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Trapping Norovirus by Glycosylated Hydrogels: a Potential Oral Antiviral Drug

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Acute gastroenteritis is a common disease in humans. Each year there are 700 million cases of acute diarrhea, including 2.5–3.2 million deaths of children under the age of five.[1,2] Recent studies indicate that the majority of acute viral gastroenteritis episodes are caused by noroviruses.[3] Noroviruses cause large-scale outbreaks of acute diarrhea through water and food contamination, resulting in public panic. The outbreaks can also bring unnecessary situations of stress, such as foreign military operations. Its highly contagious nature and high resistance to disinfectants make norovirus and its associated diseases difficult to control. They have therefore been categorized as B agents[4,5] in the NIH/CDC biodefense program.

Norovirus, also called Norwalk-like virus, has been found to recognize human histo-blood group antigens (HBGAs).[6] HBGAs are complex carbohydrates linked to glycoproteins or glycolipids located on the surface of red blood cells and mucosal epithelial cells. They are also present as free antigens in biological fluids such as blood, saliva, intestinal contents, and milk. Huang and co-workers demonstrated that different noroviruses can recognize different HBGAs.[7] The research groups of Lee,[8] Whitesides,[9] and others[10,11] have demonstrated that the polyvalent form of carbohydrate ligands, either polymer- or dendrimer-based, can significantly increase carbohydrate binding to bacterial or viral lectins. Eklind and co-workers prepared Lewis B derivatives co-polymerized with acrylamide to inhibit the growth of *Helicobacter pylori*.[12] However, there are two limitations in the application of linear polymers or dendrimers as oral antiviral drugs: 1) a large quantity of polymer or dendrimer is required to efficiently bind noroviruses located in the intestinal tract owing to the high solubility of the polymer in aqueous solution and 2) because of polydispersity, the low-molecular-weight fraction of the linear glycosylated polymer, especially species smaller than 200 nm in diameter,[13] may enter the blood vessels and cause severe side effects.

To overcome these limitations, glycosylated hydrogels were applied to trap noroviruses. Hydrogels are cross-linked polymers and are insoluble in water, but can swell from several to a few thousand times their original weight by absorbing water. Hydrogels are also stable in acidic or alkaline environments due to their covalent nature. Through these characteristics, hydrogels resemble living tissues in their physical properties, biocompatibility, and nontoxicity.[14] As a result, hydrogels are widely used as drug-delivery systems.[15–24] Herein we report a new method to trap norovirus in an HBGA-containing hydrogel (Figure 1). Glycosylated hydrogels have the potential for use as oral prophylactic antiviral drugs by trapping norovirus inside their mesh through a "caged polyvalent effect". The norovirus entrapped in the hydrogels could then be excreted from the body through the normal GI tract.

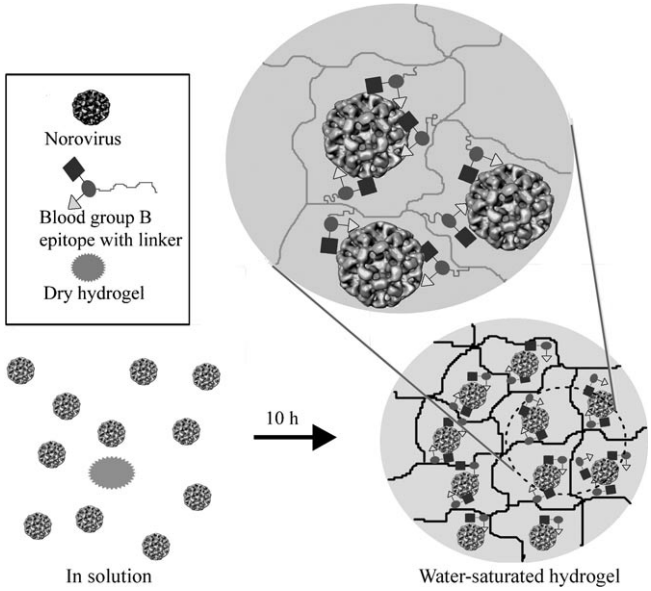

Figure 1. Schematic entrapment of norovirus particles in a hydrogel.

The acrylic-group-conjugated human HBGA type B was synthesized (Scheme 1) and combined with diallyldimethylammonium chloride (DADMAC, **2**) and acrylamide (AAm, **3**) to prepare the glycosylated poly(DADMAC)–poly(AAm) hydrogels at various densities of the cross-linking reagent *N*,*N*′-methylenebisacrylamide (Bis), as shown in Scheme 2. The hydrogel was synthesized through a free-radical polymerization in deionized

