Conformational Perturbations Induced by N-Amination and N-Hydroxylation of Peptides‖

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Abstract: Amination and hydroxylation of the amide nitrogen in a peptide chain have little influence on the local geometry, but both affect the hydrogen-bonding network, and therefore the conformational properties of the modified peptide. An experimental study in solution (IR spectroscopy and ^{1}H-NMR) and in the solid state (X-ray diffraction) has been carried out on the *N*-amino and *N*-hydroxy analogues of the two RCO-Pro-NHMe and RCO-Pro-Gly-NHiPr peptides known to adopt preferentially the γ- and β-turn structures, respectively. The *N*-amino group is a weak proton donor which does not interact significantly with the peptide chain. On the contrary, the *N*-hydroxyl group is a strong proton donor giving close contacts with the peptide carbonyls. The resulting folded conformers of an expanded γ- or β-like type, presenting an 8- or 11-membered cycle instead of a 7- or 10-membered cycle in the cognate peptides have been also analyzed by a SYBYL molecular dynamics simulation.

The studies of structure–activity relationships for biologically active peptides are largely based on peptide analogues with geometry constrained by covalent cycles or modified side chains.[1-9] Various cyclic structures designed to mimic the β- and γ-turns have also been proposed.[10-16] Another means to influence the conformational properties of a peptide is to modify the peptide bond itself.[2,17-42] Besides the probable dropping of biodegradation kinetics, this change should perturb the intramolecular hydrogen-bonding network, the electronic distribution, and the sterical hindrances, and therefore affect the conformational preferences. However, in comparison with peptides, the conformational studies

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‖ Abbreviations: Bzl, benzyl; Boc, *tert*-butyloxycarbonyl; DCCI, dicyclohexylcarbodiimide; DMAP, 4-(dimethylamino)pyridine; DMF, *N*,*N*-dimethylformamide; DMSO, dimethyl sulfoxide; Fmoc, 9-fluorenylmethyloxycarbonyl; NMM, *N*-methylmorpholine, OSu, *N*-oxysuccinimide; Piv, pivalyl; THF, tetrahydrofuran; Z, benzyloxycarbonyl.

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Table I. Free (Roman) and Bonded (Bold Italics) C=O, O–H, and N–H Stretching Frequencies (cm^{-1})[a] for the *N*-Amino and *N*-Hydrooxy Analogues of Piv-Pro-NHMe

compd	C=O			N–H	N^{β}/O^{β}–H
	Piv	Pro	Boc		
P1 (Piv-Pro-NHMe)					
b	1623^{W}	1682^{S}		3460^{W}	
	1608^{S}			***3334***^{S}	
c	1617^{S}	1673^{S}		3452^{M}	
	1597^{W}			***3326***^{M}	
d	1618^{S}	1679^{S}			
A1 (Piv-Proψ[CO–N^{α}($N^{\beta}H_2$)]NHMe)					
b	$1626^{S,e}$	1677^{S}			3360^{W}
c	$1615^{S,e}$	1674^{S}			3353^{W}
d	1618^{S}	1672^{S}			
A*1 (Piv-Proψ[CO–N^{α}(N^{β}HMe)]NHMe)					
b	1623^{S}	1670^{S}			3300^{W}
c	1613^{S}	1663^{S}			3309^{W}
d	1614^{S}	1680^{S}			
A'1 (Piv-Proψ[CO–N^{α}(N^{β}HBoc)]NHMe)					
b	1625^{W}	1681^{S}	1742^{S}		3394^{W}
	1612^{S}				***3283***^{M}
c	1610^{S}	1680^{S}	1739^{S}		3386^{S}
d	1618^{S}	1677^{S}	1727^{S}		
H1 (Piv-Proψ[CO–N^{α}(O^{β}H)]NHMe)					
b	***1589***^{S}	1655^{S}			***3205***^{B}
c	1615^{W}	1648^{S}			3500^{W}
	1589^{S}				***3205***^{B}
d	1616^{S}	1658^{S}			
H'1 (Piv-Proψ[CO–N^{α}(O^{β}Me)]NHMe)					
b	1624^{S}	1672^{S}			
c	1613^{S}	1667^{S}			
d	1615^{S}	1665^{S}			

[a] IR absorption: S, strong, M, medium; W, weak; B, broad. [b] Solvent: CCl_4. Concentrations: 5×10^{-4} M. [c] Solvent: CH_2Cl_2. Concentrations: 5×10^{-3} M. [d] Solvent: Me_2SO. Concentrations: 5×10^{-3} M. Because of N–H solvation giving rise to a very broad ill-resolved band, the N–H stretching frequencies are not indicated in this solvent. [e] A medium absorption at 1605 cm^{-1} disappearing on N^{β}-methylation is due to the $N^{\beta}H_2$ bending vibration.

of amide surrogate-containing peptide analogues are rather few in the literature.[17,20-42]

Contrary to N-methylation, N-amination and N-hydroxylation of the peptide bond have received little attention. As a matter of fact, natural peptides containing an *N*-amino or *N*-hydroxy substitution in the main chain are very few.[43-45] Nevertheless, *N*-hydroxamide is known as a particularly strong proton donor capable of chelating metal cations in natural siderophores, and this property has been used for the design of potent enzyme inhibitors.[46] The small number of *N*-amino and *N*-hydroxy analogues of bioactive peptides is due to several reasons; (i) the absence of N-hydroxylation or N-amination reagents compatible with peptides, (ii) the difficult obtention of optically pure α-hydrazino acids ($N^{\beta}H_2$–N^{α}H–C*HR–CO_2H) and α-hydroxamino acids (O^{β}H–N^{α}H–C*HR–CO_2H), and (iii) the weak reactivity of their N^{α} atom in most coupling reaction procedures.[46,47] After the pioneering work of Niedrich in the early 1970s on α-hydrazino acid-containing peptide analogues,[47] new procedures for getting optically pure α-hydroxamino acids and α-hydrazino acids have been proposed,[46,48-52] while the protecting groups and the coupling procedures have been diversified in peptide synthesis.[53] All these findings have encouraged us to consider the *N*-hydroxy and *N*-amino amide links as possible peptidomimetic groups.

In this paper, we report on the conformational properties of simple *N*-hydroxy and *N*-amino peptides deriving from the Piv-Pro-NHR (P1) and Piv-Pro-Gly-NHR (P2) peptides (Piv = Me_3C–CO, R = Me or iPr), which are known to adopt preferentially the γ- and β-turn structures, respectively.[54-57] The peptidomimetic group has been introduced either in the middle (in P2) or C-terminal (in P1 and P2) position. The O^{β}-Me/Bzl and N^{β}-Boc/Z synthetic intermediates have also been examined. The pivalyl group has a 2-fold advantage over other acyl groups to shift the C=O stretching to lower frequencies and to prevent cis–trans isomerization of the Pro-proceding amide bond.[54,57] All the derivatives have been investigated by ^{1}H-NMR and IR spectroscopy in organic solution rather than in water, which is known to solvate strongly such small molecules. Seven of them, having grown single crystals, have been examined in the solid state by X-ray diffraction. The experimental results have been complemented by a molecular dynamics simulation of the *N*-hydroxy analogues by using the SYBYL program.[58] In the following, we use the IUPAC-recommended "ψ-bracket" nomenclature, in which the bracketed group is substituted for the amide bond in the cognate peptide.[59]

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Table II. Free (Roman) and Bonded (Bold Italics) C=O, O–H, and N–H Stretching Frequencies (cm^{-1})[a] for the *N*-Amino and *N*-Hydroxy Analogues of Piv-Pro-Gly-NHiPr

