Molecular Design Strategy toward Enzyme-Activated Probes with Near-Infrared I and II Fluorescence for Targeted Cancer Imaging

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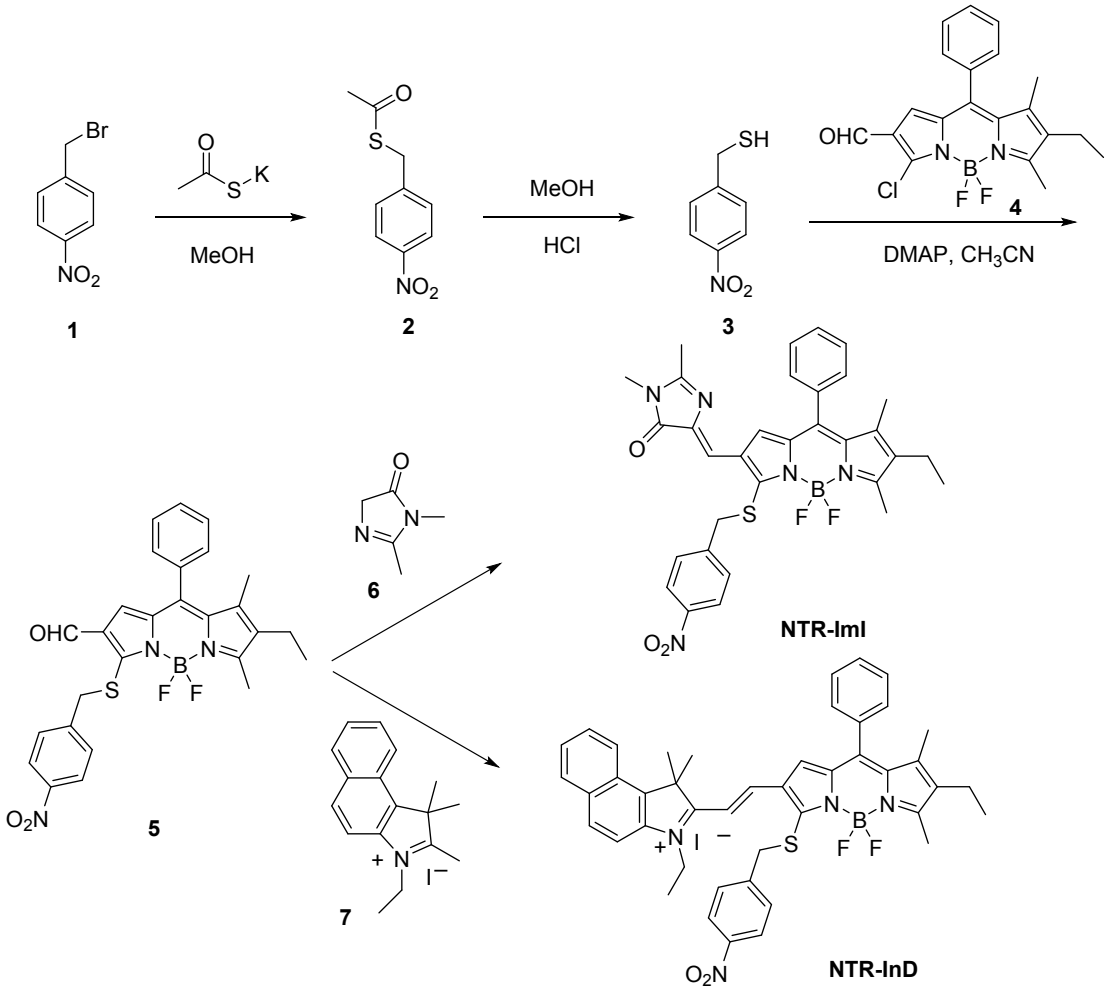
1. Design and Synthesis.



Scheme S1. The Synthetic routes for compounds NTR-ImI and NTR-InD.

Synthesis of compound 2. The solution of 4-Nitrobenzyl bromide (5.4 g, 25 mmol) and potassium thioacetate (4.3 g, 37.5 mmol) in 50 mL CH_3OH was stirred for about 12 h at room temperature. Then the solvent was removed under reduced pressure. The crude product was finally purified using silica gel flash chromatography to give compound 2 (4 g, 93%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.08-7.06 (d, 2H), 6.62-6.59 (d, 2H), 4.03 (s, 2H), 2.33 (s, 3H).

Synthesis of compound 3. After addition of 2 mL HCl (37%) to a solution of compound 2 (1.5 g, 7.1 mmol) in 25 mL MeOH, the resultant mixture was stirred for 24 h at room temperature. Then MeOH was evaporated, and the product was extracted with CH_2Cl_2, washed with water, dried

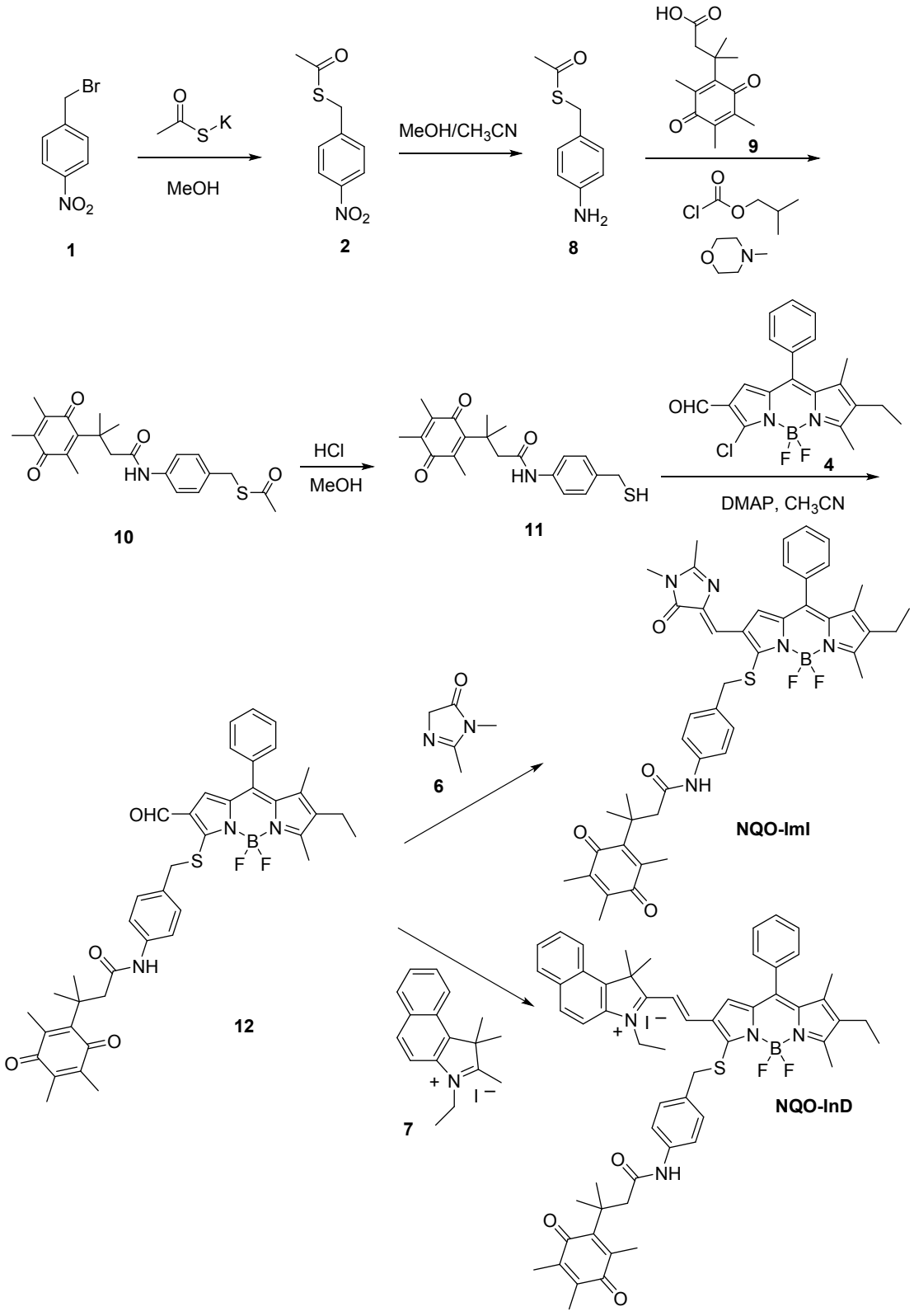
over Na_2SO_4. Finally, silica gel flash chromatography was employed to give compound 3 (1.15 g, 96%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.20-8.18 (d, 2H), 7.51-7.49 (d, 2H), 3.83-3.81 (d, 2H), 1.86-1.82 (t, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 148.53, 146.97, 128.99, 124.01, 28.44. HRMS (ESI, m/z): calculated for $C_7H_6NO_2S$ $[M-H]^-$: 168.0119, found: 168.0125.

Synthesis of compound 5. Compound 3 (87 mg, 0.51 mmol) and compound 4 (100 mg, 0.26 mmol) were dissolved in 20 mL CH_3CN, followed by addition of DMAP (38 mg, 0.31 mmol). The mixture was then stirred for 2 h at room temperature. After evaporation of the solvent, silica gel flash chromatography was used to give compound 5 (90 mg, 65%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.47 (s, 1H), 8.10-8.07 (d, 2H), 7.56-7.48 (m, 3H), 7.34-7.30 (m, 4H), 6.57 (s, 1H), 4.31 (s, 2H), 2.75 (s, 3H), 2.43-2.39 (q, 2H), 1.51 (s, 3H), 1.09-1.05 (t, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 186.00, 169.46, 147.07, 144.96, 144.12, 142.66, 140.42, 139.30, 136.43, 136.29, 133.17, 132.67, 129.93, 128.88, 128.65, 123.78, 122.06, 40.91, 30.19, 29.71, 17.26, 13.97, 12.64. HRMS (ESI, m/z): calculated for $C_{27}H_{25}BF_2N_3O_3S$ $[M+H]^+$: 520.1687, found: 520.1676.

Synthesis of compound NTR-ImI. The solution of compound 5 (100 mg, 0.19 mmol) and compound 6 (50 mg, 0.44 mmol) in 30 mL anhydrous EtOH was refluxed for 12 h. Then the solvent was removed and the crude product was purified utilizing silica gel flash chromatography. NTR-ImI was thus obtained with a high yield of 63% (70 mg). ^{1}H NMR (400 MHz, d_6-DMSO) δ 7.96-7.94 (d, 2H), 7.63-7.59 (m, 3H), 7.52-7.50 (m, 2H), 7.24-7.22 (d, 2H), 7.03 (s, 1H), 6.16 (s, 1H), 4.21 (s, 2H), 2.93 (s, 3H), 2.69 (s, 3H), 2.45-2.40 (q, 2H), 2.09 (s, 3H), 1.46 (s, 3H), 1.03-0.99 (t, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.07, 160.89, 146.69, 145.37, 143.46, 141.93, 140.75, 138.29, 137.04, 135.58, 133.19, 130.06, 129.67, 129.06, 128.72, 124.87, 123.73, 41.30, 26.37, 17.24, 15.46, 14.08, 13.78, 12.61. HRMS (ESI, m/z): calculated for $C_{32}H_{31}BF_2N_5O_3S$ $[M+H]^+$: 614.2209,

found: 614.2213.

Synthesis of compound NTR-InD. The solution of compound 5 (100 mg, 0.19 mmol) and compound 7 (103 mg, 0.28 mmol) in 30 mL anhydrous EtOH was refluxed for 12 h. Then the solvent was removed and the crude product was purified utilizing silica gel flash chromatography. NTR-InD was thus obtained with a high yield of 52% (85 mg). ^{1}H NMR (400 MHz, d_6-DMSO) δ 8.40-8.38 (d, 1H), 8.27-8.24 (d, 1H), 8.21-8.19 (d, 1H), 8.10-8.08 (d, 2H), 8.04-8.01 (d, 1H), 7.83-7.78 (m, 2H), 7.73-7.64 (m, 4H), 7.59-7.56 (m, 2H), 7.35-7.33 (d, 2H), 7.25-7.21 (d, 1H), 7.12 (s, 1H), 4.61-4.55 (q, 2H), 4.45 (s, 2H), 2.76 (s, 3H), 2.49-2.43 (q, 2H), 1.71 (s, 6H), 1.45 (s, 3H), 1.35-1.32 (t, 3H), 1.05-1.02 (t, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 181.27, 169.97, 147.10, 145.52, 145.38, 144.82, 138.56, 137.83, 137.57, 133.56, 132.02, 131.81, 130.59, 130.41, 129.94, 129.67, 128.90, 128.55, 127.32, 127.26, 124.05, 122.42, 121.43, 112.22, 109.63, 53.34, 44.27, 41.52, 31.51, 30.13, 29.70, 26.77, 17.33, 14.30, 14.10, 13.95, 12.78. HRMS (ESI, m/z): calculated for $C_{44}H_{42}BF_2N_4O_2S$ $[M\text{-}I]^+$: 739.3090, found: 739.3095.



Scheme S2. The Synthetic routes for compounds NQO-ImI and NQO-InD.

Synthesis of compound 8. To a solution of compound 2 (1.48 g, 7.08 mmol) in 30 mL MeOH/CH_3CN (v/v = 1:1) was added $SnCl_2 \cdot 2H_2O$ (17.26 g, 76.50 mmol) and the resultant mixture

was refluxed for 3 h. The reaction solution was then cooled to room temperature, wherein saturated Na_2CO_3 was added. Then the mixture was filtered, and the solution was extracted with EtOAc, washed with H_2O, dried over Na_2SO_4. Upon evaporation of the organic solvent, the crude product was purified with silica gel flash chromatography to give compound 8 in a yield of 64% (0.816 g). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.08-7.07 (d, 2H), 6.62-6.60 (d, 2H), 4.04 (s, 2H), 2.33 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 195.63, 145.57, 129.85, 127.21, 115.23, 33.23, 30.40. HRMS (ESI, m/z): calculated for $C_9H_{12}NOS$ $[M+H]^+$: 182.0640, found: 182.0648.

Synthesis of compound 10. The solution of compound 9 (0.324g, 1.3mmol) in 10 mL THF was cooled to 0 ^{0}C, then isobutyl chloroformate (176 μL, 1.3 mmol) was added. After being stirred for 5 min, N-methyl morphorline (170 μL, 1.3 mmol) was then added. The reaction mixture was further stirred for 30 min. Then compound 8 (0.155 g, 0.86 mmol) in 10 mL THF was added dropwisely. The obtained reaction mixture was stirred overnight at room temperature. Finally, silica gel flash chromatography was employed to give compound 10 (0.145 g, 40%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.34-7.31 (d, 2H), 7.20-7.18 (d, 2H), 7.09 (s, 1H), 4.05 (s, 2H), 3.00 (s, 2H), 2.33 (s, 3H), 2.15 (s, 3H), 1.95-1.94 (d, 6H), 1.48 (s, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 195.20, 191.55, 187.53, 170.20, 152.89, 143.27, 138.36, 138.27, 136.73, 133.46, 129.43, 119.97, 50.39, 38.45, 32.98, 30.34, 29.12, 14.20, 12.70, 12.17. HRMS (ESI, m/z): calculated for $C_{23}H_{27}NO_4NaS$ $[M+Na]^+$: 436.1559, found: 436.1557.

Synthesis of compound 11. After addition of 2 mL HCl (37%) to a solution of compound 10 (94.7 mg, 0.229 mmol) in 20 mL MeOH, the resultant mixture was refluxed for 2 h. Then MeOH was evaporated, and the product was extracted with CH_2Cl_2, washed with water, dried over Na_2SO_4. Finally, silica gel flash chromatography was employed to give compound 11 (27.2 mg, 32%).

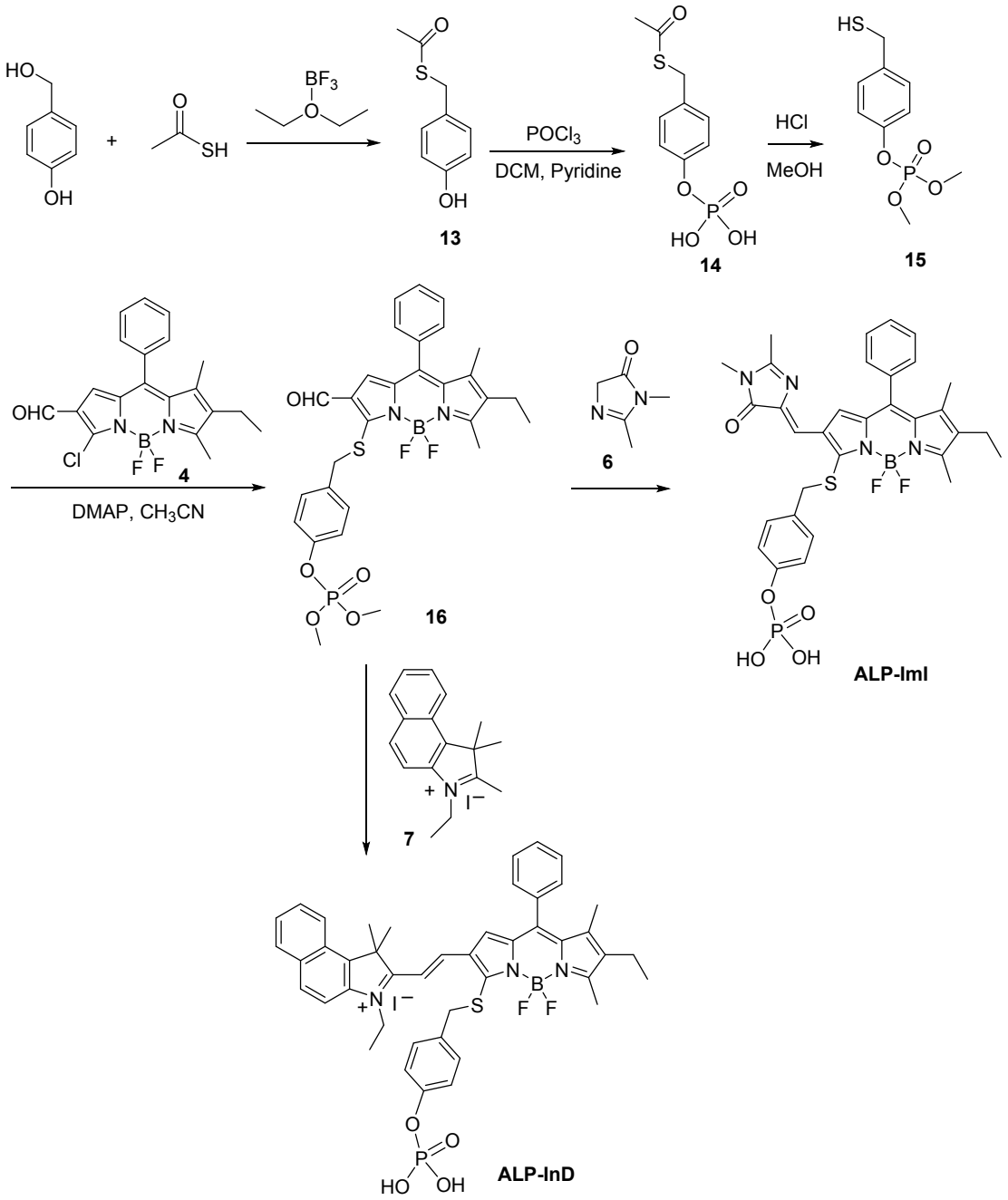
^{1}H NMR (400 MHz, d_6-DMSO) δ 9.93 (s, 1H), 7.43-7.41 (d, 2H), 7.17-7.15 (d, 2H), 3.54 (s, 2H), 2.95 (s, 2H), 2.05 (s, 3H), 1.89 (d, 6H), 1.39 (s, 6H), 1.19-1.16 (t, 3H). HRMS (ESI, m/z): calculated for: $C_{21}H_{25}NO_3NaS$ $[M+Na]^+$: 394.1453, found: 394.1434.

Synthesis of compound 12. Compound 11 (12.6 mg, 0.034 mmol) and compound 4 (19.7 mg, 0.051 mmol) were dissolved in 10 mL CH_3CN, followed by addition of DMAP (6.5 mg, 0.053 mmol). The mixture was then stirred overnight at room temperature. After evaporation of the solvent, silica gel flash chromatography was used to give compound 12 (10.3 mg, 42%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.32 (s, 1H), 7.54-7.48 (m, 3H), 7.32-7.29 (m, 2H), 7.14-7.12 (d, 2H), 7.05-7.03 (d, 2H), 6.65 (s, 1H), 4.16 (s, 2H), 2.98 (s, 2H), 2.75 (s, 3H), 2.43-2.38 (q, 2H), 2.14 (s, 3H), 1.94-1.92 (d, 6H), 1.50 (s, 3H), 1.33 (s, 6H), 1.08-1.04 (t, 3H). ^{13}C NMR (101 MHz, d_6-DMSO) δ 190.37, 186.78, 170.21, 169.09, 162.01, 154.11, 143.49, 139.87, 137.97, 137.16, 136.64, 136.05, 136.01, 134.57, 132.77, 131.52, 129.81, 129.61, 129.30, 129.17, 128.86, 128.70, 118.86, 116.52, 48.76, 37.55, 28.08, 26.52, 26.09, 22.07, 15.33, 13.79, 13.71, 12.58, 12.12, 11.73. HRMS (ESI, m/z): calculated for: $C_{41}H_{42}BF_2N_3O_4NaS$ $[M+Na]^+$: 744.2855, found: 744.2852.

Synthesis of compound NQO-ImI. The solution of compound 12 (20.3 mg, 0.028 mmol) and compound 6 (49.7 mg, 0.44 mmol) in 20 mL anhydrous EtOH was refluxed for 12 h. Then the solvent was removed and the crude product was purified utilizing silica gel flash chromatography. NQO-ImI was thus obtained with a high yield of 71% (20 mg). ^{1}H NMR (400 MHz, d_6-DMSO) δ 9.89 (s, 1H), 7.63-7.58 (m, 3H), 7.50-7.48 (m, 2H), 7.35-7.33 (d, 2H), 7.12 (s, 1H), 7.06-7.04 (d, 2H), 6.70 (s, 1H), 4.09 (s, 2H), 3.00 (s, 3H), 2.91 (s, 2H), 2.67 (s, 3H), 2.43-2.38 (q, 2H), 2.12 (s, 3H), 2.04 (s, 3H), 1.89-1.87 (d, 6H), 1.44 (s, 3H), 1.37 (s, 6H), 1.02-0.99 (t, 3H). ^{13}C NMR (101 MHz, d_6-DMSO) δ 190.37, 186.78, 170.21, 169.09, 162.01, 154.11, 143.49, 139.87, 137.97, 137.16, 136.64, 136.05,

136.01, 134.57, 132.77, 131.52, 129.17, 128.86, 128.70, 118.86, 116.52, 48.76, 39.47, 37.55, 28.08, 26.09, 15.33, 13.79, 13.71, 12.58, 12.12, 11.73. HRMS (ESI, m/z): calculated for: $C_{46}H_{49}BF_2N_5O_4S$ $[M+H]^+$: 816.3566, found: 816.3583.

Synthesis of compound NQO-InD. The solution of compound 12 (18 mg, 0.025 mmol) and compound 7 (32.3 mg, 0.089 mmol) in 20 mL anhydrous EtOH was refluxed for 12 h. Then the solvent was removed and the crude product was purified utilizing silica gel flash chromatography. NQO-InD was thus obtained with a high yield of 53% (14 mg). ^{1}H NMR (400 MHz, d_6-DMSO) δ 9.82 (s, 1H), 8.39-8.37 (d, 1H), 8.27-8.25 (d, 1H), 8.21-8.19 (d, 1H), 8.04-8.02 (d, 1H), 7.84-7.80 (d, 1H), 7.77-7.75 (m, 1H), 7.73-7.71 (m, 1H), 7.67-7.63 (m, 3H), 7.58-7.56 (m, 2H), 7.34-7.31 (d, 2H), 7.25-7.21 (d, 1H), 7.15 (s, 1H), 6.98-6.96 (d, 2H), 4.63-4.57 (q, 2H), 4.23 (s, 2H), 2.77 (s, 2H), 2.75 (s, 3H), 2.47-2.42 (q, 2H), 1.84 (s, 3H), 1.71 (s, 3H), 1.67 (s, 6H), 1.58 (s, 3H), 1.44 (s, 3H), 1.33-1.30 (t, 3H), 1.23 (s, 6H), 1.05-1.01 (t, 3H). ^{13}C NMR (101 MHz, d_6-DMSO) δ 190.00, 186.46, 181.38, 169.98, 169.67, 153.73, 144.74, 144.38, 143.07, 139.45, 137.90, 137.63, 137.33, 136.35, 135.79, 132.86, 132.19, 131.42, 130.92, 129.98, 129.83, 129.48, 128.97, 128.79, 128.19, 126.88, 126.57, 122.83, 120.30, 119.05, 112.69, 109.24, 53.04, 48.44, 41.66, 37.22, 31.11, 30.98, 29.67, 28.84, 27.66, 25.71, 21.91, 16.38, 13.77, 13.56, 13.40, 12.09, 11.99, 11.38. HRMS (ESI, m/z): calculated for: $C_{58}H_{60}BF_2N_4O_3S$ $[M-I]^+$: 941.4447, found: 941.4493.



