

Electronic Supporting Information

**Enediyne-based Protein Capture Agent:
Demonstration of Enediyne Moiety Acting As
Photoaffinity**

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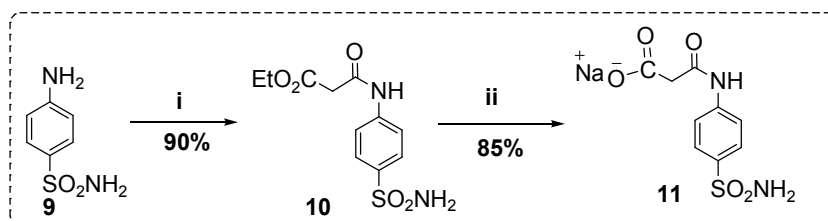
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Experimental Section

General Remarks:

All the reactions were monitored by TLC using polygram^R SILG/UV254 precoated (0.25 mm) silica gel TLC plates. Column chromatography was done with silica gel (60-120 or 230-400 mesh). NMR data were recorded at 400 MHz and 600 MHz NMR instruments in CDCl₃ unless mentioned otherwise. Proton and carbon spectra were referenced internally to solvent signals, using values of $\delta = 2.49$ for proton and $\delta = 39.5$ for carbon (middle peak) in d₆-DMSO and of $\delta = 7.26$ for proton and $\delta = 77.23$ for carbon (middle peak) in CDCl₃. The following abbreviations are used to describe peak patterns where appropriate: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, app. = apparently, and b = broad signal. All coupling constants (J) are given in Hz. Mass spectra were recorded in ESI+ mode (ion trap). All the solvents for column chromatography were distilled prior to use. For column chromatographic purifications, ethyl acetate (EA/EtOAc) and petroleum ether (PE) of boiling range 60–80 °C were used as eluents. HCA II was purified according our published protocol.⁶



Reagents and Conditions: i) Ethyl malonyl chloride, dry THF, K₂CO₃, ice-cold, 30 min; ii) NaOH solution, MeOH:THF (1:1), rt, 3 h

Synthesis of Na-salt of malonyl sulfanilamide

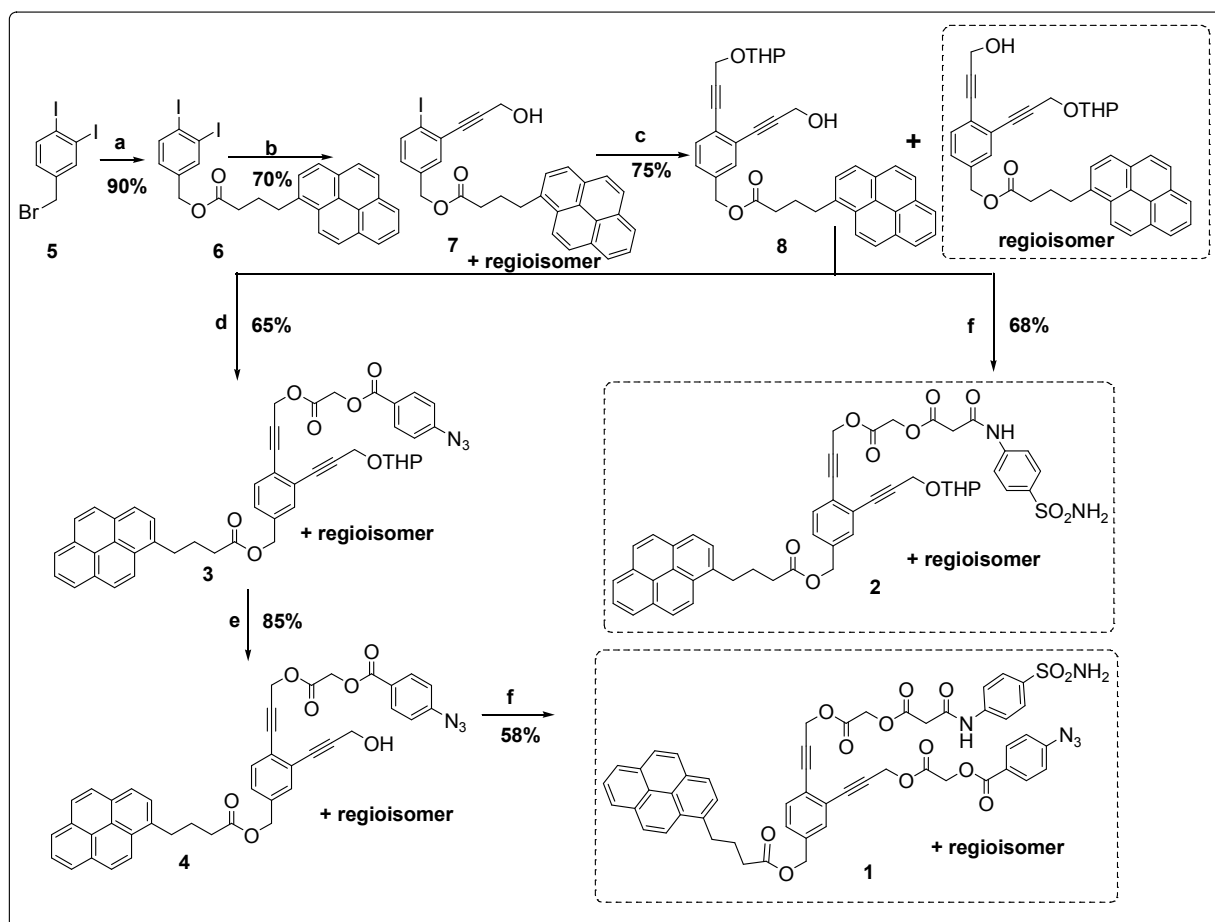
Synthesis of compound 10

To a solution of 4-amino benzenesulfonamide (500 mg, 2.90 mmol) **9** in dry THF (20 ml) at ice-cold temperature was added anhydrous K₂CO₃ (1002 mg, 7.25 mmol) followed by dropwise addition of ethyl malonyl chloride (0.34 ml, 3.48 mmol) under inert atmosphere. The reaction was then allowed to stir for

30 min at ice-cold temperature and then quenched with water and extracted with ethylacetate (20 ml×3). The combined organic layer was then washed with brine, dried with anhydrous sodium sulfate and evaporated to give the crude product **10** that was purified by repeated precipitation from a mixture of DCM-MeOH-Hexane.

Synthesis of compound **11**

N-(4-Sulfamoyl-phenyl)-malonamic acid ethyl ester **10** (500 mg, 1.75 mmol) was dissolved in MeOH+THF (1:1) (15 ml) and a solution of NaOH (105.2 mg, 2.63 mmol in 1.0 ml of water) was added and the mixture was allowed to stir at room temperature for 3 h within which all the starting materials disappeared as visualized by thin layer chromatography. The solvent was then evaporated and the crude product was purified by repeated precipitation from DCM-MeOH-Hexane. The Na-salt was then dried in vacuum and used directly for next step.



Reagents and Conditions: a) Pyrene butyric acid, Anhydrous K_2CO_3 , dry DMF, rt, overnight; b) Propargyl alcohol, $PdCl_2(PPh_3)_2$, CuI, Et_3N , dry DMF, 0 °C-25 °C, 3 h; c) mono-THP protected propargyl alcohol, $PdCl_2(PPh_3)_2$, CuI, Et_3N , dry DMF, rt, overnight; d) i) Bromoacetyl Chloride, dry DCM, Et_3N , ice-cold, 20 min, ii) Na salt of p-azido benzoic acid, dry DMF, rt, overnight; e) PPTS (cat), EtOH, 40 °C, 3 h; f) i) Bromoacetyl Chloride, dry DCM, Et_3N , ice-cold, 20 min, ii) Na salt of N-(4-Sulfamoyl-phenyl)-oxalamic acid (11), dry DMF, rt, overnight.

Synthesis of Capture compounds 1 and 2

Synthesis of compound 6

4-bromomethyl-1,2-diiodo-benzene **5** was prepared by following a reported procedure.¹² To a solution of **5** (400 mg, 0.95 mmol) in dry DMF (10 ml) was added anhydrous K_2CO_3 (197 mg, 1.42 mmol) followed by pyrene butyric acid (274 mg, 0.95mmol) under N_2 atmosphere and the mixture was stirred at room temperature overnight. The reaction was then quenched by addition of water and extracted with ethyl acetate (15 ml×2). The combined organic layer was then washed with brine, dried over anhydrous sodium sulfate, concentrated and the crude product was purified by column chromatography by using hexane-ethyl acetate as eluent.

