Electronic Supplementary Material (ESI) for Analyst.

Supporting Information

Novel Intramolecular Photoinduced Electron Transfer-Based Probes for the Human Ether-a-go-go-Related Gene (hERG) Potassium Channel

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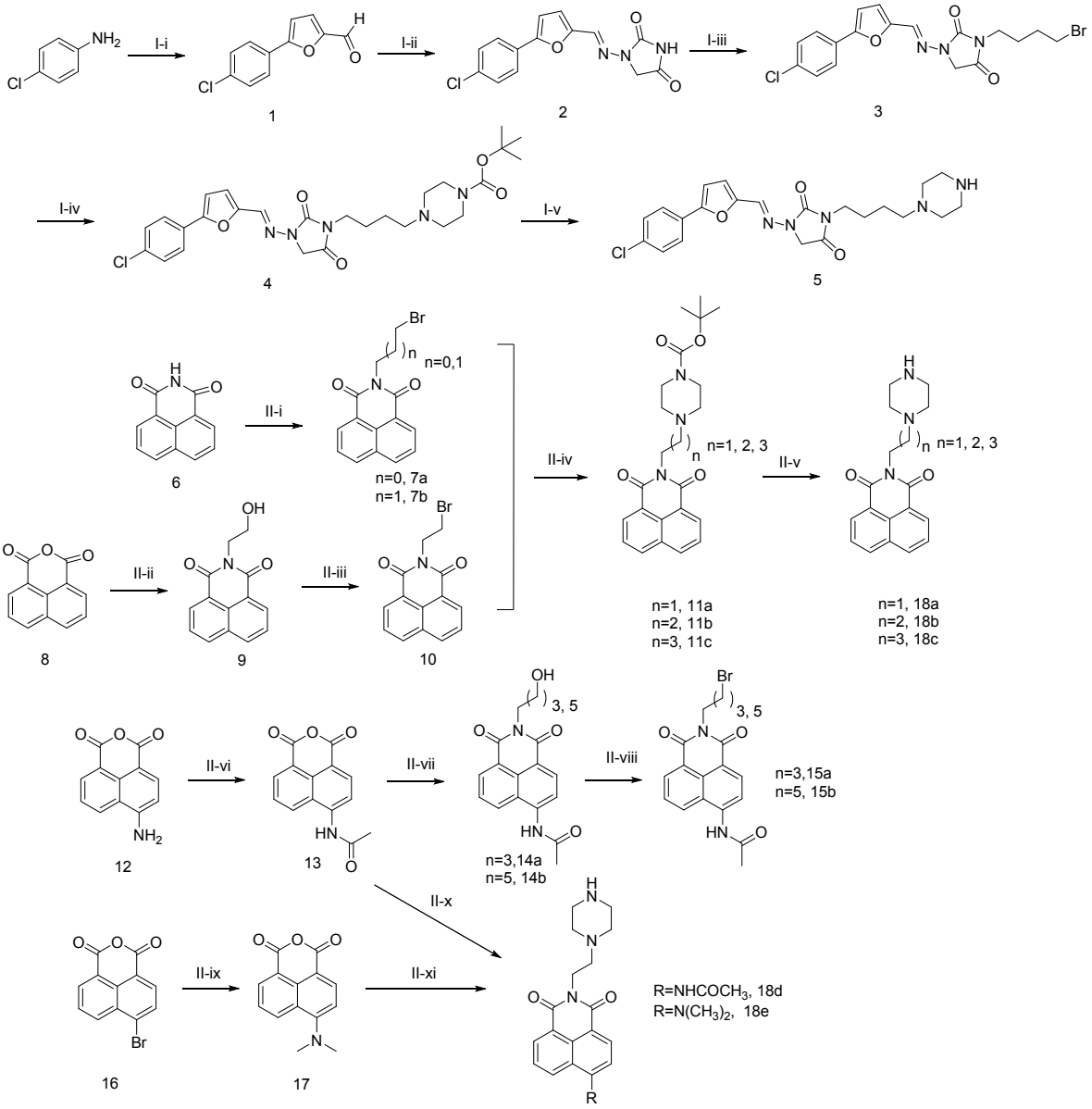
Supporting Information

1. Synthesis of the intermediate



Scheme S1. The synthesis of the intermediates

5-(4-chlorophenyl) furan-2-carbaldehyde (1) [1]

A mixture of 4-chloroaniline (2.55 g, 20 mmol), concentrated hydrochloric acid (14 mL), and water (20 mL) was heated to get a clear solution. The solution was cooled to 0 °C and was diazotized by the addition of sodium nitrite solution (13.8%, 10 mL). The cold clear solution of the diazonium salt was collected by filtration and was treated with 2-furfuraldehyde (2.88 g，30 mmol) and an aqueous solution of $CuCl_2$ (0.54 g in 2 mL of water). Then, the reaction mass was stirred at room temperature overnight and filtrated to afford brown solid. Then, the crude product was recrystallized in ethanol to afford 1 as a light brown solid in 25.4% yield. ^{1}H-NMR (300MHz, $CDCl_3$): δ 9.65 (s, 1H), 7.77 (dd, *J*=6.9, 2.1Hz, 2H), 7.41 (dd, *J*=6.9, 2.1Hz, 2H), 7.33 (d, *J*=3.9Hz, 1H), 6.84 (d, *J*=3.9Hz, 1H). ESI-MS: ($[M+H]^+$): 207.1.

(E)-1-(((5-(4-chlorophenyl) furan-2-yl) methylene) amino) imidazolidine-2,4-dione (2)

A mixture of 1-aminohydantoin hydrochloride (0.30 g, 2 mmol) and compound 1 (0.21 g, 1 mmol) in water (6 mL) was stirred at 80°C for 4 h. The reaction suspension was filtrated, washed with water and dried to afford 2 as a pale pink solid in 89.1% yield. Decomposition point: 260°C. ^{1}H-NMR (600MHz, DMSO-d6): δ 11.30 (s, 1H), 7.79 (d, *J*=7.8Hz, 1H), 7.73 (s, 1H), 7.52 (d, *J*=8.4Hz, 1H), 7.19 (d, *J*=3Hz, 1H), 6.96 (d, *J*=3.6Hz, 1H), 4.35 (s, 2H). ESI-MS: ([M+H]$^+$): 304.3.

(E)-3-(4-bromobutyl)-1-(((5-(4-chlorophenyl) furan-2-yl) methylene) amino) i2-(3-bromopropyl)-1H-benzo[de]isoquinoline-1,3(2H)-dionedine-2,4-dione (3)

A mixture of compound 3 (0.44 g, 1 mmol), 1,4-dibromobutane (0.32 g, 1.5 mmol), K_2CO_3 (0.14 g, 1 mmol) in DMF was stirred at 65 °C for 4 h. After that, water was added to the suspension, and precipitation was collected and washed with water and recrystallized in ethanol to afford 3 as a yellow solid in 65.1% yield. ^{1}H-NMR (600MHz, DMSO-d6): δ 7.80 (m, 3H), 7.55 (m, 2H), 7.18 (d, *J*= 3.3Hz, 1H), 7.00 (d, *J*=3.30lHz, 1H), 4.40 (s, 2H), 3.56-3.49 (m, 4H), 1.83-1.68 (m, 4H). ESI-MS: ([M+H]$^+$): 438.4.

(E)-tert-butyl 4-(4-(3-(((5-(4-chlorophenyl) furan-2-yl) methylene) amino)-2,5-dioxoimidazolidin-1-yl) butyl) piperazine-1-carboxylate (4)

A mixture of compound 3 (0.3 g, 0.68 mmol), tert-butyl piperazine-1-carboxylate (0.14 g, 0.75 mmol), K_2CO_3 (0.19 g, 1.37 mmol) in acetonitrile was refluxed for 4 h. Thereafter, water was added to the suspension, and precipitation was collected and recrystallized in ethanol/ethyl acetate to afford 4 as a yellow solid in 55.9% yield. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.94 (s, 1H), 7.69 (dt, *J*=8.4, 1.8Hz, 2H), 7.38 (d, *J*=8.7, 1.8Hz, 2H), 6.91 (d, *J*=3.6Hz, 1H), 6.75 (d, *J*=3.6Hz, 1H), 4.24 (s, 2H), 3.66 (t, *J*=7.2Hz, 2H), 3.43 (t, *J*=4.8Hz, 4H), 2.38-2.35 (m, 6H), 1.76-1.66 (m, 2H), 1.57-1.50 (m, 1H), 1.45 (s, 9H). ESI-MS: [M+H]$^+$: 544.5.

(E)-1-(((5-(4-chlorophenyl)furan-2-yl)methylene)amino)-3-(4-(piperazin-1-yl)butyl)imidazolidine-2,4-dione (5)

Triethylamine (1 mL) was added to a solution of compound 4 (0.2 g, 0.37 mmol) in CH_2Cl_2 (2 mL) and the reaction mixture was stirred at room temperature for 3 h. Water was added and the mixture was basified with saturated aqueous solution of $NaHCO_3$. Then, the mixture was extracted with CH_2Cl_2 (40 mL). The combined organic layer was dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure to afford compound 5 as light yellow solid in 75.0% yield. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.95 (s, 1H), 7.69 (d, *J*=8.4Hz, 2H), 7.39 (d, *J*=8.4Hz, 2H), 6.92 (d, *J*=3.6Hz, 1H), 6.75 (d, *J*=3.6Hz, 1H), 4.24 (s, 2H), 3.66 (t, *J*=7.2Hz, 2H), 2.99 (m, 3H), 2.51-2.36 (m, 8H), 1.75-1.66 (m, 2H), 1.58-1.48 (m, 1H). ESI-MS: ([M+H]$^+$): 444.5.

2--(3-brompropyl)-1H-benzo[de]isoquinoline-1,3(2H)-dione （7a）[2]

To a solution of compound 6 (2.00 g, 10.14 mmol) and anhydrous potassium carbonate (5.59 g, 40.56 mmol) in 20 mL acetonitrile, 1,3-dibromopropane (6.14 g, 30.43 mmol) was added and the mixture was refluxed for 12 h. Then, the reaction mixture was filtrated, and the filtrate was concentrated in vacuo. The crude product was further purified by silica gel chromatography (eluent: 0%-20% EtOAc in Petroleum ether) to afford compound 5a as a white solid in 44.4% yield. M.p.: 140.0~142.0°C. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 8.63 (dd, *J*=7.5, 1.2Hz, 2H), 8.24 (dd, *J*= 8.1, 1.2Hz, 2H), 7.79 (dd, *J*=8.1, 7.5Hz, 2H), 4.36 (t, *J*=7.2Hz, 2H), 3.53 (t, *J*=6.9Hz, 2H), 2.39-2.29 (m, 2H). ESI-MS: ([M+H]$^+$): 318.2.

2-(4-bromobutyl)-1H-benzo[de]isoquinoline-1,3(2H)-dione (7b)

The compound 7b was prepared following the method described for the preparation of compound 7a, employing compound 6 (2.00 g, 10.14 mmol) and 1,4-dibromobutane (8.76 g, 30.43 mmol), and the crude product was purified by silica gel chromatography (eluent: 0%-20% EtOAc in Petroleum ether) to afford compound 5b as a white solid in 55.1% yield. M.p.: 116.0~119.0°C. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 8.62 (dd, *J*=7.2, 1.2Hz, 2H), 8.24 (dd, *J*=8.4, 0.9Hz, 2H), 7.79 (t, *J*=8.1Hz, 2H), 4.26 (t, *J*=6.9Hz, 2H), 3.50 (t, *J*=6.6Hz, 2H), 2.05-1.86 (m, 4H). ESI-MS: ([M+H]$^+$): 332.3.

