# Orthogonal $^{18}F$ and $^{64}Cu$ labelling of functionalized *bis*(thiosemicarbazonato) complexes of copper

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## SUPPORTING INFORMATION

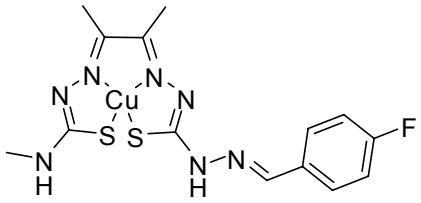
$^{1}H$ NMRs were reported on Bruker DPX 200, DPX 400, AV 400 and AV 500 spectrometers, at a frequency of 200, 400 and 500 MHz respectively. $^{13}C$ NMRs were recorded on Bruker AV 400 and AV 500 spectrometers at a frequency of 100 or 125 MHz respectively. Mass spectra (*m/z*) were obtained on a Bruker MicroTOF in Electrospray (ESI). Analytical thin layer chromatography (TLC) was performed on Merck Silica 60 $F_{254}$ plates. Crude reaction mixtures were analysed by TLC and HPLC. HPLC analysis was performed with a Gilson 322 or Dionex Ultimate 3000 systems, equipped with a NaI/PMT radiodetector and a UV-detector. Radio-TLC was performed on Macherey-Nagel Polygram Silica Plates and eluted with EtOAc or 95% aq. MeCN. Analysis was performed with a plastic scintillator/PMT detector. FSPE separation was carried out using pre-assembled Waters Sep-Pak cartridges (Waters, Milford, MA) and FluoroFlash Silica gel (Fluorous Technologies Inc., Pittsburgh, PA). Pre-assembled Sep-Pak $C_{18}$SPE cartridges (Waters, Milford, MA) were used in the same way. Copper-64 was prepared using a biomedical cyclotron with a nickel-64 target in a procedure similar to that describe by McCarthy et al.[32] The copper-64 was extracted from the nickel-64 target as $[^{64}Cu]CuCl_2$(aq) and purified from the nickel-64 using an ion-exchange column. An aqueous solution of copper-64 acetate, $[^{64}Cu]Cu(OAc)_2$, was prepared by diluting 0.2 mL of $[^{64}Cu]CuCl_2$(aq) in 0.1 mol/L HCl with 0.1 mol/L sodium acetate (1.8 mL, pH 5.5). This stock solution was used for the radiolabeling experiments. $[^{18}F]$Fluoride was produced by the cyclotron of PETNET Solutions at Mont Vernon Hospital (UK) via the $^{18}O(p,n)^{18}F$ reaction and delivered as $[^{18}F]$fluoride in $[^{18}O]$water (1-2 GBq, 1-3 mL). This target solution was passed through a QMA anion exchange resin cartridge (20 mg, Waters). $[^{18}F]$Fluoride adsorbed on the charged-resin was eluted into a reaction vial with a solution of Kryptofix 222 (15 mg) and $K_2CO_3$ (3 mg) in 1 mL acetonitrile/water (8:2). Excess water was removed under $N_2$ stream at 100-110°C, and the resulting complex was dried an additional 3 times by azeotropic distillation with 0.5 mL acetonitrile each under $N_2$ stream. The resulting dry complex of $K^{18}F$/Kryptofix 222 was further dissolved by anhydrous acetonitrile (2-4 mL) and dispensed into reaction vials containing the precursor for nucleophilic fluorination.

Log *P* values were calculated using an HPLC method, previously shown to work on this kind of complex, using a Gilson HPLC system equipped with a Hamilton PRP-1 reverse-phase column and UV/Vis detection at 254 nm.[15] The HPLC column was calibrated using a series of known *bis*(thiosemicarbazone) complexes, with known log *P* values. Electrochemistry was carried out using a CH Instruments Electrochemical Analyser. The potentiostat was controlled using a PC running CH Instruments version 2.05 electrochemical software. All cyclic voltametry measurements were recorded in

a glass cell sealed with a Teflon cap and located inside a Faraday cage at a temperature of 21°C.

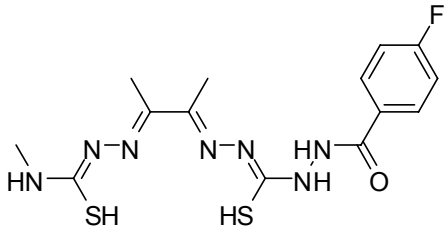
## Characterisation of Compounds

*Copper(II) Diacetyl-2-(4-N-methyl-3-thiosemicarbazonato)-3-(4-N-amino-(4-fluorobenzilidene)-3-thiosemicarbazonato),* ***4***



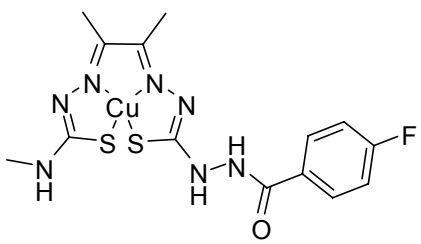
Zinc(II) Diacetyl-2-(4-*N*-methyl-3-thiosemicarbazonato)-3-(4-*N*-amino-(4-fluorobenzilidene)-3-thiosemicarbazonato) (60 mg, 0.14 mmol) was dissolved in DMF (0.2 mL) and copper (II) diacetate (74 mg, 0.41 mmol) was added, The reaction mixture turned deep purple within 1 minute, and was stirred for 1 hour at room temperature. The reaction mixture was concentrated *in vacuo* and the residue was washed with water (20 mL) and then dried *in vacuo* overnight to afford copper(II) diacetyl-2-(4-*N*-methyl-3-thiosemicarbazonato)-3-(4-*N*-amino-(4-fluorobenzilidene)-3-thiosemicarbazonato) (53 mg, 88 % yield) as a dark purple solid; *m/z* (ESI) $C_{14}H_{17}FN_7S_2Cu$ ($M+H^+$) calc. 429.0261, found 429.0249.

*Diacetyl-2-(4-N-methyl-3-thiosemicarbazone)-3-(4-N-amino-(4-fluorobenzilamide)-3-thiosemicarbazone),* ***14***



4-Fluorobenzoic acid (71 mg, 0.5 mmol) was added to a solution of HATSM/A (130 mg, 0.5 mmol) and *O*-Benzotriazole-*N*,*N*,*N*',*N*'-tetramethyl-uronium-hexafluoro-phosphate (179 mg, 0.5 mmol) in DMF (25 mL). *N*,*N*-Diisopropylethylamine (87 μL, 0.5 mmol) was then added and the reaction mixture was stirred for 16 hours at room temperature, by which time the solution had turned dark brown. A precipitate was formed upon the addition of water (25 mL) dropwise. The precipitate was collected and washed with copious amounts of water (5 x 25 mL) and then dried *in vacuo* to give diacetyl-2-(4-*N*-methyl-3-thiosemicarbazone)-3-(4-*N*-amino-(4-fluorobenzilamide)-3-thiosemicarbazone) (111 mg, 58 % yield) as a brown solid; $\delta_H$ (400 MHz, DMSO-$d_6$) 2.24 (3H, s, *Me*C=N), 2.27 (3H, s, *Me*C=N), 3.03 (3H, d, $J$ = 3.5 Hz, *Me*NH), 7.36 (1H, t, $J$ = 9.2 Hz, Me*NH*C), 7.92-8.03 (3H, m, *Ph*), 8.37-8.45 (1H, m, *Ph*), 10.16 (1H, s, C*NH*N), 10.25 (1H, s, C*NH*N), 10.65 (1H, s, C*NH*N), 10.68 (1H, s, C*NH*N); $\delta_C$ (125 MHz, DMSO-$d_6$) 13.8, 14.2, , 82.0, 83.5, 121.7, 126.4, 132.1, 135.8, 140.3; $\delta_F$ (376.56 MHz, DMSO-$d_6$) -108.4, -108.8; *m/z* (ESI) $C_{14}H_{17}FN_7OS_2$ ($M-H^+$) calc. 382.0926, found 382.0925.

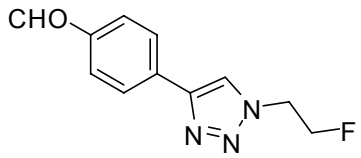
*Diacetyl-2-(4-N-methyl-3-thiosemicarbazonato)-3-(4-N-amino-(4-fluorobenzilamide)-3-thiosemicarbazonato)copper(II),* ***6***



Diacetyl-2-(4-*N*-methyl-3-thiosemicarbazone)-3-(4-*N*-amino-(4-fluorobenzilamide)-3-thiosemicarbazone) (54 mg, 0.14 mmol) was dissolved in DMF (0.2 mL) and copper (II)

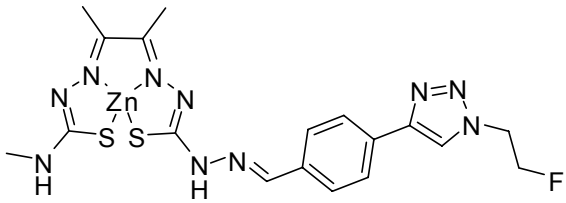
diacetate (74 mg, 0.41 mmol) was added, The reaction mixture turned deep purple within 1 minute, and was stirred for 1 hour at room temperature. The reaction mixture was concentrated *in vacuo* and the residue was washed with water (20 mL) and then dried *in vacuo* overnight to afford copper(II) diacetyl-2-(4-*N*-methyl-3-thiosemicarbazone)-3-(4-*N*-amino-(4-fluorobenzilamide)-3-thiosemicarbazone) (57 mg, 93 % yield) as a dark purple/brown solid; *m/z* (ESI) $C_{14}H_{16}FN_7OS_2Cu$ ($M\text{-}H^+$) calc. 443.0065, found 443.0061.