Synthesis of compound 7

To a degassed solution of pyrene butyric acid ester **6** (300 mg, 0.48 mmol) and propargyl alcohol (0.026 ml, 0.48 mmol) in dry DMF (10 ml) was added $PdCl_2(PPh_3)_2$ (10 mg, 0.014 mmol), dry Et_3N (0.1 ml, 0.72 mmol) and CuI (19 mg, 0.096 mmol) under inert atmosphere at 0 °C and the mixture was allowed to stir at 25 °C for 3 h. The temperature and time should be carefully maintained in order to obtain the mono propargylated product exclusively. The reaction was then quenched by addition of a saturated solution of NH_4Cl and the crude products were extracted in ethyl acetate (10 ml×2). The ethyl acetate layer was then dried over anhydrous sodium sulfate, evaporated and the purified product was obtained *via* column chromatography by using hexane-ethyl acetate as eluent.

Synthesis of compound 8

Compound 7 (200 mg, 0.34 mmol) and mono-THP protected propargyl alcohol were dissolved in dry DMF (8 ml) and degassed for 30 min. After that PdCl₂(PPh₃)₂ (7 mg, 0.01 mmol), dry Et₃N (0.07 ml, 0.51 mmol) and CuI (13 mg, 0.068 mmol) was added into the solution under N₂ atmosphere at room temperature and the mixture was stirred overnight. The reaction mixture was then poured into ethyl acetate (15 mL) and the organic layer was washed with saturated NH₄Cl solution and brine (30 mL each), dried over anhydrous Na₂SO₄. Evaporation of solvent left an oily residue from which the product was isolated by column chromatography (Silica-gel, petroleum ether-ethyl acetate mixture as eluent).

Synthesis of compound 3

To a solution of compound 8 (100 mg, 0.18 mmol) in dry DCM (5 ml) was added dry Et₃N (0.05 ml, 0.36 mmol) followed by bromoacetyl chloride (0.03 ml, 0.27 mmol) at ice cold temperature and the reaction was stirred at ice cold condition for 20 min. After that the reaction was quenched by addition of water and extracted with DCM (10 ml×2). The combined organic layer was washed with brine, dried over anhydrous Na₂SO₄ and evaporated under vacuum. The crude bromoacetylated product was then dissolved in dry DMF (5 ml) and Na-salt of p-azido benzoic acid (100 mg, 0.54 mmol) was added into it and the reaction mixture was stirred at room temperature overnight. The reaction was then quenched with water and the crude product 3 was extracted in ethyl acetate (10 ml×3). The ethyl acetate layer was washed with brine, dried over anhydrous Na₂SO₄, concentrated and purified by column chromatography by using hexane-ethyl acetate as eluent.

Synthesis of compound 2

Compound 8 (50 mg, 0.09 mmol) was taken in dry DCM (3 ml) and cooled to ice-cold temperature. To the solution was then added dry Et₃N (0.03 ml, 0.18 mmol) followed by bromoacetyl chloride (0.01 ml, 0.135 mmol) and the reaction was allowed to stir at ice cold temperature for 20 min. The reaction was then quenched with water and after performing usual work up procedure the crude bromoacetylated product was isolated. It was then dissolved in dry DMF (3 ml) and Na salt of *N*-(4-Sulfamoyl-phenyl)-

oxalamic acid **11** (75.6 mg, 0.27 mmol) was added into it under inert atmosphere and the reaction was stirred overnight at room temperature. The reaction was then quenched by diluting the reaction mixture with water and ethyl acetate. The water layer was extracted with ethyl acetate (10 ml) thrice and the combined organic layer was washed with brine, dried over anhydrous Na₂SO₄, concentrated and the crude product **2** was purified by column chromatography by using hexane-ethyl acetate as eluent.

Synthesis of compound 1

To a solution of compound **4** (40 mg, 0.06 mmol) yielded after deprotection of THP from Compound **3** in dry DCM was added dry Et₃N (0.02 ml, 0.12 mmol) and bromoacetyl chloride (0.01 ml, 0.10 mmol) in ice cold temperature and stirred at ice cold condition for 20 min. The reaction was quenched with water and extracted with DCM (5 ml×3). The crude product isolated after following usual work up procedure was dissolved in dry DMF (2 ml) and Na salt of *N*-(4-Sulfamoyl-phenyl)-oxalamic acid **11** (50 mg, 0.18 mmol) was added into it and the reaction mixture was stirred at room temperature overnight. After that the reaction mixture was quenched by addition of water, extracted the crude product **1** in ethyl acetate (5 ml×3) and the combined organic layer was washed with brine, dried over anhydrous Na₂SO₄, concentrated and purified by column chromatography by using DCM-MeOH as eluent. The product was then purified further by repeated precipitation from DCM-Hexane.

Spectral data of new compounds

N-(4-Sulfamoyl-phenyl)-malonamic acid ethyl ester (**10**)

State: colorless solid; m.p. 182-183 °C; Yield: 748 mg, 90%; R_f = 0.50 (CH₂Cl₂/MeOH 20:1); ¹H NMR (400 MHz, d₆-DMSO) δ 10.52 (s, 1H), 7.78, 7.73 (ABq, 4H, *J* = 8.2 Hz), 7.27 (s, 2H), 4.12 (q, 2H, *J* = 6.8 Hz), 3.50 (s, 2H), 1.20 (t, 3H, *J* = 6.8 Hz); ¹³C NMR (100 MHz, d₆-DMSO) δ 167.5, 164.7, 141.7, 138.7, 126.8, 118.7, 60.7, 43.7, 14.0. HRMS: calcd for C₁₁H₁₄N₂O₅S + H⁺, 287.0702; found, 287.0701.

Sodium; (4-Sulfamoyl-phenylcarbamoyl)-acetate (**11**)

State: colorless solid; Yield: 416 mg, 85%; ^1H NMR (600 MHz, d_6 -DMSO) δ 12.10 (bs, 1H), 7.73 (s, 4H), 7.07 (bs, 2H), 3.03 (s, 2H); ^{13}C NMR (150 MHz, d_6 -DMSO) δ 170.9, 168.9, 142.1, 138.1, 126.7, 118.4, 45.9. HRMS: calcd for $\text{C}_9\text{H}_{10}\text{N}_2\text{O}_5\text{S} + \text{H}^+$, 259.0389; found, 259.0384.

4-Pyrene-1-yl-butyric acid 3,4-diiodo-benzyl ester (6)

State: gummy mass; Yield: 537 mg, 90%; $R_f = 0.50$ (Hexane/EtOAc 10:1); ^1H NMR (600 MHz, CDCl_3) δ 8.23-8.18 (m, 3H), 8.10-8.03 (m, 5H), 7.80-7.78 (m, 2H), 7.64 (d, $J = 7.8$ Hz, 1H), 6.74 (dd, $J = 8.4$ Hz, 1.8 Hz, 1H), 3.32 (t, $J = 7.2$ Hz, 2H), 2.48 (t, $J = 7.2$ Hz, 2H), 2.25-2.20 (m, 2H); ^{13}C NMR (150 MHz, CDCl_3) δ 172.5, 138.9, 138.2, 137.1, 135.1, 131.0, 130.5, 129.6, 128.4 ($\times 2$), 127.2 ($\times 2$), 127.0, 126.5, 125.6, 124.7 ($\times 2$), 124.6, 122.9, 108.0, 107.4, 64.0, 33.4, 32.2, 26.3. HRMS: calcd for $\text{C}_{27}\text{H}_{20}\text{I}_2\text{O}_2 + \text{H}^+$, 630.9631; found, 630.9632.

4-Pyrene-1-yl-butyric acid 4-(3-hydroxy-prop-1-ynyl)-3-iodo-benzyl ester + 4-Pyrene-1-yl-butyric acid 3-(3-hydroxy-prop-1-ynyl)-4-iodo-benzyl ester (7)

State: buff colored sticky solid; Yield: 186 mg, 70%; $R_f = 0.50$ (Hexane/EtOAc 3:1); ^1H NMR (600 MHz, CDCl_3) δ 8.26-8.23 (m, 1H), 8.16 (t, $J = 6.6$ Hz, 2H), 8.11-8.06 (m, 2H), 8.02-7.98 (m, 3H), 7.83 (d, $J = 7.8$ Hz, 1H), 7.78 (d, $J = 8.4$ Hz, 1H), 7.44-7.42 (m, 1H), 6.95 (d, $J = 9.6$ Hz, 1H), 5.02-5.00 (m, 2H), 4.52 (t, $J = 14.4$ Hz, 2H), 3.38 (t, $J = 7.2$ Hz, 2H), 2.52-2.49 (m, 2H), 2.23-2.19 (m, 2H); ^{13}C NMR (150 MHz, CDCl_3) δ 173.3 ($\times 2$), 139.0, 138.2, 137.8, 136.2, 135.6, 135.5, 132.8, 132.4, 131.5, 131.0, 130.1, 129.5, 129.4, 129.1, 128.8, 127.6, 127.5, 126.9, 126.0, 125.2, 125.1, 124.9, 123.3, 100.9, 100.4, 91.8, 91.7, 87.3 ($\times 2$), 65.1, 64.8, 51.7 ($\times 2$), 33.9 ($\times 2$), 32.8, 26.8 ($\times 2$). HRMS: calcd for $\text{C}_{30}\text{H}_{23}\text{IO}_3 + \text{H}^+$, 559.0770; found, 559.0775.

