Supporting Information

for

Synthesis and testing of the first azobenzene mannobioside as photoswitchable ligand for the bacterial lectin FimH

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Photoisomerization studies, UV–vis spectra, NMR spectra, bioassay and docking results

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1. Data of photoisomerization

Photoirradiation experiments were carried out at room temperature by using 365 nm LED lights (Product Number: NC4U133A) from Nichia Corporation (NJSE107) with power dissipation 1.4 W and luminous flux 44 [lm]. Photoisomerization experiments with compound **6** were performed in DMSO and with compound **2** in water. Upon irradiaton, the photostationary state (PSS) was within 10 min for both compounds, as observed by UV–vis spectroscopy, The *E*→*Z* isomerisation process is reflected by a decrease of the π–π* transition and an increase in the n–π* transition band.

The kinetics of the *Z*→*E* relaxation process was determined by UV–vis spectroscopy in the dark. For determination of the rate constants, a graph of $\ln(A_\infty - A_t)$ was plotted as a function of time; where A_∞ is the absorbance of the π–π* transition at infinitive time and A_t is the absorbance at time *t* after the relaxation process was started. The negative slope *k* of the linear plot is the rate constant of the *Z*→*E* relaxation process. The half life $\tau_{1/2}$ as $\tau_{1/2} = \ln 2/k$.

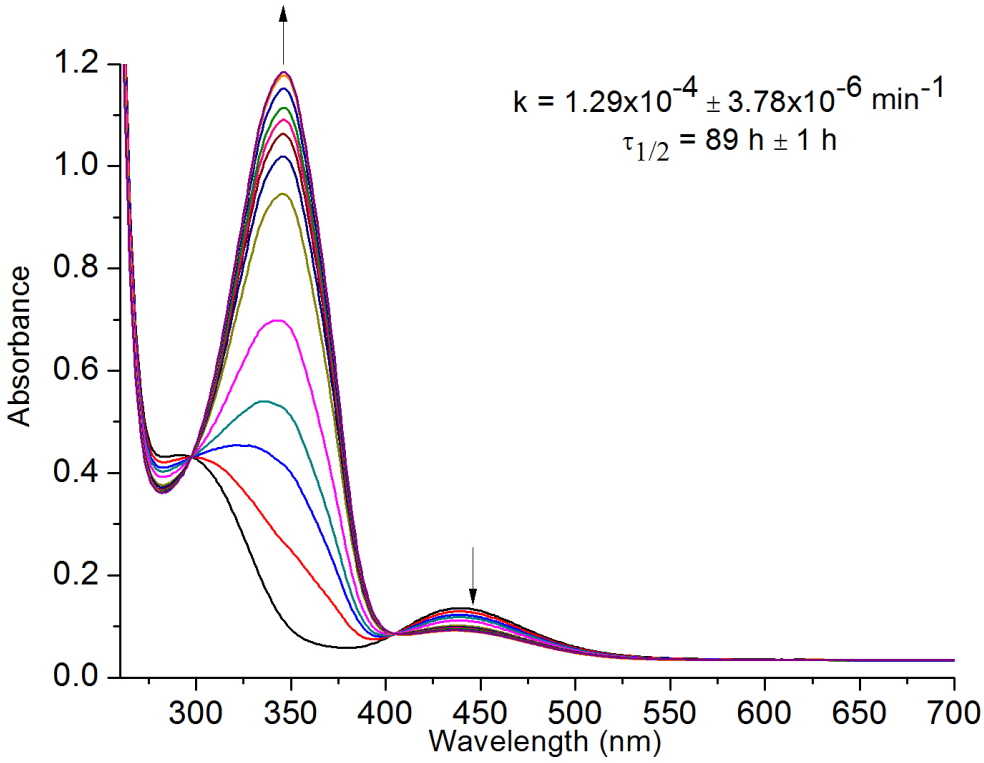

Figure S1: UV–vis spectra of thermal *Z*→*E* relaxation of mannoside **6** in DMSO (50 µM) at 18 ± 1 °C.

