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Supporting Information

Synthesis, Physicochemical Studies, Molecular Dynamics Simulations, and Metal-Ion-Dependent Antiproliferative and Antiangiogenic Properties of Cone ICL670-Substituted Calix[4]arenes

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cplu_201200141_sm_miscellaneous_information.pdf

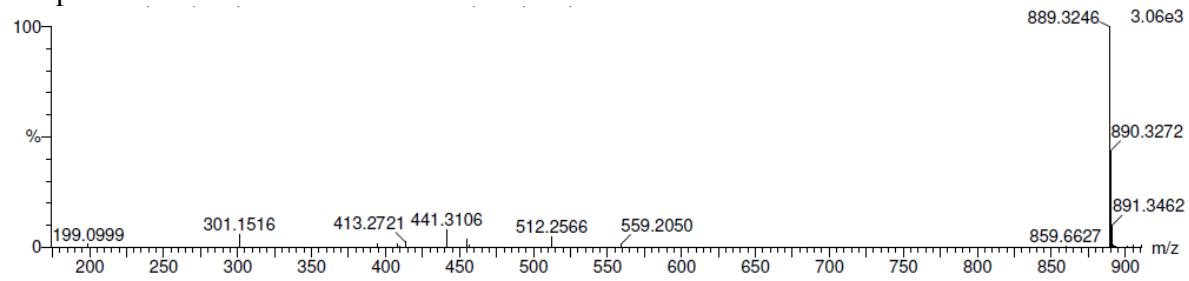
1. Synthesis of ICL670**.**

2-(2-Hydroxyphenyl)-benzo-4*H*-[1,3]-oxazin-4-one: Salicylic acid (24.9 g, 180 mmol), salicylamide (20.6 g, 150 mmol) and pyridine (1.5 mL) were refluxed in xylene (35 mL). Thionyl chloride (23.7 mL, 330 mmol) was added during 6 h. At the end of the addition, the product started to crystallize. Stirring was continued for an additional 1 hour. The xylene was evaporated under reduced pressure. The crude was suspended in EtOH (60 mL) and acetic acid (1.5 mL). The mixture was heated gently and then allowed to cool to 20°C. The precipitate was filtered and recrystallized from 2-methoxyethanol (65 mL). 17 g of the 2-(2-hydroxyphenyl)-benzo-4*H*-[1,3]-oxazin-4-one were obtained as yellow needles, yield: 47%, m.p. 203.4-204-4°C; ^{1}H NMR (500 MHz, DMSO-*d6*): δ = 7.06-7.10 (m, 2H), 7.59-7.64 (m, 2H), 7.77 (d, ^{3}J (H,H) = 8.4 Hz, 1H), 7.94 (dt, ^{3}J (H,H) = 7.8 Hz, ^{4}J (H,H) = 1.7 Hz, 1H), 8.06 (dd, ^{3}J (H,H) = 7.9 Hz, ^{4}J (H,H) = 1.4 Hz, 1H), 8.19 (dd, ^{3}J (H,H) = 8.0, ^{4}J (H,H) = 1.5, 1H), 12.89 (s, OH); ^{13}C NMR (125 MHz, DMSO-*d6*): δ = 112.35 (1C), 118.35 (1C), 118.71 (1C), 118.86 (1C), 120.46 (1C), 127.66 (1C), 128.09 (1C), 129.87 (1C), 136.89 (1C), 137.59 (1C), 154.78 (1C), 162.75 (1C), 164.29 (1C), 165.70 (1C); IR (ATR): ν 3376 (OH), 1661 (C=O), 1605 cm^{-1} (C=N); MS: *m/z* : 262 [MNa]$^{+}$.

4-[3,5-Bis(2-hydroxyphenyl)-1,2,4-triazol-1-yl]benzoic Acid (ICL670): 2-(2-Hydroxyphenyl)-4*H*-3,1-benzoxazin-4-one (2.50 g, 10.45 mmol) was added to a solution of the 4-Hydrazino-benzoic acid (1.75 g, 11.5 mmol) and NEt_3 (1.16 g, 11.5 mmol) in boiling EtOH (110 mL). The reaction mixture was refluxed for 3 h. The solution was then allowed to cool to room temperature, and H_2O was added until the first sign of precipitation. The mixture was concentrated to a total volume of 50% under reduced pressure and aqueous 6M HCl (40 mL) was added. The resulting solid was filtered and dried for 24 h in vacuo. 3.5 g of the ICL670 were obtained as white solid, yield: 90%, m.p. 260°C; ^{1}H NMR (500 MHz, DMSO-*d6*): δ = 6.84 (d, ^{3}J(H,H) = 8.3 Hz, 1H), 6.94-6.98 (m, 2H), 6.99 (d, ^{3}J(H,H) = 7.4 Hz, 1H), 7.33-7.39 (m, 2H), 7.51-7.54 (m, 3H), 7.95-7.98 (m, 2H), 8.02 (dd, ^{3}J(H,H) = 7.8 Hz, ^{4}J(H,H) = 1.7, 1H); ^{13}C NMR (125 MHz, DMSO-*d6*): δ = 114.54 (1C), 115.30 (1C), 117.03 (1CH), 117.94 (1CH), 120.33 (1CH), 120.56 (1CH), 124.22 (2CH), 127.67 (1CH), 131.16 (2CH), 131.44 (1C), 131.92 (1C), 132.33 (1CH), 132.42 (1CH), 142.06 (1C), 152.93 (1C), 156.17 (1CH), 157.22 (1C), 160.78 (1C), 167.29 (1CO); IR (ATR): ν 3080 (vOH), 1695 (C=O), 1607 cm^{-1} (C=N); MS: *m/z* : 374 [MH]$^{+}$.

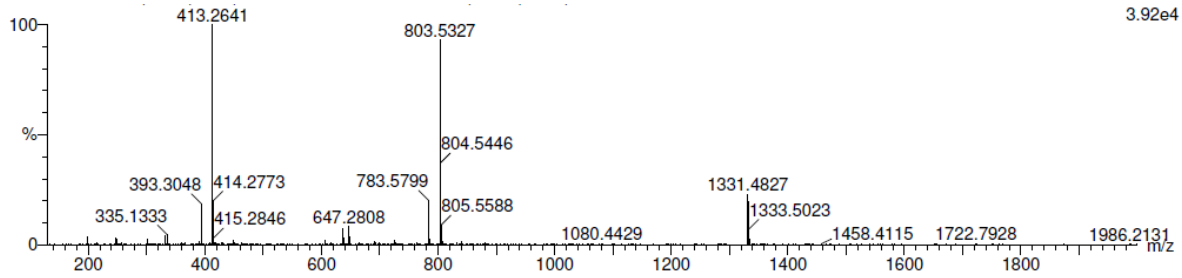
2. Mass spectra

Compound **5a:**



Minimum:				-1.5		
Maximum:		200.0	5.0	50.0		
Mass	Calc. Mass	mDa	PPM	DBE	Score	Formula
889.3246	889.3237	0.9	1.0	35.5	2	C55 H45 N4 O8
	889.3213	3.3	3.7	32.5	1	C53 H46 N4 O8 Na

Compound **5b:**



Minimum:				-100.0		
Maximum:		200.0	5.0	150.0		
Mass	Calc. Mass	mDa	PPM	DBE	Score	Formula
1331.4827	1331.4814	1.2	0.9	44.5	1	C73 H68 N10 O14 Na
	1331.4854	-2.8	-2.1	48.5	2	C78 H68 N8 O12 Na

3. Protonation and iron-binding constants in solution

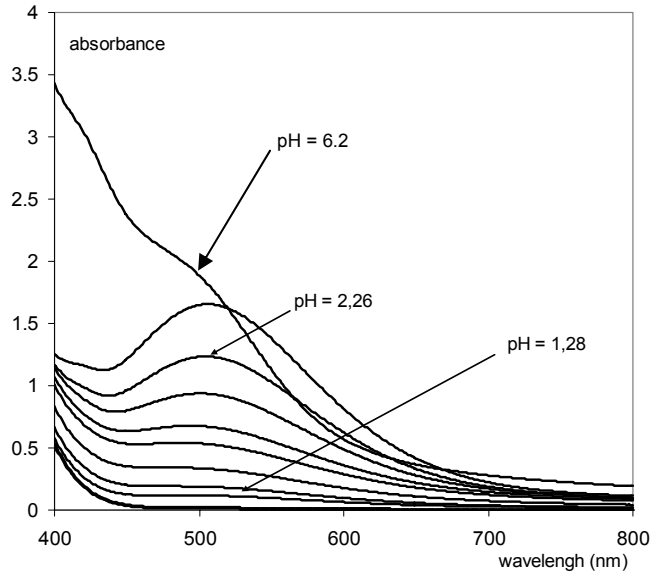


Figure SI1**:** Spectrophotometric Titration (batch experiment) with total Fe(III) = 5.10^{-4} M and total L = 1.10^{-3} M ; (0.1 M KCl, 25 °C, xDMSO = 0.5).

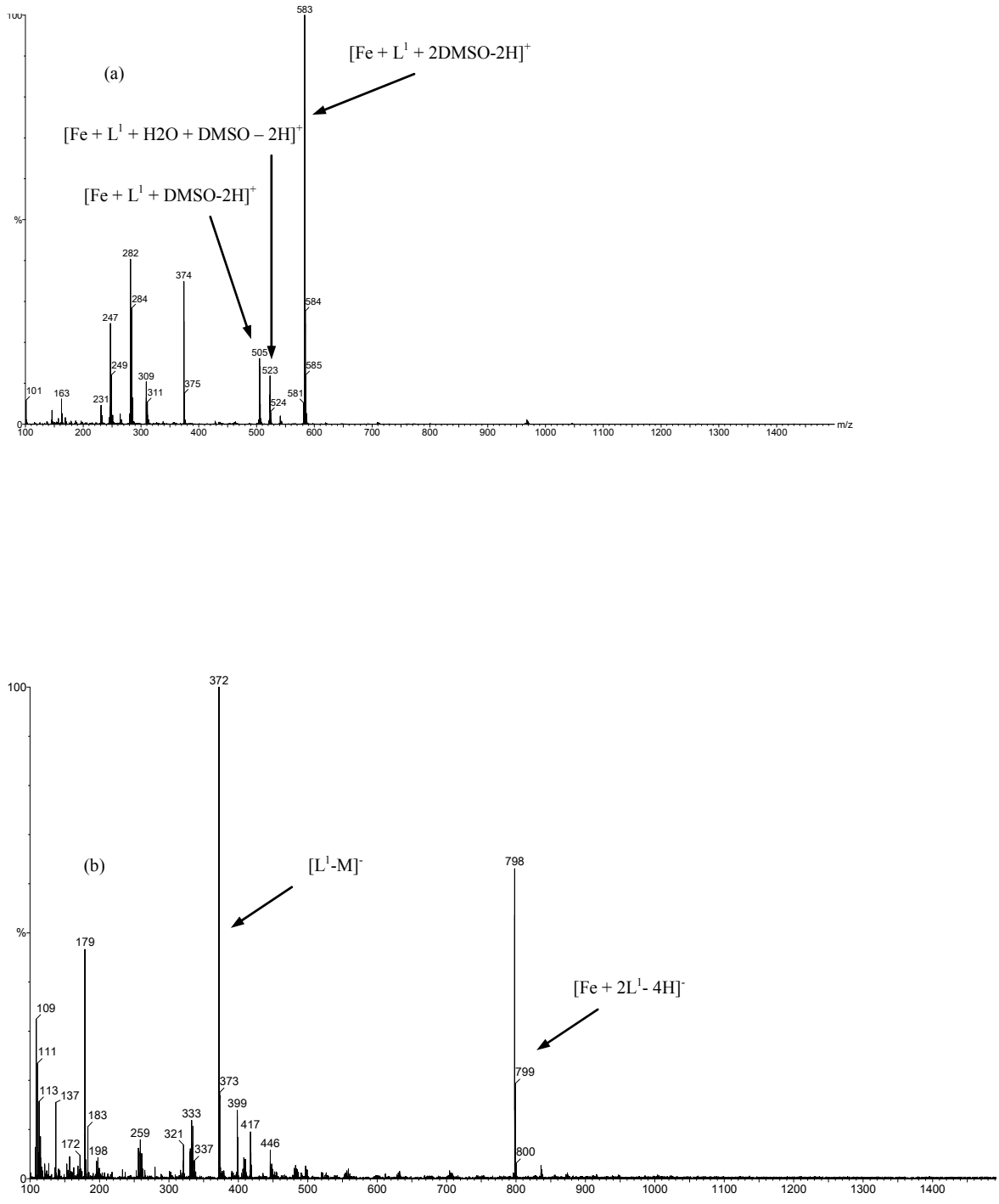


Figure SI2: ESI-MS spectra of FeL^1 solution (a) at acidic pH (b) at medium pH (b); R = 2, $C_L = 10^{-3}$ M.

