Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics.

Supporting information for

Catalytic mechanism of Phenylacetone monooxygenases for non-native linear substrates: implications on rational engineering of BVMOs to expand the substrate specificity

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Table S1

Relevant distances at the transition states for phenylacetone and 2-octanone (in the cluster models).

		distance$_{OX-C2}$	**distance$_{OX-C3}$**	**distance$_{C2-C3}$**
phenylacetone	TS1	1.70 Å		
	TS2		2.17 Å	1.82Å
2-octanone	TS1	1.76 Å		
	TS2		2.08 Å	1.84 Å

Table S2

Atom names and RESP charges for the C4a-peroxyflavin intermediate

Atom names	Charges	Atom names	Charges	Atom names	Charges
O1	-0.750689	C15	-0.417332	H8	0.038554
O2	-0.750689	C16	0.201327	H9	0.142671
O3	-0.410738	C17	-0.323534	H10	0.395651
C1	0.084146	C18	0.053932	H11	0.395651
C2	0.320625	C19	-0.344892	H12	0.071097
O4	-0.579831	C20	-0.184737	H13	0.355706
C3	-0.01102	C21	-0.233903	H14	0.370277
O5	-0.583078	N9	0.116336	H15	0.183678
C4	0.151937	C22	0.390734	H16	0.096655
O6	-0.646985	C23	-0.213015	H17	0.096655
C5	0.591447	C24	0.514661	H18	0.096655
N1	-0.392062	O9	-0.706937	H19	0.10425
C6	0.261341	C25	0.000225	H20	0.10425
N2	-0.653614	O10	-0.593283	H21	0.10425
C7	0.094783	C26	0.317474	H22	0.170087
C8	0.579045	O11	-0.647139	H23	0.083932
N3	-0.787502	C27	-0.059474	H24	0.083932
N4	-0.738769	O12	-0.391831	H25	-0.020397
C9	0.524738	O13	-0.760938	H26	0.415804
N5	-0.82368	O14	-0.760938	H27	0.098958
C10	0.642946	O15	-0.42389	H28	0.343896
N6	-0.630222	O16	-0.141027	H29	0.066462
C11	0.787411	O17	-0.642101	H30	0.41153
O7	-0.641492	H1	0.053617	H31	0.069922
N7	-0.582068	H2	0.053617	H32	0.069922
C12	0.506697	H3	0.040912	P1	0.934765
O8	-0.541782	H4	0.105558	P2	0.917385
C13	0.333224	H5	0.383648		
N8	-0.787168	H6	0.045989		
C14	0.347973	H7	0.449821		

Table S3

Atom names and RESP charges for the Criegee intermediate.

phenylacetone system		**2-octanone system**	
Atom names	Charges	Atom names	Charges
C1	-0.425002	C1	0.219062
C2	0.885316	C2	-0.245049
O3	-0.891789	C3	0.176103
C4	-0.531083	C4	-0.286100
H5	0.104667	H5	0.050675
H6	0.104667	H6	0.050675
H7	0.104667	H7	0.050675
H8	0.070907	H8	0.010408
H9	0.070907	H9	0.010408
C10	0.322029	H10	0.025735
C11	-0.247309	H11	0.025735
C12	-0.135434	H12	-0.017981
H13	0.116244	H13	-0.017981
H14	0.152541	C14	-0.369395
C15	-0.247309	H15	0.088265
H16	0.152541	H16	0.088265
C17	-0.135434	C17	-0.088617
H18	0.116244	H18	0.104482
C19	-0.203179	H19	0.104482
H20	0.113856	C20	0.619826
O21	-0.344089	O21	-0.399888
O22	-0.324033	C22	-0.513506
N23	-0.64596	H23	0.127882
C24	0.897542	H24	0.127882
O25	-0.589321	H25	0.127882
N26	-0.77406	O26	-0.386514
C27	0.63892	O27	-0.399888
O28	-0.573432	N28	-0.978717
C29	0.549815	C29	1.086765
N30	-0.849602	O30	-0.666635
C31	0.438978	N31	-0.780803
C32	-0.493329	C32	0.802332
C33	0.183485	O33	-0.610191
C34	-0.352111	C34	-0.266244
C35	0.084752	N35	-0.770616
C36	-0.206993	C36	0.270315
C37	-0.311929	C37	-0.411445
C38	-0.101144	C38	0.109325

N39	-0.021082	C39	-0.258951
C40	0.367531	C40	0.158273
C41	-0.075631	C41	-0.420326
H42	0.397387	C42	-0.297683
H43	0.429069	C43	0.105563
H44	0.206849	N44	-0.415426
H45	0.094534	C45	1.166968
H46	0.094534	C46	-0.102279
H47	0.094534	O47	-0.877972
H48	0.064676	O48	-0.877972
H49	0.064676	O49	-0.602495
H50	0.064676	C50	0.160005
H51	0.248978	C51	0.146643
H52	0.090641	O52	-0.610545
H53	0.090641	C53	0.310121
O54	-0.901302	O54	-0.668416
O55	-0.901302	C55	0.137662
O56	-0.601568	O56	-0.663971
C57	0.215019	C57	0.286616
C58	0.023578	N58	-0.094404
O59	-0.518801	C59	0.164540
C60	0.509534	N60	-0.573871
O61	-0.715073	C61	-0.070633
C62	0.036765	C62	0.802432
O63	-0.647632	N63	-0.938563
C64	0.374192	N64	-0.809399
N65	-0.160591	C65	0.589682
C66	0.230079	N66	-0.829677
N67	-0.598765	C67	0.494805
C68	-0.055015	C68	0.172046
C69	0.855343	O69	-0.753764
N70	-1.032455	C70	0.389519
N71	-0.850511	O71	-0.795780
C72	0.624398	C72	0.265170
N73	-0.794874	O73	-0.755342
C74	0.465603	C74	-0.084488
C75	0.310302	O75	-0.499113
O76	-0.745018	O76	-0.881246
C77	0.259545	O77	-0.881246
O78	-0.762265	O78	-0.606591
C79	0.334155	H79	0.052811
O80	-0.77199	H80	0.052811
C81	-0.039436	H81	0.119513
O82	-0.550714	H82	0.123451
O83	-0.889945	H83	0.371233

O84	-0.889945	H84	0.095731
O85	-0.609088	H85	0.393496
H86	0.043336	H86	0.108064
H87	0.043336	H87	0.192304
H88	0.083065	H88	0.405638
H89	0.062571	H89	0.405638
H90	0.381502	H90	0.056947
H91	0.094805	H91	0.347038
H92	0.386693	H92	0.402286
H93	0.064508	H93	0.197032
H94	0.164821	H94	0.068199
H95	0.438882	H95	0.068199
H96	0.438882	H96	0.068199
H97	0.0481	H97	0.111427
H98	0.027206	H98	0.111427
H99	0.467489	H99	0.111427
H100	-0.008152	H100	0.236678
H101	0.433651	H101	0.111723
H102	0.054057	H102	0.111723
H103	0.545836	H103	0.098470
H104	0.063743	H104	0.455186
H105	0.063743	H105	-0.030115
P106	1.449779	H106	0.437609
P107	1.512373	H107	0.066739
		H108	0.538640
		H109	0.075589
		H110	0.075589
		P111	1.432751
		P112	1.479041

