Supporting Information

Insights into the dioxygen activation and catalytic mechanism of the nickel-containing quercetinase

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Table S1. Key structural parameters and relative energies of different binding structures before and after optimization. All distances are given in angstrom and angles in degree. Relative energies (RE) are given in kcal/mol.

Initial structures	red	medium blue	yellow	purple	green	magenta	cyan	blue
d(Ni-Od)	2.40	2.00	2.40	2.40	3.00	2.39	4.88	2.51
d(Ni-Op)	2.40	2.00	2.40	2.40	3.00	2.39	4.68	2.41
θ(N69-Ni-Op-Od)	93.7	93.7	114.6	54.9	-84.0	-14.0	-93.6	-77.6
Optimized structures		$(^1R)^3R[^5R]$				$(^1R')^3R'[^5R']$		
d(Ni-Od)	3.07	3.07	3.07	3.07	2.98	2.92	2.98	2.92
d(Ni-Op)	2.14	2.14	2.14	2.14	2.15	2.07	2.15	2.07
θ(N69-Ni-Op-Od)	76.0	76.1	76.2	76.1	-45.4	-49.7	-45.1	-49.6
RE	(46.5)[a]	(47.0)	(47.0)	(21.0)	(25.0)	(25.1)	(25.0)	(19.1)
	5.5	6.4	6.2	6.2	2.4	2.5	2.4	2.8
	[0.0]	[2.2]	[2.2]	[2.2]	[0.7]	[0.6]	[0.7]	[0.6]

[a]The data shown in red, black and green represent the relative energies of the singlet, triplet and quintet states, respectively.

Table S2. Second-order perturbative estimations of some key orbital interactions in $^3R'$. Values in parenthesis present the type and constituents of orbitals.

Alpha spin orbitals			Beta spin orbitals		
Donor	Acceptor	$\Delta E^{(2)}$(kcal/mol)	Donor	Acceptor	$\Delta E^{(2)}$(kcal/mol)
$LP_{O3}(s^{0.52}p^{0.48})$	$LV_{Ni}(s)$	7.4	$LP_{O3}(s^{0.02}p^{0.98})$	$LV_{Ni}(d)$	6.0
$LP_{Op}(s^{0.79}p^{0.21})$	$LV_{Ni}(s)$	8.4	$LP_{Op}(s^{0.14}p^{0.85})$	$LV_{Ni}(d)$	12.9
			$LP_{O3}(s^{0.02}p^{0.98})$	$LV_{Ni}(d)$	6.6
			$LP_{Op}(s^{0.14}p^{0.85})$	$LV_{Ni}(d)$	2.2
			$LP_{O3}(s^{0.02}p^{0.98})$	$LV_{Ni}(s)$	5.7
			$LP_{Op}(s^{0.14}p^{0.85})$	$LV_{Ni}(s)$	9.2

Table S3. NPA charges for ^{3}R' and ^{5}R' complex.

Species	Ni	O_2	QUE
^{3}R'	1.37	-0.42	-0.24
^{5}R'	1.40	-0.21	-0.50

Table S4. Spin density for O_2-Ni^{2+}-QUE complex.

Species	Ni	O_2	C3	C2	O3	O4	QUE
^{3}R'	1.69	0.61	-0.09	-0.15	-0.07	-0.03	-0.48
^{5}R'	1.74	1.72	0.05	0.07	0.12	0.03	0.34

Table S5. Second-order perturbative estimates of some key orbital interactions in ^{3}IM1, ^{3}TS2 and ^{3}IM2 complex. Values in bracket present type and constituents of orbitals.