4-Pyrene-1-yl-butyric acid 4-(3-hydroxy-prop-1-ynyl)-3-[3-(tetrahydro-pyran-2-yloxy)-prop-1-ynyl]-benzyl ester + 4-Pyrene-1-yl-butyric acid 3-(3-hydroxy-prop-1-ynyl)-4-[3-(tetrahydro-pyran-2-yloxy)-prop-1-ynyl]-benzyl ester (8)

State: yellow gummy mass; Yield: 153 mg, 75%; $R_f = 0.30$ (Hexane/EtOAc 3:1); ^1H NMR (600 MHz, CDCl_3) δ 8.23 (d, $J = 9.0$ Hz, 1H), 8.15 (t, $J = 7.2$ Hz, 2H), 8.08 (t, $J = 8.4$ Hz, 2H), 8.01-7.97 (m, 3H), 7.81 (d, $J = 7.8$ Hz, 1H), 7.43-7.37 (m, 2H), 7.21-7.19 (m, 1H), 5.22 (merged m, 1H), 5.05 (s, 2H), 4.99-4.50 (m, 4H), 3.86 (t, $J = 10.8$ Hz, 1H), 3.63-3.61 (m, 1H), 3.36 (t, $J = 7.2$ Hz, 2H), 2.49 (t, $J = 7.2$ Hz, 2H), 2.23-2.18 (m, 2H), 1.85-1.53 (m, 6H); ^{13}C NMR (150 MHz, CDCl_3) δ 173.3, 136.5, 136.2, 135.6, 131.9, 131.7, 131.5, 131.3, 131.0 ($\times 2$), 130.1, 128.8, 128.0, 127.8, 127.6 ($\times 2$), 127.5, 126.9, 126.4, 126.0, 125.9, 125.7, 125.2 ($\times 2$), 125.1 ($\times 2$), 123.3, 95.5, 92.8, 92.7, 89.3, 89.2, 85.0, 83.7, 65.3, 61.6, 54.7, 54.6, 51.5 ($\times 2$), 33.9, 32.8, 31.7, 30.1, 26.8, 25.5, 22.8. HRMS: calcd for $\text{C}_{38}\text{H}_{34}\text{O}_5 + \text{H}^+$, 571.2485; found, 571.2484.

4-Azido-benzoic acid 3-{4-(4-pyrene-1-yl-butyryloxymethyl)-2-[3-(tetrahydro-pyran-2-yloxy)-prop-1-ynyl]-phenyl}-prop-2-ynyloxycarbonylmethyl ester + 4-Azido-benzoic acid 3-{5-(4-pyrene-1-yl-butyryloxymethyl)-2-[3-(tetrahydro-pyran-2-yloxy)-prop-1-ynyl]-phenyl}-prop-2-ynyloxycarbonylmethyl ester (3)

State: brown gummy mass; Yield: 88 mg, 65%; $R_f = 0.20$ (Hexane/EtOAc 3:1); ^1H NMR (600 MHz, CDCl_3) δ 8.25 (dd, $J = 9.3$ Hz, 2.4 Hz, 1H), 8.16 (t, $J = 7.2$ Hz, 2H), 8.11-8.07 (m, 4H), 8.02-7.98 (m, 3H), 7.84 (dd, $J = 7.8$ Hz, 1.2 Hz, 1H), 7.45 (bs, 1H), 7.42 (dd, $J = 8.4$ Hz, 3.0 Hz, 1H), 7.25-7.21 (m, 1H), 7.09-7.06 (m, 2H), 5.07-5.06 (merged s, 4H), 4.97-4.96 (m, 1H), 4.93-4.90 (merged s, 2H), 4.56-4.49 (m, 2H), 3.88-3.84 (m, 1H), 3.57-3.54 (m, 1H), 3.39 (t, $J = 7.8$ Hz, 2H), 2.51 (t, $J = 6.6$ Hz, 2H), 2.25-2.20 (m, 2H); ^{13}C NMR (150 MHz, CDCl_3) δ 173.3, 167.4, 165.2 ($\times 2$), 136.9, 136.4, 135.7, 132.6, 132.5, 132.0 ($\times 2$), 131.9, 131.8, 131.6, 131.1, 130.2, 129.0, 128.5, 128.0, 127.7 ($\times 2$), 127.6, 126.9, 126.1, 125.3, 125.2, 125.1, 125.0 ($\times 2$), 123.4, 119.2, 119.1, 96.7, 90.2, 90.1, 86.5, 86.4, 85.6, 83.9, 65.4,

62.1, 61.2 (×2), 54.8 (×2), 53.6, 34.0, 32.9, 31.8, 26.9, 25.6, 22.8, 19.0. HRMS: calcd for C₄₇H₃₉N₃O₈ + H⁺, 774.2815; found, 774.2820.

4-Pyren-1-yl-butyric acid 4-(3-{2-[2-(4-sulfamoyl-phenylcarbamoyl)-acetoxy]-acetoxy}-prop-1-ynyl)-3-[3-(tetrahydro-pyran-2-yloxy)-prop-1-ynyl]-benzyl ester + 4-Pyren-1-yl-butyric acid 3-(3-{2-[2-(4-sulfamoyl-phenylcarbamoyl)-acetoxy]-acetoxy}-prop-1-ynyl)-4-[3-(tetrahydro-pyran-2-yloxy)-prop-1-ynyl]-benzyl ester (2)

State: yellow sticky mass; Yield: 52 mg, 68%; R_f = 0.20 (Hexane/EtOAc 1:1); ¹H NMR (600 MHz, CDCl₃) δ 9.27 (d, *J* = 12.0 Hz, 1H), 8.25-8.23 (m, 1H), 8.17-8.15 (m, 2H), 8.11-8.07 (m, 2H), 8.02-7.98 (m, 3H), 7.83-7.81 (m, 3H), 7.71 (app d, *J* = 8.4 Hz, 2H), 7.44-7.39 (m, 2H), 7.23 (d, *J* = 8.4 Hz, 1H), 5.08-5.05 (merged s, 4H), 4.96-4.94 (m, 1H), 4.85 (s, 1H), 4.80 (s, 1H), 4.55-4.48 (m, 2H), 3.89-3.83 (m, 1H), 3.64 (d, *J* = 16.8 Hz, 2H), 3.56-3.54 (m, 1H), 3.39-3.36 (m, 2H), 2.52-2.49 (m, 2H), 2.24-2.17 (m, 2H), 1.86-1.63 (m, 6H); ¹³C NMR (150 MHz, CDCl₃) δ 173.4 (×2), 168.1, 168.0, 167.7 (×2), 163.0, 147.3, 141.7, 139.5, 137.5, 137.2, 136.5, 135.7, 132.6 (×2), 131.9, 131.8, 131.6, 131.1, 130.2, 128.9, 128.6, 128.0, 127.8, 127.7, 127.6, 127.0, 126.9, 125.3, 125.2, 125.1, 125.0, 123.4 (×2), 120.1, 114.3, 96.8, 90.1 (×2), 86.0 (×2), 85.9 (×2), 84.0, 65.3, 62.2, 61.5, 54.8, 54.5, 34.0, 32.9, 32.1, 31.6, 29.6, 26.9, 22.9, 19.1; ν_{max} (KBr, cm⁻¹): 3122, 3031, 2960, 2874, 1751, 1597, 1281, 1159, 1135, 845, 768; λ_{max} (CH₃CN): 236 (35030 M⁻¹ cm⁻¹), 263 (27636 M⁻¹ cm⁻¹), 273 (31515 M⁻¹ cm⁻¹), 326 (8484 M⁻¹ cm⁻¹), 341 (13333 M⁻¹ cm⁻¹), 396 (363 M⁻¹ cm⁻¹); HRMS: calcd for C₄₉H₄₄N₂O₁₁S + Na⁺, 891.2564; found, 891.2540.

