Supporting Information

for

Synthesis and Evaluation of a Library of Fluorescent Dipeptidomimetic Analogues as Substrates for Modified Bacterial Ribosomes

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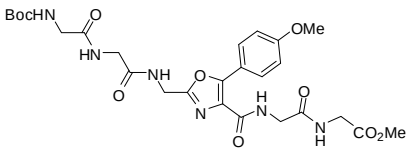
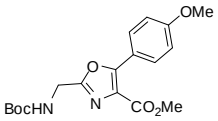
Table S1. Photophysical Properties of the Free amines Derived from Compounds 12 and 63[a,b]



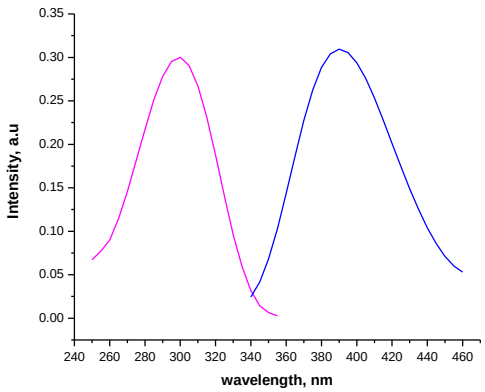
12



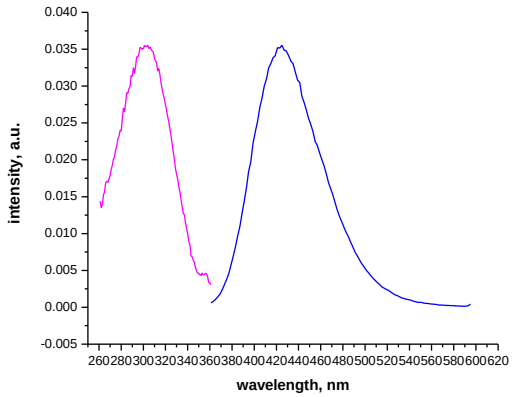
63

Compound	λ_{abs} (nm)	λ_{em} (nm)	ε ($M^{-1}cm^{-1}$)	Φ_F	Brightness
12	300±2	381±2	17400±1000	0.88±0.02	15300±1200
63	300±2	391±2	15600±1000	0.92±0.02	14400±1200

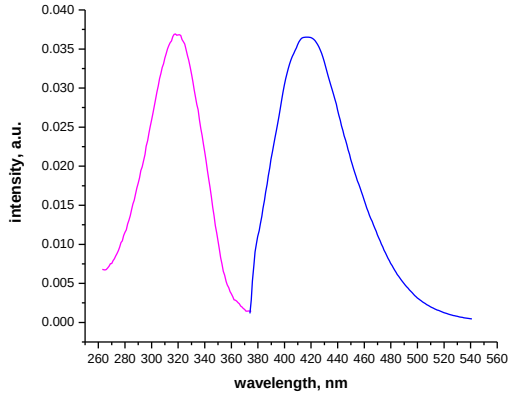
[a]Determined in MeOH. [b]Both compounds were treated with CF_3COOH to remove the Boc protecting groups prior to acquisition of photophysical data.



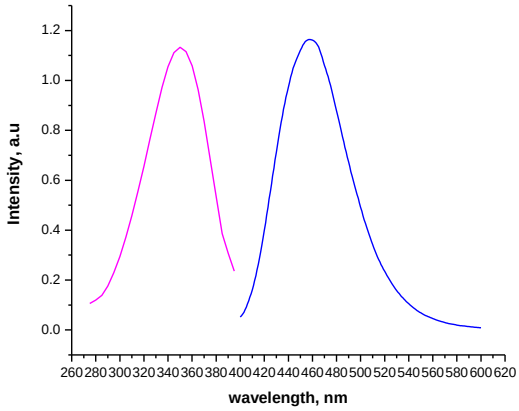
compound **1b**



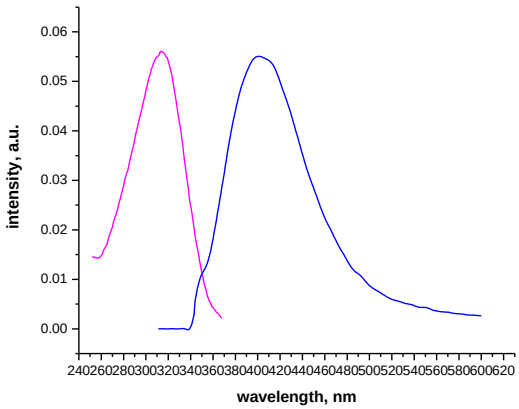
compound **2b**



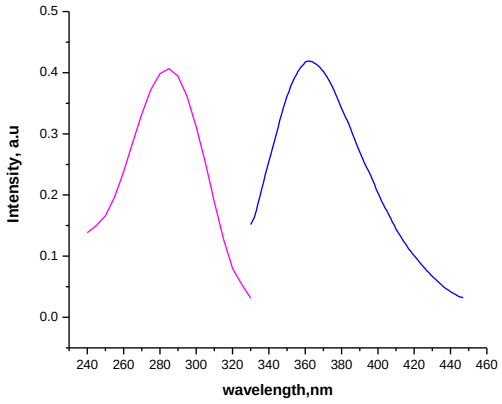
compound **3b**



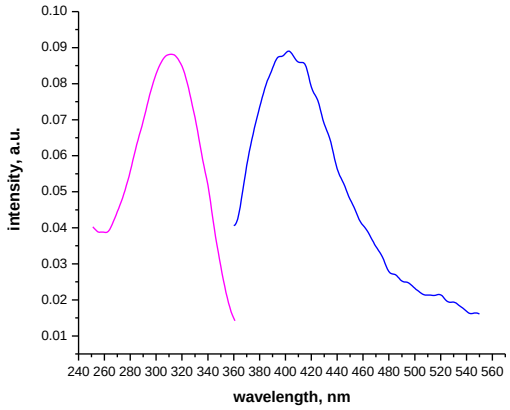
compound **4b**



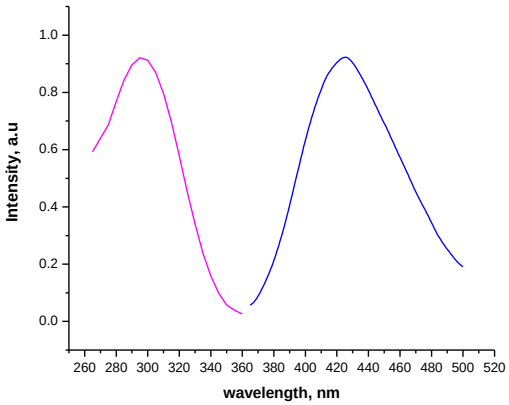
compound **5b**



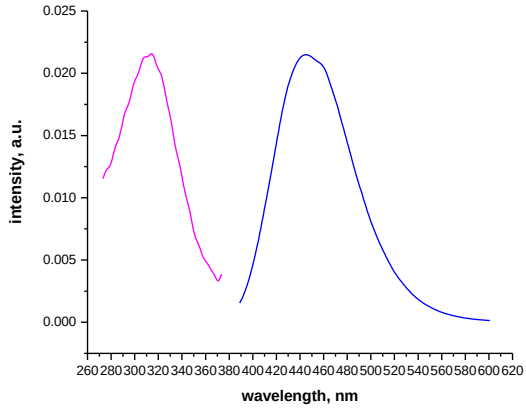
compound **6b**



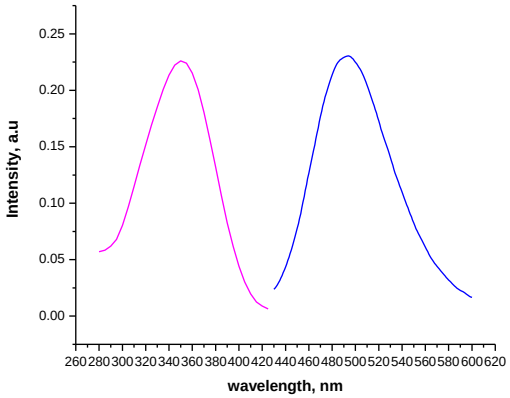
compound **7b**



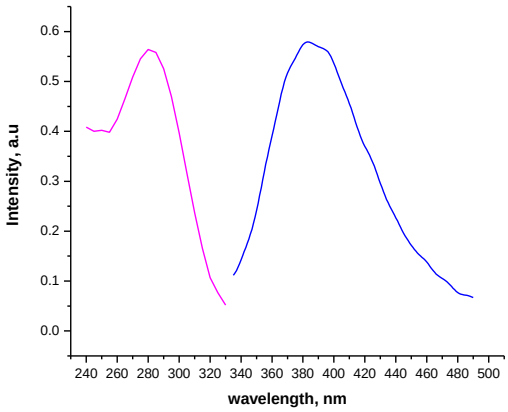
compound **8b**



compound **9b**



compound **10b**



compound **11b**

Figure S1. Absorption (pink) and emission (blue) spectra of the protected oxazoles and thiazoles in MeOH.

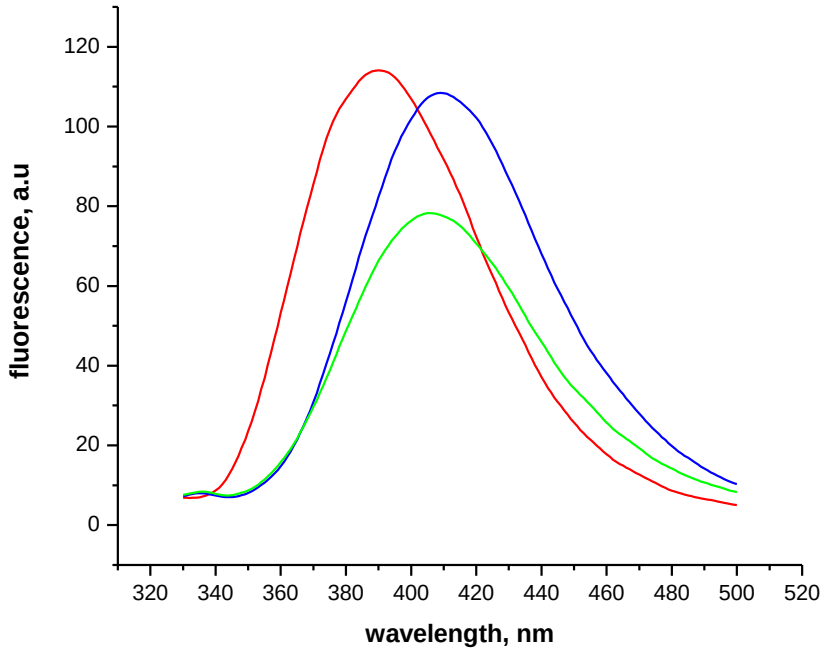


Figure S2. Fluorescence emission spectra of oxazole **1a**, protected as the *N*-pentenoyl methyl ester (**1b**) in MeOH (red trace; emission maximum 391 nm) and in 25 mM Tris-HCl, pH 7.4, containing 0.25 M NaCl (green trace; emission maximum 406 nm), versus a suppressor tRNA activated with oxazole **1a** (blue trace; emission maximum 411 nm) in 25 mM Tris-HCl, pH 7.4, containing 0.25 M NaCl, following excitation at 300 nm. The concentrations of the fluorophores employed in this experiment were similar, but not identical.

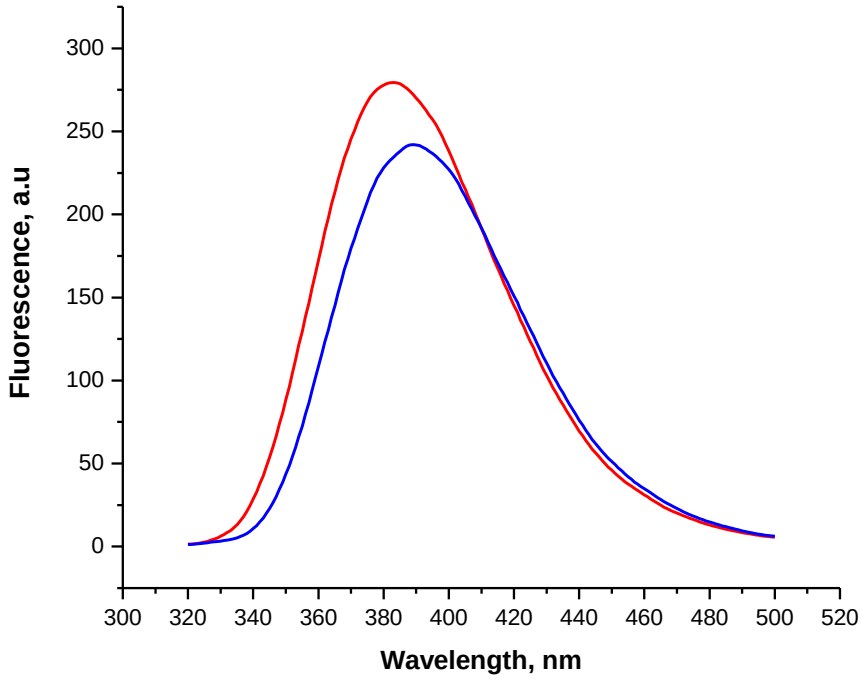


Figure S3. Fluorescence emission spectra of Boc-protected oxazole derivatives **63** (blue trace) and **12** (red trace). Both samples were present at 1.0 μM concentration in MeOH.

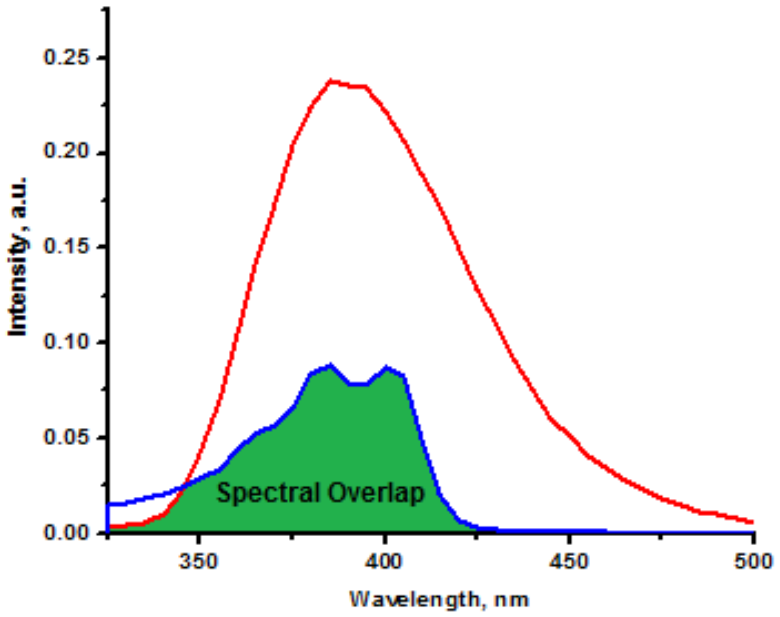


Figure S4. Fluorescence emission spectrum of oxazole **1b** (donor) in MeOH (red trace; emission maximum 391 nm) and absorption spectrum of acridon-2-ylalanine (Acd) methyl ester (acceptor) in MeOH (blue trace; absorption maxima at 385 and 401 nm). The green area represents the spectral overlap between the emission of the donor and the absorption of the acceptor, illustrating the potential for energy transfer.

