Electronic Supplementary Material (ESI) for Medicinal Chemistry Communications

Supporting Information

^{99m}Tc-labeled Benzothiazole and Stilbene Derivatives as Imaging Agents for Aβ Plaques in Cerebral Amyloid Angiopathy

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General information

All reagents used in the synthesis were commercial products and were used without further purification unless otherwise indicated. The ^{1}H-NMR spectra were obtained at 400 MHz on Bruker Avance Ⅲ NMR spectrometers in $CDCl_3$ or DMSO-d_6 solutions at room temperature with TMS as an internal standard. Chemical shifts were reported as δ values relative to the internal TMS. Coupling constants were reported in Hertz. Multiplicity is defined by s (singlet), d (doublet), t (triplet), and m (multiplet). Mass spectra were acquired under SurveyorMSQ Plus (ESI) (Waltham, MA, USA) instrument. Radiochemical purity was determined by HPLC performed on a Shimadzu SCL-20 AVP system equipped with a SPD-20A UV detector (λ = 254 nm) and Bioscan Flow Count 3200 NaI/PMT γ-radiation scintillation detector. HPLC

separations and analysis were both achieved on a Venusil MP C18 reverse phase column (Agela Technologies, 5 μm, 4.6 mm × 250 mm) eluted with a binary gradient system at a flow rate of 1.0 mL/min. Mobile phase A was water (0.1% TFA) while mobile phase B was acetonitrile (0.1% TFA). Reactions were monitored by TLC (Silica gel 60 F_{254} aluminum sheets, Merck) and compounds were visualized by illumination with a short wavelength UV lamp (λ = 254 nm or 365 nm). Column chromatography purification was performed on silica gel (54 - 74 μm) from Qingdao Haiyang Chemical Co., Ltd or alumina. Fluorescent observation was performed by the Axio Observer Z1 inverted fluorescence microscope (Zeiss, Germany) equipped with a DAPI filter set (excitation, 405 nm) and AF488 filter set (excitation, 495 nm). Normal ICR mice (five weeks, male) were used for biodistribution experiments. All protocols requiring the use of animal were approved by the animal care committee of Beijing Normal University. Post-mortem brain tissues from an autopsy-confirmed case of AD (64-year-old, female, 8 μm, temporal lobe) was kindly gifted from Dr. Jiapei Dai, which were obtained from Chinese Brain Bank Center (CBBC) by autopsy.

1. Chemistry

4-(5-(3-Bromopropoxy)benzo[d]thiazol-2-yl)-*N*,*N*-dimethylbenzenamine (2)

A mixture of **1** (540.0 mg, 2.00 mmol), 1,3-dibromoprotane (808.0 mg, 4.00 mmol) and K_2CO_3 (279.5 mg, 2.00 mmol) in CH_3CN (30 mL) was stirred at 90 ˚C. After removing the K_2CO_3, CH_3CN was removed in vacuum. The residue was

purified by silica gel column chromatography (petroleum ether/AcOEt = 6/1, v/v) to give 234.6 mg **2** as light yellow solid (yield, 30.1%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.91 (d, *J* = 8.6 Hz, 2H), 7.86 (d, *J* = 8.9 Hz, 1H), 7.33 (d, *J* = 2.0 Hz, 1H), 7.04 (d, *J* = 8.9 Hz, 1H), 6.76 (d, *J* = 8.4 Hz, 2H), 4.18 (t, *J* = 5.8 Hz, 2H), 3.64 (t, *J* = 6.4 Hz, 2H), 3.06 (s, 6H), 2.48 – 2.29 (m, 2H).

4-(5-(5-Bromopentyloxy)benzo[d]thiazol-2-yl)-*N*,*N*-dimethylbenzenamine (3)

The same method described above to prepare **2** was used, and 33.6 mg of **3** was obtained in a yield of 26.7%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.83 (d, *J* = 8.9 Hz, 2H), 7.80 (d, *J* = 9.0 Hz, 1H), 7.61 (d, *J* = 2.2 Hz, 1H), 7.05 (dd, *J* = 8.8, 2.4 Hz, 1H), 6.81 (d, *J* = 8.9 Hz, 2H), 4.05 (t, *J* = 6.3 Hz, 2H), 3.57 (t, *J* = 6.7 Hz, 2H), 3.01 (s, 6H), 1.96 – 1.81 (m, 2H), 1.80 – 1.67 (m, 2H), 1.61 – 1.48 (m, 2H).

4-(5-(3-(Bis(pyridin-2-ylmethyl)amino)propoxy)benzo[d]thiazol-2-yl)-*N*,*N*-dimethylbenzenamine (4)

A mixture of bis(pyridin-2-ylmethyl)amine (40.1 mg, 0.20 mmol), **2** (77.4 mg, 0.20 mmol), K_2CO_3 (62.5 mg, 0.45 mmol) and KI (26.6 mg, 0.16 mmol) in CH_3CN (10 mL) was heated to reflux overnight at 90 ˚C. After removing CH_3CN, water was added, the resulting mixture was extracted by dichloromethane (3 × 20 mL) and the organic layer was dried over anhydrous $MgSO_4$. The organic solvent was removed in vacuum, and the residue was purified by silica gel column chromatography (petroleum ether/AcOEt = 4/1, v/v) to give 26.0 mg **4** as light yellow solid (yield,

25.5%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.51 (d, *J* = 4.7 Hz, 2H), 7.91 (d, *J* = 8.9 Hz, 2H), 7.82 (d, *J* = 8.9 Hz, 1H), 7.61 – 7.45 (m, 4H), 7.22 (d, *J* = 2.3 Hz, 1H), 7.15 – 7.08 (m, 2H), 6.90 (dd, *J* = 8.9, 2.4 Hz, 1H), 6.75 (d, *J* = 8.9 Hz, 2H), 4.06 (t, *J* = 5.9 Hz, 2H), 3.87 (s, 4H), 3.06 (s, 6H), 2.88 – 2.70 (m, 2H), 2.15 – 1.95 (m, 2H). MS: calcd for $C_{30}H_{31}N_5OS$ (m/z) 509.2, obsd 510.4 (M + H, ES+).

4-(5-(5-(Bis(pyridin-2-ylmethyl)amino)pentyloxy)benzo[d]thiazol-2-yl)-*N*,*N*-dimethylbenzenamine (5)

The same method described above to prepare **4** was used, and 33.6 mg of **5** was obtained in a yield of 14.6%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.52 (d, *J* = 4.2 Hz, 2H), 7.90 (d, *J* = 8.9 Hz, 2H), 7.84 (d, *J* = 8.9 Hz, 1H), 7.63 (td, *J* = 7.7, 1.7 Hz, 2H), 7.54 (d, *J* = 7.8 Hz, 2H), 7.27 (s, 1H), 7.17 – 7.09 (m, 2H), 6.99 (dd, *J* = 8.9, 2.5 Hz, 1H), 6.74 (d, *J* = 8.9 Hz, 2H), 3.96 (t, *J* = 6.4 Hz, 2H), 3.83 (s, 4H), 3.04 (s, 6H), 2.59 (t, *J* = 7.2 Hz, 2H), 1.78 – 1.71 (m, 2H), 1.68 – 1.58 (m, 2H), 1.51 – 1.43 (m, 1H). MS: calcd for $C_{32}H_{35}N_5OS$ (m/z) 537.3, obsd 538.6 (M + H, ES+).

Synthesis of complex 6

A solution of **5** (50.9 mg, 0.10 mmol) and $Re(CO)_5Cl$ (36.1 mg, 0.10 mmol) in CH_3OH (10 mL) was heated to reflux for 4 h at 70 ˚C. Evaporation of the solvent under reduced pressure gave a residue, which was purified by alumina column chromatography (CH_3CN/H_2O = 30/1, v/v) to give a yellow mucoid material, which was recrystaled in dichloromethane and ethyl acetate (1/2, v/v) to give **6** as yellow

powder (yield, 26.1%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.66 (d, *J* = 5.0 Hz, 2H), 7.91 (d, *J* = 8.8 Hz, 3H), 7.87 (d, *J* = 8.7 Hz, 2H), 7.82 (s, 2H), 7.36 (s, 1H), 7.21 (s, 2H), 7.04 (d, *J* = 8.8 Hz, 1H), 6.74 (d, *J* = 8.8 Hz, 2H), 5.98 (s, 2H), 4.46 (s, 2H), 4.22 (s, 2H), 4.03 (s, 2H), 3.05 (s, 6H), 2.75 – 2.47 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 195.76, 166.99, 161.05, 155.87, 152.05, 150.71, 149.03, 140.42, 135.73, 128.63, 125.35, 124.99, 122.76, 121.39, 115.37, 111.82, 105.55, 68.39, 67.30, 65.58, 40.21, 25.64. HRMS: calcd for $C_{33}H_{31}N_5O_4SRe^+$ (m/z) 778.1627, obsd 778.1628 (M^+, ES+).

Synthesis of complex 7

The same reaction described above to prepare **6** was used, and 33.6 mg of **7** was obtained in a yield of 42.0%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.63 (d, *J* = 5.2 Hz, 2H), 7.99 (d, *J* = 7.9 Hz, 2H), 7.89 (d, *J* = 8.9 Hz, 2H), 7.85 – 7.79 (m, 3H), 7.35 (d, *J* = 2.3 Hz, 1H), 7.18 (t, *J* = 6.5 Hz, 2H), 7.03 (dd, *J* = 8.9, 2.4 Hz, 1H), 6.73 (d, *J* = 8.9 Hz, 2H), 6.05 (d, *J* = 16.5 Hz, 2H), 4.41 (d, *J* = 16.4 Hz, 2H), 4.09 (t, *J* = 6.0 Hz, 2H), 3.83 – 3.67 (m, 2H), 3.04 (s, 6H), 2.22 – 2.14 (m, 2H), 2.06 – 1.85 (m, 2H), 1.79 – 1.45 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 195.99, 166.55, 161.41, 156.48, 151.97, 150.54, 148.69, 140.27, 135.72, 128.57, 125.37, 125.16, 122.67, 121.54, 115.47, 111.80, 105.33, 71.04, 68.06, 67.17, 40.22, 28.85, 25.50, 23.72. HRMS: calcd for $C_{35}H_{35}N_5O_4SRe^+$ (m/z) 806.1932, obsd 806.1940 (M^+, ES+).

(*E*)-4-(4-(3-bromopropoxy)styryl)-*N*,*N*-dimethylbenzenamine (9)

A mixture of (*E*)-4-(4-(dimethylamino)styryl)phenol (71.7 mg, 0.30 mmol),

1,3-dibromopentane (120.0 mg, 0.60 mmol) and K_2CO_3 (41.4 mg, 0.30 mmol) in CH_3CN (15 mL) was stirred at 90 ˚C. After removing CH_3CN, water was added to the reactant, the resulting mixture was extracted by dichloromethane (3 ×20 mL) and the organic layer was dried over anhydrous $MgSO_4$. After the solvent was removed by vacuum, light pink powder **9** was obtained without further purification (yield, 62.7%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.46 (d, J = 8.4 Hz, 2H), 7.39 (d, J = 8.4 Hz, 2H), 7.22 – 6.81 (m, 4H), 6.71 (d, J = 8.5 Hz, 2H), 4.09 (t, J = 5.9 Hz, 2H), 3.67 (t, J = 6.5 Hz, 2H), 2.92 (s, 6H), 2.35 – 2.12 (m, 2H).

