Synthesis and conformational analysis of a type VIb β-turn mimetic based on an eight-membered lactam

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As part of a programme evaluating medium-ring lactams as peptide conformational constraints the *trans*-disubstituted lactam dipeptide *(3S,8R)-3-acetylamino-8-methoxycarbamoylazocan-2-one* (**3**) was selected as a template to mimic the type VI β-turn conformation of natural polypeptides. The enantioselective synthesis of the eight-membered lactam **3** is discussed. The X-ray crystal structure of the methyl ester precursor of **3** shows a classical type VIb β-turn conformation. Extensive ^{1}H NMR spectroscopic studies of the dipeptide **3** in polar solvents provided evidence for a type VI β-turn. The analysis shows an equilibrium of two folded conformations.

Introduction

Proteins and peptides are known to interact with macromolecular receptors to initiate biological events, and a multitude of such bioactive peptides have so far been discovered and characterised.[1] Many of these have been found in both neuronal and non-neuronal tissues. Representative examples include somatostatin, substance P, cholecystokinin, endorphin, enkephalin, angiotensin II, and endothelin. After binding to membrane-bound receptors, these neurotransmitters, neuromodulators, and hormones influence cell-to-cell communication and control a series of vital functions. Naturally, they are of enormous medical interest. Constrained peptide analogues (or peptidomimetics) can be made to resemble the secondary structural elements and thus become not only valuable in the design of new pharmaceuticals, but also serve to help elucidate biologically active protein conformation and to further the study of structure–activity relationships.[2–5]

The reverse turns are important to the recognition systems necessary for post-translational modification of proteins such as phosphorylation[6] and glycosylation,[7] proteolytic processing,[8] initiation of proteolytic degradation,[9] protein folding[10] and even early evolution.[11] They participate in these recognition events either actively, for example where the precise spatial orientation of pharmacophoric information is critical, or in a more passive manner by properly positioning the two peptide chains. Thus, turns are the most frequently imitated secondary structural motif.

The β-turn (Fig. 1) is the most common reverse turn found in peptides. β-Turns comprise a rather diverse group of structures and are classified according to the φ and ψ torsion angles of the $i + 1$ and $i + 2$ residues. In addition, the distance from the Cα of the first residue to the Cα of the fourth residue varies from 4–7 Å.[12–16] The $O_1 \cdots H_4$–N hydrogen bond is not an essential structural feature, but is often indicative of a β-turn structure, as shown by X-ray crystallography and NMR spectroscopy.

Conformationally restricted peptides, for example β-turn mimetics, can be obtained by limiting the number of φ, ψ-angle combinations. In this regard, the most obvious approach involves formation of a lactam by the intramolecular reaction of *N*- and *C*-termini to give a cyclic peptide. The ideal β-turn mimetic is one in which the four variable torsion angles φ_2, φ_3, ψ_2 and ψ_3 are constrained in a predictable manner such that a β-turn conformation exists over the pseudo tetrapeptide sequence.

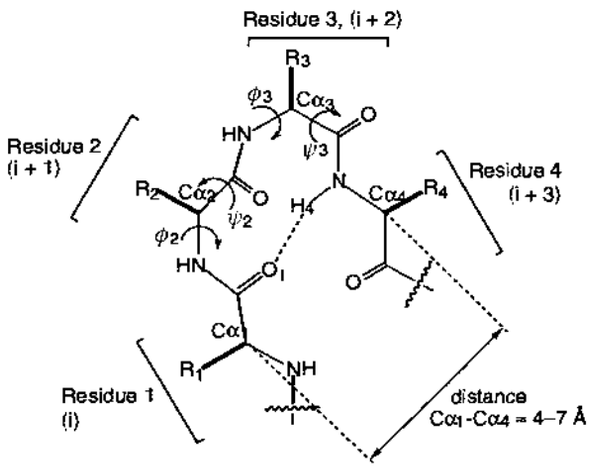

Fig. 1 β-Turn conformation.

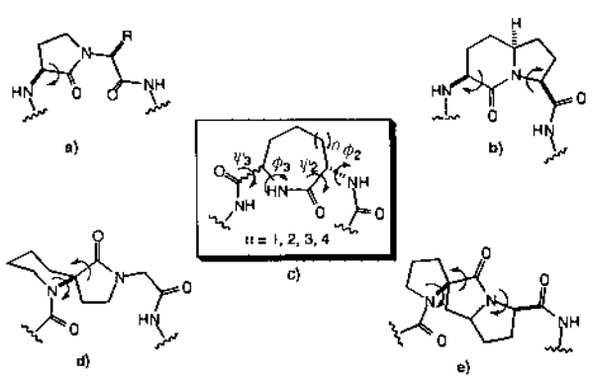

Fig. 2 Reverse turn mimetics based on (a) small lactams; (b) bicyclic lactams; (c) medium ring lactams; (d) spirocyclic lactams; (e) tricyclic lactams.

Lactams are widely used as conformational constraints in the design of peptidomimetics. Depending on the substitution pattern and the ring constitution, lactams are able to constrain covalently up to three of the four torsion angles that describe the β-turn conformation (Fig. 2). Monocyclic lactams constrain one or two dihedral angles (Fig. 2a,c); bicyclic and spirobicyclic lactams restrict two torsion angles (Fig. 2b,d); and the

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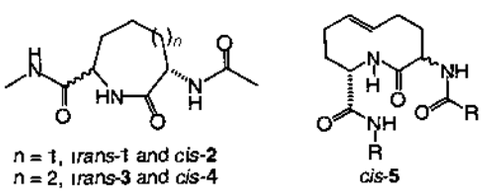
rigid structures of tricyclic lactams constrain a maximum of three angles (Fig. 2e). Two major surveys of conformationally constrained amino acids and dipeptides recently appeared in the literature. Gillespie *et al.*[17] carried out a basic conformational analysis of a compendium of compounds designed as dipeptide mimetics and Hanessian *et. al.*[18] provided an up-to-date overview of molecules related to oxo-1-azabicycloalkane amino acids (Fig. 2b) and analogues that incorporate a complete dipeptide unit only.

The work described here is part of a project that aims to synthesise and evaluate a family of disubstituted medium-ring lactams as constrained dipeptide surrogates (Fig. 2c). Since these compounds have the ability to constrain covalently two dihedral angles, ψ_2 and φ_3 and to restrict the conformational space available around ψ_3 and φ_2, they are comparable to bicyclic lactams. Particular interest has been in the design of first generation type VI β-turn mimetics, bearing the characteristic *cis*-peptide bond within this structural motif. Robinson *et al*. have shown that the incorporation of a bicyclic type VI β-turn mimetic into the reduced structure (cyclic hexapeptide) of somatostatin retained biological activity[19] and it is believed that this type VI turn induces a particular conformation in what is thought to be the molecular recognition site of somatostatin. The quest for peptidomimetic agonists and antagonists of somatostatin has generated enormous activity particularly in the pharmaceutical industry. The type VI β-turn motif has also been identified in HUN-7293, an inhibitor of cell adhesion molecule expression.[20]

We have previously reported the synthesis of the disubstituted seven-membered lactams, *trans*-**1** and *cis*-**2**[21,22] and a



further step was to evaluate the analogous eight-membered lactams *trans*-**3** and *cis*-**4**.[23] In a recent publication, Katzenellenbogen *et al.* reported the synthesis and conformational evaluation of the unsaturated ten-membered homologue *cis*-**5**.[24] Molecular mechanics conformational searching and NMR studies indicated that tetrapeptide derivatives of *cis*-**5** constrain the four relevant torsion angles to within 30° of those predicted for an ideal type I β-turn.

Results and discussion

Molecular modelling

Molecular mechanics calculations were performed on lactams *trans*-**3** and *cis*-**4**. The starting structures were randomly built in MacroModel v4.5,[25] energy-minimised and subjected to a Monte Carlo conformational search, using the implemented MM2,[26] the MM3[27] and the AMBER* force fields.[28] In all instances, the lowest energy structure was found several times, implying it to be the probable global minimum. In addition to calculations *in vacuo*, Still's continuum model for chloroform[29] was used, giving qualitatively similar results. Whereas *cis*-**4** shows an extended (with the MM3 and the AMBER force fields) or semi-extended conformation (with the MM2 force field), as deduced from the $C\alpha_1$–$C\alpha_4$ distance, calculations for *trans*-**3** reveal a closed ($C\alpha_1$–$C\alpha_4$ < 7 Å) and therefore β-turn-like conformation with all three force fields. The results strongly indicated a possible type VI β-turn conformation and so the *trans*-disubstituted eight-membered lactam dipeptide was subsequently synthesised and its conformation analysed.

