120. Synthesis of a Type-VI β-Turn Peptide Mimetic and Its Incorporation into a High-Affinity Somatostatin Receptor Ligand

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The synthesis of a *cis*-Phe-Pro dipeptide mimetic is described, which adopts a type-VIβ-turn conformation. In this mimetic, the α-positions of Phe and Pro are joined by a CH_2CH_2 bridge, thereby forming a fused bicyclic system, and fixing a geometry similar to that seen in *cis*-Phe-Pro units in protein crystal structures. The dipeptide mimetic **20** was synthesized in optically pure form starting from (*R*)-α-allylproline (**6**; *Schemes 1*, *3*, and *4*), with a free carboxylic acid and an Fmoc-protected N-terminus, thereby allowing its incorporation into linear and cyclic peptides using standard solid-phase methods. The mimetic **20** was incorporated into the cyclic somatostatin analogue cyclo(-Phe = Pro-Phe-D-Trp-Lys-Thr-), where Phe = Pro represents the mimetic. This analogue shows a high affinity (pIC_{50} 8.6) for somatostatin receptors on rat-brain cortex membranes. Based on NMR studies in aqueous solution, likely low-energy conformations for this analogue were deduced using restrained dynamic simulated annealing. The conformations found, which include a distorted type-II′ turn at D-Trp-Lys, are similar to low-energy conformations deduced elsewhere for cyclo(-Phe-Pro-Phe-D-Trp-Lys-Thr-), as well as to those seen in crystal structures of the somatostatin analogue octreotide.

Introduction. – Proline residues are of special significance amongst the 20 common proteinogenic amino acids, because they considerably restrict the local conformational freedom of a polypeptide chain [1]. Proline may, therefore, exert an important influence upon the process of protein folding and upon protein-protein, or in general, protein-ligand recognition. In particular, the frequent occurrence of proline in β-turns [2] has stimulated interest in the design and synthesis of proline analogues that more effectively stabilize this secondary structure. In this work, the synthesis of a conformational mimic of a type-VI β-turn and its incorporation into a cyclic somatostatin receptor ligand are described.

Xaa-Pro (Xaa = a proteinogenic amino acid) peptide bonds have been found in the *cis*- and the *trans*-conformation (*Fig. 1*) in protein crystal structures [3] and in numerous

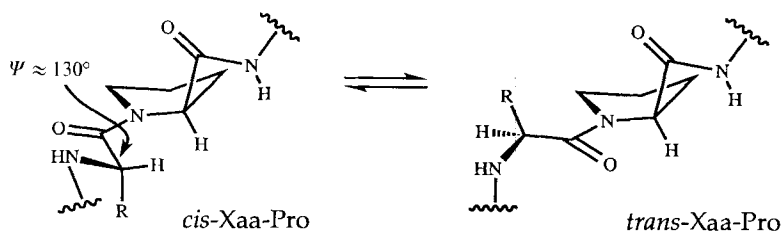
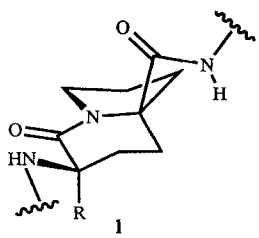


Fig. 1. cis-*and* trans-*Xaa-Pro peptide conformers*

linear and cyclic peptides. In the *cis*-conformation, the two polypeptide strands emerge in the same direction, thereby placing the *cis*-Xaa-Pro dipeptide unit at the centre of a β-turn. According to a recent analysis of the occurrence of nine standard β-turn types in proteins [2], three belong to the family of type-VI turns (namely type-VIa1, -VIa2, and -VIb), and all contain the *cis*-Xaa-Pro dipeptide unit at positions $i+1$ and $i+2$. Moreover, the ψ value of residue $i+1$ in type-VI turns is always close to 130°, to minimize a steric clash between H–C(α) of Xaa and the carbonyl O-atom of Pro (*Fig. 1*). For this reason, an obvious approach for constraining a proline-containing peptide into a type-VI β-turn is to link the α-centres of Xaa and Pro through a CH_2CH_2 bridge, as shown in **1**. We describe below a synthesis of **2** with R = $PhCH_2$, its incorporation into the cyclic somatostatin receptor ligand **3**, and an investigation of the conformational behaviour of **3** by NMR spectroscopy and dynamic simulated annealing. A preliminary report on the synthesis of **2** with R = H was described by us [4] and by others [5].



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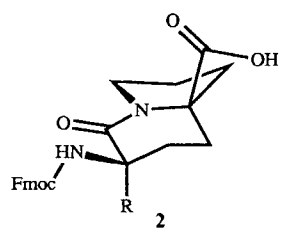

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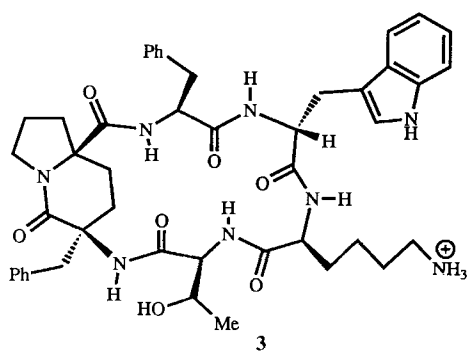

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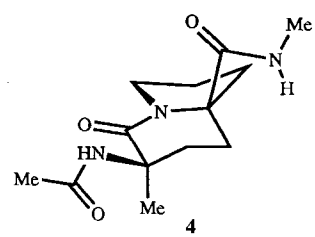

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Results and Discussion. – *Modelling Studies.* To provide an initial indication of how well **1** mimics a type-VI turn, computer-modelling experiments were undertaken on **4**. The starting structure **4** was built in MacroModel v.4.5 [6] and energy-minimized. A conformational search was then performed using the systematic unbounded multiple-minimum Monte Carlo procedure implemented in BatchMin v.4.5. [7] [8], the AMBER* force field for minimizations, with a continuum model for H_2O solvent [9], and the *Polak-Ribiere* conjugate gradient minimization mode [10] with default derivative convergence criteria (*ca.* 0.01 kcal/Å-mol). The search (1000 steps) was repeated using different starting conformations, including C(γ)-*exo*- as well as C(γ)-*endo*-puckered conformations

of the pyrrolidine ring [11]. In each case, the same low-energy conformations were found (*Fig. 2*), including the three of lowest energy **4a** ($E = -189.23$ kJ/mol), **4b** ($E = -181.91$ kJ/mol), and **4c** ($E = -181.58$ kJ/mol). The ϕ and ψ angles found in **4a–c** are similar to those classified by *Hutchinson* and *Thornton* [2] as type-VIa2, -VIa1, and -VIb turns, respectively. Superimposition of **4a–c**, on typical type-VIa2, -VIa1, and -VIb turns taken from protein crystal structures (*Brookhaven* data bank) revealed a r.m.s. deviation for the

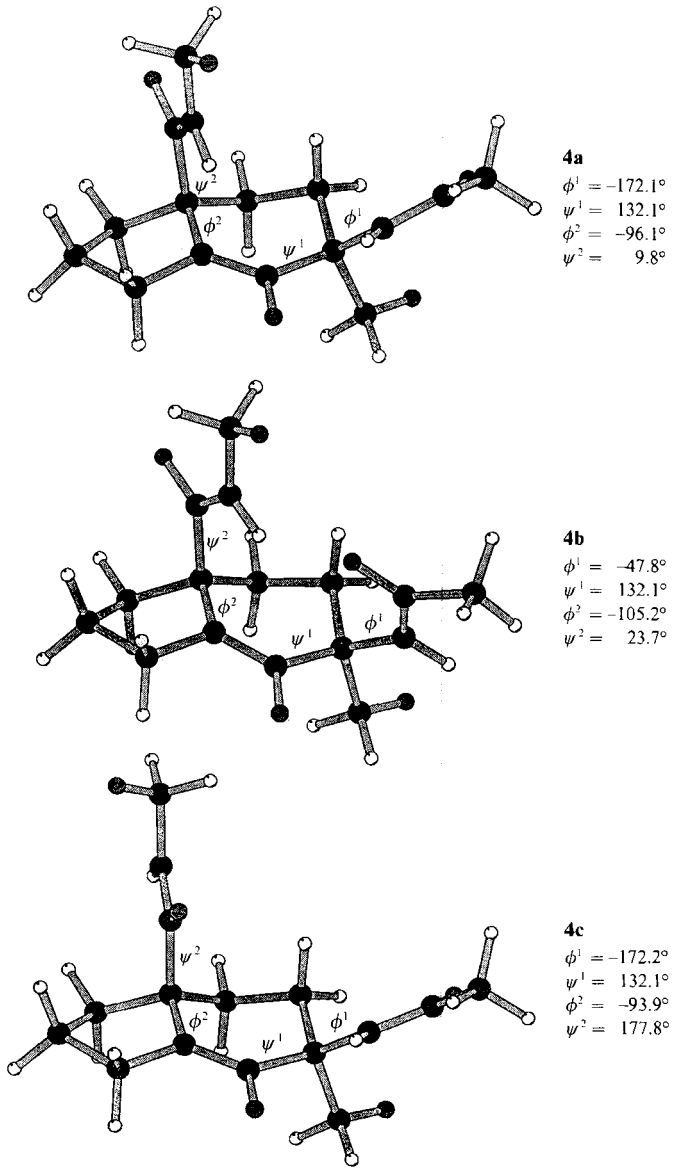


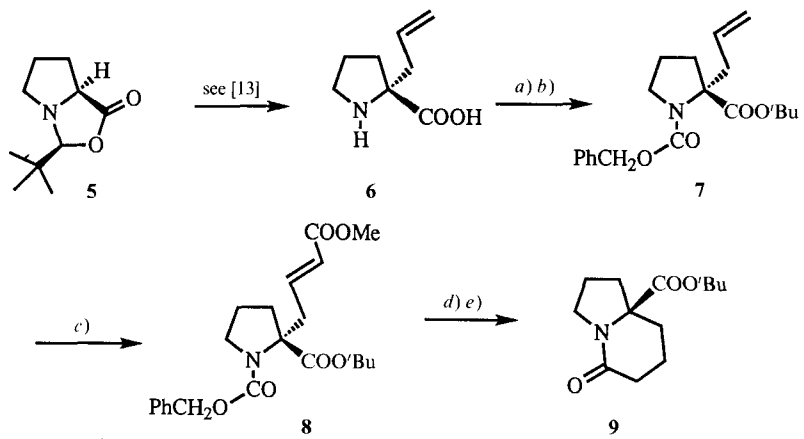
Fig. 2. *Low-energy conformations for* **4** *deduced by modelling* (see text)

backbone atoms of ≤ 0.26 Å. These results indicate that **1** should be a close structural mimic of type-VI β-turn conformations.

Synthesis. The carboxylic acid **2** is a suitable derivative for use in solid-phase peptide synthesis using (9*H*-fluoren-9-yl)methoxycarbonyl (Fmoc) chemistry [12]. A synthetic route to **2** was, therefore, established, which should allow a variety of side chains (R) to be incorporated. As an example, here R = $PhCH_2$ was chosen to provide a mimic for a *cis*-Phe-Pro dipeptide unit, suitable for elaboration into **3**.

The optically pure proline derivative **5** was alkylated with self-reproduction of chirality [13], to afford (*R*)-2-allylproline (**6**, *Scheme 1*). After conversion to **7**, ozonolytic cleavage of the C=C bond, one-pot [14] ozonide reduction, and extension of the alkyl chain by a *Wittig* reaction gave **8**. Subsequent hydrogenation and cyclization afforded the bicyclic molecule **9** in high overall yield [4].

