Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry Letters 15 (2005) 5442–5445
Bioorganic & Medicinal Chemistry Letters

Efficient synthesis of C-terminal modified peptide ketones for chemical ligations

Philippe Marceau, Corinne Buré and Agnès F. Delmas*

Centre de biophysique moléculaire, UPR 4301 CNRS †, rue Charles Sadron, 45071 Orléans, Cedex 2, France

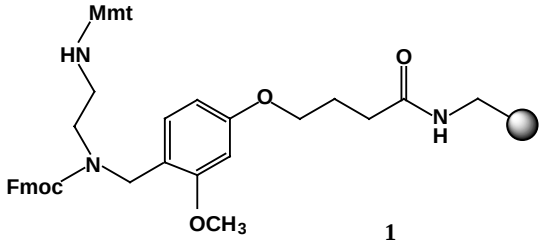
Received 21 July 2005; revised 25 August 2005; accepted 30 August 2005
Available online 30 September 2005

Abstract—Synthesis of a C-terminal modified peptide with an α-amido methylketone was efficiently carried out using a backbone-amide-type linker loading with a monofunctionalized diamine, provided that no base such as piperidine or diisopropylethylamine or a reducing agent such as triisopopylsilane was used for the synthetic pathway. The ketoxime-forming chemoselective ligation between a methylketone and an aminooxy was quantitative in 5 h at pH 2.

Aldehyde- and ketone-containing peptides are important components as inhibitors towards serine and cysteine proteases[1] and as key intermediates for protein engineering.[2] In the field of protein engineering, the use of the aldehyde or ketone group for site-specific and backbone modifications of peptides and proteins has been steadily growing ever since the appearance of chemoselective ligations in peptide chemistry, that is, the condensation of unprotected peptides in aqueous medium. Aldehyde- and ketone-containing peptides are the electrophilic partners for oxime, hydrazone and thiazolidine bond formations.[3]

The derivatization of peptides by aldehyde or keto groups has recently been reviewed.[4] An aldehyde group is generally generated by periodate oxidation of a 2-amino alcohol[5] (i.e., Ser or Thr) incorporated at the N-terminal or internal position in a peptidic sequence[3a,6] or by periodate oxidation of a diol installed at either the C-terminus[7] or an internal position thanks to a modified amino acid.[8] Alternatively, an aldehyde can also be introduced masked as an acetal at the C-terminus[9] and used for aldoxime formation. However, the inherent reactivity of aldehydes, pronounced in the acidic medium needed to remove the acetal, could be deleterious in the presence of oxime bond inducing transoximation[10] as well as the oxidation step used to unmask the aldehyde from the diol and 2-amino alcohol in a Met-containing peptide.[5] An alternative would be to use a ketone group such as levulinic acid[11] or keto-containing amino acid.[12] However, incomplete oximation reactions were noticed.[11,13] Best results were obtained when using an α-amido methylketone as generated after coupling pyruvic acid to an amine at the N-terminal or an internal position, respectively.[14] To increase the diversity in introducing the α-amido methylketone group in a peptide sequence for an oxime reaction, we describe here an efficient synthesis of a C-terminal modified peptide by pyruvic acid.

Solid-phase methods for the synthesis of peptides bearing different moieties at the C-terminus are limited due to the fact that the C-terminal residue is generally attached to the resin through its α-carboxyl group.[15] To introduce pyruvic acid at the C-terminus of a peptide sequence, we took advantage of a recent commercially available resin, that is, the NovaTag™ resin **1** (Chart 1) which was designed for bis-labelled peptides at the