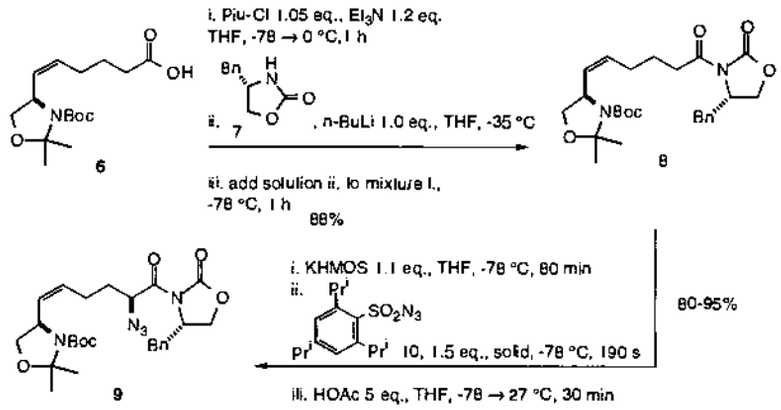
Synthesis of the *trans*-lactam dipeptide 3

The synthesis of the *trans*-disubstituted eight-membered lactam **3** proceeded with remarkable ease and efficiency, following the route optimised for the *cis*-disubstituted analogue **4**, reported in the preceding paper.[23] The most advanced common intermediate is the (*Z*)-octenoic acid **6**. This was condensed with auxiliary **7**[30] to give the carboximide **8** in 88% yield (Scheme 1). Application of the improved conditions for the electrophilic azide transfer reaction[23] yielded, after optimisation of the reaction time, α-azido carboximide **9** (ν_{N_3} 2108 cm^{-1}) in a consistently excellent (90–95%) yield. This result is undoubtedly proof of the invaluable improvement on the Evans procedure by adding the trisyl azide (**10**) (trisyl = 2,4,6-triisopropylbenzenesulfonyl) as a solid to the carboximide enolate reaction mixture rather than transfer by cannula as a concentrated precooled solution.‡

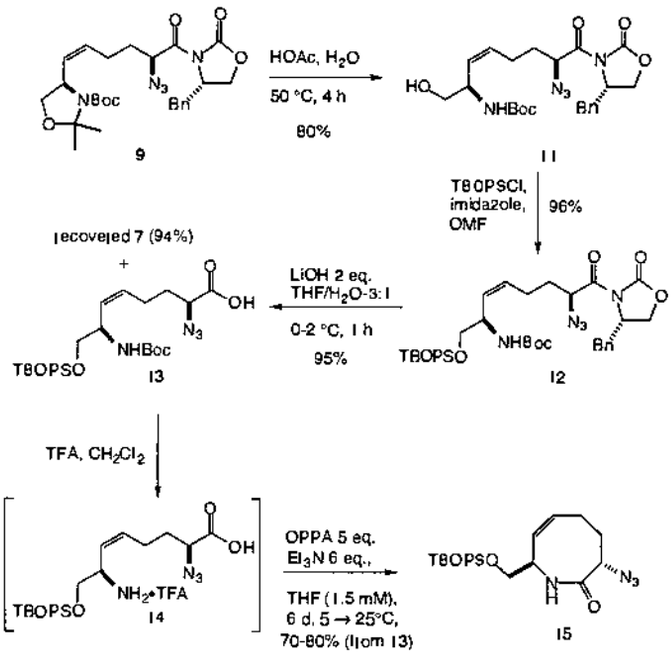
Protecting group manipulations and removel of the auxiliary, as outline in Scheme 2, were carried out in order to elaborate the α-azido carboximide **9** to the seco-acid **14**. Subsequently, the diphenylphosphoryl azide [DPPA, $(PhO)_2P(O)N_3$]-mediated lactamisation was carried under optimised conditions [DPPA 5 equiv., Et_3N 6 equiv., THF ~1.5 mM, 5–25 °C, 6 days] and gave the desired eight-membered lactam **15** (ν_{NH} 3368, ν_{azide} 2104, ν_{CO} 1660 cm^{-1}) in 70–80% yield.

The continuation of the synthesis is outlined in Scheme 3. The concomitant hydrogenation of the carbon–carbon double bond and the azide functionalities over palladium on charcoal in the presence of HCl gave the hydrochloride **16**. This was

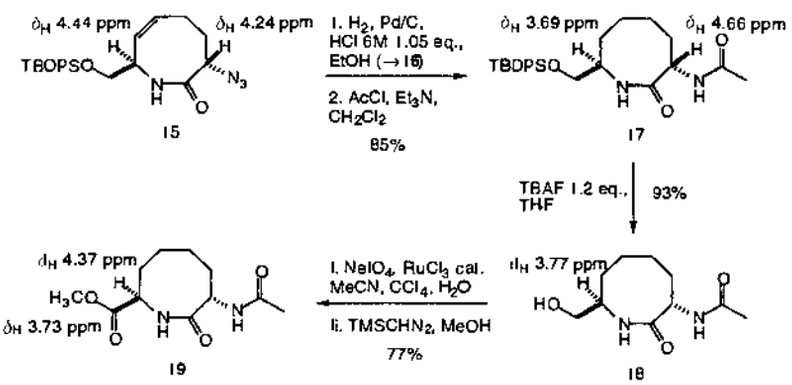
‡ For example, a batch of 13.5 g (28.5 mmol) of the carboximide **8** was subjected to the optimised conditions and yielded 13.7 g (26.7 mmol) of the azide **9**, corresponding to 94% yield.



Scheme 1



Scheme 2



Scheme 3

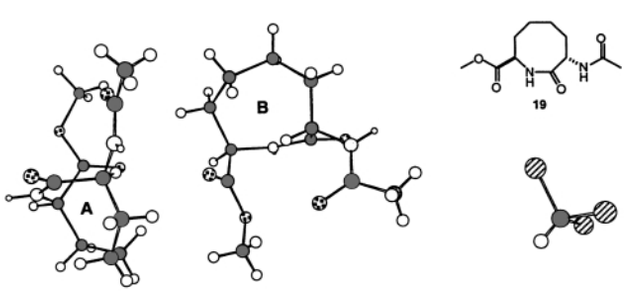


Fig. 3 Chem3D® representation of the X-ray structure of methyl ester **19**. The unit cell consists of two conformers, **A** and **B**, as well as one molecule of chloroform.§

converted in a series of steps into the methyl ester **19** (ν_{CO} 1740 cm^{-1}), the structure and absolute stereochemistry of which were unambiguously established by X-ray crystallographic analysis (see Fig. 3, for a detailed discussion of the conformation *vide infra*).

In the final step of the synthesis, the methyl ester **19** was slowly treated with methylamine at room temperature, which resulted in both the epimerisation of C-8 and amide formation to yield the corresponding methyl amides **3** and **4** in a 1 : 5 ratio (Scheme 4). However, if the reaction was carried out at lower temperature (0 °C) and the solid methyl ester **19** was added to ethanolic methylamine (8 mol dm^{-3}), the desired *trans*-disubstituted lactam dipeptide **3** could be obtained as the major product (8 : 1 ratio of **3** : **4**). The diastereoisomers were readily separable by chromatography. The absolute stereochemistry of compounds **3** and **4** follows from the X-ray crystal structure of the ester **19** and the fact that the enantiomer of compound **4** had been prepared by an independent route.[23]

In summary, the synthesis of the *trans*-disubstituted lactam dipeptide **3**, has been achieved in 11 steps, starting from (*Z*)-octenoic acid **6**, the overall yield being up to 23%. Furthermore, in addition to being extremely efficient, the synthesis delivers simple lactam precursors such as **15** which can readily be elaborated for use in peptide synthesis.

Conformational studies of the *trans*-lactam dipeptide 3

The conformation of the lactam dipeptide 3 in the solid state. It

§ Close inspection of the crystal unit cell revealed a chloroform solvate molecule (disordered over two sites) which enabled the unambiguous determination of the absolute stereochemistry of methyl ester **19**. Atomic coordinates, bond lengths and angles, and thermal parameters have been deposited at the Cambridge Crystallographic Data Centre. CCDC reference number 207/453. See http://www.rsc.org/suppdata/p1/b0/b003791p for crystallographic files in .cif format. Crystal data, analysis and refinement for **2**: empirical formula $C_{11.5}H_{18.5}Cl_{1.5}N_2O_4$; formula weight (*M*) 301.96; temperature 293(2) K; crystal system monoclinic; space group $P2_1$; unit-cell dimensions $a = 7.955(2)$ Å, $\alpha = 90°$, $b = 18.210(5)$ Å, $\beta = 90.93(2)°$, $c = 10.141(2)$ Å, $\gamma = 90°$; volume 1468.8(6) $Å^3$; $Z = 4$; $\mu = 32.59$ cm^{-1}; reflections collected 4894; independent reflections 4375 ($R_{int} = 0.0266$); final *R* indices (all data) $R1 = 0.0588$, $wR2 = 0.1419$.

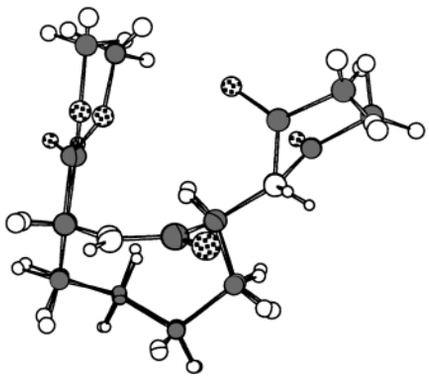
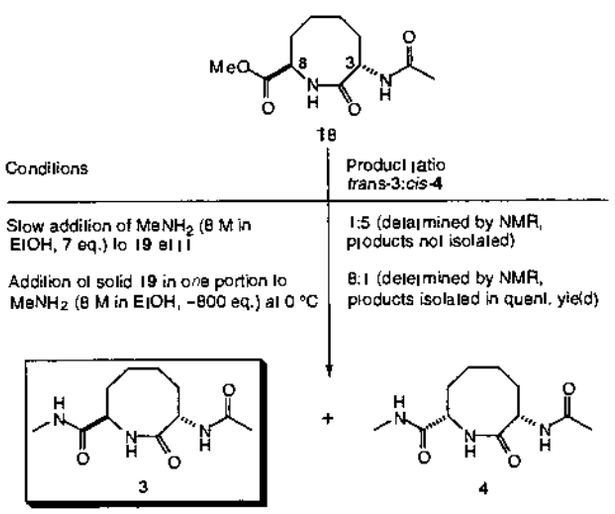


Fig. 4 Chem3D® representation of MacroModel (Amber) calculated lowest energy conformation of **19**, overlaid on conformer B of the X-ray structure, demonstrating good agreement between the two methods. [Note: this is not a least squares fit; the four atoms of the lactam functionality (*C*–*C*O–*N*H–*C*) were simply superimposed.]



Scheme 4

would have been preferable to have obtained an X-ray crystal structure of the *trans*-disubstituted lactam dipeptide **3** in order to prove that its conformation mimics a reverse turn, as predicted by Amber-based molecular mechanics calculations. Unfortunately, a single crystal suitable for X-ray crystallographic analysis could not be grown, despite many attempts using a variety of solvents and temperatures.