2-(2-hydroxyethyl)-1H-benzo[de]isoquinoline-1,3(2H)-dione (9)[3]

A mixture of compound 8 (4.00 g, 20.18 mmol) and 2-aminoethanol (1.23 g, 20.18 mmol) in ethanol (40 mL) was refluxed for 5 h. The reaction mixture was concentrated by evaporating some ethanol under in vacuo and cooled to 4°C. The solid filtrated, washed with cold ethanol to afford compound 9 as a gray solid in 95.7% yield. M.p.: 177.0~180.0°C. ^{1}H-NMR (300MHz, $CDCl_3$): δ 8.63 (dd, *J*=7.5, 1.2Hz, 2H), 8.24 (dd, *J*=8.4, 0.9Hz, 2H), 7.79 (t, *J*= 8.1Hz, 2H), 4.49 (t, *J*=8.1Hz, 2H), 4.01 (t, *J*=5.4Hz, 2H). ESI-MS: ($[M+H]^+$): 242.4.

2-(2-bromoethyl)-1H-benzo[de]isoquinoline-1,3(2H)-dione (10)[3]

PBr_3 (0.8 mL) was dissolved in dried ethyl acetate (5 mL) and then dropped into a stirred suspension of compound 9 (1.00 g, 4.15 mmol) in dried ethyl acetate in an ice bath. Then, the reaction mixture was transferred to oil bath and refluxed for 7 h. The resulting mixture was cooled, and water was added, and extracted with ethyl acetate. The combined organic layer was washed with saturated $NaHCO_3$, dried over $MgSO_4$, filtrated and the solvent was evaporated under reduced pressure to afford compound 8 as a white solid in 81.7%. M.p.: 226.0~229.0°C ^{1}H-NMR (300 MHz, DMSO-d6): δ 8.26 (dd, *J*=7.2, 1.2Hz, 2H), 8.24 (dd, *J*=8.1, 0.9Hz, 2H), 7.79 (t, *J*= 8.1Hz, 2H), 4.64 (t, *J*=7.2Hz, 2H), 3.70 (t, *J*=7.2Hz, 2H). ESI-MS: ($[M+H]^+$): 304.2.

N-(1,3-dioxo-1,3-dihydrobenzo[de]isochromen-6-yl)acetamide (13)

A mixture of compound 12 (0.90 g, 2.3 mmol), acetic acid (1 mL), and pyridine (4 mL) was refluxed for 1 h. Then, acetic anhydride (8 mL) was added, and the refluxing was continued 3 h. The resulting mixture was poured into water, and the precipitation filtrated and washed with water to afford compound 10 as a brown solid in 67.2%.

N-(2-(4-hydroxybutyl)-1,3-dioxo-2,3-dihydro-1H-benzo[de]isoquinolin-6-yl)acetamide (14a)

The compound 14a was prepared following the method described for the preparation of compound 9, employing compound 13 (1.00 g, 3.91 mmol) and 4-amino-1-butanol (0.42 g, 4.70 mmol), and the product 14a was afford as a brown solid in 50.78% yield. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 8.63 (dd, *J*=7.2, 0.9Hz, 1H), 8.59 (d, *J*=8.4Hz, 1H), 8.37 (brs, 1H), 8.19 (d, *J*=8.1Hz, 1H), 7.83-7.75 (m, 2H), 4.24 (t, *J*=7.2Hz, 2H), 3.76 (t, *J*=6.3Hz, 2H), 1.89-1.79 (m, 2H), 1.74-1.57 (m, 2H). ESI-MS: ($[M+H]^+$): 327.5.

N-(2-(6-hydroxyhexyl)-1,3-dioxo-2,3-dihydro-1H-benzo[de]isoquinolin-6-yl)acetamide (14b)

The compound 14b was prepared following the method described for the preparation of compound 9, employing compound 13 (1.00 g, 3.91 mmol) and 6-amino-1-butanol (0.46 g, 4.70 mmol), and the product 14b was afford as a brown solid in 51.08% yield. ^{1}H-NMR (300 MHz, DMSO-d6): δ 10.39 (s, 1H)， 8.72 (dd, *J*=7.8, 0.9Hz, 1H), 8.54 (dd, *J*=7.5, 0.9Hz, 1H), 8.49 (d, *J*=8.1Hz, 1H), 8.32 (d, *J*=8.1Hz, 1H), 7.91 (t, *J*=8.4Hz, 1H), 4.32 (t, *J*=5.4Hz, 1H), 4.05 (t, *J*=7.2Hz, 2H), 3.40-3.30 (m, 2H), 2.28 (s, 3H), 1.63-1.61 (m, 2H), 1.44-1.33 (m, 6H). ESI-MS: ($[M+H]^+$): 355.6.

N-(2-(4-bromohexyl)-1,3-dioxo-2,3-dihydro-1H-benzo[de]isoquinolin-6-yl)acetamide (15a)

The compound 15a was prepared following the method described for the preparation of compound 10, employing compound 14a (0.61 g, 1.87 mmol) and PBr_3 (0.60 mL), and the product 15a was afford as a brown solid in 83.56% yield. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 8.63 (d, *J*=7.2Hz, 1H), 8.60 (d, *J*=8.1Hz, 1H), 8.39 (brs, 1H), 8.20 (d, *J*=8.4Hz, 1H), 7.81-7.75 (m, 2H), 4.24 (t, *J*=6.9Hz, 2H), 3.49 (t, *J*=6.3Hz, 2H), 2.38 (s, 3H), 2.04-1.85 (m, 4H). ESI-MS: ($[M+H]^+$): 389.4.

N-(2-(6-bromohexyl)-1,3-dioxo-2,3-dihydro-1H-benzo[de]isoquinolin-6-yl)acetamide (15b)

The compound 15b was prepared following the method described for the preparation of compound 10, employing compound 14b (0.65 g, 1.98 mmol) and PBr_3 (0.65 mL), and the product 15b was afford as a brown solid in 90.24% yield. ^{1}H-NMR (300 MHz,

$CDCl_3$): δ 8.64 (dd, *J*=7.5, 0.9Hz, 1H), 8.61 (d, *J*=8.1Hz, 1H), 8.39 (brs, 1H), 8.20 (d, *J*=8.7Hz, 1H), 7.81-7.76 (m, 2H), 4.19 (t, *J*=7.5Hz, 2H), 3.42 (t, *J*=6.9Hz, 2H), 2.38 (s, 3H), 1.93-1.83 (m, 2H), 1.80-1.70 (m, 2H), 1.49-1.40 (m, 4H).ESI-MS: ($[M+H]^+$): 417.5.

6-(dimethylamino)benzo[de]isochromene-1,3-dione (17) [4]

Compound 16 (1.00 g, 3.61 mol) was dissolved in DMF (15 mL), and then dimethylamine (7 mL, 40% aqueous solution,excess) and $CuSO_4 \cdot 5H_2O$ (90 mg, 0.361mmol) were added. The solution was stirred and refluxed for 4 h, and then the solvent was removed in under vacuum. The crude product was crystallized from hot ethanol to afford compound 14 as a yellow solid in 59.8% yield. M.p.:195.0-199.0°C. ^{1}H-NMR (300MHz, $CDCl_3$): δ 8.58 (d, *J*=7.5Hz, 1H), 8.50-8.44 (m, 2H), 7.70 (t, *J*=8.4Hz, 1H), 7.12 (d, *J*=8.4Hz, 1H), 3.18 (s, 6H).

2-(2-(piperazin-1-yl)ethyl)-1H-benzo[de]isoquinoline-1,3(2H)-dione (18a)

A mixture of compound 10 (0.20 g, 0.66 mmol), tert-butyl piperazine-1-carboxylate (0.13 g, 0.79 mmol), 4-methylmorpholine (0.2 g, 1.98 mmol) in 15 mL acetonitrile was refluxed for 12 h. The resulting mixture was cooled, and water was added and extracted with CH_2Cl_2. The combined organic layer was washed with a saturated sodium chloride solution, dried over $MgSO_4$, filtrated and concentrated under reduced pressure. The crude product was purified by silica gel chromatography (eluent: 0%-20% CH_2Cl_2 in methanol) afford compound **11a** as a white solid (not very pure, still containing .by-product compound 7) in 74.1% yield.

Trifluoroacetic acid (0.5 mL) was added to a solution of compound 11a (0.2 g, 0.49 mmol) in CH_2Cl_2 (3 mL), and the reaction mixture was stirred at room temperature for 6 h. Water was added and the mixture was extracted with CH_2Cl_2. Then, the aqueous layer was basified with saturated aqueous solution of $NaHCO_3$.and extracted with CH_2Cl_2 (40 mL). The combined organic layer was dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure to afford compound 18a as white solid in 80.0% yield. ^{1}H-NMR (300MHz, DMSO-d6): δ 8.51-8.45 (m, 4H), 7.90 (t, *J*=7.8Hz, 2H), 7.47 (brs, 1H), 4.20 (t, *J*= 6.9Hz, 2H), 2.93 (brs, 4H), 2.61-2.60 (m, 6H). ESI-MS: ($[M+H]^+$): 367.3.

Tert-butyl 4-(3-(1,3-dioxo-1H-benzo[de]isoquinolin-2(3H)-yl)propyl)piperazine-1-carboxylate (11b)

The compound 11b was prepared following the method described for the preparation of compound 11a, employing compound 7a (0.50 g, 1.57 mmol), K_2CO_3 (0.43 g, 3.14 mmol) and tert-butyl piperazine-1-carboxylate (0.44 g, 2.36 mmol), and the crude product was purified by silica gel chromatography (eluent: 0%-12.5% CH_2Cl_2 in methanol) to afford compound 11b as a white solid (pure) in 60.6% yield. ^{1}H-NMR (300MHz, $CDCl_3$): δ 8.60-8.57 (m, 2H), 8.22-8.19 (m, 2H), 7.78-7.72 (m, 2H), 4.28 (t, *J*=6.9Hz, 2H), 3.33 (t, *J*=4.8Hz, 4H), 2.53 (t, *J*=6.9Hz, 2H), 2.39 (t, *J*=4.8Hz, 4H), 1.99-1.89 (m, 2H), 1.43 (s, 9H). ESI-MS: ($[M+H]^+$): 424.4.

2-(3-(piperazin-1-yl)propyl)-1H-benzo[de]isoquinoline-1,3(2H)-dione (18b)

Trifluoroacetic acid (0.8 mL) was added to a solution of compound 11b (0.34 g, 0.80 mmol) in CH_2Cl_2 (3 mL), and the reaction mixture was stirred at room temperature for 6 h. The mixture was basified with saturated aqueous solution of $NaHCO_3$.and extracted with CH_2Cl_2 (40 mL). The combined organic layer was dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure to afford compound 18b as white solid in 80.0% yield.

Tert-butyl 4-(4-(1,3-dioxo-1H-benzo[de]isoquinolin-2(3H)-yl)butyl)piperazine-1-carboxylate (11c)

The compound 11c was prepared following the method described for the preparation of compound 11a, employing compound 7b (0.50 g, 1.51 mmol), K_2CO_3 (0.42 g, 3.01 mmol) and tert-butyl piperazine-1-carboxylate (0.42 g, 2.26 mmol), and the crude product was purified by silica gel chromatography (eluent: 0%-20% CH_2Cl_2 in methanol) to afford compound 11c as a white solid (pure) in 77.3% yield. ^{1}H-NMR (300MHz, $CDCl_3$): δ 8.60 (d, *J*=7.5Hz, 2H), 8.22 (d, *J*=7.5Hz, 2H), 7.78 (m, 2H), 4.23 (t, *J*=7.2Hz, 2H), 3.43 (t, *J*=4.8Hz, 4H), 2.43-2.36 (m, 6H), 1.82-1.72 (m, 2H), 1.66-1.56 (m, 2H), 1.45 (s, 9H). ESI-MS: ($[M+H]^+$): 438.5.

2-(4-(piperazin-1-yl)butyl)-1H-benzo[de]isoquinoline-1,3(2H)-dione (18c)

The compound 18c was prepared following the method described for the preparation of compound 18b, employing compound 11c (0.20 g, 0.46 mmol) and trifluoroacetic acid (0.8 mL), and get compound 18c as a white solid in 73.3% yield.