	C=O				N–H		
compd	Piv	Pro	Gly	Z	Gly	iPr	N^{β}/O^{β}–H
P2 (Piv-Pro-Gly-NHiPr)							
b	***1612***^{M}, ***1602***^{S}	1694^{S}	1664^{S}		3443^{S}	***3340***^{S}	
c	1618^{W}	1686^{S}	1663^{S}				
	1605^{S}						
A2 (Piv-Proψ[CO–N$^{\alpha}$($N^{\beta}H_2$)]Gly-NHiPr)							
b	***1600***^{S,e}	1668^{S}	1668^{S}			***3311***^{S}	***3348***^{W}
c	1616^{M}	1669^{S}	1669^{S}				
	1600^{M}						
A'2 (Piv-Proψ[CO–N$^{\alpha}$(N^{β}HZ)]Gly-NHiPr)							
b	***1597***^{S}	1688^{S}	1667^{S}	1751^{S}		***3340***^{S}	***3365***^{S}
c	1616^{W}	1687^{S}	1664^{S}	1734^{S}			
	1606^{S}						
H2 (Piv-Proψ[CO–N$^{\alpha}$(O^{β}H)]Gly-NHiPr)							
b	1615^{W}	1668^{S}	1668^{S}			3420^{M}	3500^{W}
	1599^{M}, ***1581***^{S}					***3372***^{M}, ***3330***^{W}	***3315***^{B}, ***3205***^{B}
c	1618^{M}	1687^{M}	1663^{S}				
	1603^{M}						
H'2 (Piv-Proψ[CO–N$^{\alpha}$(O^{β}Bzl)]Gly-NHiPr)							
b	***1604***^{S}	1670^{S}	1670^{S}			***3332***^{S}	
c	1615^{M}	1668^{S}	1668^{S}				
	1603^{S}						
A3 (Piv-Pro-Glyψ[CO–N$^{\alpha}$($N^{\beta}H_2$)]NHMe)							
b	1624^{S,d}	1678^{S}	1665^{S}		***3404***^{S}		3353^{W}
c	1619^{S,d}	1680^{S}	1668^{S}				
A*3 (Piv-Pro-Glyψ[CO–N$^{\alpha}$(N^{β}HMe)]NHMe)							
b	1624^{S}	1674^{S}	1658^{S}		***3404***^{S}		3317^{W}
c	1619^{S}	1682^{S}	1656^{S}				
H3 (Piv-Pro-Glyψ[CO–N$^{\alpha}$(O^{β}H)]NHMe)							
b	1623^{M}	1691^{M}	1654^{S}		3435^{W}		3500^{W}
	1599^{M}	***1675***^{M}			***3401***^{M}		***3220***^{S}
c	1623^{S}	1680^{M}	1662^{S}				
H'3 (Piv-Pro-Glyψ[CO–N$^{\alpha}$(O^{β}Me)]NHMe)							
b	1624^{M}	1680^{M}	1664^{S}		3441^{W}		
					3410^{S}		
c	1619^{M}	1685^{M}	1668^{S}				

[a] IR absorption: strong, S; medium, M; weak, W. Concentrations: 5×10^{-3} M. [b] Solvent: CH_2Cl_2. [c] Solvent: Me_2SO. Because of N–H solvation giving rise to a very broad ill-resolved band, the N–H and O–H stretching frequencies are not indicated in this solvent. [d] A medium absorption at 1605 cm^{-1} disappearing on N^{β}-methylation is due to the $N^{\beta}H_2$ bending vibration. [e] This absorption is only slightly reduced on N^{β}-methylation and masks the $N^{\beta}H_2$ bending vibration.

Experimental Section

Synthesis. The compounds we have investigated are listed in Tables I and II. Direct coupling of Piv-Pro-OH or Piv-Pro-Gly-OH to OH–NHMe using DCCI and DMAP as coupling reagents gave the H1 and H3 *N*-hydroxypeptides containing a C-terminal *N*-methylhydroxamide group. The homologous O^{β}-methylated compounds H'1 and H'3 were prepared from MeO–NH_2 by the same procedure. Derivatives H2 and H'2 with the hydroxamide link in the central position were obtained in good yields (75%) according to Figure 1.

Although α preference has been stated for N-monosubstituted hydrazines,[60] the regioselective acylation of $N^{\beta}H_2$–N^{α}HMe and $N^{\beta}H_2$–N^{α}H–CH_2–CO–OEt, HCl (Aldrich) proved to depend on both the coupling partner and the coupling agent.[61] For example, A3 was simply obtained from Piv-Pro-Gly-OSu and $N^{\beta}H_2$–N^{α}HMe without protection. The same procedure failed for A1 and A2, probably because of the bulkier side chain of proline compared with glycine. The intermediate selective protection of the N^{β} nitrogen was thus necessary for the synthesis of A1 (Figure 2), as already described for A2.[61] All derivatives were purified by flash chromatography on silica gel, with CH_2Cl_2/isopropyl alcohol as the eluent, and characterized by ^{1}H-NMR.

^{1}H-NMR and IR Spectroscopy. The possible intramolecular hydrogen bonds were investigated by considering both the solvent sensitivity of the proton NMR signals in DMSO-d_6/C^2HCl_3 mixtures and the stretching frequencies of the proton-donating and -accepting groups. We have focused our attention on the O–H and N–H stretching frequencies (3100–3650 cm^{-1}) and the (tBu)C=O absorption in the 1580–1630 cm^{-1} domain, which is devoid of any other significant contribution (Tables I and II). The peptide concentration in various solvents (CCl_4 for P1 analogues, CH_2Cl_2 and DMSO for all derivatives) was adjusted to 5×10^{-4} M (CCl_4) and 5×10^{-3} M (CH_2Cl_2 and DMSO), for which further dilution confirmed the absence of molecular aggregation.

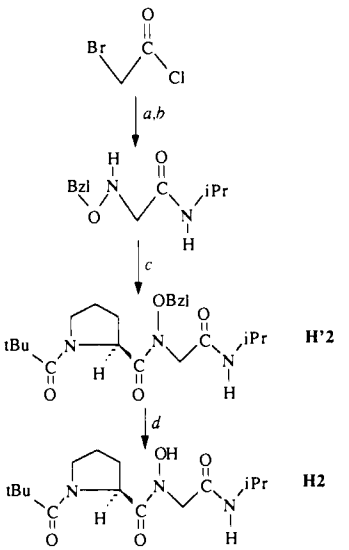

Figure 1. Synthesis of H2: *a*, $iPrNH_2$/NMM/CH_2Cl_2, −15 °C; *b*, $BzlONH_2$, HCl/NMM/DMF, 25 °C; *c*, Piv-Pro-OH/DCCI/DMAP/THF, 0 °C; *d*, H_2/5% Pd–C/MeOH.

^{1}H-NMR spectra were run on a Brüker AC-200P apparatus. Chemical shifts were measured with reference to internal Me_4Si, and spin systems were solved by COSY experiments. Contrary to isolated hydroxamides,[62] the *N*-hydroxy peptides only accommodate all-trans conformations. The

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Table III. Crystallographic Data