Scheme S3. The Synthetic routes for compounds ALP-ImI and ALP-InD.

Synthesis of compound 13. To a solution of p-hydroxybenzyl alcohol (3 g, 24.2 mmol) in thiolacetic acid (18 mL) was added $BF_3 \cdot Et_2O$ (0.45 mL). The reaction mixture was then stirred for 2 h at room temperature, followed by cooling to 0 ^{0}C. Then saturated $NaHCO_3$ was poured into the reaction system. Finally, the reaction mixture was extracted with EtOAc, washed with water, dried over Na_2SO_4, purified by using silica gel flash chromatography to afford compound 13 in a

high yield of 80% (3.534 g). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.16-7.14 (d, 2H), 6.77-6.74 (d, 2H), 4.06 (s, 2H), 2.34 (s, 3H).

Synthesis of compound 14. To a solution of compound 13 (0.514 g, 2.8 mmol) in 30 mL anhydrous CH_2Cl_2 was added pyridine (1.5 mL) and $POCl_3$ (1 mL) at 0 ^{0}C. The resultant reaction solution was further stirred for 2 h at room temperature and then poured into ice water. The system was extracted with CH_2Cl_2, dried over Na_2SO_4. Evaporation of the organic solvents gave compound 14 (0.678 g, 92%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36-7.34 (d, 2H), 7.23-7.21 (d, 2H), 4.11(s, 2H), 2.37 (s, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 194.89, 148.65, 137.13, 130.72, 120.76, 32.56, 30.37. HRMS (ESI, m/z): calculated for: $C_9H_{10}O_5PS$ $[M-H]^-$: 260.9987, found: 260.9978.

Synthesis of compound 15. After addition of 2 mL HCl (37%) to a solution of compound 14 (0.678 g, 2.58 mmol) in 20 mL MeOH, the resultant mixture was refluxed for 2 h. Then MeOH was evaporated, and the product was extracted with CH_2Cl_2, washed with water, dried over Na_2SO_4. Finally, silica gel flash chromatography was employed to give compound 15 (0.535 g, 84%). ^{1}H NMR (400 MHz, CD_3OD) δ 7.38-7.36 (d, 2H), 7.16-7.14 (d, 2H), 3.89 (s, 3H), 3.86 (s, 3H), 3.73 (s, 2H), 1.26-1.22 (t, 1H). ^{13}C NMR (101 MHz, CD_3OD) δ 150.55, 140.65, 130.72, 121.04, 55.84, 28.39. HRMS (ESI, m/z): calculated for: $C_9H_{13}O_4NaPS$ $[M+Na]^+$: 270.0170, found: 270.0169.

Synthesis of compound 16. Compound 15 (70.1 mg, 0.28 mmol) and compound 4 (38.7 mg, 0.1 mmol) were dissolved in 10 mL CH_3CN, followed by addition of DMAP (48.8 mg, 0.4 mmol). The mixture was then stirred overnight at room temperature. After evaporation of the solvent, silica gel flash chromatography was used to give compound 16 (53.7 mg, 54%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.40 (s, 1H), 7.53-7.48 (m, 3H), 7.33-7.30 (m, 2H), 7.14-7.12 (d, 2H), 7.09-7.07 (d, 2H), 6.56 (s, 1H), 4.20 (s, 2H), 3.84-3.81 (d, 6H), 2.75 (s, 3H), 2.42-2.38 (q, 2H), 1.51 (s, 3H), 1.09-1.05

(t, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 186.45, 169.03, 149.89, 149.82, 143.88, 140.51, 134.26, 132.83, 130.54, 129.81, 128.80, 128.69, 121.75, 119.89, 119.84, 54.99, 41.39, 29.71, 17.25, 13.98, 12.60. HRMS (ESI, m/z): calculated for $C_{29}H_{30}BF_2N_2O_5PSNa$ $[M+Na]^+$: 621.1572, found: 621.1572.

Synthesis of compound ALP-ImI. The solution of compound 16 (35.4 mg, 0.059 mmol) and compound 6 (40.3 mg, 0.36 mmol) in 20 mL anhydrous EtOH was refluxed for 12 h. Then the solvent was removed and redissolved in anhydrous CH_2Cl_2 (30 mL). To the CH_2Cl_2 solution was then added TMSBr (33 μL), followed by further being stirred overnight. Then CH_2Cl_2 was removed and 20 mL MeOH was added. After stirring for 30 min, ALP-ImI was obtained by silica gel flash chromatography in a high yield (28.5 mg, 72.6%). ^{1}H NMR (400 MHz, CD3OD) δ 7.69 (s, 1H), 7.65-7.60 (m, 3H), 7.49-7.43 (m, 2H), 6.92-6.91 (d, 2H), 6.89-6.87 (d, 2H), 6.70 (s, 1H), 4.11 (s, 2H), 2.54-2.48 (q, 2H), 2.04 (s, 3H), 1.58 (s, 3H), 1.12-1.08 (t, 3H). ^{13}C NMR (101 MHz, CD_3OD) δ 163.61, 162.72, 161.19, 135.64, 135.27, 134.31, 131.75, 131.26, 131.10, 130.34, 130.25, 130.14, 128.48, 123.72, 122.30, 121.43, 121.39, 121.29, 121.25, 120.84, 42.14, 30.77, 27.57, 27.33, 17.96, 14.17, 12.8. HRMS (ESI, m/z): calculated for: $C_{32}H_{32}BF_2N_4O_5PS$ $[M-H]^-$: 664.1892, found: 664.1891.

Synthesis of compound ALP-InD. The solution of compound 16 (33.9 mg, 0.057 mmol) and compound 7 (32.3 mg, 0.089 mmol) in 20 mL anhydrous EtOH was refluxed for 12 h. Then the solvent was removed and redissolved in anhydrous CH_2Cl_2 (30 mL). To the CH_2Cl_2 solution was then added TMSBr (33 μL), followed by further being stirred overnight. Then CH_2Cl_2 was removed and 20 mL MeOH was added. After stirring for 30 min, ALP-InD was obtained by silica gel flash chromatography in a high yield (32.9 mg, 63.4%). ^{1}H NMR (400 MHz, CD_3OD) δ 8.35-8.33 (d, 1H), 8.17-8.15 (d, 1H), 8.12-8.05 (m, 2H), 7.87-7.84 (d, 2H), 7.79-7.75 (t, 3H), 7.68-7.65 (m, 4H), 7.51-7.50 (m, 2H), 7.46-7.44 (m, 2H), 7.05-7.02 (m, 4H), 4.58-4.52 (q, 2H), 4.27 (s, 2H), 2.78 (s, 3H),

2.54-2.49 (q, 4H), 1.84 (s, 6H), 1.55 (s, 3H), 1.49-1.45 (t, 3H), 1.12-1.09 (t, 3H). ^{13}C NMR (101 MHz, CD_3OD) δ 183.51, 174.83, 153.78, 149.08, 148.74, 145.69, 139.31, 135.08, 134.31, 132.60, 131.27, 131.20, 131.00, 130.86, 130.23, 129.52, 128.73, 128.24, 125.87, 125.24, 124.17, 121.53, 120.02, 114.36, 113.29, 54.98, 35.91, 35.48, 33.09, 31.83, 27.10, 26.13, 23.76, 17.99, 14.46, 14.17, 14.03, 12.82. HRMS (ESI, m/z): calculated for $C_{44}H_{44}BF_2N_3O_4PS$ $[M-I]^+$: 790.2851, found: 790.2871.

2. Cells culture and imaging.

A549 cells were grown in Dulbecco's Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) at 37 °C. HT-29 cells were grown in McCoy's 5A medium supplemented with 10% fetal bovine serum (FBS) at 37 °C. Such cultures were kept in a humidified atmosphere of 5/95 CO_2/air incubator for 24 h. CCK-8 assay was performed to demonstrate the low cytotoxicity of our designed probes.

For real-time tracking of NTR activities, A549 cells were cultured with NTR-ImI (10 μM) for 0.5 h, 1 h under various oxygen concentration conditions.

For real-time tracking of NQO1 activities, HT-29 cells were loaded with NQO-ImI (20 μM) for 2 h. For assay the inhibitory effects of dicoumarol: (1) HT-29cells pretreated with 0.5 mM dicoumarol for 2h, followed by incubation with NQO-ImI (20 μM) for 2h.

The confocal imaging was performed using a Zeiss Axiovert 200M Microscope with a 20 × immersion objective. Green channel at 600–670 nm with λ_{ex} = 514 nm excitation, red channel at 700–755 nm using λ_{ex} = 633 nm, ratio image generated from red to green channel.

3. In vivo imaging.

All animal experiments were carried out in compliance with the relevant laws and institutional guidelines for the Care and Use of Research Animals established by Fudan University, and the

experimental protocols and procedures were approved by the committee.

A549 cells and HT-29 cells (5×10^6) suspended in 200 µl PBS were implanted subcutaneously into the indicated location in male mice to obtain the A549 and HT-29 subcutaneous xenograft nude mice, respectively. When the tumor was about 70 mm^3 (about 3 weeks after implantation), *in vivo* fluorescence imaging was performed for targeted cancer visualization.

Two groups of mice were randomly divided, 1) probes (30 nmol) in PBS were administered to the tumor regions and normal sites via intratumoral injection; 2) tumor-bearing mice were treated with dicoumarin (0.3 mmol) for 2 h, followed by treatment with probes. Images were acquired at various time points after injection. NIR-I image was collected using the IVIS spectrum imaging system, 640 nm was used as the excitation wavelength and the fluorescence signals were collected between 700 nm-750 nm. NIR-II Images were recorded via series II 900/1700 imaging system. The excitation wavelength was 808 nm and the NIR-II emission was collected from 900 to 1300 nm.

4. HRMS analysis.

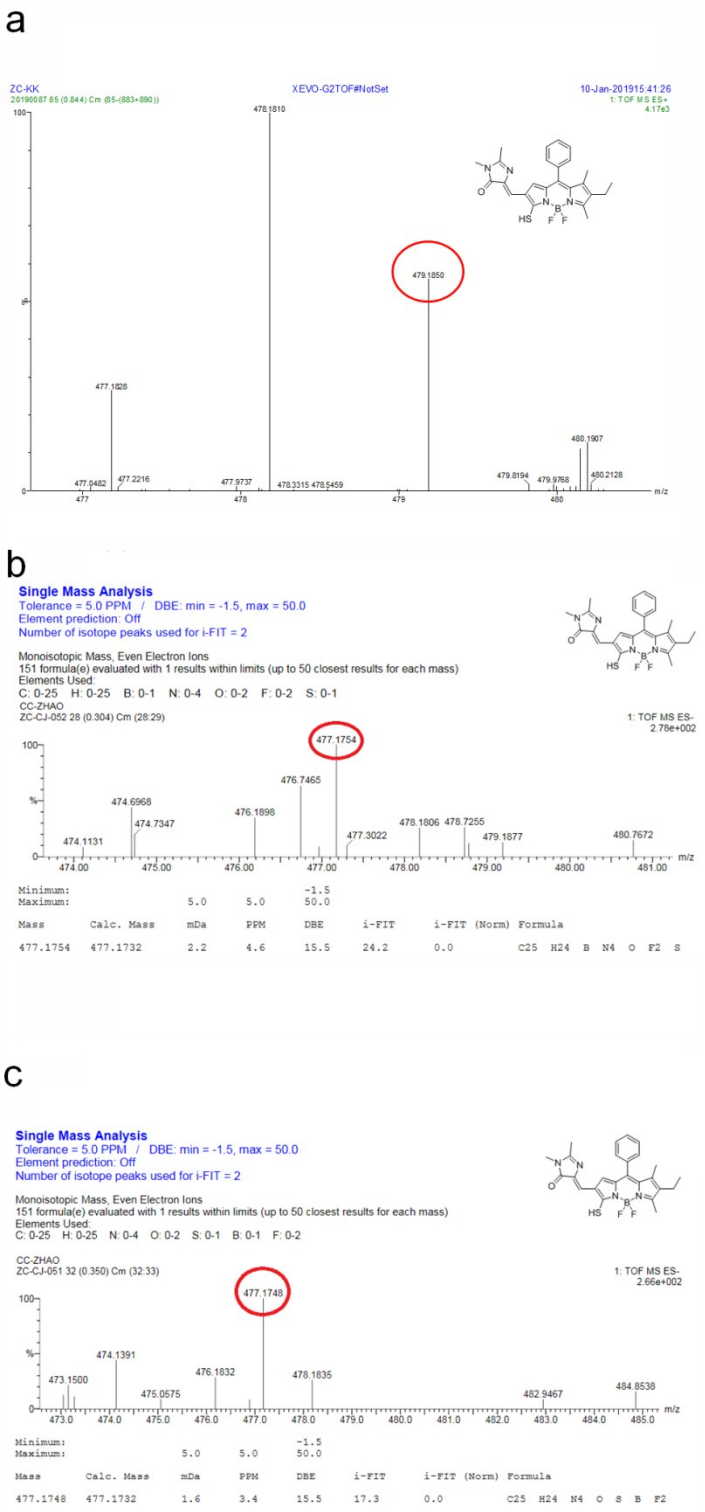


Figure S1. HRMS characterizations of the products from reactions of (a) NTR-Iml + NTR ($[M+H]^+$), (b) NQO-Iml + NQO1 ($[M-H]^-$) and (c) ALP-Iml + ALP ($[M-H]^-$).

5. Time-dependent fluorescence intensity ratio.

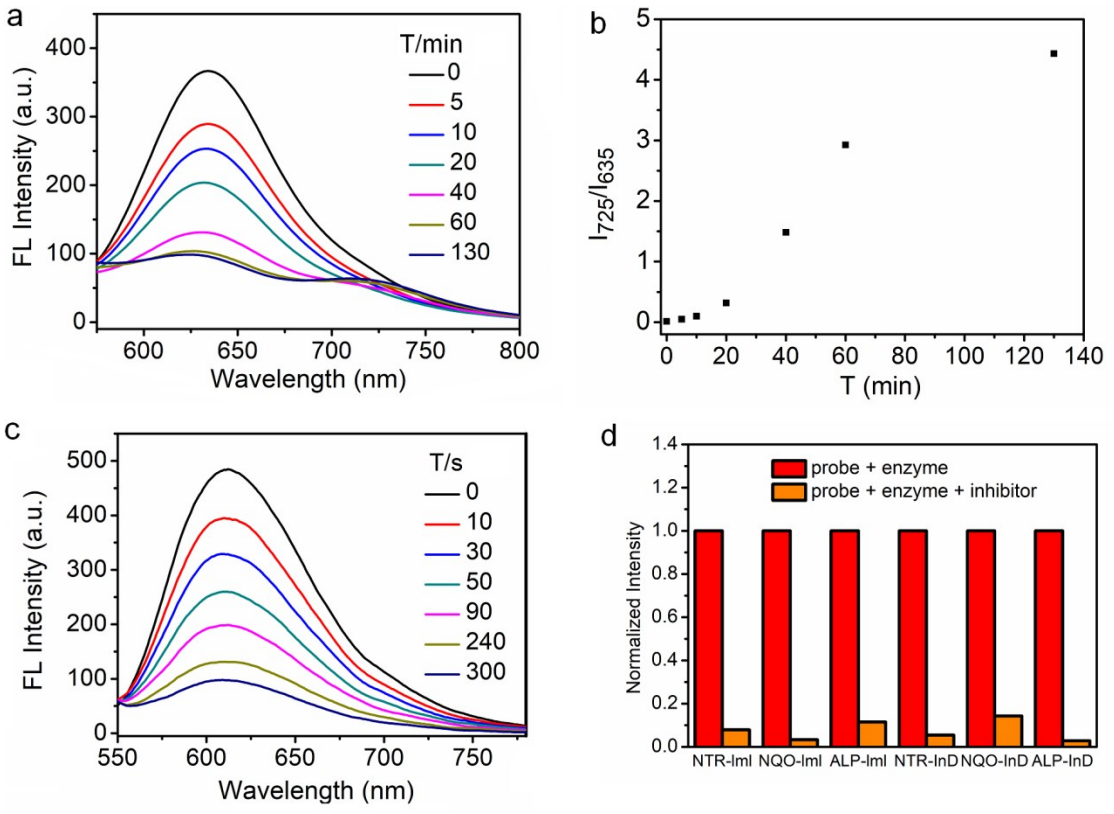


Figure S2. a) Fluorescence quenching (λ_{ex} = 557 nm) and b) Time-dependent fluorescence intensity ratio at 635 and 725 nm (I_{725}/I_{635}) of NQO-ImI (10 μM) upon addition of NQO1 (25 μg/mL) in buffer (DMSO/PBS, v/v, 2:8, pH 7.4) at 37 ^{0}C. I_{725}/I_{635} shows a 316-fold enhancement upon enzyme-catalyzed complete conversion. c) Fluorescence quenching (λ_{ex} = 535 nm) of NTR-InD upon addition of NTR (20 μg/mL) in buffer (DMSO/Tris-HCl, v/v, 2:8, pH 7.4)) at 37 ^{0}C. d) Enzymes-induced the fluorescence enhancement of probes in the absence or presence of inhibitors. Probes: 10 μM; enzyme: NTR 20 μg/mL; NQO1 25 μg/mL; ALP 0.2U/mL; the inhibitor: NTR + 2mM dicoumarol; NQO1+ 2mM dicoumarol; ALP+ 2mM Na3VO4. The fluorescence intensity at 725 nm was selected for NQO-ImI, 715 nm for NTR-ImI, 715 nm for ALP-ImI, 900 nm for NTR-InD, 900 nm for ALP-InD, 910 nm for NQO-InD.

6. NQO-ImI exhibited high specificity for NQO1.

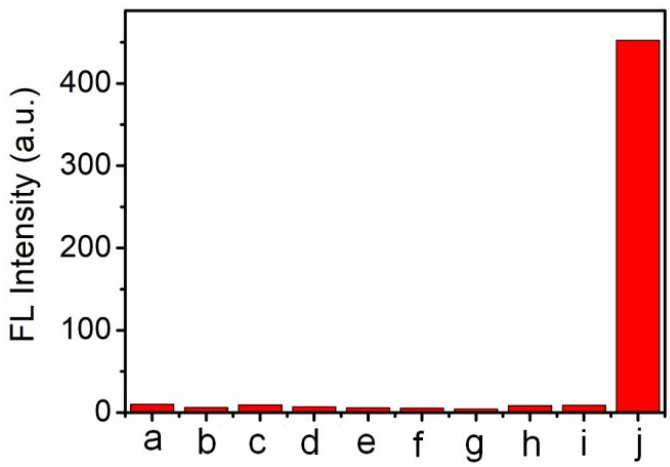


Figure S3. NQO-ImI exhibited high specificity for NQO1 activity in PBS buffer (DMSO/PBS, v/v, 2:8, pH 7.4) at 37 ^{0}C. The fluorescence intensity at 725 nm was selected for the selectivity discussion. (a) collagenase I (30 μg/mL); (b) phosphatase (30 μg/mL); (c) aprotinin (30 μg/mL); (d) lipase (30 μg/mL); (e) glucoamylase (30 μg/mL); (f) Cys (1 mM); (g) HCY (1 mM); (h) GSH (1mM); (i) NADH 500 μM); (j) NADH + NQO1. Data were collected at 150 min after addition of analytes.

7. The kinetic values of NQO-ImI against NQO1.

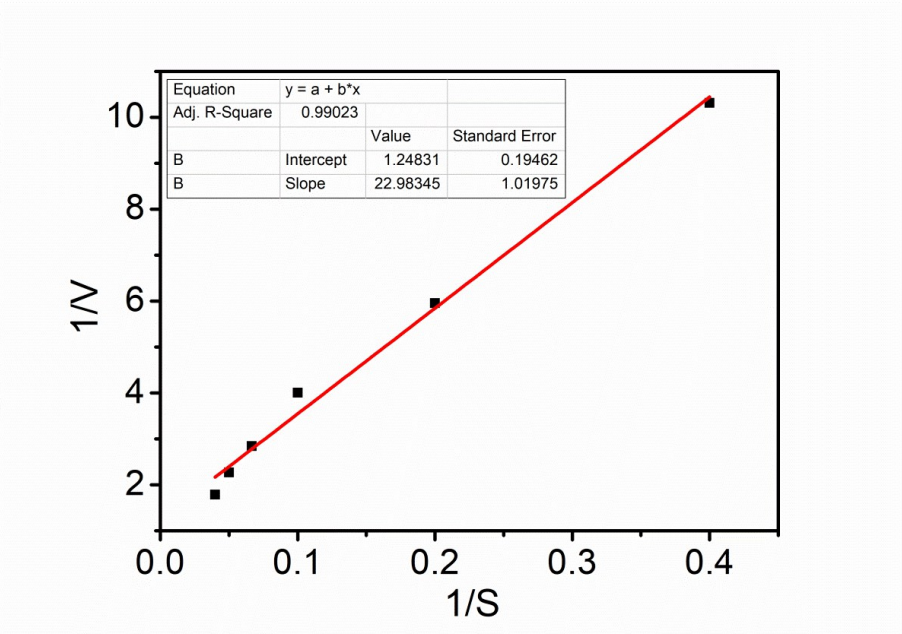


Figure S4. Plots of 1/V as function of 1/S for determining V and K_m. Utilizing Michaelis-Menten equation (1), we can get equation (2), wherein V represents the velocity and [S] represents the substrate concentration, while K_m is the Michaelis constant. The slope and the intercept can be afforded by the linear plot. Accordingly, K_m and V_{max} were determined to be 18.42 μM and 64.10 μM • min^{-1} for NQO-ImI.

$$V = V_{max} \times [S]/(K_m + [S]) \qquad (1)$$

$$1/V = (K_m/V_{max}) \times (1/[S]) + 1/V_{max} \qquad (2)$$

8. Time-dependent spectra changes of NTR-ImI in the presence of NTR.

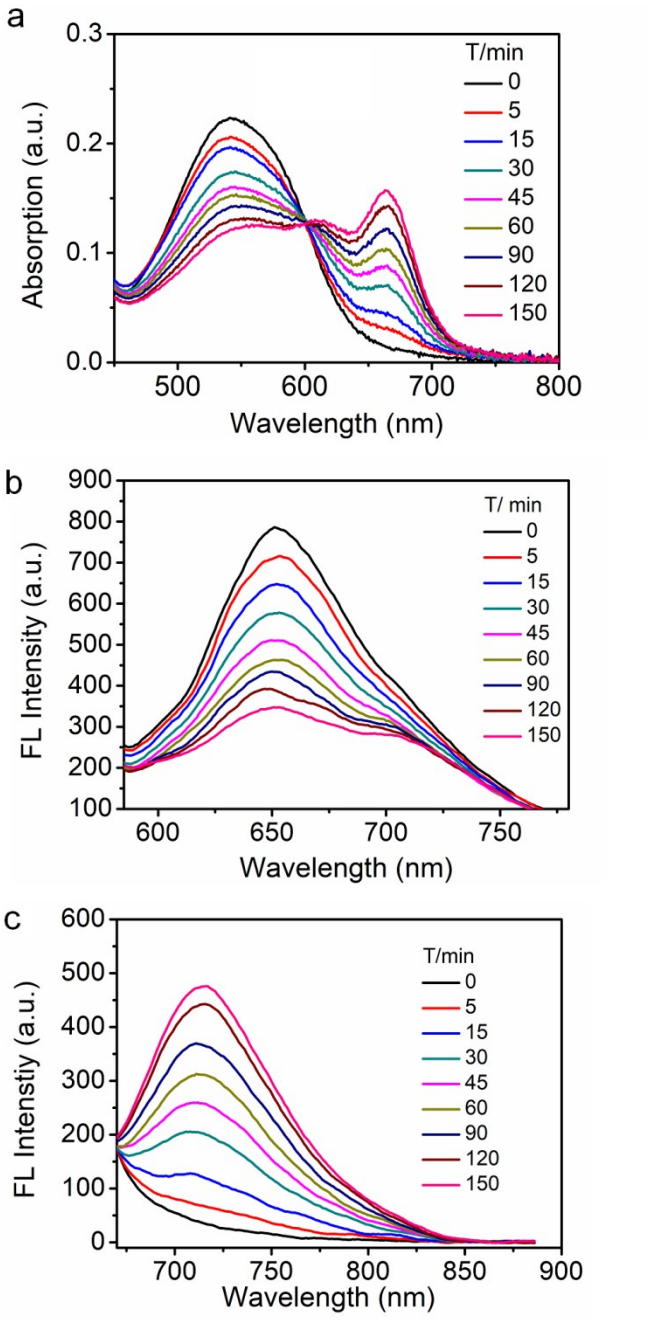


Figure S5. Time-dependent spectra changes of NTR-ImI (10 μM) in the presence of NTR (20 μg/mL). a) Absorption, b) fluorescence quenching (λ_{ex} = 520 nm) and c) NIR-I fluorescence turn-on (λ_{ex} = 640 nm) of NTR-ImI upon addition of NTR in buffer (DMSO/Tris-HCl, v/v, 2:8, pH 7.4) at 37 ^{0}C.

