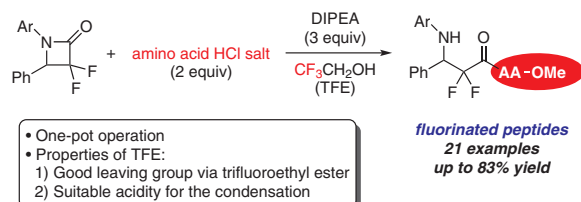


One-Pot Ring-Opening Peptide Synthesis Using α,α -Difluoro- β -Lactams

Atsushi Tarui
Masakazu Ueo
Marino Morikawa
Masahiko Tsuta
Sumika Iwasaki
Noriko Morishita
Yukiko Karuo
Kazuyuki Sato
Kentaro Kawai
Masaaki Omote*

Faculty of Pharmaceutical Sciences, Setsunan University,
Hirakata, Osaka 573-0101, Japan
omote@pharm.setsunan.ac.jp



Received: 17.06.2020
Accepted after revision: 09.07.2020
Published online: 17.08.2020
DOI: 10.1055/s-0040-1707238; Art ID: ss-2020-f0329-op

Abstract α,α -Difluoro- β -lactams successfully underwent ring-opening aminolysis with various amino acids in 2,2,2-trifluoroethanol to afford fluorine-containing peptides. In this aminolysis, it was found that 2,2,2-trifluoroethanol first attacked the α,α -difluoro- β -lactams with cleavage of lactam ring to form the corresponding open-chain 2,2,2-trifluoroethyl esters as reactive intermediates. The trifluoroethyl esters were more electrophilic compared with the corresponding methyl ester and thereby accelerated the aminolysis with various amino acids to form β -amino acid peptides with α,α -difluoromethylene unit.

Key words fluorine, peptide, β -amino acid, α,α -difluoro- β -lactam, 2,2,2-trifluoroethanol

β -Amino acids are considered important non-proteinogenic amino acids and are deliberately incorporated into peptides to change their physical properties and biological activity.¹ In drug discovery, β -amino acids have been used to mimic natural peptide-based antibiotics that have high proteolytic stability *in vivo*.² Protease resistance is an intrinsic property of β -amino acids and has been successfully exploited to produce the aminopeptidase inhibitor, bestatin³ and the more potent analogue **1**⁴ (Figure 1). We have previously focused on these β -amino acid structures, particularly 3-amino-2-hydroxy-3-phenylpropanoic acid moiety, which is found in paclitaxel (Taxol®)⁵ and docetaxel (Taxotere®).⁶ Additionally, we envisioned that a hydroxymethylene group could be replaced by a difluoromethylene unit, which may be useful because fluoroalkyl group sometimes behaves like hydroxyl groups in biological assays.⁷ However, a convenient approach to synthesize peptide **3** is still very challenging using current peptide chemistry.

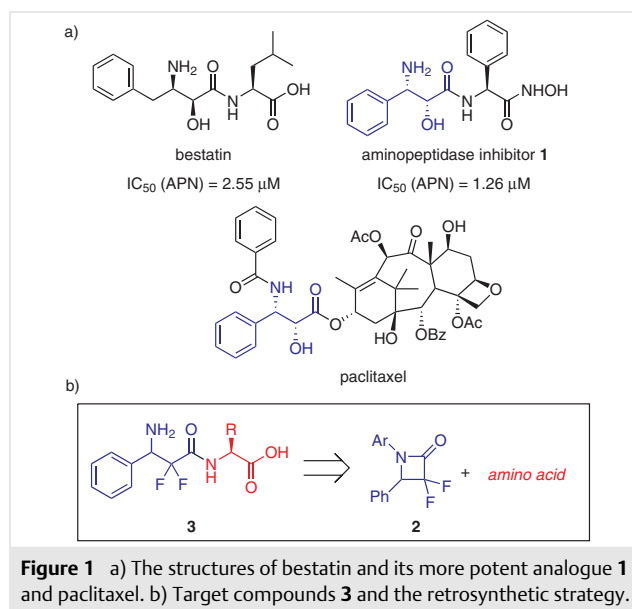


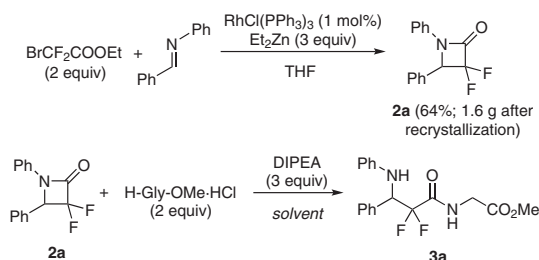
Figure 1 a) The structures of bestatin and its more potent analogue **1** and paclitaxel. b) Target compounds **3** and the retrosynthetic strategy.

Regarding the synthesis of β -amino acid-containing peptides, recent progress in ribosome or enzyme engineering allows β -amino acid incorporation into polypeptides.^{8,9} However, in most cases, organic synthesis is still required to prepare highly functionalized β -amino acids and incorporate them into peptides. To date, there have been several reports on the synthesis of functionalized β -amino acids and related structures,¹⁰ including those with fluorine substituents adjacent to the carbonyl (β^2 -)¹¹ or to the amino (β^3 -)¹² positions. β -Lactam synthon method has been also known for the construction of β -amino acid substructure.^{10a,11a,c,e,f,13} However, to the best of our knowledge, there are only few examples on the one-pot synthesis of

functionalized β -amino acid-containing peptides, except for conventional peptide synthesis, which involves sequential, step-by-step condensation reactions of activated amino acid derivatives.^{11c,13c} In this context, we hypothesized that α,α -difluoro- β -lactams **2** ought to be a good precursor for the one-pot synthesis of **3** via a ring-opening nucleophilic attack by various amino acids, although the product would be a diastereomixture.

For this approach, the synthetic drawback remained in the reactivity of both **2** and the amino acid. Particularly, the nucleophilicity of the nitrogen atom in the amino acid is significantly lower than commonly used amino compounds, so the direct reaction of **2** and amino acid would be suppressed. To check the reactivity of **2** towards amino acids, **2a** was reacted with glycine methyl ester hydrochloride in the presence of *N,N*-diisopropylethylamine (DIPEA) as a non-nucleophilic base (Table 1). The desired substrate **2** was synthesized by our previously reported Rh-catalyzed imino-Reformatsky reaction of ethyl bromodifluoroacetate using diethylzinc in a gram scale reaction.¹⁴

Table 1 Synthesis of α,α -Difluoro- β -lactam **2a** and Solvent Screen for Ring-Opening Peptide Synthesis Using **2a** as an α,α -Difluoro- β -amino Acid Unit^a



Entry	Solvent	Time (h)	Yield (%) ^b 3a	Recovery (%) 2a
1	DMA	20	45	52
2	NMP	24	61	36
3	DMSO	21	75	23
4	DMF	48	88	6
5	MeOH	20	92	0
6	CF ₃ CH ₂ OH	2	84	0
7 ^c	MeOH	4	49	50

^a Reaction was done with the concentration of 0.67 M (0.2 mmol/0.3 mL) of **2a**.

^b Isolated yields.

^c TFE and **2a** were used in equimolar amounts.

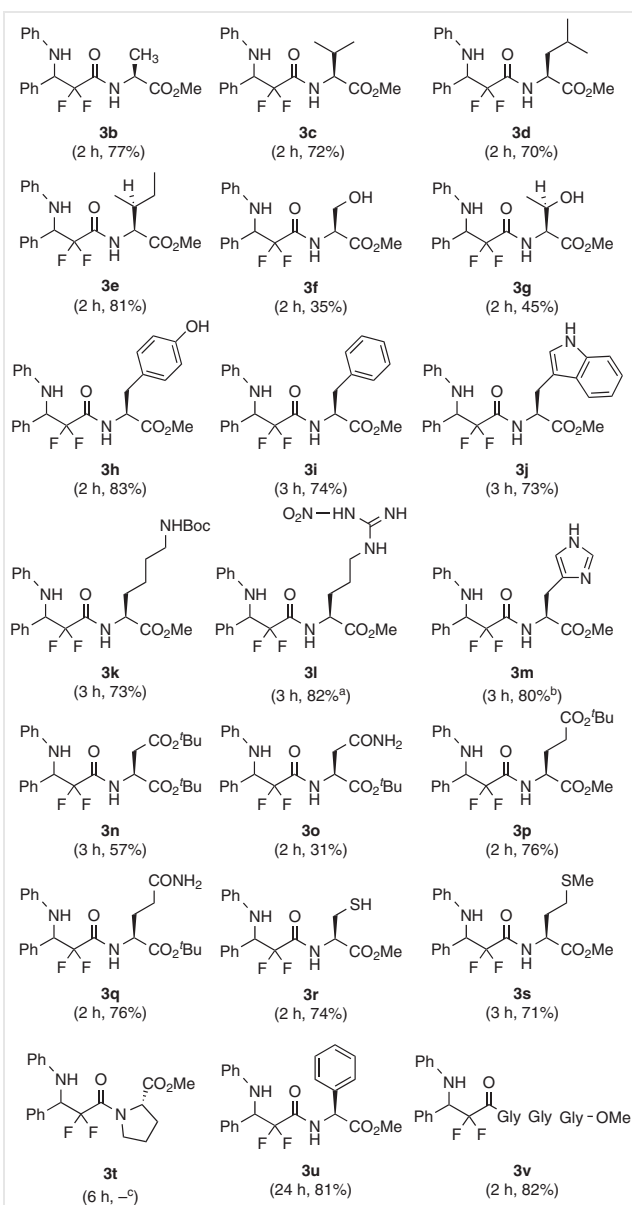
The ring-opening aminolysis with glycine proceeded to give the product of the open-chain peptide **3a**. In this reaction, polar aprotic solvents commonly used in S_N2-type reactions seemed to decrease the reactivity and gave **3a** with very slow reaction times, which were impractical for use (Table 1, entries 1–4). In contrast, methanol, a protic solvent, accelerated the reaction to give **3a** in 92% yield; how-

ever, the reaction rate was still slow (20 h). A dramatic improvement in reaction efficiency was observed when it was performed in 2,2,2-trifluoroethanol (TFE) solution; **3a** was provided in 84% yield after two hours reaction time. However, when equimolar amount of TFE was used in methanol, a significant decrease in yield of **3a** was observed along with unreacted **2a** (entry 7). This reaction was very simple to perform, and there was no need for any activation process prior to the reaction, suggesting that this new approach was practical and effective for preparing α,α -difluorinated β -amino acid containing peptides.