(*E*)-4-(4-(5-bromopentyloxy)styryl)-*N*,*N*-dimethylbenzenamine (10)

The same method described above to prepare **9** was used, and 98.3 mg of **10** was obtained in a yield of 87.8%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.44 (d, J = 8.7 Hz, 2H), 7.38 (d, J = 8.8 Hz, 2H), 7.19 – 6.50 (m, 4H), 6.71 (d, J = 8.8 Hz, 2H), 3.97 (t, J = 6.3 Hz, 2H), 3.57 (t, J = 6.7 Hz, 2H), 2.92 (s, 6H), 1.87 – 1.71 (m, 2H), 1.82 – 1.64(m, 2H), 1.61 – 1.46 (m, 2H).

(*E*)-4-(4-(3-(bis(pyridin-2-ylmethyl)amino)propoxy)styryl)-*N*,*N*-dimethylbenzenamine (11)

The same method described above to prepare **4** was used, and 48.0 mg of **11** was obtained in a yield of 35.2%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.51 (d, J = 4.5 Hz, 2H), 7.64 – 7.57 (m, 2H), 7.56 – 7.46 (m, 4H), 7.45 – 7.30 (m, 2H), 7.18 – 7.11 (m, 2H), 6.89 (d, J = 7.4 Hz, 2H), 6.77 (d, J = 8.7 Hz, 2H), 6.72 (d, J = 8.8 Hz, 2H), 4.01 (t, J =

6.0 Hz, 2H), 3.91 (s, 4H), 2.98 (s, 6H), 2.88 – 2.70 (m, 2H), 2.09 – 1.98 (m, 2H). MS: calcd for $C_{31}H_{34}N_4O$ (m/z) 478.3, obsd 479.5 (M + H, ES+).

(*E*)-4-(4-(3-(bis(pyridin-2-ylmethyl)amino)pentyloxy)styryl)-*N*,*N*-dimethylbenzenamine (12)

The same method described above to prepare **4** was used, and 46.0 mg of **12** was obtained in a yield of 30.1%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.52 (d, *J* = 4.4 Hz, 2H), 7.65 (t, *J* = 7.1 Hz, 2H), 7.60 – 7.47 (m, 2H), 7.38 (d, *J* = 7.5 Hz, 2H), 7.19 – 7.10 (m, 2H), 6.88 (d, *J* = 6.3 Hz, 2H), 6.83 (d, *J* = 8.7 Hz, 2H), 6.71 (d, *J* = 8.8 Hz, 2H), 3.92 (t, *J* = 6.4 Hz, 2H), 3.86 (s, 4H), 2.97 (s, 6H), 2.73 – 2.52 (m, 2H), 1.76 – 1.69 (m, 2H), 1.66 – 1.58 (m, 2H), 1.51 – 1.39 (m, 2H). MS: calcd for $C_{33}H_{38}N_4O$ (m/z) 506.3, obsd 507.5 (M + H, ES+).

Synthesis of complex 13

The same method described above to prepare compound **6** was used, and 34.1 mg of **13** was obtained in a yield of 43.3%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.66 (d, *J* = 5.5 Hz, 2H), 7.99 (d, *J* = 7.9 Hz, 2H), 7.81 (td, *J* = 7.8, 1.3 Hz, 2H), 7.41 (t, *J* = 8.8 Hz, 4H), 7.19 (t, *J* = 8.2 Hz, 2H), 6.89 (d, *J* = 7.8Hz, 4H), 6.75 (d, *J* = 7.9 Hz, 2H), 6.33 (d, *J* = 16.3 Hz, 2H), 4.40 (d, *J* = 16.2 Hz, 2H), 4.18 (t, *J* = 5.3 Hz, 2H), 4.07 – 3.95 (m, 2H), 2.98 (s, 6H), 2.64-2.57 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 195.88, 161.44, 157.50, 150.53, 140.28, 131.55, 127.36, 127.29, 127.04, 125.40, 125.17, 114.87, 112.90, 68.52, 67.02, 65.06, 40.76, 25.82. HRMS: calcd for $C_{34}H_{34}N_4O_4Re^+$ (m/z) 747.2110, obsd 747.2141 (M^+, ES+).

Synthesis of complex 14

The same method described above to prepare compound **6** was used, and 26.8 mg of **14** was obtained in a yield of 49.1%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.64 (d, *J* = 5.3 Hz, 2H), 7.97 (d, *J* = 7.9 Hz, 2H), 7.81 (t, *J* = 7.5 Hz, 2H), 7.40 (d, *J* = 8.5 Hz, 4H), 7.21 – 7.14 (m, 2H), 6.92 – 6.86 (m, 4H), 6.80 (s, 2H), 6.15 (d, *J* = 16.6 Hz, 2H), 4.36 (d, *J* = 15.8 Hz, 2H), 4.05 (t, *J* = 5.9 Hz, 2H), 3.81 – 3.69 (m, 2H), 2.99 (s, 6H), 2.21 – 2.12 (m, 2H), 1.97 – 1.90 (m, 2H), 1.67 – 1.60 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 195.97, 161.31, 158.09, 150.59, 149.84, 140.39, 130.99, 129.84, 127.28, 127.20, 126.73, 126.39, 125.30, 125.23, 124.17, 114.80, 112.70, 70.98, 67.49, 67.27, 40.61, 28.83, 25.46, 23.68. HRMS: calcd for $C_{36}H_{38}N_4O_4Re^+$ (m/z) 775.2423, obsd 775.2441 (M^+, ES+).

2. Preparation of [^{99m}Tc]7 and [^{99m}Tc]14

A freshly prepared solution (1 mL) of the *fac*-[^{99m}Tc$(CO)_3(H_2O)_3]^+$ precursor (pH 7)[1] was added to a vial that contained a solution of **5** (500 μg) and **13** (500 μg) in ethanol (200 μL)**,** respectively. The vial was sealed and heated for 10 min at 100 ˚C. After cooling to room temperature, the resulting mixture was extracted by dichloromethane (3 × 0.5 mL) and the solvents were removed under a stream of nitrogen gas. The residue was redissolved in 100 μL acetonitrile and purified by HPLC on a Venusil MP C18 reverse phase column (5 μm, 4.6 × 250 mm) with a binary gradient system (acetonitrile and water containing 0.1% of TFA) at 1.0 mL/min

flow rate to give [^{99m}Tc]**7** (7/3, v/v, t_R = 8.99 min, radiochemical yield of 67.8%) and [^{99m}Tc]**14** (5/5, v/v, t_R = 10.35 min, radiochemical yield of 41.7%).

3. Binding assays using the aggregated A$\beta_{(1–42)}$ peptide in solution

Peptides A$\beta_{(1-42)}$ were purchased from Osaka Peptide Institute (Osaka, Japan). Aggregation was carried out by gently dissolving the peptide [0.56 mg/mL for A$\beta_{(1-42)}$] in a buffer solution (pH = 7.4) containing 10 mM sodium phosphate and 1 mM EDTA. The solutions were incubated at 37 ˚C for 48 h with gentle and constant shaking. Inhibition studies were carried out in 12 × 75 mm borosilicate glass tubes according to the procedure described previously with some modifications.[2] 100 μL of aggregated A$\beta_{(1–42)}$ fibrils were added to the mixture containing 100 μL of radioligands ([^{125}I]IMPY) with appropriate concentration, 100 μL of inhibitors (10^{-4} - $10^{-8.5}$ M in ethanol) and 700 μL BSA (0.1 %, pH = 7.4) in a final volume of 1 mL. Nonspecific binding was defined in the presence of 100 nM IMPY. The mixture was incubated for 3 h at 37 ˚C, and then the bound and free radioactive fractions were separated by vacuum filtration through borosilicate glass fiber filters (Whatman GF/B) using a Mp-48T cell harvester (Brandel, Gaithersburg, MD). Filters containing the bound ^{125}I ligand were counted in a γ-counter (WALLAC/Wizard 1470, USA) with 70% counting efficiency. The half maximal inhibitory concentration (IC_{50}) values were determined using GraphPad Prism 5.0, and those for the inhibition constant (K_i) were calculated using the Cheng–Prusoff equation[3]: $K_i = IC_{50}/(1 + [L]/K_d)$.

4. *In vitro* fluorescent staining using brain sections of AD patient

The brain sections were deparaffinized with 2 × 20 min washes in xylene, 2 × 5 min washes in 100% ethanol, 5 min washes in 90% ethanol/water, 5 min washes in 80% ethanol/water, 5 min wash in 60% ethanol/water, running tap water for 10 min, and then incubated in PBS (0.2 M, pH = 7.4) for 30 min. The brain sections were incubated with 20% ethanol solution (1 mM) of rhenium complexes for 10 min. Finally, the sections were washed with 40% ethanol for 10 min. Fluorescent observation was performed by the Axio Observer Z1 inverted fluorescence microscope (Zeiss, Germany) equipped with DAPI (excitation, 405 nm) and AF (excitation, 495 nm) filter sets. The localization of plaques was confirmed by staining with thioflavin-S (1 mM) on the adjacent sections.

5. Determination of the partition co-efficient

The partition co-efficient of the radioligand was determined as described previously but with some modifications[4]. 1110 kBq of tracer was added to premixed suspensions containing 3.6 mL of *n*-octanol and 3.0 mL of PBS (0.05 M, pH 7.4) in a test tube. The test tube was vortexed for 3 min at room temperature, and centrifuged for 5 min (3500 rpm, Anke TDL80-2B, China). Two samples from the *n*-octanol (50 μL) and buffer (500 μL) layers were measured. The distribution coefficient was determined by calculating the ratio of cpm/mL of *n*-octanol phase versus that of PBS phase and expressed as log*D*. Samples from the *n*-octanol layer were repartitioned until consistent partitions of co-efficient values were obtained. The measurement was

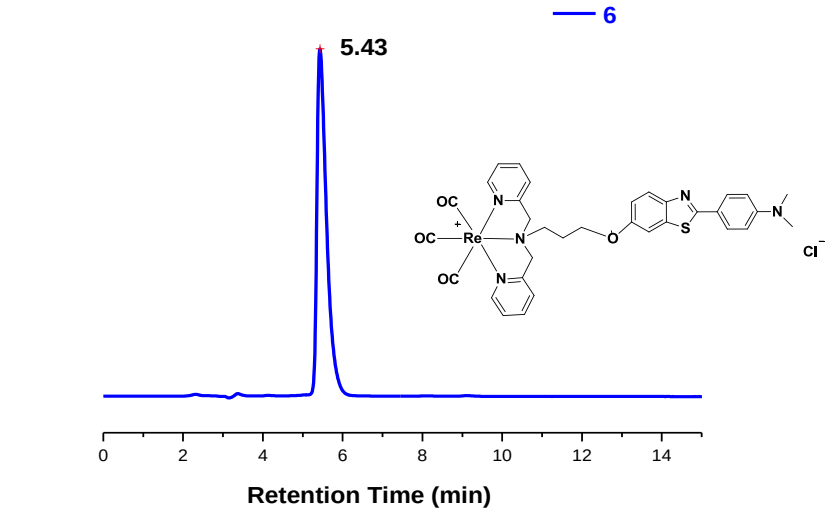
done in triplicate and repeated three times.

6. Biodistribution experiments with normal mice

The biodistribution experiments were performed in normal ICR mice (male, 5 weeks). A saline solution (100 μL, 5% EtOH) containing [^{99m}Tc]**7** (166 kBq) and [^{99m}Tc]**14** (126 kBq) injected directly into the tail vein. The mice were sacrificed at various time points post-injection. The organs of interest were removed and weighed, and the radioactivity was measured with an automatic γ-counter (Wallac 1470 Wizard, USA). The percentage dose per gram of wet tissue was calculated by a comparison of the tissue counts to suitably diluted aliquots of the injected material.

