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A new linker for the synthesis of C-terminal peptide α-oxo-aldehydes

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Abstract

A tartaric acid-based linker was elaborated on an amino PEGA resin for the synthesis of C-terminal α-oxo-aldehydes. A solid phase periodic oxidation allowed the formation of the glyoxylyl moiety and the separation of the final product from the solid support. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: C-terminal peptide α-oxo-aldehydes; periodic oxidation; tartaric acid-based linker; solid phase; chemical ligation.

Chemical ligation[1] permits the linkage of unprotected peptide fragments through the use of efficient and mild chemoselective reactions in aqueous media. Thus, proteins or more generally high molecular weight constructs can be assembled from small peptide fragments synthesized using solid phase methodologies. Peptide aldehydes are involved in thiazolidine,[2] oxime[3] and hydrazone[4] chemical ligations. A large amount of work has been devoted to the solid phase synthesis of peptides built on C-terminal α-amino aldehyde moieties.[5] However, these compounds are unstable and prone to epimerization. This drawback has led many workers to use essentially glyoxylyl-peptides in chemical ligation studies. N-terminal peptide α-oxo-aldehydes are usually obtained by periodic oxidation of a terminal serine or threonine.[6] Analogously, C-terminal peptide α-oxo-aldehydes were synthesized by performing the periodic oxidation of C-terminal lysyl peptides bearing a Ser or Thr residue on the ε-amino group. All these methods involve the formation of the desired product in homogeneous phase.

With the synthesis of chemical libraries in mind, we needed a solid phase methodology permitting the formation of a C-terminal α-oxo-aldehyde function during the separation of the product from the solid support. The strategy described in Scheme 1 was based upon the elaboration of a new linker starting from (+)-dimethyl-2,3-*O*-isopropylidene-D-tartrate.[7] The tartrate derivative was the precursor of the 1,2-diol moiety leading to a C-terminal α-oxo-aldehyde group following a periodic oxidation. This solid phase periodic oxidation was performed on a fully deprotected peptide and led simultaneously to the formation of the α-oxo-aldehyde moiety and to the cleavage of the product from the solid support. To this end, we used a solid support well solvated in aqueous media. The acetonide protecting group is stable during

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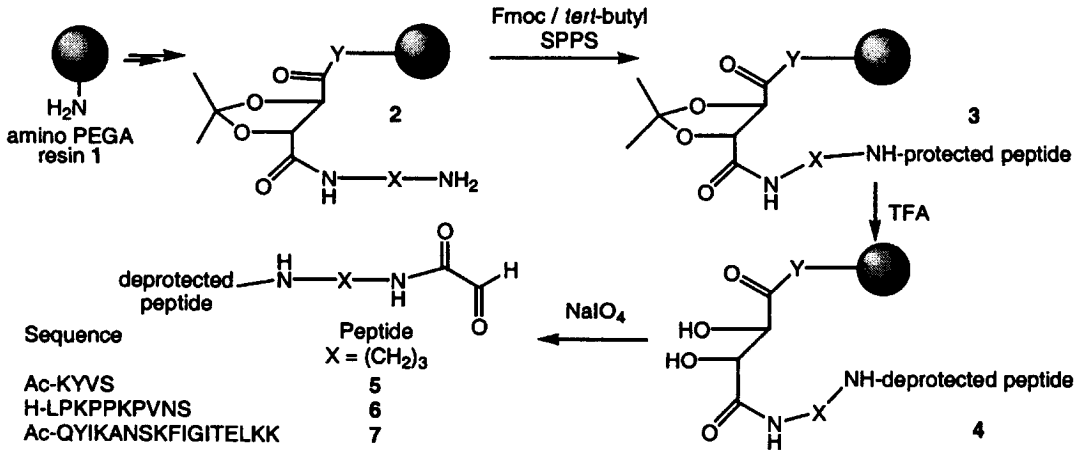
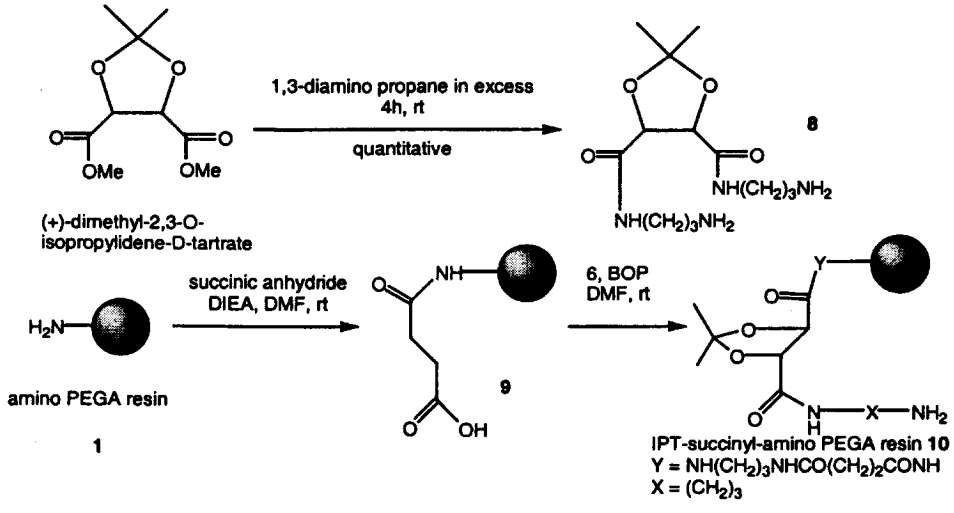
standard Fmoc/*tert*-butyl solid phase peptide synthesis. It was simultaneously and easily deprotected during the removal of the side chain protecting groups in trifluoroacetic acid.