*4-[1-(2-Fluoroethyl)-1H-[1,2,3]triazole-4-yl]benzaldehyde*, **15**



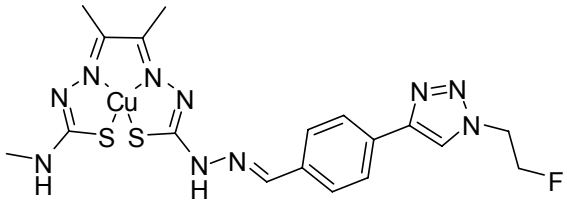

Copper(II) sulphate (0.5 M in water, 9 mL) and sodium ascorbate (0.5 M in water, 9 mL) were added to 4-ethynyl benzaldehyde (550 mg, 4.23 mmol) in DMF (5 mL), and 2-fluoroethylazide (4.5 mmol in 13.5 mL THF) was added and stirred at room temperature for 4 hours. The reaction mixture was diluted with water (10 mL) and the crude product was extracted with ethyl acetate (3 x 20 mL). The organic layer was washed with brine (20 mL), dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by flash chromatography (hexane:ethyl acetate, 1:1) to afford 4-[1-(2-fluoroethyl)-1*H*-[1,2,3]triazole-4-yl]benzaldehyde (380 mg, 41 % yield) as a white solid; $\delta_H$ (400 MHz, $CDCl_3$) 4.72-4.76 (1H, m, ½ x $CH_2CH_2N$), 4.79-4.83 (2H, m, ½ x $FCH_2CH_2N$), 4.93 (1H, t, $J$ = 4.0 Hz, ½ x $FCH_2CH_2$), 7.97 (2H, d, $J$ = 8.3 Hz, *Ph*), 8.03 (1H, s, C=*CH*N), 8.05 (2H, d, $J$ = 8.3 Hz, *Ph*), 10.05 (1H, s, PhC*H*O); $\delta_C$ (175 MHz, $CDCl_3$) 81.6 (d, $J$ = 172.6 Hz), 99.7, 126.1, 130.4, 135.9, 136.1, 146.9, 191.7; $\delta_F$ (376.56 MHz, $CDCl_3$) -221.4; *m/z* (ESI) $C_{11}H_{10}FN_3NaO$ ($M+Na^+$) calc. 242.0706, found 242.0707.

*Diacetyl-2-(4-N-methyl-3-thiosemicarbazonato)-3-(4-N-amino-(4-[1-(2-fluoroethyl)-1H-[1,2,3]triazole-4-yl]benzilidene)-3-thiosemicarbazonato)zinc(II),* ***16***



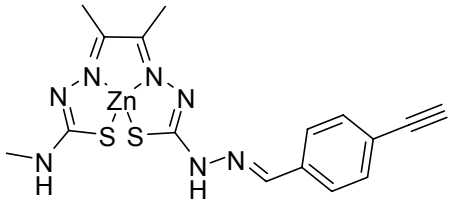

4-[1-(2-Fluoroethyl)-1*H*-[1,2,3]triazole-4-yl]benzaldehyde (93 mg, 0.43 mmol) was added to a suspension of ZnATSA/M (140 mg, 0.43 mmol) in methanol (5 mL) and the reaction mixture was heated to reflux for 2 hours. During the reaction, the solution went orange/red. The mixture was cooled and a precipitate was formed upon addition of diethyl ether (30 mL) dropwise. The precipitate was collected and washed with copious amounts of diethyl ether (5 x 20 mL) and then dried *in vacuo* to give zinc(II) diacetyl-2-(4-*N*-methyl-3-thiosemicarbazone)-3-(4-*N*-amino-(4-[1-(2-fluoroethyl)-1*H*-[1,2,3] triazole-4-yl]benzilidene)-3-thiosemicarbazone) (136 mg, 60 % yield) as an orange solid; $\delta_H$ (400 MHz, DMSO-$d_6$) 2.23 (3H, s, *Me*C=N), 2.29 (3H, s, *Me*C=N), 2.86 (3H, br s, *Me*NH), 4.78 (2H, dt, $J$ = 28.0, 4.6 Hz, $CH_2CH_2N$), 4.88 (2H, dt, $J$ = 47.2, 4.6 Hz, $CH_2CH_2F$), 7.36 (1H, br s, Me*NH*C), 7.70 (2H, d, $J$ = 8.3 Hz, *Ph*), 7.90 (2H, d, $J$ = 8.3 Hz, *Ph*), 8.14 (1H, s, N*CH*=C), 8.67 (1H, s, C*NH*N); $\delta_C$ (125 MHz, DMSO-$d_6$) 13.8, 14.2, 50.3 (d, $J$ = 20.0 Hz), 81.9 (d, $J$ = 168.8 Hz), 122.1, 125.4, 126.9, 127.6, 127.8, 128.0, 130.9, 134.8, 141.0, 146.2; $\delta_F$ (376.56 MHz, DMSO-$d_6$) -222.0; *m/z* (ESI) $C_{18}H_{22}FN_{10}S_2Zn$ ($M+H^+$) calc. 525.0756, found 525.0740.

*Diacetyl-2-(4-N-methyl-3-thiosemicarbazonato)-3-(4-N-amino-(4-[1-(2-fluoroethyl)-1H-[1,2,3]triazole-4-yl]benzilidene)-3-thiosemicarbazonato)copper(II),* ***13***



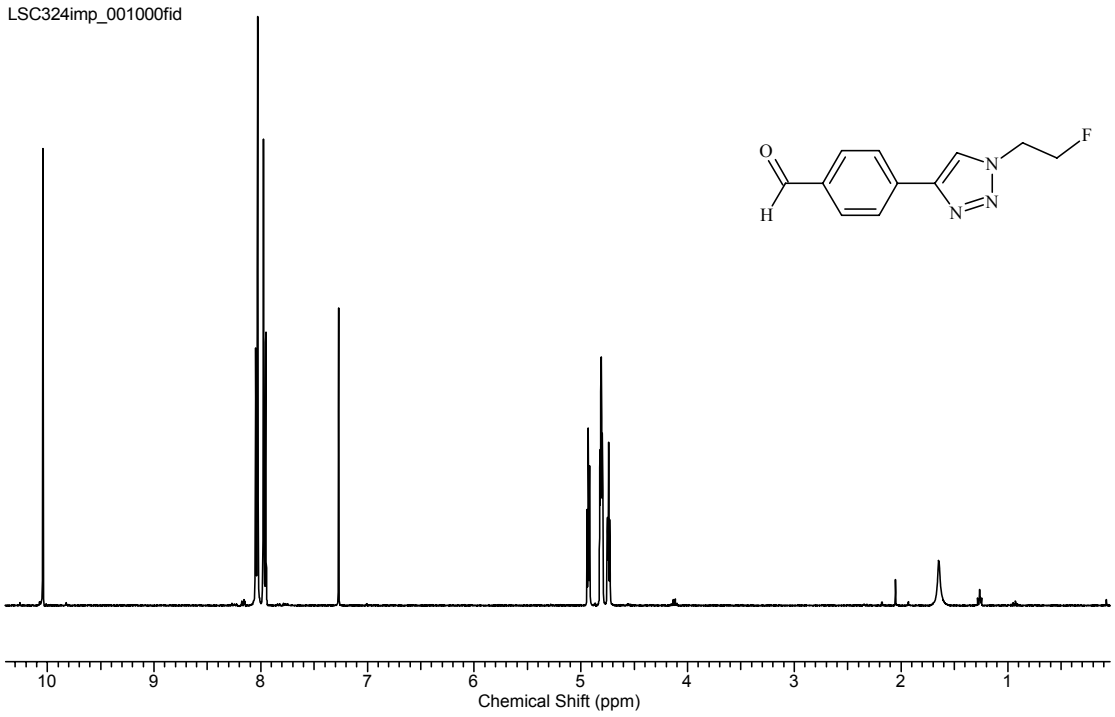
Zinc(II) diacetyl-2-(4-*N*-methyl-3-thiosemicarbazone)-3-(4-*N*-amino-(4-[1-(2-fluoroethyl)-1*H*-[1,2,3]triazole-4-yl]benzilidene)-3-thiosemicarbazone) (59 mg, 0.11 mmol) was dissolved in DMF (0.3 mL) and copper(II) diacetate (62 mg, 0.34 mmol) was added. The reaction mixture turned deep purple within 1 minute, and was stirred for 1 hour at room temperature. The reaction mixture was concentrated *in vacuo* and the residue was washed with water (20 mL) and then dried *in vacuo* overnight to afford copper(II) diacetyl-2-(4-*N*-methyl-3-thiosemicarbazone)-3-(4-*N*-amino-(4-[1-(2-fluoroethyl)-1*H*-[1,2,3]triazole-4-yl]benzilidene)-3-thiosemicarbazone) (52 mg, 90 % yield) as a dark purple solid; $\delta_F$ (376.56 MHz, DMSO-$d_6$) -222.4; *m/z* (ESI) $C_{18}H_{20}FN_{10}S_2Cu$ ($M\text{-}H^+$) calc. 522.0594, found 525.0424.

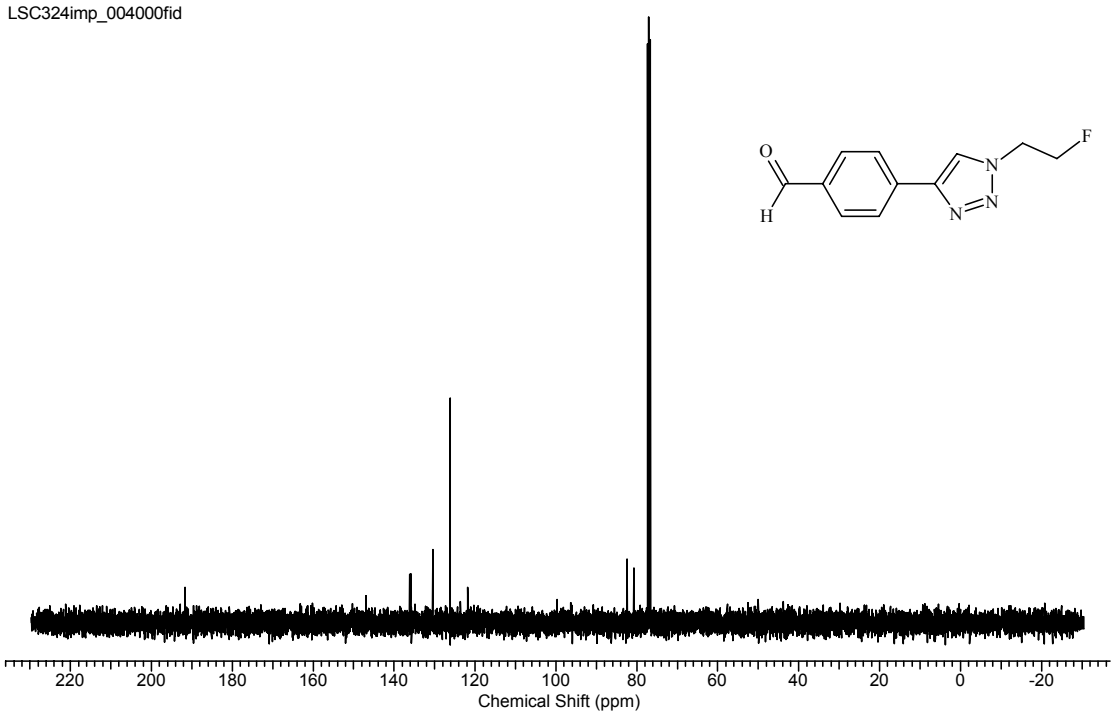
*Zinc(II) diacetyl-2-(4-N-methyl-3-thiosemicarbazone)-3-(4-N-amino-(4-ethynylbenzaldehyde)-3-thiosemicarbazone),* ***11***