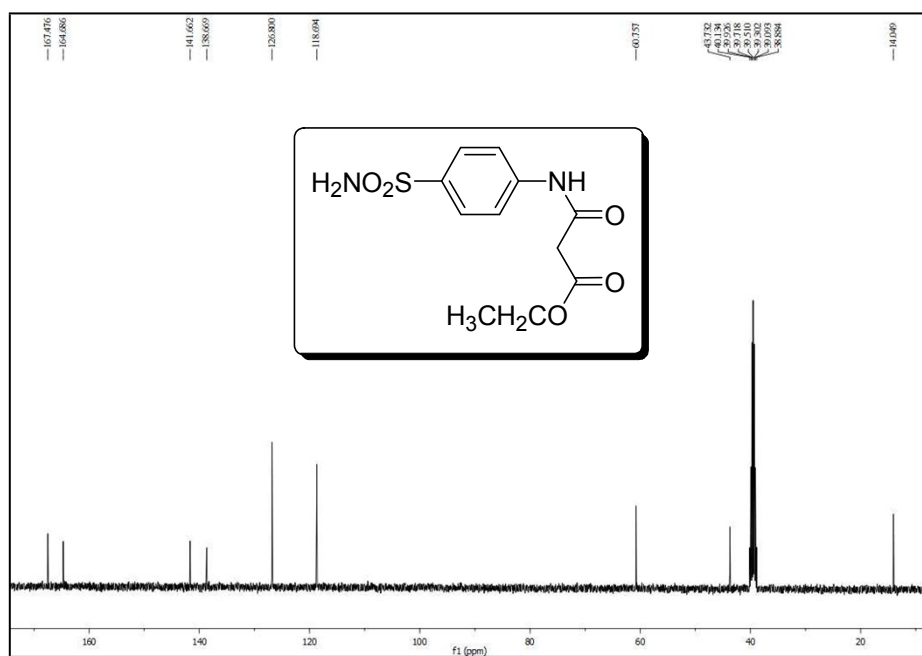
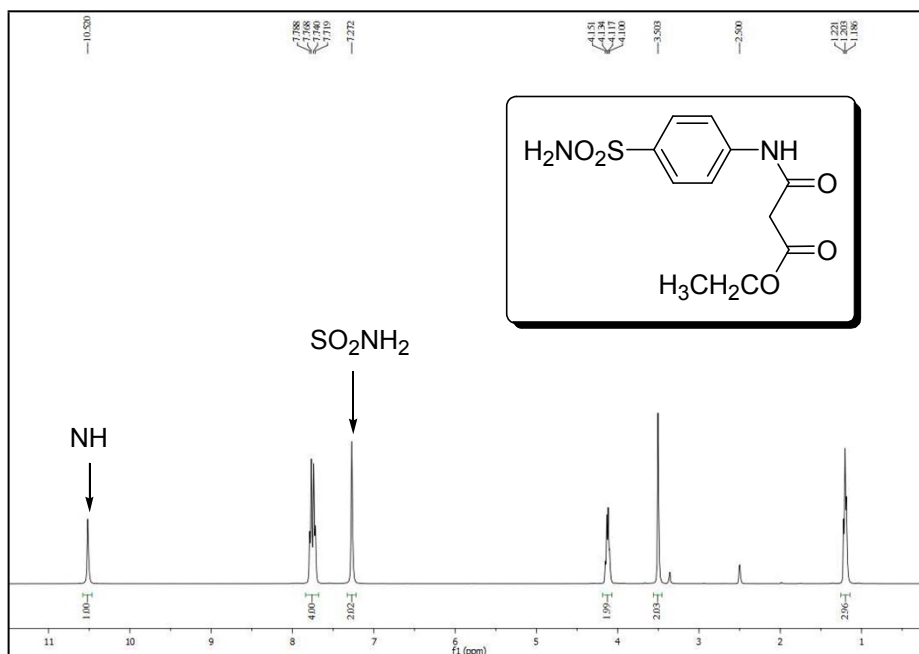
4-Azido-benzoic acid 3-[5-(4-pyrene-1-yl-butyryloxymethyl)-2-(3-{2-[2-(4-sulfamoyl-phenylcarbamoyl)-acetoxy]-acetoxy}-prop-1-ynyl)-phenyl]-prop-2-ynyloxy carbonylmethyl ester + 4-Azido-benzoic acid 3-[4-(4-pyrene-1-yl-butyryloxymethyl)-2-(3-{2-[2-(4-sulfamoyl-phenylcarbamoyl)-acetoxy]-acetoxy}-prop-1-ynyl)-phenyl]-prop-2-ynyloxy carbonylmethyl ester (1)

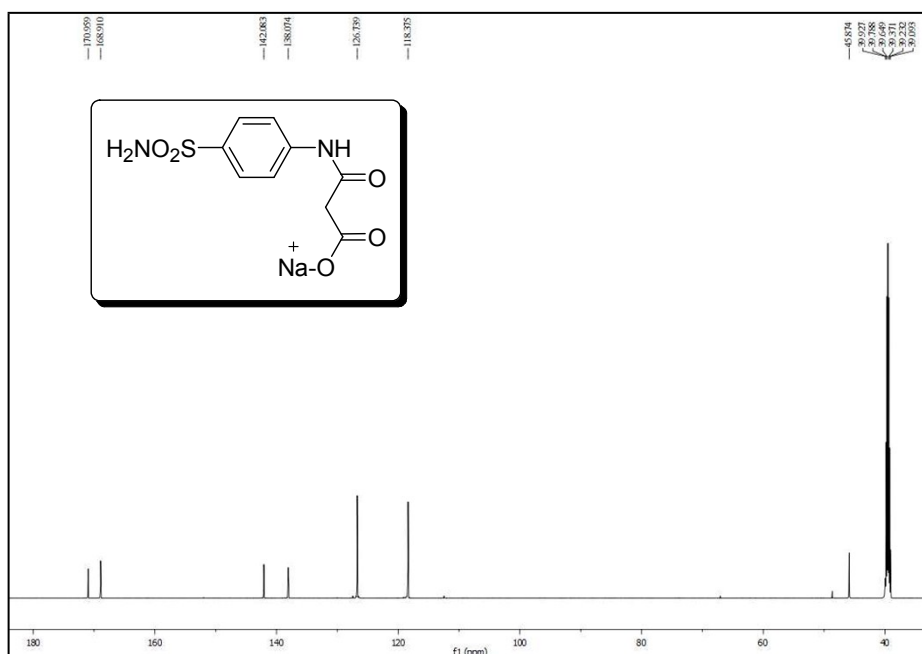
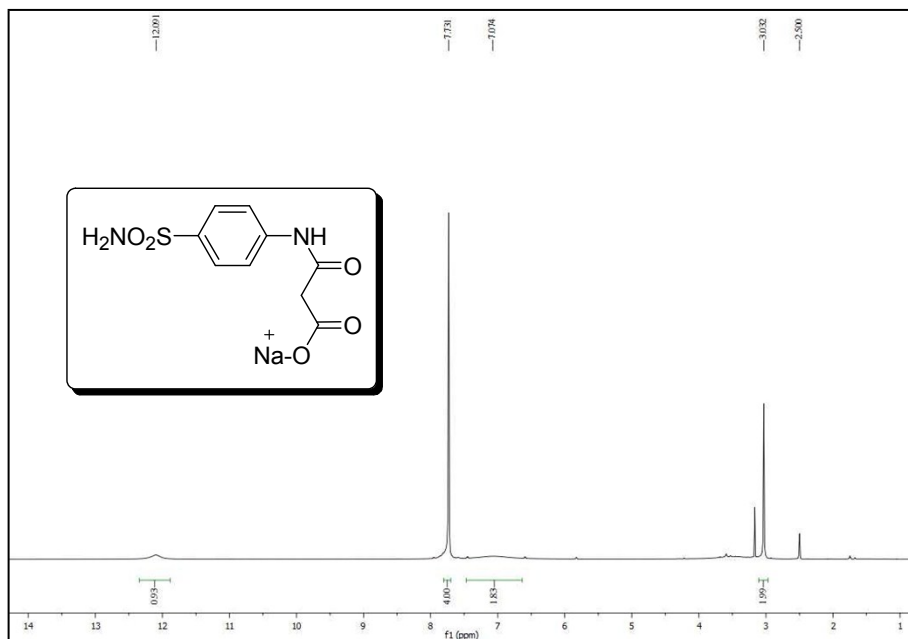
State: brown solid; Yield: 25 mg, 58%; $R_f = 0.30$ ($\text{CH}_2\text{Cl}_2/\text{MeOH}$ 10:1); ^1H NMR (600 MHz, CDCl_3) δ 9.21-9.16 (bm, 1H), 8.23-8.22 (m, 1H), 8.16-8.14 (m, 2H), 8.09-7.96 (m, 6H), 7.82-7.64 (m, 6H), 7.43-7.37 (m, 2H), 7.24-7.22 (m, 1H), 7.03-7.02 (m, 2H), 5.06-4.76 (m, 10H), 3.64-3.60 (bm, 2H), 3.37-3.35 (bm, 2H), 2.50-2.49 (m, 2H), 2.20-2.17 (bm, 2H); ^{13}C NMR (150 MHz, CDCl_3) δ 173.4 ($\times 2$), 167.7, 167.5, 165.4, 165.3, 163.1, 145.7, 145.6, 141.7, 141.6, 139.5, 137.4, 137.2, 137.1, 135.7, 132.7, 132.6, 132.0, 131.8, 131.6, 131.1, 130.2, 128.9, 128.5 ($\times 2$), 127.7 ($\times 2$), 127.6, 127.0, 126.1, 125.3, 125.2, 125.0, 123.7, 123.4, 120.4, 120.0, 119.2, 116.1, 114.3, 87.0, 86.9 ($\times 2$), 86.8, 85.4, 85.3, 85.1 ($\times 2$), 65.2, 61.2 ($\times 2$), 53.9, 53.6, 33.9, 32.8, 29.9, 26.9; ν_{max} (KBr, cm^{-1}): 3123, 3035, 2961, 2929, 2870, 2120, 1752, 1597, 1410, 1282, 1160, 1138, 846, 767; λ_{max} (CH_3CN): 238 ($36125 \text{ M}^{-1} \text{ cm}^{-1}$), 264 ($28500 \text{ M}^{-1} \text{ cm}^{-1}$), 273 ($33125 \text{ M}^{-1} \text{ cm}^{-1}$), 326 ($9375 \text{ M}^{-1} \text{ cm}^{-1}$), 341 ($14375 \text{ M}^{-1} \text{ cm}^{-1}$), 399 ($750 \text{ M}^{-1} \text{ cm}^{-1}$); HRMS: calcd for $\text{C}_{53}\text{H}_{41} \text{N}_5\text{O}_{13}\text{S} + \text{Na}^+$, 1010.2319; found, 1010.2346.

