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Supporting Information

Escherichia coli β-Galactosidase Inhibitors through Modifications at the Aglyconic Moiety: Experimental Evidence of Conformational Distortion in the Molecular Recognition Process

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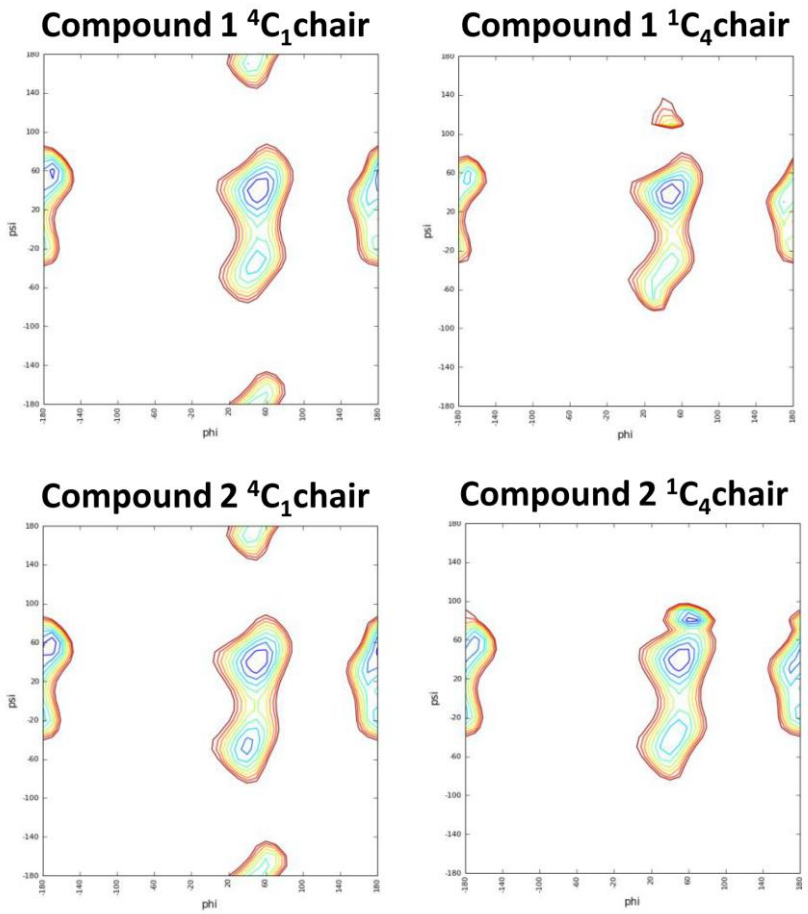

Figure S1: Coordinate scans of compound **1** (above) and compound **2** (below) fixing for the alternative chair conformation for the pentopyranose ring. Calculations were performed using the force field *OPLS_2005*.