4-Ethynylbenzaldehyde (176 mg, 1.35 mmol) was added to a suspension of ZnATSA/M (440 mg, 1.35 mmol) in methanol (10 mL) and the reaction mixture was heated to reflux for 2 hours. During the reaction, the solution went orange/red. The mixture was cooled and a precipitate was formed upon addition of diethyl ether (30 mL) dropwise. The precipitate was collected and washed with copious amounts of diethyl ether (5 x 20 mL) and then dried *in vacuo* to give zinc(II) diacetyl-2-(4-*N*-methyl-3-thiosemicarbazone)-3-(4-*N*-amino-(4-[4-ethynylbenzaldehyde)-3-thiosemicarbazone) (470 mg, 80 % yield) as an orange/red solid; $\delta_H$ (400 MHz, DMSO-$d_6$) 2.23 (3H, s, *Me*C=N), 2.28 (3H, s, *Me*C=N), 2.85 (3H, br s, *Me*NH), 4.30 (1H, s, C≡C*H*), 7.36 (1H, br s, Me*NH*C), 7.50 (2H, d, *J* = 8.5 Hz, *Ph*), 7.60 (2H, d, *J* = 8.5 Hz, *Ph*), 8.10 (1H, s, C*NH*N), 11.38 (1H, br s, N=*CH*C); $\delta_C$ (125 MHz, DMSO-$d_6$) 13.8, 14.2, , 82.0, 83.5, 121.7, 126.4, 132.1, 135.8, 140.3; *m/z* (ESI) $C_{16}H_{18}N_7S_2Zn$ ($M+H^+$) calc. 525.0357, found 525.0351.

## NMR Spectra of Novel Compounds



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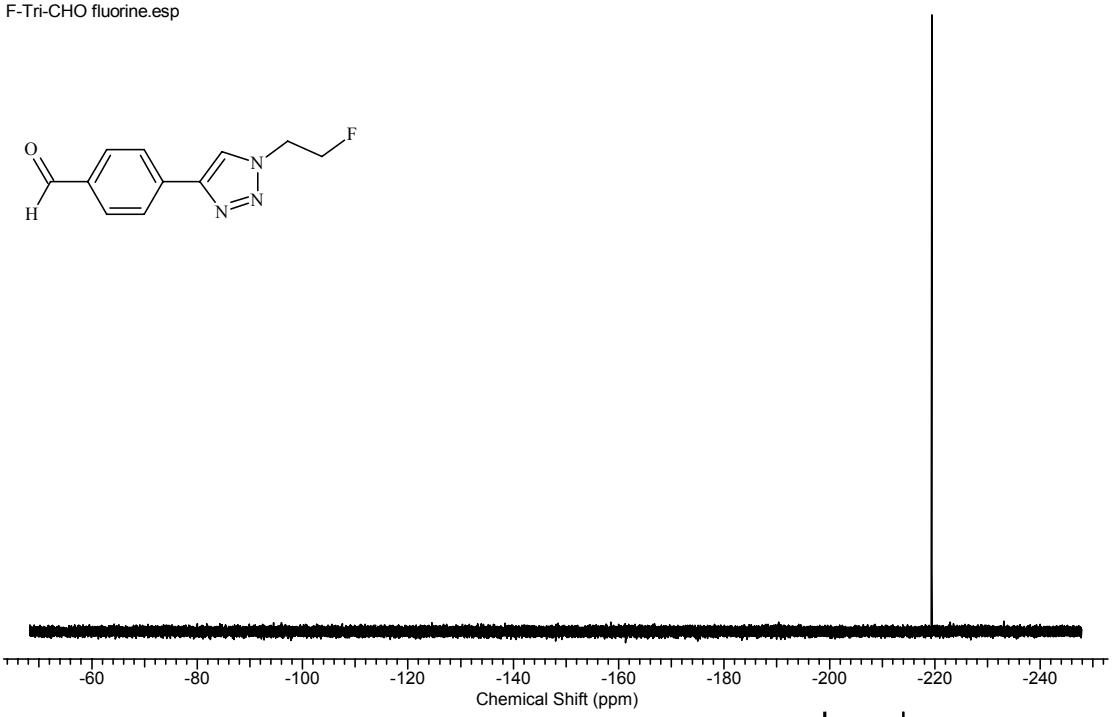


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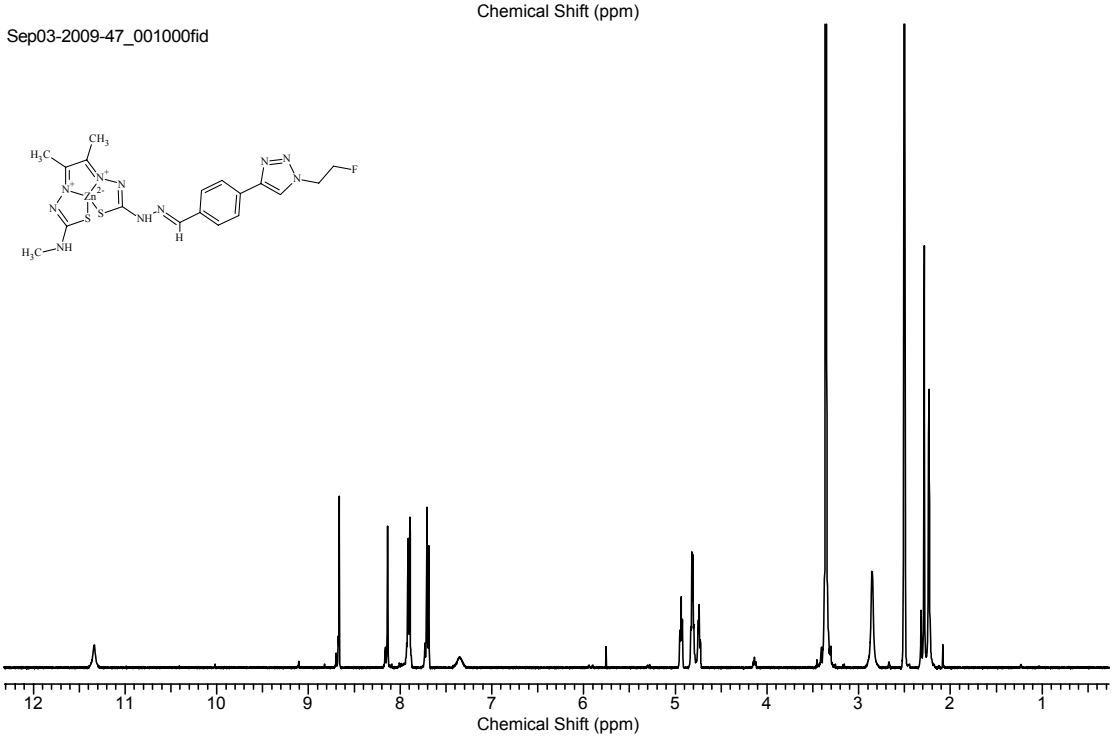

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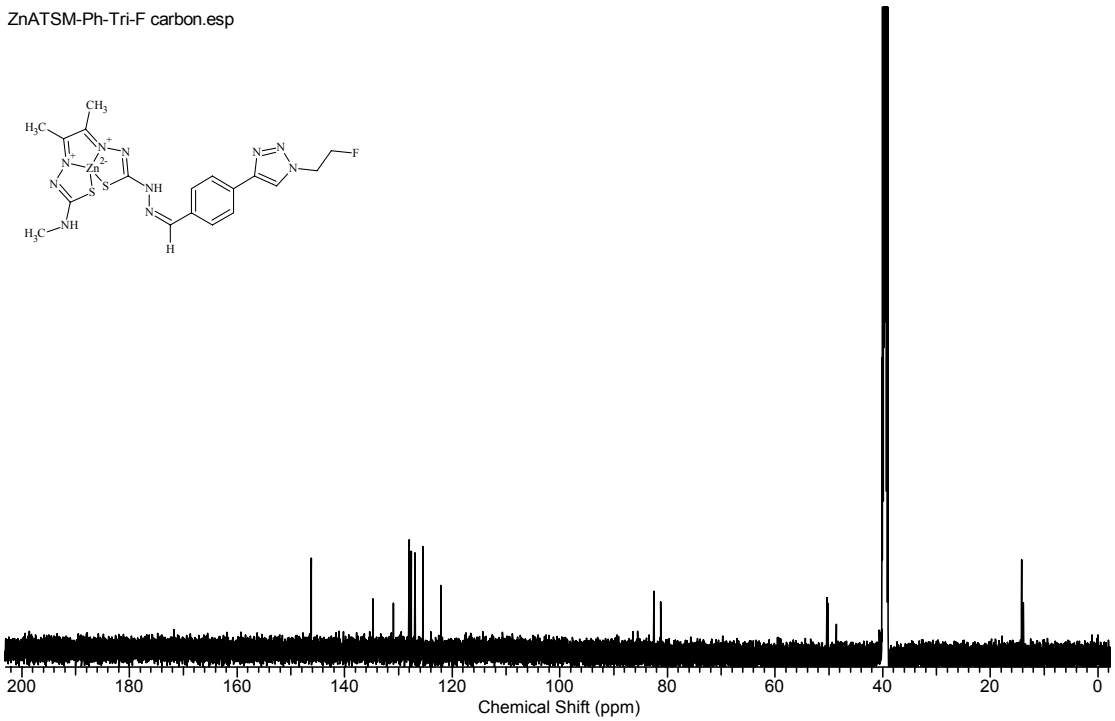


