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(2*R*)- and (2*S*)-3-Fluoroalanine and Their *N*-Methyl Derivatives: Synthesis and Incorporation in Peptide Scaffolds

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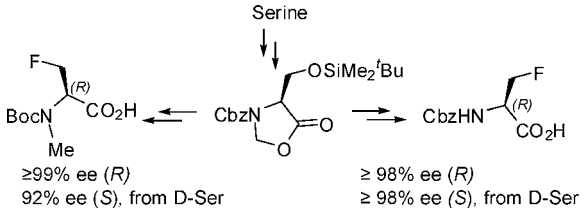
ABSTRACT



A convergent synthetic methodology has been developed to access both (2*S*)- and (2*R*)-3-fluoroalanine and their corresponding *N*-methyl analogues, in optically pure form, through a common oxazolidinone intermediate that can be obtained from L- or D-serine. In addition, a procedure for incorporation of these unnatural amino acids in peptide scaffolds is also disclosed herein that minimizes the occurrence of *β*-elimination during amide bond formation.

Fluorine substitution as a means of modifying the physicochemical properties of a biologically active molecule is a strategy that finds great utility in medicinal chemistry and in biomedical applications.[1]

In the context of a medicinal chemistry program, we required access to both enantiomers of *N*-methyl-3-fluoroalanine.[2] To the best of our knowledge, no reports for the chiral synthesis of *N*-alkyl derivatives of 3-fluoroalanines have appeared in the literature.[3] Therefore, we attempted to adapt some of the known procedures for the synthesis of 3-fluoroalanine, or related derivatives, and subsequently apply the known strategies to prepare the *N*-methyl derivatives as well.[4] As these efforts proved futile, we devised a convergent strategy, using oxazolidinone intermediates, to gain access to both *N*-methylated and primary analogues of 3-fluoroalanines in enantiopure form. A summary of these efforts is provided herein, as well as optimal amide bond formation conditions for efficient incorporation of such amino acids in peptide scaffolds.

Fluorodehydroxylation in the side chain of serine can be effected through reaction with sulfur tetrafluoride in liquid hydrogen fluoride at low temperature with little racemization.[5] However, given the extremely corrosive nature of the

(1) For a selection of review articles on this topic, see: (a) Welch, J. T. *Tetrahedron* **1987**, *43*, 3123−3197. (b) Imperiali, B. *Adv. Biotechnol. Processes* **1988**, *10*, 97−129. (c) Welch, J. T.; Eswarakrishnan, S. *Fluorine in Bioorganic Chemistry*; John Wiley & Sons: New York, 1991. (d) Filler, R.; Kobayashi, Y.; Yagupolskii, L. M. *Organofluorine Compounds in Medicinal Chemistry and Biomedical Applications*; Elsevier: New York, 1993. (e) O'Hagan, D.; Rzepa, H. S. *Chem. Commun.* **1997**, 645−652. (f) Park, K. B.; Kitteringham, N. R.; O'Neill, P. M. *Annu. Rev. Pharmacol. Toxicol.* **2001**, *41*, 443−470. (g) Ismail, F. M. D. *J. Fluorine Chem.* **2002**, *118*, 27−33. (h) Böhm, H.-S.; Banner, D.; Bendels, S.; Kansy, M.; Kuhn, B.; Müller, K.; Obst-Sander, U.; Stahl, M. *ChemBioChem* **2004**, *5*, 637−643. (i) Thayer, A. M. *C&EN* **2006**, *June 5*, 15−24.

(2) For a few recent review articles on the synthesis of fluorinated amino acids, see: (a) Sutherland, A.; Willis, C. L. *Nat. Prod. Rep.* **2000**, *17*, 621−631. (b) Qiu, X.-L.; Meng, W.-D.; Qing, F.-L. *Tetrahedron* **2004**, *60*, 6711−6745. For a review on *β*-fluoro-α-amino acids, see: Sewald, N.; Burger, K. In *Fluorine Containing Amino Acids*; Kukhar, V. P., Soloshonok, V. A., Eds.; John Wiley & Sons: New York, 1995; Chapter 4, pp 139−220.

(3) For a report on the racemic synthesis of *N*-alkylated fluoroamino acids, in very low yields, see ref 6a.