7. Purity and HPLC chromatograms of key target compounds

Compd	Flow rate (mL/min)	Mobile phase ACN % (0.1% TFA)	Agela Technologies, 5 μm	Retention time (RT, min)	Purity (%)
6	1	70	4.6 × 250 mm	5.43	99.42
7	1	70	4.6 × 250 mm	7.52	99.38
[^{99m}Tc]**7**	1	70	4.6 × 250 mm	8.99	95.93
13	1	50	4.6 × 250 mm	5.63	96.85
14	1	50	4.6 × 250 mm	8.85	96.96
[^{99m}Tc]**14**	1	50	4.6 × 250 mm	10.35	96.52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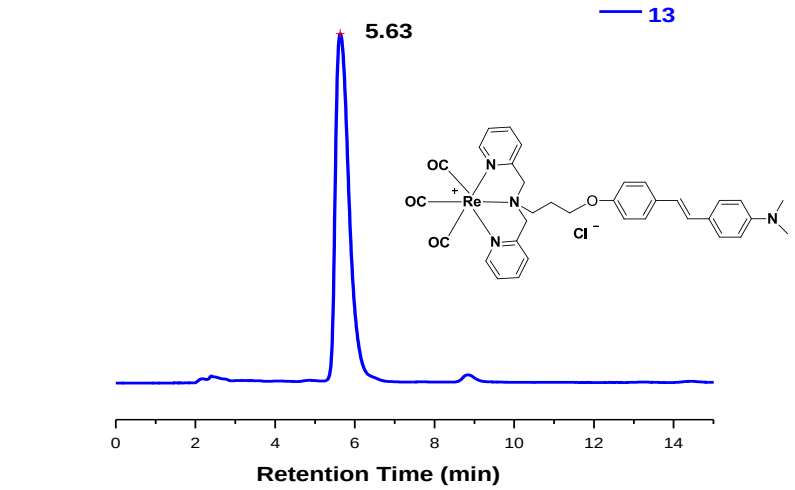
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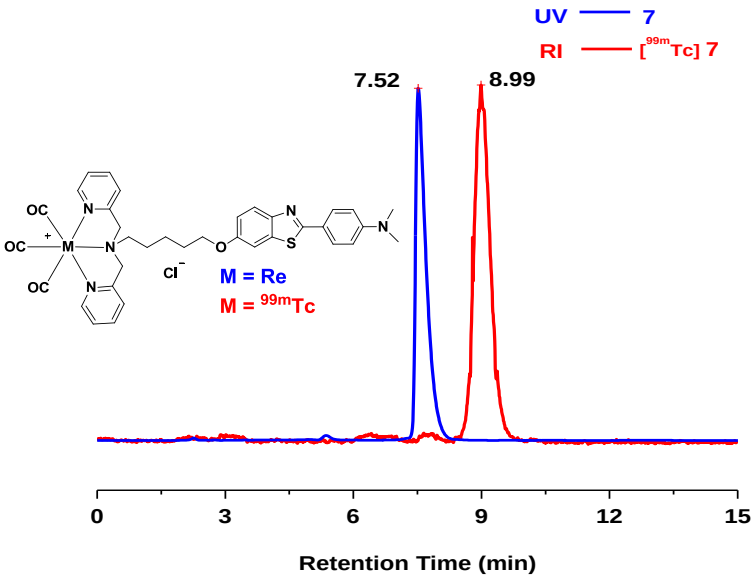
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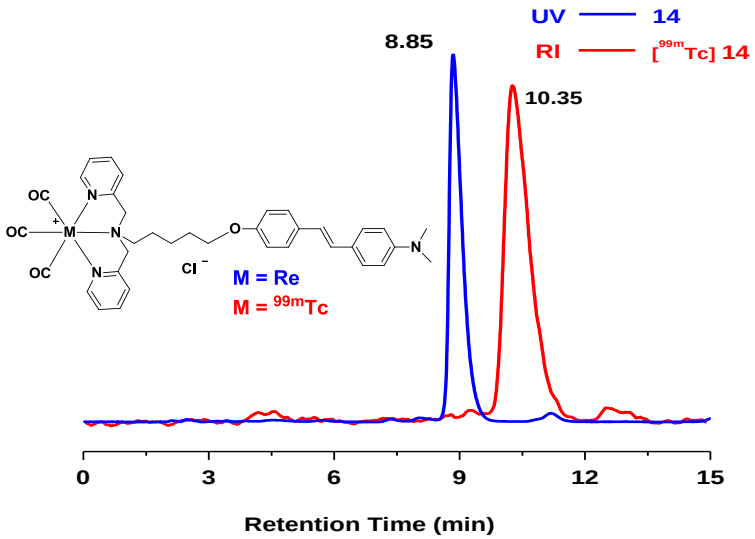
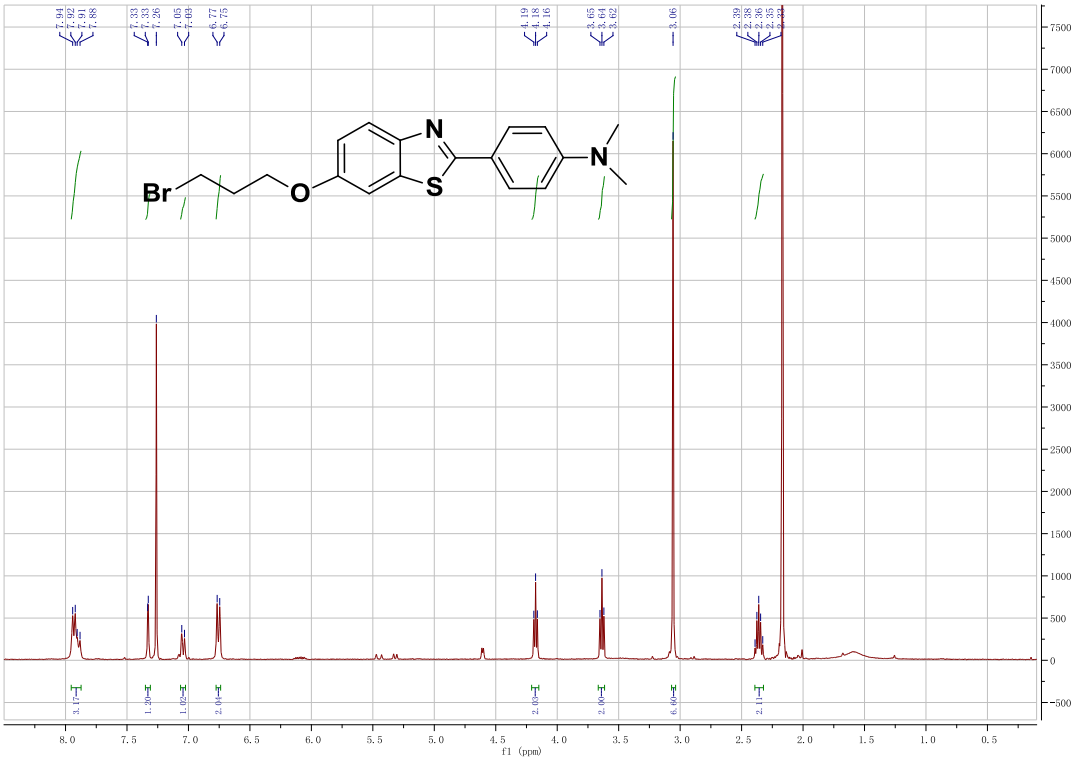


8. ^{1}H-NMR, ^{13}C-NMR, MS and HRMS data of synthesized compounds

^{1}H-NMR for compound **2**



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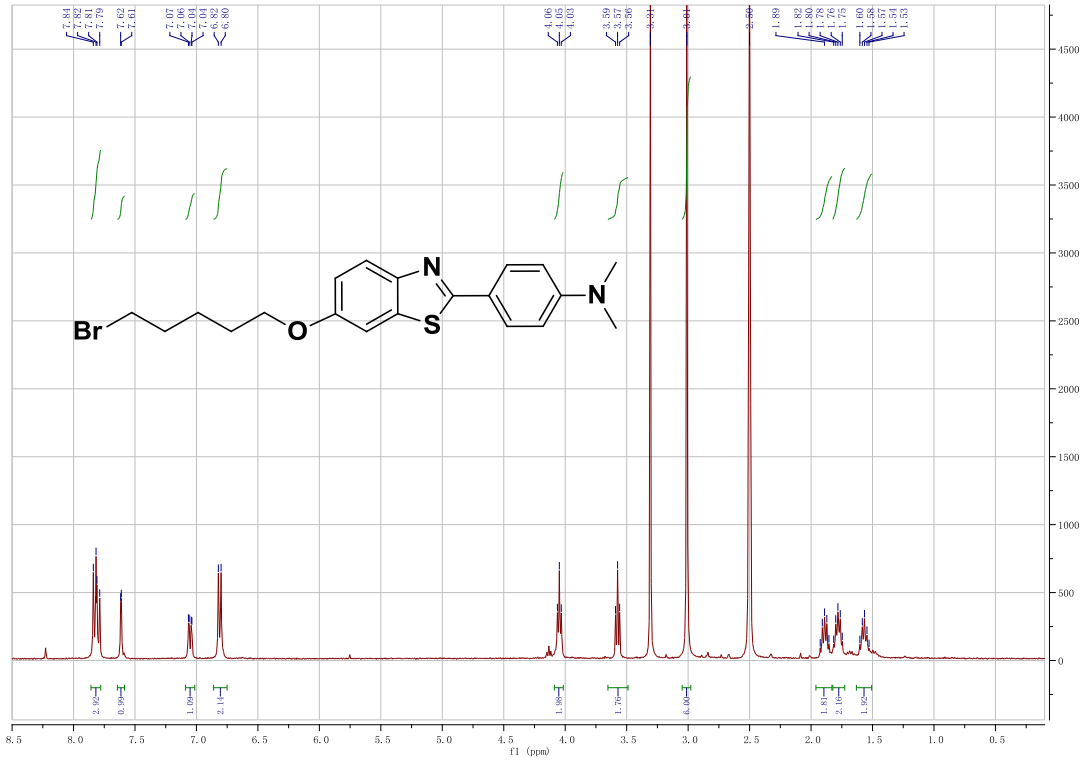
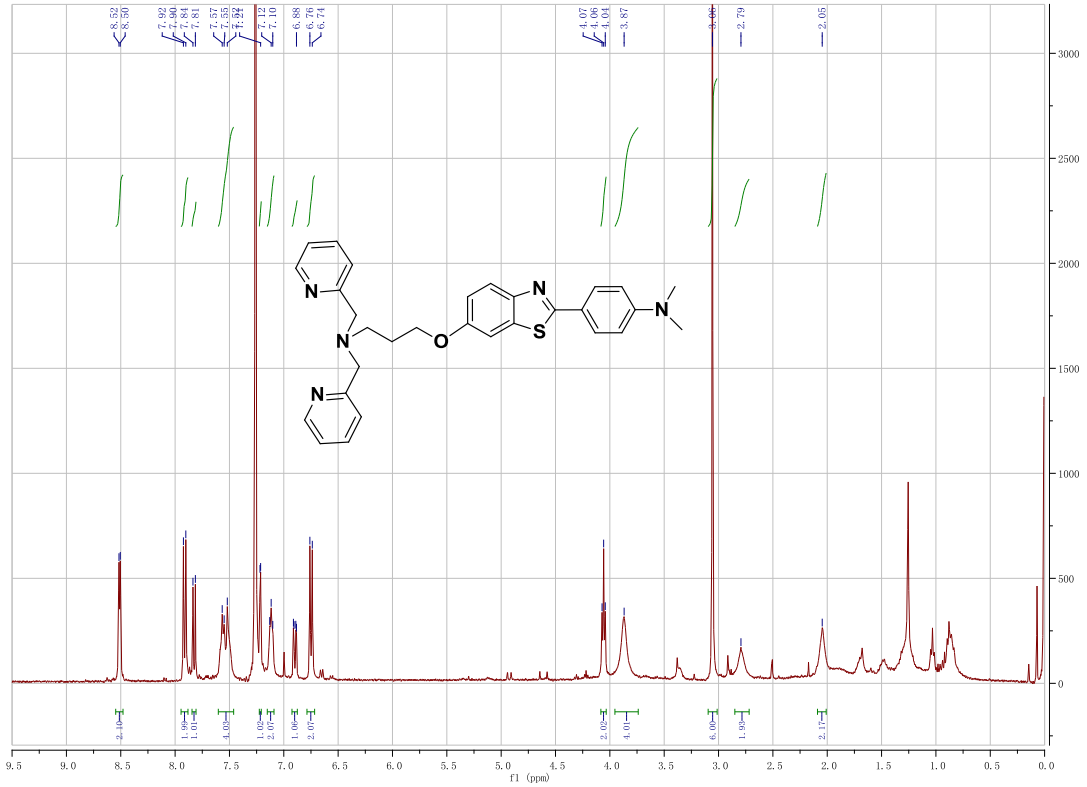
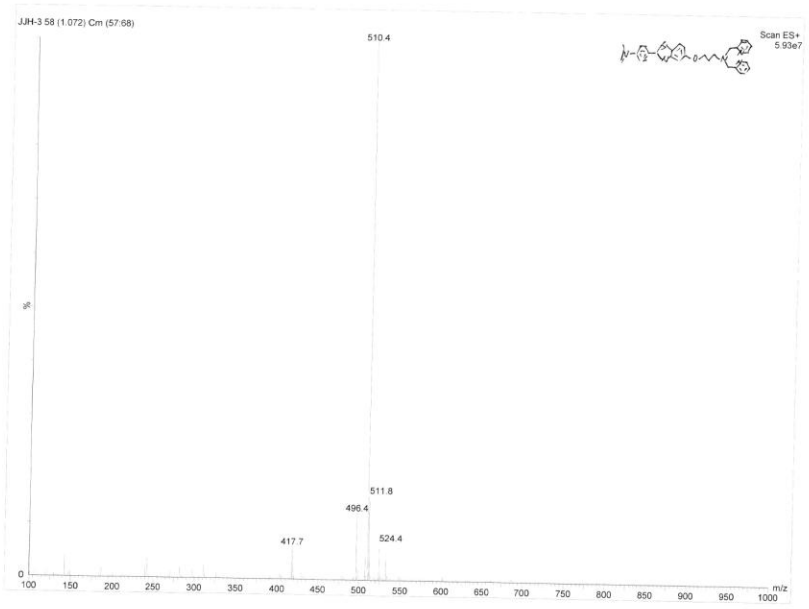