9. NTR-ImI exhibited high specificity for NTR activity.

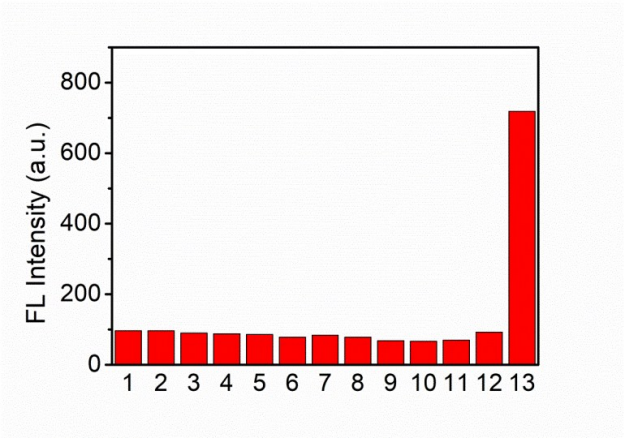


Figure S6. NTR-ImI exhibited high specificity for NTR activity in buffer (DMSO/Tris-HCl, v/v, 2:8, pH 7.4) at 37 ^{0}C. The fluorescence intensity at 715 nm was selected for the selectivity discussion. (1) none; (2) phosphatase (30 μg/mL); (3) collagenase I (30 μg/mL); (4) tyrosinase (30 μg/mL); (5) lipase (30 μg/mL); (6) aprotinin (30 μg/mL); (7) NAD(P)H:quinone oxidoreductase isozyme 1 (30 μg/mL); (8) BSA (10 mg/mL); (9) Cys (1 mM); (10) HCY (1 mM); (11) GSH (1 mM); (12) NADH (500 μM); (13) NADH + NTR. Data were collected at 150 min after addition of analytes.

10. The kinetic values of NTR-ImI against NTR.

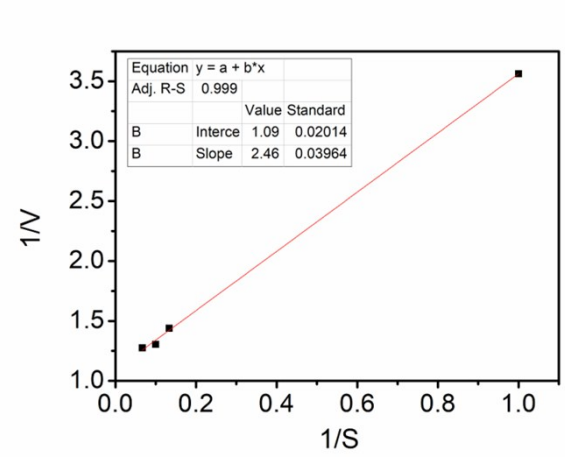


Figure S7. Plots of 1/V as function of 1/S for determining V and K_m. Utilizing Michaelis-Menten equation (1) and (2) in Figure S4, K_m and V_{max} were determined to be 2.51 μM and 0.92 μM • min^{-1} for NTR-ImI.

11. Time-dependent spectra changes of ALP-ImI in the presence of ALP.

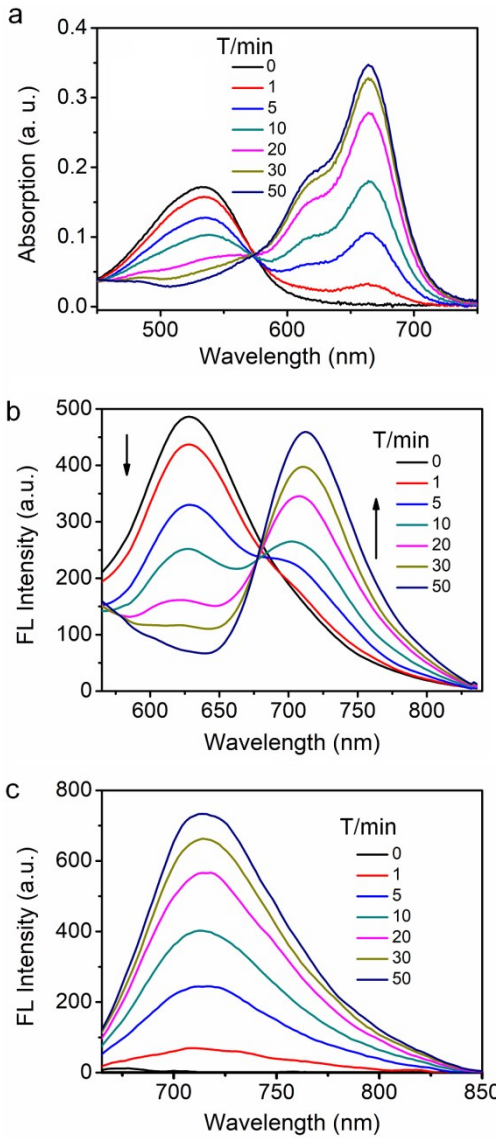


Figure S8. Time-dependent spectra changes of ALP-ImI (10 μM) in the presence of ALP (150 mU/mL). a) Absorption, b) ratiometric fluorescence changes (λ_{ex} = 530 nm) and c) NIR-I fluorescence turn-on (λ_{ex} = 640 nm) of ALP-ImI upon addition of ALP in buffer (DMSO/Tris-HCl, v/v, 2:8, pH 7.4) at 37 ^{0}C. Fortunately, the ratiometric fluorescence changes could be observed by single excitation at 530 nm when ALP-ImI incubated with ALP, which may presumably due to the high activity of ALP and the good solubility of ALP-ImI in the testing conditions.

12. ALP-ImI exhibited high specificity for ALP activity.

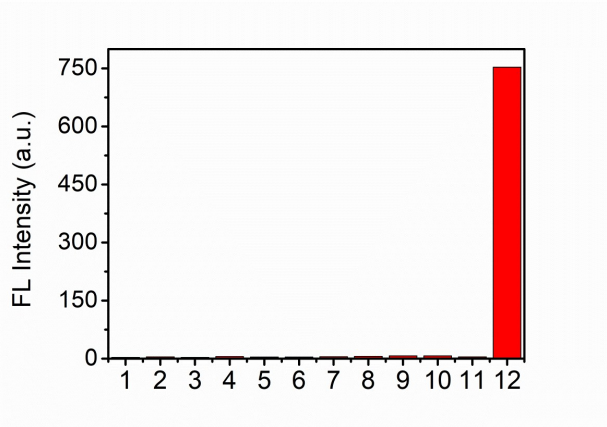


Figure S9. ALP-ImI exhibited high specificity for ALP activity in buffer (DMSO/Tris-HCl, v/v, 2:8, pH 7.4) at 37 ^{0}C. The fluorescence intensity at 715 nm was selected for the selectivity discussion. (1) none; (2) aprotinin (30 μg/mL); (3) tyrosinase (30 μg/mL); (4) lipase (30 μg/mL); (5) collagenase I (30 μg/mL); (6) BSA (10mg/mL); (7) GSH (1 mM); (8) NAD(P)H:quinone oxidoreductase isozyme 1 (30 μg/mL); (9) Cys (1 mM); (10) HCY (1 mM); (11) NADH (500 μM); (12) ALP (30 μg/mL). Data were collected at 60 min after addition of analytes.

13. The kinetic values of ALP-ImI against ALP.

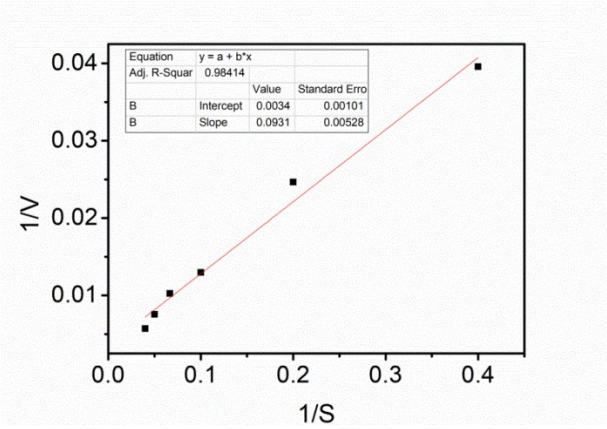


Figure S10 Plots of 1/V as function of 1/S for determining V and K_m. Utilizing Michaelis-Menten equation (1) and (2) in Figure S4, K_m and V_{max} were determined to be 26.69 μM and 286.53 μM • min^{-1} for ALP-ImI.

14. pH effects.

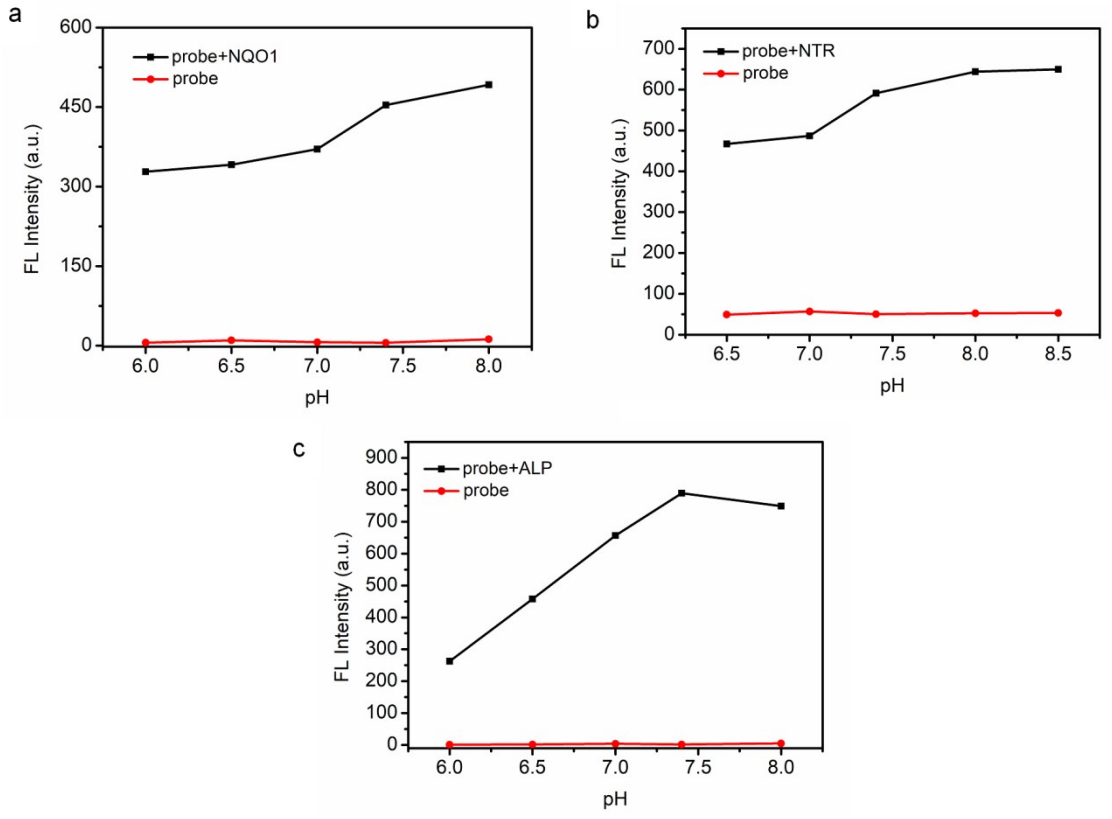


Figure S11. pH effects on the optical response of probes toward various enzymes. (a) NQO-ImI with NQO1, λ_{ex} = 650 nm, λ_{em} = 725 nm, data were collected at 150 min after addition of NQO1. (b) NTR-IMI with NTR, λ_{ex} = 640 nm, λ_{em} = 715 nm, data were collected at 150 min after addition of NTR. (c) ALP-IMI with ALP, λ_{ex} = 640 nm, λ_{em} = 715 nm, data were collected at 60 min after addition of ALP.

15. NTR-InD exhibited high specificity for NTR activity.

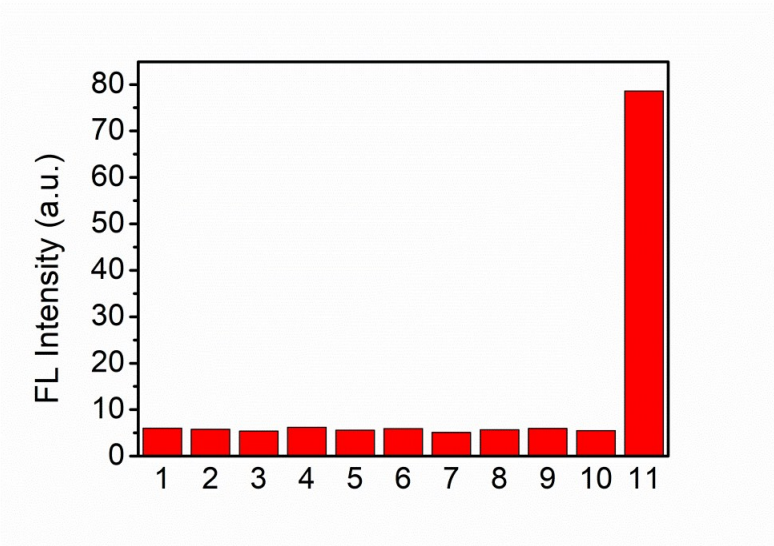


Figure S12. NTR-InD exhibited high specificity for NTR activity in buffer (DMSO/Tris-HCl, v/v, 2:8, pH 7.4) at 37 ^{0}C. The fluorescence intensity at 900 nm was selected for the selectivity discussion. (1) phosphatase (30 μg/mL); (2) aprotinin (30 μg/mL); (3) lipase (30 μg/mL); (4) collagenase I (30 μg/mL); (5) NAD(P)H:quinone oxidoreductase isozyme 1 (30 μg/mL); (6) NADH (500 μM); (7) BSA (10 mg/mL); (8) Cys (1mM); (9) HCY (1mM); (10) GSH (1mM); (11) NTR + NADH. Data were collected at 5 min after addition of analytes.

16. Time-dependent spectra changes of ALP-InD in the presence of ALP.

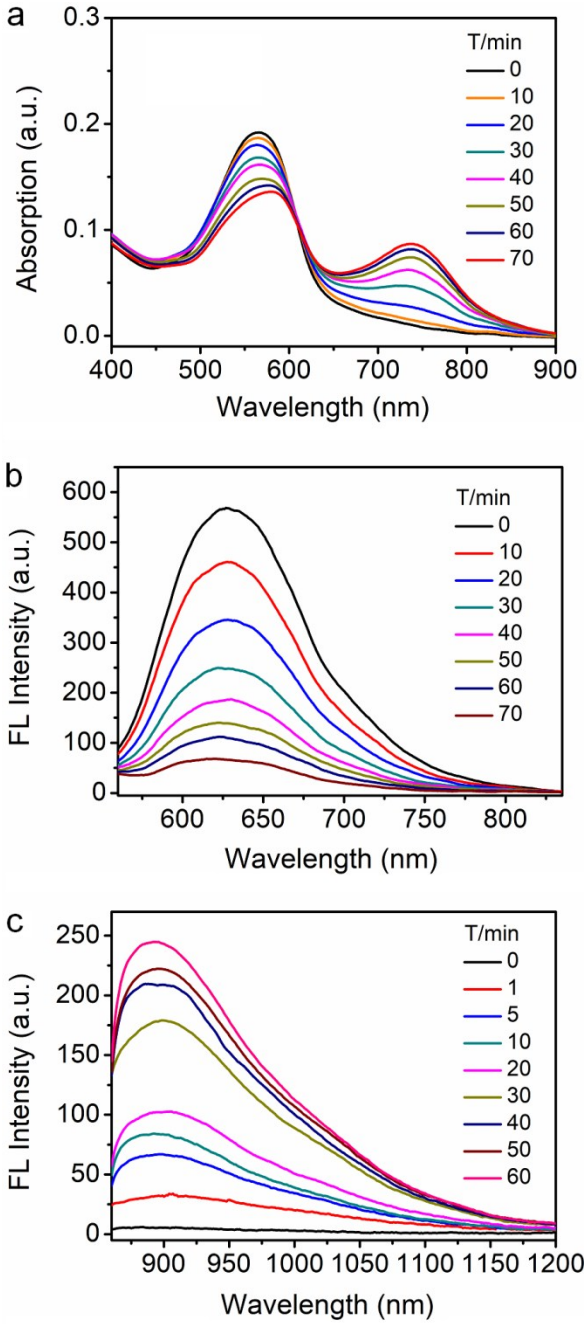


Figure S13. Time-dependent spectra changes of ALP-InD in the presence of ALP. a) Absorption, b) fluorescence quenching (λ_{ex} = 520 nm) and c) NIR-II fluorescence turn-on (λ_{ex} = 730 nm) of ALP-InD upon addition of ALP (200 mU/mL) in buffer (DMSO/Tris-HCl, v/v, 4:6, pH 7.4) at 37 ^{0}C.

17. ALP-InD exhibited high specificity for ALP activity.

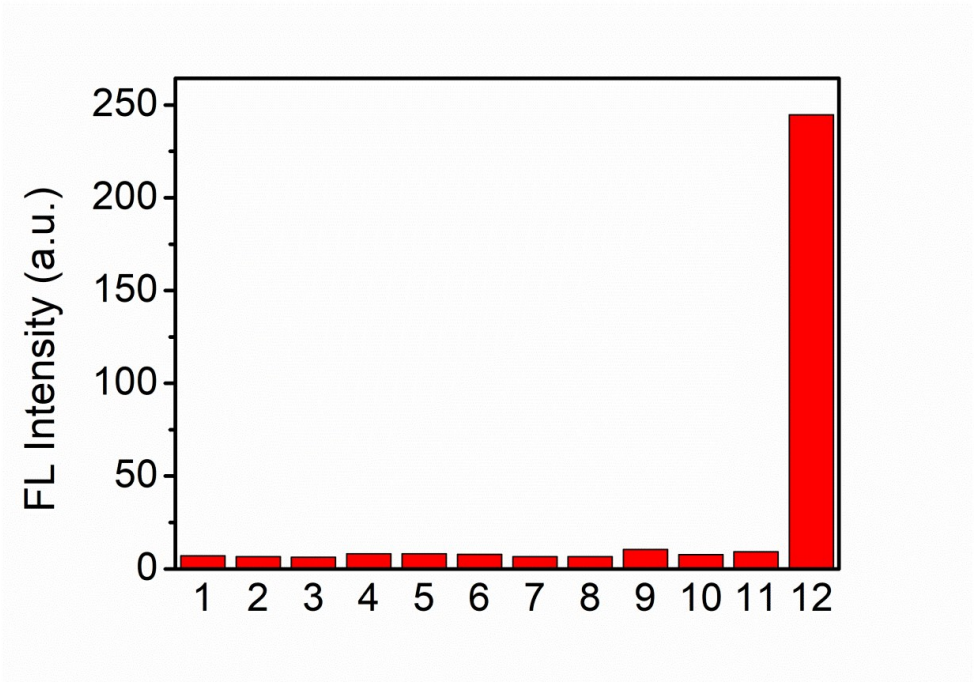


Figure S14. ALP-InD exhibited high specificity for ALP activity in buffer (DMSO/Tris-HCl, v/v, 4:6, pH 7.4) at 37 ^{0}C. The fluorescence intensity at 900 nm was selected for the selectivity discussion. (1) BSA (10 mg/mL); (2) NADH (500 μM); (3) NAD(P)H:quinone oxidoreductase isozyme 1 (30 μg/mL); (4) tyrosinase (30 μg/mL); (5) aprotinin (30 μg/mL); (6) lipase (30 μg/mL); (7) collagenase I (30 μg/mL); (8) NTR (30 μg/mL); (8) Cys (1mM); (9) HCY (1mM); (11) GSH (1mM); (12) ALP (30 μg/mL). Data were collected at 60 min after addition of analytes.

18. Time-dependent spectra changes of NQO-InD in the presence of NQO1.

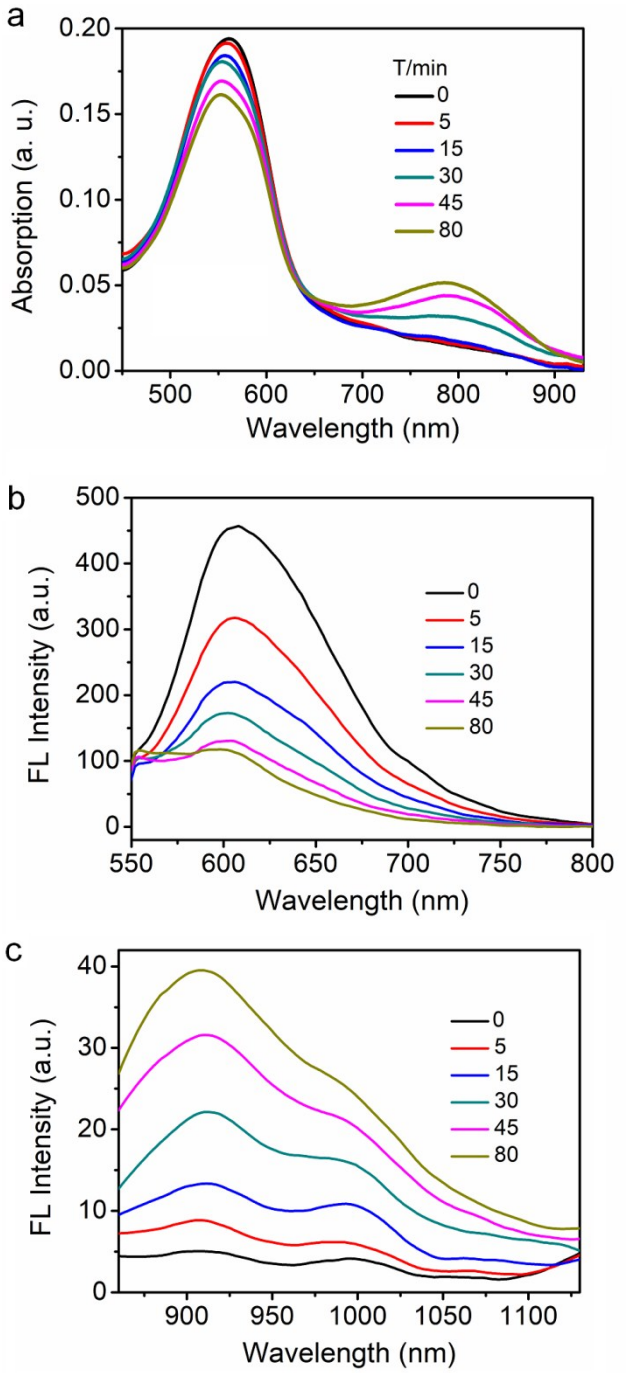


Figure S15. Time-dependent spectra changes of NQO-InD in the presence of NQO1. a) Absorption, b) fluorescence quenching (λ_{ex} = 520 nm) and c) NIR-II fluorescence turn-on (λ_{ex} = 760 nm) of NQO-InD upon addition of NQO1 (60 μg/mL) in buffer (DMSO/PBS, v/v, 2:8, pH 7.4) at 37 ^{0}C.

