SUPPORTING INFORMATION (SI)

A multidisciplinary structural approach for the identification of *Haemophilus influenzae* type b capsular polysaccharide protective epitope

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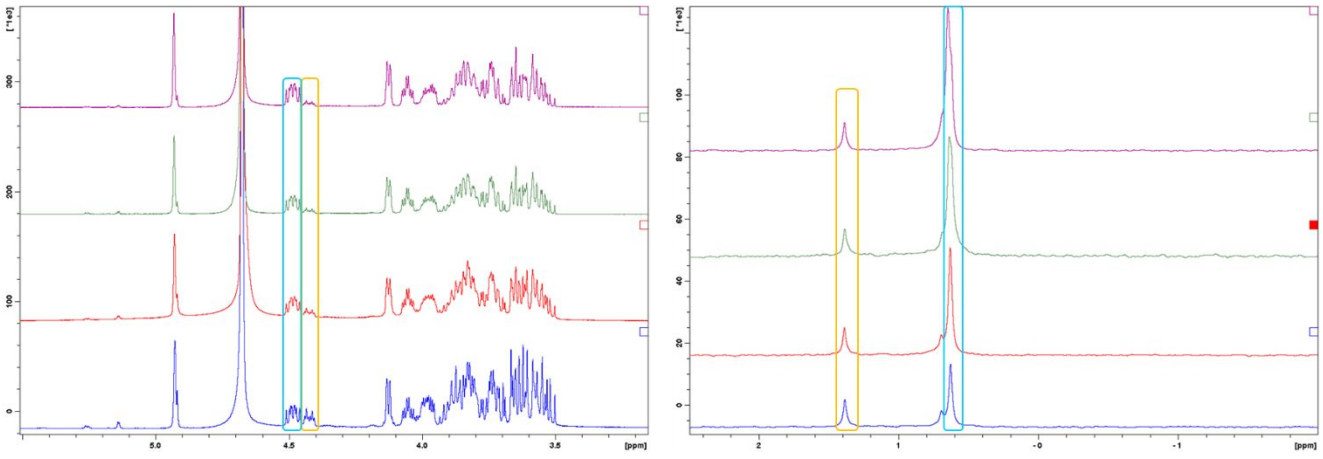
A.



B.

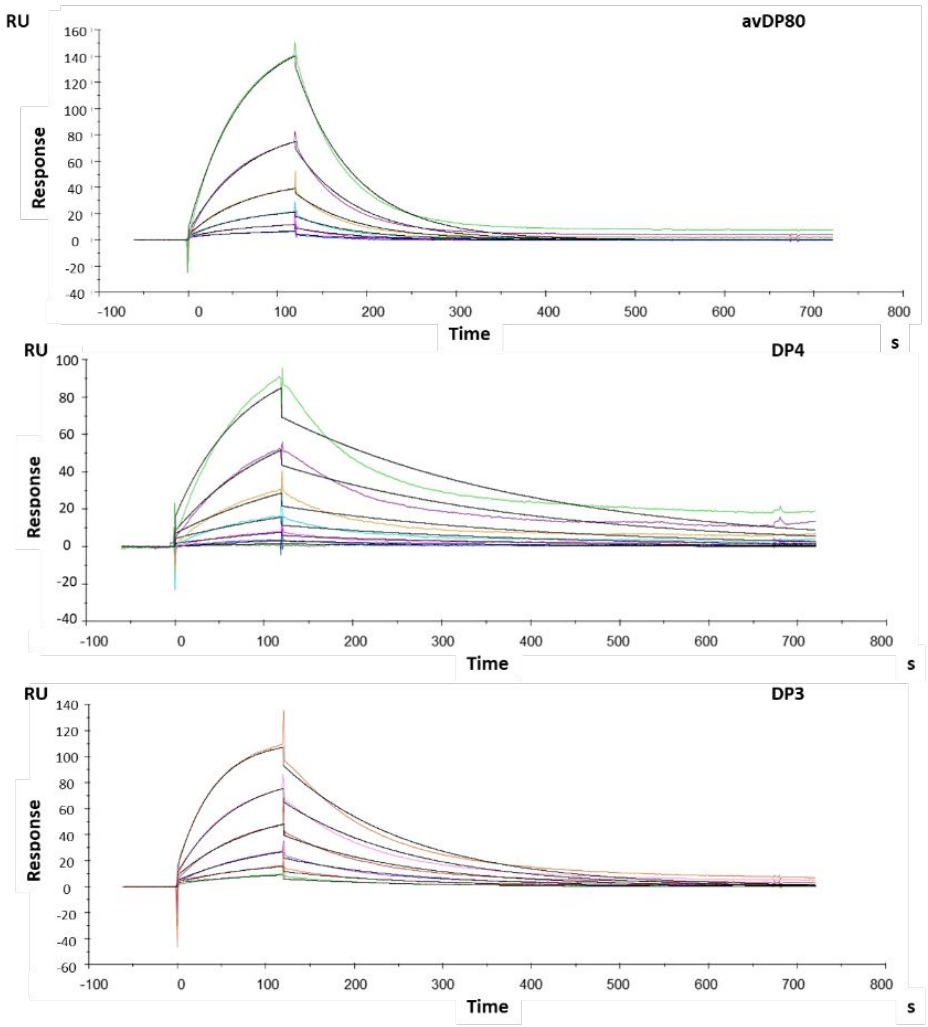
OS	Formula	Exact Mass	Calculated $[M-H]^{-1}$	Detected $[M-H]^{-1}$	Calculated $[M-2H]^{-2}$	Detected $[M-2H]^{-2}$	Calculated $[M-3H]^{-3}$	Detected $[M-3H]^{-3}$	Calculated $[M-4H]^{-4}$	Detected $[M-4H]^{-4}$
DP2	$C_{20}H_{40}O_{23}P_2$	710.14	709.14	709.13	354.06	354.06	235.71	n.d.	176.53	n.d.
DP3	$C_{30}H_{59}O_{34}P_3$	1056.21	1055.20	1055.20	527.10	527.10	351.06	351.06	263.04	n.d.
DP4	$C_{40}H_{78}O_{45}P_4$	1402.28	1401.27	1401.25*	700.13	700.13	466.42	466.42	349.56	349.53
DP5	$C_{50}H_{97}O_{56}P_5$	1748.34	1747.34	1747.34*	873.16	873.16	581.77	581.78	436.08	436.07