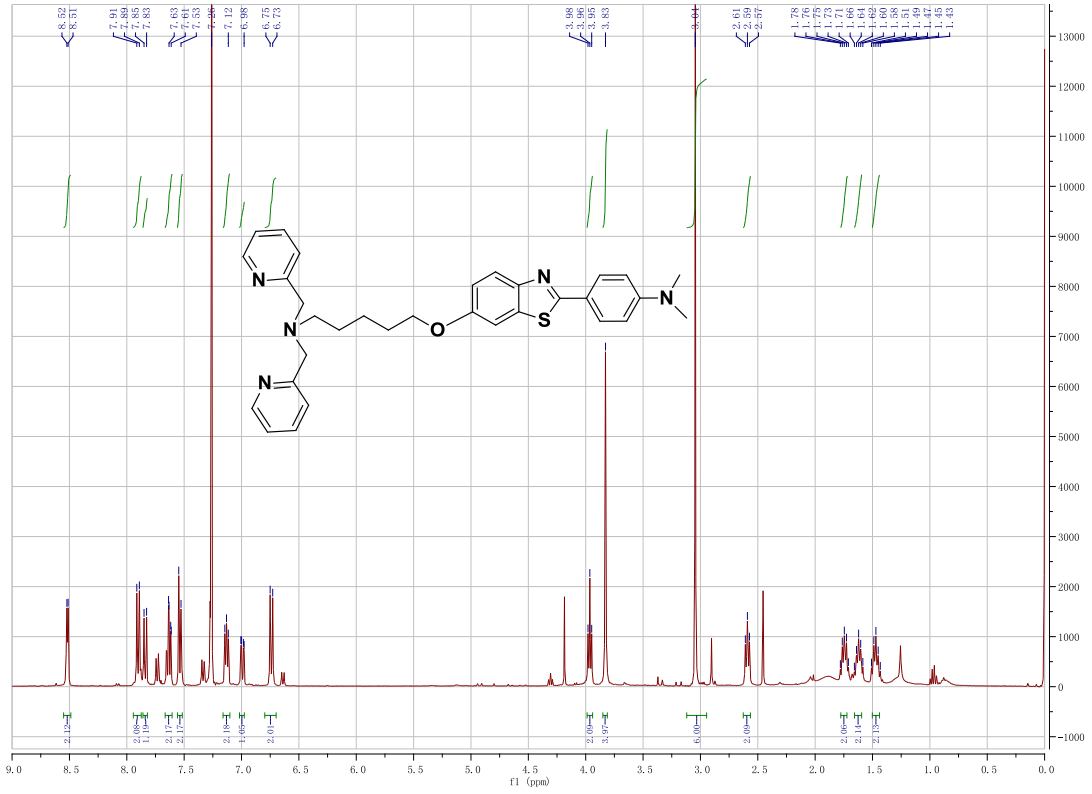
^{1}H-NMR for compound **4**



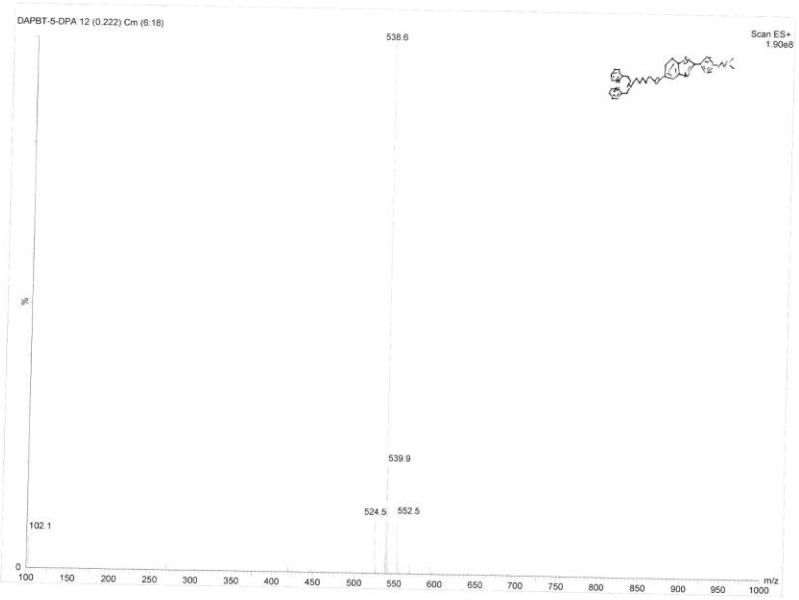
MS for compound **4**



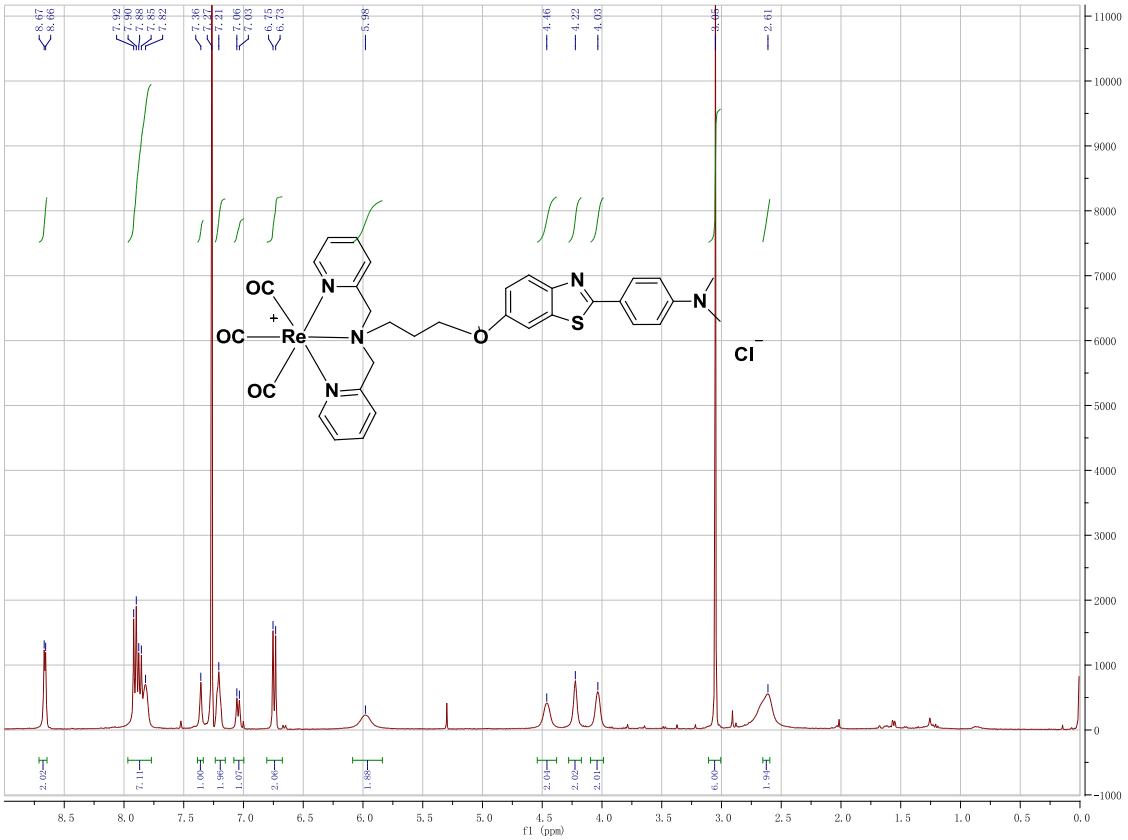
^{1}H-NMR for compound **5**



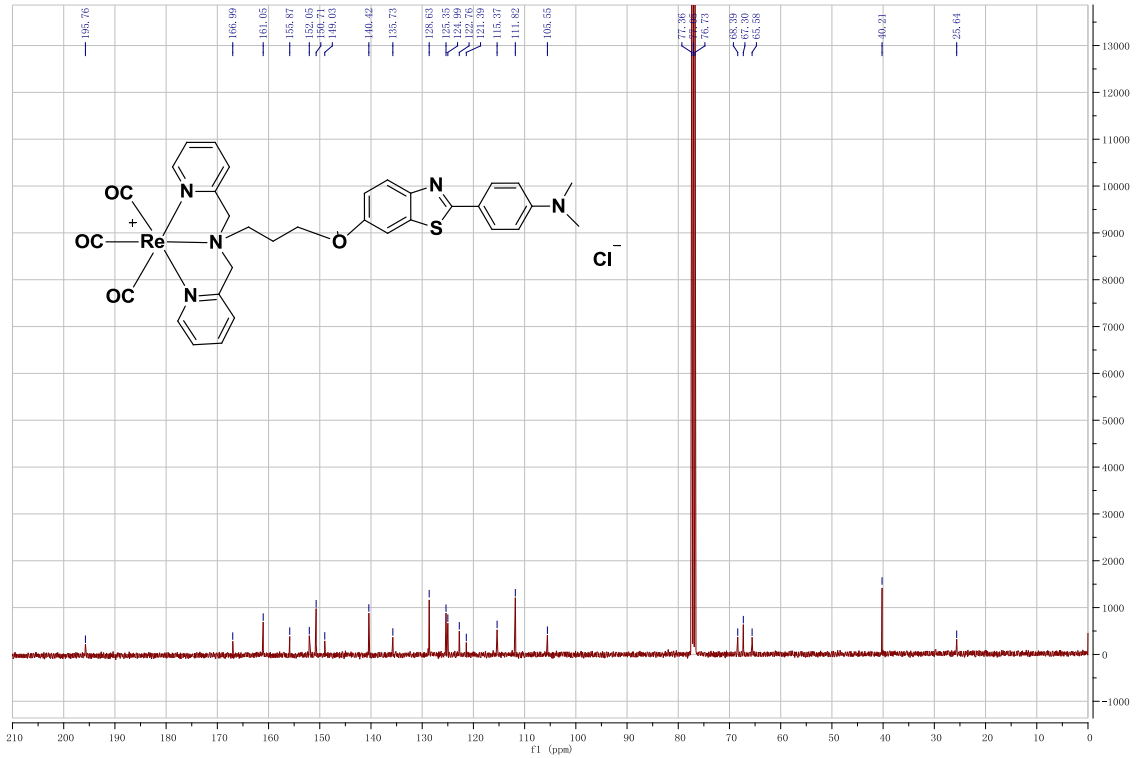
MS for compound **5**



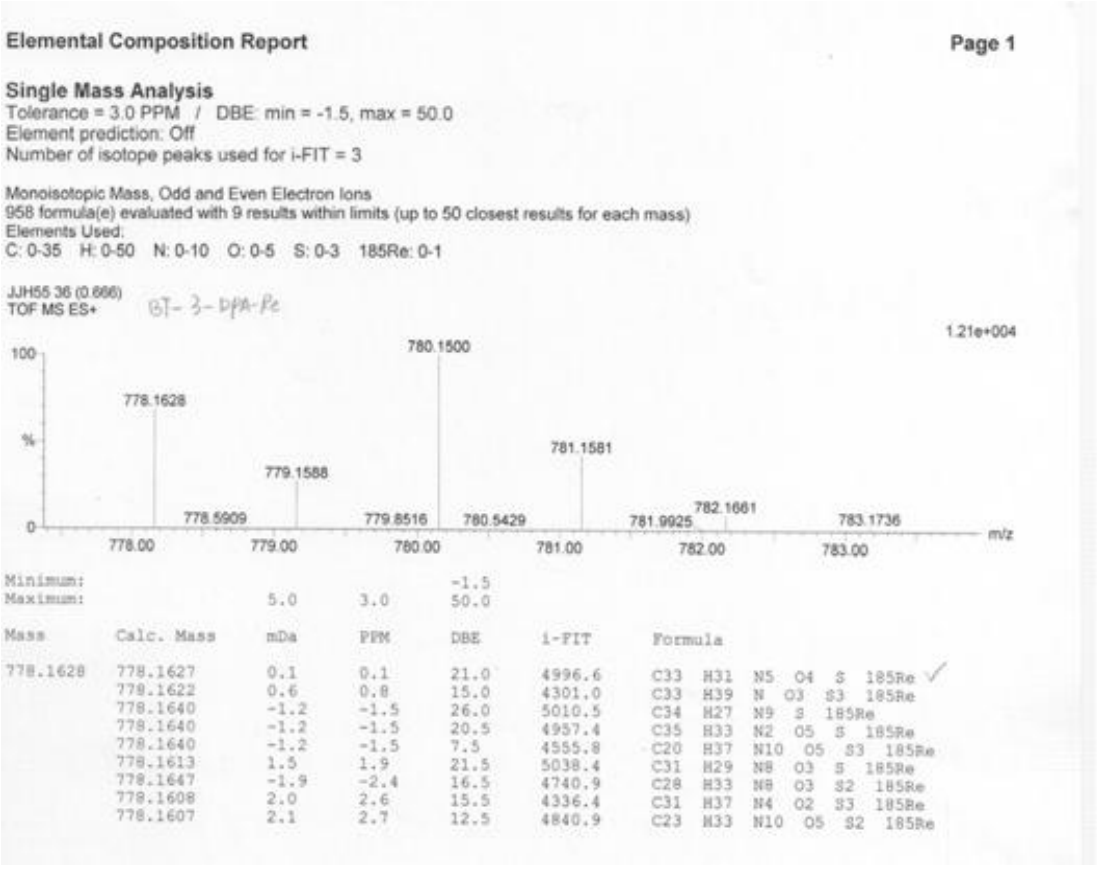
^{1}H-NMR for compound **6**



^{13}C-NMR for compound **6**



HRMS for compound **6**



Elemental Composition Report Page 1

Single Mass Analysis
Tolerance = 3.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

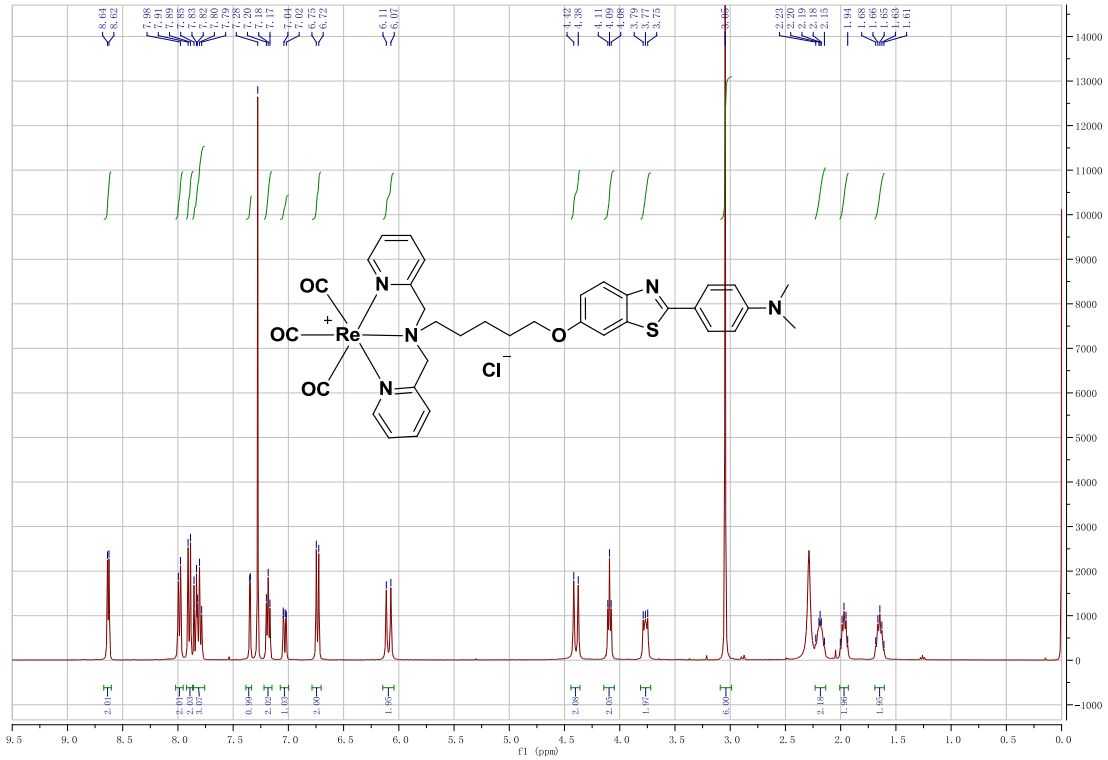
Monoisotopic Mass, Odd and Even Electron Ions
958 formula(e) evaluated with 9 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-35 H: 0-50 N: 0-10 O: 0-5 S: 0-3 185Re: 0-1

JJH55 36 (0.666)