19. NQO-InD exhibited high specificity for NQO1 activity.

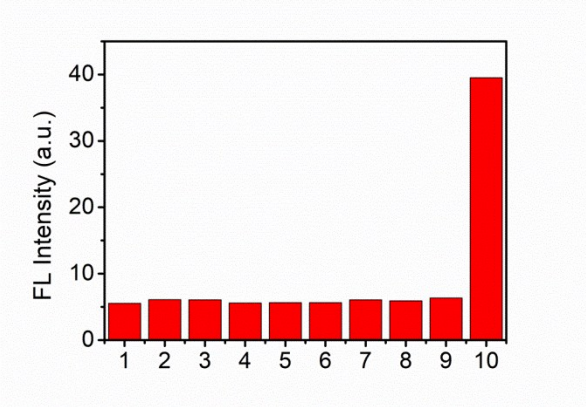


Figure S16. NQO-InD exhibited high specificity for NQO1 activity in buffer (DMSO/PBS, v/v, 2:8, pH 7.4) at 37 ^{0}C. The fluorescence intensity at 910 nm was selected for the selectivity discussion. (1) phosphatase (30 μg/mL); (2) aprotinin (30 μg/mL); (3) lipase (30 μg/mL); (4) collagenase I (30 μg/mL); (5) NTR (30 μg/mL); (6) NADH (500 μM); (7) BSA (10 mg/mL); (8) Cys (1 mM); (9) GSH (1 mM); (10) NAD(P)H:quinone oxidoreductase isozyme 1 (30 μg/mL). Data were collected at 90 min after addition of analytes.

20. Fluorescence quantum yields.

Table S1. The fluorescence quantum yields of the designed probes.

Compound	NTR-ImI	NQO-ImI	ALP-ImI	NTR-InD	NQO-InD	ALP-InD	ImI-BOD-S	InD-BOD-S
ϕ	1.8%	1.7%	1.4%	3.9%	1.9%	5.1%	7.4%	0.048%

Note: The fluorescence quantum yields for NTR-ImI, NTR-InD, ALP-ImI, ALP-InD, ImI-BOD-S and InD-BOD-S were obtained in solution of DMSO/Tris-HCl, v/v, 2:8, pH 7.4, and those for NQO-ImI and NQO-InD in solution of DMSO/PBS, v/v, 2:8, pH 7.4.

21. The stability.

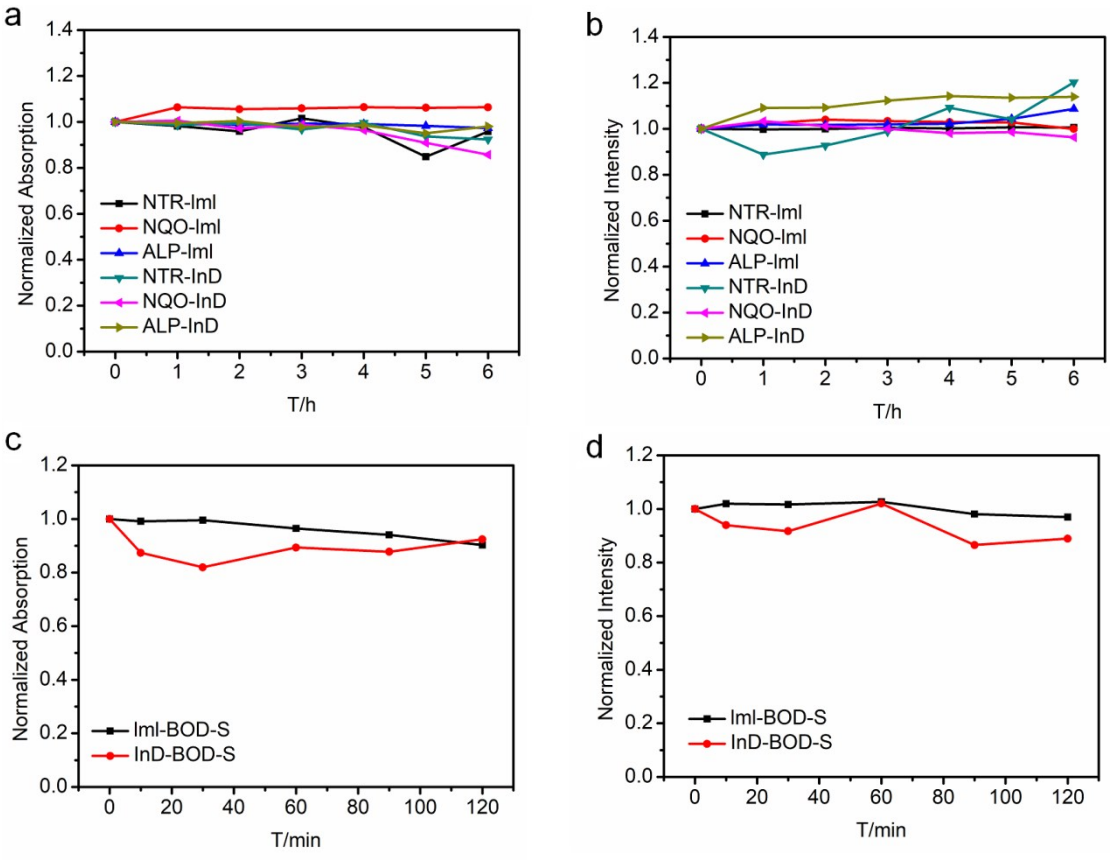


Figure S17. (a) Time dependent absorption changes (normalized) of probes in buffer solutions: NTR-Iml (1 μM), λ_{ab} = 557 nm; NQO-Iml (10 μM), λ_{ab} = 557nm; ALP-Iml (10 μM), λ_{ab} = 557 nm; NIR-InD (1 μM), λ_{ab} = 553 nm; NQO-InD (1 μM), λ_{ab} = 553 nm; ALP-InD (10 μM), λ_{ab} = 553 nm. (b) Time dependent fluorescence changes of probes in buffer solutions: NTR-Iml (1 μM), λ_{em} = 636 nm; NQO-Iml (10 μM), λ_{em} = 636 nm; ALP-Iml (10 μM), λ_{em} = 636 nm; NTR-InD (1 μM), λ_{em} = 595 nm; NQO-InD (1 μM), λ_{em} = 595 nm; ALP-InD (10 μM), λ_{em} = 595 nm. (c) Time dependent absorption changes of products in buffer: Iml-BOD-S, λ_{ab} = 675 nm; InD-BOD, λ_{ab} = 744nm. (d) Time dependent fluorescence changes of products in buffer: Iml-BOD-S, λ_{em} = 720 nm; InD-BOD, λ_{em} = 900nm.

22. The photostability.

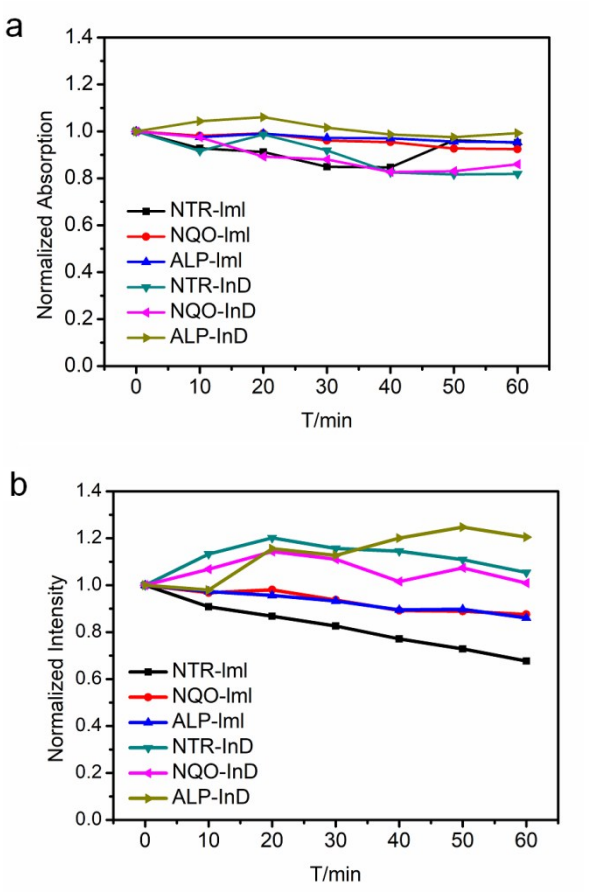


Figure S18. (a) Time course of absorption changes (normalized) of probes in buffer under continuous irradiation at 530nm with an LED lamp (100 mW): NTR-Iml, λ_{ab} = 566 nm; NQO-Iml, λ_{ab} = 566 nm; ALP-Iml, λ_{ab} = 566 nm; NTR-InD, λ_{ab} = 553 nm; NQO-InD, λ_{ab} = 553 nm; ALP-InD, λ_{ab} = 553 nm. (b) Time course of fluorescence changes (normalized) of probes in buffer under continuous irradiation at 530nm with an LED lamp (100 mW): NTR-Iml, λ_{em} = 636 nm; NQO-Iml, λ_{em}= 636 nm; ALP-Iml, λ_{em} = 636 nm; NTR-InD, λ_{em}= 595 nm; NQO-InD, λ_{em} = 595 nm; ALP-InD, λ_{em} = 595 nm.

23. CCK-8 assay and cell imaging.

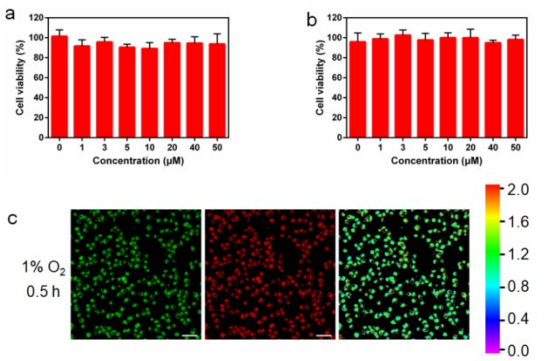


Figure S19. (a) and (b) The cytotoxicity of our designed probes toward living cells by CCK-8 assay. (a) A549 cells with various concentrations of NTR-ImI for 12 h. (b) HT-29 cells with various concentrations of NQO-ImI for 12 h. (c) A549 cells cultured with NTR-ImI (10 μM) under 1% oxygen concentration condition for 0.5 h.

24. The superiority of NIR-II over NIR-I imaging.

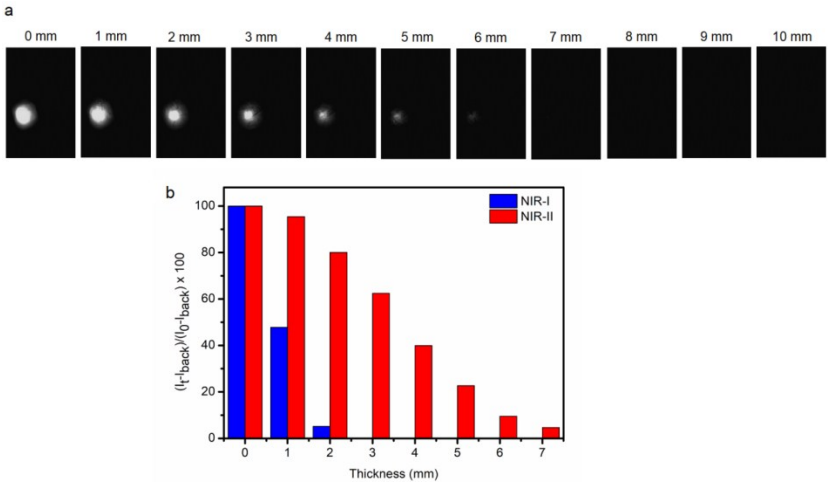


Figure S20. (a) The bright NIR-II signal of NTR-InD treated mice could be clearly visualized even through a 7 mm pork tissue. (b) The correlation of fluorescence signal ratio changes and the thickness of pork tissues. I_t is the fluorescence intensity of samples in different pork tissue thicknesses. I_{back} represents the background fluorescence intensity of pork tissue. I_0 is the fluorescence intensity of the original sample without pork tissue.

25. Targeted cancer visualization in HT-29 tumor-bearing mouse model.

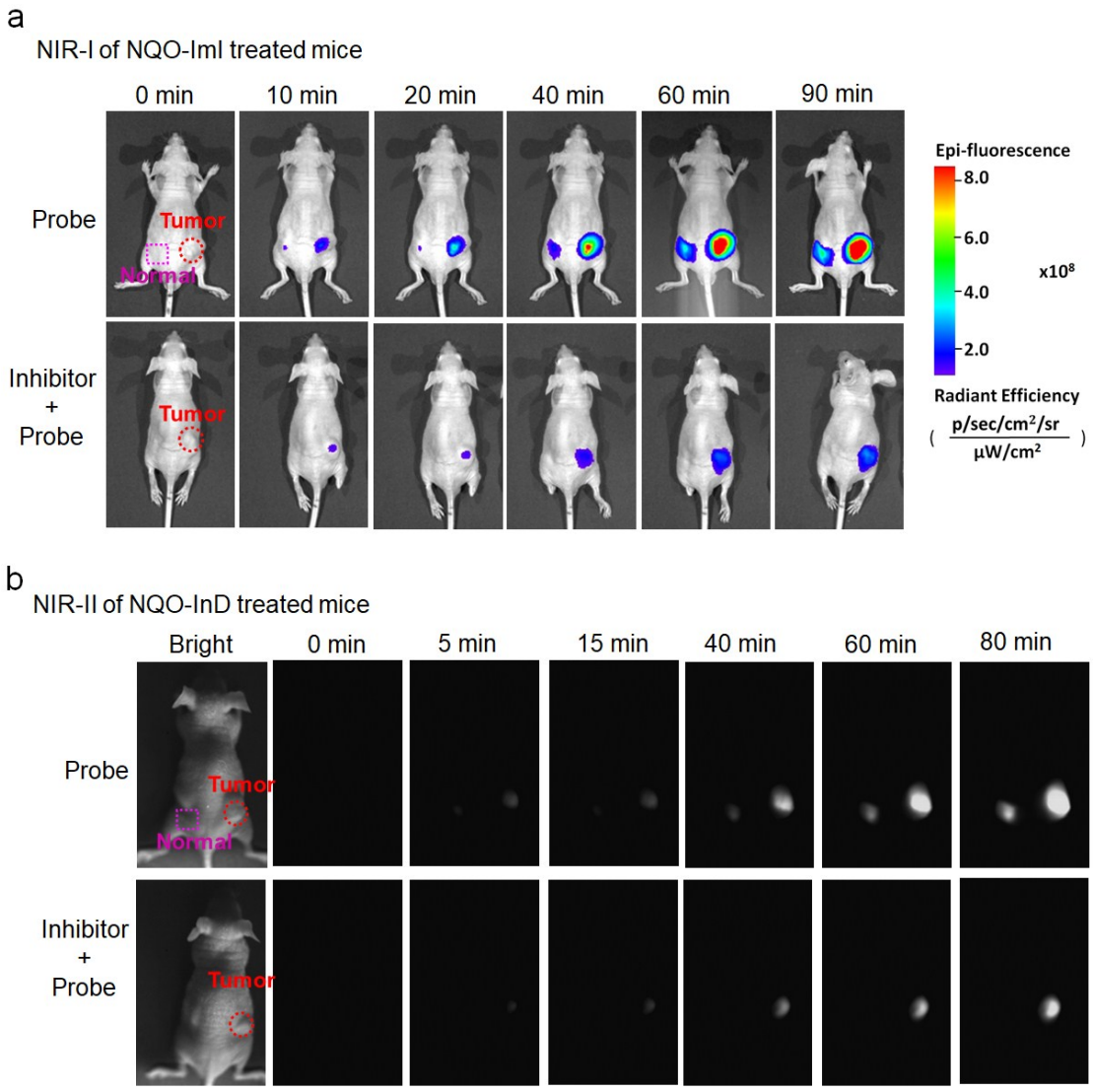


Figure S21. Targeted cancer visualization in HT-29 tumor-bearing mouse model. (a) Time-dependent NIR-I imaging of mice injected with NQO-ImI (30 nmol) or NQO-ImI + dicoumarol (0.3 mmol). (b) Time-dependent NIR-II imaging of mice injected with NQO-InD (30 nmol) or NQO-InD + dicoumarol (0.3 mmol).

26. NMR and MS characterizations.

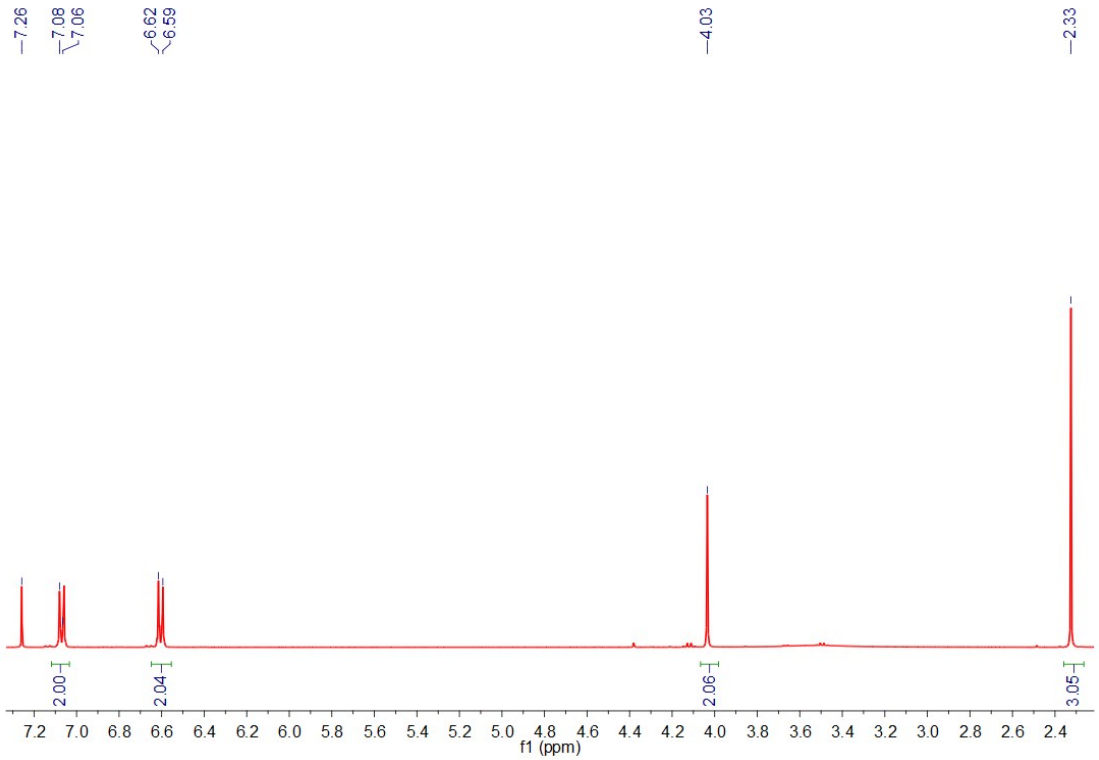


Figure S22. ^{1}H NMR spectrum for compound 2.

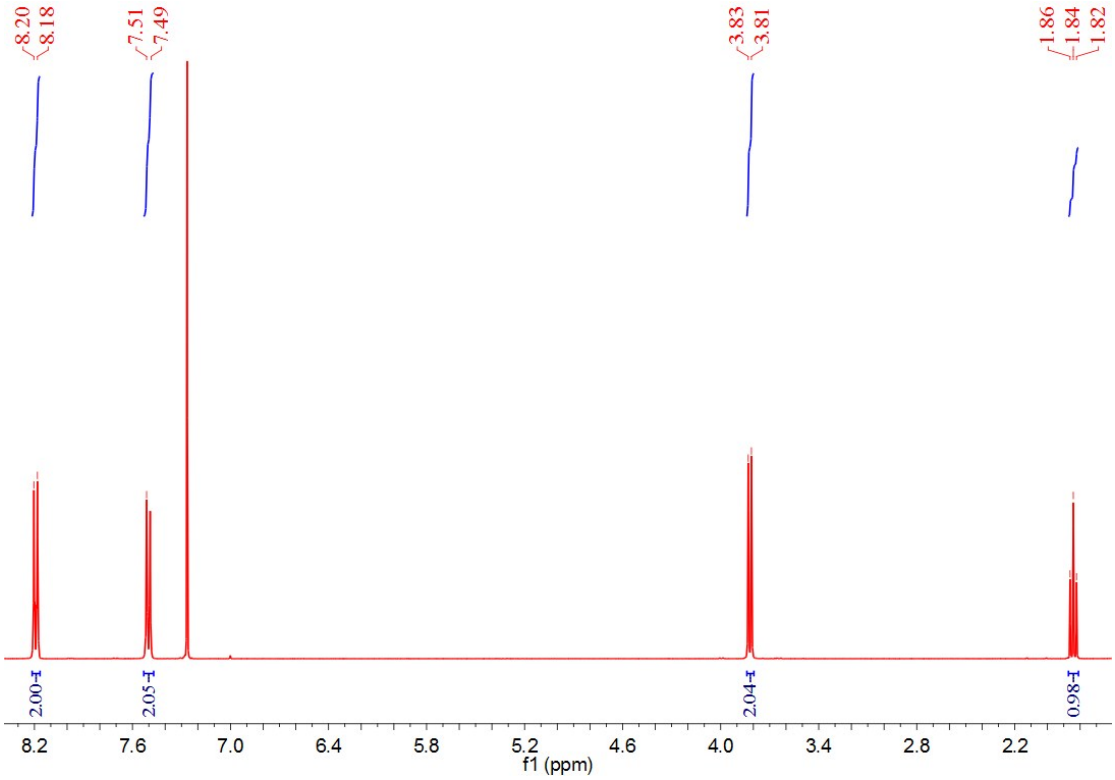


Figure S23. ^{1}H NMR spectrum for compound 3.

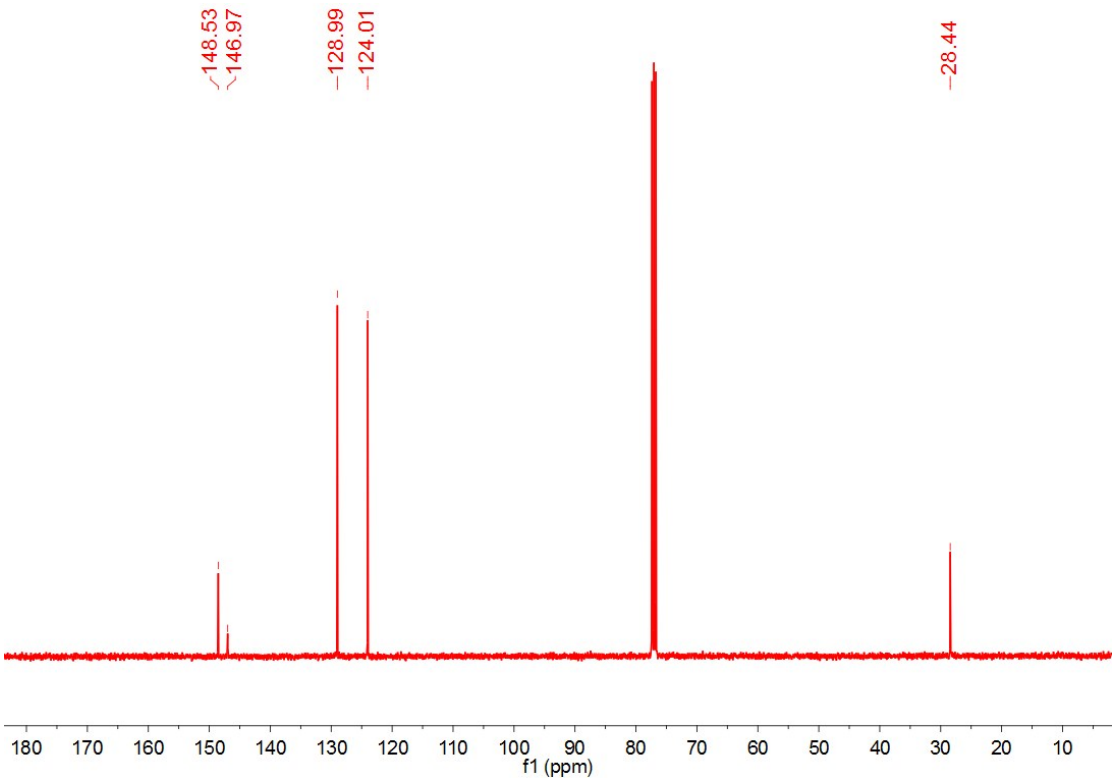


Figure S24. ^{13}C NMR spectrum for compound 3.

