Electronic supplementary information for:

How inverting β-1,4-galactosyltransferase-1 can quench a high charge of by-product UDP^{3-} in catalysis: QM/MM study of enzymatic reaction with native and UDP-5' thio galactose substrate

J. Kóňa

[a] Institute of Chemistry, Center for Glycomics, Slovak Academy of Sciences, Dúbravská cesta 9, 84538 Bratislava, Slovak Republic,

Table S1. Overview of inverting GTs of which catalytic mechanisms were proposed based on DFT calculations (References are numbered according to the Reference section in the article).

inverting GT	**predicted mechanism**	**model, approach and DFT method used**
GnT-I (EC 2.4.1.101)	stepwise S_Ni-like via oxocarbenium ion intermediate[1]	organic model static QM calculations[1] B3LYP
GnT-I	concerted S_N2-like[2]	active-site model static QM calculations[2] B3LYP
GnT-I	concerted S_N2-like[3]	enzyme model static QM/MM calculations[3] BP86
β4Gal-T1 (EC 2.4.1.38)	concerted S_N2-like[4]	enzyme model static QM/MM calculations[4] BP86
β4Gal-T1	stepwise S_Ni-like via oxocarbenium ion intermediate[a]	enzyme model static QM/MM calculations[a] M06-2X, PWB6K
OGT (EC 2.4.1.255)	concerted S_N2-like[5]	enzyme model static QM/MM calculations[5] B3LYP[b]
OGT	concerted S_N2-like[6]	enzyme model QM/MM MD simulations[6] PBE
C2GnT (EC 2.4.1.102)	concerted S_N2-like[7]	enzyme model static QM/MM calculations[7] B3LYP[b]
GlfT2 (EC 2.4.1.288)	concerted S_N2-like[8]	enzyme model QM/MM MD simulations[8] PBE
POFUT1 (EC 2.4.1.221)	S_N1-like via ion-pair TS[9]	organic model static QM calculations[9] B3LYP

[a] This work.
[b] Also the MPW1K and M06-2X functional were used on optimized B3LYP geometries (the MPW1K//B3LYP and M06-2X//B3LYP approaches).

Table S2. Conformations of the mannose ring of the donor substrate (UDP-Gal) according to the Cremer-Pople parameters, and selected interatomic distances (d) (in Å) and valence (φ) and dihedral angles (ϕ) for transition states and intermediates optimized at the DFT-M06-2X/OPLS2005 level.

UDP-Gal	**1**	**TS12**	**2**	**TS23**	**3**	**TS34**	**4**
Ring conformation	$^4C_1$	$^4H_3$	$^4H_3$	$^4H_3$	$^4H_3$	^4E	$^4H_3$
d($C1_D$-$O1_D$)	1.475	2.349	2.675	2.872	3.534	3.484	3.535
d($C1_D$-$O4_A$)	3.186	2.577	2.426	2.304	1.445	1.455	1.440
d($O4_A$-$H4_A$)	0.994	1.009	1.019	1.030	1.650	1.736	1.700
d($H4_A$-O_{Asp318})	1.716	1.614	1.563	1.525	1.001	0.994	0.994
d($O5_D$-$C1_D$)	1.374	1.262	1.258	1.261	1.386	1.390	1.388
d($O5_D$-$O4_A$)	3.073	2.922	2.885	2.829	2.335	2.345	2.349
d($O1_D$-$P1_D$)	1.604	1.532	1.530	1.531	1.530	1.567	1.589
d($O1_D$-$H2_D$)	2.321	1.683	1.581	1.540	1.599	1.075	0.991
d($O2_D$-$H2_D$)	0.970	0.985	0.997	1.003	0.99	1.376	1.682
d($O2_D$-H_{Asp252})	2.576	2.558	2.639	2.708	3.118	1.272	0.983
d(O_{Asp252}-H_{Asp252})	1.025	1.007	1.004	1.005	1.010	1.225	2.086
φ($O1_D$-$C1_D$-$O4_A$)	164.5	160.5	160.8	160.3	163.4	168.4	170.4
φ($O5_D$-$C1_D$-$C2_D$)	114.3	124.3	125.0	124.7	117.2	116.2	116.0
φ($O5_D$-$C1_D$-$H1_D$)	106.9	115.6	115.6	115.4	105.3	105.1	104.8
φ(O_{Asp252}-H_{Asp252}-$O2_D$)	102.3	112.9	115.6	116.8	122.3	163.6	151.6
φ($O2_D$-$H2_D$-$O1_D$)	109.0	142.3	153.4	158.9	166.0	161.9	157.3
ϕ($O5_D$-$C2_D$-$C1_D$-$O1_D$)	-122.6	-100.2	-92.3	-88.1	-68.2	-65.8	-62.6
ϕ($O5_D$-$C2_D$-$C1_D$-$O4_D$)	69.7	99.3	107.1	111.5	126.0	125.6	126.5
ϕ($C2_D$-$C1_D$-$O5_D$-$C5_D$)	-45.0	-12.5	-3.5	1.2	-3.3	29.4	30.9
ϕ($C3_D$-$C2_D$-$C1_D$-$O5_D$)	53.1	26.4	19.1	15.2	-42.8	-19.1	-13.4

