Supporting Information

From Poly(vinylimidazole) to Cationic Glycopolymers and Glyco-particles: Effective Antibacterial Agents with Enhanced Biocompatibility and Selectivity

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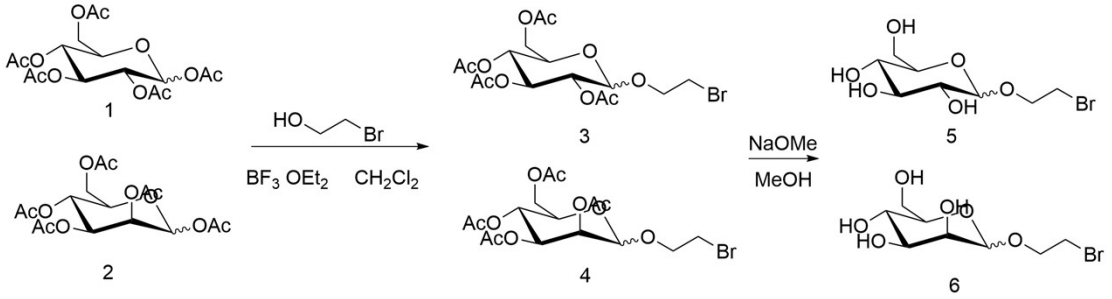
Experimental:

Materials

1-Vinylimidazole (Vi, 99%) and styrene (St, 99%) were purchased from Shanghai Aladdin Bio-Chem Technology Co., Ltd. 2, 2'-Azobis (2-methylpropionitrile) (AIBN, Aladdin, 98%) was recrystallized from methanol (Merk, 99.9%) before use. *O*-ethyl *S*-(1-phenylethyl) carbonodithioate (EPDC) was prepared following previously described route elsewhere.[1] 2-bromoethanol and 6-bromohexan-1-ol were purchased from Shanghai Aladdin Bio-Chem Technology Co., Ltd. (2R,3R,4S,5S,6R)-2-(2-bromoethoxy)-6-(hydroxymethyl)tetrahydro-2H-pyran-3,4,5-triol (A_2-GluBr), (2R,3S,4S,5S,6R)-2-(2-bromoethoxy)-6-(hydroxymethyl)tetrahydro-2H-pyran-3,4,5-triol (A_2-ManBr), (2R,3R,4S,5S,6R)-2-((6-bromohexyl)oxy)-6-(hydroxymethyl)tetrahydro-2H-pyran-3,4,5-triol (A_6-GluBr), (2R,3S,4S,5S,6R)-2-((6-bromohexyl)oxy)-6-(hydroxymethyl)tetrahydro-2H-pyran-3,4,5-triol (A_6-ManBr) were prepared following previously described route elsewhere.[2] Gram-negative bacterium *E. coli* (ATCC25922) and Gram-positive bacterium *S. aureus* (ATCC29223) were purchased from Nanjing Bianzhen Biological Technology *Co. Ltd*. 1-Vinylimidazole was treated by basic alumina column before use. Other chemicals were used as they were purchased.

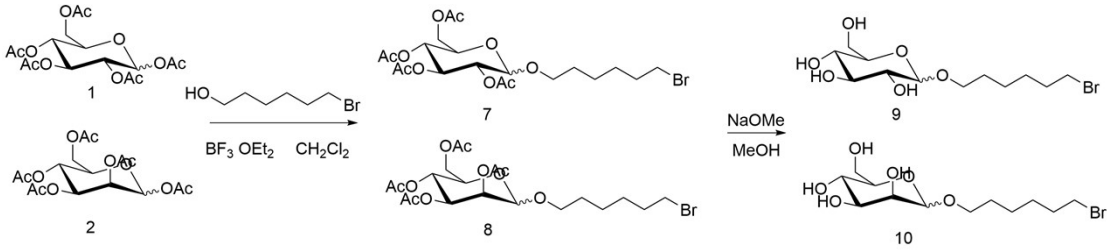
Synthesis of bromoalkyl glycosides



The Standard Procedure was followed by use of D-Glucopyranose (or D-Mannopyranose) penta-*O*-acetate (0.513 g, 1.31 mmol, 1.0 equiv), 2-bromoethanol (1.06 g, 1.41 mmol, 1.0 equiv), molecular sieves 4Å (1.00 g), CH_2Cl_2 (3.0 mL), and $BF_3{\cdot}OEt_2$ (1.0 mL, 7.96 mmol, 5.9 equiv). The crude residue was purified by column chromatography to give product **3** or **4** (0.272 g, 0.597 mmol) in 45% yield as white solid.

Product **3**: ^{1}H NMR (400 MHz, $CDCl_3$) δ = 5.22 (t, J = 9.5 Hz, 1H), 5.08 (t, J = 9.7 Hz, 1H), 5.01 (dd, J = 9.6, 8.0 Hz, 1H), 4.57 (d, J = 8.0 Hz, 1H), 4.26 (dd, J = 12.3, 4.7 Hz, 1H), 4.19 - 4.12 (m, 2H), 3.85 - 3.78 (m, 1H), 3.71 (ddd, J = 9.9, 4.7, 2.4 Hz, 1H), 3.48 - 3.43 (m, 2H), 2.09 (s, 3H), 2.07 (s, 3H), 2.02 (s, 3H), 2.01 ppm (s, 2H).

Product **4**: ^{1}H NMR (400MHz, $CDCl_3$), δ = 5.45 (1H, t, J = 9. 6Hz); 5. 15(1H, m, J = 4Hz); 5.02 (1H, t, J = 9.6Hz); 4.80-4. 83 (1H, m); 4.19-4.23 (1H, m); 4.04-4. 15 (2H, m); 3. 92-4. 00 (1H, m); 3. 75-3. 85 (1H, m); 3. 49 (2H, t, J = 6Hz); 1.91-2.11 (12H, m).