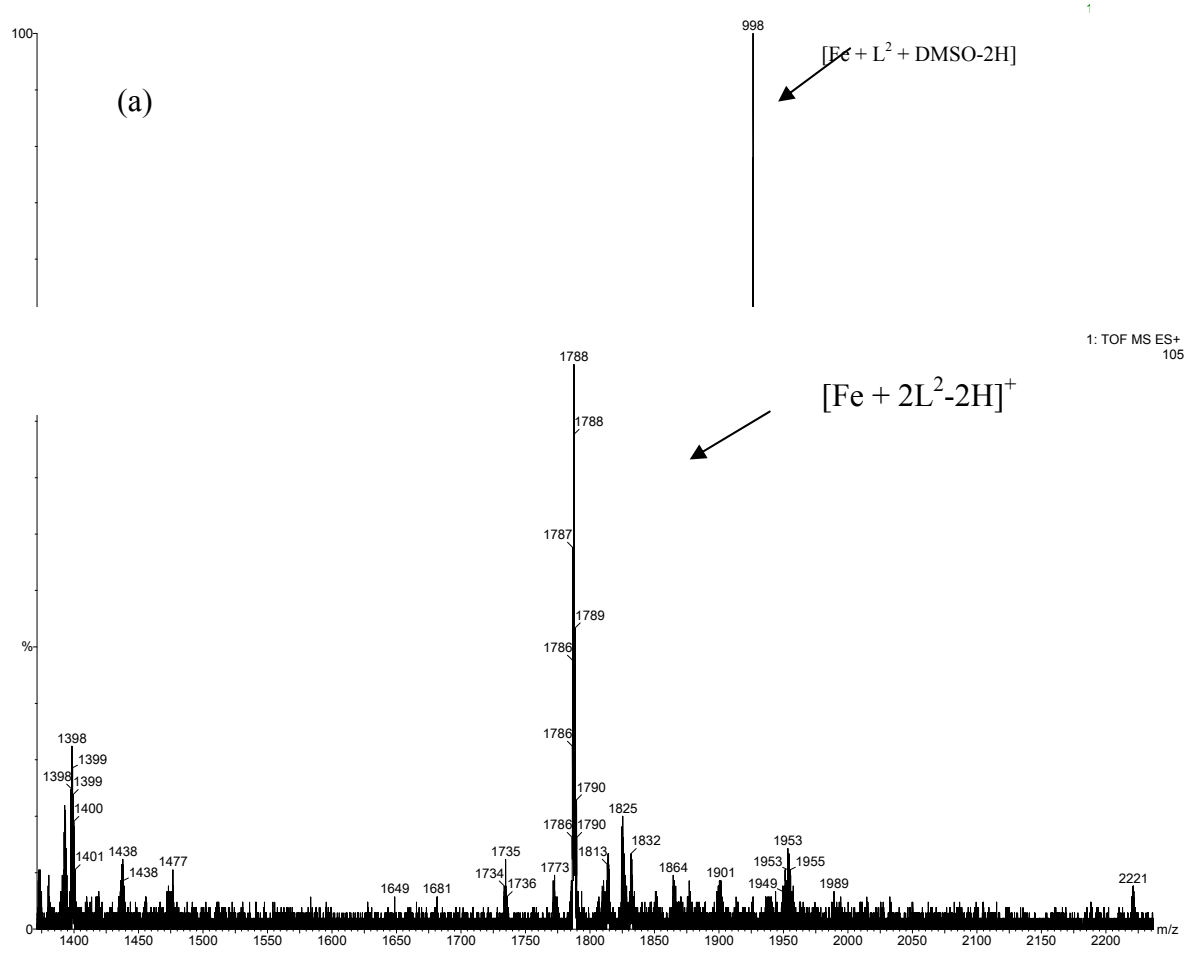
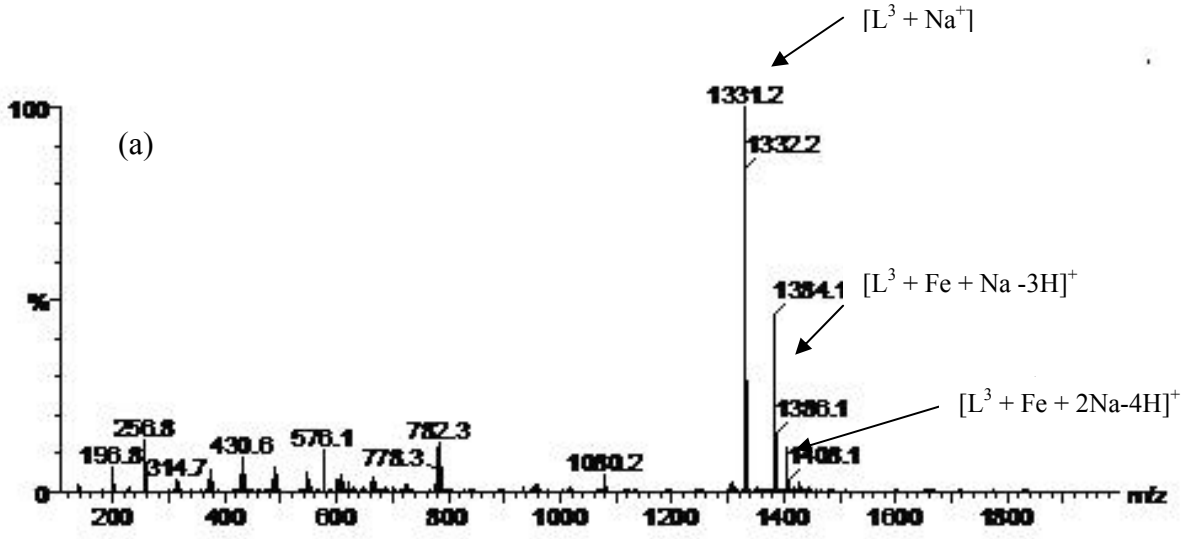


Figure SI3: ESI-MS spectra of FeL^2 solution (a) at acidic pH (b) at medium pH (b); R = 2, $C_L = 10^{-3}$ M.

Fi


(a)
[L3 + Na+]
1331.2
1332.2
[L3 + Fe + Na -3H]+
1384.1
[L3 + Fe + 2Na-4H]+
1386.1
1408.1
256.8
196.8
314.7
430.6
576.1
778.3
782.3
1060.2
100
%
0
200
400
600
800
1000
1200
1400
1600
1800
m/z

4. Molecular Modeling

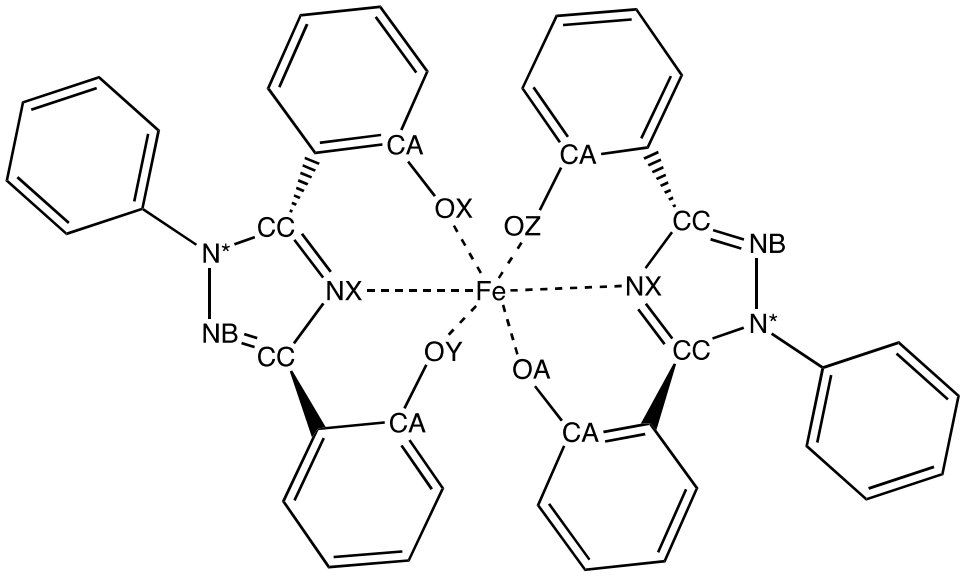


Figure SI5. Type definition for key atoms in [Fe-ICL670]$^-$, [Fe-**5a**]$^-$ and [Fe-**5b**]$^-$ when using the q4md-FeB force field.

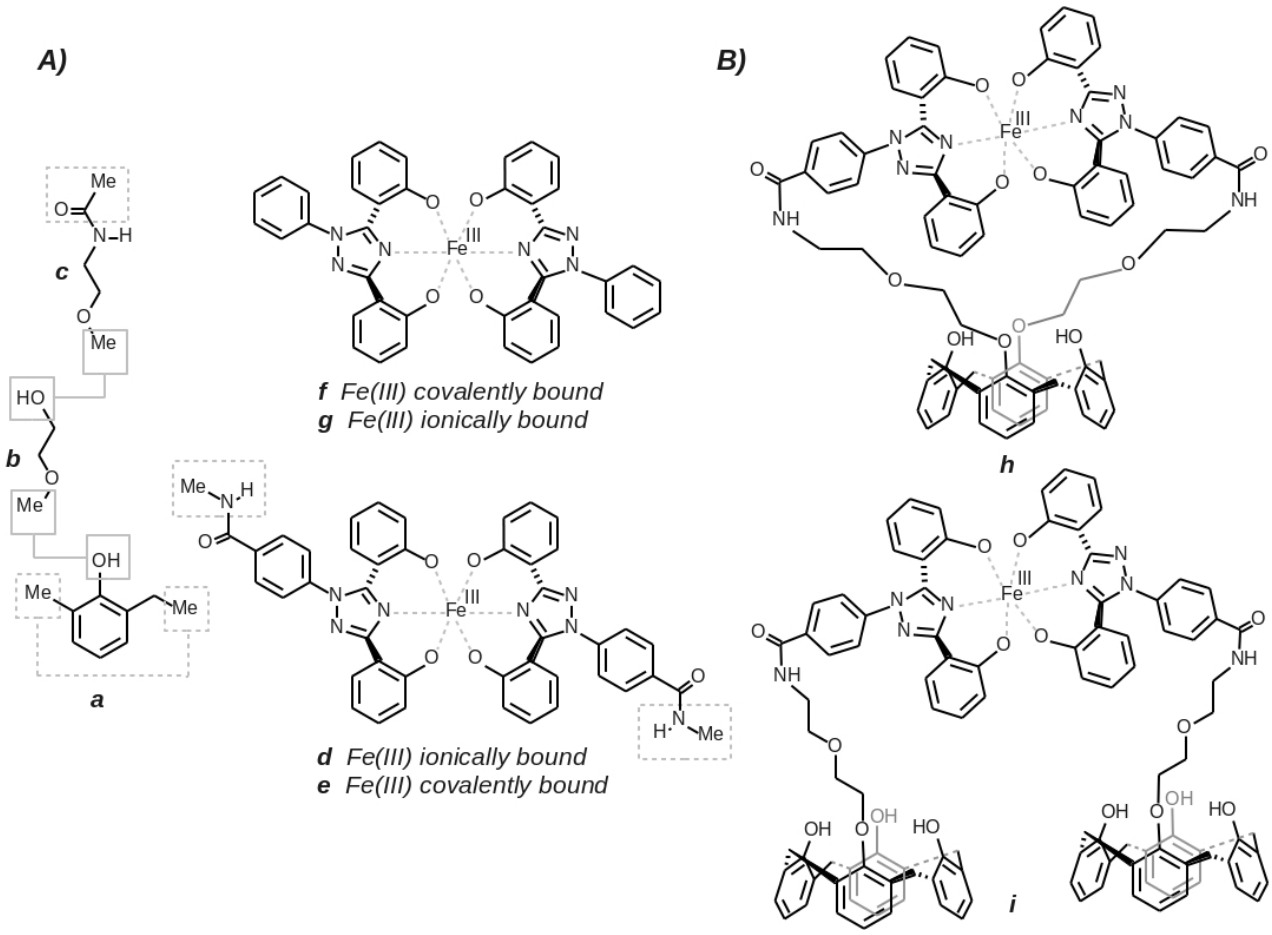
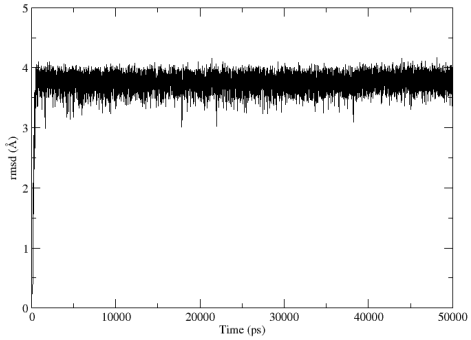
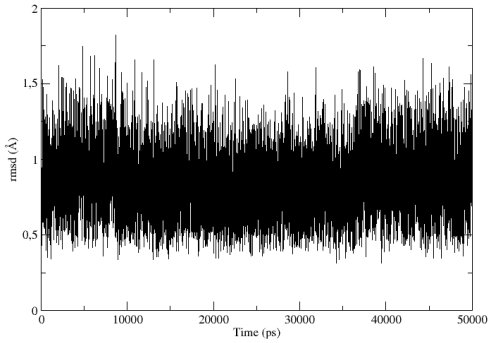


Figure SI6. Charge derivation (involving multiple orientations, multiple conformations and multiple molecules) and FFTopDB building for ICL670 and calix[4]arene- ICL670-based Fe(III) systems have been automatically carried out using the R.E.D. IV program available in "R.E.D. Server "Development". a) Description of the different building blocks involved in ESP charge derivation and FFTopDB building; dashed and gray boxes: intra-molecular charge constraints within each selected building block; plain and gray boxes: inter-molecular charge constraints defined between pairs of building blocks, b) building of the calix[4]arene-ICL670 Fe(III) chelators obtained from total synthesis using the FFTopDB.

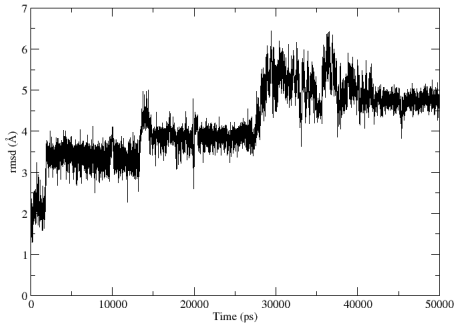
a)



b)



c)



d)

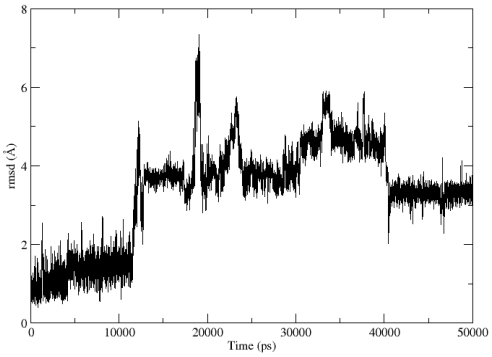


Figure SI7. Root mean square deviation (RMSD) for [Fe-**5b**]$^-$ using the (a) the q4md-FeB force field and (b) the q4md-FeF force field and for [Fe-**5a**]$^-$ using the (c) the q4md-FeB force field and (d) the q4md-FeF force field. The curves are extracted from representative 50 ns MD simulations, taking into account the heavy atoms and with the initial structure taken as the reference.

Bond type	Stretching force constant ($kcal.mol^{-1}.Å^{-2}$)	Equilibrium bond length (Å)
NX-Fe	40.0	2.16
OX-Fe	80.0	1.97
OY-Fe	80.0	1.97
OZ-Fe	80.0	1.97
OA-Fe	80.0	1.97
Bond Angle	Bending force constant ($kcal.mol^{-1}.rad^{-2}$)	Equilibrium bond length (Å)
NX-Fe-NX	50.0	180.0
OX-Fe-OY	95.0	180.0
OX-Fe-OZ	75.0	90.0
OX-Fe-OA	75.0	90.0
OY-Fe-OZ	75.0	90.0
OY-Fe-OA	75.0	90.0
OZ-Fe-OA	95.0	180.0
NX-Fe-OX	85.0	90.0
NX-Fe-OY	85.0	90.0
NX-Fe-OZ	85.0	90.0
NX-Fe-OA	85.0	90.0
CC-NX-Fe	100.0	126.3
CA-OX-Fe	70.0	135.4
CA-OY-Fe	70.0	131.0
CA-OZ-Fe	70.0	135.4
CA-OA-Fe	70.0	131.0

Table SI1. Bond stretching and angle bending parameters related to iron, obtained from the $[Fe\text{-}ICL670]^-$ system. See Fig. SI2 for atom type definition.
These values were taken from the QM optimized geometry and are reported along with their corresponding force constants extracted from the Hessian matrix.
We did not develop parameters for torsion angles, as they are often ignored for metal sites (the energy barriers have been set to a value of zero in our cases).

