The hydrazide/hydrazone click reaction as a biomolecule labeling strategy for $M(CO)_3$ (M = Re, ^{99m}Tc) radiopharmaceuticals.

Tanushee Ganguly[1], Benjamin B. Kasten[1], Dejan-Krešimir Bučar[2], Leonard R. MacGillivray[2], Clifford E. Berkman[1], Paul D. Benny[1*]

[1]Department of Chemistry, Washington State University, Pullman WA 99164, USA

[2]Department of Chemistry, University of Iowa, Iowa City, IA 52242-1294, USA

Received (in XXX, XXX) Xth XXXXXXXXX 200X, Accepted Xth XXXXXXXXX 200X
First published on the web Xth XXXXXXXXX 200X
DOI: 10.1039/b000000x

Supplemental Information

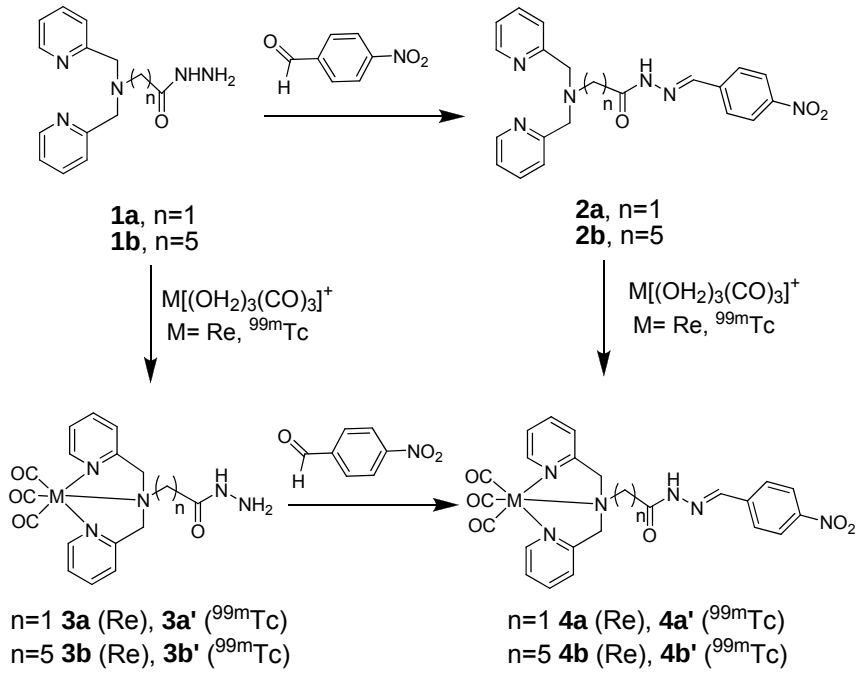
Preparation of Compounds

Materials. All reagents of ACS grade or higher were used as received without further purification. $Re(CO)_3(H_2O)_3OTf$, $Re(CO)_5OTf$, and $Re(CO)_5Br$ were prepared according to reported literature procedures[1,2].

Instrumentation. NMR spectra were recorded at 293 K on a Varian Mercury Vx 300 spectrometer using 5 mm NMR tubes. ^{1}H NMR spectra peak positions were referenced using trimethylsilane. ^{13}C NMR spectra peak positions were referenced using trimethylsilane and/or solvent peaks. Spectra were processed using Varian VMR 6.1 software. IR spectra were recorded on a Thermo Nicolett 6700 FTIR with an ATR cell and analyzed with OMNIC 7.1a software. UV/Vis spectra were recorded on a Varian Cary 50 Bio and analyzed with Cary WinUV 3.00 software. Solutions of the compounds were prepared in UV/Vis quality methanol and measured in quartz cuvettes. Separation and identification of compounds were conducted on a Perkin Elmer Series 200 High Pressure Liquid Chomatograph (HPLC) with an Agilent Zorbex 30 cm SB-C18 column. The reverse phase gradient system begins with 0.1% trifluoroacetic acid (TFA) aqueous eluent gradually shifting to methanol according to the following method, 0-3.0 min (100% TFA), 3.0-9.0 min (75% TFA, 25% MeOH), 9.0-20.0 min (25% to 100% MeOH linear gradient), 20.0-25.0 min (100% MeOH) at a flow rate of 1.0 mL/min.

(1)Alberto, R., Schibli, R., Waibel, R., Abram, U., and Schubiger,A. P. (1999) *Coord. Chem. Rev. 192*, 901–919.
(2) He, H. Y., Lipowska, M., Xu, X. L., Taylor, A. T., Carlone,M., and Marzilli, L. G. (2005) *Inorg. Chem. 44*, 5437–5446.

Figure S1: Parallel synthesis approaches *Route 1* (1→2→4, *click, then chelate*) and *Route 2* (1→3→4, *prelabel, then click*) for the formation of $M(CO)_3^+$, (M= Re, ^{99m}Tc) DPA-hydrazone complexes.



Synthesis of 2-(bis(pyridin-2-ylmethyl)amino)acetohydrazide, 1a: The ethyl ester precursor of **1a** (0.2516 g, 0.927 mmol) and a 35% hydrazine solution in water (1.019 mL, 11.2 mmol) were refluxed in EtOH (1.5 mL) for 20 h. The resulting solution was concentrated by rotary evaporation, dissolved in CH_2Cl_2 and washed with water. The organic phases were combined, purified by liquid column chomatography (silica gel, 10% MeOH in CH_2Cl_2), concentrated by rotary evaporation, and dried *in vacuo* to yield the desired product **1a** (0.178 g, 71%). **Anal. calcd.** ($C_{14}H_{19}N_5O_2$): C, 58.13; H, 6.57; N, 24.22. **Found:** C, 58.41; H, 6.41; N, 24.13. **^{1}H NMR** [δ (ppm), CD_3CN] 9.45 (s, 1H), 8.56 (d, 2H), 7.71 (dd, 2H), 7.37 (d, 2H), 7.25 (dd, 2H), 3.81 (s, 4H), 3.30 (s, 1H), 2.04-2.4 (broad singlet, 2H). **^{13}C NMR** [δ (ppm), $CDCl_3$] 171.5, 158.4, 149.7, 136.8, 123.4, 122.7, 60.7, 57.5. **UV** (λ_{max}, 262 nm) ε_{max}, 8130 M^{-1} cm^{-1}. **IR** (cm^{-1}): 3310, 1665, 1591. **Mass Spec:** 272.1.

Synthesis of 2-(bis(pyridin-2-ylmethyl)amino)-N'-(4-nitrobenzylidene)acetohydrazide, 2a: A solution of **1a** (0.0852 g, 0.314 mmol) and *p*-nitrobenzaldehyde (PNB) (0.0522 g, 0.345 mmol) was made in EtOH (3 ml) and the pH of the solution was adjusted to slightly acidic (≈4) with 0.1 M HCl. The solution was refluxed with stirring at

55°C for 3 h. The resulting solution was dried by rotary evaporation and purified by liquid column chomatography (silica gel, 20% MeOH in CH_2Cl_2), concentrated by rotary evaporation, and dried *in vacuo* to yield the desired product **2a** (0.103 g, 81%). **Anal. calcd (**$C_{21}H_{20}N_6O_3$): C, 62.37; H, 4.98; N, 20.78. **Found**: C, 61.67; H, 4.95; N, 20.08. **^{1}H NMR** [δ (ppm), CD_3CN] 12.62 (s, 1H), 8.65 (d, 2H), 8.51 (s, 1H), 8.29 (d, 2H), 7.99 (d, 2H), 7.71 (dd, 2H), 7.34 (d, 2H), 7.25 (dd, 2H), 3.92 (s, 4H), 3.52 (s, 2H). **^{13}C NMR** [δ (ppm), $CDCl_3$] 168.7, 158.1, 149.4, 148.7, 145.4, 140.5, 137.1, 128.4, 124.2, 123.7, 123.0, 60.4, 58.0. **UV**: (λ_{max}, 322 nm) ε_{max}, 20,800 M^{-1} cm^{-1}. **IR (**cm^{-1}): 1672, 1516, 1337. **Mass Spec:** 405.2.