Scheme 1

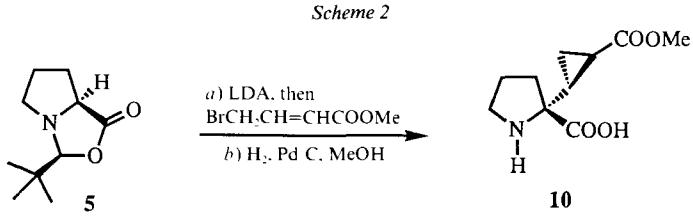


5 6 7 8 9

a) $PhCH_2OCOCl$, NaOH, EtOH; 96%. *b*) 2-Methylprop-1-ene, H_2SO_4; 93%. *c*) O_3, then $Ph_3PCHCOOMe$ (2 equiv.); 100%. *d*) H_2, Pd; 93%. *e*) 4-(Dimethylamino)pyridine, toluene, reflux; 79%.

Although this route from **1** to **9** is reliable and efficient, attempts were made to shorten it by alkylating **5** with methyl (*E*/*Z*)-4-bromobut-2-enoate. After cleavage of the N,O-acetal, however, the cyclopropane derivative **10** was obtained as sole diastereoisomer in 56% yield (*Scheme 2*), rather than the desired product of an S_N2 alkylation.

Scheme 2



5 10

Although such *Michael*-initiated ring-closures are well known [15], it is noteworthy that the formation of **10**, whose structure was confirmed by X-ray crystallography (*Fig. 3*), occurred with high stereoselectivity.

Electrophilic amination of the enolate derived from **9** with di(*tert*-butyl) azodicarboxylate afforded a 1:9 mixture **11a**/**11b** (*Scheme 3*). The major diastereoisomer was

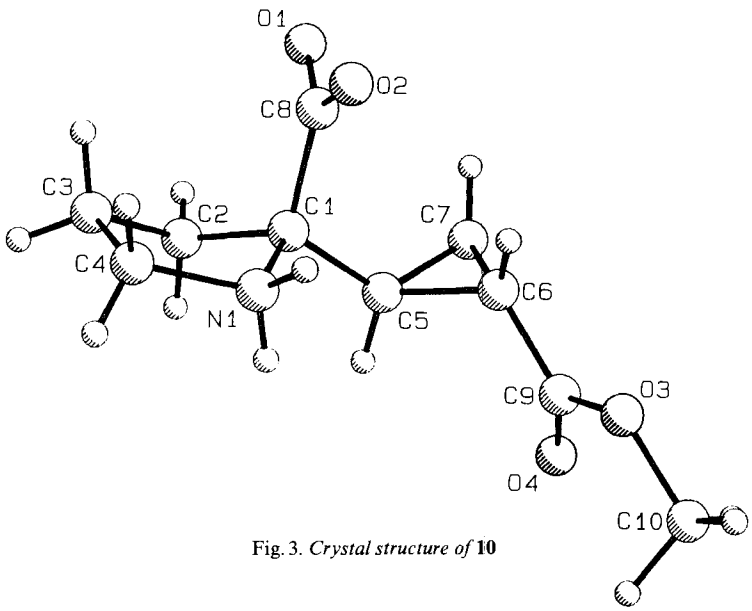
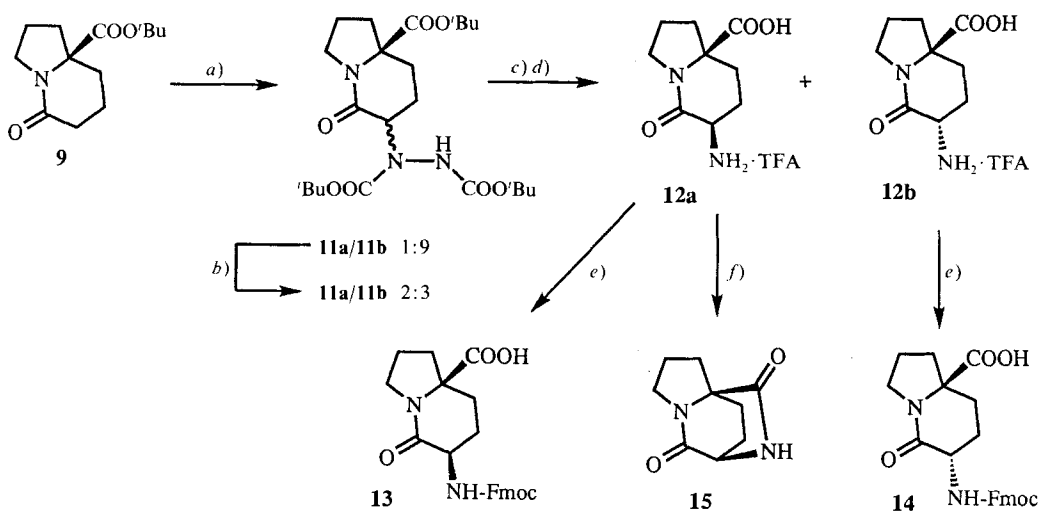


Fig. 3. *Crystal structure of* **10**

Scheme 3

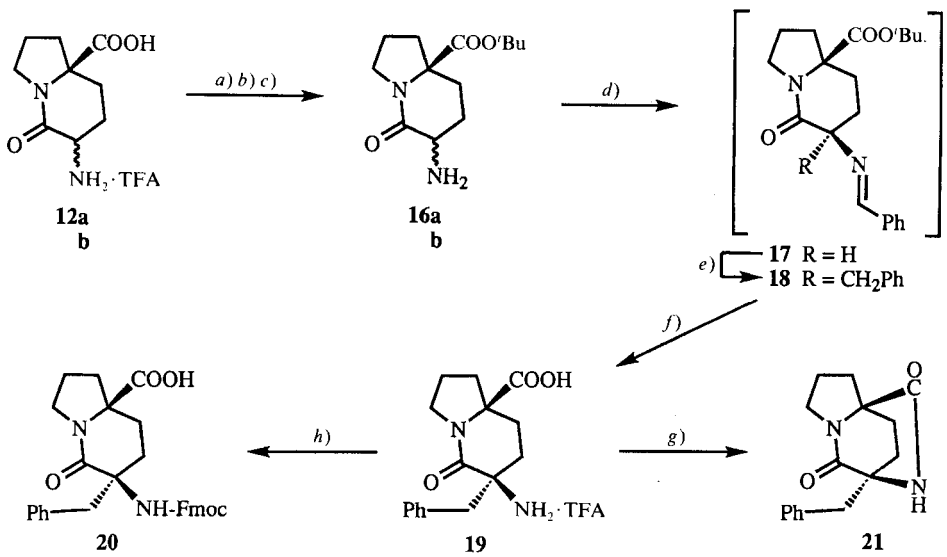


a) LDA, THF, then *t*BuOOCN=NCOO*t*Bu; 88%. *b*) LDA, THF, then *t*BuCOOH. *c*) CF_3COOH (TFA), CH_2Cl_2. *d*) H_2, Pt, H_2O; 68%. *e*) Fmoc-Cl, dioxan, aq. Na_2CO_3; 61%. *f*) $SOCl_2$, MeOH; 100%.

shown later to be the *trans*-isomer, as expected from electrophilic attack on the least hindered side of the enolate. An attempt to increase the proportion of *cis*-isomer by treatment of this mixture with LDA and then pivalic acid gave **11a**/**11b** in a 2:3 ratio, which were separated by flash chromatography (FC). The *cis*-isomer **11a** was converted in straightforward steps *via* **12a** into **13** and into **15**, the latter providing proof for the *cis* relative configuration. Similarly, **11b** was converted *via* **12b** into **14**. The derivatives **13** and **14** may be viewed as mimics for a *cis*-Gly-Pro dipeptide unit, and are suitable for use in solid-phase peptide synthesis [4].

The introduction of a side chain into **12a** was accomplished by conversion *via* **16a** into imine **17**, followed by enolate alkylation (→**18**; *Scheme 4*). After imine and ester hydrolysis, **19** was obtained as a single diastereoisomer, which could by cyclized to **21** or be protected with Fmoc-Cl to afford **20**. The obtention of **21** from **19** proves the relative configuration shown, and is again consistent with alkylation on the least hindered face of the enolate derived from **17**. The same reaction sequence was also completed starting from **12b**.

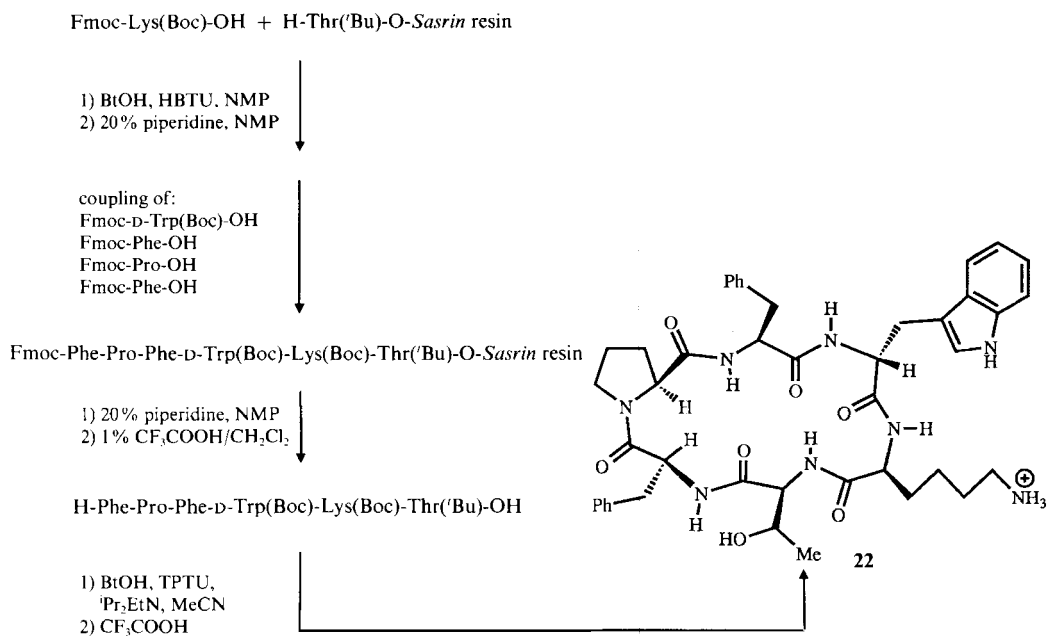
Scheme 4



a) $PhCH_2OCOCl$, EtOH, NaOH; 80%. *b*) 2-Methylprop-1-ene, H_2SO_4; 93%. *c*) H_2, Pd/C, MeOH; 96%. *d*) PhCHO, 4 Å molecular sieve, CH_2Cl_2. *e*) LDA, THF, then $PhCH_2Br$. *f*) CF_3COOH (TFA), H_2O; 83% from **16a**. *g*) $PhCH_2OCOCl$, then CH_2N_2, then H_2, Pd/C, MeOH; 31%. *h*) Fmoc-Cl, dioxan, Na_2CO_3; 61%.

The well known cyclic hexapeptide somatostatin analogue **22** [16] was prepared by the route shown in *Scheme 5*. The linear hexapeptide H-Phe-Pro-Phe-D-Trp(Boc)-Lys(Boc)-Thr(*t*Bu)-OH was assembled on *Sasrin* resin [17] and then cleaved from the resin with 1% CF_3COOH in CH_2Cl_2. Cyclization was performed at high dilution, in 65% yield, in MeCN with TPTU and BtOH activation [18]. The protecting groups were removed with CF_3COOH in the presence of H_2O, phenol, thioanisole, and ethanedithiol as scavengers.

