111. Synthesis of a Novel Tricyclic Dipeptide Template and Its Incorporation into a Cyclic Peptide Mimetic Containing an NPNA Motif

by **Marc E. Pfeifer**, **Anthony Linden**, and **John A. Robinson***

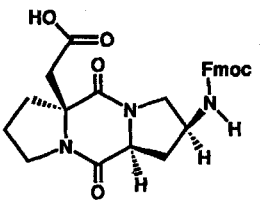
Institute of Organic Chemistry, University of Zurich, Winterthurerstrasse 190, CH-8057 Zurich

(8. IV. 97)

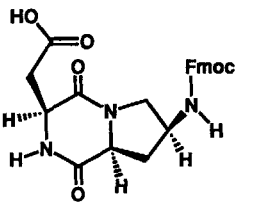
A novel tricyclic dipeptide template, formally derived by coupling (2*S*,4*S*)-4-aminoproline (Pro(NH_2)) and (*S*)-2-(carboxymethyl)proline (Pro(CH_2COOH)) as a diketopiperazine, has been synthesized in a form suitable for solid-phase peptide synthesis using Fmoc chemistry. This template was incorporated into the cyclic molecule cyclo(-Ala[1]-Asn[2]-Pro[3]-Asn[4]-Ala[5]-Ala[6]-Temp-) (Temp = template), whose conformation in H_2O was studied by NMR methods. Average solution structures derived by restrained simulated annealing point to a highly populated βI-turn within the Asn-Pro-Asn-Ala motif and also indicate which conformations are likely to be preferred by the template.

1. Introduction. – Organic templates are currently of interest in the design of small-molecule peptide and protein mimetics. They are also of interest as scaffolds upon which a variety of pharmacophore groups may be attached in a combinatorial-chemistry approach to ligand discovery. In our previous work [1–3], readily available *trans*-4-hydroxy-L-proline (Pro(OH)) [4] has been used as a building block for the design of novel diketopiperazine-based templates. Such cyclic dipeptides derived from Pro(OH) provide convenient access to molecules of defined geometry, that are relatively rigid and readily functionalized. Here, we describe the synthesis of the tricyclic template **1**, with an (9*H*-fluoren-9-yl)methoxycarbonyl (Fmoc)-protected amino and a free carboxy group, to which a peptide chain may be attached by solid-phase peptide synthesis. This template is closely related to **2**, described in earlier work [1], but includes an additional pyrrolidine ring, which may influence the conformation of loops attached to the template, and also provides an extended C-backbone for incorporating new functional groups. Here, **1** was used to synthesize the cyclic peptide **3**, containing the Asn-Pro-Asn-Ala (NPNA) motif, and the preferred conformation of **3** in aqueous solution was studied by NMR methods and compared to that deduced for **4** in earlier work.

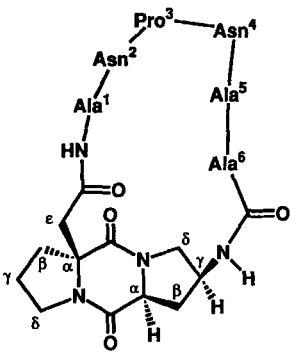
2. Results and Discussion. – 2.1. *Synthesis.* For the synthesis of **1**, (benzyloxy)carbonyl (Z)-protected proline methyl ester was alkylated non-stereoselectively and the resulting methyl ester saponified to afford (±)-**5** (*Scheme 1*). After coupling to (2*S*,4*R*)-Pro(OH) methyl ester the diastereoisomeric dipeptides **6** and **7** could be conveniently separated by column chromatography. The enantiomerically pure (*S*)-configurated ester (–)-**5** (and the corresponding diacid (–)-**9**) was also prepared from (*S*)-2-allylproline (**8**) [5] (*Scheme 2*) and used for the synthesis of **7**. This stereoselective route, however, proved less convenient for the production of **7** on a large scale (> 0.1 mol), but allowed an unambiguous assignment of the configuration of both dipeptides **6** and **7** derived from (±)-**5** (see *Exper. Part*). This configurational assignment was confirmed after formation



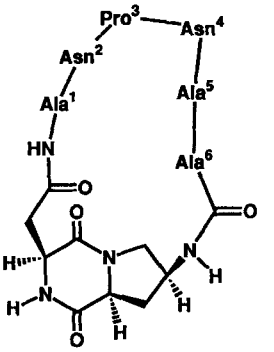
1



2



3

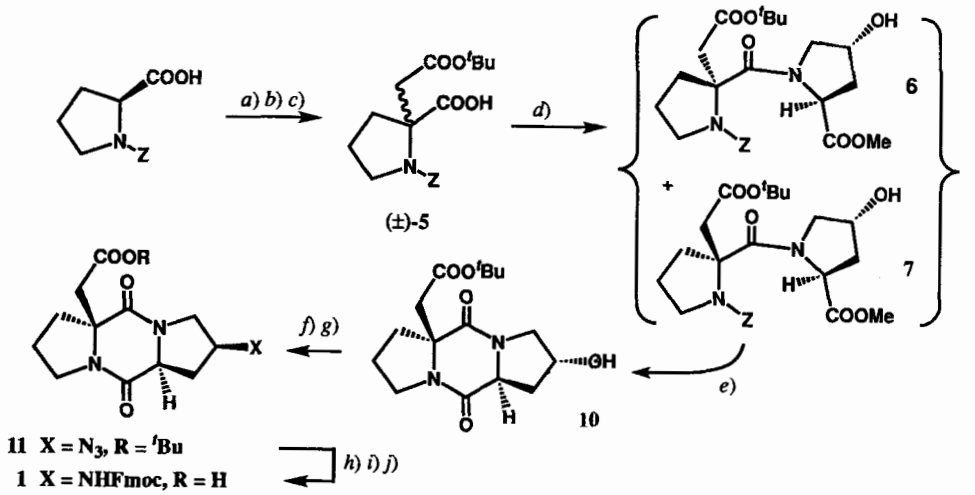


4

of azide **11**, whose structure was solved by X-ray crystallography (*Fig. 1*). This proved all the more important, since we observed that removal of the Z-protecting group from **7** in MeOH as solvent (step *e* in *Scheme 1*) led to an epimerization at C(α) in the Pro(OH) building block, and hence to the isolation also of the *cis*-diastereoisomer of **10** (*cis*/*trans* 3:1). It is not known whether the epimerization occurs before or after ring closure. This problem did not arise using AcOEt as solvent for the hydrogenolysis. Similarly, reduction of azide **11** in MeOH (step *h* in *Scheme 1*) or addition of the Fmoc group (step *i* in *Scheme 1*) using Fmoc-Cl in aqueous Na_2CO_3 solution dioxane, gave predominantly a *cis*-diastereoisomer of **1**, epimeric at C(α) in the Pro(OH)-derived unit. Using the conditions shown in *Scheme 1*, the epimerization was avoided. These observations are consistent with earlier studies, which established that *cis*-configurated cyclo(-Pro-Pro-) and cyclo(-Pro(OH)-Pro(OH)-) are more stable thermodynamically than their *trans*-isomers [6–9]. In all cases, the epimers could be easily distinguished by their consistently different and characteristic ^{1}H-NMR chemical shifts (particularly for H–C(α) in the Pro(NH_2) unit and the methylene protons of the carboxymethyl substituent), and their relative configurations were clearly indicated by steady-state 1D-NOE difference experiments (see *Exper. Part*). Fortunately, the Fmoc group could be removed from the *tert*-butyl ester of **1** using piperidine in DMF without affecting the configuration of the molecule (no epimer was detected by HPLC analysis of the product), thus allowing the use of **1** in solid-phase peptide synthesis.

The cyclic peptide **3** was prepared following the route shown in *Scheme 3*. The linear peptide **12** was constructed on the solid-phase using Fmoc chemistry, and isolated after

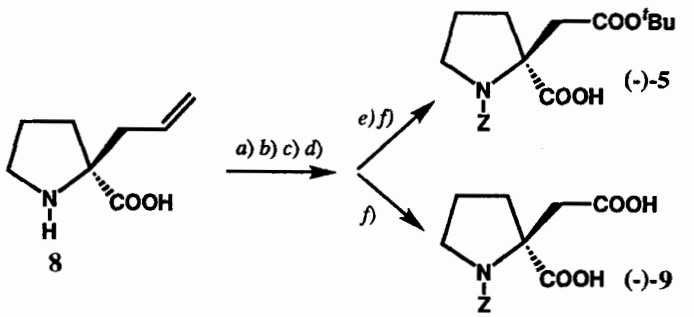
Scheme 1[a])



a) $SOCl_2$, MeOH; 100%. *b*) LDA (1.3 equiv.), −78°, then $BrCH_2COO^tBu$ (1.2 equiv.); 80%. *c*) $MeOH/H_2O$, $LiOH \cdot H_2O$ (10 equiv.); 83%. *d*) H-Pro(OH)-OMe · HCl (1.1 equiv.), $^iPr_2EtN$, HBTU, CH_2Cl_2, and separation of diastereoisomers; 42%. *e*) H_2, Pd/C, AcOEt; 83%. *f*) Tos-Cl, Py; 91%. *g*) NaN_3, DMF, 78°; 91%, *h*) H_2, Pd/C, AcOEt; 100%. *i*) Fmoc-Cl (1.4 equiv.), $^iPr_2EtN$, CH_2Cl_2; 98%. *j*) H_2O/CF_3COOH 1:5; 100%.

[a]) See *Exper. Part* for abbreviations of reagents.

Scheme 2



a) $PhCH_2OCOCl$, 1M Na_2CO_3, dioxane; 82%. *b*) $SOCl_2$, MeOH; 88%. *c*) O_3, acetone, −20°. *d*) H_2SO_4/CrO_3, acetone, 0°; 50%. *e*) 2-Methylprop-1-ene, conc. H_2SO_4 soln. *f*) $LiOH \cdot H_2O$, $MeOH/H_2O$.

reversed-phase HPLC in 40% yield. The coupling efficiency of **1** was essentially comparable to that achieved with the standard protected amino acids. Cyclization in solution also proceeded in good yield (77%). The protecting groups were then removed and the product purified by reversed-phase HPLC.

