J|A|C|S
COMMUNICATIONS

Published on Web 07/14/2004

Just Add Water: A New Fluorous Capping Reagent for Facile Purification of Peptides Synthesized on the Solid Phase

Vittorio Montanari and Krishna Kumar*

Department of Chemistry, Tufts University, Medford, Massachusetts 02155

Received April 12, 2004; E-mail: krishna.kumar@tufts.edu

Solid-phase peptide synthesis (SPPS) has become a mainstay method for the synthesis of small- to medium-length peptides and proteins.[1] With the advent of native chemical ligation methods, the lengths achievable by SPPS have been significantly extended.[2−6] However, the tedious aspect of SPPS remains the removal of shorter peptide products, resulting from the failure of one or more of the chain extension steps (deletion sequences), from the desired full-length product. To facilitate purification, various new reagents have been developed with the intention of tagging deletion sequences or, alternatively, the full-length product, so that they may have different elution profiles during chromatographic purification. Several purification handles have been described that take advantage of selective conditions under which retention time on ion-exchange, hydrophobic interaction, affinity, and other types of chromatographic columns could be varied.[7−12] Still, sequences short of just one residue ($n - 1$ products) are notoriously difficult to separate.

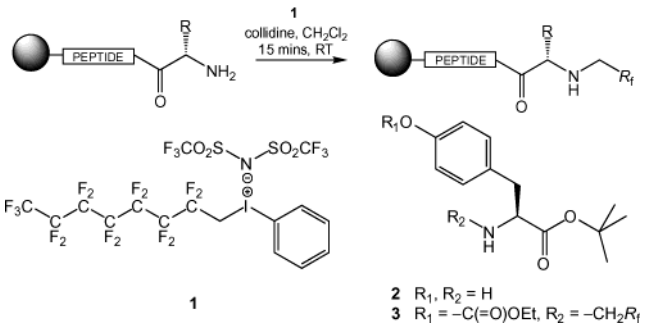
Recently, fluorous compounds have been employed in protein design,[13−21] reaction acceleration,[22,23] catalysis,[24−26] combinatorial chemistry, and organic separation methodology.[27−29] Among the canonical amino acid side chain functionalities, none are expected to extensively interact with fluorous chromatographic materials. Thus, an appendage that is highly fluorinated should be unique in its physical properties, compared to most peptide products.[30] In this report, we introduce a versatile new fluorous reagent that is useful as a handle for the efficient removal of deletion products formed during SPPS.

Automated peptide synthesizers typically use a capping step to terminate chains resulting from incomplete coupling steps. One approach is to make the capping reagent orthogonal in its properties to aid purification. The following design elements were central to our development of a new capping reagent. First, the reagent should be aggressive in reactivity toward free amines of α-amino acids to deliver a fluoroalkyl chain. Furthermore, the reactivity of the amine must be eliminated with respect to further peptide coupling steps. In addition, the new tag should be stable to subsequent peptide coupling reactions, the deprotection steps during synthesis, and the final cleavage of the peptide chain from the resin. We report here that trivalent iodonium compound **1** possesses all these relevant properties and functions as a useful tag for *t*-Boc-based SPPS.[31] It is compatible with the solvent systems of peptide synthesis and efficient at tagging free amines with an R_f ($C_7F_{15}-$) group. Once free amines have been alkylated in this manner, they are unreactive in further peptide coupling steps and stable to both deprotection in neat trifluoroacetic acid and to the final cleavage step in anhydrous HF.

The trivalent iodonium compound **1** has reactivity analogous to earlier reagents that were mainly employed in a two-phase organic−water solvent system with sodium bicarbonate as the base.[32−34] Compound **1** is a fluorous trivalent iodonium salt, and its reactivity has been fine-tuned to be compatible with peptide synthesis.[35] In model reactions, for example with the *tert*-butyl carboxyl ester of tyrosine **2**, 2 equiv of compound **1** in the presence of collidine/CH_2Cl_2 resulted in quantitative monoalkylation of the amino group in 10 min or less. The purified alkylated product, when treated with reagent **1** for an additional 30 min under identical conditions, did not react further. The deactivated amine is also inert to peptide coupling conditions. Upon reaction with 4 equiv of Boc-L-alanine, 3.6 equiv of HBTU as a coupling reagent, and 6 equiv of diisopropylethylamine in DMF for 30 min, the monoalkylated product **3** was recovered unchanged. In summary, the monofluoroalkylated α-amino acids survive all reactions that they would be subject to during peptide synthesis using *t*-Boc chemistry.

