Supporting Information

Clickable, hydrophilic ligand for *fac*-$[M^{I}(CO)_3]^+$ (M = Re/^{99m}Tc) applied in an *S*-functionalized α-MSH peptide

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Contents: Radiolabeling yields for **4a**. *In vitro* stability of **4a**, **9a´**, and **10a´**. Log P analysis of **2a**, **4a**, **9a´** and **10a´**. RP-HPLC chromatograms of all peptides. X-ray crystallography experimental procedures and tables with crystal data, structure refinement, bond lengths, bond angles, and hydrogen bonds for **4**. Additional crystallographic material is available as a cif file free of charge via the Internet at http://pubs.acs.org.

Table S1. Radiolabeling yields of **4a** based on starting concentration of **3**. Reactions were heated at 70 °C in 10 mM pH 7.2 PBS and then allowed to cool briefly before aliquots were removed for analysis by radio-HPLC using HPLC method 1.

Concentration of 4	Time	
	30 min	60 min
1×10^{-4} M	>99%	>99%
1×10^{-5} M	40%	63%
1×10^{-6} M	2%	19%

Table S2. Stability analysis of RP-HPLC purified **4a** in the presence of 1 mM histidine or cysteine in 10 mM pH 7.4 PBS or in mouse serum following incubation at 37 °C. Aliquots of the solutions were removed at the time points shown and analyzed by radio-HPLC using HPLC method 1 or HPLC method 6 (see Experimental Procedures for details).

	2 h	4 h	24 h
Histidine	>99%	>99%	>99%
Cysteine	>99%	>98%	>98%
Serum	>99%	>99%	>99%

Table S3. Stability analysis of RP-HPLC purified **9a´** and **10a´** in the presence of 1 mM histidine or cysteine in 10 mM pH 7.4 PBS following incubation at 37 °C. Aliquots of the solutions were removed at the time points shown and analyzed by radio-HPLC using HPLC method 6.

Peptide	Amino Acid	Time		
		2 h	4 h	18 h
9a´	**Histidine**	>99%	>99%	>99%
	Cysteine	>99%	>99%	84%
10a´	**Histidine**	>99%	>99%	>99%
	Cysteine	>99%	>99%	>99%

Table S4. Stability analysis of RP-HPLC purified **9a´** and **10a´** in mouse serum following incubation at 37 °C. Aliquots of the solutions were removed at the time points shown, filtered through a 10 kDa centrifugal filter, and the filtrates were analyzed by radio-HPLC using HPLC method 2.

Peptide	Time	
	1 h	6 h
9a´	97%	91%
10a´	96%	93%

Table S5. Log P analysis of **2a**, **4a**, **9a´** and **10a´**. HPLC-purified solutions of **2a**, **4a**, **9a´**, or **10a´** (0.1 μCi in 2-15 μL, MeOH removed under a stream of N_2 following isolation) were added to triplicate sets of tubes after an equal volume of the aqueous phase was removed, and the tubes were vortexed (1 min) and centrifuged (2,000 × g, 5 min). Aliquots (50 μL) from each layer were removed and counted to determine the ratio of radioactivity present in the octanol layer compared to the aqueous layer.

Complex	Activity in octanol (cpm)	Activity in PBS (cpm)	Calculated Log P (octanol/PBS)
2a	8717	14910	-0.233110597
	8847	15252	-0.236530769
	8985	15103	-0.225545141
4a	327	24595	-1.876299074
	348	24547	-1.848419179
	350	23664	-1.830020112
9a´	1833	8367	-0.659407304
	2137	9829	-0.662704813
	2122	11283	-0.725679209
10a´	52	5586	-2.031097588
	63	6749	-2.029898879
	129	13352	-2.014956613

