Electronic Supplementary Information

Investigation of Zr(IV) and ⁸⁹Zr(IV) complexation with hydroxamates: Progress towards designing a better chelator than desferrioxamine B for immuno-PET imaging

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Preparation of the cold complexes

Acetohydroxamic acid (AHA) and all other reagents and solvents were obtained commercially and used without further purification unless otherwise noted. *N*-methyl acetohydroxamic acid (Me-AHA) was prepared according to the literature procedure.¹ An additional purification over silica gel using ethyl acetate/methanol (99/1) was necessary to obtain Me-AHA with satisfactory purity before use for complexation. ¹H NMR and ¹³C NMR spectra were recorded on a Bruker Advance 300 MHz instrument, and chemical shifts are reported in ppm on the δ scale relative to TMS or residual solvent (except in D₂O spectra where ACN was added as reference). Electrospray ionization-mass spectra (ESI-MS) were acquired using an Agilent LC / MSD system equipped with a multimode ion. Elemental analyses were performed by Galbraith Lab. Inc. (Knoxville, TN) using combustion analysis method for C, H, and N and inductively coupled plasma-atomic emission spectroscopy (ICP-OES) method for Zr.

Zr(AHA)₄:



To a solution of zirconium (IV) acetylacetonate (525 mg, 1.08 mmol) in 40 mL dry MeOH under nitrogen, was added a solution of acetohydroxamic acid (363 mg, 4.86 mmol) in 5 mL dry MeOH. The reaction mixture was refluxed under nitrogen for 15 h. After cooling to room temperature, the white precipitate was filtered, washed with MeOH and dried *in vacuo* to afford a white powder (350 mg, 85 %), mp > 300 °C.

¹H NMR (D₂O, 300 MHz, ppm): δ 4.80 (s, 3H). ¹³C NMR (D₂O, 75 MHz, ppm): δ 15.38, 166.95. ESI-MS: m/z = 312.0 [M - AHA]⁺; 344.0 [M + MeOH – AHA]⁺. Elemental analyses: Calculated for C₈H₁₆N₄O₈Zr : C, 24.80; H, 4.16; N, 14.46; Zr, 23.54 %. Found: C, 24.98; H, 4.39; N, 13.73; Zr, 23.67 %.

Our attempts to obtain suitable crystals for X-ray diffraction with this complex were unsuccessful.

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Zr(Me-AHA)₄:



To a solution of zirconium (IV) acetylacetonate (239 mg, 0.49 mmol) in 20 mL dry MeOH under nitrogen, was added a solution of *N*-methylacetohydroxamic acid (179 mg, 2.01 mmol) dissolved in 10 mL dry MeOH. The reaction mixture was refluxed under nitrogen for 15 h. After cooling to room temperature, the solution was evaporated *in vacuo*, the residue was dissolved in chloroform and extracted twice with water. The aqueous layers were combined and evaporated *in vacuo* to afford a white solid (208 mg, 96 %), mp > 300 °C.

¹H NMR (CDCl₃, 300 MHz, ppm): δ 2.05 (s, 3H), 3.22 (s, 3H). ¹³C NMR (CDCl₃, 75 MHz, ppm): δ 17.46, 39.28, 161.72. ESI-MS: m/z = 354.0 [M – Me-AHA]⁺; 436.0 [M + 2MeOH + H₂O –Me- AHA]⁺. Elemental analyses: Calculated for C₈H₁₆N₄O₈Zr : C, 32.42; H, 5.45; N, 12.63; Zr, 20.57 %. Found: C, 31.05; H, 5.35; N, 11.32; Zr, 22.46 %.

Slow evaporation from a mixture of dichloromethane/nitrobenzene (5:1) over several days afforded crystals suitable for X-ray diffraction.

Crystallographic study of Zr(Me-AHA)₄

Crystallographic data was collected on a 0.572 x 0.258 x 0.097 mm³ crystal. The crystal was monoclinic in space group *Cc*, with unit cell dimensions a = 17.8295(12), b = 14.3111(14), c = 13.2223(8) Å, and β = 129.061(2)°. Data was 97.6% complete to 29.16° θ (~ 0.73 Å) with an average redundancy of 3.86. The structure was solved by direct methods and refined by full-matrix least squares on F² values using the programs found in the SHELXTL suite (Bruker, SHELXTL v6.10, 2000, Bruker AXS Inc., Madison, WI). Corrections were applied for Lorentz, polarization, and absorption effects. Parameters refined included atomic coordinates and anisotropic thermal parameters for all non-hydrogen atoms. Hydrogen atoms on carbons were included using a riding model [coordinate shifts of C applied to H atoms] with C-H distance set at 0.96 Å.