n.d.=not detected

*obtained from the deconvoluted spectrum

Figure S1. A) ^{1}H-NMR spectra (left) and ^{31}P-NMR spectra (right) of the produced Hib oligosaccharide fragments: DP2 (blue), DP3 (red), DP4 (green), DP5 (purple). Signals of H3-ribose within the chain and at the reducing terminal units are indicated in light blue and yellow boxes, respectively (left panel). The relative intensity of the reducing terminal H3-ribose signal decreased with increasing chain length. Signals of phosphate groups of terminal RU and within the chain are circled yellow and light blue, respectively (right panel). **B)** DP2-DP5 mass detected by negative mode ESI-MS.

A.



B.

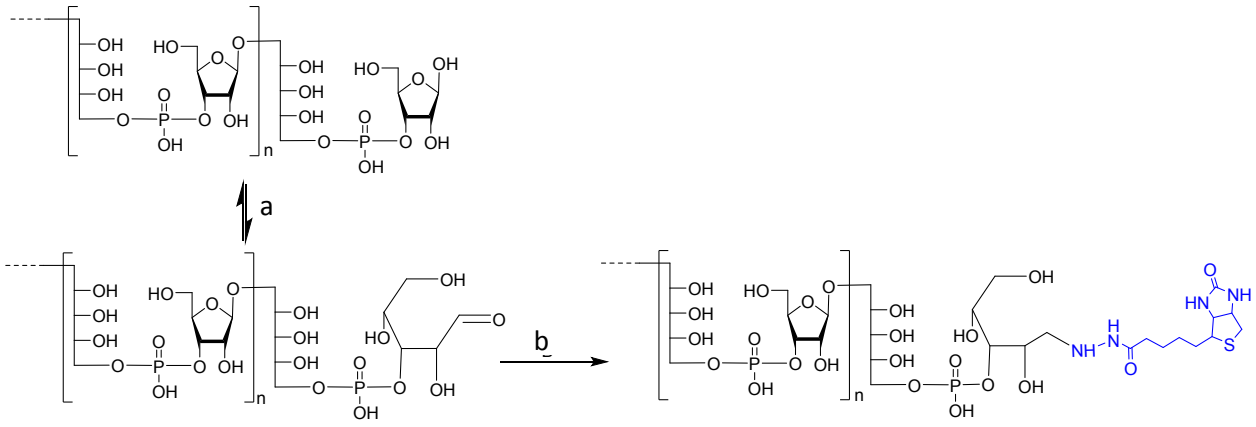


Figure S2. A) Binding kinetics of Fab CA4 to avDP80, DP4 and DP3 fragments by SPR. **B)** Sugar forms in water [25] (a) and biotinylation (b)