Scheme 5 [a])

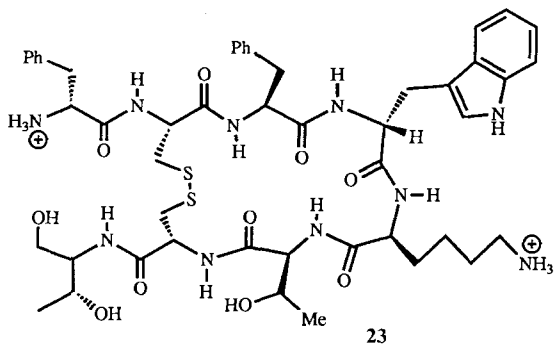


[a]) For abbreviations, see *Exper. Part.*

Exactly the same method was then used to assemble the analogue **3**, except that turn mimetic **20** was incorporated instead of the corresponding Phe-Pro dipeptide unit. Although 4 equiv. of the other Fmoc-protected amino acids were used in each coupling cycle, only 1 equiv. of **20** was used, and **H-19**-Phe-D-Trp(Boc)-Lys(Boc)-Thr(tBu)-OH was obtained after purification by HPLC in 48% yield. Cyclization under the same conditions as used for **22** and deprotection with CF_3COOH afforded the desired cyclic peptide **3** in 9% yield.

Biological Assays. Radioligand binding studies to SRIF receptors on rat-brain cortex membranes were carried out using a previously described method [19]. The pIC_{50} values ($-\log IC_{50}$) obtained for **3**, **22**, and the somatostatin analogue **23** (also known as SMS 201–995 or octreotide) were 8.6, 9.0, and 9.6, respectively. These results show that **3** is a high-affinity somatostatin receptor ligand and provide unambiguous evidence that a type-VI turn conformation at *cis*-Phe-Pro is present in the biologically active (*i.e.*, receptor-bound) conformation of **22** [20].

NMR Studies. ^{1}H-NMR Spectra of **3** were recorded exclusively in aqueous solution (H_2O/D_2O 9:1) at pH 4.5. The chemical shifts and resonance line widths changed significantly with concentration above 1 mM, indicating pronounced aggregation. However, at or below 1 mM, no significant concentration-dependent changes in either line widths or chemical shifts were observed, suggesting that in this range, aggregation is not a serious problem.



23

H-Ala[1]-Gly[2]-Cys[3]-Lys[4]-Asn[5]-Phe[6]-Phe[7]-Trp[8]-Lys[9]-Thr[10]-Phe[11]-Thr[12]-Ser[13]-Cys[14]-OH

Somatostatin

The cyclic hexapeptide **22** has previously been the subject of an extensive conformational analysis using NMR methods, both in H_2O and DMSO solution [20] [21]. Less conformational averaging was seen in H_2O than in DMSO, and the existence of a *cis*-Phe-Pro peptide bond was clearly demonstrated. Also from NMR studies, *Veber* [20] proposed a type-II′ β-turn conformation around Trp-D-Lys (corresponding to a type-II turn in somatostatin) and a type-VI β-turn at the *cis*-Phe-Pro peptide bond in the biologically most active conformation of **22**. These conclusions were confirmed and extended in studies of other analogs by *Kessler* and coworkers [22].

The ^{1}H-NMR assignments for **3** given in *Table 1* were derived at 1 mM concentration and 278 K using standard 2D-NMR methods [23] at 600 MHz. Individual spin systems were assigned using TOCSY and DQF-COSY spectra, while analysis of ROESY spectra afforded sequential assignments. The ROE intensities involving the four protons in the

Table 1. *Assignment of the ^{1}H-NMR Spectrum of* **3** *in H_2O/D_2O 9:1.* pH 4.5, 278 K.

Residue[a])	NH	H–C(α)	H–C(β)[b])	Others
Pro[6]	–	–	0.76, 2.18	0.83, 1.69 ($CH_2(\gamma)$); 3.30, 3.61 ($CH_2(\delta)$)
Phe[7]	8.55	4.89	3.12	7.38 (2 H_o); 7.44 (2 H_m); 7.36 (H_p)
Trp[8]	8.48	4.45	3.09	10.19 (NH); 7.21 (H–C(2)); 7.63 (H–C(4)); 7.23 (H–C(5)); 7.31 (H–C(6)); 7.54 (H–C(7))
Lys[9]	8.29	3.62	1.30, 1.61	0.30, 0.48 ($CH_2(\gamma)$); 1.32 ($CH_2(\delta)$); 2.75 ($CH_2(\varepsilon)$)
Thr[10]	7.48	4.38	4.17	1.24 ($CH_3(\gamma)$)
Phe[11]	[c])	–	2.97, 3.40	7.26 (2 H_o); 7.42 (2 H_m); 7.40 (H_p)
CH_2CH_2	–	–	2.12, 1.94 1.82, 0.85	

[a]) The superscript numbers refer to the location of the residues in somatostatin (see structure). The mimetic template in **3** is referred to as Phe[11]-Pro[6] with a CH_2CH_2 unit linking the C(α) centres. Assignments within the CH_2CH_2 unit could not be made with confidence (see text).

[b]) A single value for a CH_2 group indicates degenerate chemical shifts; no stereospecific assignments were made.

[c]) The Phe[11] NH resonance was not observable, most likely due to bleaching through presaturation of the H_2O resonance.

CH_2CH_2 unit of the turn mimetic, connecting C(α) of Phe[11] and Pro[6], were not sufficiently different to allow unambiguous resonance assignments. The Phe[11] NH resonance was not observed. Noteworthy are the upfield-shifted resonances for $CH_2(\gamma)$ of Lys[9], reflecting proximity of the lysine side chain and the indole nucleus [20], and the upfield shifted $H-C(\gamma)$ and $H-C(\beta)$ of Pro[6]. The temperature coefficients for the observable peptide NH resonances (*Table 2*) revealed a value close to zero for the Thr[10] NH, indicating substantial shielding from the solvent, possibly due to intramolecular H-bonding.

Table 2. *Temperature Coefficients of the Amide Chemical Shifts for* **3**

Residue NH	Lys[9]	Thr[10]	Phe[11]	Phe[7]	Trp[8]
Temp. coeff.[a]) $-\Delta\delta/\Delta T$ [ppb/K]	5.2	0.0	n.d.	4.6	9.6

[a]) Determined over the range 5–25°.

Compound **3** yielded near-zero NOESY cross-peak intensities at 600 MHz, since its molecular size falls in the range where the transition from positive to negative NOE's occurs. Thus, for the analysis of NOE connectivities, a series of ROESY spectra were measured. Only ROE connectivities between adjacent residues were observed in ROESY spectra, due in part to the low peptide concentration used. However, ROE connectivities were found between the aromatic protons of Phe[11] and one proton of the Pro[6] $CH_2(\delta)$, as well as the CH_2CH_2 unit of the mimetic (*Fig. 4*). ROE Connectivities were also found between the Phe[7] ring and the other proton of the Pro[6] $CH_2(\delta)$ (*Fig. 4*), indicating a possible clustering of the hydrophobic Phe[11], mimetic, and Phe[7] rings in a sandwich arrangement (*vide infra*).

To determine from the NMR data likely average solution structures for **3**, the ROE connectivities were used to derive distance restraints as input for structure calculations using the simulated annealing (SA) protocol [24] with the DISCOVER program (*Biosym*, San Diego) and the CVFF force field.

Cross-peak volumes were quantitated in ROESY spectra with 50, 100, 200, and 300 ms mixing times using the FELIX software (*Biosym*, San Diego). The ROESY cross-peak intensities were corrected for resonance offset effects, as described by *Griesinger* and *Ernst* [25] for medium-size molecules. Distances were then estimated by setting the $d_{\beta\beta}$ distance in Phe[11] to 1.75 Å and calculating other distances according to the r^{-6} relationship, using three models: *1*) assuming a linear NOE build-up to 200 ms mixing time and using the cross-peak volumes at 200 ms to determine distances; *2*) fitting a straight line to the build-up curve from which distances were then extracted; *3*) fitting the function $v = a_0 \cdot e^{a1 \cdot x}$ to the build-up curve and then extracting distances (r) from the linear term ($r = k \cdot a_0$). The agreement between the distances calculated in these ways was excellent, indicating the absence of spin-diffusion effects in the ROE's used. These distances were then extended by 10%, the necessary pseudoatom corrections [23] were applied for non-stereospecifically assigned protons at prochiral centres and for Me groups, and the resulting values were used as input upper distance restraints (*Table 3*). Lower distance restraints were set to 2.0 Å throughout. NOE Restraint violations were penalized with a weighting factor of 15 kcal/Å^2/mol with an upper limit of 250 kcal/Å^2/mol.

The calculations were initiated with an arbitrary starting conformation. Trajectories were inspected to ensure that the starting coordinates were effectively randomized during the initial phases of the SA protocol. Typically, 30 calculations were performed differing in the values of the random number seed, to generate different initial velocities for all atoms. The *van der Waals* potential was set to a quartic form during the SA procedure [24] and changed to the normal *Lennard-Jones* potential for the final energy minimization. The coulombic term was set to zero in the force field. Constraints were applied to avoid inversions at chiral centres and the conversion of *trans*- to *cis*-peptide bonds or *vice versa*.

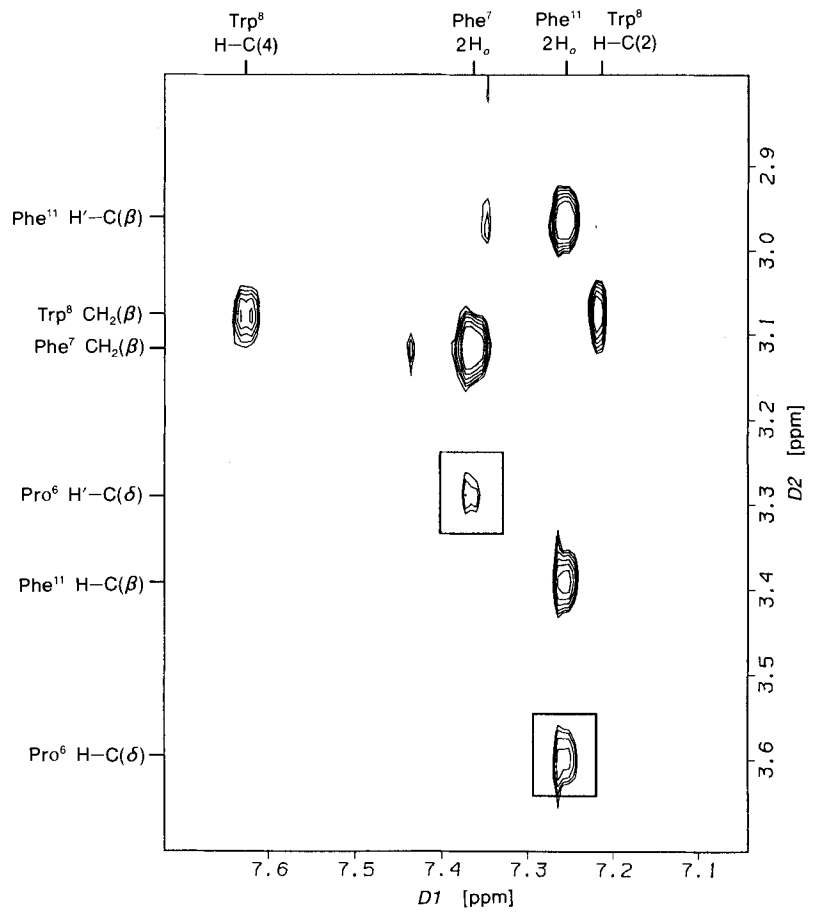


Fig. 4. *Portion of a 2D ROESY spectrum of* **3**. The chemical shifts and assignments are given on the axes. The boxed cross-peaks show ROE connectivities between Pro^6 and the aromatic rings of Phe^7 and Phe^{11}.

Table 3. *Summary of Input Constraints for the Structure Calculations and the Violations, r.m.s. Deviations, and Energies of the Final 13 SA Structures*

Input to structure calculations		
^{1}H,^{1}H-distance restraints		
total	33	
intra-residue	22	
sequential	11	
medium-range	0	
total restraints/residue	5.5	
Results of structure calculations[a])		
Energy [kcal/mol]		
total	243.494 ± 4.141	234.106 ... 248.984
nonbond	134.096 ± 3.184	129.203 ... 138.161
restraint	2.806 ± 1.198	1.317 ... 4.238

Table 3 (cont.)