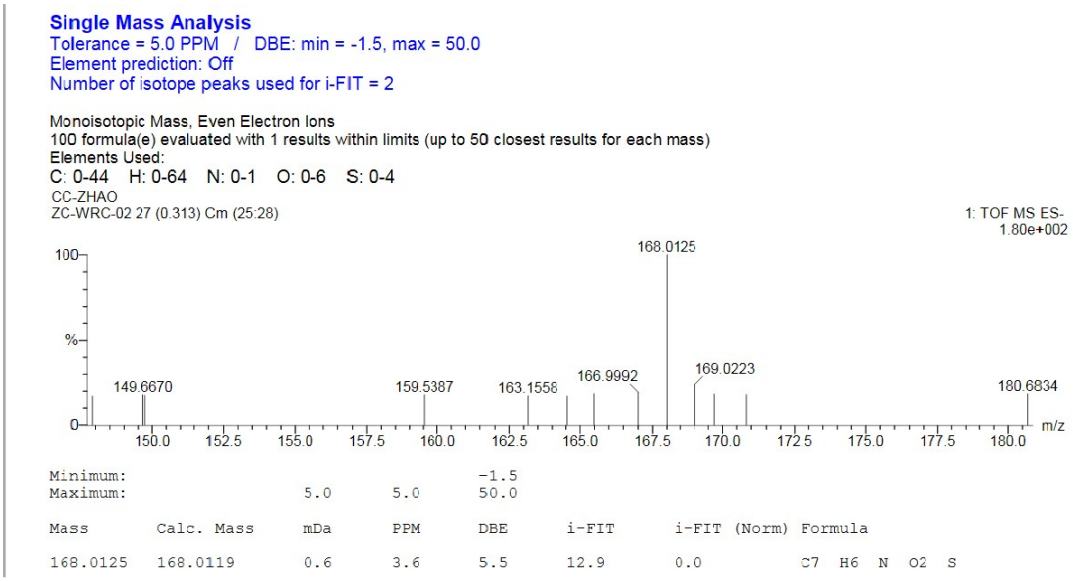


Figure S25. HRMS spectrum for compound 3.

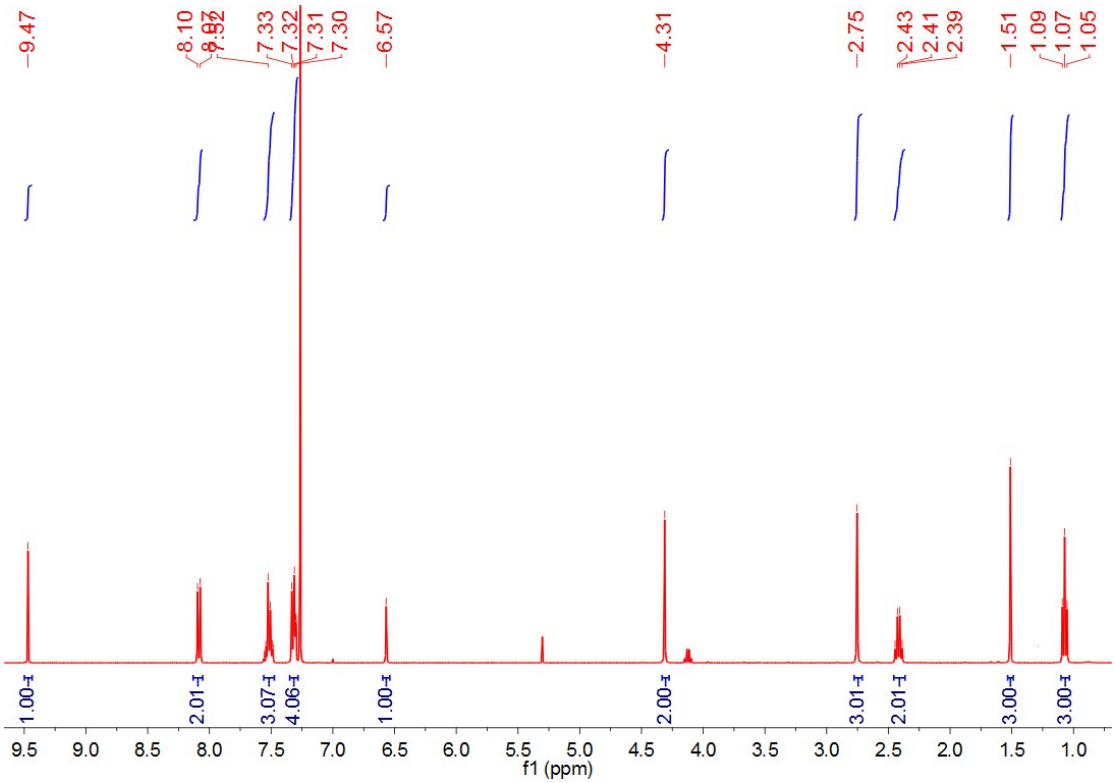


Figure S26. ^{1}H NMR spectrum for compound 5.

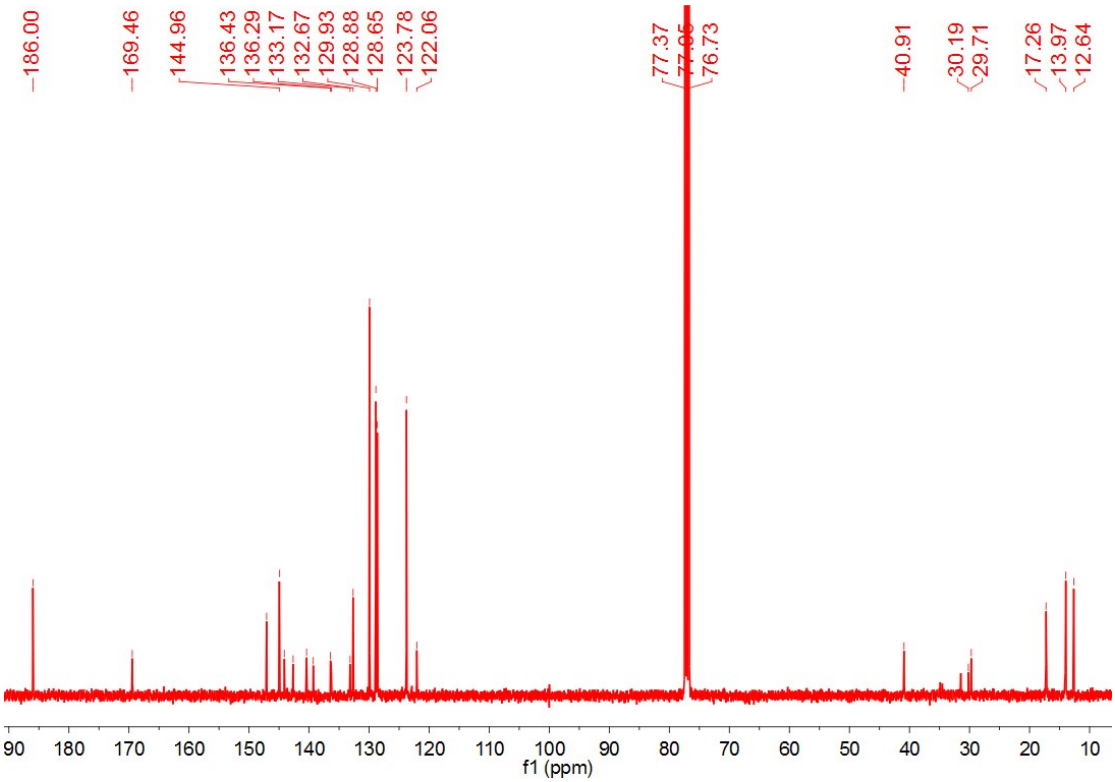


Figure S27. ^{13}C NMR spectrum for compound 5.

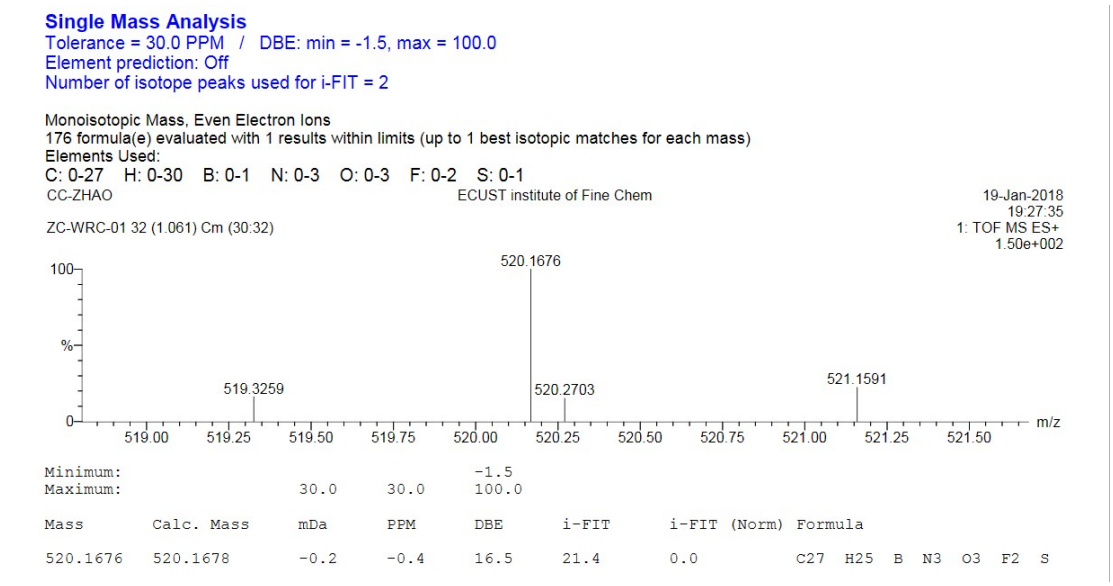


Figure S28. HRMS spectrum for compound 5.

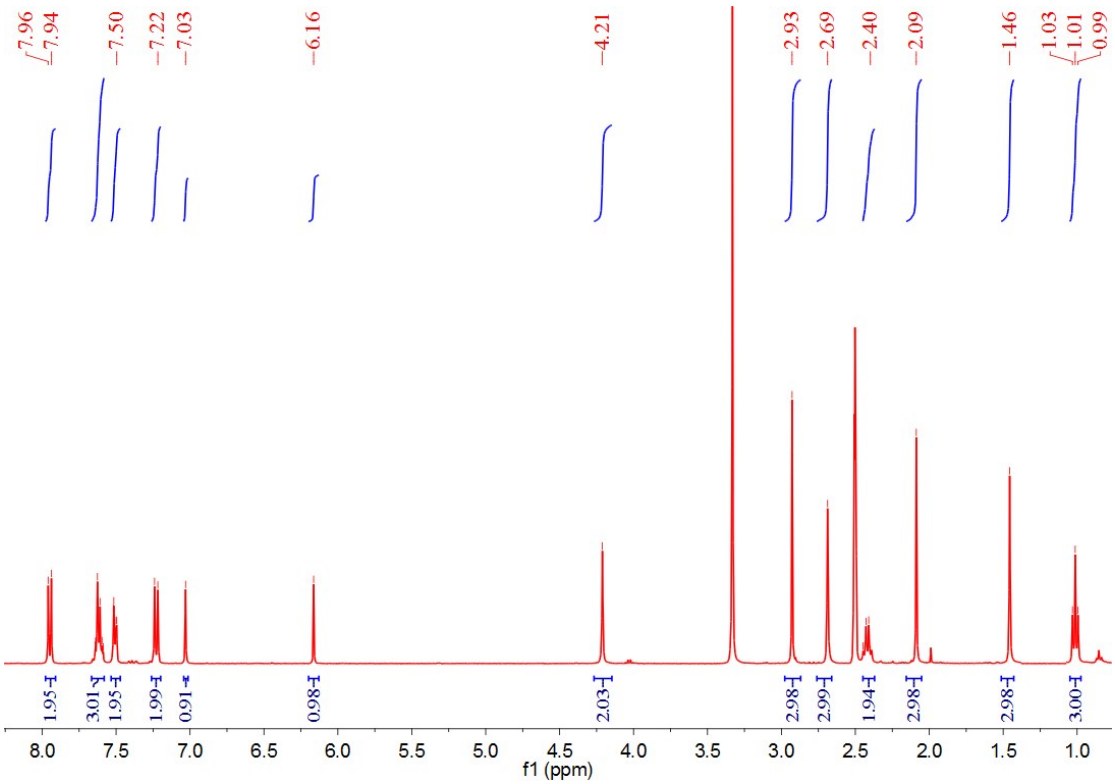


Figure S29. ^{1}H NMR spectrum for compound NTR-ImI.

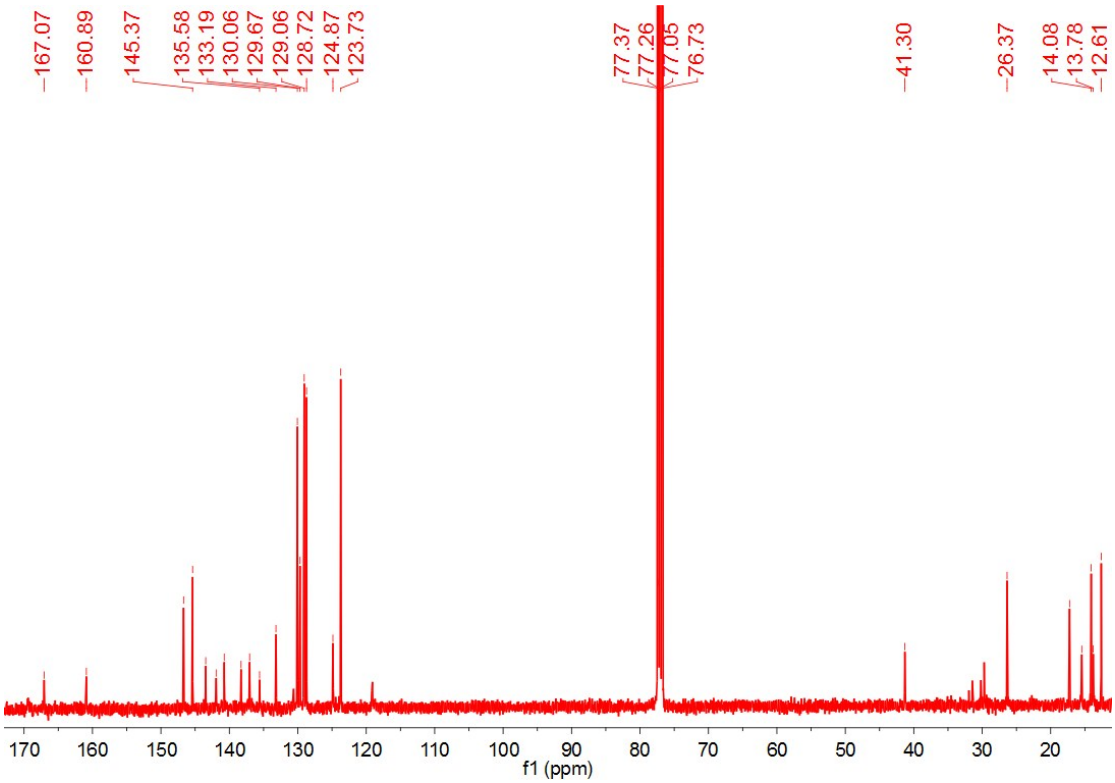


Figure S30. ^{13}C NMR spectrum for compound NTR-ImI.

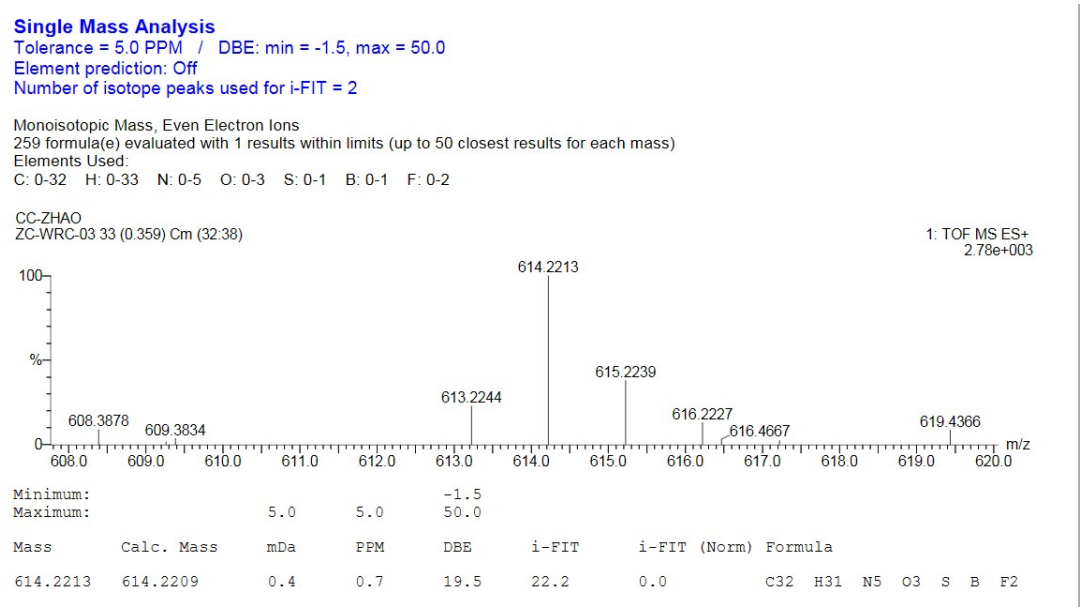


Figure S31. HRMS spectrum for compound NTR-ImI.

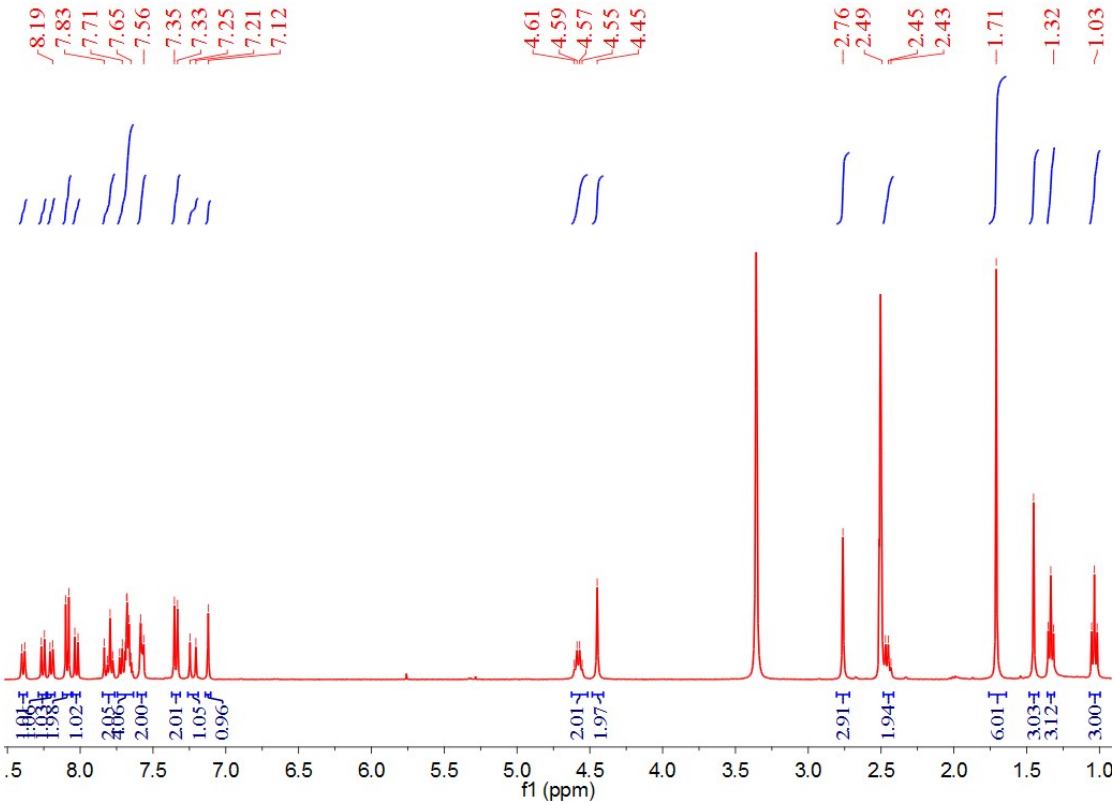


Figure S32. ^{1}H NMR spectrum for compound NTR-InD.

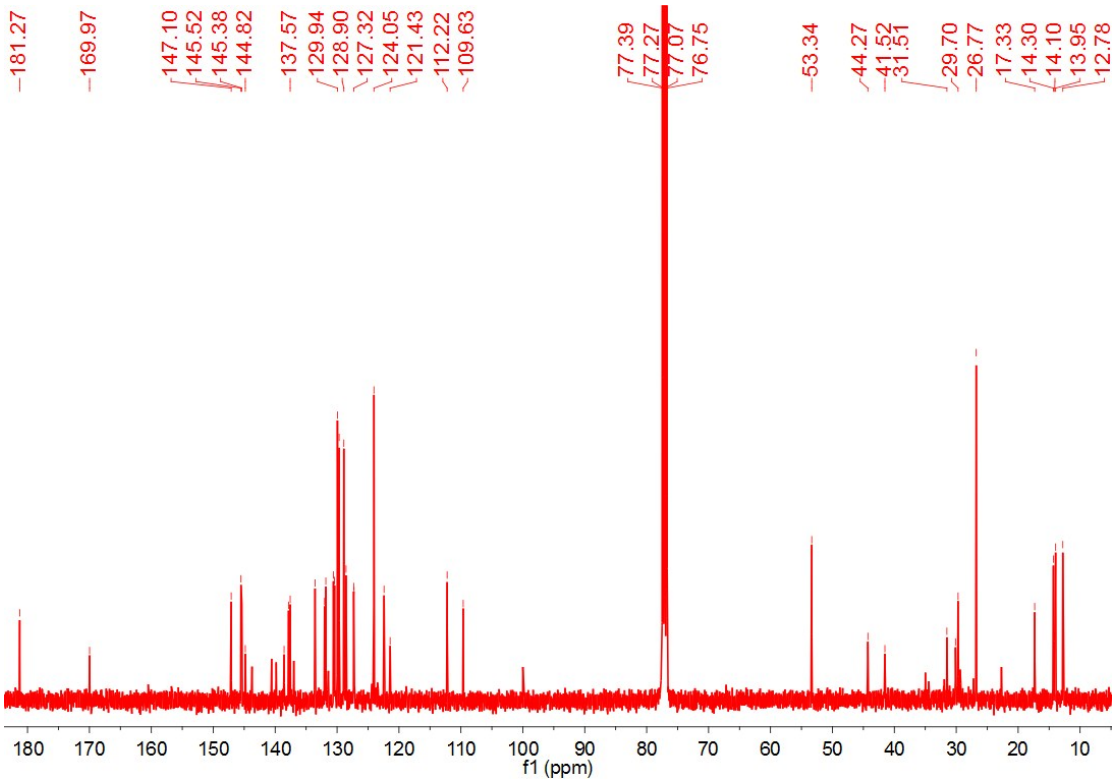
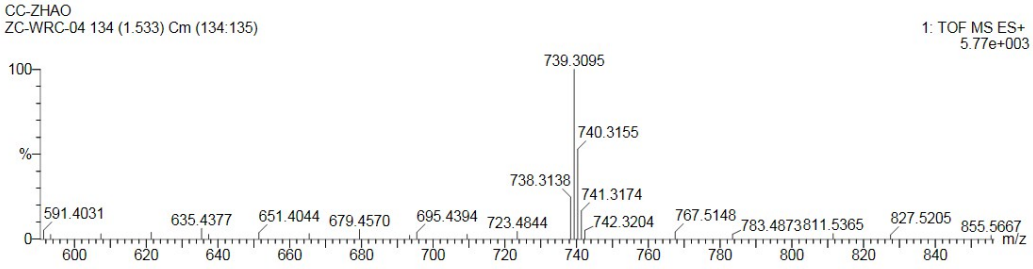


Figure S33. ^{13}C NMR spectrum for compound NTR-InD.

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

Monoisotopic Mass, Even Electron Ions
132 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-45 H: 0-45 B: 0-1 N: 0-5 O: 0-2 F: 0-2 S: 0-1
CC-ZHAO
ZC-WRC-04 134 (1.533) Cm (134:135)



Minimum:				-1.5									
Maximum:		5.0	5.0	50.0									
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula						
739.3095	739.3090	0.5	0.7	25.5	24.5	0.0	C44	H42	B	N4	O2	F2	S

Figure S34. HRMS spectrum for compound NTR-InD.