Table S3. Conformations of the mannose ring of the thio donor substrate (UDP-5S-Gal) according to the Cremer-Pople parameters, and selected interatomic distances (d) (in Å) and valence (φ) and dihedral angles (ϕ) for transition states and intermediates optimized at the DFT-M06-2X/OPLS2005 level.

UDP-5S-Gal	**1**	**TS12**	**2**	**TS23**	**3**	**TS34**	**4**
Ring conformation	$^4C_1$	$^4H_3/^4C_1$	$^4H_3$	$^4H_3$	$^1S_3$	$^{1,4}B/^1S_3$	$^1S_3$
d($C1_D$-$O1_D$)	1.463	2.334	2.695	3.053	3.543	3.486	3.717
d($C1_D$-$O4_A$)	3.359	2.794	2.646	2.266	1.444	1.445	1.431
d($O4_A$-$H4_A$)	0.995	1.005	1.011	1.041	1.574	1.717	1.641
d($H4_A$-O_{Asp318})	1.688	1.620	1.585	1.472	1.007	0.989	0.996
d($S5_D$-$C1_D$)	1.788	1.631	1.619	1.632	1.794	1.798	1.805
d($S5_D$-$O4_A$)	2.990	2.948	2.996	3.027	2.724	2.737	2.736
d($O1_D$-$P1_D$)	1.607	1.529	1.526	1.528	1.528	1.537	1.599
d($O1_D$-$H2_D$)	2.364	1.705	1.623	1.568	1.766	1.320	0.976
d($O2_D$-$H2_D$)	0.970	0.983	0.992	1.002	0.986	1.101	2.308
d($O2_D$-H_{Asp252})	2.504	2.353	2.381	2.591	2.948	1.261	0.978
d(O_{Asp252}-H_{Asp252})	1.029	1.006	1.001	1.002	1.007	1.192	2.174
φ($O1_D$-$C1_D$-$O4_A$)	167.1	160.7	162.9	166.8	171.2	169.5	173.1
φ($S5_D$-$C1_D$-$C2_D$)	112.9	127.3	128.8	127.7	115.8	113.5	112.9
φ($S5_D$-$C1_D$-$H1_D$)	105.0	113.5	113.6	113.3	102.3	102.9	101.8
φ(O_{Asp252}-H_{Asp252}-$O2_D$)	100.3	111.9	115.2	116.7	123.6	162.1	158.9
φ($O2_D$-$H2_D$-$O1_D$)	99.0	142.1	153.9	165.2	162.8	165.2	121.8
ϕ($S5_D$-$C2_D$-$C1_D$-$O1_D$)	-127.2	-107.2	-94.1	-76.2	-57.1	-60.6	-52.0
ϕ($S5_D$-$C2_D$-$C1_D$-$O4_D$)	58.6	88.2	100.7	117.5	131.4	130.3	131.1
ϕ($C2_D$-$C1_D$-$S5_D$-$C5_D$)	-50.2	-19.4	-7.2	12.2	38.3	40.8	44.3
ϕ($C3_D$-$C2_D$-$C1_D$-$S5_D$)	60.1	31.5	20.0	4.1	-13.4	-32.0	-24.1

Table S4 Total DFT-QM/MM(OPLS2005) energies of optimized intermediates and transition states for the reactions with the native (UDP-Gal) and thio donor substrate (UDP-5S-Gal) in the presence of the Mn^{2+} ion co-factor (in a.u.)

UDP-Gal	M06-2X	PWB6K	B3LYP	MPW1K	PBE0	BP86
1	-5400.276456	-5404.555738	-5402.008746	-5400.759478	-5396.984152	-5402.099216
TS12	-5400.258326	-5404.537699	-- [a]	-- [a]	-- [a]	-5402.092915
2	-5400.259380	-5404.537418	-5401.994392	-5400.732979	-5396.966606	-- [a]
TS23	-5400.259197	-5404.536635	-- [a]	-- [a]	-- [a]	-- [a]
3	-5400.302679	-5404.581962	-5402.033096	-5400.784809	-5397.008407	-5402.123608
TS34	-5400.278814	-5404.549505	-5402.000212	-5400.748348	-5396.977003	-5402.10103
4	-5400.304551	-5404.580419	-5402.027825	-5400.776896	-5397.004206	-5402.117605
UDP-5S-Gal						
1	-5723.234154	-5727.702357	-5724.970781	-5723.753962	-5719.870860	-5725.088499
TS12	-5723.211645	-5727.675543	-5724.955248	-5723.728470	-5719.851593	-5725.079374
2	-5723.214528	-5727.677501	-5724.958325	-5723.729731	-5719.853529	-- [a]
TS23	-5723.211994	-5727.673470	-5724.958183	-5723.729566	-5719.854076	-- [a]
3	-5723.254254	-5727.721855	-5724.990277	-5723.773698	-5719.891664	-5725.118236
TS34	-5723.239344	-5727.694602	-5724.960869	-5723.745365	-5719.866202	-5725.091156
4	-5723.263147	-5727.729551	-5724.994378	-5723.778577	-5719.897701	-5725.113412