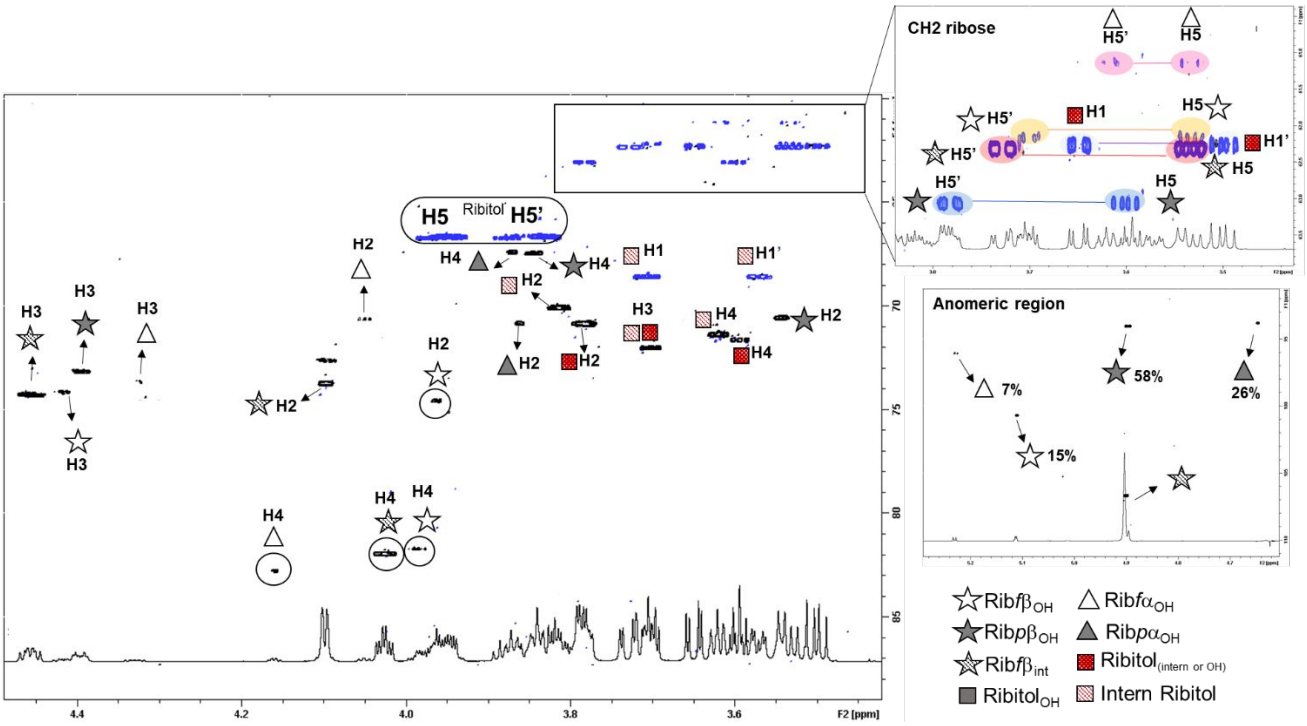


Figure S3. ^{1}H-^{13}C full HSQC spectrum of Hib DP3 1 mM in D_2O with the relative peak assignment. The experiment was acquired with 500 µL of sample transferred into a 5 mm NMR tube. The assignment for Hib DP2 is identical to the one here reported for DP3.

