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Chemistry of Hydrazinopeptides: a New Hydroperoxydeamination process

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Abstract:
The *N*-alkyl hydrazino group of hydrazinopeptides **1** is oxidized by air in bicarbonate buffers into the corresponding hydroperoxy moiety in high yield.

Keywords: hydrazinopeptides / *N*-electrophilic amination / hydroperoxydeamination

The solid phase *N*-electrophilic amination of primary amino groups allows the synthesis of hydrazinopeptides such as **1**, in which the ε-amino group of Lys[1] was replaced by a hydrazino moiety.[1] This methodology was easily adapted to an automatic solid phase peptide synthesizer, permitting the rapid synthesis of a large variety of hydrazinopeptides.[2] Both Boc / benzyl and Fmoc / *tert*-butyl strategies can be used, though the latter leads to better yields and purer compounds.[3]

The unique chemical properties of the hydrazino group permit the introduction of various modifications into peptides while working on completely deprotected fragments. We report in this letter the unprecedented oxidation of *N*-alkylhydrazines **1** into hydroperoxides **2** by molecular oxygen in bicarbonate buffers (Fig. 1). Standard approaches to the synthesis of hydroperoxides are often non-applicable to sensitive molecules.[4] In connection with the isolation of a number of biologically active peroxidic compounds from natural sources, there is a need for new and efficient methods which allow the mild incorporation of the peroxide linkage into highly functionalized structures.

Hydrazinopeptide **4** (Scheme 1) corresponds to the pseudo-substrate sequence 15-28 of PKC-α and was synthesized using our solid phase *N*-amination protocol.[1] The substitution of the hydrazino group of compound **4** by a hydroperoxy moiety was effected by simply dissolving peptide **4** in 0.1 M ammonium carbonate buffer at pH 8.90. After 17 hours at room temperature, peptide **4** was cleanly transformed into a slightly more hydrophobic structure **5**, whose molecular weight by ES-MS corresponded to the starting hydrazinopeptide plus 2 mass units. 2D-NMR analysis of the new compound revealed that the modification occured on the side chain of the first amino acid. In particular, H^{ε} (2H, 4.00 ppm) and C^{ε} (79.0 ppm) were shifted upfield to a large extent, indicating the presence of an electron-withdrawing group at the terminus of the side chain. All the analytical data were compatible with the proposed structure **5**, which was isolated with a 42% yield

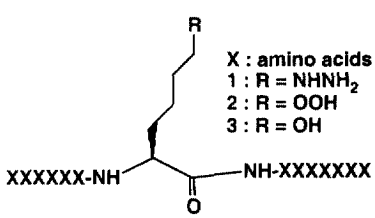

Figure 1

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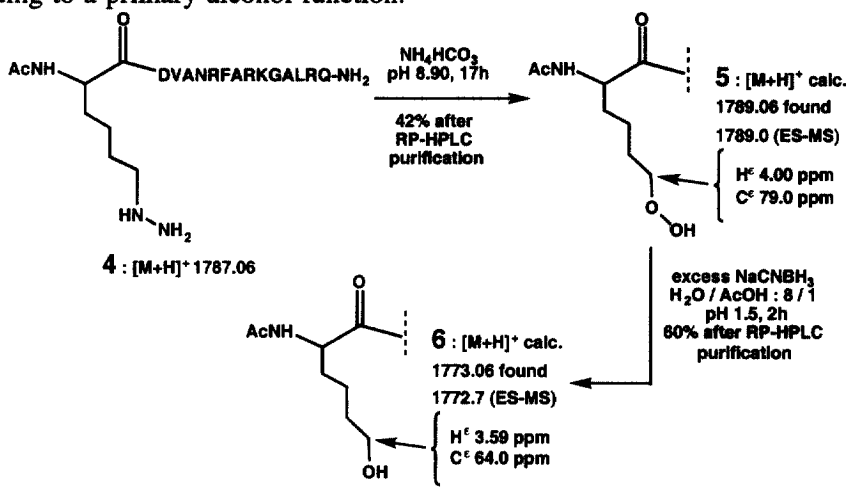
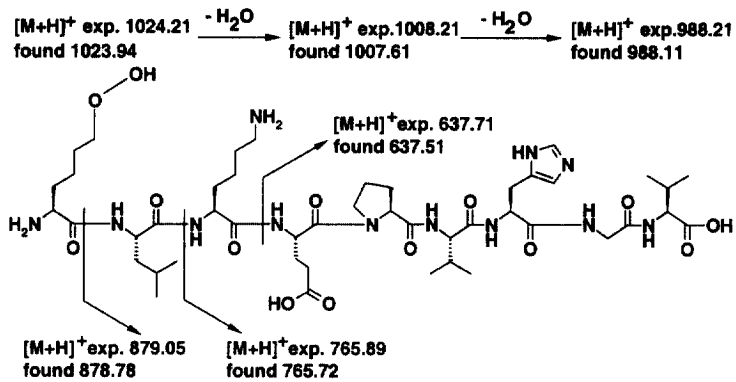
after RP-HPLC purification. Compound **5** was stable in H_2O / AcOH : 8 / 1, but addition of an excess of $NaCNBH_3$ resulted in the immediate reduction of the hydroperoxy group into the corresponding alcohol **6**, the NMR spectra of which displayed the chemical shifts corresponding to a primary alcohol function.