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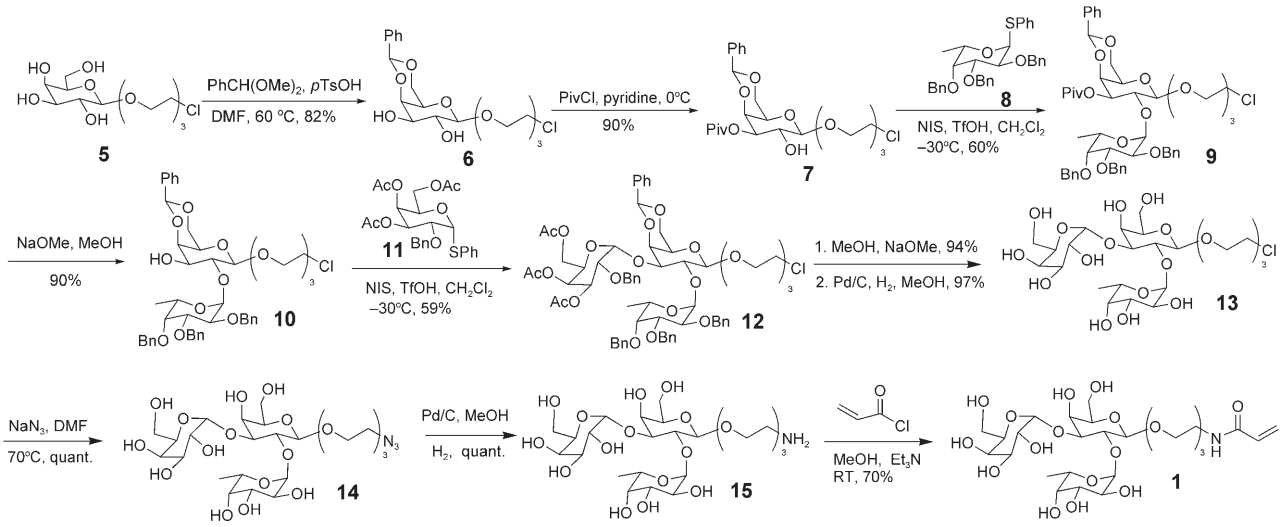


Scheme 1. Synthesis of the blood group B epitope derivative **1**. Bn = benzyl; DMF = *N*,*N*-dimethylformamide; NIS = *N*-iodosuccinimide; Piv = pivaloyl; Tf = trifluoromethanesulfonyl; *p*TsOH = *para*-toluenesulfonic acid.

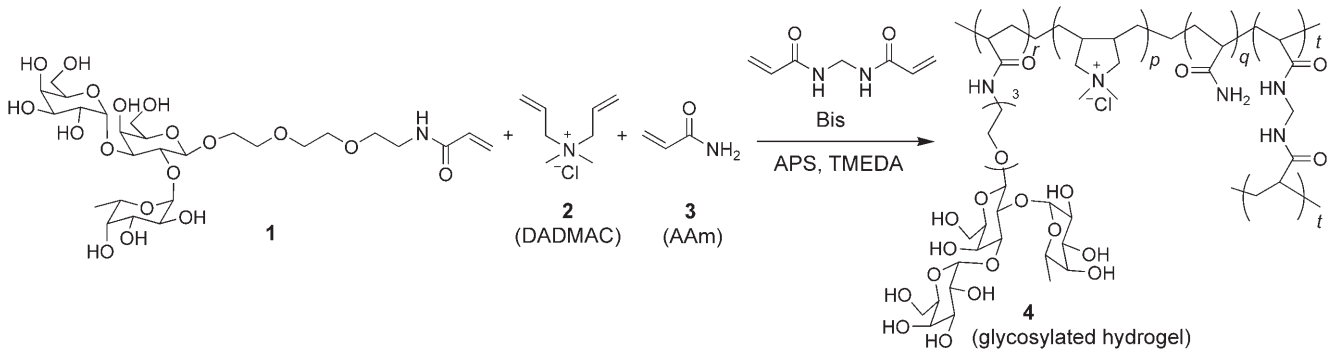
aqueous solution with ammonium persulfate (APS) and *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TMEDA) as the initiators at 37 °C for 24 h (Scheme 2). Swelling ratio, a parameter in direct proportion to the mesh size of the hydrogel,[25] was determined by calculating the ratio between the mass of hydrogel saturated with deionized water and that of dry hydrogel. All hydrogels were immersed in deionized water for 48 h. The water was changed every 12 h to wash out residual initiators, unreacted monomers, and linear polymers before performing the trapping experiments.

The optimal conditions for the largest mesh size of the non-glycosylated hydrogels were obtained by varying the cross-linker density and the molar ratio of DADMAC/AAm, while maintaining a constant total monomer concentration of 3 M. The results indicate that the hydrogel synthesized with a DADMAC/AAm ratio of 7:3 and Bis at 0.2 mol% has the largest mesh size (highest swelling ratio of 300). The glycosylated hydrogel was obtained in at the milligram scale by adopting these optimized conditions (swelling ratio of 320).

To test the hydrogel's ability to trap noroviruses, recombinant virus-like particles (VLPs, the protein shells of norovirus) from strain VA387 and a type B blood sugar binder[7] were used to interact with the hydrogel in solution. The concentration of VLPs was determined by enzyme-linked immunosorbent assays (ELISA)[26] (see Experimental Section) before and after trapping with the glycosylated hydrogels by using the procedure described by Jones and co-workers,[27] with the non-glycosylated hydrogels as control.

Two experiments were performed to determine the effects of trapping VLPs in the glycosylated hydrogels. First, we wished to determine how mesh size affects the entrapment of VLPs (Figure 2). Because the diameter of the VLPs is the same as a norovirus (about 20 nm[28]), it is important to know whether the mesh size of the hydrogel can accommodate the VLPs. Thus, glycosylated hydrogels of similar mass (about 1 mg) but with various proportions of cross-linker were immersed in a solution of VLPs (1.0×10^{-4} μg μL^{-1}) overnight. The concentration of VLPs in solution was calculated through the calibration curve obtained by ELISA. Only a minor difference in entrapment was observed between glycosylated hydrogels prepared with various cross-linker densities, ranging from 0.75 to 2.0% (Figure 2). This result indicates that the mesh size of the hydrogels in this range is sufficient to accommodate the VLPs derived from strain VA387.