	[Fe-ICL670]$^-$	[Fe-**5a**]$^-$	[Fe-**5b**]$^-$	X-Ray
Bond type (Å)				
NX-Fe	1.92 / 1.92	1.95 / 1.95	1.95 / 1.96	2.11 / 2.11
OX-Fe	1.86	1.86	1.85	1.97
OY-Fe	1.88	1.87	1.87	2.00
OZ-Fe	1.86	1.86	1.85	1.97
OA-Fe	1.88	1.87	1.86	2.00
Bond Angle (°)				
NX-Fe-NX	176.0	175.7	175.4	174.6
OX-Fe-OY	170.9	170.3	170.7	166.2
OX-Fe-OZ	91.3	91.0	91.2	92.1
OX-Fe-OA	90.2	89.7	89.7	90.8
OY-Fe-OZ	90.2	89.9	90.5	90.8
OY-Fe-OA	89.5	90.7	89.9	89.7
OZ-Fe-OA	170.9	170.4	171.2	166.2
NX-Fe-OX	85.5 / 95.2	85.2 / 95.8	85.6 / 96.9	83.7 / 100.1
NX-Fe-OY	86.1 / 93.2	85.8 / 93.2	85.8 / 91.7	82.5 / 93.7
NX-Fe-OZ	95.2 / 85.5	95.5 / 85.1	94.7 / 85.6	100.1 / 83.7
NX-Fe-OA	93.2 / 86.1	93.5 / 85.9	93.5 / 86.2	93.7 / 82.5
CC-NX-Fe	130.8 / 130.8	129.4 / 129.2	129.3 / 126.3	125.7 / 125.8
CA-OX-Fe	135.3	135.6	135.4	126.4
CA-OY-Fe	129.2	132.7	132.5	124.8
CA-OZ-Fe	135.3	135.8	136.7	126.4
CA-OA-Fe	129.1	132.6	136.1	124.8

Table SI2. Bond distances and angle values involved in the iron coordination center of [Fe-ICL670]$^-$ and [Fe-**5a**]$^-$ and [Fe-**5b**]$^-$ systems. These values, averaged from a 50 ns MD simulation, are compared to those measured on the crystallographic structure of [Fe-ICL670]$^-$ (abbreviated as X-Ray in the table). See Fig. SI2 for atom type definition.

			COMPLEX			
	ELE[a]	VDW[b]	INT[c]	SUR[d]	GB[e]	GBtot[f]
[Fe-ICL670]$^-$	-2084	143	107	7	-70	-1897
[Fe-**5a**]$^-$	-1956	143	260	11	-87	-1629
[Fe-**5b**]$^-$	-1956	144	217	10	-76	-1661
			RECEPTOR			
	ELE[a]	VDW[b]	INT[c]	SUR[d]	GB[e]	GBtot[f]
[Fe-ICL670]$^-$	257	49	107	7	-921	-501
[Fe-**5a**]$^-$	346	51	260	11	-884	-216
[Fe-**5b**]$^-$	343	51	217	10	-878	-257
			LIGAND (*i.e.* Fe(III))			
	ELE[a]	VDW[b]	INT[c]	SUR[d]	GB[e]	GBtot[f]
[Fe-ICL670]$^-$	0	0	0	1	-1046	-1045
[Fe-**5a**]$^-$	0	0	0	1	-1046	-1045
[Fe-**5b**]$^-$	0	0	0	1	-1046	-1045
			Delta (COMPLEX – (RECEPTOR + LIGAND))			
	ΔELE	ΔVDW	ΔINT	ΔSUR	ΔGB	ΔGBtot
[Fe-ICL670]$^-$	-2341	49	0	-1	1898	-351
[Fe-**5a**]$^-$	-2301	92	0	-1	1844	-366
[Fe-**5b**]$^-$	-2299	93	0	-1	1848	-359

Table SI3. MM-GBSA enthalpy values (kcal.mol^{-1}) obtained over 50 ns MD simulations. [a] Internal electrostatics. [b] van der Waals energy. [c] Internal energy: bond, angle, dihedral. [d] Hydrophobic part of the solvation energy (GBSUR). [e] Electrostatic part of the solvation energy (GBCAL). [f] Sum of ELE + VDW + INT + GBSUR + GBCAL.

Charge derivation and force field topology database building. Atomic charges computed using the Hartree-Fock (HF)/6-31G* level of theory, are generally of a large magnitude and overestimate the gas phase dipole moment of small molecules.[1] For example, the computed dipole moment of water is around 20% larger than the experimental value, which approximately corresponds to the dipole moment enhancement of a water molecule in the liquid phase. The enhanced magnitude of the charges serves as a correction for the lack of explicit treatment of polarization in the additive force field model. However, Density Functional Theory (DFT) has now become the preferred method for electronic structure theory for transition metals. Indeed, it is well accepted that quantitatively accurate electronic structure calculations for transition metals must include electron correlation.[2] Thus, in this work we decided to calculate charges from DFT-optimized geometries and molecular electrostatic potentials (MEP). Considering the size of the complexes reported here, the double-zeta 6-31G* basis set associated with the most popular hybrid functional, *i.e.* B3LYP were selected.[3] Moreover, St.-Amant *et al.* have shown that DFT charges generally have 5-10% smaller magnitudes compared to the Hartree-Fock charges when computed using basis sets of similar size.[1a] Considering that cations generally display low polarizabilities, DFT may be a good choice for performing calculations for positively charged iron complexes. For MD simulations with the AMBER force field model, RESP charges are widely used. However, the use of the default hyperbolic constraints in the two stage RESP fitting approach leads to a drastic decrease of the Fe(III) charge value in the studied complexes. In this case the charge on the iron ion could have a value between 0.38-0.45 *e*, which is very different from the Mulliken charge value of 1.26 *e*. At the same time, the ESP charge varies between 1.29 and 1.33 *e*, which is in better agreement with the Mulliken charge. Consequently the ESP model was selected to derive charges for MD simulations. Surprisingly, this observation is exactly the opposite to that reported by Autenrieth *et al.* for heme complexes.[3] On the other hand, Hoops *et al.* concluded that the outcome of the ESP fitting model is highly dependent on the complex under study.[4] DFT-based computations, RESP and ESP charge fitting as well as force field library building were carried out using the latest version of the R.E.D. IV program available through "R.E.D. Server Development".[5] For transition metal complexes, this approach allows rigorously deriving reproducible charge values. It associates specific algorithms required to (i) generate representative optimized geometries and (ii) to control molecular orientation before MEP computation.

In one approach the cation and the organic molecule interact by non-bonded interactions (electrostatic and van der Waals) in which iron has a formal charge value of +3 *e*. In the second approach, the metal is connected to the organic molecule by explicit bonds to ensure that the ion stays bound. In this case, the charges on the metal ion are derived by quantum chemical calculations performed on the metal-organic molecule complex.

[1] a) A. St.-Amant, W. D. Cornell, P. A. Kollman, T. A. Halgren, *J. Comput. Chem.* **1995**, *16*, 1483-1506; b) C. I. Bayly, P. Cieplak, W. D. Cornell, P. A. Kollman, *J. Phys. Chem.* **1993**, *97*, 10269-10280.

[2] C. J. Cramer, D. G. Truhlar, *Phys. Chem. Chem. Phys.* **2009**, *11*, 10757-10816.

[3] F. Autenrieth, E. Tajkhorshid, J. Baudry, Z. Luthey-Schulten, *J. Comput. Chem.* **2004**, *25*, 1613-1622.

[4] S. C. Hoops, K. W. Anderson, K. M. Merz, *J. Am. Chem. Soc.* **1991**, *113*, 8262-8270.

[5] a) F.-Y. Dupradeau, A. Pigache, T. Zaffran, C. Savineau, R. Lelong, N. Grivel, D. Lelong, D. Rosanski, P. Cieplak, *Phys. Chem. Chem. Phys.* **2010**, *12*, 7821-7839; b) E. Vanquelef, S. Simon, G. Marquant, E. Garcia, G. Klimerak, J. C. Delepine, P. Cieplak, F. Y. Dupradeau, *Nucleic Acids Res.* **2011**, *39*, W511-W517.

Cartesian coordinates (in Å) of the $[FeL^2_2]^-$ and $[FeL^3]^-$ complex obtained after a geometry optimization at the B3LYP/6-31G* level of theory.

N	-2.387105	-4.133052	-0.319847
N	-2.684197	-4.454278	0.986842
C	-3.610842	-3.566260	1.326850
N	-3.920095	-2.707263	0.297745
C	-3.162246	-3.083610	-0.735629
C	-4.228328	-3.515458	2.647184
C	-5.162434	-2.486285	2.987000
O	-5.531706	-1.534923	2.169498
C	-5.728093	-2.528171	4.290772
H	-6.440104	-1.747292	4.541589
C	-5.387409	-3.512999	5.202204
H	-5.841305	-3.507240	6.191786
C	-4.463493	-4.515381	4.861353
H	-4.195181	-5.289323	5.575636
C	-3.899467	-4.503895	3.594300
H	-3.186592	-5.270471	3.303962
C	-3.231986	-2.504065	-2.066293
C	-3.579535	-1.115876	-2.175149
O	-3.806887	-0.362409	-1.141662
C	-3.624192	-0.565008	-3.487741
H	-3.876513	0.487883	-3.571637
C	-3.389687	-1.342038	-4.608078
H	-3.451541	-0.887730	-5.595367
C	-3.091378	-2.712064	-4.490040
H	-2.937474	-3.324809	-5.373702
C	-3.018385	-3.276037	-3.225674
H	-2.807804	-4.336980	-3.123225
C	-1.215697	-4.697168	-0.892209
C	-0.906131	-6.038979	-0.638800
H	-1.590061	-6.634594	-0.045098
C	0.283188	-6.575023	-1.122219
H	0.539634	-7.612537	-0.935571
C	1.183739	-5.787473	-1.853502
C	0.847485	-4.451058	-2.115792
H	1.501378	-3.814721	-2.704783
C	-0.334855	-3.902557	-1.634816
H	-0.562095	-2.859606	-1.811369
C	2.465760	-6.420700	-2.302545
O	2.609939	-7.642001	-2.353731
N	-4.254822	3.405451	0.251631
N	-5.287225	3.270809	-0.654489
C	-5.766926	2.060734	-0.404270
N	-5.101674	1.423247	0.621962
C	-4.172169	2.283571	1.033711
C	-6.880382	1.470880	-1.137856
C	-7.161707	0.072205	-1.042557

O	-6.468170	-0.764919	-0.314651
C	-8.267897	-0.419430	-1.787019
H	-8.480966	-1.481440	-1.707324
C	-9.036492	0.412354	-2.584162
H	-9.874514	-0.004094	-3.140584
C	-8.744425	1.783085	-2.680577
H	-9.348005	2.436847	-3.304620
C	-7.674272	2.292456	-1.959162
H	-7.429541	3.349656	-2.011164
C	-3.289896	2.072921	2.169927
C	-2.827234	0.739076	2.424748
O	-3.117432	-0.265414	1.653781
C	-1.972174	0.561929	3.550547
H	-1.614312	-0.443953	3.747564
C	-1.626765	1.622293	4.372962
H	-0.984460	1.441413	5.232861
C	-2.104512	2.921335	4.121537
H	-1.851094	3.744224	4.783810
C	-2.930049	3.130726	3.025700
H	-3.322223	4.124951	2.827335
C	-3.250273	4.370280	-0.029120
C	-3.606453	5.634477	-0.515738
H	-4.656240	5.883807	-0.620871
C	-2.611805	6.528363	-0.903051
H	-2.870439	7.499947	-1.311116
C	-1.254187	6.189529	-0.802349
C	-0.917726	4.921004	-0.306016
H	0.111034	4.577660	-0.261594
C	-1.900340	4.016898	0.071410
H	-1.619895	3.029212	0.412370
C	-0.232040	7.150424	-1.334091
O	-0.535696	8.086324	-2.073241
Fe	-4.760241	-0.737761	0.584771
C	2.731600	2.630203	-0.206259
C	3.366732	3.566681	0.628965
C	2.806472	3.814481	1.886922
H	3.282781	4.538781	2.544035
C	1.661323	3.136999	2.303012
H	1.216837	3.345573	3.271740
C	1.095150	2.161982	1.484093
H	0.243644	1.591712	1.845182
C	1.626666	1.872000	0.218967
C	4.667565	4.246184	0.217365
H	4.746707	4.255499	-0.872323
H	4.630001	5.296141	0.528656
O	3.313660	2.403269	-1.454885
C	2.582869	2.800422	-2.621758
H	1.515173	2.849491	-2.387469
H	2.743047	2.019069	-3.373086
C	3.031181	4.138132	-3.193388

H	2.489714	4.291228	-4.142089
H	4.109294	4.121588	-3.422013
O	2.730667	5.171594	-2.275472
C	2.578722	6.461092	-2.849895
H	1.787073	6.438638	-3.615095
H	3.512636	6.795500	-3.332084
C	2.187444	7.425472	-1.725999
H	1.897145	8.389356	-2.149957
H	3.039922	7.578197	-1.054647
N	1.069575	6.917605	-0.952526
H	1.265714	6.072310	-0.436561
C	2.502170	-1.260935	0.032021
C	1.247574	-0.621373	0.112162
C	0.229017	-1.210817	0.866027
H	-0.753905	-0.746142	0.907064
C	0.452151	-2.401837	1.561147
H	-0.353720	-2.862712	2.123831
C	1.708818	-2.998302	1.505958
H	1.890195	-3.925231	2.046529
C	2.751396	-2.450420	0.746062
O	3.420747	-0.658339	-0.779435
H	4.288544	-1.103515	-0.777797
C	1.041479	0.712966	-0.588063
H	1.499620	0.673872	-1.577314
H	-0.034481	0.873118	-0.725628
C	6.123600	-1.549685	0.810371
C	5.183744	-2.350394	1.482700
C	5.200792	-2.351106	2.881820
H	4.476328	-2.959844	3.417060
C	6.115312	-1.570097	3.584467
H	6.123095	-1.585244	4.671112
C	6.991679	-0.736082	2.892635
H	7.657491	-0.076893	3.443721
C	7.002017	-0.690766	1.493386
C	4.115138	-3.131495	0.726744
H	4.002603	-4.116875	1.193045
H	4.447236	-3.311596	-0.300260
O	6.090145	-1.553632	-0.585359
C	7.141918	-2.255982	-1.256598
H	8.110301	-1.953773	-0.838568
H	7.091718	-1.926497	-2.298268
C	7.046781	-3.773265	-1.200135
H	6.908458	-4.111017	-0.161133
H	8.006626	-4.187171	-1.557162
O	5.989966	-4.224830	-2.026685
C	5.881714	-5.642312	-2.063138
H	5.646620	-6.035368	-1.061746
H	6.839271	-6.084429	-2.387818
C	4.786414	-6.020501	-3.051746
H	5.045142	-5.612659	-4.038236