2.2. *NMR Studies.* The conformation of **3** was investigated in aqueous solution at pH 5 and 300 K using NMR methods. Under these conditions, the amide NH region of the ^{1}H-NMR spectrum reveals a major and a minor conformer (6:1), which interconvert slowly on the chemical-shift time scale. This was clearly evident from the NH region of

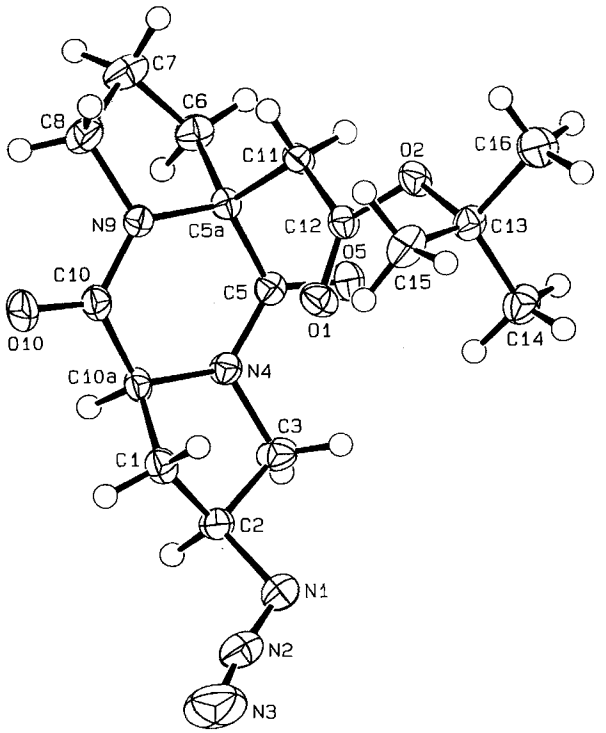
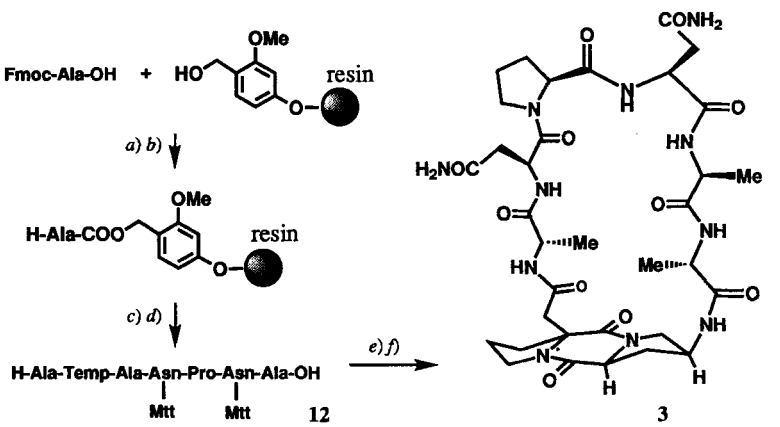


Fig. 1. *ORTEP Plot with 50% probability ellipsoids of the molecular structure of* **11**

ROESY spectra, which contained cross-peaks with the same phase as the diagonal, but of opposite sign compared to ROE cross-peaks, due to chemical exchange between the major and minor forms. Exchange cross-peaks were also evident in TOCSY spectra of **3**. A similar occurrence of major and minor forms of the cyclic peptide **4** was noted in earlier work [1]. We speculate that the two forms might correspond to *cis*/*trans* rotamers at the Asn^2-Pro^3 peptide bond. However, a full assignment of the minor form of **3** was problematic due to spectral overlap, although the peptide NH resonances are given along with assignments for the major form (see *Table 1*). The chemical shifts and line widths were invariant upon dilution over the range 0.5–20 mM.

Evidence for a stable secondary structure in **3** (major) was first obtained from the temperature coefficients of NH resonances (*Table 2*) and relative H/D exchange rates (*Fig. 2*). The temperature coefficient for the Ala^5NH is very low compared to the random-coil value for alanine [10] (*Table 2*), and this amide proton also has a relatively slow rate of exchange in D_2O (*Fig. 2*). A similar but less pronounced trend in both parameters is seen also for the Asn^4NH proton. In earlier work, stable βI-turns were identified in the NPNA motif of **4** [1] and in linear peptides containing tandemly repeated $NP^{Me}NA$ motifs (P^{Me} = (*S*)-2-methylproline) [11]. The temperature coefficient for Ala^5NH in **3** (major) is lower than that observed for the corresponding residue in **4** or the Ala residues in linear peptides containing $NP^{Me}NA$ motifs, suggesting that the βI-turn is present and more highly populated in **3**. The minor conformer of **3** has a different pattern of amide

Scheme 3[a])



a) Fmoc-Ala-OH (4 equiv.), *Tentagel-S-AC*, DCC (4 equiv.), DMAP, DMF. *b*) 20% piperidine/DMF. *c*) HBTU (3 equiv.), HOBt (3 equiv.), DMF, $^{i}Pr_2EtN$, and Fmoc-protected amino acid (3 equiv.) or **1** (2 equiv.) in each coupling step, 20% piperidine/DMF for Fmoc removal. *d*) 1% CF_3COOH/CH_2Cl_2. *e*) HATU (1.5 equiv.), HOAt (1.5 equiv.), 1% $^{i}Pr_2EtN$/DMF. *f*) CF_3COOH/H_2O/thioanisol/phenol/ethane-1,2-dithiol/$^{i}Pr_3SiH$ 80:5:5:5:2.5:3.5; 22% over all steps.

[a]) See *Exper. Part* for abbreviations of reagents.

Table 1. *^{1}H-NMR* (600 MHz) *Chemical Shifts for* **3** *at 300 K in 10% D_2O/H_2O at pH 5.0*

	Chemical shift [ppm][a])			
Residue[b])	NH	H–C(α)	$CH_2(\beta)$ or Me(β)	Others
Ala[1]	8.31 (8.07[c]))	4.26	1.33	
Asn[2]	8.27 (7.51[c]))	4.97	2.73, 3.13	7.68 (NH, (E)); 7.07 (NH, (Z))
Pro[3]	–	4.37	1.91, 2.35	2.02 ($CH_2(\gamma)$); 3.76 ($CH_2(\delta)$)
Asn[4]	8.47 (8.54[c]))	H_2O[d])	2.72, 2.89	7.66 (NH, (E)); 6.97 (NH, (Z))
Ala[5]	7.68 (8.51[c]))	4.43	1.33	
Ala[6]	8.23 (7.88[c]))	4.27	1.36	
Pro(NH_2)[7]	8.42 (7.74[c]))	4.39	1.89, 2.68[e])	4.63 (H–C(γ)); 3.77, 3.69[c]) ($CH_2(\delta)$)
Pro(CH_2COOH)[8]	–	–	2.11	2.11 ($CH_2(\gamma)$); 3.99, 3.48[e]) ($CH_2(\delta)$); 2.83, 3.00 ($CH_2(\varepsilon)$)

[a]) Chemical shifts are measured relative to internal TSP (= sodium 3-(trimethylsilyl)(D_4)propanoate).

[b]) Pro(NH_2)[7] and Pro(CH_2COOH)[8] are the 4-aminoproline and 2-(carboxymethyl)proline moieties of the template, respectively. The Pro(NH_2)[7]NH refers to NH–C(γ), and $CH_2(\varepsilon)$ of Pro(CH_2COOH)[8] refers to the CH_2 of the 2-(carboxymethyl) substituent (see **3**).

[c]) Assignment for the minor conformer.

[d]) Resonance lies under the signal of H_2O.

[e]) Stereoheterotopic H-atoms in the order *pro-R*, *pro-S*.

temperature coefficients (see *Table 2*), but the same relative rates of exchange of NH protons as seen for the major form. This reflects the different time scales of the exchange and chemical-shift data and the likely absence of the same βI-turn in the NPNA motif in the minor form. In this context, it is noteworthy that the chemical shift of

Ala^{5}NH in the major conformer is significantly upfield from the random-coil value expected under these conditions [10], whereas in the minor form, the NHs of Asn^2, Pro(NH$_2$)^7, and Ala^6 are upfield-shifted and have temperature coefficients smaller than random-coil values.

$^3J(^1H,^1H)$ coupling constants measured for the major conformer of **3** (see *Table 3*) are closely similar to those observed earlier for **4** [1], with the Asn^4 $^3J(\alpha,NH)$ value being typical of a residue at the $(i + 2)$ position of a βI-turn. The minor form, however, has a low $^3J(\alpha,NH)$ value for Ala^5, indicating again (see also *Table 2*) that the major and minor conformations are significantly different. Further evidence for a stable βI-turn in the major conformer was found in ROESY spectra, which revealed several long-range NOEs (*i.e.*, between nonadjacent residues), *e.g.*, connecting protons in Asn^2 with others in Ala^5 and Pro(NH$_2$)^7.

2.3. *Structure Calculations.* The evidence described above points to a well-populated secondary structure in the major form of **3**, so average solution conformations were

Table 2. *Amide ^{1}H-NMR Chemical Shift Temperature Coefficients $\Delta\delta/\Delta$T* [ppb/K] *for the Major and Minor Conformers of* **3** *and the Major Conformer of* **4**[a])

	Ala^1	Asn^2	NH (*trans*)[b])	NH (*cis*)[b])	Asn^4	NH (*trans*)[b])	NH (*trans*)[b])	Ala^5	Ala^6	Pro(NH$_2$)^7
major conformer **3**	−7.3	−6.5	−5.9	−6.3	−5.1	−6.7	−6.0	−0.6	−7.2	−7.9
minor conformer **3**	−7.3	−2.7	−4.8	−5.1	−8.3	−6.7	−6.3	−8.2	−3.1	−3.8
major conformer **4**[c])	−7.3	−6.0	−3.9	−5.8	−5.0	−6.3	−5.8	−2.9	−5.7	−6.0

[a]) The coefficients for backbone and side-chain amide NHs are given. Pro(NH$_2$)^7 is the 4-aminoproline moiety in the template. All measurements were made under the same conditions on an *AMX600* spectrometer in H_2O/D_2O 9:1, pH 5.0, over the temperature range 278–315 K. Probe temperature was calibrated by the method of *Van Geet* [15]. The variation in $\Delta\delta/\Delta T$ over several measurements was *ca.* ± 10%.