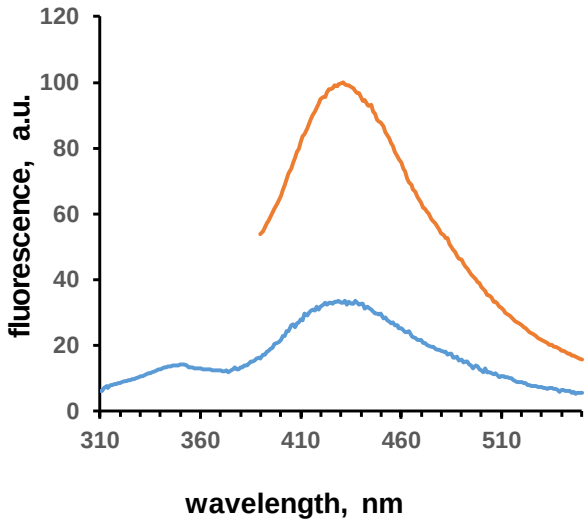
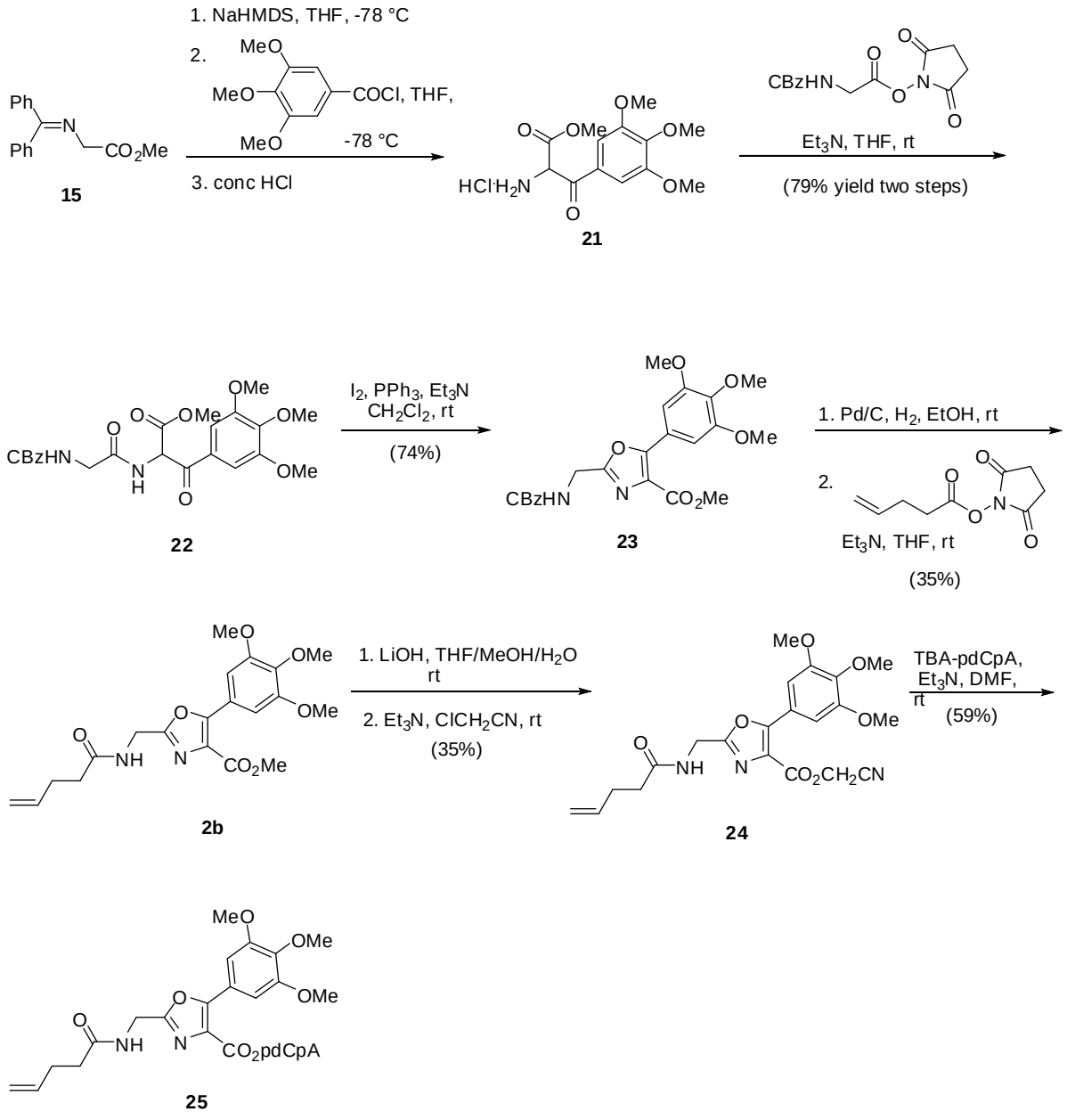
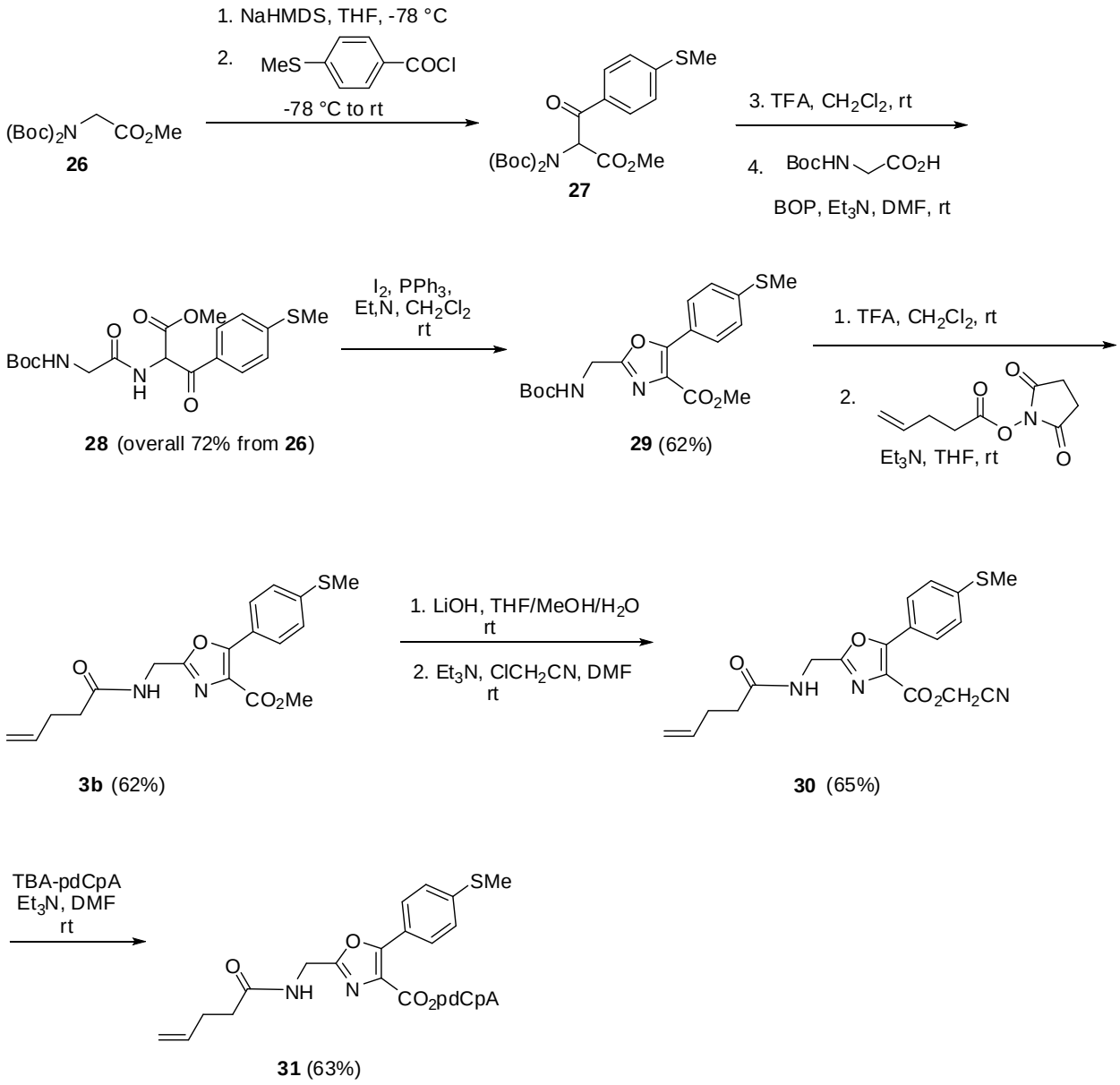


Figure S5. Emission spectra of GFP66Gly39Acd following excitation at 296 nm (blue) or 370 nm (orange).

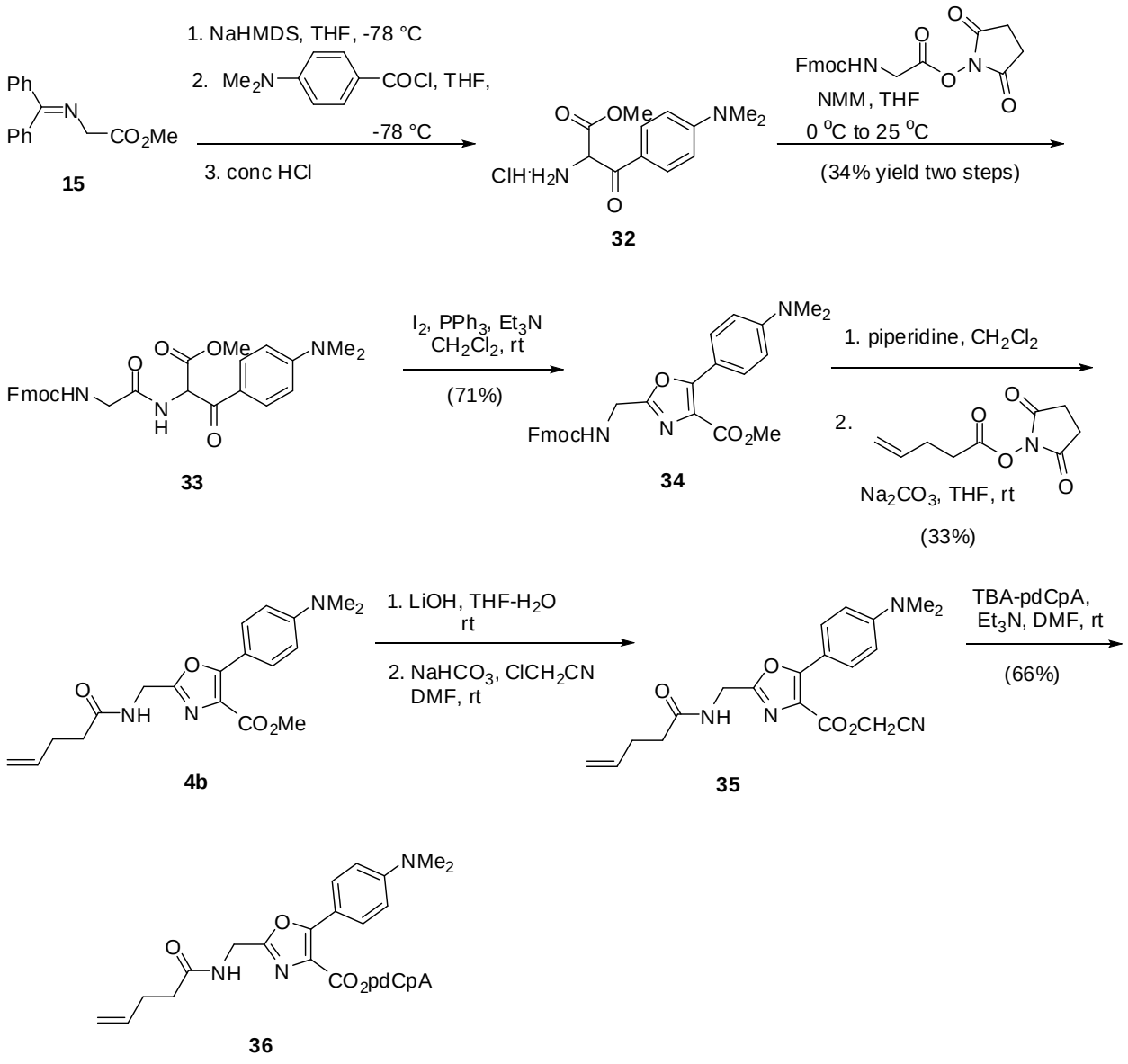
Scheme S1. Synthetic Route Used for the Preparation of the pdCpA Derivative of Dipeptidomimetic 2a.



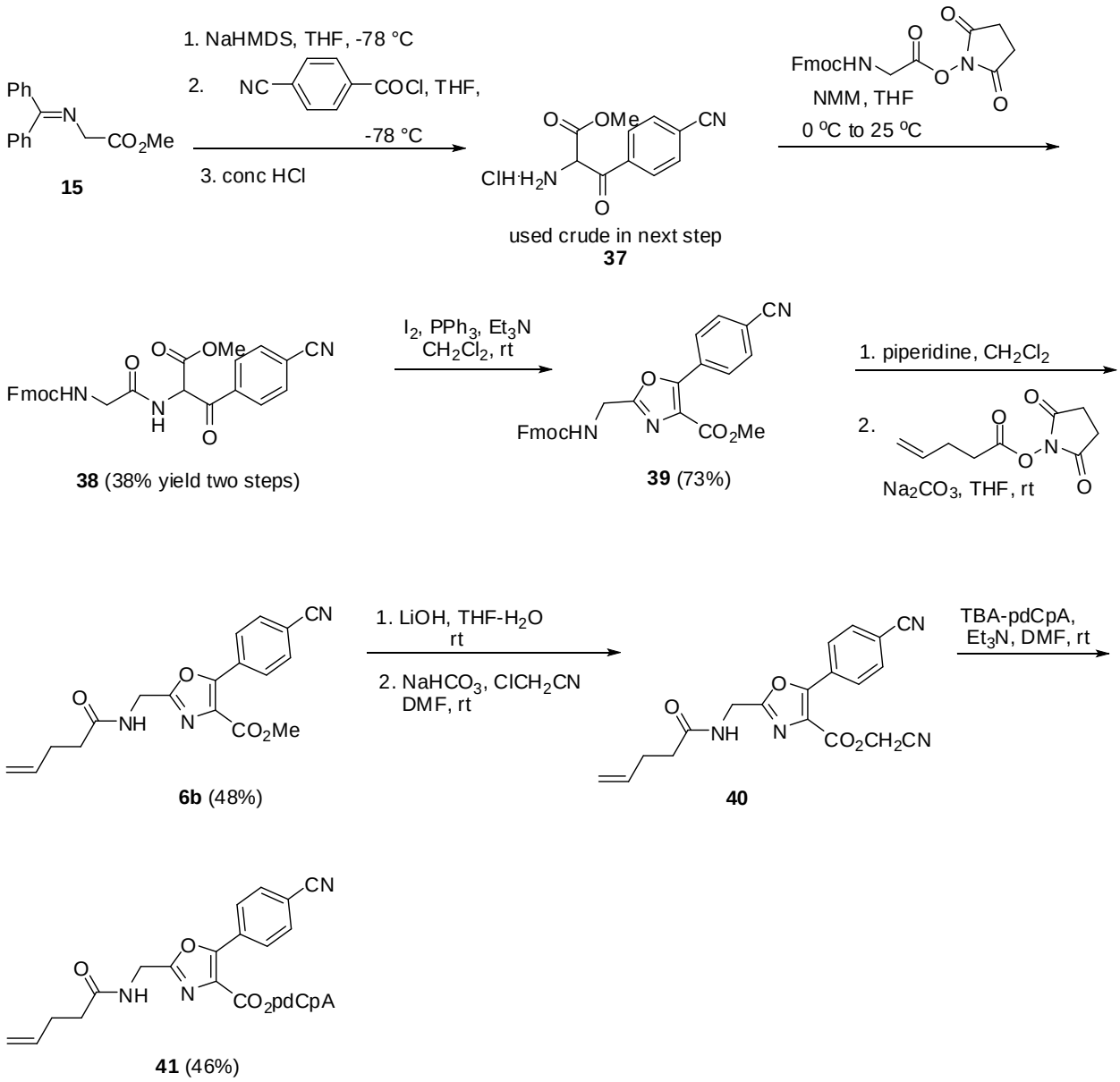
Scheme S2. Synthetic Route Used for the Preparation of the pdCpA Derivative of Dipeptidomimetic 3a.



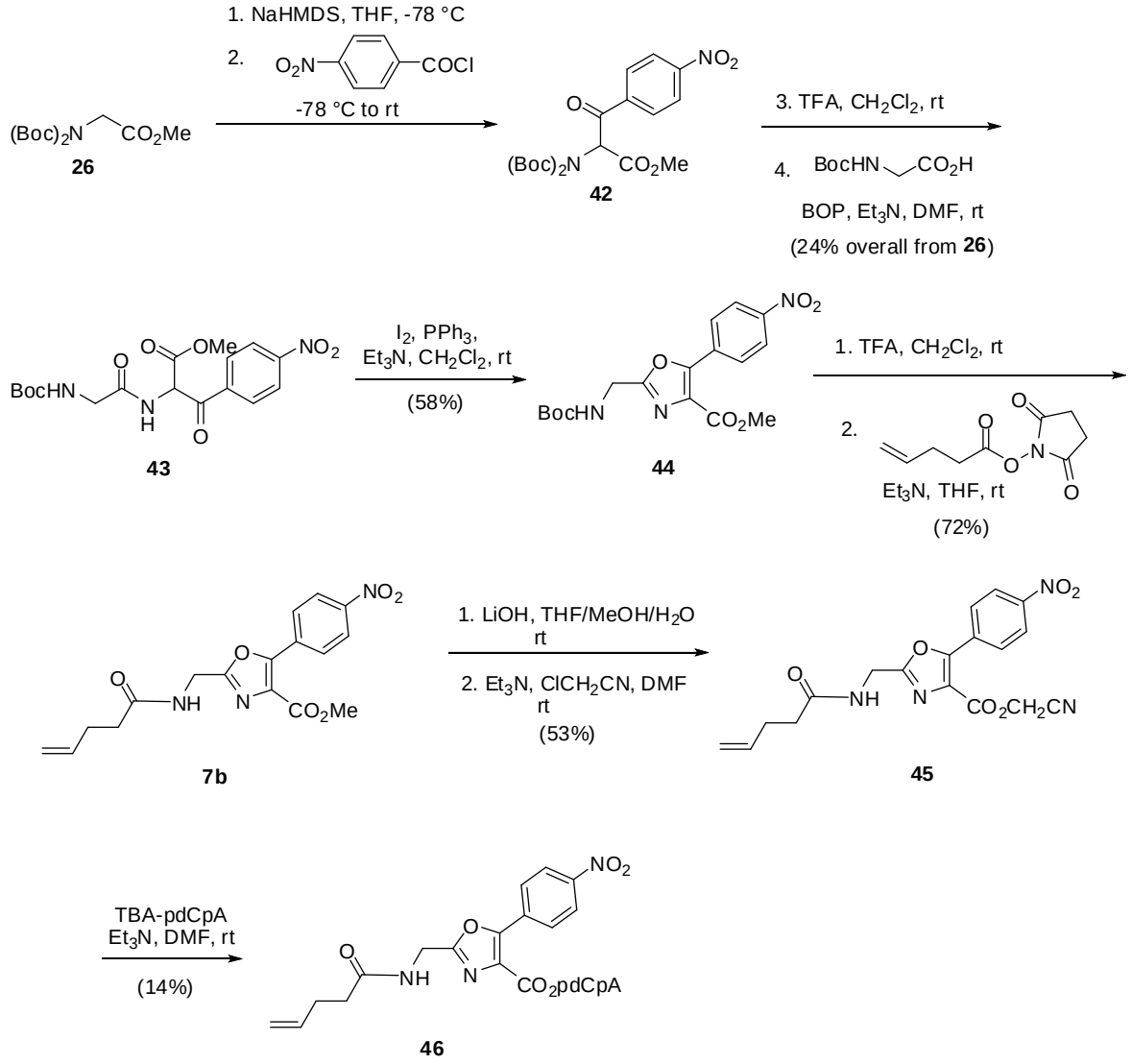
Scheme S3. Synthetic Route Used for the Preparation of the pdCpA Derivative of Dipeptidomimetic 4a.