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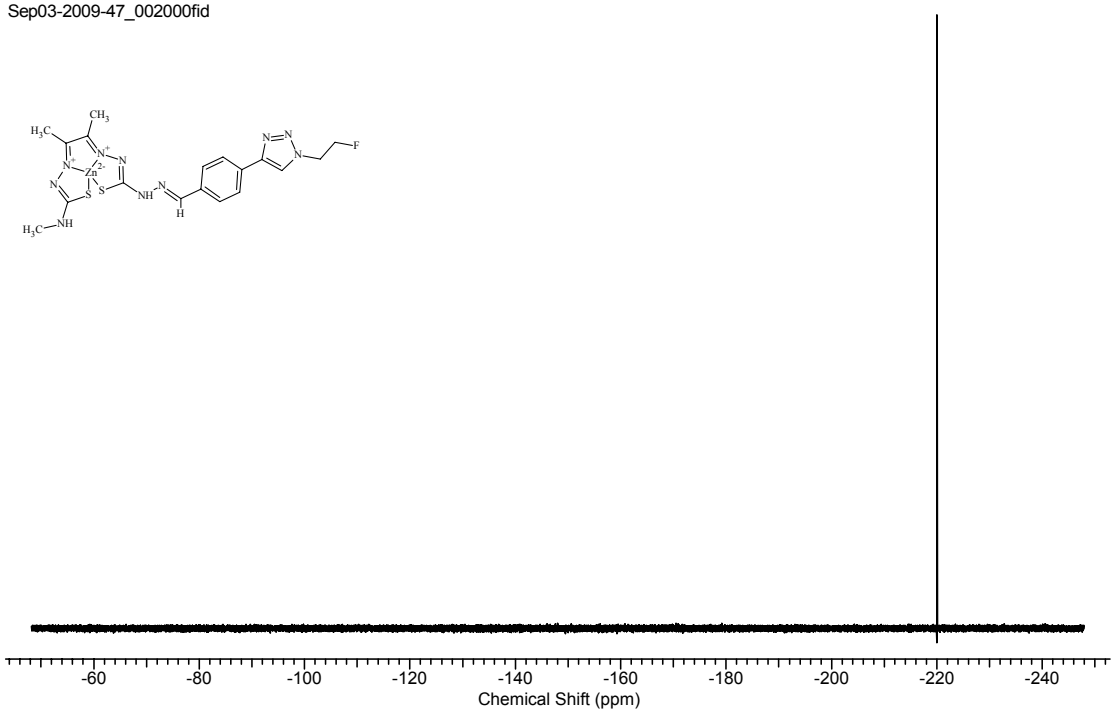

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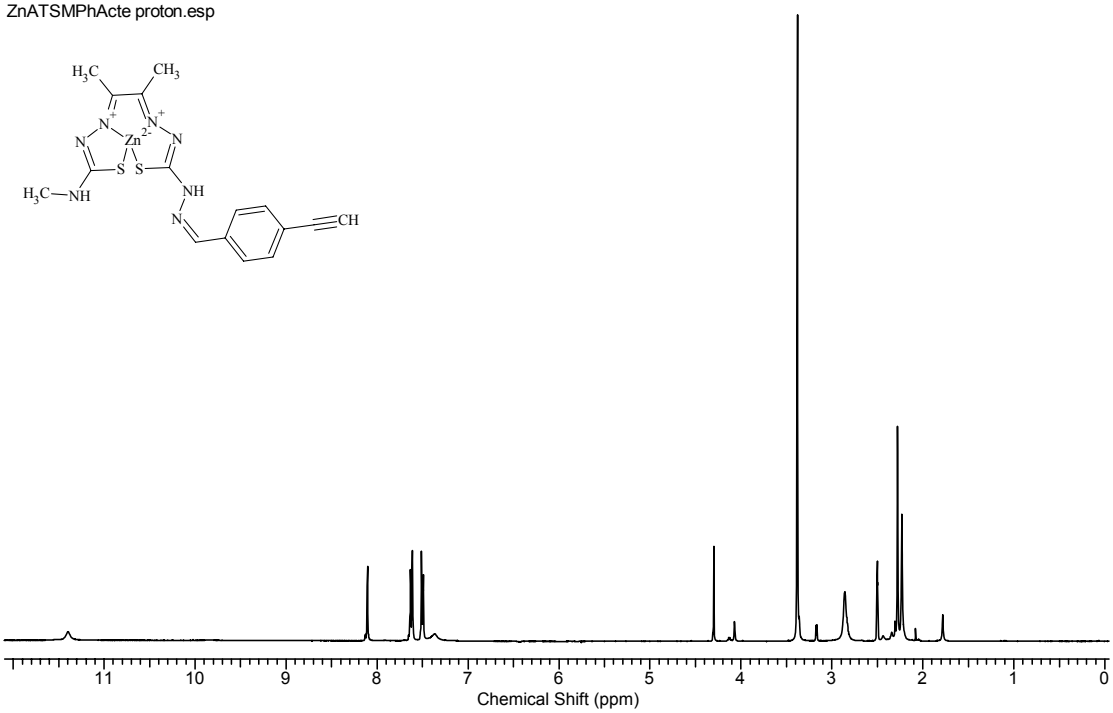


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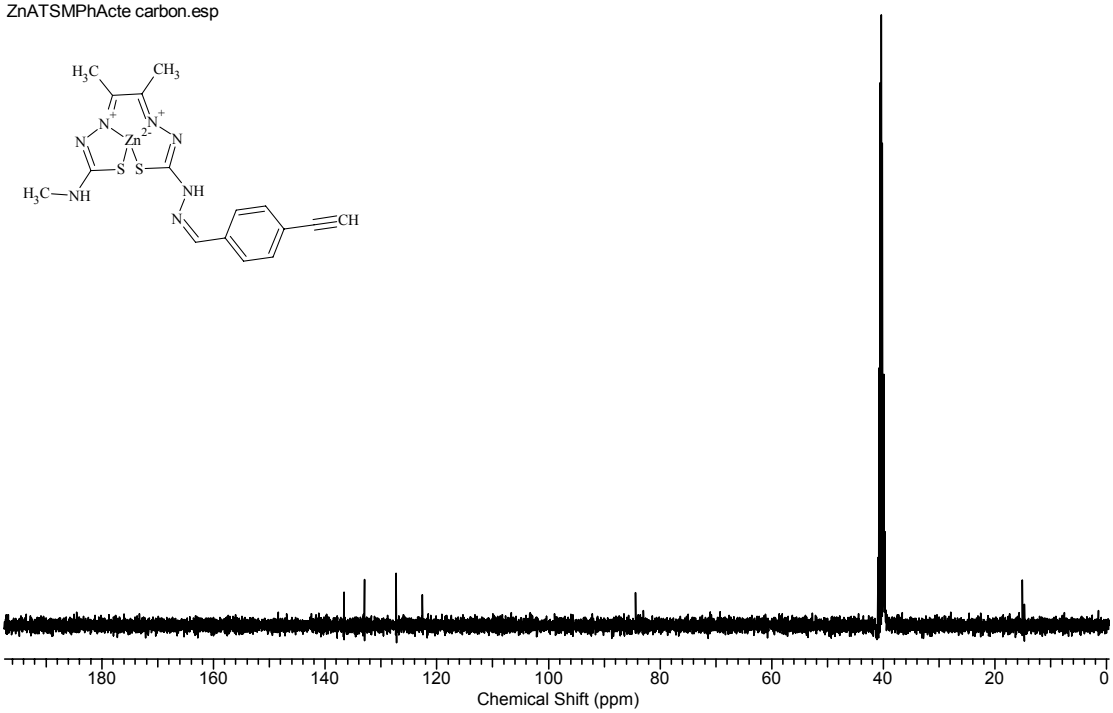

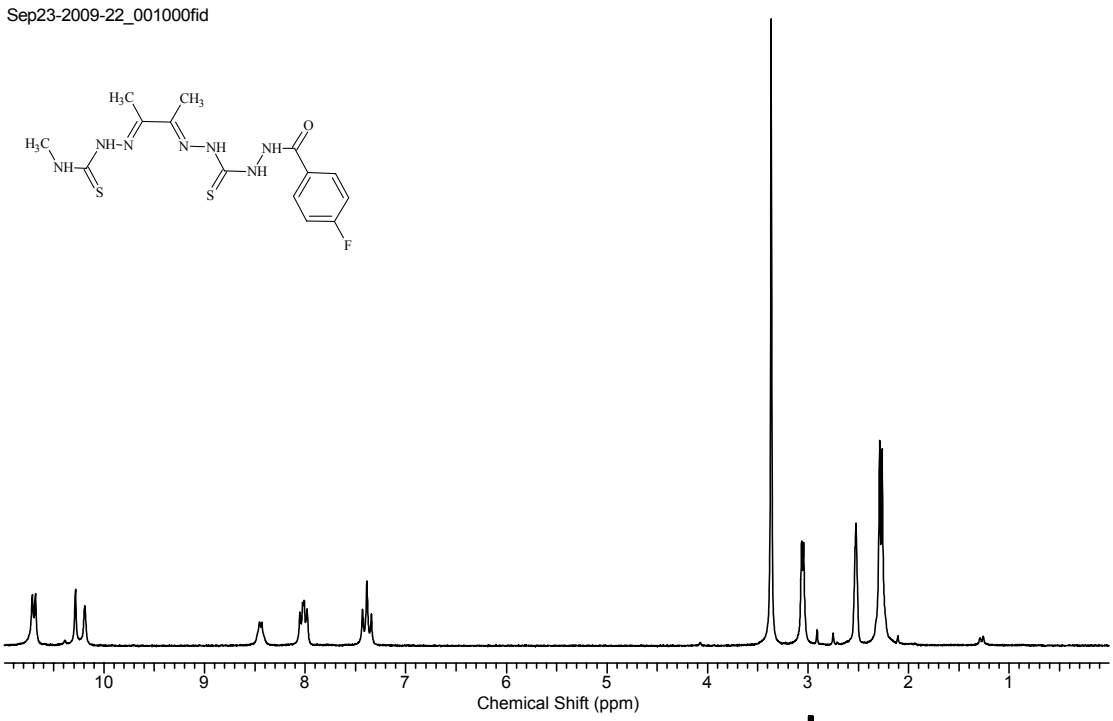
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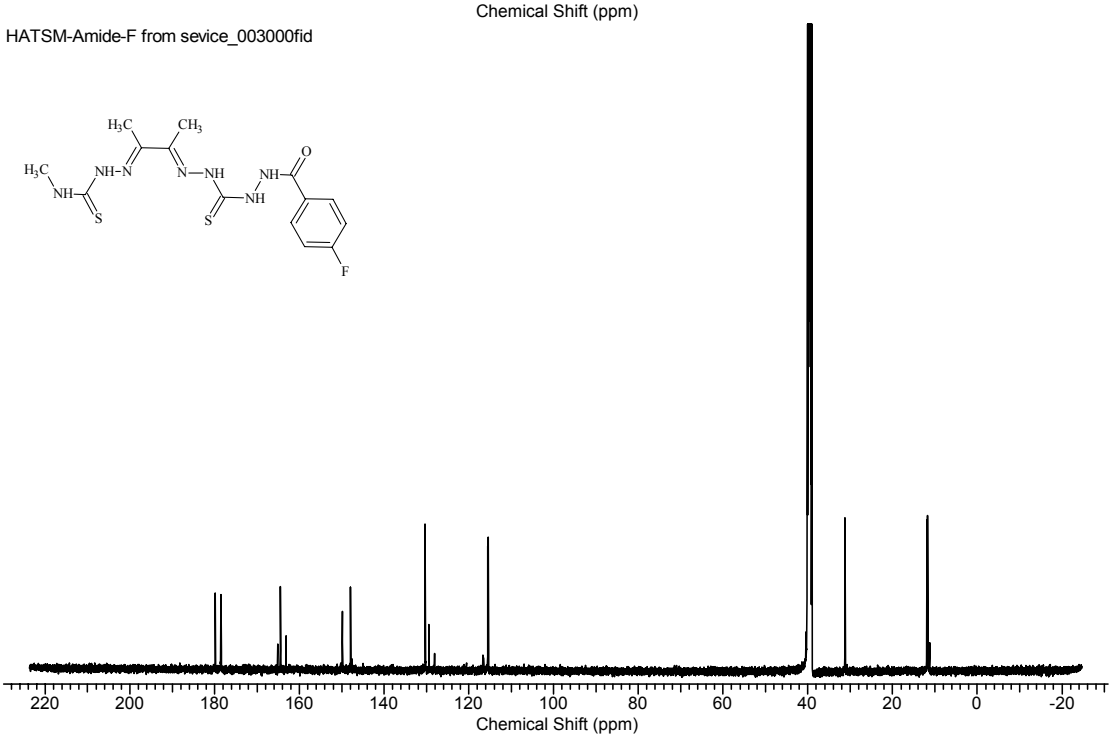
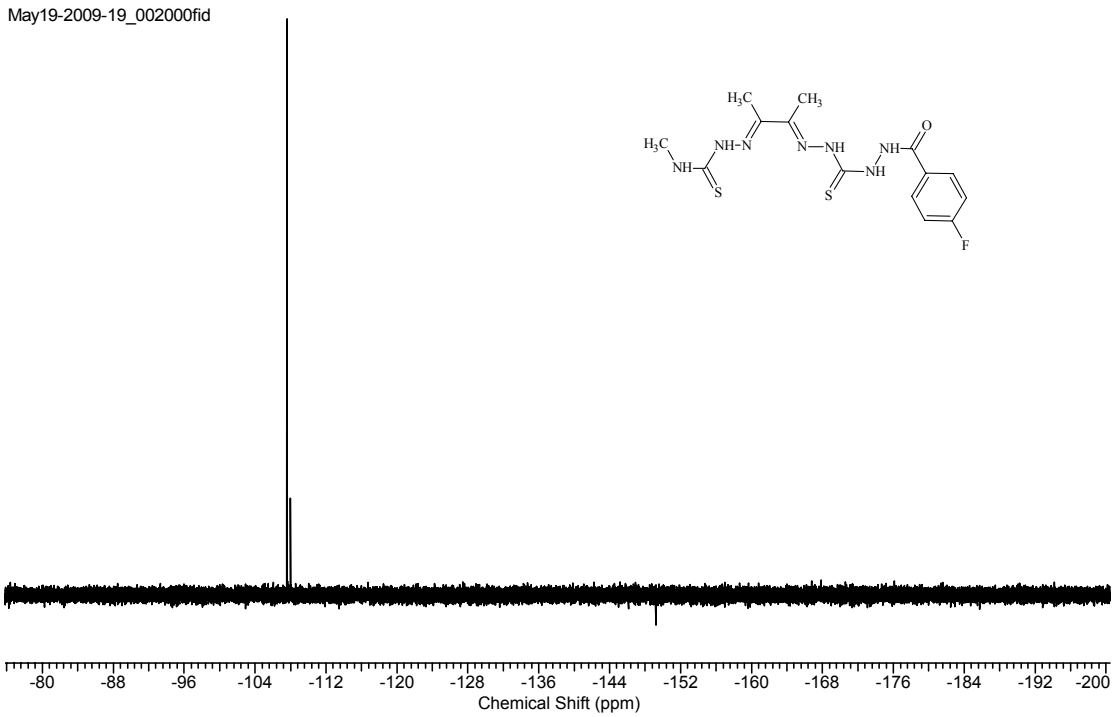
Chemical Shift (ppm)


