Supplementary Material (ESI) for Organic & Biomolecular Chemistry

Aldolase Activity of Serum Albumins

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Electronic Supplementary Informations

Table of Contents

1. **General experimental protocol**
2. **HPLC data**
3. **Kinetic data**
4. **Stereoselectivity**
5. **Hammett plot**
6. **Production and purification of the MBP-IIa-103 peptide construct**
7. **Transition state geometries**

1. General experimental protocol

HSA and BSA were from Sigma-Aldrich, codes A1653 and A3912, respectively. Reference aldol products were obtained by the reaction of aldehydes with lithiated acetone, or by NaOH – Mg^{2+} catalyzed reactions as described in: H.O. House, D.S. Crumrine, A.Y. Teranishi, H.D. Holmsted, *J. Am. Chem. Soc.* 1973, **95**, 3310–3324.
Both the albumin-catalyzed and the backgroud reactions were run at 37.0 °C, in 10% acetone (1.36 M) in 100 mM phosphate buffer pH 7.5, 200 mM NaCl, and at substrate concentrations ranging from 0.50 to 2 mM. The catalyzed reactions were run in the presence of 200 μM albumin. Reaction rates were measured by HPLC following the appearance of the aldol product and the disappearance of aldehyde.

2. HPLC data

HPLC analyses were carried out with a HP1100 series instrument equipped with a C18 Phenomenex Luna 5m 150 x 4.60 mm column using mixtures of acetonitrile and water as eluant, at 0.5 ml/min, with spectrophotometric detection at 214 nm. All the aldol reactions were followed by monitoring both the appearance of the aldol product and the disappearance of the aldehyde, according to the conditions reported in table S1. The appearance of dehydration products at high conversions was also monitored by HPLC.

Aldehyde	Eluant		rt Aldol	rt Aldehyde	rt Enone
	CH_3CN	H_2O(TFA 0.1%)	(min)	(min)	(min)
1a	40%	60%	6.3	15.1	23.2
1b	35%	65%	5.7	9.4	12.2
1c	45%	55%	7.5	4.2	10.6
1d	20%	80%	5.1	8.2	9.5
1e	30%	70%	4.4	7.8	8.4

Table S1

3. Kinetic data

0.2mM albumin in phospate buffer (Na_2HPO_4 0.05M, NaCl 0.5M), pH 7.5 (890 μl) is incubated at 37°C for 15 min. 10 μl of an aldehyede solution in acetonitrile, and 100 μl of acetone are then added. The sample is sealed and kept at 37°C. Initial velocities are obtained by measuring the concentrations of aldehyede and aldol products during the first 5% reaction. All the measurments were quadruplicated, and the observed velocities are given in tables S2 – S6.

[1a] (mM)	V_{uncat} (nM/s)	V_{HSA} (nM/s)	V_{BSA} (nM/s)	V_{BSA} + warfarin (1,2 and 3 mM) (nM/s)		
0.50	0.141 ± 0.021	6.45 ± 0.12	3.62 ± 0.39	3.43 ± 0.04	2.58 ± 0.14	2.17 ± 0.09
0.75	0.242 ± 0.020	9.46 ± 0.17	5.12 ± 0.09			
1.00	0.310 ± 0.006	12.0 ± 2.1	6.71 ± 0.11	6.02 ± 0.56	4.92 ± 0.73	3.96 ± 0.37
1.50	0.354 ± 0.004	14.4 ± 0.3	9.15 ± 0.25			
1.75	0.438 ± 0.031	14.3 ± 0.2	7.80 ± 0.90			
2.00	0.313 ± 0.045	17.0 ± 1.7	8.46 ± 0.11			

Table S2

[1b] (mM)	V_{uncat} (nM/s)	V_{BSA} (nM/s)
1.00	1.30 ± 0.09	5.59 ± 0.96
1.50	1.43 ± 0.29	7.26 ± 0.65
2.00	2.18 ± 0.32	8.33 ± 0.29
3.00	3.02 ± 0.05	13.7 ± 1.7
5.00	3.36 ± 0.41	15.3 ± 0.13
7.00	1.62 ± 0.21	15.4 ± 2.8

