Synthesis and Solution Conformation of β-Hairpin Mimetics Utilizing a Template Derived from (2*S*,3*R*,4*R*)-Diaminoproline

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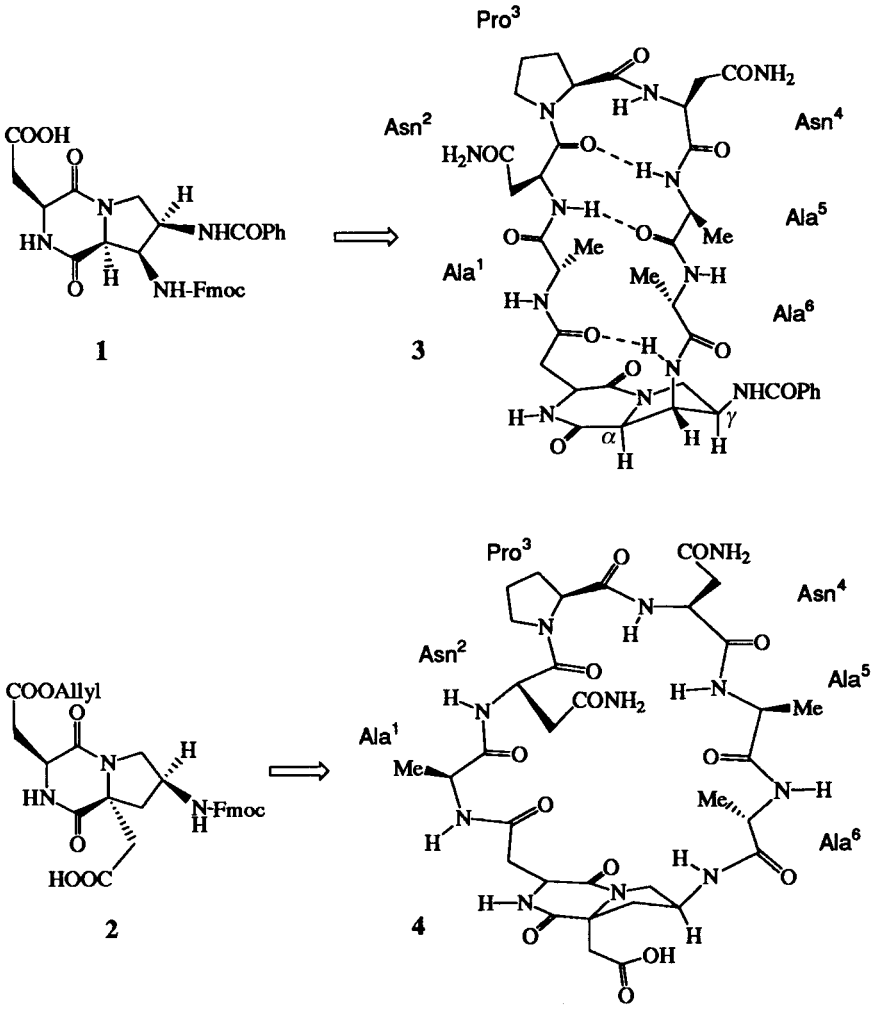
A novel template was synthesized for stabilizing β-hairpin conformations in cyclic peptide mimetics. The template is a diketopiperazine derived formally from L-aspartic acid and (2*S*,3*R*,4*R*)-diaminoproline, the latter being available by an efficient synthetic route from vitamin C. The template was incorporated by solid-phase peptide synthesis into a cyclic loop mimetic containing the sequence (-Ala-Asn-Pro-Asn-Ala-Ala-template-). This mimetic was shown by NMR to adopt a stable β-hairpin conformation in (D_6)DMSO solution. The template may prove to be generally useful for creating small-molecule mimetics of hairpin loops on proteins of diverse function.

1. Introduction. – Conformationally defined synthetic molecules that mimic surface epitopes on proteins are potentially valuable reagents in biology, as well as in drug and vaccine design [1]. Short linear peptides are inherently flexible molecules, especially in aqueous solution, and so are often poor mimics of the secondary structures (turns, α-helices, β-strands) found on the surfaces of folded proteins. To circumvent this folding problem, much attention has been focused on the design of templates that constrain peptide chains into biologically relevant secondary structures [2]. We report here a novel bicyclic template **1**, comprising a diketopiperazine derived from L-aspartic acid and (2*S*,3*R*,4*R*)-diaminoproline, which in the context of a cyclic peptide mimetic can stabilize β-hairpin conformations.

The design of **1** stems from our earlier studies with the related template **2** [3][4], which was used to generate the loop mimetic **4** containing the motif Asn-Pro-Asn-Ala (NPNA). The biological interest in the NPNA motif stems from its occurrence in a tandemly repeated form (as $NPNA)_{\approx 37}$) in the circumsporozoite (CS) protein of the malaria parasite *Plasmodium falciparum*, where it is believed to prefer a β-turn, or turn-like conformation [5][6]. Linear synthetic peptides containing tandemly repeated NPNA motifs were evaluated in the late 1980s as potential malaria vaccine candidates [7]; however, their efficacy was found to be low [8]. Such linear peptides in H_2O are conformationally flexible [5], whereas in the intact CS protein, the NPNA repeats presumably adopt a folded conformation. The structure of the native CS protein is unknown. NMR Studies showed that the NPNA motif in **4** adopts a stable β-turn conformation, and immunological studies showed that **4** can elicit anti-sporozoite antibodies in mice [3]. In the solution structure of **4**, the amido N-atom in the 4-aminoproline moiety of the template prefers a pseudo-equatorial position (an E_γ ring pucker), as shown in *Fig. 1*, which leads preferentially to a bulged loop conformation in aqueous solution, rather than to a stable β-hairpin geometry.



Since β-hairpins frequently play important roles in protein-protein recognition, and templates that specifically stabilize this secondary structure could be generally useful in peptide-mimetic design, we set out to modify **2** by introducing an additional axial amino group at the 3-position of the proline moiety, as in **1**, which could then be used to more accurately anchor a peptide loop in a β-hairpin geometry. We describe below the synthesis of this new template, and of the mimetic **3** containing the ANPNAA loop. Conformational studies with NMR and MD support the conclusion that **3** adopts a stable β-hairpin geometry, rather than the bulged loop conformation deduced earlier for **4**, as shown in *Fig. 1*. Some aspects of this work were described earlier in a communication [9].

2. Results and Discussion. – 2.1. *Synthesis of the Template.* A convenient gram-scale synthesis of (2*S*,3*R*,4*R*)-diaminoproline was established by exploiting a known route to

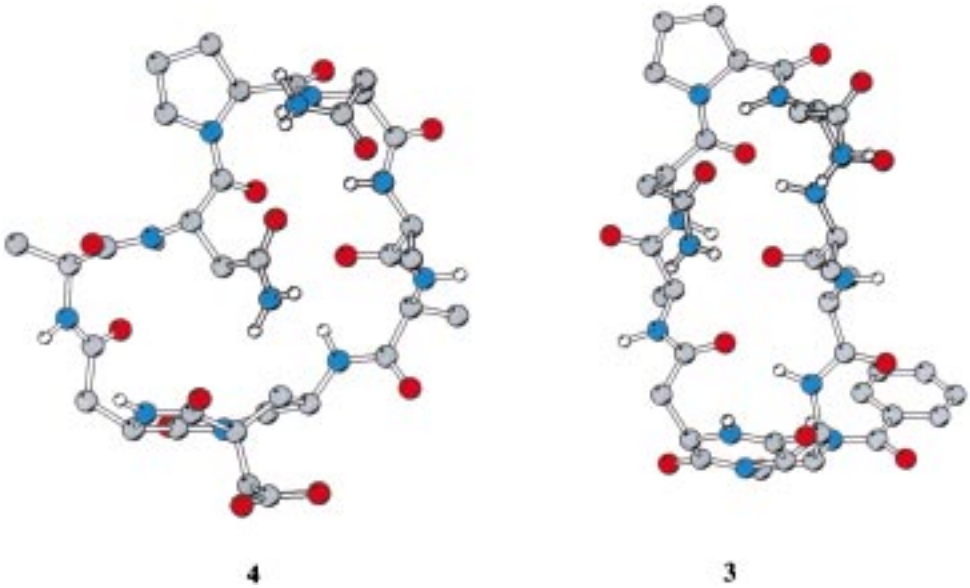


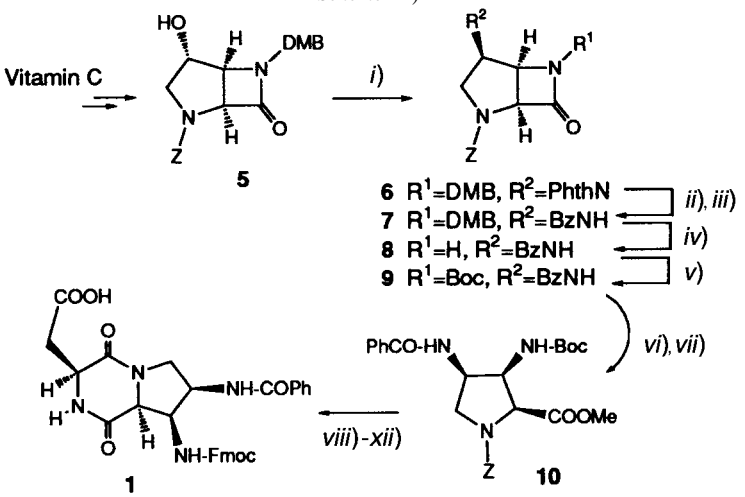
Fig. 1. *Average solution structures of* **3** (this work) *and* **4** (see [3]) *deduced by NMR and simulated annealing* (see text). All H-atoms apart from amide NHs are omitted for clarity. N-Atoms blue, O-atoms red, C-atoms grey, and amide H-atoms white.

the bicyclic β-lactam **5** from vitamin C [10–12], which has been implemented already on a multi-kilogram scale in a commercial synthesis of β-lactamase inhibitors. As shown in *Scheme 1*, **5** was converted to **6** by a *Mitsunobu* reaction [13] and subsequently into **9** *via* **7** and **8** by exchange of protecting groups. The β-lactam ring was then opened to yield, after esterification, the orthogonally protected (2*S*,3*R*,4*R*)-diaminoproline derivative **10**. Coupling to Z-Asp(O^{t}Bu)-OH, cyclization to afford the diketopiperazine, and further manipulation of the protecting groups gave **1** in good overall yield.

The intermediate **6** was also converted to the orthogonally protected 3,4-diaminoproline derivative **12** (*Scheme 2*). However, hydrolysis of the β-lactam in **11** (steps *iii*) and *iv*)) led partially to opening of the phthalimide protecting group, thereby reducing the yield and complicating the purification of **12**. The structure of **12** could be confirmed, however, by X-ray crystallography. In the crystal structure (*Fig. 2*), the pyrrolidine ring has a C(β)-*endo* envelope conformation ($\chi_1 = -38.9°$), *i.e.* C(β) is above the plane defined by the other four ring atoms ($^{\beta}E$). This places the carbamate N–C(β) N-atom in axial position (as desired for **3**) and the γ-amido substituent in an equatorial position. The carbamate NH group forms an intramolecular H-bond with the ester carbonyl O-atom (H-bond length 2.44 Å and donor-H-acceptor angle 117°).

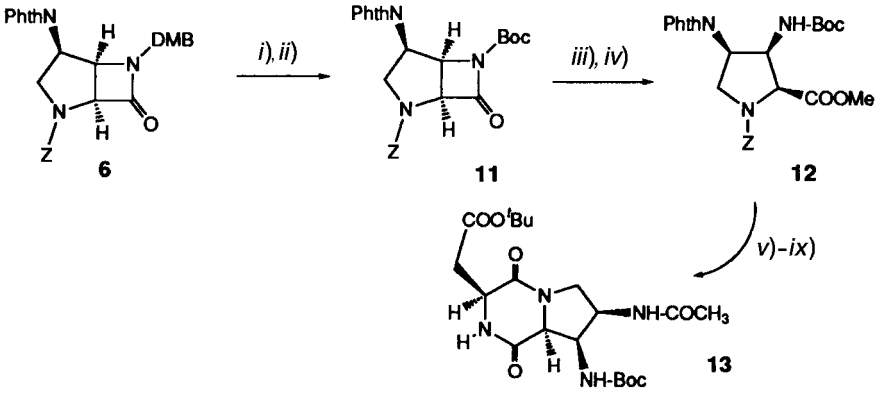
The intermediate **12** could then be converted to the diketopiperazine **13**, as shown in *Scheme 2*. It is potentially a useful feature of the template that the γ-amino substituent in the proline moiety can be functionalized with a variety of acyl groups (benzoyl in **1**, acetyl in **13**). For the present purpose, however, only the derivative **1** was carried forward for further studies.

Scheme 1 [1])



DMB = 2,4-dimethoxybenzyl, Z = $PhCH_2OCO$, Phth = phthalimido, Bz = PhCO, Boc = ^{t}BuOCO, Fmoc = (9*H*-fluoren-9-ylmethoxy)carbonyl. *i*) PhthH, Ph_3P, THF, DEAD; 61%. *ii*) $MeNHNH_2$, DMF, 80°. *iii*) Bz_2O, Et_3N, CH_2Cl_2; 66% (2 steps). *iv*) $K_2S_2O_8$, Na_2HPO_4, MeCN/H_2O, 78°; 77%. *v*) Boc_2O, Et_3N, DMAP, CH_2Cl_2; 60%. *vi*) Na_2CO_3, THF/H_2O. *vii*) CH_2N_2, Et_2O; 98% (2 steps). *viii*) H_2, Pd/C, DMF; 96%. *ix*) Z-Asp(O^{t}Bu)-OH, HATU, HOAt, ^{i}Pr$_2$EtN, CH_2Cl_2; 80%. *x*) H_2, Pd/C, DMF; 100%. *xi*) CF_3COOH, CH_2Cl_2, 89%. *xii*) Fmoc-ONSu, ^{i}Pr$_2$EtN, CH_2Cl_2; 60%.

