SUPPORTING INFORMATION

Computational and experimental analyses converge to reveal a coherent, yet malleable aptamer structure that controls chemical reactivity

Tianjiao Wang[§‡], Julie A. Hoy[†], Monica H. Lamm[¶] and Marit Nilsen-Hamilton[§‡*]

[§]Department of Biochemistry, Biophysics and Molecular Biology, [†]Macromolecular X-ray Crystallography Facility, Office of Biotechnology, and [¶]Department of Chemical and Biological Engineering, Iowa State University, Ames, IA 50011

[‡]Ames Laboratory, Ames, IA 50011

*Correspondence addressed to: marit@iastate.edu

CONTENTS:

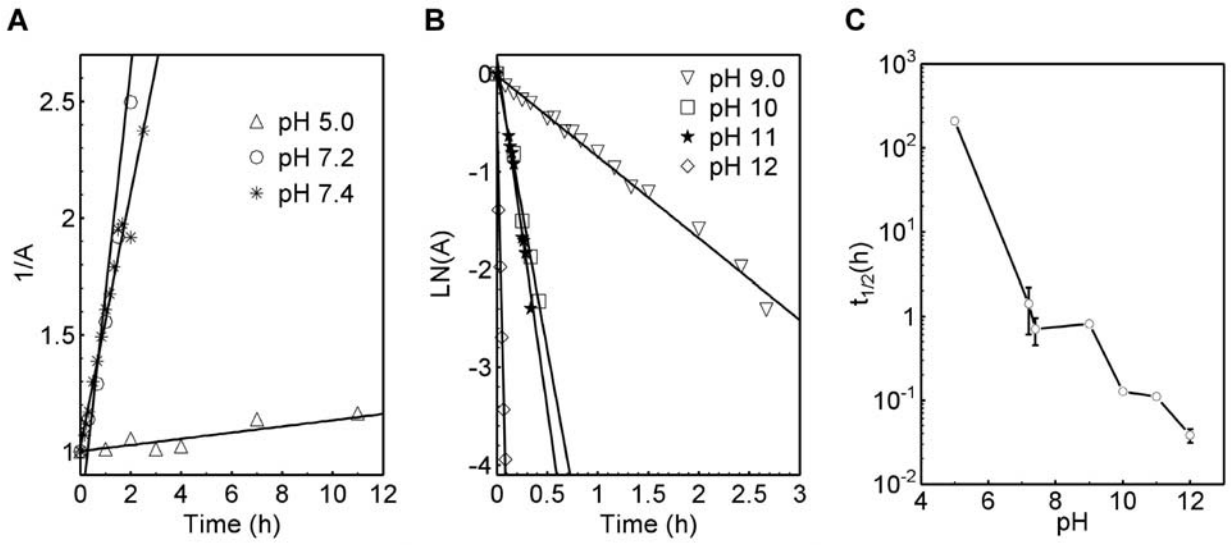


Figure S1. The pH dependence of MG oxidation. A time course of loss of MG color was determined at each of a range of pHs using the buffers 16.7 mM acetate, pH 5.0; 100 mM KCl, 5 mM $MgCl_2$, 10 mM HEPES, pH 7.2; 9.8 mM Tris, pH 9.0; 0.1 mM NaOH, pH 10; 1 mM NaOH, pH 11 or 10 mM NaOH, pH 12. From these time courses the half-lives ($t_{1/2}$) were determined and plotted against pH (C). MG oxidation is a second-order reaction at pH ≤7.4 (A) but fits to a first order equation at pH ≥ 9.0 (B). We interpret this latter first order fit as reflecting a pseudo-first order kinetics due to the large concentration of OH^- at the higher pHs. This experiment was repeated three times for each pH. The standard deviations of the half-lives ($t_{1/2}$) (C) are shown as error bars.

