Electronic Supplementary Material (ESI) for Chemical Communications

Fluorous Ligand Capture (FLC): A Chemoselective Solution-Phase Strategy for Isolating ^{99m}Tc-Labelled Compounds in High Effective Specific Activity

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Supplementary Information

Materials and methods

Reagents were purchased from Sigma-Aldrich and Fluorous Technologies and were used without further purification. Fluorous solid-phase extractions were conducted using Fluorous Technologies Inc. FluoroFlash® 2 g SPE cartridges. $[Re(CO)_3(OH_2)_3]Br$,[1] bispyridyl valeric acid (**5**),[2] and dpK-GGG-LTVSPWY peptide (**7**)[3] were prepared according to literature procedures. ^1H and ^{13}C NMR spectra were recorded on Bruker AV200 spectrometers. ^1H NMR chemical shifts are reported in ppm relative to the residual proton signal of the deuterated solvents. Coupling constants (*J*) are reported in Hertz (Hz). ^{13}C chemical shifts are reported in ppm relative to the carbon signal of the solvent. Low resolution electrospray ionization mass spectroscopy experiments were performed on a Waters/Micromass Quattro Ultima instrument. High resolution mass spectra were obtained on a Waters/Micromass Global Q-ToF spectrometer. Infrared spectra were obtained on a Bio-Rad FTS-40 Fourier transform IR spectrometer. All non-radioactive products were purified using a Biotage SP1 normal phase automated purification system. Analytical HPLC was conducted using an Agilent 1100 series HPLC and Phenomenex 5 µm C18 column (4.6 x 250 mm). The elution protocols were: Method A: Solvent A = water (with 10 mM ammonium formate), Solvent B = methanol: Gradient elution 0 min., 20% B; 20 min., 90% B. Method B: Solvent A = water (with 0.1% trifluoroacetic acid, TFA), Solvent B = acetonitrile (0.1% TFA): Gradient elution 0 min., 5% B; 20 min., 60% B. For all experiments the flow rate was 1.0 mL min^{-1} and monitoring was performed at 254 nm.

Calibration curves

Calibration curves for compounds **5** (t_R = 10.7 min.) and **7** (t_R = 13.0 min.) were prepared using an Agilent 1100 series UV-HPLC employing elution methods A and B respectively with a Phenomenex Gemini NX 5 µm C18 column (4.6 x 250 mm). The actual concentrations of **5** were 0, 5, 20, 35, and 50 µM and 50 µL of each sample was injected. The actual concentrations of **7** were 0, 45, 225, 450, and 670 nM and 50 µL was injected for each.

Preparation of $C_{29}H_{24}F_{17}NO_4$ (2)

3-(Perfluorooctyl)propyl amine (1.01 g, 2.1 mmol) was added to a 20 mL microwave vial, followed by acetonitrile (14 mL) and diisopropylethyl amine (1.8 mL, 10 mmol). To the stirring solution, benzyl bromoacetate (0.69 mL, 4.4 mmol) was added dropwise. The vial was sealed and heated to 135 ˚C for 5 min with stirring in a Biotage Initiator 60 microwave reactor. The pale red liquid was loaded onto a 100 g SNAP column and purified using the Biotage SP1 system with a gradient of 7-60% ethyl acetate/hexanes over 10 column volumes. The solvent was removed by rotary evaporation to give a colourless oil (1.60 g, 98%). FTIR NaCl disc (cm^{-1}) 3036, 2957, 1746, 1498, 1456, 1242, 1212. ^1H NMR (200 MHz, $CDCl_3$) δ 7.37 (s, 10H), 5.17 (s, 4H), 3.63 (s, 4H), 2.84 (t, *J*= 6.6, 2H), 2.15 (m, 2H), 1.78 (m, 2H). ^{13}C NMR (50 MHz, $CDCl_3$) δ 171.0, 135.7, 128.7, 128.5, 66.5, 55.1, 53.1, 28.4 (t, $^3J_{C-F}$= 22.3 Hz), 18.9. TLC (30% ethyl acetate in hexane) R_f 0.52. HRMS-ESI (*m/z*) for $[C_{29}H_{24}F_{17}NO_4]$ calcd 774.1512, found 774.1507.

Preparation of $C_{15}H_{12}F_{17}NO_4$ (3)

Compound **2** (1.60 g, 2.1 mmol) was dissolved in methanol (10 mL) and added to a suspension of 10% Pd/C (160 mg) in methanol (15 mL) in a 50 mL round bottom flask. The flask was sealed and flushed with Ar for 20 minutes, then two 5 minute flushes with H_2 after which the reaction was left stirring overnight under H_2. The solution was then filtered through a celite plug and washed with methanol (3 x 5

mL). The filtrate was concentrated by rotary evaporation leaving a white powder (1.09 g, 87%). m.p. = 161˚C decomp. FTIR KBr (cm^{-1}) 3438, 1734, 1636, 1240, 1149. ^{1}H NMR (200 MHz, CD_3OD) δ 3.76 (s, 4H), 3.20 (t, *J*= 7.2, 2H), 2.35 (m, 2H), 1.98 (m, 2H). ^{13}C NMR (50 MHz, DMSO-d_6) δ 172.6, 54.8, 52.5, 27.4, 18.4. HRMS-ESI (*m/z*) for $[C_{15}H_{12}F_{17}NO_4]$ calcd 594.0573, found 594.0580.

