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# 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.



Automated solid-phase synthesis has revolutionized the construction of oligonucleotides[1] and peptides.[2] Indeed, the availability of reasonably priced, "off-the-shelf" oligos and peptides has become the central pillar on which modern molecular and cell biology have thrived. Within these two classes of ubiquitous biopolymers, solid-phase oligonucleotide synthesis is clearly more efficient, where optimized protocols allow the preparation of ca. 100mers routinely. However, the relative diversity of chemical functionality in amino acid side chains and protecting groups in peptides makes the synthetic effort more demanding, limiting the length to ca. 50 residues. Furthermore, accumulation of deletion products, especially those missing a single residue ($n$ – 1 products), are frequent, thus complicating the purification of the desired full-length product.[3] Nevertheless, several strategies have been devised to solve this general problem with varying outcomes in the ease of implementation. The more promising of these approaches have been the introduction of handles that provide an avenue for the facile removal of unwanted products, mainly those due to failed couplings.[4–6]

We and others have demonstrated the use of fluorous capping during solid-phase synthesis of peptides,[7–9] oligonucleotides,[10] and carbohydrates.[11–15] In addition, fluorous compounds and solvents have found use in protein design,[16–27] reaction acceleration,[28] catalysis,[29–34] combinatorial chemistry,[35] proteomics[36] and organic separation methodology.[34,37–45] Our approach for peptide purification by use of fluorous technology comprised treating the solid support with reagent **1**, which caps all unconsumed residual terminal amines resulting from incomplete reaction that may participate in further couplings steps, giving rise to undesired deletion products.[7] The fluoroalkyl tag ($R_f CH_2$–) appended in this manner renders amines unreactive in all subsequent steps of peptide synthesis. At the termination of the synthesis, the crude peptide can then be purified by simple centrifugation or by fluorous flash chromatography, which results in the removal of all tagged deletions (Figure 1).

The trivalent iodonium salts **1** and **2** are extremely aggressive electrophiles that react rapidly and quantitatively with amines. Indeed, in the absence of a primary amine, the hindered base (2,4,6-collidine), commonly employed under our capping conditions to neutralize the acid [bis(trifluoromethylsulfonyl)imide], is itself consumed in less than 3 minutes.[46] Capping of unreacted amines during the automated synthesis can be troublesome as secondary structure formation or resin occlusion of amines may make these terminal reactive groups inaccessible. The potent reactivity of **1** and **2** makes the reagents attractive for complete fluorous tagging of recalcitrant amines, which fail to react during the coupling step.

Peptide synthesis protocols have embraced two major protecting group strategies – using either *tert*-butoxycarbonyl (*t*Boc) or 9-fluorenylmethoxycarbonyl (Fmoc) functionalities. In automated solid-phase peptide synthesis (SPPS), Fmoc chemistry predominates, because the deprotection step is performed with base (piperidine in DMF) rather than with corrosive trifluoroacetic acid. Furthermore, in *t*Boc-based synthesis, final cleavage of the peptide from the resin is frequently accomplished with neat anhydrous HF, which requires specialized equipment and safety protocols. In a recent report, we have shown the versatility

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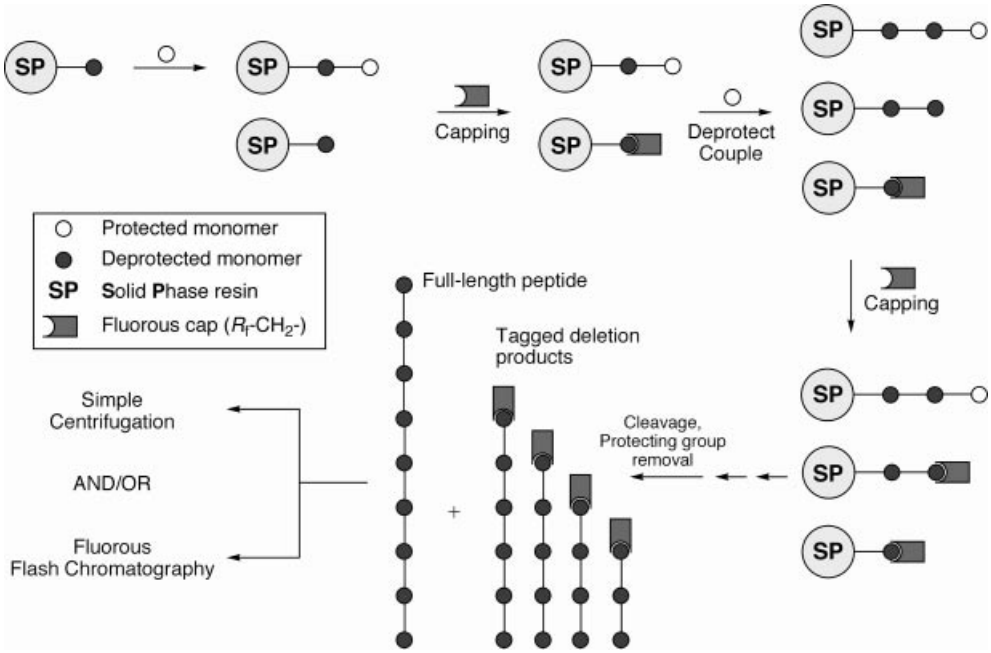