Synthesis of Re-1a, 3a-OTf: $Re(CO)_5OTf$ (0.0495g, 0.104 mmol) was dissolved in 4 mL MeOH, and a solution of **1a** (0.032 g, 0.118 mmol) in MeOH (1 mL) was added. The reaction was refluxed with stirring at 63°C for 3 h and subsequently dried by rotary evaporation. The residue was briefly washed with chloroform, dissolved in a water/methanol solution (9:1), and purified by preparative RP-HPLC using water/methanol gradient. Pure product elutes out at 70% MeOH which on evaporation yielded **3a** (0.033 g, 46%). **Anal. calcd** ($C_{19}H_{18}F_6N_5O_{10}ReS_2$): C, 27.15; H, 2.16; N, 8.33. **Found:** C, 27.21; H, 2.17; N, 8.37. **^{1}H NMR** [δ (ppm), CD_3CN] 8.79 (d, 2H), 7.91 (dd, 2H), 7.50 (d, 2H), 7.34 (dd, 2H), 5.15 (d, J=16.7 Hz, 2H), 4.87 (d, J=16.9 Hz, 2H), 4.44 (s, 2H). **^{13}C NMR** [δ (ppm), CD_3CN] 167.5, 161.0, 152.5, 140.8, 126.1, 124.1, 68.9, 68.2. **UV**: (λ_{2nd}, 264 nm) ε_{max}, 11,600 M^{-1} cm^{-1}. **IR (**cm^{-1}**)** : 2030, 1925, 1680, 1260, 1040. **Mass Spec:** 542.

Synthesis of Re-2a, 4a:

***Route 1 'click, then chelate'*: 2a** (0.022 g, 0.054 mmol) and $Re(CO)_5OTf$ (0.027 g, 0.057 mmol) were dissolved in 7 mL MeOH and the solution was refluxed with stirring at 55 °C for 20 h and allowed to cool. The solution was centrifuged and the supernatant allowed to slowly concentrate by evaporation to 0.5 mL, yielding orange-colored crystals of **4a** which were rinsed with chilled MeOH. A second crop of crystals were obtained by slow diffusion of pentane into a CH_2Cl_2/MeOH mixture containing the remaining supernatant; the crystals were re-dissolved in MeOH to remove excessive solvent from the crystal lattice and subsequently dried in vacuo. Combined yield: 45%.

***Route 2 'prelabel, then click'*: 3b** (0.047 g, 0.068 mmol) was dissolved in 9:1 EtOH/H_2O and PNB (0.010 g, 0.068 mmol) was added. The pH of the solution was adjusted to 4 with 0.1 M HCl, heated at 50 °C with stirring for 1.5 h, and allowed to cool. The solution was dried by rotary evaporation and the residue was briefly washed with chilled benzene (2 x 1 mL) to yield **4a** (93%).

One pot synthesis: PNB (0.0196 g, 0.131 mmol), $Re(CO)_5OTf$ (0.0592 g, 0.125 mmol), and **1a** (0.0349g, 0.131mmol) were dissolved in 4 mL MeOH, and the solution was refluxed with stirring at 65 °C for 2.5 h and was allowed to cool. The resulting solution containing an orange-colored precipitate was concentrated by rotary evaporation and the precipitate collected by filtration. This material was recrystallized repeatedly from MeOH to yield orange-colored needles of **4a** (Combined yield, 0.026 g, 24.5%). X-ray quality crystals were grown by diffusion of benzene into a solution of **4a** in acetonitrile. **Anal. calcd (**$C_{25}H_{20}F_3N_6O_9ReS$): C, 36.45; H, 2.45; N, 10.20. **Found:** C, 36.87; H, 2.10; N, 9.67. **^{1}H NMR** [δ (ppm), CD_3CN] *10.28*, 9.98 (s, 1 H), 8.81 (d, 2 H), 8.28 (d, 2 H), *8.26*, 8.10 (s, 1H), 7.94 (d, 2H), 7.93 (dd, 2H), 7.51 (d, 2H), 7.35 (dd, 2 H), 5.26 (d, *J* =16.9 Hz, 2H), 5.15, *4.67* (s, 2 H), 4.89 (d, *J* = 17.2 Hz, 2 H). **^{13}C NMR** [δ (ppm), CD_3CN] 169.5, 161.3, 152.5, 149.2, 143.2, 140.8, 140.3, 128.7, 128.3, 126.1, 124.6, 124.1, 68.5, 68.0. **UV**: (λ_{max}, 313 nm) ε_{max}, 30,500 M^{-1} cm^{-1}. **IR (**cm^{-1}) 2029, 1931, 1690, 1518, 1344, 1254, 1030. **Mass Spec:** 675.1.

Synthesis of 6-(bis(pyridin-2-ylmethyl)amino)hexanehydrazide, 1b : The ethylester precursor of 1b (1.0 g, 2.92 mmol) and a 35% hydrazine solution in water (1.406 mL,15 equiv) were refluxed in EtOH (15 mL) for 30 h. The resulting solution was concentrated by rotary evaporation, dissolved in CH_2Cl_2 and washed with water. The organic phases were combined, purified by liquid column chomatography (silica gel, 10% MeOH in CH_2Cl_2), concentrated by rotary evaporation, and dried *in vacuo* to yield the desired product **1b** (0.958 g, 64.3%). **Anal calcd** ($C_{18}H_{26}N_5O$): C, 64.60 ; H, 8.06; N, 20.36. **Found:** C, 64.32; H, 7.41; N, 20.92. **^{1}H NMR** [δ (ppm), $CDCl_3$] 8.43 (m, 2H), 7.80 (m, 2H), 7.64 (m, 2H), 7.28 (m, 2H), 3.77 (s, 4H), 2.52 (t, 2H), 2.09 (t, 2H), 1.55 (m, 4H), 1.35 (m, 2H). **^{13}C NMR** [δ (ppm), $CDCl_3$] 176.5, 157.4, 148.7, 139.6, 124.1, 120.7, 61.3, 56.4, 34.6, 28.0, 27.2, 25.6. **IR (oil, cm^{-1})** 3270, 2931, 2858, 1651, 1590. **UV**: (λ_{max}, 252 nm) ε_{max}, 36,000 M^{-1} cm^{-1}. **Mass Spec:** 328.2, 350.2.