The Standard Procedure was followed by use of D-Glucopyranose (or D-Mannopyranose) penta-*O*-acetate (2.02 g, 5.18 mmol, 1.0 equiv), 6-bromohexanol (1.06 g, 5.88 mmol, 1.1equiv), molecular sieves 4Å (3.10 g), CH_2Cl_2 (7.0 mL), and $BF_3{\cdot}OEt_2$ (6.0 mL, 31.8 mmol, 6.1 equiv). The crude residue was purified by column chromatography to give product **7** or **8** (0.821 g, 1.60 mmol) in 31% yield as white solids.

Product **7**: ^{1}H-NMR (400MHz, $CDCl_3$): δ = 5.47 (t, 1H, J = 9.6 Hz); 5.06 (d, 1H, J = 4.0 Hz); 5.04 (t, 1H, J = 9.6 Hz); 4.85 (dd, 1H, J = 10.4 Hz); 4.25 (dd, 1H, J =4.4 Hz); 4.09 (dd, 1H, J = 2.4 Hz J = 12.0 Hz); 4.00 (m, 1H); 3.69 (dt, 1H); 3.46-3.36 (m, 3H); 2.09-2.01 (12H); 1.87 (m, 2H); 1.62 (m, 2H); 1.47 (m, 2H); 1.39 (m, 2H),

Product **8**: ^{1}H NMR (400MHz, $CDCl_3$) δ = 5.22-5.40 (m, 3H); 4.80 (d, J = 1.52 Hz, 1H); 4.30 (m, 1H); 4.07-4.14 (m,1H), 4.00 (m, 1H); 3.70 (dt, J = 9.6, 6.5 Hz, 1H); 3.37-3.51 (m, 3H); 2.20 (s,3H); 2.10 (s, 3H); 2.05(s, 3H); 2.00 (s, 3H); 1.88 (m, 2H); 1.39-1.70 (m, 6H).

A_2-GluBr. The Standard Procedure was followed by use of **3** (0.272 g, 0.597 mmol), dry MeOH (2.0 mL), and NaOMe (4.6 M, 15 mL). The crude residue was purified by column chromatography to give **5** (0.153 g, 0.533 mmol) in 88% yield; ^{1}H NMR (MeOD, 500 MHz): δ 4.83 (d, *J* = 1.7 Hz, 1H), 3.95-3.89 (m, 1H), 3.86-3.82 (m, 2H), 3.77-3.70 (m, 2H), 3.67-3.55 (m, 3H), 3.42 (t, *J* = 4.9 Hz, 2H) ppm.

A_2-ManBr. The Standard Procedure was followed by use of **4** (0.272 g, 0.597 mmol), dry MeOH (2.0 mL), and NaOMe (4.6 M, 15 mL). The crude residue was purified by column chromatography to give **6** (0.159 g, 0.554 mmol) in 92% yield; ^{1}H NMR (MeOD, 500 MHz): δ 4.83 (d, *J* = 1.7 Hz, 1H), 3.95-3.89 (m, 1H), 3.86-3.82 (m, 2H), 3.77-3.70 (m, 2H), 3.67-3.55 (m, 3H), 3.42 (t, *J* = 4.9 Hz, 2H) ppm.

A_6-GluBr. The Standard Procedure was followed by use of **7** (0.821 g, 1.60 mmol), dry MeOH (5.0 mL), and NaOMe (4.6 M, 25 mL). The crude residue was purified by column chromatography to give **9** (0.500g, 1.46 mmol) in 91% yield: ^{1}H NMR (MeOD, 500 MHz) δ 4.26 (d, *J* = 7.6 Hz, 1 H), 3.94–3.86 (m, 2 H) 3.66 (dd, *J* = 11.8, 5.0 Hz, 1 H), 3.59–3.53 (m, 1 H), 3.45 (t, *J* = 6.8 Hz, 2 H), 3.39–3.32 (m, 3 H), 3.18 (t, *J* = 8.4 Hz, 1 H), 1.90–1.83 (m, 2 H), 1.68–1.61 (m, 2 H), 1.52–1.41 (m, 4 H) ppm.

A_6-ManBr. The Standard Procedure was followed by use of **8** (0.821 g, 1.60 mmol), dry MeOH (5.0 mL), and NaOMe (4.6 M, 25 mL). The crude residue was purified by column chromatography to give **10** (0.510 g, 1.49 mmol) in 93% yield: ^{1}H NMR (MeOD, 500 MHz) δ 4.26 (d, *J* = 7.6 Hz, 1 H), 3.94–3.86 (m, 2 H) 3.66 (dd, *J* = 11.8,

5.0 Hz, 1 H), 3.59–3.53 (m, 1 H), 3.45 (t, *J* = 6.8 Hz, 2 H), 3.39–3.32 (m, 3 H), 3.18 (t, *J* = 8.4 Hz, 1 H), 1.90–1.83 (m, 2 H), 1.68–1.61 (m, 2 H), 1.52–1.41 (m, 4 H) ppm.

Synthesis of Poly(vinylimidazole) (PVi)

Poly(vinylimidazole) were obtained *via* RAFT polymerization of 1-vinylimidazole by the following general method.[3] In a typical reaction, Vi (4.23 g, 450 mmol), AIBN (147.8 mg, 0.9 mmol), and 203.7 mg (0.9 mmol) of EPDC were taken in a 50 ml vial containing 30 ml of ethanol, and the solution was deoxygenated by the nitrogen bubbling for 10 min with N_2 and agitated at 70°C for 30 hr. The polymerization of reaction was stop through reaction vessel quickly cooled in liquid nitrogen. To obtain high-purity polymer products, other impurities could be removed by dialysis (MWCO 1000Da) against water/methanol (4:1) for two days and pure water for another day following lyophilization. The resulting product was further dried under vacuum at ambient temperature to yield PVi with RAFT pendant groups as a light yellow powder. The sample for ^{1}H NMR was directly diluted with D_2O.

Synthesis of a series of PVi-based glycopolymer and anionic exchange.

In a typical reaction, PVi (188.2 mg, ~ 2 mmol Vi unit), 2-bromoethanol (500 mg, 4 mmol), and 3 ml of DMSO were added to a 25 mL round-bottomed flask. The resulting mixture was bubbled for 15 min with N_2 and stirred at 80 °C for two days. DMSO and excess 2-bromoethanol were removed by dialysis against water for two days following lyophilization.

