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An Improved Synthesis of Fmoc-*N*-methyl-α-amino Acids

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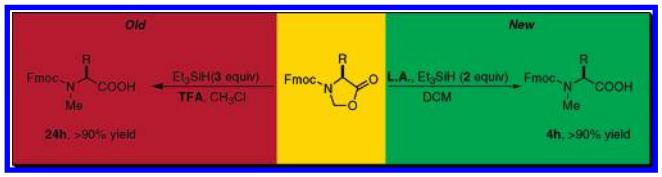
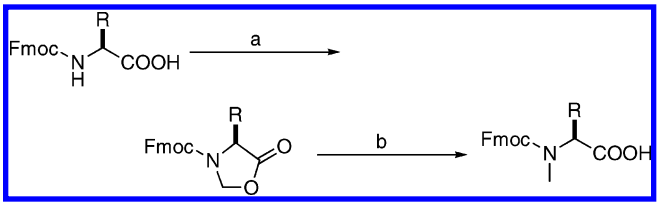


A highly efficient and environmentally more benign synthesis of Fmoc-*N*-methyl-α-amino acids from the corresponding Fmoc-amino acid, via intermediate 5-oxazolidinones, has been developed by using Lewis acid catalysis for the reductive opening of the oxazolidinone ring.

N-Methylated α-amino acids are widespread in nature, as part of larger peptidic natural products. They also find broad application for the design of biologically active substances in medicinal chemistry.[1] *N*-Methylation of amino acids is known to increase membrane permeability, proteolytic stability, and conformational rigidity.[2] Recently, several peptides incorporating one or more *N*-methylated amino acids have been reported as potent inhibitors of amyloidosis formation, e.g., as inhibitors of the Aβ-peptide aggregation related to Alzheimer's disease and the IAPP aggregation associated with diabetes type II.[3]

Several procedures exist for the synthetic preparation of *N*-methylated amino acids; however, many of these methods suffer from limitations in terms of yield or racemization or use large excesses of, often, expensive reagents.[4] One of the mildest and most general procedures for producing *N*-methyl amino acids is based on the reduction of 5-oxazolidinones. The preparation of 5-oxazolidinones from *N*-protected amino acids and paraformaldehyde was originally suggested by Ben-Ishai.[5] Freidinger et al.[6] later developed the methodology for reducing the 5-oxazolidinone with triethylsilane and trifluoroacetic acid (TFA), Scheme 1.

SCHEME 1. Freidinger Synthesis of *N*-Methylated Amino Acids via Reduction of 5-Oxazolidinones[a]



[a] Reagents and conditions: (a) $(CH_2O)_n$, *p*-toluenesulfonic acid (cat.), toluene, azeotropic removal of water; (b) Et_3SiH, CF_3COOH, $CHCl_3$.

This methodology is known to be free of racemization and may be applied to *acid-stable N*-carbamate protected amino acids, i.e., CBz- and Fmoc-protected. This process also tolerates functionalized side chains, provided that they are protected with non-acid-labile protecting groups.

Although powerful, this method suffers from the use of large excess, typically 3 equiv or more, of triethylsilane and consumes large quantities of TFA. Silane reducing agents are costly; however, attempts to replace them with other hydride donors has proven difficult, as other reducing agents may cause cleavage of the carbamate protecting group or may be too weak to affect the reduction of the intermediate *N*-acyliminium ion. The trifluoroacetic acid used for the ionic hydrogenation is also expensive, extremely corrosive, and very destructive to tissue of mucous membranes.[7]

Because of these limitations, we became interested in developing a more efficient methodology for the reduction of the intermediate 5-oxazolidinone. Initially, we attempted the method developed by Reddy et al.[8] for the reduction of Cbz- and Boc-protected oxazolidinones with $NaCNBH_3$ and TMSCl; however, upon attempting this methodology we failed to reduce the Fmoc-protected oxazolidinones investigated. Instead, we focused our attention on using various Lewis acids as replacements for the trifluoroacetic acid used to affect the ionic hydrogenation reaction.

