Supporting Material for:

Fluorogenic Peptide Substrates for Serine and Threonine Phosphatases

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Contents

1. General Methods

1.1. General Experimental Procedures

All experiments were conducted using anhydrous conditions under an atmosphere of nitrogen. All solvents were distilled and stored under argon before use. All reagents and enzymes were used as received. Aqueous solutions of sodium chloride (brine) were saturated. Analytical thin layer chromatography (TLC) plates were visualized by ultraviolet irradiation or ninhydrin staining solutions. Flash column chromatography was carried out under a positive pressure of nitrogen. ^{1}H NMR spectra were recorded on 300 MHz or 400 MHz spectrometers. Data are presented as follows: chemical shift (in ppm on the δ scale relative to δ = 0.00 ppm for the protons in TMS), multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad), coupling constant (*J*/Hz), which was taken directly from the spectra and are uncorrected, and integration. ^{13}C NMR spectra were recorded at 75 or 100 MHz, and all chemical shift values are reported in ppm on the δ scale, with an internal reference of δ 77.0 or 49.0 for $CDCl_3$ or CD_3OD, respectively. Low- resolution mass spectra were measured using liquid chromatography mass spectrometery (LC-MS). High-resolution mass spectra were measured using electron impact (EI) or fast atom bombardment (FAB) ionization. A rainin HPLC system was used with reverse phase C8 column (Phenomenex, OOG-4167-NO, 250 × 10.00 mm) and UV detection (λ = 340 nm) for peptide purification. Compound 1b has been reported in the literature.[1]

1.2. General Methods for Peptide Synthesis

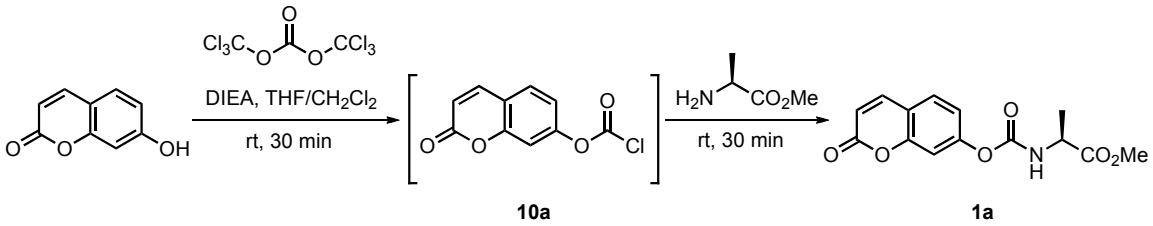
Syntheses of peptides **4a-b** and **5** were accomplished using standard manual Fmoc solid phase peptide synthesis techniques on Rink Amide AM resin (VWR 80013-820). All Fmoc deprotection steps were performed using 20% piperidine in DMF (10 mL, 2 × 30 min). The Fmoc amino acids and coupling reagents (Fmoc-Ala-OH, Fmoc-Val-OH, Fmoc-Glu-OH, HBTU, HOBt) were purchased from Novabiochem and used in 4 fold excess relative to the resin loading. DIEA was used as the base in the coupling reactions: 8 fold excess for Ala, Val or Glu coupling and 12 fold excess for compound **19** coupling. The reaction mixture was shaken using a Wrist Action Shaker (Burrell, Model 75). After each step, the resin was washed with DMF (10 × 10 mL), CH_2Cl_2 (15 × 10 mL), and DMF (10 × 10 mL).

The acylation step employed six equiv *p*-nitrophenylchloroformate **10b** (15 mL CH_2Cl_2) or freshly synthesized **10a** (15 mL, 1:1 THF/CH_2Cl_2) and 12 equiv DIEA. The reaction was allowed to shake at room temperature for 12 h. The resulting resin was washed with CH_2Cl_2 (500 mL), and then dried under high vacuum for 24 h.

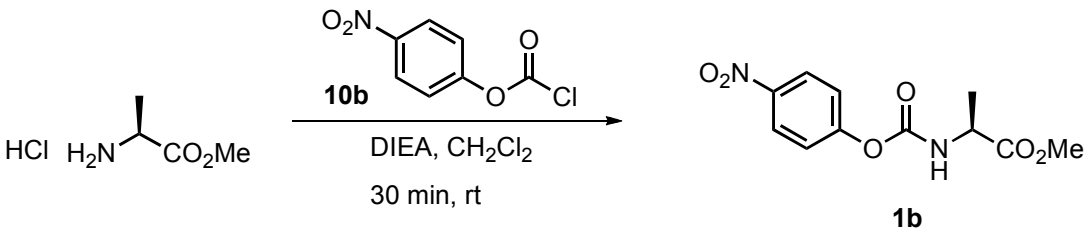
The cleavage of the peptide and the global deprotection of the amino acid side chains were achieved by stirring the dried resin with the cleavage cocktail containing 94% trifluoroacetic acid (TFA), 3% H_2O and 3% triisopropylsilane (TIS). After stirring the reaction for 30 min, the resin was removed by filtration. The solution was concentrated by rotary evaporation. To the resulting residue, 10 mL cold ether was added to precipitate the crude peptide. The suspension was centrifuged and the white solid was washed with ether (3 × 10 mL). The crude peptide was dissolved in a minimum amount

of DMSO and purified by RP-HPLC (Phenomenex, OOG-4167-NO, 250 × 10.00 mm) using a CH_3CN/H_2O solvent system.

2. Preparation and Characterization of Compounds

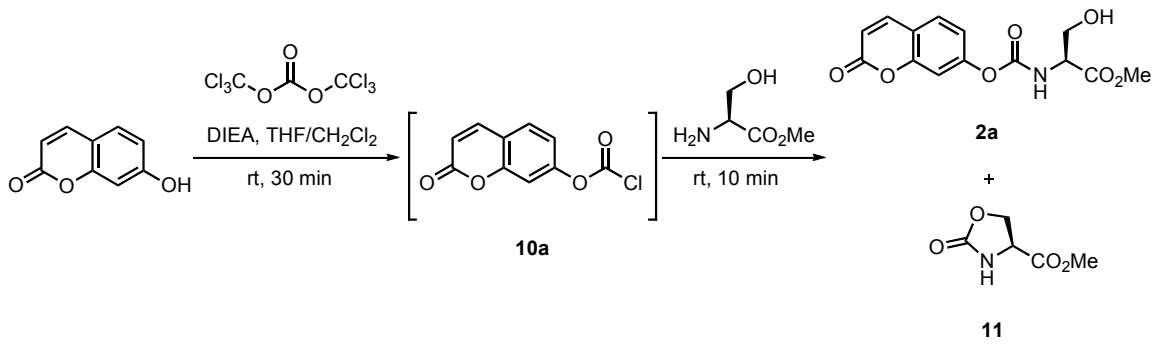


Carbamate 1a. To a solution of triphosgene (99 mg, 0.33 mmol) in CH_2Cl_2 (10 mL), was slowly added 7-hydroxycoumarin (162 mg, 1.0 mmol) and DIEA (176 μL, 130 mg, 1.0 mmol) as a solution in THF (10 mL). The reaction was stirred at room temperature for 30 min to generate the intermediate chloroformate **10a**. In a separate flask containing CH_2Cl_2 (10 mL), H_2N-Ala-OMe hydrochloride (208 mg, 1.5 mmol) was mixed with DIEA (263 μL, 195 mg, 1.5 mmol). The mixture was stirred for 5 min at room temperature and then dropped into the flask containing chloroformate **10a**. The reaction mixture was stirred at room temperature for another 30 min and partitioned between CH_2Cl_2 (50 mL) and saturated NH_4Cl (50 mL). The organic layer was washed one more time with saturated NH_4Cl (30 mL), and dried over $MgSO_4$. The solvent was removed by rotary evaporation and purified using flash chromatography (1:1 EtOAc/hexanes) to provide compound **1a** (168 mg, 0.55 mmol, 55%) as a white solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.51-1.53 (d, J = 6.0 Hz, 3H), 3.81 (s, 3H), 4.40-4.50 (m, 1H), 5.85-5.95 (d, J = 6.6 Hz, 1H), 6.30-6.40 (d, J = 9.0 Hz, 1H), 7.10-7.13 (dd, J = 2.1, 6.0 Hz, 1H), 7.14-7.17 (d, J = 3.0 Hz, 1H), 7.40-7.50 (d, J = 9.0 Hz, 1H), 7.60-7.70 (d, J = 9.0 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 18.6, 50.0, 52.9, 110.3, 115.9, 116.4, 118.4, 128.6, 143.1, 153.0, 153.6, 154.8, 160.7, 173.2; HRMS-FAB (M + Na^+) calcd for $C_{14}H_{13}NO_6$ 314.0635, found 314.0652.

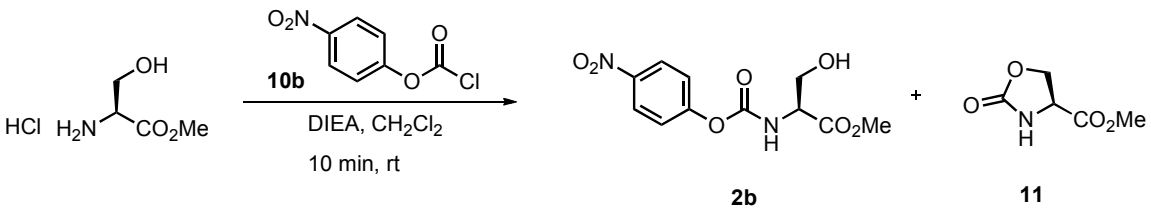


1b

Carbamate 1b. To H_2N-Ala-OMe hydrochloride (208 mg, 1.5 mmol) in CH_2Cl_2 (10 mL) was added DIEA (402 µL, 299 mg, 2.3 mmol), and *p*-nitrophenyl chloroformate **10b** (1.5 mmol, 303 mg). The reaction mixture was stirred at room temperature for 30 min, and then partitioned between CH_2Cl_2 (50 mL) and saturated NH_4Cl (50 mL). The organic layer was washed one more time with saturated NH_4Cl (30 mL), and dried over $MgSO_4$. The solvent was removed by rotary evaporation and the crude product was purified using flash chromatography (1:1 EtOAc/hexanes) to provide compound **1b** (230 mg, 0.83 mmol, 55%) as a white solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.52-1.56 (d, J = 7.2 Hz, 3H), 3.83 (s, 3H), 4.40-4.52 (m, 1H), 5.70-5.80 (d, J = 6.6 Hz, 1H), 7.30-7.40 (d, J = 9.0 Hz, 2H), 8.20-8.30 (d, J = 9.3 Hz, 2H); HRMS-EI (M + H^+) calcd for $C_{11}H_{13}N_2O_6$ 268.0695, found 268.0701.



Carbamate 2a. To a solution of triphosgene (99 mg, 0.33 mmol) in CH_2Cl_2 (10 mL), was slowly added 7-hydroxycoumarin (162 mg, 1.0 mmol) and DIEA (176 μL, 130 mg, 1.0 mmol) as a solution in THF (10 mL). The reaction was stirred at room temperature for 30 min to generate the intermediate chloroformate **10a**. In a separate flask containing CH_2Cl_2 (10 mL), H_2N-Ser-OMe hydrochloride (232 mg, 1.5 mmol) was mixed with DIEA (263 μL, 195 mg, 1.5 mmol). The mixture was stirred for 5 min at room temperature and then dropped into the flask containing chloroformate **10a**. The reaction mixture was stirred at room temperature for another 10 min and partitioned between CH_2Cl_2 (50 mL) and saturated NH_4Cl (50 mL). The organic layer was washed one more time with saturated NH_4Cl (30 mL), and dried over $MgSO_4$. The solvent was removed by rotary evaporation and purified using flash chromatography[2] to provide a 2:1 mixture of compound **2a** and compound **11**. Compound **2a**: ^{1}H NMR (300 MHz, D_2O) δ 3.74 (s, 3H), 3.85-3.95 (m, 2H), 4.35-4.45 (m, 1H), 6.37-6.48 (d, J = 9.9 Hz, 1H), 7.10-7.19 (dd, J = 3.0, 9.0 Hz, 1H), 7.20-7.26 (d, J = 3.0 Hz, 1H), 7.60-7.70 (d, J = 9.0 Hz, 1H), 7.95-8.05 (d, J = 9.0 Hz, 1H); MS-EI ($M + H^+$) calcd for $C_{14}H_{14}NO_7$ 308, found 308. Compound **11**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.81 (s, 3H), 4.40-4.50 (dd, J = 6.0, 9.0 Hz, 1H), 4.51-4.58 (dd, J = 6.0, 9.0 Hz, 1H), 4.59-4.68 (m, 1H); EI ($M + H^+$) calcd for $C_5H_8NO_4$ 146, found 146.



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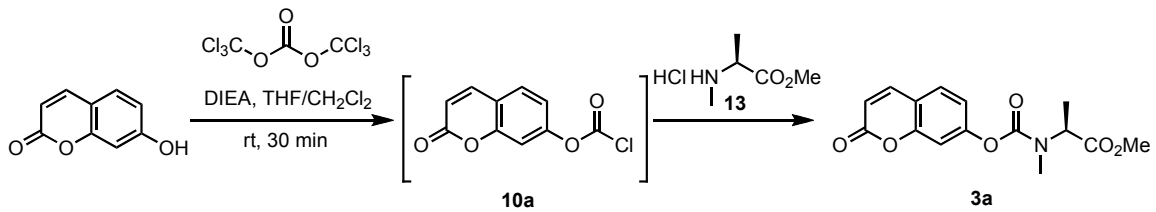
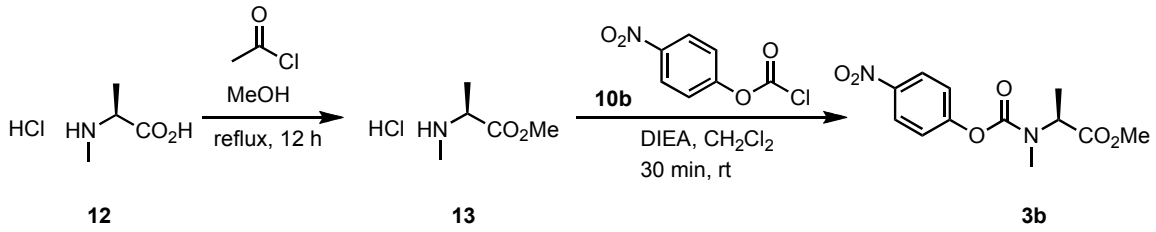
Carbamate 2b. To H_2N-Ser-OMe hydrochloride (232 mg, 1.5 mmol) in CH_2Cl_2 (10 mL) was added DIEA (402 μL, 299 mg, 2.3 mmol), and *p*-nitrophenyl chloroformate (1.5 mmol, 303 mg). The reaction mixture was stirred at room temperature for 10 min, and then partitioned between CH_2Cl_2 (50 mL) and saturated NH_4Cl (50 mL). The organic layer was washed one more time with saturated NH_4Cl (30 mL), and dried over $MgSO_4$. The solvent was removed by rotary evaporation and purified using flash chromatography[2] (1:1 to 4:1 EtOAc/hexanes) to provide a 1:2 mixture of compound **11** and compound **2b**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.85 (s, 3H), 3.95-4.07 (dd, *J* = 3.0, 12.0 Hz, 1H), 4.08-4.20 (dd, *J* = 3.0, 12.0 Hz, 1H), 4.48-4.58 (m, 1H), 6.15-6.30 (d, *J* = 6.0 Hz, 1H), 7.30-7.40 (d, *J* = 9.0 Hz, 2H), 8.20-8.30 (d, *J* = 9.3 Hz, 2H).



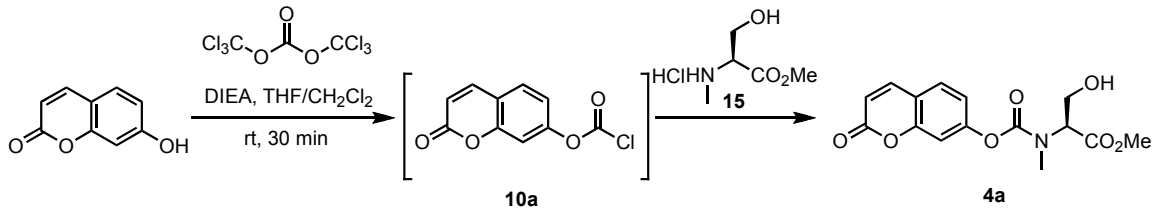
Carbamate 3a. To a solution of triphosgene (99 mg, 0.33 mmol) in CH_2Cl_2 (10 mL), was slowly added 7-hydroxycoumarin (162 mg, 1.0 mmol) and DIEA (176 μL, 130 mg, 1.0 mmol) as a solution in THF (10 mL). The reaction was stirred at room temperature for 30 min to generate the intermediate chloroformate **10a**. In a separate flask containing CH_2Cl_2 (10 mL), *N*-methylalanine methyl ester hydrochloride **13**[3] (176 mg, 1.5 mmol) was mixed with DIEA (263 μL, 195 mg, 1.5 mmol). The mixture was stirred for 5 min at room temperature and then dropped into the flask containing chloroformate **10a**. The reaction mixture was stirred at room temperature for another 30 min then was loaded directly onto a flash chromatography column. The product was eluted with 1:1 EtOAc/hexanes to afford compound **3a** (268 mg, 0.88 mmol, 88%) as a 3:2 mixture of *cis/trans* conformers: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.51-1.54 (d, J = 9.0, 1.8H), 1.56-1.59 (d, J = 9.0, 1.2H), 3.01 (s, 1.2H), 3.09 (s, 1.8H), 3.78 (s, 2H), 3.80 (s, 1H), 4.83-4.92 (m, 1H), 6.37-6.40 (d, J = 9.0, 1H), 7.00-7.20 (m, 2H), 7.46-7.49 (d, J = 9.0, 1H), 7.68-7.71 (d, J = 9.0, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 9.8, 10.4, 26.2, 26.5, 47.4, 47.6, 49.6, 50.1, 105.4, 110.8, 111.3, 111.4, 113.49, 113.53, 123.37, 123.43, 137.9, 148.6, 148.8, 149.0, 149.2, 149.7, 155.5, 166.8, 166.9; HRMS-FAB ($M + Na^+$) calcd for $C_{15}H_{15}NaNO_6$ 328.0797, found 328.0790.



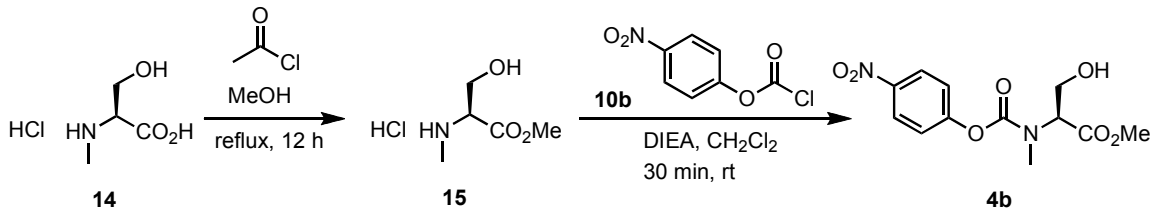
12 **13** **3b**

Carbamate 3b. To a solution of *N*-methylalanine hydrochloride **12b** (500 mg, 3.60 mmol) in MeOH (30 mL) at 0 ºC, was added acetyl chloride (2.5 mL, 2.76 g, 36 mmol). The reaction was heated under reflux for 12 hr and then cooled to room temperature. The solvent was removed by rotary evaporation and the resulting oil was dried under high vacuum overnight to yield the methyl ester hydrochloride **13**[3] (550 mg, 3.6 mmol, 99%).

To a solution of methyl ester hydrochloride **13** (190 mg, 1.24 mmol) in CH_2Cl_2 (5 mL) was added DIEA (430 µL, 322 mg, 2.48 mmol), and *p*-nitrophenyl chloroformate **10b** (250 mg, 1.24 mmol). The reaction was allowed to stir at room temperature for 30 min, and then was loaded directly onto a flash chromatography column. The product was eluted with 1:1 EtOAc/hexanes to afford compound **3b** (311 mg, 1.1 mmol, 90%) as a 3:2 mixture of *cis/trans* conformers: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.52-1.54 (d, J = 8.0, 1.8H), 1.57-1.59 (d, J = 8.0, 1.2H), 3.02 (s, 1.2H), 3.10 (s, 1.8H), 3.78 (s, 3H), 4.83-4.87 (dd, J = 8.0, 15.0 Hz, 0.4H), 4.87-4.93 (dd, J = 8.0, 16.0 Hz, 0.6H), 7.28-7.30 (d, J = 8.0, 0.8H), 7.33-7.35 (d, J = 8.0, 1.2H), 8.22-8.38 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.6, 15.3, 31.0, 31.4, 52.4, 52.5, 54.6, 55.1, 115.4, 122.18, 122.22, 124.99, 125.03, 126.0, 144.85, 144.91, 153.2, 153.7, 155.9, 156.1, 171.6, 171.7; HRMS-FAB (M + H^+) calcd for $C_{12}H_{15}N_2O_6$ 283.0930, found 283.0936.

