Virus-Glycopolymer Conjugates by Copper(I) Catalysis of Atom Transfer Radical Polymerization and Azide-Alkyne Cycloaddition

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Supporting Information

General Procedure for Modification of CPMV with Chemical Reagents. Organic reagents were introduced into a solution of virus, such that the final solvent mixture was composed of 80% buffer and 20% DMSO. Unless otherwise specified, "buffer" refers to 0.1 M phosphate, pH 7.0. Purification of larger quantities of derivatized virus (>1 mg) was performed by ultracentrifugation over a 0-40% sucrose gradient, pelleting of the recovered virus, and solvation of the resulting material in buffer. Mass recoveries of derivatized viruses were typically 60-80%; all such samples were composed of >95% intact particles as determined by analytical size-exclusion FPLC. Virus concentrations were measured by absorbance at 260 nm; virus at 0.10 mg/mL gives a standard absorbance of 0.80. Fluorescein concentrations were obtained by measurement of absorbance at 495 nm, applying a calibrated extinction coefficient of 70,000. Each data point is the average of values obtained from three independent parallel reactions; loading values (the number of units attached to the virus) are subject to an experimental error of ±10%. The average molecular weight of the CPMV virion is 5.6×10^6.

Syntheses.

2-[2-(2-Azidoethoxy)ethoxy]ethanol: A mixture of 2-[2-(2-chloroethoxy)ethoxy]ethanol (5.00 g, 29.7 mmol), sodium azide (9.6 g, 150 mmol) and a pinch of potassium iodide in water (50 mL) was stirred at 80 °C for 24 h. The reaction mixture was extracted with ether, and the organic solution was washed with brine and then dried over anhydrous Na_2SO_4. The solvent was evaporated and the product was dried under vacuum to give a colorless oil. ^{1}H NMR ($CDCl_3$, δ) 3.3-3.8 (m, 10H), 2.4 (m, 2H); ESI-MS *m/z* = 198.1 (M+Na); IR (KBr, cm^{-1}) 2100.

*2-Bromo-2-methylpropionic acid 2-[2-(2-Azidoethoxy)ethoxy]ethyl ester (**1**):* A solution of 2--bromoisobutyryl bromide (2.9 g, 12.6 mmol) and triethylamine (1.3 g, 12.8 mmol) in THF (20 mL) was cooled to 0 °C in a 3-necked round-bottomed flask. A solution of 2-[2-(2-azidoethoxy)ethoxy]ethanol (2.0 g, 11.4 mmol) in THF (20 mL) was added dropwise with stirring. The reaction mixture was then stirred at room temperature for 4 h, filtered, and the solvent was removed by rotatory evaporation. The crude product was added to a cooled (ice bath) 5% aqueous (Na_2CO_3) solution and the resulting mixture was extracted with ethyl acetate (3 x 100 mL). The combined organic layers were washed with water, dried over anhydrous (Na_2SO_4), and evaporated to provide **1** as a yellow oil. ^{1}H NMR ($CDCl_3$, δ) 4.2 (t, 2H), 3.4-3.8 (m, 8H), 3.2, (m, 2H), 1.9 (s, 6H), ESI-MS *m/z* = 346 (M+Na); IR (KBr, cm^{-1}) 2100.

*Poly(methacryloxy ethylglucoside) (**3**).* Methacryloxy ethylglucoside (2.48 g, 8.5 mmol), 2,2'-bipyridine (0.0882 g, 0.56 mmol), and **1** (0.091 g, 0.28 mmol) were dissolved in 3:2 methanol/water (20 mL) in a Shlenk flask. Nitrogen was bubbled vigorously through the mixture for 15 minutes and CuBr (0.0405 g, 0.282 mmol) was added. The mixture was maintained under a positive pressure of nitrogen at room temperature overnight. Exposing the reaction mixture to air stopped the polymerization. The methanol was removed under reduced pressure and 10 mL of water was added to the reaction mixture. Excess copper was removed using the commercial copper binding resin Cuprisorb™ and the resulting solution was washed with ethyl acetate (3 x 15 mL) to remove unreacted initiator and bipyridine. The resulting aqueous polymer solution was lyophilized overnight to afford a white flaky powder. The presence of the

