Electronic Supplementary Information for

A glucose-based molecular rotor inhibitor of glycogen phosphorylase as a probe of cellular enzymatic function

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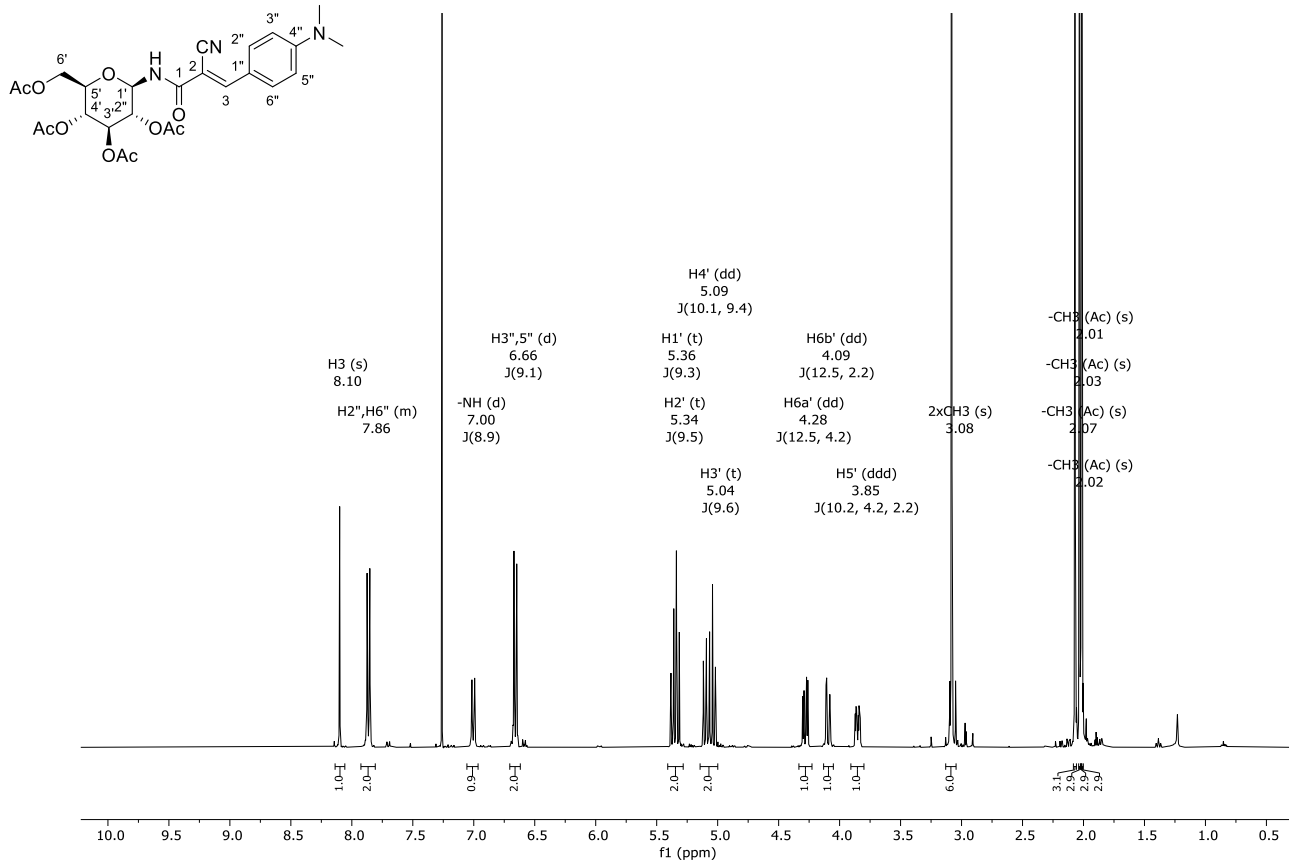


Figure S1. ^{1}H NMR spectrum of compound **2b** (400 MHz, $CDCl_3$).

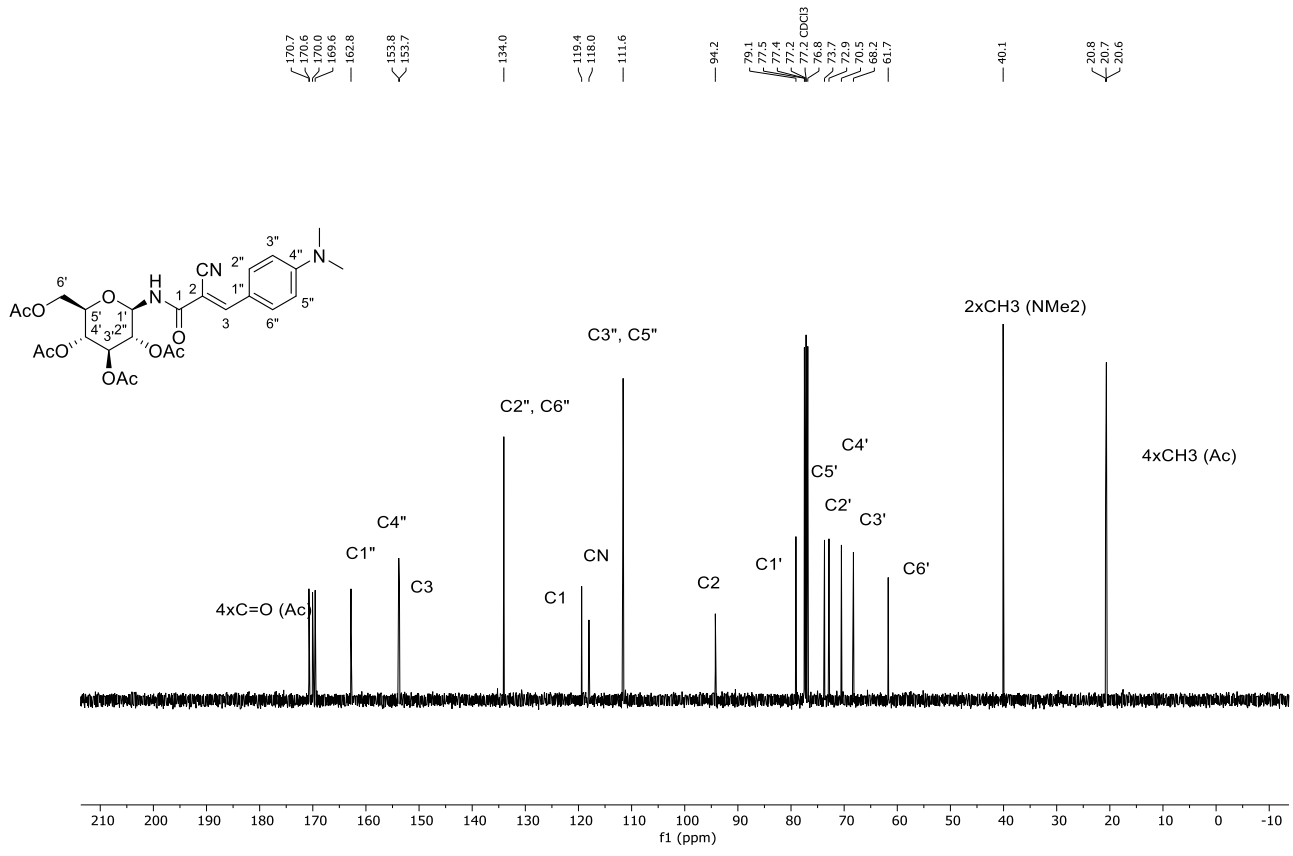
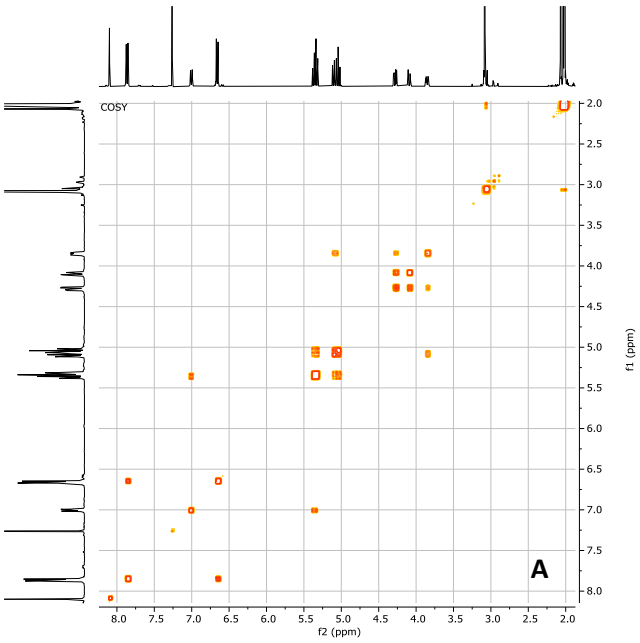
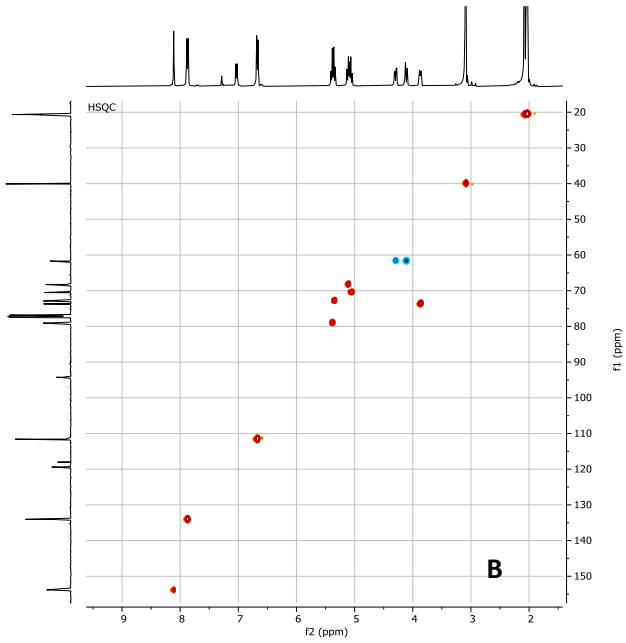
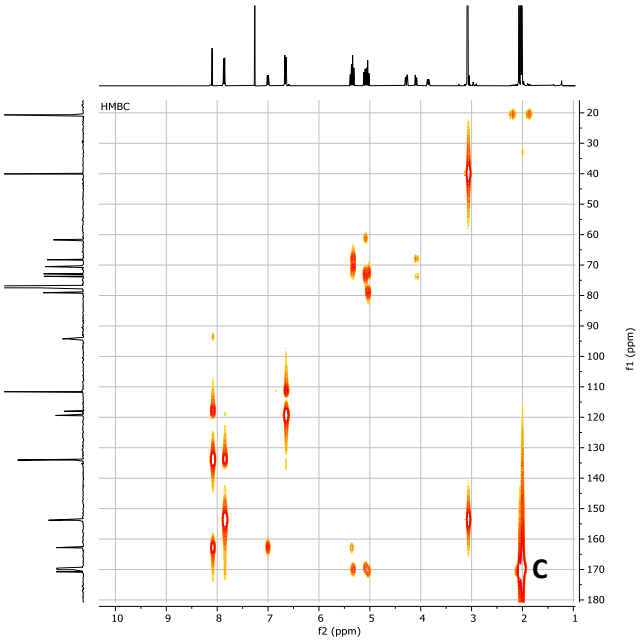


Figure S2. ^{13}C NMR spectrum of compound **2b** (101 MHz, $CDCl_3$).







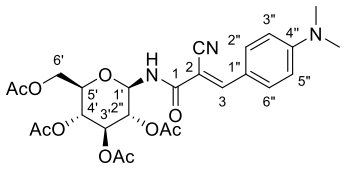


Figure S3. COSY (**A**), HSQC (**B**) and HMBC (**C**) NMR spectra of compound **2b** ($CDCl_3$).

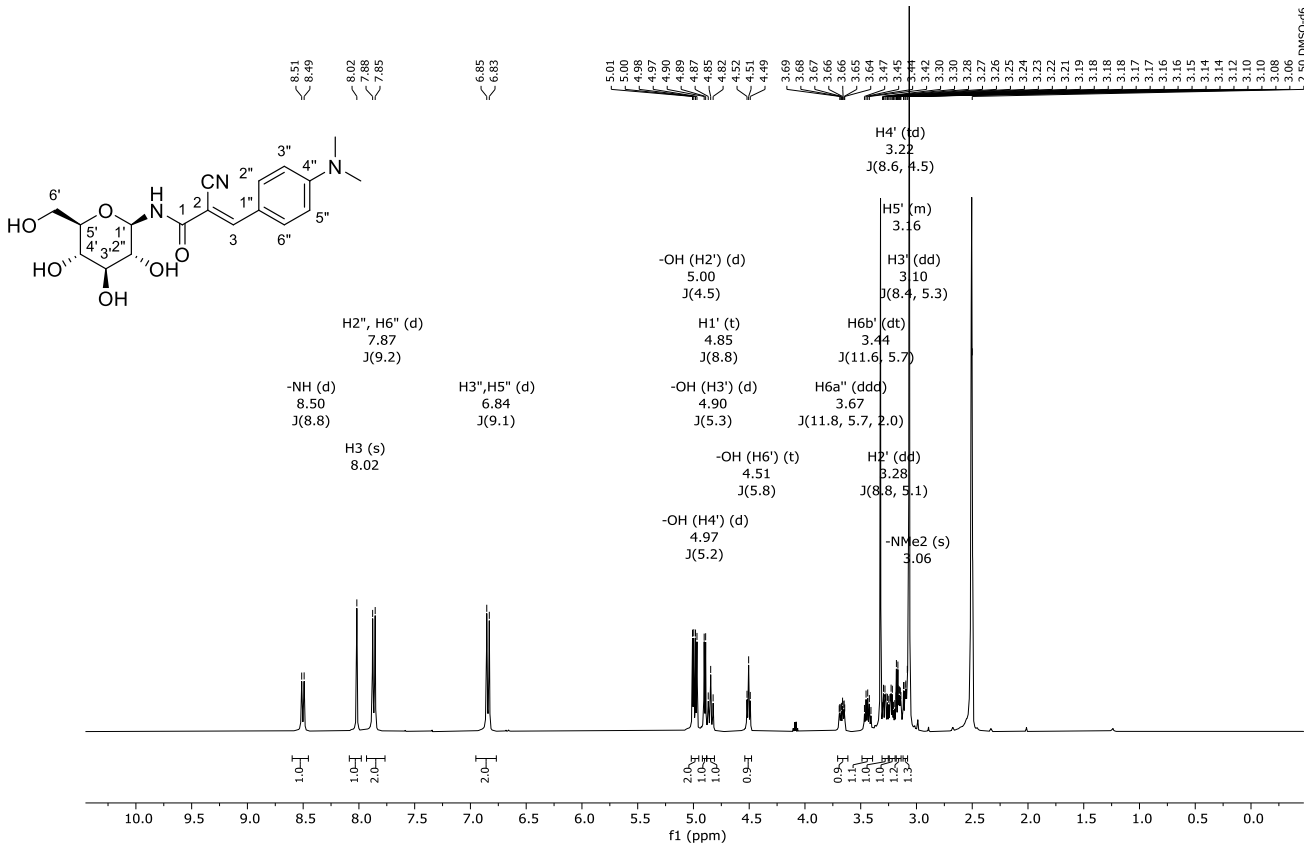


Figure S4. ^{1}H NMR spectrum of compound **2a** (400 MHz, DMSO-*d6*).