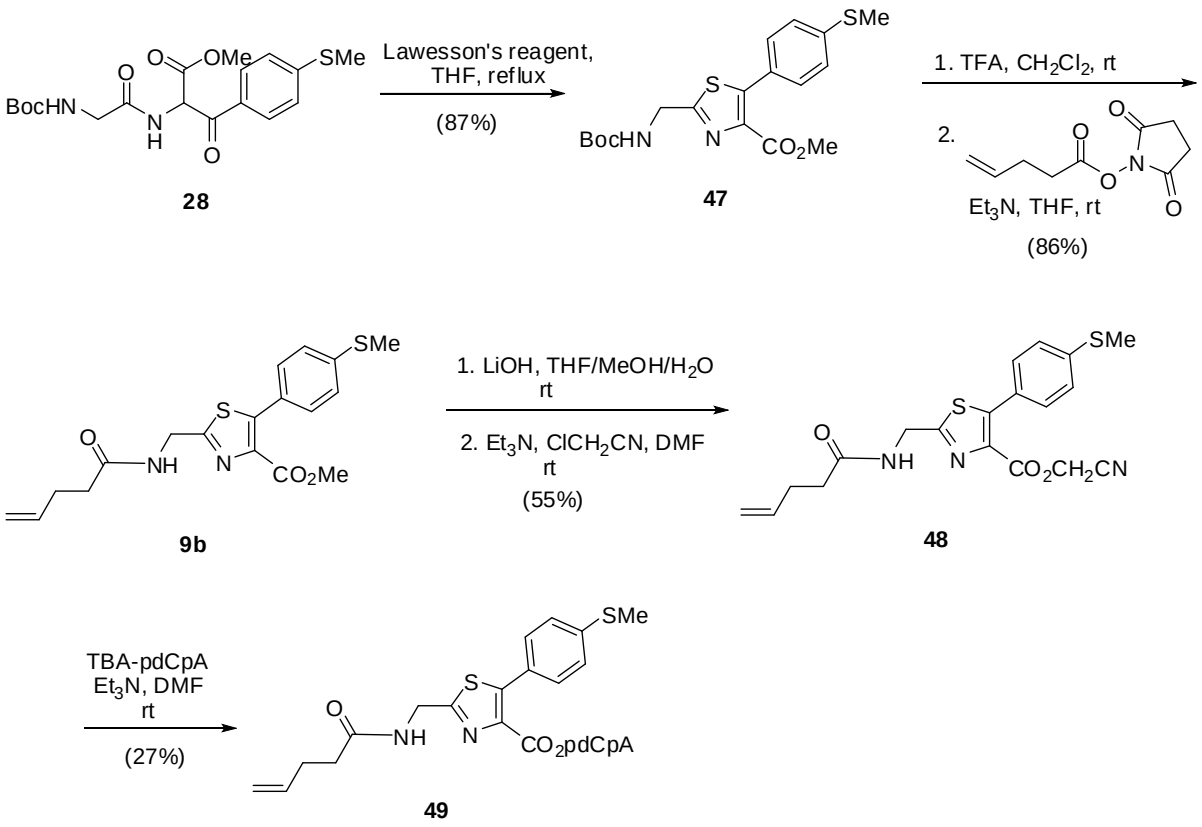
Scheme S4. Synthetic Route Used for the Preparation of the pdCpA Derivative of Dipeptidomimetic 6a.



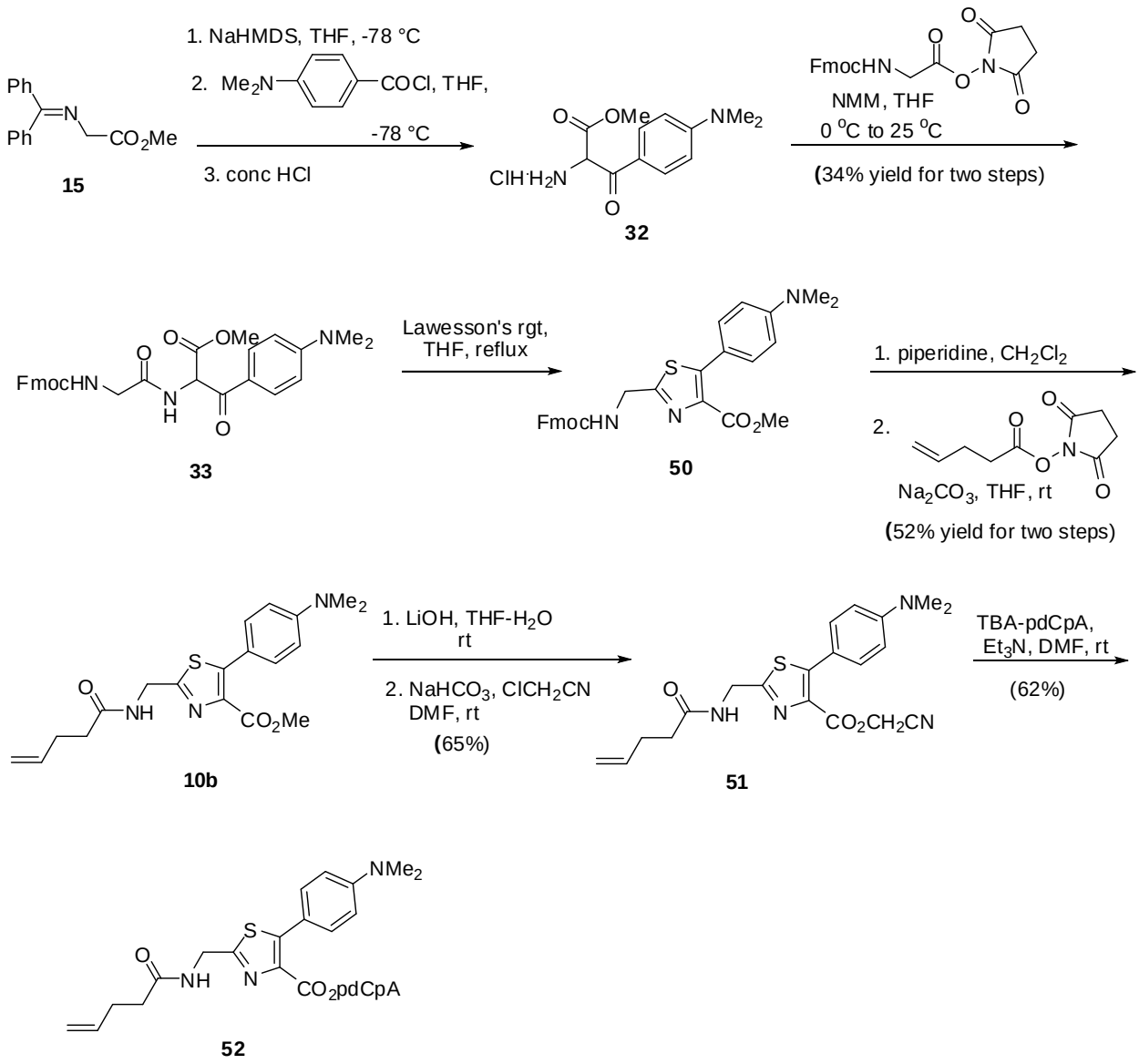
Scheme S5. Synthetic Route Used for the Preparation of the pdCpA Derivative of Dipeptidomimetic 7a.



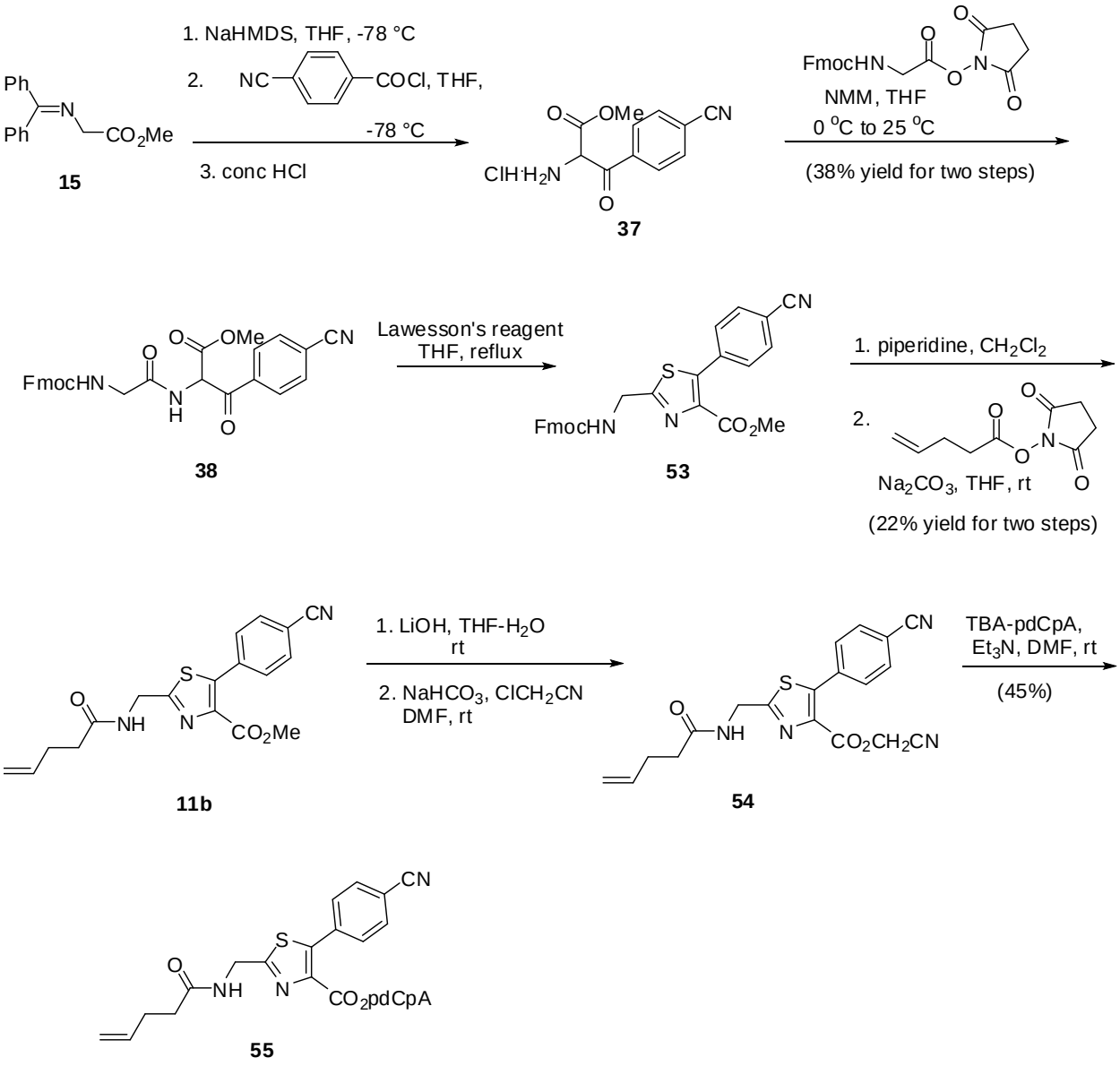
Scheme S6. Synthetic Route Used for the Preparation of the pdCpA Derivative of Dipeptidomimetic 9a.



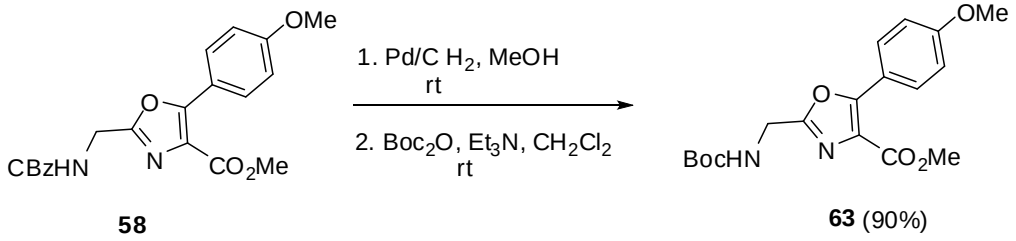
Scheme S7. Synthetic Route Used for the Preparation of the pdCpA Derivative of Dipeptidomimetic 10a.



Scheme S8. Synthetic Route Used for the Preparation of the pdCpA derivative of Dipeptidomimetic 11a.

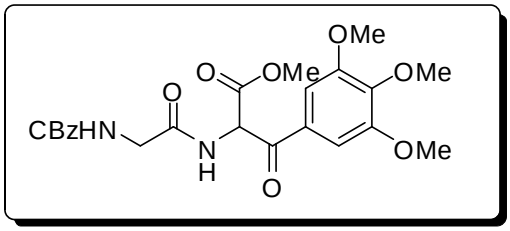


Scheme S9. Synthetic Route Used for the Preparation of Compound 63.



58

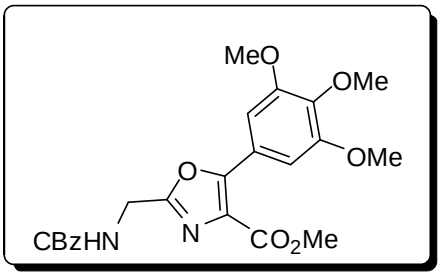
63 (90%)



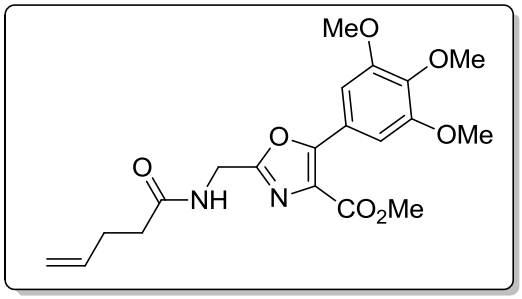
Methyl 2-(2-(Benzyloxycarbonyl)acetamido)-3-oxo-3-(3,4,5-trimethoxyphenyl)propanoate (22). To a solution of 3.00 g (11.8 mmol) of imine ester **15** in 100 mL of THF was added 14.2 mL (14.2 mmol) of 1 M NaHMDS solution in THF at –78 °C. After 30 min, 2.73 g (11.8 mmol) of 3,4,5-trimethoxybenzoyl chloride dissolved in 15 mL of THF was added to the reaction mixture, which was stirred at –78 °C for 2 h. The reaction mixture was treated with conc HCl solution until pH 2 was reached. The solvent was concentrated under diminished pressure to obtain the amine salt **21** as a colorless solid. This material was used for the next reaction without further purification.

To a solution of the amine salt **21** in 150 mL of THF were added 7.25 g (23.7 mmol) of CBz-Gly-OSu and 8.30 mL (6.05 g, 59.2 mmol) of Et_3N. The reaction mixture was stirred overnight at room temperature. The reaction mixture was diluted with 300 mL of water and extracted with two 50-mL portions of EtOAc. The organic phase was dried ($MgSO_4$) and concentrated under diminished pressure. The residue was purified by chromatography on a silica gel column (10 × 4 cm). Elution with 1:1 hexanes–ethyl acetate gave the desired product **22** as a colorless solid: yield 4.43 g (79% overall yield from imine **15**); silica gel TLC R_f 0.50 (1:1 hexanes–ethyl acetate); ^{1}H NMR ($CDCl_3$) δ 3.71 (s, 3H), 3.87-3.93 (m, 7H), 3.94 (s, 3H), 4.01 (s, 1H), 5.12 (s, 2H), 5.58 (br s, 1H), 6.16 (d, 1H, J = 4.0 Hz), 7.31-7.34 (m, 5H), 7.39 (s, 2H) and 7.51 (d, 1H, J = 8.0 Hz); ^{13}C NMR ($CDCl_3$) δ 44.5, 53.5, 56.4, 56.5, 57.9, 61.2, 67.4, 106.6, 107.2, 107.4,

128.3, 128.4, 128.7, 128.8, 136.3, 144.1, 153.0, 153.1, 153.3, 156.8, 167.4, 169.3 and 189.8; mass spectrum (APCI), *m*/*z* 475.1721 $(M+H)^+$ ($C_{23}H_{27}N_2O_9$ requires *m*/*z* 475.1716).

