A Fluorous Capping Strategy for Fmoc-Based Automated and Manual Solid-Phase Peptide Synthesis

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Just add water: Peptides synthesized by the use of standardized Fmoc protocols with commercial automated synthesizers can be purified from deletion products by simple centrifugation of aqueous solutions. The deletion products are capped with fluorous trivalent iodonium salts. At the end of the synthesis, the crude peptide is dissolved in water and centrifuged, and the deletion products precipitate leaving only the full length peptide in solution. Protocols for generalized use of this strategy are reported.

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SHORT COMMUNICATION

Figure 1. Generalized fluorous capping strategy. Amino acids that fail to couple leave an unprotected terminal amino group that is capped with reagent 1 (or 2) in standard peptide synthesis solvents. All products lacking one (or more) residues are therefore tagged with a fluorous tag that is easily removed at the end of the synthesis by simple centrifugation or by fluorous flash chromatography.

of reagent 1 during manual tBoc-solid-phase peptide synthesis.[7] This work was motivated by the need to make our strategy general and establish the utility of trivalent iodonium salts as robust capping reagents during routine Fmoc synthesis.

Here, we describe two related reagents 1 and 2, that differ only in the length of the perfluoroalkyl chains, that are equally efficient in automated Fmoc peptide synthesis. The capping protocol can be included as a programmable step in commercially available synthesizers using standard solvents. As a control experiment, the fluoroalkylated compound 3 (Figure 2) was stirred in piperidine/DMF (1:1) for 20 hours at 20 °C and recovered in 91% yield after workup and chromatography. Furthermore, when the fluoroalkylated tyrosine derivative 4 was treated with 4 equiv. of Fmoc-l-ala, 3.6 equiv. of HBTU as coupling agent and 6 equiv. of DIEA in DMF for 45 min, it was recovered unchanged. When 4 was treated with an excess of 2 under typical capping conditions, it was again recovered intact. These preliminary experiments established that the capping reagents and tagged products generated from them are compatible with the solvents and reagents normally employed during Fmoc peptide synthesis. In particular, there were no problems with elimination reactions which lead to reactive compounds that might result in undesired impurities.

We tested the capping reagents by both manual and automated Fmoc synthesis. Model peptides of length 10 (P1), 14 (P2) and 10 (P3) residues, which are analogs of acyl carrier peptide 65–74, of insulin-like growth factor 28–41 and bombesin 5–13, respectively, were prepared, and incomplete couplings were intentionally introduced at selected positions (marked with asterisks and shown in bold in Figure 2). The peptides were prepared in tandem using identical conditions, and were either capped with Ac2O or with fluorous capping reagent 2. To ensure identical levels of incomplete couplings, 20% of the resin by weight was removed following deprotection of the Fmoc group, but prior to amino acid coupling. The incoming amino acid was coupled to the remainder (80%) of the solid support. The resin was then washed, and the previously removed portion lacking the terminal amino acid returned to the synthesis vessels followed by the capping step. The capping step was programmed as an additional step in the synthesizer, which involved prewashing of the resin (2 × 3 min DMF followed by 2 × 3 min CH2Cl2), addition of 10 equiv. of reagent 1 or 2 in CH2Cl2, followed by addition of 2,4,6-collidine in CH2Cl2 which was stored separately. After 10 minutes of shaking this step was repeated. After two washing cycles
with DMF, standard cycles resumed. Peptides P1 and P2 were synthesized in this manner using reagent 2 with capping carried out only at the sites of incomplete coupling. At the end of the synthesis and subsequent to cleavage and deprotection, crude peptides were simply dissolved in 1% AcOH, centrifuged at 14000 rpm for 5 minutes and then analyzed by reversed-phase HPLC. Figure 3 shows that in both cases, a dramatic improvement in the purity of the crude peptide is observed. The fluorous-tagged deletion products tend to precipitate out of aqueous solutions, and the supernatant contains mostly the desired product. We have noted previously, that for longer peptides passage through fluorous silica gel may sometimes be necessary but for the shorter peptides employed here, mere centrifugation is enough to remove most unwanted products.

Peptide P3 was synthesized by manual Fmoc synthesis with capping reagent 2. A new protocol was employed that involved soaking the resin with the iodonium salt solution, which could be recovered by simple draining. The base collidine was then added in a separate step. In this manner, the solution of iodonium salt could be recycled several times. Because the salt and the base are employed in two consecutive steps, other bases besides collidine may in principle be used in this protocol. Successful capping and purification by simple centrifugation gave >95% pure P3 (see supporting information; for supporting information see also the footnote on the first page of this article). We note that the reagents are cost-effective and competitive with Ac2O in that each capping step adds ca. $6 to the overall materials cost resulting in substantial financial benefit in purification of peptides (Figure 4).

We note that nineteen of the twenty canonical amino acids are compatible with the capping protocol, however, the relatively uncommon amino acid methionine is alkylated rapidly by 1 or 2 to yield the corresponding sulfonium salt. Nevertheless, our protocol is still applicable to peptides.

![Figure 3](https://www.eurjoc.org/doi/10.1002/eurjoc.200660003)
20 amino acids are compatible with our capping method. We envision that these reagents will find extensive use in solid-phase peptide synthesis. For instance, when the pentapeptide P3 synthesized manually using capping reagent 1 was treated with the reagent 2, no reaction was observed (see supporting information). Methods for conversion of Met(sulfoxide) to methionine are well established, and with the inclusion of this additional step, all 20 amino acids are compatible with our capping method.

In summary, these results demonstrate robust and efficient fluoros tagging of deletion products that accumulate during automated and manual Fmoc peptide synthesis. The purification is achieved in a facile manner by simple centrifugation or by fluoros flash chromatography for larger products. We envision that these reagents will find broad use in solid-phase peptide and combinatorial chemistry where terminal amines are coupled to reaction partners.

Supporting Information Available (see footnote on the first page of this article): Summary of mass spectroscopic data (LC ESI-MS) of crude peptide mixtures and capped fragments; synthesis of reagent 1 and 2 and generating analytical data.

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[46] A 1:1 mixture of 2,4,6-collidine and 1 in CDCl3 was followed by NMR spectroscopy. In 3 minutes, 50% of collidine was alkylated.

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