Support Information for:

QM/MM Investigations of Catalytic Mechanism for the Processive Endoglucanase Cel9G from *Clostridium cellulovorans*

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Content

Principal component analysis (PCA)

The principal component analysis (PCA)[1-3] was used in this work in helping identify the principal conformation during the 200 ns of molecular dynamics (MD) simulation. The PCA were carried out by the CPPTRAJ module of AMBER 16[4]. The entire trajectory was aligned against a reference structure in order to remove overall global and translation motion before the PCA is applied. Then the covariance matrix of C_α was carried out from the trajectory. The first two eigenvectors of C_α trajectory are known as PC1 and PC2. To determine the preferred conformation, further free energy landscapes calculation, carried out by the equation $\mu(PC1, PC2) = -k_B T \ln P(PC1, PC2)$.[3] Where P(PC1,PC2), T, and k_B are the probability density function, the absolute temperature, and the Boltzmann constant, respectively. The local minima in the free energy landscape helps us locate the representative conformers during MD simulations.

Reference
1. B. Hess, *Phys. Rev. E*, 2000, **62**, 8438-8448.
2. M. A. Balsera, W. Wriggers, Y. Oono and K. Schulten, *J. Phys. Chem.*, 1996, **100**, 2567-2572.
3. G. G. Maisuradze, A. Liwo and H. A. Scheraga, *J. Mol. Biol.*, 2009, **385**, 312-329.
4. R. M. B. D.A. Case, D.S. Cerutti, T.E. Cheatham, III, T.A. Darden, R.E. Duke, T.J. Giese, H. Gohlke, A.W. Goetz, N. Homeyer, S. Izadi, P. Janowski, J. Kaus, A. Kovalenko, T.S. Lee, S. LeGrand, P. Li, C., T. L. Lin, R. Luo, B. Madej, D. Mermelstein, K.M. Merz, G. Monard, H. Nguyen, H.T. Nguyen, I. and A. O. Omelyan, D.R. Roe, A. Roitberg, C. Sagui, C.L. Simmerling, W.M. Botello-Smith, J. Swails, R.C. Walker, J. Wang, R.M. Wolf, X. Wu, L. Xiao and P.A. Kollman (2016), *AMBER 2016*, University of California, San Francisco.

Table S1. Box size and total atom number of each model.

Model	Box Size	Atom Number
Cel9G/G6	117 Å × 85 Å × 121 Å	101551
GH9/G6	91 Å × 83 Å × 100 Å	62374
Cel9G	117 Å × 85 Å × 120 Å	100969
GH9	89 Å × 83 Å × 98 Å	59989
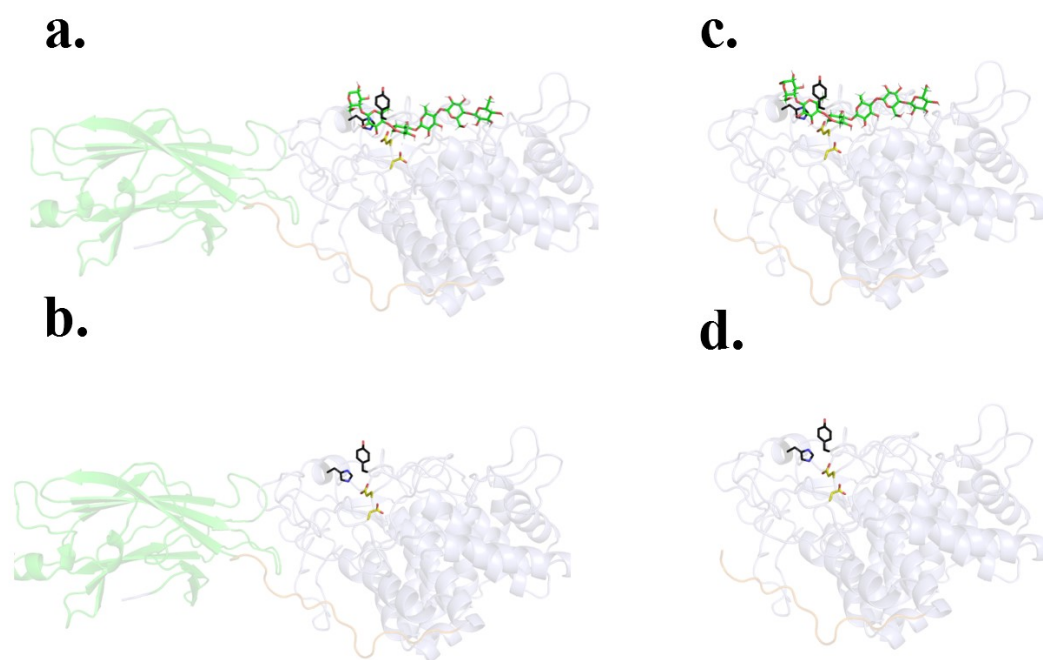
Figure S1. Initial structures of the four models investigated in this work.