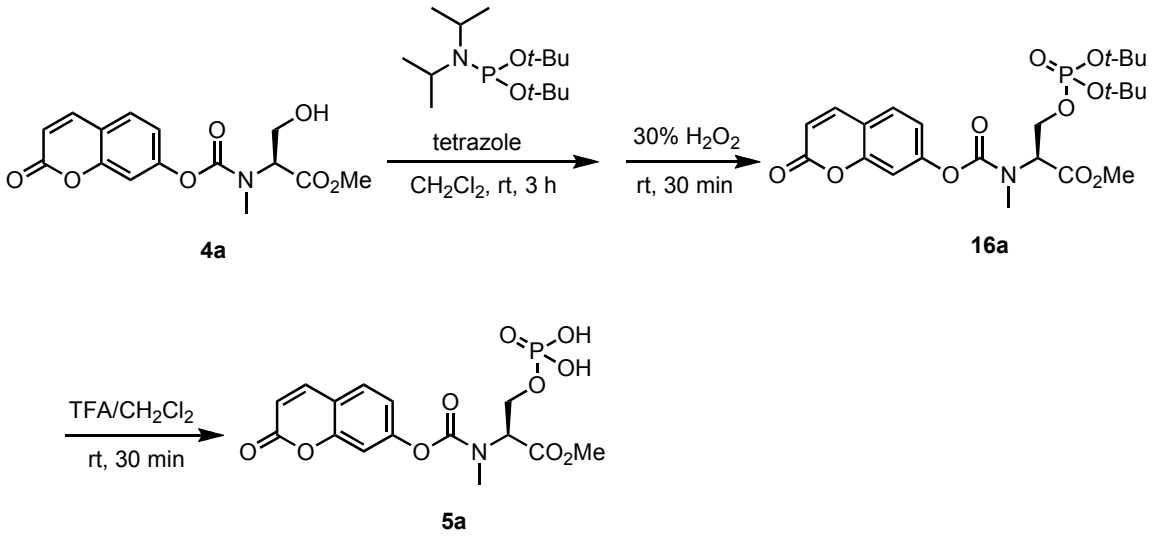


Carbamate 4a. To a solution of triphosgene (99 mg, 0.33 mmol) in CH_2Cl_2 (10 mL), was slowly added 7-hydroxycoumarin (162 mg, 1.0 mmol) and DIEA (176 μL, 130 mg, 1.0 mmol) as a solution in THF (10 mL). The reaction was stirred at room temperature for 30 min to generate the intermediate chloroformate **10a**. In a separate flask containing CH_2Cl_2 (10 mL), *N*-methylalanine methyl ester hydrochloride **15**[3] (200 mg, 1.5 mmol) was mixed with DIEA (263 μL, 195 mg, 1.5 mmol). The mixture was stirred for 5 min at room temperature and then dropped into the flask containing chloroformate **10a**. The reaction mixture was stirred at room temperature for another 30 min, then was loaded directly onto a flash chromatography column. The product was eluted with 2:1-4:1 EtOAc/hexanes to afford compound **4a** (280 mg, 0.9 mmol, 90%) as a 1:1 mixture of *cis/trans* conformers: ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.54-2.58 (dd, J = 8.0, 10.0 Hz, 0.33H), 2.64-2.68 (dd, J = 4.0, 8.0 Hz, 0.66H), 3.09 (s, 1H), 3.20 (s, 2H), 3.82 (s, 2H), 3.84 (s, 1H), 3.95-4.10 (m, 1H), 4.11-4.25 (m, 1H), 4.67-4.70 (dd, J = 6.0, 8.0 Hz, 0.66H), 4.72-4.77 (dd, J = 8.0, 12.0 Hz, 0.33H), 6.38-6.41 (d, J = 8.0 Hz, 1H), 7.00-7.25 (m, 2H), 7.46-7.50 (m, 1H), 7.65-7.75 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 33.6, 34.0, 52.7, 52.8, 60.9, 61.9, 62.2, 110.3, 110.4, 115.9, 116.4, 116.5, 118.4, 118.5, 128.5, 128.6, 143.0, 153.7, 153.8, 154.7, 160.6, 169.9, 170.2; HRMS-FAB (M + Na^+) calcd for $C_{15}H_{15}NaNO_7$ 344.0746, found 344.0741.



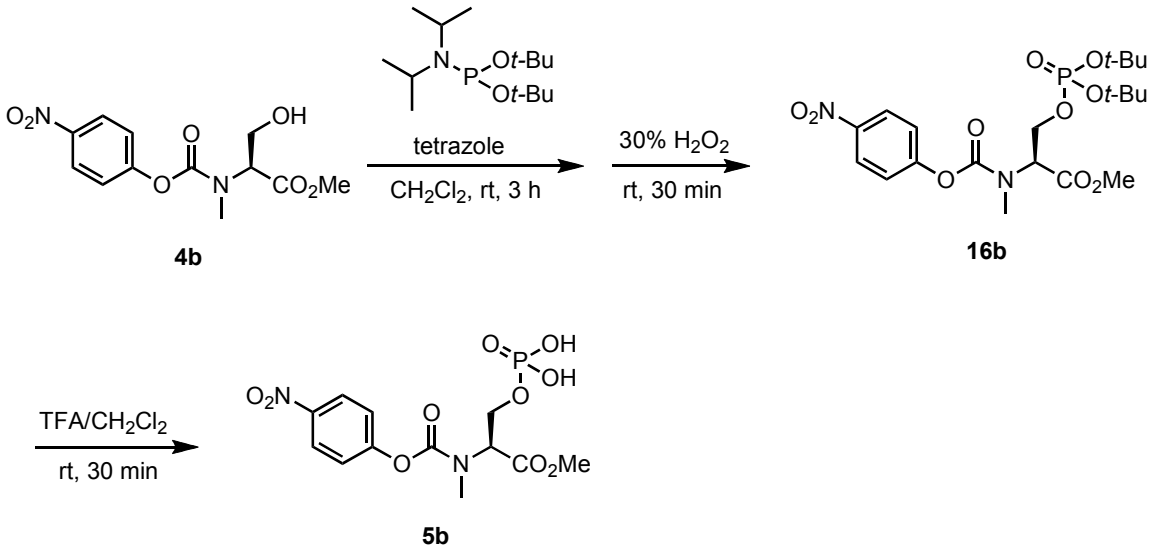
Carbamate 4b. To a solution of *N*-methylserine hydrochloride **14** (500 mg, 3.2 mmol) in MeOH (30 mL) at 0 ºC, was added acetyl chloride (2.3 mL, 2.5 g, 32.2 mmol). The reaction was heated under reflux for 12 hr and then cooled to room temperature. The solvent was removed by rotary evaporation, and the crude product was recrystalized in acetone to yield the methyl ester hydrochloride **15**[3] (506 mg, 2.98 mmol, 93%) as a white solid.

To a solution of methyl ester hydrochloride **15** (50 mg, 0.30 mmol) in CH_2Cl_2 (5 mL) was added DIEA (105 µL, 78 mg, 0.60 mmol), and *p*-nitrophenyl chloroformate **10b** (60 mg, 0.30 mmol). The reaction was allowed to stir at room temperature for 30 min, and then was loaded directly onto a flash chromatography column. The product was eluted with 2:1-4:1 EtOAc/hexanes to afford compound **4b** (81 mg, 0.27 mmol, 90%) as a mixture of 2:1 *cis/trans* conformers: ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.59 (br s, 0.4 H), 2.70 (br s, 0.6H), 3.07 (s, 1H), 3.18 (s, 2H), 3.81 (s, 3H), 3.90-4.10 (m, 1H), 4.11-4.20 (m, 1H), 4.60-4.70 (dd, *J* = 8.0, 10.0 Hz, 0.6H), 4.71-4.80 (dd, *J* = 8.0, 9.0 Hz, 0.6H), 7.27-7.28 (d, *J* = 4.0, 0.8H), 7.33-7.35 (d, *J* = 8.0, 1.2H), 8.20-8.30 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 33.2, 33.6, 52.6, 62.7, 60.5, 61.7, 61.9, 122.1, 122.2, 124.99, 125.04, 144.9, 153.3, 154.2, 155.7, 155.9, 169.6, 169.8; HRMS-FAB (M + Na^+) calcd for $C_{12}H_{14}NaN_2O_7$ 321.0699, found 321.0690.



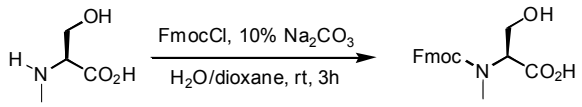
Carbamate 5a. The synthesis of compound **5a** began with compound **4a**.[4] To a solution of compound **4a** (175 mg, 0.55 mmol) in CH_2Cl_2 (15 mL) were added 1H-tetrazole (3.6 mL, 0.45 M solution in CH_3CN, 1.64 mmol) and di-*t*-butyl diisopropylphosphoramidite (214 μL, 0.68 mmol). The reaction mixture was stirred at room temperature for additional 3 h. The reaction solution was then cooled to 0 ºC, followed by the addition of 30% H_2O_2 (220 μL, 2.2 mmol). The reaction mixture was slowly warm to room temperature over 30 min, and saturated Na_2SO_3 (10 mL) was added. After stirring vigorously for 30 min, the mixture was partitioned between CH_2Cl_2 (50 mL) and saturated Na_2SO_3 (40 mL). The organic layer was washed with Na_2SO_3 (2 × 50 mL), and dried over $MgSO_4$. The solvent was removed by rotary evaporation. The crude material was purified by flash chromatography (4:1 EtOAc/hexanes) to yield compound **16a**. The resulting compound **16a** was dissolved in 1:3 TFA/CH_2Cl_2 (5 mL) at room temperature. The reaction was allowed to stir for 20 min and the solvent was removed by rotary evaporation. The crude product was purified by flash chromatography (10% MeOH in CH_2Cl_2) to afford compound **5a** (120 mg, 0.3 mmol, 55%) as a 3:2 mixture of *cis*/*trans* conformers: ^{1}H NMR (400 MHz, CD_3OD) δ 3.08-3.09 (d, J = 4.0 Hz, 1.2H),

3.22-3.22 (d, J = 8.0 Hz, 1.9H), 3.70-3.79 (m, 3H), 4.25-4.55 (m, 2H), 4.91-4.99 (m, 0.6H), 5.00-5.10 (m, 0.4H), 6.30-6.45 (m, 1H), 7.00-7.20 (m, 2H), 7.50-7.65 (m, 1H), 7.82-7.98 (m, 1H); ^{13}C NMR (100 MHz, CD_3OD) δ 30.5, 34.0, 24.3, 53.1, 53.3, 62.1, 63.8, 64.1, 111.1, 116.3, 116.4, 117.9, 118.0, 119.7, 130.1, 130.3, 144.99, 145.04, 155.0, 155.2, 155.4, 155.6, 155.7, 155.8, 156.0, 162.2, 162.3, 170.3, 170.4, 170.5; MALDI-TOF ($M + H^+$) calcd for $C_{12}H_{15}N_2O_{10}P$ 402.1, found 402.3; LC-MS ($M - H^+$) calcd for $C_{12}H_{13}N_2O_{10}P$ 400, found 400.



Carbamate 5b. The synthesis of compound **5b** began with compound **4b**.[4] To a solution of compound **4b** (215 mg, 0.72 mmol) in CH_2Cl_2 (15 mL) were added 1H-tetrazole (4.8 mL, 0.45 M solution in CH_3CN, 2.2 mmol) and di-*t*-butyl diisopropylphosphoramidite (290 μL, 0.90 mmol). The reaction mixture was stirred at room temperature for additional 3 h. The reaction solution was then cooled to 0 ºC, followed by the addition of 30% H_2O_2 (270 μL, 2.9 mmol). The reaction mixture was slowly warm to room temperature over 30 min, and saturated Na_2SO_3 (5 mL) was added. After stirring vigorously for 30 min, the mixture was partitioned between CH_2Cl_2 (50 mL) and saturated Na_2SO_3 (50 mL). The organic layer was washed with Na_2SO_3 (2 × 50 mL), and dried over $MgSO_4$. The solvent was removed by rotary evaporation. The crude material was purified by flash chromatography (4:1 EtOAc/hexanes) to yield compound **16b**. The resulting compound **16b** was dissolved in 1:3 TFA/CH_2Cl_2 (10 mL) at room temperature. The reaction was allowed to stir for 20 min and the solvent was removed by rotary evaporation. The crude product was purified by flash chromatography (10% MeOH in CH_2Cl_2) to afford compound **5b** (163 mg, 0.43 mmol, 60%) as a 3:2 mixture of *cis*/*trans* conformers: ^{1}H NMR (400 MHz, CD_3OD) δ 3.07-3.08 (d, J = 4.0 Hz, 1.2H),

3.21-3.22 (d, J = 4.0 Hz, 1.8H), 3.75-3.79 (m, 3H), 4.31-4.37 (d, J = 8.0, 15.0 Hz, 1H), 4.39-4.48 (m, 1H), 4.91-4.99 (m, 0.6H), 5.10-5.16 (m, 0.4H), 7.33-7.39 (m, 2H), 8.21-8.26 (m, 2H); ^{13}C NMR (100 MHz, CD_3OD) δ 34.3, 34.4, 53.1, 53.2, 62.1, 63.8, 64.0, 123.7, 126.1, 146.5, 155.2, 155.7, 157.3, 157.4, 170.3, 170.4; MALDI-TOF ($M + H^+$) calcd for $C_{12}H_{15}N_2O_{10}P$ 379.1, found 379.5; LC-MS ($M - H^+$) calcd for $C_{12}H_{13}N_2O_{10}P$ 377, found 377.



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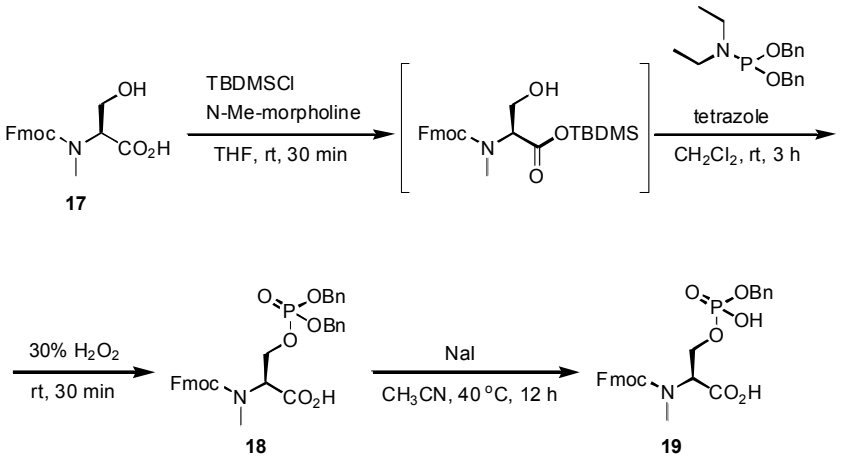
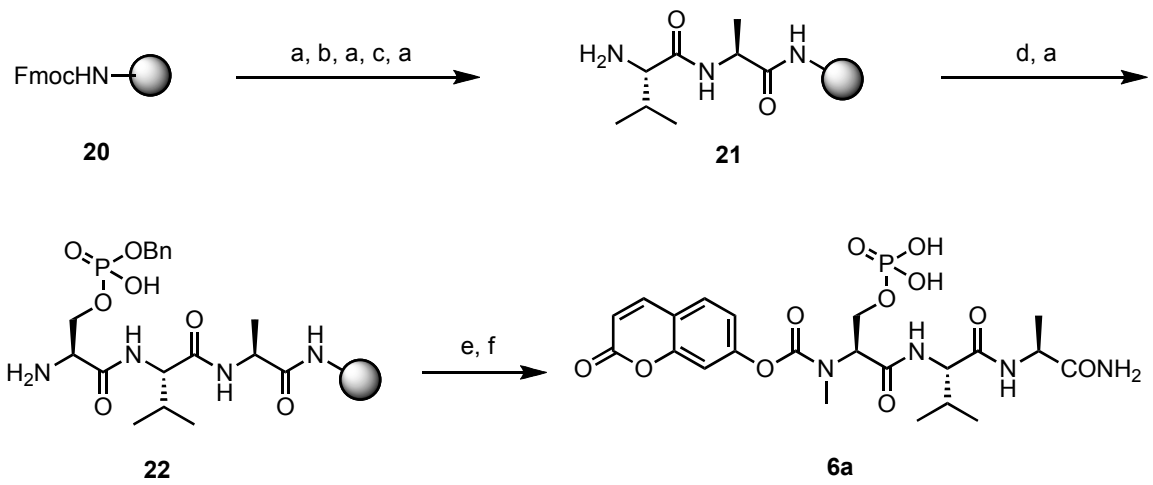
Fmoc-*N*-Me-Ser-OH 17. To a solution of *N*-Methyl-Ser-OH hydrochloride (340 mg, 2.0 mmol) in THF (10 mL) and 10% Na_2CO_3 (10 mL) was added FmocCl (516 mg, 2.0 mmol).[5] The reaction was stirred at room temperature for 3h, and then partitioned between ether (150 mL) and H_2O (200 mL). After it was washed one more time with ether (150 mL), the aqueous layer was adjusted to pH 2 with 12 N HCl. The resulting suspension was extracted with EtOAc (3 × 150 mL). The combined organic layers were dried over $MgSO_4$. The solvent was removed by rotary evaporation to yield compound **17** (666 mg, 1.9 mmol, 95%) as a 2:1 mixture of *cis*/*trans* conformers: ^{1}H NMR (400 MHz, CD_3OD) δ 2.98 (s, 2H), 2.99 (s, 1H), 3.80-4.00 (m, 2H), 4.20-4.30 (m, 1H), 4.31-4.45 (m, 2H), 4.70-4.74 (m, 1H), 7.25-7.35 (m, 2H), 7.36-7.45 (m, 2H), 7.60-7.70 (m, 2H), 7.75-7.85 (m, 2H); ^{13}C NMR (100 MHz, CD_3OD) δ 32.1, 32.5, 60.5, 60.6, 62.5, 62.8, 69.1, 69.3, 120.9, 126.1, 128.2, 128.8, 142.5, 142.6, 145.17, 145.24, 158.4, 158.8, 172.5, 172.6; HRMS-FAB ($M + Na^+$) calcd for $C_{19}H_{19}NaNO_5$ 364.1161, found 364.1168.



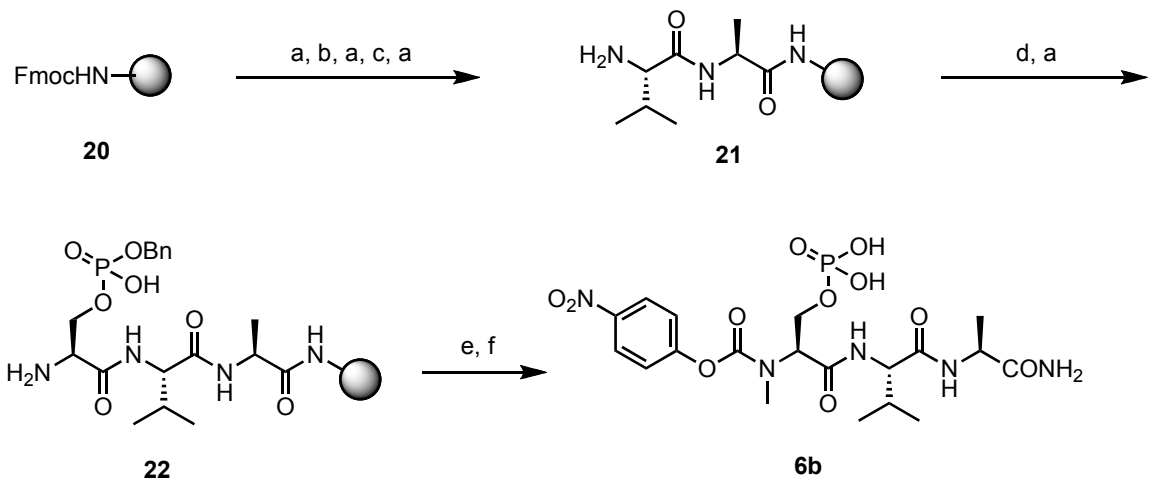
Fmoc-*N*-Me-Ser(PO(OBn)OH)-OH 19. To a solution of compound **17** (2.3 g, 6.8 mmol) in THF (15 mL) were added *N*-methyl-morpholine (480 mL, 6.8 mmol, dissolved in 4 mL of THF) and TBDMSCl (980 mg, 6.8 mmol, dissolved in 5 mL of THF). After stirring the reaction mixture for 30 min, 1H-tetrazole (70 mL, 0.45 M solution in CH_3CN, 32 mmol) and dibenzyl diethylphosphoramidite (4.0 mL, 14 mmol) were added.[6] The reaction mixture was stirred at room temperature for an additional 3 h, cooled to 0 ºC, and then 30% H_2O_2 (1.8 mL, 18 mmol) was added. The reaction mixture was slowly warm to room temperature over 30 min; saturated Na_2SO_3 (45 mL) was then added. After stirring vigorously for 30 min, the mixture was partitioned between ether (300 mL) and saturated Na_2SO_3 (200 mL). The organic layer was washed with Na_2SO_3 (2 × 150 mL), and then extracted with 5% $NaHCO_3$ (3 × 150 mL). The pH value of the combined aqueous layers was adjusted to 3.0 with 2 N HCl, and the resulting suspension was extracted with CH_2Cl_2 (3 × 150 mL). The combined organic layers were dried over $MgSO_4$. The solvent was removed to yield compound **18** (2.9 g, 4.8 mmol, 72%): LC-MS (M + Na^+) calcd for $C_{33}H_{32}NaNO_8P$ 624, found 624.