1

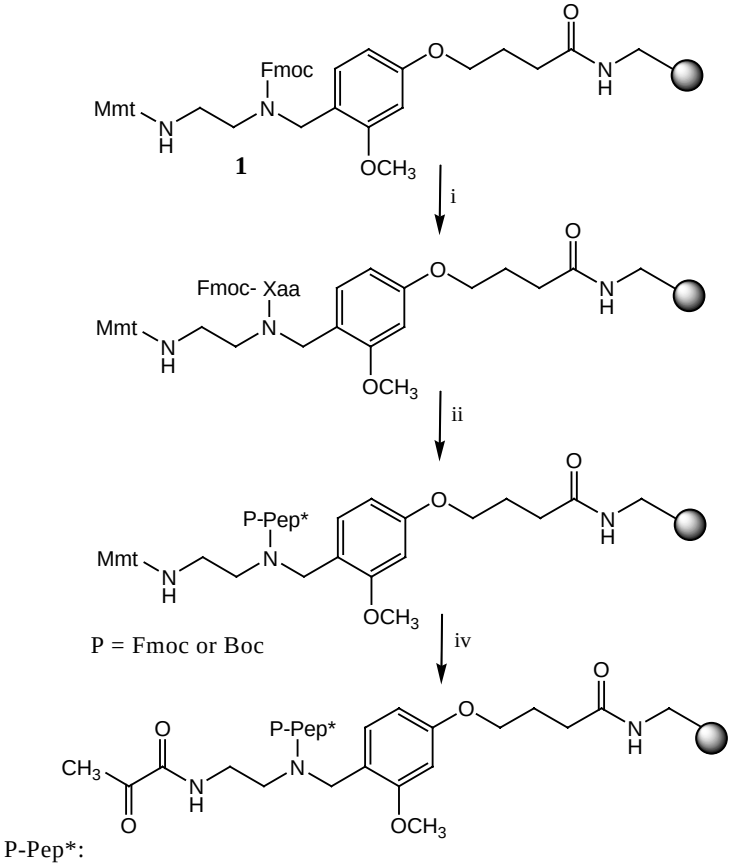
Chart 1. NovaTag™ resin.

Keywords: Oxime ligation; Ketone; C-terminal modified peptide.
* Corresponding author. Tel.: +33 2 38 25 55 77; fax: +33 2 38 63 15 17; e-mail: delmas@cnrs-orleans.fr
† Affiliated with the University of Orléans and INSERM.

0960-894X/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2005.08.105

N- and C-terminal sides for FRET experiments.[16] The linker of the NovaTag™ resin is based on the 4-(3-formyl-2-methoxyphenoxy)-butanoic acid (FMPB) linker, a backbone-amide-type linker, substituted by reductive amination with mono-protected ethylenediamine.

The optimization of the keto-peptide synthesis on NovaTag™ resin **1** (Scheme 1) was first developed with the model peptide **4** (Table 1). Solid-phase elongation was carried out as already described[16] with, first, the deprotection of Fmoc by 20% piperidine in DMF followed by the coupling of Fmoc-Gly-OH using HBTU[17]/HOBt/DIEA as coupling reagent.[18] The completion of the acylation was evaluated using the chloranil test to check for free secondary amines.[19] Even with a double coupling, the yield was only 50%, whereas it was quantitative with only one coupling when HATU[20] was used. After completion of the elongation following the Fmoc/*t*Bu strategy, the very acid-labile Mmt (methoxytrityl) group was removed with 1 M HOBt in trifluoroethanol/CH_2Cl_2. As a first approach, pyruvic acid was introduced using HBTU/HOBt/DIEA as coupling reagent to afford peptidyl-resin **2** (Scheme 1). The reaction was checked with the ninhydrin test.[21] Peptidyl-resin **2** was treated with the reagent B (TFA, H_2O, phenol and TIS, 88/5/5/2)[22] to release peptide **4**. Analyses of crude keto-peptide **4** by analytical HPLC and mass spectrometry revealed a major product with a Δm of +2 Da attributed to the reduction of the keto group by TIS (see Supporting information). This was confirmed by treatment of the peptidyl-resin **2** with a TFA cleavage cocktail without TIS (TFA, H_2O and phenol, 90/5/5). However, the crude keto-peptide **4** corresponding to only 73% purity was accompanied by a by-product attributed to the dimer. According to the introduction of pyruvic acid in the presence of DIEA and to the susceptibility of the keto group to a Claisen reaction under basic conditions, pyruvic acid was introduced further using DCC/NHS as coupling reagent to give peptidyl-resin **2**. Under these conditions, the purity of the target keto-peptide **1** was increased to 83% although a dimer was still present, likely due to the piperidine treatment for N^{α}-Fmoc removal. According to this hypothesis and to the best conditions deciphered with the synthesis of keto-peptide **4**, keto-peptide **5** (Table 1) was assembled ending with N^{α}-Boc protection. Peptidyl-resin **3** (Scheme 1) was then treated with TFA, H_2O and phenol (90/5/5) to release peptide **5** in 95% purity. After purification by semi-preparative HPLC, keto-peptide **5** was obtained with an overall yield of 50%.



