Electronic Supplementary Material (ESI) for RSC Advances.


# Exploring Interfacial Dynamics in Homodimeric S-ribosylhomocysteine Lyases (LuxS) from *Vibrio Cholerae* Through Molecular Dynamics Simulation

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*Simulation protocol*: All systems were subjected to enzyme minimization steps prior to equilibration to remove close-ranged intra- as well as inter-molecular interactions using the PMEMD module implemented in AMBER18 [1, 2]. To keep the coordination bond between the iron and inhibitors, distance restraints were applied throughout the simulation time from minimization to production MD. Initially 500 steps of minimization were carried out for the water molecules while applying restraints on the enzyme using a force constant of 25 kcal.mol$^{-1}$.Å$^{-1}$. Afterwards, the force constant was gradually released after every 100 steps, thus a total of 1000 steps of restrained minimization were performed followed by an unrestrained minimization for 500 steps. After the minimization, the systems were subjected to heating and equilibration which involved thermal equilibration using the Langevin thermostat at 300 K. During the equilibration phase, pressure was maintained at $\sim$1 atm using a relexation time of 1 ps in the pressure coupling algorithm [3]. Finally, simulations for productions were performed in the isothermal-isobaric (NPT) ensemble for a total of 200 ns for all the three systems with the sampling of the simulation trajectories performed every 2 ps using PMEMD module implemented with CUDA executed with the help of GPU based workstation. The first 100 ns MD simulation was considered as equilibration stage for all the systems and the final 100 ns simulation was for production MD. During the whole simulation time, bonds involving H-atoms were constrained employing the SHAKE algorithm [3] with a time interval of 2 fs, and the long-ranged electrostatic interactions were treated by the Particle Mesh Ewald (PME) algorithm [4] with the associated cutoff distance set to 10 Å. In addition, for the case of complexes, the metal-ligand distance was attempted to keep restrained within range between 1.9 and 2.0 Å as for the metal-water

coordination distance in the ligand-free enzyme along with certain parameters for the NMR restraint method, whereas the rest of the system was set free during the whole simulation time that is during equilibration as well as the production runs (overall simulation time). The simulation trajectories were analyzed with help of the cpptraj module implemented in AMBERTOOLS18 [5, 1], VMD [6] and Chimera [7] were employed for the visualization of the simulation data.

*Free energy simulation protocol*: MD simulations for the free energy calculation were carried out for both complexes of the LuxS enzyme with the RHC and KRI inhibitors following an appropriate protocol required for the simulation at each $\lambda$ point. Prior to the simulations, minimization of the systems was carried out twice, first using the steepest descent algorithm, and second the L-BFGS algorithm for the minimization in order to avoid instabilities of the systems during ensemble averaging in the simulations. The systems were then equilibrated for 100 ps using a time-step of 2 fs using first the canonical and later the isothermal-isobaric ensembles. Finally, a $\sim$1 ns simulation was performed for each $\lambda$-value to collect data for the estimation of the free energy contribution. Again the particle Mesh Ewald (PME) algorithm was used for the treatment of non-bonded interactions using a cutoff distance of 12 Å for the Coulombic as well as the van der Waals interactions. A constant temperature was maintained at $\sim$298 K using the Langevin dynamics integrator with a coupling constant of 1.0 ps. The pressure was controlled using the Parinello-Rahman barostat fixed at 1 bar along with a coupling constant of 1.0 ps and a compressibility value for water set to 4.5 x $10^{-5}$ $bar^{-1}$ for all simulations. The trajectories obtained

from the AMBER simulation package [1, 10] were converted to GROMACS format for the estimation of the binding free energy through the thermodynamic integration (TI) method. GROMACS 2018.3 [11] was used for all free energy simulations performed after which the energy difference between the two states was estimated using the Benettet Acceptance Ratio (BAR) module of the GROMACS program enabling a straightforward data treatment [12]. The free energy estimates were determined using BAR with bisection, with the precision of the output set and an error estimate taking into account time correlations is made by splitting the data into blocks and determining the free energy differences over those blocks and assuming the blocks are independent. The final error estimate is determined from the average variance over 5 blocks. A range of block numbers for error estimation were also obtained. In general, the results are split in two parts: the last part contains the final results in kJ/mol, together with the error estimate for each part and the total. The first part contains detailed free energy difference estimates and phase space overlap measures in units of kT (together with their computed error estimate).

