compare well with the preferred solid state conformation, obtained by X-ray crystallography. The lactam **4** was not a turn mimic in its own right but could be used as an external constraint to prepare the cyclic peptide **29** containing the integrin recognition motif GLDV. High-resolution NMR measurements were consistent with this compound having a single backbone conformation.

Reverse turns connect elements of protein secondary structure and usually occur on the surface of the protein. They can, therefore, be important in molecular recognition processes.[1,2] Synthetic molecules which mimic these turns, such as the classic 5/6 bicyclic lactam BTD, **1**, prepared by Nagai,[3–5] have been used to replace β-turns in various proteins without affecting their overall biological properties.[4–6] BTD, **1**, has been shown to be a type II′ β-turn mimic from the angles ψ_2 (−161°) and ϕ_3 (−69.4°) in the X-ray crystal structure.[7] One of us has used BTD[8] as a strong structural constraint on which to build a cyclic GLDV motif. This was shown to be a type I β-turn and inhibited the interaction of the integrin $\alpha_4\beta_1$ with vascular cell adhesion molecule-1.[8]

Amino acid 7/5-bicyclic lactams of type **2** have found use as dipeptide surrogates in angiotensin-converting enzyme (ACE) inhibitors[9] and have more recently been used as external constraints for tripeptide RGD turns which were inhibitors of the $\alpha_V\beta_3$ integrin receptor.[10] The 7/5 bicyclic lactam **3** was synthesised using an electrochemical approach and conformational analysis showed that the (4*S*,7*S*,10*S*)-stereoisomer had families of minimum conformations with torsion angles close to those of classical β-turns.[11] In continuation of our work on externally constrained GLDV turns,[8] we have now developed a new and stereoselective synthesis of the 7/5 bicyclic lactam, **4**, via a route involving Eschenmoser coupling. The crystal structure of our new constraint will indicate its promise as a turn mimic, and attach-



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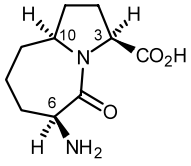

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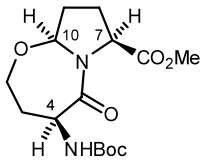

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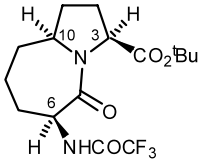

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Keywords: β-turn; protein mimetic; amino acid; bicyclic lactam.
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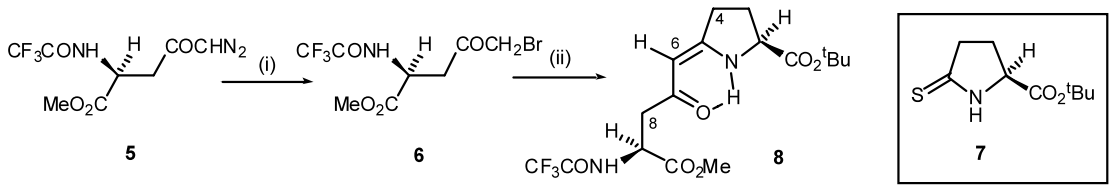
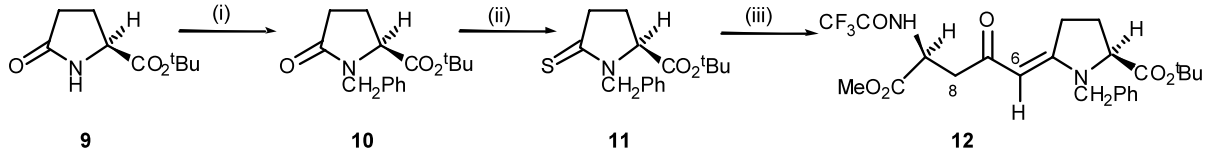


Scheme 1. *Reagents and conditions*: (i) $HBr/CHCl_3$, rt, 89%; (ii) **7**/MeCN, reflux, then Ph_3P/Et_3N, 25%.



