Pergamon

Tetrahedron Letters 40 (1999) 7315–7318

TETRAHEDRON LETTERS

A novel and mild solid phase hydroperoxydeamination reaction

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Received 31 May 1999; accepted 20 July 1999

Abstract

The unnatural amino acid Lys(NH_2), easily incorporated into a peptidyl-PEGA resin by *N*-electrophilic amination of the ε-amino group, was cleanly transformed on the solid phase into 6-hydroxynorleucine by air oxidation in the presence of bicarbonate ions followed by reduction of the hydroperoxy intermediate with a water soluble phosphine.

Keywords: hydroperoxydeamination; *N*-electrophilic amination; solid phase; PEGA resin; 6-hydroxynorleucine; HR MAS NMR.

The introduction of unnatural amino acids into peptides is a method of both modifying their biological activity and enhancing their stability toward proteases. In this context, we were interested in the synthesis of peptides containing 6-norleucine derivatives. For example, we have recently reported that 6-hydrazinonorleucine (or Lys(NH_2)) containing peptides are more stable in PBS diluted human serum than their corresponding lysyl and arginyl counterparts.[1]

One way to access a large variety of 6-norleucine derivatives is to derivatize 6-hydroxynorleucine containing peptidyl-resins using the available solid phase chemistry of primary alcohols.[2] The preparation of 6-hydroxynorleucine being quite difficult,[3] we focused our efforts on the solid phase transformation of lysine into 6-hydroxynorleucine using mild conditions compatible with the peptide chain. Despite intense investigations devoted to the deamination of amines, the conversion of primary-alkyl primary-amines into primary alcohols remains a difficult task.[4] Indeed, the classical method involves the nitrosation of the amine with nitrosonium ion followed by the displacement of the diazonium intermediate with a nucleophile such as water. However, the generation and subsequent rearrangement of carbocations usually competes with the nucleophilic substitution and results in complex mixtures of alcohols and olefins. Moreover, nitrosonium ion and other nitrosating agents are known to react with amides and carbamates.[5]

We have recently described a new hydroperoxydeamination reaction which allows the mild convertion of hydrazinopeptides **1** into alcohols **3** via the hydroperoxy intermediate **2** (Fig. 1).[6] This two step transformation involved the air oxidation of compound **1** in aqueous bicarbonate buffer followed by reduction of the hydroperoxy moiety with a phosphine. Hydrazinopeptides **1** are easily available using

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0040-4039/99/$ - see front matter
PII: S0040-4039(99)01414-8

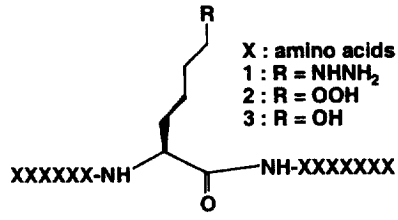
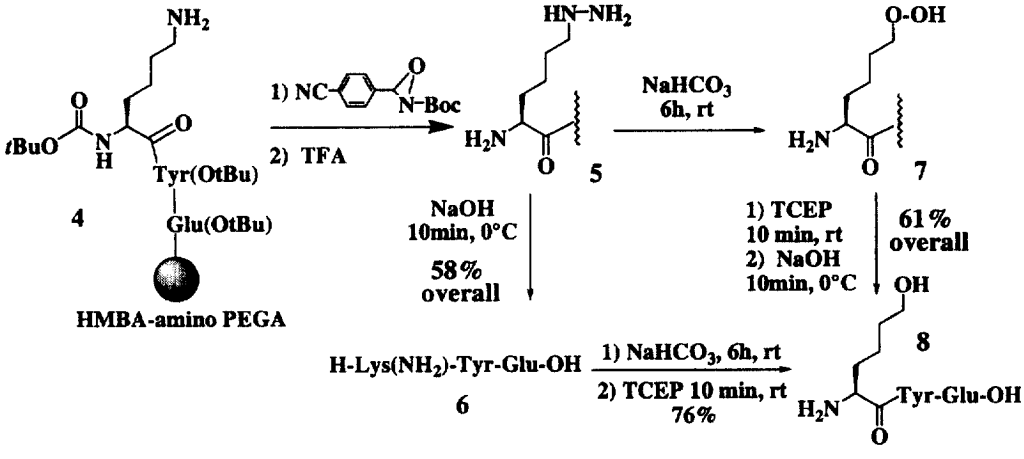


Figure 1.

the solid phase *N*-electrophilic amination of the ε-amino group with the commercially available *N*-Boc-3-(4-cyanophenyl)oxaziridine.[7,8] We report in this letter that the adaptation of this *N*-electrophilic amination/hydroperoxydeamination/reduction sequence on the solid phase is a simple and high yielding process for the transformation of Lys into 6-hydroxynorleucine (Scheme 1).