Preparation of $C_{15}H_{10}F_{17}NO_4Cu$(II) (4)

Compound **3** (1.07 g, 1.8 mmol) was dissolved in ethanol (20 mL). To this stirring solution, a concentrated solution of copper(II) bromide (0.80 g in 20 mL H_2O) was added dropwise and the clear solution became blue. Once the solution began to turn yellow, the addition of $CuBr_2$ was stopped and the mixture was stirred for 2 days at room temperature. After this time, a pale blue powder was collected by suction filtration from the yellow solution. After washing with cold ethanol (3 x 50 mL) and DCM (25 mL), the blue powder was dried under high vacuum overnight to give **4** (1.08 g, 91%) m.p. = 254 - 255˚C. FTIR KBr (cm^{-1}) 3250, 1637, 1594, 1203, 1152. HRMS-ESI (*m/z*) calcd 671.9978, found 672.0005 $[M+NH_4]^+$.

Preparation of $[C_{17}H_{21}N_3O_2{}^{99m}Tc(CO)_3]^+$ (6a)

Compound **5**[2] (0.2 mL, 5 mg/mL in CH_3CN) was added to a crimp sealed microwave vial and purged with argon for 15 min. To this, $[^{99m}Tc(CO)_3(H_2O)_3]^+$ was added (740 MBq, 0.8 mL, pH = 10) and the mixture was heated to 150˚C for 2 min with stirring. Compound **4** was dissolved in DMF (10 mg in 0.5 mL) and then added to the reaction mixture. This solution was stirred for 5 min and then loaded onto a 2 g Fluoro Flash SPE cartridge, which had been prewashed with 4 mL DMF and 8 mL of a fluorophobic solution (20% water in methanol). After loading the sample onto the cartridge, another 8 mL fluorophobic wash was conducted to elute the radiolabeled complex. The amount of ligand removal was measured by analytical HPLC using Method A: t_R = 12.9 min, which corresponds to the Re standard **6b**;[4] Yield: 675 MBq, 91 %.

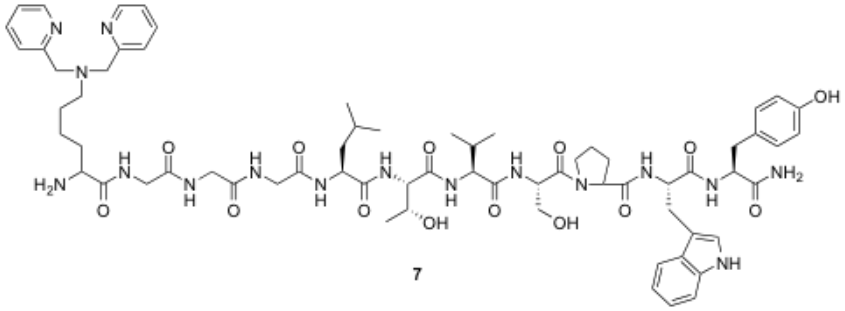
Solid Phase Ligand Capture

The solid-phase analogue of **4** was prepared.[5] Three Amberlite IRC-784 resins of different particle sizes were used (20 – 75, 75 – 150, and 150 – 300 μm, 5g of each). The resins were loaded with copper by shaking a suspension of the resins in saturated solutions of $CuSO_4$ (100 g in water) for 24 hr. After filtering and thorough washing with water and THF, the resins were dried under vacuum. The resins were then loaded into standard solid-phase extraction tubes (100 mg) and activated immediately prior to use with an acidic saline solution (pH = 4). The mixture containing **5** (1 mg) and **6a** (74-185 mBq) was dripped onto the resin slowly, where approximately 30% of the activity was eluted during the loading process and another 40 – 50% was eluted with a 3 mL saline wash. Yield: 73 % (20 – 75 μm); 78 % (75 – 150 μm); 79 % (150 – 300 μm).

Combined Solid Phase and Fluorous Ligand Capture

Fluorous silica preloaded with **4** was also used to purify the model reaction mixture. Compound **4** (5 mg) was preloaded onto a 2g F-SPE cartridge using 2mL of a slightly acidic 1:1 mixture of MeCN and DMF containing 5 drops of 1N HCl. The reaction mixture containing **5** (1 mg) and **6b** (274 MBq) was added subsequently. Following a gradient elution using 4 mL of MeOH in water increasing from 0% to 80% in increments of 10%, **6b** was selectively eluted with 99% of the ligand removed and 90% of the activity was recovered (80% yield, 10% TcO_4^-).

Peptide Synthesis (Dpk-GGG-LTVSPWY (**7**) and [Re(CO)$_3$-Dpk-GGG-LTVSPWY]$^+$ **(8b)**)



7

Peptide **7** was prepared using a modified literature procedure[3,6] employing a standard Fmoc coupling protocol on a CEM Liberty solid-phase synthesis system. Rink amide resin (100 mg) was added to a 20 mL BD vial and was then added to the microwave reactor of the system by automated flushes with DMF prior to the addition of the first Fmoc protected amino acid as a 0.1 mol/L solution in DMF. Following each coupling reaction using a 0.1 mol/L solution of HATU in DMF, the Fmoc group was removed using a solution of 20% piperidine in DMF. This process was repeated for each amino acid in the sequence. Once the coupling reactions were complete, the resin was filtered and washed with cold DMF followed by cleaving the peptide from the resin [cleavage cocktail = TFA (3.8 mL), triisopropyl silane (0.1 mL), and water (0.1 mL)]. The resin was shaken in the cleavage cocktail for 4 hours at room temperature and 350 rpm before filtering the suspension through glass wool into cold diethyl ether (40 mL). The resulting heterogeneous solution was centrifuged at 3000 rpm for 10 min. at 4 ˚C, forming a white pellet. After decanting the diethyl ether, the process was repeated twice more. Once completed, the white solid was dissolved into 10% CH_3CN in water and lyophilized, yielding a white solid. The peptide **7** was then purified using semipreparative HPLC using a Phenomenex Gemini-NX C-18 column (250 x 10 mm) and Method B: t_R = 13.0 min (ESI $[M+2Na]^{2+}$: m/z 704). The same procedure was also used to produce the Re standard **8b**, which exhibited a retention time of 15.2 min.