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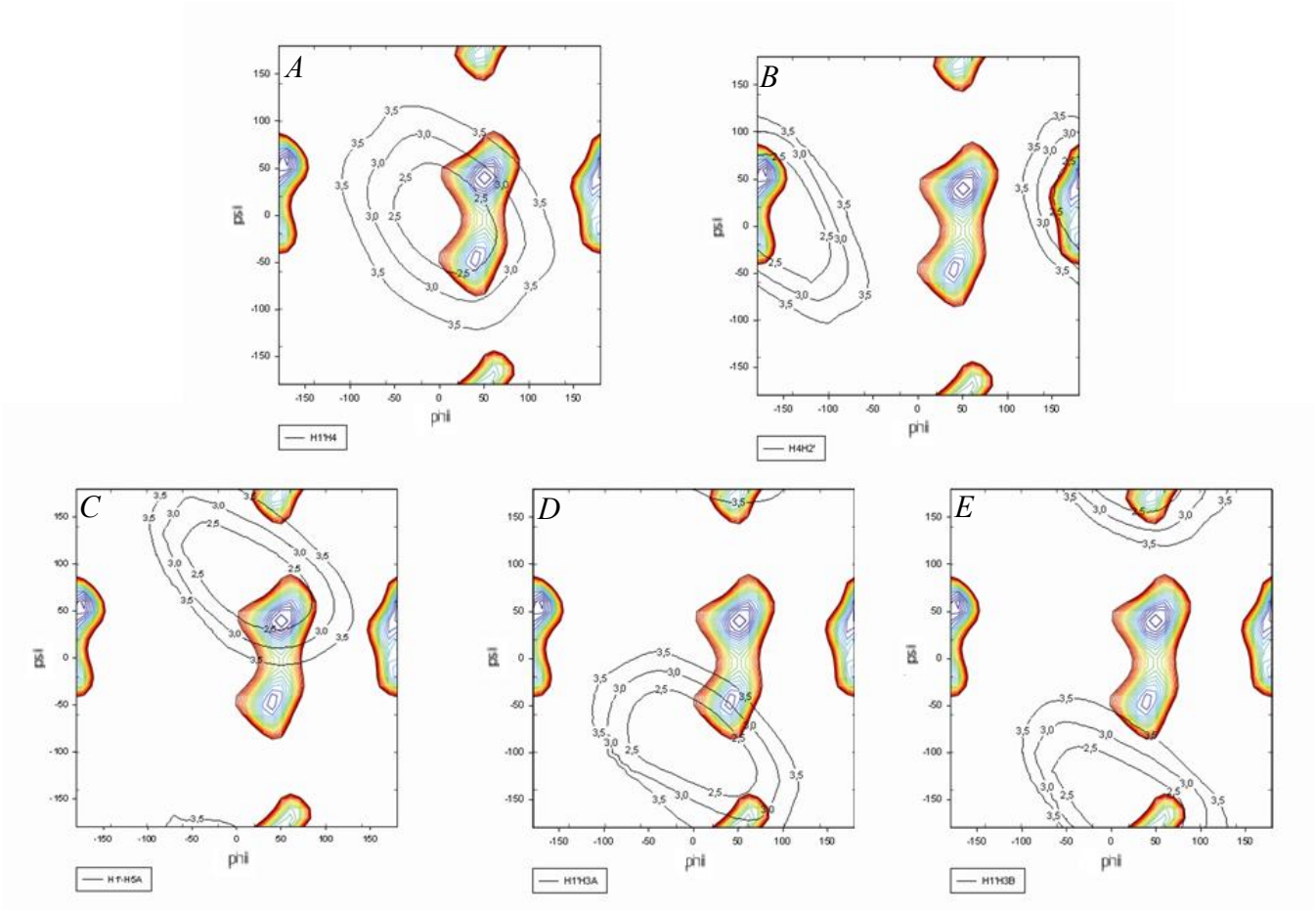

Figure S2: Representation of proton distances in the coordinate scan of compound **2** with fixed $^{4}C_{1}$ conformation for the pentopyranose. *A*, H-1' and H-4 pentose; *B*, H-2' and H-4 pentose; *C*, H-1' Gal and H-5eq pentose; *D*, H-1' Gal and H-3eq pentose; *E*, H-1' Gal and H-3ax pentose.

