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Synthesis of glyoxylyl peptides using a phosphine labile α,α′-diaminoacetic acid derivative

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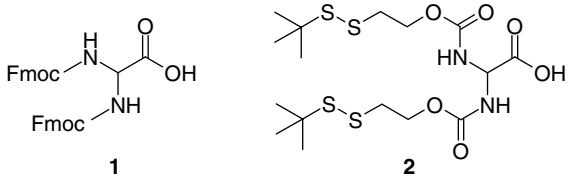
Abstract—We describe in this letter the preparation of a novel protected α,α′-diaminoacetic acid derivative that acts as a masked glyoxylic acid equivalent. The reagent could easily be introduced on a peptide chain using standard Fmoc/*tert*-butyl solid-phase methods and resisted to the TFA treatment allowing the deprotection and cleavage of the peptide. Unmasking of the glyoxylyl group was performed in solution in the presence of a phosphine.

The use of α-oxo aldehyde functionalized peptides for the convergent synthesis of large molecular constructs or hybrid materials has been the subject of numerous reports over the past decade.[1] Despite the interest of this modification, there is a lack of general methods allowing the synthesis of glyoxylyl peptides using mild and non-oxidizing experimental conditions. Recently, a Fmoc-protected α,α′-diaminoacetic acid derivative **1** (Scheme 1) acting as a masked glyoxylic acid equivalent was prepared in one step from glyoxylic acid. Compound **1** could be introduced on a peptide chain after Fmoc/*tert*-butyl solid-phase peptide synthesis. Deprotection and cleavage of the peptide from the solid support using trifluoroacetic acid were followed by unmasking of the glyoxylyl group in the presence of a base such as DBU.[2]

Here we present the preparation and use of α,α′-diaminoacetic acid derivative **2** (Scheme 1), which as for **1** could easily be introduced into a peptide using standard Fmoc/*tert*-butyl protocols. Interestingly, unmasking occurred under mild reducing conditions in aqueous solution.



Scheme 1. α,α′-Diaminoacetic acid derivatives **1** and **2**.

Glyoxylic acid derivative **2** was prepared as depicted in Scheme 2. *S*-*tert*-Butylthio group was first introduced on 2-mercaptoethanol using di-*tert*-butyl 1-(*tert*-butylthio)-1,2-hydrazine dicarboxylate.[3] Disulfide **3** was then converted to carbamate **5** by reaction with *p*-nitrophenyl chloroformate to give carbonate **4**, followed by ammonia treatment. Reaction of glyoxylic acid hydrate with 2 equiv of carbamate **5** in refluxing toluene and in the presence of a catalytic amount of *p*-toluenesulfonic acid afforded bis[2-(*tert*-butyldisulfanyl)ethoxycarbonylamino] acetic acid **2** with a 54% yield following silica gel chromatography.

Derivative **2** was then coupled to the N-terminus of peptididyl resin **6**, which was prepared using standard Fmoc/*tert*-butyl protocols[4] (Scheme 3). Deprotection and cleavage of the peptide were performed in concentrated trifluoroacetic acid, which left the α,α′-diaminoacetyl moiety unaffected. Peptide **7** was isolated in 40% yield after RP-HPLC purification.

Deprotection of the bis[2-(*tert*-butyldisulfanyl)ethoxy carbonylamino]acetyl moiety is based on the reductive cleavage of disulfide bonds. Generation of the free thiols was expected to induce intramolecular thiirane formation and then decarboxylation. Once generated, the α,α′-diaminoacetyl group is known to hydrolyze

