Supporting Information

Glyco-Macroligand Microarray with Controlled Orientation and Glycan Density

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1. Syntheses of *O*-cyanate chain-end functionalized glycopolymers

Four kinds of glycopolymers with different ratios of pendant glycan and molecular weights (**1a, 1b, 1c** and **1d**) were obtained by using different ratios of glycomonomer (GM) to acryl amide (AA) as shown in the Table 1 and were characterized by ^{1}H NMR below (Fig. S1, S2, S3, S4, respectively).

Table S1. *O*-cyanate chain-end functionalized glycopolyner

Glycopolymer	GM/AA[a]	GM/AA[b]	Mn(g/mol)[c]
1a	1/1	$(1/18)_{12}$	20,700
1b	1/10	$(1/30)_{12}$	38,800
1c	1/20	$(1/54)_4$	17,200
1d	1/50	$(1/51)_4$	16,300

a. Ratio of GM and AA added. *b. Ratio of* GM and AA determined by ^{1}H NMR. *c.* Molecular weight determined by ^{1}H NMR analysis (D_2O).

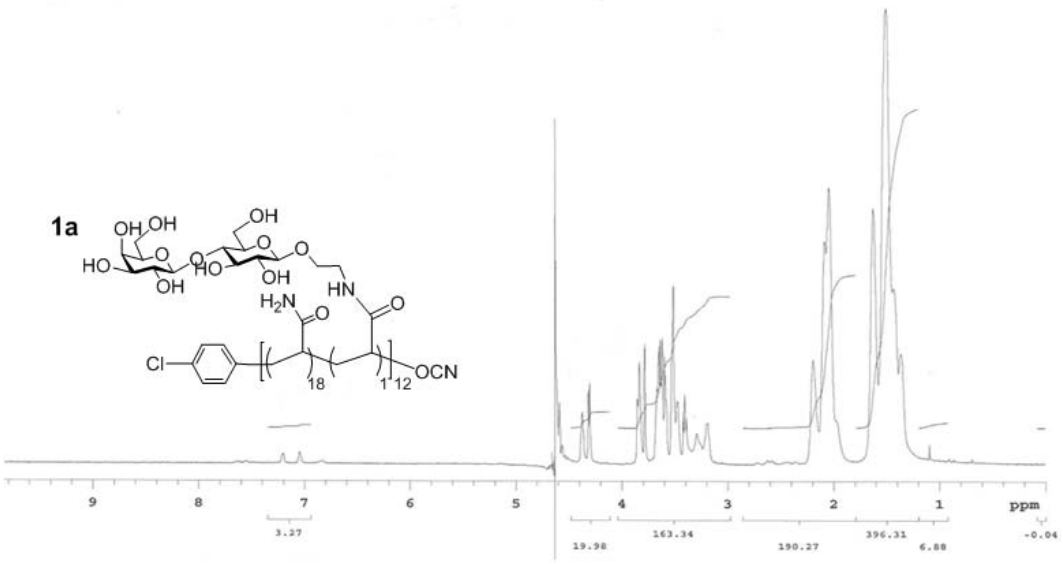

Figure S1. ^{1}H NMR spectrum of glycopolymer **1a** in D_2O

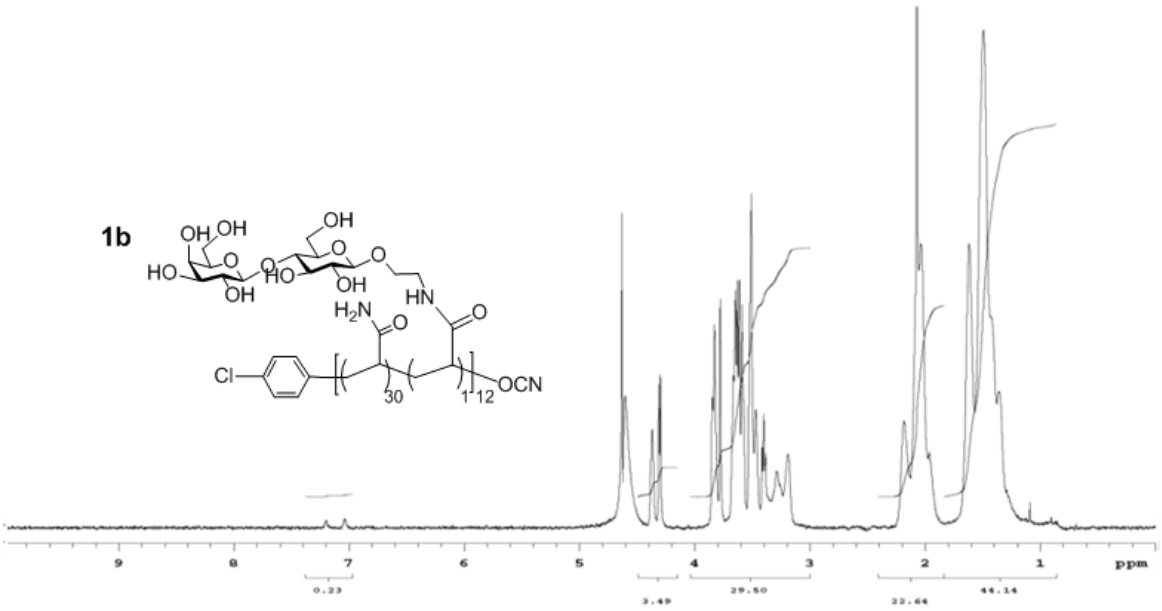

Figure S2. ^{1}H NMR spectrum of glycopolymer **1b** in D_2O

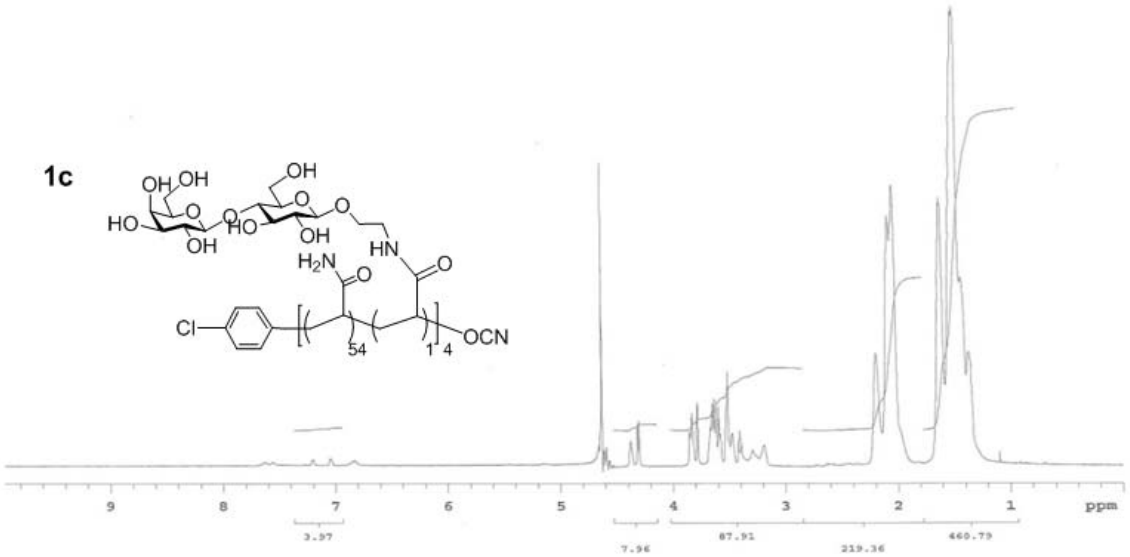

Figure S3. ^{1}H NMR spectrum of glycopolymer **1c** in D_2O

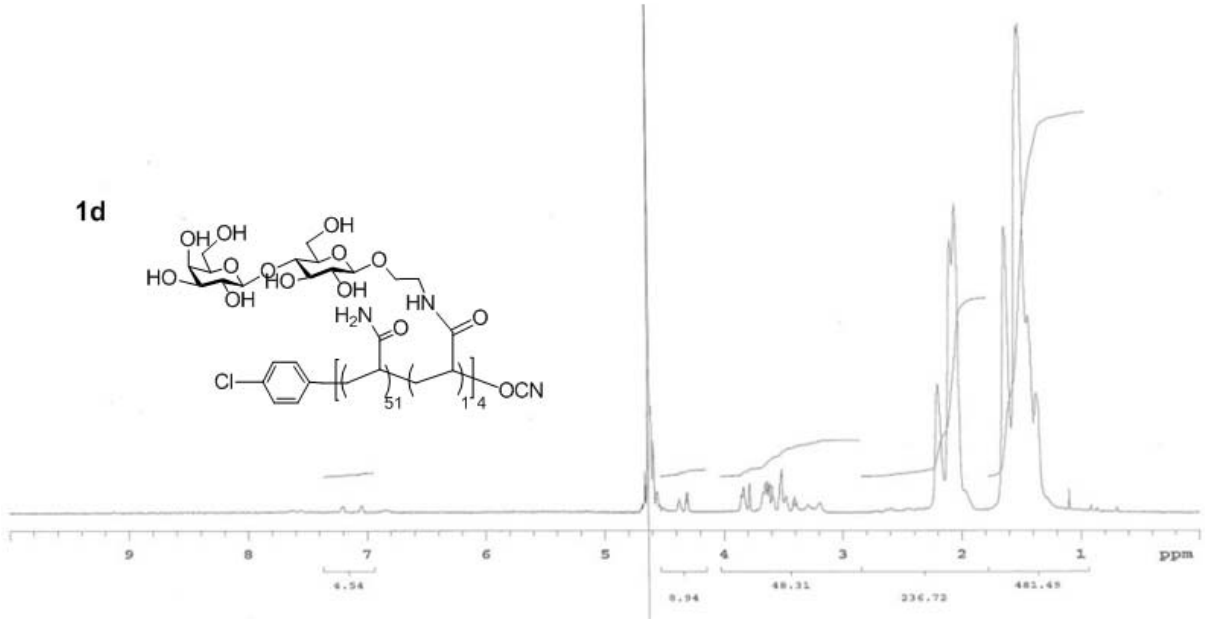

Figure S4. ^{1}H NMR spectrum of glycopolymer **1d** in D_2O

2. Characterization of boronic acid ligands

ARS assay. Qualitative determination of boronic acid conjugation to both BSA and Lysozyme were performed by Alizarin Red S assay. Briefly, Alizarin Red S (ARS) exhibits change in fluorescence intensity and color by the addition of boronic acid. As shown in Figure 5, ARS (a) showed a color change from pink to yellow when bound to boronic acid (b) and also a shift in wavelength from 510 nm to 460 nm by UV absorption in PBS (pH 7.4) buffer; and by adding free galactose, galactose -boronic acid complex was formed to release ARS indicated by a color change from yellow to pink (c) with a shift in wavelength to 510 nm from 460 nm. The same pattern of fluorescence intensity and color change was observed by both BSA-BA and Lysozyme-BA conjugates as shown in Figure S5A: ARS plus Lyz (d) no color change, ARS plus Lyz-BA (e) color change from pink to yellow and ARS-Lyz-BA plus galactose (f) color change back to pink, Figure S5B: ARS plus BSA (g) no color change, ARS plus BSA-BA (h) color change from pink to yellow and ARS-BSA-BA plus galactose (i) color change back to pink.

