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Synthesis and Spectroscopic Properties of Fluorinated Coumarin Lysine Derivatives

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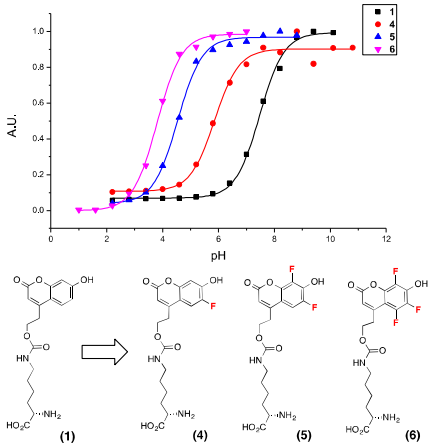
TOC Graphics



ABSTRACT: The site-selective incorporation of fluorescent amino acids into proteins has emerged as a valuable alternative to expressible protein reporters. For successful application, a robust and scalable yet flexible route to non-natural amino acids is required. This work describes an improved synthesis of coumarin-conjugated lysine derivatives where fluorinated variants are accessed. These analogues can be utilized at low pH and should find application probing biological processes that operate under acidic conditions.

Fluorescently labeled proteins are valuable tools for studying biological processes. Green fluorescent protein is universally employed,[1] but has limitations in terms of its size and that it can only be placed at the termini of the studied protein, which can perturb protein function. The site-selective incorporation of non-natural

fluorescent amino acids is a promising alternative to expressible protein tags due to their significantly smaller size, higher photo efficiency and the option to introduce them at internal positions of the protein of interest (Figure 1A).[2-4] Unnatural amino acids can be introduced in response to a tactically placed stop codon, typically the least commonly used amber codon, TAG, by orthogonal tRNA/aminoacyl-tRNA synthetase pairs.[5] Amber suppression studies have been successfully demonstrated in prokaryotic,[6] eukaryotic systems[7] and in living organisms.[8]

The coumarin structural motif is commonly incorporated into probes for biological study due to its desirable photo-physical properties: high quantum yields, photostability (low bleaching) and reasonable Stokes shifts. Coumarins are also amendable to functionalization, typically at the 3- or 4-positions.[9] 7-Hydroxycoumarins exhibit pH dependent photophysical properties, dictated by the pK_a of the phenolic substituent (**1**, pK_a = 7.7) and therefore operate more efficiently under neutral to basic conditions. To investigate biological systems that operate under reduced pH, for example the lysosomal and endosomal networks (pH ≈ 5), coumarins that exhibit greater phenolic acidity are desirable. Aryl ring fluorination is an effective strategy to achieve this, as is evident by the phenol pK_a of diFMU (**3**) (pK_a = 4.7), in comparison to its parent derivative umbelliferone (**2**) (pK_a = 7.8) (Figure 1B).[10,11]

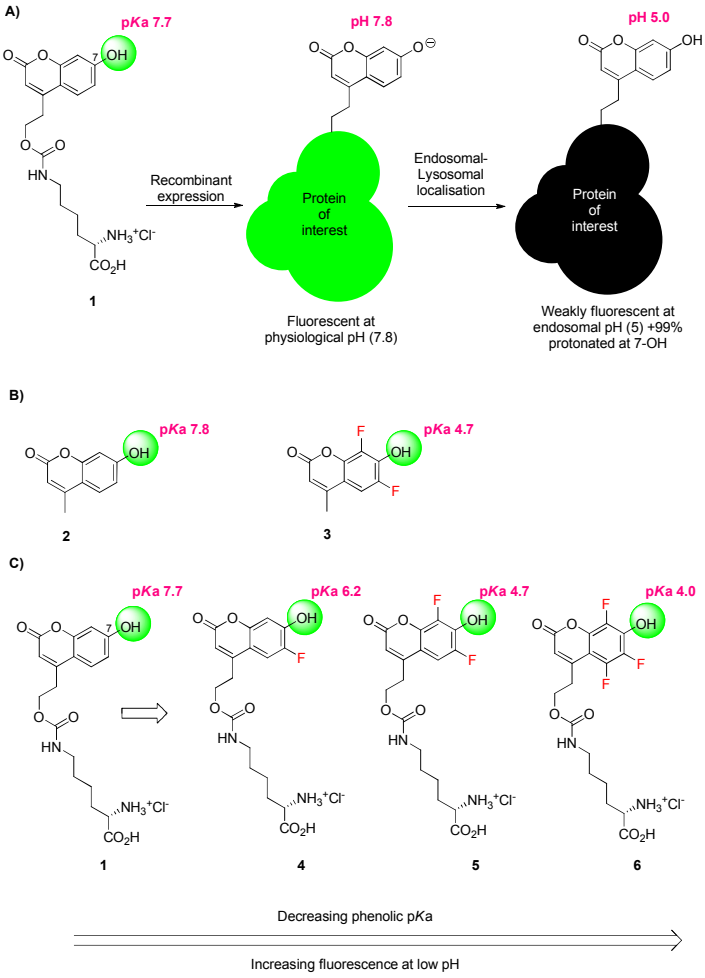


Figure 1. Dependence of fluorescence on phenolic coumarin pK_a.

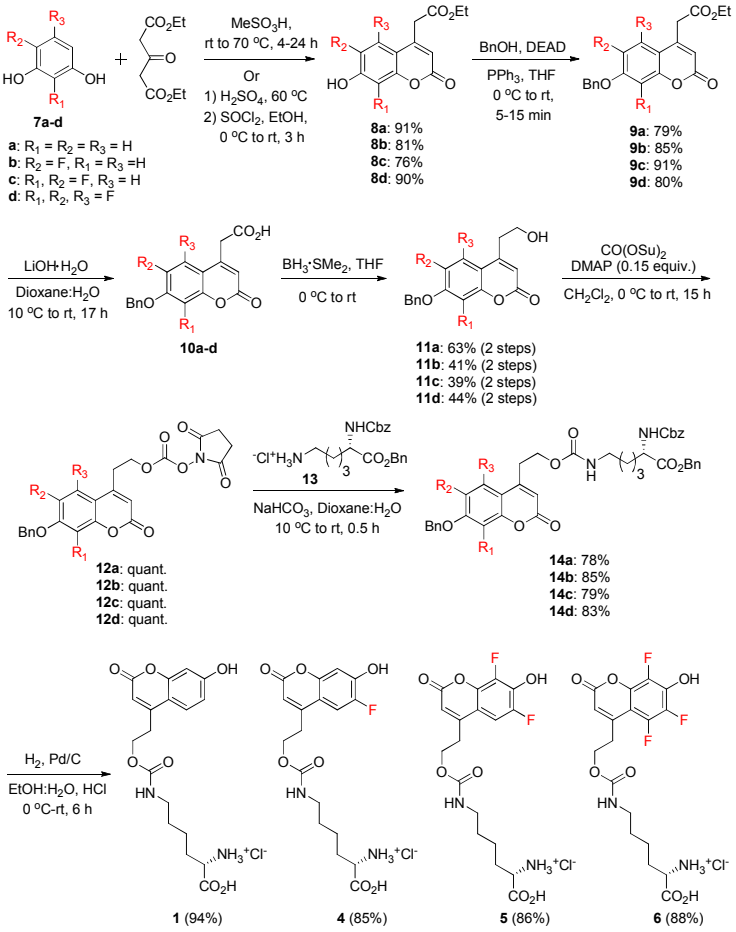
In 2014, Deiters' group reported the synthesis of the novel coumarin functionalized lysine **1**. Using an engineered pyrrolysyl-tRNA synthetase system, this amino acid was incorporated into proteins in both bacteria and mammalian cells, where it was used as a fluorescent probe for protein localization.[12]

Given the successful application of **1**, and the effect of coumarin fluorination on phenolic pK_a, we envisaged that fluorinated variants **4-6** would find application in elucidating bacterial translocation systems, as well as protein trafficking in the endosome at lower pH (Figure 1C). However, for the required orthogonal tRNA/aminoacyl-tRNA synthetase mutagenesis studies, and further application in expression systems that often exhibit low incorporation efficiency, ample amounts of

amino acids **1** and **4-6** would be required. The current route to **1** is reported only on the milligram scale, including a low yielding step (33%), which reduces the overall synthetic efficiency of the route.[12] Furthermore, it utilizes the methoxymethyl (MOM) as a phenolic protecting group, which is installed with chloromethyl methyl ether, a known carcinogen not acceptable in most laboratories. We hereby report a modified approach that enables gram scale syntheses of coumarin-functionalized lysines **1** and fluorinated analogues **4-6**. Key facets of this route include the use of *N*,*N*'-disuccinimidyl carbonate to unify the coumarin and lysine moieties, and a benzyl-based protecting group strategy that allows easily manipulated synthetic intermediates and global deprotection by hydrogenation to afford the desired compounds as their hydrochloride salts.