To obtain sugar-containing polymer, PVi (188.2 mg, ~ 2 mmol Vi unit), bromine-containing mannose (A_2-ManBr, 1.1 g, 4 mmol) or glucose (A_2-GluBr, 1.1 g, 4 mmol) and 3 ml DMSO were added to a 25 mL round-bottomed flask. The resulting mixture was bubbled for 15 min with N_2 and stirred at 80 °C for five days. DMSO and excess bromine-containing sugar were removed by dialysis against water for two days following lyophilization.

To obtain sugar-containing polymers with different anion, 1 equiv. of polymers, 1.2 equiv. of organic lithium salts ($LiPF_6$, $LiTf_2N$) and 2 ml of H_2O added to a 25 mL round-bottomed flask. Obvious precipitation was immediately watched when the two reactants were mixed. The suspension continued to be stirred for 12 hours at room temperature. The product was separated by centrifugation, then washed with pure water several times. Finally, the product was further dried under vacuum at ambient temperature.

Synthesis of PS_{45}-*b*-PVi_{15}, PS_{45}-*b*-PVi_{15}-Glu, formation of micelles

PS macromolecular chain transfer agent was obtained by the following general method *via* RAFT polymerization. In a typical reaction, St (4 ml, 35 mmol), AIBN (9 mg, 0.055 mmol), and 150 mg (0.65 mmol) of EPDC were taken in a 50 ml vial containing 2 ml of DMF, and the solution was deoxygenated by the nitrogen bubbling for 10 min with N_2 and agitated at 70°C for 30 hr. The polymerization of reaction was stop through reaction vessel quickly cooled with liquid nitrogen. The white pure solid product was obtained after precipitation in methanol. Block polymer PS_{45}-*b*-PVi_{15} was prepared

using PS macromolecular as chain transfer agent, using a ratio of [Vi] / [macroCTA] / [AIBN] = 1700: 10: 1. To obtain high-purity polymer products, other impurities could be removed by dialysis (MWCO 1000Da) against water/methanol (4:1) for two days and pure water for another day following lyophilization. Micelles were prepared by 100mg of pure product and 200 mg of bromosaccharide dissolving in 5mL DMF at 80 °C for 12 hours. A certain amount of heterogeneous suspension was dissolved in 10mL THF or 10mL water for subsequent characterization tests after dialysis in corresponding solvent.

Bacteria culture

The Gram-negative bacterium *E. coli* and Gram-positive bacterium *S. aureus* were cultured in an MHB broth medium overnight at 37 °C with continuous shaking at 180 rpm. The germ concentration could be determined by measured with a microplate reader (SpectraMax, M3, Molecular Devices, CA, USA) at a wavelength of 600 nm (OD_{600}). Then, 1 mL of the obtained bacterial suspension centrifuge tube. *S. aureus* and *E. coli* were gathered by centrifugation (at 6000 rpm for 5 min). They were thoroughly washed with phosphate buffer saline three times (PBS, 0.3 mM KH_2PO_4, pH 7.2 ± 0.2). The remaining precipitate was resuspended in 1 mL of PBS buffer, waiting for further dilution. For *S. aureus*, a suspension with an optical density of 0.3 at 600 nm, corresponding to 10^7 CFU/mL, was prepared. For *E. coli*, a suspension with an optical density of 0.2 at 600 nm, corresponding to 10^8 CFU/mL, was designed. These stock solutions were then diluted to ∼1×10^6 CFU/mL in fresh MHB.

Antibacterial test

Minimum Inhibitory Concentration (MIC) Determination. Minimum inhibition concentrations (MICs) were measured following the standard broth dilution method with minor modification.[4] Stock solutions of different cationic glycopolymers were prepared in the MHB medium at a concentration of 1024 $\mu g \cdot mL^{-1}$. The solutions were 2-fold serially diluted in MHB medium, and 100 μL of each dilution was placed in each well of 96-well microplates (Corning, ThermoScientific) followed by the addition of 100 μL of the bacterial suspension. The plate was mixed in a shaker incubator for 10 min before incubated at 37 °C for 24 h, and the absorbance at 600 nm was measured with a microplate reader spectrophotometer (SpectraMax, M3, Molecular Devices, CA, USA). A positive control without polymer and a negative control without bacteria were included. MIC was determined as the compound's lowest concentration that inhibited bacteria's growth by more than 90%. All tests were done in three independent tests with duplicate per test.

Comparison of sterilization effect of cationic glycopolymers after counter ion exchange was measured quantitatively using plate count method for killing two typical bacteria *E. coli.* and *S. aureus*. A certain amount of cationic glycopolymers stock solution were added to 500 μL of bacterial suspension (1×10^6 CFUs per mL) in a sterile centrifuge tube to reach a final concentration at 0.5 mg/mL, respectively. The suspension was shaken at 37 °C for 90 min with a rotation speed of 180 rpm. Subsequently, the bacterial

suspension was diluted 100 times (1×10^4 CFU/mL) before seeding (10 μL) on a Luria agar (L-agar) medium. The culture was incubated at 37 °C for 12 h in the dark.

Lectin-binding assay

The turbidimetric assay was adopted to measure the binding ability with ConA, which was carried out following a previously described procedure by Kiessling.[5] ConA (1 mg mL^{-1}) was fully dissolved in HBS buffer (HEPES 10 mM, NaCl 150 mM, and $CaCl_2$ 1 mM, adjusted to pH 7.4 and filtered with 0.2 μm nylon filters). ConA's exact concentration was determined by using the UV absorbance at 280 nm [A = 1.37 × (mg mL^{-1} ConA)]. The solution was then diluted to 2 μM. Cationic glycopolymers in HBS buffer (50 μL, 200 μM) and an extra 500 μL HBS buffer were added into a 5 mL micro-cuvette. Then the Con A solution (500 μL) was rapidly injected into the cuvette. The absorbance of the mixture was quickly recorded at 420 nm for 60 min every 3 s. The relative rate of interaction was determined by a linear fit of the initial aggregation's steepest portion.