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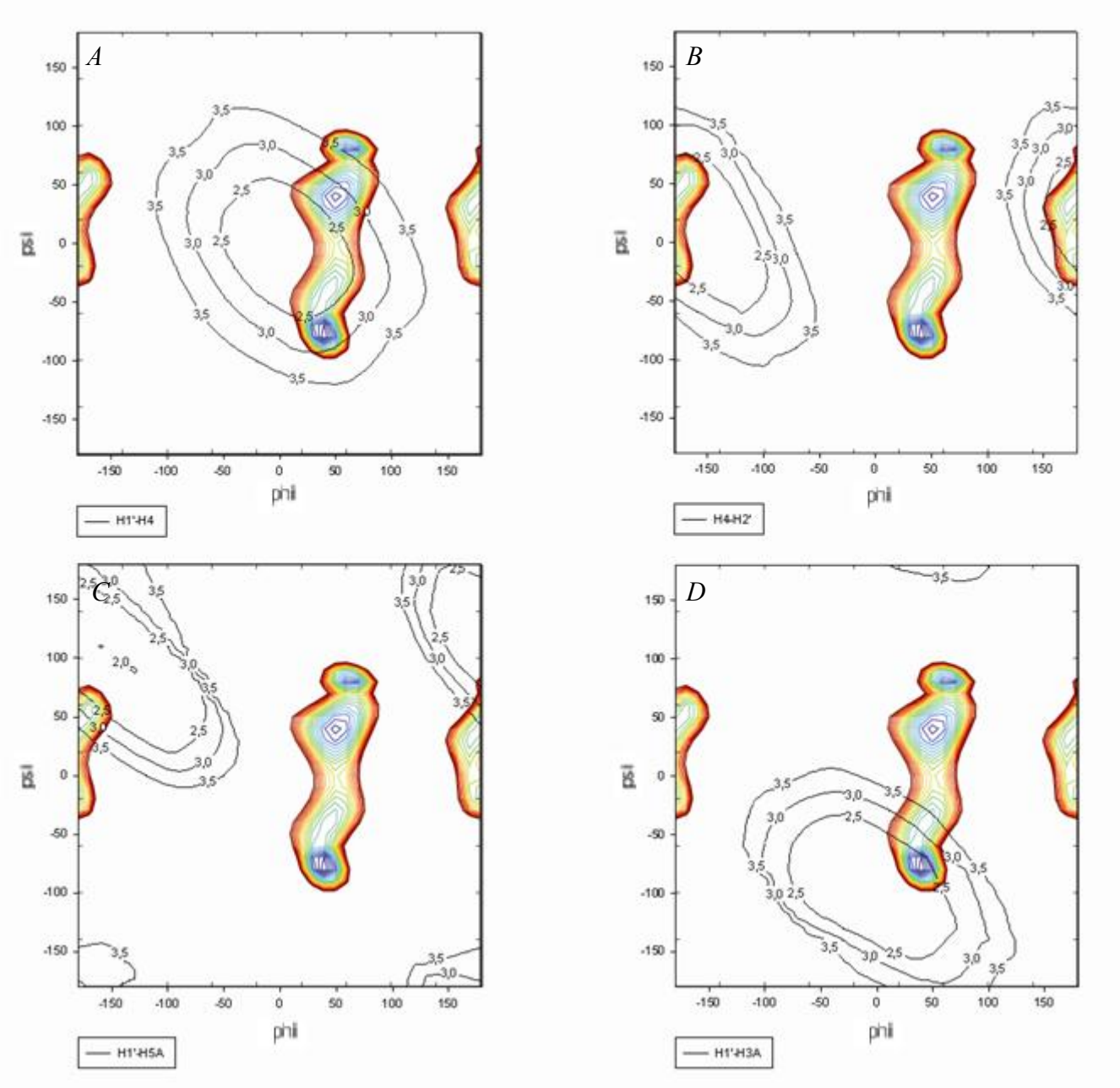

Figure S3: Representation of proton distances of the coordinate scan of compound **2** with fixed $^{1}C_{4}$ conformation for the pentopyranose ring. Distances between: *A*, H-1' Gal and H-4 pentose; *B*, H-2' Gal and H-4 pentose; *C*, H-1' and H-5 pentose; *D*, H-1' Gal and H-3ax pentose.

Table S1. Absolute STD fractions for compounds **1** or **2** with ligand:enzyme 100:1 molar ratio (20 millimolar phosphate buffer with $MgCl_2$ at pH 7.2 at 298 K). These values were employed to build the saturation curves of Figures 4B and 5B.

Compound 1	**0.5 second**	**1.0 second**	**1.5 seconds**	**2.0 seconds**	**2.5 seconds**
aromatic	0.21	0.37	0.45	0.54	0.54
CH2	not measured	not measured	not measured	not measured	not measured
CH2'	not measured	not measured	not measured	not measured	not measured
H1'	0.23	0.37	0.45	0.5	0.51
H1	0.25	0.35	0.47	0.49	0.52
H5a	0.27	0.38	0.47	0.52	0.51
H4'	0.33	0.48	0.54	0.56	0.56
H5' + H6's	0.27	0.4	0.44	0.47	0.48
H3'	0.12	0.16	0.17	0.17	0.14
H2	not measured	not measured	not measured	not measured	not measured
H2'	0.16	0.21	0.24	0.25	0.24
H5b	0.24	0.33	0.4	0.42	0.41
H4	0.27	0.38	0.4	0.45	0.45
H3a	0.33	0.41	0.45	0.48	0.49
H3b	0.36	0.4	0.45	0.49	0.51

Compound 2	**0.5 second**	**1.0 second**	**1.5 seconds**	**2.0 seconds**	**2.5 seconds**
aromatic	0.19	0.22	0.3	0.36	0.4
CH2	not measured	not measured	not measured	not measured	not measured
H1	not measured	not measured	not measured	not measured	not measured
CH2	0.11	0.13	0.2	0.19	0.24
H1'	0.11	0.14	0.21	0.21	0.24
H5a	0.09	0.14	0.16	0.2	0.2
H2	0.08	0.16	0.18	0.22	0.26
H4'	0.1	0.16	0.22	0.25	0.27
H5' + H6's	0.07	0.1	0.17	0.18	0.18
H3'	0.07	0.11	0.13	0.13	0.15
H5b	not measured	0.1	0.13	0.16	0.17
H2'	not measured	0.17	0.22	0.27	0.29
H4	not measured	0.18	0.21	0.2	0.2
H3a	not measured	0.1	0.16	0.17	0.18
H3b	0.15	0.15	0.17	0.18	0.18

