Supporting Information

Detection of ligand binding to glycopolymers using Saturation Transfer Difference NMR

*Janet Muzulu and Amit Basu**

Department of Chemistry, Brown University, Providence RI, USA 02912

abasu@brown.edu

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1. Glycopolymer synthesis

All reagents and solvents were purchased from commercial suppliers and used as received. Dry solvents (methanol and dichloromethane) were obtained using a commercially available solvent purification system from GlassContour based on reported purification protocols.[1] Reactions were carried out under nitrogen atmosphere. Thin layer chromatography was carried out on Merck silica gel 60 F254 precoated glass plates and compounds were visualized under a UV lamp. Centrifugation of precipitated polymers was carried out using an Adams physician's compact centrifuge. Regenerated cellulose dialysis membrane 1000 MWCO (6 Spectra/Por) from Spectrum was used for dialysis. Purified water for dialysis was obtained from an EMD Millipore Direct-Q 3 Tap to Pure and Ultrapure Water Purification system. ^{1}H NMR experiments of the polymer backbone and glycopolymers were recorded on a Bruker Avance Ultra-Shield 400 MHz with an automatic tuning and matching BBO probe. Spectra were processed using Bruker Topspin software (version 3.6.2). Coupling constants are given in hertz (Hz), and chemical shifts are given in parts per million (ppm).

All STD and DOSY NMR spectra were recorded on a Bruker Avance Ultra-Shield 600 MHz instrument at 296 K with an automatic tuning and matching BBO probe. Samples for STD and DOSY NMR were prepared by combining an appropriate amount of the PBA and glycopolymer stock solutions in a 0.1 M sodium phosphate buffer in D_2O, corrected to pH 7.2. The final concentration of the PBA in each sample was 1 mM, while that of the glycopolymers was 100 μM. STD and DOSY NMR experiments were carried out in duplicate. Indole STD NMR samples were prepared in D_2O, and the final concentration of indole was 3 mM, while that of **Gal90** was 50 μM.

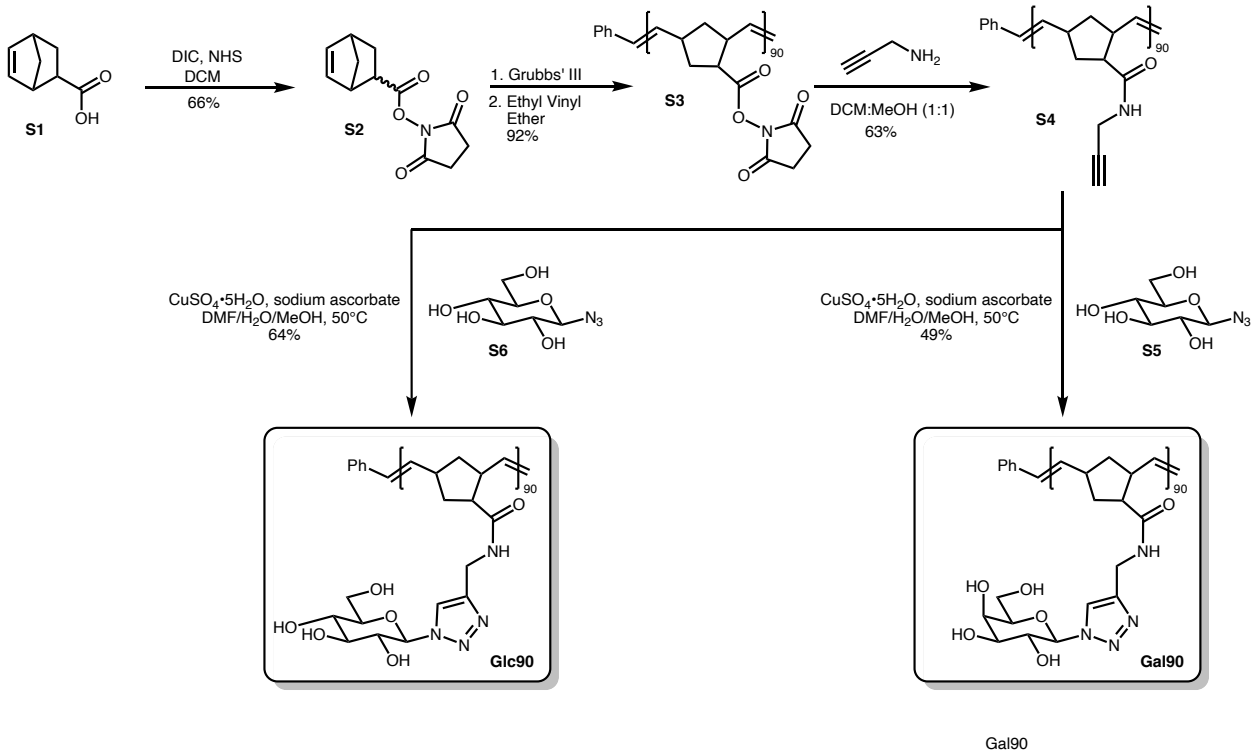

Figure S1 –Synthesis of **Gal90** and **Glc90**

Synthesis of S3: S3 was prepared using a variation of the protocol reported by Okoth *et al.*[2] A solution of Grubbs' 3rd generation catalyst (0.02 g, 0.023 mmol) in 5 mL dry CH_2Cl_2 was added rapidly via a syringe to a solution of **S2**[2] (0.48 g, 2.05 mmol) in 10 mL dry CH_2Cl_2 in a capped vial at -78°C. The reaction was allowed to warm to room temperature, whereupon the solution turned from bright green to brown after about 2 minutes. After 40 minutes ethyl vinyl ether (5.22 mmol, 0.5 ml) was added via a syringe, and the reaction solution was transferred using a Pasteur pipette to 4 different test tubes each containing ether (10 mL), forming a light brown precipitate. The test tubes were centrifuged and the ether decanted, and the precipitate was washed by adding 10 mL of ether into the test tubes and agitating the mixture with a Pasture pipette then centrifuging and decanting the ether. The washing process was repeated until the ether was clear. The precipitate was collected and dried under vacuum to afford **S3** as a gray solid (483 mg, 0.02 mmol) in 92% yield; ^{1}H NMR (600 MHz, acetone-d_6) δ 7.36–7.29 (m, 5H, *H*-Ph) 5.39–5.76 (m, 191 H, *H*-alkene) 1.12-3.42 (m, 1105 H, *H*-aliphatic).

Synthesis of S4: To a solution of **S3** (400 mg, 18 μmol) in 10 mL dry CH_2Cl_2: MeOH (1:1) in a vial was added propargyl amine (225 μL, 180 mg, 3 mmol) and the mixture stirred at room temperature. After 24 h more propargyl amine (225 μL, 180 mg, 3 mmol) was added and the mixture stirred at room temperature for another 24 h. The contents in the vial were transferred to a dialysis tube and dialyzed against CH_2Cl_2: MeOH (1:1) (400 mL) for 2 days with the solvent reservoir changed every 12 h. After dialysis, excess solvent was removed under vacuum to afford **S4** as a thin brown film (193 mg, 0.01 mmol) in 63% yield. ^{1}H NMR (400 MHz, 1:1 CD_3OD:DMSO-d_6) δ 7.08-7.27 (m, 5 H, *H*-Ph) 5.36–5.21 (m, 196 H, *H*-alkene) 3.78 (m, 202 H, -C*H*$_2$-NH-), (m, 193 H, *H*-alkyne), 3.1-1.9 (m, 834 H, *H*-aliphatic).

Synthesis of Gal90 and Glc90: To a solution of **S4** (25 mg, 1.6 μmol) in DMF (2 mL) in a vial was added a solution of either **S5** or **S6**[3, 4] (40 mg, 0.2 mmol) in MeOH (2 mL), $CuSO_4$•$5H_2O$ (8 mg, 0.03 mmol) in H_2O (0.5 mL), sodium-ascorbate (12 mg, 0.01 mmol) in H_2O (0.5 mL) and the mixture stirred at 50 °C. 1 h after completion of the reaction (approximately 3 h as judged by TLC after staining with a triazole-forming fluorogenic dye)[5] 1 g of CupriSorb® was added and the mixture stirred overnight at 50 °C. The CupriSorb® was filtered off and the filtrate transferred to a dialysis tube and dialyzed against H_2O (400 mL) for 3 days with the solvent reservoir changed every 12 h. After dialysis the solution was transferred to a scintillation vial and frozen in liquid nitrogen. Water was removed by lyophilization to afford the Gal or Glc glycopolymers as puffy white solids.

Gal90: (26 mg, 0.74 μmol, 47% yield; ^{1}H NMR (400 MHz, D_2O): δ 8.05 (br s,1 H, *H*-triazole) 5.57 (br s, 1 H, *H*-1) 5.18 (br s, 2H, *H*-alkene) 3.98-3.52 (m, 8 H; *H*-2, *H*-3, *H*-4, *H*-5, *H*-6, *H*-6*'*, -C*H*$_2$NH-), 3.39-0.39 (m, 7 H, *H*-aliphatic)

Glc90: 33 mg, 0.97 μmol, 61% yield; ^{1}H NMR (400 MHz, D_2O): δ 7.97 (br s ,1 H, *H*-triazole) 5.57 (br s, 1 H, *H*-1) 5.16 (br s, 2H, *H*-alkene) 4.26-3.59 (m, 8 H; *H*-2, *H*-3, *H*-4, *H*-5, *H*-6, *H*-6', - C*H*$_2$NH -), 3.10-0.53 (m, 7 H, *H*-aliphatic)

2. Polymer NMR spectra

[ppm]	Integral [rel]$_{expected}$	Integral [rel]$_{observed}$
7.36 (H_{phenyl})	5	5
5.56 (H_{alkene})	180	191
3.31 - 1.47 ($H_{aliphatic}$)	1170	1105

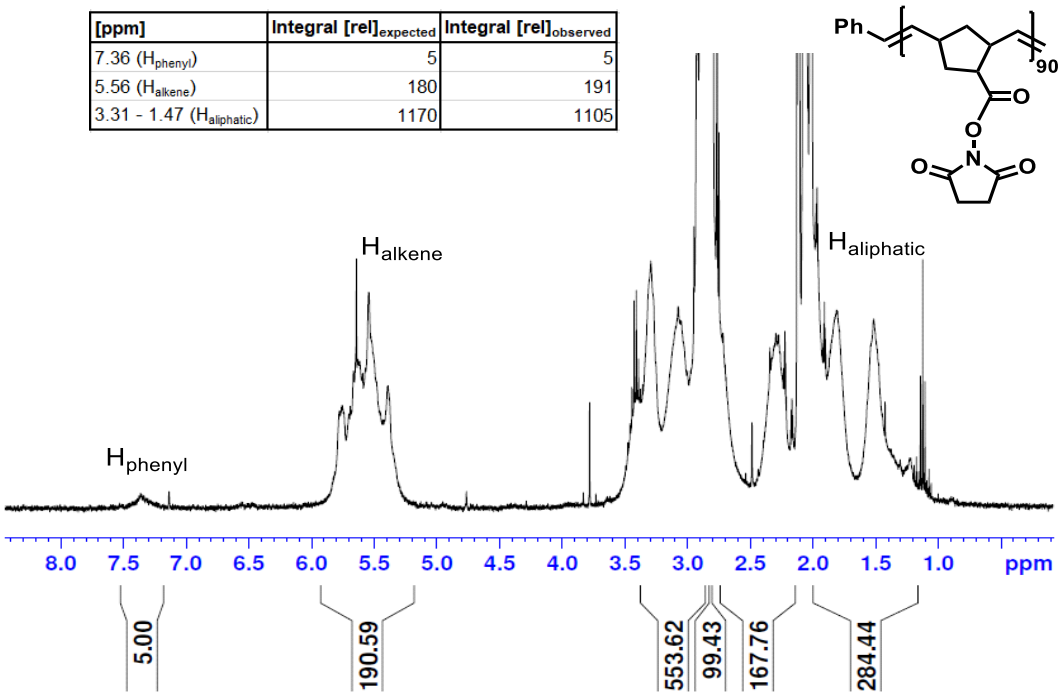

Figure S2 – NHS-ester Polymer **S3** [^{1}H NMR (600 MHz, acetone-d_6)]