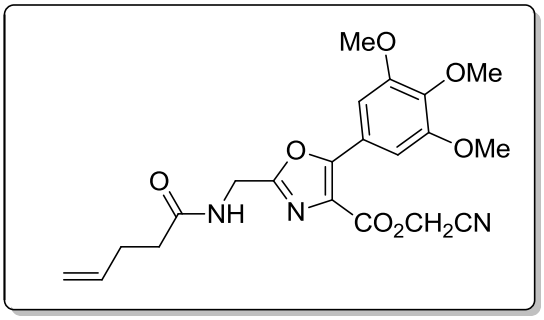


Methyl 2-((Benzyloxycarbonyl)methyl)-5-(3,4,5-trimethoxyphenyl)oxazole-4-carboxylate (23). To a stirred solution of 1.10 g (4.19 mmol) of triphenylphosphine and 1.06 g (4.17 mmol) of iodine in 50 mL of CH_2Cl_2 was added 1.16 mL (0.84 g, 8.34 mmol) of Et_3N. The dark yellow solution was stirred for 5 min and 0.99 g (2.08 mmol) of ketoamide **22** was added to the reaction mixture, which was stirred at room temperature for 2 h. The reaction mixture was concentrated under diminished pressure and the residue was purified by chromatography on a silica gel column (10 × 1 cm). Elution with 1:1 hexanes–ethyl acetate gave the desired product **23** as a colorless oil: yield 0.70 g (74%); silica gel TLC R_f 0.50 (1:1 hexanes–ethyl acetate); ^{1}H NMR ($CDCl_3$) δ 3.81-3.92 (m, 12H), 4.56 (d, 2H, *J* = 4.0 Hz), 5.10 (s, 2H), 5.63 (br s, 1H), 7.23-7.29 (m, 5H) and 7.24 (s, 2H); ^{13}C NMR ($CDCl_3$) δ 38.5, 52.6, 56.4, 56.5, 61.2, 67.5, 103.8, 106.1, 107.4, 121.8, 126.4, 128.3, 128.5, 128.7, 128.7, 132.2, 136.3, 140.3, 153.3, 156.0, 156.4, 158.9 and 162.6; mass spectrum (APCI), *m*/*z* 457.1617 $(M+H)^+$ ($C_{23}H_{25}N_2O_8$ requires *m*/*z* 457.1611).



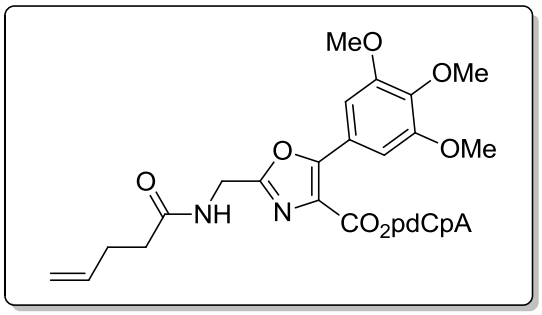
Methyl 2-(Pent-4-enamidomethyl)-5-(3,4,5-trimethoxyphenyl)oxazole-4-carboxylate (2b). To a solution of 0.36 g (0.79 mmol) of CBz-protected amine **23** in 25 mL of EtOH was added 100 mg of 10% Pd/C. The suspension was stirred overnight under 1 atm of H_2. The reaction mixture was filtered through a Celite pad and the filtrate was concentrated under diminished pressure to obtain the deprotected amine. This material was used in next step without further purification.

To the solution of deprotected amine in 8 mL of THF were added 0.23 g (1.18 mmol) of 4-pentenoyloxysuccinimide and 0.33 mL (0.24 g, 2.37 mmol) of Et_3N. The reaction mixture was stirred overnight at room temperature and then concentrated under diminished pressure. The residue was purified by chromatography on a silica gel column (10 × 1 cm). Elution with 1:10 MeOH–ethyl acetate gave the desired product **2b** as a colorless oil: yield 0.11 g (35% yield over two steps); silica gel TLC R_f 0.70 (1:10 MeOH–ethyl acetate); ^{1}H NMR ($CDCl_3$) δ 2.34-2.42 (m, 4H), 3.86-3.93 (m, 12H), 4.61-4.66 (m, 2H), 4.97-5.08 (m, 2H), 5.81-5.82 (m, 1H), 6.35 (br s, 1H) and 7.40-7.43 (s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 29.6, 35.8, 36.8, 52.7, 56.6, 56.6, 61.2, 106.1, 116.0, 121.8, 126.4, 128.8, 132.2, 137.0, 140.4, 153.3, 156.1, 158.9, 162.6 and 172.6; mass spectrum (APCI), *m*/*z* 405.1656 $(M+H)^+$ ($C_{20}H_{25}N_2O_7$ requires *m*/*z* 405.1662).

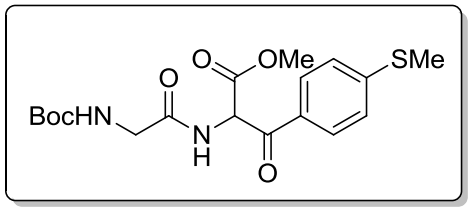


Cyanomethyl 2-(Pent-4-enamidomethyl)-5-(3,4,5-trimethoxyphenyl)oxazole-4-carboxylate (24). To a solution of 0.22 g (0.55 mmol) of ester **2b** in 5 mL of MeOH and 5 mL of THF was added 1.11 mL (1.11 mmol) of 1 M LiOH aqueous solution. The solution was stirred overnight at room temperature and then concentrated under diminished pressure to obtain the free acid which was used in the next step without further purification.

To a solution of the acid in 100 μL of chloroacetonitrile was added 0.15 mL (0.11 g, 1.11 mmol) of Et_3N. The reaction mixture was stirred overnight at room temperature and then concentrated under diminished pressure. The residue was purified by chromatography on a silica gel column (10 × 1 cm). Elution with 1:10 MeOH–ethyl acetate gave the desired product **24** as a colorless solid: yield 80.0 mg (35% yield over two steps); silica gel TLC R_f 0.70 (1:10 MeOH–ethyl acetate); ^{1}H NMR ($CDCl_3$) δ 2.39-2.43 (m, 4H), 3.87-3.92 (m, 9H), 4.65-4.66 (m, 2H), 4.95 (s, 2H), 4.99-5.09 (m, 2H), 5.80-5.86 (m, 1H), 6.33 (br s, 1H) and 7.40-7.42 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 29.6, 35.7, 41.5, 49.1, 56.6, 56.6, 61.3, 106.2, 114.4, 116.0, 116.1, 121.1, 124.5, 128.8, 132.3, 137.0, 141.0, 153.5, 158.0, 160.7 and 172.8; mass spectrum (APCI), *m*/*z* 430.1607 $(M+H)^+$ ($C_{21}H_{24}N_3O_7$ requires *m*/*z* 430.1614).



2-(Pent-4-enamidomethyl)-5-(3,4,5-trimethoxyphenyl)oxazole-4-carboxylic Acid pdCpA Ester (25). To a solution of 6.40 mg (15.0 μmol) of cyanomethyl ester **24** in 100 μL of 9:1 DMF–Et_3N was added 4.00 mg (3.00 μmol) of the tris(tetrabutylammonium) salt of pdCpA. The reaction mixture was sonicated at room temperature for 2.5 h. The reaction mixture was purified by C_{18} reversed-phase HPLC (250 × 10 mm) using a gradient of 1 to 65% acetonitrile in 50 mM ammonium acetate, pH 4.5, over a period of 1 h. The retention time of the desired product was 19.5 min. The fractions containing the product were lyophilized to afford **25** as a colorless solid: yield 1.8 mg (59%); mass spectrum (ESI), *m/z* 1007.2334 $(M-H)^-$ ($C_{38}H_{45}N_{10}O_{19}P_2$ requires *m/z* 1007.2338).

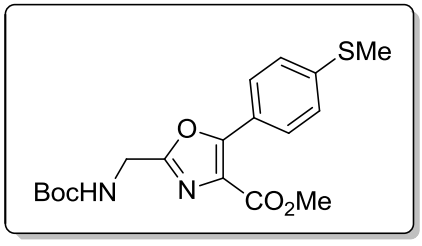


Methyl 2-(2-((*tert*-Butoxycarbonyl)amino)acetamido)-3-(4-(methylthio)phenyl)-3-oxopropanoate (28). To a solution of 0.53 g (1.82 mmol) of Boc-protected glycine methyl ester **26** in 30 mL of THF was added 2.18 mL (2.18 mmol) of 1 M NaHMDS solution in THF at –78 °C. After 30 min, 0.36 g (1.91 mmol) of 4-thiomethylbenzoyl chloride was added to the reaction mixture, which was stirred at –78 °C for an additional 2 h. The reaction mixture was quenched

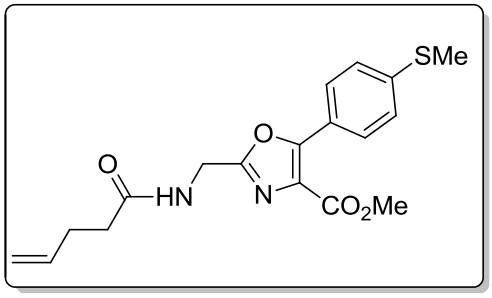
with excess aq NH_4Cl solution and extracted with two 50-mL portions of EtOAc. The organic phase was dried ($MgSO_4$) and concentrated under diminished pressure. The residue was purified by chromatography on a silica gel column (10 × 4 cm). Elution with 3:1 hexanes–ethyl acetate gave the ketoester **27** as a colorless foamy solid.

To the solution of ketoester **27** in 10 mL of CH_2Cl_2 was added 10 mL of CF_3COOH and the reaction mixture was stirred overnight at room temperature. The reaction mixture was concentrated under diminished pressure to obtain the amine, which was used in next step without further purification.

To a solution of 0.63 g (3.64 mmol) of Boc-Gly-OH in 15 mL of DMF was added 1.61 g (3.64 mmol) of BOP, 1.51 mL (1.10 g, 10.91 mmol) of Et_3N and the amine obtained from previous step. The reaction mixture was stirred overnight at room temperature. The reaction mixture was concentrated under diminished pressure and the residue was purified by chromatography on a silica gel column (10 × 4 cm). Elution with 1:1 hexanes–ethyl acetate gave the ketoamide **28** as a colorless oil: yield 0.52 g (72% overall yield from **26**); silica gel TLC R_f 0.50 (1:1 hexanes–ethyl acetate); ^{1}H NMR ($CDCl_3$) δ 1.44 (s, 9H), 2.51 (s, 3H), 3.69 (s, 3H), 3.93 (br s, 2H), 5.54 (t, 1H, J = 10.0 Hz), 6.18 (d, 1H, J = 10.0 Hz), 7.26 (d, 2H, J = 10.0 Hz), 7.68 (d, 1H, J = 10.0 Hz) and 7.99 (d, 2H, J = 8.0 Hz); ^{13}C NMR ($CDCl_3$) δ 14.6, 28.4, 44.1, 53.3, 57.7, 80.2, 125.0, 130.0, 130.1, 148.4, 156.1, 167.3, 169.8 and 190.1; mass spectrum (APCI), m/z 397.1429 $(M+H)^+$ ($C_{18}H_{25}N_2O_6S$ requires m/z 397.1433).

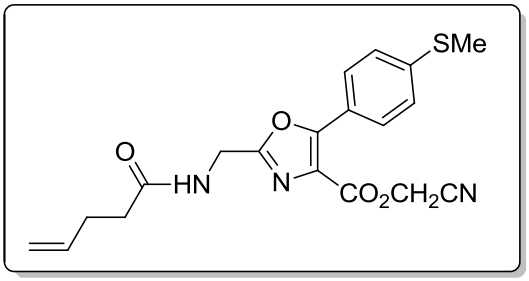


Methyl 2-(((*tert*-Butoxycarbonyl)amino)methyl)-5-(4-(methylthio)phenyl)oxazole4-carboxylate (29). To a stirred solution of 0.87 g (3.31 mmol) of triphenylphosphine and 0.83 g (3.30 mmol) of iodine in 50 mL of CH_2Cl_2 was added 0.92 mL (0.67 g, 6.60 mmol) of Et_3N. The dark yellow solution was stirred for 5 min and then 0.65 g (1.65 mmol) of ketoamide **28** was added to the reaction mixture, which was stirred at room temperature for an additional 2 h. The reaction mixture was concentrated under diminished pressure and the residue was purified by chromatography on a silica gel column (10 × 1 cm). Elution with 1:1 hexanes–ethyl acetate gave the desired product **29** as a yellow solid: yield 0.38 g (62%); silica gel TLC R_f 0.50 (1:1 hexanes–ethyl acetate); ^{1}H NMR ($CDCl_3$) δ 1.39 (s, 9H), 2.44 (s, 3H), 3.85 (s, 3H), 4.45 (d, 2H, J = 4.0 Hz), 5.37 (br s, 1H), 7.21 (d, 2H, J = 8.0 Hz) and 7.91 (d, 2H, J = 8.0 Hz); ^{13}C NMR ($CDCl_3$) δ 15.1, 28.4, 38.0, 52.4, 80.4, 123.0, 125.5, 126.2, 128.6, 142.4, 155.7, 155.8, 159.4 and 162.5; mass spectrum (APCI), m/z 379.1339 $(M+H)^+$ ($C_{18}H_{23}N_2O_5S$ requires m/z 379.1328).



Methyl 5-(4-(Methylthio)phenyl)-2-(pent-4-enamidomethyl)oxazole-4-carboxylate (3b). To a solution of 0.39 g (1.02 mmol) of Boc-protected amine **29** in 10 mL of CH_2Cl_2 was added 10 mL of CF_3COOH. The reaction mixture was stirred overnight and then concentrated under diminished pressure to obtain the deprotected amine, which was used in next step without further purification.

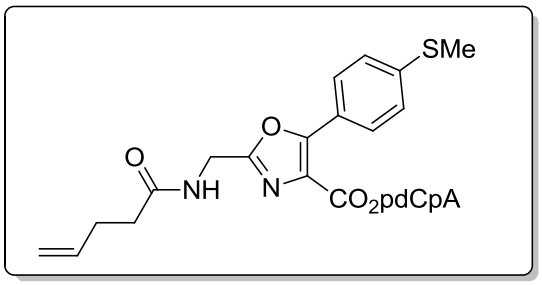
To the solution of deprotected amine in 10 mL of THF were added 0.50 g (2.56 mmol) of 4-pentenoyloxysuccinimide and 3 mL of saturated aq $NaHCO_3$. The reaction mixture was stirred overnight at room temperature and then concentrated under diminished pressure. The residue was purified by chromatography on a silica gel column (10 × 1 cm). Elution with 1:10 MeOH–ethyl acetate gave the desired product **3b** as a colorless solid: yield 0.23 g (62%); silica gel TLC R_f 0.75 (1:10 MeOH–ethyl acetate); ^{1}H NMR ($CDCl_3$) δ 2.30-2.38 (m, 4H), 2.47 (s, 3H), 3.87 (s, 3H), 4.59 (d, 2H, J = 4.0 Hz), 4.93-5.04 (m, 2H), 5.75-5.79 (m, 1H), 6.58 (br s, 1H), 7.24 (d, 2H, J = 8.0 Hz) and 7.91 (d, 2H, J = 8.0 Hz); ^{13}C NMR ($CDCl_3$) δ 15.1, 29.5, 36.7, 49.2, 52.5, 115.9, 122.8, 125.5, 126.2, 128.7, 137.0, 142.6, 156.0, 159.1, 162.5 and 172.7; mass spectrum (APCI), m/z 361.1232 $(M+H)^+$ ($C_{18}H_{21}N_2O_4S$ requires m/z 361.1222).



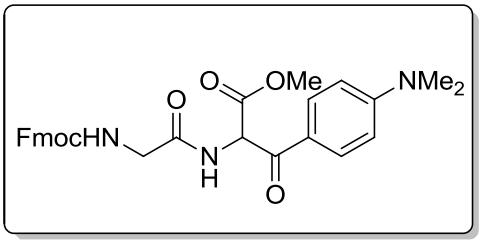
Cyanomethyl 5-(4-(Methylthio)phenyl)-2-(pent-4-enamidomethyl)oxazole-4-carboxylate (30). To a solution of 0.18 g (0.50 mmol) of ester **3b** in 5 mL of MeOH and 5 mL of THF was added 1.25 mL (1.25 mmol) of 1 M LiOH aqueous solution. The solution was stirred overnight at room temperature, then concentrated under diminished pressure to obtain the free acid, which was used in next step without further purification.

