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TFA-NHS as bifunctional protecting agent: simultaneous protection and activation of amino carboxylic acids

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Abstract—Reaction of amino carboxylic acids with *N*-trifluoroacetoxy succinimide yields the corresponding trifluoroacetyl protected active NHS esters.

Protected amino carboxylic acid active esters are the key intermediates in the synthesis of combinatorial libraries of organic compounds and peptides.[1] In addition, these compounds are widely used for the extension of linker arms.[2–4] In our projects related to the synthesis of fluorescent dye labeled nucleotide terminators, trifluoroacetyl protected aminocaproic acid *N*-hydroxysuccinimidyl active esters are the key intermediates for extension of the nucleoside/nucleotide side chains. Typically the synthesis of the protected active esters is carried out by a two step procedure.[5] In the first step, the amino functionality is protected by treating with ethyl trifluoroacetate and in the second step, carboxylic acid is converted to the corresponding NHS ester by treating with *N*-hydroxysuccinimide in the presence of DCC or EDC. After the synthesis, the product is purified by lengthy column chromatography. In our continued efforts[6–8] to improve upon the energy transfer (ET) dye terminator chemistry for DNA sequencing, we needed to develop a procedure in which simultaneous protection and activation of the fluorescent dye can be accomplished in high yields.

Herein we report a simple and efficient one-step process for the preparation of *N*-TFA protected carboxylic acid NHS esters (Fig. 1) utilizing *N*-trifluoroacetoxy succinimide (TFA-NHS). TFA-NHS has been known for several years[9]and extensively used in the preparation of NHS esters of carboxylic acids.[10–14]

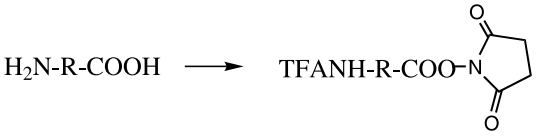

Figure 1.

However, to the best of our knowledge, this reagent has not been utilized for simultaneous protection and activation of the amino and carboxylic acid groups giving rise to the *N*-TFA protected active carboxylic acid NHS esters. Thus, aminocaproic acid on reaction with 6 equiv. of TFA-NHS in dichloromethane in the presence of pyridine gave the TFA-aminocaproic acid-NHS ester in almost quantitative yield. The product isolated is identical to the sample prepared by other methods and characterized by ^{19}F and ^{1}H NMR spectral data. A variety of organic amino acids have been chosen to test the generality of the simultaneous protection/activation. The results indicated that amine and acid functionalities could be protected and activated with equal efficiency.

In a typical synthesis, amino carboxylic acid (3 mmol) was suspended either in dichloromethane and pyridine (15 mL, 2:1) or pyridine (15 mL) to which TFA-NHS (18 mmol) was added under an argon atmosphere at ambient temperature. The reaction mixture was stirred at room temperature for 2–4 h, diluted with dichloromethane (50 mL) and washed with water (3×50 mL). The organic layer was dried over anhydrous sodium sulfate and evaporated. The residue was coevaporated with toluene to give the protected active NHS ester in good yield (Fig. 2). The product obtained by this method is found to be pure[15] and can be used in the conjugation reactions without any further purification.

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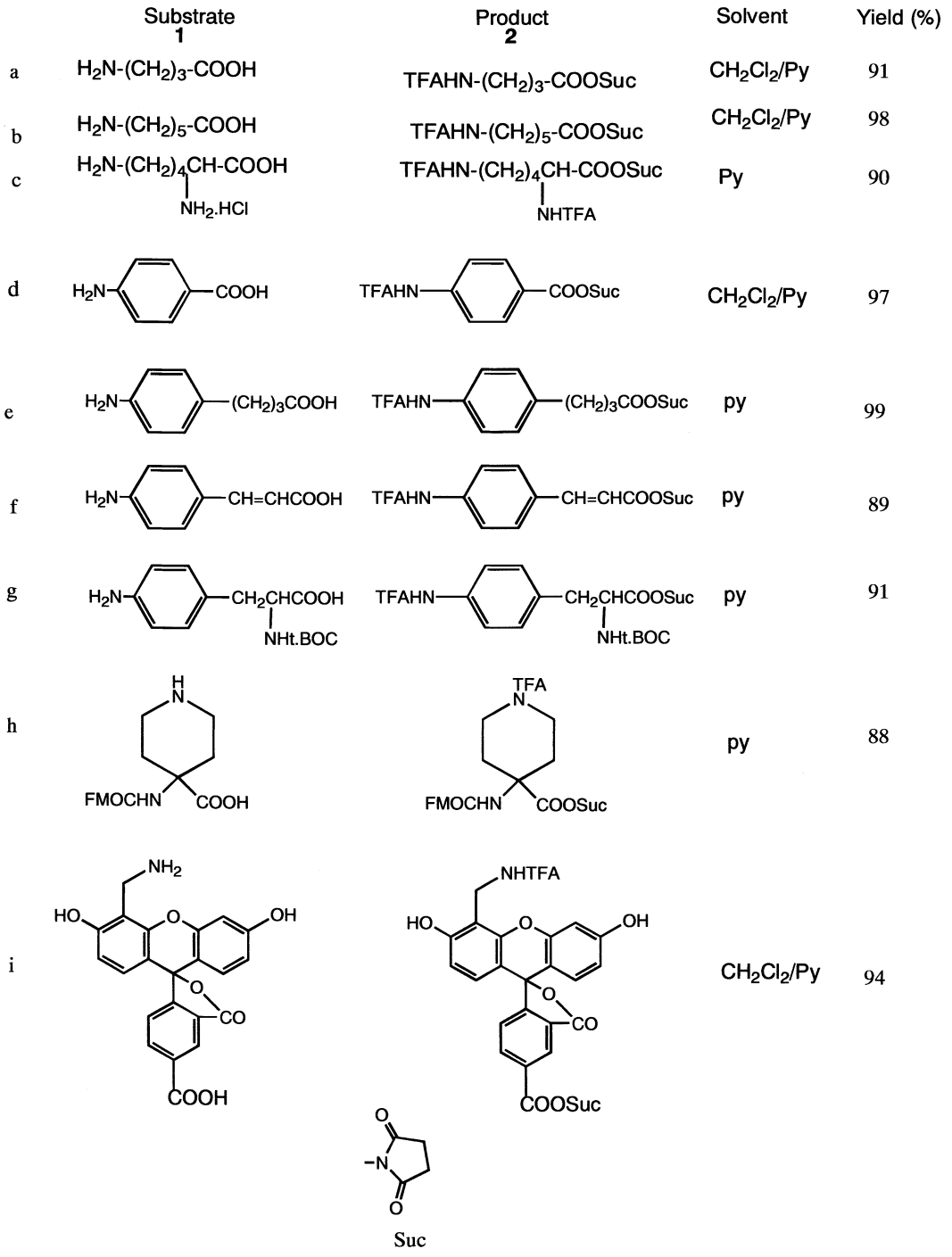