Scheme 2 [1])



DMB = 2,4-dimethoxybenzyl, Z = $PhCH_2OCO$, Phth = phthalimido, Boc = ^{t}BuOCO. *i*) $K_2S_2O_8$, Na_2HPO_4, MeCN/H_2O, 78°. *ii*) Boc_2O, Et_3N, DMAP, CH_2Cl_2; 65% (2 steps). *iii*) Na_2CO_3, THF/H_2O. *iv*) CH_2N_2, Et_2O; 62% (2 steps). *v*) H_2, Pd/C; 100%. *vi*) Z-Asp(O^{t}Bu)-OH, HATU, HOAt, ^{i}Pr$_2$EtN, CH_2Cl_2; 80%. *vii*) H_2, Pd/C; 95%. *viii*) $MeNHNH_2$, 80°, EtOH. *ix*) Ac_2O; 70% (2 steps).

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[1]) Abbreviations: DEAD, diethyl diazenedicarboxylate; DMAP = 4-(dimethylamino)pyridine; DMF, dimethylformamide; HBTU, *O*-(1*H*-benzotriazol-1-yl)-*N,N,N′,N′*-tetramethyluronium hexafluorophosphate; HATU, *O*-(7-aza-1*H*-benzotriazol-1-yl)-*N,N,N′,N′*-tetramethyluronium hexafluorophosphate; HOBt, 1-hydroxy-1*H*-benzotriazole; HOAt, 1-hydroxy-7-aza-1*H*-benzotriazole; CIP, 2-chloro-1,3-dimethylimidazolidinium hexafluorophosphate; Mtt, 4-methyltrityl.

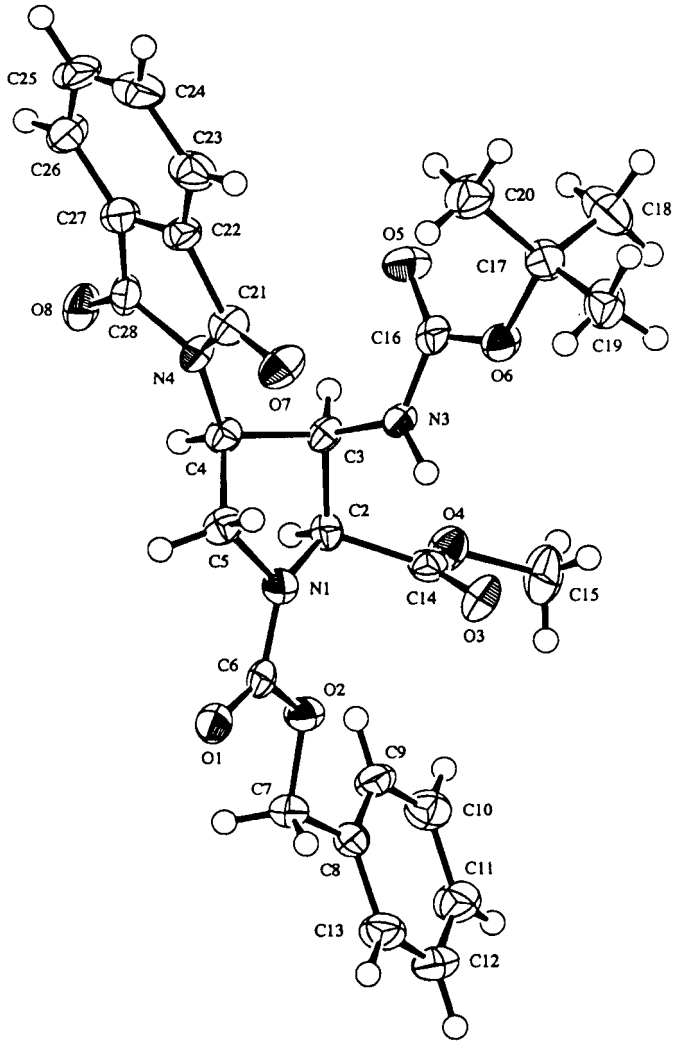


Fig. 2. *X-Ray crystal structure of* **12**

2.2. *Synthesis of the Loop Mimetic.* To evaluate the hairpin-inducing properties of the template, the target **3** containing the sequence -Ala-Asn-Pro-Asn-Ala-Ala- was chosen, so that a direct comparison of its preferred conformation could be made with that deduced for **4** in earlier studies [3][4]. The template **1** could be incorporated into the cyclic peptide **3** by standard solid-phase methods and Fmoc ((9*H*-fluoren-9-ylmethoxy)carbonyl) chemistry [14]. For example, **1** was coupled to *Tentagel-S-AC* resin, and the peptide chain was elaborated to afford H-Ala-Asn(Mtt)-Pro-Asn(Mtt)-Ala-Ala-template-resin[1]). After cleavage from the resin with 1% CF_3COOH in CH_2Cl_2, the linear precursor was cyclized in solution with HATU/HOAt[1]) in DMF, and all side-chain protecting groups were then removed with CF_3COOH/CH_2Cl_2 35 : 60

and $^{i}Pr_3SiH$ (5% *v*/*v*). After purification by HPLC, the cyclic peptide **3** was obtained from **1** in an unoptimized 11% yield.

2.3. *Conformational Studies.* The conformation of **3** was studied in DMSO solution rather than H_2O, due to the limited solubility of **3** in the latter solvent. ^3J Coupling constants, amide temperature coefficients, and amide proton H/D exchange rates, as well as NOEs were measured by NMR spectroscopy. From distance restraints derived from NOE intensities, average structures were then calculated by dynamic simulated annealing. A typical solution structure was also used as a starting point for MD simulations in explicit DMSO solvent. The results of these experiments and simulations are presented below.

The ^{1}H-NMR spectrum of **3** shows a single major species (> 98%) on the chemical-shift time scale in (D_6)DMSO solution, whereas **4** in D_2O showed *ca.* 10% of a minor conformer due to *cis*/*trans* isomerism at the Asn-Pro peptide bond. The spectrum was assigned by standard methods [15] at 305 K, a temperature at which the amide-proton resonances are optimally resolved (*Table 1*). The chemical shifts and line-widths were essentially invariant over the concentration range 21 – 0.6 mM, indicating an absence of significant aggregation under these conditions. The amide-proton temperature coefficients, relative H/D-exchange rates, and key NOE connectivities measured for **3** are summarized in *Fig. 3*, and coupling constants are collected in *Table 2*.

H/D-Exchange. The NH–C(β) amide proton of the diaminoproline moiety of the template in **3** exchanges very slowly with deuterium in (D_6)DMSO/(D_4)MeOH 9:1, with a half-life of several days, whereas under the same conditions, the half-lives for Ala^1 and Ala^6 NHs are around 30 min, and the aspartate NH of the template and the side-chain amide protons of Asn^2 and Asn^4 exchange completely within a few minutes. By contrast, with mimetic **4**, the NH–C(γ) amide proton of the template exchanged relatively quickly [3], in accord with the absence of a stable intramolecular H-bond to this NH group in **4**. A further interesting comparison arises with the disulfide-bridged analogue **14**, also studied in earlier work [3]. In this molecule, the disulfide bridge fixes the loop backbone into a stable β-hairpin conformation. The corresponding NH–C(γ)

Table 1. *^{1}H-NMR* (600 MHz) *Chemical Shifts for* **3** *at 305 K* in *(D_6)DMSO*

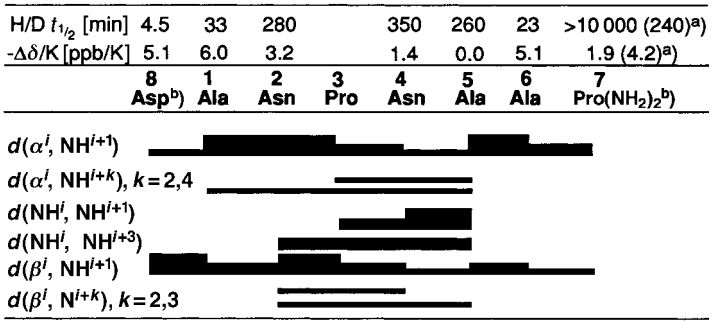
Residue[b])	Chemical shift [ppm][a])			
	NH	H–C(α)	CH_2(β)[c]) or Me(β)	Others
Ala^1	8.55	4.74	1.22	
Asn^2	8.50	4.84	2.83, 2.79	7.74 (NH(δ),(*E*); 7.02 (NH(δ),(*Z*))
Pro^3	–	4.01	2.17, 1.56	1.92, 1.80 (CH_2(γ)); 3.80, 3.42 (CH_2(δ))
Asn^4	7.95	4.52	2.48, 2.32	7.13 (NH(δ),(*E*)); 6.77 (NH(δ),(*Z*))
Ala^5	7.26	4.37	1.08	
Ala^6	8.48	4.77	1.30	
Pro(NH_2)$_2^7$[d])	8.24	4.60	4.69	4.78 (CH(γ)); *3.54*, *3.94*[b]) (CH_2(δ))
				8.22 (HNCOPh); 7.86, 7.49, 7.42 (arom. H)
Asp^8	8.33	4.18	*2.77*, *2.98*[c])	

[a]) Chemical shifts are measured relative to internal $SiMe_4$.
[b]) Pro(NH_2)^7 and Asp^8 are the 3,4-diaminoproline and aspartatic acid moieties of the template.
[c]) Stereospecific assignments are in *italics*, in the order *pro-R*, *pro-S*.
[d]) The Pro(NH_2)$_2^7$NH and HNCOPh refer to NH–C(β) and NH–C(γ), respectively.

Table 2. *Coupling Constants*[a]) [Hz] *for* **3**

Residue[b])	$^3J(\alpha,NH)$	$^3J(\alpha,\beta)$	Others
Ala[1]	7.5	6.8	
Asn[2]	7.6	5.0, 4.1	$^2J(\beta,\beta')=16.1$
Pro[3]	–	10.0, 7.4[c])	n.d.
Asn[4]	9.4	10.3, 3.9[c])	$^2J(\beta,\beta')=14.6$
Ala[5]	7.0	6.8	
Ala[6]	9.1	6.9	
$Pro(NH_2)_2$[7]	8.9[d])	3.5	$^3J(NH-C(\gamma)H)=7.0$, $^3J(\beta,\gamma)=4.3$, $^3J(\gamma,\delta_{pro\text{-}S})=10.1$, $^3J(\gamma,\delta_{pro\text{-}R})=9.6$, $^2J(\delta,\delta')=11.6$, $^5J(\alpha,\alpha')=2.0$
Asp[8]	<2.0	*4.1*, *2.6*[e])	$^2J(\beta,\beta')=16.9$

[a]) Measured from 1D ^{1}H-NMR spectra and/or E.COSY experiments.
[b]) $Pro(NH_2)_2$[7] and Asp[8] are the 3,4-diaminoproline and aspartic acid moieties of the template.
[c]) Stereospecific assignments not available.
[d]) Refers to the $^3J(NH-C(\beta),H)$ coupling.
[e]) Given in the order *pro-R*, *pro-S*.

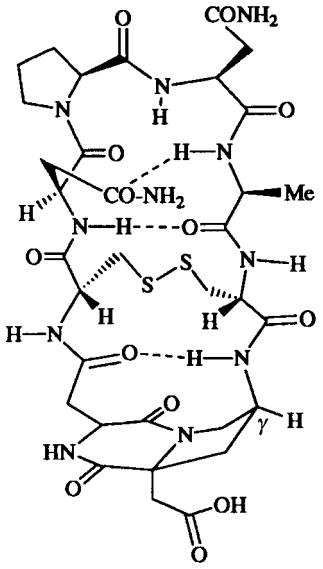


[a]) The values in brackets refer to the HN-C(γ) amide, the others to the HN-C(β) amide.
[b]) $Pro(NH_2)_2^7$ and Asp[8] are the 3,4-diaminoproline and aspartic acid moieties of the template.

Fig. 3. *Summary of NOEs, H/D exchange rates, and amide temperature coefficients measured for* **3** *by NMR*. The temperature coefficients ($-\Delta\delta/T$) in ppb/K were determined over the range 295–320 K in (D_6)DMSO; relative H/D exchange rates of peptide NH protons were determined by monitoring residual peak intensities after dissolution in (D_6)DMSO + 10% CD_3OD. The half-lives (H/D $t_{1/2}$ in min) were derived by fitting residual peak intensities to an exponential function.

amide proton of **14** again exchanges very slowly, mirroring the behaviour of the NH–C(β) amide proton of **3**. These data support the conclusions of structure calculations (see also below) that both **3** and **14** adopt similar β-hairpin conformations, with the NH–C(β) amide proton in **3** and the NH–C(γ) amide proton in **14** involved in intramolecular H-bonding across the hairpin.

Coupling Constants. The side chains of Asn[2] and Asn[4] in **3** have preferred χ_1 values, since non-averaged $^3J(\alpha,\beta)$ values are seen for Asn[2] (5.0 and 4.1 Hz) and for Asn[4] (3.9 and 10.3 Hz) (*Table 2*). A similar pattern was observed earlier for **14** (*i.e.*, Asn[2], $^3J(\alpha,\beta)$ 3.9 and 5.4 Hz; Asn[4], $^3J(\alpha,\beta)$ 5.4 and 10.1 Hz), whereas in **4**, high and low $^3J(\alpha,\beta)$ values



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were found for the methylene protons of both Asn[2] and Asn[4] [3]. The $^3J(\alpha,NH)$ for Ala[1], Asn[2], and Ala[5] of **3** are lower than expected for an ideal β-hairpin, although the Ala[6] and Asn[4] $^3J(\alpha,NH)$ values are close to those expected (*i.e.*, >9.0 Hz) for a β-strand and a type-I β-turn, respectively.