However, an early indication of how well the lactam dipeptide **3** would mimic the type VI β-turn motif was provided by the X-ray crystal structure of its precursor, the methyl ester **19** (Fig. 3). Crystals of the ester **19** suitable for X-ray crystallographic analysis were grown in chloroform–hexane. The crystal structure showed that the methyl ester **19** crystallised in two non-superimposable conformers, **A** and **B**, differing only in their side-chain geometries.§ Examination of the relevant dihedral angles shows that both solid state conformations of methyl ester **19** clearly resemble a classical type VIb β-turn. The torsion angles in **19** are shown in Table 1 and are compared with those for the idealised standard type VIb β turn.[12,14] Furthermore, the conformations found in the crystal structure of methyl ester **19** are in extremely good agreement with those predicted by molecular mechanics calculations using MacroModel v5.5, utilising the Amber force field and parameters for *in vacuo* simulations (Fig. 4). In fact, the conformation of the lactam ring was predicted near-perfectly, and only minor deviations were found in the geometry of the substituents with respect to the cyclic scaffold. The deviations from the idealised standard type VIb β-turn in the crystal **19** are largely within the limits defined by Wilmot and Thornton.[14,15] The value for ψ_3 exceeds only slightly the allowed deviation, and this arises in the flexible side chain of **19**.

Table 1 Relevant torsion angles defining a type VI β turn for the ester **19** and the dipeptide **3**

Compound	φ_2	ψ_2	φ_3	ψ_3
Ester **19** conformer **A**[a]	−98	146	−53	−46
Ester **19** conformer **B**	−82	146	−57	−53
Ideal type VIb β-turn[b]	−120	120	−60	0
Dipeptide **3** conformer **A**[c]	−163	146	−59	−20
Dipeptide **3** conformer **B**[c]	−163	−147	−58	142
Dipeptide **3** conformer **C**[c]	173	−52	152	−136
Dipeptide **3** conformer **D**[c]	−52	−48	154	−136

[a] Taken from the X-ray structure. [b] Taken from Wilmot and Thornton.[14,15] [c] Macromodel calculated values (see text).

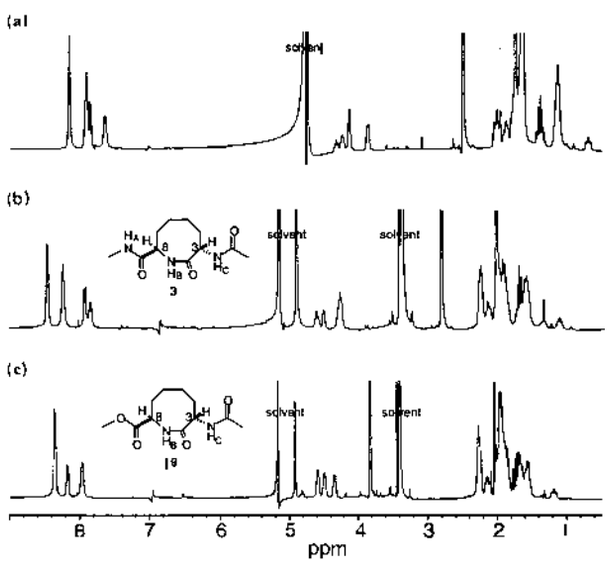


Fig. 5 ^{1}H NMR spectra of (a) dipeptide **3** in H_2O at 275 K; (b) dipeptide **3** in CD_3OH at 275 K and (c) methyl ester **19** in CD_3OH at 275 K. Assignment of the relevant signals is listed in Table 2.

The solution conformation of the lactam dipeptide 3. The conformational analysis of the lactam **3** in solution was considered next. However, evaluation under the conditions used previously for the analysis of the *cis*-lactam dipeptide *ent*-**4**[23] was near-impossible, owing to the poor solubility of **3** in chlorinated solvents. ^{1}H NMR spectroscopy was employed, with the measurements being conducted in polar solvents, such as H_2O (with 10% D_2O), CD_3OH, CF_3CD_2OH and DMSO-d_6 (Table 2). Inspection of the ^{1}H NMR spectra of dipeptide **3** at ambient temperature shows that all the signals are broad and largely uninterpretable, regardless of solvent. However, when the solution was cooled to 275 K it was observed that the spectra of aqueous (Fig. 5, trace a) and methanolic (Fig. 5, trace b) samples showed two distinct sets of resonances for the lactam NH and each of the ring protons, H-3 and H-8, indicating the presence of two lactam conformations in approximately a 1 : 1 ratio. Significantly, the ^{1}H NMR spectrum of dipeptide **3** greatly resembled that of the methyl ester **19** (Fig. 5, trace c), showing conservation of chemical shifts, signals and multiplicity. This behaviour is firm evidence for conformational similarity between the two structures. The corresponding NOESY spectra for both compounds (Fig. 6) showed cross peaks between all amide hydrogens and the two ring protons H-3 and H-8. The NOE observations do not accommodate a unique ring conformation, but can be

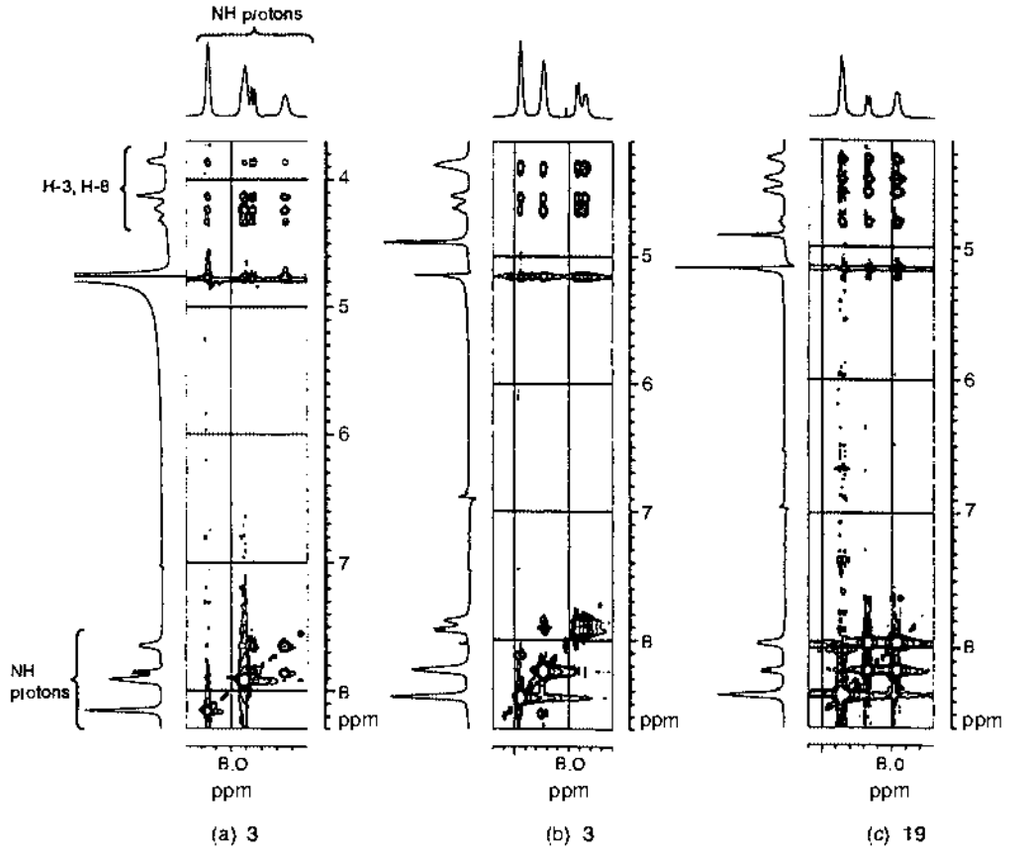


Fig. 6 Extracts from the NOESY spectra of (a) dipeptide **3** in H_2O at 275 K, (b) dipeptide **3** in CD_3OH at 275 K and (c) methyl ester **19** in CD_3OH at 275 K, showing the existence of cross peaks between each NH proton and both H-3 and H-8.

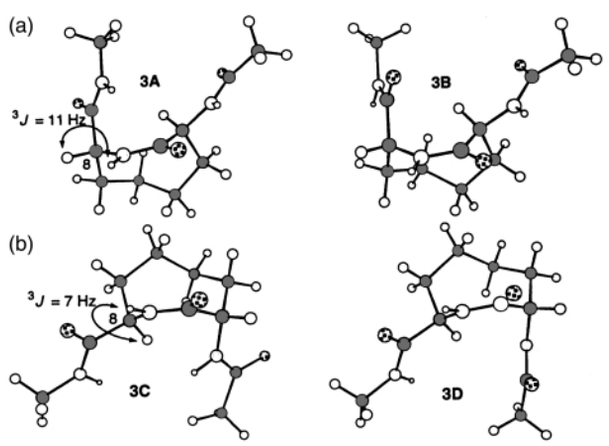


Fig. 7 The four lowest energy conformers of lactam dipeptide **3**, generated with MacroModel v5.5, the Amber force field and parameter for the simulation of a water solution. (a) Two conformers (**3A** and **3B**) are folded above the 'plane' of the ring, mimicking a type VIb β-turn. (b) Two conformers (**3C** and **3D**) are folded below the 'plane' of the ring.

explained by the existence of two slowly interconverting ring conformers (as predicted by molecular mechanics calculations and shown later in Fig. 7) together with the effect of NH proton exchange with solvent. The existence of *cis–trans* lactam rotamers is discounted based on the low likelihood of this phenomenon in eight-membered lactams.[31–33]