Hemolysis test

Stock solutions of sample compounds were prepared in PBS at a 16384 μg / mL concentration, serially diluted by 2-fold, and 100 μL of each dilution was placed in a 96-well plate.[4] Fresh sterile defibrinated sheep's blood was dispersed in PBS at a concentration of 8% (v/v), and 100 μL of the blood suspension was added to each compound solution. The blood suspension was added to blank PBS as negative hemolysis control and PBS containing 0.1% (v/v) Triton X-100 as positive hemolysis

control. The plate was incubated at 37 °C for 1 h to allow the interaction between the compounds and the blood cells. Each mixture was centrifuged at 6500 rpm for 5 min, and the optical absorbance of the supernatant was measured at 570 nm wavelength under a UV microplate reader (SpectraMax, M3, Molecular Devices, CA, USA). The percentage of hemoglobin release was calculated as $[(A - A_0)/(A_{total} - A_0)] \times 100\%$, where A, A_0, and A_{total} are the optical absorbance of the supernatant from the incubated sample, negative control and positive control, respectively.

Characterization

^{1}H NMR spectra were recorded with a Bruker AV 500M spectrometer using deuterated solvents obtained from Aladdin. Monomer conversion for Vi was calculated by comparing the integral of vinyl protons from the polymer backbones. Fourier transform infrared (FTIR) spectra were recorded on a Nicolet iS5 FTIR spectrometer using an iD7 diamond attenuated total reflectance optical base. Thermo Scientific K-Alpha+ performed the X-ray photoelectron survey spectrum with Al Kα radiation. The binding activities of sugar-containing polymers with ConA were tested by measuring the absorbance at 420 nm using a SHIMADZU UV-2600 UV/Vis spectrophotometer. The number-average molecular weight (M_n) and molecular weight distribution (M_w / M_n) were determined by Waters 1515 size exclusion chromatography (SEC) in *N, N*-dimethylformamide (DMF) at 40 °C with a flow rate of 1.00 mL min^{-1}, which was equipped with 2414 refractive index (RI) and 2489 UV detectors, a 20 μm guard column (4.6 mm × 30 mm, 100-10K) followed by three Styragel columns (HR_1, HR_3, and HR_4)

and autosampler. Narrow linear polystyrene standards in the range of 540 to 7.4×10^5 g mol^{-1} were used to calibrate the system. All samples were passed through 0.22 μm nylon filter before analysis. Particle size test was analysis by Zetasizer Nano ZS90. The polymer morphology was characterized by Transmission electron microscope (TEM, FEI Talos F200x, USA). TEM samples were prepared by drop-casting aliquots (ca. 10 μL) of the solutions onto carbon-coated copper grids which were placed on a piece of filter paper to absorb excess solvent and dry in the air. Then 10 μL of phosphotungstic acid solution (1%) was drop-casting on the copper grids, and rinse gently three times with pure water after a few minutes.

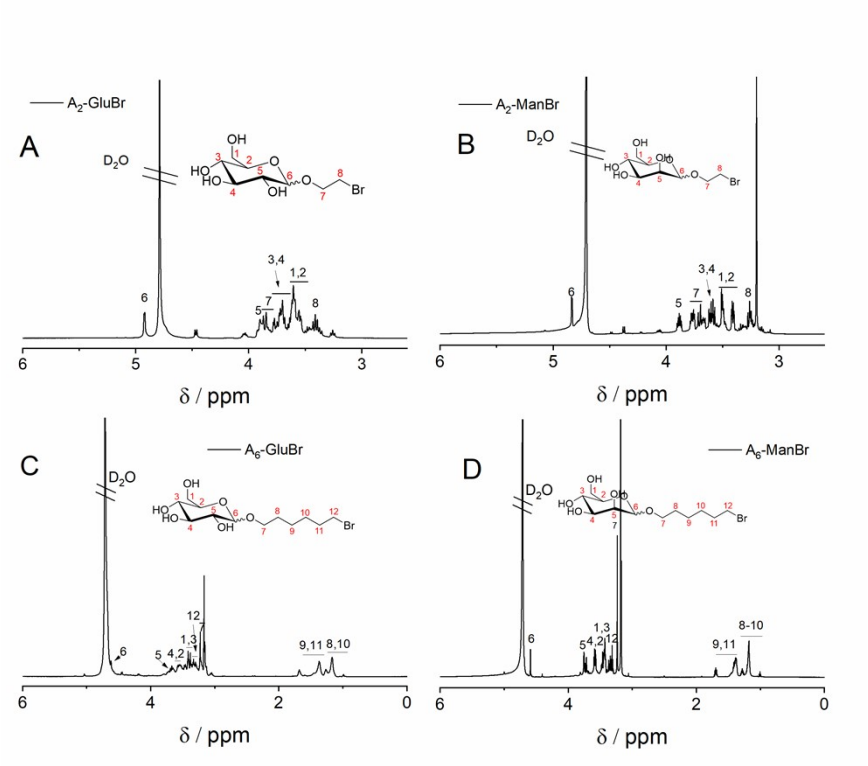


Figure S1. The ^{1}H NMR spectra of (A) A_2-GluBr, (B) A_2-ManBr, (C) A_6-GluBr, (D) A_6-ManBr using deuteroxide as solvents.