Compound **18** was dissolved in CH_3CN (40 mL). To this solution was added NaI (1.5 g, 9.6 mmol). The reaction was stirred at 45 ºC for 12 h under an atmosphere of argon. The suspension was centrifuged and the resulting solid was washed with dry CH_3CN (4 × 15 mL) to provide the white solid **19** (2.3 g, 4.4 mmol, 91%) as a 3:2 mixture of *cis*/*trans* conformers: ^{1}H NMR (400 MHz, CD_3OD) δ 3.00 (s, 1.2H), 3.02 (s, 1.8H), 4.10-4.45 (m, 5H), 4.75-4.82 (m, 1H), 4.90-4.97 (m, 1H), 7.20-7.40 (m, 9H), 7.55-7.65 (m, 2H), 7.72-7.85 (m, 2H); ^{13}C NMR (100 MHz, CD_3OD) δ 32.2, 32.9, 61.8, 62.0, 62.1, 64.1, 68.2, 68.3, 69.1, 69.4, 120.8, 120.9, 126.2, 126.36, 126.40, 128.2, 128.3, 128.4, 128.5, 128.7, 128.8, 129.2, 139.7, 139.8, 142.5, 142.6, 145.2, 145.3, 158.4, 158.6, 173.0; MALDI-TOF (M - H^+) calcd for $C_{26}H_{25}NO_8P$ 510.1, found 509.9; LC-MS (M - H^+) calcd for $C_{26}H_{25}NO_8P$ 510, found 510.



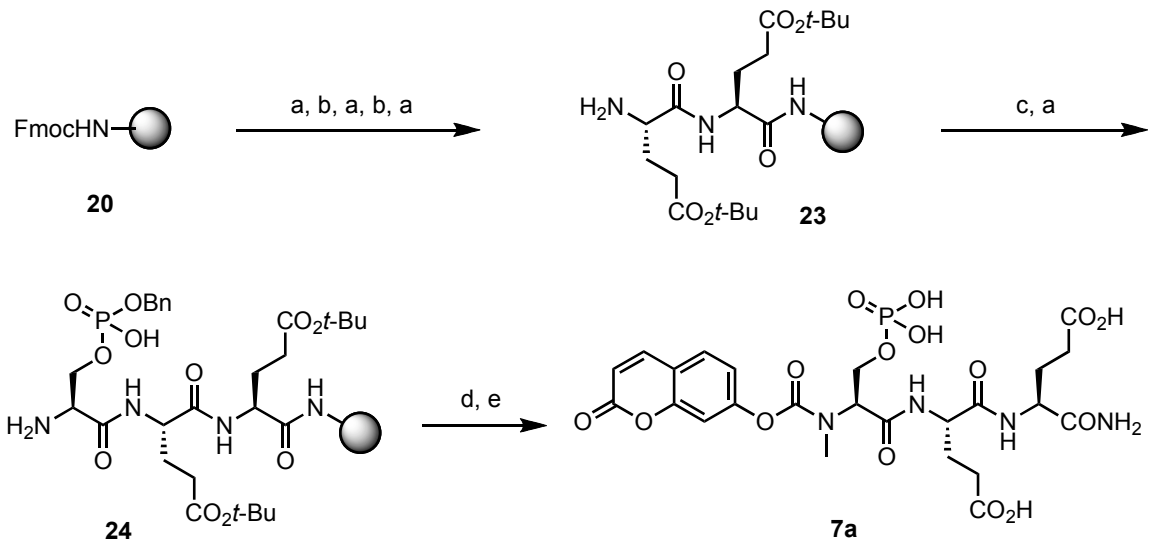
(a) piperidine/DMF (20%), 2 × 30 min; (b) Fmoc-Ala-OH, HBTU, HOBt, DIEA, DMF, 2 × 1 h; (c) Fmoc-Val-OH, HBTU, HOBt, DIEA, DMF, 2 × 1 h; (d) **19**, HBTU, HOBt, DIEA, DMF, 2 × 1 h; (e) **10a**, DIEA, CH_2Cl_2/THF (1:1); (f) TFA/TIS/H_2O (94/3/3), 30 min.

Peptide 6a. Compound **6a** was synthesized using general method described in section 1.2 (3.0 mg, 5.6 μmol, 8%) as a 3:2 mixture of *cis*/*trans* conformers: ^{1}H NMR (400 MHz, CD_3OD) δ 0.85-1.05 (m, 6H), 1.29-1.51 (m, 3H), 2.05-2.20 (m, 1H), 3.05 (s, 1.2H), 3.25 (s, 1.8H), 4.15-4.25 (m, 1H), 4.26-4.60 (m, 3H), 5.10-5.20 (m, 1H), 6.40-6.42 (d, J = 9.2 Hz, 1H), 7.10-7.25 (m, 2H), 7.60-7.70 (m, 1H), 7.95-7.97 (d, J = 9.2 Hz, 1H); MALDI-TOF (M - H^+) calcd for $C_{22}H_{28}N_4O_{11}P$ 555.2, found 555.0; LC-MS (M + Na^+) calcd for $C_{22}H_{29}NaN_4O_{11}P$ 579, found 579; RP-HPLC 15% CH_3CN (0.1% TFA) in H_2O (0.1% TFA), retention time 12.5 min.



(a) piperidine/DMF (20%), 2 × 30 min; (b) Fmoc-Ala-OH, HBTU, HOBt, DIEA, DMF, 2 × 1 h; (c) Fmoc-Val-OH, HBTU, HOBt, DIEA, DMF, 2 × 1 h; (d) **19**, HBTU, HOBt, DIEA, DMF, 2 × 1 h; (e) **10b**, DIEA, CH_2Cl_2; (f) TFA/TIS/H_2O (94/3/3), 30 min.

Peptide 6b. Compound **6b** was synthesized general method described in section 1.2 (3.7 mg, 7.0 μmol, 10% calculated based on the amount of resin) as a mixture of *cis*/*trans* conformers: ^{1}H NMR (400 MHz, CD_3OD) δ 0.94-1.05 (m, 6H), 1.29-1.31 (d, J = 7.2 Hz, 1.8H), 1.35-1.37 (d, J = 6.8 Hz, 1.2H), 2.05-2.20 (m, 1H), 3.12 (s, 1.2H), 3.26 (s, 1.8H), 4.10-4.55 (m, 4H), 7.40-7.50 (d, J = 8.8 Hz, 2H), 8.20-8.30 (d, J = 8.0 Hz, 2H); MALDI-TOF (M - H^+) calcd for $C_{19}H_{27}N_5O_{11}P$ 532.1, found 531.9; LC-MS (M + Na^+) calcd for $C_{19}H_{28}NaN_5O_{11}P$ 556, found 556; RP-HPLC 16% CH_3CN (0.1% TFA) in H_2O (0.1% TFA), retention time 16.3 min.



(a) piperidine/DMF (20%), 2 × 30 min; (b) Fmoc-Glu(O*t*Bu)-OH, HBTU, HOBt, DIEA, DMF, 2 × 1 h; (c) **19**, HBTU, HOBt, DIEA, DMF, 2 × 1 h; (d) **10a**, DIEA, CH_2Cl_2/THF (1:1); (e) TFA/TIS/H_2O (94/3/3), 30 min.

Peptide 7a. Compound **7a** was synthesized general method described in section 1.2 (3.0 mg, 5.6 μmol, 8% calculated based on the amount of resin) as a mixture of *cis*/*trans* conformers: ^{1}H NMR (400 MHz, CD_3OD) δ 1.85-2.05 (m, 2H), 2.10-2.30 (m, 2H), 2.35-2.50 (m, 4H), 3.10 (s, 1.6H), 3.26 (s, 1.4 H), 4.30-4.46 (m, 3H), 4.47-4.60 (m, 1H), 4.95-5.20 (m, 1H), 6.40-6.42 (d, J = 9.2 Hz, 1H), 7.10-7.25 (m, 2H), 7.60-7.70 (m, 1H), 7.95-7.97 (d, J = 9.2 Hz, 1H); MALDI-TOF (M - H^+) calcd for $C_{22}H_{28}N_4O_{11}P$ 555.2, found 555.0; LC-MS (M + Na^+) calcd for $C_{22}H_{29}NaN_4O_{11}P$ 579, found 579; RP-HPLC 14% CH_3CN (0.1% TFA) in H_2O (0.1% TFA), retention time 9.3 min.

2. Enzyme Assays and Kinetic Studies.

2.1. Standard Fluorescence and Absorbance Curves.

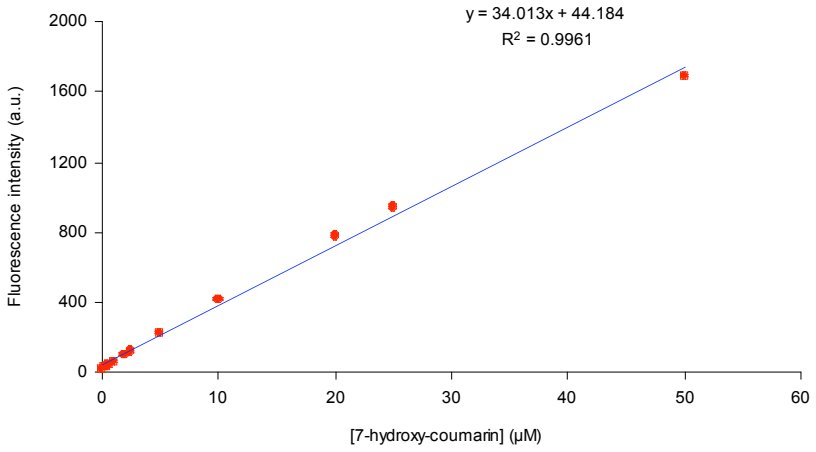


Figure S1. Standard fluorescence curve of umbelliferone at different concentrations.

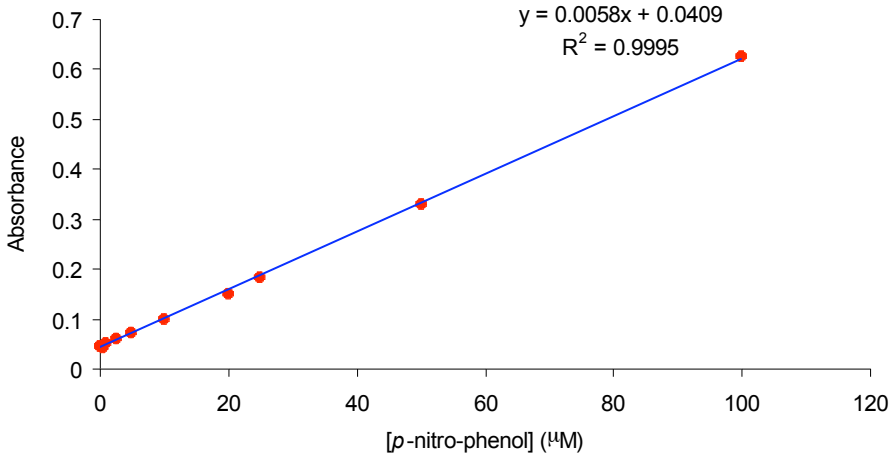


Figure S2. Standard absorbance curve of *p*-nitrophenol at different concentrations.

2.2. Optimization of the pH Value of Phosphatase Assays.

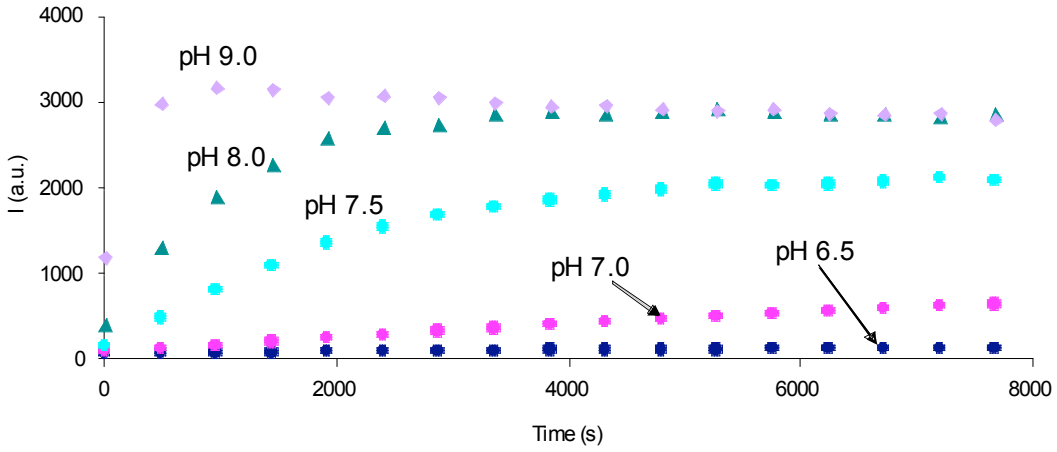


Figure S3. Time courses of cyclization reactions of **4a** under different pH values. The initial concentration of **4a** for each measurement was 100 μM. The increase in fluorescence intensity (I, λ_{em} = 460 nm) was recorded. pH 6.5 (10 mM phosphate buffer), pH 7.0 (10 mM phosphate buffer), pH 7.5 (10 mM tris buffer), pH 8.0 (10 mM tris buffer), pH 9.0 (10 mM tris buffer).

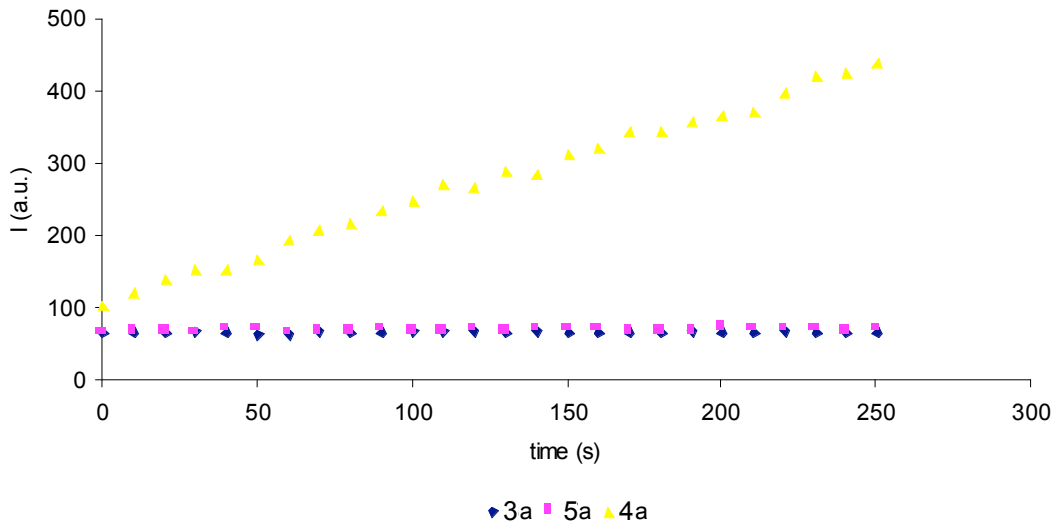


Figure S4. Generation of fluorescence intensity (I, λ_{em} = 460 nm) from compounds **3a**, **4a** and **5a** in 10 mM tris buffer (pH 8.0). The initial concentrations of **3a**, **4a** and **5a** were 100 μM. Based on these results, the initial cyclization rates of compound **3a**, **4a** and **5a** were measured to be 0.3 nM·s^{-1}, 115 nM s^{-1} and 0.5 nM·s^{-1}, respectively.

Table S1. Enzyme concentrations used in different substrate assays.

enzyme	**5a**	**5b**	**6a**	**6b**	**7a**
ALP (μM)	0.016	0.016	0.0033	0.0016	0.0054
λ-PPase (μM)	2.0	2.0	1.0	1.0	1.0
VHR (μM)	1.0	1.0	0.20	0.20	0.20

2.3. Alkaline Phosphatase (ALP) Assay

ALP was purchased from Sigma (P6774, ALP from bovine intestinal mucosa) and used as received in all assays. The activity of ALP was measured in 384-well microtiter plates with compounds **5a-b**, **6a-b** or **7a** as substrates. The concentration of ALP stock solution was determined by Bradford Protein Assay.[7] The amount of ALP used in each assay was optimized as shown in Figure S5 and the final concentration of ALP for each measurement is listed in Table S1. The assays were run in duplicate or triplicate. The enzymatic reaction mixture (50 μL) consisted of 10 mM Tris buffer (pH 8.0) and 1 mM $MgCl_2$. Substrates were added as solutions in DMSO. The overall concentration of DMSO was 10% for each measurement. The enzymatic reaction was run at 37 ºC and initiated by the addition of substrates. For fluorogenic substrates **5a**, **6a** and **7a**, the fluorescence emission generated from the reaction was measured using a Galaxy microplate reader (FLUOStar, BMG Labtechnologies) with an excitation filter of 340 nm and an emission filter of 460 nm. For phenolic substates **5a**, **6a** and **7a**, the absorbance at 405 nm was measured using the same plate reader. The fluorescence intensities were converted to units of product formation using a standard umbelliferone (Figure S1) or *p*-nitrophenol curve (Figure S2). The K_m and V_{max} values of different substrates were calculated using KaleidaGraph and are listed in Table S2.

Table S2. K_m and V_{max} measurements of substrates **5a-b**, **6a-b** and **7a** for ALP.

substrate	K_m (μM)		V_{max} (μM)	
	1	2	1	2
5a	340 ± 96	420 ± 95	2.6 ± 0.3	2.8 ± 0.3
5b	830 ± 450	810 ± 350	10 ± 3	10 ± 2
6a	38 ± 8	41 ± 14	18 ± 1	18 ± 3
6b	360 ± 92	440 ± 140	12 ± 1	13 ± 2
7a	59 ± 18	80 ± 20	13 ± 1	14 ± 2

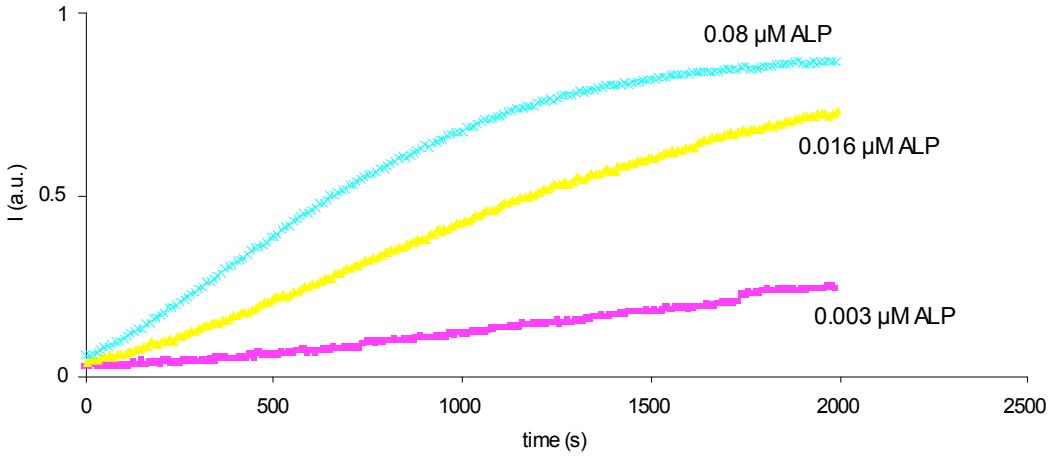


Figure S5. Increase in fluorescence intensity (I, λ_{em} = 460 nm) for the treatment of compound **6a** at the indicated concentration of ALP at 37ºC in 10 mM tris buffer (pH 8.0).

2.4. Dual Specificity Phosphatase VHR Assay

Dual specificity phosphatase VHR was purchased from Biomol International (SE333) and used as received in all assays. The activity of VHR was measured in 384-well microtiter plates with compounds **5a-b**, **6a-b** or **7a** as substrates. The concentration of the VHR stock solution was determined by Bradford Assay.[7] The amount of VHR used in each assay was optimized as shown in Figure S6 and the final concentration of VHR for each measurement was listed in Table S1. The assays were run in duplicate or triplicate. The enzymatic reaction mixture (50 μL) consisted of 10 mM Tris buffer (pH 8.0), 1 mM dithiothreitol (DTT), 1 mM ethylenediaminetetraacetic acid (EDTA) and 0.033% bovine serum albumin (BSA). Substrates were added as solutions in DMSO. The overall concentration of DMSO was 10% for each measurement. The enzymatic reactions were run at 30 ºC and initiated by the addition of substrates. For fluorogenic substrates **5a**, **6a** and **7a**, the fluorescence emission generated from the reaction was measured using a Galaxy microplate reader (FLUOStar, BMG Labtechnologies) with an excitation filter of 340 nm and an emission filter of 460 nm. For phenolic substates **5a**, **6a** and **7a**, the absorbance at 405 nm was measured using the same plate reader. The fluorescence intensities were converted to units of product formation using a standard umbelliferone (Figure S1) or *p*-nitrophenol curve (Figure S2). The K_m and V_{max} values of different substrates were calculated using KaleidaGraph.

Table S3. K_m and V_{max} measurements of substrates **5a-b**, **6a-b** and **7a** for VHR.

substrate	K_m (μM)		V_{max} (μM)	
	1	2	1	2
5a	NA*	NA*	NA*	NA*
5b	NA*	NA*	NA*	NA*
6a	200 ± 63	240 ± 92	8.2 ± 2	8.6 ± 2
6b	820 ± 220	760 ± 200	18 ± 3	16 ± 2
7a	NA**	NA**	NA**	NA**

* High enzyme loading (1.0 μM) has to be used in order to detect optical signal. The optical signal increased linearly with respect to substrate concentrations (0-2000 μM). ** No fluorescence signal was detected in an assay with 0.2 μM enzyme and 1000 μM compound **7a**.