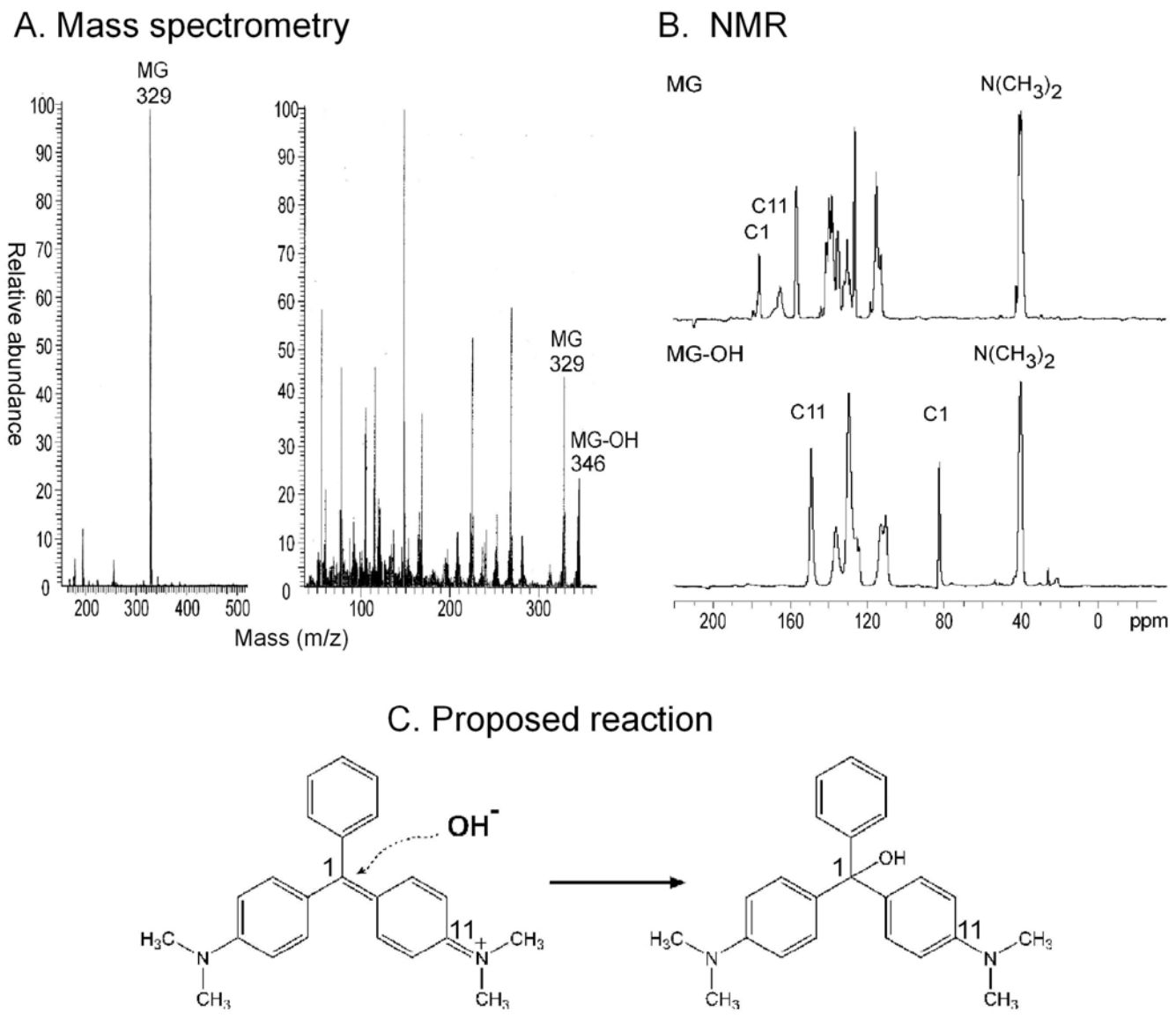


Figure S2. MG bleaching is due to its hydroxylation. (A, left) The mass spectrum was determined by ESI of MG (molecular mass of 329 Da). (A, right) The mass spectrum of MG-OH was determined by EI (molecular mass 346 Da). (B) The NMR spectra of MG (top) and MG-OH (bottom). (C) MG bleaching is proposed to proceed by an OH^- attack on the C1 of MG, forming the carbinol base. C1 and C11 are labeled to indicate their positions in MG and MG-OH.