Scheme 1. Solid-phase peptide synthesis of the C-terminal α-amido methylketone modified peptide using the NovaTag™ resin. (i) a, 20% piperidine in DMF; b, Fmoc-Thr(*t*Bu)-OH, HATU, DIEA. (ii) Fmoc/*t*Bu elongation. (iii) 1 M HOBt in TFE/DCM. (iv) CH_3–CO–COOH, coupling reagent (see text).

Table 1. Sequences used in this study

Pyruvic acid-containing peptide	
4	H-Tyr-Lys-Ala-Gly-Leu-Gly-NH-$(CH_2)_2$-NH-CO-CO-CH_3
5	H-Pro-Pro-Ala-His-Gly-Val-Thr-Ser-Ala-Pro-Asp-Thr-Arg-Pro-Ala-Pro-Gly-Ser-Thr-NH-$(CH_2)_2$-NH-CO-CO-CH_3
Aoa-containing peptide	
6	H_2N-O-CH_2-CO-Ala-Leu-Lys-Trp-Ser-Leu-Ala-OH
7	H_2N-O-CH_2-CO-ala-Lys-Cha-Val-Ala-ala-Trp-Asn-Leu-Lys-Ala-ala-NH_2

ala, D-Ala; Cha, cyclohexylalanine.

Condensation via an aldehyde–nucleophile coupling has been shown to be pH dependent.[23,24] The oximation reaction with an α-amido aldehyde as the electrophile proceeds faster at a lower pH and very slowly with a pH close to neutral.[3b,25] Accordingly, the kinetics of the oximation with α-amido methylketone **4** and amino-oxy-containing peptide **6** was followed during 37 h by micro-HPLC at pH 2 and 4.6.[26] The keto-peptide was 1.5 times in excess over the Aoa-peptide. Every 40 min, the aliquots were analyzed by HPLC. Figure 1 shows the percentage of the ligated peptide as a function of time. Over time (20 h), the reaction performed at pH 2 came close to completion, whereas the oxime formation realized at pH 4.6 only reached about 50% after 20 h. Based on these data, keto peptide **5** was condensed to aminoxy-containing peptide **7** at pH 2. The reaction was quantitative after 5 h as judged by analytical HPLC and mass spectrometry with the total disappearance of

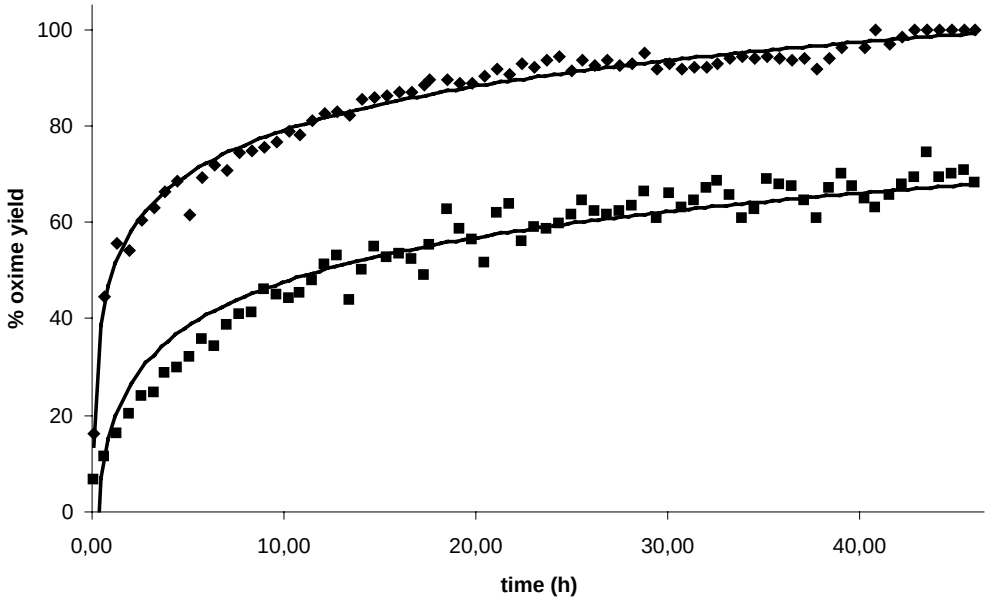


Figure 1. Kinetic of the oximation reaction between keto-peptide **5** and Aoa-peptide **6** at pH 4.6 (■) and at pH 1 (♦).