# References


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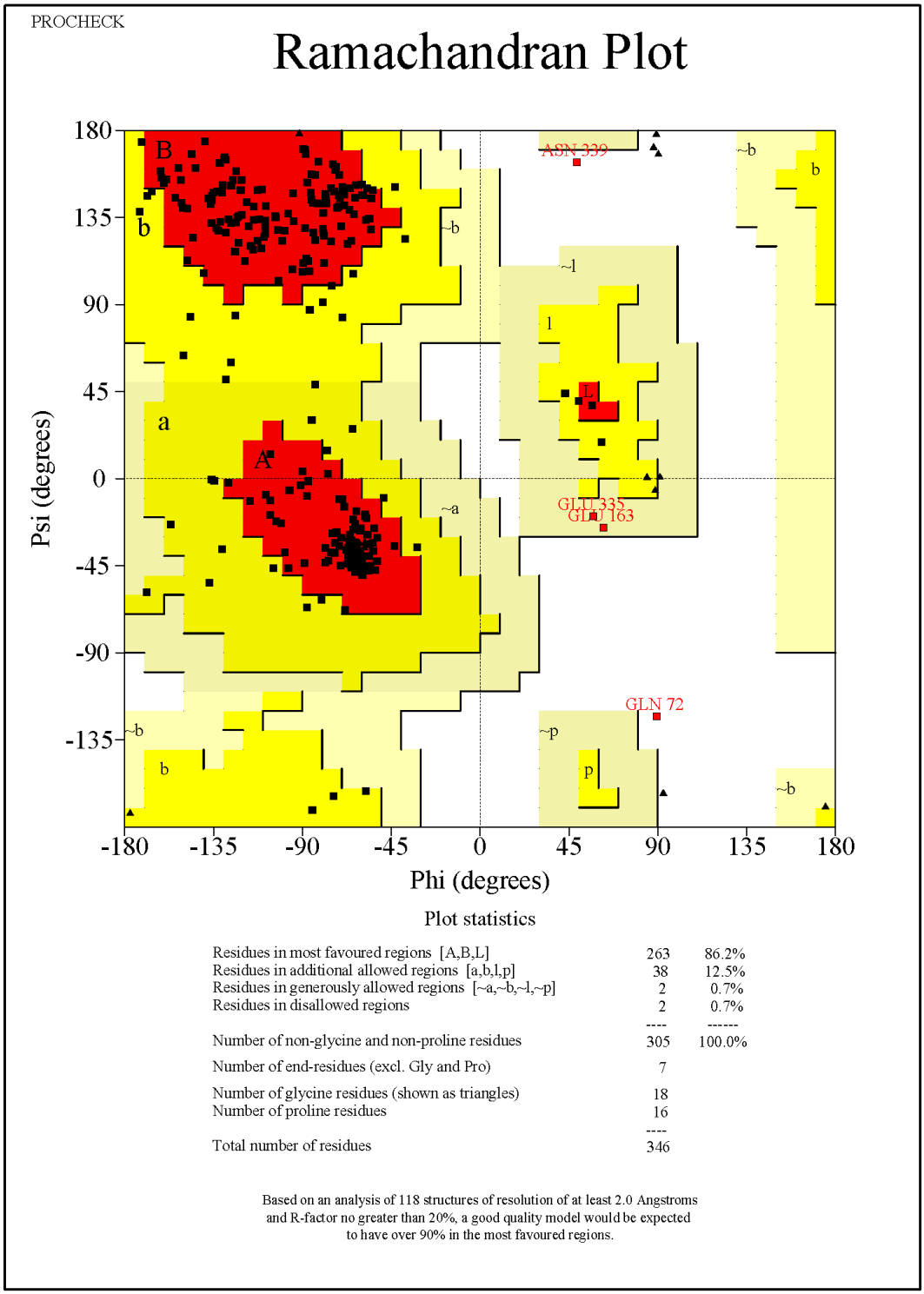
Figure S1: Ramachandran plot for the modelled LuxS enzyme obtained from PROCHECK Program



## Ligand parameter files obtained from GAFF

**RHC inhibitor**

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**prepin file**

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   0    0    2


This is a remark line
molecule.res
MOL   XYZ  0
CHANGE     OMIT DU   BEG
  0.0000
   1  DUMM  DU    M       999.000     999.0     -999.0          .00000
   2  DUMM  DU    M       999.000    -999.0      999.0          .00000
   3  DUMM  DU    M      -999.000     999.0      999.0          .00000
   4  O1    o     M         3.261000   -7.261000    4.097000   -0.719564
   5  C1    c     M         2.196000   -7.328000    3.419000    0.668787
   6  O2    o     E         1.932000   -8.041000    2.413000   -0.719564
   7  C2    c3    M         1.079000   -6.384000    3.894000    0.211496
   8  N1    n4    3         1.039000   -5.089000    3.121000   -0.577087
   9  H1    hn    E         0.779000   -5.285000    2.145000    0.364494
  10  H2    hn    E         0.310000   -4.469000    3.503000    0.364494
  11  H17   hn    E         1.926000   -4.632000    3.189000    0.364494
  12  H3    hx    E         1.262000   -6.093000    4.936000    0.022014
  13  C3    c3    M        -0.326000   -6.993000    3.778000   -0.163932
  14  H4    hc    E        -0.542000   -7.269000    2.737000    0.064226
  15  H5    hc    E        -1.057000   -6.219000    4.044000    0.064226
  16  C4    c3    M        -0.502000   -8.223000    4.671000    0.094729
  17  H6    h1    E         0.001000   -9.088000    4.228000    0.082610
  18  H7    h1    E        -0.072000   -8.067000    5.667000    0.082610
```

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 19 | S1 | ss | M | -2.268000 | -8.637000 | 4.850000 | -0.241420 |
| 20 | C5 | c3 | M | -2.718000 | -7.395000 | 6.113000 | -0.526807 |
| 21 | H8 | h1 | E | -2.503000 | -7.832000 | 7.094000 | 0.160840 |
| 22 | H9 | h1 | E | -2.080000 | -6.519000 | 5.986000 | 0.160840 |
| 23 | C6 | c3 | M | -4.200000 | -7.002000 | 6.006000 | 0.591398 |
| 24 | O3 | oh | S | -4.985000 | -8.078000 | 6.502000 | -0.620803 |