NOE violations		
number > 0.1 Å	2.153 ± 1.625	0 ... 4
number > 0.2 Å	0.307 ± 0.480	0 ... 1
maximum [Å]	0.164 ± 0.061	0.067 ... 0.247
sum [Å]	0.462 ± 0.252	0.125 ... 0.799
Pairwise r.m.s. deviation	0.687 ± 0.199	0.227 ... 1.066

[a]) Results are listed as average ± standard deviation and range of observed values (low ... high).

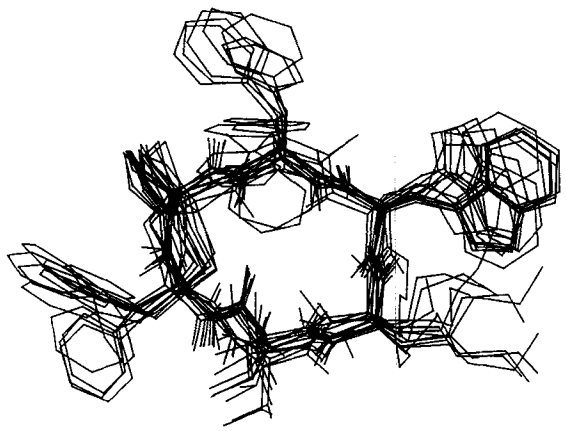


Fig. 5. *Backbone superimposition of the final 13 lowest-energy SA-derived structures*

The calculations yielded 13 out of 30 structures that have no NOE violations larger than 0.25 Å, and all of which are within 15 kcal/mol of the lowest-energy conformer (*Table 3*). The r.m.s. deviation for the backbone N, C(α), and C′ backbone atoms in all six residues is 0.69 ± 0.2 Å (*Fig. 5*). These 13 structures form a family of closely related conformations, consisting essentially of two β-turns joined at Thr[10] and Phe[7]; residues Phe[7]-D-Trp[8]-Lys[9]-Thr[10] are in a distorted type-II′ turn conformation, while residues Phe[11]-Pro[6] (*i.e.*, the mimetic; see *Table 1*) adopt type-VI β-turns (*Table 4*). The peptide backbone in all 13 SA structures adopts a cup shape (*Fig. 6*), somewhat similar to those found in earlier studies of somatostatin analogues [21] [26]. A regular flat β-sheet conformation with its characteristic H-bonding pattern was not found. For comparison, a series of SA calculations were performed without applying the NOE-derived distance constraints. These afforded essentially the same family of backbone conformers, but with a wider spread of side-chain torsion angles for all amino-acid residues (data not shown).

It is noteworthy, that a recurring motif in the restrained SA structures is a clustering of the hydrophobic aromatic rings of Phe[11] and Phe[7] above and below the bicyclic mimetic. However, we do not view this as a rigid sandwich, but rather as one possible arrangement of these side chains in a dynamic equilibrium with other folded forms. Similar hydrophobic clusters were deduced for the solution conformation of **22**, with the proline ring sandwiched between the Ph rings of Phe[7] and Phe[11] [20], and more recently for a type-VI turn in a family of linear peptides containing a *cis*-Tyr-Pro-Tyr motif in aqueous solution [27].

Table 4. *Backbone* (ϕ and Ψ) *and Side-chain* (χ_1) *Torsion Angles Found in the Final 13 SA Structures*

Structure	Pro[6]			Phe[7]			Trp[8]		
	ϕ	Ψ	χ_1	ϕ	Ψ	χ_1	ϕ	Ψ	χ_1
1	−104.7	−1.4	+36.2	−70.0	−4.9	+67.2	+156.6	−99.9	−106.5
2	−103.9	−30.4	+32.7	−94.8	+126.7	−64.9	+69.3	−124.5	+82.3
3	−99.6	−3.0	+33.1	−163.1	+131.2	+51.3	+93.7	−100.9	−94.5
4	−99.5	−13.9	+33.9	−88.5	+107.6	−67.2	+82.0	−119.0	+82.1
5	−96.9	−126.3	+33.0	+60.7	−21.7	+68.9	+152.2	−85.8	+82.9
6	−98.4	−15.6	+33.4	−158.2	+145.1	+48.9	+98.5	−98.4	−108.3
7	−111.4	+27.6	+37.7	−137.4	+104.3	−63.0	+75.5	−107.6	+80.3
8	−106.6	+36.5	+37.9	−134.0	+105.5	+53.2	+70.6	−115.1	+80.6
9	−122.2	+12.5	+37.8	−93.8	+99.6	−69.1	+95.6	−113.8	+82.1
10	−109.1	+30.6	+37.4	−154.7	+119.1	+59.3	+67.0	−113.3	+81.6
11	−107.3	+34.9	+37.8	−149.2	+125.1	+58.0	+80.4	−124.0	−89.6
12	−99.0	−27.9	+32.9	−151.7	+123.1	+55.7	+132.2	−93.8	−104.7
13	−113.0	+38.0	+39.0	−138.4	−83.7	+54.4	−70.0	−119.8	−90.1

Structure	Lys[9]			Thr[10]			Phe[11]		
	ϕ	Ψ	χ_1	ϕ	Ψ	χ_1	ϕ	Ψ	χ_1
1	−66.0	−64.8	−63.7	−89.9	+128.2	+52.8	−45.9	+134.9	+77.4
2	−81.2	+123.9	+72.0	+69.3	+145.4	−155.1	−47.1	+130.4	+177.3
3	−97.7	+95.0	−74.0	+42.5	+94.1	−167.6	+60.9	+120.0	−172.4
4	−73.9	−57.7	−64.8	−70.5	−85.7	−174.2	+176.1	+124.3	+169.2
5	−73.5	−84.4	−72.6	−86.6	+117.8	−173.3	−33.7	+122.7	−174.6
6	−71.0	−67.8	−72.6	−139.0	−135.1	−172.9	−63.4	+126.0	−179.5
7	−81.0	+148.8	−61.7	+67.3	−95.2	−167.8	+179.8	+133.5	+176.2
8	−72.4	−61.9	−62.1	−83.1	+126.3	−172.3	−52.0	+132.3	−178.1
9	−100.1	−38.3	−64.4	−66.5	−83.6	+56.7	+177.8	+102.3	+164.7
10	−81.9	+115.5	−67.9	+66.5	+141.4	−157.2	−45.7	+136.5	+62.0
11	−84.0	−54.8	+56.2	−85.6	+131.1	+55.1	−48.4	+135.5	+60.4
12	−138.8	+114.2	+74.5	+53.6	+90.9	−167.65	+52.8	+123.5	+87.6
13	−72.0	−55.5	+77.5	−91.6	+148.0	+53.5	−46.4	+139.6	+61.9

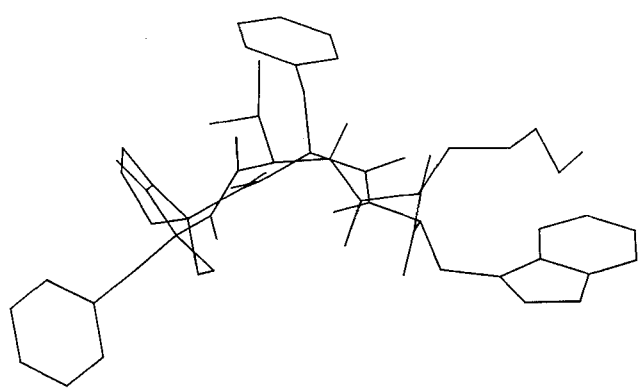


Fig. 6. *A side view of one typical SA structure, showing the cup-shaped conformation of the backbone*

The residues in both turn regions in the SA structures of **3** have ϕ and ψ angles (*Table 4*) close to the mean values of low-energy type-II′ or type-VI β-turns, as determined through analyses of protein X-ray crystal structures [2]. The Trp[8] ϕ and ψ angles cluster around 70 to 90°, and −90 to −120°, respectively, which are close to the canonical values of 60° and −120° for a type-II′ turn. For Lys[9], the observed ϕ angles (*ca.* −85°) are those expected for a type-II′ turn, but ψ is found in either the β region (*ca.* 120°, 5 instances) or the α region (*ca.* 60°, 8 instances) of the $\phi\psi$ space, rather than the typical value for a type-II′ turn of *ca.* 0°. This leads to a distortion in the type-II′ turn, with no consistent H-bonding pattern found between Thr[10] NH and Phe[7] CO. However, the peptide-amide chemical-shift temperature coefficients (*Table 2*) reveal a strongly solvent-shielded Thr[10] NH, suggesting that this H-bond may be substantially populated under the conditions of the NMR experiments.

Crystal structures of the synthetic somatostatin analogue octreotide **23** were reported recently [28]. Three crystallographically independent molecules were observed in the unit cell. One molecule adopts a β-sheet-like structure, whereas the other two possess different irregular backbone conformations. However, all three have a type-II′ β-turn at D-Trp-Lys. The backbone atoms (N, C(α), C′) of the β-sheet-like crystal structure of **23**, from N of Phe[7] to C′ of Thr[10] can be superimposed on the corresponding part of a typical SA structure (*e.g.* structure *8* in *Table 4*) of **3** with an r.m.s. deviation of only 0.28 Å (*Fig. 7*).

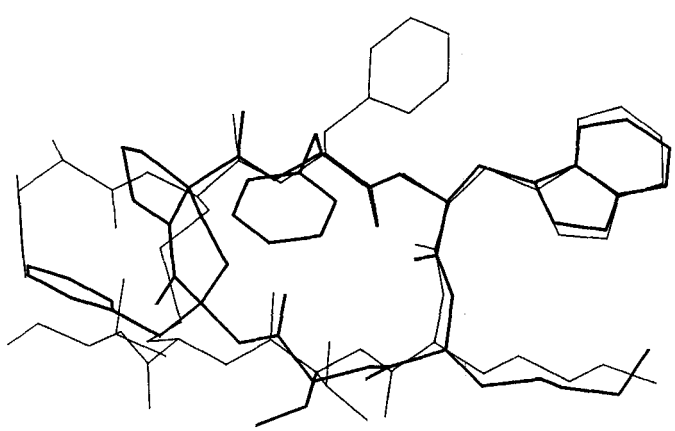


Fig. 7. *Backbone superimposition of one SA structure of* **3** (thick lines) *and a crystal structure of octreotide* **23** (thin lines)

In the region of the type-VI-turn mimetic in **3**, the SA structures contain two major sub-families of type-VI turns. One sub-family (*e.g.* structures *1*, *2*, *6*, *8*, *10*, *11* and *13*, *Table 4*) superimpose closely on Tyr[92]-Pro[93] (a type-VIa1 turn) in the crystal structure of ribonuclease A, while another (*e.g.* structures *4*, *7*, and *9*) superimposes closely on Tyr[95]-Pro[96] (a type-VIb turn) in the crystal structure of adenylate kinase. These two families of SA structures thus have conformations similar to **4b** and **4a**, respectively. These comparisons suggest that the bicyclic system in **3** indeed fulfills its intended function as a type-VI-turn mimetic, and that the NMR-derived SA structures for **3** resemble likely low-energy conformers of this molecule in aqueous solution.

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Experimental Part

General. THF was freshly distilled from Na/benzophenone under N_2. All other solvents were distilled before use. Flash chromatography (FC): *Merck* silica gel *60* (230–400 mesh). HPLC: see below (peptide synthesis). M.p.: *Büchi-SMP-20* apparatus, uncorrected. $[\alpha]_D$: *Perkin-Elmer-241* polarimeter. UV Spectra: *Varian-Cary-3* spectrophotometer; in nm (ε in $dm^3mol^{-1}cm^{-1}$). IR Spectra: *Perkin-Elmer-781* spectrophotometer; in cm^{-1}. NMR Spectra: *Bruker-AC300* or *Bruker-AMX600* for ^1H, and *Varian-XL200* or *Bruker-ARX300* spectrometers for ^{13}C; δ in ppm rel. to $SiMe_4$ as internal standard in $CDCl_3$ or sodium 3-trimethylsilyl(D_4)propionate in D_2O, J in Hz. MS: chemical ionization (CI) on a *Varian-MAT-90* or electrospray ionization (ES) on a *Finnigan-TSQ-700* spectrometer; m/z (rel. %).