HATSM-Amide-F from sevice_003000fid
Chemical Shift (ppm)


May19-2009-19_002000fid
-80
-88
-96
-104
-112
-120
-128
-136
-144
-152
-160
-168
-176
-184
-192
-200
Chemical Shift (ppm)

***In Vitro***

To measure the stability of the labeled complexes, 5 μCi of the required $^{64}$Cu complex was incubated in 1 mL of fresh mouse serum at 37°C, where at selected time points the amount of intact complex was determined using radio-TLC. To determine the amount of protein-bound $^{64}$Cu, 50 μL fractions were removed and 200 μL of ethanol added to precipitate the proteins present. The sample was then centrifuged until the precipitated proteins formed a pellet. The supernatant was then carefully removed from the pellet and the pellet washed with 500 μL ethanol, and the sample centrifuged again. The combined supernatants and the pellet were counted on a gamma counter to determine the amount of protein-bound activity.

For radiotracer uptake experiments;

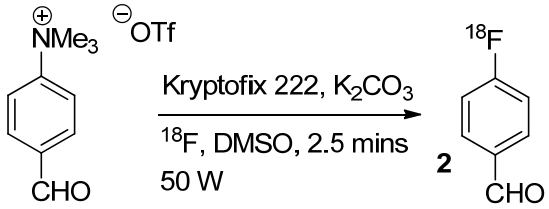
Method a – suspensions of HT1080 cells ($5\times10^6$ cells/mL) were incubated in glass vessels in a humidified atmosphere under anoxic or normoxic conditions with 5 % $CO_2$ and the balance $N_2$ (with a constant flow of gas) at 37$^o$C. The medium used was minimum essential medium with the Eagle-Spinner modification (Earle's salts and sodium bicarbonate), without calcium chloride and L-glutamine, and supplemented with L-glutamine (0.292 g/L), penicillin-streptomycin, and nonessential amino acids. After 1 hour, the oxygen conditions had reached equilibrium (probed with an Oxford Optronics Oxylab $pO_2$ tissue oxygenation monitor), and the $N_2$-purged radiotracer was added. Samples (1 mL) were taken by use of a long needle syringe at 1, 5, 15, 30, 45 and 60 min, and three 300-μL portions of each sample were dispensed into Eppendorf tubes. The tubes were spun to pellet the cells, and the supernatant liquid was removed. The activities of the cell pellet and of the supernatant liquid were measured with a γ-counter (Hidex Triathler). The uptake of $^{64}$Cu as a percentage of the activity injected was calculated and plotted. A control experiment was performed with the radiotracer in minimum essential medium under normoxic conditions to assess the amount of $^{64}$Cu adhering to the plastic Eppendorf tubes; this value was measured and subtracted from each data point.

Method b - The EMT6 mammary carcinoma cell suspension (10 mL), taken from a homogenous 50 mL cell suspension at a concentration of $5\times10^6$ cells/mL, was equilibrated in a three-necked, glass round-bottomed flask at 37°C under anoxic (95% $N_2$, 5% $CO_2$) and normoxic (75% $N_2$, 20% $O_2$, 5% $CO_2$) conditions by passing a continuous flow of warmed humidified gas over the cells, with all remaining variables kept constant. After 30 min, when the vessels had reached equilibrium, 50 μCi of the radiopharmaceutical was added. Then at 1, 5, 15, 30 and 60 min, triplicate samples of 200 μL of cell suspension were removed, the suspension centrifuged and the percentage uptake of the compound into the cells calculated. As a control, the compounds were put through the identical methods without cells present to determine the extent to which the compounds adhere to vials due to the lipophilicity of the compounds. No appreciable adherence of any of the compounds was noted. A protein assay was not required as the cell studies were all performed on the same homogenous cell mixture of precisely known cellular concentration.

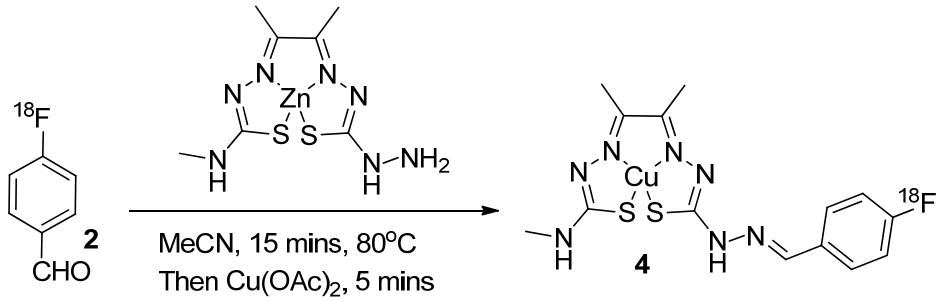
Method c - 5 × 105 HT1080 cells were seeded per 60 mm glass petri dish using 5 mL 5 % FCS DMEM supplemented with L-glutamine (0.292 g/L) and penicillin–streptomycin, and cultured for 5 hours in a humidified atmosphere of 5 % $CO_2$ at 37°C. The petri dish lids were removed and the petri dishes were transferred to air tight perspex boxes. The boxes were placed into a 37°C incubator under continuous humidified as flow for 18 hours using normoxic or anoxic conditions, respectively 21 % $O_2$ and 5 % $CO_2$, or 0 % $O_2$ and 5 % $CO_2$ and the balance made up with $N_2$.

Following overnight incubation, the radiotracers were diluted with PBS and 150 μL were added through a septum to each confluent petri dish. The petri dishes were incubated for a further 3 hours at 37°C with a continuous humidified gas flow. After the incubation the supernatant was removed to counting tubes followed by a 2 mL PBS wash. The cells of each petri dish were lysed using 2 mL of 1 M NaOH at room temperature for 15 minutes. The NaOH solution was transferred to counting tubes followed by a 2 mL wash. The cell and supernatant fractions were measured using a γ-counter (Hidex Triathler). The $^{18}F$ activity associated with the cell was calculated as the percentage of the total activity added per Petri dish. Each compound was tested using both normoxic and anoxic conditions in triplicate.