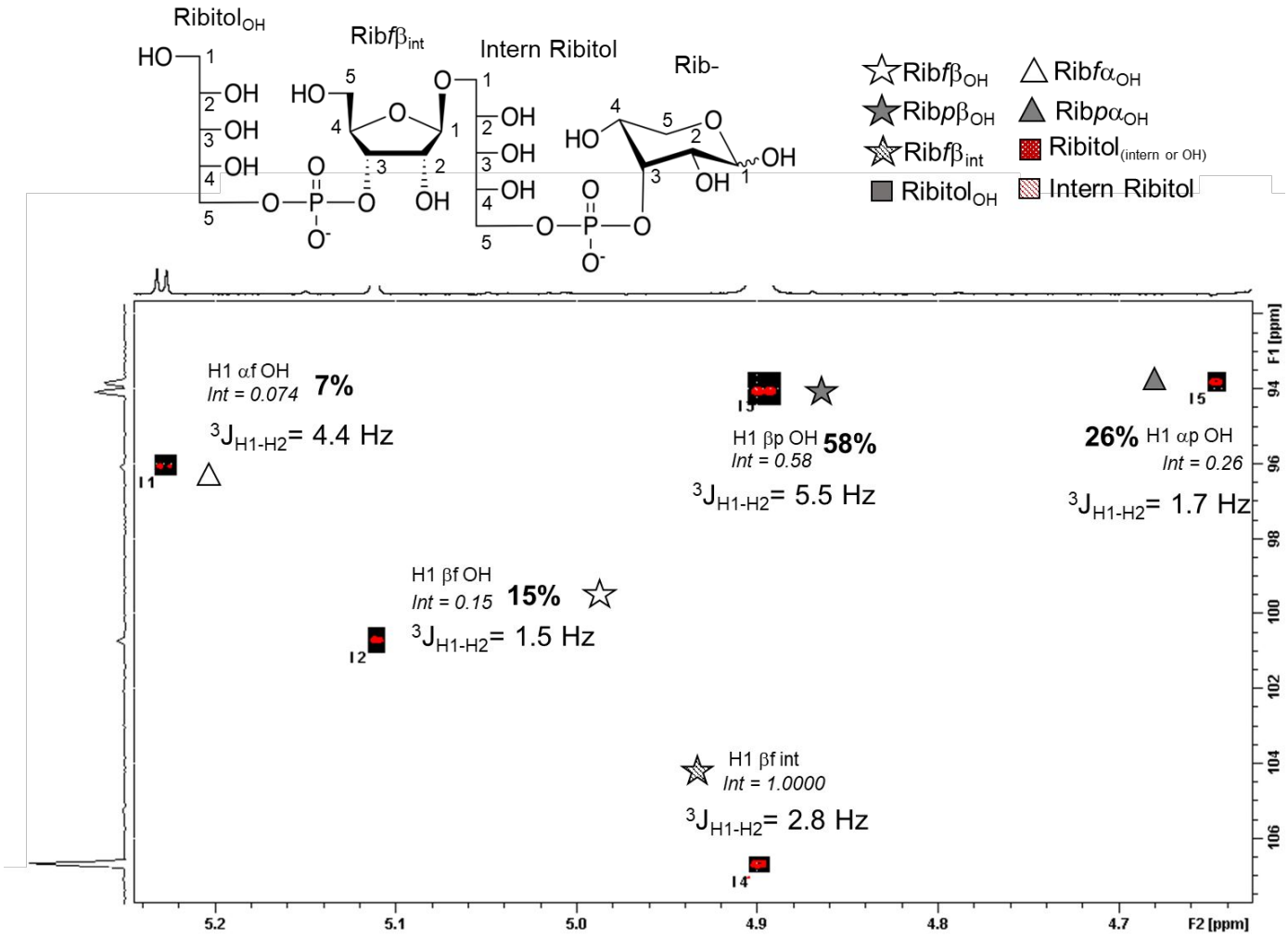


Figure S4. Zoom of the ^{1}H-^{13}C HSQC spectrum of Hib DP3 1 mM in D_2O on the anomeric region. The peak assignment is reported, together with the calculated $^3J_{H1-H2}$ couplings, the integrals for each signal and the relative percentage for each anomer. The experiment was acquired with 500 µL of sample transferred into a 5 mm NMR tube.

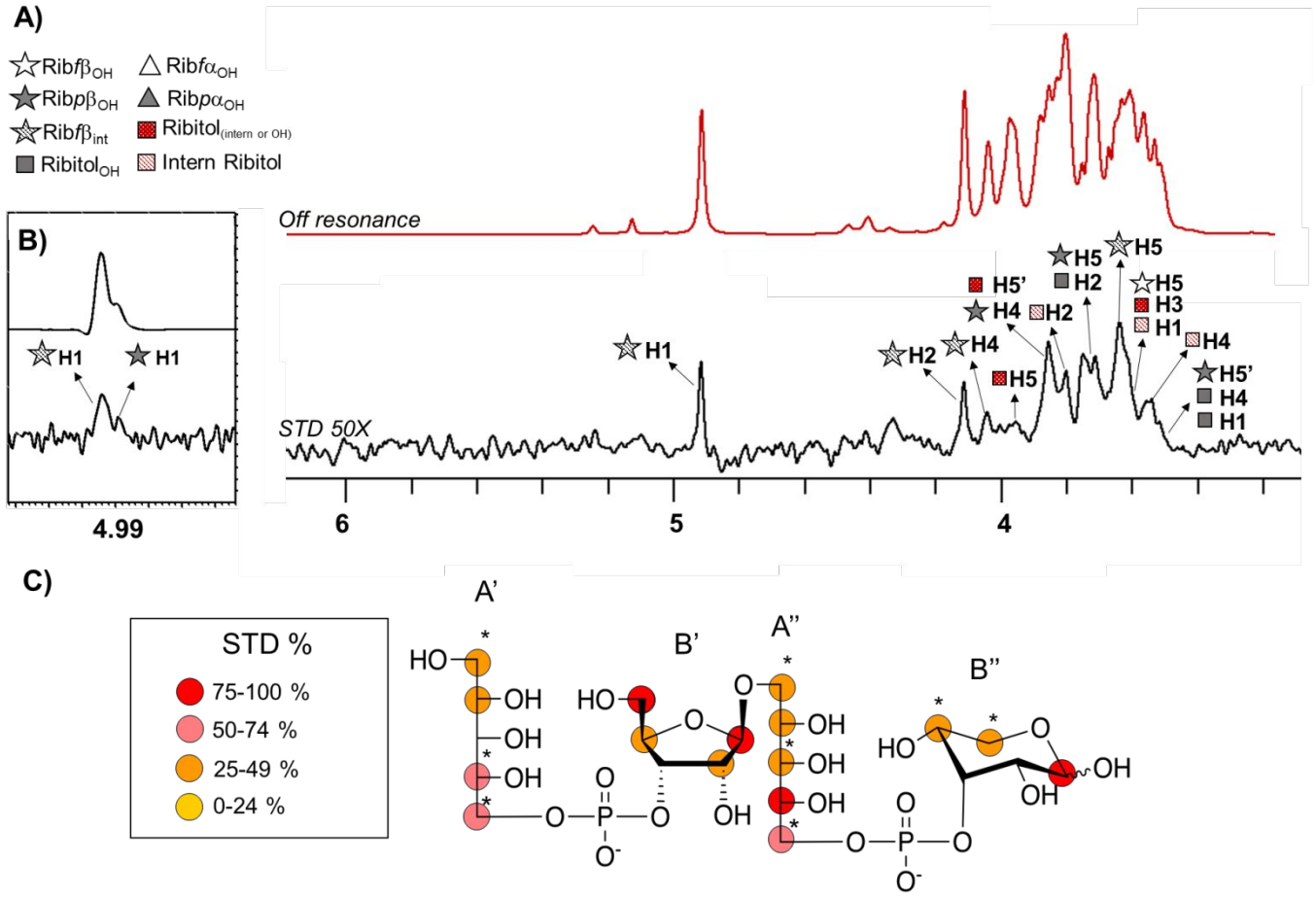


Figure S5. A) STD NMR experiments performed for the complex Hib DP2-CA4 hmAb. Off-resonance spectrum (irradiation at δ 100 ppm, top panel) and STD spectra obtained from aliphatic irradiation (δ 0.6 ppm) and processed applying exponential line-broadening. The main ^{1}H NMR signals are annotated in the STD spectrum and the pictorial notation for each residue is reported. The antibody/ligand molar ratio was 1:50, with the CA4 hmAb being at a concentration of 10 μM. The STD NMR experiments were acquired with 2 s of saturation time, 3 s of relaxation delay, and 2880 scans at 298 K. **B**) Expansion of the STD in the anomeric region of protons H1 Ribfβ$_{int}$ and Rib*p*β$_{OH}$ processed omitting the exponential multiplication for resolution enhancement. **C**) Representation of the epitope map disclosed by the analysis of the STD-NMR experiments of Hib DP2-CA4 hmAb and Hib DP3-CA4 hmAb complexes. The color legend of associated to STD% values is also reported.