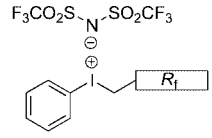

Figure 1. Generalized fluorous capping strategy. Amino acids that fail to couple leave an unprotected terminal amino group that is capped with reagent **1** (or **2**) in standard peptide synthesis solvents. All products lacking one (or more) residues are therefore tagged with a fluorous tag that is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

of reagent **1** during manual *t*Boc-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.

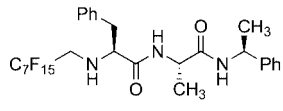
Here, we describe two related reagents **1** and **2**, that differ only in the length of the perfluoroalkyl chains, that are equally efficient in automated Fmoc peptide synthesis. The capping protocol can be included as a programmable step in commercially available synthesizers using standard solvents. 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-alanine, 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 5–14, 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

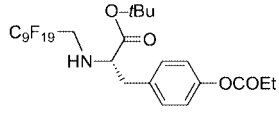
**P1** $NH_2$–**V***EAAIDYIDA–$CONH_2$
**P2** $NH_2$–PT*GY*GSSSRRAPET–$CONH_2$
**P3** $NH_2$–G**N***Q**W***AVGHLC–$CONH_2$




**1** $R_f = C_7F_{15}$–
**2** $R_f = C_9F_{19}$–




**3**




**4**

Figure 2. Sequences of peptides **P1**–**P3**; structures of reagents **1** and **2**; and of model compounds **3** and **4**. Sites of intentional incomplete couplings in **P1**–**P3** are shown in bold and marked with asterisks.

identical conditions, and were either capped with $Ac_2O$ 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 $CH_2Cl_2$), addition of 10 equiv. of reagent **1** or **2** in $CH_2Cl_2$, followed by addition of 2,4,6-collidine in $CH_2Cl_2$ 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[7] 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 $Ac_2O$ 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

