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# Synthesis and Characterization of α,ω-End Orthogonally Functionalizable Glycopolymers from Native Glycans

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## Abstract

Glycopolymers have been employed as biomimetic glycoconjugates in both biological and biomedical research and applications. Among them, chain-end functionalized glycopolymers are very often explored for protein modification, microarray, biosensor, bioprobe and other applications. Herein, we report a straightforward synthesis of α,ω-end orthogonally functionalizable glycopolymers. Specifically, glycopolymers with an alkyne or azide group at one end and an *O*-cyanate on the other end were synthesized *via* cyanoxyl-mediated free-radical polymerization from native glycans without protection and deprotection. The alkyne chain-end can react with azide-containing molecules *via* click chemistry. The azide chain-end can react with alkyne-containing molecules *via* click chemistry or copper free click chemistry. On the other hand, *O*-cyanate can react with an amine group *via* isourea bond, affording a site-specific bioconjugation as well. Furthermore, chain-end heterofunctionalizations of the glycopolymers were demonstrated *via* sequential or one-pot click chemistry and isourea bond formation, respectively. Finally, end-to-end dimerization of the glycopolymers was demonstrated *via* chain-end click chemistry. These α,ω-end orthogonally functionalizable glycopolymers will be useful in many biological and biomedical research applications.

***Keywords:*** *N*-glycan · glycosylamine · glycopolymer · sialylglycan · cyanoxyl-mediated free radical polymerization · click chemistry

## Introduction

Carbohydrate recognition events are crucial in many biological processes and have come to the forefront of biological and biomedical research, aiming to uncover the molecular mechanisms of many physiological and pathological processes, and discover potential therapeutic targets or diagnostic mechanisms for various diseases. Consequently, synthesis of carbohydrate-containing molecules has become the key research topics for both studying carbohydrate recognition and developing therapeutic and diagnostic tools.[1,2] Glycopolymers that contain multiple copies of sugar moieties have been employed as natural glycoprotein and proteoglycan mimics, which have been

used in many biological investigations and biomedical research and applications.[2–7] In particular, chain-end functionalized glycopolymers have been explored for protein modification, microarray, biosensor, bioprobe, and other applications.[8–11] Several types of glycopolymers have been synthesized with single chain-end functional group such as fluorescein, carboxylic acid, biotin, amine, hydrazide, *O*-cyanate, maleimide, pyridine disulfide, and DPPE.[3,7,12–24] Furthermore, dual chain-end functionalization has been used to immobilize a glycopolymer on one chain-end while also introducing fluorescence onto the other.[22]

Cyanoxyl-mediated free radical polymerization (CMFRP) has been used for straightforward synthesis of glycopolymers with chain-end functionalization using aryl amines as initiators.[8,25–27] CMFRP using unprotected monomers can be performed in aqueous systems that allows for a wide range of functional groups to be used such as carboxylic acid, amine, hydroxyl, and sulfate groups. Glycopolymers made by this method have previously been shown to have low polydispersity (PDI< 1.5).[15] Azide and alkyne groups are attractive chain-end groups of glycopolymers since they allow for functionalization of the glycopolymers using bioorthogonal click chemistry which react in a high yield and with site-specificity.[28-30] In this report, dual chain-end functionalized glycopolymers with an alkyne or azide on one end and an *O*-cyanate on the other end were synthesized using CMFRP methods (Scheme 1). The chain-end functional groups of the glycopolymers were then functionalized *via* click chemistry with the alkyne and azide functional groups, combined with isourea bond formation from reacting the *O*-cyanate group with an amine. Additionally, heterofunctionalization of the glycopolymers with two different fluorescent probes (Bodipy and Cy5) was performed in a one-pot reaction, illustrating that each of these reactions can be classified as orthogonal because each reaction proceeds with high specificity for the functional

group it is meant to modify. Furthermore, end-to-end glycopolymer dimers were also synthesized *via* click chemistry.