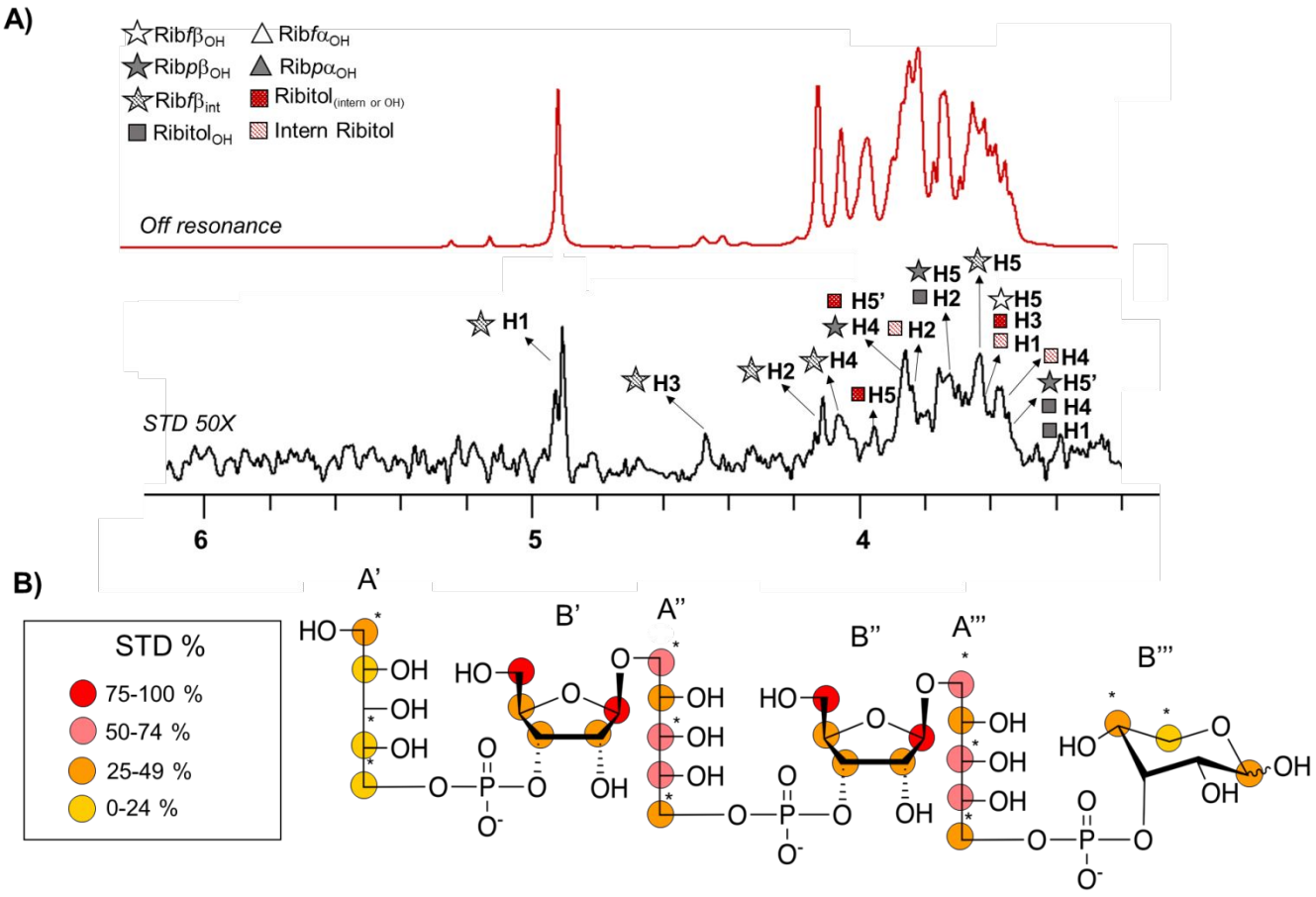
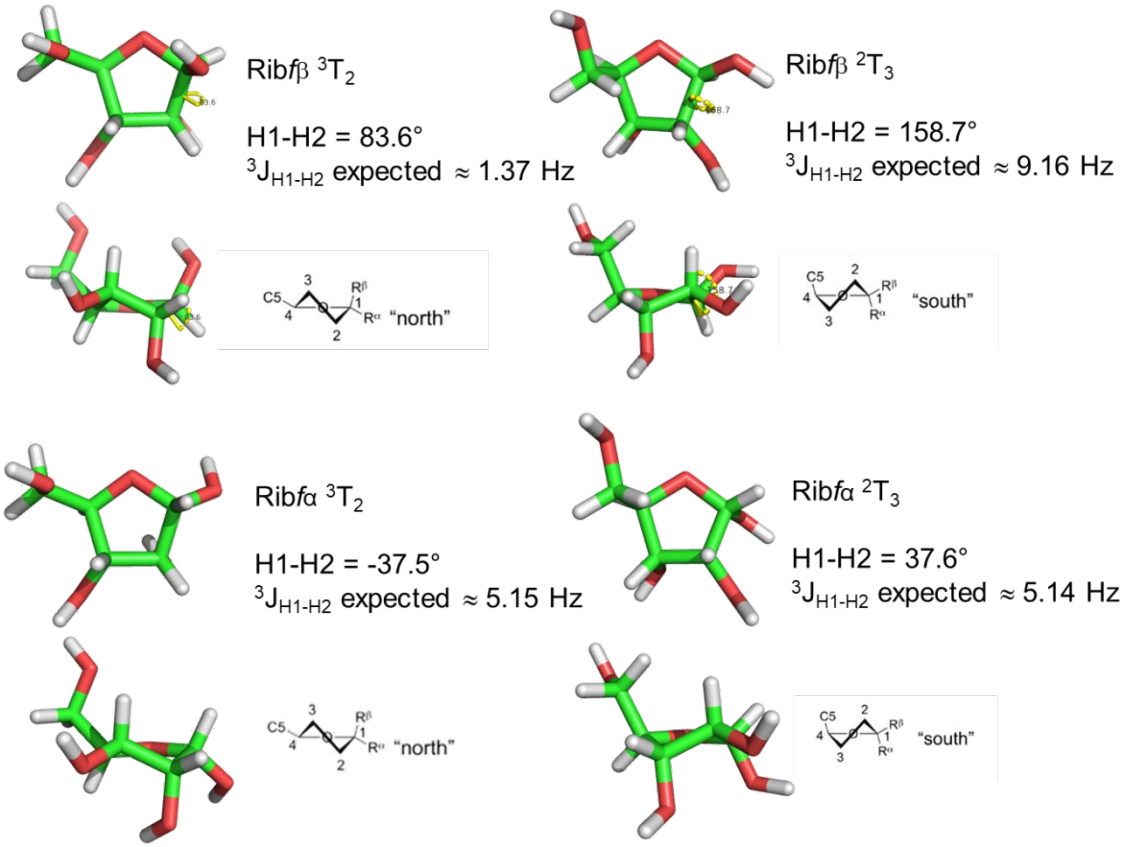