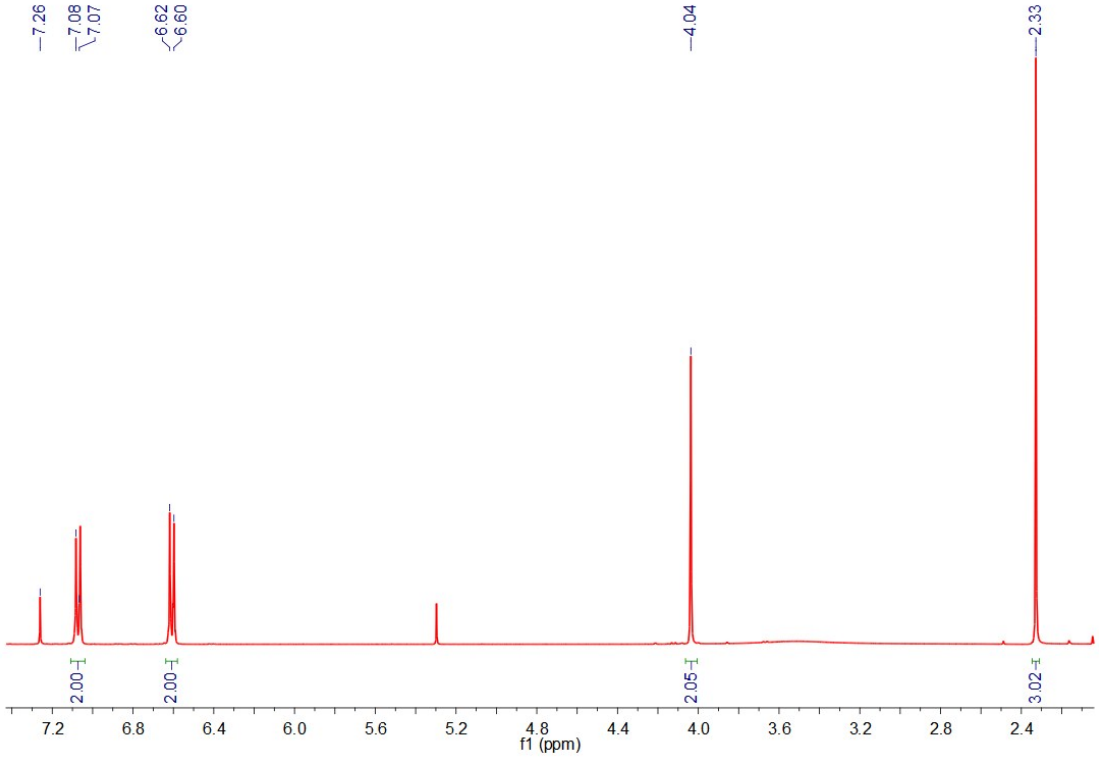


Figure S35. ^{1}H NMR spectrum for compound 8.

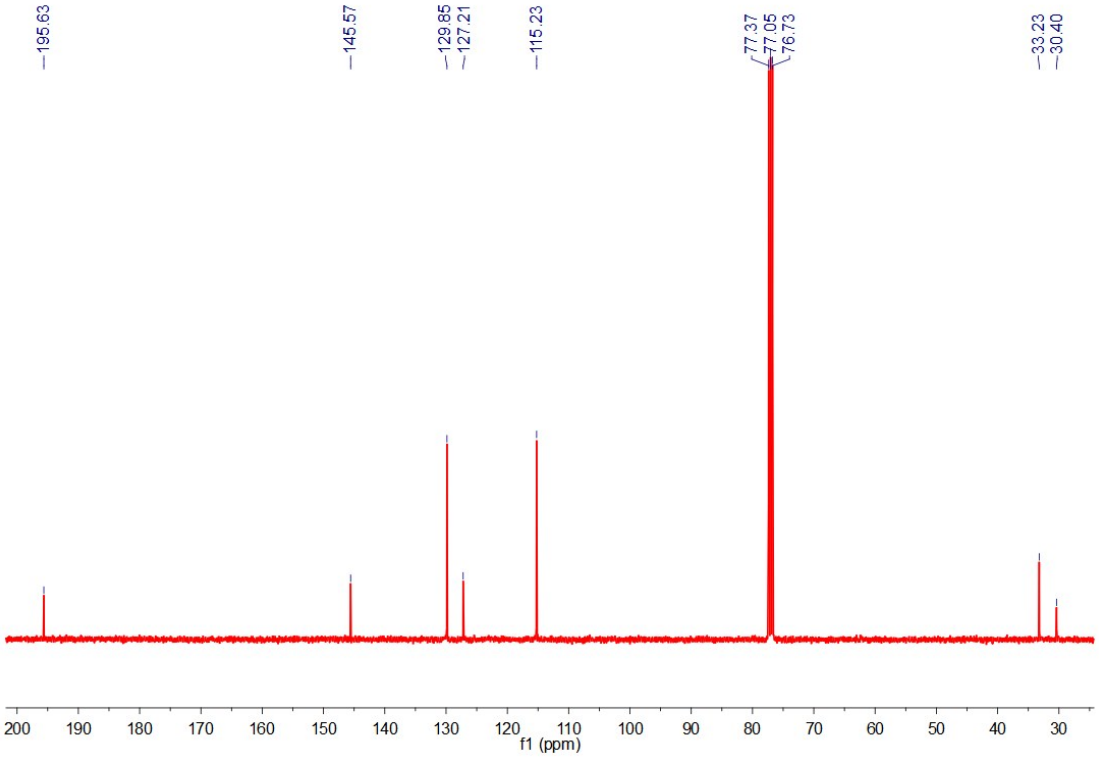


Figure S36. ^{13}C NMR spectrum for compound 8.

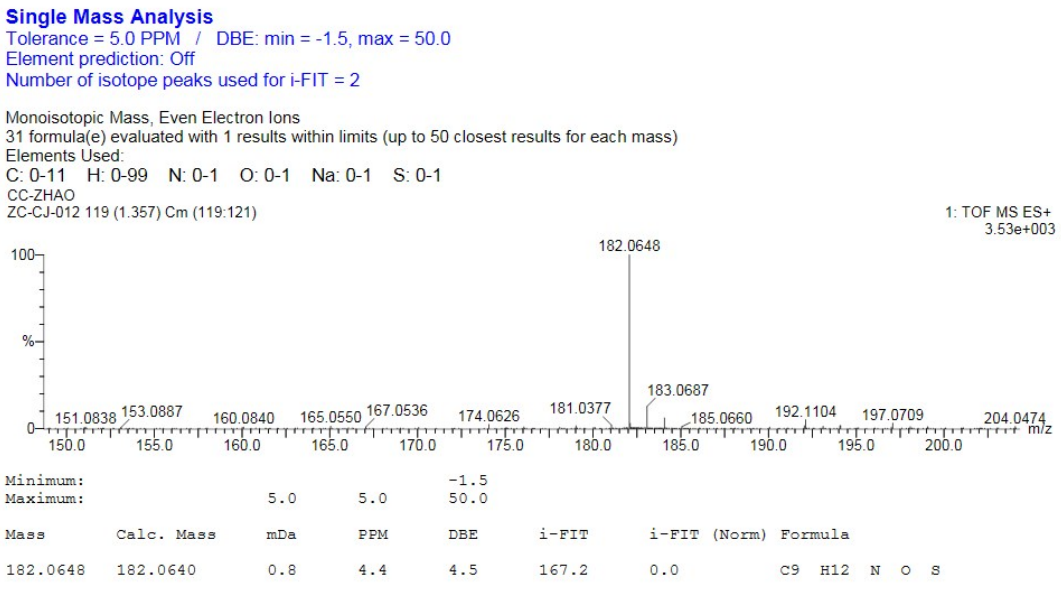


Figure S37. HRMS spectrum for compound 8.

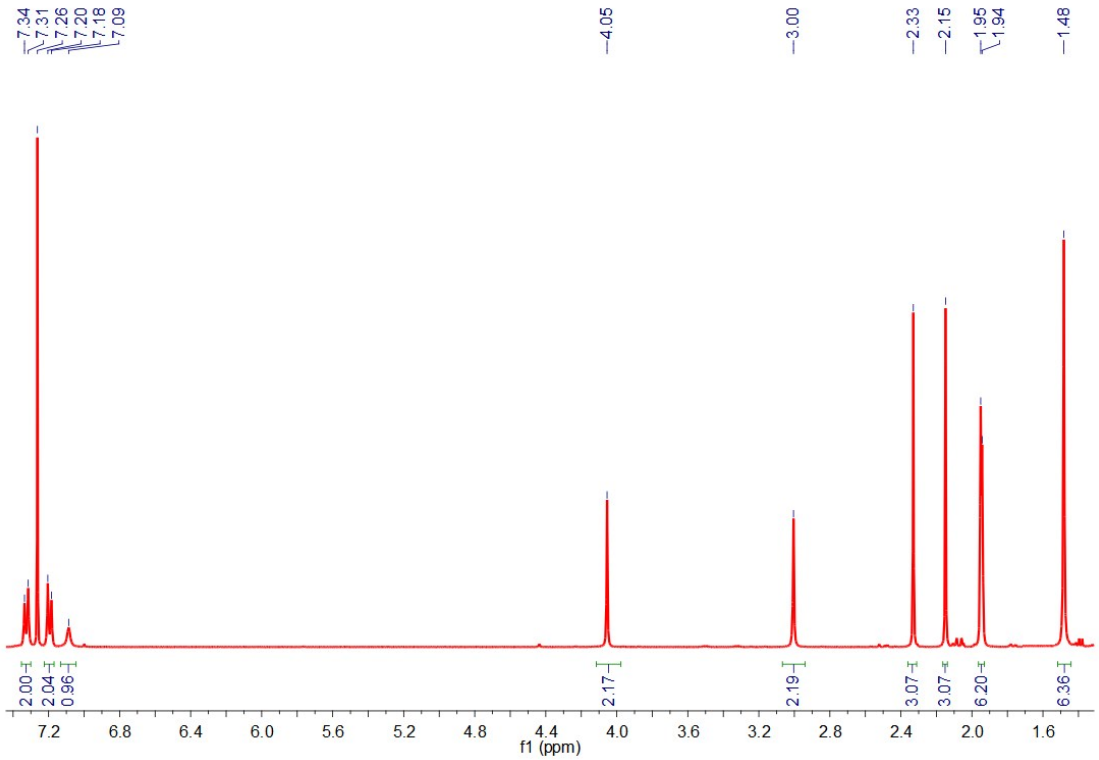


Figure S38. ^{1}H NMR spectrum for compound 10.

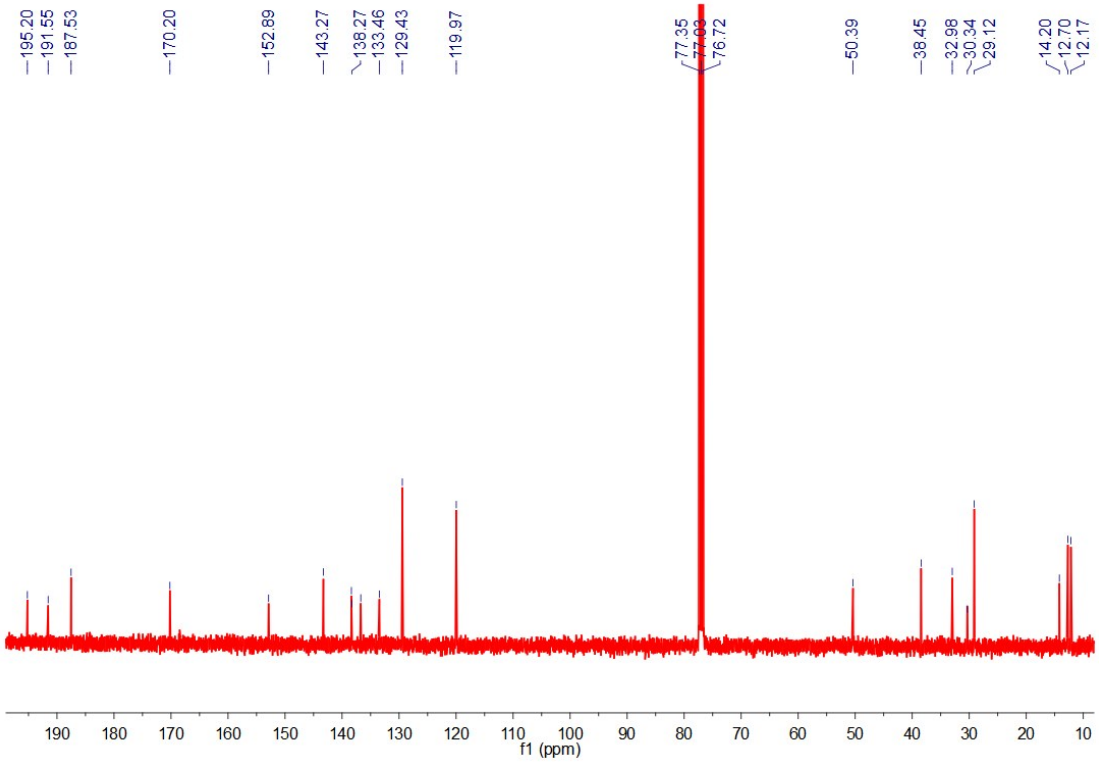
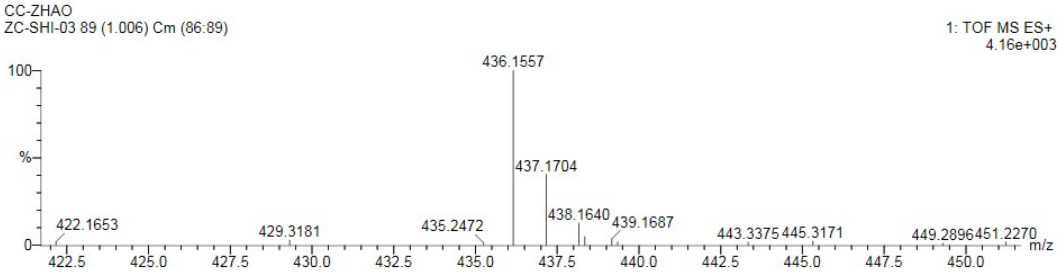


Figure S39. ^{13}C NMR spectrum for compound 10.

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

Monoisotopic Mass, Even Electron Ions
156 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-23 H: 0-30 N: 0-4 O: 0-4 Na: 0-1 S: 0-2
CC-ZHAO
ZC-SHI-03 89 (1.006) Cm (86:89)



Minimum:				-1.5			
Maximum:		5.0	5.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
436.1557	436.1559	-0.2	-0.5	10.5	20.7	0.0	C23 H27 N O4 Na S

Figure S40. HRMS spectrum for compound 10.

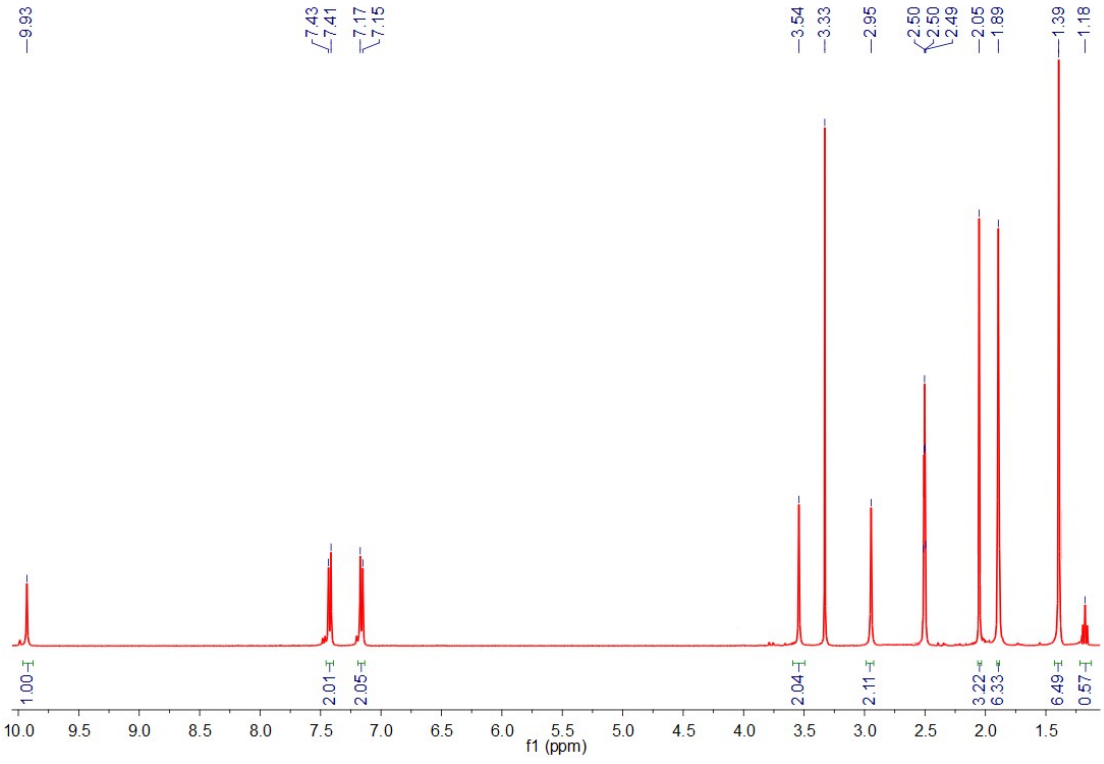
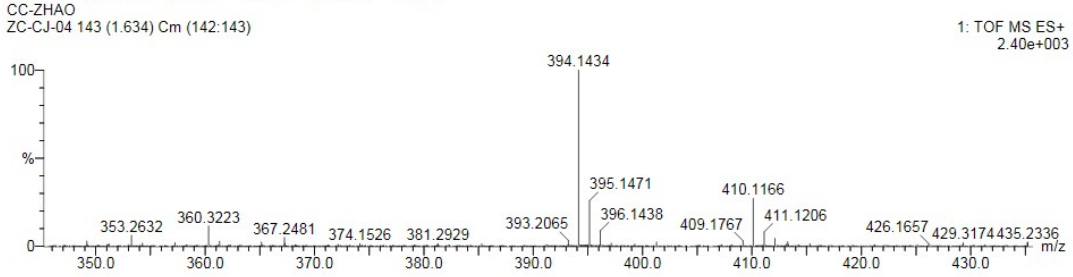


Figure S41. ^{1}H NMR spectrum for compound 11.

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

Monoisotopic Mass, Even Electron Ions
126 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-23 H: 0-30 N: 0-4 O: 0-4 Na: 0-1 S: 0-1
CC-ZHAO
ZC-CJ-04 143 (1.634) Cm (142:143)



Minimum:				-1.5			
Maximum:		5.0	5.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
394.1434	394.1453	-1.9	-4.8	9.5	102.0	0.0	C21 H25 N O3 Na S

Figure S42. HRMS spectrum for compound 11.

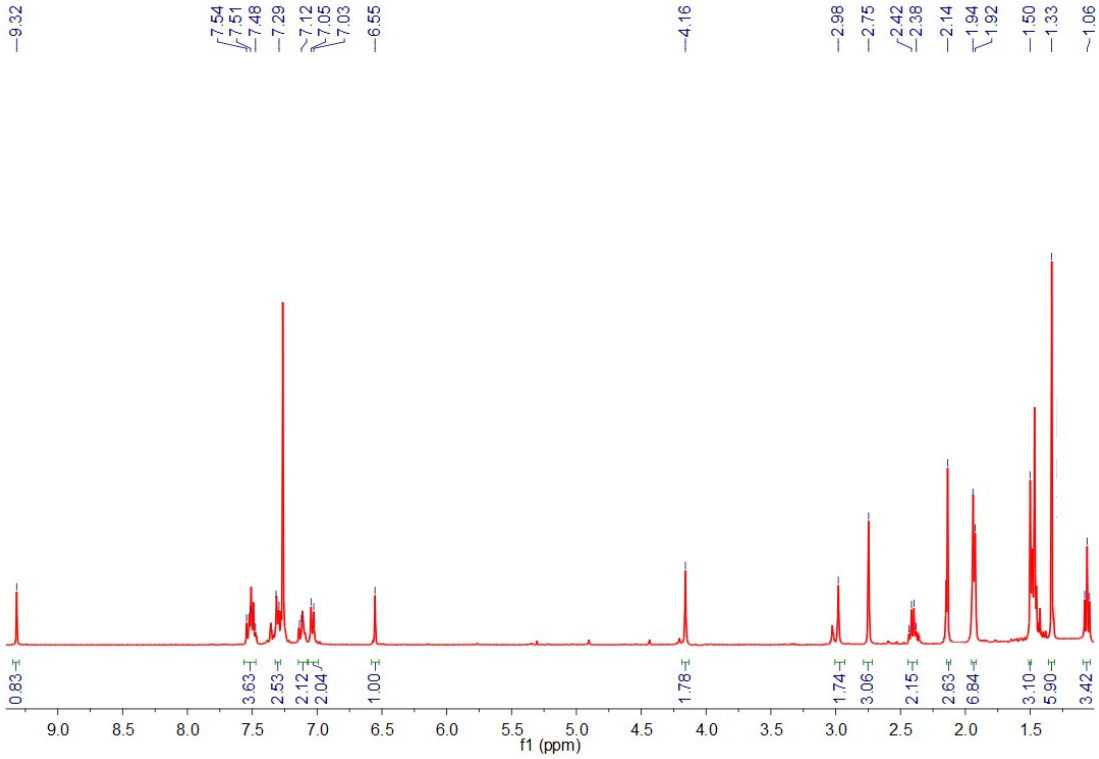


Figure S43. ^{1}H NMR spectrum for compound 12.

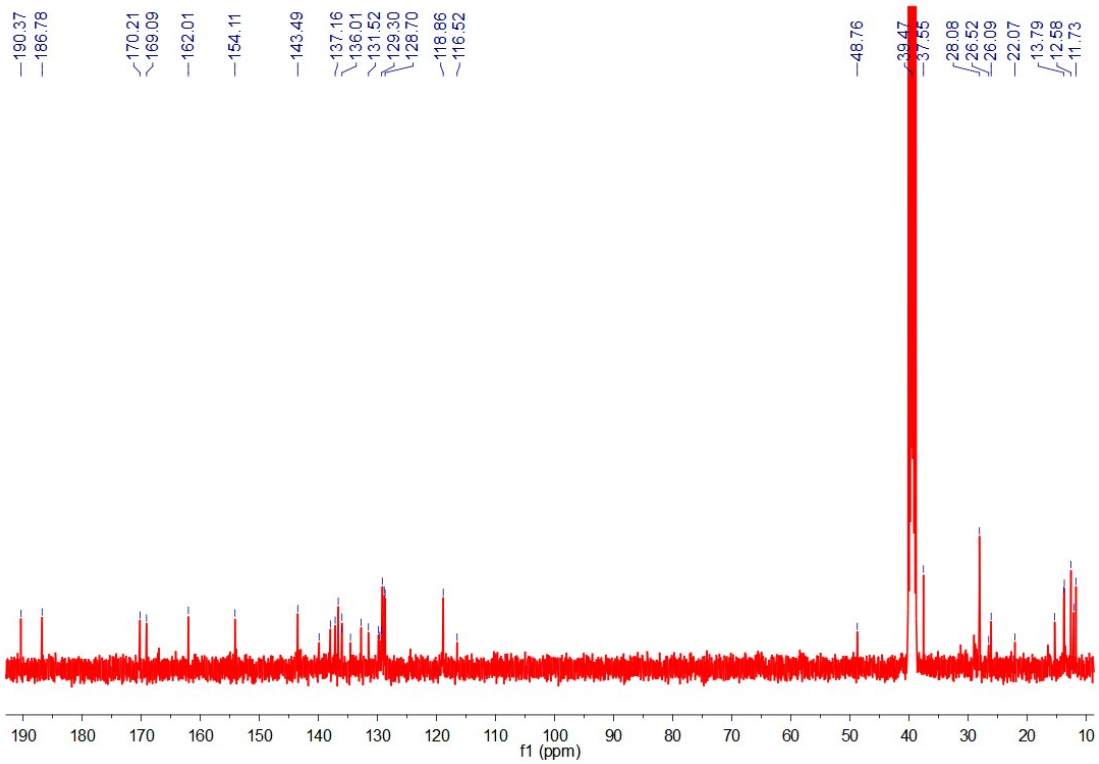
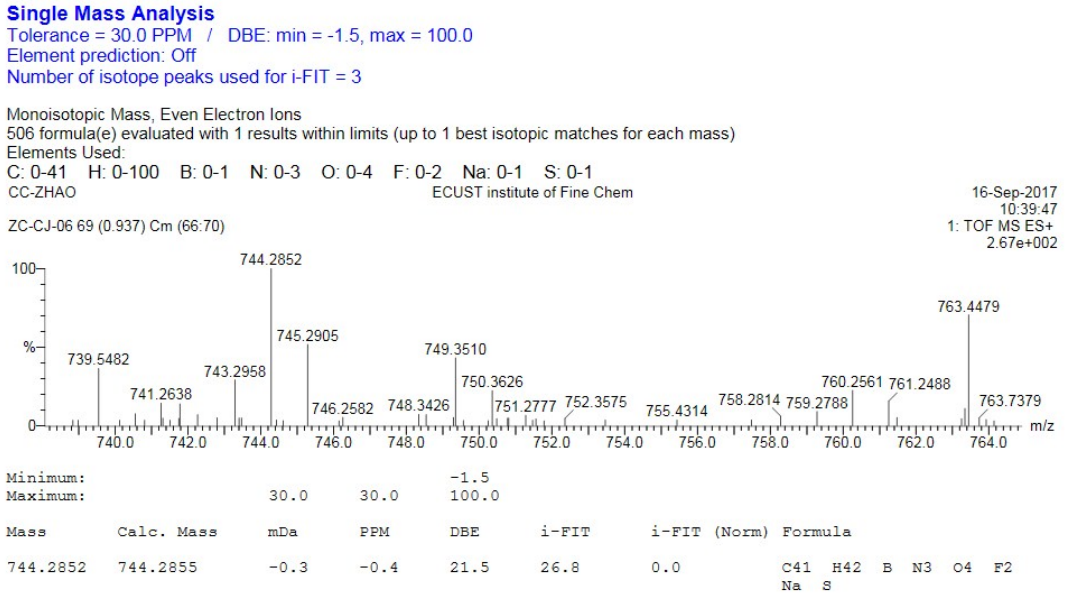


Figure S44. ^{13}C NMR spectrum for compound 12.