Figure S1

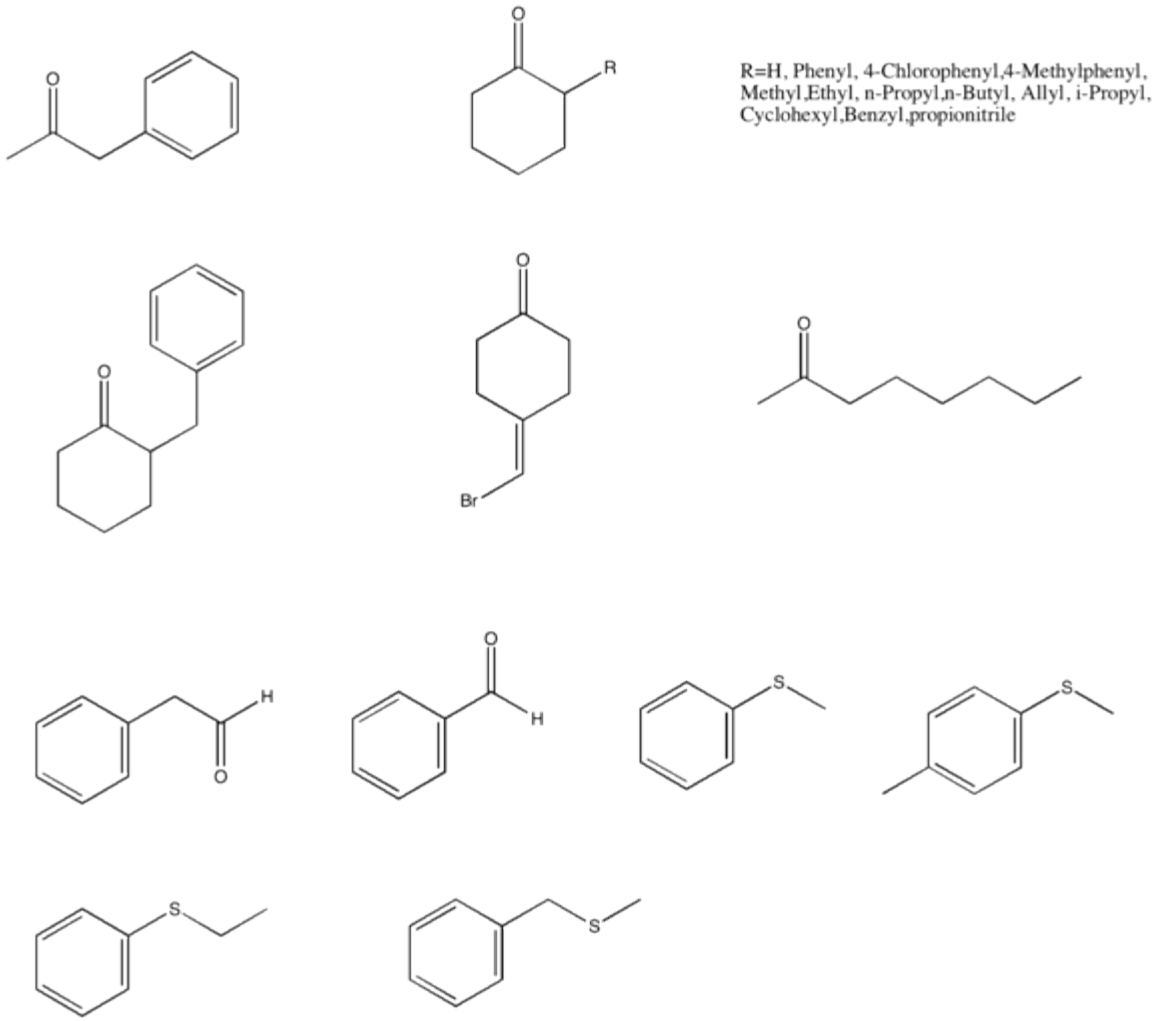


Figure S1 Best characterized substrates in previous studies. Here we only present the substrates for which quantitative data is available and for which the conversion or k_{cat}/K_M values are higher, excluding those with low conversions.

Figure S2

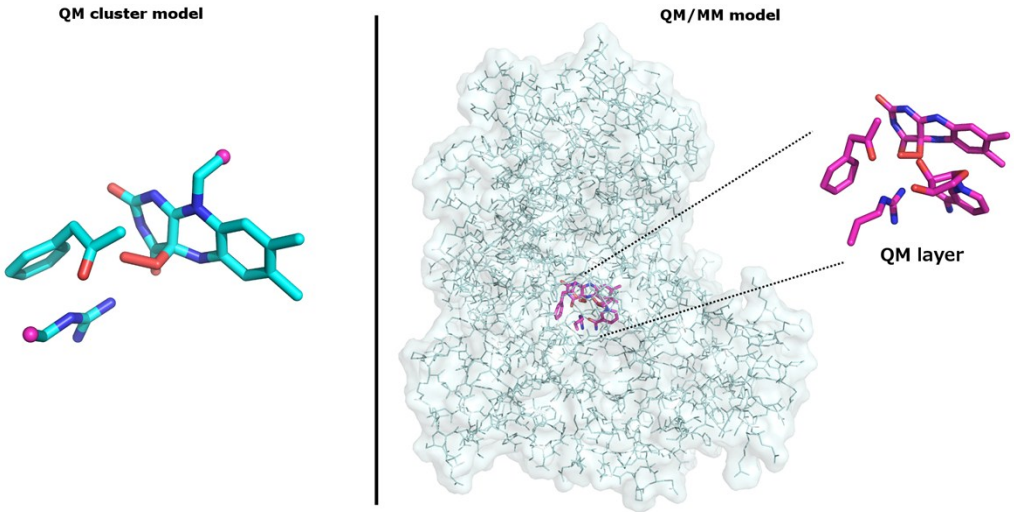


Figure S2 Comparison of the QM cluster model (left) vs. the QM/MM model (right). The phenylacetone cluster model is constituted by 73 atoms, while the 2-octanone cluster model includes 78 atoms. For the QM/MM model the QM layer of the phenylacetone:PAMO complex is composed by 123 atoms while the one of 2-octanone:PAMO complex is composed by 128 atoms.

