

“Amide Resonance” in the Catalysis of 1, 2- α -L-Fucosidase from *Bifidobacterium bifidum*

Jingli Liu¹, Min Zheng¹, Chunchun Zhang^{2*} and Dingguo Xu^{1*}

¹Key Laboratory of Green Chemistry and Technology, Ministry of Education, College of Chemistry, Chengdu, Sichuan 610064, P. R. China, ²Testing & Analytical Center, Sichuan University, Chengdu, Sichuan, 610064, P. R. China.

Supporting Information:

1. Full citation of Ref. 36 and 48.
2. Selected geometric parameters for stationary points for the hydrolysis of 2'-Fucosyllactose catalyzed by N423G AfcA at the SCC-DFTB/MM level of theory.
3. Snapshot for the stationary states along the reaction coordinate for the hydrolysis of 2'-Fucosyllactose molecule catalyzed by N423G AfcA from *B. bifidum* calculated using SCC-DFTB/MM approach.
4. Minimum energy path scan of the truncated active site model calculated at the SCC-DFTB level of theory, and energetic profile at B3LYP/6-31+G(d,p) level of theory.
5. Cartesian coordinates of stationary states for truncated active site model optimized by DFT.

Ref.36 MacKerell Jr., A. D.; Bashford, D.; Bellott, M.; Dunbrack Jr., R. L.; Evanseck, J. D.; Field, M. J.; Fischer, S.; Gao, J.; Guo, H.; Ha, S.; Joseph-McCarthy, D.; Kuchnir, L.; Kuczera, K.; Lau, F. T. K.; Mattos, C.; Michnick, S.; Ngo, T.; Nguyen, D. T.; Prodhom, B.; Reiher III, W. E.; Roux, B.; Schlenkrich, M.; Smith, J. C.; Stote, R.; Straub, J.; Watanabe, M.; Wiorkiewicz-Kuczera, J.; Yin, D.; Karplus, M. *J. Phys. Chem.* **1998**, *B102*, 3586-3616.

Ref.48 Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Montgomery, J. A., Jr., T. V.; Kudin, K. N.; Burant, J. C.; Millam, J. M.; Iyengar, S. S.; Tomasi, J.; Barone, V.; Mennucci, B.; Cossi, M.; Scalmani, G.; Rega, N.; Petersson, G. A.; Nakatsuji, H.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Klene, M.; Li, X.; Knox, J. E.; Hratchian, H. P.; Cross, J. B.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Ayala, P. Y.; Morokuma, K.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Zakrzewski, V. G.; Dapprich, S.; Daniels, A. D.; Strain, M. C.; Farkas, O.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Ortiz, J. V.; Cui, Q.; Baboul, A. G.; Clifford, S.; Cioslowski, J.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Challacombe, M.; Gill, P. M. W.; Johnson, B.; Chen, W.; Wong, M.

W.; Gonzalez, C.; Pople, J. A.; Gaussian, Inc.: Pittsburgh, PA, 2003.

TableS1 Selected geometric parameters for stationary points for the hydrolysis of 2'-Fucosyllactose catalyzed by N423G AfcA at the SCC-DFTB/MM level of theory.

Distances (Å) Angles(degree)	SCC-DFTB/CHARMM		
	ES	TS	EP
C1(Fuc)···O _w	2.95	1.91	1.43
C1(Fuc)···O5(Fuc)	1.43	1.32	1.46
C1(Fuc)···O1(Fuc)	1.50	2.26	3.21
H _{ε2} (E566)···O1(Fuc)	1.82	1.07	0.99
H _w ···O _w)	0.98	1.06	1.78
H _w ···O _{δ1} (N421)	1.83	1.46	1.01
H _{ε2} (E566)··· O _{ε2} (E566)	1.00	1.46	1.86
C _γ (N421)···N _{δ2} (N421)	1.36	1.35	1.32
C _γ (N421)···O _{δ1} (N421)	1.25	1.27	1.30
N _{ε2} (H678)···HO2(Fuc)	1.89	1.97	1.84
N _{ε2} (H419)···HO3(Gal)	1.84	1.94	2.18
H _{ε1} (W414)···O4(Glc)	1.95	1.95	2.18
C1(Fuc)···O1(Fuc)···C2(Gal)	116.59	-	-
O1(Fuc)···C1(Fuc)···O _w	152.46	-	-
τ ₁	64.68	53.67	58.31
τ ₂	-61.71	-68.88	-53.98
τ ₃	51.95	51.70	56.93
τ ₄	-47.52	-21.48	-64.00
τ ₅	50.27	8.24	67.78
τ ₆	-58.29	-25.23	-65.47
O5(Fuc)···C1(Fuc)···C2(Fuc)···H1(Fuc)	119.07	-163.02	-120.57