## Experimental

### Materials and methods

All solvents and reagents were purchased from commercial sources and were used as received. Lactose was purchased from Sigma (USA). α2,3-Sialolactose (α2,3-Sia-Lact) and α2,6-sialolactose (α2,6-Sia-Lact) were purchased from BIOSYN (USA). DBCO-Cy5 and lactose were purchased from Sigma (USA). Bodipy-FL azide, Bodipy amine, and Cy5 amine were purchased from Lumiprobe Corp (USA). Deionized water with a resistivity of 18 MΩ $cm^{-1}$ was used as a solvent in all polymerization reactions and dialysis experiments. Dialysis was performed using cellulose membranes with a molecular weight cutoff of 3 kDa and 12 kDa with water as solvent. $^1H$ NMR spectra were measured at room temperature with a Bruker AVIII HD 400 MHz spectrometer and $D_2O$ was used as deuterated solvent.

**Synthesis of lactosylamine and lactosyl acrylamide**. Lactosylamine and lactosyl acrylamide were synthesized as previously reported.[8]

**Synthesis of alkyne chain-end functionalized *N*-lactose polymer.** 4-Ethynylaniline (7.61 mg, 0.06 mmol) and sodium nitrite ($NaNO_2$, 7 mg, 0.10 mmol) were dissolved in 2mL of $H_2O$-THF (1:1) in a three-necked flask. The mixture solution was cooled in an ice bath for 30 min and then 14μL of $HBF_4$ solution (48 wt%, 0.028 mmol) was added to react for 2 h. Then, a degassed mixture of lactosyl acrylamide (172 mg, 0.43 mmol), acrylamide (75 mg, 1.05 mmol) and NaOCN (6 mg, 0.09 mmol) dissolved in 1.0 mL of $H_2O$ was added into the flask containing the diazonium salt. The reaction solution was kept in an oil bath at 60 °C to react for 20 h. The resulting mixture was

dialyzed (MWCO 3 kDa) against deionized water for 2 days to remove the impurity and then freeze-dried to yield alkyne chain-end functionalized *N*-lactosyl polymer. The molecular weight ($M_n$) was about 120,600 as determined by $^1$H NMR spectrum.

**Synthesis of azide chain-end functionalized *N*-lactose polymer.** 4-Azidoaniline (12.0 mg, 0.09 mmol) and sodium nitrite ($NaNO_2$, 10 mg, 0.14 mmol) were dissolved in 4 mL of $H_2O$-THF (1:1, v/v) in a three-necked flask. The mixture solution was cooled in an ice bath for 30 min and then 20 μL of $HBF_4$ solution (48 wt%, 0.04 mmol) was added to react for 2 h. Then, a degassed mixture of lactosyl acrylamide (420 mg, 1.05 mmol), acrylamide (280 mg, 3.92 mmol) and NaOCN (20 mg, 0.3 mmol) dissolved in 4.0 mL of $H_2O$ was added into the flask containing the diazonium salt. The reaction solution was kept in an oil bath at 60 °C to react for 20 h. The resulting mixture was dialyzed (MWCO 3 kDa) against deionized water for 2 days to remove the impurity and then freeze-dried to yield azide chain-end functionalized *N*-lactose polymer. The molecular weight ($M_n$) of the glycopolymer was about 80,200 as determined by comparing the integration of the anomeric proton of Gal with polymer backbone protons (-CHCO- and -$CH_2$-) in $^1$H NMR spectrum.

**Chain-end functionalization of alkyne-*N*-lactose polymer.** Functionalization of alkyne-*N*-lactose polymer was done using Cy5-amine and Bodipy-azide. For functionalization with Bodipy-azide , 5 mg glycopolymer was dissolved in PBS and 0.25 mg Bodipy-azide solubilized in DMSO was added dropwise to the reaction mixture, $CuSO_4$ (0.1 mM), THPTA (0.5 mM), and sodium ascorbate (0.5 mM) were also added to the reaction as they are needed for the CuAAC reaction. The reaction was left for 4 h at rt and then the reaction mixture was purified by dialysis (MWCO 3 kDa) for 2 days. The remaining solution was then dried using lyophilization, and Bodipy-lactose polymer, a light-yellow powder, was obtained (4.16 mg, 83.2% yield). For functionalization with Cy5-amine, 5 mg glycopolymer was dissolved in 0.1 M $NaHCO_3$ (pH 8.3) buffer, then 0.25 mg