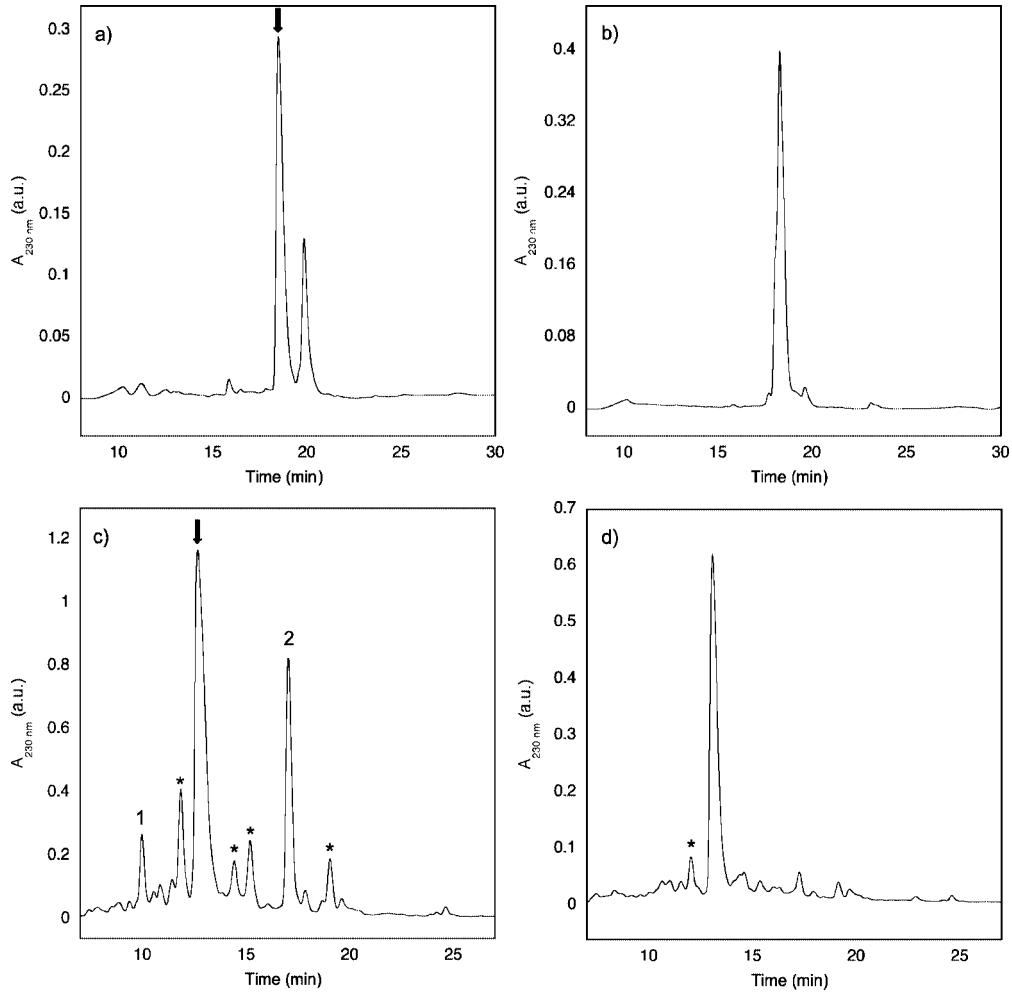



Figure 3. Analytical reverse-phase HPLC analysis of crude peptides after fluorous capping: (a) reverse-phase (Vydac $C_{18}$) HPLC chromatogram of peptide **P1** synthesized using an automated synthesizer with one incomplete coupling at position marked with an asterisk (also shown in bold) as shown in Figure 2 and capping with $Ac_2O$; (b) peptide **P1** made under identical conditions as in (a) but with fluorous capping reagent **2**; (c) peptide **P3** synthesized manually using $Ac_2O$ capping (see intentional deletions in Figure 2) and (d) using fluorous capping reagent **2**. Samples in (a)–(d) were dissolved in 1% AcOH and centrifuged at 14,000 rpm for 5 min and then the supernatant injected. Chromatogram peaks marked with arrows are full length peptides and those marked with asterisks are unknown impurities. In (c), peaks marked 1 and 2 are the acetyl capped products of increasing mass.

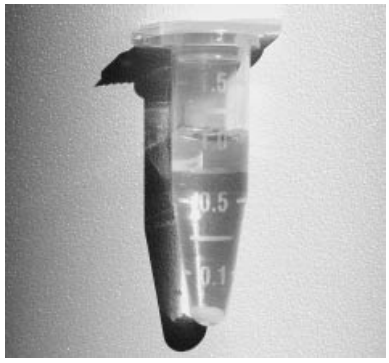


Figure 4. Photograph of precipitate resulting from centrifugation of a 1% AcOH solution of crude peptide **P3** synthesized manually using capping reagent **2**. The supernatant contains largely the full-length peptide. Similar treatment of the $Ac_2O$ capped crude peptide mixture does not result in any observable precipitate.

containing methionine as the alkylation reaction is circumvented by the use of methionine sulfoxide during the course of the synthesis. For instance, when the pentapeptide Ac–NH–Phe–Ala–Met(O)–Ala–Phe–CO–NH–Resin was treated with the reagent **1** under typical capping conditions, it did not react (see supporting information). Methods for conversion of Met(sulfoxide) to methionine are well established,[47] 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 fluorous 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 fluorous flash chromatography for longer 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 **2** and accompanying analytical data.

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