Synthesis of 6-(bis(pyridin-2-ylmethyl)amino)-N'-(4-nitrobenzylidene)hexanehydrazide, 2b : A solution of **1b** (0.130 g, 0.397 mmol) and PNB (0.060 g, 0.397 mmol, 1.0 equiv) was made in 10 mL EtOH. The pH of the solution was adjusted to 4 by adding 0.1 M HCl and the solution was refluxed for 1 h. The reaction progress was monitored by HPLC with product formation shown by the peak at 19.4 min. The reaction mixture was purified by liquid column chomatography (silica gel, 10% MeOH in CH_2Cl_2). Eluent was concentrated by rotary evaporation and dried *in vacuo* to yield the desired product **2b** (0.139 g, 76%). **Anal calcd** ($C_{25}H_{28}N_6O_3$): C, 65.4; H, 6.1; N, 18.2. **Found:** C, 64.68; H, 5.61; N, 17.51. **^{1}H NMR** [δ (ppm), $CDCl_3$] 8.51 (m, 2H), 7.64 (m, 2H), 7.53 (m, 2H), 7.12 (m, 2H), 4.08 (q, 2H), 3.79 (s, 4H), 2.38 (t, 2H), 2.24 (t, 2H), 1.55 (m, 4H), 1.31 (m, 2H), 1.22 (t, 3H). **^{13}C NMR** [δ (ppm), $CDCl_3$] 173.9, 160.2, 149.1, 136.5, 123.0, 122.0, 60.7, 60.4, 54.4, 34.5, 27.0, 27.0, 25.0, 14.4. **IR (oil, cm^{-1})** 1660, 1583, 1508, 1336. **UV**: (λ_{max}, 252 nm) ε_{max}, 11,500 M^{-1} cm^{-1}. **Mass Spec:** 328.2, 368.2.

Synthesis of Re-1b, 3b : To a solution of **1b** (0.130g, 0.397mmol) in 3mL MeOH, $Re(H_2O)_3(CO)_3OTf$ (5.1 mL, 0.397 mmol) was added. Additional MeOH was added to redissolve any precipitate which had formed. The pH was adjusted to 6 by addition of a few drops of sat. $NaHCO_3$ and the reaction mixture was stirred at 50^{o}C for 30 min. Major peak formation at 17.4 min was observed using RP-HPLC. The reaction was dried *in vacuo* and purified using preparative RP-HPLC with MeOH/water as the solvent system. Product elutes out at 60% MeOH and is obtained as a white, hygroscopic solid **3b** (87%). **Anal calcd** ($C_{22}H_{25}F_3N_5O_7ReS$): C, 35.24 ; H, 3.76; N, 9.34. **Found:** C, 35.89; H, 3.17; N, 8.97. **^{1}H NMR** [δ (ppm), CD_3OD] 8.862 (d, 2H), 7.932 (dt, 2H), 7.389 (d, 2H), 7.359 (dt, 2H), 4.882 (dd, 4H), 3.816 (m, 2H), 2.408 (t, 2H), 1.985 (m, 2H), 1.767 (m, 2H), 1,486 (m, 2H). **^{13}C NMR** [δ (ppm), CD_3OD] 176.66, 161.00, 151.34, 140.44, 125.70, 1223.45, 70.57, 67.57, 32.79, 28.84, 25.97, 24.63. **IR (hygroscopic solid, cm^{-1})** 2029, 1913, 1675. **UV**: (λ_{max}, 311 nm) ε_{max}, 21,500 M^{-1} cm^{-1}. **Mass Spec:** 598.1, 638.2.

Synthesis of Re-2b, 4b:

***Route 1 'click, then chelate'*:** To a solution of **2b** (0.08 g, 0.191 mmol) in 5mL MeOH, $Re(CO)_5OTf$ (0.062 g, 0.191mmol) was added. The reaction mixture was refluxed at 80 $^\circ$C for two days. Major peak formation at 21.08 min was observed using RP-HPLC. The reaction was dried down and purified using preparative RP-HPLC with methanol/water as the solvent system. Product elutes out at 90% MeOH and on concentrating down is obtained as a shiny yellow solid, **4b** (57%).

***Route 2 'prelabel, then click'*: A solution of **3b** (0.046 g, 0.075 mmol) and PNB (0.013 g, 0.075 mmol) was made by dissolving in 5 mL EtOH and the pH of the solution adjusted to 4 by adding 0.1 M HCl and heated at 50°C for 4 h. The reaction progress was monitored by HPLC with product formation shown by the peak at 21.1 min. The reaction mixture was purified by RP-HPLC with MeOH/water as the solvent system. Product elutes out at 90% MeOH and on concentrating down gives a bright yellow solid, **4b (**62%). **Anal calcd** ($C_{29}H_{34}F_3N_6O_9ReS$): C, 39.45; H, 3.54; N, 9.52. **Found:** C, 40.99; H, 3.37; N, 9.99. **^{1}H NMR** [δ (ppm), CD_3OD] 8.85 (d, 2H), 8.27 (dd, 2H), 8.00 (t, 2H), 7.93 (m, 3H), 7.56 (m, 2H), 7.35 (m, 2H), 4.88 (dd, 4H), 3.83 (m, 2H), 2.85 (t, 1H), 2.44 (t, 1H), 2.00 (m, 2H), 1.87 (m, 2H), 1.55 (m, 2H). **^{13}C NMR** [δ (ppm), CD_3OD] 176.5, 160.9, 160.9, 151.9, 148.8, 141.6, 128.2, 127.5, 125.6, 123.8, 123.7, 123.4, 70.4, 67.5, 34.0, 32.0, 26.2, 24.7, 24.6, 24.3. **IR (solid, cm^{-1})** 2005, 1905, 1681. **UV**: (λ_{max}, 305 nm) ε_{max}, 47,000 M^{-1} cm^{-1}. **Mass Spec:** 731.2

Figure S2. Chromatographic trace of compounds, Re (3a and 4a) by UV (220 nm) and ^{99m}Tc (3a´ and 4a´) by radio (γ) HPLC

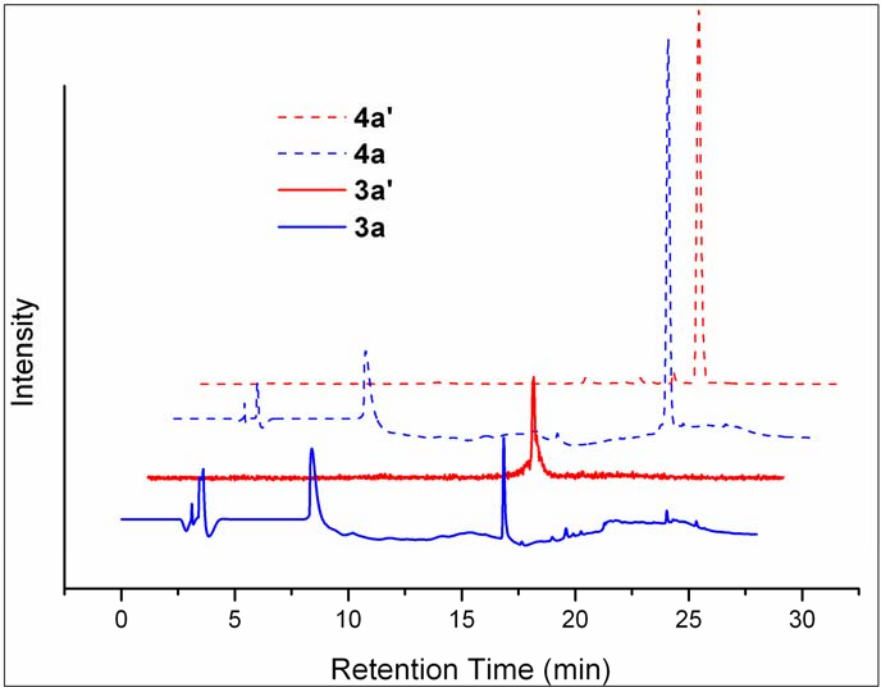
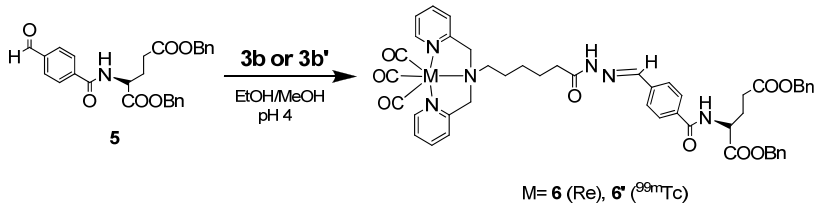