With the optimized conditions in hand, the substrate scope of the reaction was examined. The results are shown in Figure 2. All the reactions were completed within 2–3 hours. It is noteworthy that this reaction could accommodate α -amino acids with bulky substituents. Not only simple amino acids, such as alanine, but also bulkier amino acids, valine, leucine, and isoleucine, all successfully reacted with **2a** to give peptides **3c–e** in good yields. Hydroxy groups on aliphatic carbons disturbed the reaction to some extent, and their yield decreased to around 35% (\rightarrow **3f** and **3g**). However, the less nucleophilic phenolic group did not disturb the reaction process, and **3h** was obtained in good yield. Other aromatic amino acids and basic amino acids, which were masked amino nucleophiles on the side chain, were also compatible (\rightarrow **3i–l**). It is worth mentioning that histidine was able to participate in the reaction without any protection on nitrogen atom to give **3m** in good yield. Ester, amido, and sulfide substituents, and even nucleophilic thiol in the case of cysteine, were compatible with the reaction (\rightarrow **3n–s**).

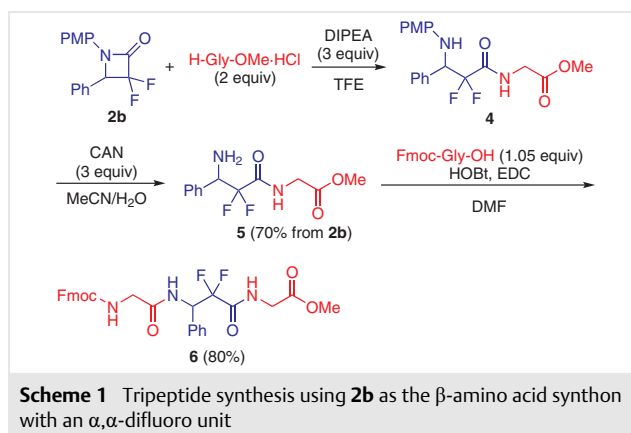
Unfortunately, in the case of proline, the reaction was unfavorable, and only a small amount of the product **3t** was observed as the reaction progressed; furthermore, it did not appear to be since it decomposed in the purification process. In the case of another bulky amino acid, phenyl glycine, the reaction proceeded but longer reaction time was needed to obtain **3u**, which is an analogue of aminopeptidase inhibitor **1**, in 81% yield. It is interesting to note that the tripeptide, H-Gly-Gly-Gly-OMe was able to react with **2a** to give **3v** in good yield, highlighting the good performance of this reaction as a convenient method for one-pot peptide synthesis.

To demonstrate the high practical potential of this method, we proceeded to synthesize the tripeptide **6** that contained α,α -difluoro- β -amino acid unit between two glycine terminals. As shown in Scheme 1, *N*-PMP- α,α -difluoro- β -lactam (**2b**) was used instead of **2a** to obtain a free nitrogen atom for consequent peptide elongation. As expected, the reaction of **2b** with H-Gly-OMe occurred in the same manner, and peptide **4** was obtained. The crude product **4** was treated with ceric ammonium nitrate to remove the PMP group and to give **5** in good yield (from **2b**).¹⁵ For the following peptide elongation at the *N*-terminus of **5**, Fmoc-Gly-OH was condensed with 1-hydroxybenzotriazole

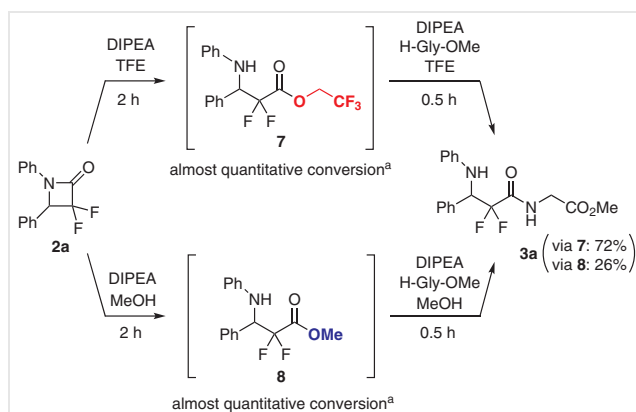


(HOBt) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) to give **6** in good yield.^{11d,16} This reaction scheme exhibits the potential utility of our method by showing its capability of preparing polypeptides containing an α,α -difluoro- β -amino acid unit at the desired position.

To elucidate the reaction mechanism, we tried to understand how TFE accelerated the reaction between **2a** and the α -amino acids. For this purpose, a kinetic study of the reaction was performed in both the TFE and methanol solution



(Scheme 2). When **2a** was dissolved in TFE or methanol, respectively, the ring-opening alcoholysis of **2a** occurred at almost the same rate in both cases. After confirming that both conversions to TFE ester **7** or methyl ester **8** were completed in two hours (monitored by TLC), H-Gly-OMe and DIPEA were added to each solution to take place the subsequent aminolysis.¹⁷ Interestingly, large differences were observed in the reaction rate; the aminolysis in TFE solution was obviously faster than that in methanol solution, and gave **3a** in 72% yield (compared to 26% in methanol solution). The acceleration effect could be attributed to the TFE group being a much better leaving group for aminolysis, because of the existence of a strong electronegative trifluoromethyl group; pK_a (TFE) = 12 versus pK_a (MeOH) = 16.¹⁸ In addition, the moderate acidity of TFE could work as an acidic catalyst for aminolysis process. We consequently ascribed the rate acceleration in TFE solution to the synergistic effects of an enhanced leaving group and acid catalysis, both resulting from TFE.



In conclusion, we have developed a convenient method for the synthesis of β -amino acid peptides containing an α,α -difluoromethylene unit by using α,α -difluoro- β -lactams **2a** and **2b**. This ring-opening reaction allows access to a wide range of fluorinated β -amino acid peptides. Most α -amino acids, except for proline, were successfully used in this transformation to provide the fluorinated dipeptides in good yields. Additionally, the reaction mechanism was examined by comparing the reactivity in TFE and methanol solutions, which demonstrated the activation process in TFE. Further studies, for example, on the synthesis of particular peptide-based pharmaceuticals and analogues in diastereomerically pure form, are ongoing in our laboratory with enantiomerically pure **2**.

NMR spectra were obtained from a solution in DMSO- d_6 or $CDCl_3$ using 400 MHz for 1H NMR, 100 MHz for ^{13}C , 376 MHz for ^{19}F NMR. DMSO- d_6 solution of NMR samples were recorded at 40 °C, unless noted otherwise. Chemical shifts of 1H and ^{13}C NMR are reported in ppm downfield of TMS ($^1H = 0.00$) and DMSO- d_6 ($^1H \delta = 2.49$, $^{13}C \delta = 39.5$). Chemical shifts of ^{19}F NMR are reported in ppm from $CFCl_3$ as an internal standard. ^{13}C NMR spectra were obtained with 1H decoupling. All data are reported as follows: chemical shifts, multiplicity (standard abbreviations), coupling constants (Hz), and relative integration value. The measurement of all NMR spectra were obtained from the diastereomixture of peptide products. HRMS experiments were measured on a double-focusing mass spectrometer with an ionization mode of EI or positive-FAB using glycerin as a matrix. All experiments were carried out under an argon atmosphere in flame-dried glassware using standard inert techniques for introducing reagents and solvents unless otherwise noted. All commercially available materials were used as received without further purification. Solvents were heated to reflux over CaH_2 (DMF) and Mg metal (TFE and MeOH) under an argon atmosphere and collected by distillation just before use.

Note: All aromatic carbons could not be observed in ^{13}C NMR spectra of the products **3a–v** and **6** reported below, probably due to overlapping.

α,α -Difluoro- β -lactams **2a,b**; General Procedure

Ethyl bromodifluoroacetate (20 mmol) and the corresponding imine (10 mmol) were added to a solution of $RhCl(PPh_3)_3$ (1 mol%) in THF (60 mL) at 0 °C and stirred for 0.5 h. A 1.0 M solution of Et_2Zn in hexane (30 mmol) was slowly added to the solution via a dropping funnel, and then the whole mixture was stirred at the same temperature. The reaction was quenched with aq 10% HCl, the mixture was extracted with EtOAc, and the combined extracts were washed with brine and dried ($MgSO_4$). The solvent was removed in vacuo and the residue was purified by flash column chromatography to give the corresponding α,α -difluoro- β -lactams **2a,b**.

Ring-Opening Peptide Synthesis; General Procedure

To a vial containing difluoro- β -lactam **2a** (51.9 mg, 0.2 mmol) and amino acid ester hydrochloride (0.4 mmol) in TFE (0.3 mL) was added DIPEA (0.10 mL, 0.6 mmol) at r.t. and the mixture was stirred at r.t. for

2 h. The mixture was poured into H_2O and then extracted with EtOAc. The combined organic phases were washed with brine, dried ($MgSO_4$), and concentrated in vacuo. The crude mixture was purified with flash column chromatography to give the pure product **3**.

Methyl [2,2-Difluoro-3-phenyl-3-(phenylamino)propanoyl]-glycinate (**3a**)

Eluent: EtOAc/hexane (3:7); yield: 58.5 mg (84%); colorless solid; mp 153.0–154.0 °C; $R_f = 0.2$ (EtOAc/hexane, 3:7).

1H NMR (DMSO- d_6 , 400 MHz): $\delta = 9.07$ (m, 1 H), 7.52 (d, $J = 7.2$ Hz, 2 H), 7.36–7.29 (m, 3 H), 7.02 (t, $J = 8.0$ Hz, 2 H), 6.73 (d, $J = 7.6$ Hz, 2 H), 6.55 (t, $J = 7.4$ Hz, 1 H), 6.27 (d, $J = 10.4$ Hz, 1 H), 5.28 (ddd, $J = 19.5$, 10.4, 9.4 Hz, 1 H), 3.88 (m, 2 H), 3.58 (s, 3 H).

$^{13}C\{^1H\}$ NMR (DMSO- d_6 , 100 MHz): $\delta = 169.1$, 163.3 (t, $J = 28.8$ Hz), 146.6, 135.2, 128.7, 128.6, 128.0, 117.2, 115.9 (dd, $J = 260.3$, 254.3 Hz), 113.7, 58.0 (dd, $J = 27.5$, 21.7 Hz), 51.7, 40.7.

^{19}F NMR (DMSO- d_6 , 376 MHz): $\delta = -107.0$ (dd, $J = 252.9$, 9.4 Hz, 1 F), -117.3 (dd, $J = 252.9$, 19.5 Hz, 1 F).

MS (EI): $m/z = 348$ [M] $^+$.

HRMS (EI): m/z [M] $^+$ calcd for $C_{18}H_{18}F_2N_2O_3$: 348.1285; found: 348.1288.

Methyl [2,2-Difluoro-3-phenyl-3-(phenylamino)propanoyl]-L-alaninate (**3b**)

Eluent: EtOAc/hexane (3:7); yield: 56.1 mg (77%); dr 1:1.3; colorless solid.

1H NMR (DMSO- d_6 , 400 MHz): $\delta = 8.00$ (t, $J = 6.0$ Hz, 1 H), 7.53–7.50 (m, 2 H), 7.36–7.26 (m, 3 H), 7.04–7.00 (m, 2 H), 6.75–6.71 (m, 2 H), 6.57–6.53 (m, 1 H), 6.30–6.27 (m, 1 H), 5.34–5.22 (m, 1 H), 4.37–4.28 (m, 1 H), 3.57 (s, 1.7 H), 3.54 (s, 1.3 H), 1.27 (d, $J = 6.8$ Hz, 1.3 H), 1.19 (d, $J = 6.8$ Hz, 1.7 H).