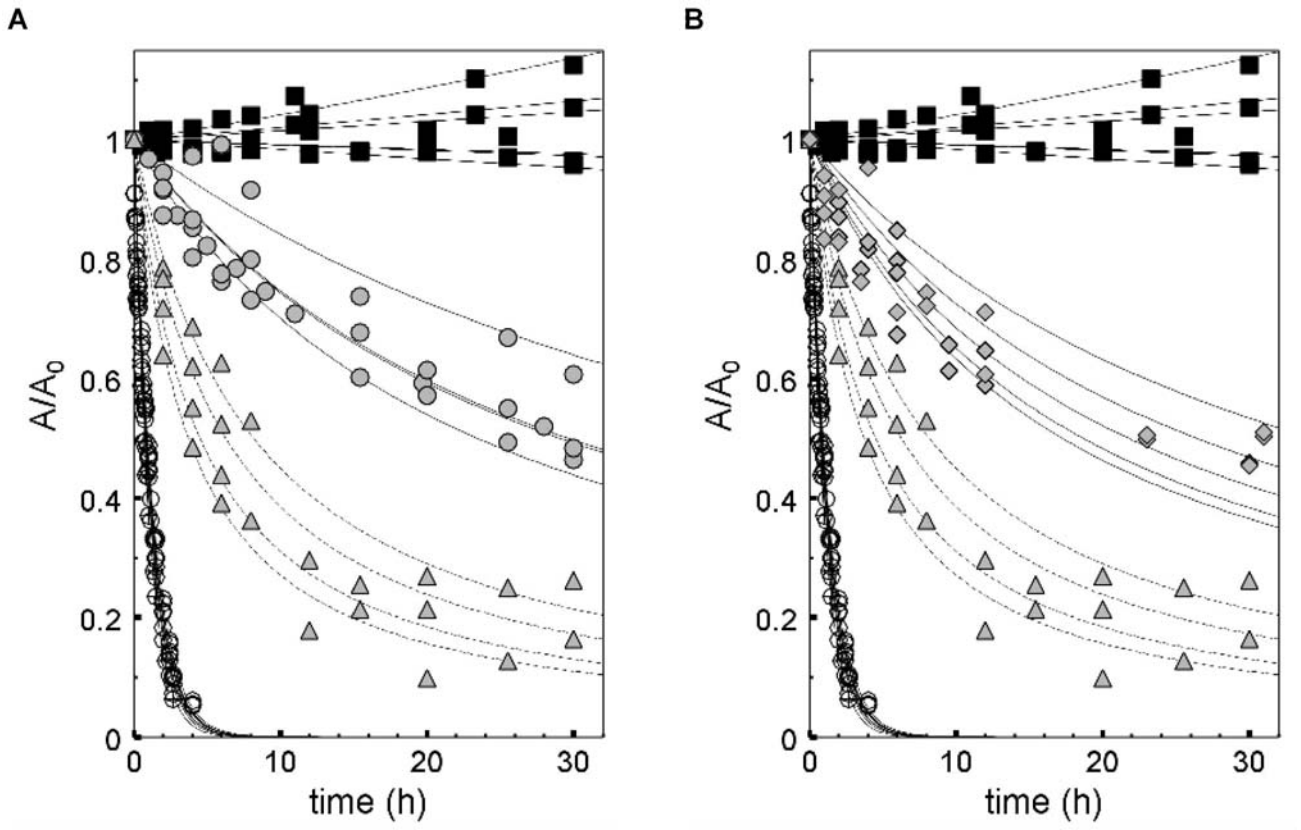


Figure S3. Variations around the MGA binding pocket compromise its ability to protect MG. MG oxidation in the presence of the MGA variants at concentrations equivalent to 10-15 times Kd was measured spectrophotometrically. The concentrations of interacting components were: 3 or 30 μM MG (○); 3 μM MG, 10 μM MGA (■); 3 μM MG, 20-30 μM MGA(A31C) (gray-filled ○); 30 μM MG, 140-200 μM MGA(U25C) (gray-filled ◊) and 30 μM MG, 3 mM MGA(G8C, G24C, G29C) (gray-filled Δ). The figure shows all the results of 4-9 independent experiments. For clarity, MG in the presence of MGA(A31C) or MGA(U25C), for which half-lives were determined to be statistically identical, is presented in A and B respectively. The data for MG in the presence or absence of MGA or MGA(G8C, G24C, G29C) are displayed in both panels to allow for comparisons.