[a] a stationary point along a reaction coordinate of catalysis of β4Gal-T1 was not possible to localized by the DFT method.

Table S5 Total DFT-QM/MM(OPLS2005) energies of optimized intermediates and transition states for the reactions with the native (UDP-Gal) and thio donor substrate (UDP-5S-Gal) in the presence of Mg^{2+} ion co-factor (in a.u.)

UDP-Gal	M06-2X	PWB6K	MP2//M06-2X	MP2//PWB6K
1	-5496.44190	-5500.87992645	-5484.042417	-5484.025025
TS12	-5496.42436	-5500.86092260	-5484.014041	-5483.998870
2	-5496.42567	-5500.86120963	-5484.014806	-5483.999427
TS23	-5496.42542	-5500.86018761	-5484.009997	-5483.997980
3	-5496.46900	-5500.90601615	-5484.067505	-5484.061821
TS34	-5496.44612	-5500.87144878	-5484.031476	-5484.022829
4	-5496.46969	-5500.90306227	-5484.066752	-5484.054643
UDP-5S-Gal				
1	-5819.400719	-5824.02114362	-5806.632061	-5806.61357258
TS12	-5819.375945	-5823.99920908	-5806.608903	-5806.59238738
2	-5819.380169	-5824.00165795	-5806.613602	-5806.60229925
TS23	-5819.378491	-5823.99662084	-5806.602757	-5806.58793832
3	-5819.419021	-5824.04056309	-5806.653744	-5806.64549628
TS34	-5819.405996	-5824.01874914	-5806.628972	-5806.61705754
4	-5819.430360	-5824.05280034	-5806.671597	-5806.65626942

Table S6. Conformations of the mannose ring of the donor substrates (UDP-Gal and UDP-5S-Gal) according to the Cremer-Pople parameters (φ, ϕ and Q). Meridian (φ) and azimuthal (ϕ) angles are in degrees, and radius Q is the deviation from perfectly flat six-membered ring (Q=0) for transition states and intermediates optimized at the DFT-M06-2X/OPLS2005 level.

UDP-Gal	**1**	**TS12**	**2**	**TS23**	**3**	**TS34**	**4**
Ring conformation	$^4C_1$	$^4H_3$	$^4H_3$	$^4H_3$	$^4H_3$	^4E	$^4H_3$
φ	173.0	206.8	209.3	210.6	215.2	229.9	221.7
ϕ	17.8	41.1	47.2	50.3	66.3	73.0	71.5
Q	0.54	0.52	0.53	0.54	0.61	0.59	0.61
UDP-5S-Gal							
Ring conformation	$^4C_1$	$^4H_3/^4C_1$	$^4H_3$	$^4H_3$	$^1S_3$	$^{1,4}B/^1S_3$	$^1S_3$
φ	152.2	217.1	217.6	214.5	210.2	226.1	215.0
ϕ	2.2	33.4	44.6	60.5	80.1	84.6	84.9
Q	0.62	0.52	0.53	0.58	0.75	0.72	0.76

Table S7. Atomic ESP charges (q) of selected atoms in the transition states and intermediates optimized at the DFT-M06-2X/OPLS2005 level for the reactions with both of the donor substrates (UDP-Gal and UDP-5S-Gal).