Scheme 1

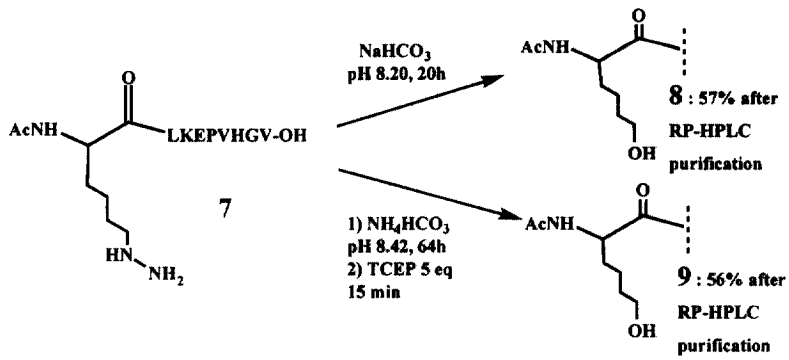
The size of peptide **4** rendered it unsuitable for a study aimed at defining the parameters important for the reaction. Peptide **7** (Scheme 2, HIV-1 POL 477-484 derived sequence) being preferred, it was easily oxidized in an aqueous ammonium carbonate buffer to give peptide **8** with a 57% yield following RP-HPLC purification (15 mg scale).[5] The ES-MS fragmentation pattern given by peptide **8** is depicted in Fig. 2.



peptide 8

Figure 2

The hydroperoxopeptide obtained following oxidation of the hydrazinopeptide by molecular oxygen in a bicarbonate buffer was also directly reduced by tris(2-carboxyethyl)phosphine hydrochloride. By use of this one-pot procedure, peptide **9** was isolated with a 56% yield following RP-HPLC purification.



Scheme 2

Initially we checked the influence of the buffer's pH and found that the yield of the reaction was insensitive to the pH of the medium in the range 8.08-8.61. The best kinetics were obtained at pH 8.45. The presence of the bicarbonate ion, however, was critical. Indeed, peptide **7** proved to be stable for days in aqueous ammonium formate. On the other hand, the ammonium ion was not important since the oxidation proceeded well in aqueous sodium bicarbonate (Fig. 3). Finally, the reaction was slowed significantly when peptide **7** was dissolved in sodium bicarbonate under nitrogen. The oxidation of the hydrazino moiety appears to be a highly efficient process since the formation of the hydroperoxo compound continued in the presence of traces of oxygen. However, the addition of excess DTT to the reaction mixture inhibited completely the oxidation for a few days. The oxidation described here represents an unprecedented method of introducing the primary hydroperoxo or alcohol moiety onto highly functionalized structures by the transformation of a primary amino group. Collazo and coworkers described a new synthesis of primary hydroperoxides starting from *N*-alkyl-*N*-tosyl hydrazines.[4c] The oxidant was molecular oxygen in the presence of potassium ethoxide. As for the reaction described here, the use of an inert atmosphere did not preclude the base-catalyzed oxidation of *N*-alkyl-*N*-tosyl hydrazines. The mechanism illustrated in Scheme 3 shares some common features with that proposed by Collazo *et al.* The first step involves the carbonation of the hydrazine on the more nucleophilic alkylated nitrogen atom.[6] Unlike the starting *N*-alkyl hydrazine, intermediate **10** is supposed to be highly susceptible to oxidation by molecular oxygen, thus explaining the importance of the bicarbonate ion. As proposed by Collazo *et al.*, the formation of radical **10** may be favored due to resonance stabilization. Reaction of radical **10** with molecular oxygen followed by hydrogen abstraction may lead to the hydroperoxy intermediate **12**. At this stage and in the case of *N*-alkyl-*N*-tosyl hydrazines, Collazo proposed the base-promoted elimination of p-toluenesulfinate leading to the key intermediate **15** with a N=N double bond. In our case, however, such an elimination is not possible and an additional oxidation step is supposed to occur. The formation of **15** may thus occur *via* the

