JOC Article

Design and Synthesis of Novel Fluoropeptidomimetics as Potential Mimics of the Transition State during Peptide Hydrolysis

Subhash C. Annedi,[†] Weiyong Li,[†] Sheeba Samson,[†] and Lakshmi P. Kotra*[,†,‡]

Faculty of Pharmacy, Molecular Design and Information Technology Center, and Department of Chemistry, University of Toronto, 19 Russell Street, Toronto, Ontario, M5S 2S2, Canada

p.kotra@utoronto.ca

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α-Fluoroamino acids were targeted in our ongoing efforts to design novel fluoropeptidomimetics (**1**) as potential protease inhibitors. α-Fluoroglycine derivative (**2**) and α-fluoro-*β*-aminoethanethiol derivatives (**3**−**9**) were synthesized for the first time en route to obtain the peptidomimetic moiety **1**. The stability of **2**−**9** was investigated under organic as well as aqueous conditions. The stability of **3**−**9** under acidic and basic conditions, the effect of substitution at C-2 position, and potential biological activities are discussed.

Introduction

Peptidomimetics have been a focus of investigation for several years and some transition-state analogues that mimic the transition state during the hydrolysis of a peptide bond have been successful therapeutic agents.[1] The transition-state analogue strategy is based on the hypothesis that enzymes bind tighter to their transition states than to their corresponding substrate or the products.[2] Such strategies involving peptidomimetics include the hydroxyethylene moiety, where the tetrahedral oxyanion species during peptide hydrolysis is substituted with a hydroxyethylene moiety, and the latter is more stable than the oxyanion species under physiological conditions for therapeutic development.[3,4]

The mechanism of hydrolysis of a peptide bond by a serine protease involves an oxyanion species (Figure 1).[4] This species is formed two times during the hydrolysis of one peptide bond, once during the acylation of the protease and then again during the deacylation of the acyl−enzyme species by a water molecule. Fluoropeptidomimetics, such as the derivatives of α-fluoroethylamine or α-fluoroethanethiol or α-fluoroethanol (Figure 1), are designed to mimic the oxyanion by a fluorine atom and the −NH− in the peptide bond by a −S− or −O−. In these compounds the sp^2-hybridized carbonyl group (Scheme 1) in the peptide moiety such as in **10** (planar) is substituted by an sp^3-tetrahedral carbon with a fluorine atom generating a peptidomimetic such as **1**. The latter tetrahedral carbon with a fluorine substitution may mimic the transition state during the hydrolysis of peptide bond by proteases. The design of the peptidomimetic moiety **1** is based on the hypothesis that the fluorine atom will occupy the position of the oxyanion in the active site of serine proteases and interact with the oxyanion hole via hydrogen bonds. This interaction may result in the elimination of fluorine facilitated by the lone pair electrons on the heteroatom (X = NH, S, O). This would generate a reactive species such as **11** in the active site of the protease, leading to the formation of a covalent bond between the species **11** and a nucleophilic residue in the vicinity such as a serine. Key moiety **12** was designed as the prototype moiety with X = NH or S, which may be derivatized to obtain the intended compounds such as **1**. α-Fluoroglycine derivatives (**13**) and 2-amino-α-fluoroethanethiol derivatives (**14**) are good starting points to prepare target compounds such as **1** (X = NH, S). Preparation of compounds such as **13** and **14** has been of interest to some research groups in the

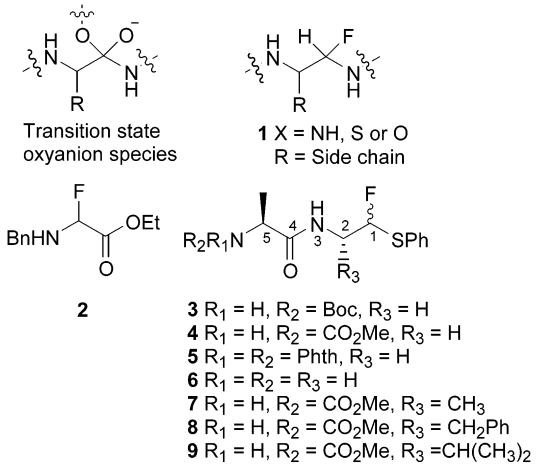

FIGURE 1.

* Corresponding author: Tel. (416) 978-2882. Fax. (416) 978-8511.
† Faculty of Pharmacy, Molecular Design and Information Technology Center.
‡ Department of Chemistry.

(1) Radzicka, A.; Wolfenden, R. *Methods Enzymol.* **1995**, *249*, 284−312.
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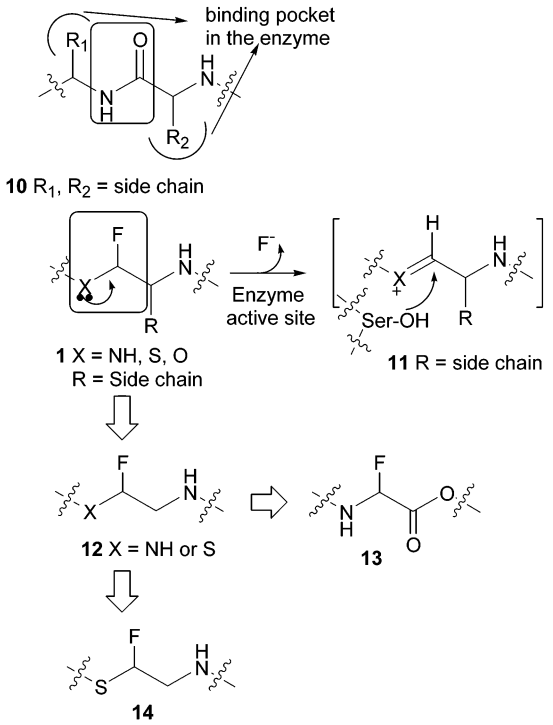
SCHEME 1



past.[5] Several attempts were reported in the literature to obtain both α-fluoro amino acids as well as α-fluoro-*β*-aminoethanethiol derivatives.[6] Many attempts failed due to the chemical instability of the "−NH−CHF−" moiety. Recently, Bailey et al. reported the synthesis of trimethylammonium α-fluoroglycine; however, no success has been reported so far in preparing α-fluoroglycine without a quaternary amine.[7] This report describes our successful synthesis of α-fluoroglycine (**2**) containing the −NH−CHF− moiety, several α-fluoro-*β*-aminoethanethiol derivatives **3**−**9**, and their chemistry. Some insight into the stability of these compounds and their biological compatibility and potential biological activities are revealed for the first time.

