Structural Comparison of Homologous Reduced Peptide, Reduced Azapeptide, Iminoazapeptide, and Methyleneoxypeptide Analogues

R. Vanderesse,*,† V. Grand,†,⊥ D. Limal,†,∥ A. Vicherat,† M. Marraud,† C. Didierjean,‡ and A. Aubry‡

Contribution from the Laboratory of Macromolecular Physical Chemistry, UMR 7568 CNRS-INPL, ENSIC-INPL, BP 451, 54001 Nancy, France, and Laboratory of Crystallography and Modeling of Mineral and Biological Materials, ESA-7036, University Henri Poincaré of Nancy, BP 236, 54509 Vandoeuvre, France

Received March 19, 1998

Abstract: The homologous RCO-Pro-Xaa-NHR′ model pseudodipeptides containing the reduced peptide ($C^{\alpha}H_2NHC^{\alpha}$), reduced azapeptide ($C^{\alpha}H_2NHN^{\alpha}$), methyleneoxy ($C^{\alpha}H_2OC^{\alpha}$), and iminoazapeptide ($C^{\alpha}H{=}NN^{\alpha}$) surrogate of the middle amide group have been prepared. Their structural analysis has been carried out in solution by ^{1}H NMR and IR spectroscopy and in the solid state by X-ray diffraction. The last three fragments, not protonated in the pH range 2−12, and the reduced fragment in its neutral amine form induce quite similar molecular structures characterized by a hydrogen bond between NHR′ and the N/O atom replacing the amide NH group and closing a five-membered cycle. The neutral amine or protonated ammonium state of the reduced amide fragment, with a pK_a value of about 7, depends on the environment. Protonation induces a conformational transition due to the strong proton donating properties of the ammonium group which interacts with the RCO carbonyl.

Various amide surrogates have been used for the design of peptidase inhibitors.[1] The reduced amide bond CH_2−NH, considered as mimicking the $C(OH)_2$−NH transient state of the amide bond CO−NH during enzymatic cleavage, is frequently used,[2] probably because of its easy introduction in a peptide chain by reductive amination of a peptide aldehyde.[3] However, there is some NMR indication that the pK_a of a reduced amide is at about neutral pH,[4] and the question arises about the neutral or ionic state of a reduced peptide at the physiological pH.

The reduced peptides have been the subject of a limited number of experimental[4,5] and theoretical[6] conformational analyses. The crystal structures of the N^{α}-Z and N^{α}-Boc-Proψ[CH_2NH]Leu-Gly-NH_2 reduced derivatives of the oxytocin C-terminal tripeptide,[7] containing a reduced Pro-Leu amide bond, have been solved,[8] as well as the structures of reduced protease inhibitors in renin[2b,d] or endothiapepsin[9] complexes. Surprisingly, most of these studies do not take into account the possibility for the reduced amide bond to be protonated at the physiological pH. This point is of importance since experimental results have demonstrated that protonation of a reduced amide link affects considerably its conformational properties.[5e,10]

† ENSIC-INPL.

‡ University Henri Poincaré of Nancy.

⊥ Present address: Laboratory for Scientific Police, BP 2162, 31021 Toulouse Cedex 2, France.

∥ Present address: IBMC, 67084 Strasbourg Cedex, France.

¶ Abbreviations: Boc, *tert*-butyloxycarbonyl; DIEA, diisopropylethylamine; ICF, isobutyl chloroformate; NMM, *N*-methylmorpholine; PE, petroleum ether; Piv, pivaloyl; TFA, trifluoroacetic acid; Z, benzyloxycarbonyl.

(1) Gante, J. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 1699−1720 and references therein.

(2) (a) Szelke, M.; Leckie, B. J.; Tree, M.; Brown, A.; Grant, J.; Hallett, A.; Hughes, M.; Jones, D. M.; Lever, A. F. *Hypertension, Suppl. II* **1982**, *4*, 52−69. (b) Epps, D. E.; Mao, B.; Staples, D. J.; Sawyer, T. K. *Int. J. Pept. Protein Res.* **1988**, *31*, 22−34. (c) Sawyer, T. K.; Pals, D. T.; Mao, B.; Maggiora, L. L.; Staples, D. J.; De Vaux, A. E.; Shostarez, H. J.; Kinner, J. H.; Smith, C. W. *Tetrahedron* **1988**, *44*, 661−673. (d) Sawyer, T. K.; Pals, D. T.; Mao, B.; Staples, D. J.; De Vaux, A. E.; Maggiora, L. L.; Affholter, J. A.; Kati, W.; Duchamp, D.; Hester, J. B.; Smith, C. W.; Saneii, H. H.; Kinner, J.; Handschumacher, M.; Carlson, W. *J. Med. Chem.* **1988**, *31*, 18−30. (e) Kaltenbronn, J. S.; Hudspeth, J. P.; Lunney, E. A.; Michniewicz, B. M.; Nicolaides, E. D.; Repine, J. T.; Roark, W. H.; Stier, M. A.; Tinner, F. J.; Woo, P. K. W.; Essenburg, A. D. *J. Med. Chem.* **1990**, *33*, 838−845.

(3) (a) Szelke, M.; Leckie, B.; Hallet, A.; Jones, D. M.; Sueiras, J.; Atrasch, B.; Lever, A. F. *Nature* **1982**, *299*, 555−557. (b) Martinez, J.; Bali, J. P.; Rodriguez, M.; Castro, B.; Magous, R.; Laur, J.; Lignon, M. F. *J. Med. Chem.* **1985**, *28*, 1874−1879.

(4) Aumelas, A.; Rodriguez, M.; Heitz, A.; Castro, B.; Martinez, J. *Int. J. Pept. Protein Res.* **1987**, *30*, 596−604.

(5) (a) Di Bello, C.; Scatturin, A.; Vertuani, G.; D′Auria, G.; Gargiulo, M.; Paolillo, L.; Trivellone, G.; Gozzoni, L.; De Castiglione, R. *Biopolymers* **1991**, *31*, 1397−1408. (b) Kazmierski, W. M.; Ferguson, R. D.; Knapp, R. J.; Lui, G. K.; Yamamura, H. I.; Hruby, V. J. *Int. J. Pept. Protein Res.* **1992**, *39*, 401−414. (c) Vander Elst, P.; Elseviers, M.; De Cock, E.; Van Marsenille, M.; Tourwé, D.; Van Binst, G. *Int. J. Pept. Protein Res.* **1986**, *27*, 633−642. (d) Ma, S.; Spatola, A. F. In *Peptides: Chemistry and Biology*; Smith, J. A., Rivier, J. E., Eds.; ESCOM: Leiden, The Netherlands, 1992; pp 777−778. (e) Ma, S.; Spatola, A. F. *Int. J. Pept. Protein Res.* **1993**, *41*, 204−206.

(6) (a) Dauber-Osguthorpe, P.; Jones, D. K.; Campbell, M. M.; Osguthorpe, D. J. *Tetrahedron Lett.* **1990**, *31*, 917−920. (b) Dauber-Osguthorpe, P.; Campbell, M. M.; Osguthorpe, D. J. *Int. J. Pept. Protein Res.* **1991**, *38*, 357−377.

(7) In the following, we use Spatola's nomenclature for pseudopeptides where the bracketed group is substituted for the amide CO-NH group (Spatola, A. F. In *Chemistry and Biochemistry of Amino Acids, Peptides and Proteins*; Weinstein, B., Ed.; Marcel Dekker Inc.: New York, 1983; Vol. 7, pp 267−357). In addition, an amino acid analogue is noted by putting in quotes the corresponding three-letter code, and the methylene carbon in a reduced amino acid analogue is noted C_r. The AzAla and AzGly codes specify the aza analogues of alanine and glycine, respectively.

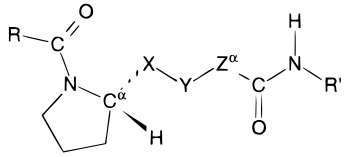
(8) Toniolo, C.; Valle, G.; Crisma, M.; Kaltenbronn, J. S.; Repine, J. T.; Van Binst, G.; Elseviers, M.; Tourwé, D. *Pept. Res.* **1989**, *2*, 332−337.

(9) Cooper, J.; Foundling, S.; Hemming, A.; Blundell, T.; Jones, D. M.; Hallett, A.; Szelke, M. *Eur. J. Biochem.* **1987**, *169*, 215−221.

