

Electronic Supporting Information

1,3,5-Trisubstituted Benzenes as Fluorescent Photoaffinity Probes for Human Carbonic Anhydrase II Capture

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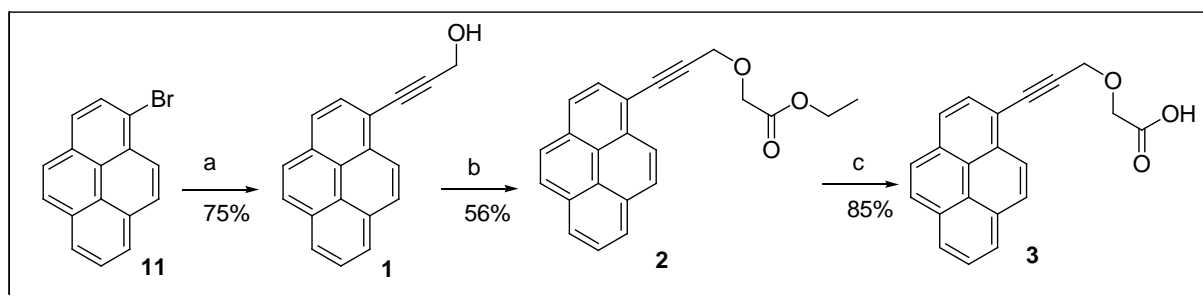
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Experimental Section

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 obtained with 200 MHz and 400 MHz Bruker NMR instruments. Proton and carbon spectra were referenced internally to solvent signals, using values of $\delta_{\text{H}} = 7.26$ ppm for proton and $\delta_{\text{C}} = 77.0$ ppm for carbon (middle peak) in CDCl_3 . 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 (70 eV). Melting points were determined in open capillary tubes and are uncorrected.



Reagents and Conditions: a) $\text{Pd}(\text{PPh}_3)_4$, CuI , Propargyl alcohol, $n\text{BuNH}_2$, reflux, 24h; b) NaH , Bromoethylacetate, dry THF, 3h; c) NaOH , MeOH, rt, 15h.

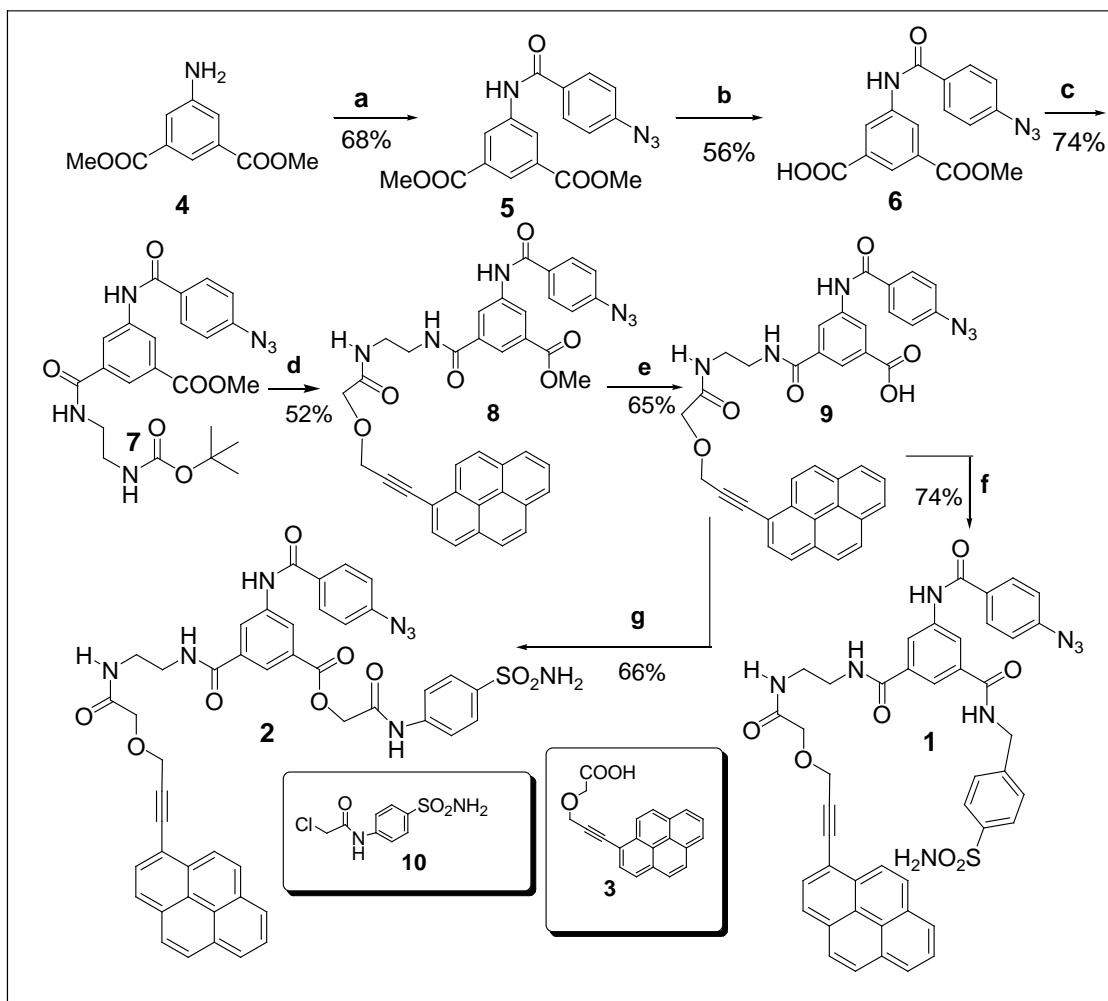
Synthesis of Fluorescent hand

3-Pyren-1-yl-prop-2-yn-1-ol (12): To a solution of 1-Bromopyrene **11** (700 mg, 1.78 mmol) in $n\text{BuNH}_2$ (60 mL) was added propargyl alcohol (0.54 mL, 8.9 mmol), $\text{Pd}(\text{PPh}_3)_4$ (62 mg, 0.05 mmol) under N_2 . The reaction mixture was refluxed for 10 h at 90°C and cooled to room temperature. 6M HCl (20 mL) was added and stirred for 30 min. Aqueous layer was extracted with EtOAc (100 mL \times 2), and the organic layer was washed with H_2O and brine, dried over MgSO_4 and concentrated in vacuo. The residue was purified by column chromatography (Hexane/EtOAc 3:1) to afford pyrenyl propargyl alcohol **12** as an orange gummy mass; yield 370 mg (95%); $R_f = 0.35$ (Hexane/EtOAc 2:1); $^1\text{H-NMR}$ (400 MHz, CDCl_3): 8.53 (1H, d, $J = 9.0$ Hz), 8.19-8.17 (2H, m), 8.12 (1H, d, $J = 9.0$ Hz), 8.10-7.99 (5H,

m), 4.75 (2H, s); ^{13}C -NMR (100 MHz, CDCl_3): 131.9, 131.3, 131.1, 130.9, 129.7, 128.2, 127.2, 126.2, 125.6, 125.5, 125.3, 124.3, 124.2, 124.1, 116.9, 92.7, 84.7, 51.9; MS: m/z 257; $[\text{MH}^+]$.

(3-Pyren-1-yl-prop-2-ynyloxy)-acetic acid ethyl ester (13): Compound **12** (300 mg, 1.17 mmol) in dry THF (5 mL) was added dropwise to a suspension of NaH (60 % content, 46 mg) in THF (45 mL) at 0 °C under nitrogen atmosphere. The stirring was continued at this temperature for 3 h after which a solution of bromoacetic acid ethyl ester (0.25 mL, 2.33 mmol) in THF (1 mL) was added dropwise. The solution was allowed to warm to r.t and the stirring was continued for another 3 h. 2M HCl (10 mL) was added, the organic layer was separated and the aqueous layer was extracted with EtOAc (50 mL x 2). The combined organic layer was washed with brine, dried over anhydrous Na_2SO_4 and concentrated in vacuo. The residue was purified by column chromatography to give the desired product **13**; yellow gummy mass; yield 224 mg (56%); $R_f = 0.40$ (Hexane/EtOAc 7:1); ^1H -NMR(400 MHz, CDCl_3): 8.53 (1H, d, $J = 9.0$ Hz), 8.23-8.15 (3H, m), 8.13-8.08 (3H, m), 8.04-8.00 (2H, m), 4.79 (2H, s), 4.28 (2H, q, $J = 7.0$ Hz), 1.32 (3H, t, $J = 7.0$ Hz); ^{13}C -NMR (100 MHz, CDCl_3): 170.0, 132.1, 131.5, 131.1, 130.9, 129.8, 128.4, 128.3, 127.1, 126.2, 125.7, 125.6, 125.2, 124.4, 124.3, 124.2, 116.7, 89.3, 86.3, 66.5, 61.1, 59.4, 14.2; MS: m/z 343 $[\text{MH}^+]$.

(3-Pyren-1-yl-prop-2-ynyloxy)-acetic acid (3): A mixture of NaOH (25 mg, 0.61 mmol.) in MeOH (1 mL) was dropwise added to a solution of compound **13** (176 mg, 0.50 mmol) and methanol (7 mL) and stirred at r.t. for 15 h. The solvents were evaporated in vacuo, water (10 mL) added, pH adjusted to 1 with 2M HCl. It was extracted with EtOAc (2 x 25 mL) and the organic layer was dried over Na_2SO_4 . It was concentrated and purified by precipitation from hexane-ethylacetate to give the desired compound **3** as a yellow sticky solid; yield 122 mg (76%); ^1H -NMR (400 MHz, CDCl_3): 8.51 (1H, d, $J = 9.0$ Hz), 8.24-8.20 (2H, m), 8.18 (1H, d, $J = 9.0$ Hz), 8.14-8.09 (3H, m), 8.06-8.02 (2H, m), 4.79 (2H, s), 4.46 (2H, s); ^{13}C -NMR (100 MHz, CDCl_3): 172.8, 132.1, 131.6, 131.1, 130.9, 129.8, 128.6, 128.4, 127.1, 126.3, 125.74, 125.7, 125.1, 124.4, 124.3, 124.1, 116.3, 88.5, 86.9, 66.0, 59.6; MS: m/z 314 $[\text{M}^+]$.



Reagents and conditions: a) *p*-Azidobenzoic acid, BOP-reagent, diisopropylethylamine (DIPEA), CH₂Cl₂ reflux, 24 h; b) NaOH, MeOH, 50 °C, 3 h; c) MonoBOC protected ethylenediamine, BOP-reagent, DIPEA, CH₂Cl₂, 40 °C, 48 h; d) (i) CF₃CO₂H, CH₂Cl₂, 0 °C - rt, 20 min; (ii) Acid 3, BOP-reagent, DIPEA, CH₂Cl₂:DMF (5:1), rt, 20 h; e) NaOH, MeOH, 50 °C, 6 h; f) 4-Aminomethyl-benzenesulfonamide, BOP-reagent, DIPEA, CH₂Cl₂:DMF (5:1), 40 °C, 48 h; g) K₂CO₃, 2-Chloro-N-(4-sulfamoylphenyl)acetamide 10, dry DMF, rt, 20 h.

Synthesis of capture compounds 1 and 2.

5-(4-Azido-benzoylamino)-isophthalic acid dimethyl ester (5): 5-Amino-isophthalic acid dimethyl ester 5 (641 mg, 3.07 mmol) and 4-Azidobenzoic acid (500 mg, 3.07 mmol) were taken in dry DCM (35 mL). BOP-reagent (1.4 gm, 3.07 mmol) was added followed by DIPEA (0.3 mL, 6.14 mmol). The solution was refluxed for 24 h. 2M HCl solution (10 mL)

was added and extracted with EtOAc (25 mL x 2). The combined organic layer was washed with brine, dried over anhydrous Na₂SO₄ and concentrated in vacuo. The crude solid was purified to give the desired product **5** as a buff coloured sticky solid; yield 738 mg (68%); R_f = 0.50 (Hexane/EtOAc 2:1) ¹H-NMR (200 MHz, CDCl₃): 8.52 (2H, s), 8.42 (1H, s), 8.35 (1H, s), 7.92 (2H, d, *J* = 8.5 Hz), 7.09 (2H, d, *J* = 8.5 Hz), 3.92 (6H, s); ¹³C-NMR (50 MHz, CDCl₃): 165.9, 164.9, 144.5, 138.4, 131.4, 130.5, 129.1, 126.6, 125.4, 119.3, 52.6; MS: m/z 355 [MH⁺].

5-(4-Azido-benzoylamino)-isophthalic acid monomethyl ester (6): A mixture of NaOH (23 mg, 0.56 mmol) in MeOH (1 mL) was added dropwise to a solution of compound **5** (200 mg, 0.56 mmol) in methanol (15 mL) and stirred at 50 °C for 3 h. The solvents were evaporated in vacuo, water (10 mL) added, pH adjusted to 1 with 2M HCl. It was extracted with EtOAc (2 x 25 mL). The organic layer was separated and dried over Na₂SO₄. It was concentrated and the target compound **6** was isolated by precipitation from hexane-ethyl acetate as a white solid; yield 107 mg (56%); ¹H-NMR (200 MHz, CD₃OD): 8.65-8.61 (1H, m), 8.41 (1H, s), 8.03 (2H, dd, *J* = 2.0, 9.0 Hz), 3.98 (6H, s); ¹³C-NMR (100 MHz, CD₃OD): 167.2, 166.5, 166.2, 144.2, 139.5, 131.9, 131.1, 130.8, 129.5, 125.9, 125.8, 125.4, 118.9, 51.7; MS: m/z 363 [MNa⁺].