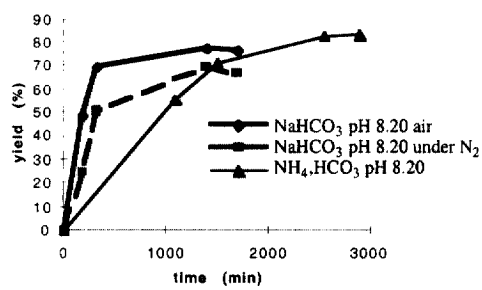
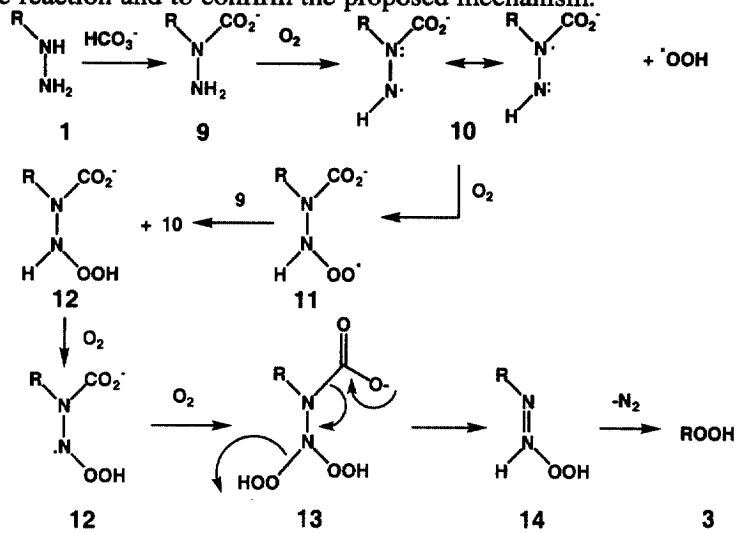


Fig. 3 : Progress curves of the oxidation of peptide 7 in bicarbonate buffers. Yields were estimated using the peak areas obtained by C18 RP-HPLC (1 mL/min, 215 nm, linear gradient 0-40% acetonitrile in water in 30 min, 0.05% TFA).

bis-hydroperoxy structure **14** by the simultaneous elimination of carbon dioxide and hydrogen peroxide. DTT in excess has been shown to be an efficient inhibitor of the oxidation of hydrazinopeptides **1**. This may be due to the ability of DTT to trap dissolved oxygen through the formation of a cyclic disulfide. Alternately, DTT may quench the radicals involved in the hypothetical chain radical process described by Scheme 3.

In conclusion, we have described a new and mild access to primary hydroperoxides starting from the corresponding hydrazino analogs. This modification was performed on peptides bearing diverse functional groups. Further studies are currently in progress to define the scope of the reaction and to confirm the proposed mechanism.



Scheme 3

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[5] Typical experimental procedures : 14.4 mg (9.75 µmol) of peptide **7** were dissolved in 14.4 mL of aqueous 0.1 M $NaHCO_3$ buffer (pH 8.20). After 20h at rt, the reaction medium was acidified to pH 5.5 with 100 µL of AcOH and purified directly by RP-HPLC on a C18 hyperprep column (8 µ, 300 Å, 15 x 500 mm; solvent A : H_2O containing 0.05% TFA, solvent B : MeCN / H_2O : 4 / 1 containing 0.05% TFA, linear gradient 0-50% B in 150 min, detection at 215 nm). Peptide **8** : 7.64 mg (57%). ES-MS (peptide dissolved in H_2O / MeCN: 4 / 1 containing 0.1% of formic acid at 5 µL per min) $[M+H]^+$ exp. 1024.21 found 1023.94, see fig. 2 for the typical fragments obtained using an orifice plate voltage of 60V. NMR (^1H 300 Mhz or ^{13}C 75 MHz) in H_2O / D_2O : 9 / 1 (pH 2.69) using 3-(trimethylsilyl)[2,2,3,3-d_4] propionic acid, sodium salt as an internal standard. For the unnatural amino acid, δ in ppm : Hε 4.00 Cε 76.7; Hδ 1.70; Hγ 1.42; Hβ 1.90; Hα 4.00 Cα 53.4.
Peptide **9** was obtained after reduction of the hydroperoxide **8** with TCEP (5 eq) in the ammonium bicarbonate buffer used for the oxidation. Starting from 10.5 mg of peptide **7**, 5.4 mg (56.4%) of peptide **9** were obtained after RP-HPLC purification as described before. ES-MS $[M+H]^+$ exp. 1007.22 found 1007.50. NMR in H_2O / D_2O : 9 / 1 (pH 2.56). For the unnatural amino acid NMR (^1H 600 MHz or ^{13}C 150 MHz) : Hε 3.61; Hδ 1.57; Hγ 1.39; Hβ 1.88; Hα 4.00.

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