Supporting Information

First Enzymatic Hydrolysis/Thio-Michael Addition Cascade Route to Synthesis of AChE Inhibitors

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CAUTION! All of the compounds described here (and especially the most potent bivalent inhibitors) are potentially neurotoxic. They must be handled with extreme care by trained personnel.

List of contents

I. General information and materials

All solvents were dried following standard procedures; DIEA and CH_2Cl_2: distillation over CaH_2; THF and toluene: distillation over sodium and benzophenone. Anhydrous DMF stocked over 4 Å molecular sieves was purchased from Carlo Erba or Alfa Aesar. MeOH: drying over activated 3 Å molecular sieves. Acrylic acid: distillation under reduced pressure and Argon atmosphere. The HPLC-gradient grade acetonitrile (CH_3CN) and methanol (MeOH) were purchased from VWR. Aq. buffers (used for IC_{50} determinations and *in situ* click chemistry experiments) and mobile-phases for HPLC were prepared using water purified with a Milli-Q system (purified to 18.2 MΩ.cm).

^{1}H and ^{13}C NMR spectra (C13CPD experiments) were recorded on either a Bruker DPX 200 or a Bruker DPX 300 spectrometers. Chemical shifts are expressed in parts per million (ppm) from $CDCl_3$ (δH = 7.26, δC = 77.16) or CD_3OD (δH = 3.31, δC = 49.00).[1] Multiplicities are described as s (singlet), d (doublet), dd (doublet of doublets), t (triplet), q (quadruplet), dt (doublet of triplets), dq (doublet of quadruplets), td (triplet of doublets), m (multiplet), br (broad). ^{1}J values are expressed in Hz.

Low-resolution mass spectra were obtained with a Finnigan LCQ Advantage MAX (ion trap) apparatus equipped with an electrospray ionization source. High-resolution mass spectra (HRMS) were obtained with a Waters LCT Premier XE mass spectrometer.

IR spectra were recorded with a universal attenuated total reflectance (ATR) sampling accessory on a Perkin-Elmer FTIR Spectrum 100 spectrometer. Column chromatography purifications were performed on silica gel (40-63 μm). Thin-layer chromatography (TLC) was carried out on silica gel aluminium sheets. Compounds were visualized by one of the two following methods: 1) illumination with a short wavelength UV lamp (λ = 254 nm) or 2) staining with a basic $KMnO_4$ solution in water.

Analytical RP-HPLC was performed on a Thermo Electron Surveyor instrument equipped with a PDA detector and a Thermo Hypersil GOLD C_{18} column (5 μm, 2.1 × 100 mm) at a flow rate of 0.25 mL/min (System A) with CH_3CN and 0.1% aqueous TFA system [100% TFA (5 min), linear gradient from 0 to 67% of CH_3CN (20 min), then a linear gradient to 100% of CH_3CN (25 min)] and UV detection at between 220 and 360 nm. UV-Vis detection at specified wavelengths or with the "Max Plot" mode, *i.e.* chromatogram at maximum absorbance of each compound, was used to determine the compounds purity. Several

[1] 1. G. R. Fulmer, A. J. M. Miller, N. H. Sherden, H. E. Gottlieb, A. Nudelman, B. M. Stoltz, J. E. Bercaw and K. I. Goldberg, *Organometallics*, 2010, **29**, 2176-2179.

chromatographic systems were used for the semi-preparative HPLC purification steps on one of the three RP-HPLC columns (C_{18}, Thermo Hypersil GOLD, 1) 5 µm, 21.2 × 250 mm; flow rate of 12 mL/min; 2) 5 µm, 10 × 250 mm; flow rate of 4 mL/min; 5 µm, 10 × 100 mm; flow rate of 4 mL/min; Systems B). System B: with MeOH and trifluoroacetic acid 0.1% (TFA 0.1%, pH 2.0) as eluents [95% TFA (5 min), then linear gradient from 5% to 10% of MeOH (5 min), then a linear gradient to 40% of MeOH (90 min), then a linear gradient to 70% of MeOH (60 min), then a linear gradient to 100% of MeOH (40 min)] and UV detection at 228 and 260 nm. System C: with CH_3CN and trifluoroacetic acid 0.1% (TFA 0.1%, pH 2.0) as eluents [95% TFA (5 min), then linear gradient from 5% to 10% of CH_3CN (5 min), then a linear gradient to 70% of CH_3CN (185 min)] and UV detection at 259 and 327 nm. System D: with CH_3CN and trifluoroacetic acid 0.1% (TFA 0.1%, pH 2.0) as eluents [95% TFA (5 min), then linear gradient from 5% to 10% of CH_3CN (5 min), then a linear gradient to 25% of CH_3CN (40 min), then a linear gradient to 50% of CH_3CN (130 min), then a linear gradient to 65% of CH_3CN (40 min)] and UV detection at 259 and 300 nm. System E: with CH_3CN and trifluoroacetic acid 0.1% (TFA 0.1%, pH 2.0) as eluents [100% TFA (10 min), then linear gradient from 0% to 10% of CH_3CN (7.5 min), then a linear gradient to 70% of CH_3CN (110 min)] and UV detection at 256 and 327 nm.

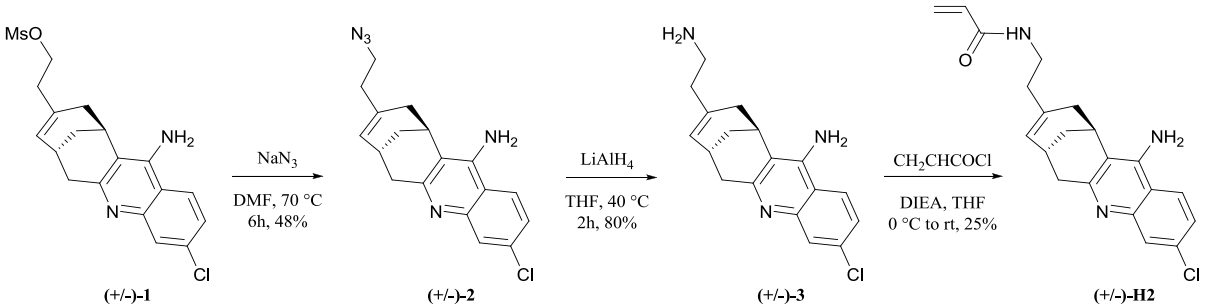
LCMS/SIM experiments were performed on a Thermo Scientific Surveyor Plus instrument; RP-HPLC separations were achieved with a 5 µm, 2.1 × 50 mm Thermo Hypersil GOLD C_{18} column equipped with a C_{18} guard column. The solvent system consisted of a linear gradient of CH_3CN in aqueous formic acid 0.1% (FA 0.1%, pH 2.7) at a flow rate of 0.25 mL/min. ESI detection in the positive mode (Full scan, 150-2000 a.m.u., SIM mode centered on the expected molecular weight of compound and its doubly charged ion).

System	Sheath gaz flow (arb u)	Aux/sweep gaz flow (arb u)	Spray voltage (KV)	Capillary temp (°C)	Capillary voltage (KV)	Tube lens offset (KV)
F1	30	20	7	332	3	-10
F2	10	20	5	360	10	0
F3	25	20	7.5	260	42	35
F4	25	15	6.5	270	31	5
F5	25	15	7	300	17	0
F6	25	20	7.5	350	3	-5
F7	20	10	5.5	320	25	40
F8	20	15	5.5	300	42	35

II. Experimental details

Huprine (±)-**1** and (±)-**6** were synthesized according to the procedures previously described by our group.[2,3]

II.1 Synthetic procedures

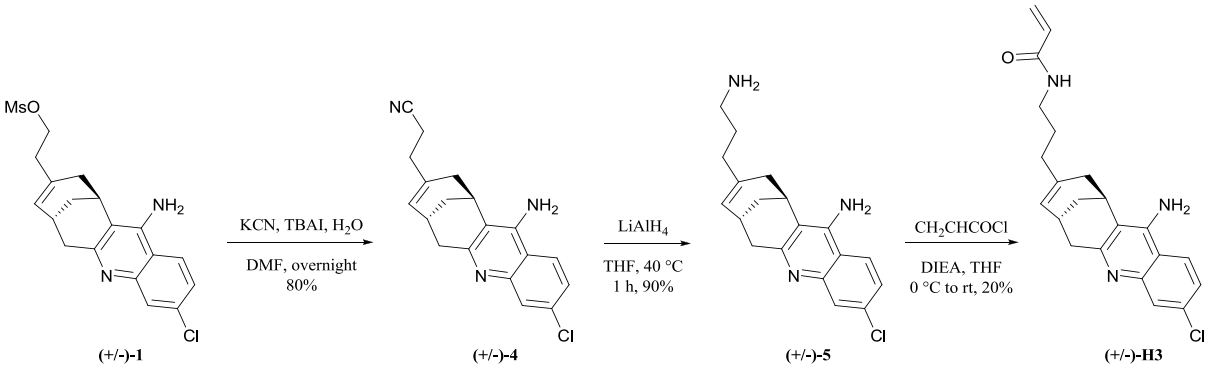


A suspension of mesylate (±)-**1** (500 mg, 1.27 mmol) and sodium azide (331 mg, 5.1 mmol, 4 equiv.) in dry DMF (6 mL) was stirred under argon at 70 °C for 8 h. The reaction mixture was then cooled to r.t. and water (15 mL) was added under stirring. The aqueous phase was extracted with EtOAc (3 × 30 mL) and the combined organic layers were washed with water, dried over $MgSO_4$ and concentrated under reduced pressure. Purification of the crude reaction mixture by chromatography over silica gel (EtOAc) afforded the desired huprine (±)-**2** as pale yellow solid (210 mg, 48%). ^1H and ^{13}C were in agreement with those given in the literature.[4]

To a suspension of lithium aluminium hydride (35 mg, 0.9 mmol, 2 equiv.) in anhydrous THF (3 mL), a solution of huprine (±)-**2** (150 mg, 0.45 mmol,) in anhydrous THF (2 mL) was added dropwise and the solution was heated to 40 °C for 1 h. Water (0.2 mL) was added at 0 °C followed by the addition of NaOH solution 5M (0.2 mL) and water again (0.4 mL) and the mixture was allowed to stir at room temperature for 15 min. Na_2SO_4 was then added and the resulting cake was filtered on celite, washed with EtOAc and the filtrate evaporated to dryness. The desired product (±)-**3** needed no further purification and was obtained as yellow solid (155 mg, 90%). ^1H and ^{13}C were in agreement with those given in the literature.[4]

To a solution of amine (±)-**3** (80 mg, 0.26 mmol) and DIEA (98 µL, 0.56 mmol, 2.15 equiv.) in anhydrous THF (6 mL) at 0 ºC was added acryloyl chloride (25 µL, 0.31 mmol, 1.19 equiv.) then the reaction was allowed to return to room temperature for 1 h. The reaction

mixture was hydrolysed by addition of a solution of sat. aq. $NaHCO_3$ (5 mL). The solution was next extracted with EtOAc (3 × 5 mL) and the combined organic layers were washed with brine, dried over $MgSO_4$, and evaporated to dryness. The residue was then purified by preparative HPLC (system B) and the TFA salt of the desired product (±)-**H2** was obtained as white solid (25 mg, 20%). ^{1}H NMR: (300 MHz, MeOD) δ = 1.87-2.16 (m, 5H), 2.51 (dd, *J* = 4.4 Hz, *J* = 17.4 Hz, 1H), 2.78-2.84 (m, 2H), 3.07-3.23 (m, 2H), 3.33-3.40 (m, 2H), 5.40 (dd, *J* = 1.9 Hz, *J* = 10.1 Hz, 1H), 5.65 (d, *J* = 5.4 Hz, 1H), 5.76 (dd, *J* = 1.9 Hz, *J* = 17.0 Hz, 1H), 5.94 (dd, *J* = 10.1 Hz, *J* = 17.0 Hz, 1H), 7.59 (dd, *J* = 1.9 Hz, *J* = 9.1 Hz, 1H), 7.69 (d, *J* = 1.9 Hz, 1H), 8.35 (d, *J* = 9.1 Hz, 1H); ^{13}C NMR: (50 MHz, MeOD) δ = 27.4 (CH), 28.1 (CH), 29.1 (CH_2), 33.4 (CH_2), 35.8 (CH_2), 37.8 (CH_2), 38.1 (CH_2), 115.3 (C), 115.6 (C), 119.1 (CH), 126.2 (CH), 126.5 (CH), 127.3 (CH), 127.6 (CH), 131.5 (CH_2), 135.9 (CH), 139.7 (C), 140.4 (C), 152.7 (C), 156.9 (C), 167.5 (C); MS (ESI+) m/z (%): 368.20 (100), 370.20 (35) $[M+H]^+$; HPLC: t_R=15.5 min (purity > 93%); System A. IC_{50} *m*-AChE = 64.9 ± 0.1 nM.

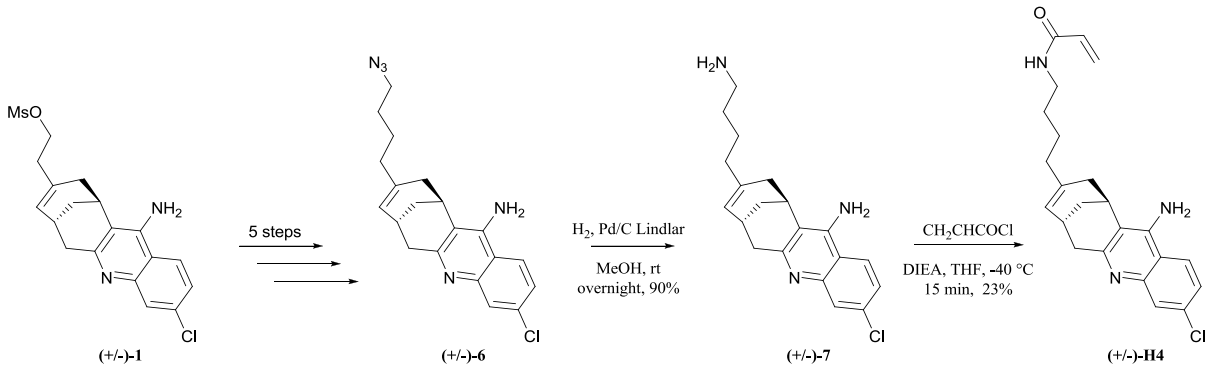


To a solution of mesylate (±)-**1** (1.96 g, 4.99 mmol) in DMF (24 mL) was added potassium cyanide (1.6 g, 24.9 mmol, 5 equiv.), TBAI (83 mg, 0.22 mmol, 4 mol%) and water (50 µL). The resulting mixture was heated overnight at 70 °C. After full consumption of the starting material, the reaction mixture was next cooled to 0 °C and the reaction was stopped by addition of water (30 mL) and EtOAc (10 mL) and the solution was extracted with EtOAc (2 × 30 mL). The combined organic layers were washed with water (40 mL) dried over $MgSO_4$ and filtered, and the solvent was removed under reduced pressure. The resulting residue was purified by chromatography over silica gel (EtOAc) to yield the corresponding cyano derivative (±)-**4** as yellow solid (907 mg, 57%). ^{1}H and ^{13}C were in agreement with those given in the literature.[4]

To a suspension of lithium aluminium hydride (35.1 mg, 0.9 mmol, 2 equiv.) in anhydrous THF (3.5 mL), a solution of the huprine (±)-**4** (150 mg, 0.45 mmol) in anhydrous THF (1.5

mL) was added dropwise and the solution was heated at 40 °C for 1 h. Water (1.2 mL) was added at 0 °C followed by the addition of sodium hydroxide solution 10M (1.2 mL) and water again (3.5 mL) and the mixture was allowed to stir at room temperature for 15 min. $MgSO_4$ was then added and the resulting cake was filtered on celite, washed with EtOAc and the filtrate evaporated to dryness. The desired product (±)-**5** needed no further purification and was obtained as off-white solid (141 mg, 90%). ^{1}H NMR: (300 MHz, MeOD) δ = 1.34-1.44 (m, 2H), 1.84 (t, *J* = 7.5 Hz, 1H), 1.91-2.08 (m, 3H), 1.31-2.47 (m, 3H), 2.68 (s, 1H), 2.83 (d, *J* = 17.4 Hz, 1H), 3.03 (dd, *J* = 5.4 Hz, *J* = 17.4 Hz, 1H), 3.32 (s, 1H), 5.54 (d, *J* = 5.1 Hz, 1H), 7.25 (dd, *J* = 2.0 Hz, *J* = 9.0 Hz, 1H), 7.66 (d, *J* = 2.0 Hz, 1H), 8.01 (d, *J* = 9.0 Hz, 1H); ^{13}C NMR: (75 MHz, $CDCl_3$) δ = 28.4 (CH), 29.7 (CH), 30.4 (CH_2), 31.4 (CH_2), 34.6 (CH_2), 35.6 (CH_2), 40.4 (CH_2), 41.9 (CH_2), 115.6 (C), 117.1 (C), 124.6 (CH), 124.8 (CH), 126.0 (CH), 126.4 (CH), 135.5 (C), 137.5 (C), 148.2 (C), 149.8 (C), 159.3 (C); IR (neat) cm^{-1}: 3336, 3196, 2922, 1645, 1608, 1558, 1488, 1422; Melting point: 114-116 °C (degradation). MS (ESI+) m/z (%): 328.3 (100), 330.1 (35) $[M+H]^+$; HPLC t_R=14.4 (purity >95%); System A. IC_{50} *m*-AChE = 30.4 ± 2.0 nM.

To a solution of amine (±)-**5** (180 mg, 0.55 mmol) and DIEA (211 µL, 1.21 mmol, 2.2 equiv.) in anhydrous THF (13 mL) at 0 °C was added acryloyl chloride (54 µL, 0.66 mmol, 1.2 equiv.) then the reaction was allowed to return to room temperature for 3 h. The reaction mixture was hydrolysed by addition of water (5 mL) and a solution of sat. aq. $NaHCO_3$ (5 mL). The solution was extracted with EtOAc (3 × 15 mL) and the combined organic layers were washed with brine, dried over $MgSO_4$, and evaporated to dryness. The residue was then purified by preparative HPLC (system C) to afford the TFA salt of the desired product (±)-**H3** as yellow solid (49 mg, 20%). ^{1}H NMR: (200 MHz, MeOD) δ = 1.44-1.59 (m, 2H), 1.89-2.14 (m, 5H), 2.53 (dd, *J* = 4.1 Hz, *J* = 17.6 Hz, 1H), 2.82-2.92 (d, *J* = 18.2 Hz, 1H), 2.95-3.11 (m, 2H), 3.21 (dd, *J* = 5.5 Hz, *J* = 17.9 Hz, 1H), 3.40 (s, 1H), 5.56-5.62 (m, 2H), 6.12-6.15 (m, 2H), 7.57 (dd, *J* = 1.9 Hz, *J* = 9.1 Hz, 1H), 7.73 (d, *J* = 1.9 Hz, 1H), 8.34 (d, *J* = 9.1 Hz, 1H); ^{13}C NMR: (50 MHz, MeOD) δ = 27.5 (CH), 27.9 (CH), 28.1 (CH_2), 29.3 (CH_2), 33.9 (CH_2), 35.3 (CH_2), 35.9 (CH_2), 39.7 (CH_2), 115.3 (C), 115.4 (C), 119.3 (CH), 125.4 (CH), 126.3 (CH), 126.4 (CH), 127.7 (CH), 131.9 (CH), 137.8 (CH), 139.5 (C), 140.4 (C), 153.0 (C), 156.7 (C), 167.9 (C); MS (ESI+) m/z (%): 382.27 (100) $[M+H]^+$; HPLC t_R = 16.63 (purity >92%); System A; IC_{50} *m*-AChE = 56.9 ± 0.9 nM.

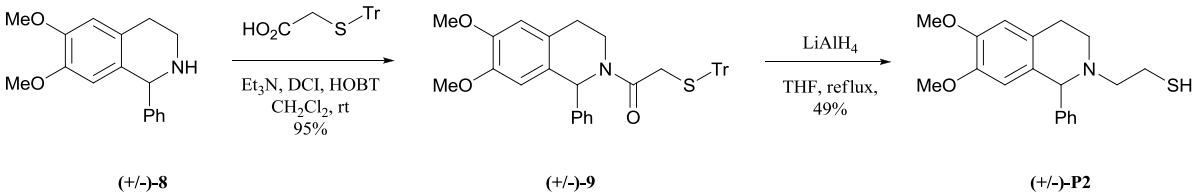


Compound (±)-**6** was obtained following the procedure described in the literature starting from mesylate (±)-**1**.[3]

To a solution of azide (±)-**6** (106 mg, 0.29 mmol) in anhydrous MeOH (3.5 mL) under Argon atmosphere was added Pd/C 5% Lindlar (30.7 mg, 0.014 mmol, 5 mol%) and the solution was purged with Argon then with H_2 and left under H_2 atmosphere at normal pressure overnight at rt. The reaction was filtered over celite, washed with MeOH and evaporated to dryness. The desired product (±)-**7** was obtained after recrystallization in CH_2Cl_2 as yellow solid (95 mg, 90%). ^{1}H NMR: (300 MHz, MeOD) δ = 1.14-1.34 (m, 4H), 1.83-2.13 (m, 5H), 2.36-2.48 (m, 3H), 2.71 (s, 1H), 2.84 (td, *J* = 1.6 Hz, *J* = 17.5 Hz, 1H), 3.06 (dd, *J* = 5.4 Hz, *J* = 16.6 Hz, 1H), 3.33 (s, 1H), 5.54 (d, *J* = 4.4 Hz, 1H), 7.29 (dd, *J* = 2.0 Hz, *J* = 9.0 Hz, 1H), 7.68 (d, *J* = 2.0 Hz, 1H), 8.04 (d, *J* = 9.0 Hz, 1H); ^{13}C NMR: (75 MHz, $CDCl_3$) δ = 25.6 (CH_2), 28.4 (CH), 29.7 (CH), 30.4 (CH_2), 32.8 (CH_2), 34.4 (CH_2), 37.9 (CH_2), 40.5 (CH_2), 42.2 (CH_2), 115.6 (C), 117.1 (C), 124.6 (CH), 124.8 (CH), 126.0 (CH), 126.4 (CH), 135.5 (C), 137.6 (C), 148.1 (C), 149.7 (C), 159.4 (C); IR (neat) cm^{-1}: 3478, 3342, 3171, 2924, 1646, 1607, 1571, 1487, 1419; Melting point: 68-70 °C (degradation); MS (ESI+) m/z (%): 342.33 (100), 344.27 (35) $[M+H]^+$; HPLC t_R=14.8 (purity >92%); System A.

To a solution of amine (±)-**7** (50 mg, 0.15 mmol) and DIEA (56 µL, 0.32 mmol, 2.13 equiv.) in anhydrous THF (3.5 mL) at -40 ºC was added acryloyl chloride (12 µL, 0.15 mmol, 1 equiv.) dropwise and the solution was allowed to stir a -40 °C for 15 min. The reaction mixture was hydrolysed by addition of a solution of sat. aq. $NaHCO_3$ (5 mL). The solution was next extracted with EtOAc (3 × 5 mL) and the combined organic layers were washed with brine, dried over $MgSO_4$, and evaporated to dryness. The residue was then purified by preparative HPLC (system D) and the TFA salt of the desired product (±)-**H4** was obtained as yellow solid (18.3 mg, 23%). ^{1}H NMR: (300 MHz, MeOD) δ = 1.29-1.36 (m, 5H), 1.89-2.12 (m, 5H), 2.51 (dd, *J* = 6.6 Hz, *J* = 17.2 Hz, 1H), 2.81 (s, 1H), 2.86 (d, *J* = 17.8 Hz, 1H), 3.06-3.11 (m, 2H), 3.21 (dd, *J* = 6.1 Hz, *J* = 17.3 Hz, 1H), 3.39 (s, 1H), 5.59-5.63 (m, 2H), 6.12-

6.14 (m, 2H), 7.59 (dd, J = 1.9 Hz, J = 9.1 Hz, 1H), 7.72 (d, J = 1.9 Hz, 1H), 8.34 (d, J = 9.1 Hz, 1H); ^{13}C NMR: (50 MHz, MeOD) δ = 25.7 (CH_2), 27.5 (CH), 28.1 (CH), 29.4 (CH_2), 29.6 (CH_2), 33.9 (CH_2), 36.0 (CH_2), 37.6 (CH_2), 40.0 (CH_2), 115.3 (C), 115.5 (C), 119.2 (CH), 125.2 (CH), 126.3 (CH), 126.4 (CH), 127.8 (CH), 132.0 (CH_2), 138.4 (CH), 139.5 (C), 140.5 (C), 153.1 (C), 156.7 (C), 167.9 (C); MS (ESI+) m/z (%):398.20 (40) $[M+H]^+$, 396.13 (100) $[M/2+H]^{2+}$; HPLC t_R=17.0 (purity >94%); System A; IC_{50} *m*-AChE = 5.4 ± 0.5 nM.

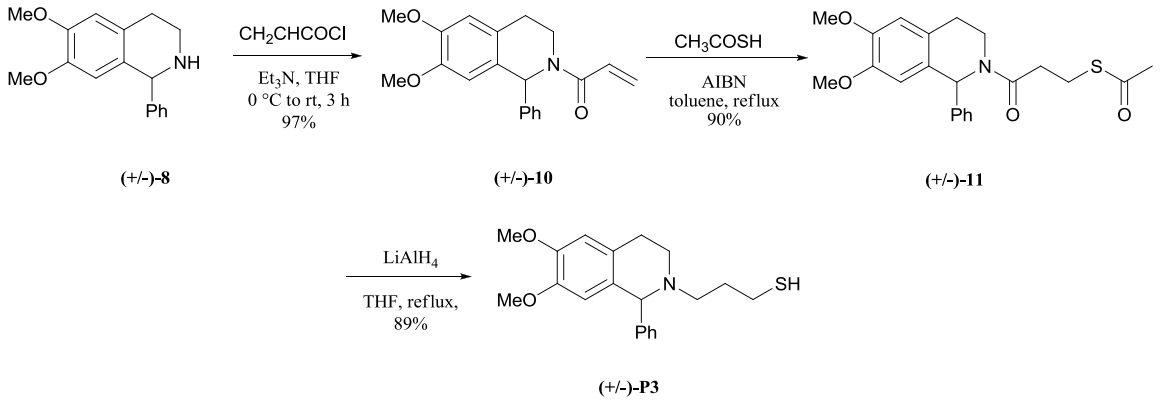


(+/-)-8 **(+/-)-9** **(+/-)-P2**

To a solution of trityl-protected mercaptoacetic acid (804 mg, 3 mmol) in anhydrous CH_2Cl_2 (30 mL) was added PIQ (±)-**8** (1 g, 3 mmol, 1 equiv.), Et_3N (0.9 mL, 6.3 mmol, 2.1 equiv.), HOBT (485 mg, 3.6 mmol), and finally DCI (0.56 mL, 3.6 mmol, 1.2 equiv.) after Argon bubbling of the solution. The mixture was allowed to stir at room temperature for 24 h (TLC monitoring). The reaction mixture was evaporated and purified by chromatography over silica gel (CH_2Cl_2/EtOAc, 80:20) to yield the corresponding PIQ (±)-**9** as white solid (1.75 g, 100%) as a mixture of two rotamers. ^{1}H NMR: (200 MHz, $CDCl_3$) δ = 2.48-2.64 (m, 1H), 2.75-2.93 (m, 1H), 2.98 (s, 2H), 3.08-3.20 (m, 2H), 3.73 (s, 2.6H), 3.78 (s, 0.4H), 3.87 (s, 3H), 5.43 (s, 0.13H), 6.37 (s, 0.13H), 6.47 (s, 0.87H), 6.60 (s, 0.87H), 6.63 (s, 0.13H), 6.78 (s, 0.87H), 7.14-7.24 (m, 6H), 7.28-7.32 (m, 4H), 7.44-7.49 (m, 6H); ^{13}C NMR: (50 MHz, $CDCl_3$) δ = 28.9 (CH_2), 35.2 (CH_2), 39.8 (CH_2), 54.9 (CH), 55.9 (CH_3), 56.0 (CH_3), 67.2 (C), 111.0 (CH), 111.3 (CH), 126.4 (C), 126.5 (C), 126.9 (3 CH), 127.5 (CH), 128.1 (6 CH), 128.3 (2 CH), 128.9 (2 CH), 129.6 (6 CH), 142.3 (C), 144.1 (3 C), 147.7 (C), 148.1 (C), 167.0 (C); IR (neat) cm^{-1}: 2938, 1630, 1519, 1423, 1245, 1114, 702; Melting point: 178-180°C; MS (ESI+) m/z (%): 1170.80 (100) $[2M]^+$; HPLC t_R=29.5 (purity >92%); System A.

To a suspension of lithium aluminium hydride (60 mg, 1.54 mmo, 3 equiv.l) in anhydrous THF (3 mL), a solution of PIQ (±)-**9** (300 mg, 0.51 mmol) in anhydrous THF (2 mL) was added dropwise and the solution was heated to reflux overnight. Water (0.5 mL) was added at 0°C followed by the addition of NaOH solution 5M (0.5 mL) and water again (1.5 mL) and the mixture was allowed to stir at room temperature for 15 min. $MgSO_4$ was then added and the resulting cake was filtered on celite, washed with EtOAc and the filtrate evaporated to dryness. The crude reaction mixture was then purified over silica gel (CH_2Cl_2)

and the desired product **P2** was obtained as yellow oil (100 mg, 59%). ^{1}H NMR: (300 MHz, $CDCl_3$) δ = 2.56-2.70 (m, 4H), 2.72-2.84 (m, 2H), 2.93-3.03 (m, 1H), 3.09-3.17 (m, 1H), 3.61 (s, 3H), 3.86 (s, 3H), 4.56 (s, 1H), 6.19 (s, 1H), 6.61 (s, 1H), 7.22-7.33 (m, 5H); ^{13}C NMR: (75 MHz, $CDCl_3$) δ = 22.6 (CH_2), 28.1 (CH_2), 46.9 (CH_2), 55.9 (CH_3), 55.9 (CH_3), 57.1 (CH_2), 67.8 (CH), 111.0 (CH), 111.8 (CH), 126.8 (C), 127.4 (CH), 128.3 (2 CH), 129.7 (2 CH), 129.8 (C), 143.8 (C), 147.3 (C), 147.6 (C); IR (neat) cm^{-1}: 2933, 2831, 1610, 1512, 1463, 1252, 1134, 1095, 1014, 972, 917, 742, 701; MS (ESI+) m/z (%): 330.13 (100) $[M+H]^+$; HPLC t_R=16.3 (purity >95%); System A.



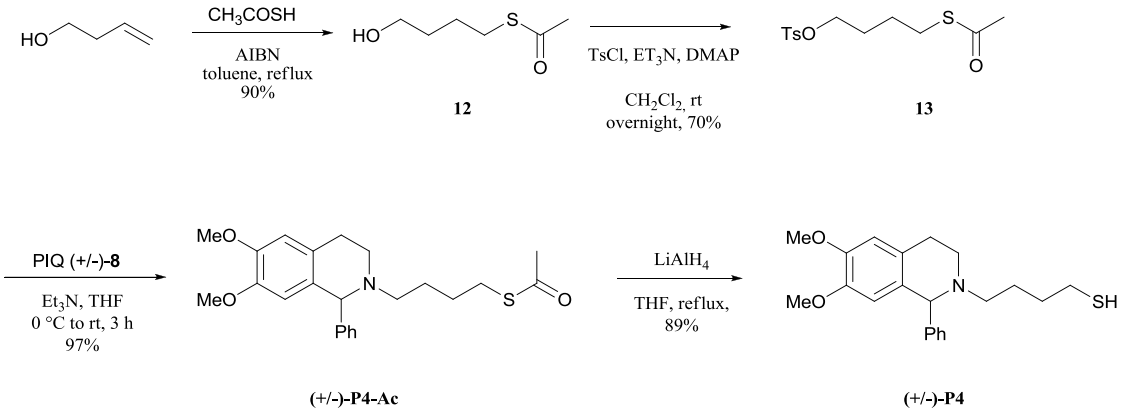
(+/-)-8 (+/-)-10 (+/-)-11

(+/-)-P3

To a solution of PIQ (±)-**8** (269 mg, 1 mmol) and Et_3N (300µL, 2.1 mmol, 2.1 equiv.) in anhydrous THF (20 mL) at 0 °C, acryloyl chloride (97 µL, 1.2 mmol, 1.2 equiv.) was added dropwise and the solution was stirred at room temperature until full consumption of the starting material. The solution was cooled to 0 °C and a sat. aq. solution of $NaHCO_3$ (10 mL) was added and the solution extracted with EtOAc (3 × 20 mL). The combined organic layers were washed with brine (30 mL), dried over $MgSO_4$ and evaporated to dryness. The crude reaction mixture was then purified over silica gel (CH_2Cl_2/Et_2O, 100:0 to 98:2) to yield the corresponding product (±)-**10** as white solid (mixture of two rotamers) (313 mg, 97%). ^{1}H NMR: (300 MHz, $CDCl_3$) δ = 2.75 (d, *J* = 16.3 Hz, 1H), 2.89-3.00 (m, 1H), 3.11-3.26 (m, 0.25H), 3.36-3.46 (m, 0.75H), 3.76 (s, 3H), 3.76-3.88 (m, 1H), 3.89 (s, 3H), 5.73 (d, *J* = 10.4 Hz, 1H), 6.09 (s, 0.2H), 6.37 (d, *J* = 16.7 Hz, 1H), 6.55-6.66 (m, 2.8H), 6.86-6.89 (m, 0.25H), 6.95 (s, 0.75H), 7.19-7.34 (m, 5H); ^{13}C NMR: (75 MHz, $CDCl_3$) δ = 28.9 (CH_2), 39.7 (CH_2), 55.1 (CH), 56.0 (CH_3), 56.0 (CH_3), 111.2 (CH), 111.4 (CH), 126.4 (C), 126.9 (C), 127.5, 127.8, 128.2, 128.3, 128.9 (6 CH, CH_2), 142.4 (C), 147.7 (C), 148.2 (C), 165.3 (C); Melting point: 186-188 °C); IR (neat) cm^{-1}: 2930, 2334, 1646, 1613, 1515, 1446, 1233, 1114, 948, 788, 745, 707; MS (ESI+) m/z (%): 324.09 (100) $[M+H]^+$; Elem. Anal. calcd for $C_{20}H_{21}NO_3$: C = 74.28, H = 6.54, N = 4.33. Found: C = 73.80, H = 6.57, N = 4.39.

A mixture of acrylamide (±)-**10** (50 mg, 0.15 mmol), thioacetic acid (22 µL, 0.31 mmol) and AIBN (50.8 mg, 0.31 mmol, 2.06 equiv.) in anhydrous toluene (1 mL) under Argon atmosphere was heated to 100 °C until full consumption of the starting material. A solution of sat. aq. $NaHCO_3$ (2 mL) was added to the reaction after cooling to room temperature and the resulting mixture was extracted with EtOAc (3 × 3 mL). The combined organic layers were washed with brine (5 mL), dried over $MgSO_4$ and concentrated *in vacuo*. The crude reaction mixture residue was purified by flash column chromatography over silica gel (CH_2Cl_2) to afford the desired thioacetate **11** as off-white solid (mixture of two rotamers) (55.4 mg, 90%). ^{1}H NMR: (300 MHz, $CDCl_3$) δ = 2.29 (s, 0.6H), 2.31 (s, 2.4H), 2.60-2.81 (m, 2.8H), 2.85-2.98 (m, 1.2H), 3.11-3.25 (m, 2.2H), 3.30-3.70 (m, 1.8H), 3.75 (s, 2.4H), 3.81 (s, 0.6H), 3.88 (s, 3H), 5.95 (s, 0.2H), 6.52 (s, 0.8), 6.62 (s, 0.2H), 6.65 (s, 0.8H), 6.67 (s, 0.2H), 6.86 (s, 0.8H), 7.14-7.28 (m, *5*H); ^{13}C NMR: (75 MHz, $CDCl_3$) δ = 24.9 (CH_2), 28.7 (CH_2), 30.7 (CH_2), 33.8 (CH_2), 39.4 (CH_2), 54.9 (CH), 56.0 (CH_3), 56.1 (CH_3), 111.2 (CH), 111.4 (CH), 126.4 (C), 127.0 (C), 128.4 (CH), 128.7 (2 CH), 128.9 (2 CH), 142.5 (C), 147.8 (C), 148.3 (C), 169.3 (C), 196.6 (C); Melting point: 124-126 °C; IR (neat) cm^{-1}: 2938, 2828, 1692, 1637, 1514, 1435, 1237, 1113, 697; MS (ESI+) m/z (%): 400.13 (100) $[M+H]^+$.

To a suspension of lithium aluminium hydride (8.6 mg, 0.23 mmol, 2.8 equiv.) in anhydrous THF (1 mL) cooled to 0 °C, a solution of PIQ (±)-**11** (30 mg, 0.08 mmol) in anhydrous THF (0.5 mL) was added dropwise and the mixture was allowed to return to room temperature until full consumption of the starting material. Water (0.1 mL) was added at 0 °C followed by the addition of NaOH solution 5M (0.1 mL) and water again (0.3 mL) and the mixture was allowed to stir at room temperature for 15 min. $MgSO_4$ was then added and the resulting cake was filtered on celite, washed with EtOAc and the filtrate evaporated to dryness. The crude reaction mixture was then purified over silica gel (CH_2Cl_2) and the desired thiol (±)-**P3** was obtained as yellow oil (18 mg, 67%). ^{1}H NMR: (300 MHz, $CDCl_3$) δ = 1.61-1.72 (m, 1H), 1.79-1.91 (m, 1H), 2.33-2.57 (m, 4H), 2.60-2.69 (m, 1H), 2.71-2.80 (m, 1H), 2.95-3.05 (m, 1H), 3.12-3.19 (m, 1H), 3.59 (s, 3H), 3.85 (s, 3H), 4.44 (s, 1H), 6.16 (s, 1H), 6.60 (s, 1H), 7.21-7.35 (m, *5*H); ^{13}C NMR: (75 MHz, $CDCl_3$) δ = 22.5 (CH_2), 28.5 (CH_2), 31.4 (CH_2), 47.2 (CH_2), 52.4 (CH_2), 55.9 (2 CH_3), 68.6 (CH), 110.9 (CH), 111.9 (CH), 127.0 (C), 127.4 (CH), 128.4 (2 CH), 129.8 (2 CH), 130.3 (C), 144.5 (C), 147.2 (C), 147.5 (C); MS (ESI+) m/z (%): 344.13 (100) $[M+H]^+$; HPLC t_R = 16.70 (purity > 97%); System A.