The synthesis commenced with the Pechmann condensation of resorcinols **7a-d** with diethyl 1,3-acetonedicarboxylate to afford the requisite coumarin scaffolds **8a-d** (Scheme 1). Reaction of compounds **7a-c** proceeded smoothly in methane sulfonic acid resulting in coumarin esters **8a-c** (76-91%). For 2,4,5-trifluoro resorcinol **7d**, we found that sulfuric acid was more suitable to affect the Pechmann reaction. As some of the corresponding acid was detected along with the desired product, the crude reaction mixture was treated with $SOCl_2$/EtOH for re-esterification, which after purification afforded coumarin ester **8d** in 90% yield. During evaluation of the previously reported route to **1**,[12] we found that the borane-mediated reduction of a coumarin acid that lacked protection of the C-7 phenolic functionality was problematic due to over-reduction of the coumarin system, particularly on larger scales.

Scheme 1. Synthesis of coumarin-lysines 1, 4-6.



It was rationalized that this problem could be circumvented *via* 7-OH benzylation of the appropriate coumarin ester prior to reduction. However, standard conditions for phenolic benzylation (BnBr, K_2CO_3, DMF) resulted in complex reaction mixtures, which included compounds indicative of over-alkylation; mono-benzylation at the α-carbon of the ethyl ester was observed (see Supporting Information). Introducing the phenolic benzyl-protecting group under Mitsunobu condition solved this issue and the corresponding benzylated coumarins **9a-d** were isolated in excellent yields (79-91%), Attempts were made to directly reduce ethyl ester **9a** to the requisite alcohol **11a** by employing ester-selective reducing agents (e.g. $Zn(BH_4)_2$ or $LiBH_4$). However, in both cases the reactions were sluggish, while increasing temperature or reaction times yielded complex reaction mixtures. Alternatively, ester hydrolysis using lithium

hydroxide followed by acidification gave the corresponding coumarin acids, which on reduction with $BH_3 \cdot Me_2S$, yielded coumarin alcohols **11a-d** (63-44%) over two steps. To access an intermediate suitable for linking the coumarin alcohol and lysine moieties together, the previously reported route utilized *p*-nitrophenyl chloroformate to form the corresponding mixed carbonate.[12] We found that this reagent difficult to control on larger scales, resulting in inconsistent yields. To remedy this, we decided to employ *N*,*N*'-disuccinimidyl carbonate $(SuO)_2CO$ as a synthetic lynchpin between the coumarin and lysine motifs. Treatment of coumarin alcohol **11c** with $(SuO)_2CO$ under standard conditions (DIPEA in MeCN) provided the corresponding elimination product in close to quantitative yield (see SI). After careful investigation, it was found that a sub-stoichiometric amount (20 mol%) of 4-dimethylaminopyridine (DMAP) in CH_2Cl_2 successfully promoted the formation of mixed OSu-carbonates **12a-d** in high yields and purity after aqueous workup. It should be noted that these intermediates are reasonably stable and could be stored at -20°C without noticeable decomposition. Unsymmetrical carbonates **12a-d** were coupled to Cbz-protected lysine benzyl ester **13** under mild conditions (dioxane/water, $NaHCO_3$), providing the fully protected coumarin-lysine derivatives **14a-d** in high yields. With the fully protected amino acid, the hydroxy-succinimide byproduct can be easily removed by aqueous workup. Furthermore, these fully protected derivatives can be purified to absolute homogeneity by column chromatography at this stage, prior to global deprotection. Final deprotections of **14a-d** were conducted using standard conditions (Pd/C, $EtOH/H_2O$, H_2) with the inclusion of aq. HCl (1 eq.). After filtration, the reaction mixtures were concentrated to dryness and triturated with diethyl ether to provide the corresponding HCl-salts **1**, **4-6** in excellent yields (Scheme 1).

With the four coumarin-functionalized lysines in hand, we investigated their spectroscopic properties from a range of pH values (1.0 – 10.8) to elucidate the effect of fluorination. The phenolic pK_a's of compounds **1** and **4-6** were determined by examining the effect of pH on absorbance at 360 nm, revealing a clear relationship between increasing fluorine substitution and decreasing pK_a (Table 1). The compounds displayed favorable photophysical properties, including high quantum yields, long lifetimes and well-separated emission peaks.

Table 1. Spectroscopic data.[a]

Compound	pKa[b]	em_{max} (nm)	Φ_f	τ (ns)
1	7.7	456	0.56	5.5
4	6.2	453	0.64	5.0
5	4.7	462	0.70	5.5
6	4.0	467	0.58	5.6

[a] For the deprotonated, fluorescent state. [b] Determined with absorption at 360 nm

Examining the normalized total fluorescence emission of **1** from pH 2.2 – 10.8 showed that the non-fluorinated amino acid derivative only showed significant fluorescence at pH values greater than 6.0 (Figure 2) when excited at 360 nm. In comparison, the fluorinated analogues **4-6** demonstrated fluorescence at lower pH ranges. For applications in biological settings that operate at reduced pH's, compounds **5** and **6** in particular would be suitable.

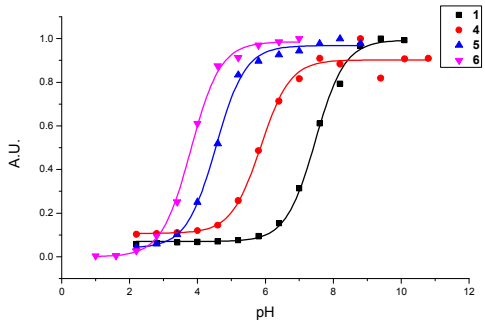


Figure 2. Normalized summed fluorescence emission of coumarin derivatives **1**, **4-6** with sigmoidal curve fit.

The site selective incorporation of non-natural fluorescent amino acids into proteins is a powerful tool for studying biological processes. For effective application of this technology, robust and scalable synthetic methodologies are required to access the requisite amino acids. Furthermore, these probes must operate efficiently in the cellular locations of study. The current research describes the scalable synthesis of four coumarin-functionalized lysines, including three fluorinated derivatives (**4-6**). The latter compounds displayed desirable spectroscopic properties at low pH and will find use for probing protein function in acidic environments.