[b]) *cis* and *trans* refer to the position rel. to the C=O group.

[c]) The values for **4** are from *Bisang et al.* [1].

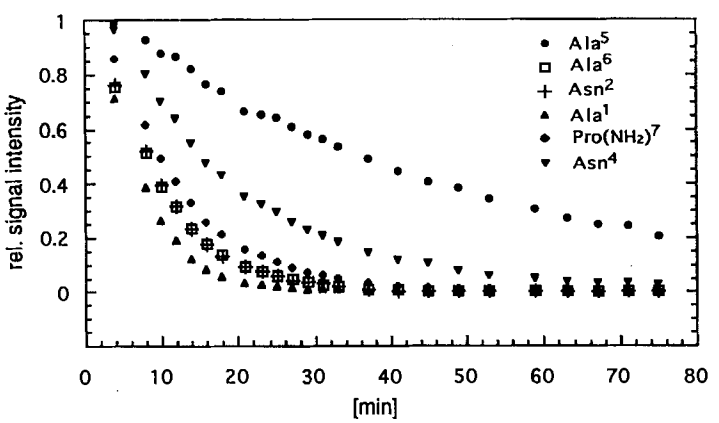


Fig. 2. *H/D Exchange of the peptide NH protons in* **3** (major conformer), *measured at 600 MHz in D_2O, pD*5* (uncorrected reading) at 300 K. Each point represents the residual signal intensity *vs.* time, measured immediately after dissolving the sample.

Table 3. *^{1}H-NMR Coupling Constants* J[a]) [Hz] *for* **3**

Residue	$^3J(\alpha, NH)$[b])	$^3J(\alpha, \beta)$	Others
Ala^1	6.0 (6.1)	7.0	
Asn^2	7.6 (8.7)	4.5, 9.1[c])	$J(\beta,\beta') = 14.4$
Pro^3	–	5.8, 8.5[c])	n.d.
Asn^4	8.8 (8.4)	4.7, 10.3[c])	$J(\beta,\beta') = 15.1$
Ala^5	6.7 (3.5)	7.0	
Ala^6	5.6 (6.7)	7.0	
Pro$(NH_2)^7$	(7.9 (7.5))[d])	8.8, 5.9[e])	$J(\beta_{pro\text{-}R}, \gamma) = 8.5$, $J(\beta_{pro\text{-}S}, \gamma) = 6.2$, $J(\beta,\beta') = 12.0$, $J(\gamma, \delta_{pro\text{-}R}) = 9.1$, $J(\gamma, \delta_{pro\text{-}S}) = 6.9$, $J(\delta, \delta') = 12.6$
Pro$(CH_2COOH)^8$	–	–	n.d.

[a]) Measured from 1D spectra and/or E.COSY spectra.
[b]) Values for the minor isomer measured from 1D spectra are given in parentheses.
[c]) Assignments of stereoheterotopic H-atoms not available.
[d]) Refers to the $^3J(NH-C(\gamma), H-C(\gamma))$ coupling.
[e]) Given in the order *pro-R*, *pro-S*.

calculated using distance restraints derived from ROESY spectra with spin-lock periods of 75, 150, 225, and 300 ms and a dynamic simulated annealing (SA) protocol described in detail in [1]. The NOE connectivities used for the SA calculations and their classification are given in *Table 4*. These NOEs were used to derive 43 upper distance restraints, which from 50 SA calculations yielded 25 structures that show no distance violations > 0.2 Å, and all of which lie within 10 kcal/mol of the minimum-energy structure. The 25 SA structures fall into a family with tightly clustered ϕ and ψ angles (*Fig. 3*) which is typified by the lowest-energy structure shown in *Fig. 4*.

As found for **4** in earlier work [1], the NPNA motif in the SA structures of **3** again populates preferentially a type-I β-turn, with ϕ and ψ for Pro^3 and ϕ for Asn^4 being close to those values found in 'ideal' βI-turns in protein crystal structures [12]. Indeed, the lowest-energy SA structures determined for **3** here and **4** in earlier work could be superimposed over their backbone atoms (N, C(α), C) with an r.m.s. deviation of only *ca.* 0.12 Å, showing that the SA calculations lead to essentially identical backbone conformations. Thus within the limited accuracy of the structure calculations, the templates **1** and **2** appear to exert essentially the same influence on the conformation of the ANPNAA hexapeptide loop in **3** and **4**. On the other hand, we have previously used the relative magnitude of the Ala^5 amide temperature coefficient as a measure of the extent of βI-turn formation in **4** [1] [11], and on this basis, the value closer to zero for **3** suggests that the βI-turn is more highly populated in **3** than in **4** in aqueous solution (*cf. Table 2*).

An optimum geometry for H-bonding (*i.e.*, H-bond length < 2.5 Å and donor-H-acceptor angle > 135°) between the Ala^{5}NH and the Asn^2-(backbone CO) groups was not observed in the final 25 SA structures, rather it is the side-chain CO group of Asn^2 that is most frequently found in a position suitable for H-bonding to Ala^{5}NH, as illustrated in the lowest-energy SA structure shown in *Fig. 4*. This would explain the low temperature coefficient and the slow exchange of the Ala^{5}NH proton. However, rotation of the Asn^2 side chain about the C(β)–C(γ) bond (χ_2 torsion) may bring either the side chain amide *trans* NH proton into a H-bonding position with the Ala^{5}CO group, or the

Table 4. *The Strong, Medium, and Weak $^1H,^1H$-NOE Connectivities*[a]) *Used for the Derivation of Distance Restraints in Simulated Annealing Calculations*

Strong NOEs (up to 2.5 Å)		Weak NOEs (up to 5.0 Å)	
Ala^6NH	$Ala^5H-C(\alpha)$	$Asn^4H_{cis}-N(\delta)$	$Pro^3H-C(\delta)$
Pro^7NH	$Ala^6H-C(\alpha)$	$Asn^4H_{trans}-N(\delta)$	$Pro^3H-C(\gamma)$
Asn^2NH	$Ala^1H-C(\alpha)$	$Asn^4H_{trans}-N(\delta)$	$Pro^3H'-C(\beta)$
$Pro^3H-C(\delta)$	$Asn^2H-C(\alpha)$	Asn^4NH	$Asn^2H''-C(\beta)$
		Asn^4NH	$Asn^2H-C(\alpha)$
Medium NOEs (up to 3.0 Å)		Asn^4NH	$Pro^3H-C(\gamma)$
Ala^1NH	$Pro^8H'-C(\varepsilon)$	$Asn^4H''-C(\beta)$	$Ala^5H-C(\beta)$
Asn^4NH	Ala^5NH	$Asn^2H_{cis}-N(\delta)$	$Pro^3H-C(\gamma)$
$Asn^4H_{cis}-N(\delta)$	$Asn^4H'-C(\beta)$	$Asn^2H_{cis}-N(\delta)$	$Pro^7H_{pro\text{-}R}-C(\beta)$
Ala^1NH	$Ala^1H-C(\beta)$	$Asn^2H_{trans}-N(\delta)$	$Pro^3H-C(\delta)$
Ala^1NH	$Pro^8H''-C(\varepsilon)$	$Asn^2H_{trans}-N(\delta)$	$Ala^5H-C(\beta)$
Pro^7NH	$Pro^7H_{pro\text{-}S}-C(\delta)$	$Asn^2H''-C(\beta)$	$Ala^5H-C(\beta)$
Pro^7NH	$Pro^7H_{pro\text{-}R}-C(\beta)$	Pro^7NH	$Asn^2H''-C(\beta)$
Ala^6NH	$Ala^6H-C(\beta)$	$Pro^7H_{pro\text{-}R}-C(\beta)$	$Ala^6H-C(\alpha)$
$Asn^2H''-C(\beta)$	$Pro^7H_{pro\text{-}R}-C(\beta)$	$Pro^7H_{pro\text{-}R}-C(\beta)$	$Asn^2H'-C(\beta)$
		$Pro^7H_{pro\text{-}S}-C(\beta)$	$Ala^5H-C(\beta)$
Weak NOEs (up to 5.0 Å)		Ala^5NH	$Ala^6H-C(\alpha)$
$Asn^2H'-C(\beta)$	$Pro^3H-C(\delta)$	Ala^5NH	$Asn^2H''-C(\beta)$
Ala^5NH	$Ala^5H-C(\beta)$	Ala^1NH	$Pro^8H_{pro\text{-}S}-C(\delta)$
Pro^7NH	$Pro^7H_{pro\text{-}R}-C(\delta)$	Ala^1NH	$Asn^2H'-C(\beta)$
Asn^4NH	$Asn^4H'-C(\beta)$	$Asn^2H''-C(\beta)$	$Pro^3H-C(\delta)$
$Asn^4H_{cis}-N(\delta)$	Asn^4NH	$Asn^2H_{cis}-N(\delta)$	$Ala^5H-C(\beta)$
$Asn^4H_{cis}-N(\delta)$	$Pro^3H-C(\gamma)$	Asn^4NH	$Asn^2H''-C(\beta)$
$Asn^4H_{cis}-N(\delta)$	$Pro^3H'-C(\beta)$		

[a]) Pro^7 refers to $Pro(NH_2)^7$ and Pro^8 to $Pro(CH_2COOH)^8$. See **3**.