Table S3

[1c] (mM)	V_{uncat} (nM/s)	V_{BSA} (nM/s)
0.64	3.11 ± 0.02	14.9 ± 0.1
1.06	4.94 ± 0.06	34.7 ± 6.2
1.16	5.38 ± 1.13	25.1 ± 0.6
1.91	8.60 ± 0.11	43.6 ± 0.3
1.93	8.69 ± 0.27	37.3 ± 0.1
2.67	11.8 ± 1.7	53.9 ± 1.3
3.60	15.9 ± 1.3	62.6 ± 1.1
4.59	20.1 ± 2.3	68.1 ± 0.8

Table S4

[1d] (mM)	V_{uncat} (nM/s)	V_{BSA} (nM/s)
0.50	18.7 ± 4.3	49.3 ± 2.2
1.00	28.1 ± 1.7	95.0 ± 3.9
2.00	58.8 ± 3.4	181.0 ± 9.3
2.50	72.2 ± 8.8	202.3 ± 15.3
3.00	88.9 ± 5.5	296.5 ± 18.2
4.00	110.0 ± 7.7	273.1 ± 37.5

Table S5

[1c] (mM)	V_{uncat} (nM/s)	V_{BSA} (nM/s)
0.50	8.14 ± 0.51	25.8 ± 0.9
1.00	17.8 ± 1.7	56.9 ± 5.8
1.50	29.2 ± 0.8	95.7 ± 5.2
2.00	60.8 ± 2.3	127.3 ± 6.7
3.00	52.4 ± 0.9	193.1 ± 4.9
4.00	119.2 ± 1.0	239.8 ± 9.6
7.00	120.0 ± 9.8	319.2 ± 8.9
10.0	162.6 ± 8.7	325.0 ± 34.2

Table S6

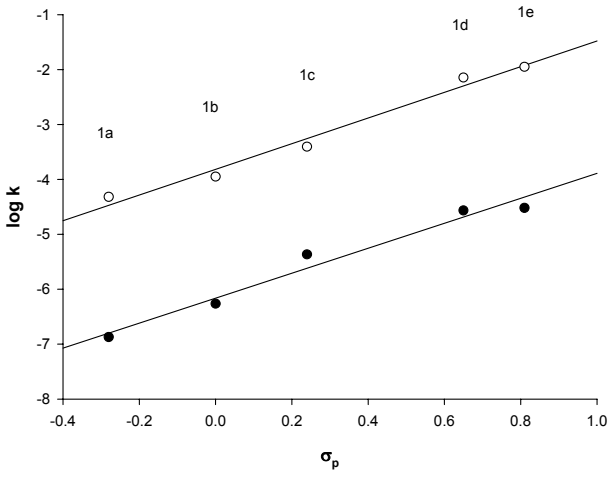
4. Stereoselectivity

Reference (S)-**2a** was obtained as follows: aldehyede **1a** (8 mg, 0.042 mmol) in 1 mL DMF and 2.2 mL acetone was slowly added to 40 mL of phospate buffer pH 7.4 (Na_2HPO_4 0.05M e NaCl 0.5M). 10 mg of antibody 38C2, were then added and the reaction was kept at room temperature for seven days, under an inert athmosphere and in the dark. After this time the mixture was extracted with ethyl acetate. The organic layers were washed with aq. sodium metabisulfite and brine, and then dried over anhydrous $NaSO_4$. The solvent was removed and the crude aldol was purified on silica.

Samples of **2a** from albumin-catalyzed reactions were obtained as follows: aldehyde **1a** (1.86 g, 10 mmol) was dissolved in 100 ml acetonitrile and added to 900 ml of a 200 μM albumin solution in phosphate buffer. 100 ml of acetone were added and the mixture was kept at room temperature for seven days, under an inert athmosphere and in the dark. After this time the mixture was extracted with ethyl acetate. The organic layers were washed with aq. sodium metabisulfite and brine, and then dried over anhydrous $NaSO_4$. The solvent was removed and the crude aldol was purified on silica.
$Eu(hfc)_3$ NMR experiments were performed in $CDCl_3$ on a Varian 500 MHz spectrometer. The signals at 5.3 and 5.6 ppm, due to the hydroxymethine protons of aldol **2a,** after addition of 0.25 eq $Eu(hfc)_3$, were used for measuring the ee: aldol **2a** was dissolved at known concentration in $CDCl_3$. A solution of [tri (Hepta-fluoro-propyl-Hydroxymethylene)-D-canforate]-europium-(III) ($Eu(hfc)_3$) in $CDCl_3$ was then prepared at the same concentration. The NMR spectrum of pure aldol was recorded, and then 0.1 eq increments of $Eu(hfc)_3$ were added until the optimal resolution was acheived.