Figure S3: Peptide model system for the hydrazone click



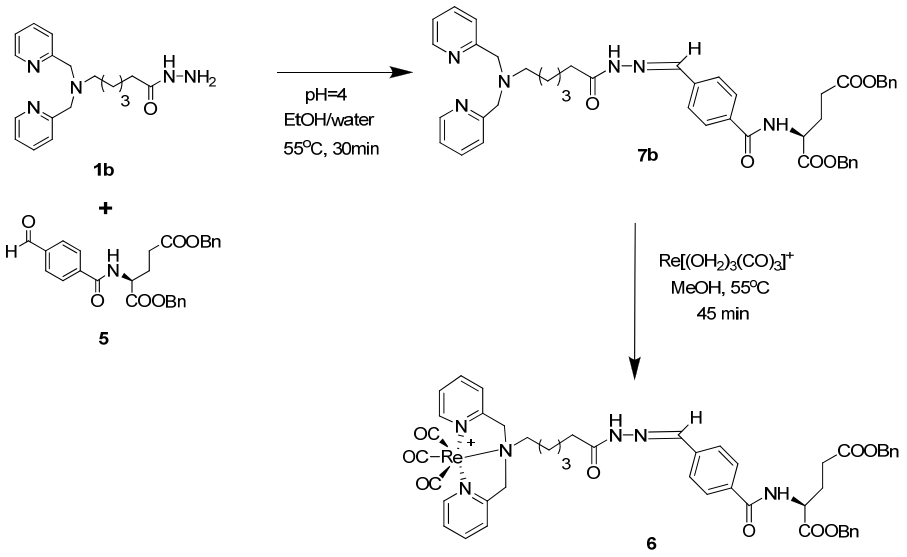
Synthesis of (R)-dibenzyl 2-(4-formylbenzamido)pentanedioate, 5: The synthesis of **5** was carried out according to a known literature procedure.[3] The product was purified using liquid column chomatography (silica gel, 30% EtOAc in CH_2Cl_2). Product was recrystallized from CH_2Cl_2/hexanes mixture as a white solid (46%). **Anal calcd** ($C_{27}H_{25}NO_6$): C, 70.58; H, 5.48; N, 3.05. **Found:** C, 70.22; H, 5.47; N, 3.02. **^{1}H NMR** [δ (ppm), $CDCl_3$] 10.07 (s, 1H), 7.93 (s, 1H), 7.32 (m, 10H), 5.20 (s, 2H), 5.08 (s, 2H), 4.82 (m, 1H), 2.52 (m, 2H), 2.34 (m, 1H), 2.16 (m, 1H). **^{13}C NMR** [δ (ppm), $CDCl_3$] 191.7, 173.4, 171.7, 166.2, 138.8, 135.6, 135.2, 130.0, 128.9, 128.8, 128.5, 128.4, 128.0, 67.0, 52.9, 30.6, 26.9. **IR (solid, cm^{-1})**3280, 1750, 1726, 1646, 1530, 1202. **UV/VIS** (λ_{max}, 237 nm) ε_{max}, 21,000 M^{-1} cm^{-1}. **Mass Spec:** 492.3, 514.5.

(3)Poulsen et al. (2006) *J. Am Soc Mass Spec. 17*, 1074-1080

Synthesis of compound 6 via "prelabel, then click" approach (Paper, *Scheme 2*): A solution of **3b** (0.04 g, 0.0652 mmol) and **5** (0.029 g, 0.0652 mmol) was made in a 8:2 ethanol/water mixture and the pH was adjusted to 4 by addition of 1M HCl. Dried molecular sieves (4Å units) were added and the reaction heated to 55 °C. Reaction progress was monitored by RP-HPLC with consumption of **3b** and **5** and formation of a major peak at 22.1 min observed in 40 min. The reaction mixture was filtered though a celite bed, concentrated and purified using preparative RP-HPLC. 0.1%TFA/ MeOH was used as mobile phase and product was eluted at 90% MeOH as a white powder (61%). **Anal calcd** ($C_{49}H_{48}F_3N_6O_{12}ReS$): C, 49.53; H, 4.07; N, 7.07. **Found:** C, 49.54; H, 4.09; N, 7.51. **^{1}H NMR** [δ (ppm), CD_3OD] 8.63 (t, 2H), 8.32 (s, 1H), 7.93 (s, 1H), 7.72 (m, 6H), 7.58 (m, 2H), 7.54 (m, 2H), 7.32 (d, 10H), 7.20 (t, 2H), 5.17 (s, 1H), 5.16 (dd, 4H), 4.77 (m, 1H), 4.49 (m, 2H), 3.70 (m, 2H), 2.80 (m, 1H), 2.48 (m, 3H), 2.29 (m, 1H), 2.17 (m, 1H), 1.95 (m, 4H), 1.48 (m, 2H). **^{13}C NMR** [δ (ppm), $CDCl_3$] 173.3, 172.0, 170.8, 167.0, 166.9, 160.8, 151.2, 151.1, 146.9, 140.6, 137.8, 135.7, 135.4, 134.6, 127.6, 127.3, 125.7, 124.5, 124.2, 71.3,

67.5, 66.8, 52.7, 30.6, 26.9, 25.6, 24.8, 23.2. **IR (oil, cm^{-1})** 2005, 1905, 1681, 1530, 1202. **UV/VIS:** (λ_{max}, 311 nm) ε_{max}, 36,900 M^{-1} cm^{-1}. **Mass Spec:** 1039.4.