Preparation of [^{99m}Tc(CO)$_3$-Dpk-GGG-LTVSPWY]$^+$ (8a)

Compound **7** (0.2 mL, 5 mg/mL in 1:1 CH_3CN in water) was added to a crimp sealed microwave vial and purged with Ar for 15 min. To this, $[^{99m}Tc(CO)_3(H_2O)_3]^+$ was added (370 MBq, 0.8 mL, pH = 7) and the mixture was heated to 60˚C for 10 min in a Biotage microwave reactor with stirring. Compound **4** was dissolved in DMF (20 mg in 0.4 mL) as a pale blue solution and then added to the reaction mixture. This solution was sonicated for 10 min and then loaded onto a 2 g Fluoro Flash SPE cartridge, which had been prewashed with 4 mL DMF and 8 mL of a fluorophobic solution (20% water in CH_3CN). After loading the sample onto the cartridge, another 8 mL fluorophobic wash was conducted to elute the radiolabeled complex. The amount of ligand removal was measured by analytical HPLC using Method A: t_R = 15.6 min; Yield: 330 MBq, 89%.

References:

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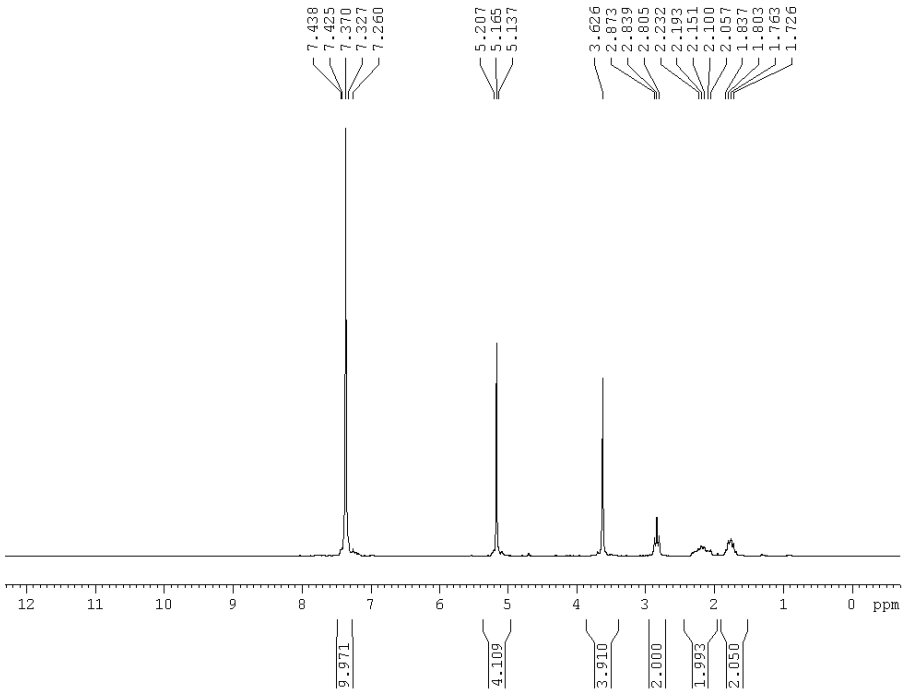

Figure S1: ^{1}H NMR of compound 2 (200 MHz, $CDCl_3$)

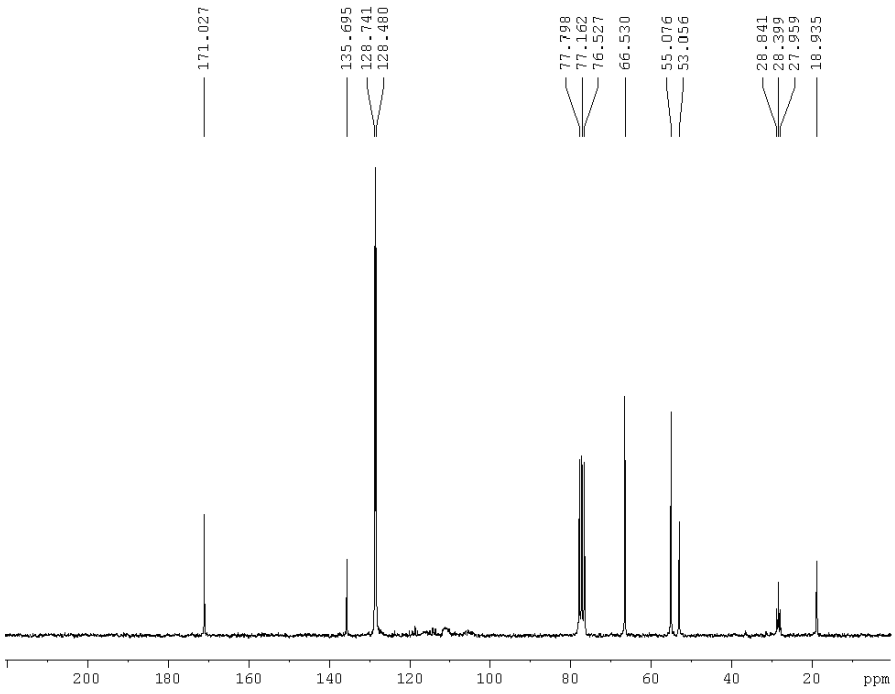

Figure S2: ^{13}C NMR of compound 2 (50 MHz, $CDCl_3$).