[ppm]	Integral [rel]$_{expected}$	Integral [rel]$_{observed}$
7.28 (H_{phenyl})	5	5
5.20 (H_{alkene})	180	197

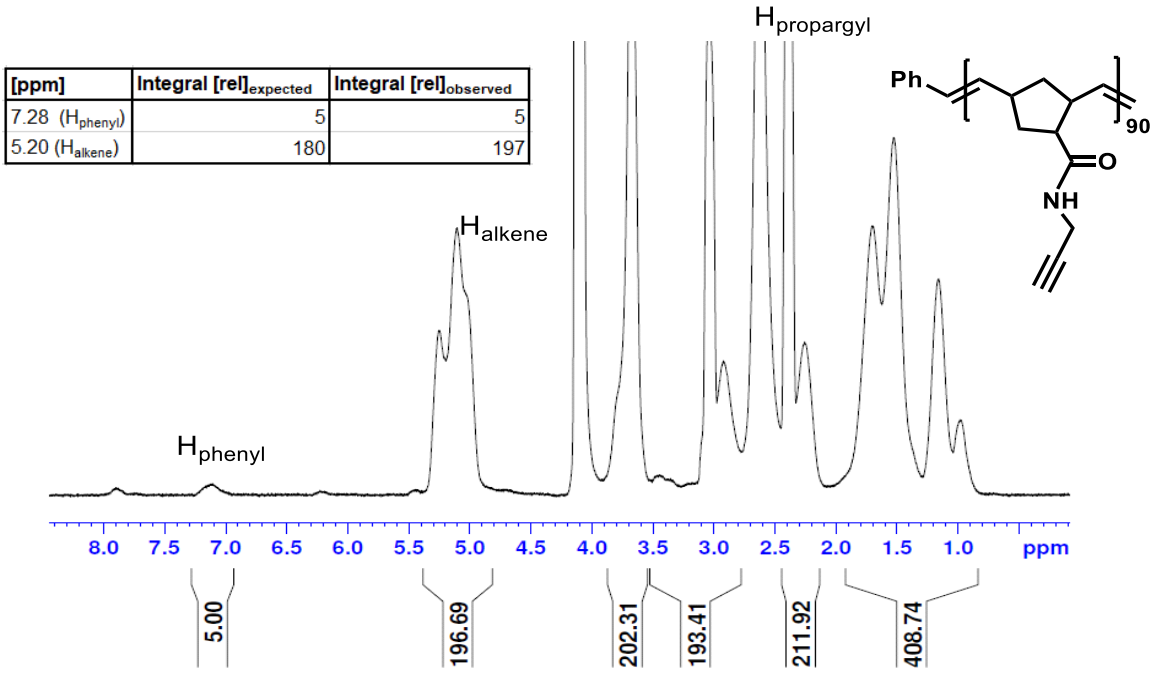

Figure S3 – Alkyne Polymer **S4** [^{1}H NMR (600 MHz, 1:1 DMSO-d_6/CD_3OD)]

[ppm]	Integral [rel]$_{expected}$	Integral [rel]$_{observed}$
7.97 (H_{phenyl})	1	1
5.57 (H_1)	1	1
5.16 (H_{alkene})	2	2
4.26 - 3.59 (H_2-H_6, -C**H$_2$**NH-)	8	8
3.10 - 0.53 ($H_{aliphatic}$)	7	7

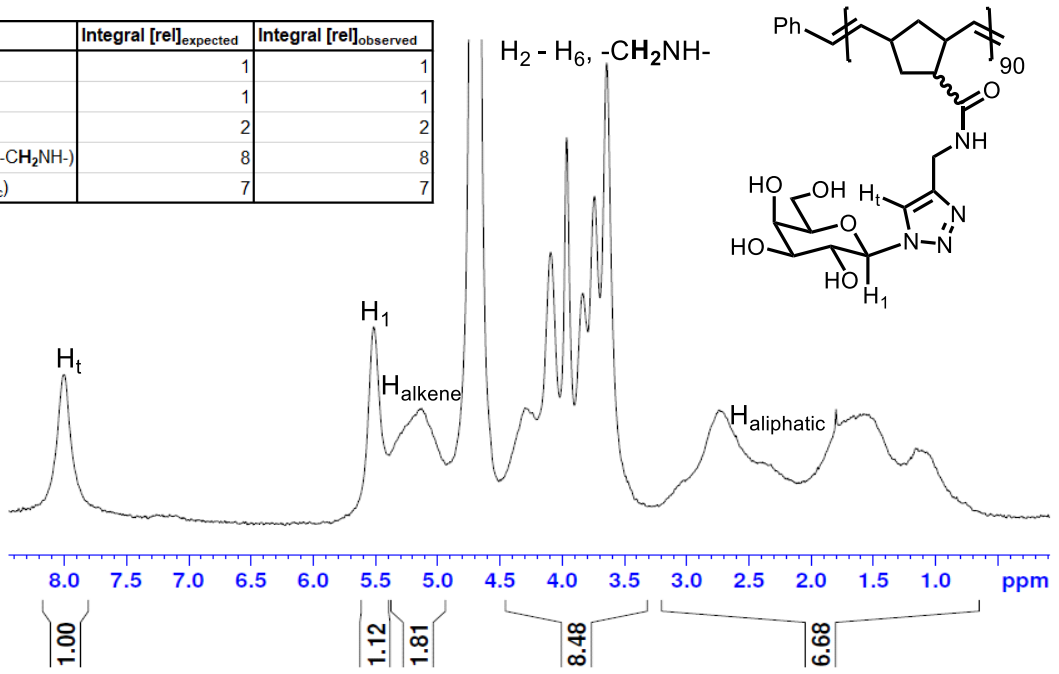

Figure S4 – Galactose glycopolymer **Gal90** [^{1}H NMR (600 MHz, D_2O)]

[ppm]	Integral [rel]$_{expected}$	Integral [rel]$_{observed}$
7.97 (H_{phenyl})	1	1
5.57 (H_1)	1	1
5.16 (H_{alkene})	2	2
4.26 - 3.59 (H_2-H_6, -C**H$_2$**NH-)	8	12
3.10 - 0.53 ($H_{aliphatic}$)	7	9

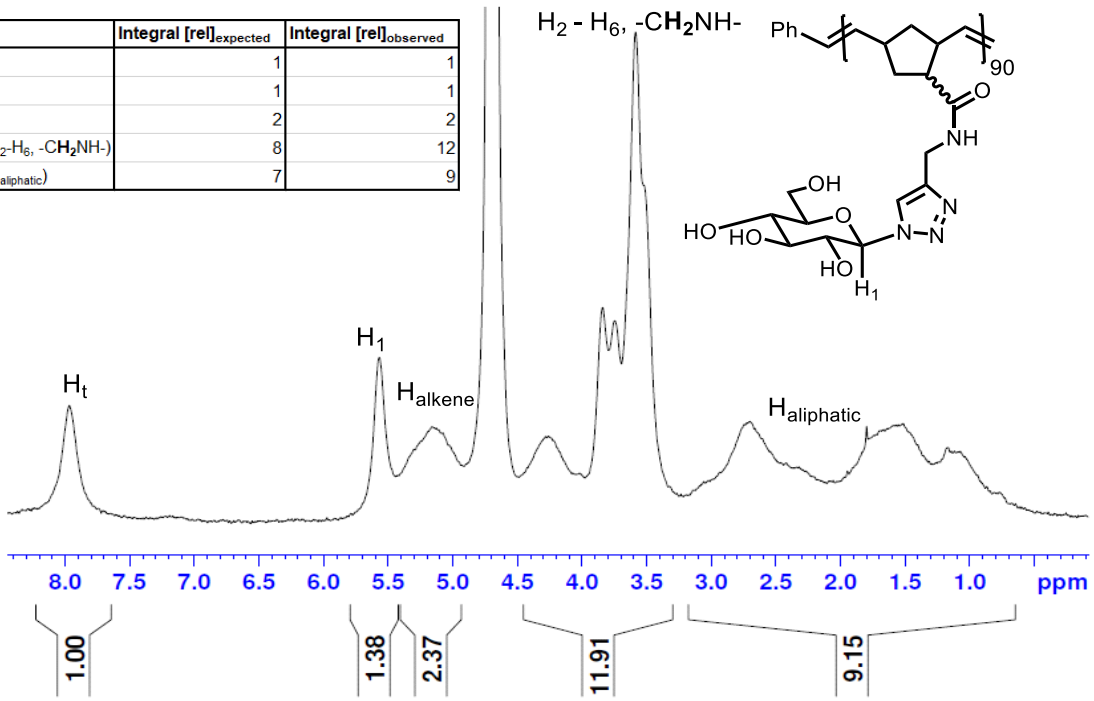

Figure S5 – Glucose glycopolymer **Glc90** [^{1}H NMR (600 MHz, D_2O)]

3. STD NMR experimental protocols and spectra for control and optimization experiments

STD NMR[6] STD experiments were performed using the stddifesgp.3 sequence from the Bruker pulse sequence library. The excitation sculpting with gradients water suppression sequence was employed. Eight dummy scans were collected at the beginning of each STD experiment. The on-resonance irradiation of the glycopolymer was applied at 1.07 ppm or 3.75 ppm (for DEEP-STD NMR experiments). Off-resonance irradiation was applied at 40 ppm. 1D STD NMR spectra were multiplied by an exponential line- broadening function of 2.5 Hz prior to Fourier transformation. The irradiation power of the selective pulses in the STD NMR experiment was set to 14 Hz for entries 1 – 6 in Table 1 (in text), 19 Hz for entries 7 – 9, 11 and 1.4 Hz for entry 10. Selective pre-saturation of the glycopolymer was achieved by a train of Gauss-shaped pulses of 25 ms length each, separated by a 1.5 ms delay. The saturation time was varied from 1 s to 8 s. For indole-**Gal90** STD NMR studies, duplicate experiments were conducted with an on-resonance irradiation at 3.75 ppm and were performed over 256 scans. A single experiment was conducted with the on-resonance irradiation at 1.07 ppm and was performed over 512 scans.