Scheme 1. Reactivity and Structure of Fluorous Capping Reagent **1**, Model Compounds (**2** and **3**), and Sequences of Peptides **P1**, **P2**, and **P3**

P1: Ac–NH–VE**A***AI**D***YI***DA–CONH$_2$
P2: Ac–NH–RA**V***KV**Y***ADA**A***EDESAEAFALEF–CONH$_2$
P3: Ac–NH–P**T***GYG**S***SSRRAPET–CONH$_2$



To demonstrate the versatility and utility of reagent **1**, we employed it in capping amines from intentionally incomplete couplings during SPPS. We used this approach on peptides of length 10 (**P1**), 21 (**P2**), and 14 residues (**P3**), which are variants of the acyl carrier peptide 65−74, adrenocorticotropic hormone 18−39, and insulin-like growth factor 28−41, respectively (Scheme 1). Peptides were synthesized using the in situ neutralization protocol using *t*-Boc chemistry on MBHA (methylbenzhydrylamine) resin.[36] To ensure incomplete coupling at selected sites, we used 0.8 equiv of amino acid and 0.72 equiv of HBTU and ran the coupling step for 20 min. This was followed by a 1 min flow wash with DMF. The resin was then washed thoroughly with CH_2Cl_2 using a combination of shake and flow washes. The capping step was performed with a solution containing 2 equiv of **1** in CH_2Cl_2 with collidine as a base for 15 min and was repeated once with 1 equiv of **1**. The resin was thoroughly washed with CH_2Cl_2, and the cycle of deprotection and coupling steps was then resumed in a normal manner. Capping steps were performed only at sites where couplings were incomplete.

The capped peptides were removed in one of two ways. In the case of the smaller peptide **P1**, the crude product obtained from the cleavage reaction was simply dissolved in 1% AcOH solution

10.1021/ja0479033 CCC: $27.50

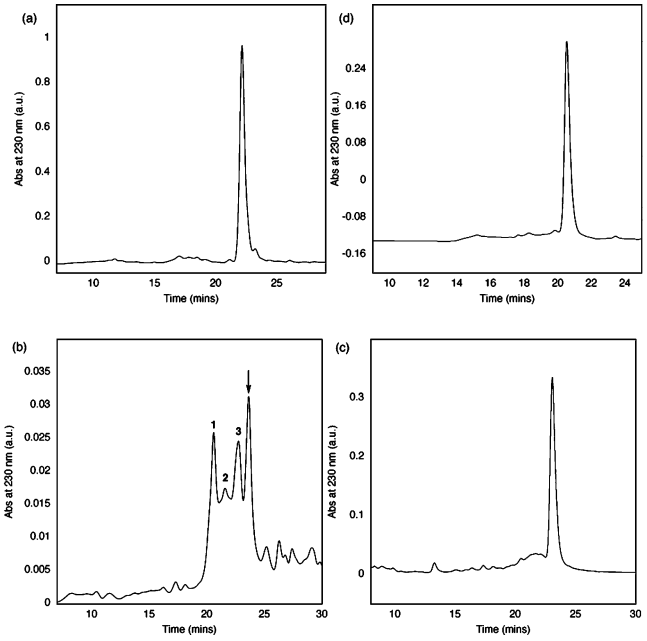


Figure 1. Fluorous tagging-aided purification. Counterclockwise from top left: (a) reverse-phase HPLC chromatogram (Vydac C18) of ACP analogue **P1** synthesized using incomplete couplings at positions marked by asterisks (and shown in bold) in the sequence in Scheme 1 and capping with **1** (the crude product was dissolved in 1% AcOH and centrifuged and the supernatant injected); (b) chromatogram of peptide **P2** synthesized using Ac_2O capping (full-length product is marked with an arrow, and peaks 1−3 are acetylated products of increasing mass); (c) peptide **P2** synthesized using fluorous capping; (d) chromatogram of **P3** obtained from synthesis employing capping reagent **1**. Samples in b−d were subjected to filtration through fluorous silica gel prior to injection on HPLC.