Cy5-amine solubilized in 0.1 M $NaHCO_3$ (pH 8.3) buffer was added dropwise and the reaction was left for 4 h at rt. The reaction mixture was purified by dialysis (MWCO 3 kDa) for 2 days and then the remaining solution was lyophilized yielding a green powder, alkyne-Lact-Cy5 polymer (4.51 mg, 79% yield). For dual functionalization of alkyne-*N*-lactose polymer, a one-pot reaction was used, first, 5 mg of glycopolymer was dissolved in 0.1M $NaHCO_3$ buffer (pH 8.3), then 0.25 mg of Cy5-amine and Bodipy-azide were added dropwise into the reaction mixture which contained $CuSO_4$ (0.1 mM), THPTA (0.5 mM), and sodium ascorbate (0.5 mM). The reaction was left for 4 h at rt and then purified using dialysis (MWCO 3 kDa) for 2 days and the resulting solution was lyophilized to yield a light brown powder (4.7 mg, 94% yield).

**Facile synthesis of α2,6-sialolactosyl amine and α2,3-sialolactosyl amine from free sugars *via* Kochetkov method and their *N*-acryloyl derivatives as glycomonomers.** Sialyllactose (500 mg, 800 mM) and ammonium bicarbonate (75 mg, 10 mM) were dissolved in 5 mL of aqueous ammonia and allowed to react 48 h at 40$^o$ C. The product was lyophilized and used for the next step without purification. $H_2O$ and methanol (1:1, v/v) was added to dissolve the dried product and to this mixture was added 1.0 g of sodium bicarbonate. The solution was allowed to cool in an ice bath for approximately 30 minutes, before adding a solution of 0.430 mL acryloyl chloride (5 mM) and 2.33 mL tetrahydrofuran (30 mM) was added dropwise. This solution was stirred for 1 h and the product dried by lyophilization prior to separation on C-18 column. Separation of *N*-acryloyl derivatives was done on C-18 silica gel dry pack column using gradient elution system from 2:3 methanol/$H_2O$→100% methanol.

**Synthesis of alkyne-chain-end functionalized α2,6-sialolactose polymer and α2,3-sialolactose polymer.** 4-Ethynylaniline (7.6 mg, 0.04 mmol) and sodium nitrite ($NaNO_2$, 8 mg, 0.12 mmol) were dissolved in 2.0 mL of $H_2O$-THF (1:1) in a three-necked flask. The mixture solution was

cooled in an ice bath for 30 min and then 12 μL of $HBF_4$ solution (48 wt%, 0.028 mmol) was added to react for 2 h. Then, a degassed mixture of *N*-sialyl-(prop-2-enoyl)-β-D-lactosyl acrylamide (187 mg, 0.27 mmol), acrylamide (70 mg, 0.99 mmol) and NaOCN (6 mg, 0.09 mmol) dissolved in 1.0 mL of $H_2O$ was added into the flask containing the diazonium salt. The reaction solution was kept in an oil bath at 60 °C to react for 20 h. The resulting mixture was dialyzed (MWCO 3 kDa) against deionized water for 2 days to remove the impurity and then freeze-dried to yield alkyne chain-end functionalized *N*-α2,6-sialolactose polymer and *N*-α2,3-sialolactose polymer. The molecular weight ($M_n$) was about 76,800 for α2,3-sialolactose polymer and 20,300 for α2,6-sialolactose polymer, as determined comparing the integration of the anomeric proton of Gal and C3-equatorial proton of Neu5Ac with polymer backbone protons (-CHCO- and $-CH_2-$) in $^1$H NMR spectrum.

$^1$H NMR spectrum.