Scheme 1.

The 2,3-*O*-isopropylidene-D-tartrate based linker (IPT) was elaborated as described in Scheme 2. Compound **8** was synthesized in one step by slow addition of (+)-dimethyl-2,3-*O*-isopropylidene-D-tartrate to an excess of 1,3-diamino propane at room temperature. The excess of diamine was easily removed by evaporation under reduced pressure. The poly(ethyleneglycol) dimethylacrylamide copolymer (PEGA resin) was a well adapted solid support for our strategy.[8] Indeed, PEGA resins were found to swell efficiently in a broad range of solvents (toluene to water). Moreover, the PEGA resins were shown to be superior to many of the existing polymer supports for peptide synthesis. Thus, commercially available amino PEGA resin **1** (0.4 mmol/g) was reacted with succinic anhydride to give resin **9**, whose carboxylic acid group was activated with BOP in the presence of an excess of the tartric acid derivative **8**. The charge of the IPT-succinyl-amino PEGA resin **10** was found to be 0.20 mmol/g, as determined by spectrophotometric analysis of the fulvene-piperidine adduct following derivatization with Fmoc-Gly-OH.[9]



Scheme 2.

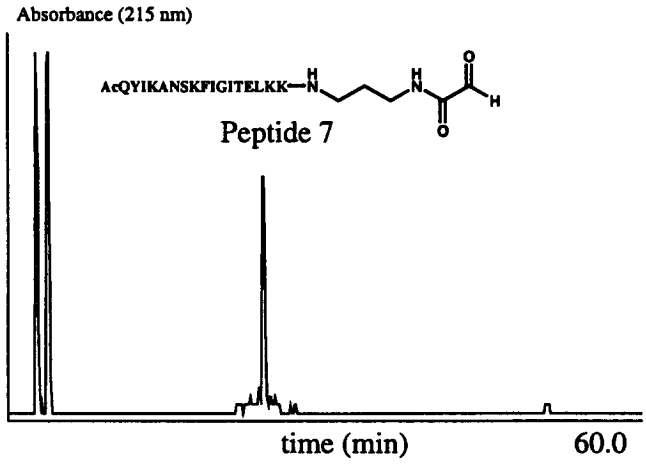
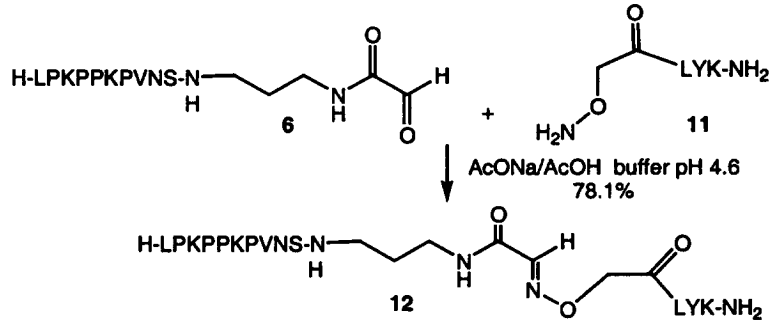


Figure 1. RP-HPLC profile of the crude peptide **7** (C18 Vydac column, eluent A: water containing 0.05% TFA, eluent B: water/acetonitrile: 1/4 containing 0.05% TFA, linear gradient 0–100% B in 60 min, flow 1 mL/min)

In order to evaluate the compatibility of the IPT linker with Fmoc SPPS, sequence Ac-KYVS was assembled upon resin **10** using the HBTU/HOBt/DIEA activation. The peptidyl-resin was then deprotected in trifluoroacetic acid over 2 h. Finally, the AcKYVS peptide linked to the IPT-succinyl-amino PEGA resin was treated with an excess of sodium periodate in a water–acetic acid mixture containing *p*-nitrophenol as an internal standard. The release of peptide **5** was monitored by RP-HPLC following the quenching of a small aliquot of the reaction medium with ethylene glycol. The periodic oxidation appeared to be very fast since all of the product was released following only 30 seconds of reaction at room temperature. Peptide **5** was isolated with a 38% yield following RP-HPLC purification. Similarly, the nonapeptide **6** was obtained with a 26% yield.