and centrifuged at 14 000 rpm for 10 min. This method produced a pellet presumably of the material that was capped with the fluorous tag.[37] The supernatant was injected after further acidification with 0.1% trifluoroacetic acid (TFA) on a Vydac C18 reverse-phase column and eluted with a gradient of CH_3CN in water (0.1% TFA). Figure 1a shows the results with the smaller peptide **P1**. When the capping was carried out with **1**, all deletion sequences precipitated after centrifugation, leaving only the full-length product in solution. While plain addition of water followed by centrifugation was sufficient for removal of small peptides (8−10 residues in length) that were fluoroalkylated, longer tagged peptides needed passage through fluorous flash silica gel in aqueous solvents to be efficiently excluded.

Figure 1b shows the chromatogram obtained from the synthesis of **P2** with Ac_2O as the capping reagent, while Figure 1c shows the same synthesis carried out with **1** as the capping reagent. Both samples were subjected to fluorous flash silica filtration using CH_3CN/H_2O (1:4, 1% AcOH) solvent. In the case of the products tagged with **1**, all the fluorous material is retained on the column, leaving mostly the desired full-length product, while the acetic anhydride-capped products closely mirror the elution profile of the final product. A short filtration removes almost all the deletion products generated during the synthesis of the 21 residue peptide using reagent **1**, thus greatly simplifying purification. Peptide **P3** gave similar results (see Figure 1d). The elution solvent for the fluorous flash column in the case of peptide **P3** was 1% AcOH.

In summary, we have developed an efficient and robust fluorous tagging reagent that has the potential to help with the synthesis of routine and difficult peptide and protein sequences. Since a majority of automated synthesizers work using Fmoc chemistry, a modified protocol capable of introduction as a routine step would be desirable. Studies along these lines are currently being pursued in our laboratories and will be reported shortly.

Acknowledgment. This work was supported in part by NIH (GM65500) and NSF (CHE-0236846 and CHE-0320783). K.K. is a DuPont Young Professor. We thank A. Kennan for help with ESI-MS experiments and M. d'Alarcao for helpful discussions.