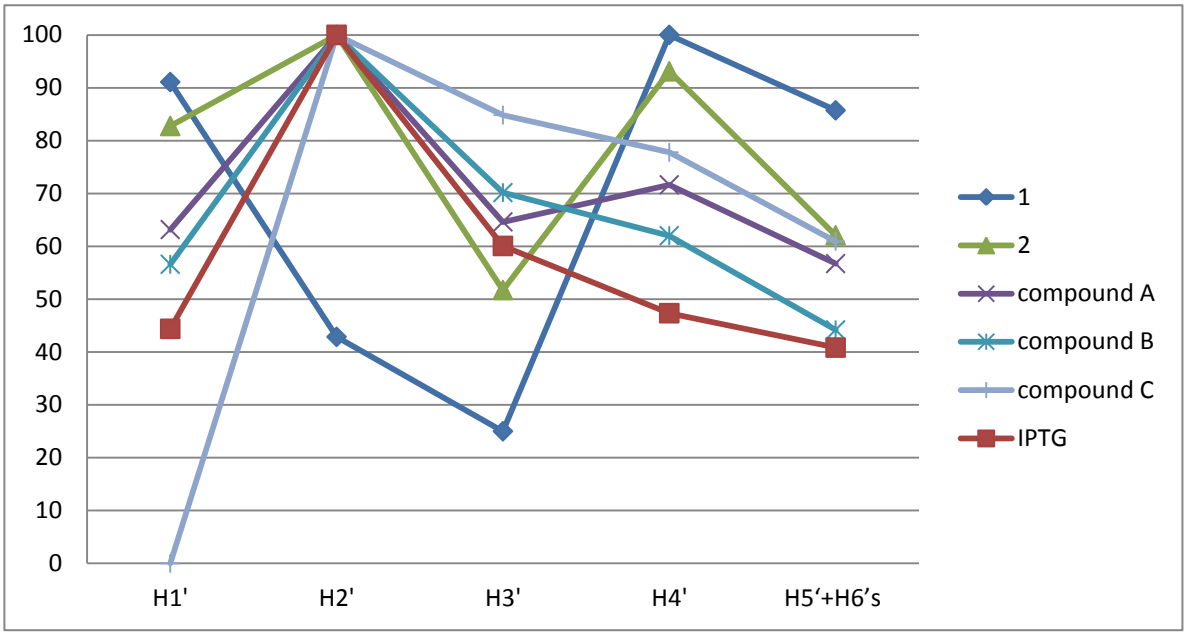

Figure S4. Comparison of STD pattern of non-reducing galactose from several compounds. STD values are normalized to the highest saturated proton of galactose residue. Compound A, B and C are thiodisaccharides, with β-galactose in their non-reducing end, whose glycosidic bond is β-(1-4). Compound A - (2-Propyl 3-deoxy-4-S-(β-D-Galactopyranosyl)-4-thio-α-D-lyxo-hexopyranoside). Compound B - (2-Propyl 3-deoxy-4-S-(β-D-Galactopyranosyl)-4-thio-α-D-xylo-hexopyranoside). Compound C - (2-Propyl 4-S-(β-D-Galactopyranosyl)-4-thio-α-D-gulopyranoside).[25]