Peptide Synthesis: *General.* Peptide synthesis: *ABI 430A* with *FastMoc*® method (HBTU and BtOH) [18]. *O*-(Benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HBTU), 4-(dimethylamino)-pyridine, *O*-(1,2-dihydro-2-oxopyridin-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate (TPTU), and benzotriazol-1-ol (BtOH) were anal. grade. *N*,*N*-Dimethylformamide (DMF) and 1-methylpyrrolidin-2-one (NMP) were dried ($MgSO_4$) and redistilled from ninhydrin, and $^iPr_2EtN$ was redistilled from ninhydrin prior to use. HPLC: dual pump *Pharmacia* system and *Waters RCM-μBondapak*®-C_{18} cartridges (10 μm, 125 Å, 25 × 100 mm) for prep. and (8 × 10 mm) anal. separations; solvents: *A*, H_2O + 0.1% CF_3COOH, and *B*, MeCN with 0.1% CF_3COOH; UV detection at 226 and 254 nm.

(R)-N-[(Benzyloxy)carbonyl]-2-(prop-2-enyl)proline. Benzyl chloroformate (0.88 g, 5.2 mmol) was added dropwise at 0° to a soln. of **6** [13] (610 mg, 3.9 mmol) in 2N aq. NaOH (4.5 ml) and EtOH (7.5 ml) and stirred at r.t. The addition of benzyl chloroformate, 2N NaOH, and EtOH was repeated after 19 h and 36 h. After 72 h, 1N NaOH was added and the mixture extracted with Et_2O. The aq. phase was acidified to pH 2 (conc. HCl) and extracted with Et_2O. The org. phase was dried ($MgSO_4$) and evaporated. FC (CH_2Cl_2/MeOH) gave the product as a yellow oil (1.09 g, 96%). $[\alpha]_D^{25} = +27.5$ ($c = 1.0$, CH_2Cl_2). IR ($CHCl_3$): 2800–2400*w*, 1750*s*, 1700*s*. ^1H-NMR (300 MHz, $CDCl_3$): 1.78–2.44 (*m*, 4 H); 2.56–2.98 (*m*, 2 H); 3.34–3.74 (*m*, 2 H); 4.99–5.19 (*m*, 4 H); 5.85–5.72 (*m*, 1 H); 7.24–7.37 (*m*, 5 H). ^{13}C-NMR (50 MHz, $CDCl_3$): 22.5; 22.9; 35.3; 37.1; 37.7; 38.9; 48.6; 49.2; 67.1; 67.3; 68.5; 119.4; 119.5; 127.6; 127.8; 127.9; 128.3; 128.4; 132.4; 132.6; 136.1; 136.4; 155.2; 177.9; 179.6. CI-MS (C_4H_{10}): 290.2 (75, $[M + H]^+$), 246.2 (100, $[M + H - CO_2]^+$). Anal. calc. for $C_{16}H_{19}NO_4$ (289.33): C 66.42, H 6.62, N 4.84; found: C 66.74, H 6.76, N 4.66.

tert-*Butyl (R)-N-[(Benzyloxy)carbonyl]-2-(prop-2-enyl)prolinate* (**7**). At −78° 2-methylprop-1-ene (45 ml) was added to the foregoing product (2.57 g, 8.9 mmol) in CH_2Cl_2 (25 ml) with conc. H_2SO_4 (5 drops). After 4 d at r.t. under pressure, the excess 2-methylprop-1-ene was evaporated, the H_2SO_4 neutralized with 5% aq. $NaHCO_3$ soln., the soln. extracted with CH_2Cl_2, the extract washed with 5% aq. $NaHCO_3$ soln. and brine, dried ($MgSO_4$), and evaporated, and the brown oil submitted to FC (hexane/AcOEt): 7 (2.85 g, 93%). Colourless oil, $[\alpha]_D^{25} = +29.1$ ($c = 0.5$, CH_2Cl_2). IR ($CHCl_3$): 1730*s*, 1700*s*. ^1H-NMR (300 MHz, $CDCl_3$): 1.34, 1.39 (2*s*, 9 H); 1.77–1.90 (*m*, 2 H); 1.99–2.15 (*m*, 2 H); 2.54–2.59 (*m*, 1 H); 2.91–3.09 (*m*, 1 H); 3.39–3.49 (*m*, 1 H); 3.61–3.75 (*m*, 1 H); 5.00–5.30 (*m*, 4 H); 5.54–5.77 (*m*, 1 H); 7.26–7.36 (*m*, 5 H). ^{13}C-NMR (50 MHz, $CDCl_3$): 22.5; 23.1; 27.7; 27.8; 35.5; 37.2; 38.0; 39.1; 48.4; 49.3; 66.4; 67.1; 67.6; 68.4; 80.9; 81.1; 118.6; 118.9; 127.7; 127.9; 128.0; 128.3; 133.2; 133.7; 154.4; 173.0; 173.1. CI-MS (C_4H_{10}); 346.4 (24, $[M + H]^+$), 290.3 (100). Anal. calc. for $C_{20}H_{27}NO_4$ (345.44): C 69.54, H 7.88, N 4.06; found: C 69.99, H 8.22, N 4.06.

tert-*Butyl (R)-N-[Benzyloxy)carbonyl]-2-[(E)-3-(methoxycarbonyl)prop-2-enyl]prolinate* (**8**). A soln. of **7** (19.72 g, 57.1 mmol) in dry CH_2Cl_2 (120 ml) was saturated with O_3 at −78°. After 20 min, the excess O_3 was displaced with a N_2 stream, and [(methoxycarbonyl)methylidene]triphenylphosphorane (43.9 g, 131.3 mmol) was added at −78°. The soln. was warmed to r.t. overnight, added to H_2O, and extracted with CH_2Cl_2, the org. phase dried ($MgSO_4$) and evaporated, the resulting solid triturated in hexane and filtered, and the filtrate evaporated: **8** (23.0 g, 100%). Colourless oil. $[\alpha]_D^{25} = +51.8$ ($c = 0.7$, CH_2Cl_2). IR (CH_2Cl_2): 1720*s*, 1701*s*, 1657*w*. ^1H-NMR (300 MHz, $CDCl_3$): 1.32, 1.36 (2*s*, 9 H); 1.69–2.12 (*m*, 4 H); 2.68–3.25 (*m*, 2 H); 3.40–3.76 (*m*, 2 H); 3.69, 3.70 (2*s*, 3 H); 5.01–5.05 (*m*, 1 H); 5.17–5.19 (*m*, 1 H); 5.75–5.88 (*m*, 1 H); 6.78–6.93 (*m*, 1 H); 7.20–7.38 (*m*, 5 H). ^{13}C-NMR (50 MHz, $CDCl_3$): 22.4; 23.1; 27.7; 35.8; 37.5; 36.7; 37.8; 48.3; 49.2; 51.4; 66.6; 67.3; 67.4; 68.2; 81.4; 81.6; 124.2; 124.5; 127.7; 127.8; 128.1; 128.4; 136.9; 143.7; 144.5; 154.3; 166.6; 172.4. CI-MS (C_4H_{10}): 404.4 (45, $[M + H]^+$), 348.3 (100). Anal. calc. for $C_{22}H_{29}NO_6$ (403.48): C 65.49, H 7.24, N 3.47; found: C 65.24, H 7.02, N 3.32.

tert-*Butyl (6S)-2-Oxo-1-azabicyclo[4.3.0]nonane-6-carboxylate* (= tert-*Butyl (8aS)-Perhydro-5-oxoindolizine-8a-carboxylate*; **9**). A soln. of **8** (5.11 g, 12.7 mmol) in MeOH (150 ml) was stirred with Pd-black (140 mg) for 12 h under H_2 and then filtered through *Celite* and evaporated to give tert-*Butyl (S)-2-[(3-(methoxycarbonyl)propyl]prolinate* (3.18 g, 93%). Colourless oil. $[\alpha]_D^{25} = -26.2$ ($c = 1.0$, CH_2Cl_2). IR (CH_2Cl_2): 3340*w*, 1724*s*, 1626*s*. ^{1}H-NMR (300 MHz, $CDCl_3$): 1.38–1.53 (*m*, 2 H); 1.44 (*s*, 9 H); 1.55–1.84 (*m*, 4 H); 2.03–2.13 (*m*, 2 H); 2.21–2.34 (*m*, 2 H); 2.85–3.00 (*m*, 2 H); 3.63 (*s*, 3 H). ^{13}C-NMR (50 MHz, $CDCl_3$): 18.6; 20.4; 20.6; 24.9; 27.8; 27.9; 30.2; 32.0; 34.1, 36.1, 38.0; 39.3; 44.9; 46.4; 51.3; 69.2; 80.9; 81.9; 173.6; 176.0. CI-MS (C_4H_{10}): 272.4 (100, $[M + H]^+$). Anal. calc. for $C_{14}H_{25}NO_4$ (271.36): C 61.97, H 9.29, N 5.16; found: C 61.77, H 9.41, N 5.35.

The foregoing product (2.59 g, 9.6 mmol) and 4-(dimethylamino)pyridine (233 mg, 1.9 mmol) in dry toluene (200 ml) was refluxed under N_2 for 12 h. The soln. was washed with dil. aq. HCl soln., dried ($MgSO_4$), and evaporated to give a yellow oil from which **9** crystallized on trituration with hexane: 1.81 g (79%). M.p. 65–66°. $[\alpha]_D^{25} = -44.0$ ($c = 1.0$, CH_2Cl_2). IR ($CHCl_3$): 1726*s*, 1626*s*. ^{1}H-NMR (600 MHz, $CDCl_3$): 1.41–1.48 (*m*, 2 H); 1.45 (*s*, 9 H); 1.56–1.73 (*m*, 3 H); 1.80–1.92 (*m*, 2 H); 2.26–2.33 (*m*, 1 H); 2.40–2.50 (*m*, 3 H); 3.50–3.54 (*m*, 1 H); 3.65–3.70 (*m*, 1 H). ^{13}C-NMR (50 MHz, $CDCl_3$): 18.6; 20.3; 27.8; 30.2; 31.9; 38.0; 44.9; 69.8; 81.9; 168.8; 172.4. CI-MS (C_4H_{10}): 240.4 (100, $[M + H]^+$). Anal. calc. for $C_{13}H_{21}NO_3$ (239.32): C 65.25, H 8.84, N 5.85; found: C 65.39, H 8.84, N 6.09.

(2R,1'S,2'S)-2-[(2-(Methoxycarbonyl)cyclopropyl]proline (**10**). A soln. of **5** (14.8 g, 80.9 mmol) in dry THF (100 ml) was added to lithium diisopropylamide (LDA; 85.0 mmol) in hexane (50 ml) and THF (250 ml) at −78°. After 30 min, methyl (*E*/*Z*)-4-bromobut-2-enoate (12.5 ml, 104.6 mmol) in THF (100 ml) was added and the mixture allowed to warm to 21° over 3 h. The soln. was extracted with CH_2Cl_2 and sat. aq. NaCl soln., the org. phase dried ($MgSO_4$) and evaporated, and the residue submitted to FC (hexane/AcOEt 7:3) gave *methyl (1R,2R,3'S,7'aR)-2-[3-(*tert-*butyl)tetrahydro-1-oxo-1H,3H-pyrrolo[1,2-c]oxazol-7a-yl]cyclopropane-1-carboxylate* (16.8 g, 74%). Yellow oil. $[\alpha]_D^{25} = +26.4$ ($c = 1.2$, CH_2Cl_2). IR (CH_2Cl_2): 3020*m*, 1771*s*, 1722*s*. ^{1}H-NMR (300 MHz, $CDCl_3$): 0.89 (*s*, 9 H); 1.11 (*dt*, $J = 9.3, 4.5$, 1 H); 1.24 (*ddd*, $J = 8.8, 6.8, 4.5$, 1 H); 1.63 (*dt*, $J = 8.8, 4.5$, 1 H); 1.67–1.73 (*m*, 1 H); 1.78 (*ddd*, $J = 9.3, 6.8, 4.5$, 1 H); 1.82–1.89 (*m*, 1 H); 1.91–2.02 (*m*, 1 H); 2.16–2.25 (*m*, 1 H); 2.78–2.86 (*m*, 1 H); 2.96–3.05 (*m*, 1 H); 3.65 (*s*, 3 H); 4.16 (*s*, 1 H). ^{13}C-NMR (50 MHz, $CDCl_3$): 11.2; 18.1; 24.3; 24.7; 28.9; 36.1; 37.5; 51.7; 57.2; 71.0; 104.5; 173.8; 175.6. CI-MS (C_4H_{10}): 282.4 (100, $[M + H]^+$). Anal. calc. for $C_{15}H_{23}NO_4$ (281.35): C 64.04, H 8.24, N 4.98; found: C 63.96, H 8.51, N 4.76.