(10) (a) Grand, V.; Aubry, A.; Dupont, V.; Vicherat, A.; Marraud, M. *J. Pept. Sci.* **1996**, *2*, 381−391. (b) Geyer, A.; Müller, G.; Kessler, H. *J. Am. Chem. Soc.* **1994**, *116*, 7735−7743.

S0002-7863(98)00937-8 CCC: $15.00
Published on Web 09/09/1998

Chart 1. General Formula of the Investigated Reduced Dipeptide (X-Y-Z^{α} = $C_rH_2NHC^{\alpha}HMe$), Methyleneoxypeptide (X-Y-Z^{α} = $C_rH_2OC^{\alpha}H_2$), Iminoazapeptide (X-Y-Z^{α} = $C_rH{=}NN^{\alpha}Me$), and Reduced Azapeptide (X-Y-Z^{α} = $C_rH_2NHN^{\alpha}Me$) Analogues.



A neutral amine is essentially a proton-accepting site by its nitrogen lone pair and a weak donating site, whereas an ammonium is exclusively a strong proton donor. In particular, the ammonium group is capable of interacting with the peptide carbonyls and favoring β- and γ-like turns in the chain.[10a]

The phosphonamide, phosphinic, sulfinamide, and sulfonamide groups[11] have been also proposed as surrogates of the amide tetragonal transient state, but their difficult synthesis is an obstacle to a general use. A noncleavable, easy to obtain, nonprotonable peptide surrogate would be of interest to the design of bioresistant peptide analogues. The lower pK_a value of 3.53 for protonated semicarbazide[12] indicates that it is not protonated at the physiological pH. We therefore have examined the structural properties of the RCO-Pro-Xaa-NHR′ dipeptide analogues containing a modified Pro-Xaa link and having the general formula in shown Chart 1, where RCO = Boc or Piv, R′ = H or ^{i}Pr, X-Y-Z^{α} = $C_rH_2NHC^{\alpha}$ (neutral reduced peptide), $C_rH_2N^+H_2C^{\alpha}$ (protonated reduced peptide), $C_rH_2NHN^{\alpha}$ (reduced azapeptide), $C_rH_2OC^{\alpha}$ (methyleneoxypeptide), or $C_rH{=}NN^{\alpha}$ (iminoazapeptide), where C_r denotes the "reduced" carbon replacing the peptide carbonyl. The structural analysis has been carried out on the pseudopeptides listed in Table 1 and on the compounds reproducing the N- and C-terminal part of the molecules by using ^{1}H NMR, IR spectroscopy, and X-ray diffraction. Such small molecules are readily solvated by water, where they do not exhibit defined spectroscopic data. We therefore have considered organic solvents (CH_2Cl_2, $CHCl_3$, MeCN, and DMSO) with increasing polarity for the spectroscopic experiments.

Experimental Procedures

Synthesis. The reduced dipeptide **1** was obtained by the reductive amination procedure[3b] by coupling the aldehyde **5**[13] to the L-alanine methyl ester (Figure 1). To make the chromatographic purification easier and to prevent undesirable side reactions during further acylation steps, the reduced amide was Z-protected to give **6**. The ester function in **6** was saponified and converted into the isopropylamide group in **7** by the mixed anhydride method. The Piv group was substituted for Boc in **1′**, and the Z group was eliminated by catalytic hydrogenolysis to yield the neutral reduced dipeptide **1**, which was then protonated quantitatively to $\mathbf{1}^+$ by $PF_6^-{\cdot}Et_2O^+H$ (Aldrich 17,638-9). The PF_6^- anion was selected to obtain an ammonium salt soluble in nonpolar media and for its weak interaction with ammonium anions,[14] thus allowing the ammonium group to participate in intramolecular interactions.[15] The methyleneoxypeptide **2** was prepared from the commercially available Boc-prolinol **10** (Merck 818348), which was treated by NaH and reacted with *N*-isopropyl-bromoacetamide (**9**) (Figure 2).[16] The synthesis of the iminoazadipeptides **3** and **3′** and the reduced azadipeptide **4** is summarized in Figure 3. Coupling of Boc-prolinal **5**[16] either to semicarbazide **11** or to the substituted semicarbazide **13** gave the iminoazadipeptides **3′** and **14**, respectively. Substitution of the Piv group for Boc in **3**, followed by catalytic reduction of the imine

Table 1. List of the Pseudopeptides Investigated with the Derivatives Reproducing the N- or C-Terminal Part of the Molecules[a]

compd	code	techniques	ref
Neutral Reduced Peptides			
Piv-Proψ[CH_2NH]Ala-NH^{i}Pr	**1**	IR, ^{1}H NMR, X-ray	*b*
Piv-Proψ[CH_2NZ]Ala-NH^{i}Pr	**1′**	X-ray	*b*
Piv-Proψ[CH_2NH]NH^{i}Pr	**1N**	IR	10a
^{n}Bu$_2$NCH$_2$CONHMe	**1C**	IR	15
Protonated Reduced Peptides[c]			
Piv-Proψ[$CH_2N^+H_2$]Ala-NH^{i}Pr	$\mathbf{1}^+$	IR, ^{1}H NMR	*b*
Piv-Proψ[$CH_2N^+H_2$]NH^{i}Pr	$\mathbf{1}^+$**N**	IR	10a
^{n}Bu$_2N^+$HCH$_2$CONHMe	$\mathbf{1}^+$**C**	IR	15
Methylenoxypeptide			
Piv-Proψ[CH_2O]Gly-NH^{i}Pr	**2**	IR, ^{1}H NMR	*b*
MeOCH$_2$CONH^{i}Pr	**2C**	IR, ^{1}H NMR	*b*
Iminoazapaptides			
Piv-Proψ[CH═N]AzAla-NH^{i}Pr	**3**	IR, ^{1}H NMR, X-ray	*b*
Boc-Proψ[CH═N]AzGly-NH$_2$	**3′**	X-ray	*b*
^{i}PrCH═NNMeCONH^{i}Pr	**3C**	IR, ^{1}H NMR	*b*
Reduced Azapeptide			
Piv-Proψ[CH_2NH]AzAla-NH^{i}Pr	**4**	IR, ^{1}H NMR, X-ray	*b*
^{i}BuNHNMeCONH^{i}Pr	**4C**	IR, ^{1}H NMR	*b*

[a] For nomenclature, see ref 7. [b] This work. [c] PF_6^- is the associated anion.

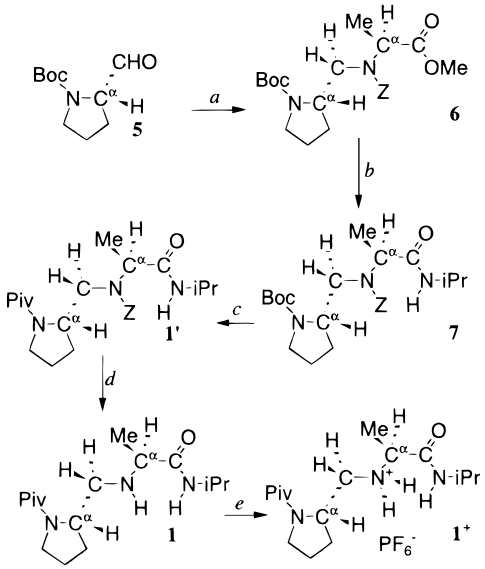


Figure 1. Synthesis of the neutral **1** and protonated $\mathbf{1}^+$ reduced dipeptide by reductive amination and intermediate Z protection (**1′**) of the reduced amide bond. *a*: (1) HCl·H−Ala-OMe, (2) $NaBH_3CN$, (3) ZCl/NMM; *b*: (1) 1 N NaOH/acetone, (2) ICF/NMM, (3) ^{i}PrNH$_2$; *c*: (1) HCl/AcOEt, (2) Piv-Cl/NMM; *d*: H_2/5% Pd−C; *e*: $Et_2O^+H.PF_6^-$.