**Synthesis of azide chain-end functionalized α2,6-sialolactose polymer and α2,3-sialolactose polymer.** 4-Azidoaniline (8 mg, 0.05 mmol) and sodium nitrite ($NaNO_2$, 6 mg, 0.09 mmol) were dissolved in 2 mL of $H_2O$-THF (1:1) in a three-necked flask. The mixture solution was cooled in an ice bath for 30 min and then 24 μL of $HBF_4$ solution (48 wt%, 0.056 mmol) was added to react for 2 h. Then, a degassed mixture of *N*-sialyl-(prop-2-enoyl)-β-D-lactosyl acrylamide (200 mg, 0.29 mmol), acrylamide (120 mg, 1.69 mmol) and NaOCN (4 mg, 0.06 mmol) dissolved in 3.0 mL of $H_2O$ was added into the flask containing the diazonium salt. The reaction solution was kept in an oil bath at 60 °C to react for 20 h. The resulting mixture was dialyzed (MWCO 3 kDa) against deionized water for 2 days to remove the impurity and then freeze-dried to yield azide chain-end functionalized *N*-α2,6-sialolactose polymer and *N*-α2,3-sialolactose polymer. The molecular weight ($M_n$) was about 13,500 for *N*-α2,3-sialolactose polymer and about 11,760 for *N*-α2,6-

sialolactose polymer, as determined comparing the integration of the anomeric proton of Gal and C3-equatorial proton of Neu5Ac with polymer backbone protons (-CHCO- and -$CH_2$-) in $^1$H NMR spectrum.

**Chain-end functionalization of alkyne chain-end functionalized α2,6-sialolactose polymer (**alkyne-α2,6-Sia-Lact polymer**).** Functionalization of alkyne-α2,6-Sia-Lact polymer was done using Cy5-amine and Bodipy-azide. For functionalization with Bodipy-azide, 5 mg of glycopolymer was dissolved in PBS and 0.25 mg of Bodipy-azide dissolved in DMSO was added dropwise to the reaction mixture, $CuSO_4$ (0.1 mM), THPTA (0.5 mM), and sodium ascorbate (0.5 mM) were also added to the reaction as they are needed for the CuAAC reaction. The reaction was left for 4 h at rt and then the reaction mixture was purified using dialysis (MWCO 3 kDa) for 2 days. The remaining solution was then dried using lyophilization, and Bodipy-α2,6-Sia-Lact polymer, a light brown powder (4.32 mg, 76%) was obtained. For functionalization with Cy5-amine, 5 mg of glycopolymer was dissolved in 0.1 M $NaHCO_3$ (pH 8.3) buffer, then 0.25 mg of Cy5-amine dissolved in 0.1 M $NaHCO_3$ (pH 8.3) buffer was added dropwise, and the reaction was left for 4 h at rt. The reaction mixture was purified using dialysis (MWCO 3kDa) for 2 days and then the remaining solution was lyophilized yielding a green powder, alkyne-α2,6-Sia-Lact-Cy5 polymer (4.46 mg, 78% yield). For dual functionalization of alkyne-α2,6-Sia-Lact polymer, a one-pot reaction was used, first 5 mg of glycopolymer was dissolved in 0.1 M $NaHCO_3$ buffer (pH 8.3), then 0.25 mg of Cy5-amine and Bodipy-azide were added dropwise into the reaction mixture which contained $CuSO_4$ (0.1 mM), THPTA (0.5 mM), and sodium ascorbate (0.5 mM). The reaction was left for 4 h at rt and then purified using dialysis (MWCO 3 kDa) for 2 days and the resulting solution was lyophilized to yield a brown powder (4.72 mg, 73% yield).

