Pyridine Inhibitor Binding to the 4Fe-4S Protein *A. aeolicus* IspH (LytB): A HYSCORE Investigation

Supporting Information

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Full citation for reference 21:

M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, G. Scalmani, V. Barone, B. Mennucci, G. A. Petersson, H. Nakatsuji, M. Caricato, X. Li, H. P. Hratchian, A. F. Izmaylov, J. Bloino, G. Zheng, J. L. Sonnenberg, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, J. A. Montgomery, Jr., J. E. Peralta, F. Ogliaro, M. Bearpark, J. J. Heyd, E. Brothers, K. N. Kudin, V. N. Staroverov, R. Kobayashi, J. Normand, K. Raghavachari, A. Rendell, J. C. Burant, S. S. Iyengar, J. Tomasi, M. Cossi, N. Rega, J. M. Millam, M. Klene, J. E. Knox, J. B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R. E. Stratmann, O. Yazyev, A. J. Austin, R. Cammi, C. Pomelli, J. W. Ochterski, R. L. Martin, K. Morokuma, V. G. Zakrzewski, G. A. Voth, P. Salvador, J. J. Dannenberg, S. Dapprich, A. D. Daniels, O. Farkas, J. B. Foresman, J. V. Ortiz, J. Cioslowski, and D. J. Fox, Gaussian 09, Revision A.01, Gaussian, Inc., Wallingford CT, 2009.

Supplementary Methods

Materials. All chemicals were purchased from Sigma-Aldrich (St. Louis, MO) and were used as provided. BL-21(DE3) competent cells were purchased from Stratagene (La Jolla, CA). Syntheses of **7**, **8**, **9**, **10**, **11** were reported previously.[1] The *A. aeolicus* plasmid was provided by Hassan Jomaa and Jochen Wiesner.

***A. aeolicus* IspH Protein Production and Purification.** Reconstituted *A. aeolicus* IspH was prepared as reported previously.[2] Anaerobically purified *A. aeolicus* IspH was prepared using a Coy Vinyl Anaerobic Chamber (Coy Laboratories, Grass Lake, MI) with an oxygen level < 2 ppm, and all buffers were degassed on a Schlenk line. Cell pellets were re-suspended in 100 mM Tris-HCl, 150 mM NaCl buffer (pH 8.0). Lysozyme, Benzonase nuclease (EMD Chemicals, San Diego, CA) and phenylmethanesulfonyl fluoride were added, and stirred for 1.5 hour at 10 °C followed by sonication (Fisher Scientific Sonic Dismembrator, Model 500) with 4 pulses, each 7 sec duration and 35% power. The cell lysate was then centrifuged at 11,000 rpm at 10 °C for 30 min. The supernatant was purified by using His-tag affinity chromatography. Fractions having a brown color were collected and desalted in pH 8.0 buffer containing 100 mM Tris-HCl and 150 mM NaCl. *A. aeolicus* IspH was used as purified without reconstitution.

To prepare [u-^{15}N]-labeled *A. aeolicus* IspH, *E. coli* BL-21 (DE3) cells harboring an *A. aeolicus* IspH plasmid were grown in M9 minimal media (12.8 g Na_2HPO_4-$7H_2O$, 3 g KH_2PO_4, and 0.5 g NaCl in 1000 mL distilled H_2O, supplemented with 2 mL 1M $MgSO_4$, 100 µL 1M $CaCl_2$, 4 g glucose, 1 g $^{15}NH_4Cl$ and 5 mL 100X MEM vitamin solution (Sigma, St Louis, MO)). When the OD_{600} reached 0.6, 400 µg/L anhydrotetracycline was added to induce the overexpression of *A. aeolicus* IspH. Cells were then allowed to grow at 28 °C for 20 hours, then harvested by centrifugation and stored in -80 °C until purification. The ^{15}N-labeled *A. aeolicus* IspH protein was purified and reconstituted according to published procedures.[2]

HYSCORE Sample Preparation. All samples were prepared inside a Coy Vinyl Anaerobic Chamber with an oxygen level < 2 ppm. IspH was typically 1.0-2.0 mM in pH 8.0 Tris-HCl buffer (50 mM Tris-HCl, 150 mM NaCl), and glycerol was added to 40% (v/v). 20 equivalents of sodium dithionite were added as a reducing agent, and ligands were added as follows: **5**, 50 eq.; **6**, 50 eq.; **7**, 10 eq.; **8**, 50 eq.; **9**, 30 eq.; **10**, 30 eq.; **11**, 10 eq.). A 80 µL samples were then transferred into EPR tubes (706-PQ-9.50, Wilmad Labglass, Vineland, NJ), then frozen in liquid nitrogen after a 5-minute incubation with dithionite and ligands.