	Alpha spin orbitals			Beta spin orbitals		
	Donor	Acceptor	$\Delta E^{(2)}$ (kcal/mol)	Donor	Acceptor	$\Delta E^{(2)}$ (kcal/mol)
^{3}IM1	$LP_{C43}(p)$	$BD^*_{C41\text{-}C42}(p\text{-}p)$	55.4	$LP_{C43}(p)$	$BD^*_{C41\text{-}C42}(p\text{-}p)$	55.4
	$LP_{Op}(s^{0.04}p^{0.96})$	$LV_{Ni}(s)$	9.0	$LP_{Op}(s^{0.08}p^{0.92})$	$LV_{Ni}(s^{0.03}d^{0.97})$	28.9
	$LP_{Ni}(d)$	$BD^*_{C54\text{-}C58}(p\text{-}p)$	0.3	$LP_{Ni}(d)$	$BD^*_{Op\text{-}Od}$ $(s^{0.14}p^{0.86}\text{-}s^{0.09}p^{0.91})$	0.5
^{3}TS2	$LP_{C43}(p)$	$BD^*_{C41\text{-}C42}(p\text{-}p)$	57.5	$LP_{C43}(p)$	$BD^*_{C41\text{-}C42}(p\text{-}p)$	57.5
	$LP_{Op}(s^{0.25}p^{0.75})$	$LV_{Ni}(s)$	15.5	$LP_{Op}(s^{0.02}p^{0.98})$	$LV_{Ni}(s^{0.04}d^{0.96})$	15.2
	$LP_{Ni}(d)$	$BD^*_{Op\text{-}Od}$ $(s^{0.11}p^{0.89}\text{-}s^{0.08}p^{0.92})$	0.8	$LP_{Ni}(d)$	$BD^*_{Op\text{-}Od}$ $(s^{0.11}p^{0.89}\text{-}s^{0.09}p^{0.91})$	0.9
^{3}IM2	$LP_{C43}(p)$	$BD^*_{C41\text{-}C42}(p\text{-}p)$	53.4	$LP_{C43}(p)$	$BD^*_{C41\text{-}C42}(p\text{-}p)$	53.4
	$LP_{Op}(s^{0.23}p^{0.77})$	$LV_{Ni}(s)$	13.8	$LP_{Op}(s^{0.11}p^{0.89})$	$LV_{Ni}(s^{0.05}p^{0.95})$	27.2
	$LP_{Ni}(d)$	$BD^*_{Op\text{-}Od}$ $(s^{0.13}p^{0.87}\text{-}s^{0.08}p^{0.92})$	0.6	$LP_{Ni}(d)$	$BD^*_{Op\text{-}Od}$ $(s^{0.12}p^{0.88}\text{-}s^{0.08}p^{0.92})$	0.3

Table S6. Spin density for O_2 -Fe^{2+}-QUE complex.

Species	Fe	dioxygen	C3	C2	O3	O4	QUE
^{5}R'	3.63	0.47	-0.08	-0.08	-0.01	-0.04	-0.29
^{7}R'	3.68	1.78	0.05	0.06	0.14	0.03	0.33

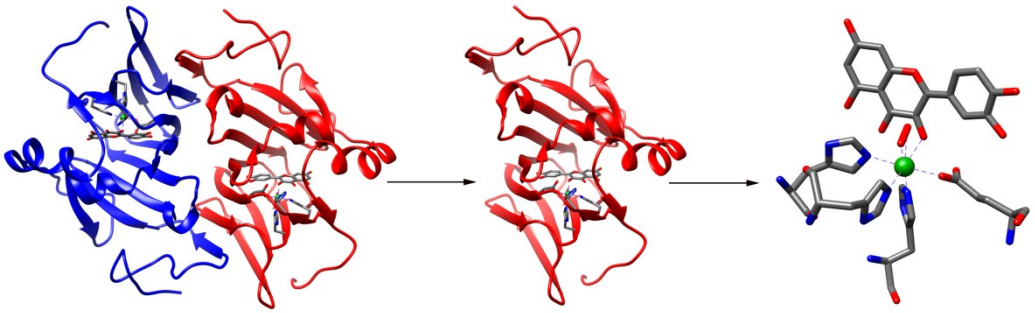

Figure S1. Left: Crystal structure of Ni-QueD^{FLA} (pdb code:5FLJ), two chains represent the dimer; Middle: the selected chain C; Right: Active site in Ni-QueD^{FLA}.