	Piv-Proψ-[CO–NOH]-NHMe (H1)	Piv-Proψ-[CO–NOH]-Gly-NHiPr (H2)	Piv-Pro-Glyψ-[CO–NOH]-NHMe (H3)	Piv-Proψ-[CO–N(NHMe)]-NHMe (A*1)	Piv-Proψ-[CO–N(NH_2)]-Gly-NHiPr (A2)	Piv-Pro-Glyψ-[CO–N(NH_2)]-NHMe (A3)	Piv-Proψ-[CO–N(NHZ)]-Gly-NHiPr (A'2)
space group	$P2_1$	$P2_12_12_1$	$P\bar{1}$	$P2_1$	$P2_1$	$P2_1/c$	$P2_1$
Z	4	4	2	4	2	4	2
a, Å	8.801(1)	9.553(1)	7.267(2)	12.162(2)	5.976(2)	15.525(2)	9.730(1)
b, Å	10.147(2)	10.890(2)	9.775(2)	9.938(2)	9.362(1)	8.538(1)	11.108(1)
c, Å	15.334(2)	16.859(3)	12.086(2)	12.232(3)	16.154(1)	12.254(2)	11.227(1)
α, deg			72.54(2)				
β, deg	105.68(1)		86.50(2)	103.16(2)	91.59(2)	111.06(1)	91.56(1)
γ, deg			68.71(2)				
d(calcd), g cm^{-3}	1.15	1.19	1.24	1.11	1.15	1.24	1.22
no. of independent reflections	2631	1915	2879	2708	1761	2724	2423
no. of unique reflections	1893[a]	1819[b]	2174[c]	2180[c]	1664[a]	2312[c]	2279[c]
final R	0.052	0.046	0.065	0.046	0.057	0.054	0.042
final R_w	0.058	0.057	0.083	0.057	0.064	0.070	0.058
w	$1.927/(\sigma^2(F) + 0.0016F^2)$	$1/(\sigma^2(F) + 0.0077F^2)$	$1.311/(\sigma^2(F) + 0.0021F^2)$	$3.40/(\sigma^2(F) + 0.0005F^2)$	$1/(\sigma^2(F) + 0.076F^2)$	$1/(\sigma^2(F) + 0.003F^2)$	$2.55/(\sigma^2(F) + 0.0036F^2)$
residual peak height max, e Å^{-3}	0.22	0.12	0.26	0.16	0.20	0.56	0.16
min, e Å^{-3}	–0.19	–0.19	–0.40	–0.16	–0.28	–0.33	–0.23

[a] $I > 1.5\sigma(I)$. [b] $I > \sigma(I)$. [c] $I > 3\sigma(I)$.

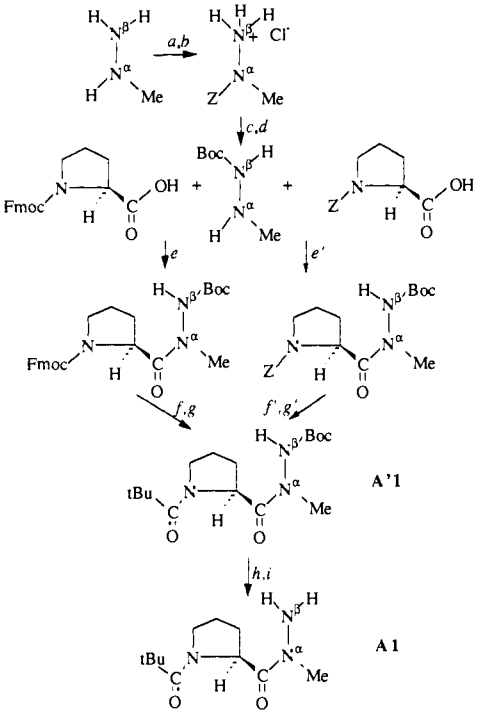

Figure 2. Synthesis of A1: *a*, ZCl/NMM/acetone, 0 °C; *b*, HCl 3N/AcOEt and fractional crystallization; *c*, Boc_2O/DMAP/NMM/THF, 0 °C; *d*, H_2/5% Pd–C/MeOH; *e*, $SOCl_2/CH_2Cl_2$ (acid chloride procedure[53]); *e'*, DCCI/CH_2Cl_2 (symmetric anhydride procedure[53]); *f*, Et_2NH/CH_2Cl_2; *g*, tBuCOCl/NaOH; *f'*, H_2/5% Pd–C/MeOH; *g'*, tBuCOCl/NaOH; *h*, HCl 3N/AcOEt; *i*, NMM.

same holds partly true for the *N*-amino peptides, since small amounts (never exceeding 30%) of cis conformers in chloroform-rich C^2HCl_3/DMSO-d_6 mixtures are observed for A3 having a C-terminal *N*-amino group.

IR spectra were scanned on a Brüker IFS-85 apparatus in the Fourier transform mode using a cell path length of 2 mm (CCl_4), 0.5 mm (CCl_4 and CH_2Cl_2), or 0.2 mm (DMSO). The IR frequencies in low polar solvents (Tables I and II) were assigned on the basis of previous results on similar compounds[54,56,63] and from the following considerations: (i) Free peptide N–Hs give a sharp absorption at 3400–3460 cm^{-1}, and bonded N–Hs give a stronger and broader contribution at 3300-3380 cm^{-1}. (ii) Free peptide C=O vibrators have a strong and sharp absorption at 1660–1690 cm^{-1} (but pivalamide C=O stretchings are shifted to 1580–1630 cm^{-1}), with a frequency shift of 10–30 cm^{-1} in the bonded state. (iii) Hydroxamides in the trans (*E*) conformation exhibit a free O–H stretching frequency at 3500–3550 cm^{-1}, and the C=O stretching is lowered by about 15 cm^{-1} with reference to amides.[62] (We have verified the absence of any additional absorption in the pivalamide C=O domain). (iv) *N*-amino amides have a very weak N–H absorption at 3330 cm^{-1} and a C=O stretching frequency similar to that of classical amides. (v) In *N*-amino amides, the very weak contribution at 1600 cm^{-1}, which is eliminated by methylation of the *N*-amino group, is attributed to the $N^\beta H_2$ bending mode. In a strong solvating medium such as DMSO, the nonbonded, solvated N–H and O–H absorptions are considerably enhanced and shifted to lower frequencies to such an extent that they mask the intramolecularly bonded contributions whereas the stretching frequencies of the bonded and nonbonded carbonyls are only little affected.

X-ray Diffraction. Seven of the *N*-hydroxy and *N*-amino peptides have grown single crystals from AcOEt/iPr_2O solutions (Table III). X-ray data were collected at room temperature on an Enraf Nonius CAD 4 automatic diffractometer with a graphite monochromator, operating with the Cu Kα radiation (λ = 1.540 51 Å) in the θ–2θ scanning mode ($\theta \leq 70°$). Intensity data were corrected for Lorentz and polarization effects and for decay when three standard reflexions decreased by more than 5%. Due to the small size (<0.3 mm) of the crystals, the absorption was neglected. Cell dimensions obtained by refinement from a set of 25 high-angle reflections are indicated in Table III together with other crystal data. Note that H3 and A3 were crystallized in the racemic form.

The structures were solved by using the computer program MULTAN-80[64] and refined by a full-matrix least-squares procedure.[65] Atom scattering factors used were those listed in the *International Tables for X-Ray Crystallography*.[66] *E*-maps revealed all the non-hydrogen atoms and the existence of two nonequivalent molecules in the cells of H1 and A*1. All hydrogens were located in difference maps. Refinement was carried out with anisotropic temperature factors for the non-hydrogen atoms and fixed isotopic thermal factors for the hydrogens. Final residual factors with weighting schemes and final residual electron densities are indicated in Table III. Following Taylor and Kennard's recommendations,[67] OH and NH hydrogens were replaced at 1.03 Å from O and N in the direction obtained by refinement. Fractional coordinates of non-hydrogen and hydrogen atoms, equivalent thermal parameters and

(63) Aubry, A.; Cung, M. T.; Marraud, M. *J. Am. Chem. Soc.* **1985**, *107*, 7640–7647.
(64) Main, P.; Fiske, S.; Hull, S. E.; Lessinger, L.; Germain, G.; Declerc, J. P.; Woolfson, M. M. *MULTAN 80, A system of computer programs for the automatic solution of crystal structures from X-ray diffraction data*; Universities of York and Louvain: York, England, and Louvain, Belgium, 1980.
(65) Sheldrick, G. M. *Programs for crystal structure determination*; University of Cambridge: Cambridge, England, 1976.
(66) *International Tables for X-Ray Crystallography*; Kynoch Press: Birmingham, England, 1974; Vol. IV.
(67) Taylor, R.; Kennard, O. *Acta Crystallogr., Sect. B* **1983**, *39*, 133–138.