TOF MS ES+ BT- 3-DPA-Re
1.21e+004

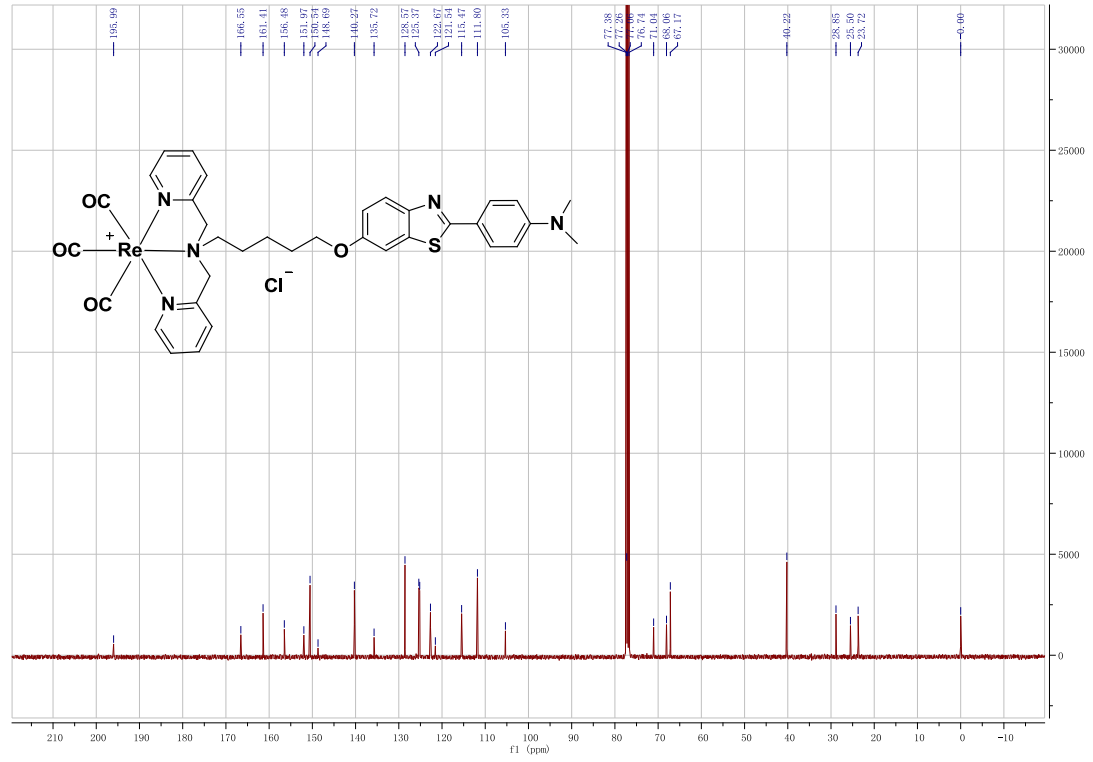
780.1500
778.1628
781.1581
779.1588
778.5909
779.8516
780.5429
781.9925
782.1661
783.1736
m/z

Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
Minimum:				-1.5		
Maximum:		5.0	3.0	50.0		
778.1628	778.1627	0.1	0.1	21.0	4996.6	C33 H31 N5 O4 S 185Re ✓
	779.1622	0.6	0.8	15.0	4301.0	C33 H39 N O3 S3 185Re
	778.1640	-1.2	-1.5	26.0	5010.5	C34 H27 N9 S 185Re
	778.1640	-1.2	-1.5	20.5	4957.4	C35 H33 N2 O5 S 185Re
	778.1640	-1.2	-1.5	7.5	4555.8	C20 H37 N10 O5 S3 185Re
	778.1613	1.5	1.9	21.5	5038.4	C31 H29 N8 O3 S 185Re
	778.1647	-1.9	-2.4	16.5	4740.9	C28 H33 N8 O3 S2 185Re
	778.1608	2.0	2.6	15.5	4336.4	C31 H37 N4 O2 S3 185Re
	778.1607	2.1	2.7	12.5	4840.9	C23 H33 N10 O5 S2 185Re