To a solution of the acid in 1 mL of DMF were added 100 μL of chloroacetonitrile and 0.21 mL (0.16 g, 1.51 mmol) of Et_3N. The reaction mixture was stirred overnight at room temperature, then concentrated under diminished pressure. The residue was purified by chromatography on a

silica gel column (10 × 1 cm). Elution with 1:10 MeOH–ethyl acetate gave the desired product **30** as a yellow solid: yield 0.12 g (65%); silica gel TLC R_f 0.75 (1:10 MeOH–ethyl acetate); ^{1}H NMR ($CDCl_3$) δ 2.36-2.45 (m, 4H), 2.42 (s, 3H), 4.65 (d, 2H, J = 8.0 Hz), 4.94 (s, 2H), 4.99-5.10 (m, 2H), 5.80-5.84 (m, 1H), 6.26 (br s, 1H), 7.31 (d, 2H, J = 8.0 Hz) and 7.96 (d, 2H, J = 8.0 Hz); ^{13}C NMR ($CDCl_3$) δ 15.2, 29.6, 35.7, 36.8, 49.0, 114.3, 116.1, 122.2, 124.4, 125.7, 128.9, 137.0, 143.8, 158.0, 159.5, 160.6 and 172.7; mass spectrum (APCI), *m*/*z* 386.1176 $(M+H)^+$ ($C_{19}H_{20}N_3O_4S$ requires *m*/*z* 386.1175).



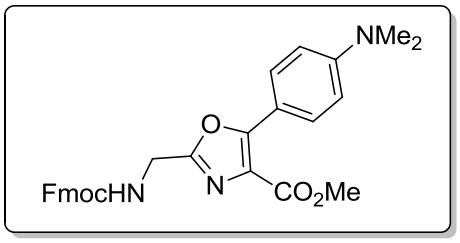
5-(4-(Methylthio)phenyl)-2-(pent-4-enamidomethyl)oxazole-4-carboxylic Acid pdCpA Ester (31). To a stirred solution containing 6.60 mg (5.00 μmol) of pdCpA tetrabutylammonium salt in 100 μL of 9:1 anhydrous DMF–Et_3N was added 9.50 mg (25.0 μmol) of cyanomethyl ester **30**. The reaction mixture was sonicated for 6 h. The reaction mixture was then purified by C_{18} reversed-phase HPLC (250 x 10 mm) using a gradient of 1% to 65% acetonitrile in 50 mM ammonium acetate, pH 4.5, over a period of 1 h. The retention time of the desired product was 20.3 min. The fractions containing the product were lyophilized to afford **31** as a colorless solid: yield 3.0 mg (63%); mass spectrum (ESI), *m/z* 963.1908 $(M-H)^-$ ($C_{36}H_{41}N_{10}O_{16}P_2S$ requires *m/z* 963.1898).



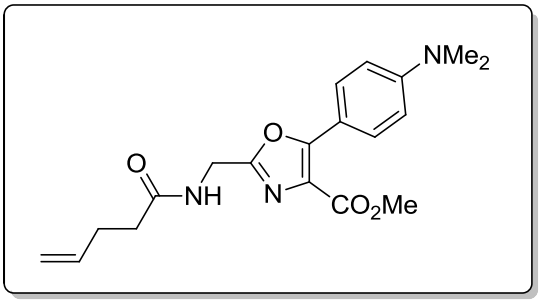
Methyl 2-(2-(((9H-Fluoren-9-yl)methoxy)carbonylamino)acetamido)-3-(4-(dimethylamino)phenyl)-3-oxopropanoate (33). A solution of 0.50 g (2.00 mmol) of **15** in 5 mL of anhydrous THF was cooled to –78 ^{o}C under argon atmosphere and 2.00 mL (2.00 mmol) of 1 M sodium bis(trimethylsilyl)amide in THF was added dropwise while maintaining the temperature at –78 ^{o}C. After 30 min, the resulting yellow solution was added via cannula to a stirred solution of 0.43 g (2.00 mmol) of 4-dimethylaminobenzoyl chloride in 3 mL of anhydrous THF at –78 ^{o}C. The reaction mixture was stirred at –78 ^{o}C for 2h. The yellow reaction mixture was acidified with concentrated HCl until pH ~2 was reached, and was then concentrated under diminished pressure. Crude product **32** was utilized for the next reaction without further purification.

To a solution of the crude product in 10 mL of anhydrous THF at 0 ^{o}C was added 0.79 g (2.00 mmol) of Fmoc-glycine *N*-hydroxysuccinimide ester followed by the dropwise addition of 0.22 mL (0.20 g, 2.00 mmol) of *N*-methylmorpholine. The yellow reaction mixture was stirred at 25 ^{o}C for 2 h and concentrated under diminished pressure. The crude product was purified on a silica gel column (15 × 2 cm). Elution with 1:1 ethyl acetate−hexanes afforded **33** as a yellowish solid: yield 0.35 g (34%); silica gel TLC R_f 0.27 (1:1 ethyl acetate−hexanes); ^{1}H NMR ($CDCl_3$) δ 3.07 (s, 6H), 3.68 (s, 3H), 4.02 (br s, 2H), 4.21 (s, 1H), 4.39 (d, 2H, J = 6.8 Hz), 5.50 (br s, 1H), 6.08 (d, 1H, J = 7.2 Hz), 6.64 (d, 2H, J = 8.8 Hz), 7.28 (t, 2H, J = 7.4 Hz), 7.37 (t, 2H, J = 7.4 Hz), 7.48 (d, 1H, J = 7.2 Hz), 7.59 (d, 2H, J = 7.6 Hz), 7.73 (d, 2H, J = 7.6 Hz) and 8.00 (d, 2H, J = 8.8 Hz); ^{13}C NMR ($CDCl_3$) δ 44.3, 47.2, 53.4, 55.7, 57.7, 67.4, 114.2, 120.0, 125.2, 126.8,

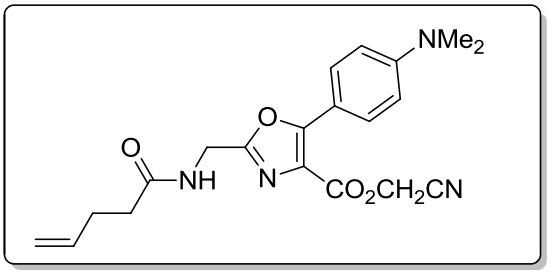
127.2, 127.8, 132.2, 141.4, 143.9, 156.6, 164.9, 167.3, 169.0 and 189.1; mass spectrum (APCI), *m/z* 516.2060 $(M + H)^+$ ($C_{29}H_{30}N_3O_6$ requires *m/z* 516.2069).



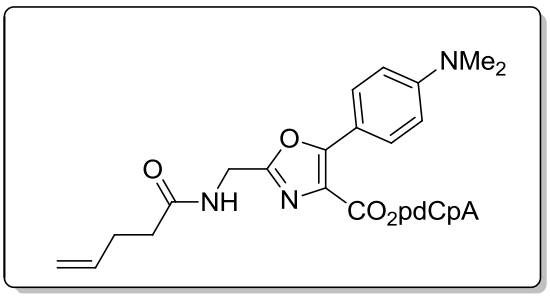
Methyl 2-((((9H-Fluoren-9-yl)methoxy)carbonylamino)methyl)-4-(4-(dimethylamino)phenyl)oxazole-5-carboxylate (34). To a stirred solution of 0.29 g (1.12 mmol) of triphenylphosphine and 0.28 g (1.12 mmol) of iodine in 10 mL of anhydrous CH_2Cl_2 was added 0.15 mL (0.11 g, 1.12 mmol) of triethylamine. The dark yellow solution was stirred for 5 min and 0.29 g (0.56 mmol) of **33** dissolved in 5 mL of anhydrous CH_2Cl_2 was added dropwise. The reaction mixture was stirred for 30 min at 25 ^oC under an argon atmosphere and was then concentrated under diminished pressure. The residue was purified on a silica gel column (15 × 2 cm). Elution with 2:3 ethyl acetate−hexanes afforded **34** as a yellow solid: yield 0.20 g (71%); silica gel TLC R_f 0.43 (1: 1 ethyl acetate−hexanes); ^1H NMR ($CDCl_3$) δ 3.02 (s, 6H), 3.91 (s, 3H), 4.23 (t, 1H, *J* = 5.8 Hz), 4.44 (d, 2H, *J* = 6.8 Hz), 4.57 (d, 2H, *J* = 5.6 Hz), 5.67 (br s, 1H), 6.71 (d, 2H, *J* = 8.8 Hz), 7.29 (t, 2H, *J* = 7.4 Hz), 7.38 (t, 2H, *J* = 7.2 Hz), 7.60 (d, 2H, *J* = 7.2 Hz), 7.74 (d, 2H, *J* = 7.6 Hz) and 7.98 (d, 2H, *J* = 8.8 Hz); ^{13}C NMR ($CDCl_3$) δ 38.2, 40.0, 47.0, 52.0, 67.2, 111.2, 113.7, 119.9, 123.7, 125.0, 127.0, 127.6, 128.5, 129.6, 141.2, 143.7, 151.5, 156.1, 157.4 and 162.7; mass spectrum (APCI), *m/z* 498.1930 $(M + H)^+$ ($C_{29}H_{28}N_3O_5$ requires *m/z* 498.1951).



Methyl 4-(4-(Dimethylamino)phenyl)-2-(pent-4-enamidomethyl)oxazole-5-carboxylate (4b).To a stirred solution of 0.20 g (0.40 mmol) of **34** in 4 mL of anhydrous CH_2Cl_2 was added dropwise 77.0 μL (66.0 mg, 0.78 mmol) of piperidine. The reaction mixture was stirred at 25 ^oC under an argon atmosphere for 2 h and was then concentrated under diminished pressure. The residue was dissolved in 5 mL of anhydrous THF and 0.15 g (0.78 mmol) of 4-pentenoyloxysuccinimide followed by 83.0 mg (0.78 mmol) of Na_2CO_3 was added. The reaction mixture was stirred at room temperature for 3 h under argon atmosphere and was then concentrated under diminished pressure. The residue was purified on a silica gel column (7 × 2 cm). Elution with 7:3 ethyl acetate−hexanes afforded **4b** as a pale yellow solid: yield 48.0 mg (33% over two steps); silica gel TLC R_f 0.19 (7:3 ethyl acetate−hexanes); ^1H NMR ($CDCl_3$) δ 2.35-2.42 (m, 4H), 3.02 (s, 6H), 3.90 (s, 3H), 4.61 (d, 2H, J = 5.6 Hz), 4.97-5.08 (m, 2H), 5.79-5.85 (m, 1H), 6.39 (br s, 1H), 6.71 (d, 2H, J = 8.8 Hz) and 7.96 (d, 2H, J = 8.8 Hz); ^{13}C NMR ($CDCl_3$) δ 29.3, 35.5, 36.6, 40.0, 52.1, 111.2, 113.7, 115.7, 123.7, 129.6, 136.7, 151.6, 157.45, 157.50, 162.7 and 172.2; mass spectrum (APCI), *m/z* 358.1680 $(M + H)^+$ ($C_{19}H_{24}N_3O_4$ requires *m/z* 358.1689).

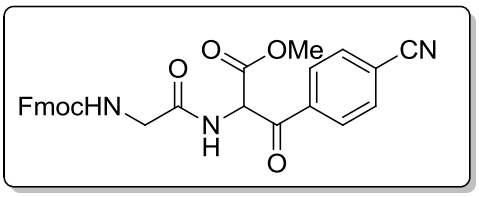


Cyanomethyl 4-(4-(Dimethylamino)phenyl)-2-(pent-4-enamidomethyl)oxazole-5-carboxylate (35). To a stirred solution of 15.0 mg (0.04 mmol) of **4b** in 0.40 mL 3:1 THF–water was added 0.08 mL of 1 M LiOH. The reaction mixture was stirred at 25 ^{o}C for 3 h. The reaction was diluted with MeOH, dried over anh Na_2SO_4 and concentrated under diminished pressure. The crude product was dissolved in 2 mL of anhydrous DMF and 10.0 mg (0.09 mmol) of $NaHCO_3$ was added followed by 13.0 µL (16.0 mg, 0.21 mmol) of $ClCH_2CN$. The reaction mixture was stirred at 25 ^{o}C for 3 h under an argon atmosphere. The reaction mixture was concentrated under diminished pressure and used in the next step without further purification.



4-(4-(Dimethylamino)phenyl)-2-(pent-4-enamidomethyl)oxazole-5-carboxylic Acid pdCpA Ester (36). A solution containing 6.0 mg (16 µmol) of cyanomethyl ester **35** and 8.0 mg (6.0 µmol) of the tris(tetrabutylammonium) salt of pdCpA in 100 µL of 9:1 DMF–Et_3N was subjected to sonication at room temperature for 2.5 h. The reaction mixture was purified by C_{18} reversed-phase HPLC (250 × 10 mm) using a gradient of 1 to 65% acetonitrile in 50 mM ammonium

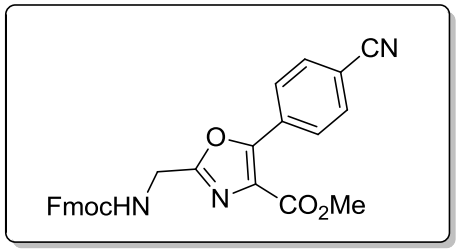
acetate, pH 4.5, over a period of 45 min. The fraction eluting at 19.5 min was collected and lyophilized to afford **36** as a yellow solid: yield 3.7 mg (66%); mass spectrum (ESI), *m/z* 960.2440 $(M - H)^-$ ($C_{37}H_{44}N_{11}O_{16}P_2$ requires *m/z* 960.2443).



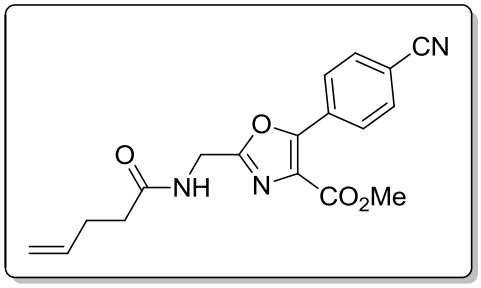
Methyl 2-(2-(((9H-Fluoren-9-yl)methoxy)carbonylamino)acetamido)-3-(4-cyanophenyl)-3-oxopropanoate (**38).** A solution of 1.00 g (4.00 mmol) of **15** in 10 mL of anhydrous THF was cooled to −78 °C under argon atmosphere and 4.00 mL (4.00 mmol) of 1 M sodium bis(trimethylsilyl)amide in THF was added dropwise while maintaining the temperature at −78 °C. After 30 min, the resulting yellow solution was added via cannula to a stirred solution of 0.66 g (4.00 mmol) of 4-cyanobenzoyl chloride in 3 mL of anhydrous THF at −78 °C. The mixture was stirred at −78 °C for 2 h. The yellow mixture was acidified with concentrated HCl until pH ~ 2 and was concentrated under diminished pressure. The crude product (**37**) was utilized for the next reaction without further purification.

To a solution of crude product **37** in 10 mL of anhydrous THF at 0 °C was added 1.58 g (4.00 mmol) of Fmoc-glycine *N*-hydroxysuccinimide ester followed by the dropwise addition of 0.44 mL (0.40 g, 4.00 mmol) of *N*-methylmorpholine. The yellow mixture was stirred at 25 °C for 2 h and then concentrated under diminished pressure. The crude product was purified on a silica gel column (15 × 2 cm). Elution with 1:1 ethyl acetate−hexanes afforded **38** as a colorless oil: yield 0.60 g (38%); silica gel TLC R_f 0.3 (1:1 ethyl acetate−hexanes); ^{1}H NMR ($CDCl_3$) δ 3.69 (s, 3H), 4.00 (d, 2H, J = 5.6 Hz), 4.20 (t, 1H, J = 7.0 Hz), 4.39 (d, 2H, J = 6.8 Hz), 5.55 (t, 1H, J = 5.4 Hz), 6.15 (d, 1H, J = 7.2 Hz), 7.27 (t, 2H, J = 7.4 Hz), 7.37 (t, 2H, J = 7.4 Hz), 7.47 (br s,

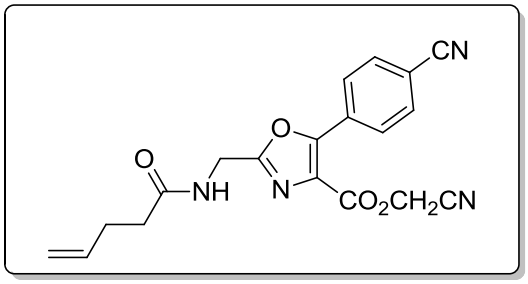
1H), 7.56 (d, 2H, *J* = 7.2 Hz), 7.73-7.77 (m, 4H) and 8.14 (d, 2H, *J* = 7.2 Hz); mass spectrum (APCI), *m/z* 498.1662 $(M + H)^+$ ($C_{28}H_{24}N_3O_6$ requires *m/z* 498.1665).