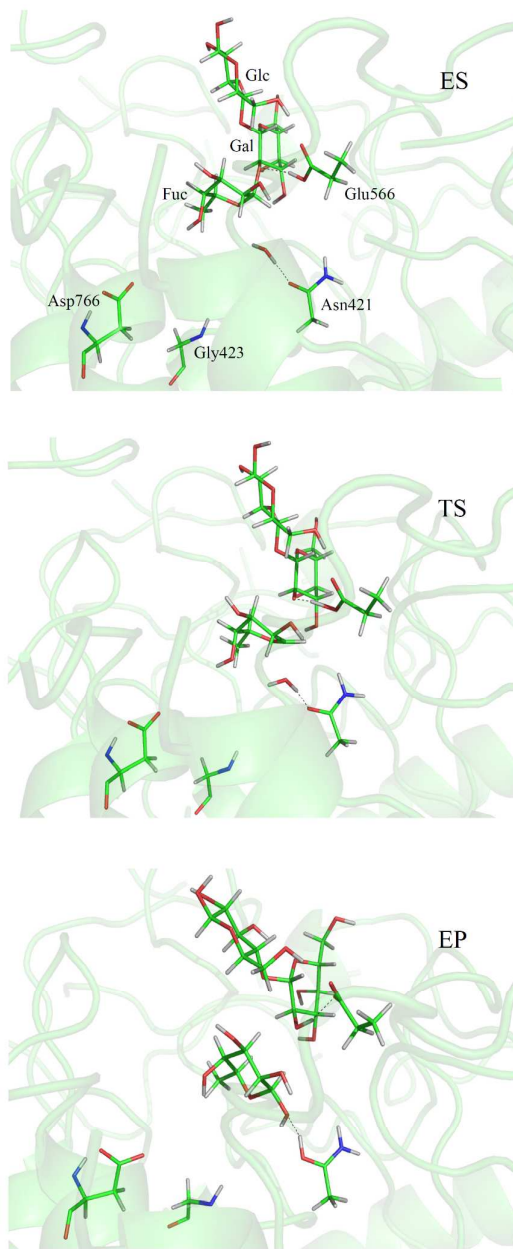


Figure S1. Snapshot for the stationary states along the reaction coordinate for the hydrolysis of 2'-Fucosyllactose molecule catalyzed by N423G AfcA from *B. bifidum* calculated using SCC-DFTB/MM approach.