| 25 | H10 | ho | E | -4.802000 | -8.169000 | 7.453000 | 0.415802 |
| 26 | H11 | h1 | E | -4.460000 | -6.847000 | 4.952000 | 0.102413 |
| 27 | C7 | c3 | M | -4.480000 | -5.702000 | 6.787000 | -0.004201 |
| 28 | O4 | oh | S | -3.671000 | -4.677000 | 6.188000 | -0.660131 |
| 29 | H12 | ho | E | -3.710000 | -3.896000 | 6.765000 | 0.425536 |
| 30 | H13 | h1 | E | -4.136000 | -5.813000 | 7.824000 | 0.042683 |
| 31 | C8 | c3 | M | -5.930000 | -5.177000 | 6.792000 | 0.405812 |
| 32 | O5 | oh | S | -6.538000 | -5.376000 | 5.521000 | -0.734700 |
| 33 | H14 | ho | E | -7.421000 | -4.970000 | 5.552000 | 0.466893 |
| 34 | H15 | h1 | E | -5.895000 | -4.091000 | 6.948000 | -0.021687 |
| 35 | C9 | c | M | -6.816000 | -5.750000 | 7.893000 | 0.162232 |
| 36 | H16 | h4 | E | -6.320000 | -5.675000 | 8.864000 | 0.068791 |
| 37 | O6 | o | M | -7.158000 | -7.107000 | 7.655000 | -0.397522 |

LOOP

IMPROPER

C2 O1 C1 O2

C8 H16 C9 O6

DONE

STOP

**frcmod file**

Remark line goes here

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c3 12.010  0.878

n4 14.010  0.530

hn 1.008  0.161

hx 1.008  0.135

hc 1.008  0.135

h1 1.008  0.135

ss 32.060  2.900

oh 16.000  0.465

ho 1.008  0.135

h4 1.008  0.135

BOND

c -o  637.70  1.218

c -c3  313.00  1.524

c3-n4  283.30  1.511

c3-hx  338.70  1.091

c3-c3  300.90  1.538

hn-n4  373.20  1.030

c3-hc  330.60  1.097

c3-h1  330.60  1.097

c3-ss  215.90  1.839

c3-oh  316.70  1.423

ho-oh  371.40  0.973

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ANGLE

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c3-c -o  67.400  123.200

c -c3-n4  65.500  110.730

c -c3-hx  46.900  108.850

c -c3-c3  63.300  111.040

c3-n4-hn  45.900  110.110

c3-c3-hc  46.300  109.800

c3-c3-c3  62.900  111.510

hx-c3-n4  48.600  108.010

c3-c3-n4  64.200  114.210

hn-n4-hn  40.600  108.300

c3-c3-hx  46.200  110.560

c3-c3-h1  46.400  109.560

c3-c3-ss  61.300  110.270

hc-c3-hc  39.400  107.580

c3-ss-c3  60.200  99.240

h1-c3-h1  39.200  108.460

h1-c3-ss  42.100  108.760

c3-c3-oh  67.500  110.190

c3-oh-ho  47.400  107.260

h1-c3-oh  50.900  110.260

c3-c -h4  45.600  114.640

c -c3-oh  68.300  108.790

c -c3-h1  47.000  108.220

h4-c -o  54.200  120.700

DIHE

o -c -c3-n4  6  0.000  180.000  2.000

o -c -c3-hx  6  0.000  180.000  2.000