In order to evaluate the IPT-succinyl-amino PEGA resin's ability to lead to larger C-terminal peptide α-oxo-aldehydes, synthesis of peptide **7** was undertaken. Fig. 1, which corresponds to the RP-HPLC trace of the crude peptide **7** released in the periodic oxidation mixture, illustrates the efficiency of the process.[10] The usefulness of the C-terminal peptide α-oxo-aldehydes synthesized in this study in oxime chemical ligation is illustrated in Scheme 3. Indeed, peptide **6** reacted rapidly with peptide **11** derivatized with an α-aminooxyacetyl moiety in buffered aqueous medium. The corresponding oxime product **12** was isolated with a 78% yield following RP-HPLC purification. In conclusion, we have developed a new and efficient tartaric acid-based linker for the synthesis of C-terminal peptide α-oxo-aldehydes. A mild, solid phase periodic oxidation leads to the formation of the α-oxo-aldehyde moiety and to the liberation of the final product in solution. These C-terminal peptide α-oxo-aldehydes are useful partners in oxime chemical ligation.



Scheme 3.

Acknowledgements

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9. Typical experimental procedure: IPT linker: 2 mL (9.16 mmol) of (+)-dimethyl-2,3-*O*-isopropylidene-D-tartrate (ACROS) were added over 45 min to 10 mL (120 mmol) of 1,3-diaminopropane. The mixture was stirred for 5 h and 30 min at rt. The excess of diamine was removed under reduced pressure to give **6** in quantitative yield. ^{1}H 300 MHz NMR (DMSO-d_6, TMS): 8.19 (t, 2H, J=5.5 Hz, CONH), 4.46 (s, 2H, COCH), 3.15 (m, 4H, CH_2NHCO), 2.50 (m, 4H, CH_2NH_2), 1.48 (q, 4H, J=6.6 Hz, $NHCH_2CH_2$), 1.39 (s, 6H, $(CH_3)_2C$). ^{13}C 75 MHz NMR (DMSO-d_6, TMS): 171.5, 111.5, 77.6, 39.0, 36.4, 32.6, 26.2. IR (neat, cm^{-1}): 3356.8, 2940.3, 1660.7, 1537.6, 1084.6. TOF-PDMS: [M+H] exp. 303.38 found 302.3. IPT-succinyl-amino PEGA resin **10**: 0.4 mmol of amino PEGA resin (0.4 mmol/g, Novabiochem) were swelled in the minimal volume of DMF (9.6 mL). Succinic anhydride was coupled twice (30 min and 1 h) by adding 400.3 mg (4.0 mmol) of succinic anhydride, 697 µL (4.0 mmol) of DIEA and 2 mL of DMF to the beads. The resin was washed with DMF (2×2 min), CH_2Cl_2 (2×2 min) and NMP (2×2 min). 1209 mg (4.0 mmol) of **8** dissolved in 1 mL of NMP were added to the succinyl-amino PEGA resin swelled in the minimal volume of NMP. 265.4 mg (0.6 mmol) of BOP reagent was then added in one portion and the resin was shaken for 1 h. The resin was washed with DMF (4×2 min), CH_2Cl_2 (2×2 min) and Et_2O (2×2 min), and dried in vacuo. Periodic oxidation: The periodic oxidations were performed by swelling the dry peptidyl-resins (0.1 mmol) with 5 mL of H_2O/acetic acid, 2/1 by v. for 15 min. 128.3 mg (6 equiv.) of sodium periodate dissolved in 2 mL of H_2O/acetic acid, 5/1 were added in one portion and the suspension was stirred for 2 min. The resins were filtered and washed twice with 6 mL (1 min) of water. The combined solutions were immediately added to 200 µL of ethyleneglycol and injected on a C18 RP-HPLC Hyperprep (15×300 mm) column.
10. Peptide **7** was synthesized using standard Fmoc/*t*-Bu chemistry. The deprotection of the peptidyl-resin was performed using TFA/H_2O/TIS, 95/2.5/2.5 by v. for 3 h at room temperature. The resin was washed with CH_2Cl_2 (4×2 min) and diethylether (2×2 min), and then dried under reduced pressure. The resin was then treated as described above. To the pure fractions of peptide **7**, 500 mg of (+)-D-mannitol (Sigma) were added before the lyophilization step to avoid the aggregation of the peptide. The content of peptide **7** (3.3%) was determined by quantitative amino acid analysis using ninhydrin detection following total acid hydrolysis with 6N HCl/phenol, 10/1 at 110°C over 24 h (6.1% yield). ES-MS $[M+H]^+$ calcd 2136.6 and 2154.6 for the hydrate, found 2135.0 and 2155.0.