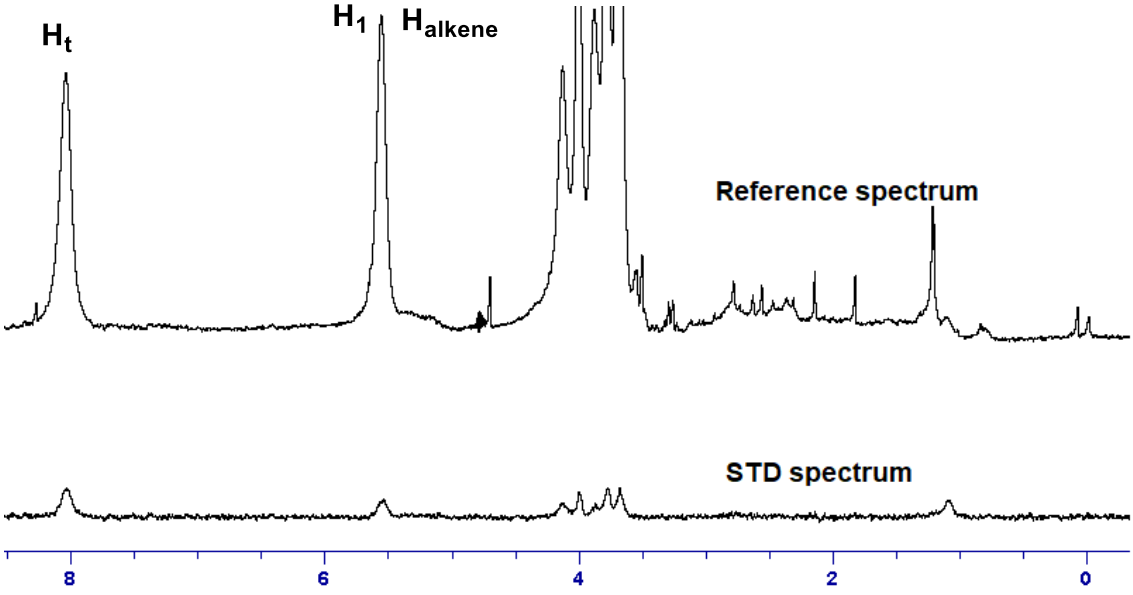

Figure S6 – **Gal90** spectra: Reference and STD (bottom) spectra obtained using optimal parameters (Entry 11 in Table 1 in the text).

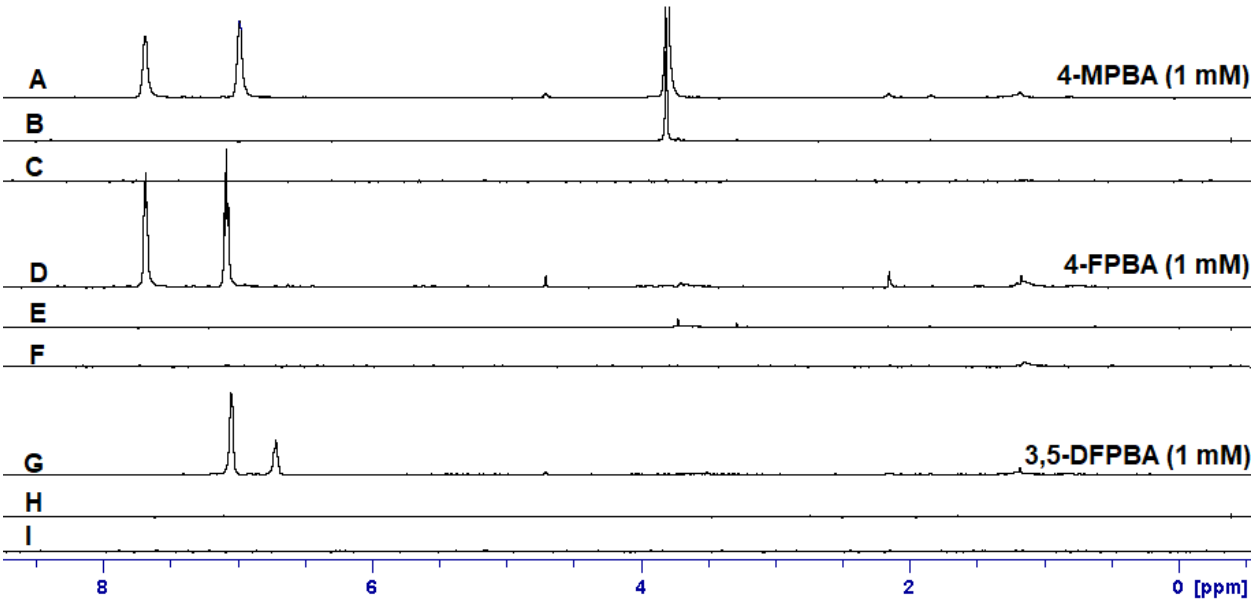

Figure S7 – STD NMR negative control experiments. Reference spectra of 4-MPBA, 4-FPBA and 3,5-DFPBA respectively (**A, D** &**G**). Corresponding STD spectra of the PBAs (**C, F** & **I**) after application of on-resonance irradiation at 1.07 ppm. Corresponding STD spectra of the PBAs (**B**, **E** &**H**) after application of on-resonance irradiation at 3.75 ppm. All solutions are 1 mM PBA in 0.1 M phosphate buffer at pH 7.2.

All spectra in Figure S7 were acquired using optimized parameters – 25 ms Gauss shaped pulse with a pulse power of -47.5 dB at a saturation time of 1 s. The difference spectra (**C**, **F** and **I**) show the cancellation of the small molecule resonances as there is no macromolecule to receive saturation from. The difference spectra **E** and **H** show the cancellation of the small molecule resonances while spectrum **B** shows that the methoxy protons of 4-MPBA are irradiated by the applied pulse since they appear in the same region as the on-resonance irradiation frequency. The aromatic protons of interest, however, are completely cancelled.

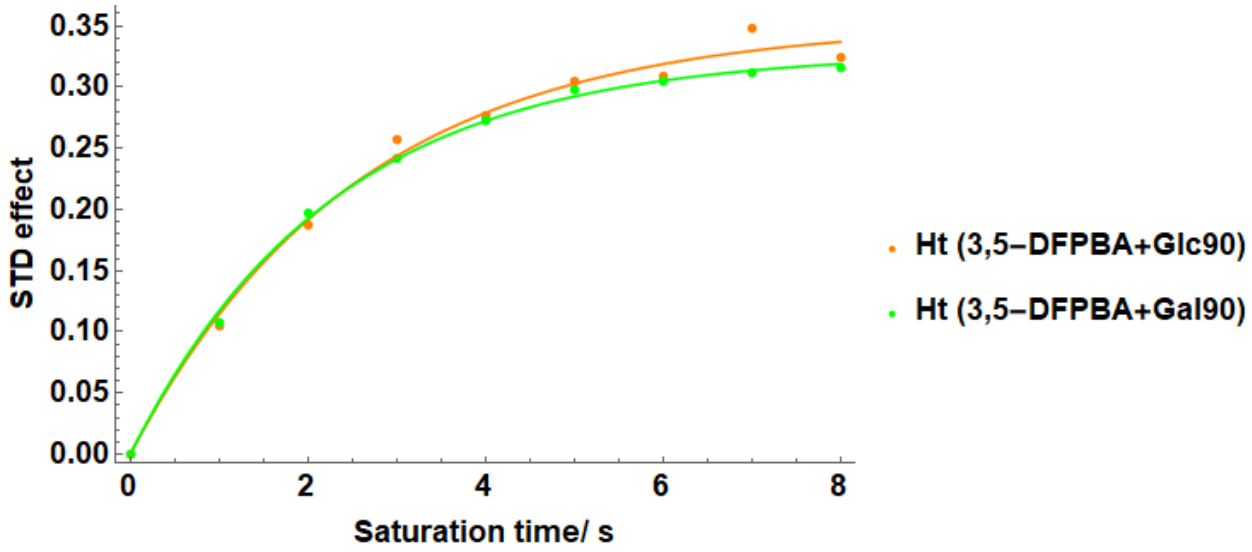

Figure S8 – STD build-up curves of the triazole proton (Ht) of **Gal90** (100 µM) in the presence of 3,5-DFPBA (1 mM). Maximum STD effects of 0.34 (green) effect of 0.39 (orange) are observed for **Gal90** and **Glc90**, respectively. On-resonance frequency – 1.07 ppm. The increase in the STD effect of the triazole proton with increasing saturation time confirms that spin diffusion occurs successfully in **Gal90** and **Glc90.**

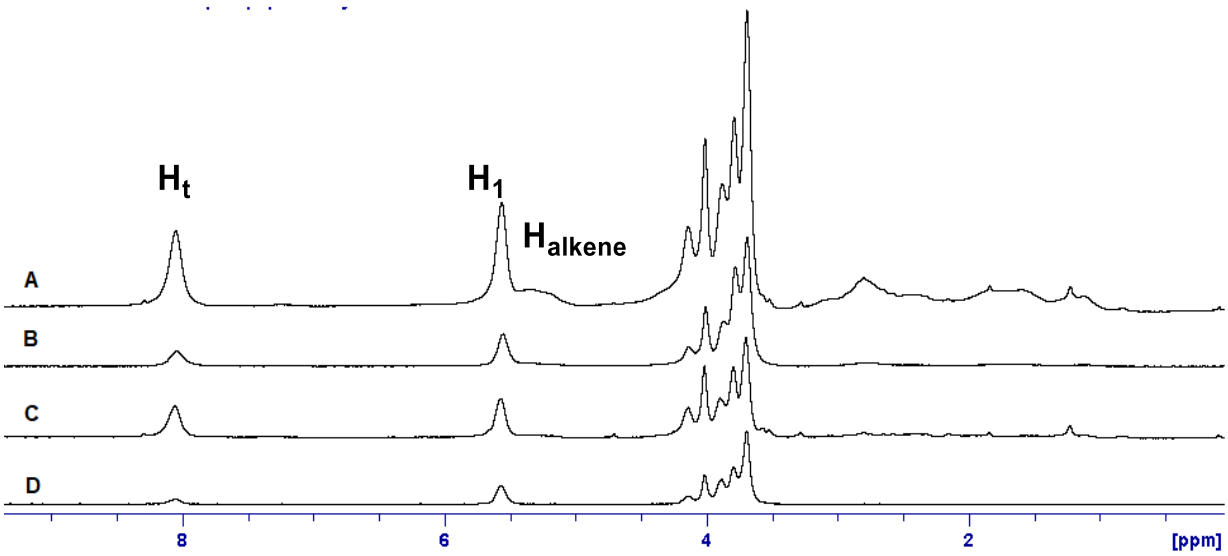

Figure S9 – Spin diffusion studies with **Gal90** after application of on-resonance irradiation at 3.75 ppm with a saturation time of 1 s. A – Reference spectrum; B – STD NMR spectrum using excitation parameters found in Table 1, Entry 8; C – Reference spectrum with a 50 ms spin lock filter, and D – corresponding STD NMR spectrum with a 50 ms spin lock filter. The envelopes of the STD spectra are similar to those of the corresponding reference spectra, indicating effective saturation of the macromolecule after on-resonance irradiation at 3.75 ppm.