(a) Cel9G/G6; (b) apo Cel9G; (c) GH9/G6; and (d) apo GH9. The CBM3c module were shown in green cartoon. The GH9 module of Cel9G were shown in light bule cartoon. G6 (green), H125 (black), Y416(black), D55 (yellow) and E420 (yellow) were shown in stick.
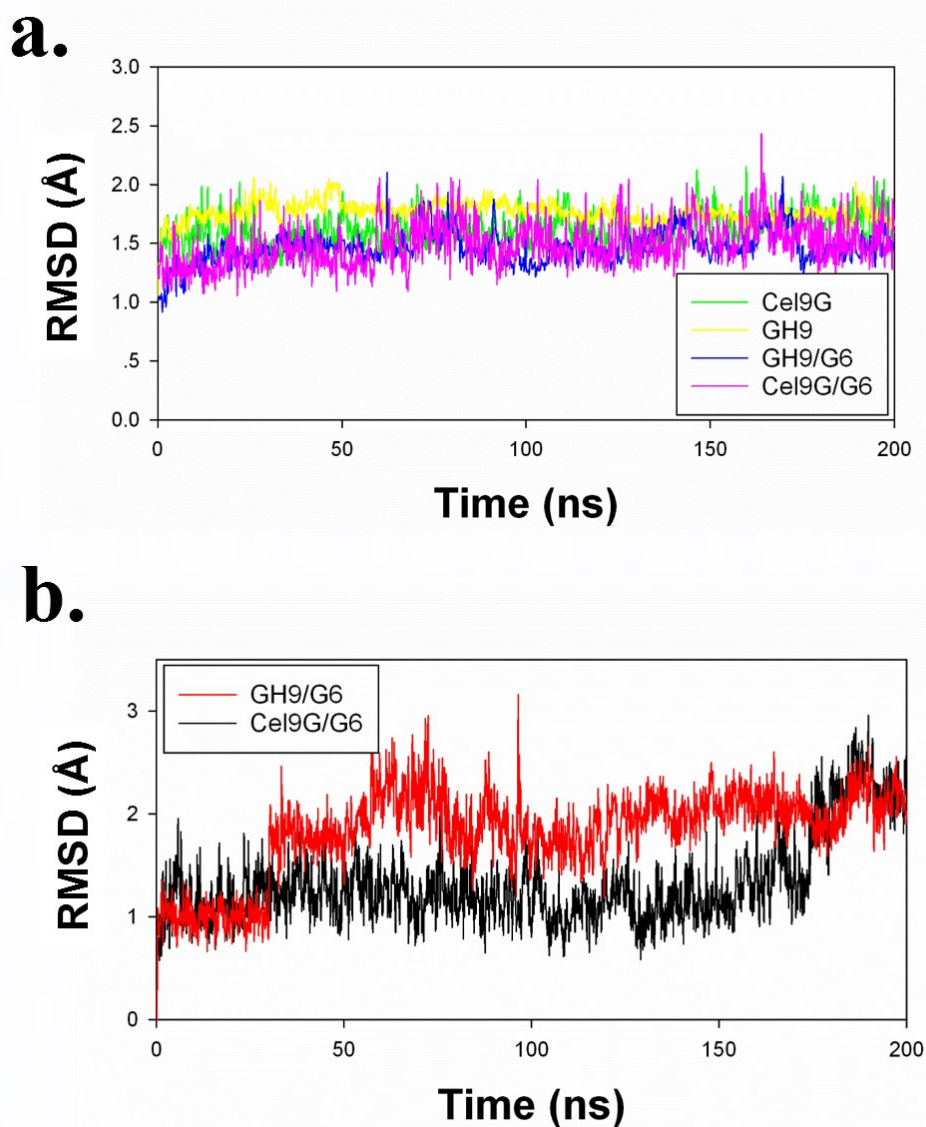
Figure S2. (a) Root-mean-square deviations (RMSD) of Cα atom compared to the crystal structure (PDB ID: 1KFG) during the 200 ns of classic molecular dynamics of each model. Crystal structure (PDB ID: 1KFG) of endoglucanase Cel9G from *Clostridium cellulolyticum* contain a Glycoside Hydrolase family 9 (GH9) catalytic domain and a Carbohydrate-Binding Module family 3c (CBM3c) domain. (D. Mandelman, A. Belaich, J. P. Belaich, N. Aghajari, H. Driguez and R. Haser, J. Bacteriol., 2003, 185, 4127-4135) (b) RMSD of heavy atoms of substrate during the 200 ns MD simulation.
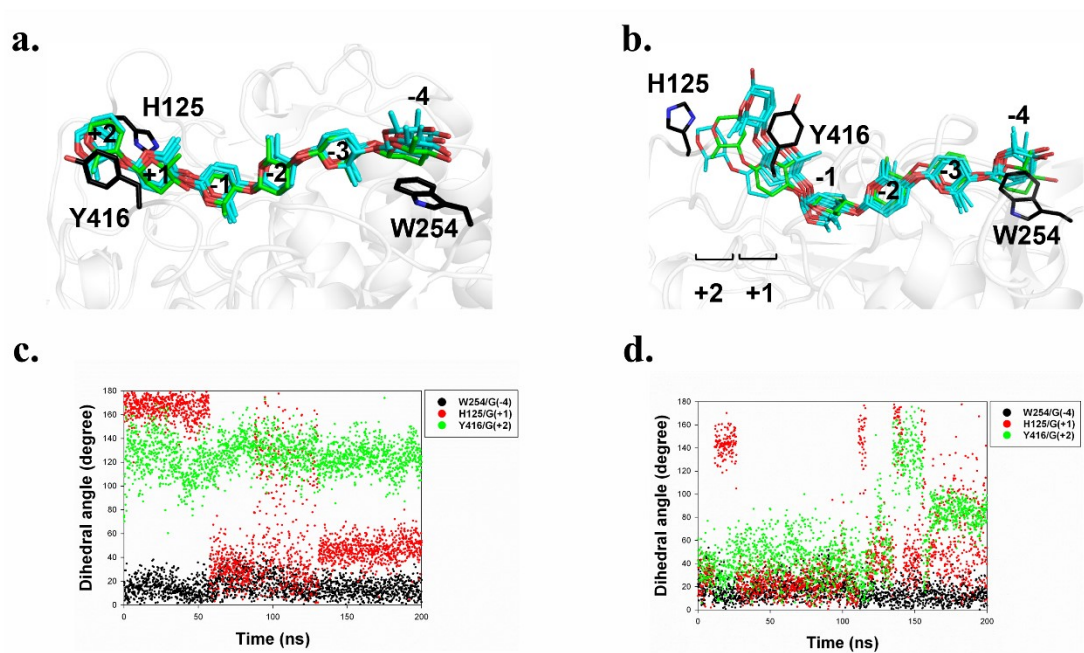
Figure S3. Stability of substrate bound in Cel9G/G6 and apo-GH9/G6 system.