The second experiment evaluated the maximum amount of VLPs trapped by using hydrogels prepared with the same



Scheme 2. Synthesis of the blood group B epitope glycosylated hydrogel **4**.

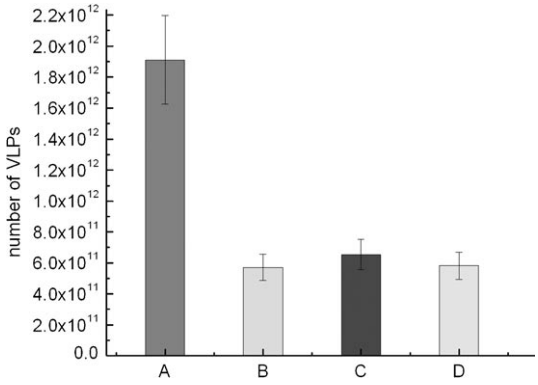


Figure 2. Absorption of VLPs by hydrogels of different cross-linker (Bis) density: A) negative control (no hydrogel); B) glycosylated hydrogel with 0.75% Bis; C) 1.0% Bis; D) 2.0% Bis.

cross-linker density (1.0 mol% relative to total monomers) in varying concentrations of VLPs in solution (Figure 3). It was found that the amount of VLPs remaining in solution treated with the glycosylated hydrogels decreased significantly. The

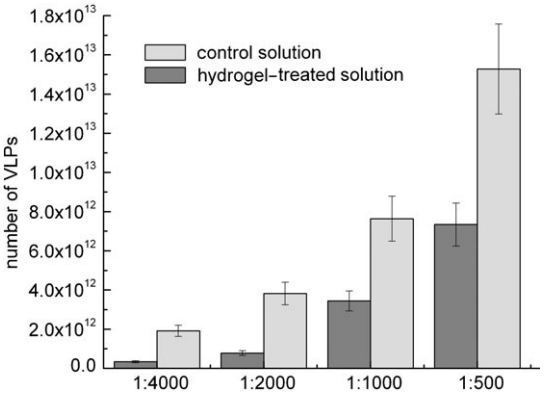


Figure 3. Immersion of the glycosylated hydrogel (5 mol%) in various concentrations of VLPs in solution; all solutions were diluted from a solution of VLPs (0.8 $\mu g\,\mu L^{-1}$).

entrapment of VLPs increased in proportion with their concentration, which may indicate diffusion-controlled trapping instead of surface absorption. The trapping capacity of the hydrogel is about 24 μg VLPs per mg glycosylated hydrogel in VLP solution (8.0×10^{-4} $\mu g\,\mu L^{-1}$, 1:1000). Therefore, in practice, multiple doses of hydrogel can be applied to completely entrap the virus.

The entrapment of the protein shell of norovirus (VLPs) by these glycosylated cross-linked hydrogels in aqueous solution indicates that the mesh size may be greater than 20 nm and can thus accommodate the norovirus inside the hydrogel. Furthermore, the insolubility of the hydrogel in water simplifies the purification procedure. These results, along with the biocompatibility of poly(DADMAC–AAm) hydrogels,[29] suggest that the glycosylated hydrogels reported herein have potential for use as prophylactic drugs against norovirus.

Experimental Section

VLPs from strain VA387, polyvalent rabbit pre- and post-immune sera (solid-phase antibody), and polyvalent guinea pig post-immune sera were gifts from Dr. Xi Jiang. Alkaline phosphatase (AP)-conjugated goat anti-guinea pig IgG and AP yellow liquid substrate were purchased from Sigma. All other reagents, unless specified, were obtained from commercial sources and used without further purification.

Synthesis of 1-chloro-3,6-dioxa-8-octyl-4,6-*O*-benzylidene-β-D-galactoside (6): Benzaldehyde dimethyl acetal (1.8 mL, 11.7 mmol) and *p*-toluenesulfonic acid (100 mg, 0.53 mmol) were added to a solution of galactoside **5** (3.5 g, 10.6 mmol) in DMF (100 mL) at room temperature. The mixture was warmed to 60 °C and stirred under decreased pressure for 5 h. The cooled solution was diluted with CH_2Cl_2, washed with saturated Na_2CO_3 aqueous solution, dried over Na_2SO_4, and concentrated under vacuum. The residue was purified by column chromatography with hexanes/EtOAc (1:4) to provide **6** in 82% yield as a white solid; ^{1}H NMR (500 MHz, $CDCl_3$, 25 °C): $\delta=3.46$ (br, 1H), 3.62–3.80 (m, 13H), 4.05–4.11 (m, 2H), 4.19 (d, $J=3.5$ Hz, 1H), 4.31 (dd, $J=12.5$, 1.0 Hz, 1H), 4.36 (d, $J=7.7$ Hz, 1H), 5.54 (s, 1H), 7.35–7.36 (m, 3H), 7.50–7.51 ppm (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): $\delta=42.83$, 66.70, 68.36, 69.18, 70.33, 70.37, 70.48, 71.11, 71.29, 72.61, 75.45, 76.81, 101.31, 103.23, 126.78, 129.12, 130.41, 138.01 ppm; HRMS: calcd for $C_{19}H_{27}ClO_8Na$ [M+Na^+] 441.1287, found 441.1288.