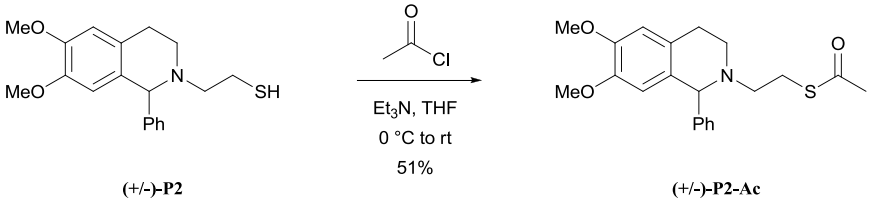
A mixture of 3-buten-1-ol (250 µL, 2.95 mmol), thioacetic acid (300 µL, 4.26 mmol, 1.44 equiv.) and AIBN (415 mg, 2.95 mmol, 1 equiv.) in anhydrous toluene (15 mL) under Argon atmosphere was heated to 100 °C until full consumption of the starting material. A solution of sat. aq. $NaHCO_3$ (10 mL) was added to the reaction after cooling to room temperature and the resulting mixture was extracted with EtOAc (3 × 15 mL). The combined organic layers were washed with brine (20 mL), dried over $MgSO_4$ and concentrated *in vacuo*. The crude reaction mixture residue was purified by flash column chromatography over silica gel (CH_2Cl_2) to afford the desired thioacetate **12** as yellow oil (393.5 mg, 90%). ^{1}H NMR: (300 MHz, $CDCl_3$) δ = 1.60-1.69 (m, 4H), 2.31 (s, 3H), 2.89 (t, *J* = 7.0 Hz, 2H), 3.65 (t, *J* = 6.0 Hz, 2H); ^{13}C NMR: (75 MHz, $CDCl_3$) δ = 26.1 (CH_2), 28.9 (CH_2), 30.7 (CH_3), 31.6 (CH_2), 62.3 (CH_2), 196.3 (C); IR (neat) cm^{-1}: 3358, 2936, 1687, 1354, 1132, 1054, 954, 623.

To a solution of alcohol **12** (100 mg, 0.67 mmol), Et_3N (190 µL, 1.35 mmol, 2 equiv.), and DMAP (24.7 mg, 0.2 mmol, 0.3 equiv.) in anhydrous CH_2Cl_2 (1.6 mL) under Argon atmosphere at 0 °C was added TsCl (141.5 mg, 0.74 mmol, 1.1 equiv.) then the mixture was allowed to stir at room temperature overnight. An aq. solution of HCl 2M (2 mL) was added to the solution which was next extracted with CH_2Cl_2 (3 × 5 mL). The combined organic layers were washed with brine, dried over $MgSO_4$, and evaporated to dryness. The resulting residue was purified by chromatography over silica gel (CH_2Cl_2) to yield the corresponding tosylate **13** as yellow oil (140 mg, 70%). ^{1}H NMR: (300 MHz, $CDCl_3$) δ = 1.52-1.62 (m, 2H), 1.63-1.72 (m, 2H), 2.27 (s, 3H), 2.42 (s, 3H), 2.78 (t, *J* = 6.9 Hz, 2H), 3.99 (t, *J* = 6.0 Hz, 2H), 7.32 (d, *J* = 8.0 Hz, 2H), 7.75 (d, *J* = 8.0 Hz, 2H). ^{13}C NMR: (75 MHz, $CDCl_3$) δ = 21.7 (CH_3), 25.6 (CH_2), 27.8 (CH_2), 28.2 (CH_2), 30.6 (CH_3), 69.8 (CH_2), 127.9 (2 CH), 129.9 (2 CH), 133.0 (C), 144.9 (C), 195.6 (C); IR (neat) cm^{-1}: 2925, 1687, 1353, 1173, 926, 814, 662; MS (ESI+) m/z (%): 303,1 (10) $[M+H]^+$; 320.1 (100) $[M+H_2O]^+$; 325.1 (50) $[M+Na]^+$.

A mixture of the tosylate **13** (237 mg, 0.79 mmol, 1 equiv.), PIQ (±)-**8** (211.2 mg, 0.79

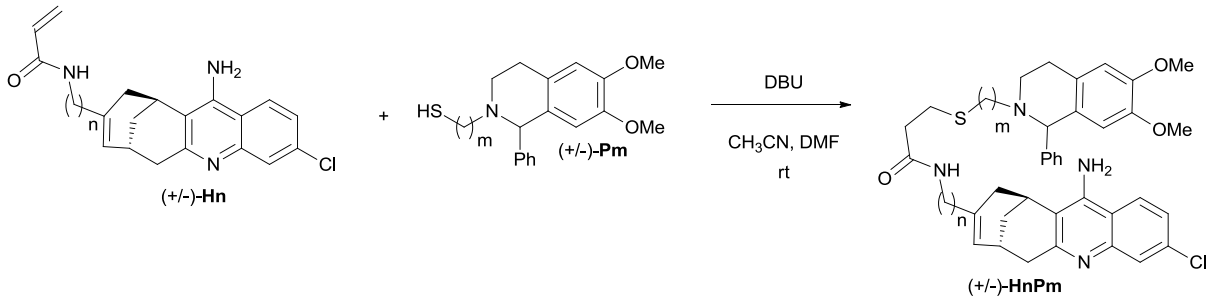
mmol), and K_2CO_3 (325 mg, 2.36 mmol, 3 equiv.) in dry CH_3CN (5 mL) was refluxed until full consumption of the starting material (NMR monitoring). The solution was cooled to room temperature, filtered over celite, washed with EtOAc and evaporated to dryness. The resulting residue was purified by chromatography over silica gel (CH_2Cl_2/Et_2O, 100:0 to 95:5) to yield the corresponding product (±)-**P4-Ac** as yellow oil (245 mg, 78%). ^{1}H NMR: (300 MHz, $CDCl_3$) A: δ = 1.43-1.59 (m, 4H), 2.30 (s, 3H), 2.26-2.34 (m, 1H), 2.46-2.58 (m, 2H), 2.72-2.81 (m, 3H), 2.93-3.03 (m, 1H), 3.10-3.17 (m, 1H), 3.60 (s, 3H), 3.85 (s, 3H), 4.46 (s, 1H), 6.17 (s, 1H), 6.60 (s, 1H), 7.20-7.32 (m, 5H). ^{13}C NMR: (75 MHz, $CDCl_3$) δ = 26.1 (CH_2), 27.3 (CH_2), 28.4 (CH_2), 29.1 (CH_2), 30.8 (CH_3), 47.0 (CH_2), 53.5 (CH_2), 55.9 (CH_3), 55.9 (CH_3), 68.3 (CH), 110.9 (CH), 111.9 (CH), 127.1 (C), 127.2 (CH), 128.2 (2 CH), 129.7 (2 CH), 130.3 (C), 144.4 (C), 147.1 (C), 147.5 (C), 196.1 (C); IR (neat) cm^{-1}: 2937, 1687, 1512, 1251, 1221, 1131, 1098, 1026, 742, 701, 624; MS (ESI+) m/z (%):400.12 (100) $[M+H]^+$. HPLC t_R = 17.9 (purity > 92%); System A.

To a suspension of lithium aluminium hydride (8.6 mg, 0.23 mmol, 2.87 equiv.) in anhydrous THF (1 mL) cooled to 0 °C, a solution of (±)-**P4-Ac** (30 mg, 0.08 mmol) in anhydrous THF (0.5 mL) was added dropwise and the mixture was allowed to return to room temperature until full consumption of the starting material. Water (0.1 mL) was added at 0 °C followed by the addition of sodium hydroxide solution 5M (0.1 mL) and water again (0.3 mL) and the mixture was allowed to stir at room temperature for 15 min. $MgSO_4$ was then added and the resulting cake was filtered on celite, washed with EtOAc and the filtrate evaporated to dryness. The crude reaction mixture was then purified over silica gel (CH_2Cl_2) and the desired thiol (±)-**P4** was obtained as yellow oil (18 mg, 67%). ^{1}H NMR: (300 MHz, $CDCl_3$) δ = 1.49-1.62 (m, 4H), 2.26-2.34 (m, 1H), 2.37-2.44 (m, 2H), 2.47-2.60 (m, 2H), 2.73-2.81 (m, 1H), 2.95-3.05 (m, 1H), 3.13-3.20 (m, 1H), 3.60 (s, 3H), 3.85 (s, 3H), 4.47 (s, 1H), 6.17 (s, 1H), 6.61 (s, 1H), 7.23-7.32 (m, 5H); ^{13}C NMR: (75 MHz, $CDCl_3$) δ = 24.5 (CH_2), 25.6 (CH_2), 28.4 (CH_2), 31.7 (CH_2), 47.0 (CH_2), 53.4 (CH_2), 55.9 (CH_3), 55.9 (CH_3), 68.3 (CH), 110.9 (CH), 111.8 (CH), 127.0 (C), 127.2 (CH), 128.2 (2 CH), 129.6 (2 CH), 130.3 (C), 144.4 (C), 147.1 (C), 147.5 (C); MS (ESI+) m/z (%): 358.1 (100) $[M+H]^+$.



(+/-)-P2 (+/-)-P2-Ac

To a mixture of (±)-**P2** (25 mg, 0.08 mmol) and Et_3N (21 µL, 0.15 mmol, 1.87 equiv.) in anhydrous THF (1.5 mL) was added acetyl chloride (14.2 µL, 0.09 mmol, 1.12 equiv.) dropwise at 0 °C and the reaction was allowed to stir at room temperature until full consumption of the starting material. A sat. aq. solution of $NaHCO_3$ (5 mL) was added to the solution which was next extracted with EtOAc (3 × 5 mL). The combined organic layers were washed with brine, dried over $MgSO_4$, and evaporated to dryness. The resulting residue was purified by chromatography over silica gel (CH_2Cl_2) to yield the corresponding thioacetate (±)-**P2-Ac** as dark yellow oil (28.2 mg, 51%). ^{1}H NMR: (300 MHz, $CDCl_3$) δ = 2.8 (s, 3H), 2.54-2.61 (m, 1H), 2.65-2.82 (m, 3H), 2.88-2.98 (m, 2H), 3.02-3.11 (m, 1H), 3.16-3.23 (m, 1H), 3.60 (s, 3H), 3.85 (s, 3H), 4.57 (s, 1H), 6.18 (s, 1H), 6.60 (s, 1H), 7.23-7.29 (m, 5H); ^{13}C NMR: (75 MHz, $CDCl_3$) δ = 27.1 (CH_2), 28.2 (CH_3), 30.7 (CH_2), 47.1 (CH_2), 53.4 (CH_2), 55.9 (2 CH_3), 67.6 (CH), 111.0 (CH), 111.8 (CH), 126.9 (C), 127.4 (CH), 128.3 (2 CH), 129.7 (2 CH), 129.9 (C), 143.9 (C), 147.2 (C), 147.6 (C), 196.1 (C); MS (ESI+) m/z (%): 372.13 (100) $[M+H]^+$.



General procedure for the thio-Michael reaction between thiol derivatives and acrylamides. (Synthesis of **Hn-Pm**, **14**, and **15**)

To a solution of acrylamide (1 eq.) and DBU (1.2 eq.) in anhydrous CH_3CN and DMF (10/1, v/v) bubbled with Argon and under Argon atmosphere was added a solution of the thiol (±)-**Pm** (1.2 eq.) then the mixture was allowed to stir at room temperature with light protection until full consumption of thiol **Pm** (analytic HPLC monitoring). A large excess of DTT was next added (15 eq.) and the solution was stirred for 15 min. The mixture was directly purified by preparative HPLC (Systems D (**Hn-Pm**) and E (14, 15) and the TFA salt of the desired product was obtained as a solid.

(±)-**H2-P2**

Yield: White solid, 24%

^{1}H NMR: (300 MHz, MeOD) δ = 1.91-2.09 (m, 5H), 2.20-2.39 (m, 2H), 2.48-2.57 (m, 1H), 2.60-2.66 (m, 2H), 2.80 (s, 1H), 2.85 (d, *J* = 18.2 Hz, 1H), 2.91-3.00 (m, 2H), 3.06-3.27 (m, 6H), 3.38 (s, 1H), 3.40-3.48 (m, 2H), 3.62 (s, 3H), 3.65-3.83 (m, 1H), 3.87 (s, 3H), 5.61 (d, *J* = 5.4 Hz, 1H), 5.79 (s, 1H), 6.00 (s, 1H), 6.33 (s, 1H), 7.34-7.37 (m, 2H), 7.47-7.49 (m, 3H), 7.59 (td, *J* = 2.0 Hz, *J* = 9.1 Hz, 1H), 7.75 (d, *J* = 2.0 Hz, 1H), 8.36 (d, *J* = 9.1 Hz, 1H); ^{13}C NMR: (75 MHz, MeOD) δ = 26.9 (CH_2), 27.4 (CH), 27.8 (CH), 28.1 (CH_2), 29.1 (CH_2), 33.8 (CH_2), 35.8 (CH_2), 36.3 (CH_2), 37.9 (CH_2), 38.5 (CH_2), 58.4 (CH_3), 58.5 (CH_3), 67.0 (CH), 112.3 (2 CH), 115.4 (C), 115.4 (C), 119.2 (CH), 124.7 (C), 126.4 (CH), 126.8 (CH), 127.7 (CH), 130.4 (2 CH), 131.4 (2 CH), 131.8 (CH), 135.9 (C), 139.6 (C), 140.5 (C), 150.1 (C), 151.2 (C), 152.9 (C), 156.8 (C), 162.7 (C), 163.1 (C), 173.3 (C); MS (ESI+) m/z (%):697.3 (100) $[M+H]^+$, 349.2 (35) $[M/2+H]^{2+}$; HRMS (ESI+): Calc. for $C_{40}H_{45}N_4SO_3Cl$ $[M+H]^+$: 697.2979; found: 697.2965; HPLC t_R = 17.9 (purity >93%); System A; IC_{50} *m*-AChE = 11.4 ± 0.7 nM.

(±)-**H2-P3**

Yield: White solid, 40%

^{1}H NMR: (300 MHz, MeOD) δ = 1.92-2.10 (m, 7H), 2.15-2.33 (m, 2H), 2.51-2.59 (m, 5H), 2.80 (s, 1H), 2.86 (d, *J* = 18.1 Hz, 1H), 3.07-3.26 (m, 7H), 3.39 (s, 1H), 3.42-3.49 (m, 1H), 3.60 (s, 3H), 3.65-3.74 (m, 1H), 3.86 (s, 3H), 5.63 (d, *J* = 5.5 Hz, 1H), 5.74 (s, 1H), 6.33 (s, 1H), 6.92 (s, 1H), 7.33-7.37 (m, 2H), 7.48-7.51 (m, 3H), 7.59 (dd, *J* = 2.0 Hz, *J* = 9.1 Hz, 1H), 7.74 (d, *J* = 2.0 Hz, 1H), 8.36 (d, *J* = 9.1 Hz, 1H); ^{13}C NMR: (75 MHz, MeOD) δ = 25.0 (2 CH_2), 27.4 (CH), 28.1 (CH), 28.2 (CH_2), 29.1 (CH_2), 29.5 (CH_2), 33.8 (CH_2), 35.8 (CH_2), 36.8 (CH_2), 37.9 (CH_2), 38.4 (CH_2), 56.4 (CH_3), 56.5 (CH_3), 112.4 (2 CH), 115.4 (C), 115.5 (C), 119.2 (CH), 126.4 (CH), 126.9 (CH), 127.7 (CH), 130.5 (2 CH), 131.4 (2 CH), 135.9 (C), 139.6 (C), 140.5 (C), 150.1 (C), 151.2 (C), 152.9 (C), 156.9 (C), 173.5 (C); MS (ESI+) m/z (%):711.3 (100) $[M+H]^+$, 356.3 (45) $[M/2+H]^{2+}$; HRMS (ESI+): Calc. for $C_{41}H_{47}N_4SO_3Cl$ $[M+H]^+$: 711.3136; found: 711.3140; HPLC t_R = 17.9 (purity >97%); System A; IC_{50} *m*-AChE = 10.6 ± 0.1 nM.

(±)-**H2-P4**

Yield: White solid, 14%.

^{1}H NMR: (300 MHz, MeOD) δ = 1.54-1.63 (m, 2H), 1.93-2.09 (m, 7H), 2.16-2.32 (m, 2H), 2.49-2.58 (m, 5H), 2.80 (s, 1H), 2.85 (d, *J* = 18.2 Hz, 1H), 3.05-3.27 (m, 7H), 3.39 (s, 1H), 3.42-3.52 (m, 1H), 3.60 (s, 3H), 3.65-3.76 (m, 1H), 3.86 (s, 3H), 5.64 (d, *J* = 4.1 Hz, 1H),

5.74 (s, 1H), 6.33 (s, 1H), 6.91 (s, 1H), 7.33-7.36 (m, 2H), 7.49-7.50 (m, 3H), 7.59 (dd, J = 1.9 Hz, J = 9.1 Hz, 1H), 7.74 (d, J = 1.9 Hz, 1H), 8.36 (d, J = 9.1 Hz, 1H); ^{13}C NMR: (75 MHz, MeOD) δ = 24.2 (2 CH_2), 27.3 (CH_2), 27.4 (CH), 28.1 (CH_2), 28.3 (CH), 29.1 (CH_2), 31.9 (CH_2), 33.8 (CH_2), 35.8 (CH_2), 37.0 (CH_2), 38.0 (CH_2), 38.4 (CH_2), 46.7 (CH_2), 54.1 (CH_2), 56.4 (CH_3), 56.5 (CH_3), 67.9 (CH), 112.4 (2 CH), 115.4 (C), 115.5 (C), 119.2 (CH), 124.9 (C), 126.5 (CH), 126.9 (CH), 127.7 (CH), 127.8 (C), 130.5 (2 CH), 131.4 (2 CH), 131.7 (CH), 134.3 (C), 136.0 (C), 139.6 (C), 140.5 (C), 150.2 (C), 151.2 (C), 152.9 (C), 156.9 (C), 173.6 (C); MS (ESI+) m/z (%):725.07 (90), 727.20 (50) $[M+H]^+$, 363.2 (100) $[M/2+H]^{2+}$; HRMS (ESI+): Calc. for $C_{42}H_{49}N_4SO_3Cl$ $[M+H]^+$: 725.3292; found: 725.3273; HPLC t_R = 18.2 (purity >96%); System A; IC_{50} *m*-AChE = 14.6 ± 1.1 nM.

(±)-**H3-P2**

Yield: White solid, 22%.

^{1}H NMR: (300 MHz, MeOD) δ = 1.41-1.50 (m, 2H), 1.88 (t, J = 7.6 Hz, 2H), 1.94-2.11 (m, 3H), 2.40 (t, J = 7.0 Hz, 2H), 2.52 (dd, J = 4.9 Hz, J = 17.7 Hz, 1H), 2.74 (t, J = 6.9 Hz, 2H), 2.80 (s, 1H), 2.87 (d, J = 19.0 Hz, 1H), 2.90-3.08 (m, 4H), 3.18-3.28 (m, 3H), 3.41-3.55 (m, 4H), 3.61 (s, 3H), 3.64-3.788 (m, 1H), 3.87 (s, 3H), 5.60 (dd, J = 0.6 Hz, J = 5.3 Hz, 1H), 5.80 (s, 1H), 6.40 (s, 1H), 6.92 (s, 1H), 7.34-7.38 (m, 2H), 7.46-7.51 (m, 3H), 7.59 (dd, J = 1.9 Hz, J = 9.1 Hz, 1H), 7.74 (d, J = 1.9 Hz, 1H), 8.35 (d, J = 9.1 Hz, 1H); ^{13}C NMR: (75 MHz, MeOD) δ = 24.1 (CH_2), 26.9 (CH_2), 27.5 (CH), 28.0 (CH_2), 28.1 (CH, CH_2), 29.3 (CH_2), 34.1 (CH_2), 35.4 (CH_2), 35.9 (CH_2), 36.5 (CH_2), 39.9 (CH_2), 53.2 (CH_2), 56.4 (CH_3), 56.5 (CH_3), 67.1 (CH), 112.4 (2 CH), 115.3 (C), 115.4 (C), 119.3 (CH), 122.5 (C), 124.7 (C), 125.3 (CH), 126.3 (CH), 127.7 (CH), 130.4 (2 CH), 131.4 (2 CH), 131.9 (CH), 137.9 (C), 139.6 (C), 140.5 (C), 150.2 (C), 151.3 (C), 153.1 (C), 156.8 (C), 162.2 (C), 162.7 (C), 173.6 (C); MS (ESI+) m/z (%): 711.19 (35) $[M+H]^+$, 356.29 (100) $[M/2+H]^{2+}$; HRMS (ESI+): Calc. for $C_{41}H_{47}N_4SO_3Cl$ $[M+H]^+$: 711.3130; found: 711.3110. HPLC t_R=18.7 (purity >96%); System A; IC_{50} *m*-AChE = 0.9 ± 0.1 nM.

(±)-**H3-P3**

Yield: White solid, 55%.

^{1}H NMR: (300 MHz, MeOD) δ = 1.41-1.52 (m, 2H), 1.90 (t, J = 7.7 Hz, 2H), 1.98-2.21 (m, 5H), 2.36 (t, J = 7.1 Hz, 2H), 2.49 (dd, J = 1.6 Hz, J = 4.1 Hz, 1H), 2.57 (t, J = 6.8 Hz, 2H), 2.69 (t, J = 7.1 Hz, 2H), 2.81 (s, 1H), 2.87 (d, J = 18.2 Hz, 1H), 2.92-3.01 (m, 2H), 3.18-3.28 (m, 4H), 3.33-3.67 (m, 3H), 3.60 (s, 3H), 3.64-3.79 (m, 1H), 3.86 (s, 3H), 5.61 (d, J = 5.2 Hz,

1H), 5.75 (s, 1H), 6.34 (s, 1H), 6.91 (s, 1H), 7.34-7.37 (m, 2H), 7.49-7.51 (m, 3H), 7.59 (dd, *J* = 1.9 Hz, *J* = 9.1 Hz, 1H), 7.74 (d, *J* = 1.9 Hz, 1H), 8.35 (d, *J* = 9.1 Hz, 1H); ^{13}C NMR: (75 MHz, MeOD) δ = 24.2 (CH_2), 25.0 (CH_2), 27.5 (CH), 28.0 (CH_2), 28.1 (CH), 28.3 (CH_2), 29.3 (CH_2), 29.4 (CH_2), 34.0 (CH_2), 35.3 (CH_2), 35.9 (CH_2), 36.9 (CH_2), 39.8 (CH_2), 53.5 (CH_2), 56.4 (CH_3), 56.5 (CH_3), 68.2 (CH), 112.4 (2 CH), 115.3 (C), 115.4 (C), 119.3 (CH), 124.8 (C), 125.4 (CH), 126.4 (CH), 127.7 (CH), 130.5 (2 CH), 131.4 (2 CH), 131.6 (CH), 137.9 (C), 139.6 (C), 140.5 (C), 150.1 (C), 151.2 (C), 153.1 (C), 156.7 (C), 173.8 (C); MS (ESI+) m/z (%):725.2 (20) $[M+H]^+$, 363,4 (100) $[M/2+H]^{2+}$; HRMS (ESI+): Calc. for $C_{42}H_{49}N_4SO_3Cl$ $[M+H]^+$: 725.3287; found: 731.3290; HPLC t_R=18.6 (purity >99%); System A; IC_{50} *m*-AChE = 3.2 ± 0.1nM.

(±)-**H3-P4**

Yield: White solid, 45%.

^{1}H NMR: (300 MHz, MeOD) δ = 1.43-1.53 (m, 2H), 1.55-1.65 (m, 2H), 1.88-2.12 (m, 7H), 2.36 (t, *J* = 7.2 Hz, 2H), 2.49 (d, *J* = 4.5 Hz, 1H), 2.54 (t, *J* = 6.9 Hz, 2H), 2.68 (t, *J* = 7.1 Hz, 2H), 2.81 (s, 1H), 2.87 (d, *J* = 18.2 Hz, 1H), 2.93-3.02 (m, 2H), 3.12-3.25 (m, 5H), 3.40 (s, 1H), 3.43-3.53 (m, 1H), 3.60 (s, 3H), 3.67-3.79 (m, 1H), 3.86 (s, 3H), 5.61 (d, *J* = 5.3 Hz, 1H), 5.74 (s, 1H), 6.34 (s, 1H), 6.91 (s, 1H), 7.34-7.37 (m, 2H), 7.49-7.51 (m, 3H), 7.59 (dd, *J* = 1.9 Hz, *J* = 9.1 Hz, 1H), 7.74 (d, *J* = 7.9 Hz, 1H), 8.35 (d, *J* = 9.1 Hz, 1H); ^{13}C NMR: (75 MHz, MeOD) δ = 24.2 (2 CH_2), 27.3 (CH_2), 27.5 (CH), 28.0 (CH_2), 28.1 (CH), 28.4 (CH_2), 29.3 (CH_2), 31.9 (CH_2), 34.0 (CH_2), 35.3 (CH_2), 35.9 (CH_2), 37.1 (CH_2), 39.8 (CH_2), 46.1 (CH_2), 54.1 (CH_2), 56.4 (CH_3), 56.5 (CH_3), 67.9 (CH), 112.4 (2 CH), 115.3 (C), 115.4 (C), 119.3 (CH), 123.0 (C), 124.8 (C), 125.4 (CH), 126.3 (CH), 127.7 (CH), 130.5 (2 CH), 131.4 (2 CH), 131.6 (CH), 137.1 (C), 137.9 (C), 139.6 (C), 140.5 (C), 150.1 (C), 151.2 (C), 153.1 (C), 156.7 (C), 173.9 (C); MS (ESI+) m/z (%):739.2 (20) $[M+H]^+$, 370.1 (100) $[M/2+H]^{2+}$; HRMS (ESI+): Calc. for $C_{43}H_{51}N_4SO_3Cl$ $[M+H]^+$: 739.3443; found: 739.3429; HPLC t_R = 18.9 (purity >96%); System A; IC_{50} *m*-AChE = 4.0 ± 0.2 nM.

(±)-**H4-P2**

Yield: Pale pink solid, 30%.

^{1}H NMR: (300 MHz, MeOD) δ = 1.22-1.36 (m, 4H), 1.82-2.10 (m, 5H), 2.40 (t, *J* = 6.9 Hz, 2H), 2.50 (dd, *J* = 4.5 Hz, *J* = 17.7 Hz, 1H), 2.75 (t, *J* = 6.9 Hz, 2H), 2.80 (s, 1H), 2.86 (d, *J* = 18.3 Hz, 1H), 2.93-3.04 (m, 4H), 3.14-3.31 (m, 3H), 3.36-3.54 (m, 4H), 3.61 (s, 3H), 3.66-3.82 (m, 1H), 3.86 (s, 3H), 5.58 (d, *J* = 4.8 Hz, 1H), 5.80 (s, 1H), 6.40 (s, 1H), 6.92 (s, 1H),

7.35-7.38 (m, 2H), 7.47-7.49 (m, 3H), 7.59 (dd, J = 1.9 Hz, J = 9.1 Hz, 1H), 7.75 (d, J = 1.9 Hz, 1H), 8.35 (d, J = 9.1 Hz, 1H); ^{13}C NMR: (75 MHz, MeOD) δ = 25.7 (CH_2), 26.9 (CH_2), 27.5 (CH), 28.0 (CH), 28.1 (CH_2), 29.3 (CH_2), 29.7 (CH_2), 34.0 (CH_2), 36.0 (CH_2), 36.5 (CH_2), 37.7 (CH_2), 40.2 (CH_2), 53.2 (CH_2), 56.4 (CH_3), 56.5 (CH_3), 67.2 (CH), 112.4 (2 CH), 115.3 (C), 115.5 (C), 119.2 (CH), 124.8 (C), 125.0 (CH), 126.4 (CH), 127.8 (CH), 130.4 (2 CH), 131.4 (2 CH), 131.9 (CH), 138.4 (C), 139.5 (C), 140.5 (C), 150.1 (C), 151.2 (C), 153.1 (C), 156.7 (C), 173.6 (C); MS (ESI+) m/z (%):725.1 (80) $[M+H]^+$, 363,3 (80) $[M/2+H]^{2+}$; HRMS (ESI+): Calc. for $C_{42}H_{49}N_4SO_3Cl$ $[M+H]^+$: 725.3292; found: 725.3275; HPLC t_R=18.7 (purity >93%); System A; IC_{50} *m*-AChE = 1.6 ± 0.1 nM.

(±)-**H4-P3**

Yield: Pale pink solid, 20%.

^{1}H NMR: (300 MHz, MeOD) δ = 1.28-1.35 (m, 4H), 1.90 (t, J = 7.7 Hz, 2H), 1.87-2.14 (m, 7H), 2.37 (t, J = 7.1 Hz, 2H), 2.51 (dd, J = 1.2 Hz, J = 16.2 Hz, 1H), 2.57 (t, J = 6.8 Hz, 2H), 2.71 (t, J = 7.1 Hz, 2H), 2.80 (s, 1H), 2.86 (d, J = 18.1 Hz, 1H), 3.01 (t, J = 6.3 Hz, 2H), 3.17-3.27 (m, 4H), 3.39-3.54 (m, 3H), 3.60 (s, 3H), 3.65-3.82 (m, 1H), 3.86 (s, 3H), 5.59 (d, J = 4.9 Hz, 1H), 5.75 (s, 1H), 6.34 (s, 1H), 6.91 (s, 1H), 7.34-7.37 (m, 2H), 7.48-7.51 (m, 3H), 7.59 (dd, J = 1.9 Hz, J = 9.1 Hz, 1H), 7.75 (d, J = 1.9 Hz, 1H), 8.35 (d, J = 9.1 Hz, 1H); ^{13}C NMR: (75 MHz, MeOD) δ = 24.0 (CH_2), 25.0 (CH_2), 25.8 (CH_2), 27.5 (CH), 28.1 (CH), 28.3 (CH_2), 29.4 (CH_2), 29.5 (CH_2), 29.7 (CH_2), 30.8 (CH_2), 34.0 (CH_2), 36.0 (CH_2), 37.0 (CH_2), 37.7 (CH_2), 40.1 (CH_2), 53.6 (CH_2), 56.4 (CH_3), 56.5 (CH_3), 68.2 (CH), 112.4 (2 CH), 115.3 (C), 115.4 (C), 119.2 (CH), 124.8 (C), 125.1 (CH), 126.4 (CH), 127.8 (CH), 130.5 (2 CH), 131.4 (2 CH), 131.6 (CH), 138.5 (C), 139.6 (C), 140.5 (C), 150.1 (C), 151.2 (C), 153.1 (C), 156.7 (C), 173.8 (C); MS (ESI+) m/z (%):739.3 (80) $[M+H]^+$, 370,3 (100) $[M/2+H]^{2+}$; HRMS (ESI+): Calc. for $C_{42}H_{49}N_4SO_3Cl$ $[M+H]^+$: 739.3449; found: 739.3443; HPLC t_R=18.8 (purity >88%); system A; IC_{50} *m*-AChE = 2.1 ± 0.4 nM.

(±)-**H4-P4**

Yield: Pale pink solid, 10%.

^{1}H NMR: (300 MHz, MeOD) δ = 1.30-1.34 (m, 4H), 1.56-1.66 (m, 2H), 1.88-2.11 (m, 7H), 2.36 (t, J = 7.2 Hz, 2H), 2.47-2.57 (m, 1H), 2.55 (t, J = 7.0 Hz, 2H), 2.70 (t, J = 7.2 Hz, 2H), 2.80 (s, 1H), 2.86 (d, J = 18.0 Hz, 1H), 3.02 (t, J = 5.8 Hz, 2H), 3.12-3.25 (m, 5H), 3.39-3.54 (m, 3H), 3.60 (s, 3H), 3.68-3.78 (m, 1H), 3.86 (s, 3H), 5.59 (d, J = 4.9 Hz, 1H), 5.74 (s, 1H), 6.33 (s, 1H), 6.91 (s, 1H), 7.34-7.37 (m, 2H), 7.49-7.51 (m, 3H), 7.59 (dd, J = 2.0 Hz, J = 9.1

Hz, 1H), 7.74 (d, *J* = 2.0 Hz, 1H), 8.35 (d, *J* = 9.1 Hz, 1H); ^{13}C NMR: (75 MHz, MeOD) δ = 24.2 (CH_2), 25.8 (CH_2), 27.3 (CH), 27.5 (CH), 28.1 (CH_2), 28.4 (CH_2), 29.4 (CH_2), 29.7 (CH_2), 31.9 (CH_2), 34.0 (CH_2), 36.0 (CH_2), 37.2 (CH_2), 37.7 (CH_2), 40.1 (CH_2), 46.7 (CH_2), 54.0 (CH_2), 56.4 (CH_3), 56.5 (CH_3), 67.9 (CH), 112.4 (CH), 112.6 (CH), 115.3 (C), 115.5 (C), 119.2 (CH), 124.8 (C), 125.0 (CH), 126.4 (CH), 127.8 (CH), 130.5 (2 CH), 131.4 (2 CH), 131.6 (CH), 137.2 (C), 138.5 (C), 139.5 (C), 140.5 (C), 150.1 (C), 151.2 (C), 153.1 (C), 156.7 (C), 162.6 (C), 167.0 (C), 173.9 (C); MS (ESI+) m/z (%):753.2 (60) $[M+H]^+$, 377,3 (80) $[M/2+H]^{2+}$; HRMS (ESI+): Calc. for $C_{44}H_{53}N_4SO_3Cl$ $[M+H]^+$: 753.3605; found: 753.3608; HPLC t_R=19.1 (purity >95%); System A; IC_{50} *m*-AChE = 3.2 ± 0.7 nM.

(±)-**PIQ 14**

Yield: yellow solid, 30%.

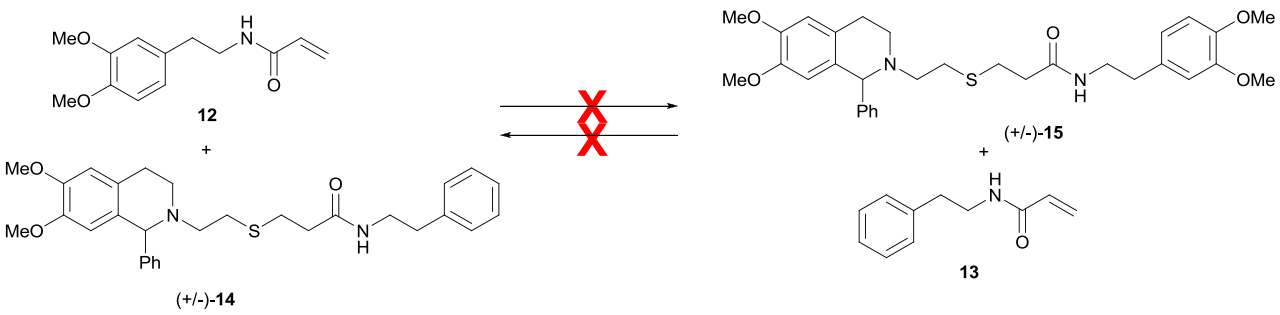
^{1}H NMR: (300 MHz, $CDCl_3$) δ = 2.47 (t, *J* = 7.3 Hz, 1H, 2.76 (t, *J* = 7.5 Hz, 1H), 2.83 (t, *J* = 7.5 Hz, 1H), 2.93-2.98 (m, 2H), 3.15-3.21 (m, 2H), 3.24-3.34 (m, 2H), 3.37-3.58 (m, 2H), 3.49 (dd, *J* = 6.3 Hz, *J* = 13.9 Hz, 2H), 3.70 (s, 3H), 3.91 (s, 3H), 5.45 (br s, 1H), 6.29 (s, 1H), 6.71 (s, 1H), 7.20-7.42 (m, 10H); ^{13}C NMR: (75 MHz, $CDCl_3$) δ = 24.8 (CH_2), 27.7 (CH_2), 35.6 (CH_2), 36.6 (CH_2), 41.0 (CH_2), 52.6 (CH_2), 56.1 (CH_3), 56.2 (CH_3), 111.8 (2 CH), 122.6 (C), 126.5 (C), 128.6 (4 CH), 128.9 (4 CH), 129.4 (2 CH), 130.6 (C), 139.1 (C), 149.0 (C), 149.8 (C), 171.9 (C); Melting point: Degradation (54-56 °C); IR (neat) cm^{-1}: 3287, 2935, 1663, 1517, 1454, 1260, 1196, 1175, 1119, 700; MS (ESI+) m/z (%): 505.13 (100) $[M+H]^+$; HPLC t_R=19.8 (purity >98%); System A.