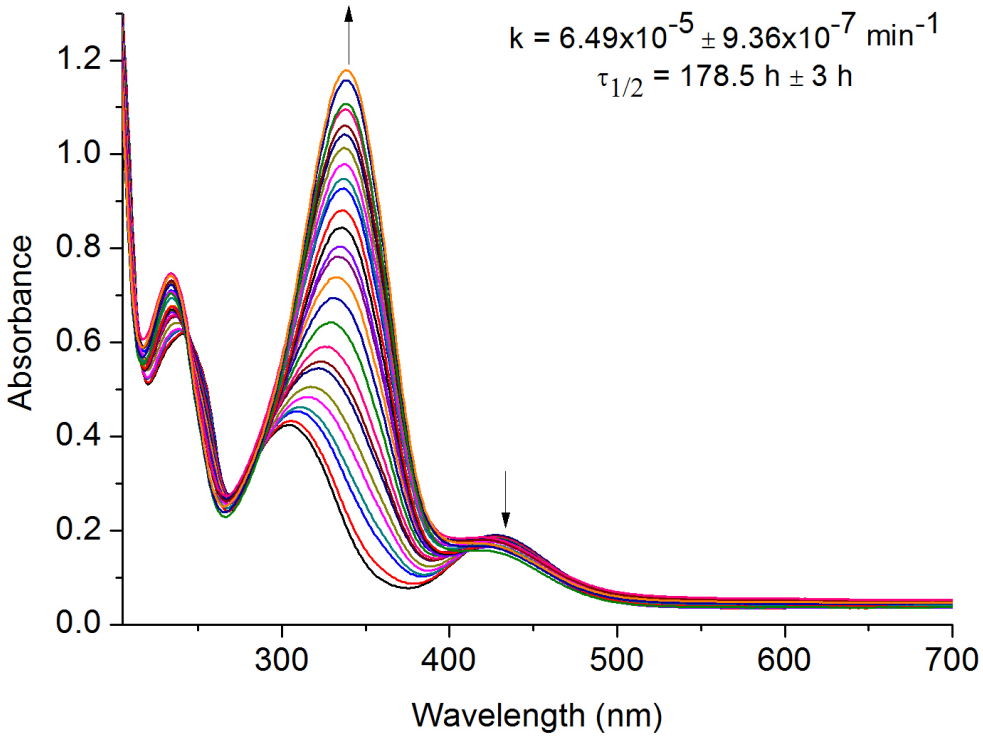

Figure S2: UV–vis spectra of thermal *Z*→*E* relaxation of the mannobioside **2** in water (65 µM) at 18 ± 1 °C.

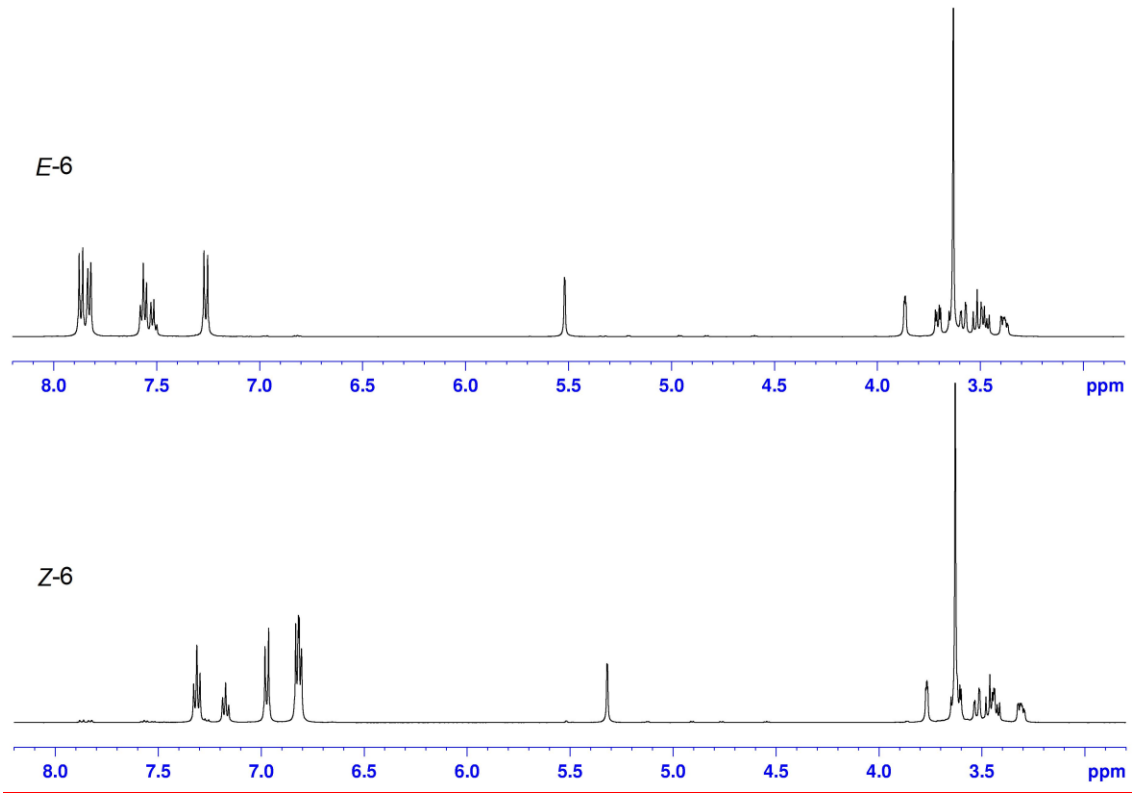

Figure S3: ^{1}H NMR spectra of (*E*)-**6** and (*Z*)-**6** in DMSO-d_6 (500 MHz).