Figure S4: Synthesis of compound 6 via "click, then chelate" approach:



Synthesis of (R,E)-dibenzyl 2-(4-((2-(6-(bis(pyridin-2-ylmethyl)amino)hexanoyl)hydrazono)methyl)benzamido)pentanedioate, 7b: A solution of **1b** (0.142 g, 0.435 mmol) and **5** (0.200 g, 0.435 mmol) was made in a 8:2 ethanol/water mixture. The pH was adjusted to 4 by addition of dil. HCl. Dried molecular sieves (4Å units) were added. The reaction mixture was heated to 50 °C and progress monitored by RP-HPLC. Consumption of **1b** and **5** and formation of a major peak at 22.3 min was observed in 25 min. The reaction mixture was filtered though a celite bed, concentrated and recrystallized from methanol to yield **7b** as a pale yellow solid (84%). **Anal calcd** ($C_{45}H_{48}N_6O_6.CH_3OH$): C, 68.98 ; H, 5.49; N, 10.49 **Found:** C, 68.94; H, 5.73; N, 10.68. **^{1}H NMR** [δ (ppm), $CDCl_3$] 9.16 (s, 1H), 8.50 (dd, 2H), 7.81 (d, 2H), 7.69 (m, 3H), 7.54 (m, 2H), 7.25 (m, 10H), 7.19 (d, 1H), 7.11 (dt, 2H), 5.20 (s, 2H), 5.07 (s, 2H), 3.79 (s, 4H), 2.58 (t, 2H), 2.38 (m, 4H), 2.35 (m, 4H), 2.34 (m, 1H), 1.68 (s, 2H), 1.63 (m, 4H), 1.39 (m, 2H). **^{13}C NMR** [δ (ppm), $CDCl_3$] 173.3, 172.0, 166.7, 160.2, 149.17, 137.3, 136.6, 135.7, 135.3, 134.7, 128.9, 128.8, 128.6, 128.5, 128.4, 127.8, 127.3, 123.0, 122.1, 67.7,

66.9, 60.6, 54.6, 52.7, 32.8, 30.6, 27.3, 27.2, 24.8. **IR (oil, cm^{-1})** 3274, 3066, 2933, 1746, 1720, 1628, 1540, 1261. **UV/VIS:** (λ_{max}, 311 nm) ε_{max}, 36,900 M^{-1} cm^{-1}. **Mass Spec:** 769.5, 791.5.

Synthesis of Re-7b, 6 via "click to chelate approach": A solution of **7b** (0.02 g, 0.026 mmol) was made in a MeOH/water mixture and $Re(H_2O)_3(CO)_3OTf$ (0.260 ml, 0.026 mmol) was added and heated to 55 °C for 45 min to yield a major peak at 22.1 min as monitored by RP-HPLC. The reaction mixture was dried down and purified using preparative RP-HPLC. 0.1%TFA/ MeOH was used as mobile phase and product was eluted at 90% MeOH (66%).

Thermal Stability of 4b:

0.005 g of **4b** was dissolved in 0.5 mL MeOH and 1.5 mL PBS buffer (0.1M, pH=7.4) and heated at different temperatures for different time periods. The stability was monitored by RP-HPLC. Heating for 30 min at 70, 80 and 90 °C showed no decomposition of **4b**. Heating at 90 °C overnight shows 3% decomposition.

pH stability of 4b:

0.002 g of **4b** was dissolved in 2 drops of MeOH and volume increased to 1 mL with water. The solution was then divided into two 500 µL parts and pH adjusted by dropwise addition of 1 M HCl to yield a final **pH of 0 and 2**. The solutions were stirred at room temperature and analyzed using RP-HPLC at different time points.

- At pH 0, 55 % of **4b** remains undecomposed and 36% gets hydrolyzed to the hydrazide, **3b,** in 30 min. 47% of **4b** remains after the solution was left overnight at pH 0.
- At pH 2, 61 % of **4b** remains undecomposed and 39% gets hydrolyzed to the hydrazide in 60 min. 55% of **4b** remains after the solution was left overnight at pH 2.

0.002 g of **4b** was dissolved in 2 drops of MeOH and volume increased to 1 mL with water. The solution was then divided into two 500 µL parts and pH adjusted by dropwise addition of 1M NaOH to yield a final **pH of 10 and 12** was achieved. The solutions were stirred at room temperature and analyzed using RP-HPLC at different time points.

- At pH 10, no decomposition was observed after 60 min.
- At pH 12, no decomposition was observed upto 10 h.

Radiolabeling data for 1a, 1b and formation of 6´ using ^{99m}Tc:

Preparation of $[^{99m}Tc(CO)_3(H_2O)_3]^+$ precursor: The generator obtained $^{99m}TcO_4^-$ (11.43 mCi) was added to the Isolink kit and heated to 95 °C for 20 min. It was then cooled, acidified to pH 4 with 1 M HCl and then neutralized to pH 7.4 after 10 min by addition of 1 M NaOH.

1a/1b +^{99m}Tc rxn (to produce 3a´/3b´): 100 µL of a 10^{-3} M **1a (or 1b)** solution in water and 800 µL of a 0.1 M sodium phosphate pH 7.4 buffer were combined in a sealed vial and the solution was de-gassed under nitrogen for 10 min. 100 µL of the prepared $^{99m}Tc(CO)_3(H_2O)_3^+$ solution was added and the vial heated at 50 °C for 30 min. The solution was cooled in an ice bath and aliquots were removed for reaction analysis and purification by HPLC which indicated the formation of the desired complex **3a´** (retention time: 16.8-17.1 min, radiochemical yields of 34-46%) and **3b´** (retention time: 17.9-18.7 min, radiochemical yields of 52-70%).

3a´/3b´+PNB rxn (to produce 4a´/4b´): 100 µL of the peak-purified **3a´ (or 3b´)** solution, 170 µL of a 0.1 M sodium acetate pH 4 buffer, and 30 µL of various concentrations of PNB in MeOH to yield the final aldehyde concentrations shown below (10^{-4}-10^{-6} M PNB) were combined in a sealed vial and heated for the times and temperatures shown below in *Table S1*. The solutions were cooled in an ice bath and aliquots were removed for reaction analysis by HPLC which indicated the formation of the desired complex **4a´** (retention time: 21.9 min) and **4b´** (retention time: 22.2 min) in the radiochemical yields shown in *Table S1*.

2a/2b +^{99m}Tc rxn (to produce 4a´/4b´): Varying amounts of a 10^{-3} M solution of **2a (or 2b)** in MeOH were added to sealable vials containing varying amounts of a 0.1 M sodium phosphate pH 7.4 buffer to yield a final volume of 900 µL, the vials were sealed and de-gassed under nitrogen for 10 min, and 100 µL of the prepared $^{99m}Tc(CO)_3(H_2O)_3^+$ solution was added to yield the final **2a (or 2b)** concentrations shown in *Table S1* (10^{-4}-10^{-6}). The vials were heated for the times and temperatures shown below, cooled in an ice bath, and aliquots were removed for analysis by HPLC which indicated the formation of the desired complex **4a´** (retention time: 21.8 min) and **4b´** (retention time: 21.7 min) in the radiochemical yields shown below.

One pot 1a+Tc+PNB rxn: The first procedure **1a**+Tc (or **1b**+Tc) was followed as above, except the vial was heated at 60 °C. The vial was cooled in an ice bath 2-3 min and the PNB solution was added to give the desired final PNB concentration (10^{-4} M). The vial was then heated for an additional 30 min at 60 °C, cooled in an ice bath, and an aliquot was removed for analysis by HPLC which indicated the formation of the desired complex **4a´** in the radiochemical yield shown below.

3b´+5 rxn: 100 µL of the peak-purified **3b´** solution, 170 µL of a 0.1 M sodium acetate pH 4 buffer, and 30 µL of various concentrations of **5** in MeOH to yield the final aldehyde concentrations shown below (10^{-4}-10^{-6} M PNB) were combined in a sealed vial and heated for the times and temperatures shown below in *Table S1*. The solutions were cooled in an ice bath and aliquots were removed for reaction analysis by HPLC which indicated the formation of the desired complex **4b´** (retention time: 22.4-22.5 min) in the radiochemical yields shown below.