5-(4-Azido-benzoylamino)-N-(2-tert-butoxycarbonylamino-ethyl)-isophthalamic acid methyl ester (7): Compound **6** (80 mg, 0.24 mmol) and BOP-reagent (107 mg, 0.24 mmol) were taken in a mixed solvent of CH₂Cl₂ (10 mL) and DMF (2 mL). DIPEA (0.1 mL, 0.48 mmol) was added and stirred for 5 min. Mono Boc-protected ethylene diamine (39mg, 0.24 mmol) was added and stirred for 48 h. 2M HCl (10 mL) was added into the reaction mixture, the organic layer was isolated and the aqueous layer was extracted with EtOAc (100 mL x 2). The combined organic layer was washed with brine, dried over anhydrous Na₂SO₄ and concentrated in vacuo. The solid is purified by column chromatography to give the desired product **7** as yellow gummy mass; Yield 84 mg (74%); R_f = 0.50 (CH₂Cl₂/MeOH 25:1) ¹H-NMR (400 MHz, CDCl₃): 8.57 (1H, s), 8.40 (1H, bs), 8.24 (1H, s), 8.19 (1H, s), 7.93 (2H, d, *J* = 8.5 Hz), 5.11 (1H, bs), 3.93 (3H, s), 3.55 (2H, m), 3.40 (2H, m), 1.43 (9H, s); ¹³C-NMR (100 MHz, CDCl₃): 166.0, 164.8, 144.2, 138.6, 135.3, 131.5, 130.5, 129.1, 124.0, 123.6, 123.3, 119.3, 119.2, 80.2, 52.5, 31.9, 30.2, 28.3; MS: m/z 483 [MH⁺].

5-(4-Azido-benzoylamino)-N-{2-[2-(3-pyren-1-yl-prop-2-ynyloxy)-acetylamino]-ethyl}-isophthalamic acid methyl ester (8): Compound **7** (102 mg, 0.22 mmol) was taken in 3 mL

of CH₂Cl₂ and 0.05 mL of TFA in ice. Then it is stirred at 0 °C for 10 min and for further 50 min at r.t. It was concentrated in vacuo and benzene (4 mL) was added and again concentrated. Addition of benzene and evaporation was repeated thrice. The isolated amine was taken in 2 mL CH₂Cl₂ and 8 mL DMF. Acid **3** (50 mg, 0.16 mmol) was added followed by the addition of BOP-reagent (84 mg, 0.2 mmol) and DIPEA. The mixture was stirred for 20 h. 2M HCl (5 ml) was added and the aqueous layer was extracted with EtOAc (20 ml x 2). The combined organic layer was washed with brine, dried over anhydrous Na₂SO₄ and concentrated in vacuo. The solid is purified by column chromatography to give the desired product **8** 72 mg; yellow gummy mass; yield 52%; R_f = 0.35 (DCM/MeOH 25:1); ¹H-NMR(400 MHz, d₆-DMSO): 10.58 (1H, s), 8.84 (2H, s), 8.59 (1H, s), 8.42 (1H, d, *J* = 9.2 Hz), 8.29-7.90 (6H, m), 7.19-7.15 (2H, m), 4.75 (2H, s), 4.19 (2H, s), 3.85 (3H, s), 3.42 (4H, s); ¹³C-NMR (100 MHz, d⁶-DMSO): 169.5, 166.2, 166.1, 165.0, 162.7, 143.4, 140.2, 136.1, 131.7, 131.4, 131.1, 131.0, 130.8, 130.5, 130.1, 129.1, 128.8, 127.5, 127.0, 126.4, 126.3, 125.2, 125.0, 124.5, 123.9, 123.7, 123.1, 119.3, 116.6, 91.6, 85.4, 69.3, 59.4, 52.7, 36.7, 31.5; MS: m/z 679 [MH⁺].

5-(4-Azido-benzoylamino)-N-{2-[2-(3-pyren-1-yl-prop-2-ynyloxy)-acetylamino]-ethyl}-N'-(4-sulfamoyl-benzyl)-isophthalamide (1**):** A mixture of NaOH (6 mg, 0.12 mmol.) in MeOH (2 mL) was added dropwise to a solution of compound **8** (72 mg, 0.1 mmol) in methanol (4 mL) and stirred at 50 °C for 6 h. The solvents were evaporated in vacuo, water (10 mL) added, pH adjusted to 1 with 2M HCl. EtOAc (25 mL) was added, the organic layer was isolated and dried over Na₂SO₄. It was concentrated and purified by precipitation from hexane ethyl acetate to give compound **9**; yield: 45 mg (65 %) yield.

Compound **9** (22 mg, 0.033mmol) was taken in 7 mL of DMF followed by the addition of BOP- reagent (18 mg, 0.039 mmol) and DIPEA (0.03 mL, 0.132 mmol). To this mixture, 4-Aminomethyl benzenesulphonamide (11 mg, 0.05 mmol) was added and stirred for 48 h at 40 °C. 2M HCl (4 mL) was added into the reaction mixture, the organic layer was isolated and the aqueous layer was extracted with EtOAc (10 ml x 2). The combined organic layer was washed with brine, dried over anhydrous Na₂SO₄ and concentrated in vacuo. The crude solid is purified by column chromatography to give the desired product **1** as a white sticky mass; yield 20 mg (74%); R_f = 0.40 (CH₂Cl₂/MeOH 10:1); ¹H-NMR (400 MHz, d₆-DMSO): 10.52 (1H, s), 9.21 (1H, t, *J* = 5.5 Hz), 8.66 (1H, bs), 8.42 (1H, d, *J* = 9.0 Hz), 8.39-8.27 (4H, m), 8.26-8.07 (5H, m), 8.02 (2H, d, *J* = 8.5 Hz), 7.77 (2H, d, *J* = 8.5 Hz), 7.49 (2H, d, *J* = 8.5 Hz), 7.22 (2H, d, *J* = 8.5 Hz), 4.72 (2H, s), 4.53 (2H, d, *J* = 5.5 Hz), 4.12 (3H, s), 3.16 (2H, s), 3.14 (3H, s). ¹³C-NMR (100 MHz, CDCl₃): 169.6, 166.8, 166.7, 166.2, 144.2, 143.6, 143.2, 139.9, 135.9, 135.6, 131.9, 131.6, 131.3, 131.2, 131.0, 131.4, 129.5, 129.0, 128.3, 127.8, 127.4, 126.7, 126.6, 126.3, 125.5, 125.2, 124.2, 123.9, 122.9, 122.7, 121.7,

119.7, 116.8, 91.9, 85.5, 69.4, 59.5, 43.1, 38.5, 31.9. HRMS: Calcd for $C_{45}H_{36}N_8O_7S+Na^+$ 855.2325, found 855.2346.

2-Chloro-N-(4-sulfamoyl-phenyl)-acetamide (10): To a solution of sulphanilamide (350 mg, 2.03 mmol) in dry THF (10 mL) at 0 °C, K_2CO_3 (561 mg, 4.06 mmol) was added. Chloroacetyl chloride (0.2 mL, 2.44 mmol) was added drop wise and the mixture was stirred for 30 min. Water (7 mL) was added into the reaction mixture, the organic layer was separated and the aqueous layer was extracted with EtOAc (50 mL x 2). The combined organic layer was washed with brine, dried over anhydrous Na_2SO_4 and concentrated in vacuo. The crude solid was purified by column chromatography to give the desired product as white solid; Yield 425 mg (84%); mp 217 °C; $R_f = 0.50$ ($CH_2Cl_2/MeOH$ 30:1); 1H -NMR (400 MHz, d_6 -DMSO): 10.96 (1H, s), 7.76 (4H, s), 7.27 (2H, s), 4.28 (2H, s); ^{13}C -NMR (100 MHz, $CDCl_3$): 165.8, 142.0, 139.6, 127.5, 119.6, 44.2. MS: m/z 249 [MH^+].

5-(4-Azido-benzoylamino)-N-{2-[2-(3-pyren-1-yl-prop-2-ynyloxy)-acetyl-amino]-ethyl}-isophthalamic acid (4-sulfamoyl-phenyl carbamoyl)-methyl ester (2): Compound **9** (22 mg, 0.033 mmol) was taken in DMF (5 mL) followed by the addition of K_2CO_3 (6 mg, 0.04 mmol) and stirred for 30 min. The sulfonamide **10** (1.2 eq) was added to this mixture and stirred for 20 h. 10mL 2M HCl was added to it and the aqueous layer was extracted with EtOAc (7 mL x 4).). The combined organic layer was washed with brine, dried over anhydrous Na_2SO_4 and concentrated in vacuo. The solid is purified by column chromatography to give the desired product **2** as a white gummy mass; Yield 19 mg (66%); $R_f = 0.50$ ($CH_2Cl_2/MeOH$ 15:1); 1H -NMR (400 MHz, d_6 -DMSO): 10.60 (2H, s), 8.83 (1H, s), 8.65 (1H, s), 8.57 (1H, s), 8.45 (1H, d, $J = 8.5$ Hz), 8.34 (1H, d, $J = 7.0$ Hz), 8.29 (2H, d, $J = 9.5$ Hz), 8.23-8.04 (8H, m), 7.76 (4H, bs), 7.25 (4H, bs), 4.99 (2H, s), 4.72 (2H, s), 4.14 (2H, s), 3.32 (4H, s); ^{13}C -NMR (100 MHz, $CDCl_3$): 169.4, 166.2, 165.4, 146.5, 143.9, 141.7, 139.1, 136.4, 136.1, 135.6, 134.9, 133.9, 133.6, 132.3, 131.9, 131.6, 131.4, 130.8, 130.1, 129.7, 129.2, 128.8, 128.5, 127.6, 127.2, 126.4, 126.3, 125.2, 125.0, 124.2, 123.7, 119.4, 119.3, 116.5, 91.7, 85.3, 69.2, 59.3, 56.2, 38.3, 34.7; HRMS: Calcd for $C_{46}H_{36}N_8O_9S+Na^+$ 899.2224, found 899.2259.

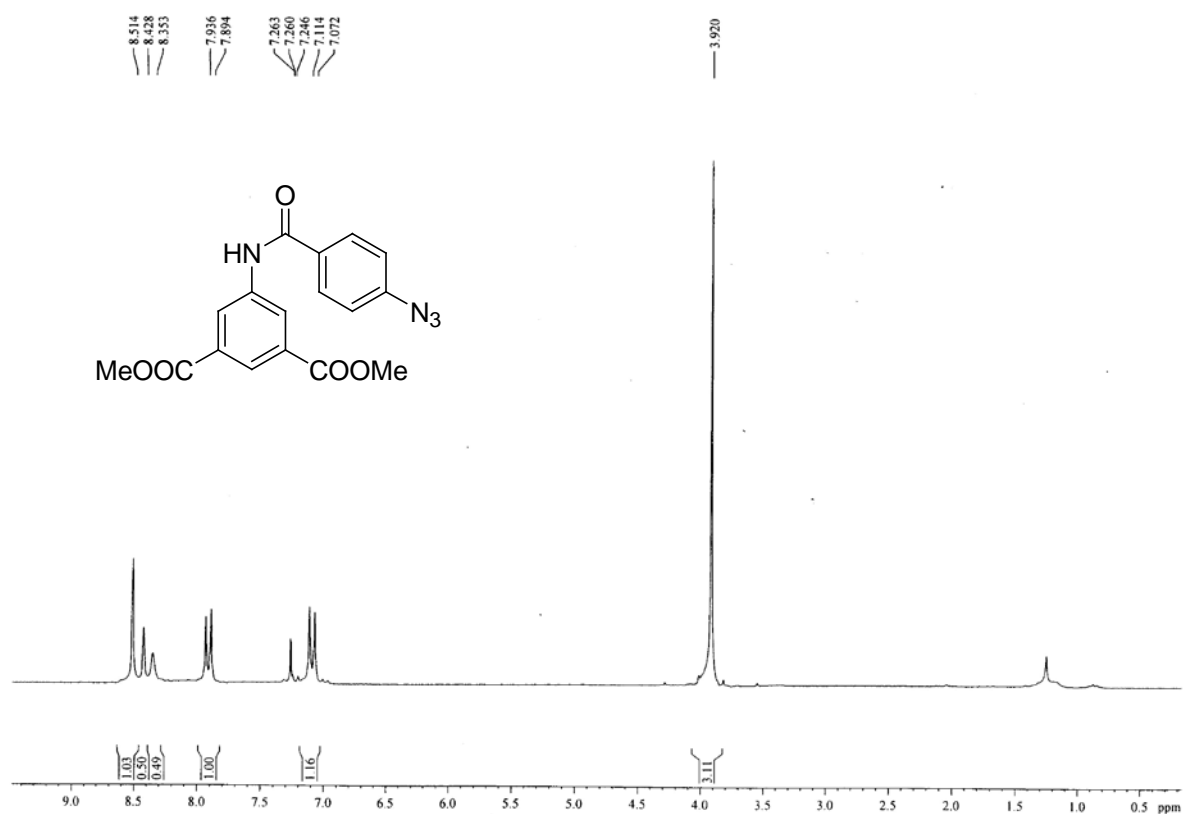
Purification of HCA II: The *Escherichia coli* strain BL21(DE3)pLysS carrying the plasmid pACA/HCAII (a kind gift from Professor Sven Lindskog, Umea University, Sweden) were grown in Luria Bertani media containing 0.333X M9 salts, 0.37% glucose and 55.6 μM $ZnSO_4$ at 37°C. At late-log phase, the cells were induced with 0.25 mM IPTG followed by the addition of 0.45 mM $ZnSO_4$ and further grown at 30 °C for 6 h. The over-expressed protein was purified at 4 °C following some modifications of the previously published procedure [J. E. Jackman, K. M. Jr Merz, C. A. Fierke, *Biochemistry*, 1996. **35**, 16421–

16428. The cells from one litre culture were harvested and resuspended in 50 mL of lysis buffer, Buffer A (50 mM Tris; pH 8.0, 50 mM NaCl, 10 mM EDTA, 1 mM DTT, 1 mM PMSF, 0.2 mM ZnSO₄), disrupted by MICROSON™ (MISONIX) ultrasonic cell disrupter at 40s pulse at regular intervals of 60s and then clarified by centrifugation. The supernatant was fractionated with 10% streptomycin sulphate and again centrifuged. The supernatant was dialyzed against Buffer B (10 mM Tris; pH 8.0, 0.2 mM ZnSO₄, 1 mM DTT). The dialysate was loaded onto pre-equilibrated Q-sepharose anion exchange column from which the flowthrough was collected for further purification. The flowthrough was dialyzed against Buffer C (10 mM Tris; pH 7.0, 0.1 mM ZnSO₄, 1 mM DTT). The dialysate was applied to pre-equilibrated cation exchange S-sepharose column. The protein was eluted by a linear gradient of 0-0.5M (NH₄)₂SO₄ in 10 mM Tris; pH 7.0. The pooled peak fractions were concentrated to be further purified by size-exclusion chromatography using Superdex 75 prep-grade matrix in a 16/70 C column (GE Healthcare Biosciences) equilibrated with Buffer D (20 mM HEPES; pH 7.2) on an ÄKTA prime Plus system (GE Healthcare Biosciences). Fractions of 2 mL were collected at a flow rate of 1 mL/min. The purity of the fractions containing HCAII was checked by 12% SDS PAGE and the protein content was estimated by the method of Bradford [M.M. Bradford, Rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding, *Anal. Biochem.* 1976, **72**, 248 -254]. SDS-PAGE revealed that HCAII obtained was ≥98% pure.