The foregoing product (5.22 g) in MeOH (100 ml) and 10% Pd/C (2.0 g) were stirred under H_2 for 16 h. The mixture was filtered through *Celite* and evaporated, the residue partitioned between CH_2Cl_2 and H_2O, and the aq. phase evaporated: **10** (2.84 g, 76%). Colourless crystals. Recrystallization from MeOH afforded crystals for X-ray analysis (*Fig. 3*). M.p. > 230°. $[\alpha]_D^{25} = +71.1$ ($c = 1.0$, MeOH). IR (KBr): 3045*w* (br.), 2400*m* (br.), 1738*s*, 1625*s*. ^{1}H-NMR (300 MHz, D_2O): 1.22, 1.26 (2*d*, $J = 7.5, 7.5$, 2 H); 1.74–1.90 (*m*, 4 H); 1.93–2.06 (*m*, 1 H); 2.18–2.27 (*m*, 1 H); 3.20–3.36 (*m*, 2 H); 3.63 (*s*, 3 H). ^{13}C-NMR (50 MHz, D_2O): 12.6; 19.0; 23.6; 25.1; 32.3; 45.7; 53.0; 74.5; 174.0; 175.8. CI-MS (NH_3): 214.2 (100, $[M + H^+]$). Anal. calc. for $C_{10}H_{15}NO_4$ (281.35): C 56.33, H 7.09, N 6.57; found: C 56.03, H 7.10, N 6.41.

tert-*Butyl (3R,6R)- and (3S,6R)-3-*{N,N'-*Bis[(*tert-*butyl)oxycarbonyl]hydrazino*}*-2-oxo-1-azabicyclo-[4.3.0]nonane-6-carboxylate* (**11a** and **11b**, resp.). To a soln. of LDA (9.3 mmol) in THF (28 ml) and hexane (6 ml) was added **9** (2.02 g, 8.4 mmol) in THF (28 ml) at −78°. After 30 min, a soln. of di(*tert*-butyl) azodicarboxylate (2.53 g, 11.0 mmol) in THF (28 ml) was added with stirring at −78°. Then AcOH (1.34 ml) was added, the mixture warmed to 21° and extracted with Et_2O/0.02M aq. phosphate buffer (pH 7), and the org. phase washed with 5% aq. $NaHCO_3$ soln., dried ($MgSO_4$), and evaporated. FC (hexane/EtOAc 40:60) gave **11a**/**11b** 1:9 (3.47 g, 88%). This mixture in THF (40 ml) was added to a soln. of LDA (15.5 mmol) in THF (40 ml) and hexane (8 ml) at −78°. After 30 min, pivalic acid (3.77 g, 36.9 mmol) in THF (40 ml) was added, the mixture warmed to 21° and extracted with Et_2O/0.02M aq. phosphate buffer (pH 7), and the org. phase washed with 5% aq. $NaHCO_3$ soln., dried ($MgSO_4$), and evaporated. FC (hexane/EtOAc 7:3) gave **11b** and more polar **11a** (2:3 ratio).

11a: Colourless oil (1.40 g, 35%). $[\alpha]_D^{25} = -10.6$ ($c = 0.5$, CH_2Cl_2). IR ($CHCl_3$): 3380*w*, 1720*s*, 1650*s*. ^{1}H-NMR (300 MHz, $CDCl_3$): 1.37, 1.38, 1.40 (3*s*, 27 H); 1.55–2.52 (*m*, 8 H); 3.43–3.59 (*m*, 2 H); 4.6, 4.8 (2 br. *s*, 1 H); 6.0, 6.4 (2 br. *s*, 1 H). ^{13}C-NMR (50 MHz, $CDCl_3$): 20.7; 23.3; 27.7; 28.0; 31.1; 37.7; 44.8; 58.2; 70.1; 80.6; 81.4; 82.2; 155.3; 155.5; 166.0; 172.3. CI-MS (NH_3): 470.5 (100, $[M + H]^+$), 414.4 (27, $[M + H - 1$-methylpropene$]^+$).

11b: Colourless oil (2.07 g, 52%). $[\alpha]_D^{25} = -14.4$ ($c = 0.5$, CH_2Cl_2). IR (CH_2Cl_2): 3380*w*, 1730*s*, 1655*s*. ^{1}H-NMR (300 MHz, $CDCl_3$): 1.42, 1.44 (2*s*, 27 H); 1.57–2.10 (*m*, 6 H); 2.34–2.46 (*m*, 2 H); 3.46–3.70 (*m*, 2 H); 4.5, 4.8 (2 br. *s*, 1 H); 6.2, 6.6 (2 br. *s*, 1 H). ^{13}C-NMR (50 MHz, $CDCl_3$): 21.4; 23.1; 27.7; 28.0; 30.5; 38.5; 45.5; 55.5; 69.1; 80.5; 81.2; 82.3; 155.2; 155.4; 167.3; 171.9. CI-MS (NH_3): 470.2 (100 $[M + H]^+$), 414.2 (43, $[M + H - 1$-methylpropene$]^+$). Anal. calc. for $C_{23}H_{39}N_3O_7$ (469.58): C 58.83, H 8.37, N 8.95; found: C 58.55, H 8.27, N 9.00.

(3R,6R)-3-Amino-2-oxo-1-azabicyclo[4.3.0]nonane-6-carboxylic Acid–Trifluoroacetic Acid (**12a**). For 1.5 h, **11a** (266 mg, 0.57 mmol) was stirred in CF_3COOH (20 ml) and CH_2Cl_2 (4 ml). After evaporation, the oil was redissolved in H_2O and lyophilized. After HPLC (5–50% *B* in *A*), *(3R,6R)-3-hydrazino-2-oxo-1-azabicyclo-[4.3.0]nonane-6-carboxylic acid–trifluoroacetic acid* was obtained (156 mg). $[\alpha]_D^{25} = -5.4$ ($c = 0.7$, H_2O). ^{1}H-NMR (300 MHz, D_2O): 1.36–1.49 (*m*, 1 H); 1.52–1.86 (*m*, 4 H); 2.02–2.08 (*m*, 1 H); 2.29–2.33 (*m*, 1 H); 2.41–2.46 (*m*, 1 H); 3.31–3.47 (*m*, 2 H); 3.64 (*dd*, $J = 11.2$, 6.6, 1 H). ^{13}C-NMR (50 MHz, D_2O): 20.5; 23.0; 29.6; 36.9; 45.6; 56.6; 70.6; 169.6; 176.9. ES-MS: 214.0 ($[M + H]^+$).

The foregoing product (318 mg, 1.0 mmol) was stirred with PtO_2 (100 mg) in H_2O (20 ml) under H_2 for 11 h. The soln. was then filtered through *Celite* and lyophilized. HPLC (5–30% *B* in *A*) gave **12a** (244 mg, 68% from **11a**). White powder. $[\alpha]_D^{25} = -23.5$ ($c = 0.5$, H_2O). IR (KBr): 3160*m*, 1705*s*, 1655*s*. ^{1}H-NMR (300 MHz, D_2O): 1.57–1.90 (*m*, 5 H); 2.13–2.20 (*m*, 1 H); 2.29–2.35 (*m*, 1 H); 2.45–2.50 (*m*, 1 H); 3.31–3.48 (*m*, 2 H); 3.84 (*dd*, $J = 11.5$, 6.7, 1 H). ^{13}C-NMR (50 MHz, D_2O): 20.6; 23.6; 29.4; 36.9; 45.8; 49.9; 70.9; 166.0; 176.7. CI-MS (NH_3): 199.1 (18, $[M + H]^+$), 198.1 (21, M^+), 181.1 (100, $[M + H - H_2O]^+$).

(3S,6R)-3-Amino-2-oxo-1-azabicyclo[4.3.0]nonane-6-carboxylic Acid–Trifluoroacetic Acid (**12b**). As described for **12a**, with **11b** (193 mg, 0.41 mmol) and TFA. HPLC (5–50% *B* in *A*) gave *(3S,6R)-3-hydrazino-2-oxo-1-azabicyclo[4.3.0]nonane-6-carboxylic acid–trifluoroacetic acid* (112 mg). $[\alpha]_D^{25} = -44.0$ ($c = 0.8$, H_2O). ^{1}H-NMR (300 MHz, D_2O): 1.57–1.70 (*m*, 3 H); 1.74–1.86 (*m*, 2 H); 1.94–2.08 (*m*, 1 H); 2.30–2.40 (*m*, 2 H); 3.32–3.52 (*m*, 2 H); 3.58 (*dd*, $J = 8.2$, 4.8, 1 H). ^{13}C-NMR (50 MHz, D_2O): 21.1; 22.5; 29.0; 37.8; 46.2; 54.6; 69.9; 169.6; 176.8. CI-MS (NH_3): 214.1 (100, $[M + H]^+$).

The foregoing product (116 mg, 0.4 mmol) was reduced with Pt, as for **12a**. HPLC (5–30% *B* in *A*) gave **12b** (92 mg, 69% from **11b**). White powder. $[\alpha]_D^{25} = -37.6$ ($c = 0.4$, H_2O). IR (KBr): 3510*s*, 3400*m*, 2960*s* (br.), 1730*s*, 1670*s*. ^{1}H-NMR (300 MHz, D_2O): 1.59–1.93 (*m*, 5 H); 2.13–2.27 (*m*, 1 H); 2.35–2.44 (*m*, 2 H); 3.38–3.57 (*m*, 2 H); 3.84 (*dd*, $J = 9.4$, 5.7, 1 H). ^{13}C-NMR (50 MHz, D_2O): 21.3; 22.8; 28.9; 38.1; 46.5; 47.5; 69.9; 166.8; 176.7. CI-MS (NH_3): 199.0 (100, $[M + H]^+$). Anal. calc. for $C_{11}H_{15}F_3N_2O_5$ (312.25): C 42.31, H 4.84, N 8.97, F 18.25; found: C 42.17, H 5.11, N 9.20, F 18.06.