N-(1,3-dioxo-2-(2-(piperazin-1-yl)ethyl)-2,3-dihydro-1H-benzo[de]isoquinolin-6-yl)acetamide (18d)

A mixture of compound 13 (0.30 g, 1.18 mmol) and 1-(2-aminoethyl)-piperazine (0.18 g, 1.41 mmol) in 15 mL ethanol was refluxed for 2 h and then the solvent was removed in under vacuum. The crude product was purified by silica gel chromatography (eluent: 0%-20% CH_2Cl_2 in methanol) to afford compound 18d as a browm solid (pure) in 40.20% yield. ^{1}H-NMR (300MHz, $CDCl_3$): δ 8.64-8.59 (m, 2H), 8.40 (s, 1H), 8.21 (d, *J*=7.5Hz, 1H), 7.82-7.77 (m, 2H), 4.35 (t, *J*=6.9Hz, 2H), 2.93 (t, *J*=4.8Hz, 4H), 2.73 (t, *J*=6.9Hz, 2H), 2.64 (brs, 4H), 2.38 (s, 3H). ESI-MS: ([M+H]$^+$): 367.3.

6-(dimethylamino)-2-(2-(piperazin-1-yl)ethyl)-1H-benzo[de]isoquinoline-1,3(2H)-dione (18e)

The compound 18e was prepared following the method described for the preparation of compound 18d, employing compound 17 (0.20 g, 0.83 mmol) and 1-(2-aminoethyl)-piperazin (0.14 g, 1.08 mmol). The crude product was purified by silica gel chromatography (eluent: 0%-20% CH_2Cl_2 in methanol) to afford compound 18e as a yellow solid (pure) in 37.3% yield. ^{1}H-NMR (300MHz, $CDCl_3$): δ 8.56 (d, *J*=7.2Hz, 1H), 8.47 (d, *J*=8.1Hz, 1H), 8.46 (d, *J*=7.8Hz, 1H), 7.69 (t, *J*=8.1Hz, 1H), 7.14 (d, *J*=8.4Hz, 1H) 4.32 (t, *J*=6.3Hz, 2H), 3.11 (s, 6H), 3.04 (t, *J*=4.5Hz, 4H), 2.77-2.70 (m, 6H). ESI-MS: ([M+H]$^+$): 353.6.

2. Absorption and emission spectra of probes

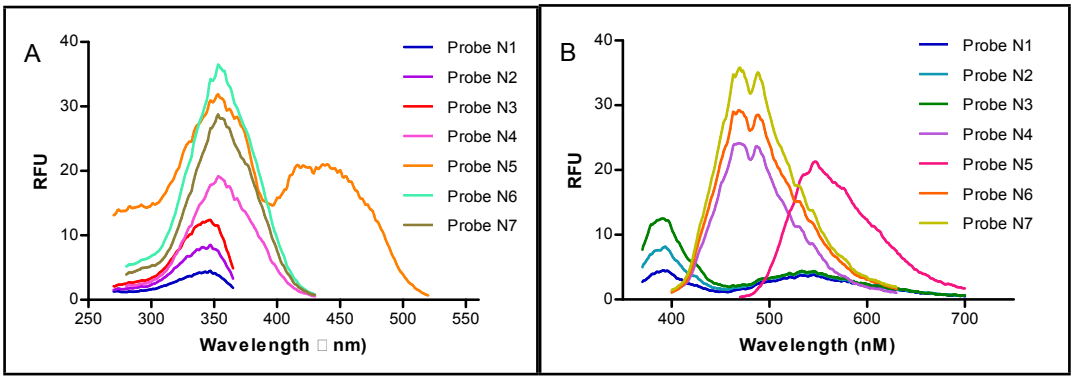


Figure S1. A) Fluorescent excitation spectra of probe N1-7 (20 at emission wavelength 392, 394, 390, 460, 545, 470, 469 nm respectively; B) Fluorescence emission spectra of probe N1-7 at excitation wavelength 345, 346, 345, 355, 440, 354, 353 nm in Tris-HCl buffer (50 mM Tris-HCl, 10 mM KCl, 1 mM $MgCl_2$, pH= 7.4)

3. *h*ERG potassium channel inhibition assay[5]

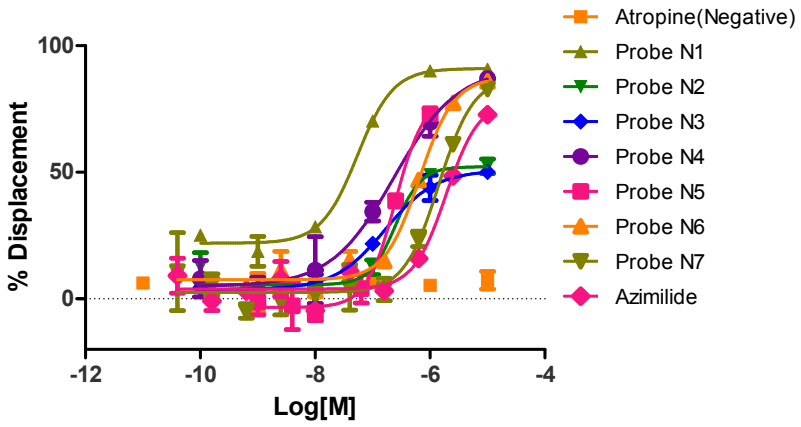


Figure S2. The competitive binding curves of probe N1-7 that were determined by radio-ligand binding assay.

4. Fluorescent properties of the synthesized probes under different pH conditions

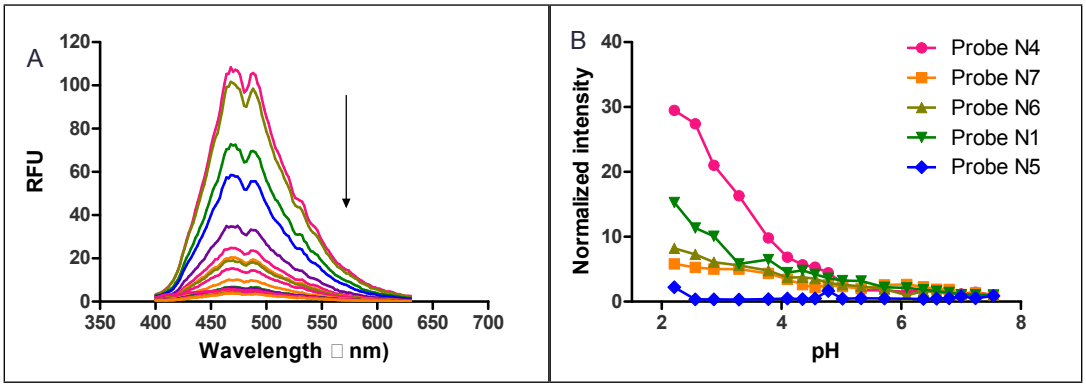


Figure S3. A: Fluorescent emission spectra changes of probe N4 (10 μM) in Britton-Robinson buffer at different pH values; B: Fluorescent intensity (normalized based on the last point which is seen as 1) changes of probe N1, N4-7 at their maximum wavelength, 460 nm for probe N4, 6, 7 (excited at 355 nm), 396 nm for probe N1, (excited at 355 nm), 545 nm for probe N5, (excited at 440 nm) with pH values.

5. Cell membrane preparation[5,6]

HEK 293 cell line stably transfected with hERG gene was obtained as a gift from the Li group at the University of Hong Kong and cultured in Dulbecco's modified eagle's medium (DMEM) supplemented with 10% fetal bovine serum and 400 μg/mL G418 (Sigma). The establishment of the hERG-transfected HEK293 cell line was described in their previous paper.[7] Briefly, the vector of hERG/pcDNA3 generously provided by Dr. G. Robertson (University of Wisconsin, Madison, WI, USA) was transfected into HEK 293 cells (ATCC, Manassas, VA, USA) using 10 ml Lipofectamine 2000TM (Invitrogen, Hong Kong) with 4 mg hERG/pcDNA3 plasmid, and selected using 1000 mg/ml G418 (Sigma–Aldrich, St. Louis, MO). Colonies were picked with cloning cylinders and examined for channel expression by whole-cell current recordings as previously reported.[8] The significant tail current of hERG channel was observed.

As we know, although the hERG-HEK293 we use was a stably transfected cell line, with the cells passaged, the transfected gene may be lost. Therefore, to guarantee the quality of the extracted cell membrane, we examined the hERG channel current changes with the increase of the cell generation. The results showed that the hERG channel current was weak after about 6 times of passages. Therefore, to obtain cell membrane pellets for PET assay and FP assay, we only collected membrane from the first

sixth generations of cells. Before collecting the membrane, hERG-transfecting HEK 293 cells were cultured in 10-cm dishes. When they reached 90% confluency, cells were collected and washed with PBS buffer (pH 7.4) three times by centrifugation for 4 min at 800 RMP .The cell pellets were resuspended in assay buffer (50 mM Tris-HCl, 1 mM MgCl2,10 mM KCl, pH 7.4, at 4 °C) and stored in -80°C. Until use, the cell suspension was thawed at 4 °C and was lysed by passing 20-30 times through 27G ½ needle on an ice bath. The cell lysates were centrifuged at 40,000 g for 20 min (4 °C). The supernatant was discarded and the pellet was suspended in assay buffer and homogenized using 27G ½ needle and centrifuged at 40,000 g for 20 min (4 °C). The supernatant was discarded and the obtained membrane pellets was resuspended in assay buffer and aliquoted in 1.5 mL tube and stored in -80 °C before use. Protein concentration was determined using a Braford kit as manufacturer's instructions (M2031, Mbchem)

6. References

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7. Characterization data for probes