Table IV. Main Torsional Angles with Estimated Standard Deviations (deg)

atoms	angle	H1(A)	H1(B)	H2	H3	A*1(A)	A*1(B)	A2	A3	A'2
C'_0–N_1–C^α_1–C'_1	ϕ_1	−70.8(5)	−64.6(6)	−60.8(2)	−75.9(3)	−75.2(3)	−75.1(3)	−60.0(3)	−66.5(2)	−54.6(2)
N_1–C^α_1–C'_1–N_2	ψ_1	158.7(4)	167.6(4)	141.0(2)	156.5(2)	160.6(2)	156.0(2)	141.8(2)	158.2(2)	137.3(2)
C'_1–N_2–C^α_2–C'_2	ϕ_2			97.3(3)	119.8(3)			97.5(3)	140.6(2)	95.7(2)
N_2–C^α_2–C'_2–N_3	ψ_2			−21.1(3)	−176.2(2)			−19.3(4)	−171.0(2)	−6.9(3)
C^α_0–C'_0–N_1–C^α_1	ω_0	177.5(5)	167.5(5)	−176.4(2)	−177.7(3)	177.8(3)	−179.9(3)	−176.8(3)	176.3(1)	176.5(2)
C^α_1–C'_1–N_2–C^α_2	ω_1	173.6(5)	179.0(6)	−171.3(6)	171.9(2)	178.5(3)	178.7(3)	−176.8(2)	177.2(2)	−179.2(2)
C^α_1–C'_1–N_2–X_2[a]	ω'_1	4.2(6)	7.7(7)	−10.2(3)		−2.7(4)	−1.3(4)	0.5(3)		0.8(3)
C'_1–N_2–N'_2–C(N'$_2$)						−108.4(4)	−108.0(4)			
C'_1–N_2–X_2–H[a]		−88(3)	−84(3)	105(2)		138(2)	123(2)	−127(2)		−66(2)
C'_1–N_2–N'_2–K								136(2)		
C^α_2–C'_2–N_3–C^α_3	ω_2			179.6(2)	−178.6(3)			−179.5(3)	177.9(2)	176.3(2)
C^α_2–C'_2–N_3–X_3[b]	ω'_2				−2.3(4)				−4.3(3)	
C'_2–N_3–X_3–H[b]					92(2)				−119(2)	
C'_2–N_3–N'_3–K									112(2)	

[a] X_2 = O'_2 in H1 and H2 and N'_2 in A*1, A2, and A'2. [b] X_3 = O'_3 in H3 and N'_3 in A_3.

Table V. D–H···O Hydrogen Bond Distances (Å) and Angles (deg)[a]

compd	symmetry code	D···O	H···O	D–H···O
H1 (Piv-Proψ[CO–N(O^βH)]NHMe)				
O^β(*A*)–H···O(Piv, *B*)	*x*, *y*, *z*	2.646(5)	1.77(5)	141(4)
O^β(*B*)–H···O(Piv, *A*)	*x*, *y*, *z*	2.592(6)	1.64(6)	151(5)
H2 (Piv-Proψ[CO–N(O^βH)]Gly-NHiPr)				
(iPr)N–H···O(Piv)[b]	*x*, *y*, *z*	3.005(2)	2.00(3)	164(2)
O^β–H···O(Gly)	$-x, -^1/_2 + y, -^1/_2 - z$	2.596(3)	1.60(2)	161(2)
H3 (Piv-Pro-Glyψ[CO–N(O^βH)]NHMe)				
(Gly)N–H···O(Gly)[c]	*x*, *y*, *z*	2.488(2)	1.71(2)	101(1)
(Gly)N–H···O(Piv)	$1-x, 1-y, 1-z$	2.960(3)	1.93(3)	174(2)
O^β–H···O(Pro)	$1-x, -y, 1-z$	2.620(3)	1.60(3)	170(3)
A*1 (Piv-Proψ[CO–N(N^βHMe)]NHMe)				
N^β(*A*)–H···O(Pro, *A*)	$1-x, ^1/_2 + y, 1-z$	2.997(4)	1.99(3)	166(3)
N^β(*B*)–H···O(Pro, *B*)	$1-x, -^1/_2 + y, -z$	3.001(4)	2.07(3)	148(2)
A2 (Piv-Proψ[CO–N($N^\beta H_2$)]Gly-NHiPr)				
(iPr)N-H···O(Piv)[b]	*x*, *y*, *z*	3.104(4)	2.10(3)	165(3)
N^β–H···O(Pro)	$1-x, y, z$	3.021(3)	2.07(3)	152(3)
N^β–K···O(Gly)	$1-x, -^1/_2 + y, 2-z$	3.017(3)	2.19(3)	136(3)
A3 (Piv-Pro-Glyψ[CO–N($N^\beta H_2$)]NHMe)				
(Gly)N–H···O(Gly)[c]	*x*, *y*, *z*	2.727(3)	2.62(3)	85(2)
(Gly)N–H···O(Gly)	$-x, 1-y, -z$	3.009(2)	2.00(2)	165(2)
N^β–H···O(Gly)	$-x, -^1/_2 + y, ^1/_2 - z$	3.039(3)	2.05(2)	160(2)
N^β–K···O(Piv)	$x, ^3/_2 - y, ^1/_2 + z$	3.037(2)	2.04(2)	162(2)
A'2 (Piv-Proψ[CO–N(N^βHZ)]Gly-NHiPr)				
(iPr)N–H···O(Piv)[b]	*x*, *y*, *z*	3.077(3)	2.11(3)	156(2)
N^β–H···O(Gly)	$2-x, ^1/_2 + y, -z$	2.862(4)	1.94(3)	147(2)

[a] The hydrogen atom was moved in the observed D–H direction until the D–H bond length was equal to 1.03 Å. [b] Intramolecular $i + 3 \rightarrow i$ hydrogen bond. [c] Intramolecular $i \rightarrow i$ hydrogen bond.

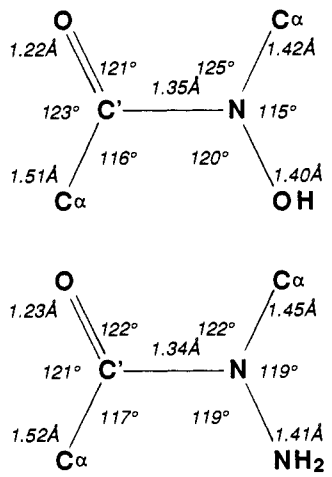

Figure 3. Average dimensions of the *N*-hydroxy and *N*-amino amide links deduced from the present X-ray data.

anisotropic temperature parameters for the non-hydrogen atoms, interatomic distances, and bond angles have been deposited as supplementary material.

Theoretical Analysis. The folded structures of the *N*-acetyl analogues of the *N*-hydroxy peptides were studied by molecular dynamics and energy minimization by using version 5.4 of the SYBYL program[58] running on an IRIS 4D35G graphic station. First, we defined the force field of the *N*-hydroxamide group and introduce the *N*-hydroxy glycine residue in the building library. The bond lengths and bond angles were selected from the crystal structures solved in the present work (Figure 3), and the energetical features assigned to the CO–N^α–O^β–H fragment were taken equal to those available for the CO–N–N moiety. We also had to introduce the additional ν dihedral angle in the CO–N^α–N/O^β–H fragment. The interaction between 1–4 non-bonded atoms was divided by 2 in order to avoid overestimation of the short-range contacts. The partial electrostatic charges were calculated by MOPAC[68] for the minimized extended conformer, and the electrostatic constant was taken equal to twice the interatomic distance.