ENDOR/HYSCORE Spectroscopy. HYSCORE spectra were obtained on a Bruker ElexSys E-580-10 FT-EPR X-band EPR spectrometer using an Oxford Instruments CF935 cryostat. HYSCORE used a four-pulse sequence $\pi/2_{mw} - \tau - \pi/2_{mw} - t_1 - \pi_{mw} - t_2 - \pi/2_{mw}$ – echo; $\pi/2_{mw}$ = 16 ns and π_{mw} = 32 ns, 256 points for both t_1 and t_2, each at 16 ns steps. Time-domain data were baseline corrected using a 3rd order polynomial, then Hamming windowed, followed by zero-filling, and 2D-Fourier transformation. HYSCORE spectra were simulated using the EasySpin program package.[3]

Quantum Mechanical Calculations. All QM calculations were performed with Gaussian 09 software.[4] For all systems, geometry optimization was carried out using the BPW91 functional, a Wachters+f[5, 6] basis set for first-row transition metals, 6-311+G*[7] for other atoms. The optimized structures were then used to calculate the ^{14}N nuclear quadrupole coupling constants using BPW91[8, 9] or B3LYP[10, 11] functionals, and the aforementioned Wachters, 6-311+G* or TZVP[12] basis sets. There was little difference between the different calculational results. The QM calculations give the electric field gradient (*eq*) in atomic units; a conversion factor of eQ/h = 4.8027 MHz/a.u. (Q = 0.02044 barn [13]) for ^{14}N was used to calculate the e^2qQ/h value, in MHz. Atomic coordinates after geometric optimization of all model compounds are given in Table S2.