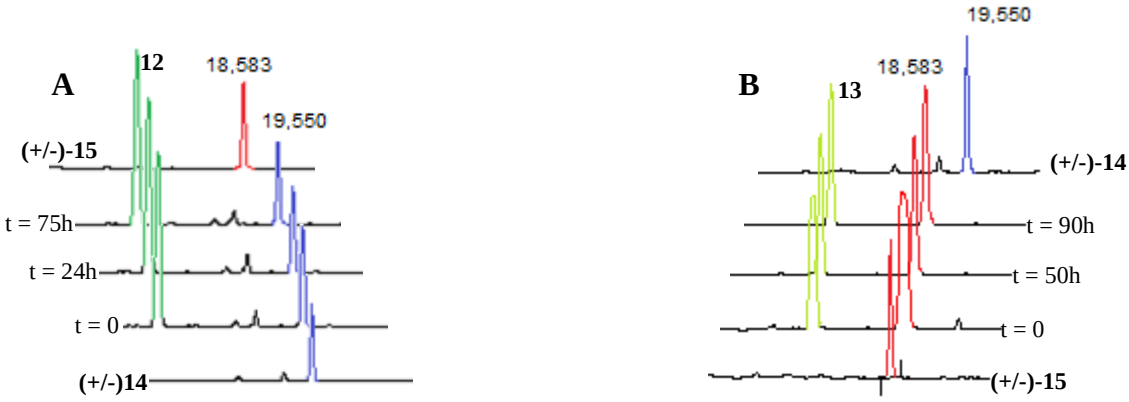
(±)-**PIQ 15**

Yield: white solid, 38%.

^{1}H NMR: (300 MHz, $CDCl_3$) δ = 2.47 (t, *J* = 7.5 Hz, 2H), 2.75-2.82 (m, 4H), 2.89-2.98 (m, 2H), 3.12-3.37 (m, 4H), 3.45-3.62 (m, 4H), 3.71 (s, 3H), 3.84 (s, 3H), 3.87 (s, 3H), 3.92 (s, 3H), 5.44 (br s, 1H), 6.32 (s, 1H), 6.72 (s, 1H), 6.76-6.83 (m, 3H), 7.23-7.30 (m, 2H), 7.41-7.43 (m, 3H); ^{13}C NMR: (75 MHz, $CDCl_3$) δ = 24.6 (CH_2), 27.8 (CH_2), 35.2 (CH_2), 36.6 (CH_2), 41.1 (CH_2), 52.6 (CH_2), 56.0 (2 CH_3), 56.1 (CH_3), 56.2 (CH_3), 68.0 (CH), 110.8 (2 CH), 111.5 (CH), 112.3 (CH), 120.9 (CH), 122.7 (C), 126.5 (C), 129.4 (2 CH), 130.5 (2 CH), 131.8 (CH), 147.6 (C), 149.0 (C), 171.4 (C); MS (ESI+) m/z (%): 565.1 (100) $[M+H]^+$; HPLC t_R=18.6 (purity >95%); System A.

II.2 Irreversibility tests for addition of acrylamide to thioether derivatives





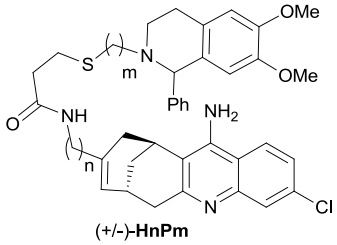
The left hand figure A is an overlay of the HPLC traces for the addition of acrylamide **12** (green) to thioether (±)-**14** (blue). As expected, the conjugate addition product (±)-**15** (red) was not formed. The reaction mixture was also analysed by mass spectrometry (Full scan and SIM mode) in order to detect any traces of the conjugate product, but none were found. Furthermore, the reverse reaction was also tested, in order to eliminate any effect of the difference in reactivity of the two acrylamide derivatives **13** and (±)-**14**. The right hand figure B is an overlay of the HPLC traces for the addition of acrylamide **13** (light green) to thioether (±)-**15** (red). The conjugate addition product (±)-**14** (blue) was not formed as it could not be detected neither by HPLC nor by mass spectrometry. This demonstrates the irreversibility of the addition of thiols to acrylamide derivatives.

II.3 Molecular modelling

We performed a molecular docking analysis of the final heterodimeric inhibitors to predict the effect of the substitution and the spacer arm length on binding to AChE. The structure of human AChE was prepared from the crystal structure of human AChE in complex with

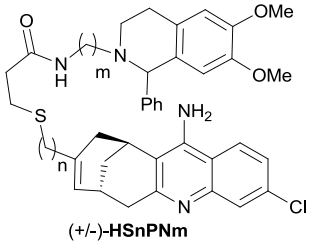
huprine W and fasciculin 2.[5] Missing *N*-terminal residues were rebuilt and minimized with MODELLER 9v8.[6] Crystallographic water molecules were conserved in the model. We then performed molecular docking calculations using Autodock Vina[7] conserving the key structural water molecules in the template.

The variation between the different heterodimeric inhibitors is small given the general accuracy of the scoring function (about 2 kcal.mol^{-1}).[7] Nonetheless, the results of the docking simulations show that the heterodimeric inhibitors (+/-)-**HnPm** (with acrylamide function on the huprine derivatives and the thiol on the PIQ derivatives) are better than the opposite derivatives (+/-)-**HSnPNm (**with the thiol on the huprine derivatives and the acrylamide function on the PIQ derivatives).



(+/-)-**HnPm**

m \ n	**Stabilisation energy (Kcal/mol)** 2	**Stabilisation energy (Kcal/mol)** 3	**Stabilisation energy (Kcal/mol)** 4	**Stabilisation energy (Kcal/mol)** 5
2	-13.5	-14.1	-13.3	-13.0
3	-13.3	-13.4	-12.8	-12.6
4	-12.8	-13.3	-12.6	-12.2



(+/-)-**HSnPNm**

m \ n	**Stabilisation energy (Kcal/mol)** 2	**Stabilisation energy (Kcal/mol)** 3	**Stabilisation energy (Kcal/mol)** 4
2	-12.6	-12.7	-12.6
3	-12.2	-12.7	-12.6
4	-12.2	-12.1	-12.2

II.4 AChE inhibition assay

The inhibitory activity was evaluated spectrophotometrically using a UV-vis Varian Cary 50 scan spectrophotometer equipped with a microplate reader at 25 °C by the method of Ellman using mouse AChE[4, 8] and acetylthiocholine iodide (0.50 mM) as substrate.[9] The reaction was performed in the presence of 40 pM of AChE in a final volume of 200 µL of 0.1 M phosphate-buffered solution (pH 7.4) containing 0.025% (25 mg per 100 mL) of bovine serum albumin (BSA) and 300 µM 5,5'- dithiobis-2-nitrobenzoic acid (DTNB) solution used to produce the yellow anion of 5-thio-2-nitrobenzoic acid. The different derivatives were pre-incubated with the enzyme at 25 °C for 90 min before UV absorbance measurement. One sample without inhibitor was always present to yield 100% of cholinesterase activity. One blank sample containing only DTNB was always present to yield the minimum absorbance of non-specific hydrolysis. The rate of change of absorbance ($\Delta Abs\text{min}^{-1}$), which reflects the rate of hydrolysis of acetylthiocholine, was recorded at 414 nm for 20 min (kinetics mode). These experiments were done in triplicate, the values were averaged and minimum absorbance subtracted. Data from concentration-response experiments of the inhibitors were analysed by nonlinear regression based on a four parameter logistic model using XLFit (IDBS), which gave estimates of the IC_{50} values (concentration of drug producing 50% of enzyme activity inhibition). DTNB and acetylthiocholine iodide were purchased from Sigma.

II.5 *In situ* click reaction experiments

- **Preparation of ligands solutions**

10 mM methanolic solutions of acylation site ligand and peripheral site ligand were first prepared. These stock solutions were diluted with ammonium citrate buffer (2.0 mM ammonium citrate, 100 mM NaCl, pH 7.4) to afford 100 µM aqueous solutions (containing 1% of MeOH) of each ligand.

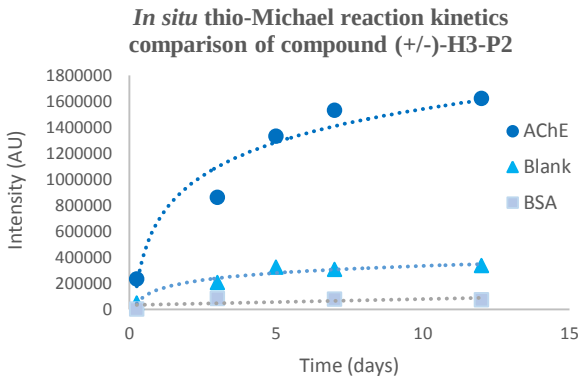
- **Enzymatic inhibitor assembly**

To ~1µM solutions of AChE in 2.0 mM ammonium citrate buffer (100 mM NaCl, pH 7.3-7.5), the previously prepared acylation site ligands huprine components (**Hn**) in ammonium citrate buffer were added followed by the corresponding peripheral site ligand coupling partner (**Pm or Pm-Ac**) (5 eq.). The resulting solutions were thoroughly mixed. The final concentrations were: AChE, 1 µM; huprine component, 4.6 µM, $PIQA_n$ component, 24 µM

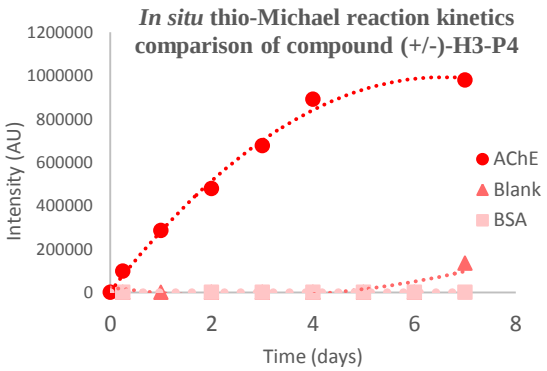
with a total volume of 1 mL. Each reaction mixture was allowed to stand in a laboratory oven at 37 °C. The enzyme was found to be stable in these experimental conditions.[11]

At regular intervals, aliquots of 50 µL were sampled and added to 50 µL of MeOH (for denaturation of the enzyme), and then the mixture was vortexed for 3 min, placed in an ultra sound bath for 5 min and lastly centrifuged. Finally, 20 µL of the solution were analysed by LC/MS-SIM following system F. (Assembly of (±)-**H2P2**: F4; (±)-**H2P3**; (±)-**H2P4**: F6; (±)-**H3P2**: F1; (±)-**H3P3**: F3; (±)-**H3P4** and (±)-**H4P2**: F2; (±)-**H4P3**: F7; (±)-**H4P4**: F8). Parallel control experiments were conducted in the absence of AChE (buffer alone with ligands). Non-specific templated reactions of the successful click experiments (that is (±)-**H3** with (±)-**P2** and (±)-**P4**) were also conducted in the presence of BSA instead of AChE. An additional control experiment was conducted with (±)-**H3** and (±)-**P4** in the presence of AChE by addition of the potent inhibitor huprine X.

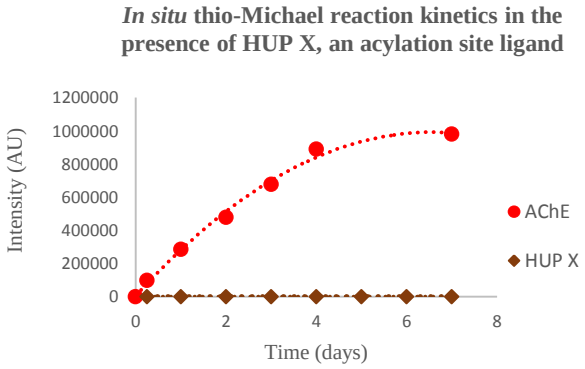
A



B



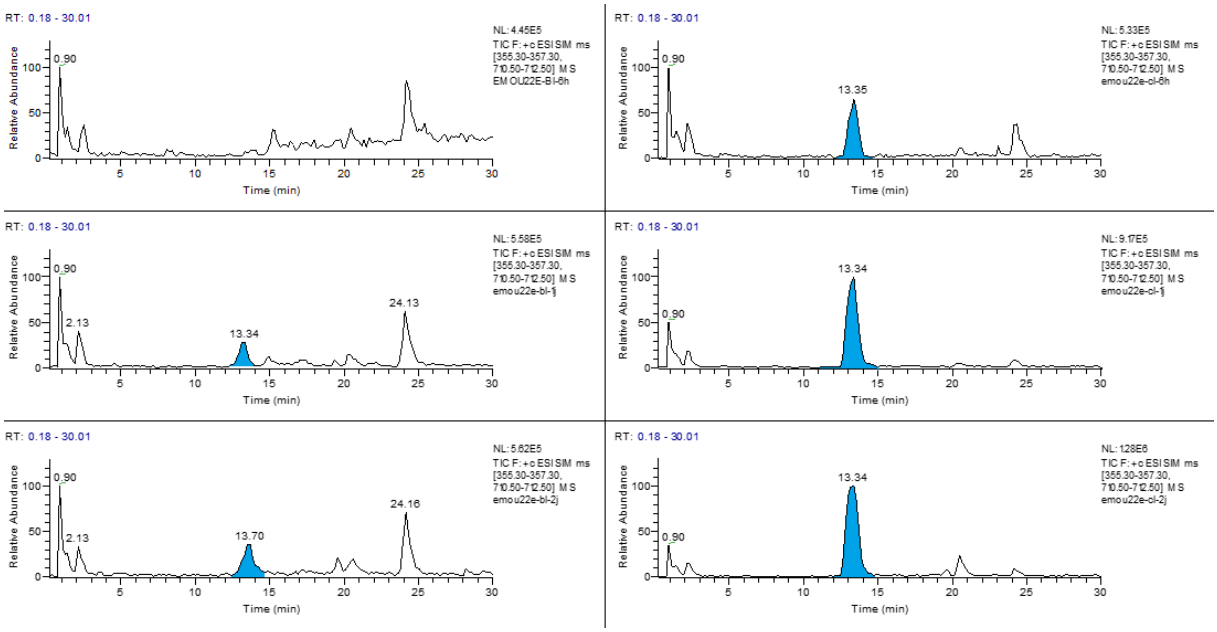
C



The upper left hand figure A represents the kinetics comparison for the assembly of heterodimer (±)-**H3-P2** in the presence and the absence of *m*-AChE and in the presence of BSA. The upper right hand figure B represents the kinetics comparison for the assembly of heterodimer (±)-**H3-P4** in the presence and the absence of *m*-AChE and in the presence of BSA. The lower figure C shows that no *in situ*

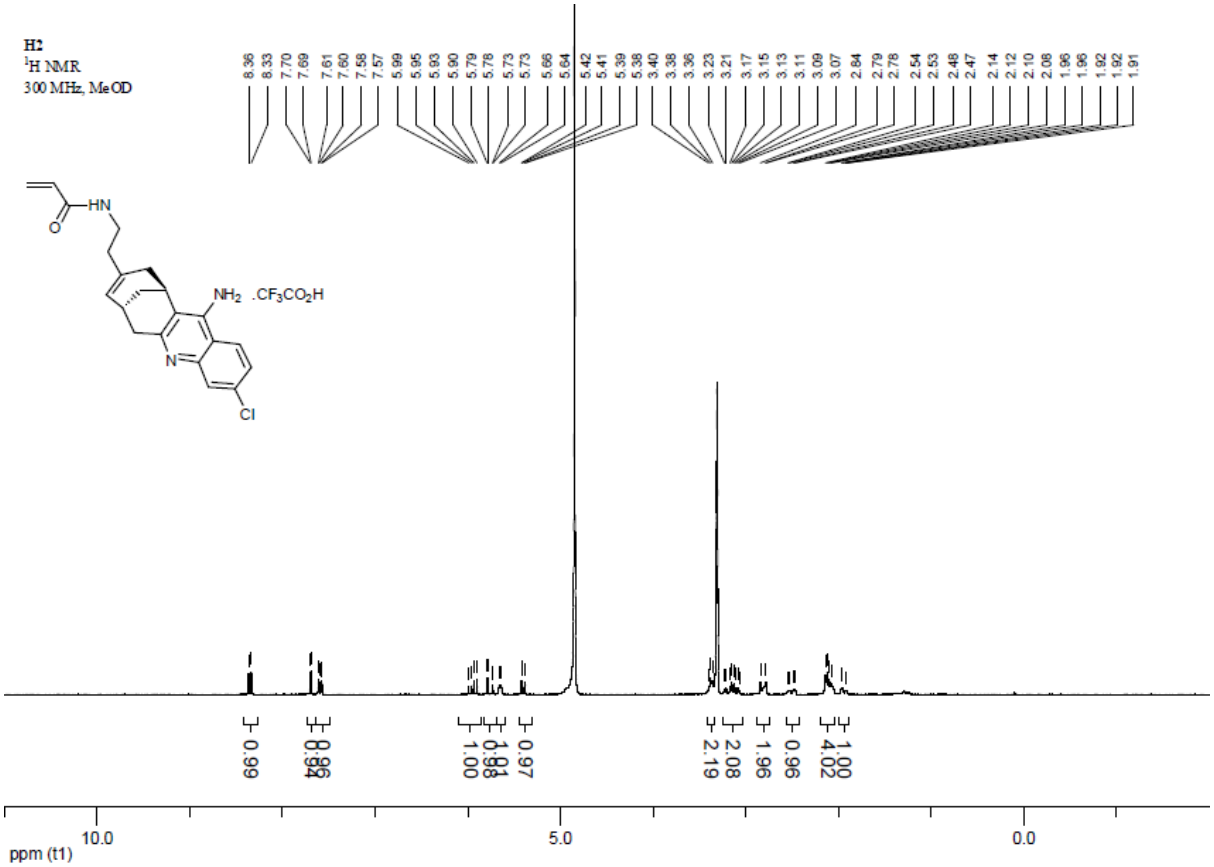
reaction occurred between (±)-**H3** and (±)-**P4** in the presence of the acylation site inhibitor (±)-huprine X.

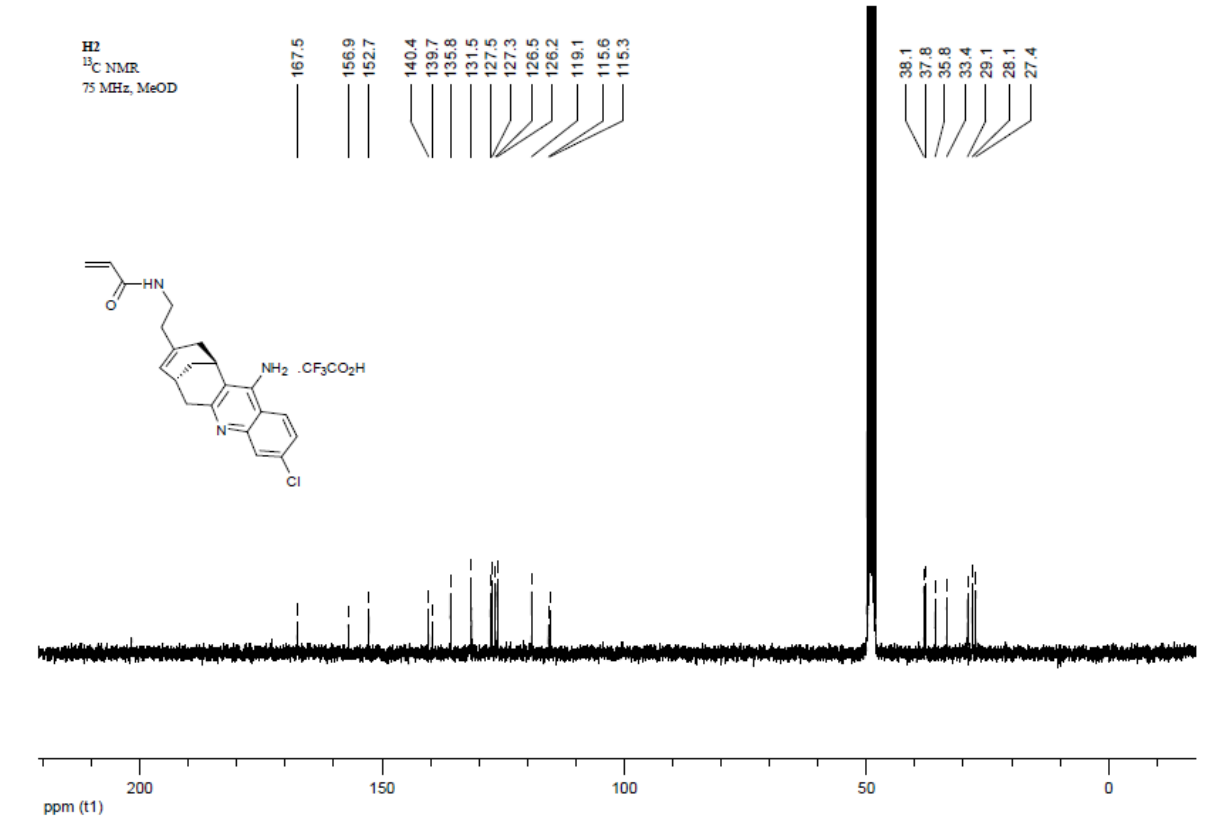
- **Double catalysis experiments**

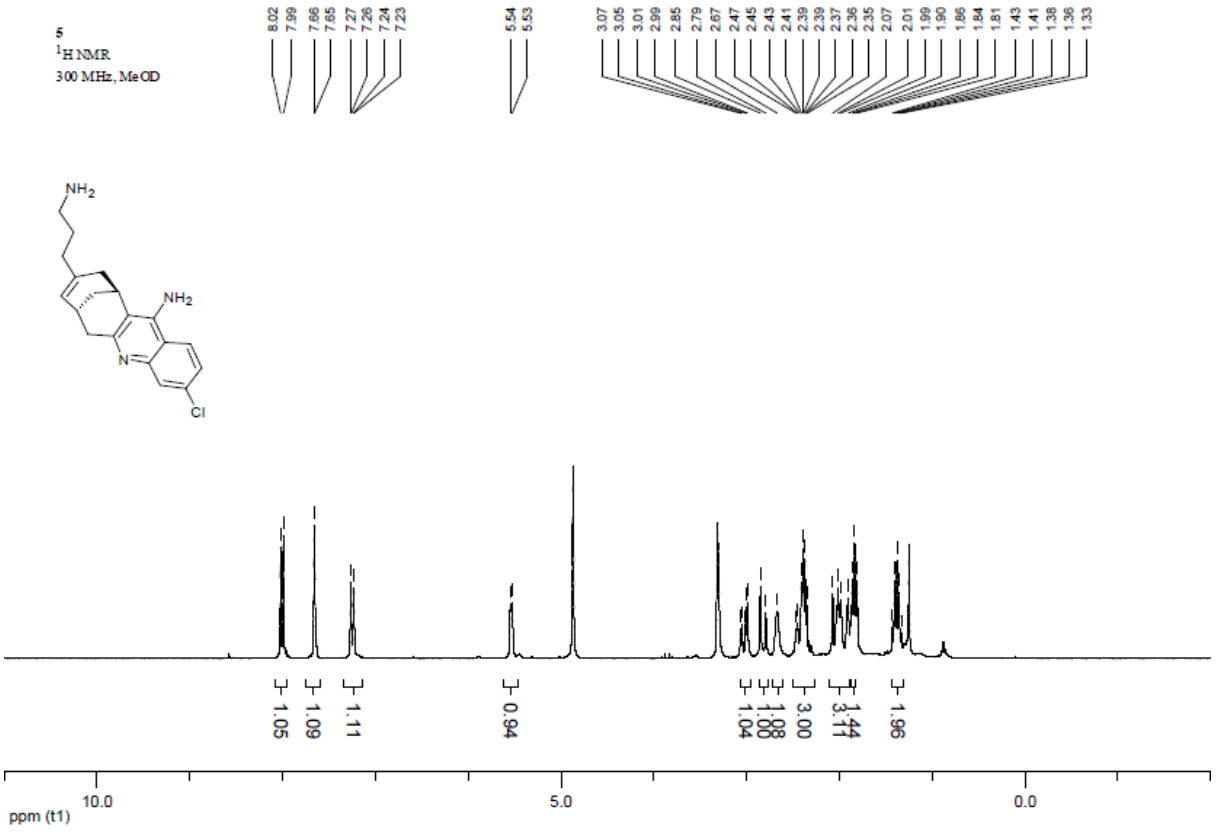


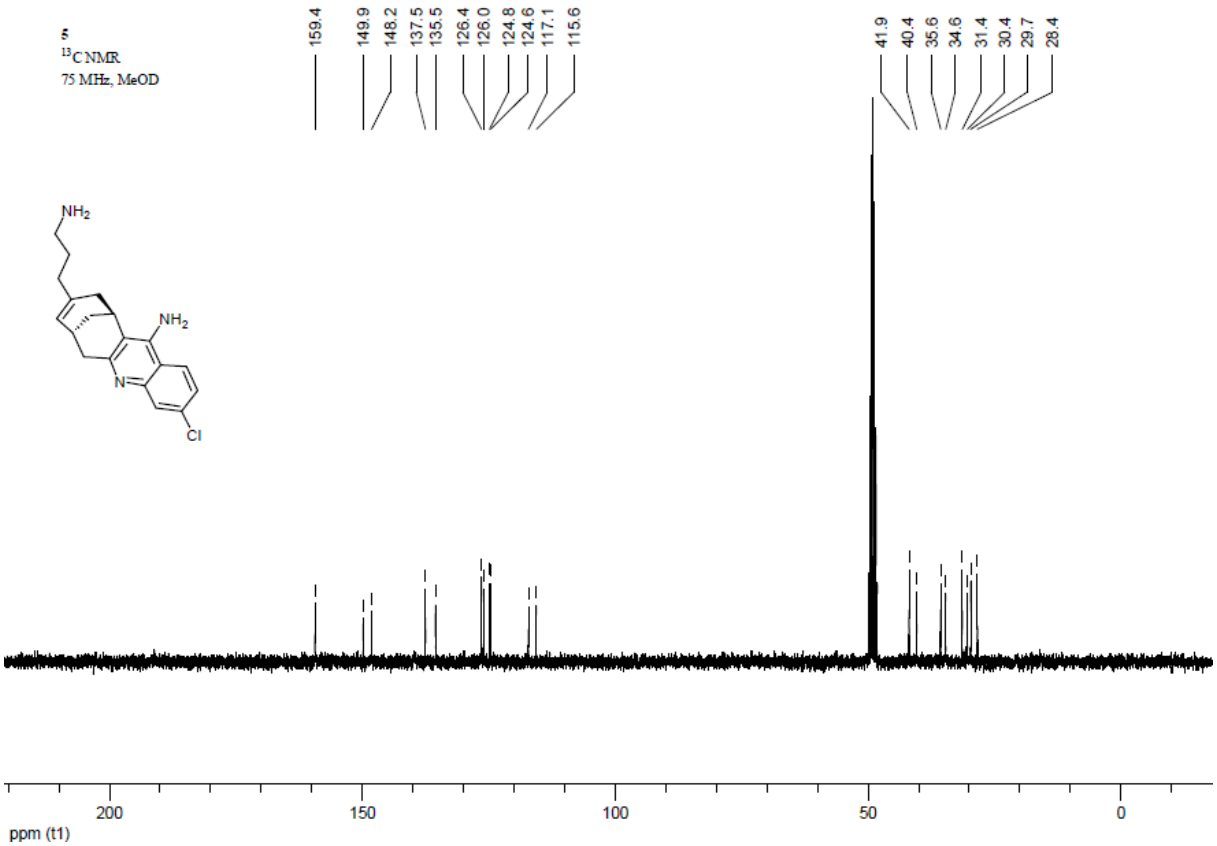
LCMS/SIM traces of the blank (left) and click (right) experiments of (±)-**H3** and (±)-**P2-Ac** at different times of incubation (6 hours, 1 and 2 days) showing the amplified formation of the desired product in the presence of AChE.

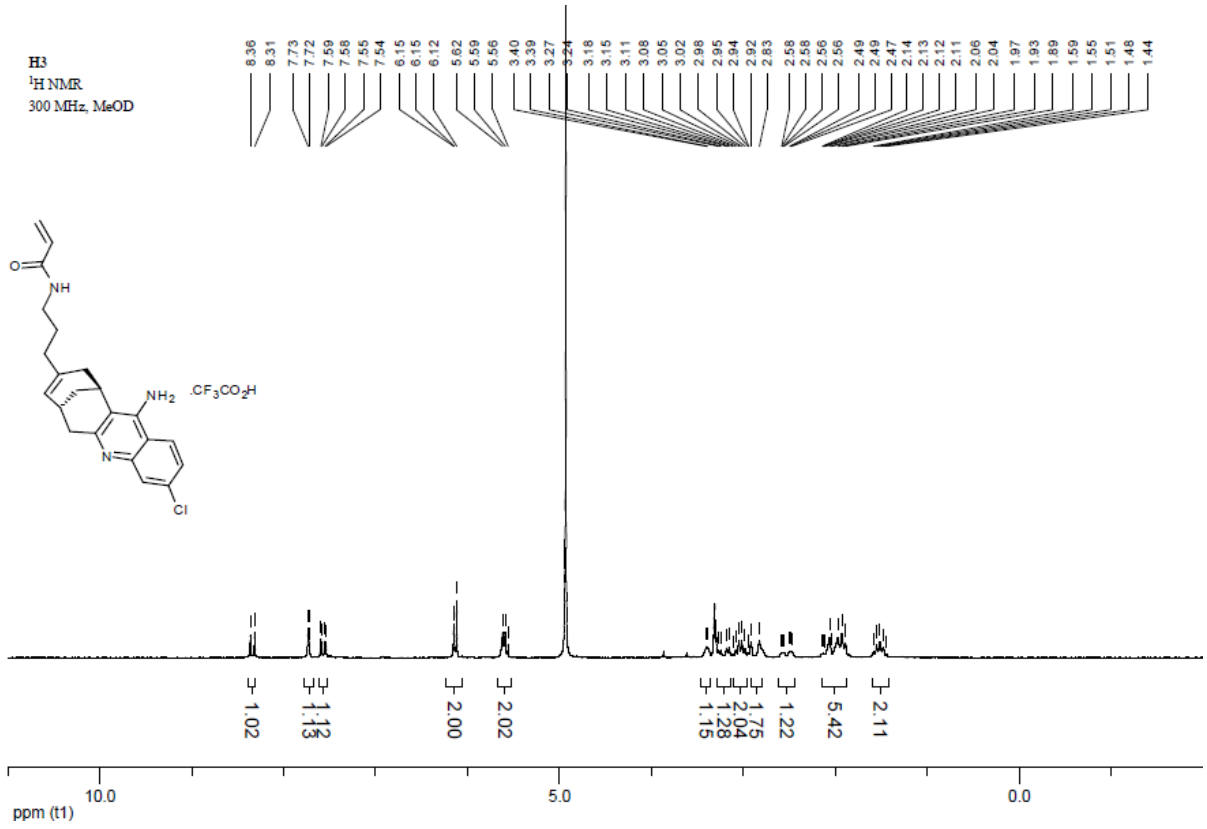
III. Copies of ^{1}H and ^{13}C NMR spectra

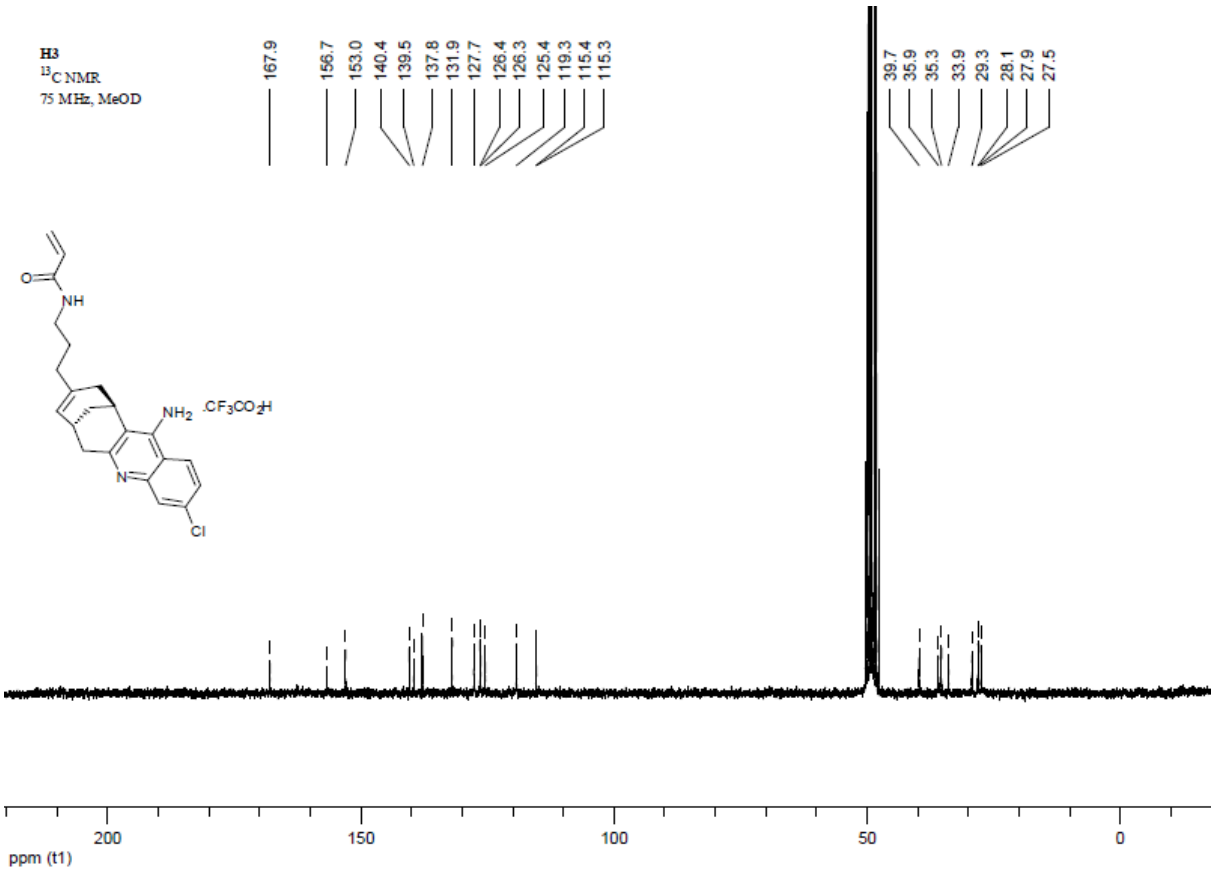


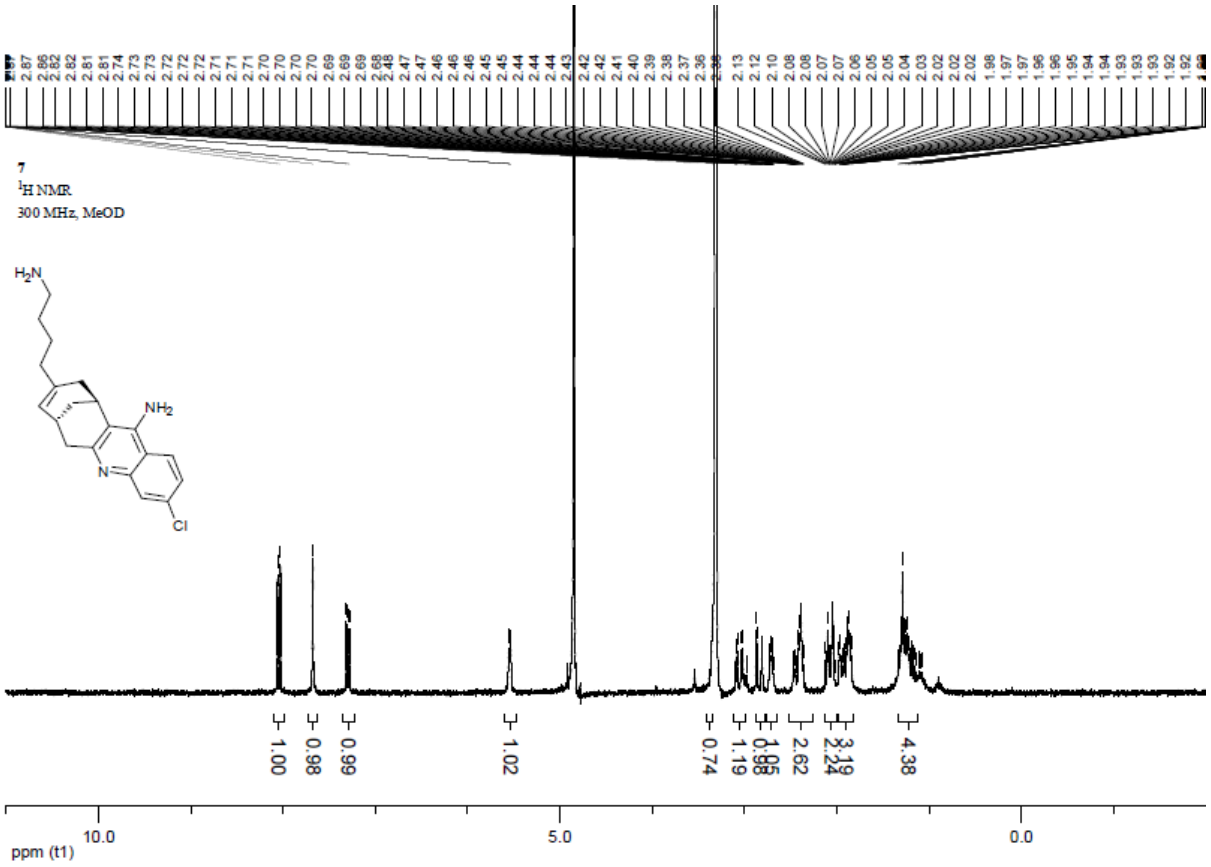


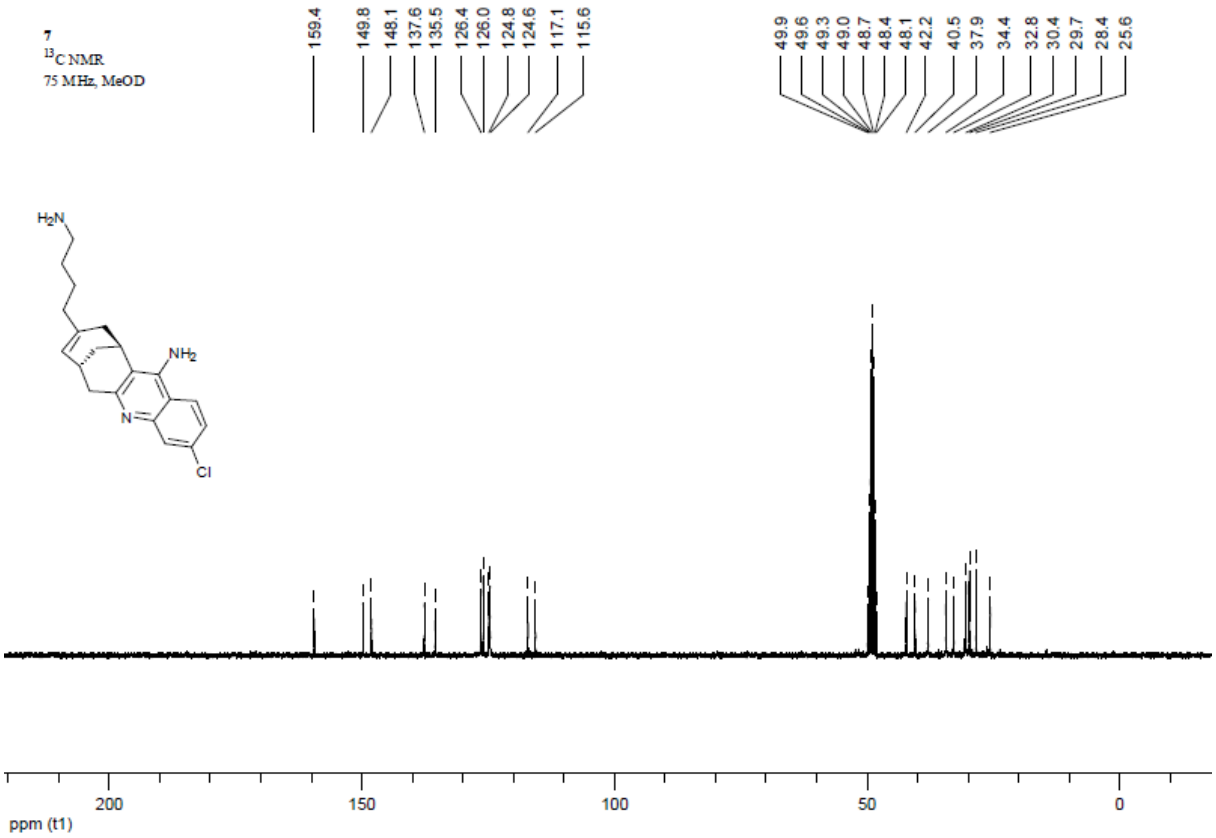

5
1H NMR
300 MHz, MeOD
8.02
7.99
7.66
7.65
7.27
7.26
7.24
7.23
5.54
5.53
3.07
3.05
3.01
2.99
2.85
2.79
2.67
2.47
2.45
2.43
2.41
2.39
2.37
2.36
2.35
2.07
2.01
1.99
1.90
1.86
1.84
1.81
1.43
1.41
1.38
1.36
1.33
NH2
NH2
N
Cl
1.05
1.09
1.11
0.94
1.04
1.00
3.00
3.11
1.96
10.0
5.0
0.0
ppm (t1)