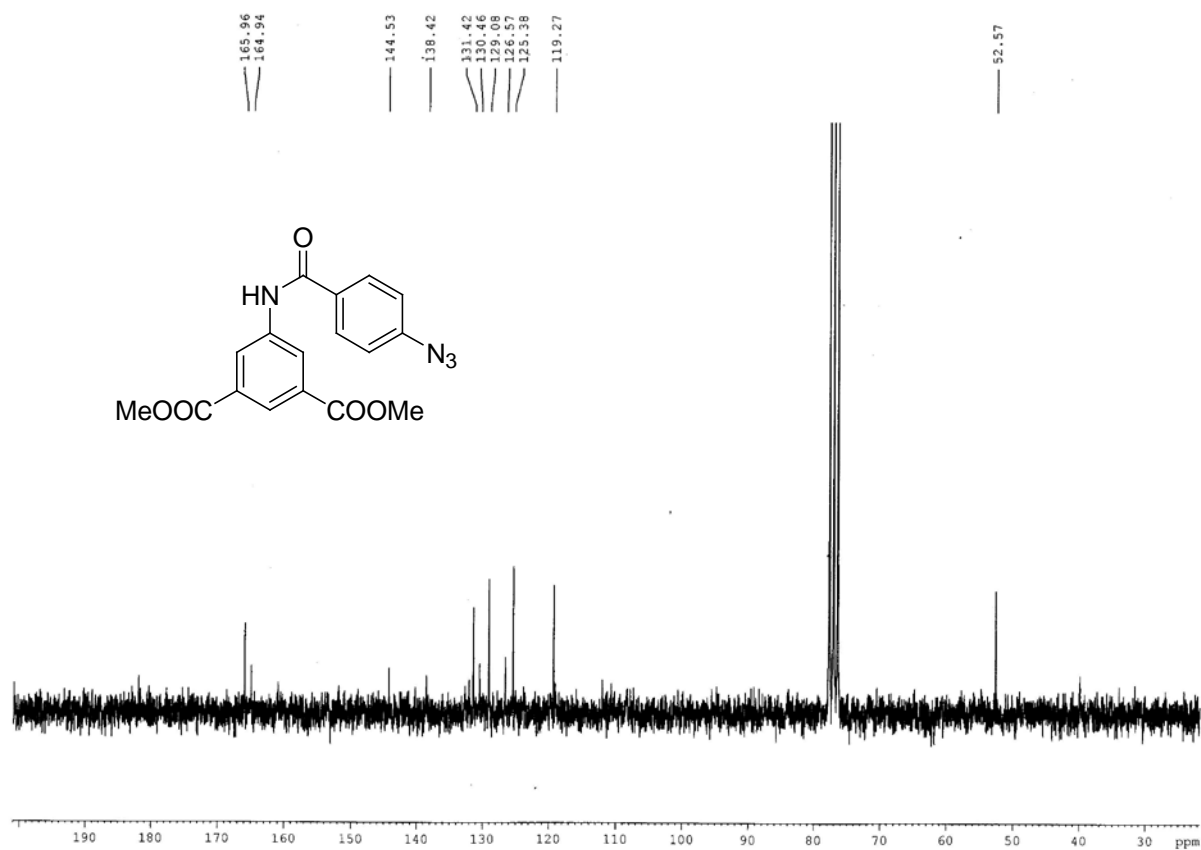
SDS-polyacrylamide gel electrophoresis: For SDS-polyacrylamide gel analyses, the samples were mixed with 1X Laemmli buffer prepared by mixing 63 mM Tris-HCl ((pH 6.8), 2% SDS, 10% glycerol, 0.1% 2-mercaptoethanol and 0.0005% Bromophenol blue (U. K. Laemmli, Cleavage of structural proteins during the 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.

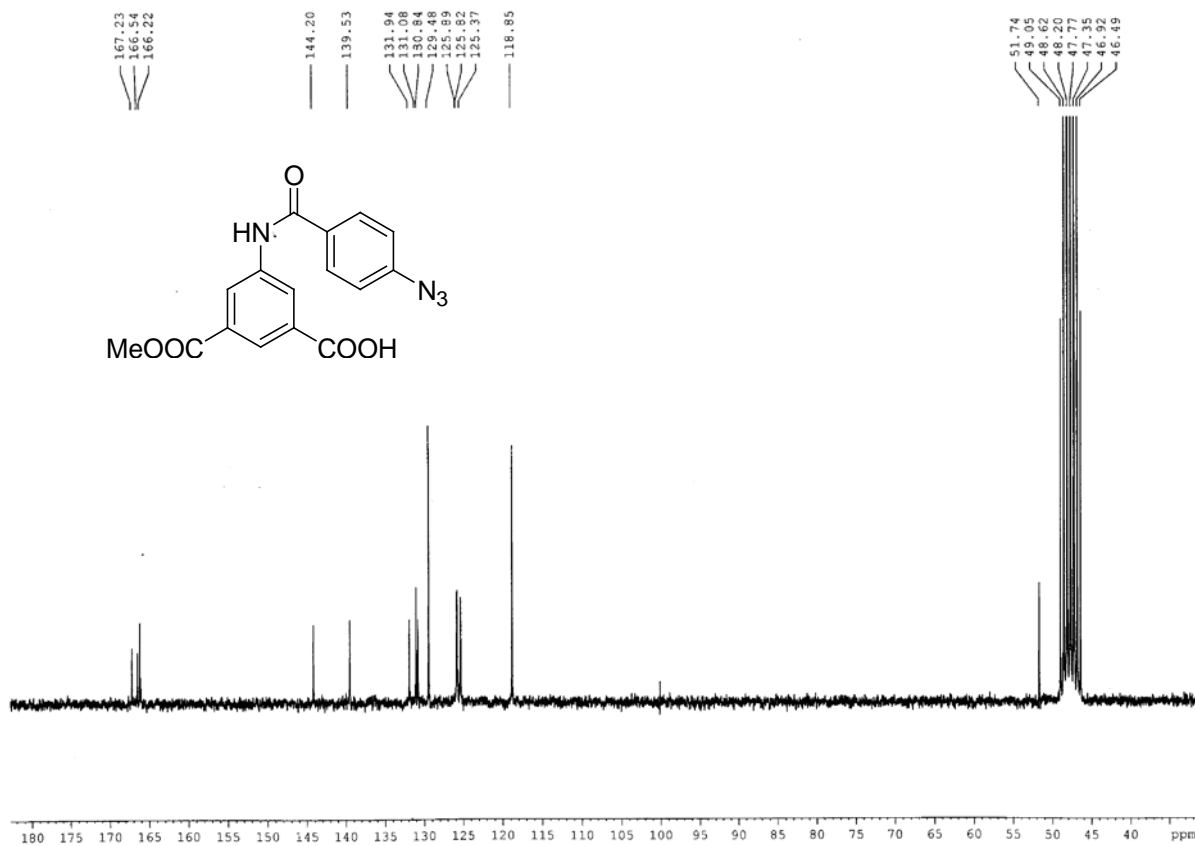
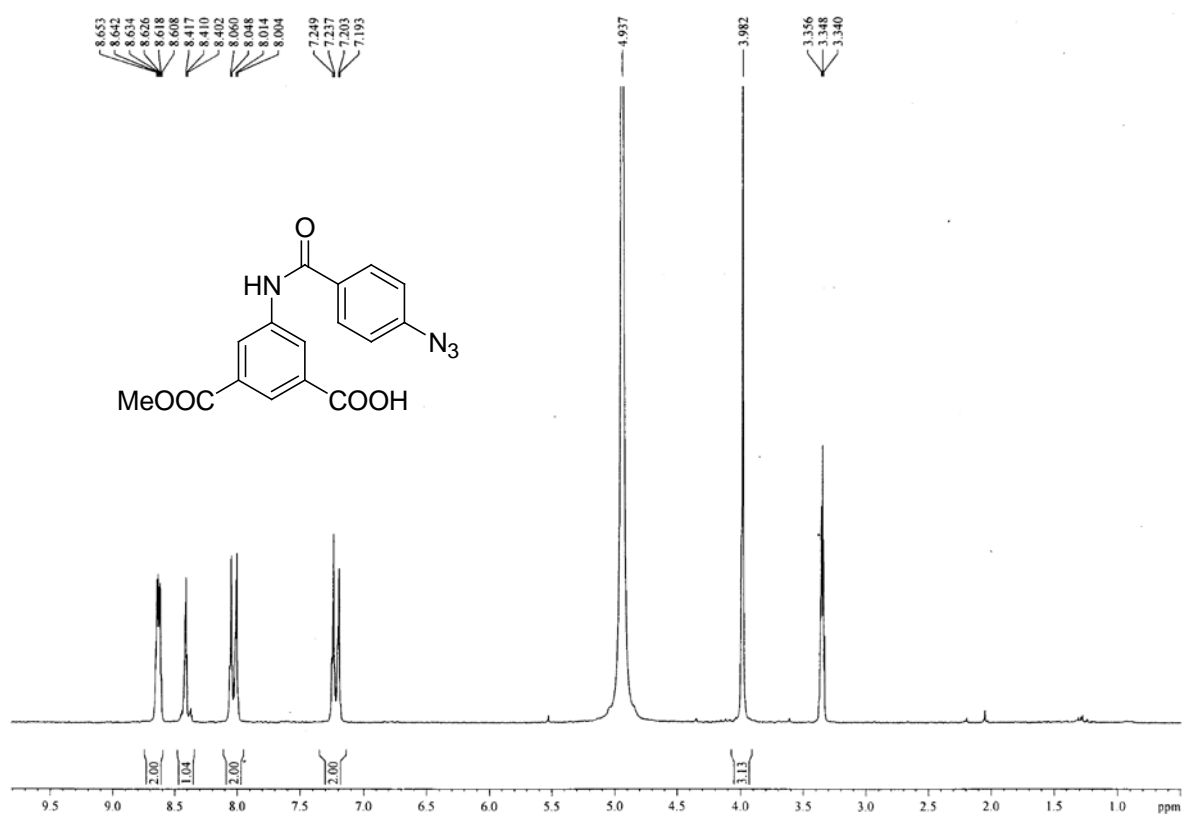
Matrix Assisted Laser Desorption and Ionization Spectrometry (MALDI analyses): The molecular mass of the protein-capture compound complex was determined by mass spectrometry. For mass spectrometric studies, 2 μ L sample was mixed thoroughly with 4 μ L sinapinic acid (α -cyano-4-hydroxycinnamic acid) as matrix. From the mixture, 2 μ L was spotted onto 100 well stainless steel MALDI plate and allowed to be air dried prior to MALDI analysis by the Voyager DE ProTM mass spectrometer equipped with 337 nm N₂ laser (Applied Biosystem, USA). The spectra were recorded in the linear and positive ion mode with an accelerating voltage 20 kV and an average of 100 laser shots with a grid voltage of 90% visualization.



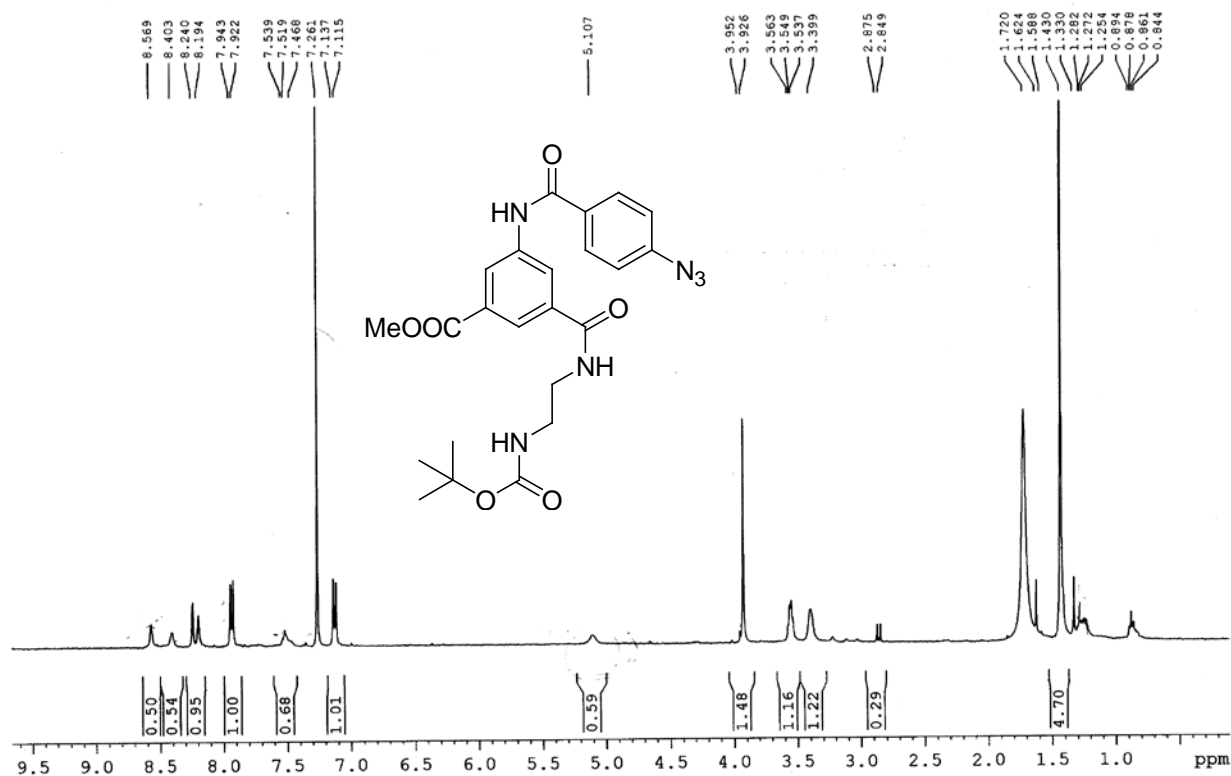
¹H-NMR (200 MHz, CDCl₃) of compound **8**