$^{13}C\{^1H\}$ NMR (DMSO- d_6 , 100 MHz): $\delta = 171.7$, 162.6 (t, $J = 28.9$ Hz), 162.5 (t, $J = 28.9$ Hz), 146.6, 146.5, 135.5, 135.5, 135.1, 135.1, 128.6, 128.6, 128.0, 117.1, 117.0, 115.9 (dd, $J = 257.2$, 256.2 Hz), 115.8 (dd, $J = 257.2$, 254.3 Hz), 113.4, 58.1 (t, $J = 21.2$ Hz), 57.9 (t, $J = 22.2$ Hz), 51.8, 51.8, 47.7, 47.6, 16.3.

^{19}F NMR (DMSO- d_6 , 376 MHz): $\delta = -107.5$ (dd, $J = 251.4$, 9.4 Hz, 0.43 F), -108.9 (dd, $J = 250.3$, 10.5 Hz, 0.59 F), -115.9 (dd, $J = 250.7$, 18.8 Hz, 0.58 F), -117.1 (dd, $J = 251.1$, 19.9 Hz, 0.42 F).

MS (EI): $m/z = 362$ [M] $^+$.

HRMS (EI): m/z [M] $^+$ calcd for $C_{19}H_{20}F_2N_2O_3$: 362.1442; found: 362.1446.

Methyl [2,2-Difluoro-3-phenyl-3-(phenylamino)propanoyl]-L-valinate (**3c**)

Eluent: EtOAc/hexane (3:7); yield: 56.6 mg (72%); dr 1:1.2; colorless solid.

1H NMR (DMSO- d_6 , 400 MHz): $\delta = 8.83$ (d, $J = 8.2$ Hz, 0.54 H), 8.66 (d, $J = 8.4$ Hz, 0.46 H), 7.55–7.52 (m, 2 H), 7.35–7.26 (m, 3 H), 7.03–7.00 (m, 2 H), 6.75–6.70 (m, 2 H), 6.56–6.53 (m, 1 H), 6.35 (d, $J = 11.0$ Hz, 0.47 H), 6.34 (d, $J = 10.4$ Hz, 0.56 H), 5.40 (ddd, $J = 19.7$, 10.4, 9.7 Hz, 0.56 H), 5.30 (ddd, $J = 22.2$, 11.0, 7.3 Hz, 0.47 H), 4.21 (dd, $J = 8.4$, 7.8 Hz, 0.46 H), 4.17 (dd, $J = 8.2$, 7.8 Hz, 0.54 H), 3.6 (s, 1.56 H), 3.57 (s, 1.32 H), 2.16–1.98 (m, 1 H), 0.85 (dd, $J = 15.6$, 6.9 Hz, 2.9 H), 0.74 (dd, $J = 11.4$, 6.9 Hz, 3.3 H).

$^{13}\text{C}\{^1\text{H}\}$ NMR (DMSO- d_6 , 100 MHz): δ = 170.9, 170.8, 163.1 (t, J = 28.9 Hz), 162.9 (t, J = 29.5 Hz), 146.5, 146.3, 135.3, 135.1, 128.7, 128.6, 128.5, 127.9, 127.9, 117.1, 117.0, 115.9 (dd, J = 256.7, 253.3 Hz), 115.8 (dd, J = 256.2, 255.3 Hz), 113.5, 113.4, 58.2–57.5 (m), 57.8, 51.7, 29.7, 29.5, 18.7, 18.6, 18.2, 18.1.

^{19}F NMR (DMSO- d_6 , 376 MHz): δ = -106.2 (dd, J = 250.7, 7.3 Hz, 0.47 F), -107.9 (dd, J = 251.4, 9.7 Hz, 0.56 F), -115.3 (dd, J = 251.4, 19.7 Hz, 0.56 F), -117.5 (dd, J = 250.7, 22.2 Hz, 0.47 F).

MS (EI): m/z = 390 [M] $^+$.

HRMS (EI): m/z [M] $^+$ calcd for $\text{C}_{21}\text{H}_{24}\text{F}_2\text{N}_2\text{O}_3$: 390.1755; found: 390.1758.

Methyl [2,2-difluoro-3-phenyl-3-(phenylamino)propanoyl]-L-leucinate (3d)

Eluent: EtOAc/hexane (1:4); yield: 57.0 mg (70%); dr 1:1; colorless solid.

^1H NMR (DMSO- d_6 , 400 MHz): δ = 8.98 (d, J = 8.2 Hz, 0.5 H), 8.91 (d, J = 7.8 Hz, 0.5 H), 7.53–7.51 (m, 2 H), 7.36–7.27 (m, 3 H), 7.04–7.00 (m, 2 H), 6.72–6.70 (m, 2 H), 6.56–6.53 (m, 1 H), 6.34 (d, J = 10.5 Hz, 0.5 H), 6.27 (d, J = 10.5 Hz, 0.5 H), 5.37–5.22 (m, 1 H), 4.38–4.28 (m, 1 H), 3.60 (s, 1.5 H), 3.56 (s, 1.5 H), 1.72–1.20 (m, 3 H), 0.80 (d, J = 6.0 Hz, 1.5 H), 0.68 (d, J = 6.4 Hz, 3 H), 0.62 (d, J = 6.4 Hz, 1.5 H).

$^{13}\text{C}\{^1\text{H}\}$ NMR (acetone- d_6 , 100 MHz): δ = 172.8, 172.7, 164.2 (t, J = 28.5 Hz), 164.0 (t, J = 28.5 Hz), 147.6, 147.3, 136.3, 136.2, 136.1, 129.7, 129.6, 129.2, 129.1, 129.1, 118.7, 118.6, 117.2 (dd, J = 260.9, 257.0 Hz), 117.0 (dd, J = 260.6, 254.8 Hz), 114.6, 60.1 (dd, J = 25.5, 22.1 Hz), 59.7 (dd, J = 27.9, 22.1 Hz), 52.5, 52.5, 51.5, 51.4, 40.9, 40.7, 25.0, 24.9, 23.3, 23.2, 21.5, 21.3.

^{19}F NMR (DMSO- d_6 , 376 MHz): δ = -106.7 (dd, J = 250.3, 8.3 Hz, 0.5 F), -109.4 (dd, J = 250.7, 10.8 Hz, 0.5 F), -115.0 (dd, J = 250.7, 18.8 Hz, 0.5 F), -117.9 (dd, J = 250.3, 21.3 Hz, 0.5 F).

MS (EI): m/z = 404 [M] $^+$.

HRMS (EI): m/z [M] $^+$ calcd for $\text{C}_{22}\text{H}_{26}\text{F}_2\text{N}_2\text{O}_3$: 404.1911; found: 404.1912.

Methyl [2,2-Difluoro-3-phenyl-3-(phenylamino)propanoyl]-L-isoleucinate (3e)

Eluent: EtOAc/hexane (3:7); yield: 65.6 mg (81%); dr 1:1.1; colorless solid.

^1H NMR (DMSO- d_6 , 400 MHz): δ = 8.86 (d, J = 8.2 Hz, 0.5 H), 8.67 (d, J = 8.2 Hz, 0.42 H), 7.54–7.51 (m, 2 H), 7.35–7.28 (m, 3 H), 7.03–6.99 (m, 2 H), 6.73–6.69 (m, 2 H), 6.56–6.52 (m, 1 H), 6.37–6.32 (m, 1 H), 5.34–5.45 (m, 1 H), 5.40 (ddd, J = 19.8, 10.8, 9.5 Hz, 0.52 H), 5.28 (ddd, J = 21.9, 11.0, 7.8 Hz, 0.48 H), 4.25–4.16 (m, 1 H), 3.61 (s, 1.48 H), 3.57 (s, 1.36 H), 1.89–1.74 (m, 1 H), 1.45–0.91 (m, 2 H), 0.81–0.62 (m, 6 H).

$^{13}\text{C}\{^1\text{H}\}$ NMR (DMSO- d_6 , 100 MHz): δ = 171.1, 170.9, 162.9 (t, J = 29.4 Hz), 162.8 (t, J = 29.4 Hz), 146.5, 146.3, 135.3, 135.1, 128.7, 128.6, 128.6, 128.5, 128.0, 127.9, 117.1, 117.0, 115.9 (dd, J = 259.1, 253.4 Hz), 115.8 (dd, J = 257.2, 255.3 Hz), 113.4, 113.4, 57.8 (dd, J = 27.9, 22.1 Hz), 57.7 (dd, J = 28.4, 21.2 Hz), 56.7, 56.6, 51.7, 39.50, 35.8, 35.7, 24.5, 24.4, 15.0, 14.9, 10.6, 10.3.

^{19}F NMR (DMSO- d_6 , 376 MHz): δ = -106.4 (dd, J = 250.3, 7.8 Hz, 0.48 F), -107.8 (dd, J = 251.4, 9.5 Hz, 0.52 F), -115.5 (dd, J = 251.1, 19.8 Hz, 0.52 F), -117.5 (dd, J = 250.3, 21.9 Hz, 0.48 F).

MS (EI): m/z = 404 [M] $^+$.

HRMS (EI): m/z [M] $^+$ calcd for $\text{C}_{22}\text{H}_{26}\text{F}_2\text{N}_2\text{O}_3$: 404.1911; found: 404.1919.

Methyl [2,2-Difluoro-3-phenyl-3-(phenylamino)propanoyl]-L-serinate (3f)

Eluent: EtOAc/hexane (1:1); yield: 26.3 mg (35%); dr 1:1; colorless solid.

^1H NMR (DMSO- d_6 , 400 MHz): δ = 8.77–8.75 (m, 1 H), 7.59–7.56 (m, 2 H), 7.40–7.31 (m, 3 H), 7.08–7.04 (m, 2 H), 6.79–6.76 (m, 2 H), 6.61–6.57 (m, 1 H), 6.36–6.33 (m, 1 H), 5.42–5.30 (m, 1 H), 5.10–5.04 (m, 1 H), 4.46–4.39 (m, 1 H), 3.80–3.63 (m, 2 H), 3.61 (s, 1.5 H), 3.59 (s, 1.5 H).

$^{13}\text{C}\{^1\text{H}\}$ NMR (DMSO- d_6 , 100 MHz): δ = 169.9, 169.8, 163.2–162.6 (m), 146.6, 146.5, 135.2, 135.1, 128.7, 128.6, 128.6, 128.0, 117.2, 117.1, 115.9 (dd, J = 258.6, 254.4 Hz), 115.8 (dd, J = 259.1, 254.3 Hz), 113.6, 113.5, 60.7, 58.1 (dd, J = 27.2, 21.7 Hz), 57.9 (dd, J = 26.7, 21.8 Hz), 54.9, 54.8, 51.9.

