Synthesis and conformational analysis of a dimerising eight-membered lactam dipeptide

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The eight-membered lactam dipeptide *(3R,8R)-3-acetylamino-8-methoxycarbamoylazocan-2-one* was prepared from the L-serine derived *(4S)-3-tert-butyloxycarbonyl-4-formyl-2,2-dimethyl-1,3-oxazolidine* in 12 steps and 27% overall yield. An extensive conformational analysis both in the solid state and in solution, using NMR, IR and vapour pressure osmometry, was conducted. It was shown that the constrained dipeptide exists in a semi-extended conformation and exhibits a head-to-tail self-recognition (K_{dim} 100 ± 20 dm^3 mol^{-1} in $CDCl_2CDCl_2$). This dimerisation appears to be a general phenomenon in a series of *cis*-disubstituted medium-ring lactam dipeptides.

Introduction

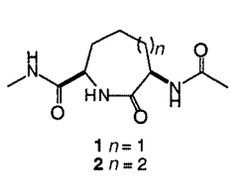
Self-assembly is an important phenomenon in biology where it occurs with great efficiency to afford a very high level of specificity and architectural control under mild conditions.[1–3] Examples include RNA, DNA, membranes, proteins, *etc*. Much of the structure and function of many biomolecules or bioassemblies is derived from non-covalent interactions such as hydrogen bonding and hydrophobic and ionic interactions. The study of non-covalent interactions has occupied a central role in modern organic chemistry and is exemplified with the advent of supramolecular chemistry.[4,5]

As part of a programme to design disubstituted medium-ring lactam β-turn mimetics, we have encountered a remarkable example of amide self-recognition. The *cis*-disubstituted seven-membered lactam **1** was found to self-associate in non-polar organic solvents in a highly ordered head-to-tail manner. Although simple lactams are known to dimerise weakly in organic solvents, the relatively large dimerisation constant for **1** (K_{dim} 150 ± 50 dm^3 mol^{-1}) is due to the high degree of co-operativity upon binding. We have now extended this work and report here the synthesis and comprehensive study of the conformational behaviour of the homologous eight-membered lactam dipeptide **2**.

Results and discussion

Synthesis of eight-membered lactam dipeptide 2

Following the basic strategy used to make the seven-membered homologue **1**,[6,7] the major disconnections outlined in Fig. 1 were envisaged. The nitrogen functionality could be introduced as an azide, with the stereochemistry being directed by the existing stereocentre and the medium-ring conformational bias. Failing this, however, the α-position would alternatively be directed using Evans's chiral auxiliary,[8] with the electrophilic azide transfer occurring on the open chain precursor. The lactam was to be formed by means of cyclisation of the open chain precursors. The *Z*-double bond should not only help to bring the reacting moieties closer together for the lactamisation, but also provide a convenient disconnection point through Wittig type chemistry.



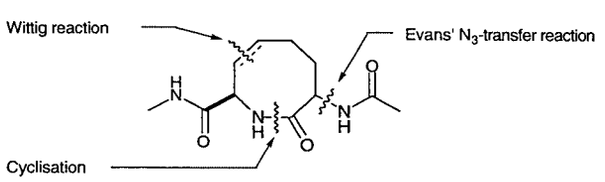
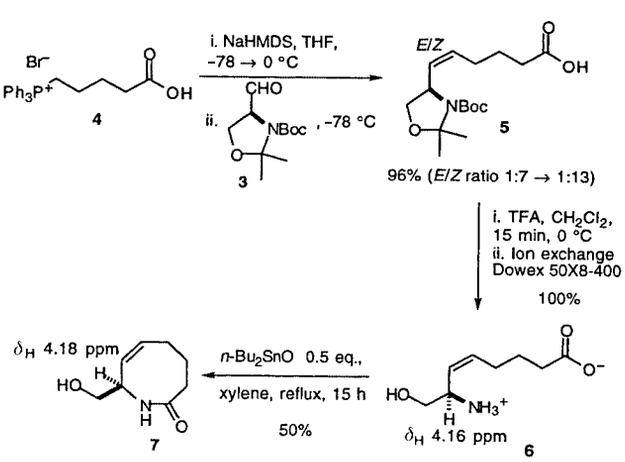


Fig. 1 Relevant disconnections of the target molecule **2**.

Wittig reaction of the Garner aldehyde **3**, available from Boc-L-serine,[9,10] and commercially available (4-carboxybutyl)-triphenylphosphonium bromide **4** provided olefins **5** in excellent yield (Scheme 1). The *Z*:*E* ratio of the crude product mixture was found to be 7:1, as determined by ^{1}H NMR, and recrystallisation from carbon tetrachloride afforded crystals with an



Scheme 1

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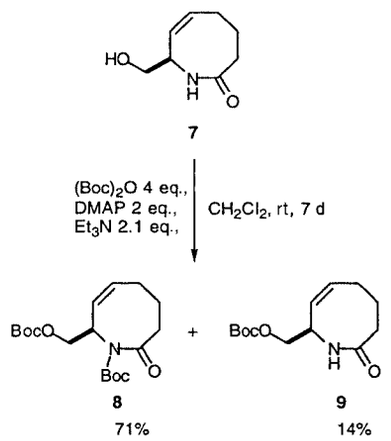
increased ratio of 13:1. Further such recrystallisations from carbon tetrachloride afforded virtually pure *Z*-isomer.

The crude mixture of *N*,*O*-acetonides **5** was deprotected with excess TFA in DCM at 0 °C and the resulting trifluoroacetate was subjected to ion exchange chromatography on Dowex 50X8-400 resin to afford the free amino acids **6** in quantitative yield. The cyclisation of amino acid **6** to the eight-membered hydroxymethyl lactam **7** (ν_{NH} 3383, ν_{CO} 1657 cm^{-1}) was performed using dibutyltin oxide in refluxing *p*-xylene.[6,7,11] It is interesting to note that the yield of the eight-membered lactam never exceeded 50%, whereas the corresponding seven-membered lactam was obtained in up to 89% yield.[6,7] Protection of the hydroxy and amido functionalities was achieved by treatment of the lactam **7** with di-*tert*-butyl dicarbonate, triethylamine and DMAP in DCM (Scheme 2). This gave the bis(Boc) lactam **8** in 71% yield together with the mono(Boc) lactam **9** (14%, ν_{NH} 3387 cm^{-1}).

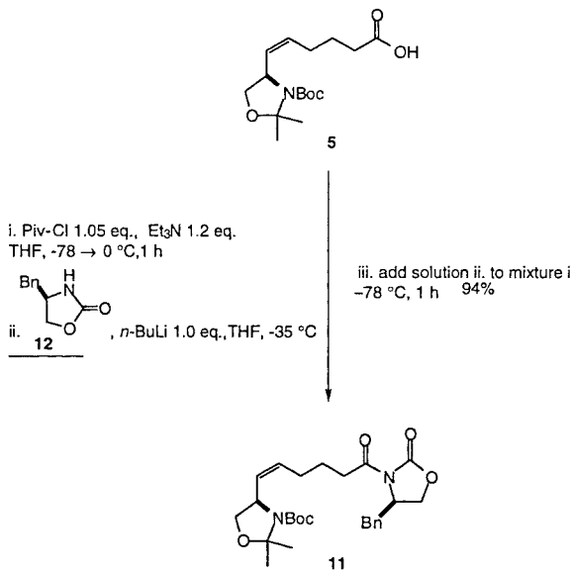


Scheme 2

Introduction of the nitrogen functionality using the azidation of the lactam enolate from **8** required considerable investigation. In parallel, early success was realised with the alternative strategy using the acyclic precursor. Effort was therefore directed towards the preparation of the open chain precursor. The control of diastereoselectivity in the azidation of carboximides[8] was expected to be superior to that achieved by the conformational bias of the eight-membered lactam.

Carboximide **11**‡ (ν_{CO} 1780 cm^{-1}) was prepared by acylation of oxazolidinone **12** with the mixed anhydride derived from the octenoic acid **5** (Scheme 3).[12] If a mixture of *E*- and *Z*-isomers of **5** was used, the product could easily be recrystallised from diethyl ether–hexane to give the desired *Z*-isomer in over 95% diastereomeric excess. This finding avoided the need to recrystallise the octenoic acid **5** from toxic carbon tetrachloride.

Applying Evans's optimised conditions§ to the carboximide **11** gave the desired α-azido carboximide **13** in moderate yield (20–40%), together with some recovered starting material (Scheme 4). However, by cooling the trisyl azide solution to approximately −95 °C before transferring it *via* an insulated steel cannula to the enolate solution (at −78 °C), the α-azido carboximide **13** (ν_{N_3} 2108 cm^{-1}) could be isolated in 45–82% yield.¶ Moreover, this could be further improved by adding trisyl azide as a *solid* to the vigorously stirred enolate solution at −78 °C, thus giving higher and more reproducible yields (75–95%). This observation could be of general synthetic interest to chemists who are involved in azide transfer reactions.



Scheme 3

Saponification of the α-azido imide **13** was carried out with 2 equiv. of lithium hydroxide in THF–water (3:1) (Scheme 5)[8] and the α-azido acid **15** was isolated in up to 97% yield. The auxiliary **12** was generally recovered in nearly quantitative yield. Deprotection of α-azido acid **15** to give the corresponding α-azido amino acid **16** was carried out using TFA. An attempt to cyclise the α-azido amino acid **16**, using di-*n*-butyltin oxide[6,7,11] in refluxing xylene failed to yield any of the expected lactam **17** since the substrate decomposed in boiling xylene.

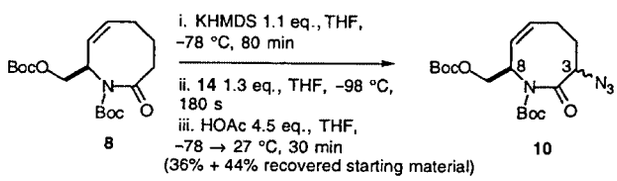
Diphenylphosphoryl azide [$(PhO)_2P(O)N_3$, DPPA, **18**], a reagent introduced by Yamada and co-workers[13] for the formation of the amide bonds in peptide synthesis, and more recently used by Qian[14] and Cathala[15] for the formation of macrocyclic lactams, showed promise in this context. Thus, the acyclic precursor **16** was treated with 2.5 equiv. of triethylamine for ten minutes followed by 2.5 equiv. of DPPA overnight at ambient temperature (Scheme 6) to afford the azido lactam **17** (ν_{NH} 3376, ν_{CO} 1673, ν_{N_3} 2107 cm^{-1}) in 40% yield.

X-Ray crystallographic analysis of azide **17**‖ revealed a pseudo-boat conformation in the solid state (Fig. 2) and proved

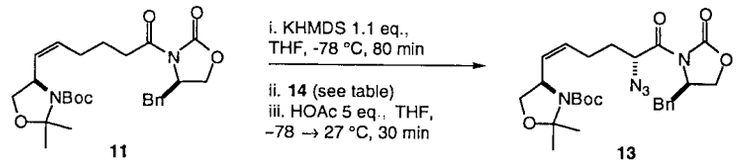
‡ The ^1H NMR spectrum (400 MHz, $CDCl_3$, 295 K) of **11** was largely uninterpretable, owing to the hindered rotation of the Boc group.[9,12] However, at 70 °C, in C_6D_6, these signals coalesced to a single set.

§ This involves the addition of a pre-cooled (−78 °C) solution of trisyl azide (**14**) to the stirred enolate solution at −78 °C (trisyl = 2,4,6-triisopropylbenzenesulfonyl).

¶ Azidation of the lactam **8** required the use of the precooled (−95 °C) trisyl azide technique which gave an inseparable mixture of starting material **8** [44%, δ_H(H-8) 5.03 ppm] and the two product diastereoisomers **10** [36%; ν_{N_3} 2108 cm^{-1}]; ratio of diastereoisomers 1:1.4, as deduced from the ^1H NMR spectrum; δ_H(H-8) 5.20 ppm for the 3*S* isomer and 5.08 ppm for the 3*R* isomer, was obtained. This result shows that the diastereoselectivity of the electrophilic azide transfer reaction to the eight-membered lactam enolate is not very high, which justifies the use of the auxiliary to control the stereochemistry of the azide centre.



‖ Atomic coordinates, bond lengths and angles, and thermal parameters for compounds **17** and **2** have been deposited at the Cambridge Crystallographic Data Centre. CCDC reference number 207/452. See http://www.rsc.org/suppdata/p1/b0/b003789n for crystallographic files in .cif format. Crystal data, analysis and refinement for **17**: empirical formula $C_8H_{12}N_4O_2$; formula weight (*M*) 196.22; temperature 293(2) K; crystal system orthorhombic; space group $P2_12_12_1$; unit-cell dimensions a = 12.033(2) Å, α = 90°, b = 12.7680(10) Å, β = 90°, c = 6.244(2) Å, γ = 90°; volume 959.3(4) $Å^3$; Z = 4; μ = 1.01 cm^{-1}; reflections collected 1409; independent reflections 1385 (R_{int} = 0.0135); final *R* indices (all data) $R1$ = 0.1165, $wR2$ = 0.1892.