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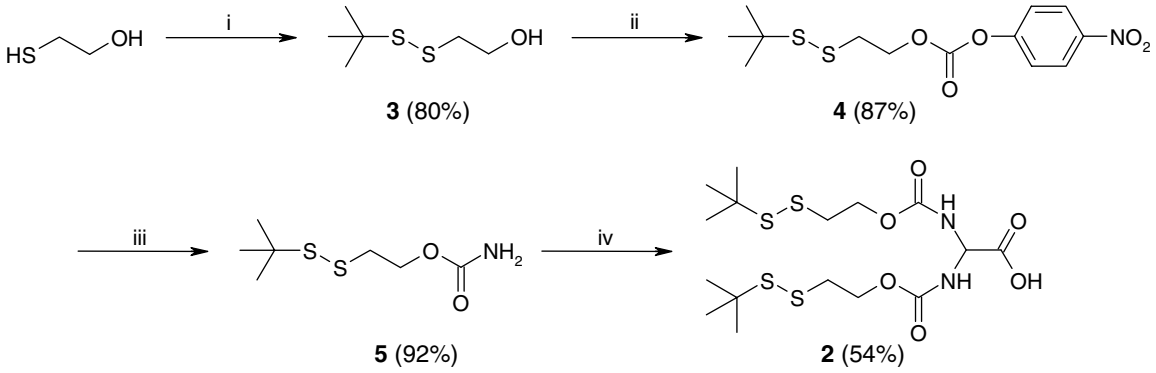
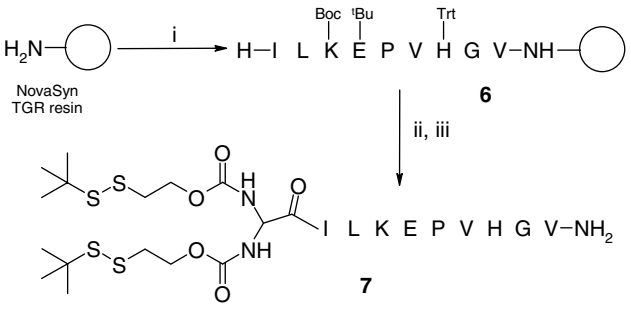


Scheme 2. Synthesis of glyoxylic acid derivative **2**. Reagents and conditions: (i) di-*tert*-butyl 1-(*tert*-butylthio)-1,2-hydrazine dicarboxylate, Et_3N, DMF, rt, overnight; (ii) 4-nitrophenyl chloroformate, Et_3N, THF, 0 °C → rt, overnight; (iii) NH_4OH 32%, CH_3CN, rt, 1 h; (iv) glyoxylic acid monohydrate (0.5 equiv), PTSA, toluene, reflux (Dean–Stark trap), 2 h.



Scheme 3. Synthesis of peptide **7** using derivative **2**. Reagents and conditions: (i) Fmoc/*t*-Bu solid-phase peptide synthesis using TBTU/HOBt/DIEA activation in DMF; (ii) **2** (1.2 equiv), PyBOP/DIEA, DMF, 1 h; (iii) TFA/H_2O/anisole (95/2.5/2.5 by vol).

spontaneously in aqueous medium to give the α-oxo aldehyde function.[2]

Deprotection was first attempted using dithiothreitol (DTT) as reducing agent in 0.1 M Tris–HCl buffer (pH 8.3). The reaction was found to be poorly efficient as shown by RP-HPLC monitoring. Alternately and as shown in Figure 1, the use of tris(2-carboxyethyl)phosphine hydrochloride (TCEP), which is known to be generally more efficient than DTT for the reduction of disulfide bonds,[5] permitted the clean conversion of peptide **7** (22.49 min) into glyoxylyl peptide **10** (11.74 min).[6] The product formed in this reaction after 21 h at rt was found to be identical by RP-HPLC to a reference glyoxylyl peptide obtained by periodic oxidation of the corresponding seryl precursor[7] and displayed the expected analytical characteristics. The monitoring of the reaction revealed also two intermediate peaks at 19.15 and 15.03 min, which corresponded probably to derivatives **8** and **9**, respectively (Scheme 4). Indeed, the same products could be formed, isolated and characterized by mass spectrometry when the reaction was performed at pH 5.5, experimental conditions, which permitted the reduction of the disulfide bonds but not the intramolecular nucleophilic substitution.

In conclusion, we have prepared a novel protected α,α′-diaminoacetic acid derivative that can be easily introduced into peptide using standard Fmoc/*tert*-butyl methods and deprotected in aqueous solution to give a glyoxylyl group. The glyoxylyl group was generated in the presence of a phosphine at pH 8.3. This reagent is thus complementary to $(FmocNH)_2CHCO_2H$ derivative