EXPERIMENTAL SECTION

General

All reactions were performed in oven-dried apparatus and under a nitrogen atmosphere. Unless otherwise mentioned, all chemicals, reagents and solvents were used as obtained from commercial suppliers. Melting points were obtained in open capillary tubes and are uncorrected. NMR spectra were recorded on Bruker AVANCE (at 400 MHz or 600 MHz) spectrometers. ^{1}H NMR chemical shifts are reported in δ values relative to tetramethylsilane and referenced to the residual solvent peak

($CDCl_3$: δ_H = 7.26 ppm, DMSO-d_6: δ_H = 2.50 ppm and ^{13}C NMR chemical shift values are relative to tetramethylsilane (δ = 0.00 ppm), $CDCl_3$ (δ = 77.16 ppm), DMSO-d_6 (δ = 39.50 ppm). Infrared spectra were recorded as thin films. High resolution mass spectra (HRMS) were recorded using electron spray ionization time-of-flight (ESI-tof) mode. Optical rotations were measured using a 1 mL cell with a 1 dm path length and are reported as $[\alpha]_D^{20}$ (c = g/100 mL, solvent).

Ethyl 2-(7-hydroxy-2-oxo-2H-chromen-4-yl)acetate (8a).[13] To a 0 °C cooled and well stirred mixture of resorcinol **7a** (11.0 g, 100.0 mmol) and diethyl 1,3-acetonedicarboxylate (22.2 g, 110.0 mmol) was added $MeSO_3H$ (100 mL) dropwise over a period of 10 minutes, and the resultant reaction solution was stirred at 50 °C for 4 h. It was thereafter allowed to cool to room temperature and poured onto crushed ice. The obtained yellowish precipitate was filtered, washed with cold water (3 × 50 mL) and pet. ether (3 × 30 mL). The obtained solid was dried under high vacuum to get compound **8a** as a yellowish solid (22.5 g, 91%). Mp 150–152 °C (lit[14] 155–157 °C); ^1H NMR (600 MHz, DMSO-d_6) δ 10.59 (s, 1H), 7.51 (d, J = 8.8 Hz, 1H), 6.80 (dd, J = 8.7, 2.4 Hz, 1H), 6.73 (d, J = 2.3 Hz, 1H), 6.23 (s, 1H), 4.11 (q, J = 7.1 Hz, 2H), 3.93 (s, 2H), 1.18 (t, J = 7.1 Hz, 3H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 169.2, 161.3, 160.1, 155.0, 149.6, 126.7, 113.0, 112.1, 111.2, 102.3, 60.9, 36.9, 14.0.

Ethyl 2-(6-fluoro-7-hydroxy-2-oxo-2H-chromen-4-yl)acetate (8b). The experimental procedure was similar to that for compound **8a**, except that the resultant reaction solution was heated at 70 °C for 24 h. After filtration, washing and drying, compound **8b** (4.30 g, 81%) was obtained as an off-white solid. Mp 160–163 °C; ^1H NMR (600 MHz, DMSO-d_6) δ 11.16 (s, 1H), 7.55 (d, J = 11.5 Hz, 1H), 6.92 (d, J = 7.3 Hz, 1H), 6.32 (s, 1H), 4.12 (q, J = 7.1 Hz, 2H), 3.95 (s, 2H), 1.18 (t, J = 7.0 Hz,

3H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 169.0, 159.9, 150.6, 149.2 (d, J = 2.5 Hz), 149.1 (d, J = 14.4 Hz), 148.01 (d, J = 239.6 Hz), 113.2, 111.9 (d, J = 21.3 Hz), 110.7 (d, J = 7.6 Hz), 104.6 (d, J = 3.1 Hz), 60.8, 36.7, 14.0; ^{19}F NMR (376 MHz, DMSO-d_6) δ -139.15 (dd, J = 11.4, 7.3 Hz); IR (neat) 3176, 2985, 1739, 1681cm^{-1}; HRMS (ESI) calcd for $C_{13}H_{12}FO_5^+$ $(M + H)^+$, 267.0663; found, 267.0674.

Ethyl 2-(6,8-difluoro-7-hydroxy-2-oxo-2H-chromen-4-yl)acetate (8c). The experimental procedure was similar to that for compound **8a**, except that the resultant reaction solution was stirred at room temperature overnight. After filtration, washing and drying, compound **8c** (4.00 g, 76%) was obtained as an off-white solid. Mp 143–145 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.58 (s, 1H), 7.47 (d, J = 11.3 Hz, 1H), 6.43 (s, 1H), 4.12 (q, J = 7.1 Hz, 2H), 3.97 (s, 2H), 1.19 (t, J = 7.1 Hz, 3H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 168.9, 158.6, 149.2, 148.4 (dd, J = 238.6. 5.2 Hz), 139.6 (d, J = 9.1 Hz), 138.3 (dd, J = 244.6, 6.8 Hz), 137.7 (dd, J = 18.0, 12.8 Hz), 114.3, 110.2 (d, J = 9.2 Hz), 106.6 (dd, J = 21.9, 3.0 Hz), 61.0, 36.7, 13.9; ^{19}F NMR (376 MHz, DMSO-d_6) δ -136.02 (d, J = 9.2 Hz), -153.55 (d, J = 9.3 Hz); IR (neat) 3476,1712, 1637 cm^{-1}; HRMS (ESI) calcd for $C_{13}H_{11}F_2O_5^+$ $(M + H)^+$, 285.0569; found, 285.0567.

Ethyl 2-(5,6,8-trifluoro-7-hydroxy-2-oxo-2H-chromen-4-yl)acetate (8d). To a 0 °C cooled and well stirred mixture of resorcinol **7d**[15] (1.64 g, 10.0 mmol) and diethyl 1,3-acetonedicarboxylate (2.42 g, 12.0 mmol) was added conc. H_2SO_4 (10 mL) slowly over a period of 10 minutes. The resultant reaction solution was stirred at 60 °C overnight. It was thereafter allowed to cool to room temperature, poured onto crushed ice, and extracted with ethyl acetate (3 × 50 mL). The combined organic phases were washed with water (2 × 50 mL), brine (50 mL), dried over Na_2SO_4 and concentrated under reduced pressure. The obtained residue was dissolved in EtOH (12.8 mL), cooled to 0 °C, and $SOCl_2$ (4.76 g, 40.0 mmol) was added dropwise. The resultant

reaction solution was stirred at room temperature for 3 h. The volatiles were removed under reduced pressure and the residue was extracted with EtOAc (3 × 40 mL), washed with water (2 × 30 mL), brine, dried over Na_2SO_4 and concentrated under reduced pressure. The obtained residue was triturated with pet. ether to give compound **8d** (2.70 g, 90%) as an off-white solid. Mp 201–203 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.27 (br s, 1H), 6.47 (s, 1H), 4.13 (q, J = 7.1 Hz, 2H), 3.98 (d, J = 4.3 Hz, 2H), 1.18 (t, J = 7.1 Hz, 3H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 169.0, 157.8, 146.6 (m), 143.5 (ddd, J = 249.3, 12.2, 3.5 Hz), 138.7 (td, J = 14.0, 4.3 Hz), 138.4 (ddd, J = 10, 8, 2 Hz), 137.9 (dd, J = 256.3, 5.3 Hz), 136.3 (ddd, J = 241.2, 5, 3 Hz), 115.7, 100.9 (d, J = 13.4 Hz), 60.8, 39.3, 14.0; ^{19}F NMR (376 MHz, DMSO-d_6) δ -144.68 (ddd, J = 21.9, 9.5, 4.6 Hz), -158.62 (dd, J = 9.8, 5.4 Hz), -162.09 (dd, J = 21.9, 5.3 Hz); IR (neat) 3226, 2986, 1728, 1647 cm^{-1}; HRMS (ESI) calcd for $C_{13}H_{10}F_3O_5^+$ $(M + H)^+$, 303.0475; found, 303.0476.