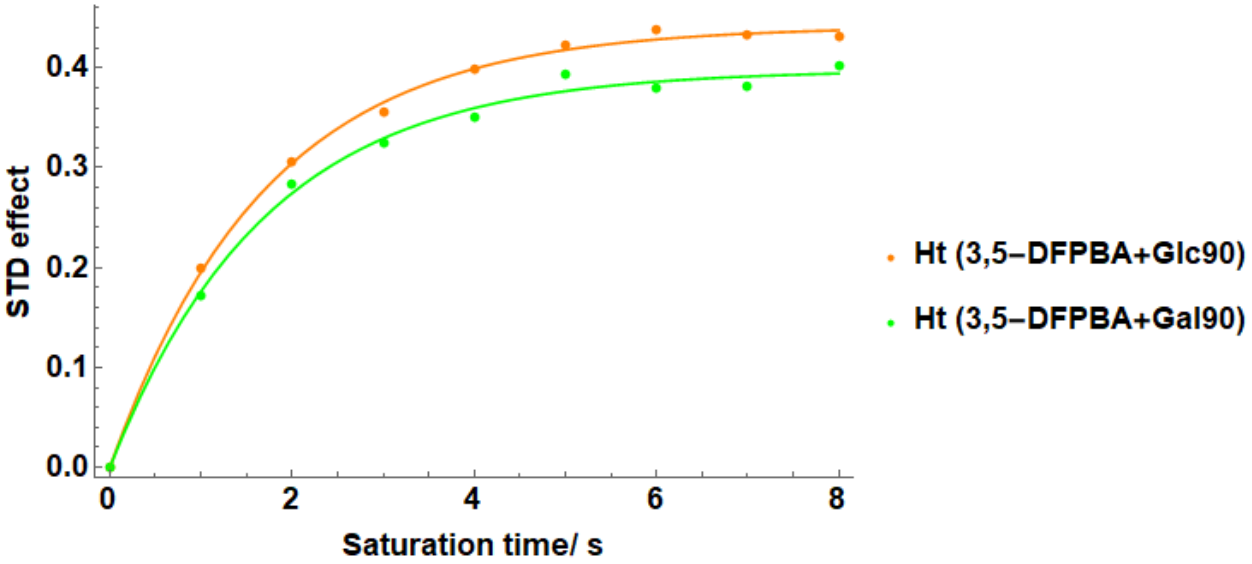

Figure S10 – STD build-up curves of the triazole proton (Ht) of **Gal90 (100 µM)** in the presence of 3,5-DFPBA (1 mM). Maximum STD effects of 0.40 (green) effect of 0.44 (orange) are observed for **Gal90** and **Glc90**, respectively. On-resonance frequency – 3.75 ppm. The increase in the STD effect of the triazole proton with increasing saturation time confirms that spin diffusion occurs successfully in **Gal90** and **Glc90.**

4. PBA STD NMR spectra

Stacked STD plots of the PBA•glycopolymer mixtures at saturation times varying from 1 to 8 seconds are shown in Figures S11 – S13. These plots were obtained after application of the saturation pulse at 1.07 ppm. Stacked plots of the PBA•glycopolymer mixtures at varying saturation times with a saturation pules applied at 3.75 ppm are shown in Figures S15 – S18.

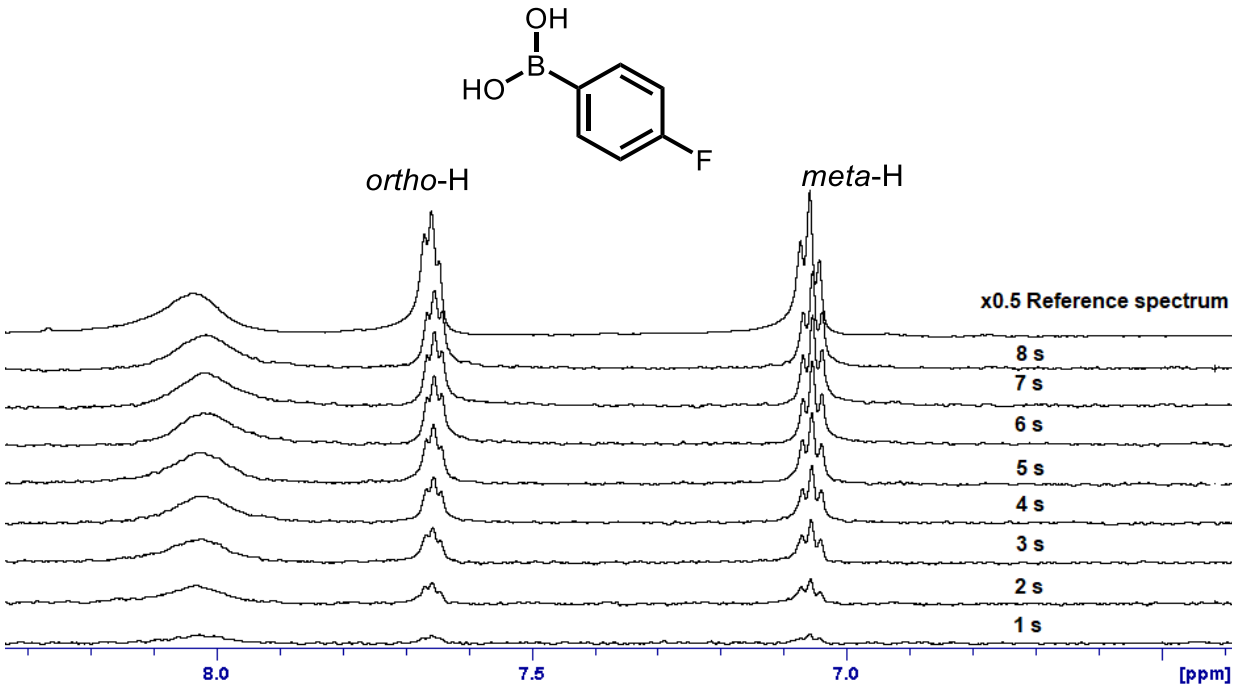

Figure S11 – STD spectra of 4-FPBA (1 mM) in the presence of **Gal90** (100 µM) in D_2O obtained after the application of on-resonance irradiation at 1.07 ppm. The reference spectrum is scaled x0.5.

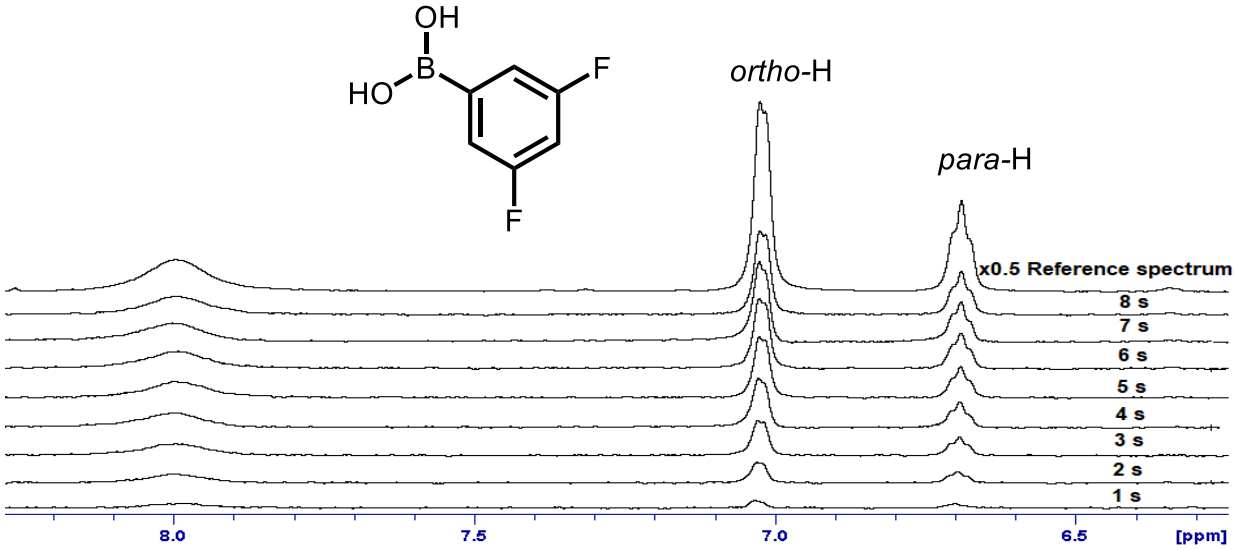

Figure S12 – STD spectra of 3,5-DFPBA (1 mM) in the presence of **Glc90** (100 µM) in D_2O obtained after the application of on-resonance irradiation at 1.07 ppm. The reference spectrum is scaled x0.5.

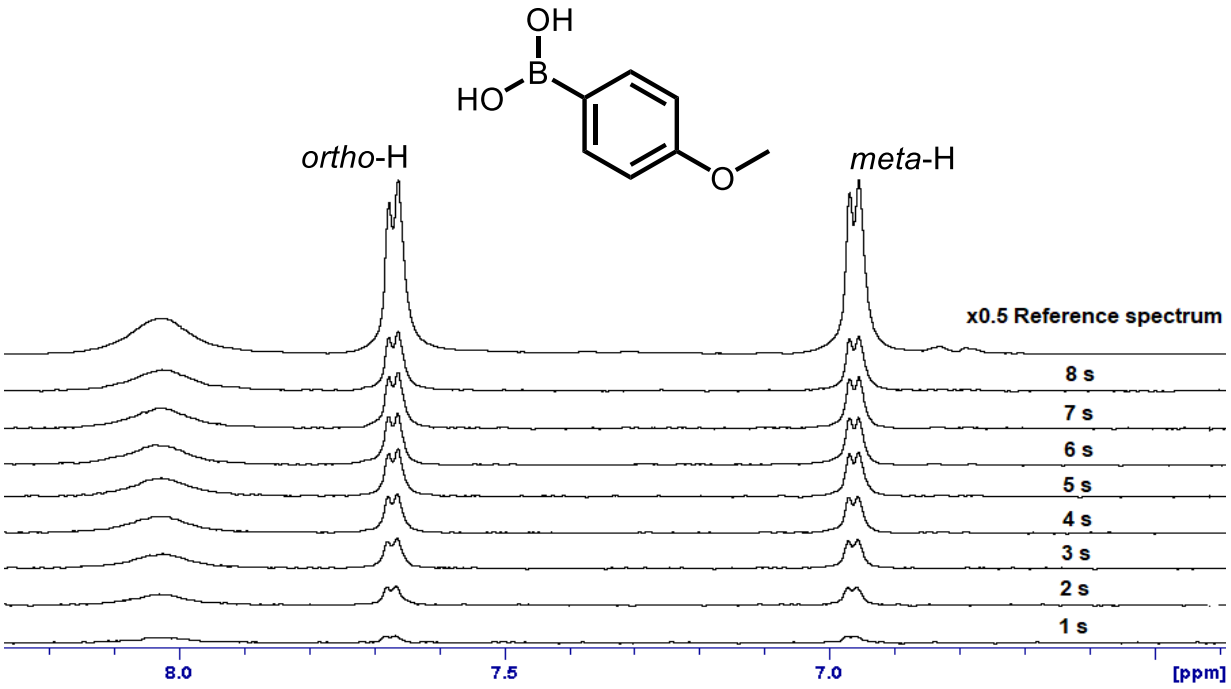

Figure S13 – STD spectra of 4-MPBA (1 mM) in the presence of **Gal90** (100 μM) in D_2O obtained after the application of on-resonance irradiation at 1.07 ppm. The reference spectrum is scaled x0.5.