The molecular dynamics simulation was run by starting from the extended structures for 100 ps at 350 K, generating 100 000 conformers. We verified on the Ramachandran maps that the whole permitted conformational space was explored, and after the first 20 ps, for stabilization of the temperature, one of every 10 conformers set was retained to analyze the different subsets corresponding to particular conformational features. Every subset was submitted to energy minimization, and the probability of existence of each conformation was estimated from the relative number of conformers generated during the dynamics simulation having a molecular energy of less than 10 kcal with reference to the energy minimum, in the conformational space defined by a given H···O distance in the 1.5–2.5-Å range.

Crystal Structures

Dimensions. Both *N*-hydroxamide and *N*-amino amide peptidomimetic groups are nearly planar and adopt the trans

(68) Stewart, J. J. P. *J. Comput.-Aided Mol. Des.* **1990**, *4*, 1–105.

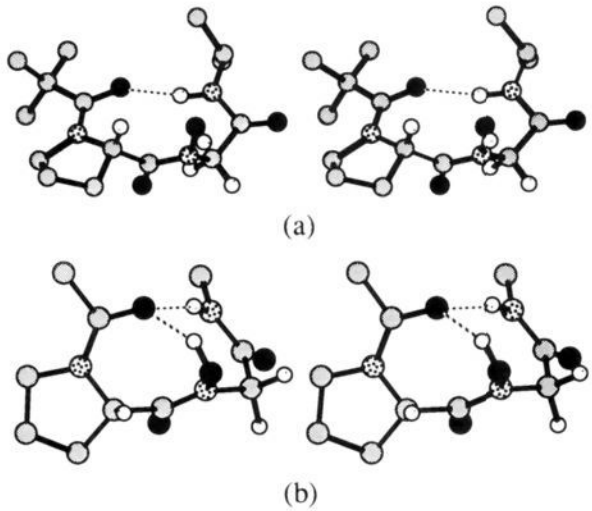

Figure 4. Stereoviews of the βII-folded crystal molecular structure of H2 (a) and of the SYBYL-minimized folded conformer for the acetyl analogue of H2, associating the βII- and expanded γ⁻-like turns (b). The hydrogen bonds are represented by broken lines.

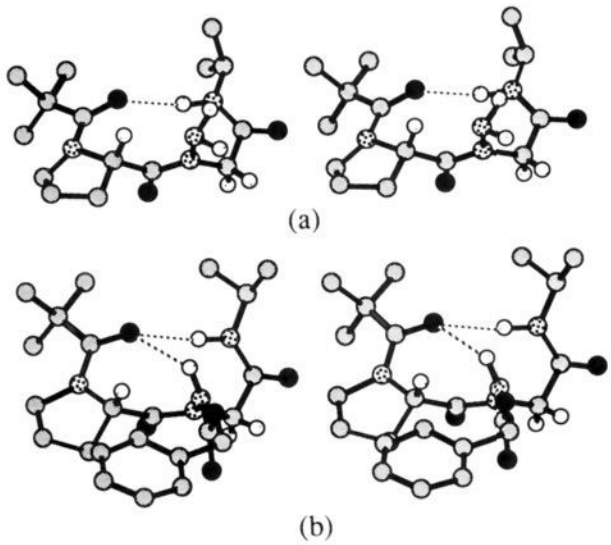

Figure 5. Stereoviews of the βII-folded conformation assumed by the *N*-amino A2 (a) and *N*-benzyloxycarbonylamino A′2 (b) peptides in the solid state. The intramolecular hydrogen bonds are indicated by broken lines. In A′2, the N^{β}···O(Piv) distance (3.58 Å) in the solid state exceeds the upper limit for hydrogen bonding, but a weak interaction occurs in inert solvents (see text).

conformation with respect to the main chain, with the |ω| angle (C^{α}–C′–N–C^{α}) in the 170–180° range (Table IV). Their dimensions are very similar to those of the standard peptide group,[69] especially in the case of the *N*-amino amide group (Figure 3). The N-hydroxamide group only differs by the somewhat shorter N–C^{α} bond and the wider C′–N–C^{α} angle. The O^{β} and N^{β} atoms are clearly in the sp^3 electronic state with bond angles of 100–110°. The O–H bond is practically perpendicular to the amide plane, while the N^{β}–H bonds are oriented in such a way that the amide plane bisects the H–N^{β}–H angle, with two ν (C′–N^{α}–N^{β}–H) dihedral angles of about ±120° (Table IV). N^{β}-acylation in A′2 has only very little influence on the amide dimensions. The *N*-acylamino amide (*N,N′*-diacylhydrazine) fragment presents two planar and nearly orthogonal amide groups as already reported for two "hydrazino" valine derivatives.[70]

(69) Benedetti, E. In *Peptides; Proceedings of the Fifth American Peptide Symposium*; Goodman, M.; Meienhofer, J., Eds.; Wiley & Sons: New York, 1977; pp 257–273.

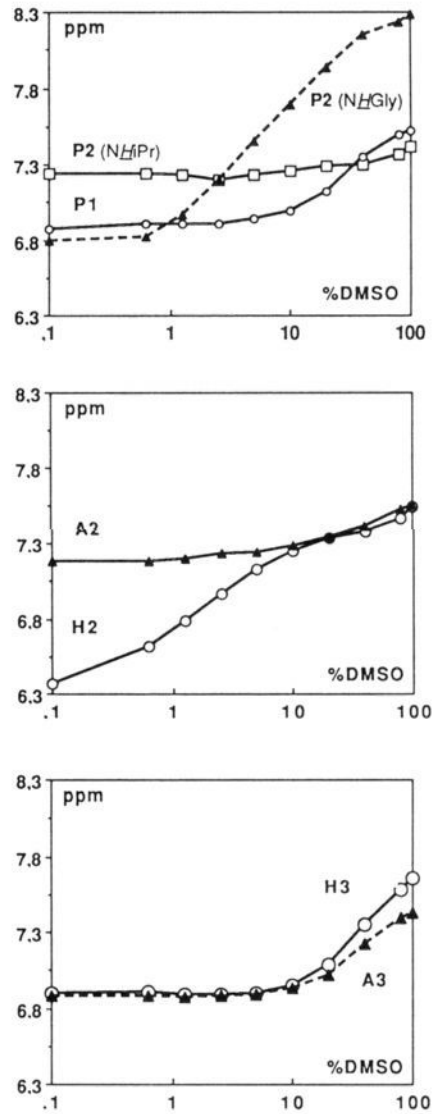

Figure 6. Influence of Me_2SO-d_6 content in C^2HCl_3/Me_2SO-d_6 mixtures on the amide N*H* proton resonances for P1 and P2, A2 and H2, and A3 and H3.

Interatomic Interactions. The short inter- and intramolecular interatomic distances between polar groups are listed in Table V. Half of the contacts between polar sites are classical interactions between amide N–H and C═O groups, with N···O distances ranging from 2.9 to 3.1 Å. Both the $O^{\beta}H$ and $N^{\beta}H_2$ groups act as a proton donor to amide oxygens. The former gives a single very short O^{β}···O contact (2.6 Å), and hence a stronger interaction than the latter, having two longer N^{β}···O contacts (3.0 Å). In no case does the O^{β} or N^{β} atom behave like a proton acceptor. However, we have recently solved the crystal structure of the Bocψ[CO–NH–N]Pro-Glyψ[CO–N^{α}(O^{β}H)]NHMe modified peptide and observed an intermolecular N–H···O^{β} interaction with a N···O^{β} distance of 2.98 Å (not published).