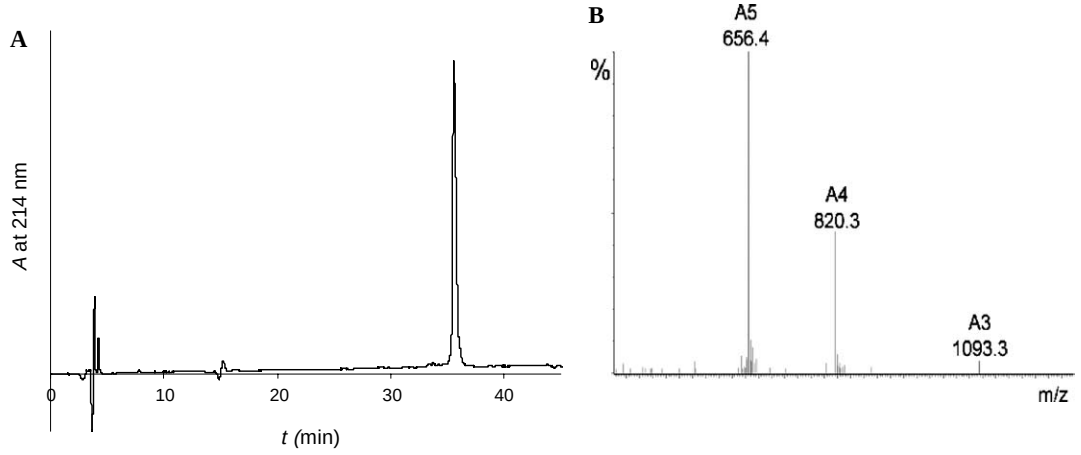


Figure 2. (A) Analytical HPLC chromatogram of a ligated product obtained from keto-peptide **5** and Aoa-peptide **7.** (B) Electrospray mass spectrum of the ligated product.

Aoa-peptide **7**. The ligated product was purified by HPLC (Fig. 2) and obtained with an overall yield of 45% based on Aoa-peptide **7** which was in limited quantity. This low yield is likely due to non-specific adsorption on semi-preparative column of the ligated peptide which contains the hydrophobic peptide **7** as already noticed with amphipathic/hydrophobic peptide.[10d,27]

In summary, a C-terminal modified peptide with an α-amido methylketone was obtained in a very good yield using NovaTag™ as the resin. Elongation was performed using Fmoc/*t*Bu strategy ending with N^{α}-Boc protection. Pyruvic acid was coupled at the C-terminus using a coupling reagent which did not necessitate a base as DCC/NHS and the peptide was released with a TFA cleavage cocktail without TIS. The keto-peptide was efficiently ligated to an aminoxy-containing peptide in 5 h at pH 2. These data extend the diversity in introducing aldehyde and ketone groups onto peptides as well as the great versatility of the oxime ligation.

Supplementary data

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.bmcl.2005.08.105.