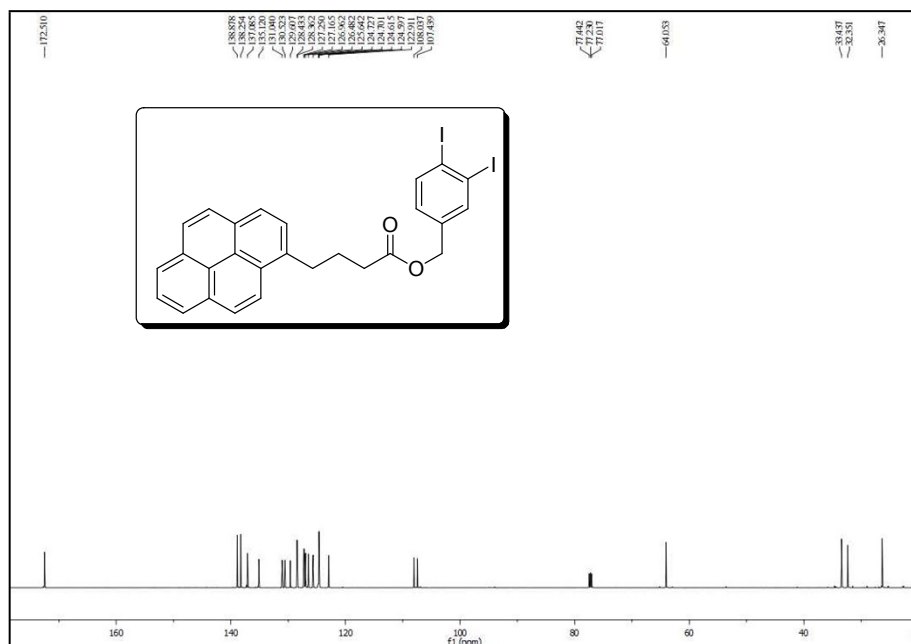
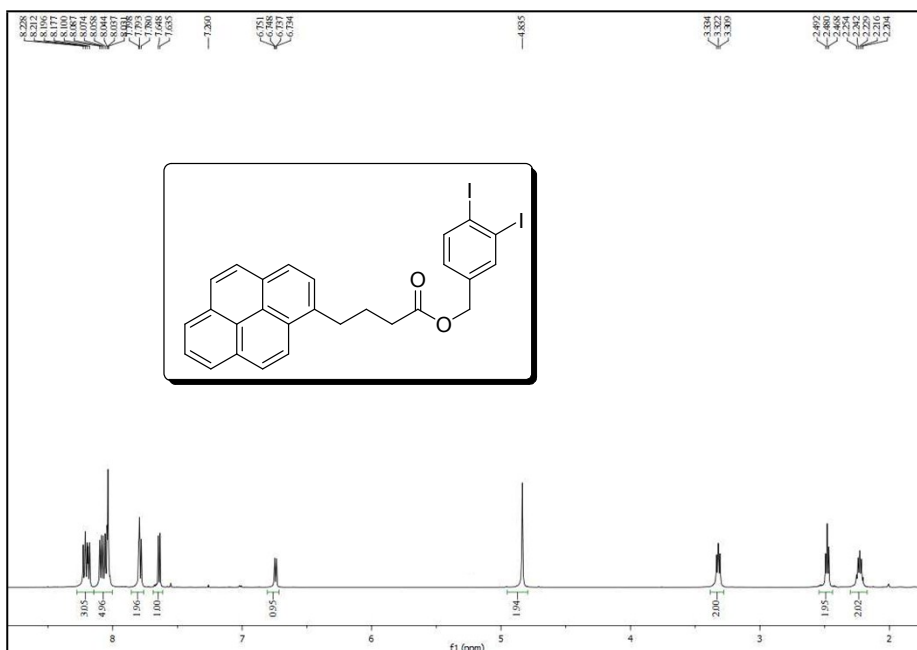
Trypsin digestion and Matrix Assisted Laser Desorption Ionization Spectrometry (MALDI analyses): The molecular mass of the protein-capture compound complex was determined by mass spectrometry after digesting the protein bands with Trypsin. In brief, protein bands were chopped from SDS-PAGE and emerged in 100 μL of 100 mM Ammonium bicarbonate and 100 % Acetonitrile in 1:1 (V/V). The samples were agitated occasionally for 30 min. Another 500 μL of Acetonitrile was added to each of the sample followed by 15 min incubation at room temperature. Total volume of liquid supernatant were decanted and the gel pieces were air dried for 15 min. 40 μL of Trypsin (200 ng, sequencing grade, Roche Life Science) was added to each samples and kept for 1h 30 min in ice and then in 37 $^\circ\text{C}$ for overnight. Total solutions were taken in fresh tubes and lyophilized. For mass spectrometric studies, the lyophilized samples were dissolved in 10 μL of 0.1 % Trifluoro acetic acid and 2 μL from the solution was mixed thoroughly with 2 μL of HCCA (α -cyano-4-hydroxycinnamic acid) as matrix. From the mixture, 2 μL was spotted onto 384 well stainless steel MALDI plate and allowed to be air dried prior to MALDI analysis by the ultraFlextreme MALDI Time-of-Flight Mass Spectrophotometer in positive ion mode. The instrument was calibrated for the mass range 600-3500 Da

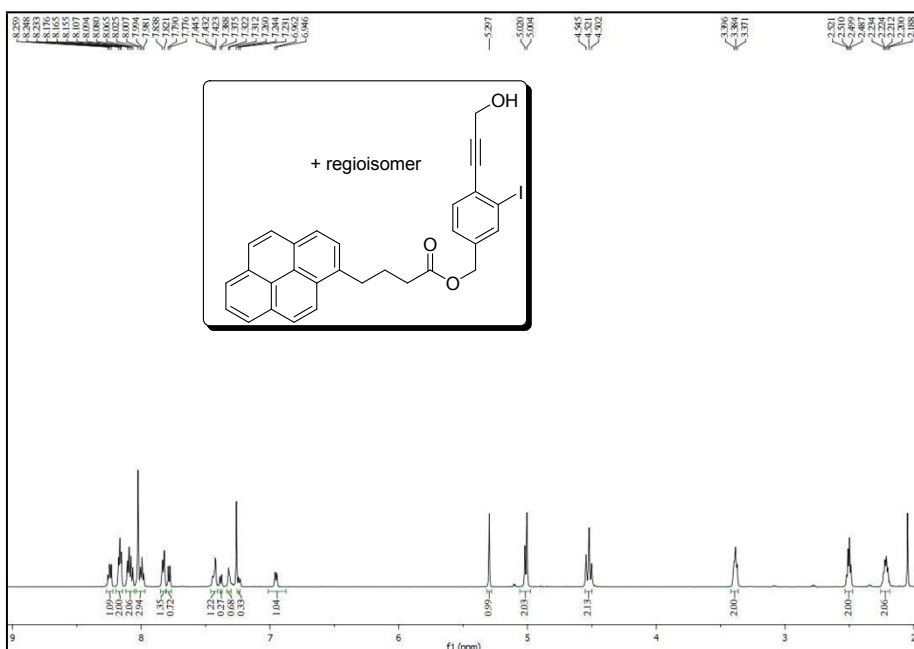
using a standard calibration kit that contains Bradykinin, Angiotensin II, Angiotensin I, Substance_P, Bombesin, Renin_substrate, ACTH_clip (1-17), ACTH_clip (18-39), Somatostatin. By using this kit whole mass range was calibrated in positive ion mode. UV Laser: Smartbeam II (N₂, NdYag), 355 nm wavelength, Laser rep rate 2 KHz, Reflector mode.