azide was confirmed by the modified ninhydrin test[1] and by the presence of the azide peak in the IR spectrum (2100 cm^{-1}). ^1H NMR (D2O, δ) 3.0-4.2 (m, 10H), 1.9 (m, 3 H), 0.7-1.1, (m, 2H). GPC was performed using polyethylene glycol and poly(*N,N*-dimethylacrylamide) calibration samples under standard conditions in water: M_n = 13,000, M_w = 10,000, polydispersity = 1.30.

*5-(3,5-Bis-prop-2-ynyloxy-benzoylamino)-2-(6-hydroxy-3-oxo-9,9a-dihydro-3H-xanthen-9-yl)-benzoic acid (**4**).* A mixture of fluorescein amine (1.53 g, 4.4 mmol) and sodium bicarbonate (0.8 g, 9.5 mmol) in dry THF (30 mL) was cooled in an ice bath and stirred under N_2 for 15 min. 3,5-Bis-prop-2-ynyloxy-benzoyl chloride (1.2 g, 4.84 mmol) in dry THF (40 mL) was added dropwise and the mixture was stirred overnight at room temperature. The solid bicarbonate was removed by filtration and the solvent was evaporated to give **4** as an orange solid, which was purified by column chromatography (silica gel, eluent 95:5 EtOAc:MeOH). ^1H NMR (CD_3OD, δ) 8.4 (s, 1H), 8.2 (d, 2H), 7.3 (m, 3H), 6.8-7 (m, 3H), 6.6-6.8 (m, 4H), 4.8 (d, 4H) (s, 6 H), 3.1 (t, 2H). ESI-MS *m/z* = 560.1 (MH^+); UV-VIS (0.1 M phosphate, pH 7) λ_{max} 494 nm, ε = 64,000. Note that the reaction conditions used here, while convenient, may be adjusted to provide greater rates of cycloaddition by the use of a ligand for Cu(I).[2]

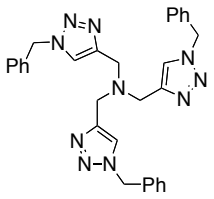
*Polymer **5***. A solution of **4** (120 mg, 0.214 mmol) in THF (2 mL) was added to a solution of **3** (107 mg, 0.0082 mmol) in H_2O (2 mL), followed by the addition of 2 mL *t*-BuOH. Sodium ascorbate (13 mg, 0.065 mmol) was added, followed by copper sulfate (8 mg, 0.032 mmol). The reaction mixture was capped (but not otherwise protected from oxygen) and stirred for 48 h at room temperature. The solvents were removed by rotary evaporation, water (10 mL) was added, and the most of the excess **4** was removed by extraction with ethyl acetate. The aqueous phase was concentrated by evaporation and the remaining residual **4** was removed by column chromatography over Sephadex G-15, eluting with water. The complete conversion of the azide to the alkyne end group was confirmed by the modified ninhydrin test[1] and by the disappearance of the azide peak (2100 cm^{-1}) in the IR spectrum. ^1H NMR (D_2O, δ) 3.0-4.2 (m, 10H), 1.9 (3 H), 0.7-1.1, (2H); the aromatic end-group signals were not easily observed.

