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The non Protonable Reduced Aza-Peptide Fragment

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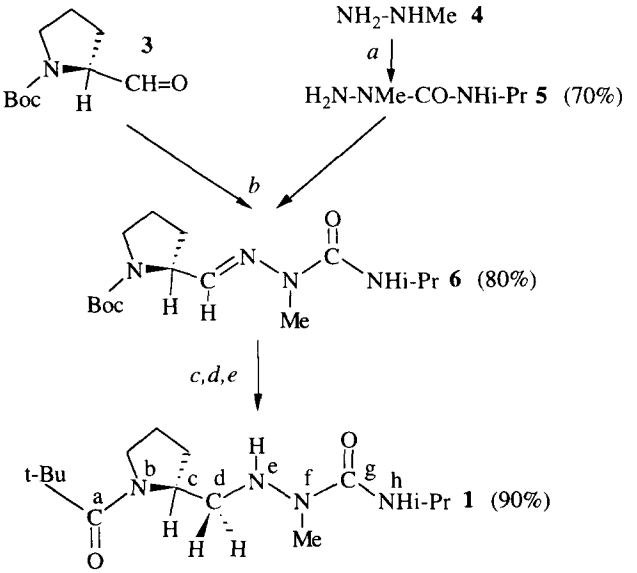
Abstract: The reduced aza-peptide fragment (C^{α}-CH_2-NH-N^{α}R-CO-NH-C^{α}) is obtained by reduction of the semicarbazone moiety (C^{α}-CH=N-N^{α}R-CO-NH-C^{α}) in an imino aza-peptide. It differs from the aminomethylene link (C^{α}-CH_2-NH-C^{α}HR-CO-NH-C^{α}) found in the reduced peptides by the absence of protonation in water in the 2-12 pH range. The reduced aza-analogue of the Pro-Ala dipeptide has been studied in solution by ^{1}H-NMR and IR spectroscopy, and in the solid state by X-ray diffraction. Its structure is quite similar to that of the neutral reduced dipeptide analogue in solution.

Among the mimics of the $C(OH)_2$-NH transient state of the amide bond during enzymatic cleavage, the so-called reduced amide bond, or aminomethylene moiety CH_2-NH, is frequently used for the design of peptidase inhibitors, probably because of its easy introduction in a peptide chain.[1,2] However, the question arises about the actual chemical nature of the reduced amide bond: there is some NMR indication that the pKa of a protonated reduced amide bond is near neutrality,[3,4] and the ionic or neutral form of its amine function at the physiological pH is then questionable and depends upon the local environment.

Taking into account that the pKa (3.53)[5] of the protonated semicarbazide is much lower than that of the CH_2-N^+H_2-CH moiety, it was worth to investigate the structural properties of the semicarbazide link C^{α}-CH_2-NH-N^{α}R-CO-NH-C^{α}, deriving from the semicarbazone fragment C^{α}-CH=N-N^{α}R-CO-NH-C^{α} in imino aza-peptides,[6] and giving access to a new family of pseudopeptides that we propose to call reduced aza-peptides. The structure of the semicarbazone reveals to be rigidly cis-planar with a 5-membered pseudocycle closed by a N-H···N hydrogen bond,[6] but reduction of the imine bond is expected to restore some conformational flexibility. We have prepared the reduced aza-dipeptide **1** (Scheme 1), and compared its conformational properties to that of the reduced dipeptide analogue **2** (Scheme 2). Both have been submitted to IR and ^{1}H-NMR experiments in solution and have grown single crystals which have been studied by X-ray diffraction.

Derivative **1** (Scheme 1) was synthesized by catalytic hydrogenation of the corresponding imino aza-dipeptide[6] with in addition Boc to Piv exchange in order to prevent cis conformation of the Pro-preceding amide bond.[7] The reduced dipeptide **2** (Scheme 2) was prepared by one-pot reductive amination,[1,2] using the