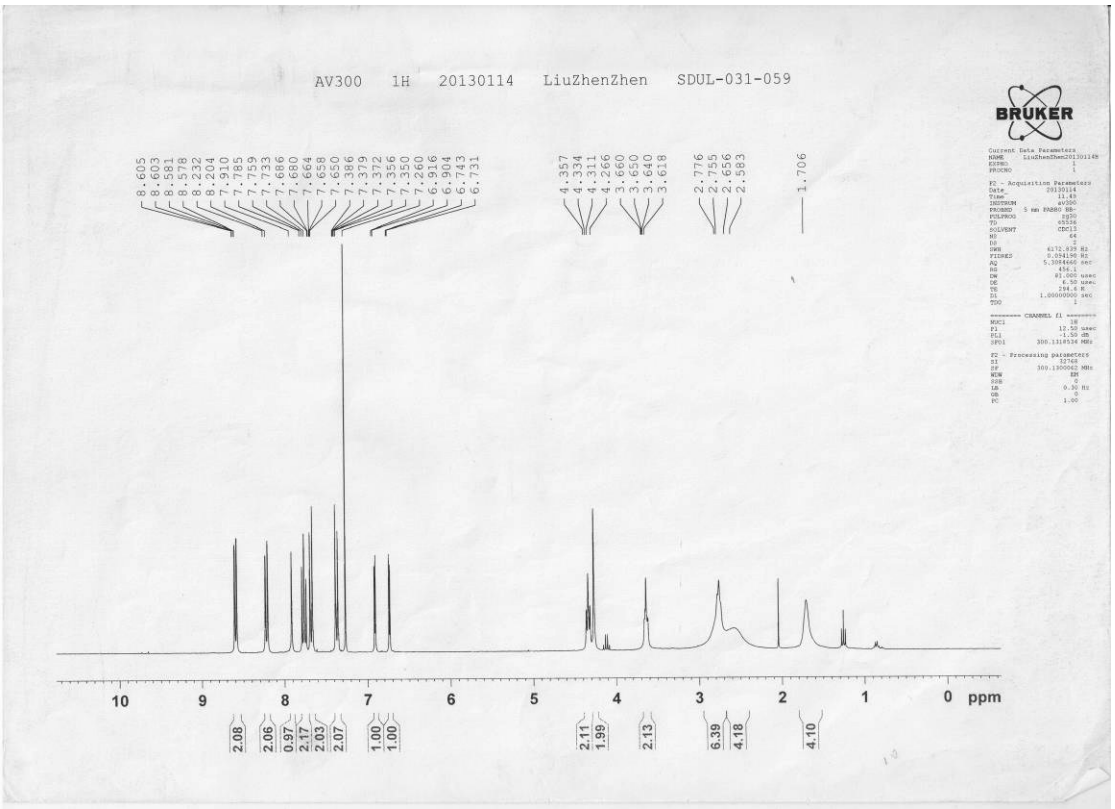

Figure S4. ^{1}H-NMR spectra of probe N1

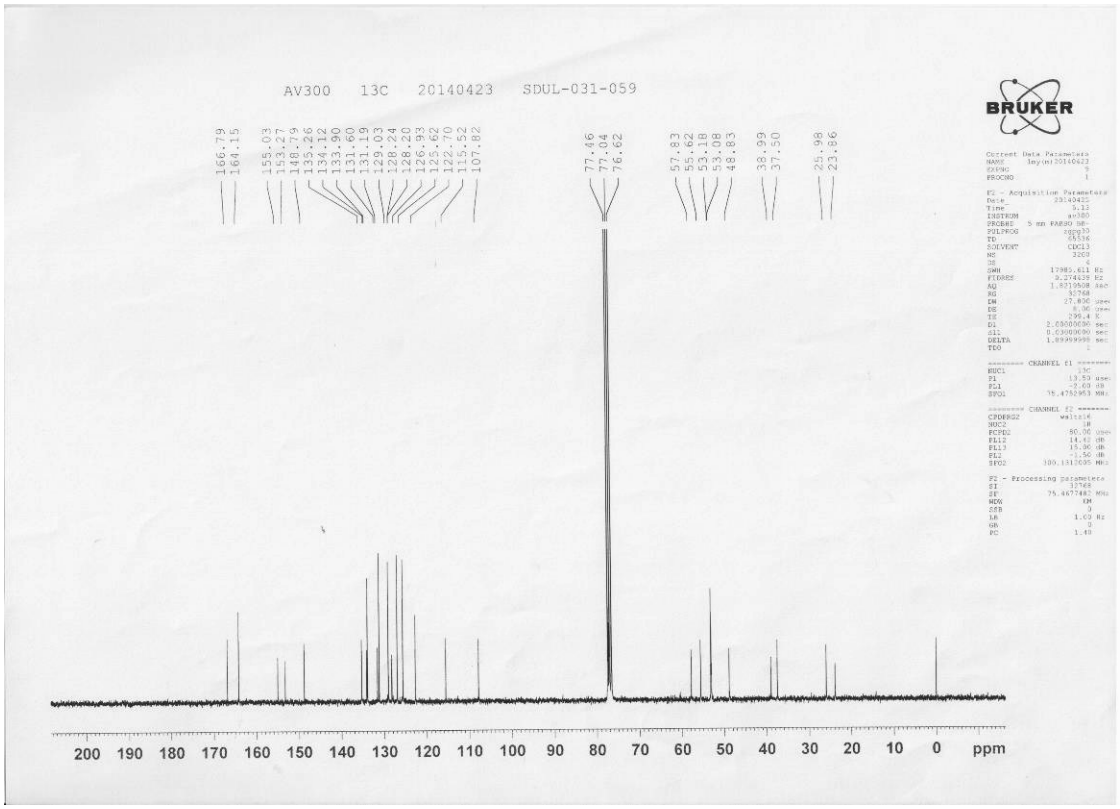

Figure S5. ^{13}C-NMR spectra of probe N1

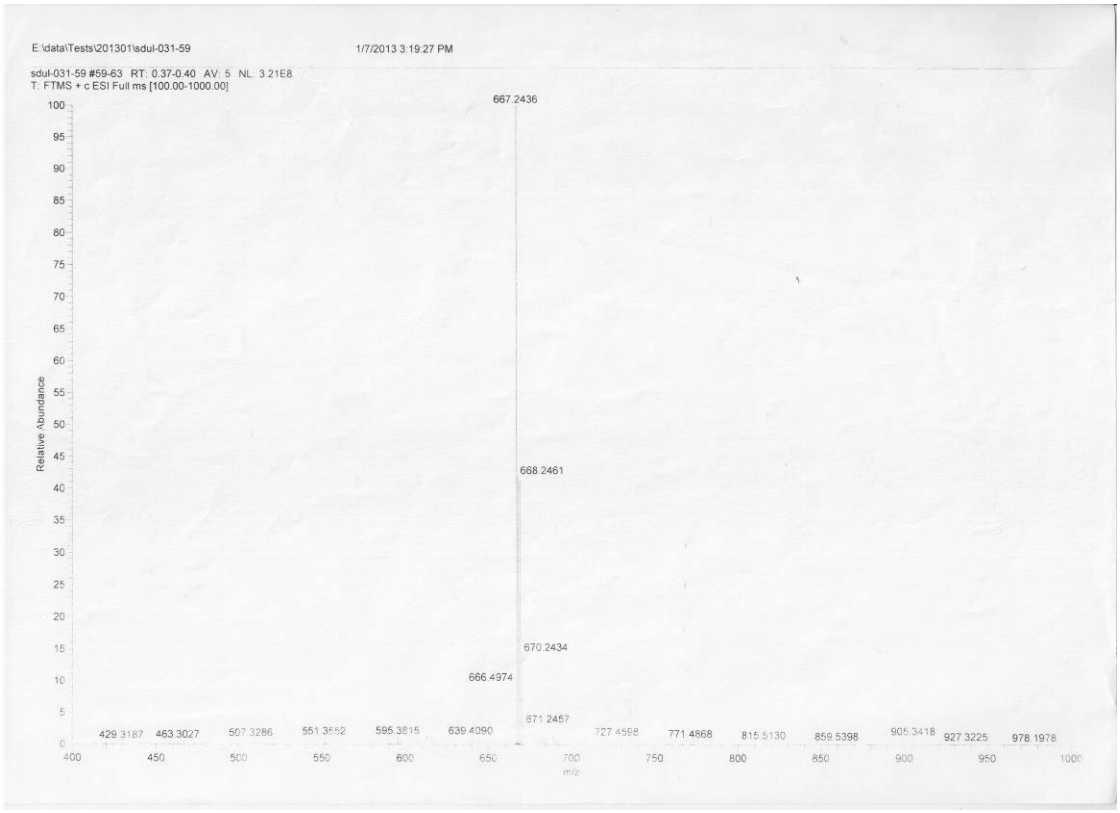

Figure S6. ESI-HRMS spectra of probe N1

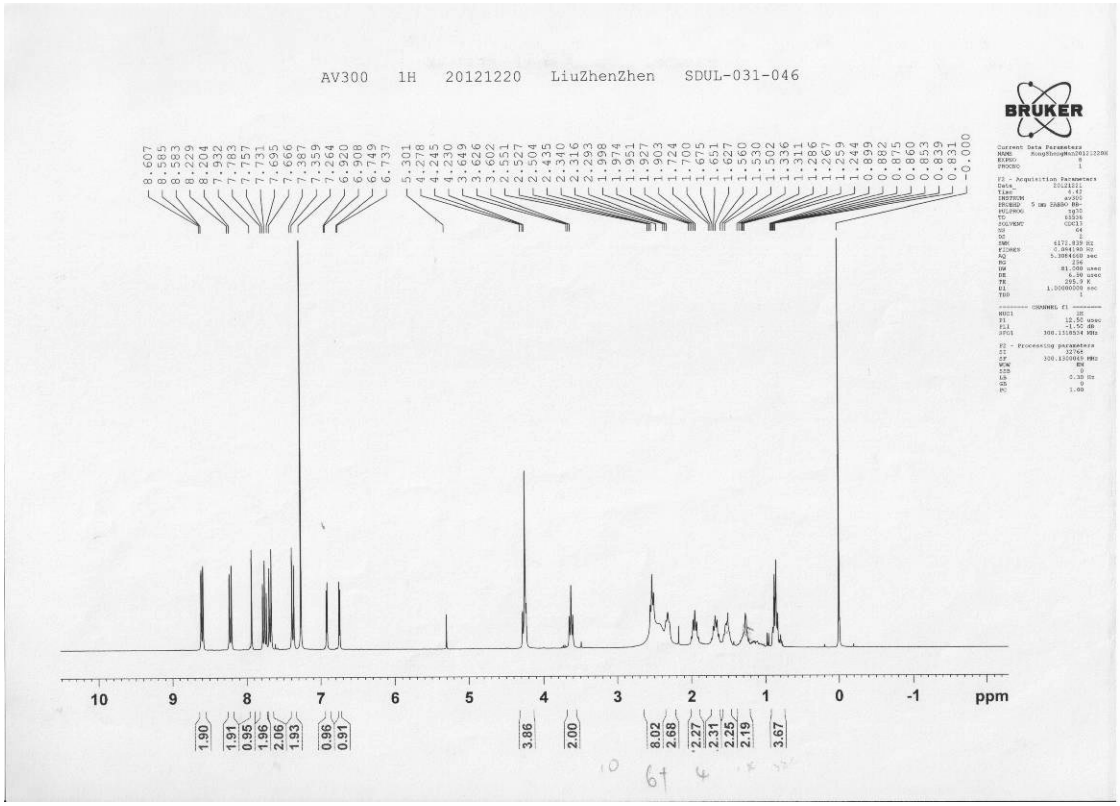

Figure S7. ^{1}H-NMR spectra of probe N2