The $^3J(\gamma,\delta)$ coupling constants within the proline moiety of the template in **3** both have large values (9.6 and 10.1 Hz), as expected for a preferred $^{\beta}E$ envelope conformation, with the β-amido group axial and the γ-benzamido substituent equatorial. This conformation gives rise to small and large dihedral angles for $H-C(\gamma)-C(\delta)-H_{pro\text{-}R}$ and $H-C(\gamma)-C(\delta)-H_{pro\text{-}S}$, respectively, and hence to large $^3J(\gamma,\delta)$ values for both couplings. In comparison, the $^3J(\gamma,\delta)$ values in **4** (5.7 and 8.9 Hz) were close to the averaged values expected from rapid flipping, whereas in **14** two small values (<2.0 and 4.2 Hz) were found [3], as expected for the axial position of the γ-amido N-atom. The two small $^3J(\alpha,\beta)$ values for the aspartate moiety of the template in **3** indicate a preferred χ_1 torsion of around $+60°$, thus placing the C(γ) carbonyl group above the diketopiperazine ring, as found earlier for **4** and **14** [3]. Finally, the long-range $^5J(\alpha,\alpha')$ coupling (*Table 2*) is well precedented and indicates a significant puckering of the diketopiperazine ring [16][17].

NOEs and Structure Calculations. A summary of key NOEs observed in NOESY spectra of **3** in (D_6)DMSO is shown in *Fig. 3*. A cross-strand $H-C(\alpha)/H-C(\alpha)$ NOE between Ala[1] and Ala[6] could not be observed due to the small chemical-shift difference between these $H-C(\alpha)$ resonances (*Table 1*). However, several other NOEs were observed that strongly indicate a stable β-hairpin conformation, in particular between Asn^2NH^i/Ala^5NH^{i+3} and $Ala^1H-C(\alpha^i)/Ala^5NH^{i+4}$. NOESY Experiments also revealed for Ala[1]/Asn[2] as well as for Ala[5]/Ala[6] strong $H-C(\alpha^i)/NH^{i+1}$ NOEs, but an absence of NH^i/NH^{i+1} NOEs, as would be expected in the proposed β-strands. A β-turn in the

NPNA motif was indicated by a relatively strong Asn^4NH^i/Ala^5NH^{i+1} NOE, as well as by NOEs between $Asn^2H-C(\beta)$ and Ala^5NH (*Fig. 3*).

Since the NMR data provide evidence for a preferred conformation, average solution structures were determined by dynamic simulated annealing (SA), using distance restraints derived from NOE build-up curves. From 30 structures generated in this way, the energies of 15 were within 20 kcal/mol of the global energy minimum and showed no distance restraint violations >0.3 Å. Of this set, 8 structures were within 10 kcal/mol of the energy minimum, and all possessed essentially the same backbone β-hairpin conformation. The remaining seven possessed a distorted hairpin, and were not considered further because the $NH-C(\beta)$ was directed out towards solvent rather than participating in intramolecular H-bonding, as expected on the grounds of the very slow H/D exchange rate of this amide proton (*Fig. 3*).

The final 8 structures superimposed over the backbone C-, C(α)-, and N-atoms in residues Ala^1–Ala^6 with an average pairwise r.m.s.d. of only 0.11 Å (*Fig. 4*). Three cross-strand H-bonds were observed, one involving the template $Asp^8C(\gamma)O/Pro(NH_2)_2{}^7NH-C(\beta)$ groups, as well as backbone Asn^2NH/Ala^5CO and Asn^2CO/Ala^5NH. The three amide NH groups involved in these H-bonds are also those that have the slowest H/D exchange rates (*Fig. 3*), whereas the Ala^1 and Ala^6 peptide NHs point outwards from the hairpin, are not involved in cross-strand H-bonding, and have

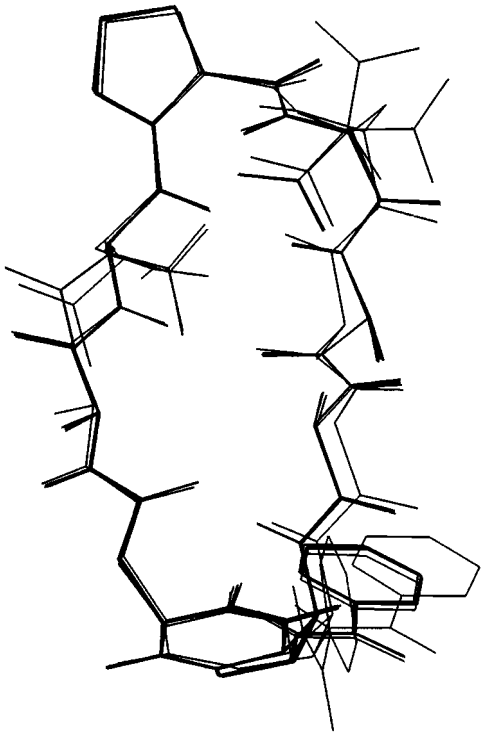

Fig. 4. *Superimposition over the backbone N-, C(α)-, and C-atoms of the final solution structures for* **3** *determined by simulated annealing* (see text)

relatively fast H/D exchange rates. The Asn[4] peptide NH forms H-bonds with the side-chain carbonyl O-atom of Asn[2]. The Asn[2]-Pro[3]-Asn[4]-Ala[5] motif is locked into close to a type-I β-turn conformation. The Asn[2] side-chain conformation in the minimum energy structure (*Fig. 1*) also has both H–C(α)–C(β)–H torsion angles at *ca.* 60°, which is consistent with the two small $^3J(\alpha,\beta)$ values (*Table 2*) found for Asn[2]. With this χ_1 angle, the Asn[2] side-chain amide CO group can H-bond with either the Asn[4] or Ala[5] peptide NH groups (*Fig. 1*).

The average solution structure deduced for **3** was compared with that deduced for the disulfide-bridged loop mimetic **14** in earlier work. As shown in *Fig. 5*, the backbone conformations of the two mimetics are essentially identical.

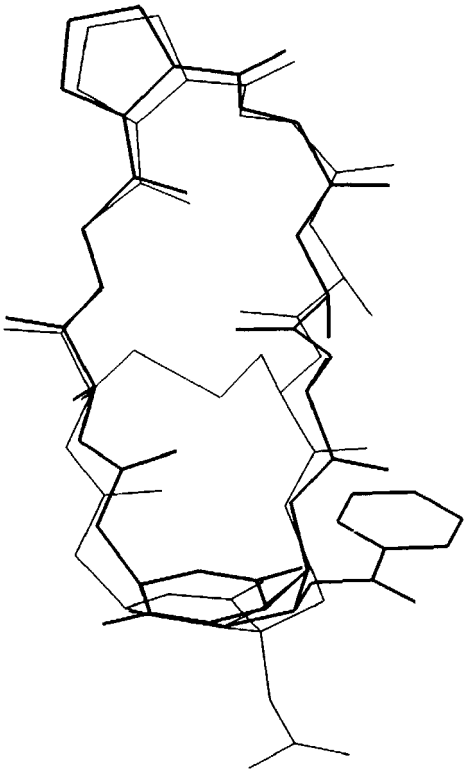

Fig. 5. *Superimposition over the backbone N-, C(α)-, and C-atoms of the solution structures for* **3** *and* **14** (see [3]) *determined by simulated annealing* (see text)

MD Simulations. Simulations of **3** were carried out in explicit DMSO solvent at 300 K to explore how the structural properties inferred from NOEs might be influenced by motional averaging. The minimum-energy structure found for **3** by SA was used as a starting point for 2-ns MD simulations, both with and without time-averaged distance restraints (TA-DR) [18][19]. The results of both simulations were similar, suggesting that the restraints are well satisfied by structures at or near a local or global energy minimum in the force field used.

In the 2-ns simulation with TA-DR, only two of 39 average restraint violations were within the range 0.5–1.0 Å, the others were <0.5 Å. The average ^3J coupling constants showed average violations for several backbone $^3J(\alpha,\mathrm{NH})$ values of *ca.* 1.6 Hz. The reasons for the discrepancies are unknown, but conceivably may reflect an insufficiently long simulation time or an inadequate representation of the mimetic in the force field. The Pro[3] and Asn[4] ϕ/ψ angles remain for most of the simulation with TA-DR within $\pm 30^\circ$ of the values expected for a βI-turn conformation (*Fig. 6*). And three well-populated cross-strand backbone-backbone H-bonds are found in the restrained and unrestrained trajectories (*Fig. 7*). The population of all three simultaneously would correspond to a class 2:2 β-hairpin, the most abundant β-hairpin type found in high-resolution protein crystal structures [20]. However, the most frequently found turn types in 2:2 β-hairpins in proteins are the type-I′ and -II′, and less so the type-I turn [21][22]. Here, type-I′ and -II′ turns are not possible due to the presence of proline ($\phi \approx -60^\circ$) in the $i+1$ position. The Asn[2]CO/Ala[5]NH H-bond is only poorly populated in the unrestrained simulation. Instead, the side-chain CO of Asn[2] frequently acts as an H-bond acceptor for the main-chain NHs of Asn[4] and Ala[5] (*Figs. 1* and *7*). There is again a good correlation between the involvement of amide NHs in intramolecular H-bonding, indicated experimentally by relatively slow H/D exchange rates and the population of H-bonds during the MD simulation (*Figs. 3* and *7*). On the other hand, the significantly slower rate of H/D exchange seen for the template NH–C(β), in comparison to all others (*Fig. 3*), is not so well reflected in the different H-bonding populations in the restrained simulation (*Fig. 7*).

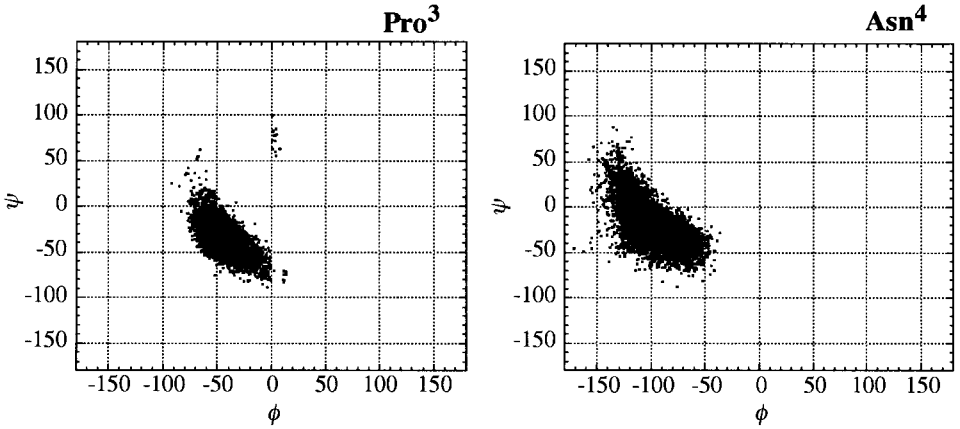

Fig. 6. *Variations in ϕ and ψ torsion angles for Pro[3] and Asn[4] during the MD simulation with TA-DR*

Large variations of the ring pucker in the diaminoproline moiety of the template in **3** were not observed during either simulation, unlike in simulations reported earlier with **4** [3]. The χ_1 torsion angle (N–C(α)–C(β)–C(γ)) remains close to -35° throughout both simulations (*Fig. 8,a*), with the $^{\beta}E$ ring pucker and the NH–C(β) substituent in an axial position and the peptide loop in a β-hairpin conformation. For comparison, an unrestrained MD simulation was also performed under the same conditions but starting with an energy-minimized structure adopting an inverted $^{\gamma}E$

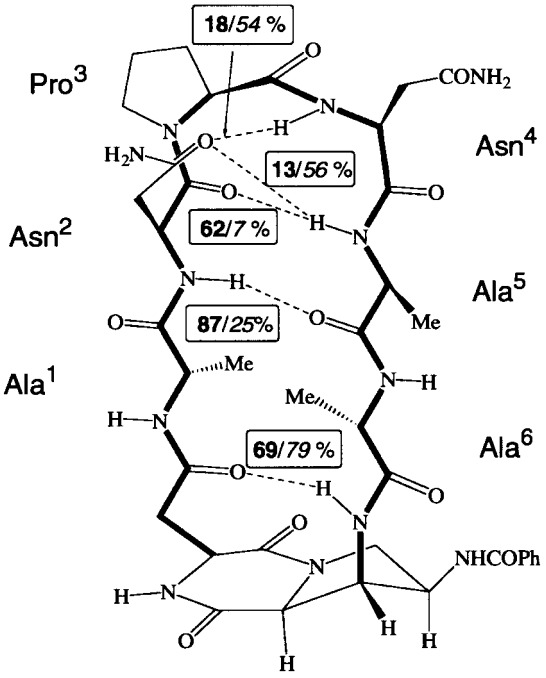

Fig. 7. *Population of H-bonds during the MD simulation with TA-DR*. The H-bonds are shown by broken lines and the percent population over the entire 2-ns simulation with TA-DR (in bold) and without restraints (in italics) are given.

conformation ($\chi_1 \approx 20°$; C(γ)-*endo*) within the diaminoproline moiety of the template (*i.e.*, with the NH–C(β) substituent in an equatorial position (*Fig. 8,b*)) and a bulged-loop backbone conformation. This structure was derived by computer modelling, using the SA structure deduced for **4** in earlier work, and exchanging templates. About 200 ps into the simulation of this model system, the pyrrolidine ring of the template flipped back to the $^{\beta}E$ conformation ($\chi_1 \approx -35°$; C(β)-*endo*; *Fig. 8,b*), and the loop backbone into a 2:2 β-hairpin geometry, as seen in the simulations starting from the NMR solution structure of **3**.