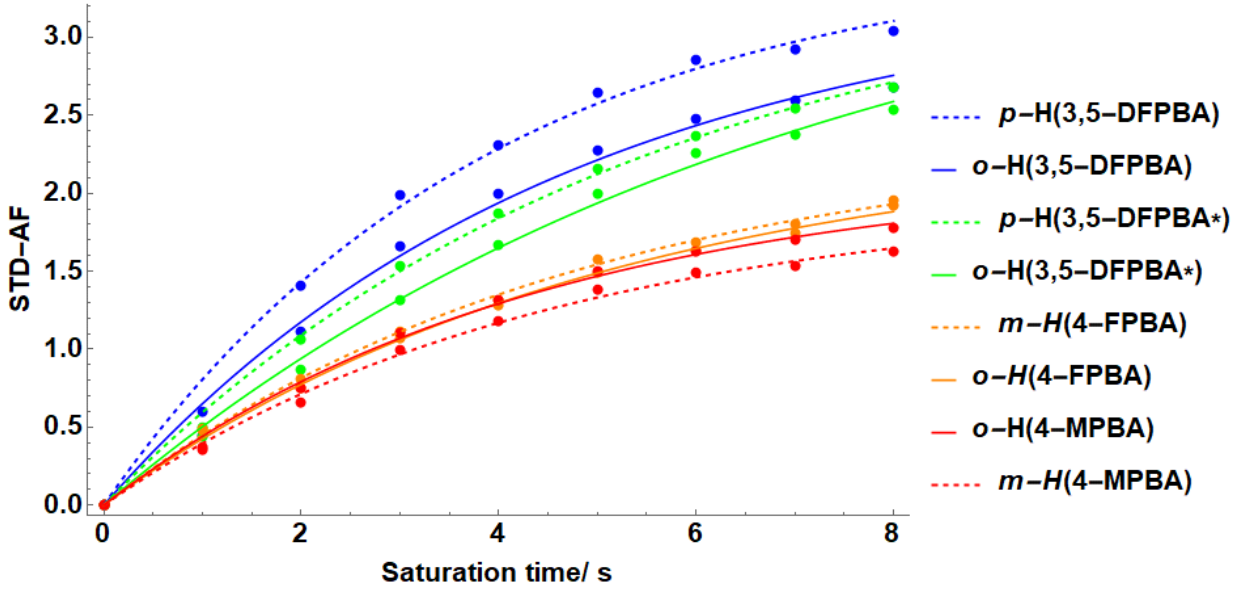

Figure S14 – STD build up curves for PBAs in the presence of **Gal90**. (*Build up curves in the presence of **Glc90**.) On resonance frequency – 1.07 ppm

Entry	PBA Proton	STD-AF_{max}	k_{sat} [s^{-1}]
3,5-DFPBA/Gal90	*o* -H	3.20± 0.15	0.21± 0.0
	p -H	3.43± 0.13	0.26± 0.0
3,5-DFPBA/Glc90	*o*- H	3.74± 0.08	0.15± 0.01
	p- H	3.55± 0.06	0.19± 0.0
4FPBA/Gal90	*o* -H	2.31± 0.05	0.19± 0.01
	m- H	2.27± 0.10	0.21± 0.0
4MPBA/Gal90	o-H	2.04± 0.11	0.23± 0.01
	m -H	1.83± 0.15	0.23± 0.01

Table S1 – Curve fit parameters for data in Figure S14

Each STD-AF buildup curve is fitted to the equation – $STD\text{-}AF_{tsat} = STD\text{-}AF_{max}(1 - e^{-k_{sat} \times t_{sat}})$. STD-$AF_0$ values are obtained from multiplying the STD-AF_{max} value with the k_{sat} value. Values for k_{sat} and STD-AF_{max} are derived by least-squares fitting using Mathematica.

Entry	PBA Proton	STD-AF_0	% amplification
3,5-DFPBA/Gal90	*o* -H	0.69± 0.04	78
	p -H	0.88± 0.04	100
3,5-DFPBA/Glc90	*o*- H	0.56± 0.03	84
	p- H	0.67± 0.02	100
4FPBA/Gal90	*o* -H	0.44± 0.04	93
	m- H	0.48± 0.03	100
4MPBA/Gal90	o-H	0.46± 0.03	100
	m -H	0.42± 0.02	91

Table S2 – Binding epitope maps. On-resonance frequency – 1.07 ppm.

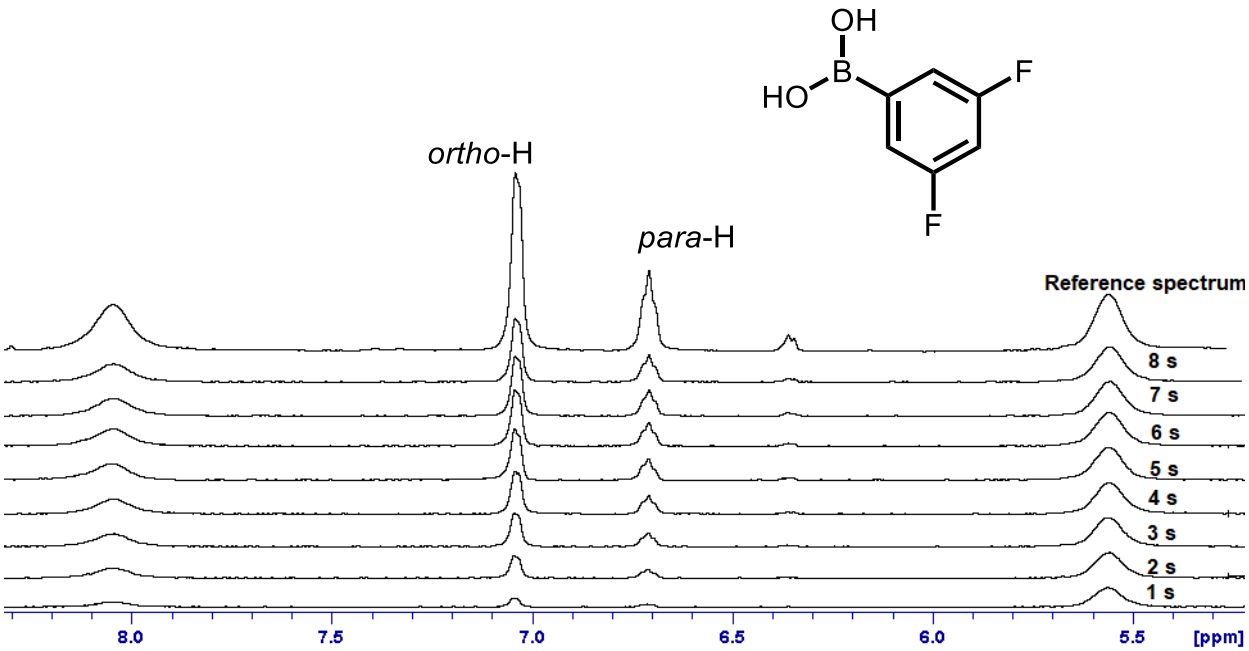

Figure S15 – STD spectra of 3,5-DFPBA (1 mM) in the presence of **Gal90** (100 μM) in D_2O obtained after the application of on-resonance irradiation at 3.75 ppm. Intensity of the 3,5-DFPBA peaks increases with increasing saturation time. The reference spectrum is scaled x1.

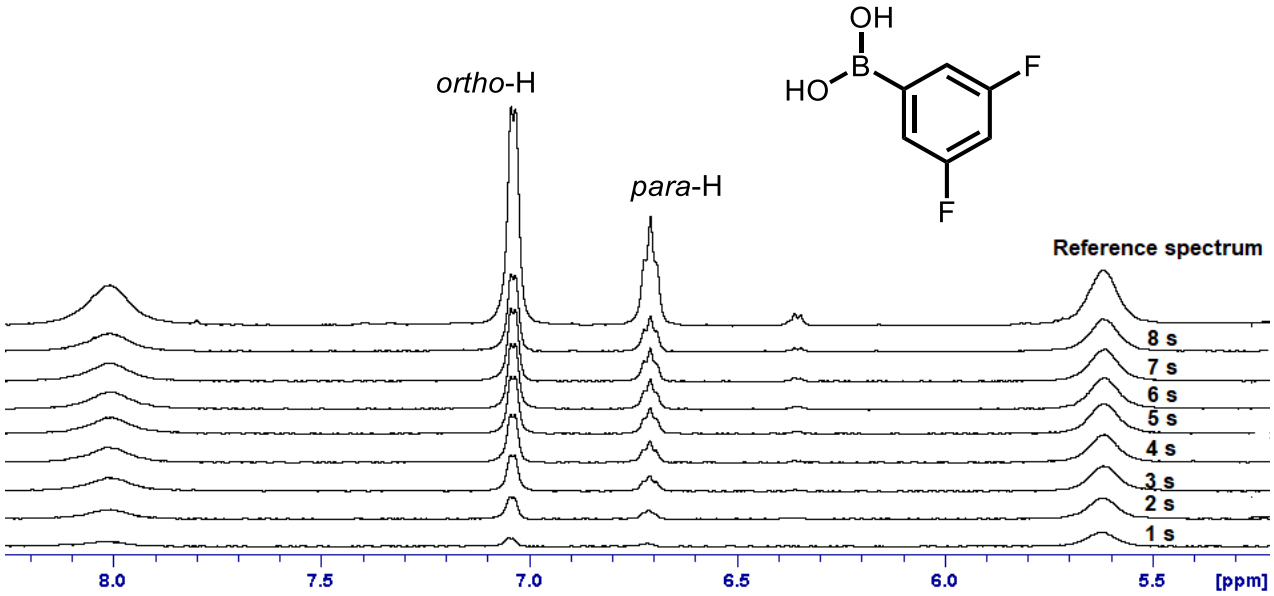

Figure S16 – STD spectra of 3,5-DFPBA (1 mM) in the presence of **Glc90** (100 μM) in D_2O obtained after the application of on-resonance irradiation at 3.75 ppm. Intensity of the 3,5-DFPBA peaks increases with increasing saturation time. The reference spectrum is scaled x1.