| | | | | |
|---|---|---|---|---|
| o -c -c3-c3 | 6 | 0.000 | 180.000 | 2.000 |
| c -c3-n4-hn | 9 | 1.400 | 0.000 | 3.000 |
| c -c3-c3-hc | 9 | 1.400 | 0.000 | 3.000 |
| c -c3-c3-c3 | 9 | 1.400 | 0.000 | 3.000 |
| c3-c3-c3-h1 | 9 | 1.400 | 0.000 | 3.000 |
| c3-c3-c3-ss | 9 | 1.400 | 0.000 | 3.000 |
| hc-c3-c3-n4 | 9 | 1.400 | 0.000 | 3.000 |
| c3-c3-c3-n4 | 9 | 1.400 | 0.000 | 3.000 |
| hx-c3-n4-hn | 9 | 1.400 | 0.000 | 3.000 |
| c3-c3-n4-hn | 9 | 1.400 | 0.000 | 3.000 |
| hc-c3-c3-hx | 9 | 1.400 | 0.000 | 3.000 |
| c3-c3-c3-hx | 9 | 1.400 | 0.000 | 3.000 |
| c3-c3-ss-c3 | 3 | 1.000 | 0.000 | 3.000 |
| h1-c3-c3-hc | 9 | 1.400 | 0.000 | 3.000 |
| hc-c3-c3-ss | 9 | 1.400 | 0.000 | 3.000 |
| h1-c3-ss-c3 | 3 | 1.000 | 0.000 | 3.000 |
| oh-c3-c3-ss | 9 | 1.400 | 0.000 | 3.000 |
| h1-c3-c3-ss | 9 | 1.400 | 0.000 | 3.000 |
| c3-c3-oh-ho | 1 | 0.160 | 0.000 | -3.000 |
| c3-c3-oh-ho | 1 | 0.250 | 0.000 | 1.000 |
| c3-c3-c3-oh | 9 | 1.400 | 0.000 | 3.000 |
| c3-c3-c3-c3 | 1 | 0.180 | 0.000 | -3.000 |
| c3-c3-c3-c3 | 1 | 0.250 | 180.000 | -2.000 |
| c3-c3-c3-c3 | 1 | 0.200 | 180.000 | 1.000 |
| h1-c3-c3-oh | 1 | 0.000 | 0.000 | -3.000 |
| h1-c3-c3-oh | 1 | 0.250 | 0.000 | 1.000 |
| h1-c3-c3-h1 | 9 | 1.400 | 0.000 | 3.000 |
| oh-c3-c3-oh | 1 | 0.144 | 0.000 | -3.000 |
| oh-c3-c3-oh | 1 | 1.175 | 0.000 | 2.000 |
| h1-c3-oh-ho | 3 | 0.500 | 0.000 | 3.000 |

h4-c -c3-c3 6 0.000 180.000 2.000

c -c3-c3-oh 9 1.400 0.000 3.000

c -c3-c3-h1 9 1.400 0.000 3.000

h4-c -c3-oh 6 0.000 180.000 2.000

o -c -c3-oh 6 0.000 180.000 2.000

c -c3-oh-ho 3 0.500 0.000 3.000

h4-c -c3-h1 6 0.000 180.000 2.000

o -c -c3-h1 1 0.800 0.000 -1.000

o -c -c3-h1 1 0.000 0.000 -2.000

o -c -c3-h1 1 0.080 180.000 3.000

IMPROPER

c3-o -c -o 1.1 180.0 2.0 Using general improper torsional angle X- o- c- o, penalty score= 3.0)

c3-h4-c -o 10.5 180.0 2.0 Using general improper torsional angle X- X- c- o, penalty score= 6.0)

NONBON

o 1.6612 0.2100

c 1.9080 0.0860

c3 1.9080 0.1094

n4 1.8240 0.1700

hn 0.6000 0.0157

hx 1.1000 0.0157

hc 1.4870 0.0157

h1 1.3870 0.0157

ss 2.0000 0.2500

oh 1.7210 0.2104

ho 0.0000 0.0000

h4 1.4090 0.0150

**KRI inhibitor**

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**prepin file**

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   0    0    2

