Available online at www.sciencedirect.com
SCIENCE DIRECT®
Tetrahedron Letters 45 (2004) 8603–8606
Tetrahedron Letters

Efficient and direct solid phase synthesis of ketomethylenimino and ketomethylenamino peptides

Elise Bernard and Régis Vanderesse*

Laboratoire de Chimie Physique Macromoléculaire, UMR CNRS-INPL 7568, Groupe ENSIC, 1 rue Grandville, BP 451, 54001 NANCY Cedex, France

Received 14 September 2004; revised 22 September 2004; accepted 28 September 2004

Abstract—The aim of this study is the synthesis of pseudopeptides on solid supports, in order to quickly obtain modified peptides. We report a convenient step-by-step synthesis of ketomethylenimino ψ[CO–CH=N] and ketomethylenamino ψ[CO–CH_2–NH] peptides. The key is the reaction between the free amino terminus of the supported peptide and a glyoxal-modified amino acid, leading to a ketomethylenimino bond, which can be reduced to a ketomethylenamino bond.

Several diseases involve a protease imbalance and it is of great importance to find new protease inhibitors. Human Leucocyte Elastase (HLE) is a serine protease that hydrolyzes a wide variety of proteins, including collagen and elastin. One of its natural inhibitors is α1-proteinase inhibitor (α1-PI) and its imbalance with HLE may induce various chronic inflammatory diseases such as acute respiratory distress syndrome (ARDS) or pulmonary emphysema.[1,2] This explains the increasing need of efficient inhibitors, resistant to hydrolysis caused by the enzyme. A possible class of enzyme inhibitors is the pseudopeptides. They can be very interesting tools for scientific and mechanistic investigation and have been widely used for this purpose for a long time. Our group has extensively studied, on model pseudopeptides, the structural influence of different amide bond surrogates[3] and of the nitrogen substitution for the $C^{\alpha}H$ group.[4] These peptide surrogates present the advantage of allowing structural modulation of the peptide backbone with retention of the side chains generally required for biological activity. Furthermore, it is possible to obtain (i) a control of the structural flexibility, (ii) an increased selectivity for the binding site, (iii) increased resistance to hydrolysis, and (iv) improved permeability through various biological barriers.

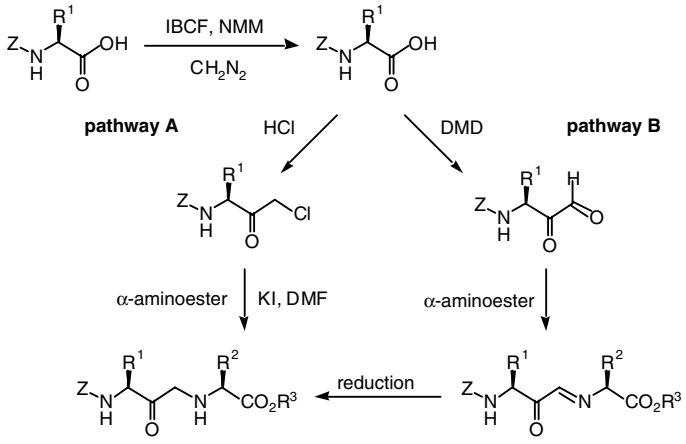
The replacement of a natural peptide bond with a ketomethylenamino bond ψ[CO–CH_2–NH] is of great interest due to its increased flexibility, and therefore a potentially better adaptability to the binding site of an enzyme, and to a putative better resistance to hydrolysis. The ketomethylenamino link has been introduced by Meyer et al.[5] for the design of ACE inhibitors. It has later been used as such or in various *N*-alkylated or reduced forms to give ACE,[6] furin,[7] and HIV[8] protease inhibitors.

Herein, we present the incorporation of ketomethylenimino and ketomethylenamino moieties in a hexapeptide, derived from the target sequence of α1-PI, H-Ala^1-Ala^2-Pro^3-Val^4-Ala^5-Ala^6-OH.[7] HLE cleaves this peptide at the Val^4-Ala^5 position.[9] Therefore, we developed a concise and general method for the direct incorporation of ketomethylenimino and ketomethylenamino moieties into Wang-resin-supported peptides.