Molecular Conformations. Exactly as the cognate peptide P2,[55] H2, A2, and A′2 adopt in the solid state the classical βII-folded conformation in which the (iPr)N—H···O═C(tBu) hydrogen bond closes a 10-membered cycle (Figures 4 and 5a). The modified middle amide bond does not participate in the stabilization of the β-turn but is engaged in intermolecular interactions (Table V). The *N*-hydroxy peptide H1 assumes an open structure, forming cyclic dimers of two nonequivalent molecules connected by two short O^{β}—H···O═C(tBu) hydrogen bonds. In comparison,

(70) Aubry, A.; Bayeul, D.; Mangeot, J. P.; Vidal, J.; Sterin, S.; Collet, A.; Lecoq, A.; Marraud, M. *Biopolymers* **1991**, *31*, 793–801.

the *N*-methylamino analogue A*1 forms chains of open molecules connected by longer N^{β}—H···O=C(Pro) hydrogen bonds. Both *N*-hydroxy peptide H3 and *N*-amino peptide A3 also adopt more or less extended conformations (Table IV) with a stretched Gly residue presenting an $i \rightarrow i$ intramolecular hydrogen bond (Table V).

Conformations in Solution

The conformational preferences of these simple molecules are essentially governed by the existence of intramolecular hydrogen bonds which have been investigated by IR and ^{1}H-NMR experiments in various organic solvents with increasing polarity (CCl_4, CH_2Cl_2, $CHCl_3$, DMSO). The involvement of a N–H or O–H site in hydrogen bonding is denoted by the shift to lower frequencies of its stretching vibration and by the low sensitivity of the corresponding ^{1}H-NMR signal to environmental factors.[56,63,71] For example, the signal of a hydrogen-bonded, solvent-protected N*H* or O*H* proton is not very sensitive to the addition of DMSO-d_6 in a C^2HCl_3 solution whereas the resonance of a nonbonded, solvent-accessible N*H* or O*H* proton is progressively shifted to lower fields (Figure 6). A rapid equilibrium between open and folded conformers gives rise to a moderate variation of the averaged ^{1}H resonance but is characterized by a typical IR pattern exhibiting both free and bonded contributions. ^{1}H-NMR also gives an estimation of the folding ratio of these simple molecules by considering the shift of the C-terminal N*H* resonance when going from C^2HCl_3 to DMSO-d_6.[56,63]

Before considering the modified sequences, one must put forward the spectroscopic arguments in favor of the γ- and β-folded structures adopted by P1 and P2 in solution, respectively, which are of interest to the present study of their *N*-hydroxy and *N*-amino analogues.

Piv-Pro-NHMe (P1) and Piv-Pro-Gly-NHiPr (P2) Peptides. The γ-turn is characterized in P1 by the low frequencies of both the (tBu)C=O and (Me)N–H stretching vibrations in CCl_4 (Table I). The very weak residual free absorptions at 1623 and 3460 cm^{-1} denote the high occurrence of the γ-turn for P1 in this inert medium. However, this structure is very sensitive to solvation, even by the weak proton donor CH_2Cl_2, and completely disappears in DMSO (Table I). The β-turn in P2 is also characterized by the low frequencies of both (tBu)C=O and (iPr)N–H vibrators in CH_2Cl_2 (Table II). This fact is corroborated by the low solvent sensitivity of the (iPr)N*H* resonance compared with the steeper variation for (Gly)N*H*. A previous IR study of the Piv-L-Pro-L-Ala-NHMe and Piv-L-Pro-D-Ala-NHMe diastereoisomers has shown that the type I and II β-turns in CH_2Cl_2 can be discriminated by the bonded (tBu)C=O stretching at 1610 and 1601 cm^{-1}, respectively.[63] The splitting of the (tBu)C=O stretching into a major contribution at 1604 and a weaker one at 1610 cm^{-1} for P2 in CH_2Cl_2 denotes an equilibrium between the major βII- and minor βI-turns. The retention of only the former frequency in DMSO indicates the lower sensitivity to solvation of the type II β-turn.

***N*-Amino (A1) and *N*-Hydroxy (H1) Analogues of Piv-Pro-NHMe (P1).** In a low solvating medium (CCl_4), the IR data for A1 and H1 differ essentially by the (tBu)C=O frequency which is typical of a free vibrator for A1 and of a bonded vibrator for both P1 and H1. However, the very low frequency for H1 (1589 cm^{-1} instead of 1608 cm^{-1} for P1) denotes a stronger hydrogen bond in H1 than the classical $i + 2 \rightarrow i$ interaction (γ-turn) in P1. Therefore, contrary to the *N*-amino group, the *N*-hydroxyl site is capable of interacting strongly with the preceding carbonyl to close an 8-membered cycle, and thus gives a very broad and composite absorption at about 3200 cm^{-1} in CCl_4 and CH_2Cl_2 (Table I). In a strong solvating medium (DMSO), both the N—H···O=C(tBu) hydrogen bond in P1 and the O^{β}—H···O=C-(tBu) interaction in H1 vanish, and all three derivatives exhibit the same free (tBu)C=O frequency.

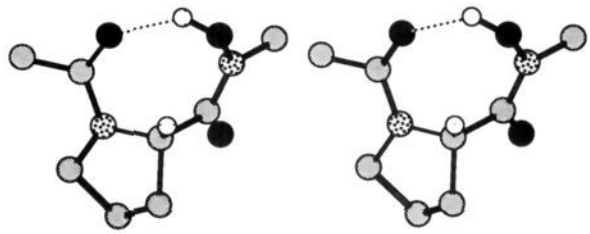

Figure 7. Stereoview of the SYBYL-minimized conformation of the γ^--like expanded turn in Ac-Proψ[CO–N(O^{β}H)]NHMe. The O^{β}—H···O=C hydrogen bond closing an 8-membered cycle is indicated by a broken line.

N^{β}-Boc acylation in A′1 confers an enhanced proton donor character on the N^{β}–H bond, which becomes capable of interacting with (tBu)C=O, as denoted by the two N^{β}–H stretching components at 3394 cm^{-1} (free state, sharp peak) and at 3283 cm^{-1} (bonded state, broad peak) in CCl_4. However, the great solvent sensitivity of the $N^{\beta}H$ resonance (2.23 ppm compared with 0.65 ppm for N*H* (Me) in P1 when going from C^2HCl_3 to DMSO-d_6) is typical of an easily accessible site for solvation by weakly polar solvent molecules.

With reference to the γ-turn in peptides characterized by an N—H···O=C hydrogen bond closing a 7-membered cycle, the conformation resulting from the N^{β}/O^{β}—H···O=C(tBu) interaction in A′1 and H1, closing an 8-membered cycle, will be called an expanded γ-like turn in the following (Figure 7).

***N*-Amino (A2) and *N*-Hydroxy (H2) Analogues of Piv-Pro-Gly-NHiPr (P2).** The low (tBu)C=O and (iPr)N–H stretching frequencies for A2 in CH_2Cl_2 (Table II) and the low solvent sensitivity of the (iPr)N*H* resonance (Figure 6b) are typical of a β-turn structure which is of type II on the basis of the (tBu)C=O contribution at 1600 cm^{-1}. The percentage of βII-turn structures in CH_2Cl_2 can be estimated to be 85% from the (iPr)N*H* resonance shift (0.38 ppm) from C^2HCl_3 to DMSO-d_6.[56] N^{β}-Z acylation in A′2 results in two N^{β}–H and (iPr)N–H stretching frequencies typical of hydrogen-bonded vibrators (Table II) in a particular conformation associating in the same molecule a βII-turn and an expanded γ-like turn and resembling the crystal molecular structure depicted in Figure 5b. However, the great solvent sensitivity of the $N^{\beta}H$ resonance (2.33 ppm when going from C^2HCl_3 to DMSO-d_6) confirms the destabilizing effect of solvation of the expanded γ-like turn as already pointed out for A′1.