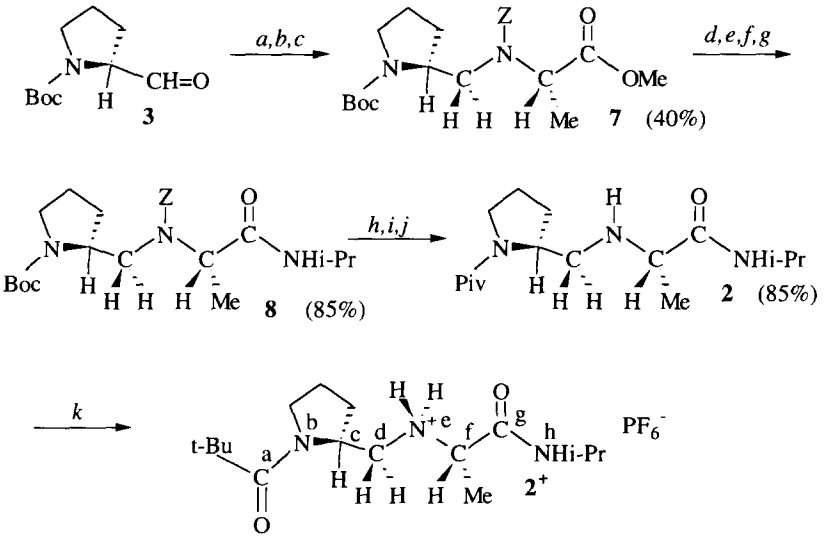
temporary Z-protection of the amino group in order to make the chromatographic purification easier, and to avoid side reactions during further acylation steps. Saponification of the ester **7** and introduction of the isopropylamide in **8** by the mixed anhydride method, followed by Boc to Piv exchange and Z-hydrogenolysis gave **2** in the neutral form. The protonated form 2^+ was associated with the PF_6^- anion in order to minimize ammonium-anion interactions.[8] All derivatives are chromatographically pure and give satisfactory ^{1}H-NMR data.



Scheme 1.[9] *a*: i-PrN=C=O / THF / reflux / overnight; *b*: EtOH / AcONa / r.t. / overnight; *c*: TFA / r.t. / 15 min.; *d*: PivCl / NEt(i-Pr)$_2$ / DCM / 0°C; *e*: H_2 / Pd-C10% / MeOH - AcOH / r.t. / 96h..

The ^{1}H-NMR spectrum of **1** in water is practically invariant in the 2-12 pH range whereas the resonances of **2** are significantly shifted at about pH 6-8. It ensues that the semicarbazide group in **1** is not protonated under the physiological conditions. On the contrary, the protonated reduced amide bond C^dH_2-$N^eH_2^+$ in 2^+ presents a pKa value of about 7, and protonation of the reduced amide link in aza-peptide analogues has been shown to affect considerably the conformational properties.[4,10,11]

The crystal stucture of derivatives **1** and **2** have been solved by X-ray diffraction.[12] Molecule **1** (ϕ_1, ψ_1, ω, ϕ_2, ψ_2 = -78°, 172°, -70°, 128°, -7°) exhibits a rather extended conformation in which the C-terminal N^hH is hydrogen bonded to the N^e nitrogen ($N^e\cdots N^h$ = 2.62 Å) (Fig. 1). Molecule **2** (ϕ_1, ψ_1, ω, ϕ_2, ψ_2 = -81°, 159°, -79°, -70°, 103°) is folded by an intramolecular hydrogen bond between the C-terminal N^hH and the C^aO carbonyl ($N^h\cdots O$ = 2.94 Å), in a similar way to the β-turn structure in the peptides (Fig. 1).[13] The N^eH interacts with the AzAla-C^gO in **1** ($N^e\cdots O$ = 3.02 Å), and with the Piv-C^aO in **2** ($N^e\cdots O$ = 3.14 Å) of a neighbour molecule. In both cases, its nitrogen atom is pyramidal with the same R-chirality.



Scheme 2.[9] *a*: HCl. H-Ala-OMe / MeOH:AcOH (99:1) with molecular sieves 3-4 Å / r.t. / 1 h; *b*: $NaBH_3CN$ / r.t.; *c*: ZCl / NMM / THF / -10°C; *d*: NaOH 1N / acetone / 0°C / 2h; *e*: HCl 1N / r.t.; *f*: i-BuOCOCl / NMM / $CHCl_3$ /-15°C; *g*: iPr-NH_2 / NMM / $CHCl_3$ / -10°C; *h*: TFA / r.t. / 15 min.; *i*: PivCl / NMM / DCM / 0°C; *j*: H_2 / Pd-C 5% / MeOH / r.t.; *k*: $Et_2O^+H.PF_6^-$ / H_2O / r.t.