(11) (a) Elliott, R. L.; Marks, N.; Berg, M. J.; Portoghese, P. S. *Biochemistry* **1985**, *28*, 1208−1216. (b) Bartlett, P. A.; Marlowe, C. K. *Biochemistry* **1987**, *26*, 8553−8561. (c) Parson, W. H.; Patchett, A. A.; Bull, H. G.; Schoen, W. R.; Taub, D.; Davidson, J.; Combs, P. L.; Springer, J. P.; Gadebusch, H.; Weissberger, B.; Valiant, M. E.; Mellin, T. N.; Busch, R. D. *J. Med. Chem.* **1988**, *31*, 1772−1778. (d) Morgan, B. P.; Scholtz, J. M.; Ballinger, M. D.; Zipkin, I. D.; Bartlett, P. A. *J. Am. Chem. Soc.* **1991**, *113*, 297−307. (e) Phillips, M. A.; Kaplan, A. P.; Rutter, W. J.; Bartlett, P. A. *Biochemistry* **1992**, *31*, 959−963. (f) Moree, W. J.; Van der Marel, G. A.; Liskamp, R. M. J. *Tetrahedron Lett.* **1991**, *32*, 409−412. (g) Merricks, D.; Sammes, P. G.; Walker, E. R. H.; Henrick, K.; McPartlin, M. *J. Chem. Soc., Perkin Trans. 1* **1991**, 2169−2176. (h) Calvagni, A.; Gavuzzo, E.; Lucente, G.; Mazza, F.; Pinnen, F.; Pochetti, G.; Rossi, D. *Int. J. Pept. Protein Res.* **1991**, *37*, 167−173.

(12) Goddard, D. R.; Lodam, B. D.; Ajavi, S. O.; Campbell, M. J. *J. Chem. Soc. (A)* **1969**, 506−512.

(13) Fehrentz, J. A.; Castro, B. *Synthesis* **1983**, 676−678.

(14) Moreno-Gonzalez, E.; Marraud, M.; Néel, J. *J. Chim. Phys., Phys.-Chim. Biol.* **1977**, *74*, 563−571.

(15) Moreno-Gonzalez, E.; Marraud, M. *J. Chim. Phys., Phys.-Chim. Biol.* **1980**, *77*, 149−155.

(16) Breton, P.; Monsigny, M.; Meyer, R. *Int. J. Pept. Protein Res.* **1990**, *35*, 346−351.

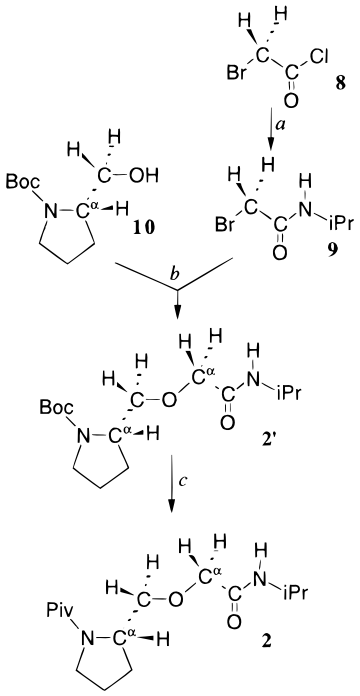


Figure 2. Synthesis of the methyleneoxy peptide **2**.[16] *a*: $^{i}PrNH_2$; *b*: NaH/THF; *c*: (1) H_2/5% Pd−C, (2) Piv-Cl/NMM.

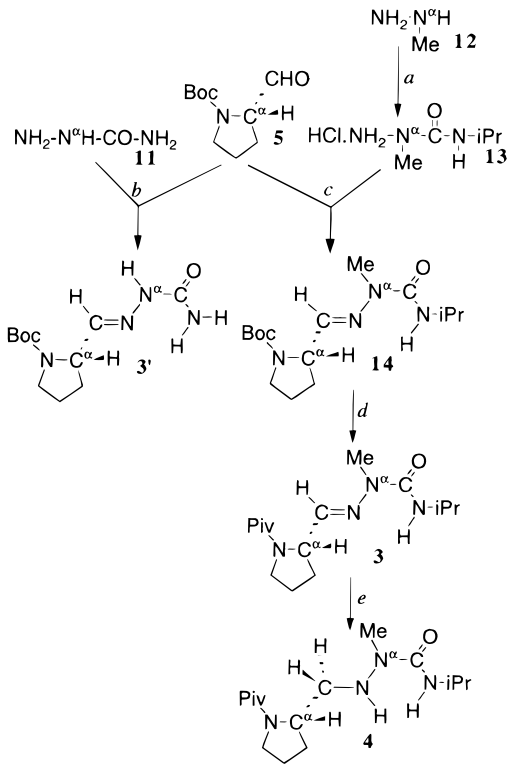


Figure 3. Synthesis of the iminoazapeptides **3** and **3′** by condensation of Boc-prolinal and a semicarbazide, and reduction of **3** into the reduced azadipeptide **4**: (*a*) (1) O═C═N^{i}Pr, (2) HCl/AcOEt; (*b*) AcO^-Na^+/EtOH/molecular sieves/room temperature (rt); (*c*) NMM/AcO^-Na^+/EtOH/molecular sieves/rt; (*d*) (1) TFA/CH_2Cl_2 40/60, (2) Piv-Cl/NMM; (*e*) H_2/5% Pd−C/EtOH/AcOH.

bond, gave the reduced azadipeptide **4**. The Piv group in **1**−**4** was introduced to exclude the cis conformation of the Pro-preceding amide bond.[17] All intermediate and final derivatives were purified by flash chromatography and characterized by ^{1}H NMR and IR spectroscopy. The melting points were uncorrected. R_f values were measured by thin-layer chromatography (Merck, Silicagel 60 F_{254} plates 5735).

Piv-Proψ[C_rH_2NZ]Ala-NH^{i}Pr. 1′ (Figure 1) was purified by silica gel flash chromatography (AcOEt/PE 60/40) and recrystallized from AcOEt/PE. White crystals: mp = 118−120 °C, R_f = 0.36 (^{i}PrOH/CH_2Cl_2 3/97) or 0.31 (AcOEt/PE 50/50), 20% overall yield. IR (CH_2Cl_2, cm^{-1}): 3280 (^{i}Pr-*NH*); 1701 (Z-*CO*); 1675 (Ala-*CO*); 1611 (Piv-*CO*). ^{1}H NMR (DMSO-d_6, ppm), the two conformers I (35%) and II (65%) denote *cis*−*trans* isomerization of the Z−N amide bond: 0.86 (I) and 1.03 (II) (d, ^3J = 6.7 Hz, 6H, ^{i}Pr-(CH_3)$_2$); 0.95 (I) and 1.14 (II) (s, 9H, Piv-(CH_3)$_3$); 1.39 (d, ^3J = 7.2 Hz, Ala-C$^{\beta}H_3$); 1.54−2.09 (m, 4H, "Pro"-C$^{\beta}H_2$ + C$^{\gamma}H_2$); 3.26 (B) and 3.41 (A) (*AB*X, $^2J_{AB}$ = 14.1 Hz, $^3J_{BX}$ = 7.8 Hz, $^3J_{AX}$ = 5.7 Hz, 2H, "Pro"-C$_r$$H_2$); 3.58 (m, 2H, "Pro"-C$^{\delta}H_2$); 3.79 (m, 1H, ^{i}Pr-C*H*); 4.09 (q, ^3J = 7.2 Hz, 1H, Ala-C$^{\alpha}H$); 4.33 (m, 1H, "Pro"-C$^{\alpha}H$); 4.85 (I) and 5.20 (II) (b, 2H, Z-CH_2); 7.33 (b, 5H, Z-C$_6H_5$); 7.48 (I) and 7.89 (II) (b, 1H, ^{i}Pr−N*H*).

Piv-Proψ[C_rH_2NH]Ala-NH^{i}Pr. 1 (Figure 1) was crystallized from AcOEt/PE. White crystals: mp = 82−84 °C, R_f = 0.50 (^{i}PrOH/CH_2Cl_2 10/90), 90% yield. IR (DMSO, cm^{-1}): 3280 (^{i}Pr*NH*), 1613 (Piv-*CO*), 1665 (Ala-*CO*). ^{1}H NMR ($CDCl_3$, ppm): 1.15 and 1.16 (d, ^3J = 6.7 Hz, 6H, ^{i}Pr-(CH_3)$_2$); 1.25 (s, 9H, Piv-(CH_3)$_3$); 1.27 (d, ^3J = 6.7 Hz, Ala-C$^{\beta}H_3$); 1.72 (m, 1H, "Pro"-C$^{\beta}H$); 1.80−2.02 (m, 3H, "Pro"-C$^{\beta}H$ + C$^{\gamma}H_2$); 2.36 (b, 1H, Ala-N*H*); 2.36 (B) and 2.83 (A) (*AB*X, $^2J_{AB}$ = 11.4 Hz, $^3J_{BX}$ = 7.7 Hz, $^3J_{AX}$ = 4.1 Hz, 2H, "Pro"-C$_r$$H_2$); 3.16 (q, ^3J = 6.7 Hz, 1H, Ala-C$^{\alpha}H$); 3.49−3.74 (m, 2H, "Pro"-C$^{\delta}H_2$); 4.05 (m, 1H, ^{i}Pr-C*H*); 4.17 (m, 1H, "Pro"-C$^{\alpha}H$); 7.16 (d, ^3J = 7.5 Hz, 1H, ^{i}Pr−N*H*).