Minimum:				-1.5			
Maximum:		30.0	30.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
744.2852	744.2855	-0.3	-0.4	21.5	26.8	0.0	C41 H42 B N3 O4 F2 Na S

Figure S45. HRMS spectrum for compound 12.

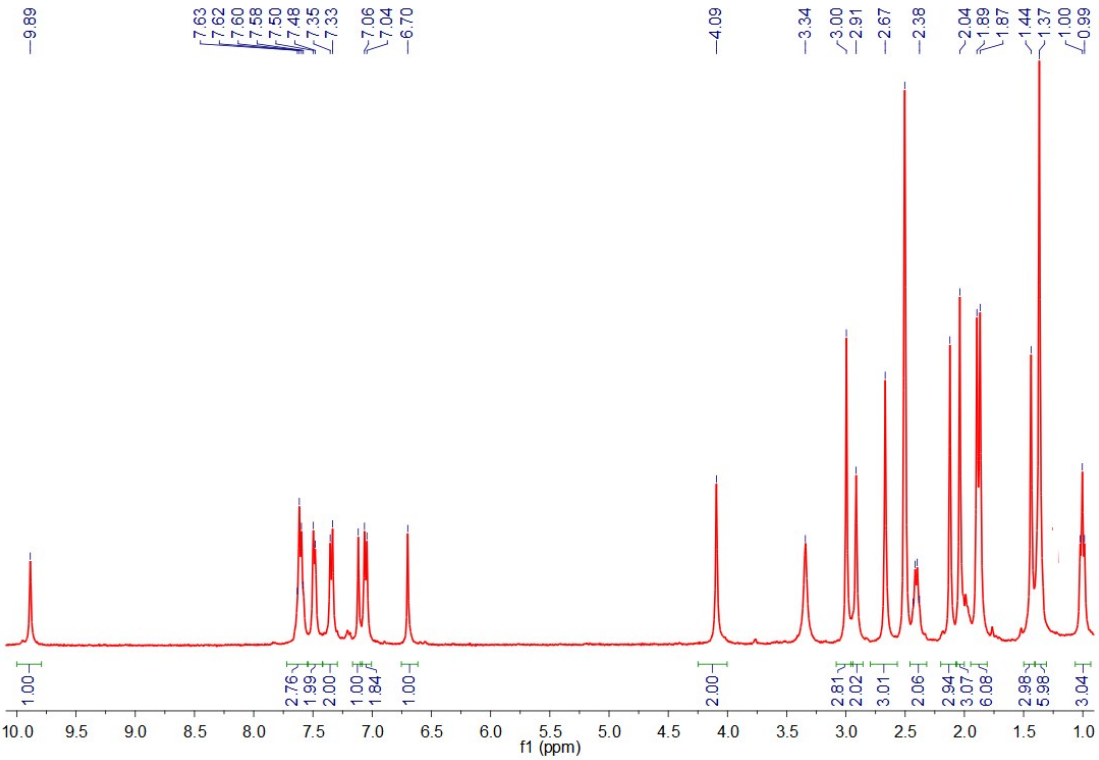


Figure S46. ^{1}H NMR spectrum for compound NQO-ImI.

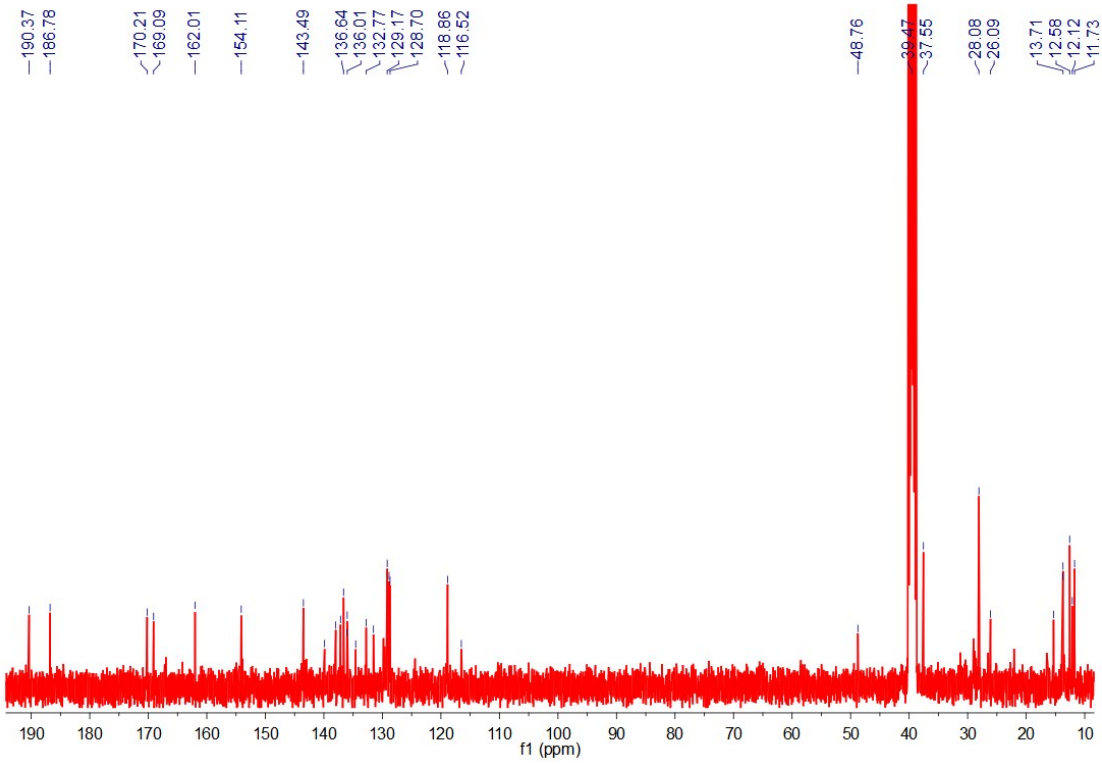
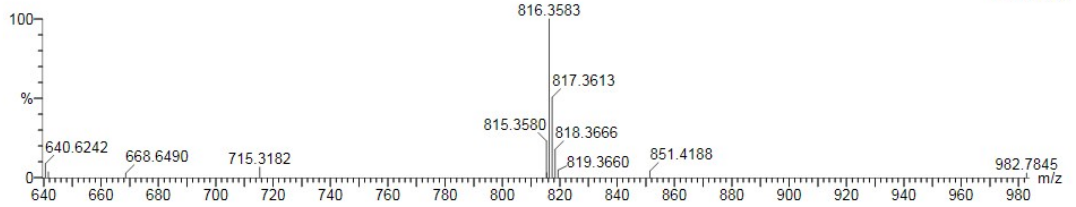


Figure S47. ^{13}C NMR spectrum for compound NQO-ImI.

Single Mass Analysis
Tolerance = 30.0 PPM / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
396 formula(e) evaluated with 1 results within limits (up to 1 best isotopic matches for each mass)
Elements Used:
C: 0-46 H: 0-120 N: 0-5 O: 0-4 S: 0-1 B: 0-1 F: 0-2

CC-ZHAO ECUST institute of Fine Chem 27-Nov-2017 20:27:27
ZC-CJ-51 26 (0.411) Cm (26:27) 1: TOF MS ES+ 1.71e+003



Minimum:				-1.5			
Maximum:		30.0	30.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
816.3583	816.3566	1.7	2.1	24.5	19.4	0.0	C46 H49 N5 O4 S B F2

Figure S48. HRMS spectrum for compound NQO-ImI.

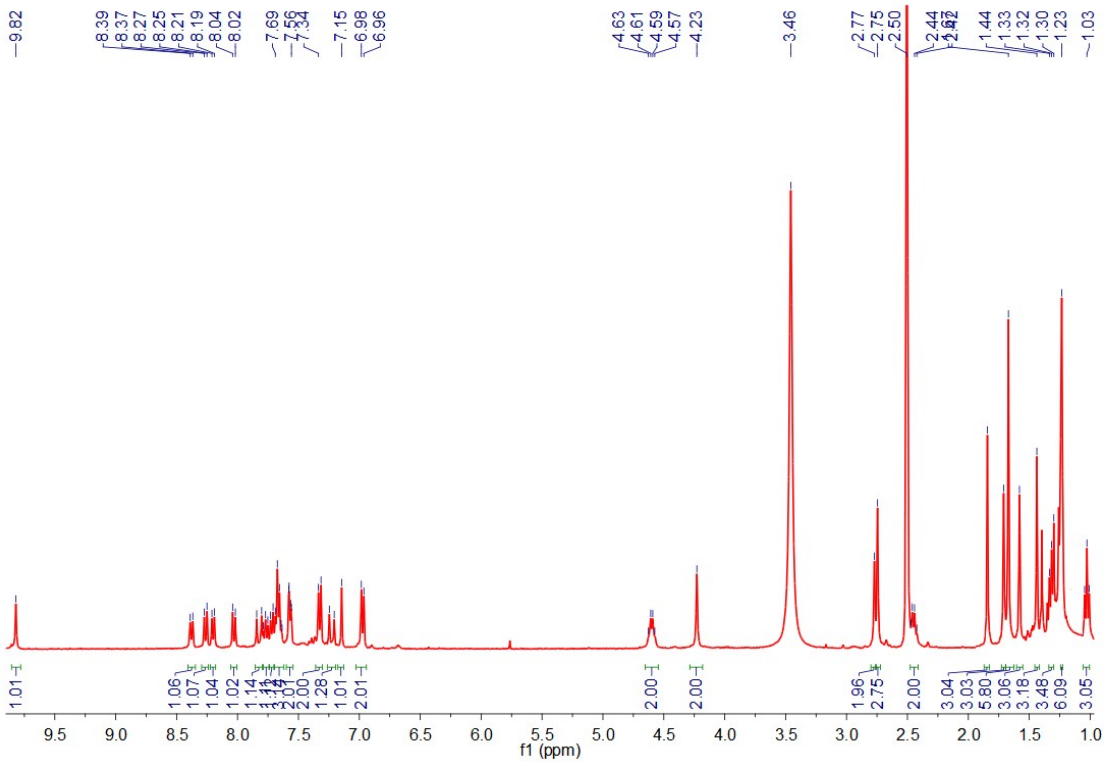


Figure S49. ^{1}H NMR spectrum for compound NQO-InD.

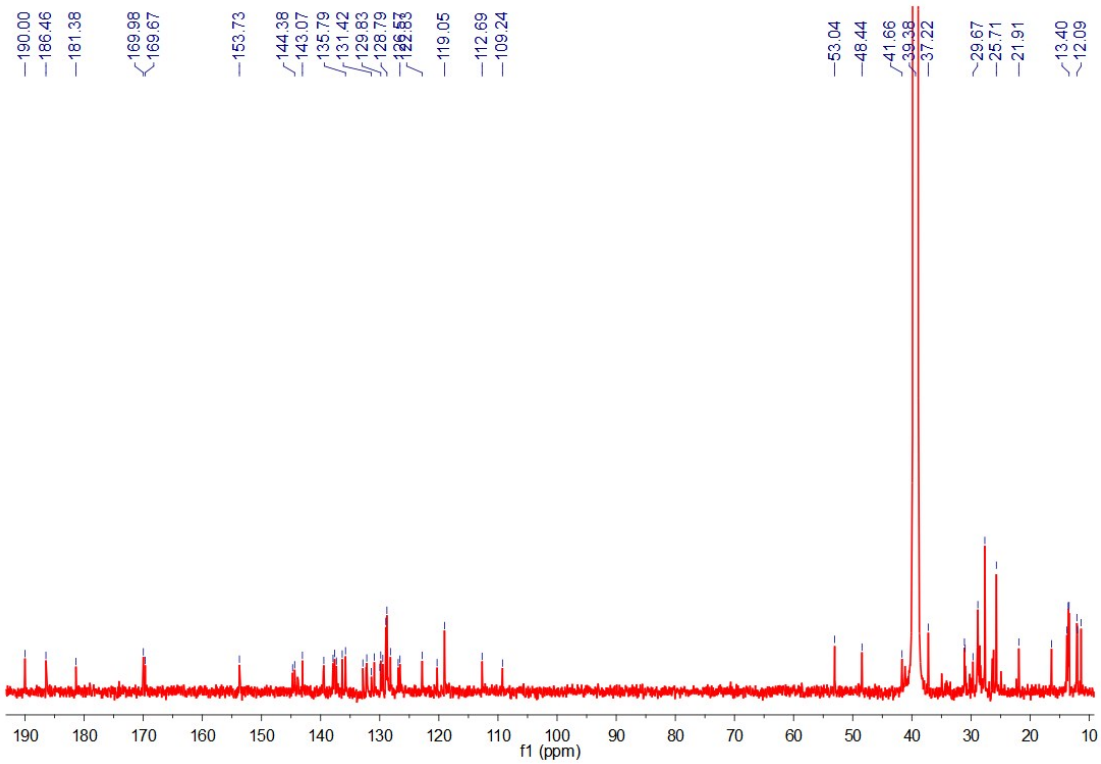


Figure S50. ^{13}C NMR spectrum for compound NQO-InD.

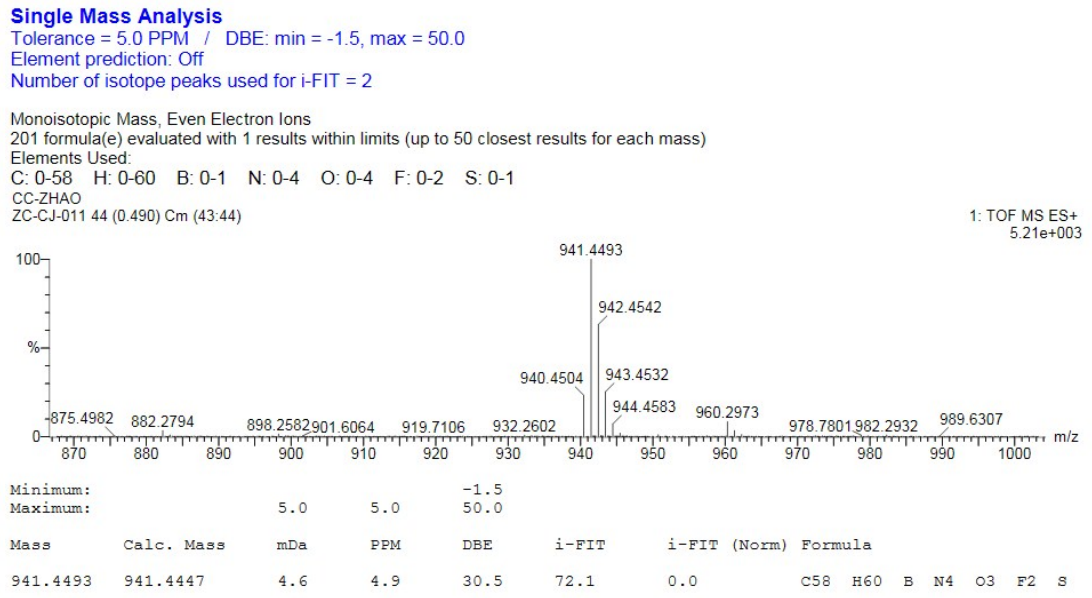


Figure S51. HRMS spectrum for compound NQO-InD.

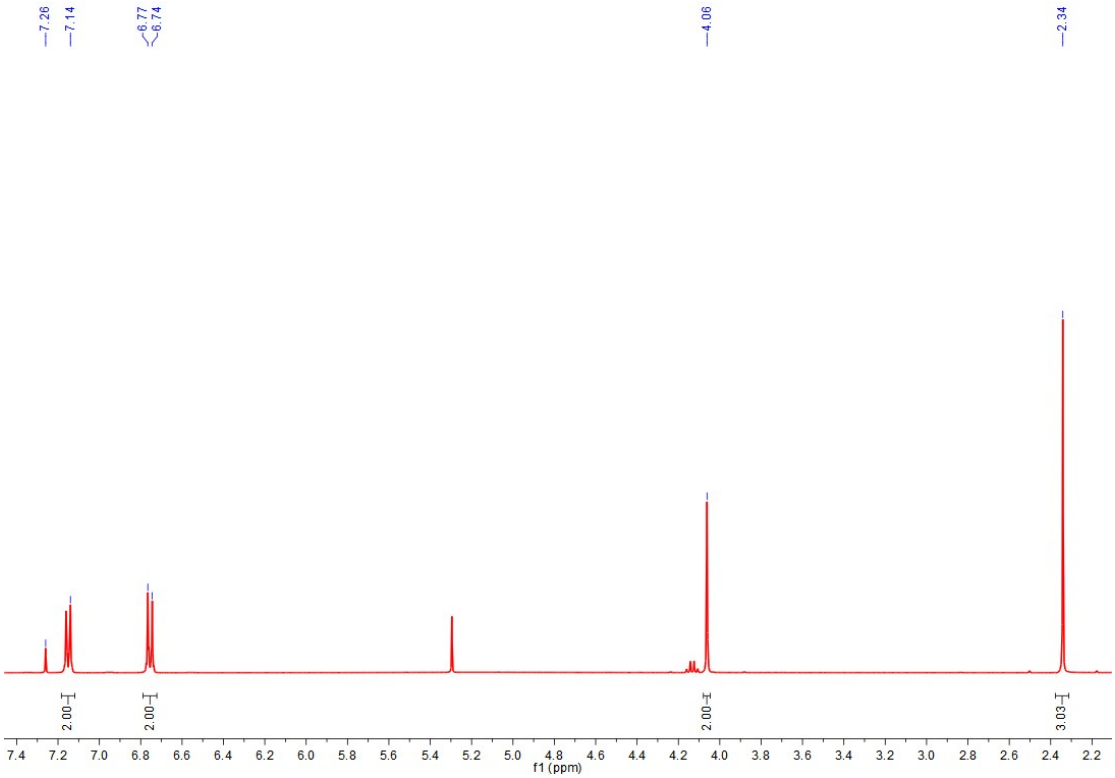


Figure S52. ^{1}H NMR spectrum for compound 13.

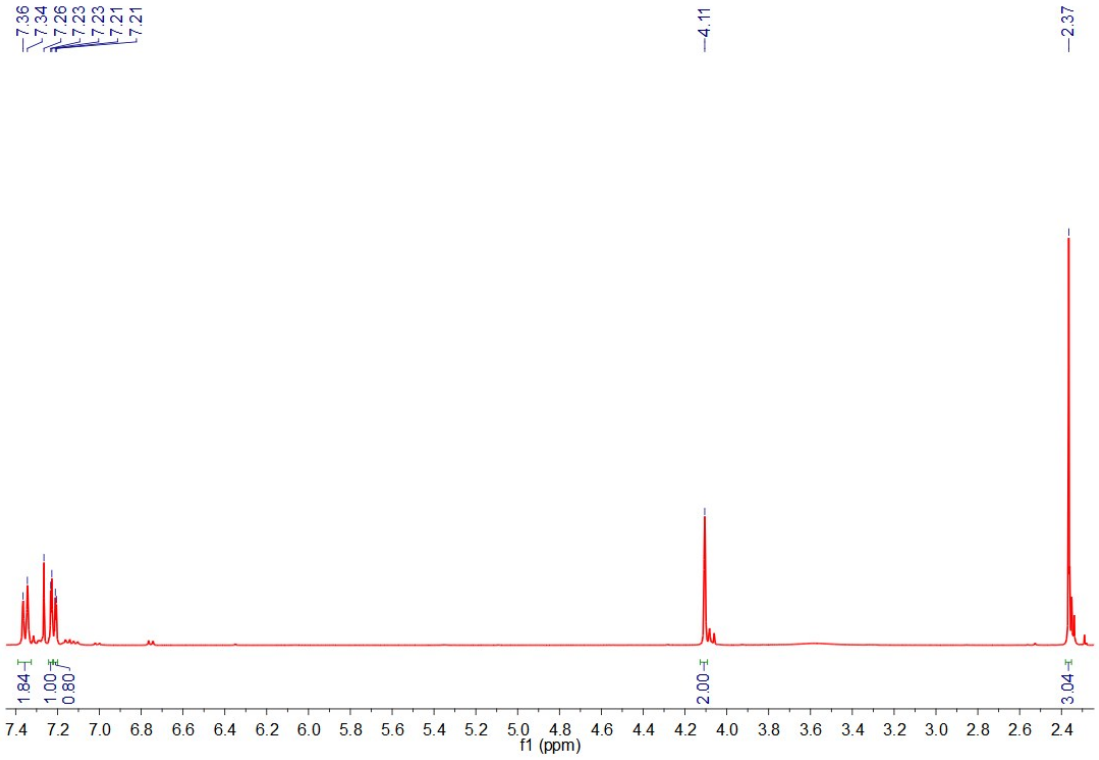


Figure S53. ^{1}H NMR spectrum for compound 14.

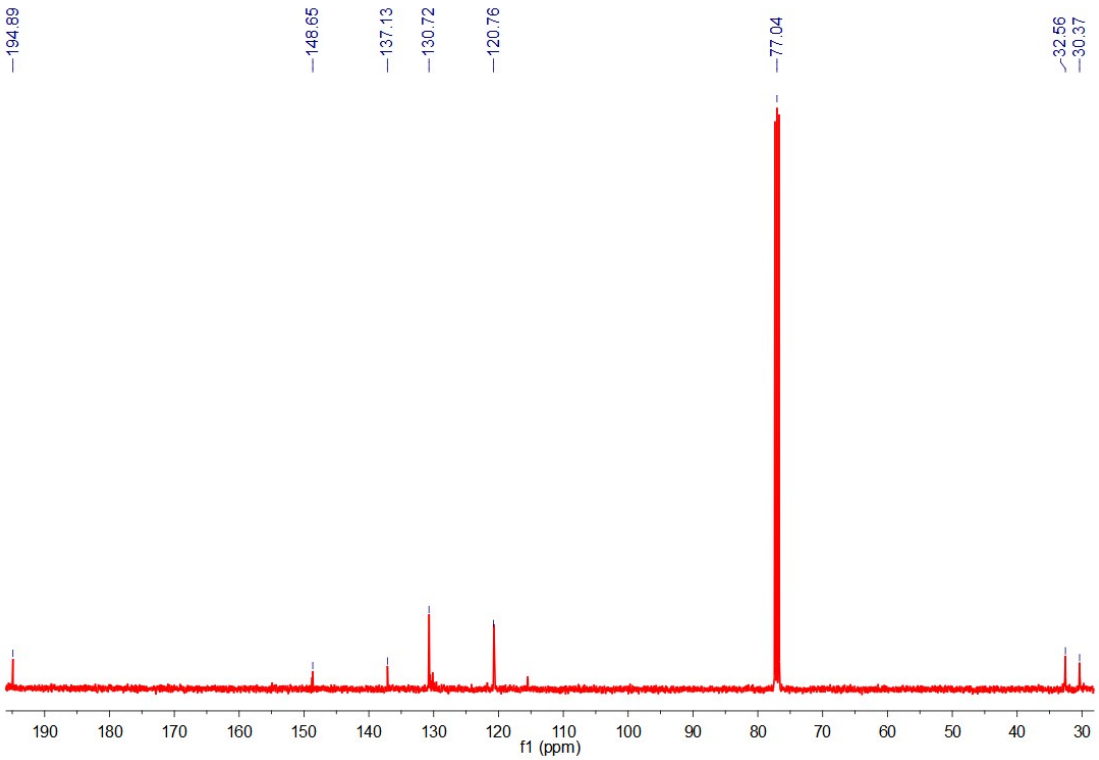
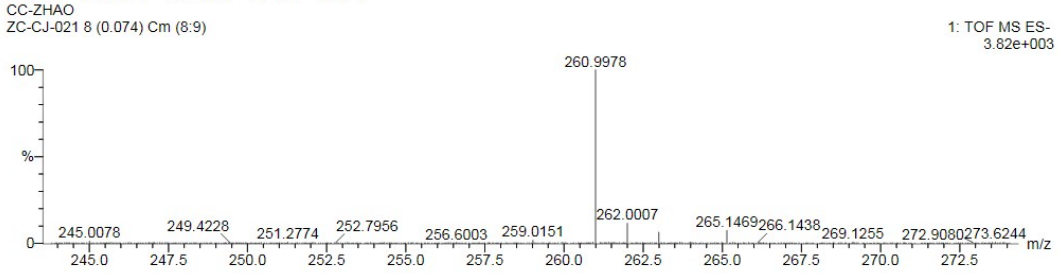


Figure S54. ^{13}C NMR spectrum for compound 14.