Conditions for ii.	Yield	Remarks
14 1.2 eq., THF, -78 °C, 220 s	20–40%	Incomplete reaction
1.2 eq., THF, -95 to -100 °C, 220 s	45–82%	Sometimes incomplete reaction
1.5 eq., solid, -78 °C, 200 s	75–95%	Usually complete reaction

Scheme 4

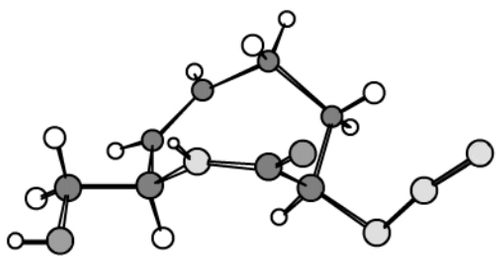
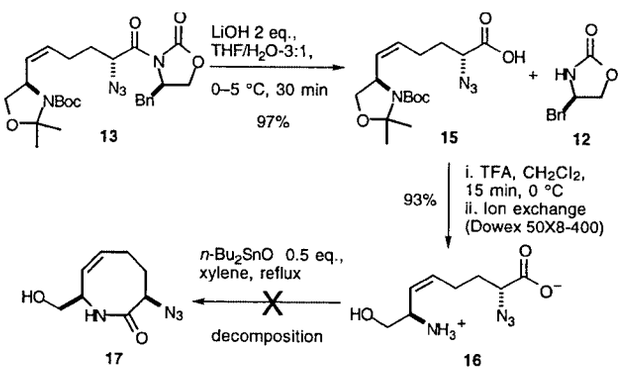
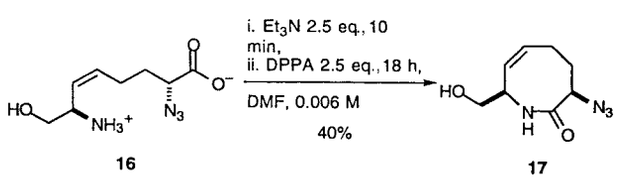


Fig. 2 Chem3D® representation of the single crystal X-ray structure of *cis*-azidolactam **17**.



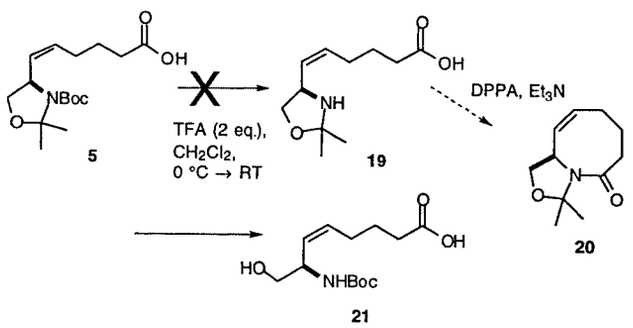
Scheme 5



Scheme 6

the overall relative stereochemistry of this molecule beyond doubt.

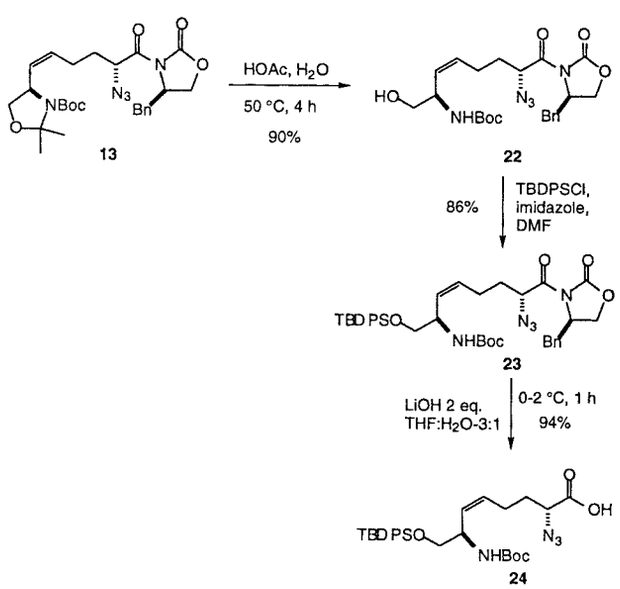
During the course of these synthetic studies, Nakagawa *et al.*[16] published a paper describing a convenient route for the preparation of optically active azocin-2-ones from the serine-derived aldehyde **3**. Although no experimental details were given, the yield for the DPPA-mediated cyclisation of seco-acid **19** to eight-membered lactam **20** was reported to be as high as 78%. This improvement (compared with our 40% yield for cyclisation of **10**) appears to be due to the protection of the hydroxymethyl moiety. However, we were unable to prepare **19** by selective removal of the Boc protecting group from **5** (Scheme 7). Treatment of *N*-Boc-amino acid **5** with two equivalents of TFA in DCM resulted in removal of the acetonide to provide the hydroxy amino acid **21** instead of the required secondary amine **19**. Other procedures to remove the Boc



Scheme 7

protecting group selectively were then explored,[17,18] but in each one the isopropylidene acetal proved to be more labile than the Boc group.

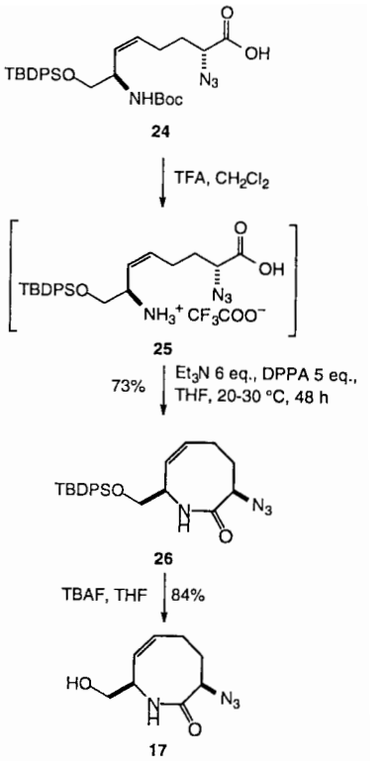
To overcome the problem with the free hydroxy group we adopted an alternative protecting group strategy. Acetic acid–water-mediated cleavage of the *N*,*O*-isopropylidene acetal gave the alcohol **22** in 90% yield (Scheme 8). Silylation of the alcohol



Scheme 8

22 with *tert*-butyldiphenylsilyl chloride (TBDPSCl) and imidazole in DMF gave the TBDPS ether **23**. The auxiliary was removed as described earlier and the *Z*-azidooctenoic acid **24** was isolated in 94% yield.

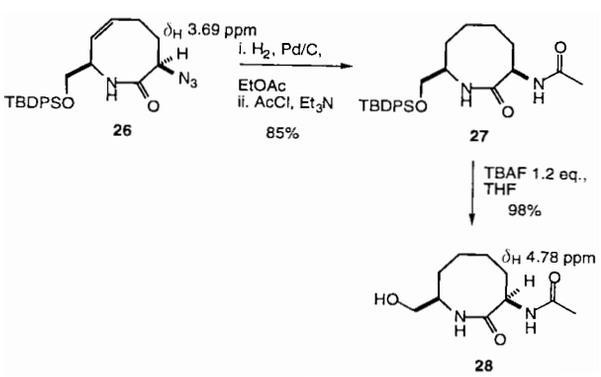
The amine functionality in **24** was exposed by cleaving the Boc group with TFA in DCM (Scheme 9). The amino acid TFA salt **25** was directly subjected to cyclisation conditions. Thus,



Scheme 9

treatment of seco-acid **25** with 6 equiv. of triethylamine and 5 equiv. of DPPA provided the lactam **26** (ν_{NH} 3378, ν_{N_3} 2107, ν_{CO} 1676 cm^{-1}) in 73% yield. Removal of the TBDPS group gave compound **17**, identical to that isolated earlier.

Concomitant reduction of the azide functionality and the carbon–carbon double was achieved by catalytic hydrogenation (Scheme 10), and the resulting amine was directly exposed to



Scheme 10

acetyl chloride and triethylamine to afford the *N*-acetyl derivative **27**. TBAF-Mediated deprotection of the alcohol moiety gave the hydroxymethyl lactam **28** in 83% yield (from **26**).

The hydroxymethyl lactam **28** was then oxidised to the carboxylic acid **29** following Sharpless's ruthenium tetraoxide procedure (Scheme 11).[19] The acid **29** was not isolated, but was treated directly with trimethylsilyldiazomethane[20] to give the methyl ester **30** in 75% yield. Treatment of the methyl ester **30** with methylamine[21] gave the *cis*-disubstituted lactam dipeptide **2** in quantitative yield.

The structure of **2** was initially confirmed by ^{1}H NMR spectroscopy, whereby a strong NOE[22,23] was observed between the two C*H*N-groups ($\delta_{H\text{-}3}$ 4.74 and $\delta_{H\text{-}8}$ 4.45 ppm) in the ring. Later, the structure was underpinned by an X-ray structure (Fig. 3).

In summary, the synthesis of the *cis*-disubstituted lactam dipeptide **2**, has been achieved in 12 steps, starting with the

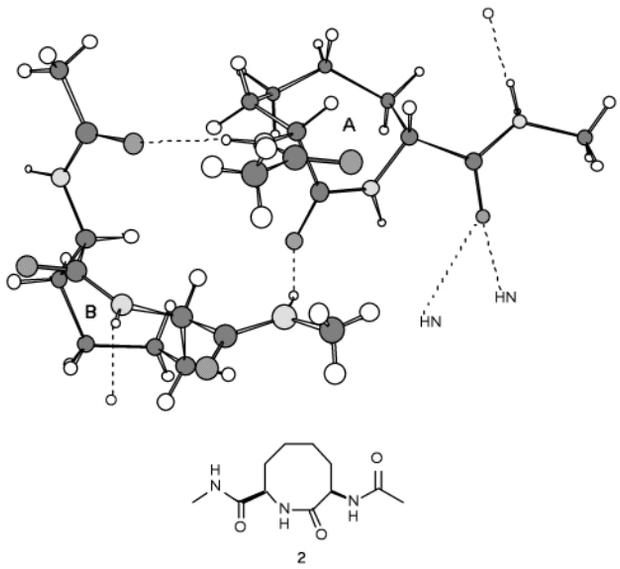
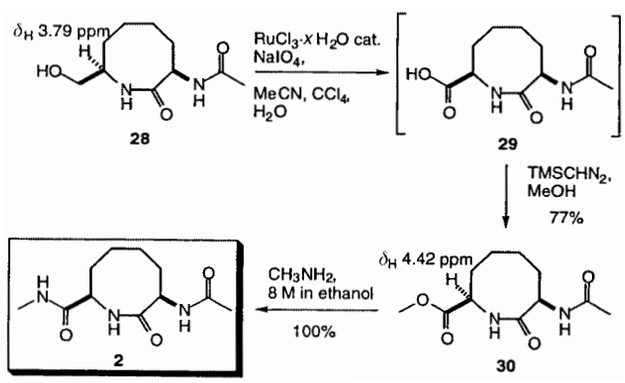


Fig. 3 Two conformers, **A** and **B**, as found by X-ray crystallographic analysis of *cis*-lactam dipeptide **2** represented with Chem3D®.



Scheme 11

L-serine derived aldehyde **3**. The overall yield for the sequence is 22–27%.

Conformational analysis

X-Ray structure. Single crystals of the lactam dipeptide **2** were grown from solution in chloroform–hexane and were readily analysed. The crystal structure** is shown in Fig. 3. The solid state analysis revealed a dimerisation of two slightly different conformers of lactam dipeptide **2** in the crystal unit cell. The dimers are further cross-linked through a highly complex hydrogen bonding network involving most CONH functionalities.

Solution NMR studies. The crystallographic analysis of the lactam **2** points to important interactions in the solid state. An analogous seven-membered lactam also showed a propensity to dimerise in solution,[6] and it was of interest to study the potential dimerisation of the eight-membered lactam **2**. Spectroscopic techniques have found wide application in the conformational analysis of peptides and proteins in solution. Gellman *et al.* have systematically investigated a variety of

** Crystal data, analysis and refinement for **2**: empirical formula $C_{11}H_{19}N_3O_3$; formula weight (*M*) 241.29; temperature 293(2) K; crystal system orthorhombic; space group $P2_12_12_1$; unit-cell dimensions $a = 14.871(3)$ Å, $\alpha = 90°$, $b = 18.478(5)$ Å, $\beta = 90°$, $c = 9.682(3)$ Å, $\gamma = 90°$; volume 2660.5(12) Å^3; $Z = 8$; $\mu = 0.89$ cm^{-1}; reflections collected 1994; independent reflections 1994 ($R_{int} = 0.0000$); final *R* indices (all data) $R1 = 0.2021$, $wR2 = 0.2724$.

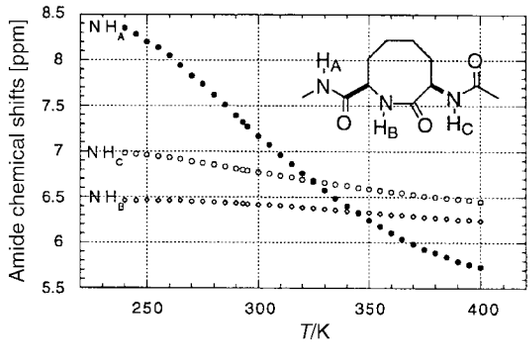


Fig. 4 Amide proton chemical shifts of **2** (*c* 0.02 mol dm^{-3} in $CDCl_2CDCl_2$) as a function of temperature.

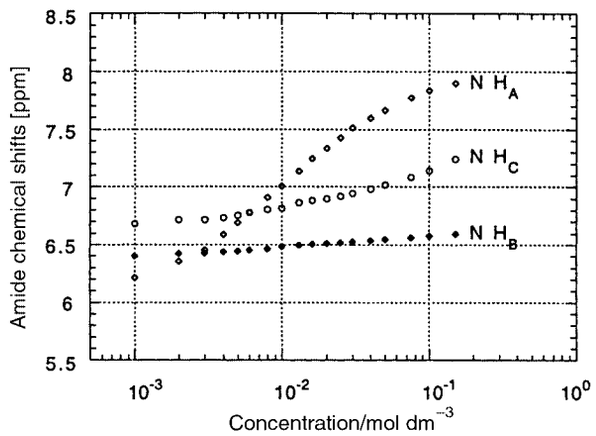


Fig. 5 Amide proton chemical shifts of **2** (in $CDCl_3$ at 293 K) as a function of concentration (see Fig. 4 for assignments).

small molecules, ranging from simple diamides to oligopeptides, using IR and ^{1}H NMR spectroscopy.[24–31] Indeed, it was shown that this provided both qualitative and quantitative information on thermodynamic relationships.[27] Based on this precedent, the conformational behaviour of the lactam dipeptides described previously was probed primarily using variable temperature ^{1}H NMR, but complemented by variable concentration NMR and IR, as well as vapour pressure osmometry and NOE measurements.