Results and Discussion

We first started investigating the synthesis of α-fluoro-α-amino acids, particularly glycine derivatives, because these compounds could serve as precursors to synthesize target intermediates such as **1**. Many unsuccessful attempts were made, which included the fluorination of *N*-diprotected α-amino acids, conversion of the amide ketone into difluoro derivative, or reduction of the amide ketone in to the corresponding alcohol. In the course of these investigations, treatment of benzylamine with ethyl bromofluoroacetate in anhydrous DMF at 0 °C for 15 min gave α-fluoro-*N*-benzyl ethyl glycine **2** in 21% yield (Scheme 2). The same reaction in other solvents such as THF, DMSO, and $CHCl_3$ did not yield an isolable product, but a complex mixture was obtained as observed on TLC. In our experiments, a prolonged reaction time either reduced the yield or resulted in no product isolation. Compound **2** was characterized using ^{1}H, ^{13}C, and ^{19}F NMR and mass spectral analyses. This reaction was monitored by conducting the experiment in an NMR tube using DMF-d_7 as the solvent and recording ^{1}H NMR spectra at various time points. A characteristic peak at 4.85 ppm for the methylene protons of benzyl moiety were observed as the reaction progressed (Figure 2). A gradual appearance of this characteristic peak at 4.85 ppm up to 120 min was noted, implying the formation of the α-fluoro analogue **2** in situ. On the basis of the TLC and isolated yields (21%), the reaction did not appear to go to completion; thus, in the NMR experiments, we did not observe completion of the reaction, even up to 120 min.

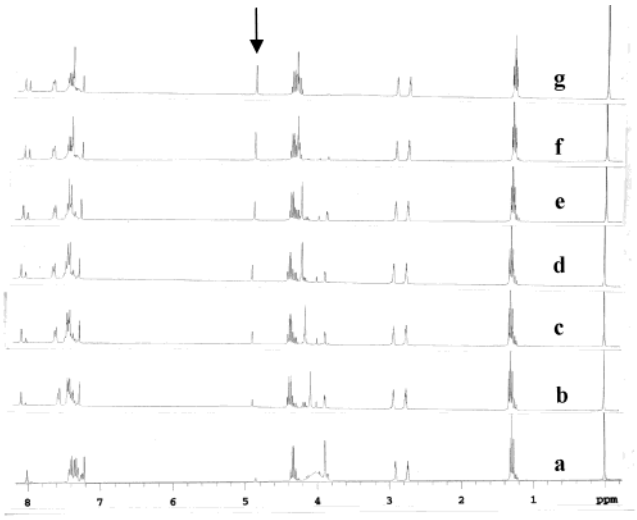
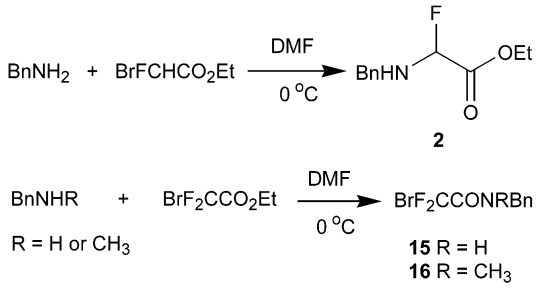


FIGURE 2. ^{1}H NMR spectra during the reaction between benzylamine and ethyl bromofluoroacetate at $t = 0$ (a), 5 (b), 10 (c), 20 (e), 60 (f), and 120 min (g). The reaction was carried out in deuterated dimethyl formatminde. The methylene peak is shown by an arrow.

SCHEME 2



We attempted the reduction of ethyl ester **2** to obtain the alcohol en route to the amino derivative **12**. Reduction of ethyl ester **2** using $NaBH_4$ or DIBAL-H at 0 °C did not yield the desired product. Conceivably, these reaction conditions are causing the fluoride elimination leading toward decomposed products. Following the synthesis of **2**, we were interested in exploring α,α-difluoroglycine derivatives. Ethyl bromodifluoroacetate was treated with benzylamine to obtain compound **15** as the only product

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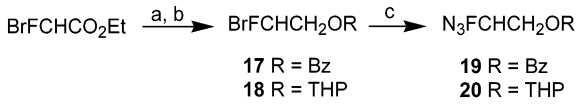
SCHEME 3[a]



[a] Reagents: (a) $NaBH_4$, MeOH; (b) BzCl, Et_3N, CH_2Cl_2 or DHP, PTSA, Et_2O; (c) NaN_3, DMSO, 50 °C.

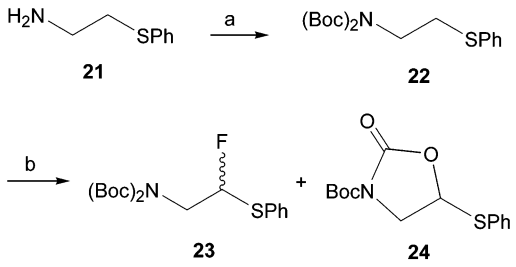
(Scheme 2), but not a difluoro derivative of **2**. No further attempts were made in this direction. Thus, compound **2** could not be utilized in further reactions due to its poor stability (vide infra).

The stability of compound **2** was investigated to better understand its utility in subsequent reactions. During the purification of compound **2**, column chromatography appeared to decrease the yields of the isolated product. Compound **2** was relatively stable in $CDCl_3$ at room temperature, when the ^1H NMR spectrum of **2** was monitored for up to 5 h. The ^1H peaks characteristic for compound **2** were consistent, although some noise peaks were observed in the region between 2 and 5 ppm, as the time elapsed to 5 h. In the ^1H NMR experiments using $CDCl_3/D_2O$ (9:1) as the solvent, we did not see ^1H peaks characteristic of compound **2** and the compound appeared to have decomposed.

Due to the observed instability of compound **2** containing a secondary amine, we were interested in studying its derivatives containing various groups on the amino moiety of α-fluoroglycine. Thus, the synthesis of α-fluoroglycine analogues was investigated using several primary and secondary amines such as benzyl carbamate, *N*-benzyl methylamine, and *N*-(CBz)benzylamine, and fully protected α-fluoroglycine derivatives were the anticipated products. When ethyl bromodifluoroacetate was treated with *N*-benzyl methylamine, amide **16** was isolated as a major product (Scheme 2), where as in the cases with benzyl carbamate or *N*-CBz-benzylamine, starting material was recovered. In cases where a relatively strong base (such as NaOH) was used to activate the amine, decomposition of the starting material was observed.