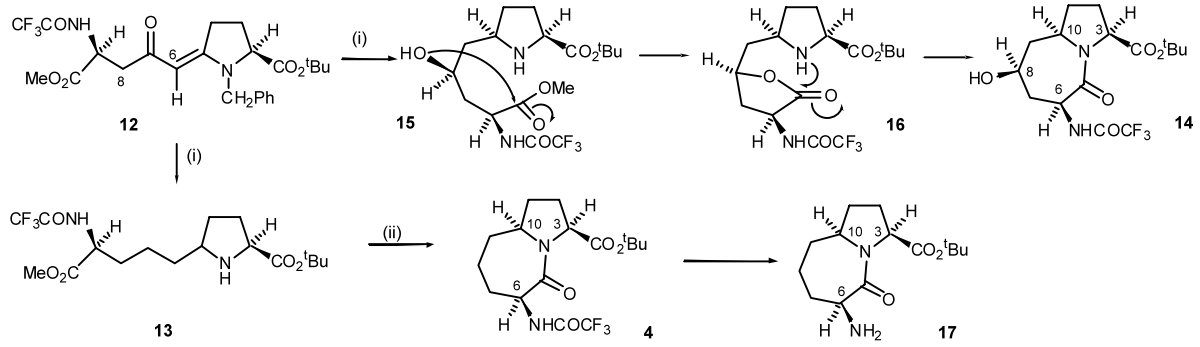
Scheme 2. *Reagents and conditions:*(i) $NaHMDS/PhCH_2Br/THF/-78°C$, 64%; (ii) $P_4S_{10}/THF/rt$, 95%; (iii) **6**/$Ph_3P/Et_3N/CH_3CN$, rt, 91%.

ment of the GLDV recognition motif to it will allow it to be assessed as an external turn constraint. GLDV is present in the integrin family of cell adhesion proteins.[12]

As a first step in our synthesis of the bicyclic lactam **4**, we attempted to prepare the bromide **6** by the method used by Weygand[13] to prepare the corresponding ethyl ester bromide but without success. Modification of the method by reacting the diazoketone **5**[14] with HBr in $CHCl_3$, however, gave the bromide **6**,† mp 117–8°C, $[\alpha]_D^{30}$ +3 (*c* 1.01, CH_2Cl_2), in 89% yield, as shown in Scheme 1. The bromide was heated at reflux with the thiolactam **7**[15] in CH_3CN, followed by addition of Ph_3P and triethylamine to give the desired product **8**† as an oil, $[\alpha]_D^{30}$ +12.3 (*c* 1, $CHCl_3$). This was shown to be the *Z*-isomer, since irradiation of the olefinic proton, H-6, caused enhancements to all four protons at C-4 and C-8 in the ^1H NMR spectrum. The hydrogen bond between the N–H of the vinylogous amide and its carbonyl group, as shown, presumably accounts for this specificity. The yield of the product **8** could not be optimised above 25%.

Since sulfur contraction reactions have been reported to be more effective when the thiolactam is trisubstituted,[16] we prepared the *N*-benzyl thiolactam **11** as shown in Scheme 2. This compound had previously been prepared by Rapoport[17] by a synthesis involving *N*-alkylation of glutamic acid prior to cyclisation to avoid the danger of racemisation. We were able to alkylate *tert*-butyl pyroglutamate **9**[18] directly to the *N*-benzyl derivative **10** in 64% yield and reaction with P_4S_{10} gave the thiolactam **11** in 95% yield with spectroscopic and optical properties similar to those reported by Rapoport.[17] The sulfur contraction reaction with the bromide **6**, using Ph_3P/Et_3N in CH_3CN then gave the vinylogous amide **12**,† as an oil, $[\alpha]_D^{28}$ +122.6 (*c* 1.025, $CHCl_3$), in 91% yield.