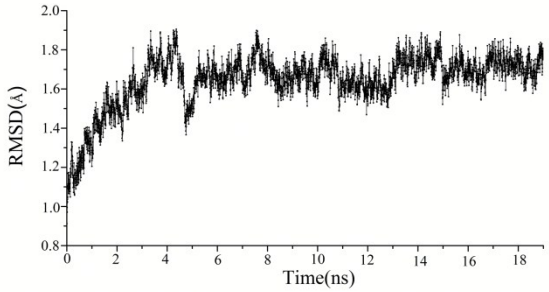

Figure S2. RMSD for the backbone atoms of the enzyme-substrate complex (M=Ni) in 19 ns molecular dynamics simulation.

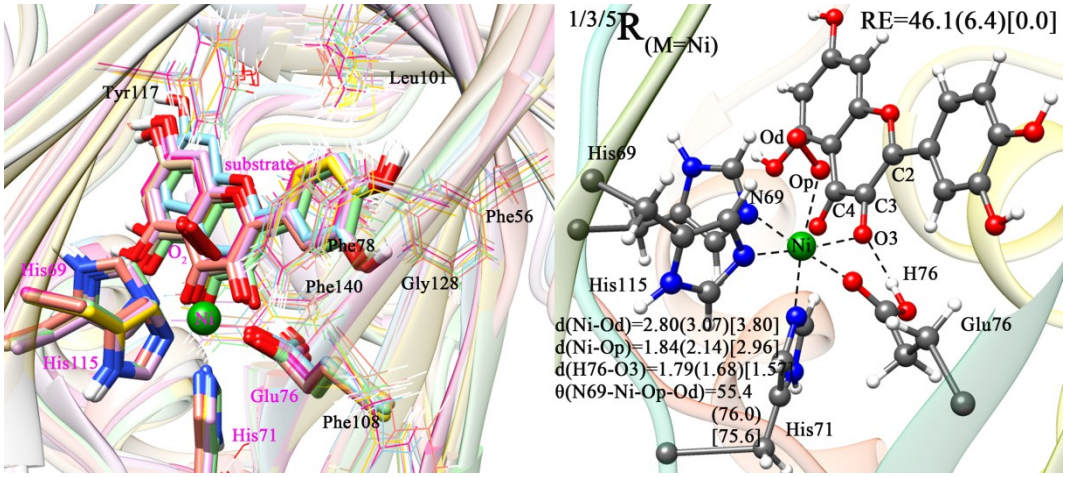

Figure S3. Left: Overlap of eight QM/MM-optimized active site pockets from 10 to 19 ns. The calculated RMSD values of these optimized structures range from 0.66 to 1.20 Å, and the RMSD value at 19 ns corresponds to the lowest one. Besides, the RMSDs of the QM region were also calculated, which are quite small, indicting the high consistence of QM region. The QM region atoms are shown in stick model. Right: Optimized structures and key parameters of the snapshot derived from 19 ns. All distances are given in angstrom and angles in degree. Relative Gibbs free

energies (RE) are given in kcal/mol. The RMSD values of backbone atoms of the whole system relative to the average one, and the RMSDs of QM region are listed here:

snapshots	10 ns	11 ns	13 ns	14 ns	16 ns	17 ns	18 ns	19 ns
Whole system	0.80	0.81	1.20	0.72	1.04	0.90	0.67	0.66
QM region	(0.31)	(0.56)	(0.14)	(0.41)	(0.19)	(0.10)	(0.29)	(0.18)

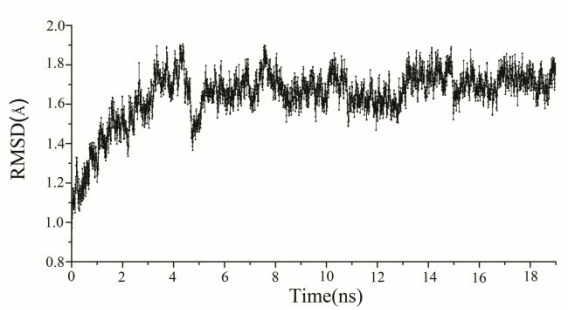

Figure S4. RMSD for the backbone atoms of the enzyme-substrate complex (M=Fe) in 19 ns molecular dynamics simulation.