Computational study

The quantum chemical study with Gaussian 09^2 utilized the polarizable continuum model to incorporate the solvent effect of water or CHCl₃.





Figure S1. Geometry-optimized $Zr(Me-AHA)_4$ at the level of B3LYP/DGDZVP in the solvent reaction field of water. Quantum chemical calculation was done on the X-ray structure described in the manuscript. Atoms are colored as follows: white, hydrogen; green, carbon; blue, nitrogen; red, oxygen; gray, zirconium. Coordinates available in Table S1

Figure S2. Geometry-optimized $Zr(Me-AHA)_4$ at the level of B3LYP/DGDZVP in the solvent reaction field of water. Quantum chemical calculation was done after the replacement of Hf by Zr and NBPHA by Me-AHA in the X-ray structure of Hf(NBPHA)₄; see reference 9 in the main paper. Atoms are colored as follows: white, hydrogen; green, carbon; blue, nitrogen; red, oxygen; gray, zirconium. Coordinates available in Table S2.

Table S1. Coordinates of the geometry optimized Zr(Me-AHA)4 (Figure S1) at the B3LYP/DGDZVP level in the reaction field of water; Gibbs Free Energy at 298.15 K = -4833.243588 hartrees.

Atom	Coordinates (Angstroms)		
	Х	Y	Z
_			
Zr	0.000001	0.054566	0.000000
С	-2.477822	-2.408858	-2.906464
Н	-2.036759	-1.977318	-3.805145
Н	-3.555809	-2.224265	-2.920564
Н	-2.316516	-3.490582	-2.920159
0	-0.915947	-0.877452	-1.864641
Ν	-2.160069	-2.102090	-0.471866
С	-1.822876	-1.766945	-1.707921
0	-1.496657	-1.465514	0.545907
С	-3.144378	-3.078906	-0.034900

Н	-2.647307	-3.849963	0.560767
н	-3.627559	-3.540902	-0.894627
н	-3.898312	-2.579945	0.580589
С	3.144368	-3.078918	0.034898
Н	2.647292	-3.849973	-0.560769
Н	3.627547	-3.540917	0.894624
Н	3.898303	-2.579962	-0.580592
0	1.496654	-1.465518	-0.545908
Ν	2.160064	-2.102096	0.471865
С	1.822873	-1.766951	1.707920
0	0.915948	-0.877454	1.864640
С	2.477817	-2.408867	2.906463
Н	2.036759	-1.977321	3.805144
Н	3.555805	-2.224284	2.920559
Н	2.316500	-3.490589	2.920160
С	3.646217	2.817081	-0.192631
Н	3.488110	3.866087	-0.459173
Н	4.499021	2.444471	-0.767208
Н	3.886326	2.757462	0.869071
0	1.825274	1.380029	0.520876
С	2.407834	1.990541	-0.440828
Ν	1.884840	1.874381	-1.651847
0	0.750062	1.112889	-1.771459
С	2.346882	2.460683	-2.899399
Н	1.560123	3.099294	-3.311130
Н	2.571333	1.661930	-3.612065
Н	3.243167	3.055318	-2.728797
0	-0.750058	1.112889	1.771459
С	-2.346876	2.460684	2.899401
Н	-3.243157	3.055324	2.728799
Н	-1.560114	3.099289	3.311134
Н	-2.571333	1.661930	3.612065
Ν	-1.884836	1.874382	1.651849
С	-2.407830	1.990543	0.440829
0	-1.825272	1.380031	-0.520874
С	-3.646213	2.817083	0.192635
Н	-3.488106	3.866089	0.459175
Н	-4.499017	2.444474	0.767212
Н	-3.886323	2.757464	-0.869068

Table S2. Coordinates of the geometry optimized Zr(Me-AHA)4 (Figure S2) at the B3LYP/DGDZVP level in the reaction field of water; Gibbs Free Energy at 298.15 K = -4833.242494 hartrees.