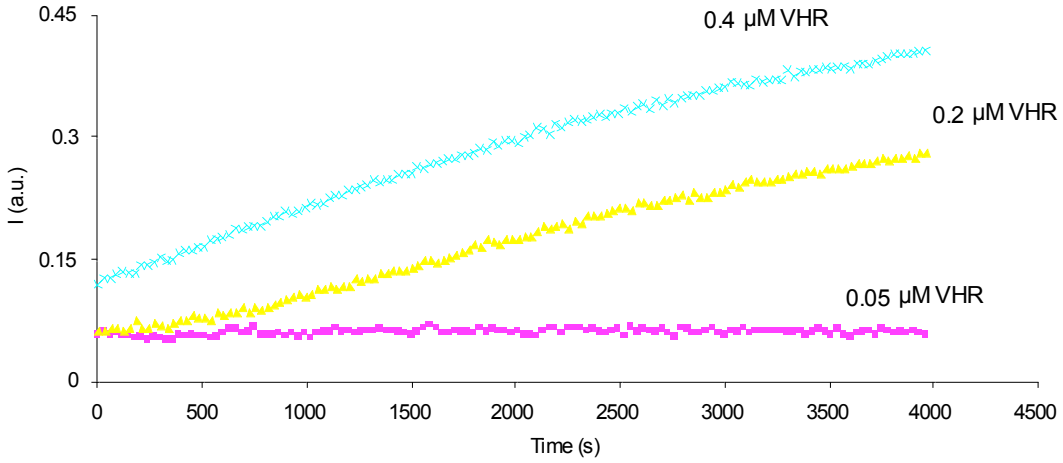


Figure S6. Increase in fluorescence intensity (I, λ_{em} = 460 nm) for the treatment of compound **6a** at the indicated concentration of VHR at 30ºC in 10 mM Tris buffer (pH 8.0).

2.5. Bacteriophage λ Protein Phosphatase (λ-PPase) Assay

λ-PPase was purchased from New England Biolabs Int. (P0753) and used as received in all assays. The activity of λ-PPase was measured in 384-well microtiter plates with compounds **5a-b**, **6a-b** or **7a** as substrates. The concentration of λ-PPase stock solution was determined by Bradford Assay.[7] The amount of VHR used in each assay was optimized as shown in Figure S7 and the final concentration of ALP for each measurement was listed in Table S1. The assays were run in duplicate or triplicate. The enzymatic reaction mixture (50 μL) consisted of 10 mM Tris buffer (pH 8.0) and 2 mM $MnCl_2$ solution. Substrates were added as solutions in DMSO. The overall concentration of DMSO was 10% for each measurement. The enzymatic reactions were run at 30 ºC and initiated by the addition of substrates. For fluorogenic substrates **5a**, **6a** and **7a**, the fluorescence emission generated from the reaction was measured using a Galaxy microplate reader (FLUOStar, BMG Labtechnologies) with an excitation filter of 340 nm and an emission filter of 460 nm. For phenolic substates **5a**, **6a** and **7a**, the absorbance at 405 nm was measured using the same plate reader. The fluorescence intensities were converted to units of product formation using a standard umbelliferone (Figure S1) or *p*-nitrophenol curve (Figure S2). The K_m and V_{max} values of different substrates were calculated using KaleidaGraph.

Table S4. K_m and V_{max} measurements of substrates **5a-b**, **6a-b** and **7a** for λ-PPase.

substrate	K_m (μM)		V_{max} (μM)	
	1	2	1	2
5a	NA*	NA*	NA*	NA*
5b	NA*	NA*	NA*	NA*
6a	130 ± 40	100 ± 30	17 ± 3	15 ± 2
6b	820 ± 310	720 ± 340	18 ± 4	19 ± 3
7a	160 ± 42	180 ± 30	3.1 ± 0.3	3.2 ± 0.2

* High enzyme loading (2.0 μM) has to be used in order to detect optical signal. The optical signal was increased linearly with respect to substrate concentrations (0-2000 μM).

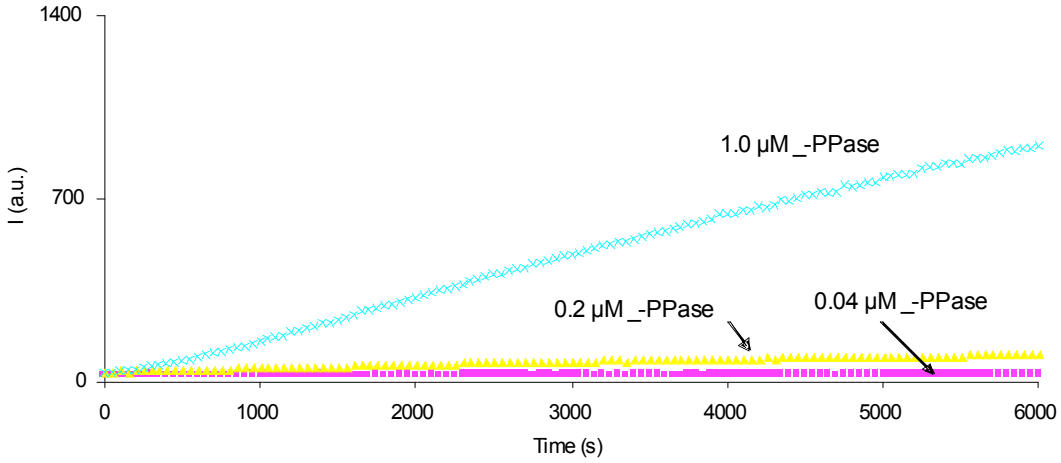


Figure S7. Increase in fluorescence intensity (I, λ_{em} = 460 nm) for the treatment of compound **6a** at the indicated concentration of λ-PPase at 30ºC in 10 mM Tris buffer (pH 8.0, 2 mM $MnCl_2$).

3. NMR Characterization Data.

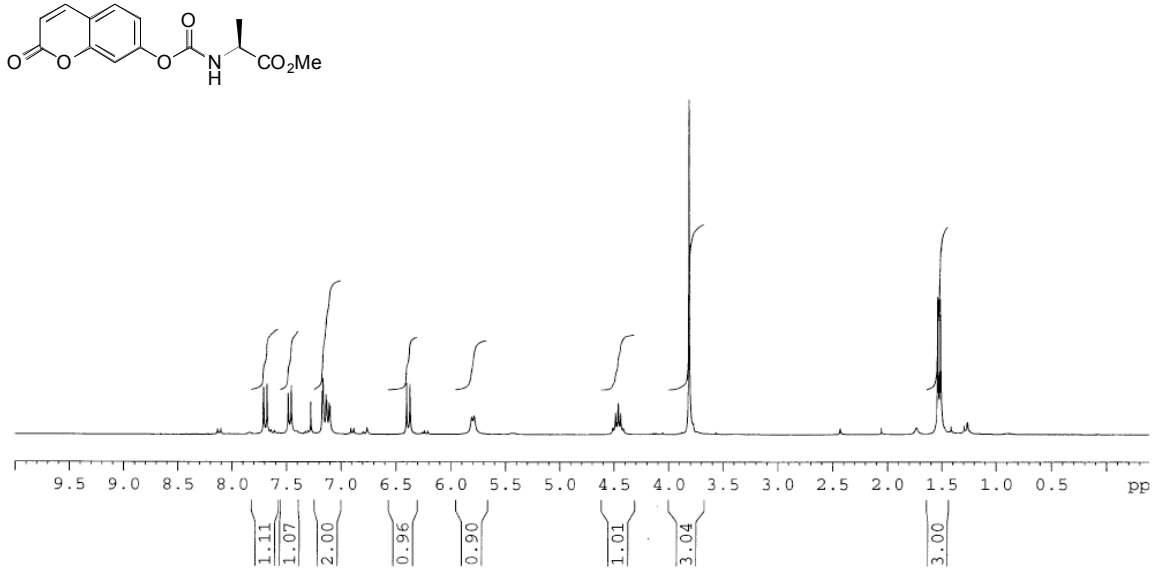


Figure S8. ^{1}H NMR spectrum for compound **1a**.

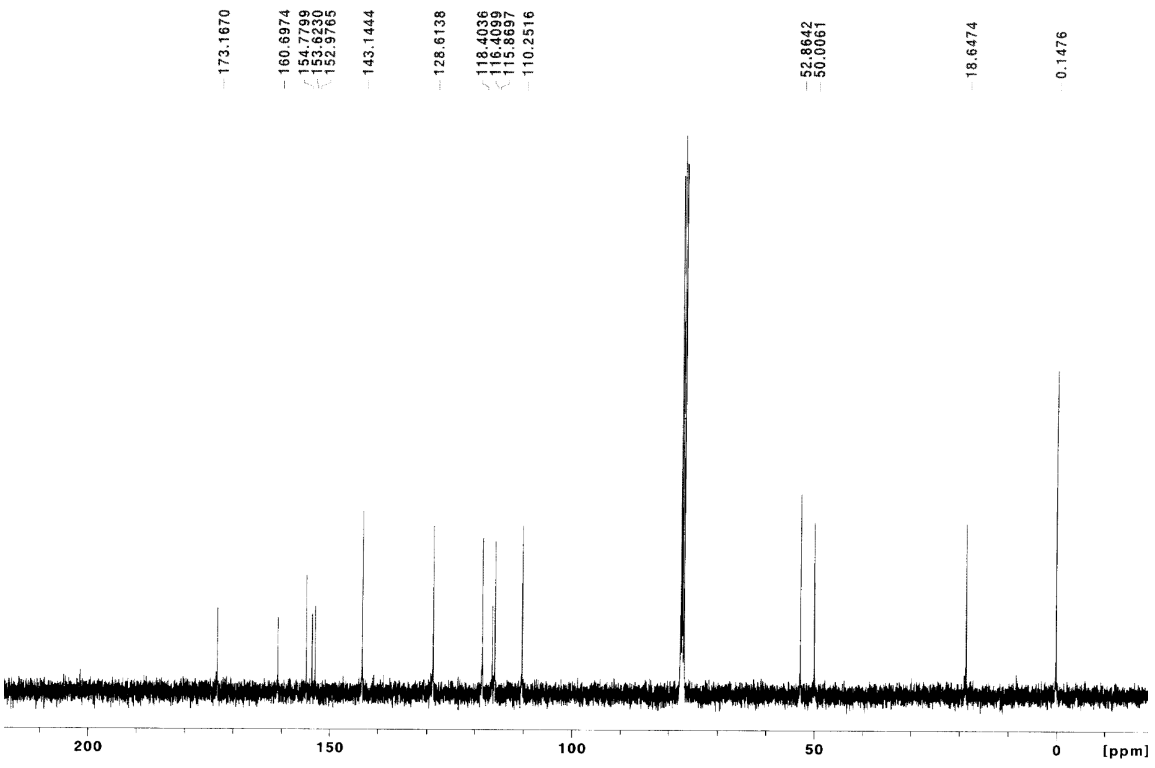


Figure S9. ^{13}C NMR spectrum for compound **1a**.

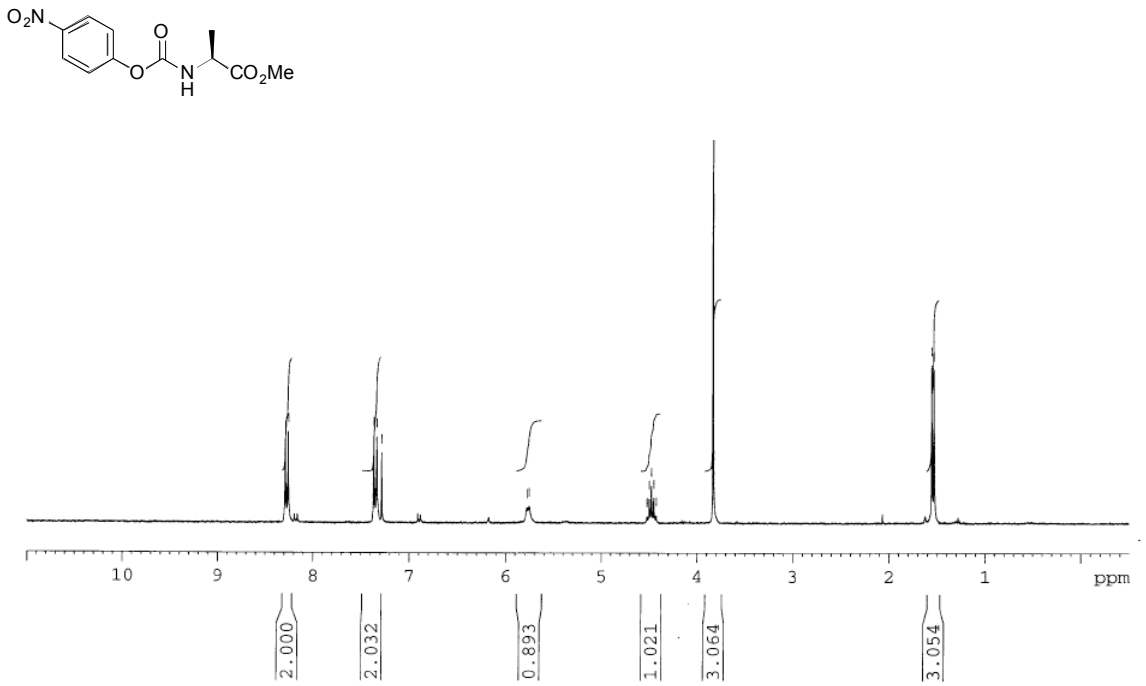


Figure S10. ^{1}H NMR spectrum for compound **1b** (mixture of *cis/trans* conformers).

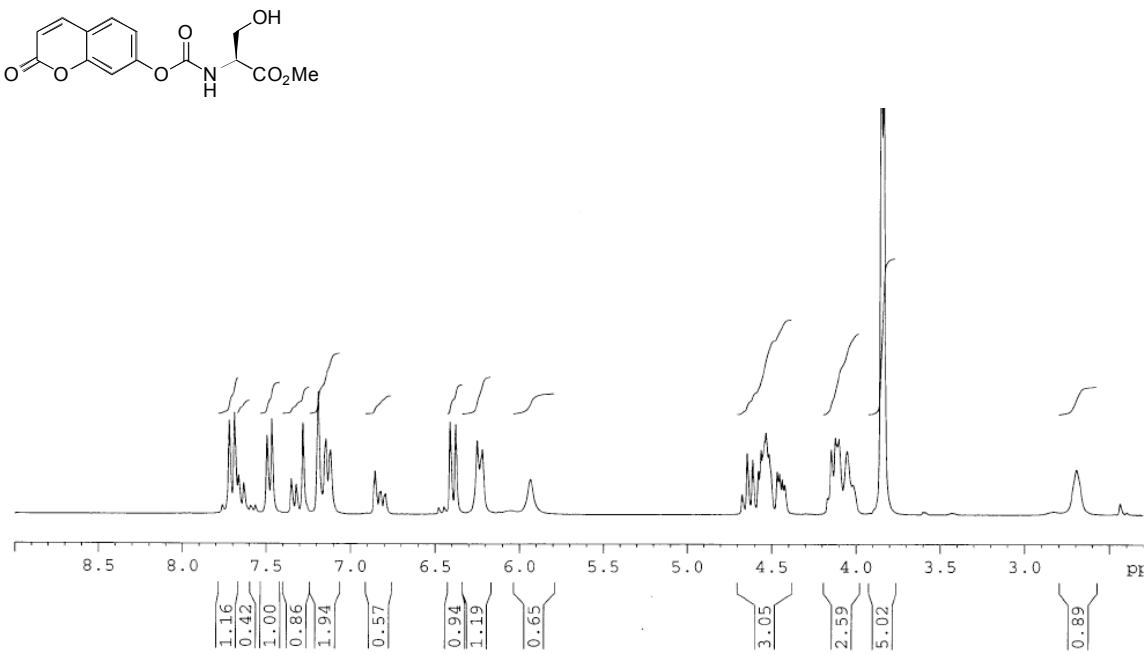


Figure S11. ^{1}H NMR spectrum for a mixture of compound **2a** and **11**.

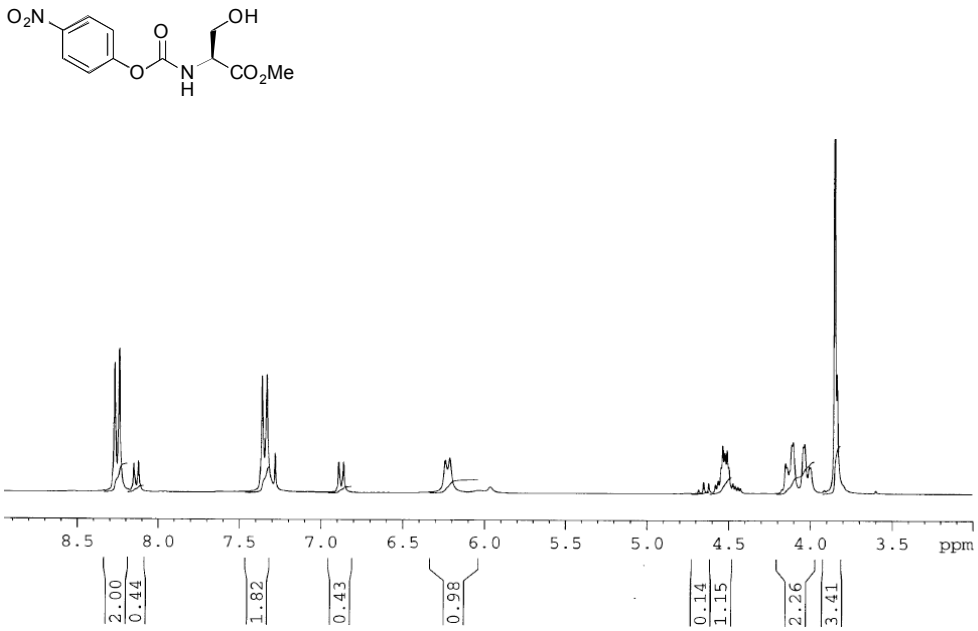


Figure S12. [1]H NMR spectrum for a mixture of compound **2b** and **11**.

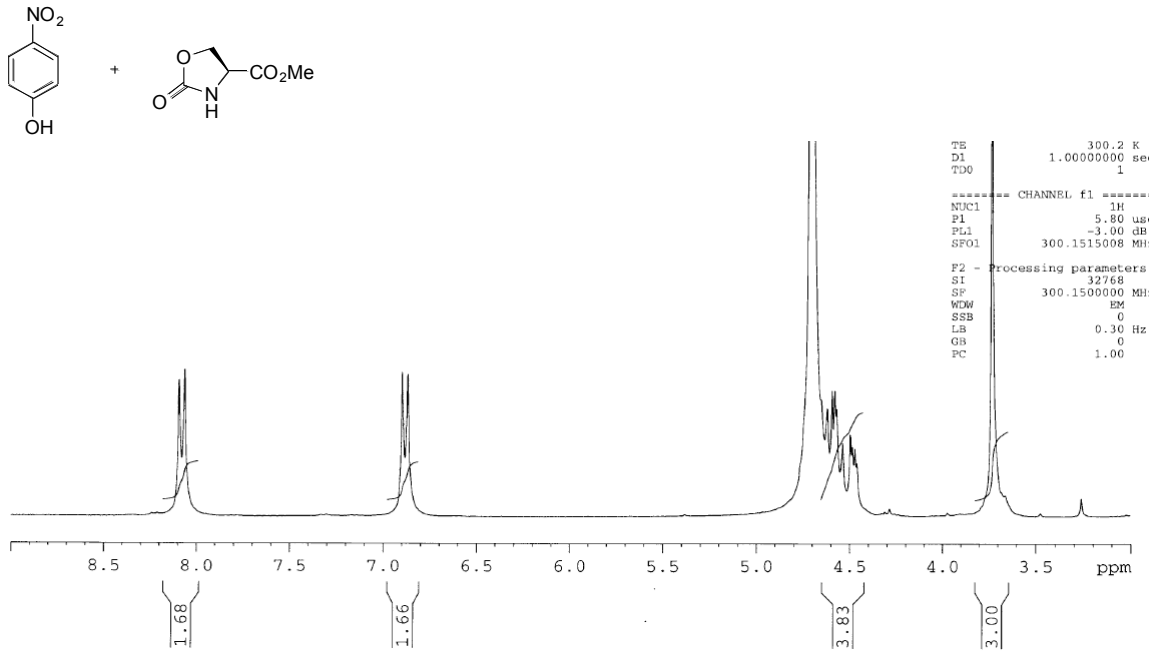
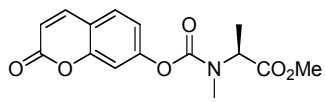


Figure S13. [1]H NMR spectrum right after treating compound **2b** in phosphate buffer (pH 7.5), the formation of compound **11** and *p*NPP.



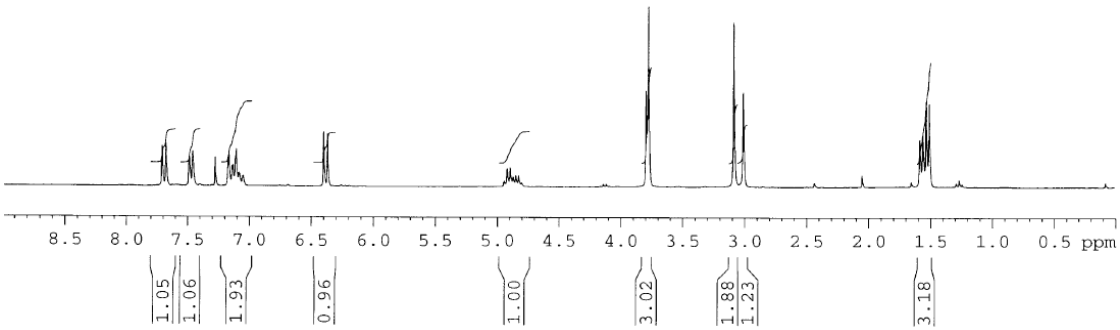


Figure S14. [1]H NMR spectrum for compound **3a** (mixture of *cis/trans* conformers).