Table S1: Half lives ($t_{1/2}$) of MG in the presence and absence of MGA and its variants

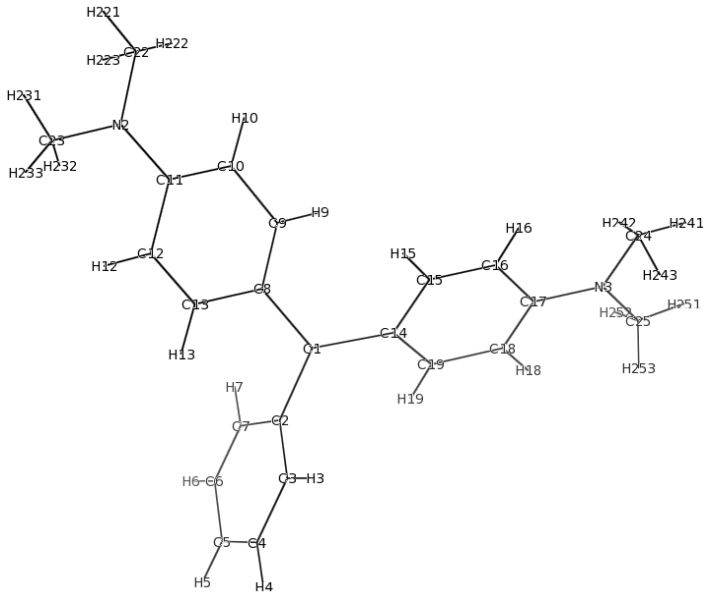
treatments	$t_{1/2}$ (h)										
	Experiment numbers									mean	std
	1	2	3	4	5	6	7	8	9		
none	0.70	0.81	0.86	0.85	0.87	0.77	0.79	0.92	0.88	0.83	0.07
MGA	U.D.	U.D.	U.D.	U.D.	10040	U.D.				U.D.	
MGA(A31C)	27	53	23	30						33	13
MGA(U25C)	21	21	33	17	19					22	4.3
MGA(G8C,G24C, G29C)	6.3	3.7	4.5	8.2						5.7	2.4

U.D.: undetectable. The values of $t_{1/2}$ were derived from Figure S3. The averages of the individual estimates vary slightly from those shown in Table 1. This is because of the different means of obtaining the estimates. In Table 1, the averages at each time point for all experiments were first obtained using all time points for which multiple values were available and the resulting curve of average values was fitted to obtain a half-life. Here the data for each experiment were fit separately to obtain the values given for each experiment number and these individual values were averaged to obtain the mean half-life for each condition.

Table S2: P values of Tukey multiple comparisons between MG treatments from Table S1

	MGA	MGA(A31C)	MGA(U25C)	MGA(G8C,G24C,G29C)
None	$<10^{-8}$	$<10^{-8}$	$<10^{-8}$	0.175
MGA		$<10^{-8}$	$<10^{-8}$	$<10^{-8}$
MGA(A31C)			0.327	$<10^{-8}$
MGA(U25C)				$<10^{-8}$

The topology file and parameter file of malachite green that were added to top_all27_prot_na.rtf and par_all27_prot_na.prm respectively for MD simulation.



Malachite green topology file:

RESI MGR 1.00

GROUP ! central carbon

ATOM C1 CT 0.133

GROUP ! ring A

ATOM C14 CA -0.146
ATOM C15 CA 0.152
ATOM H15 HP 0.035
ATOM C16 CA -0.504
ATOM H16 HP 0.208
ATOM C17 CA 0.573
ATOM C18 CA -0.419
ATOM H18 HP 0.219
ATOM C19 CA 0.054
ATOM H19 HP 0.128
ATOM N3 NH2 -0.326
ATOM C24 CT3 -0.138
ATOM H241 HA 0.168
ATOM H242 HA 0.273
ATOM H243 HA -0.074
ATOM C25 CT3 0.096
ATOM H251 HA -0.048
ATOM H252 HA 0.074
ATOM H253 HA 0.052

GROUP !ring B

ATOM C8 CA -0.120
ATOM C9 CA 0.115
ATOM H9 HP 0.060
ATOM C10 CA -0.501
ATOM H10 HP 0.197
ATOM C11 CA 0.564
ATOM C12 CA -0.467
ATOM H12 HP 0.199
ATOM C13 CA 0.108
ATOM H13 HP 0.027
ATOM N2 NH2 -0.434
ATOM C22 CT3 0.071
ATOM H221 HA 0.069
ATOM H222 HA 0.151
ATOM H223 HA -0.073
ATOM C23 CT3 0.061
ATOM H231 HA -0.049
ATOM H232 HA 0.104
ATOM H233 HA 0.074