Piv-Proψ[$C_rH_2N^+H_2$]Ala-NH^{i}Pr.PF$_6^-$. 1$^+$ (Figure 1) was recovered as a white powder by lyophilization. IR (DMSO, cm^{-1}): 3280 (^{i}Pr-*NH*), 1616 (Piv-*CO*), 1685 (Ala-*CO*). ^{1}H NMR ($CDCl_3$, ppm): 1.19 and 1.22 (2d, ^3J = 6.6 Hz, 6H, ^{i}Pr-(CH_3)$_2$); 1.29 (s, 9H, Piv-(CH_3)$_3$); 1.52 (d, ^3J = 6.7 Hz, Ala-C$^{\beta}H_3$); 1.50 (m, 1H, "Pro"-C$^{\beta}H$); 1.80−2.30 (m, 3H, "Pro"-C$^{\beta}H$ + C$^{\gamma}H_2$); 3.11 (b, 2H, "Pro"-C$_r$$H_2$); 3.48 (q, ^3J = 6.7 Hz, 1H, Ala-C$^{\alpha}H$); 3.60 and 3.86 (2m, 2H, "Pro"-C$^{\delta}H_2$); 4.03 (m, 1H, ^{i}Pr-C*H*); 4.42 (m, 1H, "Pro"-C$^{\alpha}H$); 7.27 (b, 1H, ^{i}Pr-N*H*); 8.18 and 9.78 (2b, 2H, Ala-N$^+H_2$).

Piv-Proψ[C_rH_2O]Gly-NH^{i}Pr. 2 (Figure 2) was recovered as a colorless oil after silica gel chromatography with AcOEt/PE (30/70): R_f = 0.35; 50% overall yield. IR (CH_2Cl_2, cm^{-1}): 3414 (^{i}Pr-*NH*), 1614 (Piv-*CO*), 1673 (Ala-*CO*). ^{1}H NMR ($CDCl_3$, ppm): 1.20 (d, ^3J = 6.6 Hz, 6H, ^{i}Pr-(CH_3)$_2$); 1.26 (s, 9H, Piv-(CH_3)$_3$); 1.40−2.00 (m, 4H, "Pro"-C$^{\beta}H_2$ + C$^{\gamma}H_2$); 3.40−3.80 (m, 4H, "Pro"-C$^{\delta}H_2$ + C$_r$$H_2$); 3.96 (m, 2H, Gly-C$^{\alpha}H_2$); 4.13 (m, 1H, ^{i}Pr-C*H*); 4.37 (m, 1H, "Pro"-C$^{\alpha}H$); 6.64 (b, 1H, ^{i}Pr-N*H*).

MeOCH$_2$CONH^{i}Pr. 2C was obtained as a colorless oil from $MeOCH_2CO_2H$ (Aldrich 19,455-7) by the mixed anhydride method using ICF, NMM, and $^{i}PrNH_2$. It was purified by silica gel chromatography using ^{i}PrOH/CH_2Cl_2 as eluent (R_f = 0.40). IR (CH_2Cl_2, cm^{-1}): 3411 (*NH*), 1674 (*CO*). ^{1}H NMR ($CDCl_3$, ppm): 1.19 (d, ^3J = 6.5 Hz, 6H, ^{i}Pr-(CH_3)$_2$); 3.42 (s, 3H, OCH_3); 3.87 (s, 2H, CH_2); 4.14 (m, 1H, ^{i}Pr-C*H*); 6.33 (b, 1H, ^{i}Pr-N*H*).

Boc-Proψ[CH═N]AzGly-NH$_2$ (3′) (Figure 3). A solution of Boc-prolinal **5** (6.5 mmol), semicarbazide **11** (9.75 mmol), and AcONa (26 mmol) in EtOH (15 mL) was stirred overnight at room temperature in the presence of molecular sieves. **3′** was purified by silica gel flash chromatography with ^{i}PrOH/CH_2Cl_2 (5/95) as the eluent and crystallized from EtOH. White solid: mp = 161 °C, R_f = 0.38 (^{i}PrOH/CH_2Cl_2 5/95), 85% yield. IR (CH_2Cl_2, cm^{-1}): 1567 (*C*═N), 1687 (AzGly-*CO*); 1700 (Boc-*CO*); 3461, 3528, 3410, and 3355 (*NH*$_2$ + *NH*). ^{1}H NMR ($CDCl_3$, ppm), I (30%) and II (70%) are two conformers due to *cis*−*trans* isomerization of the Boc-"Pro" amide link: 1.41 (I) and 1.46 (II) (s, 9H, Boc-(CH_3)$_3$); 1.60−2.20 (m, 4H, "Pro"-C$^{\beta}H_2$ + C$^{\gamma}H_2$); 3.41 (m, 2H, "Pro"-C$^{\delta}H_2$); 4.30 (I) and 4.40 (II) (m, 1H, "Pro"-C$^{\alpha}H$); 5.50 (b, 2H, NH_2); 7.00 (I) and 7.08 (II) (m, 1H, N═C*H*); 9.17 (I) and 9.51 (II) (b, 1H, N-N*H*).

Piv-Proψ[CH═N]AzAla-NH^{i}Pr (3) (Figure 3). To methylhydrazine (**12**) (20 mmol) in Et_2O (30 mL) was added O═C═N^{i}Pr (20 mmol) in Et_2O (10 mL) under stirring at −5 °C, and the solution was allowed to come back to room temperature. **13** is precipitated by HCl-saturated

(17) (a) Nishihara, H.; Nishihara, K.; Uefuji, T.; Sakota, N. *Bull. Chem. Soc. Jpn* **1975**, *48*, 553−555. (b) Aubry, A.; Cung, M. T.; Marraud, M. *J. Am. Chem. Soc.* **1985**, *107*, 7640−7647. (c) Liang, G. B.; Rito, C. J.; Gellman, S. H. *Biopolymers* **1992**, *32*, 293−301.

Table 2. Crystallographic Data

pseudopeptide	**1**[a]	**1′**[a]	**3**[a]	**3′**[a]	**4**[b]
space group	$P2_12_12_1$	$P2_12_12_1$	$P1$	$P2_12_12_1$	$P2_1/c$
Z	4	4	1	12	4
cell dimensions					
a (Å)	9.337(1)	11.095(1)	5.951(1)	10.022(1)	10.083(2)
b (Å)	9.458(1)	25.617(3)	9.387(1)	16.485(2)	18.553(1)
c (Å)	20.425(3)	8.787(1)	15.920(2)	25.765(3)	10.282(1)
α (deg)			92.98(1)		
β (deg)			98.20(1)		111.19(2)
γ (deg)			105.58(1)		
calcd density (g cm^{-3})	1.10	1.15	1.14	1.24	1.11
no. of total reflns	1785	2722	3139	4374	3632
no. of significant reflns	1529[c]	2265[c]	1744[c]	3136[c]	3357[d]
residual factors					
R	0.055	0.054	0.060	0.046	0.073[e]
R_w	0.059	0.059	0.067	0.055	
w	$1/[\sigma^2(F) + 0.005F^2]$	$2.953/[\sigma^2(F) + 0.0007F^2]$	$0.3774/[\sigma^2(F) + 0.0180F^2]$	$0.0462/[\sigma^2(F) + 0.0023F^2]$	
residual peak height					
max (e Å^{-3})	0.20	0.17	0.32	0.24	0.34
min (e Å^{-3})	−0.25	−0.25	−0.25	−0.30	−0.36

[a] Refined by SHELX86.[19] [b] Refined by SHELXL93.[20] [c] $I > 3\ \sigma(I)$. [d] $I > 2\ \sigma(I)$. [e] w$R = 0.219$.