(a). Five snapshots (40, 80, 120, 160, and 200 ns; cyan stick.) from Cel9G/G6 system overlap with crystal structure (PDB ID: 1KFG/1K72, green stick). (b) Five snapshots (40, 80, 120, 160, and 200 ns; cyan stick.) from apo-GH9/G6 system overlap with crystal structure (PDB ID: 1KFG/1K72, green stick). (c) Dihedral angle between the plane of sugar ring and the plane of aromatic residue in the MM MD of GH9/G6 system. (d) Dihedral angle between the plane of sugar ring and the plane of aromatic residue in the QM/MM MD of Cel9G/G6 system. Plane definition: Sugar ring (C1, C3, C5); H125 (CG, ND1, NE2); Y416 (CZ, CD1, CD2); W254 (CD1, CD2, CH2). 1KFG/1K72: Crystal structure of Cel9G complexed with Cello-oligosaccharides. Ligand in 1KFG occupy -1, -2, -3, and -4 binding site, and ligand in 1K72 occupy +1/+2 binding site. (D. Mandelman, A. Belaich, J. P. Belaich, N. Aghajari, H. Driguez and R. Haser, J. Bacteriol., 2003, 185, 4127-4135)
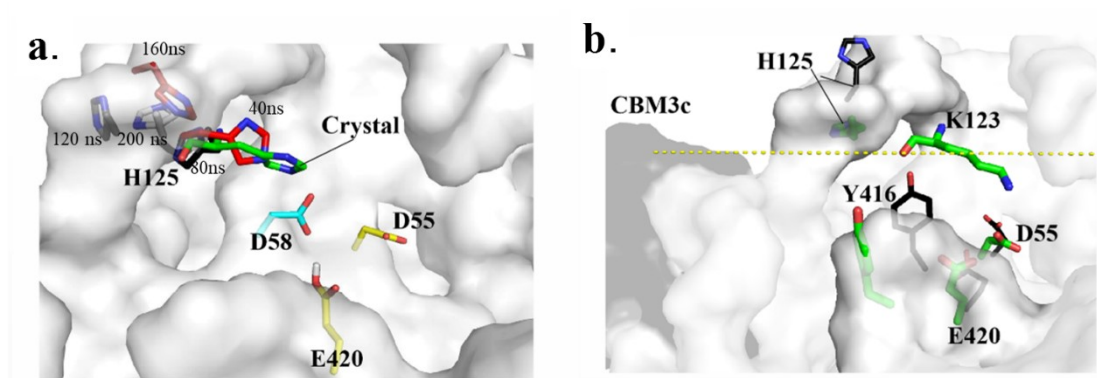
Figure S4. Conformation of H125 in apo GH9 and apo Cel9G system.

(a). Overlap of the five snapshots from the molecular dynamic of apo-GH9 system (40, 80, 120, 160, and 200 ns). (b). Overlap of the principal conformation from apo Cel9G system (black) and apo-GH9 (green).

Main chain of crystal structure (PDB ID: 1KFG) was shown in white surface, and E420/D55 of crystal structure were shown in yellow stick. H125 and D55 from crystal structure were also shown in stick in Figure S4b (green, cyan).
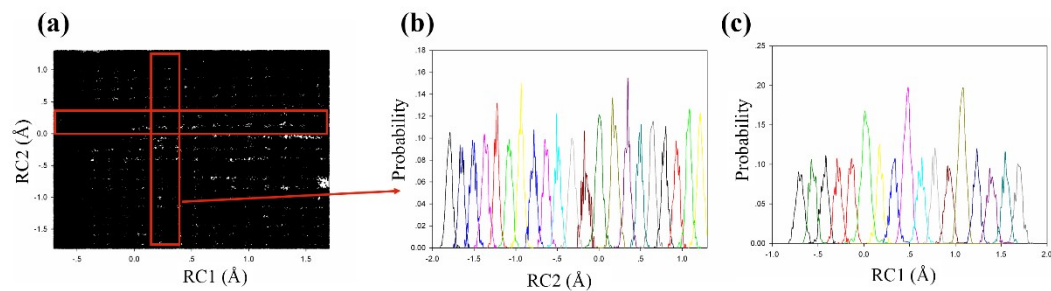
Figure S5. (a)The sampling along RC1 and RC2 from the umbrella sampling simulation of Cel9G/G6 system. (b). The probability distribution along the RC2 to show the overlap between different sampling windows when RC1=0.15Å. (c) The probability distribution along RC1 to show the overlap between different sampling windows when RC2=0.15Å.

Reaction coordinates were defined in Scheme 2. There are 17 grids along RC1 and 21 grids along RC2 which result in 357 (17×21) umbrella sampling windows in total.
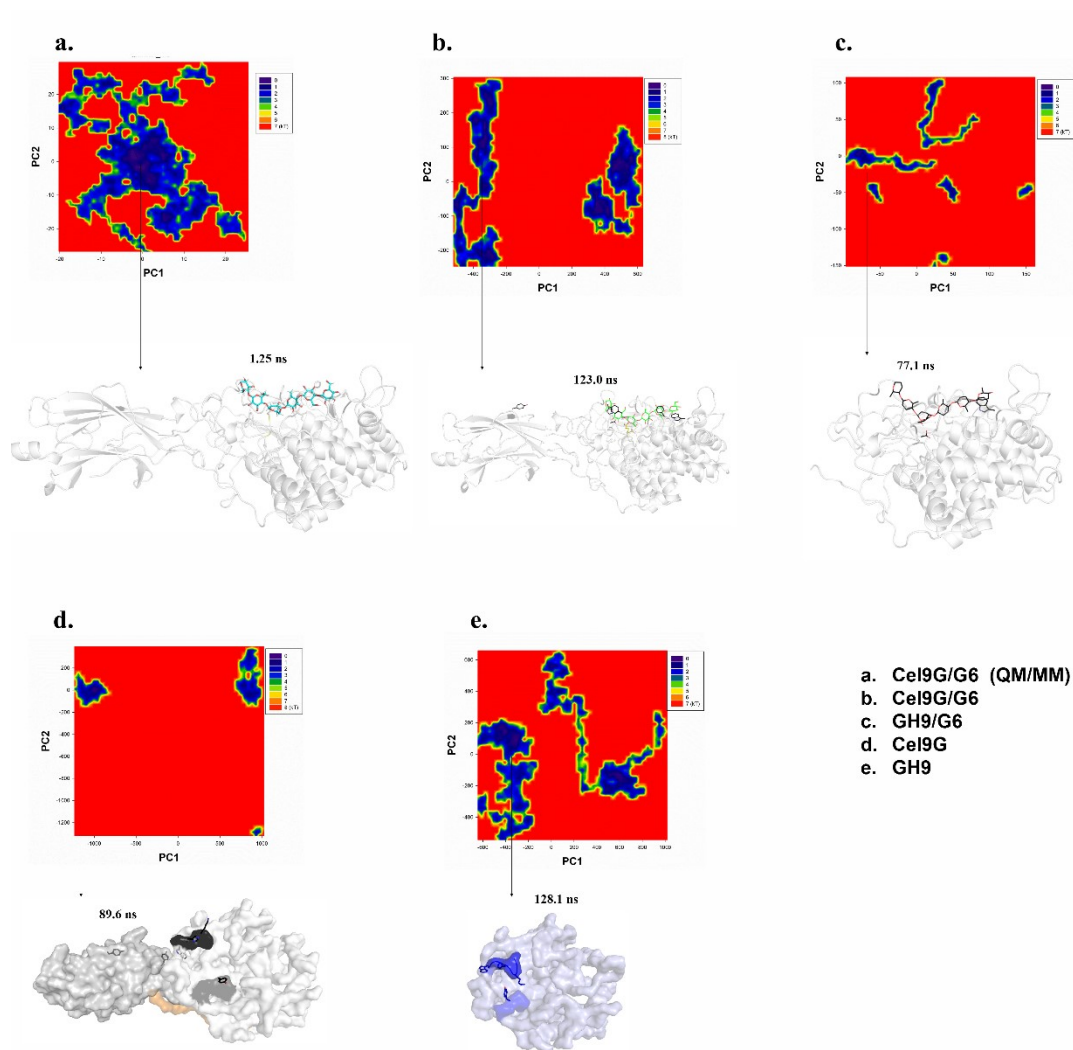
Figure S6. Free energy landscapes and representative snapshots extracted from the Principal Component Analysis (PCA).