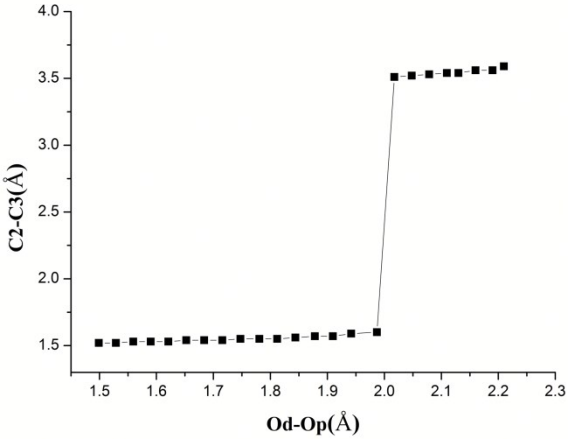

Figure S5. Variation of bond lengths for Op-Od bond and C2-C3 bond.

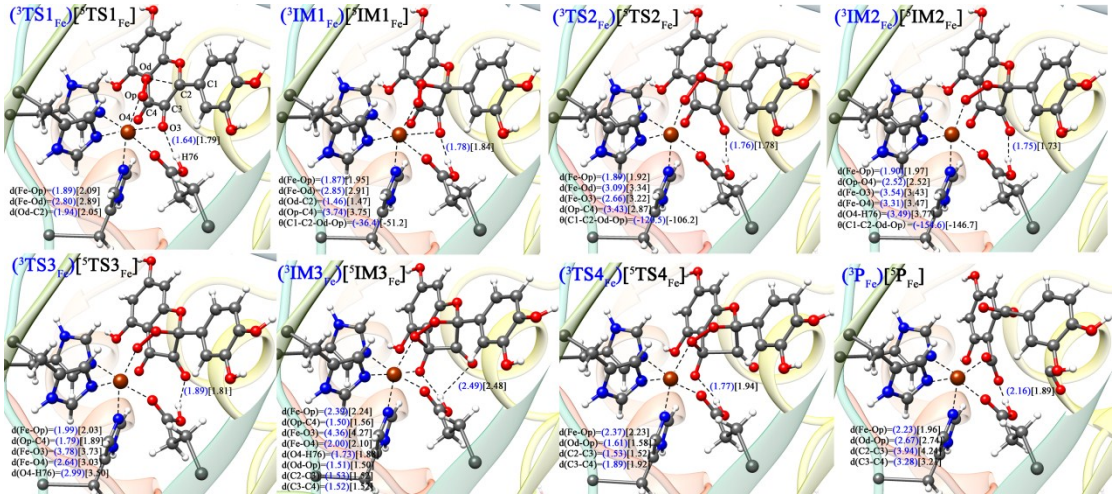

Figure S6. Optimized structures of transition states and intermediates. All distances are given in angstrom and angles in degree.

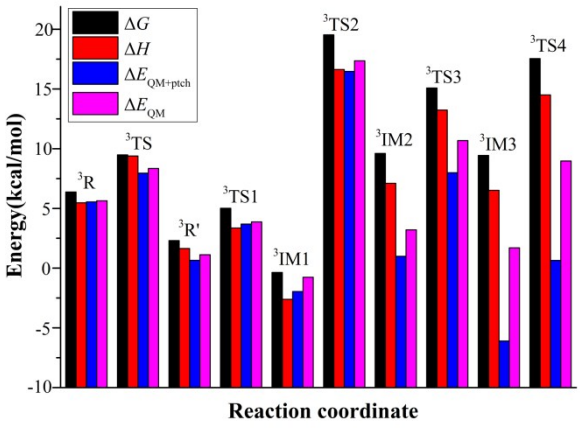

Figure S7. Calculated ΔG (the relative free energies, black), ΔH (enthalpies, red), $\Delta E_{\mathrm{QM+ptch}}$ (QM region energies calculated at the field of MM point charges, blue) and ΔE_{QM} (QM region energies without point charges, purple) at triplet state for Ni-QueD$^{\mathrm{FLA}}$. All energy items of ^{5}R are set to zero.