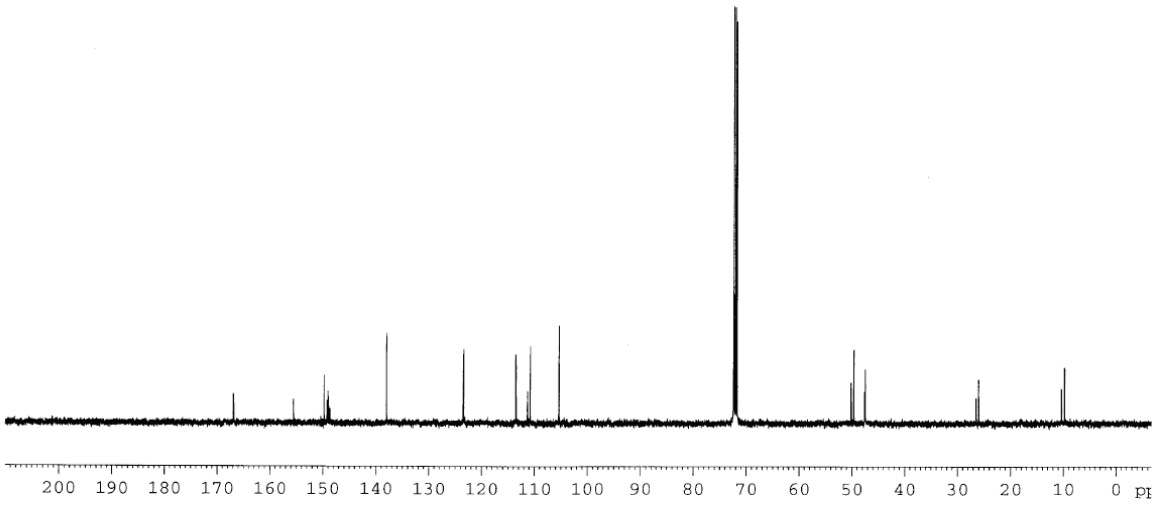
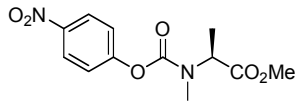


Figure S15. [13]C NMR spectrum for compound **3b** (mixture of *cis/trans* conformers).



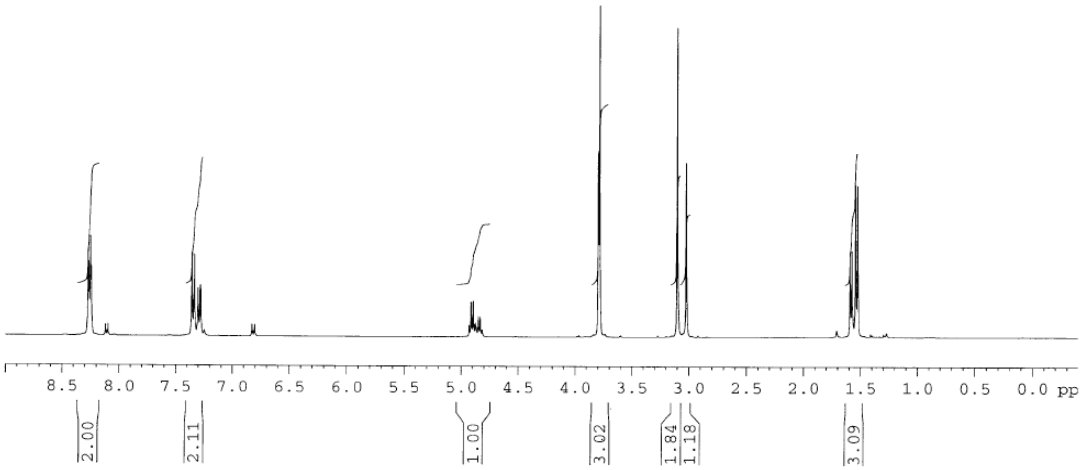


Figure S16. ^{1}H NMR spectrum for compound **3b** (mixture of *cis/trans* conformers).

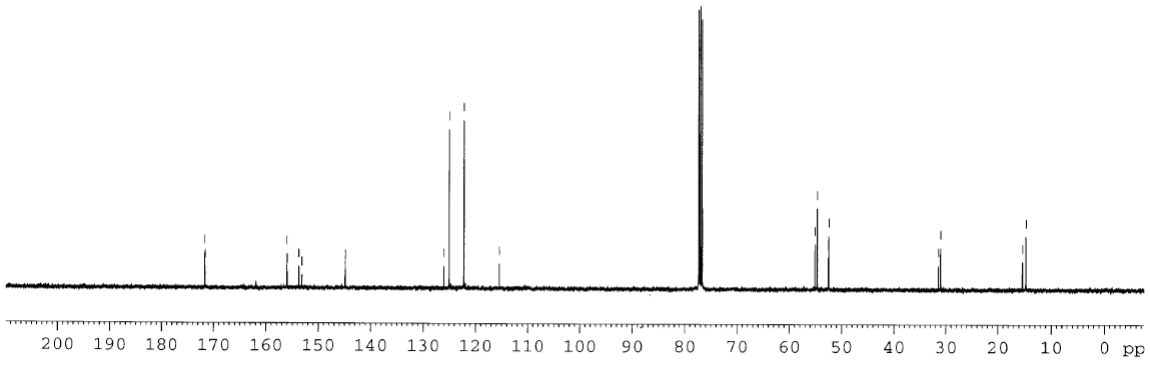


Figure S17. ^{13}C NMR spectrum for compound **3b** (mixture of *cis/trans* conformers).

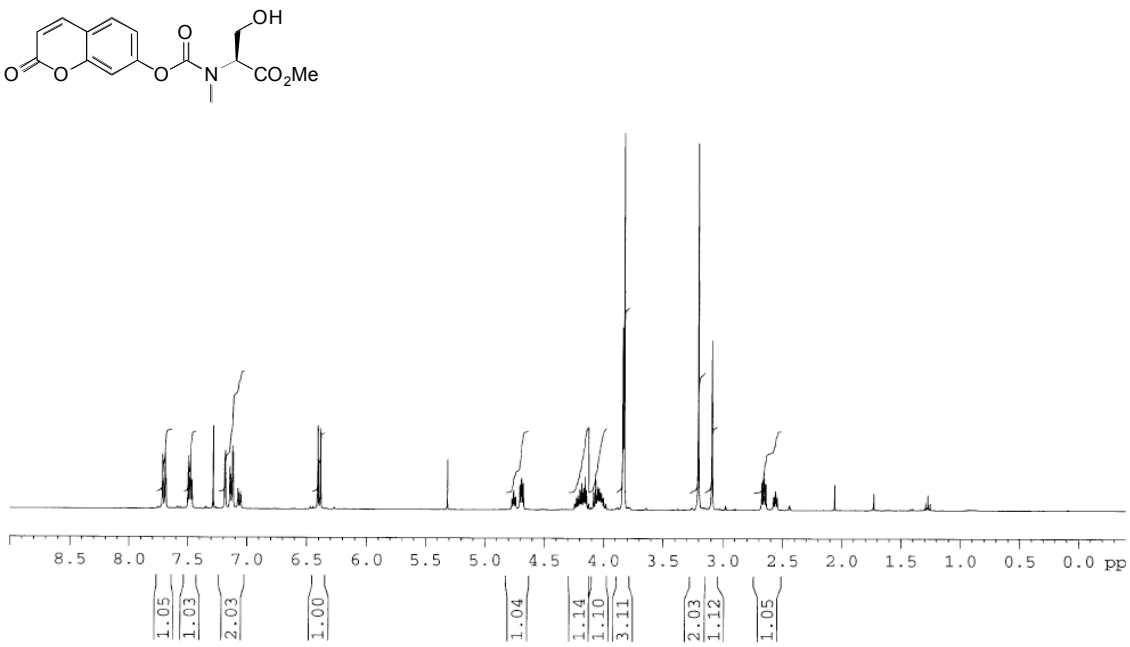


Figure S18. ^{1}H NMR spectrum for compound **4a** (mixture of *cis/trans* conformers).

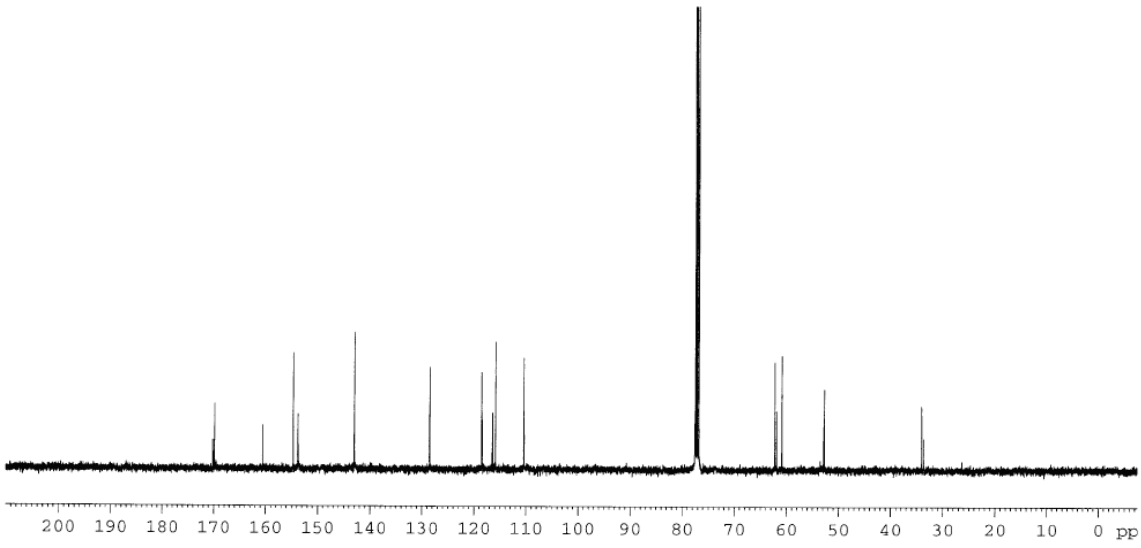
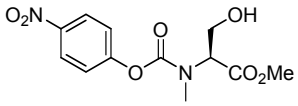


Figure S19. ^{13}C NMR spectrum for compound **4a** (mixture of *cis/trans* conformers).



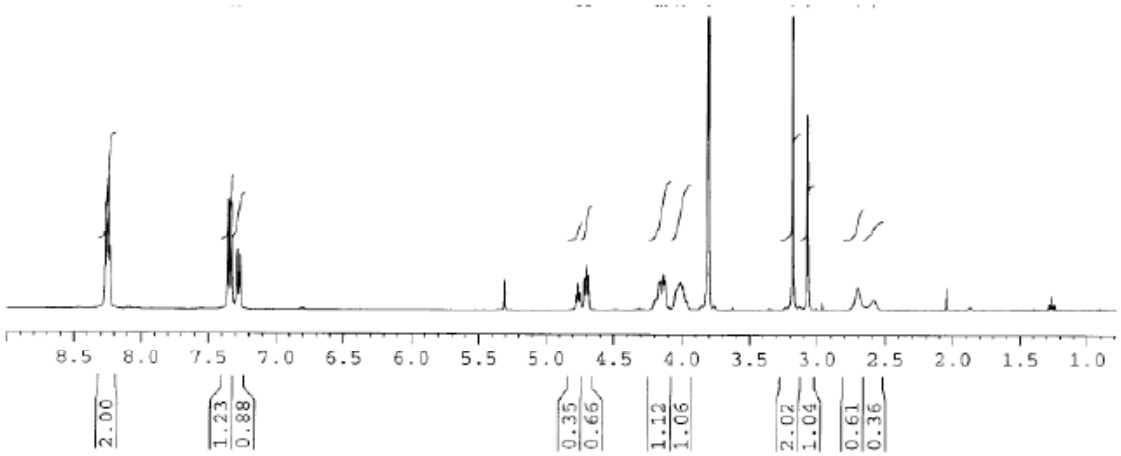


Figure S20. ^{1}H NMR spectrum for compound **4b** (mixture of *cis/trans* conformers).

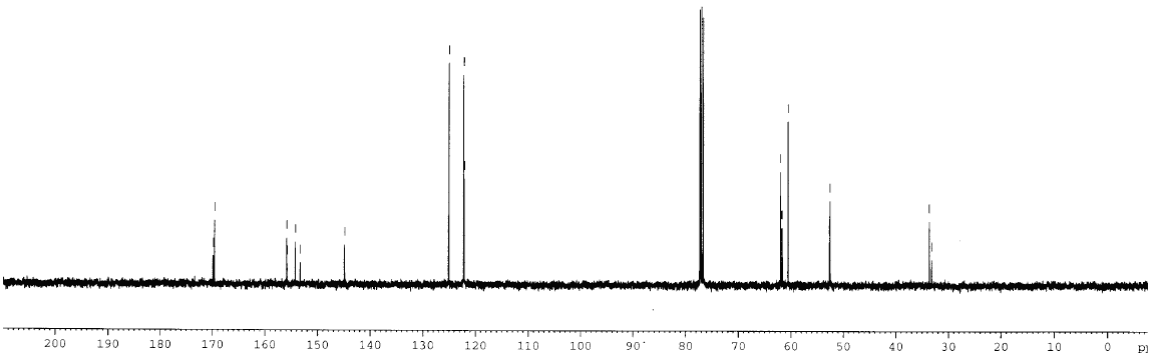
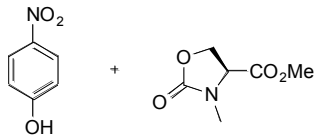


Figure S21. ^{13}C NMR spectrum for compound **4b** (mixture of *cis/trans* conformers).



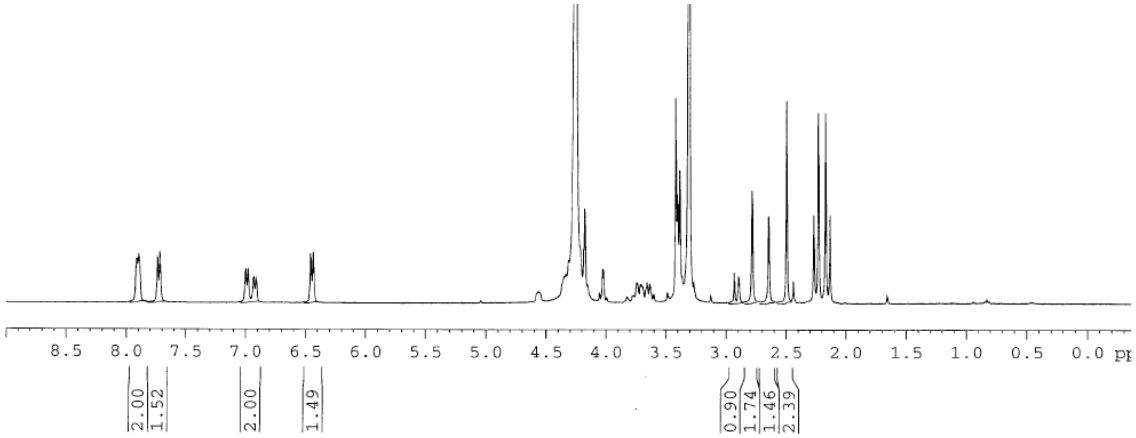


Figure S22. ^{1}H NMR spectrum after treating compound **4b** in Tris buffer (pH 8.0) for 8 min.

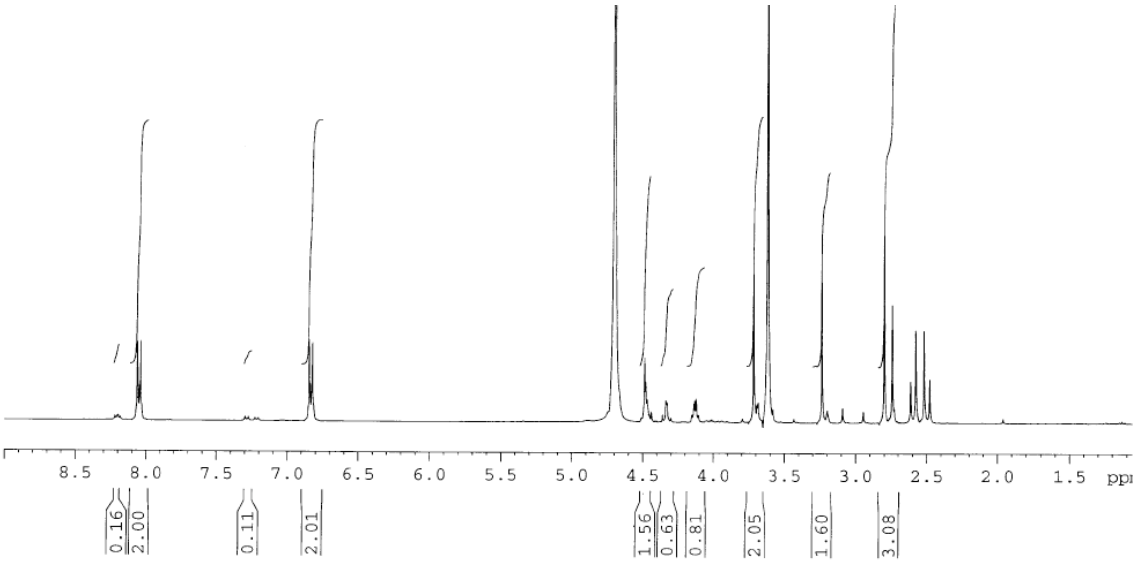


Figure S23. ^{1}H NMR spectrum after treating compound **4b** in Tris buffer (pH 8.0) for 60 min, the formation of the cyclized compound and *p*NPP.

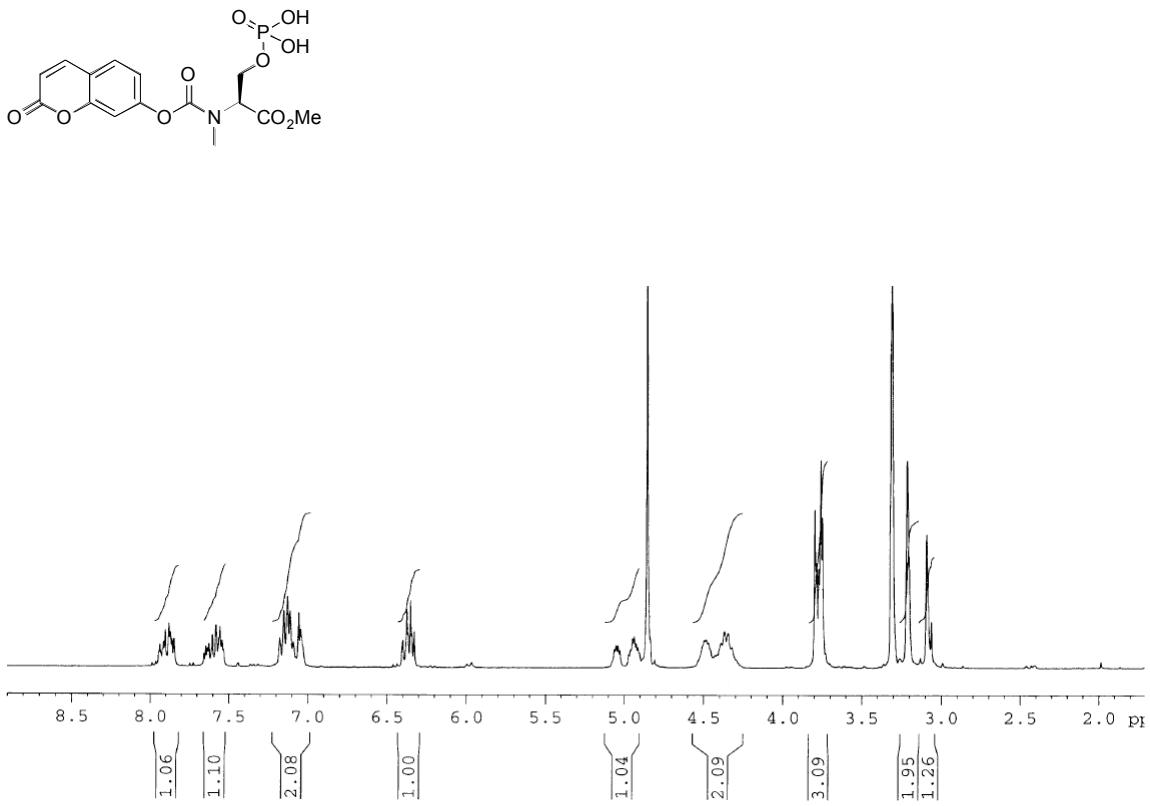


Figure S24. [1]H NMR spectrum for compound **5a** (mixture of *cis/trans* conformers).

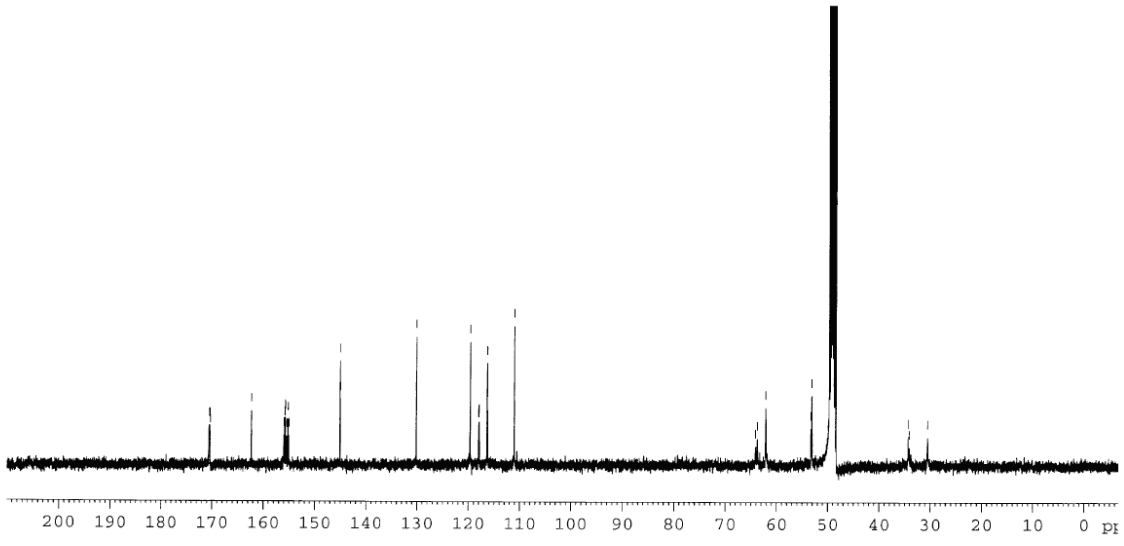


Figure S25. [13]C NMR spectrum for compound **5a** (mixture of *cis/trans* conformers).