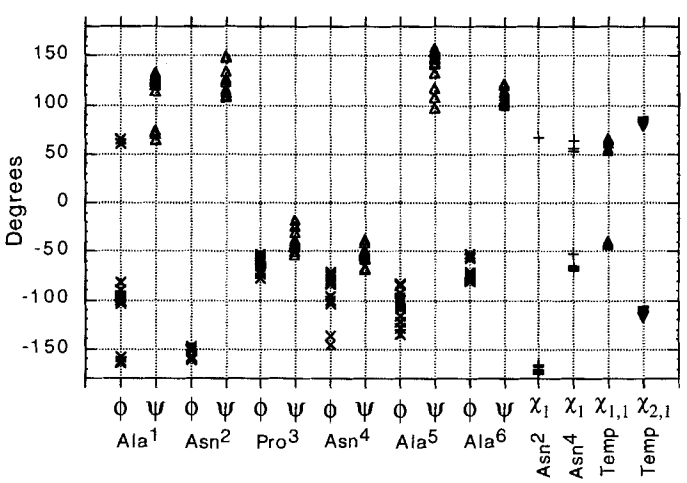


Fig. 3. *The distribution of ϕ and ψ angles, as well as χ_1 in Asn^2 and Asn^4, and the $\chi_{1,1}$ and $\chi_{2,1}$* (C(α)–C(ε) and C(ε)–CO) *torsions in the Pro(CH_2COOH) unit of the template, found in the final 25 SA structures of* **3**

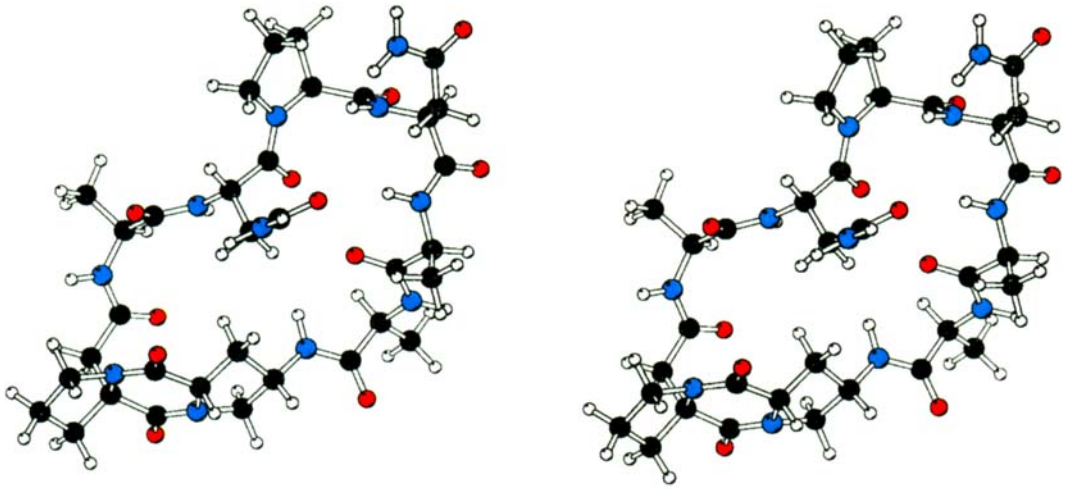


Fig. 4. *Stereodiagram of the lowest-energy SA structure found for* **3**. O-Atoms in red, N-atoms in blue. The H-bond length (2.16 Å) and donor-H-acceptor angle (161°) for the Asn^2(side chain CO)/Ala^5NH interaction indicate a short strong H-bond, whereas the same parameters for the Asn^2(backbone CO)/Ala^5NH (3.93 Å and 113°, resp.) represent a non-optimal geometry for H-bonding in this structure.

Asn^2 side-chain CO into H-bonding distance of the Asn^4 backbone NH or the template NH–C(γ). In fact, all these geometries are observed in the final 25 SA structures. The formation of H-bonds between the Asn^2 peptide CO, its side-chain $CONH_2$ group, and peptide bonds involving Asn^4, Ala^5, or $Pro(NH_2)^7$, should certainly contribute to the stability of the backbone conformation deduced for **3** and **4** in aqueous solution.

3. Conclusions. – An expedient route for the synthesis of template **1** on a gram scale is presented (*Scheme 1*). A tendency for the *trans*-fused diketopiperazine in **1** to epimerize under basic/hydroxylic conditions was observed, which in some cases may be problematic. However, this did not prevent the use of **1** here in peptide synthesis using the Fmoc chemistry. The application of **1** for the production of a cyclic template-bound peptide is also demonstrated with the efficient synthesis of **3**. The average solution structures derived for **3** by SA satisfy well all distance constraints and provide at least a qualitative explanation for the temperature coefficients and relative exchange rates of NH protons in **3**. These properties are accounted for by invoking a single family of closely related structures, which includes a βI-turn in each NPNA motif. However, **3** is certainly not rigid, and restrained and unrestrained molecular-dynamics simulations with explicit H_2O as solvent and time-dependent averaging of distance and angle restraints may yield a more detailed picture of the dynamic behaviour of this molecule in aqueous solution [13]. An important objective in the design of loop mimetics is to produce molecules with a stable and definable backbone conformation. This goal appears to have been achieved in the synthesis of **3** and **4**. The main interest now focuses on assessing the utility of such templates in the design of biologically active protein loop mimetics.

The authors thank the *Swiss National Science Foundation* for financial support.

Experimental Part

General. See [1]. NOE Difference: irradiation proton → affected signal (intensity).

Peptide Synthesis: General. Peptide Synthesis: *Biolynx™ 4175* continuous flow peptide synthesizer. *O*-(1*H*-Benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HBTU), 4-(dimethylamino)-pyridine (DMAP), *O*-(1*H*-1,2,3,-triazolo[4,5-*b*]pyridin-1-yl) *N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HATU), 1-hydroxybenzotriazole (HOBt), 1-hydroxy-1*H*-1,2,3-triazolo[4,5-*b*]pyridine (HOAt) were anal. grade. *N*,*N*-Dimethylformamide (DMF) was dried ($MgSO_4$) and distilled from ninhydrin, CH_2Cl_2 distilled from CaH_2, and $^iPr_2EtN$ was redistilled from ninhydrin and KOH prior to use. HPLC: dual pump *Pharmacia* system, *Waters-RCM-μBondapak®-C18* cartridges (10 μm, 125 Å, 25 × 100 mm and 8 × 10 mm) for prep. and anal. separations resp.; solvents *A*, H_2O + 0.1% CF_3COOH, and *B*, MeCN with 0.1% CF_3COOH; UV detection at 223 and 278 nm.

Methyl (S)-1-[(Benzyloxy)carbonyl]prolinate. Thionyl chloride (2.9 ml, 40 mmol) was added to a soln. of (*S*)-1-[(benzyloxy)carbonyl]proline (5 g, 20 mmol) in anh. MeOH (50 ml) at −10° within 10 min. After stirring at r.t. for 1 h, the soln. was refluxed for 2 h. The solvent was evaporated ant the residue extracted with CH_2Cl_2 from 0.1N NaOH. The org. layer was dried (Na_2SO_4) and evaporated to give a product that was used without further purification (4.9 g, 100%). Clear oil. TLC (hexane/AcOEt 4:6 + 0.1% CF_3COOH): R_f 0.67. $[\alpha]_D^{20} = -54.6$ (c = 0.48, $CHCl_3$). IR (NaCl): 3150*w*, 3100*w*, 2962*m*, 2944*s*, 2879*m*, 1740*s*, 1704*s*. ^{1}H-NMR (300 MHz, $CDCl_3$): 7.37–7.27 (*m*, 5 H); 5.28–5.02 (*m*, 2 H); 4.36 (2*dd*, 1 H); 3.74–3.38 (*m* + 2*s*, 5 H); 2.27–1.16 (*m*, 1 H); 2.03–1.95 (*m*, 3 H). ^{13}C-NMR (75 MHz, $CDCl_3$): 173.2, 173.1, 154.8, 154.2, 136.7, 136.6, 128.6, 128.4, 128.4, 127.93, 127.9, 127.8, 67.0, 66.9, 59.2, 58.8, 52.2, 52.0, 46.9, 46.4, 30.9, 29.9, 24.3, 23.5. CI-MS (NH_3): 281.2 (25, $[M + NH_4]^+$), 265.3 (16), 264.2 (100, $[M + H]^+$).

*(±)-Methyl 1-[(Benzyloxy)carbonyl]-2-{[(*tert*-butoxy)carbonyl]methyl}prolinate.* A soln. of the foregoing product (4 g, 15.2 mmol) in THF (20 ml) was added to a soln. of LDA/THF complex (17 ml, 1.3 equiv., 19.8 mmol) in THF (160 ml) and hexane (40 ml) at −78°. After 0.5 h, *tert*-butyl bromoacetate (2.8 ml, 18.2 mmol) was slowly added to the mixture before warming to r.t. The soln. was extracted with CH_2Cl_2. The org. layer was dried (Na_2SO_4) and evaporated to give an orange oil. FC (hexane/AcOEt 4:6, R_f 0.69) gave the product as an oil (4.6 g, 80%). TLC (hexane/AcOEt 4:6): R_f 0.69. IR (NaCl): 3010*m*, 2669*m*, 2875*m*, 1735*s*, 1720*s*, 1705*s*. ^{1}H-NMR (300 MHz, $CDCl_3$): 7.35–7.27 (*m*, 5 H); 5.22, 5.00 and 5.14, 5.09 (2 × 2*d*, 2 H); 3.81–3.67, 3.52–3.43 (2*s*, + 2*m*, 5 H); 3.10, 2.98 and 3.00, 2.81 (2 × 2*d*, 2 H); 2.71–2.48, 2.23–2.14, 2.03–1.85 (*m*, 4 H); 1.42, 1.40 (*s*, 9 H). ^{13}C-NMR (75 MHz, $CDCl_3$): 173.5, 173.3, 169.7, 169.5, 154.0, 153.7, 136.6, 136.1, 128.5, 128.2, 127.8, 127.7, 127.5, 80.5, 80.1, 66.9, 66.5, 66.4, 65.6, 52.3, 52.1, 48.6, 47.8, 40.5, 38.9, 37.2, 35.7, 27.8, 27.7, 23.0, 22.4. CI-MS (NH_3): 395.3 (20, $[M + NH_4]^+$), 379.3 (20), 378.2 (100, $[M + H]^+$).