(4) For recent reviews of synthetic methodologies for the preparation of *N*-methyl-α-amino acids, see: (a) Sagan, S.; Karoyan, P.; Lequin, O.; Chassaing, G.; Lavielle, S. *Curr. Med. Chem.* **2004**, *11*, 2799−2822. (b) Aurelio, L.; Brownlee, R. T. C.; Hughes, A. B. *Chem. Rev.* **2004**, *104*, 5823−5846 and references cited therein. For a review on the use of *N*-methyl-substituted amino acids in macrocyclic peptidomimetics, see: (c) Fairlie, D.; Abbenante, G.; March, D. R. *Curr. Med. Chem.* **1995**, *2*, 654−686.

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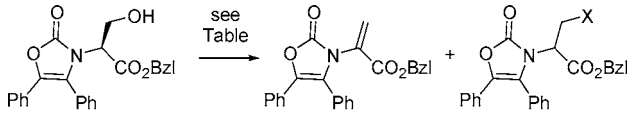
reagents employed in this approach, we looked for more user-friendly synthetic procedures. Several other literature reports exist on the synthesis of 3-fluoroalanines or related derivatives (excluding *N*-alkylated derivatives). Of these reports,[2] the majority include the use of nucleophilic fluorinating agents,[6] although other approaches such as use of electrophilic fluorination[7] and enzymatic chiral resolution[8] are also precedented. One of the commonly used nucleophilic fluorination reagents for the fluorodehydroxylation reaction is DAST.[9,10a] Nonetheless, it is well appreciated that reagents such as DAST can also cause β-elimination to result in formation of dehydroalanine derivatives as byproducts.[11a,12] The work of Pansare and Vederas is notable in this regard, as they were able to successfully use DAST to prepare several β-fluoro-α-amino acids, albeit by employing 4,5-diphenyl-4-oxazolin-2-one as the amine protective group (cf. **1**, Table 1).[12] However, with substrates such as **1**, we were unable to extend their approach (entry 1) to form 3-fluoroalanines despite attempting different reaction conditions (e.g., entries 2 and 3).[13] In all cases, β-elimination byproduct **2** or a derivative, i.e., **3**, prevailed as depicted below; the desired product **4** was not detected under any of the experimental conditions attempted.

Table 1. Screening of Fluorodehydroxylation Reaction Conditions on Substrate **1**

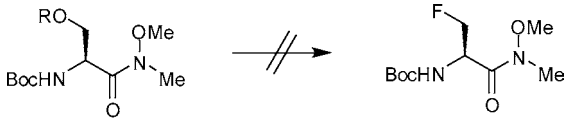


entry	reagents and conditions	HPLC yields, % 1	2	3
1	DAST (1.1 equiv), CH_2Cl_2, −78 °C → rt, 7 h	6	55	20[a]
2	Deoxo-Fluor (1.3 equiv), CH_2Cl_2, −78 °C → rt, 7 h	52	44	0
3	$nC_4H_9SO_2F$ (2 equiv), iPr$_2$NEt (4.5 equiv), $Et_3N(HF)_3$ (1.5 equiv), MeCN, 45 °C, 24 h	0	100	0

[a] In addition, 18% of an intractable byproduct was detected.

Other potential approaches were investigated in hopes of finding a synthetic route that obviates β-elimination during the nucleophilic fluorodehydroxylation of serine (cf. Schemes 1 and 2). One such effort consisted of employing the corresponding Weinreb amide of serine, **5a**,**b** (Scheme 1), for the fluorination chemistry—a strategy that has been effective in circumventing β-elimination under Mitsunobu conditions.[14] Another attempt consisted of testing the utility

Scheme 1. Attempted Use of Weinreb Amide Derivatives of Serine To Effect Fluorodehydroxylation[a]



[a] Reagents and conditions: (a) DAST, CH_2Cl_2; (b) Deoxo-Fluor, CH_2Cl_2; (c) $nC_4H_9SO_2F$, iPr$_2$NEt, $Et_3N(HF)_3$; (d) TBAF, THF. (With substrate **5a**, conditions a−c and with **5b** conditions a and c−d were attempted.)

(5) (a) Kollonitsch, J.; Marburg, S.; Perkins, L. M. *J. Org. Chem.* **1979**, *44*, 771−777. (b) Reider, P. J.; Conn, R. S. E.; Davis, P.; Grenda, V. J.; Zambito, A. J.; Grabowski, E. J. J. *Org. Chem.* **1987**, *52*, 3326−3334.