Variable temperature (VT) and variable concentration (VC) ^{1}H NMR data were collected in either deuterated 1,1,2,2-tetrachloroethane ($CDCl_2CDCl_2$) or deuterated chloroform ($CDCl_3$), both of which are known to have similar properties, and it is not unreasonable to assume that the behaviour of lactam **2** is very similar in both solvents. Fig. 4 shows the VT NMR plot of the amide proton chemical shifts of **2** (H_A, H_B and H_C were assigned based on COSY and NOE spectra) at a concentration of 10^{-2} mol dm^{-3}. Compared with those of protons H_B and H_C, the shift of proton H_A shows a much larger temperature dependence and is thus most probably locked in a hydrogen bond.

In order to establish whether the interaction was *intra*- or *inter*-molecular, a plot of the amide proton chemical shifts of **2** over a concentration range of 1.5×10^{-1} to 10^{-3} mol dm^{-3} in $CDCl_3$ at 293 K (Fig. 5) was obtained. From these data the concentration dependence of $\delta(H_A)$ can clearly be seen, and it was found to be significantly larger than the variation of $\delta(H_B)$, $\delta(H_C)$ of the non- or only very weakly hydrogen-bonded protons H_B and H_C. The combined temperature and concentration dependence of $\delta(H_A)$ in $CDCl_2CDCl_2$ (Fig. 6) clearly delineates limiting values for the amide chemical shifts, these being ~8.5 ppm for a hydrogen-bonded species and ~5.5 ppm for a non-hydrogen-bonded species. Since one would expect an *intra*-molecular hydrogen bond to be concentration independent, H_A of lactam **2** most probably experiences *inter*-molecular

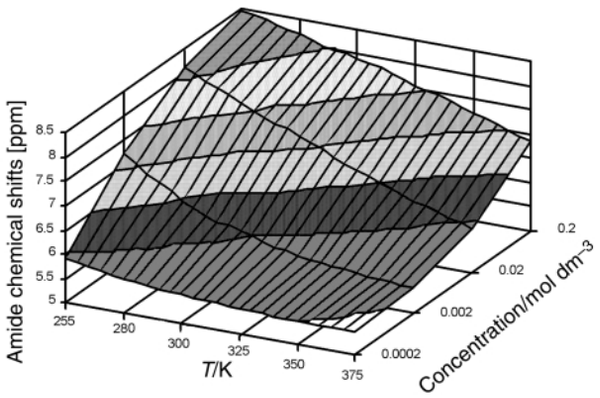


Fig. 6 Amide proton chemical shifts of NH_A of **2** in $CDCl_2CDCl_2$ as a function of temperature and concentration.

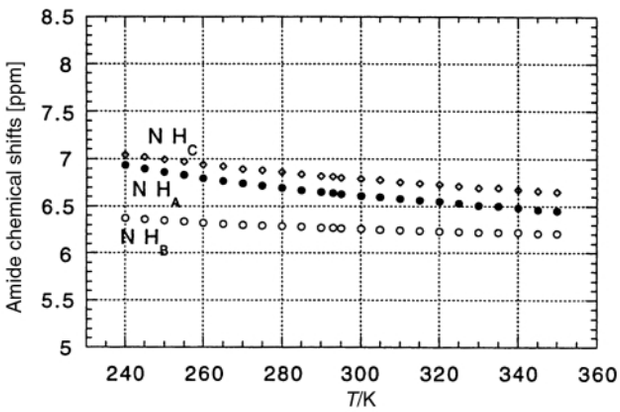


Fig. 7 Amide proton chemical shifts of **2** (*c* 0.01 mol dm^{-3} in CD_3CN) as a function of temperature.

interactions. Further evidence for this assumption came *via* a range of ^{1}H NMR spectra of **2**, recorded at different temperatures in a polar solvent (deuterated acetonitrile, Fig. 7). Under these conditions, the chemical shifts of all three amide protons showed a small, similar temperature dependence, which can be attributed to a uniform interaction between the solvent and all three amide protons.

Vapour pressure osmometry. Reinforcing evidence for the dimerisation of **2** was provided in the first instance by vapour pressure osmometry (VPO) experiments. The principles and practice of VPO have been extensively reviewed[32] and self-association studies using this technique have been described relatively frequently in recent years.[33–35]

The osmotic behaviour of the lactam dipeptide **2** was measured relative to that of a series of standard solutions of benzil in $CDCl_3$.†† The data from the VPO plot (Fig. 8) show that lactam dipeptide **2** associates to form a dimer at low concentrations in the range of 0.001–0.1 mol dm^{-3}. Further aggregation is observed if the concentration is increased above 0.1 mol dm^{-3}. The dimerisation of other dipeptides in dilute $CHCl_3$ has also been studied by VPO techniques.[35]

Physicochemical interpretation of the VT and VC ^{1}H NMR data. With firm evidence of the existence of dimerisation of dipeptide **2** in hand, an estimate of the relevant thermodynamic

†† It is important to realise that these standard solutions are not perfect and other factors may contribute to deviations from ideality. Therefore, not all of the observed differences in osmotic behaviour between the compound **2** and the standard can be attributed solely to associations, but it is not unreasonable to attribute large differences to associations, especially when there is independent evidence, for example from NMR measurements, that such associations do exist.

Table 1 Results of the curve fitting analysis of VT and VC NMR data for the dimerisation of *cis*-**2**

T/K	K_{dim}/dm^3 mol^{-1}	δ_{mon} (ppm)[a]	δ_{dim} (ppm)[b]
275	~236	~5.49	~8.55
293	~100	~5.51	~8.44
295	~90	~5.52	~8.43
315	~45	~5.51	~8.30
335	~23	~5.50	~8.19
355	~13	~5.48	~8.07
365	~9	~5.47	~8.04
375	~7	~5.46	~8.00

[a] δ_{mon} is the calculated limiting amide chemical shift for *cis*-**2** in monomeric state. [b] δ_{dim} is the calculated limiting amide chemical shift for *cis*-**2** in dimeric state.

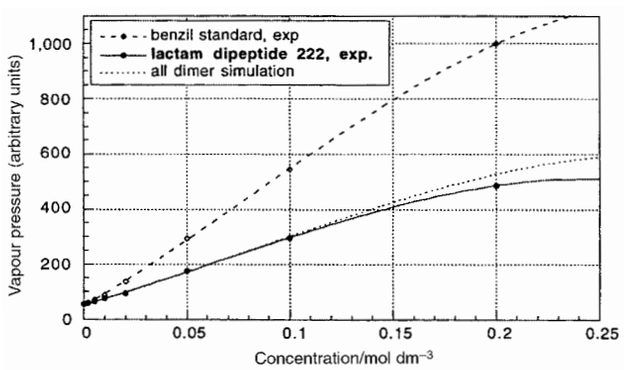


Fig. 8 Plots of vapour pressure *versus* concentrations of solutions of benzil (non-associating VPO reference standard) and the dimerising eight-membered *cis*-lactam **2** in $CDCl_3$. Cubic curves were fitted to all data points. The dashed curve represents the benzil standard, and the dotted curve simulates dimerisation (gradient 0.5, K_{dim} infinite). The solid curve for the behaviour of **2** is almost superimposable on the latter curve.

constants was obtained from an analysis of the VT and VC ^{1}H NMR data. The curve fitting analysis was performed using a Pascal fit routine implemented in KaleidaGraph™ (Version 3.0.4, by Abelbeck Software) and furnished the two limiting values for the amide chemical shifts (δ_{mon} and δ_{dim}, ppm) as well as the dimerisation constant (K_{dim}, dm^3 mol^{-1}, Table 1).

At 298 K, the estimated dimerisation constant for **2** is 84 ± 20 dm^3 mol^{-1}. This is in relatively good agreement with the estimated dimerisation constant for the seven-membered analogue **1** (150 ± 50 dm^3 mol^{-1}) in CD_2Cl_2,[6,7] despite the reservation that the solvent in this case was $CDCl_2CDCl_2$.

Values for ΔH_{dim} (−29.7 kJ mol^{-1}) and ΔS_{dim} (−136 J mol^{-1} K^{-1}) at 298 K can be estimated from a van't Hoff analysis (Fig. 9) and application of the Gibbs–Helmholtz equation.

Variable concentration FTIR spectroscopy. FTIR was employed to examine the properties of the amide protons involved in the hydrogen-bonding event. The four different signals occurring between 3500 and 3250 cm^{-1} (Fig. 10) in the spectrum of *cis*-lactam **2**, were assigned on the basis of a comparative examination of the IR spectra of intermediates in the synthesis of **2**. Table 2 summarises the various amide NH stretches, using the designation NH_A for the methyl amide, NH_B for the lactam and NH_C for the acetamide stretching frequencies.

Variable concentration IR measurements were performed and Fig. 10 shows the variation in the NH stretch region over a range of concentrations in $CDCl_3$. The findings are in remarkable agreement with the interpretation of the vapour pressure osmometry data. Indeed, all dilute samples (<10^{-1} mol dm^{-3}) show four distinct stretch bands that can be attributed to two species (monomer and dimer) of **2** as in Table 2.

Table 2 Wavenumber (cm^{-1}) of the different amide stretch bands in the IR spectra of derivatives of lactam **2**

	ν/cm^{-1}		
Compound	NH_A	NH_B	NH_C
2[a]	3452/3292	3368	3408
7	—	3376	—
26	—	3378	—
27	—	3383	3416
28	—	3380	3416
30	—	3379	3416

[a] The comparison of the above data strongly suggests the assignment of the amide stretch bands in *cis*-lactam **2** as following: 3452 ($NH_{A,\ monomer}$), 3408 (NH_C), 3368 (NH_B) and 3292 cm^{-1} ($NH_{A,\ dimer}$).

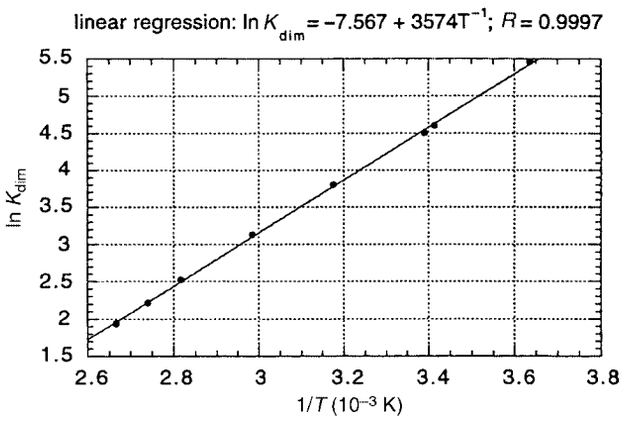


Fig. 9 Van't Hoff plot (ln K_{dim} *vs.* 1/*T*) of a set of K_{dim} values at a temperature range from 275 to 375 K.

Increasing the concentration drives the dynamic equilibrium to favour the dimer. This behaviour can be seen by the almost complete disappearance of the sharp NH_A-stretch band at ~3450 cm^{-1} (representing the monomeric, or the non-hydrogen-bonded form of **2**) at a level of 10^{-1} mol dm^{-3} (Fig. 10d) by which stage the broad NH_A-stretch band at ~3290 cm^{-1} (representing the dimeric or hydrogen bonded species of **2**) becomes predominant. The spectra of samples at higher concentration (>10^{-1} mol dm^{-3}), however, become highly complex and it is largely impossible to assign the bands, which supports the hypothesis that further aggregation above a concentration of 10^{-1} mol dm^{-3} occurs. Closer inspection of the spectrum of **2** in the solid state (KBr disc), implies that most amide hydrogens are involved in hydrogen bonding, which was clearly seen in the crystal structure of **2** (Fig. 3).

Variable concentration NOE.‡‡ Intermolecular NOEs have been used in the determination of the geometry of host–guest complexes of β-cyclodextrin and 1-bromoadamantane[37] and for chiral recognition models.[38] In the present case, NOE experiments also provide further evidence for the existence of dimerisation of the lactam dipeptide **2**. In order to obtain an idea of the possible conformation the postulated head-to-tail dimer might adopt, molecular mechanics calculations§§ were performed using MacroModel v5.5.[39–41] Within an energy range of 1 kcal eleven conformers were found; the presumed global minimum structure is shown in Fig. 11.

‡‡ The NMR results described in this section were obtained using a gradient NOE experiment[22,36] in order to circumvent subtraction errors that occur in NOE difference spectroscopy.

§§ Monte Carlo conformational searches[39] were performed with the Amber force field[40] and parameters suitable for chloroform solvent effects in a dilute solution.[41] Several starting orientations were selected and the resulting overall lowest energy structures were examined.

