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One-pot conversion of benzyl carbamates into fluorenylmethyl carbamates

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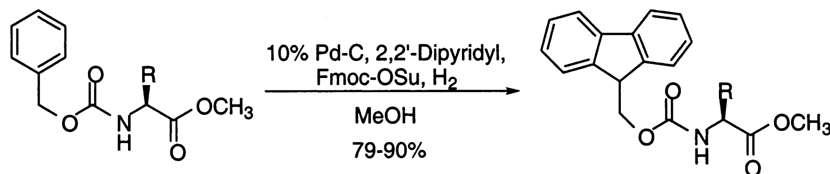
Abstract

A simple one-pot procedure smoothly converted *N*-benzyloxycarbonyl groups into *N*-fluorenylmethoxycarbonyl groups via hydrogenation with a poisoned catalyst in the presence of Fmoc-OSu. Functional groups such as *t*-butyl esters, *t*-butyl ethers, and *N*-Boc were stable under the reaction conditions. © 2000 Elsevier Science Ltd. All rights reserved.

In the course of de novo protein design it is often desirable to incorporate unnatural amino acid residues into a protein to impart unique structural or functional features. While some unnatural residues are commercially available, many must be prepared by the investigators wishing to exploit their use. We have taken advantage of chemistry developed by Vederas in which an N^{α}-benzyloxycarbonyl (Cbz)-protected β-lactone derived from *N*-Cbz-Serine can be opened at the β-carbon with a variety of nucleophiles to afford *N*-Cbz-protected amino acid residues bearing unnatural side chains.[1] In order to utilize the resulting unnatural residues in N^{α}-fluorenylmethoxycarbonyl (Fmoc)-based solid phase peptide synthesis (SPPS), the Cbz-protecting group must be replaced with the base labile Fmoc-protecting group.[2]

We present here, a simple one-pot procedure which smoothly converts *N*-Cbz groups into *N*-Fmoc groups in very good yield via hydrogenation with a poisoned catalyst in the presence of *N*-(9-fluorenylmethoxycarbonyloxy)succinimide (Fmoc-OSu).



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In the past, the Cbz to Fmoc transformation had been effected in a multi-step procedure involving catalytic hydrogenation of the Cbz-functionality, isolation of the liberated amine and subsequent reaction of the amine with an Fmoc-donor reagent such as Fmoc-OSu, 9-fluorenylmethyl chloroformate, or 9-fluorenylmethyl pentafluorophenyl carbonate.

A one-pot procedure to effect this transformation should find utility not only for those wishing to synthesize amino acid residues amenable to Fmoc-based SPPS but could potentially find use in the combinatorial synthesis of Fmoc-protected small molecules. The Cbz protecting group is advantageous in many respects to the Fmoc group for the combinatorial synthesis of amines due to its marked stability under diverse reaction conditions. However, if libraries of molecules are to be later incorporated into peptides or proteins, replacement of the Cbz group with Fmoc is necessary and a one-pot transformation would facilitate this process by eliminating additional steps.

To our knowledge, there have been no previous reports of a one-pot procedure for the conversion of *N*-Cbz into *N*-Fmoc. This may be due to the fact that the Fmoc-protecting group is only marginally stable under the conditions of catalytic hydrogenation. Williams has shown that Fmoc-Gly is cleaved by catalytic hydrogenation under various conditions ($t_{1/2}=3$–33 h).[3] Therefore, a simple one-pot procedure involving removal of the Cbz group via Pd–C catalyzed hydrogenation in the presence of an Fmoc donor reagent such as Fmoc-OSu is rendered ineffective because cleavage of the desired Fmoc product, as well as the Fmoc-OSu reagent competes with product formation.[4]

However, Hirota reported that olefin, benzyl ester, *N*-Cbz, and nitro functionalities could be chemoselectively hydrogenated (5% Pd–C, H_2) in the presence of a phenolic benzyl-protecting group by poisoning the catalyst with sub-stoichiometric amounts of 2,2′-dipyridyl.[5] We wondered whether a 2,2′-dipyridyl poisoned Pd–C catalyst could selectively reduce an N^{α}-Cbz group while leaving an Fmoc group intact. If this selectivity could be realized, then a simple one-pot transformation of *N*-Cbz into *N*-Fmoc via catalytic hydrogenation in the presence of an Fmoc-donor reagent could be possible.