Synthesis of 1-chloro-3,6-dioxa-8-octyl-2-*O*-trimethylacetyl-4,6-*O*-benzylidene-β-D-galactoside (7): Trimethylacetyl chloride (0.40 mL, 3.34 mmol) was added to a solution of galactoside **6** (1.0 g, 2.78 mmol) in pyridine (15 mL). The mixture was stirred at 0 °C for 2 h, then slowly warmed to room temperature and stirred overnight. Pyridine was removed under vacuum. The residue was dissolved in CH_2Cl_2, then washed with dilute HCl (1 M) and saturated Na_2CO_3 (aq). The organic phase was dried with Na_2SO_4, filtered, and concentrated. The crude product was purified by flash column chromatography with EtOAc/MeOH (5:1) to give **7** (90%) as a white solid; ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): $\delta=1.23$ (s, 9H), 3.49 (s, 1H), 3.58–3.76 (m, 11H), 3.98–4.08 (m, 3H), 4.30 (dd, $J=12.4$, 1.2 Hz, 1H), 4.34 (d, $J=3.6$ Hz, 1H), 4.42 (d, $J=7.6$ Hz, 1H), 4.79 (dd, $J=10.0$, 3.6 Hz, 1H), 5.48 (s, 1H), 7.24–7.32 (m, 3H), 7.44–7.47 ppm (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): $\delta=14.20$, 21.04, 25.61, 39.00, 42.57, 66.59, 67.96, 68.14, 68.53, 69.10, 70.29, 70.45, 70.56, 71.36, 73.31, 73.54, 100.52, 103.65, 125.98, 128.03, 128.73, 137.83, 178.41 ppm; HRMS: calcd for $C_{24}H_{35}ClO_9Na$ [M+Na^+] 525.1862, found 525.1869.

Synthesis of 1-chloro-3,6-dioxa-8-octyl-*O*-(2,3,4,6-tetra-*O*-benzyl-α-L-fucopyranosyl)-(1→2)-2-*O*-trimethylacetyl-4,6-*O*-benzylidene-β-D-galactoside (9): Under N_2 atmosphere, compound **8** (0.65 g, 1.24 mmol) and molecular sieves (4 Å, 4.0 g) were mixed with a solution of compound **7** (0.50 g, 1.13 mmol) in CH_2Cl_2 (8.0 mL). After stirring at −30 °C for 0.5 h, NIS (0.28 g, 1.24 mmol) and trifluoromethanesulfonic acid (16 μL, 0.12 mmol) were added, and stirring was continued for 1 h. The solution was then allowed to warm to room temperature slowly and treated with saturated Na_2CO_3 (aq) and $Na_2S_2O_3$. The organic phase was collected, dried over Na_2SO_4, then concentrated under vacuum. Flash chromatography with hexanes/EtOAc (2:1) provided **9** in 59% yield; ^{1}H NMR (500 MHz, $CDCl_3$, 25 °C): $\delta=1.13$–1.15 (m, 12H), 3.51 (s, 1H), 3.57–3.71 (m, 12H), 3.99 (dd, $J=15.0$, 2.0 Hz, 1H), 4.08–4.12 (m, 3H), 4.28 (dd, $J=10.0$, 8.0 Hz, 1H), 4.34 (d, $J=15$ Hz, 1H), 4.44 (dd, $J=12.5$, 7.0 Hz, 1H), 4.48 (d, $J=4.0$ Hz, 1H), 4.60 (d, $J=7.5$ Hz, 1H), 4.62 (d, $J=11.0$ Hz, 1H), 4.70–4.81 (m, 4H), 5.00 (d, $J=12.0$ Hz, 1H),

5.09 (dd, $J=8.0$, 3.0 Hz, 1H), 5.43 (d, $J=3.5$ Hz, 1H), 5.49 (s, 1H), 7.27–7.39 (m, 18H), 7.49 ppm (dd, $J=3.0$, 2.0 Hz, 2H); ^{13}C NMR (125 MHz, $CDCl_3$, 25 °C): $\delta=16.69$, 27.07, 38.90, 42.78, 66.13, 66.49, 68.19, 69.04, 70.34, 70.38, 70.65, 71.37, 72.78, 73.11, 73.45, 74.72, 76.03, 77.75, 79.88, 96.97, 100.70, 101.72, 126.06, 127.41, 127.48, 127.56, 127.59, 128.06, 128.22, 128.35, 128.44, 128.81, 137.63, 138.35, 138.73, 138.90, 178.07 ppm; HRMS: calcd for $C_{51}H_{63}ClO_{13}Na$ [M+Na^+] 941.3849, found 941.3844.

Synthesis of 1-chloro-3,6-dioxa-8-octyl-*O*-(2,3,4,6-tetra-*O*-benzyl-α-L-fucopyranosyl)-(1→2)-4,6-*O*-benzylidene-β-D-galactoside (10): The trimethylacetyl group on disaccharide **9** (0.5 g, 0.55 mmol) was removed by adding sodium methoxide (6.0 mg, 0.11 mmol) to a solution of **9** in anhydrous methanol (5 mL) and stirred overnight. After neutralization with Dowex 50WX2–100 (H^+) resin, the solution was concentrated under decreased pressure. The crude product was purified by flash column chromatography with hexanes/EtOAc (1:4) to give **10** (90%); ^{1}H NMR (500 MHz, $CDCl_3$, 25 °C): $\delta=1.13$ (d, $J=6.5$ Hz, 3H), 3.44 (br, 1H), 3.57–3.75 (m, 12H), 3.85 (d, $J=5.5$ Hz, 2H), 3.97–4.01 (m, 2H), 4.06–4.10 (m, 2H), 4.18–4.22 (m, 2H), 4.31 (d, $J=12.0$ Hz, 1H), 4.44 (d, $J=7.0$ Hz, 1H), 4.67 (d, $J=11.5$ Hz, 1H), 4.74–4.84 (m, 4H), 4.98 (d, $J=12.0$ Hz, 1H), 5.23 (d, $J=3.5$ Hz, 1H), 5.56 (s, 1H), 7.24–7.25 (m, 3H), 7.29–7.41 (m, 15H), 7.53–7.55 ppm (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): $\delta=16.77$, 29.70, 42.77, 66.63, 66.86, 67.97, 69.26, 70.44, 70.47, 70.63, 71.36, 72.91, 73.54, 73.76, 74.75, 75.49, 77.21, 77.63, 77.83, 79.78, 83.67, 99.43, 101.40, 101.82, 126.54, 127.35, 127.51, 127.56, 127.82, 128.16, 128.19, 128.31, 128.40, 128.42, 129.10, 137.71, 137.83, 138.68, 138.80 ppm; HRMS: calcd for $C_{46}H_{55}ClO_{12}Na$ [M+Na^+] 857.3274, found 857.3291.