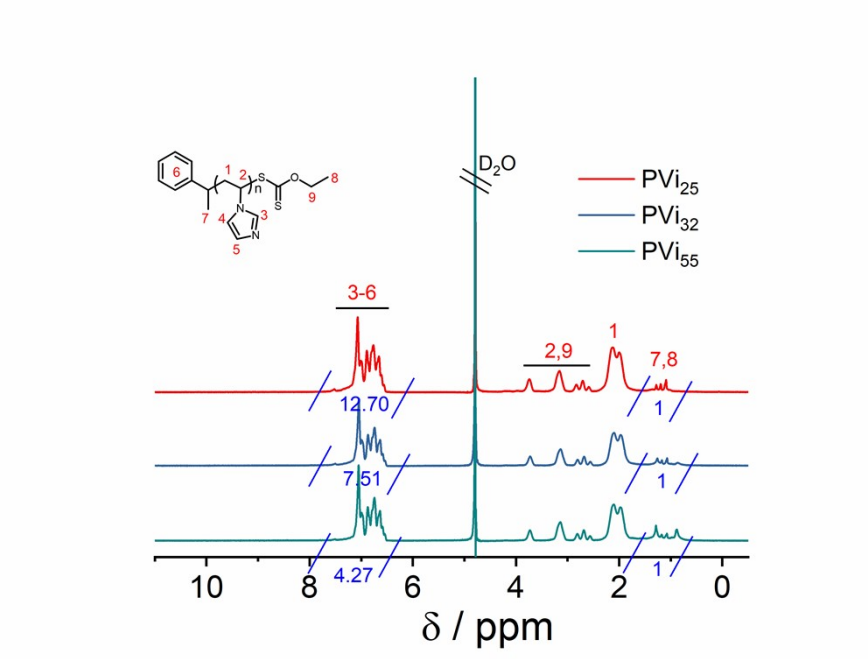


Figure S2. The ^{1}H NMR spectra of three different precursors.

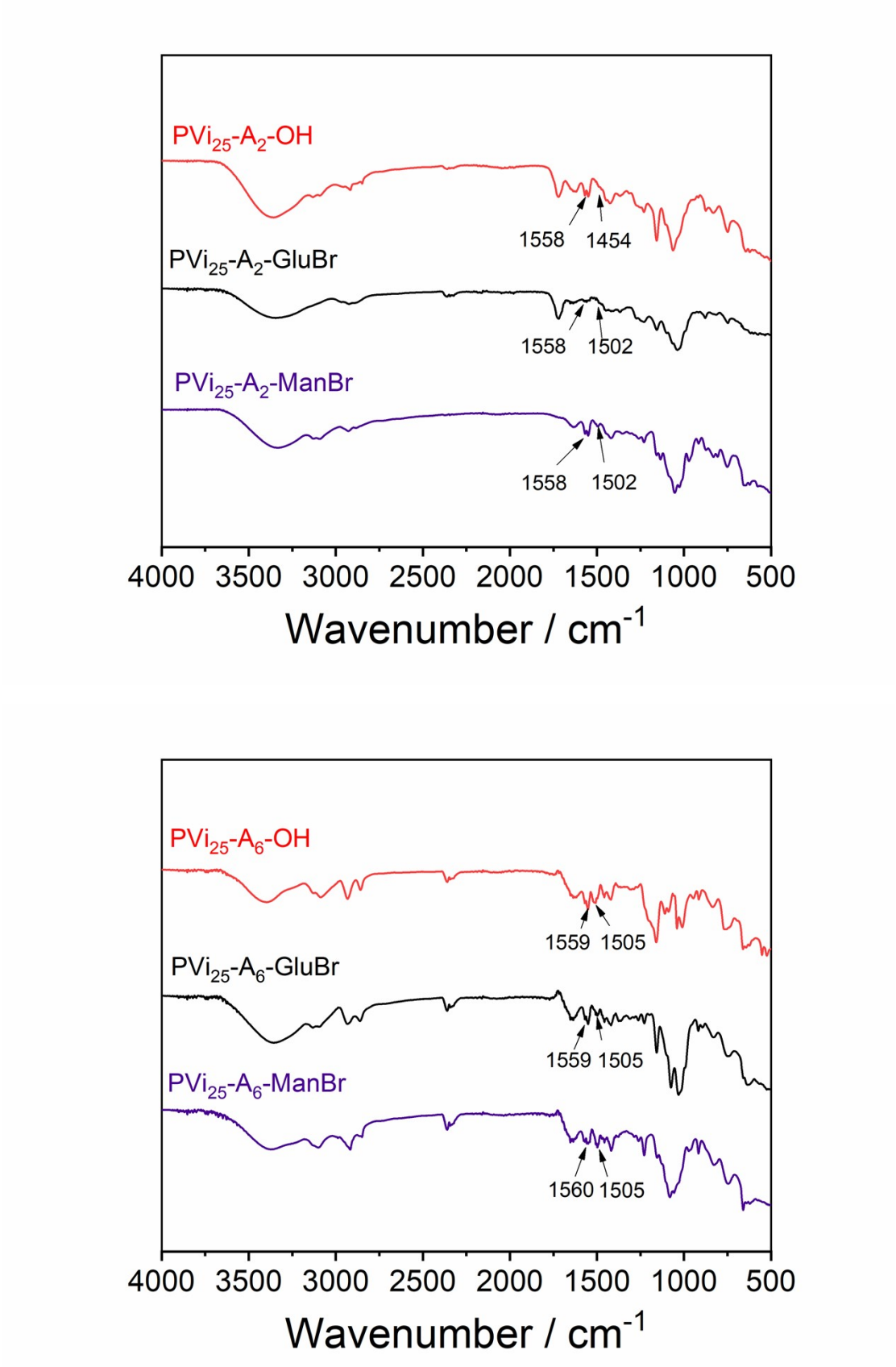


Figure S3. The FTIR spectra of cationic glycopolymers.