When the olefinic proton, H-6, in the *N*-benzyl derivative **12** was irradiated, both of the protons H-8 and one of the benzylic CH_2 protons showed enhancement, implying that, unlike the N-H analogue **8**, the *N*-benzyl compound **12** had *E*-geometry. In order to reduce the vinylogous amide and hydrogenolyse the *N*-benzyl group in **12**, it was necessary to hydrogenate the compound at a pressure of 60–200 psi for 50 hours using 10% palladium on activated carbon (75% by weight) in methanol containing trifluoroacetic acid as shown in Scheme 3. The resultant single diastereoisomeric product **13**† was obtained as an oil, $[\alpha]_D^{28}$ +8.5 (*c* 1,



Scheme 3. *Reagents and conditions*: (i) H_2/Pd-C/60–200 psi, MeOH, TFA, 65%; (ii) $^tBuMgCl/Et_2O/0°C$, 29%; (iii) K_2CO_3/MeOH/Δ, 86%.

† These compounds had the appropriate analytical and spectroscopic properties.

$CHCl_3$), in 65% yield but NOE experiments were inconclusive as to its stereochemistry. Interestingly, in some hydrogenation reactions, a small yield of a by-product was obtained. This proved to be the bicyclic lactam alcohol **14**,† mp 141–3°C, $[\alpha]_D^{28}$ +14.6 (*c* 1, $CHCl_3$). The (3*S*,6*S*,8*S*,10*S*)-stereochemistry of this compound was implied from NOE studies, since irradiation at H-3 caused enhancement of H-10 and irradiation at H-10 caused enhancements at both H-8 and H-6. It was not possible to optimise the yield of the bicyclic compound **14** but the mechanism suggested in Scheme 3 would account for its formation. Here, incomplete reduction to the alcohol **15** would be followed by lactonisation, giving the product **16** from which final cyclisation would give the product **14**.

The amino ester **13** was cyclised using *t*BuMgCl in ether at 0°C, giving the bicyclic lactam **4**,† mp 127–9°C, $[\alpha]_D^{28}$ −20.6 (*c* 1, $CHCl_3$), in 29% yield. Calculations of the conformational preferences[19] of the compound **4**, BTD, **1**, and compound, **3**, are shown in Figure 1. Except for minor conformers, those torsional minima found for compound **4** are not compatible with β-turns, and this agrees with the X-ray crystal structure analysis (Fig. 2)[20] which showed torsion angles ϕ_3 −86.5° and ψ_2 +169° which are highlighted in Figure 1. The conformational minima found for BTD, **1**, are almost entirely β-turns, whilst the dispersion of minima indicate a more flexible scaffold than our compound **4**. The torsion angles found for the X-ray structure of **1**[7] are somewhat shifted relative to those calculated but this is likely to be due to the combination of a flexible scaffold and strong lattice interactions, since the crystal structure was that of the free acid. The oxa compound **3** is seen to have more rigidity than compound **1** whilst maintaining three distinct local minima that enable the scaffold to mimic both the torsional regions displayed by compound **4** and the region associated with β-turns.

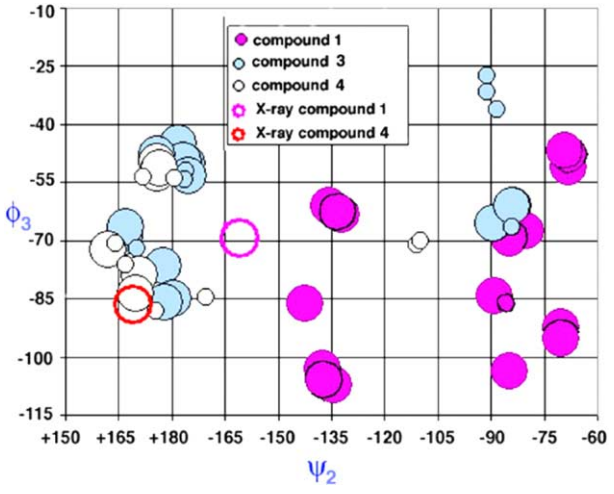


Figure 1. Calculated conformations based on energy minimisation for compounds **1**, **3**, and **4**. Large circles denote conformations within 5 kCal of minimum energy, smaller circles within 12 kCal. X-ray derived angles for **1** and **4** are also shown.