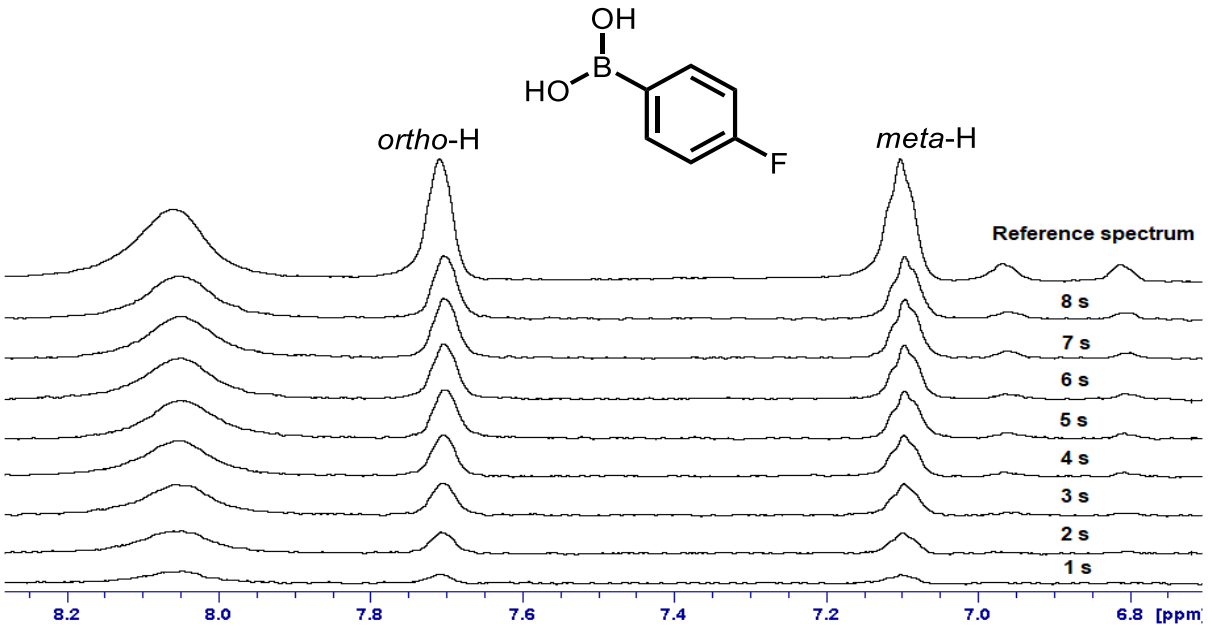

Figure S17 – STD spectra of 4-FPBA (1 mM) in the presence of **Gal90** (100 µM) in D_2O obtained after the application of on-resonance irradiation at 3.75 ppm. Intensity of the 4-FPBA peaks increases with increasing saturation time. The reference spectrum is scaled x0.5.

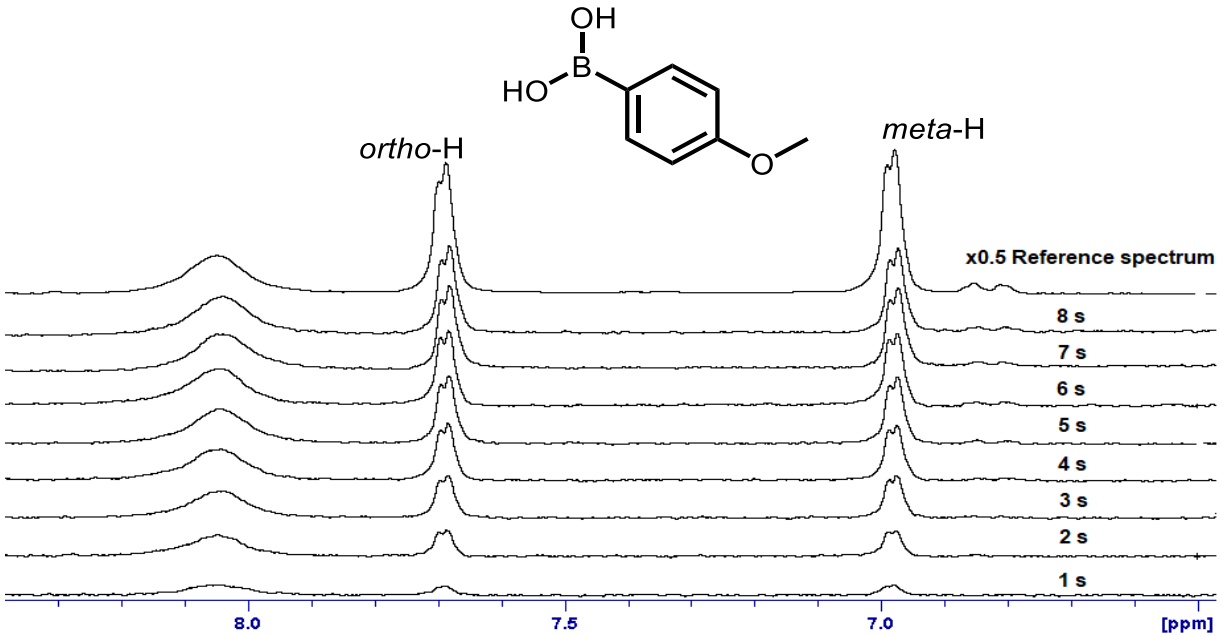

Figure S18 – STD spectra of 4-MPBA (1 mM) in the presence of **Gal90** (100 µM) in D_2O obtained after the application of on-resonance irradiation at 3.75 ppm. Intensity of the 4-MPBA peaks increases with increasing saturation time. The reference spectrum is scaled x0.5.

Entry	PBA Proton	STD-AF_{max}	k_{sat} [s^{-1}]
3,5-DFPBA/Gal90	*o* -H	3.83± 0.03	0.35± 0
	p -H	3.81± 0.13	0.31± 0
3,5-DFPBA/Glc90	*o*- H	4.09± 0.01	0.26± 0.01
	p- H	3.94± 0.01	0.23± 0.01
4FPBA/Gal90	*o* -H	3.00± 0.31	0.28± 0.02
	m- H	2.95± 0.27	0.27± 0.02
4MPBA/Gal90	o-H	2.48± 0.10	0.23± 0.01
	m -H	2.40± 0.20	0.23± 0.02

Table S3 – Curve fit parameters for data in Figure 5 with 3.75 ppm on resonance frequencies

Each STD-AF buildup curve is fitted to the equation: $STD-AF_{tsat} = STD-AF_{max}(1 - e^{-k_{sat} \times t_{sat}})$, and the STD-$AF_0$ values are obtained from multiplying the STD-AF_{max} value with the k_{sat} value. Values for k_{sat} and STD-AF_{max} are derived by least-squares fitting using Mathematica.

Entry	PBA Proton	STD-AF_0	% amplification
3,5-DFPBA/Gal90	*o*- H	1.33± 0.02	100
	p- H	1.19± 0.04	89
3,5-DFPBA/Glc90	*o*- H	1.05± 0.01	100
	p- H	0.91± 0.03	87
4FPBA/Gal90	*o* -H	0.83± 0.2	100
	m- H	0.80± 0.2	95
4MPBA/Gal90	*o* -H	0.58± 0.03	100
	m -H	0.54± 0.02	93

Table S4 – STD NMR data summary with binding epitope maps of each small molecule. Data was obtained after application of on-resonance irradiation at 3.75 ppm.

5. DOSY experimental protocols and spectra

DOSY NMR[7] DOSY experiments were performed using the ledbpg2s pulse sequence, with stimulated echo, longitudinal eddy current compensation, bipolar gradient pulses, and two spoil gradients. The experiments were conducted with a linear gradient (52.5 $G.cm^{-1}$) stepped between 2% and 95%. 16 1D ^{1}H spectra were collected with a gradient duration of 1-ms and an echo delay of 150 ms for the PBAs in the presence or absence of the glycopolymers. The 1D ^{1}H spectra were processed and automatically baseline corrected. The diffusion dimension, zero-filled to 1k, was exponentially fitted according to pre-set windows for the diffusion dimension. Diffusion data was obtained using regression analysis and the T1/T2 module in TopSpin. Here, the decay in peak integral was fitted to obtain a decay time proportional to D for one peak and one diffusivity at a time. The data points showed peak areas as a function of increasing gradient strength. DOSY analysis was only used to generate pseudo-2D plots – i.e. chemical shift on the *x* axis and log D on the *y* axis. Comparison of diffusion coefficients in the presence or absence of glycopolymer was measured by taking the water signal as a standard. The average log D measurements reported for the PBAs in their free and bound states were determined by first obtaining log *D* values of the individual resonances of each PBA, which were averaged to obtain a log *D* value for the molecule. These measurements were carried out in duplicate, and the mean log *D* values from the replicates is reported. Errors are propagated as the standard deviation of each average.