UDP-Gal	**1**	**TS13**	**2**	**TS23**	**3**	**TS34**	**4**
q(C1)	-0.18	-0.11	-0.07	0.07	-0.52	-0.21	0.06
q(O1)	-0.38	-0.66	-0.64	-0.69	-0.47	-0.59	-0.32
q(O4)	-0.99	-1.00	-0.99	-0.98	-0.31	-0.49	-0.53
q(H4)	0.58	0.64	0.65	0.66	0.47	0.48	0.49
q(O_{Asp318})	-0.87	-0.90	-0.90	-0.90	-0.72	-0.74	-0.73
q(O5)	-0.32	-0.16	-0.16	-0.15	-0.47	-0.40	-0.50
q(C2)	0.42	0.60	0.69	0.78	1.09	0.66	0.48
q(O2)	-0.73	-0.77	-0.82	-0.87	-1.03	-1.04	-0.85
q(H2)	0.40	0.36	0.39	0.45	0.55	0.44	0.05
q(O_{asp252})	-0.74	-0.73	-0.73	0.74	-0.76	-0.81	-0.80
q(H_{asp252})	0.49	0.51	0.51	0.51	0.54	0.57	0.49
q(P1)	1.21	1.08	1.05	1.04	1.03	1.15	1.06
q(Mn)	1.39	1.29	1.27	1.28	1.34	1.41	1.42
UDP-5S-Gal							
q(C1)	-0.46	-0.26	-0.25	-0.36	-0.43	-0.30	-0.09
q(O1)	-0.28	-0.69	-0.69	-0.71	-0.62	-0.68	-0.48
q(O4)	-0.94	-0.96	-0.97	-0.93	-0.31	-0.41	-0.44
q(H4)	0.55	0.61	0.64	0.70	0.50	0.54	0.49
q(O_{Asp318})	-0.84	-0.87	-0.88	-0.91	-0.75	-0.77	-0.74
q(S5)	0.14	0.27	0.33	0.28	-0.17	-0.11	-0.22
q(C2)	0.41	0.38	1.06	0.72	0.97	0.43	0.48
q(O2)	-0.72	-0.69	-0.72	-0.83	-0.95	-0.89	-0.94
q(H2)	0.40	0.32	0.36	0.45	0.52	0.42	0.26
q(O_{asp252})	-0.73	-0.71	-0.71	-0.73	-0.73	-0.83	0.81
q(H_{asp252})	0.47	0.50	0.50	0.51	0.52	0.65	0.51
q(P1)	1.21	1.10	0.41	1.06	0.82	1.05	1.05
q(Mn)	1.38	1.26	1.25	1.27	1.32	1.39	1.41

Table S8. A list of the selected iGTs with available X-ray structures, their EC family numbers, putative candidates for the general acid in catalysis of iGTs, distances (in Å) between the O_{acid} oxygen of ionizable amino acid and the O_P oxygen of the phosphate group of the bound nucleotide in iGT, angles (in degrees) among the C_{acid}, O_{acid} and O_P atoms, and catalytic activities of the mutated enzymes.

iGT	Fold	Family	PDB ID	Ionizable amino acid	$d(O_{acid}\text{-}O_P)$	$\varphi(C_{acid}\text{-}O_{acid}\text{-}O_P)$	No catalytic activity
β4Gal-T1	GT-A	EC 2.4.1.90	1O0R[10]	Asp252	3.4	144.5	D252N[11]
β4Gal-T7	GT-A	EC 2.4.1.133	3LW6[12]	Asp145	3.9	159.7	n.a.[a]
GlcAT-1	GT-A	EC 2.4.1.135	1KWS[13]	Asp194 or His308	4.1 3.3	143.8 146.4	D194A/D195A[14] H308A[14]
GlcAT-P	GT-A	EC 2.4.1.17	1V84[14]	His311	3.3	89.8	H311A[15]
GnT-1	GT-A	EC 2.4.1.101	1FOA[16]	Glu211	4.7	108.2	n.a.[a]

[a] n.a. – not available

Table S9. FMO interaction energies (in kcal mol^{-1}) between the donor substrate UDP-Gal (or UDP-5S-Gal) and selected active-site residues of the Michaelis complexes **1** in radius of 15Å calculated by the wB97X-D method. In case of the transition and the intermediate states, interaction energies between the transferred galactose ring (oxocarbenium ion) or the thiogalactose ring (thiocarbenium ion) and the enzyme residues are compiled (The most significant differences in energies between the native and thio substrates are typed in bold).