Until now[8,10] ketomethylenamino peptides have been synthesized in homogeneous phase via the incorporation of the modified peptide building blocks that require laborious purification steps with concomitant racemization at the stereogenic center. One method (Pathway A, Scheme 1) involves the formation of a chloromethylketone.[8] A second method uses the formation of the glyoxal of an *N*-protected amino acid obtained via the oxidation of its corresponding diazo derivative by dimethyldioxirane (DMD) (Pathway B, Scheme 1). The imino bond formed is reduced by $SiCl_3H$.[10]

Keywords: Solid phase peptide synthesis; Pseudopeptide; Glyoxal; Ketomethylenimino; Ketomethylenamino.
* Corresponding author. Tel.: +33 3 83 17 52 04; fax: +33 3 83 37 99 77; e-mail: regis.vanderesse@ensic.inpl-nancy.fr

0040-4039/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetlet.2004.09.164



Scheme 1. General synthesis of a ketomethylenamino dipeptide.

These two methods are aimed at the preparation of *N*-Z-protected pseudodipeptides, which are incompatible with direct solid phase synthesis (SPPS). These approaches entail: (i) the preparation and purification of the Z-pseudodipeptide ester; (ii) the substitution of the Z group for a Boc or an Fmoc group; (iii) the saponification of the C-terminus ester, and (iv) the coupling of the building block on the growing chain. To minimize the number of the steps, we developed a direct method in only one step via SPPS. An Fmoc aminoglyoxal is coupled to the free N-terminus of a peptide linked to the resin to form the ketomethylenimino bond, which can be reduced, in situ, to the ketomethylenamino bond. The ketomethylenimino link has been introduced at the Val^4-Ala^5 position and the ketomethylenamino at the Ala^2-Pro^3, Pro^3-Val^4, and Val^4-Ala^5 positions.

Initially, we synthesized the Fmoc aminoglyoxals Fmoc-Ala-CHO, Fmoc-Pro-CHO, and Fmoc-Val-CHO by the Groarke[10] method with 90% overall yields (Scheme 2).

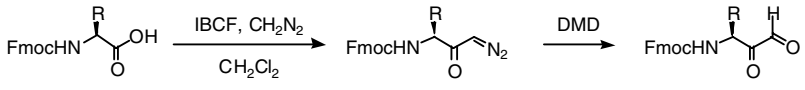
The methylene group of the pseudopeptidic link ψ[CO–CH_2–NH] was then included via homologization on the C-terminus part by action of diazomethane[12] on the mixed anhydride of the *N*-Fmoc protected original amino acid. The diazo compounds were synthesized in good to excellent yields (Table 1). Purification was achieved via silica gel column flash chromatography. The diazo compounds were oxidized into the corresponding glyoxal immediately prior to use. The oxidizing agent DMD is prepared via the action of Oxone® on acetone in the presence of $NaHCO_3$.[13] The DMD solution quantitatively oxidizes the diazo compound in 10 min at 0 °C. After removal of the solvent, the glyoxal must be used without further purification, due to its low stability.

These Fmoc aminoglyoxals are then directly reacted with the growing peptide linked to the Wang-resin, leading first to the ketomethylenimino link. This short step synthesis of ketomethylenimino derivatives is advantageous in view of the fragility of this class of compounds. To form the ketomethylenamino bond, a few drops of acetic acid are added prior to 3 equiv of $NaBH_3CN$, added portionwise for 1 h, in order to reduce the imino bond. After stirring overnight, a Kaiser test clearly indicates the complete coupling. The following peptide couplings, as well as the final cleavage of the pseudopeptide from the resin, are achieved according to the methods known in the literature (Scheme 3).[14] The overall yield of the final crude pseudopeptides ranges from 59% to 90%. They were characterized by nuclear magnetic resonance (^{13}C, ^1H, COSY, TOCSY NMR) and mass spectroscopy. COSY and TOCSY NMR experiments confirmed the inclusion of either the ketomethylenimino link ($\delta_H = 7.99$ for CO–CH=N) or the ketomethylenamino link ($\delta_H = 3.68$ for CO–CH_2–NH). Purity of hexapseudopeptides tested is >99% after HPLC.