Atom	Coordinates (Angstroms)		
	Х	Y	Z
Zr	-0.000020	-0.000048	-0.000080
0	-1.867248	0.391858	-1.320890
С	-2.481022	-0.641813	-1.750949
0	-0.714854	-1.847917	-0.895803
Ν	-1.934612	-1.830847	-1.529585
0	1.867361	-1.320596	-0.392723
С	2.480898	-1.751363	0.640774
0	0.714650	-0.896786	1.847253
Ν	1.934362	-1.530679	1.829893
0	-1.867342	-0.391635	1.320698
С	-2.480907	0.642134	1.750812
0	-0.714531	1.847923	0.895655
Ν	-1.934275	1.831078	1.529474
0	1.867603	1.320135	0.392612
С	2.481161	1.750960	-0.640850
0	0.714848	0.896597	-1.847379
Ν	1.934600	1.530412	-1.829984
С	2.415637	1.936313	-3.140187
С	3.787974	2.494440	-0.507401
С	3.787726	-2.494828	0.507395
С	2.415639	-1.936086	3.140158
С	-3.788031	-0.508700	-2.494129
С	-2.416377	-3.141355	-1.933620
С	-3.787717	0.509214	2.494378
С	-2.415269	3.141585	1.934439
Н	3.716872	3.507371	-0.913835
Н	4.597733	1.974323	-1.027310
Н	4.035353	2.556791	0.552451
Н	2.522874	1.051183	-3.773484
Н	3.379769	2.434954	-3.051599
Н	1.693364	2.619790	-3.596402
Н	3.717428	-3.506987	0.915851
Н	4.597833	-1.973395	1.025469
Н	4.034055	-2.559091	-0.552587
Н	2.522588	-1.050739	3.773198
Н	3.379978	-2.434336	3.051619
Н	1.693676	-2.619721	3.596634
Н	-2.523589	-3.773447	-1.047623

Н	-1.694563	-3.598797	-2.616760
Н	-3.380674	-3.052962	-2.431978
Н	-3.718514	-0.918879	-3.505636
Н	-4.033629	0.551353	-2.560007
Н	-4.598272	-1.025323	-1.971448
Н	-4.597699	1.027561	1.973031
Н	-3.717167	0.917662	3.506529
Н	-4.034399	-0.550683	2.558695
Н	-2.521865	3.774395	1.048889
Н	-1.693323	3.598061	2.618094
Н	-3.379733	3.053446	2.432518



Z (0.0 kcal/mol)

E (0.4 kcal/mol)

Figure S3. Geometry-optimized Z and E isomers of N-methyl acetohydroxamic acid (Me-AHA) at the level of B3LYP/6-31G* in the solvent reaction field of CHCl₃. Values in parentheses represent the Gibbs free energy at 298.15 K relative to that of the Z isomer. The amide bond isomerization, Z to E, can be done by varying the dihedral angle, ϕ , centered on the C–N bond. Atoms are colored as follows: white, hydrogen; green, carbon; blue, nitrogen; red, oxygen. Coordinates of both isomers are available in Tables S3 and S4.

Table S3. Coordinates of the geometry optimized Z isomer of Me-AHA (Figure S3) at the level of B3LYP/6-31G* in the reaction field of $CHCl_3$; Gibbs free energy at 298.15 K = -323.613745 hartrees.

Atom	Coordinates (Angstroms)		
	Х	Y	Z
С	-0.724734	-0.192446	-0.017409
С	-1.674180	0.985548	-0.022866
0	-1.105467	-1.370569	0.078275
Ν	0.603463	0.055726	-0.159526
0	1.425304	-1.080115	-0.022616
С	1.341350	1.275976	0.080928
Н	0.735490	-1.781135	0.078978
Н	-1.629696	1.526045	0.929686
н	-1.452782	1.693377	-0.826749
Н	-2.685190	0.597811	-0.155856
н	1.720262	1.308439	1.109831
Н	2.185621	1.325703	-0.612324
н	0.688735	2.130687	-0.096069

Table S4. Coordinates of the geometry optimized E isomer of N-methylacetohydroxamic acid (Figure S3) at the level of B3LYP/6-31G* in the reaction field of $CHCl_3$; Gibbs free energy at 298.15 K = -323.613104 hartrees.

