Pergamon

Tetrahedron Letters 39 (1998) 4171–4174

TETRAHEDRON
LETTERS

N-Linked Solid Phase Peptide Synthesis

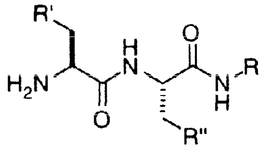
Roger Léger,* Rose Yen, Miles W. She, Ving J. Lee and Scott J. Hecker

Microcide Pharmaceuticals Inc., 850 Maude Ave., Mountain View CA 94043

Received 9 March 1998; revised 3 April 1998; accepted 5 April 1998

Abstract: The synthesis of *N*-linked peptides on solid phase is accomplished using a resin bound Boc equivalent. The formation of the peptide bond is done by an activated 2,4-dinitrophenyl ester intermediate and an unprotected amino acid at the C-terminus. Further extension of the peptide and cleavage of the resin is done using mild conditions. © 1998 Elsevier Science Ltd. All rights reserved.

In the course of our investigation of biologically active peptides, we needed a library of dipeptides having C-terminus capping groups. Thus, solid phase synthesis[1] is an excellent strategy to generate a large number of analogs having the AA_1-AA_2-Cap motif (Figure 1). Specifically, we were interested in analogs with two structural constraints on the dipeptide: (i) aromatic amino acid residue at the AA_1 position and (ii) dibasic amino acids at the AA_2 position. The capping group could derive from any amine, especially those of low nucleophilicity.



AA_1-AA_2-Cap

R = Capping Group
R' = Aryl Group
R"= Basic Function

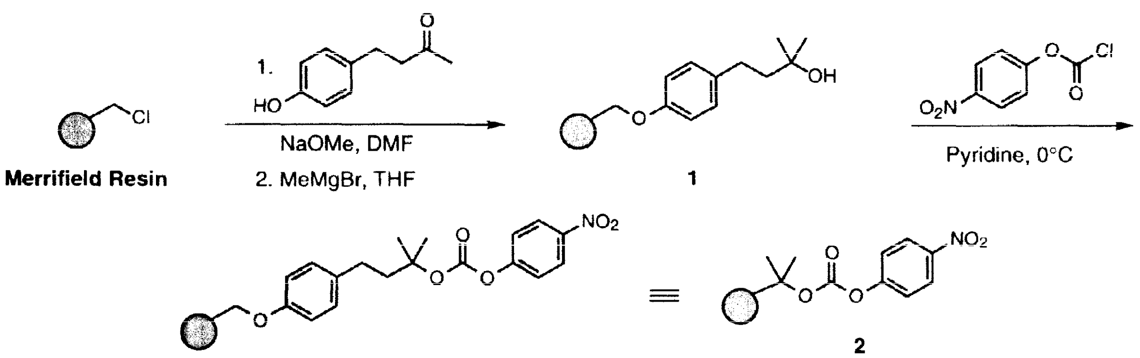
Figure 1

While there are many examples of *N*-linkers for solid phase synthesis,[1,2] the scarcity of easily cleavable *N*-linkers prompted us to devise conditions where an unprotected amino acid monomer could be attached to a solid support by a functional equivalent to the *tert*-butoxy carbonyl protecting group (Boc). Herein we communicate the use of a solid phase Boc group equivalent to prepare dipeptides.

To prepare the Boc resin we needed a modified Wang resin[3] containing a tertiary alcohol linker **1** (Scheme 1). To accomplish this, Merrifield resin was initially treated with 4-(*p*-hydroxyphenyl)-2-butanone in the presence of freshly prepared sodium methoxide followed by the formation of the tertiary alcohol using methyl magnesium bromide[4] giving **1**. The carbonate resin **2** was prepared from the tertiary alcohol **1** and *p*-nitrophenyl chloroformate in pyridine at 0°C. The selection of *p*-nitrophenyloxy group on the resin-bound carbonate, was (i) due to its greater reactivity compared to phenyl or imidazole[5] and (ii) visual readout in the subsequent reaction.[6]