Figure S6. STD NMR experiments performed for the complex Hib DP3-CA4 hmAb. Off-resonance spectrum (irradiation at δ 100 ppm, top panel) and STD spectra obtained from aliphatic irradiation (δ 0.6 ppm) and processed applying exponential line-broadening. The main ^{1}H NMR signals are annotated in the STD spectrum and the pictorial notation for each residue is reported. The antibody/ligand molar ratio was 1:50, with the CA4 hmAb being at a concentration of 10 μM. The STD NMR experiments were acquired with 2 s of saturation time, 3 s of relaxation delay, and 2880 scans at 298 K. **B**) Representation of the epitope map disclosed by the analysis of the STD-NMR experiments of Hib DP3-CA4 hmAb and Hib DP3-CA4 hmAb complexes. The color legend associated to the STD% values is also reported.

A)



B)

	Experimental $^3J_{H1-H2}$	$p_{Rf\beta}$ 3T2	$p_{Rf\beta}$ 2T3
Internal Ribfβ	2.8 Hz	0.82	0.18
Reducing Ribfβ	1.5 Hz	0.98	0.02

	Experimental $^3J_{H1-H2}$	$p_{Rf\beta}$ 3T2	$p_{Rf\beta}$ 2T3
Reducing Ribfα	4.4 Hz	0.5	0.5

Figure S7. A) Study of the interconversion of ring conformations performed through J coupling analysis. Representation of the possible $^3T_2$ and $^2T_3$ conformations for Ribfβ (on the left) and Ribfα (on the right) with the $^3J_{H1-H2}$ expected values reported. **B**) Table reporting the measured experimental $^3J_{H1-H2}$ and the relative population for each moiety.

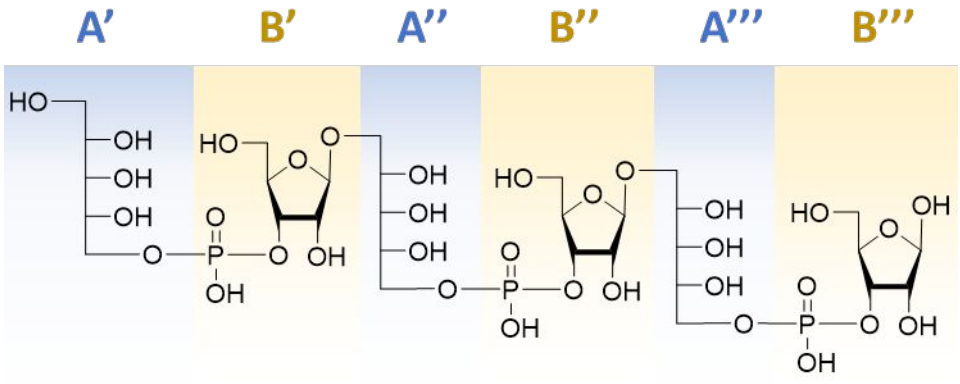


Figure S8. Ribitol and Ribose moieties of the DP3 fragment are individually indicated as A’, B’, A’’, B’’, A’’’, B’’’ starting from the Ribitol of the first repeating unit.