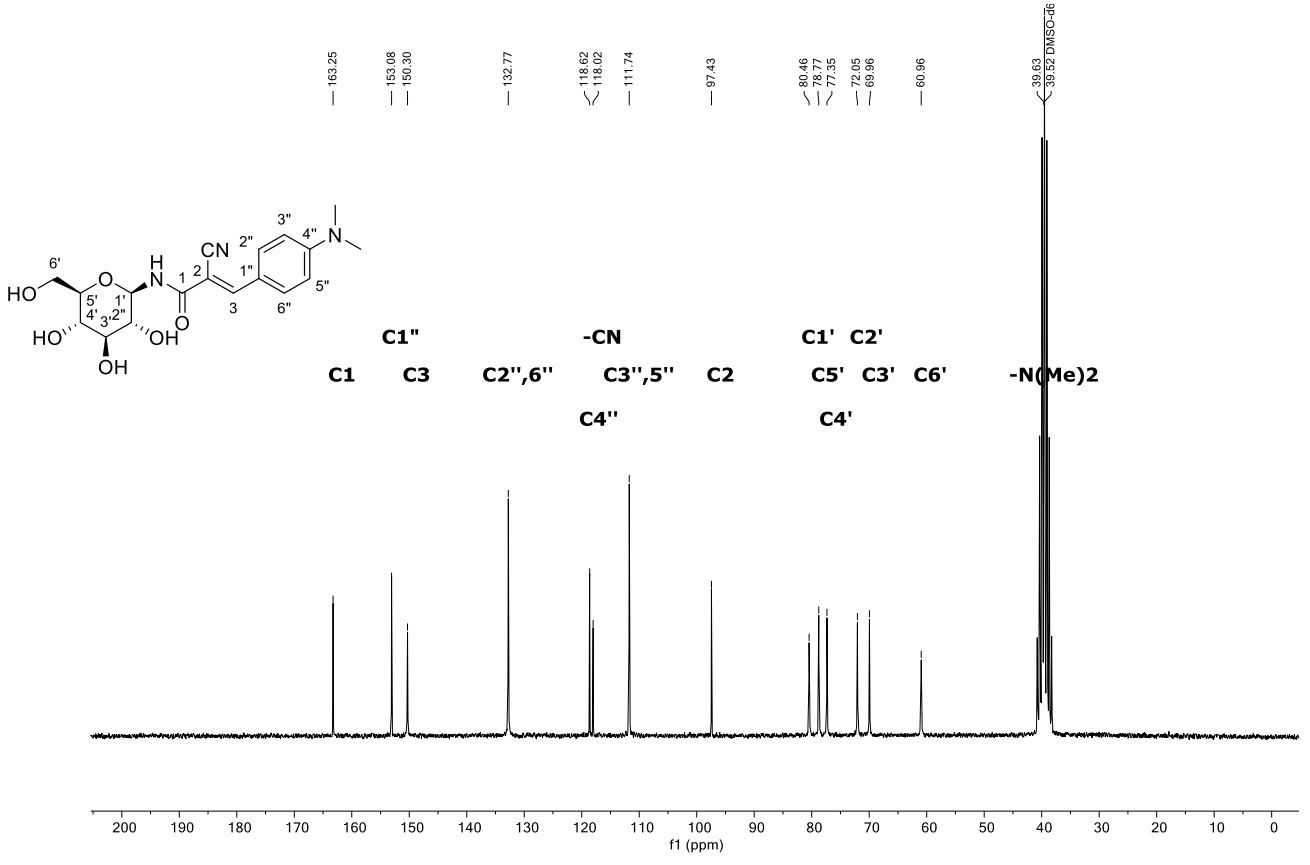
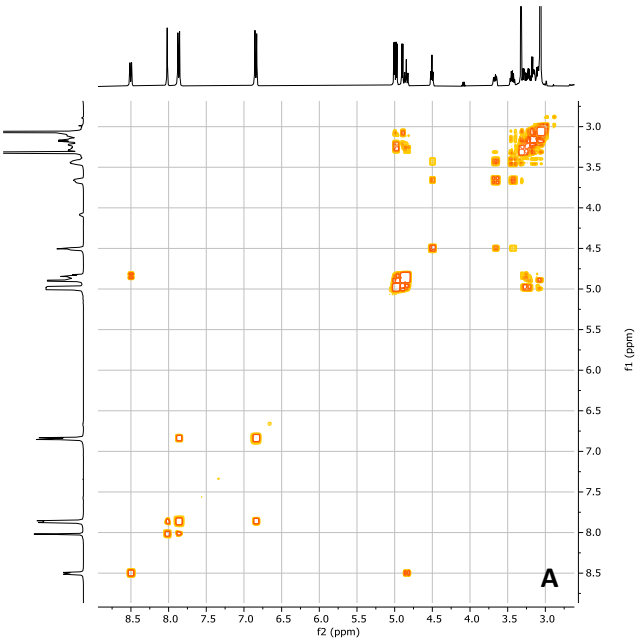
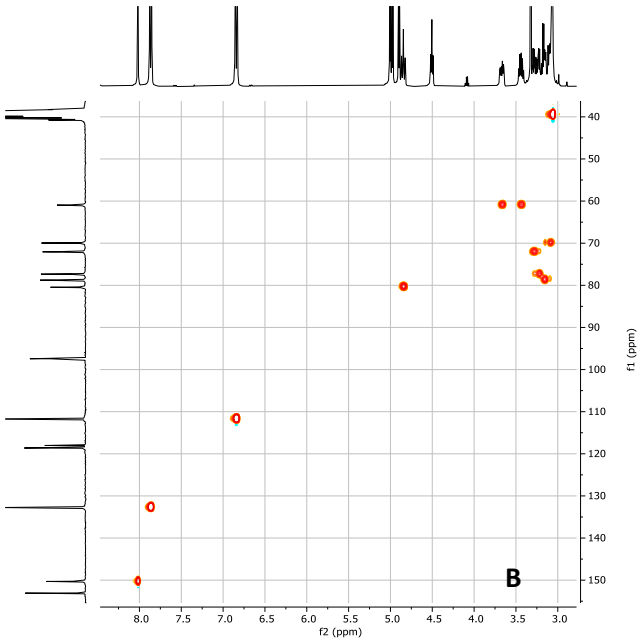
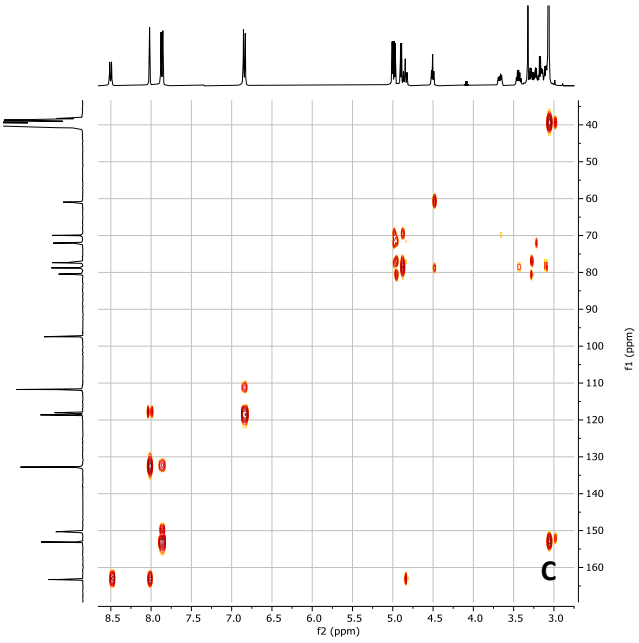


Figure S5. ^{13}C NMR spectrum of compound **2a** (50 MHz, DMSO-*d6*).







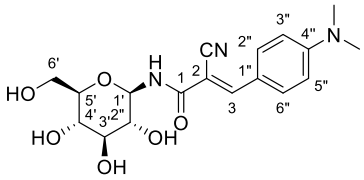


Figure S6. COSY (**A**), HSQC (**B**) and HMBC (**C**) NMR spectra of compound **2a** (DMSO-*d6*).

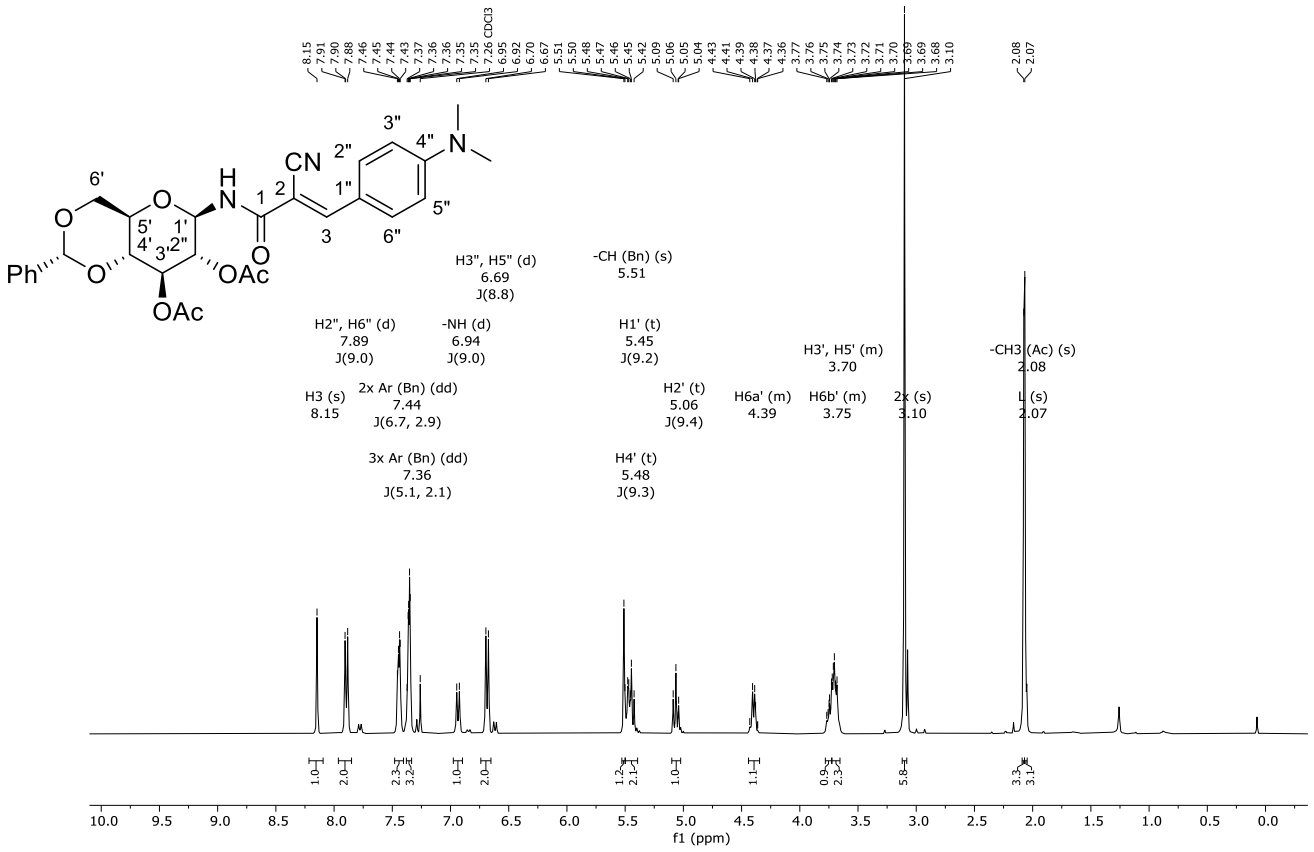


Figure S7. ^{1}H NMR spectrum of compound **7** (400 MHz, $CDCl_3$).