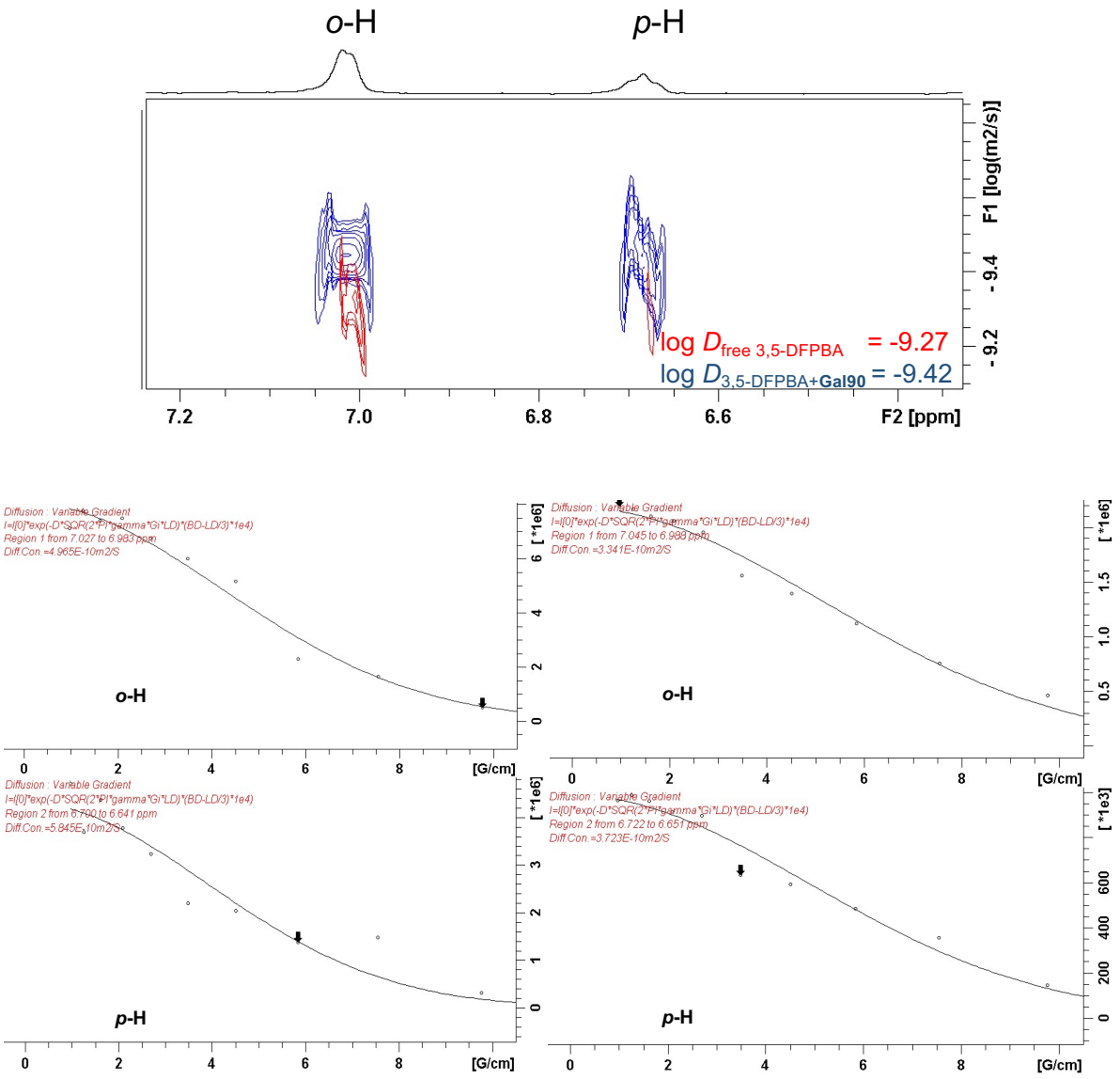

Figure S19 – Top: DOSY 2D spectrum of (red): free 3,5-DFPBA (1 mM) and (blue): 3,5-DFPBA (1 mM) in the presence of **Gal90** (100 µM) in phosphate buffer (0.1 M) in D_2O, pH 7.23 at room temperature. (Zoomed in view of PBA resonances.) Bottom: Correlation curves of field strength vs. integrated peak area for free 3,5-DFPBA (left) and 3,5-DFPBA in the presence of **Gal90** (right)

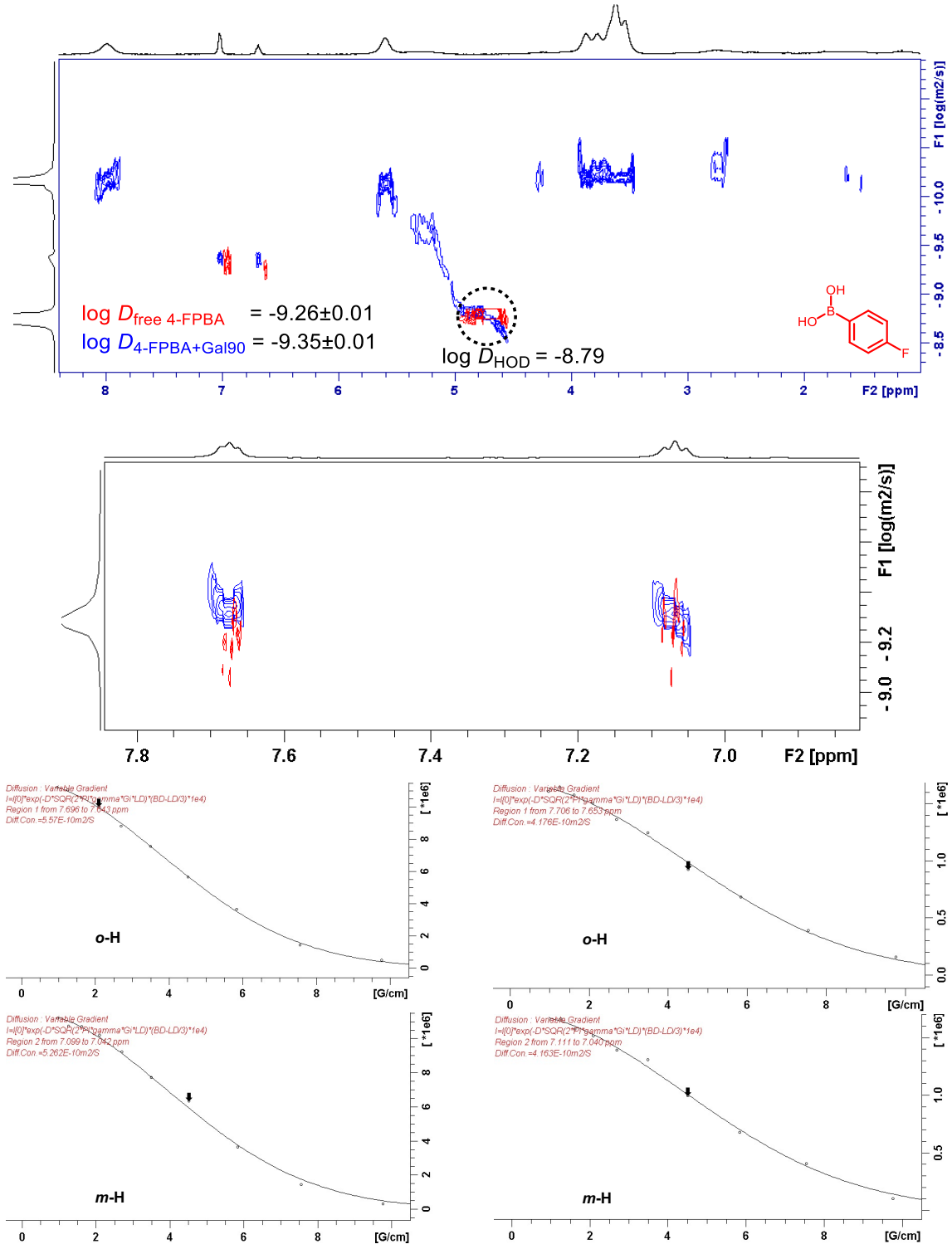

Figure S20 – Top: DOSY 2D spectrum of (red): free 4-FPBA (1 mM) and (blue): 4-FPBA (1 mM) in the presence of **Gal90** (100 µM) in phosphate buffer (0.1 M) in D_2O, pH 7.23 at room temperature. Middle: Zoomed in view of PBA resonances. Bottom: Correlation curves of field strength vs. integrated peak area for free 4-FPBA (left) and 4-FPBA in the presence of **Gal90** (right)

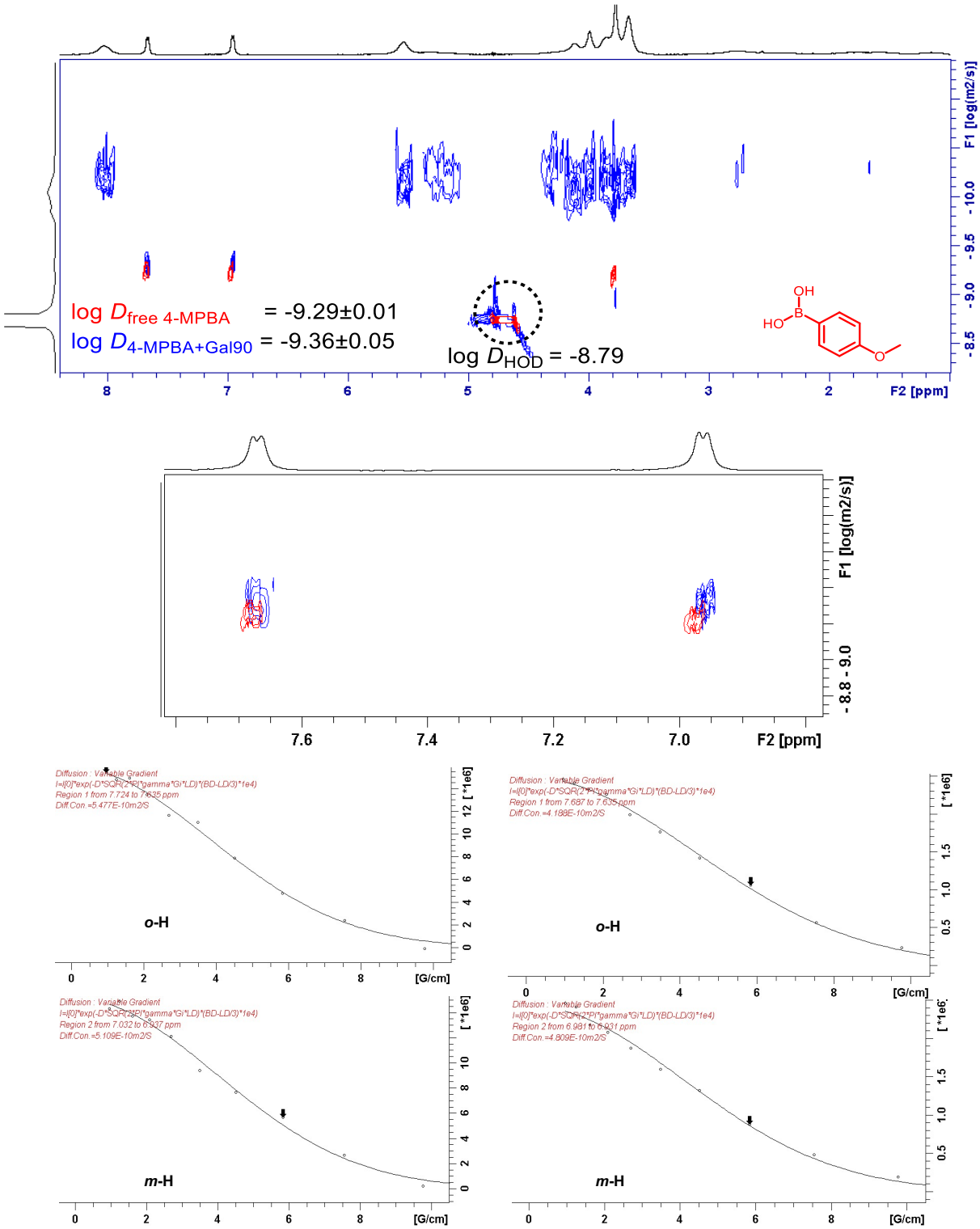

Figure S21 – Top: DOSY 2D spectrum of (red): free 4-MPBA (1 mM) and (blue): 4-MPBA (1 mM) in the presence of **Gal90** (100 µM) in phosphate buffer (0.1 M) in D_2O, pH 7.23 at room temperature. Middle: Zoomed in view of PBA resonances. Bottom: Correlation curves of field strength vs. integrated peak area for free 4-MPBA (left) and 4-MPBA in the presence of **Gal90** (right)

