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Hydrazino and N-Amino Peptides. Chemical and Structural Aspects

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Abstract: The regioselective acylation of the nitrogens in hydrazino acetic acid has been studied to obtain the hydrazide and N-amino amide peptidomimetic groups. Their conformational influence on the β-turn structure has also been considered.

Among the peptidomimetic groups that have been introduced in pseudopeptide analogues of bioactive peptides,[1] the hydrazide (CO-NH-NH) and N-amino amide (CO-N(NH_2)) groups have received little attention,[2] probably because of the difficulties in obtaining chiral α-hydrazino acids and in coupling the hydrazine nitrogens regioselectively. When using the common procedures of peptide synthesis, the direct coupling of an α-hydrazino acid ester ($N^{\beta}H_2$-$N^{\alpha}H$-CHR-CO_2Et) with an N-protected amino acid essentially leads to the N^{β}-acylation, especially in the case of R ≠ H. If R = H, variable but always small amounts of N^{α}-acylation are observed depending on the experimental conditions.[2-4]

We have studied the regioselective reactivity of the nitrogens in hydrazino acetic acid (R = H), the hydrazino analogue of glycine, with regards to the usual protections (Table 1) and coupling procedures of peptide synthesis. In a first approach, we prepared the two analogues **1** and **2** of the dipeptide **3** containing the Pro-Gly sequence and known to adopt the so-called β-folded conformation in both the solution[5] and the solid state.[6] We also considered the conformational influence of the hydrazide and N-amino amide peptidomimetic groups on this particular structure characterized by the N-H···O=C hydrogen bond closing a 10-membered cycle, and known for its biological significance.[7]

1 ^{t}BuCO-Pro-$N^{\beta}H$-$N^{\alpha}H$-CH_2-CO-$NH^{i}Pr$

2 ^{t}BuCO-Pro-$N^{\alpha}(N^{\beta}H_2)$-CH_2-CO-$NH^{i}Pr$

3 ^{t}BuCO-Pro-NH-CH_2-CO-$NH^{i}Pr$

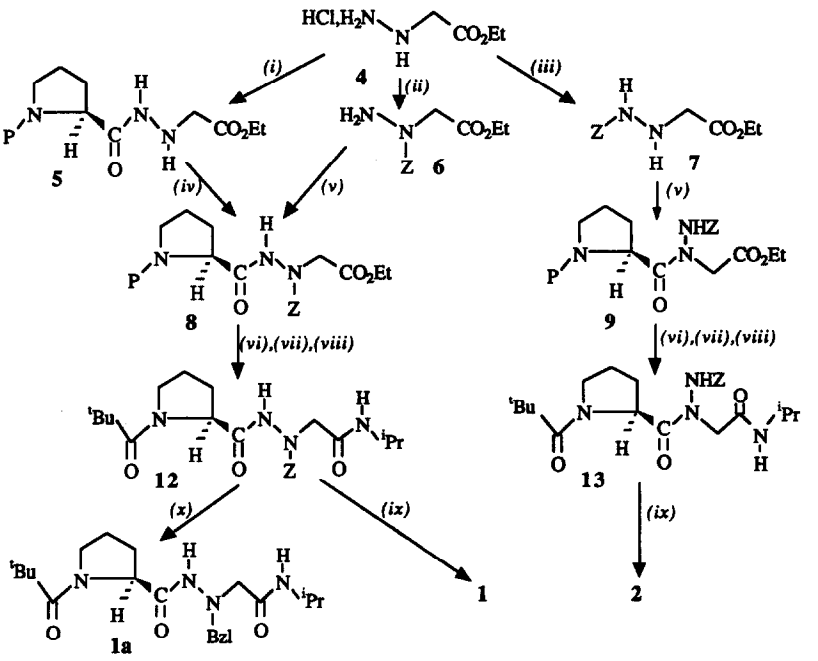
The synthetic procedures for preparing **1** and **2** are summarized in Scheme 1. The hydrazide bond in **5** was obtained with a 80% yield by direct coupling of the non-protected hydrazine **4** (Aldrich) with the O-succinimidyl

ester of either Boc-Pro-OH or Fmoc-Pro-OH, leading to no detectable N^{α}-isomer formation. However, further chemical modification of the N- or C-termini required the Z-protection of the non-acylated N^{α} nitrogen. We therefore have investigated the regioselective N-protection of **4** before a further coupling. This was achieved in 80% yield by using either Z_2O or Z-OSu for a preferential N^{α} or N^{β}-protection, respectively, provided the temperature is carefully controlled (selective crystallization of **7** in AcOEt).