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(1) (a) Fairlie, D. P.; Abbenante, G.; March, D. R. *Curr. Med. Chem.* **1995**, *2*, 654. (b) Cody, W. L.; He, J. X.; Reily, M. D.; Haleen, S. J.; Walker, D. M.; Reyner, E. L.; Stewart, B. H.; Doherty, A. M. *J. Med. Chem.* **1997**, *40*, 2228. (c) Haviv, F.; Fitzpatrick, T. D.; Swenson, R. E.; Nichols, C. J.; Mort, N. A.; Bush, E. U.; Diaz, G.; Bammert, G.; Nguyen, A.; Nellans, H. N.; Hoffman, D. J.; Johnson, E. S.; Greer, J. *J. Med. Chem.* **1993**, *36*, 363. (d) Vitoux, B.; Aubry, A.; Cung, M. T.; Marraud, M. *Int. J. Pept. Protein Res.* **1986,** *27*, 617.

(2) (a) Ostresh, J. M.; Husar, G. M.; Blondelle, S.; Dorner, B.; Weber, P. A.; Houghten, R. A. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 11138. (b) Miller, S. M.; Simon, R. J.; Ng, S.; Zuckermann, R. N.; Kerr, J. M.; Moos, W. H. *Drug. Dev. Res.* **1995**, *35*, 20.

(3) (a) Hughes, E.; Burke, R. M.; Doig, A. J. *J. Biol. Chem.* **2000**, *275*, 25109. (b) Doig, A. J.; Hughes, E.; Burke, R. M.; Su, T. J.; Heenan, R. K.; Lu, J. *Biochem. Soc. Trans.* **2002**, *30*, 537. (c) Gordon, D. J.; Sciarretta, L.; Meredith, S. C. *Biochemistry* **2001**, *40*, 8237 (d) Gordon, D. J.; Tappe, R.; Meredith, S. C. *J. Pept. Res.* **2002**, *60*, 37. (e) Kapurniotu, A.; Schmauder, A.; Tenidis, K. *J. Mol. Biol.* **2002**, *315*, 339.

(4) (a) Aurelio, L.; Brownlee, R. T. C.; Hughes, A. B. *Chem. Rev.* **2004**, *104*, 5823. (b) Aurelio, L.; Box, J. S.; Brownlee, R. T. C.; Hughes, A. B.; Sleebs, B. E. *J. Org. Chem.* **2003**, *68*, 2652.

(5) Ben-Ishai, D. *J. Am. Chem. Soc.* **1957**, *79*, 5736.

(6) (a) Freidinger, R. M.; Hinkle, J. S.; Perlow, D. S.; Arison, B. H. *J. Org. Chem.* **1983**, *48*, 77. (b) Aurelio, L.; Brownlee, R. T. C.; Hughes, A. B.; Sleebs, B. E. *Aust. J. Chem.* **2000**, *53*, 425.

(7) *Encyclopedia of Reagents for Organic Synthesis*; Paquette, L. A., Ed.; John Wiley & Sons Ltd.: Chicester, 1995; Vol. 7, p 5132.

(8) Reddy, G. V.; Iyengar, D. S. *Chem. Lett.* **1999**, 299.

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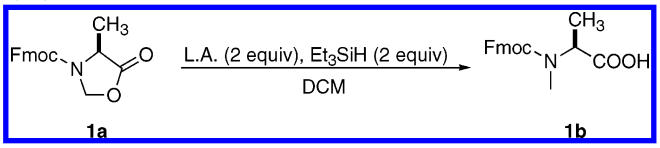
TABLE 1. Initial Investigation of Lewis Acid Catalysts for the Silane Reduction of Fmoc Alanine Oxazolidinone (1a)



1a 1b

entry	Lewis acid catalyst	time (h)	isolated yield (%)
1	*a*	22	96
2	$AlCl_3$	4	90
3	$ZnBr_2$	24	95
4	$ZnCl_2$	72	72
5	$FeCl_3$	0.5	84
6	$CuBr_2$	72	12
7	$CuCl_2$	72	
8	LiCl	72	

[a] Reaction run under standard conditions,[6] TFA/$CHCl_3$ 1:1 v/v, 3 equiv of Et_3SiH.