AcOEt and crystallized from EtOH (white solid, mp = 177 °C, 80% yield). Treatment of **5** by **13** using the same procedure as for **3′** gave **14**. The Boc group was cleaved by TFA, and the Piv group was introduced by PivCl and DIEA to give **3**, which was crystallized from AcOEt. White solid: mp = 70 °C. $R_f = 0.48$ (^{i}PrOH/CH_2Cl_2, 5/95), 85% yield. IR (CH_2Cl_2, cm^{-1}): 1516 (*C*═N); 1618 (Piv-*CO*); 1672 (AzAla-*CO*); 3417 (*NH*). ^{1}H NMR ($CDCl_3$, ppm): 1.16 (d, 6H, ^{i}Pr-(CH_3)$_2$); 1.28 (s, 9H, Piv-(CH_3)$_3$); 1.90−2.10 (m, 4H, "Pro"-C$^\beta H_2$ + C$^\gamma H_2$); 3.14 (s, 3H, AzAla-C$^\beta H_3$); 3.70 (m, 2H, "Pro"-C$^\delta H_2$); 3.94 (m, 1H, ^{i}Pr-C*H*); 4.86 (m, 1H, "Pro"-C$^\alpha H$); 6.23 (d, $^3J = 7.6$ Hz, 1H, N*H*); 6.79 (d, $^3J = 4.0$ Hz, 1H, N═C*H*).

^{i}PrCH═NNMeCONH^{i}Pr. 3C was prepared from isobutyraldehyde and **13** using the same procedure as for **3** to give a colorless oil by silica gel flash chromatography with AcOEt/PE (25/75) as the eluent ($R_f = 0.41$). IR (CH_2Cl_2, cm^{-1}): 1516 (*C*═N); 1669 (AzAla-*CO*); 3413 (*NH*). ^{1}H NMR ($CDCl_3$, ppm): 1.06 (d, $^3J = 6.9$ Hz, 6H, N^{i}Pr-(CH_3)$_2$); 1.31 (d, $^3J = 6.8$ Hz, 6H, C^{i}Pr-(CH_3)$_2$); 2.34 (m, 1H, C^{i}Pr-C*H*); 3.07 (s, 1H, N-CH_3); 3.90 (m, 1H, ^{i}Pr-C*H*); 6.27 (d, $^3J = 6.0$ Hz, 1H, N*H*); 6.66 (d, $^3J = 5.1$ Hz, 1H, ═C*H*).

Piv-Proψ[C$_r$H$_2$NH]AzAla-NH^{i}Pr (4) (Figure 3). Catalytic hydrogenation (96 h) on Pd−C 10% (10 mg) under atmospheric pressure of **3** (40 mg, 0.14 mmol) in MeOH (10 mL) and AcOH (0.2 mL) gave **4**, which was crystallized from AcOEt. White solid: mp = 78 °C, R_f = 0.40 (^{i}PrOH/CH_2Cl_2, 5/95), 90% yield. IR (CH_2Cl_2, cm^{-1}): 1612 (Piv-*CO*); 1653 (AzAla-*CO*); 3404 (*N-H*). ^{1}H NMR ($CDCl_3$, ppm): 1.16 (d, $^3J = 6.5$ Hz, 6H, ^{i}Pr-(CH_3)$_2$); 1.28 (s, 9H, Piv-(CH_3)$_3$); 1.80−2.10 (m, 4H, "Pro"-C$^\beta H_2$ + C$^\gamma H_2$); 2.62 (B) and 3.12 (A) (*ABX*, J_{AB} = 11.3 Hz, $J_{AX} = 4.5$ Hz, $J_{BX} = 7.0$ Hz, 2H, "Pro"-C$_r H_2$); 3.04 (s, 3H, AzAla-C$^\beta H_3$); 3.55 (m, 2H, "Pro"-C$^\delta H_2$); 3.64 (m, 1H, N-N*H*); 4.20 (m, 1H, "Pro"-C$^\alpha H$); 6.19 (d, $^3J = 7.7$ Hz, 1H, N*H*).

^{i}BuNHNMeCONH^{i}Pr. 4C was obtained from **3C** by the same procedure as for **4** from **3**. Purification by silica gel chromatography gave a colorless oil with AcOEt/PE (50/50) as the eluent ($R_f = 0.26$). IR (CH_2Cl_2, cm^{-1}): 1669 (*CO*); 3413 (*NH*). ^{1}H NMR ($CDCl_3$, ppm): 0.94 (d, $^3J = 6.7$ Hz, 6H, ^{i}Pr-(CH_3)$_2$); 1.12 (d, $^3J = 6.6$ Hz, 6H, ^{i}Bu-(CH_3)$_2$); 1.64 (m, 1H, ^{i}Bu-C*H*); 2.58 (d, $^3J = 6.6$ Hz, 2H, ^{i}Bu-CH_2); 3.02 (s, 3H, N-CH_3); 3.85 (m, 1H, ^{i}Pr-C*H*); 6.24 (d, $^3J = 7.2$ Hz, 1H, N*H*).

^{1}H NMR and FTIR Spectroscopy. Spectroscopic experiments were carried out on the neutral reduced peptide **1** and its ionic form **1**$^+$, the methyleneoxypeptide **2**, the iminoazapeptide **3**, and the reduced azapeptide **4**, all containing the Piv group, which is known to favor the trans isomer of the Piv-Pro amide bond.[17] IR spectra were scanned in the Fourier transform mode on a Bruker IFS-25 apparatus using a cell path length of 0.5 mm to investigate the NH (3200−3500 cm^{-1}), CO (1580−1720 cm^{-1}), and N + H (2500−3300 cm^{-1}) stretching frequencies. The peptide concentration was 0.005 M, and further dilution confirmed the absence of molecular aggregation. The NH and CO stretching frequencies were assigned on the basis of previous studies on related peptides.[17b] In particular, the free amide NH is expected to give a sharp absorption in the 3400−3450 cm^{-1} domain, and the free Piv-*CO*, a strong contribution at 1610−1625 cm^{-1} in CH_2Cl_2. Previous studies on ammonia and protonated reduced peptides have shown that the N^+H_2 stretching frequencies greatly depend on the solvent and the associated anion.[10a,15] For example, the ^{n}Bu$_2$N$^+$H$_2$ group exhibits a sharp peak at 3246 cm^{-1} when associated with the PF_6^- anion in a low polar solvent (CH_2Cl_2) but gives rise to a multicomponent absorption shifted down to 2700 cm^{-1} when solvated in a strong aprotic medium. The ^{n}Bu$_2$N$^+$H$_2$ cation has also a weak absorption at 1596 cm^{-1} in CH_2Cl_2, while the PF_6^- anion has no visible absorption in the 2200−2800 and 3100−3600 cm^{-1} domains.

^{1}H NMR spectra were run on a Bruker AC-200P apparatus with Me_4Si as internal reference, and the spin systems were solved by COSY and HOHAHA experiments. The solvent accessibility, and therefore the free or hydrogen-bonded character of the amide and ammonium protons in these small molecules, was investigated by considering their resonance shift in $CDCl_3$/DMSO-d_6 mixtures with increasing DMSO-d_6 content.[17b] The signal of a hydrogen-bonded, solvent-shielded N*H* is only slightly sensitive to DMSO content, whereas the signal of a free, solvent-exposed N*H* is downfield shifted from small DMSO-d_6 contents.

X-ray Diffraction. Single crystals of the reduced dipeptides **1** and **1′**, the iminoazadipeptides **3** and **3′**, and the reduced azadipeptide **4** were grown by slow evaporation of an EtOH/AcOEt solution. The X-ray diffraction data were collected on an Enraf Nonius CAD-4 four-circle diffractometer in the $\omega/2\theta$ scan mode. The independent reflections were measured at room temperature in the 1−70° θ range using Cu Kα radiation ($\lambda = 1.541\ 78$ Å) monochromatized by a graphite crystal. During data collection, two standard reflections were measured every 2 h to check the stability of the crystal. Intensities were corrected for Lorentz and polarization effects, but no absorption correction was applied. Crystallographic data are given in Table 2. The crystal structures were solved by direct methods using SHELXS90.[18] The *E* maps revealed the whole molecules apart from the hydrogen atoms (three independent molecules of **3′** are present in the cell), and the structures were refined through the least-squares procedure with the complete matrix of normal equations using SHELX86[19] for **1**, **1′**, **3**, and **3′** and SHELXL93[20] for **4**. Heavy atoms were affected by anisotropic thermal factors, and hydrogen atoms were located on *E* map differences and affected by an isotropic thermal factor of 4 Å^2. The residual *R* factors are indicated in Table 2. The NH hydrogen

(18) Sheldrick, G. M. *SHELX86. Program for the solution of crystal structures*; University of Göttingen: Göttingen, Germany, 1986.
(19) Sheldrick, G. M. *Acta Crystallogr., Sect. A* **1990**, *46*, 467−473.
(20) Sheldrick, G. M. *SHELXL93. Programs for the refinement of crystal structures*; University of Göttingen: Göttingen, Germany, 1993.