Table 1. Regioselective N-Protection (%) of **4** by the Z or Boc Group.

Reagent [8]	Temperature (°C)	N^{α}-protection	N^{β}-protection	N^{α},N^{β}-diprotection
Z_2O [a]	0	80	10	5
Z-Cl / DMAP [a]	-5	65	10	15
Z-OSu [b]	0	10	85	0
Boc_2O [a]	0	80	10	5
Boc-OSu [b]	0	50	40	0

[a] reagent - hydrazine equimolar ratio. [b] 20% excess of the reagent.



Scheme 1[8]. *(i)* Boc- or Fmoc-Pro-OSu / NMM / THF / 0°C; *(ii)* Z_2O / NMM / THF / 0°C; *(iii)* Z-OSu / NMM / THF / 0°C; *(iv)* Z-Cl / $NaHCO_3$ / DMAP / MeCN / -10°C, *(v)* $(Boc\text{-}Pro)_2O$ / NMM / CH_2Cl_2 / -10°C or Fmoc-Pro-Cl / $NaHCO_3$ / CH_2Cl_2 / -10°C; *(vi)* P = Boc: HCl 3N / AcOEt, P = Fmoc: Et_2NH 4N/ CH_2Cl_2; *(vii)* ^tBuCO-Cl / $NaHCO_3$ / CH_2Cl_2 / 0°C; *(viii)* a) NaOH 1N aq / acetone / 0°C, b) ^iBuOCO-Cl /NMM / $^iPrNH_2$ / THF / -15°C; *(ix)* H_2 / 5%Pd-C / MeOH; *(x)* spontaneous decarboxylation during crystallization in $^iPr_2O$ / CH_2Cl_2 solution.

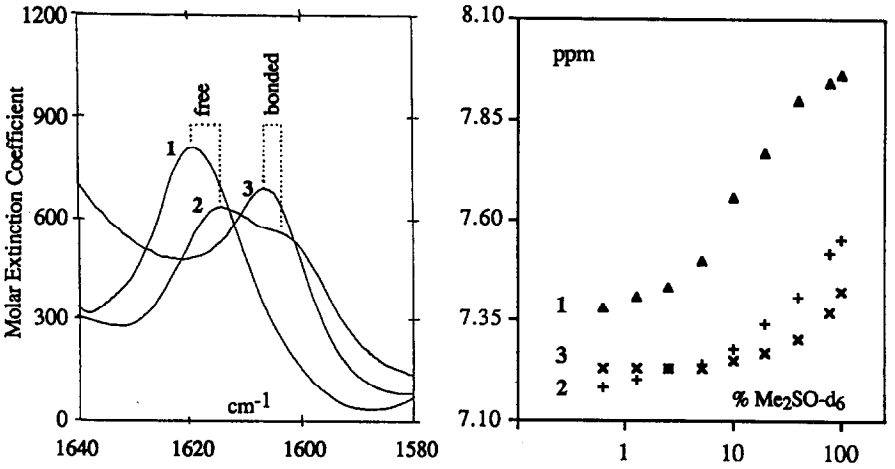


Figure 1. (^{t}Bu)C=O infrared stretching absorption in Me_2SO (left), and solvent sensitivity of the (^{i}Pr)NH proton NMR signal in $CDCl_3$ / Me_2SO-d_6 mixtures (right) for derivatives **1-3**.

N^{α} or N^{β}-protection in **6** and **7** results in reduced nucleophilic properties for the non-acylated nitrogen. Its coupling to give either the hydrazide or the N-amino amide link, requires strong activation of the carboxylic group via the $(Boc\text{-}Pro)_2O$ symmetric anhydride[9] or the Fmoc-Pro-Cl acid chloride.[10] The Boc or Fmoc protecting group of proline has been removed classically, and the pivaloyl group has been introduced via the pivaloyl chloride. The ethyl ester has been hydrolyzed by aqueous sodium hydroxide in acetone, and the C-terminal isopropylamide has been introduced by the mixed anhydride method. All derivatives in Scheme 1 are chromatographically pure and give satisfactory spectroscopic data.