Further molecular mechanics calculations were performed with a continuum model for water, which offered insight into the preferred conformations of the dipeptide **3**.[25,28,29] Of the four lowest energy conformations (within 4 kJ mol^{-1}) found by the Monte Carlo search, two (**3A**, **3B**) were found to be folded above the 'plane' of the eight-membered ring (Fig. 7a) and are indicative of the type VI β-turn. The torsion angles calculated for these conformers (see Table 1) were very similar to those of a type VIb-turn. The remaining two conformers (**3C**, **3D**) are folded below the 'plane' of the lactam ring (Fig. 7b) and, although their torsion angles (Table 1) were found to be inconsistent with those of a classical type VI turn,[12,14] they meet the general criteria for such turns.¶

In all the ^1H NMR spectra of the methyl amide **3** and the methyl ester **19** the vicinal coupling constant ^3J between the NH_B and H-8 of one conformer is quite large (11 Hz, compare Table 2). A *J*-value of this magnitude suggests that the corresponding dihedral angle H_B–N–C-8–H-8 is either close to 0 or 180°. In the crystal structure of the methyl ester **19** this dihedral angle is approximately 10° which is in very good agreement with the theoretical value. Similarly, the values of the corresponding torsion angles in the models depicted in Fig. 7a are 5 and 6° respectively, which is good evidence for the relatively large coupling constant. Furthermore, in the ^1H NMR spectrum of **19** (in CD_3OH) and of **3** [in CD_3OH (37%)–CF_3D_2OH (63%)] the ^3J-value between the NH_B and H-8 of the second conformer was found to be 7 Hz, which would imply that the corresponding dihedral angle H_B'–N′–C-8′–H-8′ was close to either 30 or 150°. Indeed, in the models of the conformers folded below the plane of the lactam ring (**3C**, **3D**; Fig. 7b), these dihedral angles were found to be in very good agreement (148 and 149°).

In DMSO-d_6 (0.015 mol dm^{-3}, 290 K, Fig. 8), the ratio of the two conformers is about 2:1. Irradiation of the methyl amide resonance (NH_A, broad quartet, δ 7.87 ppm) showed strong 1D-selective gradient NOEs to the methyl group and to the ring proton H-3 respectively, and somewhat weaker NOEs to lactam amide, NH_B, and the ring proton H-8. This observation is consistent with both the solid state conformation of the methyl ester **19** and the corresponding calculated structure (Fig. 9).

¶ These criteria are: the central *cis*-peptide bond, the $C\alpha_1$–$C\alpha_4$ distance (≤7 Å), and the overall torsion angle τ ($C\alpha_1$–$C\alpha_2$–$C\alpha_3$–$C\alpha_4$, $-90 \leq 0 \leq 90$).[34]

Table 2 Chemical shifts (δ, ppm), multiplicity and, in parentheses, coupling constants (J, Hz) of a selection of amide and ring protons of lactams **3** and **19** in a variety of solvents. The corresponding spectra are shown in Figs. 5 and 13

	3				**19**
	H_2O, 275 K	CD_3OH, 275 K	63% CF_2CD_2OH–37% CD_3OH, 275 K	DMSO-d_6, 290 K	CD_3OH, 275 K
NH_A	7.91, br q (4)	8.14, br m	7.96, br m	7.87, br q (4)	N/A
NH_A'	Under NH_A	Under NH_A	7.74, br q (2)	Under NH_A	N/A
NH_B	7.85, d (11)	7.83, d (11)	7.40, d (11)	7.67, d (11)	8.17, d (11)
NH_B'	7.64, br s	7.76, br s	7.45, br d (7)	7.38, br s	7.97, br d (7)
NH_C	8.15, br m	8.35, br m	7.93, br d (2)	7.96, br d (7)	8.36, br m
NH_C'	Under NH_C	Under NH_C	7.84, br d (4.5)	8.02, br m	Under NH_C
H-3	4.12, br m	4.43, br m	4.51, br m	4.30, br m	4.59, m
H-3′	3.85, dd (6, 5.5)	4.18, br m	4.22, br dt (6, 6)	Under H-3	4.34, dt (6, 6)
H-8	4.32, br t (11)	4.53, br t (11)	4.62, br td (11, 3)	4.38, br t (11)	4.81, dt (11, 4)
H-8′	4.23, br m	4.20, br m	4.26, br m	4.01, br m	4.49, br m

In DMSO-d_6 the major conformer exists in a type VIb turn, whereas in H_2O and CD_3OH both conformers are equally populated and the cross peaks in the NOESY spectra (*vide supra*) originating from both conformers show that they are in a rapid equilibrium (NOE mixing time 1.2–1.5 s).

The calculations also predicted H-bonded structures amongst the lowest energy conformations, the first (Fig. 10) being found within 7.4 kJ mol^{-1} of the global minimum. This prompted the experimental investigation of the possible presence of an intramolecular H-bond in more detail.‖ An initial variable-temperature experiment on the lactam dipeptide **3** in DMSO-d_6 (Fig. 11) showed that the amide proton NH_A (see Fig. 5b) has a significantly smaller temperature coefficient ($-\Delta\delta/\Delta T = 4.5$ ppb K^{-1}) than the amide protons NH_B and NH_C (5.6 and 5.9 ppb K^{-1}, respectively). Although the accepted value for the temperature coefficient delineating amide protons that are shielded from the solvent from those that are exposed to the solvent is about 4 ppb K^{-1},[35,38] NH_A is consistently clearly different from the other two, suggesting it to be probably involved in an intramolecular hydrogen bond between H_A and O_1.**,††

Clearly at elevated temperatures only an average of two conformers of **3** can be observed and so a solvent titration experiment[35,38,39] was used to examine the individual conformations at a lower temperature. Thus, a solution of dipeptide **3** in CD_3OH (0.015 mol dm^{-3}) at 275 K was titrated with a solution in CF_3CD_2OH (0.015 mol dm^{-3}), and the result is shown in Fig. 12. This solvent combination was selected as a result of the insolubility of compound **3** in $CHCl_3$.

In the ^{1}H NMR spectrum of dipeptide **3** in CF_3CD_2OH or in CD_3OH with more than 15% v/v CF_3CD_2OH at 275 K both the methyl amide NHs and the acetamide NHs appear as two sets of signals for each resonance (Fig. 13b and Table 2), and are designated NH_A, NH_A' and NH_C, NH_C', indicative of the two fully distinguishable conformations. During the titration the chemical shifts of NH_C and NH_C' and lactam protons, NH_B and NH_B', greatly shifted upfield, as did the NH_A' resonance, whereas the signal for NH_A (the second conformer) moved only slightly upfield.‡‡ These results represent firm evidence that the amide proton NH_A is shielded from the solvent and, by implication, is involved in an intramolecular H-bond. Although none of the included observations, when taken in isolation, confirms the unambiguous existence of an intramolecular hydrogen bond, all the data together provide a strong indication for this phenomenon.

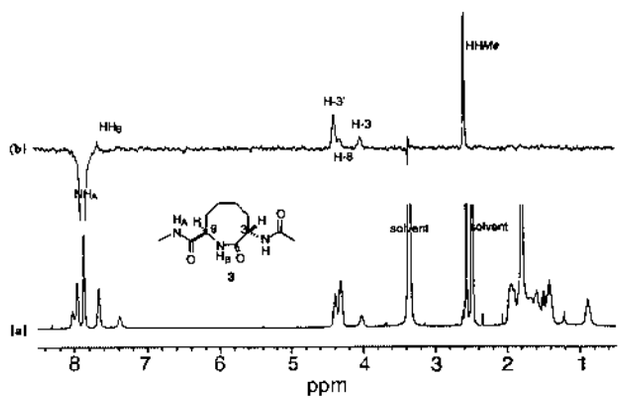


Fig. 8 (a) ^{1}H NMR spectra of dipeptide **3** in DMSO-d_6 at 290 K. (b) Corresponding 1D-selective gradient NOE spectrum (irradiated at the amide NH_A at 7.87 ppm).

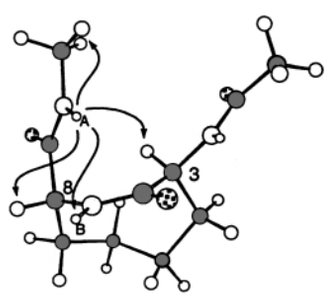


Fig. 9 NOEs observed between NH_A and the methyl group, NH_B and to both ring protons, H-3 and H-8 in the NMR spectrum (DMSO-d_6) of **3**. The MacroModel-calculated structure was used in this representation.

‖ One of the most important steps in determining the secondary structure of small peptides using ^{1}H NMR spectroscopy is the separation of amide protons into two groups according to whether they are exposed to the solvent or shielded from the solvent either sterically or *via* hydrogen bonds.[35] The three most common methods of accomplishing this delineation are through the temperature dependence of the peptide proton chemical shifts,[36–38] by deuterium proton exchange rates[36,38] and by solvent titration measurements.[37–39]

** Since the experiment was carried out at relatively high concentration (0.1 mol dm^{-3}), some aggregation might have had an influence on the outcome of the result.

†† The NMR spectrum of lactam dipeptide **3** in DMSO-d_6 at ambient temperature shows the presence of two conformers, and signal coalescence occurred only above 323 K. The relatively high barrier implied by the coalescence temperature would be consistent with the need to re-organise hydrogen-bonded structures in a viscous solvent. The relatively small temperature dependence of NH_A, however, became only prominent between 320 and 420 K, a temperature range somewhat irrelevant since physiological conditions are ultimately of more interest.

‡‡ The change in chemical shifts ($\delta_{CD3OH} - \delta_{CF3CD2OH}$) of the acetamides and of the two lactams were in the range 0.9–1.2 ppm; however, the NH_A' difference was 0.90 ppm whereas that of NH_A was a mere 0.54 ppm.

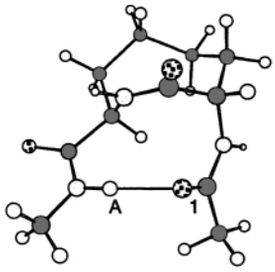


Fig. 10 The calculated lowest energy conformer of lactam dipeptide **3** possessing an apparent intramolecular H-bond between NH_A and O_1 (obtained from above-described conformational search).