*(±)-1-[(Benzyloxy)carbonyl]-2-{[(*tert*-butoxy)carbonyl]methyl}proline (= (±)-1-Benzyl 2-Hydrogen 2-[2-(*tert*-Butoxy)-2-oxoethyl]pyrrolidine-1,2-dicarboxylate; (±)-5).* To a soln. of the foregoing product (3.2 g, 8.5 mmol) in MeOH/H_2O 3:1 (80 ml) was added with cooling LiOH · H_2O (3.6 g, 10 equiv., 85 mmol). After stirring for 15 h and acidifying, the aq. soln. was extracted with CH_2Cl_2 and the org. layer dried (Na_2SO_4) and evaporated. (±)-**5** (2.6 g, 83%). Off-white paste. TLC (CH_2Cl_2/MeOH/AcOH 90:9:1): R_f 0.79. IR (NaCl): 3600–2700*s* (br.), 3015*m*, 2900*m*, 1700*s* (br.), 1410*s*. ^{1}H-NMR (300 MHz, $CDCl_3$): 10.23 (br. *s*, 1 H); 7.34–7.26 (*m*, 5 H); 5.15–5.11 (*m*, 2 H); 3.76–3.68 (*m*, 1 H); 3.52–3.43 (*m*, 1 H); 3.16, 2.97, 3.01, 2.79 (2 × 2*d*, 2 H); 2.69–2.18, 2.05–1.96 (*m*, 4 H), 1.40 (*s*, 9 H). ^{13}C-NMR (75 MHz, $CDCl_3$): 176.6, 169.7, 155.0, 136.2, 128.3, 127.9, 127.7, 80.8, 67.2, 66.7, 48.2, 39.0, 35.7, 27.9, 22.9. CI-MS (NH_3): 364.2 ($[M + H]^+$).

tert-*Butyl (2R)-and (2S)-1-[(Benzyloxy)carbonyl]-2-{[(2S,4R)-4-hydroxy-2-(methoxycarbonyl)pyrrolidin-1-yl]carbonyl}pyrrolidine-2-acetate* (**6** and **7**, resp.). To a soln. of (±)-**5** (5 g, 14 mmol), methyl (2*S*,4*R*)-4-hydroxyprolinate hydrochloride [1] (2.2 g, 15.4 mmol) and $^iPr_2EtN$ (7.2 ml, 42 mmol) in CH_2Cl_2 (25 ml) was added HBTU (5.3 g, 14 mmol). After stirring for 4.5 h at r.t., the soln. was diluted with CH_2Cl_2 (25 ml), washed with 10% aq. citric acid soln. (3 × 50 ml), 5% aq. $NaHCO_3$ soln. (50 ml), and brine (50 ml), dried (Na_2SO_4), and evaporated. The diastereoisomeric dipeptides **6** and **7** were separated by FC (Et_2O/^{i}PrOH, 95:5 → 90:10): 3.0 g (45%) of **6** and 2.8 g (42%) **7** as white solids.

6: TLC (Et_2O/^{i}PrOH, 95:5): R_f 0.56. $[\alpha]_D^{21} = +8.7$ (c = 0.94, MeOH). IR (NaCl): 3660*w*, 3600*m*, 3650–3100*m* (br.), 2950*s*, 1744*s*, 1704*s*, 1640*s*, 1502*s*. ^{1}H-NMR (600 MHz, CD_3OD): 7.42, 7.33 (2*m*, 5 H); 5.35, 5.04 (*m*, 2 H); 4.55, 4.50 (2*m*, 1 H); 4.44, 4.28 (2*m*, 1 H); 3.70, 3.63 (2*s*, 3 H); 3.65 (*m*, 1 H); 3.60–3.55 (2*m*, 1 H); 3.44 (*m*, 1 H); 3.41 (*m*, 2 H); 3.15 (*m*, 2 H); 3.02 (*d*, 1 H); 2.89 (*d*, 1 H); 2.79 (*d*, 1 H); 2.59 (*m*, 1 H); 2.38 (*m*, 1 H); 2.23 (*m*, 1 H); 2.18 (*dd*, 1 H); 2.12 (*dd*, 1 H); 2.03 (*m*, 2 H); 1.90 (*m*, 1 H); 1.83 (*m*, 1 H); 1.42, 1.37 (2*s*, 9 H). ^{13}C-NMR (75 MHz, CD_3OD): 174.2, 173.9, 172.0, 155.8, 138.1, 138.0, 129.6, 129.5, 129.4, 129.1, 129.0, 81.9, 81.8, 71.9, 71.8, 69.4, 68.7, 68.6, 68.1, 61.7, 61.5, 56.7, 56.5, 52.6, 49.7, 48.9, 42.9, 41.0, 37.8, 37.2, 36.0, 35.7, 28.3, 28.2, 23.5, 22.7. CI-MS (NH_3): 508.2 (19.0, $[M + NH_4]^+$), 492.3 (26.3), 491.2 (100, $[M + H]^+$).

7: TLC (Et_2O/^{i}PrOH, 95:5): R_f 0.74. $[\alpha]_D^{21} = -40.2$ ($c = 0.99$, MeOH). IR (NaCl): 3700*m*, 3420*m*, 2975*m*, 1746*s*, 1704*s*, 1645*s*. ^{1}H-NMR (600 MHz, CD_3OD): 7.39–7.30 (*m*, 5 H); 5.15–5.06 (2*m*, 2 H); 4.55–4.22 (2*m*, 2 H); 3.74, 3.17 (2*m*, 2 H); 3.68, 3.66 (2*s*, 3 H); 3.66–3.53 (*m*, 2 H); 3.05, 2.75 (2*m*, 2 H); 2.60 (*m*, 1 H); 2.41–2.30 (*m*, 1 H); 2.08–1.98 (*m*, 3 H); 1.90, 1.83 (2*m*, 1 H); 1.42, 1.40 (2*s*, 9 H). ^{13}C-NMR (75 MHz, CD_3OD): 175.5, 173.8, 171.9, 155.6, 138.1, 138.0, 129.6, 129.4, 129.3, 129.2, 129.1, 81.9, 81.8, 71.1, 70.8, 68.6, 68.4, 68.8, 68.5, 61.3, 55.5, 52.6, 49.6, 48.9, 42.9, 40.9, 37.0, 36.6, 36.4, 35.3, 28.2, 23.6, 22.9. CI-MS (NH_3): 508.2 ($[M + NH_4]^+$), 491.2 ($[M + H]^+$).

tert-*Butyl* *(2*R,*5a*S,*10a*S*)-Octahydro-2-hydroxy-5,10-dioxo-1*H,*5*H*-dipyrrolo[1,2*-a*:1′,2′-*d*]pyrazine-5a-acetate* (**10**). The dipeptide **7** (5.5 g, 11 mmol) was stirred in AcOEt (200 ml) with 10% Pd/C (0.5 g) under H_2 for 1.5 d. The mixture was then filtered through *Celite*, the filtrate evaporated, and the residue submitted to FC (CH_2Cl_2/MeOH 9:1, R_f 0.68): **10** (3.0 g, 83%). White solid. M.p. 161–162°. $[\alpha]_D^{21} = +19.6$ ($c = 1.01$, MeOH). IR (KBr): 3340*s* (br.), 2975*m*, 2896*m*, 1735*s*, 1675*s*, 1640*s*. ^{1}H-NMR (600 MHz, $CDCl_3$): 4.60 (*t*, 1 H); 4.48 (*dd*, 1 H); 4.18 (*dd*, 1 H); 4.14 (*ddd*, 1 H); 3.30 (*ddd*, 1 H); 3.28 (*d*, 1 H); 3.08 (*d*, 1 H); 2.56 (br.*s*, 1 H); 2.54 (*d*, 1 H); 2.36 (*ddd*, 1 H); 2.29 (*dd*, 1 H); 2.10 (*m*, 1 H); 2.00 (*m*, 3 H); 1.36 (*s*, 9 H). NOE Difference: 4.60 (H–(2)) → 4.18 ($H_{pro\text{-}S}$–C(3), 4.2), 2.36 ($H_{pro\text{-}S}$–C(1), 4.0); 4.48 (H–C(10a)) → 3.28 ($H_{pro\text{-}R}$–C(3), 1.0), 2.29 ($H_{pro\text{-}R}$–C(1), 3.7); 3.08 (*H*CHCOO^{t}Bu) → 2.54 (HC*H*COO^{t}Cu, 18.0), 2.10 ($H_{pro\text{-}R}$–C(6), 2.4); 2.10 ($H_{pro\text{-}R}$–C(6) → 3.08 (*H*CH-COO^{t}BU, 3.1), 2.05–1.90 ($H_{pro\text{-}S}$–C(6), H′–C(7), 17.8). ^{13}C-NMR (75 MHz, CD_3OD): 169.1, 166.9, 165.2, 81.5, 68.4, 66.8, 59.3, 55.2, 43.5, 41.6, 39.2, 35.5, 28.1, 19.9. CI-MS (NH_3): 342.3 ($[M + NH_4]^+$), 326.4 (16.4), 325.4 (100, $[M + H]^+$). Anal. calc. for $C_{16}H_{24}N_2O_5$ (324.4): C 59.2, H 7.5, N 8.6; found: C 59.1, H 7.4, N 8.6.

tert-*Butyl* *(2*R,*5a*S,*10a*R*)-Octahydro-2-hydroxy-5,10-dioxo-1*H,*5*H*-dipyrrolo[1,2*-a*:1′,2′-*d*]pyrazine-5a-acetate*. As described for **10**, but conducting the hydrogenation in MeOH as solvent gave the 10a-epimer of **10**. FC (CH_2Cl_2/MeOH 9:1, R_f 0.62) yielded a white solid (2.2 g, 60%). IR (KBr): 3350*s* (br.), 2980*m*, 2894*m*, 1735*s*, 1655*s*, 1650*s*. ^{1}H-NMR (600 MHz, $CDCl_3$): 4.51 (*sext.*, 1 H); 4.42 (*t*, 1 H); 3.80 (*m*, 2 H); 3.48 (*dd*, 1 H); 3.44 (*ddd*, 1 H); 2.75 (br. *s*, 1 H); 2.73 (*d*, 1 H); 2.57 (*d*, 1 H); 2.52 (*m*, 1 H); 2.42 (*m*, 1 H); 2.32 (*m*, 2 H); 1.97 (*m*, 2 H); 1.44 (*s*, 9 H). CI-MS (NH_3): 325.3 (37.7, $[M + H]^+$), 286.3 ($[M + NH_4 - {}^t\text{Bu}]^+$), 269.3 (100, $[M + H - {}^t\text{Bu}]^+$).