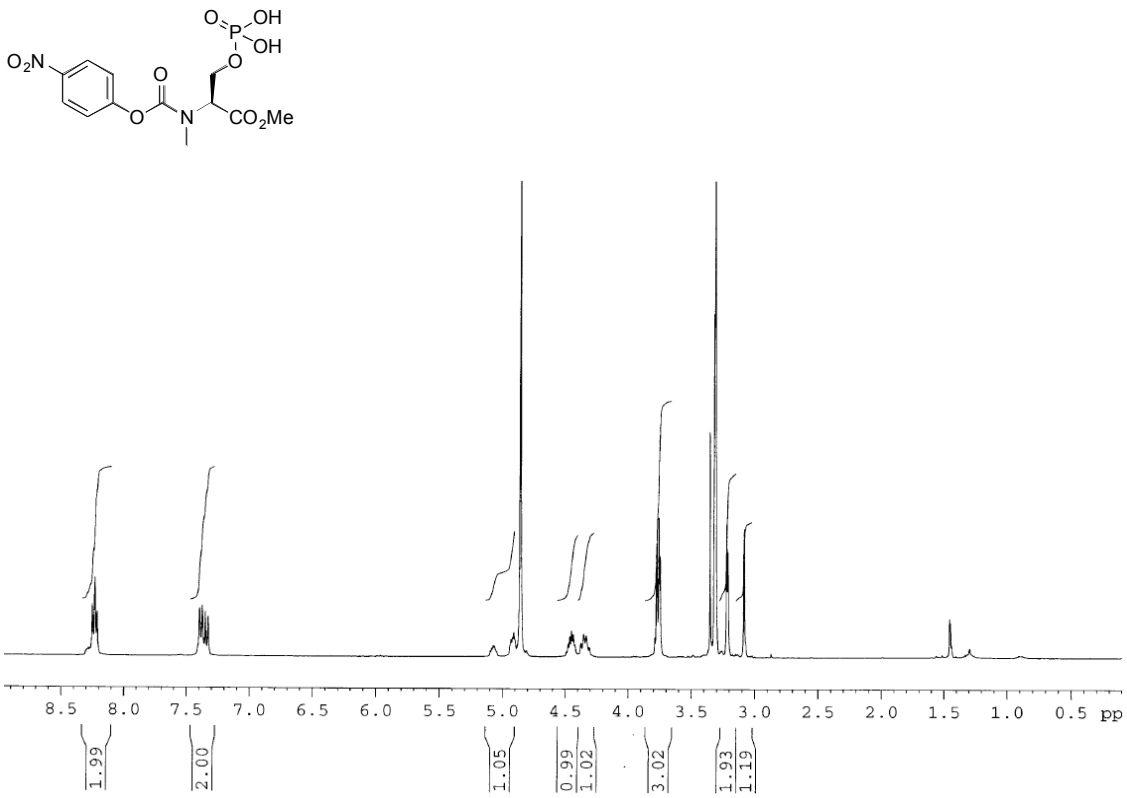


Figure S26. ^{1}H NMR spectrum for compound **5b** (mixture of *cis/trans* conformers).

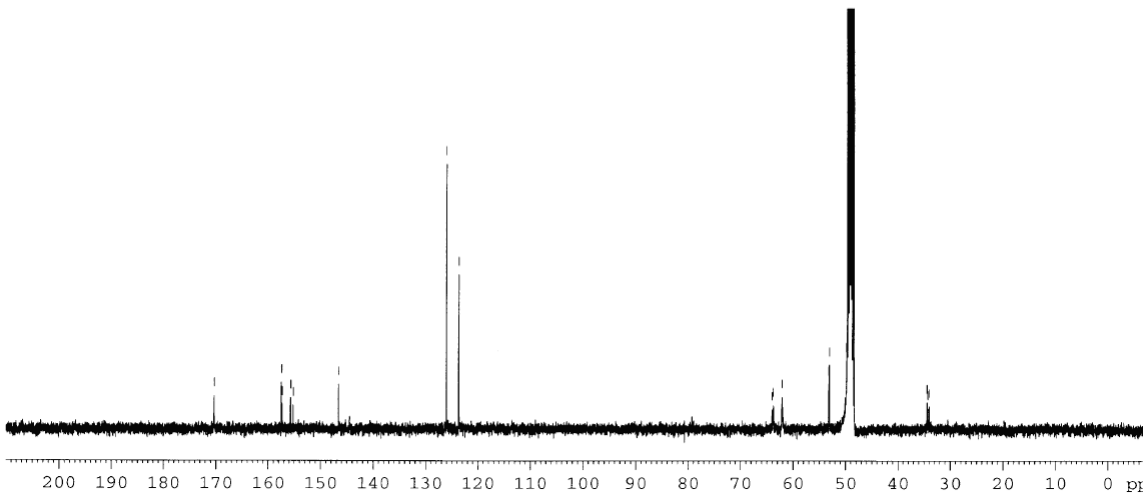


Figure S27. ^{13}C NMR spectrum for compound **5b** (mixture of *cis/trans* conformers).

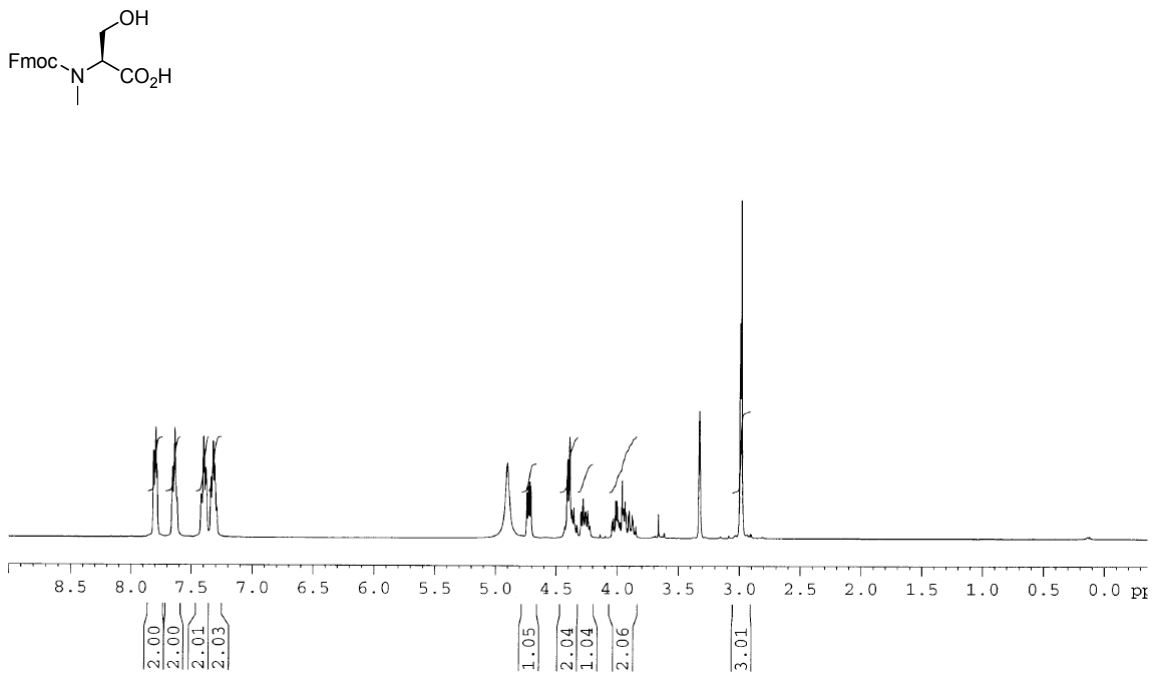


Figure S28. ^{1}H NMR spectrum for compound **17** (mixture of *cis/trans* conformers).

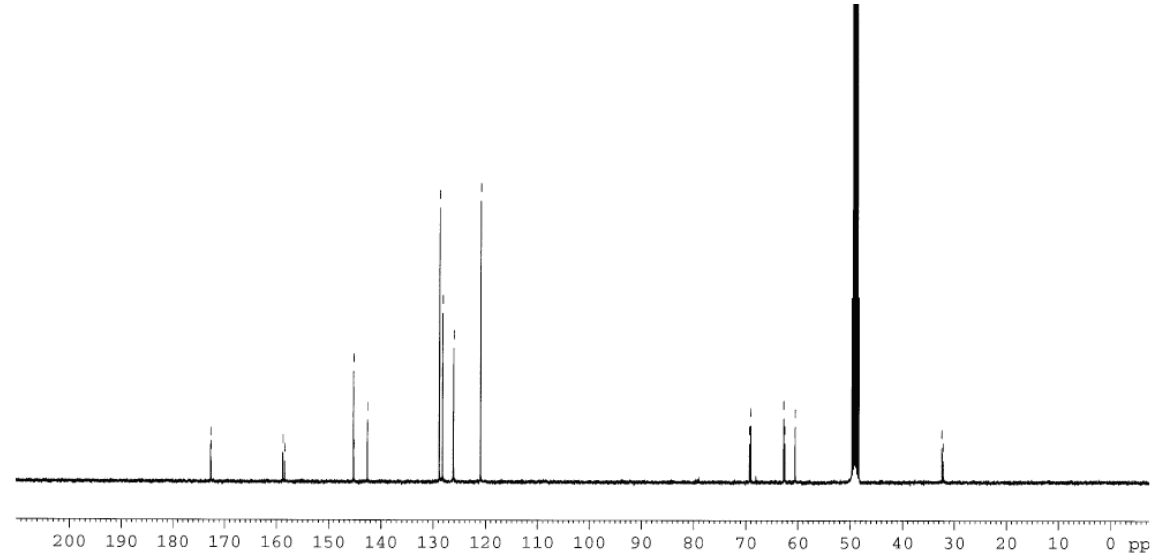


Figure S29. ^{13}C NMR spectrum for compound **17** (mixture of *cis/trans* conformers).

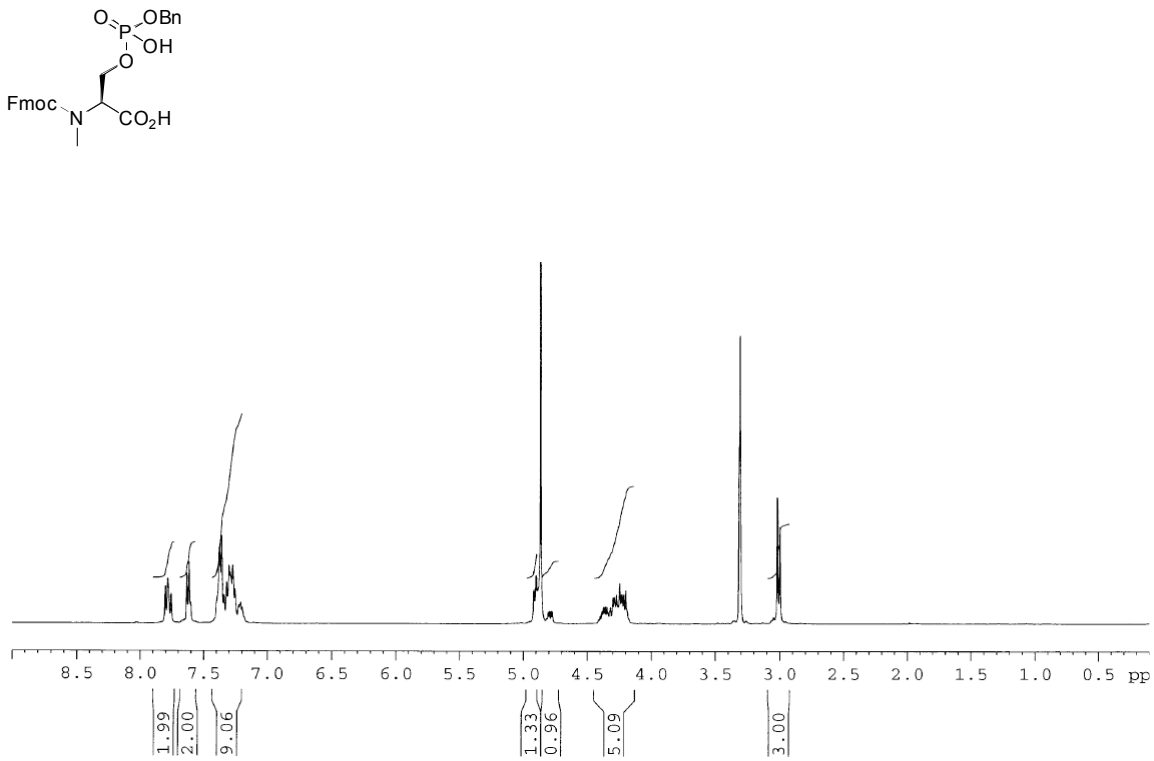


Figure S30. [1]H NMR spectrum for compound **19** (mixture of *cis/trans* conformers).

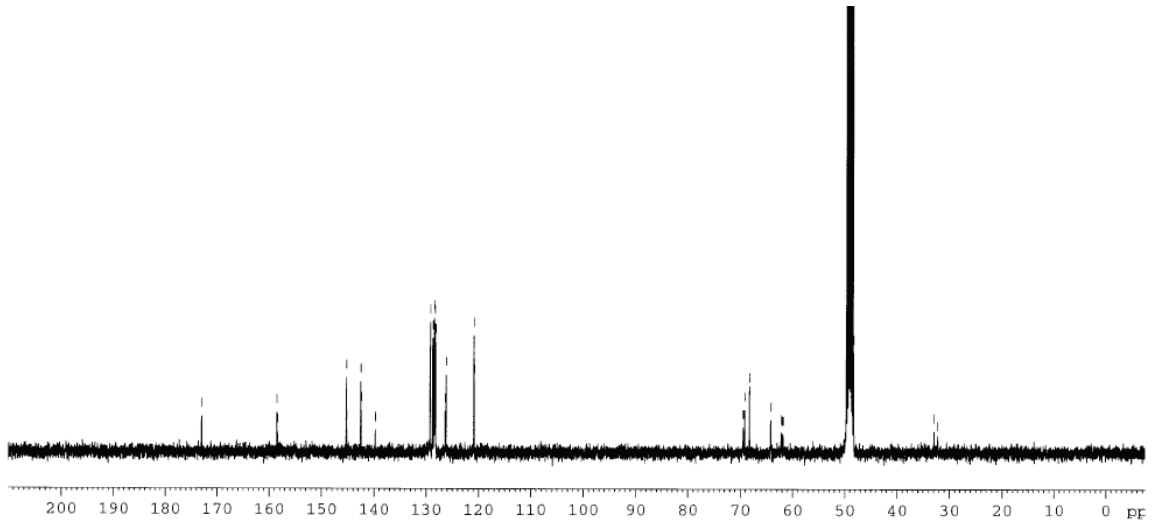


Figure S31. [13]C NMR spectrum for compound **19** (mixture of *cis/trans* conformers).

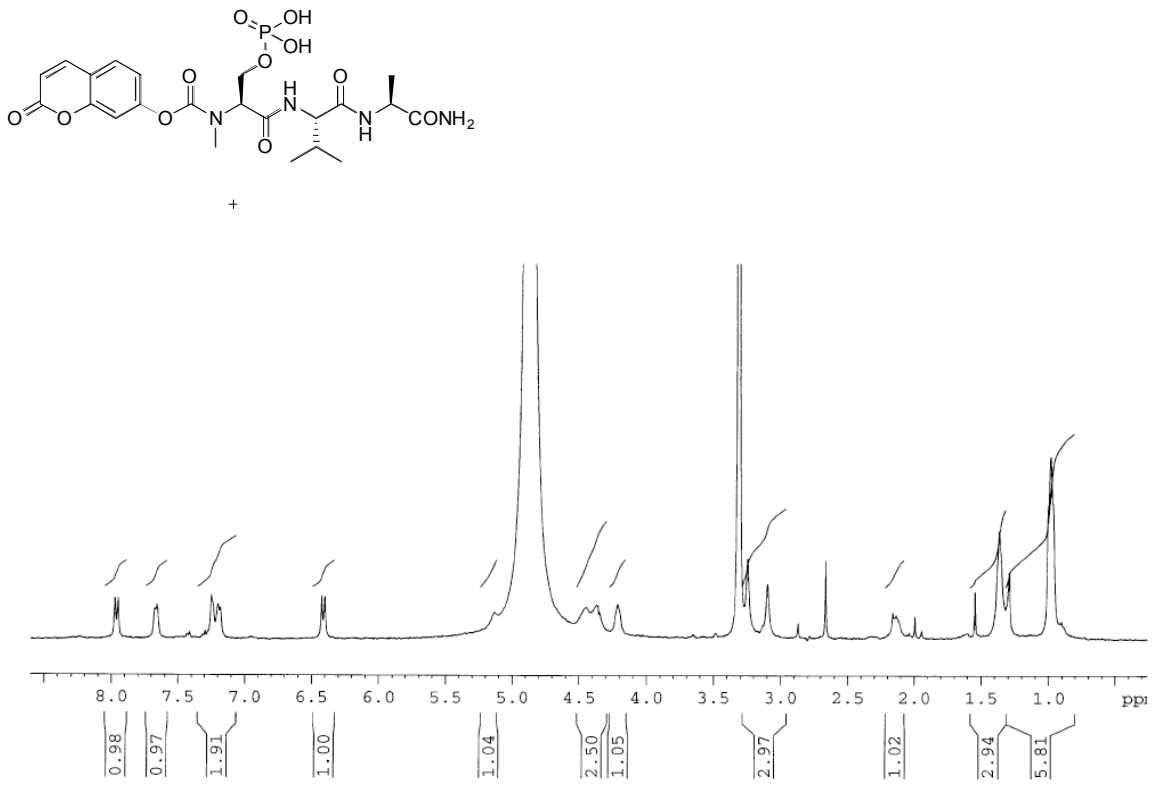


Figure S32. ^{1}H NMR spectrum for compound **6a** (mixture of *cis/trans* conformers).

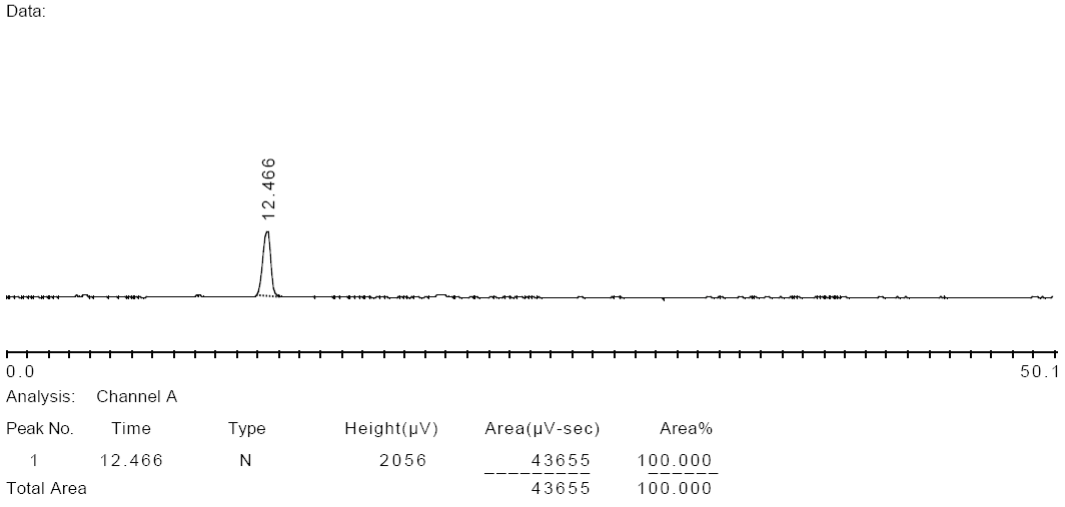


Figure 33. RP-HPLC trace of compound **6a**.

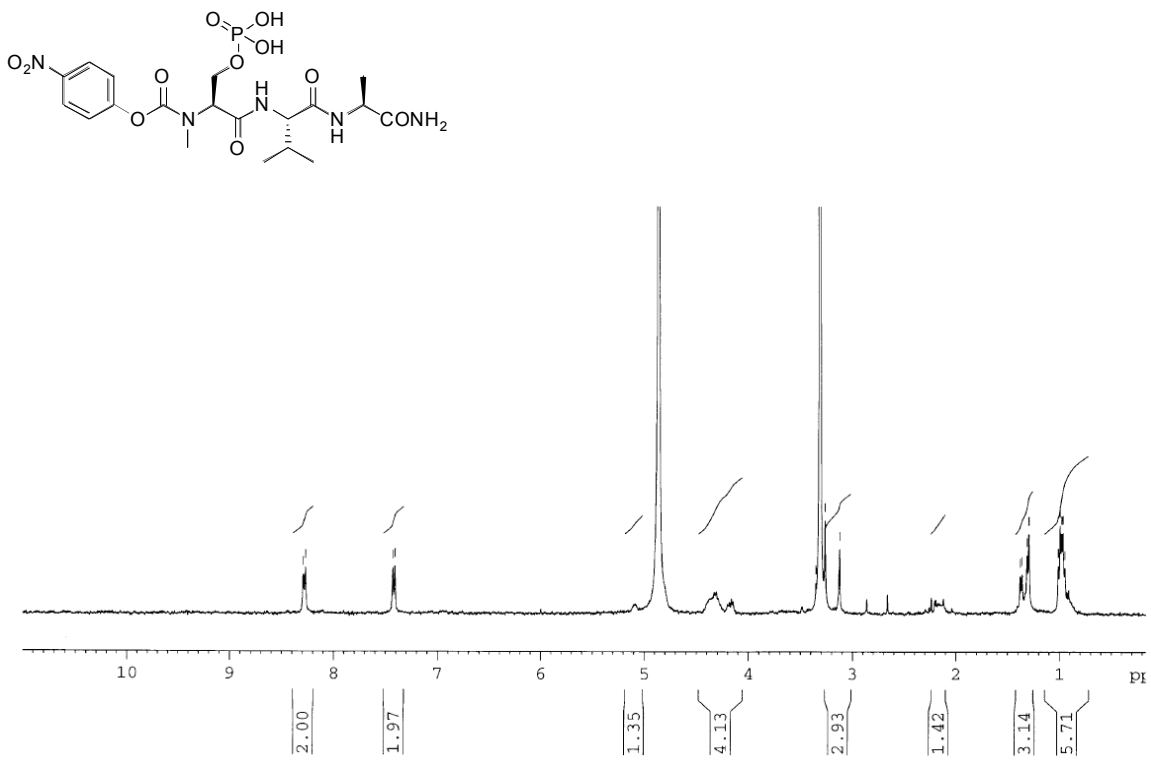


Figure S34. ^{1}H NMR spectrum for compound **6b** (mixture of *cis/trans* conformers).