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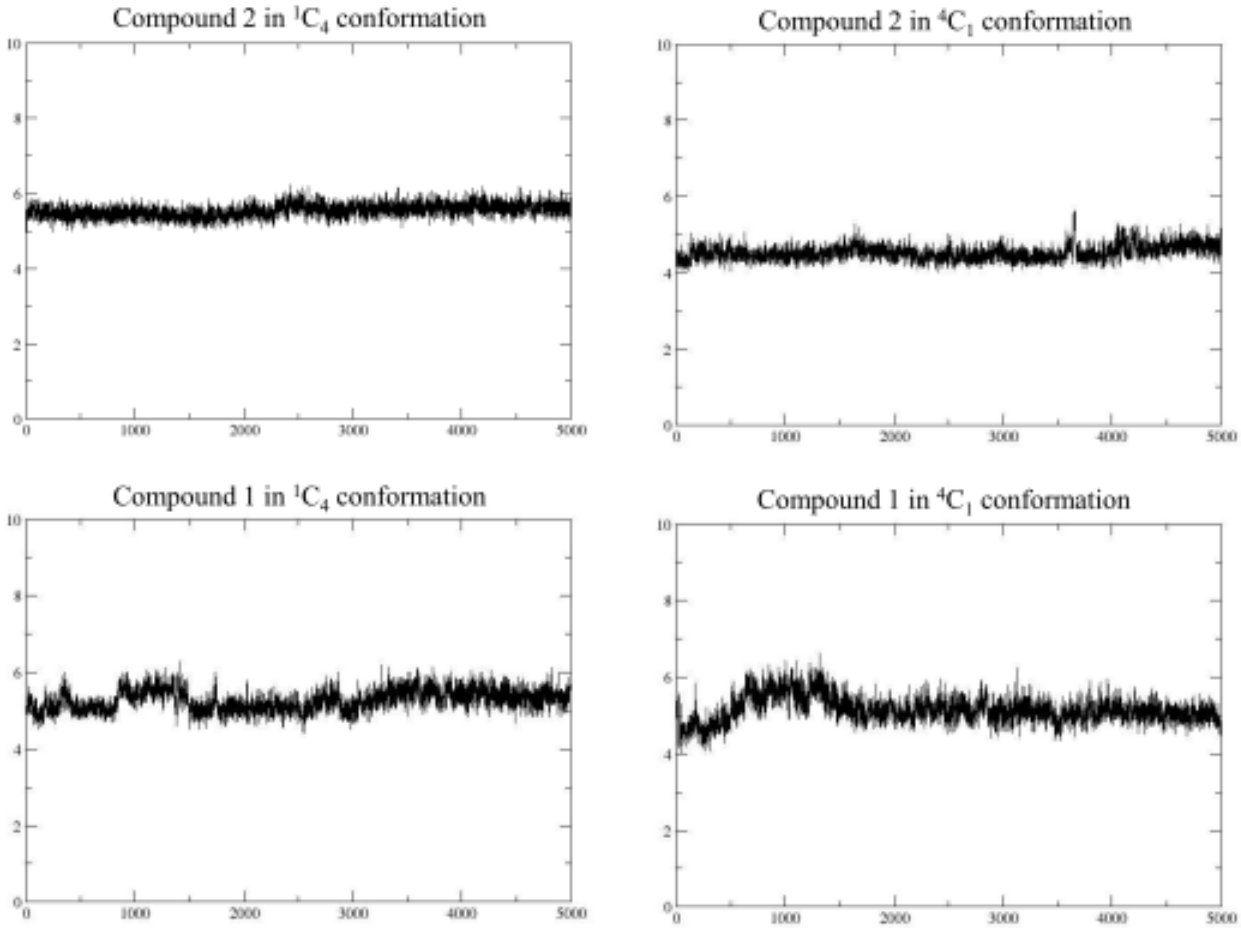

Figure S5: Trajectory of distance between galactose centroid and Trp^{567} indol centroid for compounds **1** and **2**, with the pentopyranose in both alternative conformations (5000 steps of 2 picoseconds each).