1	**1** UDP-Gal	**1** UDP-5S-Gal	**TS12** Gal^{1+}	**TS12** $5S\text{-}Gal^{1+}$	**2** Gal^{1+}	**2** $5S\text{-}Gal^{1+}$	**TS23** Gal^{1+}	**TS23** $5S\text{-}Gal^{1+}$
UDP^{3-}-Mn^{2+}-L[a]	--	--	-204.45	-205.04	**-193.52**	**-185.64**	**-188.93**	**-174.66**
Mn^{2+}-L[b]	**-577.41**	**-573.02**	--	--	--	--	--	--
GlcNAc(Accceptor)	6.48	6.65	**-17.76**	**-13.46**	**-20.34**	**-15.93**	**-22.82**	**-30.18**
Glu136	16.22	16.15	-9.36	-9.31	-9.40	-9.33	-9.42	-9.42
Glu137	22.26	22.12	-12.33	-12.32	-12.37	-12.33	-12.39	-12.43
Glu148	32.89	32.76	-14.47	-14.43	-14.41	-14.31	-14.36	-14.23
Asp154	20.81	20.68	-9.66	-9.54	-9.65	-9.51	-9.63	-9.48
Lys156	-18.28	-18.21	8.67	8.59	8.67	8.56	8.65	8.54
Glu159	20.40	20.30	-9.89	-9.82	-9.89	-9.80	-9.87	-9.77
Lys164	-18.48	-18.38	9.54	9.51	9.56	9.50	9.55	9.52
Lys166	-15.60	-15.57	8.41	8.37	8.43	8.37	8.43	8.40
Arg170	-19.64	-19.56	10.65	10.61	10.65	10.61	10.68	10.64
Asp175	16.44	16.36	-8.65	-8.61	-8.66	-8.60	-8.66	-8.63
Lys181	-15.63	-15.55	9.09	9.03	9.13	9.05	9.14	9.11
Arg189	-55.58	-55.53	21.45	21.17	21.43	21.12	21.38	20.97
Arg191	-125.77	-124.62	33.85	33.49	33.48	33.06	33.18	32.25
Glu193	28.46	28.31	-12.43	-12.31	-12.40	-12.26	-12.36	-12.18
Lys196	-26.25	-26.13	11.29	11.17	11.25	11.11	11.22	11.02
Arg208	-22.92	-22.76	11.36	11.24	11.36	11.31	11.35	11.32
Asp212	20.42	20.32	-10.96	-10.93	-10.99	-10.93	-10.99	-10.96
Gln223	34.87	34.80	-14.80	-14.55	-14.79	-14.50	-14.76	-14.42
Arg228	-49.19	-48.67	52.83	52.67	53.37	53.15	53.59	53.59
Lys230	-24.78	-24.57	17.88	17.66	17.98	17.69	18.03	17.79
Lys237	-18.59	-18.48	12.30	12.16	12.37	12.19	12.40	12.27
Glu238	19.32	19.18	-11.24	-11.13	-11.28	-11.14	-11.29	-11.18
Lys241	-19.38	-19.26	11.37	11.27	11.41	11.29	11.42	11.33
Asp242	17.50	17.39	-9.55	-9.48	-9.58	-9.48	-9.58	-9.51
Asp244	17.80	17.71	-10.25	-10.20	-10.29	-10.22	-10.31	-10.28
Asp252	-33.69	-33.65	-19.06	-19.01	-18.84	-18.59	-18.53	-17.85
Val253	-14.45	-14.38	2.25	2.21	2.31	2.33	2.29	2.26
Asp260	26.05	25.93	-13.08	-13.11	-13.09	-13.09	-13.08	-13.12
Arg265	-21.20	-21.10	11.77	11.76	11.81	11.78	11.82	11.87
Arg271	-24.56	-24.41	13.95	13.97	14.01	14.00	14.03	14.12
Asp278	47.24	46.98	-21.10	-21.18	-20.97	-20.96	-20.86	-20.79
Lys279	-122.60	-121.95	55.80	55.78	53.05	53.85	52.19	52.03
Tyr289	-1.87	-2.70	6.07	4.43	6.30	5.26	6.49	6.46
Gly292	-7.20	-6.69	0.01	-0.24	0.17	-0.11	0.33	0.06
Lys298	-13.66	-13.63	8.13	8.07	8.17	8.08	8.19	8.14
Trp314	**-38.97**	**-42.03**	1.13	-0.23	-5.43	-5.49	0.55	-1.21
Gly315	-9.27	-7.81	2.33	2.86	4.26	4.24	2.64	3.26
Gly316	-4.46	-4.31	7.00	6.81	7.15	6.90	7.28	7.26
Glu317	9.62	7.19	-113.41	-113.30	-114.49	-114.21	-115.30	-115.51

