Pergamon

Tetrahedron Letters, Vol. 36, No. 43, pp. 7871-7874, 1995
Elsevier Science Ltd
Printed in Great Britain
0040-4039/95 $9.50+0.00

0040-4039(95)01646-5

Improved solid phase synthesis of C-terminal peptide aldehydes

Jean-Alain Fehrentz[1], Marielle Paris[1], Annie Heitz[2], Jiri Velek[1], Chuan-Fa Liu[3], François Winternitz[1] & Jean Martinez[1]*

[1]Laboratoire de Chimie et Pharmacologie de Molécules d'Intérêt Biologique, Associé au CNRS, Faculté de Pharmacie, 15 av. C. Flahault, 34060 Montpellier.[2]Unité Mixte CNRS, Faculté de Pharmacie, Montpellier, France.
[3]Present address : Department of Microbiology and Immunology, Vanderbilt University, Nashville, Tennessee, USA.

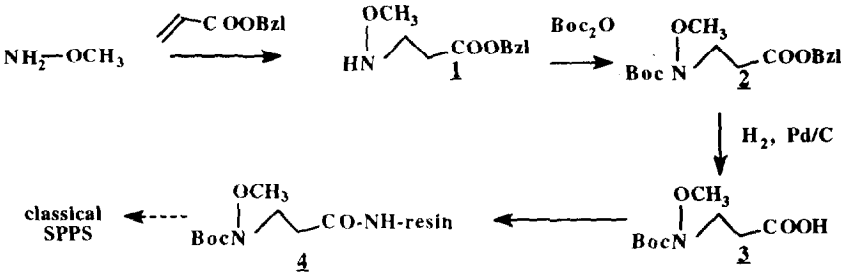
Abstract : A new linker based on the Weinreb amide was developed in order to synthesize from peptidyl-resin the corresponding aldehydic peptides by reduction with LiAlH This new reaction was tested with N-protected amino-residues and with tripeptides to obtain the corresponding aldehydes without racemisation.

Peptide C-terminal aldehydes (PAs) are of great interest due to their inhibitory properties as transition-state analogs towards numerous classes of proteolytic enzymes. Indeed, since the discovery of leupeptin[1], a natural product which is a potent inhibitor of trypsin, many other classes of enzymes have been inhibited by PAs, such as aspartyl proteases (HIV protease[2], renin[3]), seryl and thiol proteases[4,5], prohormone convertases[6], cysteyl proteases[7,8]. Further, PAs can be used in pseudo-peptide chemistry in order to modify either the C-terminus part of the peptide or the carbonyl moiety (i.e. reduced bond).

Various methods for the solution synthesis of PAs have been described[4,5,9-13] but only one report concerns solid phase synthesis[14]. This rather complicated procedure has not yet been widely applied.

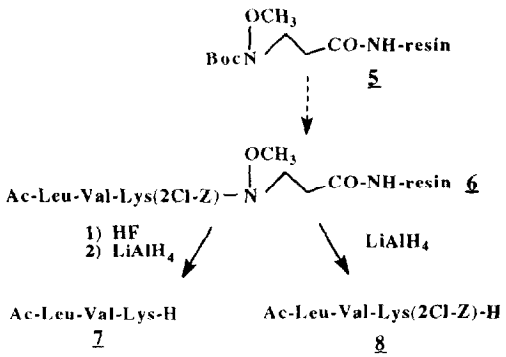
On the basis of the Weinreb amide[15,16], we have investigated a simple alternative route for the preparation of PAs and side-chains protected PAs in solid phase synthesis. We have synthesized a new linker which is stable under classic Fmoc or Boc solid phase strategies and during liquid HF cleavage but which reacted with $LiAlH_4$ to yield after hydrolysis, the desired corresponding aldehyde.

The synthesis of the linker was performed as shown in the scheme 1[17]. Methoxy-amine was reacted with benzyl acrylate and the resulting alkylated methoxy-amine **1** was protected by the tert-butyloxycarbonyl group. After deprotection of compound **2** by hydrogenolysis, the linker **3** was coupled to the solid support (i.e. MBHA resin) with an activating agent to yield the substituted resin **4**. After deprotection of the N-terminal Boc, elongation by classical solid phase synthesis (Boc or Fmoc strategies) was possible.



Scheme 1. Synthesis of the functionalized solid support. NH-resin = β-Ala-MBHA

In order to validate this methodology, we synthesized several N-protected amino-aldehydes by this method. These compounds were purified according to the procedure described by Ho and Ngu[19] on silica gel column, with organic solvents in the presence of 0.1% pyridine as eluent to prevent racemisation during purification. As reported in Table 1, the optical purity of the α-amino aldehydes thus obtained was similar to that obtained by conventional solution synthesis[16]. We have also synthesized tripeptide aldehydes on the solid support as indicated in scheme 2.