H	4.720578	-7.108231	-3.131137
N	3.475573	-5.553750	-2.648476
H	3.381010	-4.572556	-2.431317
C	6.363846	2.334738	0.477362
C	7.484270	1.758869	1.115355
C	8.174408	2.501506	2.074600
H	9.051571	2.064581	2.548218
C	7.756459	3.784282	2.430801
H	8.302091	4.355314	3.176915
C	6.623873	4.320271	1.821932
H	6.281502	5.314633	2.101467
C	5.907689	3.619354	0.843067
O	5.796286	1.574036	-0.493887
H	4.982289	1.957239	-0.877997
C	7.883309	0.331565	0.777386
H	7.814920	0.187558	-0.301961
H	8.929965	0.178862	1.071502

The q4md-FeB and FeF force fields

```
# The q4md-FeB and q4md-FeF Force Fields
#
##############################################################################
#                                                                            #
#                       Script file for Leap/xleap                           #
#                                                                            #
##############################################################################

addAtomTypes {
        { "H"   "H" "sp3" }
        { "HO"  "H" "sp3" }
        { "HS"  "H" "sp3" }
        { "H1"  "H" "sp3" }
        { "H2"  "H" "sp3" }
        { "H3"  "H" "sp3" }
        { "H4"  "H" "sp3" }
        { "H5"  "H" "sp3" }
        { "HW"  "H" "sp3" }
        { "HC"  "H" "sp3" }
        { "HA"  "H" "sp3" }
        { "HP"  "H" "sp3" }
        { "OH"  "O" "sp3" }
        { "OS"  "O" "sp3" }
        { "O"   "O" "sp2" }
        { "O2"  "O" "sp2" }
        { "OW"  "O" "sp3" }
        { "CT"  "C" "sp3" }
        { "CH"  "C" "sp3" }
        { "C2"  "C" "sp3" }
        { "C3"  "C" "sp3" }
        { "C"   "C" "sp2" }
        { "C*"  "C" "sp2" }
        { "CA"  "C" "sp2" }
        { "CB"  "C" "sp2" }
        { "CC"  "C" "sp2" }
        { "CN"  "C" "sp2" }
        { "CM"  "C" "sp2" }
        { "CK"  "C" "sp2" }
        { "CQ"  "C" "sp2" }
        { "CD"  "C" "sp2" }
        { "CE"  "C" "sp2" }
        { "CF"  "C" "sp2" }
        { "CG"  "C" "sp2" }
        { "CP"  "C" "sp2" }
        { "CI"  "C" "sp2" }
        { "CJ"  "C" "sp2" }
        { "CW"  "C" "sp2" }
        { "CV"  "C" "sp2" }
        { "CR"  "C" "sp2" }
        { "CA"  "C" "sp2" }
        { "CY"  "C" "sp2" }
        { "C0"  "C" "sp2" }
        { "MG"  "Mg" "sp3" }
        { "N"   "N" "sp2" }
        { "NA"  "N" "sp2" }
        { "N2"  "N" "sp2" }
        { "N*"  "N" "sp2" }
        { "NP"  "N" "sp2" }
        { "NQ"  "N" "sp2" }
        { "NB"  "N" "sp2" }
        { "NC"  "N" "sp2" }
        { "NT"  "N" "sp3" }
        { "N3"  "N" "sp3" }
        { "S"   "S" "sp3" }
        { "SH"  "S" "sp3" }
        { "P"   "P" "sp3" }
        { "LP"  ""  "sp3" }
        { "F"   "F"  "sp3" }
        { "CL"  "Cl" "sp3" }
        { "BR"  "Br" "sp3" }
        { "I"   "I"  "sp3" }
        { "FE"  "Fe" "sp3" }
        { "EP"  ""   "sp3" }
```

```
        { "NX"  "N"  "sp2" }
        { "OX"  "O"  "sp3" }
        { "OY"  "O"  "sp3" }
        { "OZ"  "O"  "sp3" }
        { "OA"  "O"  "sp3" }
        { "FE"  "Fe" "sp3" }
        { "OW"  "O"  "sp3" }
        { "HW"  "H"  "sp3" }
        { "IP"  "Na" "sp3" }
        { "CL"  "Cl" "sp3" }
}

parm99 = loadamberparams parm99.dat
CalixICL = loadamberparams script3.ff

# Load water & ions
loadOff ions94.lib
loadOff solvents.lib
HOH = TP3
WAT = TP3

alias e edit
alias q quit
alias c charge
alias i impose
alias seq sequence

verbosity 2

# Loading mol2 file
PHP = loadmol2 mm1-o1.FG1.mol2
# General information
set PHP name "PHP"
set PHP head PHP.1.C6
set PHP tail PHP.1.C1
set PHP.1 connect0 PHP.1.C6
set PHP.1 connect1 PHP.1.C1
set PHP.1 restype undefined
set PHP.1 name "PHP"
# set FF atom types
set PHP.1.C1  type C
set PHP.1.C2  type CA
set PHP.1.C3  type CA
set PHP.1.H3  type HA
set PHP.1.C4  type CA
set PHP.1.H4  type HA
set PHP.1.C5  type CA
set PHP.1.H5  type HA
set PHP.1.C6  type CA
set PHP.1.CM  type CT
set PHP.1.HM1 type HC
set PHP.1.HM2 type HC

PHO = loadmol2 mm1-o1.FG2.mol2
# General information
set PHO name "PHO"
set PHO head PHO.1.CM
set PHO tail PHO.1.C6
set PHO.1 connect0 PHO.1.CM
set PHO.1 connect1 PHO.1.C6
set PHO.1 restype undefined
set PHO.1 name "PHO"
# set FF atom types
set PHO.1.C1  type C
set PHO.1.C2  type CA
set PHO.1.C3  type CA
set PHO.1.H3  type HA
set PHO.1.C4  type CA
set PHO.1.H4  type HA
set PHO.1.C5  type CA
set PHO.1.H5  type HA
set PHO.1.C6  type CA
set PHO.1.OX  type OH
set PHO.1.HX  type HO
set PHO.1.CM  type CT
set PHO.1.HM1 type HC
set PHO.1.HM2 type HC
```

```
O13 = loadmol2 mm2-o1.FG1.mol2
# General information
set O13 name "O13"
set O13 head O13.1.O1
set O13 tail O13.1.C3
set O13.1 connect0 O13.1.O1
set O13.1 connect1 O13.1.C3
set O13.1 restype undefined
set O13.1 name "O13"
# set FF atom types
set O13.1.O1  type OS
set O13.1.C2  type CT
set O13.1.H21 type H1
set O13.1.H22 type H1
set O13.1.C3  type CT
set O13.1.H31 type H1
set O13.1.H32 type H1

O24 = loadmol2 mm3-o1.FG1.mol2
# General information
set O24 name "O24"
set O24 head O24.1.O1
set O24 tail O24.1.N
set O24.1 connect0 O24.1.O1
set O24.1 connect1 O24.1.N
set O24.1 restype undefined
set O24.1 name "O24"
# set FF atom types
set O24.1.O1  type OS
set O24.1.C2  type CT
set O24.1.H21 type H1
set O24.1.H22 type H1
set O24.1.C3  type CT
set O24.1.H31 type H1
set O24.1.H32 type H1
set O24.1.N   type N
set O24.1.H   type H

DFB = loadmol2 mm5-o1.FG2.mol2
# General information
set DFB name "DFB"
set DFB head DFB.1.C
set DFB tail DFB.2.C
# set FF atom types
set DFB.1.N1  type "N*"
set DFB.1.N2  type NB
set DFB.1.C3  type CC
set DFB.1.N4  type NX
set DFB.1.C5  type CC
set DFB.1.CB1 type CA
set DFB.1.CB2 type CA
set DFB.1.OB2 type OX
set DFB.1.CB3 type CA
set DFB.1.HB3 type HA
set DFB.1.CB4 type CA
set DFB.1.HB4 type HA
set DFB.1.CB5 type CA
set DFB.1.HB5 type HA
set DFB.1.CB6 type CA
set DFB.1.HB6 type HA
set DFB.1.CC1 type CA
set DFB.1.CC2 type CA
set DFB.1.OC2 type OY
set DFB.1.CC3 type CA
set DFB.1.HC3 type HA
set DFB.1.CC4 type CA
set DFB.1.HC4 type HA
set DFB.1.CC5 type CA
set DFB.1.HC5 type HA
set DFB.1.CC6 type CA
set DFB.1.HC6 type HA
set DFB.1.CX1 type CA
set DFB.1.CX2 type CA
set DFB.1.HX2 type HA
set DFB.1.CX3 type CA
set DFB.1.HX3 type HA
```

```
set DFB.1.CX4 type CA
set DFB.1.C   type C
set DFB.1.O   type O
set DFB.1.CX5 type CA
set DFB.1.HX5 type HA
set DFB.1.CX6 type CA
set DFB.1.HX6 type HA
set DFB.2.N1  type "N*"
set DFB.2.N2  type NB
set DFB.2.C3  type CC
set DFB.2.N4  type NX
set DFB.2.C5  type CC
set DFB.2.CB1 type CA
set DFB.2.CB2 type CA
set DFB.2.OB2 type OZ
set DFB.2.CB3 type CA
set DFB.2.HB3 type HA
set DFB.2.CB4 type CA
set DFB.2.HB4 type HA
set DFB.2.CB5 type CA
set DFB.2.HB5 type HA
set DFB.2.CB6 type CA
set DFB.2.HB6 type HA
set DFB.2.CC1 type CA
set DFB.2.CC2 type CA
set DFB.2.OC2 type OA
set DFB.2.CC3 type CA
set DFB.2.HC3 type HA
set DFB.2.CC4 type CA
set DFB.2.HC4 type HA
set DFB.2.CC5 type CA
set DFB.2.HC5 type HA
set DFB.2.CC6 type CA
set DFB.2.HC6 type HA
set DFB.2.CX1 type CA
set DFB.2.CX2 type CA
set DFB.2.HX2 type HA
set DFB.2.CX3 type CA
set DFB.2.HX3 type HA
set DFB.2.CX4 type CA
set DFB.2.C   type C
set DFB.2.O   type O
set DFB.2.CX5 type CA
set DFB.2.HX5 type HA
set DFB.2.CX6 type CA
set DFB.2.HX6 type HA
set DFB.3.Fe  type FE

DFF = loadmol2 mm4-o1.FG2.mol2
# General information
set DFF name "DFF"
set DFF head DFF.1.C
set DFF tail DFF.2.C
# set FF atom types
set DFF.1.N1  type "N*"
set DFF.1.N2  type NB
set DFF.1.C3  type CC
set DFF.1.N4  type NB
set DFF.1.C5  type CC
set DFF.1.CB1 type CA
set DFF.1.CB2 type CA
set DFF.1.OB2 type O
set DFF.1.CB3 type CA
set DFF.1.HB3 type HA
set DFF.1.CB4 type CA
set DFF.1.HB4 type HA
set DFF.1.CB5 type CA
set DFF.1.HB5 type HA
set DFF.1.CB6 type CA
set DFF.1.HB6 type HA
set DFF.1.CC1 type CA
set DFF.1.CC2 type CA
set DFF.1.OC2 type O
set DFF.1.CC3 type CA
set DFF.1.HC3 type HA
set DFF.1.CC4 type CA
set DFF.1.HC4 type HA
```

```
set DFF.1.CC5 type CA
set DFF.1.HC5 type HA
set DFF.1.CC6 type CA
set DFF.1.HC6 type HA
set DFF.1.CX1 type CA
set DFF.1.CX2 type CA
set DFF.1.HX2 type HA
set DFF.1.CX3 type CA
set DFF.1.HX3 type HA
set DFF.1.CX4 type CA
set DFF.1.C   type C
set DFF.1.O   type O
set DFF.1.CX5 type CA
set DFF.1.HX5 type HA
set DFF.1.CX6 type CA
set DFF.1.HX6 type HA
set DFF.2.N1  type "N*"
set DFF.2.N2  type NB
set DFF.2.C3  type CC
set DFF.2.N4  type NB
set DFF.2.C5  type CC
set DFF.2.CB1 type CA
set DFF.2.CB2 type CA
set DFF.2.OB2 type O
set DFF.2.CB3 type CA
set DFF.2.HB3 type HA
set DFF.2.CB4 type CA
set DFF.2.HB4 type HA
set DFF.2.CB5 type CA
set DFF.2.HB5 type HA
set DFF.2.CB6 type CA
set DFF.2.HB6 type HA
set DFF.2.CC1 type CA
set DFF.2.CC2 type CA
set DFF.2.OC2 type O
set DFF.2.CC3 type CA
set DFF.2.HC3 type HA
set DFF.2.CC4 type CA
set DFF.2.HC4 type HA
set DFF.2.CC5 type CA
set DFF.2.HC5 type HA
set DFF.2.CC6 type CA
set DFF.2.HC6 type HA
set DFF.2.CX1 type CA
set DFF.2.CX2 type CA
set DFF.2.HX2 type HA
set DFF.2.CX3 type CA
set DFF.2.HX3 type HA
set DFF.2.CX4 type CA
set DFF.2.C   type C
set DFF.2.O   type O
set DFF.2.CX5 type CA
set DFF.2.HX5 type HA
set DFF.2.CX6 type CA
set DFF.2.HX6 type HA
set DFF.3.Fe  type FE

ICLB = loadmol2 mm6-o1.mol2
# General information
set ICLB name "ICLB"
# set FF atom types
set ICLB.1.N1  type "N*"
set ICLB.1.N2  type NB
set ICLB.1.C3  type CC
set ICLB.1.N4  type NX
set ICLB.1.C5  type CC
set ICLB.1.CB1 type CA
set ICLB.1.CB2 type CA
set ICLB.1.OB2 type OX
set ICLB.1.CB3 type CA
set ICLB.1.HB3 type HA
set ICLB.1.CB4 type CA
set ICLB.1.HB4 type HA
set ICLB.1.CB5 type CA
set ICLB.1.HB5 type HA
set ICLB.1.CB6 type CA
set ICLB.1.HB6 type HA
```

```
set ICLB.1.CC1 type CA
set ICLB.1.CC2 type CA
set ICLB.1.OC2 type OY
set ICLB.1.CC3 type CA
set ICLB.1.HC3 type HA
set ICLB.1.CC4 type CA
set ICLB.1.HC4 type HA
set ICLB.1.CC5 type CA
set ICLB.1.HC5 type HA
set ICLB.1.CC6 type CA
set ICLB.1.HC6 type HA
set ICLB.1.CX1 type CA
set ICLB.1.CX2 type CA
set ICLB.1.HX2 type HA
set ICLB.1.CX3 type CA
set ICLB.1.HX3 type HA
set ICLB.1.CX4 type CA
set ICLB.1.HX4 type HA
set ICLB.1.CX5 type CA
set ICLB.1.HX5 type HA
set ICLB.1.CX6 type CA
set ICLB.1.HX6 type HA
set ICLB.2.N1  type "N*"
set ICLB.2.N2  type NB
set ICLB.2.C3  type CC
set ICLB.2.N4  type NX
set ICLB.2.C5  type CC
set ICLB.2.CB1 type CA
set ICLB.2.CB2 type CA
set ICLB.2.OB2 type OZ
set ICLB.2.CB3 type CA
set ICLB.2.HB3 type HA
set ICLB.2.CB4 type CA
set ICLB.2.HB4 type HA
set ICLB.2.CB5 type CA
set ICLB.2.HB5 type HA
set ICLB.2.CB6 type CA
set ICLB.2.HB6 type HA
set ICLB.2.CC1 type CA
set ICLB.2.CC2 type CA
set ICLB.2.OC2 type OA
set ICLB.2.CC3 type CA
set ICLB.2.HC3 type HA
set ICLB.2.CC4 type CA