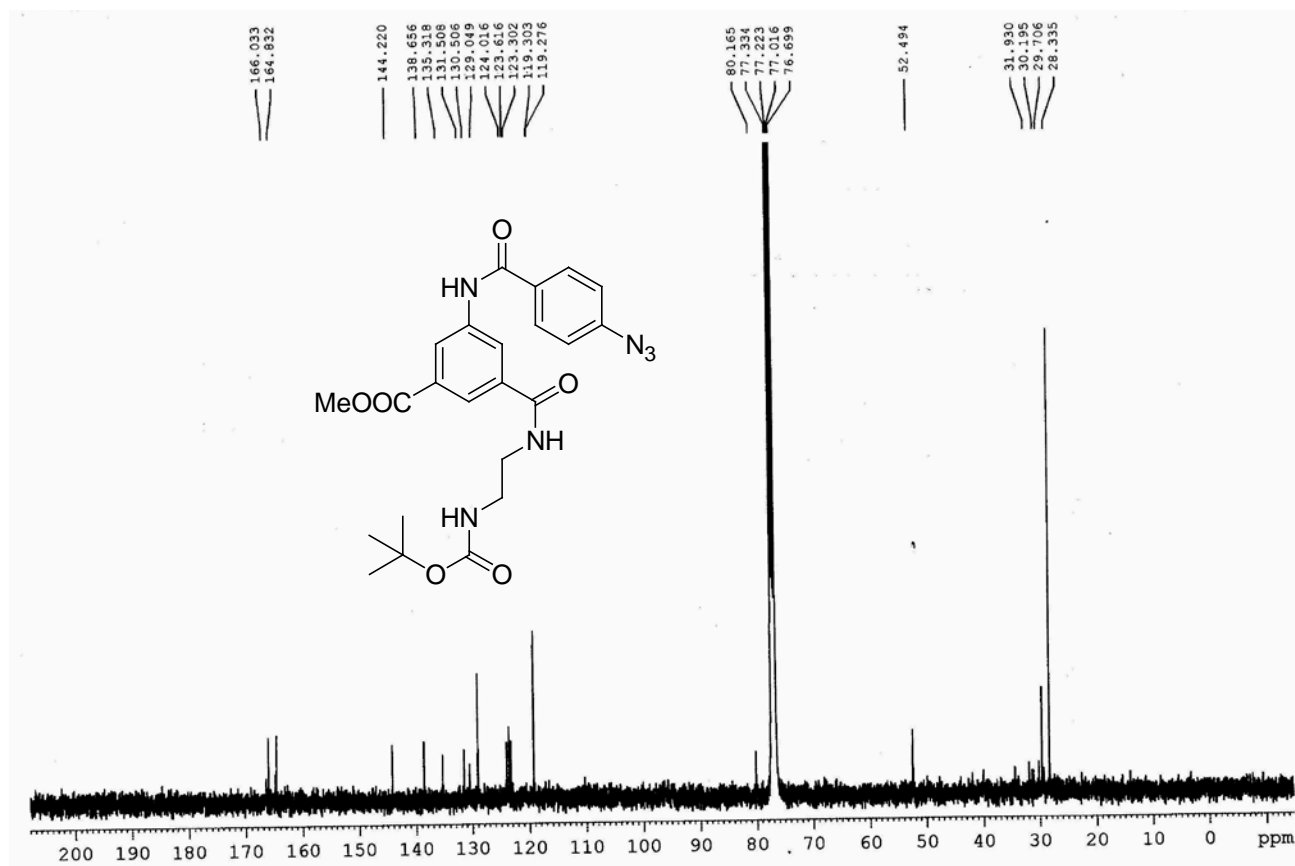
¹³C-NMR (50 MHz, CDCl₃) of compound **8**