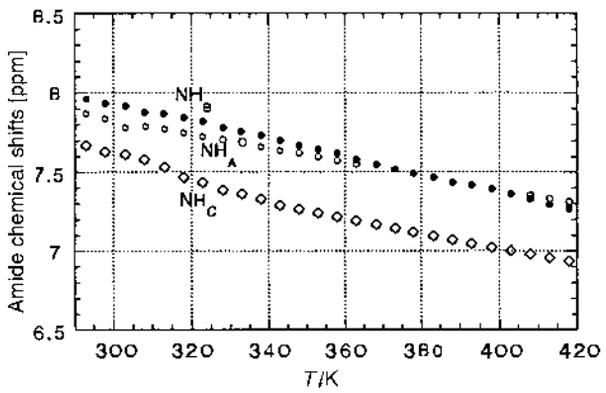


Fig. 11 Amide chemical proton NMR shifts of **3** (*c* 0.1 mol dm^{-3} in DMSO-d_6) as a function of temperature.

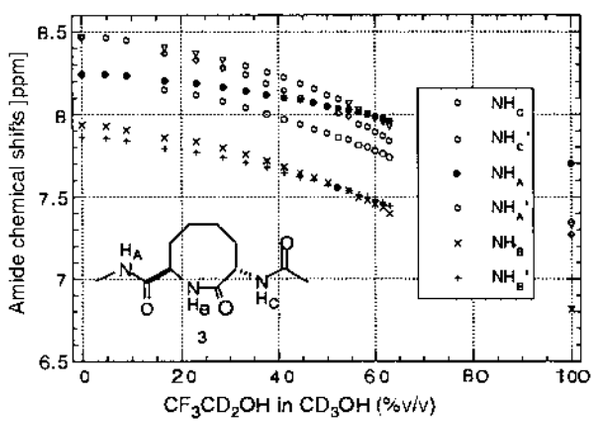


Fig. 12 Solvent titration measurements: amide proton NMR chemical shifts of both conformers of **3** (*c* 0.015 mol dm^{-3} in CD_3OH) as a function of added solution of **3** in CF_3CD_2OH (*c* 0.015 mol dm^{-3}).

Conclusions

The crystal structure of the methyl ester **19** provided an initial indication that the lactam **3** may exist in a type VIb β-turn conformation. Subsequent ^{1}H NMR studies, including NOE, VT experiments and solvent titration, together with in-depth molecular mechanics calculations, strongly suggest that dipeptide **3** in polar solvents exists in an equilibrium between two closed conformations (Fig. 14). One conformer, the major component in DMSO, appears to be the first genuine mimic of a type VIb β-turn. The other conformer was found to contain a stable intramolecular H-bond between NH_A and O_1, although the torsion angles are not consistent with those of a classical type VIa β-turn, which is known to possess such a stabilising H-bond. Furthermore, the fact that lactam dipeptide **3** is folded in polar solvents is a very encouraging result since, ultimately, the turn-like conformation has to exist in water, the relevant solvent for biological systems.

In summary, it has been demonstrated that the semi-flexible conformation of eight-membered lactams can be used as a

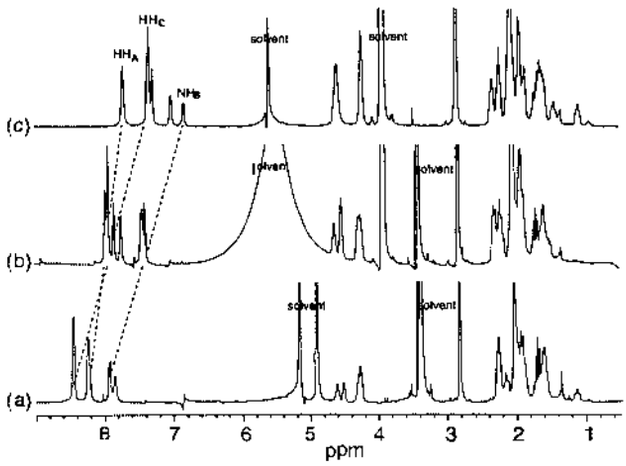


Fig. 13 A selection of ^{1}H NMR spectra of the solvent titration measurements at 275 K: (a) dipeptide **3** in 100% CD_3OH; (b) in a mixture of 63% CF_3CD_2OH and 37% CD_3OH (v/v); and (c) in 100% CF_3CD_2OH.

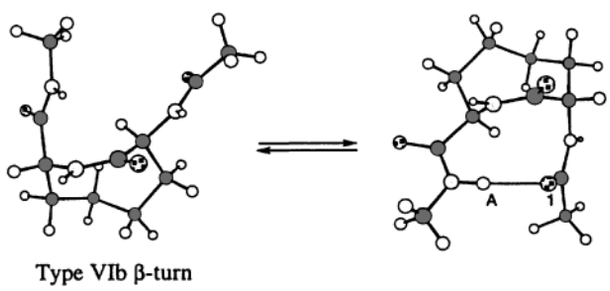


Fig. 14 Equilibrium of the two conformations of lactam dipeptide **3** in polar solvents.

novel template to mimic type VI reverse turns. Several examples of type VI β-turn mimetics based on bicyclic lactams templates have been reported,[19,40] but most of these adopt a type VIa conformation. In addition, all these azabicyclic structures contain the natural proline moiety, a feature which is clearly not present in the design reported herein. Furthermore, the eight-membered lactam does not appear to be as rigid as the bicyclic templates and would certainly have advantageous properties in structure–activity relationship studies, being capable of adapting to receptor conformations ('induced fit').

Experimental

General experimental conditions appear in the preceding paper.[23]

(4*S*)-3-{6-(*Z*,4*R*)[(4*R*)-3-*tert*-Butyloxycarbonyl-2,2-dimethyl-1,3-oxazolidin-4-yl]-1-oxohex-5-enyl}-4-phenylmethyl-1,3-oxazolidin-2-one 8

To a mechanically stirred solution of carboxylic acid **6** (20.9 g, 62.6 mmol, *E*:*Z* ratio 1:10) and triethylamine (10.5 cm^3, 75.1 mmol) in THF (400 cm^3), cooled to −70 °C, was added dropwise within 10 minutes pivaloyl chloride (8.1 cm^3, 65.7 mmol). The resulting white suspension was allowed to warm to 0 °C and stirred for 45 minutes during which the mixed anhydride formed. The resulting thick white suspension was re-cooled to −75 °C.

To a solution of (4*S*)-4-phenylmethyloxazolidin-2-one (**7**, 11.4 g, 62.6 mmol) and pyreneacetic acid (2 mg, indicator) in THF (150 cm^3), stirred at −40 °C, was added dropwise *n*-butyllithium (*ca.* 1.6 mol dm^{-3} in hexane) until the reaction mixture turned red (42 cm^3 required, 1 equiv.). The resulting solution was stirred for 0.5 h at −40 °C, cooled to −70 °C and added rapidly by cannula to the above stirred suspension containing the mixed anhydride. The residual lithiated oxazolidinone was

rinsed in with THF (2 × 5 cm^3) and the resulting mixture stirred at −70 °C for 70 minutes. After warming to 0 °C, the mixture was poured into phosphate buffer pH 7 (350 cm^3) and extracted with ethyl acetate (400 and 2 × 100 cm^3). The organic layers were each washed with aqueous sodium bicarbonate (half-sat., 200 cm^3) and brine (200 cm^3), combined, dried ($MgSO_4$) and concentrated *in vacuo*. The residual pale yellow oil crystallised from ether–hexane (3:2, 500 cm^3, two crops) to give the *carboximide* **8** (26.1 g, 88%, *E*:*Z* ratio <1:20, as determined by ^{1}H NMR) as white needles; mp 111.5–112 °C; R_F 0.51 (hexane–ethyl acetate 1:1); $[a]_D^{20}$ +20.5 (*c* 2.65, $CHCl_3$) (Found: C, 66.0; H, 7.8; N, 5.8. $C_{26}H_{36}N_2O_6$ requires C, 66.08; H, 7.68; N, 5.93%); $\nu_{max}(CHCl_3)/cm^{-1}$ 3067w (HPh), 1781s (carboximide CO), 1689s (carbamate CO); δ_H(400 MHz; C_6H_6; 343 K) 1.42 (9H, s, CMe_3), 1.63 [3H, s, C(Me)*Me*], 1.67 [3H, s, C(*Me*)Me], 1.80 (2H, m, CH_2), 2.21 (2H, br m, =CHCH_2), 2.41 (1H, dd, *J* 13.4, 9.4, C*H*HPh), 2.87 (1H, ddd, *J* 16.6, 8.0, 6.7, C*H*HCON), 2.99–3.07 (2H, m, CH*H*Ph and CH*H*CON), 3.37 (1H, t, *J* 8.5, OC*H*H auxiliary), 3.54–3.59 (2H, m, OCH*H* auxiliary and OC*H*H), 3.89 (1H, dd, *J* 8.6, 6.3, OCH*H*), 4.22 (1H, ddt, *J* 8.5, 3.2, 1.1, CHN auxiliary), 4.61 (1H, br m, CHN), 5.39 (1H, dt, *J* 10.6, 7.1, =C*H*CH$_2$), 5.49 (1H, dd, *J* 10.6, 9.0, =C*H*CHN), 6.90 (2H, dd, *J* 7.5, 1.5, Ph) and 6.99–7.09 (3H, m, Ph); δ_C(100 MHz; C_6H_6; 343 K) 24.9 and 27.3 (2 × CH_2), 28.6 (C*Me*$_2$ and C*Me*$_3$), 35.3 (*C*H$_2$COO), 36.2 (PhCH_2), 55.0 and 55.1 (CHN and CHN auxiliary), 65.9 (OCH_2 auxiliary), 69.2 (OCH_2), 79.3 (O*C*Me$_3$), 94.1 (O*C*Me$_2$), 127.3, 129.0, 129.6 and 136.1 (CH=CH and Ph), 152.1 and 153.3 (2 × NCOO) and 172.8 (CON); *m*/*z* (CI) 473 [(M + H)$^+$, 4%], 373 [(M − Boc + H)$^+$, 43], 242 (13), 196 (100), 178 (34) and 58 (26) [Found: (M + H)$^+$ 473.2652. $C_{26}H_{37}N_2O_6$ requires *M*, 473.2651].