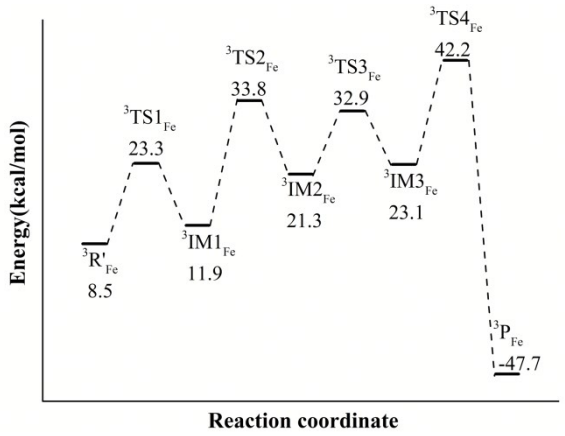

Figure S8. Calculated QM/MM energy profiles at triplet state for the oxidative ring-cleaving reaction of quercetion by Fe-QueD^{FLA}. The energy of $^5R'_{Fe}$ is set to zero.

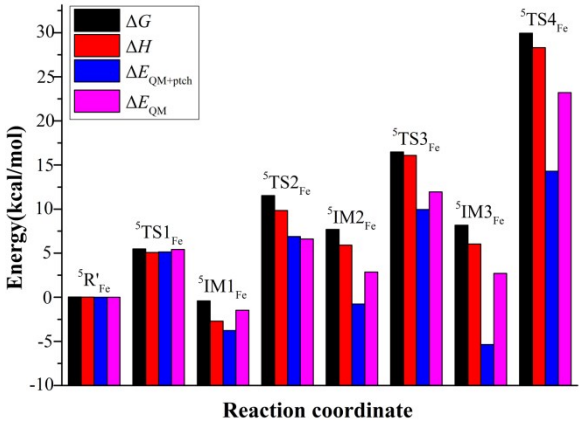

Figure S9. Calculated Δ*G* (the relative free energies, black), Δ*H* (enthalpies, red), $\Delta E_{QM+ptch}$ (QM region energies calculated at the field of MM point charges, blue) and ΔE_{QM} (QM region energies without point charges, purple) at quintet state for Fe-QueD^{FLA}. All energy items of $^5R'_{Fe}$ are set to zero.

Coordinates of optimized species at the level of UB3LYP/BSI (LANL2DZ basis set for metal ion and 6-31G(d,p) for non-metal atoms).

The coordinate of reactant model R (M=Ni, triplet state)

C	9.203	-5.128	3.883
H	8.712	-6.065	4.044
H	9.293	-4.607	4.813
N	8.427	-2.925	2.836
H	8.981	-2.278	3.406
C	8.402	-4.306	2.929

C	7.644	-2.563	1.793
H	7.507	-1.541	1.482
N	7.105	-3.630	1.219
C	7.572	-4.715	1.922
H	7.303	-5.722	1.647
C	5.793	-9.562	-0.937
H	6.095	-9.619	-1.961
H	4.889	-10.118	-0.800
N	4.842	-7.720	0.539
H	4.353	-8.377	1.153
C	5.560	-8.133	-0.565
C	4.883	-6.370	0.614
H	4.411	-5.765	1.377
N	5.603	-5.886	-0.394
C	6.004	-6.974	-1.150
H	6.564	-6.864	-2.064
C	4.052	-5.430	-4.247
H	4.766	-6.221	-4.338
H	3.063	-5.838	-4.264
C	4.273	-4.700	-2.941
O	5.321	-4.105	-2.668
O	3.254	-4.808	-2.105
H	3.470	-4.337	-1.252
C	11.367	-3.136	-2.655
H	11.164	-2.187	-3.106
H	11.850	-3.773	-3.365
N	9.950	-5.104	-1.893
H	10.650	-5.835	-2.031
C	10.083	-3.763	-2.215
C	8.700	-5.309	-1.436
H	8.357	-6.276	-1.111
N	8.004	-4.181	-1.460
C	8.858	-3.211	-1.945
H	8.547	-2.181	-2.028
C	4.670	-0.583	5.364
C	4.709	-1.702	4.530
C	4.329	-1.606	3.161
C	3.980	-0.326	2.694
C	3.948	0.807	3.502
C	4.277	0.659	4.847
O	4.198	1.779	5.613
O	5.111	-2.903	4.998
H	4.916	-0.688	6.417
H	3.659	1.767	3.093