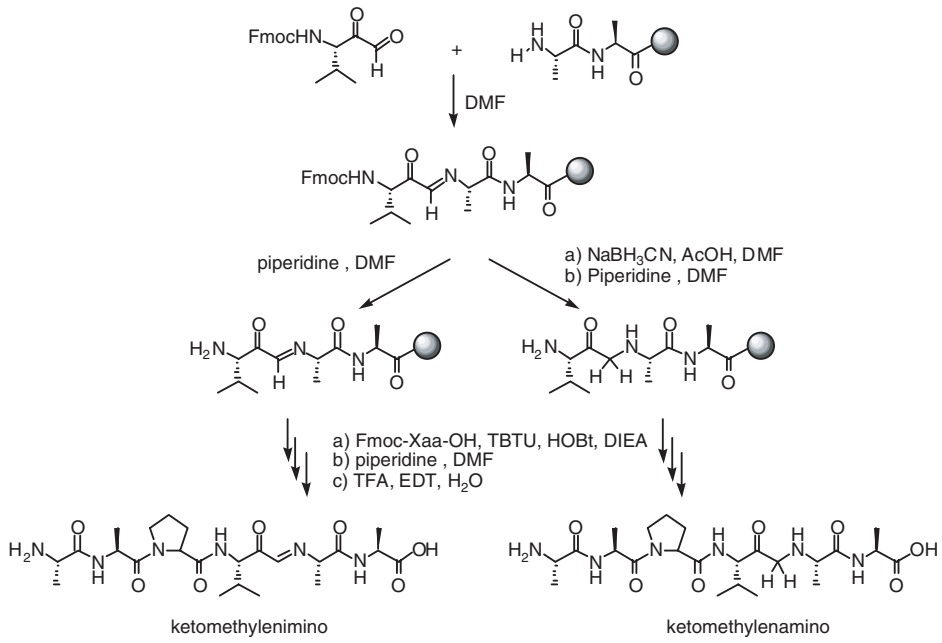
Table 1. Yields for the preparation of the modified amino acids

Fmoc-amino acid	Diazo (%)	Glyoxal (%)
Fmoc-Ala-OH	92	>99
Fmoc-Pro-OH	93	>99
Fmoc-Val-OH	98	>99

In conclusion, we propose an efficient method for the synthesis of ketomethylenimino ψ[CO–CH=N] and ketomethylenamino ψ[CO–CH_2–NH] peptides on a solid support. This method uses *N*-protected aminoglyoxals as reagents, readily obtained by a diazotation–oxidation pathway. In this way, we avoid the purification, transprotection, and nonfree racemization saponification steps needed for the pseudodipeptide building-block method previously described. The hexapseudopeptide H-Ala-Ala-Pro-Valψ[CO–CH_2–NH]-



Scheme 2. Synthesis of Fmoc-α-aminoglyoxals.[11]



Scheme 3. Solid phase synthesis of the ketomethylenimino and ketomethylenamino peptides (example given for the Val^4-Ala^5 position).

Ala-Ala-OH exhibits inhibition toward HLE (IC_{50}: 1.90×10^{-4} M) and is currently being studied by NMR in order to determine its structure when bound to its receptor.

Acknowledgements

Pr. Michèle Reboud-Ravaux (Institut Jacques Monod, University of Paris VI/VII) is gratefully acknowledged for the enzymatic tests on HLE. We would also like to thank Mathilde Achard and Olivier Fabre for their technical assistance and Dr. Matthew Wilkinson (University of Amsterdam) for the revision of the English text.