set ICLB.2.HC4 type HA
set ICLB.2.CC5 type CA
set ICLB.2.HC5 type HA
set ICLB.2.CC6 type CA
set ICLB.2.HC6 type HA
set ICLB.2.CX1 type CA
set ICLB.2.CX2 type CA
set ICLB.2.HX2 type HA
set ICLB.2.CX3 type CA
set ICLB.2.HX3 type HA
set ICLB.2.CX4 type CA
set ICLB.2.HX4 type HA
set ICLB.2.CX5 type CA
set ICLB.2.HX5 type HA
set ICLB.2.CX6 type CA
set ICLB.2.HX6 type HA
set ICLB.3.Fe  type FE

ICLF = loadmol2 mm7-o1.mol2
# General information
set ICLF name "ICLF"
# set FF atom types
set ICLF.1.N1  type "N*"
set ICLF.1.N2  type NB
set ICLF.1.C3  type CC
set ICLF.1.N4  type NB
set ICLF.1.C5  type CC
set ICLF.1.CB1 type CA
set ICLF.1.CB2 type CA
set ICLF.1.OB2 type O
set ICLF.1.CB3 type CA
set ICLF.1.HB3 type HA
set ICLF.1.CB4 type CA
```

```
set ICLF.1.HB4 type HA
set ICLF.1.CB5 type CA
set ICLF.1.HB5 type HA
set ICLF.1.CB6 type CA
set ICLF.1.HB6 type HA
set ICLF.1.CC1 type CA
set ICLF.1.CC2 type CA
set ICLF.1.OC2 type O
set ICLF.1.CC3 type CA
set ICLF.1.HC3 type HA
set ICLF.1.CC4 type CA
set ICLF.1.HC4 type HA
set ICLF.1.CC5 type CA
set ICLF.1.HC5 type HA
set ICLF.1.CC6 type CA
set ICLF.1.HC6 type HA
set ICLF.1.CX1 type CA
set ICLF.1.CX2 type CA
set ICLF.1.HX2 type HA
set ICLF.1.CX3 type CA
set ICLF.1.HX3 type HA
set ICLF.1.CX4 type CA
set ICLF.1.HX4 type HA
set ICLF.1.CX5 type CA
set ICLF.1.HX5 type HA
set ICLF.1.CX6 type CA
set ICLF.1.HX6 type HA
set ICLF.2.N1  type "N*"
set ICLF.2.N2  type NB
set ICLF.2.C3  type CC
set ICLF.2.N4  type NB
set ICLF.2.C5  type CC
set ICLF.2.CB1 type CA
set ICLF.2.CB2 type CA
set ICLF.2.OB2 type O
set ICLF.2.CB3 type CA
set ICLF.2.HB3 type HA
set ICLF.2.CB4 type CA
set ICLF.2.HB4 type HA
set ICLF.2.CB5 type CA
set ICLF.2.HB5 type HA
set ICLF.2.CB6 type CA
set ICLF.2.HB6 type HA
set ICLF.2.CC1 type CA
set ICLF.2.CC2 type CA
set ICLF.2.OC2 type O
set ICLF.2.CC3 type CA
set ICLF.2.HC3 type HA
set ICLF.2.CC4 type CA
set ICLF.2.HC4 type HA
set ICLF.2.CC5 type CA
set ICLF.2.HC5 type HA
set ICLF.2.CC6 type CA
set ICLF.2.HC6 type HA
set ICLF.2.CX1 type CA
set ICLF.2.CX2 type CA
set ICLF.2.HX2 type HA
set ICLF.2.CX3 type CA
set ICLF.2.HX3 type HA
set ICLF.2.CX4 type CA
set ICLF.2.HX4 type HA
set ICLF.2.CX5 type CA
set ICLF.2.HX5 type HA
set ICLF.2.CX6 type CA
set ICLF.2.HX6 type HA
set ICLF.3.Fe  type FE

C1 = sequence { PHP O13 O24 }
set C1 name "C1"
set C1 head C1.1.CM
set C1 tail C1.1.C6
set C1.1 connect0 C1.1.CM
set C1.1 connect1 C1.1.C6

CLX = sequence { C1 PHO C1 PHO }
CLX2 = sequence { C1 PHO PHO PHO }
```

```
i CLX {1 4 5 8} {
  {"C6" "C1" "C2" "CM"  176.4}
  {"CM" "C6" "C1" "C2" -176.2}
  {"C2" "CM" "C6" "C3"  -80.6}
  {"C3" "C2" "CM" "C6"   83.1}
  {"C2" "CM" "C6" "C1"   92.6}
  {"C1" "C2" "CM" "C6"  -92.6}
}

i CLX2 {1 4 5 6} {
  {"C6" "C1" "C2" "CM"  176.4}
  {"CM" "C6" "C1" "C2" -176.2}
  {"C2" "CM" "C6" "C3"  -80.6}
  {"C3" "C2" "CM" "C6"   83.1}
  {"C2" "CM" "C6" "C1"   92.6}
  {"C1" "C2" "CM" "C6"  -92.6}
}

bond CLX.1.CM CLX.8.C6
bond CLX2.1.CM CLX2.6.C6

# Prepare geometry
i CLX {1 5} {{"C2" "C1" "O1" "C2" 270.0}}
i CLX {3 7} {{ "O1" "C2" "C3" "N" -90.0 }}
set CLX head CLX.3.N
set CLX tail CLX.7.N

set CLX2 head CLX2.3.N

5bICLb = seq { DFB CLX }
bond C2ICLb.1.C C2ICLb.10.N
5bICLf = seq { DFF CLX }
bond C2ICLf.1.C C2ICLf.10.N

XX = sequence { DFB CLX2 }
YY = sequence { DFF CLX2 }
set XX tail XX.1.C
set YY tail YY.1.C

5aICLb = sequence { XX CLX2 }
5aICLf = sequence { YY CLX2 }

c DFB
c DFF
c 5aICLb
c 5aICLf
c 5bICLb
c 5bICLf

# See http://q4md-forcefieldtools.org/Tutorial/leap.php


###############################################################################
#                                                                             #
#           The frcmod (script3.ff) file for  Leap/xleap                      #
#                                                                             #
###############################################################################

MASS
OX     16.00                          new oxygen atom type (connecting Fe)
OY     16.00                          new oxygen atom type (connecting Fe)
OZ     16.00                          new oxygen atom type (connecting Fe)
OA     16.00                          new oxygen atom type (connecting Fe)
NX     14.01                          new nitrogen atom type (connecting Fe)
FE     55.85

BOND
NX-CC  440.0    1.371                 adapted from GAFF (same as na-cc)
N*-CC  440.0    1.371                 adapted from GAFF (same as na-cc)
N*-CA  470.0    1.350                 adapted from GAFF (same as na-ca)
N*-NB  535.0    1.350                 adapted from GAFF (same as na-nc)
CA-CC  410.0    1.434                 adapted from GAFF (same as ca-cc)
CA-OX  570.0    1.323                 from parm99 (same as C -OS)
CA-OY  570.0    1.323
CA-OZ  570.0    1.323
CA-OA  570.0    1.323
CA-O   570.0    1.229                 from parm99 (same as C -O)
```

```
NX-FE   40.0     2.15                derived from B3LYP calculation
OX-FE   80.0    1.970                derived from B3LYP calculation
OY-FE   80.0    1.970                derived from B3LYP calculation
OZ-FE   80.0    1.970                derived from B3LYP calculation
OA-FE   80.0    1.970                derived from B3LYP calculation

ANGL
N*-NB-CC    75.0      102.97          adapted from GAFF (same as cc-nc-na)
N*-CC-NX    70.0      125.32          adapted from GAFF (same as na-cc-nc)
N*-CC-NB    70.0      125.32          adapted from GAFF (same as na-cc-nc)
NB-CC-NX    70.0      125.32          adapted from GAFF (same as na-cc-nc)
NB-CC-NB    70.0      125.32          adapted from GAFF (same as na-cc-nc)
N*-CC-CA    70.0      115.05          adapted from GAFF (same as ca-cc-nd)
N*-CA-CA    70.0      118.34          adapted from GAFF (same as ca-ca-na)
NB-CC-CA    70.0      115.05          adapted from GAFF (same as ca-ca-nd)
CC-NX-CC    70.0      109.90          adapted from GAFF (same as ca-na-CC)
CC-NB-CC    70.0      109.90          adapted from GAFF (same as ca-na-CC)
CA-CC-NX    70.0      115.05          adapted from GAFF (same as ca-cc-nd)
CA-N*-NB    70.0      117.85          adapted from GAFF (same as ca-na-nc)
CA-N*-CC    70.0      113.15          adapted from GAFF (same as ca-na-cc)
CC-N*-NB    70.0      113.02          adapted from GAFF (same as cc-na-nc)
CA-CA-CC    65.0      120.10          adapted from GAFF (same as ca-ca-cc)
CA-CA-CC    65.0      120.10          adapted from GAFF (same as ca-ca-cc)
CA-CA-OX    70.0      119.94          adapted from GAFF (same as ca-ca-oh)
CA-CA-OY    70.0      119.94
CA-CA-OZ    70.0      119.94
CA-CA-OA    70.0      119.94
CA-CA-O     70.0      123.43          adapted from GAFF (same as ca-ca-o)
CA-C -O     70.0      123.44          adapted from GAFF (same as ca-c -o)
CA-C -N     70.0      112.03          adapted from GAFF (same as ca-c -n)
CT-CA-C     65.0      118.06          adapted from GAFF (same as c -ca-c3)
CA-CT-CA    65.0      112.47          adapted from GAFF (same as ca-c3-ca)
CA-C -OS    70.0      115.54          adapted from GAFF (same as ca-c -os)
CC-NX-FE   100.0      126.30          derived from B3LYP calculation
CA-OX-FE    70.0      135.40          derived from B3LYP calculation
CA-OY-FE    70.0      131.00          derived from B3LYP calculation
CA-OZ-FE    70.0      135.40          derived from B3LYP calculation
CA-OA-FE    70.0      131.00          derived from B3LYP calculation
NX-FE-OX    85.0       90.00          derived from B3LYP calculation
NX-FE-OY    85.0       90.00          derived from B3LYP calculation
NX-FE-OZ    85.0       90.00          derived from B3LYP calculation
NX-FE-OA    85.0       90.00          derived from B3LYP calculation
NX-FE-NX    50.0      180.00          derived from B3LYP calculation
OX-FE-OY    95.0      180.00          derived from B3LYP calculation
OX-FE-OZ    75.0       90.00          derived from B3LYP calculation
OX-FE-OA    75.0       90.00          derived from B3LYP calculation
OY-FE-OZ    75.0       90.00          derived from B3LYP calculation
OY-FE-OA    75.0       90.00          derived from B3LYP calculation
OZ-FE-OA    95.0      180.00          derived from B3LYP calculation

DIHEDRAL
X- CC-NX-X    4    6.800 180.00   2.000    adapted from GAFF (same as X- cc-na-X)
X- CC-N*-X    4    6.800 180.00   0.000    adapted from GAFF (same as X- cc-na-X)
X- CA-N*-X    4    6.800 180.00   2.000    adapted from GAFF (same as X- cc-na-X)
X- N*-NB-X    2    9.600 180.00   2.000    adapted from GAFF (same as X- na-nc-X)
X -CA-OX-X    2    1.800 180.00   2.000    adapted from GAFF (same as X- CA-oh-X)
X -CA-OY-X    2    1.800 180.00   2.000
X -CA-OZ-X    2    1.800 180.00   2.000    adapted from GAFF (same as X- ca-oh-X)
X -CA-OA-X    2    1.800 180.00   2.000
X -NX-FE-X    1    0.00    0.00   2.0      estimated
X -OX-FE-X    1    0.00    0.00   2.0      estimated
X -OY-FE-X    1    0.00    0.00   2.0
X -OZ-FE-X    1    0.00    0.00   2.0
X -OA-FE-X    1    0.00    0.00   2.0
X -CA-CC-X    4    4.000 180.000  2.000    adapted from GAFF (same as X- ce-ce-X)

IMPROPER

NONB
  NX         1.8240    0.1700            OPLS
  OX         1.6612    0.2100            OPLS
  OY         1.6612    0.2100            OPLS
  OZ         1.6612    0.2100            OPLS
  OA         1.6612    0.2100            OPLS
  FE         1.2000    0.0500            Amber contrib...
```

```
################################################################################
#                            The eight .mol2 files                             #
#                                                                              #
################################################################################

@<TRIPOS>MOLECULE
CAL
   12    12     1     0     1
SMALL
USER_CHARGES
@<TRIPOS>ATOM
  1 C1          0.431905   -0.653001    0.000003 C         1 CAL        0.2416 ****
  2 C2         -0.810960    0.006720    0.000000 C         1 CAL       -0.0152 ****
  3 C3         -0.801940    1.403569   -0.000006 C         1 CAL       -0.1892 ****
  4 H3         -1.744782    1.941490   -0.000009 H         1 CAL        0.1354 ****
  5 C4          0.395073    2.123658   -0.000010 C         1 CAL       -0.1799 ****
  6 H4          0.377472    3.209834   -0.000016 H         1 CAL        0.1357 ****
  7 C5          1.609163    1.443017   -0.000008 C         1 CAL       -0.1892 ****
  8 H5          2.545562    1.996434   -0.000011 H         1 CAL        0.1354 ****
  9 C6          1.650592    0.044067   -0.000001 C         1 CAL       -0.0152 ****