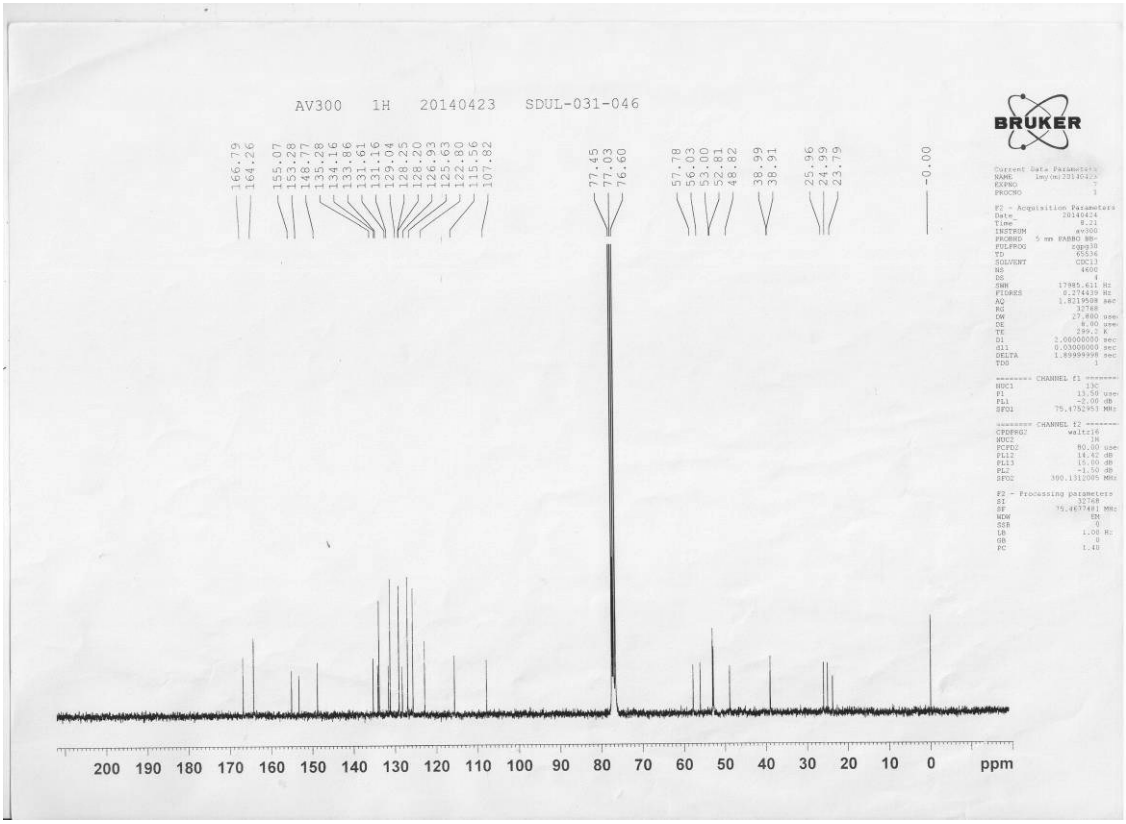

Figure S8. ^{13}C-NMR spectra of probe N2

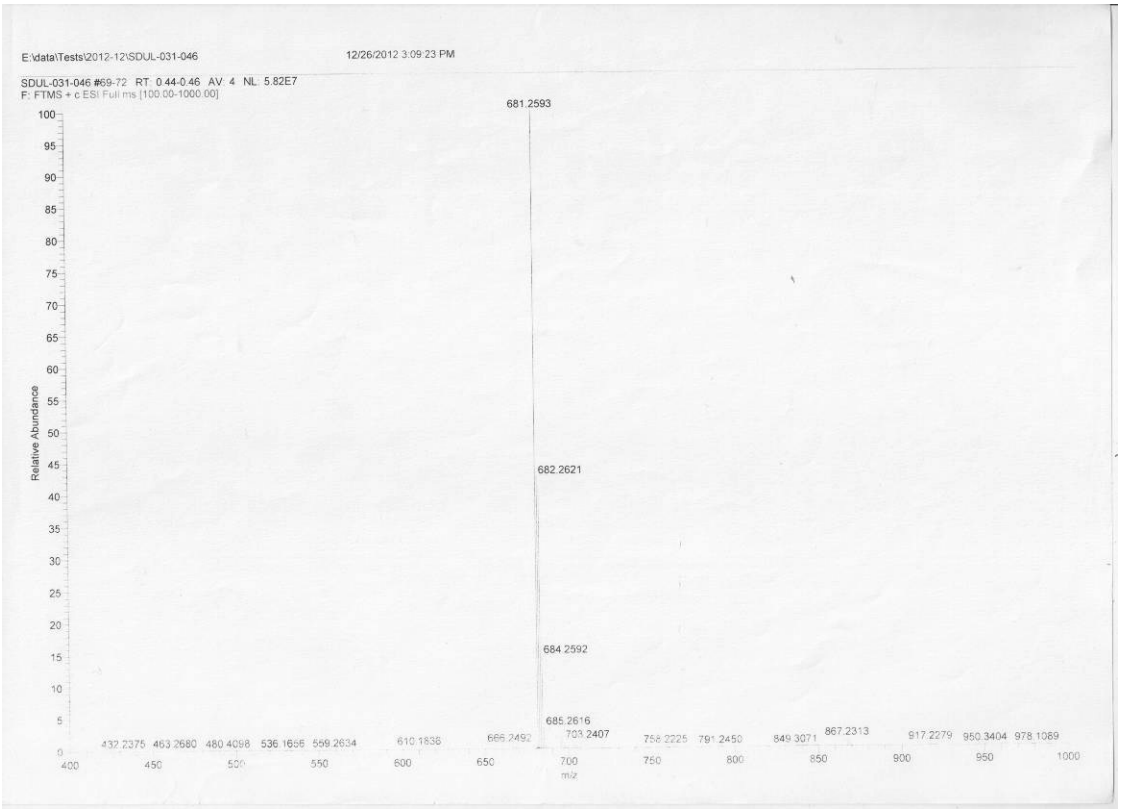

Figure S9. ESI-HRMS spectra of probe N2

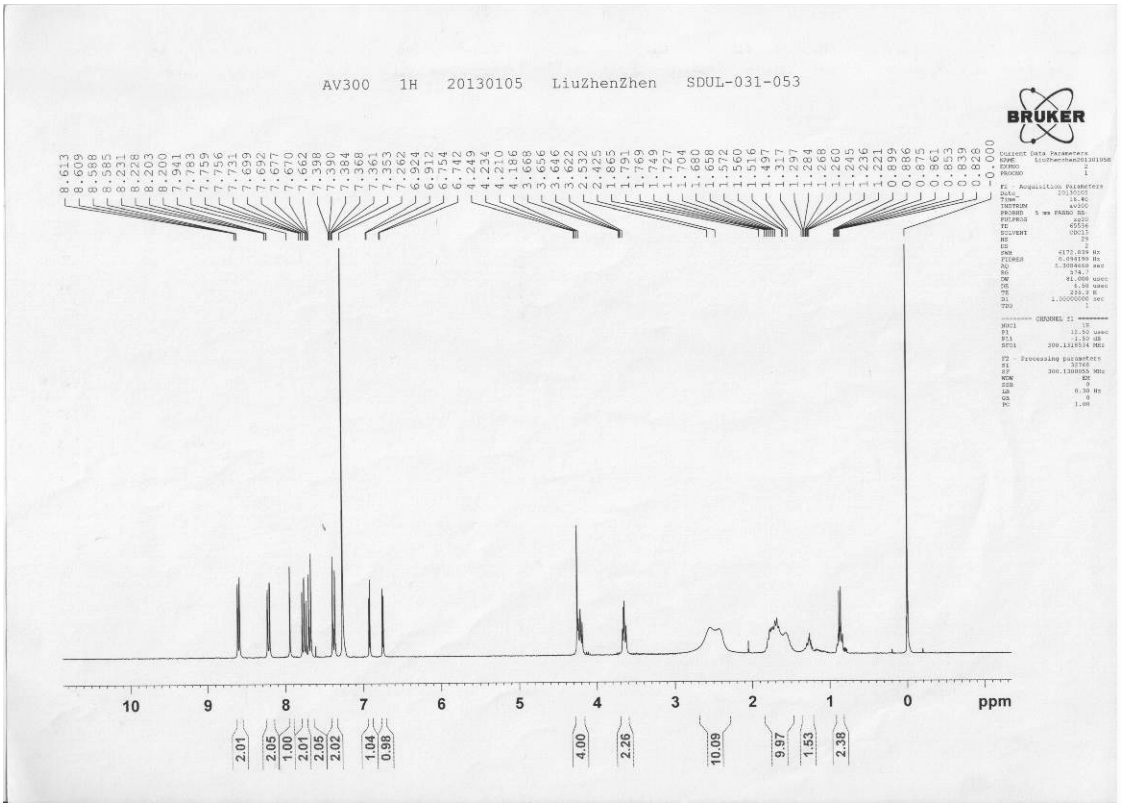

Figure S10. ^{1}H-NMR spectra of probe N3

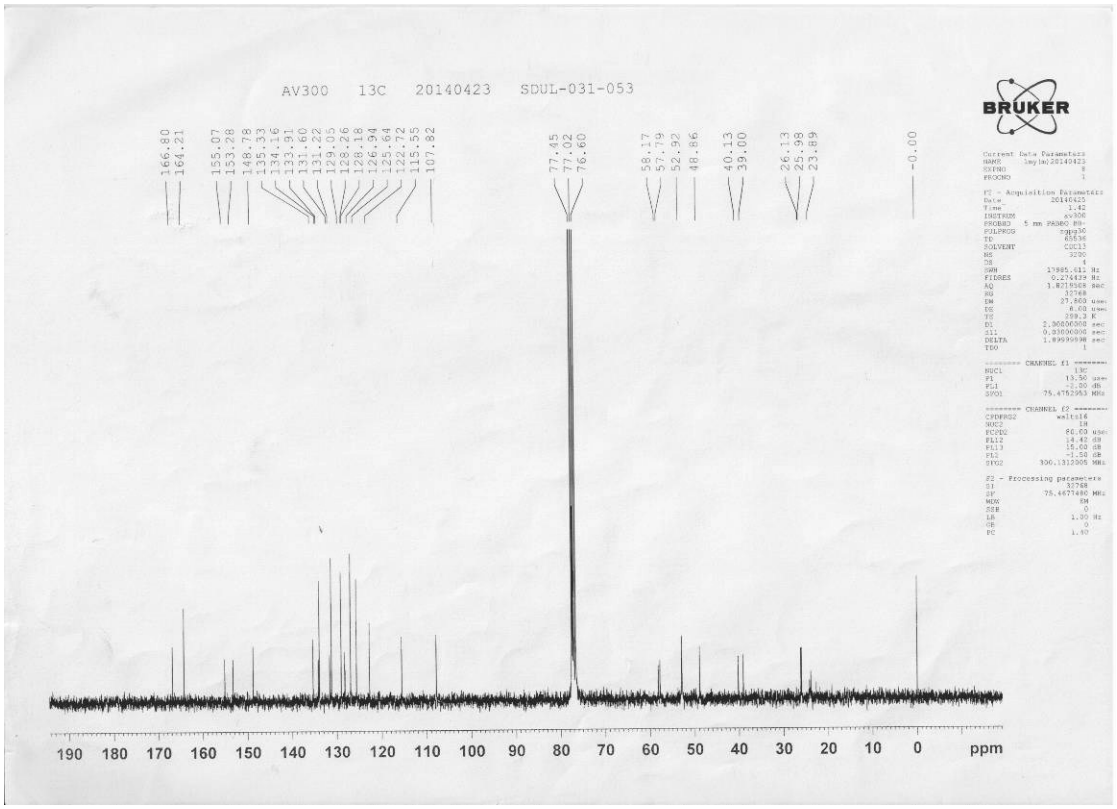

Figure S11. ^{13}C-NMR pectra of probe N3

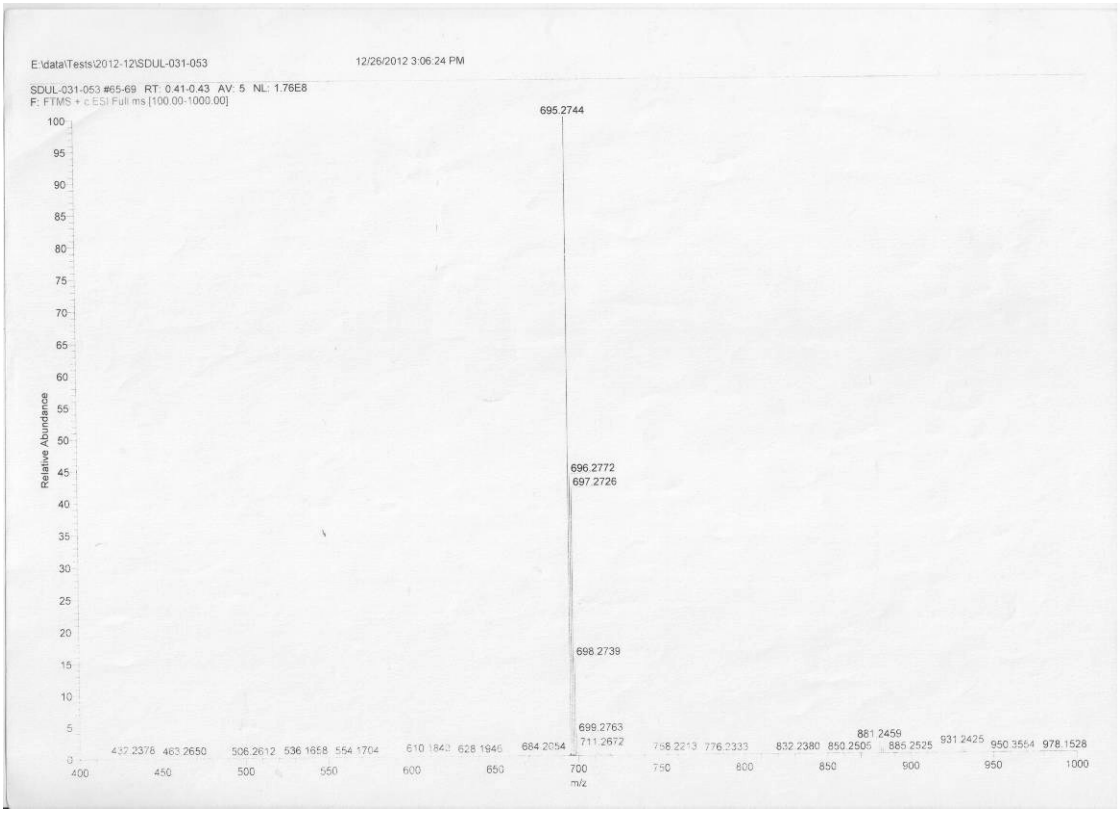