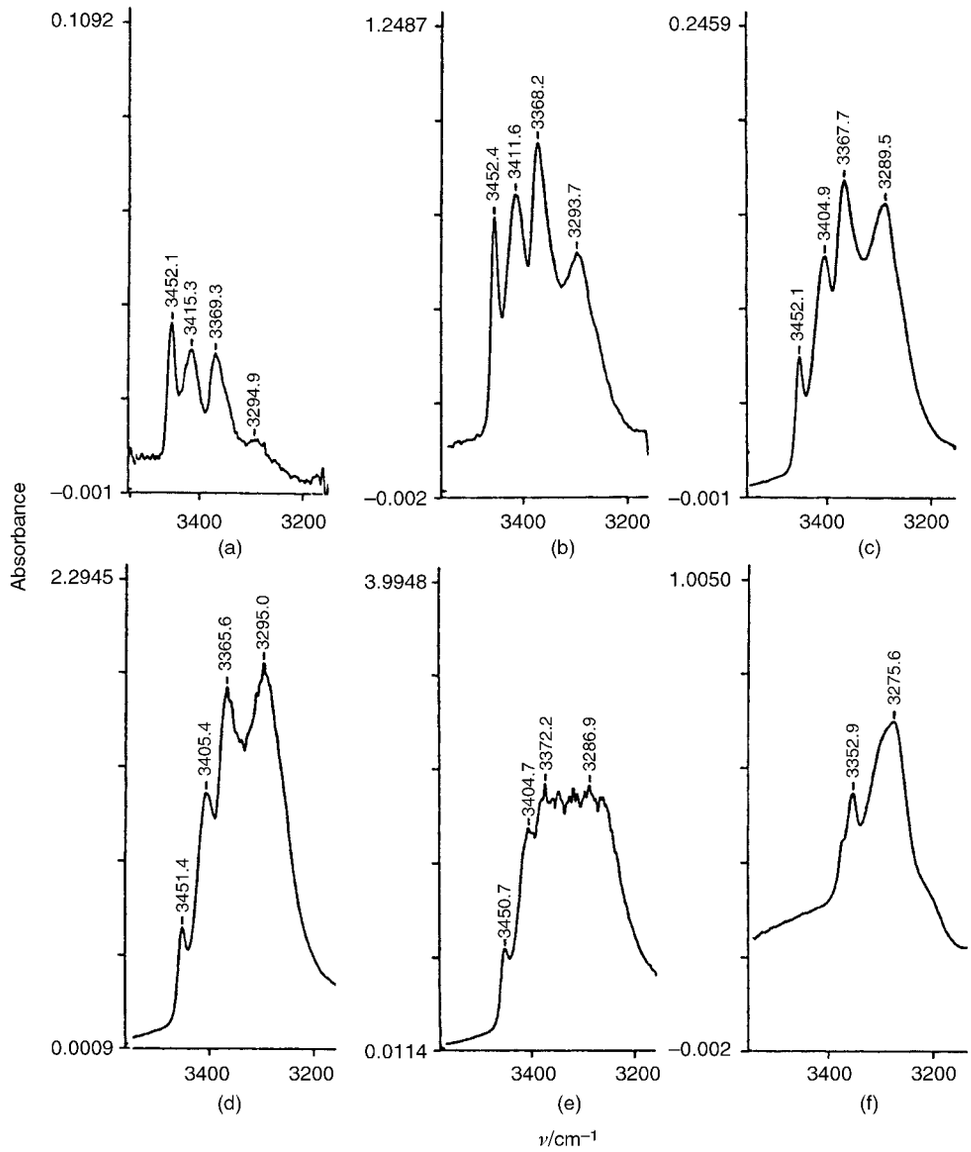


Fig. 10 FTIR spectral data for *cis*-lactam **2** showing the NH stretch regions at a range of concentrations in $CDCl_3$ and in the solid state at ambient temperature: (a) 0.002 mol dm^{-3} [maxima at 3452 ($NH_{A,\ monomer}$), 3415 (NH_C), 3369 (NH_B) and 3294 cm^{-1} ($NH_{A,\ dimer}$)]; (b) 0.01 mol dm^{-3}; (c) 0.05 mol dm^{-3}; (d) 0.1 mol dm^{-3}; (e) 0.2 mol dm^{-3} (the observed bands cannot be assigned) and (f) solid state IR (KBr disc).

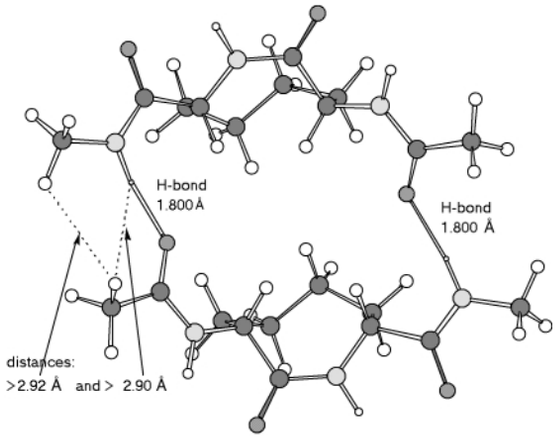


Fig. 11 Lowest energy conformation of dimerising **2** obtained by Monte Carlo conformational searches using MacroModel v5.5 with Amber force field and parameters for the simulation of dilute chloroform solution.

NOE measurements were performed over a series of concentrations in $CDCl_3$. At 0.1 mol dm^{-3} (Fig. 12) intermolecular NOEs between the acetyl COMe of one molecule and both, *Me*NH and NH_A of the second molecule in the dimer were observed. Irradiating the *Me*NH of the second molecule showed a reciprocal NOE to the acetyl COMe of the first molecule. In a control experiment, an intramolecular negative NOE was observed between the COMe and the adjacent NH_C of the first molecule as well as between the *Me*NH and the adjacent NH_A of the methyl amide in the second molecule.

Although the intermolecular enhancements are modest (up to 1.3%) in comparison with intramolecular enhancements (up to 6.6%), they are reproducible and specific.

Owing to significant changes in solution viscosity at higher concentrations, the comparison of absolute enhancement values between experiments is probably not valid. At relatively high dilutions (<0.05 mol dm^{-3}, Fig. 13), however, viscosity changes should be minimal, and the NOE values from different experiments may be directly compared. Given this restriction, a concentration dependence was indeed observed with intermolecular NOEs, providing support for the molecular self-recognition phenomenon initially proposed.

Conclusions from the conformational analysis. The conformational analysis of **2** in apolar solvents revealed that in dilute solution only one amide group is significantly involved in hydrogen bonding as a dimer, and that the data are best

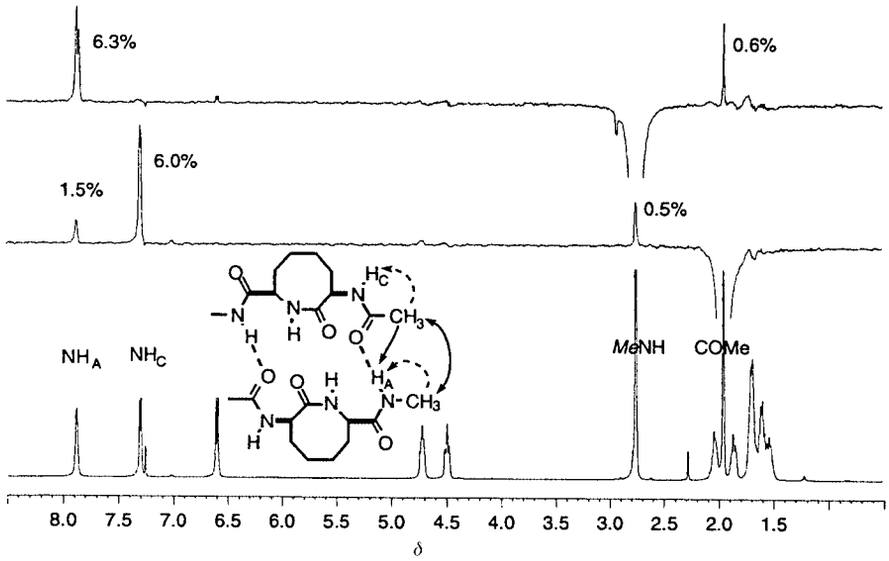


Fig. 12 1D-Selective NOE measurements of lactam **2** in $CDCl_3$ (0.1 mol dm^{-3}).

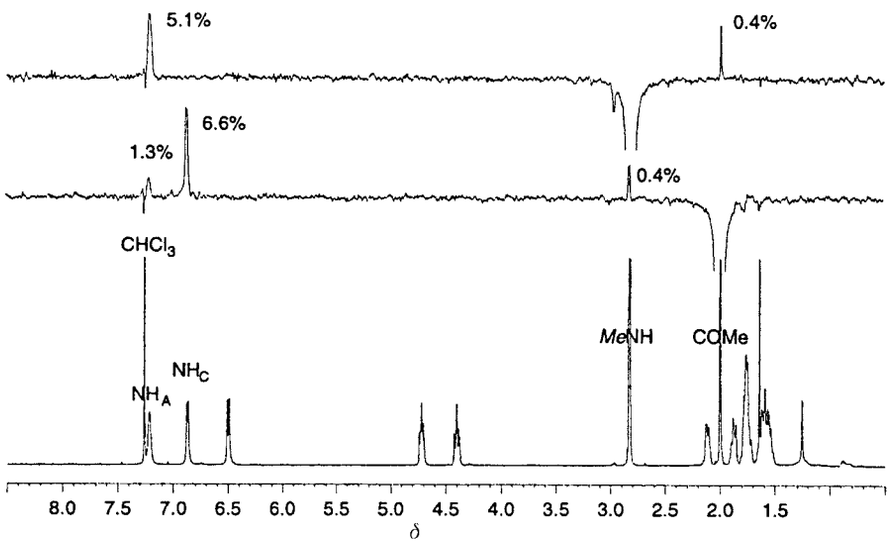


Fig. 13 1D-Selective NOE measurements of lactam **2** in $CDCl_3$ (0.02 mol dm^{-3}).

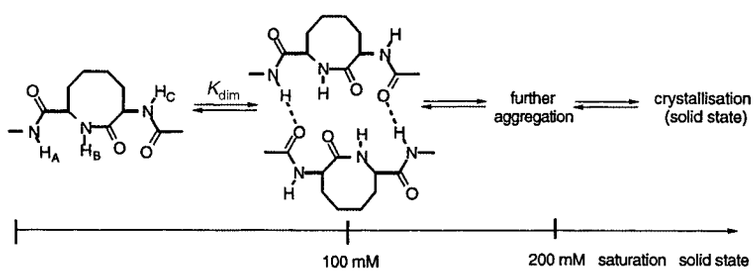


Fig. 14 Concentration-dependent behaviour of lactam dipeptide **2** in $CDCl_3$.

accommodated by assuming that **2** exists in a dynamic equilibrium between a monomer and a head-to-tail dimer (Fig. 14). In concentrated solutions the lactam **2** further aggregates in a less well defined manner.

The strength of this dimerisation ($K_{dim} = 84 \pm 20$ mol dm^{-3} at 298 K in $CDCl_2CDCl_2$) is not only comparable to that of the seven-membered homologue **1**,[6,7] but also to that of the rather important base pairing of 9-ethyladenine and 1-cyclohexyluracil ($K_{AU} = 100 \pm 20$ mol dm^{-3} at 298 K in $CDCl_3$) as determined by IR spectroscopy.[42] The present study also provides evidence that the described example of self-recognition between relatively unconstrained amides in preference to a pre-oriented lactam appears to be a relatively general phenomenon for *cis*-disubstituted medium-ring lactam dipeptides. The association offers the opportunity for the synthesis of novel molecules involving self-assembly based on this recognition phenomenon.

Experimental

General

^{1}H NMR spectra were recorded on Bruker WM-400 (400 MHz), AM-400 (400 MHz) and DRX-500 (500 MHz) instruments, using deuterochloroform (or other indicated solvent) as reference or internal deuterium lock. The chemical shift data for each signal are given in units of δ relative to tetramethyl-

silane (TMS) where δ(TMS) = 0 ppm. The multiplicity of the signal is indicated as: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad, dd = doublet of doublets, dt = doublet of triplets, *etc*. and coupling constants *J* are quoted in Hz. All two dimensional (2-D) spectra were recorded on a Bruker DRX-500 spectrometer, fitted with gradient coils. Double Quantum Filtered (DQF), magnitude COSY and HMQC heteronuclear correlation spectra were typically acquired with 256 slices in F_1 and 2048 points in F_2. 1-D Gradient NOE spectra[22,36] were acquired using standard Gauss selective pulses and mixing times (τ_m) of the order of 1.2 s. For the acquisition of ^{1}H spectra of samples in H_2O, CD_3OH or CF_3CD_2OH the OH peak was suppressed using either the 'Watergate' or the 'Pre-saturation' pulse programme.[43] ^{13}C NMR spectra were recorded on a Bruker AM-400 (100 MHz) instrument, using internal deuterium lock and proton decoupling. The chemical shift data for each signal are given in units of δ relative to TMS where δ(TMS) = 0 ppm. In most cases an attached proton test (APT) was run to aid assignment of signals. IR spectra were recorded on a Perkin-Elmer 1600 FTIR series spectrophotometer. The sample was prepared as a thin liquid film, a KBr disc or as a solution in the solvent indicated. Calibration in each case was made relative to polystyrene at 1603 cm^{-1}. The relative intensities are indicated as: s = strong, m = medium, w = weak and sh = shoulder. Mass spectra were recorded at the EPSRC Mass Spectrometry Service Centre, University of Swansea (Dr J. C. Ballantine) or at the Cambridge University Chemical Laboratory. In Swansea, Electron Impact (EI) and Chemical Ionisation (CI) low resolution spectra were recorded on a VG model 12-253 under ACE conditions. Accurate mass measurements for EI and CI were made on a +VG ZAB-E instrument. In Cambridge, EI and CI low resolution and accurate mass spectra were obtained on a KRATOS MS-890. Electrospray spectra were determined with either an ES Bruker FTICR or a VG-BioQ instrument. All CI measurements were obtained with NH_3 as the carrier gas. Microanalyses were carried out by the staff of the University Chemical Laboratory Microanalytical Department, Cambridge. Melting points were determined using a Büchi 510 melting point apparatus (open capillaries used) and are uncorrected. Optical rotations were measured using a Perkin-Elmer 241 polarimeter, in a cell of 1 dm path length. The concentration (*c*) is expressed in g/100 cm^3 (equivalent to g/0.1 dm^3). Specific rotations, denoted as $[\alpha]_D^T$, imply units of deg cm^2 g^{-1} (T = temp./°C). Analytical TLC was carried out on pre-coated 0.25 mm thick Merck 60 F_{254} silica plates. Visualisation was achieved by absorption of UV light, and either spraying with ninhydrin solution or dipping with basic potassium permanganate followed by thermal development. Flash chromatography[44] was carried out using Merck Kieselgel 60 (230–400 mesh). Non-aqueous reactions were carried out under an atmosphere of dry nitrogen or argon where appropriate. THF was distilled from potassium in a recycling still, using benzophenone ketyl as indicator. Other dry solvents and reagents were purified and dried where necessary by standard techniques.[45] Ether refers to diethyl ether. Brine refers to a saturated solution of sodium chloride in water.