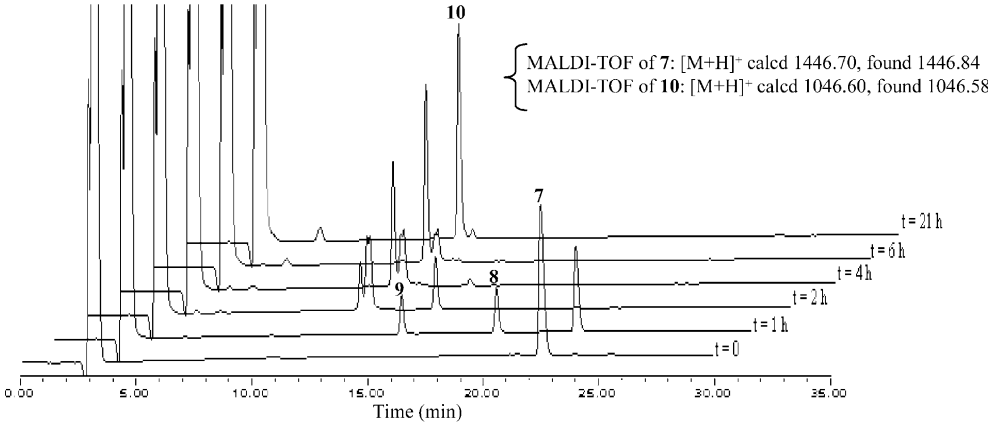
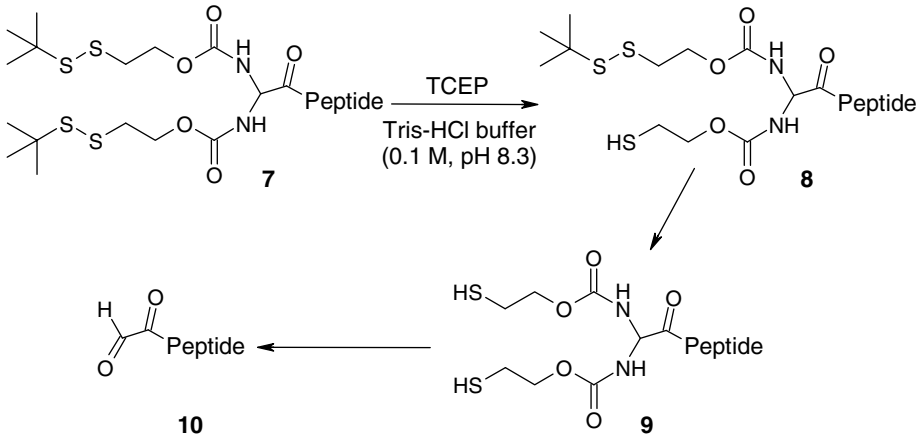


Figure 1. RP-HPLC monitoring of the conversion of peptide **7** into glyoxylyl peptide **10** in the presence of TCEP in 0.1 M Tris–HCl buffer pH 8.3. C18 Delta Pak 3.9 × 300 mm column, eluent A: water containing 0.05% TFA by vol, eluent B: acetonitrile/water 4/1 by vol containing 0.05% TFA by vol, linear gradient 0–100% B in 30 min, 1 mL/min, detection at 215 nm.



Scheme 4. Unmasking of peptide **7** in the presence of TCEP in 0.1 M Tris–HCl buffer pH 8.3.

1, whose unmasking requires a base such as DBU in an organic solvent (DMF).

Acknowledgements

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References and notes

1. Melnyk, O.; Fehrentz, J.-A.; Martinez, J.; Gras-Masse, H. *Peptide Sci.* **2000**, *55*, 165–186.
2. Far, S.; Melnyk, O. *Tetrahedron Lett.* **2004**, *45*, 1271–1273.
3. Wünsch, E.; Moroder, L.; Romani, S. *Hoppe-Seyler's Z. Physiol. Chem.* **1982**, *363*, 1461–1464.
4. Fields, G. B.; Noble, R. L. *Int. J. Pept. Protein Res.* **1990**, *35*, 161–214.
5. Getz, E. B.; Xiao, M.; Chakrabarty, T.; Cooke, R.; Selvin, P. R. *Anal. Biochem.* **1999**, *273*, 73–80.
6. Typical deprotection procedure: the peptide (3.0 μmol) was dissolved in 5 mL of 0.1 M Tris–HCl buffer (pH 8.33). Tris(2-carboxyethyl)phosphine hydrochloride (86 mg, 0.3 mmol, 100 equiv) was added and the mixture was stirred at room temperature for 21 h. Aliquots (95 μL) of the reaction mixture were mixed with 5 μL of acetic acid and injected on a C18 Delta Pak column (3.9 × 300 mm, experimental conditions specified in Fig. 1) for HPLC monitoring.
7. Geoghegan, K. F.; Stroh, J. G. *Bioconjugate Chem.* **1992**, *3*, 138–146.