^{19}F NMR (DMSO- d_6 , 376 MHz): δ = -107.1 (dd, J = 251.4, 8.7 Hz, 0.5 F), -107.3 (dd, J = 251.9, 9.4 Hz, 0.5 F), -116.7 (dd, J = 251.4, 19.7 Hz, 0.5 F), -117.1 (dd, J = 251.9, 20.3 Hz, 0.5 F).

MS (EI): m/z = 378 [M] $^+$.

HRMS (EI): m/z [M] $^+$ calcd for $\text{C}_{19}\text{H}_{20}\text{F}_2\text{N}_2\text{O}_4$: 378.1391; found: 378.1389.

Methyl [2,2-Difluoro-3-phenyl-3-(phenylamino)propanoyl]-L-threoninate (3g)

Eluent: EtOAc/hexane (3:2); yield: 35.0 mg (45%); dr 1:1; colorless solid.

^1H NMR (DMSO- d_6 , 400 MHz): δ = 8.39 (d, J = 8.7 Hz, 0.5 H), 8.26 (d, J = 8.2 Hz, 0.5 H), 7.61–7.59 (m, 2 H), 7.42–7.33 (m, 3 H), 7.08–7.05 (m, 2 H), 6.81–6.76 (m, 2 H), 6.61–6.58 (m, 1 H), 6.46–6.42 (m, 1 H), 5.48–5.32 (m, 1 H), 5.12 (d, J = 6.4 Hz, 0.5 H), 5.02 (d, J = 6.4 Hz, 0.5 H), 4.40 (dd, J = 14.4, 8.4 Hz, 0.5 H), 4.39 (dd, J = 14.1, 8.7 Hz, 0.5 H), 4.25–4.13 (m, 1 H), 3.65 (s, 1.5 H), 3.64 (s, 1.5 H), 1.10 (d, J = 6.4 Hz, 1.5 H), 0.92 (d, J = 6.4 Hz, 1.5 H).

$^{13}\text{C}\{^1\text{H}\}$ NMR (DMSO- d_6 , 100 MHz): δ = 169.9, 169.8, 163.2 (t, J = 28.9 Hz), 146.6, 146.4, 135.2, 135.2, 135.0, 128.7, 128.6, 128.6, 127.9, 117.1, 117.0, 116.1 (dd, J = 257.7, 254.8 Hz), 116.0 (dd, J = 260.6, 255.7 Hz), 113.6, 66.0, 65.9, 58.0, 57.9 (dd, J = 26.0, 21.2 Hz), 57.6 (dd, J = 28.4, 22.2 Hz), 51.9, 51.9, 19.7, 19.5.

^{19}F NMR (DMSO- d_6 , 376 MHz): δ = -105.60 (dd, J = 250.3, 6.9 Hz, 0.5 F), -108.45 (dd, J = 251.4, 10.5 Hz, 0.5 F), -115.47 (dd, J = 251.4, 19.5 Hz, 0.5 F), -118.66 (dd, J = 250.3, 22.8 Hz, 0.5 F).

MS (EI): m/z = 392 [M] $^+$.

HRMS (EI): m/z [M] $^+$ calcd for $\text{C}_{20}\text{H}_{22}\text{F}_2\text{N}_2\text{O}_4$: 392.1548; found: 392.1549.

Methyl [2,2-Difluoro-3-phenyl-3-(phenylamino)propanoyl]-L-tyrosinate (3h)

Eluent: EtOAc/hexane (3:7); yield: 75.1 mg (83%); dr 1:1; colorless solid.

^1H NMR (DMSO- d_6 , 400 MHz): δ = 9.13 (br s, 0.5 H), 9.12 (br s, 0.5 H), 8.96 (d, J = 7.3 Hz, 0.5 H), 8.94 (d, J = 7.3 Hz, 0.5 H), 7.50–7.28 (m, 5 H), 7.05–6.88 (m, 4 H), 6.72–6.54 (m, 5 H), 6.26 (d, J = 9.2 Hz, 0.5 H), 6.23 (d, J = 8.6 Hz, 0.5 H), 5.28 (ddd, J = 18.8, 10.5, 8.6 Hz, 1 H), 5.21 (ddd, J = 19.5, 9.2, 8.3 Hz, 1 H), 4.43–4.37 (m, 1 H), 3.54 (s, 1.5 H), 3.50 (s, 1.5 H), 3.00–2.81 (m, 2 H).

$^{13}\text{C}\{^1\text{H}\}$ NMR (DMSO- d_6 , 100 MHz): δ = 170.9, 170.8, 162.8 (t, J = 28.9 Hz), 162.7 (t, J = 28.9 Hz), 156.0, 155.9, 146.6, 146.5, 135.2, 135.1, 129.9, 129.8, 128.7, 128.7, 128.6, 128.0, 127.9, 126.9, 126.8, 117.2,

117.1, 115.8 (dd, $J = 259.1, 254.3$ Hz), 115.7 (dd, $J = 258.7, 254.5$ Hz), 115.1, 115.0, 113.6, 113.5, 58.0 (dd, $J = 27.0, 22.2$ Hz), 54.2, 54.1, 51.8, 51.8, 35.3, 35.2.

^{19}F NMR (DMSO- d_6 , 376 MHz): $\delta = -106.9$ (dd, $J = 253.2, 8.3$ Hz, 0.5 F), -108.3 (dd, $J = 251.1, 10.5$ Hz, 0.5 F), -115.9 (dd, $J = 251.4, 18.8$ Hz, 0.5 F), -116.4 (dd, $J = 252.9, 19.5$ Hz, 0.5 F).

MS (EI): $m/z = 454$ [M] $^+$.

HRMS (EI): m/z [M] $^+$ calcd for $\text{C}_{25}\text{H}_{24}\text{F}_2\text{N}_2\text{O}_4$: 454.1704; found: 454.1705.

Methyl [2,2-Difluoro-3-phenyl-3-(phenylamino)propanoyl]-L-phenylalaninate (3i)

Eluent: EtOAc/hexane (3:7); yield: 64.9 mg (74%); dr 1:1; colorless solid.

^1H NMR (DMSO- d_6 , 400 MHz): $\delta = 9.11$ (d, $J = 7.8$ Hz, 1 H), 7.55–7.06 (m, 12 H), 6.78–6.60 (m, 3 H), 6.30 (d, $J = 10.6$ Hz, 0.5 H), 6.28 (d, $J = 10.3$ Hz, 0.5 H), 5.33 (ddd, $J = 20.2, 10.6, 8.7$ Hz, 0.5 H), 5.26 (ddd, $J = 18.8, 10.3, 10.1$ Hz, 0.5 H), 4.57–4.51 (m, 1 H), 3.60 (s, 1.5 H), 3.55 (s, 1.5 H), 3.10 (dd, $J = 14.0, 5.3$ Hz, 0.5 H), 3.06 (dd, $J = 13.9, 5.2$ Hz, 0.5 H), 2.99 (dd, $J = 14.0, 9.3$ Hz, 0.5 H), 2.95 (dd, $J = 13.9, 9.1$ Hz, 0.5 H).

$^{13}\text{C}\{^1\text{H}\}$ NMR (DMSO- d_6 , 100 MHz): $\delta = 170.7, 162.8$ (t, $J = 28.9$ Hz), 162.8 (t, $J = 29.5$ Hz), 146.5, 136.9, 136.8, 135.1, 135.0, 128.9, 128.8, 128.6, 128.6, 128.5, 128.1, 128.0, 127.9, 127.9, 126.4, 126.3, 117.1, 117.1, 115.8 (dd, $J = 260.1, 254.3$ Hz), 115.6 (dd, $J = 260.1, 254.3$ Hz), 113.6, 113.5, 57.9 (dd, $J = 27.0, 22.1$ Hz), 57.9 (dd, $J = 28.3, 22.1$ Hz), 53.8, 53.7, 51.9, 51.8, 35.9, 35.8.

^{19}F NMR (DMSO- d_6 , 376 MHz): $\delta = -106.67$ (dd, $J = 253.6, 8.7$ Hz, 0.5 F), -107.94 (dd, $J = 251.4, 10.1$ Hz, 0.5 F), -116.3 (dd, $J = 251.4, 18.8$ Hz, 0.5 F), -116.6 (dd, $J = 253.6, 20.2$ Hz, 0.5 F).

MS (EI): $m/z = 438$ [M] $^+$.

HRMS (EI): m/z [M] $^+$ calcd for $\text{C}_{25}\text{H}_{24}\text{F}_2\text{N}_2\text{O}_3$: 438.1755; found: 438.1764.

Methyl [2,2-Difluoro-3-phenyl-3-(phenylamino)propanoyl]-L-tryptophanate (3j)

Eluent: EtOAc/hexane (3:7); yield: 104.2 mg (73%); dr 1:1; colorless solid.

^1H NMR (DMSO- d_6 , 400 MHz): $\delta = 10.8$ (s, 0.5 H), 10.7 (s, 0.5 H), 9.0 (d, $J = 7.3$ Hz, 0.5 H), 8.9 (d, $J = 7.8$ Hz, 0.5 H), 7.5–6.9 (m, 12 H), 6.7–6.5 (m, 3 H), 6.3 (d, $J = 10.5$ Hz, 1 H), 5.2–5.3 (m, 1 H), 4.5 (m, 1 H), 3.5 (s, 1.5 H), 3.5 (s, 1.5 H), 3.3–3.1 (m, 2 H).

$^{13}\text{C}\{^1\text{H}\}$ NMR (DMSO- d_6 , 100 MHz): $\delta = 171.1, 171.0, 162.7$ (t, $J = 29.0$ Hz), 146.6, 146.5, 136.1, 136.0, 135.2, 135.1, 135.0, 135.0, 128.6, 128.5, 128.0, 127.9, 127.9, 126.9, 126.8, 123.6, 123.4, 120.9, 120.9, 118.4, 118.3, 117.8, 117.7, 117.1, 117.1, 115.8 (dd, $J = 259.2, 254.9$ Hz), 115.8 (dd, $J = 259.2, 255.0$ Hz), 113.5, 113.4, 111.4, 111.3, 109.1, 109.0, 58.1 (dd, $J = 26.7, 21.9$ Hz), 53.3, 53.2, 51.8, 51.8, 26.4, 26.3.

^{19}F NMR (DMSO- d_6 , 376 MHz): $\delta = -107.98$ (dd, $J = 251.4, 10.1$ Hz, 0.5 F), -109.19 (dd, $J = 250.7, 10.8$ Hz, 0.5 F), -115.3 (dd, $J = 250.3, 18.3$ Hz, 0.5 F), -116.0 (dd, $J = 251.3, 19.2$ Hz, 0.5 F).

MS (EI): $m/z = 477$ [M] $^+$.

HRMS (EI): m/z [M] $^+$ calcd for $\text{C}_{27}\text{H}_{25}\text{F}_2\text{N}_3\text{O}_3$: 477.1864; found: 477.1868.