(6) For a few specific references, in addition to those cited in ref 2, see: (a) Cohen, A.; Bergmann, E. D. *Tetrahedron* **1966**, *22*, 3545−3547. (Racemic synthesis of *N*,*N*-dimethyl 3-fluoroalanine in overall ca. 11% yield, using 1,1,2-trifluoro-2-chloroethyl-diethylamine as fluorinating reagent.) (b) Gershon, H.; McNeil, M. W.; Bergmann, E. D. *J. Med. Chem.* **1973**, *16*, 1407−1409. (Racemic synthesis of 3-fluoroalanines, using liquid HF, in poor yield.) (c) Groth, U.; Schöllkopf, U. *Synthesis* **1983**, 673−675. (DAST was used in this asymmetric synthesis for fluorodehydroxylation. Fluoroalanine, however, cannot be obtained by this route.) (d) Yang, D.; Kuang, L.-R.; Cherif, A.; Tansey, W.; Li, C.; Lin, W. J.; Liu, C.-W.; Kim, E.; Wallace, S. *J. Drug Targeting* **1993**, *1*, 259−267. ([^{18}F]Fluoroalanine prepared in 0.5−1.% yield by side chain displacement in Boc-Ser(OTs)-OMe with K^{18}F.)

(7) In addition to those cited in ref 2, for a few specific references see: (a) Davis, F. A.; Sriajan, V.; Titus, D. A. *J. Org. Chem.* **1999**, *64*, 6931−6934. (Electrophilic fluorination with *N*-fluorobenzenesulfonimide, followed by an interesting but lengthy asymmetric synthesis with chiral sulfinimines. Fluoroalanine was not provided in the examples synthesized, presumably because fluorine alone is a poor stereodirecting group, as stated by the authors.) (b) Gerus, I. I.; Kolomeitsev, A. A.; Kolycheva, M. I.; Kukhar, V. P. *J. Fluorine Chem.* **2000**, *105*, 31−33. (Racemic synthesis of 3-fluoroalanine; no characterization data offered.)

(8) (a) Schmitt, L.; Boniface, J. J.; Davis, M. M.; McConnel, H. M. M. *J. Mol. Biol.* **1999**, *286*, 207−218. (b) Gonçalves, L. P. B.; Antunes, O. A. C.; Pinto, G. F.; Oestericher, E. G. *J. Fluorine Chem.* **2003**, *124*, 219−227. (c) Gonçalves, L. P. B.; Antunes, O. A. C.; Oestericher, E. G. *Org. Process. Res. Dev.* **2006**, *10*, 673−677.

(9) Abbreviations used herein: DAST, (diethylamino)sulfur trifluoride; Deoxo-Fluor, bis(2-methoxyethyl)aminosulfur trifluoride; MorphoCDI, *N*-cyclohexyl-*N*′-(2-morpholinoethyl)carbodiimide methyl-*p*-toluenesulfonate; DPPA, diphenylphosphoryl azide; NMM, *N*-methylmorpholine; IBCF, isobutyl chloroformate; EDCI, 1-ethyl-3-(3′-dimethylaminopropyl)carbodimide hydrochloride; HOAt, 7-aza-1-hydroxybenzotriazole; Ms, methanesulfonyl; TBSCl, *tert*-butyldimethylsilyl chloride; TBS, *tert*-butyldimethylsilyl; TBAF, *tert*-butylammonium fluoride; PTSA, *p*-toluenesulfonic acid.

(10) (a) Middleton, W. J. *J. Org. Chem.* **1975**, *40*, 574−578. (b) Deoxo-Fluor is a more thermally stable variant of DAST; see: Lal, G. S.; Pez, G. P.; Pesaresi, R. J.; Prozonic, F. M.; Cheng, H. *J. Org. Chem.* **1999**, *64*, 7048−7054. (c) For a recent publication on new methodologies for direct conversion of alcohols to fluorides see: Yin, J.; Zarkowsky, D. S.; Thomas, D. W.; Zhao, M. M.; Huffman, M. A. *Org. Lett.* **2004**, *6*, 1465−1468. (Cf. Conditions used in entry 3, Table 1.)

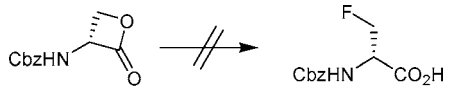
(11) (a) Somekh, L.; Shanzer, A. *J. Org. Chem.* **1983**, *48*, 907−908. The authors used DAST/pyridine to effect stereospecific synthesis of α,β-dehydroamino acids. (b) Use of fluorotrimethylsilanes to effect S_N2 fluorodehydroxylation of serine side chain resulted in a cyclodehydration reaction instead, leading to the formation of corresponding oxazoline, see: Choi, D.; Stables, J. P.; Kohn, H. *J. Med. Chem.* **1996**, *39*, 1907−1916.