Figure S3

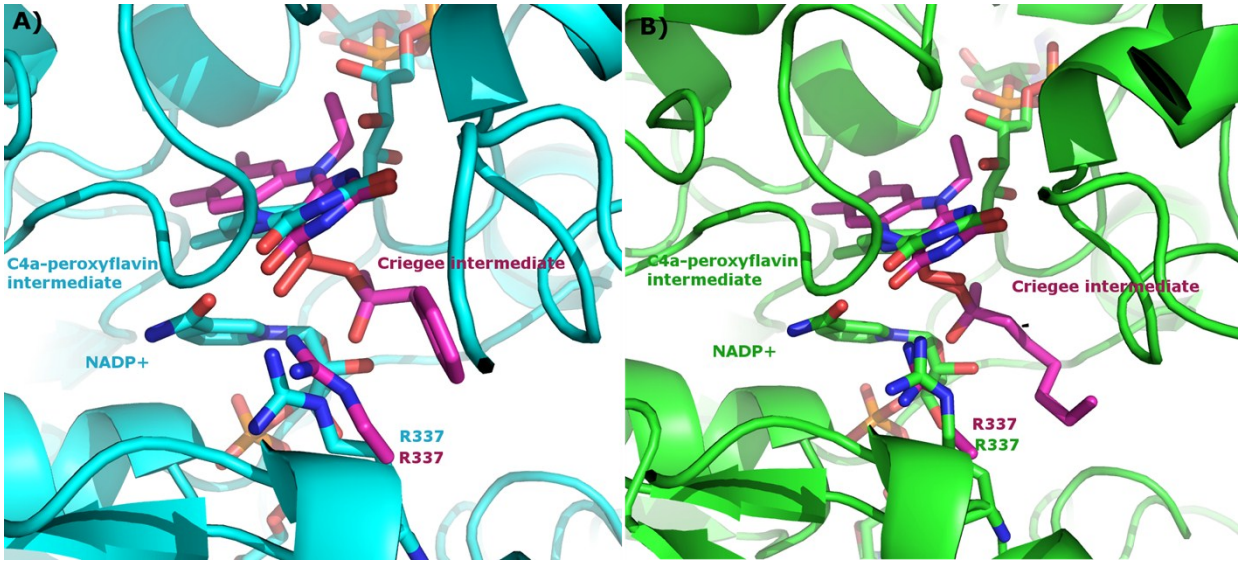
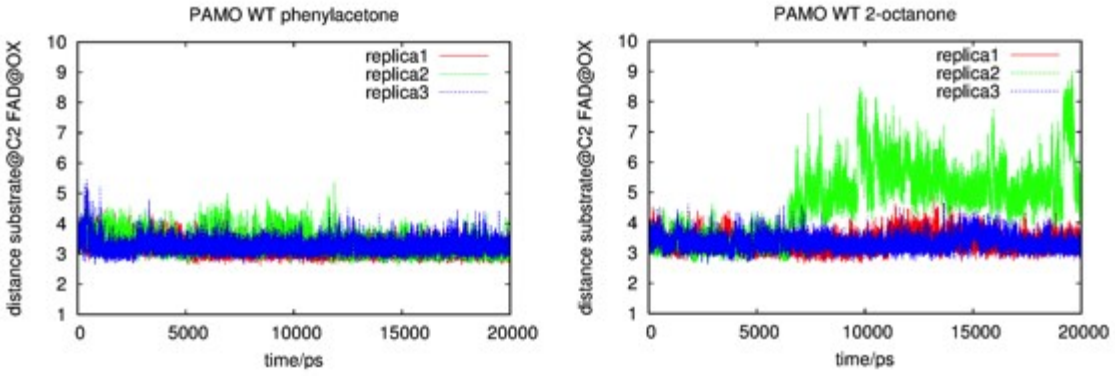
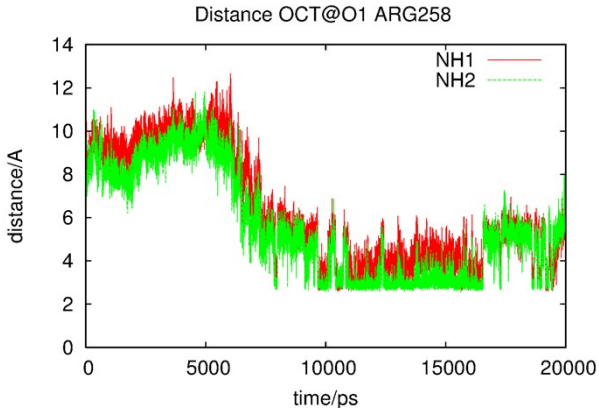


Figure S3 The optimized structure of the Criegee intermediate (cluster model) superimposed with the crystal structure of the WT PAMO in complex with the modelled C4a-peroxyflavin intermediate: A) phenylacetone system; B) 2-octanone system.

Figure S4



(a)



(b)

Figure S4 (a) Distance between the peroxy-FAD oxygen and the carbonyl carbon of the substrates during the molecular dynamics simulations of the WT PAMO (left panel). For phenylacetone the distance was retained around 3.18±0.24 Å in replica 1, 3.36±0.36 Å in replica 2 and 3.27±0.26 in replica 3 (right panel). 2-octanone is also able to engage in a catalytic relevant distance as shown in replicas 1 and 3, however it can easily move away from the peroxy moiety of the cofactor to at a distance up to 9 Å in replica 2. (b) Distance between NH1 or NH2 of R258 and the carbonyl oxygen atom of the 2-octanone substrate in the MD simulation of the WT PAMO (replica 2).

Figure S5

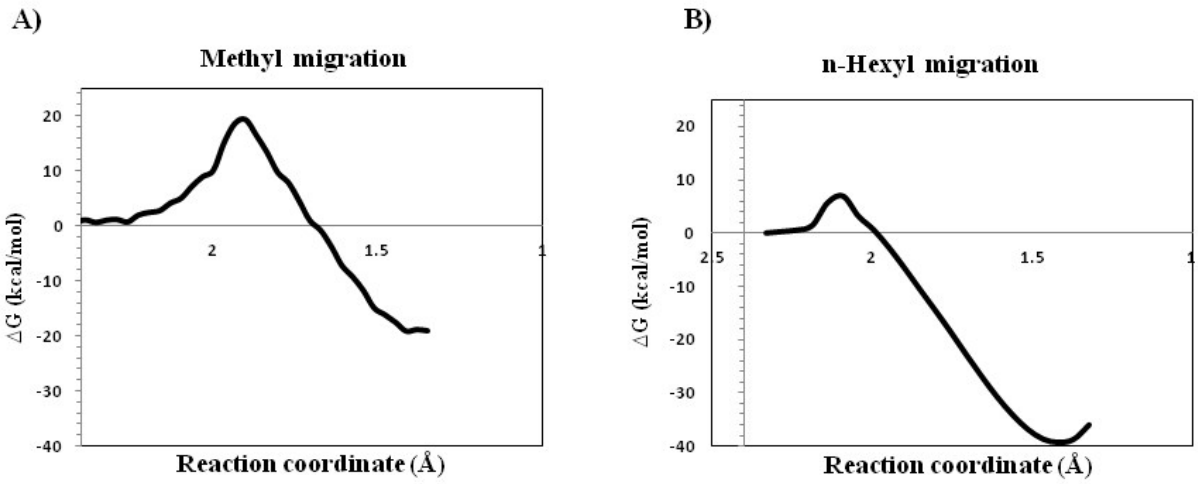


Figure S5 QM/MM (PM3/Amber_parm99SB) Gibbs free energy profile for the WT-PAMO-catalyzed transformation of 2-octanone. The energy barrier associated with the decay of the Criegee intermediate to yield the "abnormal" product via methyl migration (a) is much higher than that associated with "normal" product via n-hexyl migration (b).