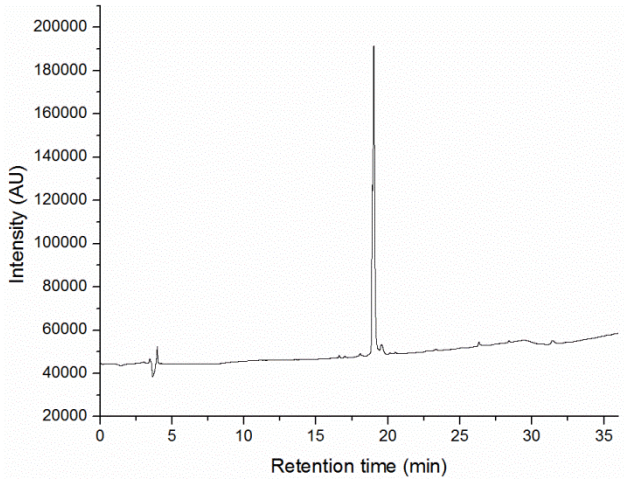

Figure S1. RP-HPLC chromatogram of peptide **5** (UV absorbance, 238 nm) with t_R 19.0 min using HPLC method 3.

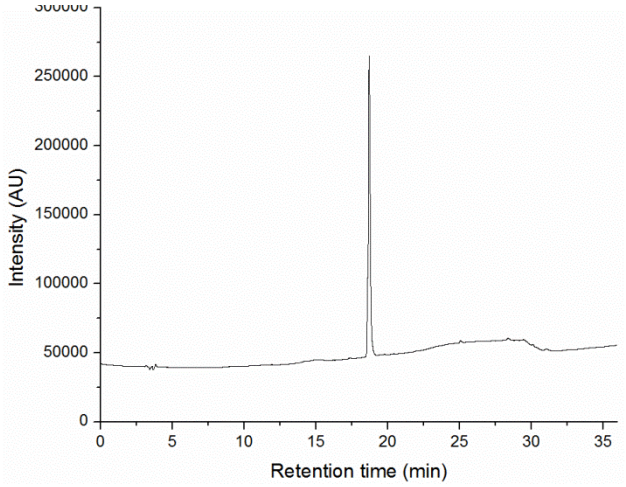

Figure S2. RP-HPLC chromatogram of peptide **6** (UV absorbance, 238 nm) with t_R 18.7 min using HPLC method 3.

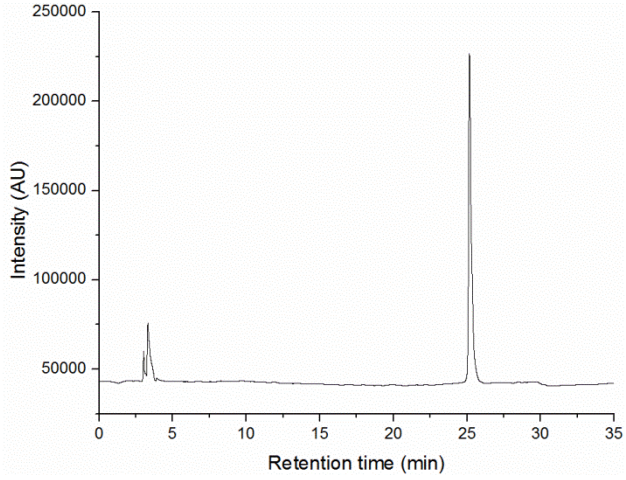

Figure S3. RP-HPLC chromatogram of peptide **7** (UV absorbance, 238 nm) with t_R 25.2 min using HPLC method 4.

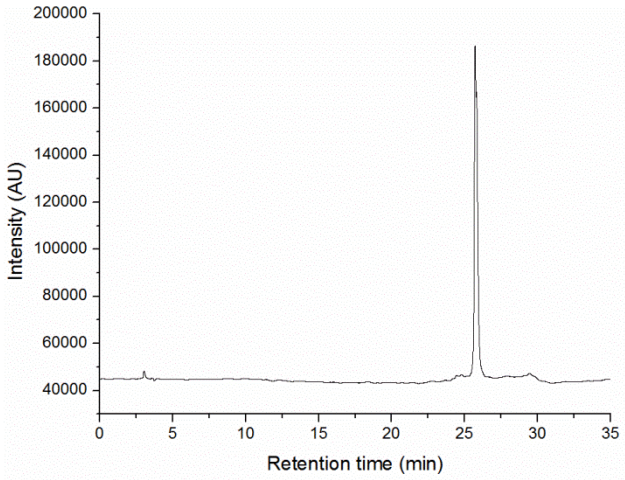

Figure S4. RP-HPLC chromatogram of peptide **8** (UV absorbance, 238 nm) with t_R 25.7 min using HPLC method 4.