Ethyl 2-(7-(benzyloxy)-2-oxo-2H-chromen-4-yl)acetate (9a).[13] To a cooled 0 °C solution of compound **8a** (6.20 g, 25.0 mmol) in anhydrous THF (250 mL) were added benzyl alcohol (4.59 g, 42.5 mmol), diethyl azodicarboxylate (DEAD, 40% in toluene, 16.03 mL, 35.0 mmol) and PPh_3 (9.83 g, 37.5 mmol). The resultant reaction solution was stirred at 0 °C for 15 min. After this, volatiles were removed under reduced pressure and the residue was purified by silica gel column chromatography (5–50% EtOAc in pet. ether) to get compound **9a** (6.65 g, 79%) as a white solid. Mp 138–140 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.48 (d, J = 8.8 Hz, 1H), 7.44–7.31 (m, 5H), 6.92 (dd, J = 8.8, 2.5 Hz, 1H), 6.88 (d, J = 2.5 Hz, 1H), 6.21 (s, 1H), 5.11 (s, 2H), 4.18 (q, J = 7.1 Hz, 2H), 3.70 (s, 2H), 1.24 (t, J = 7.1 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.8, 161.9, 160.9, 155.5, 148.2, 135.78, 128.8, 128.4, 127.6, 125.7, 113.9, 113.2, 112.7, 102.2, 70.54, 61.79, 38.30, 14.13.

Ethyl 2-(7-(benzyloxy)-6-fluoro-2-oxo-2H-chromen-4-yl)acetate (9b). The experimental procedure was similar to that for compound **9a**. After column purification, compound **9b** (1.52 g, 85%) was obtained as a white solid. Mp 126–128 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.46– 7.26 (m, 6H), 6.94 (d, *J* = 7.1 Hz, 1H), 6.27 (s, 1H), 5.18 (s, 2H), 4.19 (q, *J* = 7.2 Hz, 2H), 3.67 (s, 2H), 1.26 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 168.5, 160.4, 151.0 (d, *J* = 1.9 Hz), 150.2 (d, *J* = 12.7 Hz), 149.32 (d, *J* = 245.6 Hz), 147.7 (d, *J* = 2.7 Hz), 135.1, 128.9, 128.6, 127.5, 115.0, 111.8 (d, *J* = 7.4 Hz), 110.8 (d, *J* = 21.5 Hz), 103.2 (d, *J* = 2.0 Hz), 71.4, 61.9, 38.3, 14.1; ^{19}F NMR (376 MHz, $CDCl_3$) δ -136.75 (dd, *J* = 11.2, 7.1 Hz); IR (neat) 1728, 1626, 1563 cm^{-1}; HRMS (ESI) calcd for $C_{20}H_{18}FO_5^+$ $(M + H)^+$, 357.1133; found, 357.1136.

Ethyl 2-(7-(benzyloxy)-6,8-difluoro-2-oxo-2H-chromen-4-yl)acetate (9c). The experimental procedure was similar to that for compound **9a**. After column purification, compound **9c** (0.34 g, 91%) was obtained as a white solid. Mp 92–94 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.46–7.41 (m, 2H), 7.40–7.30 (m, 3H), 7.09 (dd, *J* = 10.9, 2.2 Hz, 1H), 6.36 (s, 1H), 5.33 (s, 2H), 4.20 (q, *J* = 7.1 Hz, 2H), 3.67 (s, 2H), 1.26 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 168.2, 158.8, 151.8 (dd, *J* = 246.5, 4.4 Hz), 147.2 (t, *J* = 2.7 Hz), 143.6 (dd, *J* = 253.7, 6.0 Hz), 140.1 (dd, *J* = 10.5, 2.7 Hz), 138.4 (dd, *J* = 15.6, 10.8 Hz), 135.7, 129.0, 128.8, 128.4, 117.1, 114.1 (d, *J* = 9.2 Hz), 105.9 (dd, *J* = 22.6, 3.7 Hz), 76.3 (t, *J* = 3.8 Hz), 62.2, 38.4, 14.2; ^{19}F NMR (376 MHz, $CDCl_3$) δ -130.42 (d, *J* = 5.3 Hz), -145.07 (d, *J* = 5.2 Hz).; IR (neat) 1734, 1629, 1511 cm^{-1}; HRMS (ESI) calcd for $C_{20}H_{17}F_2O_5^+$ $(M + H)^+$, 375.1039; found, 375.1039.

Ethyl 2-(7-(benzyloxy)-5,6,8-trifluoro-2-oxo-2H-chromen-4-yl)acetate (9d). To anhydrous THF (10 mL) at 0 °C were added diethyl azodicarboxylate (DEAD, 40% in

toluene, 0.5 mL, 1.1 mmol), PPh_3 (335 mg, 1.3 mmol), compound **8d** (302 mg, 1.0 mmol), and benzyl alcohol (0.13 mL, 1.2 mmol). The resultant reaction solution was stirred at 0 °C for 5 min, after which volatiles were removed under vacuum. The residue was purified by silica gel column chromatography (5–50% EtOAc in pet. ether) to get compound **9d** (314 mg, 80%) as a white solid. Mp 141–143 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.44 (d, *J* = 7.3 Hz, 2H), 7.42–7.34 (m, 3H), 6.24 (s, 1H), 5.37 (s, 2H), 4.21 (q, *J* = 7.1 Hz, 2H), 3.80 (d, *J* = 4.3 Hz, 2H), 1.26 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 168.6, 157.9, 145.6 (q, *J* = 2.2 Hz), 144.0 (ddd, *J* = 252.2, 13.7, 4.2 Hz), 141.1 (ddd, *J* = 247.6, 16.0, 4.5), 139.9 (dt, *J* = 250.2, 4.0 Hz), 139.0 (d, *J* = 3.6 Hz), 138.9 (dd, *J* = 4.6, 3.1 Hz), 135.4, 129.2, 128.9, 128.4, 118.0, 105.0 (d, *J* = 12.9 Hz), 76.5, 61.9, 40.5 (d, *J* = 8.8 Hz), 14.2; ^{19}F NMR (376 MHz, $CDCl_3$) δ -143.38 (ddt, *J* = 20.2, 10.6, 4.5 Hz), -151.45 (d, *J* = 10.7 Hz), -156.29 (d, *J* = 20.7 Hz); IR (neat) 1719, 1644, 1503 cm^{-1}; HRMS (ESI) calcd for $C_{20}H_{16}F_3O_5^+$ $(M + H)^+$, 393.0944; found, 393.0945.

7-(Benzyloxy)-4-(2-hydroxyethyl)-2H-chromen-2-one (11a).[13] To a cooled solution of **9a** (7.30 g, 21.6 mmol) in dioxane:H_2O (130 mL, 5:1) was added LiOH:H_2O (2.90 g, 69.1 mmol) and the resultant reaction solution was stirred at room temperature for 17 h. All volatiles were removed under reduced pressure, and the residue was dissolved in water. The resultant solution was cooled to 0 °C and then acidified with HCl (6 M, 15.0 mL) to pH 2–3. The aqueous layer was extracted with EtOAc (3 × 100 ml), and the combined organic layers were washed with water (2 × 100 ml) and brine (100 ml), and dried over Na_2SO_4. The organic phase was concentrated under reduced pressure to give carboxylic acid **10a** (6.60 g) as a yellow solid. The crude product was used as-is for the following reaction procedure.

The obtained acid was dissolved in anhydrous THF (198 mL) and cooled to 0 °C. $BH_3 \cdot SMe_2$ (1.78 g, 23.4 mmol) was added dropwise, and resultant reaction solution was stirred at room temperature for 16 h. The reaction mixture was cooled to 0 °C and quenched with MeOH (60 mL). All volatiles were removed under vacuum, and the residue was purified by silica gel column chromatography (35–90% EtOAc in pet. ether) to give alcohol **11a** (4.00 g, 63% over two steps) as a yellowish solid. Mp 130–132 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.55 (d, *J* = 8.9 Hz, 1H), 7.43–7.31 (m, 5H), 6.93 (dd, *J* = 8.8, 2.5 Hz, 1H), 6.87 (d, *J* = 2.5 Hz, 1H), 6.20 (s, 1H), 5.10 (s, 2H), 3.97 (t, *J* = 6.4 Hz, 2H), 2.98 (t, *J* = 6.5 Hz, 2H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 161.8, 161.5, 155.6, 153.5, 135.9, 128.9, 128.5, 127.6, 125.6, 113.2, 113.2, 112.1, 102.2, 70.6, 60.9, 34.9.