tert-*Butyl* *(2*R,*5a*S,*10a*S*)-Octahydro-5,10-dioxo-2-(tosyloxy)-1*H,*5*H-*di*[1,2*-a*:1′,2′-*d*]pyrazine-5a-acetate*. To a soln. of **10** (1 g, 3.1 mmol) in pyridine (10 ml) was added tosyl chloride (3.56 g, 18.7 mmol) at 0°, and the soln. was stirred for 3 h. After dilution with CH_2Cl_2 (100 ml), the soln. was washed with 10% aq. citric acid soln., sat. aq. $NaHCO_3$ soln., and brine. The org. phase was dried ($MgSO_4$) and evaporated. FC (CH_2Cl_2/Et_2O 1:1, R_f 0.65) gave a white solid (1.3 g, 91%). M.p. 154–155°. $[\alpha]_D^{21} = +15.0$ ($c = 0.99$, $CHCl_3$). IR (KBr): 2980*w*, 2930*w*, 1720*s*, 1658*s*, 1450*s*, 1362*s*. ^{1}H-NMR (300 MHz, $CDCl_3$): 7.80 (*d*, 2 H); 7.37 (*d*, 2 H); 5.16 (*m*, 1 H); 4.35 (*dd*, 1 H); 4.25 (*dd*, 1 H); 4.11 (*m*, 1 H); 3.38 (*d*, 1 H); 3.29 (*m*, 1 H); 3.04 (*d*, 1 H); 2.53 (*d*, 1 H); 2.46 (*s*, 3 H); 2.43 (*m*, 2 H); 2.17–1.87 (*m*, 4 H); 1.34 (*s*, 9 H). ^{13}C-NMR (75 MHz, $CDCl_3$): 169.3, 167.2, 163.7, 145.3, 133.6, 130.1, 127.8, 81.7, 78.0, 66.6, 59.3, 52.8, 43.5, 41.6, 36.5, 35.5, 28.1, 21.7, 19.8. EI-MS: 478.3 (15.9, M^+), 422.3 (24.4, $[M - {}^t\text{Bu}]^+$). Anal. calc. for $C_{23}H_{30}N_2O_7S$: C 57.7, H 6.3, N 5.9, S 6.7; found: C 57.9, H 6.2, N 6.0, S 7.0.

tert-*Butyl* *(2*S,*5a*S,*10a*S*)-2-Azidooctahydro-5,10-dioxo-1*H,*5*H*-dipyrrolo[1,2*-a*:1′,2′-*d*]pyrazine-5a-acetate* (**11**). The foregoing product (1.18 g, 2.47 mmol) in DMF (50 ml) and NaN_3 (9.7 g, 150 mmol) was stirred at 78° for 3 h. The DMF was evaporated and the residue partitioned between Et_2O and H_2O. The aq. layer was extracted with Et_2O and the combined org. layers dried ($MgSO_4$) and evaporated: **11** (0.78 g, 91%). Fine colourless needles. M.p. 144–145°. TLC (CH_2Cl_2/MeOH 95:5): R_f 0.61. $[\alpha]_D^{21} = +61.6$ ($c = 0.97$, MeOH). IR (KBr): 2970*m*, 2920*m*, 2094*s*, 1732*s*, 1660*s*, 1450*s*. ^{1}H-NMR (400 MHz, CD_3OD): 4.34–4.26 (*m*, 2 H); 4.00 (*m*, 1 H); 3.76 (*dd*, 1 H); 3.60 (*dd*, 1 H); 3.38–3.29 (*m*, 1 H); 2.95 (*d*, 1 H); 2.75 (*d*, 1 H); 2.61 (*ddd*, 1 H); 2.35 (*ddd*, 1 H); 2.02 (*m*, 4 H); 1.42 (*s*, 9 H). ^{13}C-NMR (75 MHz, CD_3OD): 171.2, 168.9, 166.5, 83.0, 68.4, 61.8, 59.1, 51.6, 45.2, 42.3, 36.5, 35.8, 28.7, 20.9. CI-MS (NH_3): 367.2 (9.3, $[M + NH_4]^+$), 351.5 (13.6), 350.2 (100, $[M + H]^+$). Anal. calc. for $C_{16}H_{23}N_5O_4$: C 55.0, H 6.6, N 20.1; found: C 55.2, H 6.8, N 20.0.

tert-*Butyl* *(2*S,*5a*S,*10a*S*)-2-Aminooctahydro-5,10-dioxo-1*H,*5*H*-dipyrrolo[1,2*-a*:1′,2′-*d*]pyrazine-5a-acetate*. Azide **11** (0.64 g, 1.83 mmol) was stirred for 15 h in AcOEt (25 ml) with 10% Pd/C (60 mg) under H_2. The suspension was filtered through *Celite* and the filtrate evaporated: colourless solid (0.59 g, 100%). M.p. 118–119°. TLC (CH_2Cl_2/MeOH 4:1): R_f 0.5. $[\alpha]_D^{21} = +24.4$ ($c = 1.0$, MeOH). IR (NaCl): 3506*s*, 3360*s*, 3280*m*, 2926*s*, 2890*m*, 1727*s*, 1660*s*, 1650*s*, 1480*s*. ^{1}H-NMR (600 MHz, CD_3OD): 4.80 (br. *s*, 1 H); 4.25 (*dd*, 1 H); 4.04 (*ddd*, 1 H); 3.70 (*dd*, 1 H); 3.60 (*m*, 1 H); 3.49 (*dd*, 1 H); 3.34 (*ddd*, 1 H); 3.02 (*d*, 1 H); 2.77 (*d*, 1 H); 2.58 (*ddd*, 1 H); 2.08–1.93 (*m*, 3 H); 1.42 (*s*, 9 H). NOE Difference: 4.25 (H–C(10a)) → 3.60 (H–C(2), 2.8), 3.49 ($H_{pro\text{-}R}$–C(3), 2.1), 2.58 ($H_{pro\text{-}S}$–C(1), 7.4); 3.60 (H–C(2)) → 4.25 (H–C(10a), 3.1), 2.58 ($H_{pro\text{-}S}$–C(1), 4.6); 3.49 ($H_{pro\text{-}R}$–C(3)) → 4.25 (H–C(10a), 2.7), 3.70 ($H_{pro\text{-}S}$–C(3), 25.4), 3.60 (H–C(2), 5.5); 3.02 (*H*CHCO$_2{}^t$Bu) → 2.77 (HC*H*CO$_2{}^t$Bu), 24.4), 2.07 (H–C(6 or 7), 4.7); 2.77 (HC*H*CO$_2{}^t$Bu) → 3.34 (H–C(8), 3.0), 3.02 (*H*CHCO$_2{}^t$Bu, 25.4), 2.07 (H–C(6 or 7), 4.7). ^{13}C-NMR (75 MHz, CD_3OD): 169.8, 168.5, 167.0, 83.1, 68.4, 61.8, 55.3, 50.7, 45.1, 42.6, 39.3, 36.3, 30.3, 20.7. ESI-MS: 346.1 ($[M + Na]^+$), 324.6 ($[M + H]^+$), 268.3 ($[M + H - {}^t\text{Bu}]^+$).

tert-*Butyl (2S,5aS,10aR)-2-Aminooctahydro-5,10-dioxo-1*H,5H-*dipyrrolo[1,2-a:1',2'-d]pyrazine-5a-acetate.* Azide **11** (0.64 g, 1.83 mmol) in MeOH (25 ml) was stirred for 15 h with 10% Pd/C (60 mg) under H_2. The suspension was filtered through *Celite* and the filtrate evaporated: pure colourless oil (0.59 g, 100%). TLC (CH_2Cl_2/MeOH 4:1): R_f 0.63. $[\alpha]_D^{21} = +75.1$ ($c = 0.96$, MeOH). IR (NaCl): 3440*s* (br.), 3360*s* (br.), 2974*s*, 2944*s*, 2892*s*, 2820*s*, 1720*s*, 1655*s*, 1425*s*. ^{1}H-NMR (600 MHz, CD_3OD): 4.74 (*dd*, 1 H); 3.79 (*dd*, 1 H); 3.70 (*m*, 2 H); 3.45 (*ddd*, 1 H); 3.22 (*d*, 1 H); 2.80 (*d*, 1 H); 2.69 (*d*, 1 H); 2.34 (*ddd*, 1 H); 2.25 (*ddd*, 1 H); 2.17 (*m*, 2 H); 2.05 (*m*, 1 H); 1.94 (*m*, 1 H); 1.45 (*s*, 9 H). ^{13}C-NMR (75 MHz, CD_3OD): 170.5, 169.5, 169.3, 83.2, 69.5, 59.9, 55.3, 49.9, 46.8, 42.8, 38.4, 35.3, 28.7, 22.4. ESI-MS: 346.3 ($[M + Na]^+$), 324.6 ($[M + H]^+$).

tert-*Butyl (2S,5aS,10aS)-2-{[(9H-Fluoren-9-yl)methoxycarbonyl]amino}octahydro-5,10-dioxo-1*H,5H-*dipyrrolo[1,2a:1',2'-d]pyrazine-5a-acetate.* To a soln. of amine (0.9 g, 2.78 mmol) in CH_2Cl_2 (30 ml) was added $^iPr_2EtN$ (476 µl) and Fmoc-Cl (0.72 g, 1.0 equiv., 2.78 mmol). After stirring at r.t. for 1 h, the emulsion was partitioned between CH_2Cl_2 (50 ml) and 10% aq. citric acid soln. (50 ml), and the org. layer was washed with brine (50 ml), dried ($MgSO_4$), and evaporated. FC (CH_2Cl_2/MeOH, 95:5) afforded a white solid (1.3 g, 87%). M.p. 87.5–91°. TLC (CH_2Cl_2/MeOH 95:5): R_f 0.39. $[\alpha]_D^{21} = -2.9$ ($c = 1.02$, $CHCl_3$). IR (KBr): 3680–3200*m* (br.), 3382*m*, 3062*m*, 3030*w*, 1714*s*, 1658*s*, 1522*m*, 1479*w*, 1449*s*. ^{1}H-NMR (600 MHz, $CDCl_3$): 7.76 (*d*, 2 H); 7.57 (*t*, 2 H); 7.40 (*t*, 2 H); 7.32 (*t*, 1 H); 7.29 (*t*, 1 H); 6.19 (*d*, 1 H); 4.52 (*m*, 1 H); 4.35 (*d*, 2 H); 4.26 (*d*, 1 H); 4.19 (*m*, 2 H); 4.14 (*t*, 1 H); 3.35 (*ddd*, 1 H); 3.29 (*dd*, 1 H); 3.23 (*d*, 1 H); 2.86 (*ddd*, 1 H); 2.66 (*d*, 1 H); 2.14 (*m*, 2 H); 2.06–1.95 (*m*, 3 H); 1.43 (*s*, 9 H). ^{13}C-NMR (75 MHz, $CDCl_3$): 170.4, 166.4, 164.5, 156.1, 144.2, 141.4, 127.8, 127.3, 125.3, 120.1, 82.8, 67.1, 66.7, 59.9, 53.2, 48.8, 47.4, 43.8, 41.8, 36.7, 36.1, 28.4, 20.0. ESI-MS: 568.3 ($[M + Na]^+$), 512.2 ($[M + Na - {}^tBu]^+$), 490.4 ($[M + H - {}^tBu]^+$).