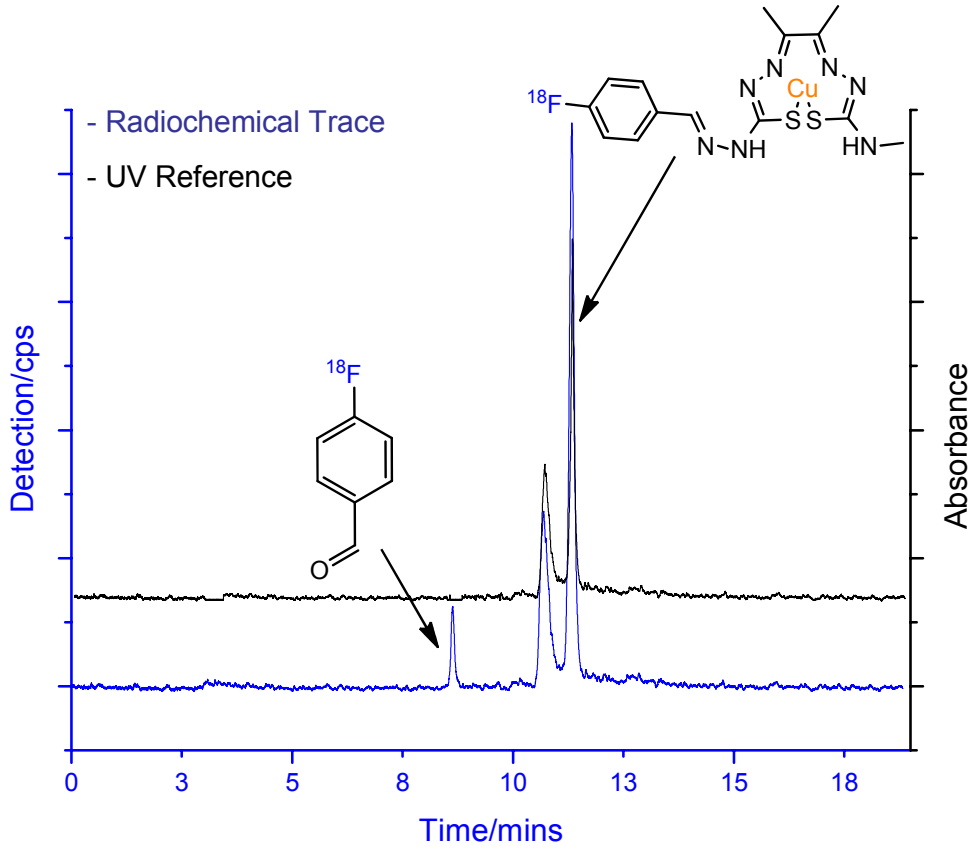
## Radiochemical Procedures



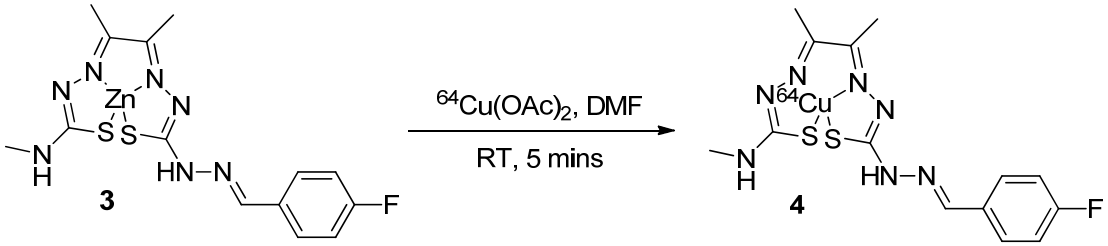

In a sealed reaction vial, 0.3 mL of $K^{18}F$/Kryptofix 222 in anhydrous DMSO (20-100 MBq) was added to the precursor (10 mg) and heated for 150 seconds at 50 W. The reaction was then diluted with water (5 mL) and passed through a SepPak C18 cartridge. The cartridge was washed with water (5 mL) and then eluted with methanol (2 mL). Analysis by radio-TLC (acetonitrile:water 95:5) indicated a 60 % RCY of compound **2**. Analysis by reverse-phase HPLC (Vydac, 300A, C18, 100 mm x 4.6 mm, MeCN/$H_2O$ gradient, 1 mL/min) gave a retention time of 8.21 minutes.(Vaidyanathan and Zalutsky 2006)



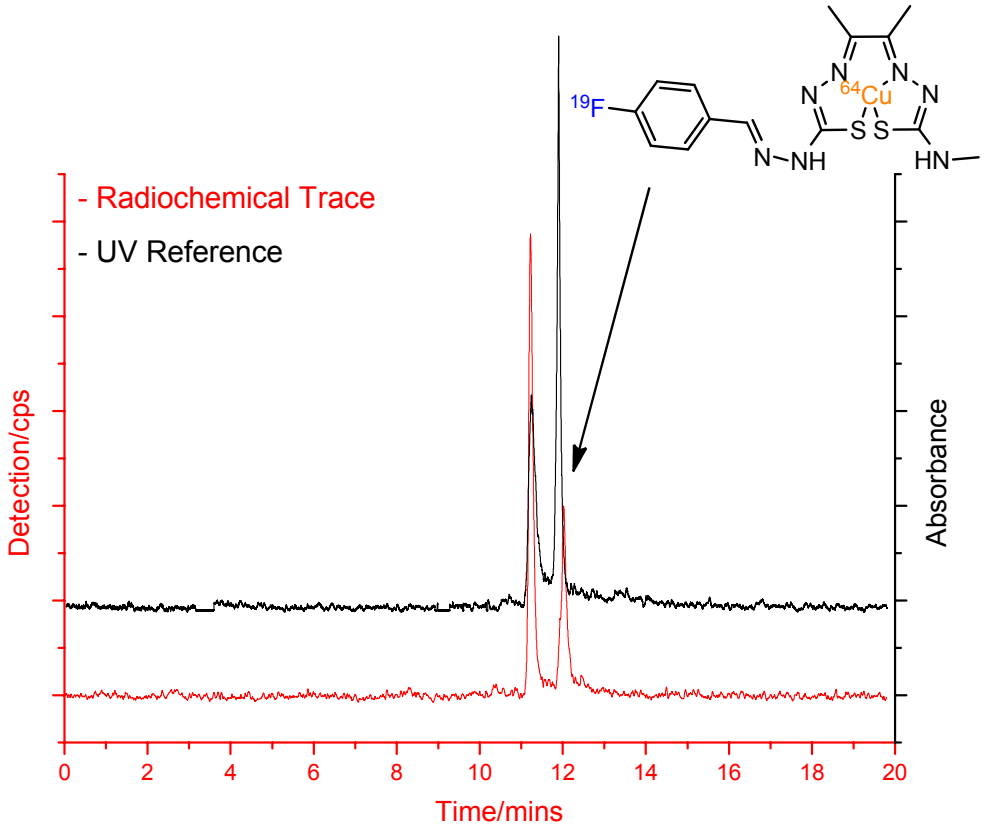

In a sealed reaction vial, the SPE purified solution of compound **2** (20-100 MBq) in MeOH (2 mL) was reacted with diacetyl-2-(4-*N*-methyl-3-thiosemicarbazonato)-3-(4-*N*-amino-3-thiosemicarbazonato)zinc(II) monomethanol (5 mg) and heated to 80°C for 15 mins. $Cu(OAc)_2$ (5 mg) was then added and the reaction was stirred for a further 5 mins at room temperature. Analysis by reverse-phase HPLC (Vydac, 300A, C18, 100 mm x 4.6 mm, MeCN/$H_2O$ gradient, 1 mL/min) gave a retention time of 10.69 and 11.33 minutes. Comparison with a cold reference HPLC trace (Trace 1) confirmed this to be the two stereoisomers of complex **4**. The conversion to the product was measured as 94 %, giving an overall RCY of 56 % over the two steps.



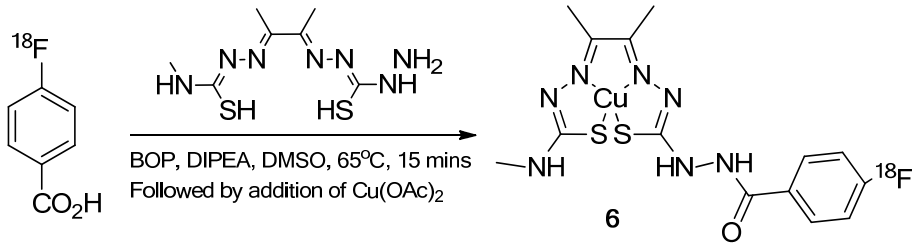

**Trace 1.** Radiochemical HPLC trace of [$^{18}$F]**4** (blue) with UV cold reference sample (black).



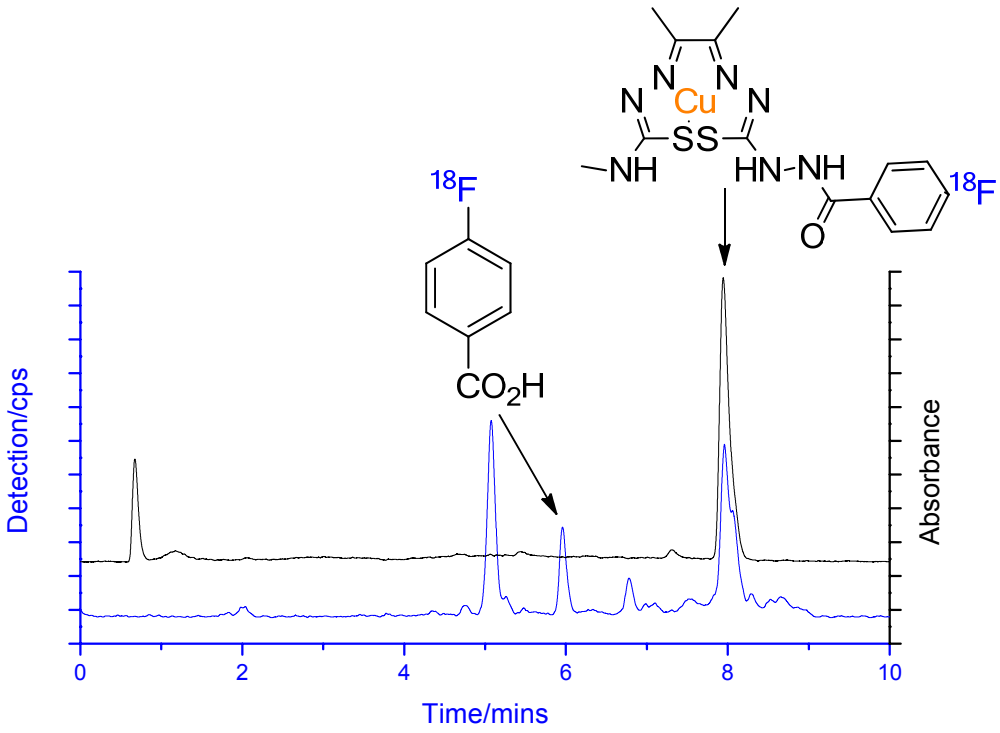

The copper-64 complex **4** was prepared by reacting [$^{64}$Cu]Cu(OAc)$_2$ (200 µL, < 10 MBq) with 100 µL of a standard solution of 0.5 mg of complex **3** dissolved in 1mL of DMF and water (400 µL) in a 2 mL reaction vial. The reaction was stirred at room temperature for 15 mins and then analysed by reverse-phase radio-HPLC (Vydac, 300A, C18, 100 mm x 4.6 mm, MeCN/$H_2O$ gradient, 1 mL/min) gave a retention time of 10.69 and 11.33 minutes. Comparison with a cold reference HPLC trace confirmed this to be the two stereoisomers of complex **5**.