7-(Benzyloxy)-6-fluoro-4-(2-hydroxyethyl)-2H-chromen-2-one (11b). The experimental procedure was similar to that for compound **11a**. After column purification, compound **11b** (650 mg, 41% over two steps) was obtained as a yellowish solid. Mp 112–114 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.49–7.30 (m, 6H), 6.92 (d, *J* = 7.1 Hz, 1H), 6.26 (s, 1H), 5.18 (s, 2H), 3.98 (t, *J* = 6.3 Hz, 2H), 2.93 (td, *J* = 6.3, 1.0 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 161.1, 152.9 (d, *J* = 2.7 Hz), 151.0 (d, *J* = 1.9 Hz), 150.1 (d, *J* = 12.8 Hz), 149.44 (d, *J* = 245.4 Hz), 135.2, 129.0, 128.7, 127.6, 113.2, 112.4 (d, *J* = 7.2 Hz), 110.7 (d, *J* = 21.3 Hz), 103.3 (d, *J* = 2.0 Hz), 71.5, 60.7, 34.9; ^{19}F NMR (376 MHz, $CDCl_3$) δ -136.96 (dd, *J* = 11.3, 7.2 Hz); IR (neat) 3387, 1760, 1722, 1564 cm^{-1}; HRMS (ESI) calcd for $C_{18}H_{16}FO_4^+$ $(M + H)^+$, 315.1027; found, 315.1034.

7-(Benzyloxy)-6,8-difluoro-4-(2-hydroxyethyl)-2H-chromen-2-one (11c). The experimental procedure was similar to that for compound **11a**. After column purification, compound **11c** (1.90 g, 39% over two steps) was obtained as a yellowish

solid. Mp 109–111 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.48–7.41 (m, 2H), 7.40–7.31 (m, 3H), 7.15 (dd, *J* = 11.1, 2.3 Hz, 1H), 6.35 (s, 1H), 5.33 (s, 2H), 3.99 (t, *J* = 6.2 Hz, 2H), 2.92 (td, *J* = 6.3, 1.1 Hz, 2H), 1.75 (br s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 159.4, 152.6 (t, *J* = 2.7 Hz), 151.8 (dd, *J* = 244, 4.4 Hz), 143.5 (dd, *J* = 253.4, 6.0 Hz), 140.0 (dd, *J* = 10.3, 2.5 Hz), 138.1 (dd, *J* = 15.7, 10.7 Hz), 135.7, 129.0, 128.7, 128.4, 115.0, 114.6 (d, *J* = 8.9 Hz), 105.8 (dd, *J* = 22.4, 3.7 Hz), 76.3 (t, *J* = 3.8 Hz), 60.4, 34.7; ^{19}F NMR (376 MHz, $CDCl_3$) δ -130.78 (dd, *J* = 11.1, 5.4 Hz), -145.25 (d, *J* = 5.2 Hz).; IR (neat) 3423, 1724, 1630, 1567 cm^{-1}; HRMS (ESI) calcd for $C_{18}H_{15}F_2O_4^+$ $(M + H)^+$, 333.0933; found, 333.0940.

7-(Benzyloxy)-5,6,8-trifluoro-4-(2-hydroxyethyl)-2H-chromen-2-one (11d). The experimental procedure was similar to that for compound **11a**. After column purification, compound **11d** (430 mg, 44% over two steps) was obtained as a yellowish solid. Mp 111–113 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.43 (d, *J* = 6.6 Hz, 2H), 7.41–7.32 (m, 3H), 6.31 (s, 1H), 5.37 (s, 2H), 3.97 (t, *J* = 6.0 Hz, 2H), 3.11–3.07 (m, 2H), 1.94 (br 2, 1H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 158.2, 151.0, 144.2 (ddd, *J* = 253.3, 13.6, 3.9 Hz), 141.3 (ddd, *J* = 247.3, 16.0, 4.6 Hz), 139.9 (dt, *J* = 250.3, 3.8 Hz), 139.1 (dd, *J* = 17.9, 2.9 Hz), 138.7 (td, *J* = 12.1, 3.4 Hz), 135.3, 129.1, 129.0, 128.4, 116.3, 105.1 (d, *J* = 12.5 Hz), 76.4, 60.5 (d, *J* = 4.0 Hz), 37.8 (d, *J* = 9.0 Hz); ^{19}F NMR (376 MHz, $CDCl_3$) δ -142.57 – -142.84 (m), -151.45 (d, *J* = 10.8 Hz), -156.27 (d, *J* = 20.5 Hz); IR (neat) 3424, 1745, 1642 cm^{-1}; HRMS (ESI) calcd for $C_{18}H_{14}F_3O_4^+$ $(M + H)^+$, 351.0839; found, 351.0830.

2-(7-(Benzyloxy)-2-oxo-2H-chromen-4-yl)ethyl 2,5-dioxopyrrolidin-1-yl carbonate (12a). To a cooled 0 °C suspension of *N*,*N*′-disuccinimidyl carbonate (3.63 g, 14.2 mmol) in anhydrous CH_2Cl_2 (135 ml) were added compound **11a** (4.0 g, 13.5 mmol) and 4-dimethylaminopyridine (0.25 g, 2.03 mmol). The resultant reaction

mixture was allowed to warm to room temperature, and stirred overnight. The mixture was partitioned between CH_2Cl_2 (100 ml) and water (100 ml), separated, and the aqueous layer was extracted with CH_2Cl_2 (2 × 70 mL). The combined organic layers were washed with 5% citric acid (2 × 70 ml), saturated $NaHCO_3$ (2 × 100 ml), water (2 × 100 ml) and brine (100 ml), and dried over Na_2SO_4. The solution was concentrated under reduced pressure to give carbonate **12a** (5.8 g, quantitative) as an off white solid. Mp 70–72 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.52 (d, *J* = 8.8 Hz, 1H), 7.46–7.33 (m, 5H), 6.97 (dd, *J* = 8.8, 2.5 Hz, 1H), 6.91 (d, *J* = 2.6 Hz, 1H), 6.19 (s, 1H), 5.13 (s, 2H), 4.60 (t, *J* = 6.9 Hz, 2H), 3.18 (t, *J* = 6.9 Hz, 2H), 2.83 (s, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.6, 162.1, 160.7, 155.7, 151.6, 150.3, 135.8, 128.9, 128.5, 127.6, 125.2, 113.5, 112.9, 112.5, 102.5, 70.6, 68.4, 30.8, 25.6; IR (neat) 1812, 1788, 1737, 1611 cm^{-1}; HRMS (ESI) calcd for $C_{23}H_{20}NO_8^+$ $(M + H)^+$, 438.1183; found, 438.1185.