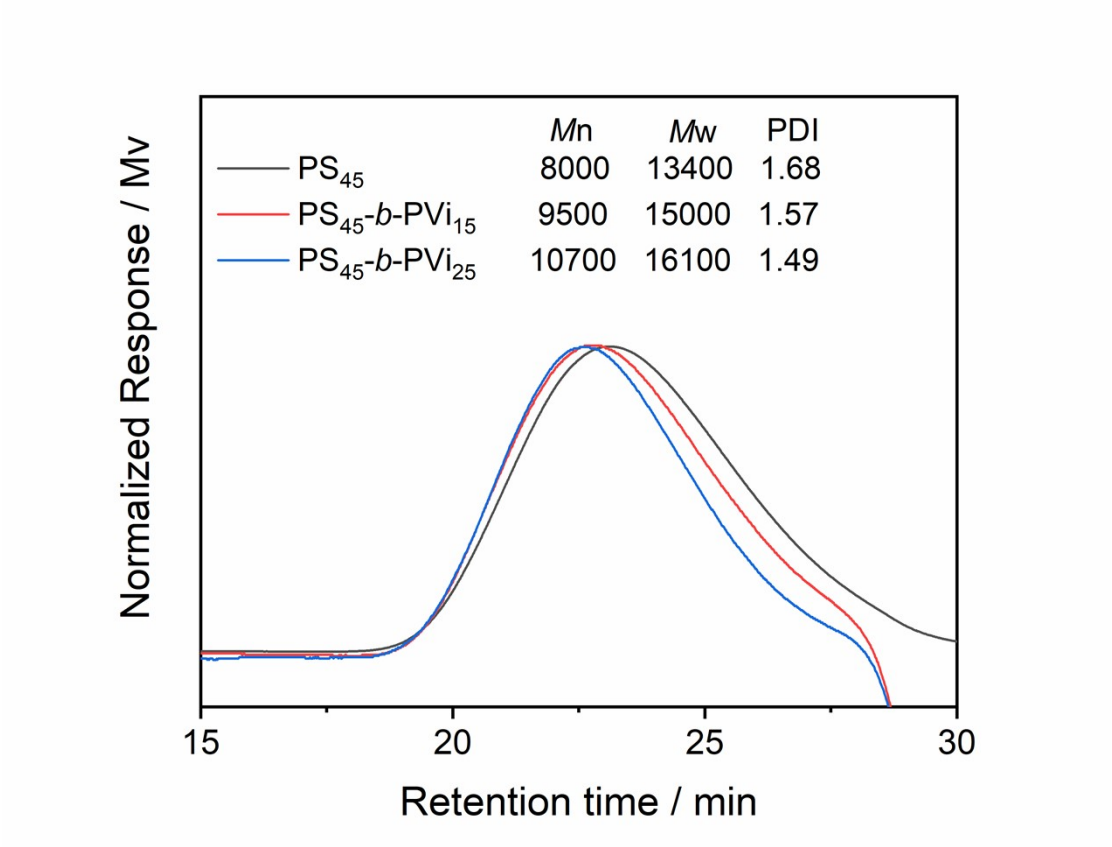


Figure S4. The GPC elution traces of block copolymers.

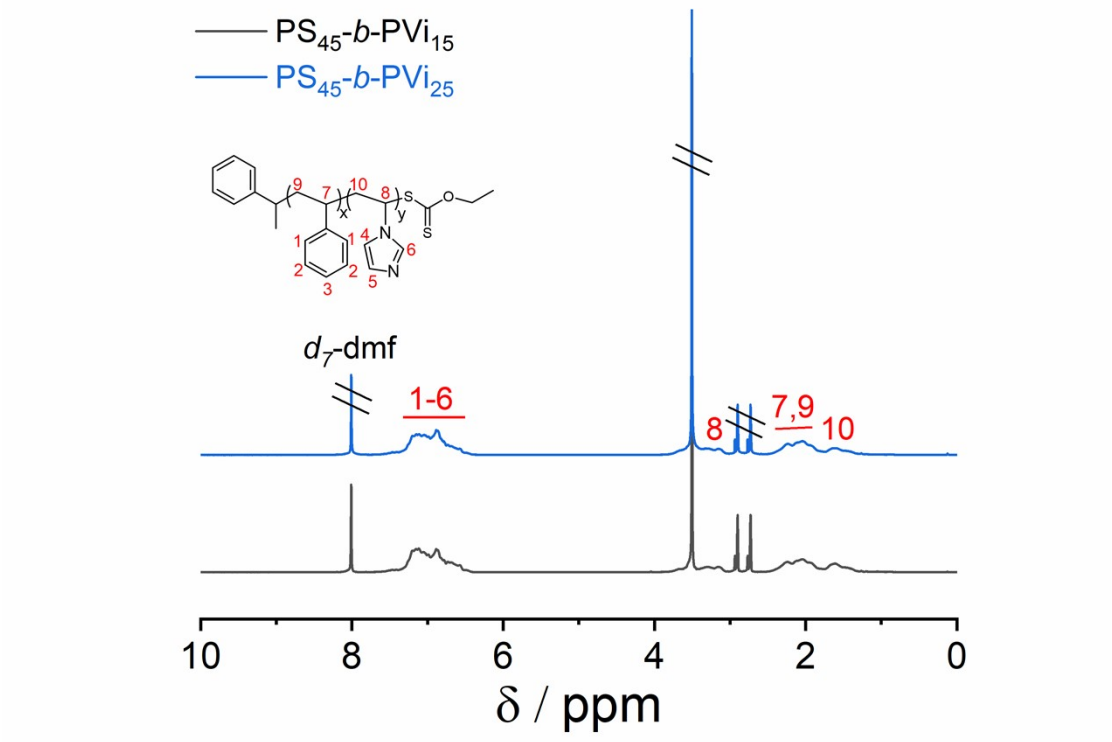


Figure S5 The ^{1}H NMR spectra of two different precursors.