In summary, the simulations indicate only a limited motional averaging within the backbone of the mimetic **3**, and reinforce the conclusion that the molecule adopts a well-defined β-hairpin conformation.

3. Conclusions. – An efficient synthetic route to the novel template **1** has been developed. The template can be incorporated into a linear peptide-mimetic precursor using solid-phase Fmoc chemistry, with subsequent solution-phase macrocyclization to afford mimetic **3**. NMR Spectra and MD simulations of **3** indicate a well defined β-hairpin conformation in DMSO solution. The deduced average solution structures (*Fig. 1*) are well satisfied by the NOEs and amide H/D-exchange rates, and comprise a well-populated class 2:2 β-hairpin conformation with a type-I β-turn in the NPNA motif at the tip of the hairpin loop. It seems that the template may be useful for

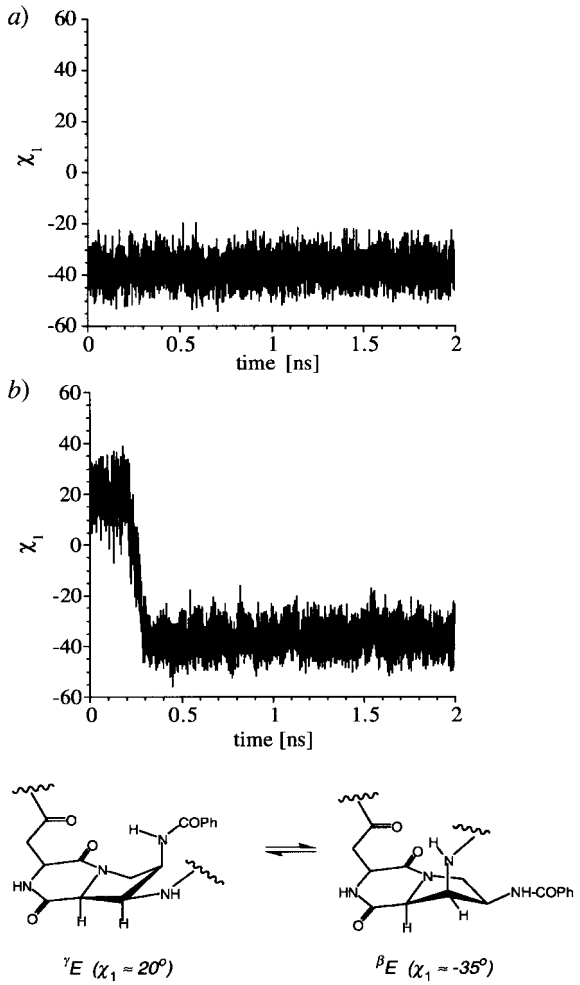

Fig. 8. a) χ_1 *Torsion angle in the template* (N–C(α)–C(β)–C(γ)) *monitored during the course of an MD simulation with TA-DR, starting from the solution SA structure, and* b) *as in* a) *but starting from a structure with the template in a* $^{\gamma}$E ($\chi_1 \approx 20°$) *conformation*

inducing β-hairpin conformations in six (or more) residue loops of diverse sequence, although no systematic investigation of the dependence of loop conformation on peptide sequence has so far been carried out. We have shown in other studies [23] that a template comprising a heterochiral diproline unit (D-Pro-L-Pro) is very effective in stabilizing β-hairpin conformations in octapeptide loops. Given the wide occurrence of β-hairpins in proteins of diverse function, both **1** and the D-Pro-L-Pro unit may prove to be generally useful in hairpin-mimetic design.

The authors thank the *Swiss National Science Foundation* for financial support and Dr. *Pflieger*, *F. Hoffmann-La Roche Ltd.*, Basel, for a generous gift of compound **5**.

Experimental Part

General. See [24].

Benzyl (1S,4R,5R)-6-(2,4-Dimethoxybenzyl)-4-(1,3-dihydro-1,3-dioxo-2H-isoindol-2-yl)-7-oxo-2,6-diazabicyclo[3.2.0]heptane-2-carboxylate (**6**). To a suspension of alcohol **5** (20 g, 48.5 mmol), triphenylphosphine (31.8 g, 121.2 mmol), and phthalimide (21.4 g, 145.4 mmol) in anh. THF (500 ml), diethyl diazenedicarboxylate (DEAD; 9.1 ml, 58.1 mmol) was slowly added at −30° under Ar. After stirring at −30° for 3 h, the soln. was stirred at r.t. for 16 h. The soln. was washed with sat. aq. $NaHCO_3$ soln. and brine, dried ($MgSO_4$), and evaporated and the product purified by FC (hexane/AcOEt 4:6 → 3:7): **6** (16.0 g, 61%). TLC (hexane/AcOEt 4:6): R_f 0.44. White solid. M.p. 56–58°. $[\alpha]_D^{20} = -55.1$ ($c = 0.490$, AcOEt). IR (KBr): 2940*w*, 2832*w*, 1765*s*, 1712v*s*, 1611*m*, 1589*m*, 1506*m*. ^{1}H-NMR (300 MHz, (D_6)acetone): 7.89–7.80 (*m*, 4 H); 7.44–7.28 (*m*, 5 H); 6.81 (*d*, $J = 8.3$, 1 H); 6.22 (*d*, $J = 2.4$, 1 H); 6.19 (*dd*, $J = 8.3$, 2.4, 1 H); 5.24 (br. *d*, $J = 3.7$, 1 H); 5.17 (*s*, 2 H); 4.55–4.36 (*m*, 4 H); 4.30 (*d*, $J = 15.0$, 1 H); 3.98 (*d*, $J = 15.0$, 1 H); 3.64 (*s*, 3 H); 3.50 (*s*, 3 H). ^{13}C-NMR (75 MHz, (D_6)acetone): 169.0 (*s*); 166.0 (*s*); 161.6 (*s*); 159.0 (*s*); 154.5 (*s*); 138.0 (*s*); 135.2 (*d*); 132.8 (*s*); 131.3 (*d*); 129.4 (*d*); 128.8 (*d*); 128.7 (*d*); 124.2 (*d*); 117.2 (*s*); 105.5 (*d*); 99.3 (*d*); 67.75 (*d*); 67.71 (*t*); 59.3 (*d*); 58.3 (*d*); 55.7 (*q*); 55.5 (*q*); 43.7 (*t*); 40.6 (*t*). ESI-MS: 564.2 ($[M + Na]^+$), 542.2 ($[M + H]^+$).

Benzyl (1S,4R,5R)-4-(Benzoylamino)-6-(2,4-dimethoxybenzyl)-7-oxo-2,6-diazabicyclo[3.2.0]heptane-2-carboxylate (**7**). To a soln. of **6** (8 g, 14.8 mmol) in DMF (250 ml), methylhydrazine (3.9 ml, 5 equiv., 74.1 mmol was added). After stirring at 80° for 1.5 h, DMF was evaporated, and CH_2Cl_2 (250 ml), Et_3N (2.1 ml, 15.1 mmol), and benzoic anhydride (3.5 g, 15.5 mmol) were added. After stirring overnight, the suspension was washed with 10% aq. citric acid soln. and brine, dried ($MgSO_4$), and evaporated. Purification by FC (hexane/AcOEt 3:7; R_f 0.66) gave **7** (5.0 g, 66% over 2 steps). White solid. M.p. 142–143°. TLC (hexane/AcOEt 2:8): R_f 0.76. $[\alpha]_D^{20} = -43.4$ ($c = 0.53$, AcOEt). IR (KBr): 3285*s*, 3054*w*, 3020*w*, 3000*w*, 3020*w*, 2930*m*, 2835*w*, 1755v*s*, 1729v*s*, 1712v*s*, 1660*s*, 1629v*s*, 1585*s*, 1535v*s*, 1506*s*. ^{1}H-NMR (300 MHz, (D_6)acetone): 7.91–7.24 (*m*, 11 H); 6.99 (*d*, $J = 8.2$, 1 H); 6.45 (*d*, $J = 2.3$, 1 H); 6.41 (*dd*, $J = 8.2$, 2.3, 1 H); 5.17 (br. *s*, 3 H); 4.61–4.51 (*m*, 1 H); 4.54 (*d*, $J = 15.1$, 1 H); 4.39–4.19 (*m*, 2 H); 4.17 (*d*, $J = 15.1$, 1 H); 3.76 (*s*, 3 H); 3.61 (*s*, 3 H); 3.55 (*dd*, $J = 10.8$, 10.2, 1 H). ^{13}C-NMR (75 MHz, (D_6)acetone): 167.7 (*s*); 166.1 (*s*); 161.9 (*s*); 159.3 (*s*); 154.5 (*s*); 138.0 (*s*); 135.1 (*s*); 132.4 (*d*); 131.4 (*d*); 129.4 (*d*); 129.3 (*d*); 128.8 (*d*); 128.4 (*d*); 117.2 (*s*); 105.5 (*d*); 99.2 (*d*); 68.5 (*d*); 67.6 (*t*); 58.6 (*d*); 55.8 (*q*); 55.7 (*q*); 51.1 (*d*); 47.5 (*t*); 41.3 (*t*). ESI-MS: 538.3 ($[M + Na]^+$), 516.3 ($[M + H]^+$). Anal. calc. for $C_{29}H_{29}N_3O_6$ (515.56): C 67.6, H 5.7, N 8.2; found: C 67.5, H 5.7, N 8.2.

Benzyl (1S,4R,5R)-4-(Benzoylamino)-7-oxo-2,6-diazabicyclo[3.2.0]heptane-2-carboxylate (**8**). To a refluxing soln. of **7** (4.4 g, 8.5 mmol) in MeCN (75 ml) and H_2O (10 ml), a soln. of $K_2S_2O_8$ (9.2 g, 4 equiv., 34.0 mmol) and $Na_2HPO_4 \cdot 2H_2O$ (12.2 g, 8 equiv., 68.5 mmol) in H_2O (50 ml) was added in 4 portions under vigorous stirring every 5 min. Upon conversion (TLC control, 55 min), the orange soln. was immediately cooled and the two-phase system made slightly alkaline by adding crystalline $NaHCO_3$. The aq. phase was extracted with AcOEt, the org. phase washed successively with sat. aq. $NaHCO_3$ soln. and brine, dried ($MgSO_4$), and evaporated, and the residue purified by FC (hexane/AcOEt 1:1): **8** (2.4 g, 77%). Off-white solid. M.p. 92–94°. TLC (hexane/AcOEt 1:4): R_f 0.37. $[\alpha]_D^{20} = -4.9$ ($c = 0.51$, AcOEt). IR (KBr): 3295*m* (br.), 3060*w*, 2950*w*, 1769v*s*, 1722*s*, 1710*s*, 1696*s*, 1680*s*, 1665*s*, 1645*s*, 1602*m*, 1579*m*, 1534*s*. ^{1}H-NMR (300 MHz, (D_6)acetone): 7.92–7.28 (*m*, 10 H); 5.26 (br. *s*, 1 H); 5.15 (br. *s*, 2 H); 4.54–4.48 (*m*, 2 H); 4.47 (br. *dd*, 1 H); 3.42 (*dd*, $J = 10.8$, 10.2, 1 H). ^{13}C-NMR (75 MHz, (D_6)acetone): 167.9 (*s*); 166.3 (*s*); 154.4 (*s*); 138.0 (*s*); 135.3 (*s*); 132.4 (*d*); 129.4 (*d*); 129.2 (*d*); 128.8 (*d*); 128.4 (*d*); 69.8 (*d*); 67.6 (*t*); 56.6, 54.7 (*d*); 51.4 (*d*); 50.7 (*d*); 47.7 (*t*). ESI-MS: 388.2 ($[M + Na]^+$).

*2-Benzyl 6-(*tert*-Butyl) (1S,4R,5R)-4-(Benzoylamino)-7-oxo-2,6-diazabicyclo[3.2.0]heptane-2,6-dicarboxylate* (**9**). To **8** (1.46 g, 4.0 mmol), Et_3N (558 ml, 4.0 mmol), and 4-(dimethylamino)pyridine (DMAP; 0.49 g, 4.0 mmol) in anh. CH_2Cl_2 (50 ml), $(Boc)_2O$ (1.74 g, 8.0 mmol) was added. After stirring for 1 h, the org. phase was washed with 1% aq. citric acid soln. and brine, dried ($MgSO_4$), and evaporated, and the residue purified by FC (hexane/AcOEt 3:2): **9** (1.12 g, 60%). Yellowish solid. TLC (hexane/AcOEt 1:1): R_f 0.5. M.p. >65° (dec.). $[\alpha]_D^{21} = -49.6$ ($c = 0.482$, AcOEt). IR (KBr): 3342*s* (br.), 3060*m*, 3030*w*, 2978*m*, 2930*m*, 1812v*s*, 1727v*s*, 1712v*s*, 1692v*s*, 1665v*s*, 1602v*s*, 1578*s*, 1550v*s*, 1536v*s*. ^{1}H-NMR (300 MHz, (D_6)acetone): 8.08 (br. *d*, $J = 6.4$, 1 H); 7.95 (*d*, $J = 7.3$, 2 H); 7.57–7.29 (*m*, 8 H); 5.31 (*d*, $J = 4.8$, 1 H); 5.12 (*s*, 2 H); 4.87 (*dd*, $J = 5.0$, 4.8, 1 H); 4.68 (*dddd*, $J = 11.0$, 10.7, 6.4, 5.0, 1 H); 4.34 (br. *m*, 1 H); 3.36 (*dd*, $J = 11.0$, 10.7, 1 H); 1.29 (*s*, 9 H). NOE: 5.31 (H–C(1)) → 4.87 (H–C(5)); 4.87 (H–C(5)) → 5.31 (H–C(1)); 4.68 (H–C(4)) → 4.87 (H–C(5)); 4.34 ($H_{pro\text{-}R}$–C(3)) → 4.68 (H–C(4)); 3.36 ($H_{pro\text{-}S}$–C(3)) → 4.34 ($H_{pro\text{-}R}$–C(3)). ^{13}C-NMR (75 MHz, (D_6)acetone): 167.4 (*s*); 163.9 (*s*); 154.4 (*s*); 154.1 (*s*); 149.6 (*s*); 137.7 (*s*); 135.1 (*s*); 132.4 (*d*); 129.3 (*d*); 129.2 (*d*); 128.9 (*d*);

128.3 (*d*); 84.0 (*s*); 68.0 (*d*); 67.9 (*t*); 59.9 (*d*); 58.9 (*d*); 51.2 (*d*); 50.5 (*d*); 48.1 (*t*); 28.1 (*q*). ESI-MS: 488.2 ($[M+Na]^+$), 410.1 ($[M+H-{}^t\mathrm{Bu}]^+$), 388.1 ($[M+Na-{}^t\mathrm{Bu}-CO_2]^+$).