Synthesis of 1-chloro-3,6-dioxa-8-octyl-*O*-(2,3,4,6-tetra-*O*-benzyl-α-L-fucopyranosyl)-(1→2)-*O*-(2-*O*-benzyl-3,4,6-tri-*O*-acetyl-α-D-galactopyranosyl)-(1→3)-4,6-*O*-benzylidene-β-D-galactoside (12): Disaccharide **10** (0.2 g, 0.24 mmol), compound **11** (0.14 g, 0.29 mmol), and molecular sieves (4 Å, 2.0 g) were mixed in anhydrous CH_2Cl_2 (10 mL). The mixture was cooled to −30 °C and stirred for 0.5 h under N_2 atmosphere. Then NIS (64 mg, 0.29 mmol) and trifluoromethanesulfonic acid (5 μL, 0.058 mmol) were added and stirred for another 1 h.[11,30] Afterward, the reaction was slowly warmed to room temperature, treated with saturated Na_2CO_3 (aq) and $Na_2S_2O_3$; the organic phase was collected, dried over Na_2SO_4, then concentrated under vacuum. The crude product was purified by flash column chromatography with hexanes/EtOAc (1:1) to give α-galactoside **12** as a colorless oil (59%). The galactoside donor gave this thermodynamically preferred α-galactoside in CH_2Cl_2 by controlling the temperature below −30 °C. Similar results were published in which CH_2Cl_2 was used as solvent.[11,30] ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): $\delta=1.18$ (d, $J=6.4$ Hz, 3H), 1.98 (s, 3H), 2.00 (s, 3H), 2.10 (s, 3H), 3.15 (s, 1H), 3.52 (dd, $J=12.0$, 4.0 Hz, 1H), 3.60–3.74 (m, 13H), 3.80 (dd, $J=9.6$, 4.0 Hz, 1H), 3.88–3.95 (m, 2H), 4.02–4.07 (m, 2H), 4.12–4.19 (m, 2H), 4.24–4.30 (m, 2H), 4.32–4.37 (m, 3H), 4.43–4.48 (m, 2H), 4.67 (d, $J=11.2$ Hz, 1H), 4.81 (dd, $J=17.2$, 10.0 Hz, 2H), 4.90 (d, $J=12.0$ Hz, 1H), 4.98 (d, $J=12.0$ Hz, 1H), 5.09 (d, $J=12.0$ Hz, 1H), 5.20 (d, $J=2.8$ Hz, 1H), 5.31 (dd, $J=16.0$, 8.0 Hz, 1H), 5.37 (d, $J=3.2$ Hz, 1H), 5.49 (s, 1H), 5.67 (d, $J=4.0$ Hz, 1H), 7.04–7.06 (m, 2H), 7.24–7.32 (m, 19H), 7.46–7.53 ppm (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): $\delta=16.80$, 20.65, 20.71, 20.80, 42.79, 62.75, 66.24, 67.59, 67.95, 68.90, 69.27, 69.58, 70.40, 70.67, 71.37, 71.71, 71.85, 72.60, 73.30, 73.56, 74.16, 74.69, 76.30, 77.85, 78.45, 80.53, 94.77, 97.43, 101.14, 101.79, 126.37, 127.28, 127.43, 127.58, 127.85, 128.07, 128.15, 128.16, 128.20, 128.33, 128.93, 137.64, 137.77, 138.83, 138.90, 139.40, 169.73, 169.90, 170.60 ppm; HRMS: calcd for $C_{65}H_{77}ClO_{20}Na$ [M+Na^+] 1235.4589, found 1235.4617.