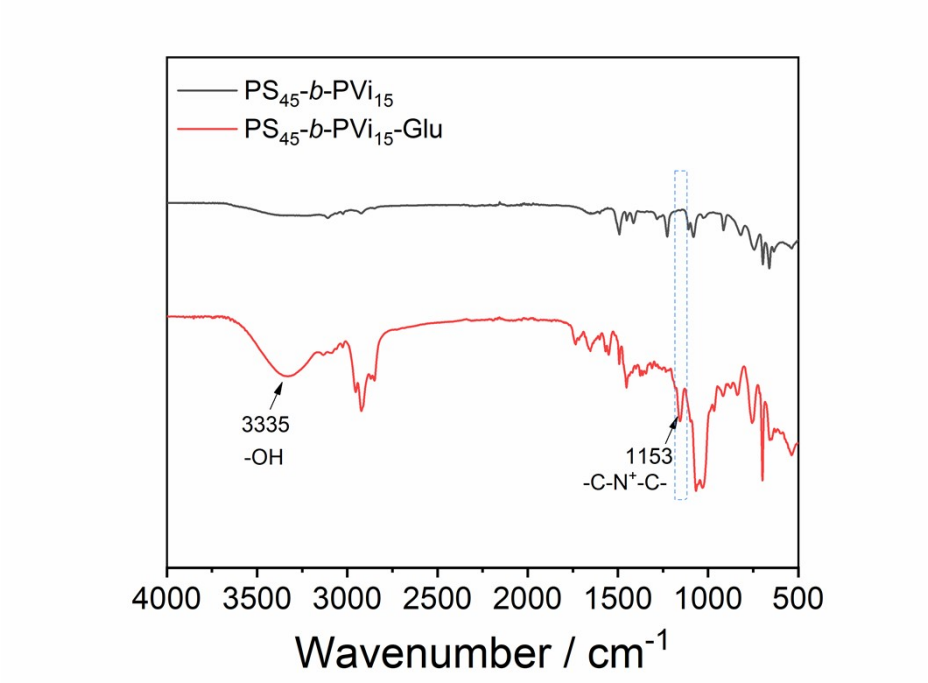


Figure S6. The FT-IR spectra of block polymers and cationic polymeric nanoparticle.

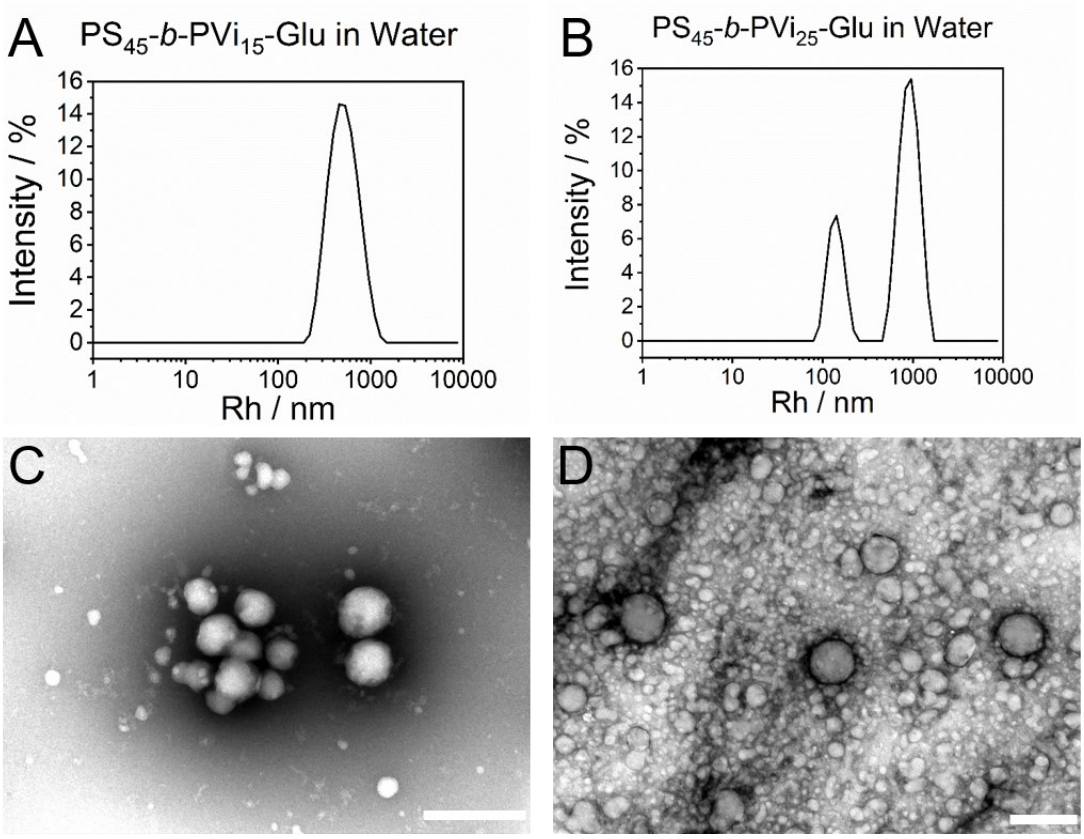


Figure S7. Particle size distribution by DLS (A, B), TEM (C: PS_{45}-*b*-PVi_{15}-Glu; D: PS_{45}-*b*-PVi_{25}-Glu, scale bar: 200 nm) images of glyconanoparticles in water.

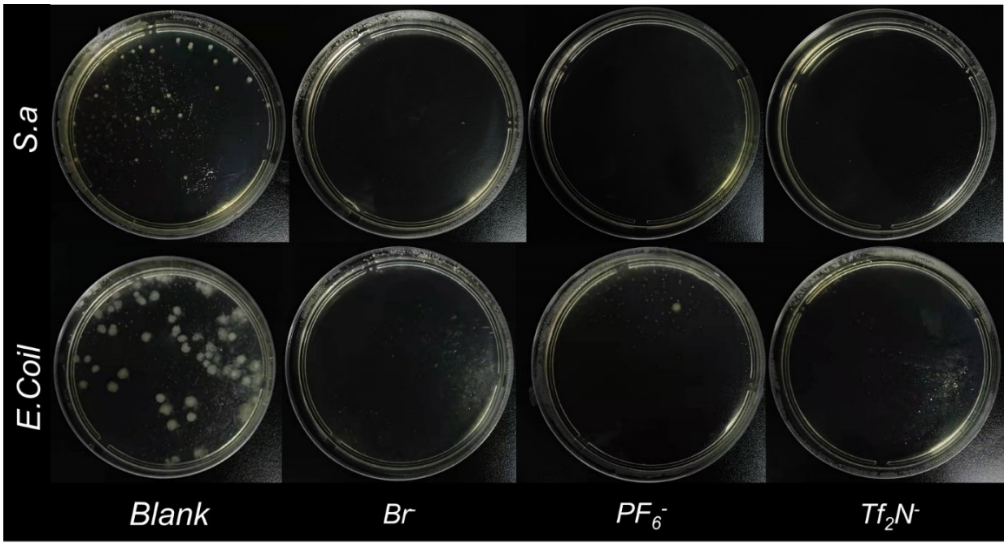


Figure S8. The actual picture of sugar-containing with different counter ion exchanges for killing *E. coli* and *S. aureus* (polymer concentration: 0.5 mg / mL), [bacterial concentration: 1×10^6 CFU mL^{-1}, and all sample was dilute 100 times before coating. Incubation condition: 37 °C, 12 hours].