2-(7-(Benzyloxy)-6-fluoro-2-oxo-2H-chromen-4-yl)ethyl-2,5-dioxopyrrolidin-1-yl carbonate (12b). The experimental procedure was similar to that for compound **12a**. The compound **12b** (455 mg, quantitative) was obtained as an off-white solid. Mp 172–175 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.45 (d, *J* = 7.6 Hz, 2H), 7.41 (t, *J* = 7.5 Hz, 2H), 7.36 (t, *J* = 7.2 Hz, 1H), 7.32 (d, *J* = 11.0 Hz, 1H), 6.97 (d, *J* = 6.9 Hz, 1H), 6.26 (s, 1H), 5.21 (s, 2H), 4.60 (t, *J* = 6.8 Hz, 2H), 3.14 (t, *J* = 6.8 Hz, 2H), 2.84 (s, 4H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 168.5, 160.4, 151.6, 151.2, 150.4 (d, *J* = 4.6 Hz), 149.7 (d, *J* = 2.7 Hz), 149.6 (d, *J* = 241.6 Hz), 135.2, 129.0, 128.8, 127.6, 114.1, 111.6 (d, *J* = 7.1 Hz), 110.3 (d, *J* = 21.4 Hz), 103.6, 71.6, 68.0, 30.9, 25.6; ^{19}F NMR (376 MHz, $CDCl_3$) δ -136.36 (dd, *J* = 11.0, 7.1 Hz); IR (neat) 1812, 1788, 1739, 1628 cm^{-1}; HRMS (ESI) calcd for $C_{23}H_{19}FNO_8^+$ $(M + H)^+$, 456.1089; found, 456.1071.

2-(7-(Benzyloxy)-6,8-difluoro-2-oxo-2H-chromen-4-yl)ethyl-2,5-dioxopyrrolidin-1-yl carbonate (12c). The experimental procedure was similar to that for compound **12a**. The compound **12c** (1.85 g, quantitative) was obtained as an off white solid. Mp 85–88 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.46–7.42 (m, 2H), 7.38–7.33 (m, 3H), 7.13 (dd, *J* = 10.9, 2.0 Hz, 1H), 6.34 (s, 1H), 5.33 (s, 2H), 4.59 (t, *J* = 6.7 Hz, 2H), 3.12 (t, *J* = 6.7 Hz, 2H), 2.82 (s, 4H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 168.6, 158.6, 151.9 (dd, *J* = 241.6, 4.3 Hz), 151.5, 149.4 (t, *J* = 2.7 Hz), 143.7 (dd, *J* = 254.0, 5.9 Hz), 140.2 (dd, *J* = 10.2, 2.5 Hz), 138.4 (dd, *J* = 15.6, 10.7 Hz), 135.7, 128.9, 128.8, 128.4, 115.9, 113.8 (d, *J* = 8.8 Hz), 105.3 (dd, *J* = 22.6, 3.4 Hz), 76.3 (t, *J* = 3.8 Hz), 67.8, 30.8, 25.5; ^{19}F NMR (376 MHz, $CDCl_3$) δ -130.14 (d, *J* = 5.7 Hz), -144.66 (d, *J* = 5.9 Hz); IR (neat) 1813, 1789, 1741, 1570 cm^{-1}; HRMS (ESI) calcd for $C_{23}H_{18}F_2NO_8^+$ $(M + H)^+$, 474.0995; found, 474.0983.

2-(7-(Benzyloxy)-5,6,8-trifluoro-2-oxo-2H-chromen-4-yl)ethyl-2,5-dioxopyrrolidin-1-yl carbonate (12d). The experimental procedure was similar to that for compound **12a**. The compound **12d** (490 mg, quantitative) was obtained as an off white solid. Mp 162–164 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.44 (d, *J* = 7.1 Hz, 2H), 7.41–7.36 (m, 3H), 6.31 (s, 1H), 5.39 (s, 2H), 4.62 (t, *J* = 6.0 Hz, 2H), 3.20–3.36 (m, 2H), 2.82 (s, 4H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 168.5, 157.5, 151.6, 147.8, 143.9 (ddd, *J* = 252.0, 13.5, 4.1 Hz), 141.2 (ddd, *J* = 241.6, 15.7, 4.4 Hz), 140.0 (dt, *J* = 250.7, 3.9 Hz), 139.3–138.9 (m, presumably overlap of 2 C and one of the triplets from the dt at 140.0 ppm), 135.3, 129.2, 129.0, 128.4, 117.7, 104.4 (d, *J* = 12.6 Hz), 76.5 (t, *J* = 3.8 Hz), 68.0, 34.1 (d, *J* = 8.7 Hz), 25.6; ^{19}F NMR (376 MHz, $CDCl_3$) δ -143.8 (dd, *J* = 20.7, 10.7 Hz), -150.9 (d, *J* = 10.7 Hz), -156.0 (d, *J* = 20.6 Hz); IR (neat) 1789, 1742, 1643 cm^{-1}; HRMS (ESI) calcd for $C_{23}H_{17}F_3NO_8^+$ $(M + H)^+$, 492.0901; found, 492.0871.

(*S*)-Benzyl-6-((2-(7-(benzyloxy)-2-oxo-2H-chromen-4-yl)ethoxy)carbonylamino)-2-(benzyloxycarbonylamino)hexanoate (14a). To a cooled solution of lysine hydrochloride **13** (5.41 g, 13.3 mmol) in dioxane:H_2O (8:2, 266 mL) were added $NaHCO_3$ (2.2 g, 26.6 mmol) and carbonate **12a** (5.8 g, 13.3 mmol). The resultant reaction mixture was slowly allowed to warm to room temperature and stirred for 0.5 h. After the completion of the reaction, EtOAc (150 mL) and water (80 mL) were added, and the organic layer was separated. The aqueous layer was extracted with EtOAc (3 × 70 mL), and the combined organic layers were washed with water (2 × 70 mL) and brine (100 mL), dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (35–90% EtOAc in pet. ether) to give carbamate **14a** (7.1 g, 78%) as a yellowish sticky solid; $[\alpha]^{20}_D$ = -2.6 (*c* = 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.55 (d, *J* = 8.6 Hz, 1H), 7.46–7.27 (m, 15H), 6.93 (dd, *J* = 8.8, 2.5 Hz, 1H), 6.87 (d, *J* = 2.5 Hz, 1H), 6.14 (s, 1H), 5.53 (d, *J* = 8.2 Hz, 1H), 5.25–5.02 (m, 6H), 4.88–4.80 (m, 1H), 4.46–4.35 (m, 1H), 4.30 (t, *J* = 6.7 Hz, 2H), 3.15–3.03 (m, 2H), 2.98 (t, *J* = 6.6 Hz, 2H), 1.91–1.76 (m, 1H), 1.75–1.60 (m, 1H), 1.55–1.38 (m, 2H), 1.37–1.24 (m, 2H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 172.3, 161.8, 161.2, 156.3, 156.1, 155.5, 152.3, 136.3, 135.8, 135.4, 128.8, 128.7, 128.57, 128.56, 128.5, 128.4, 128.2, 128.1, 127.6, 125.5, 113.2, 112.9, 112.3, 102.3, 70.5, 67.2, 67.1, 62.3, 53.8, 40.5, 32.1, 31.7, 29.3, 22.3; IR (neat) 3332, 3033, 1720, 1611 cm^{-1}; HRMS (ESI) calcd for $C_{40}H_{41}N_2O_9^+$ $(M + H)^+$, 693.2807; found, 693.2810.