***Table S1*: Radiolabelling data compilation for compounds 1a, 1b and 7b.**

Reaction		Concentration (M)	Temperature (°C)		
			50 *	60 *	90 *
1)	1a+Tc	10^{-4}	46/-	n/a	n/a
2)	Peak Purif. 3a'+PNB	10^{-4}	n/a	60/70	48/56
		10^{-5}	n/a	-/21.60	18/24
		10^{-6}	n/a	-/13	-/0
3)	2a+Tc	10^{-4}	90/-		79/-
		10^{-5}	21/-		66/-
		10^{-6}	3/-		15/-
4)	One Pot 1a+Tc+PNB	10^{-4}		-/24	
5)	1b+Tc	10^{-4}	52.47/-		70.51/86.40
		10^{-5}			19.05/33.24
		10^{-6}			8.27/12.39
6)	Peak Purif. 3b'+PNB	10^{-4}		82.19/90.88	
		10^{-5}		-/39.18	
		10^{-6}		-/10.57	
7)	2b+Tc	10^{-4}	94.60/-		97.35/-
		10^{-5}	53/-		90.91/-
		10^{-6}	3.40/-		23.64/-
8)	One Pot 1b+Tc+PNB	10^{-4}		-/53.43	
9)	Peak Purif. 3b'+5	10^{-4}		70/66	54/-
		10^{-5}		-/31	40/47
		10^{-6}		-/18	-/21

Legend: * = % Yield 30min/60min and Tc=^{99m}Tc

Single crystal X-ray diffraction studies.

The X-ray diffraction data of compound **4a** was collected on a Nonius KappaCCD single-crystal X-ray diffractometer at 125(2) K using MoK_α radiation (λ = 0.7107 Å). Data collection, cell refinement, and data reduction were performed using Collect[1] and HKL Scalepack/Denzo,[2] respectively. Structure solution was accomplished with the aid of SHELXS-97, whereas refinement by full-matrix least-squares based on F^2 was conducted using SHELXL-97.[3] All non-hydrogen atoms were refined anisotropically. Hydrogen atoms attached to C-atoms were fixed in geometrically constrained riding positions, and refined isotropically on the basis of the corresponding C-atoms [U_{iso}(H) = 1.2 × U_{eq}(C)]. The hydrogen atom bound to the N-atom was located in the Fourier map and isotropically refined on the basis of the corresponding N-atom [U_{iso}(H) = 1.2 × U_{eq}(N)]. The N-H distance was refined using a distance restraint (*i.e.* DFIX command in SHELX). In addition, constraints and restraints (*i.e.* EADP, DELU, SIMU commands in SHELX) were utilized to achieve a model with reasonable bond distances and thermal parameters. Crystallographic data of compound **4a** is listed in *Table S2*. Selected bond distances and angles are listed in *Table S3*.

[1] Hooft, R. W. W., Nonius BV, Delft, The Netherlands, 1998.
[2] Otwinowski, Z.; Minor, W. in *Methods in Enzymology*, ed. Carter , C. W. Jr and Sweet, R. M., 1997, Vol. 276 (*Macromolecular Crystallography*, Part A), pp. 307-326.
[3] G. M. Sheldrick, *Acta Cryst.* **2008**, *A64*, 112-122.

Figure S5: Molecular structure of 4a. Hydrogen atoms are omitted for clarity.

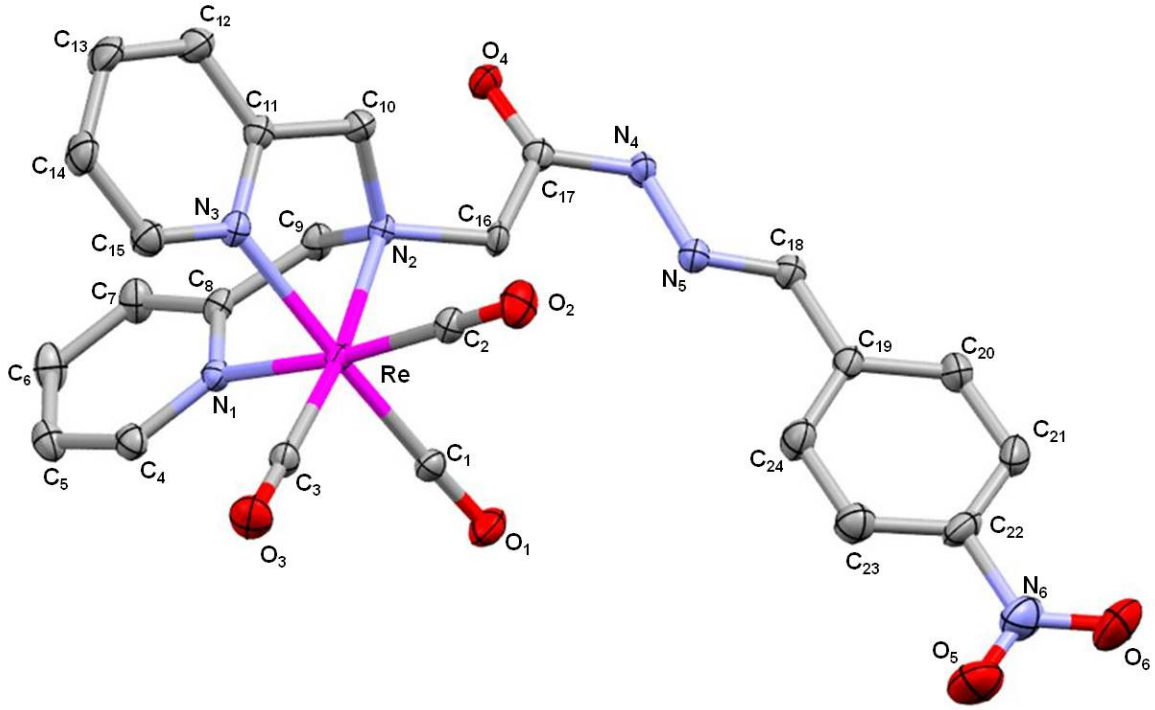


Table S2. Crystal data and structure refinement for 4a.

Identification code	4a	
Empirical formula	C34 H29 F3 N6 O9 Re S	
Formula weight	940.89	
Temperature	125(2) K	
Wavelength	0.71073 Å	
Crystal system	triclinic	
Space group	*P* -1	
Unit cell dimensions	a = 10.9711(12) Å	α= 92.749(5)°.
	b = 11.6456(13) Å	β= 109.576(5)°.
	c = 15.6740(17) Å	γ = 101.608(5)°.
Volume	1833.7(3) $Å^3$	
Z	2	
Density (calculated)	1.704 Mg/m^3	
Absorption coefficient	3.447 mm^{-1}	
F(000)	930	
Crystal size	0.22 x 0.16 x 0.16 mm^3	
Theta range for data collection	2.99 to 25.00°.	
Index ranges	-13<=h<=13, -13<=k<=13, -18<=l<=18	
Reflections collected	12482	
Independent reflections	6421 [R(int) = 0.0165]	
Completeness to theta = 25.00°	99.7 %	
Max. and min. transmission	0.6085 and 0.5176	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	6421 / 13 / 485	
Goodness-of-fit on F^2	1.046	
Final R indices [I>2sigma(I)]	R1 = 0.0230, wR2 = 0.0572	
R indices (all data)	R1 = 0.0242, wR2 = 0.0578	
Largest diff. peak and hole	0.780 and -1.390 $e.Å^{-3}$	

Table S2. Crystallographic data for compound **4a**.