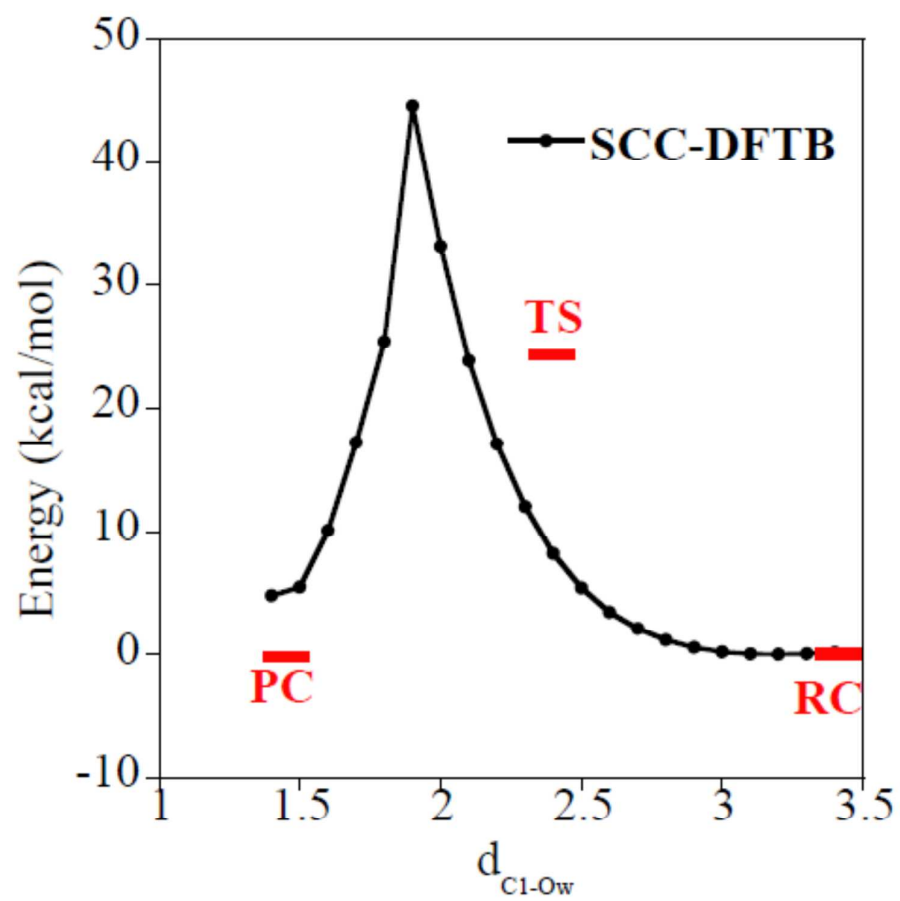
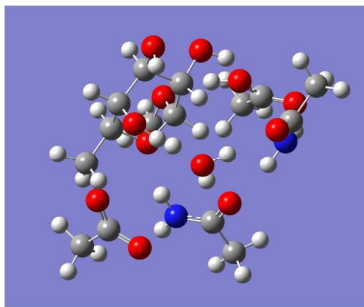


Figure S2. Minimum energy path scan of the truncated active site model calculated at the SCC-DFTB level of theory (black dot line), and energetic profile (red bar) at B3LYP/6-31+G(d,p) level of theory.

Cartesian coordinates for stationary states of the hydrolysis of 2'fucosyllactose calculated at the B3LYP/6-31+G(d,p) level of theory.

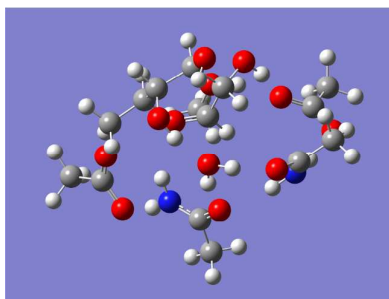
A. RC



C	-0.16910500	-0.13225900	0.09600700
H	-0.11402600	-0.10448900	1.18876000
H	0.85232800	-0.24016700	-0.28003000
C	-0.73052100	1.19464200	-0.39235400
O	-0.11907300	2.25071100	-0.17316500
N	-1.90929600	1.14289400	-1.04931900
H	-2.32876000	1.99654300	-1.42859900
H	-2.31545200	0.25103900	-1.30468400
C	-5.33601000	3.67957400	-2.91835600
H	-5.55205500	2.71456000	-2.44884600
H	-5.98510000	3.82512200	-3.78481900
H	-5.53819900	4.45587100	-2.17310200
C	-3.86489600	3.73386500	-3.28859500
O	-2.98914900	3.51289200	-2.40807600
N	-3.57652700	4.02449700	-4.55092200
H	-2.59883800	4.02576500	-4.82943500
H	-4.29826400	4.12494200	-5.31399700
C	-3.50875100	-1.56428600	-4.93698200
H	-2.93985500	-2.04082400	-5.74105800
H	-3.88149200	-0.60631100	-5.31409400
C	-2.64286300	-1.33198000	-3.71789100
O	-1.42391000	-0.80490200	-3.92523200
O	-2.99141200	-1.59838000	-2.58212000
H	-1.25640200	-0.39701900	-4.81307200
C	-5.57521700	4.40476400	-8.95047900
H	-5.85214700	5.46456900	-9.01232400
H	-6.50540500	3.82931700	-8.89488900
C	-4.72869700	4.18416900	-7.68248400
O	-3.48889900	4.02294100	-7.81829500
O	-5.37153900	4.20449100	-6.58029200
H	-5.01500100	4.13510700	-9.84994900
C	-2.00079500	1.38541900	-6.58800100
H	-1.94388900	2.34022600	-7.11214600