(4*S*)-3-{(*Z*,2*S*)-2-Azido-6-[(4*R*)-3-*tert*-butyloxycarbonyl-2,2-dimethyl-1,3-oxazolidin-4-yl]-1-oxohex-5-enyl}-4-phenylmethyl-1,3-oxazolidin-2-one 9

A flame dried 4-neck round bottom flask (1 dm^3), equipped with a mechanical stirrer, thermometer and a septum was flushed with nitrogen. THF (320 cm^3) was added and cooled to −75 °C. Then was added, *via* syringe, potassium hexamethyldisilazide (0.5 mol dm^{-3} in toluene, 63 cm^3, 31.5 mmol) and the inner temperature was held at −72 to −68 °C. To the resulting yellowish solution was added, *via* insulated steel cannula, a pre-cooled (−78 °C) solution of carboximide **8** (13.47 g, 28.5 mmol) in THF (115 and 5 cm^3 rinse) within 20 minutes. Stirring was continued at −72 °C for 100 min, in order to allow the enolate to form.

Under positive argon pressure the septum was exchanged for a solid addition tube containing trisyl azide. To the potassium enolate solution, stirred vigorously at −72 °C, was added within 30 s solid trisyl azide (**10**, 11.5 g, 37.1 mmol), which caused the mixture to become bright yellow and the inner temperature to rise to −67 °C. After 190 s from the start of the addition, the reaction mixture was quenched with glacial acetic acid (50% v/v in THF, 16 cm^3, 143 mmol), during which the yellow colour discharged. The cooling bath was immediately replaced by a warm water bath at 50 °C causing the reaction mixture to warm to 26 °C within 3 minutes. An immediate white precipitation of potassium acetate was observed and the suspension was stirred at 25–27 °C for 20 min. The mixture was then partitioned between aqueous ammonium chloride (half-sat., 300 cm^3) and ethyl acetate (200 cm^3) the aqueous phase extracted with ethyl acetate (2 × 100 cm^3) and the organic layers each washed with aqueous sodium bicarbonate (half-sat., 250 cm^3) and brine (300 cm^3). The combined organic phases were dried ($MgSO_4$) and concentrated *in vacuo* to give a crude pale brown oil. Purification by flash chromatography on silica gel (250 g), eluting with ethyl acetate–hexane (0:1 to 1:2, eluant gradient) gave the *azide* **9** (13.72 g, 94%) as a very pale yellow foam; R_F 0.52 (hexane–ethyl acetate 1:1); $[a]_D^{22}$ +119 (*c* 1.28, $CHCl_3$) (Found: C, 60.5; H, 6.7; N, 13.9. $C_{26}H_{35}N_5O_6$ requires C, 60.80; H, 6.87; N, 13.64%); $\nu_{max}(CHCl_3)/cm^{-1}$ 3067w (HPh), 2108s (N_3), 1782s (carboximide CO), 1689s (carbamate CO); δ_H(400 MHz; C_6H_6; 343 K) 1.41 (9H, s, CMe_3), 1.53 [3H, s, C(Me)*Me*], 1.63 [3H, s, C(*Me*)Me], 1.78–2.04 (2H, m, CH_2), 2.34–2.45 (2H, m, =CHCH_2), 2.38 (1H, dd, *J* 13.5, 9.2, C*H*HPh), 2.95 (1H, dd, *J* 13.5, 3.2, CH*H*Ph), 3.45 (1H, br t, *J* 9.0, OC*H*H auxiliary), 3.56 (1H, dd, *J* 8.6, 2.9, OC*H*H), 3.58 (1H, dd, *J* 9.0, 3.3, OCH*H* auxiliary), 3.90 (1H, br m, OCH*H*), 4.20 (1H, dt, *J* 8.6, 3.2, CHN auxiliary), 4.84 (1H, br m, CHN), 5.11 (1H, br m, CHN_3), 5.37 (1H, dt, *J* 10.7, 7.4, =C*H*CH$_2$), 5.50 (1H, ddt, *J* 10.7, 9.5, 1.2, =C*H*CHN), 6.87–6.92 (2H, m, Ph) and 7.00–7.10 (3H, m, Ph); δ_C(100 MHz; C_6H_6; 343 K) 24.3 (CH_2), 24.9 and 27.3 (C*Me*$_2$), 28.6 (C*Me*$_3$), 31.3 (CH_2), 37.6 (PhCH_2), 54.8 and 55.4 (CHN and CHN auxiliary), 60.4 (CHN_3), 66.4 (OCH_2 auxiliary), 69.0 (OCH_2), 79.4 (O*C*Me$_3$), 94.0 (O*C*Me$_2$), 127.5, 129.1, 129.6 and 135.4 (Ph and CH=CH), 152.0 (2 × NCOO) and 170.7 (CON); *m*/*z* (CI) 486 [(M − N_2 + H)$^+$, 96%], 430 (23), 386 [(M − Boc − N_2 + H)$^+$, 8], 195 (100), 178 (31) and 58 (96) [Found: (M − N_2 + H)$^+$ 486.2604. $C_{26}H_{36}N_3O_6$ requires *M*, 486.2604].

(4*S*)-3-[(*Z*,2*R*,7*S*)-2-Azido-7-(*tert*-butyloxycarbonylamino)-8-hydroxy-1-oxooct-5-enyl]-4-phenylmethyl-1,3-oxazolidin-2-one 11

A solution of *N*,*O*-acetonide **9** (1.41 g, 2.74 mmol) in acetic acid–water (10:1, 22 cm^3) was warmed to 40–50 °C and stirred for 5 h. The solvents were removed under reduced pressure and residual traces of acetic acid and water removed by azeotropic distillation with toluene (3 × 20 cm^3). The crude material was purified by flash chromatography on silica gel, eluting with light hexane–ethyl acetate (2:1 to 1:1, eluant gradient) to give the *alcohol* **11** (936 mg, 72%) as a colourless oil [also eluted was recovered starting material (226 mg, 16%)]; R_F 0.16 (hexane–ethyl acetate 1:1); $[a]_D^{21}$ +91.7 (*c* 1.82, $CHCl_3$); $\nu_{max}(CHCl_3)/cm^{-1}$ 3500–3200 br w (OH), 3441m (NH), 3067w (HPh), 2109s (N_3), 1782s (carboximide CO), 1706s (carbamate CO); δ_H(400 MHz; $CDCl_3$) 1.43 (9H, s, CMe_3), 1.92 (2H, br q, *J* 7, CH_2), 2.37 (2H, br q, *J* 7, =CHCH_2), 2.53 (1H, br s, OH), 2.83 (1H, dd, *J* 13.5, 3.0, C*H*HPh), 3.33 (1H, dd, *J* 13.5, 3.0, CH*H*Ph), 3.60 (2H, br t, *J* 5.7, OCH_2 auxiliary), 4.22 (1H, dd, *J* 9.1, 2.7, OC*H*H), 4.29 (1H, br t, *J* 9.1, OCH*H*), 4.44 (1H, m, CHN), 4.69 (1H, br tt, *J* 8.7, 3.0, CHN auxiliary), 4.79 (1H, br d, *J* 5, NH), 4.98 (1H, br t, *J* 7, CHN_3), 5.38 (1H, br t, *J* 10.5, =C*H*CHN), 5.59 (1H, br dt, *J* 10.5, 7.6, =C*H*CH$_2$) and 7.18–7.37 (5H, m, Ph); δ_C(100 MHz; $CDCl_3$) 24.3 (CH_2), 28.4 (C*Me*$_3$), 30.8 (CH_2), 37.6 (PhCH_2), 50.4 (CHNH), 55.5 (CHN auxiliary), 59.9 (CHN_3), 66.0 (CH_2OH), 66.7 (OCH_2), 79.8 (O*C*Me$_3$), 127.6, 128.6, 129.1, 129.5, 131.3 and 134.8 (CH=CH and Ph), 152.9 and 156.0 (NCOO carbamate and OCON auxiliary) and 170.7 (CON); *m*/*z* (CI) 446 [(M − N_2 + H)$^+$, 3%], 195 (100), 178 (26) and 147 (49) [Found: (M − N_2 + H)$^+$ 474.2353. $C_{23}H_{32}N_5O_6$ requires *M*, 474.2352].