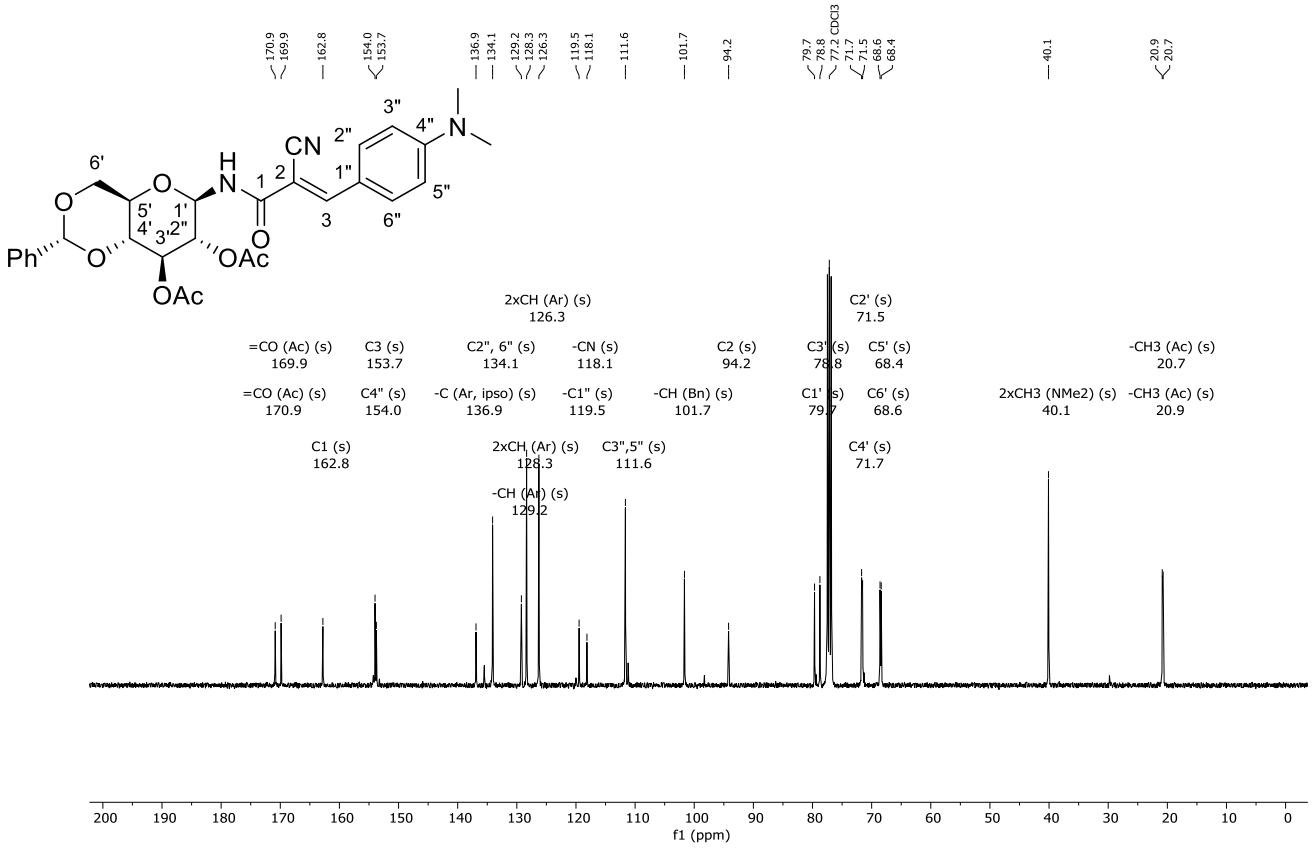


Figure S8. ^{13}C NMR spectrum of compound **7** (50 MHz, $CDCl_3$).

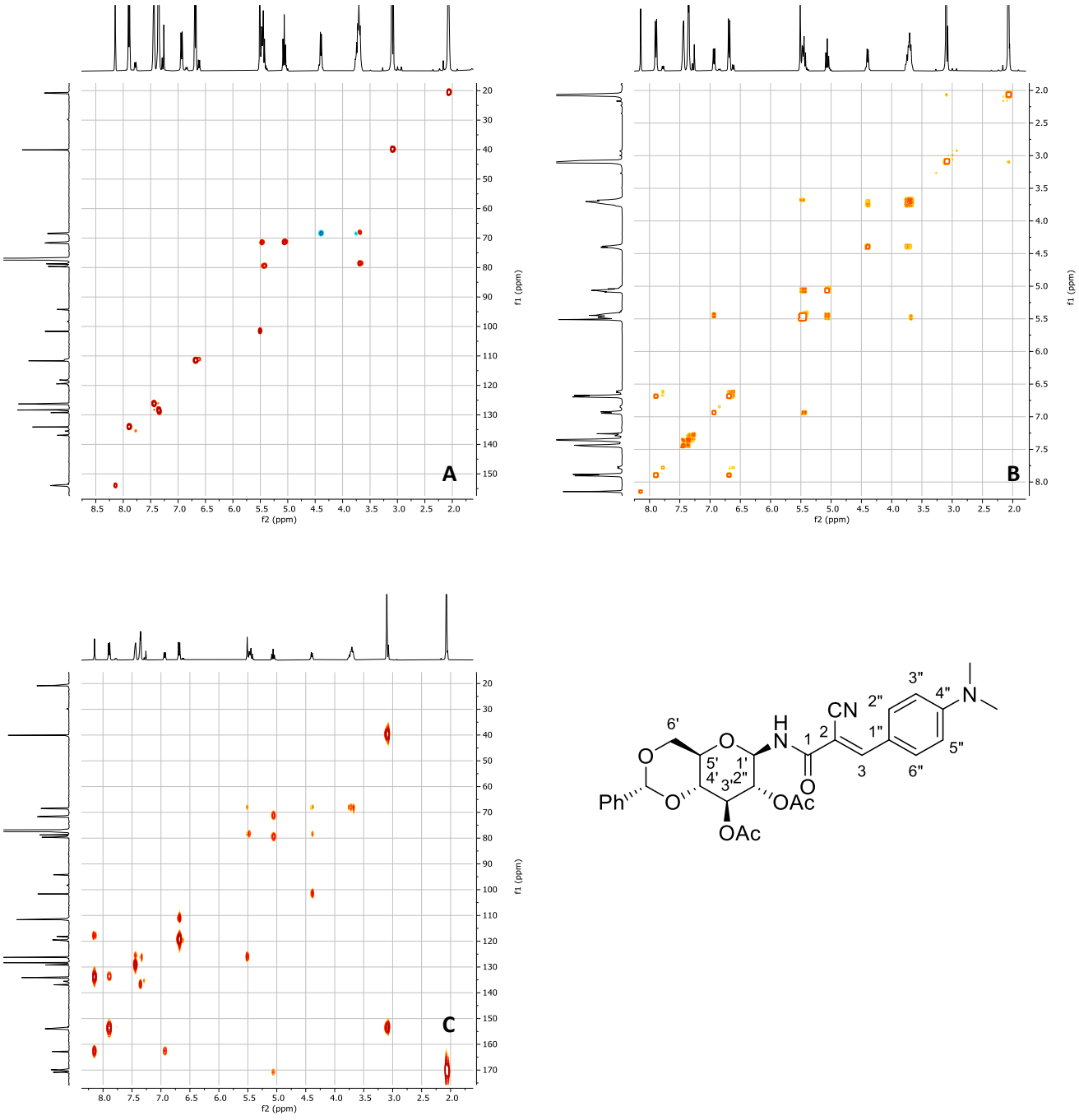


Figure S9. COSY (**A**), HSQC (**B**) and HMBC (**C**) NMR spectra of compound **7** ($CDCl_3$).

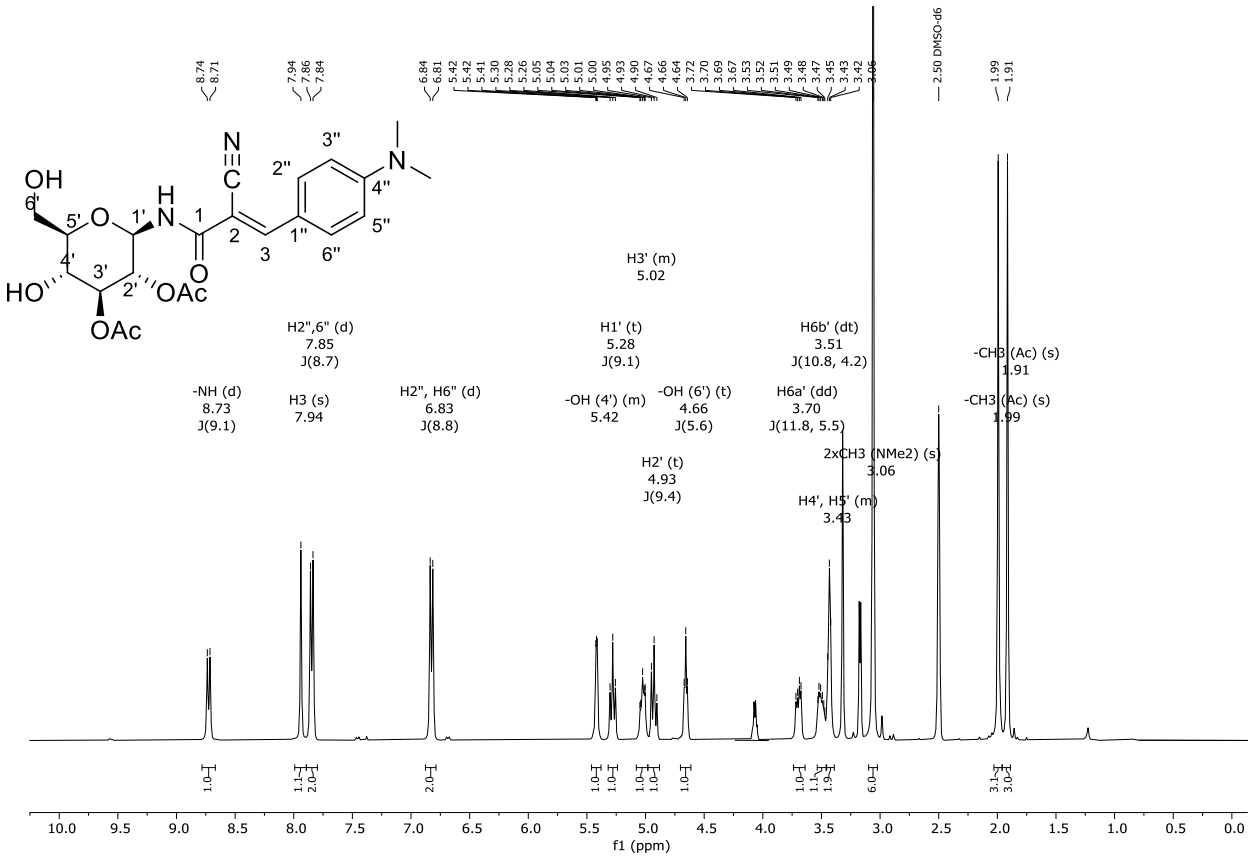


Figure S10. ^{1}H NMR spectrum of compound **2d** (400 MHz, DMSO-d_6).

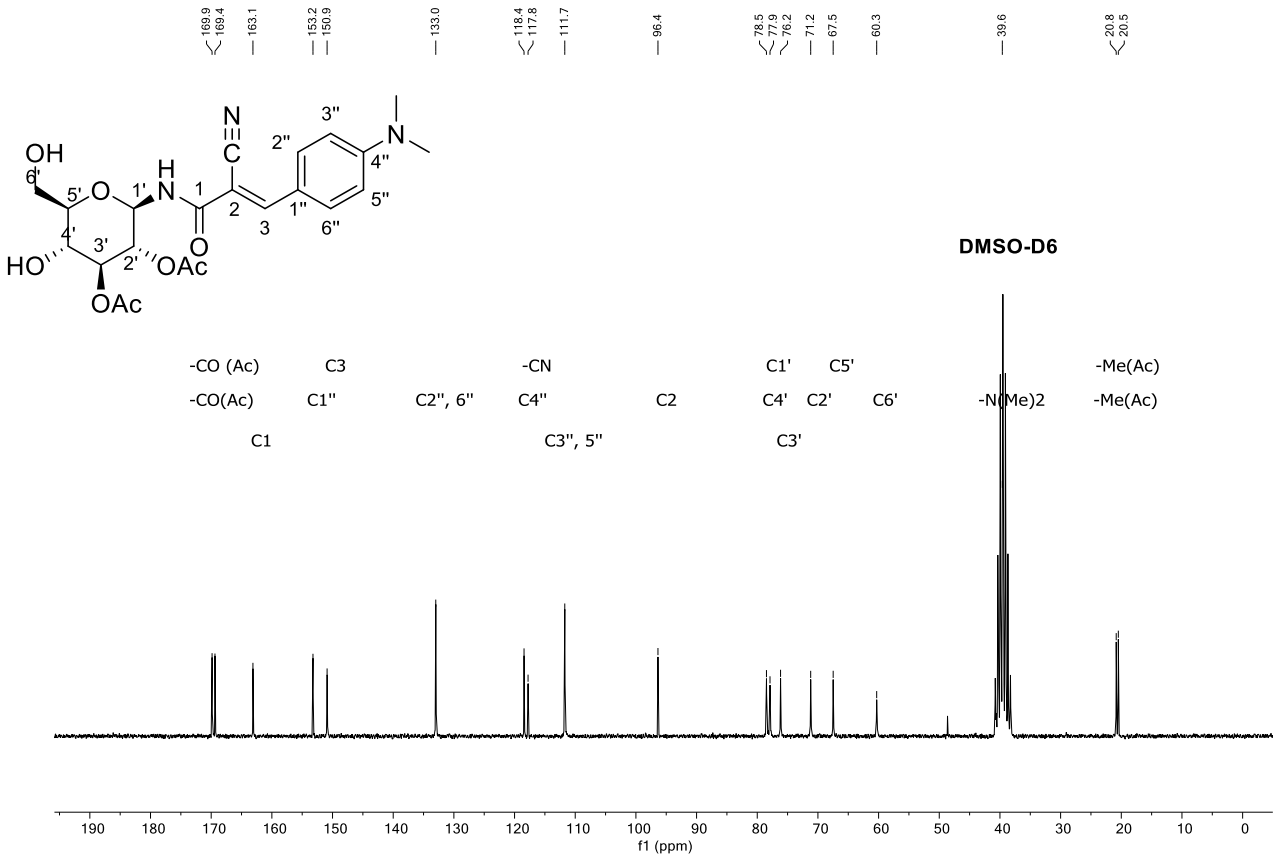


Figure S11. ^{13}C NMR spectrum of compound **2d** (100 MHz, DMSO-d_6).