C	-0.55749300	2.01242800	-4.76848000
H	-1.33521600	1.87672900	-4.00678000
O	-0.80346600	1.08347100	-5.83785100
C	0.30106500	3.88752200	-6.04483300
H	0.38502500	3.24113300	-6.93133700
O	-0.71617000	3.34554700	-5.17372800
C	0.84175400	1.74356300	-4.14592400
H	0.80791800	2.21014700	-3.15382700
O	1.13677800	0.35740700	-4.03083600
H	0.51357500	-0.04945500	-3.41343700
C	1.98855200	2.39914600	-4.92894200
H	2.16968900	1.81292900	-5.83737300
O	3.19984800	2.37848700	-4.18272100
H	3.16152100	3.16437000	-3.61156200
C	1.63890000	3.85236200	-5.30463300
H	2.42357300	4.22651500	-5.97461500
O	1.68976500	4.69017800	-4.15669200
H	0.93255900	4.53095100	-3.54207700
C	-0.17385700	5.26878500	-6.46625400
H	-0.25210900	5.92824100	-5.59771600
H	-1.14854400	5.19342600	-6.95818100
H	0.54442500	5.70796200	-7.16751900
O	-0.25828700	4.35145200	-2.15232700
H	-0.10953300	3.76864600	-1.38221100
H	-1.20276800	4.20768000	-2.36833500
H	-2.11468400	0.57072900	-7.30695000
H	-2.87522900	1.43452000	-5.92676200
H	-4.35853100	-2.18760900	-4.65871900
H	-0.76533900	-0.99448100	-0.21358300

B. TS

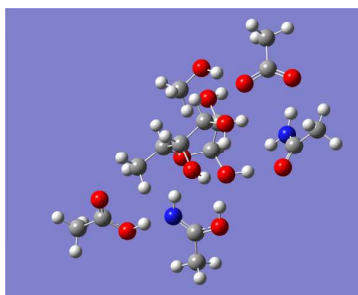


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H	0.00000000	0.00000000	1.09569000
H	1.04221600	0.00000000	-0.32975200
C	-0.67070100	1.27339700	-0.47800100
O	-0.09283800	2.37715900	-0.34299000

N	-1.88877100	1.13602400	-1.01991100
H	-2.38661900	1.96131100	-1.34675800
H	-2.20711700	0.20336300	-1.33484800
C	-5.55743000	4.02978300	-2.69786700
H	-5.82113700	3.01522800	-2.38293300
H	-6.25699100	4.36912900	-3.46465100
H	-5.63078100	4.67513300	-1.81705000
C	-4.12386400	4.03111100	-3.18805300
O	-3.20210300	3.66788000	-2.40381500
N	-3.91550200	4.41865200	-4.43756900
H	-2.97742400	4.38402600	-4.81667300
H	-4.65444200	4.69854400	-5.14542700
C	-2.85102200	-2.76314700	-3.99347800
H	-2.03847400	-3.26964500	-4.52544300
H	-3.58329700	-2.45138000	-4.74677200
C	-2.30450300	-1.53008300	-3.26981200
O	-1.69847700	-0.66802600	-3.99999000
O	-2.48671200	-1.43212100	-2.03287100
H	-1.89401300	0.03806300	-5.64269800
C	-5.34732200	5.64118000	-8.76849600
H	-5.83719100	6.61488500	-8.65383800
H	-6.12368500	4.92315600	-9.05679900
C	-4.72512100	5.21490200	-7.42836100
O	-3.48969900	4.97194300	-7.39229600
O	-5.53350800	5.13911500	-6.44418100
H	-4.59085100	5.69866700	-9.55525500
C	-3.18239900	1.20177400	-6.59400400
H	-3.12150600	2.18067900	-7.07665500
C	-0.67148400	2.34714700	-4.41861400
H	-1.54810900	1.90049700	-3.95786900
O	-1.85836300	0.75617500	-6.31158500
C	0.17229800	3.96418300	-6.00770900
H	0.26187500	3.33049000	-6.89638400
O	-0.91561200	3.29558700	-5.22419600
C	0.69132900	1.72790500	-4.15148500
H	0.82898000	1.79965200	-3.06428500
O	0.68328800	0.38741900	-4.58628000
H	-0.10914800	-0.08976400	-4.22547900
C	1.84844700	2.47310500	-4.85053200
H	2.06417300	1.91614900	-5.76869500
O	3.03350200	2.45842500	-4.07271800
H	2.97570800	3.22393500	-3.47687600
C	1.47667500	3.92476500	-5.21240800
H	2.25916900	4.31257300	-5.87591800