Variable temperature and concentration NMR experiments

All VT NMR spectra were recorded on either a Bruker AM-400 or a DRX-500 spectrometer. The deuterated solvents were dried by passage through a short column of alumina before use and stored over activated powdered (0.5–5.0 μm) Linde type 4 Å molecular sieve. The NMR tubes were flushed with nitrogen and sealed with a cap or a rubber septum. The data acquisition was performed in the presence of activated powdered molecular sieve. The low temperature experiments were performed by cooling the probe and the sample to the minimum temperature before raising the temperature of the probe by the desired amount, waiting for ten minutes and refining *Z* and *Z*^{2} shims before acquisition. The high temperature experiments were performed by increasing the temperature of the probe by the desired amount, and waiting ten minutes before acquisition. Spectra are referenced to the residual solvent peak at 295 K. Variable concentration experiments were performed on a Bruker AM-400 spectrometer. Sample preparation was as normal, followed by sequential serial dilution, using micro pipettes.

Variable concentration IR experiments and vapour pressure osmometry

Substrates used for preparation of solutions for variable concentration IR experiments were vacuum desiccated at rt in the presence of P_4O_{10} for several days. The solvent was distilled off P_4O_{10} and stored over activated 4 Å Linde type molecular sieve. A standard sample was prepared and lower concentration samples were obtained by serial dilution of the standard sample. IR measurements were performed on a Perkin-Elmer 1600 series FTIR spectrometer, using a 1 mm cell with sodium chloride windows. Spectra of 16 scans with 4 cm^{-1} resolution were obtained. Solvent subtraction was carried out by using the spectra of the neat solvent. A smooth function over a number of data points was applied in order to enhance the appearance of the spectra.

For the vapour pressure osmometry experiments, a WESCOR 5500 Vapor Pressure Osmometer, which operates at a fixed temperature of 37 °C, was employed and the same sample solutions as prepared for IR spectroscopy were used for these measurements.

Experimental procedures

(*Z*,4*R*)-3-*tert*-Butyloxycarbonyl-4-(5-carboxypent-1-enyl)-2,2-dimethyl-1,3-oxazolidine 5. Commercially available (4-carboxybutyl)triphenylphosphonium bromide **4** (5.42 g, 12.24 mmol), dried at rt *in vacuo* for 20 h, was suspended in THF (100 cm^3) and cooled to −70 °C. The stirred solution was treated slowly with NaHMDS (1.0 mol dm^{-3} in THF, 24.5 cm^3, 24.5 mmol) and the mixture warmed to rt for 1 h, causing a deep orange colour. This was then re-cooled to −70 °C and a solution of the aldehyde **3** (2.34 g, 10.2 mmol) in THF (22 cm^3 + 2 × 2 cm^3 rinse) was added *via* cannula. The orange colour was substantially discharged upon warming to rt. The mixture was stirred for further 1 h, before it was poured into aqueous ammonium chloride (sat., 120 cm^3) and THF removed *in vacuo*. The aqueous solution was extracted with ethyl acetate (200 and 2 × 100 cm^3), the combined organic layers dried ($MgSO_4$) and the solvent removed *in vacuo* to afford a pale yellow oil. Purification by flash chromatography on silica gel, eluting with hexane–ethyl acetate–acetic acid (50 : 50 : 1) gave the *acid* **5** (3.09 g, 96%, *E* : *Z* ratio 1 : 7, as determined by ^{1}H NMR) as pale yellow crystals; mp 115–118 °C; R_F 0.28 (hexane–ethyl acetate–acetic acid 50 : 50 : 1); five-fold recrystallisation of a sample from carbon tetrachloride afforded the desired *Z*-isomer in a 97% purity; mp 124–124.5 °C; $[\alpha]_D^{18}$ +66.4 (*c* 0.67, $CHCl_3$) (Found: C, 61.3; H, 8.9; N, 4.4. $C_{16}H_{27}NO_5$ requires C, 61.32; H, 8.68; N, 4.47%); ν_{max}($CHCl_3$)/cm^{-1} 3100–2500br m (COOH), 1707s (acid CO), 1698s (carbamate CO); δ_H(400 MHz; C_6H_6; 343 K) 1.41 (9H, s, CMe_3), 1.50–1.64 (2H, m, CH_2), 1.57 [3H, s, C(Me)*Me*], 1.66 [3H, s, C(*Me*)Me], 1.95–2.09 (2H, m, CH_2COO), 2.14 (2H, br t, *J* 7.2, =CHC*H*$_2$), 3.48 (1H, dd, *J* 8.6, 3.3, OC*H*H), 3.80 (1H, dd, *J* 8.6, 6.4, OCH*H*), 4.52 (1H, br m, CHN), 5.22 (1H, dt, *J* 10.0, 7.2, =C*H*CH$_2$) and 5.44 (1H, dd, *J* 10.0, 9.4, =C*H*CHN); δ_C(100 MHz; C_6H_6; 343 K) 25.0 (CH_2), 26.9 (=CH*C*H$_2$), 28.6 (C*Me*$_2$ and C*Me*$_3$), 33.2 (*C*H$_2$COO), 54.9 (CHN), 69.1 (OCH$_2$), 79.5 (O*C*Me$_3$), 94.1 (O*C*Me$_2$), 152.1 (NCOO) and 177.6 (COO); *m*/*z* (CI) 314 [(M + H)$^+$, 64%], 275 (42), 258 (68), 214 [(M − Boc + H)$^+$, 63] and 58 (100) [Found: (M + H)$^+$ 314.1967. $C_{16}H_{28}NO_5$ requires *M*, 314.1967].

(*Z*,7*R*)-7-Amino-8-hydroxyoct-5-enoic acid 6. A solution of oxazolidine **5** (*E*:*Z* ratio 1:7, 1.855 g, 5.92 mmol) in DCM (30 cm^3) was cooled to 0–3 °C, treated with TFA (15 cm^3) and stirred for 20 minutes. The reaction mixture was concentrated *in vacuo*, taken up in water (20 cm^3) and subjected to ion exchange chromatography according to the following procedure: A slurry of Dowex 50X8-400 (30 g) in aqueous sodium hydroxide (2 mol dm^{-3}) was packed into a column and eluted with aqueous sodium hydroxide (2 mol dm^{-3}, 100 cm^3) under gentle pressure. The resin was then washed with water (100 cm^3) and aqueous hydrochloric acid (2 mol dm^{-3}, 100 cm^3). The column was eluted with water until the effluent reached pH 6–7. The solution containing the crude amino acid TFA salt was applied to the column and eluted with water. When the effluent returned to pH 6–7, elution was continued with aqueous ammonia (1 mol dm^{-3}). The ninhydrin positive fractions were collected, evaporated and azeotropically dried with toluene to give free *amino acid* **6** (1.03 g, 100%, *E*:*Z* ratio 1:7) as an off-white solid; mp 163–164 °C; R_F 0.58 (*n*-butanol–acetic acid–water 8:3:3); $[\alpha]_D^{21}$ −16.3 (*c* 0.36, MeOH); ν_{max}(KBr)/cm^{-1} 3420br m (OH), 3075m (NH_3^+), 3300–2300br m (COO), 2171s (amino acid), 1639m (amino acid I), 1543s (amino acid II, COO^- ionised), 1399s (COO^- symmetric valence); δ_H(400 MHz; D_2O) 1.57–1.67 (2H, m, CH_2), 2.07–2.18 (4H, m, CH_2COO and =CHCH_2), 3.58 (1H, dd, *J* 12.1, 7.5, OC*H*H), 3.70 (1H, dd, *J* 12.1, 4.6, OCH*H*), 4.16 (1H, m, CHN), 5.45 (1H, dd, *J* 10.3, 10.0, =C*H*CHN) and 5.84 (1H, dd, *J* 10.3, 8.3, =C*H*CH_2); δ_C(100 MHz; D_2O) 27.8 (CH_2), 29.5 (=CHCH_2), 39.4 (CH_2COO), 52.6 (CHN), 64.4 (CH_2OH), 123.9 and 140.9 (CH=CH) and 185.9 (COO); *m*/*z* (CI) 174 [$(M + H)^+$, 100%], 158 (7) and 142 (6) [Found: $(M + H)^+$ 174.1130. $C_8H_{16}NO_3$ requires *M*, 174.1130].

(8*R*)-8-Hydroxymethyl-1,2,3,4,5,8-hexahydroazocin-2-one 7. A stirred volume of xylene (230 cm^3) was refluxed for 1 h under Dean–Stark conditions, cooled to approximately 100 °C and amino acid **6** (*E*:*Z* ratio 1:7, 980 mg, 5.66 mmol) and di-*n*-butyltin oxide (698 mg, 2.80 mmol) were added. The mixture was refluxed under Dean–Stark conditions for 15 h, cooled to rt and concentrated *in vacuo* to give a dark brown gum. Purification by flash chromatography on silica gel, eluting with ethyl acetate–methanol (10:1) gave *lactam* **7** (406 mg, 46%) as a pale yellow solid; mp 55–58 °C (ethyl acetate–hexane); R_F 0.24 (ethyl acetate–methanol 10:1), $[\alpha]_D^{18}$ +42.7 (*c* 1.48, $CHCl_3$) (Found: C, 61.8; H, 8.4; N, 8.6. $C_8H_{13}NO_2$ requires C, 61.91; H, 8.44; N, 9.03%); ν_{max}($CHCl_3$)/cm^{-1} 3448br m sh (OH), 3382m (NH), 3073w (HPh), 1657s (amide CO); δ_H(400 MHz; $CDCl_3$) 1.64–1.83 (2H, m, CH_2), 2.06–2.15 (1H, m, =CHC*H*H), 2.31 (1H, ddt, *J* 11.8, 4.4, 1.1, NCOC*H*H), 2.37–2.50 (1H, m, =CHC*H*H), 2.61 (1H, dt, *J* 11.8, 5.6, NCOCH*H*), 3.60 (1H, dd, *J* 11.7, 7.4, OC*H*H), 3.73 (1H, dd, *J* 11.7, 3.6, OCH*H*), 4.18 (1H, m, CHN), 5.46 (1H, dd, *J* 11.7, 4.5, =C*H*CHN), 5.75 (1H, ddt, *J* 11.7, 8.1, 1.9, =C*H*CH_2) and 7.45 (1H, br d, *J* 3, NH); δ_C(100 MHz; $CDCl_3$) 23.9 (CH_2), 24.5 (=CHCH_2), 33.0 (CH_2CON), 56.2 (CHN), 64.1 (CH_2OH), 129.7 and 130.1 (CH=CH) and 177.9 (CON); *m*/*z* (CI) 173 [$(M + NH_4)^+$, 13%], 156 [$(M + H)^+$, 100], 140 (5), 138 (5) and 124 (5) [Found: $(M + H)^+$ 156.1025. $C_8H_{14}NO_2$ requires *M*, 156.1024].

(8*R*)-1-*tert*-Butyloxycarbonyl-8-(*tert*-butyloxycarbonyloxymethyl)-1,2,3,4,5,8-hexahydroazocin-2-one 8. A solution of lactam **7** (157 mg, 1.01 mmol), di-*tert*-butyl dicarbonate (932 mg, 4.14 mmol), DMAP (251 mg, 2.03 mmol) and triethylamine (0.3 cm^3, 0.138 mmol) in DCM (50 cm^3) was stirred at rt for 7 d. The reaction mixture was evaporated *in vacuo* and purified by flash chromatography on silica gel, eluting with hexane–ethyl acetate (10:1 to 0:1, eluant gradient) to give the *bis(Boc) lactam* **8** (255 mg, 71%) as colourless crystals; mp 108–110 °C (ethyl acetate–hexane); R_F 0.68 (ethyl acetate–methanol 10:1), $[\alpha]_D^{18}$ −66 (*c* 0.44, $CHCl_3$) (Found: C, 60.7; H, 8.3; N, 3.8. $C_{18}H_{29}NO_6$ requires C, 60.83; H, 8.22; N, 3.94%); ν_{max}($CHCl_3$)/cm^{-1} 1740s (carbonate CO), 1698s (carbamate CO); δ_H(400 MHz; $CDCl_3$) 1.46 (9H, s, CMe_3), 1.51 (9H, s, CMe_3), 1.80–1.90 (2H, m, CH_2), 2.20–2.32 (2H, m, CH_2), 2.49 (1H, ddd, *J* 11.6, 4.9, <1, NCOC*H*H), 2.85 (1H, ddd, *J* 11.6, 11.5, 6.2, NCOCH*H*), 4.30 (2H, d, *J* 7.3, OCH_2), 5.03 (1H, m, CHN), 5.48 (1H, dd, *J* 11.3, 3.0, =C*H*CHN) and 5.64 (1H, ddt, *J* 11.3, 8.6, 2.5, =C*H*CH_2); δ_C(100 MHz; $CDCl_3$) 21.7 (CH_2), 25.6 (=CHCH_2), 27.7 and 28.1 (2 × CMe_3), 37.2 (CH_2CON), 53.7 (CHN), 68.1 (CH_2O), 82.2 and 83.0 (2 × OCMe_3), 126.9 and 130.7 (CH=CH), 153.2 and 153.3 (OCOO and NCOO) and 178.6 (CON); *m*/*z* (CI) 356 [$(M + H)^+$, 17%], 256 [$(M - Boc + H)^+$, 90], 217 (27), 200 (100), 199 (41), 182 (11), 156 [$(M - 2Boc + H)^+$, 40], 138 (12) and 124 (17) [Found: $(M + H)^+$ 356.2073. $C_{18}H_{30}NO_6$ requires *M*, 356.2073].