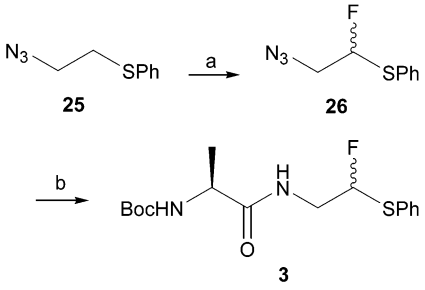
An alternate approach to compound **12** was considered by reducing ethyl bromofluoroacetate and then condensing with an amine. Ethyl bromofluoroacetate was subjected to reduction at room temperature to give the corresponding alcohol, and the alcohol was protected with either a benzoyl or a THP moiety to obtain compound **17** or **18** (Scheme 3).[8] Substitution of the bromo moiety in compound **17** or **18** with various primary and secondary amines [$BnNH_2$, $(CBz)_2NH$, CBzNHMe] under various conditions [$(i\text{-}Pr)_2EtN$, DMAP, NaOH, K_2CO_3, NaH and NaOEt] resulted in either the recovery or decomposition of starting material. Treatment of compounds **17** and **18** with NaN_3 in DMSO at 50 °C, however, gave the corresponding azides **19** and **20**, respectively, in good yields. Reduction of the azido moiety in compounds **19** and **20** was not successful under several conditions (Pd−C/H_2; Pd−C/H_2/Boc_2O; TPP/THF/H_2O; $SnCl_2$/MeOH; Raney nickel; SmI_2/MeOH/THF), and no product could be isolated. These observations did not bode well for us to introduce the substituents to stabilize the α-fluoroglycine template to synthesize biologically suitable, oxyanion-mimicking peptidomimetics.

SCHEME 4[a]



[a] Reagents: (a) (i) $(Boc)_2O$, Et_3N, Dioxane:H_2O; (ii) $(Boc)_2O$, DMAP, CH_3CN; (b) Selectfluor, Et_3N, CH_3CN.

SCHEME 5[a]



[a] Reagents: (a) Selectfluor, Et_3N, CH_3CN; (b) (i) TPP, THF:H_2O, rt, (ii) BocHN-L-Ala-OH, EDAC, HOBt, CH_2Cl_2.

Then we turned our attention to the synthesis of α-fluoro-β-aminoethanethiol derivatives such as **1** (X = S). Thus, 2-bromoethylamine was first converted to *S*-phenyl derivative **21** (Scheme 4).[9] Compound **21** upon reaction with $(Boc)_2O$ followed by fluorination with Selectfluor at room temperature gave the required α-fluorothio derivative **23** in 20% yield and a cyclic product **24** in 44% yield.[10] To avoid the cyclic byproduct, the target fluoro peptide was obtained through an azide intermediate (**26**) as well as *N*-phthaloyl derivative (**28**). Accordingly, the azide **25** upon fluorination with Selectfluor gave the α-fluoro derivative **26** (Scheme 5).[11] Reduction of the azide **26** to an amine was conveniently carried out by TPP and the amine was coupled to BocNH-L-Ala-OH to give the dipeptide **3** in 41% overall yield from **26**. The deprotection of the Boc group in **3** could not be achieved using standard CF_3CO_2H conditions and the fluorine signature was not found in the ^1H NMR spectrum of the product. This indicated that the α-fluorothio moiety was not stable in acidic media. Due to the acid labile nature of this α-fluorothio moiety, methyl carbamate or phthaloyl group was used as a protecting group for the amine in the subsequent dipeptide synthesis.

Compound **27** upon treatment with Selectfluor (Scheme 6) gave the fluoro derivative **28**.[12] The phthaloyl group

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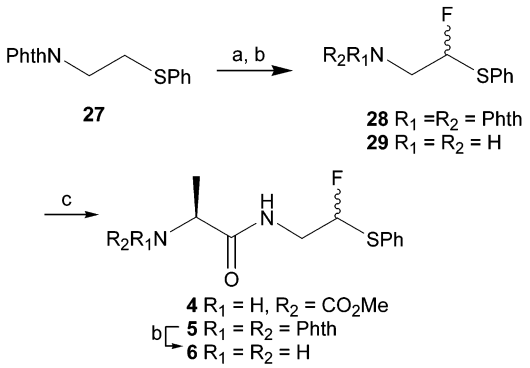
SCHEME 6[a]



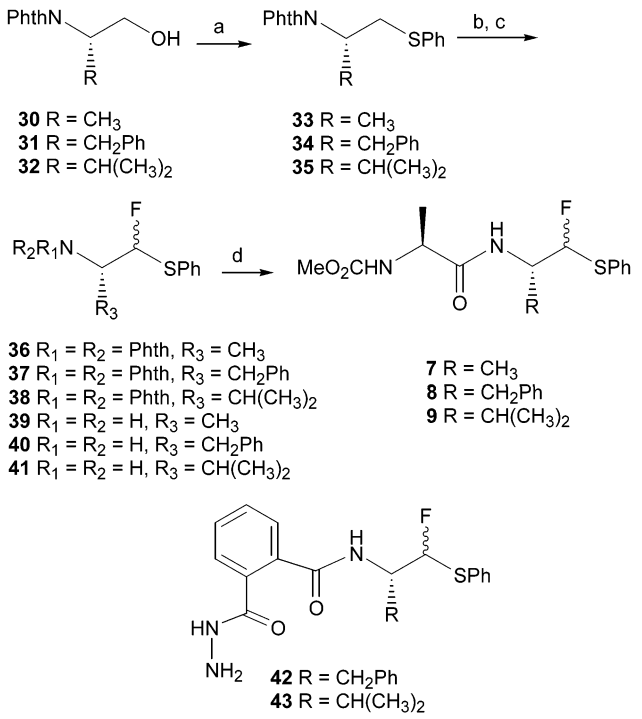
[a] Reagents: (a) Selectfluor, Et_3N, CH_3CN; (b) NH_2–NH_2–H_2O, MeOH; (c) $MeCO_2HN$-L-Ala-OH or PhthN-L-Ala-OH, EDAC, HOBt, CH_2Cl_2.

was removed by hydrazine monohydrate in methanol at room temperature to obtain compound **29**.[13] The α-fluoro monomer **29** was coupled with $MeCO_2NH$-L-Ala-OH and PhthN-L-Ala-OH to give the dipeptides **4** and **5**, respectively. Deprotection of the methyl carbamate in **4** was not successful,[14] whereas the phthaloyl deprotection of **5** was conveniently achieved by hydrazine monohydrate to give compound **6**.