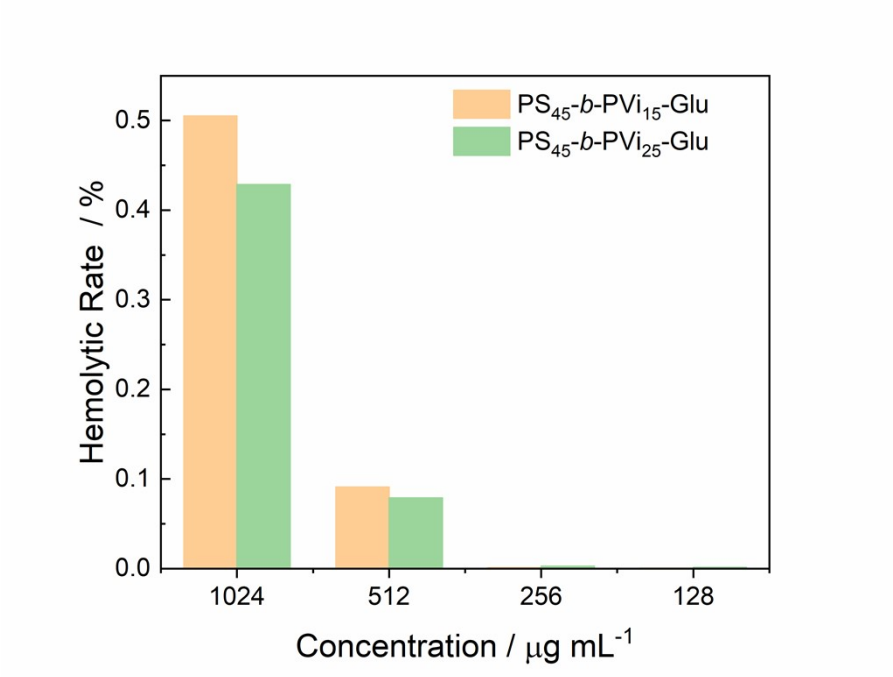


Figure S9 Hemolysis of red blood cells from defibrinated sheep blood.

Table S1. Degree of polymerization (calculated from ^{1}H NMR) and Number-average Molecular Weight (M_n, determined by ^{1}H NMR) for three polymers precursor.

Polymer	Vi/AIBN/CTA	Yield (%)	$M_{n,\ NMR}$	DP_{NMR}
PVi_{25}	50:1:1	35.1	2300	25
PVi_{32}	100:1:1	37.5	3000	32
PVi_{55}	200:1:1	32.4	5100	55

Table S2. Quaternization of polymerization (calculated from ^{1}H NMR) for Partially Quaternized polymers.

Quaternization of Composition	QP
PVi_{25}-A_2-OH	100%
PVi_{25}-A_2-GluBr	60.0%
PVi_{25}-A_2-ManBr	74.6%
PVi_{25}-A_6-GluBr	4.2%
PVi_{25}-A_6-ManBr	12.7%

Table S3. Element concentration of PVi-ManX as measured by XPS.

Polymer	C1s	O1s	N1s	F1s	Br3d	P2p	S2p
PVi_{25}-A_2-ManBr	74.8	15.9	7.8	0.1	1.4	-	-
PVi_{25}-A_2-ManPF_6	65.9	14.3	14.0	5.6	0.1	0.1	
PVi_{25}-A_2-ManTf_2N	48.0	23.1	13.4	11.5	0.1		4.0

Note: "-" was defined as no element detected in the corresponding polymer.

Table S4. Solubility of cationic glycopolymers in different solvents.

Polymer	X: Anion	Acetone	MeOH	DMF	DMSO	H_2O
PVi-$_{25}$-A_2-ManX	Br	-	+	-	+	+
	PF_6	-	+	-	+	-
	Tf_2N	+	x	-	x	-

Note: x was defined as slightly soluble, and "+" "-" was defined as soluble and insoluble, respectively.

Table S5. Weight-average molecular weight (M_w, determined by GPC) and Number-average molecular weight (M_n, determined by GPC) for three polymers precursor.

Polymer	$M_{n,GPC}$	$M_{w,GPC}$	PDI
PS_{45}	8000	13400	1.68
PS_{45}-*b*-PVi_{15}	9500	15000	1.57
PS_{45}-*b*-PVi_{25}	10700	16100	1.49

Table S6. Table S6. Particle size, zeta potential and PdI of nanoassembly.

Polymer	Z-Average (d.nm)	PdI	ζ
PS_{45}-*b*-PVi_{15}-Glu in water	487.4	0.253	29.4
PS_{45}-*b*-PVi_{25}-Glu in water	585.8	0.888	31.4
PS_{45}-*b*-PVi_{15}-Glu in THF	212.4	0.134	--
PS_{45}-*b*-PVi_{25}-Glu in THF	257.3	0.280	--
PS_{45}-*b*-PVi_{15}-Glu in DMF	210.7	0.136	--
PS_{45}-*b*-PVi_{25}-Glu in DMF	260.5	0.274	--

Table S7. Initial binding rate for different cationic glycopolymers with ConA

Polymer	k_i (Abs/s)[a]
PVi_{32}-A_6-GluBr	0.000375
PVi_{32}-A_6-ManBr	0.00045
PVi_{55}-A_6-GluBr	0.00101
PVi_{55}-A_6-ManBr	0.0023

Note: [a] The initial binding rate (k_i) was calculated using the absorbance plot's initial linear region slope.

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