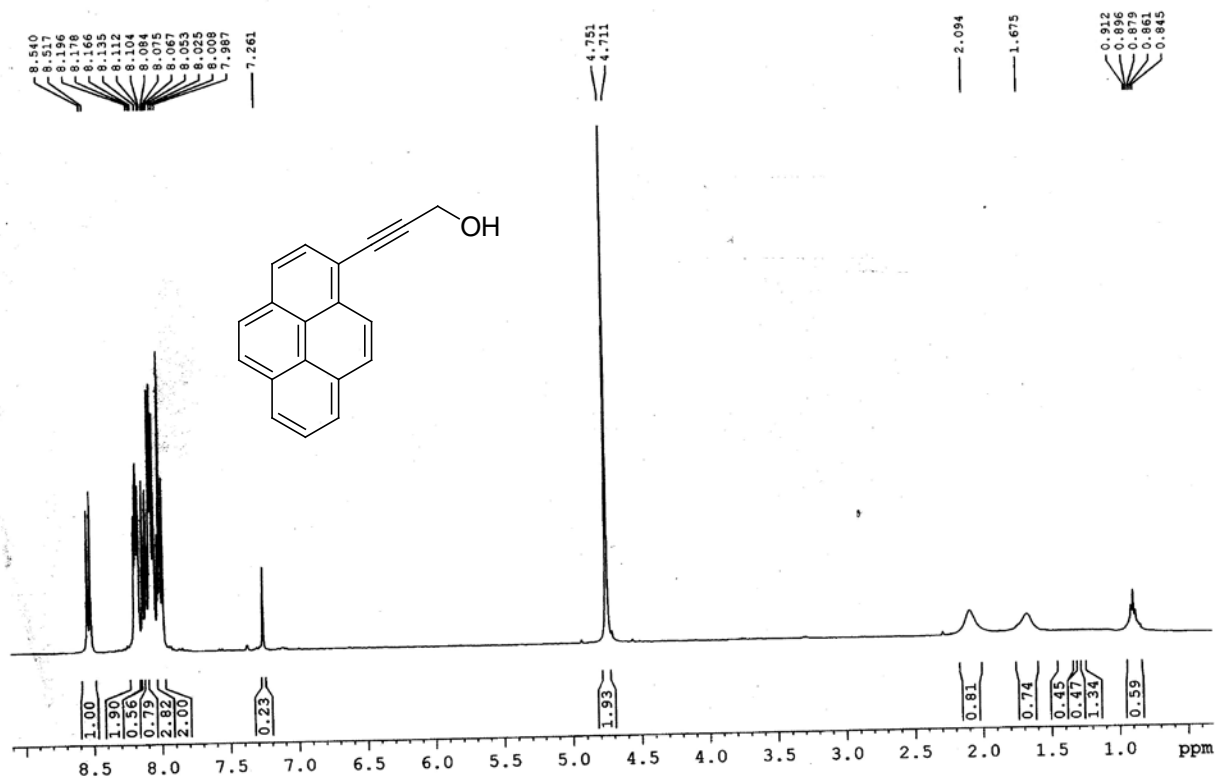
¹³C-NMR (100 MHz, CD₃OD) of compound 9



¹H-NMR(400 MHz, CDCl₃) of compound **10**

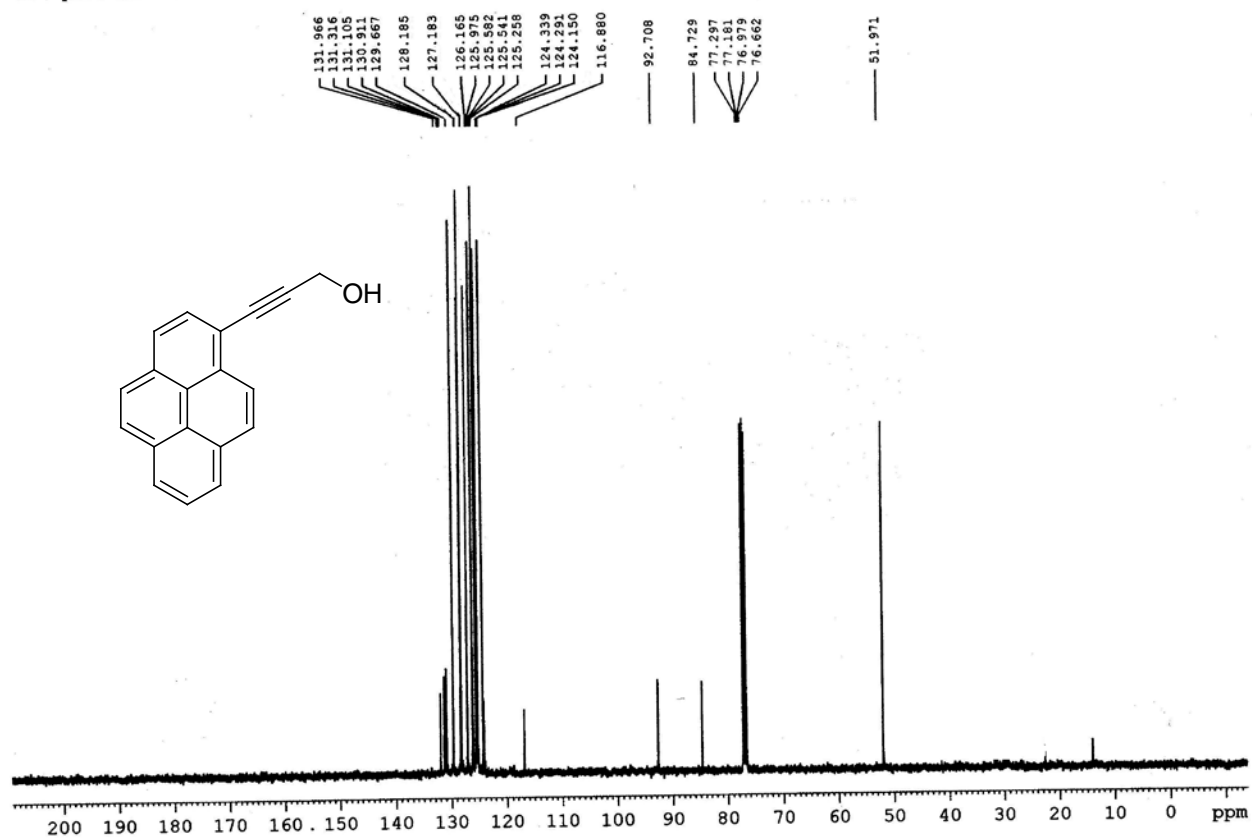


¹³C-NMR (100 MHz, CDCl₃) of compound **10**

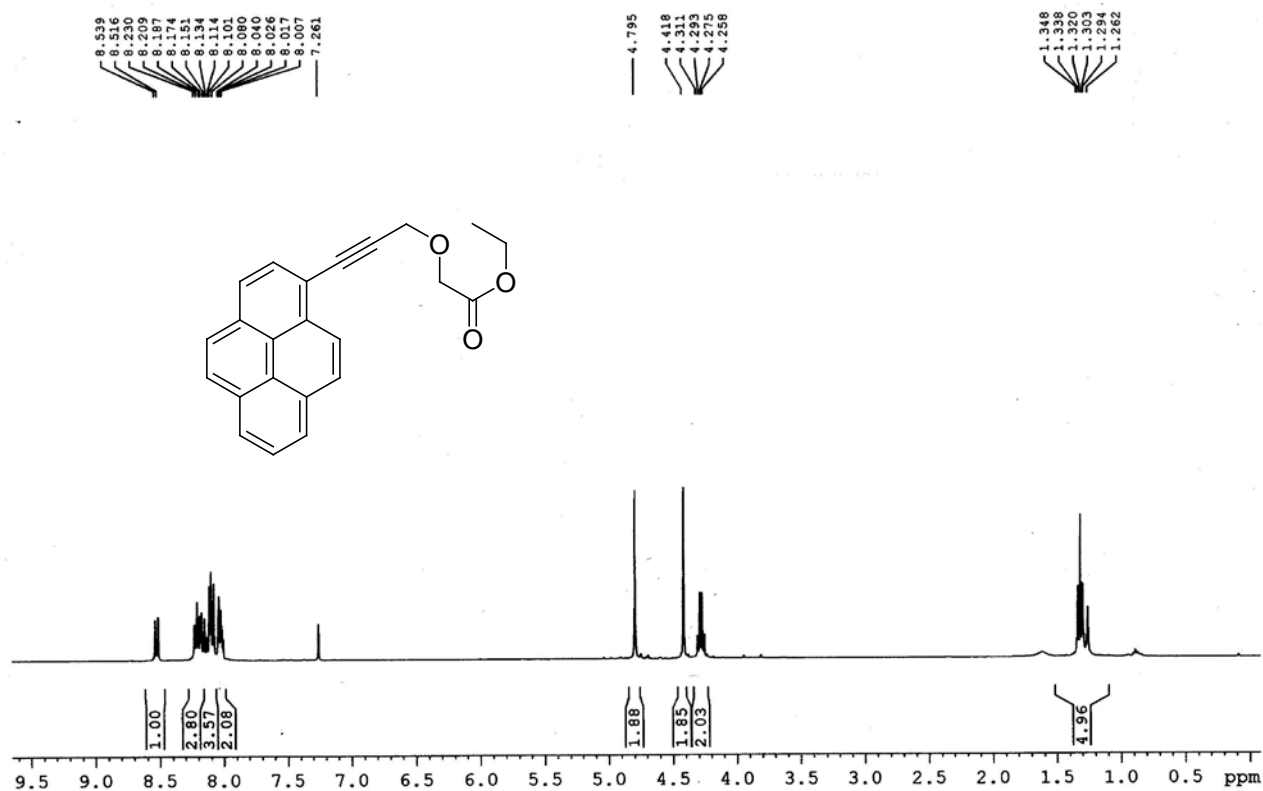


¹H-NMR(400 MHz, CDCl₃) of compound 4

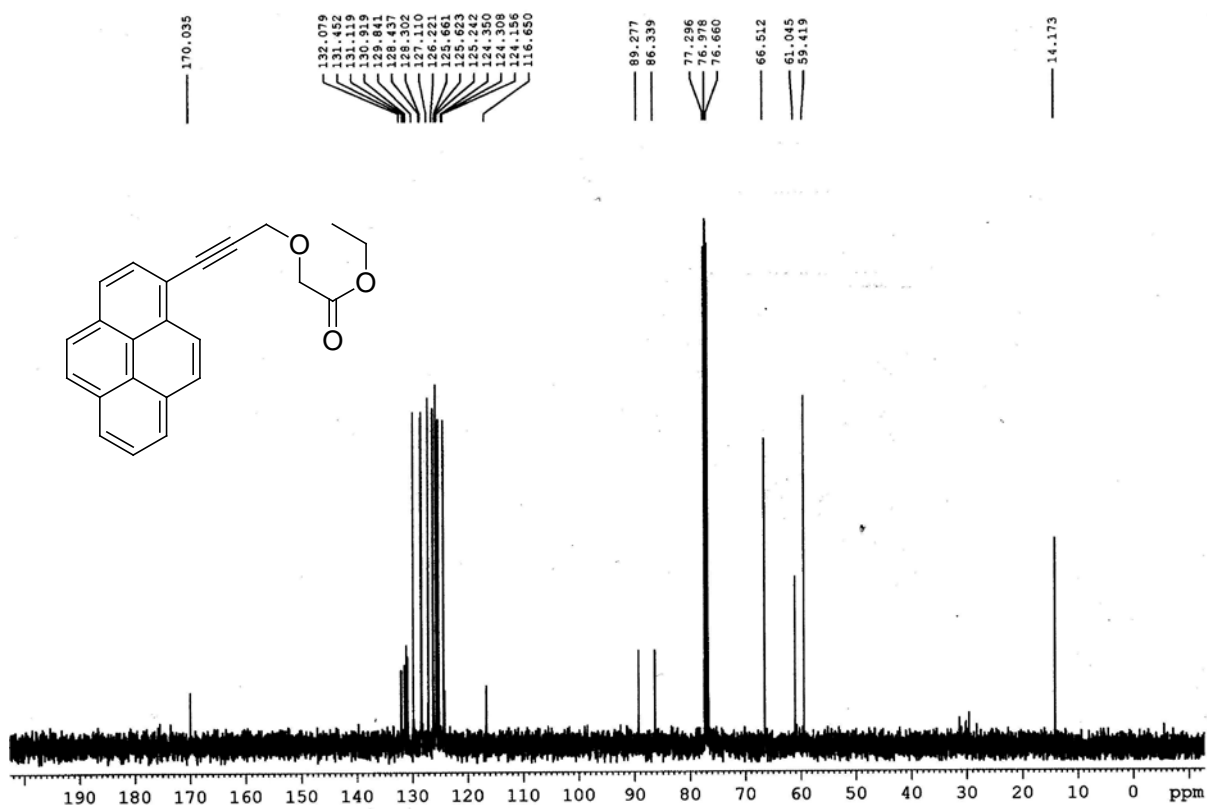
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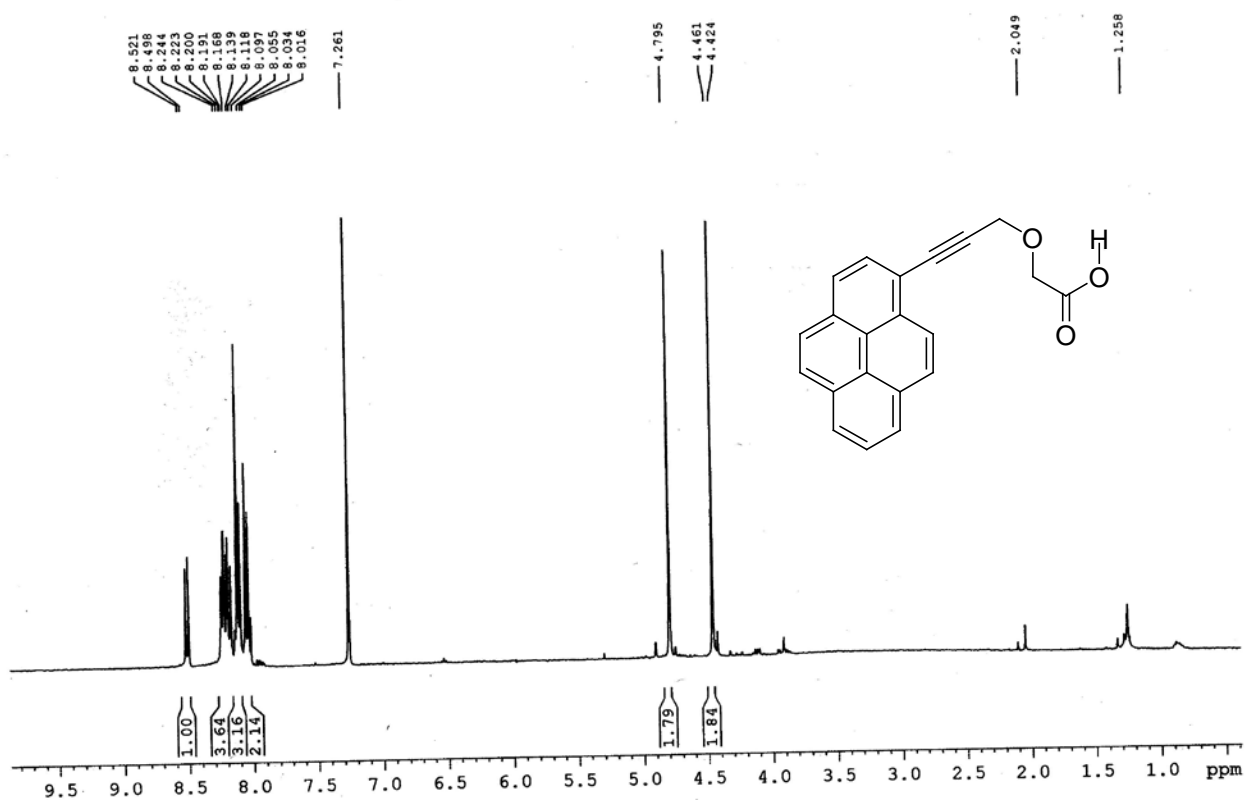
¹³C-NMR (100 MHz, CDCl₃) of compound 4