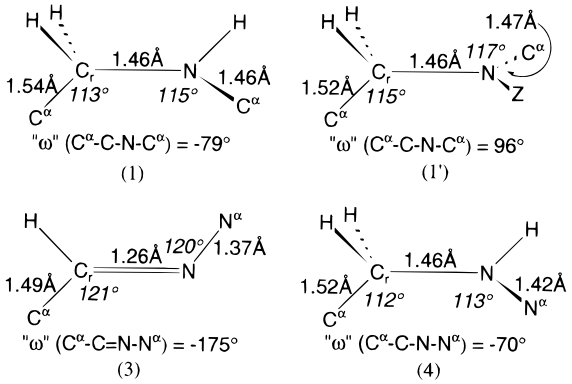


Figure 4. Dimensions of the amide surrogate in **1** (neutral reduced amide), **1′** (Z-protected reduced amide), **3** (semicarbazone fragment), and **4** (semicarbazide fragment).

Table 3. Main Torsional Angles (deg)[a]

	reduced peptides		iminoazapeptides				reduced azapeptide
angle	1	**1′**	3	**3′$_A$**[b]	**3′$_B$**[b]	**3′$_C$**[b]	4
ω_0	175	180	173	−1	−2	−1	177
"Pro"							
"ϕ1"	−80	−93	−73	−72	−69	−68	−78
"ψ_1"	158	72	−21	134	135	−100	172
"ω_1"	−79	96	−175	176	175	180	−70
"Ala"							
"ϕ_2"	−70	−141	−178	−177	−176	−179	128
"ψ_2"	103	41	−1	−6	−10	4	−7
"ω_2"	178	−178	168				−177

[a] The torsional angles are defined by considering the atoms of the main chain, in a way similar to that in peptides.[23] [b] Three independent molecules per asymmetric unit.

atoms were replaced at 1.03 Å from N in the direction obtained by refinement.[21] Crystal data, fractional coordinates for the hydrogen and non-hydrogen atoms, equivalent thermal parameters and anisotropic temperature parameters for the non-hydrogen atoms, interatomic bond lengths and bond angles, and torsional angles have been deposited as Supporting Information.

Crystal Structures

$C^\alpha \rightarrow N^\alpha$ substitution has little influence on the dimensions of the reduced amide bond in **1** and **4** (Figure 4). The sp^3 character of the reduced amide nitrogen results in the same staggered conformation of the $C^\alpha C_r N C^\alpha$ group ("ω" = −70° and −79°, respectively) and confers the same *R*-chirality on the amine nitrogen. Acylation of the reduced amide nitrogen in **1′** restores the sp^2 character of the nitrogen, and the C_r-N bond rotates by 175° in comparison with **1** (Figure 4). The same fragment in the reduced analogue Boc-Proψ[C_rH_2NH]Leu-Gly-NH_2 of the oxytocin C-terminal tripeptide assumes a nearly trans conformation ("ω" = −157°) with a *S*-chiral sp^3 nitrogen.[8] A trans planar conformation is also observed for the imine group in **3** and **3′** (Figure 4), but the $C_r{=}N$ and $N-N^\alpha$ bonds are 0.1 Å shorter than the corresponding bonds in the peptides.[22]

The main conformational angles are listed in Table 3. The two reduced peptides **1** and **1′** adopt a folded structure (Figure 5a,b) with an intramolecular (^{i}Pr)*NH* to Piv-*CO* hydrogen bond (N···O = 2.94 and 2.85 Å, respectively) closing a 10-membered cycle. However, they differ in the conformation of the Ala residue, in the gauche$^-$ or gauche$^+$ conformation of the middle reduced amide bond, and in the value of the "Pro"-"ψ" angle. Both folded structures are intermediate between types I (trans middle amide bond) and VI (cis middle amide bond) of the peptide β-turn.[24] The β-like structure of **1** also differs from the crystal molecular structure of Boc-Proψ[C_rH_2NH]Leu-Gly-NH_2, where Leu-*NH* interacts with Boc-*CO* in a γ-like turn and Gly-*NH* interacts with the reduced amide nitrogen to close a five-membered cycle,[8] similar to that observed for example in the crystal structure of lidocaine $Et_2NCH_2CONH(2,6\text{-}Me_2Ph)$.[25] The molecular conformation of the reduced azapeptide **4** (Figure 5c) is related partly to that of **1** (same conformation of the "Pro" residue) and partly to that of Boc-Proψ[C_rH_2NH]Leu-Gly-NH_2 (same five-membered cycle).[8]

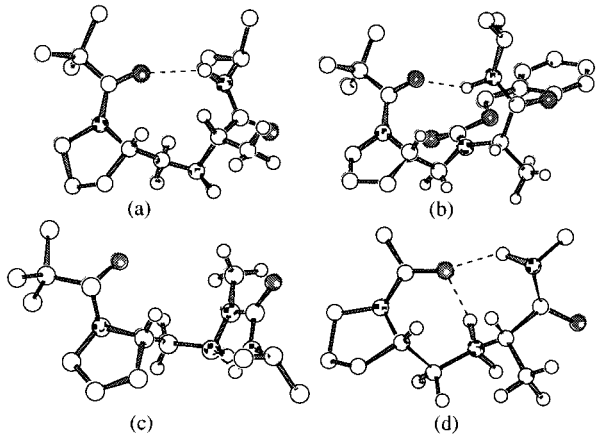


Figure 5. Crystal molecular conformations of the neutral reduced dipeptide **1** (a), the Z-protected reduced dipeptide **1′** (b), and the reduced azapeptide **4** (c). Major conformation in solution of the protonated reduced peptide $\mathbf{1}^+$ (d). The intramolecular hydrogen bonds are indicated by broken lines.

It ensues that the neutral reduced amide acts in three different ways: (i) as an intramolecular acceptor from the following amide NH in Boc-Proψ[C_rH_2NH]Leu-Gly-NH_2 (N···N = 2.65 Å) and in the reduced azapeptide **4** (N···N = 2.62 Å), (ii) as an intramolecular donor to Boc-*CO* in Boc-Proψ[C_rH_2NH]Leu-Gly-NH_2 (N···O = 3.08 Å),[8] and (iii) as an intermolecular donor to Ala-*CO* in **1** (N···O = 3.02 Å) and to Piv-*CO* in **4** (N···O = 3.14 Å) (Table 4).

In the iminoazapeptide series, due to the presence of three independent molecules in the cell of **3′**, four different molecular structures have been found, but molecules **3′$_A$** and **3′$_B$** actually adopt very similar structures (Table 3). In all cases, the semicarbazone fragment is practically planar with a cis NN^α-CON system, in such a way that the C-terminal N−H bond is directed toward the lone pair of the imine nitrogen (N···N = 2.60−2.65 Å) and that the only degree of freedom appears to be the rotation of the "Pro" $C^\alpha-C_r$ bond (Figure 6). The $N^\alpha H$ bond in **3′** is a proton donor to the C-terminal carbonyl of a neighbor molecule ($N^\alpha{}_A\cdots O_A$ = 2.91 Å, $N^\alpha{}_B\cdots O_B$ = 2.84 Å, and $N^\alpha{}_C\cdots O_A$ = 2.91 Å) (Table 4).

Conformations in Solution

Before investigating the conformational properties of the pseudopeptide analogues **1**−**4**, we have considered the influence

(21) Taylor, R.; Kennard, O. *Acta Crystallogr., Sect. B* **1983**, *39*, 133−138.

(22) Benedetti, E. In *Chemistry and Biochemistry of Amino Acids, Peptides and Proteins*; Weinstein, B., Ed.; Marcel Dekker Inc.: New York, 1982; Vol. 6, pp 105−184.

(23) IUPAC-IUB Commission on Biochemical Nomenclature *Eur. J. Biochem.* **1984**, *138*, 9−37.