Figure S6

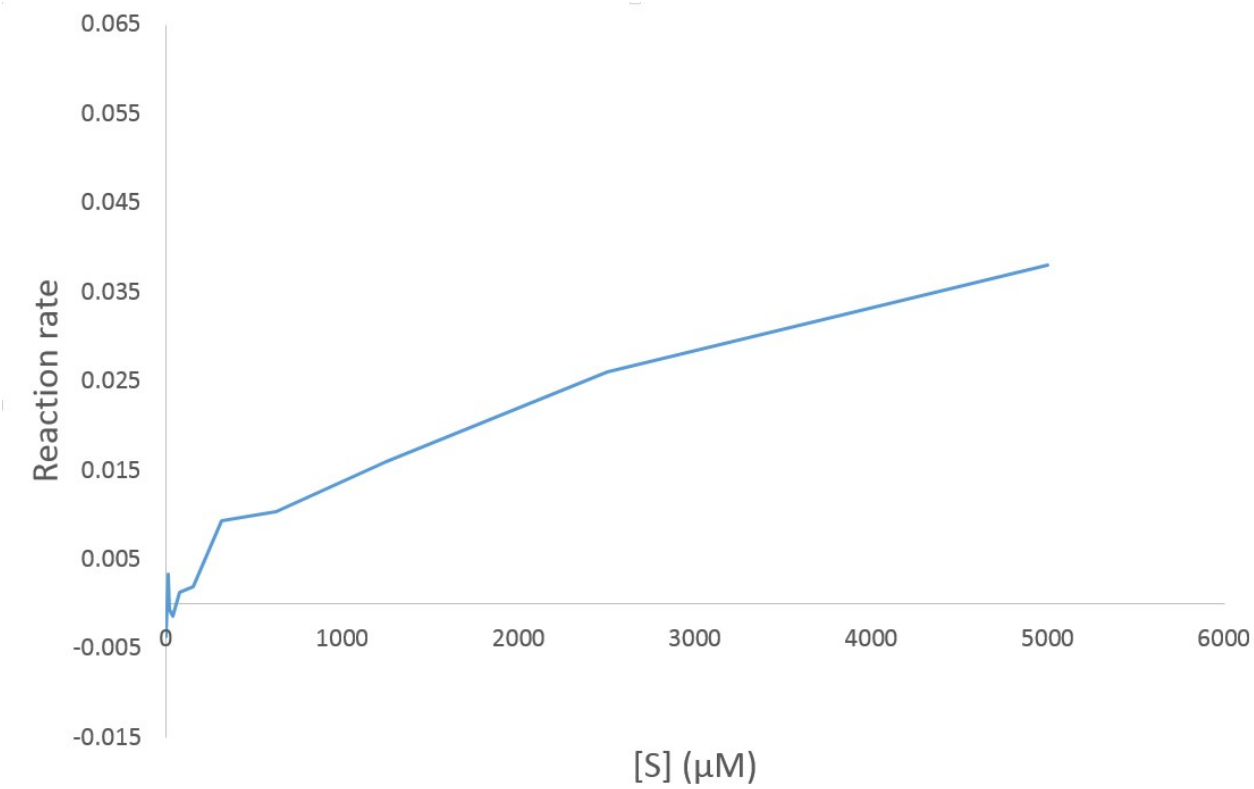


Figure S6 Michaelis-Menten kinetic curve for the transformation of 2-octanone catalyzed by the WT PAMO.

Optimized Coordinates of the stationary points from DFT calculations (B3LYP/6-31g(d))

Phenylacetone

RC

H	-5.895313	-3.142791	2.516347
C	-4.943792	-2.708706	2.190540
H	-4.838783	-1.707093	2.618089
H	-4.121165	-3.325435	2.567911
C	-4.903096	-2.633730	0.667184
H	-4.990749	-3.639497	0.234163
H	-5.751600	-2.031591	0.308605
N	-3.649806	-2.036350	0.222761
H	-3.086684	-1.533860	0.919580
C	-3.264531	-1.991787	-1.057690
N	-4.096903	-2.561702	-1.998461
H	-5.087523	-2.517946	-1.797599
H	-3.875008	-2.332303	-2.957887
N	-2.095452	-1.494288	-1.397531
H	-1.466648	-0.834031	-0.703306
H	-1.874023	-1.398636	-2.382707
C	-2.276670	1.537471	1.741615
C	-1.550163	0.264218	2.196596
O	-2.136841	-0.806095	2.333619
C	-0.136448	0.452581	2.708657
H	0.296479	1.388697	2.344888
H	-0.152481	0.455442	3.807813
H	0.475055	-0.385548	2.364985
H	-1.576603	2.182296	1.205818
H	-2.549162	2.058640	2.674410
C	-3.506544	1.271150	0.916116
C	-4.705335	0.867205	1.518502
C	-5.833886	0.584483	0.747579
H	-6.759493	0.283049	1.232267
H	-4.749972	0.780621	2.601222
C	-3.457402	1.386104	-0.478087
H	-2.531905	1.710720	-0.940044
C	-4.580417	1.091961	-1.254322
H	-4.528328	1.190520	-2.336385
C	-5.771794	0.687944	-0.645734
H	-6.652152	0.477010	-1.248996
H	4.310189	3.613541	-0.313616
H	4.977372	3.925207	1.306260
N	1.171224	2.574051	-0.233635
C	0.069020	2.773917	-1.029900
O	-0.760148	3.647871	-0.848204
N	-0.062015	1.950152	-2.192558
C	0.353162	0.651865	-2.234073
O	0.010804	-0.153616	-3.089979
C	1.324409	0.251503	-1.104668

N	2.409463	-0.471884	-1.673216
C	3.479298	-0.774389	-0.832698
C	4.275076	-1.904383	-1.028886
C	5.379475	-2.172538	-0.220969
C	6.218299	-3.403683	-0.457564
C	5.695508	-1.279758	0.820971
C	6.881505	-1.538052	1.718079
C	4.899605	-0.149275	1.016508
C	3.791067	0.123471	0.206375
N	2.995538	1.286988	0.367814
C	1.843730	1.461120	-0.335992
C	3.469806	2.372987	1.246002
C	4.636312	3.141488	0.619444
O	0.604841	-0.574625	-0.193718
O	-0.694939	0.075878	-0.039729
H	-0.825073	2.203152	-2.811583
H	2.090619	-1.212204	-2.290416
H	4.016323	-2.588694	-1.834239
H	7.263954	-3.139495	-0.668740
H	5.836054	-3.986534	-1.302346
H	6.231809	-4.053598	0.428434
H	6.782702	-2.495837	2.248071
H	6.990567	-0.746673	2.467819
H	7.816503	-1.592983	1.142998
H	5.167925	0.530197	1.816289
H	2.620693	3.035323	1.409420
H	3.747364	1.931783	2.208074
H	5.477821	2.475461	0.400759

TS1

H	34.139302	48.533277	50.371676
C	34.880002	48.332000	51.153001
H	34.462516	48.622471	52.122638
H	35.090680	47.257288	51.176689
C	36.154168	49.127389	50.869412
H	36.562071	48.847142	49.888337
H	35.907504	50.198835	50.840071
N	37.178579	48.891463	51.884512
H	36.977207	48.414947	52.819078
C	38.410686	49.357267	51.761617
N	38.751295	50.121825	50.675083
H	38.016299	50.707785	50.302554
H	39.654024	50.575484	50.717089
N	39.361360	49.007940	52.633950
H	39.062497	48.589021	53.521987
H	40.206773	49.559538	52.717425
C	36.708336	49.359451	55.852285
C	37.440997	48.061095	55.378464
O	37.160427	47.606234	54.211703
C	37.527449	47.030489	56.506228
H	37.861755	47.487697	57.444697
H	36.531286	46.593711	56.649974
H	38.222100	46.237226	56.219113