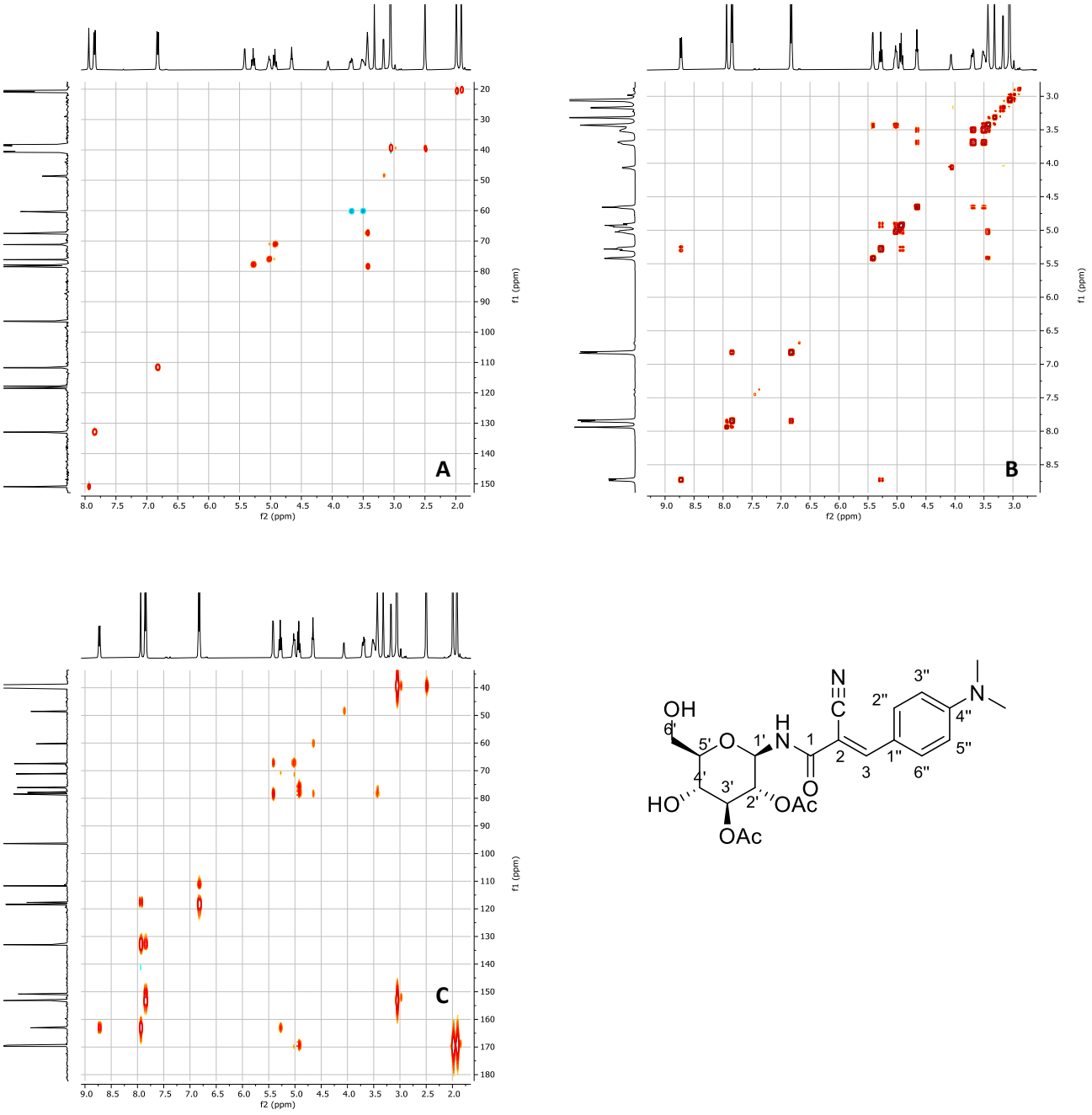
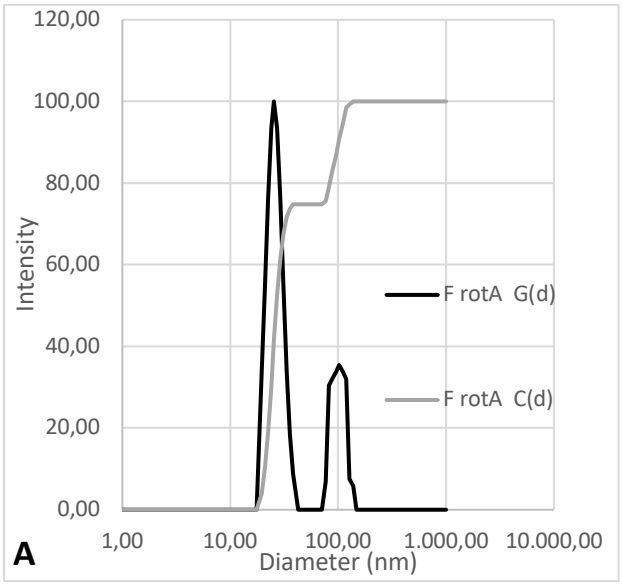
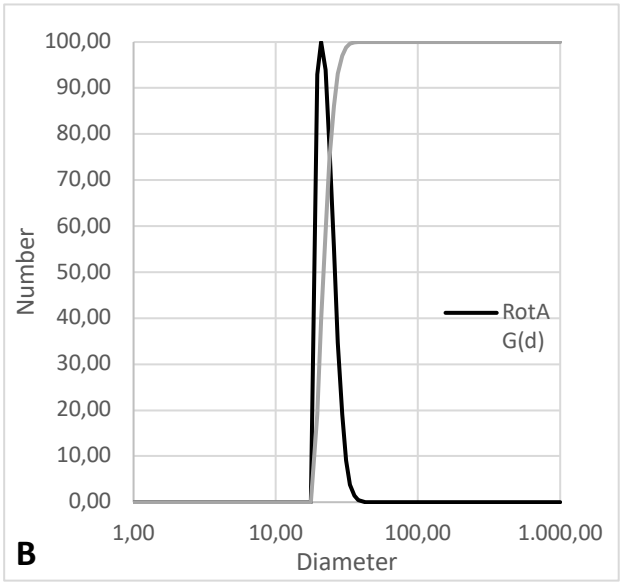


Figure S12. COSY (**A**), HSQC (**B**) and HMBC (**C**) NMR spectra of compound **2d** (DMSO-d_6).



A



B

C

pH	Degrees	Diameter (nm)	Intensity	Polydispersity	Count Rate (kcps)
7.4	90°	21 102	100 35	0.190	313.64

Figure S13. Dynamic Light Scattering analysis results for PEO_{228}-*b*-$PHIS_{44}$ - **RotA** nanoparticles. Shown are (**A**) the intensity measurement and (**B**) population analysis of the main peak at 21 nm. The second peak in (**A**) is of negligible population and is believed to be due to aggregation of smaller particles occurring during the measurement. (**C**) Experimental parameters and results of the measurements.

Enzyme kinetic and crystallographic studies

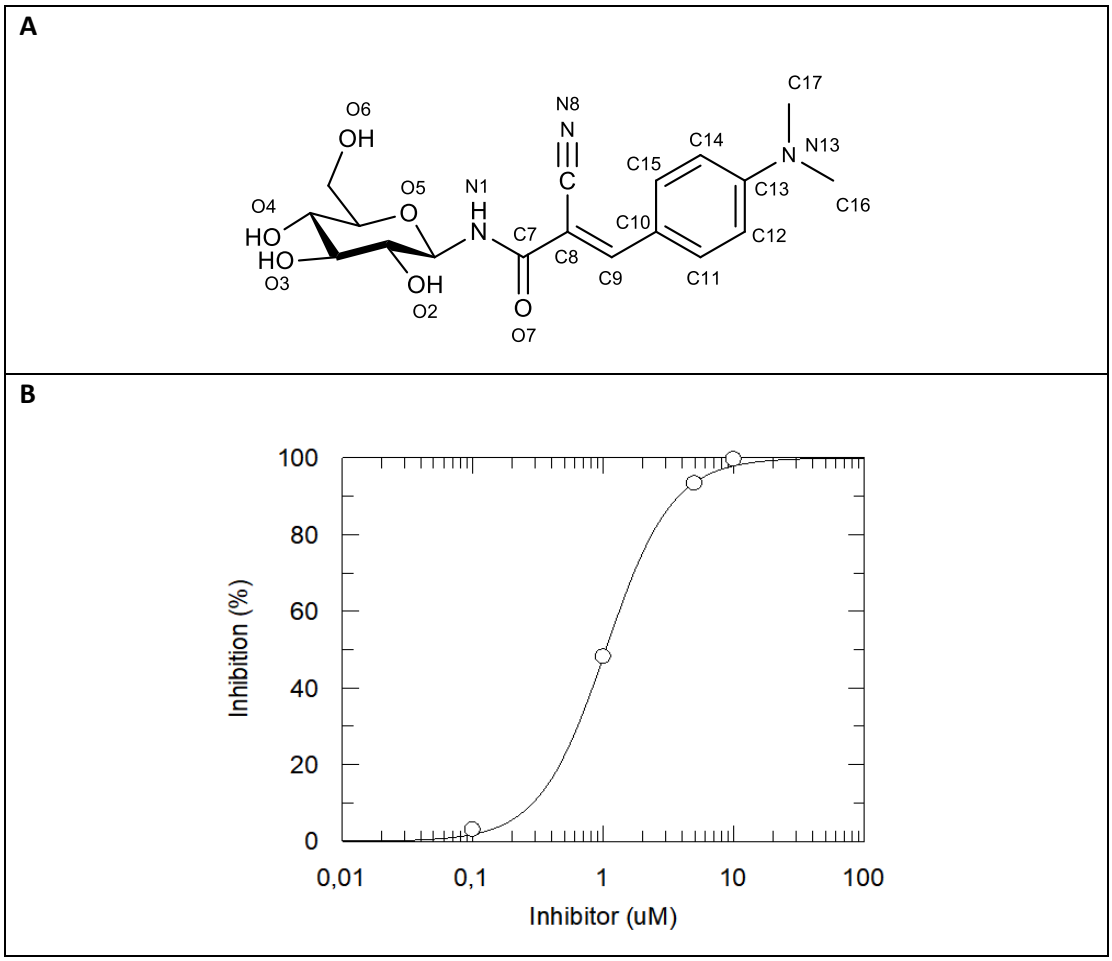


Figure S14: **(A)** Skeletal formula and numbering of **RotA** showing the numbering system used. **(B)** Schematic representation of the % inhibition of RMGP*b* activity calculated in the direction of glycogen synthesis in the presence of 2 mM glucose-1-phosphate, 1 % glycogen, 1 mM AMP and 0.1, 1, 5 and 10 µM inhibitor concentration. The IC50 calculated was 1.041 ± 0.035 µM.

Table S1. X-ray crystallographic analysis of RMGP*b* in complex with **RotA**.

Data collection & processing statistics	
Experiment	T-state RMGP*b* crystals soaked with 10 mM of **RotA** in 20% (v/v) DMSO, pH 6.8, exposed at room temperature
Beamline	P13, PETRA III (DESY/EMBL-Hamburg, Germany)
Wavelength (Å)	0.9763
Oscillation range (°)	0.2
No of images (°)	1800 (360)
Space group	$P4_32_12$
Unit cell dimensions (a, b, c) (Å), (α, β, γ)(°)	a=b=128.6 Å, c=116.7 Å α=β=γ=90°
No of molecules per asymmetric unit	1
Resolution (Å)	128.6 – 1.80
Highest resolution bin (Å)	1.83 – 1.80
[*]No of observations	1850583 (91125)
[*]No of unique reflections	90796 (4430)
[*]Multiplicity	20.4 (20.6)
[*]Completeness (%)	100.0 (100.0)
[a,*]R_{merge} (I)	0.076 (1.884)
[a,*]R_{pim} (I)	0.017 (0.437)
[a,*]$<I/\sigma(I)>$	21.4 (1.7)
[a,*]$CC_{1/2}$	0.999 (0.769)
Wilson B value (Å^2)	31.54
Refinement statistics and model quality	
Resolution range (Å)	30.0 – 1.80
No of reflections used (free)	86023 (4661)
Residues included	(13 – 251) (258 – 314) (325 – 836)
No of protein atoms	6614
No of water molecules	250
No of heteroatoms	27 (**RotA**), 15 (PLP), 4 (CO_3-901), 4 (CO_3-902), 4 (CO_3-903), 4 (BME)
[b]Final R (R_{free})	0.159 (0.180)
r.m.s deviation in	

bond lengths (Å)	0.005
bond angles (°)	1.312
Molprobity analysis	
Ramachandran favoured / outliers (%)	97.87 / 0.00
Clashscore all atoms	1.06 (100th percentile)
Overall score	0.84 (100th percentile)
Poor rotamers outliers (%)	0.71
Average B (Å^2) for protein residues	(13 - 251) (258 - 314) (325-836)
Overall	40.9
Ca, C, N, O	37.8
Side chain	43.8
Average B (Å^2) for heteroatoms	30.0 (**RotA**), 28.7 (PLP), 63.6 (CO3-901), 57.2 (CO3-902), 52.4 (CO3-903), 61.9 (BME)
Average B (Å^2) for water molecules	43.2

*Numbers in parentheses refer to the highest resolution bin unless stated otherwise,

[a]Indicators for assessing the collected data quality as described in Karplus & Diederichs (2016)[1].