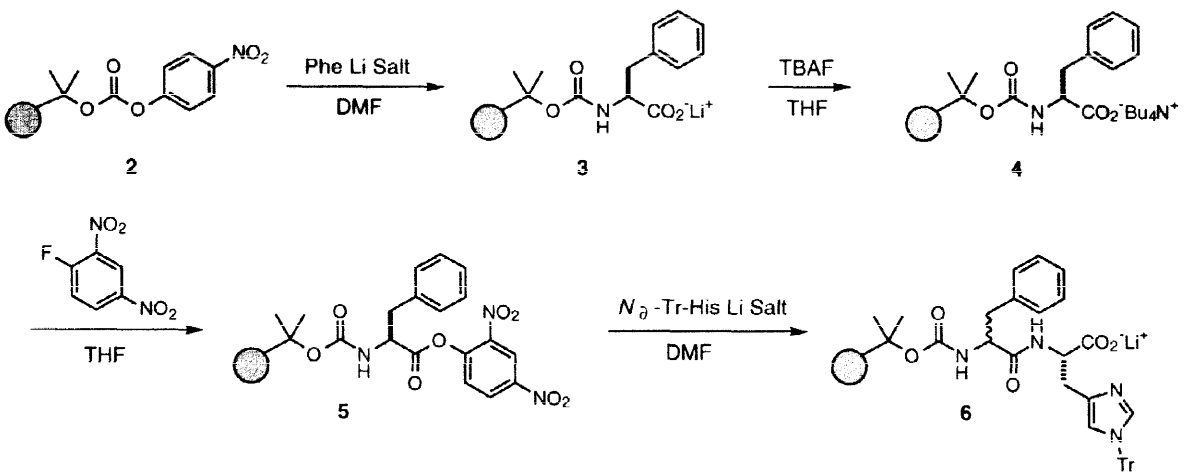
0040-4039/98/$19.00 © 1998 Elsevier Science Ltd. All rights reserved.
PII: S0040-4039(98)00777-1

Scheme 1



Attachment of the first amino acid was difficult, specifically we wanted to attach unfunctionalized phenylalanine in the absence of base. This is further complicated by the low solubility of phenylalanine in either DMF or mixtures of DMF and water; water also reduced the swelling of the beads. Adding bistrimethylsilyl trifluoroacetamide (BSTFA)[2b] to form the trimethylsilyl ester did facilitate the coupling but it never went to completion. The most practical method (Scheme 2) was the reaction of **2** with freshly prepared phenylalanine lithium salt[7] (5 equivalents) in DMF at room temperature to give **3**. The excess reagent was washed off with water leaving the polymeric lithium salt **3** unable to swell in organic solvents.[8] To increase the permeability and the reactivity of the lithium salt in DMF, **3** was conveniently converted to the tetrabutylammonium salt **4** *in situ* with tetrabutylammonium fluoride.

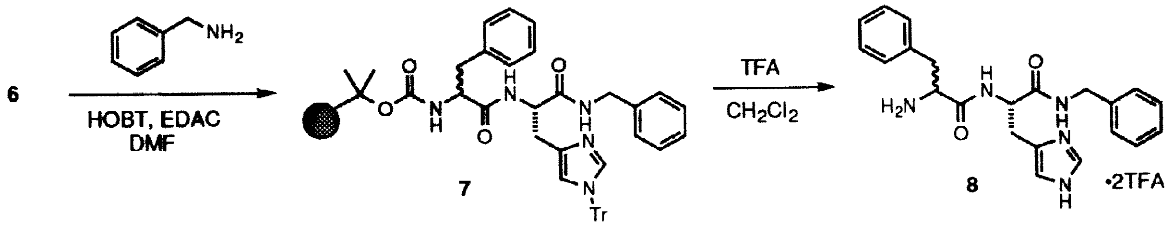
Scheme 2



The highly nucleophilic intermediate **4** is activated as the 2,4-dinitrophenyl ester **5** by treatment with 2,4-dinitro-1-fluorobenzene.[9] Indeed, **5** reacts smoothly with the lithium salt of N_{∂}-tritylhistidine to give **6** which has the same physical properties as **3** in terms of solubility. At this point it became impossible to repeat the methodology using 2,4-dinitro-1-fluorobenzene due to the reaction of the amide bond with the reagent. Attempts to generate a 2,4-dinitrophenyl ester afforded compounds containing two 2,4-dinitrophenyl groups.