H	37.212706	49.763156	56.736208
H	35.708131	49.026469	56.160672
C	36.577835	50.411974	54.783796
C	35.400360	50.527200	54.034022
C	35.279618	51.478518	53.018469
H	34.350212	51.558394	52.459183
H	34.567759	49.865316	54.260460
C	37.639105	51.282143	54.493470
H	38.544178	51.216746	55.086976
C	37.532446	52.220728	53.466370
H	38.363240	52.892869	53.261333
C	36.352274	52.322847	52.721346
H	36.260200	53.072143	51.938014
H	41.691031	48.527817	60.668451
H	40.695405	47.546591	61.769625
N	40.238416	49.644062	58.106137
C	40.231230	50.792522	57.351132
O	39.645449	51.813399	57.655070
N	41.022808	50.813208	56.154522
C	41.401964	49.718624	55.446451
O	41.889318	49.755823	54.322132
C	41.128998	48.375000	56.146999
N	42.286453	47.558218	56.064802
C	42.222991	46.334914	56.740187
C	42.956578	45.225881	56.318847
C	42.924923	44.018930	57.016699
C	43.725622	42.835205	56.534514
C	42.128457	43.922378	58.173725
C	42.061788	42.631907	58.953626
C	41.398468	45.034450	58.597383
C	41.429516	46.248226	57.900452
N	40.734377	47.401516	58.345617
C	40.706606	48.538226	57.601498
C	40.123299	47.415725	59.686835
C	41.175547	47.568536	60.784150
O	40.061980	47.739194	55.429992
O	38.994655	48.752907	55.394148
H	41.059378	51.709750	55.680854
H	42.675614	47.538420	55.127033
H	43.560034	45.313126	55.417707
H	44.442019	42.501078	57.297881
H	44.285026	43.079177	55.625051
H	43.075618	41.976537	56.316690
H	41.680813	41.808554	58.333441
H	41.405279	42.730816	59.824926
H	43.055396	42.324363	59.308515
H	40.805864	44.938960	59.499193
H	39.428366	48.254439	59.705470
H	39.546326	46.493689	59.799829
H	41.915160	46.761743	60.736184

INT

H	6.969843	-2.896075	-0.785551
C	5.880084	-2.780168	-0.915198
H	5.701668	-2.147803	-1.791849
H	5.447168	-3.768246	-1.107908
C	5.234730	-2.152079	0.323766
H	5.437683	-2.772681	1.205575
H	5.657695	-1.154339	0.498355
N	3.783109	-2.070581	0.163928
H	3.324450	-1.873817	-0.750828
C	2.922384	-2.007969	1.181783
N	3.370353	-1.816755	2.461106
H	4.217973	-1.271225	2.549291
H	2.645586	-1.562075	3.123712
N	1.628319	-2.221697	0.959369
H	1.377309	-2.148818	-0.061940
H	0.973200	-1.793228	1.606714
C	1.892581	0.729457	-2.319622
C	1.039504	-0.469057	-1.784065
O	1.697905	-1.586153	-1.589844
C	-0.227631	-0.625397	-2.640483
H	-0.793776	0.311884	-2.710083
H	0.075302	-0.941195	-3.645605
H	-0.865849	-1.401435	-2.210041
H	1.270370	1.631222	-2.344400
H	2.135327	0.461486	-3.355264
C	3.163405	0.990867	-1.556773
C	4.378445	0.440698	-1.985636
C	5.567625	0.696036	-1.298173
H	6.502190	0.272279	-1.658617
H	4.386532	-0.179679	-2.878798
C	3.164820	1.793065	-0.406385
H	2.239462	2.250814	-0.075377
C	4.346397	2.036218	0.295221
H	4.324881	2.676899	1.174036
C	5.555864	1.491073	-0.149062
H	6.481356	1.699362	0.382861
H	-4.013473	3.737979	0.430522
H	-4.698335	4.100992	-1.170997
N	-1.087052	2.708244	0.514226
C	0.042259	2.863121	1.275549
O	0.751745	3.850611	1.270584
N	0.395317	1.810315	2.189447
C	-0.074802	0.545353	2.164417
O	0.340742	-0.363924	2.883993
C	-1.154141	0.272520	1.101662
N	-2.238503	-0.422965	1.699109
C	-3.324629	-0.684422	0.854053
C	-4.148859	-1.793790	1.038629
C	-5.249658	-2.030667	0.215843
C	-6.121253	-3.241725	0.434390
C	-5.528533	-1.125865	-0.826909
C	-6.707176	-1.352479	-1.741872
C	-4.704963	-0.013428	-1.007001

C	-3.601406	0.228841	-0.180929
N	-2.789937	1.385870	-0.311056
C	-1.674167	1.546939	0.443288
C	-3.239283	2.504387	-1.161747
C	-4.369931	3.294329	-0.505064
O	-0.563299	-0.576975	0.116168
O	0.555924	0.210613	-0.448317
H	1.199474	2.005078	2.776853
H	-1.935745	-1.187245	2.294719
H	-3.915003	-2.489851	1.841481
H	-7.162998	-2.952364	0.630597
H	-5.766713	-3.837775	1.281954
H	-6.136492	-3.886604	-0.455002
H	-6.620945	-2.309432	-2.275055
H	-6.787514	-0.555018	-2.488422
H	-7.650442	-1.389787	-1.179386
H	-4.947597	0.676587	-1.805794
H	-2.371135	3.142343	-1.323083
H	-3.538979	2.087628	-2.127125
H	-5.227835	2.648604	-0.287231

TS2

H	-6.985425	-2.660890	0.667722
C	-5.925345	-2.741610	0.933055
H	-5.776086	-2.254249	1.902075
H	-5.658224	-3.800080	1.023737
C	-5.072500	-2.058040	-0.141379
H	-5.209301	-2.581097	-1.095110
H	-5.391118	-1.015902	-0.264480
N	-3.649816	-2.118198	0.191608
H	-3.329807	-1.869552	1.134577
C	-2.660011	-2.120470	-0.712951
N	-2.920206	-1.944957	-2.036354
H	-3.759650	-1.428614	-2.260192
H	-2.106553	-1.694482	-2.598153
N	-1.420210	-2.392237	-0.309553
H	-1.289121	-2.226165	0.708829
H	-0.652301	-1.994947	-0.847027
C	-1.857976	1.316340	2.141682
C	-0.877455	-0.200508	1.945682
O	-1.579075	-1.263246	2.137625
C	0.337697	-0.004388	2.861405
H	0.742506	1.010714	2.779056
H	0.061808	-0.220155	3.898276
H	1.105429	-0.713420	2.534434
H	-1.333059	2.174152	1.725429
H	-1.779587	1.311356	3.232326
C	-3.259141	1.190807	1.693426
C	-4.185027	0.486936	2.482643
C	-5.536952	0.459036	2.139227
H	-6.247355	-0.061608	2.776453
H	-3.829387	-0.018560	3.375885
C	-3.707190	1.818022	0.519071