Figure S12. ESI-HRMS spectra of probe N3

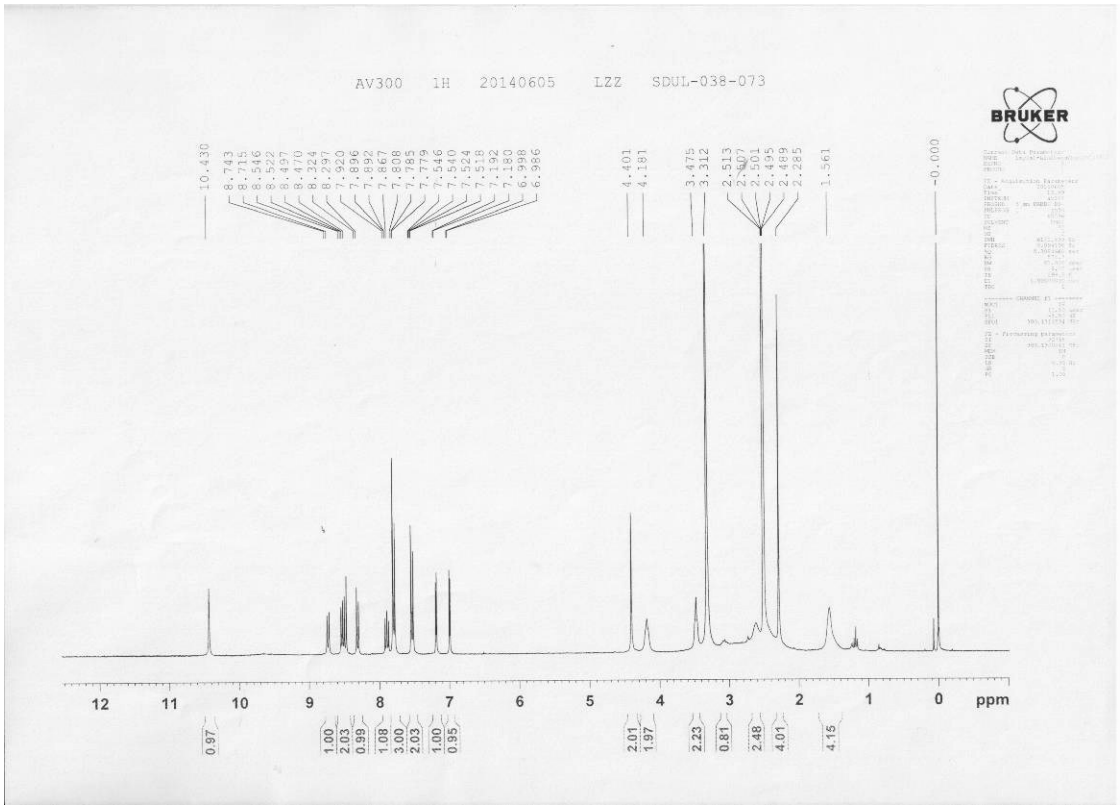

Figure S13. ^{1}H-NMR spectra of probe N4

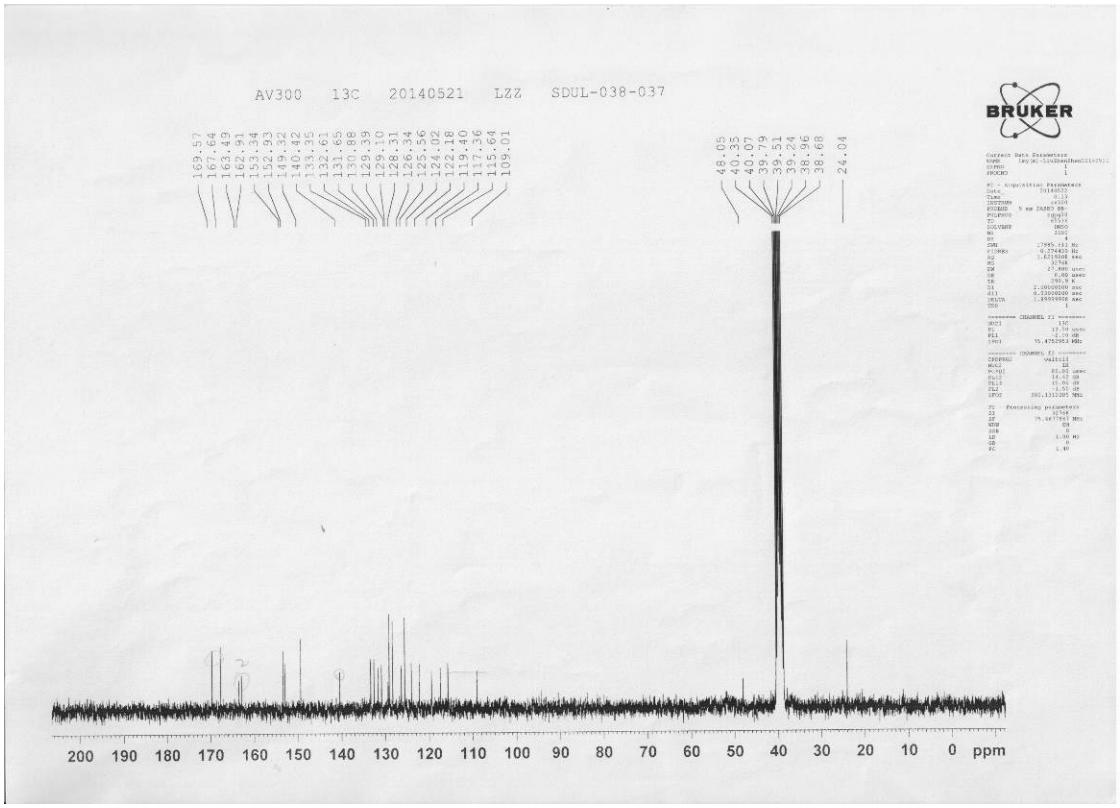

Figure S14. ^{13}C-NMR spectra of probe N4

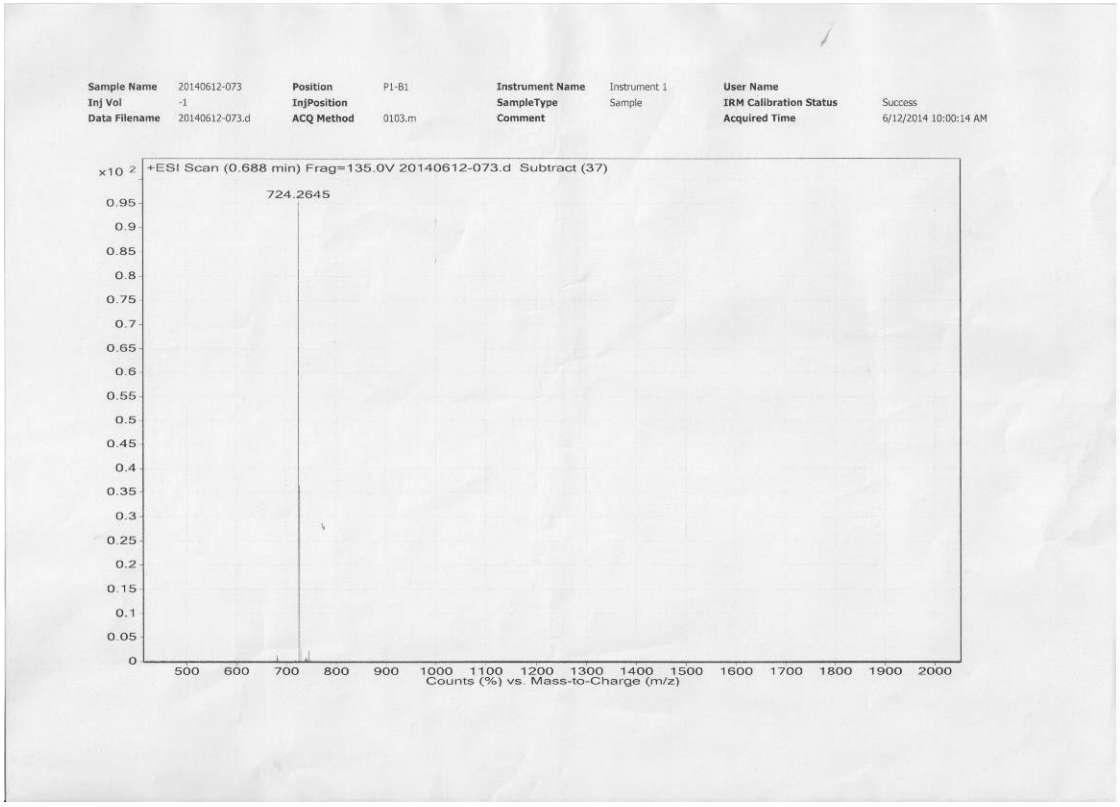

Figure S15. ESI-HRMS spectra of probe N4

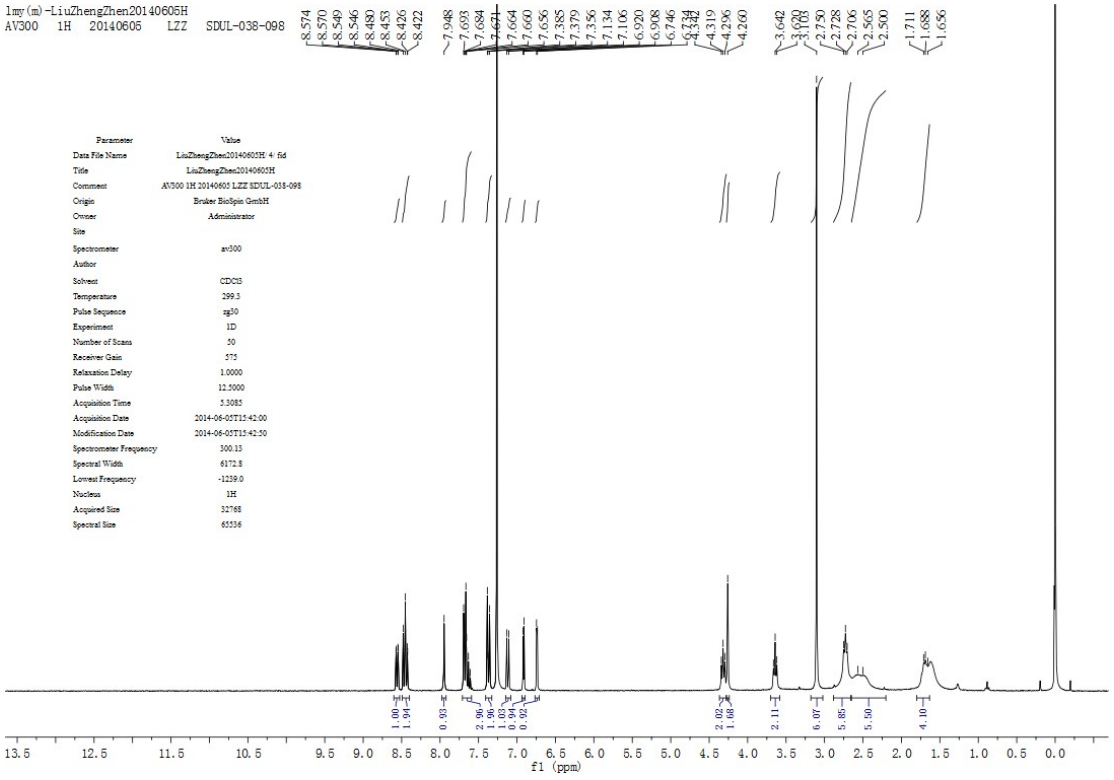