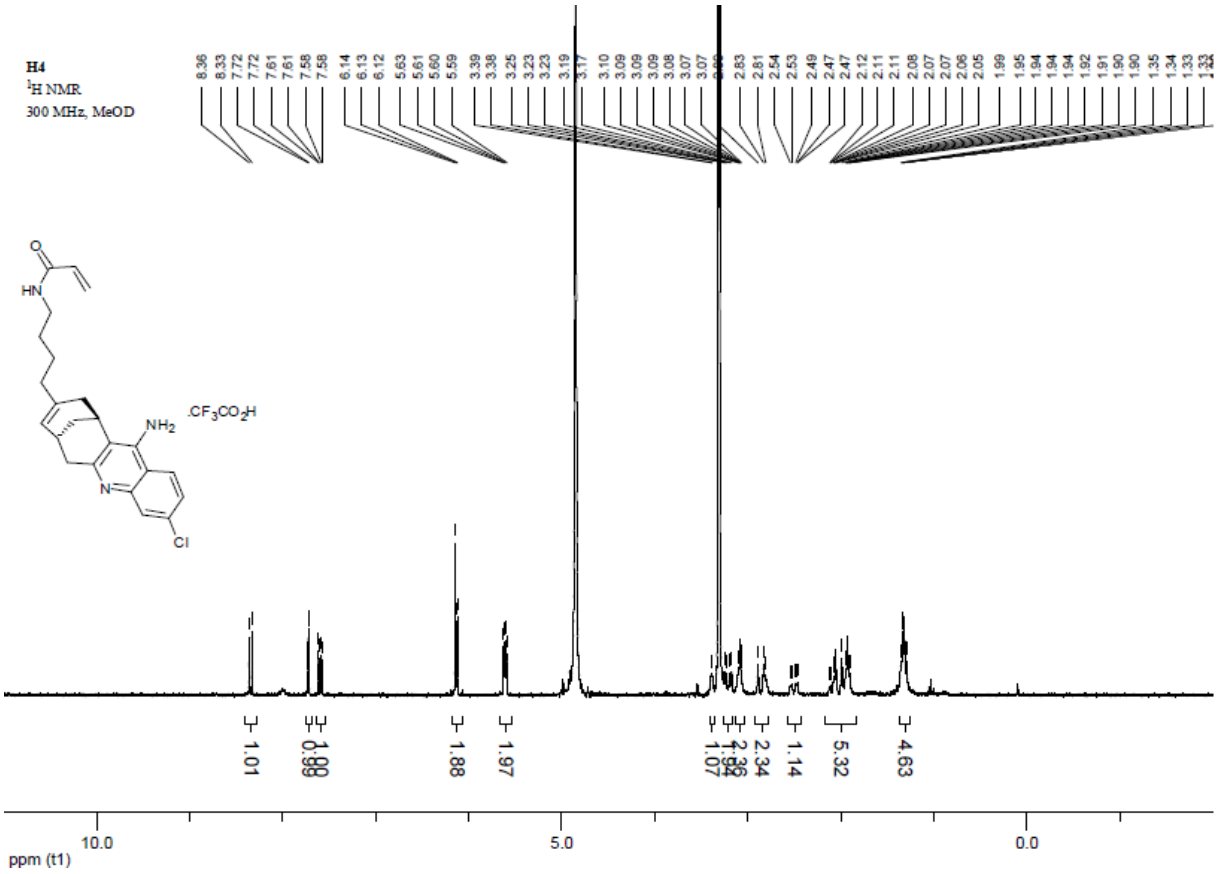

5
13C NMR
75 MHz, MeOD
159.4
149.9
148.2
137.5
135.5
126.4
126.0
124.8
124.6
117.1
115.6
41.9
40.4
35.6
34.6
31.4
30.4
29.7
28.4
NH2
NH2
N
Cl
200
150
100
50
0
ppm (t1)

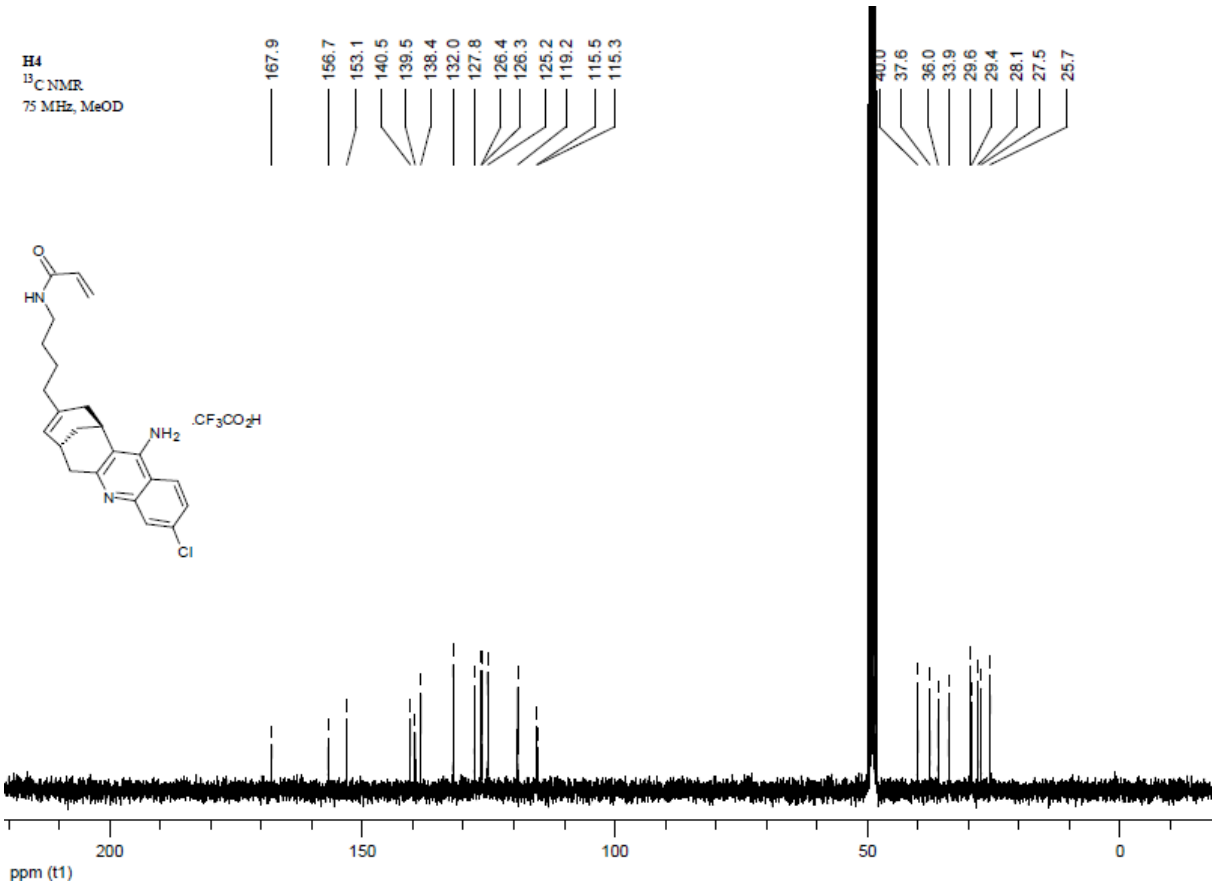

H3
1H NMR
300 MHz, MeOD
8.36
8.31
7.73
7.72
7.59
7.58
7.55
7.54
6.15
6.15
6.12
5.62
5.59
5.56
3.40
3.39
3.27
3.24
3.18
3.15
3.11
3.08
3.05
3.02
2.98
2.95
2.94
2.92
2.83
2.58
2.58
2.56
2.56
2.49
2.49
2.47
2.14
2.13
2.12
2.11
2.06
2.04
1.97
1.93
1.89
1.59
1.55
1.51
1.48
1.44
NH
NH2
.CF3CO2H
N
Cl
1.02
1.13
1.12
2.00
2.02
1.15
1.28
2.04
1.75
1.22
5.42
2.11
10.0
5.0
0.0
ppm (t1)

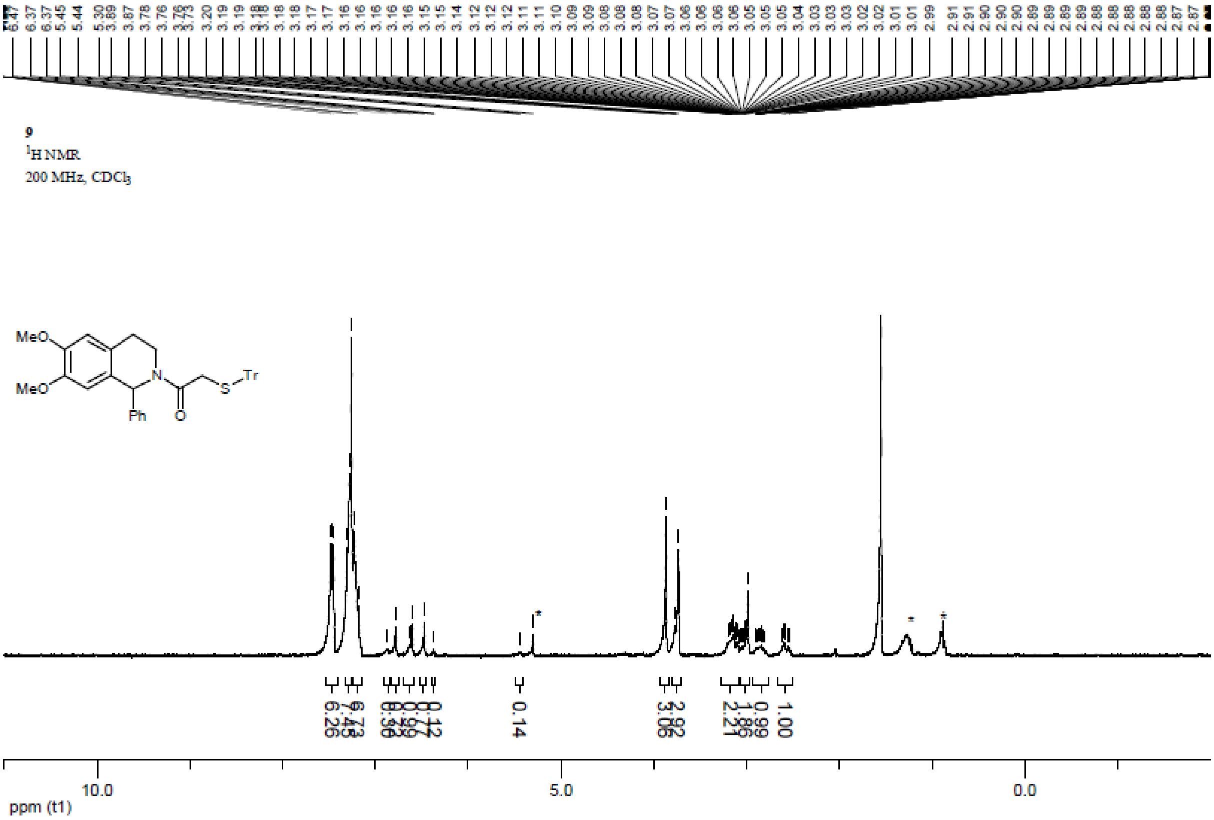

H3
13C NMR
75 MHz, MeOD
167.9
156.7
153.0
140.4
139.5
137.8
131.9
127.7
126.4
126.3
125.4
119.3
115.4
115.3
39.7
35.9
35.3
33.9
29.3
28.1
27.9
27.5
NH
NH2
.CF3CO2H
N
Cl
200
150
100
50
0
ppm (t1)

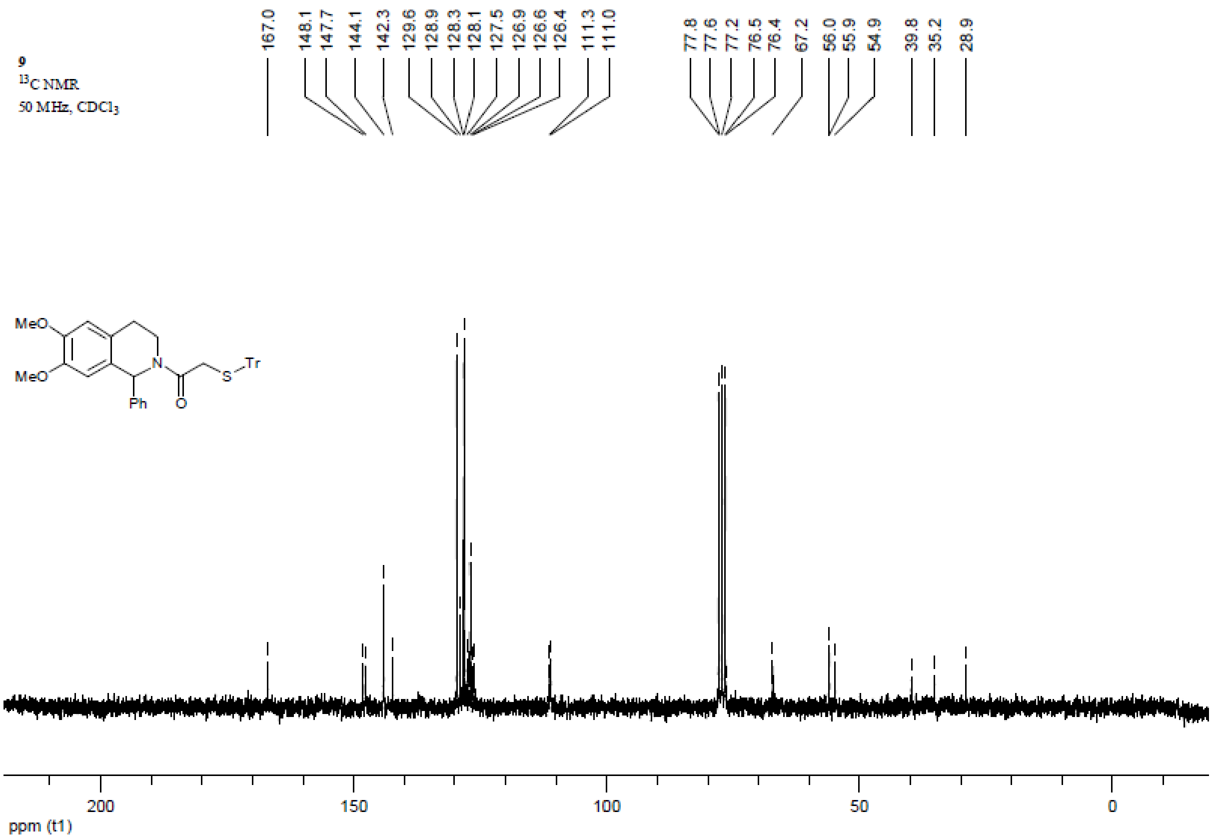

7
1H NMR
300 MHz, MeOD
H2N
NH2
N
Cl
1.00
0.98
0.99
1.02
0.74
1.19
2.92
4.38
10.0
5.0
0.0
ppm (t1)

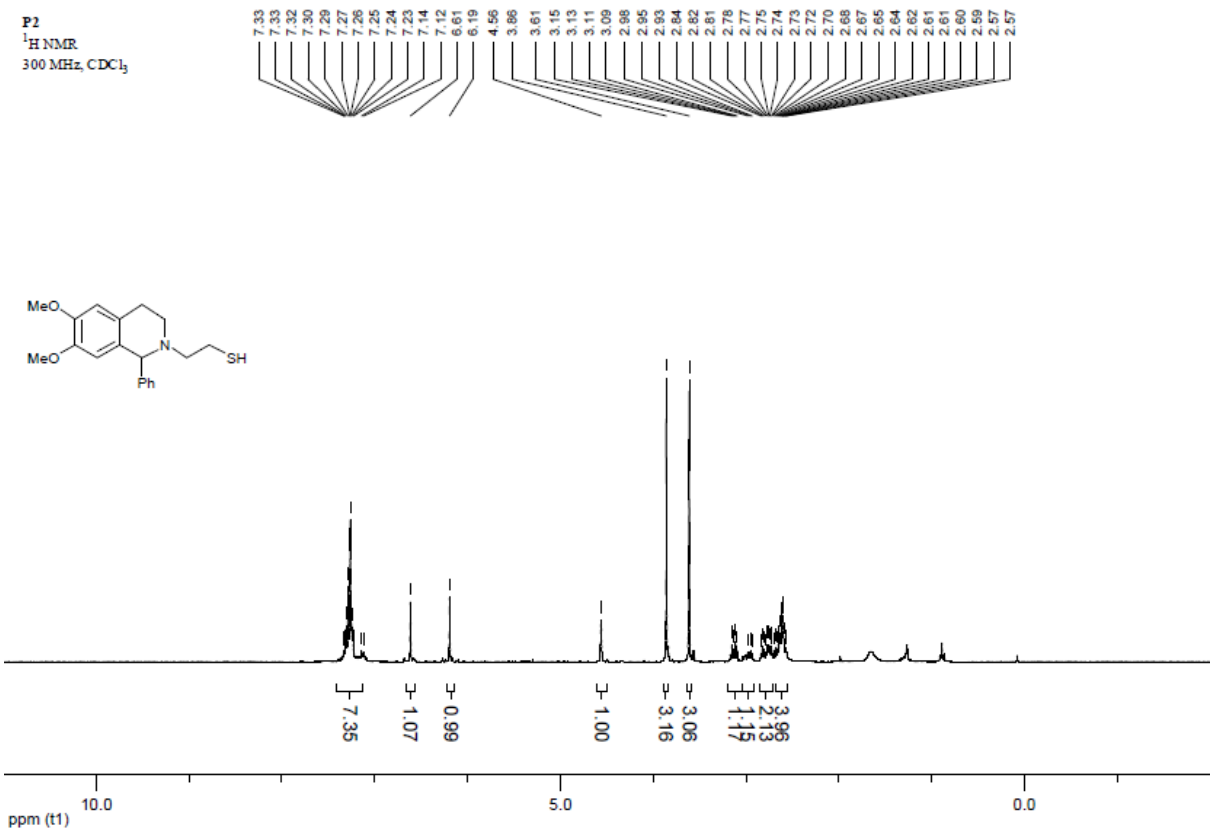

7
13C NMR
75 MHz, MeOD
159.4
149.8
148.1
137.6
135.5
126.4
126.0
124.8
124.6
117.1
115.6
49.9
49.6
49.3
49.0
48.7
48.4
48.1
42.2
40.5
37.9
34.4
32.8
30.4
29.7
28.4
25.6
H2N
NH2
N
Cl
200
150
100
50
0
ppm (t1)

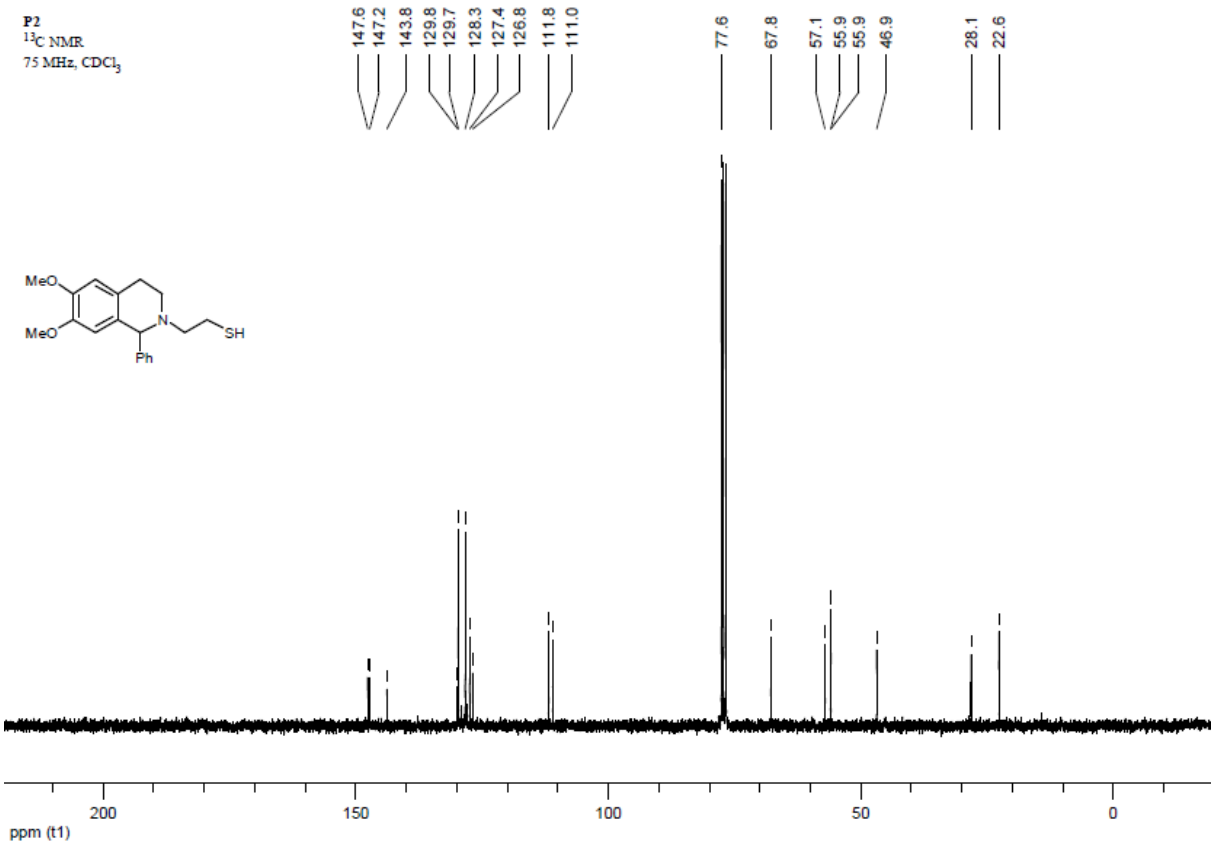

H4
^{1}H NMR
300 MHz, MeOD
HN
O
NH$_2$
.CF$_3$CO$_2$H
N
Cl
1.01
0.99
1.88
1.97
1.07
2.34
1.14
5.32
4.63
10.0
5.0
0.0
ppm (t1)

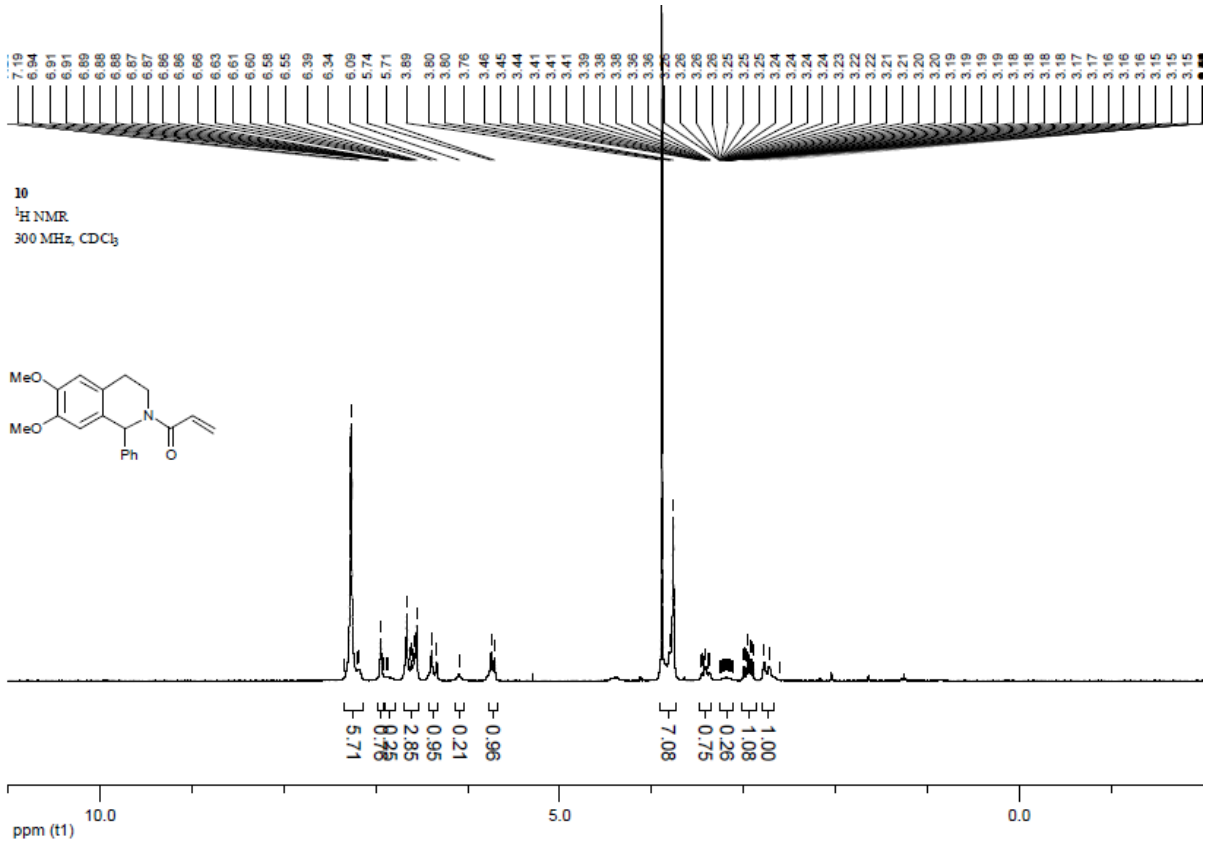

H4
^{13}C NMR
75 MHz, MeOD
167.9
156.7
153.1
140.5
139.5
138.4
132.0
127.8
126.4
126.3
125.2
119.2
115.5
115.3
40.0
37.6
36.0
33.9
29.6
29.4
28.1
27.5
25.7
HN
O
NH$_2$
.CF$_3$CO$_2$H
N
Cl
200
150
100
50
0
ppm (t1)

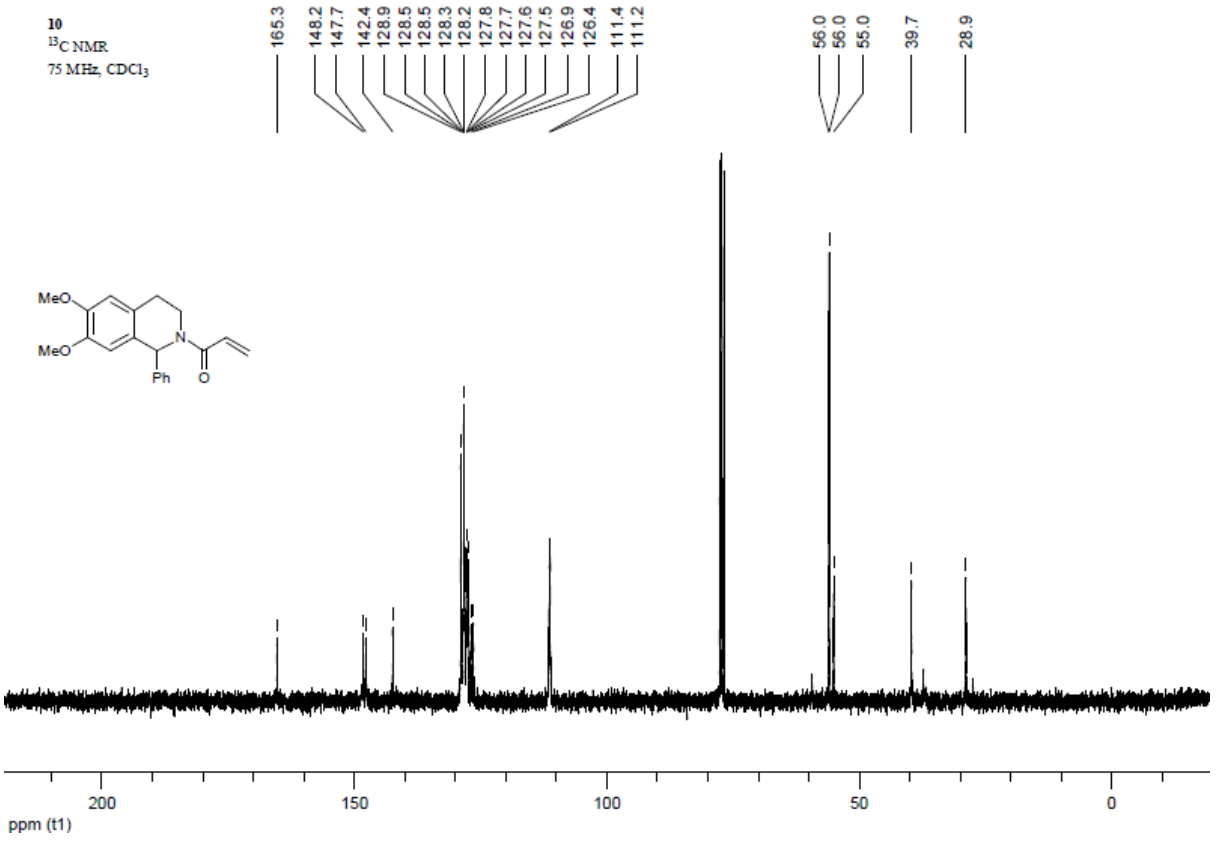

9
1H NMR
200 MHz, CDCl3
MeO
MeO
N
S
Tr
Ph
O
10.0
5.0
0.0
ppm (t1)


167.0
148.1
147.7
144.1
142.3
129.6
128.9
128.3
128.1
127.5
126.9
126.6
126.4
111.3
111.0
77.8
77.6
77.2
76.5
76.4
67.2
56.0
55.9
54.9
39.8
35.2
28.9
9
13C NMR
50 MHz, CDCl3
MeO
MeO
N
S
Tr
Ph
O
200
150
100
50
0
ppm (t1)

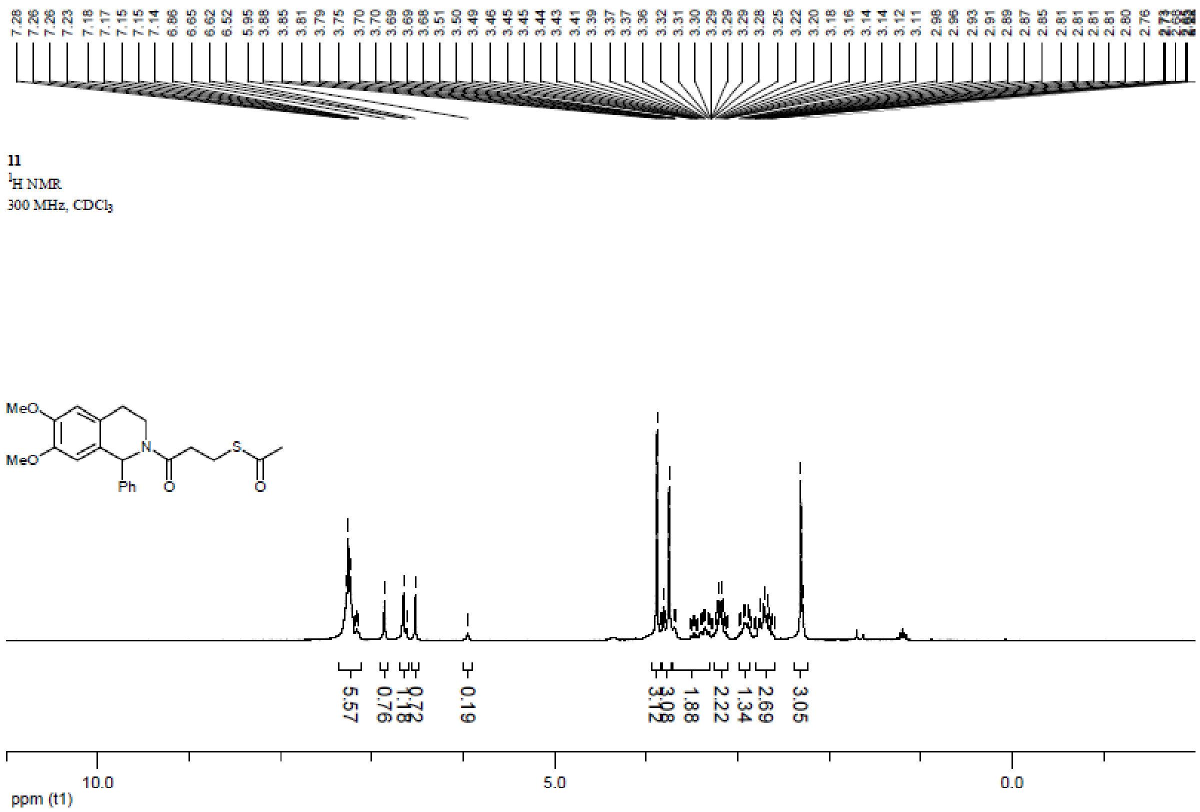

P2
1H NMR
300 MHz, CDCl3
7.33 7.33 7.32 7.30 7.29 7.27 7.26 7.25 7.24 7.23 7.14 7.12 6.61 6.19 4.56 3.86 3.61 3.15 3.13 3.11 3.09 2.98 2.95 2.93 2.84 2.82 2.81 2.78 2.77 2.75 2.74 2.73 2.72 2.70 2.68 2.67 2.65 2.64 2.62 2.61 2.61 2.60 2.59 2.57 2.57
MeO
MeO
N
SH
Ph
7.35
1.07
0.99
1.00
3.16
3.06
1.15
2.13
3.96
10.0
5.0
0.0
ppm (t1)


P2
13C NMR
75 MHz, CDCl3
147.6 147.2 143.8 129.8 129.7 128.3 127.4 126.8 111.8 111.0 77.6 67.8 57.1 55.9 55.9 46.9 28.1 22.6
MeO
MeO
N
SH
Ph
200
150
100
50
0
ppm (t1)