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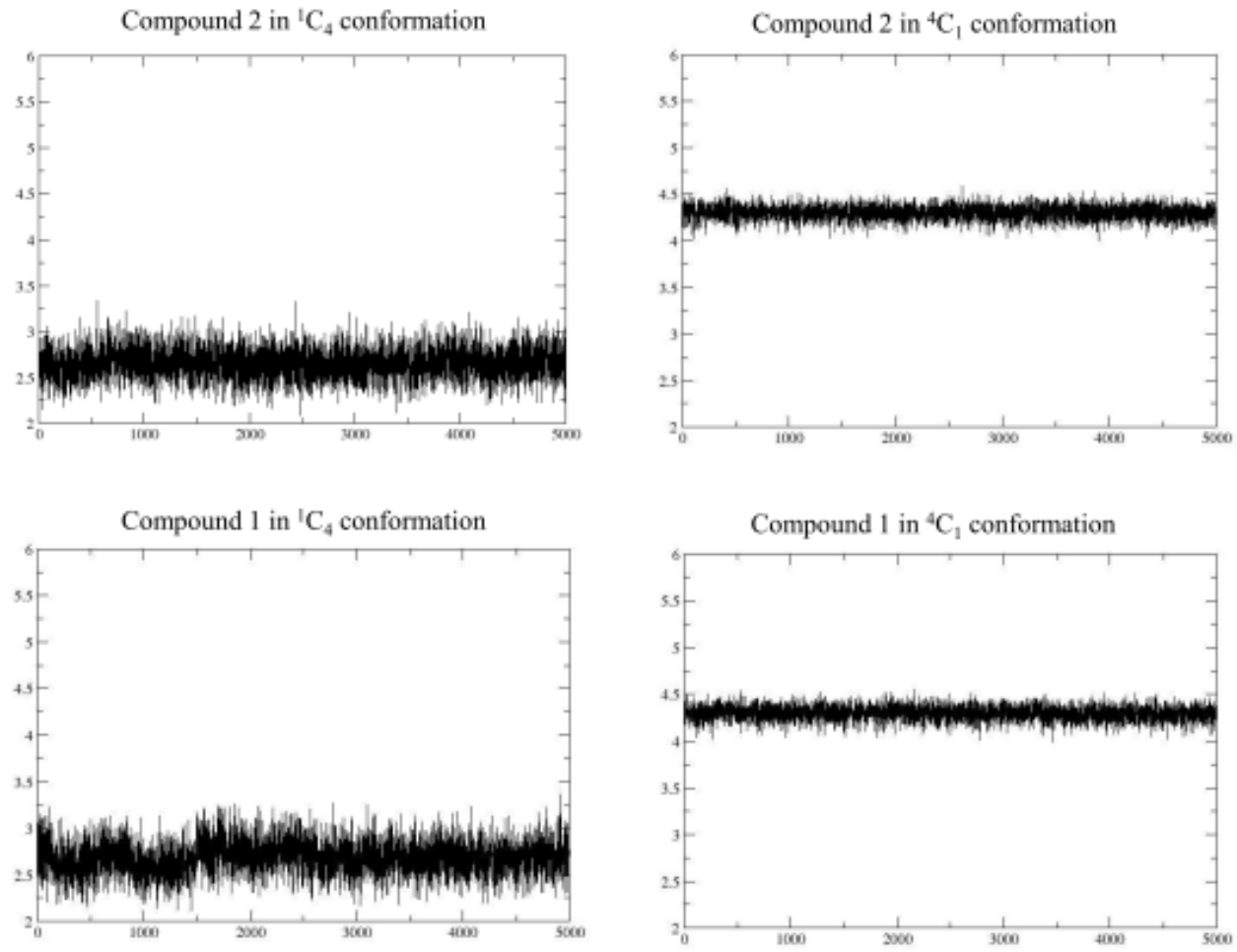

Figure S6: Trajectory of distance between protons H3a and H5a along the dynamic runs for compounds **1** and **2** with the conformation of the pentopyranose fixed as $^1C_4$ or $^4C_1$ (5000 steps of 2 picoseconds each).

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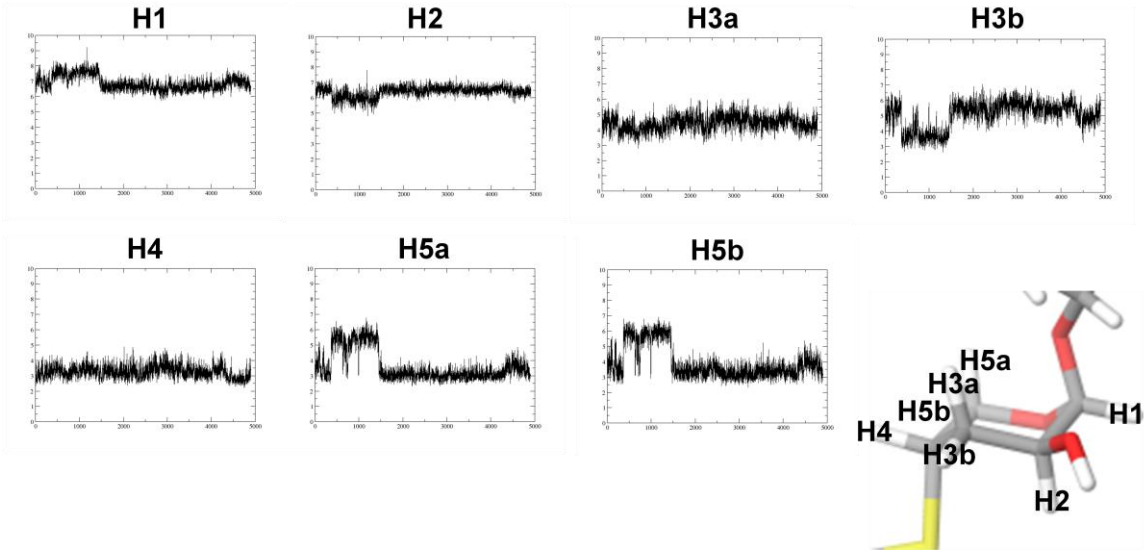

Figure S7: Trajectory of the distances between pentopyranose protons in the $^{1}C_{4}$ conformation of **2** to the Trp^{999} of β-galactosidase from *E.coli*, and molecular representation of the pentose residue (5000 steps of 2 picoseconds each).