TABLE 2. Investigation of Lewis Acid Catalysts for the Silane Reduction of Fmoc-oxazolidinones with Nonfunctionalized and Functionalized Side Chains

entry	oxazolidinone derived from	Fmoc-*N*-Me-AA	Lewis acid catalyst (2 equiv)	isolated yield (%)
1	Fmoc-L-Gly-OH (**2a**)	**2b**	$AlCl_3$	94
2	Fmoc-L-Phe-OH (**3a**)	**3b**	$AlCl_3$	90
3	Fmoc-L-Leu-OH (**4a**)	**4b**	$AlCl_3$	92
4	Fmoc-L-Val-OH (**5a**)	**5b**	$AlCl_3$	94
5	Fmoc-L-Lys(Boc)-OH (**6a**)	**6b**	$ZnBr_2$	
6	Fmoc-L-Ser(OtBu)-OH (**7a**)	**7b**	$ZnBr_2$	
7	Fmoc-L-Asp(Bz)-OH (**8a**)	**8b**	$AlCl_3$	65
8	Fmoc-D-Asp(OAll)-OH (**9a**)	**9b**	$AlCl_3$	92
9	Fmoc-L-Ser(OBz)-OH (**10a**)	**10b**	$AlCl_3$	72
10	Fmoc-L-Lys(Cbz)-OH (**11a**)	**11b**	$AlCl_3$	80

Initially, we used the Fmoc-protected alanine oxazolidinone (**1a**) to determine which Lewis acids could compare to the TFA method in terms of yield (Table 1).[9] The Fmoc-protected oxazolidinone was treated with 2 equiv of the Lewis acid and 2 equiv of triethylsilane in dichloromethane (DCM). The solvent was removed, and the Fmoc-protected *N*-methylated amino acid (**1b**) was taken up in aqueous sodium carbonate solution. The aqueous layer was extracted with diethyl ether to remove nonacidic impurities and acidified with hydrochloric acid, and the product was extracted with DCM. We found that this was needed in the case of $AlCl_3$ since a triethylsilane derivative was present in the proton NMR spectra. This extraction process decreased the yield somewhat because some product was present in the ether phase. The lowering in yields was more evident with other amino acids having more lipophilic side chains. Alternatively, it was found that the product could be purified via flash chromatography to give the *N*-methylated amino acid in a higher yield, although with the disadvantage of chromatography in terms of time and solvent consumption. The yields reported in Tables 1 and 2 are based on purification by chromatography.

We found that $AlCl_3$ and $ZnBr_2$ gave reactions with negligible side product formation (entries 2 and 3). The $AlCl_3$ reaction needed 4 h for completion and the $ZnBr_2$ required 22 h. The use of $FeCl_3$ as Lewis acid resulted in reaction completion in just 30 min (entry 5). However, this catalyst also led to various side products that could not be removed by extraction alone. The other Lewis acids investigated did not affect the reaction in a reasonable time. Thus, $AlCl_3$ or $ZnBr_2$ appeared to be the most promising catalysts for further study of functionalized amino acid derivatives.

An early report claimed that zinc bromide in DCM could facilitate the *N*-Boc deprotection of secondary amines in the presence of primary amines,[10] whereas a more recent publication claims otherwise.[11] Nevertheless, we decided to test the possibility of using Lewis acids to mediate the reduction of oxazolidinone rings in the presence of acid-labile protecting groups, Table 2.