tert-*Butyl (2S,5aS,10aR)-2-{[(9H-Fluoren-9-yl)methoxycarbonyl]amino}octahydro-5,10-dioxo-1*H,5H-*dipyrrolo[1,2-a:1',2'-d]pyrazine-5a-acetate.* The amine (0.59 g, 1.82 mmol) in 1M aq. Na_2CO_3 (35 ml) and dioxane (30 ml) was stirred with Fmoc-Cl (0.67 g, 2.55 mmol) at 0° for 1 h. The emulsion was then partitioned between AcOEt (100 ml) and 1M aq. Na_2CO_3 (100 ml), the aq. layer extracted with AcOEt (100 ml), the pooled org. phase dried ($MgSO_4$) and evaporated, and the product purified by FC (CH_2Cl_2/MeOH 95:5): white solid (0.98 g, 98%). M.p. 172–173°. TLC (CH_2Cl_2/MeOH 95:5): R_f 0.33. $[\alpha]_D^{21} = +67.9$ ($c = 1.00$, $CHCl_3$). IR (KBr): 3700–3200*m* (br.), 3305*s*, 3055*m*, 2972*m*, 1722*s*, 1660*s*, 1644*s*, 1530*s*, 1449*s*, 1430*s*. ^{1}H-NMR (600 MHz, (D_6) DMSO): 7.88 (*d*, 2 H); 7.68 (br. *d*, 3 H); 7.40 (*t*, 2 H); 7.32 (*t*, 2 H); 4.55 (*t*, 1 H); 4.37 (*m*, 2 H); 4.22 (*m*, 1 H); 4.04 (br. *s*, 1 H); 3.68 (*dd*, 1 H); 3.55 (*ddd*, 1 H); 3.28 (*ddd*, 1 H); 3.21 (*d*, 1 H); 2.68 (*d*, 1 H); 2.56 (*d*, 1 H); 2.14 (*m*, 4 H); 2.08–1.95 (*m*, 4 H); 1.89–1.74 (*m*, 2 H); 1.35 (*s*, 9 H). ^{13}C-NMR (75 MHz, $CDCl_3$): 168.6, 167.1, 166.0, 155.6, 143.7, 141.3, 127.7, 127.0, 124.8, 119.9, 82.1, 67.6, 66.5, 58.0, 51.8, 48.5, 47.2, 45.0, 42.1, 35.2, 34.6, 27.9, 20.9. ESI-MS: 568.6 ($[M + Na]^+$), 512.5, 490.6 ($[M + H - {}^tBu]^+$). Anal. calc. for $C_{31}H_{34}N_3O_6$ (545.6): C 68.2, H 6.3, N 7.7; found: C 68.5, H 6.2, N 7.9.

*(2S,5aS,10aS)-2-{[(9H-Fluoren-9-yl)methoxycarbonyl]amino}octahydro-5,10-dioxo-1*H,5H-*dipyrrolo[1,2-2:1',2'-d]pyrazine-5a-acetic Acid* (**1**). The *tert*-butyl ester (0.89 g, 1.64 mmol) was stirred in H_2O (5 ml) and CF_3COOH (15 ml) at 0° for 1 h. The CF_3COOH was evaporated, the product precipitated by addition of H_2O and lyophilized. The product was redissolved in dioxan and lyophilized: **1** (0.80 g, 100%). White solid. M.p. 54–55°. TLC (CH_2Cl_2/MeOH 95:5): R_f 0.39. $[\alpha]_D^{21} = -4.3$ ($c = 0.81$, MeOH). IR ($CHCl_3$): 3700–2800*s* (br.), 3061*m*, 2958*m*, 1780*m*, 1720*s*, 1642*s*, 1531*m*, 1450*s*. ^{1}H-NMR (600 MHz, (D_6)DMSO): 7.88 (*d*, 2 H); 7.69 (*d*, 2 H); 7.48 (*d*, 1 H); 7.41 (*t*, 2 H); 7.32 (*t*, 2 H); 4.30 (*m*, 2 H); 4.21 (*m*, 1 H); 4.16 (*m*, 1 H); 4.15 (*dd*, 1 H); 3.88 (*m*, 1 H); 3.53 (*m*, 1 H); 3.46 (*m*, 1 H); 3.23 (*m*, 1 H); 2.87 (*d*, 1 H); 2.70 (*d*, 1 H); 2.38 (*m*, 1 H); 1.93–1.80 (*m*, 5 H). ^{13}C-NMR (75 MHz, $CDCl_3$): 173.8, 168.6, 166.8, 158.4, 145.4, 142.8, 129.0, 128.4, 127.8, 121.1, 68.3, 68.1, 61.4, 52.9, 50.2, 48.6, 44.9, 41.1, 36.4, 35.2, 20.8. ESI-MS: 512.3 ($[M + Na]^+$), 534.4 ($[M + 2Na - H]^+$), 490.4 ($[M + H]^+$).

Cyclo(-Ala-Asn-Pro-Asn-Ala-Ala-Temp-) (**3**). Fmoc-Ala-OH (4× excess) was coupled to *Tentagel™-S-AC* resin [5] (*Rapp Polymere* Tübingen; 0.24 mmol/g) using dicyclohexylcarbodiimide and DMAP for activation in DMF. The solid-phase peptide synthesis was performed on a 0.50-mmol scale by chain elongation with Fmoc-Asn(Mtt)-OH, Fmoc-Pro-OH, Fmoc-Asn(Mtt)-OH, Fmoc-Ala-OH, **1**, Fmoc-Ala-OH (1.5 mmol each; except for **1** (1.0 mmol)), using HOBt/HBTU for activation. The completion of each coupling cycle was monitored by ninhydrin or isatin tests. Upon completion of chain assembly, the N-terminal Fmoc protecting group was removed with 20% piperidine/DMF, and the resin was washed with MeOH and CH_2Cl_2. Cleavage of the peptide from the resin was repeated three times each with 1% CF_3COOH/CH_2Cl_2 (60 ml) for 15 min. Each filtrate was neutralized with pyridine (2 ml) in CH_2Cl_2 (20 ml), and the combined layers were evaporated to give linear, protected peptide **12** (123 mg, 40%) after purification by HPLC (C_{18} column, 20% solvent-*B* in *A* for 5 min, then gradient 20–62% over 0.5 min, 62–95% over 24.5 min, and 95% for 5 min; $A = H_2O + 0.1\%$ CF_3COOH, $B = MeCN + 0.1\%$ CF_3COOH). ESI-MS: 1340.9 ($[M + Na]^+$), 1318.9 ($[M + H]^+$). For cyclization, the linear peptide (66 mg, 50.2 µmol) in 1% $^iPr_2EtN$/DMF was stirred with HOAt/HATU (1.5 equiv.) overnight at r.t. Evaporation and

purification by HPLC (C_{18} column, 50% solvent *A* for 5 min, then gradient 50–75% over 0.5 min, then 75–95% over 12.5 min, and 95% for 4 min) afforded side-chain-protected cyclic peptide (47.4 mg, 77%). ESI-MS: 1322.7 ($[M + Na]^+$), 1302.2 ($[M + H]^+$). The protected cyclic peptide (47 mg) was treated with a soln. containing CF_3COOH/H_2O/thioanisol/phenol/ethanedithiol/$^iPr_3SiH$ 80:5:5:5:2.5:3.5 (10.3 ml) at r.t. for 3 h. Evaporation, precipitation with $^iPr_2O$, and purification by HPLC (C_{18} column, 10% solvent/*A* for 3 min, then gradient 10–35% over 0.5 min, 35–52% over 12.5 min, 52–95% over 0.5 min, and 95% for 3.5 min) gave **3** (16 mg, 73%). ^{1}H-NMR (600 MHz, D_2O/H_2O 1:9, pH 5.0, 300 K): *Table 1*. ESI-MS: 810.4 ($[M + Na]^+$), 788.7 ($[M + H]^+$). Amino-acid analysis (molar ratio): Ala 3.00, Asx 1.87, Pro 0.98 (modified proline derivatives from template not determined).

Methyl (S)-1-[(Benzyloxy)carbonyl]-2-(prop-2-enyl)prolinate. To a soln. of Z-protected **8** [5] [14] (2.3 g, 7.95 mmol) in dry MeOH (25 ml) was added thionyl chloride (1.15 ml, 16.4 mmol) at −5°. After stirring 1 h at r.t., the mixture was refluxed for 2 h. The solvent was then evaporated, the residue partitioned between CH_2Cl_2 and 0.25N aq. NaOH, the org. layer dried ($MgSO_4$) and evaporated, and the product purified by FC (Et_2O/CH_2Cl_2 1:9): oil (2.12 g, 88%). TLC (CH_2Cl_2/Et_2O 9:1): R_f 0.78. $[\alpha]_D^{21} = -40.5$ ($c = 1.01$, CH_2Cl_2). IR (NaCl): 2979*m*, 2952*m*, 2879*m*, 1738*s*, 1700*s*. ^{1}H-NMR (300 MHz, $CDCl_3$): 7.36–7.26 (*m*, 5 H); 5.79–5.63 (*m*, 1 H); 5.32–5.00 (*m*, 4 H); 3.89–3.66 and 3.47–3.38 (2*s* + 2*m*, 5 H); 3.14–2.63 (*m*, 2 H); 2.19–1.80 (*m*, 4 H). ^{13}C-NMR (75 MHz, $CDCl_3$): 174.6, 174.5, 154.2, 136.9, 136.3, 133.2, 132.9, 128.4, 128.1, 128.0, 127.8, 127.6, 119.2, 119.0, 68.0, 66.6, 67.2, 67.0, 52.3, 52.1, 49.1, 48.3, 39.4, 38.0, 37.0, 35.6, 23.1, 22.6. CI-MS (NH_3): 321.2 (13.8, $[M + NH_4]^+$), 304.2 (100, $[M + H]^+$).