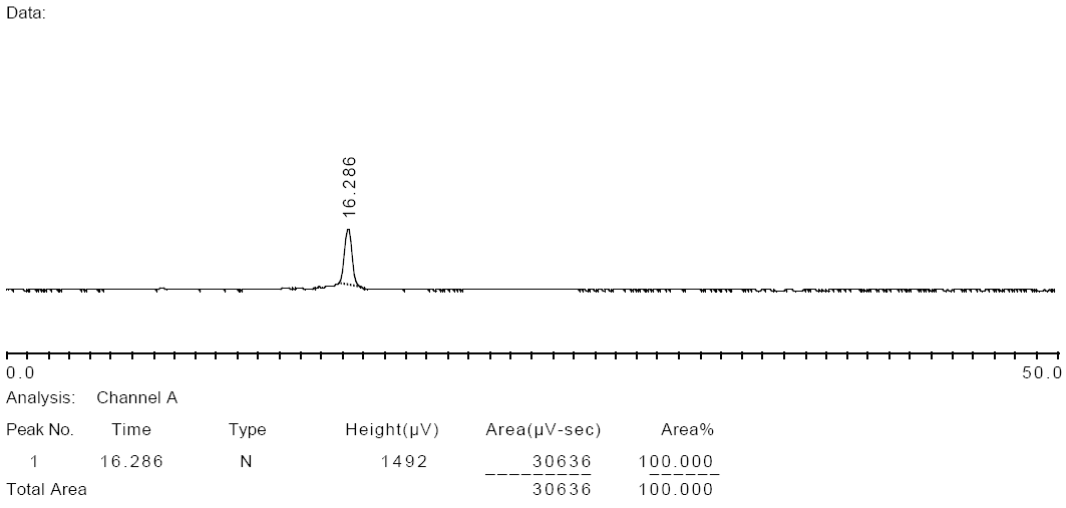


Figure S35. RP-HPLC trace of compound **6b**.

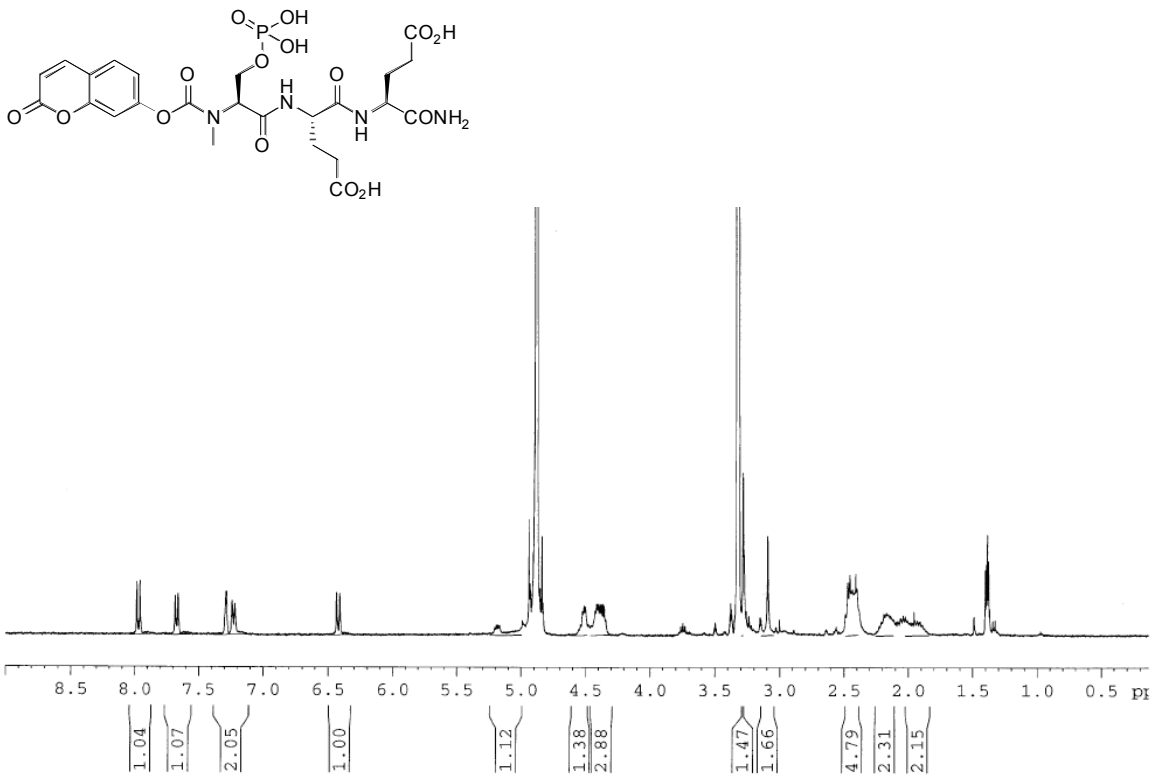


Figure S36. ^{1}H NMR spectrum for compound **7a** (mixture of *cis/trans* conformers).

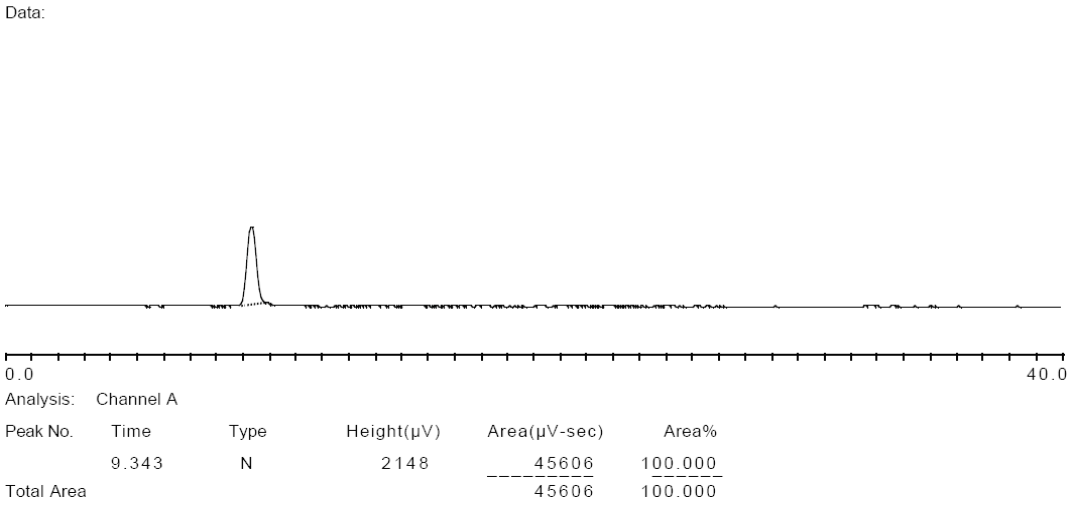


Figure S37. RP-HPLC trace of compound **7a**.

4. Traces of K_m and V_{max} Calculations.

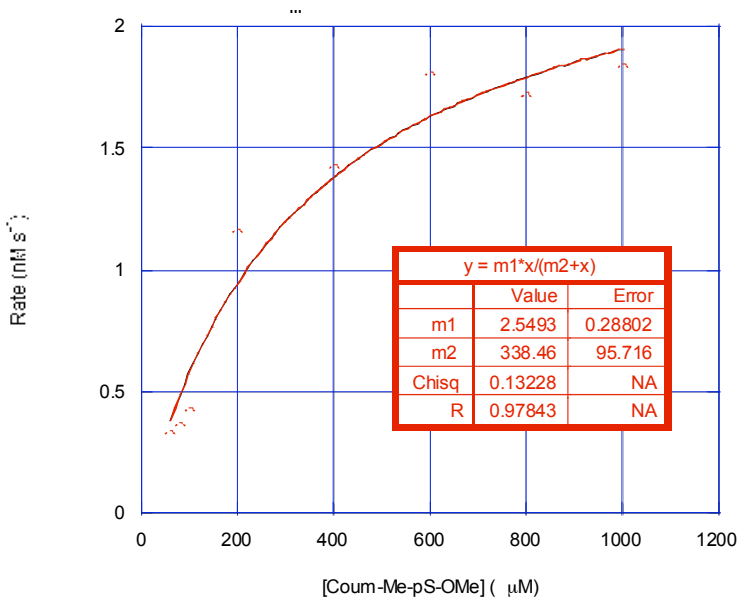


Figure S38. K_m and V_{max} calculation of compound **5a** to ALP, trace 1.

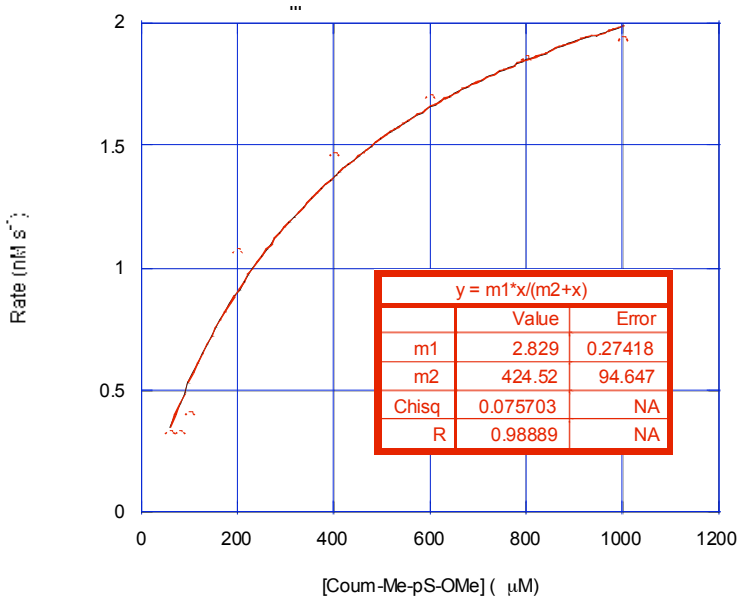


Figure S39. K_m and V_{max} calculation of compound **5a** to ALP, trace 2.

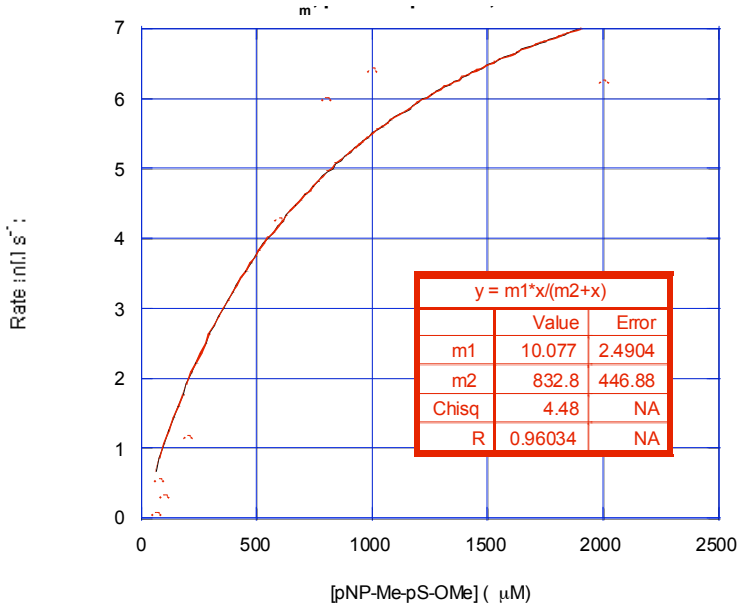


Figure S40. K_m and V_{max} calculation of compound **5b** to ALP, trace 1.

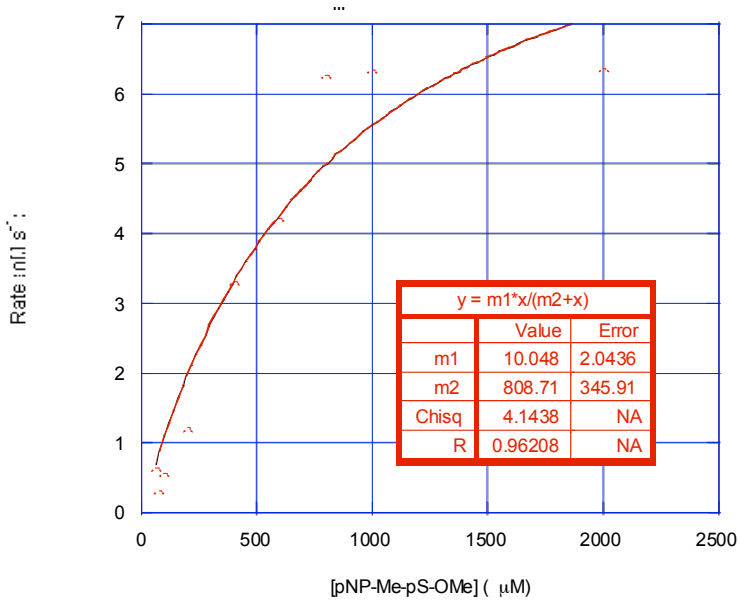


Figure S41. K_m and V_{max} calculation of compound **5b** to ALP, trace 2.

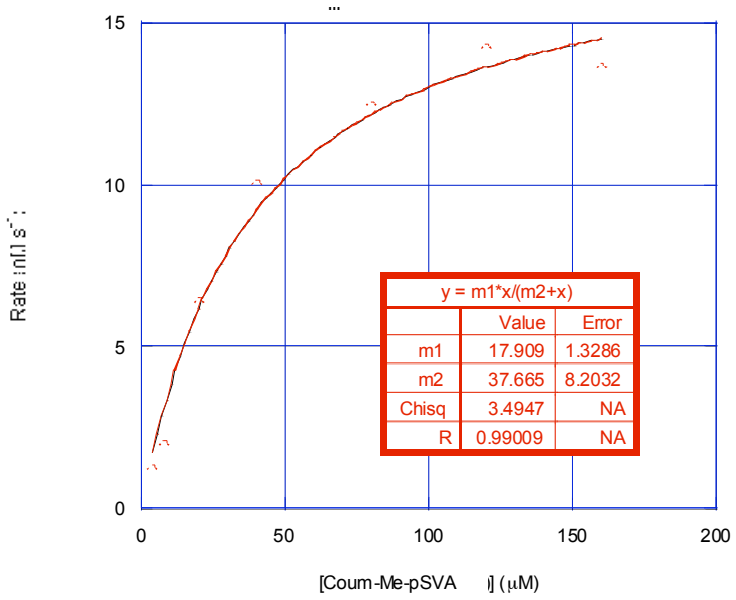


Figure S42. K_m and V_{max} calculation of compound **6a** to ALP, trace 1.

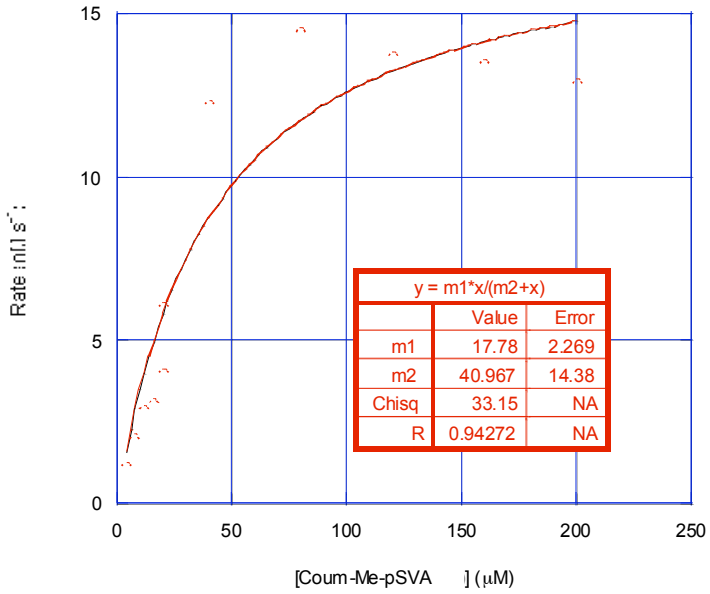


Figure S43. K_m and V_{max} calculation of compound **6a** to ALP, trace 2.

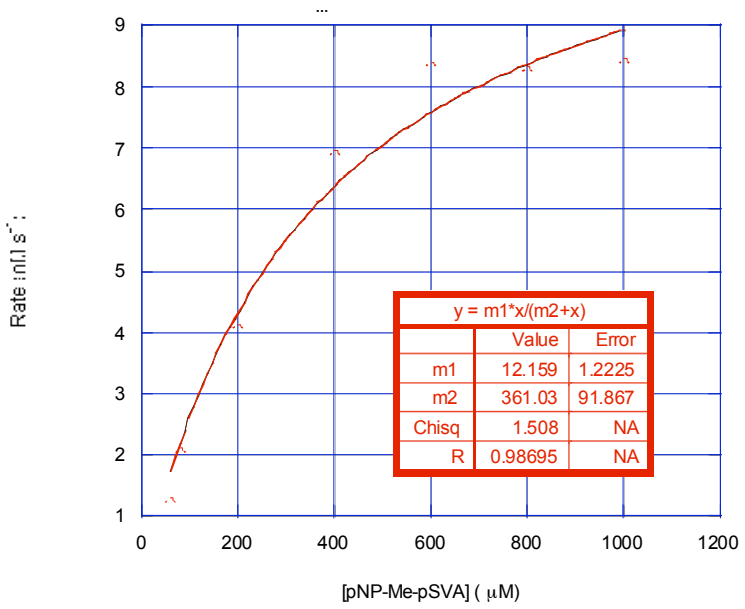


Figure S44. K_m and V_{max} calculation of compound **6b** to ALP, trace 1.

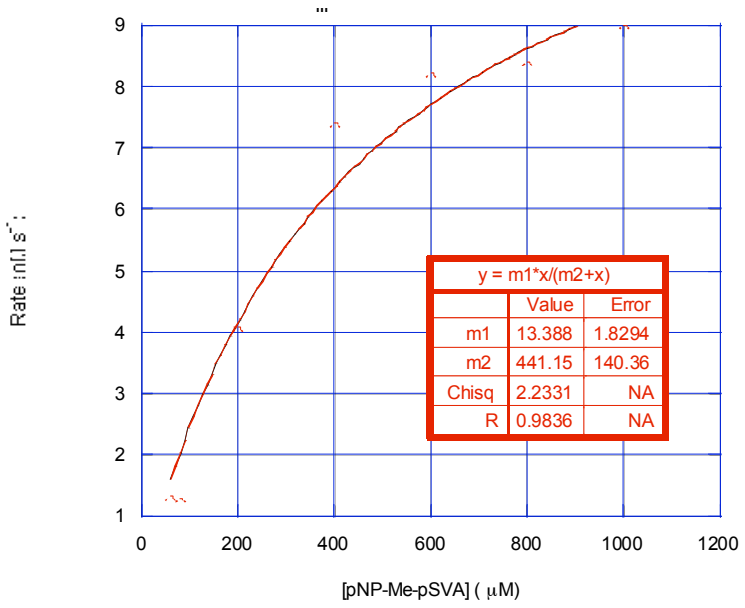


Figure S45. K_m and V_{max} calculation of compound **6b** to ALP, trace 2.

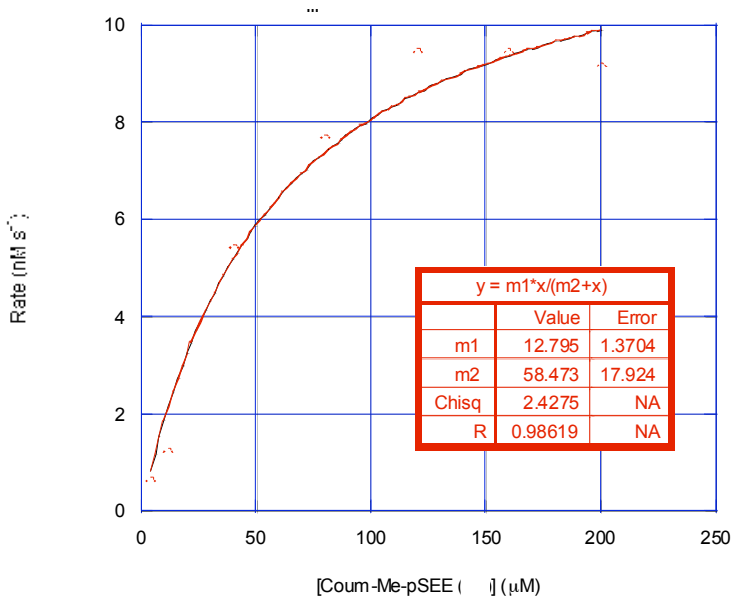


Figure S46. K_m and V_{max} calculation of compound **7a** to ALP, trace 1.