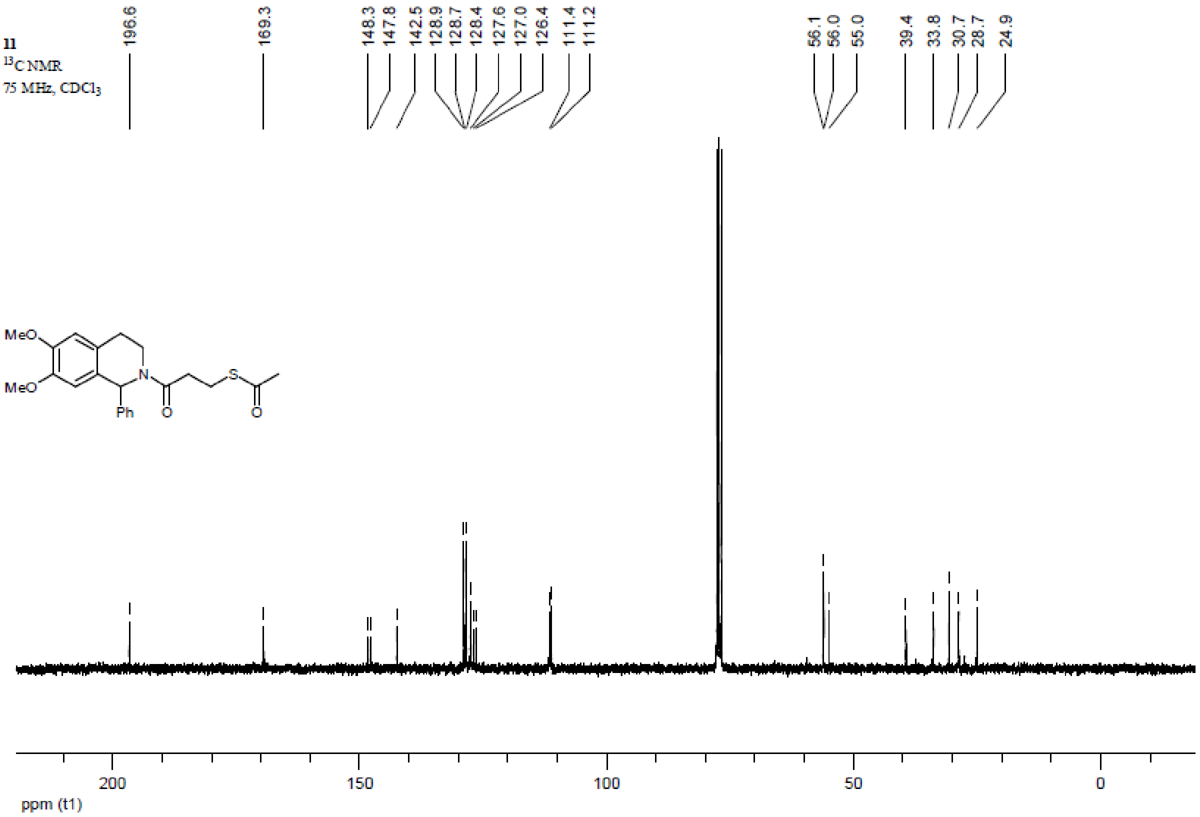

10
^{1}H NMR
300 MHz, $CDCl_3$
MeO
MeO
N
Ph
O
5.71
2.85
0.95
1.21
0.96
7.08
0.75
0.26
1.08
1.00
10.0
5.0
0.0
ppm (t1)

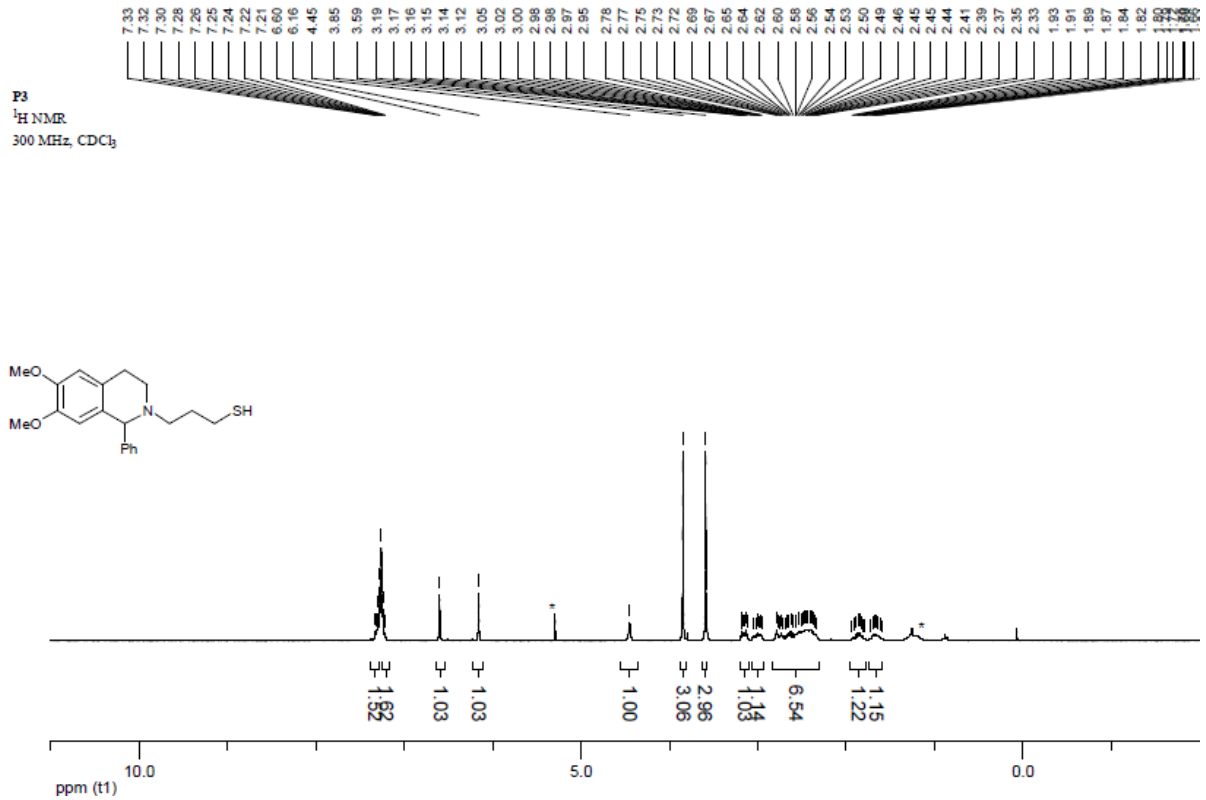

10
^{13}C NMR
75 MHz, $CDCl_3$
165.3
148.2
147.7
142.4
128.9
128.5
128.5
128.3
128.2
127.8
127.7
127.6
127.5
126.9
126.4
111.4
111.2
56.0
56.0
55.0
39.7
28.9
MeO
MeO
N
Ph
O
200
150
100
50
0
ppm (t1)

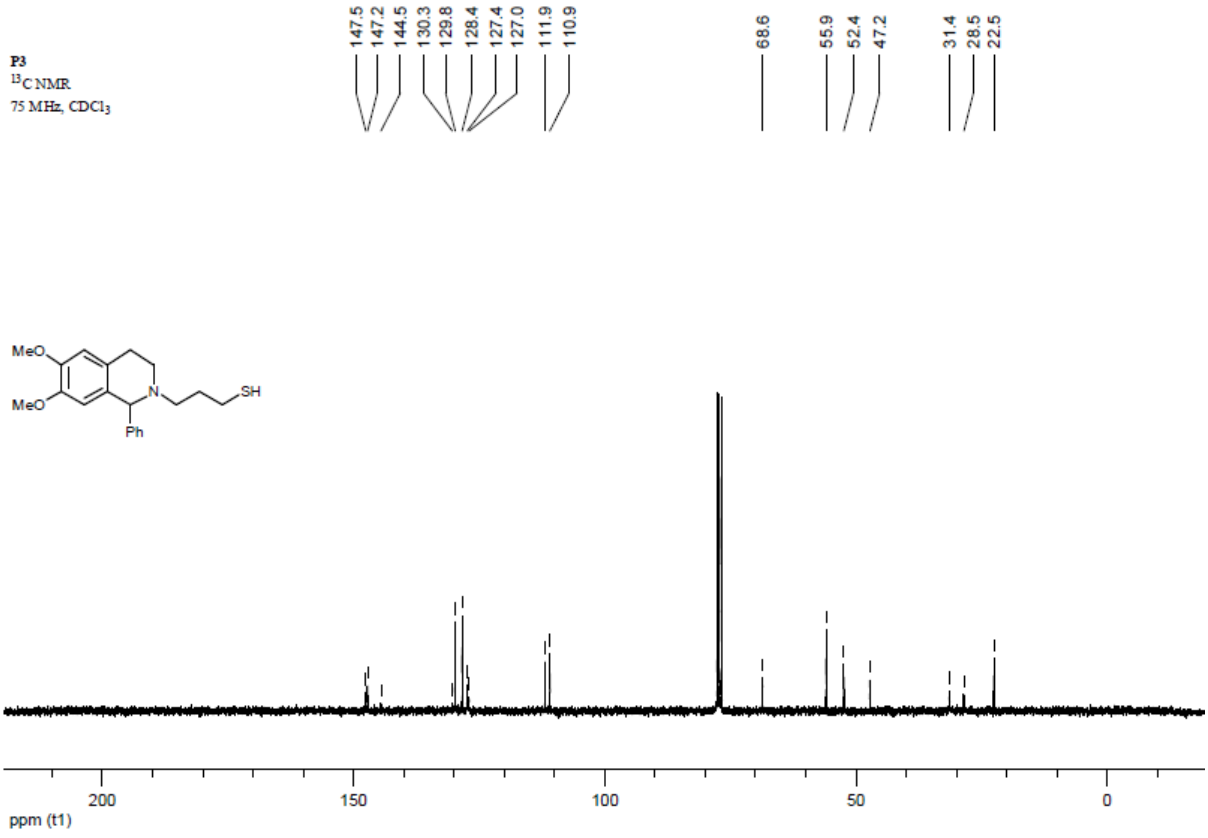
11
^{1}H NMR
300 MHz, $CDCl_3$

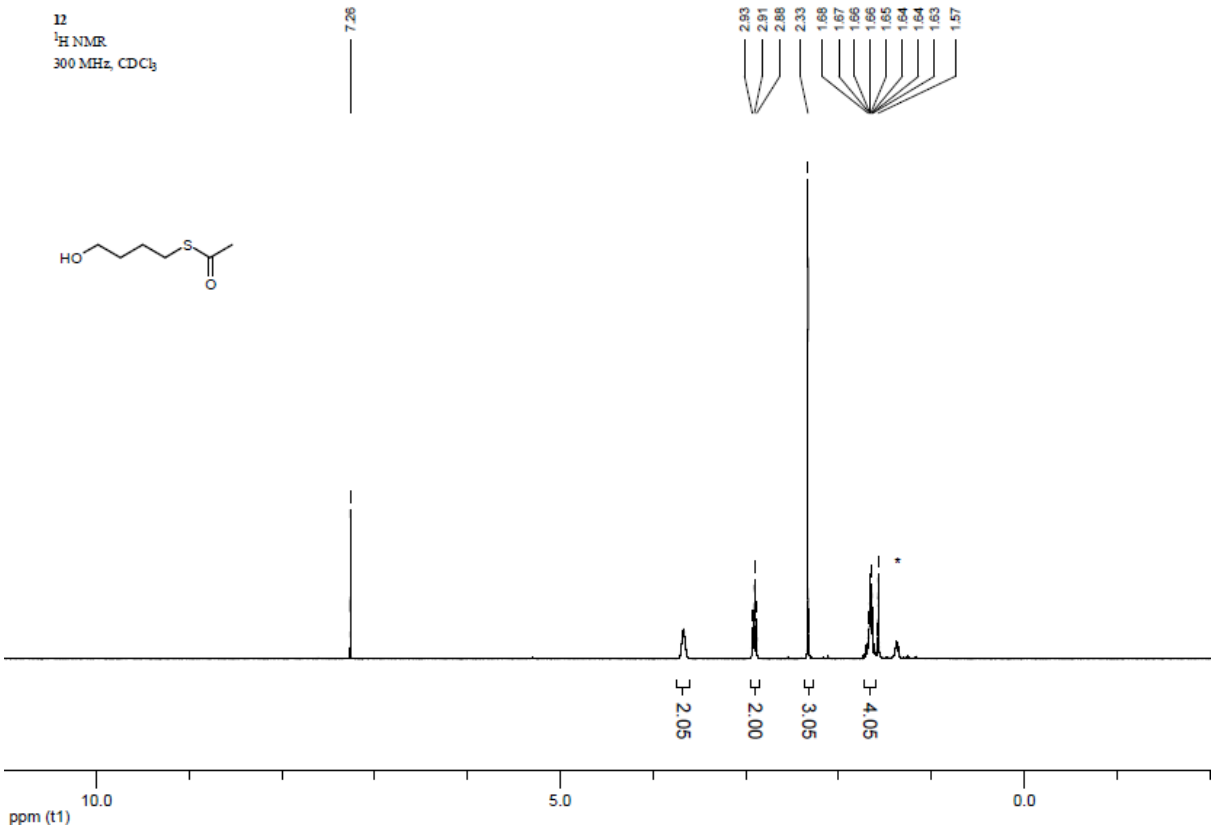


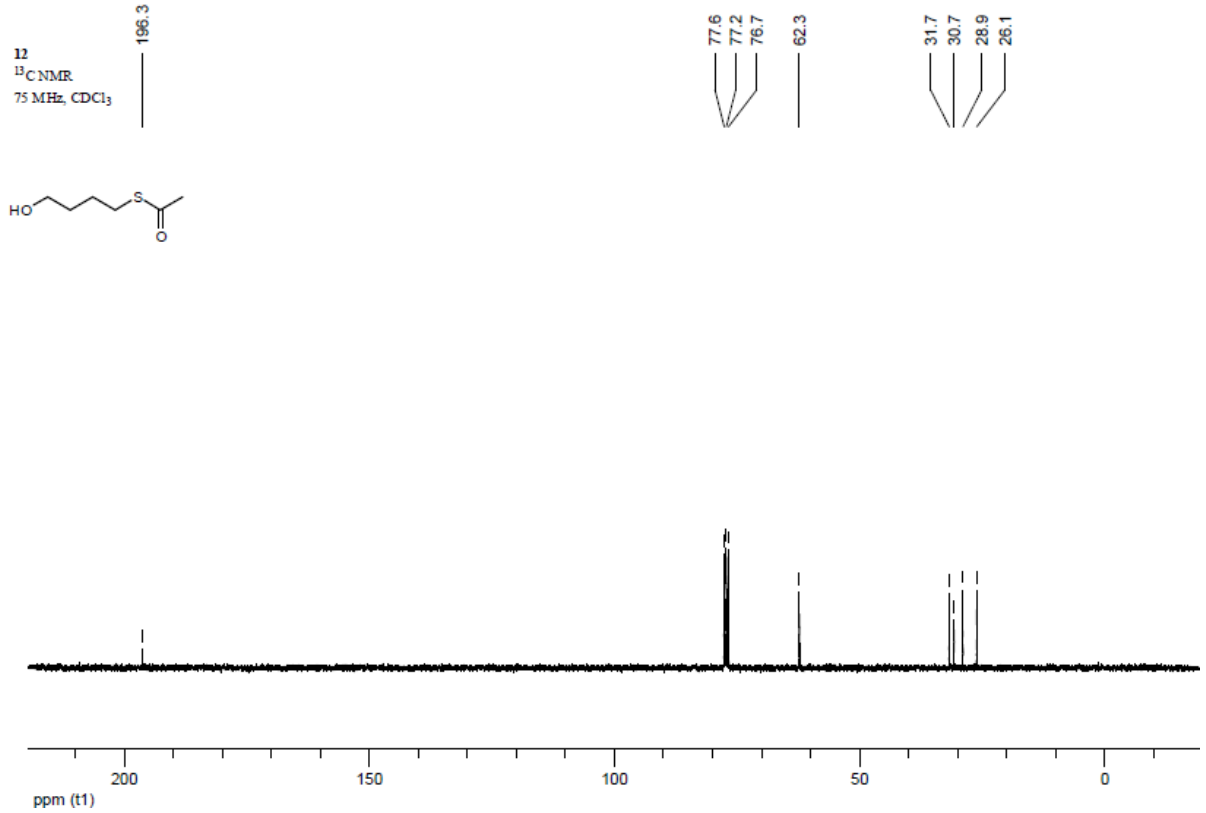
11
^{13}C NMR
75 MHz, $CDCl_3$

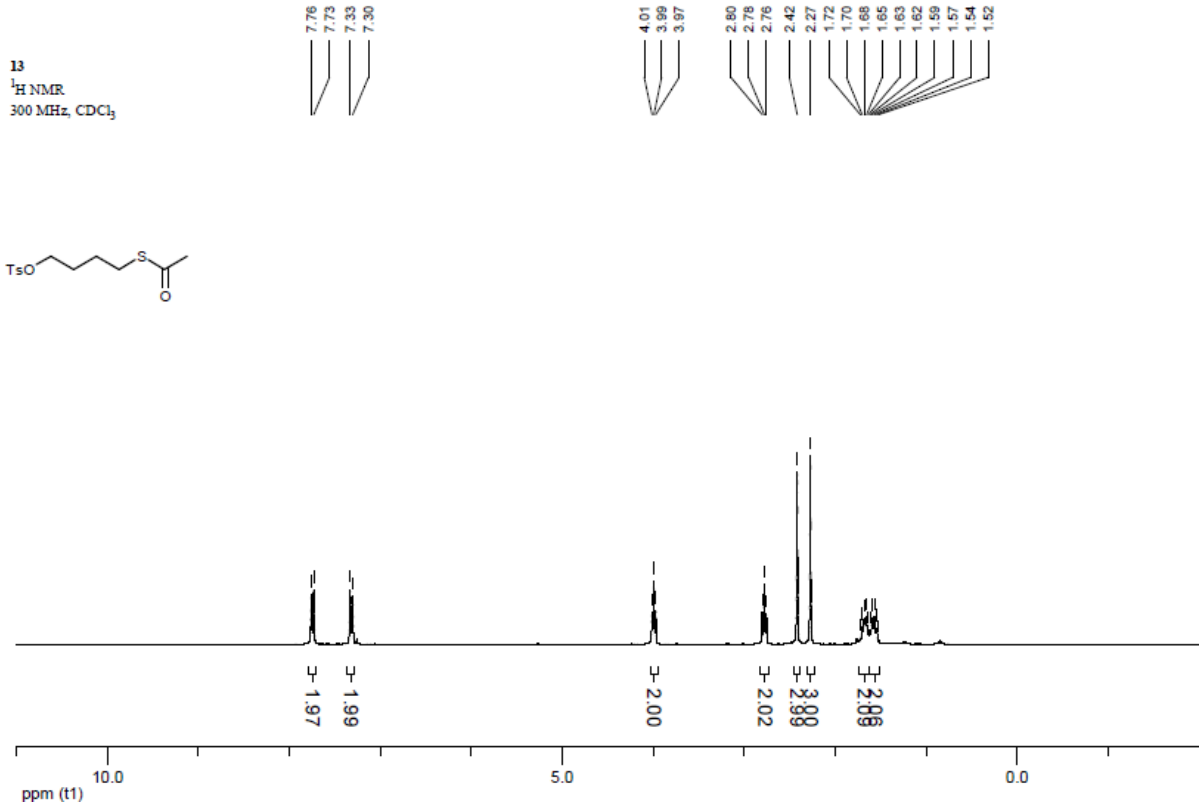


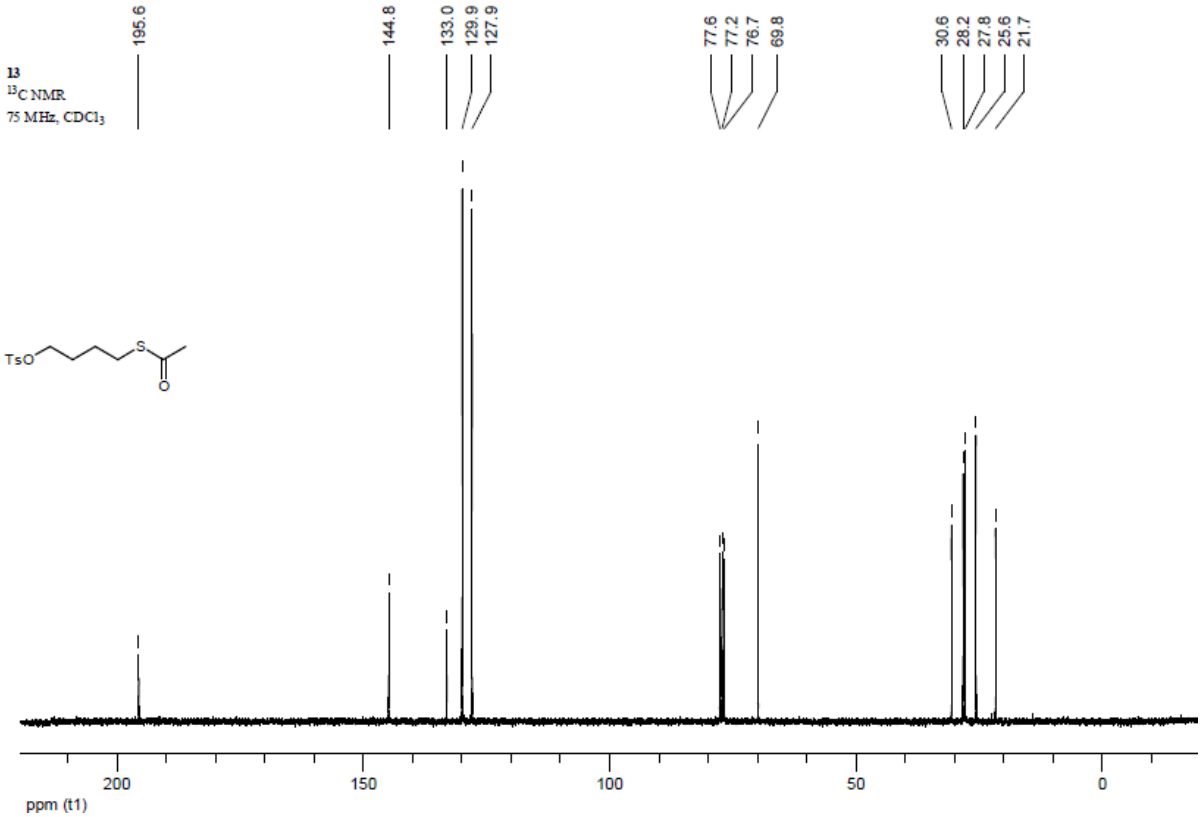

P3
1H NMR
300 MHz, CDCl3
MeO
MeO
N
SH
Ph
1.92
1.03
1.03
1.00
3.06
2.96
1.03
1.14
6.54
1.22
1.15
10.0
5.0
0.0
ppm (t1)

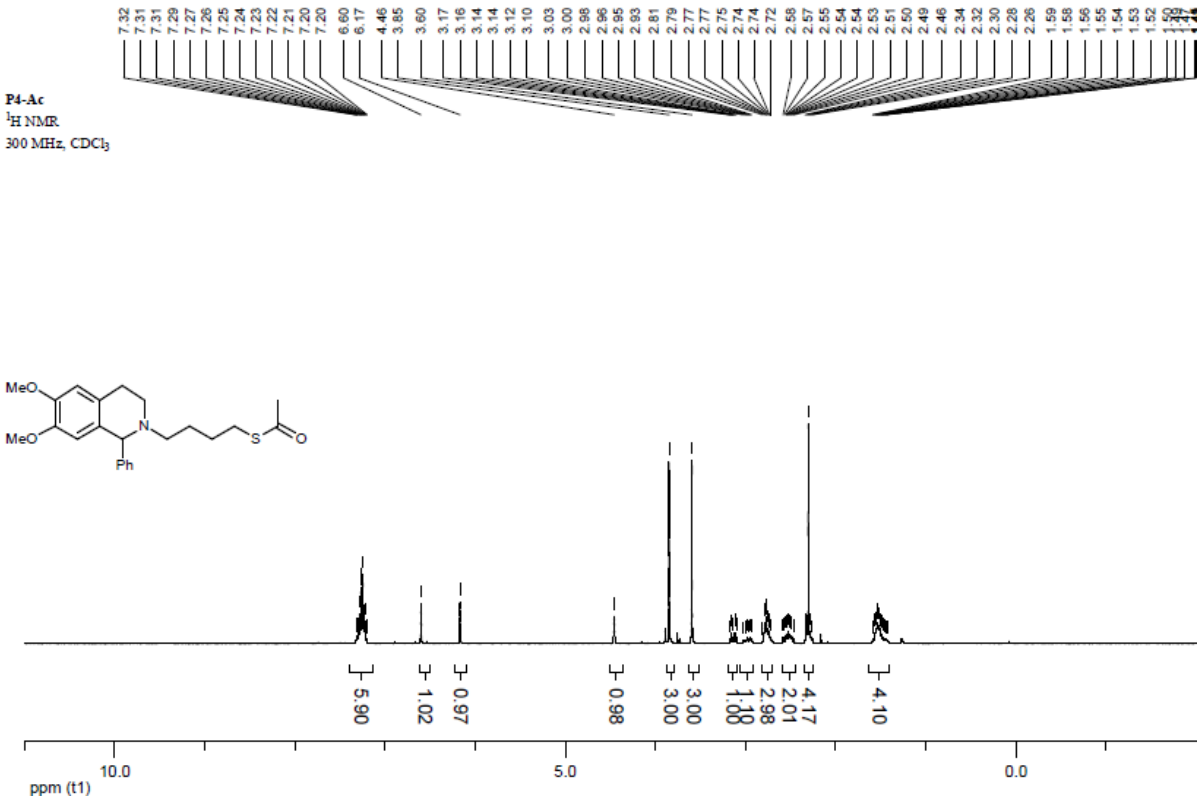

P3
13C NMR
75 MHz, CDCl3
147.5
147.2
144.5
130.3
129.8
128.4
127.4
127.0
111.9
110.9
68.6
55.9
52.4
47.2
31.4
28.5
22.5
MeO
MeO
N
SH
Ph
200
150
100
50
0
ppm (t1)

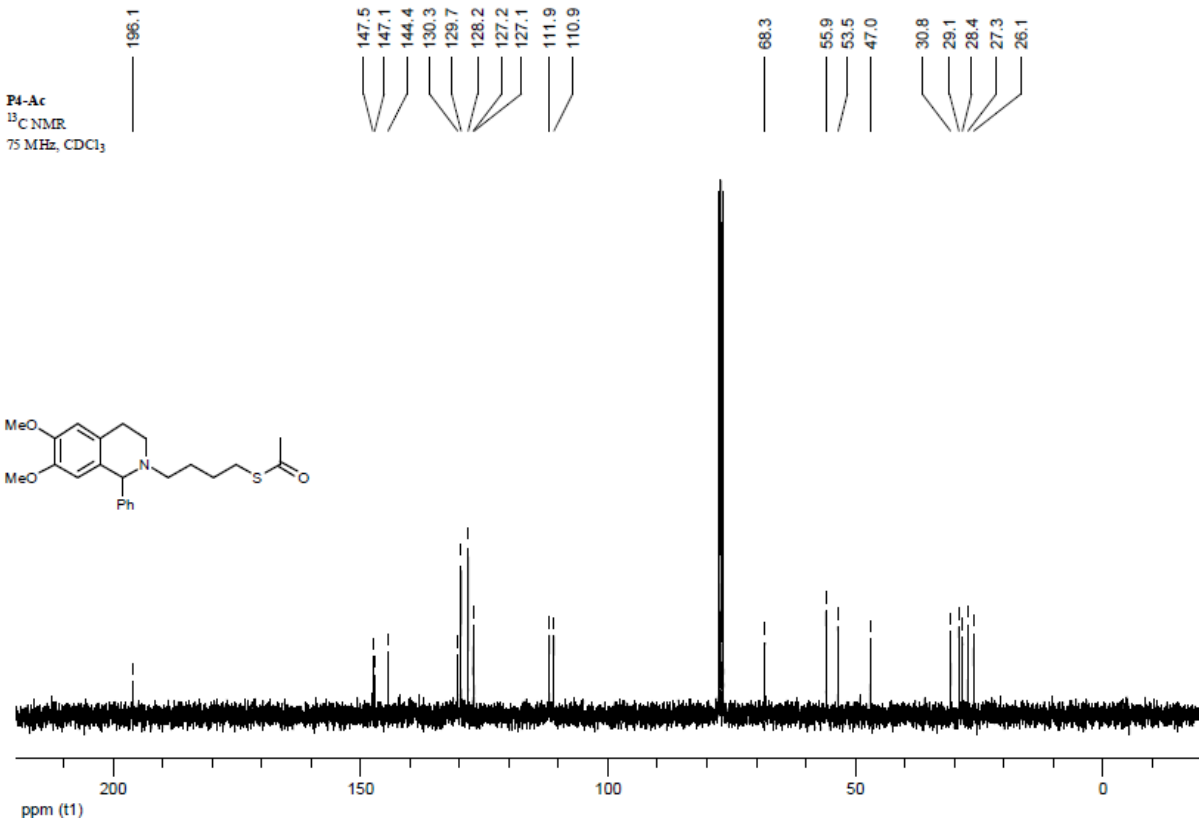

12
^{1}H NMR
300 MHz, CDCl$_3$
7.26
2.93
2.91
2.88
2.33
1.68
1.67
1.66
1.66
1.65
1.64
1.64
1.63
1.57
HO
S
O
2.05
2.00
3.05
4.05
*
10.0
5.0
0.0
ppm (t1)

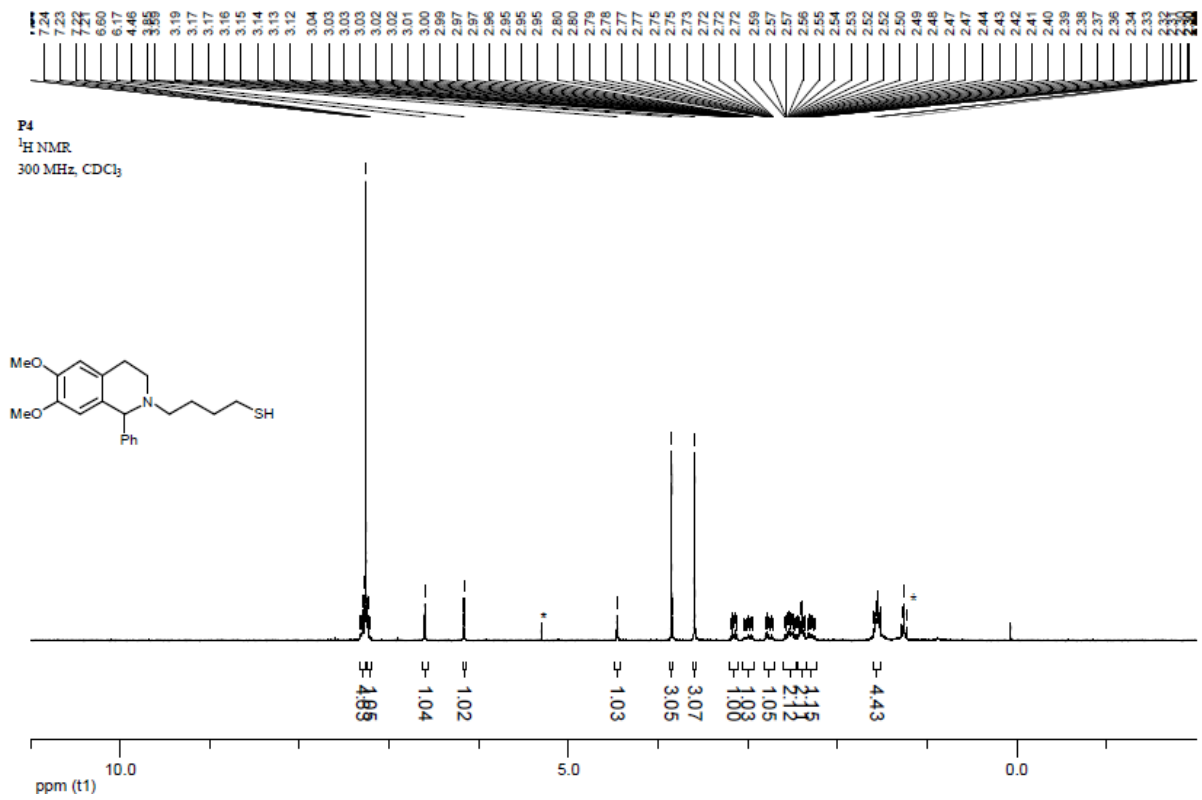

12
^{13}C NMR
75 MHz, CDCl$_3$
196.3
77.6
77.2
76.7
62.3
31.7
30.7
28.9
26.1
HO
S
O
200
150
100
50
0
ppm (t1)

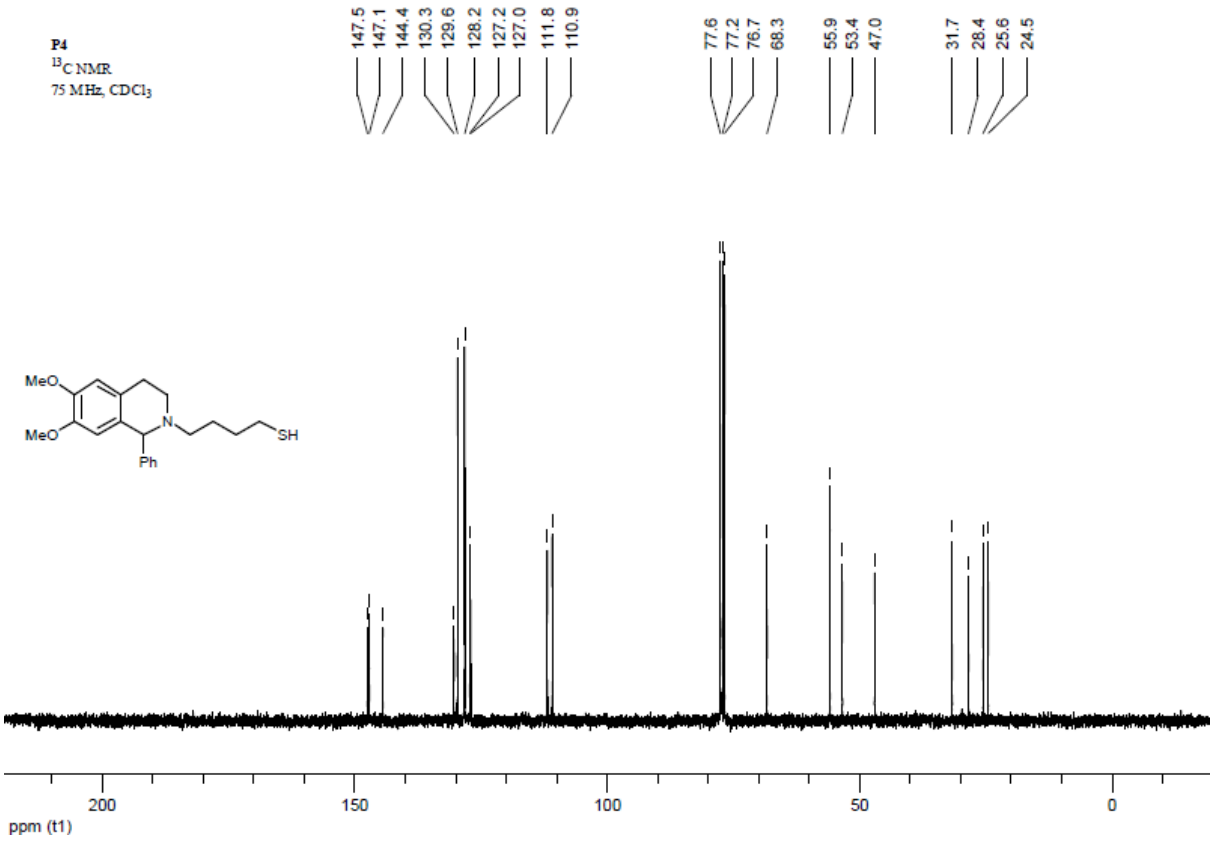

13
1H NMR
300 MHz, CDCl3
7.76
7.73
7.33
7.30
4.01
3.99
3.97
2.80
2.78
2.76
2.42
2.27
1.72
1.70
1.68
1.65
1.63
1.62
1.59
1.57
1.54
1.52
TsO
S
O
1.97
1.99
2.00
2.02
3.00
2.08
10.0
5.0
0.0
ppm (t1)

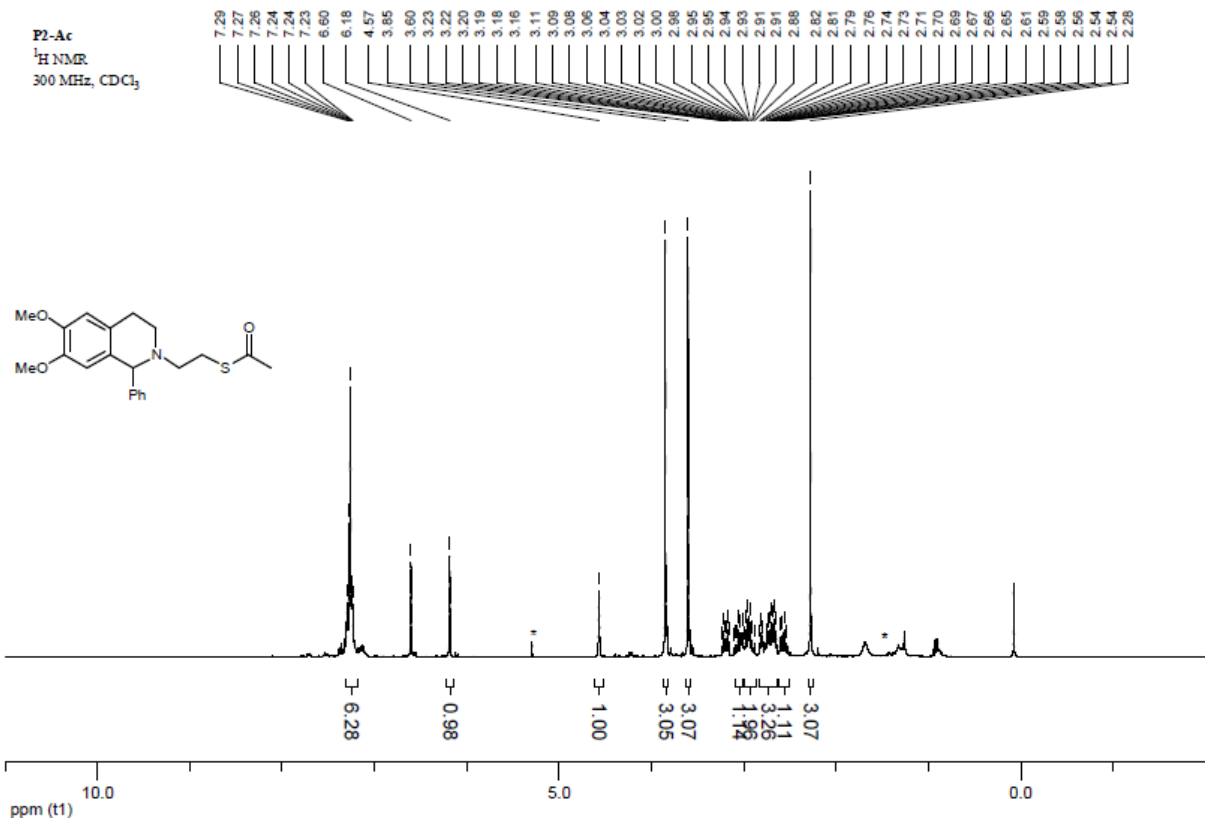

13
13C NMR
75 MHz, CDCl3
195.6
144.8
133.0
129.9
127.9
77.6
77.2
76.7
69.8
30.6
28.2
27.8
25.6
21.7
TsO
S
O
200
150
100
50
0
ppm (t1)