(12) Pansare, S. V.; Vederas, J. C. *J. Org. Chem.* **1987**, *52*, 4804−4810. The following points are noteworthy. (i) The use of 4,5-diphenyl-4-oxazolin-2-one as the amine protective group was reported as a means of suppressing the intramolecular attack of the carbamate oxygen (e.g., with Boc or Cbz) on the activated β-carbon. (ii) All the successful examples cited by the authors bear an alkyl substituent at β-carbon, *unlike* the case with **1**. (iii) Elimination byproducts were attenuated but not abrogated; nevertheless, apart from cases in which the β-carbon substituent was extremely bulky (e.g., iPr group), the fluorodehydroxylation product predominated.

(13) We did not have much success with direct fluorination of protected serine derivatives, using DAST or similar reagents, either.

(14) See for example: Panda, G.; Rao, N. V. *Synlett* **2004**, 714−716.

Scheme 2. Attempted Use of the β-Lactone Derived from Serine to Nucleophilic Fluorination Reaction[a]



7 **11b**

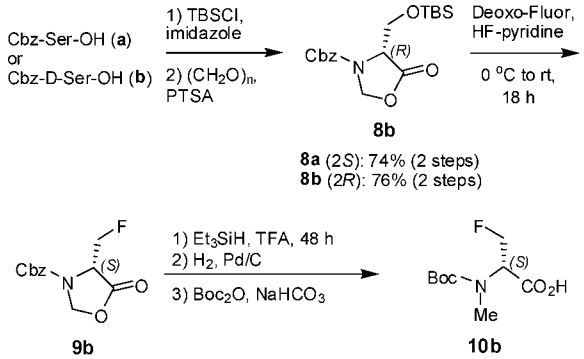
[a] Reagents and conditions: (a) TBAF, THF; (b) HF-pyridine; (c)[16] $Et_3N(HF)_3$ (4 equiv), 110 °C, 18 h.

of the β-lactone derived from serine, **7** (Scheme 2), under nucleophilic fluorination conditions.[15] None of these efforts proved fruitful: either no reaction was observed, or an intractable mixture, which occasionally contained traces of the desired product, was obtained (cf. Schemes 1 and 2).

The utility of oxazolidinone derivatives of amino acids as synthetic intermediates both for the synthesis of *N*-alkylated amino acids[17] and as a protective group strategy is well established.[18] We thus decided to investigate the potential utility of such oxazolidinone derivatives as a common intermediate for accessing both 3-fluoroalanine and its *N*-methyl derivatives in enantiopure form.

As the oxazolidinone **8** showed little propensity toward β-elimination during fluorodehydroxylation, we were able to successfully employ this approach for the synthesis of the title compounds (Scheme 3). Starting with Cbz-Ser-OH or

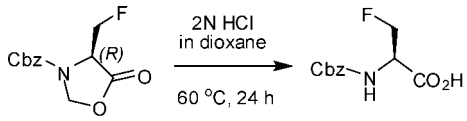
Scheme 3. Synthesis of Boc-*N*-Me-(2*R*)-3-fluoroalanine and Boc-*N*-Me-(2*S*)-3-fluoroalanine



Cbz-D-Ser-OH, the TBS[9] protection of the side chain alcohol, followed by the formation of the oxazolidinone with paraformaldehyde, was effected through standard conditions to obtain intermediates **8a** and **8b**, respectively.[19] A number of reaction conditions were then explored for the conversion of the silyl-protected oxazolidinones (Scheme 3, **8a**,**b**) to their corresponding fluoro congeners (Scheme 3, **9a**,**b**). Initially, we carried out the fluorodehydroxylation reaction after having isolated the free alcohol by treatment of the silylated oxazolidinones (**8a**, **8b**) with HF-pyridine. Upon additional optimization studies, we observed that desilylation and fluorodehydroxylation steps can be conducted in a one-pot manner through treatment of the silylated intermediate with HF-pyridine (8 equiv) and Deoxo-Fluor (2 equiv).[10b,20] The fluorinated oxazolidinones (**9a**, **9b**) were then reduced by using the triethylsilane/TFA conditions reported by Freidinger[17] and co-workers, although rather sluggishly, requiring 2 days for its completion.[21] During this prolonged acidolytic reductive ring-opening, the Cbz protective group was partially cleaved. As we needed an acid labile protective group, such as Boc, in the final product, we simply proceeded to the hydrogenolytic deprotection of the remaining Cbz, followed by protection of the secondary amine by the Boc protective group to obtain both antipodes of *N*-methyl-3-fluoroalanine (Scheme 3, **10a**,**b**) in ≥92% ee (chiral HPLC) and with an overall yield of ≥50%.