The MD time of each representative snapshot were also labelled.

(a) From the QM/MM molecular dynamic simulation of Cel9G/G6 system. (b) From the MM molecular dynamic simulation of Cel9G/G6 system (c). From the MM molecular dynamic simulation of GH9/G6 system. (d). From the MM molecular dynamic simulation of apo-Cel9G system. (e). From the MM molecular dynamic simulation of apo-GH9 system.
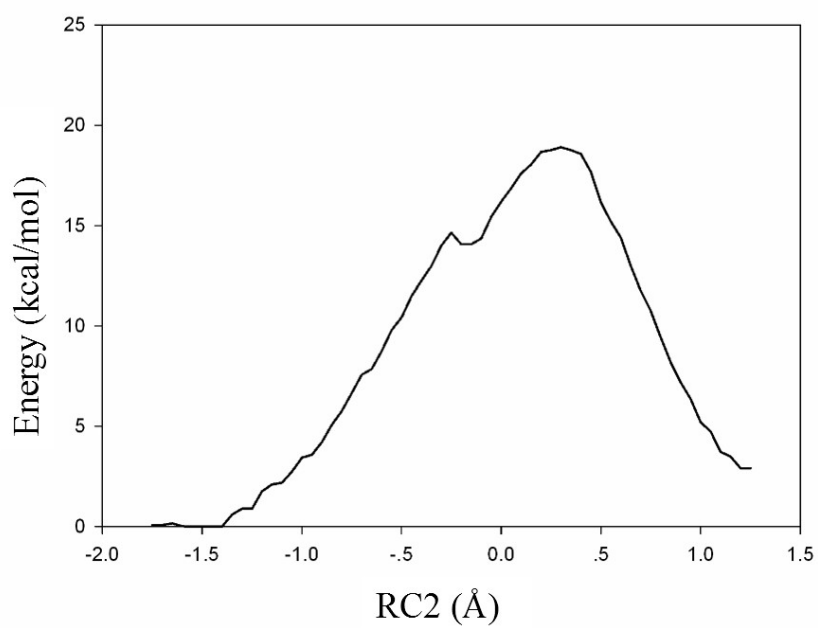
Figure S7. Minimum energy path (MEP) of the hydrolysis reaction in Cel9G/G6 system.

This MEP was extracted from the free energy landscape shown in Figure 5a.
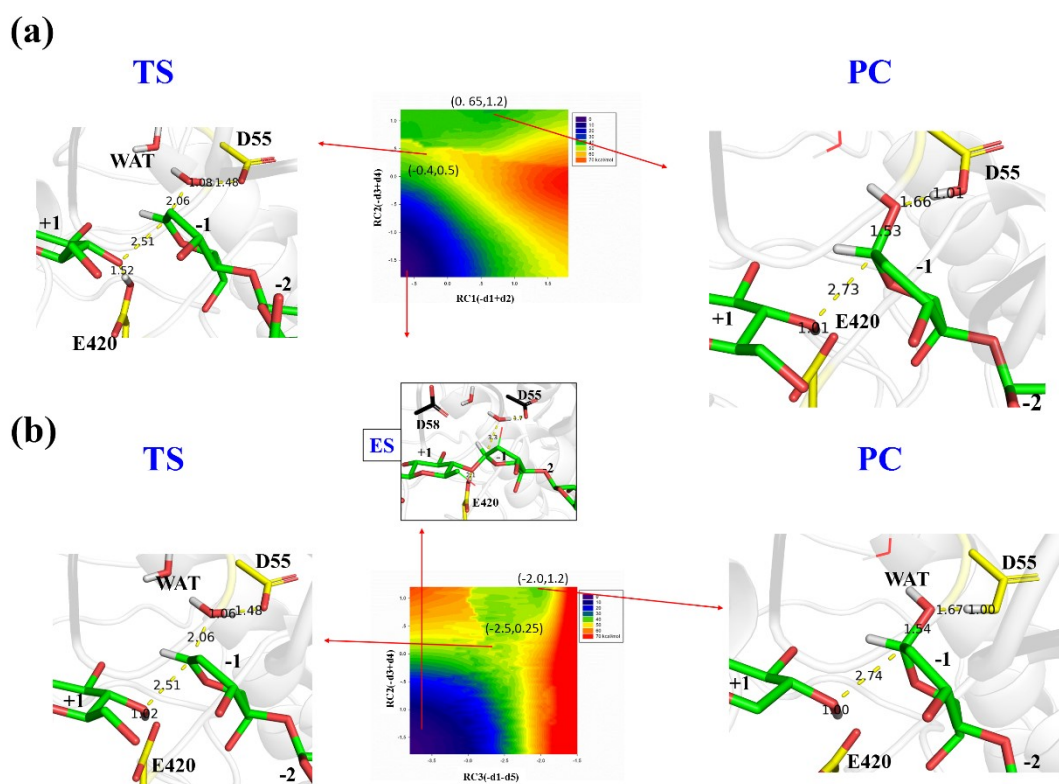
Figure S8. 2D Potential energy surfaces (PES) with respect to different reaction coordinates calculated using SCC-DFTB/AMBER method. (a) PES using RC1= -d1+d2, RC2 = -d3+d4 as the reaction coordinates. Snapshots of transition state and enzyme-product complexes are also included. (b) PES using RC3 = -d1-d5, RC2 = -d3+d4 as the reaction coordinates. Similarly, Snapshots of transition state and enzyme-product complexes are also included for comparison.