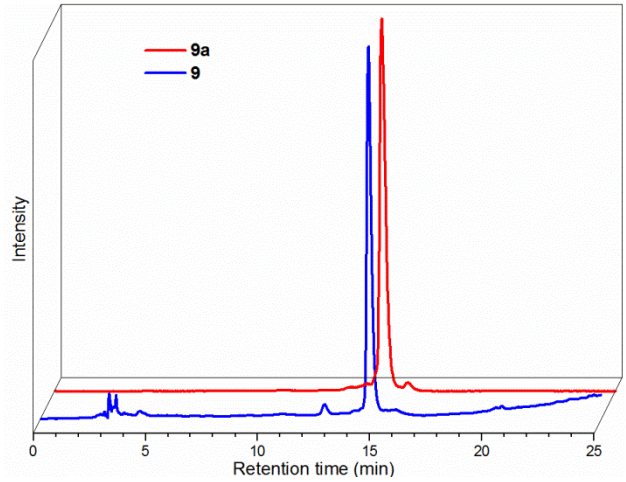

Figure S5. Normalized and offset RP-HPLC chromatograms of peptides complexed with *fac*-$[M^I(CO)_3]^+$ (M = Re, ^{99m}Tc) produced by the *click, then chelate* route: **9** (UV absorbance, 254 nm; lower blue trace) at t_R 14.6 min and **9a** (radiodetector, counts per minute (cpm); higher red trace) at t_R 14.6 min using HPLC method 6.

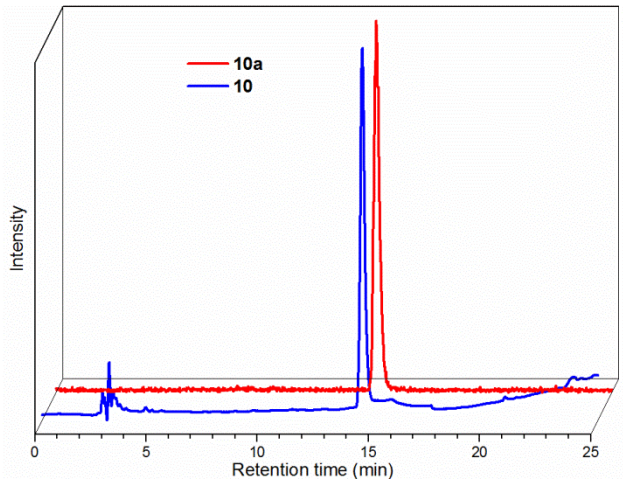

Figure S6. Normalized and offset RP-HPLC chromatograms of peptides complexed with *fac*-$[M^{I}(CO)_3]^+$ (M = Re, ^{99m}Tc) produced by the *click, then chelate* route: **10** (UV absorbance, 254 nm; lower blue trace) at t_R 14.4 min and **10a** (radiodetector, counts per minute (cpm); higher red trace) at t_R 14.4 min using HPLC method 6.

X-ray crystallography experimental procedure for 4. Intensity data on a crystal of **4** were obtained at -100 °C on a Bruker APEX II CCD Area Detector system using the ω scan technique with Mo Kα radiation from a graphite monochromator. Intensities were corrected for Lorentz and polarization effects. Equivalent reflections were merged, and absorption corrections were made using the multi-scan method. Space group, lattice parameters and other relevant information are given in the supplementary material as a cif file (CCDC deposition number: 975046). The structure was solved by direct methods followed by full-matrix least-squares refinement using the SHELX package[1] with the aid of the program X-Seed.[2] All non-hydrogen atoms were refined with anisotropic thermal parameters. The hydrogen atoms were placed at calculated positions and included in the refinement using a riding model, with fixed isotropic *U*. Disordered solvent believed to be ethyl acetate could not be reasonably modeled. Squeeze was used to remove the effects of this disorder from the structure factors and found an estimated electron count of 105, in reasonable agreement with one half ethyl acetate per molecule, this being added to the cell contents. The final difference map contained no features of chemical significance.

Table S6. Crystal data and structure refinement for **4**.