Scheme 2. Synthesis of a tripeptide aldehyde. NH-resin = β-Ala-MBHA

As previously described[13], due to the presence of several amide functions, the amount of $LiAlH_4$ equivalents has to be increased with the length of the peptide. After hydrolysis and treatment, the resulting crude peptide aldehydes were purified either by flash chromatography on silica gel as described above or by RP HPLC[14,20]. The crude, silica gel- and RP-HPLC-purified compounds were studied by high field ^1H NMR to detect epimerisation. This can be easily measured by the integration of the aldehydic proton signal(s). As expected, the crude revealed the presence of a single aldehydic proton signal, indicating the absence of epimerisation; some impurities were present (traces). Surprisingly, both purified tripeptide aldehydes showed two aldehydic proton peaks corresponding to the two diastereomers, indicating some epimerisation during chromatography.

The results of these experiments are reported in Table 1 and shown in Figure 1. This procedure is quite interesting for the preparation of PAs. The synthesis of the linker is easy and all the methodology and advantages of solid phase peptide synthesis are available to synthesize peptide aldehydes on solid support. By using this methodology, it should be possible to prepare large peptide aldehydes, as well as protected peptide aldehydes that might be useful for the synthesis of large pseudopeptides with a reduced peptide bond. On the other hand, this linker might be useful for the synthesis of combinatorial librairies.

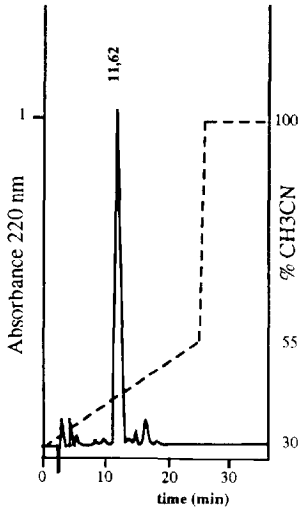


Figure 1. RP HPLC chromatogram of the crude tripeptide aldehyde Boc-Phe-Val-Ala-H.

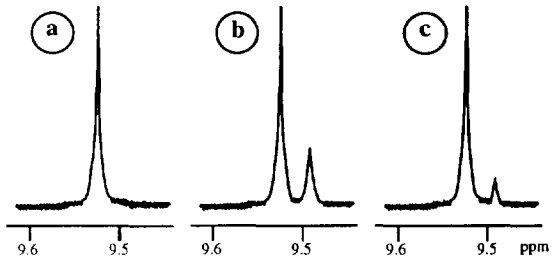


Figure 2. ^1H NMR aldehydic proton signal of crude (a), silica gel purified (b) and RP HPLC purified (c) tripeptide aldehyde Boc-Phe-Val-Ala-H. The double peak is due to some epimerisation.

Table 1. Physical characteristics of amino-acid and peptide aldehydes.

Aldehyde	[α]D (c=1,MeOH)/literature	Yield[d]
Boc-Ala-H	- 32 /- 34.1[a]	40
Boc-Phe-H	- 46 /- 44.4[a]	40
Boc-Phe-Val-Ala-H	- 21[b]	40
Boc-Leu-Leu-Lys(2Cl-Z)-H	- 50[c]	40
Ac-Leu-Val-Lys(2Cl-Z)-H	- 42[b]	25
Z-Val-Phe-H	-45[b]	30

a. Reference 16; b. After RP C18 HPLC purification; c. Purified by crystallization in diethyl ether; d. The yields are calculated after purification (silica gel for N-protected α-amino aldehydes and HPLC for PAs) based on the substitution of the commercial resin.

As the linker is stable in HF, side-chain protecting groups can be cleaved in a Boc strategy, before the reduction. In the case of compound **7**, unfortunately we only observed the formation of the cyclized

carbinolamine as described by McConnell et al[21]. However, protected peptide aldehydes can be prepared in solid phase as shown in table 1. This procedure will improve the preparation of large synthetic pseudopeptides.

References and notes :