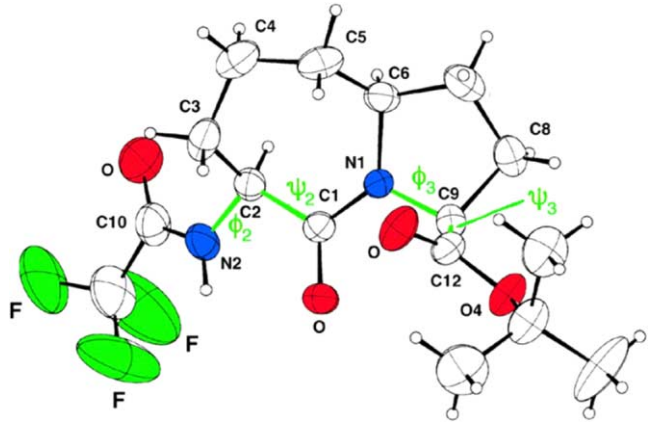
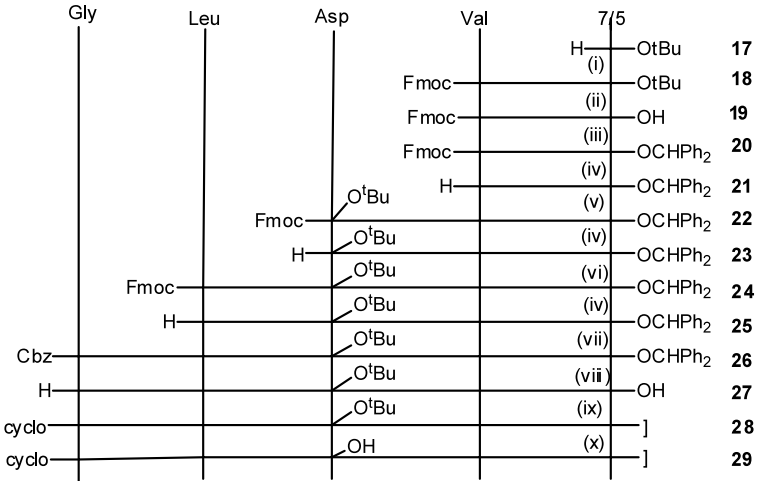
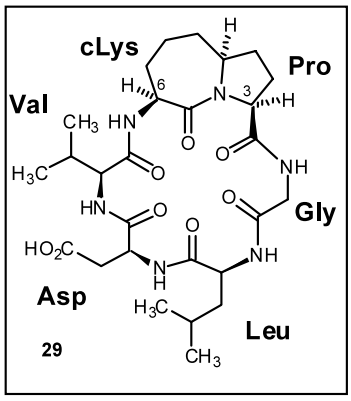


Figure 2. X-ray crystal structure of the bicyclic lactam **4**.

We now wished to examine the usefulness of our turn mimic as an external constraint by adding GLDV across the amino and carboxyl groups. Treatment of the lactam **4** with K_2CO_3 in methanol at reflux, as shown in Scheme 3, gave the free amine **17**† in 86% yield and reaction of this with Fmoc-valine, TBTU and DIPEA gave the product **18**,† mp 129–131°C, $[\alpha]_D^{28}$ −24.4 (*c* 1.1, $CHCl_3$), in 80% yield, as shown in Scheme 4. Given the need to protect the β-carboxyl of the aspartate residue in GLDV as the *tert*-butyl ester to prevent intramolecular cyclisation reactions, we now needed to change the orthogonality of the protecting groups. The Fmoc-*tert*-butyl ester **18** was therefore deprotected using TFA to give the acid **19**,† mp 131–2°C, $[\alpha]_D^{28}$ −69.6 (*c* 0.47, $CHCl_3$), in quantitative yield. Reprotection using diphenyldiazomethane yielded the ester **20**† as an oil, $[\alpha]_D^{30}$ −32.6 (*c* 1, $CHCl_3$) in 64% yield. This was now sequentially deprotected with piperidine and reacted in turn with Fmoc-Asp(O′Bu)-OH, Fmoc-Leu-OH and Cbz-Gly-OH to give the protected tetrapeptide **26**,† as summarised in Scheme 4. Hydrogenolysis, cyclisation and final deprotection were then carried out, as in Scheme 4, and the final cyclic peptide **29** was purified by repeated reverse phase HPLC.