Identification code	**4**	
Empirical formula	$C_{22}H_{18}N_3O_8Re$	
Formula weight	638.59	
Temperature	173(2) K	
Wavelength	0.71073 Å	
Crystal system, space group	Monoclinic, $P2_1/c$	
Unit cell dimensions	a = 8.3712(11) Å	α = 90°
	b = 14.8036(19) Å	β = 90.475(2) °
	c = 18.193(2) Å	γ = 90°
Volume	2254.5(5) $Å^3$	
Z, Calculated density	4, 1.881 Mg/m^3	
Absorption coefficient	5.444 mm^{-1}	
F(000)	1240	
Crystal size	0.55 x 0.15 x 0.15 mm	
Theta range for data collection	1.77 to 27.53 deg.	
Limiting indices	-10<=h<=10, -19<=k<=19, -23<=l<=23	
Reflections collected / unique	25000 / 5110 [R(int) = 0.0279]	
Completeness to theta = 27.53	98.5%	
Absorption correction	Semi-empirical from equivalents	
Max. and min. transmission	0.50 and 0.29	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	5110 / 0 / 281	
Goodness-of-fit on F^2	1.088	
Final R indices [I>2σ(I)]	R1 = 0.0194, wR2 = 0.0467	
R indices (all data)	R1 = 0.0244, wR2 = 0.0480	
Largest diff. peak and hole	0.944 and -0.682 e. $Å^{-3}$	

Table S7. Bond lengths [Å] and angles [°] for **4**.

Re(1)-C(18)	1.918(3)	C(12)-H(12)	0.95
Re(1)-C(20)	1.925(3)	C(15)-C(16)	1.466(5)
Re(1)-C(19)	1.939(3)	C(15)-H(15A)	0.99
Re(1)-N(3)	2.163(2)	C(15)-H(15B)	0.99
Re(1)-N(1)	2.176(2)	C(16)-C(17)	1.163(5)
Re(1)-N(2)	2.227(2)	C(17)-H(17)	0.95
O(1)-C(13)	1.219(3)		
O(2)-C(13)	1.295(3)	C(18)-Re(1)-C(20)	89.90(12)
O(2)-H(2)	0.84	C(18)-Re(1)-C(19)	89.18(12)
O(3)-C(14)	1.220(3)	C(20)-Re(1)-C(19)	91.16(11)
O(4)-C(14)	1.281(3)	C(18)-Re(1)-N(3)	95.79(10)
O(5)-C(18)	1.151(3)	C(20)-Re(1)-N(3)	172.94(10)
O(6)-C(19)	1.147(3)	C(19)-Re(1)-N(3)	93.11(10)
O(7)-C(20)	1.155(3)	C(18)-Re(1)-N(1)	95.61(10)
N(1)-C(5)	1.347(4)	C(20)-Re(1)-N(1)	94.00(10)
N(1)-C(1)	1.348(3)	C(19)-Re(1)-N(1)	172.97(9)
N(2)-C(6)	1.492(3)	N(3)-Re(1)-N(1)	81.30(8)
N(2)-C(7)	1.496(3)	C(18)-Re(1)-N(2)	171.10(10)
N(2)-C(15)	1.514(3)	C(20)-Re(1)-N(2)	95.63(11)
N(3)-C(12)	1.352(3)	C(19)-Re(1)-N(2)	97.66(10)
N(3)-C(8)	1.354(3)	N(3)-Re(1)-N(2)	78.23(8)
C(1)-C(2)	1.376(4)	N(1)-Re(1)-N(2)	77.09(8)
C(1)-H(1)	0.95	C(13)-O(2)-H(2)	109.5
C(2)-C(3)	1.379(4)	C(5)-N(1)-C(1)	118.6(2)
C(2)-C(13)	1.513(4)	C(5)-N(1)-Re(1)	115.33(17)
C(3)-C(4)	1.376(5)	C(1)-N(1)-Re(1)	125.84(17)
C(3)-H(3)	0.95	C(6)-N(2)-C(7)	110.8(2)
C(4)-C(5)	1.381(4)	C(6)-N(2)-C(15)	108.6(2)
C(4)-H(4)	0.95	C(7)-N(2)-C(15)	107.8(2)
C(5)-C(6)	1.500(4)	C(6)-N(2)-Re(1)	106.05(16)
C(6)-H(6A)	0.99	C(7)-N(2)-Re(1)	110.90(15)
C(6)-H(6B)	0.99	C(15)-N(2)-Re(1)	112.74(17)
C(7)-C(8)	1.503(4)	C(12)-N(3)-C(8)	118.3(2)
C(7)-H(7A)	0.99	C(12)-N(3)-Re(1)	124.72(17)
C(7)-H(7B)	0.99	C(8)-N(3)-Re(1)	117.02(17)
C(8)-C(9)	1.389(4)	N(1)-C(1)-C(2)	122.5(2)
C(9)-C(10)	1.371(3)	N(1)-C(1)-H(1)	118.7
C(9)-H(9)	0.95	C(2)-C(1)-H(1)	118.7
C(10)-C(11)	1.391(4)	C(1)-C(2)-C(3)	118.3(3)
C(10)-H(10)	0.95	C(1)-C(2)-C(13)	119.0(2)
C(11)-C(12)	1.383(4)	C(3)-C(2)-C(13)	122.7(3)
C(11)-C(14)	1.516(4)	C(4)-C(3)-C(2)	119.7(3)