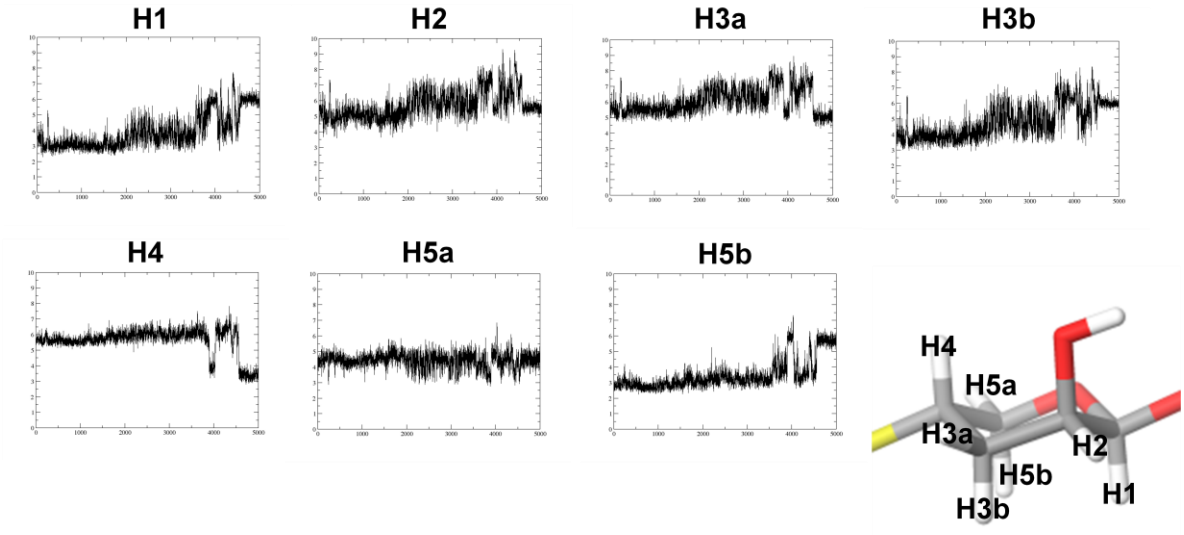

Figure S8: Trajectory of the distances between pentopyranose protons in the $^{4}C_{1}$ conformation of **2** to the Trp^{999} of β-galactosidase from *E.coli*, and molecular representation of the pentose residue (5000 steps of 2 picoseconds each).