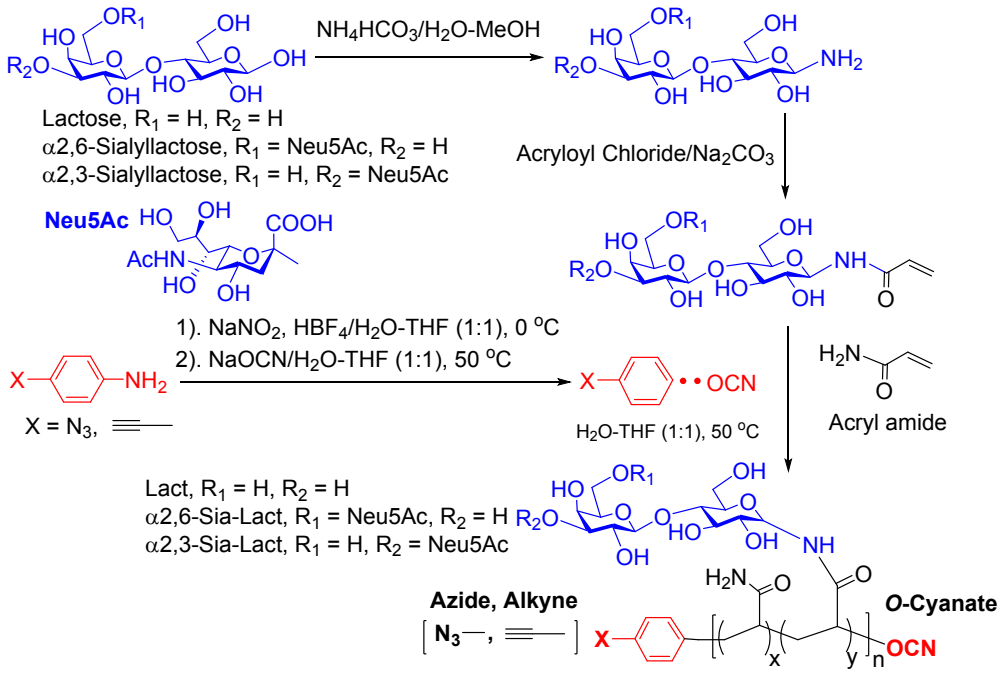
**Chain-end functionalization of azide chain-end functionalized α2,6-sialolactose polymer (**azide-α2,6-Sia-Lact polymer**).** Functionalization of azide-α2,6-Sia-Lact polymer was done using strain promoted azide-alkyne cycloaddition (SPAAC)[31] with DBCO-Cy5 and isourea bond formation using Bodipy-amine (Scheme 2). Functionalization with just DBCO-Cy5 was done by first dissolving 10 mg of azide-α2,6-Sia-Lact polymer in PBS (pH 7.4), then 0.5 mg of DBCO-Cy5 in a small amount of DMSO was added dropwise and the reaction was left to react for 4 h at rt. The reaction mixture was then purified using dialysis (MWCO 3 kDa) for 2 days and the remaining solution was dried using lyophilization and Cy5-α2,6-Sia-Lact polymer was obtained as a blue powder (8.2 mg, 82% yield). Functionalization with just Bodipy-Fl amine was done by dissolving 10 mg of azide-α2,6-Sia-Lact polymer in 0.1 M $NaHCO_3$ (pH 8.3) buffer, then 0.5 mg of Bodipy-Fl amine dissolved in a small amount of DMSO was added dropwise to the reaction solution as well. The reaction was left for 4 h at rt and then the reaction mixture was purified with dialysis (MWCO 3 kDa). The remaining solution was dried by lyophilization yielding azide-α2,6-Sia-Lact-Bodipy polymer (7.8 mg, 78% yield). Dual functionalization of azide-α2,6-Sia-Lact polymer was done in a one-pot reaction using 10 mg glycopolymer dissolved in 0.1 M $NaHCO_3$ (pH 8.3) buffer, then 0.5 mg DBCO-Cy5 and Bodipy-amine in a small amount of DMSO were added dropwise and the reaction was left to react for 4 h at rt. The reaction mixture was purified by dialysis (MWCO 3 kDa) for 2 days and the remaining solution was dried by lyophilization yielding a blue powder (9.2 mg, 92% yield).

**Dimerization of azide chain-end functionalized α2,6-sialolactose polymer.** For azide-α2,6-Sia-Lact-Bodipy polymer and alkyne-Lact-Cy5 polymer dimer, 3 mg of azide-α2,6-Sia-Lact-Bodipy polymer and 3 mg of alkyne-Lact-Cy5 polymer were dissolved in PBS with $CuSO_4$ (0.1 mM), THPTA (0.5 mM), and sodium ascorbate (0.5 mM) and left to react for 4 h at rt. The reaction

mixture was purified by dialysis (MWCO 12-14 kDa) for 2 days and the resulting solution was lyophilized to yield a brown powder (4.1 mg). For dimerization of azide-α2,6-Sia-Lact-Bodipy polymer and alkyne-α2,6-Sia-Lact-Cy5 polymer, 4 mg of each polymer was added to PBS with $CuSO_4$ (0.1 mM), THPTA (0.5 mM), and sodium ascorbate (0.5 mM) and left to react for 4 h at rt. The reaction mixture was purified with dialysis (MWCO 12-14 kDa) and the resulting solution was lyophilized to give a brown powder (5.2 mg, 83% yield).