¹H-NMR(400 MHz, CDCl₃) of compound 5

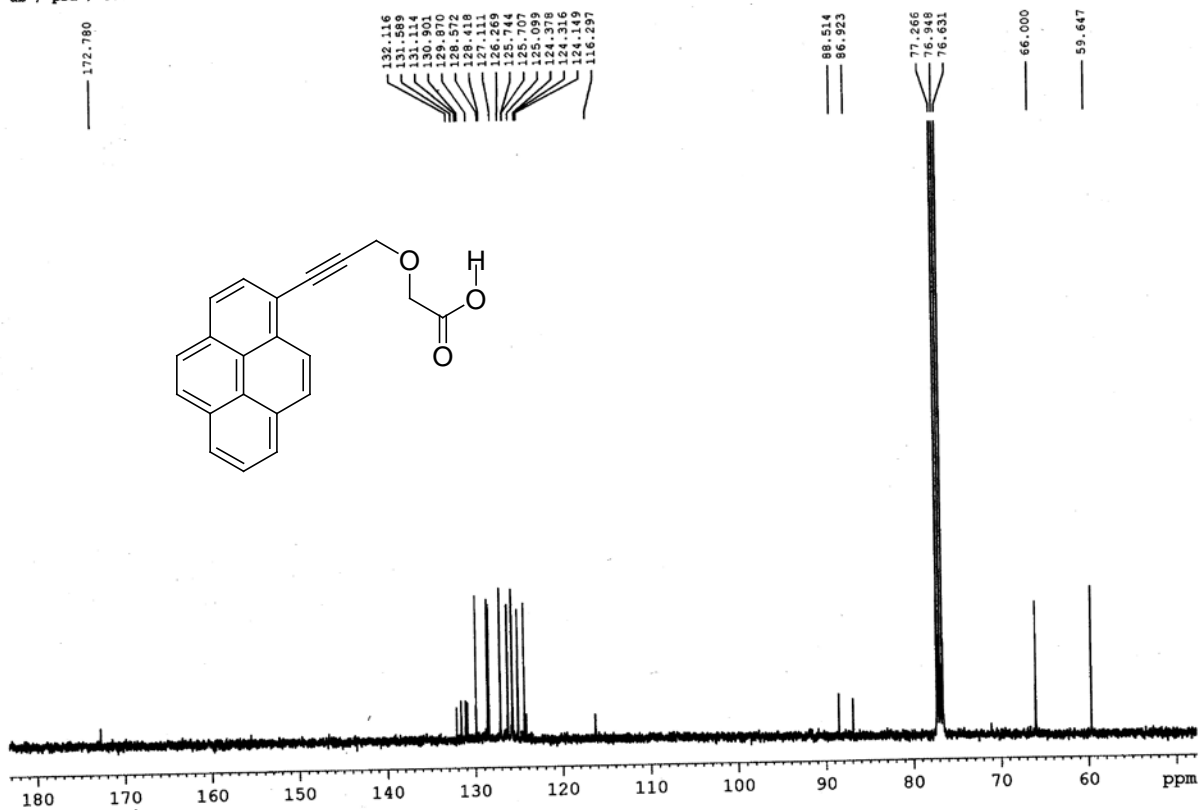


¹³C-NMR (100 MHz, CDCl₃) of compound 5

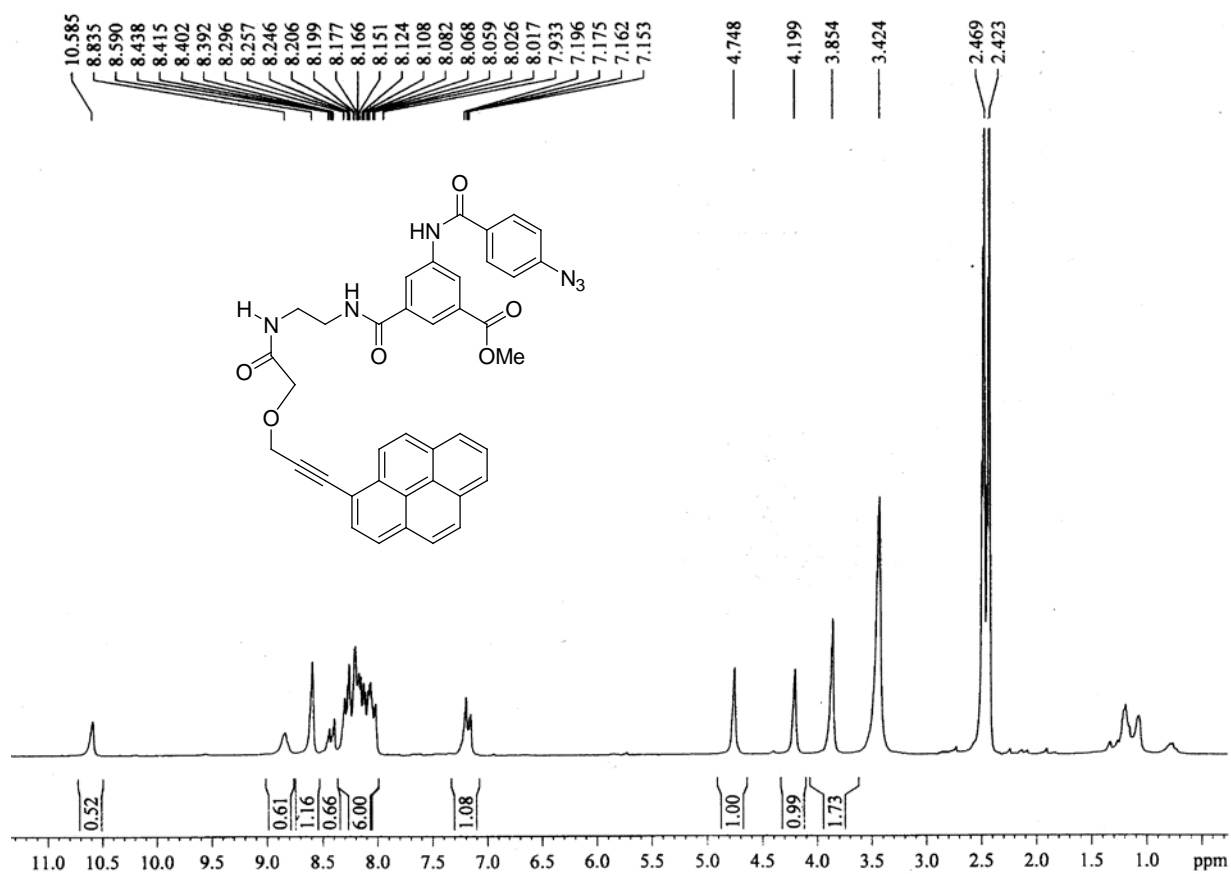


¹H-NMR(400 MHz, CDCl₃) of compound 6

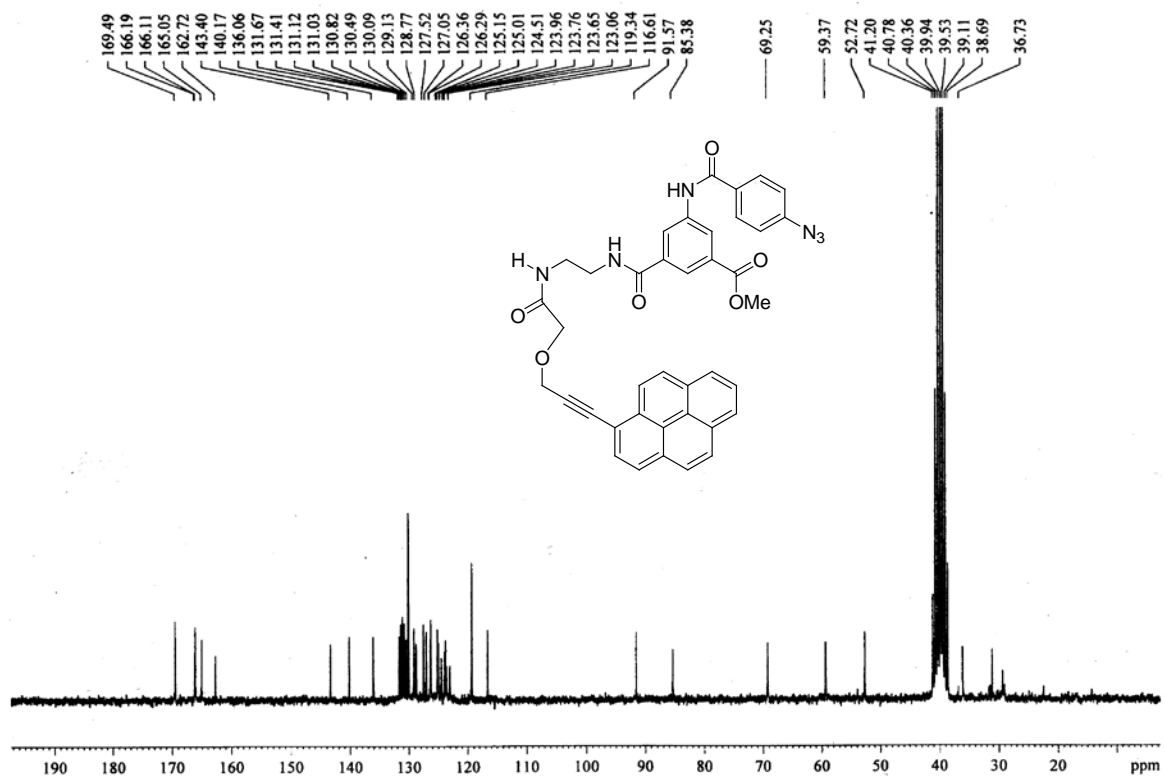
ab / ppa / 332 - 13c



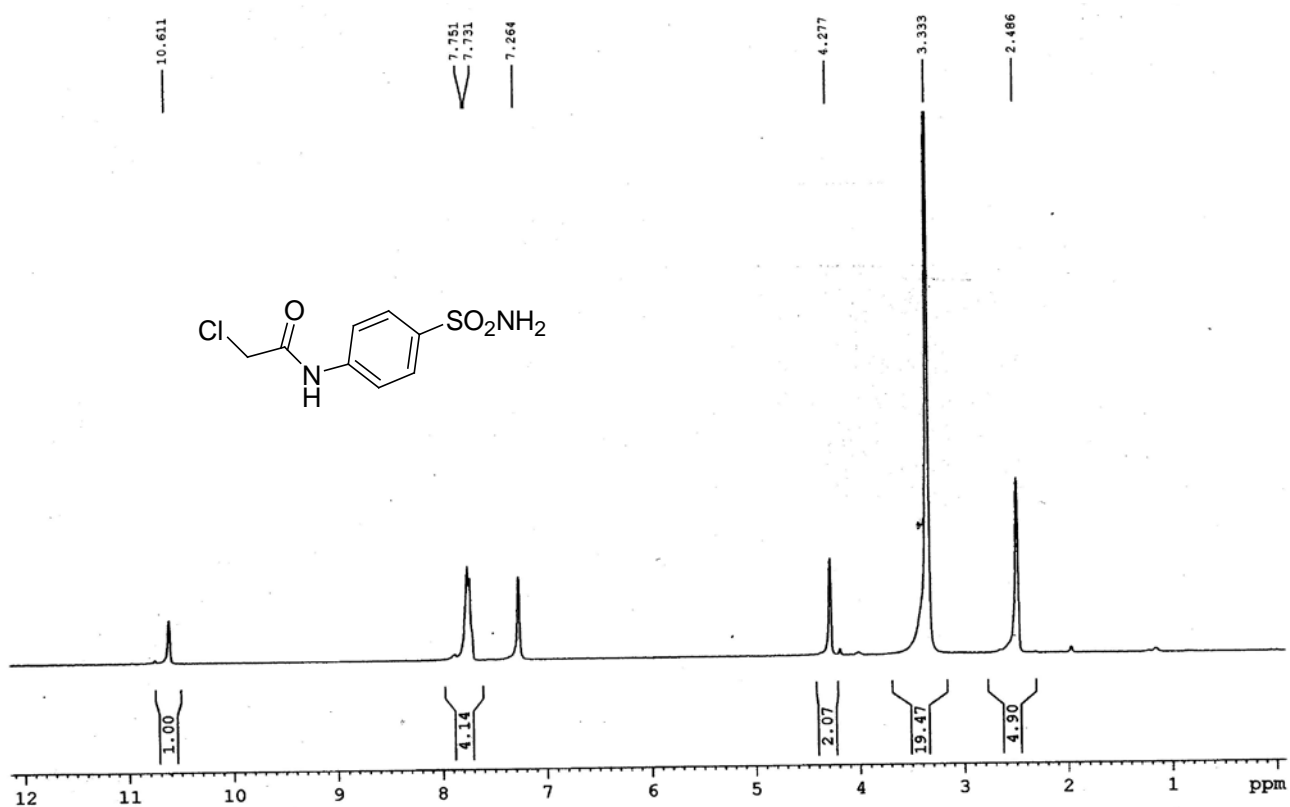
¹³C-NMR (100 MHz, CDCl₃) of compound 6



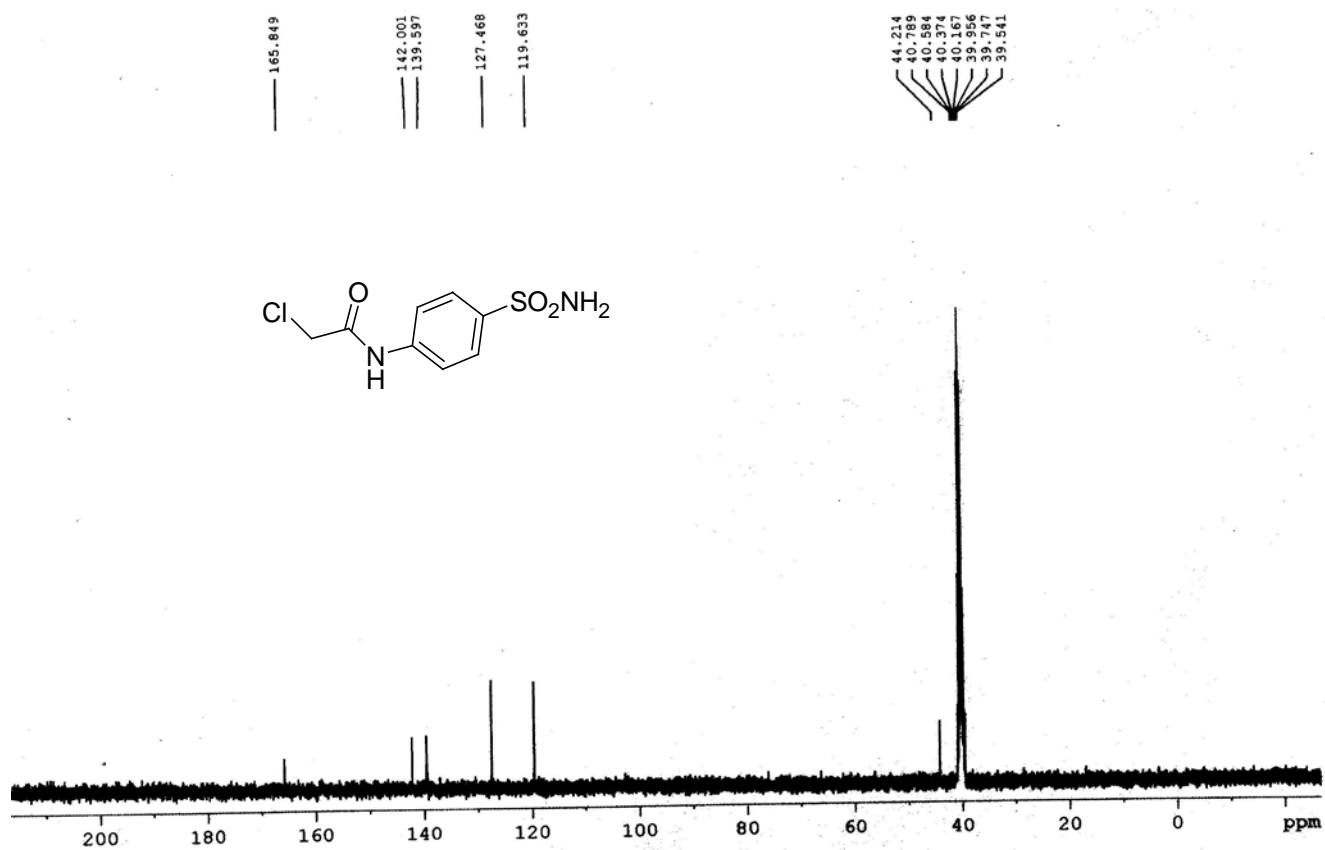
¹H-NMR(400 MHz, d₆-DMSO) of compound **11**



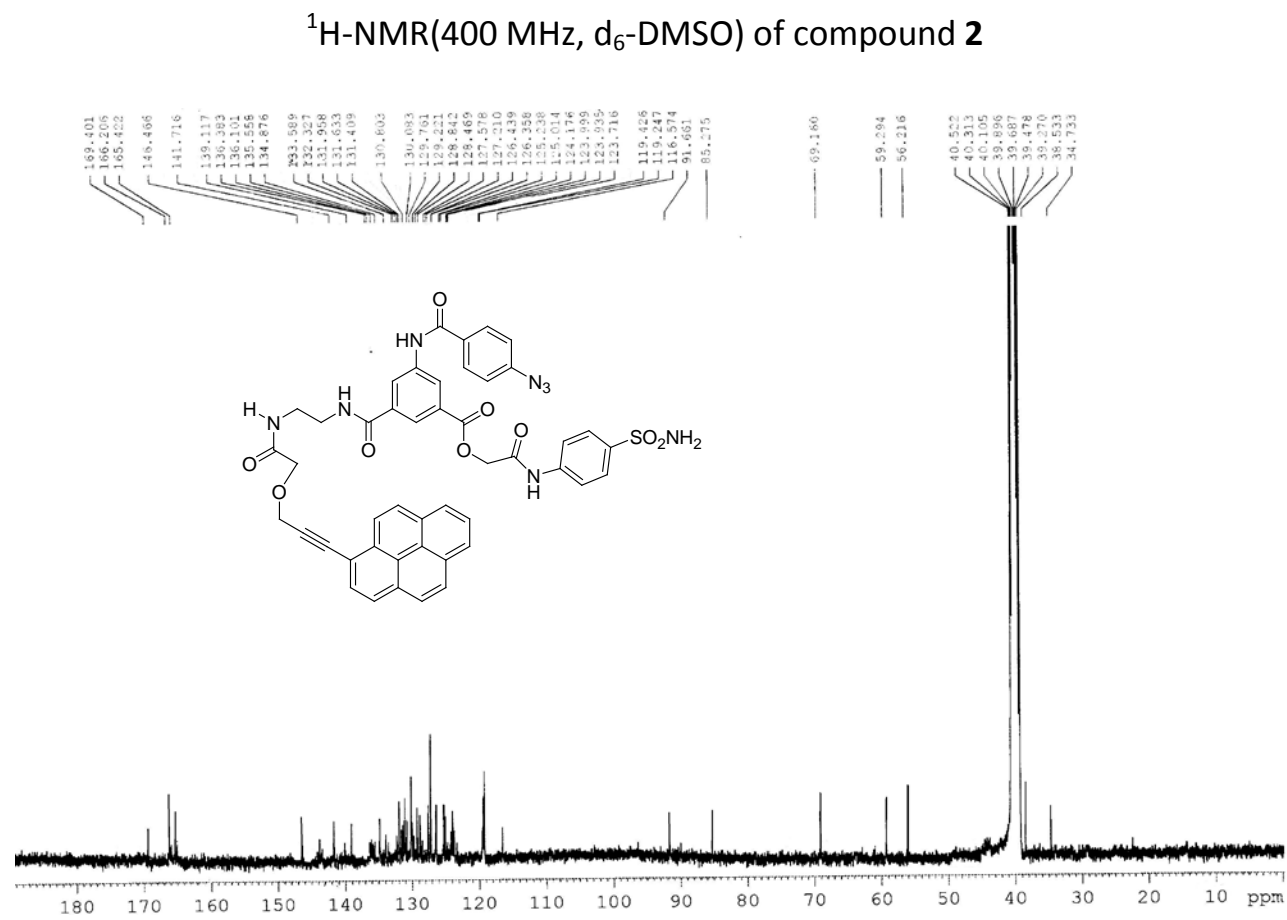
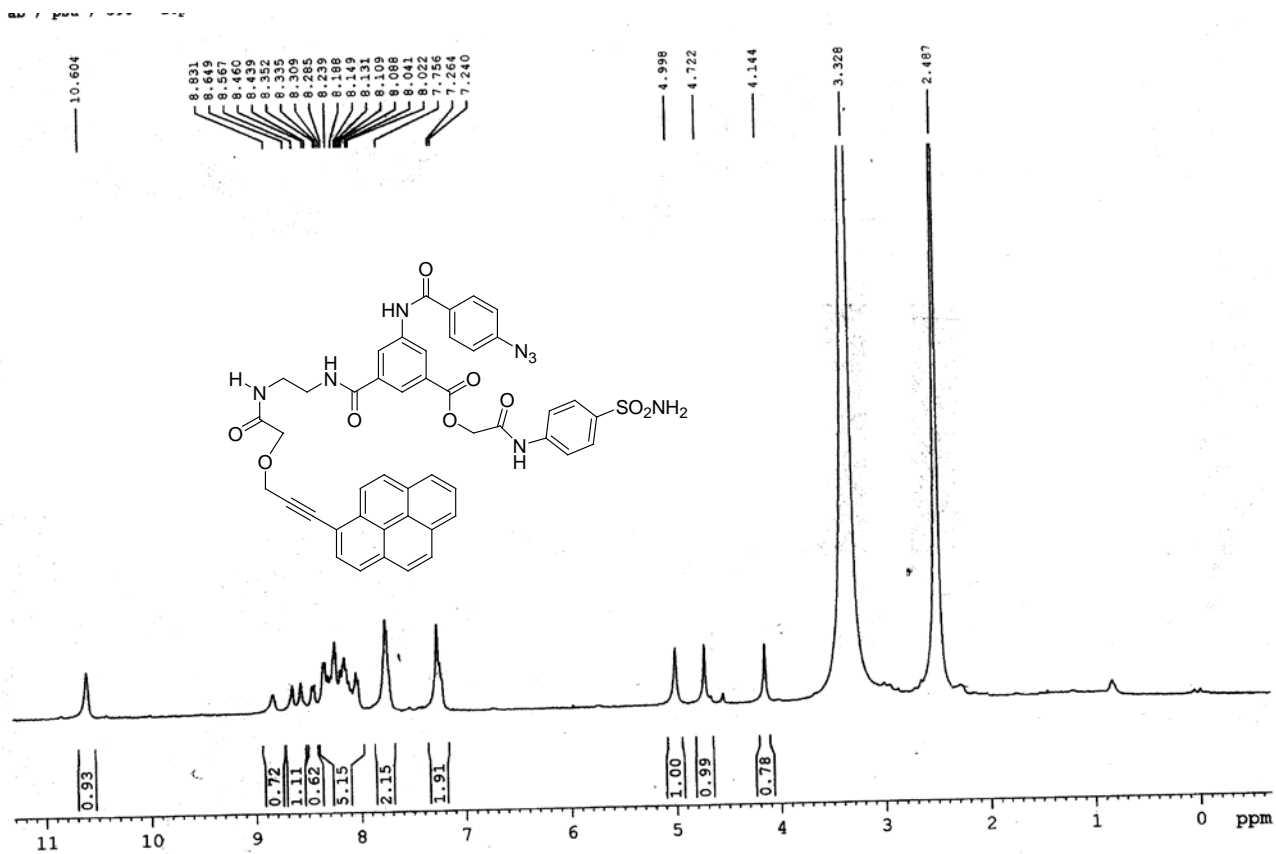
¹³C-NMR (100 MHz, d₆-DMSO) of compound **11**