SDS-polyacrylamide gel electrophoresis: For SDS-polyacrylamide gel analysis, the samples were mixed with 6X Laemmli buffer (1X buffer composition was 63 mM Tris-HCl ((pH 6.8), 2% SDS, 10% glycerol, 0.1% 2-mercaptoethanol and 0.01% Bromophenol blue) (U. K. Laemmli, Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 1970, **227**, 680-685) and heated at 95 °C for 5 min. 20 µL sample from each mixture was loaded in each well of 12% discontinuous SDS-PAGE (Mini-PROTEAN 3, Multi casting chamber, BioRad Instruments). The electrophoresis was done under denaturing condition. The stacking and resolving gels were composed of 5% (w/v) and 12% (w/v) acrylamide with Tris (pH 6.8 and pH 8.8) respectively, and 0.1% SDS. The composition of electrophoresis buffer was 0.025 M Tris, 0.2 M glycine, pH 8.3 and 0.1% SDS. An electric potential of 160 volt was applied to run the gel until the bromphenol blue dye reached the end of the resolving gel. The gel was visualized under UV light in UVP gel documentation system. The position of the fluorescent bands was confirmed by staining the gels with 0.1% (w /v) Coomassie Brilliant Blue R-250 in 50% (v /v) methanol and 10% (v /v) acetic acid and destained with methanol / acetic acid.

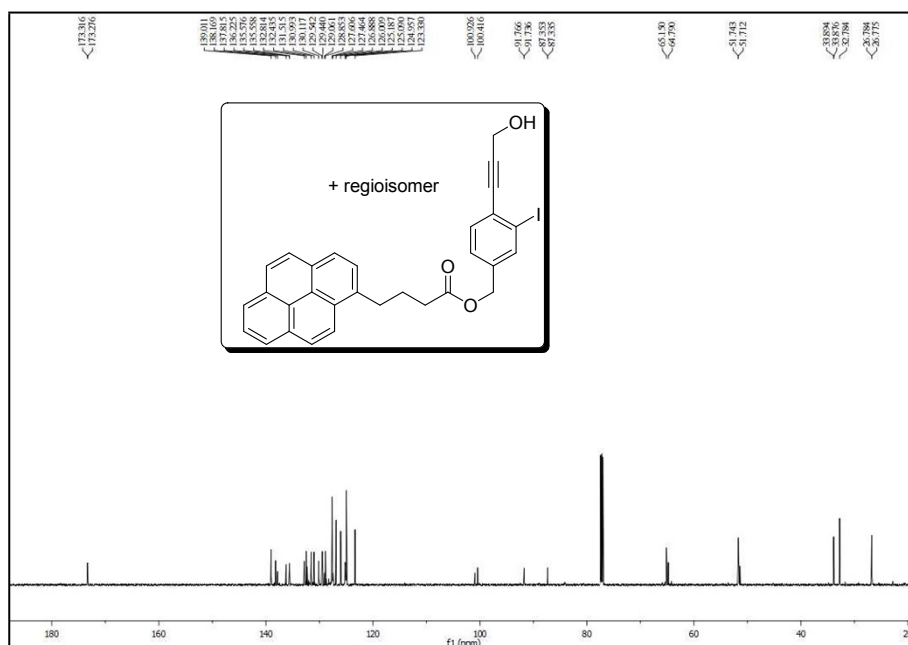




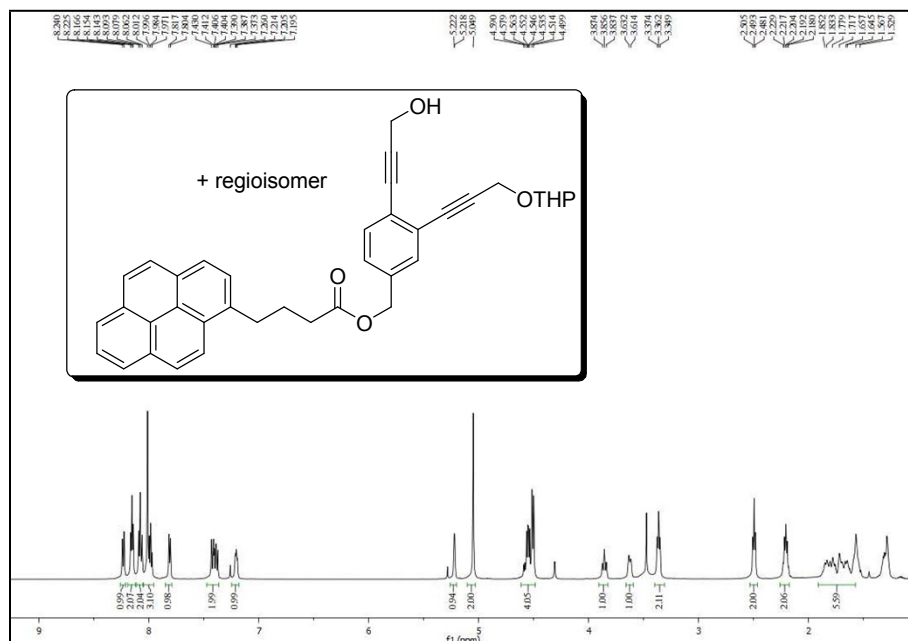




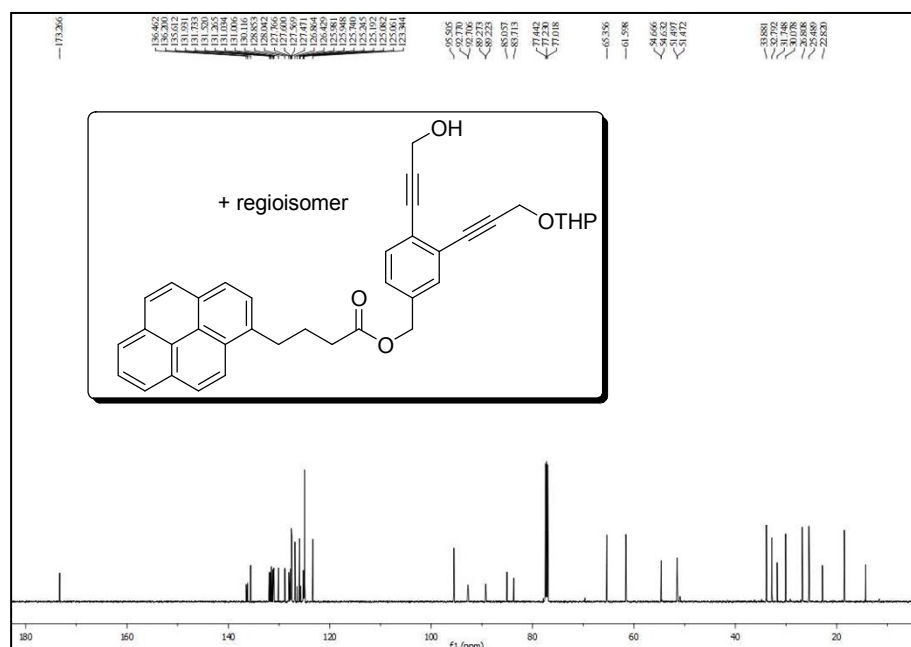
¹H-NMR (600 MHz, CDCl₃) spectrum of compound 7



