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A novel α,α′-diaminoacetic acid derivative for the introduction of the α-oxo aldehyde functionality into peptides

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Abstract—A Fmoc-protected α,α′-diaminoacetic acid derivative acting as a masked glyoxylic acid equivalent was prepared in one step from glyoxylic acid and introduced into peptide chains after solid-phase peptide elongation. Deprotection and cleavage of the peptide from the solid support using trifluoroacetic acid was followed by unmasking of the glyoxylyl group in the presence of a base. This reagent allowed the synthesis of a glyoxylyl peptide using nonoxidizing conditions.

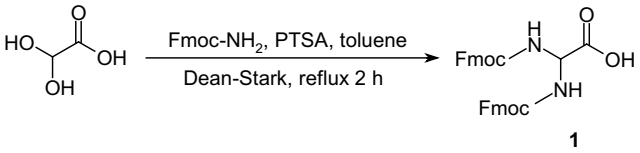
The use of aldehyde chemistry for the convergent synthesis of large peptidic constructs has been the subject of increasing attention over the past decade. The ability of the aldehyde function to react chemoselectively with a variety of naturally occurring amino acids is of widespread interest since it permits the linkage of unprotected peptide fragments under mild conditions in aqueous media. Indeed, aldehyde-functionalized peptides can be ligated chemoselectively with the β-aminothiol moiety of cysteine or the β-aminoalcohol moiety of serine or threonine to give the thiazolidine[1] or pseudoproline linkages, respectively.[2] Alternately, stable hydrazones[3] or oximes[4] can also be formed by reaction with hydrazine or hydroxylamine derivatives. In this context, the glyoxylyl group has been intensively used due to its stability and reactivity. Recently, we exploited this function for the fabrication of peptide microarrays by site-specific ligation of glyoxylyl peptides onto semicarbazide-functionalized glass slides.[5,6]

The glyoxylyl group can be generated in solution via periodate oxidation of a terminal serine or threonine residue.[7] However, oxidation of peptides by periodate is often observed, especially for those containing cysteine or methionine residues, and only a few alternatives to periodate oxidation have been proposed.[8] Therefore, an efficient and general method for the introduction of the α-oxo aldehyde group is needed, that can be applied to the synthesis of large libraries of glyoxylyl peptides.

Our strategy is based upon the elaboration of a masked glyoxylic acid derivative suitable for the Fmoc/*tert*-butyl solid-phase peptide synthesis. Since the glyoxylic group is unstable during the TFA treatment used to deprotect and cleave the peptide from the solid support, we decided to unmask the aldehyde function in the final stage of the synthesis using nonoxidizing and nonacidic experimental conditions. We demonstrate here that Fmoc-protected α,α′-diaminoacetic acid derivative **1** (Scheme 1) responds to all these specifications.

Bis-(9*H*-fluoren-9-ylmethoxycarbonylamino)-acetic acid **1** was obtained in 59% yield by reacting glyoxylic acid monohydrate with 2 equiv of 9*H*-fluorenylmethyl carbamate in refluxing toluene in the presence of a catalytic amount of *p*-toluenesulfonic acid.[9]

The utility of derivative **1** was examined using the model sequence ILKEPVHGV, which was assembled on a NovaSyn® TGR resin (Scheme 2) using standard Fmoc/



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Scheme 1. Synthesis of bis-(9*H*-fluoren-9-ylmethoxycarbonylamino)-acetic acid **1**.

Keywords: α-Oxo aldehyde; Peptide; Oxime.
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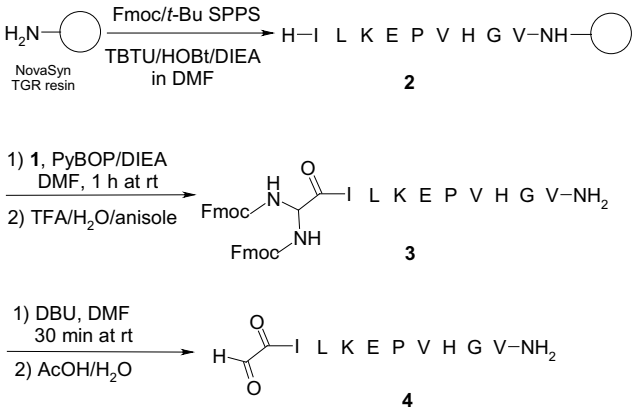


Scheme 2. Synthesis of *N*-glyoxylyl peptide **4** using derivative **1**.

tert-butyl protocols.[10] Acid **1** was activated using PyBOP/DIEA in DMF. The peptidyl resin was then treated with TFA to give peptide **3** in 42% yield following RP-HPLC purification (Fig. 1A). The Hα proton of the $(FmocNH)_2CHCO$ moiety was situated at 5.74 ppm (DMF-d_7, 5.59 ppm for **1**) in the ^1H NMR spectrum of **3**, and was correlated with Cα at 62.0 ppm (60.8 ppm for **1**) in the HSQC spectrum (Fig. 2).