Methyl N^6 -(tert-Butoxycarbonyl)- N^2 -[2,2-difluoro-3-phenyl-3-(phenylamino)propanoyl]-L-lysinate (3k)

Eluent: EtOAc/hexane (3:7); yield: 76.2 mg (73%); dr 1:1; colorless solid.

^1H NMR (DMSO- d_6 , 400 MHz): $\delta = 8.9$ (d, $J = 7.8$ Hz, 0.5 H), 8.8 (d, $J = 8.2$ Hz, 0.5 H), 7.5 (m, 2 H), 7.4–7.3 (m, 3 H), 7.0–6.9 (m, 2 H), 6.7 (m, 2 H), 6.6 (br s, 1 H), 6.5 (m, 2 H), 6.3 (m, 1 H), 5.4–5.2 (m, 1 H), 4.3–4.2 (m, 1 H), 3.6 (s, 1.5 H), 3.5 (s, 1.5 H), 2.9–2.7 (m, 2 H), 1.8–1.5 (m, 2 H), 1.4 (s, 9 H), 1.3–0.9 (m, 4 H).

$^{13}\text{C}\{^1\text{H}\}$ NMR (DMSO- d_6 , 100 MHz): $\delta = 171.4, 171.3, 162.9$ (t, $J = 28.9$ Hz), 162.9 (t, $J = 28.9$ Hz), 155.4, 146.6, 146.4, 135.2, 135.2, 135.1, 128.7, 128.6, 128.0, 117.1, 117.1, 115.9 (dd, $J = 257.5, 256.7$ Hz), 115.8 (dd, $J = 261.0, 253.4$ Hz), 113.4, 77.2, 58.0 (dd, $J = 26.4, 21.7$ Hz), 57.8 (dd, $J = 27.8, 21.5$ Hz), 52.1, 52.0, 51.8, 51.8, 39.2,^{a,b} 29.9,^a 29.9,^a 28.9,^a 28.7,^a 28.1, 22.4,^a 22.3.^a These signals were observed as reverse image in DEPT 135. ^b This signal overlapped with solvent peaks (DMSO- d_6).

^{19}F NMR (DMSO- d_6 , 376 MHz): $\delta = -107.0$ (dd, $J = 250.5, 6.1$ Hz, 0.5 F), -109.4 (dd, $J = 251.2, 9.4$ Hz, 0.5 F), -115.1 (dd, $J = 251.2, 17.7$ Hz, 0.5 F), -117.3 (dd, $J = 250.5, 21.0$ Hz, 0.5 F).

MS (EI): $m/z = 519$ [M] $^+$.

HRMS (EI): m/z [M] $^+$ calcd for $\text{C}_{27}\text{H}_{35}\text{F}_2\text{N}_3\text{O}_5$: 519.2545; found: 519.2546.

Methyl N^2 -[2,2-Difluoro-3-phenyl-3-(phenylamino)propanoyl]- N^6 -nitro-L-argininate (3l)

Amino acid (1 equiv) and DIPEA (2 equiv) were used; eluent: EtOAc/hexane (4:1); yield: 80.5 mg (82%); dr 1:1.2; colorless solid.

^1H NMR (DMSO- d_6 , 400 MHz): $\delta = 8.9$ (m, 1 H), 8.4 (s, 1 H), 7.9 (s, 2 H), 7.5 (m, 2 H), 7.4–7.3 (m, 3 H), 7.0 (m, 2 H), 6.7–6.5 (m, 3 H), 6.3 (m, 1 H), 5.3–5.2 (m, 1 H), 4.3–4.2 (m, 1 H), 3.6 (s, 1.6 H), 3.5 (s, 1.4 H), 3.1–2.9 (m, 2 H), 1.9–1.2 (m, 4 H).

$^{13}\text{C}\{^1\text{H}\}$ NMR (DMSO- d_6 , 100 MHz): $\delta = 171.2, 171.1, 163.0$ (t, $J = 28.9$ Hz), 162.9 (t, $J = 29.5$ Hz), 159.2, 159.2, 146.6, 146.4, 135.2, 135.2, 135.1, 135.1, 128.7, 128.6, 128.0, 117.1, 117.1, 116.0 (dd, $J = 253.3, 248.1$ Hz), 113.4, 113.4, 58.3–57.6 (m), 51.9, 51.9, 51.8, 39.9,^{a,b} 27.4,^a 27.3,^a 24.6.^a These signals were observed as reverse image in DEPT 135. ^b This signal overlapped with solvent peaks (DMSO- d_6).

^{19}F NMR (DMSO- d_6 , 376 MHz): $\delta = -107.2$ (d, $J = 250.4$ Hz, 0.4 F), -109.7 (dd, $J = 249.5, 10.8$ Hz, 0.6 F), -114.9 (dd, $J = 249.5, 17.7$ Hz, 0.6 F), -117.1 (dd, $J = 250.4, 20.2$ Hz, 0.4 F).

MS (FAB): $m/z = 493$ [M + H] $^+$.

HRMS (FAB): m/z [M + H] $^+$ calcd for $\text{C}_{22}\text{H}_{27}\text{F}_2\text{N}_6\text{O}_5$: 493.2011; found: 493.2014.

Methyl [2,2-Difluoro-3-phenyl-3-(phenylamino)propanoyl]-L-histidininate (3m)

The reaction was carried out on a 0.3 mmol scale. Histidine methyl ester 2 HCl (2 equiv) and DIPEA (5 equiv) were used; eluent: EtOAc/hexane (3:7) \rightarrow EtOH/ CHCl_3 (5:95); yield: 73.3 mg (80%); dr 1:1.1; colorless solid.

^1H NMR (DMSO- d_6 , 400 MHz): $\delta = 11.8$ (s, 1 H), 9.1 (d, $J = 7.8$ Hz, 0.5 H), 9.1 (d, $J = 7.8$ Hz, 0.5 H), 7.6–7.3 (m, 6 H), 7.0 (m, 2 H), 6.8 (s, 0.5 H), 6.7 (m, 2.5 H), 6.5 (m, 1 H), 6.3 (m, 1 H), 5.3–5.2 (m, 1 H), 4.6–4.4 (m, 1 H), 3.51 (s, 1.5 H), 3.5 (s, 1.5 H), 2.9 (d, $J = 6.4$ Hz, 2 H), 2.9 (d, $J = 6.4$ Hz, 2 H).

$^{13}\text{C}\{^1\text{H}\}$ NMR (DMSO- d_6 , 100 MHz): $\delta = 170.6, 162.7$ (t, $J = 28.9$ Hz), 162.6 (t, $J = 28.6$ Hz), 146.6, 146.5, 135.2, 135.1, 135.0, 134.9, 134.9, 128.6, 128.6, 128.6, 128.6, 127.9, 117.1, 117.0, 115.8 (dd, $J = 259.6, 256.3$ Hz), 113.5, 58.1 (dd, $J = 27.0, 23.1$ Hz), 57.9 (dd, $J = 27.0, 23.1$ Hz), 52.5, 52.4, 51.8, 28.4.

^{19}F NMR (DMSO- d_6 , 376 MHz): $\delta = -107.3$ (dd, $J = 252.1, 8.7$ Hz, 0.47 F), -108.1 (dd, $J = 251.8, 9.2$ Hz, 0.53 F), -116.34 (dd, $J = 251.8, 18.8$ Hz, 0.53 F), -117.21 (dd, $J = 252.1, 19.9$ Hz, 0.47 F).

MS (EI): $m/z = 428$ [M] $^+$.

HRMS (EI): m/z [M] $^+$ calcd for $\text{C}_{22}\text{H}_{22}\text{F}_2\text{N}_4\text{O}_3$: 428.1660; found: 428.1668.

Di-tert-butyl [2,2-Difluoro-3-phenyl-3-(phenylamino)propanoyl]-L-aspartate (3n)

Eluent: EtOAc/hexane (1:9); yield: 58.0 mg (57%); dr 1:1.1; colorless solid.

^1H NMR (DMSO- d_6 , 400 MHz): $\delta = 8.9$ (m, 1 H), 7.5 (m, 2 H), 7.3–7.2 (m, 3 H), 7.0 (m, 2 H), 6.7 (m, 2 H), 6.5 (m, 1 H), 6.3 (d, $J = 11.0$ Hz, 1 H), 5.3–5.2 (m, 1 H), 4.5 (dd, $J = 15.0, 6.7$ Hz, 0.5 H), 4.5 (dd, $J = 13.8, 6.5$ Hz, 0.5 H), 2.7–2.6 (m, 1 H), 2.5–2.4 (m, 1 H),^a 1.3 (s, 4.5 H), 1.3 (s, 4.5 H), 1.3 (s, 4.5 H).^a This signal overlapped with solvent peaks (DMSO- d_6).

$^{13}\text{C}\{^1\text{H}\}$ NMR (DMSO- d_6 , 100 MHz): $\delta = 168.8, 168.7, 168.6, 162.6$ (t, $J = 28.9$ Hz), 162.5 (t, $J = 28.9$ Hz), 146.6, 146.5, 135.2, 135.1, 128.7, 128.5, 128.0, 117.19, 115.8 (dd, $J = 260.1, 254.3$ Hz), 115.8 (dd, $J = 259.6, 254.7$ Hz), 113.7, 113.6, 81.3, 81.3, 80.5, 80.4, 58.2–57.5 (m), 49.4, 49.3, 36.4, 36.3, 27.5, 27.5, 27.3, 27.3.

^{19}F NMR (DMSO- d_6 , 376 MHz): $\delta = -106.5$ (dd, $J = 253.2, 8.3$ Hz, 0.48 F), -107.6 (dd, $J = 252.1, 9.4$ Hz, 0.52 F), -116.9 (dd, $J = 252.1, 19.5$ Hz, 0.52 F), -117.5 (dd, $J = 253.2, 20.9$ Hz, 0.48 F).

MS (EI): $m/z = 504$ [M] $^+$.

HRMS (EI): m/z [M] $^+$ calcd for $\text{C}_{27}\text{H}_{34}\text{F}_2\text{N}_2\text{O}_5$: 504.2436; found: 504.2439.

tert-Butyl [2,2-Difluoro-3-phenyl-3-(phenylamino)propanoyl]-L-asparaginate (3o)

Eluent: MeOH/ CHCl_3 (5:95); yield: 28.8 mg (31%); dr 1:1.2; colorless solid.

^1H NMR (DMSO- d_6 , 400 MHz): $\delta = 8.7$ (m, 1 H), 7.4 (m, 2 H), 7.3–7.2 (m, 4 H), 6.9 (m, 2 H), 6.8 (br d, $J = 21.5$ Hz, 1 H), 6.6 (m, 2 H), 6.5 (m, 1 H), 6.2 (d, $J = 10.6$ Hz, 0.45 H), 6.2 (d, $J = 10.4$ Hz, 0.55 H), 5.2 (ddd, $J = 20.2, 10.4, 9.6$ Hz, 1 H), 4.5–4.4 (m, 1 H), 2.5–2.3 (m, 2 H),^a 1.3 (s, 9 H).^a This signal overlapped with solvent peaks (DMSO- d_6).