(24) Rose, G. D.; Gierasch, L. M.; Smith, J. A. *Adv. Protein Chem.* **1985**, *37*, 1−109.

(25) Hanson, A. W.; Banner, D. W. *Acta Crystallogr., Sect. B* **1974**, *30*, 2486−2488.

Table 4. Dimensions (Å, deg) of the Hydrogen Bonds

atoms	symmetry code	N···O/N	H···O/N	N−H···O/N
		Reduced Peptide **1**		
(^{i}Pr)*NH* to Piv-*CO*		2.94	1.93	166
Ala-*NH* to Piv-*CO*	$-x, {}^1/_2 + y, {}^1/_2 - z$	3.14	2.14	162
		Reduced Peptide **1′**		
(^{i}Pr)*NH* to Piv-*CO*	a	2.84	1.91	150
		Iminoazapeptide **3**		
(^{i}Pr)*NH* to AzAla-*N*	b	2.59	2.14	104
		Iminoazapeptide **3′** [c]		
NH_{2A} to AzGly-N_A	b	2.64	2.29	98
NH_{2B} to AzGly-N_B	b	2.64	2.22	103
NH_{2C} to AzGly-N_C	b	2.61	2.24	99
NH_{2A} to Boc-CO_A	$1 - x, y, z$	2.91	1.88	177
NH_{2B} to Boc-CO_B	$1 + x, y, z$	2.84	1.85	162
NH_{2C} to AzGly-CO_A	${}^1/_2 + x, {}^7/_2 - y, -z$	2.91	1.94	156
AzGly-$N^{\alpha}H_A$ to AzGly-CO_B	$-{}^1/_2 + x, {}^3/_2 - y, -z$	2.92	1.92	165
AzGly-$N^{\alpha}H_B$ to AzGly-CO_B	${}^1/_2 + x, {}^7/_2 - y, -z$	2.89	1.86	177
AzGly-$N^{\alpha}H_C$ to AzGly-CO_B	x, y, z	2.92	1.92	162
		Reduced Azapeptide **4**		
(^{i}Pr)*NH* to AzAla-*N*	b	2.61	2.05	112
AzAla-*NH* to AzAla-*CO*	$x, 1.5 - y, -0.5 + z$	3.02	2.12	145

[a] Intramolecular hydrogen bond closing a 10-membered cycle [b] Intramolecular hyrogen bond closing a five-membered cycle [c] Three independent molecules per asymmetric unit.

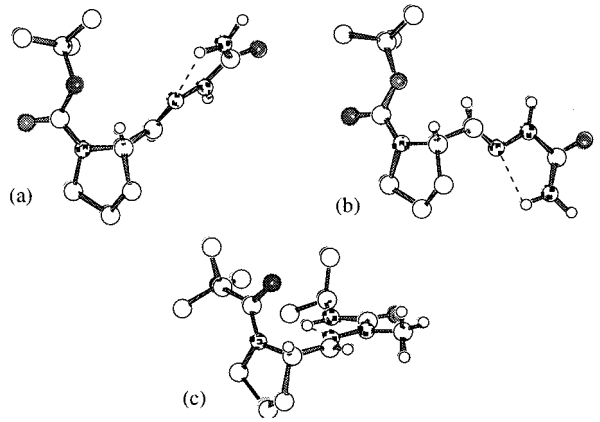


Figure 6. Crystal molecular conformation of the iminoazadipeptides $\mathbf{3'}_\mathbf{A}$ (a), $\mathbf{3'}_\mathbf{C}$ (b), and **3** (c). The intramolecular hydrogen bonds are indicated by broken lines.

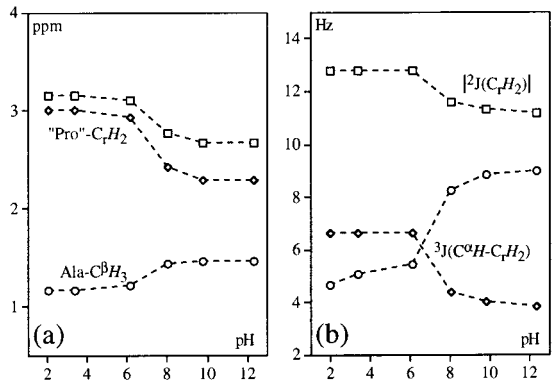


Figure 7. Influence of the pH on the ^{1}H NMR data (chemical shifts and coupling constants) for the "Pro" $C^{\alpha}H$-$C_rH_AH_B$ and AzAla-$C^{\beta}H_3$ systems in the reduced peptide $\mathbf{1}/\mathbf{1}^+$ in water.

of the pH in the 2−12 range on the ^{1}H NMR data collected in water. Only the resonances of **1** are significantly shifted at about pH 6−8, while those of the other three derivatives are not affected (Figure 7). It ensues that the semicarbazone and semicarbazide groups are essentially neutral in the 2−12 pH range, whereas the pK_a value of the reduced amide bond in **1** is

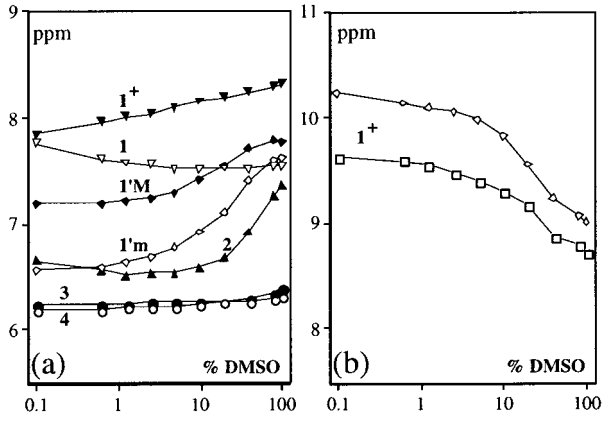


Figure 8. Influence of DMSO-d_6 content in $CDCl_3$/DMSO-d_6 mixtures on the C-terminal N*H* resonance (a) for the neutral reduced peptides **1**, **1′M** (major conformer), **1′m** (minor conformer), the protonated reduced peptide $\mathbf{1}^+$, the methyleneoxypeptide **2**, the iminoazapeptide **3**, and the reduced azapeptide **4** and for the Ala-N^+H_2 resonances (b) for $\mathbf{1}^+$.

about 7. Therefore, under the physiological conditions, a reduced peptide may assume a neutral or protonated form depending on the environment. A previous study has shown that protonation induces particular conformational properties in dipeptides containing a C-terminal reduced amide bond.[10a] The same probably holds true for **1** since the coupling constants in the "Pro" $C^{\alpha}HC_rH_2$ system, which are related to the rotational state of this bond, vary significantly with the pH (Figure 7).

In all four neutral pseudodipeptides **1**−**4**, the small variation of the (^{i}Pr)N*H* resonance with DMSO-d_6 content in $CDCl_3$/DMSO-d_6 mixtures (Figure 8) is typical of a solvent-protected proton[17b] which is therefore intramolecularly hydrogen-bonded. This is corroborated by the low (^{i}Pr)*NH* stretching frequency in CH_2Cl_2 (Table 4), inferior to the value expected for a free vibrator (3425 cm^{-1}).[17b] In DMSO, the (^{i}Pr)*NH* absorption is shifted to a very low-frequency denoting its solvated state, except for **3**, where it retains the same frequency and sharp profile as in CH_2Cl_2.

