Pergamon

Tetrahedron Letters, Vol. 38, No. 42, pp. 7307-7310, 1997

0040-4039/97 $17.00 + 0.00

PII: S0040-4039(97)01774-7

Solid Phase Synthesis of Fmoc N-Methyl Amino Acids: Application of the Fukuyama Amine Synthesis

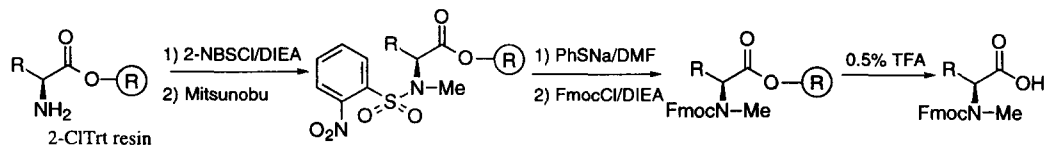
Lihu Yang* and Kuenley Chiu†

Modular Synthesis, Department of Medicinal Chemistry
Merck Research Laboratories, PO Box 2000, Rahway, NJ 07065

Abstract: N-Methyl amino acids and their Fmoc derivatives are synthesized in high yield and purity on solid support using the Fukuyama amine synthesis protocol.

The incorporation of N-methyl amino acids into biologically active peptides has been widely used to study conformation and biological activity.[1] An N-methylated peptide amide bond often exhibits higher resistance to proteolysis and thus may result in improved oral activity and enhanced duration of action.[2] N-methylated peptides are generally synthesized by incorporation of protected N-methylated amino acids in solution or solid phase.[3,4] Several methods have been used in the synthesis of N-methyl amino acids and their derivatives in solution. The direct alkylation procedure developed by Benoiton has been widely used in the synthesis of Cbz or Boc protected N-methyl amino acids.[5] Fmoc protected N-methyl amino acids have been prepared following the method of Freidinger[6] or modifications[7] through the acidic reduction of oxazolidinones or methylol. Grieco developed a method for selective N-methylation via a retro aza Diels-Alder reaction of amino acids and small peptides.[8] Although these methods can be used in a variety of amino acids, the strong basic and acidic conditions required exclude their use in some sensitive systems, such as tryptophan and Boc orthogonally protected lysine analogs. Other methods for the preparation of N-methyl amino acids or esters include the reduction of N-formyl amino acid esters[9] and reductive alkylation of N-benzyl amino acids followed by hydrogenolysis.[10]

Recently, a new amine protection/alkylation protocol was introduced by Fukuyama and co-workers, and was found to be effective in the preparation of secondary amines from primary amines without either primary or tertiary amine contamination.[11] They also demonstrated the synthesis of mono alkylated phenylalanines based on this strategy. Adaptation of this method to solid phase peptide synthesis should prove extremely valuable. Herein we report the solid phase synthesis of N-methyl amino acids or their N-Fmoc protected analogs by applying the Fukuyama amine synthesis.



We investigated the scope of the reaction by using amino acids preloaded on the 2-Cl-trityl resin,[12] since it has been widely used for fully protected peptide fragment synthesis. A number of bases and solvents were investigated for the sulfonylation with 2-nitrobenzenesulfonyl chloride (2-NBSCl). Using the standard sulfonylation reaction procedures such as dichloromethane (DCM) as solvent and N,N-diisopropylethylamine (DIEA) as base resulted in darkened resin and incomplete reaction, even after prolonged reaction time and the addition of excess reagents, presumably because 2-NBSCl is unstable under such conditions. The use of bases such as pyridine or 2,6-lutidine in DCM resulted in substantial resin cleavage during the sulfonylation. The best results were obtained by using 2-NBSCl (4 equivalents) and N,N-diisopropylethylamine (DIEA, 6 equivalents) in THF or THF/DCM (2:1). The reaction was monitored by the Kaiser ninhydrin test and generally went to completion in less than three hours. A much longer reaction time (1 day) only deteriorated the purity of the product slightly. DMAP (1 equiv.) accelerates the sulfonylation greatly but with slightly less pure product in the case of Trp. Alkylation of the resulting sulfonamide[13] was accomplished under Mitsunobu conditions, which were recently applied in solid phase synthesis for the alkylation of phenols.[14,15] The removal of the sulfonyl protecting group was accomplished by using the commercially available thiophenol sodium salt (or lithium salt) in DMF (0.5 M). Cleavage from resin at this stage gave the N-methyl amino acids. The Fmoc protecting group was introduced by treatment with FmocCl (4 equiv.) and DIEA (6 equiv.) in DCM for 2 hours. The intermediates or final N-Fmoc N-methylated amino acids were cleaved from the resin under the usual acidic conditions (0.5% TFA/DCM), which is mild enough to retain all side chain protecting groups such as t-Bu Boc.[16] The purity of the Fmoc protected amino acids is quite good (generally >90%). If necessary, a reverse phase HPLC purification yields pure product.