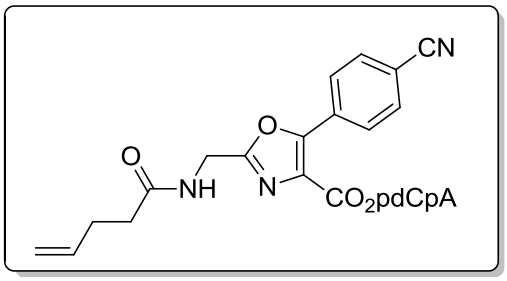
Methyl 2-((((9H-Fluoren-9-yl)methoxy)carbonylamino)methyl)-4-(4-cyanophenyl)oxazole-5-carboxylate (39). To a stirred solution of 0.21 g (0.80 mmol) of triphenylphosphine and 0.2 g (0.8 mmol) of iodine in 10 mL of anhydrous CH_2Cl_2 was added 0.11 mL (83.0 mg, 0.80 mmol) of triethylamine. The dark yellow solution was stirred for 5 min and 0.20 g (0.40 mmol) of **38** dissolved in 5 mL of anhydrous CH_2Cl_2 was added dropwise. The reaction mixture was stirred for 30 minutes at 25 ^oC under argon atmosphere and was then concentrated under diminished pressure. The residue was purified on a silica gel column (15 × 2 cm). Elution with 1:1 ethyl acetate−hexanes afforded **39** as a pale yellow solid: yield 0.14 g (73%); silica gel TLC R_f 0.5 (1:1 ethyl acetate−hexanes); ^1H NMR ($CDCl_3$) δ 3.95 (s, 3H), 4.22 (t, 1H, *J* = 6.0 Hz), 4.45 (d, 2H, *J* = 6.8 Hz), 4.61 (d, 2H, *J* = 5.6 Hz), 5.52 (br s, 1H), 7.28 (t, 2H, *J* = 7.4 Hz), 7.38 (t, 2H, *J* = 7.4 Hz), 7.58 (d, 2H, *J* = 7.2 Hz), 7.71-7.76 (m, 4H) and 8.20 (d, 2H, *J* = 8.4 Hz); ^{13}C NMR ($CDCl_3$) δ 29.7, 38.2, 47.0, 52.7, 67.4, 113.7, 118.2, 120.0,124.9, 127.0, 127.8, 128.7, 130.4, 132.2, 141.3, 143.6, 153.5, 156.1, 160.2 and 161.9; mass spectrum (APCI), *m/z* 479.1479 $(M + H)^+$ ($C_{28}H_{22}N_3O_5$ requires *m/z* 479.1490).



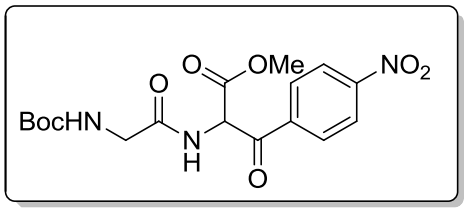
Methyl 4-(4-Cyanophenyl)-2-(pent-4-enamidomethyl)oxazole-5-carboxylate (6b). To a stirred solution of 0.14 g (0.29 mmol) of **39** in 4 mL of anhydrous CH_2Cl_2 was added 59.0 μL (51.0 mg, 0.60 mmol) of piperidine dropwise. The reaction mixture was stirred at 25 ^oC under argon atmosphere for 2 h and was concentrated under diminished pressure. The residue was dissolved in 5 mL of anhydrous THF and 0.11 g (0.58 mmol) of 4-pentenoylsuccinimide followed by 37.0 mg (0.44 mmol) of Na_2CO_3 was added. The mixture was stirred at room temperature for 3 h under argon atmosphere and was concentrated under diminished pressure. The residue was purified on a silica gel column (7 × 2 cm). Elution with 7:3 ethyl acetate−hexanes yielded **6b** as a pale yellow solid: yield 47.0 mg (48% over two steps); silica gel TLC R_f 0.29 (7:3 ethyl acetate−hexanes); ^{1}H NMR ($CDCl_3$) δ 2.34-2.40 (m, 4H), 3.92 (s, 3H), 4.64 (d, 2H, J = 6.0 Hz), 4.96-5.12 (m, 2H), 5.77-5.81 (m, 1H), 6.45 (br s, 1H), 7.72 (d, 2H, J = 8.8 Hz) and 8.18 (d, 2H, J = 8.8 Hz); ^{13}C NMR ($CDCl_3$) δ 29.3, 35.5, 43.2, 52.4, 115.8, 125.7, 127.7, 128.8, 136.7, 136.9, 141.0, 155.7, 159.1, 162.2, 172.1 and 172.8; mass spectrum (APCI), *m/z* 340.1210 $(M + H)^+$ ($C_{18}H_{18}N_3O_4$ requires *m/z* 340.1219).



Cyanomethyl 4-(4-Cyanophenyl)-2-(pent-4-enamidomethyl)oxazole-5-carboxylate (40). To a stirred solution of 16.0 mg (0.05 mmol) of **6b** in 0.4 mL of 3:1 THF-water was added 0.05 mL of 1 N LiOH. The mixture was stirred at 25 ^{o}C for 2 h. The aqueous layer was diluted with MeOH. The organic layer was dried over anhydrous Na_2SO_4 and was concentrated under diminished pressure. The crude product was dissolved in 2 mL of anhydrous DMF and 12.0 mg (0.14 mmol) of $NaHCO_3$ was added followed by 15.0 μL (18.0 mg, 0.24 mmol) of $ClCH_2CN$. The reaction mixture was stirred at 25 ^{o}C for 3 h under argon atmosphere. The mixture was concentrated under diminished pressure. The crude product was utilized in the next step without further purification.



4-(4-Cyanophenyl)-2-(pent-4-enamidomethyl)oxazole-5-carboxyl pdCpA (41). A solution containing 7.0 mg (~20 μmol) of the crude cyanomethyl ester **40** and 6.0 mg (4.4 μmol) of the tris(tetrabutylammonium) salt of pdCpA in 100 μL of 9:1 DMF–Et_3N was subjected to sonication at room temperature for 4 h. The reaction mixture was purified by C_{18} reversed phase HPLC (250 x 10 mm) using a gradient of 1% to 65% acetonitrile in 50 mM ammonium acetate, pH 4.5, over a period of 45 min. The fraction eluting at 19 min was collected and lyophilized to afford **41** as a colorless solid: yield 1.9 mg (46%); mass spectrum (ESI), *m/z* 942.1973 $(M - H)^-$ ($C_{36}H_{38}N_{11}O_{16}P_2$ requires *m/z* 942.1968).

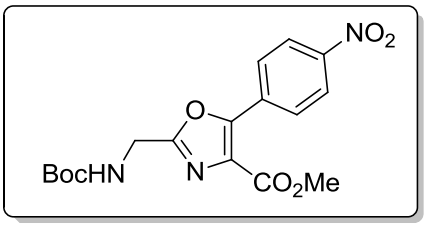


Methyl 2-(2-((*tert*-Butoxycarbonyl)amino)acetamido)-3-(4-nitrophenyl)-3-oxopropanoate (43). To a solution of 2.00 g (6.92 mmol) of Boc-protected glycine methyl ester **26** in 50 mL of THF was added 6.92 mL (6.92 mmol) of 1M NaHMDS solution in THF at –78 °C. After 30 min, 1.28 g (6.92 mmol) of 4-nitrobenzoyl chloride was added to the reaction mixture and stirred at –78 °C for 2 h. The reaction mixture was quenched with excess aqueous NH_4Cl solution and extracted with two 50-mL portions of EtOAc. The organic phase was dried ($MgSO_4$) and concentrated under diminished pressure. The residue was purified by chromatography on a silica gel column (10 × 4 cm). Elution with 3:1 hexanes–ethyl acetate gave the keto-ester **42** as a white foamy solid.

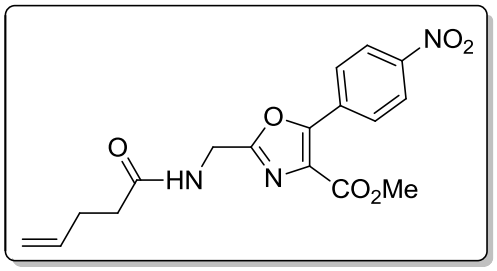
To the solution of keto-ester **42** in 25 mL of CH_2Cl_2 was added 25 mL of CF_3COOH and the reaction was stirred overnight at room temperature. The reaction mixture was concentrated under diminished pressure to obtain the amine which was used in next step without purification.

To a solution of 1.45 g (8.30 mmol) of Boc-Gly-OH in 100 mL of DMF was added 3.67 g (8.30 mmol) of BOP, 2.88 mL (1.66 g, 20.8 mmol) of Et_3N and the amine (obtained from previous step). The reaction mixture was stirred overnight at room temperature. The reaction mixture was concentrated under diminished pressure and the residue was purified by chromatography on a silica gel column (10 × 4 cm). Elution with 1:1 hexanes–ethyl acetate gave the ketoamide **43** as a colorless solid: yield 0.65 g (24% overall yield from **26**); silica gel TLC R_f 0.50 (1:1 hexanes–ethyl acetate); ^{1}H NMR ($CDCl_3$) δ 1.42 (s, 9H), 3.70 (s, 3H), 3.92 (br s, 2H), 5.28 (t, 1H, J = 10.0 Hz), 6.17 (d, 1H, J = 10.0 Hz), 7.30 (d, 2H, J = 10.0 Hz), 7.52 (d, 1H, J = 10.0 Hz) and 8.15

(d, 2H, J = 8.0 Hz); mass spectrum (APCI), m/z 396.1426 $(M+H)^+$ ($C_{17}H_{22}N_3O_8$ requires m/z 396.1407).

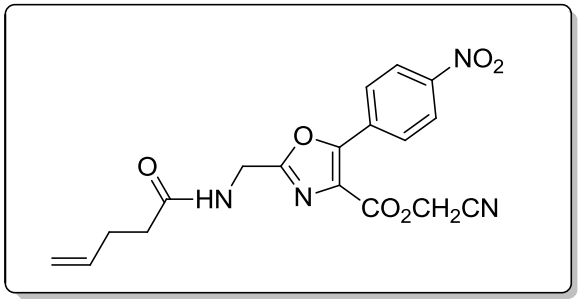


Methyl 2-(((*tert*-Butoxycarbonyl)amino)methyl)-5-(4-nitrophenyl)oxazole-4-carboxylate (44). To a stirred solution of 0.96 g (3.67 mmol) of triphenylphosphine and 0.93 g (3.67 mmol) of iodine in 50 mL of CH_2Cl_2 was added 1.02 mL (0.74 g, 7.34 mmol) of Et_3N. The dark yellow solution was stirred for 5 min and 0.73 g (1.83 mmol) of keto-amide **43** was added to the reaction mixture and stirred at rt for 2 h. The reaction mixture was concentrated under diminished pressure and the residue was purified by chromatography on a silica gel column (10 × 1 cm). Elution with 1:1 hexanes–ethyl acetate gave the desired product **44** as a yellow solid: yield 0.40 g (58%); silica gel TLC R_f 0.50 (1:1 hexanes–ethyl acetate); ^{1}H NMR ($CDCl_3$) δ 1.43 (s, 9H), 3.97 (s, 3H), 4.53 (d, 2H, J = 4.0 Hz), 5.31 (br s, 1H) and 8.27 (s, 4H); ^{13}C NMR ($CDCl_3$) δ 28.5, 38.2, 52.9, 80.8, 123.9, 129.2, 129.3, 132.6, 148.6, 153.3, 155.7, 161.2 and 162.2; mass spectrum (APCI), m/z 378.1305 $(M+H)^+$ ($C_{17}H_{20}N_3O_7$ requires m/z 378.1301).



Methyl 5-(4-Nitrophenyl)-2-(pent-4-enamidomethyl)oxazole-4-carboxylate (7b). To a solution of 0.40 g (1.06 mmol) of Boc-protected amine **44** in 10 mL of CH_2Cl_2 was added 10 mL of TFA. The reaction mixture was stirred overnight and concentrated under diminished pressure to obtain the deprotected amine which was used in next step without purification.

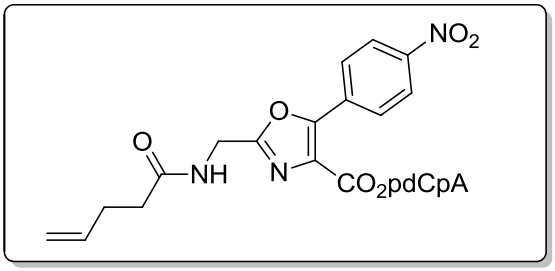
To the solution of deprotected amine in 15 mL of THF were added 0.42 g (2.13 mmol) of 4-pentenoyloxysuccinimide and 5 mL of aqueous saturated $NaHCO_3$. The reaction mixture was stirred overnight at rt and concentrated under diminished pressure. The residue was purified by chromatography on a silica gel column (10 × 1 cm). Elution with 1:10 MeOH–ethyl acetate gave the desired product **7b** as a colorless solid: yield 0.28 g (72%); silica gel TLC R_f 0.75 (1:10 MeOH–ethyl acetate); ^{1}H NMR ($CDCl_3$) δ 2.34-2.43 (m, 4H), 3.94 (s, 3H), 4.67 (d, 2H, J = 8.0 Hz), 4.97-5.08 (m, 2H), 5.78-5.82 (m, 1H), 6.38 (br s, 1H) and 8.28 (s, 4H); ^{13}C NMR ($CDCl_3$) δ 29.5, 35.6, 36.8, 53.0, 115.8, 116.1, 123.9, 129.2, 129.4, 132.5, 136.9, 148.6, 153.4, 160.8, 162.1 and 172.8; mass spectrum (APCI), m/z 360.1199 $(M+H)^+$ ($C_{17}H_{18}N_3O_6$ requires m/z 360.1196).



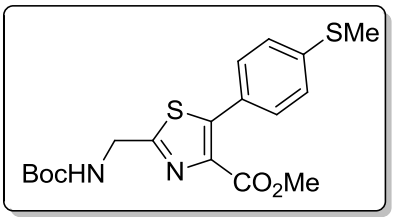
Cyanomethyl 5-(4-Nitrophenyl)-2-(pent-4-enamidomethyl)oxazole-4-carboxylate (45). To a solution of 65.0 mg (0.18 mmol) of ester **7b** in 4 mL of MeOH and 4 mL of THF was added 0.36 mL (0.36 mmol) of 1 M LiOH aqueous solution. The solution was stirred overnight at rt and

concentrated under diminished pressure to obtain the acid which was used in next step without purification.

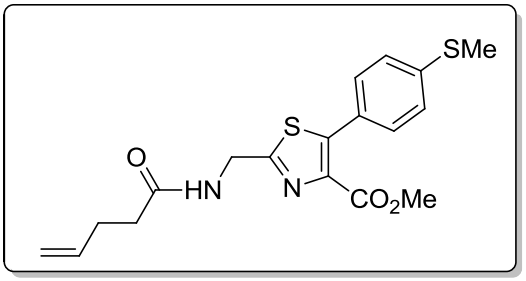
To a solution of the acid in 3.00 mL of DMF were added 100 μL of chloroacetonitrile and 0.20 mL (0.14 g, 1.51 mmol) of Et_3N. The reaction mixture was stirred overnight at rt, concentrated under diminished pressure. The residue was purified by chromatography on a silica gel column (10 × 1 cm). Elution with 1:10 MeOH–ethyl acetate gave the desired product **45** as a yellow solid: yield 37.0 mg (53%); silica gel TLC R_f 0.75 (1:10 MeOH–ethyl acetate); ^{1}H NMR ($CDCl_3$) δ 2.39-2.54 (m, 4H), 4.69 (d, 2H, J = 4.0 Hz), 4.98 (s, 2H), 5.01-5.11 (m, 2H), 5.81-5.88 (m, 1H), 6.15 (br s, 1H), 8.27 (d, 2H, J = 4.0 Hz) and 8.35 (d, 2H, J = 12.0 Hz); mass spectrum (APCI), m/z 385.1153 $(M+H)^+$ ($C_{18}H_{17}N_4O_6$ requires m/z 385.1148).



5-(4-Nitrophenyl)-2-(pent-4-enamidomethyl)oxazole-4-carboxylic acid pdCpA ester (46). To a stirred solution containing 18.0 mg (13.5 μmol) of pdCpA tetrabutylammonium salt in 100 μL of 9:1 anhydrous DMF–Et_3N was added 13.0 mg (33.7 μmol) of cyanomethyl ester **45**. The reaction mixture was sonicated for 6 h. The reaction mixture was purified by C_{18} reversed phase HPLC (250 x 10 mm) using a gradient of 1% to 65% acetonitrile in 50 mM ammonium acetate, pH 4.5, over a period of 1 h. The retention time of the desired product was 25.3 min. The fractions containing the product were lyophilized to afford **46** as a colorless solid: yield 1.8 mg (14%); mass spectrum (ESI), m/z 962.1874 $(M-H)^-$ ($C_{35}H_{38}N_{11}O_{18}P_2$ requires m/z 962.1871).