(*S*)-Benzyl-6-((2-(7-(benzyloxy)-6-fluoro-2-oxo-2H-chromen-4-yl)ethoxy)carbonylamino)-2-(benzyloxycarbonylamino)hexanoate (14b). The experimental procedure was similar to that for compound **14a**. After column purification, compound **14b** (0.6 g, 85%) was obtained as a yellowish solid. Mp 78–

80 °C; $[\alpha]^{20}_D$ = -3.0 (*c* = 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.44 (d, *J* = 7.6 Hz, 2H), 7.40 (t, *J* = 7.4 Hz, 2H), 7.37–7.30 (m, 12H), 6.92 (d, *J* = 7.0 Hz, 1H), 6.19 (s, 1H), 5.48 (d, *J* = 8.3 Hz, 1H), 5.21–5.05 (m, 6H), 4.76 (t, *J* = 6.0 Hz, 1H), 4.44–4.36 (m, 1H), 4.30 (t, *J* = 6.6 Hz, 2H), 3.17–2.99 (m, 2H), 2.93 (t, *J* = 6.6 Hz, 2H), 1.89–1.78 (m, 1H), 1.74–1.62 (m, 1H), 1.53–1.41 (m, 2H), 1.37–1.26 (m, 2H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 172.3, 160.8, 156.14 (d, *J* = 5.0 Hz), 151.9, 151.0 (d, *J* = 1.5 Hz), 150.1 (d, *J* = 12.7 Hz), 149.5 (d, *J* = 245.5 Hz), 136.3, 135.4, 135.2, 129.0, 128.74, 128.70, 128.62, 128.60, 128.4, 128.3, 128.2, 127.6, 113.4, 112.1 (d, *J* = 7.3 Hz), 110.6 (d, *J* = 21.3 Hz), 103.4 (d, *J* = 1.8 Hz), 71.5, 67.19 (d, *J* = 15.0 Hz), 62.1, 53.8, 40.6, 32.2, 31.7, 29.3, 22.3; ^{19}F NMR (376 MHz, $CDCl_3$) δ -136.7; IR (neat) 3332, 2949, 1719 cm^{-1}; HRMS (ESI) calcd for $C_{40}H_{40}FN_2O_9^+$ $(M + H)^+$, 711.2712; found, 711.2721.

(*S*)-Benzyl-6-((2-(7-(benzyloxy)-6,8-difluoro-2-oxo-2H-chromen-4-yl)ethoxy)carbonylamino)-2-(benzyloxycarbonylamino)hexanoate (14c). The experimental procedure was similar to that for compound **14a**. After column purification, compound **14c** (1.9 g, 79%) was obtained as a yellowish sticky solid. $[\alpha]^{20}_D$ = -2.8 (*c* = 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.44 (d, *J* = 6.9 Hz, 2H), 7.41–7.27 (m, 13H), 7.15 (d, *J* = 10.8 Hz, 1H), 6.27 (s, 1H), 5.46 (d, *J* = 7.9 Hz, 1H), 5.32 (s, 2H), 5.22–5.12 (m, 2H), 5.08 (s, 2H), 4.76 (br s, 1H), 4.47– 4.35 (m, 1H), 4.29 (s, 2H), 3.11 (s, 2H), 3.02–2.81 (m, 2H), 1.91–1.76 (m, 1H), 1.75–1.60 (m, 1H), 1.55–1.40 (m, 2H), 1.40–1.24 (m, 2H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 172.3, 159.0, 156.1, 152.7 (d, *J* = 4.2 Hz), 151.3, 151.1 (d, *J* = 4.2 Hz), 144.4 (d, *J* = 5.8 Hz), 142.8 (d, *J* = 6.0 Hz), 140.1 (d, *J* = 10.1 Hz), 138.2 (dd, *J* = 15.8, 10.9 Hz), 136.3, 135.7, 135.4, 128.9, 128.77, 128.75, 128.6, 128.45, 128.41, 128.3, 128.2, 115.3, 114.3 (d, *J* = 8.9 Hz), 105.6 (dd, *J* = 24.2, 2.6 Hz), 76.2 (t, *J* = 3.7 Hz), 67.3, 67.1, 61.8,

53.8, 40.6, 32.2, 31.7, 29.3, 22.2; ^{19}F NMR (376 MHz, $CDCl_3$) δ -130.46 (d, J = 5.4 Hz), -145.18 (d, J = 5.3 Hz); IR (neat) 3332, 2949, 1724 cm^{-1}; HRMS (ESI) calcd for $C_{40}H_{39}F_2N_2O_9^+$ $(M + H)^+$, 729.2618; found, 729.2610.

(*S*)-Benzyl-6-((2-(7-(benzyloxy)-5,6,8-trifluoro-2-oxo-2H-chromen-4-yl)ethoxy)carbonylamino)-2-(benzyloxycarbonylamino)hexanoate (14d). The experimental procedure was similar to that for compound **14a**. After column purification, compound **14d** (756 mg, 83%) was obtained as a yellowish sticky solid. $[\alpha]^{20}_D$ = -2.6 (c = 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.47–7.27 (m, 15H), 6.20 (s, 1H), 5.52 (d, J = 8.0 Hz, 1H), 5.37 (s, 2H), 5.16 (q, J = 12.3 Hz, 2H), 5.08 (s, 2H), 4.81 (br. s, 1H), 4.52–4.26 (m, 3H), 3.20–3.01 (s, 4H), 1.92–1.77 (m, 1H), 1.73–1.62 (m, 1H), 1.52–1.21 (m, 4H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 172.3, 158.0, 156.1, 150.2, 144.0 (ddd, J = 253.3, 13.1, 3.8 Hz), 141.2 (ddd, J = 247.8, 15.9, 4.2 Hz), 139.8 (dt, J = 250.0, 3.3 Hz), 139.1-138.5 (m, presumably overlap of 2 C and one of the triplets from the dt at 139.8 ppm), 136.3, 135.4, 135.3, 129.1, 128.7 (d, J = 19.2 Hz), 128.54, 128.52, 128.4, 128.3, 128.2, 128.1, 116.3, 104.8 (d, J = 12.6 Hz), 98.9, 76.4 (t, J = 3.4 Hz), 67.2, 67.0, 66.1 (d = 19.9 Hz), 61.7 (d, J = 3.4 Hz), 61.0, 53.8, 40.5, 34.6 (d, J = 8.9 Hz), 32.0, 29.3, 22.3; ^{19}F NMR (376 MHz, $CDCl_3$) δ -142.78, -142.81, -142.83, -142.86, -151.41, -151.44, -156.13, -156.18; IR (neat) 3359, 2943, 1723, 1700 cm^{-1}; HRMS (ESI) calcd for $C_{40}H_{38}F_3N_2O_9^+$ $(M + H)^+$, 747.2524; found, 747.2533.

(*S*)-2-amino-6-(((2-(7-hydroxy-2-oxo-2H-chromen-4-yl)ethoxy)carbonyl)amino)hexanoic acid hydrochloride (1).[12] To a cooled suspension of carbamate **14a** (7.10 g, 10.3 mmol) in EtOH:H_2O (9:1, 103 mL) was added conc. HCl (0.96 mL, 1 eq.) followed by Pd/C (10% w/w, 0.71 g). The reaction

mixture was evacuated and back-filled with H_2, and was stirred at room temperature under an H_2 atmosphere (1 atm). After completion, the reaction solution was filtered through a small pad of celite and washed with EtOH:H_2O (1:1, 2 × 50 mL). The filtrate was concentrated under reduced pressure, and the residue was triturated with Et_2O. The obtained solid was dissolved in H_2O (60 mL) and lyophilized to give compound **1** (4.00 g, 94%) as a white solid. Mp 185–188 °C; $[\alpha]_D^{20}$ = +10 (*c* = 1.0, DMSO); ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.32 (br s, 3H), 7.68 (d, *J* = 8.7 Hz, 1H), 7.17 (br s, 1H), 6.84 (d, *J* = 8.7 Hz, 1H), 6.76 (s, 1H), 6.11 (s, 1H), 4.31–4.21 (m, 2H), 3.84–3.70 (m, 1H), 3.08–3.00 (m, 2H), 2.99–2.90 (m, 2H), 1.84–1.70 (m, 2H), 1.45–1.20 (m, 4H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 171.0, 161.4, 160.2, 156.0, 155.1, 153.5, 126.4, 113.1, 111.1, 110.4, 102.5, 61.6, 51.8, 31.0, 29.6, 28.8, 21.6; IR (neat) 3371, 2933, 1688, 1602 cm^{-1}; HRMS (ESI) calcd for $C_{18}H_{23}N_2O_7^+$ $(M + H)^+$, 379.1500; found, 379.1524.