- Rac. **2a:** ^{1}H-NMR 500 MHz($CDCl_3$) (δ): 0.87-1.25 (m, C=OC$\underline{H}_3^{A+B}$), 2.01-2.46(m, 2H, C$\underline{H}_2^{A+B}$), 3.66-3.99 (m, 6H, OC$\underline{H}_3^{A+B}$), **5.35 (s, 1H, C$\underline{H}^{A}$-OH), 5.63 (s, 1H, C$\underline{H}^{B}$-OH)**, 6.46-8.74 (m, 12H, $\underline{H}$-Ar $^{A+B}$).
- **S-2a** from the 38C2 – catalyzed aldol reaction: ^{1}H-NMR 500 MHz($CDCl_3$) (δ): 0.85-1.26 (m, C=OC$\underline{H}_3^{A}$), 2.35 (m, 2H, C$\underline{H}_2^{A}$), 3.98 (m, 6H, OC$\underline{H}_3^{A}$), **5.31 (s, 1H, C$\underline{H}^{A}$-OH)**, 6.56-8.35 (m, 12H, $\underline{H}$-Ar ^{A}).
- **2a** from the BSA-catalyzed reaction: ^{1}H-NMR 500MHz($CDCl_3$) (δ): 0.87-1.44 (m, C=OC$\underline{H}_3^{A+B}$), 2.22-2.58 (m, C$\underline{H}_2^{A+B}$), 3.82-3.97 (m, OC$\underline{H}_3^{A+B}$), **5.38 (s, 1H, C$\underline{H}^{A}$-OH), 5.67 (s, 0.43H, C$\underline{H}^{B}$-OH)**, 6.92-7.97 (m, $\underline{H}$-Ar $^{A+B}$).
- **2a** from the HSA-catalyzed reaction: ^{1}H-NMR 500MHz($CDCl_3$) (δ): 0.87-1.44 (m, C=OC$\underline{H}_3^{A+B}$), 2.22-2.58 (m, C$\underline{H}_2^{A+B}$), 3.82-3.97 (m, OC$\underline{H}_3^{A+B}$), **5.32 (s, 0.20H, C$\underline{H}^{A}$-OH), 5.61 (s, 1H, C$\underline{H}^{B}$-OH)**, 6.92-7.97 (m, $\underline{H}$-Ar $^{A+B}$). HPLC: 7.3 min (27.7 %), 12.2 min (72.3 %).

5. Hammett plot



Hammett plot for the uncatalyzed (●) and BSA-catalyzed (○) aldol reactions of aldehydes **1a-e** and acetone. The usual value of σ_p for the methoxy group is used for **2a**.

6. Production and purification of the MBP-IIa-103 peptide construct

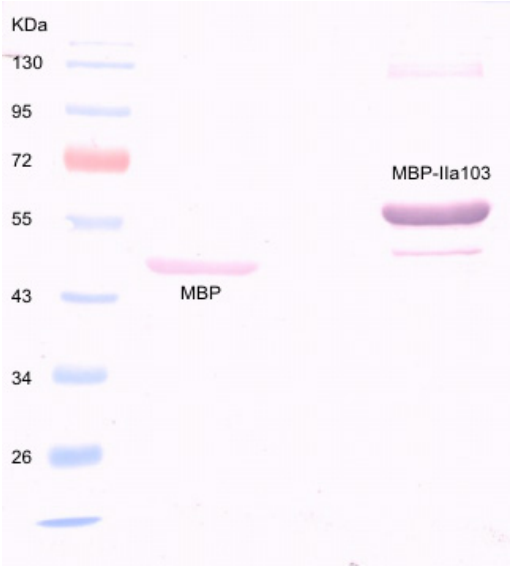
A DNA strand encoding for the IIa-103 peptide was synthesized and the cloned sequence was expressed using the pMAL™ protein fusion and purification system to give the following resulting fusion protein (IIa-103 in red):