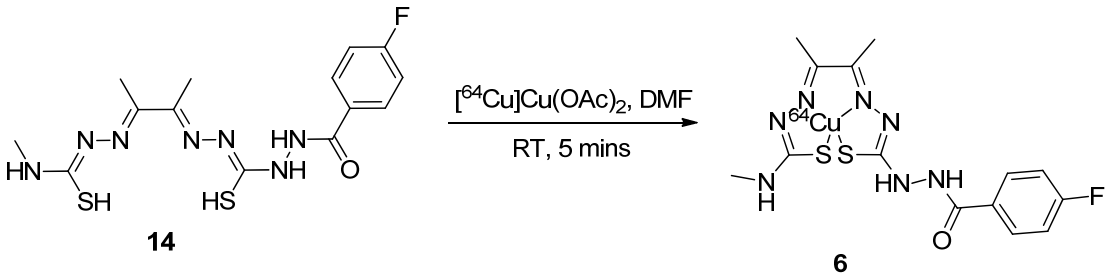

**Trace 2.** Radiochemical HPLC trace of [$^{64}$Cu]**4** (red) with UV reference sample (black).




In a sealed reaction vial, the SPE purified solution of compound **5** (20-100 MBq) in MeCN (1 mL) was reacted with $H_2$ATSM/A (3 mg), *O*-benzotriazole-*N*,*N*,*N*',*N*'-tetramethyl-uronium-hexafluoro-phosphate (3 mg) and *N*,*N*-Diisopropylethylamine (30 µL) in DMSO (0.3 mL) and heated to 80°C for 15 mins. Copper(II) acetate (5 mg) was then added and the reaction was stirred for a further 5 mins at room temperature. Analysis by reverse-phase HPLC (Phenomenex Gemini NX, C18, 250 mm x 4.6 mm, 0.1 % TFA in MeCN/$H_2O$ gradient, 1 mL/min, $t_R$ = 8.5 minutes). Comparison with a cold reference HPLC trace confirmed this to be complex **6**. The conversion to the product was measured as 45 %, giving an overall RCY of 32 % over the two steps.

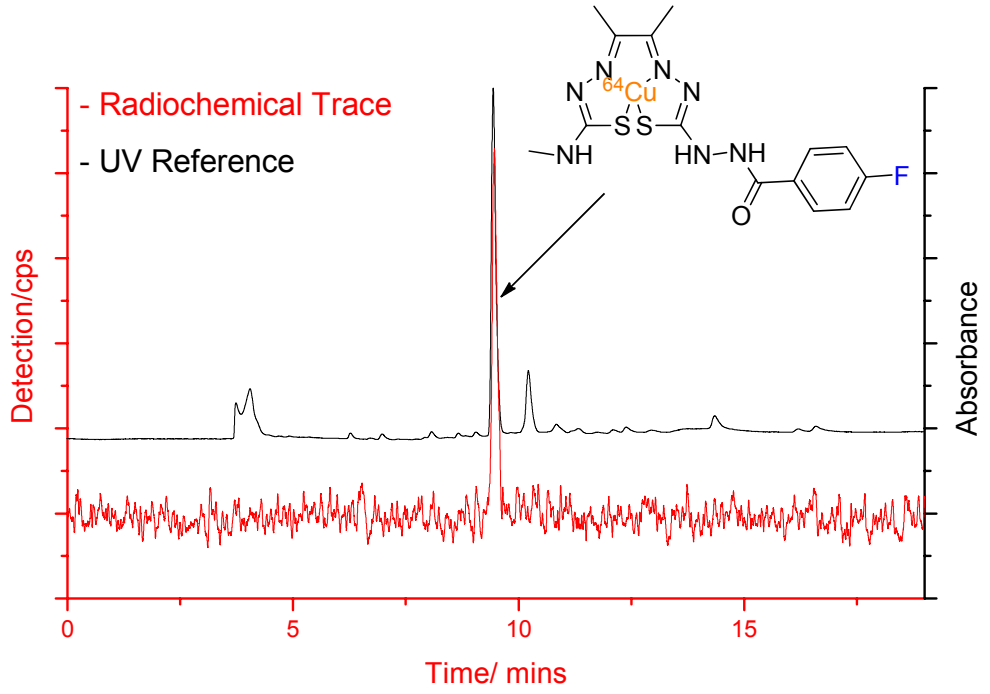



**Trace 3.** Radiochemical HPLC trace of [$^{18}$F]**6** (blue) with UV reference sample (black).

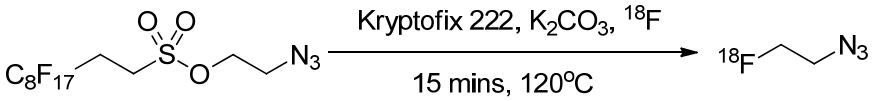



**14** **6**

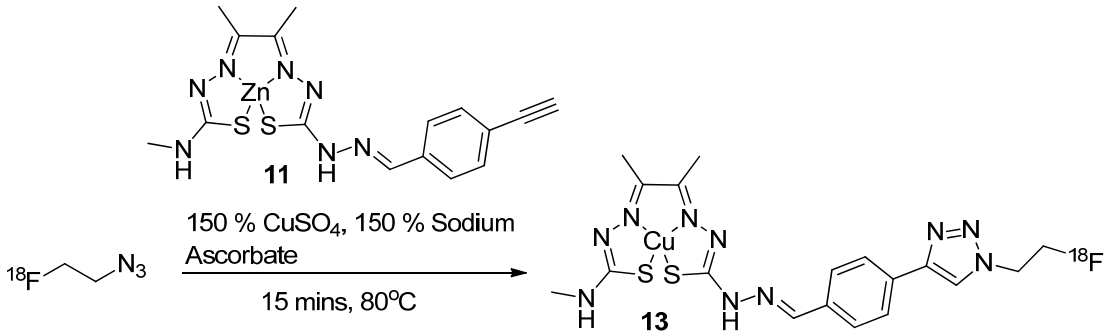
The copper-64 complex **6** was prepared by reacting [$^{64}$Cu]Cu(OAc)$_2$ (200 µL, < 10 MBq) with 100 µL of a standard solution of 0.5 mg of complex **13** dissolved in 1mL of DMF and water (400 µL) in a 2 mL reaction vial. The reaction was stirred at room temperature for 15 mins and then analysed by reverse-phase radio-HPLC (Grace, Platinum C18, 250 mm x 4.6 mm, MeCN/$H_2O$ gradient, 1 mL/min, $t_R$ = 9.7 minutes) (Trace 4). Comparison with a cold reference HPLC trace confirmed this to be complex **6**.



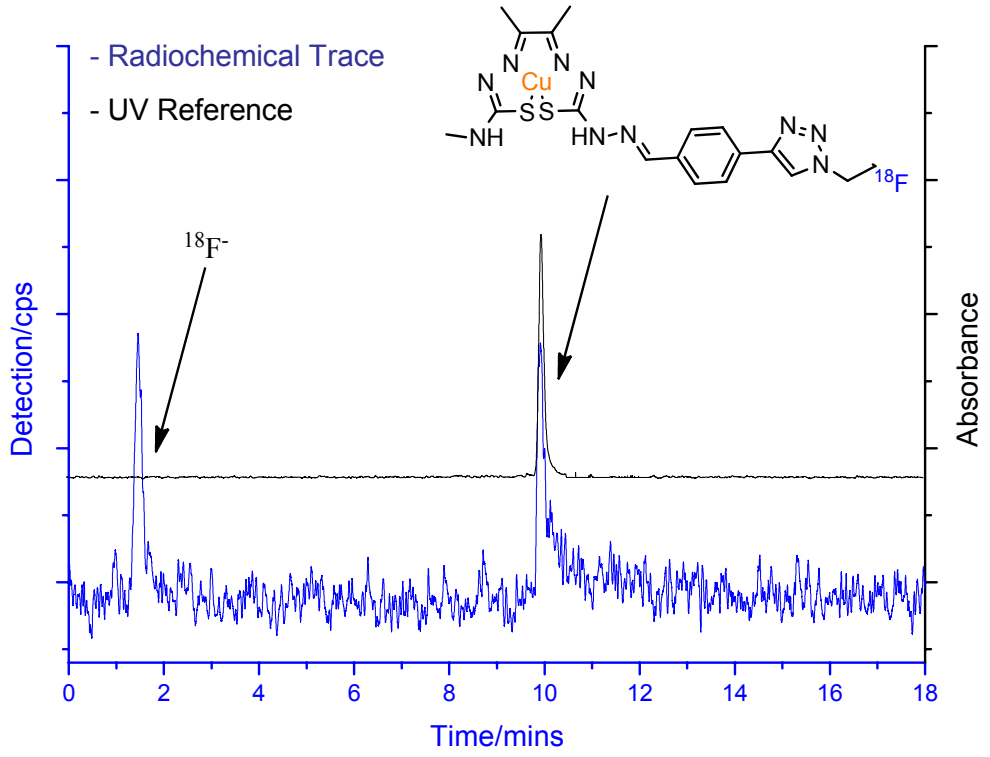

**Trace 4.** Radiochemical HPLC trace of [$^{64}$Cu]-**6** (red) with UV reference sample (black).



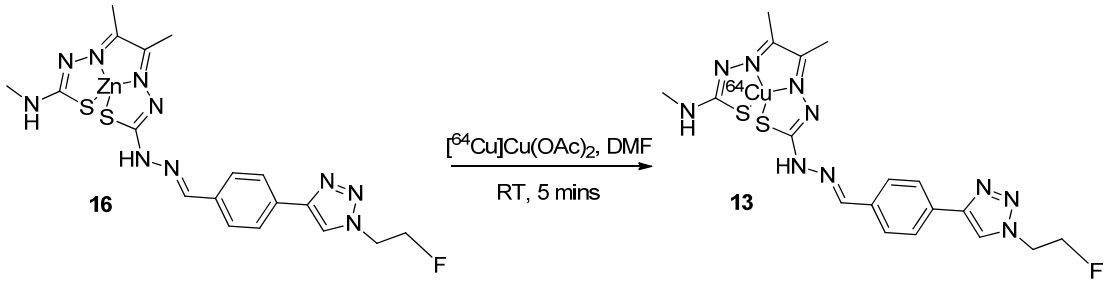

In a sealed reaction vial, 0.3 mL of K$^{18}$F/Kryptofix 222 in anhydrous MeCN (20-100 MBq) was added to 2-azidoethyl 1*H*,1*H*,2*H*,2*H*-perfluorodecane-1-sulfonate (10 mg) and heated for 15 minutes at 120°C. Determination of the radiochemical yield was by a further reaction. An aliquot (10 µL) of the crude reaction mixture was combined with *N*-propargylbenzamide (5 mg), $CuSO_{4(aq)}$ (50 µL) and sodium ascorbate$_{(aq)}$ (50 µL) and heated for 15 minutes at 80°C. Analysis by HPLC (Zorbax SB, C18, 250 x 4.6 mm, MeCN/$H_2O$ gradient, 1 mL/min) gave a retention time of 6.70 minutes. Comparison with the cold reference HPLC trace confirmed the product to be successfully labelled [$^{18}$F]*N*-benzyl-3-[1-(2-fluoroethyl)-1H-[1,2,3]triazol-4-yl]propionamide. Analysis by radio-TLC (acetonitrile:water 95:5) indicated an 84 % RCY.(Glaser and Arstad 2007) Purification was carried out by FSPE as described in the General Procedures.(Bejot R., Fowler T. et al. 2009)