$^{13}\text{C}\{^1\text{H}\}$ NMR (DMSO- d_6 , 100 MHz): $\delta = 171.1, 171.0, 169.1, 162.4$ (t, $J = 28.9$ Hz), 162.3 (t, $J = 28.9$ Hz), 146.6, 146.5, 135.2, 135.1, 128.7, 128.7, 128.6, 128.0, 117.2, 115.9 (dd, $J = 259.1, 255.3$ Hz), 115.9 (dd, $J = 260.6, 253.8$ Hz), 113.7, 113.6, 80.9, 58.1 (dd, $J = 26.7, 22.3$ Hz), 57.8 (dd, $J = 28.0, 21.8$ Hz), 49.6, 49.5, 35.9, 35.9, 27.4.

^{19}F NMR (DMSO- d_6 , 376 MHz): $\delta = -106.6$ (dd, $J = 252.3, 8.7$ Hz, 0.45 F), -108.0 (dd, $J = 251.3, 10.1$ Hz, 0.55 F), -116.5 (dd, $J = 251.3, 19.1$ Hz, 0.55 F), -117.8 (dd, $J = 252.3, 20.6$ Hz, 0.45 F).

MS (EI): $m/z = 447$ [M] $^+$.

HRMS (EI): m/z [M] $^+$ calcd for $\text{C}_{23}\text{H}_{27}\text{F}_2\text{N}_3\text{O}_4$: 447.1970; found: 447.1974.

5-tert-Butyl 1-Methyl [2,2-Difluoro-3-phenyl-3-(phenylamino)propanoyl]-L-glutamate (3p)

Eluent: EtOAc/hexane (1:4); yield: 72.4 mg (76%); dr 1:1.1; colorless solid.

^1H NMR (DMSO- d_6 , 400 MHz): $\delta = 9.0$ (d, $J = 8.2$ Hz, 0.52 H), 8.9 (d, $J = 8.2$ Hz, 0.48 H), 7.5 (m, 2 H), 7.4–7.3 (m, 3 H), 7.0 (m, 2 H), 6.7 (m, 2 H), 6.5 (m, 1 H), 6.3 (d, $J = 10.6$ Hz, 0.52 H), 6.3 (d, $J = 10.6$ Hz, 0.48 H), 5.4–5.2 (m, 1 H), 4.4–4.3 (m, 1 H), 3.6 (s, 1.57 H), 3.6 (s, 1.43 H), 2.3–1.7 (m, 4 H), 1.3 (s, 4.71 H), 1.3 (s, 4.28 H).

$^{13}\text{C}\{^1\text{H}\}$ NMR (DMSO- d_6 , 100 MHz): $\delta = 171.2, 171.2, 171.0, 170.9, 162.9$ (t, $J = 28.9$ Hz), 162.9 (t, $J = 29.0$ Hz), 146.6, 146.4, 135.2, 135.2, 135.0, 128.7, 128.7, 128.6, 128.0, 117.1, 117.1, 115.9 (dd, $J = 261.0, 255.9$ Hz), 115.9 (dd, $J = 261.4, 253.4$ Hz), 113.5, 113.4, 79.6, 58.1 (dd, $J = 26.0, 22.2$ Hz), 57.9 (dd, $J = 28.2, 21.9$ Hz), 51.9, 51.92, 5.17, 30.7, 30.6, 27.6, 25.6, 25.5.

^{19}F NMR (DMSO- d_6 , 376 MHz): $\delta = -106.8$ (dd, $J = 251.3, 8.3$ Hz, 0.48 F), -109.5 (dd, $J = 250.7, 10.8$ Hz, 0.52 F), -115.0 (dd, $J = 250.7, 18.1$ Hz, 0.52 F), -117.7 (dd, $J = 251.3, 21.0$ Hz, 0.48 F).

MS (EI): $m/z = 476$ [M] $^+$.

HRMS (EI): m/z [M] $^+$ calcd for $\text{C}_{25}\text{H}_{30}\text{F}_2\text{N}_2\text{O}_5$: 476.2123; found: 476.2124.

tert-Butyl [2,2-Difluoro-3-phenyl-3-(phenylamino)propanoyl]-L-glutamate (3q)

Eluent: EtOAc/hexane (3:2); yield: 72.2 mg (76%); dr 1:1; colorless solid.

^1H NMR (DMSO- d_6 , 400 MHz): $\delta = 8.9$ (d, $J = 7.1$ Hz, 0.5 H), 8.9 (d, $J = 7.1$ Hz, 0.5 H), 7.5 (m, 2 H), 7.4–7.2 (m, 3 H), 7.1–7.0 (m, 3 H), 6.7 (m, 3 H), 6.5 (m, 1 H), 6.3 (d, $J = 10.4$ Hz, 1 H), 6.3 (d, $J = 10.5$ Hz, 1 H), 5.3–5.2 (m, 1 H), 4.1–4.0 (m, 1 H), 2.2–1.7 (m, 4 H), 1.3 (s, 4.5 H), 1.3 (s, 4.5 H).

$^{13}\text{C}\{^1\text{H}\}$ NMR (DMSO- d_6 , 100 MHz): $\delta = 173.3, 173.3, 169.8, 169.8, 162.8$ (t, $J = 28.9$ Hz), 146.6, 146.4, 135.2, 135.2, 135.1, 128.7, 128.6, 128.6, 128.0, 117.1, 117.1, 115.8 (dd, $J = 257.7, 255.5$ Hz), 115.8 (dd, $J = 260.6, 253.7$ Hz), 113.6, 80.8, 58.1 (dd, $J = 26.8, 22.2$ Hz), 57.8 (dd, $J = 28.0, 21.8$ Hz), 52.8, 30.9, 30.9, 27.4, 25.9, 25.9.

^{19}F NMR (DMSO- d_6 , 376 MHz): $\delta = -106.5$ (dd, $J = 252.7, 8.3$ Hz, 0.5 F), -108.47 (dd, $J = 251.6, 10.5$ Hz, 0.5 F), -115.8 (dd, $J = 251.6, 18.8$ Hz, 0.5 F), -117.3 (dd, $J = 252.7, 20.2$ Hz, 0.5 F).

MS (EI): $m/z = 461$ [M] $^+$.

HRMS (EI): m/z [M] $^+$ calcd for $\text{C}_{24}\text{H}_{29}\text{F}_2\text{N}_3\text{O}_4$: 461.2126; found: 461.2131.

Methyl [2,2-Difluoro-3-phenyl-3-(phenylamino)propanoyl]-L-cysteinate (3r)

Eluent: EtOAc/hexane (1:4); yield: 58.1 mg (74%); dr 1:1.1; colorless solid.

^1H NMR (DMSO- d_6 , 400 MHz): $\delta = 9.1$ (d, $J = 8.2$ Hz, 0.48 H), 9.0 (d, $J = 7.8$ Hz, 0.52 H), 7.5 (m, 2 H), 7.4–7.3 (m, 3 H), 7.0 (m, 2 H), 6.7 (m, 2 H), 6.5 (m, 1 H), 6.3 (d, $J = 10.5$ Hz, 0.52 H), 6.3 (d, $J = 10.4$ Hz, 0.48 H), 5.4–5.2 (m, 1 H), 4.5–4.4 (m, 1 H), 3.5 (s, 1.44 H), 3.5 (s, 1.56 H), 2.9–2.7 (m, 2 H), 2.4 (t, $J = 8.5$ Hz, 0.52 H), 2.2 (t, $J = 8.7$ Hz, 0.48 H).

$^{13}\text{C}\{^1\text{H}\}$ NMR (DMSO- d_6 , 100 MHz): $\delta = 169.6, 162.9$ (t, $J = 28.9$ Hz), 162.9 (t, $J = 29.8$ Hz), 146.6, 146.4, 135.1, 135.0, 128.7, 128.6, 128.0, 117.2, 117.2, 115.9 (dd, $J = 260.6, 254.2$ Hz), 115.9 (dd, $J = 257.4, 257.4$ Hz), 113.5, 58.0 (dd, $J = 27.1, 21.2$ Hz), 57.8 (dd, $J = 26.8, 21.1$ Hz), 54.9, 54.8, 52.1, 24.5, 24.5.

^{19}F NMR (DMSO- d_6 , 376 MHz): $\delta = -107.1$ (dd, $J = 251.2, 8.7$ Hz, 0.52 F), -108.2 (dd, $J = 251.8, 9.8$ Hz, 0.48 F), -116.0 (dd, $J = 251.8, 18.8$ Hz, 0.48 F), -117.4 (dd, $J = 251.2, 20.6$ Hz, 0.52 F).

MS (EI): $m/z = 394$ [M] $^+$.

HRMS (EI): m/z [M]⁺ calcd for C₁₉H₂₀F₂N₂O₃S: 394.1163; found: 394.1158.

Methyl [2,2-Difluoro-3-phenyl-3-(phenylamino)propanoyl]-L-methioninate (3s)

Eluent: EtOAc/hexane (3:7); yield: 60.9 mg (71%); dr 1:1.1; colorless solid.

¹H NMR (DMSO-*d*₆, 400 MHz): δ = 9.0 (d, *J* = 7.8 Hz, 0.52 H), 8.9 (d, *J* = 7.8 Hz, 0.48 H), 7.5 (m, 2 H), 7.4–7.3 (m, 3 H), 7.0 (m, 2 H), 6.7 (m, 2 H), 6.5 (m, 1 H), 6.3 (d, *J* = 10.7 Hz, 0.52 H), 6.3 (d, *J* = 10.8 Hz, 0.48 H), 5.4–5.2 (m, 1 H), 4.5–4.4 (m, 1 H), 3.6 (s, 1.57 H), 3.6 (s, 1.43 H), 2.4–1.8 (m, 7 H).

¹³C{¹H} NMR (DMSO-*d*₆, 100 MHz): δ = 171.1, 171.0, 163.0 (t, *J* = 28.9 Hz), 162.9 (t, *J* = 29.8 Hz), 146.6, 146.4, 135.2, 135.2, 135.0, 128.7, 128.7, 128.6, 128.0, 117.2, 117.1, 115.9 (dd, *J* = 255.3, 254.3 Hz), 115.9 (dd, *J* = 261.0, 256.4 Hz), 113.4, 113.4, 58.2–57.6 (m), 52.0, 52.0, 50.9, 50.9, 29.9, 29.8, 29.3, 29.2, 14.3, 14.2.

¹⁹F NMR (DMSO-*d*₆, 376 MHz): δ = –106.7 (dd, *J* = 251.1, 7.9 Hz, 0.48 F), –109.4 (dd, *J* = 250.7, 10.8 Hz, 0.52 F), –115.1 (dd, *J* = 250.7, 18.8 Hz, 0.52 F), –117.9 (dd, *J* = 251.1, 21.3 Hz, 0.48 F).