Table 1
One-pot conversion of benzyl carbamates into fluorenylmethyl carbamates

Entry	Substrate	Product	Time (h)	Yield[a] (%)	% ee[b]
1	N^{α}-Cbz-Ala-OMe	N^{α}-Fmoc-Ala-OMe	3.5	90	≥99
2	N^{α}-Cbz-Phe-OMe	N^{α}-Fmoc-Phe-OMe	15.0	88	≥99
3	N^{α}-Cbz-Asp(*t*But)-OMe	N^{α}-Fmoc-Asp(*t*But)-OMe	4.5	89	–
4	N^{α}-Cbz-Leu-OMe	N^{α}-Fmoc-Leu-OMe	15.0	89	≥99
5	N^{α}-Cbz-Pro-OMe	N^{α}-Fmoc-Pro-OMe	3.5	79	≥99
6	N^{α}-Cbz-Lys(Boc)-OMe	N^{α}-Fmoc-Lys(Boc)-OMe	7.0	88	–
7	N^{α}-Cbz-Gly-Gly-OMe	N^{α}-Fmoc-Gly-Gly-OMe	4.0	84	–
8	N^{α}-Cbz-Thr(*t*But)-Ala-OMe	N^{α}-Fmoc-Thr(*t*But)-Ala-OMe	4.0	83	≥99[c]

[a] Each one-pot conversion was performed in triplicate according to the procedure described in the text. Yields represent the average isolated yield.

[b] % ee values were experimentally determined by chiral HPLC using either Chiralcel OD or Chiralpak AS columns (0.46 × 25 cm). Products from the one-pot conversions were eluted by linear gradients of hexanes and isopropanol at 20°C and a flow-rate of 1 mL/min, monitoring at 280 nm. Fmoc-D-products were synthesized and used as standards to demonstrate that resolution of possible enantiomers was achieved with the HPLC conditions employed.

[c] % ee value determined via RP-HPLC using a Vydac C4 peptide/protein column with water and 90% acetonitrile in water each containing 0.1% TFA as elution solvents.

Table 1 shows that the desired selectivity can be achieved. Treatment of N^{α}-Cbz-protected methyl esters of various amino acids and dipeptides with 10% Pd–C, H_2, and 2,2′-dipyridyl in the presence of Fmoc-OSu using methanol as solvent, efficiently converted the *N*-Cbz group into *N*-Fmoc.

Substrates of varying hydrophobicity and size were transformed in very good yields with convenient reaction times and *tert*-butyl ester, *tert*-butyl ether, and *N*-Boc functionalities were perfectly stable under the reaction conditions. In addition, a simple workup affords a product which is greater than 98% pure by NMR without performing column chromatography. A convenient feature of this reaction is that Fmoc-OSu is sparingly soluble in methanol but goes into solution upon reacting with the liberated amine, allowing one to visually detect when the transformation is nearing completion.

All of the *N*-Fmoc protected products were characterized by $^{1}H/^{13}C$ NMR and high resolution mass spectroscopy.[6] Chiral HPLC was employed to assess racemization for amino acids whose D-isomers were commercially available (Table 1). A representative chromatograph (Fig. 1) is shown for the one-pot transformation of *N*-Cbz-Ala-OMe into *N*-Fmoc-Ala-OMe demonstrating that greater than 99% ee had been achieved.

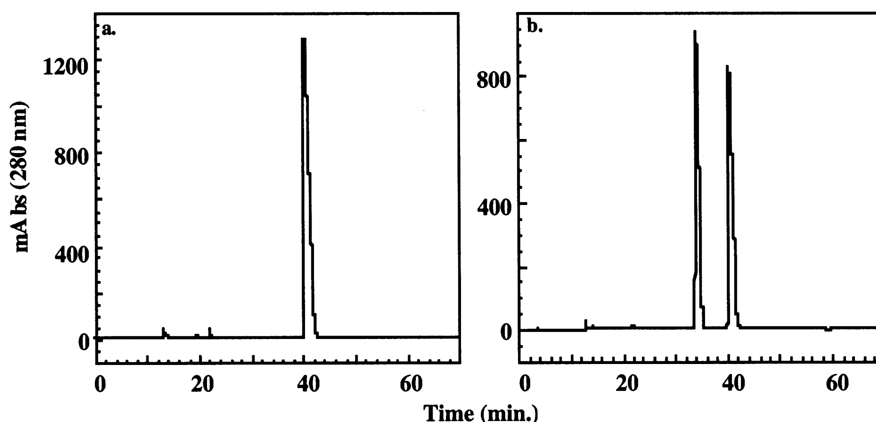

Figure 1. (a) Chiral HPLC analysis of Fmoc-L-Ala-OMe isolated from a typical one-pot conversion reaction. There is no observable racemization of the C^{α}-carbon of the amino acid residue. (b) Conjection of isolated Fmoc-L-Ala-OMe and Fmoc-D-Ala-OMe. HPLC conditions are described in Table 1