		Fmoc-AA	FmocN-Me AA				
entry	amino acids	Rt (min.)[17]	Rt (min.)	purity†	yield†	MW	MS found[18]
1	Ala	5.42	6.65	98	86%	325	$M+H^+$, $M+NH_4^+$
2	Val	7.32	8.58	96	95%	353	$M+H^+$, $M+NH_4^+$
3	Phe	8.56	9.34	90	80%	401	$M+H^+$, $M+NH_4^+$
4	Trp	8.20	8.60	88	100%	440	$M+H^+$, $M+NH_4^+$
5	Trp(Boc)	12.31	13.11	96	98%	540	$M+H^+$, $M+NH_4^+$
6	Lys(Boc)	8.58	9.45	90	100%	482	$M+H^+$, $M+NH_4^+$
7	Ser(tBu)	8.49	9.39	98	100%	397	$M+H^+$, $M+NH_4^+$
8	Asp(tBu)	8.51	9.38	90	96%	425	$M+H^+$, $M+NH_4^+$

†: purity & crude yield after cleavage.

As can be seen from the table, the procedure works very well with aliphatic and aromatic amino acids (Ala, Val, Phe, Trp) to give the corresponding N-methyl amino acids in high yield and purity[19]. Side chain protected amino acids such as Lys(Boc), Trp(Boc), Ser(tBu), Asp(tBu), gave equally good results. It should be noted that many of these N-methyl amino acids would not be obtainable by the Freidinger method in their side chain protected forms.

In summary, the Fukuyama amine synthesis is a reliable method for the solid phase conversion of amino acids to their N-methylated forms in high yield and purity. The procedure is especially valuable for the preparation of N-methylated amino acids that are difficult to synthesize by conventional methods. In addition, this methodology can be easily adapted for the preparation of other N-alkyl amino acids providing a good alternative to reductive alkylation.

The following is a description of the general procedure:

1) Sulfonylation: Trp-2-Cl-Trityl resin was swelled in THF (2 mL/100mg resin) and DIEA (6 equiv.). 2-Nitrobenzenesulfonyl chloride (1 M in DCM, 4 equiv.) was introduced slowly with agitation. The resulting mixture was agitated at room temperature for 3 h until Kaiser ninhydrin test is negative. Double coupling was carried out if necessary. With other amino acids, 1 equiv. of DMAP in THF/DCM (2:1) was most effective. The resin was then drained and washed with THF (2x), MeOH (2x), DCM (2x), THF (2x).

2) N-methylation: The resin-bound sulfonamide was mixed with triphenylphosphine (2 M in THF, 5 equiv.) and methanol (10 equiv.) in THF (1.5 mL/100mg of resin). DEAD (1 M in THF, 5 equiv.) was introduced slowly and the resulting reaction mixture was agitated for 1 h. The resin was then washed with THF (4x) and DCM (3x). Refer to note 13 for direct alkylation.

3) Removal of the protective group: The resin-bound methylated sulfonamide was swelled in DMF (1 mL/100mg resin) and treated with NaSPh/DMF solution (1M, 1 mL/100 mg resin) for 1 hour and this was repeated once. The resin was drained and washed with DMF (4x), methanol (4x) and DCM (4x).