Fmoc-protected *N*-methyl amino acids with simple aliphatic or aromatic side-chains (**1b**−**5b**) were readily synthesized with $AlCl_3$ as the Lewis acid. Typically, the reaction time for all these reactions was less than 4 h and the crude product obtained after workup was sufficiently pure to be used in solid-phase peptide synthesis without further purification by flash chromatography. Amino acid derivatives with acid-sensitive *tert*-butoxycarbonyl and *tert*-butyl side chain protecting groups of Fmoc-lysine- and Fmoc-serine-derived oxazolidinones were cleaved during the reduction using relatively mild zinc bromide as the Lewis acid (Table 2, entries 5 and 6). However, it should be stated that the side chain protecting groups can readily be put back on, making the methodology overall more efficient than previously reported syntheses of these derivatives.[4] The use of less acid-labile protecting groups made it possible to synthesize functionalized Fmoc-protected *N*-methylated amino acids in respectable yields, even with the more active Lewis acid $AlCl_3$ (entries 7−10, **8b**−**11b**). The benzyl ester protecting groups of Fmoc-aspartic acid benzyl ether protecting group of Fmoc-serine were cleaved during prolonged reduction; however, the extent of deprotection could be reduced by quenching the reaction as soon as TLC showed complete consumption of starting material, giving the amino acids **8b** and **10b** in 65% and 72% yield, respectively. The Fmoc-lysine oxazolidinone with a Cbz side chain protecting group gave good yields of the *N*-methylated protected lysine **11b**.

In conclusion, the use of Lewis acids in the reduction of amino acid oxazolidinones for the preparation of Fmoc-protected *N*-methyl amino acids offers excellent yields, as compared to the use of TFA. The new methodology allows large-scale preparation of Fmoc-protected *N*-methyl amino acids, as a simple extraction procedure is sufficient for producing amino acids suitable for solid-phase synthesis. Furthermore, it was possible to reduce the amount of triethylsilane to 2 equiv, as compared to 3 for the TFA method, and the reaction can be completed in only 4 h, as compared to a minimum of 22 h with the use of TFA. In addition to being more environmentally benign, the new methodology also tolerates a larger variety of functionalization in the molecule and thus

(9) Other Lewis acids, e.g., $TiCl_4$ and $BF_3{\cdot}OEt_2$, also effectively mediated the ring-opening reaction but lead to a rapid cleavage of acid-sensitive side chain protecting groups.

(10) Nigam, S. C.; Mann, A.; Taddel, M.; Wermuth, C. G. *Synth. Commun.* **1989**, *19*, 3139.

(11) Kaul, R.; Brouillette, Y.; Sajjadi, Z.; Hansford, K. A.; Lubell, W. D. *J. Org. Chem.* **2004**, *69*, 6131.

offers a shorter route to side-chain-protected *N*-methylated amino acid derivatives.

Experimental Section

Typical Experimental Procedure. *N*-Methyl-Fmoc-α-alanine (1b). To a solution of the Fmoc-proteced oxazolidinone **1a** (0.32 g, 1.0 mmol) and anhydrous $AlCl_3$ (0.26 g, 2.0 mmol) in dry DCM (20 mL) was added triethylsilane (0.32 mL, 2.0 mmol). The reaction was stirred at ambient temperature until TLC (1:3 ethyl acetate/hexane) showed the absence of starting material. An additional amount of DCM (20 mL) was added, and the organic phase was washed with 1 M HCl (20 mL). The organic phase was dried over anhydrous magnesium sulfate and concentrated in vacuo. The crude product was purified via column chromatography on silica gel (ethyl acetate and hexane) to give product **1b** (0.29 g, 90%). The sample was identical to the authentic sample described in ref 6.

A detailed description of equipment and experiments can be found in Supporting Information.

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Supporting Information Available: Experimental details for oxazolidinone formation; detailed description for the synthesis and characterization of all listed Fmoc-*N*-methyl-amino acids, including copies of HPLC traces with UV, MS, and evaporative light scattering (ELSD) detection and electrospray mass spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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