*Methyl (2*S*,3*R*,4*R*)-4-(Benzoylamino)-1-[(benzyloxy)carbonyl]-3-{[(*tert*-butoxy)carbonyl]amino}prolinate* (**10**). To a soln. of **9** (1.2 g, 2.6 mmol) in THF (65 ml) and H_2O (20 ml), crystalline Na_2CO_3 (0.68 g, 6.4 mmol) was added portionwise. When the turbid soln. had cleared (a few ml of H_2O may be added), THF was evaporated and the aq. soln. acidified to pH 4.5 with dil. aq. AcOH soln. The white suspension was extracted with AcOEt and the org. phase washed with brine, dried (Na_2SO_4), and evaporated. The crude product (free carboxylic acid; ESI-MS: 989.6 ($[2M+Na]^+$), 788.4 ($[2(M-{}^t\mathrm{Bu}-CO_2)+Na]^+$), 506.2 ($[M+Na]^+$), 484.2 ($[M+H]^+$), 428.1 ($[M+H-{}^t\mathrm{Bu}]^+$), 384.0 ($[M+H-{}^t\mathrm{Bu}-CO_2]^+$)) in AcOEt (20 ml) was titrated with CH_2N_2 in Et_2O. The org. phase was evaporated: **10** (1.26 g, 98%). White solid. TLC (hexane/AcOEt 1:1): R_f 0.55. M.p. 68–70°. $[\alpha]_D^{20}=+11.1$ ($c=0.5$, AcOEt). IR (KBr): 3349*s* (br.), 3065*m*, 3033*m*, 2979*s*, 2899*m*, 1715v*s*, 1668v*s*, 1603*m*, 1581*s*, 1536v*s*. ^{1}H-NMR (300 MHz, (D_6)acetone; (*E*)/(*Z*)-isomers): 7.86 (br. *d*, 3 H); 7.58–7.31 (*m*, 8 H); 6.31 (2 br. *d*, 1 H); 5.19–4.94, 4.81–4.66 (2*m*, 5 H); 3.94–3.66 (*m*, 5 H); 1.36, 1.35 (2*s*, 9 H). ^{13}C-NMR (75 MHz, (D_6)acetone; (*E*)/(*Z*)-isomers): 173.6, 172.9 (2*s*); 167.2 (*s*); 156.4 (*s*); 154.8, 154.4 (2*s*); 137.7, 137.6 (2*s*); 135.3 (*s*); 132.4 (*d*); 129.3 (*d*); 128.9 (*d*); 128.7 (*d*); 128.6 (*d*); 128.2 (*d*); 79.8 (*s*); 67.7 (*t*); 62.4 (*d*); 62.0 (*d*); 55.2 (*d*); 54.3 (*d*); 53.0 (*q*); 52.3 (*t*); 52.1 (*t*); 51.6 (*d*); 50.7 (*d*); 28.5 (*q*). ESI-MS: 520.4 ($[M+Na]^+$), 498.3 ($[M+H]^+$), 442.3 ($[M+H-{}^t\mathrm{Bu}]^+$), 398.2 ($[M+H-{}^t\mathrm{Bu}-CO_2]^+$).

*Methyl (2*S*,3*R*,4*R*)-4-(Benzoylamino)-3-{[(*tert*-butoxy)carbonyl]amino}prolinate.* A soln. of **10** (1.18 g, 2.37 mmol) in DMF (80 ml) was stirred with 10% Pd/C (0.2 g) under H_2 for 1.5 d. DMF was evaporated, the residue suspended in AcOEt, the mixture filtered through *Celite*, and the filtrate evaporated: white solid (0.83 g, 96%). TLC (CH_2Cl_2/MeOH 95:5): R_f 0.32. M.p. 73–74°. $[\alpha]_D^{20}=-4.4$ ($c=0.39$, AcOEt). IR (KBr): 3365*m* (br.), 2978*w*, 2931*w*, 1745*m*, 1730*m*, 1710*s*, 1694*s*, 1680*s*, 1670*s*, 1645*s*, 1604*s*, 1579*m*, 1540*s*, 1490*m*. ^{1}H-NMR (600 MHz, (D_6)acetone): 7.88 (*d*, *J* = 7.1, 2 H); 7.77 (br. *d*, *J* = 6.8, 1 H); 7.56–7.43 (*m*, 3 H); 5.92 (br. *d*, *J* = 7.8, 1 H); 4.80 (*dddd*, *J* = 7.1, 6.8, 6.1, 5.7, 1 H); 4.56 (*ddd*, *J* = 7.8, 6.4, 6.1, 1 H); 4.08 (*d*, *J* = 6.4, 1 H); 3.69 (*s*, 3 H); 3.30 (*dd*, *J* = 10.6, 7.1, 1 H); 3.13 (*dd*, *J* = 10.6, 5.7, 1 H); 1.33 (*s*, 9 H). ^{13}C-NMR (150 MHz, (D_6)acetone): 174.0 (*s*); 167.0 (*s*); 156.1 (*s*); 135.4 (*s*); 132.1 (*d*); 129.0 (*d*); 128.1 (*d*); 79.0 (*s*); 63.6 (*d*); 55.5 (*d*); 52.6 (*d*); 52.3 (*q*); 50.2 (*t*); 28.4 (*q*). ESI-MS: 386.3 ($[M+Na]^+$), 364.3 ($[M+H]^+$), 308.3 ($[M+H-{}^t\mathrm{Bu}]^+$).

*Methyl {N^2-[(Benzyloxy)carbonyl]-O^4-(*tert*-butyl)-L-aspart-1-yl}-(2*S*,3*R*,4*R*)-4-(benzoylamino)-3-{[(*tert*-butoxy)carbonyl]amino}prolinate.* To the foregoing product (0.79 g, 2.17 mmol), Z-Asp(O^{t}Bu)-OH (0.84 g, 2.6 mmol), HATU (0.99 g, 2.6 mmol), and HOAt (0.3 g, 2.2 mmol) in anh. CH_2Cl_2 (25 ml), $^i\mathrm{Pr}_2$EtN (1.1 ml, 6.43 mmol) was added, and the soln. was stirred at r.t. for 24 h[1]). After washing with sat. aq. $NaHCO_3$ soln. and brine, the org. phase was dried ($MgSO_4$) and evaporated and the residue purified by FC (hexane/AcOEt 2:3): white solid (1.16 g, 80%). TLC (hexane/AcOEt 1:1): R_f 0.56. M.p. 97–98°. $[\alpha]_D^{20}=-21.2$ ($c=0.48$, AcOEt). IR (KBr): 3320*m* (br.), 3060*w*, 2975*m*, 2930*m*, 1739v*s*, 1728v*s*, 1714v*s*, 1660v*s*, 1650v*s*, 1602*m*, 1580*m*, 1534v*s*, 1503*s*, 1486*s*. ^{1}H-NMR (300 MHz, (D_6)acetone): 7.96 (*d*, *J* = 9.1, 1 H); 7.84 (*d*, *J* = 7.3, 2 H); 7.52–7.39 (*m*, 3 H); 7.32–7.25 (*m*, 5 H); 6.73 (*d*, *J* = 8.6, 1 H); 6.18 (br. *d*, 1 H); 5.03–4.98 (*s* + *m*, 3 H); 4.78–4.59 (*m*, 3 H); 4.05–3.96 (*m*, 2 H); 3.68 (*s*, 3 H); 2.73 (*dd*, *J* = 16.3, 7.9, 1 H); 2.50 (*dd*, *J* = 16.3, 6.2, 1 H); 1.32, 1.31 (2*s*, 18 H). ^{13}C-NMR (75 MHz, (D_6)acetone): 172.6 (*s*); 170.9 (*s*); 170.2 (*s*); 167.2 (*s*); 156.6 (*s*); 156.3 (*s*); 137.9 (*s*); 135.4 (*s*); 132.4 (*d*); 129.3 (*d*); 128.8 (*d*); 128.5 (*d*); 128.3 (*d*); 81.3 (*s*); 79.8 (*s*); 67.2 (*t*); 62.1 (*d*); 53.5 (*d*); 52.9 (*q*); 52.7 (*t*); 52.0 (*d*); 50.0 (*d*); 38.7 (*t*); 28.5 (*q*); 28.2 (*q*). ESI-MS: 691.5 ($[M+Na]^+$), 669.4 ($[M+H]^+$), 613.4 ($[M+H-{}^t\mathrm{Bu}]^+$), 557.4 ($[M+H-2\,{}^t\mathrm{Bu}]^+$); 513.3 ($[M+H-2\,{}^t\mathrm{Bu}-CO_2]^+$).

tert-*Butyl (3*S*,7*R*,8*R*,8a*S*)-7-(Benzoylamino)-8-{[(*tert*-butoxy)carbonyl]amino}octahydro-1,4-dioxopyrrolo[1,2-*a*]pyrazine-3-acetate.* The foregoing product (1.14 g, 1.7 mmol) in DMF (100 ml) was stirred with 10% Pd/C (0.2 g) under H_2 for 5 d. DMF was evaporated, the residue suspended in CH_2Cl_2, the mixture filtered through *Celite*, and the filtrate evaporated: white solid (0.86 g, 100%). TLC (CH_2Cl_2/MeOH 9:1): R_f 0.59. M.p. 144–146°. $[\alpha]_D^{20}=-18.8$ ($c=0.48$, AcOEt). IR (KBr): 3350*s* (br.), 2980*m*, 2930*m*, 1783*m*, 1770*m*, 1712v*s*, 1693v*s*, 1682v*s*, 1668v*s*, 1660v*s*, 1645v*s*, 1580*m*, 1533v*s*, 1487v*s*. ^{1}H-NMR (600 MHz, (D_6)acetone): 7.85 (br. *d*, *J* = 7.2, 3 H); 7.51 (*t*, *J* = 7.2, 1 H); 7.42 (*t*, *J* = 7.2, 2 H); 7.31 (br. *s*, 1 H); 6.41 (*d*, *J* = 7.7, 1 H); 4.82 (*m*, 1 H); 4.67 (*m*, 2 H); 4.36 (*dd*, *J* = 4.5, 4.1, 1 H); 3.85 (*dd*, *J* = 11.8, 9.0, 1 H); 3.61 (*dd*, *J* = 11.8, 10.6, 1 H); 3.00 (*dd*, *J* = 17.3, 4.1, 1 H); 2.82 (*dd*, *J* = 17.3, 4.5, 1 H); 1.45 (*s*, 9 H); 1.34 (*s*, 9 H). ^{13}C-NMR (75 MHz, (D_6)acetone): 171.5 (*s*); 167.7 (*s*); 165.0 (*s*); 164.5 (*s*); 157.4 (*s*); 135.5 (*s*); 132.1 (*d*); 129.1 (*d*); 128.2 (*d*); 82.2 (*s*); 79.6 (*s*); 61.8 (*d*); 55.0 (*d*); 52.7 (*d*); 51.9 (*d*); 48.0 (*t*); 38.0 (*t*); 28.4 (*q*); 28.3 (*q*). ESI-MS: 525.4 ($[M+Na]^+$), 503.3 ($[M+H]^+$), 447.3 ($[M+H-{}^t\mathrm{Bu}]^+$), 403.3 ($[M+H-{}^t\mathrm{Bu}-CO_2]^+$). Anal. calc. for $C_{25}H_{34}N_4O_7$ (502.56): C 59.7, H 6.8, N 11.2; found: C 59.4, H 6.9, N 11.0.