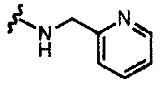
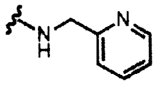
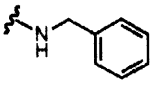
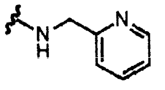
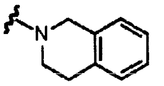
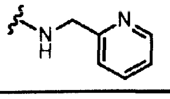
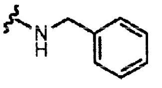
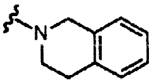
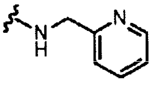
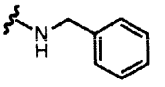
A normal peptide coupling procedure using the amine, EDAC and HOBT in DMF was used to accomplish the capping step (Scheme 3). Even though **6** was insoluble in DMF, the presence of the weak acid HOBT was sufficient to protonate the salt and to swell the polymer to an acceptable state. Since the progress of the capping step could not be followed by i.r. spectroscopy, the reaction was followed by HPLC.[10] The reaction conditions were optimized when 5 equivalents of each reagent were used with shaking at ambient temperature for 10-16h.

Scheme 3



Cleavage of the capped peptide from resin 7 was done under mild conditions (20% TFA in dichloromethane) at ambient temperature for 1h. Under these conditions, peptide 8 trifluoroacetate salt was obtained in 78% yield overall with 42% racemization of the phenylalanine.[11] Representative examples are shown in Table 1.

Table 1. Examples of Final Compounds of AA_1-AA_2-Cap Motif Prepared from Solid Phase Synthesis

Entry	Peptide AA_1-AA_2	Capping Amine	Overall Yield (%)[a]	Racemization of AA_1 (%)		ESMS[d] (M+H)
				HPLC[b]	NMR[c]	
1	Phe-His		68		40	393.3
2	*p*-MeOPhe-His		71		37	423.2
3	Phe-Lys		61	48		383.3
4	Phe-Lys		55		61	384.2
5	Phe-Lys		53	71		409.3
6	*p*-MeOPhe-Lys		53		18	414.3
7	Phe-Orn		52	68		369.2
8	Phe-Orn		37	100		395.3
9	Phe-Orn		73		75	370.2
10	*p*-MeOPhe-Orn		69	87		399.2

a) Isolated yield of TFA salt relative to a theoretical loading of 0.7 mmol/g. b) Racemization calculated from HPLC integration; Amberchrom stationary phase, 0 to 75% gradient of acetonitrile in 0.1% aqueous TFA over 45 min. c) Sample not separable by HPLC, racemization is estimated by NMR. d) Electrospray Ionization Mass Spectrometry.

The entries shown in Table 1 have variations at the second amino acid and the capping groups. Entries 1-2 contain a histidine, entries 3-6 contain a lysine and entries 7-10 have an ornithine.[12] The capping groups comprise those from aminomethylpyridine (entries 1,2,4,6 and 9), benzylamine (entries 3, 7 and 10) and a secondary amine in the likes of 1,2,3,4-tetrahydroisoquinoline (entries 5 and 8). The overall yields are good, but there is a high level of racemization of the first amino acid. Since similar levels of racemization are found, it is postulated that ester **5** is epimerized[13] during the addition of the second amino acid lithium salt since it is difficult to epimerize the deprotonated acids **4** or **6**. The racemization of the amino acid at the AA_1 position was not a problem for us since most of the diastereomers were separable by HPLC and could be used individually or as a mixture for biological analysis. Nevertheless, the compound in entry 7 was separated by preparative HPLC and subjected to rat serum peptidase (L-amino acid specific) where the minor isomer was stable and thus attributed the D,L stereochemistry, while the major component was totally consumed within 15 min.

In summary, we provide an alternative to the C-terminal attachment strategy for solid phase peptide synthesis. Further improvements are possible, specifically, less harsh coupling conditions and will be reported in the future.

Acknowledgments. We wish to thank Burton G. Christensen for helpful discussions. We also wish to thank Richard M. Fronko for his help with NMR spectra and May D. Lee for HPLC conditions.