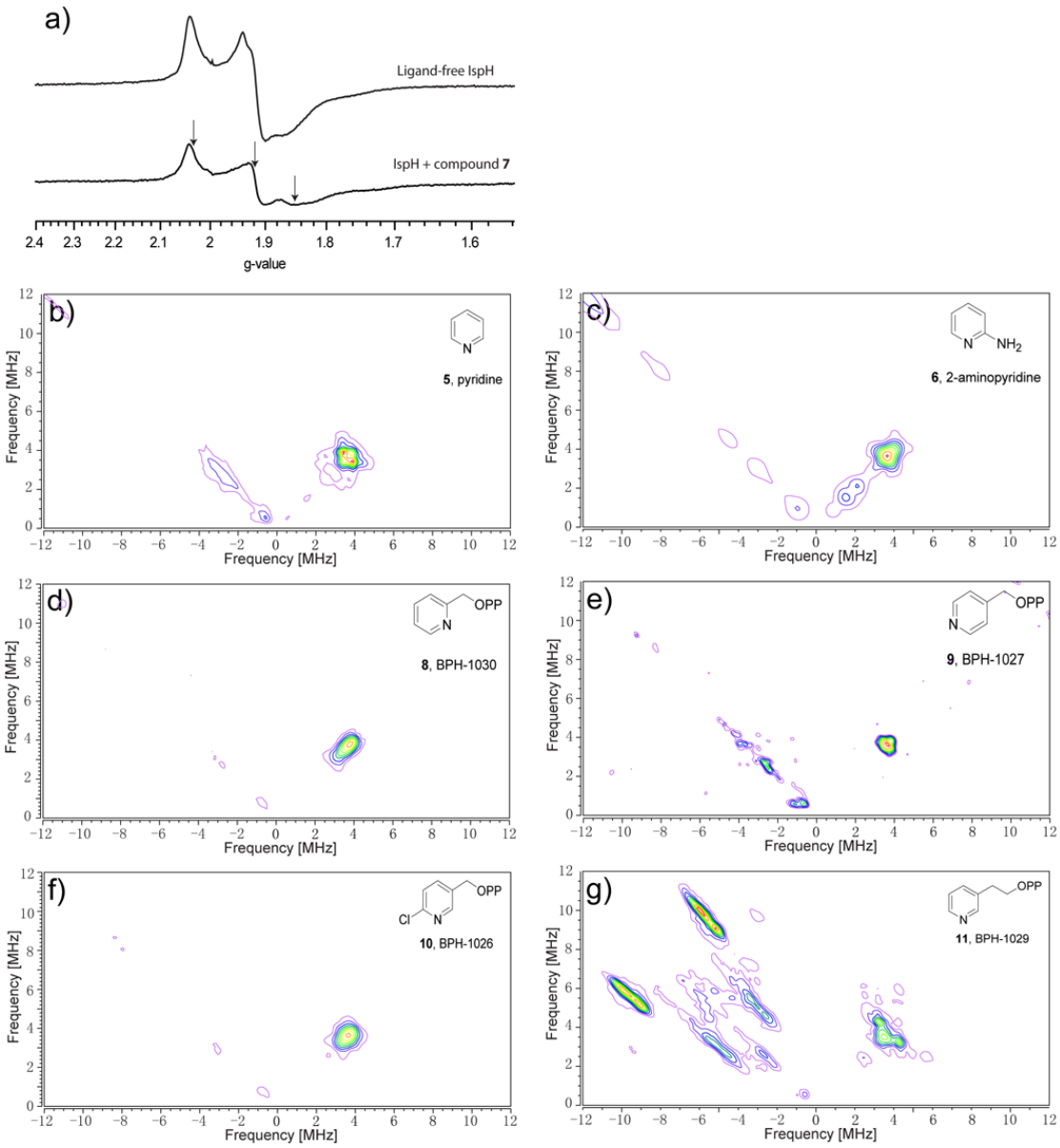

Figure S1. Continuous-wave EPR of ligand-free IspH and IspH + **7** **(a)**, and HYSCORE spectra of IspH + pyridine ligands. In (a), arrows indicate the magnetic field positions for collecting field-dependent HYSCORE of IspH + **7**. HYSCORE spectra were taken at g_2; τ = 136 ns.

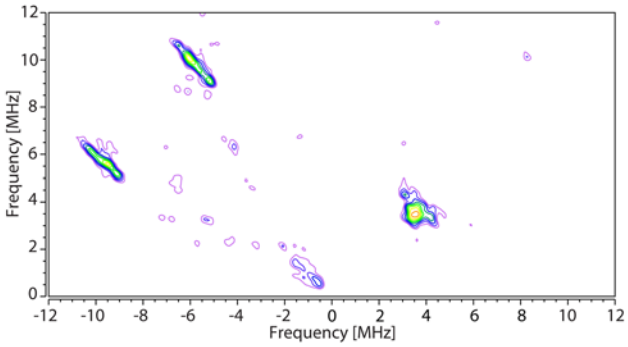

Figure S2. HYSCORE spectrum of anaerobically purified *A. aeolicus* IspH + **7**. Microwave frequency = 9.68 GHz; magnetic field = 360 mT (g_2=1.921); τ = 136ns.