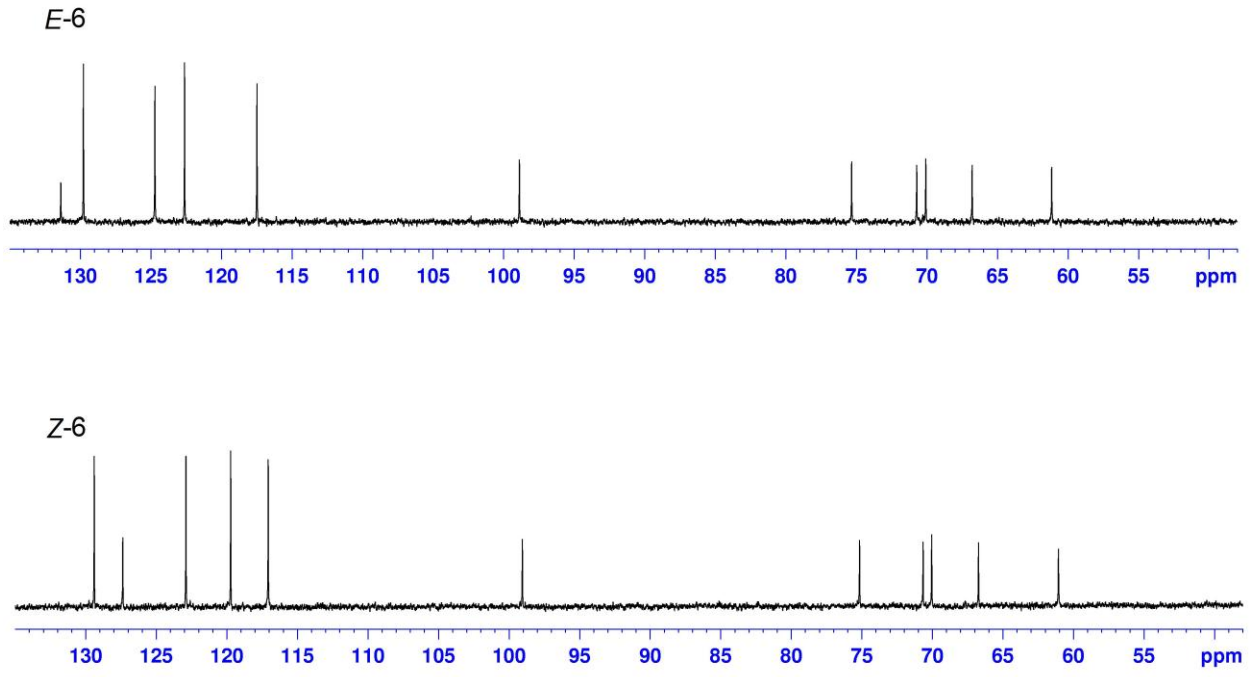

Figure S4: ^{13}C NMR spectra of (*E*)-**6** and (*Z*)-**6** in DMSO-d_6 (125 MHz)**.**

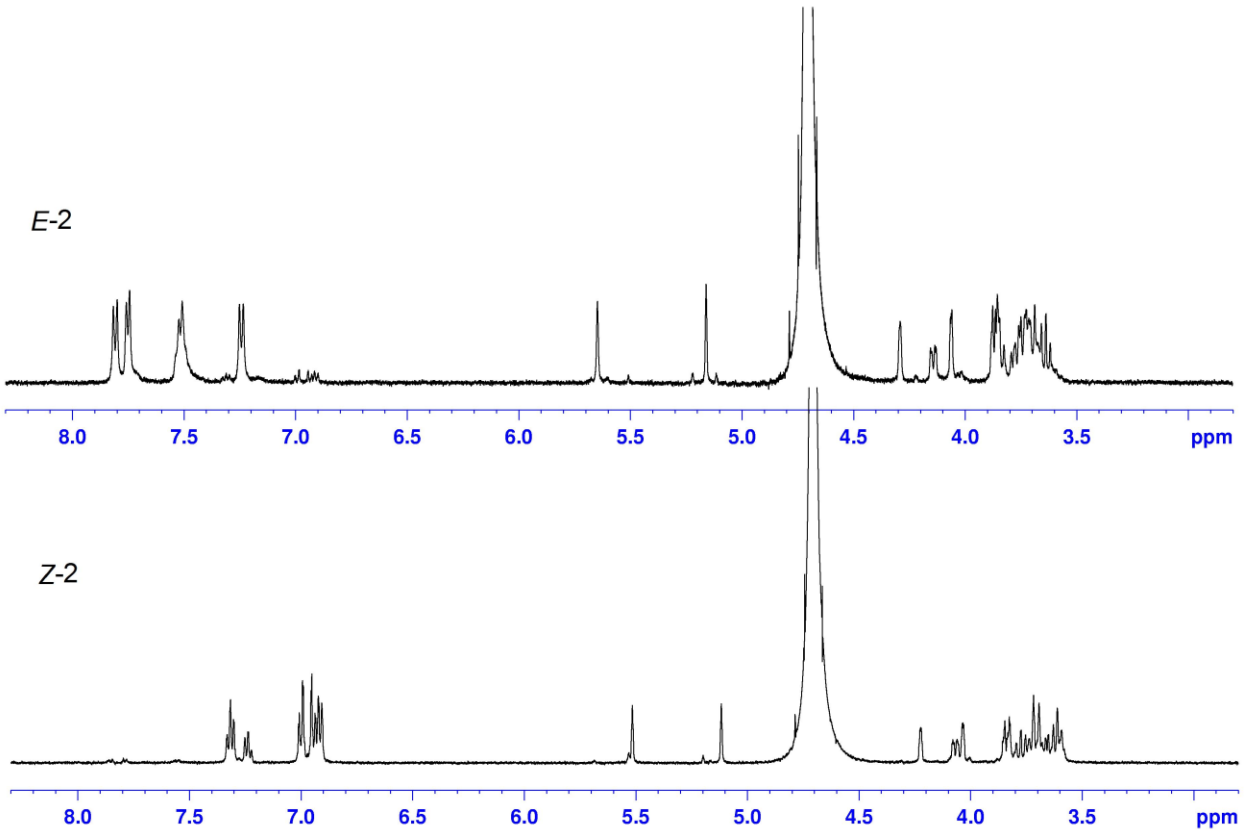

Figure S5: ^{1}H NMR spectra of (*E*)-**2** and (*Z*)-**2** in D_2O (500 MHz).