Synthesis of 1-chloro-3,6-dioxa-8-octyl-*O*-(α-L-fucopyranosyl)-(1→2)-*O*-(-α-D-galactopyranosyl)-(1→3)-β-D-galactoside (13): Compound **12** (0.10 g, 0.082 mmol) was treated with sodium methoxide (4.4 mg, 0.082 mmol) in methanol (4 mL). After neutralization with Dowex 50WX2-100 (H^+) resin, the solution was concentrated under decreased pressure. The crude product was purified by flash column chromatography with EtOAc/MeOH (10:1). The deacetylated compound (0.090 g, 0.082 mmol) in methanol solution (2.0 mL) was then hydrogenated (276 kPa) at room temperature in the presence of palladium hydroxide on carbon (20%, 8.7 mg) for 24 h. The mixture was diluted with methanol (5.0 mL) and filtered through a nylon filter (pore size: 0.2 μm, Fisher). The residue was then purified by C_{18} reversed-phase column chromatography with H_2O/MeOH (10:1) and lyophilized to give **13** as a white solid (0.050 g, 91% total yield); ^{1}H NMR (500 MHz, MeOD, 25 °C): $\delta=1.13$ (d, $J=6.5$ Hz, 3H), 3.48 (t, $J=6.0$ Hz, 1H), 3.56–3.82 (m, 20H), 3.87–3.91 (m, 3H), 3.96–4.00 (m, 1H), 4.10–4.12 (m, 2H), 4.39 (t, $J=3.5$ Hz, 1H), 4.48 (dd, $J=11.2$, 4.5 Hz, 1H), 5.11 (d, $J=3.5$ Hz, 1H), 5.22 ppm (d, $J=2.0$ Hz, 1H); ^{13}C NMR (125 MHz, MeOD, 25 °C): $\delta=15.34$, 42.48, 61.08, 61.95, 64.23, 66.14, 68.31, 68.63, 68.71, 69.88, 70.03, 70.13, 70.20, 70.36, 71.14, 71.65, 72.06, 72.49, 74.69, 78.48, 94.65, 98.69, 102.05 ppm; HRMS: calcd for $C_{24}H_{43}ClO_{17}Na$ [M+Na^+] 661.2081, found 661.2085.

Synthesis of 1-azido-3,6-dioxa-8-octyl-*O*-(α-L-fucopyranosyl)-(1→2)-*O*-(-α-D-galactopyranosyl)-(1→3) -β-D-galactoside (14): Sodium azide (0.152 g, 2.35 mmol) was added to a solution of **13** (50 mg, 0.078 mmol) in DMF (4.0 mL). The mixture was stirred overnight at 70 °C, then cooled to room temperature. The solution was concentrated under decreased pressure. The product was desalted by reversed-phase column chromatography with MeOH/H_2O (1:5) to give **14** in quantitative yield. ^{1}H NMR (400 MHz, MeOD, 25 °C): $\delta=1.13$ (d, $J=6.4$ Hz, 3H), 3.34 (t, $J=4.8$ Hz, 2H), 3.49 (t, $J=6.0$ Hz, 1H), 3.57–3.74 (m, 16H), 3.78 (dd, $J=10.0$, 2.8 Hz, 2H), 3.87 (t, 1H), 3.90 (d, $J=4.0$ Hz, 2H), 3.95–4.01 (m, 1H), 4.10 (m, 2H), 4.38 (m, 1H), 4.49 (dd, $J=12.8$, 6 Hz, 1H), 5.12 (d, $J=3.2$ Hz, 1H), 5.23 ppm (s, 1H); ^{13}C NMR (100 MHz, MeOD, 25 °C): $\delta=15.33$, 50.37, 61.06, 61.94, 64.21, 66.14, 68.32, 68.62, 68.70, 69.76, 70.03, 70.11, 70.14, 70.22, 70.36, 71.64, 72.06, 72.50, 74.68, 78.44, 94.61, 98.69, 102.04 ppm; HRMS: calcd for $C_{24}H_{43}N_3O_{17}Na$ [M+Na^+] 668.2485, found 668.2486.

Synthesis of 1-amino-3,6-dioxa-8-octyl-*O*-(α-L-fucopyranosyl)-(1→2)-*O*-(-α-D-galactopyranosyl)-(1→3)-β-D-galactoside (15): A solution of **14** (30 mg, 0.047 mmol) in methanol (2.0 mL) was hydrogenated (276 kPa) at room temperature with palladium hydroxide on carbon (20%, 2 mg) as catalyst for 24 h. The mixture was diluted with methanol (5.0 mL) and filtered through a nylon filter (pore size: 0.2 μm, Fisher). The product was lyophilized to give **15** as a white solid in quantitative yield; ^{1}H NMR (400 MHz, MeOD, 25 °C): $\delta=1.13$ (d, $J=6.4$ Hz, 3H), 2.83 (br, 1H), 3.25–3.29 (m, 2H), 3.74–3.79 (m, 18H), 3.85–3.88 (m, 3H), 3.95–4.00 (m, 1H), 4.08 (br, 2H), 4.38–4.39 (m, 1H), 4.44–4.49 (m, 1H), 5.10 (br, 1H), 5.21 ppm (br, 1H); ^{13}C NMR (100 MHz, MeOD, 25 °C): $\delta=15.33$, 40.27, 61.07, 61.93, 64.22, 66.15, 68.27, 68.61, 68.66, 69.87, 70.01, 70.08, 70.26, 70.69, 71.66, 72.06, 72.19, 72.45, 74.67, 78.38, 94.62, 98.69, 98.77, 102.05 ppm; HRMS: calcd for $C_{24}H_{45}NO_{17}Na$ [M+Na^+] 642.2580, found 642.2602.