 10 CM         -2.077641   -0.828191    0.000005 C         1 CAL        0.0004 ****
 11 HM1        -2.052695   -1.500210   -0.868962 H         1 CAL        0.0427 ****
 12 HM2        -2.052694   -1.500202    0.868978 H         1 CAL        0.0427 ****
@<TRIPOS>BOND
    1     1     2 1
    2     1     9 1
    3     2     3 1
    4     2    10 1
    5     3     4 1
    6     3     5 1
    7     5     6 1
    8     5     7 1
    9     7     8 1
   10     7     9 1
   11    10    11 1
   12    10    12 1
@<TRIPOS>SUBSTRUCTURE
      1  CAL             1 ****               0 ****  ****

--------------------------------------------------------------------------------

@<TRIPOS>MOLECULE
CAL
   14    14     1     0     1
SMALL
USER_CHARGES
@<TRIPOS>ATOM
  1 C1          0.431905   -0.653001    0.000003 C         1 CAL        0.2416 ****
  2 C2         -0.810960    0.006720    0.000000 C         1 CAL       -0.0152 ****
  3 C3         -0.801940    1.403569   -0.000006 C         1 CAL       -0.1892 ****
  4 H3         -1.744782    1.941490   -0.000009 H         1 CAL        0.1354 ****
  5 C4          0.395073    2.123658   -0.000010 C         1 CAL       -0.1799 ****
  6 H4          0.377472    3.209834   -0.000016 H         1 CAL        0.1357 ****
  7 C5          1.609163    1.443017   -0.000008 C         1 CAL       -0.1892 ****
  8 H5          2.545562    1.996434   -0.000011 H         1 CAL        0.1354 ****
  9 C6          1.650592    0.044067   -0.000001 C         1 CAL       -0.0152 ****
 10 OX          0.388185   -2.027215    0.000009 O         1 CAL       -0.5632 ****
 11 HX          1.292757   -2.373587    0.000010 H         1 CAL        0.4180 ****
 12 CM         -2.077641   -0.828191    0.000005 C         1 CAL        0.0004 ****
 13 HM1        -2.052695   -1.500210   -0.868962 H         1 CAL        0.0427 ****
 14 HM2        -2.052694   -1.500202    0.868978 H         1 CAL        0.0427 ****
@<TRIPOS>BOND
    1     1     2 1
    2     1     9 1
    3     1    10 1
    4     2     3 1
    5     2    12 1
    6     3     4 1
    7     3     5 1
    8     5     6 1
    9     5     7 1
   10     7     8 1
   11     7     9 1
   12    10    11 1
   13    12    13 1
   14    12    14 1
@<TRIPOS>SUBSTRUCTURE
```

```
      1  CAL              1 ****               0 ****  ****

-------------------------------------------------------------------------------
---------

@<TRIPOS>MOLECULE
O13
    7     6     1     0     1
SMALL
USER_CHARGES
@<TRIPOS>ATOM
  1 O1          -1.141287    0.507431    0.000001 O         1 O13       -0.3221 ****
  2 C2          -0.025641   -0.360467   -0.000001 C         1 O13        0.1483 ****
  3 H21         -0.027666   -1.012428    0.889156 H         1 O13        0.0269 ****
  4 H22         -0.027668   -1.012427   -0.889158 H         1 O13        0.0269 ****
  5 C3           1.229620    0.498282   -0.000002 C         1 O13        0.1551 ****
  6 H31          1.221044    1.147089    0.889129 H         1 O13        0.0040 ****
  7 H32          1.221043    1.147089   -0.889133 H         1 O13        0.0040 ****
@<TRIPOS>BOND
    1     1     2 1
    2     2     3 1
    3     2     4 1
    4     2     5 1
    5     5     6 1
    6     5     7 1
@<TRIPOS>SUBSTRUCTURE
      1  O13              1 ****               0 ****  ****

-------------------------------------------------------------------------------

@<TRIPOS>MOLECULE
O24
    9     8     1     0     1
SMALL
USER_CHARGES
@<TRIPOS>ATOM
  1 O1           2.124518    0.339551   -0.341031 O         1 O24       -0.3609 ****
  2 C2           1.510579   -0.777608    0.282975 C         1 O24        0.1134 ****
  3 H21          2.170401   -1.660169    0.233944 H         1 O24        0.0364 ****
  4 H22          1.310241   -0.560843    1.345164 H         1 O24        0.0364 ****
  5 C3           0.208034   -1.057193   -0.452187 C         1 O24        0.0013 ****
  6 H31         -0.327228   -1.889114    0.011569 H         1 O24        0.0640 ****
  7 H32          0.433562   -1.328800   -1.492288 H         1 O24        0.0640 ****
  8 N           -0.675618    0.093165   -0.412110 N         1 O24       -0.4145 ****
  9 H           -0.273110    0.983538   -0.667267 H         1 O24        0.2716 ****
@<TRIPOS>BOND
    1     1     2 1
    2     2     3 1
    3     2     4 1
    4     2     5 1
    5     5     6 1
    6     5     7 1
    7     5     8 1
    8     8     9 1
@<TRIPOS>SUBSTRUCTURE
      1  O24              1 ****               0 ****  ****

-------------------------------------------------------------------------------

 @<TRIPOS>MOLECULE
ICL-ICL-FE
   79    84     3     0     1
SMALL
USER_CHARGES
@<TRIPOS>ATOM
  1 N1           4.170414   -0.513113   -0.296944 N         1 ICL        0.1191 ****
  2 N2           3.975636   -1.680176   -1.000507 N         1 ICL       -0.5270 ****
  3 C3           2.716803   -1.594686   -1.410066 C         1 ICL        0.8593 ****
  4 N4           2.112782   -0.424560   -1.012532 N         1 ICL       -1.1427 ****
  5 C5           3.037296    0.261861   -0.334997 C         1 ICL        0.6025 ****
  6 CB1          2.061562   -2.629554   -2.200951 C         1 ICL       -0.5790 ****
  7 CB2          0.714275   -2.469295   -2.654174 C         1 ICL        0.7520 ****
  8 OB2         -0.019914   -1.418666   -2.402202 O         1 ICL       -1.0014 ****
  9 CB3          0.167157   -3.518211   -3.444115 C         1 ICL       -0.3698 ****
 10 HB3         -0.854667   -3.390344   -3.789362 H         1 ICL        0.1650 ****
 11 CB4          0.896858   -4.652002   -3.756962 C         1 ICL       -0.0546 ****
 12 HB4          0.439248   -5.433635   -4.361248 H         1 ICL        0.0955 ****
```

```
 13 CB5         2.217745   -4.802598   -3.300886 C         1 ICL       -0.2607 ****
 14 HB5         2.790856   -5.693611   -3.543557 H         1 ICL        0.1131 ****
 15 CB6         2.780048   -3.794128   -2.532846 C         1 ICL        0.0684 ****
 16 HB6         3.799766   -3.884644   -2.169522 H         1 ICL        0.0607 ****
 17 CC1         2.861420    1.610426    0.178294 C         1 ICL       -0.5208 ****
 18 CC2         1.530020    2.048206    0.504434 C         1 ICL        0.7867 ****
 19 OC2         0.479656    1.304234    0.357081 O         1 ICL       -0.9757 ****
 20 CC3         1.399915    3.360636    1.046731 C         1 ICL       -0.4210 ****
 21 HC3         0.397847    3.681397    1.315336 H         1 ICL        0.1636 ****
 22 CC4         2.487139    4.201958    1.187513 C         1 ICL        0.0113 ****
 23 HC4         2.339806    5.204974    1.584204 H         1 ICL        0.0927 ****
 24 CC5         3.775323    3.786988    0.800796 C         1 ICL       -0.3161 ****
 25 HC5         4.621855    4.464286    0.872076 H         1 ICL        0.1294 ****
 26 CC6         3.945005    2.505488    0.301821 C         1 ICL        0.0216 ****
 27 HC6         4.931714    2.189549   -0.022657 H         1 ICL        0.0835 ****
 28 CX1         5.370828   -0.390112    0.446947 C         1 ICL        0.1370 ****
 29 CX2         6.570881   -0.834698   -0.123433 C         1 ICL       -0.1837 ****
 30 HX2         6.553503   -1.246813   -1.125657 H         1 ICL        0.1499 ****
 31 CX3         7.752702   -0.754469    0.603961 C         1 ICL       -0.0472 ****
 32 HX3         8.690192   -1.097024    0.178731 H         1 ICL        0.0900 ****
 33 CX4         7.765353   -0.223115    1.901126 C         1 ICL       -0.1246 ****
 34 CX5         6.549883    0.185738    2.472181 C         1 ICL       -0.0472 ****
 35 HX5         6.511428    0.531853    3.501633 H         1 ICL        0.0900 ****
 36 CX6         5.358379    0.097164    1.759840 C         1 ICL       -0.1837 ****
 37 HX6         4.421602    0.395539    2.215330 H         1 ICL        0.1499 ****
 38 C           9.084361   -0.143759    2.609724 C         1 ICL        0.4655 ****
 39 O          10.051036   -0.824569    2.272411 O         1 ICL       -0.4515 ****
 40 N1         -4.170416    0.513140   -0.296898 N         2 ICL        0.1191 ****
 41 N2         -3.975644    1.680251   -1.000382 N         2 ICL       -0.5270 ****
 42 C3         -2.716808    1.594798   -1.409943 C         2 ICL        0.8593 ****
 43 N4         -2.112781    0.424647   -1.012488 N         2 ICL       -1.1427 ****
 44 C5         -3.037293   -0.261825   -0.335001 C         2 ICL        0.6025 ****
 45 CB1        -2.061570    2.629725   -2.200753 C         2 ICL       -0.5790 ****
 46 CB2        -0.714287    2.469496   -2.653999 C         2 ICL        0.7520 ****
 47 OB2         0.019898    1.418842   -2.402118 O         2 ICL       -1.0014 ****
 48 CB3        -0.167171    3.518471   -3.443863 C         2 ICL       -0.3698 ****
 49 HB3         0.854650    3.390628   -3.789127 H         2 ICL        0.1650 ****
 50 CB4        -0.896871    4.652289   -3.756617 C         2 ICL       -0.0546 ****
 51 HB4        -0.439264    5.433966   -4.360846 H         2 ICL        0.0955 ****
 52 CB5        -2.217754    4.802854   -3.300521 C         2 ICL       -0.2607 ****
 53 HB5        -2.790865    5.693886   -3.543119 H         2 ICL        0.1131 ****
 54 CB6        -2.780055    3.794326   -2.532553 C         2 ICL        0.0684 ****
 55 HB6        -3.799770    3.884817   -2.169214 H         2 ICL        0.0607 ****
 56 CC1        -2.861408   -1.610423    0.178197 C         2 ICL       -0.5208 ****
 57 CC2        -1.530005   -2.048216    0.504309 C         2 ICL        0.7867 ****
 58 OC2        -0.479647   -1.304227    0.357007 O         2 ICL       -0.9757 ****
 59 CC3        -1.399893   -3.360682    1.046520 C         2 ICL       -0.4210 ****
 60 HC3        -0.397823   -3.681454    1.315105 H         2 ICL        0.1636 ****
 61 CC4        -2.487112   -4.202019    1.187246 C         2 ICL        0.0113 ****
 62 HC4        -2.339772   -5.205060    1.583871 H         2 ICL        0.0927 ****
 63 CC5        -3.775298   -3.787033    0.800554 C         2 ICL       -0.3161 ****
 64 HC5        -4.621825   -4.464341    0.871788 H         2 ICL        0.1294 ****
 65 CC6        -3.944988   -2.505501    0.301664 C         2 ICL        0.0216 ****
 66 HC6        -4.931698   -2.189546   -0.022795 H         2 ICL        0.0835 ****
 67 CX1        -5.370831    0.390079    0.446981 C         2 ICL        0.1370 ****
 68 CX2        -6.570887    0.834691   -0.123372 C         2 ICL       -0.1837 ****
 69 HX2        -6.553510    1.246873   -1.125569 H         2 ICL        0.1499 ****
 70 CX3        -7.752709    0.754397    0.604012 C         2 ICL       -0.0472 ****