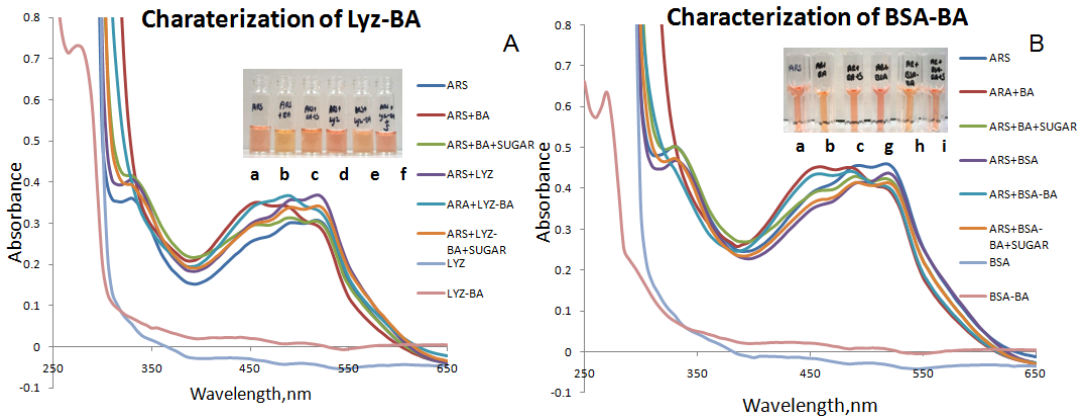

Figure S5. ARS assay of Lysozyme-BA (**A**) and BSA-BA (**B**) monitored by UV-Vis Spectroscopy

Also quantitative determination of boronic acid in BSA-BA and Lysozyme-BA conjugates was performed by monitoring ARS assay through fluorescence spectroscopy. Solutions of BSA-BA and Lysozyme-BA were added to 1 mM ARS solution in PBS (pH7.4) buffer and fluorescence intensity was recorded at 378 nm. From the calibration curve made with standard ARS and APBA below (Figure S6), the ratio of BA and protein obtained was determined as shown in Table 2S.

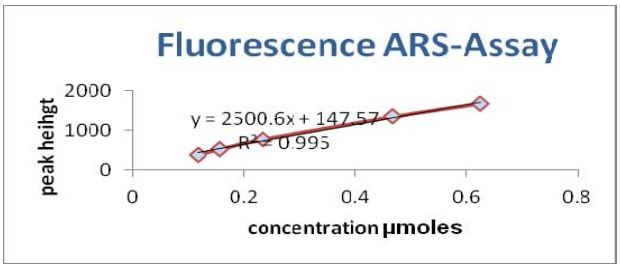

Figure S6. Calibration Curve of boronic acid by ARS assay monitored by Fluorescence Spectroscopy

Table S2. Quantification of BSA-BA and Lyz-BA conjugates from ARS assay by fluorescence spectroscopy

Protein-BA	Protein/APBA[a] (mole)	Protein/BA[b](mole)
Lyz-BA	1/33	1/4
BSA-BA	1/87	1/54

a. Ratio of protein and APBA added. b. Ratio of BA and protein determined from ARS assay.

3. Determination of boronic acid ligand detached from the glass slide with ARS assay

Detachment of boronic acid ligand from the glycopolymer was performed by incubating the glass slide with 7.4 pH PBS buffer for 30 min followed by 7.4 pH PBS buffer with 1 mM glucose for 10 min. Boronic acid ligands detached from the immobilized glycopolymer were confirmed by ARS assay, in which ARS was added to the releasing PBS buffer solution and reacted with the detached boronic acid ligand and showed coloring change from red to brown. (Figure S7).

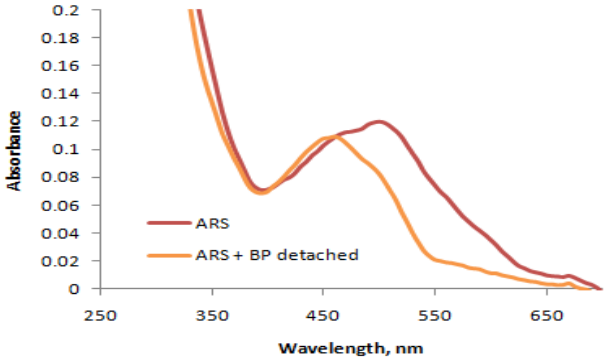

Figure S7. UV spectra of ARS and ARS upon boronic acid ligand detached from the glass slide in PBS buffer (pH7.4). BP: polyacrylamide-boronic acid