H	4.474	1.640	6.540
H	5.376	-2.814	5.926
C	4.237	-2.742	2.238
C	3.996	-2.399	0.789
C	3.646	-1.080	0.433
O	3.603	-0.119	1.402
O	4.347	-3.917	2.581
O	4.149	-3.358	-0.068
C	3.269	-0.535	-0.868
C	3.129	0.862	-1.024
C	2.828	1.413	-2.266
C	2.658	0.586	-3.374
C	2.764	-0.812	-3.231
C	3.060	-1.361	-1.995
O	2.574	-1.620	-4.315
O	2.418	1.005	-4.648
H	3.266	1.520	-0.176
H	2.739	2.490	-2.375
H	3.132	-2.430	-1.913
H	2.490	-1.047	-5.094
H	2.233	1.951	-4.696
O	7.127	-0.954	-0.858
O	6.128	-1.705	-0.619
Ni	6.108	-3.846	-0.648
H	10.176	-5.300	3.474
H	12.005	-2.998	-1.807
H	6.561	-9.971	-0.315
H	4.170	-4.746	-5.061

The coordinate of reactant model R' (M=Ni, triplet state)

C	9.214	-5.166	3.887
H	8.728	-6.107	4.039
H	9.300	-4.653	4.822
N	8.422	-2.956	2.870
H	8.972	-2.312	3.446
C	8.410	-4.339	2.940
C	7.637	-2.586	1.832
H	7.469	-1.560	1.547
N	7.109	-3.647	1.239

C	7.586	-4.739	1.924
H	7.329	-5.744	1.631
C	5.799	-9.569	-0.932
H	6.099	-9.619	-1.957
H	4.898	-10.130	-0.796
N	4.836	-7.740	0.552
H	4.348	-8.404	1.161
C	5.559	-8.143	-0.553
C	4.870	-6.391	0.634
H	4.395	-5.793	1.401
N	5.589	-5.896	-0.368
C	5.998	-6.978	-1.128
H	6.558	-6.858	-2.042
C	4.064	-5.438	-4.256
H	4.787	-6.219	-4.359
H	3.080	-5.858	-4.263
C	4.291	-4.712	-2.950
O	5.351	-4.148	-2.668
O	3.259	-4.787	-2.122
H	3.483	-4.318	-1.273
C	11.373	-3.147	-2.645
H	11.158	-2.196	-3.087
H	11.855	-3.774	-3.364
N	9.979	-5.135	-1.903
H	10.686	-5.858	-2.060
C	10.098	-3.788	-2.200
C	8.734	-5.361	-1.440
H	8.402	-6.338	-1.132
N	8.027	-4.241	-1.439
C	8.869	-3.254	-1.911

H	8.541	-2.230	-1.995
C	4.662	-0.566	5.351
C	4.704	-1.679	4.510
C	4.312	-1.578	3.145
C	3.938	-0.301	2.690
C	3.897	0.825	3.506
C	4.248	0.673	4.845
O	4.167	1.789	5.619
O	5.123	-2.879	4.968
H	4.920	-0.674	6.401
H	3.589	1.783	3.108
H	4.457	1.647	6.541
H	5.394	-2.793	5.894
C	4.235	-2.709	2.215
C	3.998	-2.354	0.768
C	3.603	-1.041	0.429
O	3.530	-0.093	1.405
O	4.348	-3.886	2.548
O	4.176	-3.286	-0.106
C	3.233	-0.500	-0.875
C	3.082	0.896	-1.030
C	2.800	1.446	-2.277
C	2.663	0.619	-3.390
C	2.782	-0.778	-3.247
C	3.058	-1.327	-2.007
O	2.621	-1.585	-4.337
O	2.440	1.035	-4.667
H	3.201	1.553	-0.179
H	2.701	2.522	-2.387
H	3.143	-2.395	-1.927