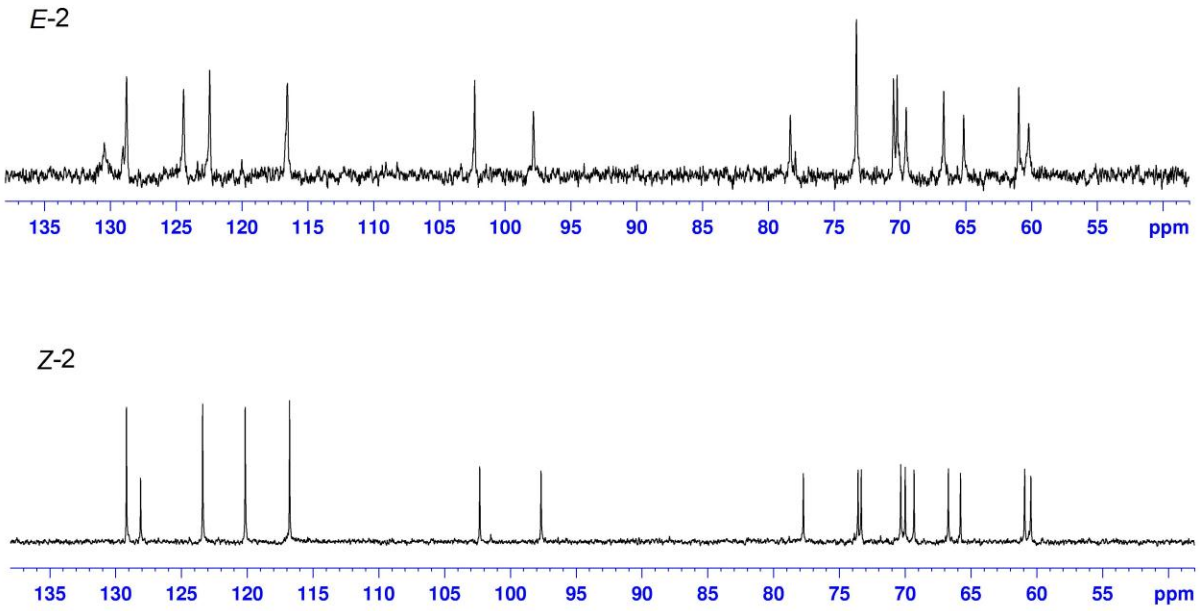

Figure S6: ^{13}C NMR spectra of (*E*)-**2** and (*Z*)-**2** in D_2O (125 MHz).

2. Bioassays

Media and buffer solutions: Carbonate buffer solution (pH 9.6): sodium carbonate (1.59 g) and sodium hydrogen carbonate (2.52 g) were dissolved in double-distilled water (1.00 L). PBS buffer solution (pH 7.2): sodium chloride (8.00 g), potassium chloride (200 mg), sodium hydrogen phosphate dihydrate (1.44 g) and potassium dihydrogenphosphate (200 mg) were dissolved in double-distilled water (1.00 L). PBST buffer solution (pH 7.2): PBS buffer + 0.05% v/v Tween® 20. LB medium: tryptone (10.0 g), sodium chloride (10.0 g) and yeast extract (5.00 g) were dissolved in distilled, deionised water (1.00 L); after autoclaving, ampicillin (100 mg) and chloramphenicol (50.0 mg) were added. pH values were adjusted by using 0.1 M HCl or 0.1 M NaOH.

Cultivation of bacteria: *E. coli* bacteria (strain pPKL1162) [1] were grown on LB medium overnight at 37 °C in a sterilized tube. After centrifugation and washing with PBS buffer (2 × 2.00 mL) the bacteria pellet was suspended to a concentration of 2.00 mg/mL in PBS buffer.

GFP assay: The published assay [2] was adapted and modified as follows: Black (nunc Maxisorp) plates were treated with a solution of mannan from *Saccharomyces cerevisiae* (1.2 mg/mL in carbonate buffer, pH 9.5; 100 µL/well) and allowed to dry at 37 °C overnight. The plates were washed with PBST (3 × 150 µL/well). Before use the wells were blocked with BSA (5% in PBS, 120 µL/well) for 2 h at 37 °C and then washed with PBST (3 × 150 µL/well). Serial dilutions of the examined inhibitor mannobioside **2** ((*E*)- or (*Z*)-configured, respectively) were prepared in the plates (50 µL/well). The bacteria suspension (2 mg bacteria/mL, 50 µL/well) was added and the plates were agitated (80 rpm) and incubated for 1 h at 37 °C. After washing with PBS (2 × 150 µL) the wells were filled with PBS (100 µL/well) and the fluorescence intensity (485 nm/535 nm) was determined.