Scheme 1.

For this purpose, we needed a solid support well solvated in organic and aqueous solvents such as the poly(ethyleneglycol) dimethylacrylamide copolymer (PEGA resin).[9] The model sequence Lys-Tyr-Glu was elaborated on an HMBA-amino PEGA resin using the Fmoc/*t*Bu strategy. Fmoc-L-Glu(O*t*Bu)-OH was anchored to the polymer using MSNT activation (quantitative, 0.38 mmol/g).[10] The other amino acids were activated with HBTU/HOBt/DIEA.[11] The last residue was introduced as Boc-L-Lys(Fmoc)-OH. The deprotection of the ε-amino group with piperidine was followed by our automated *N*-electrophilic amination protocol[7] (quantitative as shown by a negative TNBS test).[12] The peptidyl resin was then completely deprotected in TFA/anisole/water.

The HR MAS NMR TOCSY spectrum of the deprotected peptidyl resin **5** in DMF-d_7 is presented in Fig. 2a. All the spin systems were precisely detected. In particular, H^{α} and H^{ε} of $Lys^1(NH_2)$ were situated at 4.20 and 3.18 ppm, respectively. At this stage, the hydrazinopeptide was cleaved from the solid support to compare the solid phase hydroperoxydeamination with the same reaction performed in solution. Peptide **6** was isolated with a 58% yield following RP-HPLC purification. The hydroperoxydeamination was performed in sodium bicarbonate buffer at pH 8.20, as this medium was found to be kinetically more favorable than ammonium bicarbonate.[6] The one-pot reduction of the hydroperoxo moiety with tris(carboxyethyl)phosphine led cleanly to peptide **8** with a 76% yield (44% overall yield). Analogously, peptidyl resin **5** was subjected to aqueous sodium bicarbonate to give resin **7**, whose HR MAS NMR TOCSY spectrum in DMF-d_7 is presented in Fig. 2b. The spectrum of resin **7** suffered from line broadening, however the spin system of 6-hydroperoxynorleucine was clearly detected (H^{α} and H^{ε} at 3.91 ppm). The absence of the $Lys^1(NH_2)$ signals proved that the hydroperoxydeamination reaction had

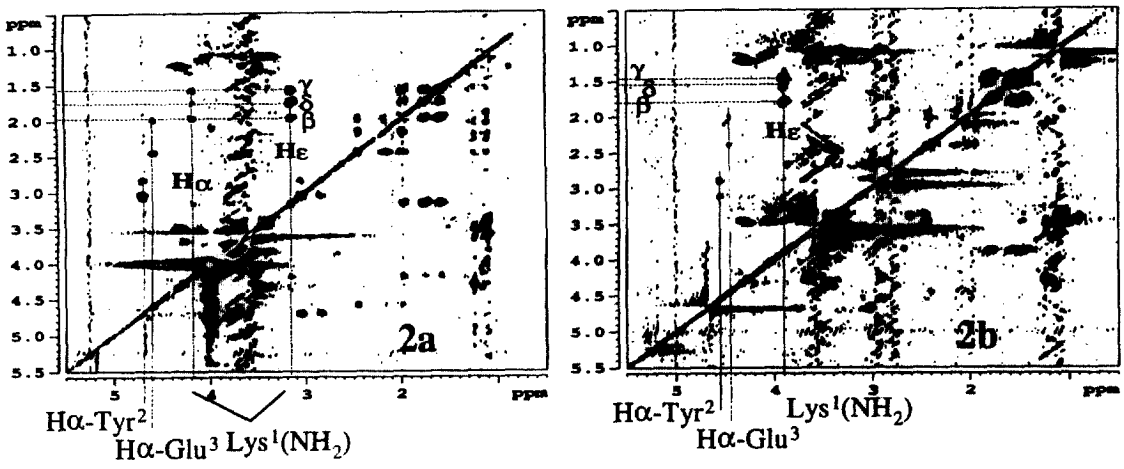


Figure 2. HR MAS NMR TOCSY spectra in DMF-d_7 at 300 MHz using tetramethylsilane as the internal standard: (a) resin **5**; and (b) resin **7**