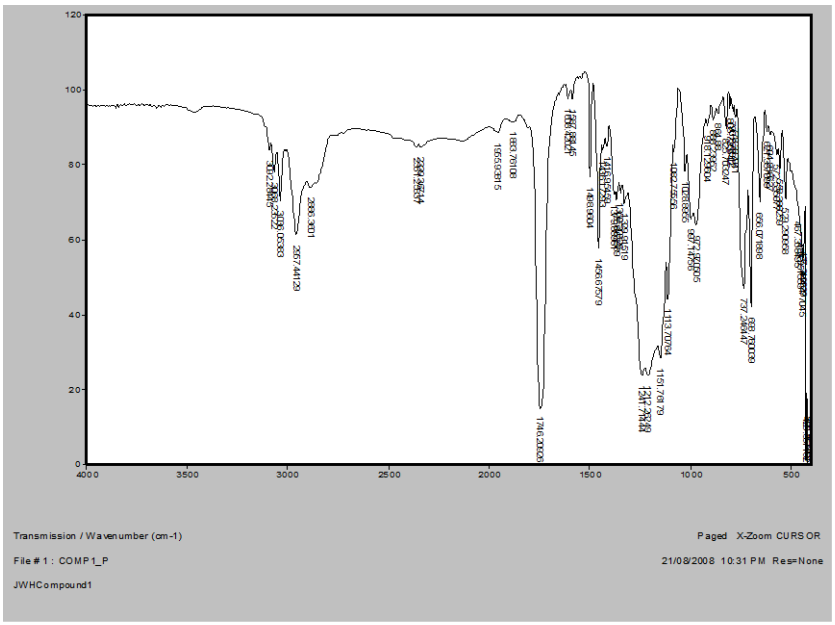

Figure S3: IR of compound 2 (NaCl, neat).

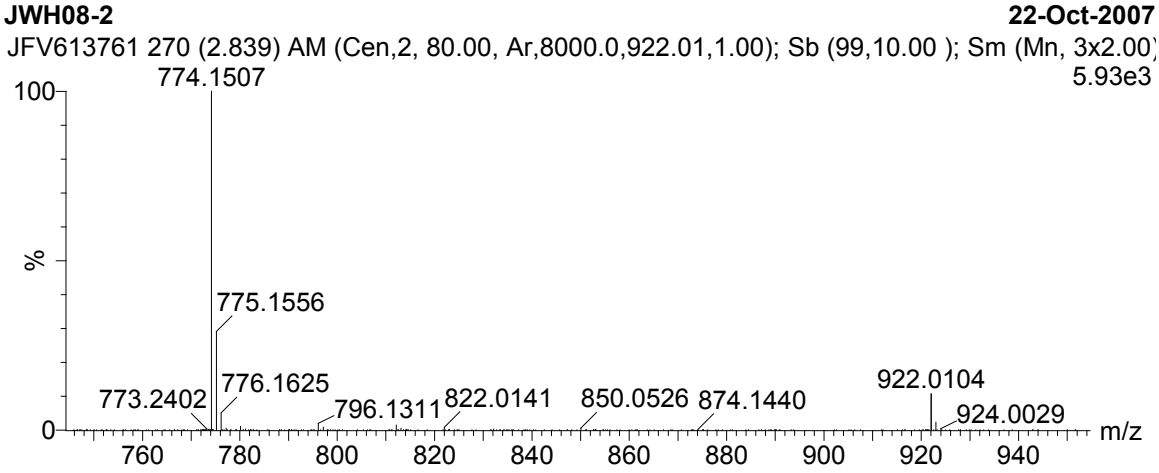

Figure S4: HRMS of compound 2 (ESI).

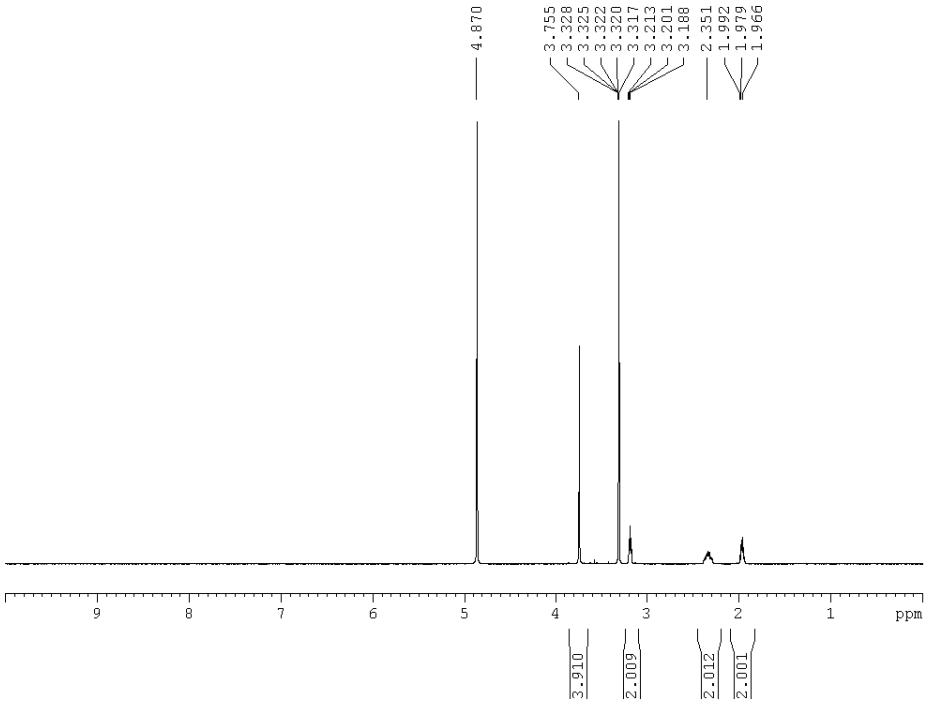

Figure S5: ^{1}H NMR of compound 3 (200 MHz, CD_3OD).