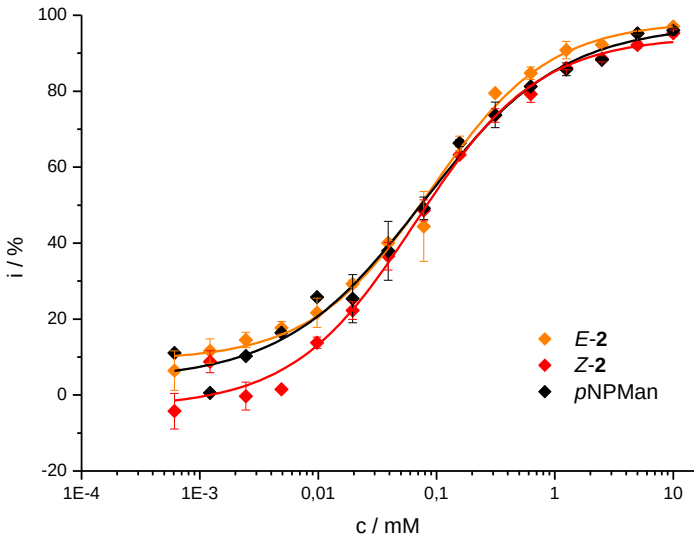

Figure S7: Inhibition curves obtained in inhibition of adhesion of *E. coli* to a mannan-coated surface. The isomers (*E*)- and (*Z*)-**2** were tested on one microtiter plate together with *p*NPMan. The sigmoidal concentration–response curves were fitted by nonlinear regression.

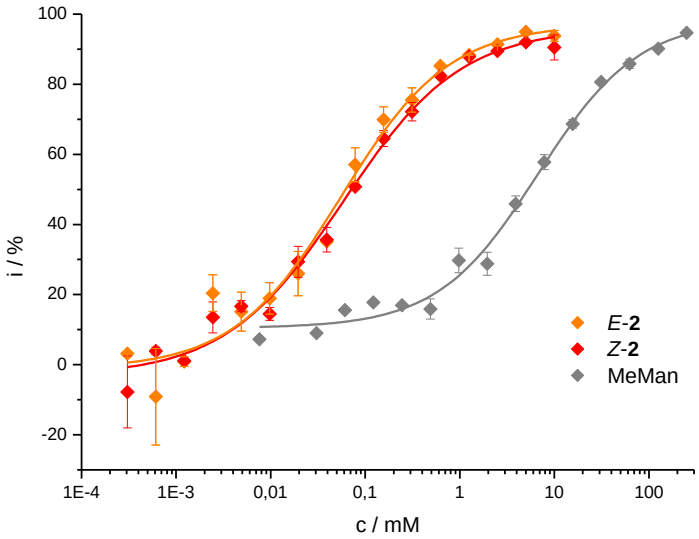

Figure S8: Inhibition curves obtained in inhibition of adhesion of *E. coli* to a mannan-coated surface. The isomers (*E*)- and (*Z*)-**2** were tested on one microtiter plate together with MeMan. The sigmoidal concentration–response curves were fitted by nonlinear regression.

3. Docking studies

Computer-aided docking was performed by using FlexX flexible docking and consensus scoring, as implemented in Sybyl 6.9 [3] as described previously [4]. Docking was based on two published X-ray structures of the bacterial lectin FimH [5] (1KLF (open-gate) and 1UWF (closed-gate structure)). The torsion angle of the azobenzene moiety was manually set up to 180° for the (*E*)- and to 90° for the (*Z*)-isomer and then the structures were minimized by using the Tripos force field and Gasteiger–Hückel charges. Thirty conformations and FlexX scoring values were obtained for each ligand and listed in Tables S1–S4.

Table S1: Scoring values for docking of *E*-configured azobenzene mannobioside **2** into the closed-gate structure of FimH.