References and Notes

1. (a) Früchtel, J. S.; Jung, G., *Angew. Chem. Int. Engl.* **1996**, *35*, 17-42. (b) Hermkens, P. H. H.; Ottenheijm, H. C. J.; Rees, D. C., *Tetrahedron* **1997**, *53*, 5643-5678.
2. (a) Hauske, J. R.; Dorff, P., *Tetrahedron Lett.* **1995**, *36*, 1589-1592. (b) Dressman, B. A.; Spangle, L. A.; Kaldor, S. W., *Tetrahedron Lett.* **1996**, *37*, 937-940. (c) Kaljuste, K.; Undén, A., *Tetrahedron Lett.* **1996**, *37*, 3031-3034. (d) Rotella, D. P., *J. Am. Chem. Soc.* **1996**, *118*, 12246-12247. (e) Nugiel, D. A.; Waker, D. A.; Nemeth, G. A., *Tetrehedron Lett.* **1997**, *38*, 5789-5790. (f) Youngman, M. A.; Dax, S. L., *Tetrahedron Lett.* **1997**, *38*, 6347-6350. (g) Wang, F.; Hauske, J. R., *Tetrahedron Lett.* **1997**, *38*, 6529-6532. (h) Furth, P. S.; Reitmen, M. S.; Gentles, R.; Cook, A. F., *Tetrahedron Lett.* **1997**, *38*, 6643-6646. (i) Garigipati, R. S., *Tetrahedron Lett.* **1997**, *38*, 6807-6810. (j) Kay, C.; Murray, P. J.; Sandow, L.; Holmes, A. B., *Tetrahedron Lett.* **1997**, *38*, 6941-6944. (k) Brady, S. F.; Stauffer, K. J.; Lumma, W. C.; Smith, G. M.; Ramjit, H. G.; Lewis, S. D.; Lucas, B. J.; Gardell, S. J.; Lyle, E. A.; Appleby, S. D.; Cook, J. J.; Holahan, M. A.; Stranieri, M. T.; Lynch, J. J. Jr.; Lin, J. H.; Chen, I.-W.; Vastag, K.; Naylor-Olsen, A. M.; Vacca, J. P., *J. Med. Chem.* **1998**, *41*, 401-406.
3. Wang, S. S., *J. Am. Chem. Soc.* **1973**, *95*, 1328-1333.
4. Stewart, M. J.; Young, J. D., *Solid Phase Peptide Synthesis*, 2nd Ed., Pierce Chemical Company, Rockford, Illinois 1984, pp 12-13.
5. Both phenyl carbonate and imidazole carbamate were prepared and test reactions were done by (1) treating a known amount of **2** with benzylamine and (2) cleaving off the benzylamine and weighing it as its TFA salt. Thus *p*-nitrophenyl carbonate was found to give the best loading of 0.7 mmol/g.
6. The *p*-nitrophenoxy group provided an excellent chromophore for monitoring the reaction. The carbonyl peak in the i.r. spectrum (KBr pellet) shifted from 1756 cm^{-1} for the carbonate to approximately 1685 cm^{-1} for carbamates.
7. The lithium salts are prepared by dissolving the amino acid and LiOH (1 eq.) in water and lyophilizing the solution.
8. At this point, the phenylalanine was cleaved to measure the loading and it was found to be 0.7 mmol/g.
9. Takahashi, S.; Kobuko, M.; Satake, K., *Chem. Lett.* **1983**, 1445-1448.
10. PRLPS column with 20 to 60 % gradient of acetonitrile in 0.1% aqueous TFA over 20 min.
11. After seperation by preparative HPLC: Compound **8** (major) L,L diastereomer, mass spectrum (ES, M+H) 392.3, NMR (400 MHz, D_2O) δ 8.50 (s, 1H), 7.43 (m, 3H), 7.29 (m, 2H), 7.16 (s, 1H), 4.65 (t, J = 7.6 Hz, 1H), 4.43 (d, J = 14.8 Hz, 1H), 4.33 (dd, J = 8.0, 6.4 Hz, 1H), 4.24 (d, J = 15.2 Hz, 1H), 3.30 (dd, J = 13.6, 6.4 Hz, 1H), 3.19 (m, 2H). Compound **8** (minor) D,L diastereomer, mass spectrum (ES, M+H) 392.3, NMR (400 MHz, D_2O) δ 8.47 (s, 1H), 7.34 (m, 3H), 7.18 (m, 3H), 4.40 (m, 4H), 3.135 (m, 4H).
12. Lysine and ornithine peptides were prepared from ε– and δ–Boc protected amino acids respectively, with a free α amino function.
13. Epimerization may take place through an azlactone intermediate. Benoiton, N. L., Chen, F. M. F., *Can. J. Chem.* **1981**, *59*, 384-389.