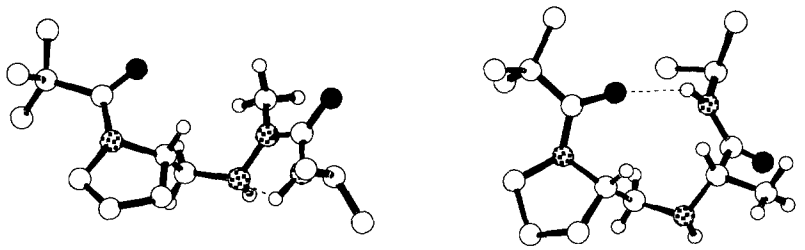


Fig. 1. Crystal structures of the reduced aza-dipeptide **1** (left) and the neutral reduced dipeptide **2** (right).

For both neutral pseudodipeptides, the small solvent sensitivity of the C-terminal amide N^hH resonance (0.11 ppm (**1**) and -0.22 ppm (**2**) going from $CDCl_3$ to DMSO-d_6), and the low amide N^h-H stretching frequency (3402 cm^{-1} (**1**) and 3350 cm^{-1} (**2**) in DCM) are typical of a hydrogen bonded site. In DMSO, the amide N^h-H absorption is shifted down to 3280 cm^{-1}, a value denoting a totally solvated state. Moreover and surprisingly, the Piv C^a=O frequency is practically independent of the solvated or non solvated state of the amide N^hH, indicating that the Piv carbonyl is not engaged in any intramolecular interaction. Therefore, the only possible partner of the amide N^hH is the N^eH amine nitrogen of the reduced amide link. The resulting N^h-H···N^e interaction closing a 5-membered pseudocycle is present in the crystal structures of **1** and of the reduced peptide Boc-Proψ[CH_2-NH]Leu-Gly-NH_2.[14]

As expected, protonation induces noticeable changes in the 2^+ IR data . The Piv C^a=O is engaged in a strong interaction denoted by its very low stretching frequency (1588 cm^{-1} in DCM), and the very broad and composite absorption near 2800 cm^{-1} is typical of a bonded ammonium[7] while the C-terminal N^hH is still

partly protected from solvation (resonance shift of 0.46 ppm when going from $CDCl_3$ to DMSO-d_6). We therefore might conclude that on one side the Piv carbonyl and the $N^eH_2^+$ in **2**$^+$ are intramolecularly hydrogen bonded forming a γ-like turn, and that on the other side part of the molecules also present an amide N^hH to Piv C^aO interaction resulting in a β-like turn.[13]

The amine pKa of the protonated semicarbazide moiety is much lower[5] than that of the aminomethylene moiety in reduced peptides, so that the former is not protonated at the physiological pH. Their structure being similar structures, the semicarbazide moiety appears as a possible substitute for the cognate reduced sequence for the design of a neutral mimic of the amide hydrolysis transient state.

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9. The following abbreviations are used: Ac, acetyl; Boc, *tert*-butyloxycarbonyl; DCM, dichloromethane; DMSO, dimethylsulfoxide; DMSO-d_6, hexadeuterated dimethylsulfoxide; NMM, N-methylmorpholine; Piv, pivalyl ; TFA, trifluoroacetic acid; THF, tetrahydrofuran; Z, benzyloxycarbonyl.
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12. Single crystals of **1** and **2** have been obtained by slow cooling of an EtOH / AcOEt solution. **1**: $P2_1/c$; a = 10.083(2)Å, b = 18.553(1)Å, c = 10.282(1)Å, β = 111.19(2)°; Z = 4; $d_{calc.}$ = 1.11 g.cm^{-3}; 3632 reflections; R = 0.073. **2**: $P2_12_12_1$; a = 9.337(1)Å, b = 9.458(1)Å, c = 20.425(3)Å; Z = 4; $d_{calc.}$ = 1.10 g.cm^{-3}; 1785 reflections; R = 0.055. The reduced amide in **1** and **2** have quite similar dimensions (C^dH_2-N^e = 1.46 Å), but the α-nitrogen (N^f) in **1** is nearly planar whereas the α-carbon (C^f) in **2** is tetragonal. The N^f-Me bond in **1** (1.43 Å) is shorter than the C^f-Me bond in **2** (1.53 Å). Due to the absence of electronic conjugation, the C^dH_2-N^e in **1** and **2** is 0.14 Å longer than the standard peptide bond one,[15] and allows a staggered conformation instead of the trans planar arrangement.
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