No.	Total Score	Match-Score	Lipo-Score	Ambig-Score	Clash-Score	Rot-Score	RMS-Value	Simil. Index	#Match	Avg. Volume	Max. Volume	Frag. No.
1	-20.362	-25.378	-12.299	-8.232	1.947	18.200	0.000	-1.000	9	0.045	0.666	1
2	-19.480	-28.399	-10.811	-8.923	5.053	18.200	5.165	-1.000	10	0.193	2.362	1
3	-18.806	-23.699	-12.232	-8.767	2.292	18.200	0.816	-1.000	8	0.049	0.840	1
4	-18.641	-25.472	-11.712	-8.529	3.472	18.200	0.563	-1.000	10	0.127	1.369	1
5	-18.416	-24.813	-11.531	-8.295	2.622	18.200	0.825	-1.000	9	0.053	0.710	1
6	-18.401	-25.638	-10.708	-7.968	2.313	18.200	1.574	-1.000	9	0.072	1.215	1
7	-18.063	-23.870	-11.586	-9.123	2.917	18.200	0.904	-1.000	9	0.099	1.916	1
8	-18.047	-28.399	-9.790	-8.512	5.053	18.200	4.341	-1.000	10	0.193	2.362	1
9	-17.876	-28.399	-9.822	-8.309	5.053	18.200	4.908	-1.000	10	0.193	2.362	1
10	-17.872	-28.399	-9.817	-8.309	5.053	18.200	4.922	-1.000	10	0.193	2.362	1
11	-17.859	-28.399	-9.810	-8.306	5.056	18.200	4.911	-1.000	10	0.193	2.362	1
12	-17.832	-27.788	-10.267	-8.836	5.459	18.200	3.758	-1.000	10	0.237	2.362	1
13	-17.800	-28.928	-7.061	-8.124	2.714	18.200	3.619	-1.000	14	0.096	1.580	1
14	-17.744	-27.788	-9.883	-9.132	5.459	18.200	3.807	-1.000	10	0.237	2.362	1
15	-17.676	-28.399	-9.579	-8.351	5.053	18.200	4.356	-1.000	10	0.193	2.362	1
16	-17.563	-29.810	-5.148	-7.249	1.044	18.200	7.255	-1.000	10	0.067	1.818	1
17	-17.563	-29.810	-5.148	-7.249	1.044	18.200	7.015	-1.000	10	0.067	1.818	1
18	-17.563	-29.810	-5.148	-7.249	1.044	18.200	5.507	-1.000	10	0.067	1.818	1
19	-17.563	-29.810	-5.148	-7.249	1.044	18.200	6.413	-1.000	10	0.067	1.818	1
20	-17.563	-29.810	-5.148	-7.249	1.044	18.200	7.271	-1.000	10	0.067	1.818	1
21	-17.563	-29.810	-5.148	-7.249	1.044	18.200	7.057	-1.000	10	0.067	1.818	1
22	-17.563	-29.810	-5.148	-7.249	1.044	18.200	5.813	-1.000	10	0.067	1.818	1
23	-17.563	-29.810	-5.148	-7.249	1.044	18.200	5.763	-1.000	10	0.067	1.818	1
24	-17.563	-29.810	-5.148	-7.249	1.044	18.200	7.050	-1.000	10	0.067	1.818	1
25	-17.563	-29.810	-5.148	-7.249	1.044	18.200	6.432	-1.000	10	0.067	1.818	1
26	-17.563	-29.810	-5.148	-7.249	1.044	18.200	7.000	-1.000	10	0.067	1.818	1
27	-17.563	-29.810	-5.148	-7.249	1.044	18.200	6.404	-1.000	10	0.067	1.818	1
28	-17.563	-29.810	-5.148	-7.249	1.044	18.200	5.785	-1.000	10	0.067	1.818	1
29	-17.563	-29.810	-5.148	-7.249	1.044	18.200	5.823	-1.000	10	0.067	1.818	1
30	-17.563	-29.810	-5.148	-7.249	1.044	18.200	7.052	-1.000	10	0.067	1.818	1

Table S2: Scoring values for docking of *E*-configured azobenzene mannobioside **2** into the open-gate structure of FimH.

No.	Total Score	Match-Score	Lipo-Score	Ambig-Score	Clash-Score	Rot-Score	RMS-Value	Simil. Index	#Match	Avg. Volume	Max. Volume	Frag. No.
1	-28.809	-34.291	-10.848	-9.010	1.740	18.200	0.000	-1.000	16	0.040	0.577	1
2	-28.340	-34.839	-11.028	-9.298	3.224	18.200	0.553	-1.000	17	0.112	1.245	1
3	-28.331	-34.037	-10.560	-9.118	1.784	18.200	0.312	-1.000	17	0.042	0.600	1
4	-28.006	-34.637	-11.348	-8.848	3.227	18.200	0.451	-1.000	16	0.115	1.271	1
5	-27.439	-34.726	-12.221	-9.279	5.186	18.200	0.753	-1.000	19	0.188	1.635	1
6	-27.439	-34.726	-12.221	-9.279	5.186	18.200	0.754	-1.000	19	0.188	1.635	1
7	-27.310	-34.110	-12.764	-9.021	4.986	18.200	0.973	-1.000	19	0.195	1.803	1
8	-27.310	-34.110	-12.764	-9.021	4.986	18.200	0.977	-1.000	19	0.195	1.803	1
9	-27.105	-34.470	-11.724	-9.320	4.810	18.200	0.821	-1.000	19	0.174	1.591	1
10	-27.105	-34.470	-11.724	-9.320	4.810	18.200	0.820	-1.000	19	0.174	1.591	1
11	-26.932	-33.398	-9.896	-9.136	1.898	18.200	0.692	-1.000	18	0.050	0.357	1
12	-26.374	-32.093	-11.116	-9.247	2.482	18.200	2.173	-1.000	14	0.103	2.432	1
13	-26.374	-32.093	-11.116	-9.247	2.482	18.200	2.178	-1.000	14	0.103	2.432	1
14	-26.027	-32.323	-11.843	-9.705	4.243	18.200	0.715	-1.000	19	0.154	1.414	1
15	-25.828	-32.093	-10.673	-9.376	2.713	18.200	2.142	-1.000	14	0.109	2.432	1
16	-25.656	-31.832	-11.030	-9.314	2.920	18.200	2.217	-1.000	14	0.121	2.499	1
17	-25.269	-32.753	-9.472	-9.062	2.417	18.200	0.774	-1.000	17	0.062	0.408	1
18	-25.180	-32.257	-12.177	-9.206	4.860	18.200	2.309	-1.000	16	0.210	1.894	1
19	-25.136	-36.193	-4.934	-8.667	1.058	18.200	6.207	-1.000	15	0.020	0.233	1
20	-25.028	-36.127	-4.994	-8.590	1.083	18.200	6.155	-1.000	15	0.021	0.239	1
21	-24.949	-36.035	-5.107	-8.583	1.175	18.200	6.188	-1.000	15	0.021	0.233	1
22	-24.836	-31.393	-10.343	-9.182	2.482	18.200	2.013	-1.000	13	0.103	2.432	1
23	-24.836	-31.393	-10.343	-9.182	2.482	18.200	2.010	-1.000	13	0.103	2.432	1
24	-24.666	-31.393	-10.173	-9.182	2.482	18.200	2.012	-1.000	13	0.103	2.432	1
25	-24.666	-31.393	-10.173	-9.182	2.482	18.200	2.007	-1.000	13	0.103	2.432	1
26	-24.462	-32.257	-11.944	-9.259	5.397	18.200	2.333	-1.000	16	0.240	1.894	1
27	-24.379	-28.723	-10.438	-9.887	1.068	18.200	0.784	-1.000	17	0.042	0.743	1
28	-24.110	-31.788	-9.435	-9.119	2.631	18.200	0.894	-1.000	16	0.075	0.833	1
29	-24.097	-31.488	-8.155	-8.762	0.709	18.200	1.267	-1.000	19	0.010	0.155	1
30	-24.059	-32.062	-11.949	-9.376	5.727	18.200	2.277	-1.000	16	0.264	1.894	1