H	-2.998671	2.369521	-0.093340
C	-5.058769	1.781495	0.171540
H	-5.398011	2.297255	-0.723841
C	-5.981054	1.114799	0.986340
H	-7.036758	1.107386	0.726144
H	3.571366	4.029889	-0.872356
H	4.345250	4.573271	0.635522
N	0.805866	2.612198	-0.592036
C	-0.392409	2.575651	-1.250041
O	-1.235690	3.455496	-1.201551
N	-0.666833	1.455371	-2.105071
C	-0.003031	0.276963	-2.104033
O	-0.341191	-0.704054	-2.772466
C	1.159438	0.177684	-1.104231
N	2.300734	-0.376051	-1.789767
C	3.470198	-0.450700	-1.029523
C	4.422467	-1.450224	-1.233924
C	5.610126	-1.492055	-0.504444
C	6.619552	-2.586160	-0.746957
C	5.847579	-0.502145	0.468636
C	7.118566	-0.516066	1.282470
C	4.895439	0.498312	0.673144
C	3.704738	0.545060	-0.060653
N	2.753260	1.586463	0.094022
C	1.561370	1.547024	-0.552087
C	3.117356	2.803390	0.843108
C	4.067935	3.692885	0.043682
O	0.746250	-0.666579	-0.083853
O	-0.629453	0.278570	0.679470
H	-1.533380	1.516371	-2.629047
H	2.057366	-1.216010	-2.306578
H	4.221204	-2.214578	-1.981647
H	7.595094	-2.171099	-1.035922
H	6.287723	-3.263491	-1.541188
H	6.788440	-3.181188	0.161331
H	7.218465	-1.449400	1.853922
H	7.142897	0.319175	1.990968
H	8.007987	-0.444166	0.640758
H	5.107316	1.255493	1.418531
H	2.187963	3.331209	1.053014
H	3.554999	2.492337	1.795812
H	4.980411	3.150527	-0.227344

PC

H	6.999072	2.302910	0.544425
C	6.023706	2.392758	1.033082
H	5.914306	1.561159	1.739122
H	5.989463	3.336559	1.589055
C	4.914831	2.334009	-0.014434
H	5.009603	3.164773	-0.725162
H	4.999671	1.393508	-0.573226
N	3.606470	2.431794	0.633112
H	3.545768	2.125857	1.603882

C	2.444400	2.353709	-0.049621
N	2.470157	2.252834	-1.392843
H	3.298231	1.878661	-1.833026
H	1.584137	2.191774	-1.906231
N	1.281745	2.450009	0.590652
H	1.342924	2.496323	1.601760
H	0.378647	1.981914	0.249123
C	3.387912	-1.557643	0.694269
C	2.283007	-0.013439	2.281865
O	2.404721	1.013225	2.935611
C	0.984706	-0.761572	2.160739
H	1.100433	-1.819697	1.914520
H	0.445285	-0.649493	3.104904
H	0.370547	-0.269319	1.382304
H	2.418972	-1.707565	0.216949
H	3.634405	-2.444259	1.290645
C	4.446921	-1.257279	-0.334254
C	5.771634	-1.034273	0.062701
C	6.738243	-0.671280	-0.875590
H	7.762756	-0.496170	-0.558080
H	6.034945	-1.122379	1.113470
C	4.104361	-1.140386	-1.686029
H	3.090071	-1.388324	-1.986228
C	5.071944	-0.773328	-2.627091
H	4.802303	-0.692062	-3.677191
C	6.387128	-0.528855	-2.222259
H	7.138574	-0.243121	-2.953539
H	-3.825789	-3.343700	-1.411779
H	-4.544602	-4.196969	-0.025150
N	-0.997367	-1.915369	-0.751591
C	0.179312	-1.717062	-1.401936
O	1.086749	-2.544815	-1.468209
N	0.389608	-0.477600	-2.078876
C	-0.372020	0.643545	-1.938552
O	-0.086900	1.708504	-2.498523
C	-1.451815	0.550849	-0.861884
N	-2.635299	1.236078	-1.377249
C	-3.783099	1.113700	-0.610922
C	-4.756748	2.117085	-0.556487
C	-5.943632	1.958072	0.155611
C	-6.970380	3.063077	0.186716
C	-6.168902	0.752967	0.849067
C	-7.442848	0.542719	1.630832
C	-5.198388	-0.249168	0.802757
C	-4.004863	-0.091927	0.088180
N	-3.029647	-1.122345	0.000556
C	-1.833212	-0.906686	-0.593232
C	-3.351825	-2.470541	0.504455
C	-4.303901	-3.208873	-0.435579
O	-0.898767	1.037243	0.270711
O	3.372798	-0.414819	1.594994
H	1.228948	-0.413376	-2.643673
H	-2.414239	2.179051	-1.682684
H	-4.570536	3.045287	-1.093127
H	-7.934447	2.724250	-0.218081

H	-6.639253	3.927193	-0.399379
H	-7.163484	3.400296	1.214744
H	-7.556228	1.292659	2.426680
H	-7.461146	-0.448919	2.096792
H	-8.330264	0.632269	0.988177
H	-5.398707	-1.172234	1.333415
H	-2.407695	-3.006936	0.587080
H	-3.774329	-2.363487	1.507877
H	-5.235560	-2.650015	-0.574678

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RC

H	6.977629	-1.847745	-2.155660
C	6.063487	-1.507447	-1.653824
H	6.181563	-1.681970	-0.576304
H	5.963357	-0.428232	-1.804027
C	4.843834	-2.242692	-2.221858
H	4.751701	-2.027564	-3.290405
H	4.957369	-3.327717	-2.113476
N	3.583691	-1.842057	-1.609578
H	3.104819	-1.050051	-2.048864
C	3.050955	-2.391812	-0.508895
N	3.809803	-3.303198	0.188780
H	4.809074	-3.151331	0.204404
H	3.423711	-3.611458	1.070697
N	1.822004	-2.112134	-0.145046
H	1.161755	-1.354294	-0.640400
H	1.524506	-2.352070	0.791146
C	4.161673	1.619293	0.788854
C	4.044405	0.173906	1.295745
C	4.474884	-0.015538	2.758173
C	3.457220	0.520498	3.771722
H	3.219443	1.574836	3.573440
H	3.838902	0.458427	4.798023
H	2.533158	-0.071202	3.724909
H	5.446930	0.475206	2.910315
H	4.631840	-1.086406	2.953422
H	4.676789	-0.455663	0.655416
H	3.017722	-0.190659	1.166426
H	5.179883	1.975644	1.001975
H	3.470851	2.279348	1.330395
C	3.890566	1.732292	-0.721780
H	4.309594	2.672192	-1.104211
H	4.405357	0.919214	-1.246535
C	2.395019	1.680021	-1.059388
H	1.879938	0.918146	-0.456668
H	1.911358	2.627312	-0.807763
C	2.068591	1.282798	-2.483907
O	2.717232	0.427767	-3.083961
C	0.841988	1.909717	-3.093211
H	0.731782	1.611488	-4.139361
H	-0.003689	1.531158	-2.502343