Atom	Coordinates (Angstroms)		
	х	Y	Z
С	0.690213	-0.355537	-0.032295
С	1.785580	0.689148	0.040680
0	0.910868	-1.563735	-0.006425
Ν	-0.594657	0.122085	-0.181112
0	-0.797313	1.482445	0.130516
С	-1.766686	-0.701155	0.064778
Н	-1.007242	1.900359	-0.724396
Н	1.700337	1.425373	-0.763717
Н	1.729586	1.236185	0.987630
Н	2.746466	0.176733	-0.025754
Н	-2.106173	-0.599583	1.102749
Н	-2.574148	-0.401571	-0.609593
Н	-1.489309	-1.736503	-0.130839

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NMR Study



Figure S4A. ¹H NMR spectrum of Me-AHA in CDCl_{3.}



Figure S4B. ¹³C NMR spectrum of Me-AHA in CDCl_{3.}



Figure S4C. 2D proton/carbon NMR spectrum of Me-AHA in CDCl₃.



Figure S5A. ¹H NMR spectrum of Zr(Me-AHA)₄ in CDCl_{3.}



Figure S5B. ¹³C NMR spectrum of Zr(Me-AHA)₄ in CDCl_{3.}

Table S4. Assignment of ¹H NMR chemical shifts for the Z and E rotamers of Me-AHA with respect to TMS in $CDCl_3$.

La Chemic		ft (δ) in ppm
	Z	E
C-CH ₃	2.11	2.13
N-C H ₃	3.37	3.24
0-Н	8.91	

Table S5. Assignment of ¹³C NMR chemical shifts for the *Z* and *E* rotamers of Me-AHA with respect to TMS in $CDCl_3$.

¹³ C	Chemical shift (δ) in ppm	
Ľ	Z	E
C- C H ₃	19.02	20.17
N-CH ₃	36.89	36.14
0= C	165.05	172.54

Potentiometric study of the acid-base and Zr(IV) complexation properties of AHA and Me-AHA

Potentiometric titrations were performed in a glass-jacketed titration cell thermostatized at 25 ± 0.1 °C and closed under an inert gas atmosphere, using a Metrohm 702 SM Titrino titration stand connected to a Metrohm 6.0233.100 combined glass electrode and controlled by the Metrohm Tinet 2.4 software. The titrant was a carbonate-free KOH solution at approximately 0.1 M prepared from a commercial ampoule of analytical grade, and its exact concentration was obtained by titration of a standard HNO₃ solution. Stocks solutions of AHA and Me-AHA were prepared at ~1.5×10⁻³ M. A stock solution of zirconium (IV) cations was prepared at ~0.05 M from the analytical grade salts ZrCl₄ and was standardized by complexometry against an EDTA (ethylenediaminetetraacetic acid) standard solution (use of an excess of EDTA and titration of this excess with $Bi(NO_3)$ using xylenol orange as indicator). Sample solutions contained ~0.03 mmol of ligand in a total volume of 30 mL, with the ionic strength kept at 0.10 M using KNO₃ as background electrolyte. Complexation titrations were run by addition of approximately 0.25 equivalents of zirconium (IV) relative to the ligand amount. The $[H^{\dagger}]$ of the solutions was determined by measurement of the electromotive force of the cell, $E = E^{0} + Q \log[H^{+}] + E_{i}$. The term pH is defined as $-\log [H^+]$, and a value of $K_w = [H^+][OH^-] = 10^{-13.778}$ was taken from the literature³ for our experimental conditions. E^{o_1} and Q were determined by titrating a standard HNO₃ solution at the same ionic strength. The liquid-junction potential, E_i, was found to be negligible under the experimental conditions used. Each titration consisted of 150-200 equilibrium points in the range of pH 2.0-12.0, and at least two replicate titrations were performed for each individual system. The potentiometric data were refined with the Hyperquad software,⁴ and speciation diagrams were plotted using the Hyss software.⁵ The overall equilibrium (formation) constants β_i^{H} and β_{MmHhLl} are defined by $\beta_{MmHhLl} = [M_m H_h L_i]/[M]^m [H]^h [L]^l$ and $\beta_{MH-1L} = \beta_{ML(OH)} \times K_w$, while stepwise equilibrium constants are given by $K_{MmHhLl} = [M_m H_h L_i]/[M_m H_{h-1} L_i][H]$ and correspond to the difference in log units between overall constants of sequentially protonated (or hydroxide) species.