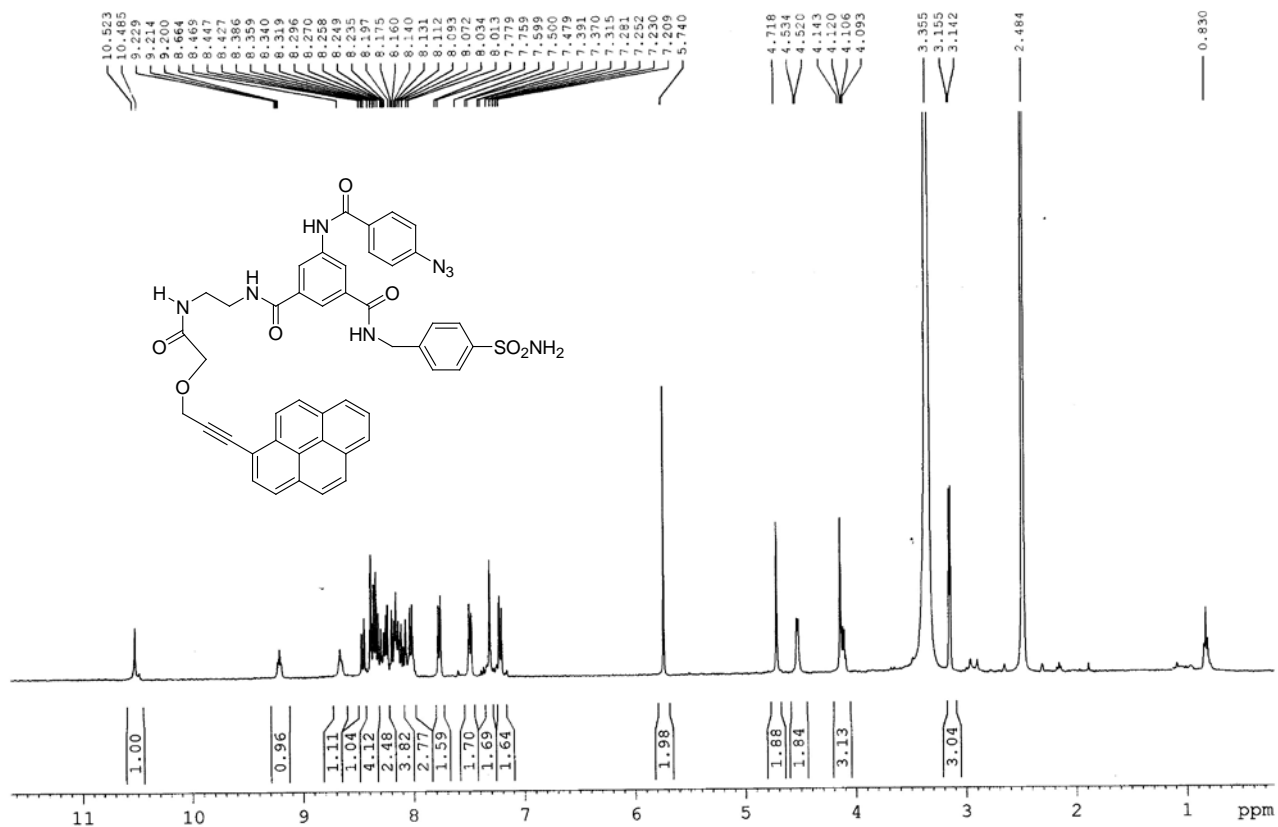
$^1\text{H-NMR}$ (400 MHz, d_6 -DMSO) of compound 13



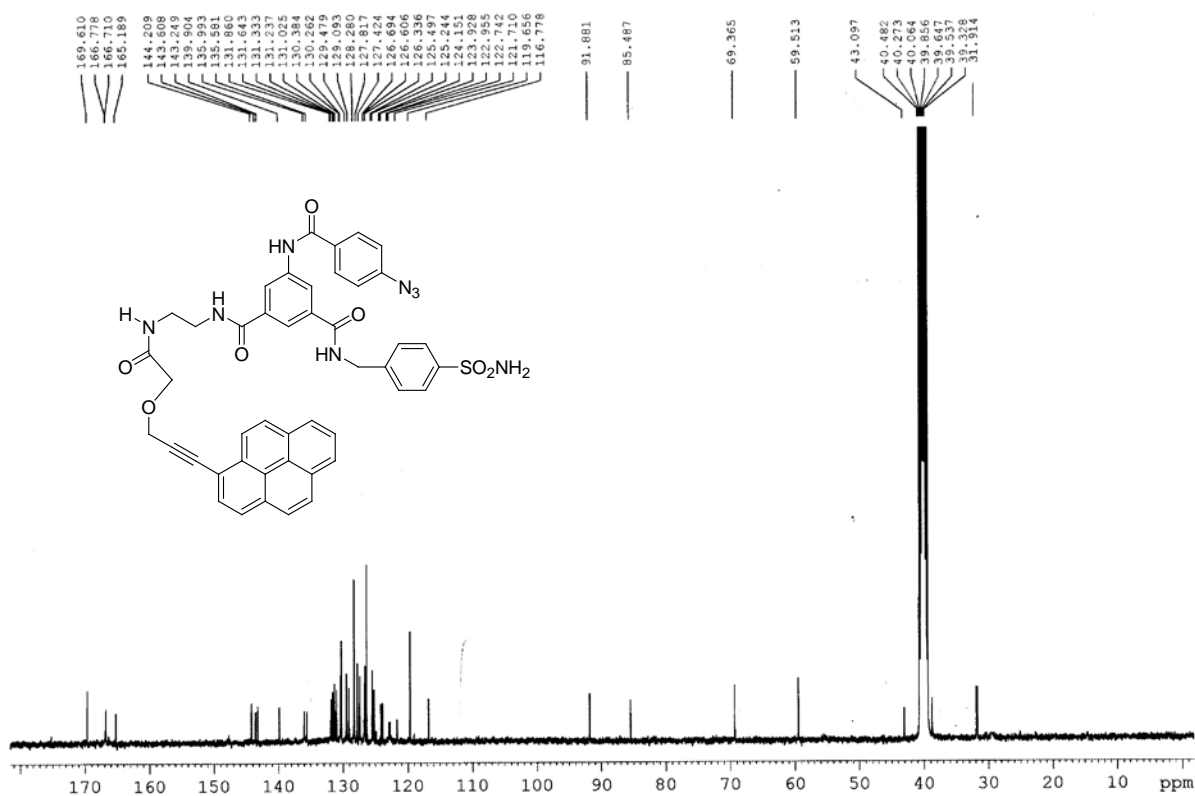
$^{13}\text{C-NMR}$ (100 MHz, d_6 -DMSO) of compound 13



¹³C-NMR (100 MHz, d₆-DMSO) of compound 2

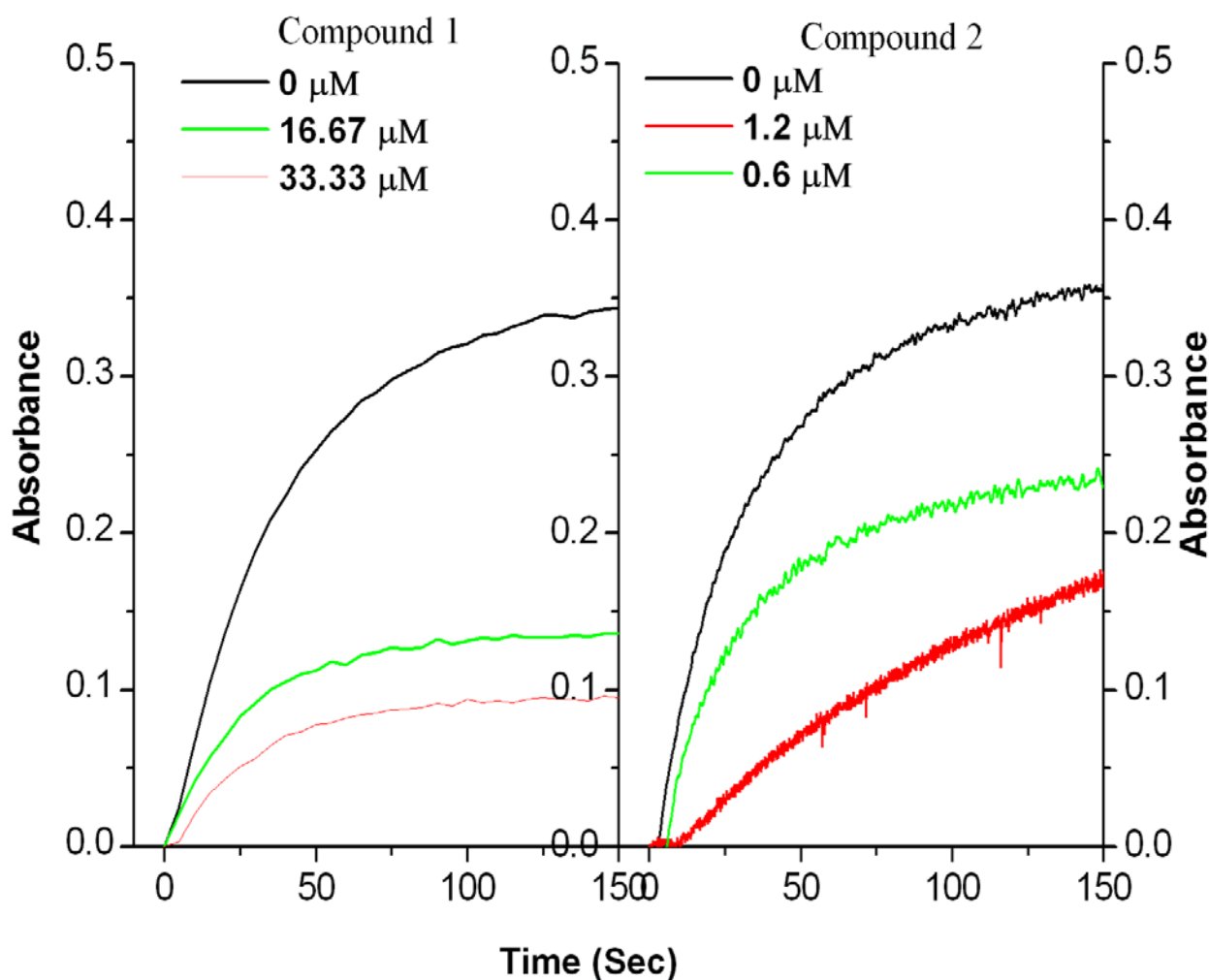


¹H-NMR (400 MHz, d₆-DMSO) of compound 1



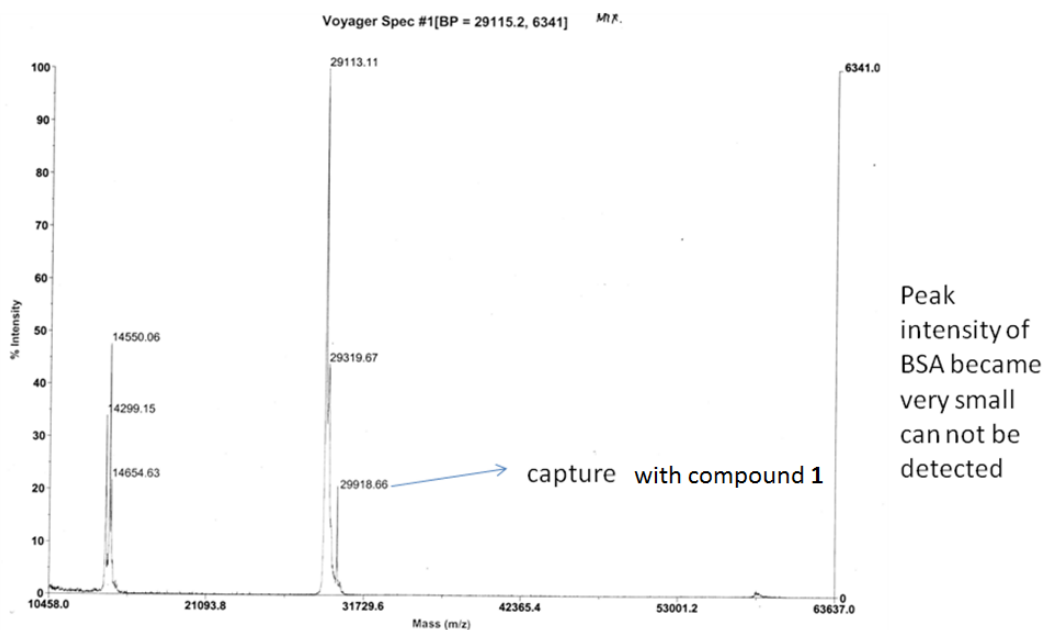
¹³C-NMR (100 MHz, d₆-DMSO) of compound 1

At 1.67 mM Substrate (PNPA) Concentration Inhibition Kinetics of CC1 and CC2 :



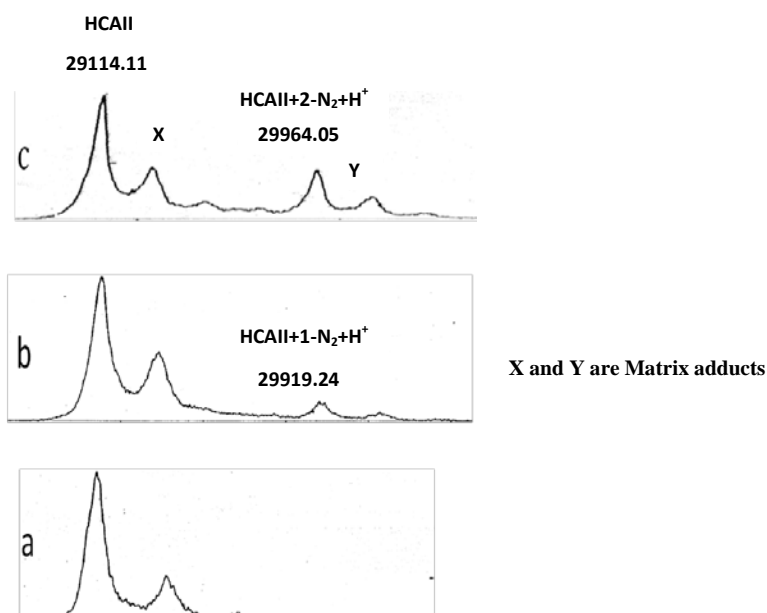
References:

1. de Leval et al., *J. Med. Chem.*, **2004**, *47*, 2796.
2. S. M. Gould, D. S. Tawfik, *Biochemistry.*, **2005**, *44*, 5444.