References and notes

1. Thompson, R. C. *Methods Enzymol.* **1977**, *46*, 220.
2. (a) Walker, M. A. *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 1069; (b) Hang, H. C.; Bertozzi, C. R. *Acc. Chem. Res.* **2001**, *34*, 727.
3. (a) Rose, K. *J. Am. Chem. Soc.* **1994**, *116*, 30; (b) Shao, J.; Tam, J. P. *J. Am. Chem. Soc.* **1995**, *117*, 3893.
4. Melnyk, O.; Fehrentz, J. A.; Martinez, J.; Gras-Masse, H. *Biopolymers* **2000**, *55*, 165.
5. Geoghegan, K. F.; Stroh, J. G. *Bioconjugate Chem.* **1992**, *1*, 114.
6. Spetzler, J. C.; Hoeg-Jensen, T. *Tetrahedron Lett.* **2002**, *43*, 2303.
7. (a) Zhang, L.; Torgerson, T. R.; Liu, X. Y.; Timmons, S.; Colosia, A. D.; Hawiger, J.; Tam, J. P. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 9184; (b) Melnyk, O.; Fruchart, J. S.; Grandjean, C.; Gras-Masse, H. *J. Org. Chem.* **2001**, *66*, 4153; (c) Xu, Q.; Lam, K. S. *Tetrahedron Lett.* **2002**, *43*, 4435.
8. Spetzler, J. C.; Hoeg-Jensen, T. *J. Peptide Sci.* **2001**, *7*, 537.
9. (a) Jensen, K. J.; Alsina, J.; Songster, M. F.; Vagner, J.; Albericio, F.; Barany, G. *J. Am. Chem. Soc.* **1998**, *120*, 5441; (b) Lelièvre, D.; Chabane, H.; Delmas, A. *Tetrahedron Lett.* **1998**, *39*, 9675; (c) Guillaumie, F.; Kappel, J. C.; Kelly, N. M.; Barany, G.; Jensen, K. J. *Tetrahedron Lett.* **2000**, *41*, 6131.
10. (a) Depuy, C. H.; Ponder, B. W. *J. Am. Chem. Soc.* **1959**, *81*, 4629; (b) Hershbeg, E. B. *J. Org. Chem.* **1948**, *13*, 542; (c) Lelièvre, D.; Buré, C.; Laot, F.; Delmas, A. *Tetrahedron Lett.* **2001**, *42*, 235; (d) Cremer, G. A.; Bureaud, N.; Lelièvre, D.; Piller, V.; Piller, F.; Delmas, A. *Chem. Eur. J.* **2004**, *10*, 6353.
11. Canne, L. E.; Ferré d'Amaré, A. R.; Burley, S. K.; Kent, S. B. H. *J. Am. Chem. Soc.* **1995**, *115*, 2998.
12. Marcaurelle, L. A.; Bertozzi, C. R. *Tetrahedron Lett.* **1998**, *39*, 7279.
13. (a) Mitchell, J. P.; Roberts, K. D.; Langley, J.; Koentgen, F.; Lambert, J. N. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 2785; (b) Renaudet, O.; Dumy, P. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 3619.
14. Kochendoerfer, G. G.; Tack, J. M.; Cressman, S. *Bioconjugate Chem.* **2002**, *13*, 474.
15. Alsina, J.; Albericio, F. *Biopolymers* **2003**, *71*, 454.
16. Beythien, J.; White, P. D. *Tetrahedron Lett.* **2005**, *46*, 101.
17. *Abbreviations:* DCC, dicyclohexylcarbodiimide; DIEA, diisopropylethyl amine; HATU, *O*-(benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate; HBTU, 2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate; HOBt, 1-hydroxybenzotriazole; Mmt, 4-methoxytrityl; NHS, *N*-hydroxysuccinimide; TFA, trifluoroacetic acid; TFE, trifluoroethanol; TIS, triisopropylsilane.
18. Dourtoglou, V.; Gross, B.; Lambropoulou, V.; Zioudrou, C. *Synthesis* **1984**, 572.
19. Vojkovsky, T. *Pept. Res.* **1995**, *8*, 236.
20. Carpino, L. A. *J. Am. Chem. Soc.* **1993**, *115*, 4397.
21. Kaiser, E.; Colescott, R. L.; Bossinger, C. D.; Cook, P. I. *Anal. Biochem.* **1970**, *34*, 595.
22. Solé, N. A.; Barany, G. *J. Org. Chem.* **1992**, *57*, 5399.
23. Jencks, W. P. *J. Am. Chem. Soc.* **1959**, *81*, 475.
24. (a) Sayer, J. M.; Pinsky, B.; Schonbrunn, A.; Washtien, W. *J. Am. Chem. Soc.* **1974**, *96*, 7998; (b) Chenevier, P.; Bourel-Bonnet, L.; Roux, D. *J. Am. Chem. Soc.* **2003**, *125*, 16261.
25. Rose, K.; Zeng, W.; Regamey, P. O; Chernushevich, I. V; Standing, K. G; Gaertner, H. F *Bioconjugate Chem.* **1996**, *7*, 552.
26. The oxime formation was followed by micro-HPLC using a CAP-LC system (Waters), a Symmetry column (C18 3.5 μm, 00.32×50 mm) and a flow rate of 5 μl/min. The detection was followed by a diode array from 200 to 350 nm. Samples were maintained at 25 °C. Peptide concentrations were determined at 220 nm for **5** with a ε of 13970 and at 280 nm for **6** with a ε of 5600 (Trp). Final concentration of keto-peptide was 1.2 mM. For pH 4.6, 0.1 M acetate buffer was used and for pH 2, H_2O containing 0.1% TFA was used.
27. Lelièvre, D.; Turpin, O.; El Kassouli, S.; Delmas, A. *Tetrahedron* **2002**, *58*, 5525.