Table S3: Scoring values for docking of *Z*-configured azobenzene mannobioside **2** into the closed-gate structure of FimH.

No.	Total Score	Match-Score	Lipo-Score	Ambig-Score	Clash-Score	Rot-Score	RMS-Value	Simil. Index	#Match	Avg. Volume	Max. Volume	Frag. No.
1	-21.629	-32.441	-6.000	-8.104	1.316	18.200	0.000	-1.000	16	0.023	0.368	1
2	-21.028	-31.583	-6.370	-8.124	1.449	18.200	0.562	-1.000	14	0.027	0.373	1
3	-20.667	-29.688	-8.669	-7.905	1.996	18.200	1.280	-1.000	15	0.069	1.365	1
4	-20.367	-29.176	-8.595	-8.544	2.347	18.200	1.314	-1.000	15	0.091	1.397	1
5	-20.343	-27.224	-11.410	-7.122	1.813	18.200	3.973	-1.000	9	0.036	0.505	1
6	-20.294	-30.810	-8.664	-7.539	3.119	18.200	2.711	-1.000	15	0.103	1.239	1
7	-19.913	-28.876	-9.322	-6.770	1.454	18.200	4.235	-1.000	12	0.029	0.272	1
8	-19.572	-31.351	-5.484	-7.834	1.497	18.200	1.277	-1.000	13	0.029	0.433	1
9	-19.433	-31.283	-5.756	-7.819	1.824	18.200	1.265	-1.000	13	0.036	0.436	1
10	-19.289	-31.357	-6.031	-8.700	3.198	18.200	0.913	-1.000	13	0.124	1.623	1
11	-19.261	-30.218	-8.659	-7.725	3.742	18.200	2.711	-1.000	14	0.144	1.726	1
12	-19.256	-28.248	-9.447	-6.788	1.627	18.200	4.389	-1.000	11	0.034	0.392	1
13	-19.074	-29.401	-8.115	-7.754	2.596	18.200	1.463	-1.000	15	0.085	1.293	1
14	-19.073	-30.200	-7.072	-6.499	1.098	18.200	2.802	-1.000	15	0.017	0.213	1
15	-19.073	-30.200	-7.072	-6.499	1.098	18.200	2.726	-1.000	15	0.017	0.213	1
16	-18.928	-28.939	-8.841	-6.950	2.203	18.200	3.917	-1.000	12	0.070	0.948	1
17	-18.657	-29.088	-7.827	-7.390	2.048	18.200	3.044	-1.000	13	0.050	0.699	1
18	-18.636	-29.088	-7.806	-7.390	2.048	18.200	3.025	-1.000	13	0.050	0.699	1
19	-18.544	-29.238	-6.309	-7.606	1.010	18.200	2.447	-1.000	13	0.018	0.163	1
20	-18.544	-29.238	-6.309	-7.606	1.010	18.200	2.536	-1.000	13	0.018	0.163	1
21	-18.532	-29.157	-10.540	-6.618	4.184	18.200	2.877	-1.000	11	0.190	2.247	1
22	-18.369	-27.137	-9.210	-7.423	1.801	18.200	2.706	-1.000	10	0.040	0.506	1
23	-18.219	-28.909	-7.796	-7.162	2.048	18.200	2.076	-1.000	12	0.048	0.699	1
24	-18.171	-28.909	-7.796	-7.113	2.048	18.200	2.070	-1.000	12	0.048	0.699	1
25	-18.063	-29.804	-6.325	-6.930	1.396	18.200	2.757	-1.000	13	0.025	0.214	1
26	-18.046	-29.467	-6.656	-6.676	1.153	18.200	3.441	-1.000	13	0.018	0.197	1
27	-18.046	-29.467	-6.656	-6.676	1.153	18.200	3.375	-1.000	13	0.018	0.197	1
28	-18.012	-30.467	-6.022	-7.753	2.630	18.200	1.721	-1.000	15	0.117	2.095	1
29	-17.962	-28.449	-8.869	-6.443	2.198	18.200	4.259	-1.000	10	0.052	0.496	1
30	-17.885	-28.909	-7.796	-6.828	2.048	18.200	2.666	-1.000	12	0.048	0.699	1