Synthesis of 1-acrylamido-3,6-dioxa-8-octyl-*O*-(α-L-fucopyranosyl)-(1→2)-*O*-(-α-D-galactopyranosyl)-(1→3)-β-D-galactoside (1): A solution of **15** (30 mg, 0.048 mmol) was dissolved in methanol

(4 mL). The mixture was stirred at −30 °C for 0.5 h, after which triethylamine (30 μL) was added. Acryloyl chloride (8 μL, 0.11 mmol) was then injected into the mixture and stirred for another 2 h at −30 °C. After slowly warming the mixture to room temperature, the solution was concentrated under vacuum. The crude product was purified by reversed-phase column chromatography with H_2O/MeOH (5:1) and lyophilized to give **1** as a white solid (70%); ^{1}H NMR (400 MHz, MeOD, 25 °C): δ = 1.12 (d, J = 6.4 Hz, 3H), 3.40 (t, J = 5.2 Hz, 3H), 3.45–3.81 (m, 18H), 3.85–3.90 (m, 3H), 3.95–3.99 (m, 1H), 4.08–4.11 (m, 2H), 4.36–4.38 (m, 1H), 4.47 (dd, J = 13.2, 6.8 Hz, 1H), 5.10 (d, J = 3.6 Hz, 1H), 5.21 (d, J = 2.8 Hz, 1H), 5.58–5.62 (m, 1H), 6.13–6.26 ppm (m, 2H); ^{13}C NMR (100 MHz, MeOD, 25 °C): δ = 15.29, 39.02, 61.07, 61.94, 64.22, 66.11, 68.28, 68.62, 68.70, 69.14, 69.88, 69.99, 70.04, 70.33, 71.66, 71.99, 72.46, 74.72, 78.50, 94.66, 98.66, 102.07, 125.35, 130.64, 166.87 ppm; HRMS: calcd for $C_{27}H_{47}NO_{18}Na$ [M+Na^+] 696.2685, found 696.2701.

Synthesis of DADMAC–AAm hydrogels: The density of cross-linking reagent (Bis) and the molar ratio of DADMAC/AAm were varied, with a constant total monomer concentration of 3.0 mol L^{-1} to achieve the optimal hydrogel swelling ratio. First, the cross-linker density was changed from 0.1 to 0.6 mol% based on the total monomer concentration. After the addition of 1 mol% APS (relative to total monomer) in a solution volume of 100 μL, the solution was bubbled with pure N_2 for 15 min. TMEDA (1 mol%) was then added, and the solution was sealed in a glass tube. Second, after setting the reaction at 37 °C overnight, synthesized hydrogels were cut into round disks and immersed in deionized water (250 mL) for 48 h. The water was changed every 12 h. Finally, the water-saturated hydrogels were dried at room temperature for 12 h and lyophilized for 12 h. Alternatively, the ratios of DADMAC/AAm were varied from 10:0 to 4:6 with a constant density of 0.2 mol%. Dry hydrogel disks were obtained by following a similar procedure. The optimal ratio of DADMAC/AAm was 7:3.

Synthesis of glycosylated hydrogels: The glycosylated hydrogels were synthesized under the optimized conditions for DADMAC–AAm hydrogels, but with variation in cross-linker density. First, a solution of DADMAC/AAm (7:3, 100 μL) was prepared, to which APS (1 mol%) was added. The solution was purged with N_2 for 15 min, then blood group B sugar monomer (2 mg) and water were transferred to 10 μL of the solution to obtain monomer solutions (3 mol L^{-1}) containing 5 mol% sugars. After the addition of 1 mol% TMEDA, the solutions were transferred to a glass tube and sealed. The dry hydrogels were obtained by applying the same method for the preparation of DADMAC–AAm hydrogels.

Determination of hydrogel swelling ratio: Swelling ratios of the hydrogels were calculated by Equation (1):

$$\text{swelling ratio} = \frac{\text{mass of water-saturated hydrogel}}{\text{mass of dry hydrogel}} - 1 \qquad (1)$$

The hydrogels were immersed in water for 12 h to ensure saturation with deionized water. They were then removed and carefully dried with Kimwipe lab tissues. The mass of these hydrogels was set as the mass of water-saturated hydrogels.

Preparation of recombinant norovirus VLPs: Procedures for the production of VLPs in insect cell culture have been published previously by Jiang and co-workers.[7] Briefly, cDNA from the 3′ end of the genome containing the viral capsid gene (ORF2) and ORF3 were cloned from the viral RNA extracted from stool specimens. The recombinant baculoviruses carrying the viral capsid genes were constructed from the cloned cDNAs using a 'Bac-to-Bac' expression system, according to the manufacturer's instructions (Invitrogen Life Technologies, Carlsbad, CA, USA). Norovirus VLPs were produced in Sf9 or H5 insect cell cultures. VLPs were partially purified by sucrose gradient centrifugation and stored at −70 °C. Protein concentrations were determined by measuring OD_{280} and by comparison with a bovine serum albumin standard in SDS-PAGE.

ELISA procedure: An ELISA plate (Dynex Immulon) was coated with a working solution of the polyvalent rabbit pre- and post-immune sera (diluted by 1% blotto-PBS to 1:2000, 100 μL well^{-1}) and incubated at 4 °C overnight. Each well was blocked by a solution of blotto-PBS (5%) and placed at 37 °C for 1 h. After washing with PBS-T washing solution, VLPs (100 μL well^{-1}) were added (except for the 'blank' control), followed by incubation at 37 °C for 1 h. Wells were washed five times with PBS-T washing solution, and a working solution of polyvalent guinea pig post-immune sera (diluted with 1% blotto-PBS to 1:5000, 100 μL well^{-1}) was added. The solution was settled at 37 °C for 1.5 h. Each well was washed five times completely with PBS-T washing solution before the addition of AP-conjugated goat anti-guinea pig IgG (diluted with 1% blotto-PBS to 1:5000, 100 μL well^{-1}). Plates were then incubated at 37 °C for 1 h. After washing each well, AP yellow liquid substrate (100 μL well^{-1}) was added, and the absorbance was measured at λ = 405 nm in a microplate reader (Bio-Rad).