Next, we examined the conversion of peptide **3** into glyoxylyl peptide **4** in the presence of base.[11] DBU was found to be more efficient than piperidine and permitted the isolation of peptide **4** in 54% yield following acidification of the reaction medium and RP-HPLC purification.[12]

Peptide **4** was found to be identical by MALDI-TOF, RP-HPLC (Fig. 1B and C) and ^1H NMR (Fig. 2) to a reference compound obtained by standard periodic oxidation of H–SILKEPVHGV–NH_2 peptide (Scheme 3).[7]

To verify the reactivity of the α-oxo aldehyde function generated by using derivative **1**, oxime formation with *O*-benzylhydroxylamine was undertaken as described in Scheme 4. Oxime formation with 2 equiv of *O*-benzylhydroxylamine was complete after 4 h of reaction, yielding oxime **6** in 46% yield following purification by RP-HPLC.

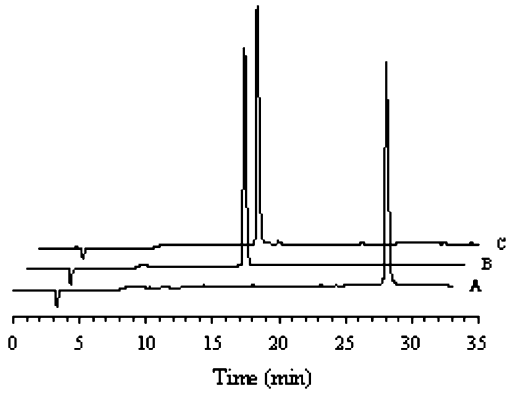
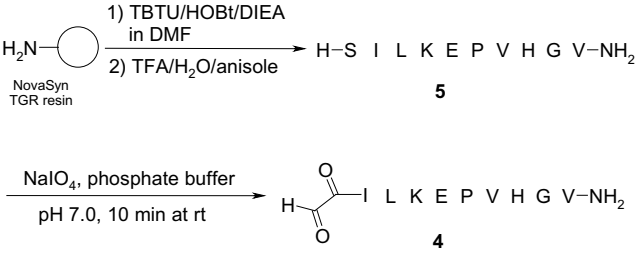


Figure 1. RP-HPLC traces of (A) purified peptide **3**, (B) **4** obtained with reagent **1** and (C) **4** obtained by periodic oxidation of a N-terminal serine. C18 Nucleosil 4.6×250 mm column, eluent A: water containing 0.05% TFA by vol, eluent B: acetonitrile/water 4/1 by vol containing 0.05% TFA by vol, linear gradient 0–100% B in 30 min, 1 mL/min, detection at 215 nm.



Scheme 3. Synthesis of glyoxylyl peptide **4** by periodic oxidation of a N-terminal serine.

In conclusion, we have synthesized a novel Fmoc-protected α,α′-diaminoacetic acid derivative, which allows

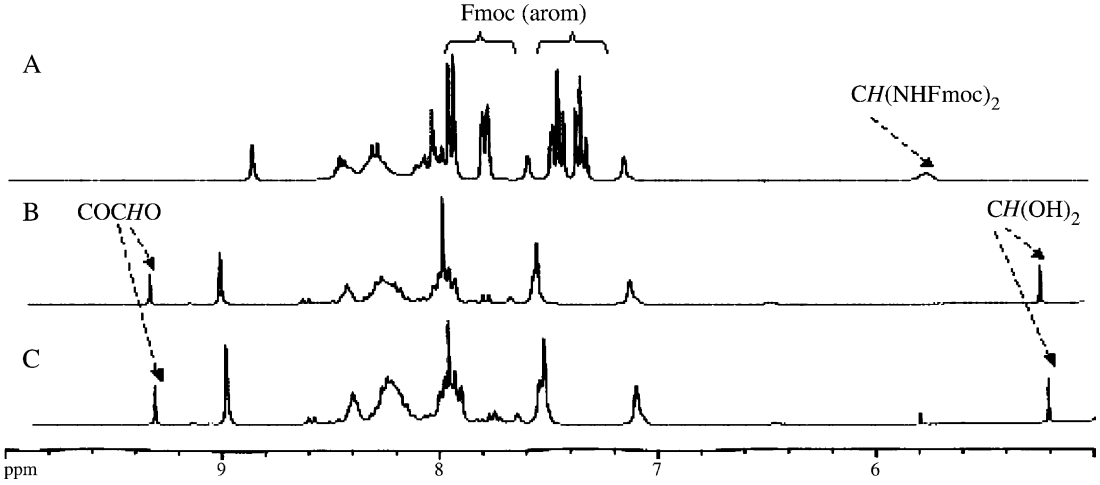
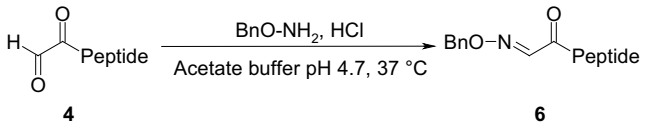


Figure 2. ^1H NMR spectra of peptide **3** (A), peptide **4** synthesized using reagent **1** (B) and the peptide prepared by periodic oxidation of a N-terminal Ser (C).