MS (EI): m/z = 422 [M]⁺.

HRMS (EI): m/z [M]⁺ calcd for C₂₁H₂₄F₂N₂O₃S: 422.1476; found: 422.1475.

Methyl (S)-2-[2,2-Difluoro-3-phenyl-3-(phenylamino)propanamido]-2-phenylacetate (3u)

Eluent: EtOAc/hexane (1:4); yield: 68.7 mg (81%); dr 1:2; colorless solid.

¹H NMR (DMSO-*d*₆, 400 MHz): δ = 9.4 (d, *J* = 7.3 Hz, 0.33 H), 9.3 (d, *J* = 7.3 Hz, 0.67 H), 7.5–7.4 (m, 2 H), 7.4–7.2 (m, 8 H), 7.0 (m, 2 H), 6.7 (m, 2 H), 6.5 (m, 1 H), 6.4 (m, 1 H), 5.5 (m, 1 H), 5.4–5.2 (m, 1 H), 3.6 (s, 1 H), 3.6 (s, 2 H).

¹³C{¹H} NMR (DMSO-*d*₆, 100 MHz): δ = 169.8, 162.6 (t, *J* = 28.9 Hz), 146.5, 146.3, 135.4, 135.3, 135.2, 135.0, 128.7, 128.6, 128.6, 128.3, 128.3, 128.1, 128.0, 127.9, 127.6, 127.6, 117.1, 115.8 (dd, *J* = 259.6, 253.8 Hz), 113.5, 113.4, 57.8 (dd, *J* = 27.8, 22.3 Hz), 56.2, 56.1, 52.3, 52.3.

¹⁹F NMR (DMSO-*d*₆, 376 MHz): δ = –106.6 (dd, *J* = 250.7, 7.9 Hz, 0.67 F), –107.0 (dd, *J* = 252.5, 8.1 Hz, 0.33 F), –116.3 (d, *J* = 252.5, 20.0 Hz, 0.37 F), –117.0 (dd, *J* = 250.7, 21.4 Hz, 0.67 F).

MS (EI): m/z = 424 [M]⁺.

HRMS (EI): m/z [M]⁺ calcd for C₂₄H₂₂F₂N₂O₃: 424.1598 found: 424.1607.

Methyl [2,2-Difluoro-3-phenyl-3-(phenylamino)propanoyl]-glycylglycylglycinate (3v)

This product was purified without flash column chromatography. The reaction mixture was diluted with CHCl₃, then the precipitated solid was collected via suction filtration. The filtrate was washed with cold EtOAc; yield: 75.9 mg (82%); colorless solid; mp 206.0–207.0 °C.

¹H NMR (DMSO-*d*₆, 400 MHz): δ = 8.8 (t, *J* = 5.5 Hz, 1 H), 8.2 (t, *J* = 5.7 Hz, 1 H), 8.1 (t, *J* = 5.7 Hz, 1 H), 7.5 (m, 2 H), 7.4–7.3 (m, 3 H), 7.0 (m, 2 H), 6.7 (m, 2 H), 6.5 (m, 1 H), 6.3 (d, *J* = 10.5 Hz, 1 H), 5.3 (ddd, *J* = 20.6, 10.5, 8.7 Hz, 1 H), 3.9–3.7 (m, 6 H), 3.6 (s, 3 H).

¹³C{¹H} NMR (DMSO-*d*₆, 100 MHz): δ = 170.7, 169.6, 168.6, 163.7 (t, *J* = 28.9 Hz), 147.1, 135.8, 129.4, 129.3, 128.6, 117.9, 116.6 (dd, *J* = 260.6, 254.3 Hz), 114.3, 58.7 (dd, *J* = 27.9, 22.1 Hz), 52.2, 42.6, 42.3, 41.1.

¹⁹F NMR (DMSO-*d*₆, 376 MHz): δ = –106.6 (dd, *J* = 251.2, 8.7 Hz, 1 F), –117.8 (dd, *J* = 251.2, 20.6 Hz, 1 F).

MS (EI): m/z = 462 [M]⁺.

HRMS (EI): m/z [M]⁺ calcd for C₂₂H₂₄F₂N₄O₅: 462.1715; found: 462.1716.

Peptide Elongation Procedure for the Synthesis of Tripeptides 6

Methyl (3-Amino-2,2-difluoro-3-phenylpropanoyl)glycinate (5)

To a vial containing difluoro-β-lactam **2b** (145 mg, 0.5 mmol) and glycine methyl ester hydrochloride (126 mg, 1.0 mmol) in TFE (2.5 mL) was added DIPEA (0.26 mL, 1.5 mmol) at r.t. Then, the mixture was stirred at r.t. for 2 h, poured into H₂O, and extracted with EtOAc. The combined organic phases were washed with brine, dried (MgSO₄), and concentrated in vacuo. The crude fluorinated dipeptide **4** was used to the oxidative deprotection reactions without further purification. To a vial containing the crude dipeptide **4** in MeCN (10 mL) was added a solution of ceric ammonium nitrate (548 mg, 1.0 mmol) in H₂O (1.1 mL) at 0 °C over 10 min under air. The mixture was stirred at r.t. for 2 h. The mixture was poured into sat. aq NaHCO₃, and extracted with EtOAc. The combined organic phases were washed with sat. aq Na₂SO₃ (2 ×) and brine, dried (MgSO₄), and concentrated in vacuo. The crude mixture was purified by flash column chromatography (70% EtOAc in hexane) to give the pure product **5** as a colorless liquid; yield: 77 mg (70% based on **2b**).

¹H NMR (CDCl₃, 400 MHz): δ = 7.4–7.3 (m, 5 H), 7.1 (br s, 1 H), 4.6 (dd, *J* = 16.3, 10.2 Hz, 1 H), 4.1 (dd, *J* = 18.3, 5.3 Hz, 1 H), 3.9 (dd, *J* = 18.3, 5.2 Hz, 1 H), 3.7 (s, 3 H), 1.8 (br s, 2 H).

¹³C{¹H} NMR (CDCl₃, 100 MHz): δ = 169.1, 163.9 (t, *J* = 29.4 Hz), 136.0, 128.6, 128.5, 128.0, 116.0 (dd, *J* = 257.7, 255.8 Hz), 57.7 (dd, *J* = 25.0, 23.1 Hz), 52.6, 41.0.

¹⁹F NMR (CDCl₃, 376 MHz): δ = –111.9 (dd, *J* = 255.0, 10.1 Hz, 1 F), –118.7 (dd, *J* = 255.0, 16.3 Hz, 1 F).

MS (EI): m/z = 272 [M]⁺.

HRMS (EI): m/z [M]⁺ calcd for C₁₂H₁₄F₂N₂O₃: 272.0972; found: 272.0979.

Methyl (3-[2-(((9H-Fluoren-9-yl)methoxy)carbonyl)amino]-acetamido)-2,2-difluoro-3-phenylpropanoyl)glycinate (6)

To a vial containing dipeptide **5** (54 mg, 0.2 mmol), Fmoc-glycine (62 mg, 0.21 mmol), and HOBt (29.7 mg, 0.22 mmol) in DMF (1 mL) was added EDC (42 mg, 0.22 mmol) in DMF (1.5 mL) at r.t. Then, the mixture was stirred at r.t. for 22 h. The mixture was poured into aq 10% HCl and extracted with EtOAc. The combined organic phases were washed with aq 10% HCl, sat. aq NaHCO₃ (2 ×) and brine, dried (MgSO₄), and concentrated in vacuo. The obtained solid was collected by suction filtration and washed with cold Et₂O to give the pure product **6** as a colorless solid; yield: 88.6 mg (80%); mp 185.5–189.0 °C.

¹H NMR (DMSO-*d*₆, 400 MHz, 80 °C): δ = 8.9 (br s, 1 H), 8.6 (br d, *J* = 9.6 Hz, 1 H), 7.8 (m, 2 H), 7.7 (m, 2 H), 7.4–7.2 (m, 10 H), 5.7 (td, *J* = 14.8, 9.8 Hz, 1 H), 4.3–4.1 (m, 3 H), 3.9–3.7 (m, 4 H), 3.6 (s, 3 H).

¹³C{¹H} NMR (DMSO-*d*₆, 100 MHz): δ = 168.9, 168.9, 162.7 (t, *J* = 28.9 Hz), 156.3, 143.7, 140.6, 134.1, 128.4, 128.2, 128.1, 127.5, 126.9, 125.1, 119.9, 115.3 (t, *J* = 257.2 Hz), 65.6, 53.7 (t, *J* = 24.1 Hz), 51.7, 46.5, 43.0, 40.6.

¹⁹F NMR (DMSO-*d*₆, 376 MHz, at 80 °C): δ = –111.1 (s, 2 F).

MS (FAB): m/z = 552 [M + H]⁺.

HRMS (FAB): m/z $[M + H]^+$ calcd for $C_{29}H_{28}F_2N_3O_6$: 552.1946; found: 552.1946.

Kinetic Study on the Stepwise Synthesis of **3a** via Alcoholysis Intermediate (Scheme 2)

To a vial containing difluoro- β -lactam **2a** (51.9 mg, 0.2 mmol) in the corresponding alcoholysis solvent (0.3 mL) was added DIPEA (0.035 mL, 0.2 mmol) at r.t. and stirred at r.t. for 2 h. The full conversion of **2a** to **7** or **8** was monitored by TLC analysis. To the reaction mixture were added glycine methyl ester hydrochloride (50.0 mg, 0.4 mmol) and DIPEA (0.07 mL, 0.4 mmol) and the mixture was stirred at r.t. for 0.5 h. The mixture was poured into H_2O and then extracted with EtOAc. The combined organic phases were washed with brine, dried ($MgSO_4$), and concentrated in vacuo. The crude mixture was purified with flash column chromatography to give the pure product **3a**.

Alcoholysis of **2a** for the Structural Determination of Intermediates **7** and **8**

2,2,2-Trifluoroethyl 2,2-Difluoro-3-phenyl-3-(phenylamino)propanoate (**7**)

To a vial containing difluoro- β -lactam **2a** (51.9 mg, 0.2 mmol) in TFE (0.3 mL) was added DIPEA (0.035 mL, 0.2 mmol) at r.t. and the mixture was stirred at r.t. for 2 h. The mixture was poured into H_2O and extracted with EtOAc. The combined organic phases were washed with brine, dried ($MgSO_4$), and concentrated in vacuo. The crude mixture was purified by flash column chromatography (10% EtOAc in hexane) to give the pure product **7** as a colorless solid; yield: 40.9 mg (59%); mp 125.0–128.0 °C.

1H NMR ($CDCl_3$, 400 MHz): δ = 7.42–7.35 (m, 5 H), 7.16–7.12 (m, 2 H), 6.78–6.75 (m, 1 H), 6.66–6.64 (m, 2 H), 5.14 (ddd, J = 19.9, 10.3, 7.4 Hz, 1 H), 4.64–4.46 (m, 2 H), 4.37 (d, J = 10.1 Hz, 1 H).