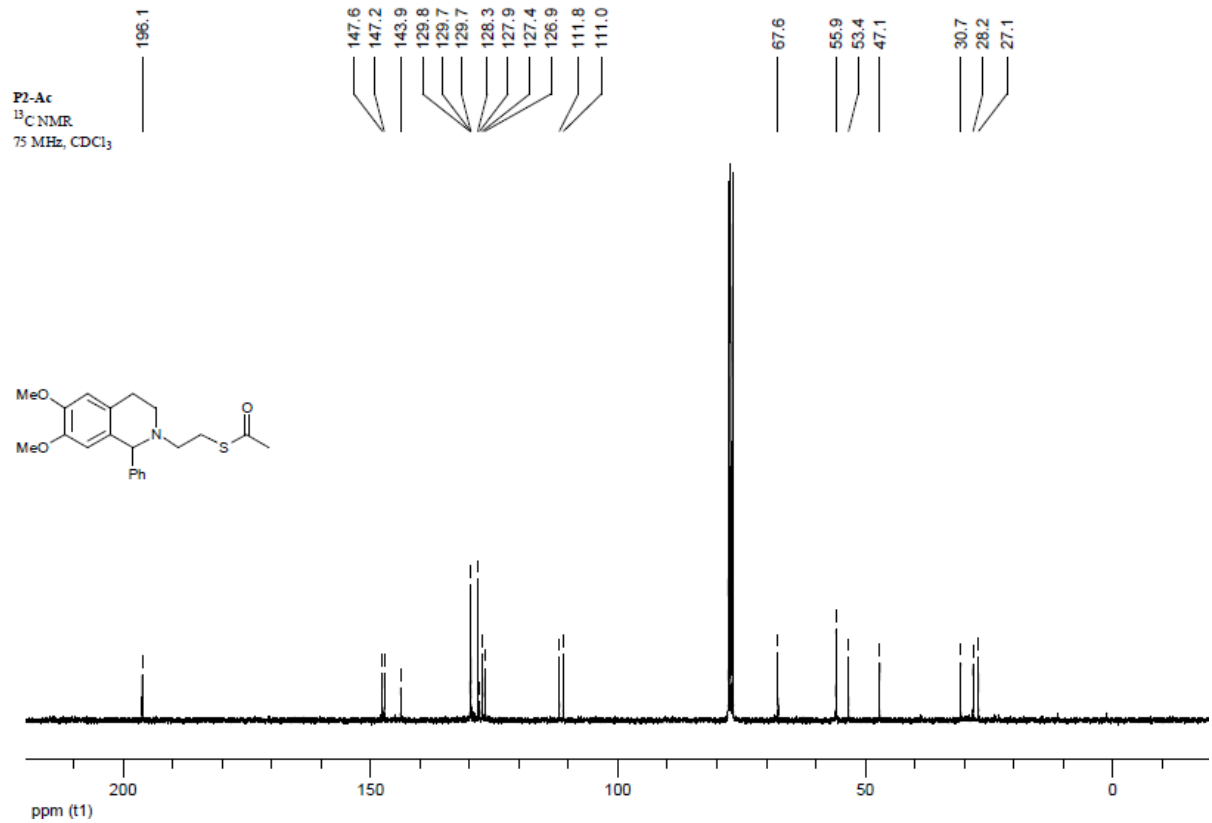

P4-Ac
1H NMR
300 MHz, CDCl3
MeO
MeO
N
S
O
Ph
5.90
1.02
0.97
0.98
3.00
3.00
1.00
2.98
2.01
4.17
4.10
10.0
5.0
0.0
ppm (t1)

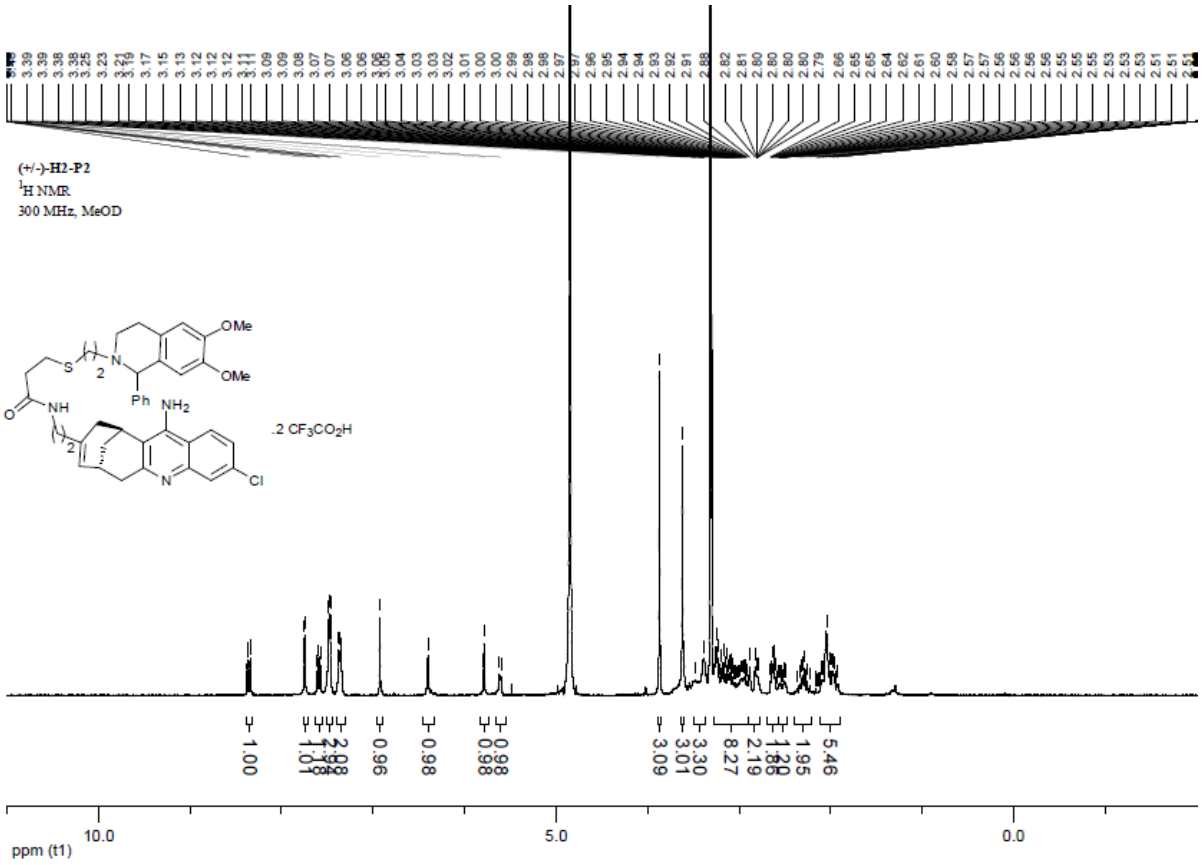

P4-Ac
13C NMR
75 MHz, CDCl3
196.1
147.5
147.1
144.4
130.3
129.7
128.2
127.2
127.1
111.9
110.9
68.3
55.9
53.5
47.0
30.8
29.1
28.4
27.3
26.1
MeO
MeO
N
S
O
Ph
200
150
100
50
0
ppm (t1)

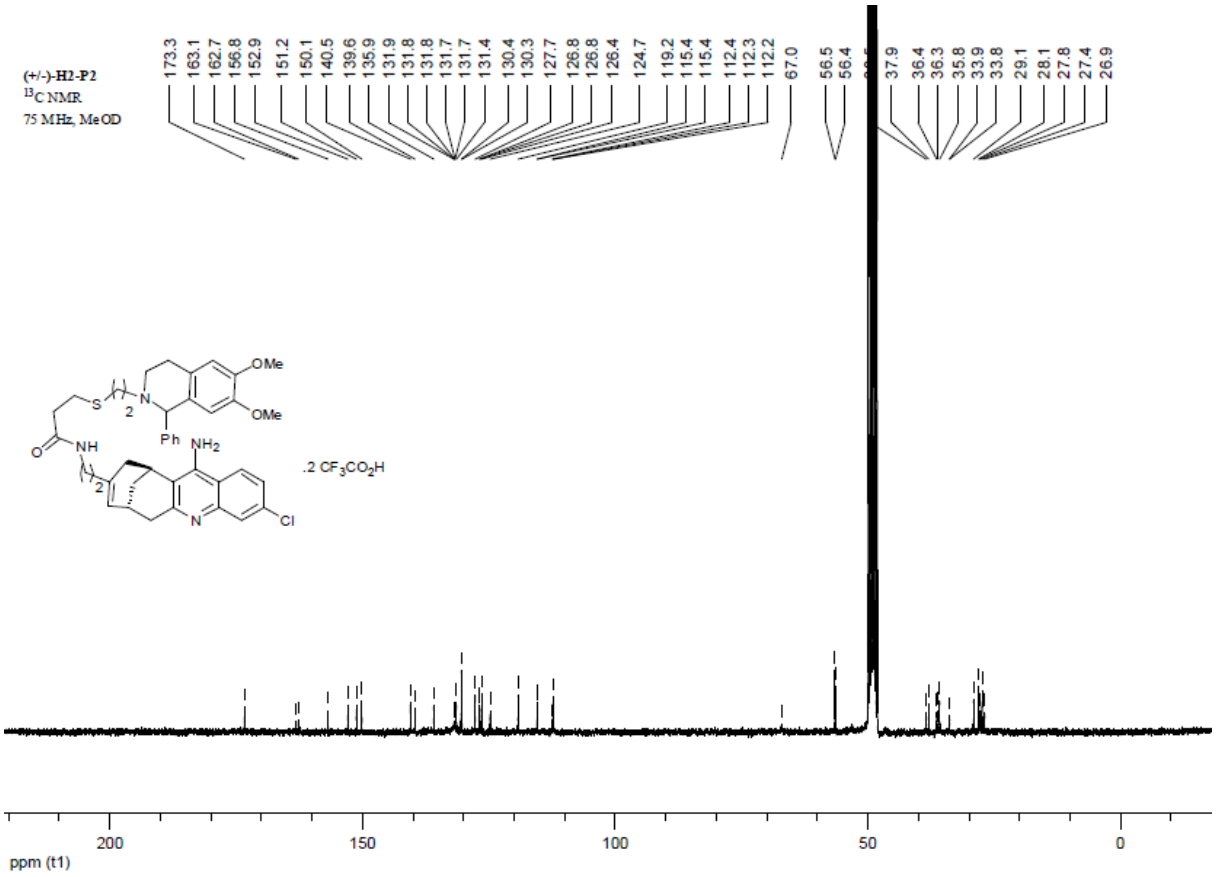

P4
1H NMR
300 MHz, CDCl3
MeO
MeO
N
SH
Ph
1.00
1.04
1.02
1.03
3.05
3.07
1.00
1.05
2.12
1.15
4.43
10.0
5.0
0.0
ppm (t1)

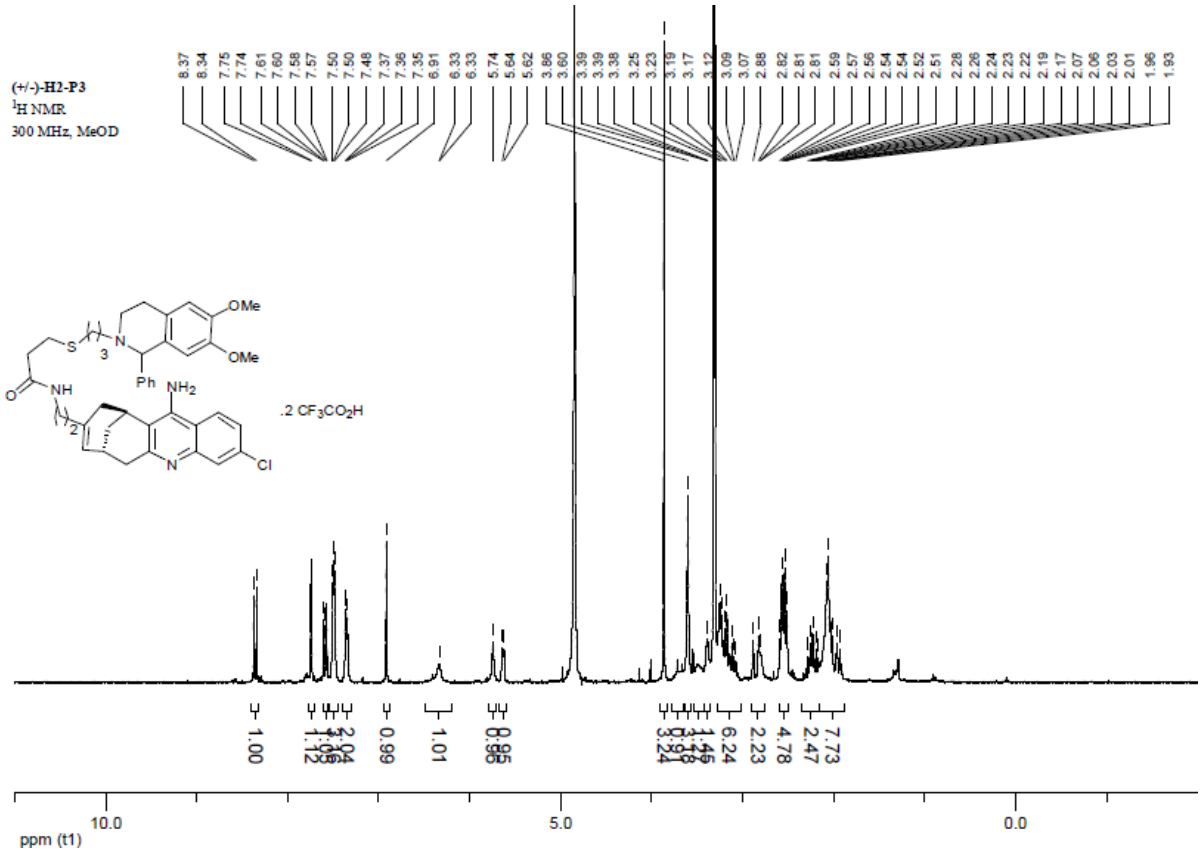

P4
13C NMR
75 MHz, CDCl3
147.5
147.1
144.4
130.3
129.6
128.2
127.2
127.0
111.8
110.9
77.6
77.2
76.7
68.3
55.9
53.4
47.0
31.7
28.4
25.6
24.5
MeO
MeO
N
SH
Ph
200
150
100
50
0
ppm (t1)

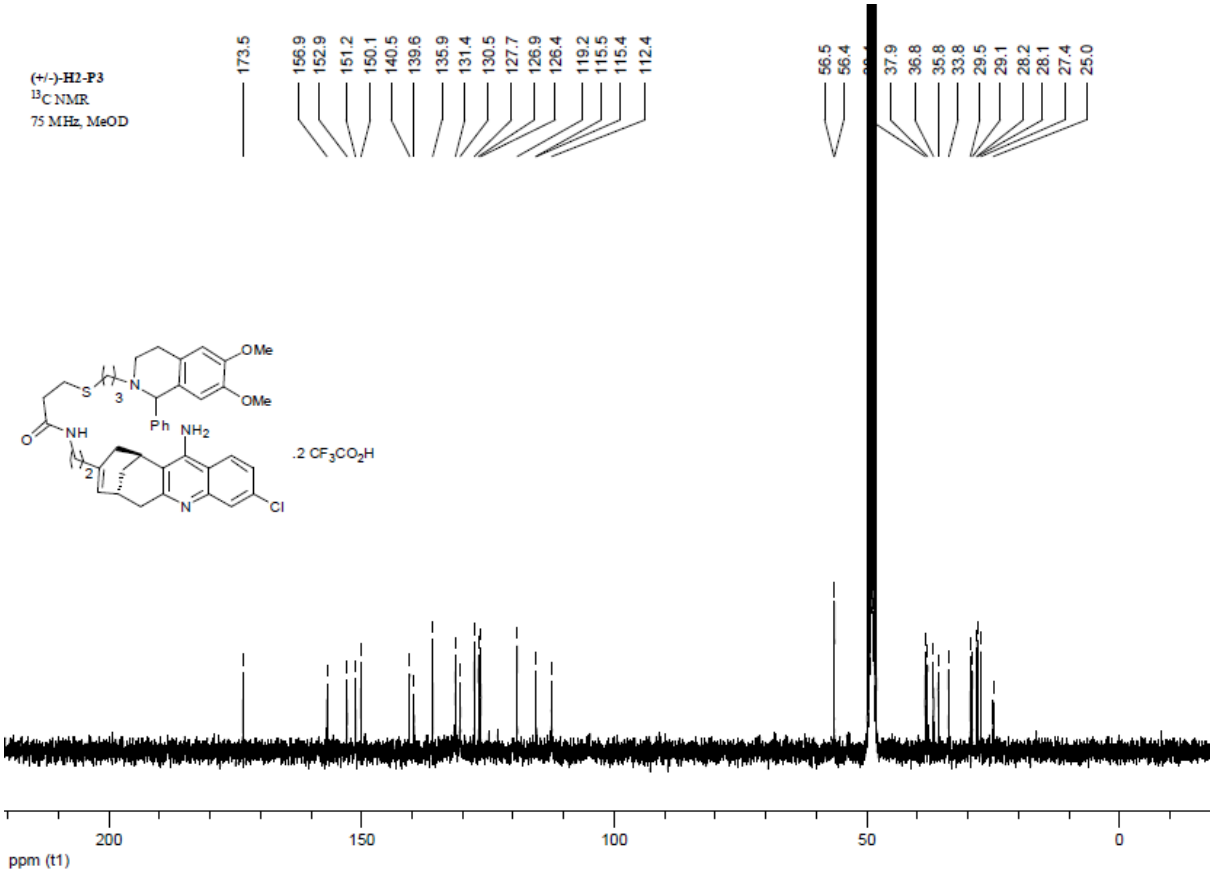

P2-Ac
1H NMR
300 MHz, CDCl3
MeO
MeO
N
S
O
Ph
6.28
0.98
1.00
3.05
3.07
1.11
3.26
3.07
10.0
5.0
0.0
ppm (t1)

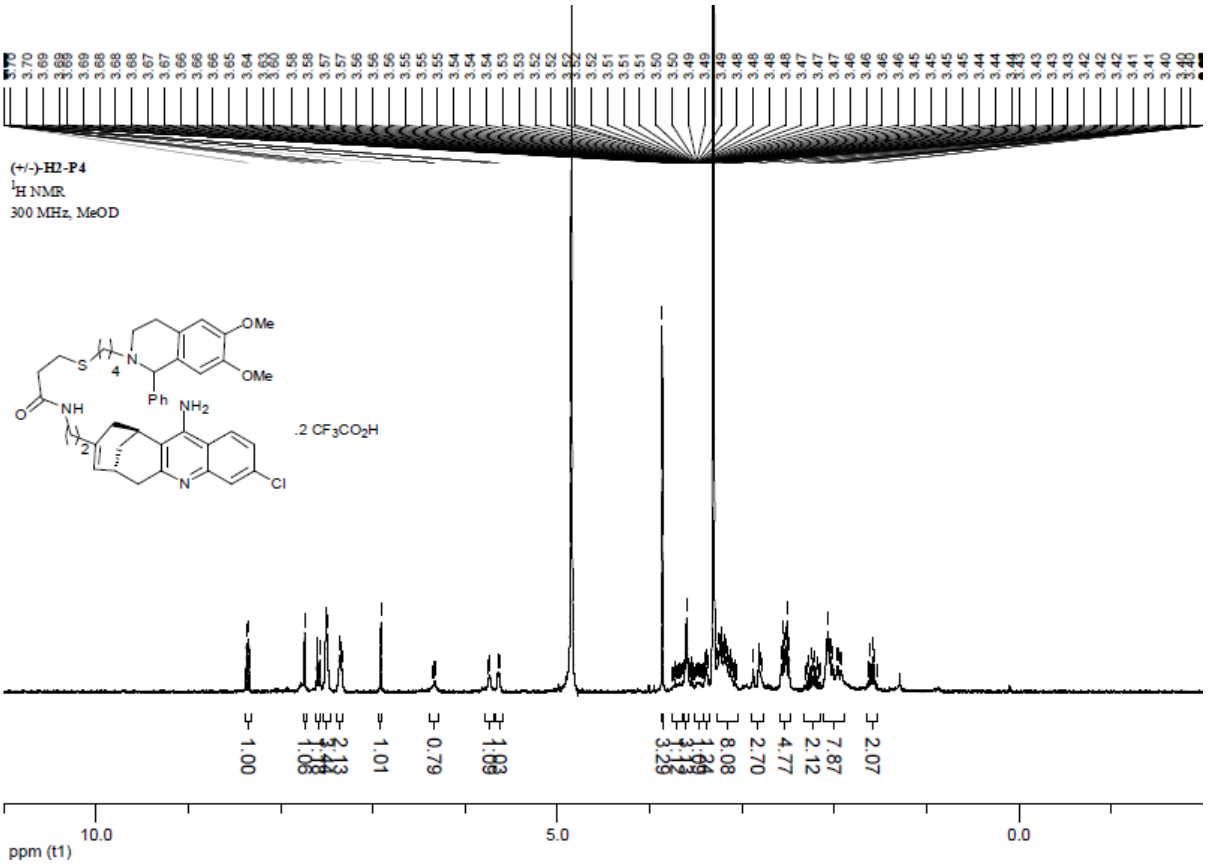

P2-Ac
13C NMR
75 MHz, CDCl3
196.1
147.6
147.2
143.9
129.8
129.7
129.7
128.3
127.9
127.4
126.9
111.8
111.0
67.6
55.9
53.4
47.1
30.7
28.2
27.1
MeO
MeO
N
S
O
Ph
200
150
100
50
0
ppm (t1)

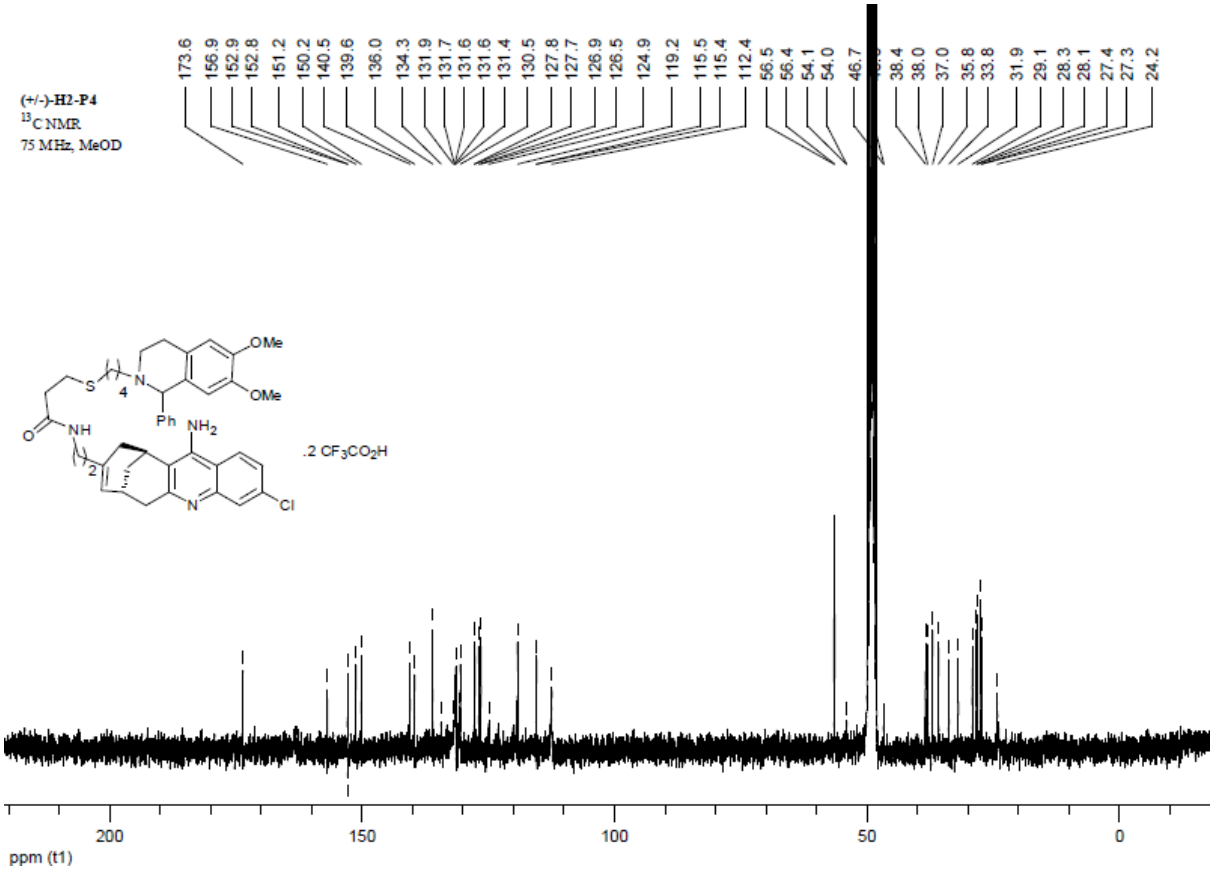

(+/-)-H2-P2
1H NMR
300 MHz, MeOD
OMe
OMe
Ph
NH2
NH
.2 CF3CO2H
Cl
1.00
0.96
0.98
0.98
0.98
3.09
3.01
8.27
2.19
1.95
5.46
10.0
5.0
0.0
ppm (t1)

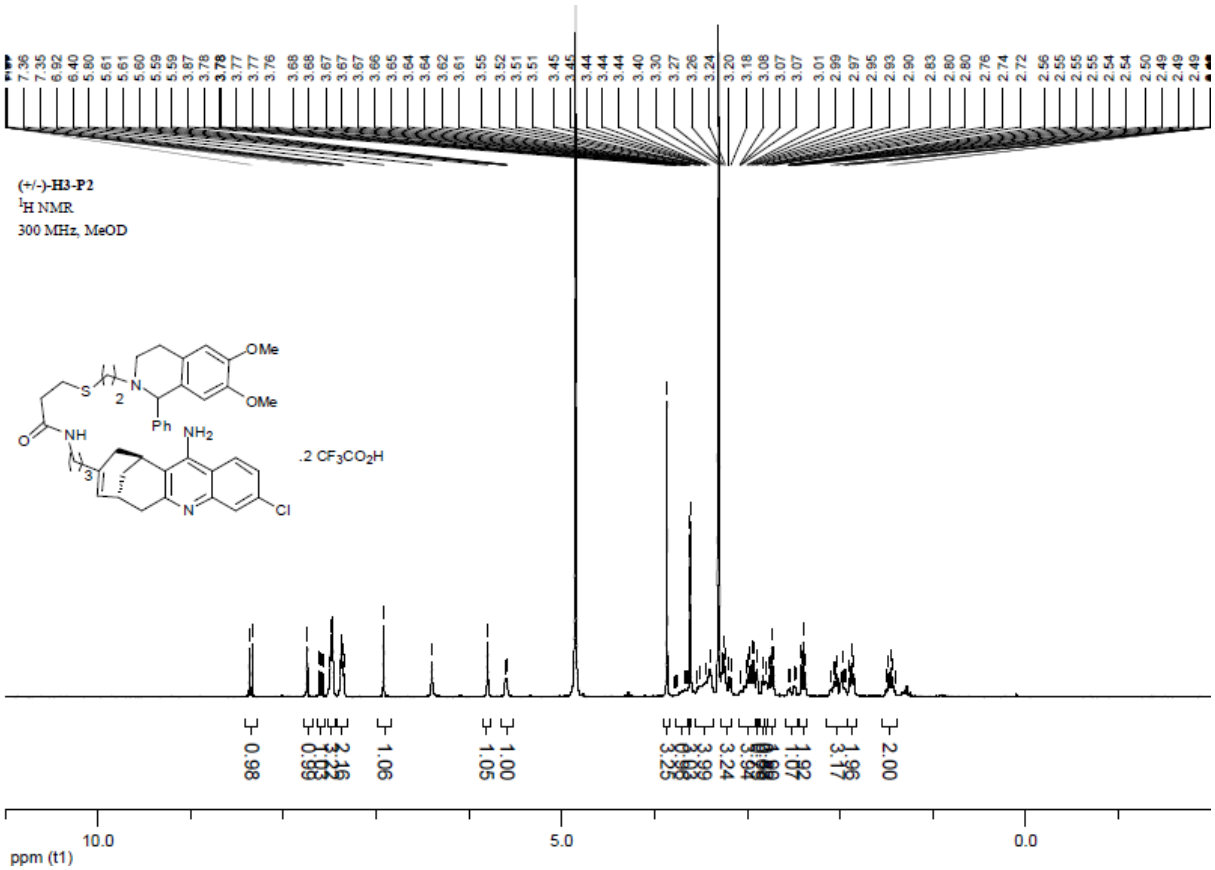

(+/-)-H2-P2
13C NMR
75 MHz, MeOD
173.3
163.1
162.7
156.8
152.9
151.2
150.1
140.5
139.6
135.9
131.9
131.8
131.8
131.7
131.7
131.4
130.4
130.3
127.7
126.8
126.8
126.4
124.7
119.2
115.4
115.4
112.4
112.3
112.2
67.0
56.5
56.4
37.9
36.4
36.3
35.8
33.9
33.8
29.1
28.1
27.8
27.4
26.9
OMe
OMe
Ph
NH2
NH
.2 CF3CO2H
Cl
200
150
100
50
0
ppm (t1)

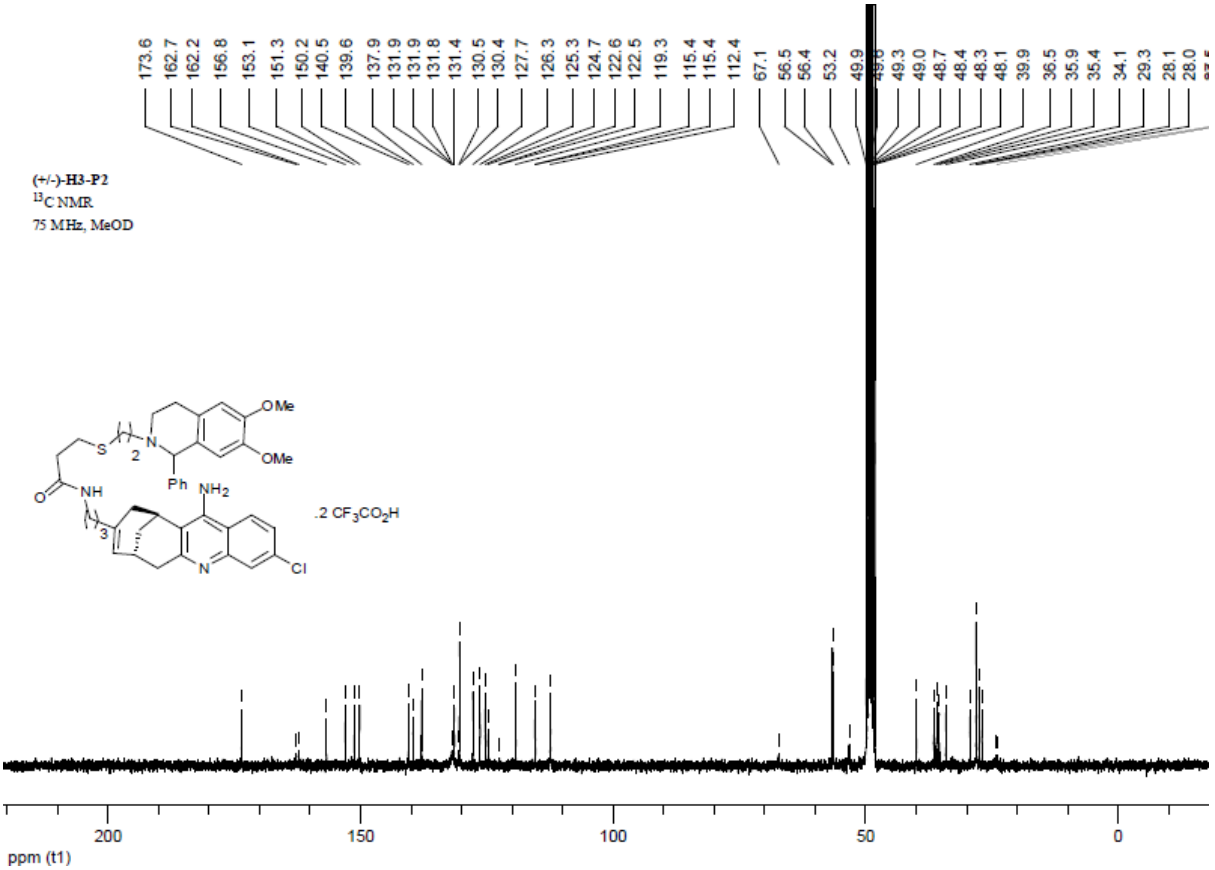

(+/-)-H2-P3
1H NMR
300 MHz, MeOD
8.37 8.34 7.75 7.74 7.61 7.60 7.58 7.57 7.50 7.50 7.48 7.37 7.36 7.35 6.91 6.33 6.33 5.74 5.64 5.62 3.86 3.60 3.39 3.39 3.38 3.25 3.23 3.19 3.17 3.12 3.09 3.07 2.88 2.82 2.81 2.81 2.59 2.57 2.56 2.54 2.54 2.52 2.51 2.28 2.26 2.24 2.23 2.22 2.19 2.17 2.07 2.06 2.03 2.01 1.96 1.93
OMe
OMe
Ph
NH2
NH
.2 CF3CO2H
Cl
1.00
0.99
1.01
9.24
2.23
4.78
2.47
2.73
10.0
5.0
0.0
ppm (t1)

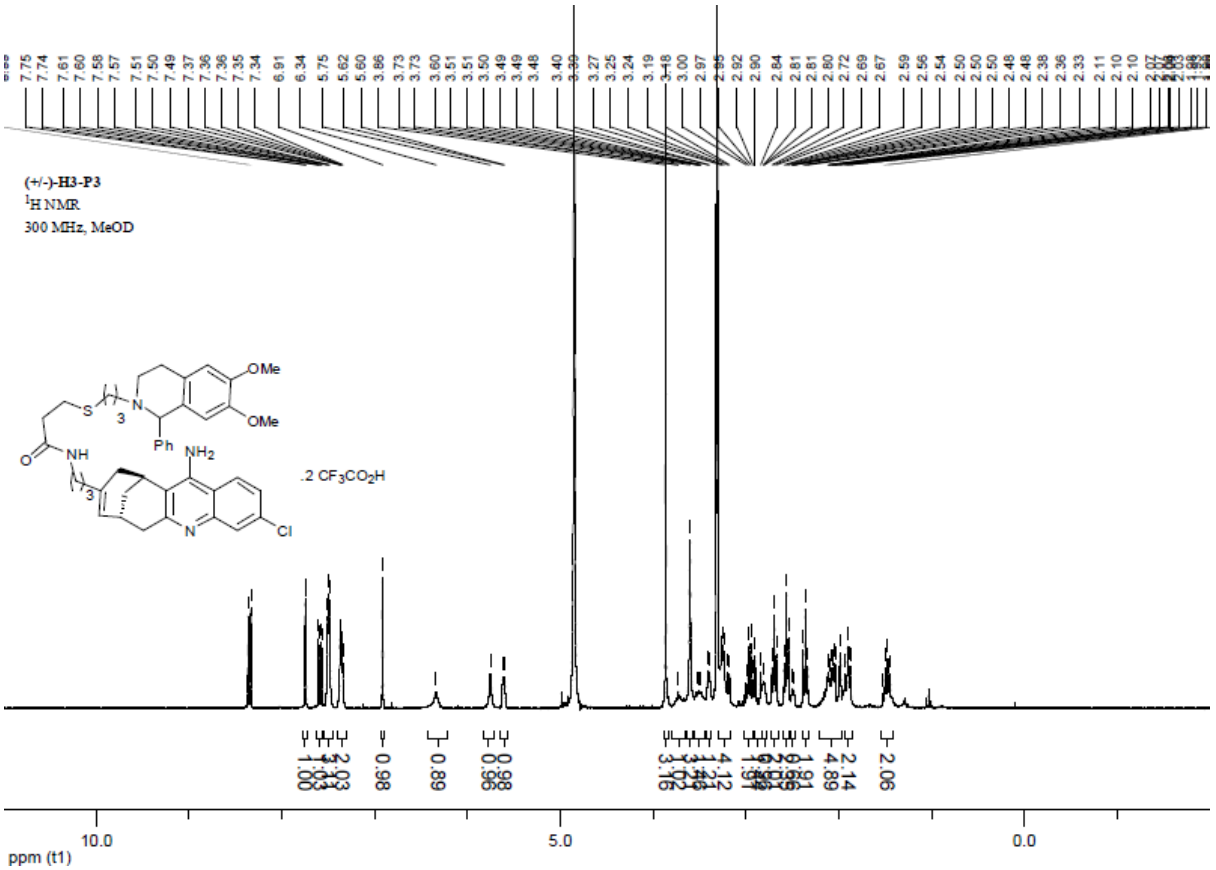

(+/-)-H2-P3
13C NMR
75 MHz, MeOD
173.5 156.9 152.9 151.2 150.1 140.5 139.6 135.9 131.4 130.5 127.7 126.9 126.4 119.2 115.5 115.4 112.4
56.5 56.4 37.9 36.8 35.8 33.8 29.5 29.1 28.2 28.1 27.4 25.0
OMe
OMe
Ph
NH2
NH
.2 CF3CO2H
Cl
200
150
100
50
0
ppm (t1)