^{1}H-NMR for compound **7**



^{13}C-NMR for compound **7**



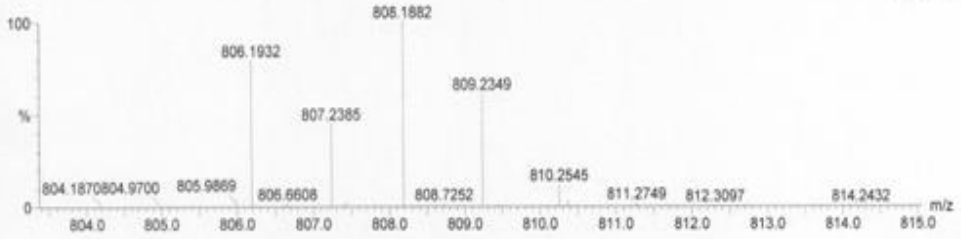
HRMS for compound **7**

Elemental Composition Report Page 1

Single Mass Analysis
Tolerance = 3.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

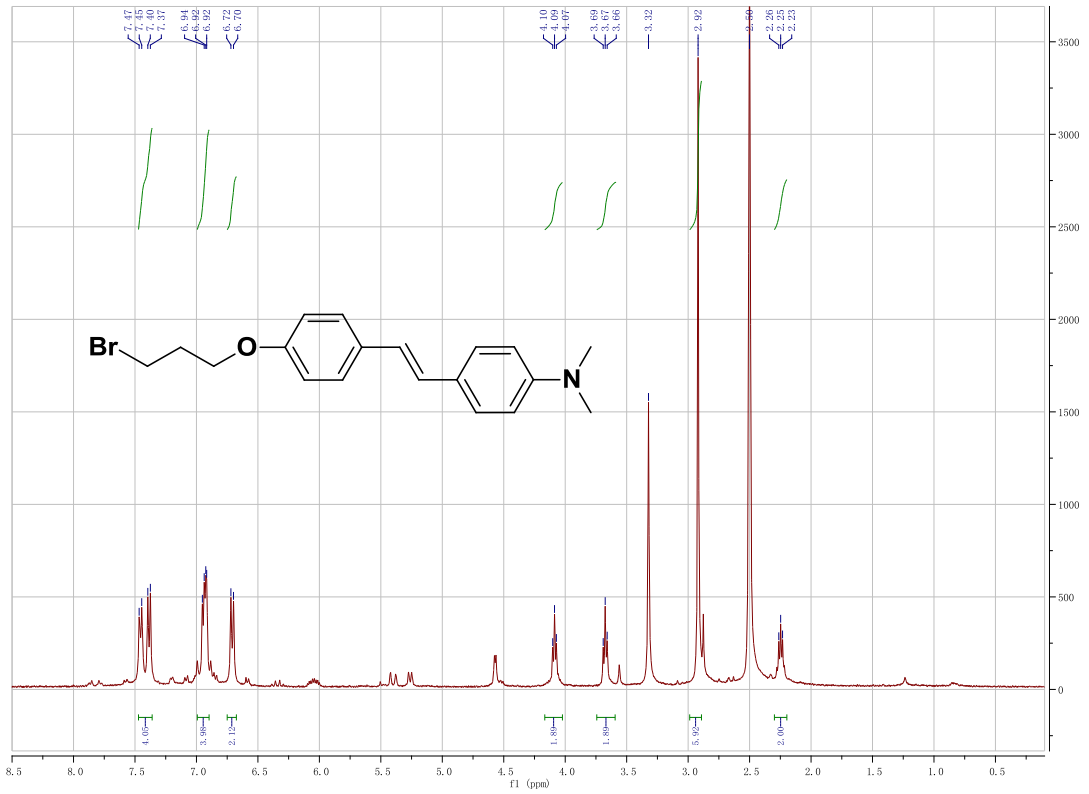
Monoisotopic Mass, Odd and Even Electron Ions
1559 formula(e) evaluated with 5 results within limits (up to 50 best isotopic matches for each mass)
Elements Used:
C: 0-40 H: 0-40 N: 0-5 O: 0-10 S: 0-5 185Re: 0-1

bt-5-dpa-re.3 (0.051)
TOF MS ES+ 5.30e+003

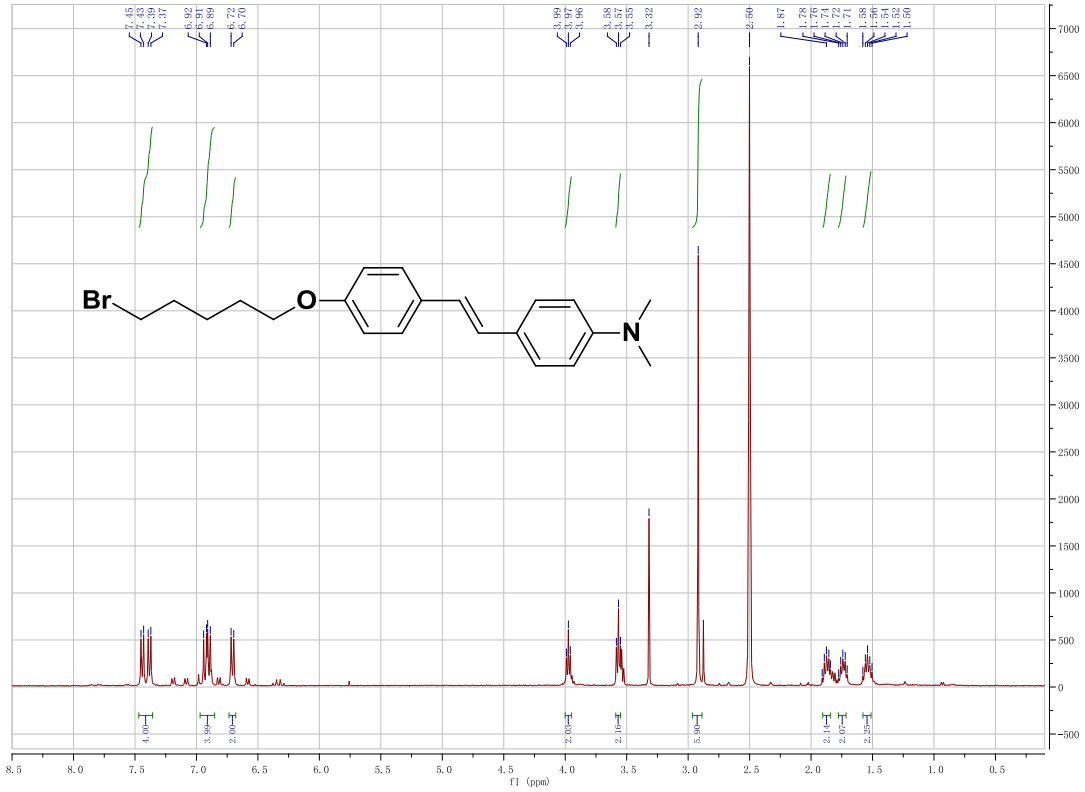


Minimum:		5.0	3.0	-1.5		
Maximum:				50.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
806.1932	806.1953	-2.1	-2.6	20.5	2075.7	C37 H37 N2 O5 S 185Re
	✓806.1940	-0.8	-1.0	21.0	2112.7	C35 H35 N5 O4 S 185Re
	806.1926	0.6	0.7	16.0	2137.3	C34 H39 N O8 S 185Re
	806.1913	1.9	2.4	16.5	2177.4	C32 H37 N4 O7 S 185Re
	806.1919	1.3	1.6	25.5	2209.7	C40 H33 N2 O5 185Re