 71 HX3        -8.690202    1.096967    0.178800 H         2 ICL        0.0900 ****
 72 CX4        -7.765359    0.222952    1.901139 C         2 ICL       -0.1246 ****
 73 CX5        -6.549885   -0.185923    2.472171 C         2 ICL       -0.0472 ****
 74 HX5        -6.511432   -0.532107    3.501600 H         2 ICL        0.0900 ****
 75 CX6        -5.358381   -0.097286    1.759842 C         2 ICL       -0.1837 ****
 76 HX6        -4.421602   -0.395681    2.215314 H         2 ICL        0.1499 ****
 77 C          -9.084369    0.143526    2.609726 C         2 ICL        0.4655 ****
 78 O         -10.051050    0.824351    2.272464 O         2 ICL       -0.4515 ****
 79 Fe          0.000000    0.000049   -1.075960 FE        3 FE         3.0000 ****
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SMALL
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  2 N2          3.975636   -1.680176   -1.000507 N         1 ICL        -0.4858 ****
  3 C3          2.716803   -1.594686   -1.410066 C         1 ICL         0.5248 ****
  4 N4          2.112782   -0.424560   -1.012532 N         1 ICL        -0.3840 ****
  5 C5          3.037296    0.261861   -0.334997 C         1 ICL         0.2470 ****
  6 CB1         2.061562   -2.629554   -2.200951 C         1 ICL        -0.4095 ****
  7 CB2         0.714275   -2.469295   -2.654174 C         1 ICL         0.6626 ****
  8 OB2        -0.019914   -1.418666   -2.402202 O         1 ICL        -0.7010 ****
  9 CB3         0.167157   -3.518211   -3.444115 C         1 ICL        -0.3742 ****
 10 HB3        -0.854667   -3.390344   -3.789362 H         1 ICL         0.1518 ****
 11 CB4         0.896858   -4.652002   -3.756962 C         1 ICL        -0.0289 ****
 12 HB4         0.439248   -5.433635   -4.361248 H         1 ICL         0.0935 ****
 13 CB5         2.217745   -4.802598   -3.300886 C         1 ICL        -0.3015 ****
 14 HB5         2.790856   -5.693611   -3.543557 H         1 ICL         0.1243 ****
 15 CB6         2.780048   -3.794128   -2.532846 C         1 ICL         0.0508 ****
 16 HB6         3.799766   -3.884644   -2.169522 H         1 ICL         0.0709 ****
 17 CC1         2.861420    1.610426    0.178294 C         1 ICL        -0.3247 ****
 18 CC2         1.530020    2.048206    0.504434 C         1 ICL         0.6801 ****
 19 OC2         0.479656    1.304234    0.357081 O         1 ICL        -0.6806 ****
 20 CC3         1.399915    3.360636    1.046731 C         1 ICL        -0.4302 ****
 21 HC3         0.397847    3.681397    1.315336 H         1 ICL         0.1663 ****
 22 CC4         2.487139    4.201958    1.187513 C         1 ICL         0.0183 ****
 23 HC4         2.339806    5.204974    1.584204 H         1 ICL         0.0898 ****
 24 CC5         3.775323    3.786988    0.800796 C         1 ICL        -0.3052 ****
 25 HC5         4.621855    4.464286    0.872076 H         1 ICL         0.1279 ****
 26 CC6         3.945005    2.505488    0.301821 C         1 ICL        -0.0315 ****
 27 HC6         4.931714    2.189549   -0.022657 H         1 ICL         0.0938 ****
 28 CX1         5.370828   -0.390112    0.446947 C         1 ICL         0.1543 ****
 29 CX2         6.570881   -0.834698   -0.123433 C         1 ICL        -0.1849 ****
 30 HX2         6.553503   -1.246813   -1.125657 H         1 ICL         0.1414 ****
 31 CX3         7.752702   -0.754469    0.603961 C         1 ICL        -0.0438 ****
 32 HX3         8.690192   -1.097024    0.178731 H         1 ICL         0.0912 ****
 33 CX4         7.765353   -0.223115    1.901126 C         1 ICL        -0.1370 ****
 34 CX5         6.549883    0.185738    2.472181 C         1 ICL        -0.0438 ****
 35 HX5         6.511428    0.531853    3.501633 H         1 ICL         0.0912 ****
 36 CX6         5.358379    0.097164    1.759840 C         1 ICL        -0.1849 ****
 37 HX6         4.421602    0.395539    2.215330 H         1 ICL         0.1414 ****
 38 C           9.084361   -0.143759    2.609724 C         1 ICL         0.4708 ****
 39 O          10.051036   -0.824569    2.272411 O         1 ICL        -0.4536 ****
 40 N1         -4.170416    0.513140   -0.296898 N         2 ICL         0.1694 ****
 41 N2         -3.975644    1.680251   -1.000382 N         2 ICL        -0.4858 ****
 42 C3         -2.716808    1.594798   -1.409943 C         2 ICL         0.5248 ****
 43 N4         -2.112781    0.424647   -1.012488 N         2 ICL        -0.3840 ****
 44 C5         -3.037293   -0.261825   -0.335001 C         2 ICL         0.2470 ****
 45 CB1        -2.061570    2.629725   -2.200753 C         2 ICL        -0.4095 ****
 46 CB2        -0.714287    2.469496   -2.653999 C         2 ICL         0.6626 ****
 47 OB2         0.019898    1.418842   -2.402118 O         2 ICL        -0.7010 ****
 48 CB3        -0.167171    3.518471   -3.443863 C         2 ICL        -0.3742 ****
 49 HB3         0.854650    3.390628   -3.789127 H         2 ICL         0.1518 ****
 50 CB4        -0.896871    4.652289   -3.756617 C         2 ICL        -0.0289 ****
 51 HB4        -0.439264    5.433966   -4.360846 H         2 ICL         0.0935 ****
 52 CB5        -2.217754    4.802854   -3.300521 C         2 ICL        -0.3015 ****
 53 HB5        -2.790865    5.693886   -3.543119 H         2 ICL         0.1243 ****
 54 CB6        -2.780055    3.794326   -2.532553 C         2 ICL         0.0508 ****
 55 HB6        -3.799770    3.884817   -2.169214 H         2 ICL         0.0709 ****
 56 CC1        -2.861408   -1.610423    0.178197 C         2 ICL        -0.3247 ****
 57 CC2        -1.530005   -2.048216    0.504309 C         2 ICL         0.6801 ****
 58 OC2        -0.479647   -1.304227    0.357007 O         2 ICL        -0.6806 ****
 59 CC3        -1.399893   -3.360682    1.046520 C         2 ICL        -0.4302 ****
 60 HC3        -0.397823   -3.681454    1.315105 H         2 ICL         0.1663 ****
 61 CC4        -2.487112   -4.202019    1.187246 C         2 ICL         0.0183 ****
 62 HC4        -2.339772   -5.205060    1.583871 H         2 ICL         0.0898 ****
 63 CC5        -3.775298   -3.787033    0.800554 C         2 ICL        -0.3052 ****
 64 HC5        -4.621825   -4.464341    0.871788 H         2 ICL         0.1279 ****
 65 CC6        -3.944988   -2.505501    0.301664 C         2 ICL        -0.0315 ****
 66 HC6        -4.931698   -2.189546   -0.022795 H         2 ICL         0.0938 ****
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 67 CX1         -5.370831    0.390079    0.446981 C         2 ICL         0.1543 ****
 68 CX2         -6.570887    0.834691   -0.123372 C         2 ICL        -0.1849 ****
 69 HX2         -6.553510    1.246873   -1.125569 H         2 ICL         0.1414 ****
 70 CX3         -7.752709    0.754397    0.604012 C         2 ICL        -0.0438 ****
 71 HX3         -8.690202    1.096967    0.178800 H         2 ICL         0.0912 ****
 72 CX4         -7.765359    0.222952    1.901139 C         2 ICL        -0.1370 ****
 73 CX5         -6.549885   -0.185923    2.472171 C         2 ICL        -0.0438 ****
 74 HX5         -6.511432   -0.532107    3.501600 H         2 ICL         0.0912 ****
 75 CX6         -5.358381   -0.097286    1.759842 C         2 ICL        -0.1849 ****
 76 HX6         -4.421602   -0.395681    2.215314 H         2 ICL         0.1414 ****
 77 C           -9.084369    0.143526    2.609726 C         2 ICL         0.4708 ****
 78 O          -10.051050    0.824351    2.272464 O         2 ICL        -0.4536 ****
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  2 N2          4.049095    1.495508    0.452418 N         1 ICL       -0.5367 ****
  3 C3          2.783760    1.469035    0.851162 C         1 ICL        0.6581 ****
  4 N4          2.129259    0.330417    0.446492 N         1 ICL       -0.5172 ****
  5 C5          3.028518   -0.398273   -0.224257 C         1 ICL        0.4003 ****
  6 CB1         2.173073    2.533933    1.639416 C         1 ICL       -0.4571 ****
  7 CB2         0.818771    2.434305    2.089394 C         1 ICL        0.6660 ****
  8 OB2         0.039595    1.417537    1.836449 O         1 ICL       -0.6949 ****
  9 CB3         0.317703    3.507571    2.877764 C         1 ICL       -0.3788 ****
 10 HB3        -0.709869    3.426033    3.220190 H         1 ICL        0.1479 ****
 11 CB4         1.096736    4.607744    3.192312 C         1 ICL       -0.0175 ****
 12 HB4         0.672974    5.409549    3.795096 H         1 ICL        0.0884 ****
 13 CB5         2.424023    4.698527    2.739479 C         1 ICL       -0.3097 ****
 14 HB5         3.036209    5.563125    2.982960 H         1 ICL        0.1210 ****
 15 CB6         2.941913    3.664962    1.972776 C         1 ICL        0.0614 ****
 16 HB6         3.965575    3.708928    1.611687 H         1 ICL        0.0701 ****
 17 CC1         2.792336   -1.734415   -0.748637 C         1 ICL       -0.4029 ****
 18 CC2         1.443058   -2.111615   -1.075922 C         1 ICL        0.6852 ****
 19 OC2         0.424941   -1.323855   -0.923634 O         1 ICL       -0.6831 ****
 20 CC3         1.254430   -3.413570   -1.625286 C         1 ICL       -0.4153 ****
 21 HC3         0.238388   -3.688304   -1.892649 H         1 ICL        0.1614 ****
 22 CC4         2.303916   -4.300155   -1.777147 C         1 ICL       -0.0022 ****
 23 HC4         2.112111   -5.292693   -2.181428 H         1 ICL        0.0923 ****
 24 CC5         3.609735   -3.943015   -1.394092 C         1 ICL       -0.3017 ****
 25 HC5         4.427752   -4.653085   -1.478423 H         1 ICL        0.1233 ****
 26 CC6         3.835839   -2.673518   -0.885713 C         1 ICL        0.0335 ****
 27 HC6         4.837290   -2.402213   -0.566890 H         1 ICL        0.0621 ****
 28 CX1         5.407463    0.134929   -0.968675 C         1 ICL        0.3083 ****
 29 CX2         6.617796    0.447350   -0.340313 C         1 ICL       -0.2297 ****
 30 HX2         6.600227    0.801393    0.684202 H         1 ICL        0.1479 ****
 31 CX3         7.814406    0.316716   -1.044003 C         1 ICL       -0.0714 ****
 32 HX3         8.752630    0.560444   -0.552153 H         1 ICL        0.1073 ****
 33 CX4         7.810586   -0.125774   -2.368798 C         1 ICL       -0.1859 ****
 34 HX4         8.745262   -0.229970   -2.913677 H         1 ICL        0.1185 ****
 35 CX5         6.596822   -0.425708   -2.992209 C         1 ICL       -0.0714 ****
 36 HX5         6.581335   -0.757547   -4.026963 H         1 ICL        0.1073 ****