Density Functional Theory Calculation

To independently check the mechanism addressed by SCC-DFTB/AMBER method, we then applied density functional theory (DFT) calculation for the truncated model. The truncated model investigated in this work contains a celloteraose molecule for saving the computational cost, an active site water molecule, the side chain groups of D55 and E420. Total number of atoms is 114. To mimic the enzyme environment, we then fixed two carbon atoms of the side chain groups shown in Figure S9, which are labelled by stars. M062x functional and a standard basis set of 6-31+G(d) were employed in all geometry optimization. The transition state was confirmed by frequency calculation with only one imaginary frequency. The intrinsic reaction coordinate (IRC) approach was applied to connect the reactant and product. To estimate the bulk solvent effects on the reaction, we performed single-point energy calculations with the polarized continuum model using the integral equation formalism variant (IEFPCM) in water solvent. All calculations were carried out using the Gaussian09 suite of program.[1] The calculated transition state was plotted in Figure S9 and the energetic profile was also included in Figure S10. Clearly, the cleavage of the glycosidic bond can be observed, which is accordance with the attacking at the anomeric carbon atom by water molecule. In addition, with the cleavage of glycosidic bond of C1-O4, the proton on E420 is also transferred to the O4 atom. Elongation of $H-O_{wat}$ distance (1.12 Å at the transition state) also means the D55 may serve as the general base. Moreover, the energy barrier height is calculated about 19.8 kcal/mol, which is very close to our QM/MM calculation. Interestingly, the conformation of G(-1) ring shows a $^4H_5/^5E$ conformer, which is a little bit different from $^4H_5/^4E$ conformer in SCC-DFTB/AMBER approach. This may be reasonable if considering the truncated model lacks the protein environment effects.

Reference

1. M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, G. Scalmani, V. Barone, G. A. Petersson, H. Nakatsuji, X. Li, M. Caricato, A. Marenich, J. Bloino, B. G. Janesko, R. Gomperts, B. Mennucci, H. P. Hratchian, J. V. Ortiz, A. F. Izmaylov, J. L. Sonnenberg, D. Williams-Young, F. Ding, F. Lipparini, F. Egidi, J. Goings, B. Peng, A. Petrone, T. Henderson, D. Ranasinghe, V. G. Zakrzewski, J. Gao, N. Rega, G. Zheng, W. Liang, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, K. Throssell, J. A. Montgomery, Jr., J. E. Peralta, F. Ogliaro, M. Bearpark, J. J. Heyd, E. Brothers, K. N. Kudin, V. N. Staroverov, T. Keith, R. Kobayashi, J. Normand, K. Raghavachari, A. Rendell, J. C. Burant, S. S. Iyengar, J. Tomasi, M. Cossi, J. M. Millam, M. Klene, C. Adamo, R. Cammi, J. W. Ochterski, R. L. Martin, K. Morokuma, O. Farkas, J. B. Foresman, and D. J. Fox, 2009, Gaussian 09, Revision A.1, Gaussian, Inc., Wallingford CT.
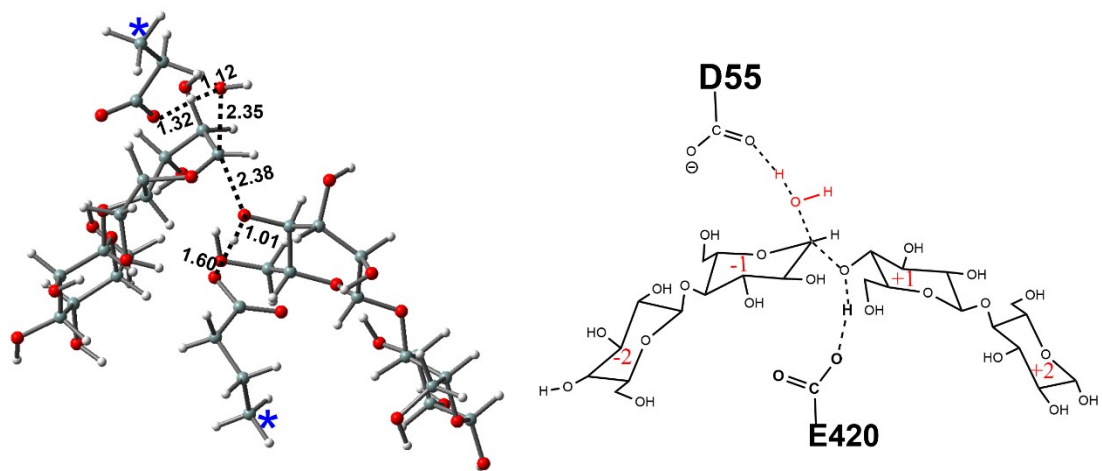
Figure S9. Transition state calculated at the M06-2X/g-31+G(d) level.
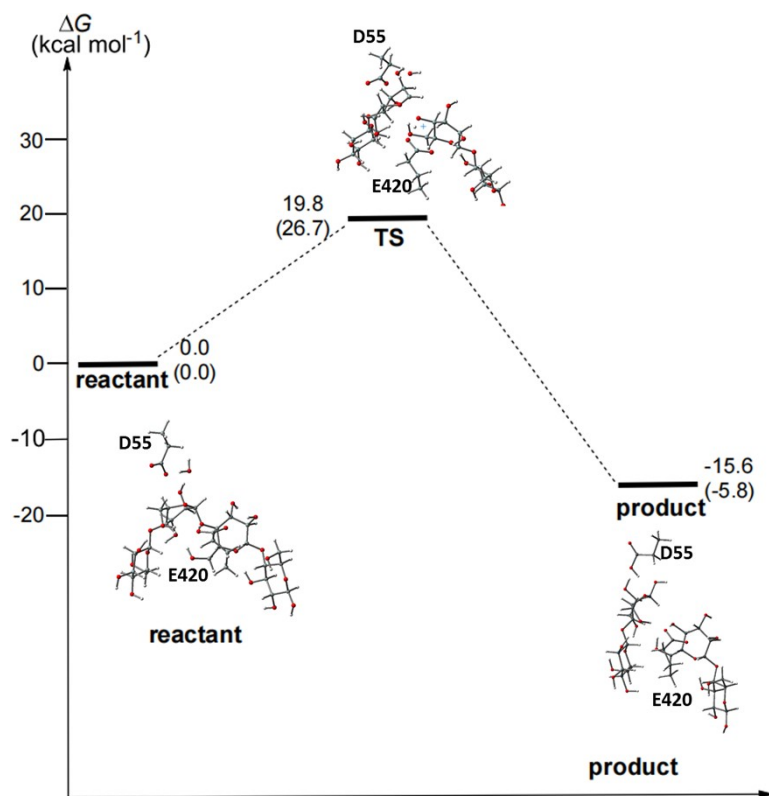
Figure S10. Gibbs free energy profile of the studied reaction at the M06-2X/g-31+G(d) level. The values in parentheses were calculated at the IEFPCM-M06-2X/6-311++G(d,p)//M06-2X/g-31+G(d) level.