High-resolution NMR spectroscopic data were obtained to establish whether the constrained peptide **29** adopted a dominant conformation in solution. Inter-proton distance constraints derived from 2D-ROESY data were incorporated into molecular dynamics calculations, but were not sufficient to define a unique solution conformation of the backbone. However, additional NMR spectral parameters including the wide range of amide proton temperature coefficients, the non-'conformationally averaged' amide to α-H coupling constants and the approximately 0.4 ppm difference in the glycine methylene proton chemical shifts together with their 'non-equal' vicinal coupling constants to the glycine amide proton (Table 1) were consistent with a single backbone conformation.





Scheme 4. *Reagents and conditions;* (i) TBTU/DIPEA/DMF/Fmoc-Val-OH, 80%; (ii) CF_3CO_2H/CH_2Cl_2, quant; (iii) Ph_2CHN_2/CH_2Cl_2, 64%; (iv) piperidine/DMF, >82%; (v) TBTU/DIPEA/Fmoc-Asp(O′Bu)-OH, 88%; (vi) TBTU/DIPEA/DMF/Fmoc-Leu-OH, 82%; (vii) TBTU/DIPEA/Cbz-Gly-OH, 81%; (viii) H_2/Pd-C, 92%; (ix) TBTU/DMAP/DMF; (x) CF_3CO_2H/ TES.

Table 1. ^{1}H NMR data for **29** in 90% H_2O:10% D_2O (ref δ 4.70) at 298 K

AA unit	NH	αH	βH	γH	δH	εH	$^3J_{NH,\alpha H}$ (Hz)	$\Delta\delta/\Delta T$ (ppb/K)
Gly (G)	8.68	3.62 and 4.01	–	–	–	–	5.4 and 7.0	−7.8
Leu (L)	8.40	4.78	1.42 and 1.75	1.50	0.83 and 0.89	–	9.0	−2.8
Asp (D)	8.58	4.53	2.81 and 2.91	–	–	–	5.5	−4.3
Val (V)	7.24	4.35	2.36	2.40	0.77 and 0.84	–	9.5	−2.6
cLys (cK)	7.53	4.45	1.61	1.88	1.39	4.48	5.6	−1.7
Pro (P)	–	4.47	1.94 and 2.10	1.61 and 1.67	–	–	–	–

The concentration of monomeric peptide was approximately 0.6 mM; a substantial amount of aggregated peptide material was also present.

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19. **Conformational analysis**: All calculations were performed with Sybyl 6.9, available from Tripos Inc., 1699 South Hanley Rd., St.Louis, MI, 63144, USA. Random searching was performed with 1000 cycles and an energy cut-off

of 20 kCal. Energy minimisations were performed with the Tripos forcefield with Gasteiger-Hückel charges and 1000 iterations.

20. **Crystal data**: compound **4**, $C_{16}H_{23}F_3N_2O_4$, M = 364.4, monoclinic, space group $P2_1$ (No 4), $a = 5.485(2)$, $b = 11.970(4)$, $c = 14.437(5)$ Å, $V = 947.9(6)$ Å^3, $Z = 2$, $D_{calc} = 1.28$ mg/m^3, μ (Mo-Kα) 0.11 mm^{-1}, $T = 293(2)$ K, 1757 independent reflections, 1295 with $I > 2\sigma(I)$, final residuals were R1 = 0.058, wR2 = 0.152; Data collection was carried out using a Nonius CAD4 diffractometer, structure analysis using program package WinGX, and refinement using SHELXL-97. The atomic coordinates are available on request from The Director, Cambridge Crystallography Data Centre, University Chemical Laboratory, Lensfield Road, Cambridge, CB2 1EW (deposition number CCDC 216301).