Methyl (S)-1-[(Benzyloxy)carbonyl]-2-(carboxymethyl)prolinate. The foregoing product (0.5 g, 1.65 mmol) was dissolved in acetone (10 ml) and saturated with ozone at −20° for 20 min. The soln. was then titrated with *Jones* reagent (2.67 g of CrO_3 in 2.3 ml of conc. H_2SO_4, diluted with H_2O to 10 ml) at 10–15°. The aq. soln. was extracted with CH_2Cl_2 (4 × 40 ml), the combined org. phase dried ($MgSO_4$) and evaporated, and the product purified by FC (CH_2Cl_2/MeOH 9:1): oil (0.26 g, 50%). TLC (CH_2Cl_2/Et_2O 9:1) R_f 0.71. IR (NaCl): 3680–2700*m* (br.), 2947*m*, 1740*s*, 1690*s*. ^{1}H-NMR (300 MHz, $CDCl_3$): 7.35–7.27 (*m*, 5 H); 5.13 (2*m*, 2 H); 3.77–3.35 (2*s* + *m*, 5 H); 3.17–3.07 (2*m*, 2 H); 2.64–1.68 (*m*, 4 H). ^{13}C-NMR (75 MHz, $CDCl_3$): 181.2, 174.1, 173.8, 154.6, 137.1, 136.1, 128.5, 128.3, 128.2, 128.0, 127.7, 67.5, 67.0, 66.5, 66.0, 52.9, 52.6, 48.8, 48.0, 39.4, 38.5, 37.5, 36.2, 23.2, 22.7.

(S)-1-[(Benzyloxy)carbonyl]-2-(carboxymethyl)proline (= *(S)-1-Benzyl 2-Hydrogen 2-(Carboxymethyl)-pyrrolidine-1,2-dicarboxylate*; (−)-**9**). The foregoing methyl ester (0.16 g, 0.5 mmol) in MeOH (10 ml), H_2O (5 ml), and $LiOH \cdot H_2O$ (0.2 g, 4.77 mmol) were stirred at r.t. for 6 h. Lyophilization and purification by HPLC (C_{18} column, gradient 20–50% solvent *B* over 20 min (see **3**)) gave (−)-**9** (0.15 g, 100%). White gum. $[\alpha]_D^{21} = -39.0$ ($c = 1.00$, MeOH). IR (NaCl): 3700–2300*s* (br.), 2980*s*, 1780–1600*s* (br.). ^{1}H-NMR (300 MHz, $CDCl_3$): 9.93 (br. *s*, 2 H); 7.35–7.27 (*m*, 5 H); 5.10 (*m*, 2 H); 3.72, 3.44 (2*m*, 2 H); 3.18, 3.01 (2*m*, 2 H); 2.56–1.88 (*m*, 4 H). ^{13}C-NMR (75 MHz, $CDCl_3$): 178.4, 177.6, 175.7, 174.9, 155.0, 154.3, 136.3, 135.8, 128.5, 128.0, 127.6, 67.8, 67.4, 66.4, 65.6, 48.6, 48.1, 39.2, 38.2, 37.6, 36.2, 23.2, 22.6.

(R)-1-[(Benzyloxy)carbonyl]-2-(carboxymethyl)proline ((+)-**9**). To establish its absolute configuration, dipeptide **6** (0.11 g, 0.22 mmol) was subjected to hydrolysis in 6N aq. HCl (5 ml) at 80°. After evaporation, the residue was treated with [(benzyloxy)carbonyl]-chloride, and the obtained product was purified by HPLC (C_{18} column, 20–50% solvent *B* over 20 min (see **3**)): (+)-**9** (68 mg, 99%). White gum. $[\alpha]_D^{21} = +42.8$ ($c = 0.78$, MeOH; *cf.* (−)-**9** above).

NMR and SA Calculations. ^{1}H-NMR spectra of **3** were recorded on an *AMX600* spectrometer in 90% H_2O/D_2O, pH 5, 300 K. Structure calculations were performed using data derived from cross-peak volumes quantitated in ROE spectra (ROESY) with 75, 150, 225, and 300 ms mixing times, by the method described previously [2].

Crystal Structure Determinations of **11** [2]. All measurements were conducted at low temperature on a *Rigaku-AFC5R* diffractometer fitted to a 12 kW rotating anode generator. The intensities were collected using ω scans, and three standard reflections, which were measured after every 150 reflections, remained stable throughout the data collection. The intensities were corrected for *Lorentz* and polarization effects, but not for absorption. The structure was solved by direct methods using SHELXS86 [16] which revealed the positions of all non-H-atoms.

---

[2]) Crystallographic data (excluding structure factors) for the structure reported in this paper have been deposited with the *Cambridge Crystallographic Data Centre* as supplementary publication No. CCDC-10/51. Copies of the data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CD2 1EZ, UK. (fax: + 44-(0)1223-336033 or email: teched@ccdc.cam.ac.uk).

The enantiomorph was assigned on the basis of the known (*S*) configuration at C(2). The non-H-atoms were refined anisotropically, while the H-atoms were located in difference electron density maps and their positions were refined together with individual isotropic displacement parameters. A correction for secondary extinction was applied. Refinement was carried out on *F* using full-matrix least-squares procedures which minimized the function $\sum w(|F_o| - |F_c|)^2$, where $1/w = [\sigma^2(F_o) + (0.005F_o)^2]$. All calculations were performed using the TEXSAN crystallographic software package [17]. The data collection and refinement parameters are listed in *Table 5*, and a view of the molecule is shown in *Fig. 1*.

Table 5. *Crystallographic Data for* **11**

Crystallized from	AcOEt
Empirical formula	$C_{16}H_{23}N_5O_4$
Formula weight	349.39
Crystal colour, habit	colourless, needle
Crystal dimensions [mm]	$0.12 \times 0.20 \times 0.43$
Temperature [K]	173(1)
Crystal system	orthorhombic
Space group	$P2_12_12_1$
Z	4
Reflections for cell determination	22
2θ range for cell determination [°]	31–40
Unit cell parameters *a*[Å]	8.567(4)
b[Å]	34.041(3)
c[Å]	5.974(6)
V[Å^3]	1742(2)
D_x[g cm^{-3}]	1.332
μ(MoK_α) [mm^{-1}]	0.0978
$2\theta_{max}$ [°]	60
Total reflections measured	3405
Symmetry-independent reflections	3271
Reflections observed [$I > 2\sigma(I)$]	2454
Parameters	319
Final *R*	0.0394
R_w	0.0327
Goodness of fit *s*	1.346
Secondary extinction coefficient	$8.2(9) \cdot 10^{-7}$
Final Δ_{max}/σ	0.0001
Δ_ρ(max; min) [e Å^{-3}]	0.21; −0.19

REFERENCES

[1] C. Bisang, C. Weber, J. A. Robinson, *Helv. Chim. Acta* **1996**, *79*, 1825.
[2] R. Beeli, M. Steger, A. Linden, J. A. Robinson, *Helv. Chim. Acta* **1996**, *79*, 2235.
[3] F. Emery, C. Bisang, M. Favre, L. Jiang, J. A. Robinson, *J. Chem. Soc., Chem. Commun.* **1996**, 2155.
[4] P. Remuzon, *Tetrahedron* **1996**, *52*, 13803.
[5] D. Seebach, M. Boes, R. Naef, W. B. Schweizer, *J. Am. Chem. Soc.* **1983**, *105*, 5390.
[6] U. Schmidt, A. Nikiforov, *Monatsh. Chem.* **1975**, *106*, 313.

[2]) Crystallographic data (excluding structure factors) for the structure reported in this paper have been deposited with the *Cambridge Crystallographic Data Centre* as supplementary publication No. CCDC-10/51. Copies of the data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CD2 1EZ, UK. (fax: + 44-(0)1223-336033 or email: teched@ccdc.cam.ac.uk).

[7] E. Eguchi, A. Kakuta, *J. Am. Chem. Soc.* **1974**, *96*, 3985.
[8] E. Eguchi, A. Kakuta, *Bull. Chem. Soc. Jpn.* **1974**, *47*, 2277.
[9] M. A. Sanner, C. Weigelt, M. Stansberry, K. Killeen, W. F. Michne, D. W. Kessler, R. K. Kullnig, *J. Org. Chem.* **1992**, *57*, 5264.
[10] G. Merutka, H. J. Dyson, P. E. Wright, *J. Biomol. NMR* **1995**, *5*, 14.
[11] C. Bisang, C. Weber, J. Inglis, C. A. Schiffer, W. F. van Gunsteren, I. Jelesarov, H. R. Bosshard, J. A. Robinson, *J. Am. Chem. Soc.* **1995**, *117*, 7904.
[12] E. G. Hutchinson, J. M. Thornton, *Protein Sci.* **1994**, *3*, 2207.
[13] A. P. Nanzer, A. E. Torda, C. Bisang, C. Weber, J. A. Robinson, W. F. van Gunsteren, *J. Mol. Biol.* **1997**, *267*, 1012.
[14] D. Gramberg, C. Weber, R. Beeli, J. Inglis, C. Bruns, J. A. Robinson, *Helv. Chim. Acta* **1995**, *78*, 1588.
[15] A. L. Van Geet, *Anal. Chem.* **1970**, *42*, 679.
[16] G. M. Sheldrick, SHELXS86. *Acta Crystallogr., Sect. A* **1990**, *46*, 467.
[17] TEXSAN, Single Crystal Structure Analysis Software, Version 5.0, Molecular Structure Corporation, The Woodlands, Texas, 1989.