(8*R*)-8-(*tert*-Butyloxycarbonyloxymethyl)-1,2,3,4,5,8-hexahydroazocin-2-one 9. The *mono(Boc) lactam* **9** was also isolated in the aforementioned experiment (33 mg, 13%) as a pale yellow gum; R_F 0.47 (ethyl acetate–methanol 10:1), $[\alpha]_D^{18}$ +38.1 (*c* 1.67, $CHCl_3$); ν_{max}($CHCl_3$)/cm^{-1} 3387m (NH), 1742s (carbonate CO), 1660s (lactam CO); δ_H(400 MHz; $CDCl_3$) 1.45 (9H, s, CMe_3), 1.56–1.84 (2H, m, CH_2), 2.17 (1H, ddd, *J* 14.3, 7.5, 3.1, =CHC*H*H), 2.35–2.46 (2H, m, =CHCH*H* and NCOC*H*H), 2.55 (1H, ddd, *J* 12.3, 11.4, 4.5, NCOCH*H*), 4.05 (1H, dd, *J* 11.3, 7.5, OC*H*H), 4.14 (1H, dd, *J* 11.3, 4.2, OCH*H*), 4.36 (1H, m, CHN), 5.49 (1H, dd, *J* 11.4, 5.5, =C*H*CHN), 5.80 (1H, ddt, *J* 11.4, 8.1, 1.8, =C*H*CH_2) and 6.09 (1H, br d, *J* 5.5, NH); δ_C(100 MHz; $CDCl_3$) 23.3 (CH_2), 25.2 (=CHCH_2), 27.6 (CMe_3), 33.6 (CH_2CON), 52.2 (CHN), 67.3 (CH_2O), 82.1 (OCMe_3), 128.8 and 131.4 (CH=CH), 153.2 (OCOO) and 176.5 (CON); *m*/*z* (CI) 256 [$(M + H)^+$, 100%], 200 (25), 199 (32), 182 (11), 156 [$(M - Boc + H)^+$, 21] and 138 (14) [Found: $(M + H)^+$ 256.1549. $C_{13}H_{22}NO_4$ requires *M*, 256.1549].

(4*R*)-3-{6-[(*Z*,4*R*)-3-*tert*-Butyloxycarbonyl-2,2-dimethyl-1,3-oxazolidin-4-yl]-1-oxohex-5-enyl}-4-phenylmethyl-1,3-oxazolidin-2-one 11. To a stirred solution of carboxylic acid **5** (*E*:*Z* ratio 1:13, 2.426 g, 9.74 mmol) and triethylamine (1.30 cm^3, 9.36 mmol) in THF (50 cm^3), cooled to −78 °C, was added dropwise, within 10 minutes, pivaloyl chloride (1.0 cm^3, 8.09 mmol). The resulting white suspension was warmed to 0 °C, stirred for 1.5 h to allow the mixed anhydride to form and then re-cooled to −78 °C. A solution of (4*R*)-phenylmethyloxazolidin-2-one (**12**) (1.372 g, 7.74 mmol) and diphenyl acetic acid (1 mg, indicator) in THF (15 cm^3), stirred at −40 °C, was treated dropwise with *n*-butyllithium (*ca*. 1.6 mol dm^{-3} in hexane) until the reaction mixture turned yellow (4.85 cm^3 required, 1 equiv.). The resulting solution was stirred for 0.5 h, cooled to −78 °C and then added rapidly by cannula to the above stirred mixture containing the mixed anhydride. The residual lithiated oxazolidinone was rinsed in with THF (2 × 3 cm^3) and the resulting mixture stirred at −78 °C for 45 min. After warming to 0 °C, the mixture was poured into phosphate buffer pH 7 (100 cm^3) and THF evaporated *in vacuo*. The aqueous mixture was extracted with DCM (3 × 100 cm^3), the combined organic layers each washed with aqueous sodium bicarbonate (5%, 100 cm^3) and brine (half-sat., 200 cm^3), dried ($MgSO_4$) and concentrated *in vacuo*. The residual pale yellow oil was purified by flash chromatography on silica gel, eluting with hexane–ethyl acetate (3:1) to give the *carboximide* **11** (3.43 g, 94%) as a colourless oil; R_F 0.52 (hexane–ethyl acetate 1:1); a sample was crystallised from ether–hexane to give the pure *Z*-isomer as colourless needles; mp 76.5–77 °C; $[\alpha]_D^{20}$ +106 (*c* 2.17, $CHCl_3$) (Found: C, 66.2; H, 7.7; N, 5.9. $C_{26}H_{36}N_2O_6$ requires C, 66.08; H, 7.68; N, 5.93%); ν_{max}($CHCl_3$)/cm^{-1} 3067w (HPh), 1781s (carboximide CO), 1689s (carbamate CO); δ_H(400 MHz; C_6H_6; 343 K) 1.43 (9H, s, CMe_3), 1.58 [3H, s, C(Me)*Me*], 1.69 [3H, s, C(*Me*)Me], 1.70–1.88 (2H, m, CH_2), 2.15–2.31 (2H, m, =CHCH_2), 2.38

(1H, dd, *J* 13.4, 9.4, C*H*HPh), 2.89 (1H, m, C*H*HCON), 3.04 (2H, m, CH*H*Ph and CH*H*CON), 3.31 (1H, dd, *J* 9.0, 8.4, OC*H*H auxiliary), 3.53 (1H, dd, *J* 9.0, 3.0, OCH*H* auxiliary), 3.58 (1H, dd, *J* 8.6, 3.3, OC*H*H), 3.90 (1H, dd, *J* 8.6, 6.4, OCH*H*), 4.20 (1H, m, CHN auxiliary), 4.63 (1H, m, CHN), 5.40 (1H, dt, *J* 9.3, 7.5, =C*H*CH_2), 5.52 (1H, br dd, *J* 10.5, 9.3, =C*H*CHN), 6.89 (2H, d, *J* 7.0, Ph) and 7.01–7.07 (3H, m, Ph); δ_C(100 MHz; C_6H_6; 343 K) 24.8 (CH_2), 27.2 (=CH*C*H_2), 28.6 (C*Me*$_2$ and C*Me*$_3$), 35.3 (*C*H_2COO), 36.1 (Ph*C*H_2), 55.0 and 55.1 (CHN and CHN auxiliary), 65.8 (OCH_2 auxiliary), 69.0 (OCH_2), 79.3 (O*C*Me$_3$), 94.6 (O*C*Me$_2$), 127.3, 129.0, 129.5 and 136.1 (Ph), 152.2 and 153.3 (NCOO carbamate and OCON auxiliary) and 172.8 (CON); *m*/*z* (CI) 473 [(M + H)$^+$, 31%], 374 (24), 373 [(M − Boc + H)$^+$, 100] and 196 (19) [Found: (M + H)$^+$ 473.2652. $C_{26}H_{37}N_2O_6$ requires *M*, 473.2651].

(4*R*)-3-{(*Z*,2*R*)-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 13. A flame dried 50 cm^3 2-neck round bottom flask, fitted with a septum and a solid addition tube, containing trisyl azide, was flushed with nitrogen. THF (7.5 cm^3) was added, cooled to −78 °C and KHMDS (0.5 mol dm^{-3} in toluene, 2.85 cm^3, 1.43 mmol) was added *via* syringe. Then, within 10 minutes, a pre-cooled (−78 °C) solution of carboximide **11** (*E*:*Z* ratio 1:13, 601 mg, 1.27 mmol) in THF (7.5 cm^3 + 2 cm^3 rinse) was added *via* an insulated steel cannula and stirring was continued at −78 °C for 80 minutes, in order to allow the enolate to form.

To the above solution of potassium enolate, vigorously stirred at −78 °C, was added in one portion, solid trisyl azide (**14**) (591 mg, 1.91 mmol) causing the mixture to become yellow. After 180 s, the reaction mixture was quenched with glacial acetic acid (50% v/v in THF, 0.7 cm^3, 6.1 mmol), the cooling bath removed and the flask immediately placed in a water bath at 28 °C. Stirring was continued for 0.5 h and a white precipitate of potassium acetate was observed. The mixture was partitioned between aqueous ammonium chloride (half-sat., 50 cm^3) and ethyl acetate (50 cm^3), the aqueous phase extracted with ethyl acetate (2 × 50 cm^3) and the organic layers washed with aqueous sodium bicarbonate (half-sat., 50 cm^3) and brine (50 cm^3). The combined organic phases were dried ($MgSO_4$) and evaporated *in vacuo*. The crude residue was purified by flash chromatography on silica gel, eluting with ethyl acetate–hexane (1:4) to give the *azide* **13** (602 mg, 92%, *E*:*Z* ratio 1:13) as a very pale yellow oil; R_F 0.52 (hexane–ethyl acetate 1:1). A sample could be crystallised from ether–hexane to give the pure *Z*-azido-isomer; mp 87–88 °C; $[a]_D^{22}$ −6.3 (*c* 1.15, $CHCl_3$) (Found: C, 60.8; H, 6.9; N, 13.9. $C_{26}H_{35}N_5O_6$ requires C, 60.80; H, 6.87; N, 13.64%); ν_{max}($CHCl_3$)/cm^{-1} 3052w (HPh), 2108s (N_3), 1783s (carboximide CO), 1690s (carbamate CO); δ_H(400 MHz; C_6H_6; 343 K) 1.43 (9H, s, CMe_3), 1.56 [3H, s, C(Me)*Me*], 1.67 [3H, s, C(*Me*)Me], 1.78–1.92 (1H, m, C*H*HCHN_3), 1.94–2.08 (1H, m, CH*H*CHN_3), 2.28–2.44 (2H, m, =CHCH_2), 2.33 (1H, dd, *J* 13.6, 9.2, C*H*HPh), 2.93 (1H, dd, *J* 13.6, 3.2, CH*H*Ph), 3.35 (1H, t, *J* 9.1, OC*H*H auxiliary), 3.52 (1H, dd, *J* 9.1, 3.2, OCH*H* auxiliary), 3.54 (1H, dd, *J* 8.6, 3.1, OC*H*H), 3.85 (1H, dd, *J* 8.6, 6.3, OCH*H*), 4.15 (1H, ddt, *J* 9.2, 8.2, 3.2, CHN auxiliary), 4.62 (1H, m, CHN), 5.18 (1H, br q, CHN_3), 5.38 (1H, br dt, *J* 10.7, 7.5, =C*H*CH_2), 5.51 (1H, ddt, *J* 10.7, 9.2, 1.4, =C*H*CHN), 6.82–6.88 (2H, m, Ph) and 6.98–7.12 (3H, m, Ph); δ_C(100 MHz; C_6H_6; 343 K) 24.4 (CH_2), 28.6 (C*Me*$_2$ and C*Me*$_3$), 31.6 (=CH*C*H_2), 37.6 (Ph*C*H_2), 54.9 and 55.4 (CHN and CHN auxiliary), 60.7 (CHN_3), 66.4 (OCH_2 auxiliary), 69.0 (OCH_2), 79.5 (O*C*Me$_3$), 94.3 (O*C*Me$_2$), 127.5, 129.1, 129.6 and 134.4 (Ph), 152.0 and 153.0 (NCOO carbamate and OCON auxiliary) and 170.8 (CON); *m*/*z* (CI) 514 [(M + H)$^+$, 2%], 486 [(M − N_2 + H)$^+$, 10%], 195 (100), 178 (28), 100 (15) and 59 (72) [Found: (M + H)$^+$ 514.2670. $C_{26}H_{36}N_5O_6$ requires *M*, 514.2665].

(4*R*)-4-[(1*Z*,5*R*)-5-Azido-5-carboxypent-1-enyl]-3-*tert*-butyloxycarbonyl-2,2-dimethyl-1,3-oxazolidine 15. To a solution of carboximide **13** (150 mg, 0.29 mmol) in THF–water (3:1), stirred at 0 °C, was added solid lithium hydroxide (hydrate, 25 mg, 0.59 mmol). After stirring for 45 minutes at 0–2 °C, excess aqueous sodium bicarbonate (0.5 mol dm^{-3}, 2 cm^3) was added to the cloudy reaction mixture and THF removed under reduced pressure. The residual mixture was extracted with DCM (4 × 30 cm^3), the organic phases each back extracted with water (2 × 10 cm^3), combined, dried ($MgSO_4$) and evaporated *in vacuo* to give the recovered chiral auxiliary **12** (51 mg, 98%) as a white solid; mp 80–83 °C. The combined aqueous phases were acidified to pH *ca.* 1–2 by dropwise addition of aqueous hydrochloric acid (2 mol dm^{-3}, 2 cm^3) and extracted with ethyl acetate (4 × 40 cm^3). The combined organic layers were dried ($MgSO_4$) and evaporated *in vacuo* to give the *acid* **15** (101 mg, 97%) as colourless needles; mp 95.5–96.5 °C (carbon tetrachloride–hexane); R_F 0.31 (hexane–ethyl acetate–acetic acid 25:25:1); $[a]_D^{22}$ +54.5 (*c* 0.53, $CHCl_3$) (Found: C, 54.0; H, 7.3; N, 15.8. $C_{16}H_{26}N_4O_5$ requires C, 54.22; H, 7.39; N, 15.81%); ν_{max}($CHCl_3$)/cm^{-1} 3100–2500br w (COOH), 2109s (N_3), 1719m (acid CO), 1698s (carbamate CO); δ_H(400 MHz; C_6H_6, 343 K) 1.30–1.90 (2H, m, CH_2), 1.41 (9H, s, CMe_3), 1.53 [3H, s, C(Me)*Me*], 1.63 [3H, s, C(*Me*)Me], 2.07–2.33 (2H, m, =CHCH_2), 3.48 (1H, dd, *J* 8.7, 3.3, OC*H*H), 3.80 (1H, br m, CHN_3), 3.82 (1H, dd, *J* 8.7, 6.3, OCH*H*), 4.58 (1H, br m, CHN), 5.22 (1H, dt, *J* 10.2, 7.5, =C*H*CH_2), 5.45 (1H, dd, *J* 10.7, 9.2, =C*H*CHN) and 8.49 (1H, br s, COOH); δ_C(100 MHz; C_6H_6, 343 K) 24.0 (CH_2), 28.5 (C*Me*$_2$ and C*Me*$_3$), 31.6 (=CH*C*H_2), 55.0 (CHN), 61.7 (CHN_3), 66.9 (OCH_2), 80.3 (O*C*Me$_3$), 94.1 (O*C*Me$_2$), 152.3 (NCOO) and 174.3 (COO); *m*/*z* (CI) 355 [(M + H)$^+$, 8%], 299 (47), 283 (68), 255 [(M − Boc + H)$^+$, 17], 239 (100), 225 (29), 167 (28), 152 (38), 139 (60), 108 (46), 82 (77) and 57 (69).