This is a remark line
molecule.res
MOL   XYZ  0
CHANGE     OMIT DU   BEG
  0.0000
   1  DUMM  DU    M       999.000     999.0      -999.0          .00000
   2  DUMM  DU    M       999.000    -999.0       999.0          .00000
   3  DUMM  DU    M      -999.000     999.0       999.0          .00000
   4  O1    o     M        47.433000   36.726000   47.275000   -0.741706
   5  C1    c     M        46.526000   37.474000   47.573000    0.761110
   6  O2    o     E        46.467000   38.658000   47.287000   -0.741706
   7  C2    c3    M        45.862000   37.164000   48.934000    0.325018
   8  N1    n4    3        46.303000   38.209000   49.982000   -0.790951
   9  H1    hn    E        47.321000   38.118000   50.127000    0.415689
  10  H2    hn    E        46.182000   39.132000   49.516000    0.415689
  11  H3    hn    E        45.798000   38.159000   50.871000    0.415689
  12  H4    hx    E        46.261000   36.191000   49.229000    0.007682
  13  C3    c3    M        44.326000   37.048000   48.783000   -0.311195
  14  H5    hc    E        44.103000   36.366000   47.950000    0.039805
  15  H6    hc    E        43.897000   38.020000   48.505000    0.039805
  16  C4    c3    M        43.650000   36.498000   50.057000    0.594536
  17  H7    h1    E        44.110000   35.536000   50.319000   -0.125855
  18  H8    h1    E        43.773000   37.185000   50.902000   -0.125855
  19  S1    ss    M        41.858000   36.196000   49.763000   -0.257505
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```

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 21 | H9 | h1 | E | 41.723000 | 38.624000 | 49.516000 | 0.139804 |
| 22 | H10 | h1 | E | 40.118000 | 37.876000 | 49.571000 | 0.139804 |
| 23 | C6 | c3 | M | 40.976000 | 38.217000 | 51.531000 | 0.403623 |
| 24 | O3 | oh | S | 40.031000 | 37.334000 | 52.141000 | -0.707418 |
| 25 | H11 | ho | E | 39.290000 | 37.869000 | 52.499000 | 0.440148 |
| 26 | H12 | h1 | E | 41.952000 | 38.059000 | 52.006000 | -0.064771 |
| 27 | C7 | c3 | M | 40.571000 | 39.699000 | 51.755000 | -0.001443 |
| 28 | O4 | oh | S | 39.456000 | 40.069000 | 50.917000 | -0.657221 |
| 29 | H13 | ho | E | 38.640000 | 40.021000 | 51.467000 | 0.445226 |
| 30 | H14 | h1 | E | 41.429000 | 40.332000 | 51.498000 | 0.094620 |