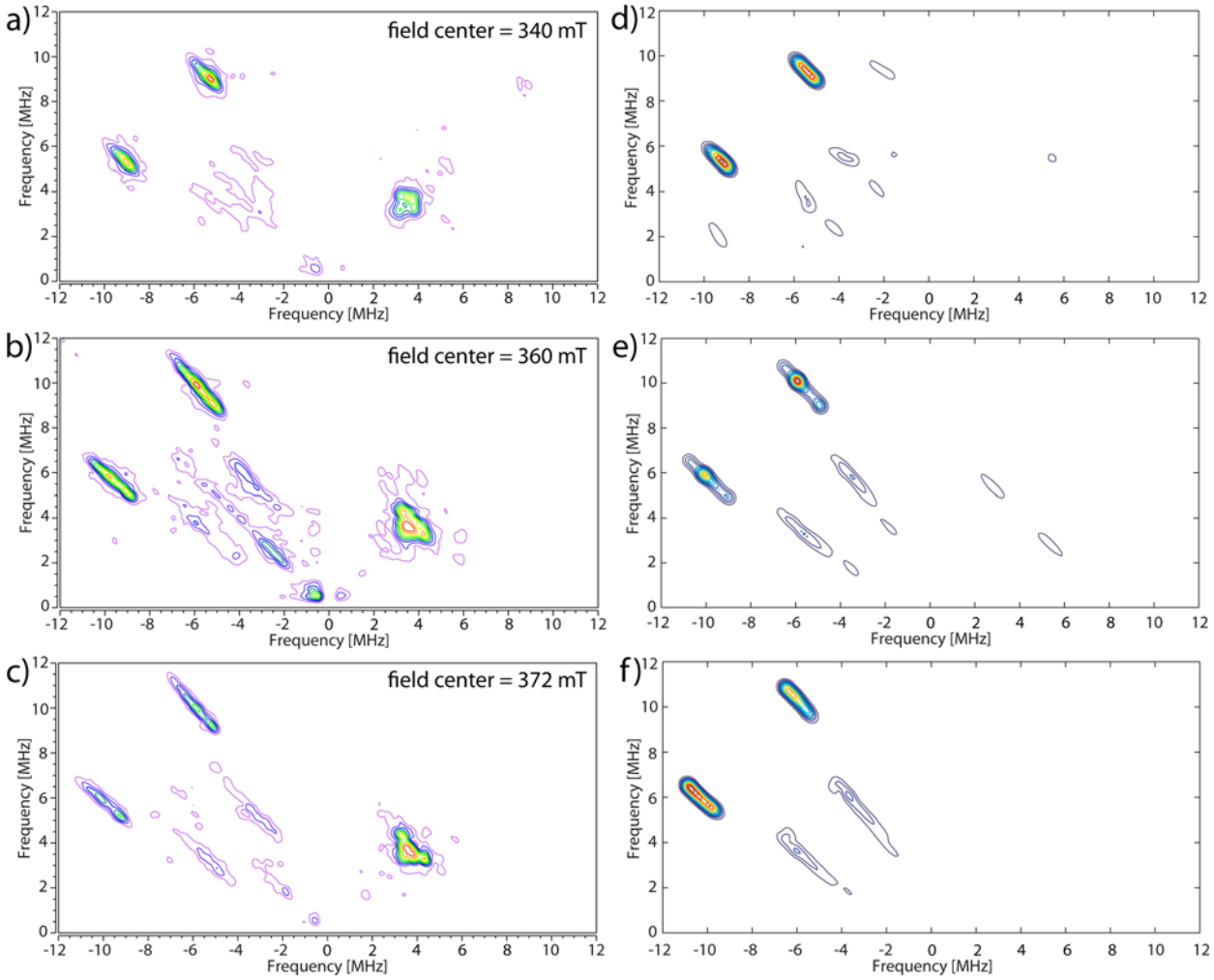

Figure S3. HYSCORE spectra of *A. aeolicus* IspH + **7** at three different magnetic field strengths and their computer simulations. (a), 340 mT; (b), 360 mT; (c), 372 mT. (d), simulation of (a); (e), simulation of (b); (f), simulation of (c). Microwave frequency = 9.66 GHz; τ = 136ns. Simulation parameters are: $A_{ii}(^{14}N)$ = [6.2 7.6 8.4] MHz, $\alpha = 10^{\circ}$, $\beta = 30^{\circ}$, $\gamma = 10^{\circ}$ for the hyperfine interaction and e^2qQ/h = 3.0 MHz, η = 0.2, $\alpha = 45^{\circ}$, $\beta = 60^{\circ}$, $\gamma = 35^{\circ}$ for the quadrupole interaction.