The Piv-*CO* frequency is practically independent of the peptide bond surrogate and of the solvated (DMSO) or non-

Table 5. Amide *NH* and Piv-*CO* Stretching Frequencies for the Pseudodipeptides and their N- and C-Terminal Derivatives in Solution[a]

compd		solvent	amide *NH*	Piv-*CO*
neutral reduced peptide	**1**	CH_2Cl_2	**3350**[b]/3420[w]	1611[s]
		DMSO	3280	1613[s]
	1N	CH_2Cl_2		1610[s]
		DMSO		1611[s]
	1C	CH_2Cl_2	**3368**[b]	
protonated reduced peptide	$\mathbf{1}^+$	CH_2Cl_2	**3340**[m]/3410[m]/3395[m]	**1588**[s]
		DMSO	3280[b]	1616[s]
	$\mathbf{1}^+\mathbf{N}$	CH_2Cl_2		1602[m]/**1584**[m]
		DMSO		1615[s]
	$\mathbf{1}^+\mathbf{C}$	CH_2Cl_2	3420[s]/3400[w]	
methylenoxypeptide	**2**	CH_2Cl_2	3414[s]	1614[s]
		DMSO	3263[b]	1615[s]
	2C	CH_2Cl_2	3411[s]	
iminoazapeptide	**3**	CH_2Cl_2	**3417**[s]	1619[s]
		DMSO	**3412**[s]	1616[s]
	3C	CH_2Cl_2	**3414**[s]	
reduced azapeptide	**4**	CH_2Cl_2	**3402**[s]	1612[s]
		DMSO	3251[b]	1613[s]
	4C	CH_2Cl_2	3413	

[a] Broad and strong (b), sharp and strong (s), medium (m), and weak (w) absorptions. Values in bold characters denote intramolecularly hydrogen-bonded vibrators.

solvated (CH_2Cl_2) state of the (^{i}Pr)*NH* site, suggesting that the Piv-*CO* is not engaged in any intramolecular interaction. This hypothesis is supported by the fact that a pseudopeptide and the molecule reproducing its C-terminal part (namely **1**/**1C**, **2**/**2C**, **3**/**3C**, and **4**/**4C**) give very similar NH stretching frequencies, independent of the presence of the Piv-*CO*. Therefore, the only possible partner of the (^{i}Pr)*NH* is the peptide surrogate itself, i.e. the sp^3 nitrogen in **1** and **4**, the ether oxygen in **2**, and the imine nitrogen in **3**. This interaction closes a five-membered pseudocycle as in the crystal structures of **3** and **3′** (Figure 6), **4** (Figure 5c), and Boc-Proψ[C_rH_2NH]Leu-Gly-NH_2.[8] When not possible in the Z-containing reduced peptide **1′**, the N*H* proton is more sensitive to DMSO solvation for both cis and trans isomers of the Z−N amide bond (Figure 8).

Protonation of the reduced amide in $\mathbf{1}^+$ induces noticeable changes in the IR data (Table 4), especially for the Piv-*CO* stretching frequency which shifts from 1616 to 1588 cm^{-1}, a low value typical of a Piv carbonyl engaged in a strong interaction. No absorption expected for the free N^+H_2 group when associated with PF_6^- in CH_2Cl_2 is observed at about 3245 cm^{-1}, but a very broad and composite absorption at about 2800 cm^{-1} denotes the bonded character of the ammonium. The same holds true when comparing the neutral and protonated derivatives **1N** and $\mathbf{1}^+\mathbf{N}$. We therefore conclude that the Piv carbonyl and the ammonium in $\mathbf{1}^+$ are hydrogen bonded in a γ-like folded structure.[10a]

The (^{i}Pr)*NH* absorption for $\mathbf{1}^+$ is also modified by protonation (Table 4) and the (^{i}Pr)N*H* proton is slightly more accessible to DMSO solvation (Figure 8). The contribution at 3420 cm^{-1} in CH_2Cl_2 is due to the free N−H vibrator, and the contribution at 3400 cm^{-1}, also present for $\mathbf{1}^+\mathbf{C}$, can be attributed to the PF_6^- bonded amide NH.[10a] The component at 3340 cm^{-1}, absent from $\mathbf{2C}^+$, is typical of a hydrogen-bonded NH, and the only possible partner is the Piv carbonyl. It results that molecules $\mathbf{1}^+$ are all folded by a N^+H_2 to Piv-*CO* interaction in a γ-like turn and that part of them also present a ^{i}PrNH to ^{t}BuCO interaction in a β-like turn (Figure 5d). The high Piv-*CO* stretching frequency in DMSO (Table 5) indicates that this structure is not retained in DMSO, and the upfield-shifted Ala-N^+H_2 resonances (Figure 8) confirm the solvated free character of the ammonium group.

The "Pro" $C^{\alpha}HC_rH_AH_B$ spin system has been solved for **1** and $\mathbf{1}^+$ in MeCN-d_3 and for **4** in $CDCl_3$, where the proton resonances are very sharp, by selective irradiation and chemical exchange of the N*H* proton with water traces (Table 6). The very small coupling constant of 1.6 Hz for $\mathbf{1}^+$ indicates a rigid conformation of the "Pro" $C^{\alpha}-C_r$ bond placing the "Pro"-$C^{\alpha}H$ and "Pro"-C_rH_B protons in an orthogonal disposition corresponding to "ψ_1" = 90° or 150°. Only the former value is compatible with the γ-like folded conformation assumed by $\mathbf{1}^+$ (Figure 5d). The vicinal coupling constants for the neutral derivatives **1** and **4** denote the higher flexibility of the $C^{\alpha}C_rN$ fragment than for $\mathbf{1}^+$, in agreement with the IR evidence that the only intramolecular interaction concerns the C-terminal moiety in both derivatives.

Table 6. ^{1}H NMR Data for the Peptide Surrogate in the Neutral **1** and Protonated $\mathbf{1}^+$ Reduced Dipeptides (MeCN-d_3) and the Reduced Azadipeptide **4** ($CDCl_3$)

compd	**1**	$\mathbf{1}^+$	**4**
	Chemical Shifts (ppm)		
"Pro"-$C^{\alpha}H$	4.10	4.23	4.21
"Pro"-$C_rH_AH_B$	2.67 (A)	2.93 (A)	3.12 (A)
	2.32 (B)	3.10 (B)	2.62 (B)
"Ala"-N*H* or N^+H_2	1.80[a]	8.40[a]	3.59
"Ala"-$C^{\alpha}H$	2.99	3.59	
"Ala"-$C^{\beta}H_3$	1.13	1.38	3.05
	Coupling Constants (Hz)		
$^3J(C^{\alpha}HC_rH_AH_B)$	3.8 (A)	8.3 (A)	4.7 (A)
	8.3 (B)	1.6 (B)	7.0 (B)
$^2J(C_rH_AH_B)$	−11.3	−13.4	−11.3
$^3J(C_rH_AH_BNH)$	b	b	5.0 (A)
			7.4 (B)

[a] Broad signal. [b] Nonmeasurable.

Conclusion

The neutral $C^{\alpha}C_rH_2NHC^{\alpha}$ and $C^{\alpha}C_rH_2NHN^{\alpha}$ fragments in **1** and **4**, respectively, have quite similar dimensions. Moreover, the reduced peptide **1**, the methyleneoxypeptide **2**, and the reduced azapeptide **4** share the same conformational properties in low polar solvents. Both nitrogen in **1** and **4** and the oxygen in **2** act as an accepting group from the contiguous peptide NH so as the intramolecular N−H···N/O hydrogen bond closes a five-membered pseudocycle. This conformation is not retained in strong aprotic DMSO medium. There is no indication for the existence in solution of the folded structure, related to the β-turn in peptides with an $i + 3 \rightarrow i$ interaction closing a 10-

membered pseudocycle, which is observed in the solid state for the reduced peptide **1**. Although this β-like folded structure is probably the consequence of the packing forces in the crystal, it must be considered as potentially accessible to the reduced peptide, methyleneoxypeptide, and reduced azapeptide motifs.

Contrary to the three above flexible peptide surrogates, the semicarbazone in the iminoazapeptides **3** is a rigid fragment assuming a planar cis structure with a N−H···N hydrogen bond closing a five-membered pseudocycle. This interaction is stable enough to be retained in a strong solvating medium such as DMSO. The only degree of freedom is then the rotation about the single bonds contiguous to the semicarbazone motif.

Protonation of a reduced peptide induces noticeable conformational changes due to the strong proton donating properties of the ammonium group in $\mathbf{1}^+$. The low pK_a value of the semicarbazide in **4**, compared with the value of about 7 for the reduced amide in **1**, indicates that the former is not protonated in water under the physiological conditions whereas the latter is at least partly protonated. Therefore, the electronic state of a reduced azapeptide is probably independent of the environment, whereas that of a reduced peptide is hardly predictable and should depend on the nature of the neighboring groups.

Acknowledgment. The authors dedicate the present work to the memory of D. Bayeul. The participation of L. David is greatly acknowledged.

Supporting Information Available: Crystal data, fractional coordinates for the hydrogen and non-hydrogen atoms, equivalent thermal parameters and anisotropic temperature parameters for the non-hydrogen atoms, interatomic bond lengths and bond angles, and torsional angles (38 pages, print/PDF). See any current masthead page for ordering information and Web access instructions.

JA980937O