1. Aoyagi, T., Takeuchi, T., Matsuzaki, A., Kawamura, M., Kondo, M., Hamada, M., Maeda, K. and Umezawa, H.*J. Antibiot* **1969**, *22*, 283-286.
2. Sarrubi, E., Seneci, P.F.and Angelastro, M.R. *FEBS Letters* **1992**, *319*, 253-256.
3. Fehrentz, J.A., Heitz, A., Castro, B., Cazaubon, C. and Nisato, D. *FEBS Letters* **1984**, *167*, 273-276.
4. McConnell, R.M., York, J.L., Frizzel, D. and Ezell, C. *J. Med. Chem.* **1993**, *36*, 1084-1089.
5. Bajusz, S., Szell, E., Badgy, D., Barabas, E., Horvath, G., Dioszegi, M., Fittler, Z., Szabo, G., Juhasz, A., Tomori, E. and Szilagyi, G. *J. Med. Chem.* **1990**, *33*, 1729-1735.
6. Basak, A., Jean, F., Seidah, N.G. and Lazure, C,*Int. J. Peptide Protein Res.* **1994**, *44*, 253-261.
7. Chapman, K.T., *Bioorganic & Medicinal Chemistry Letters*, **1992**, *2*, 613-618.
8. Graybill, T.L., Dolle, R.E., Helaszek, C.T., Miller, R.E. and Ator, M.A., *Int J.Peptide Protein Res.* **1994**, *44*, 173-182.
9. Kawamura, K., Kondo, S., Maeda, K. and Umezawa, H., *Chem. Pharm. Bull.* **1969**, *17*, 1902-1909.
10. Someno, T. and Ishii, S. *Chem. Pharm. Bull.* **1986**, *34*, 1748-1754.
11. Westerik, and Wolfenden, *J. Biol. Chem.* **1972**, *247*, 8195-8197.
12. Ito, A., Takahashi, R. and Baba, Y. *Chem. Pharm. Bull.* **1975**, *23*, 3081-3087.
13. Fehrentz, J.A., Heitz, A. and Castro, B., *Int. J. Peptide Protein Res.* **1985**, *26*, 236-241.
14. Murphy, A.M., Dagnino, R., P.L. Vallar, Trippe, A.J., Sherman, S.L., Lumpkin, R.H., Tamura, S.Y. and Webb, T.R., *J. Am. Chem. Soc.* **1992**, *114*, 3156-3157.
15. Nahm, S. and Weinreb, S., *Tetrahedron Letters* **1981**, *22*, 3815-3818.
16. Fehrentz, J.A. and Castro, B., *Synthesis* **1983**, 676-678.
17. Synthesis of the linker : 9.65 g (60 mmoles) of benzyl acrylate were added to a solution containing the methoxy-amine chlorhydrate (60 mmoles : 5 g) in acetonitrile (50 mL) in the presence of 50 mL of diisopropylethylamine (DIPEA). After 48 hours at 52°C, the oily residue was dissolved with ehtyl acetate, washed with saturated solutions of $NaHCO_3$ and NaCl. After drying with sodium sulfate, the solution was concentrated and gave an oil (crude yield : 11 g = 88%). The compound was purified on a silica gel chromatography (ethyl acetate/hexane : 5/5) to yield the pure 3-amino-N-methoxy-propionic acid benzyl ester (7.49g = 60%). 33mmoles (7.3 g) of Boc2O were then added to a solution of dioxanne/water (2/1) containing 33 mmoles (7 g) of the precedent compound in the presence of NaOH 1M in order to maintain the pH between 12 and 13. After 3 hours, the solvent was evaporated, the mixture dissolved in ethyl acetate and washed with $KHSO_4$ aqueous solution (5%). After drying and concentration, an oily residue is obtained (yield : 10 g = 98%). This 3-amino-N-*tert*-butyloxycarbonyl-N-methoxy-propionic acid benzyl ester was hydrogenolysed with Pd/c in EtOH 95% in 3 hours. After filtration of the catalyst and evaporation of the solvent, the linker is quantitatively obtained (6.8 g) and ready to be coupled to the resin.
18. Typical experiment of reduction ; synthesis of Boc-Phe-Val-Ala-H : 1.5 g of peptidyl-resin (0.66 mmole) were suspended in anhydrous THF and placed in an ice bath. $LiAlH_4$ (114 mg : 5 molar equivalents) were added and the reaction was stirred for 30 min. The reaction was then hydrolysed with a $KHSO_4$ aqueous solution (5%). The resulting mixture was filtrated in order to eliminate the resin which was washed twice with dichloromethane. The liquid phases were gathered, diluted with dichloromethane and washed with a $KHSO_4$ aqueous solution, satured solutions of $NaHCO_3$ and NaCl. After drying and evaporation a white powder was obtained (crude yield : 180 mg = 71%). This aldehydic peptide was then purified either on a silica gel chromatography with solvents containing 0.1% pyridine or by reversed phase HPLC in gradient mode using CH_3CN/H_2O/TFA : x/y/0.1% as solvent system.
19. Ho, P.T. and Ngu, K., *J. Org. Chem.* **1993**, *58*, 2313-2316.
20. Shuman, R.T., Rothenberger, R.B., Campbell, C.S., Smith, G.F., Gifford-Moore, D.S. and Gesellchen, P.D., *J. Med. Chem.* **1993**, *36*, 314-319.
21. McConnell, R.M., Barnes, G.E., Hoyong, C.F. and Gunn, J.M., *J. Med. Chem.* **1990**, *33*, 86-93.

(*Received in France* 13 *July* 1995; *accepted* 1 *September* 1995)