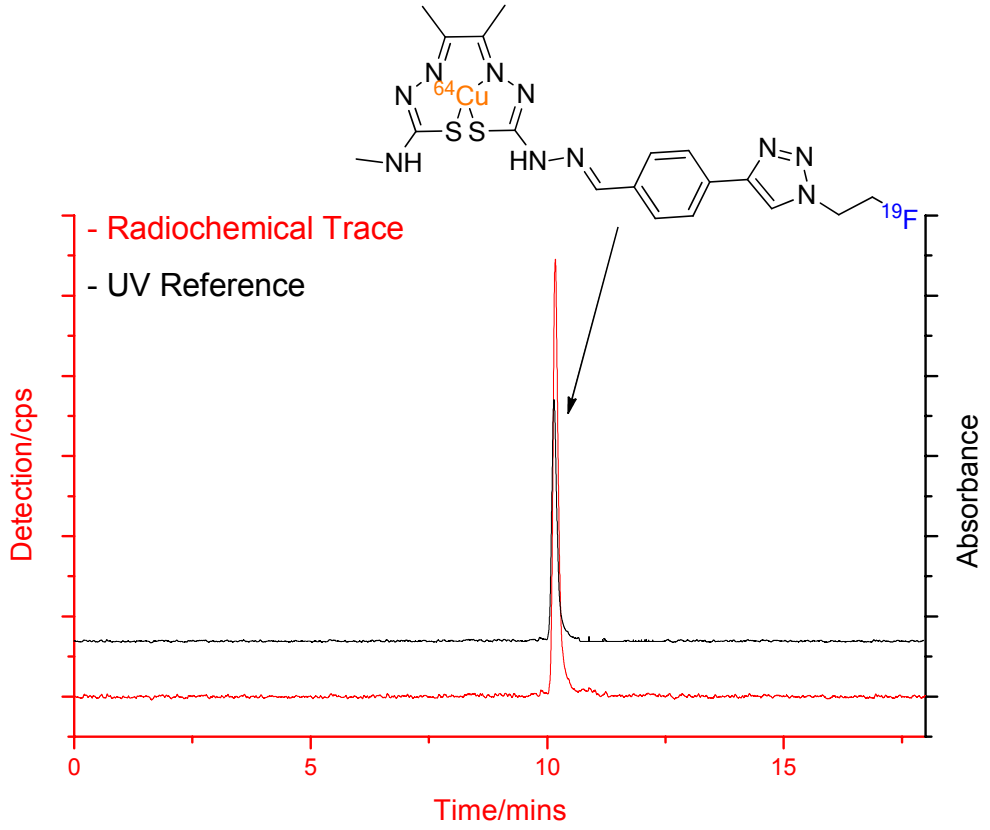

In a sealed reaction vial, the FSPE purified solution of 2-[$^{18}$F]Fluoroethylazide (20-100 MBq) in MeCN/$H_2O$ (7:3, 0.5 mL) was reacted with complex **11** (5 mg), 0.5 M $CuSO_{4(aq)}$ (50 µL) and 0.5 M sodium ascorbate$_{(aq)}$ (50 µL) and heated for 15 minutes at 80°C. Analysis by reverse-phase HPLC (Vydac, 300A, C18, 100 mm x 4.6 mm, MeCN/$H_2O$ gradient, 1 mL/min) gave a retention time of 9.90 minutes. Comparison with a cold reference HPLC trace (Trace 5) confirmed this to be complex **13**. All 2-[$^{18}$F]fluoro-1-ethylazide was consumed according to HPLC analysis, and therefore the RCY was calculated to be 84 % over the two steps.




**Trace 5.** Radiochemical HPLC trace of [$^{18}$F]**13** (blue) with UV reference sample (black).




The copper-64 complex **13** was prepared by reacting [$^{64}$Cu]Cu(OAc)$_2$ (200 µL, < 10 MBq) with 100 µL of a standard solution of 0.5 mg of complex **16** dissolved in 1mL of DMF and water (400 µL) in a 2 mL reaction vial. The reaction was stirred at room temperature for 15 mins and then analysed by reverse-phase radio-HPLC (Vydac, 300A, C18, 100 mm x 4.6 mm, MeCN/$H_2O$ gradient, 1 mL/min) (Trace 6) gave a retention time of 10.17 minutes. Comparison with a cold reference HPLC trace confirmed this to be complex **13**.




**Trace 6.** Radiochemical HPLC trace of [$^{64}$Cu]**13** (red) with UV reference sample (black).

## Specific Activity

Specific activities of [$^{18}$F]4-fluorobenzaldehyde was measured after nucleophilic radiofluorination of 4-formyl-*N*,*N*,*N*-trimethylanilinium trifluoromethanesulfonate. The reaction mixtures were analyzed by HPLC (MeCN/$H_2O$, 1 mL/min, 20 µL injected, linear increase from 25% acetonitrile to 30% acetonitrile in 20 minutes). The concentrations of the radioactive compounds were determined using calibration curves obtained by analysis of the UV absorbance (254 nm) of the relevant non-radioactive 4-fluorobenzaldehyde.

The specific activity of [$^{18}$F]-4-fluorobenzaldehyde was 34-90 GBq/µmol (n=2) at the end of synthesis.

The calibration curve (absorbance to concentration) for 4-fluorobenzaldehyde is shown in Figure 1.

log(Absorbance) = 3.78762+0.92427 log(Concentration)
$R^2$=0.99937
Concentration: mg/mL
Absorbance: AU

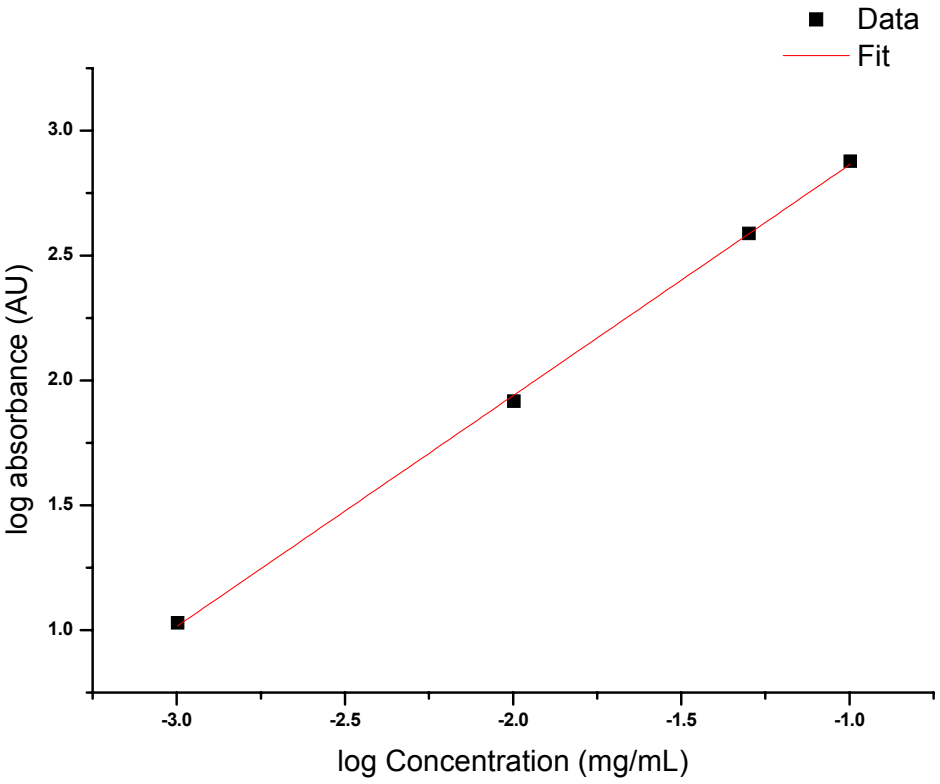



**Figure 1** Calibration Curve for 4-Fluorobenzaldehyde

## References

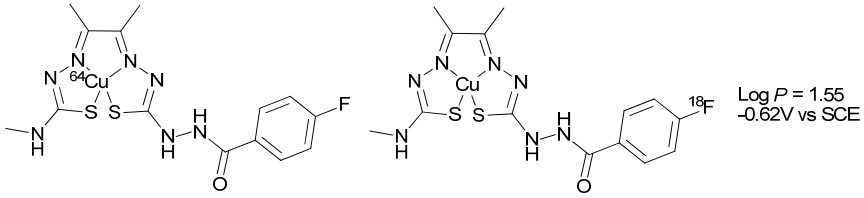
Bejot R., Fowler T., et al. (2009). "Fluorous Synthesis of $^{18}$F Radiotracers with the $^{18}$Fluoride Ion: Nucleophilic Fluorination as the Detagging Process." Angewandte Chemie International Edition **48**(3): 586-589.

Glaser, M. and E. Arstad (2007). ""Click Labeling" with 2-[18F]Fluoroethylazide for Positron Emission Tomography." Bioconjugate Chem. **18**(3): 989-993.

Vaidyanathan, G. and M. R. Zalutsky (2006). "Synthesis of N-succinimidyl 4-[18F]fluorobenzoate, an agent for labeling proteins and peptides with 18F." Nat. Protocols **1**(4): 1655-1661.

## Table of Contents






For the first time, $^{18}$F-labelled Cu(ATSM) analogues have been prepared. These compounds are designed to serve as new hypoxia selective PET tracers. These new fluorinated complexes can be either metal-radiolabelled ($^{64}$Cu) or labelled at the ligand ($^{18}$F) and can therefore be used as mechanistic probes to enhance our understanding of the mode of action of this family of hypoxia selective radiotracers.