H	2.548	-1.011	-5.116
H	2.242	1.979	-4.720
O	6.111	-0.980	-0.132
O	6.447	-1.855	-1.001
Ni	6.122	-3.867	-0.631
H	10.189	-5.329	3.478
H	12.017	-3.010	-1.801
H	6.570	-9.977	-0.313
H	4.164	-4.749	-5.068

The coordinate of reactant R'_{Fe} (quintet state)

C	9.217	-5.106	3.901
H	8.712	-6.036	4.054
H	9.317	-4.596	4.836
N	8.475	-2.881	2.877
H	9.039	-2.245	3.448
C	8.428	-4.263	2.958
C	7.693	-2.500	1.840
H	7.548	-1.471	1.551
N	7.133	-3.551	1.259
C	7.585	-4.649	1.952
H	7.293	-5.649	1.674
C	5.807	-9.581	-0.942
H	6.129	-9.629	-1.960
H	4.906	-10.147	-0.825
N	4.834	-7.759	0.542
H	4.358	-8.428	1.155
C	5.551	-8.156	-0.568
C	4.852	-6.410	0.621
H	4.376	-5.817	1.391

N	5.548	-5.907	-0.394
C	5.964	-6.987	-1.154
H	6.513	-6.863	-2.074
C	3.974	-5.437	-4.303
H	4.713	-6.200	-4.420
H	3.001	-5.882	-4.285
C	4.213	-4.700	-3.007
O	5.272	-4.125	-2.748
O	3.195	-4.771	-2.159
H	3.430	-4.296	-1.319
C	11.400	-3.130	-2.658
H	11.198	-2.177	-3.102
H	11.891	-3.759	-3.370
N	9.976	-5.111	-1.941
H	10.676	-5.843	-2.082
C	10.115	-3.764	-2.236
C	8.724	-5.321	-1.493
H	8.374	-6.295	-1.193
N	8.030	-4.191	-1.499
C	8.891	-3.214	-1.962
H	8.580	-2.185	-2.051
C	4.646	-0.549	5.312
C	4.681	-1.656	4.464
C	4.276	-1.549	3.104
C	3.896	-0.269	2.660
C	3.858	0.852	3.485
C	4.222	0.693	4.819
O	4.147	1.803	5.602
O	5.109	-2.858	4.913
H	4.915	-0.662	6.359

H	3.542	1.811	3.096
H	4.447	1.652	6.520
H	5.385	-2.773	5.838
C	4.208	-2.673	2.163
C	3.979	-2.298	0.729
C	3.574	-1.000	0.399
O	3.483	-0.054	1.379
O	4.341	-3.853	2.485
O	4.196	-3.224	-0.171
C	3.214	-0.458	-0.912
C	3.118	0.939	-1.087
C	2.845	1.479	-2.342
C	2.663	0.641	-3.439
C	2.722	-0.755	-3.273
C	2.989	-1.294	-2.027
O	2.518	-1.574	-4.349
O	2.453	1.042	-4.725
H	3.268	1.603	-0.246
H	2.791	2.556	-2.470
H	3.026	-2.365	-1.930
H	2.455	-1.006	-5.134
H	2.299	1.992	-4.798
O	6.137	-0.828	-0.191
O	6.549	-1.732	-0.979
Fe	6.077	-3.796	-0.659
H	12.030	-2.998	-1.803
H	10.187	-5.289	3.488
H	6.567	-9.985	-0.307
H	4.037	-4.750	-5.120