4) Fmoc introduction: The resin was swelled in DCM and DIEA (6 equiv.) and FmocCl (4 equiv.) was added. The resulting mixture was agitated at room temperature for 4 h and drained, washed with DCM (2x), MeOH (2x), DCM (2x), ether (2x) and dried under nitrogen.

5) Product cleavage from resin: The resin-bound N-methyl amino acids or Fmoc analogs were treated with 0.5% TFA in DCM for 5 minutes. The resin was washed with 0.5% TFA in DCM, and the solution was evaporated to give the product.

Acknowledgments: We would like to thank Professor T. Fukuyama of the University of Tokyo, Dr. T. Rano and Mrs. N. Kevin of Merck for helpful discussions, and Ms. A. Bernick for performing mass spectral analysis.

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Presented in part at the Fifteenth American Peptide Symposium, June 14-19, 1997, Nashville, Tennessee.

† Summer intern student from Harvard University, June-August 1996.

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13 Direct alkylation with methyl iodide (4 equiv.) and fine K_2CO_3 powder (10 equiv.) also gave good results (DMF, 6 h). The deprotection can be carried out by draining the reaction and adding thiophenol (6 equiv.) and DMF. After 3 hours, the reaction was drained and the inorganic salt was removed by washing with a mixture of water and toluene.

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15 It should be noted that replacing methanol with other alcohols such as ethanol and benzyl alcohol produced N-alkyl amino acid derivatives, providing an alternative way for reductive alkylation.

16 H^1 NMR data (400 MHz, CD_3OD, ppm) for selected N-Methyl amino acid TFA salt: 1) N-Me-TrpOH: 7.60 (d, J=8 Hz, 1 H); 7.38 ((d, J=8 Hz, 1 H); 7.22 (s, 1H); 7.13-7.11 (m, 1 H); 7.07-7.04 (m, 1 H); 4.26 (t, J=5.9 Hz, 1 H); 3.48 (d, J=5.9 Hz, 2 H); 2.69 (s, 3 H). 2): N-Me-Trp(Boc)OH: 8.13 (d, J=8 Hz, 1H); 7.65 (s, 1H); 7.63 (d, 1H); 7.36-7.25 (m, 2H); 4.31 (t, J=5.9 Hz, 1H); 3.44 (ABq, 2H); 2.73 (s, 3H), 1.67 (s, 9H). 3): N-Me-Lys(Boc)OH: 3.94 (t, J=6.4 Hz, 1H); 3.05 (t, J=6.7 Hz, 2 H), 2.73 (s, 3 H); 1.97-1.92 (m, 2 H); 1.60-1.40 (m, 4 H); 1.42 (s, 9 H). 4): N-Me-Ser(t-Bu)OH: 4.09 (t, J=3.2 Hz, 1 H);3.93 (dd, J=3.2, 9.5 Hz, 1H); 3.85 (dd, J=3.2, 9.5 Hz, 1H), 2.73 (s, 3 H), 1.22 (s, 9 H). 5): N-Me-Asp(t-Bu)OH: 4.82 (t, J=4.2 Hz, 1 H); 3.01 (ABq, 2 H); 2.77 (s, 3 H); 1.22 (s, 9 H).

17 Analysis was performed using a Partisil ODS-3 RAC II column (100x4.6 mm, 5 μm) eluting with a linear gradient of 40-80% acetonitrile in water containing 0.1% TFA as buffer over 15 minutes at 1 mL/min., the HPLC is equipped with PDA detector and the results are plotted at 278 nm with band width of 8 nm.

18 MS was performed on Finnigan TSQ700 Mass SpectrometerElectrospray Ionization (ESI) in 82%ACCN:18% aq 1.3mM TFA/1.3mM Ammonium Formate.

19 All the N-methyl amino acid derivatives gave satisfactory NMR and MS. No racemization is observed. The purified Fmoc-N-Me-PheOH is identical with the known material in specific rotation ($[\alpha]_D$-55 (c=2, ethanol), tested side by side with material obtained from Bachem).

(*Received in USA* 19 *June* 1997; *revised* 18 *August* 1997; *accepted* 19 *August* 1997)