The one-pot procedure presented here nicely complements the suite of previously established one-pot conversions known to transform other *N*-protection groups commonly used in peptide synthesis. For example, the conversions of Fmoc into Cbz[7] or *N*-*tert*-butyloxycarbonyl (Boc)[8] and Cbz into Boc[9,10] are known. We are currently extending the scope of this reaction to include the conversion of Cbz into 2,7-di-*tert*-butyl-Fmoc, a promising alternative to the Fmoc-protection group which has been reported to show enhanced solubility characteristics.[11]

Representative procedure: A 50 mL three necked round-bottomed flask equipped with a rubber septum and two stopcock adapters (one used for balloon attachment and the other used as a gas outlet) was charged with 2,2′-dipyridyl (24.8 mg, 0.16 mmol) and 3.8 mL of methanol. The resulting solution was stirred and 10% Pd–C (9.2 mg, 2.7 mol%), a solution of N^{α}-Cbz-phenylalanine methyl ester (100 mg, 0.32 mmol) in 3.8 mL of methanol and Fmoc-OSu (107.6 mg, 0.32 mmol) were added. The flask was flushed with H_2 and subsequently stirred under a balloon of H_2. TLC (ethyl acetate/hexanes, 1:1) was used to monitor the disappearance of

Fmoc-OSu (15 h), after which time the reaction mixture was filtered and evaporated to dryness. The resulting oily solid was dissolved in 20 mL of ethyl acetate and washed with 10% aqueous HCl (3×20 mL), water (1×20 mL), dried (Na_2SO_4) and evaporated to afford 112.9 mg (0.28 mmol, 88%) of N^{α}-Fmoc-phenylalanine methyl ester as a white solid. For residues containing acid sensitive side chain protecting groups (entries 3, 6 and 8), 0.1 M aqueous citric acid is used in the workup rather than 10% aqueous HCl.

Acknowledgements

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4. In our hands, when Cbz-Ala-OMe (1.0 equiv.) is hydrogenated via 10% Pd–C (2.7 mol%), H_2 (balloon) in the presence of Fmoc-OSu (1.0 equiv.) using methanol as solvent, the desired Fmoc-protected product is formed in very low yield. The main products of this reaction are H_2N-Ala-OMe and 9-methylfluorene, which is probably formed upon Fmoc-OSu decomposition to dibenzofulvene and subsequent saturation. Catalytic transfer hydrogenation was also attempted under similar conditions with various hydrogen donors (1,4-cyclohexadiene, formic acid, or ammonium formate) with little success due to either Fmoc-OSu instability and/or extremely slow Cbz-deprotection rates.
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6. Representative NMR and mass spectroscopic data is given for entries 2 and 8.
   N^{α}-Fmoc-Phe-OMe: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.76 (d, $J=7.5$ Hz, 2H), 7.56 (t, $J=6.7$ Hz, 2H), 7.40 (td, $J=7.5$, 0.6 Hz, 2H), 7.33–7.27 (m, 5H), 7.08 (d, $J=6.5$ Hz, 2H), 5.26 (d, $J=8.1$ Hz, 1H), 4.67 (m, 1H), 4.44 (dd, $J=10.6$, 7.1 Hz, 1H), 4.34 (dd, $J=10.6$, 6.9 Hz, 1H), 4.20 (t, $J=7.0$ Hz, 1H), 3.73 (s, 3H), 3.11 (m, 2H); ^{13}C NMR ($CDCl_3$, 400 MHz) δ 172.1, 155.7, 144.0, 141.5, 135.9, 129.5, 128.8, 127.9, 127.4, 127.3, 125.3, 120.2, 67.1, 54.9, 52.6, 47.3, 38.4; HRMS (CI) m/z 402.1705 [$(M+H)^+$, calcd for $C_{25}H_{24}NO_4$, 402.1705].
   N^{α}-Fmoc-Thr(*t*But)-Ala-OMe: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.78 (br s, 1H), 7.74 (d, $J=7.8$ Hz, 2H), 7.58 (d, $J=7.4$ Hz, 2H), 7.38 (m, 2H), 7.29 (m, 2H), 5.97 (d, $J=5.0$ Hz, 1H), 4.50 (t, $J=7.1$ Hz, 1H), 4.36 (d, $J=7.3$ Hz, 2H), 4.21–4.15 (m, 3H), 3.73 (s, 3H), 1.42 (d, $J=7.2$ Hz, 3H), 1.29 (s, 9H), 1.08 (d, $J=6.4$ Hz, 3H); ^{13}C NMR ($CDCl_3$, 400 MHz) δ 173.2, 169.4, 156.2, 144.0, 141.5, 127.9, 127.3, 125.4, 120.2, 75.8, 67.2, 66.9, 58.5, 52.6, 48.5, 47.4, 28.3, 18.4, 16.7; HRMS (CI) m/z 483.2476 [$(M+H)^+$, calcd for $C_{27}H_{35}N_2O_6$, 483.2495].
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