Figure S16. ^{1}H-NMR spectra of probe N5

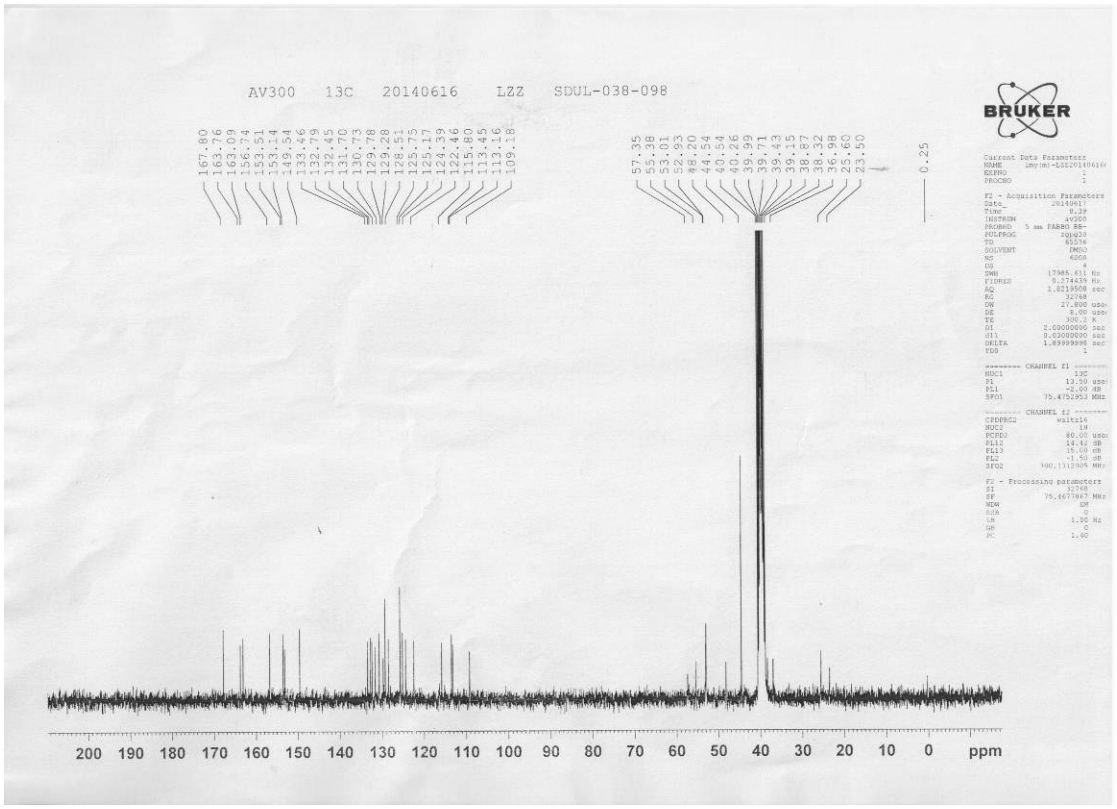

Figure S17. ^{13}C-NMR spectra of probe N5

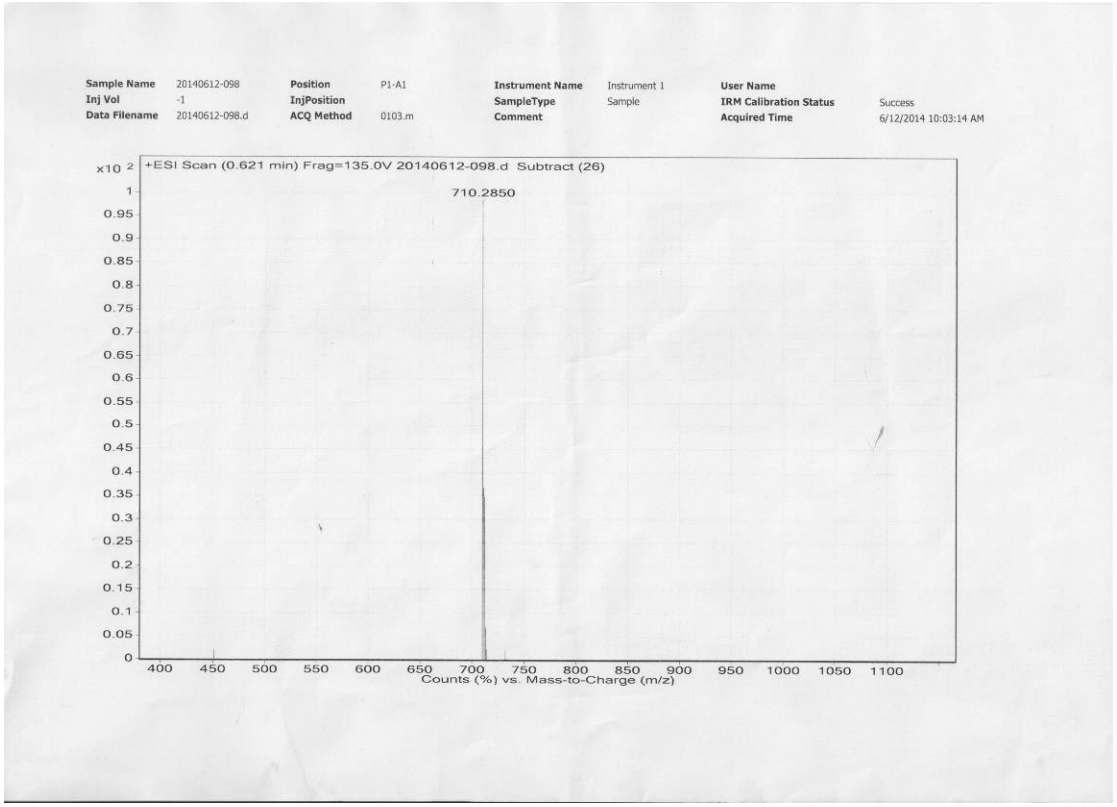

Figure S18. ESI-HRMS spectra of probe N5

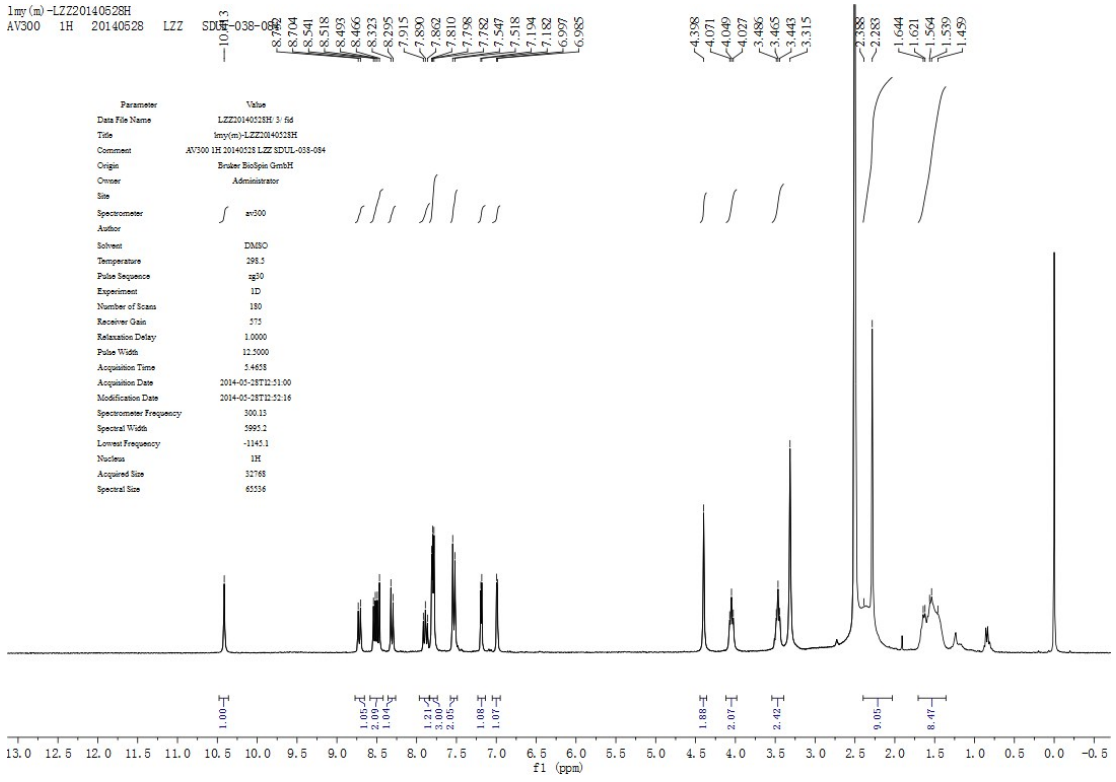

Figure S19. ^{1}H-NMR spectra of probe N6

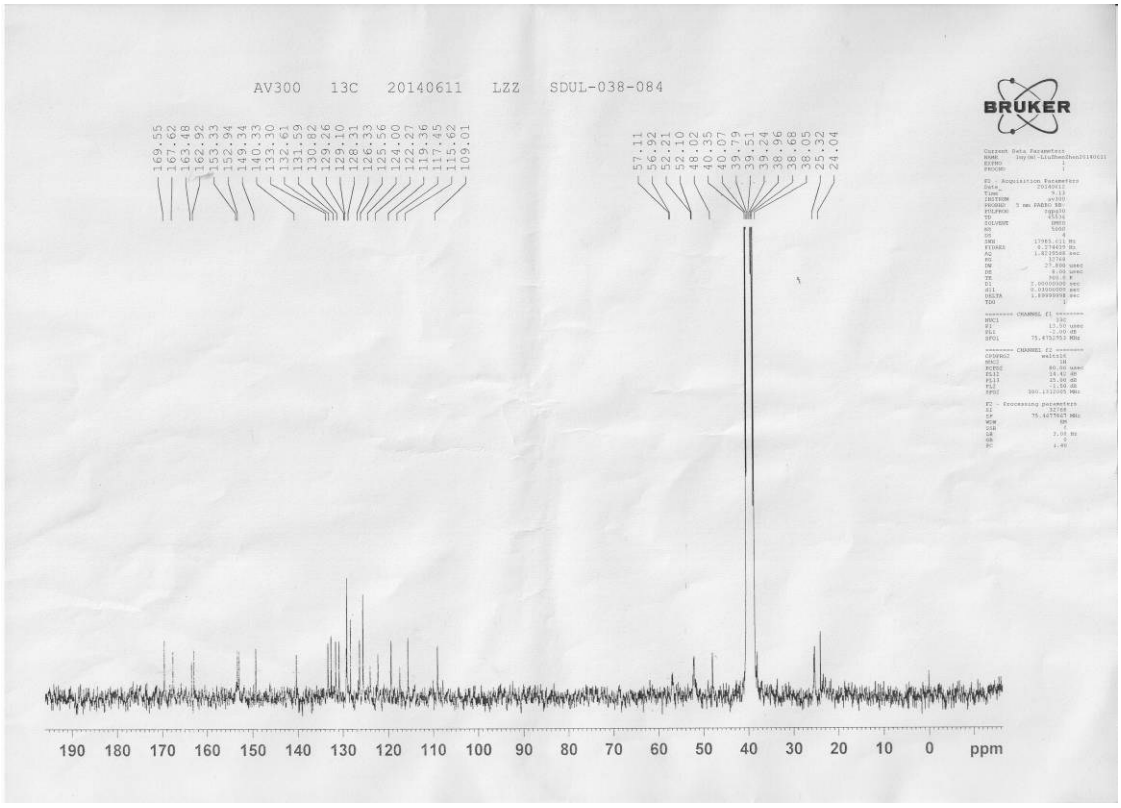

Figure S20. ^{13}C-NMR spectra of probe N6