Table S2. Selected Geometric Parameters of the transition states and product complexes obtained from different levels of theory.[a]

Distance (Å) or Angles (°)	SCC-DFTB (PES_a)[a]		SCC-DFTB (PES_b)[b]		DFT[d]	
	TS	PC	TS	PC	TS	PC
d1	1.48	1.01	1.48	1.00	1.32	0.99
d2	1.08	1.66	1.06	1.67	1.12	2.42
d3	2.06	1.53	2.06	1.54	2.35	1.40
d4	2.51	2.73	2.51	2.74	2.38	3.22
d5	1.52	1.01	1.02	1.00	1.01	1.00
ϕ	293°	308°	292°	308°	279°	269°
θ	55°	38°	53°	35°	73°	88°

[a] Angles of ϕ and θ denote the Cremer-Pople coordinates.

[b] Geometries extracted from SCC-DFTB/AMBER PES calculation using RC1=-d1+d2 and RC2=-d3+d4 as the reaction coordinates.

[c] Geometries extracted from SCC-DFTB/AMBER PES calculation using RC3=-d1-d5 and RC2=-d3+d4 as the reaction coordinates.

[d] Geometries extracted from the truncated model using the DFT calculation at the M062x/6-31+G(D) level of theory.

Gaussian input

Model **Reactant**

Cartesian Coordinates (Angstroms)

Charge = -1 Multiplicity = 1

  C,0,-3.7439019245,4.2652093283,6.1079504653
  H,0,-4.0454476914,3.3483664373,6.6335705756
  C,0,-2.2419815403,4.1325573081,5.7745308727
  O,0,-1.4623579921,5.0073586789,6.1680608625
  O,0,-1.9270345686,3.0930118926,5.0985297812
  C,0,0.8398471013,4.5263106557,-3.0303515684
  H,0,-0.157334345,4.4634122316,-3.4771295484
  C,0,0.7136048262,4.625066743,-1.5067873359
  H,0,1.6921388115,4.6581715459,-1.0182575169
  H,0,0.1725835944,5.5372060665,-1.2254015871
  C,0,-0.0750611948,3.4636347086,-0.9541595185
  O,0,-1.0757634091,3.0192359249,-1.4910868917
  O,0,0.4269161168,2.9456409766,0.16023287
  H,0,-0.1105875182,2.1623946195,0.4585805554
  H,0,1.1767512268,-0.1076081028,-9.5694451021
  O,0,0.5767042808,0.6354917584,-9.3926751815
  C,0,-0.1984803315,0.2766309895,-8.2933199144
  H,0,-1.0558279584,-0.346646708,-8.6095331782
  O,0,-0.6788499471,1.4745275736,-7.7376511305
  C,0,-1.5625774429,1.2676149674,-6.6411796842
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  C,0,-1.9832369718,2.6505716094,-6.168943375
  H,0,-2.6101448121,2.5569051562,-5.2776879004
  H,0,-2.5575131982,3.1429264803,-6.9650907052
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  H,0,-0.2676086176,3.4450738086,-6.5854088562
  C,0,-0.8640930596,0.4867256523,-5.5221945073
  H,0,-0.0727547091,1.116670096,-5.0905247514
  C,0,-0.247703857,-0.7997706901,-6.0543728385

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H,0,-5.1616342975,5.5469242544,7.1529311907

H,0,-3.5368521589,5.4878841754,7.8810432301

H,0,6.8123302333,-1.4557908526,1.3657729819

HF = -3170.3377388 (A.U.)

Zero-point correction = 0.953864 (A.U.)

Total Gibbs free energy = -3169.491735 (A.U.)

Model **TS**

Cartesian Coordinates (Angstroms)

Charge = -1 Multiplicity = 1

C,0,-7.1000596379,5.2484066924,4.4245596498

H,0,-7.4300219985,4.3297207395,4.9267499151

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O,0,-4.7889837811,5.8991948938,4.4265878367

O,0,-5.350246722,4.0270542547,3.3455283372

C,0,-2.4767460444,5.1397685258,-4.1788663839

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H,0,-2.9725613753,5.3136667109,-2.0688823475
C,0,-3.2110117929,3.2731100322,-2.5787198985
O,0,-3.9377009563,2.8379071683,-3.493032685
O,0,-3.0340162926,2.6857629417,-1.4530736566
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H,0,-6.5709026748,-0.1753437036,-8.2511488759
C,0,-5.5889740225,1.7398800188,-8.0806189746
H,0,-6.0415864056,2.0235372896,-7.1260882842
H,0,-6.2004562839,2.1391536969,-8.9019378782
O,0,-4.2855486202,2.2852610743,-8.1096491787
H,0,-3.8589465222,1.9599863794,-8.9179539354
C,0,-4.8461460835,-0.4090257934,-6.9813177774
H,0,-3.890791364,0.1058435625,-6.8050382498
C,0,-4.5919665551,-1.8937272189,-7.2131311615
H,0,-5.5684249107,-2.4064358522,-7.2581069032
O,0,-3.7758474996,-2.4910566219,-6.2292182303
H,0,-4.0333150688,-2.1413115927,-5.3529990755
C,0,-3.8826445225,-2.0704042858,-8.5409667056
H,0,-2.9108715005,-1.553498932,-8.5054485067
O,0,-3.7034592409,-3.4347777843,-8.8699871639
H,0,-3.3367142854,-3.8729238087,-8.0849903826
O,0,-5.7164536884,-0.2290517336,-5.8746321006
C,0,-5.1410324382,0.2472781954,-4.7042866007
H,0,-4.3728432601,1.0049785594,-4.9188047376
O,0,-4.5286284234,-0.8598545201,-4.0501914943
C,0,-3.844458407,-0.4447840577,-2.8713256659
H,0,-3.2017676219,0.4204920552,-3.0919919092