| 31 | C8 | c | M | 40.229000 | 39.960000 | 53.232000 | 0.681652 |
| 32 | O5 | o | E | 39.125000 | 39.679000 | 53.667000 | -0.578321 |
| 33 | N2 | n | M | 41.262000 | 40.388000 | 54.049000 | -0.434031 |
| 34 | H15 | hn | E | 42.187000 | 40.580000 | 53.687000 | 0.347394 |
| 35 | O6 | oh | M | 41.031000 | 40.759000 | 55.402000 | -0.397052 |
| 36 | H16 | ho | E | 40.768000 | 39.922000 | 55.843000 | 0.423501 |

LOOP

IMPROPER

C2 O1 C1 O2

C7 N2 C8 O5

C8 H15 N2 O6

DONE

STOP

**frcmod file**

Remark line goes here

MASS

o  16.000        0.434

c  12.010        0.616

c3 12.010        0.878

n4 14.010        0.530

hn 1.008         0.161

hx 1.008         0.135

hc 1.008         0.135

h1 1.008         0.135

ss 32.060        2.900

oh 16.000        0.465

ho 1.008         0.135

n  14.010        0.530

BOND

c -o   637.70   1.218

c -c3  313.00   1.524

c3-n4  283.30   1.511

c3-hx  338.70   1.091

c3-c3  300.90   1.538

hn-n4  373.20   1.030

c3-hc  330.60   1.097

c3-h1  330.60   1.097

c3-ss  215.90   1.839

c3-oh  316.70   1.423

ho-oh  371.40   0.973

c -n   427.60   1.379

hn-n   403.20   1.013

n -oh  400.20   1.406

ANGLE

o -c -o    77.900     130.250

c3-c -o    67.400     123.200

c -c3-n4   65.500     110.730

c -c3-hx   46.900     108.850

c -c3-c3   63.300     111.040

c3-n4-hn   45.900     110.110

c3-c3-hc   46.300     109.800

c3-c3-c3   62.900     111.510

hx-c3-n4   48.600     108.010

c3-c3-n4   64.200     114.210

hn-n4-hn   40.600     108.300

c3-c3-hx   46.200     110.560

c3-c3-h1   46.400     109.560

c3-c3-ss   61.300     110.270

hc-c3-hc   39.400     107.580

c3-ss-c3   60.200      99.240

h1-c3-h1   39.200     108.460

h1-c3-ss   42.100     108.760

c3-c3-oh   67.500     110.190

c3-oh-ho   47.400     107.260

h1-c3-oh   50.900     110.260

c3-c -n    66.800     115.180

c -c3-oh   68.300     108.790

c -c3-h1   47.000     108.220

c -n -hn   48.300     117.550

c -n -oh   68.200     115.510

n -c -o    74.200     123.050

ho-oh-n 50.500 101.290

hn-n -oh 50.400 110.740

DIHE

| | | | | |
|---|---|---|---|---|
| o -c -c3-n4 | 6 | 0.000 | 180.000 | 2.000 |
| o -c -c3-hx | 6 | 0.000 | 180.000 | 2.000 |
| o -c -c3-c3 | 6 | 0.000 | 180.000 | 2.000 |
| c -c3-n4-hn | 9 | 1.400 | 0.000 | 3.000 |