M K I K T G A R I L A L S A L T T M M F S A S A L A K I E E G K L V I W I N G D K G Y N G L A E V G K K F E K D T G I K
V T V E H P D K L E E K F P Q V A A T G D G P D I I F W A H D R F G G Y A Q S G L L A E I T P D K A F Q D K L Y P F T
W D A V R Y N G K L I A Y P I A V E A L S L I Y N K D L L P N P P K T W E E I P A L D K E L K A K G K S A L M F N L Q E
P Y F T W P L I A A D G G Y A F K Y E N G K Y D I K D V G V D N A G A K A G L T F L V D L I K N K H M N A D T D Y S I
A E A A F N K G E T A M T I N G P W A W S N I D T S K V N Y G V T V L P T F K G Q P S K P F V G V L S A G I N A A S P
N K E L A K E F L E N Y L L T D E G L E A V N K D K P L G A V A L K S Y E E E L A K D P R I A A T M E N A Q K G E I M P
N I P Q M S A F W Y A V R T A V I N A A S G R Q T V D E A L K D A Q T N S S S N N N N N N N N N N L G D D D D K V
P E F

A S S A K Q R L K C A S L Q K F G E R F K A W A V A R L S Q R F P K A E F A E V S K L V T D L T K V H T E C C H G D L L E C A D D R A D L A K Y I C E N Q D S I S S K L K E C C E K P L L E K S H C I A E V E

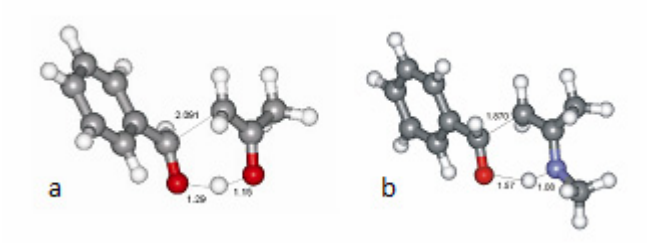
Theor. MW: **57195.9**

0.5 µg of the pMAL vector DNA were digested in 20 µl of 1X NEBuffer 2 (Biolab) with 10 units of Xmn I and 10 units of Hind III at 37°C for 1 hour. The enzymes were then inactivated by incubating at 65°C for 10 minutes. 0.5 µg of the IIa-103 fragment DNA were similarly digested in 20 µl of 1X NEBuffer 2 with 10 units of Hind III, and then the enzyme was inactivated by incubating at 65°C for 10 minutes. After isolation of the two DNA strands, ligation was carried out by mixing 2 µl pMAL digest (40 ng) and 1 µl IIa-103 digest (20 ng) and heating the DNA mixture at 45°C for 5 minutes. 1 µl (~ 400 units) of T4 DNA Ligase were then added and the mixture was incubated at 16°C for 3 hours and heated at 65°C for 5 minutes. The ligation reaction products were then mixed with 25 µl competent NM522 strain, incubated on ice for 5 minutes and heated to 42°C for 2 minutes. After growing colonies at 37°C overnight, the cells were induced with 0.3 mM IPTG, and the presence of induced construct expression was checked. 1 liter culture broth was then inoculated with an overnight culture of cells and the culture was grown up to 2 x 10^8 cells/ml. IPTG was added to a final concentration of 0.3 mM, and the cells were further incubated at 37°C for 2 hours. The cells were then harvested by centrifugation at 4000 x g for 20 minutes and resuspended in 50 ml of 20 mM Tris, 200 mM NaCl, 1mM EDTA, pH 7.4 (column buffer. The sample was frozen overnight at –20°C and thawed in cold water. The sample was then sonicated in pulses of 15 seconds and the release of proteins was monitored using the Bradford assay. The sample was then centrifuged at 9,000 x g for 30 minutes and the supernatant was diluted extract with. The protein extract was then affinity purified over an amylose resin column, and eluted with 10 mM maltose. The fusion protein containing fractions were finally dyalized against PBS, and the purity was cheched by sds:



7. Transition state geometries

Transition state geometries for the aldol reaction of acetone enol (a) and of the acetone methylenamine (b) with benzaldehyde.