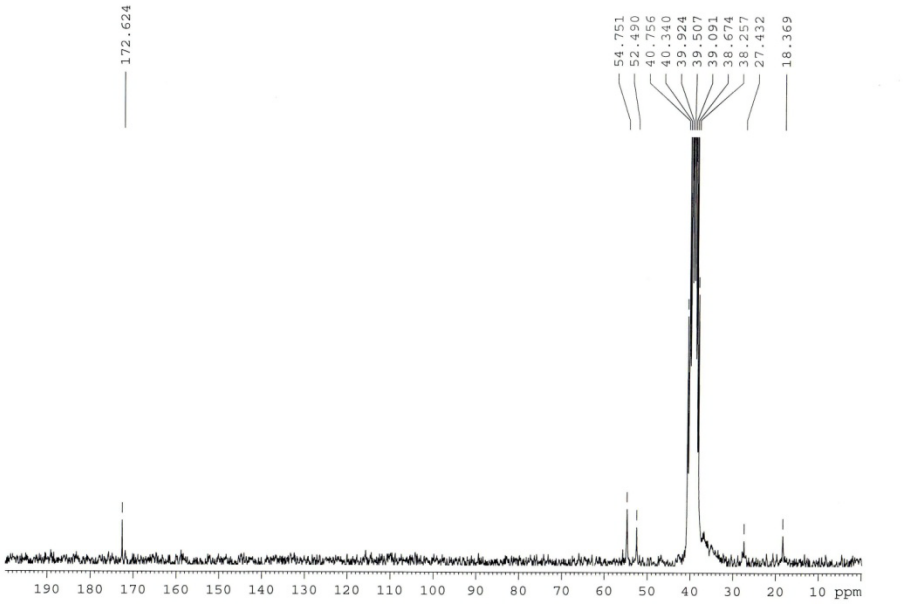

Figure S6: ^{13}C NMR of compound 3 (50 MHz, DMSO-d_6).

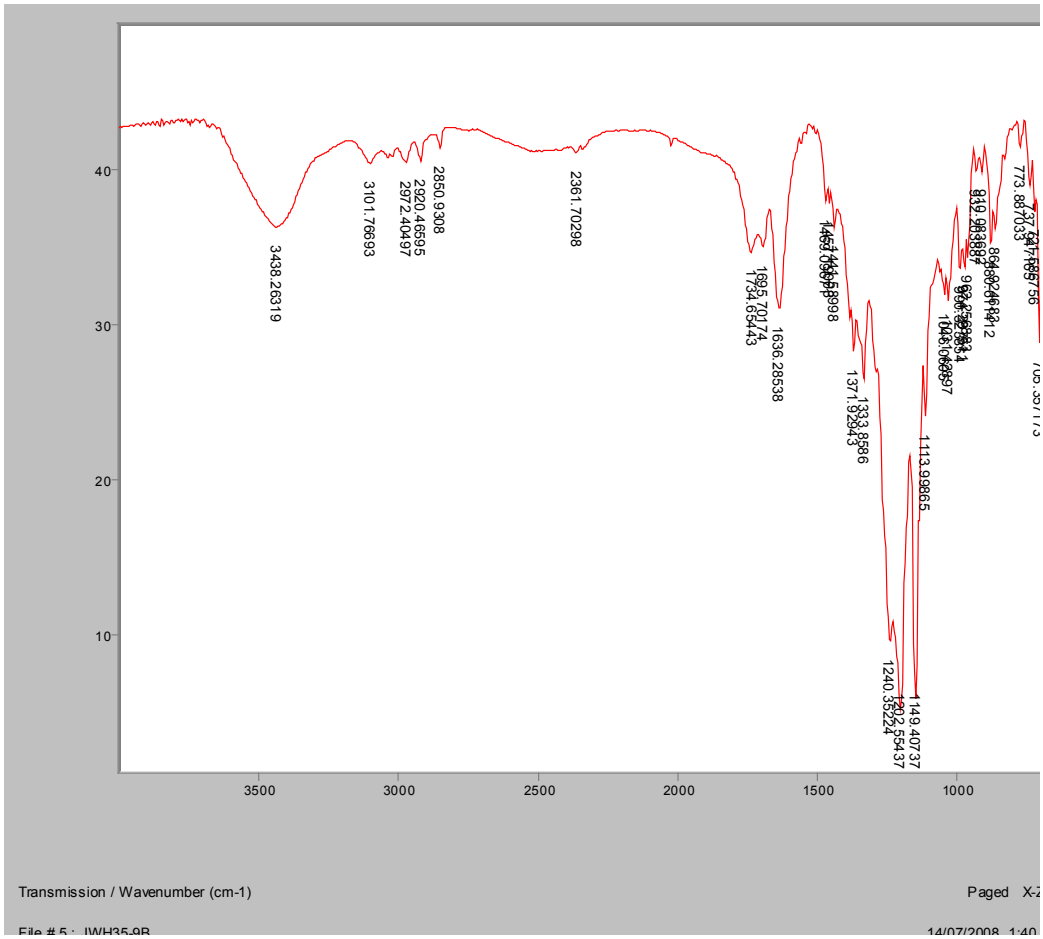

Figure S7: IR of compound 3 (KBr).