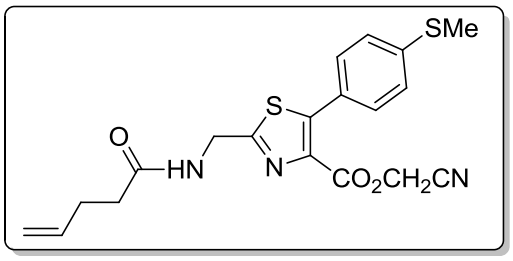


Methyl 2-(((*tert*-Butoxycarbonyl)amino)methyl)-5-(4-(methylthio)phenyl)thiazole-4-carboxylate (47). To a solution of 1.72 g (4.35 mmol) of keto-amide **28** in 80 mL of THF was added 2.46 g (6.09 mmol) of Lawesson's reagent and the mixture was refluxed for 4 h. The reaction mixture was cooled to room temperature, concentrated under diminished pressure and the residue was purified by chromatography on a silica gel column (10 × 1 cm). Elution with 1:1 hexanes–ethyl acetate gave the desired product **47** as a colorless oil: yield 1.49 g (87%); silica gel TLC R_f 0.50 (1:1 hexanes–ethyl acetate); ^{1}H NMR ($CDCl_3$) δ 1.47 (s, 9H), 2.51 (s, 3H), 3.84 (s, 3H), 4.61 (d, 2H, J = 4.0 Hz), 5.32 (br s, 1H), 7.26 (d, 2H, J = 8.0 Hz) and 7.41 (d, 2H, J = 8.0 Hz); ^{13}C NMR ($CDCl_3$) δ 15.6, 28.6, 42.7, 52.6, 80.8, 125.8, 126.7, 130.5, 139.4, 141.0, 147.7, 155.9, 162.6 and 167.6; mass spectrum (APCI), m/z 395.1100 $(M+H)^+$ ($C_{18}H_{23}N_2O_4S_2$ requires m/z 395.1099).



Methyl 5-(4-(Methylthio)phenyl)-2-(pent-4-enamidomethyl)thiazole-4-carboxylate (9b). To a solution of 1.30 g (3.30 mmol) of Boc-protected amine **47** in 25 mL of CH_2Cl_2 was added 25 mL of TFA. The reaction mixture was stirred overnight and concentrated under diminished pressure to obtain the deprotected amine which was used in next step without purification.

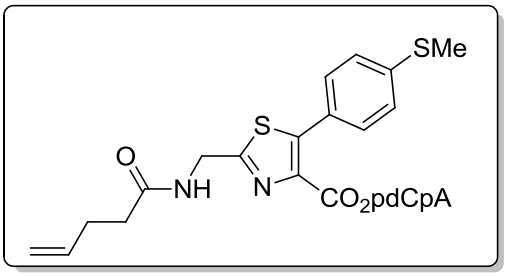
To the solution of deprotected amine in 50 mL of THF were added 1.63 g (8.25 mmol) of 4-pentenoyloxysuccinimide and 20 mL of satd aq $NaHCO_3$. The reaction mixture was stirred overnight at room temperature and concentrated under diminished pressure. The residue was purified by chromatography on a silica gel column (10 × 1 cm). Elution with 1:10 MeOH–ethyl acetate gave the desired product **9b** as a colorless solid: yield 1.06 g (86%); silica gel TLC R_f 0.75 (1:10 MeOH–ethyl acetate); ^{1}H NMR ($CDCl_3$) δ 2.39 (s, 4H), 2.49 (s, 3H), 3.80 (s, 3H), 4.71 (d, 2H, J = 4.0 Hz), 4.96-5.06 (m, 2H), 5.76-5.83 (m, 1H), 7.23 (d, 2H, J = 4.0 Hz), 7.34 (d, 2H, J = 4.0 Hz) and 7.44 (br s, 1H); ^{13}C NMR ($CDCl_3$) δ 15.1, 29.3, 35.2, 41.1, 52.1, 115.6, 115.6, 125.3, 126.1, 130.0, 136.7, 138.7, 140.7, 147.3, 162.3, 166.6 and 173.0; mass spectrum (APCI), m/z 377.0991 $(M+H)^+$ ($C_{18}H_{21}N_2O_3S_2$ requires m/z 377.0994).



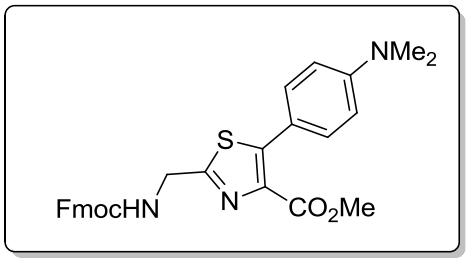
Cyanomethyl 5-(4-(Methylthio)phenyl)-2-(pent-4-enamidomethyl)thiazole-4-carboxylate (48). To a solution of 0.42 g (1.12 mmol) of ester **9b** in 5 mL of MeOH and 5 mL of THF was added 2.79 mL (2.79 mmol) of 1 M LiOH aqueous solution. The solution was stirred overnight at rt, concentrated under diminished pressure to obtain the acid which was used in next step without purification.

To a solution of the acid in 3 mL of DMF were added 100 μL of chloroacetonitrile and 0.50 mL (0.36 g, 3.60 mmol) of Et_3N. The reaction mixture was stirred overnight at rt, concentrated under diminished pressure. The residue was purified by chromatography on a silica gel column (10 × 1 cm). Elution with 1:10 MeOH–ethyl acetate gave the desired product **48** as a yellow solid: yield

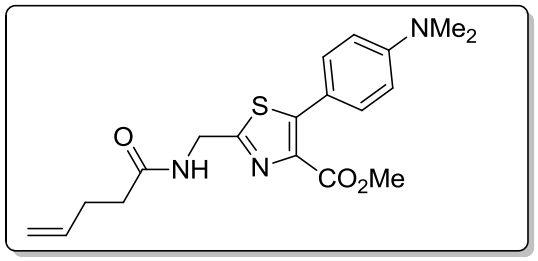
0.25 g (55%); silica gel TLC R_f 0.75 (1:10 MeOH–ethyl acetate); ^{1}H NMR ($CDCl_3$) δ 2.33-2.37 (m, 4H), 2.47 (s, 3H), 4.67 (d, 2H, J = 4.0 Hz), 4.81 (s, 2H), 4.95-5.04 (m, 2H), 5.77-5.78 (m, 1H), 6.73 (br s, 1H), 7.22 (d, 2H, J = 8.0 Hz) and 7.33 (d, 2H, J = 8.0 Hz); ^{13}C NMR ($CDCl_3$) δ 15.4, 29.5, 34.1, 35.6, 49.3, 114.3, 116.1, 125.7, 125.8, 130.3, 130.4, 136.9, 141.8, 150.6, 160.3, 166.8 and 173.1; mass spectrum (APCI), *m*/*z* 402.0958 $(M+H)^+$ ($C_{19}H_{20}N_3O_4S$ requires *m*/*z* 402.0946).



5-(4-(Methylthio)phenyl)-2-(pent-4-enamidomethyl)thiazole-4-carboxylic acid pdCpA ester (49). To a stirred solution containing 7.50 mg (18.0 μmol) of pdCpA tetrabutylammonium salt in 100 μL of 9:1 anhydrous DMF–Et_3N was added 10.0 mg (7.00 μmol) of cyanomethyl ester **48**. The reaction mixture was sonicated for 6 h. The reaction mixture was purified by C_{18} reversed phase HPLC (250 x 10 mm) using a gradient of 1% to 65% acetonitrile in 50 mM ammonium acetate, pH 4.5, over a period of 1 h. The retention time of the desired product was 24.2 min. The fractions containing the product were lyophilized to afford **49** as a colorless solid: yield 2.0 mg (27%); mass spectrum (ESI), *m/z* 979.1682 $(M-H)^-$ ($C_{36}H_{41}N_{10}O_{15}P_2S_2$ requires *m/z* 979.1682).

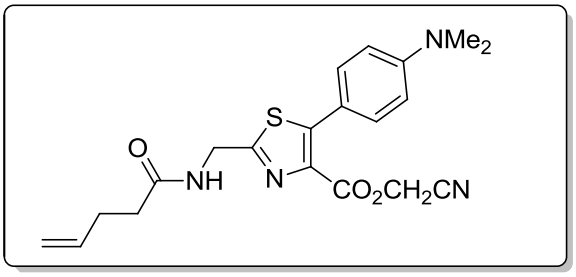


Methyl 2-((((9H-Fluoren-9-yl)methoxy)carbonylamino)methyl)-4-(4-(dimethylamino)phenyl)thiazole-5-carboxylate (50). To a stirred solution of 0.16 g (0.31 mmol) of **33** in 5 mL of anhydrous THF was added 0.25 g (0.62 mmol) of the Lawesson's reagent. The mixture was heated to reflux under argon atmosphere for 1 h. The yellow reaction mixture was diluted with 20 mL saturated $NaHCO_3$ solution. The aqueous layer was extracted with two 25-mL portions of ethyl acetate. The organic layer was dried over anhydrous $MgSO_4$ and was concentrated under diminished pressure. Crude product **50** was utilized in the next reaction without further purification.

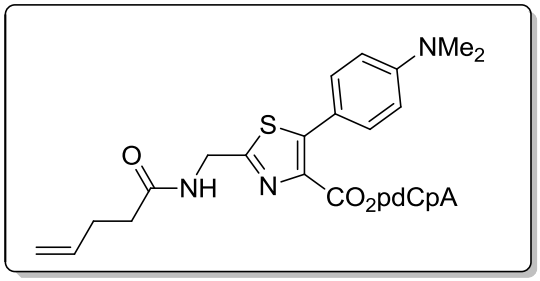


Methyl 4-(4-(Dimethylamino)phenyl)-2-(pent-4-enamidomethyl)thiazole-5-carboxylate (10b).To a stirred solution of the crude **50** in 4 mL of anhydrous CH_2Cl_2 was added 64.0 μL (55.0 mg, 0.65 mmol) of piperidine dropwise. The reaction mixture was stirred at 25 ^oC under argon atmosphere for 2 h and was concentrated under diminished pressure. The residue was dissolved in 5 mL of anhydrous THF and 0.26 g (1.32 mmol) of 4-pentenoyloxysuccinimide was added followed by 83.0 mg (0.78 mmol) of Na_2CO_3. The mixture was stirred at room temperature for 3 h under argon atmosphere and was concentrated under diminished pressure. The residue was purified on a silica gel column (7 × 2 cm). Elution with 7:3 ethyl acetate−hexanes afforded **10b** as a pale yellow solid: yield 60.0 mg (52% over three steps); silica gel TLC R_f 0.17 (7:3 ethyl acetate−hexanes); ^1H NMR ($CDCl_3$) δ 2.33-2.42 (m, 4H), 3.00 (s, 6H), 3.84 (s, 3H), 4.70 (d, 2H, J = 6.0 Hz), 4.99-5.09 (m, 2H), 5.78-5.85 (m, 1H), 6.40 (br s,

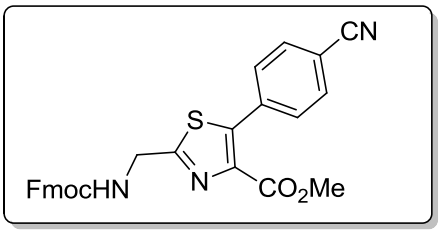
1H), 6.69 (d, 2H, J = 8.8 Hz) and 7.38 (d, 2H, J = 8.8 Hz); ^{13}C NMR ($CDCl_3$) δ 29.3, 35.5, 40.2, 40.9, 52.1, 111.3, 115.8, 116.9, 130.9, 136.7, 137.4, 149.7, 150.9, 162.6, 163.9 and 172.4; mass spectrum (APCI), *m/z* 396.1348 $(M + H)^+$ ($C_{19}H_{24}N_3O_3NaS$ requires *m/z* 396.1358).



Cyanomethyl 4-(4-(Dimethylamino)phenyl)-2-(pent-4-enamidomethyl)thiazole-5-carboxylate (51). To a stirred solution of 17.0 mg (0.05 mmol) of **10b** in 0.4 mL of 3:1 THF-water was added 0.06 mL (0.06 mmol) of 1 N LiOH. The mixture was stirred at 25 ^{o}C for 4.5 h. The yellow aqueous layer was diluted with MeOH. The organic layer was dried over anhydrous Na_2SO_4 and was concentrated under diminished pressure. The crude product was dissolved in 2 mL of anhydrous DMF and 9.0 mg (0.11 mmol) of $NaHCO_3$ was added followed by 25.0 μL (30.0 mg, 0.40 mmol) of $ClCH_2CN$. The reaction mixture was stirred at 25 ^{o}C for 3 h under argon atmosphere. The mixture was concentrated under diminished pressure and was purified on a silica gel column (7 × 1 cm). Elution with 3:2 ethyl acetate−hexanes afforded **51** as a bright yellow solid: yield 12.0 mg (65%); silica gel TLC R_f 0.7 (ethyl acetate); ^{1}H NMR ($CDCl_3$) δ 2.36-2.44 (m, 4H), 3.02 (s, 6H), 4.71 (d, 2H, J = 2.8 Hz), 4.87 (s, 2H), 5.00-5.10 (m, 2H), 5.79-5.84 (m, 1H), 6.29 (br s, 1H), 6.71 (d, 2H, J = 8.4 Hz) and 7.39 (d, 2H, J = 8.4 Hz); ^{13}C NMR ($CDCl_3$) δ 29.3, 35.5, 40.1, 41.0, 48.5, 111.3, 114.2, 115.9, 116.1, 131.0, 135.0, 136.6, 151.2, 152.8, 160.3, 164.3 and 172.4; mass spectrum (ESI), *m/z* 421.1316 $(M + H)^+$ ($C_{20}H_{22}N_4O_3NaS$ requires *m/z* 421.1310).

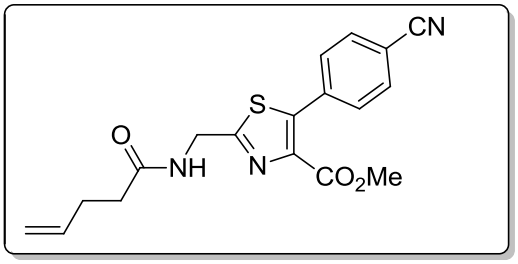


4-(4-(Dimethylamino)phenyl)-2-(pent-4-enamidomethyl)thiazole-5-carboxylic Acid pdCpA Ester (52). A solution containing 6.0 mg (15 μmol) of cyanomethyl ester **51** and 5.7 mg (4.2 μmol) of the tris(tetrabutylammonium) salt of pdCpA in 100 μL of 9:1 DMF–Et_3N was subjected to sonication at room temperature for 4 h. The reaction mixture was purified by C_{18} reversed phase HPLC (250 x 10 mm) using a gradient of 1% to 65% acetonitrile in 50 mM ammonium acetate, pH 4.5, over a period of 45 min. The fraction eluting at 19.5 min was collected and lyophilized to afford **52** as a yellow solid: yield 2.5 mg (62%); mass spectrum (ESI), *m/z* 976.2213 $(M - H)^-$ ($C_{37}H_{44}N_{11}O_{15}P_2S$ requires *m/z* 976.2214).

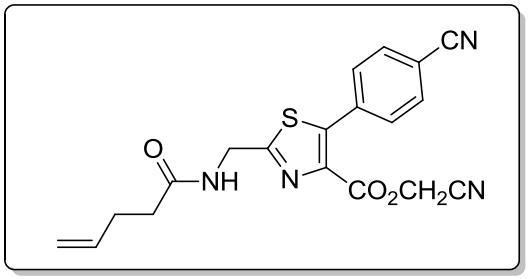


Methyl 2-((((9H-Fluoren-9-yl)methoxy)carbonylamino)methyl)-4-(4-cyanophenyl)thiazole-5-carboxylate (53). To a stirred solution of 0.30 g (0.60 mmol) of **38** in 5 mL of anhydrous THF was added 0.49 g (1.20 mmol) of the Lawesson's reagent. The mixture was heated to reflux under an argon atmosphere for 1 h. The yellow reaction mixture was diluted with 20 mL of satd aq $NaHCO_3$ solution. The aqueous layer was extracted with two 25-mL portions of ethyl acetate.

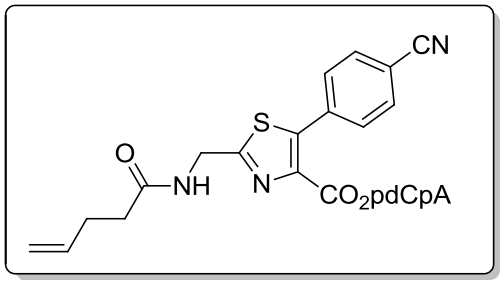
The organic layer was dried over anh $MgSO_4$ and was concentrated under diminished pressure. Crude product **53** was utilized in the next reaction without further purification.