 37 CX6         5.393022   -0.287941   -2.302565 C         1 ICL       -0.2297 ****
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 38 HX6         4.448146   -0.507425   -2.786467 H         1 ICL         0.1479 ****
 39 N1         -4.195435   -0.320706   -0.247944 N         2 ICL         0.0320 ****
 40 N2         -4.049041   -1.495549    0.452450 N         2 ICL        -0.5367 ****
 41 C3         -2.783710   -1.469019    0.851200 C         2 ICL         0.6581 ****
 42 N4         -2.129258   -0.330372    0.446532 N         2 ICL        -0.5172 ****
 43 C5         -3.028546    0.398274   -0.224226 C         2 ICL         0.4003 ****
 44 CB1        -2.172981   -2.533886    1.639462 C         2 ICL        -0.4571 ****
 45 CB2        -0.818680   -2.434202    2.089432 C         2 ICL         0.6660 ****
 46 OB2        -0.039545   -1.417406    1.836474 O         2 ICL        -0.6949 ****
 47 CB3        -0.317568   -3.507439    2.877814 C         2 ICL        -0.3788 ****
 48 HB3         0.710002   -3.425858    3.220236 H         2 ICL         0.1479 ****
 49 CB4        -1.096558   -4.607639    3.192376 C         2 ICL        -0.0175 ****
 50 HB4        -0.672763   -5.409421    3.795168 H         2 ICL         0.0884 ****
 51 CB5        -2.423843   -4.698478    2.739549 C         2 ICL        -0.3097 ****
 52 HB5        -3.035994   -5.563098    2.983041 H         2 ICL         0.1210 ****
 53 CB6        -2.941776   -3.664941    1.972837 C         2 ICL         0.0614 ****
 54 HB6        -3.965437   -3.708950    1.611752 H         2 ICL         0.0701 ****
 55 CC1        -2.792421    1.734425   -0.748613 C         2 ICL        -0.4029 ****
 56 CC2        -1.443159    2.111681   -1.075902 C         2 ICL         0.6852 ****
 57 OC2        -0.425006    1.323971   -0.923607 O         2 ICL        -0.6831 ****
 58 CC3        -1.254590    3.413643   -1.625272 C         2 ICL        -0.4153 ****
 59 HC3        -0.238560    3.688421   -1.892639 H         2 ICL         0.1614 ****
 60 CC4        -2.304116    4.300181   -1.777136 C         2 ICL        -0.0022 ****
 61 HC4        -2.112355    5.292726   -2.181422 H         2 ICL         0.0923 ****
 62 CC5        -3.609918    3.942985   -1.394076 C         2 ICL        -0.3017 ****
 63 HC5        -4.427967    4.653018   -1.478408 H         2 ICL         0.1233 ****
 64 CC6        -3.835964    2.673481   -0.885689 C         2 ICL         0.0335 ****
 65 HC6        -4.837402    2.402135   -0.566862 H         2 ICL         0.0621 ****
 66 CX1        -5.407463   -0.135038   -0.968657 C         2 ICL         0.3083 ****
 67 CX2        -6.617784   -0.447509   -0.340299 C         2 ICL        -0.2297 ****
 68 HX2        -6.600205   -0.801543    0.684219 H         2 ICL         0.1479 ****
 69 CX3        -7.814397   -0.316931   -1.043995 C         2 ICL        -0.0714 ****
 70 HX3        -8.752613   -0.560696   -0.552148 H         2 ICL         0.1073 ****
 71 CX4        -7.810590    0.125551   -2.368793 C         2 ICL        -0.1859 ****
 72 HX4        -8.745268    0.229703   -2.913677 H         2 ICL         0.1185 ****
 73 CX5        -6.596835    0.425534   -2.992200 C         2 ICL        -0.0714 ****
 74 HX5        -6.581358    0.757367   -4.026956 H         2 ICL         0.1073 ****
 75 CX6        -5.393034    0.287824   -2.302549 C         2 ICL        -0.2297 ****
 76 HX6        -4.448165    0.507347   -2.786448 H         2 ICL         0.1479 ****
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  3 C3          2.783760    1.469035    0.851162 C         1 ICL        0.9909 ****
  4 N4          2.129259    0.330417    0.446492 N         1 ICL       -1.2680 ****
  5 C5          3.028518   -0.398273   -0.224257 C         1 ICL        0.7580 ****
  6 CB1         2.173073    2.533933    1.639416 C         1 ICL       -0.6225 ****
  7 CB2         0.818771    2.434305    2.089394 C         1 ICL        0.7502 ****
  8 OB2         0.039595    1.417537    1.836449 O         1 ICL       -0.9833 ****
  9 CB3         0.317703    3.507571    2.877764 C         1 ICL       -0.3704 ****
 10 HB3        -0.709869    3.426033    3.220190 H         1 ICL        0.1595 ****
 11 CB4         1.096736    4.607744    3.192312 C         1 ICL       -0.0470 ****
 12 HB4         0.672974    5.409549    3.795096 H         1 ICL        0.0913 ****
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 13 CB5         2.424023    4.698527    2.739479 C         1 ICL       -0.2654 ****
 14 HB5         3.036209    5.563125    2.982960 H         1 ICL        0.1092 ****
 15 CB6         2.941913    3.664962    1.972776 C         1 ICL        0.0746 ****
 16 HB6         3.965575    3.708928    1.611687 H         1 ICL        0.0610 ****
 17 CC1         2.792336   -1.734415   -0.748637 C         1 ICL       -0.5925 ****
 18 CC2         1.443058   -2.111615   -1.075922 C         1 ICL        0.7845 ****
 19 OC2         0.424941   -1.323855   -0.923634 O         1 ICL       -0.9696 ****
 20 CC3         1.254430   -3.413570   -1.625286 C         1 ICL       -0.4043 ****
 21 HC3         0.238388   -3.688304   -1.892649 H         1 ICL        0.1585 ****
 22 CC4         2.303916   -4.300155   -1.777147 C         1 ICL       -0.0095 ****
 23 HC4         2.112111   -5.292693   -2.181428 H         1 ICL        0.0951 ****
 24 CC5         3.609735   -3.943015   -1.394092 C         1 ICL       -0.3116 ****
 25 HC5         4.427752   -4.653085   -1.478423 H         1 ICL        0.1248 ****
 26 CC6         3.835839   -2.673518   -0.885713 C         1 ICL        0.0846 ****
 27 HC6         4.837290   -2.402213   -0.566890 H         1 ICL        0.0510 ****
 28 CX1         5.407463    0.134929   -0.968675 C         1 ICL        0.3063 ****
 29 CX2         6.617796    0.447350   -0.340313 C         1 ICL       -0.2334 ****
 30 HX2         6.600227    0.801393    0.684202 H         1 ICL        0.1564 ****
 31 CX3         7.814406    0.316716   -1.044003 C         1 ICL       -0.0731 ****
 32 HX3         8.752630    0.560444   -0.552153 H         1 ICL        0.1067 ****
 33 CX4         7.810586   -0.125774   -2.368798 C         1 ICL       -0.1784 ****
 34 HX4         8.745262   -0.229970   -2.913677 H         1 ICL        0.1167 ****
 35 CX5         6.596822   -0.425708   -2.992209 C         1 ICL       -0.0731 ****
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 41 C3         -2.783710   -1.469019    0.851200 C         2 ICL        0.9909 ****
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 43 C5         -3.028546    0.398274   -0.224226 C         2 ICL        0.7580 ****
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 45 CB2        -0.818680   -2.434202    2.089432 C         2 ICL        0.7502 ****
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 47 CB3        -0.317568   -3.507439    2.877814 C         2 ICL       -0.3704 ****
 48 HB3         0.710002   -3.425858    3.220236 H         2 ICL        0.1595 ****
 49 CB4        -1.096558   -4.607639    3.192376 C         2 ICL       -0.0470 ****
 50 HB4        -0.672763   -5.409421    3.795168 H         2 ICL        0.0913 ****
 51 CB5        -2.423843   -4.698478    2.739549 C         2 ICL       -0.2654 ****
 52 HB5        -3.035994   -5.563098    2.983041 H         2 ICL        0.1092 ****
 53 CB6        -2.941776   -3.664941    1.972837 C         2 ICL        0.0746 ****
 54 HB6        -3.965437   -3.708950    1.611752 H         2 ICL        0.0610 ****
 55 CC1        -2.792421    1.734425   -0.748613 C         2 ICL       -0.5925 ****
 56 CC2        -1.443159    2.111681   -1.075902 C         2 ICL        0.7845 ****
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 63 HC5        -4.427967    4.653018   -1.478408 H         2 ICL        0.1248 ****
 64 CC6        -3.835964    2.673481   -0.885689 C         2 ICL        0.0846 ****
 65 HC6        -4.837402    2.402135   -0.566862 H         2 ICL        0.0510 ****
 66 CX1        -5.407463   -0.135038   -0.968657 C         2 ICL        0.3063 ****
 67 CX2        -6.617784   -0.447509   -0.340299 C         2 ICL       -0.2334 ****
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 71 CX4        -7.810590    0.125551   -2.368793 C         2 ICL       -0.1784 ****
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5.1. Effect of ligands on cell viability and cytotoxicity in proliferating HepaRG cell cultures.

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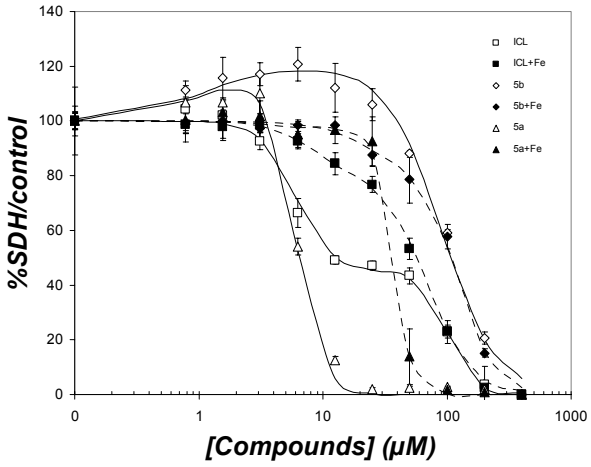

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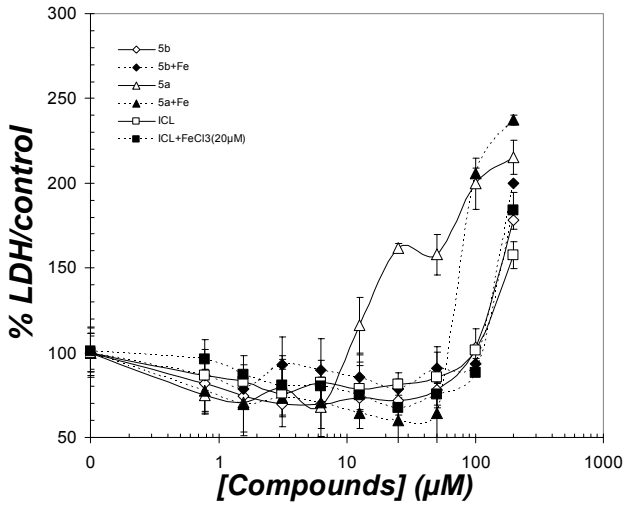

Figure SI8. Effect of ligands on cell viability (a, SDH activity) and cytotoxicity (b, LDH release in supernatant) in proliferating HepaRG cell cultures. Four days after seeding, HepaRG cells were maintained in culture for 72 h with various concentrations of Fe(III) chelators. Data expressed as a percent of the control (absence of compound) are averaged over three independent experiments.

5.2. ICL670, **5a** and **5b** effects on HUVEC and MDA-MB-231 cell survival and proliferation.

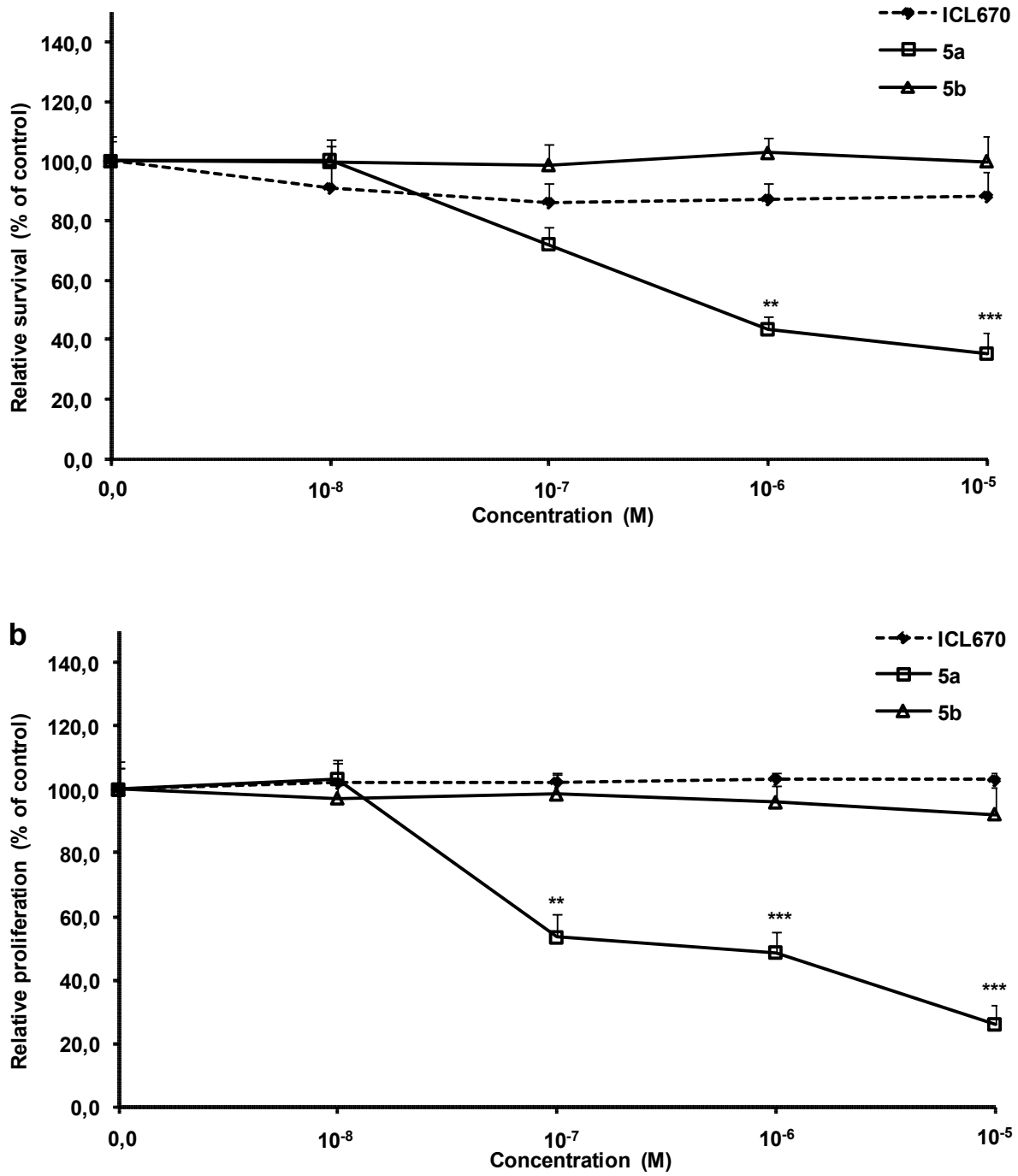

Figure SI9. ICL670, **5a** and **5b** effects on HUVEC survival and proliferation using BrdU assay. HUVEC were treated for 48 h with 10^{-8} to 10^{-5}M of ICL670 (curve with black diamonds), **5a** (curve with empty squares) or **5b** (curve with empty triangles). Two serum conditions were tested: 1% FBS for cell survival assay (a) and 10% FBS for cell proliferation assay (b). Untreated cells were used as basal control (0.0). Relative survival in the presence of ICL670, **5a** or **5b** was normalized to the untreated controls after background subtraction. Values represent the mean ± SEM for each concentration tested (n = 3). **p<0.01 ***p<0.001 *vs* control.

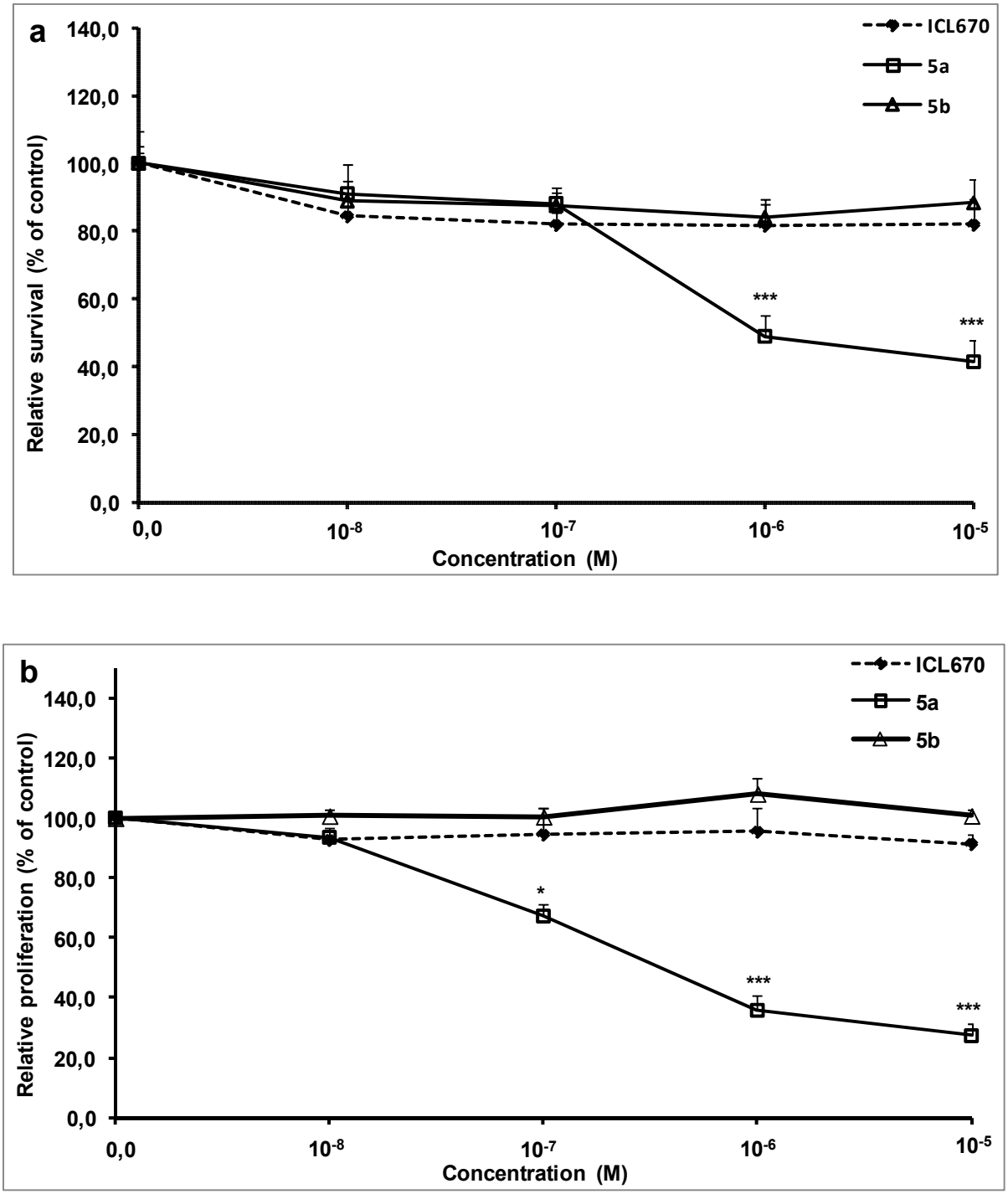

Figure SI10. ICL670, **5a** and **5b** effects on MDA-MB-231 cell survival and proliferation using BrdU assay.

MDA-MB-231 were treated for 48 h with 10^{-8} to 10^{-5} M of ICL670 (curve with black diamonds), **5a** (curve with empty squares) or **5b** (curve with empty triangles). Two serum conditions were tested: 0% FBS for cell survival assay (a) and 10% FBS for cell proliferation assay (b). Untreated cells were used as basal control (0.0). Relative survival in the presence of ICL670, **5a** or **5b** was normalized to the untreated controls after background subtraction. Values represent the mean ± SEM for each concentration tested (n = 3). *p<0.05, **p<0.01 ***p<0.001 *vs* control.