Transition state coordinates

1b + acetone

Center Number	Atomic Number	Atomic Type	Coordinates (Angstroms) X	Y	Z
1	1	0	-0.924989	-0.269947	-1.520105
2	8	0	-3.037421	-1.007791	-0.132521
3	8	0	-0.901163	-1.490777	0.829561
4	6	0	-2.707045	0.208121	-0.442024
5	6	0	-1.415113	0.479223	-0.909588
6	1	0	-1.129749	1.507434	-1.110996
7	6	0	-0.430054	-0.292450	0.765038
8	1	0	-2.083535	-1.451284	0.323420
9	6	0	-3.706749	1.281014	-0.104736
10	1	0	-3.976279	1.224719	0.956592
11	1	0	-3.335720	2.281378	-0.340721
12	1	0	-4.626842	1.104475	-0.674654
13	1	0	-0.797268	0.457233	1.485843
14	6	0	0.989246	-0.078107	0.358679
15	6	0	1.717256	-1.117180	-0.236435
16	6	0	1.615154	1.152248	0.598942
17	6	0	3.049181	-0.921319	-0.594797
18	1	0	1.225710	-2.071954	-0.393902
19	6	0	2.947738	1.347315	0.239792
20	1	0	1.054357	1.956568	1.071244
21	6	0	3.666885	0.310743	-0.360522
22	1	0	3.610678	-1.731931	-1.051804
23	1	0	3.426562	2.303901	0.431243
24	1	0	4.706756	0.460304	-0.638570

1c + acetone

Center Number	Atomic Number	Atomic Type	Coordinates (Angstroms) X	Y	Z
1	1	0	1.666490	0.173665	-1.512044
2	8	0	3.901851	0.889810	-0.320273
3	8	0	1.856113	1.539134	0.747038
4	6	0	3.493851	-0.324978	-0.526197
5	6	0	2.157082	-0.556223	-0.878612
6	1	0	1.813882	-1.580004	-0.995462
7	6	0	1.331407	0.362784	0.786508
8	1	0	3.014286	1.411118	0.152150
9	6	0	4.468524	-1.422098	-0.198998
10	1	0	4.827315	-1.312761	0.831256
11	1	0	4.034848	-2.415175	-0.338393
12	1	0	5.345225	-1.327370	-0.850971
13	1	0	1.703860	-0.356714	1.535455
14	6	0	-0.121965	0.197471	0.485349

15	6	0	-0.840891	1.241316	-0.110961
16	6	0	-0.789647	-0.986028	0.823970
17	6	0	-2.200616	1.104120	-0.377323
18	1	0	-0.320374	2.164013	-0.346267
19	6	0	-2.149367	-1.138655	0.563178
20	1	0	-0.242274	-1.797456	1.298900
21	6	0	-2.842785	-0.088794	-0.040253
22	1	0	-2.762099	1.912005	-0.834615
23	1	0	-2.668536	-2.054097	0.826009
24	17	0	-4.559405	-0.269545	-0.372948

1d + acetone

Center Number	Atomic Number	Atomic Type	Coordinates (Angstroms) X	Y	Z
1	1	0	1.511177	0.151376	-1.507057
2	8	0	3.754037	0.884603	-0.341381
3	8	0	1.709921	1.544316	0.739407
4	6	0	3.344423	-0.333273	-0.528185
5	6	0	2.001070	-0.566783	-0.859389
6	1	0	1.660064	-1.592769	-0.962823
7	6	0	1.192601	0.367354	0.786646
8	1	0	2.884172	1.413584	0.113689
9	6	0	4.327200	-1.423672	-0.209735
10	1	0	4.715844	-1.295917	0.807317
11	1	0	3.889274	-2.418367	-0.319883
12	1	0	5.184404	-1.341025	-0.888897
13	1	0	1.558732	-0.343016	1.548093
14	6	0	-0.263390	0.194064	0.481044
15	6	0	-0.980406	1.237048	-0.120511
16	6	0	-0.926884	-0.991460	0.824508
17	6	0	-2.336128	1.094356	-0.389373
18	1	0	-0.457694	2.158453	-0.354761
19	6	0	-2.282571	-1.146070	0.560451
20	1	0	-0.376420	-1.797182	1.304838
21	6	0	-2.995926	-0.101393	-0.053020
22	1	0	-2.895058	1.901396	-0.852252
23	1	0	-2.797505	-2.063491	0.826636
24	6	0	-4.394438	-0.254004	-0.328728
25	7	0	-5.528852	-0.380198	-0.554480