C,0,-2.9844124736,-1.6209214554,-2.4144646006

H,0,-2.3280734739,-1.9134592488,-3.241074479

H,0,-3.6425873877,-2.4750729632,-2.1861806239

O,0,-2.1543108275,-1.3078212895,-1.3185965595

H,0,-2.7175660156,-1.0402398838,-0.5634909722

C,0,-4.8947107862,-0.0442598666,-1.8336402795

H,0,-5.4125129164,-0.9606688519,-1.5059656178

C,0,-5.9307280482,0.9604309607,-2.3574792592

H,0,-5.5144424129,1.9545915642,-2.1924339745

O,0,-7.0989107744,0.9259640441,-1.5405640013

H,0,-7.5661141238,0.0911499347,-1.695115198

C,0,-6.2613428928,0.8620281713,-3.8581407732

H,0,-7.1417472532,0.2190678979,-3.9952146935

O,0,-6.5958276406,2.12229122,-4.3929926213

H,0,-5.8276418848,2.7026689535,-4.2213172575

O,0,-4.2463448219,0.4994410616,-0.7045196419

C,0,-5.2437600538,1.5301718299,1.1894773681

H,0,-6.0763370236,1.5900267902,0.4984390861

O,0,-4.4924900721,2.543071428,1.2304713468

C,0,-3.2797222705,2.4916614569,2.073436636

H,0,-3.6340934991,2.4856841663,3.1077434955

C,0,-2.4773000732,3.7483573444,1.7936136479

H,0,-1.8231472287,3.9211989282,2.652330615

H,0,-3.1749701077,4.5910453182,1.727930489

O,0,-1.6426595862,3.6389000199,0.6575403854

H,0,-2.1671059268,3.4124293117,-0.152310421

C,0,-2.593462454,1.2050543122,1.62979833

H,0,-2.5474592607,1.2143623396,0.537646948

C,0,-3.4074468103,0.0002490354,2.0866808957

H,0,-3.2216817459,-0.1443560024,3.1593663972

O,0,-3.0628616859,-1.1696578796,1.3686525806

H,0,-2.100430873,-1.2972933156,1.4772687942

C,0,-4.9313769939,0.253394098,1.9773821629

H,0,-5.3796910617,-0.5738981084,1.4111529423

O,0,-5.4276916845,0.3281883313,3.2891147473
H,0,-6.2229520597,0.9085940955,3.2921963071
O,0,-1.2687016558,1.1709945815,2.1299801126
C,0,-0.3310500235,0.7220892692,1.213728447
H,0,-0.6461328831,0.9751725246,0.1851342849
O,0,-0.2654979345,-0.6988137174,1.3202322866
C,0,0.5845899329,-1.2952320776,0.3394708673
H,0,0.216812829,-1.0288488835,-0.6663588317
C,0,0.45145905,-2.796569817,0.5316350464
H,0,1.1394678697,-3.3135686465,-0.1421532855
H,0,-0.5778765672,-3.0896277906,0.2821951851
O,0,0.7956803492,-3.186419218,1.8469727908
H,0,0.2776551297,-2.6392991441,2.4575545276
C,0,2.0032988464,-0.7784416684,0.5646436448
H,0,2.367155246,-1.1524111394,1.5278993432
C,0,2.0517890822,0.748142502,0.5634806563
H,0,1.808686429,1.1059236354,-0.4562415689
O,0,3.3373381839,1.191506045,0.9347752384
H,0,3.9757994338,0.6538471197,0.4398482776
C,0,1.0189656792,1.365257552,1.5041527229
H,0,1.3000120477,1.1373687052,2.5436240991
O,0,0.9676787393,2.7528444927,1.3080837623
H,0,0.0557311952,3.0463666602,1.0931669716
O,0,2.9117402292,-1.2881203169,-0.405744581
O,0,-6.9165195503,2.3256147912,2.6382620626
H,0,-7.7202924915,2.6499670106,2.2118071195
H,0,-6.2946565292,3.182205239,2.9937229613
H,0,-7.6695272728,5.2988591063,3.4860631082
H,0,-2.1083359799,6.1734104263,-4.1680615852
C,-1,-1.6147859509,4.2971353441,-5.1175160802
H,0,-1.994994408,3.2723894659,-5.1676629372
H,0,-1.6186471724,4.7036177152,-6.1342439816
H,0,-0.5752223913,4.2656215156,-4.7684534364
C,-1,-7.3713713991,6.4742448159,5.2855688602

H,0,-7.0511400668,7.3837505561,4.7700141253

H,0,-8.4372163499,6.5614369754,5.525474397

H,0,-6.8045257801,6.4198838903,6.2192034614

H,0,2.6050177021,-1.0128942652,-1.2846572932

The only imaginary frequency is –147.0 cm^{-1} with a 262.7 KM/Mole IR intensity.

HF = -3170.3009125 (A.U.)

Zero-point correction = 0.949857 (A.U.)

Total Gibbs free energy = -3169.460252 (A.U.)

Model **Product**

Cartesian Coordinates (Angstroms)