Methyl 4-(4-Cyanophenyl)-2-(pent-4-enamidomethyl)thiazole-5-carboxylate (11b). To a stirred solution of the crude **53** in 4 mL of anhydrous CH_2Cl_2 was added dropwise 120 μL (0.10 g, 1.20 mmol) of piperidine. The reaction mixture was stirred at 25 ^oC under an argon atmosphere for 2 h and was then concentrated under diminished pressure. The residue was dissolved in 5 mL of anhydrous THF and 0.26 g (1.32 mmol) of 4-pentenoyloxysuccinimide was added, followed by 83.0 mg (0.78 mmol) of Na_2CO_3. The reaction mixture was stirred at room temperature for 3 h under an argon atmosphere and was then concentrated under diminished pressure. The residue was purified on a silica gel column (7 × 2 cm). Elution with 1:1 ethyl acetate−hexanes yielded **11b** as a pale yellow solid: yield 47.0 mg (22% over two steps); silica gel TLC R_f 0.29 (7:3 ethyl acetate−hexanes); ^1H NMR ($CDCl_3$) δ 2.34-2.40 (m, 4H), 3.92 (s, 3H), 4.64 (d, 2H, J = 6.0 Hz), 4.96-5.12 (m, 2H), 5.77-5.81 (m, 1H), 6.45 (br s, 1H), 7.72 (d, 2H, J = 8.8 Hz) and 8.18 (d, 2H, J = 8.8 Hz); ^{13}C NMR ($CDCl_3$) δ 29.3, 35.4, 41.1, 52.5, 113.0,116.0, 118.2, 130.7, 131.9, 134.9, 136.6, 140.1, 145.3, 161.9, 167.2 and 172.6; mass spectrum (APCI), *m/z* 356.0980 $(M + H)^+$ ($C_{18}H_{18}N_3O_3S$ requires *m/z* 356.0991).

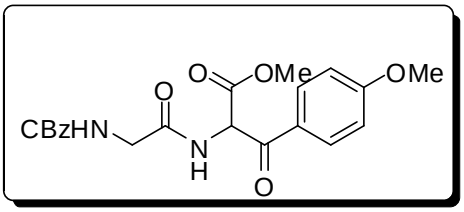


Cyanomethyl 4-(4-Cyanophenyl)-2-(pent-4-enamidomethyl)thiazole-5-carboxylate (54). To a stirred solution of 16.0 mg (0.05 mmol) of **11b** in 0.4 mL of 3:1 THF–water was added 0.05 mL (0.05 mmol) of 1 N LiOH. The reaction mixture was stirred at 25 ^{o}C for 2 h. The reaction was diluted with MeOH, dried over anh Na_2SO_4 and concentrated under diminished pressure. The crude product was dissolved in 2 mL of anhydrous DMF and 12.0 mg (0.14 mmol) of $NaHCO_3$ was added, followed by 15.0 μL (18.0 mg, 0.24 mmol) of $ClCH_2CN$. The reaction mixture was stirred at 25 ^{o}C for 3 h under an argon atmosphere. Crude product **54** was utilized in the next reaction without further purification.



4-(4-Cyanophenyl)-2-(pent-4-enamidomethyl)thiazole-5-carboxylic Acid pdCpA Ester (55). A solution containing 6.0 mg (~16 μmol) of crude cyanomethyl ester **54** and 6.0 mg (4.4 μmol) of the tris(tetrabutylammonium) salt of pdCpA in 100 μL of 9:1 DMF–Et_3N was subjected to sonication at room temperature for 4 h. The reaction mixture was purified by C_{18} reversed phase HPLC (250 × 10 mm) using a gradient of 1% to 65% acetonitrile in 50 mM ammonium acetate, pH 4.5, over a period of 45 min. The fraction eluting at 17 min was collected and lyophilized to

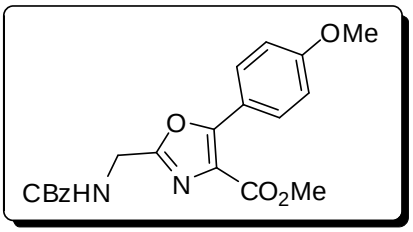
afford **55** as a colorless solid: yield 1.9 mg (45%); mass spectrum (ESI), *m/z* 958.1741 $(M + H)^+$ ($C_{36}H_{38}N_{11}O_{15}P_2S$ requires *m/z* 958.1745).



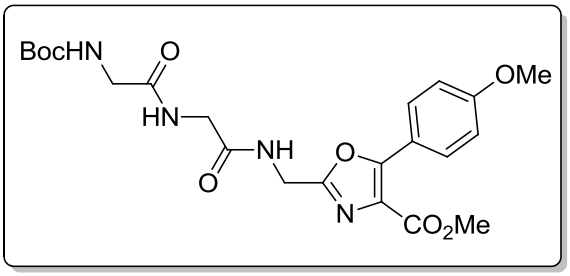
Methyl 2-(2-(Benzyloxycarbonyl)acetamido)-3-(4-methoxyphenyl)-3-oxopropanoate (56). To a solution of 0.56 g (2.23 mmol) of imine ester **15** in 100 mL of THF was added 2.45 mL (2.45 mmol) of 1 M NaHMDS solution in THF at –78 °C. After 30 min, 0.33 mL (0.42 g, 2.45 mmol) of 4-methoxybenzoyl chloride was added to the reaction mixture, which was stirred at –78 °C for 2 h. The reaction mixture was treated with 6 M aq HCl solution until pH 2 was reached. The solvent was concentrated under diminished pressure to obtain the amine salt **56** as a colorless solid; this was used in the next reaction without further purification.

To a solution of the amine salt **56** in 50 mL of THF were added 1.36 g (4.45 mmol) of CBz-Gly-OSu and 1.55 mL (1.12 g, 11.1 mmol) of Et_3N. The reaction mixture was stirred overnight at room temperature. The reaction mixture was diluted with 300 mL of water and extracted with two 50-mL portions of EtOAc. The organic phase was dried ($MgSO_4$) and concentrated under diminished pressure. The residue was purified by chromatography on a silica gel column (10 × 4 cm). Elution with 1:1 hexanes–ethyl acetate gave the desired product **57** as a colorless solid: yield 0.74 g (80% overall yield from imine **15**); silica gel TLC R_f 0.50 (1:1 hexanes–ethyl acetate); ^{1}H NMR ($CDCl_3$) δ 3.60 (s, 3H), 3.78 (s, 3H), 3.98 (br s, 2H), 5.06 (s, 2H), 5.94 (t, 1H, J = 8.0 Hz), 6.16 (d, 1H, J = 8.0 Hz), 6.87 (d, 2H, J = 8.0 Hz), 7.22-7.27 (m, 5H), 7.77-7.78 (m, 1H) and 8.03 (d, 2H, J = 8.0 Hz); ^{13}C NMR ($CDCl_3$) δ 44.2, 53.2, 55.6, 57.6, 67.0, 114.1, 114.4,

126.8, 128.0, 128.1, 128.5, 132.1, 136.4, 156.7, 164.7, 167.4, 169.4 and 189.4; mass spectrum (APCI), *m*/*z* 415.1516 $(M+H)^+$ ($C_{21}H_{23}N_2O_7$ requires *m*/*z* 415.1505).

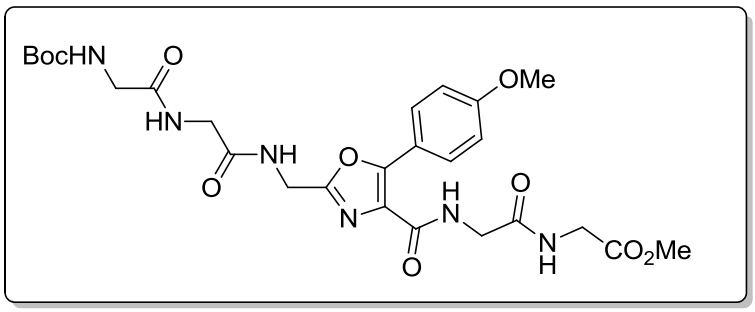


Methyl 2-((Benzyloxycarbonyl)methyl)-5-(4-methoxyphenyl)oxazole-4-carboxylate (58). To a stirred solution of 0.68 g (2.61 mmol) of triphenylphosphine and 0.66 g (2.61 mmol) of iodine in 50 mL of CH_2Cl_2 was added 0.72 mL (0.52 g, 5.22 mmol) of Et_3N. The dark yellow solution was stirred for 5 min and 0.72 g (1.74 mmol) of ketoamide **57** was added to the reaction mixture, which was stirred at room temperature for 2 h. The reaction mixture was concentrated under diminished pressure and the residue was purified by chromatography on a silica gel column (10 × 1 cm). Elution with 1:1 hexanes–ethyl acetate gave the desired product **58** as a colorless oil: yield 0.54 g (78%); silica gel TLC R_f 0.50 (1:1 hexanes–ethyl acetate); ^{1}H NMR ($CDCl_3$) δ 3.77 (s, 3H), 3.82 (s, 3H), 4.49 (d, 2H, *J* = 4.0 Hz), 5.07 (s, 2H), 5.91 (br s, 1H), 6.88 (d, 2H, *J* = 8.0 Hz), 7.24-7.27 (m, 5H) and 7.93 (d, 2H, *J* = 8.0 Hz); ^{13}C NMR ($CDCl_3$) δ 38.3, 52.2, 55.4, 67.2, 113.9, 119.1, 125.3, 128.1, 128.2, 128.5, 130.1, 136.3, 156.2, 156.4, 158.6, 161.3 and 162.5; mass spectrum (APCI), *m*/*z* 397.1392 $(M+H)^+$ ($C_{21}H_{21}N_2O_6$ requires *m*/*z* 397.1400).



Methyl 2-((2-(2-(*tert*-Butoxycarbonyl)acetamido)acetamido)methyl)-5-(4-methoxyphenyl)oxazole-4-carboxylate (60). To a solution of 0.54 g (1.36 mmol) of CBz-protected amine **58** in 50 mL of MeOH was added 50 mg of 10% Pd/C. The suspension was stirred overnight under 1 atm of H_2. The reaction mixture was filtered through a Celite pad and the filtrate was concentrated under diminished pressure to obtain the deprotected amine **59**, which was used in the next step without purification.

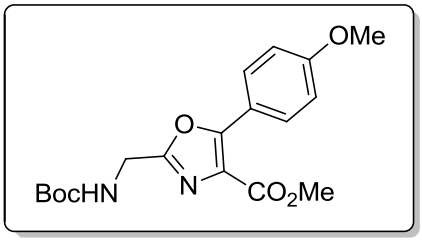
To a solution of 0.47 g (2.03 mmol) of Boc-Gly-Gly-OH in 20 mL of DMF was added 0.90 g (2.03 mmol) of BOP reagent, 0.71 mL (0.53 g, 4.06 mmol) of DIPEA and amine **59** obtained from the previous step. The reaction mixture was stirred overnight at room temperature. The reaction mixture was concentrated under diminished pressure and the residue was purified by chromatography on a silica gel column (10 × 4 cm). Elution with 1:9 methanol–ethyl acetate gave oxazole **60** as a colorless oil: yield 0.53 g (82% overall yield from **58**); silica gel TLC R_f 0.50 (1:9 methanol–ethyl acetate); ^{1}H NMR ($CDCl_3$) δ 1.34-1.39 (m, 9H), 2.58 (d, 1H, J = 8.0 Hz), 3.80 (s, 7H), 4.02 (d, 2H, J = 8.0 Hz), 4.55 (d, 2H, J = 4.0 Hz), 5.77 (br s, 1H), 6.90 (d, 2H, J = 8.0 Hz), 7.56-7.57 (m, 1H), 7.88 (d, 2H, J = 8.0 Hz), 7.98-8.00 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 28.5, 36.6, 37.0, 43.0, 44.4, 52.3, 55.6, 80.4, 114.0, 119.1, 125.2, 130.4, 156.3, 156.7, 158.9, 161.5, 162.7, 170.1 and 171.0; mass spectrum (APCI), m/z 477.1984 $(M+H)^+$ ($C_{22}H_{29}N_4O_8$ requires m/z 477.1985).



Methyl 2-(2-(2-((2-(2-(*tert*-Butoxycarbonyl)acetamido)acetamido)methyl)-5-(4-methoxyphenyl)oxazole-4-carboxamido)acetamido)acetate (12). To a solution of 0.53 g (1.11 mmol) of ester **60** in 10 mL of MeOH and 10 mL of THF was added 2.22 mL (2.22 mmol) of 1 M LiOH aqueous solution. The solution was stirred overnight at room temperature, and then concentrated under diminished pressure to obtain acid **61**, which was used in next step without further purification.

To a solution of acid **61** in 15 mL of DMF was added 0.54 g (1.22 mmol) of BOP reagent, 0.39 mL (0.29 g, 2.22 mmol) of DIPEA and 0.20 g (1.11 mmol) of amine salt **62**. The reaction mixture was stirred overnight at room temperature then concentrated under diminished pressure. The residue was purified by chromatography on a silica gel column (10 × 4 cm). Elution with 1:9 methanol–ethyl acetate gave oxazole **12** as a colorless oil: yield 0.08 g (12% overall yield from **60**); silica gel TLC R_f 0.50 (1:1 methanol–ethyl acetate); ^{1}H NMR ($CDCl_3$) δ 1.39 (s, 9H), 3.70 (s, 3H), 3.80 (s, 3H), 3.84-3.85 (m, 2H), 4.02-4.03 (m, 4H), 4.14 (d, 2H, J = 8.0 Hz), 4.38 (s, 2H), 5.73 (br s, 1H), 6.88 (d, 2H, J = 8.0 Hz), 7.41 (br s, 1H), 7.54 (br s, 1H), 7.75 (br s, 1H), 8.06 (d, 2H, J = 8.0 Hz) and 8.26 (br s, 1H); ^{13}C NMR ($CDCl_3$) δ 28.5, 29.6, 30.0, 36.6, 41.4, 43.2, 43.2, 44.6, 51.1, 52.7, 55.6, 77.0, 80.6, 114.1, 119.3, 127.3, 130.0, 153.7, 156.7, 157.6, 161.3, 162.5, 170.0, 170.6 and 171.1; mass spectrum (APCI), m/z 591.2410 $(M+H)^+$ ($C_{26}H_{35}N_6O_{10}$ requires m/z 591.2414).

Boc-deprotection was carried out in a stirred solution of 7.70 mg (0.01 mmol) of **12** in 1 mL of CH_2Cl_2 to which was added 10.0 µL (15.0 mg, 0.13 mmol) of trifluoroacetic acid. The reaction mixture was stirred overnight at room temperature and was concentrated under diminished pressure. The excess trifluoroacetic acid was removed by coevaporation of portions of toluene. The calculations were done assuming quantitative conversion.



Methyl 2-(((*tert*-Butoxycarbonyl)amino)methyl)-5-(4-methoxyphenyl)oxazole-4-carboxylate (63). To a solution of 0.22 g (0.55 mmol) of CBz-protected amine **58** in 10 mL of MeOH was added 40 mg of 10% Pd/C. The suspension was stirred overnight under 1 atm of H_2, and the reaction mixture was filtered through a Celite pad. The filtrate was concentrated under diminished pressure to obtain the deprotected amine **59** which was used in next step without further purification.

To a solution of crude amine **59** in 10 mL of CH_2Cl_2 were added 0.14 g (0.66 mmol) of Boc-anhydride and 0.19 mL (0.14 g, 1.33 mmol) of Et_3N. The reaction mixture was stirred overnight at room temperature. The reaction mixture was concentrated under diminished pressure and the residue was purified by chromatography on a silica gel column (10 × 1 cm). Elution with 1:3 hexanes–ethyl acetate gave oxazole **63** as a colorless oil: yield 0.18 g (90% for two steps); silica gel TLC R_f 0.50 (1:1 hexanes–ethyl acetate); ^{1}H NMR ($CDCl_3$) δ 1.43 (s, 9H), 3.83 (s, 3H), 3.89 (s, 3H), 4.48 (d, 2H, J = 4.0 Hz), 5.29 (br s, 1H), 6.94 (d, 2H, J = 8.0 Hz) and 8.00 (d, 2H, J = 8.0 Hz); ^{13}C NMR ($CDCl_3$) δ 28.5, 38.1, 52.4, 55.6, 76.9, 80.5, 114.1, 119.3, 125.5, 130.3, 155.7, 156.4, 159.1, 161.5 and 162.8; mass spectrum (APCI), m/z 363.1550 $(M+H)^+$ ($C_{18}H_{23}N_2O_6$ requires m/z 363.1556).

Boc-deprotection was carried out in a stirred solution of 18.0 mg (0.05 mmol) of **63** in 1 mL of CH_2Cl_2 to which was added 38.0 µL (57.0 mg, 0.50 mmol) of trifluoroacetic acid. The reaction mixture was stirred overnight at room temperature and was concentrated under diminished

pressure. The excess trifluoroacetic acid was removed by coevaporation of portions of toluene. The calculations were done assuming quantitative conversion.