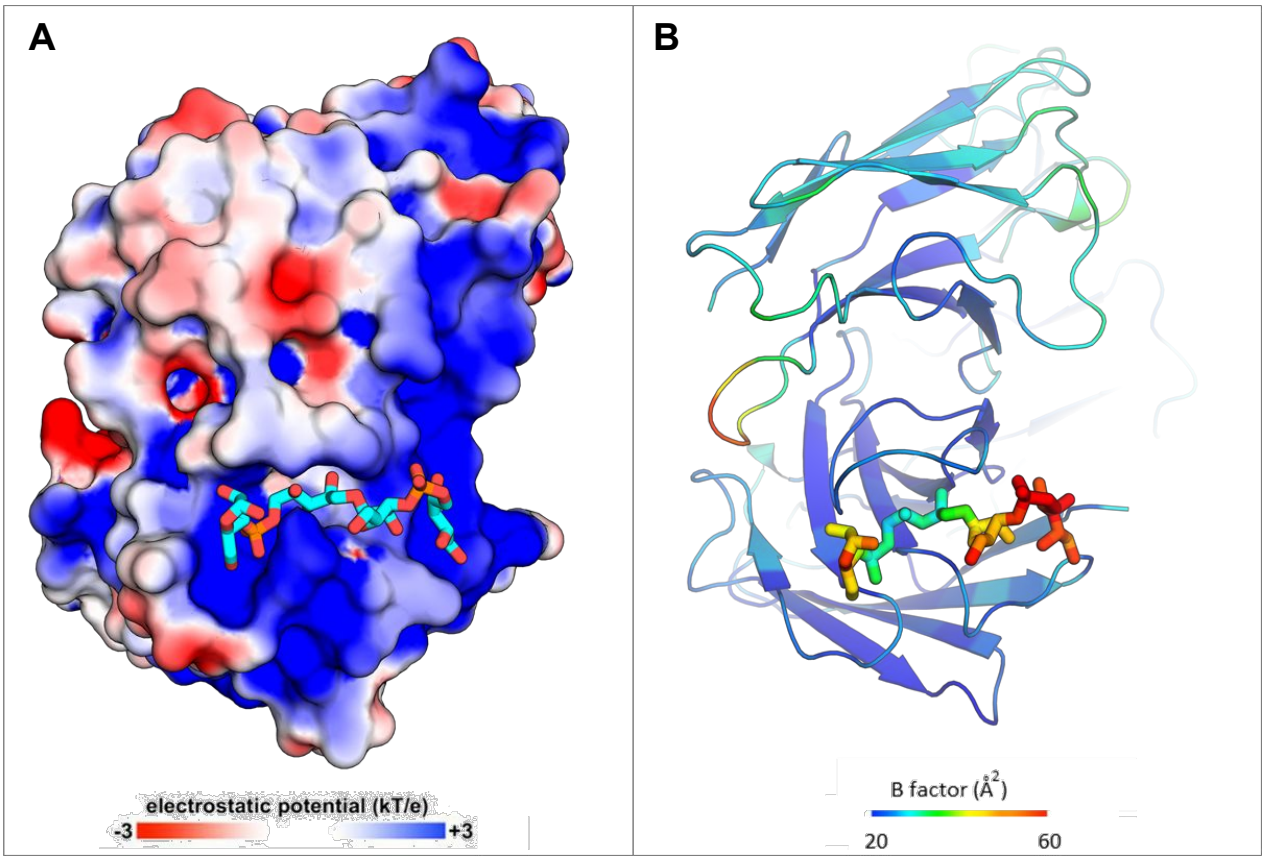


Figure S9. A) Electrostatic surface representation of the Fab CA4 (ranging from −3 kT/e [red] to +3 kT/e [blue]), calculated by APBS (adaptive Poisson-Boltzmann solver) methods [59] using Pymol. **B)** B-Factor values for Fab and Hib DP2. Crystallographic B factors represent temperature-dependent vibrations from average atomic positions. The color bar ranges from low B factor (blue, less flexible) to high B factor (red, more flexible).