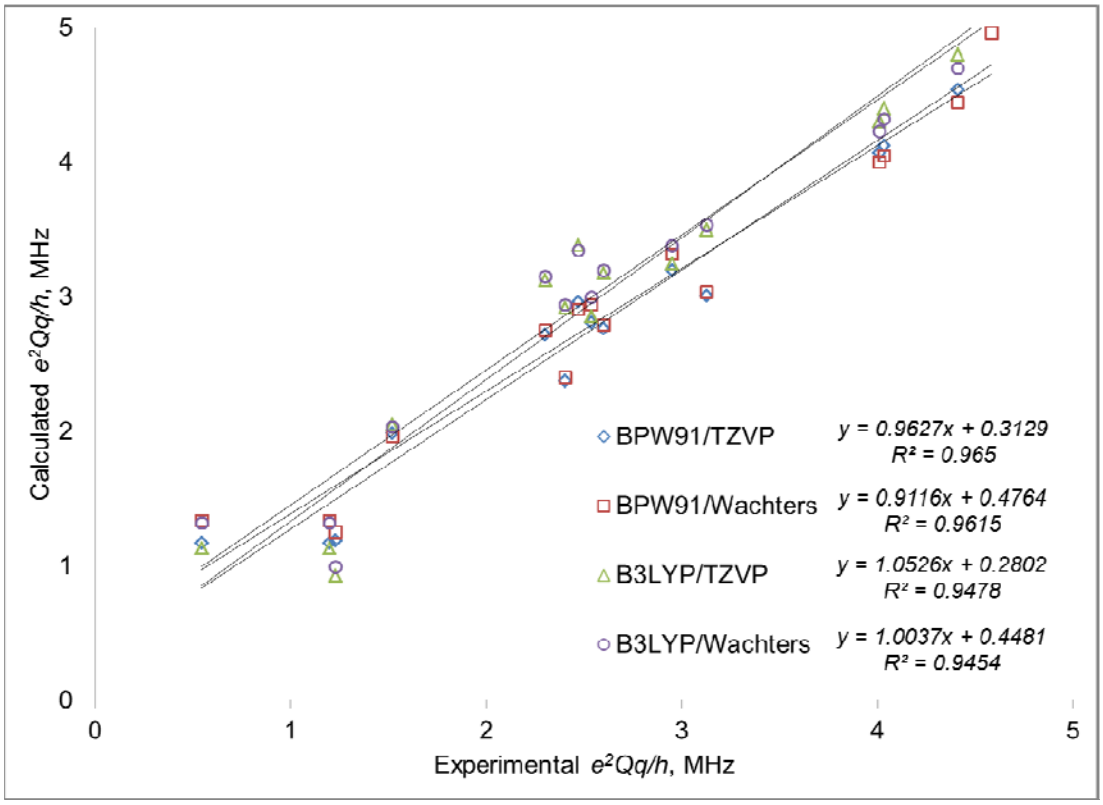

Figure S4. Correlation between experimental and computed e^2qQ/h values for model compounds, using four different basis set/functional combinations.

Table S1. Calculated ^{14}N nuclear quadrupole couplings of 16 nitrogen atoms in 13 model compounds using four different basis set/functional combinations.

Molecule	Experiment		BPW91/ TZVP		BPW91/ Wachters		B3LYP/ TZVP		B3LYP/ Wachters	
	e^2qQ/h	η	e^2qQ/h	η	e^2qQ/h	η	e^2qQ/h	η	e^2qQ/h	η
Pyridine	4.584[a]	0.396[a]	5.006	0.369	4.963	0.326	5.236	0.364	5.285	0.410
Pyridinium (HSO_4^-)	1.197[a]	0.426[a]	1.170	0.211	1.337	0.109	1.133	0.206	1.321	0.098
Imidazole(N)	4.032[b]	0.120[b]	4.124	0.050	4.051	0.002	4.406	0.108	4.322	0.055
Imidazole(NH)	2.537[b]	0.178[b]	2.812	0.177	2.942	0.218	2.855	0.165	2.998	0.209
1,2,4-Triazole(N1H)	2.95[b]	0.553[b]	3.199	0.462	3.318	0.417	3.249	0.477	3.384	0.425
1,2,4-Triazole(N2)	4.41[b]	0.728[b]	4.541	0.74	4.449	0.794	4.803	0.683	4.700	0.739
1,2,4-Triazole(N4)	4.01[b]	0.102[b]	4.070	0.052	4.004	0.001	4.305	0.087	4.231	0.033
Pyridine oxide	1.227[a]	0.426[a]	1.193	0.159	1.255	0.004	0.927	0.826	0.998	0.535
Pyridine sulfur trioxide	1.52[c]	0.15[c]	1.986	0.078	1.966	0.047	2.055	0.138	2.037	0.002
N-methylpyridinium	0.545[a]	0.07[a]	1.170	0.211	1.337	0.109	1.133	0.206	1.321	0.098
$Fe(CO)_4Py$	2.402[a]	0.319[a]	2.379	0.232	2.405	0.139	2.918	0.299	2.944	0.212
$Cr(CO)_4$(2,2'-bipyr)	3.124[a]	0.237[a]	3.011	0.094	3.041	0.026	3.502	0.203	3.538	0.137
mitoNEET cluster His	2.47[d]	0.38[d]	2.965	0.223	2.908	0.299	3.385	0.104	3.347	0.188
Rieske cluster His 1	2.6[e]	0.346[e]	2.764	0.315	2.795	0.355	3.176	0.162	3.201	0.233
Rieske cluster His 2	2.3[e]	0.391[e]	2.722	0.272	2.757	0.375	3.127	0.186	3.154	0.254
IspH + 7	3.0[f]	0.2[f]	2.268	0.530	2.363	0.534	2.571	0.160	2.877	0.331