	4a
molecular formula	$C_{34}H_{29}F_3N_6O_9ReS$
M_r	940.89
crystal system	triclinic
space group	$P\bar{1}$
a, Å	10.9711(12)
b, Å	11.6456(13)
c, Å	15.6740(17)
α, °	92.749(5)
β, °	109.576(5)
γ, °	101.608(5)
V, Å^3	1833.7(3)
Z	2
D_c/gcm^{-3}	1.704
F(000)	930
μ(Mo K$_\alpha$)/mm^{-1}	3.447
T/K	125(2)
crystal size/mm	0.22× 0.16 × 0.16
range of indices	-13 → 13 -13 → 13 -18 → 18
no. of reflections collected	12482
unique reflections	6421
R_{int}	0.0165
reflections with $I > 2\sigma(I)$	6199
no. parameters	485
$R(F)$, $F > 2\sigma$(F)	0.0230
$wR(F^2)$, $F > 2\sigma$(F)	0.0572
$R(F)$, all data	0.0242
$wR(F^2)$, all data	0.0578
Δ_r (max, min) eÅ^{-3}	0.093, -1.390
CCDC deposition number	829258

Table S3. **Bond lengths [Å] and angles [°] for 4a.**

C(26B)-C(27B)	1.3900	C(26B)-C(31B)	1.3900
C(26B)-H(26B)	0.9300	C(27B)-C(28B)	1.3900
C(27B)-H(27B)	0.9300	C(28B)-C(29B)	1.3900
C(28B)-H(28B)	0.9300	C(29B)-C(30B)	1.3900
C(29B)-H(29B)	0.9300	C(30B)-C(31B)	1.3900
C(30B)-H(30B)	0.9300	C(31B)-H(31B)	0.9300
C(26A)-C(27A)	1.3900	C(26A)-C(31A)	1.3900
C(26A)-H(26A)	0.9300	C(27A)-C(28A)	1.3900
C(27A)-H(27A)	0.9300	C(28A)-C(29A)	1.3900
C(28A)-H(28A)	0.9300	C(29A)-C(30A)	1.3900
C(29A)-H(29A)	0.9300	C(30A)-C(31A)	1.3900
C(30A)-H(30A)	0.9300	C(31A)-H(31A)	0.9300
Re(1)-C(2)	1.916(3)	Re(1)-C(3)	1.921(3)
Re(1)-C(1)	1.925(3)	Re(1)-N(1)	2.164(2)
Re(1)-N(3)	2.171(2)	Re(1)-N(2)	2.235(2)
N(1)-C(8)	1.345(4)	N(1)-C(4)	1.360(4)
N(3)-C(15)	1.343(4)	N(3)-C(11)	1.360(4)
N(2)-C(16)	1.491(3)	N(2)-C(9)	1.501(4)
N(2)-C(10)	1.509(4)	O(4)-C(17)	1.223(3)
O(1)-C(1)	1.150(4)	O(2)-C(2)	1.158(4)
N(4)-C(17)	1.359(4)	N(4)-N(5)	1.371(3)
N(4)-H(4N)	0.920(2)	O(3)-C(3)	1.149(4)
N(5)-C(18)	1.283(4)	C(5)-C(4)	1.370(4)
C(5)-C(6)	1.390(4)	C(5)-H(5)	0.9300
O(5)-N(7)	1.216(4)	N(7)-O(6)	1.234(4)
N(7)-C(22)	1.471(4)	C(16)-C(17)	1.523(4)
C(16)-H(16A)	0.9700	C(16)-H(16B)	0.9700
C(7)-C(6)	1.377(5)	C(7)-C(8)	1.390(4)
C(7)-H(7)	0.9300	C(12)-C(11)	1.380(4)
C(12)-C(13)	1.388(4)	C(12)-H(12)	0.9300
C(13)-C(14)	1.380(5)	C(13)-H(13)	0.9300
C(19)-C(20)	1.391(4)	C(19)-C(24)	1.401(4)
C(19)-C(18)	1.466(4)	C(10)-C(11)	1.499(4)
C(10)-H(10A)	0.9700	C(10)-H(10B)	0.9700

C(18)-H(18)	0.9300	C(4)-H(4)	0.9300
C(15)-C(14)	1.377(4)	C(15)-H(15)	0.9300
C(8)-C(9)	1.499(4)	C(24)-C(23)	1.381(4)
C(24)-H(24)	0.9300	C(14)-H(14)	0.9300
C(21)-C(20)	1.383(5)	C(21)-C(22)	1.384(5)
C(21)-H(21)	0.9300	C(23)-C(22)	1.382(5)
C(23)-H(23)	0.9300	C(20)-H(20)	0.9300
C(6)-H(6)	0.9300	C(9)-H(9A)	0.9700
C(9)-H(9B)	0.9700	C(33)-C(34)	1.378(5)
C(33)-C(32)	1.395(5)	C(33)-H(33)	0.9300
C(32)-C(34)#1	1.376(5)	C(32)-H(32)	0.9300
C(34)-C(32)#1	1.376(5)	C(34)-H(34)	0.9300
S(1)-O(7)	1.433(3)	S(1)-O(8)	1.441(3)
S(1)-O(9)	1.453(2)	S(1)-C(25)	1.827(4)
F(2)-C(25)	1.344(4)	F(1)-C(25)	1.332(4)
F(3)-C(25)	1.323(4)		

C(27B)-C(26B)-C(31B)	120.0	C(27B)-C(26B)-H(26B)	120.0
C(31B)-C(26B)-H(26B)	120.0	C(26B)-C(27B)-C(28B)	120.0
C(26B)-C(27B)-H(27B)	120.0	C(28B)-C(27B)-H(27B)	120.0
C(29B)-C(28B)-C(27B)	120.0	C(29B)-C(28B)-H(28B)	120.0
C(27B)-C(28B)-H(28B)	120.0	C(30B)-C(29B)-C(28B)	120.0
C(30B)-C(29B)-H(29B)	120.0	C(28B)-C(29B)-H(29B)	120.0
C(29B)-C(30B)-C(31B)	120.0	C(29B)-C(30B)-H(30B)	120.0
C(31B)-C(30B)-H(30B)	120.0	C(30B)-C(31B)-C(26B)	120.0
C(30B)-C(31B)-H(31B)	120.0	C(26B)-C(31B)-H(31B)	120.0
C(27A)-C(26A)-C(31A)	120.0	C(27A)-C(26A)-H(26A)	120.0
C(31A)-C(26A)-H(26A)	120.0	C(28A)-C(27A)-C(26A)	120.0
C(28A)-C(27A)-H(27A)	120.0	C(26A)-C(27A)-H(27A)	120.0
C(27A)-C(28A)-C(29A)	120.0	C(27A)-C(28A)-H(28A)	120.0
C(29A)-C(28A)-H(28A)	120.0	C(30A)-C(29A)-C(28A)	120.0
C(30A)-C(29A)-H(29A)	120.0	C(28A)-C(29A)-H(29A)	120.0
C(29A)-C(30A)-C(31A)	120.0	C(29A)-C(30A)-H(30A)	120.0
C(31A)-C(30A)-H(30A)	120.0	C(30A)-C(31A)-C(26A)	120.0
C(30A)-C(31A)-H(31A)	120.0	C(26A)-C(31A)-H(31A)	120.0
C(2)-Re(1)-C(3)	88.77(12)	C(2)-Re(1)-C(1)	87.83(12)