The *N*-hydroxy analogue H2 in low solvating media exhibits IR and NMR data quite different from those for A2. For example, both the IR absorption at 3420 cm^{-1} (Table II) and the medium solvent sensitivity of the (iPr)N*H* resonance (Figure 6b) are typical of a partially free (iPr)N–H site, and the β-turn content in CH_2Cl_2 is estimated to be only 55% on the basis of the (iPr)N*H* resonance shift (1.17 ppm) when going from C^2HCl_3 to DMSO-d_6.[56] We observe that the (tBu)C=O, (iPr)N–H, and O^{β}–H absorptions are split into two low components (Table II), which shows that each site is engaged in two different hydrogen bonds. We must conclude that H2 assumes three folded conformations in CH_2Cl_2: (i) a minor βII-turn, similar to the crystal molecular structure in Figure 4a and denoted by the bonded (tBu)C=O absorption at 1599 cm^{-1}, (ii) an expanded γ-like turn characterized both by the nonbonded (iPr)N–H contribution at 3420 cm^{-1} and the bonded O^{β}–H absorption at 3205 cm^{-1}, and (iii) a conformer accommodating simultaneously the βII and expanded γ-like turns (Figure 4b), as reflected by both the bonded (iPr)N–H stretching at 3372 cm^{-1} and the very low (tBu)C=O contribution at 1581 cm^{-1}. It is interesting to note that O^{β}-benzylation in H′2 leads to the same IR (Table II) and NMR (not shown) data as for P2 and A2, and therefore restores the βII-turn.

In DMSO, P2, A2, and H2 exhibit similar (tBu)C=O absorptions (Table II) and close (iPr)N*H* resonances (Figure

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Table VI. Conformational Characteristics[a] of the Molecular Dynamics Generated Turns of the Expanded β- and γ-Type in *N*-Hydroxy Peptides after Enthalpy Minimization of the Different Conformational Classes with Their Occurrences

compd	Pro		Gly		CO–NOH	(N/O$^\beta$)H···O(Ac)			enthalpy (kcal/mol)	occurrence (%)
	ϕ	ψ	ϕ	ψ	ν^b	d_8	d_{10}	d_{11}		
Ac-Proψ[CO–N(O$^\beta$H)]NHMe										
γ^--like turn	–74	99			–73	1.78			4.86	99
γ^+-like turn	–96	55			56	2.00			7.20	1
Ac-Proψ[CO–N(O$^\beta$H)]Gly-NHMe										
βII-turn	–69	109	72	28	100		1.89		4.29	45
βII/γ^--like turn	–67	101	63	32	–77	1.83	2.06		2.98	55
Ac-Pro-Glyψ[CO–N(O$^\beta$H)]NHMe										
βII$^+$-like turn	–72	76	172	–61	100			2.14	0.46	35
βII$^-$-like turn	–71	79	81	65	–100			2.02	0.56	3
βI$^-$-like turn	–71	–17	–133	66	–87			1.83	0.80	37
βI$^+$-like turn	–76	–12	–59	–50	100			1.84	0.93	25

[a] Torsional angles in deg and interatomic distances in Å. [b] C′–N$^\alpha$–O$^\beta$–H angle.

Table VII. Conformations Adopted by the Piv-Pro-Gly-NHiPr Dipeptide and the *N*-Methyl, *N*-Hydroxy, and *N*-Amino Analogues in Solution and in the Solid State[a]

compd	crystal	solution	
		CH_2Cl_2	Me_2SO
Piv-Pro-Gly-NHiPr (P2)	βII-turn[b]	βI- and βII-turn[c]	βII-turn[c]
Piv-Proψ[CO–NMe]Gly-NHiPr	βII-turn[d]	βII- and βVI-turn[e]	βII- and βVI-turn[e]
Piv-Proψ[CO–N(NH_2)]Gly-NHiPr (A2)	βII-turn[f]	βII-turn[f]	βII-turn[f]
Piv-Proψ[CO–N(OH)]Gly-NHiPr (H2)	βII-turn[f]	"γ"- and βII/"γ"-turn[f]	βII-turn[f]
Piv-Pro-Glyψ[CO–NMe]NHMe	op.[g]	γ-turn/op.[h]	op.[h]
Piv-Pro-Glyψ[CO–N(NH_2)]NHMe (A3)	op.[f]	γ-turn/op.[f]	op.[f]
Piv-Pro-Glyψ[CO–N(OH)]NHMe (H3)	op.[f]	"β"- and "γ"-turn[f]	op.[f]

[a] "β"- and "γ"-turns stand for the expanded β- and γ-like turns, respectively; op. means an open conformer. [b] Reference 55. [c] References 56 and 63. [d] Reference 74. [e] Reference 72. [f] Present work. [g] Reference 75. [h] Reference 76.

6b). This fact claims the same βII-turn structure in this aprotic solvent in which, due to easy solvation of the O$^\beta$—H bond, the expanded γ-like turn in H2 is less stable than the β-turn.

***N*-Amino (A3) and *N*-Hydroxy (H3) Analogues of Piv-Pro-Gly-NHiPr (P2).** Contrary to N-hydroxylation, N-amination of the C-terminal position in P2 leads to some amount of cis conformers in nonpolar solvents (30% in $CHCl_3$). Furthermore, the *N*-amino A3 and *N*-hydroxy H3 analogues display completely different IR absorptions in CH_2Cl_2 (Table II). Both Piv and Pro carbonyl frequencies correspond to free vibrators in A3, whereas their splitting for H3 indicates their partial involvement in an interaction with the O$^\beta$—H bond, as confirmed by the strong and very low O$^\beta$–H stretching frequency about 3220 cm^{-1}. Therefore, in CH_2Cl_2, H3 assumes two folded structures: (i) an expanded γ-like turn involving the Gly residue and characterized by the O$^\beta$—H···O=C(Pro) interaction and (ii) another conformation characterized by the O$^\beta$—H···O=C(tBu) interaction, closing an 11-membered ring, that we propose to call an expanded β-like turn (Figure 8). In DMSO, both A3 and H3 give rise to very similar IR data, and none of these folded structures is retained (Table II).

The Gly N–H stretching frequency is split into a weak component at 3435 cm^{-1}, typical of a nonbonded vibrator, and a medium component at 3401 cm^{-1}, indicating that about two-thirds of the N–H sites are involved in some weak interaction, as confirmed by the low solvent sensitivity of the N*H* resonance in C^2HCl_3/DMSO-d_6 mixtures containing less than 20% of DMSO (Figure 6c). We will see in the following that this splitting is probably related to the multiple forms of the expanded β-like turn (Table VI).

Molecular Dynamics

In order to get more conformational information on the expanded γ- and β-like turns in *N*-hydroxy peptides, we have carried out a molecular dynamics simulation for the *N*-acetyl analogues of H1, H2, and H3. Among the 8000 conformers retained from the last 80 ps of molecular dynamics, we have considered the conformers having the two following peculiarities: (i) a (N or O$^\beta$)H···O(Ac) distance (denoted by d_8, d_{10}, or d_{11} according to the number of atoms in the chelation cycle) in the 1.5–2.5-Å range and (ii) a molecular energy of less than 10 kcal above the energy minimum. When these conformers are plotted on Ramachandran maps, the points are concentrated in a few regions defining the folded conformers.

The short d_8 distances in H1 are distributed in two regions of the ϕ, ψ, ν conformational space. The torsional angles, obtained by energy minimization, of the two possible expanded γ^- and γ^+ structures (the exponent refers to the sign of the ν angle) are indicated in Table VI with their conformational energy. Practically all of these folded conformers (99%) generated by molecular dynamics are of the γ^- type (Figure 7), which is therefore the most probable form of the expanded γ-like turn in *N*-hydroxy peptides. By considering the compatibility of the short d_8 (γ-like turn) and d_{10} (β-turn) distances generated during molecular dynamics within H2, we have found that 55% of the β-folded (type II) conformers also present the (O$^\beta$)H···O(Ac) interaction (Figure 4b). The energy-minimized torsional angles for both βII and βII/γ^- conformational arrangements are indicated in Table VI.