5-(3-azidopropylamino)-5-oxopentanoic acid NHS ester **6.** To a mixture of 5-(3-azidopropylamino)-5-oxopentanoic acid (410 mg, 1.9 mmol) and *N*-hydroxysuccinimide (242 mg, 2.1 mmol) in dry CH_2Cl_2 (25 mL) was added solid 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC, 404 mg, 2.1 mmol) under nitrogen. The reaction was allowed to proceed for 12 hrs at RT. It was then washed with water (3 x 20 mL), dried over anhydrous Na_2SO_4, and the solvent was evaporated under reduced pressure to yield a white solid (417 mg, 70%). ^1H NMR ($CDCl_3$, δ) 6.2 (broad, NH),3.3-3.4 (m, 4H), 2.9 (s, 4H), 2.7 (t, 2H), 2.3 (t, 3H), 2.1 (m, 2H), 1.8 (m, 2H).

Virus azide conjugate **7**. Wild-type CPMV (24 mg, 0.25 μmol in protein asymmetric unit) was incubated with **6** (28.2 mg, 90 μmol) in 6 mL buffer containing 20% DMSO at RT for 12 hrs. The product was isolated by sucrose gradient sedimentation, ultracentrifugation pelleting, and resuspension in 0.1 M potassium phosphate buffer (pH 7.0), as previously described for similar reactions.[3]

*Virus conjugate **9***. Virus-azide **7** (4 mg, 7.1 x 10^{-4} μmol in viral capsids; approx. 0.11 μmol in azide) was incubated with **5** (140 mg, approx. 10.7 μmol) in a mixture of DMF (200 μL) and Tris buffer (pH 8, 0.1M, 1800 μL) in the presence of TCEP (4 mM), ligand **8** (4 mM), and copper sulfate (2 mM) for 24 h at 4°C. The products were purified by two successive series of sucrose gradient sedimentation, ultracentrifugation pelleting, and resuspension in 0.1 M potassium phosphate buffer (pH 7.0). The materials were shown to be free of excess **5** by size-exclusion FPLC.

The use of ligand **10**[4] – the additive originally recommended and used for a variety of bioconjugation applications[5,6] – provides less efficient reactions in demanding, quantitative situations such as the present case. For example, the optimized use of **10** rather than **8** requires the concomitant use of five times as much **5** to achieve a similar result, as follows. Virus-azide **7** (4 mg, 7.1 x 10^{-4} μmol in viral capsids; approx. 0.11 μmol in azide) was incubated with **5** (140 mg, approx. 10.7 μmol) in a mixture of DMF (200 μL) and Tris buffer (pH 8, 0.1M, 1800 µL) in the presence of tris(2-carboxyethyl)phosphine (4 mM), ligand **10** (4 mM), and copper sulfate (2 mM) for 24 h at 4°C. The product **9** was purified by two successive series of sucrose gradient sedimentation, ultracentrifugation pelleting, and resuspension in 0.1 M potassium phosphate buffer (pH 7.0). The same loading, but a slightly lower level of overall virus recovery, was observed.



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Aggregation properties with Concanavalin-A

The rate of aggregation of **9** with conA was conveniently monitored at 490 nm, where absorbance of neither the icosahedral glycoprotein assembly nor con-A was observed (Figure S1).

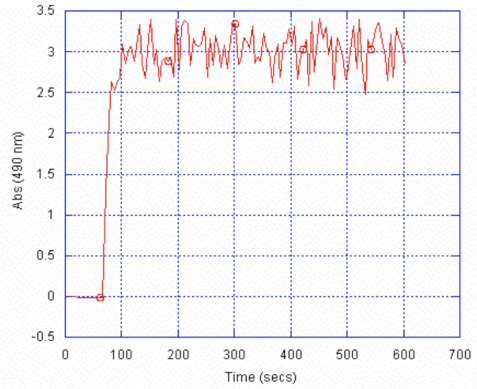

Figure S1. Time course of agglutination, monitored at 490 nm, for a mixture of con-A (0.32 mg/mL) and **9** (0.7 mg/mL) (26:1 molar ratio of con-A tetramer to virus particles, mixed at time 70 s) in PBS buffer with 0.1 mM Ca^{2+} and Mn^{2+}.

References

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