Charge = -1 Multiplicity = 1

C,0,-2.9813975148,4.7529832869,6.1978826006

H,0,-3.3616061477,3.8363267275,5.7305967598

C,0,-1.8502970337,4.3441377864,7.1250471858

O,0,-1.8728227095,4.5084530333,8.3199022394

O,0,-0.7931776128,3.7643435215,6.5363650456

C,0,1.2766903756,4.7090960151,-2.963858025

H,0,0.3783817006,5.03692819,-3.4962269437

C,0,1.0129941908,4.7359998722,-1.4586739564

H,0,1.8889070415,4.38312639,-0.8998878609

H,0,0.8422906743,5.7663860781,-1.1177281136

C,0,-0.2144499942,3.9219889845,-1.0426376045

O,0,-1.1317068416,3.7662244492,-1.8716329399

O,0,-0.217566534,3.4414889493,0.1455101452

H,0,-0.9499410137,1.956426939,0.5912736431

H,0,0.2776861842,-0.4311331697,-9.6309792605

O,0,-0.134334454,0.4393736082,-9.504383024

C,0,-0.9198002106,0.3484637968,-8.3572303158

H,0,-1.9111219946,-0.0781470879,-8.60018786

O,0,-1.0842501044,1.659308813,-7.8820888376

C,0,-1.9353929009,1.7444623858,-6.7423506525
H,0,-2.9514888899,1.4036056827,-7.0003561389
C,0,-1.9673017259,3.2152226057,-6.3529279926
H,0,-2.5368048711,3.3363228775,-5.4266008589
H,0,-2.4573108871,3.7875357709,-7.152841339
O,0,-0.660220777,3.6985778072,-6.1134290553
H,0,-0.1385393506,3.5091182349,-6.9095547974
C,0,-1.4062422196,0.8811514078,-5.5911181238
H,0,-0.4792542251,1.3308532809,-5.2057985713
C,0,-1.1212211168,-0.5417249098,-6.049467744
H,0,-2.0810765866,-1.0201756449,-6.3103526735
O,0,-0.4428320369,-1.3181989388,-5.0854794009
H,0,-0.7448742501,-1.0501728816,-4.1949736311
C,0,-0.2438316521,-0.4919202836,-7.2838241892
H,0,0.7127870452,-0.0086231677,-7.0300349876
O,0,-0.0160032071,-1.7776890591,-7.8297639594
H,0,0.258865193,-2.3556708861,-7.0997147509
O,0,-2.4055497328,0.8659586425,-4.5835863071
C,0,-1.9713567881,1.2345080161,-3.3157183986
H,0,-1.2863388572,2.092568779,-3.3722123274
O,0,-1.2758485866,0.1175045748,-2.7709185833
C,0,-0.7036434842,0.4450270122,-1.5100153982
H,0,-0.1416849478,1.3870936395,-1.5859716053
C,0,0.2677378737,-0.6704860266,-1.1486879612
H,0,0.9624740838,-0.8173536814,-1.9846889402
H,0,-0.2902352492,-1.6098653431,-1.0047728177
O,0,1.0439294606,-0.3548920486,-0.0167814734
H,0,0.4519432135,-0.1032906999,0.7198053988
C,0,-1.8292181813,0.6032521123,-0.4832783492
H,0,-2.260639316,-0.395240909,-0.3092211839
C,0,-2.9458891475,1.5532218972,-0.9485221362
H,0,-2.6515484221,2.5583920267,-0.642733576
O,0,-4.1532019688,1.2936636867,-0.24456817
H,0,-4.4758645285,0.4167775546,-0.5004884908

C,0,-3.1922392029,1.602697677,-2.4728851119
H,0,-3.9821671272,0.8844621797,-2.7327607515
O,0,-3.6550283432,2.873718225,-2.8698889191
H,0,-2.9747073031,3.5075327309,-2.5665412676
O,0,-1.2920549044,1.034702079,0.753220443
C,0,-0.9077845272,1.8118947542,3.8540013944
H,0,-1.1414691035,1.5137667641,2.8272568544
O,0,-0.4605108102,3.1758478435,3.8708446828
C,0,0.962850009,3.3813955616,3.7563842674
H,0,1.3961490585,3.3166109628,4.764754684
C,0,1.2209198905,4.7779491114,3.1972963614
H,0,2.1795860058,5.1300221677,3.5905837953
H,0,0.4323158915,5.4438414169,3.5785243178
O,0,1.3250863193,4.8650058899,1.7955418562
H,0,0.6280016664,4.3587990639,1.3005308441
C,0,1.5227896804,2.2690861487,2.8754282636
H,0,0.9212561101,2.2247885593,1.9543856927
C,0,1.4198751842,0.9241850412,3.5949662734
H,0,2.2701768285,0.8340433365,4.2859532333
O,0,1.3866507188,-0.1500355606,2.6783802251
H,0,2.1383854059,-0.0505175685,2.0617506433
C,0,0.1391239617,0.8747437469,4.4419379885
H,0,-0.262639845,-0.1465988037,4.4046892019
O,0,0.4861790837,1.2494597997,5.7588093026
H,0,-0.2924503685,1.5859821749,6.2334701386
O,0,2.8637698698,2.5828847277,2.537296815
C,0,3.2122087356,2.3358064924,1.2208334738
H,0,2.3471128652,2.5061361414,0.5519696636
O,0,3.59173201,0.9663215291,1.1206888379
C,0,3.9280271327,0.5689210798,-0.2066950067
H,0,3.0566985754,0.7313895894,-0.8654186084
C,0,4.2322603223,-0.9190699769,-0.1293464446
H,0,4.5976107958,-1.2685471726,-1.098654386
H,0,3.3067395954,-1.4511521822,0.1241966864

O,0,5.2554212229,-1.184897511,0.8127059493

H,0,4.9850130446,-0.7755603023,1.6495412983

C,0,5.1215615601,1.3962081643,-0.6797514246

H,0,5.9998157947,1.1249536757,-0.0836829986

C,0,4.859983187,2.8928788661,-0.5431042141

H,0,4.0597771957,3.1770478466,-1.2533273615

O,0,6.0345614611,3.6198026205,-0.8298522896

H,0,6.4109639463,3.2356058342,-1.637641091

C,0,4.3641683812,3.2642647175,0.848387342

H,0,5.1786689181,3.1063836233,1.5728077664

O,0,3.9523843796,4.6060563731,0.8595901045

H,0,3.0458863796,4.6857859313,1.2304702676

O,0,5.4697947784,1.0924892599,-2.0269111338

O,0,-2.0593589489,1.7626806229,4.6516117623

H,0,-2.8275196036,1.9535696476,4.094678

H,0,-0.8971307216,3.6752748902,5.5591741427

H,0,-2.5638075805,5.357313021,5.3822814852

H,0,2.079107941,5.4202306416,-3.1962690767

C,-1,1.6691075339,3.3200687804,-3.4671227582

H,0,0.8656995217,2.5996494665,-3.2753920735

H,0,1.8582383715,3.3222240385,-4.5452489995

H,0,2.5734719502,2.9594162194,-2.9579877848

C,-1,-4.0874779275,5.4971782571,6.9359622061

H,0,-3.6987059117,6.4092347868,7.3963250608

H,0,-4.8931487284,5.7683971558,6.2476186258

H,0,-4.500199992,4.8766732803,7.7351678158

H,0,4.7002076651,1.2774165676,-2.5900825598

HF = -3170.3652496 (A.U.)

Zero-point correction = 0.954653 (A.U.)

Total Gibbs free energy = -3169.516582 (A.U.)