Asp318	50.81	52.33	**-73.03**	**-70.30**	**-74.45**	**-71.32**	**-75.58**	**-73.75**
Asp319	38.25	38.64	-32.87	-31.51	-33.47	-31.95	-33.89	-33.20
Asp320	26.00	25.84	-20.36	-20.13	-20.54	-20.25	-20.66	-20.54
Arg324	-28.44	-28.30	22.89	22.60	23.10	22.74	23.21	23.11
Arg328	-17.01	-16.95	12.16	11.96	12.26	12.01	12.32	12.23
Arg334	-27.72	-27.43	17.87	17.68	18.00	17.78	18.09	18.16
Lys341	-18.50	-18.39	9.58	9.50	9.58	9.48	9.58	9.52
Arg343	-37.06	-36.88	16.25	16.25	16.19	16.13	16.13	16.03
Arg346	-23.78	-23.57	13.43	13.42	13.41	13.33	13.39	13.31
Arg349	-78.25	-77.98	**35.42**	**33.25**	**35.54**	**33.13**	**35.58**	**33.0**
Asp350	80.11	79.61	-26.68	-26.16	-26.45	-25.85	-26.28	-25.39
Lys351	-21.97	-21.95	11.08	10.78	11.09	10.75	11.08	10.78
Lys352	-43.86	-43.78	17.21	16.83	17.18	16.74	17.13	16.63
Glu354	50.14	50.16	-23.76	-22.93	-23.81	-22.85	-23.82	-22.81
Arg359	-36.28	-36.56	27.92	26.63	28.21	26.81	28.38	27.45
Asp361	18.29	18.26	-13.43	-13.13	-13.56	-13.19	-13.62	-13.42
Arg362	-24.83	-24.76	18.88	18.43	19.04	18.52	19.15	18.80
Lys367	-15.65	-15.62	11.46	11.20	11.57	11.25	11.63	11.48
Glu368	17.47	17.45	-12.39	-12.17	-12.50	-12.23	-12.56	-12.43
Asp373	25.10	24.96	-19.44	-19.17	-19.59	-19.26	-19.68	-19.50
Glu384	22.54	22.46	-11.31	-11.31	-11.32	-11.19	-11.31	-11.16
Arg387	-17.38	-17.24	9.41	9.34	9.44	9.34	9.44	9.36
Lys393	-19.19	-19.15	9.50	9.36	9.50	9.34	9.49	9.32
Asp397	29.40	29.27	-16.19	-15.97	-16.22	-15.97	-16.22	-15.95
H_2O(W2)	20.41	20.35	-7.00	-7.01	-6.80	-5.69	-6.68	-6.53
H_2O(W3)	-18.33	-18.36	0.89	1.01	0.87	-1.19	0.84	0.92
H_2O(W4)	0.94	0.96	2.20	2.26	2.25	0.25	2.30	2.36
H_2O(W7)	-13.10	-13.21	3.37	3.42	2.08	3.18	1.92	2.25
H_2O(W8)	-7.15	-12.35	-6.21	-5.85	-5.98	-7.07	-5.86	-5.49
H_2O (W12)	-16.68	-16.44	0.73	0.56	0.64	0.44	0.58	0.38
H_2O (W13)	-5.55	-5.38	3.05	3.35	2.91	3.18	2.85	3.04
H_2O (W14)	-19.74	-20.91	**-4.52**	**-7.33**	**-4.00**	**-7.07**	**-3.26**	**-6.33**

[a] (UDP^{3-}-Mn^{2+}-L) is a fragment consisted of UDP^{3-} and Mn^{2+} ion coordinated with Asp254, Met344, His347 and H_2O(W1).
[b] (Mn^{2+}-L) is a fragment consisted of Mn^{2+} ion and its ligands Asp254, Met344, His347 and H_2O(W1).