[a]. Reference 14. [b]. Reference 15. [c]. Reference 16. [d]. Reference 17. [e]. Reference 18. [f]. This work.

Table S2. QM geometry optimized coordinates (in Å) of 13 model compounds obtained by using a BPW91 funtional and a Wachters basis set for Fe.

$[(Fe_4S_4)(SMe_4)_3(pyr)]^{2-}$, model of IspH + **7**			
Atom	x	y	z
Fe	0.697405	-1.519141	1.748632
Fe	-0.015702	-0.182461	3.959295
Fe	-1.538219	-0.202396	1.769967
Fe	0.774522	1.161600	1.808083
S	1.573820	-3.459266	0.955702
S	-3.555739	-0.519003	0.888175
S	2.082533	-0.310311	3.006850
S	-1.153356	1.593588	3.016555
S	-0.033256	-0.112543	0.112040
S	-1.079980	-1.964801	3.091548
S	1.988536	3.051197	1.323568
C	-3.682411	0.590582	-0.587867
H	-2.918976	0.327846	-1.338466
H	-4.685797	0.473691	-1.039377
H	-3.543544	1.647250	-0.304165
C	0.762655	4.055774	0.373424
H	1.197245	5.050477	0.162566
H	0.508733	3.571951	-0.584845
H	-0.166359	4.191626	0.951340

C	0.118556	-4.330419	0.229090
H	-0.661316	-4.489484	0.991553
H	-0.319410	-3.745038	-0.595898
H	0.445869	-5.310914	-0.163435
N	0.087356	0.019540	5.899781
C	0.589080	1.391106	7.852330
C	0.489838	1.206626	6.482796
C	-0.221001	-1.001194	6.777851
C	-0.142196	-0.877026	8.155797
C	0.272230	0.340334	8.742391
H	0.917819	2.368101	8.225830
H	0.729250	2.008708	5.779484
H	-0.540513	-1.934359	6.305643
H	-0.407239	-1.741952	8.775495
H	0.345487	0.462052	9.827606
$Fe(CO)_4$(pyr)			
Atom	x	y	z
Fe	0.026096	0.001069	0.037612
C	1.825469	-0.041886	-0.118884
O	2.975319	-0.069256	-0.299470
C	-0.786788	1.619067	0.079081
O	-1.297389	2.663648	0.077367
C	-0.863250	-1.576273	0.075799
O	-1.423232	-2.595231	0.071938
N	0.042289	-0.001349	2.107408
C	-0.097096	0.005831	-1.727498
O	-0.184312	0.009122	-2.884101
C	-1.140918	0.025803	2.772130
C	-1.230625	0.026620	4.162406
C	-0.059558	-0.001559	4.923477
C	1.162097	-0.029585	4.247153
C	1.173315	-0.028555	2.853207
H	-2.033646	0.047359	2.145342
H	-2.216875	0.049330	4.632043
H	-0.098032	-0.001670	6.016104
H	2.112489	-0.052372	4.785766
H	2.114649	-0.050114	2.304120
$Cr(CO)_4$(2,2'-bipyr)			
Atom	x	y	z
Cr	0.453243	-0.482911	-0.491684
C	2.281405	-0.471264	-0.767528
O	3.438805	-0.459497	-0.941460
C	0.367993	-2.309023	-0.768075
O	0.310189	-3.464979	-0.942441