O	1.49243200	4.76461700	-4.07064700
H	0.73558100	4.55463200	-3.46987300
C	-0.35741200	5.33651000	-6.36674600
H	-0.41430200	5.97381800	-5.48068300
H	-1.34547100	5.26245600	-6.83382300
H	0.33778200	5.80070800	-7.07480900
O	-0.55132000	3.84259200	-2.53228600
H	-0.34411600	3.41967900	-1.65338500
H	-1.53994500	3.96089800	-2.52888800
H	-3.70177800	0.50540100	-7.26951900
H	-3.78157200	1.31053100	-5.67964500
H	-3.32276500	-3.45300000	-3.29073900
H	-0.51045800	-0.89533800	-0.36148500

C. PC



C	0.23704800	0.01310400	0.33018000
H	0.08475900	0.17245400	1.40270700
H	1.23946500	0.37290900	0.08134500
C	-0.77812900	0.84101000	-0.42961300
O	-0.76024000	2.09258100	-0.34216000
N	-1.66863400	0.16853100	-1.16578200
H	-2.32705000	0.71274400	-1.70807000
H	-1.50871100	-0.81751500	-1.49286700
C	-4.96998400	6.55949800	-2.44752600
H	-5.60814000	5.95222000	-1.79714300
H	-5.56173000	6.96779100	-3.26689200
H	-4.56726000	7.37543100	-1.83822600
C	-3.84077600	5.71804000	-2.97618100
O	-3.09870600	5.20625800	-1.98189200
N	-3.66284200	5.54894200	-4.23439000
H	-2.85982500	4.96183200	-4.47741500
H	-4.54639300	6.18820800	-5.40392500
C	-1.32463900	-3.53704400	-4.26860600
H	-0.49037900	-3.47052800	-4.97599900
H	-2.23409900	-3.67390400	-4.86251700
C	-1.41101200	-2.23616200	-3.46991500

O	-1.68314800	-1.17264700	-4.12488500
O	-1.21269100	-2.28913300	-2.22566400
H	-2.54110000	-1.36189700	-5.68037400
C	-5.71665400	7.38305200	-8.24982000
H	-6.09635700	8.32999400	-7.85284500
H	-6.57305400	6.70937600	-8.35933700
C	-4.71261600	6.77824800	-7.28533500
O	-3.57382000	6.47472300	-7.61158400
O	-5.21992900	6.61899900	-6.06782800
H	-5.24797200	7.54451500	-9.22116600
C	-3.34977600	-0.11846300	-6.96274700
H	-3.80528500	-0.19405600	-7.95665100
C	-1.07200800	2.76862500	-3.72955300
H	-1.98994400	2.16954300	-3.70217400
O	-3.03870200	-1.42919100	-6.52877700
C	-0.05871300	4.06515800	-5.55510500
H	0.07221600	3.31050300	-6.34346000
O	-1.23116200	3.67913000	-4.78685000
C	0.14679200	1.83259000	-3.87776300
H	0.29324300	1.31003200	-2.92621600
O	-0.09418700	0.91713900	-4.93080800
H	-0.65408200	0.17075500	-4.60653300
C	1.46231800	2.59734600	-4.19768000
H	1.92591600	2.03000300	-5.01146400
O	2.37884100	2.60081400	-3.11011000
H	2.28286300	3.46618500	-2.67831900
C	1.20412200	4.04113900	-4.68670000
H	2.05220600	4.37017500	-5.29696000
O	1.16136300	4.95732400	-3.58889700
H	0.37709900	4.73337900	-3.04785200
C	-0.35962000	5.42033900	-6.17585300
H	-0.40475800	6.19622800	-5.40659500
H	-1.31099700	5.40522600	-6.71401500
H	0.43382100	5.68357200	-6.88467100
O	-0.99730600	3.56891600	-2.52637900
H	-0.92823500	2.99680700	-1.70063000
H	-2.34638400	4.63160600	-2.29008700
H	-4.07043300	0.38279300	-6.29652200
H	-2.45662500	0.51838200	-7.03902700
H	-1.17515600	-4.39252500	-3.60655400
H	0.15820100	-1.05191300	0.10243700