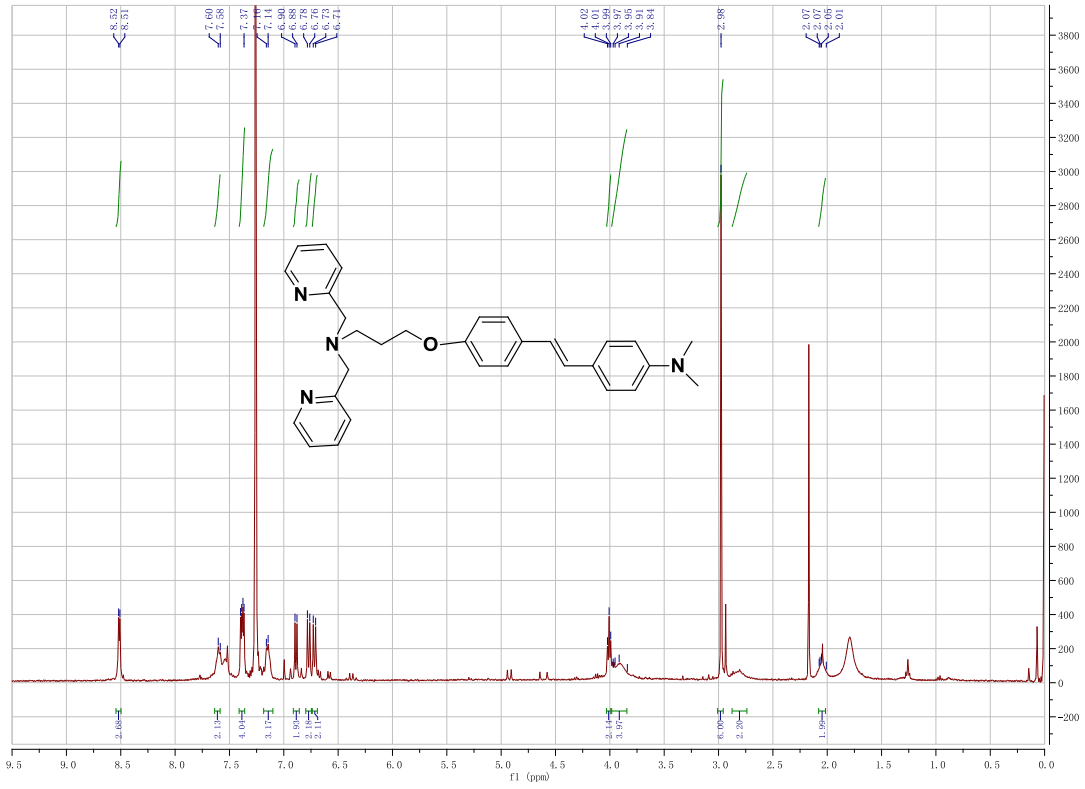
^{1}H-NMR for compound **9**



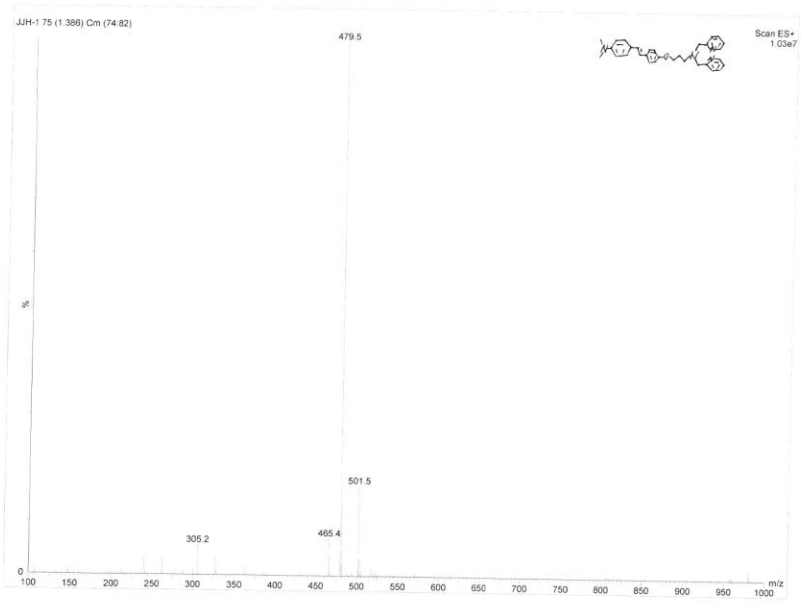
^{1}H-NMR for compound **10**



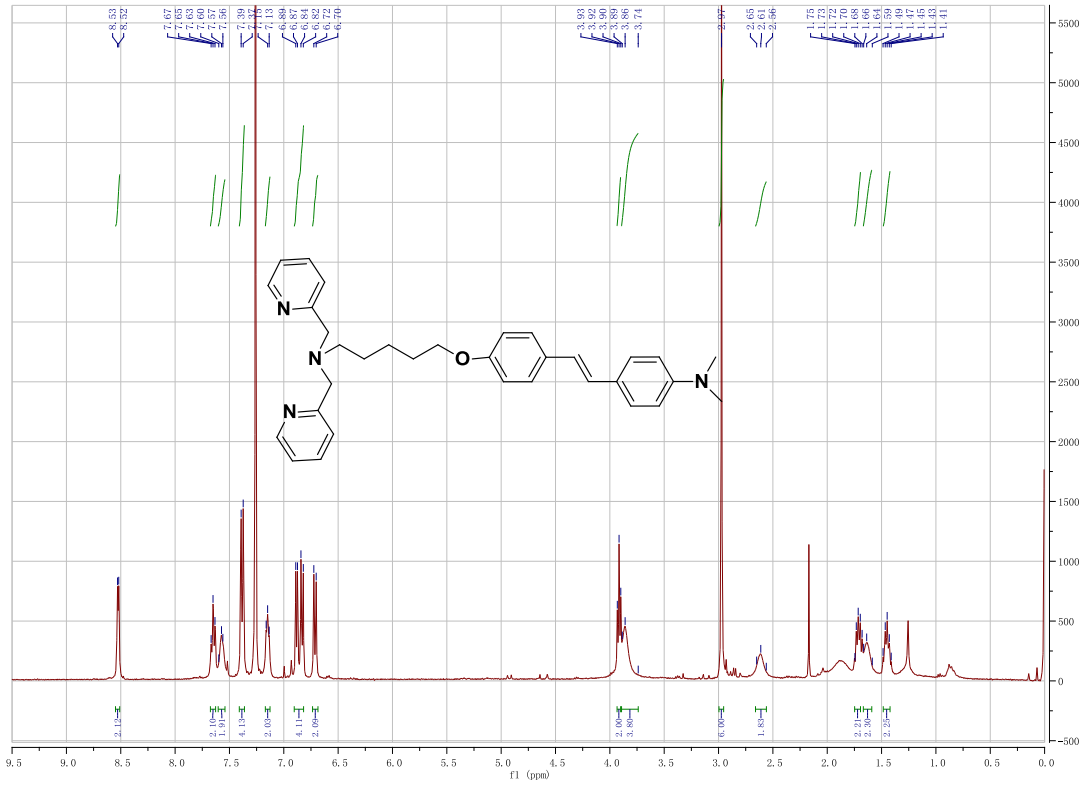
^{1}H-NMR for compound **11**



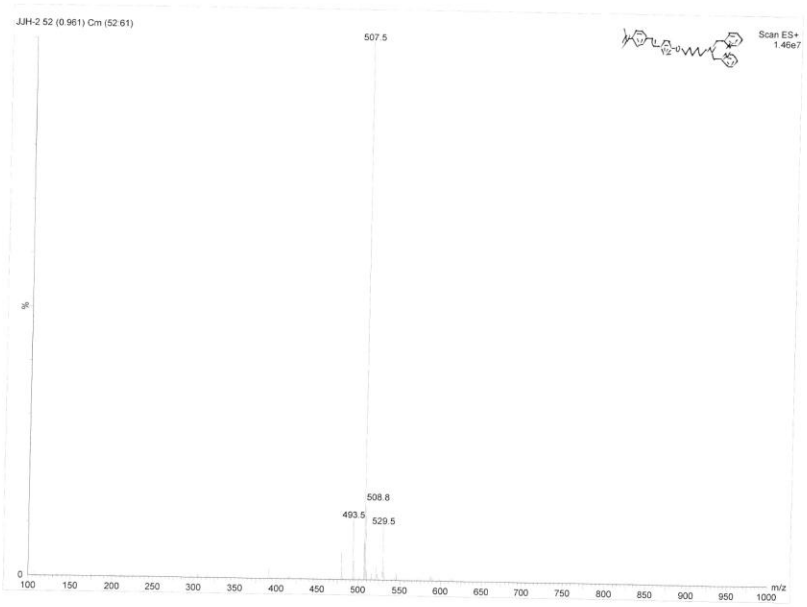
MS for compound **11**



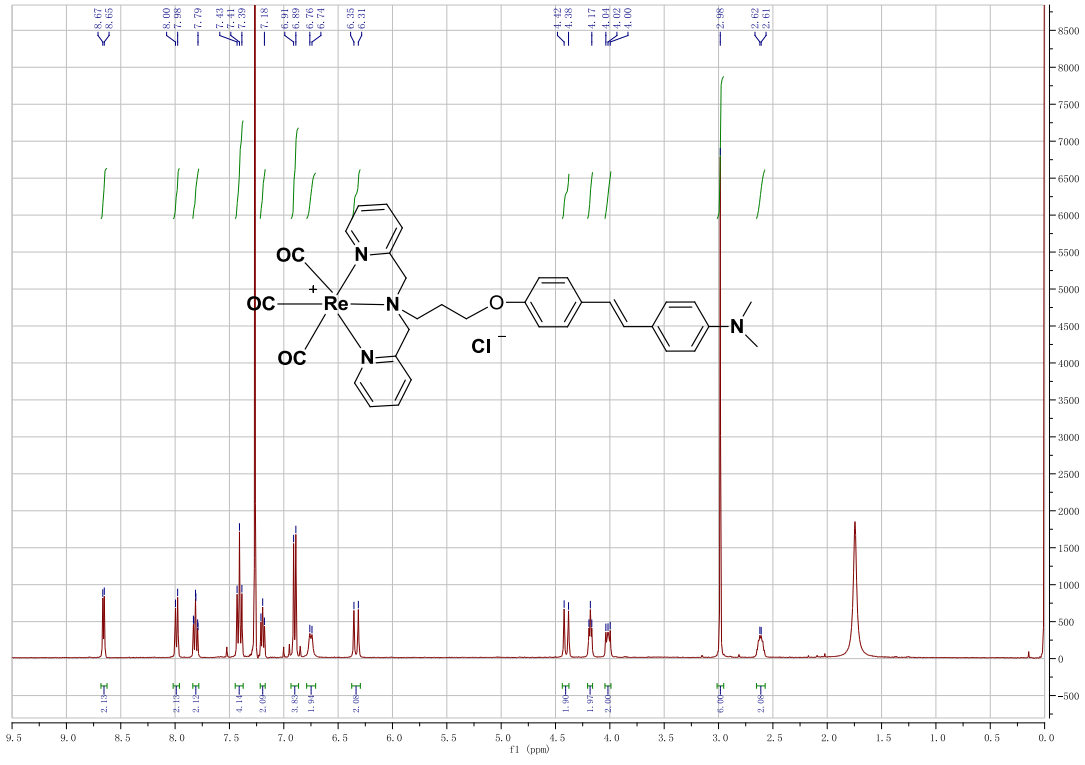
^{1}H-NMR for compound **12**



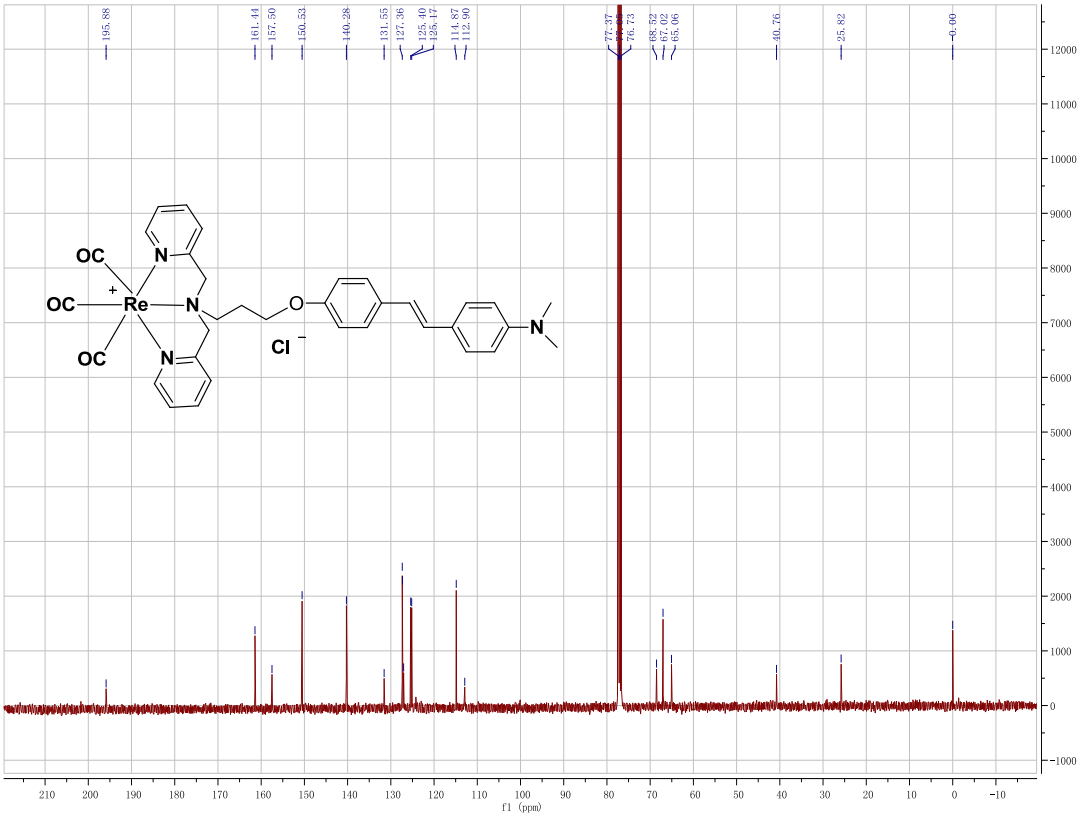
MS for compound **12**



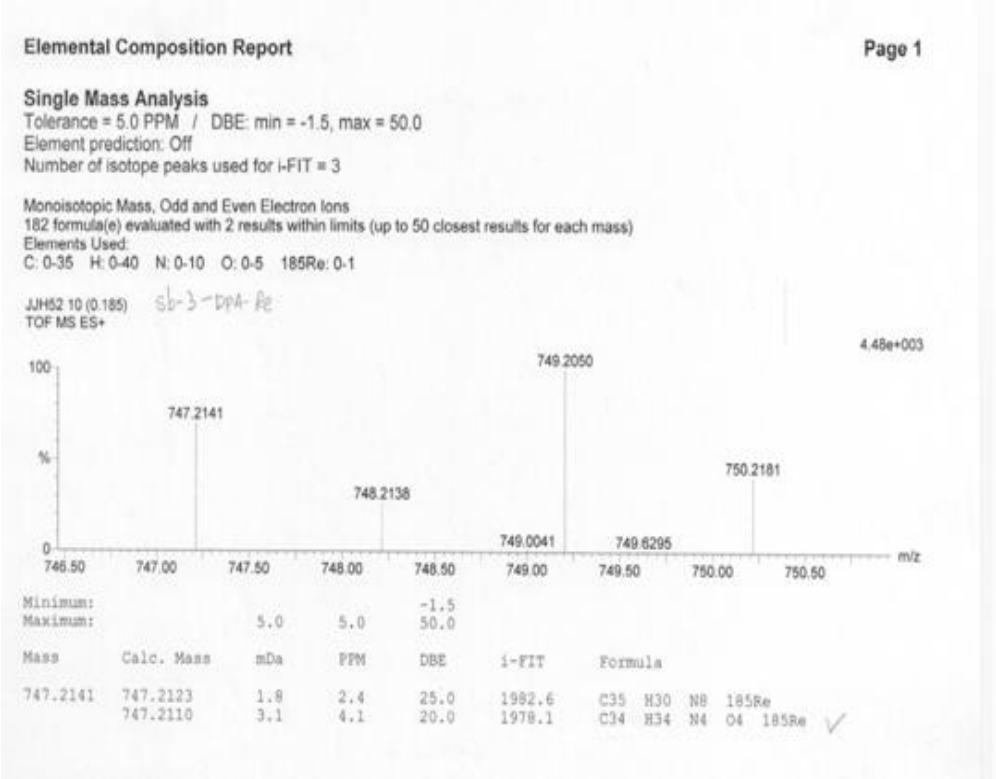
^{1}H -NMR for compound **13**



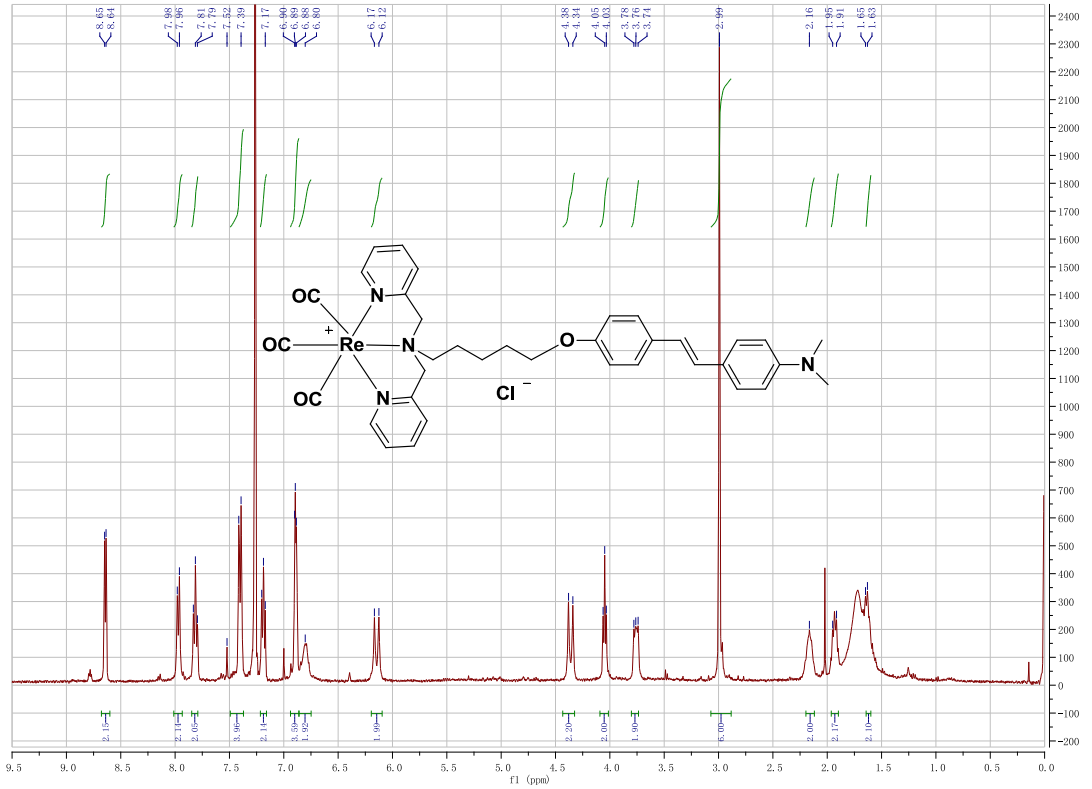
^{13}C-NMR for compound **13**



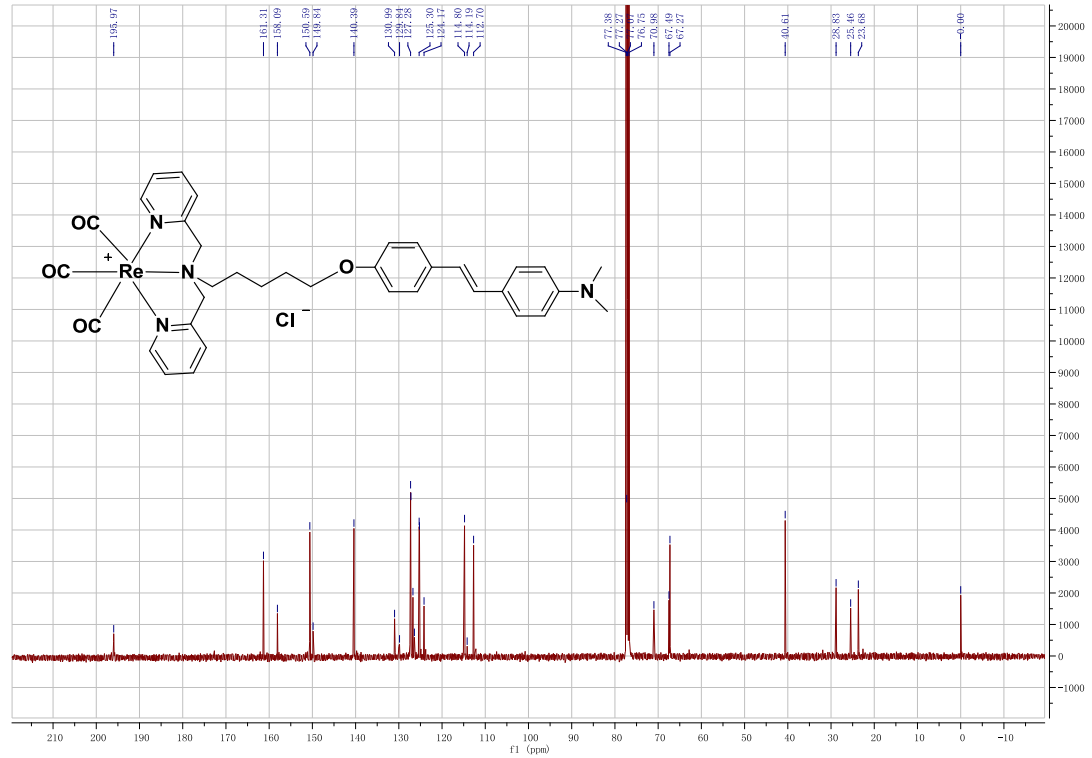
HRMS for compound **13**



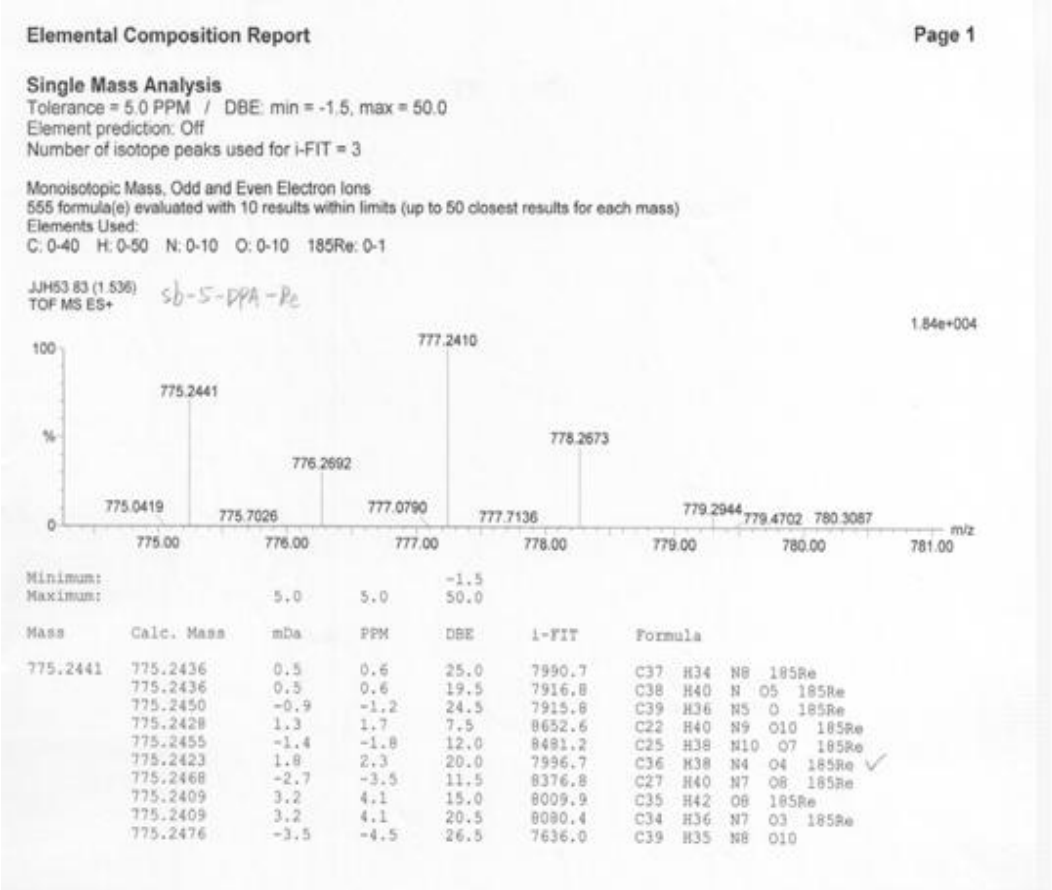
^{1}H -NMR for compound **14**



^{13}C-NMR for compound **14**



HRMS for compound **14**



Elemental Composition Report Page 1

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 = 3

Monoisotopic Mass, Odd and Even Electron Ions
555 formula(e) evaluated with 10 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-40 H: 0-50 N: 0-10 O: 0-10 185Re: 0-1

JJH53 83 (1.536)
TOF MS ES+ Sb-5-PPA-Re
1.84e+004