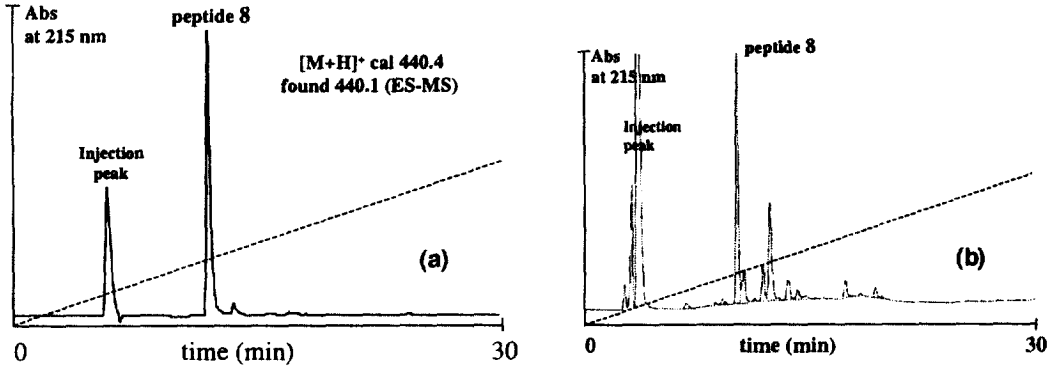
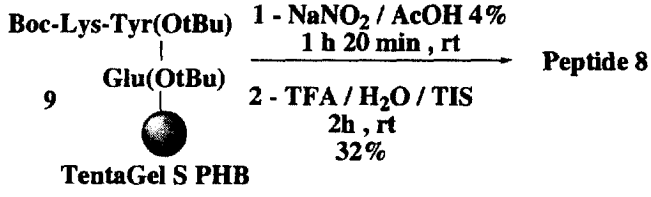


Figure 3. (a) RP-HPLC of the crude peptide **8** obtained using the full solid phase approach (Scheme 1). Eluents: see Table 1; (b) RP-HPLC of the crude peptide **8** obtained using the solid phase nitrous deamination depicted in Scheme 2

proceeded to completion. The hydroperoxide was then reduced with tris(carboxyethyl)phosphine.[13] The peptide was cleaved from the solid support as described earlier. The RP-HPLC of the crude peptide is displayed in Fig. 3, and highlights the efficiency of the process. Using the full solid phase approach, as described above, peptide **8** was isolated with a 61% overall yield following RP-HPLC purification. This value was well above those obtained by performing the hydroperoxydeamination/reduction steps in solution. Peptide **8** obtained according to Scheme 1 was found to be identical by ES-MS and NMR[14] to the compound synthesized using the solid phase nitrous deamination described in Scheme 2.



Scheme 2.

Despite the drawbacks associated with this reaction, the nitrous deamination led to peptide **8** as the major product, especially when sodium nitrite was used in a buffered water/THF mixture (see Table 1 and Fig. 3). However, as anticipated, the reaction was found to give many side products and precluded

Table 1
Solid phase nitrous deamination of peptidyl resin **9**

Entry	Reagent	Solvent	Time (min) [a]	RP-HPLC yield (%) [b] Peptide 8
1	$NaNO_2$	AcOH 4% in water/THF : 1/1	60	40
2	$NaNO_2$	AcOH 4% in water/dioxane : 1/1	80	39
3	tBuONO	AcOH-AcONa buffer/THF : 1/1	200	27
4	tBuONO	Et_3N / THF	240	22

a) The reagent (10 eq) was renewed every 20 min until the obtention of a negative Kaiser test.[15] b) C18 hyperprep column, eluant A : 0.05% TFA in water, eluant B : 0.05% TFA in acetonitrile/water : 4/1, linear gradient 0-100% B in 30 min, 1 mL/min, detection at 215 nm.

further chemistry on the solid phase. In conclusion, the recently described hydroperoxydeamination of *N*-alkylhydrazines was successfully adapted to the solid phase using a PEGA resin. The *N*-electrophilic amination hydroperoxydeamination reduction steps allowed the conversion of a primary amine into the corresponding alcohol under mild conditions. The overall process is high yielding and represents a further indication of the flexibility afforded to transformations performed on the solid phase.

Acknowledgements

We thank R. Warras, G. Montagne and J.-M. Wieruszeski for useful discussions and for performing the NMR experiments. We thank B. Coddeville and G. Ricart for the ES-MS studies (Université des Sciences et Technologies de Lille), and S. Brooks for proofreading the manuscript. We gratefully acknowledge financial support from Institut Pasteur de Lille and CNRS. The NMR facility used in this study was funded by the European Community (FEDER), the Région Nord-Pas de Calais (France), the CNRS and the Institut Pasteur de Lille.

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13. Peptide **8** linked to the HMBA-amino PEGA resin was analyzed by HR MAS NMR, but line broadening precluded further analysis.
14. NMR (^{1}H 300 MHz) in H_2O/CD_3COOD:8/2 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: H^{ε} 3.59 (t, J=6.6 Hz); H^{δ} 1.57 (m); H^{γ} 1.37 (m); H^{β} 1.87 (m); H^{α} 3.96 (t, J=6.8 Hz).