Scheme 4. Reaction of peptide **4** with *O*-benzylhydroxylamine.

the introduction of an α-oxo aldehyde functionality into a peptide. This derivative was introduced following standard Fmoc/*tert*-butyl solid-phase peptide elongation and was resistant to the acidic treatment used for the deprotection of the peptide chain. Unmasking of the glyoxylyl group was performed in solution with DBU. Thus, this method is complementary to the periodic oxidation of serine, which has to be carefully controlled for Met-containing peptides.

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9. Preparation of compound **1**: 9*H*-Fluorenylmethylcarbamate (1.00 g, 4.18 mmol), glyoxylic acid monohydrate (192 mg, 2.09 mmol) and PTSA (3.8 mg, 20 μmol) were dissolved in toluene (200 mL) and refluxed for 2 h (Dean–Stark trap). The reaction mixture was cooled to rt. The resulting precipitate was filtered, washed with toluene and dried in vacuo to give compound **1** (657 mg, 1.23 mmol) as a white powder in 59% yield. Mp 216–218 °C; IR (KBr) ν (cm^{-1}) 3301, 1726, 1690; ^1H NMR (300 MHz, DMF-d_7, TMS as internal reference) δ in ppm 4.17 (m, 6H, CH_2+CHFmoc), 5.59 (t, 1H, $J = 7.8$ Hz, C*H*–COOH), 7.19 (t, 4H, $J = 7.2$ Hz, CH_{Ar}), 7.29 (t, 4H, $J = 7.2$ Hz, CH_{Ar}), 7.65 (d, 4H, $J = 7.2$ Hz, CH_{Ar}), 7.79 (d, 4H, $J = 7.2$ Hz, CH_{Ar}), 8.13 (d, 2H, $J = 8.1$ Hz, NH); ^{13}C NMR (75 MHz, DMF-d_7) δ 47.6 (CH_2), 60.8 (*C*H–COOH), 67.3 (CH), 120.7 (CH), 126.1 (CH), 127.8 (CH), 128.4 (CH), 141.8 (C), 144.8 (C), 156.5 (CO), 170.6 (CO); FAB-MS *m*/*z* 535.3 $[M+H]^+$, 557.3 $[M+Na]^+$; HRMS (FAB) *m*/*z* calcd for $[M+H]^+$ $C_{32}H_{27}N_2O_6$ 535.1869, found 535.1865 (−0.7 ppm).
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11. Preparation of peptide **4**: Synthesis was performed on a 0.2 mmol scale using NovaSyn® TGR resin (0.29 mmol/g), Fmoc/*tert*-butyl chemistry and TBTU/HOBt/DIEA activation. Side-chain protecting groups were *t*-Bu for Glu, Boc for Lys and Trt for His. After Fmoc deprotection, the resin was washed with CH_2Cl_2 (3×3 min) and DMF (3×3 min). Derivative **1** (128 mg, 0.24 mmol) and PyBOP (125 mg, 0.24 mmol) were dissolved in DMF (2 mL) and added to the resin swelled in a minimal volume of DMF. DIEA (105 μL, 0.60 mmol) was then added and the suspension was shaken for 1 h. The resin was washed with DMF (3×3 min), CH_2Cl_2 (3×3 min) and Et_2O (3×3 min) and dried under vacuum. After cleavage from the solid support and deprotection with 10 mL of TFA/H_2O/anisole 95/2.5/2.5 (v/v/v), the crude peptide was precipitated in Et_2O/*n*-heptane (v/v). The peptide was purified by RP-HPLC to give 146 mg of peptide **3** (42%). Glyoxylyl group unmasking: peptide **3** (100 mg, 57.0 μmol) was dissolved in DMF (20 mL). DBU (80 μL, 0.57 mmol) was added dropwise to the solution. The reaction mixture was stirred at room temperature for 30 min. Acetic acid (3 mL) and water (1 mL) were added. The mixture was stirred for a further 5 min and then concentrated in vacuo. The crude residue was purified by RP-HPLC as above to give 40 mg (54%) of peptide **4**. Selected ^1H NMR data (300 MHz, DMF-d_7, TMS as internal reference) δ in ppm 5.21 (s, 0.7 H, CO–C*H*$(OH)_2$), 9.41 (s, 0.3 H, CO–C*H*O); Selected ^{13}C NMR data (75 MHz, DMF-d_7) δ 88.8 (*C*H$(OH)_2$), 189.3 (CO*C*HO); MALDI-TOF *m*/*z* $[M+H]^+$ calcd 1046.59, found 1046.52.
12. A minor by-product, which gave a peak at *m*/*z* 2090.4 (monoisotopic) in the MALDI-TOF spectrum, was also isolated in this experiment.