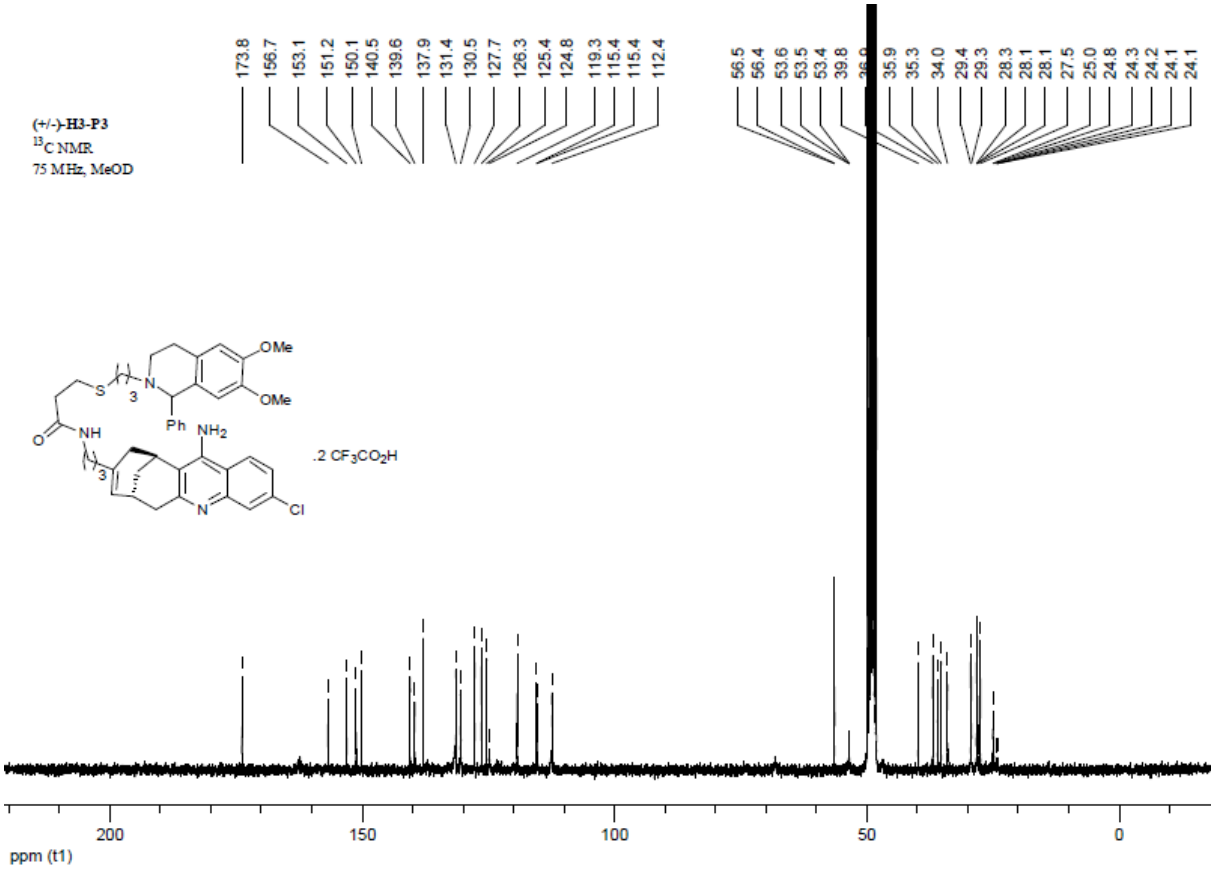

(+/-)-H2-P4
1H NMR
300 MHz, MeOD
OMe
OMe
Ph
NH2
NH
.2 CF3CO2H
Cl
1.00
1.01
0.79
2.70
4.77
2.12
7.87
2.07
8.08
10.0
5.0
0.0
ppm (t1)

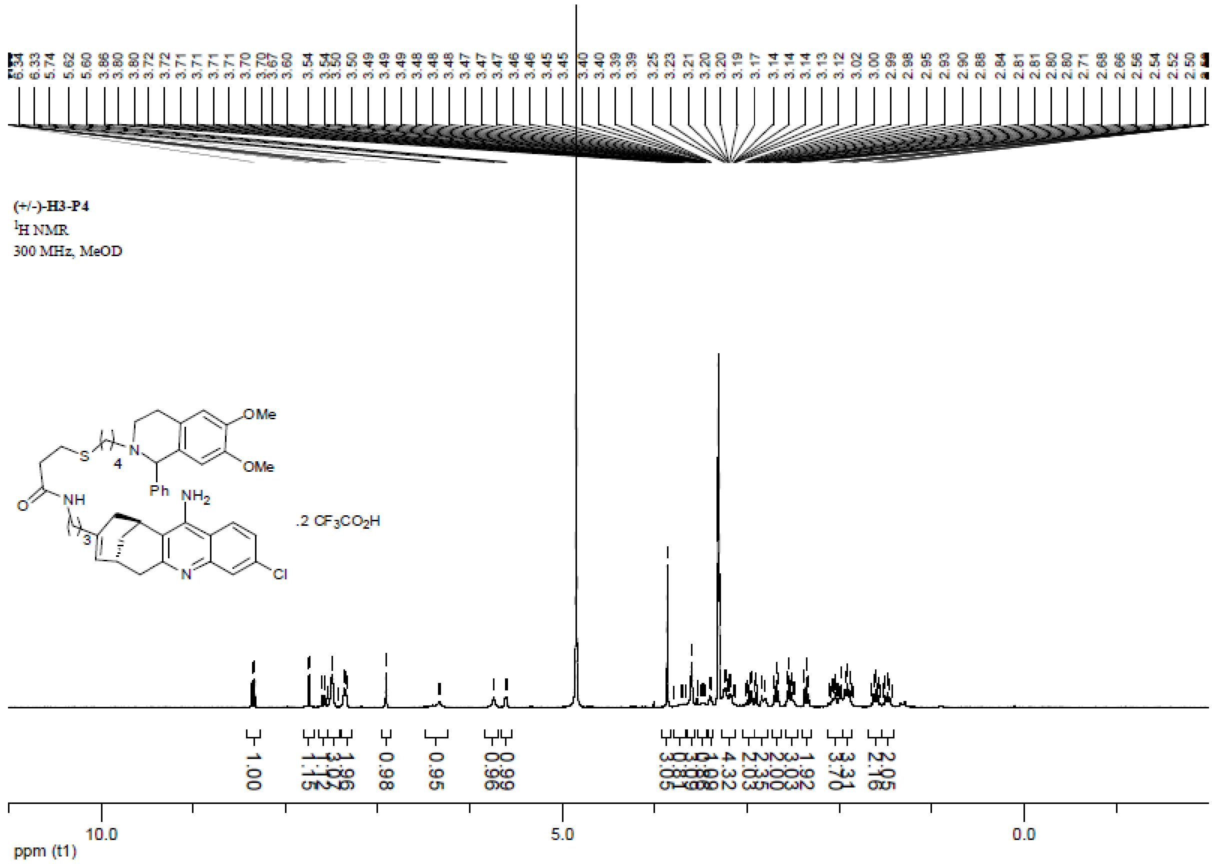

(+/-)-H2-P4
13C NMR
75 MHz, MeOD
173.6
156.9
152.9
152.8
151.2
150.2
140.5
139.6
136.0
134.3
131.9
131.7
131.6
131.6
131.4
130.5
127.8
127.7
126.9
126.5
124.9
119.2
115.5
115.4
112.4
56.5
56.4
54.1
54.0
46.7
38.4
38.0
37.0
35.8
33.8
31.9
29.1
28.3
28.1
27.4
27.3
24.2
OMe
OMe
Ph
NH2
NH
.2 CF3CO2H
Cl
200
150
100
50
0
ppm (t1)

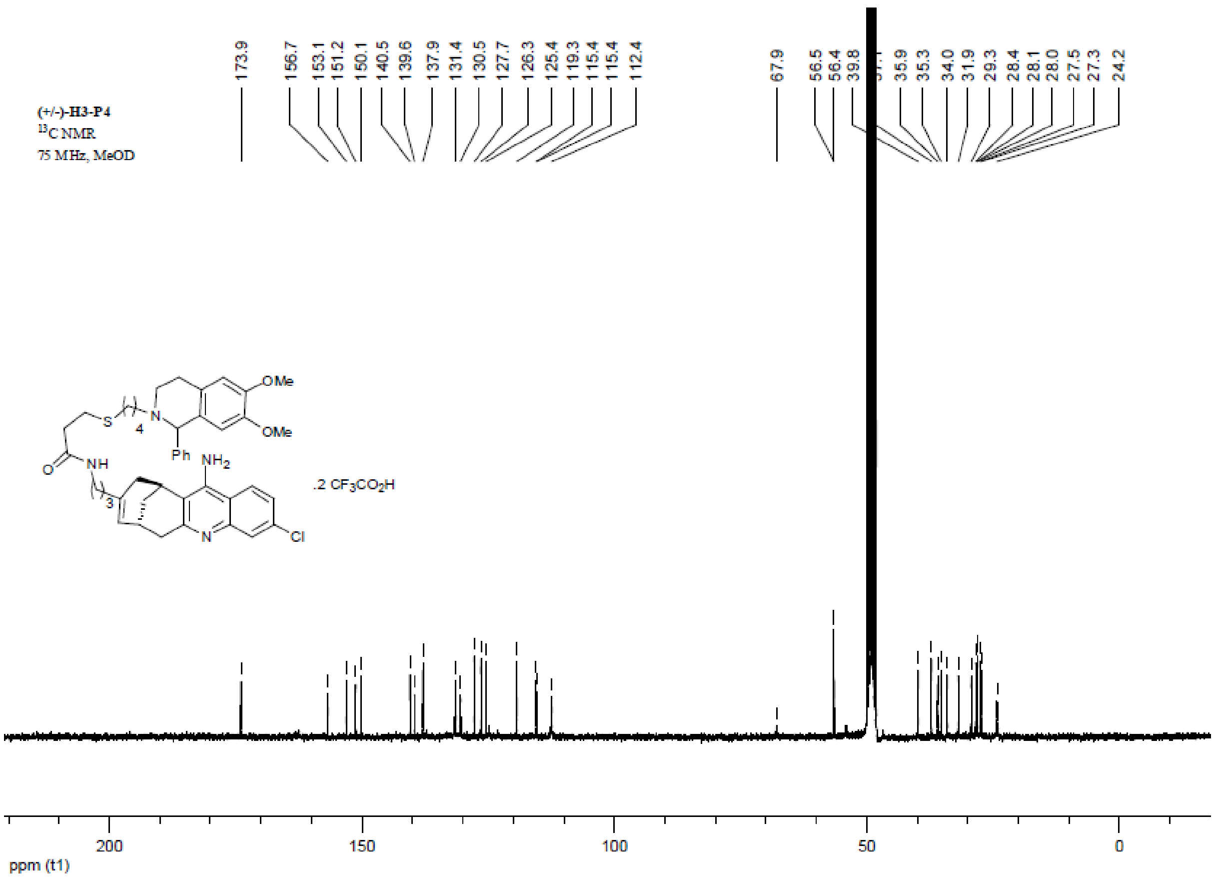

(+/-)-H3-P2
1H NMR
300 MHz, MeOD
OMe
OMe
Ph
NH2
NH
.2 CF3CO2H
Cl
0.98
0.99
1.06
1.00
1.05
3.23
1.07
3.17
1.96
2.00
10.0
5.0
0.0
ppm (t1)

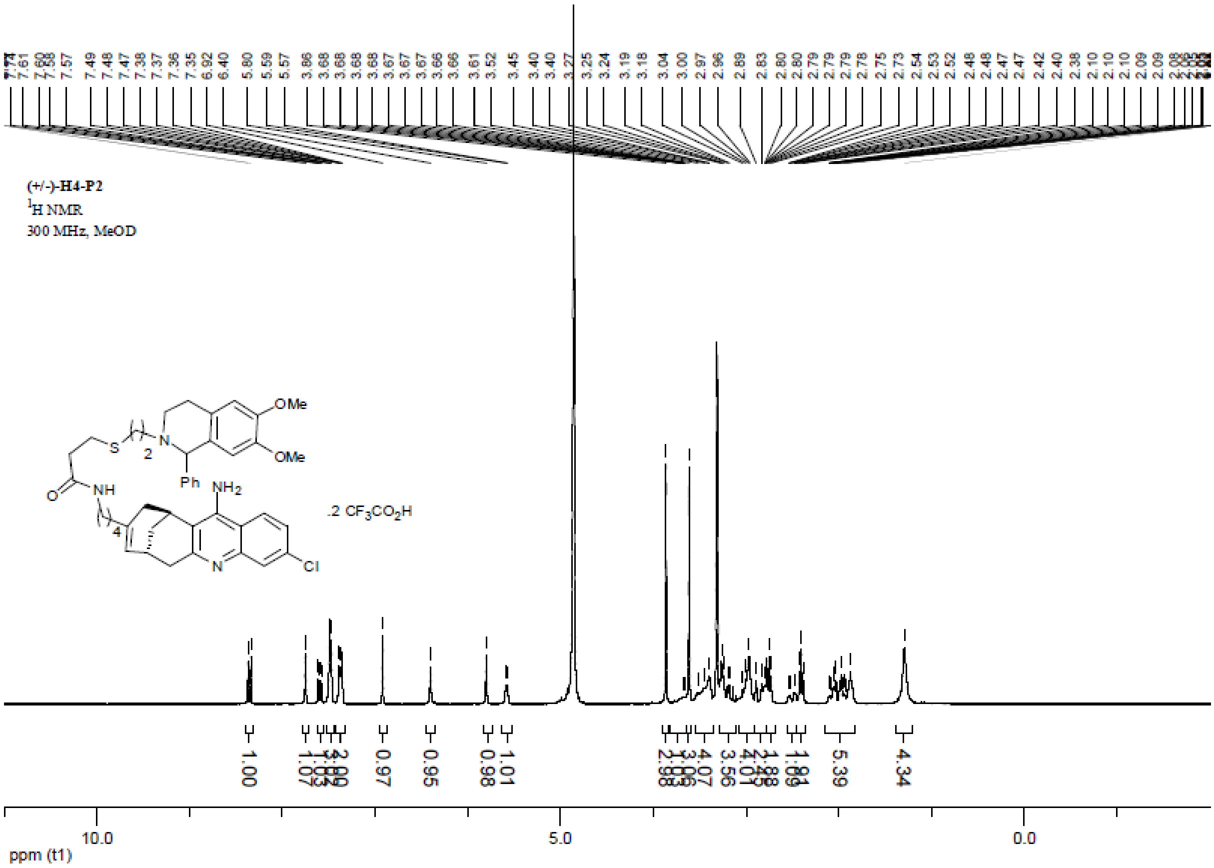

(+/-)-H3-P2
13C NMR
75 MHz, MeOD
OMe
OMe
Ph
NH2
NH
.2 CF3CO2H
Cl
200
150
100
50
0
ppm (t1)

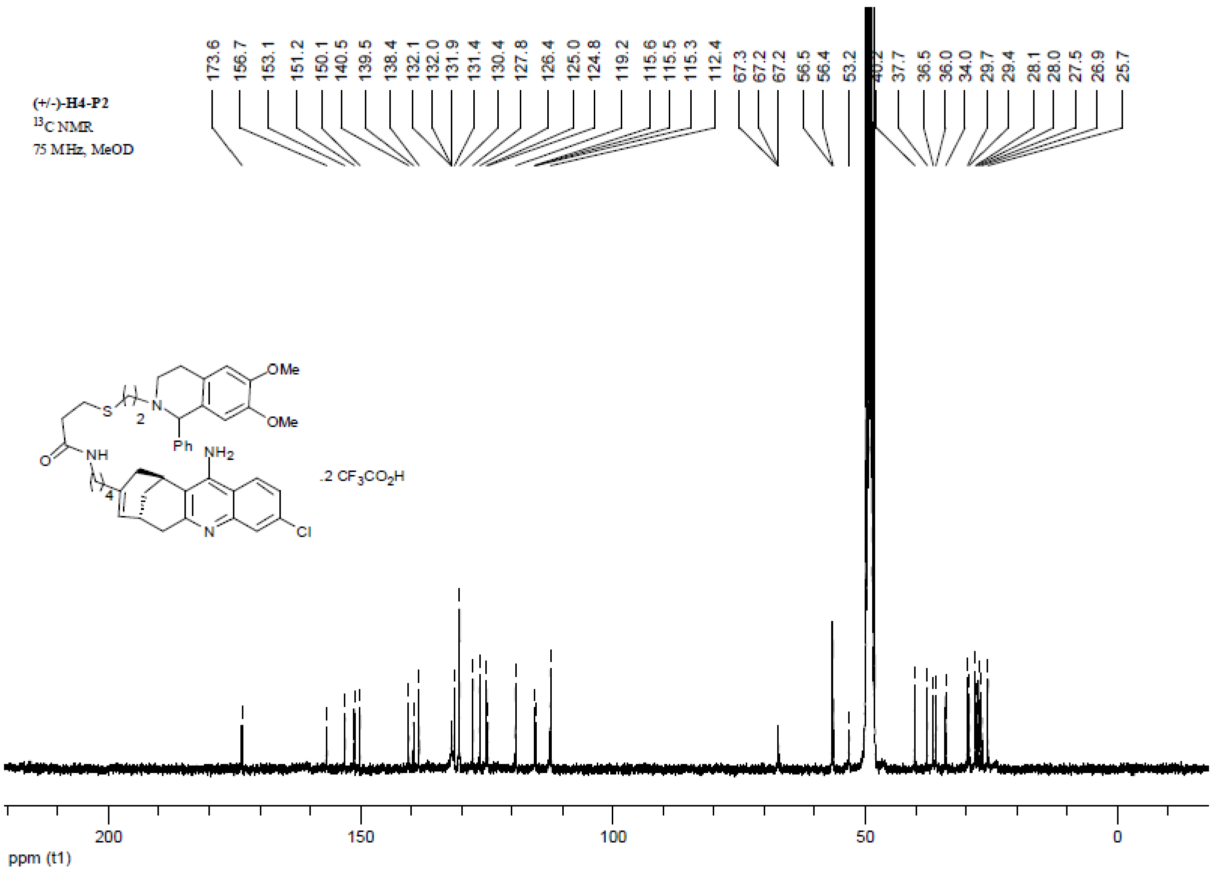

(+/-)-H3-P3
1H NMR
300 MHz, MeOD
.2 CF3CO2H
ppm (t1)

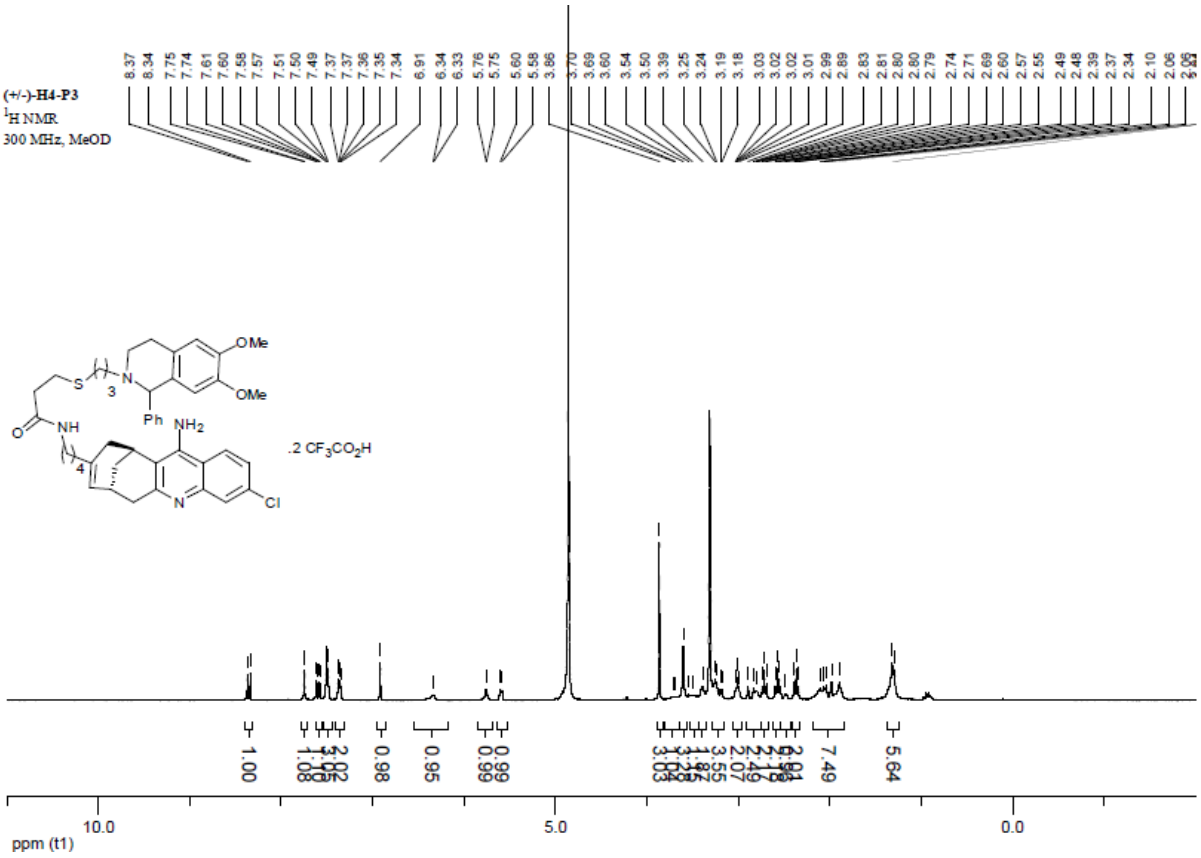

(+/-)-H3-P3
13C NMR
75 MHz, MeOD
.2 CF3CO2H
ppm (t1)

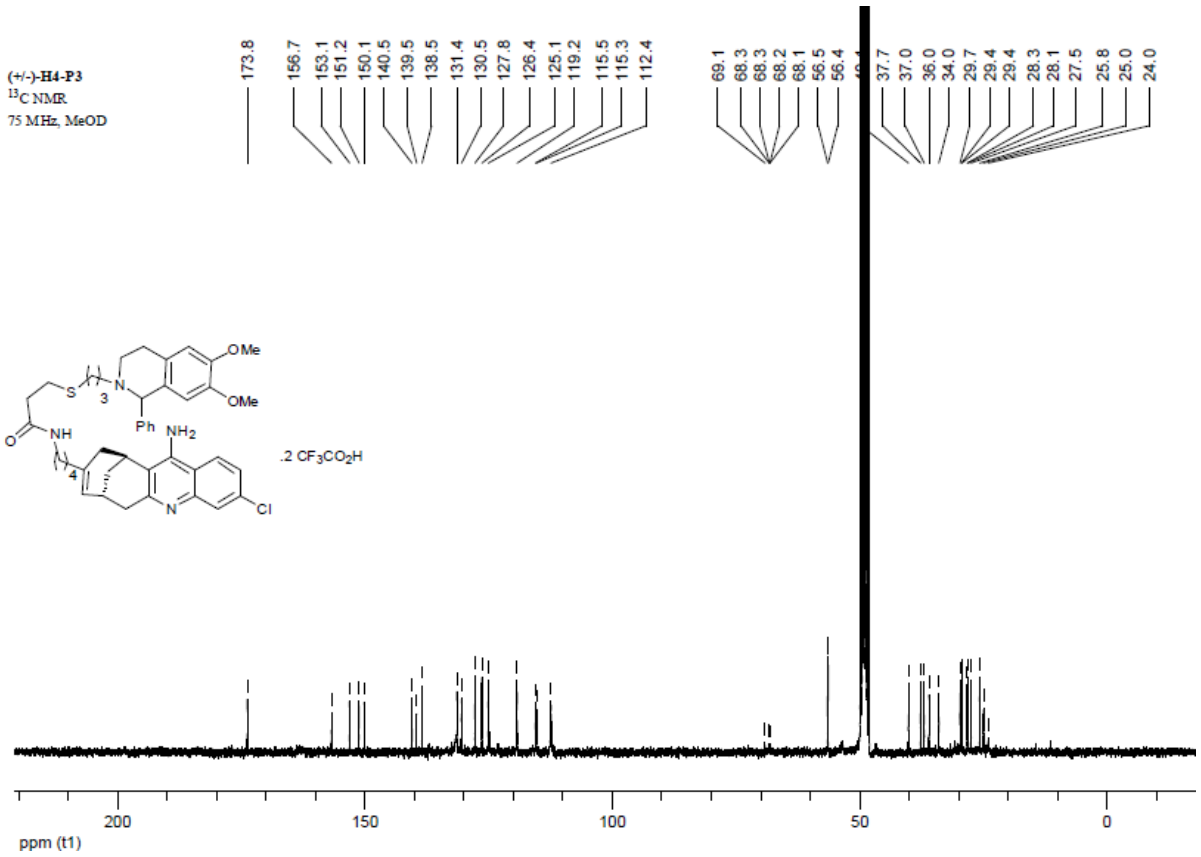

(+/-)-H3-P4
1H NMR
300 MHz, MeOD
OMe
OMe
S
N
4
Ph
NH2
O
NH
3
N
Cl
.2 CF3CO2H
1.00
0.98
0.95
10.0
5.0
0.0
ppm (t1)

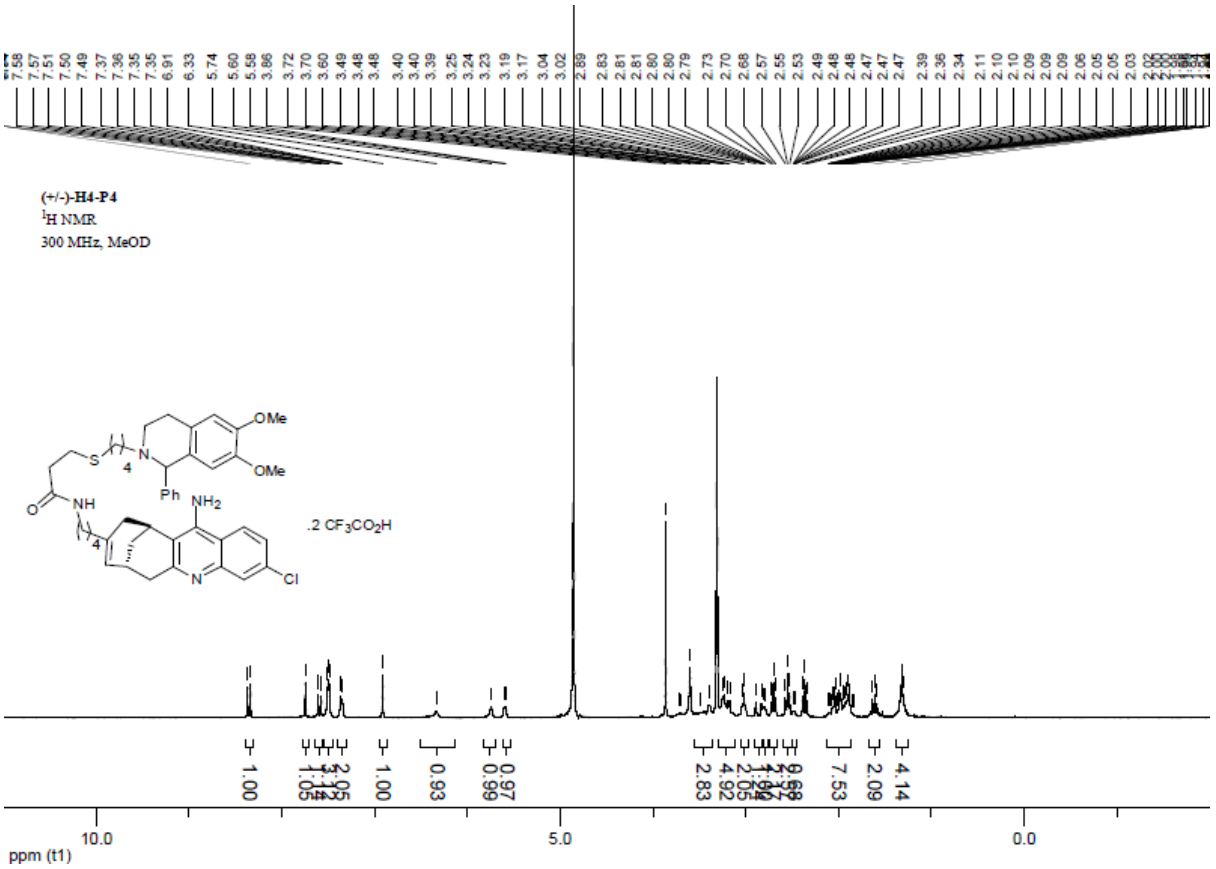

173.9
156.7
153.1
151.2
150.1
140.5
139.6
137.9
131.4
130.5
127.7
126.3
125.4
119.3
115.4
115.4
112.4
67.9
56.5
56.4
39.8
35.9
35.3
34.0
31.9
29.3
28.4
28.1
28.0
27.5
27.3
24.2
(+/-)-H3-P4
13C NMR
75 MHz, MeOD
OMe
OMe
S
N
4
Ph
NH2
O
NH
3
N
Cl
.2 CF3CO2H
200
150
100
50
0
ppm (t1)

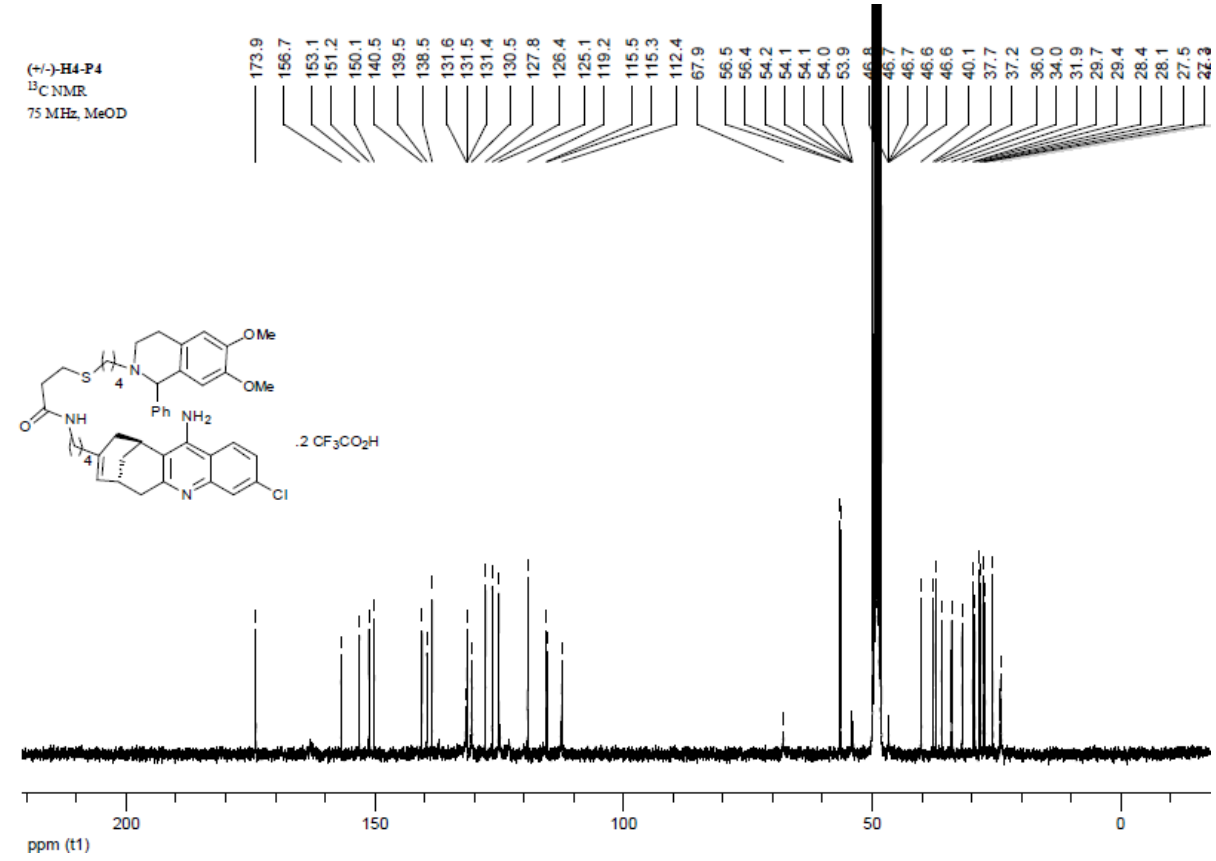

(+/-)-H4-P2
^{1}H NMR
300 MHz, MeOD
OMe
OMe
N
S
2
Ph
NH$_2$
O
NH
4
N
Cl
.2 CF_3CO_2H
10.0
5.0
0.0
ppm (t1)

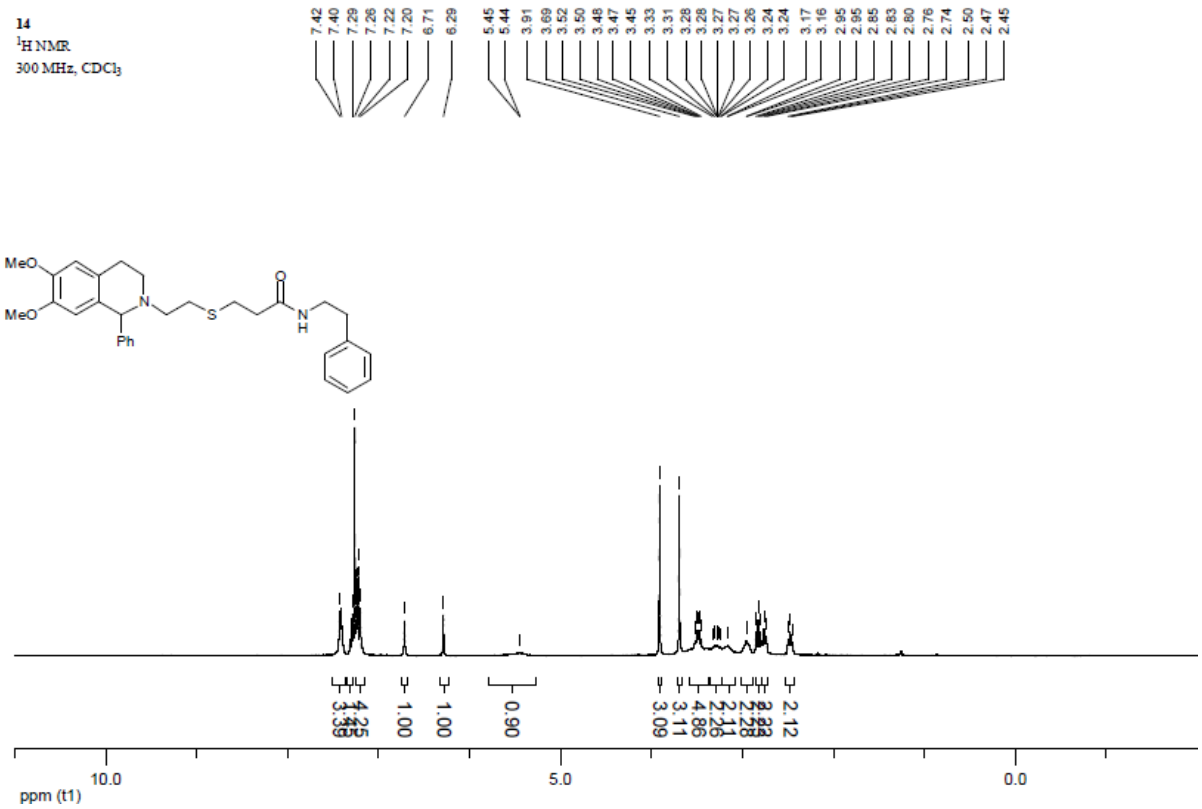

(+/-)-H4-P2
^{13}C NMR
75 MHz, MeOD
173.6
156.7
153.1
151.2
150.1
140.5
139.5
138.4
132.1
132.0
131.9
131.4
130.4
127.8
126.4
125.0
124.8
119.2
115.6
115.5
115.3
112.4
67.3
67.2
67.2
56.5
56.4
53.2
40.2
37.7
36.5
36.0
34.0
29.7
29.4
28.1
28.0
27.5
26.9
25.7
OMe
OMe
N
S
2
Ph
NH$_2$
O
NH
4
N
Cl
.2 CF_3CO_2H
200
150
100
50
0
ppm (t1)