(4*S*)-3-[2(*Z*,2*S*,7*R*)-Azido-8-(*tert*-butyldiphenylsilyloxy)-(7-(*tert*-butyloxycarbonylamino)-1-oxooct-5-enyl]-4-phenylmethyl-1,3-oxazolidin-2-one 12

A solution of alcohol **11** (932 mg, 1.97 mmol) in DMF (3 cm^3) was treated with *tert*-butyldiphenylchlorosilane (0.78 cm^3, 2.97 mmol) and imidazole (340 mg, 4.94 mmol). The colourless clear solution was stirred at rt for 23 h. TLC showed the reaction to be complete. The mixture was poured into water (20 cm^3), extracted with ethyl acetate (3 × 50 cm^3), the combined organic layers dried ($MgSO_4$) and the solvent removed under reduced pressure to give a pale yellow oil. The crude was purified by flash chromatography on silica gel, eluting with hexane–ethyl acetate (5:1 to 3:1, eluant gradient) to give the *silyl ether* **12** (1.35 g, 96%) as a white foam; R_F 0.62 (hexane–ethyl acetate

1:1); $[\alpha]_D^{25}$ +46.3 (*c* 1.0, $CHCl_3$); $\nu_{max}(CHCl_3)/cm^{-1}$ 3444m (NH), 3072w (HPh), 2108s (N_3), 1783s (carboximide CO), 1706s (carbamate CO); δ_H(500 MHz; $CDCl_3$) 1.07 (9H, s, $SiCMe_3$), 1.42 (9H, s, $OCMe_3$), 1.89 (2H, br q, *J* 7, CH_2CHN_3), 2.21–2.34 (2H, m, $=CHCH_2$), 2.83 (1H, dd, *J* 13.4, 9.6, CHHPh), 3.34 (1H, dd, *J* 13.4, 3.0, CHHPh), 3.60 (1H, dd, *J* 10.1, 4.8, OCHH), 3.73 (1H, dd, *J* 10.1, 3.9, OCHH), 4.20 (1H, dd, *J* 9.0, 2.5, OCHH auxiliary), 4.25 (1H, br t, *J* 9, OCHH auxiliary), 4.45 (1H, br m, CHN), 4.68 (1H, br t, *J* 7, CHN auxiliary), 4.83 (1H, br m, NH), 5.00 (1H, br m, CHN_3), 5.48–5.55 (2H, m, CH=CH), 7.20–7.44 (11H, m, Ph) and 7.64–7.67 (4H, m, Ph); δ_C(100 MHz; $CDCl_3$) 19.3 ($SiCMe_3$), 23.9 (CH_2), 26.6 ($SiCMe_3$), 28.4 ($OCMe_3$), 30.8 (CH_2), 37.5 ($PhCH_2$), 49.4 (CHNH), 55.4 (CHN auxiliary), 59.6 (CHN_3), 66.3 (CH_2OSi), 66.5 (OCH_2), 79.2 ($OCMe_3$), 127.4, 127.7, 129.0, 129.4, 129.7, 133.1, 135.5 and 135.6 (CH=CH and Ph), 152.7 and 155.2 (NCOO carbamate and OCON auxiliary) and 170.6 (CON); *m*/*z* (CI) 684 [$(M - N_2 + H)^+$, 86%], 610 (77), 298 (50), 287 (72), 274 (74), 196 (85) and 178 (100) [Found: $(M - N_2 + H)^+$ 684.347. $C_{39}H_{50}N_5O_6Si$ requires *M*, 684.3469].

(*Z*,2*S*,7*R*)-2-Azido-8-(*tert*-butyldiphenylsilyloxy)-7-(*tert*-butyloxycarbonylamino)oct-5-enoic acid 13

A solution of carboximide **12** (1.04 g, 1.46 mmol) in THF–water (3:1, 16 cm^3), stirred at 0 °C, was treated with solid lithium hydroxide (hydrate, 122 mg, 2.92 mmol) and after stirring for 0.5 h at 0–2 °C, TLC showed the reaction to be complete. The mixture was acidified to pH *ca.* 1–2 by dropwise addition of aqueous hydrochloric acid (2 mol dm^{-3}, 1.8 cm^3) and extracted with ethyl acetate (3 × 50 cm^3). The organic layers were washed with brine (2 × 30 cm^3), combined, dried ($MgSO_4$) and concentrated *in vacuo*. The crude material was purified by flash chromatography on silica, eluting with hexane–ethyl acetate–acetic acid (300:100:1 to 0:1:0, eluant gradient) to give the *acid* **13** (764 mg, 95%) as a colourless gum; R_F 0.25 (hexane–ethyl acetate–acetic acid 100:100:1); $[\alpha]_D^{20}$ +16.5 (*c* 0.43, $CHCl_3$); $\nu_{max}(CHCl_3)/cm^{-1}$ 3441m (NH), 3400–2400br w (COOH), 3072m (HPh), 2109s (N_3), 1715br s (acid CO and carbamate CO); δ_H(500 MHz; $CDCl_3$) 1.07 (9H, s, $SiCMe_3$), 1.46 (9H, s, $OCMe_3$), 1.76–1.84 (1H, m, CHHCHN$_3$), 1.87–1.95 (1H, br m, CHHCHN$_3$), 2.10–2.25 (2H, m, $=CHCH_2$), 3.60 (1H, dd, *J* 10.2, 5.2, OCHH), 3.69 (1H, br d, *J* 10.2, OCHH), 3.82 (1H, br m, CHN), 4.44 (1H, br m, CHN_3), 4.97 (1H, br m, NH), 5.43–5.48 (2H, br m, CH=CH), 7.38–7.44 (6H, m, Ph), 7.64–7.68 (4H, m, Ph) and 9.27 (1H, br s, COOH); δ_C(100 MHz; $CDCl_3$) 19.2 ($SiCMe_3$), 23.8 (CH_2), 26.8 ($SiCMe_3$), 28.3 ($OCMe_3$), 30.9 (CH_2), 49.6 (CHNH), 61.0 (CHN_3), 66.1 (CH_2OSi), 79.9 ($OCMe_3$), 127.7, 128.2, 129.0, 129.4, 129.8, 130.3, 133.1 and 135.5 (CH=CH and Ph), 155.7 (NCOO carbamate) and 174.1 (COOH); *m*/*z* (CI) 525 [$(M - N_2 + H)^+$, 12%], 274 (38), 196 (76), 94 (70), 58 (95) and 44 (100); also isolated was the recovered chiral auxiliary **7** (242 mg, 94%) as colourless crystals; mp 81–83 °C.

(3*S*,8*R*)-3-Azido-8-(*tert*-butyldiphenylsilyloxymethyl)-1,2,3,4,5,8-hexahydroazocin-2-one 15

A solution of Boc − amino acid **13** (159 mg, 0.287 mmol) in DCM (4 cm^3) was cooled to 0 °C, treated with TFA (2 cm^3) and the resulting pale brown solution stirred at 0 °C for 0.5 h. The solvents were removed *in vacuo* and residual traces of TFA removed by azeotropic distillation with toluene (2 × 70 cm^3). The resulting pale brown resin was dried at 0.2 mmHg for 2 h. The amino acid TFA salt **14** was dissolved in THF (92 cm^3), treated with triethylamine (0.25 cm^3, 1.73 mmol), stirred for 15 minutes, and cooled to 0 °C. Then was added diphenoxyphosphoryl azide (0.33 cm^3, 1.44 mmol) and stirring continued for 1 h. The flask was then placed in the refrigerator at 5 °C for 68 h; the mixture was then allowed to warm to rt and left for a further 72 h. The resulting pale brown reaction mixture was concentrated *in vacuo* and the crude material purified by flash chromatography on silica gel, eluting with hexane–ethyl acetate (1:0 to 3:1, eluant gradient) to give the *lactam* **15** (107 mg, 80%) as a white solid; mp 140–141 °C (ether–hexane); R_F 0.27 (hexane–ether 2:1); $[\alpha]_D^{22}$ +119.2 (*c* 0.63, $CHCl_3$) (Found: C, 66.3; H, 7.0; N, 13.0. $C_{24}H_{30}N_4O_2Si$ requires C, 66.33; H, 6.96; N, 12.89%); $\nu_{max}(CHCl_3)/cm^{-1}$ 3369w (NH), 3073w (HPh), 2104s (N_3), 1661s (lactam CO); δ_H(400 MHz; $CDCl_3$) 1.08 (9H, s, CMe_3), 1.73–1.81 (1H, m, CHHCHN$_3$), 1.94–2.00 (1H, m, CHHCHN$_3$), 2.07–2.14 (1H, m, =CHCHH), 2.58–2.66 (1H, m, =CHCHH), 3.65 (1H, dd, *J* 10.4, 5.3, OCHH), 3.77 (1H, dd, *J* 10.4, 4.4, OCHH), 4.24 (1H, br d, *J* 5.6, CHN_3), 4.44 (1H, br m, CHNH), 5.71–5.80 (2H, m, CH=CH), 6.16 (1H, br d, *J* 7.7, NH), 7.37–7.48 (6H, m, Ph) and 7.62–7.65 (4H, m, Ph); δ_C(100 MHz; $CDCl_3$) 19.3 ($SiCMe_3$), 21.4 (CHN_3CH_2), 26.9 (CMe_3), 27.6 (CH_2), 52.7 and 62.3 (CHN and CHN_3), 66.0 (OCH_2), 127.9, 129.5, 130.0, 130.5, 132.6, 132.7, 135.5 and 135.6 (CH=CH and Ph) and 173.4 (CON); *m*/*z* (CI) 435 [$(M + H)^+$, 100%], 407 [$(M - N_2 + H)^+$, 99], 377 (15), 274 (28), 151 (53), 137 (98) and 82 (36) [Found: $(M + H)^+$ 435.2216. $C_{24}H_{31}N_4O_2Si$ requires *M*, 435.2216].

(3*S*,8*R*)-3-Acetylamino-8-(*tert*-butyldiphenylsilyloxymethyl)-azocan-2-one 17

To a solution of unsaturated azido lactam **15** (210 mg, 0.488 mmol) in DCM (1 cm^3) and ethanol (95%, 6 cm^3) was added palladium on charcoal (10%, 10 mg) and aqueous hydrochloric acid (6 M, 0.082 cm^3, 0.492 mmol). The black suspension was vigorously stirred under an atmosphere of hydrogen (fitted balloon) for 18 h, whereupon TLC showed complete reduction (ninhydrin active). The mixture was filtered through a pad of Celite® and washed with ethanol (abs., 2 × 4 cm^3) and DCM (2 × 4 cm^3). The filtrate was concentrated *in vacuo* and residual traces of ethanol removed by azeotropic distillation with hexane (2 × 10 cm^3). The very pale yellow foam obtained was dried at 0.2 mmHg for 2 h.