| c -c3-c3-hc | 9 | 1.400 | 0.000 | 3.000 |
| c -c3-c3-c3 | 9 | 1.400 | 0.000 | 3.000 |
| c3-c3-c3-h1 | 9 | 1.400 | 0.000 | 3.000 |
| c3-c3-c3-ss | 9 | 1.400 | 0.000 | 3.000 |
| hc-c3-c3-n4 | 9 | 1.400 | 0.000 | 3.000 |
| c3-c3-c3-n4 | 9 | 1.400 | 0.000 | 3.000 |
| hx-c3-n4-hn | 9 | 1.400 | 0.000 | 3.000 |
| c3-c3-n4-hn | 9 | 1.400 | 0.000 | 3.000 |
| hc-c3-c3-hx | 9 | 1.400 | 0.000 | 3.000 |
| c3-c3-c3-hx | 9 | 1.400 | 0.000 | 3.000 |
| c3-c3-ss-c3 | 3 | 1.000 | 0.000 | 3.000 |
| h1-c3-c3-hc | 9 | 1.400 | 0.000 | 3.000 |
| hc-c3-c3-ss | 9 | 1.400 | 0.000 | 3.000 |
| h1-c3-ss-c3 | 3 | 1.000 | 0.000 | 3.000 |
| oh-c3-c3-ss | 9 | 1.400 | 0.000 | 3.000 |
| h1-c3-c3-ss | 9 | 1.400 | 0.000 | 3.000 |
| c3-c3-oh-ho | 1 | 0.160 | 0.000 | -3.000 |
| c3-c3-oh-ho | 1 | 0.250 | 0.000 | 1.000 |
| c3-c3-c3-oh | 9 | 1.400 | 0.000 | 3.000 |
| h1-c3-c3-oh | 1 | 0.000 | 0.000 | -3.000 |
| h1-c3-c3-oh | 1 | 0.250 | 0.000 | 1.000 |
| h1-c3-c3-h1 | 9 | 1.400 | 0.000 | 3.000 |

n -c -c3-c3 1 0.100 0.000 -4.000

n -c -c3-c3 1 0.070 0.000 2.000

oh-c3-c3-oh 1 0.144 0.000 -3.000

oh-c3-c3-oh 1 1.175 0.000 2.000

c -c3-c3-oh 9 1.400 0.000 3.000

h1-c3-oh-ho 3 0.500 0.000 3.000

c -c3-c3-h1 9 1.400 0.000 3.000

c3-c -n -hn 4 10.000 180.000 2.000

c3-c -n -oh 4 10.000 180.000 2.000

o -c -c3-oh 6 0.000 180.000 2.000

n -c -c3-oh 6 0.000 180.000 2.000

c -c3-oh-ho 3 0.500 0.000 3.000

o -c -c3-h1 1 0.800 0.000 -1.000

o -c -c3-h1 1 0.000 0.000 -2.000

o -c -c3-h1 1 0.080 180.000 3.000

n -c -c3-h1 6 0.000 180.000 2.000

c -n -oh-ho 2 3.000 0.000 2.000

o -c -n -hn 1 2.500 180.000 -2.000

o -c -n -hn 1 2.000 0.000 1.000

o -c -n -oh 4 10.000 180.000 2.000

hn-n -oh-ho 2 3.000 0.000 2.000

IMPROPER

c3-o -c -o 1.1 180.0 2.0 Using general improper torsional angle X- o- c- o, penalty score= 3.0)

c3-n -c -o 10.5 180.0 2.0 Using general improper torsional angle X- X- c- o, penalty score= 6.0)

c -hn-n -oh 1.1 180.0 2.0 Using the default value

NONBON

o 1.6612 0.2100

| | | |
|---|---|---|
| c | 1.9080 | 0.0860 |
| c3 | 1.9080 | 0.1094 |
| n4 | 1.8240 | 0.1700 |
| hn | 0.6000 | 0.0157 |
| hx | 1.1000 | 0.0157 |
| hc | 1.4870 | 0.0157 |
| h1 | 1.3870 | 0.0157 |
| ss | 2.0000 | 0.2500 |
| oh | 1.7210 | 0.2104 |
| ho | 0.0000 | 0.0000 |
| n | 1.8240 | 0.1700 |