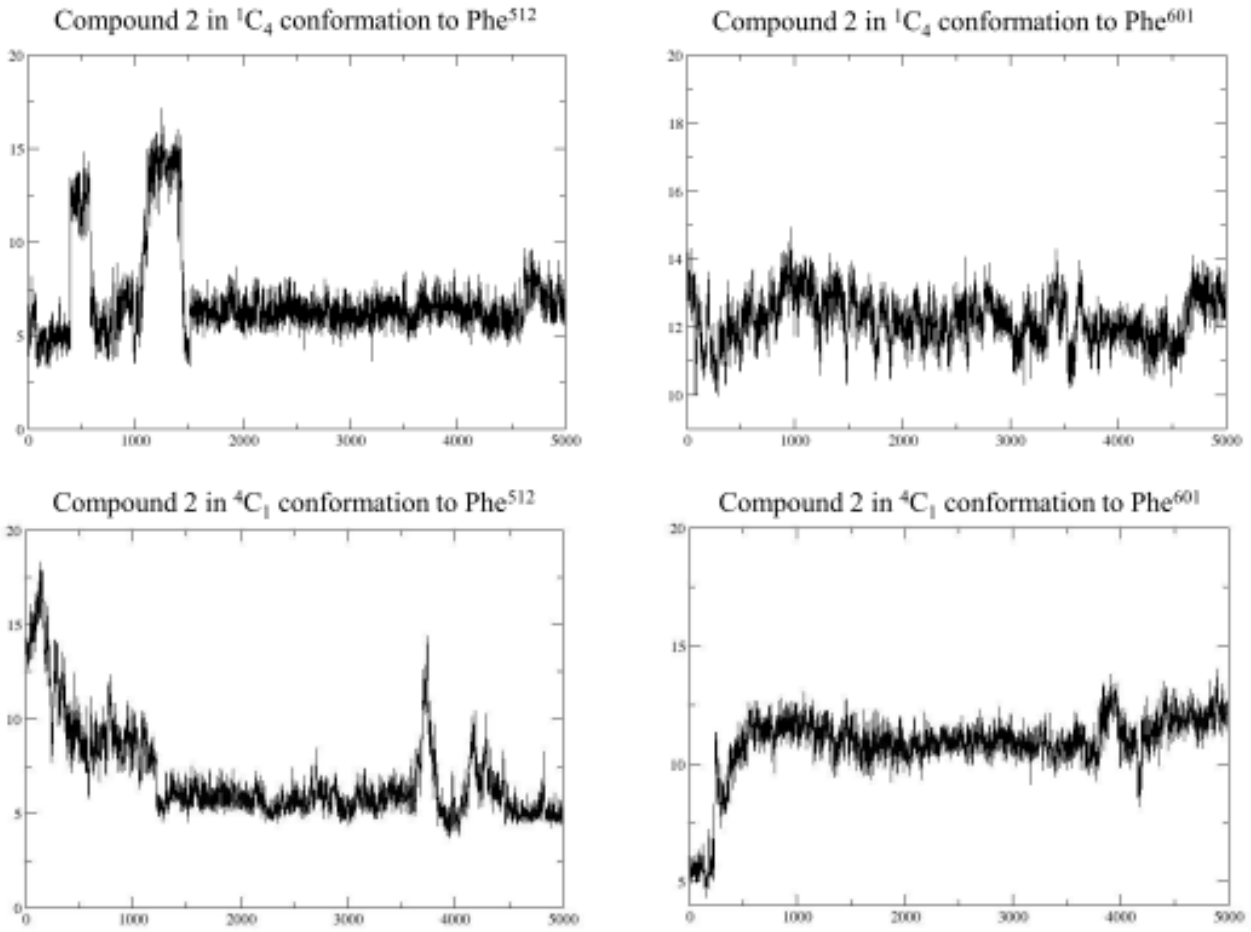

Figure S9: Trajectory of distance between aromatic protons of compound **2** to the side chain of Phe^{512} and Phe^{601} (5000 steps of 2 picoseconds each). Aromatic ring tends to get closer to Phe^{512}; $^1C_4$ conformation keeps close and $^4C_1$ conformation moves away from Phe^{601} and comes close to Phe^{512}.

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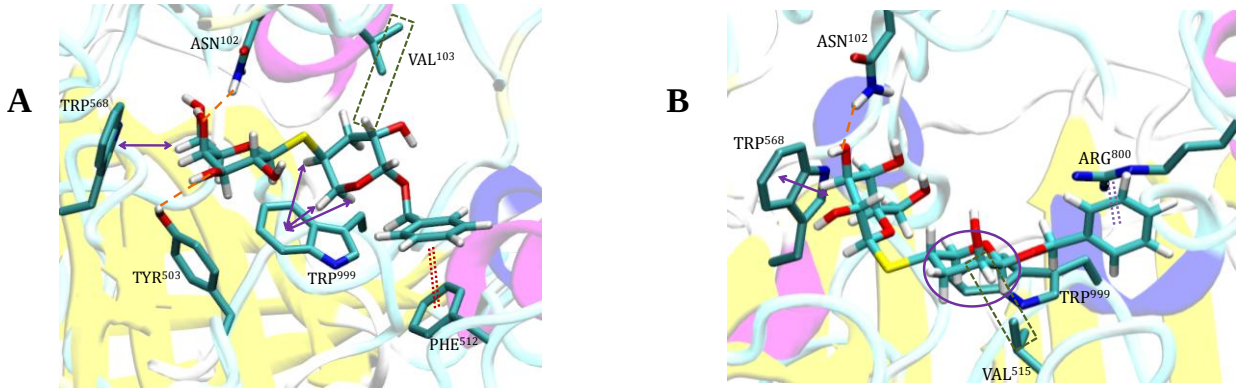

Figure S10: View of the structure of compound **2** in the catalytic site of the β-galactosidase from *E. coli* at the end of the molecular dynamics. *A*: the pentose is in the $^{1}C_{4}$ conformation; *B*: the pentose is in the $^{4}C_{1}$.

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