(*S*)-2-amino-6-(((2-(6-fluoro-7-hydroxy-2-oxo-2H-chromen-4-yl)ethoxy)carbonyl)amino)hexanoic acid hydrochloride (4). The experimental procedure was similar to that for compound **1**. Compound **4** (772 mg, 85%) was obtained as a white solid. Mp 200–203 °C; $[\alpha]^{20}_D$ = +9.8 (*c* = 1.0, DMSO); ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.36 (s, 3H), 7.69 (d, *J* = 11.7 Hz, 1H), 7.18 (t, *J* = 5.3 Hz, 1H), 7.01 (d, *J* = 7.4 Hz, 1H), 6.19 (s, 1H), 4.23 (t, *J* = 6.2 Hz, 2H), 3.81 (t, *J* = 5.6 Hz, 1H), 3.01 (t, *J* = 6.2 Hz, 2H), 2.98–2.89 (m, 2H), 1.86–1.66 (m, 2H), 1.42–1.23 (m, 4H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 171.0, 160.0, 156.0, 153.1, 150.5, 149.2 (d, *J* = 14.3 Hz), 148.2 (d, *J* = 239.8 Hz), 111.6, 111.5 (d, *J* = 21.2 Hz), 110.6 (d, *J* = 7.5 Hz), 104.7 (d, *J* = 2.9 Hz), 61.5, 51.9, 31.0, 29.6, 28.8, 21.6; ^{19}F NMR (376 MHz, DMSO-d_6) δ -139.20 (dd, *J* = 11.2, 7.9 Hz); IR (neat) 2943, 1691, 1628 cm^{-1}; HRMS (ESI) calcd for $C_{18}H_{22}FN_2O_7^+$ $(M + H)^+$, 397.1406; found, 397.1411.

(*S*)-2-amino-6-(((2-(6,8-difluoro-7-hydroxy-2-oxo-2H-chromen-4-yl)ethoxy)carbonyl)amino)hexanoic acid hydrochloride (5). The experimental procedure was similar to that for compound **1**. Compound **5** was obtained as a white solid (505 mg, 86%). Mp 198–200°C; $[\alpha]^{20}_{D}$ = +10 (*c* = 1.0, DMSO); ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.37 (s, 3H), 7.60 (d, *J* = 11.3 Hz, 1H), 7.18 (t, *J* = 5.1 Hz, 1H), 6.29 (s, 1H), 4.23 (t, *J* = 5.9 Hz, 2H), 3.79 (br. s, 1H), 3.03 (t, *J* = 5.8 Hz, 2H), 2.97–2.88 (m, 2H), 1.83–1.67 (m, 2H),), 1.43–1.20 (m, 4H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 171.1, 158.7, 156.0, 153.2, 148.7 (dd, *J* = 239.5, 4.8 Hz), 139.6 (d, *J* = 9.0 Hz), 139.5 (dd, *J* = 240.0, 6.0 Hz), 138.0 (t, *J* = 15.9 Hz), 112.5, 110.0 (d, *J* = 9.0 Hz), 106.2 (d, *J* = 21 Hz), 61.4, 51.9, 31.1, 29.7, 28.8, 21.6; ^{19}F NMR (565 MHz, DMSO-d_6) δ -135.65, -135.66, -135.68, -153.61, -153.62; IR (neat) 2944, 1693, 1632 cm^{-1}; HRMS (ESI) calcd for $C_{18}H_{21}F_2N_2O_7^+$ $(M + H)^+$, 415.1311; found, 415.1338.

(*S*)-2-amino-6-(((2-(5,6,8-trifluoro-7-hydroxy-2-oxo-2H-chromen-4-yl)ethoxy)carbonyl)amino)hexanoic acid hydrochloride (6). The experimental procedure was similar to that for compound **1**. Compound **6** was obtained as a white solid (210 mg, 88%). Mp 183–186 °C; $[\alpha]^{20}_{D}$ = +4 (*c* = 0.5, DMSO); ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.30 (s, 3H), 7.16 (t, *J* = 5.2 Hz, 1H), 6.15 (s, 1H), 4.25 (t, *J* = 5.7 Hz, 2H), 3.79 (t, *J* = 5.7 Hz, 1H), 3.12–3.04 (s, 2H), 2.97–2.88 (m, 2H), 1.85–1.60 (m, 2H), 1.44–1.15 (m, 4H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 171.0, 158.2, 155.8, 151.4, 144.1 (dd, *J* = 250.0, 13.3 Hz), 142.1, 138.9 (ddd, *J* = 241.6, 8.7, 4.8 Hz), 138.7 (t, *J* = 18.1 Hz), 137.10 (d, *J* = 237.6 Hz), 112.1, 98.7 (d, *J* = 9.9 Hz), 61.5 (d, *J* = 3.9 Hz), 52.0, 34.0 (d, *J* = 8.7 Hz), 29.7, 28.8, 21.6; ^{19}F NMR (376 MHz, DMSO-d_6) δ -144.85, -160.30, -162.58 (d, *J* = 22.3 Hz); IR (neat) 2941, 1756, 1692, 1644 cm^{-1}; HRMS (ESI) calcd for $C_{18}H_{20}F_3N_2O_7^+$ $(M + H)^+$, 433.1217; found, 433.1215.

(*S*)-6-(Benzyloxy)-5-(benzyloxycarbonylamino)-6-oxohexan-1-amine:hydrogen chloride salt (13).[16,17] Z-Lys(Boc)-OH (0.38 g, 1 mmol) and cesium carbonate (0.39 g, 1.2mmol) were suspended in DMF (10 mL), stirred for 30 min, and cooled to 0 °C. BnBr was added dropwise while stirring at 0 °C. After stirring vigorously for 2 hours at room temperature, the reaction mixture was poured into water, extracted with EtOAc, washed with 5% LiCl, water and brine, and dried over Na_2SO_4. The solvent was evaporated under reduced pressure and triturated with Et_2O to give Z-Lys(Boc)-OBn as a white solid (0.46 g, 98%). Mp 92–94 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.46–7.26 (m, 10H), 5.40 (d, *J* = 8.2 Hz, 1H), 5.25–4.98 (m, 4H), 4.63–4.26 (m, 2H), 3.05 (q, *J* = 7.0, 6.4 Hz, 2H), 1.90–1.78 (m, 1H), 1.75–1.61 (m, 1H), 1.51–1.22 (m, 13H); ^{13}C NMR (100 MHz, CDCl3) δ 172.4, 156.14, 156.07, 136.3, 135.4, 128.8, 128.6, 128.4, 128.30, 128.26, 67.3, 67.1, 53.9, 40.1, 32.3, 29.7, 28.5, 22.4.

To a cooled solution of the obtained compound (0.41 g, 0.88 mmol) in CH_2Cl_2 (3.5 mL) was added HCl in dioxane (4 M, 3.0 mL, 12.3 mmol) dropwise. The resulting mixture was stirred at room temperature for 4 h and was then concentrated under reduced pressure. After trituration with Et_2O, the title compound was obtained as white sticky solid (0.33 g, 92%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.98 (s, 3H), 7.80 (d, *J* = 7.7 Hz, 1H), 7.46 – 7.16 (m, 10H), 5.18 – 4.94 (m, 4H), 4.10–4.02 (m, 1H), 2.71 (q, *J* = 7.1 Hz, 2H), 1.76 – 1.45 (m, 4H), 1.42 – 1.29 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.2, 156.2, 136.8, 135.9, 128.4, 128.34, 128.0, 127.85, 127.78, 127.75, 66.3, 65.9, 65.5, 53.9, 38.3, 30.0, 26.4, 22.4.

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Supporting Information Available: Copies of ^{1}H NMR, ^{13}C NMR, ^{19}F NMR and related discussion. This material is available free of charge *via* the Internet at http://www.pubs.acs.org.

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