Various alkyl substitutions at the C-2 position of compound **3** were investigated. Several amino alcohols were prepared (Scheme 7) and converted into the appropriate substrates (compounds **30**−**32**) for the fluorination.[15] Compounds **30**−**32** upon treatment with diphenyl disulfide and Bu_3P yielded **33**−**35**, respectively.[16] The monomers **33**−**35** were fluorinated with Selectfluor to give the α-fluoro-*β*-aminothio derivatives **36**−**38**, respectively. Deprotection of the phthaloyl groups in **36**−**38** followed by coupling of the amines **39**−**41** with $MeCO_2$-NH-L-Ala-OH gave the desired dipeptides **7**−**9**, respectively. Compounds **42** and **43** were isolated as byproducts during the transformation of **37** and **38** into **8** and **9**, respectively, due to the incomplete cleavage of the phthaloyl moiety.

The stability of these dipeptides **3**−**9** was studied in organic and aqueous media using ^{1}H NMR spectroscopy. The ^{1}H NMR spectra of the compounds **3**−**9** were recorded in $CDCl_3$ as solvent, and no changes in the NMR spectra were observed even after several days. When the ^{1}H NMR spectra were recorded in CD_3OD/D_2O (1:1), a decrease of 10−40% of the peak intensities was observed after 24 h (Table 1). Compounds without any substitution at C-2, such as in compounds **3**−**6** (for atom numbering, see Figure 1), or with benzyl substitution (**8**) showed a loss of peak intensity of up to 40%. Whereas for those with alkyl substitutions (compounds **7** and **9**), the decomposition was slow (ca. 12%), as characterized by the decrease in ^{1}H NMR peak intensity. Alkyl substitutions at the C-2 carbon provided more stability (**7** and **9**) than without any substitution or with benzyl substitution (Table 1).

SCHEME 7[a]



[a] Reagents: (a) PhSSPh, Bu_3P, DMF; (b) Selectfluor, Et_3N, CH_3CN; (c) NH_2–NH_2–H_2O, MeOH; (d) $MeCO_2HN$-L-Ala-OH, EDAC, HOBt, CH_2Cl_2.

TABLE 1. The ^{1}H NMR Stability Study in Methanol-d_4:D_2O (1:1) after 22 h at Room Temperature[a]

compd no.	% dec obsd	compd no.	% dec obsd
3	31	**7**	11
4	35	**8**	36
5	38	**9**	12
6	a		

[a] a = not determined.

In preliminary biological evaluations, compound **8** was studied for its inhibitory activity against chymotrypsin in a time-dependent enzyme inhibition assay.[17] Com-

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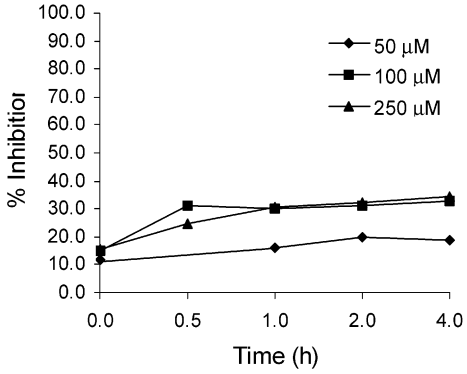

FIGURE 3. Plot of time vs percent inhibition of the activity of chymotrypsin, in a time-dependent assay. Three different concentrations of the inhibitor **8** were tested, as indicated in the graph above.

pound **8** was selected because it has a benzyl group at the pseudo-P1 position that was anticipated to fit into the S1 hydrophobic pocket of chymotrypsin. This site on chymotrypsin is the specificity-determining site and contributes toward the binding of the inhibitor.[18] In this assay, chymotrypsin lost 30% of enzyme activity at 2 h after incubation with compound **8** at 100 and 250 μM concentrations (Figure 3), and this activity was not recovered even after 24 h. These results are encouraging, and the inhibition of chymotrypsin may be due to the formation of proposed reactive species in the active site of chymotrypsin, as proposed earlier. However, further proof of these inhibition studies are needed and mechanistic investigations are in progress, to confirm the exact mode of inhibition.

Experimental Section

General. All anhydrous reactions were performed under an argon atmosphere. All solvents and reagents were obtained from commercial sources and were used as received. Chromatographic purification was performed using silica gel (60 Å, 70−230 mesh). NMR spectra were recorded at 200 or 300 MHz for ^{1}H, 75 MHz for ^{13}C, and 282 MHz for ^{19}F. Chemical shifts were reported in δ ppm using TMS standard for ^{1}H and ^{13}C NMR spectra and external CF_3CO_2H standard for ^{19}F NMR spectra. In some of the ^{13}C NMR spectra, due to the diastereomeric mixtures, the spectral values were reported in a range of chemical shifts (particularly for aromatic carbons) rather than a particular value. Mass spectra were obtained at the Department of Chemistry Mass Spectrometry facility at the University of Toronto.

(Benzylamino)fluoroacetic Acid Ethyl Ester (2). A solution of $BnNH_2$ (0.05 g, 0.46 mmol) in anhydrous DMF (1.0 mL) was treated with ethyl bromofluoroacetate (0.05 mL, 0.46 mmol) at 0 °C. After stirring for 15 min at 0 °C, the reaction mixture was treated with water and was extracted into ethyl acetate. The organic layer was washed with brine and dried (Na_2SO_4). The concentrated organic layer was purified by column chromatography (EtOAc:Hex, 1:4) to yield compound **2** as a syrup (0.02 g, 21%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.35 (t, 3H), 4.34 (q, 2H), 4.86 (s, 2H), 6.65 (d, 1H, J = 51.0 Hz), 7.26−7.37 (m, 5H), 7.72 (s, 1H); ^{1}H NMR (300 MHz, DMF-d_7) δ 1.28 (t, 3H), 4.26 (q, 2H), 4.85 (d, 2H, J = 1.5 Hz), 7.17 (d, 1H, J = 49.5 Hz), 7.24−7.37 (m, 5H), 7.93 (t, 1H, J = 0.75, 1.5 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 13.06, 42.43, 60.62, 83.28 (d, J = 264.8 Hz), 126.17, 126.34, 126.52, 127.08, 127.23, 127.41, 135.08, 161.51; ^{19}F NMR (282 MHz, $CDCl_3$) δ −70.45 (d, J = 51.0 Hz); MS-EI m/z 191 (17), 166 (62), 117 (42), 91 (100), 65 (23).