H	0.865833	3.001811	-2.993547
H	-3.447325	3.659689	0.674590
H	-4.285540	4.252547	-0.780500
N	-0.915078	2.271202	0.482720
C	0.225712	2.257928	1.240651
O	0.956887	3.223096	1.400251
N	0.573543	1.057357	1.925737
C	0.011096	-0.167171	1.761671
O	0.425715	-1.180771	2.319344
C	-1.194720	-0.221886	0.808891
N	-2.308842	-0.784756	1.510944
C	-3.498215	-0.830330	0.772927
C	-4.464244	-1.811382	0.997406
C	-5.665982	-1.834300	0.289893
C	-6.689015	-2.910930	0.553238
C	-5.905151	-0.841120	-0.678585
C	-7.192240	-0.832808	-1.466954
C	-4.940416	0.143531	-0.901201
C	-3.734678	0.170854	-0.192006
N	-2.777611	1.206389	-0.353220
C	-1.605326	1.173514	0.327994
C	-3.135807	2.441303	-1.071533
C	-4.007564	3.354951	-0.215039
O	-0.887510	-1.037986	-0.293322
O	0.236881	-0.375362	-0.966018
H	1.404118	1.120304	2.502977
H	-2.058845	-1.643631	1.992070
H	-4.262646	-2.577937	1.742881
H	-7.651470	-2.479431	0.861646
H	-6.352883	-3.593510	1.341266
H	-6.886566	-3.503619	-0.350883
H	-7.215914	0.002673	-2.175247
H	-8.067626	-0.745174	-0.807995
H	-7.319922	-1.764249	-2.036094
H	-5.155328	0.905837	-1.640499
H	-2.199965	2.938661	-1.325421
H	-3.630654	2.156706	-2.003612
H	-4.923260	2.840023	0.097208

TS1

H	43.006853	45.104675	32.743734
C	43.640003	45.982004	32.566001
H	44.411651	45.702974	31.836126
H	43.035327	46.773791	32.112859
C	44.246328	46.469755	33.892468
H	43.442059	46.745088	34.580782
H	44.814244	45.659651	34.374282
N	45.080500	47.662676	33.769460
H	44.747969	48.571707	34.316617
C	46.339754	47.628130	33.364244
N	46.933429	46.453359	32.976280
H	46.327807	45.686485	32.724890

H	47.765290	46.530963	32.407396
N	47.069515	48.746587	33.336955
H	46.712538	49.628355	33.780151
H	48.071563	48.708604	33.205850
C	42.572271	50.517038	31.333208
C	43.334526	49.313377	30.719076
C	44.269181	49.685522	29.556038
C	45.500352	50.490641	29.995023
H	45.229947	51.440286	30.469976
H	46.142203	50.719866	29.134418
H	46.093474	49.918661	30.722581
H	43.701551	50.258878	28.808977
H	44.603331	48.765776	29.054272
H	42.610369	48.565096	30.368044
H	43.932269	48.817549	31.497039
H	41.502653	50.432314	31.101345
H	42.916636	51.449261	30.863611
C	42.764836	50.638317	32.853619
H	42.067650	51.380039	33.266536
H	42.538474	49.679738	33.339348
C	44.206849	51.032897	33.184563
H	44.877815	50.380860	32.615224
H	44.407538	52.057022	32.852560
C	44.548918	50.861375	34.673212
O	44.378508	49.697981	35.187158
C	44.238475	52.054358	35.565543
H	44.559930	52.990083	35.096744
H	43.155183	52.088575	35.740120
H	44.744521	51.927195	36.526820
H	48.401038	56.846912	35.313920
H	47.208113	57.790246	36.237474
N	46.940931	54.122531	33.862354
C	47.062662	53.434384	32.676504
O	46.396463	53.657557	31.683352
N	48.107189	52.458083	32.584487
C	48.576424	51.748116	33.645156
O	49.289850	50.755825	33.556000
C	48.104475	52.259158	35.024113
N	49.209067	52.253990	35.915504
C	49.007590	52.826384	37.172314
C	49.701139	52.392502	38.302627
C	49.528830	53.002422	39.544867
C	50.290145	52.511305	40.750804
C	48.626567	54.077798	39.657609
C	48.406634	54.756559	40.987630
C	47.935575	54.512593	38.525041
C	48.112539	53.907567	37.275312
N	47.454815	54.366134	36.105560
C	47.494384	53.654231	34.944931
C	46.771607	55.674919	36.128431
C	47.760539	56.843921	36.202658
O	47.108096	51.338561	35.496016
O	46.253832	51.118865	34.330428
H	48.220071	52.039981	31.666589
H	49.720669	51.377757	35.886648

H	50.387023	51.553878	38.202728
H	50.896362	53.314167	41.192889
H	50.957814	51.683726	40.488170
H	49.607821	52.163466	41.538807
H	47.689465	55.579798	40.897826
H	49.344625	55.163645	41.390514
H	48.022375	54.051893	41.738293
H	47.253546	55.347395	38.632904
H	46.180343	55.731836	35.215515
H	46.082859	55.678402	36.979082
H	48.395922	56.775268	37.091768

INT

H	-3.474480	-6.429984	-2.386037
C	-2.881198	-5.512491	-2.477967
H	-2.090854	-5.705083	-3.216353
H	-3.521464	-4.715137	-2.865250
C	-2.313162	-5.135899	-1.097989
H	-3.143381	-5.032295	-0.392796
H	-1.670943	-5.948485	-0.732638
N	-1.571797	-3.871523	-1.025415
H	-1.910478	-3.079516	-0.400786
C	-0.251542	-3.773758	-1.229100
N	0.384712	-4.682170	-2.026051
H	-0.146386	-5.098962	-2.776623
H	1.367378	-4.531927	-2.208391
N	0.435442	-2.832281	-0.594679
H	-0.154195	-2.247628	0.057509
H	1.285682	-2.429139	-0.978999
C	-5.144002	-1.131508	-1.635888
C	-4.767170	-2.463356	-2.296600
C	-5.646005	-2.836937	-3.498057
C	-5.489305	-1.875395	-4.682181
H	-5.803669	-0.860282	-4.413973
H	-6.090898	-2.199069	-5.540500
H	-4.439576	-1.824363	-5.001562
H	-6.698672	-2.863275	-3.181114
H	-5.395172	-3.855952	-3.827272
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H	-3.715895	-2.433493	-2.614698
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H	-5.095974	-0.319297	-2.374044
C	-4.236364	-0.793174	-0.444426
H	-4.699572	-0.004689	0.162258
H	-4.138329	-1.676053	0.201446
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H	-2.465784	-0.964397	-1.694757
H	-2.854594	0.696260	-1.215760
C	-1.821774	-0.522624	0.294961
O	-1.645020	-1.792394	0.656585
C	-2.121620	0.407167	1.480055
H	-1.325011	0.310816	2.222831
H	-2.198568	1.453818	1.158683

H	-3.066973	0.100415	1.940775
H	1.957366	5.295591	0.341270
H	0.897194	6.298168	1.359621
N	0.680670	2.889327	-1.093229
C	0.789345	2.279749	-2.320513
O	0.352250	2.737263	-3.357051
N	1.533909	1.053714	-2.402203
C	1.915657	0.274964	-1.364923
O	2.478584	-0.811329	-1.484027
C	1.548021	0.822717	0.025419
N	2.683812	0.733100	0.875277
C	2.518607	1.259220	2.162267
C	3.227391	0.763116	3.255876
C	3.088993	1.316513	4.528530
C	3.864033	0.756712	5.694865
C	2.207266	2.399643	4.708034
C	2.025204	3.021916	6.071009
C	1.501300	2.897363	3.611351
C	1.640519	2.346622	2.332172
N	0.977433	2.877707	1.196054
C	1.060583	2.266929	-0.014759
C	0.345556	4.207395	1.274038
C	1.390164	5.322199	1.277645
O	0.531818	-0.024083	0.561856
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H	1.648912	0.682313	-3.339404
H	3.141414	-0.171103	0.812216
H	3.896601	-0.081141	3.103754
H	4.500335	1.524770	6.156094
H	4.504649	-0.075022	5.382632
H	3.190471	0.392279	6.482786
H	1.305706	3.847118	6.035702
H	2.973882	3.413449	6.464289
H	1.662187	2.285622	6.801443
H	0.839630	3.739778	3.772357
H	-0.304683	4.296147	0.404532
H	-0.280542	4.229315	2.169920
H	2.085268	5.209181	2.117050