Figure S6. Speciation diagrams of the ternary species obtained with Me-AHA and AHA.

⁸⁹Zr production

⁸⁹Zr was produced and purified at the National Institutes of Health, Bethesda, MD, USA, by the following procedure: Pressed pellets of yttrium metal (200 mg, 99.99 % purity; American Elements, USA) were irradiated with a proton beam of 15 MeV and a current of 20 μ A for 2–4 h on a GE PETtrace cyclotron. ⁸⁹Zr was separated from the yttrium target material by the use of hydroxamate resin as described by Holland et al.⁶ Briefly, the target material was dissolved in 4 x 0.5 mL fraction of 6M HCl. After 1 h, the undissolved solid residue was separated by filtration, the resulting solution diluted to 5 mL with deionized water and loaded onto the hydroxamate resin column. The column was then washed with 4 x 2.5 mL of 2 M HCl and 4 x 2.5 mL de-ionized water. After the solution was removed from the column, the ⁸⁹Zr was eluted with successive portions of 1 M oxalic acid. The first 0.4 mL fraction was discarded and the next 0.7 mL fraction collected for further use.

Complexation study with ⁸⁹Zr

All solutions described below were prepared with de-ionized water purified through a chelex column prior to use. Stock solutions of ⁸⁹Zr of pH ranging from 4 to 10 were prepared as follow: To 450 µL of water were added 45 μ L of the ⁸⁹Zr solution. 45 μ L of a 1M Na₂CO₃ solution were added and the pH was adjusted to the desired pH by addition of small aliquots of a 0.1 M Na₂CO₃ or 0.1 M HCl solution. To 50 μ L of stock solution (3.33 to 3.7 MBq of ⁸⁹Zr) at pH = 4, 5, 6, 7, 8, 9 or 10, were added 5 μ L of a 1 mg/mL solution of (AHA), (Me-AHA) or Desferrioxamine B (DFB) mesylate in de-ionized water. These mixtures were incubated for 30 min at 50 °C and analyzed by ITLC-SG using a 50 mM EDTA (ethylenediaminetetraacetic acid) solution in de-ionized water as eluant, and analyzed with a Typhoon 8600 scanner (GE Healthcare) in phosphorimaging mode. In this chromatographic system, the free ⁸⁹Zr is complexed to EDTA and migrates at the top of the TLC while the ⁸⁹Zr bound to the investigated ligands remains at the bottom of the TLC. The EDTA being a strong chelator to Zr(IV) (Log K ~ 29)⁷, this system does not allow to determine the percentage of ⁸⁹Zr actually bound to the investigated ligand since ligand exchange with EDTA can occur during the migration of the TLC. However this is a good system to compare the stability between these ligands since stronger ligands will have lower exchange rates, and consequently higher activity ratio remaining at the bottom of the TLC while free or exchanged ⁸⁹Zr spreads along the TLC. The percentage of the remaining activity bound to the ligand after TLC was calculated by converting the TLC scan into a chromatogram and integrating the peak corresponding to the spot at the bottom of the TLC (see Figure S7). Activity ratios were averaged out of 3 TLC for each condition. As expected, DFB was by far the best ligand with > 99 % activity remaining at the bottom of the TLC for pH ranging from 5 to 9. For the single hydroxamic acid, lower bound activity was observed but with much better results for Me-AHA with 20.5 \pm 0.4 % versus 3.5 \pm 0.6 % for AHA as best results obtained from the pH range at pH 7.



Figure S7. Radio-TLCs and corresponding chromatograms of ⁸⁹Zr solution incubated 30 min at 50 °C, pH = 7 in the presence of: top left = no ligand; top right = AHA; bottom left = Me-AHA; bottom right = Df mesylate. 10 cm long ITLC-SG were used with 50 mM EDTA and were eluted about 8 cm from the deposit (~ 1 μ L, 60-70 kBq) to the top.

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