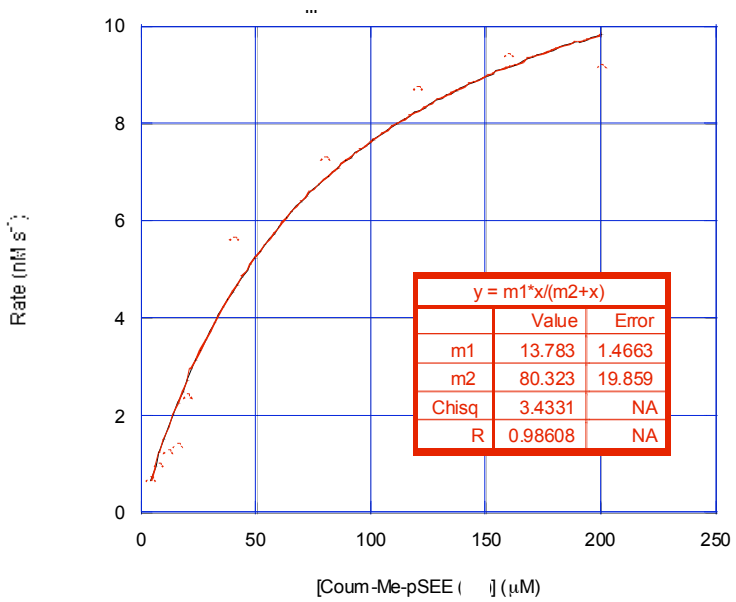


Figure S47. K_m and V_{max} calculation of compound **7a** to ALP, trace 2.

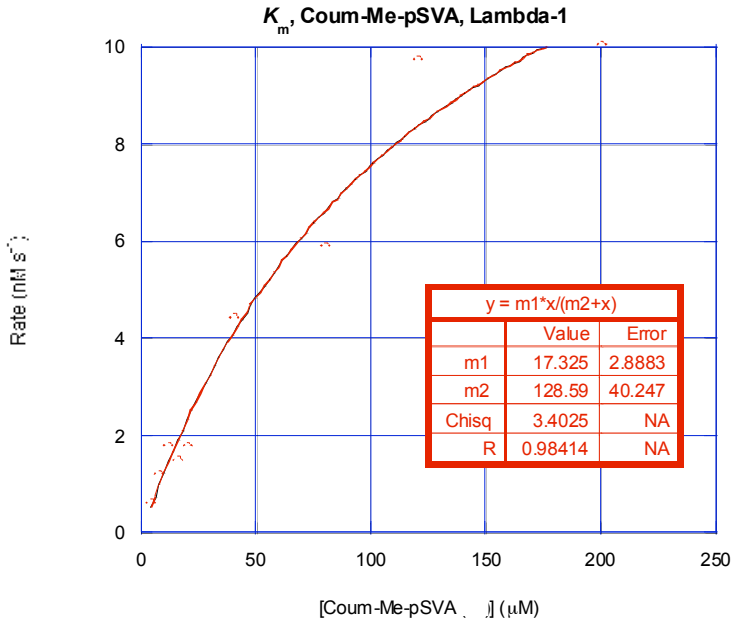


Figure S48. K_m and V_{max} calculation of compound **6a** to λ-PPase, trace 1.

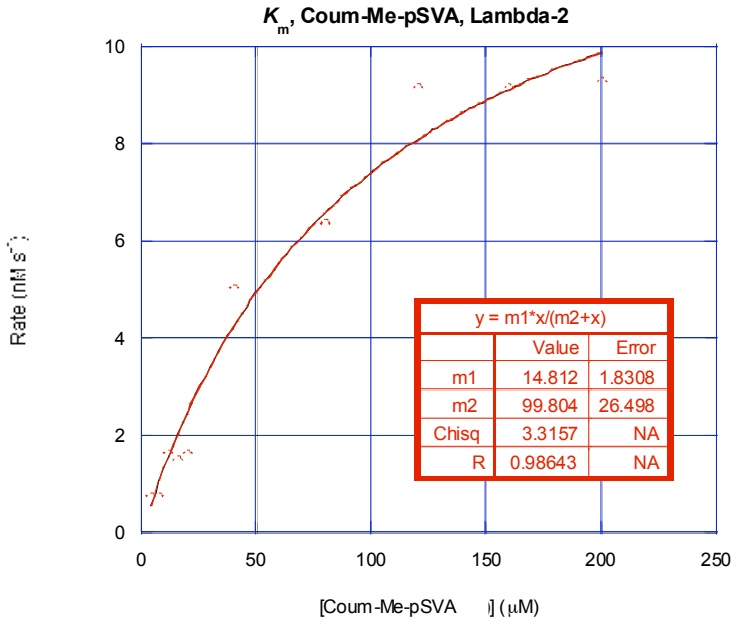


Figure S49. K_m and V_{max} calculation of compound **6a** to λ-PPase, trace 2.

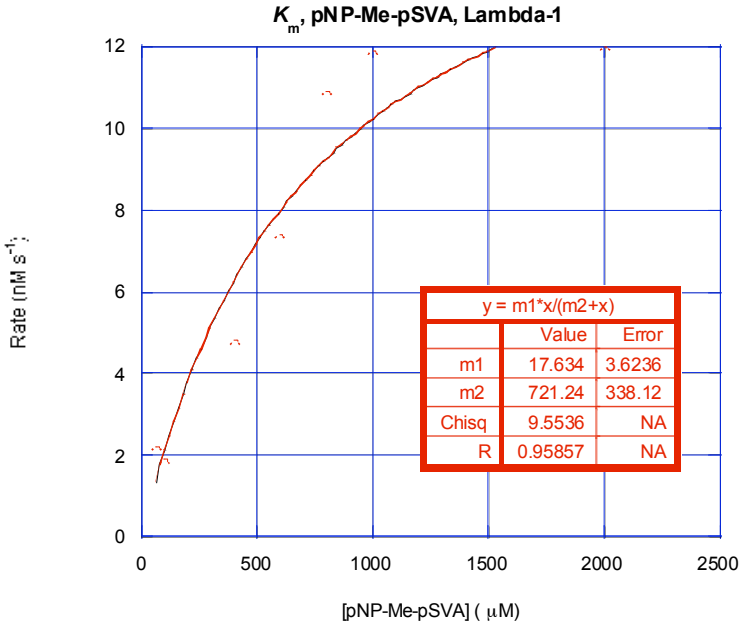


Figure S50. K_m and V_{max} calculation of compound **6b** to λ-PPase, trace 1.

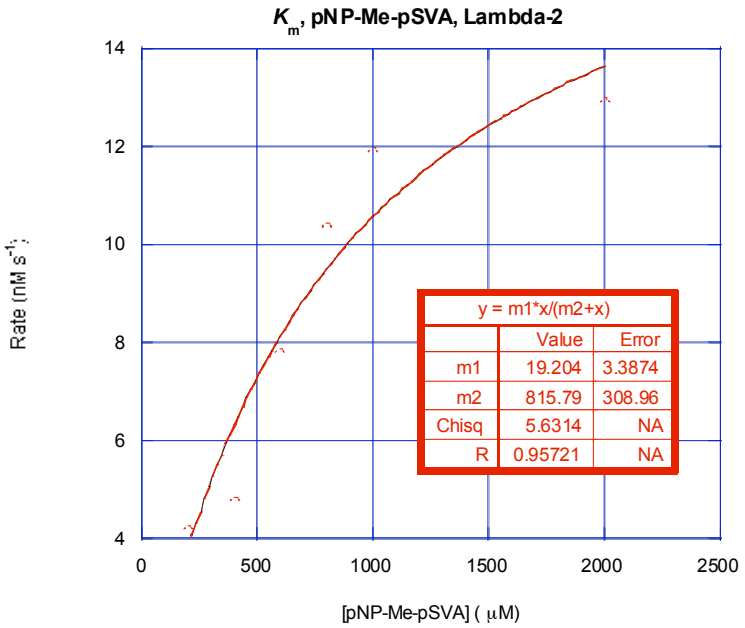


Figure S51. K_m and V_{max} calculation of compound **6b** to λ-PPase , trace 2.

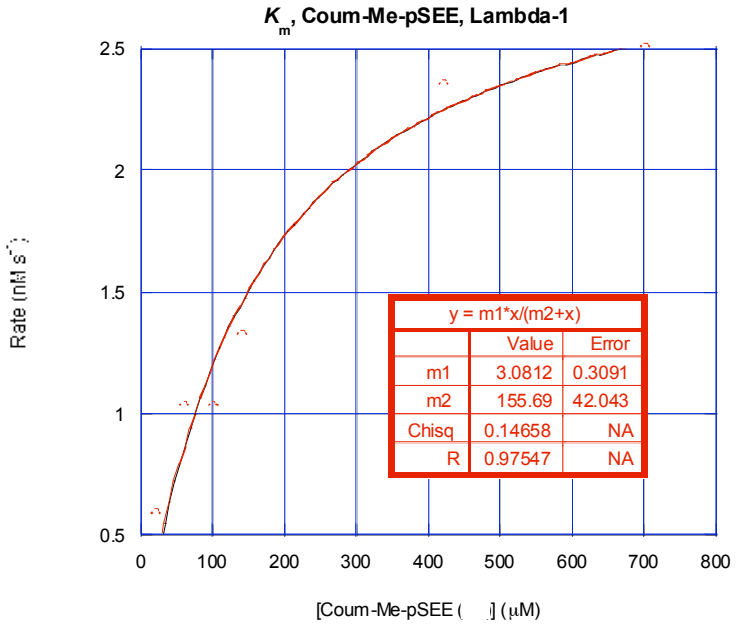


Figure S52. K_m and V_{max} calculation of compound **7a** to λ-PPase, trace 1.

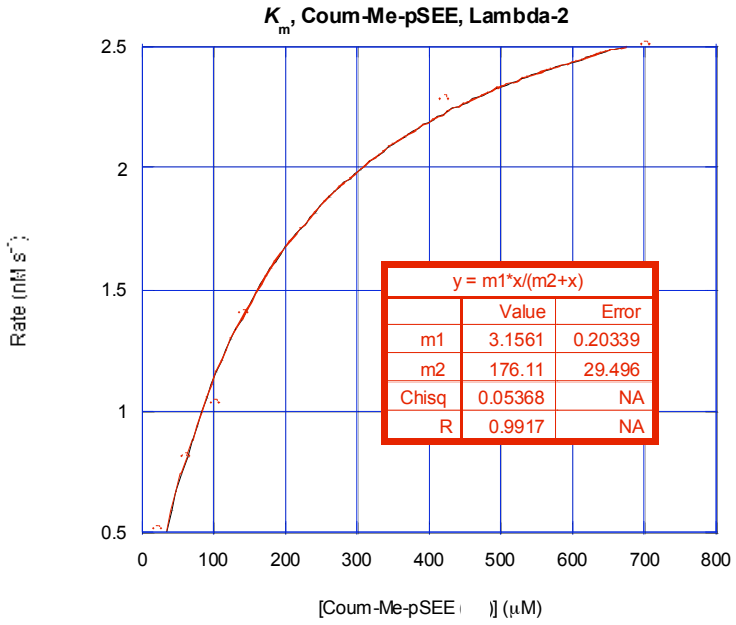


Figure S53. K_m and V_{max} calculation of compound **7a** to λ-PPase, trace 2.

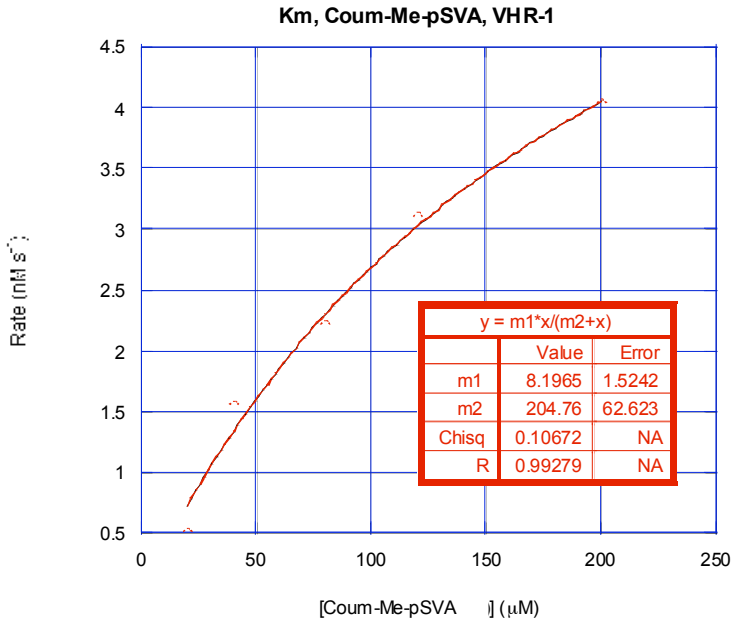


Figure S54. K_m and V_{max} calculation of compound **6a** to VHR, trace 1.

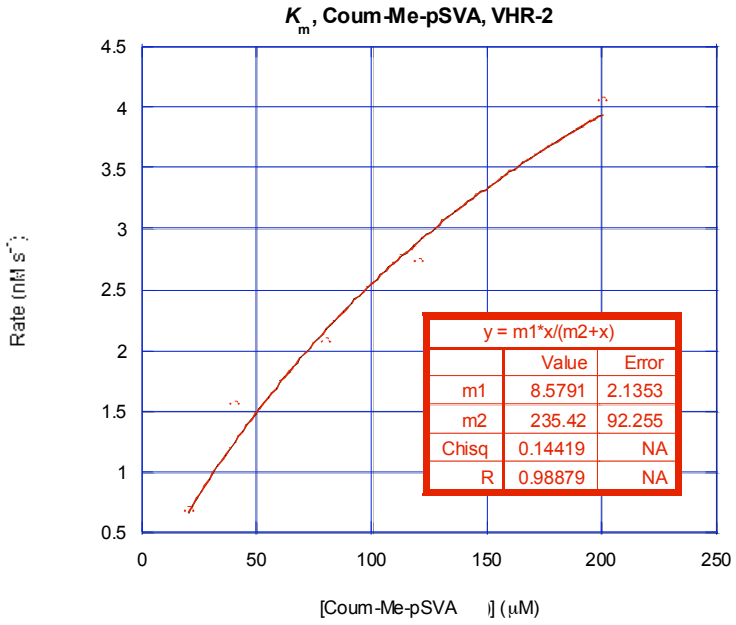


Figure S55. K_m and V_{max} calculation of compound **6a** to VHR, trace 2.

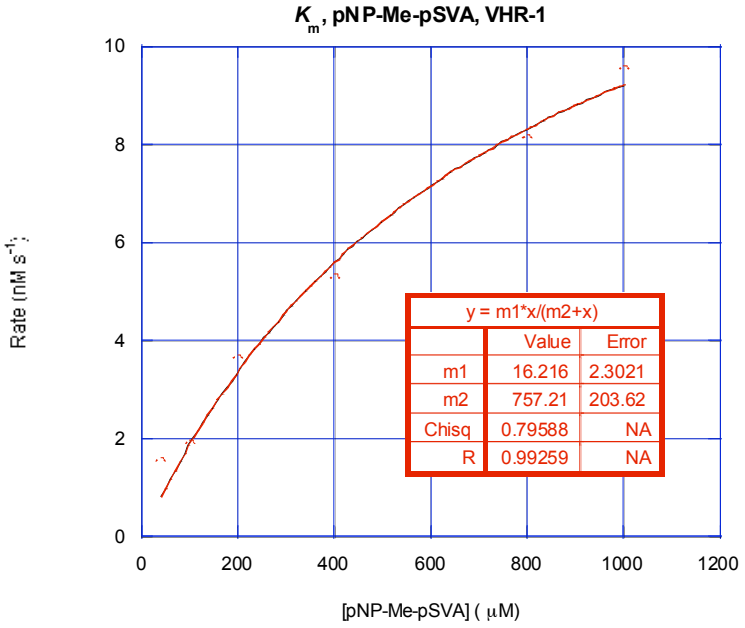


Figure S56. K_m and V_{max} calculation of compound **6b** to VHR, trace 1.

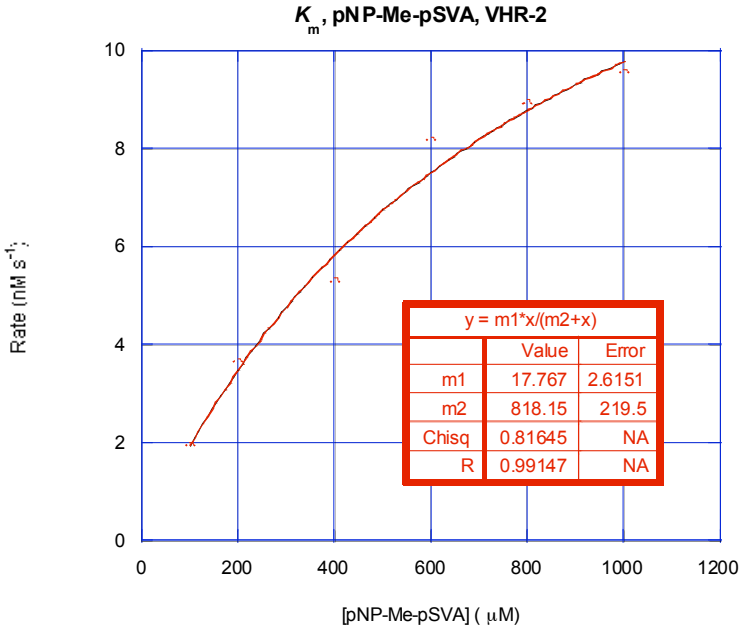


Figure S57. K_m and V_{max} calculation of compound **6b** to VHR, trace 2.

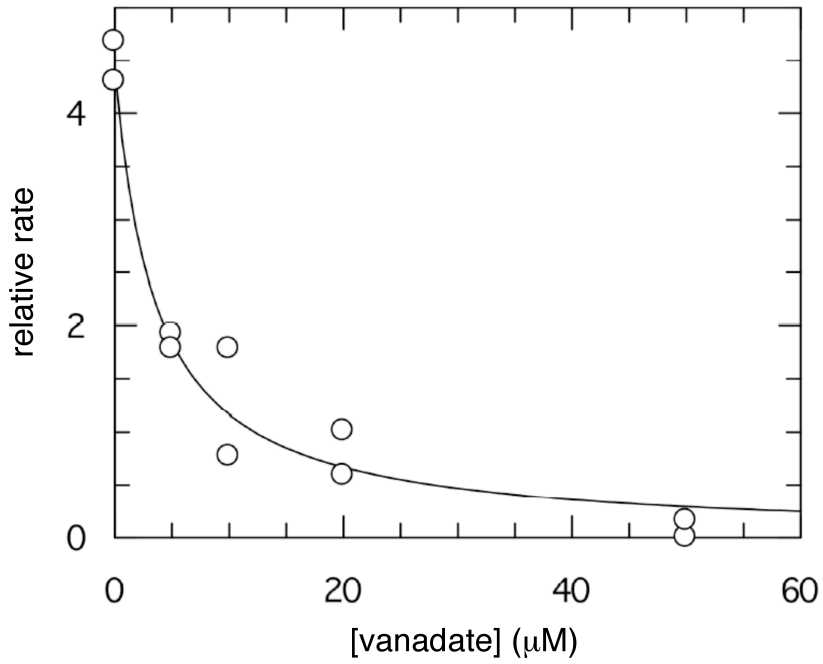


Figure S58. Inhibition of alkaline phosphatase by vanadate using compound **5b** (1 mM) as the substrate. Vanadate concentrations were 5, 10, 20 and 50 μM. The IC50 value was calculated as 1.6 ± 0.1 μM.

6. References and Notes.

[1] (a) Pope, B. M.; Sheu, S.-J.; Stanley, R. L.; Tarbell, D. S.; Yamamoto, Y. *J. Org. Chem*. **1978**, *43*, 2410-2414. (b) Sanda, F.; Ogawa, F.; Endo, T. *Nippon Setchaku Gakkaishi* **1997**, *33*, 175-180. (c) Kempf, D. J.; Norbeck, D. W.; Sham, H. L.; Zhao, C.; Sowin, T. J.; Reno, D. S.; Haight, A. R.; Cooper, A. J. Preparation of peptide analogs as retroviral protease inhibitors. Patent Application WO 93-US12326 19931216, 1994. (d) Desai, M. C.; Hong, A. Y.; Liu, H.; Xu, L.; Vivian, R. W. Preparation of peptidomimetics as modulators of pharmacokinetic properties of therapeutics by inhibiting cytochrome P450 monooxygenase. Patent Application WO 2007-US15604 20070706, 2008.

[2] The intramolecular cyclization reactions take place during the workup and flash chromatography purification steps. As a result, we were unable to isolate pure samples of compounds **2a**-**b**.

[3] D. J. Dixon, S. V. Ley, D. A. Longbottom, *J. Chem. Soc., Perkin Trans. 1*, **1999**, 2231-2232.

[4] J. S. McMurray, D. R. Coleman IV, W. Wang, M. L. Campbell, *Biopolymers*, **2001**, *60*, 3-31.

[5] L. A. Carpino, G. Y. Han, *J. Org. Chem.* **1972**, *37*, 3404-3409.

[6] S.-Z. Luo, Y.-M. Li, Z.-Z. Chen, H. Abe, L.-P. Cui, H. Nakanishi, X.-R. Qin, Y.-F. Zhao, *Lett. Pept. Sci.* **2003**, *10*, 57-62.

[7] M. M. Bradford, *Anal. Biochem.* **1976**, *72*, 248-254.