[b]Crystallographic $R = \sum | |\mathbf{F}_o| - |\mathbf{F}_c| | / \sum |\mathbf{F}_o|$, where $|\mathbf{F}_o|$ and $|\mathbf{F}_c|$ are the observed and calculated structure factor amplitudes, respectively. R_{free} is the corresponding R value for a randomly chosen 5 % of the reflections that were not included in the refinement.

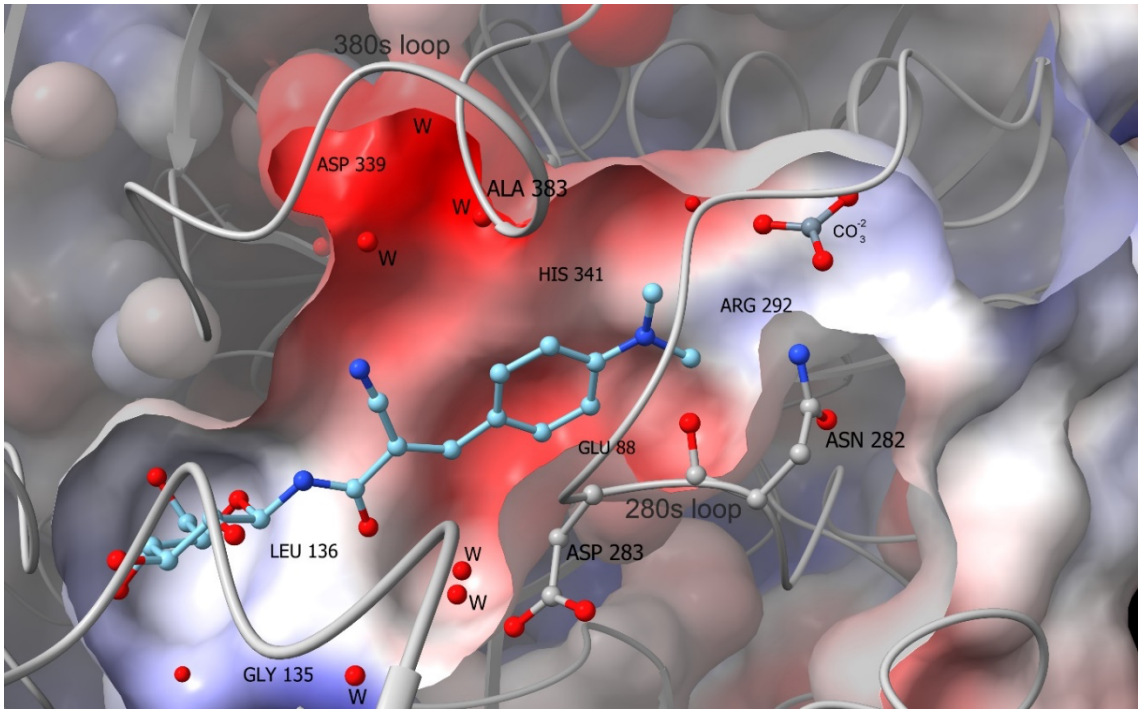


Figure S15: RMGP*b* catalytic site electrostatic (Coulomb) potential surface presentation. Coloring ranging from red for negative potential through white to blue for positive potential.

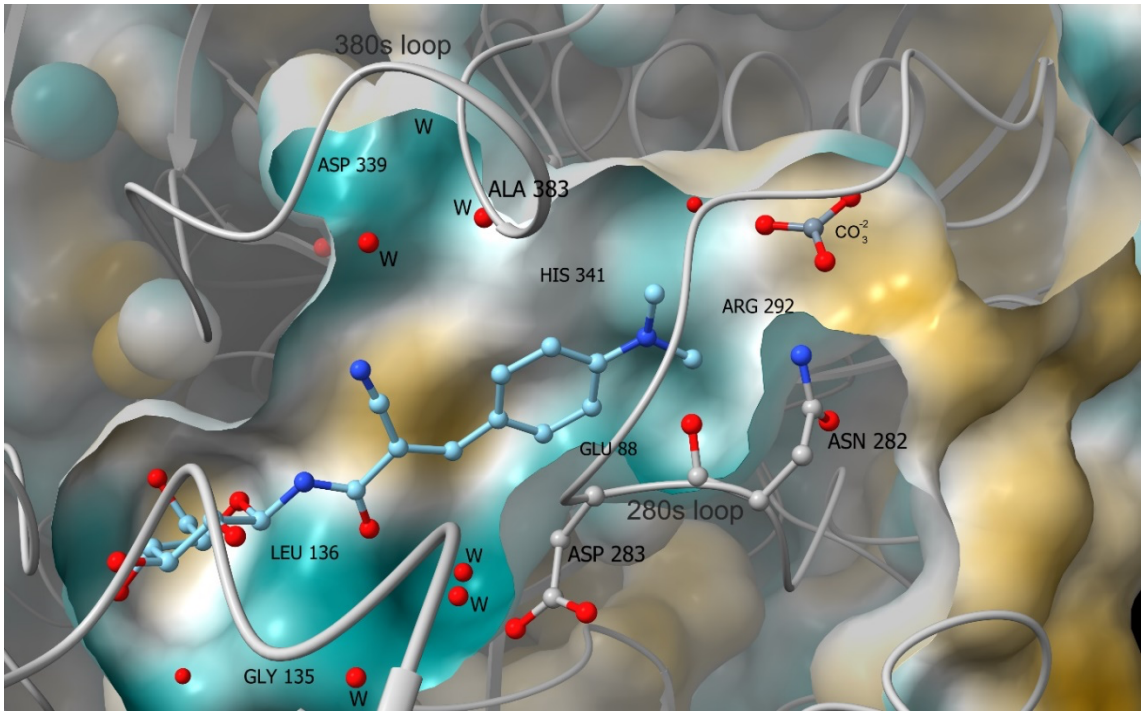


Figure S16: RMGP*b* active site hydrophobic surface representation indicating the binding mode of **RotA** at the catalytic channel. Coloring ranging from dark cyan (most hydrophilic) to white to dark goldenrod (most lipophilic).

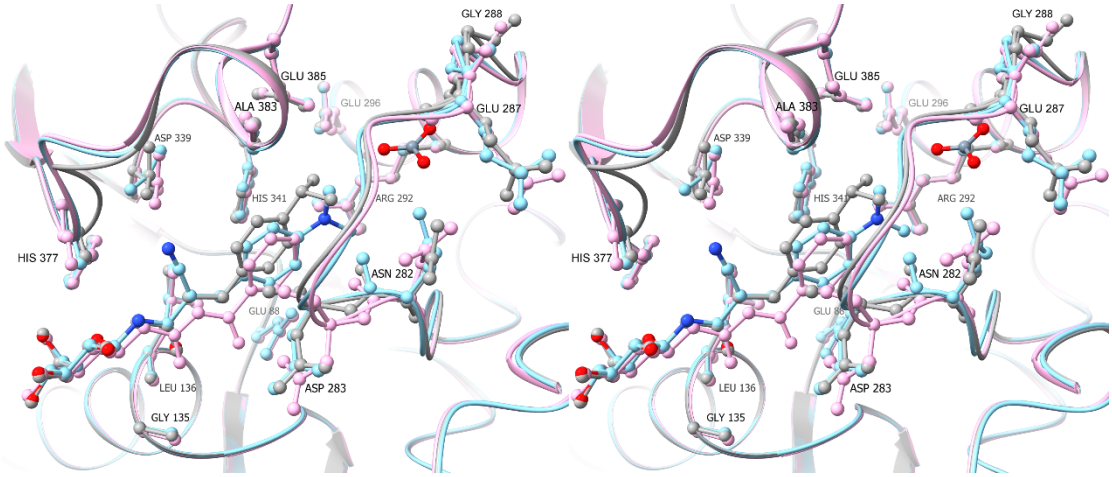


Figure S17: Stereo diagrams showing the complex structures of Bzurea (PDB ID: 1K06, depicted in pink) and **8** (PDB ID: 4MIC, depicted in light grey) superimposed onto **RotA** (PDB ID: 7Q5I, depicted in cyan) at the catalytic site of RMGP*b*.

Table S2. Hydrogen bond interactions of **RotA** at the catalytic site of RMGP*b*.

Inhibitor atom	Protein atom	Distance (Å)	Angle (°)
O2	Tyr573 OH	3.1	151.1
	Glu672 OE1	3.1	176.6
	Wat108 O	2.8	0.0
O3	Glu672 OE1	2.8	120.6
	Ser674 N	3.0	174.8
	Gly675 N	3.1	150.1
O4	Gly675 N	2.9	126.1
	Wat48 O	2.7	0.0
O6	Asn484 ND2	2.8	151.5
	His377 ND1	2.7	154.6
O7	Leu136 N	3.0	146.8
	Wat8 O	2.8	0.0
N8	Wat237 O	3.2	0.0
N13	Asn282 O	3.3	133.9
Total	**14**		

Table S3. Water-mediated hydrogen bond interactions of **RotA** at the catalytic site of RMGP*b*

Inhibitor atom	Solvent atom	Protein / solvent atom	
O2	**Wat108 O**	...Thr671 O ...Ala673 N **...Wat214 O**	 ...Thr378 OG1 … Thr671 O
O4	**Wat48 O**	...Thr676 OG1 … Llp680 OP2	
O7	**Wat8 O**	...Asp283 OD2 **...Wat12W O**	 ...Glu88 OE2 ...Gly134 N ...Gly137 N
		...Wat34 O	...Gly135 N ...Wat42 O ...Wat218 O
N8	**...Wat237 O**	...Asp 339 OD2 **...Wat 18 O**	 ...His341 NE2 ...Ala383 O ...Wat17 O

Table S4. Van der Waals interactions of **RotA** at the catalytic site of RMGP*b*

Inhibitor atom	Protein atom	No. of contacts
C1	His377 O; Wat218 O	2
C2	His377 O; Glu672 OE1; Wat108 O; Wat218 O	4
O2	His377 O; Wat218 O	2
C3	Glu672 OE1; Gly675 N; Wat48 O; Wat218 O	4
O3	Glu672 C, CG, CD; Ala673 C, N, CA, CB; Ser674 CA, C; Wat108 O	10
C4	Asn484 ND2; Gly675 N; Wat48 O	3
O4	Asn484 ND2; Ser674 CB, C; Gly675 C, O, CA; Thr676 N;	7
C5	Gly135 CA, C; Leu136 N; Wat48 O	4
O5	Leu136 CA, N; His377 CB, CG, ND1	5
C6	Gly135 O, C; Leu136 N; His377 ND1; Asn484 ND2	5
O6	Leu139 CD2; His377 CG, CE1; Val455 CB, CG1, CG2; Asn484 CG	7
C7	Leu136 N, CB; Wat8 O	3
O7	Gly135 N, C; Leu136 CA, CB; Wat12 O; Wat34 O;	6
C88	Wat237 O	1
N1	His377 CB, O	2
N8	His377 C, O; Thr378 CB, CG2	4
C9	Leu136 CB, CD1; Asp283 CB; Wat8 O	4
C10	Asp283 O, CA, CB; His341 CE1	4
C11	Glu88 OE1; Asp283 CA, CB; His341 CE1; Wat67 O;	5
C12	Glu88 OE1; Asp283 CA; His341 CE1; Wat29 O; Wat67 O	5

C13	Asn282 O; Asp283 CA; His341 CE1, NE2	4
N13	Arg292 NH2; Wat7 O; Asn282 O	3
C14	Asp 283 CA; His341 CE1, NE2; Wat18 O	4
C15	Asp283 O, CA; His341 CE1; Wat18 O	4
C16	Asn282 O, OD1, ND2; Arg292 CZ, NH1, NH2; Wat7 O; Wat29 O; CO3903 O2	9
C17	Asn282 O, ND2; Wat7 O; CO3903 O2	4
	Total	**115**

Table S5. Hydrogen bond interactions of **CO3** at the catalytic site of RMGP*b*.