Figure 2.

In conclusion we have demonstrated the practical utility of the TFA-NHS in simultaneous protection and activation of a variety of amino carboxylic acids which include not only simple organic compounds but also biomolecules such as amino acids and fluorescent dyes.

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References

1. Kassel, D. B. *Chem. Rev.* **2001**, *101*, 255 and references cited therein.
2. Urdea, M. S.; Warner, B. W.; Running, J. A.; Stempien, M.; Clyne, J.; Horn, T. *Nucleic Acids Res.* **1988**, *16*, 4937.
3. Alpha-Bazin, B.; Buillemer, S.; Sauvaigo, S.; Mathis, G. *Nucleosides Nucleotides Nucleic Acids* **2000**, *19*, 1463.
4. Kolpashchikov, D. M.; Ivanova, T. M.; Boghachev, V. S.; Nasheuer, H.-P.; Weisshart, K.; Favre, A.; Pestryakov, P. E.; Lavrik, O. I. *Bioconjugate Chem.* **2000**, *11*, 445.

5. De Vos, M. J.; Van Elsen, A.; Bollen, A. *Nucleosides Nucleotides* **1994**, *13*, 2245.
6. Nampalli, S.; Khot, M.; Kumar, S. *Tetrahedron Lett*. **2000**, *41*, 8867.
7. Rao, T. S.; Nampalli, S.; Lavrenov, K.; Zhang, W.; Xiao, H.; Nelson, J.; Kumar, S. *Nucleosides Nucleotides Nucleic Acids* **2001**, *20*, 673.
8. Nampalli, S.; Zhang, W.; Rao, T. S.; Xiao, H.; Kotra, L. P.; Kumar, S. *Tetrahedron Lett*. **2002**, *43*, 1999.
9. Sakakibara, S.; Inukai, N. *Bull. Chem. Soc. Jpn*. **1965**, *38*, 1979.
10. Adamczyk, M.; Chen, Y.-Y.; Gebler, J. C.; Johnson, D. D.; Mattingly, P. G.; Moore, J. A.; Reddy, R. E.; Wu, J.; Yu, Z. *Steroids* **2000**, *65*, 295.
11. Adamczyk, M.; Chen, Y.-Y.; Fishpaugh, J. R.; Johnson, D. D.; Mattingly, P. G.; Pan, Y.; Shreder, K.; Yu, Z. *Bioconjugate Chem*. **2000**, *11*, 714.
12. Adamczyk, M.; Fishpaugh, J.; Mattingly, P. G.; Shreder, K. *Bioorg. Med. Chem. Lett*. **1998**, *8*, 3595.
13. Brush, C. K. US patent 5,371,241, **1996**.
14. Fujii, T.; Kimura, T.; Sakakibara, S. *Bull. Chem. Soc. Jpn*. **1976**, *49*, 1595.
15. Spectral data for representative compounds: **2e**. ^{1}H NMR ($CDCl_3$): δ 2.10 (m, 2H), 2.60 (m, 2H), 2.75 (m, 2H), 2.90 (s, 4H), 7.20 (d, 2H), 7.50 (d, 2H), 7.90 (br s, 1H). ^{19}F NMR ($CDCl_3$): δ −76.08; **2i**, ^{1}H NMR (CD_3CN): δ 2.90 (s, 4H), 4.80 (s, 2H), 6.60 (m, 4H), 6.83 (s, 1H), 7.40 (s, 1H), 8.40 (d, 1H), 8.70 (s, 1H). ^{19}F NMR (CD_3CN): δ −76.66. MS: m/z 597.40 (M−H).