(5*Z*,7*S*,2*R*)-7-Amino-2-azido-8-hydroxyoct-5-enoic acid 16. Oxazolidine **15** (*E*:*Z* ratio 1:7, 75 mg, 0.21 mmol) was used in the procedure described for amino acid **6** (*vide supra*) to give the *amino acid* **16** (42.2 mg, 93%, *E*:*Z* ratio 1:7) as an off-white solid; mp 145–150 °C (decomp.); R_F 0.40 (*n*-butanol–acetic acid–water 8:3:3); $[a]_D^{21}$ −9.7 (*c* 0.53, MeOH); ν_{max}(KBr)/cm^{-1} 3384br m (OH), 3200–2300br m (COO), 3035sh (NH_3^+), 2170sh (amino acid), 2108s (N_3), 1630s (amino acid I), 1564s and 1534sh (amino acid II, COO^- ionised), 1401s (COO^- symmetric valence); δ_H(500 MHz; CD_3OD) 1.85 (2H, dq, *J* 7.5, 1.4, =CHCH_2), 2.11–2.33 (2H, m, CH_2CHN_3), 3.50 (1H, dd, *J* 11.7, 8.0, OC*H*H), 3.68 (1H, dd, *J* 11.7, 4.2, OCH*H*), 3.80 (1H, t, *J* 6.2, CHN_3), 4.09 (1H, dt, *J* 8.7, 4.2, CHN), 5.36 (1H, dd, *J* 10.7, 9.7, =C*H*CHN) and 5.84 (1H, dd, *J* 10.7, 7.5, =C*H*CH_2); δ_C(100 MHz; CD_3OD) 25.5 (CH_2), 32.6 (=CH*C*H_2), 51.6 (CHN), 63.6 (CHN_3), 66.0 (CH_2OH), 124.2 and 137.8 (CH=CH) and 185.0 (COO); *m*/*z* (ESI) 237 [(M + Na)$^+$, 18%], 215 [(M + H)$^+$, 100], 174 (21), 149 (58), 141 (37), 80 (14) and 54 (31).

(3*R*,8*R*)-3-Azido-8-hydroxymethyl-1,2,3,4,5,8-hexahydroazocin-2-one 17. *Method i.* To a solution of amino acid **16** (*E*:*Z* ratio 1:7, 13 mg, 0.06 mmol) in DMF (10 cm^3) was added triethylamine (0.022 cm^3, 0.16 mmol), the mixture was stirred for 10 minutes and diphenoxyphosphoryl azide (0.035 cm^3, 0.16 mmol) was added. The mixture was stirred at rt for 18 h and the pale yellow reaction mixture reduced *in vacuo* to afford a brownish gum. Purification by flash chromatography on silica gel, eluting with ethyl acetate–hexane (1:3 to 3:1, eluant gradient) gave the *lactam* **17** (4.7 mg, 40%) as a colourless oil.

Method ii. A solution of silyl ether **26** (90 mg, 0.207 mmol) in THF (2 cm^3) was treated with TBAF (1 mol dm^{-3} in THF, 0.23 cm^3) and the solution stirred at rt for 0.5 h. TLC showed the reaction to be complete. The reaction mixture was concentrated *in vacuo* and the resulting oil purified by flash chromatography

on silica gel, eluting with chloroform–methanol (1:0 to 10:1, eluant gradient) to give the *alcohol* **17** (34 mg, 84%) as a white solid; mp 89–90 °C (ethyl acetate–toluene–hexane); R_F 0.35 (chloroform–methanol 10:1); $[\alpha]_D^{20}$ −147.6 (*c* 1.31, $CHCl_3$); $\nu_{max}(CHCl_3)/cm^{-1}$ 3490br m (OH), 3376m (NH), 2107s (N_3), 1673s (amide CO); δ_H(400 MHz; $CDCl_3$) 1.88–2.00 (1H, m, C*H*H), 2.03–2.22 (2H, m, CH_2), 2.57–2.71 (1H, m, =CHCH*H*), 3.24 (1H, br s, OH), 3.66 (1H, dd, *J* 11.6, 7.0, C*H*HOH), 3.81 (1H, dd, *J* 11.6, 3.5, CH*H*OH), 4.03 (1H, dd, *J* 11.3, 5.9, CHN_3), 4.21 (1H, m, *J* 1.6, CHN), 5.38 (1H, br dd, *J* 11.9, 1.6, =C*H*CHN), 5.83 (1H, ddt, *J* 11.9, 8.9, 2.1, =C*H*CH_2) and 7.56 (1H, br s, NH); δ_C(100 MHz; $CDCl_3$) 22.2 and 30.5 (2 × CH_2), 56.4 (CHN), 57.7 (CHN_3), 64.2 (CH_2OH), 129.0 and 129.7 (CH=CH) and 174.7 (CON); *m*/*z* (CI) 197 [$(M + H)^+$, 51%], 169 [$(M - N_2 + H)^+$, 100], 152 (8) and 137 (9) [Found: $(M + H)^+$ 197.1039. $C_8H_{13}N_4O_2$ requires *M*, 197.1038).

(4*R*)-3-[(5*Z*,2*R*,7*R*)-2-Azido-7-(*tert*-butyloxycarbonylamino)-8-hydroxy-1-oxooct-5-enyl]-4-phenylmethyl-1,3-oxazolidin-2-one 22. A solution of acetonide (*E*:*Z* ratio 1:13, 1.22 g, 1.9 mmol) in acetic acid–water (4:1, 25 cm^3) was warmed to 40–50 °C and stirred for 4 h. TLC showed the reaction to be complete. The solvents were removed *in vacuo* and residual traces of acetic acid and water removed azeotropically with toluene (2 × 25 cm^3). The crude residue was purified by flash chromatography on silica gel, eluting with hexane–ethyl acetate (2:1 to 1:2, eluant gradient) to give the *alcohol* **22** (807 mg, 90%) as a colourless oil; R_F 0.15 (hexane–ethyl acetate 1:1); $[\alpha]_D^{22}$ −36.6 (*c* 0.94, $CHCl_3$); $\nu_{max}(CHCl_3)/cm^{-1}$ 3442br m (NH, OH), 3052w (HPh), 2109s (N_3), 1782s (carboximide CO), 1705s (carbamate CO); δ_H(400 MHz; $CDCl_3$) 1.43 (9H, s, CMe_3), 1.83–2.02 (2H, m, CH_2CHN_3), 2.28–2.44 (2H, m, =CHCH_2), 2.66 (1H, br s, OH), 2.81 (1H, dd, *J* 13.4, 9.5, C*H*HPh), 3.32 (1H, dd, *J* 13.4, 3.1, CH*H*Ph), 3.52–3.62 (2H, m, OCH_2 auxiliary), 4.22 (1H, dd, *J* 9.1, 2.8, OC*H*H), 4.28 (1H, br t, *J* 9.1, OCH*H*), 4.42 (1H, m, CHN), 4.68 (1H, m, CHN auxiliary), 4.75 (1H, br m, NH), 4.96 (1H, dd, *J* 9.2, 4.2, CHN_3), 5.38 (1H, dd, *J* 10.6, 2, =C*H*CHN), 5.51 (1H, dt, *J* 10.6, 7.6, =C*H*CH_2) and 7.19–7.36 (5H, m, Ph); δ_C(100 MHz; $CDCl_3$) 24.2 (CH_2), 28.3 (CMe_3), 30.8 (CH_2), 37.5 (PhCH_2), 50.5 (CHNH), 55.5 (CHN auxiliary), 59.8 (CHN_3), 66.2 (CH_2OH), 66.7 (OCH_2), 79.9 ($OCMe_3$), 127.5, 129.0, 129.4, 131.5 and 134.7 (CH=CH and Ph), 152.9 and 156.2 (NCOO carbamate and OCON auxiliary) and 170.8 (CON); *m*/*z* (CI) 474 [$(M + H)^+$, 1%], 446 [$(M - N_2 + H)^+$, 3%], 195 (100), 178 (39) and 44 (41) [Found: $(M - N_2 + H)^+$ 446.2291. $C_{23}H_{32}N_3O_6$ requires *M*, 446.2291].

(4*R*)-3-[(5*Z*,2*R*,7*R*)-2-Azido-8-(*tert*-butyldiphenylsilyloxy)-7-(*tert*-butyloxycarbonylamino)-1-oxooct-5-enyl]-4-phenylmethyl-1,3-oxazolidin-2-one 23. A solution of alcohol **22** (*E*:*Z*-ratio 1:13, 780 mg, 1.65 mmol) in DMF (2.5 cm^3) was treated with *tert*-butyldiphenylchlorosilane (0.65 cm^3, 2.52 mmol) and imidazole (290 mg, 4.2 mmol) and the pale yellow mixture stirred at rt for 16 h. TLC showed the reaction to be complete. The mixture was poured into water (50 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 material 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* **23** (1.01 g, 86%, *E*:*Z* ratio 1:13) as a white foam; R_F 0.57 (hexane–ethyl acetate 1:1); $[\alpha]_D^{23}$ −40.2 (*c* 1.41, $CHCl_3$); $\nu_{max}(CHCl_3)/cm^{-1}$ 3445m (NH), 3071w (HPh), 2108m (N_3), 1783s (carboximide CO), 1705s (carbamate CO); δ_H(500 MHz; $CDCl_3$) 1.07 (9H, s, $SiCMe_3$), 1.44 (9H, s, $OCMe_3$), 1.75–1.99 (2H, m, CH_2CHN_3), 2.16–2.37 (2H, m, =CHCH_2), 2.84 (1H, dd, *J* 13.5, 9.7, C*H*HPh), 3.34 (1H, dd, *J* 13.5, 3.0, CH*H*Ph), 3.61 (1H, dd, *J* 10.1, 5, OC*H*H), 3.70 (1H, dd, *J* 10.1, 4.5, OCH*H*), 4.21 (1H, dd, *J* 9.0, 2.8, OC*H*H auxiliary), 4.26 (1H, br t, *J* 9.0, OCH*H* auxiliary), 4.44 (1H, m, CHN), 4.69 (1H, m, CHN auxiliary), 4.80 (1H, br m, NH), 4.98 (1H, dd, *J* 8.7, 4.1, CHN_3), 5.48–5.55 (2H, m, CH=CH), 7.19–7.45 (11H, m, Ph) and 7.64–7.67 (4H, m, Ph); δ_C(100 MHz; $CDCl_3$) 19.3 ($SiCMe_3$), 24.1 (CH_2), 26.8 ($SiCMe_3$), 28.3 ($OCMe_3$), 31.0 (CH_2), 37.5 (PhCH_2), 55.4 (CHN auxiliary), 59.9 (CHNH), 63.4 (CHN_3), 66.2 (CH_2OSi), 66.6 (OCH_2), 79.1 ($OCMe_3$), 127.3, 127.5, 127.7, 129.0, 129.4, 129.7, 130.2, 133.2, 134.8 and 135.6 (CH=CH and Ph), 152.8 and 155.2 (NCOO carbamate and OCON auxiliary) and 170.8 (CON); *m*/*z* (CI) 684 [$(M - N_2 + H)^+$, 10%], 274 (7), 195 (100), 178 (26) and 44 (8) [Found: $(M - N_2 + H)^+$ 684.347. $C_{39}H_{50}N_5O_6Si$ requires *M*, 684.3469].

(5*Z*,2*R*,7*R*)-2-Azido-8-(*tert*-butyldiphenylsilyloxy)-7-(*tert*-butyloxycarbonylamino)oct-5-enoic acid 24. Carboximide **23** (*E*:*Z* ratio 1:13, 975 mg, 1.38 mmol) was applied to the procedure described for acid **15** and the *acid* **24** was isolated (708 mg, 94%, *E*:*Z* ratio 1:13) as a very pale yellow oil; R_F 0.33 (hexane–ethyl acetate–acetic acid 100:100:1); $[\alpha]_D^{23}$ −7.4 (*c* 0.60, $CHCl_3$); $\nu_{max}(CHCl_3)/cm^{-1}$ 3444w (NH), 3100–2500br w (COOH), 3072w (HPh), 2109s (N_3), 1714s (acid CO); δ_H(500 MHz; $CDCl_3$) 1.07 (9H, s, $SiCMe_3$), 1.45 (9H, s, $OCMe_3$), 1.74–1.95 (2H, m, CH_2CHN_3), 2.06–2.29 (2H, m, =CHCH_2), 3.59 (1H, dd, *J* 10, 5.3, OC*H*H), 3.68 (1H, br m, OCH*H*), 3.88 (1H, m, NH), 4.44 (1H, br m, CHN), 4.96 (1H, br m, CHN_3), 5.45 (2H, br m, CH=CH), 7.37–7.46 (6H, m, Ph) and 7.64–7.67 (4H, m, Ph); δ_C(100 MHz; $CDCl_3$) 19.2 ($SiCMe_3$), 23.9 (CH_2), 26.8 ($SiCMe_3$), 28.4 ($OCMe_3$), 31.1 (CH_2), 49.7 (CHNH), 61.3 (CHN_3), 66.2 (CH_2OSi), 79.9 ($OCMe_3$), 127.7, 129.0, 129.8, 130.6, 133.1 and 137.8 (CH=CH and Ph), 155.8 (NCOO carbamate) and 173.9 (COOH); *m*/*z* (CI) 553 [$(M + H)^+$, 2%], 525 [$(M - N_2 + H)^+$, 6], 481 (12), 274 (30), 208 (26), 135 (59) and 79 (100) [Found: $(M + H)^+$ 553.285. $C_{29}H_{41}N_4O_5Si$ requires *M*, 553.2846].