The crude hydrochloride **16** (0.23 g) was dissolved in DCM (6 cm^3), cooled to 0 °C and treated with acetyl chloride (0.055 cm^3, 0.73 mmol) and triethylamine (0.18 cm^3, 1.22 mmol). After stirring for 2 h, the mixture was poured into aqueous hydrochloric acid (1 mol dm^{-3}, 20 cm^3) and extracted with DCM (50 and 2 × 25 cm^3). The organic layers were each washed with aqueous sodium bicarbonate (half-sat., 20 cm^3), combined, dried ($MgSO_4$) and the solvent removed *in vacuo* to afford a pale yellow solid. Purification by flash chromatography on silica gel, eluting with hexane–ethyl acetate–methanol (1:2:0 to 0:19:1, eluant gradient) gave the *acetamide* **17** (153 mg, 85%) as a white solid; mp 180–181 °C (chloroform); R_F 0.52 (chloroform–methanol 10:1); $[\alpha]_D^{22}$ +32.4 (*c* 1.02, $CHCl_3$); $\nu_{max}(CHCl_3)/cm^{-1}$ 3416m (NH), 3374w (NH), 3073w (HPh), 1655s (amide CO); δ_H(400 MHz; $CDCl_3$; 333K) 1.07 (9H, s, CMe_3), 1.45–1.63 (4H, m, CH_2CH_2), 1.73–1.86 (2H, m, CHHCH$_2$CH$_2$CHH), 1.87–2.01 (1H, m, CHH), 1.96 (3H, s, COMe), 2.23–2.36 (1H, m, CHH), 3.60 (1H, dd, *J* 10.3, 5.6, OCHH), 3.68 (1H, dd, *J* 10.3, 3.6, OCHH), 3.69 (1H, br m, CHNH), 4.66 (1H, br m, CHNHAc), 5.70 (1H, br m, NHAc), 6.47 (1H, br d, NH), 7.34–7.43 (6H, m, Ph) and 7.60–7.64 (4H, m, Ph); δ_C(100 MHz; $CDCl_3$; 333 K) 19.3 ($SiCMe_3$), 23.1 (CH_2), 25.3 ($COMe$ and CH_2), 27.0 (CMe_3), 31.7 and 36.6 (2 × $CHNCH_2$), 53.0 and 56.4 (CHN and CHNHAc), 67.3 (OCH_2), 127.8, 129.9, 133.1, 133.2, 135.5 and 135.6 (Ph) and 169.0 and 175.1 (2 × CON); *m*/*z* (CI) 453 [$(M + H)^+$, 15%], 197 (25), 140 (28), 94 (71), 77 (83) and 44 (100) [Found: $(M + H)^+$ 453.2573. $C_{26}H_{37}N_2O_3Si$ requires *M*, 453.2573].

(3*S*,8*R*)-3-Acetylamino-8-hydroxymethylazocan-2-one 18

A solution of silyl ether **17** (136 mg, 0.30 mmol) in THF (1 cm^3) was treated with TBAF (1 mol dm^{-3} in THF, 0.33 cm^3, 0.33 mmol) and stirred at rt for 45 minutes. TLC showed the

reaction to be complete. The reaction mixture was directly subjected to flash chromatography on silica gel, eluting with ethyl acetate–methanol (1:0 to 3:1, eluant gradient) to give the *alcohol* **18** (60 mg, 93%) as a white solid; mp 188–190 °C; R_F 0.34 (chloroform–methanol 4:1); $[\alpha]_D^{19}$ +87.4 (*c* 0.44, methanol); ν_{max}(KBr)/cm^{-1} 3600–3100m (OH, NH), 3296s (NH), 1677s (CO), 1649s (CO); δ_H(400 MHz; DMSO-d_6; 373 K) 1.34–1.48 (1H, m, C*H*H), 1.48–1.69 (4H, m, CH_2CH_2), 1.69–1.82 (2H, m, CH_2), 1.85 (3H, s, Ac), 1.88–1.99 (1H, m, CH*H*), 3.42 (2H, m, OCH_2), 3.77 (1H, br m, $HOCH_2CHN$), 4.0–4.5 (1H, br s, OH), 4.39 (1H, dt, *J* 8, 6.5, C*H*NHAc), 6.71 (1H, d, *J* 9, NHAc) and 7.55 (1H, br s, NH); δ_C(100 MHz; DMSO-d_6, major conformer quoted) 22.0 (CH_2), 22.4 (CO*Me*), 26.1 (CH_2), 32.2 and 36.8 (2 × CHN*C*H_2), 53.7 and 56.6 (*C*HNHAc and CHN), 63.8 (OCH_2) and 169.1 and 178.6 (2 × CON); *m*/*z* (CI) 215 [(M + H)$^+$, 6%], 130 (54), 77 (88) and 60 (100) [Found: (M + H)$^+$ 215.1396. $C_{10}H_{19}N_2O_3$ requires *M*, 215.1396].

(3*S*,8*R*)-3-Acetylamino-8-methoxycarbonylazocan-2-one 19

To a solution of hydroxymethyl lactam **18** (75 mg, 0.35 mmol) in a mixture of acetonitrile (1.3 cm^3) and water (1.2 cm^3) was added carbon tetrachloride (1.3 cm^3), sodium periodate (300 mg, 1.4 mmol) and ruthenium trichloride hydrate (2 mg). The resulting dark brown reaction mixture was stirred vigorously at rt for 1 h. TLC showed the oxidation to be complete. The reaction mixture was cooled to 0 °C, quenched with propan-2-ol (0.28 cm^3) and the resulting black mixture stirred for 0.5 h at 0 °C. Then, methanol (3 cm^3) was added, followed by trimethylsilyldiazomethane (2 mol dm^{-3} in hexane, 1.5 cm^3) and an immediate formation of nitrogen was observed. After stirring for 15 minutes at 0 °C the dark brown suspension was treated with acetic acid (1 cm^3), filtered through a plug of silica gel, and washed with methanol (5 × 1 cm^3). The filtrate was concentrated *in vacuo,* the residual gum suspended in ethyl acetate–methanol (1:1, 0.5 cm^3) and directly subjected to flash chromatography on silica gel. Elution with ethyl acetate–methanol (1:0 to 10:1, eluant gradient) gave the *methyl ester* **19** (65 mg, 77%), as a very pale brown solid; mp 147–148 °C (ethyl acetate–hexane); R_F 0.44 (chloroform–methanol 10:1); $[\alpha]_D^{25}$ +68.9 (*c* 0.56, $CHCl_3$) (Found: C, 54.2; H, 7.5; N, 11.5. $C_{11}H_{18}N_2O_4$ requires C, 54.53; H, 7.49; N, 11.56%); ν_{max}($CHCl_3$)/cm^{-1} 3414m (NH), 3295br m (NH), 1740s (ester CO), 1659s (amide CO); δ_H(400 MHz; $CDCl_3$; 333 K) 1.47–1.83 (5H, m, CH_2CH_2C*H*H), 1.94 (3H, s, COMe), 1.98–2.22 (3H, m, C*H*H and 2 × CH*H*), 3.73 (3H, s, OMe), 4.37 (1H, br m, C*H*NH), 4.65 (1H, br dq, *J* 7.2, 2.4, C*H*NHAc) and 6.51 (2H, br m, 2 × NH); δ_C(100 MHz; $CDCl_3$) 23.0 (CO*Me*), 24.0 and 24.8 ($CH_2CH_2CH_2$), 29.6 (CHN*C*H_2), 52.5 and 55.7 (OMe, CHN and *C*HNHAc) and 169.1, 171.5 and 176.2 (*C*OOMe and 2 × CON); *m*/*z* (EI) 242 (M$^+$, 15%), 183 [(M − CO_2Me)$^+$, 18], 96 (76) and 43 (100) (Found: M$^+$ 242.1267. $C_{11}H_{18}N_2O_4$ requires *M*, 242.1266).

(3*S*,8*R*)-3-Acetylamino-8-methylcarbamoylazocan-2-one 3

Solid methyl ester **19** (11.3 mg, 0.047 mmol) was added in one portion to cold (0 °C) ethanolic methylamine (8.0 mol dm^{-3}, 5 cm^3) and the solution stirred at 0–5 °C for 1 h. The mixture was concentrated *in vacuo*, keeping the temperature at 0 °C. The crude product was purified by flash chromatography on silica gel, eluting with chloroform–methanol (1:0 to 5:1, eluant gradient) to give the *trans-lactam dipeptide* **3** (10 mg, 89%) as a white solid; mp 266–270 °C (decomp., methanol); R_F 0.16 (chloroform–methanol 10:1); $[\alpha]_D^{20}$ +50.3 (*c* 0.53, methanol); ν_{max}(KBr)/cm^{-1} 3285br s (NH), 1654s (amide CO); δ_H(400 MHz; DMSO-d_6, 333 K) 1.47–1.79 (6H, br m, CH*H*CH_2-CH_2CH*H*), 1.84 (3H, s, COMe), 1.82–2.03 (2H, br m, CH*H*, CH*H*), 2.62 (3H, d, *J* 4.6, NH*Me*), 4.27 (1H, br m, C*H*NHAc), 4.35 (1H, dt, *J* 9.7, 6.2, C*H*NH), 7.33 (1H, br m, N*H*Me), 7.69 (1H, br d, NH) and 7.74 (1H, br d, *J* 4.2, N*H*Ac); δ_C(100 MHz; DMSO-d_6, major conformer quoted) 22.4 (CH_2), 25.4 (CH_2), 25.6 (CO*Me*), 32.2 and 32.9 (2 × CHN*C*H_2), 37.7 (NHMe), 53.2 and 57.8 (CHN and *C*HNHAc) and 168.6, 171.4 and 178.4 (3 × CON); *m*/*z* (EI) 241 (M$^+$, 22%), 183 [(M − NHCOMe)$^+$, 20], 96 (86), 56 (45) and 43 (100) (Found: M$^+$ 241.1426. $C_{11}H_{19}N_3O_3$ requires *M*, 241.1426). Also isolated was the *cis-lactam* **4** (1.2 mg, 11%) as a white solid; R_F 0.27 (chloroform–methanol 10:1); $[\alpha]_D^{20}$ +2.7 (*c* 0.26, $CHCl_3$).

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