C(4)-C(3)-H(3)	120.1	C(9)-C(10)-C(11)	119.4(2)
C(2)-C(3)-H(3)	120.1	C(9)-C(10)-H(10)	120.3
C(3)-C(4)-C(5)	119.2(3)	C(11)-C(10)-H(10)	120.3
C(3)-C(4)-H(4)	120.4	C(12)-C(11)-C(10)	118.3(2)
C(5)-C(4)-H(4)	120.4	C(12)-C(11)-C(14)	120.3(2)
N(1)-C(5)-C(4)	121.5(3)	C(10)-C(11)-C(14)	121.1(2)
N(1)-C(5)-C(6)	115.6(2)	N(3)-C(12)-C(11)	122.8(2)
C(4)-C(5)-C(6)	122.8(3)	N(3)-C(12)-H(12)	118.6
N(2)-C(6)-C(5)	110.9(2)	C(11)-C(12)-H(12)	118.6
N(2)-C(6)-H(6A)	109.5	O(1)-C(13)-O(2)	126.7(3)
C(5)-C(6)-H(6A)	109.5	O(1)-C(13)-C(2)	120.9(2)
N(2)-C(6)-H(6B)	109.5	O(2)-C(13)-C(2)	112.4(2)
C(5)-C(6)-H(6B)	109.5	O(3)-C(14)-O(4)	126.6(3)
H(6A)-C(6)-H(6B)	108	O(3)-C(14)-C(11)	119.2(3)
N(2)-C(7)-C(8)	114.3(2)	O(4)-C(14)-C(11)	114.2(2)
N(2)-C(7)-H(7A)	108.7	C(16)-C(15)-N(2)	113.1(2)
C(8)-C(7)-H(7A)	108.7	C(16)-C(15)-H(15A)	109
N(2)-C(7)-H(7B)	108.7	N(2)-C(15)-H(15A)	109
C(8)-C(7)-H(7B)	108.7	C(16)-C(15)-H(15B)	109
H(7A)-C(7)-H(7B)	107.6	N(2)-C(15)-H(15B)	109
N(3)-C(8)-C(9)	121.5(2)	H(15A)-C(15)-H(15B)	107.8
N(3)-C(8)-C(7)	117.6(2)	C(17)-C(16)-C(15)	177.5(4)
C(9)-C(8)-C(7)	120.8(2)	C(16)-C(17)-H(17)	180
C(10)-C(9)-C(8)	119.7(2)	O(5)-C(18)-Re(1)	177.6(3)
C(10)-C(9)-H(9)	120.1	O(6)-C(19)-Re(1)	177.3(3)
C(8)-C(9)-H(9)	120.1	O(7)-C(20)-Re(1)	178.7(3)

Table S8. Hydrogen bonds for **4** [Å and °].

D-H...A	d(D-H)	d(H...A)	d(D...A)	<(DHA)
O(2)-H(2)...O(4)#1	0.84	1.64	2.466(3)	166.1

Symmetry transformations used to generate equivalent atoms: #1 x,-y+3/2,z+1/2

References

1. Sheldrick, G. M. (2008) A short history of SHELX. *Acta Cryst*. A64, 112-122.
2. Barbour, L. J. (2001) X-Seed - A software tool for supramolecular crystallography. *J. Supramol. Chem. 1*, 189-191.