GROUP ! ring C

```
ATOM  C2    CA     0.065
ATOM  C3    CA    -0.052
ATOM  H3    HP     0.107
ATOM  C4    CA    -0.162
ATOM  H4    HP     0.155
ATOM  C5    CA     0.052
ATOM  H5    HP     0.149
ATOM  C6    CA    -0.164
ATOM  H6    HP     0.153
ATOM  C7    CA    -0.076
ATOM  H7    HP     0.108
BOND C1 C2 C1 C8 C1 C14 C2 C3 C2 C7 C3 C4 C4 C5 C5 C6 C6 C7 C8 C9 C8 C13
BOND C9 C10 C10 C11 C11 C12 C11 N2 C12 C13 C14 C15 C14 C19 C15 C16
BOND C16 C17 C17 C18 C17 N3 C18 C19 C22 N2 C23 N2 C24 N3 C25 N3
BOND C3 H3 C4 H4 C5 H5 C6 H6 C7 H7 C9 H9 C10 H10 C12 H12 C13 H13
BOND C15 H15 C16 H16 C18 H18 C19 H19 C22 H221 C22 H222 C22 H223
BOND C23 H231 C23 H232 C23 H233 C24 H241 C24 H242 C24 H243
BOND C25 H251 C25 H252 C25 H253
IMPR  C1   C2   C8   C14 ! chirality or flatness improper    -1.90
IMPR  C2   C1   C3   C7  ! chirality or flatness improper     0.05
IMPR  C8   C1   C9   C13 ! chirality or flatness improper    -0.44
IMPR  C11  C10  C12  N2  ! chirality or flatness improper     1.19
IMPR  C14  C1   C15  C19 ! chirality or flatness improper     0.20
IMPR  C17  C16  C18  N3  ! chirality or flatness improper    -0.22
IMPR  N2   C11  C22  C23 ! chirality or flatness improper     0.18
IMPR  N3   C17  C24  C25 ! chirality or flatness improper     1.87
```

! IC of ring A below

```
IC C15  C1   *C14  C19   1.43   122.8    -177.9600 122.8     1.44
IC C1   C14  C15   C16   1.497  122.8    -177.3700 122.0     1.38
IC C16  C14  *C15  H15   1.38   122.0     179.7000 119.0     1.0814
IC C1   C14  C19   C18   1.497  122.8     177.2000 123.6     1.38
IC C18  C14  *C19  H19   1.38   123.6    -178.6900 118.2     1.0811
IC C14  C15  C16   C17   1.43   122.0    -0.1200   122.0     1.44
IC C17  C15  *C16  H16   1.44   122.0    -179.6900 119.0     1.0808
IC C17  C19  *C18  H18   1.43   120.9    -179.9300 119.55    1.0811
IC C16  C18  *C17  N3    1.44   115.6     179.5100 122.2     1.0807
IC N3   C17  C18   C19   1.360  122.2    -180.00   120.9     1.38
IC N3   C17  C16   C15   1.360  122.2     180.00   122.0     1.38
IC C24  C25  *N3   C17   1.465  115.07    180.00   122.16    1.36
IC C17  N3   C25   H251   1.36   122.16    180.00   110.4     1.1108
IC C17  N3   C25   H252  1.36   122.16    180.00   110.4     1.1108
IC C17  N3   C25   H253  1.36   122.16    180.00   110.4     1.1108
```

```
IC C17  N3   C24   H241   1.36   122.71   -180.0    110.4     1.1108
IC C17  N3   C24   H242  1.36   122.71   -180.0    110.4     1.1108
IC C17  N3   C24   H243  1.36   122.71   -180.0    110.4     1.1108
IC H251  H253 *C25  H252  1.1108 110.4     119.7    110.4     1.1108
IC H251  H253 *C25  N3    1.1108 110.4     119.7    110.4     1.482
IC H241  H243 *C24  H242  1.1108 110.4     119.7    110.4     1.1108
IC H241  H243 *C24  N3    1.1108 110.4     119.7    110.4     1.482
```