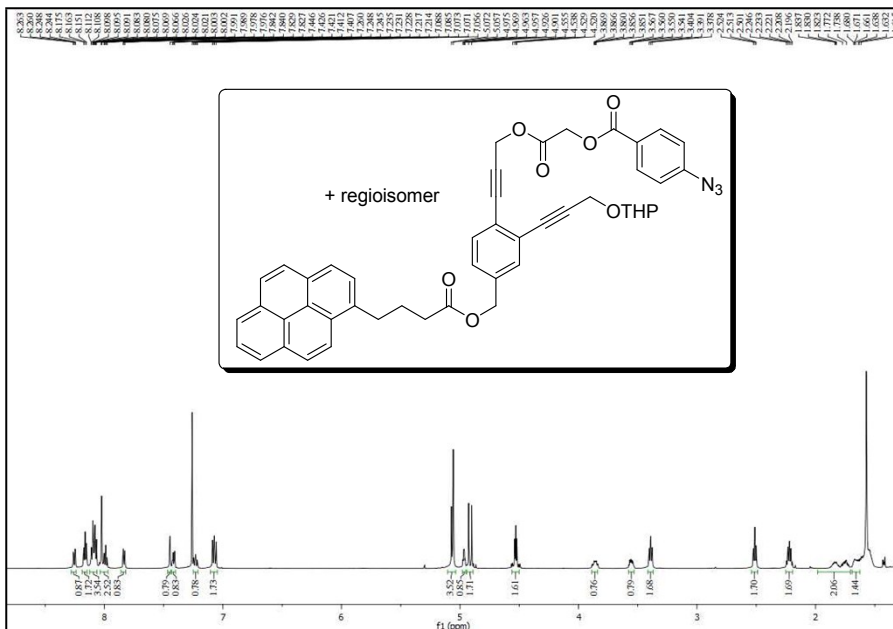
¹³C-NMR (150 MHz, CDCl₃) spectrum of compound 7



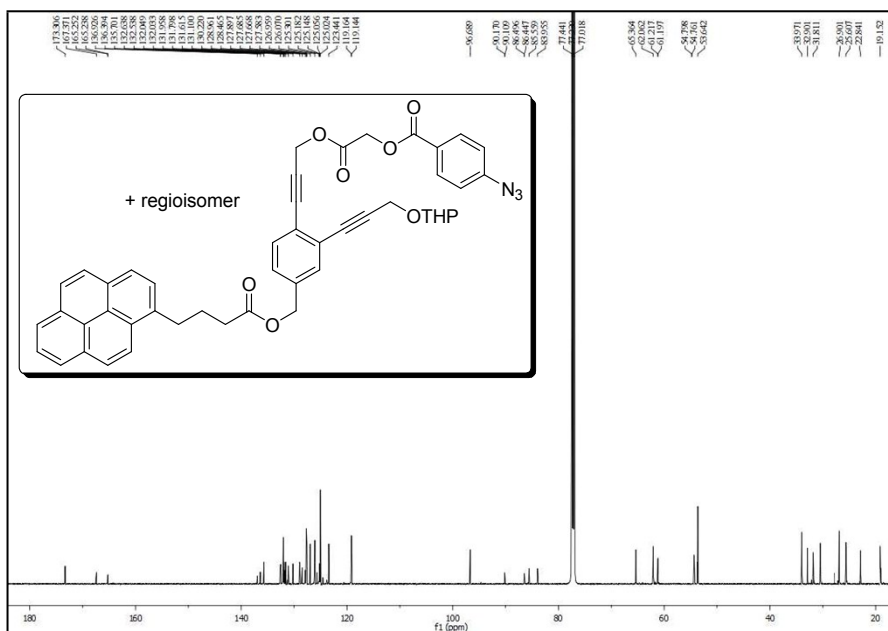
¹H-NMR (600 MHz, CDCl₃) spectrum of compound 8



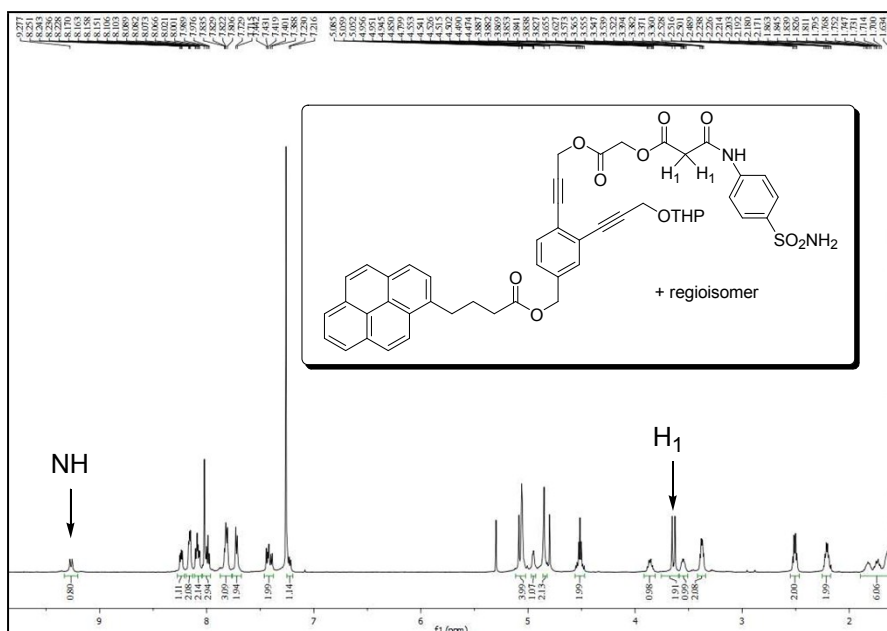
¹³C-NMR (150 MHz, CDCl₃) spectrum of compound 8



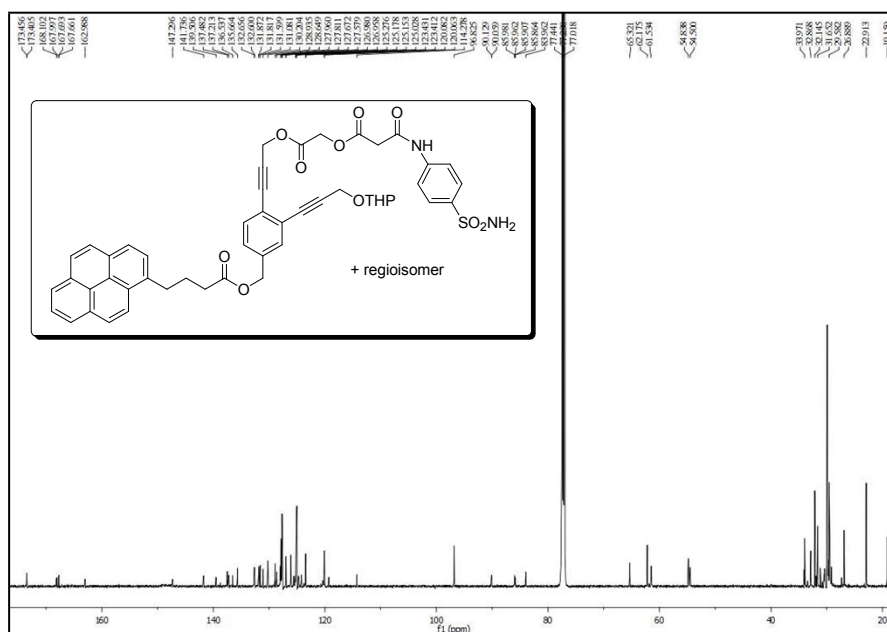
¹H-NMR (600 MHz, CDCl₃) spectrum of compound 3



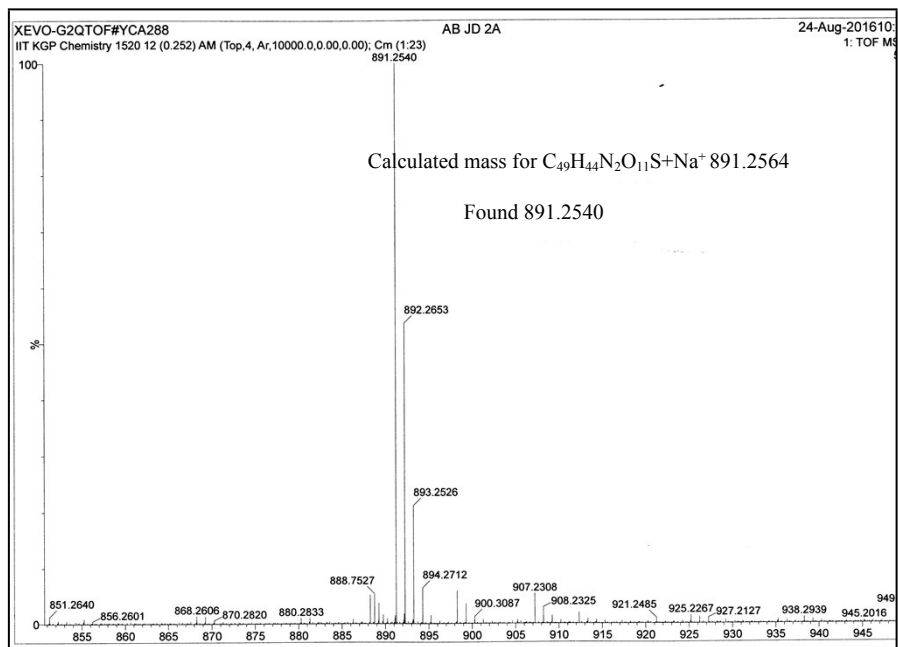
¹³C-NMR (150 MHz, CDCl₃) spectrum of compound 3