1e + acetone

Center Number	Atomic Number	Atomic Type	Coordinates (Angstroms) X	Y	Z
1	1	0	-1.897708	-0.266125	-1.482663
2	8	0	-4.166892	-0.888162	-0.302720
3	8	0	-2.145003	-1.490248	0.856478
4	6	0	-3.739136	0.308439	-0.570356
5	6	0	-2.387379	0.501998	-0.894738
6	1	0	-2.033238	1.514146	-1.067406
7	6	0	-1.617843	-0.318098	0.824756
8	1	0	-3.314886	-1.394317	0.197436
9	6	0	-4.714078	1.428930	-0.349204
10	1	0	-5.113249	1.385219	0.670898
11	1	0	-4.265373	2.407740	-0.532842
12	1	0	-5.565706	1.300111	-1.028098
13	1	0	-1.985759	0.449061	1.528204
14	6	0	-0.155300	-0.179725	0.526358
15	6	0	0.559284	-1.273776	0.018583
16	6	0	0.513109	1.025252	0.785862
17	6	0	1.920605	-1.164998	-0.242680
18	1	0	0.031328	-2.206364	-0.149408
19	6	0	1.874058	1.149959	0.529752
20	1	0	-0.036864	1.870094	1.193624
21	6	0	2.557918	0.049105	0.014268
22	1	0	2.494991	-1.996284	-0.632705

23	1	0	2.411244	2.070352	0.722132
24	7	0	3.997799	0.171984	-0.260058
25	8	0	4.534547	1.254675	-0.023952
26	8	0	4.580248	-0.813771	-0.711603

1b + methylamine-acetone enamine

Center Number	Atomic Number	Atomic Type	Coordinates (Angstroms) X	Y	Z
1	1	0	0.491693	0.065892	-1.485498
2	8	0	0.624383	-1.360195	0.801676
3	6	0	2.293750	0.582225	-0.471756
4	6	0	0.892568	0.715987	-0.711949
5	1	0	0.519571	1.735049	-0.790563
6	6	0	0.098432	-0.177633	0.726877
7	1	0	0.331926	0.534831	1.548700
8	7	0	2.746614	-0.664193	-0.380597
9	1	0	1.934974	-1.297866	-0.060291
10	6	0	4.074723	-1.006201	0.111116
11	1	0	4.195698	-0.779768	1.179140
12	1	0	4.842313	-0.465580	-0.450302
13	1	0	4.232609	-2.077064	-0.034298
14	6	0	3.178955	1.752848	-0.146345
15	1	0	3.601953	1.667626	0.863239
16	1	0	4.024739	1.807620	-0.843735
17	1	0	2.622979	2.689624	-0.217191
18	6	0	-1.368777	-0.070398	0.347186
19	6	0	-2.097596	1.099133	0.591202
20	6	0	-2.013292	-1.165289	-0.238126
21	6	0	-3.446416	1.180815	0.243673
22	1	0	-1.605690	1.948403	1.063404
23	6	0	-3.361210	-1.086028	-0.587398
24	1	0	-1.439011	-2.074026	-0.389958
25	6	0	-4.081704	0.087725	-0.350616
26	1	0	-4.003312	2.093642	0.441160
27	1	0	-3.854927	-1.944166	-1.037389
28	1	0	-5.133460	0.147583	-0.618846

1c + methylamine-acetone enamine

Center Number	Atomic Number	Atomic Type	Coordinates (Angstroms) X	Y	Z
1	1	0	1.241725	0.099068	-1.436291
2	8	0	1.514275	-1.381212	0.808286
3	6	0	3.092530	0.611386	-0.514094
4	6	0	1.678665	0.735023	-0.670557
5	1	0	1.293680	1.752070	-0.704720
6	6	0	0.974329	-0.205038	0.791022
7	1	0	1.242647	0.493024	1.614371
8	7	0	3.564892	-0.630416	-0.482332
9	1	0	2.786134	-1.287515	-0.149859
10	6	0	4.922722	-0.972591	-0.079477
11	1	0	5.099469	-0.785043	0.988149
12	1	0	5.650614	-0.400230	-0.661452
13	1	0	5.084386	-2.034816	-0.274799
14	6	0	3.984226	1.783551	-0.214358
15	1	0	4.471731	1.677296	0.763552
16	1	0	4.782905	1.866042	-0.962675
17	1	0	3.415388	2.715093	-0.225212
18	6	0	-0.512271	-0.109547	0.492597
19	6	0	-1.243051	1.048986	0.776776
20	6	0	-1.176299	-1.210453	-0.057412
21	6	0	-2.608911	1.122304	0.505375
22	1	0	-0.742069	1.906554	1.222604
23	6	0	-2.540954	-1.156299	-0.336563
24	1	0	-0.602615	-2.113247	-0.242029
25	6	0	-3.244603	0.014640	-0.054927