TS2

H	7.151910	-1.495434	1.231905
C	6.164876	-1.006797	1.277757
H	6.018172	-0.652272	2.307035
H	6.174133	-0.131637	0.621166
C	5.070741	-1.997751	0.854650
H	5.258223	-2.329157	-0.171430
H	5.106584	-2.886574	1.496834
N	3.702145	-1.470465	0.874032
H	3.191957	-1.238861	-0.004608
C	2.872980	-1.587683	1.919453
N	3.378626	-1.718603	3.179539
H	4.268386	-1.276111	3.362492
H	2.705380	-1.658200	3.933642

N	1.562880	-1.656630	1.727243
H	1.257922	-1.526607	0.752948
H	0.932700	-1.256529	2.425329
C	4.254791	2.452058	-1.922512
C	5.305140	1.745210	-1.057197
C	6.659159	2.465809	-1.007378
C	6.583795	3.845822	-0.343750
H	5.942605	4.527892	-0.913747
H	7.577277	4.303897	-0.266678
H	6.166280	3.765523	0.669097
H	7.051221	2.567951	-2.029517
H	7.377252	1.839538	-0.458708
H	5.446587	0.726743	-1.448652
H	4.925337	1.633583	-0.032452
H	4.666722	2.593034	-2.932662
H	4.047852	3.453665	-1.524105
C	2.941240	1.651849	-2.036834
H	2.312051	2.074084	-2.828156
H	3.170976	0.614840	-2.309707
C	2.180840	1.671422	-0.714244
H	2.810836	1.406053	0.133102
H	1.645560	2.603849	-0.537710
C	1.055907	0.227902	-0.911345
O	1.732416	-0.867689	-0.965746
C	0.112643	0.522501	-2.077469
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H	0.598911	0.305725	-3.032572
H	-0.746277	-0.145909	-1.948245
H	-5.075531	3.364258	1.484707
H	-5.882099	3.581352	-0.086860
N	-1.693062	2.757706	0.999845
C	-0.503385	3.072097	1.602731
O	0.161166	4.062953	1.357236
N	-0.039502	2.210254	2.657295
C	-0.360308	0.900374	2.777164
O	0.218377	0.126380	3.547686
C	-1.443173	0.395927	1.804117
N	-2.398906	-0.358539	2.599401
C	-3.526690	-0.799562	1.908725
C	-4.166946	-2.002262	2.213629
C	-5.322019	-2.410197	1.546604
C	-5.991308	-3.714290	1.903407
C	-5.846652	-1.591299	0.528851
C	-7.087331	-2.003693	-0.225544
C	-5.206899	-0.388013	0.223111
C	-4.056914	0.030199	0.901464
N	-3.430738	1.276320	0.643567
C	-2.204217	1.566158	1.161332
C	-4.149346	2.290131	-0.155025
C	-5.381605	2.854969	0.564289
O	-0.851258	-0.384102	0.843049
O	0.616365	0.720868	0.261706
H	0.800797	2.529500	3.128256
H	-1.931480	-1.067038	3.157229
H	-3.742935	-2.633443	2.992230

H	-7.027536	-3.554574	2.233213
H	-5.453611	-4.228409	2.707519
H	-6.038733	-4.389554	1.037679
H	-7.362499	-1.251616	-0.973259
H	-7.943224	-2.140207	0.450596
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H	-5.630671	0.230910	-0.558964
H	-3.432238	3.083925	-0.360127
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H	-6.093824	2.064927	0.822722

PC

H	-4.762746	3.313805	-0.421636
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H	-3.446309	2.263606	0.162954
H	-3.302534	3.040191	-1.413157
C	-2.993228	4.400869	0.240264
H	-3.192167	5.301382	-0.347679
H	-3.363819	4.597485	1.253465
N	-1.539429	4.239922	0.298473
H	-1.056225	4.316174	-0.600760
C	-0.952784	3.369891	1.141667
N	-1.621898	2.837014	2.174976
H	-2.629075	2.799097	2.141693
H	-1.165914	2.080590	2.697756
N	0.346715	3.038761	1.008114
H	0.794594	3.457014	0.198463
H	0.409822	1.973370	1.031179
C	-3.292922	-1.203606	-2.501005
C	-3.422611	-1.702237	-1.055147
C	-3.882351	-3.164243	-0.958402
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H	-2.490632	-3.964782	-2.451563
H	-3.189485	-5.189439	-1.377691
H	-1.942611	-4.089660	-0.755467
H	-4.799933	-3.289736	-1.553444
H	-4.129865	-3.387340	0.084389
H	-4.143966	-1.055117	-0.533534
H	-2.465596	-1.594170	-0.530657
H	-4.254060	-1.351213	-3.012129
H	-2.557736	-1.813255	-3.045101
C	-2.902136	0.278495	-2.599609
H	-3.034753	0.645445	-3.625707
H	-3.559660	0.872043	-1.952724
C	-1.457587	0.501554	-2.185285
H	-1.250016	0.191404	-1.157137
H	-0.787366	-0.032487	-2.869365
C	0.041419	2.410803	-2.063133
O	0.178029	3.622873	-1.926702
C	1.180932	1.436503	-1.950860
H	1.012004	0.834006	-1.047438
H	1.222018	0.756891	-2.808903
H	2.121053	1.984246	-1.867492

H	1.558978	-4.608592	0.242671
H	1.876940	-4.953699	-1.474653
N	-0.681377	-2.712608	0.965947
C	-1.660490	-2.604158	1.923161
O	-2.584158	-3.390013	2.048769
N	-1.589246	-1.517452	2.850360
C	-0.725008	-0.469006	2.806788
O	-0.777852	0.470083	3.607011
C	0.230607	-0.448544	1.611773
N	1.590627	-0.276218	2.135635
C	2.561377	-0.260105	1.137685
C	3.706169	0.538147	1.214870
C	4.685801	0.526079	0.221944
C	5.908561	1.401626	0.342698
C	4.513189	-0.312506	-0.895908
C	5.552271	-0.358831	-1.989947
C	3.368896	-1.108753	-0.980477
C	2.381297	-1.094549	0.011039
N	1.219717	-1.905372	-0.061389
C	0.237054	-1.785052	0.869964
C	1.116596	-2.966885	-1.074748
C	1.943823	-4.188205	-0.692114
O	-0.194499	0.509436	0.757377
O	-1.192359	1.933724	-2.269861
H	-2.318367	-1.502998	3.556412
H	1.645265	0.489530	2.801971
H	3.824066	1.189027	2.078981
H	6.829401	0.801790	0.349880
H	5.881620	1.994429	1.263308
H	5.992247	2.093128	-0.507357
H	5.262671	-1.060786	-2.779808
H	6.532413	-0.669899	-1.601960
H	5.697517	0.628819	-2.450040
H	3.266655	-1.758924	-1.841366
H	0.062629	-3.231953	-1.153160
H	1.430269	-2.547853	-2.035765
H	2.997305	-3.920113	-0.552997