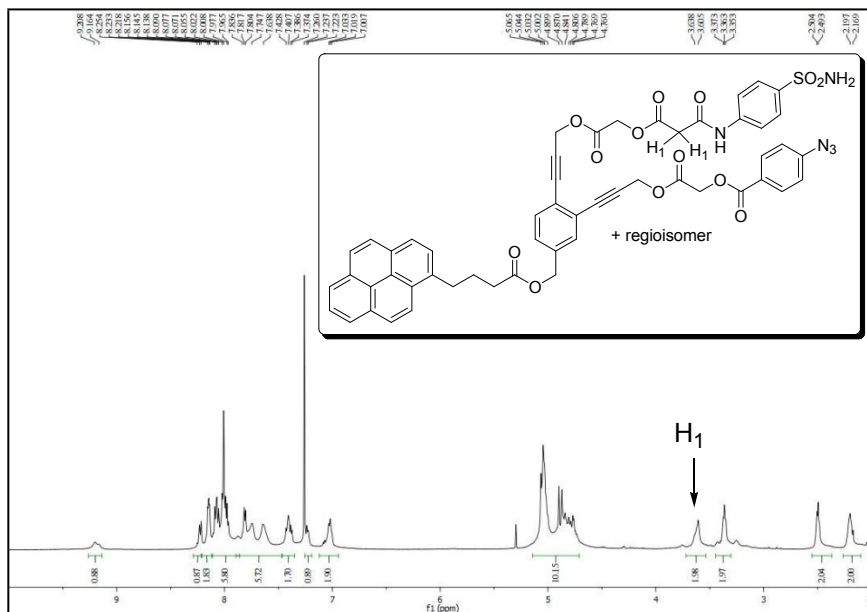
¹H-NMR (600 MHz, CDCl₃) spectrum of compound 2



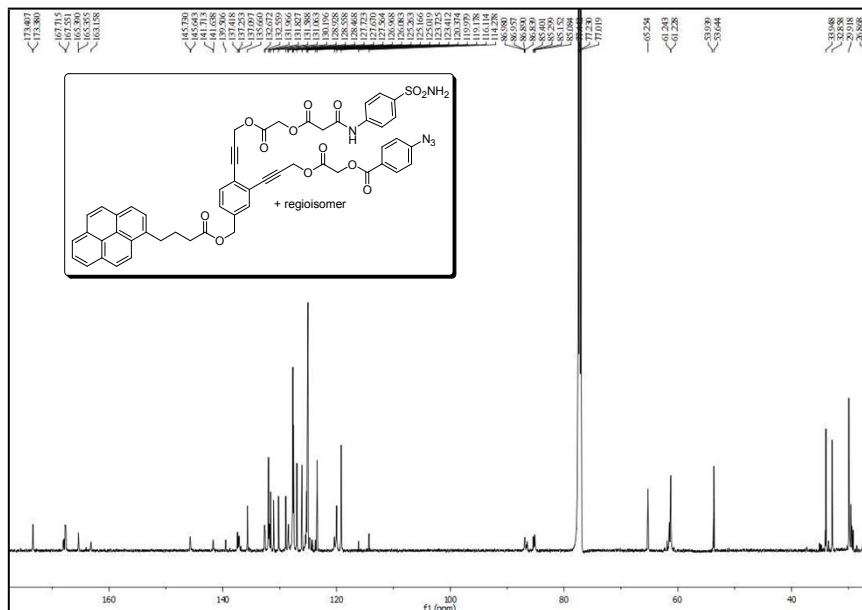
¹³C-NMR (150 MHz, CDCl₃) spectrum of compound 2



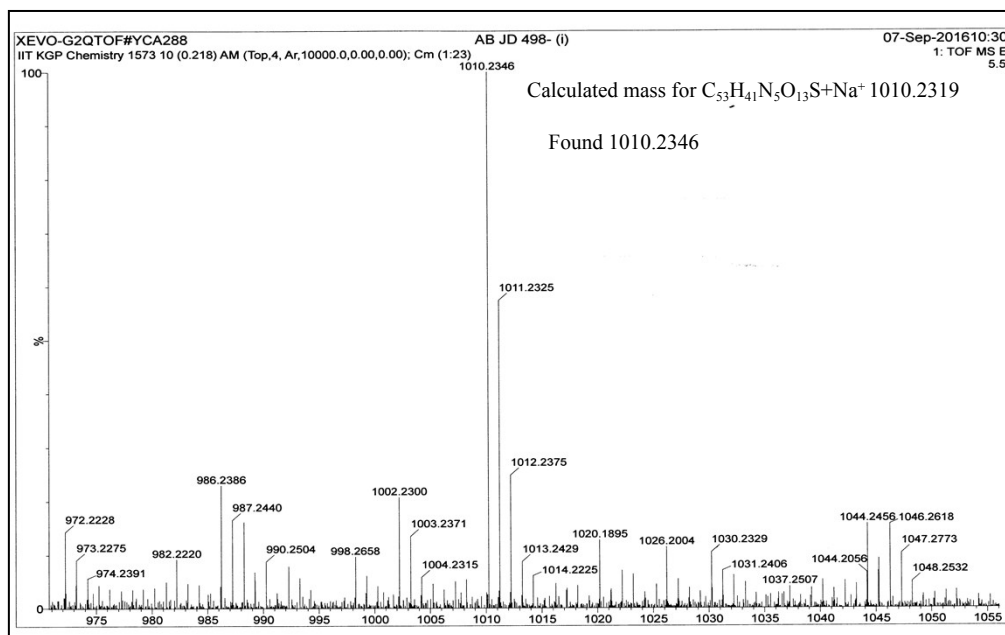
HRMS Spectrum of Compound 2



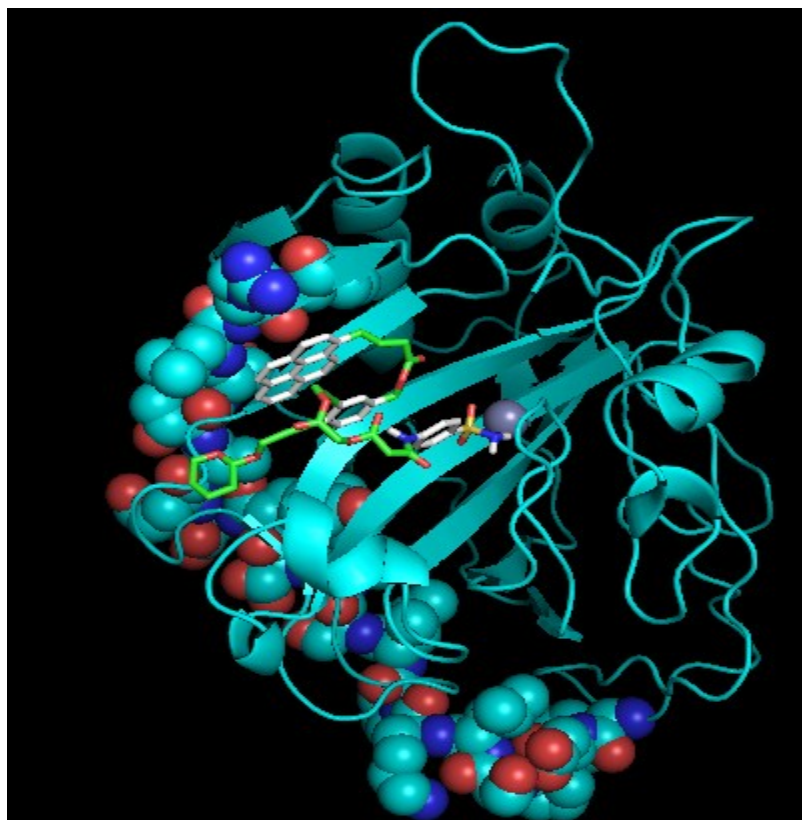
1H -NMR (600 MHz, $CDCl_3$) spectrum of compound 1



¹³C-NMR (150 MHz, CDCl₃) spectrum of compound 1



HRMS Spectrum of Compound 1



Docked image of Compound 2 with HCA II:The grey sphere represents Zn atom. The amino acid residues from 40-58 (protein portion as sphere, mass fragment 2141 in tryptic digestion of HCA II) is close to the molecule, hence we obtained a major peak at 2912/2914 after photo-crosslinking of enediyne with the protein.