*[(3*S*,7*R*,8*R*,8a*S*)-7-(Benzoylamino)-3-(carboxymethyl)octahydro-1,4-dioxopyrrolo[1,2-*a*]pyrazin-1-yl]ammonium Trifluoroacetate.* To the foregoing product (0.74 g, 1.47 mmol) in anh. CH_2Cl_2 (40 ml), CF_3COOH

(9 ml, 0.12 mol) was added at 0°, and the soln. was stirred for 5 h at r.t. After evaporation, the residue was redissolved in MeCN/H_2O and lyophilized: white solid (0.6 g, 89%). M.p. 148–150°. $[\alpha]_D^{20} = +45.6$ ($c = 0.29$, acetone). IR (KBr): 3700–2500*m* (br.), 1730*s*, 1712*w*, 1682*vs*, 1651*vs*, 1581*m*, 1536*s*, 1515*s*, 1490*m*. ^{1}H-NMR (600 MHz, (D_6)acetone): 7.95 (*d*, $J = 7.3$, 2 H); 7.55 (*t*, $J = 7.3$, 1 H); 7.45 (*t*, $J = 7.3$, 2 H); 5.35 (*dd*, $J = 5.2$, 3.3, 1 H); 5.28 (*ddd*, $J = 10.5$, 8.8, 5.2, 1 H); 5.09 (*dd*, $J = 3.3$, < 2.0, 1 H); 4.54 (br. *dd*, $J = 7.4$, 3.8, 1 H); 4.19 (*dd*, $J =$ 11.9, 8.8, 1 H); 3.95 (*dd*, $J = 11.9$, 10.5, 1 H); 3.15 (*dd*, $J = 17.6$, 3.8, 1 H); 2.85 (*dd*, $J = 17.6$, 7.4, 1 H). NOE: 5.35 (H–C(1)) → 5.09 (H–C(8a)); 5.28 (H–C(2)) → 3.95 ($H_{pro\text{-}R}$–C(3)); 5.09 (H–C(8a)) → 5.35 (H–C(1)); 4.19 ($H_{pro\text{-}S}$–C(3)) → 3.95 ($H_{pro\text{-}R}$–C(3)); 3.95 ($H_{pro\text{-}R}$–C(3)) → 5.28 (H–C(2), 4.19 ($H_{pro\text{-}S}$–C(3)). ^{13}C-NMR (75 MHz, (D_6)acetone): 173.1 (*s*); 168.2 (*s*); 165.1 (*s*); 164.6 (*s*); 161.1 (*q*); 134.0 (*s*); 132.6 (*d*); 129.2 (*d*); 128.3 (*d*); 117.1 (*q*); 61.9 (*d*); 61.4 (*d*); 52.7 (*d*); 50.4 (*d*); 47.3 (*t*); 36.5 (*t*). ESI-MS: 369.2 ($[M + Na]^+$), 347.2 ($[M + H]^+$).

*(3S,7R,8R,8aS)-7-(Benzoylamino)-8-{[(9*H*-fluoren-9-ylmethoxy)carbonyl]amino}octahydro-1,4-dioxopyrrolo[1,2-*a*]pyrazine-3-acetic Acid* (**1**). To a suspension of the foregoing product (0.58 g, 1.26 mmol) in anh. CH_2Cl_2 (45 ml), and ^{i}Pr$_2$EtN (1.28 ml, 6 equiv., 7.48 mmol), Fmoc-ONSu (1.27 g, 3 equiv., 3.76 mmol) was added, and the soln. was stirred at r.t. for 24 h. The org. phase was washed with 10% aq. citric acid soln., dried ($MgSO_4$), and evaporated. TLC (CH_2Cl_2/MeOH 9:1, +0.1% CF_3COOH): R_f 0.49. White solid (0.43 g, 60%). M.p. >186° (dec.). $[\alpha]_D^{20} = -4.6$ ($c = 0.53$, acetone). IR (KBr): 3327*m* (br.), 3065*w*, 2953*w*, 2896*w*, 1694*vs*, 1665*vs*, 1603*w*, 1579*w*, 1534*s*. ^{1}H-NMR (600 MHz, (D_6)acetone): 7.83 (*d*, $J = 7.5$, 2 H); 7.82 (*d*, $J = 6.8$, 2 H); 7.67, 7.65 (2*d*, $J =$ 7.5, 2 H); 7.42 (*t*, $J = 7.3$, 1 H); 7.38 (*t*, $J = 7.5$, 2 H); 7.36 (*t*, $J = 7.5$, 2 H); 7.30 (*t*, $J = 7.5$, 2 H); 7.27 (*t*, $J = 7.5$, 2 H); 7.23 (2*t*, $J = 7.5$, 2 H); 5.01 (*ddd*(*d*), $J = 10.1$, 9.4, 4.1, 1 H); 4.87 (*t*, $J = 4.1$, 1 H); 4.76 (*d*(*d*), $J =$ 4.1, < 2.0, 1 H); 4.49 (br. *dd*, $J = 5.3$, 5.1, 1 H); 4.23 (*dd*, $J = 9.5$, 7.6, 1 H); 4.15 (*t*, $J = 7.6$, 1 H); 4.09 (*dd*, $J = 9.5$, 7.6, 1 H); 3.88 (*dd*, $J = 11.4$, 9.4, 1 H); 3.75 (*dd*, $J = 11.4$, 10.1, 1 H); 3.08 (*dd*, $J = 17.5$, 5.3, 1 H); 3.05 (*dd*, $J = 17.5$, 5.1, 1 H). ^{13}C-NMR (150 MHz, (D_6)acetone): 172.9 (*s*); 167.8 (*s*); 165.3 (*s*); 165.1 (*s*); 157.6 (*s*); 145.1 (*s*); 145.0 (*s*); 142.0 (*s*); 135.5 (*s*); 132.1 (*d*); 129.1 (*d*); 128.50 (*d*); 128.49 (*d*); 128.2 (*d*); 128.1 (*d*); 126.6 (*d*); 126.4 (*d*); 120.7 (*d*); 67.7 (*t*); 62.3 (*d*); 55.6 (*d*); 52.5 (*d*); 51.0 (*d*); 48.0 (*d*); 47.8 (*t*); 36.6 (*t*). ESI-MS: 591.2 ($[M + Na]^+$), 569.3 ($[M + H]^+$).

*Benzyl (1S,4R,5R)-4-(1,3-Dihydro-1,3-dioxo-2*H*-isoindol-2-yl)-7-oxo-2,6-diazabicyclo[3.2.0]heptane-2-carboxylate.* To a refluxing soln. (78°) of **6** (12.0 g, 22.2 mmol) in MeCN (220 ml) and H_2O (10 ml), a soln. of $K_2S_2O_8$ (48.0 g, 8 equiv., 0.18 mol) and $Na_2HPO_4 \cdot 2\,H_2O$ (63.1 g, 16 equiv., 0.36 mol) in H_2O (60 ml), was added in portions with vigorous stirring. Upon conversion (TLC control, 30 min), the orange soln. was immediately cooled and the two-phase system made slightly alkaline by adding crystalline $NaHCO_3$. The aq. phase was extracted with AcOEt, the org. phase washed with sat. aq. $NaHCO_3$ soln. and brine, dried ($MgSO_4$), and evaporated, and the residue purified by FC (hexane/AcOEt 1:4): off-white solid (7.1 g, 82%). TLC (hexane/AcOEt 1:1): R_f 0.64. M.p. > 50° (dec.). $[\alpha]_D^{21} = +6.1$ ($c = 0.43$, AcOEt). IR (KBr): 3300*m*, 3060*w*, 3028*w*, 2944*w*, 1780*vs*, 1760*vs*, 1722*vs*, 1714*vs*, 1696*vs*, 1600*vs*, 1497*m*. ^{1}H-NMR (300 MHz, (D_6)acetone): 7.87 (*s*, 4 H); 7.46–7.30 (*m*, 6 H); 5.31 (br. *s*, 1 H); 5.19 (*s*, 2 H); 4.75–4.27 (3*m*, 4 H). ^{13}C-NMR (75 MHz, (D_6)acetone): 169.2 (*s*); 165.4 (*s*); 154.4 (*s*); 137.8 (*s*); 135.2 (*d*); 133.1 (*s*); 129.7 (*d*); 129.2 (*d*); 128.5 (*d*); 123.9 (*d*); 68.9 (*d*); 67.6 (*t*); 55.9 (br. *d*); 54.5 (br. *d*); 53.4 (br. *d*); 52.9 (br. *d*); 44.4 (br. *t*). ESI-MS: 414.1 ($[M + Na]^+$), 392.4 ($[M + H]^+$).

*2-Benzyl 6-(*tert*-Butyl) (1S,4R,5R)-4-(1,3-Dihydro-1,3-dioxo-2*H*-isoindol-2-yl)-7-oxo-2,6-diazabicyclo[3.2.0]heptane-2,6-dicarboxylate* (**11**). To the foregoing product (6.2 g, 15.8 mmol) in anh. CH_2Cl_2 (120 ml) and Et_3N (2.2 ml, 15.8 mmol), $(Boc)_2O$ (6.9 g, 31.6 mmol) and DMAP (1.93 g, 15.8 mmol) were added. After stirring for 1 h, the org. phase was washed with 1% aq. citric acid soln., sat. $NaHCO_3$ soln., and brine and evaporated. The residue was purified by FC (hexane/AcOEt 1:1): yellowish solid (5.72 g, 74%). TLC (hexane/AcOEt 1:1): R_f 0.63. M.p. 92–93°. $[\alpha]_D^{21} = -107.5$ ($c = 0.43$, AcOEt). IR (KBr): 2974*w*, 2936*w*, 1816*vs*, 1778*s*, 1725*vs*, 1711*vs*, 1696*vs*. ^{1}H-NMR (300 MHz, (D_6)acetone): 7.88 (*s*, 4 H); 7.45–7.31 (*m*, 5 H); 5.41 (br. *d*, $J = 4.9$, 1 H); 5.22–5.17 (*m*, 2 H); 4.96 (*ddd*, $J = 10.8$, 8.5, 5.5, 1 H); 4.84 (br. *dd*, $J = 5.5$, 4.9, 1 H); 4.66 (*t*, $J = 10.8$, 1 H); 4.23 (*m*, 1 H); 1.21 (*s*, 9 H). NOE: 5.41 (H–C(1)) → 4.84 (H–C(5)); 5.20 (PhCH_2) → 7.43 (H_o, *Z*); 4.96 (H–C(4)) → 4.84 (H–C(5)), 4.66 ($H_{pro\text{-}S}$–C(3)); 4.84 (H–C(5)) → 5.41 (H–C(1)), 4.96 (H–C(4)); 4.66 ($H_{pro\text{-}S}$–C(3)) → 4.23 ($H_{pro\text{-}R}$–C(3)); 4.23 ($H_{pro\text{-}R}$–C(3)) → 4.96 (H–C(4)), 4.66 ($H_{pro\text{-}S}$–C(3)). ^{13}C-NMR (75 MHz, (D_6)acetone): 168.9 (*s*); 163.0 (*s*); 154.3 (*s*); 148.6 (*s*); 137.7 (*s*); 135.3 (*d*); 132.9 (*s*); 129.8 (*d*); 129.3 (*d*); 128.6 (*d*); 124.0 (*d*); 83.5 (*s*); 67.1 (*d*); 66.9 (*t*); 59.0 (*d*); 58.1 (*d*); 51.2 (*d*); 50.7 (*d*); 43.5 (*t*); 27.8 (*q*). ESI-MS: 513.9 ($[M + Na]^+$), 457.9 ($[M + Na - {}^t$Bu$]^+$), 414.0 ($[M + Na - {}^t$Bu $- CO_2]^+$).

*Methyl (2S,3R,4R)-1-[(Benzyloxy)carbonyl]-3-{[(*tert*-butoxy)carbonyl]amino}-4-(1,3-dioxo-1,3-dihydro-2*H*-isoindol-2-yl)prolinate* (**12**). As described for **10**, with **11** (3.2 g, 6.5 mmol), THF (120 ml), H_2O (40 ml), and Na_2CO_3 (0.83 mg, 7.8 mmol) (on workup acidification to pH 4.0, and drying with $MgSO_4$): carboxylic acid. ES-MS: 564.3 ($[M + MeOH + Na]^+$), 542.3 ($[M + MeOH + H]^+$), 486.1 ($[M + MeOH + H - {}^t$Bu$]^+$), 442.1 ($[M + MeOH + H - {}^t$Bu $- CO_2]^+$). The corresponding ester was purified by FC (hexane/AcOEt 2:3). TLC

R_f 0.82. M.p. 72–74°. $[\alpha]_D^{21} = +11.7$ ($c = 0.44$, AcOEt). IR (KBr): 3420*w* (br.), 2960*w*, 2920*w*, 1775*w*, 1752*w*, 1724*s*, 1715*s*, 1610*w*, 1510*w*. ^{1}H-NMR (300 MHz, (D_6)acetone; (*E*)/(*Z*)-isomers): 7.88 (*s*, 4 H); 7.44–7.32 (*m*, 5 H); 5.59 (2 br. *d*, 1 H); 5.21–4.82 (*m*, 5 H); 4.61 (*m*, 1 H); 4.06 (*m*, 1 H); 3.72 (*s*, 3 H); 3.62 (*s*, 3 H); 1.15 (*s*, 9 H). ^{13}C-NMR (75 MHz, (D_6)acetone; (*E*)/(*Z*)-isomers): 170.4 (*s*); 169.9 (*s*); 169.2 (*s*); 155.7 (*s*); 155.3 (*s*); 154.7 (*s*); 137.8 (*s*); 137.7 (*s*); 135.4 (*d*); 132.7 (*s*); 129.3 (*d*); 128.7 (*d*); 128.6 (*d*); 128.5 (*d*); 124.0 (*d*); 79.9 (*s*); 67.6 (*t*); 62.6 (*d*); 62.3 (*d*); 54.9 (*d*); 53.9 (*d*); 52.4 (*q*); 52.2 (*d*); 51.7 (*d*); 44.6 (*t*); 44.2 (*t*); 28.2 (*q*); 28.0 (*q*). ESI-MS: 546.3 ($[M+Na]^+$), 468.3 ($[M+H-{}^t\mathrm{Bu}]^+$), 424.2 ($[M+H-{}^t\mathrm{Bu}-CO_2]^+$).