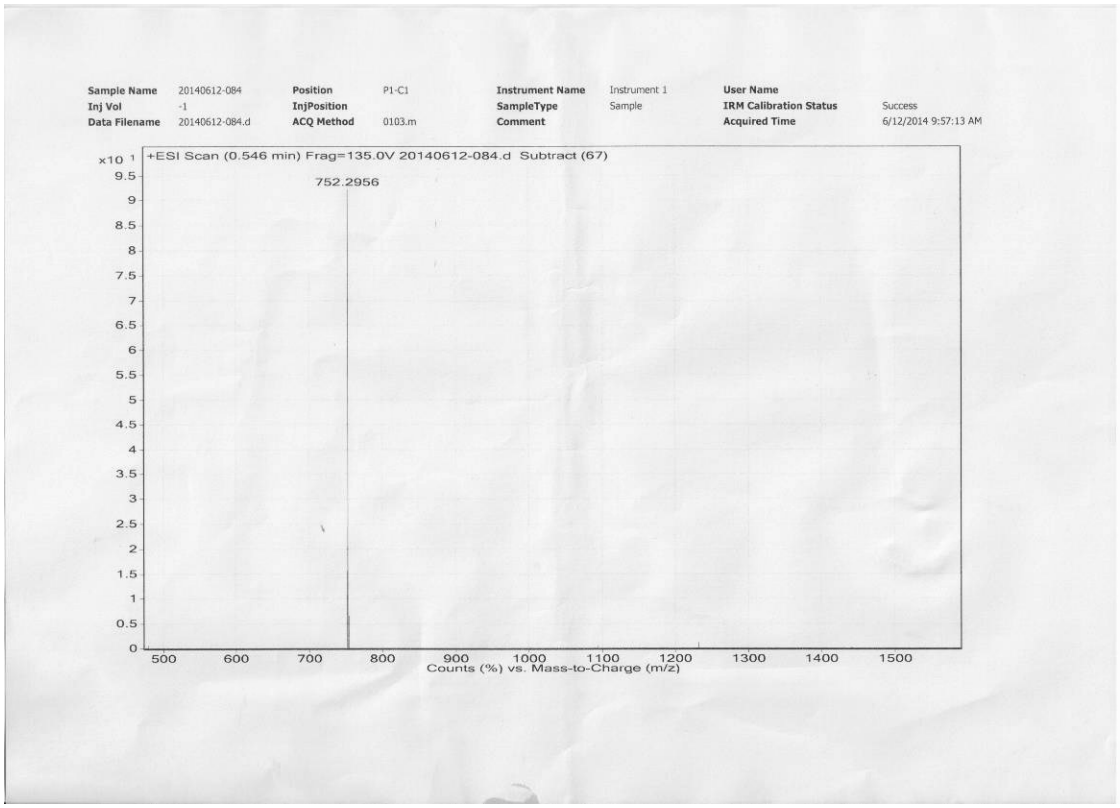

Figure S21. ESI-HRMS spectra of probe N6

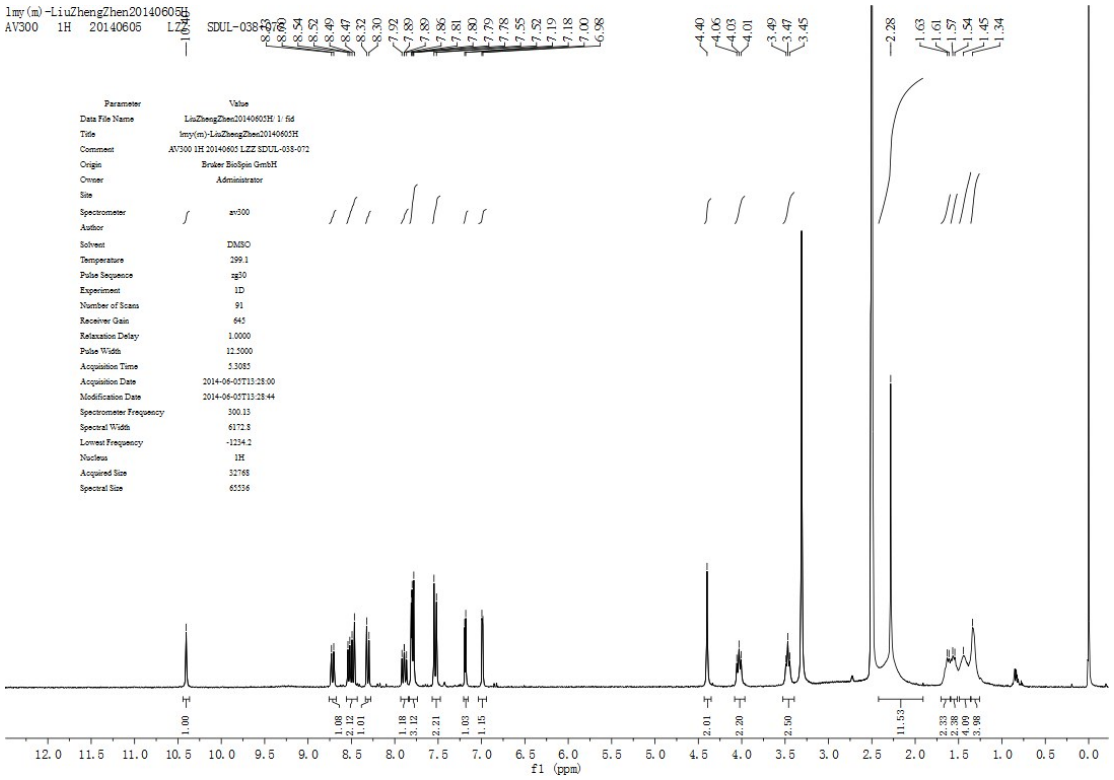

Figure S22. ^{1}H-NMR spectra of probe N7

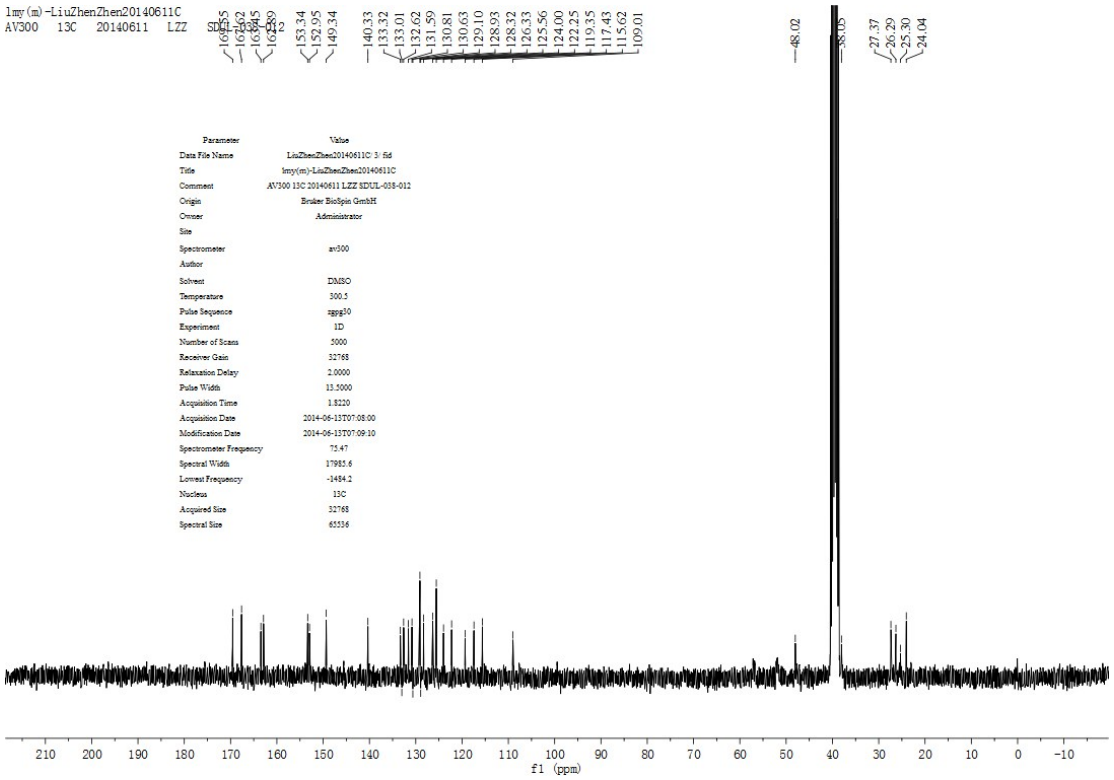

Figure S23. ^{13}C-NMR spectra of probe N7

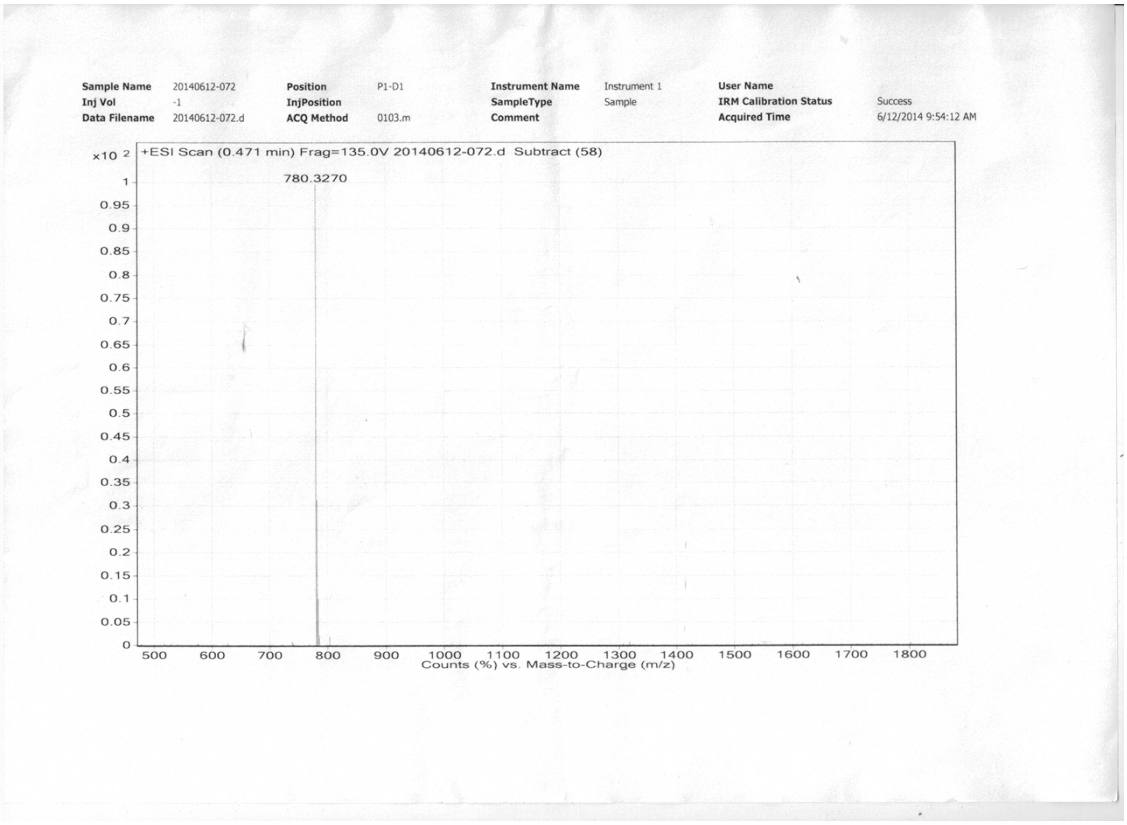

Figure S24. ESI-HRMS spectra of probe N7