$^{13}C\{^1H\}$ NMR ($CDCl_3$, 100 MHz): δ = 162.3 (dd, J = 35.0, 32.5 Hz), 144.9, 133.0, 129.4, 129.1, 128.8, 128.2, 122.1 (q, J = 277.3 Hz), 119.6, 114.5, 114.4 (dd, J = 259.4, 255.9 Hz), 61.9 (q, J = 37.6 Hz), 59.9 (dd, J = 27.2, 22.1 Hz).

^{19}F NMR ($CDCl_3$, 376 MHz): δ = –73.5 (t, J = 7.9 Hz, 3 F), –107.8 (dd, J = 259.2, 7.4 Hz, 1 F), –119.8 (dd, J = 259.2, 19.9 Hz, 1 F).

MS (EI): m/z = 359 $[M]^+$.

HRMS (EI): m/z $[M]^+$ calcd for $C_{17}H_{14}F_5NO_2$: 359.0945; found: 359.0942.

Methyl 2,2-Difluoro-3-phenyl-3-(phenylamino)propanoate (**8**)

To a vial containing difluoro- β -lactam **2a** (51.9 mg, 0.2 mmol) in MeOH (0.3 mL) was added DIPEA (0.035 mL, 0.2 mmol) at r.t. and the mixture was stirred at r.t. for 2 h. The mixture was poured into H_2O and extracted with EtOAc. The combined organic phases were washed with brine, dried ($MgSO_4$), and concentrated in vacuo. The crude mixture was purified by flash column chromatography (5% EtOAc in hexane) to give the pure product **8** as a colorless solid; yield: 54.4 mg (93%); mp 120.5–121.5 °C.

1H NMR ($CDCl_3$, 400 MHz): δ = 7.41–7.32 (m, 5 H), 7.14–7.10 (m, 2 H), 6.75–6.62 (m, 3 H), 5.11 (ddd, J = 19.0, 9.6, 7.5 Hz, 1 H), 4.44 (br d, J = 9.6 Hz, 1 H), 3.81 (s, 3 H).

$^{13}C\{^1H\}$ NMR ($CDCl_3$, 100 MHz): δ = 164.0 (dd, J = 33.2, 31.3 Hz), 145.3, 133.7, 129.3, 128.9, 128.7, 128.3, 119.2, 114.5 (dd, J = 258.2, 256.2 Hz), 114.3, 60.1 (dd, J = 27.0, 22.2 Hz), 53.6.

^{19}F NMR ($CDCl_3$, 376 MHz): δ = –108.6 (dd, J = 257.9, 7.5 Hz, 1 F), –119.2 (dd, J = 257.9, 19.0 Hz, 1 F).

MS (EI): m/z = 291 $[M]^+$.

HRMS (EI): m/z $[M]^+$ calcd for $C_{18}H_{20}F_2N_2O$: 291.1071; found: 291.1074.

Funding Information

This work was supported by JSPS KAKENHI Grant Number JP16K18855.

Acknowledgment

We thank James Murray, Ph.D. of Edanz Group (www.edanzediting.com) for editing a draft of this manuscript.

Supporting Information

Supporting information for this article is available online at <https://doi.org/10.1055/s-0040-1707238>.

References

- (a) Appella, D. H.; Christianson, L. A.; Karle, I. L.; Powell, D. R.; Samuel, H.; Gellman, S. H. *J. Am. Chem. Soc.* **1996**, *118*, 13071. (b) Gellman, S. H. *Acc. Chem. Res.* **1998**, *31*, 173. (c) Appella, D. H.; Barchi, J. J., Jr.; Durell, S. R.; Gellman, S. H. *J. Am. Chem. Soc.* **1999**, *121*, 2309. (d) Sussman, F.; Villaverde, M. C.; Estévez, J. C.; Estévez, R. *J. Phys. Chem. B* **2009**, *113*, 9669. (e) Guichard, G.; Zerbib, A.; Gal, F.-A. L.; Hoebeke, J.; Connan, F.; Choppin, J.; Briand, J.-P.; Guillet, J.-G. *J. Med. Chem.* **2000**, *43*, 3803. (f) Zhu, Y.; Wu, G.; Zhu, X.; Ma, Y.; Zhao, X.; Li, Y.; Yuan, Y.; Yang, J.; Yu, S.; Shao, F.; Lei, M. *J. Med. Chem.* **2010**, *53*, 8619. (g) Hansen, T.; Ausbacher, D.; Flaten, G.; Havelkova, M.; Strøm, M. B. *J. Med. Chem.* **2011**, *54*, 858.
- (a) Cabrele, C.; Martinek, T. A.; Reiser, O.; Berlicki, L. *J. Med. Chem.* **2014**, *57*, 9718. (b) Farmer, L. J.; Clark, M. P.; Boyd, M. J.; Perola, E.; Jones, S. M.; Tsai, A.; Jacobs, M. D.; Bandarage, U. K.; Ledebauer, M. W.; Wang, T.; Deng, H.; Ledford, B.; Gu, W.; Duffy, J. P.; Bethiel, R. S.; Shannon, D.; Byrn, R. A.; Leeman, J. R.; Rijnbrand, R.; Bennett, H. B.; O'Brien, C.; Memmott, C.; Nti-Addae, K.; Bennani, Y. L.; Charifson, P. S. *ACS Med. Chem. Lett.* **2017**, *8*, 256. (c) Hager, M. V.; Johnson, L. M.; Wootten, D.; Sexton, P. M.; Gellman, S. H. *J. Am. Chem. Soc.* **2016**, *138*, 14970.
- (a) Abe, F.; Alvord, G.; Koyama, M.; Matsuda, A.; Talmadge, J. E. *Cancer Immunol. Immunother.* **1989**, *28*, 29. (b) Chen, L.; Teng, Y.; Xu, W. *Curr. Med. Chem.* **2011**, *18*, 964.
- Zhang, X.; Zhang, L.; Zhang, J.; Feng, J.; Yuan, Y.; Fang, H.; Xu, W. *J. Enzyme Inhib. Med. Chem.* **2013**, *28*, 545.
- (a) Wani, M. C.; Taylor, H. L.; Wall, M. E.; Coggon, P.; McPhail, A. T. *J. Am. Chem. Soc.* **1971**, *93*, 2325. (b) Stierle, A.; Strobel, G.; Stierle, D. *Science* **1993**, *260*, 214.
- Guenard, D.; Gueritte-Voegelein, F.; Potier, P. *Acc. Chem. Res.* **1993**, *26*, 160.
- Meanwell, N. A. *J. Med. Chem.* **2018**, *61*, 5822.
- For ribosome engineering, see: (a) Czekster, C. M.; Robertson, W. E.; Walker, A. S.; Söll, D.; Schepartz, A. *J. Am. Chem. Soc.* **2016**, *138*, 5194. (b) Ward, F. R.; Watson, Z. L.; Ad, O.; Schepartz, A.; Cate, J. H. D. *Biochemistry* **2019**, *58*, 4494.
- For enzyme engineering, see: Zhang, D.; Chen, X.; Zhang, R.; Yao, P.; Wu, Q.; Zhu, D. *ACS Catal.* **2015**, *5*, 2220.

- (10) For examples of non-fluorinated β -amino acids, see: (a) Sun, B.; Pluta, R.; Kumagai, N.; Shibasaki, M. *Org. Lett.* **2018**, *20*, 526. (b) Nanjo, T.; Zhang, X.; Tokuhiko, Y.; Takemoto, Y. *ACS Catal.* **2019**, *9*, 10087. (c) Li, X.; You, C.; Li, S.; Lv, H.; Zhang, X. *Org. Lett.* **2017**, *19*, 5130. (d) Angelaud, R.; Zhong, Y.-L.; Maligres, P.; Lee, J.; Askin, D. *J. Org. Chem.* **2005**, *70*, 1949. (e) Nekka, I.; Bogdán, D.; Gáti, T.; Béni, S.; Juhász, T.; Palkó, M.; Paragi, G.; Tóth, G. K.; Fülöp, F.; Mándity, I. M. *Chem. Commun.* **2019**, *55*, 3061. For examples of fluorinated β -amino acids, see: (f) Remete, A. M.; Nonn, M.; Fustero, S.; Fülöp, F.; Kiss, L. *Tetrahedron* **2018**, *71*, 6367. (g) Kiss, L.; Fülöp, F. *Chem. Rec.* **2018**, *18*, 266.
- (11) (a) Straub, M. R.; Birman, V. B. *Org. Lett.* **2018**, *20*, 7550. (b) Liu, N.; Cao, S.; Shen, L.; Wu, J.; Yu, J.; Zhang, J.; Li, H.; Qian, X. *Tetrahedron Lett.* **2009**, *50*, 1982. (c) Li, X.-G.; Lähitie, M.; Kanerva, L. T. *Tetrahedron: Asymmetry* **2008**, *19*, 1857. (d) Nakayama, K.; Kawato, H. C.; Inagaki, H.; Nakajima, R.; Kitamura, A.; Someya, K.; Ohta, T. *Org. Lett.* **2000**, *2*, 977. (e) Otaka, A.; Watanabe, J.; Yukimasa, A.; Sasaki, Y.; Watanabe, H.; Kinoshita, T.; Oishi, S.; Tamamura, H.; Fujii, N. *J. Org. Chem.* **2004**, *69*, 1634. (f) Otaka, A.; Watanabe, H.; Mitsuyama, E.; Yukimasa, A.; Tamamura, H.; Fujii, N. *Tetrahedron Lett.* **2001**, *42*, 285.
- (12) Chen, M.-W.; Yang, Q.; Deng, Z.; Ding, Q.; Peng, Y. *J. Org. Chem.* **2019**, *84*, 10371.
- (13) (a) Tiong, E. A.; Gleason, J. L. *Org. Lett.* **2009**, *11*, 1725. (b) Durham, T. B.; Miller, M. J. *J. Org. Chem.* **2003**, *68*, 35. (c) Ojima, I.; Sun, C. M.; Park, Y. H. *J. Org. Chem.* **1994**, *59*, 1249.
- (14) Sato, K.; Tarui, A.; Matsuda, S.; Omote, M.; Ando, A.; Kumadaki, I. *Tetrahedron Lett.* **2005**, *46*, 7679.
- (15) (a) Jarrahpour, A.; Zarei, M. *Molecules* **2007**, *12*, 2364. (b) Hata, S.; Iguchi, M.; Iwasawa, T.; Yamada, K.; Tomioka, K. *Org. Lett.* **2004**, *6*, 1721.
- (16) Takahashi, D.; Yano, T.; Fukui, T. *Org. Lett.* **2012**, *14*, 4514.
- (17) We found it difficult to isolate the highly reactive TFE ester **7**. Careful handling was needed for the isolation.
- (18) Roberts, C. W.; McBee, E. T.; Hathaway, C. E. *J. Org. Chem.* **1956**, *21*, 1369.