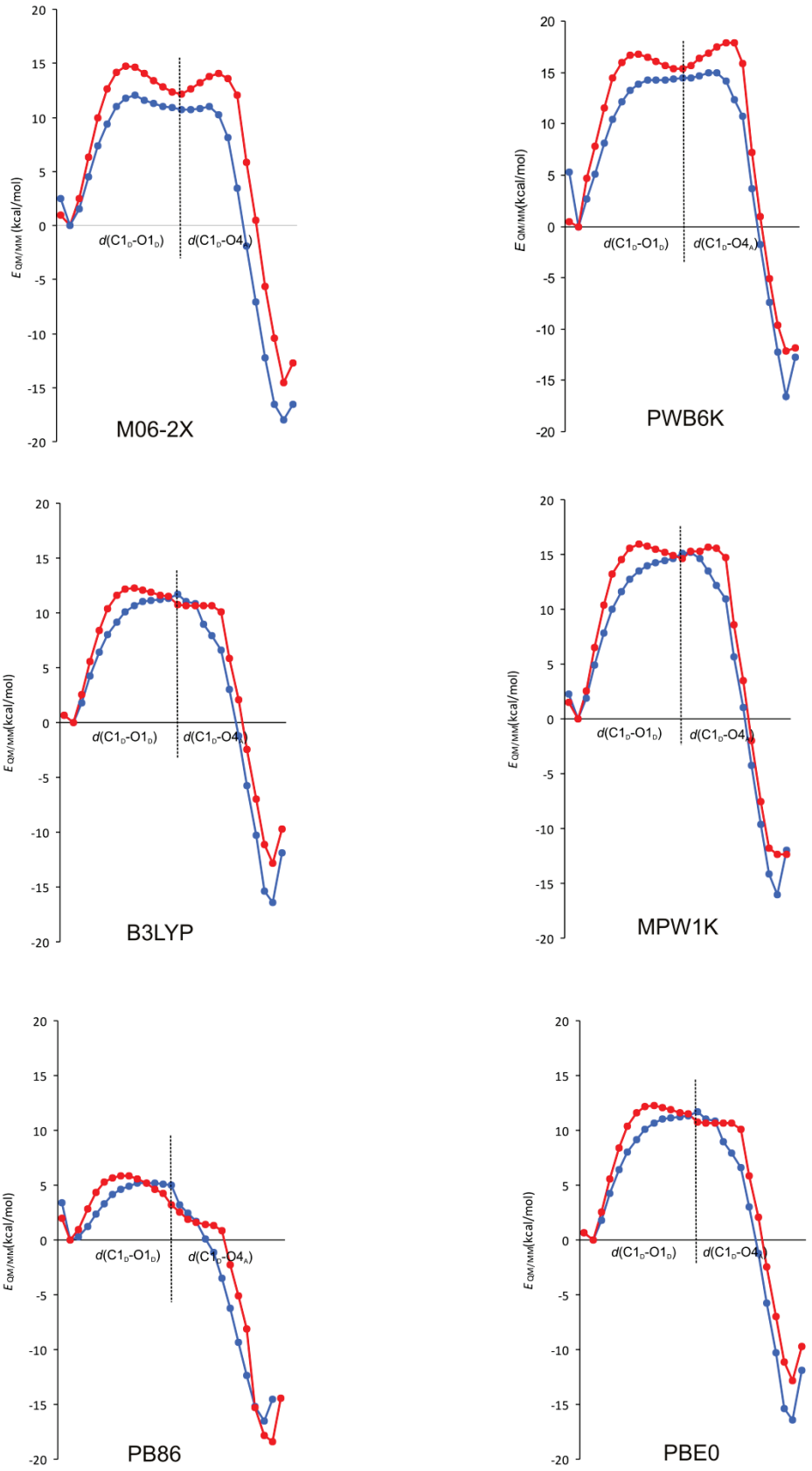

Figure S1. The QM/MM reaction profiles ($\Delta E_{QM/MM}$) for catalysis of β4GalT1 with the native donor substrate UDP-Gal (blue line) and thio UDP-5S-Gal (red line) (in kcal mol^{-1}) with the native metal ion co-factor (Mn^{2+}) along the $C1_D$-$O1_D$ and $C1_D$-$O4_A$ distance coordinates (a dash line indicates crossing points of coordinates).