(2-(Phenylsulfanyl)ethyl)carbamic Acid Di-*tert*-butyl Ester (22). A solution of compound **21** (0.5 g, 0.32 mmol) and Et_3N (1.04 mL, 0.75 mmol) in dioxane:H_2O (2.0 mL, 4:1) was treated with Boc_2O (0.71 g, 0.32 mmol) at 0 °C. After 10 min, more Et_3N (0.4 mL) was added and the mixture was stirred for another 2.5 h at room temperature. The reaction mixture was concentrated; dissolved into ethyl acetate; washed with 10% citric acid solution, 10% $NaHCO_3$ solution, and brine; and dried (Na_2SO_4). The combined organic layers were concentrated to obtain the monoprotected amino derivative as a syrup (0.692 g, 84%).

A solution of the above crude amino derivative (0.475 g, 1.87 mmol) in anhydrous CH_3CN (3.0 mL) was treated with DMAP (0.02 g, 0.18 mmol) and Boc_2O (0.4 g, 1.87 mmol) at 0 °C. The reaction mixture was stirred overnight at room temperature, concentrated, dissolved into ether, washed with water and brine, and dried (Na_2SO_4). The combined organic layers were concentrated, and the crude product was purified by column chromatography (EtOAc:Hex, 5:95) to obtain compound **22** as a syrup (0.6 g, 91%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.47 (s, 18H), 3.05−3.10 (m, 2H), 3.76−3.81 (m, 2H), 7.13−7.38 (m, 5H).

(2-Fluoro-2-(phenylsulfanyl)ethyl)carbamic Acid Di-*tert*-butyl Ester (23). A suspension of Selectfluor (0.05 g, 0.14 mmol) in anhydrous CH_3CN (2.0 mL) was treated with compound **22** (0.05 g, 0.14 mmol) in anhydrous CH_3CN (1.0 mL). After stirring for 15 min, Et_3N (0.019 mL, 0.14 mmol) was added and the mixture was stirred for another 10 min. The reaction mixture was then treated with water and extracted into ether. The organic layer was washed with saturated $NaHCO_3$ solution and brine and dried (Na_2SO_4). The concentrated organic layer was purified by column chromatography (EtOAc:Hex, 5:95) to obtain compound **23** (0.01 g, 20%) with a cyclic byproduct **24** (0.018 g, 44%). **23**: solid; mp 59−61 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.48 (s, 18H), 3.92 (ddd, 1H, J = 4.5 Hz), 4.07, 4.12 (dt, 1H, J = 8.4 Hz), 5.93 (ddd, 1H, J = 4.2, 55.0 Hz), 7.30−7.32 (m, 3H), 7.49−7.52 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 28.37, 49.38 (d, J = 27.0 Hz), 83.17, 99.29 (d, J = 219.5 Hz), 128.42, 129.13, 131.65, 132.97, 152.05; ^{19}F NMR (282 MHz, $CDCl_3$) δ −77.08 (ddd, J = 8.1, 54.8 Hz); EI-MS m/z 371 (M^+, 21), 353 (M − F, 6), 215 (33), 195 (58), 154 (100), 57 (72). **24**: solid; mp 83−85 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.49 (s, 9H), 3.80 (dd, 1H, J = 5.7, 11.2 Hz), 4.24 (dd, 1H, J = 8.7, 11.2 Hz), 5.72 (dd, 1H, J = 5.7, 8.7 Hz), 7.32−7.37 (m, 3H), 7.52−7.56 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 28.22, 48.89, 79.68, 84.31, 129.21, 129.34, 129.86, 133.70, 148.59, 150.46; EI-MS m/z 295 (M^+, 25), 178 (23), 135 (29), 110 (100), 57 (81).

(2-Azido-1-fluoroethylsulfanyl)benzene (26). A suspension of Selectfluor (5.44 g, 15.36 mmol) in anhydrous CH_3CN (30 mL) was treated with compound **25** (2.5 g, 13.96 mmol) in anhydrous CH_3CN (20 mL). After stirring for 30 min. Et_3N (1.94 mL, 13.96 mmol) was added and stirred for another 10 min. The reaction mixture was purified as described for **23** to obtain compound **26** as a syrup (1.32 g, 48%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.49−3.58 (m, 2H), 5.80 (ddd, 1H, J = 4.5, 53.0 Hz), 7.30−7.34 (m, 3H), 7.49−7.52 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 54.04 (d, J = 52.6 Hz), 99.65 (d, J = 220.4 Hz), 128.82, 129.28, 130.64, 133.19, 133.21; ^{19}F NMR (282 MHz, $CDCl_3$) δ −73.07 (ddd, J = 15.7, 19.1, 54.8 Hz).

[1-((2-Fluoro-2-(phenylsulfanyl)ethyl)carbamoyl)ethyl]-carbamic Acid *tert*-Butyl Ester (3). A solution of compound **26** (0.83 g, 4.22 mmol) in THF:H_2O (5:1, 5 mL) was treated with TPP (1.10 g, 4.22 mmol). The reaction mixture was stirred for 3 h at room temperature, dissolved in ethyl acetate, washed with water and brine, and dried (Na_2SO_4). The combined organic layers were concentrated to obtain the crude amine.

A solution of BocHN-L-Ala-OH (0.79 g, 4.22 mmol) in anhydrous CH_2Cl_2 (10 mL) was treated with EDAC (0.8 g, 4.22

(18) Scheidig, A. J.; Hynes, T. R.; Pelletier, L. A.; Wells, J. A.; Kossiakoff, A. A. *Protein Sci.* **1997**, *6*, 1806−1824.

mmol), HOBt (0.57 g, 4.22 mmol), and the above prepared crude amine at 0 °C. The reaction mixture was stirred at room temperature for 4 h; concentrated; dissolved into ethyl acetate; washed with 10% citric acid solution, 10% $NaHCO_3$ solution, water, and brine; and dried (Na_2SO_4). The organic layers were concentrated and purified by column chromatography (EtOAc:Hex, 1:9) to obtain compound **3** as a syrup (0.65 g, 45%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.35 (dd, 3H, J = 0.9, 7.0 Hz), 1.44 (s, 9H), 3.51−3.83 (m, 2H), 4.15 (brt, 1H), 4.91 (brs, 1H), 5.78 (ddd, 1H, J = 4.8, 53.9 Hz), 6.60 (brs, 1H), 7.29−7.34 (m, 3H), 7.46−7.51 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 18.64, 18.74, 28.63, 43.20 (dd, J = 25.9 Hz), 50.25, 80.36, 99.32 (dd, J = 217.8 Hz), 128.46−132.91 (aromatic), 155.43, 173.05.