*Methyl (2*S*,3*R*,4*R*)-3-[[(*tert-*Butoxy)carbonyl]amino]-4-(1,3-dihydro-1,3-dioxo-2*H*-isoindol-2-yl)prolinate.* The foregoing product (0.52 g, 1.0 mmol) was stirred in DMF (5 ml) with 10% Pd/C under H_2 for 3 d. DMF was evaporated, the residue suspended in AcOEt and filtered through *Celite*, and the filtrate evaporated: white solid (0.38 g, 100%). TLC (CH_2Cl_2/MeOH 95:5): R_f 0.44. M.p. 68–71°. $[\alpha]_D^{20} = +30.4$ ($c = 0.39$, AcOEt). IR (KBr): 3600–3200*w* (br.), 2974*m*, 1780*m*, 1757*s*, 1725v*s*, 1715v*s*, 1693*s*, 1680*s*, 1632*w*, 1566*w*, 1550*w*, 1530*m*, 1519*m*. ^{1}H-NMR (300 MHz, (D_6)acetone): 7.91 (*s*, 4 H); 6.53 (*d*, $J = 9.3$, 1 H); 5.31 (*m*, 1 H); 5.12 (*d*, $J = 6.6$, 1 H); 5.06 (*m*, 1 H); 4.76 (*dd*, $J = 12.1$, 9.0, 1 H); 3.91 (*dd*, $J = 12.1$, 9.3, 1 H); 3.82 (*s*, 3 H); 1.18 (*s*, 9 H). ^{13}C-NMR (75 MHz, (D_6)acetone): 171.6 (*s*); 169.3 (*s*); 155.6 (*s*); 135.2 (*d*); 132.7 (*s*); 123.8 (*d*); 79.3 (*s*); 64.3 (*d*); 56.5 (*d*); 54.4 (*d*); 52.1 (*q*); 46.9 (*t*); 28.0 (*q*). ESI-MS: 412.2 ($[M+Na]^+$), 390.1 ($[M+H]^+$), 356.0 ($[M+Na-{}^t\mathrm{Bu}]^+$), 334.1 ($[M+H-{}^t\mathrm{Bu}]^+$).

Methyl N^2*-[(Benzyloxy)carbonyl]-*L*-aspart-1-yl]-(2*S*,3*R*,4*R*)-3-[[(*tert-*butoxy)carbonyl]amino]-4-(1,3-dihydro-1,3-dioxo-2*H*-isoindol-2-yl)prolinate.* To the foregoing product (0.69 g, 1.77 mmol), Z-Asp(O^{t}Bu)-OH (0.68 g, 2.1 mmol), HATU (0.8 g, 2.1 mmol), and HOAt (0.24 g, 1.76 mmol) in anh. CH_2Cl_2 (20 ml), ^{i}Pr$_2$EtN (0.9 ml, 5.26 mmol) was added, and the soln. was stirred at r.t. for 24 h. After washing with 10% aq. citric acid soln., sat. aq. $NaHCO_3$ soln., and brine, the org. phase was dried ($MgSO_4$) and evaporated. The residue was purified by FC (hexane/AcOEt 1:1): white solid (0.78 g, 63%). TLC (hexane/AcOEt 1:1): R_f 0.48. M.p. 78–80°. $[\alpha]_D^{20} = +9.4$ ($c = 0.47$, AcOEt). IR (KBr): 3440–3200*w* (br.), 2979*m*, 2932*w*, 1780*m*, 1732v*s*, 1715v*s*, 1668*s*, 1661*s*, 1652*s*, 1515*s*, 1505*s*. ^{1}H-NMR (300 MHz, (D_6)acetone): 7.88 (*m*, 4 H); 7.39–7.27 (*m*, 5 H); 6.74 (*d*, $J = 8.4$, 1 H); 5.59 (br. *d*, 1 H); 5.10–4.88 (*m*, 7 H); 4.39 (*m*, 1 H); 3.68 (*s*, 3 H); 2.83 (*dd*, $J = 16.1$, 6.0, 1 H); 2.57 (*dd*, $J = 16.1$, 7.7, 1 H); 1.47 (*s*, 9 H); 1.14 (*s*, 9 H). ^{13}C-NMR (75 MHz, (D_6)acetone): 170.8 (*s*); 169.7 (*s*); 169.03 (*s*); 169.02 (*s*); 157.3 (*s*); 156.2 (*s*); 138.1 (*s*); 135.3 (*d*); 132.6 (*s*); 129.1 (*d*); 128.6 (*d*); 123.9 (*d*); 81.3 (*s*); 79.8 (*s*); 66.8 (*t*); 62.1 (*d*); 53.0 (*d*); 52.7 (*d*); 52.2 (*q*); 50.4 (*d*); 44.2 (*t*); 38.6 (*t*); 28.10 (*q*); 28.06 (*q*). ESI-MS: 717.5 ($[M+Na]^+$), 695.5 ($[M+H]^+$), 661.4 ($[M+Na-{}^t\mathrm{Bu}]^+$), 639.3 ($[M+H-{}^t\mathrm{Bu}]^+$), 605.4 ($[M+Na-2-{}^t\mathrm{Bu}]^+$), 583.4 ($[M+H-2\ {}^t\mathrm{Bu}]^+$), 539.2 ($[M+H-2\ {}^t\mathrm{Bu}-CO_2]^+$). Anal. calc. for $C_{35}H_{42}N_4O_{11}$ (694.75): C 60.5, H 6.1, N 8.1; found: C 60.3, H 6.2, N 7.9.

tert-*Butyl (3*S*,7*R*,8*R*,8a*S*)-8-[[(*tert-*Butoxy)carbonyl]amino]-7-(1,3-dihydro-1,3-dioxo-2*H*-isoindol-2-yl)octahydro-1,4-dioxopyrrolo[1,2-*a*]pyrazine-3-acetate.* The foregoing dipeptide (0.31 g, 0.45 mmol) in DMF (15 ml) was stirred with 10% Pd/C (30 mg) under H_2 for 5 d. DMF was evaporated, the residue suspended in CH_2Cl_2, the mixture filtered through *Celite*, and the filtrate evaporated: white solid (0.24 g, 95%). TLC (CH_2Cl_2/MeOH 95:5): R_f 0.33. M.p. 191° (dec.). $[\alpha]_D^{20} = +21.8$ ($c = 0.23$, AcOEt). IR (KBr): 3700–3200*m* (br.), 2977*m*, 2929*w*, 1779*m*, 1720v*s*, 1695v*s*, 1684v*s*, 1669v*s*, 1526*s*. ^{1}H-NMR (600 MHz, $CDCl_3$): 7.83 (*m*, 2 H); 7.71 (*m*, 2 H); 6.72 (br. *s*, 1 H); 6.20 (*d*, $J = 8.8$, 1 H); 5.05 (*m*, 1 H); 4.93 (*dd*, $J = 12.2$, 7.8, 1 H); 4.85 (*m*, 1 H); 4.51 (*d*, $J = 4.5$, 1 H); 4.37 (br. *dd*, 1 H); 3.64 (*dd*, $J = 12.2$, 9.6, 1 H); 3.22 (*dd*, $J = 17.2$, 6.4, 1 H); 3.18 (*dd*, $J = 17.2$, 3.4, 1 H); 1.49 (*s*, 9 H); 1.13 (*s*, 9 H). ^{13}C-NMR (75 MHz, $CDCl_3$): 170.5 (*s*); 168.3 (*s*); 164.1 (*s*); 155.9 (*s*); 134.4 (*d*); 131.9 (*s*); 123.7 (*d*); 82.8 (*s*); 79.7 (*s*); 60.2 (*d*); 54.8 (*d*); 52.3 (*d*); 51.1 (*d*); 43.4 (*t*); 37.8 (*t*); 28.3 (*q*); 28.2 (*q*). ES-MS: 551.4 ($[M+Na]^+$), 529.3 ($[M+H]^+$), 473.1 ($[M+H-{}^t\mathrm{Bu}]^+$), 429.1 ($[M+H-{}^t\mathrm{Bu}-CO_2]^+$), 373.2 ($[M+H-2\ {}^t\mathrm{Bu}-CO_2]^+$).

tert-*Butyl (3*S*,7*R*,8*R*,8a*S*)-7-Acetamido-8-[[(*tert-*butoxy)carbonyl]amino}octahydro-1,4-dioxopyrrolo[1,2-*a*]-pyrazine-3-acetate* (**13**). A soln. of the foregoing product (50 mg, 0.095 mmol) and $MeNHNH_2 \cdot H_2O$ (24 µl, 3.6 equiv., 0.34 mmol) in EtOH (2 ml) was stirred at 80° for 1 h. The solvent was evaporated, and ^{i}Pr$_2$EtN (16 µl, 0.095 mmol), CH_2Cl_2 (2 ml), and Ac_2O (18 µl, 2 equiv., 0.19 mmol) were added. After stirring for 15 min, the suspension was filtered, CH_2Cl_2 evaporated, and the residue purified by HPLC (gradient 5–95% MeCN/H_2O (+0.1% CF_3COOH) over 15 min, then 95% MeCN/H_2O (+0.1% CF_3COOH) for 5 min); t_R 11.9 min): **13** (29 mg, 70% over 2 steps). White solid. ^{1}H-NMR (300 MHz, (D_6)acetone): 4.69 (*m*, 1 H); 4.58 (*m*, 2 H); 4.34 (*dd*, 1 H); 3.67 (*dd*, $J = 11.8$, 9.0, 1 H); 3.38 (*dd*, $J = 11.8$, 10.2, 1 H); 2.97 (*dd*, $J = 17.4$, 4.0, 1 H); 2.82 (*dd*, $J = 17.4$, 4.4, 1 H); 1.87 (*s*, 3 H); 1.46 (*s*, 9 H); 1.40 (*s*, 9 H). ESI-MS: 903.5 ($[2M+Na]^+$), 463.3 ($[M+Na]^+$), 441.3 ($[M+H]^+$), 341.1 ($[M+H-{}^t\mathrm{Bu}-CO_2]^+$), 285.2 ($[M+H-2\ {}^t\mathrm{Bu}-CO_2]^+$).

*Cyclo[*L*-alanyl-*L*-alanyl-[3*S*,7*R*,8*R*,8a*S*)-7-(benzoylamino)-8-aminooctahydro-1,4-dioxopyrrolo[1,2-a]pyrazine-3-acetyl]-*L*-alanyl-*L*-asparaginyl-*L*-prolyl-*L*-asparaginyl]* (Cyclo(-Ala-Ala-temp-Ala-Asn-Pro-Asn; **3**). Fmoc-

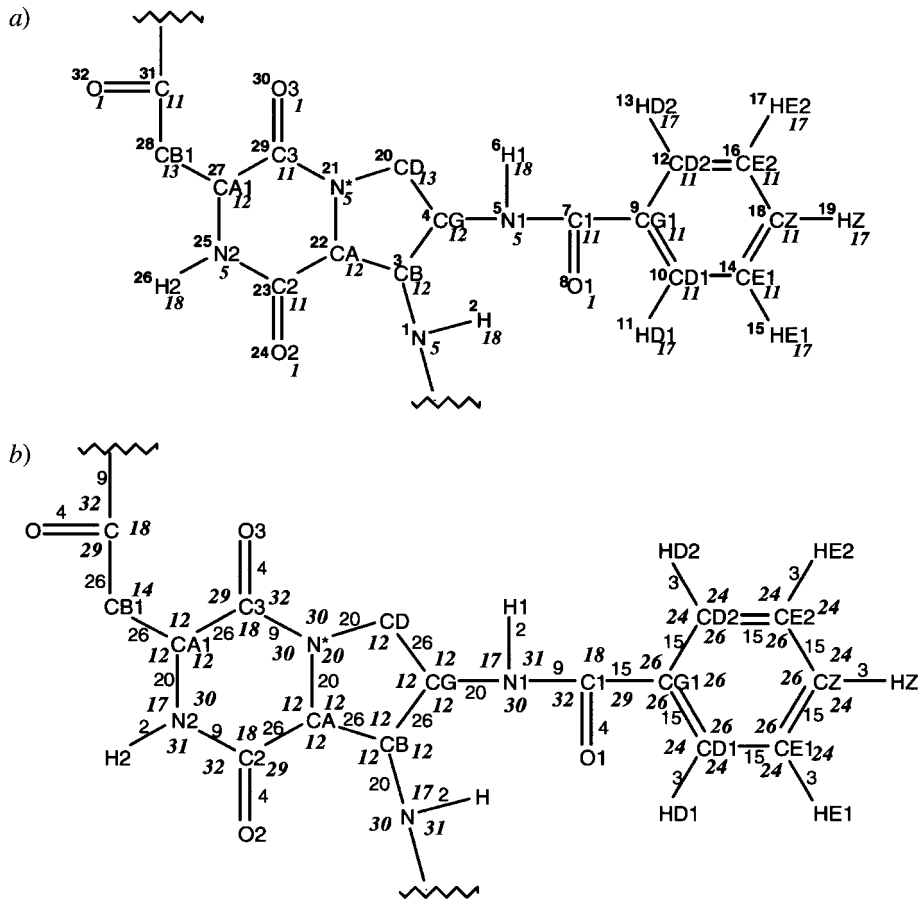

Fig. 9. *Molecular topology building block of the template used for simulations of* **3** *with GROMOS96 and the 43A1 force field.* a) Atom names X, atom numbering ^{n}X, integer atom code X_n; b) bond-type codes (thin), bond-angle-type codes (italics).

temp-OH **1** was coupled to *Tentagel*™-*S-AC* resin (1 equiv.) (*Rapp Polymere*, Tübingen; 0.27 mmol/g) using CIP[1]) for activation in pyridine/CH_2Cl_2 1:1. The solid-phase peptide synthesis was performed by chain elongation with Fmoc-Ala-OH, Fmoc-Ala-OH, Fmoc-Asn(Mtt)-OH, Fmoc-Pro-OH, Fmoc-Asn(Mtt)-OH, Fmoc-Ala-OH (0.36 mmol each) using HOBt/HBTU for activation[1]). 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 performed with 1% CF_3COOH/CH_2Cl_2 (30 ml) for 15 min, repeated four times. Each filtrate was neutralized with pyridine (3 ml in total) in CH_2Cl_2 (10 ml in total), and the combined org. phase was evaporated to give linear, protected peptide (20.2 mg, 67%) after purification by HPLC (20% MeCN/H_2O (+0.1% CF_3COOH) for 4 min, gradient 20–70% within 0.5 min, 70–95% within 10.5 min, and 95% for 5 min). ESI-MS: 1397.98 ($[M+Na]^+$), 710.57 ($[M+H+Na]^{2+}$), 699.70 ($[M+2H]^{2+}$).