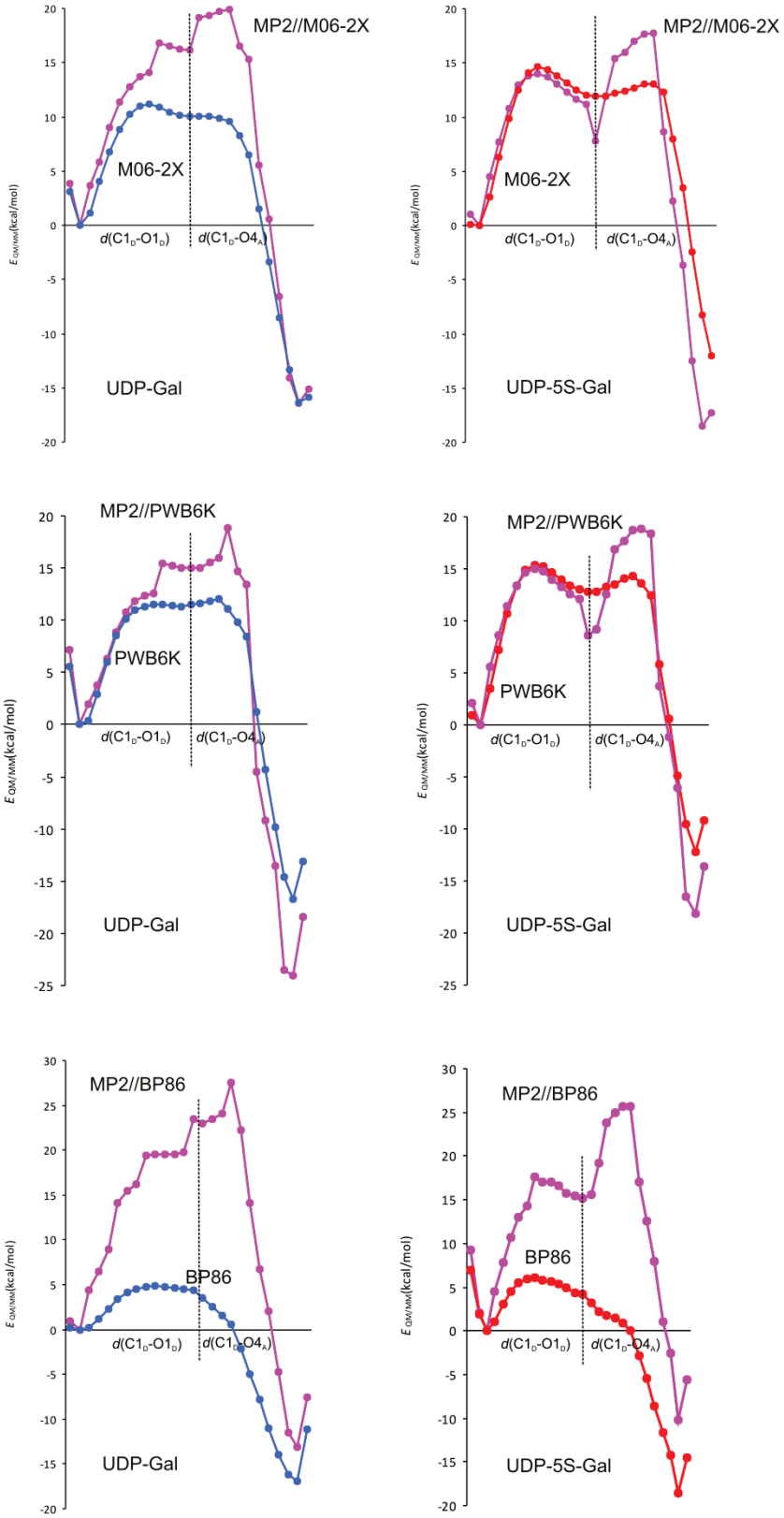

Figure S2. The QM/MM reaction profiles ($\Delta E_{QM/MM}$) for catalysis of β4GalT1 with the native donor substrate UDP-Gal (a blue line for a DFT calculation and a magenta line for a MP2//DFT calculation) and thio UDP-5S-Gal (a red line for a DFT calculation and a magenta line for a MP2//DFT calculation) (in kcal mol^{-1}) with the Mg^{2+} ion along along the $C1_D$-$O1_D$ and $C1_D$-$O4_A$ distance coordinates (a dash line indicates crossing points of coordinates).

References

1. I. Tvaroška, I. Andre and J. P. Carver, *J. Am. Chem. Soc.*, 2000, **122**, 8762-8776.

2. I. Tvaroška, I. Andre and J. P. Carver, *Glycobiology*, 2003, **13**, 559-566.

3. S. Kozmon and I. Tvaroška, *J. Am. Chem. Soc.*, 2006, **128**, 16921-16927.

4. M. Krupička and I. Tvaroška, *J. Phys. Chem. B*, 2009, **113**, 11314-11319.

5. I. Tvaroška, S. Kozmon, M. Wimmerová and J. Koča, *J. Am. Chem. Soc.*, 2012, **134**, 15563-15571.

6. M. Kumari, S. Kozmon, P. Kulhánek, J. Štepán, I. Tvaroška and J. Koča, *J. Phys. Chem. B*, 2015, **119**, 4371-4381.

7. I. Tvaroška, S. Kozmon, M. Wimmerová and J. Koča, *Chem. - Eur. J.*, 2013, **19**, 8153-8162.

8. P. Janoš, S. Kozmon, I. Tvaroška and J. Koča, *Chem. - Eur. J.*, 2018, **24**, 7051-7059.

9. E. Lira-Navarrete, J. Valero-Gonzalez, R. Villanueva, M. Martinez-Julvez, T. Tejero, P. Merino, S. Panjikar and R. Hurtado-Guerrero, *PLoS One*, 2011, **6**, e25365.

10. A. Ramakrishnan, P. V. Balaji and P. K. Qasba, *J. Mol. Biol.*, 2002, **318**, 491-502.

11. E. Boeggeman and P. K. Qasba, *Glycobiology*, 2002, **12**, 395-407.

12. B. Ramakrishnan and P. K. Qasba, *J. Biol. Chem.*, 2010, **285**, 15619-15626.

13. L. C. Pedersen, T. A. Darden and M. Negishi, *J. Biol. Chem.*, 2002, **277**, 21869-21873.

14. S. Kakuda, T. Shiba, M. Ishiguro, H. Tagawa, S. Oka, Y. Kajihara, T. Kawasaki, S. Wakatsuki and R. Kato, *J. Biol. Chem.*, 2004, **279**, 22693-22703.

15. (a) S. Gulberti, S. F. Gigleux, G. Mulliert, A. Aubry, P. Netter, J. Magdalou and M. Ouzzine, *J. Biol. Chem.*, 2003, **278**, 32219-32226; (b) K. Ohtsubo, S. Imajo, M. Ishiguro, T. Nakatani, S. Oka and T. Kawasaki, *J. Biochem.*, 2000, **128**, 283-291.

16. U. M. Unligil, S. H. Zhou, S. Yuwaraj, M. Sarkar, H. Schachter and J. M. Rini, *Embo J.*, 2000, **19**, 5269-5280.