As depicted in Scheme 4, treatment of intermediates **9a** or **9b** with HCl (2 N) in dioxane[18c] furnished the deprotection

Scheme 4. Synthesis of Cbz-(2*R*)-3-fluoroalanine and Cbz-(2*S*)-3-fluoroalanine from **9a** and **9b**



9a (2*R*) **11a** 93%; ≥ 98% ee
9b (2*S*) **11b** 99%; ≥ 98% ee

of the oxazolidinone, thus providing both (2*R*)- and (2*S*)-3-fluoroalanine in good yields and high enantiomeric excess. Our efforts in using alternative conditions[18a,b,d,f] for cleavage of the oxazolidinone met with little or no success.

With both enantiomers of *N*-Me-fluoroalanine in hand, we proceeded to the subsequent step in the synthesis of our target

(15) Both 3-chloroalanine and 3-bromoalanine, but not 3-fluoroalanine, were reported to be accessible in this manner by Vederas and co-workers; see: (a) Arnold, L. D.; Kalantar, T. H.; Vederas, J. C. *J. Am. Chem. Soc.* **1985**, *107*, 7105−7109. (b) Arnold, L. D.; May, R. G.; Vederas, J. C. *J. Am. Chem. Soc.* **1988**, *110*, 2237−2241.

(16) These conditions are similar to those reported for ring opening of epoxides to form fluorohydrins; see: Muehlbacher, M.; Poulter, C. D. *J. Org. Chem.* **1988**, *53*, 1026−1030.

(17) Freidinger, R. M.; Hinkle, J. S.; Perlow, D. S.; Arison, B. H. *J. Org. Chem.* **1983**, *48*, 77−81 Also see references cited in footnote 4.

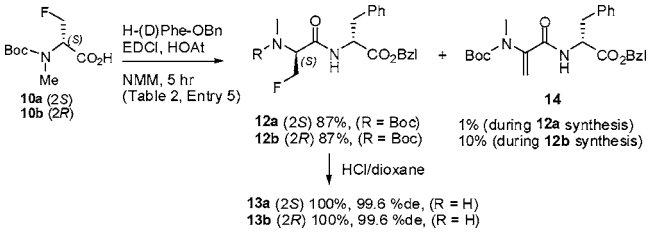
(18) See for example: (a) Itoh, M. *Chem. Pharm. Bull.* **1969**, *17*, 1679−1686. (b) Blaser, D.; Seebach, D. *Liebigs Ann. Chem.* **1991**, 1067−1078. (c) We modified the conditions reported in this reference to avoid deprotection of the Cbz protective group (cf. Scheme 4): Natalini, B.; Mattoli, L.; Pellicciari, R.; Carpenedo, R.; Chiarugi, A.; Moroni, F. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 1451−1454. (d) Thompson, M. J.; Mekhalfia, A.; Hornby, D. P.; Blackburn, G. M. *J. Org. Chem.* **1999**, *64*, 7467−7473. (e) Allevi, P.; Cribiù, R.; Anastasia, M. *Tetrahedron Lett.* **2004**, *45*, 5841−5843. (f) Allevi, P.; Anastasia, M. *Tetrahedron Lett.* **2003**, *44*, 7663−7665.

(19) (a) Aurelio, L.; Brownlee, R. T. C.; Hughes, A. B.; Sleebs, B. E. *Aust. J. Chem.* **2000**, *53*, 425−433. (b) Luo, Y.; Evindar, G.; Fishlock, D.; Lajoie, G. *Tetrahedron Lett.* **2001**, *42*, 3807−3809. (c) Aurelio, L.; Box, J. S.; Brownlee, R. T. C.; Hughes, A. B.; Sleebs, B. E. *J. Org. Chem.* **2003**, *68*, 2652−2667 and references cited therein.

(20) We obtained better results when using Deoxo-Fluor as compared to alternative fluorinating reagents such as DAST or morpholinosulfur trifluoride.