References and notes

1. Pochet, L.; Doucet, C.; Dive, G.; Wouters, J.; Masereel, B.; Reboud-Ravaux, M.; Pirotte, B. *Bioorg. Med. Chem.* **2000**, *8*, 1489–1501.
2. For more information about emphysema, see Snider, L. G. *Am. Rev. Respir. Dis.* **1992**, *146*, 1615–1622; Parfrey, H.; Mahadeva, R.; Lomas, D. A. *Int. J. Biochem. Cell Biol.* **2003**, *35*, 1009–1014.
3. (a) Vanderesse, R.; David, L.; Grand, V.; Marraud, M.; Mangeot, J.-P.; Aubry, A. *Tetrahedron Lett.* **1997**, *38*, 2669–2672; (b) Lecouvey, M.; Frochot, C.; Miclo, L.; Orlewski, P.; Marraud, M.; Gaillard, J. L.; Cung, M. T.; Vanderesse, R. *Lett. Pept. Sci.* **1997**, *4*, 359–364; (c) Vanderesse, R.; Grand, V.; Limal, D.; Vicherat, A.; Marraud, M.; Didierjean, C.; Aubry, A. *J. Am. Chem. Soc.* **1998**, *120*, 9444–9451.
4. André, F.; Boussard, G.; Marraud, M.; Didierjean, C.; Aubry, A. *Lett. Pept. Sci.* **1995**, *2*, 239–242.
5. Meyer, R. F.; Essenburg, A. D.; Smith, R. D.; Kaplan, H. R. *J. Med. Chem.* **1982**, *25*, 996–999.
6. (a) Natarajan, S.; Gordon, E. M.; Sabo, E. F.; Godfrey, J. D.; Weller, H. N.; Pluscec, J.; Rom, M. B.; Cushman, D. W. *Biochem. Biophys. Res. Commun.* **1984**, *124*, 141–147; (b) Gordon, E. M.; Natarajan, S.; Pluscec, J.; Weller, H. N.; Godfrey, J. D.; Rom, M. B.; Sabo, E. F.; Engebrecht, J.; Cushman, D. W. *Biochem. Biophys. Res. Commun.* **1984**, *124*, 148–155; (c) Gordon, E. M.; Godfrey, J. D.; Pluscec, J.; Von Langen, D.; Natarajan, S. *Biochem. Biophys. Res. Commun.* **1985**, *126*, 419–426; (d) Natarajan, S.; Gordon, E. M.; Sabo, E. F.; Godfrey, J. D.; Weller, H. N.; Pluscec, J.; Rom, M. B.; Engebrecht, J.; Cushman, D. W.; Deforrest, J. M.; Powell, J. *J. Enzyme Inhib.* **1988**, *2*, 91–97.
7. Angliker, H. *J. Med. Chem.* **1995**, *38*, 4014–4018.
8. (a) Rich, D. H.; Green, J.; Toth, M. V.; Marshall, G. R.; Kent, S. B. H. *J. Med. Chem.* **1990**, *33*, 1285–1288; (b) Rich, D. H.; Vara Prasad, J. V. N.; Sun, C.-Q.; Green, J.; Mueller, R.; Houseman, K.; MacKenzie, D.; Malkovsky, M. *J. Med. Chem.* **1992**, *35*, 3803–3812.
9. Guy, L.; Vidal, J.; Collet, A.; Amour, A.; Reboud-Ravaux, M. *J. Med. Chem.* **1998**, *41*, 4833–4843.
10. Groarke, M.; Hartzoulakis, B.; McKervey, M. A.; Walker, B.; Williams, C. H. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 153–155.
11. Typical procedure Fmoc-Val-OH (1.63 g, 4.79 mmol) was dissolved in anhydrous THF (10 mL) under nitrogen at −25 °C. *N*-Methylmorpholine (0.55 mL, 5 mmol) and isobutylchloroformate were then added to the mixture and after 5 min of stirring, the mixture was cooled down to −78 °C. Anhydrous ether (10 mL) was added and the fine suspension was filtered under N_2. Diazomethane (294 mg, 7 mmol) was added dropwise and the reaction was allowed to stir for 2 h at room temperature. After evaporation of the solvent, the residue was dissolved in CH_2Cl_2, and washed with water (3×10 mL) before drying over $MgSO_4$ and concentration. Fmoc-Val-CHN_2 were purified via flash chromatography using EtOAc/hexane (1/1, v/v). (98%) ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 0{,}90$ (d, $J = 6.7$ Hz, 3H); 0.98 (d, $J = 6.7$ Hz, 3H); 2.10 (qd, $J = 6.7$ Hz, 1H); 4.12 (m, 1H); 4.22 (t, $J = 6.6$ Hz, 1H); 4.44 (d, $J = 6.6$ Hz, 2H); 5.30 (s, 1H); 5.36 (d, $J = 8.1$ Hz, 1H); 7.30 (m, 2H); 7.40 (dd, $J = 7.3$ Hz, 2H); 7.71 (m, 2H); 7.87 (d, $J = 7.3$ Hz, 2H). ^{13}C NMR ($CDCl_3$): $\delta = 18.09$ ($CH_3{}^{\gamma}$); 20.07 ($CH_3{}^{\gamma}$); 31.77 (CH^{β}); 47.96 (CH Fmoc);