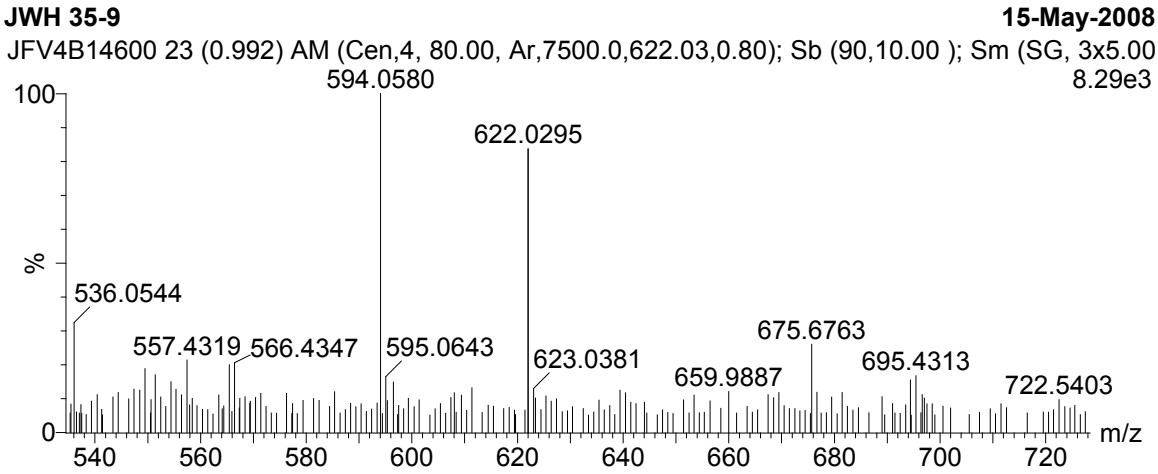

Figure S8: HRMS of compound 3 (ESI).

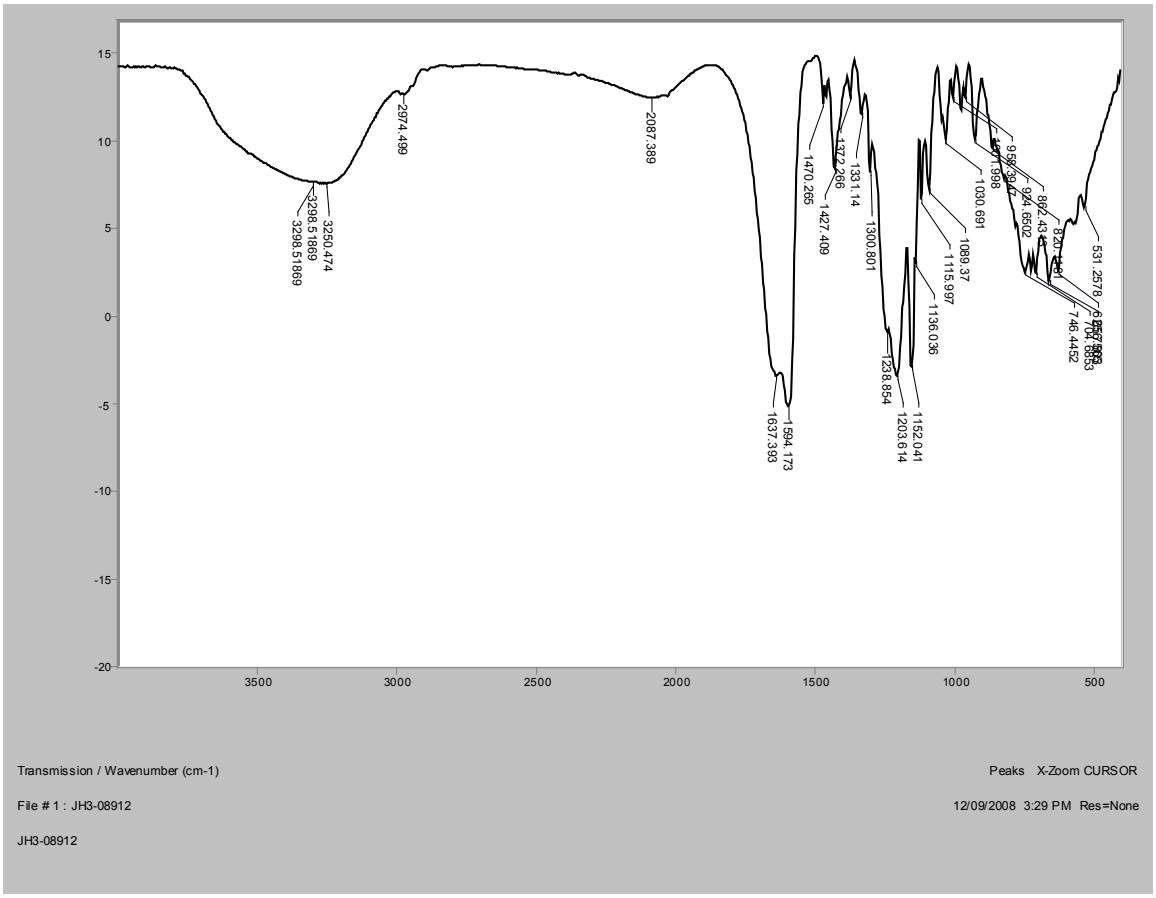

Figure S9: IR of compound 4 (KBr).