Supporting Information Available: Experimental procedures and accompanying analytical data (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Merrifield, R. B. *J. Am. Chem. Soc.* **1963**, *85*, 2149.
(2) Dawson, P. E.; Muir, T. W.; Clark-Lewis, I.; Kent, S. B. H. *Science* **1994**, *266*, 776−779.
(3) Cotton, G. J.; Muir, T. W. *Chem. Biol.* **1999**, *6*, R247−R256.
(4) Canne, L. E.; Botti, P.; Simon, R. J.; Chen, Y. J.; Dennis, E. A.; Kent, S. B. H. *J. Am. Chem. Soc.* **1999**, *121*, 8720−8727.
(5) Dawson, P. E.; Kent, S. B. H. *Annu. Rev. Biochem.* **2000**, *69*, 923−960.
(6) Kochendoerfer, G. G.; Kent, S. B. H. *Curr. Opin. Chem. Biol.* **1999**, *3*, 665−671.
(7) Funakoshi, S.; Fukuda, H.; Fujii, N. *J. Chromatogr.* **1993**, *638*, 21−27.
(8) Shogren-Knaak, M. A.; McDonnell, K. A.; Imperiali, B. *Tetrahedron Lett.* **2000**, *41*, 827−829.
(9) Shogren-Knaak, M. A.; Imperiali, B. *Tetrahedron Lett.* **1998**, *39*, 8241−8244.
(10) Villain, M.; Vizzavona, J.; Rose, K. *Chem. Biol.* **2001**, *8*, 673−679.
(11) Vizzavona, J.; Villain, M.; Rose, K. *Tetrahedron Lett.* **2002**, *43*, 8693−8696.
(12) Canne, L. E.; Winston, R. L.; Kent, S. B. H. *Tetrahedron Lett.* **1997**, *38*, 3361−3364.
(13) Bilgiçer, B.; Kumar, K. *J. Chem. Educ.* **2003**, *80*, 1275−1281.
(14) Yoder, N. C.; Kumar, K. *Chem. Soc. Rev.* **2002**, *31*, 335−341.
(15) Bilgiçer, B.; Kumar, K. *Tetrahedron* **2002**, *58*, 4105−4112.
(16) Bilgiçer, B.; Xing, X.; Kumar, K. *J. Am. Chem. Soc.* **2001**, *123*, 11815−11816.
(17) Bilgiçer, B.; Fichera, A.; Kumar, K. *J. Am. Chem. Soc.* **2001**, *123*, 4393−4399.
(18) Tang, Y.; Tirrell, D. A. *J. Am. Chem. Soc.* **2001**, *123*, 11089−11090.
(19) Wang, P.; Tang, Y.; Tirrell, D. A. *J. Am. Chem. Soc.* **2003**, *125*, 6900−6906.
(20) Tang, Y.; Ghirlanda, G.; Petka, W. A.; Nakajima, T.; DeGrado, W. F.; Tirrell, D. A. *Angew. Chem., Int. Ed.* **2001**, *40*, 1494−1496.
(21) Tang, Y.; Ghirlanda, G.; Vaidehi, N.; Kua, J.; Mainz, D. T.; Goddard, W. A.; DeGrado, W. F.; Tirrell, D. A. *Biochemistry* **2001**, *40*, 2790−2796.
(22) Myers, K. E.; Kumar, K. *J. Am. Chem. Soc.* **2000**, *122*, 12025−12026.
(23) Jenner, G.; Gacem, B. *J. Phys. Org. Chem.* **2003**, *16*, 265−270.
(24) Gladysz, J. A. *Chem. Rev.* **2002**, *102*, 3215−3216.
(25) Gladysz, J. A.; Curran, D. P. *Tetrahedron* **2002**, *58*, 3823−3825.
(26) Wende, M.; Gladysz, J. A. *J. Am. Chem. Soc.* **2003**, *125*, 5861−5872.
(27) Curran, D. P.; Luo, Z. Y. *J. Am. Chem. Soc.* **1999**, *121*, 9069−9072.
(28) Luo, Z. Y.; Zhang, Q. S.; Oderaotoshi, Y.; Curran, D. P. *Science* **2001**, *291*, 1766−1769.
(29) (a) Palmacci, E. R.; Hewitt, M. C.; Seeberger, P. H. *Angew. Chem., Int. Ed.* **2001**, *40*, 4433−4437. (b) Miura, T.; Goto, K. T.; Hosaka, D.; Inazu, T. *Angew. Chem., Int. Ed.* **2003**, *42*, 2047−2051.
(30) (a) Filippov, D. V.; van Zoelen, D. J.; Oldfield, S. P.; van der Marel, G. A.; Overkleeft, H. S.; Drijfhout, J. W.; van Boom, J. H. *Tetrahedron Lett.* **2002**, *43*, 7809−7812. (b) de Visser, P. C.; van Helden, M.; Filippov, D. V.; van der Marel, G. A.; Drijfhout, J. W.; van Boom, J. H.; Noort, D.; Overkleeft, H. S. *Tetrahedron Lett.* **2003**, *44*, 9013−9016.
(31) For synthesis and characterization of **1**, see Supporting Information.
(32) DesMarteau, D. D.; Montanari, V. *Chem. Commun.* **1998**, 2241−2242.
(33) DesMarteau, D. D.; Montanari, V. *Chem. Lett.* **2000**, 1052−1053.
(34) DesMarteau, D. D.; Pennington, W. T.; Montanari, V.; Thomas, B. H. *J. Fluorine Chem.* **2003**, *122*, 57−61.
(35) Zhdankin, V. V.; Stang, P. J. *Chem. Rev.* **2002**, *102*, 2523−2584.
(36) Schnölzer, M.; Alewood, P.; Jones, A.; Alewood, D.; Kent, S. B. H. *Int. J. Pept. Protein Res.* **1992**, *40*, 180−193.
(37) Analysis of the precipitate by ESI-MS after redissolution in $MeOH/H_2O$ revealed that it was predominantly a mixture of the monofluoroalkylated products. See Supporting Information.

JA0479033