(3R,6R)-3-{[(9H-Fluoren-9-yl)methoxycarbonyl]amino}-2-oxo-1-azabicyclo[4.3.0]nonane-6-carboxylic Acid (**13**). At 0°, **12a** (593 mg, 1.9 mmol) in 1M aq. Na_2CO_3 (37 ml) and dioxane (30 ml) was treated with Fmoc-Cl (0.78 g, 3.0 mmol) in dioxane (19 ml) and then stirred at 21° for 18 h. Et_2O was added, the mixture washed with 1M aq. Na_2CO_3, the aq. phase acidified with conc. aq. HCl soln. and extracted with AcOEt, and the org. phase dried ($MgSO_4$), stirred with active charcoal, filtered through *Celite*, and evaporated: **13** (496 mg, 61%). Colourless solid. M.p. (dec.) > 220°. $[\alpha]_D^{25} = -10.1$ ($c = 0.9$, DMSO). IR (KBr): 3330*m*, 1712*s*, 1600*s*. ^{1}H-NMR (300 MHz, (D_6)DMSO, 362 K): 1.62–2.02 (*m*, 6 H); 2.27–2.50 (*m*, 2 H); 3.30–3.38 (*m*, 1 H); 3.52–3.62 (*m*, 1 H); 3.94–4.04 (*m*, 1 H); 4.20–4.34 (*m*, 3 H); 7.02 (*d*, $J = 7.5$, 1 H); 7.31 (*t*, $J = 7.4$, 2 H); 7.40 (*t*, $J = 7.3$, 2 H); 7.70 (*d*, $J = 7.7$, 2 H); 7.84 (*d*, $J = 7.5$, 2 H). ^{13}C-NMR (50 MHz, (D_6) DMSO): 20.5; 26.3; 30.5; 37.2; 45.0; 46.7; 51.2; 65.8; 69.1; 120.1; 125.4; 127.0; 127.6; 140.7; 143.9; 156.3; 167.2; 174.7. Anal. calc. for $C_{24}H_{24}N_2O_5$ (420.47): C 68.56, H 5.75, N 6.66; found: C 68.33, H 5.94, N 6.90.

(3S,6R)-3-{[(9H-Fluoren-9-yl)methoxycarbonyl]amino}-2-oxo-1-azabicyclo[4.3.0]nonane-6-carboxylic Acid (**14**). As described for **13**, from **12b** (1.36 g, 4.4 mmol): yellow oil (1.38 g, 75%). $[\alpha]_D^{25} = -23.1$ ($c = 0.7$, MeOH). IR (KBr): 3060–3040*w*, 2950–2880*m*, 2500*w* (br.), 1710*s*, 1640*s*. ^{1}H-NMR (300 MHz, (D_6)DMSO, 363 K): 1.62–2.04 (*m*, 6 H); 2.27–2.42 (*m*, 2 H); 3.28–3.36 (*m*, 1 H); 3.57–3.66 (*m*, 1 H); 3.94 (*q*, $J = 7.4$, 1 H); 4.23 (*t*, $J = 6.7$, 1 H); 4.33 (*d*, $J = 6.7$, 2 H); 6.92 (*d*, $J = 7.0$, 1 H); 7.31 (*t*, $J = 7.4$, 2 H); 7.40 (*t*, $J = 7.1$, 2 H); 7.67 (*d*, $J = 7.4$, 2 H); 7.84 (*d*, $J = 7.5$, 2 H). ^{13}C-NMR (50 MHz, CD_3OD): 22.8; 28.1; 30.9; 40.1; 47.6; 49.0; 50.1; 68.6; 75.0; 121.2; 126.4; 128.4; 129.1; 122.0; 127.3; 129.9; 143.1; 146.0.

(1R,7R)-5,8-Diazatricyclo[5.2.2.0^{1,5}]undecane-6,9-dione (**15**). $SOCl_2$ (0.29 ml, 4.0 mmol) was added dropwise two **12a** (126.0 mg, 0.40 mmol) in dry MeOH (1 ml) at 0°. The mixture was stirred at r.t. for 1 h, then refluxed for 5 h. The solvent was evaporated, the residue evaporated twice from dry benzene, dissolved in AcOEt, and extracted with dil. aq. HCl soln. The aq. phase was made basic (pH 9–10) with 2N Na_2CO_3 and extracted with AcOEt and the aq. phase evaporated. HPLC (5–25% *B* in *A*) gave **15** (73 mg, 100%). White powder. IR (KBr): 3240*m* (br.), 1680*s*, 1465. ^{1}H-NMR (300 MHz, D_2O): 1.66–1.67 (*m*, 7 H); 2.31–2.40 (*m*, 1 H); 3.16–3.35 (*m*, 2 H); 3.95–3.97 (*m*, 1 H). CI-MS (NH_3): 198.3 (44, $[M + NH_4]^+$), 181.3 (100, $[M + H]^+$).

tert-*Butyl (3R,6R)-3-Amino-2-oxo-1-azabicyclo[4.3.0]nonane-6-carboxylate* (**16a**). At 0° **12a** (655 mg, 2.1 mmol) in 2N aq. NaOH (8 ml) and EtOH (6.8 ml) was treated dropwise with benzyl chloroformate (716 mg, 4.2 mmol). After 1 h at r.t., more 2N aq. NaOH (4 ml), EtOH (6.8 ml), and benzyl chloroformate (716 mg, 4.2 mmol) were added. After 2 h, the mixture was extracted with CH_2Cl_2, the aq. phase acidified with conc. aq. HCl soln. and extracted with CH_2Cl_2, and the org. phase dried ($MgSO_4$) and evaporated: *(3R,6R)-3-[(benzyl-*

oxy)carbonylamino]-2-oxo-1-azabicyclo[4.3.0]nonane-6-carboxylic acid (571 mg, 80%). Colourless solid. M.p. 188–189°. ^{1}H-NMR (300 MHz, CD_3OD): 1.64–2.16 (*m*, 6 H); 2.35–2.47 (*m*, 1 H) and 2.53–2.57 (*m*, 1 H); 3.41–3.65 (*m*, 2 H); 4.19 (*dd*, *J* = 11.0, 6.7, 1 H); 5.09 (*s*, 2 H); 7.24–7.38 (*m*, 5 H). CI-MS (NH_3): 333.2 (100, $[M + H]^+$).

The foregoing product (571 mg, 1.7 mmol) in CH_2Cl_2 (9.5 ml), 2-methylprop-1-ene (24 ml, 240 mmol), and 10 drops of conc. H_2SO_4 were stirred under pressure at r.t. for 40 h. The excess 2-methylprop-1-ene was evaporated, the H_2SO_4 neutralized with 5% aq. $NaHCO_3$ soln., the basic soln. extracted with CH_2Cl_2, and the org. phase dried ($MgSO_4$) and evaporated. FC (AcOEt) gave tert-*butyl (3R,6R)-3-[(benzyloxy)carbonylamino-2-oxo-1-azabicyclo[4.3.0]nonane-6-carboxylate* (620 mg, 93%). Colourless oil. ^{1}H-NMR (300 MHz, $CDCl_3$): 1.43 (*s*, 9 H); 1.22–2.07 (*m*, 5 H); 2.34–2.50 (*m*, 3 H); 3.37–3.64 (*m*, 2 H); 4.07 (*dt*, *J* = 5.6, 11.2, 1 H); 5.09 (*s*, 2 H); 5.49 (br. *s*, 1 H); 7.26–7.36 (*m*, 5 H). ^{13}C-NMR (75 MHz, $CDCl_3$): 20.6; 26.5; 27.7; 30.5; 37.4; 44.8; 52.0; 66.6; 70.3; 82.3; 127.9; 128.3; 136.3; 156.5; 167.3; 172.0. CI-MS (NH_3): 389.2 (100, $[M + H]^+$).

The foregoing product (598 mg, 1.5 mmol) in MeOH (20 ml) was stirred with 10% Pd/C (165 mg) for 1 h under H_2. The soln. was filtered through *Celite* and evaporated: **16a** (375 mg, 96%). Colourless oil. IR (CH_2Cl_2): 3690*w*, 1733*s*, 1645*s*. ^{1}H-NMR (300 MHz, $CDCl_3$): 1.41 (*s*, 9 H); 1.48–1.96 (*m*, 5 H); 2.10–2.19 (*m*, 1 H); 2.30–2.48 (*m*, 2 H); 3.18 (*dd*, *J* = 11.1, 6.8, 1 H); 3.40–3.61 (*m*, 2 H). ^{13}C-NMR (50 MHz, $CDCl_3$): 20.7; 27.6; 27.9; 31.0; 37.5; 44.5; 51.5; 70.2; 82.0; 171.4; 172.1. CI-MS (NH_3): 255.3 (43, $[M + H]^+$), 153.1 (100, $[M - CO_2{}^t\text{Bu}]^+$).

tert-*Butyl (3S,6R)-3-Amino-2-oxo-1-azabicyclo[4.3.0]nonane-6-carboxylate* (**16b**). As described for **16a**, with **12b**: *(3S,6R)-3-[(benzyloxy)carbonylamino]-2-oxo-1-azabicyclo[4.3.0]nonane-6-carboxylic acid.* Colourless oil. ^{1}H-NMR (300 MHz, $CDCl_3$): 1.57–1.87 (*m*, 5 H); 2.40–2.56 (*m*, 3 H); 3.47–3.59 (*m*, 1 H); 3.64–3.73 (*m*, 1 H); 4.08–4.13 (*m*, 1 H); 5.08 (*s*, 2 H); 6.13 (*d*, *J* = 5.2, 1 H); 6.36 (br. *s*, 1 H); 7.27–7.46 (*m*, 5 H). CI-MS (NH_3): 333.2 (100, $[M + H]^+$).

This was converted into tert-*butyl (3S,6R)-3-[(benzyloxy)carbonylamino]-2-oxo-1-azabicyclo[4.3.0]-nonane-6-carboxylate.* Colourless oil. ^{1}H-NMR (300 MHz, $CDCl_3$): 1.42 (*s*, 9 H); 1.51–1.91 (*m*, 5 H); 2.35–2.44 (*m*, 3 H); 3.44–3.52 (*m*, 1 H); 3.62–3.71 (*m*, 1 H); 3.96–4.00 (*m*, 1 H); 5.06 (*s*, 2 H); 5.90 (br. *s*, 1 H); 7.22–7.31 (*m*, 5 H). CI-MS (NH_3): 389.2 (100, $[M + H]^+$).

The foregoing product was converted into **16b**. Colourless oil. ^{1}H-NMR (300 MHz, $CDCl_3$): 1.43 (*s*, 9 H); 1.51–2.09 (*m*, 8 H); 2.31–2.43 (*m*, 2 H); 3.36 (*dd*, *J* = 7.3, 4.9, 1 H); 3.45–3.53 (*m*, 1 H); 3.63–3.73 (*m*, 1 H). ^{13}C-NMR (50 MHz, $CDCl_3$): 21.0; 27.2; 27.6; 29.0; 38.3; 45.1; 48.9; 69.3; 81.9; 171.5; 172.1. CI-MS (NH_3): 255.2 (100, $[M + H]^+$).

(3S,6R)-3-Amino-3-benzyl-2-oxo-1-azabicyclo[4.3.0]nonane-6-carboxylic Acid–Trifluoroacetic Acid (**19**). At r.t., **16a** (294 mg, 1.16 mmol) in dry CH_2Cl_2 (7 ml) was stirred with 4 Å molecular sieve (150 mg), benzaldehyde (129 µl, 1.27 mmol), and Et_3N (1.78 µl, 1.27 mmol). After 1.5 h and again at 2.5 h benzaldehyde (33 µl, 0.32 mmol) was added. After 3 h, the mixture was filtered, evaporated, and dried *in vacuo* to give tert-*butyl (3R,6R)-3-(benzylideneamino)-2-oxo-1-azabicyclo[4.3.0]nonane-6-carboxlate* (**17**). This was dissolved in dry THF (10 ml), and 1.5M LDA in cyclohexane (1.16 ml, 1.74 mmol) was added at −78°. After 30 min, benzyl bromide (355 mg, 2.08 mmol) in THF (3 ml) was added and the mixture allowed to warm to r.t. overnight. Then 0.02M aq. phosphate buffer (pH 7) was added, the mixture extracted with Et_2O, and the org. phase dried ($MgSO_4$) and evaporated: tert-*butyl (3S,6R)-3-benzyl-3-(benzylideneamino)-2-oxo-1-azabicyclo[4.3.0]nonane-6-carboxylate* (**18**). This was treated directly with CF_3COOH (26 ml) in H_2O (6 ml). for 2 h. The solvent was evaporated and the residue stirred in CF_3COOH (26 ml) and CH_2Cl_2 (6 ml) for 4 h. The soln. was then evaporated, H_2O added, and the soln. lyophilized. HPLC (5–40% *B* in *A*) afforded **19** (386 mg, 83%). White powder. $[\alpha]_D^{25} = -15.3$ (*c* = 1.0, H_2O). ^{1}H-NMR (300 MHz, D_2O): 1.14–1.37 (*m*, 2 H); 1.58–1.83 (*m*, 3 H); 2.11–2.23 (*m*, 3 H); 2.93 (*d*, *J* = 13.6, 1 H); 3.25 (*d*, *J* = 13.6, 1 H); 3.39–3.45 (*m*, 2 H); 7.05–7.08 (*m*, 2 H); 7.24–7.28 (*m*, 3 H). ^{13}C-NMR (75 MHz, $CDCl_3$): 20.6; 28.8; 29.2; 36.9; 44.4; 45.4; 58.3; 70.2; 128.1; 128.8; 129.8; 133.6; 167.0; 176.1. CI-MS (NH_3): 288.2 (26, M^+), 271.2 (100, $[M + H - H_2O]^+$).