2-(2-Fluoro-2-(phenylsulfanyl)ethyl)isoindole-1,3-dione (28). A suspension of Selectfluor (1.35 g, 3.81 mmol) in anhydrous CH_3CN (5 mL) was treated with compound **27** (0.9 g, 3.18 mmol) in anhydrous CH_3CN (5 mL). After stirring for 30 min, Et_3N (0.44 mL, 3.18 mmol) was added and the mixture was stirred for another 10 min. The reaction mixture was purified as described for **23** to obtain compound **28** as a syrup (0.3 g, 32%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.97−4.22 (m, 2H), 6.05 (ddd, 1H, J = 5.1, 54.0 Hz), 7.29−7.32 (m, 3H), 7.48−7.51 (m, 2H), 7.69−7.72 (m, 2H), 7.82−7.85 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 41.17 (d, J = 28.5 Hz), 97.42 (d, J = 220.6 Hz), 123.42, 123.49, 128.52, 129.13, 130.94, 131.70, 131.72, 132.82, 134.11, 134.16, 167.35, 167.54.

2-Fluoro-2-(phenylsulfanyl)ethylamine (29). A solution of compound **28** (0.05 g, 0.16 mmol) in anhydrous MeOH (2.0 mL) was treated with hydrazine monohydrate (0.02 mL, 0.16 mmol) and stirred overnight at room temperature. The reaction mixture was concentrated and the crude product was purified by column chromatography (EtOAc) to obtain compound **29** as a syrup (0.01 g, 36%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.48 (brs, 2H), 3.12 (brd, 2H, J = 14.4 Hz), 5.71 (dt, 1H, J = 5.7, 54.6 Hz), 7.29−7.34 (m, 3H), 7.49−7.52 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 46.34 (d, J = 25.9 Hz), 103.39 (d, J = 213.8 Hz), 128.16, 129.13, 132.18, 132.42, 132.44; ^{19}F NMR ($CDCl_3$) δ −74.66 (dt, J = 16.0, 54.1 Hz).

[1-((2-Fluoro-2-(phenylsulfanyl)ethyl)carbamoyl)ethyl]-carbamic Acid Methyl Ester (4). A solution of $MeCO_2NH$-L-Ala-OH (0.03 g, 0.20 mmol) in anhydrous CH_2Cl_2 (3.0 mL) was treated with EDAC (0.047 g, 0.24 mmol), HOBt (0.027 g, 0.20 mmol), and compound **29** (0.035 g, 0.20 mmol) at 0 °C. The same procedure as that for compound **3** was used to finally obtain compound **4** as a white solid (0.037 g, 61%): mp 89−92 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.37 (dd, 3H, J = 2.4, 2.7 Hz), 3.50−3.85 (m, 5H), 4.26 (m, 1H), 5.41 (brt, 1H, J = 5.7, 6.3 Hz), 5.70, 5.88 (2m, 1H), 6.79 (brs, 1H), 7.30−7.33 (m, 3H), 7.47−7.50 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 18.86, 19.03, 43.23 (dd, J = 25.9 Hz), 50.77, 52.75, 99.40 (dd, J = 217.5 Hz), 128.59, 129.20, 131.07, 132.96, 133.00, 133.02, 156.61, 172.67; ^{19}F NMR ($CDCl_3$) δ −74.72 to −74.41 (m).

2-(1,3-Dioxo-1,3-dihydroisoindol-2-yl)-*N*-(2-fluoro-2-(phenylsulfanyl)ethyl)propionamide (5). A solution of PhthN-L-Ala-OH (0.057 g, 0.26 mmol) in anhydrous CH_2Cl_2 (3.0 mL) was treated with EDAC (0.06 g, 0.31 mmol), HOBt (0.035 g, 0.26 mmol), and compound **29** (0.045 g, 0.26 mmol) at 0 °C. The same procedure as that for compound **3** was used to finally isolate compound **5** as a foam (0.069 g, 71%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.69 (d, 3H, J = 6.9 Hz), 3.47−3.91 (m, 2H), 4.93 (q, 1H), 5.72, 5.90 (2m, 1H, J = 54.3 Hz), 6.53 (m, 1H), 7.30−7.33 (m, 3H), 7.45−7.50 (m, 2H), 7.70−7.74 (m, 2H), 7.80−7.85 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 15.36, 15.41, 43.32 (dd, J = 25.9 Hz), 49.48, 99.23 (d, J = 218.6 Hz), 123.64−134.49 (aromatic), 167.89, 169.65, 169.68; ^{19}F NMR ($CDCl_3$) δ −75.76 to −75.13 (m).

2-Amino-*N*-(2-fluoro-2-(phenylsulfanyl)ethyl)propionamide (6). A solution of compound **5** (0.05 g, 0.13 mmol) in anhydrous MeOH (2.0 mL) was treated with hydrazine monohydrate (0.006 mL) and was stirred overnight at room temperature. The reaction mixture was concentrated and purified by column chromatography (MeOH:$CHCl_3$ 5:95) to obtain compound **6** as a syrup (0.03 g, 94%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.34 (dd, 3H, J = 2.1 Hz), 1.72 (brs, 2H), 3.48−3.85 (m, 3H), 5.73, 5.91 (2m, 1H, J = 2.7, 54.3 Hz), 7.30−7.34 (m, 3H), 7.49−7.53 (m, 2H), 7.72 (brs, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 21.84, 21.86, 42.69 (dd, J = 25.6 Hz), 50.84, 50.89, 99.68 (dd, J = 218.4 Hz), 128.66, 129.36, 131.49, 133.09, 133.09, 176.19; ^{19}F NMR ($CDCl_3$) δ −75.15 (ddd, J = 13.8, 54.1 Hz).

2-(1-Methyl-2-(phenylsulfanyl)ethyl)isoindole-1,3-dione (33). A solution of compound **30** (1.25 g, 6.09 mmol) in anhydrous DMF (3.0 mL) was treated with Bu_3P (2.27 mL, 9.14 mmol) and diphenyl disulfide (1.99 g, 9.14 mmol). The reaction mixture was stirred for 1 h at room temperature and diluted with ether. The organic layer was washed with 1 N NaOH solution and brine and dried (Na_2SO_4). The combined organic layers were concentrated and purified by column chromatography (EtOAC:Hex, 1:9) to obtain compound **33** as a syrup (1.49 g, 83%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.53 (d, 3H, J = 6.9 Hz), 3.18 (dd, 1H, J = 5.4, 13.9 Hz), 3.68 (dd, 1H, J = 9.9, 13.9 Hz), 4.44−4.56 (m, 1H), 7.03−7.09 (m, 1H), 7.13−7.18 (m, 2H), 7.29−7.33 (m, 2H), 7.63−7.68 (m, 2H), 7.71−7.76 (m, 2H).