C(3)-Re(1)-C(1)	89.96(12)	C(2)-Re(1)-N(1)	172.89(10)
C(3)-Re(1)-N(1)	97.50(11)	C(1)-Re(1)-N(1)	95.49(10)
C(2)-Re(1)-N(3)	95.76(11)	C(3)-Re(1)-N(3)	96.13(11)
C(1)-Re(1)-N(3)	172.98(10)	N(1)-Re(1)-N(3)	80.30(9)
C(2)-Re(1)-N(2)	94.81(11)	C(3)-Re(1)-N(2)	172.53(10)
C(1)-Re(1)-N(2)	96.71(10)	N(1)-Re(1)-N(2)	78.58(9)
N(3)-Re(1)-N(2)	77.01(9)	C(8)-N(1)-C(4)	118.6(2)
C(8)-N(1)-Re(1)	116.20(19)	C(4)-N(1)-Re(1)	124.72(19)
C(15)-N(3)-C(11)	118.4(2)	C(15)-N(3)-Re(1)	125.7(2)
C(11)-N(3)-Re(1)	115.73(19)	C(16)-N(2)-C(9)	109.3(2)
C(16)-N(2)-C(10)	110.8(2)	C(9)-N(2)-C(10)	111.4(2)
C(16)-N(2)-Re(1)	109.00(16)	C(9)-N(2)-Re(1)	109.50(17)
C(10)-N(2)-Re(1)	106.75(17)	C(17)-N(4)-N(5)	118.4(2)
C(17)-N(4)-H(4N)	120(2)	N(5)-N(4)-H(4N)	121(2)
C(18)-N(5)-N(4)	117.4(2)	C(4)-C(5)-C(6)	118.9(3)
C(4)-C(5)-H(5)	120.5	C(6)-C(5)-H(5)	120.5
O(5)-N(7)-O(6)	123.2(3)	O(5)-N(7)-C(22)	119.0(3)
O(6)-N(7)-C(22)	117.8(3)	O(1)-C(1)-Re(1)	178.0(3)
N(2)-C(16)-C(17)	115.1(2)	N(2)-C(16)-H(16A)	108.5
C(17)-C(16)-H(16A)	108.5	N(2)-C(16)-H(16B)	108.5
C(17)-C(16)-H(16B)	108.5	H(16A)-C(16)-H(16B)	107.5
C(6)-C(7)-C(8)	119.9(3)	C(6)-C(7)-H(7)	120.0
C(8)-C(7)-H(7)	120.0	C(11)-C(12)-C(13)	119.8(3)
C(11)-C(12)-H(12)	120.1	C(13)-C(12)-H(12)	120.1
C(14)-C(13)-C(12)	118.6(3)	C(14)-C(13)-H(13)	120.7
C(12)-C(13)-H(13)	120.7	C(20)-C(19)-C(24)	119.0(3)
C(20)-C(19)-C(18)	119.6(3)	C(24)-C(19)-C(18)	121.3(3)
C(11)-C(10)-N(2)	111.0(2)	C(11)-C(10)-H(10A)	109.4
N(2)-C(10)-H(10A)	109.4	C(11)-C(10)-H(10B)	109.4
N(2)-C(10)-H(10B)	109.4	H(10A)-C(10)-H(10B)	108.0
N(3)-C(11)-C(12)	121.3(3)	N(3)-C(11)-C(10)	116.4(2)
C(12)-C(11)-C(10)	122.3(3)	N(5)-C(18)-C(19)	118.9(3)
N(5)-C(18)-H(18)	120.6	C(19)-C(18)-H(18)	120.6
N(1)-C(4)-C(5)	122.5(3)	N(1)-C(4)-H(4)	118.8
C(5)-C(4)-H(4)	118.8	N(3)-C(15)-C(14)	122.7(3)
N(3)-C(15)-H(15)	118.6	C(14)-C(15)-H(15)	118.6

O(2)-C(2)-Re(1)	178.0(3)	O(4)-C(17)-N(4)	122.5(3)
O(4)-C(17)-C(16)	124.3(3)	N(4)-C(17)-C(16)	113.2(2)
N(1)-C(8)-C(7)	121.2(3)	N(1)-C(8)-C(9)	117.6(2)
C(7)-C(8)-C(9)	121.0(3)	O(3)-C(3)-Re(1)	177.7(3)
C(23)-C(24)-C(19)	120.8(3)	C(23)-C(24)-H(24)	119.6
C(19)-C(24)-H(24)	119.6	C(15)-C(14)-C(13)	119.1(3)
C(15)-C(14)-H(14)	120.4	C(13)-C(14)-H(14)	120.4
C(20)-C(21)-C(22)	117.9(3)	C(20)-C(21)-H(21)	121.1
C(22)-C(21)-H(21)	121.1	C(24)-C(23)-C(22)	118.2(3)
C(24)-C(23)-H(23)	120.9	C(22)-C(23)-H(23)	120.9
C(21)-C(20)-C(19)	121.2(3)	C(21)-C(20)-H(20)	119.4
C(19)-C(20)-H(20)	119.4	C(23)-C(22)-C(21)	123.0(3)
C(23)-C(22)-N(7)	117.7(3)	C(21)-C(22)-N(7)	119.3(3)
C(7)-C(6)-C(5)	118.8(3)	C(7)-C(6)-H(6)	120.6
C(5)-C(6)-H(6)	120.6	C(8)-C(9)-N(2)	114.0(2)
C(8)-C(9)-H(9A)	108.7	N(2)-C(9)-H(9A)	108.7
C(8)-C(9)-H(9B)	108.7	N(2)-C(9)-H(9B)	108.7
H(9A)-C(9)-H(9B)	107.6	C(34)-C(33)-C(32)	120.3(3)
C(34)-C(33)-H(33)	119.9	C(32)-C(33)-H(33)	119.9
C(34)#1-C(32)-C(33)	119.1(3)	C(34)#1-C(32)-H(32)	120.5
C(33)-C(32)-H(32)	120.5	C(32)#1-C(34)-C(33)	120.6(3)
C(32)#1-C(34)-H(34)	119.7	C(33)-C(34)-H(34)	119.7
O(7)-S(1)-O(8)	116.55(17)	O(7)-S(1)-O(9)	115.39(18)
O(8)-S(1)-O(9)	113.35(16)	O(7)-S(1)-C(25)	103.46(19)
O(8)-S(1)-C(25)	102.92(18)	O(9)-S(1)-C(25)	102.58(15)
F(3)-C(25)-F(1)	108.0(3)	F(3)-C(25)-F(2)	106.7(3)
F(1)-C(25)-F(2)	106.7(3)	F(3)-C(25)-S(1)	112.5(3)
F(1)-C(25)-S(1)	111.7(3)	F(2)-C(25)-S(1)	110.8(2)

Symmetry transformations used to generate equivalent atoms:

#1 -x,-y+1,-z