Carbonate atom	Protein atom	Distance (Å)	Angle (°)
O1	Asn282A ND2	2.48	146.0
	Glu287 N	2.98	142.6
O2	Wat7 O	2.38	0.0
O3	Gly288 N	3.10	141.8
Total	4		

Table S6. Water-mediated hydrogen bond interactions of **CO3** at the catalytic site of RMGP*b*

Carbonate atom	Solvent atom	Protein / solvent atom
O2	**Wat7 O**	... Arg292 NE ...Arg292 NH2 ...Glu296 OE1 ...Glu385 OE2

Table S7. Van der Waals interactions of **CO3** at the catalytic site of RMGP*b*

Carbonate atom	Protein atom	No. of contacts
C	Asn282A ND2; Arg292 CG; Wat7; Phe286 CD1; Glu287 N; Gly 288 N	6
O1	Asn282 CG, OD1; Phe286 C; Glu287 CA, CG, CB	6
O2	Asn282 ND2; Lig998 C16, C17; Arg292 CG; Phe286 CD1, CE1; Glu287 N	7
O3	Arg 292 CB, CG; Phe286 CE1; Glu287 N, C; Gly288 CA; Lys 289 N;	7
	Total	**26**

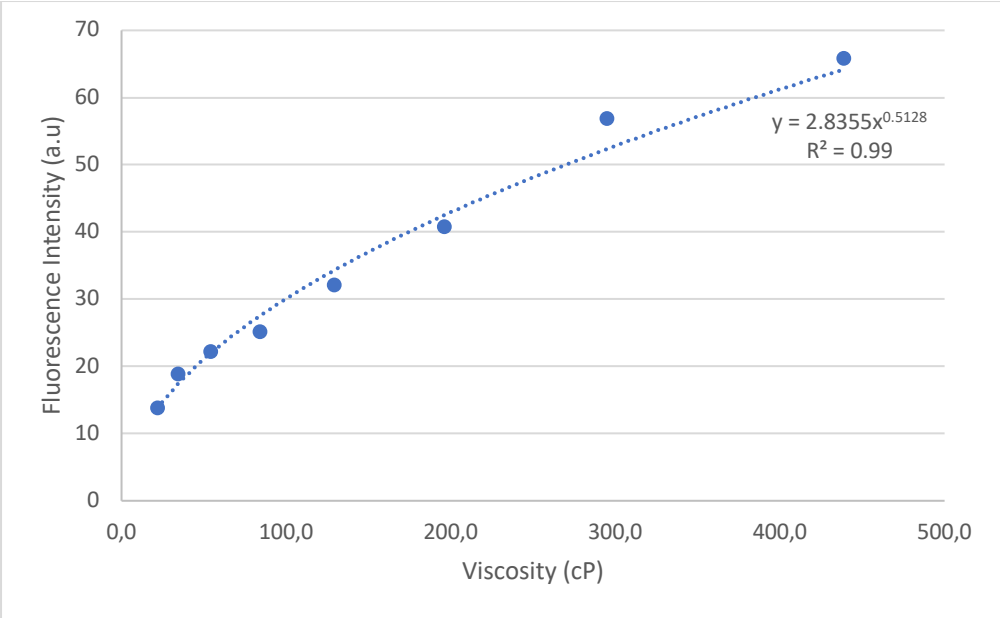


Figure S18. Viscosity-Emission intensity plot of **2b** (**AcRotA**).

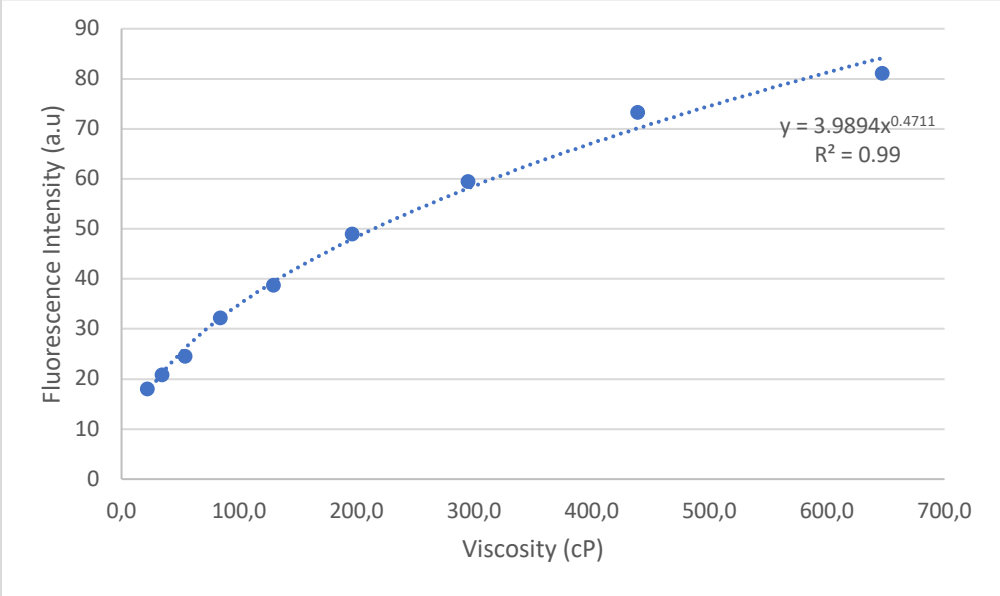


Figure S19. Viscosity-Emission intensity plot of **2a** (**RotA**).

Table S8. Final constituent concentrations of samples used in the RMGP*b*:**RotA** UV/Vis experiments, ratios 0 to 1.6.

[RMGP*b*]/[RotA] Ratio	**0.0**	**0.4**	**1.0**	**1.6**
[RotA] (µM)	29.4	29.4	29.4	29.4
[RMGP*b*] (µM)	0.0	11.5	30.0	47.2
[AMP] (µM)	5882	5882	5882	5882
DMSO (%$^{v}/_{v}$)	1.2	1.2	1.2	1.2
Glycerol (%$^{v}/_{v}$)	8.7	8.7	8.7	8.7

Table S9. Final constituent concentrations of samples used in the RMGP*b*:**RotA** UV/Vis experiments, ratios 0 to 10.0.

[RMGP*b*]/[RotA] Ratio	**0.0**	**1.6**	**2**	**5.0**	**7.5**	**10.0**
[RotA] (µM)	3.0	3.0	3.0	3.0	3.0	3.0
[RMGP*b*] (µM)	0.0	4.8	6.1	15.2	22.6	29.7
[AMP] (µM)	601	601	601	601	601	601
DMSO (%$^{v}/_{v}$)	0.1	0.1	0.1	0.1	0.1	0.1
Glycerol (%$^{v}/_{v}$)	0.6	0.6	0.6	0.6	0.6	0.6

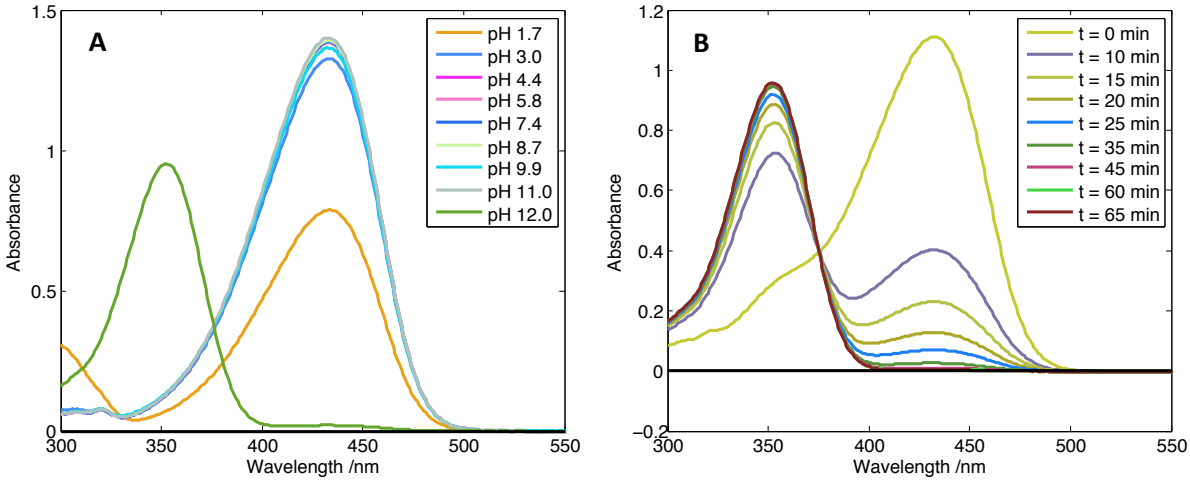


Figure S20: RotA absorption spectra in aqueous solution at (**A**) pH levels from 1.7 to 12.0 and (**B**) at pH 12.0 at various time points.

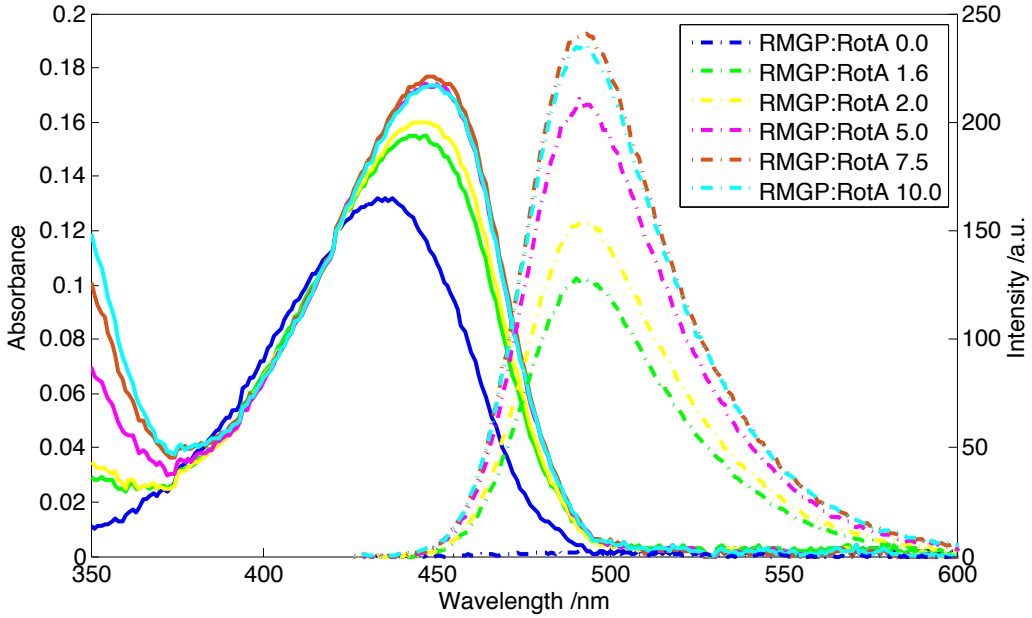


Figure S21: RMGP*b*:**RotA** complex absorption (left) and emission (right) spectra at RMGP*b*:**RotA** ratios from 0 to 10.

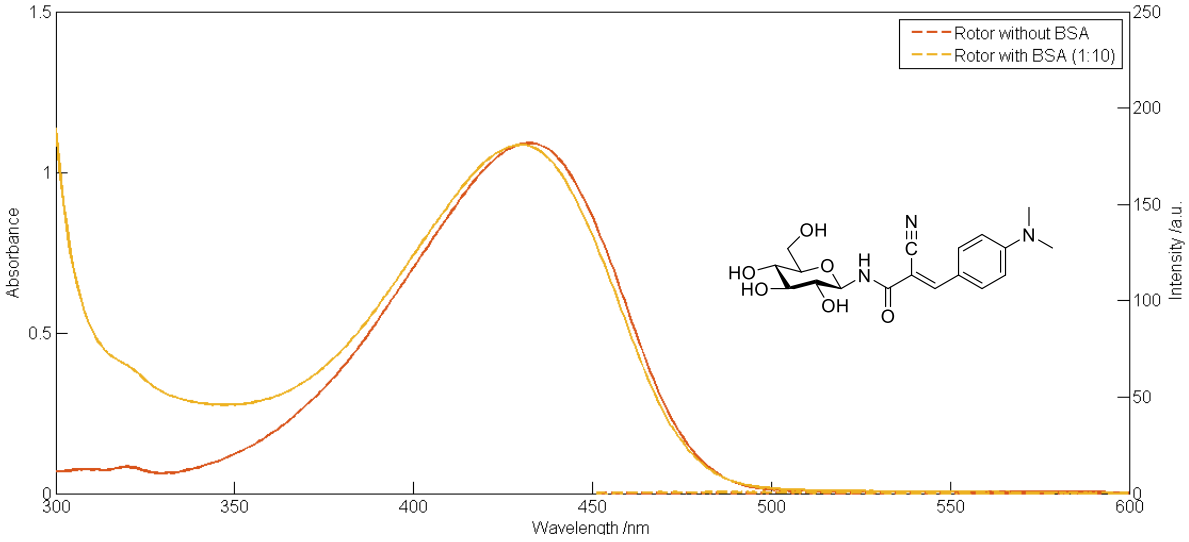


Figure S22: RotA absorption (left) and emission (right) spectra in the presence of bovine serum albumin, in Hepes buffer (pH= 7.4)

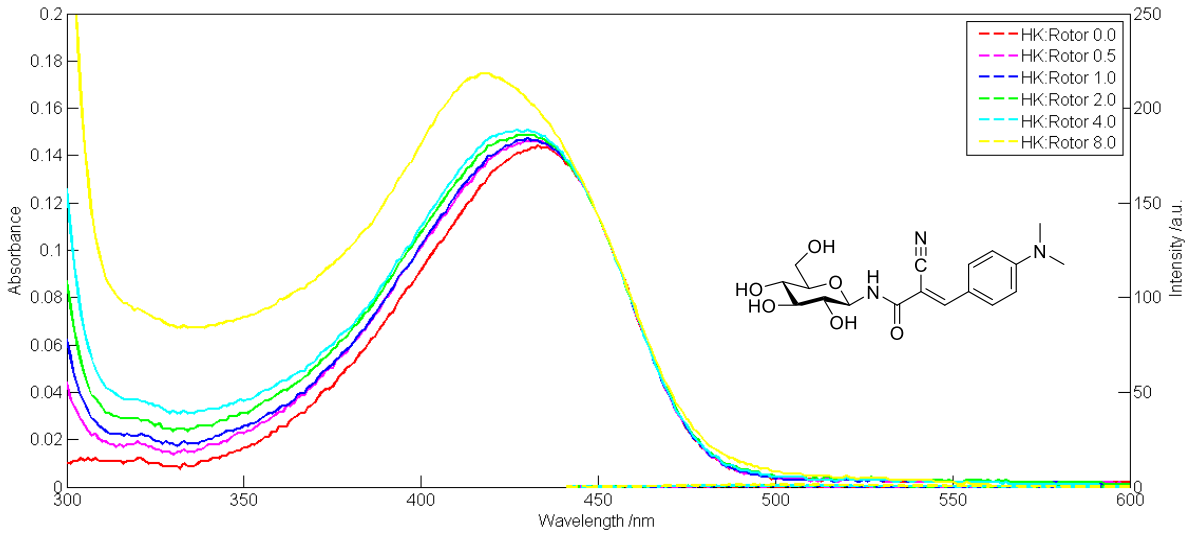


Figure S23 RotA absorption (left) and emission (right) spectra in the presence of hexokinase - III enzyme in phosphate buffer (pH= 7.4).