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[1] U. D. Parashar, S. S. Monroe, *Rev. Med. Virol.* **2001**, *11*, 243–252.
[2] I. Wilhelmi, E. Roman, A. Sanchez-Fauquier, *Clin. Microbiol. Infect.* **2003**, *9*, 247–262.
[3] D. O. Matson, G. Szucs, *Curr. Opin. Infect. Dis.* **2003**, *16*, 241–246.
[4] M. Hutson, R. L. Atmar, M. K. Estes, *Trends Microbiol.* **2004**, *6*, 279–287.
[5] L. King, T. David, K. Loren, C. Chris, P. Martin, W. Mary, B. Mike, *Clin. Infect. Dis.* **2005**, *40*, 1471–1480.
[6] M. Hutson, L. A. Robert, M. M. Donald, K. E. Mary, *J. Virol.* **2003**, *1*, 405–415.
[7] P. Huang, T. Farkas, S. Marionneau, W. Zhong, N. Ruvoen-Clouet, L. M. Ardythe, M. Altaye, K. P. Larry, S. N. David, J. LePendu, X. Jiang, *J. Infect. Dis.* **2003**, *188*, 19–31.
[8] a) Y. C. Lee, R. R. Townsend, M. R. Hardy, J. Lönngren, J. Arnarp, M. Haraldson, H. Lönn, *J. Biol. Chem.* **1983**, *258*, 199–202; b) Y. C. Lee, R. T. Lee, *Acc. Chem. Res.* **1995**, *28*, 321–327.
[9] a) M. Mammen, S.-K. Chai, G. M. Whitesides, *Angew. Chem.* **1998**, *110*, 2908–2953; *Angew. Chem. Int. Ed.* **1998**, *37*, 2754–2794; b) A. Pollak, H. Blumenfeld, M. Wax, R. L. Baughn, G. M. Whitesides, *J. Am. Chem. Soc.* **1980**, *102*, 6324–6336; c) M. Mammen, G. Dahmann, G. M. Whitesides, *J. Med. Chem.* **1995**, *38*, 4179–4190.
[10] M. Wu, J. H. Wu, J.-H. Liu, T. Singh, S. Andre, H. Kaltner, H.-J. Gabius, *Biochimie* **2004**, *86*, 317–326.
[11] J.-Q. Wang, X. Chen, W. Zhang, S. Zacharek, Y. Chen, P. G. Wang, *J. Am. Chem. Soc.* **1999**, *121*, 8174–8181.
[12] K. Eklind, R. Gustafsson, A. K. Tiden, T. Norberg, P. M. Aaberg, A. B. Astra Arcus, S. Södertälje, *J. Carbohydr. Chem.* **1996**, *15*, 1161–1178.
[13] K. Y. Win, S. S. Feng, *Biomaterials* **2005**, *26*, 2713–2722.
[14] K. S. Soppimath, T. M. Aminabhavi, A. M. Dave, S. G. Kumbar, W. E. Rudzinski, *Drug Dev. Ind. Pharm.* **2002**, *8*, 957–974.
[15] D. J. Bharali, S. K. Sahoo, S. Mozumdar, A. Maitra, *J. Colloid Interface Sci.* **2003**, *258*, 415–423.
[16] J. Blanchette, N. Kavimandan, N. A. Peppas, *Biomed. Pharmacother.* **2004**, *3*, 142–151.
[17] M. Delgado, K. J. Lee, L. Altobell III, C. Spanka, P. Wentworth Jr., K. D. Janda, *J. Am. Chem. Soc.* **2002**, *124*, 4946–4947.

[18] F. Aaron, T. Goto, M. Morishita, N. A. Peppas, *Eur. J. Pharm. Biopharm.* **2004**, *2*, 163 – 169.
[19] P. Gupta, K. Vermani, S. Garg, *Drug Discovery Today* **2002**, *7*, 569 – 579.
[20] A. Kikuchi, T. Okano, *Adv. Drug Delivery Rev.* **2002**, *1*, 53 – 77.
[21] N. A. Peppas, *Int. J. Pharm.* **2004**, *277*, 11 – 17.
[22] N. A. Peppas, W. Leobandung, *J. Biomater. Sci. Polym. Ed.* **2004**, *2*, 125 – 144.
[23] N. A. Peppas, K. M. Wood, J. O. Blanchette, *Expert Opin. Biol. Ther.* **2004**, *4*, 881 – 887.
[24] U. S. Toti, T. M. Aminabhavi, *J. Controlled Release* **2004**, *3*, 567 – 577.
[25] N. S. Patil, J. S Dordick, D. G. Rethwisch, *Biomaterials* **1996**, *17*, 2343 – 2350.
[26] A. Hale, K. Mattick, D. Lewis, M. Estes, X. Jiang, J. Green, R. Eglin, D. Brown, *J. Med. Virol.* **2000**, *1*, 99 – 103.
[27] G. Jones, M. Wortberg, S. B. Kreissig, B. D. Hammock, D. M. Rocke, *Anal. Chim. Acta* **1995**, *313*, 197 – 207.
[28] V. Prasad, M. E. Hardy, T. Dokland, J. Bella, M. G. Rossmann, M. K. Estes, *Science* **1999**, *286*, 287 – 290.
[29] C. Wandrey, J. Hernandez-Barajas, D. Hunkeler, *Adv. Polym. Sci.* **1999**, 123 – 182.
[30] D. Ramos, P. Rollin and W. Klaffke, *J. Org. Chem.* **2001**, *66*, 2948 – 2956.

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