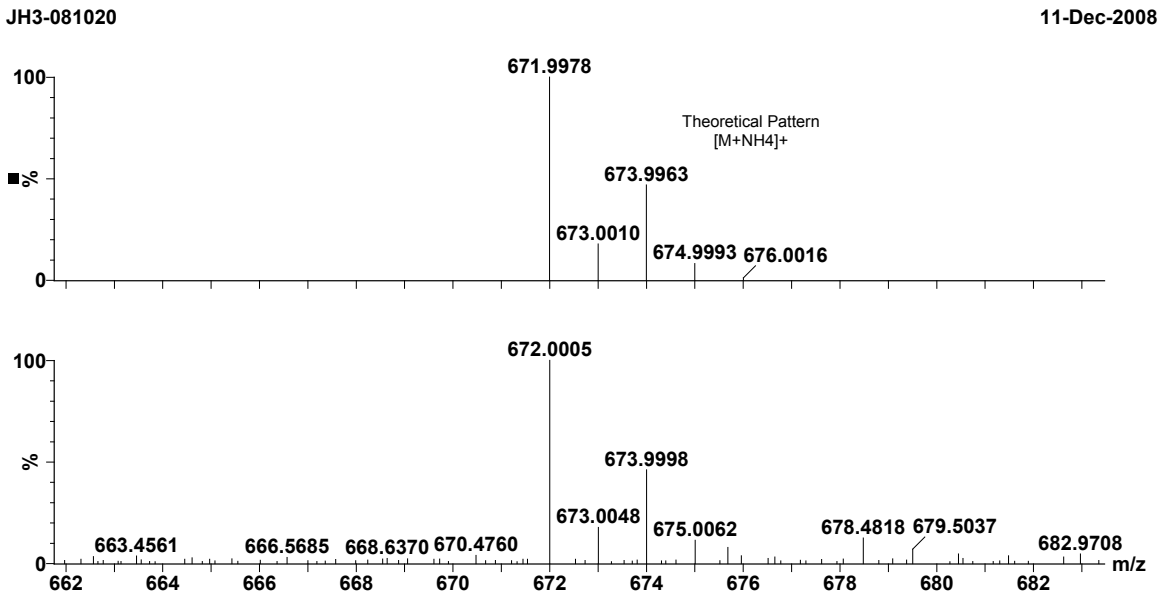

Figure S10: HRMS of compound 4 (TOF ES+).

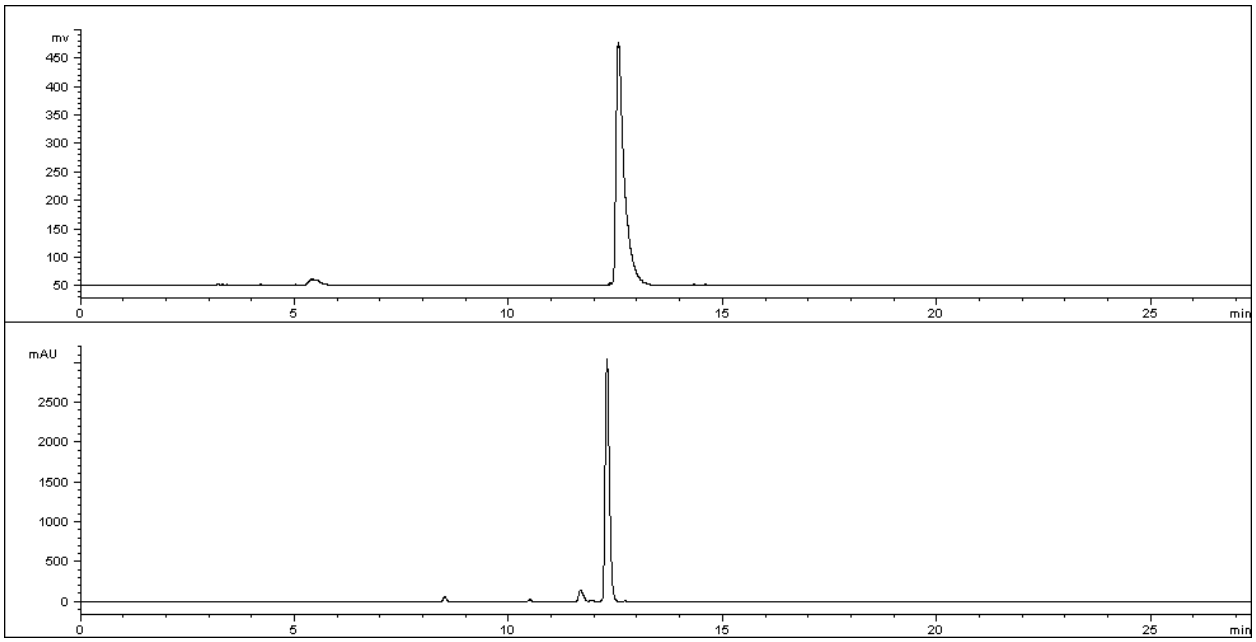

Figure S11: HPLC trace of compound 6a (top, γ) and Re standard 6b (UV, bottom). The detectors are connected in series.

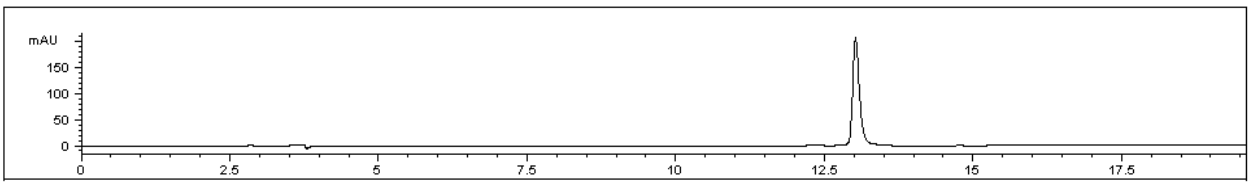

Figure S12: UV-HPLC trace of compound 7.