26	1	0	-3.175624	2.020457	0.728186
27	1	0	-3.058122	-2.011924	-0.758870
28	17	0	-4.971588	0.093765	-0.401248

1d + methylamine-acetone enamine

Center Number	Atomic Number	Atomic Type	Coordinates (Angstroms) X	Y	Z
1	1	0	1.089212	0.104253	-1.437073
2	8	0	1.363149	-1.383019	0.811779
3	6	0	2.940623	0.614954	-0.515497
4	6	0	1.527379	0.740077	-0.671933
5	1	0	1.143334	1.757422	-0.704110
6	6	0	0.825431	-0.209690	0.797074
7	1	0	1.091761	0.490514	1.618882
8	7	0	3.416211	-0.625609	-0.491160
9	1	0	2.646321	-1.291823	-0.178201
10	6	0	4.775885	-0.971297	-0.096694
11	1	0	4.952644	-0.801286	0.973639
12	1	0	5.500517	-0.386936	-0.670295
13	1	0	4.939553	-2.029527	-0.310545
14	6	0	3.832744	1.786255	-0.214950
15	1	0	4.330072	1.673274	0.757072
16	1	0	4.623395	1.876308	-0.970851
17	1	0	3.261973	2.716604	-0.213209
18	6	0	-0.659656	-0.110068	0.490381
19	6	0	-1.388664	1.051624	0.774158
20	6	0	-1.320272	-1.213457	-0.062348
21	6	0	-2.749353	1.124068	0.497448
22	1	0	-0.885403	1.905997	1.222519
23	6	0	-2.679882	-1.155949	-0.346016
24	1	0	-0.743097	-2.114576	-0.242429
25	6	0	-3.404922	0.017272	-0.070454
26	1	0	-3.313191	2.024840	0.719287
27	1	0	-3.192911	-2.012696	-0.772209
28	6	0	-4.807118	0.084009	-0.359494
29	7	0	-5.945144	0.140608	-0.596948

1e + methylamine-acetone enamine

Center Number	Atomic Number	Atomic Type	Coordinates (Angstroms) X	Y	Z
1	1	0	1.502944	-0.008600	-1.436830
2	8	0	1.811744	-1.326461	0.897775
3	6	0	3.359316	0.578900	-0.570568
4	6	0	1.943185	0.681805	-0.721825
5	1	0	1.548422	1.690852	-0.820720
6	6	0	1.261847	-0.156078	0.805643
7	1	0	1.537245	0.596480	1.577219
8	7	0	3.838478	-0.655545	-0.454363
9	1	0	3.060877	-1.285239	-0.052219
10	6	0	5.201989	-0.955969	-0.038179
11	1	0	5.389808	-0.679193	1.008100
12	1	0	5.921205	-0.430879	-0.673525
13	1	0	5.368140	-2.030041	-0.145813
14	6	0	4.244056	1.775527	-0.358069
15	1	0	4.734628	1.742578	0.623615
16	1	0	5.040548	1.810200	-1.112458
17	1	0	3.668669	2.700166	-0.433246
18	6	0	-0.228975	-0.086985	0.520967
19	6	0	-0.959845	1.082127	0.760822
20	6	0	-0.892165	-1.216483	0.029856
21	6	0	-2.329983	1.128834	0.501392
22	1	0	-0.452226	1.958960	1.160435
23	6	0	-2.261478	-1.172261	-0.231264
24	1	0	-0.313798	-2.123052	-0.119403
25	6	0	-2.984595	0.000854	0.000633

26	1	0	-2.888041	2.041824	0.694806
27	1	0	-2.769276	-2.056961	-0.608220
28	7	0	-4.428984	0.045095	-0.268969
29	8	0	-5.017387	1.068718	-0.058262
30	8	0	-4.961321	-0.944202	-0.688869