The existence in solution of the intramolecular (^{i}Pr)N-H···O=C(^{t}Bu) hydrogen bond, typical of a β-folded structure, has been investigated by considering the (^{t}Bu)C=O stretching vibration in Me_2SO solution, and the solvent sensitivity of the (^{i}Pr)NH proton NMR signal in $CDCl_3$ / Me_2SO-d_6 mixtures. From the conformational study of **3** and other similar dipeptides,[5,11] it appears that this interaction shifts the (^{t}Bu)C=O vibration to lower frequencies by nearly 20 cm^{-1}, and results in a relative solvent insensitivity for the (^{i}Pr)NH proton NMR signal. Figure 1a shows that the (^{t}Bu)CO carbonyl is free in **1**, but noticeably hydrogen-bonded in **2**. Similarly, the solvent sensitivity of the (^{i}Pr)NH proton signal in $CDCl_3$ / Me_2SO-d_6 mixtures with increasing Me_2SO-d_6 content (Figure 1b) reveals its participation in a stronger intramolecular interaction in **2** and **3** compared with **1**.

The above observations in the solution have been corroborated in the solid state by solving the crystal structures of **2** and of the N^{α}-benzyl derivative **1a** obtained by spontaneous decarboxylation of **12** during crystallization.[12] The former is found to adopt the type IIβ-folded conformation (Figure 2a) with a (^{i}Pr)N to (^{t}Bu)CO distance of 3.10 Å, against 2.97Å in **3**[6]. Although the (^{i}Pr)N to (^{t}Bu)CO distance of 4.04 Å in **1a** effectively exceeds the upper value assigned to hydrogen bonds, the molecule assumes a semi-folded conformation (Figure 2b) in which the (^{i}Pr)N-H site is hydrogen-bonded to both the Pro carbonyl and the N^{α} nitrogen. It differs from the extended conformation assumed by Boc-Pro-N^{β}H-N^{α}H-CH_2-CO_2Et in the crystal.[4]

Thus we have presented a convenient method of synthesis of hydrazino and N-amino peptides containing the hydrazino analogue of glycine. The regioselective N-protection of the α-hydrazino acid ester affords excellent

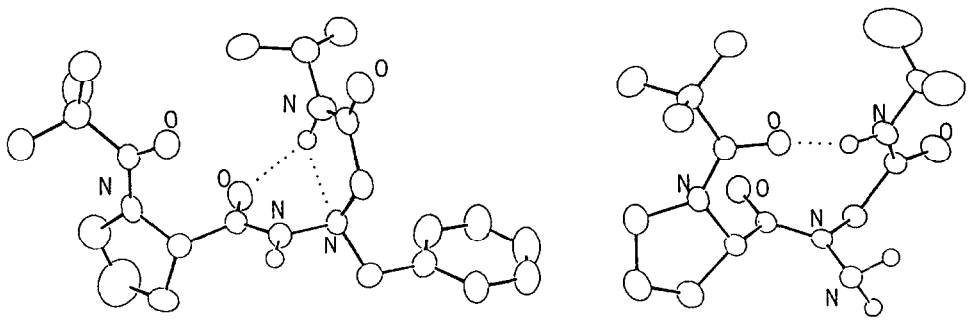


Figure 2. Crystal molecular structure of the hydrazino peptide **1a** (left), and of the N-amino peptide **2** (right).

yields, and the use of symmetric anhydrides, or acid chlorides, for the carboxyl component coupling fragment is compatible with solid phase synthesis. Work is in progress in order to extend the present procedure to α-hydrazino acids bearing a side-chain.

This work was also the opportunity of solving the second crystal structures in the hydrazino series,[4] and the first one in the N-amino peptide series, thus giving accurate dimensions of the hydrazide and N-amino amide peptidomimetic links, and some conformational preferences for the corresponding pseudopeptides.

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