(3*R*,8*R*)-3-Azido-8-(*tert*-butyldiphenylsilyloxymethyl)-1,2,3,4,5,8-hexahydroazocin-2-one 26. A solution of Boc-amino acid **24** (*E*:*Z* ratio 1:13, 426 mg, 0.77 mmol) in DCM (10 cm^3) was cooled to 0 °C, treated with TFA (5 cm^3) and stirred at 0 °C for 35 minutes. The solvents were removed *in vacuo* and the residual pale brown resin was dried at high vacuum (0.05 mmHg) for 1.5 h. The amino acid TFA salt **25** was dissolved in THF (200 cm^3) and cooled to 0 °C. Then triethylamine (0.38 cm^3, 2.70 mmol) was added, the mixture stirred for 15 minutes and diphenoxyphosphoryl azide (0.42 cm^3, 1.93 mmol) added. The stirred mixture was allowed to warm to rt. After 20 h, TLC showed only little product and more triethylamine (0.25 cm^3, 1.93 mmol) and diphenylphosphoryl azide (0.42 cm^3, 1.93 mmol) were added. Stirring was continued at 30–35 °C for a further 24 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–ether (1:0 to 3:1, eluant gradient) to give the *lactam* **26** (245 mg, 73%) as a very pale yellow oil; R_F 0.20 (hexane–ether 2:1); $[\alpha]_D^{23}$ −21.3 (*c* 0.76, $CHCl_3$); $\nu_{max}(CHCl_3)/cm^{-1}$ 3378m (NH), 3073w (HPh), 3054w (HPh), 2107s (N_3), 1676s (amide CO); δ_H(400 MHz; $CDCl_3$) 1.06 (9H, s, CMe_3), 1.78–2.16 (3H, m, =CHC*H*HCH_2), 2.55–2.68 (1H, m, =CHCH*H*), 3.56 (1H, dd, *J* 10.5, 7.1, OC*H*H), 3.76 (1H, dd, *J* 10.5, 3.5, OCH*H*), 3.96 (1H, dd, *J* 11.2, 5.6, CHN_3), 4.09 (1H, br m, C*H*NH), 5.27 (1H, dd, *J* 11, 5, CHNC*H*=), 5.76 (1H, dt, *J* 11, 7, CH_2CH=), 6.13 (1H, br m, NH), 7.37–7.47 (6H, m, Ph) and 7.57–7.65 (4H, m, Ph); δ_C(100 MHz; $CDCl_3$) 19.2 ($SiCMe_3$), 22.3 (CH_2), 26.8 (CMe_3), 30.1 (CH_2), 55.1 and 58.0 (CHN and CHN_3), 65.1 (OCH_2), 127.9, 129.1, 129.4, 129.8, 130.1, 132.4, 132.5, 135.4 and 135.5 (CH=CH and Ph) and 173.1 (CON); *m*/*z* (CI) 435 [$(M + H)^+$, 57%], 407 [$(M - N_2 + H)^+$, 48], 344 (100), 327 (45), 274 (18), 153 (19), 137 (33) and 88 (55) [Found: $(M + H)^+$ 435.2216. $C_{24}H_{31}N_4O_2Si$ requires *M*, 435.2216].

(3*R*,8*R*)-3-Acetylamino-8-(*tert*-butyldiphenylsilyloxymethyl)-azocan-2-one 27. A solution of unsaturated azido lactam **26** (29 mg, 0.07 mmol) in ethyl acetate (4 cm^3) was freeze–thaw degassed (one cycle) and palladium on charcoal (5%, 5 mg) was added. After another cycle of freeze–thaw degassing, the black suspension was vigorously stirred under an atmosphere of hydrogen (fitted balloon) for 24 h, after which TLC showed the formation of a major product (ninhydrin positive) at the expense of the starting material. The mixture was cooled to 0 °C and treated with acetyl chloride (0.01 cm^3, 0.1 mmol) and triethylamine (0.28 cm^3, 0.2 mmol). After stirring for 20 minutes, TLC showed the formation of product. The mixture was quenched by addition of aqueous hydrochloric acid (1 mol dm^{-3}, 10 cm^3) and extracted with ethyl acetate (3 × 20 cm^3). The organic phases were washed with brine (20 cm^3), combined, dried ($MgSO_4$), filtered through a pad of Celite and the solvent removed under reduced pressure to give a pale yellow oil. Purification by flash chromatography on silica gel, eluting with hexane–ethyl acetate (1:1 to 0:1, eluant gradient) gave the *acetamide* **27** (26 mg, 85%) as a pale yellow oil; R_F 0.59 (chloroform–methanol 10:1); $[a]_D^{22}$ +7.7 (*c* 0.59, $CHCl_3$); $\nu_{max}(CHCl_3)/cm^{-1}$ 3416m (NH), 3383m (NH), 3073w (HPh), 1649s (amide CO); δ_H(400 MHz; $CDCl_3$) 1.05 (9H, s, CMe_3), 1.36–1.57 (4H, m, CH_2CH_2), 1.67–1.91 (3H, m, C*H*HCH_2), 2.02 (3H, s, COMe), 2.04–2.18 (1H, m, CH*H*), 3.56 (1H, br d, *J* 10, OC*H*H), 3.72 (1H, br m, C*H*NH), 3.77 (1H, br d, *J* 10, OCH*H*), 4.70 (1H, br m, C*H*NHAc), 5.94 (1H, br d, *J* 7, N*H*Ac), 6.72 (1H, br d, *J* 9, NH), 7.32–7.47 (6H, m, Ph) and 7.54–7.65 (4H, m, Ph); δ_C(100 MHz; $CDCl_3$) 19.2 (Si*C*Me_3), 23.3 (CO*Me*), 23.7 and 24.1 (CH_2CH_2), 26.8 (C*Me*$_3$), 34.0 and 36.5 (2 × CHN*C*H_2), 49.8 and 51.9 (CHN and *C*HNHAc), 66.1 (OCH_2), 127.8, 127.9, 130.0, 132.5, 135.4 and 135.5 (Ph) and 169.0 and 173.8 (2 × CON); *m*/*z* (CI) 453 [(M + H)$^+$, 100%], 196 (8), 77 (24) and 44 (25) [Found: (M + H)$^+$ 453.2573. $C_{26}H_{37}N_2O_3Si$ requires *M*, 453.2573].

(3*R*,8*R*)-3-Acetylamino-8-hydroxymethylazocan-2-one 28. A solution of silyl ether **27** (148 mg, 0.33 mmol) in THF (4 cm^3) was treated with TBAF (1 mol dm^{-3} in THF, 0.39 cm^3) and the resulting yellow solution stirred at rt for 20 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 5:1, eluant gradient) to give the *alcohol* **28** (69 mg, 98%) as a white solid; mp 148–150 °C (ethyl acetate–hexane); R_F 0.19 (chloroform–methanol 9:1); $[a]_D^{22}$ +33.0 (*c* 0.84, $CHCl_3$); $\nu_{max}(CHCl_3)/cm^{-1}$ 3414m (NH), 3380m (NH), 3303br m (OH), 1650s (amide CO); δ_H(400 MHz; $CDCl_3$) 1.42–1.64 (5H, m, CH_2CH_2C*H*H), 1.64–1.76 (2H, m, CH_2), 1.97 (3H, s, COMe), 2.00–2.08 (1H, m, CH*H*), 3.40–3.90 (1H, br s, OH), 3.53 (1H, dd, *J* 11.4, 5.3, C*H*HOH), 3.72 (1H, dd, *J* 11.4, 3.3, CH*H*OH), 3.79 (1H, br m, C*H*NH), 4.78 (1H, br m, C*H*NHAc), 6.69 (1H, br d, *J* 9, NH) and 6.94 (1H, br d, *J* 7, N*H*Ac); δ_C(100 MHz; $CDCl_3$) 23.1 (CO*Me*), 23.8 and 24.2 (CH_2CH_2), 33.6 and 36.0 (2 × CHN*C*H_2), 49.5 and 53.1 (CHN and *C*HNHAc), 64.9 (CH_2OH) and 169.7 and 174.8 (2 × CON); *m*/*z* (CI) 215 [(M + H)$^+$, 82%], 197 (28), 140 (21), 77 (69) and 46 (100) [Found: (M + H)$^+$ 215.1396. $C_{10}H_{19}N_2O_3$ requires *M*, 215.1396].

(3*R*,8*R*)-3-Acetylamino-8-methoxycarbonylazocan-2-one 30. To a solution of hydroxymethyl lactam **28** (69 mg, 0.32 mmol) in a mixture of acetonitrile (1.2 cm^3) and water (1.1 cm^3) was added carbon tetrachloride (1.2 cm^3), sodium periodate (277 mg, 1.29 mmol) and ruthenium trichloride hydrate (2 mg). The resulting dark brown reaction mixture was stirred vigorously at rt for 40 minutes. The reaction mixture was cooled to 0 °C, quenched with propan-2-ol (0.24 cm^3) and the black mixture stirred for a further 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). An immediate formation of nitrogen was observed. After stirring for 10 minutes at 0 °C the dark brown suspension was quenched with acetic acid (1 cm^3), filtered through a pad of Hyflo and washed with methanol (5 × 1.5 cm^3). The filtrate was concentrated *in vacuo*, the residual gum suspended in ethyl acetate–methanol (1:1, 1.5 cm^3) and directly subjected to flash chromatography on silica gel. Elution with ethyl acetate–methanol (1:0 to 10:1, eluant gradient) afforded the *methyl ester* **30** (60 mg, 77%), as a white solid; mp 166–167 °C (methanol–ethyl acetate–hexane); R_F 0.41 (chloroform–methanol 10:1); $[a]_D^{22}$ +13.1 (*c* 0.86, $CHCl_3$) (Found: C, 54.4; H, 7.3; N, 11.7. $C_{11}H_{18}N_2O_4$ requires C, 54.53; H, 7.49; N, 11.56%); $\nu_{max}(CHCl_3)/cm^{-1}$ 3416m (NH), 3379m (NH), 1744s (ester CO), 1655s (amide CO); δ_H(400 MHz; $CDCl_3$) 1.48–1.67 (4H, m, CH_2CH_2), 1.68–1.85 (2H, m, 2 × C*H*H), 1.97 (3H, s, COMe), 1.98–2.14 (2H, m, 2 × CH*H*), 3.76 (3H, s, OMe), 4.42 (1H, ddd, *J* 12.1, 9.4, 3.0, C*H*NH), 4.69 (1H, ddd, *J* 11.3, 7.3, 4.1, CHNHAc), 6.30 (1H, br d, *J* 9.4, NH) and 6.68 (1H, br d, *J* 7.3, N*H*Ac); δ_C(100 MHz; $CDCl_3$) 23.2 (CO*Me*), 23.3 and 24.1 (CH_2CH_2), 35.8 and 36.0 (2 × CHN*C*H_2), 49.6 (OMe), 52.9 and 53.6 (CHN and *C*HNHAc) and 169.1, 171.7 and 172.9 (*C*OOMe and 2 × CON); *m*/*z* (EI) 242 (M$^+$, 15%), 183 (M$^+$ − CO_2Me, 28), 96 (82), 56 (58) and 43 (100) (Found: M$^+$ 242.1267. $C_{11}H_{18}N_2O_4$ requires *M*, 242.1266).

(3*R*,8*R*)-3-Acetylamino-8-methylcarbamoylazocan-2-one 2. Methyl ester **30** (9 mg, 0.037 mmol) was dissolved in ethanolic methylamine (8.0 mol dm^{-3}, 1 cm^3) and stirred at rt for 0.5 h. TLC showed the clean formation of product at the expense of the starting material. Evaporation *in vacuo* and purification by flash chromatography on silica gel, eluting with ethyl acetate–methanol (1:0 to 10:1, eluant gradient) gave the *cis-lactam* **2** (9 mg, 100%) as a white solid; mp 246–247 °C (chloroform–hexane); R_F 0.30 (chloroform–methanol 10:1); $[a]_D^{20}$ −5.5 (*c* 0.88, $CHCl_3$) (Found: C, 54.4; H, 7.8; N, 17.5. $C_{11}H_{19}N_3O_3$ requires C, 54.76; H, 7.94; N, 17.41%); $\nu_{max}(CHCl_3)/cm^{-1}$ 3452w, 3408m, 3368m and 3292br w (NHs), 1672ssh and 1644s (amide CO); δ_H(500 MHz; $CDCl_3$) 1.48–1.68 (3H, m, CH_2C*H*-HCHNHAc), 1.68–1.83 (3H, m, CH_2C*H*H), 1.83–1.94 (1H, m, COCHNCH*H*), 1.99 (3H, s, COMe), 2.04–2.15 (1H, m, CH*H*-CHNHAc), 2.81 (3H, d, *J* 4.6, NH*Me*), 4.45 (1H, br t, *J* 9.1, C*H*NH), 4.74 (1H, m, C*H*NHAc), 6.54 (1H, br d, *J* 9.1, NH), 6.97 (1H, br d, *J* 6.7, N*H*Ac) and 7.47 (1H, br q, *J* 4.6, N*H*Me); δ_C(100 MHz; $CDCl_3$) 23.4 (CH_2 and CO*Me*), 24.2 (CH_2), 26.1 (NHMe), 36.1 and 36.6 (2 × CHN*C*H_2), 50.2 and 53.8 (CHN and *C*HNHAc) and 169.6, 171.4 and 172.4 (3 × CON); *m*/*z* (EI) 241 (M$^+$, 22%), 199 (21), 155 (45), 96 (100), 84 (79) and 73 (33) (Found: M$^+$ 241.1426. $C_{11}H_{19}N_3O_3$ requires *M*, 241.1426).

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