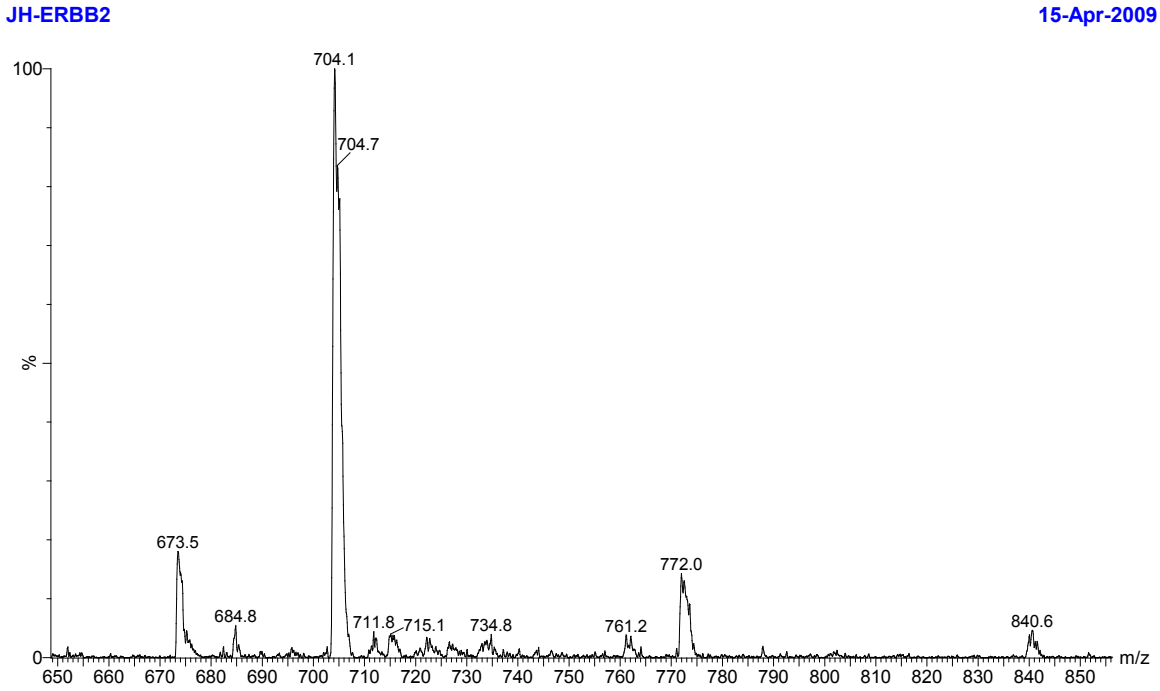

Figure S13: ESI MS of compound 7.

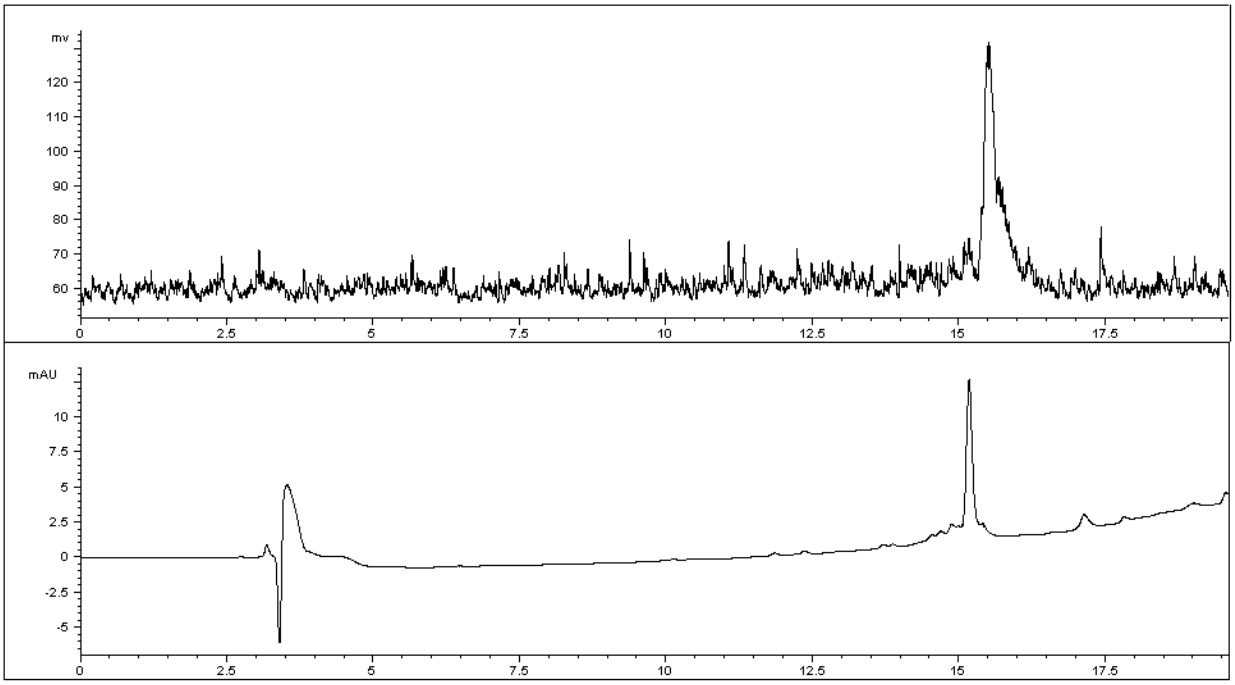

Figure S14: HPLC trace of compound 8a (top: γ; bottom: UV of Re standard).