C	0.841016	-0.887943	1.328697
O	1.138456	-1.199442	2.408112
C	0.277744	-0.300381	-2.379564
O	0.234931	-0.256948	-3.540133
N	0.270284	1.583175	-0.136529
N	-1.603771	-0.216200	-0.136971
C	-2.039285	1.054107	0.130454
C	-3.394101	1.329179	0.379830
C	-4.327479	0.296981	0.358065
C	-3.878866	-1.001352	0.084213
C	-2.524717	-1.210378	-0.154599
C	1.301032	2.463009	-0.153685
C	1.147123	3.824473	0.085416
C	-0.131977	4.325397	0.359073
C	-1.201233	3.434698	0.380354
C	-0.981348	2.069888	0.130704
H	-3.718016	2.350211	0.590804
H	-5.384320	0.499403	0.550670
H	-4.567425	-1.849076	0.054491
H	-2.139310	-2.207931	-0.371198
H	2.282177	2.037489	-0.370141
H	2.022135	4.478022	0.056063
H	-0.291361	5.389540	0.551896
H	-2.208323	3.799757	0.591164
$[(Fe_2S_2)(SMe)_3(Im)]^{2-}$, mitoNEET cluster model			
Atom	x	y	z
Fe	15.565009	-6.863056	-5.028041
Fe	13.893362	-8.003905	-3.305538
S	15.147037	-9.053765	-4.809706
S	14.092857	-5.818469	-3.699522
N	14.647719	-8.261486	-1.237200
C	15.915970	-8.494740	-0.713908
C	15.905149	-8.205919	0.636709
C	13.889179	-7.843475	-0.230662
N	14.608740	-7.795571	0.933688
C	17.054490	-8.940430	-1.569158
H	16.686014	-8.240665	1.394352
H	12.832474	-7.595106	-0.325128
H	14.279161	-7.442981	1.827121
H	16.770848	-9.820031	-2.169241
H	17.937183	-9.180776	-0.950566
H	17.325169	-8.150752	-2.295152
S	15.373509	-6.058444	-7.256472
C	13.545176	-6.095344	-7.522305

H	13.305071	-5.683788	-8.520243
H	13.031963	-5.499622	-6.750066
H	13.166201	-7.128963	-7.464845
S	17.822397	-6.355105	-4.506396
C	17.721313	-4.539352	-4.171213
H	18.717621	-4.151315	-3.888770
H	17.008769	-4.344081	-3.353879
H	17.369677	-4.004593	-5.068454
S	11.809433	-8.837906	-2.626795
C	10.628666	-8.370659	-3.974043
H	9.599072	-8.674263	-3.704538
H	10.906121	-8.868024	-4.918271
H	10.646649	-7.281415	-4.141760
$[(Fe_2S_2)(SMe)_2(Im)_2]^-$, Rieske cluster model			
Atom	x	y	z
Fe	67.395051	51.076454	0.766091
Fe	69.787283	50.246573	0.124066
S	69.318940	52.189746	1.021368
S	67.935209	49.089138	-0.111712
N	70.914613	50.270367	-1.668549
C	70.709704	51.110149	-2.765000
C	71.343443	50.566905	-3.862778
C	71.655179	49.252234	-2.100783
N	71.937776	49.388689	-3.430149
C	69.880012	52.346354	-2.673942
H	71.410014	50.898260	-4.896142
H	72.442149	48.723611	-4.008577
H	71.982549	48.411709	-1.494228
H	69.849796	52.865491	-3.645988
H	70.273597	53.031885	-1.906149
H	68.848620	52.103133	-2.361658
N	71.131010	49.059722	1.254069
C	72.329162	49.414961	1.874325
C	72.684595	48.423898	2.765932
C	70.777990	47.877784	1.760346
N	71.698026	47.454099	2.677303
C	73.005844	50.714840	1.584345
H	73.527435	48.328508	3.446475
H	69.849355	47.361784	1.523958
H	71.620459	46.621228	3.253179
H	72.309278	51.549566	1.774833
H	73.305039	50.784107	0.524441
H	73.902518	50.840564	2.212740
S	66.042618	52.357783	-0.643962