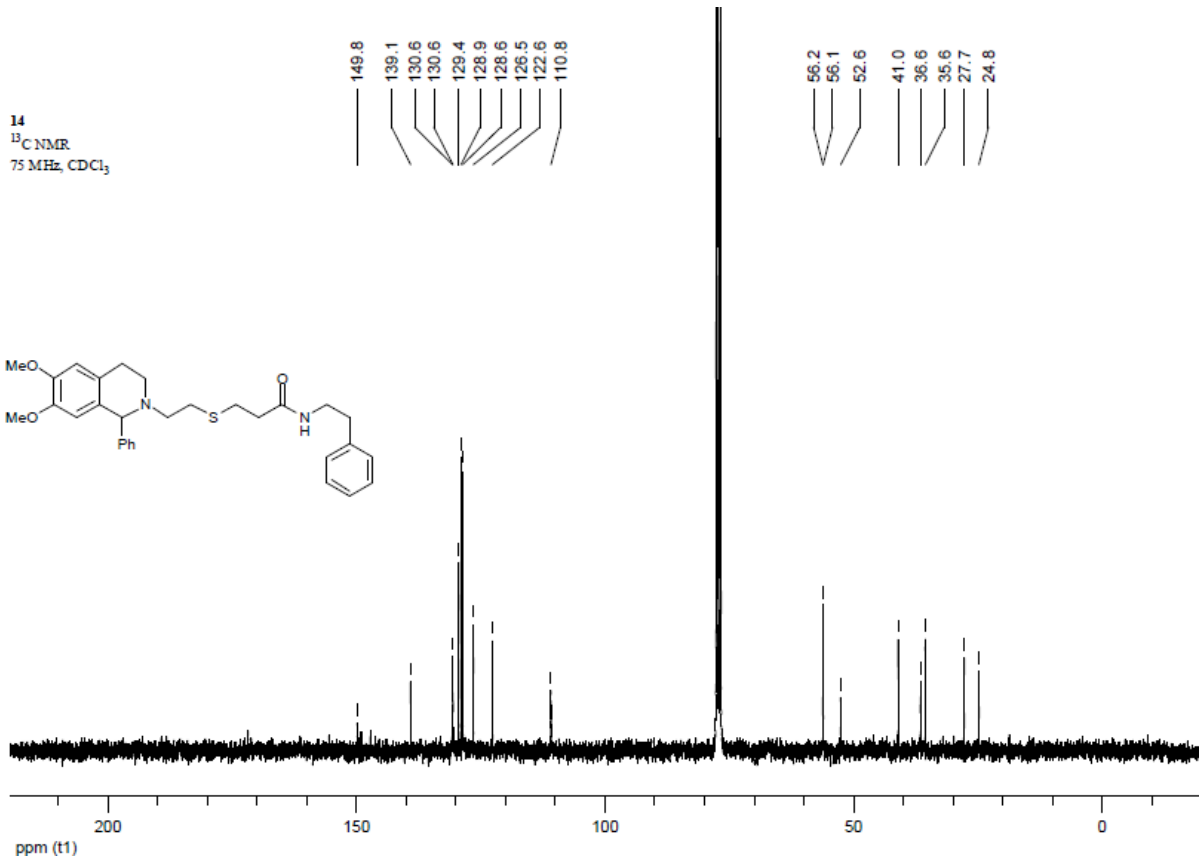

(+/-)-H4-P3
1H NMR
300 MHz, MeOD
.2 CF3CO2H
ppm (t1)

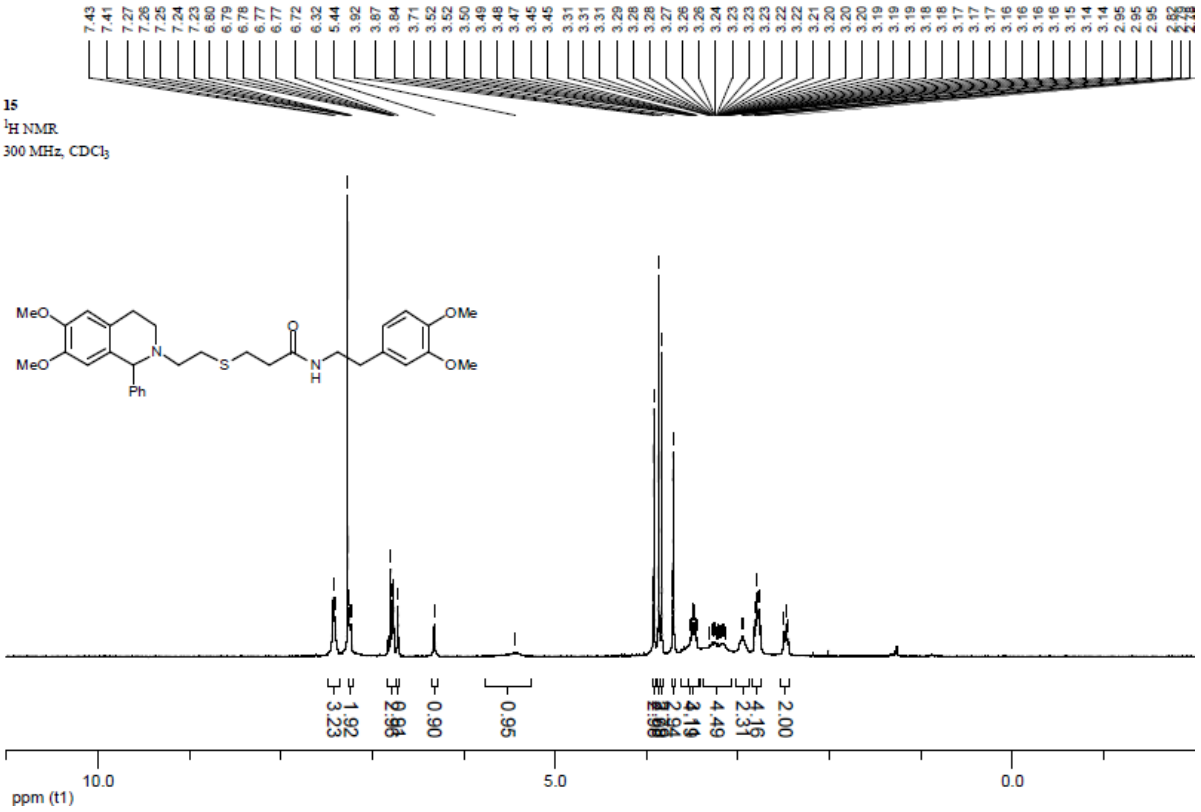

(+/-)-H4-P3
13C NMR
75 MHz, MeOD
.2 CF3CO2H
ppm (t1)

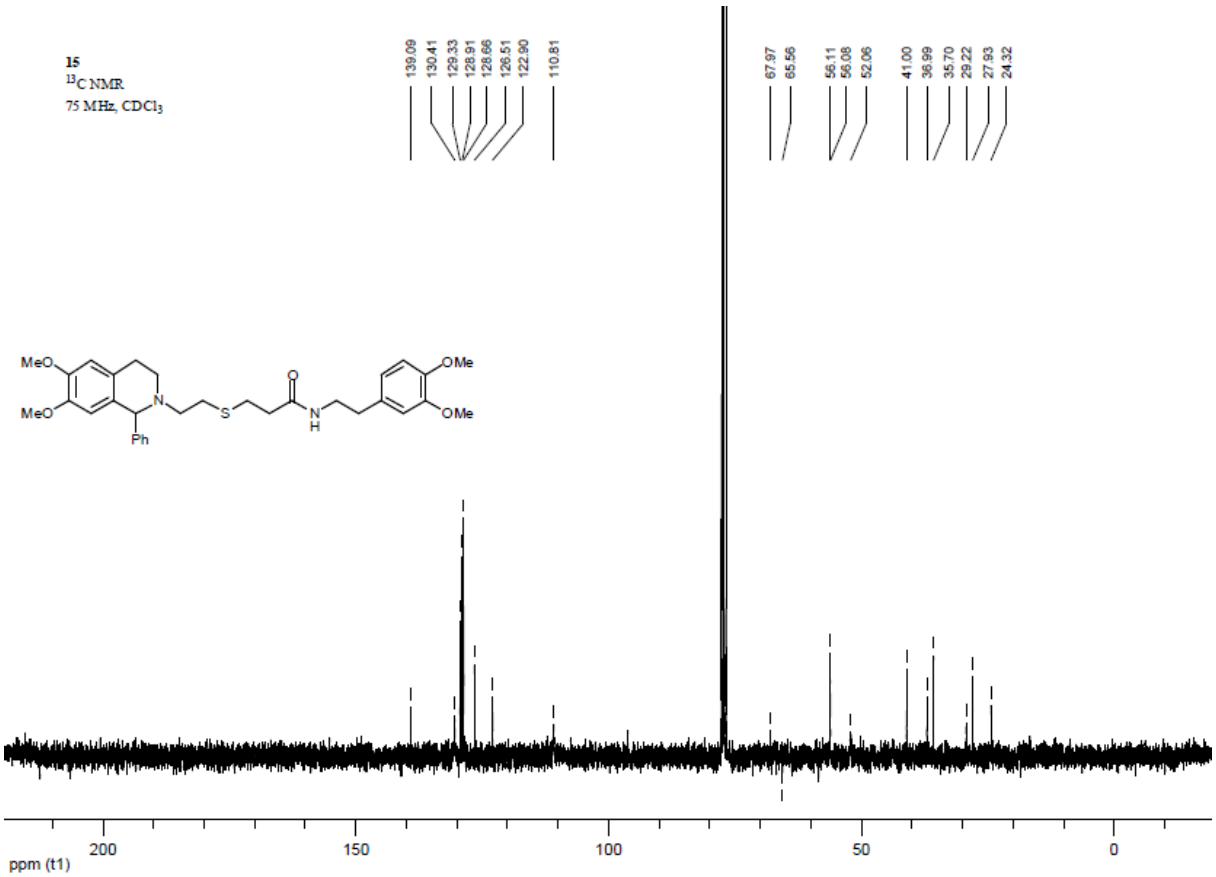

(+/-)-H4-P4
1H NMR
300 MHz, MeOD
OMe
OMe
Ph
NH2
NH
S
N
O
Cl
.2 CF3CO2H
1.00
1.08
2.05
1.00
0.93
0.99
0.97
2.83
2.92
7.53
2.09
4.14
10.0
5.0
0.0
ppm (t1)


(+/-)-H4-P4
13C NMR
75 MHz, MeOD
173.9
156.7
153.1
151.2
150.1
140.5
139.5
138.5
131.6
131.5
131.4
130.5
127.8
126.4
125.1
119.2
115.5
115.3
112.4
67.9
56.5
56.4
54.2
54.1
54.1
54.0
53.9
46.7
46.7
46.6
46.6
40.1
37.7
37.2
36.0
34.0
31.9
29.7
29.4
28.4
28.1
27.5
OMe
OMe
Ph
NH2
NH
S
N
O
Cl
.2 CF3CO2H
200
150
100
50
0
ppm (t1)


14
1H NMR
300 MHz, CDCl3
7.42 7.40 7.29 7.26 7.22 7.20 6.71 6.29
5.45 5.44 3.91 3.69 3.52 3.50 3.48 3.47 3.45 3.33 3.31 3.28 3.28 3.27 3.27 3.26 3.24 3.24 3.17 3.16 2.95 2.95 2.85 2.83 2.80 2.76 2.74 2.50 2.47 2.45
MeO
MeO
N
S
O
N
H
Ph
3.39
4.25
1.00
1.00
0.90
3.09
3.11
4.89
2.11
2.26
2.28
2.12
10.0
5.0
0.0
ppm (t1)


14
13C NMR
75 MHz, CDCl3
149.8 139.1 130.6 130.6 129.4 128.9 128.6 126.5 122.6 110.8
56.2 56.1 52.6 41.0 36.6 35.6 27.7 24.8
MeO
MeO
N
S
O
N
H
Ph
200
150
100
50
0
ppm (t1)

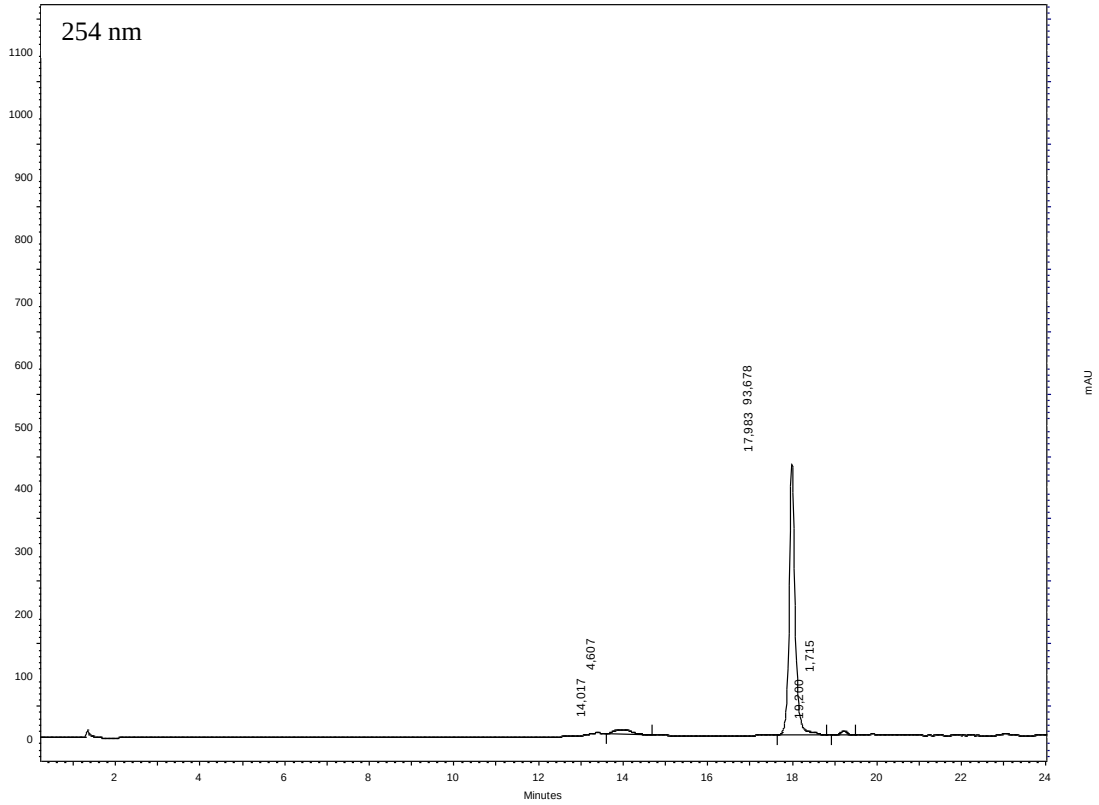




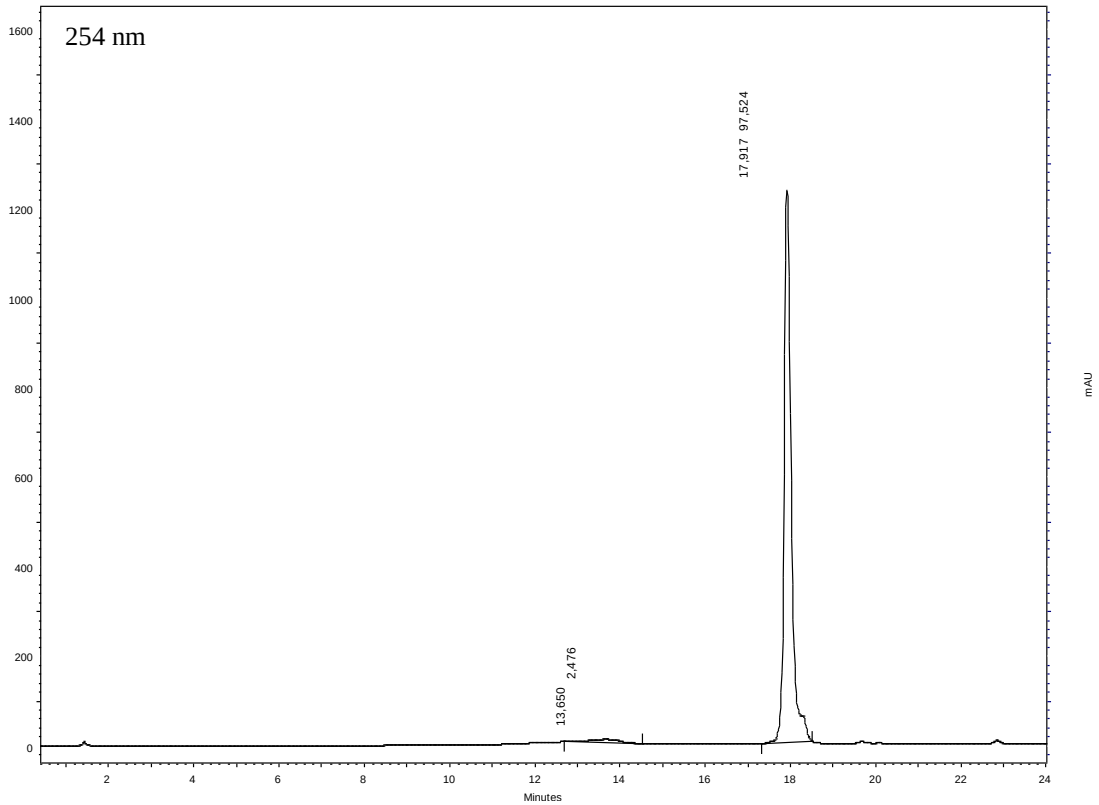
* Peaks of residual solvent, traces of grease or water in $CDCl_3$.

IV. HPLC analyses:

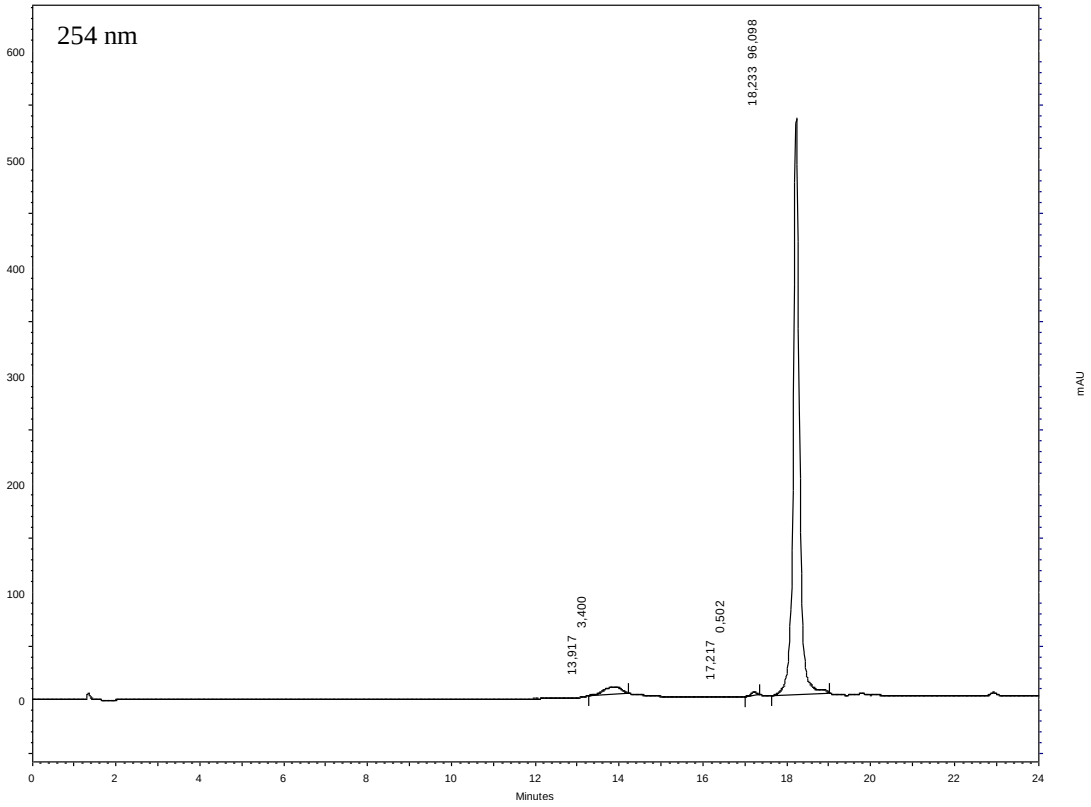
(±)-H2-P2 :



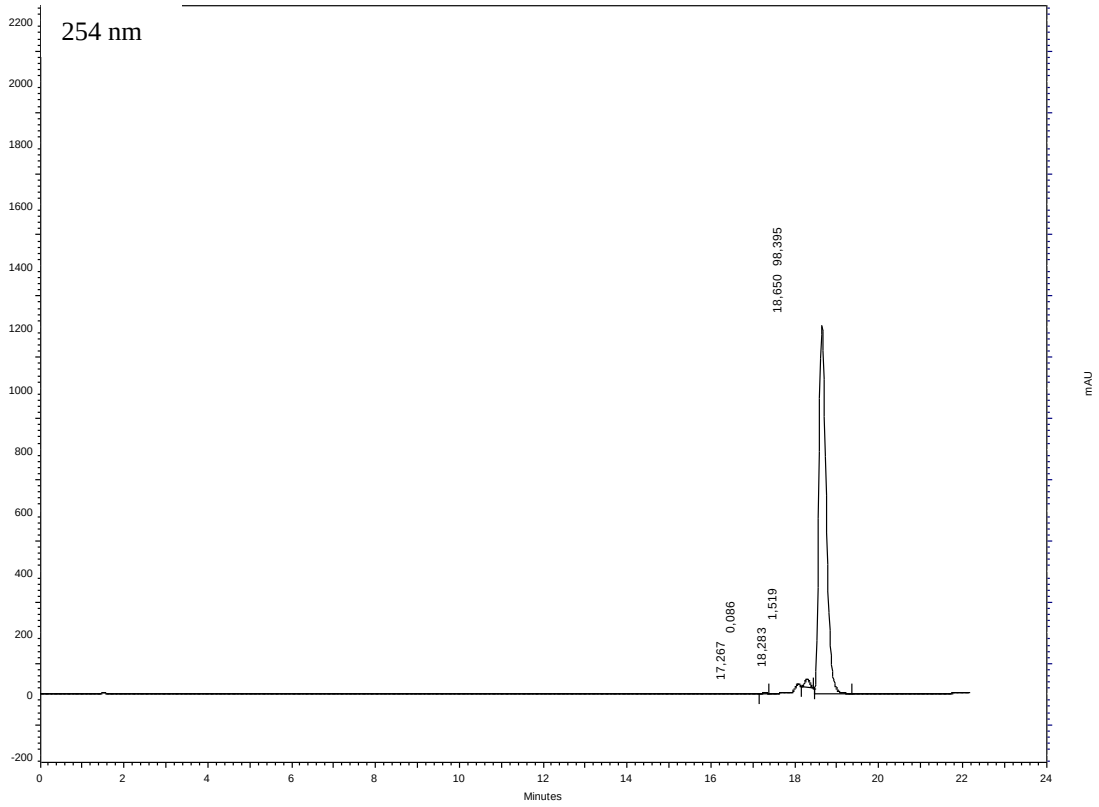
(±)-H2-P3



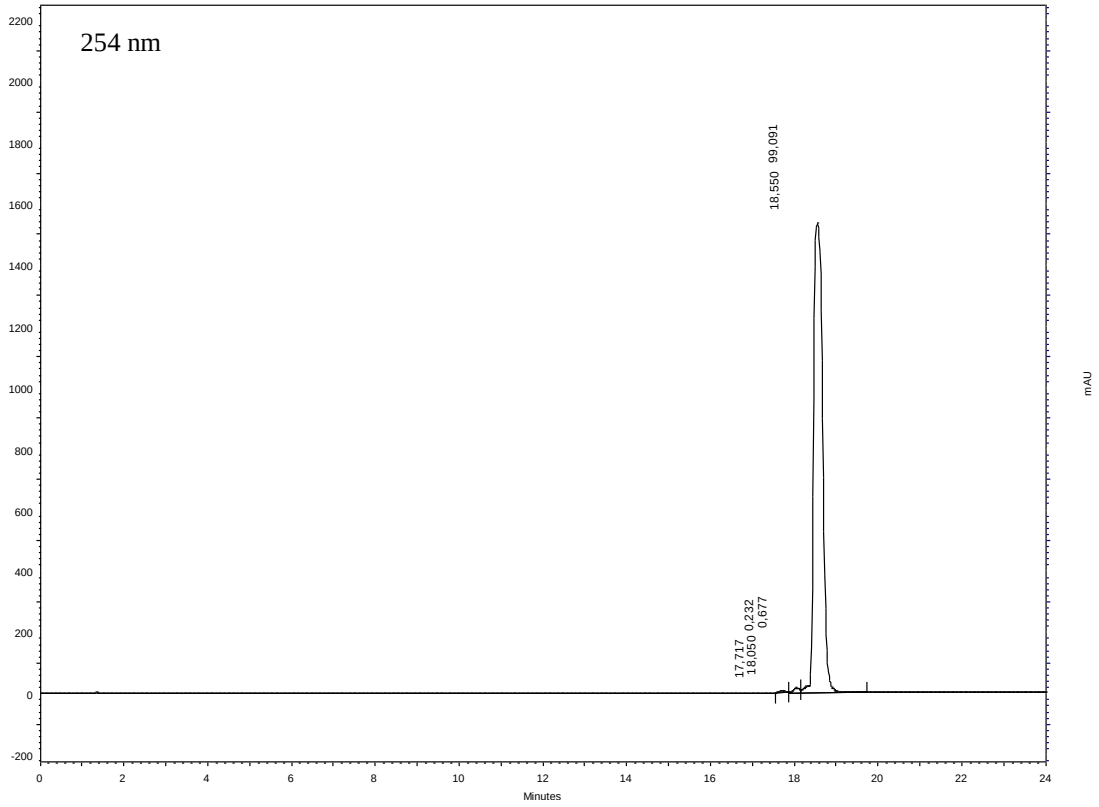
(±)-**H2-P4 :**


254 nm
18,233 96,098
13,917 3,400
17,217 0,502
mAU
Minutes

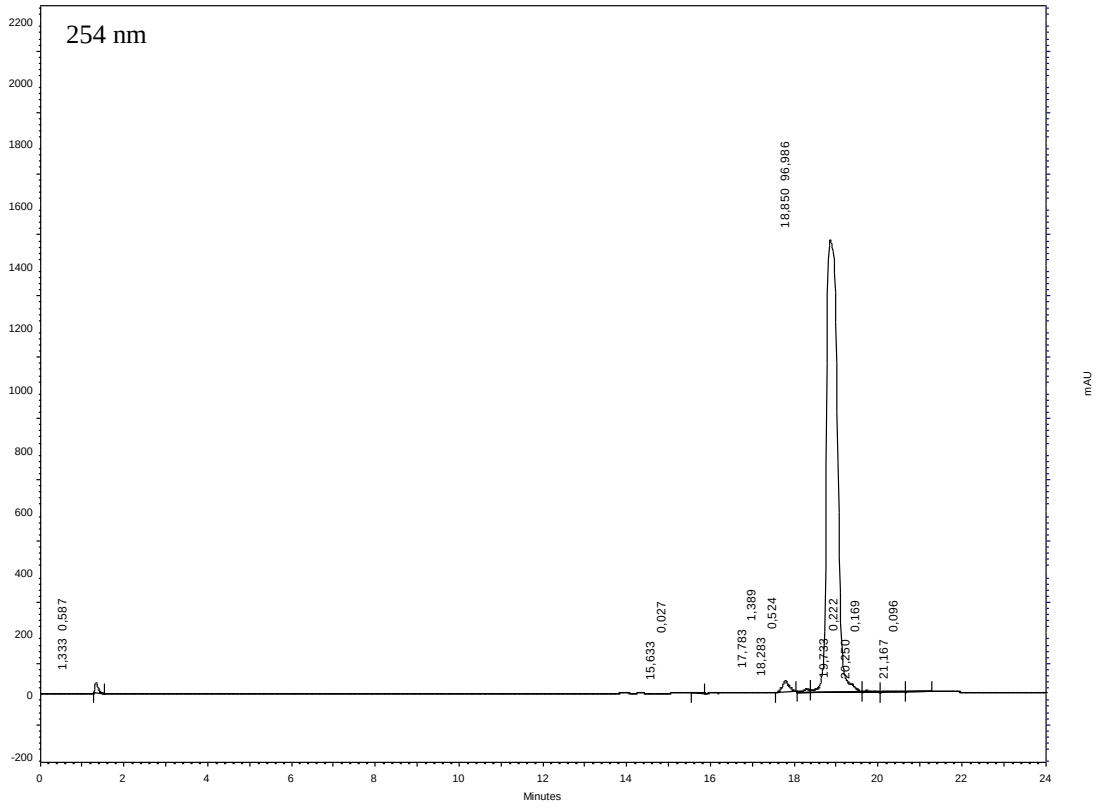
(±)-**H3-P2**


254 nm
18,650 98,395
17,267 0,086
18,283 1,519
mAU
Minutes

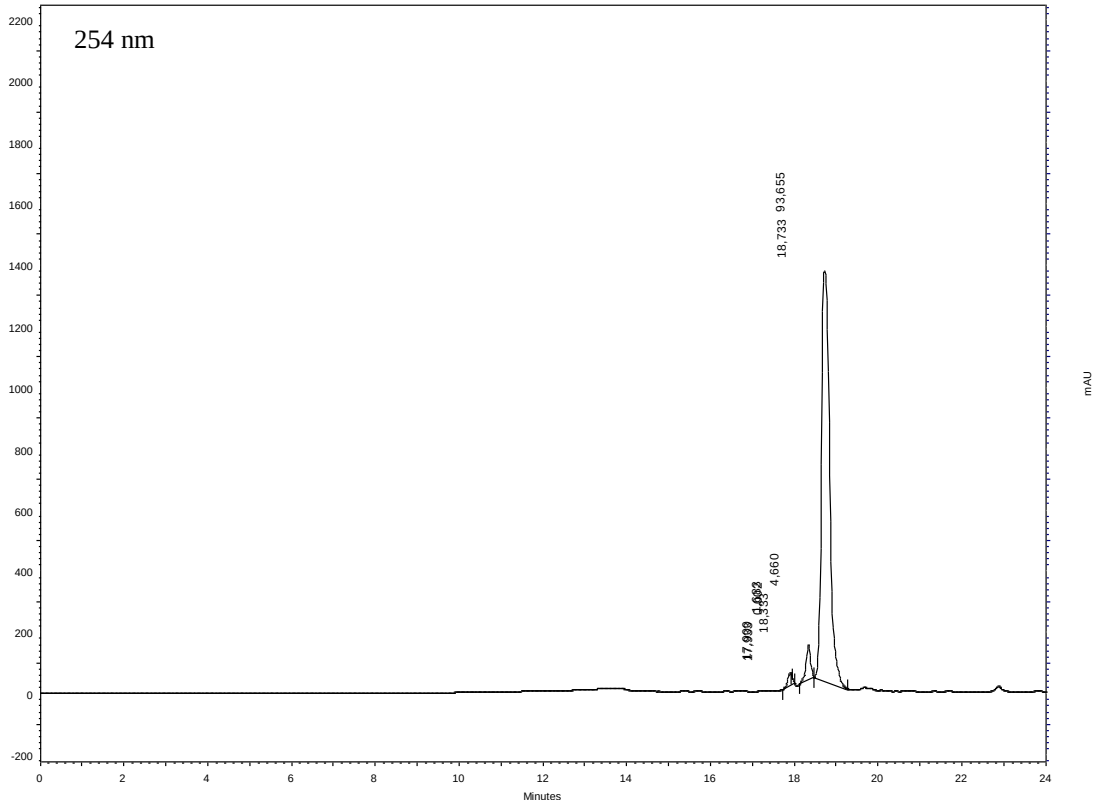
(±)-**H3-P3**


254 nm
18,550 99,091
17,717 0,232
18,050 0,677
mAU
Minutes

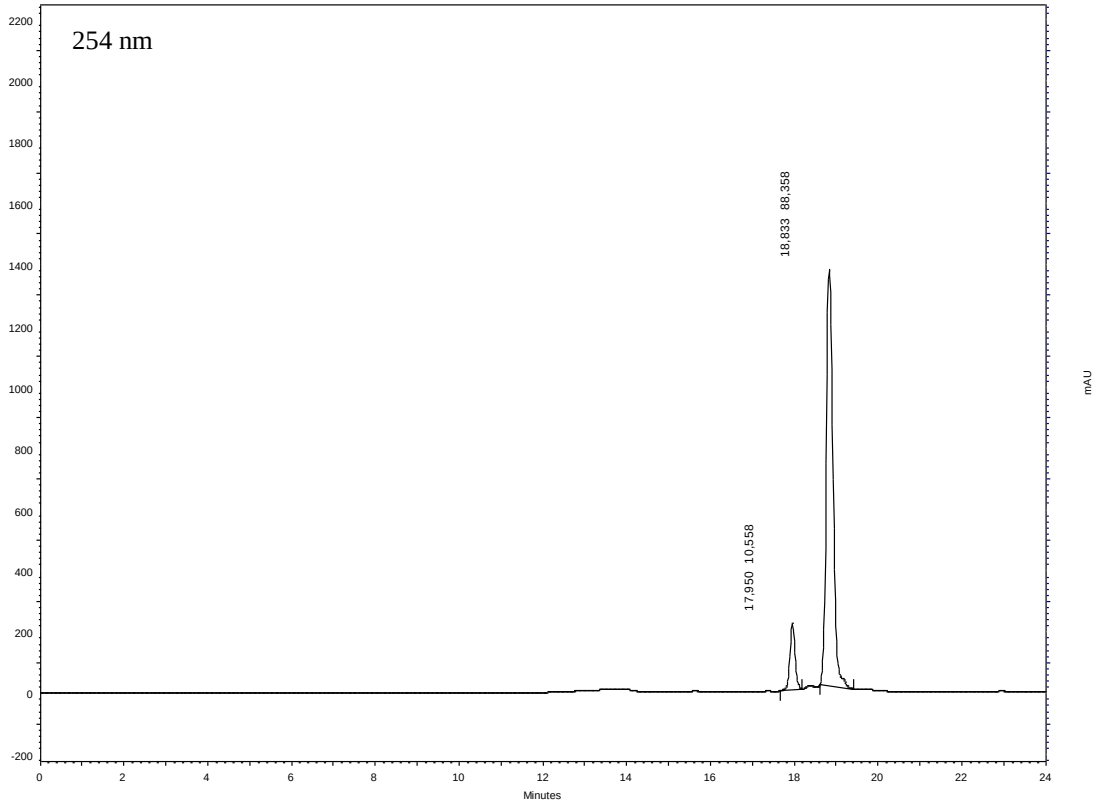
(±)-**H3-P4**


254 nm
18,850 96,986
1,333 0,587
15,633 0,027
17,783 1,389
18,283 0,524
19,733 0,222
20,250 0,169
21,167 0,096
mAU
Minutes

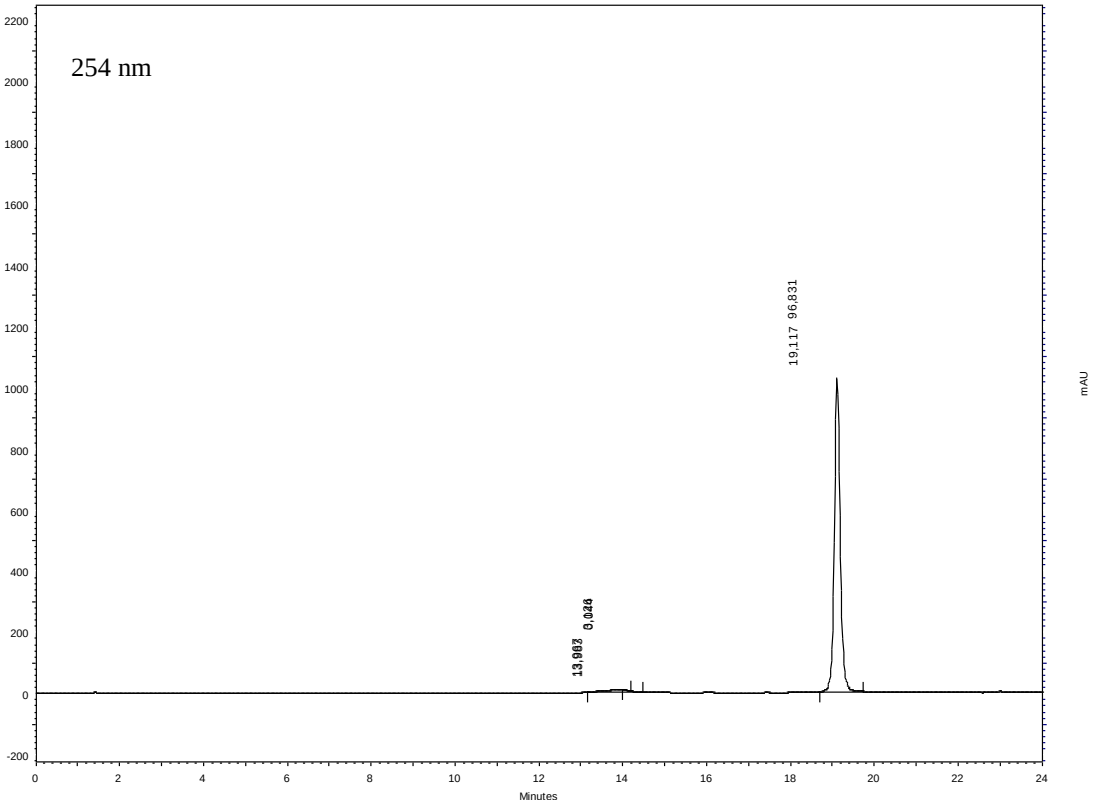
(±)-**H4-P2**


254 nm
18,733 93,655
4,660
mAU
Minutes

(±)-**H4-P3**


254 nm
18,833 88,358
17,950 10,558
mAU
Minutes

(±)-**H4-P4**



V. References

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