Table S10. Energies, structural and spectroscopic data computed to describe the S_1 excited state of **RotA**: dihedral angles δ_1 and δ_2, Energies E and free energies ΔG and ΔΔG, oscillator strength (*f*) and λ_{max} corresponding to the S_0 -> S_1 electronic transitions.

PES S_1	E (a.u.)	ΔG (a.u.)	ΔΔG (Kcal/mol)	δ_1	δ_2	λ (nm)	*f* (osc. Str.)
GS	-1315.19509	-1314.84346		176.3	179.4	363.1	1.177
M1	-1315.08355	-1314.73473	0.0	178.4	171.1	432	1.319
TS_{M1M2}	-1315.06348	-1314.71445	12.7	-91.1	173.5		
M2	-1315.07894	-1314.73077	2.5	-13.0	155.1	468.4	1.12
TS_{M2M3}	-1315.07873	-1314.72940	3.3	-9.3	138.9		
M3	-1315.10133	-1314.75001	-9.6	-0.6	90.1	2661.4	0.00

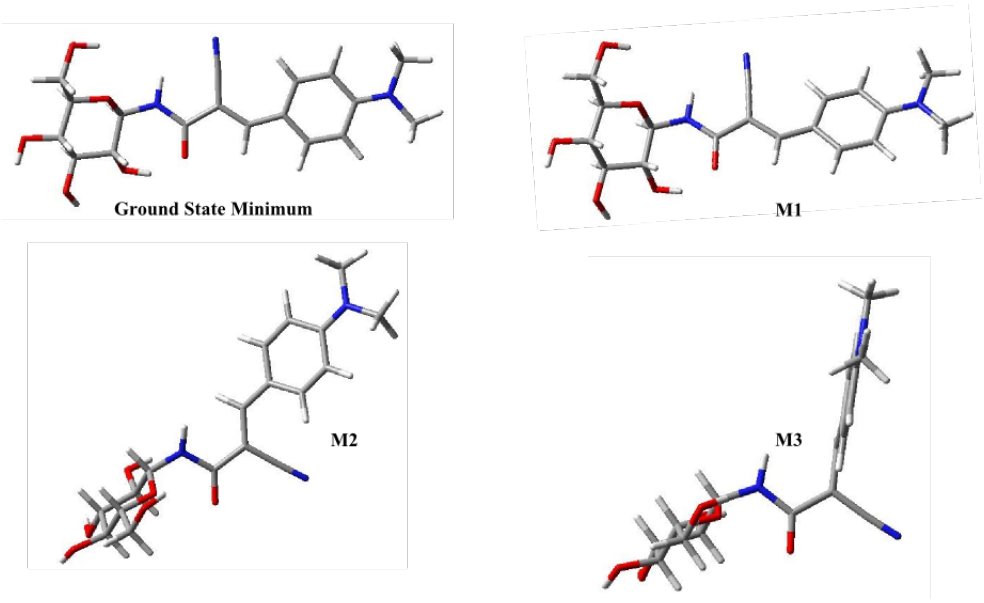


Figure S24. Geometries of the ground state minimum and of the three minima found in the excited state

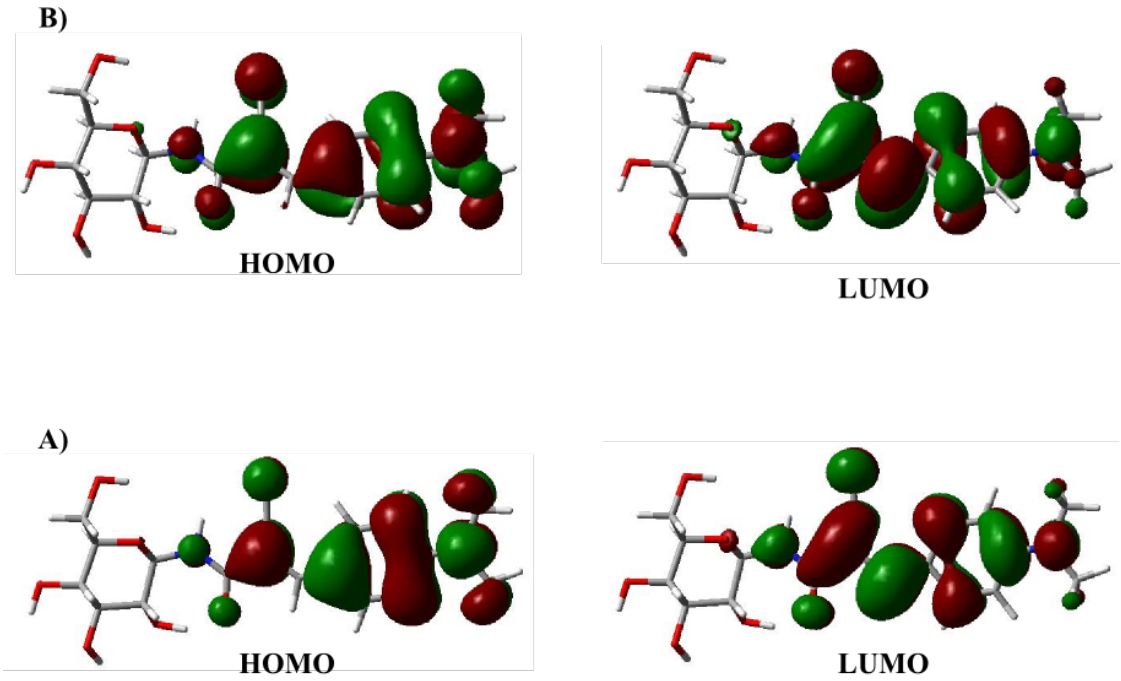


Figure S25. HOMO-LUMO orbitals responsible for (**A**) the absorption transition of Ground State (**GS**) and for (**B**) the emission from Excited State **M1**.

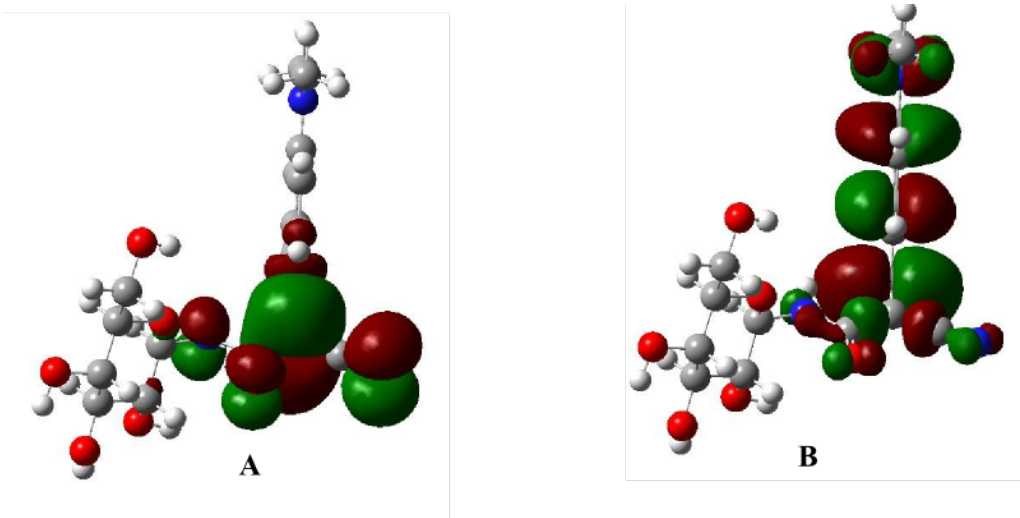


Figure S26. HOMO (**A**) and LUMO (**B**) orbitals of the **M3** minimum of the first excited state.

Table S11. Model Cartesian Coordinates

Ground State Minimum
E = -1315.19509
ΔG = -1314.843455

0 1

O	3.47611500	1.01738800	-0.16539400
C	4.90044800	1.08733100	-0.14302600
C	5.47723800	-0.23078400	-0.64006100
C	5.27655900	2.26713000	-1.01456200
C	4.92184800	-1.36642800	0.19764500
O	6.88400500	-0.16216400	-0.53508400
C	3.40009900	-1.34990000	0.21064100
O	5.40956000	-2.57391800	-0.34921500
C	2.95557900	0.02705000	0.71023700
O	2.99263600	-2.40046600	1.05327000
N	1.53099200	0.25588300	0.77262400
C	0.51371200	-0.52652200	0.33676800
O	0.64938100	-1.67656500	-0.06701200
C	-0.84740900	0.09663500	0.39618900
C	-0.94575700	1.45432700	0.81227100
N	-0.96403500	2.55447200	1.15876300
C	-1.91384000	-0.67161600	0.04319600
C	-3.31973000	-0.39544300	-0.02363900
C	-3.93124700	0.84357000	0.26707400
C	-5.29164900	1.01413300	0.17521400
C	-4.16981500	-1.45093700	-0.41523200
C	-6.14369700	-0.05535800	-0.21660100
C	-5.53325800	-1.30239900	-0.51145200
N	-7.48340200	0.11154900	-0.30511700
C	-8.33272900	-1.00061200	-0.70480900
C	-8.08491900	1.40138800	0.00017300
O	4.72078900	3.46453900	-0.51004800
H	5.24230700	1.27042200	0.88562100
H	5.16952500	-0.38618700	-1.68395400
H	5.28143300	-1.24508100	1.23034900
H	3.03361200	-1.49149200	-0.81111400
H	3.36705200	0.18935500	1.71580800
H	1.29416500	1.17653100	1.11914800
H	-3.73096200	-2.41531700	-0.64809500
H	-6.13324200	-2.14776600	-0.81586700
H	-8.24334400	-1.83733400	-0.00610200
H	-8.07558800	-1.35469900	-1.70718400
H	-9.36721900	-0.66930800	-0.71280300
H	-9.16067800	1.32783800	-0.13142400
H	-7.70843500	2.18180800	-0.66712000
H	-7.88320300	1.69813800	1.03331700
H	-5.70985600	1.98309200	0.40719000
H	-3.33520700	1.69391300	0.56916100
H	-1.62937900	-1.68253200	-0.23885100
H	2.06424900	-2.56035000	0.82811400
H	4.99737100	-3.29091500	0.14607200
H	7.22116600	-1.04655600	-0.71631900
H	4.93627200	2.07037500	-2.03926000
H	6.36049500	2.37894900	-1.02110400
H	3.77608200	3.30388700	-0.41177500

Excited StateM1
E = -1315.08358
ΔG = -1314.73473

0 1

O	-3.46472239	1.01403578	0.21009930
C	-4.88706822	1.09724332	0.16194931
C	-5.49079113	-0.24425484	0.55523752
C	-5.27390353	2.21911094	1.10291329
C	-4.92123602	-1.33358542	-0.33332046
O	-6.89372442	-0.15214735	0.41455553
C	-3.40104950	-1.32385198	-0.29686087
O	-5.43179184	-2.56756530	0.12849528
C	-2.93493751	0.07121565	-0.72038020
O	-2.96011358	-2.34157484	-1.16221094
N	-1.51409300	0.27752243	-0.77714605
C	-0.50609814	-0.50859212	-0.28251702
O	-0.68518651	-1.66841515	0.10865129
C	0.83994278	0.08243705	-0.26746585
C	0.96157438	1.43711804	-0.63879366
N	0.99760636	2.55245691	-0.95806492
C	1.90907795	-0.71900448	0.20721256
C	3.29464525	-0.45348649	0.16447822
C	3.90751310	0.63697145	-0.54401709
C	5.26226352	0.82718865	-0.53592362
C	4.18838352	-1.35022308	0.84725310
C	6.13491431	-0.05944084	0.16781532
C	5.53735058	-1.17044282	0.85617549
N	7.46926046	0.13371766	0.18591212
C	8.34307603	-0.78042675	0.91019530
C	8.06159057	1.25690007	-0.52757612
O	-4.68936840	3.44045278	0.69724837
H	-5.20514714	1.35256891	-0.85916200
H	-5.21659016	-0.46970123	1.59573400
H	-5.25091202	-1.14990993	-1.36686579
H	-3.07000430	-1.50606922	0.73020763
H	-3.34753044	0.29692882	-1.71369773
H	-1.25639069	1.19440850	-1.11680416
H	3.76165634	-2.19382305	1.37753350
H	6.15942796	-1.87469791	1.39033957
H	8.27415953	-1.79156339	0.49895604
H	8.07695507	-0.81155002	1.97010874
H	9.36749233	-0.43448480	0.81781307
H	9.13751973	1.23658316	-0.38746754
H	7.67305651	2.20506881	-0.14515772
H	7.84343269	1.19380802	-1.59753557
H	5.67249631	1.66511888	-1.08188881
H	3.29698484	1.32584111	-1.10971781
H	1.59769464	-1.65966668	0.64521257
H	-2.03718198	-2.49483312	-0.90518134
H	-4.98704153	-3.25754396	-0.37707512
H	-7.24568635	-1.04072821	0.53745212
H	-4.96317308	1.94753223	2.11998788
H	-6.35622925	2.34584812	1.08977784
H	-3.74663651	3.26744078	0.59956064