The reaction sequence described above was also performed starting with **16b**.

(3S,6R)-3-Benzyl-3-{[(9H-fluoren-9-yl)methoxycarbonyl]amino}-2-oxo-1-azabicyclo[4.3.0]nonane-6-carboxylic Acid (**20**). At 0°, **19** (150 mg, 0.37 mmol) in 1M aq. Na_2CO_3 (7.5 ml) and dioxane (6.3 ml) was treated with Fmoc-Cl (154 mg, 0.6 mmol) in dioxane (3.7 ml) and then stirred at r.t. for 19 h. The mixture was extracted with Et_2O and the aq. phase acidified with conc. aq. HCl soln. and extracted again with Et_2O. The org. phase was dried ($MgSO_4$) and evaporated: **20** (117 mg, 61%). Colourless oil. $[\alpha]_D^{25} = -37.5$ (*c* = 0.5, CH_2Cl_2). IR (CH_2Cl_2): 3435*m*, 3070*w*, 3030*w*, 2950*m*, 2900*w*, 2750*w* (br.), 1740*s*, 1650*s*, 1610*s*. ^{1}H-NMR (300 MHz, $CDCl_3$): 0.85–0.88 (*m*, 1 H); 1.09–1.27 (*m*, 1 H); 1.75–1.89 (*m*, 2 H); 2.04–2.32 (*m*, 4 H); 2.82–2.92 (*m*, 1 H); 2.30–2.38 (*m*, 1 H); 3.61–3.64 (*m*, 2 H); 4.11–4.25 (*m*, 3 H); 6.06 (*s*, 1 H); 7.10–7.73 (*m*, 13 H); 9.57 (*s*, 1 H). ^{13}C-NMR (75 MHz, $CDCl_3$): 20.6; 28.8; 29.7; 38.4; 44.7; 46.2; 46.9; 58.6; 67.2; 69.6; 119.9; 125.1; 127.0; 127.5; 127.7; 128.6; 129.9; 135.2; 141.2; 143.4; 143.9; 155.5; 170.4; 174.9.

(1R,7S)-7-Benzyl-5,8-diazatricyclo[5.2.2.0^{1,5}]undecane-6,9-dione (**21**). At 0°, **19** (62 mg, 0.15 mmol) in 2N aq. NaOH (0.35 ml) and EtOH (0.30 ml) were treated with benzyl chloroformate (36 mg, 0.21 mmol) and then stirred at r.t. After 1.5 h, additional 2N aq. NaOH (0.17 ml), EtOH (0.30 ml), and benzyl chloroformate (36 mg) were added. After 4 h, the mixture was extracted wtih CH_2Cl_2 the org. phase dried ($MgSO_4$) and evaporated, and the residue in MeOH (4 ml) treated with a soln. of CH_2N_2 in Et_2O until the yellow colour persisted. Excess CH_2N_2 was quenched with dil. AcOH in AcOEt and the soln. evaporated. The residue in MeOH (4 ml) was stirred with 10% Pd/C (32 mg) for 2 h under H_2. The soln. was filtered through *Celite* and evaporated. HPLC (5–40% *B* in *A*) gave **21** (13.0 mg, 31%). White powder. IR (KBr): 3240*m*, 1695*s*, 1670*s*. ^{1}H-NMR (300 MHz, $CDCl_3$): 1.72–2.02 (*m*, 7 H); 2.67 (*dt*, *J* = 13.4, 6.7, 1 H); 3.03 (*d*, *J* = 14.5, 1 H); 3.42 (*d*, *J* = 14.5, 1 H); 3.44–3.56 (*m*, 2 H); 5.90 (*s*, 1 H); 7.25–7.35 (*m*, 5 H). ^{13}C-NMR (75 MHz, $CDCl_3$): 24.4; 29.0; 30.2; 31.6; 36.9; 44.0; 61.2; 67.1; 127.4; 128.9; 130.4; 134.9; 169.2; 172.7. CI-MS (NH_3): 288.1 (70, $[M + NH_4]^+$), 271.1 (100, $[M + H]^+$).

*Cyclo(-Phe-Pro-Phe-*D*-Trp-Lys-Thr-)* (**22**). Fmoc Thr(^{t}Bu)-OH coupled to 2-methoxy-4-alkoxybenzyl alcohol (*Sasrin*) [17] resin (*Bachem*; 417 mg, 0.25 mmol) was used to initiate synthesis with Fmoc-Lys(Boc)-OH, Fmoc-D-Trp(Boc)-OH, Fmoc-Phe-OH, and Fmoc-Pro-OH (each 1 mmol) for chain elongation, and BtOH/HBTU for activation [18]. The completion of each coupling cycle was monitored by ninhydrin [29] or isatin [30] tests. Upon completion of the synthesis, the N-terminal Fmoc-protecting group was removed with 20% piperidine in NMP and the resin washed with MeOH, dried, and reswollen in CH_2Cl_2 for 10 min. The peptide was cleaved from the resin with 1% CF_3COOH/CH_2Cl_2. After 2 min, the soln. was filtered and quenched in pyridine (1.2 ml)/MeOH (12 ml). Evaporation and HPLC gave the side chain protected peptide H-Phe-Pro-Phe-D-Trp(Boc)-Lys(Boc)-Thr(^{t}Bu)-OH (185 mg, 62%). ES-MS: 1104.3 ($[M + Na]^+$), 1082.4 ($[M + H]^+$).

To the foregoing product (78 mg, 70 µmol) in MeCN (92 ml) was added BtOH (29 mg, 220 µmol), TPTU (64 mg, 220 µmol), and ^{i}Pr$_2$EtN (722 mg, 5.6 mmol) in MeCN (8 ml). After 17 h at r.t., the soln. was evaporated, the residue taken up in Et_2O, and the soln. washed with 1N aq. Na_2CO_3 and then dil. HCl soln., dried ($MgSO_4$), and evaporated. HPLC (30–75% *B* in *A*) gave cyclo(-Phe-Pro-Phe-D-Trp(Boc)-Lys(Boc)-Thr(^{t}Bu)-) (45 mg, 65%). ES-MS: 1086.2 ($[M + Na]^+$), 1063.1 ($[M]^+$).

The foregoing product (45 mg) was stirred in CF_3COOH/phenol/H_2O/thioanisole/ethanedithiol 82.5:5.0:5.0:5.0:2.5 at r.t. for 1.5 h. The soln. was evaporated and the residue taken up in H_2O and washed with Et_2O. After HPLC (5–50% *B* in *A*), the aq. phase afforded **22** (24 mg, 61%). ^{1}H-NMR (600 MHz, D_2O/H_2O 1:9, pH 4.5, 278 K): *Table 5*. ES-MS: 808.0 ($[M + H]^+$).

*Cyclo(-Phe=Pro-Phe-*D*-Trp-Lys-Thr-)* (**3**; Phe=Pro = CH_2–CH_2 bridged Phe-Pro). The methods described for **22** were applied except that **20** (128 mg, 0.25 mmol) was incorporated instead of the corresponding Phe and Pro. Solid-phase peptide synthesis, cleavage from the resin, and HPLC (30–75% *B*) gave H-**19**-Phe-D-Trp(Boc)-Lys(Boc)-Thr(^{t}Bu)-OH (148 mg, 48%). ES-MS: 1108.9 ($[M + H]^+$).

Table 5. *^{1}H-NMR* (600 MHz) *Chemical Shifts* [ppm] *of* **22**. D_2O/H_2O 1:9, pH 4.5, 278 K.

Residue	NH	H–C(α)	H–C(β)	H–C(γ)	H–C(δ)	Others
trans-Phe-Pro isomer						
Pro^6	–	3.16	1.44, 1.56	0.63, 0.95	3.20, 3.41	–
Phe^7	8.14	5.00	2.95	–	–	7.09, 7.27, 7.29 (arom. H)
Trp^8	8.64	4.43	2.75, 2.98	–	–	10.23 (NH); 7.12, 7.14, 7.20, 7.44, 7.53 (arom. H)
Lys^9	8.46	3.63	1.27, 1.57	0.22, 0.42	1.27	2.64 ($CH_2(\varepsilon)$); 7.46 (NH_3–C(ε))
Thr^{10}	7.44	4.35	4.07	1.11	–	–
Phe^{11}	8.50	4.05	2.75, 2.95	–	–	7.11, 7.19, 7.32 (arom. H)
cis-Phe-Pro isomer						
Pro^6	–	3.88	1.72	1.45	3.29, 3.47	–
Phe^7	7.21	4.75	3.84, 3.01	–	–	6.81, 7.14, 7.17(arom. H)
Trp^8	8.78	4.42	2.96, 3.14	–	–	10.23 (NH); 7.15, 7.20, 7.20, 7.44, 7.62 (arom. H)
Lys^9	8.39	4.13	1.16, 1.54	0.47, 0.67	1.35	2.64, 2.72($CH_2(\varepsilon)$); 7.49 (NH_3–C(ε))
Thr^{10}	7.60	4.40	3.78	0.72	–	–
Phe^{11}	8.58	4.49	2.80, 2.95	–	–	7.12, 7.23 7.30 (arom. H)

The foregoing product in MeCN (200 ml) was stirred with BtOH (58 mg, 0.43 mmol), TPTU (128 mg, 0.43 mmol), and $^iPr_2EtN$ (1.92 ml, 11.2 mmol) at r.t. for 21 h. After evaporation, the residue was taken up in Et_2O and the soln. washed with 1N aq. Na_2CO_3 and then dil. HCl soln., dried ($MgSO_4$), and evaporated. After HPLC (45–85% *B* in *A*), the intermediate was stirred in CF_3COOH/phenol/H_2O/thioanisole/ethanedithiol 82.5:5:5:5:2.5 for 1.5 h. The soln. was then evaporated and the residue extracted with Et_2O/H_2O. The aq. phase, after HPLC (20–30% *B* in *A*), gave **3** (10.4 mg, 9%). ^{1}H-NMR (600 MHz, D_2O/H_2O 1:9, pH 4.5, 278 K): *Table 1*. ES-MS: 834.1 ($[M + H]^+$).

X-Ray Structure Analysis of **10**. $C_{10}H_{15}NO_4$. Monoclinic, $P2_1$ (# 4); $a = 9.001(1)$, $b = 5.725(1)$, $c = 10.680(1)$ Å; $D = 1.32$ g/cm^3, $Z = 2$; $\mu(MoK_\alpha) = 0.956$ cm^{-1}. Data were collected on a *Nicolet-R3* diffractometer. Temp. 213 K; *Wyckoff* ω scans; scan speed 29.3° to 2.5°/min; scan witdth 0.75°; max. 2θ 60°; 2320 reflections measured, 1983 unique ($R_{int} = 0.059$); full-matrix least-squares refinement with 1677 observed reflections ($I > 3\sigma(I)$) and 213 variables; residuals $R = 0.0.438$, $R_w = 0.0500$. The structure was determined by direct methods with anisotropic refinement of all non-H-atoms.

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