The conformers with a short d_{11} distance in H3 are distributed in four subsets, suggesting that the expanded β-like turn in *N*-hydroxy peptides is more flexible than the β-turn in peptides. Energy minimization of each conformational subset gives the four standard folded conformers (Table VI and Figure 8) which differ from each other by (i) the orientation of the middle amide group, as for the classical βI- and βII-turns in peptides and (ii) the ± orientation (ν angle) of the O$^\beta$–H bond with respect to the amide plane. Hence their name of βI/II$^\pm$-like turns. All four forms have very similar conformational enthalpies, but on the basis of the occurrence of each class, the three conformers noted βI$^+$, βI$^-$, and βII$^+$ in Table VI are nearly equally probable and much more frequent than the conformer βII$^-$ (Table VI). Moreover, the compared occurrences of short d_8 [(O$^\beta$)H···O-(Gly)] and d_{11} [(O$^\beta$)H···O(Ac)] distances in H3 during molecular dynamics suggest that the expanded β-like turn is twice more frequent than the expanded γ-like turn.

Conclusion

The combined use of X-ray diffraction and ^{1}H-NMR and IR spectroscopy is a valuable tool for the analysis of the possible

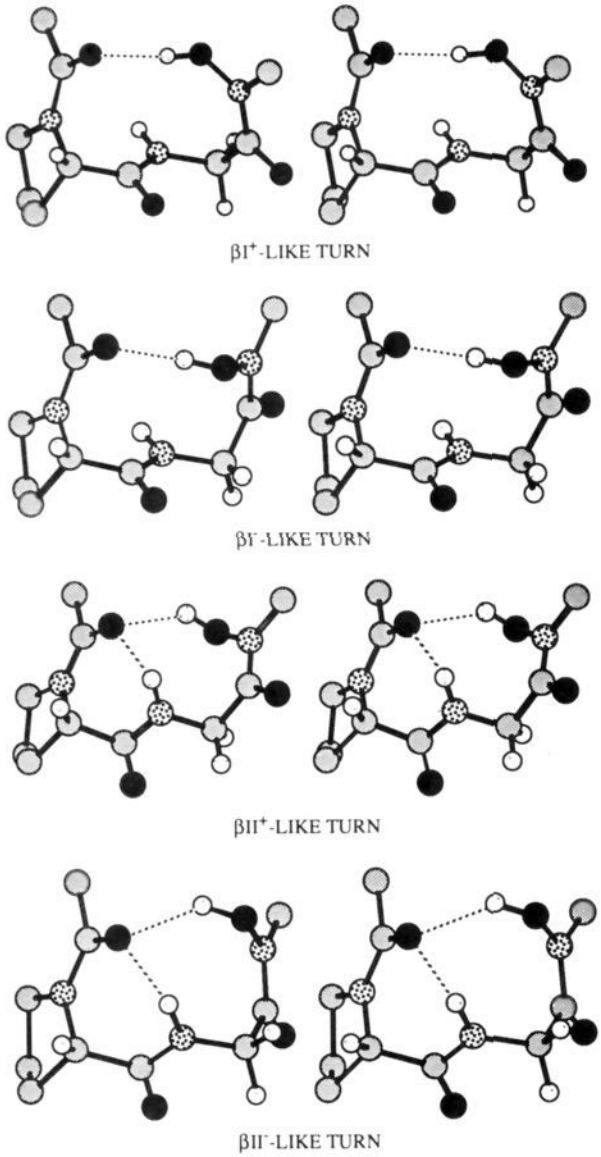

Figure 8. Stereoviews of SYBYL-minimized conformations of the expanded β-like turn in Ac-Pro-Glyψ[CO–N(O^{β}H)]NHMe. The O^{β}—H···O=C hydrogen bond closing an 11-membered cycle is indicated by a broken line. Note that both expanded βII⁺- and βII⁻-like turns are compatible with the γ-folded conformation of proline.

intramolecular hydrogen bonds which are responsible for the conformational preferences of small peptide analogues. A molecular dynamics simulation during a short time (100 ps) at 350 K allows these small molecules to visit the whole permitted conformational space and gives access to the various subsets of conformers presenting a given intramolecular hydrogen bond. The relative occurrences of the conformers generated in each subset also give an estimation of the structural preferences. In Table VII are compared the conformational states of the Piv-Pro-Gly-NHiPr dipeptide and its *N*-hydroxy, *N*-amino, and *N*-methyl analogues in solution and in the solid state. An interesting point is that, contrary to N-methylation, which tends to stabilize a very tight cis folded conformation denoted as a βVI-turn,[72,73] N-hydroxylation or N-amination of an amide bond within a peptide chain seems to have no tendency to induce a cis conformation of the modified peptide bond.

The analysis of the *N*-amino and *N*-hydroxy analogues of the Piv-Pro-Gly-NHiPr (P2) dipeptide reveals that, although N-amination and N-hydroxylation of a peptide bond have little influence on the local geometry, both substitutions affect the conformational properties of the analogue to an extent depending on their position in the chain. The conformational perturbations are essentially due to modifications of the hydrogen-bonding ability of the N-substituted peptide group. The *N*-hydroxyl bond is a strong proton donor capable of very short contacts with carbonyl oxygens whereas the *N*-amino group is a much weaker proton donor. However, acylation of the *N*-amino group enhances the proton-donating properties of the N^{β}–H bond, which then becomes capable of attractive contacts with carbonyl oxygens.

Peptide P2 is known to assume preferentially the βII-turn conformation in different solvents[54,56] and in the solid state as well.[55] The same holds true for the *N*-amino analogue A2 having a middle *N*-amino amide group. Due to its low proton-donating character, the *N*-amino group has only little tendency to interact with the peptide chain and does not interfer in the formation of the βII-turn. Due to the strong electrophilicity of the *N*-hydroxyl, the situation is quite different with the *N*-hydroxy analogue H2, which assumes different conformations depending on the environment. In low polar solvents, the βII-turn only concerns about half of the molecules and is in competition with a second conformer characterized by the interaction of the *N*-hydroxyl group with the preceding carbonyl so as to close an 8-membered cycle in a sort of an expanded γ-turn. Moreover, in most of the βII-folded conformers, this latter interaction coexists with the classical $i + 3 \rightarrow i$ hydrogen bond. In the crystal, or in a more polar solvent like DMSO, the βII-turn becomes the favored structure while the *N*-hydroxyl bond prefers intermolecular contacts with neighboring molecules or with the solvent molecules, respectively.

When N-substitution takes place at the C-terminal position of P2, the well-known β-folded conformation is excluded, but the *N*-amino A3 and *N*-hydroxy H3 analogues again behave differently in low polar solvents. Due to the weak electrophilicity of the *N*-amino group, no clear structuration appears for A3 whereas H3 assumes two different folded conformations. The *N*-hydroxyl group in position $i + 3$ is capable of interacting not only with the peptide carbonyl in position $i + 1$ to form the 8-membered cycle already found in H2 but also with the carbonyl oxygen in position i to form an 11-membered cycle. The resulting expanded γ- and β-like folded conformations have been modelized, and the most probable conformers are comparable with the classical γ- and β-turns. However, they seem to be more sensible to solvation.

The above data collected on very simple model pseudopeptides give an indication on the local conformational tendencies induced by N-amination and N-hydroxylation of a peptide chain. Of course, only short-range interactions are present in these molecules and additional conformational analyses on larger *N*-amino and *N*-hydroxy peptides with reference to the cognate peptides are required to investigate the possibility of long-range interactions. However, the conformational tendencies described in this work are of interest for the design of peptidomimetics when the side chains are required for bioactivity and cannot be modified.

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Supplementary Material Available: Tables of fractional coordinates for the hydrogen and non-hydrogen atoms, equivalent thermal parameters and anisotropic thermal parameters for the non-hydrogen atoms, and interatomic bond distances and bond angles and Ramachandran maps showing the occurrences of the expanded β- and γ-like turns in *N*-hydroxy peptides (28 pages). Ordering information is given on any current masthead page.

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