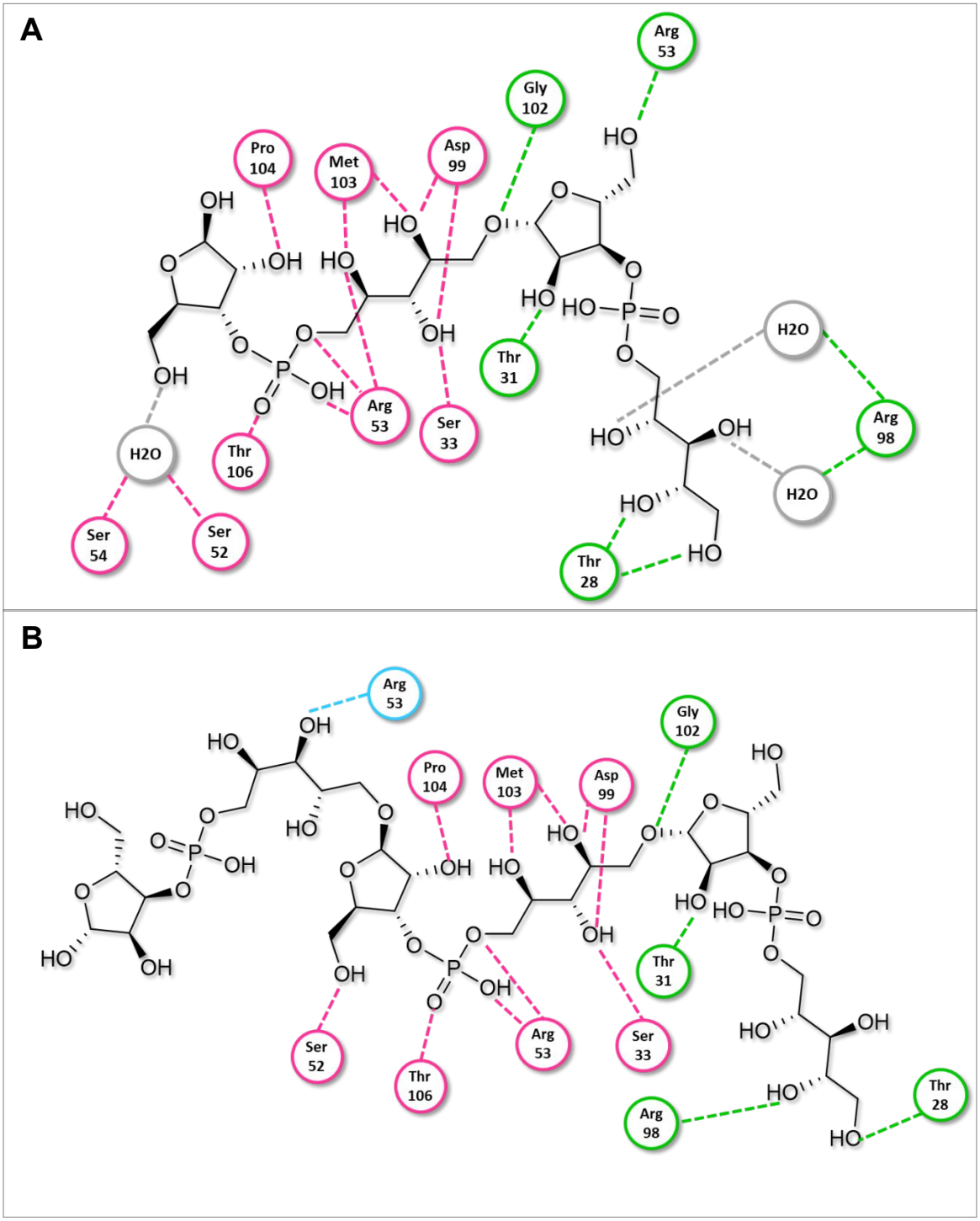


Figure S10. Schematic representation of DP2 **(A)** and DP3 **(B)** interactions. Hydrogen bonds are indicated by dotted lines. Interactions of the OS fragments with Fab residues are shown in green (Rib-ol A'-Ribf B'), pink (Rib-ol A''-Ribf B'') and light blue (Rib-ol A'''-Ribf B'''). Water-mediated interactions are represented in grey.

Table S1. Data collection and refinement statistics

	Fab CA4-DP2 (PDB id 8RDA)	**Fab CA4-DP3** (PDB id 8RDF)
Crystal		
Space group	C 2 2 2_1	C 2 2 2_1
Cell dimensions *a, b, c* (Å)	60.66, 131.59, 145.1	60.80, 131.22, 144.95
Data collection[a]		
Beamline	DLS I03	ESRF ID30A-1
Wavelength (Å)	0.976	0.965
Resolution (Å)	51.50-2.29 (2.33-2.29)	65.64- 2.74 (2.87-2.74)
Total reflections	207267 (5801)	53118 (6956)
Unique reflections	26582 (1252)	15060 (2007)
R_{merge}	0.16 (1.16)	0.18 (0.94)
R_{meas}	0.19 (1.45)	0.21 (1.11)
$I/\sigma(I)$	7.3 (1.1)	5 (1.2)
$CC_{1/2}$	0.99 (0.38)	0.94 (0.58)
Completeness (%)	99.7 (93.4)	96.8 (98.1)
Redundancy	7.8 (4.6)	3.5 (3.5)
Wilson B-factor (Å)	37.09	36.55
Refinement		
Resolution (Å)	51.50-2.29	65.64-2.74
No. reflections	26531	14988
R_{work} / R_{free}	22.6/27	22.6/27.7
No. atoms		
Protein	3307	3282
Ligands	50	68
Water	114	54
B factors		
Protein (Heavy/ Light chains)	43.78/ 47.96	40.88/46.20
Ligand (carbohydrate)	65.57	52.44
Water	44.17	37.40
R.m.s. deviations		
Bond lengths (Å)	0.002	0.003
Bond angles (°)	0.62	0.69
Clash scores	3.2	7.2
Ramachandran[#]		
Favored (%)	96.53	95.08
Allowed (%)	3.24	3.98

[a] Values in parentheses are for highest-resolution shell
[#] Measured using Molprobity