For cyclization, the linear peptide (19 mg, 12.6 mmol) in 1% $^{i}Pr_2EtN$/DMF was stirred with HOAt/HATU[1]) (2 equiv.) overnight at r.t. Evaporation of DMF and purification by HPLC (20% MeCN/H_2O (+0.1% CF_3COOH) for 4 min, gradient 20–70% within 0.5 min, 70–95% within 10.5 min, and 95% for 5 min) afforded the side-chain-protected cyclic peptide (7 mg, 40%). ESI-MS: 1379.76 ($[M+H]^+$).

The protected cyclic peptide (6 mg) was treated with $CF_3COOH/CH_2Cl_2/^{i}Pr_3SiH$ 35:60:5 (8.4 ml) at r.t. for 1 h. Evaporation, precipitation with $^{i}Pr_2O$, and purification by HPLC (2% MeCN/H_2O (+0.1% CF_3COOH) for

Table 3. *Codes for Improper Dihedrals and Proper Torsional Dihedrals Used for the GROMOS96 Molecular Topology Building Block File of the Template in* **3**

Improper dihedrals					Proper torsional dihedrals				
Atom sequence				Type code	Atom sequence				Type code
i	*j*	*k*	*l*		*i*	*j*	*k*	*l*	
1	−1	3	2	1	−2	−1	1	3	4
3	4	22	1	2	−1	1	3	4	19
4	20	3	5	2	1	3	4	5	17
5	4	7	6	1	3	4	5	7	19
7	9	5	8	1	4	5	7	9	4
9	10	12	7	1	5	7	9	10	5
10	9	12	16	1	3	4	20	21	17
12	9	10	14	1	4	20	21	22	19
9	12	16	18	1	20	21	22	23	19
9	10	14	18	1	21	22	3	1	17
10	14	18	16	1	21	22	23	25	20
12	16	18	14	1	22	23	25	27	4
10	9	14	11	1	23	25	27	29	19
12	9	16	13	1	25	27	29	21	20
14	18	10	15	1	25	27	28	31	17
16	18	12	17	1	27	28	31	33	20
18	14	16	19	1	27	29	21	22	4
21	20	22	29	1					
22	23	3	21	2					
23	22	25	24	1					
25	27	23	26	1					
27	25	29	28	2					
29	27	21	30	1					
31	28	33	32	1					

5 min, gradient 2–10% within 0.5 min, 10–40% within 39.5 min, 40–95% within 0.5 min, and 95% for 5 min) gave **3** (1.8 mg, 40%). ^{1}H-NMR (600 MHz, (D_6)DMSO, 305 K): *Tables 1* and *2*. ESI-MS: 867.68 ($[M+H]^+$). Amino acid analysis (molar ratio): Ala 3.00, Asx 3.27, Pro 0.90 (modified proline derivative from template not determined).

NMR Experiments, Structure Calculations, and Simulations. 1D and 2D ^{1}H-NMR spectra: 600 MHz; *Bruker-AMX600* spectrometer, typical peptide concentration 10 mg/ml in (D_6)DMSO; analysis of 2D spectra by 'Felix' software (*MSI*, San Diego).

To derive NOE distance restraints, it was assumed that the initial rate approximation is valid and that each peptide rotates as a single isotropic rotor. The NOEs were determined from NOESY spectra measured at 305 K with mixing times of 40, 80, 120, and 250 ms, with 2048×256 data points, and zero-filling to 4096×2048. Transformation was performed with a sine-bell function. Cross-peak volumes were determined by integration, and build-up curves were checked to ensure a smooth exponential increase in peak intensity for all NOEs used in deriving distance restraints. The relative cross-peak volumes were assumed to be proportional to r^{-6}, and were used to derive distance restraints for simulated annealing (SA) calculations, performed with the methods described in detail elsewhere [25].

For MD simulations with and without TA-DR, the GROMOS96 suite of programs was used with the 43A1 force field [26]. The dynamics of **3** in DMSO was studied at 300 K at 1 atm pressure and with periodic boundary conditions. The temp. was maintained by weak coupling ($\tau_T = 0.1$ ps) to a temp. bath. The system contained the mimic and 314 DMSO molecules in a truncated octahedral box. The upper distance restraints were the exact values obtained from NOE build-up curves, where necessary with pseudoatom corrections, a memory decay time $\tau_{dr} = 50$ ps, and a force constant $K_{dr} = 1000$ kJ mol^{-1} nm^{-2}. A molecular building block for the template was constructed using the existing GROMOS96 atom-type and force-field parameters. The parameters used are shown in *Fig. 9* and *Table 3*. The SHAKE algorithm was used to maintain bond lengths with a relative precision

Table 4. *Crystallographic Data for* **12**

Crystallized from	AcOEt
Empirical formula	$C_{27}H_{29}N_3O_8 \cdot 0.5\ H_2O$
Formula weight [g mol^{-1}]	532.55
Crystal colour, habit	colorless, prism
Crystal dimensions [mm]	$0.20 \times 0.22 \times 0.50$
Temperature [K]	173 (1)
Crystal system	monoclinic
Space group	*C*2
Z	4
Reflections for cell determination	25
2θ range for cell determination [°]	22 – 38
Unit-cell parameters *a* [Å]	27.275 (7)
b [Å]	6.017 (6)
c [Å]	17.068 (5)
β [°]	110.83 (2)
V [Å^3]	2618 (2)
D_x [g cm^{-3}]	1.351
$\mu(\mathrm{Mo}K_\alpha)$ [mm^{-1}]	0.101
$2\theta_{(\max)}$ [°]	55
Total reflections measured	3366
Symmetry-independent reflections	3295
Reflections used ($I > 2\sigma(I)$)	2303
Parameters refined	348
Final *R*	0.0591
wR	0.0522
Goodness of fit *s*	2.169
Secondary extinction coefficient	$4(5) \cdot 10^{-7}$
Final $\Delta_{\max}/\sigma$	0.0001
$\Delta\rho$ (max; min) [e Å^{-3}]	0.26; – 0.25

of 10^{-4}, and the integrator time step was 0.002 ps. Nonbonded interactions evaluated at every step were within a short range cut-off of 9 Å. For long-range interactions, calculated every 5 steps, the cut-off was 15 Å. Structures were saved for analysis every 100 steps (0.2 ps). After short simulations to relax the solute and solvent, the simulations with and without TA-DR were each run for 2 ns.

Crystal-Structure Determination for **12**[2]). All measurements were conducted at low temp. on a *Rigaku-AFC5R* diffractometer using graphite-monochromated $\mathrm{Mo}K_\alpha$ radiation (λ 0.71069 Å) and a 12-kW rotating anode generator. The intensities were collected using $\omega/2\theta$ 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 [27] which revealed the positions of all non-H-atoms of the prolinate molecule **12** plus a H_2O molecule which sits on a two-fold axis. The non-H-atoms were refined anisotropically. The unique H-atom of the H_2O molecule was located in a difference electron density map, and its position was held fixed. All other H-atoms were fixed in geometrically calculated positions ($d(\mathrm{C-H}) = 0.95$ Å). All H-atoms were assigned fixed isotropic displacement parameters with a value equal to $1.2U_{eq}$ of the atom to which each was bonded. A correction for secondary extinction was applied. Refinement was carried out on *F* using full-matrix least-squares procedures which minimized the function $\Sigma w(|F_o| - |F_c|)^2$, where $1/w = \sigma^2(F_o) + (0.005F_o)^2$. All calculations were performed using the TEXSAN crystallographic software package [28]. The data collection and refinement

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[2]) Crystallographic data (excluding structure factors) for the structure of **12** have been deposited with the *Cambridge Crystallographic Data Centre* as supplementary publication No. CCDC-136003. Copies of the data can be obtained, free of charge, on application to the CCDC, 12 Union Road, Cambridge CB21EZ, UK (fax: +44-(0)-1223-336033; email: deposit@ccdc.cam.ac.uk).

parameters are listed in *Table 4*, and a view of the molecule, produced with ORTEPII [29], is shown in *Fig. 2*. The enantiomorph was assigned from the known absolute configuration of the starting material. The H_2O molecule forms H-bonds with the (benzyloxy)carbonyl O-atom of each of two symmetry-related prolinate molecules.

REFERENCES

[1] D. P. Fairlie, M. L. West, A. K. Wong, *Curr. Med. Chem*. **1998**, *5*, 29.
[2] D. Obrecht, M. Altorfer, J. A. Robinson, *Adv. Med. Chem*. **1999**, *4*, 1.
[3] C. Bisang, L. Jiang, E. Freund, F. Emery, C. Bauch, H. Matile, G. Pluschke, J. A. Robinson, *J. Am. Chem. Soc*. **1998**, *120*, 7439.
[4] F. Emery, C. Bisang, M. Favre, L. Jiang, J. A. Robinson, *Chem. Commun.* **1996**, 2155.
[5] H. J. Dyson, A. C. Satterthwait, R. A. Lerner, P. E. Wright, *Biochemistry* **1990**, *29*, 7828.
[6] 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.
[7] D. A. Herrington, D. F. Clyde, G. Losonsky, M. Cortesia, J. R. Murphy, J. Davis, S. Baqar, A. M. Felix, E. P. Heimer, D. Gillessen, E. Nardin, R. S. Nussenzweig, V. Nussenzweig, M. R. Hollingdale, M. M. Levine, *Nature (London)* **1987**, *328*, 257.
[8] V. Nussenzweig, R. S. Nussenzweig, *Adv. Immunol*. **1989**, *45*, 283.
[9] M. E. Pfeifer, J. A. Robinson, *Chem. Commun.* **1998**, 1977.
[10] I. Heinze-Krauss, P. Angehrn, R. L. Charnas, K. Gubernator, E.-M. Gutknecht, C. Hubschwerlen, M. Kania, C. Oefner, M. G. P. Page, S. Sogabe, J.-L. Specklin, F. Winkler, *J. Med. Chem*. **1998**, *41*, 3961.
[11] C. Hubschwerlen, G. Schmid, *Helv. Chim. Acta* **1983**, *66*, 2206.
[12] C. Hubschwerlen, *Synthesis* **1986**, 961.
[13] O. Mitsunobu, M. Wada, T. Sano, *J. Am. Chem. Soc*. **1972**, *94*, 679.
[14] E. Atherton, R. C. Sheppard, 'Solid Phase Peptide Synthesis – A Practical Approach', IRL Press, Oxford, 1989.
[15] K. Wüthrich, 'NMR of Proteins and Nucleic Acids', Wiley-Interscience, New York, 1986.
[16] D. B. Davies, M. A. Khaled, *J. Chem. Soc., Perkin Trans. 2* **1976**, 187.
[17] D. B. Davies, M. A. Khaled, *J. Chem. Soc., Perkin Trans. 2* **1976**, 1238.
[18] A. P. Nanzer, W. F. van Gunsteren, A. E. Torda, *J. Biomol. NMR* **1995**, *6*, 313.
[19] A. E. Torda, R. M. Scheek, W. F. van Gunsteren, *Chem. Phys. Lett*. **1989**, *157*, 289.
[20] B. L. Sibanda, T. L. Blundell, J. M. Thornton, *J. Mol. Biol*. **1989**, *206*, 759.
[21] B. L. Sibanda, J. M. Thornton, *J. Mol. Biol*. **1993**, *229*, 428.
[22] C. Mattos, G. A. Petsko, M. Karplus, *J. Mol. Biol*. **1994**, *238*, 733.
[23] M. Favre, K. Moehle, L. Jiang, B. Pfeiffer, J. A. Robinson, *J. Am. Chem. Soc*. **1999**, *121*, 2679.
[24] D. Gramberg, C. Weber, R. Beeli, J. Inglis, C. Bruns, J. A. Robinson, *Helv. Chim. Acta* **1995**, *78*, 1588.
[25] C. Bisang, C. Weber, J. A. Robinson, *Helv. Chim. Acta* **1996**, *79*, 1825.
[26] W. F. van Gunsteren, S. R. Billeter, A. A. Eising, P. H. Hünenberger, P. Krüger, A. E. Mark, W. R. P. Scott, I. G. Tironi, 'Biomolecular Simulation: The GROMOS96 Manual and User Guide', Hochschulverlag AG an der ETH Zürich, Zürich, 1996.
[27] G. M. Sheldrick, 'SHELXS86', *Acta Crystallogr., Sect. A* **1990**, *46*, 467.
[28] 'TEXSAN: Single Crystal Structure Analysis Software, Version 5.0', Molecular Structure Corporation, The Woodlands, Texas, 1989.
[29] C. K. Johnson, 'ORTEPII. Report ORNL-5138', Oak Ridge National Laboratory, Oak Ridge, Tennessee, 1976.

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