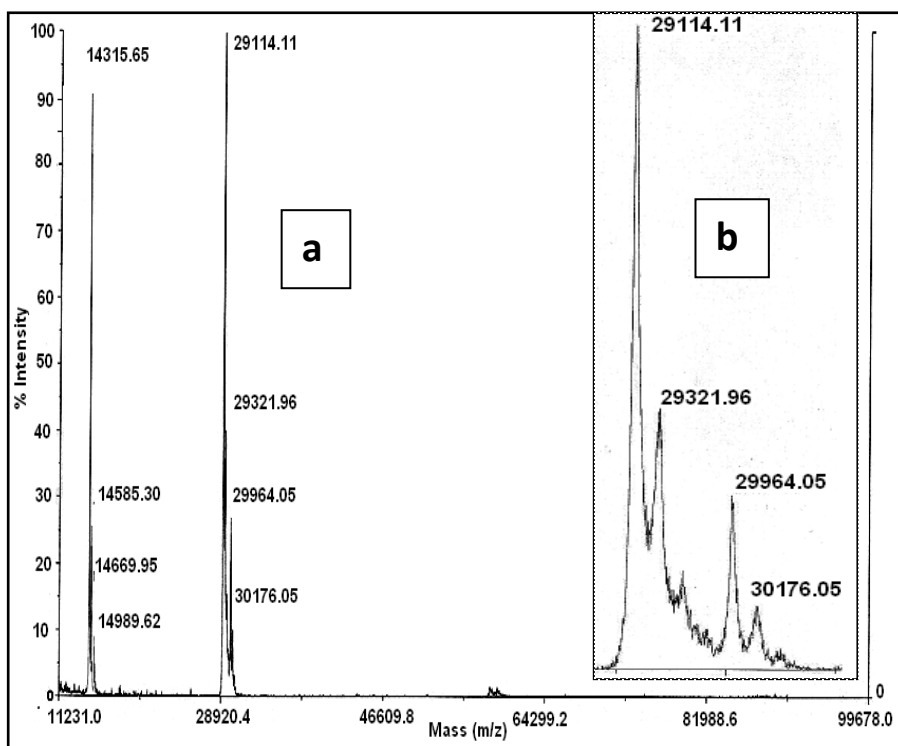


MALDI-TOF MS of enzyme HCA-II + Lysozyme + BSA + Compound 1

MALDI spectra: (a) Three proteins + compound 1, incubated and photo-reacted; (b) spectrum of expanded region in d

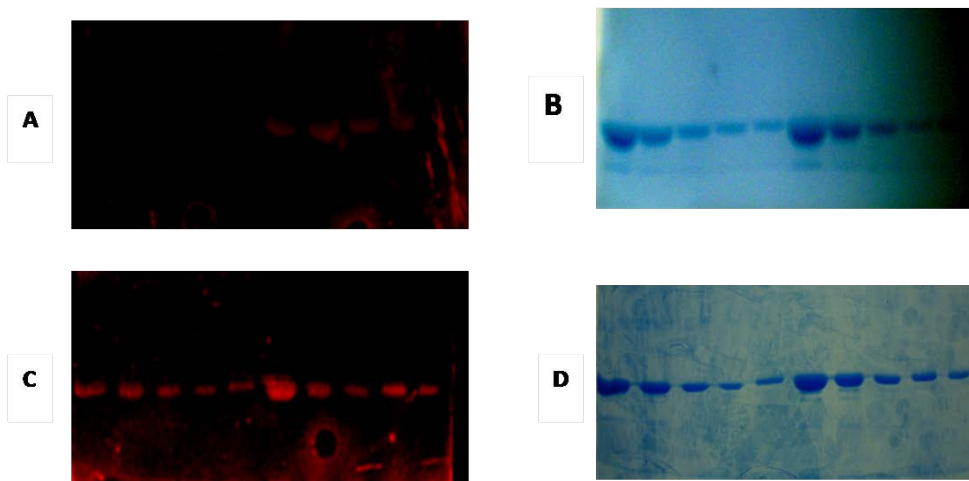


MALDI spectra: a) HCA II alone; b) HCA II + compound 1, incubated and photo-reacted; c) HCA II + compound 2, incubated and photo-reacted and crude mixture;



MALDI spectra: (a) Three proteins + compound **2**, incubated and photo-reacted; (b) spectrum of expanded region.

A comparative study to ensure protein capture enhancement due to photo-irradiation:

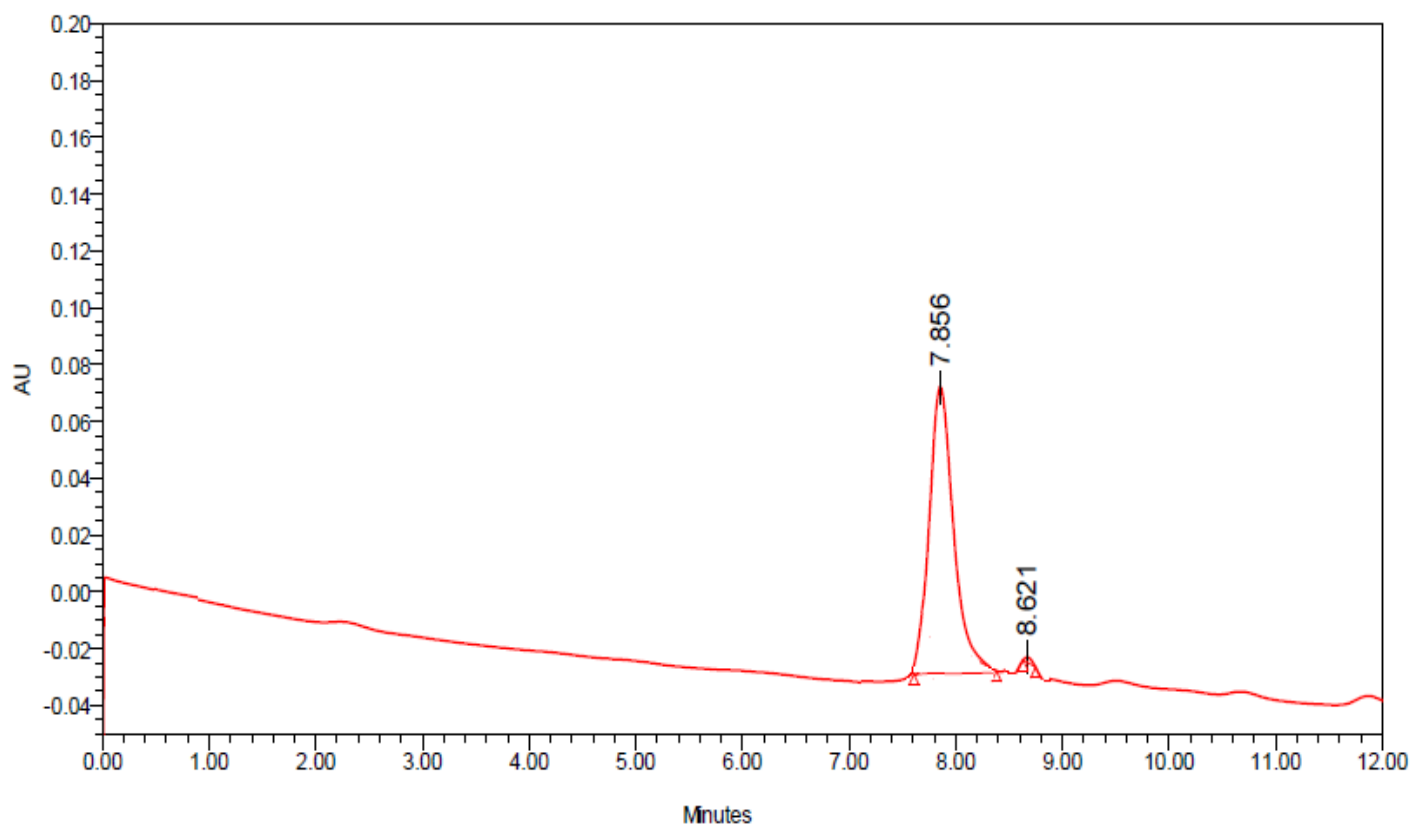


Result of gel electrophoresis analysis of capture of HCA II by **1** and **2** at different protein concentrations, as visualised by UV (left) and Coomassie blue (right). Lanes 1-5 represent incubation with **1** (100 μ M), lanes 6-10 represent incubation with **2** (100 μ M). The final concentration of protein in lanes 1-5 was 4, 6, 8, 20 and 40 μ M, respectively. HEPES buffer (pH 7.2) was used. **A**) Gel analysis without photo-irradiation under UV trans-illuminator. **B**) Gel analysis without photo-irradiation followed by coomassie staining. **C**) Gel analysis after photo-irradiation under UV trans-illuminator. **D**) Gel analysis after photo-irradiation followed by coomassie staining .

HPLC Data

HPLC data for Compound 1:

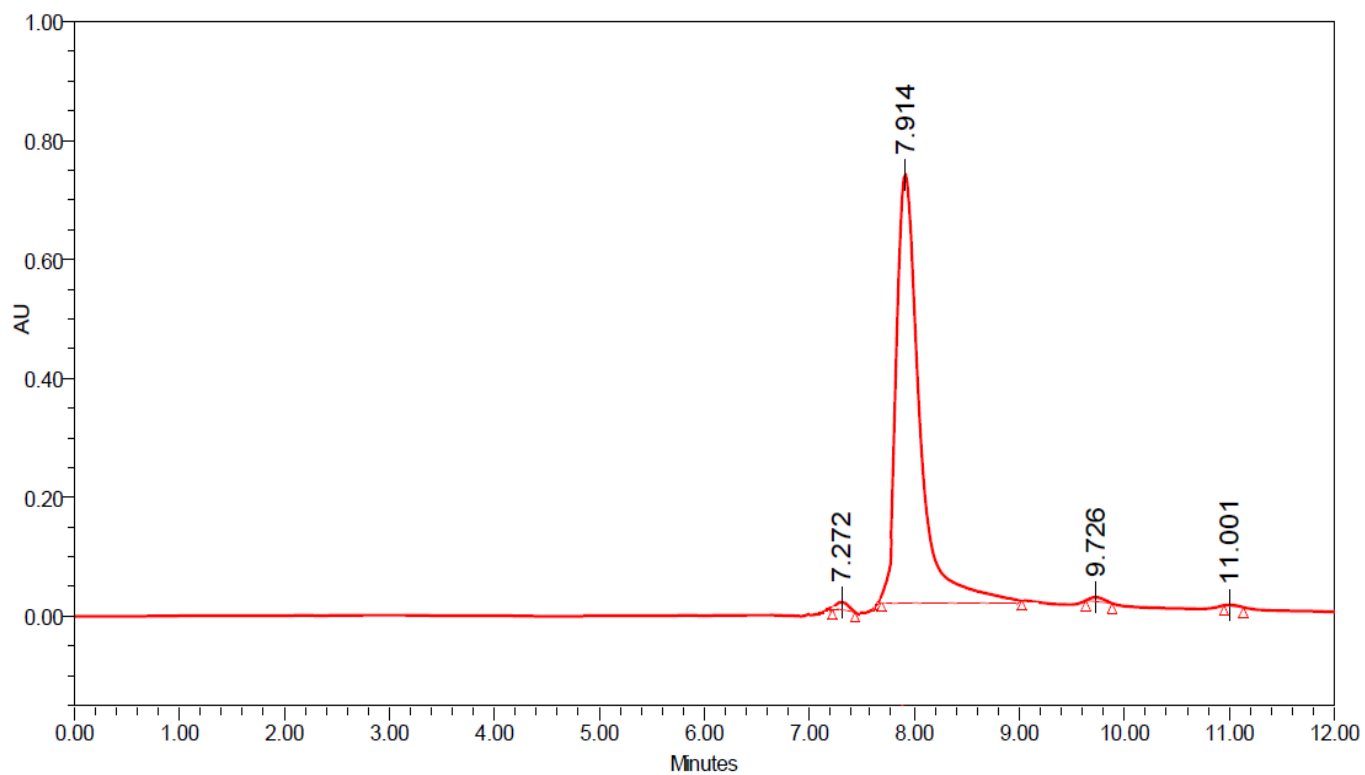
Eluent was Methanol and flow rate maintained at 0.2ml/min.



	RT	Area	% Area	Height
1	7.856	1252340	99.31	89669
2	8.621	8672	0.69	1765

HPLC data for Compound 2:

Eluent was Methanol and flow rate maintained at 0.2ml/min.



	RT	Area	% Area	Height
1	7.272	100346	0.52	12806
2	7.914	18995560	99.07	889105
3	9.726	64900	0.34	7100
4	11.001	13993	0.07	1767