neutro TS_{M1M2}
E = -1315.06348
ΔG = -1314.71445

0 1

O	-3.25308794	1.07651752	-0.19481418
C	-4.57485832	1.24034847	-0.70552095
C	-5.48572283	0.18635089	-0.09207124
C	-4.98600144	2.65600027	-0.35935072
C	-4.91040298	-1.18955708	-0.36586120
O	-6.76805911	0.32463111	-0.66755462
C	-3.47181934	-1.29260048	0.11914704
O	-5.73363073	-2.13177269	0.28949649
C	-2.67226755	-0.17006985	-0.54663735
O	-3.01791573	-2.57651193	-0.23580203
N	-1.27868745	-0.08202245	-0.16810210
C	-0.57530199	-0.79344604	0.74335326
O	-0.99802485	-1.76043725	1.36251281
C	0.84570626	-0.31134411	0.91785712
C	1.03045268	0.58021282	1.97676561
N	1.13028770	1.33436555	2.86176595
C	1.83341803	-0.91143270	0.10189671
C	3.19325062	-0.55390731	-0.01609021
C	3.79186568	0.58954838	0.62002459
C	5.11898231	0.88466493	0.45949268
C	4.05899069	-1.35078031	-0.84404264
C	5.96876815	0.07589477	-0.35469603
C	5.37717228	-1.06058904	-1.00991071
N	7.27984241	0.35949187	-0.50681927
C	8.12908067	-0.47415580	-1.34752250
C	7.86708485	1.50953916	0.16545385
O	-4.11321578	3.59455769	-0.95484458
H	-4.56277900	1.12122874	-1.79828293
H	-5.52639524	0.33657428	0.99618936
H	-4.92582648	-1.36292955	-1.45226383
H	-3.45069077	-1.14670691	1.20419197
H	-2.72198413	-0.29427786	-1.63713813
H	-0.77388346	0.66969524	-0.61857080
H	3.63822195	-2.21503750	-1.34536206
H	5.98236991	-1.69707605	-1.63993467
H	7.76538736	-0.48321984	-2.37870748
H	8.15644807	-1.50162674	-0.97416940
H	9.13700039	-0.07201755	-1.33667871
H	8.92489292	1.55602062	-0.07247480
H	7.75245432	1.42329269	1.24986773
H	7.38962595	2.43681498	-0.16491301
H	5.52266803	1.75341629	0.96059798
H	3.18892517	1.24037415	1.23677521
H	1.49178908	-1.73137497	-0.52182364
H	-2.24629358	-2.73889679	0.32646474
H	-5.32202148	-2.99485580	0.16708636
H	-7.28770376	-0.43306937	-0.37676275
H	-4.99966482	2.76138737	0.73298117
H	-5.98910973	2.84335375	-0.74174492
H	-3.22244298	3.34214860	-0.68891444

neutro M2
E = -1315.07894
ΔG = -1314.73077

0 1

O	0.36047900	-2.66988600	1.52598600
C	0.42230600	-4.03498000	1.92783800
C	1.86772500	-4.51474600	1.90818700
C	-0.20197300	-4.09160200	3.30672700
C	2.44031000	-4.31620000	0.51812100
O	1.88428800	-5.87965200	2.27194400
C	2.29988700	-2.86927200	0.08786700
O	3.79449300	-4.72239000	0.55822100
C	0.82695800	-2.45834500	0.19985600
O	2.74181400	-2.80371200	-1.25416700
N	0.59905200	-1.09838300	-0.19106500
C	1.15868100	-0.02027500	0.47192300
O	2.00414500	-0.15194200	1.35232300
C	0.68630300	1.29013300	0.01244400
C	1.14354900	2.37783700	0.78710700
N	1.49165600	3.28823700	1.41532600
C	-0.05563100	1.48559700	-1.19040000
C	-0.94185000	2.54604100	-1.47003900
C	-1.39850000	3.49198600	-0.48980300
C	-2.26545400	4.49784500	-0.81500300
C	-1.47496000	2.68898500	-2.79540800
C	-2.76394200	4.64733000	-2.14660500
C	-2.33704100	3.68919900	-3.12761100
N	-3.60211500	5.64937400	-2.47369400
C	-4.10447100	5.78383200	-3.83569300
C	-4.03295800	6.60999000	-1.46618100
O	-1.53725800	-3.62868900	3.27156600
H	-0.17133900	-4.64902300	1.23452000
H	2.45377700	-3.91326800	2.61771200
H	1.87619500	-4.94823500	-0.18397300
H	2.90475100	-2.23433200	0.73913800
H	0.25003900	-3.08368900	-0.49368000
H	-0.26878400	-0.92232700	-0.67698100
H	-1.16457200	1.98024800	-3.55470900
H	-2.69694400	3.75890800	-4.14436000
H	-4.68441700	4.90308600	-4.12487800
H	-3.27966400	5.91140300	-4.54178800
H	-4.74651300	6.65716700	-3.88840300
H	-4.68591000	7.34077700	-1.93241600
H	-3.17291400	7.13000000	-1.03564400
H	-4.58047400	6.10953700	-0.66241500
H	-2.57832100	5.18641400	-0.04281700
H	-1.06357900	3.40524000	0.53467500
H	0.08071700	0.74934500	-1.97817800
H	2.76052400	-1.87839500	-1.51871100
H	4.16461700	-4.57047700	-0.31808900
H	2.77434500	-6.20314400	2.09260400
H	0.40908500	-3.49164900	3.99345500
H	-0.21464500	-5.12283200	3.65846200
H	-1.51091800	-2.77046300	2.83436500

neutro TS_{M2M3}
E = -1315.07873
ΔG = -1314.72940

0 1

O	0.69149300	-3.28903400	1.33603100

C	1.04967300	-4.61183800	1.72429400
C	2.53095400	-4.66495100	2.07429400
C	0.15260100	-4.96064500	2.89381700
C	3.34247900	-4.19278600	0.88342100
O	2.85628400	-5.99724800	2.41279100
C	2.89293200	-2.81128500	0.44887100
O	4.70307700	-4.20341300	1.26952600
C	1.38381100	-2.84217800	0.17766200
O	3.61313900	-2.50642300	-0.72955300
N	0.86636300	-1.57344200	-0.24271700
C	0.90266400	-0.44229100	0.55117000
O	1.53134100	-0.38948300	1.60434500
C	0.16554200	0.70167700	0.00935900
C	0.11506500	1.83619300	0.84225500
N	0.01957600	2.78846600	1.49834000
C	-0.38492700	0.75295100	-1.31994500
C	-1.63977100	1.28103200	-1.65670800
C	-2.58244800	1.76284100	-0.68577700
C	-3.80389700	2.25165600	-1.05516500
C	-2.05524300	1.31877000	-3.02993200
C	-4.19742500	2.30245600	-2.42849700
C	-3.26762900	1.81106900	-3.40610400
N	-5.39124500	2.80102300	-2.79651300
C	-5.77097300	2.85981600	-4.20343400
C	-6.32795100	3.28977200	-1.79199800
O	-1.20889900	-4.88997900	2.51994500
H	0.84957400	-5.30474200	0.89364900
H	2.72333800	-3.98827700	2.91916100
H	3.17989200	-4.89375000	0.05104100
H	3.10323700	-2.09215200	1.24367000
H	1.20412500	-3.54237500	-0.64823300
H	0.14199700	-1.60221400	-0.94677400
H	-1.37005000	0.95236200	-3.78612700
H	-3.52573800	1.83123900	-4.45542200
H	-5.80629900	1.85807200	-4.63976900
H	-5.06234900	3.46852000	-4.77110300
H	-6.75600800	3.30883600	-4.28267700
H	-7.22717800	3.63903100	-2.28926700
H	-5.89353900	4.11931000	-1.22735100
H	-6.59942200	2.49280700	-1.09468200
H	-4.47703600	2.60832300	-0.28859000
H	-2.32635800	1.73257900	0.36508900
H	0.20423100	0.32147500	-2.12713200
H	3.43102900	-1.59105700	-0.96487200
H	5.21457400	-3.87525400	0.52204700
H	3.81744800	-6.04191200	2.46876400
H	0.37358800	-4.27579000	3.72271000
H	0.35799300	-5.98060200	3.21763200
H	-1.33040900	-4.02389700	2.11556900

neutro M3
E = -1315.10133
ΔG = -1314.75001

0 1

O	0.61297600	-3.13079600	1.13587900
C	0.83436200	-4.47279500	1.56338700
C	2.25115800	-4.61360500	2.10369700
C	-0.22967600	-4.75826300	2.60258100
C	3.23779600	-4.19653000	1.02975200
O	2.44735600	-5.96184100	2.47466000
C	2.93473400	-2.79219500	0.54119800
O	4.53264100	-4.28513500	1.58894600
C	1.47223300	-2.73538600	0.08179600
O	3.81429300	-2.54274800	-0.53689900
N	1.07520600	-1.43087500	-0.37447400
C	1.09193800	-0.32128400	0.41172500
O	1.56510100	-0.26197200	1.53082400
C	0.45955200	0.87483100	-0.22931200
C	0.42291400	2.04132700	0.57014400
N	0.35401400	3.02505500	1.17275900
C	-0.11220000	0.90582400	-1.54952400
C	-1.47952200	0.60483600	-1.79249300
C	-2.36514100	0.20138300	-0.76464900
C	-3.68780300	-0.09025900	-1.01808100
C	-2.01623300	0.69045500	-3.10099600
C	-4.21803600	0.00096600	-2.32842800
C	-3.33221500	0.40183100	-3.36566500
N	-5.52211700	-0.28447400	-2.58715100
C	-6.03585300	-0.19159800	-3.94391100
C	-6.40345500	-0.69859400	-1.50839300
O	-1.52263000	-4.61277700	2.05056600
H	0.70221800	-5.15402100	0.71023800
H	2.37269000	-3.94680200	2.96928900
H	3.14204400	-4.89099900	0.18178600
H	3.08959200	-2.08310800	1.35809800
H	1.35674200	-3.41457600	-0.77160700
H	0.56150900	-1.36963000	-1.24290800
H	-1.36677400	0.99486300	-3.91443200
H	-3.68959200	0.48500200	-4.38235800
H	-5.50991900	-0.87502900	-4.61717200
H	-5.94019500	0.82477600	-4.33692900
H	-7.08910400	-0.45826700	-3.94120100
H	-7.39658100	-0.87606000	-1.91157500
H	-6.47811900	0.07431100	-0.73774500
H	-6.05179700	-1.62248500	-1.03926200
H	-4.31940400	-0.39379300	-0.19533500
H	-2.00195300	0.11653900	0.25378100
H	0.51794600	1.20973500	-2.38320300
H	3.80723200	-1.60033300	-0.73002500
H	5.15670300	-4.00206500	0.91174300
H	3.38784100	-6.06549700	2.65834600
H	-0.08082300	-4.08066600	3.45308200
H	-0.12880700	-5.78568300	2.95105700
H	-1.54505100	-3.74026900	1.64250600

Western blot analysis of GP expression in A431 and HepG2 cells.

Cells at 80% confluency, were washed twice with ice-cold PBS, harvested by scraping and lysed using RIPA buffer (150 mM NaCl, 25 mM Tris pH 7.6, 0.5% sodium deoxycholate, 0.1% SDS, 1% NP-40), supplemented with 1x protease (Complete Protease Inhibitor Cocktail Tablets) and protein phosphatase (PhosSTOP Phosphatase Inhibitor Cocktail Tablets) inhibitors (both from Roche). After incubation on ice for 30 min, the lysates were centrifuged at 10,000 x g for 15 min at 4°C. Total protein concentration in the supernatant was quantified by the Bradford Assay (BioRad Laboratories, Richmond, California, USA). Equal amounts of total protein (50 µg protein per sample) were separated in 10% (w/v) SDS-PAGE gels and blotted onto nitrocellulose membranes (Porablot NCP; Macherey-Nagel, Düren, Germany). Membranes were first blocked with Tris-buffered saline containing 5% bovine serum albumin (BSA, Serva, Heidelberg, Germany) and 0.1 % Tween-20 (TBS-T buffer) for 1 h at room temperature and then incubated overnight at 4^{o}C with the appropriate primary antibodies against GP [1:1000 in TBS-T containing 5% (w/v) BSA, rabbit polyclonal antibody pAbGPBB NBP2-47446, NovusBio] and β-actin [1:2500 in TBS-T containing 5% (w/v) BSA, monoclonal antibody (MAB1501, purchased from EMD Millipore (Temecula, CA, USA)]. After washing the membranes in TBS-T, the respective anti-mouse and anti-rabbit horseradish peroxidase conjugated secondary antibody, from Millipore (Billerica, MA, USA) and Jackson (West Grove, PA, USA), respectively [1:5000 in TBS-T containing 1% (w/v) non-fat dry milk], was added for 1 h at room temperature. The blots were washed in TBS-T and the resulting bands were detected by enhanced chemiluminescence reagent (ECL; Amersham Biosciences) in an 8800 FluorChem Imaging System (Alpha Innotech Corp., San Leandro, CA, USA).

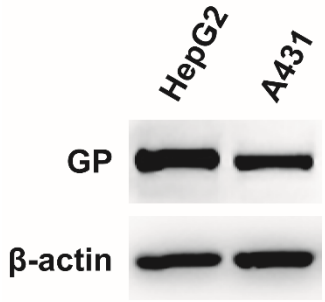


Figure S27. Representative Image of GP expression in HepG2 and A-431 cell lines using β-actin as an internal control for loading.