63.47 ($CH{=}N_2$, CH^{α}); 67.53 (CH_2 Fmoc); 120.66, 125.73, 127.77, 128.39, 142.03, 144.47 (C arom); 156.99 (C urethane); 193.88 (C=O). Diazo compound Fmoc-Val-CHN_2 (544 mg, 1.5 mol) was dissolved in the solution of DMD (50 mL, 4.5 mmol). After 10 min of stirring, the solvent was evaporated and the residue taken up in 15 mL of CH_2Cl_2 in order to remove the traces of water. The solvent was removed in vacuo and the glyoxals obtained in quantitative yields. The products are relatively unstable and should be used without delay.

12. Fales, H. M.; Jaouni, T. M.; Babashak, J. F. *Anal. Chem.* **1973**, *42*, 2302–2303.
13. Adam, W.; Bialas, J.; Hadjiarapoglou, L. *Chem. Ber.* **1991**, *124*, 2377.
14. Typical procedure To a swollen Wang-resin in DMF, the first amino acid (3 equiv) was added with TBTU (3 equiv), HOBt (3 equiv) and DIEA (9 equiv). The reaction mixture was stirred for 20 or 40 min. The resin was washed with CH_2Cl_2 (3×5 mL), MeOH (3×5 mL), and DMF (3×5 mL). A Kaiser test indicated a complete coupling. After deprotection of Fmoc group with 20% piperidine in DMF (5 mL) for 2, 5, and 8 min, the next amino acids were introduced as previously described. Then, glyoxal (3 equiv) dissolved in DMF (5 mL) was added to the peptide linked to the resin. It is then possible to obtain a ketomethylenimino or a ketomethylenamino bond. The simple condensation of the glyoxal gives rise to the ketomethylenimino bond whereas the addition of $NaBH_3CN$ (3 equiv) portionwise over 1 h as reducing agent with a few drops of acetic acid leads to the ketomethylenamino bond. The reaction mixtures were stirred overnight. The following amino acids are classically introduced. Finally, the last Fmoc group was deprotected with 20% piperidine in DMF and the resin was washed several times with CH_2Cl_2 before being dried. For the cleavage step, the peptide–resin was stirred over 2 h in a solution of ethanedithiol (250 μL) and water (500 μL) in TFA (10 mL). The peptide was then precipitated by addition of cool ether. After removal of the solvent and lyophilization of the crude material, the pseudohexapeptide was purified by HPLC with a linear gradient of A: 0.1% TFA in water and B: 0.08% TFA and 20% water in acetonitrile, from 80% A to 50% A over 30 min. H-Ala-Ala-Proψ[CO–CH=N]-Val-Ala-Ala-OH Mass: *m/z* [ES] calcd 510.28, found 511.39 for $[M + H]^+$; H-Ala-Alaψ[CO-CH_2-N]-Pro-Val-Ala-Ala-OH Mass: *m/z* [ES] calcd 512.30, found 513.44 for $[M + H]^+$ H-Ala-Ala-Proψ[CO–CH_2–NH]-Val-Ala-Ala-OH Mass: *m/z* [ES] calcd 512.30, found 513.51 for $[M + H]^+$ H-Ala-Ala-Pro-Valψ[CO–CH_2–NH]-Ala-Ala-OH Mass: *m/z* [ES] calcd 512.30, found 513.47 for $[M + H]^+$.