2-(1-Benzyl-2-(phenylsulfanyl)ethyl)isoindole-1,3-dione (34). A solution of compound **31** (0.2 g, 0.71 mmol) in anhydrous DMF (2.0 mL) was treated with Bu_3P (0.26 mL, 1.06 mmol) and diphenyl disulfide (0.23 g, 1.06 mmol). Same procedure as that for compound **33** was used to obtain compound **34** as a syrup (0.23 g, 84%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.08−3.33 (m, 3H), 3.74 (dd, 1H, J = 10.2, 14.1 Hz), 4.60−4.70 (m, 1H), 6.99−7.26 (m, 10H), 7.59−7.68 (m, 4H).

2-(2-Methyl-1-((phenylsulfanyl)methyl)propyl)isoindole-1,3-dione (35). A solution of compound **32** (1.05 g, 4.50 mmol) in anhydrous DMF (3.0 mL) was treated with Bu_3P (3.3 mL, 6.75 mmol) and diphenyl disulfide (1.47 g, 6.75 mmol). The same procedure as explained for compound **33** was used to obtain compound **35** as a solid (1.23 g, 84%): mp 89−91 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.84 (d, 3H, J = 6.6 Hz), 1.04 (d, 3H, J = 6.9 Hz), 2.34−2.46 (m, 1H), 3.32 (dd, 1H, J = 3.3, 13.8 Hz), 3.72 (dd, 1H, J = 11.7, 13.8 Hz), 3.96−4.05 (m, 1H), 6.99−7.14 (m, 3H), 7.24−7.28 (m, 2H), 7.64−7.68 (m, 2H), 7.71−7.75 (m, 2H).

2-(2-Fluoro-1-methyl-2-(phenylsulfanyl)ethyl)isoindole-1,3-dione (36). A suspension of Selectfluor (0.71 g, 2.02 mmol) in anhydrous CH_3CN (5 mL) was treated with compound **33** (0.5 g, 1.68 mmol) in anhydrous CH_3CN (5 mL). After stirring for 30 min at room temperature, Et_3N (0.23 mL, 1.68 mmol) was added and the mixture was stirred for another 10 min. The reaction mixture was purified as described for **23** to obtain compound **36** as a syrup (0.22 g, 42%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.62 (d, 3H, J = 6.9 Hz), 4.53−4.65 (m, 1H), 6.31 (ddd, 1H, J = 9.9, 52.5 Hz), 7.24−7.28 (m, 2H), 7.32−7.35 (m, 2H), 7.52−7.55 (m, 1H), 7.67−7.75 (m, 2H), 7.77−7.88 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 15.91, 16.09, 49.18 (dd, J = 21.0, 32.4 Hz), 100.99 (dd, J = 221.8 Hz), 123.37−134.78 (aromatic), 167.40, 167.75; ^{19}F NMR (282 MHz, $CDCl_3$) δ −74.15 (ddd, J = 5.3, 53.1 Hz).

2-(1-Benzyl-2-fluoro-2-(phenylsulfanyl)ethyl)isoindole-1,3-dione (37). A suspension of Selectfluor (0.2 g, 0.56 mmol) in anhydrous CH_3CN (3 mL) was treated with compound **34** (0.2 g, 0.51 mmol) in anhydrous CH_3CN (2 mL). After stirring for 30 min at room temperature, Et_3N (0.07 mL, 0.51 mmol) was added and the mixture was stirred for another 10 min. The reaction mixture was purified as described for **23** to obtain compound **37** as a syrup (0.13 g, 65%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.36−3.49 (m, 2H), 4.69−4.81 (m, 1H), 6.40 (ddd, 1H, J = 9.3, 52.8 Hz), 6.99−7.74 (m, 14H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 34.83, 35.16, 54.93 (dd, J = 20.4, 30.8 Hz), 100.41 (dd, J = 222.9 Hz), 123.27−136.61 (aromatic), 167.43, 167.71; ^{19}F NMR (282 MHz, $CDCl_3$) δ −74.91 to −74.64 and −72.49 to −72.22 (2m).

2-[1-(Fluoro(phenylsulfanyl)methyl)-2-methylpropyl]-isoindole-1,3-dione (38). A suspension of Selectfluor (1.3 g,

3.69 mmol) in anhydrous CH_3CN (15 mL) was treated with compound **35** (1.0 g, 3.07 mmol) in anhydrous CH_3CN (20 mL). After stirring for 30 min at room temperature, Et_3N (0.42 mL, 3.07 mmol) was added and the mixture was stirred for another 10 min. The reaction mixture was purified as described for **23** to obtain compound **38** as a syrup (0.31 g, 30%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.95 (dd, 3H, J = 3.6 Hz), 1.04−1.08 (m, 3H), 2.54−2.83 (m, 1H), 4.30−4.40 (m, 1H), 6.44 (ddd, 1H, J = 6.6, 53.7 Hz), 7.26−7.31 (m, 3H), 7.40−7.43 (m, 1H), 7.49−7.52 (m, 1H), 7.69−7.76 (m, 2H), 7.82−7.87 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 19.47, 19.68, 20.76, 20.84, 28.57, 29.84, 59.34 (dd, J = 20.5, 28.2 Hz), 100.64 (dd, J = 225.2 Hz), 123.45, 123.50, 128.40, 128.44, 129.06, 129.14, 131.47, 131.56, 132.60, 132.62, 133.01, 133.03, 134.09, 134.16, 168.14; ^{19}F NMR (282 MHz, $CDCl_3$) δ −73.95 (ddd, J = 5.9, 15.7, 53.0 Hz).

2-Fluoro-1-methyl-2-(phenylsulfanyl)ethylamine (39). A solution of compound **36** (0.175 g, 0.55 mmol) in anhydrous MeOH (2.0 mL) was treated with hydrazine monohydrate (0.02 mL, 0.55 mmol) and stirred for overnight at room temperature. The reaction mixture was concentrated and purified as described for **29** to obtain compound **39** as a syrup (0.095 g, 93%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.17−1.25 (m, 3H), 1.52 (brs, 2H), 3.22−3.36 (m, 1H), 5.54 (ddd, 1H, J = 5.1, 54.4 Hz), 7.24−7.33 (m, 3H), 7.46−7.51 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 19.13, 20.14, 50.79 (dd, J = 23.3 Hz), 107.61 (dd, J = 219.0 Hz), 127.49−133.43 (aromatic); ^{19}F NMR (282 MHz, $CDCl_3$) δ −77.32 to −76.98 and −72.96 to −72.63 (2m).