(21) Zhang, S.; Govender, T.; Norström, T.; Arvidsson, P. I. *J. Org. Chem.* **2005**, *70*, 6918−6920. New and improved conditions are reported in this publication. Despite multiple attempts, use of the conditions reported by Zhang et al. with oxazolidinones such as **9a**,**b** did not faciliate the reaction.

Table 2. Screening of the Amide Bond Formation Conditions for the Synthesis of N-Methylfluoroalanine Peptides[9]



		yields, %[a]	
entry	reagents and conditions	**12b**	**14**
1	MorphoCDI, Et_3N (1.5 equiv), MeCN (0.05 mM), 16 h, rt	14	0
2	DPPA, NMM (1.5 equiv), DMF (0.2 mM), 16 h, 0 °C → rt	3	5
3	IBCF, NMM (1.5 equiv), THF (0.2 mM), 16 h, −20 °C → rt	15	0
4	EDCI, HOAt, Et_3N (1.5 equiv), THF:CH_2Cl_2 (1:1, 0.2 mM), 5 h, 0 °C → rt	62[b]	10
5	EDCI, HOAt, NMM (1.5 equiv), THF: CH_2Cl_2 (1:1, 0.2 mM), 5 h, 0 °C → rt	87[b]	10

[a] Refers to partially purified HPLC yields (UV) except where indicated otherwise. [b] Isolated and purified yield.

structures, i.e., formation of the *N*-Me-fluoroalanine-containing peptides. The susceptibility of fluoroalanine toward β-elimination during the amide bond formation is documented in the literature.[22] However, the optimal 3-fluoroalanine amide bond coupling conditions reported were not very successful in the case of formation of **12b** (entry 1, Table 2). After several experiments, satisfactory conditions for the formation of **12b** were obtained through the use of EDCI/HOAt coupling reagents together with the milder base NMM (pK_a = 7.38,[23] entry 5, Table 2). Under these conditions, the amide bond formation proceeded without detectable epimerization (cf. Table 2). Once incorporated in the peptide scaffold, the fluoroalanine moiety proved stable toward further β-elimination.[24,25]

In summary, we have discovered a convergent route for the synthesis of both enantiomers of 3-fluoroalanine and their respective *N*-Me analogues through the use of oxazolidinones **8a**,**b** (cf. Scheme 3) as synthetic intermediates. Moreover, we report optimized amide coupling conditions that allow effective incorporation of such amino acids in peptide scaffolds in good yields and with minimal β-elimination and no detectable epimerization. In view of the preponderance of fluorine-containing pharmaceuticals, including fluoropeptides, the advances discussed herein should prove helpful in the context of future endeavors in this area.

Supporting Information Available: Experimental procedures and full characterization of compounds **1**, **8a**,**b**, **9a**,**b**, **10a**,**b**, **11a**,**b**, **12a**,**b**, and **13a**,**b**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(22) Mitra, A. K.; Ostashevsky, I.; Brewer, C. F. *Int. J. Peptide Protein Res.* **1983**, *22*, 495−501. The reaction times reported in their optimized conditions are fairly long (48 h at rt), attributed to a "deactivating effect" by fluorine.

(23) Carpino, L. A.; Ionescu, D.; El-Faham, A. *J. Org. Chem.* **1996**, *61*, 2460−2465.

(24) Several observations are noteworthy in this regard. (i) No β-elimination side product was detected upon treatment of **13b** with neat Et_3N (>800 equiv) for 16 h at room temperature (HPLC). (ii) In ref 22, the authors prepare Cbz-3-fluoroalanine in 82% yield in a procedure that involves treatment for 2.5 h with NaOH (2N). (iii) In the work described by Patchett et al. (see below), 21 equiv of LDA was required to eliminate HF in [3-fluoro-D-Ala[8]]cyclosporin A. (Cf.: Patchett, A. A.; Taub, D.; Hensens, O. D.; Goegelman, R. T.; Yang, L.; Dumont, F.; Peterson, L.; Sigal, N. H. *J. Antibiot.* **1992**, *45*, 94−102.) Collectively these observations tend to indicate that the tendency of 3-fluoroalanine to undergo β-elimination is a liability mainly at the activated ester stage, but the fluoropeptide itself is stable to this side reaction.

(25) For a recent report on the synthesis of 3-fluorodehydroalanines see: Zhou, H.; van der Donk, W. A. *Org. Lett.* **2001**, *3*, 593−596.