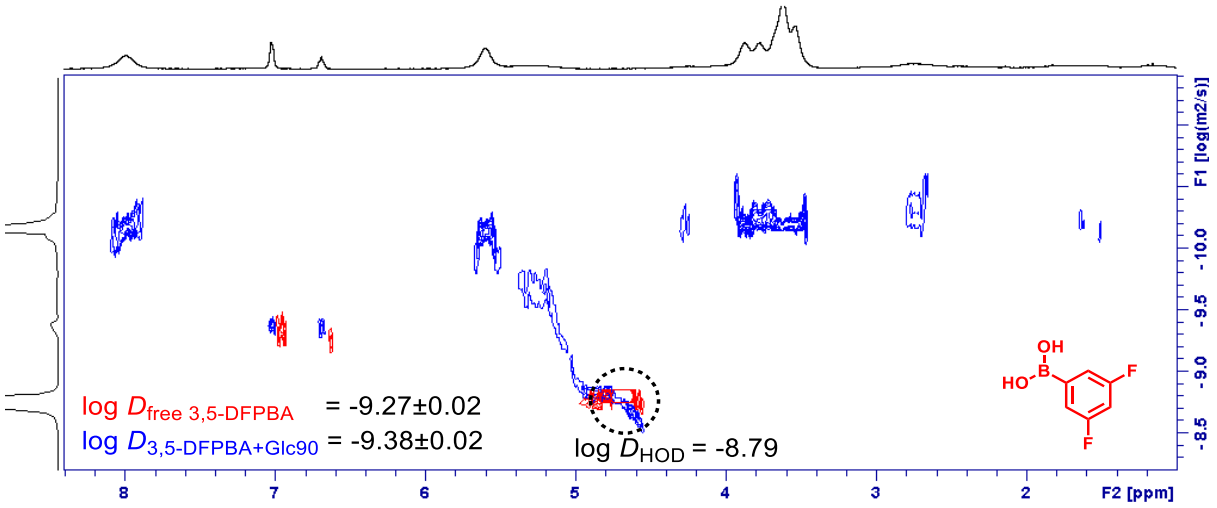

Figure S22 – DOSY 2D spectrum of (red): free 3,5-DFPBA (1 mM) and (blue): 3,5-DFPBA (1 mM) in the presence of **Glc90** (100 µM) in phosphate buffer (0.1 M) in D_2O, pH 7.23 at room temperature.

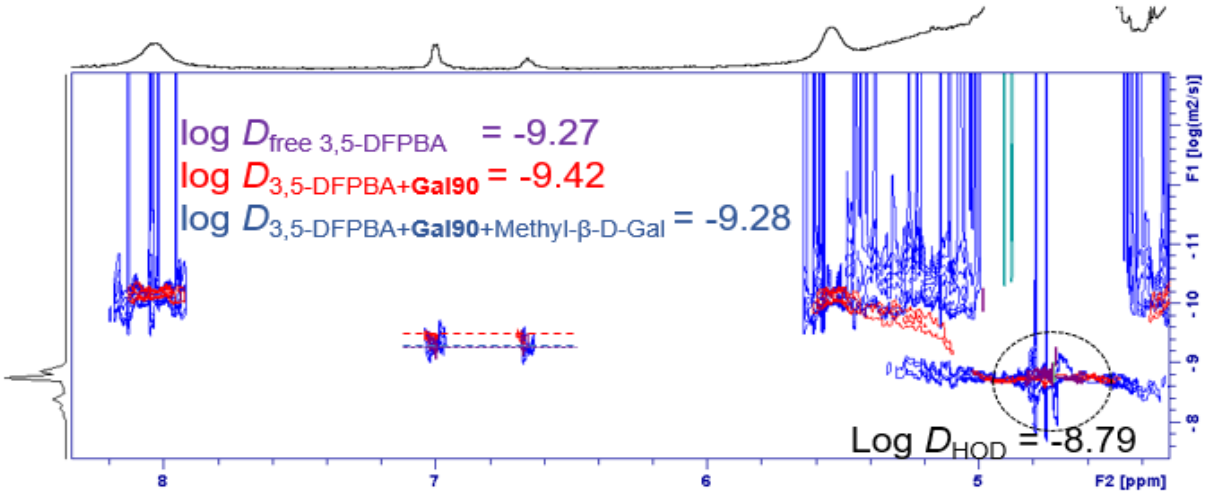

Figure S23 – DOSY competition experiment with purple signals belonging to free 3,5-DFPBA; red – 3,5DFPBA in the presence of **Gal90** (EL 10) and blue – 3,5-DFPBA (290 µM) in the presence of **Gal90** (29 µM) and methyl-β-D-Gal (29 mM). Very small changes in the log *D* of the 3,5-DFPBA are observed upon addition of the monosaccharide to the 3,5-DFPBA/**Gal90** mixture.

Entry	log $D_{\ free PBA}$	log $D_{\ avg\ mixture}$	Δ
3,5-DFPBA/Gal90	-9.27± 0.02	-9.42± 0.02	-0.15± 0.03
3,5-DFPBA/Glc90	-9.27± 0.02	-9.38± 0.02	-0.11± 0.03
4FPBA/Gal90	-9.26± 0.01	-9.35± 0.01	-0.09± 0.01
4MPBA/Gal90	-9.29±0.01	-9.36± 0.05	-0.07± 0.05

Table S5 – log *D* and Δlog *D* of PBAs in the presence/absence of glycopolymers

6. Indole + Gal90 STD NMR

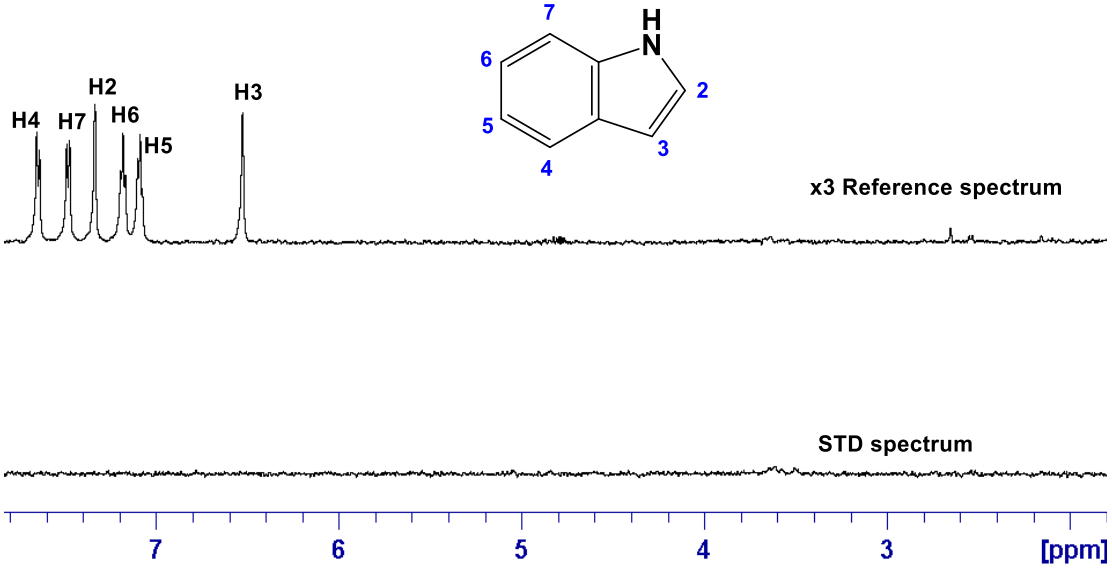

Figure S24 – STD negative control experiment. Indole (3 mM) experiences total signal cancellation in the absence of **Gal90** after on resonance irradiation at 3.75 ppm (bottom). The reference spectrum is scaled x3.

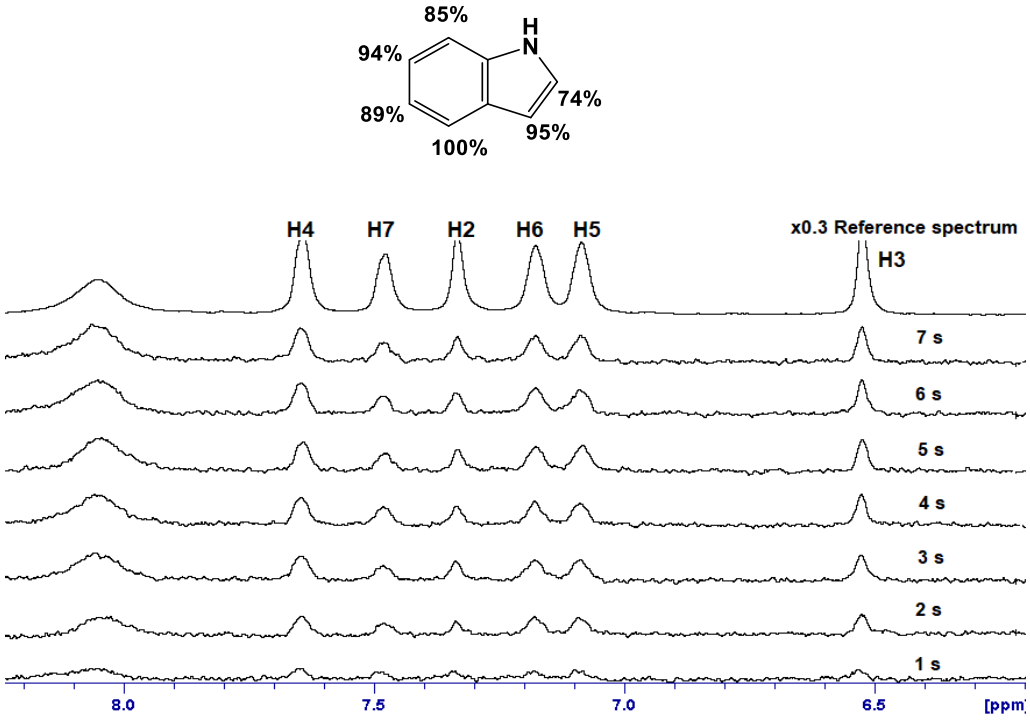

Figure S25 – STD spectra of indole (3 mM) in the presence of **Gal90** (50 µM). The on-resonance frequency was applied at 3.75 ppm. The reference spectrum is scaled x0.3. The binding epitope map is shown as an insert.

Proton	STD-AF_{max}	k_{sat} [s^{-1}]
H4	7.51± 0.01	0.50± 0.02
H3	6.63± 0.41	0.54± 0.10
H6	6.79± 0.18	0.52± 0.05
H5	6.28± 0.28	0.53± 0.09
H7	5.61± 0.02	0.56± 0.02
H2	4.87± 0.03	0.57± 0.02

Table S6 – Curve fit parameters for data in Figure 6 with 3.75 ppm on resonance frequency

Each STD-AF buildup curve is fitted to the equation: $STD-AF_{tsat} = STD-AF_{max}(1 - e^{-k_{sat} \times t_{sat}})$, and the STD-$AF_0$ values are obtained from multiplying the STD-AF_{max} value with the k_{sat} value. Values for k_{sat} and STD-AF_{max} are derived by least-squares fitting using Mathematica.

Proton	STD-AF_0	% amplification
H4	3.71± 0.2	100
H3	3.54± 0.7	95
H6	3.48± 0.3	94
H5	3.30± 0.6	89
H7	3.15± 0.1	85
H2	2.75± 0.1	74

Table S7 – Indole (3 mM) binding epitope map and STD-AF_0 values obtained in the presence of **Gal90** (50 μM) On-resonance frequency – 3.75 ppm.

9. References

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