[1-((2-Fluoro-1-methyl-2-(phenylsulfanyl)ethyl)carbamoyl)ethyl]carbamic Acid Methyl Ester (7). A solution of $MeCO_2$NH-L-Ala-OH (0.043 g, 0.29 mmol) in anhydrous CH_2Cl_2 (2.0 mL) was treated with HOBt (0.036 g, 0.27 mmol), EDAC (0.062 g, 0.32 mmol), and compound **39** (0.05 g, 0.27 mmol) in anhydrous CH_2Cl_2 (1.0 mL) at 0 °C and was purified as described for **3** to obtain compound **7** as a solid (0.065 g, 77%): mp 89−91 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.29−1.43 (m, 6H), 3.66 (s, 3H), 4.32−4.60 (m, 2H), 5.60−5.69, 5.86−5.88 (2m, 2H), 6.84 (m, 1H), 7.28−7.31 (m, 3H), 7.44−7.50 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 16.97, 19.37, 19.64, 48.61 (dd, J = 24.2 Hz), 50.05, 50.76, 52.65, 104.30 (dd, J = 221.5 Hz), 128.12−132.82 (aromatic), 156.49, 171.85, 172.16; ^{19}F NMR (282 MHz, $CDCl_3$) δ −81.27 (ddd, J = 15.7, 20.5, 53.7 Hz).

[1-((1-Benzyl-2-fluoro-2-(phenylsulfanyl)ethyl)carbamoyl)ethyl]carbamic Acid Methyl Ester (8). A solution of compound **37** (0.53 g, 1.35 mmol) in anhydrous MeOH (5.0 mL) was treated with hydrazine monohydrate (0.06 mL, 1.35 mmol) and stirred overnight at room temperature. The solvent was evaporated and the crude product was purified to obtain a nonseparable mixture of compounds **40** and the partially deprotected compound **42**, using the same procedure as described for **29**.

A solution of $MeCO_2$NH-L-Ala-OH (0.21 g, 1.45 mmol) in anhydrous CH_2Cl_2 (4.0 mL) was treated with HOBt (0.178 g, 1.32 mmol), EDAC (0.3 g, 1.58 mmol), and the mixture of compounds **40** and **42** (0.36 g, 1.32 mmol) in anhydrous CH_2Cl_2 (1.0 mL) at 0 °C. The same procedure as described for **3** was used and after chromatographic separation, compounds **8** (0.017 g, 8%) and **42** (0.21 g, 36%) were obtained. Compound **8**: solid; mp 108−110 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.26−1.40 (m, 3H), 2.88−3.16 (m, 2H), 3.66, 3.68 (2s, 3H), 4.15−4.27 (m, 1H), 4.61−4.75 (m, 1H), 5.16 (brdd, 1H), 5.78 (ddd, 1H, J = 2.7, 54.4 Hz), 6.52 (brdd, 1H), 7.19−7.52 (m, 10H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 19.10, 35.71, 37.78, 50.83, 52.74, 53.92 (dd, J = 22.5 Hz), 103.01 (dd, J = 220.9 Hz), 126.89−136.39 (aromatic), 156.35, 172.02; ^{19}F NMR (282 MHz, $CDCl_3$) δ −80.51 (ddd, J = 16.9, 18.8, 55.2 Hz); Compound **42**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.96−3.21 (m, 2H), 3.95 (brs, 2H), 4.77−4.93 (m, 1H), 5.88 (ddd, 1H, J = 3.6, 54.3 Hz), 7.05 (d, 1H, J = 8.7 Hz), 7.14−7.51 (m, 14H), 8.06 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 35.57, 37.57, 54.61 (dd, J = 22.5 Hz), 102.86 (dd, J = 220.9 Hz), 126.87−136.59 (aromatic), 168.30, 168.42, 169.31; ^{19}F NMR (282 MHz, $CDCl_3$) δ −80.39 (ddd, J = 17.4, 20.5, 54.4 Hz).

{**1-[(1-(Fluoro(phenylsulfanyl)methyl)-2-methylpropyl)-carbamoyl]ethyl**}**carbamic Acid Methyl Ester (9).** A solution of compound **38** (0.3 g, 0.87 mmol) in anhydrous MeOH (2.0 mL) was treated with hydrazine monohydrate (0.04 mL, 0.87 mmol) and was stirred overnight at room temperature. The reaction mixture was concentrated and the crude product was purified as described for **29** to give compound **41** and the partially deprotected compound **43** as a nonseparable mixture.

A solution of $MeCO_2$NH-L-Ala-OH (0.15 g, 1.03 mmol) in anhydrous CH_2Cl_2 (4.0 mL) was treated with HOBt (0.126 g, 0.938 mmol), EDAC (0.216 g, 1.12 mmol), and mixture of compounds **41** and **43** (0.2 g, 0.93 mmol) in anhydrous CH_2Cl_2 (1.0 mL) at 0 °C. The same procedure as described for **3** was used to finally obtain compound **9** (0.015 g, 8%) and compound **43** (0.12 g, 37%). **9**: solid; mp 92−94 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.95−1.03 (m, 6H), 1.42 (dd, 3H, J = 6.9, 7.2 Hz), 1.88−2.19 (m, 1H), 3.68 (s, 3H), 4.16−4.39 (m, 2H), 5.33 (m, 1H), 5.86 (ddd, 1H, J = 2.1, 5.1, 54.6 Hz), 6.37 (brs, 1H), 7.30−7.32 (m, 3H), 7.46−7.51 (m, 2H); ^{19}F NMR (282 MHz, $CDCl_3$) δ −78.86 (ddd, J = 15.2, 24.5, 54.1 Hz). **43**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.02−1.07 (m, 6H), 1.95−2.23 (m, 1H), 4.06 (brs, 2H), 4.30−4.56 (m, 1H), 5.94 (ddd, 1H, J = 2.1, 4.8, 54.4 Hz), 6.85 (t, 1H, J = 8.4 Hz), 7.30−7.68 (m, 9H), 8.01 (d, 1H, J = 18.6 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 17.95, 19.46, 19.97, 20.57, 29.42, 31.03, 58.18 (dd, J = 20.4 Hz), 103.35 (d, J = 221.5 Hz), 128.25−134.55 (aromatic), 168.61, 168.76, 169.49, 169.59; ^{19}F NMR (282 MHz, $CDCl_3$) δ −78.75 (ddd, J = 14.3, 23.4, 54.4 Hz).

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Supporting Information Available: Experimental procedures to synthesize compounds **15**−**20** and the NMR spectra for compounds **2**−**9**. This material is available free of charge via the Internet at http://pubs.acs.org.

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