Table S4: Scoring values for docking of *Z*-configured azobenzene mannobioside **2** into the open-gate structure of FimH.

No.	Total Score	Match-Score	Lipo-Score	Ambig-Score	Clash-Score	Rot-Score	RMS-Value	Simil. Index	#Match	Avg. Volume	Max. Volume	Frag. No.
1	-28.675	-36.178	-11.041	-10.107	5.052	18.200	0.000	-1.000	18	0.183	1.604	1
2	-28.101	-35.957	-11.911	-9.699	5.866	18.200	0.607	-1.000	18	0.278	1.895	1
3	-27.461	-34.986	-8.537	-9.270	1.733	18.200	1.984	-1.000	18	0.026	0.176	1
4	-27.338	-35.333	-8.159	-9.387	1.942	18.200	1.135	-1.000	18	0.047	0.658	1
5	-27.337	-36.098	-8.771	-8.701	2.633	18.200	1.827	-1.000	20	0.066	0.866	1
6	-27.336	-35.422	-8.846	-8.652	1.984	18.200	1.153	-1.000	20	0.035	0.296	1
7	-27.311	-33.089	-11.891	-9.269	3.338	18.200	1.173	-1.000	17	0.081	1.103	1
8	-26.980	-35.283	-8.233	-9.112	2.049	18.200	1.206	-1.000	17	0.044	0.567	1
9	-26.805	-32.947	-13.663	-9.641	5.846	18.200	1.635	-1.000	15	0.208	1.668	1
10	-26.779	-34.867	-8.583	-8.989	2.060	18.200	1.235	-1.000	18	0.035	0.245	1
11	-26.656	-33.244	-15.330	-9.986	8.304	18.200	1.338	-1.000	17	0.356	2.058	1
12	-26.549	-34.269	-8.961	-9.240	2.320	18.200	1.353	-1.000	17	0.054	0.507	1
13	-26.548	-34.518	-8.751	-8.639	1.760	18.200	1.811	-1.000	17	0.034	0.440	1
14	-26.414	-34.527	-8.636	-8.612	1.762	18.200	1.871	-1.000	17	0.034	0.434	1
15	-26.242	-34.448	-8.513	-8.706	1.825	18.200	1.241	-1.000	17	0.031	0.238	1
16	-26.226	-35.056	-8.329	-8.324	1.884	18.200	3.890	-1.000	17	0.033	0.275	1
17	-26.110	-32.411	-11.442	-8.774	2.917	18.200	1.181	-1.000	17	0.062	0.811	1
18	-26.072	-32.609	-14.988	-10.020	7.946	18.200	1.378	-1.000	16	0.304	1.790	1
19	-25.929	-34.907	-7.597	-9.245	2.220	18.200	1.221	-1.000	18	0.052	0.746	1
20	-25.737	-32.637	-10.894	-9.386	3.580	18.200	1.833	-1.000	16	0.086	0.998	1
21	-25.682	-33.273	-8.441	-8.614	1.047	18.200	4.184	-1.000	18	0.022	0.502	1
22	-25.442	-34.053	-8.308	-8.537	1.857	18.200	2.812	-1.000	16	0.038	0.535	1
23	-25.332	-30.941	-12.878	-10.138	5.025	18.200	1.361	-1.000	15	0.188	1.812	1
24	-25.318	-31.356	-10.634	-8.341	1.414	18.200	1.356	-1.000	19	0.019	0.134	1
25	-25.303	-33.041	-8.315	-8.586	1.039	18.200	2.651	-1.000	17	0.021	0.455	1
26	-25.300	-33.035	-8.317	-8.586	1.038	18.200	2.671	-1.000	17	0.021	0.455	1
27	-25.285	-32.073	-9.889	-9.078	2.155	18.200	2.017	-1.000	13	0.070	1.488	1
28	-25.264	-33.053	-8.295	-8.647	1.131	18.200	4.224	-1.000	17	0.022	0.485	1
29	-25.254	-32.748	-10.455	-9.964	4.313	18.200	1.356	-1.000	14	0.207	1.668	1
30	-25.249	-34.097	-10.945	-8.911	5.104	18.200	1.892	-1.000	16	0.160	1.519	1

4. References

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