Minimum:				-1.5		
Maximum:		5.0	5.0	50.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
775.2441	775.2436	0.5	0.6	25.0	7990.7	C37 H34 N8 185Re
	775.2436	0.5	0.6	19.5	7916.8	C38 H40 N O5 185Re
	775.2450	-0.9	-1.2	24.5	7915.8	C39 H36 N5 O 185Re
	775.2428	1.3	1.7	7.5	8652.6	C22 H40 N9 O10 185Re
	775.2455	-1.4	-1.8	12.0	8481.2	C25 H38 N10 O7 185Re
	775.2423	1.8	2.3	20.0	7996.7	C36 H38 N4 O4 185Re ✓
	775.2468	-2.7	-3.5	11.5	8376.8	C27 H40 N7 O8 185Re
	775.2409	3.2	4.1	15.0	8009.9	C35 H42 O8 185Re
	775.2409	3.2	4.1	20.5	8080.4	C34 H36 N7 O3 185Re
	775.2476	-3.5	-4.5	26.5	7636.0	C39 H35 N8 O10

9. References

1. R. Alberto, R. Schibli, A. Egli, A. P. Schubiger, U. Abram and T. A. Kaden, *J. Am. Chem. Soc.*, 1998, **120**, 7987-7988.

2. Z.-P. Zhuang, M.-P. Kung, A. Wilson, C.-W. Lee, K. Plössl, C. Hou, D. M. Holtzman and H. F. Kung, *J. Med. Chem.*, 2002, **46**, 237-243.

3. C. Yung-Chi and W. H. Prusoff, *Biochemical Pharmacology*, 1973, **22**, 3099-3108.

4. M. Cui, X. Wang, P. Yu, J. Zhang, Z. Li, X. Zhang, Y. Yang, M. Ono, H. Jia, H. Saji and B. Liu, *J. Med. Chem.*, 2012, **55**, 9283-9296.