! IC of ring B below

```
IC C13  C1   *C8   C9    1.42   122.4    -177.9600 122.4     1.42
IC C1   C8   C13   C12   1.52   122.4    -177.3700 123.2     1.38
IC C12  C8   *C13  H13   1.38   123.2     179.7000 118.4     1.0814
IC C1   C8   C9    C10   1.520  122.4     177.2000 122.2     1.39
IC C10  C8   *C9   H9    1.39   122.2    -178.6900 118.9     1.0811
IC C8   C13  C12   C11   1.42   123.2    -0.1200   121.3     1.42
IC C11  C13  *C12  H12   1.42   121.3    -179.6900 119.35    1.0808
IC C11  C9   *C10  H10   1.43   121.9    -179.9300 119.05    1.0811
IC C12  C10  *C11  N2    1.42   115.8     179.5100 122.1     1.387
IC N2   C11  C10   C9    1.387  122.1    -180.0    121.9     1.39
IC N2   C11  C12   C13   1.387  122.1     180.0    121.3     1.38
IC C22  C23  *N2   C11   1.537  117.26    -180     123.76    1.387
IC C11  N2   C23   H231   1.387  123.76   -180.0    110.4     1.1108
IC C11  N2   C23   H232  1.387  123.76   -180.0    110.4     1.1108
IC C11  N2   C23   H233  1.387  123.76   -180.0    110.4     1.1108
IC C11  N2   C22   H221   1.387  123.76    180.0    110.4     1.1108
IC C11  N2   C22   H222  1.387  123.76    180.0    110.4     1.1108
IC C11  N2   C22   H223  1.387  123.76    180.0    110.4     1.1108
IC H231  H233 *C23  H232  1.1108 110.4    -119.7    110.4     1.1108
IC H231  H233 *C23  N2    1.1108 110.4    -119.7    110.4     1.510
IC H221  H223 *C22  H222  1.1108 110.4    -119.7    110.4     1.1108
IC H221  H223 *C22  N2    1.1108 110.4    -119.7    110.4     1.537
```

! IC of ring C below

```
IC C7  C1   *C2  C3     1.42   121.0    -177.960  121.0     1.42
IC C1  C2   C7   C6     1.579  121.0    -177.370  120.6     1.39
IC C6  C2   *C7  H7     1.39   120.6     179.700  119.7     1.0814
IC C1  C2   C3   C4     1.579  121.0     177.200  120.9     1.39
IC C4  C2   *C3  H3     1.39   120.9    -178.690  119.55    1.0811
IC C2  C7   C6   C5     1.42   120.6    -0.1200   120.2     1.40
IC C5  C7   *C6  H6     1.40   120.2    -179.690  119.9     1.0808
IC C5  C3   *C4  H4     1.40   120.0    -179.930  120.0     1.0811
IC C6  C4   *C5  H5     1.40   120.1     179.510  119.95    1.0807
```

! IC of C1 to connect C2, C8, C14 below

IC C14 C2 *C1 C8 1.497 117.4 180.0 115.0 1.520

Malachite green parameter file:

! mgr bonds

CA CT 230.00 1.532 ! for mgr central carbon
CA NH2 240.00 1.374 ! for mgr N-methyl groups

! mgr angles

CA CT CA 40.000 120.00 35.00 2.41620 ! mgr central carbon
CT CA CA 40.000 120.00 35.00 2.41620 ! mgr central carbon
CA CA NH2 40.000 120.00 35.00 2.41620 ! mgr N-mehtyl groups
CA NH2 CT3 50.000 120.00 ! mgr N-mehtyl groups
CT3 NH2 CT3 50.000 120.00 ! mgr N-mehtyl groups

! mgr dihedrals

CA CA NH2 CT3 3.100 2 0.0
CA CT CA CA 3.10 2 180.0
CT CA CA CA 3.100 2 0.0
CT CA CA HP 3.100 2 180.0
HP CA CA NH2 3.10 2 180.0
NH2 CA CA CA 3.100 2 0.0

! mgr improper

CA CA CA NH2 60.0 0 0.0
CA CT CA CA 60.0 0 0.0
CT CA CA CA 60.0 0 0.0
NH2 CA CT3 CT3 6.0 0 0.0