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

Monoisotopic Mass, Even Electron Ions
172 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-15 H: 0-70 O: 0-15 P: 0-2 S: 0-1
CC-ZHAO
ZC-CJ-021 8 (0.074) Cm (8:9)



Minimum:				-1.5			
Maximum:		5.0	5.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
260.9978	260.9987	-0.9	-3.4	5.5	130.4	0.0	C9 H10 O5 P S

Figure S55. HRMS spectrum for compound 14.

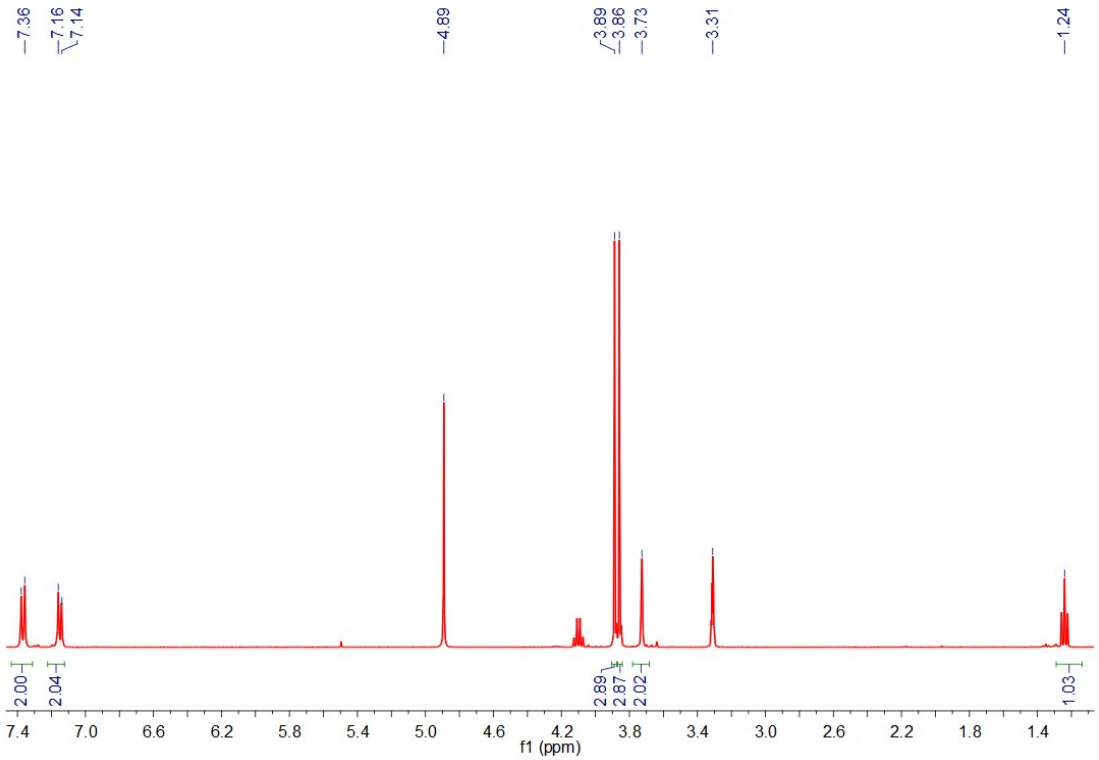


Figure S56. ^{1}H NMR spectrum for compound 15.

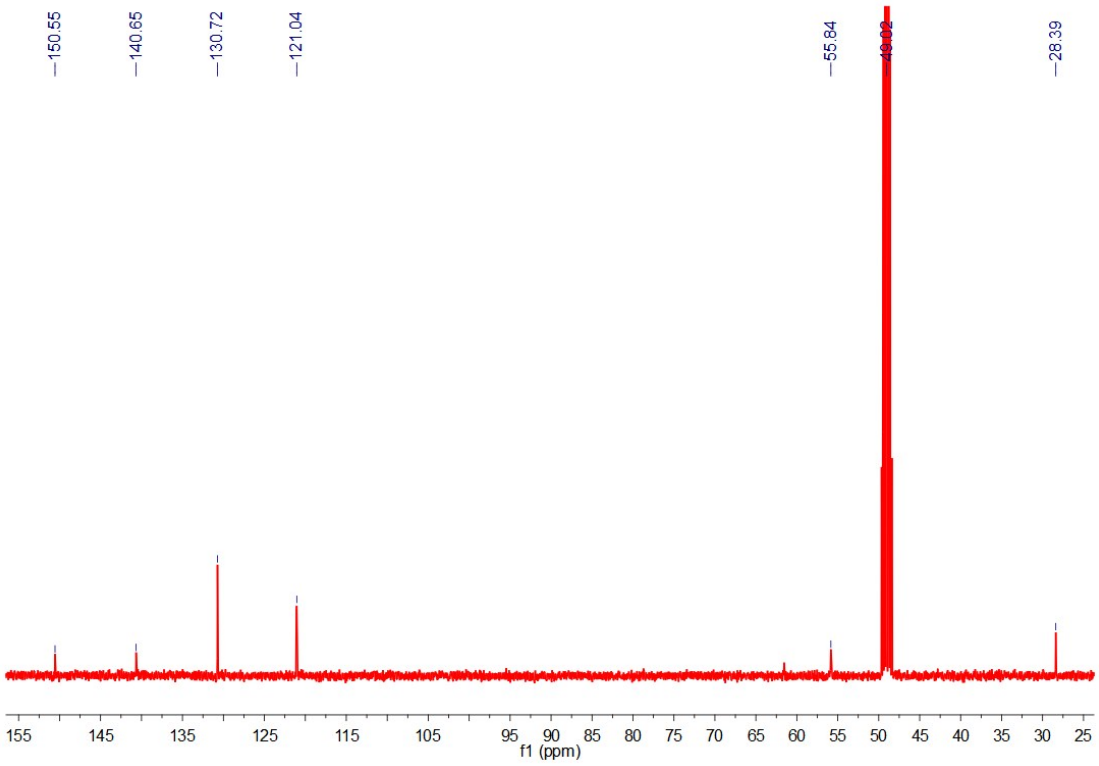


Figure S57. ^{13}C NMR spectrum for compound 15.

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

Monoisotopic Mass, Even Electron Ions
69 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 9-9 H: 0-30 O: 0-5 23Na: 0-1 P: 0-1 S: 0-2
CC-ZHAO
ZC-CJ-022 80 (0.904) Cm (76:90)

Minimum:		5.0	5.0	-1.5			
Maximum:				50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
271.0169	271.0170	-0.1	-0.4	3.5	23.3	0.0	C9 H13 O4 23Na P S

Figure S58. HRMS spectrum for compound 15.

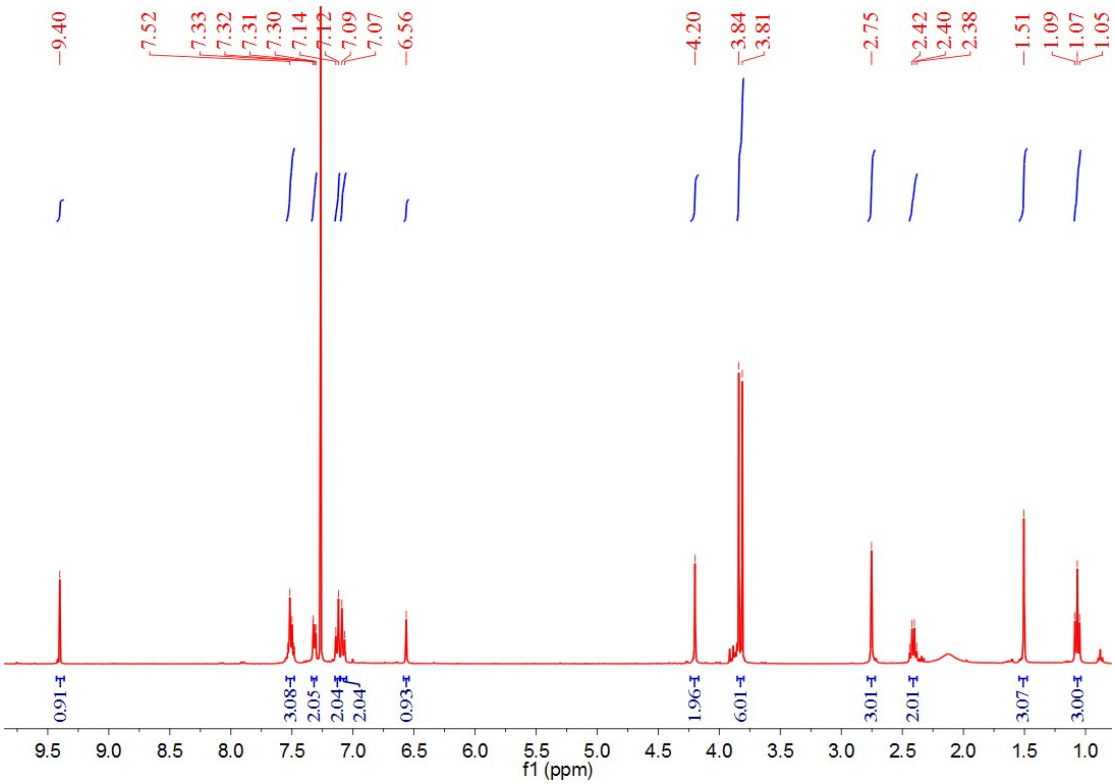


Figure S59. ^{1}H NMR spectrum for compound 16.

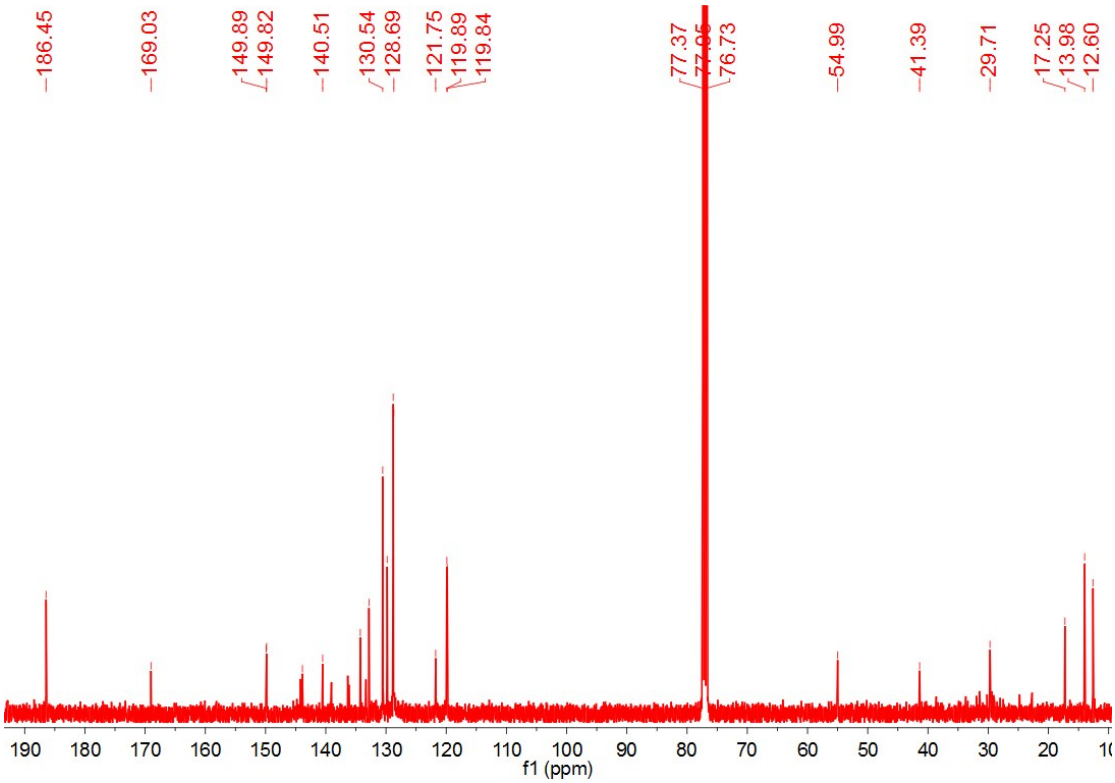


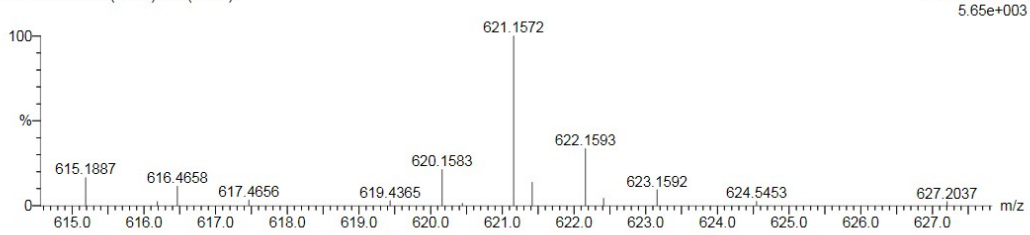
Figure S60. ^{13}C NMR spectrum for compound 16.

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

Monoisotopic Mass, Even Electron Ions
1861 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 29-29 H: 0-30 N: 0-4 O: 0-5 23Na: 0-1 S: 0-2 B: 0-1 F: 0-2 P: 0-1

CC-ZHAO
ZC-WRC-01 54 (0.610) Cm (52:56)

1: TOF MS ES+
5.65e+003



Minimum:				-1.5			
Maximum:		5.0	5.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
621.1572	621.1572	0.0	0.0	15.5	47.7	0.0	C29 H30 N2 O5 23Na S B F2 P

Figure S61. HRMS spectrum for compound 16.

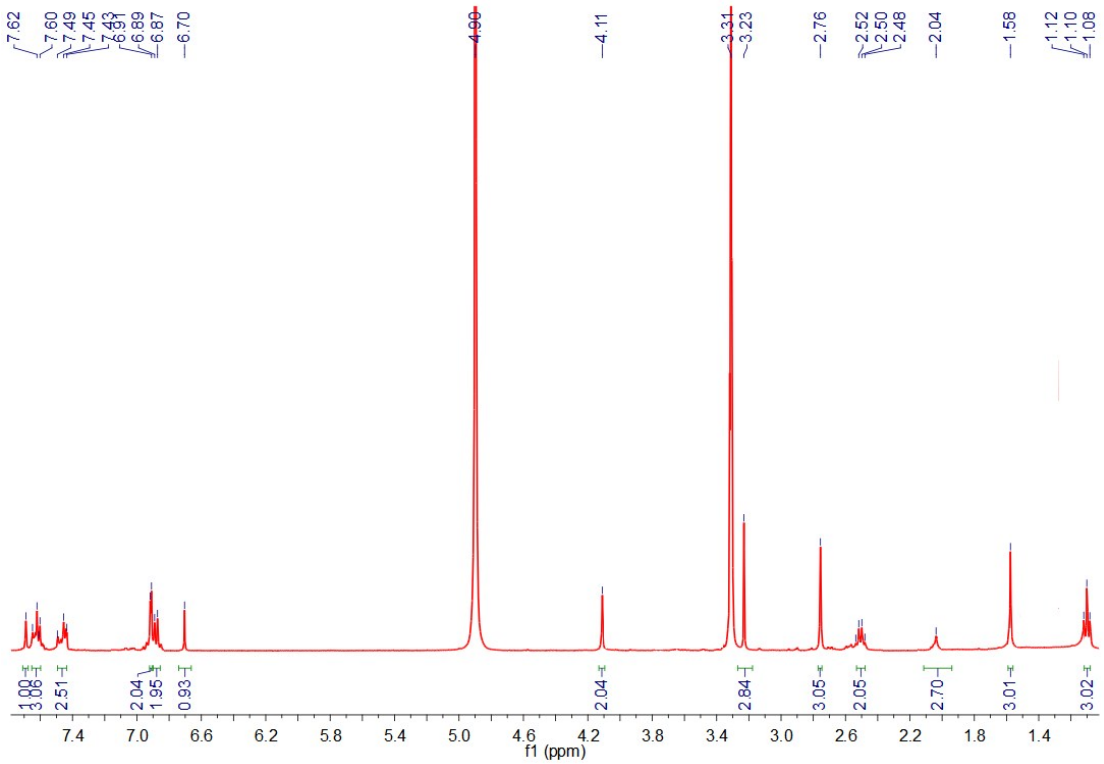


Figure S62. ^{1}H NMR spectrum for compound ALP-Iml.

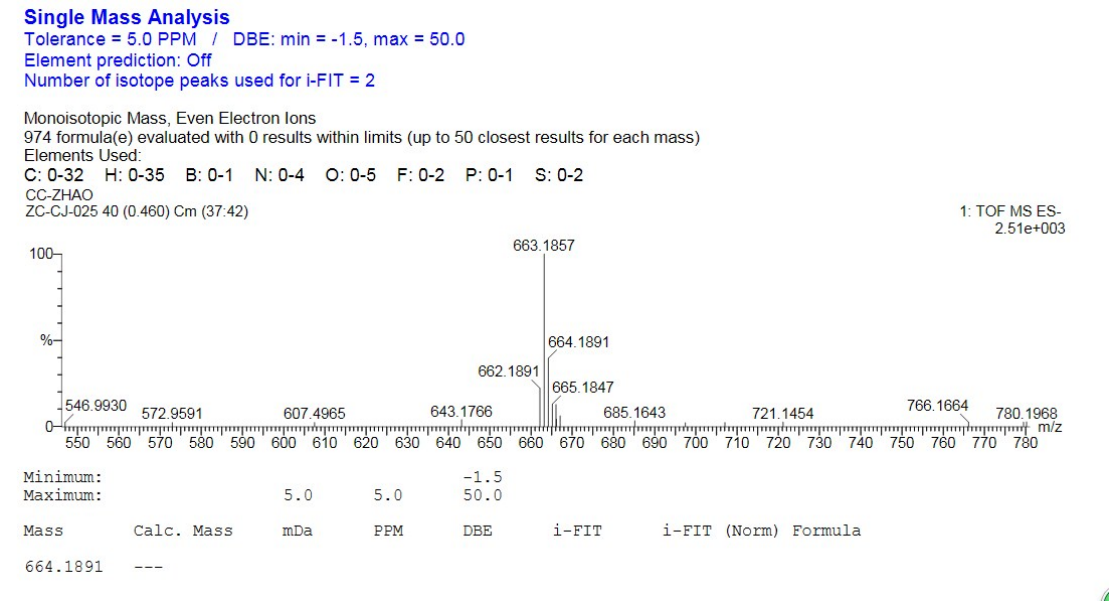


Figure S63. HRMS spectrum for compound ALP-Iml.

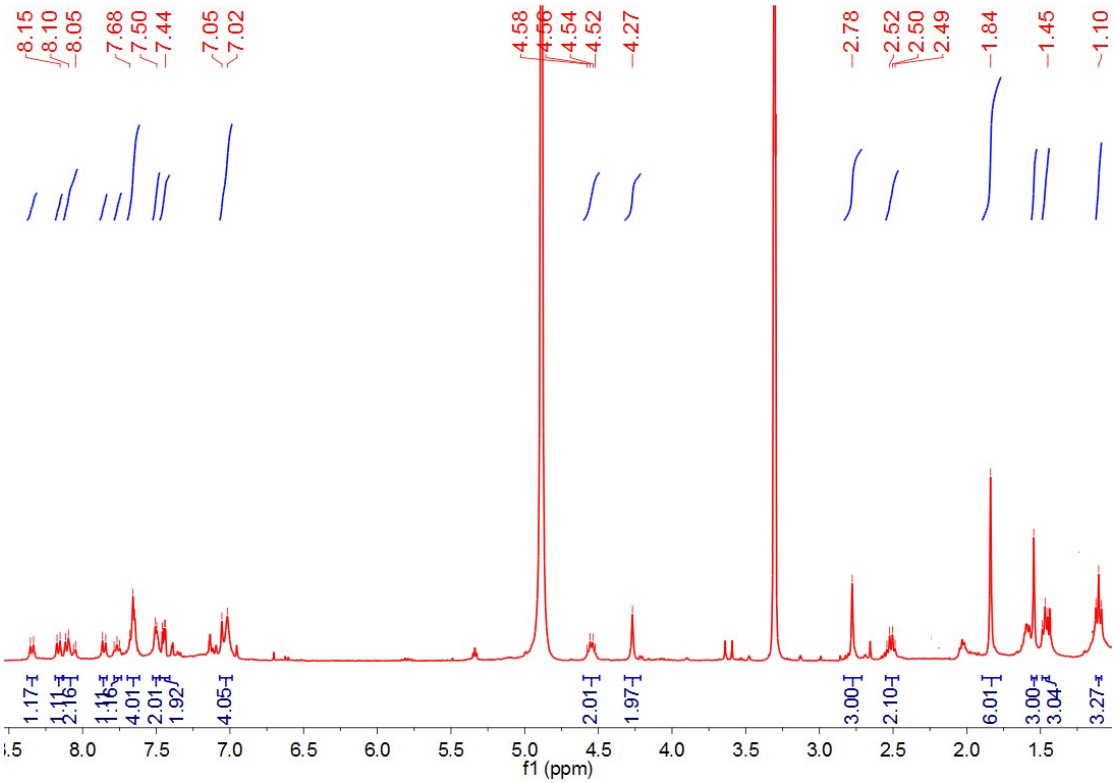


Figure S64. ^{1}H NMR spectrum for compound ALP-InD.

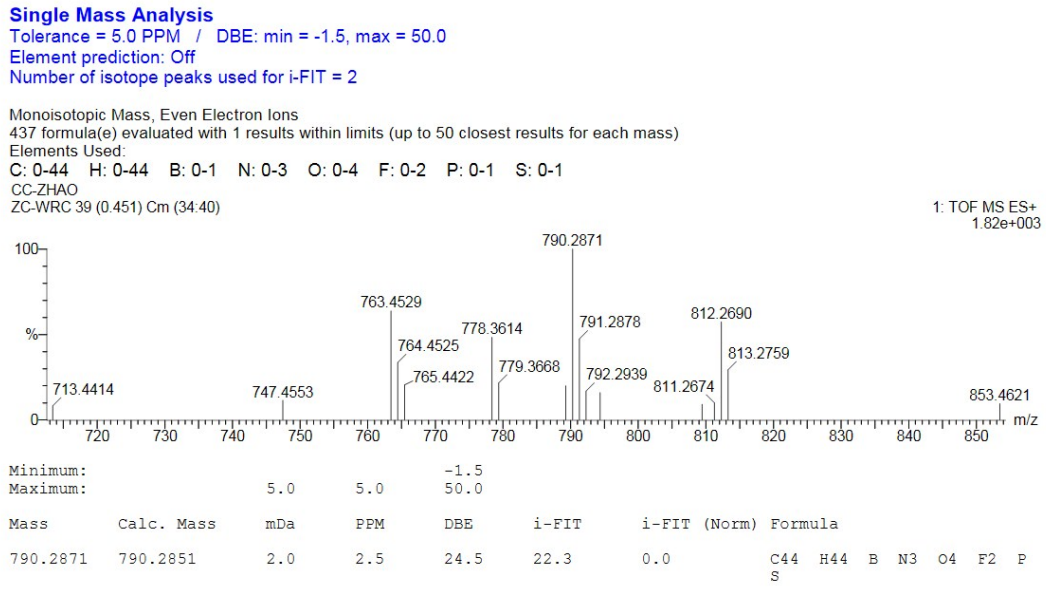


Figure S65. HRMS spectrum for compound ALP-InD.