C	64.593703	51.237310	-0.883032
H	63.814715	51.763249	-1.461754
H	64.180040	50.937904	0.093316
H	64.894064	50.328441	-1.428796
S	66.190799	50.731591	2.725388
C	65.924853	52.466198	3.302180
H	65.279528	52.458293	4.197628
H	65.441237	53.061235	2.511056
H	66.886284	52.939724	3.557888
Pyridine			
Atom	x	y	z
C	1.145736	0.686898	0.000000
N	0.000000	1.390722	0.000000
C	-1.145736	0.686898	0.000000
C	-1.201972	-0.712227	0.000000
C	0.000000	-1.427396	0.000000
C	1.201972	-0.712227	0.000000
H	2.066968	1.278924	0.000000
H	-2.066968	1.278924	0.000000
H	-2.166265	-1.225265	0.000000
H	0.000000	-2.519985	0.000000
H	2.166265	-1.225265	0.000000
Pyridinium			
Atom	x	y	z
C	1.193979	0.686365	0.000000
N	0.000000	1.332069	0.000000
C	-1.193979	0.686365	0.000000
C	-1.214138	-0.701221	0.000000
C	0.000000	-1.402121	0.000000
C	1.214138	-0.701221	0.000000
H	0.000000	2.353969	0.000000
H	2.086612	1.310218	0.000000
H	-2.086612	1.310218	0.000000
H	-2.171722	-1.221146	0.000000
H	0.000000	-2.493351	0.000000
H	2.171722	-1.221146	0.000000
N-methylpyridinium			
Atom	x	y	z
C	1.189510	0.682776	-0.001677
N	0.011174	1.360776	-0.002018
C	-1.174380	0.690694	-0.001594
C	-1.205232	-0.695819	0.000277
C	-0.000043	-1.409734	0.001243
C	1.208831	-0.706209	0.000459

H	2.095539	1.286441	-0.002208
H	-2.073688	1.305630	-0.002293
H	-2.169477	-1.203728	0.001147
H	-0.004512	-2.500673	0.002734
H	2.169483	-1.220838	0.001432
C	-0.002330	2.846232	-0.004728
H	1.026443	3.216264	0.006292
H	-0.529175	3.205461	0.886984
H	-0.509083	3.202248	-0.909421
Pyridine oxide			
Atom	x	y	z
C	1.186103	0.683712	0.000000
N	0.000000	1.395721	0.000000
C	-1.186103	0.683712	0.000000
C	-1.198250	-0.702466	0.000000
C	0.000000	-1.427532	0.000000
C	1.198250	-0.702466	0.000000
O	0.000000	2.674576	0.000000
H	2.071734	1.315327	0.000000
H	-2.071734	1.315327	0.000000
H	-2.164935	-1.209200	0.000000
H	0.000000	-2.517509	0.000000
H	2.164935	-1.209200	0.000000
Pyridine sulfur trioxide			
Atom	x	y	z
C	1.156504	0.651417	0.001795
N	-0.019213	1.304287	0.003756
S	-0.017490	3.401658	0.007699
O	-1.480061	3.582675	0.003299
C	-1.185950	0.639462	0.003952
C	-1.214382	-0.755791	0.001871
C	-0.003485	-1.455382	-0.000204
C	1.200760	-0.741412	-0.000357
O	0.708778	3.600748	1.272240
O	0.717156	3.605223	-1.250968
H	2.051580	1.274637	0.001697
H	-2.080338	1.264220	0.005446
H	-2.172573	-1.276308	0.001819
H	0.003146	-2.546992	-0.001905
H	2.164568	-1.251442	-0.002140
Imidazole			
Atom	x	y	z
C	0.867718	0.889317	0.000000
N	-0.428931	1.365904	0.000000

C	-1.195647	0.288639	0.000000
N	-0.448902	-0.863901	0.000000
C	0.884036	-0.489857	0.000000
H	1.716819	1.567928	0.000000
H	-2.282665	0.278509	0.000000
H	-0.802594	-1.814856	0.000000
H	1.687165	-1.219683	0.000000
1,2,4-Triazole			
Atom	x	y	z
C	0.947322	0.669955	0.000000
N	-0.364993	1.060876	0.000000
N	-1.132684	-0.002851	0.000000
N	-0.305333	-1.081896	0.000000
C	1.004069	-0.711203	0.000000
H	1.754753	1.395123	0.000000
H	-0.706889	-2.014408	0.000000
H	1.820755	-1.424596	0.000000

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