## Results and Discussion

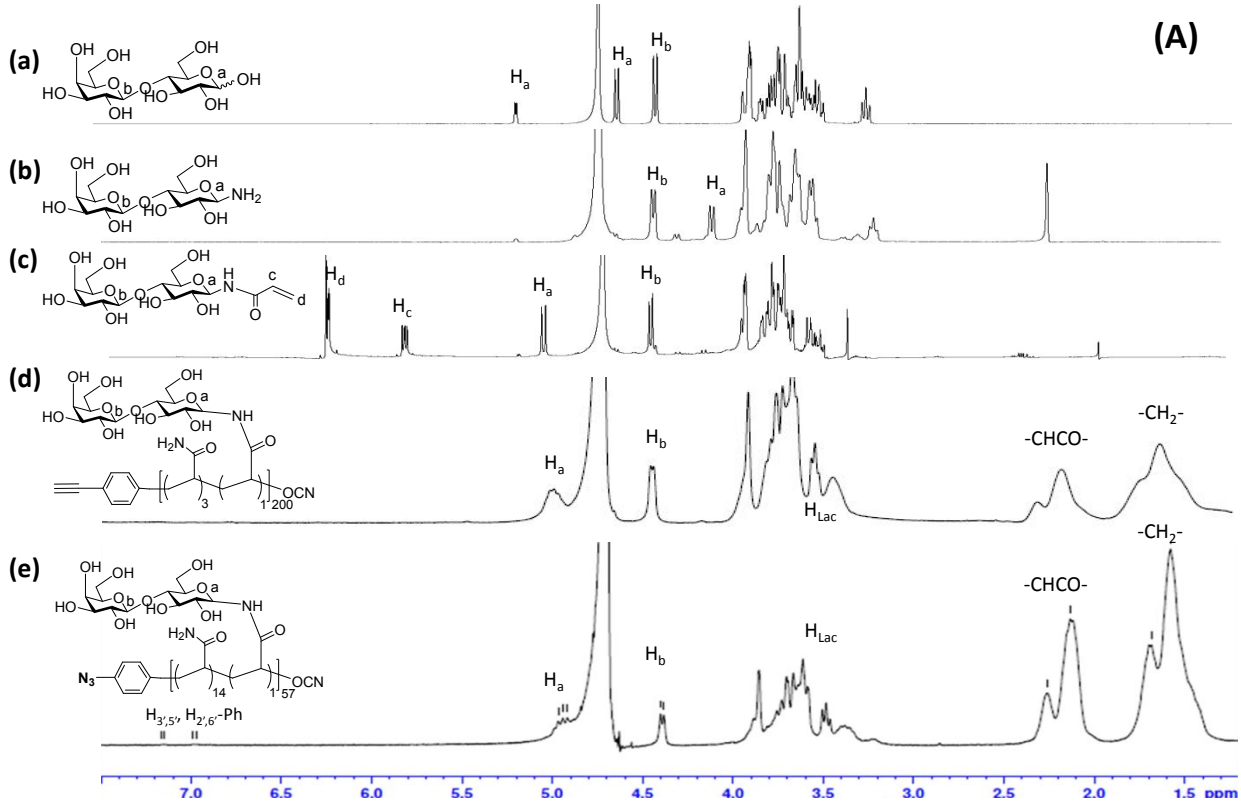
**Synthesis of α,ω-End Orthogonally Functionalizable Glycopolymers.** The glycopolymers were synthesized from *N*-acryloyl glycomonomers, which were made by converting native glycans with the free hydroxyl group on the reducing end to glycosylamines (Scheme 1). Briefly, free lactose, α2,3-sialyllactose and α2,6-sialyllactose were treated with ammonium bicarbonate in ammonium hydroxide to yield glycosylamines quantitatively. Next, *N*-acryloyl glycomonomers were made by amidation of the glycoslyamines with acryloyl chloride and sodium carbonate in a methanol-water (1:1) mixture at 0 $^{o}$C. To introduce alkyne and azide functionality into the chain-end of the glycopolymer, aryl alkyne amine (4-ethynylaniline) and aryl azide amine (4-azidoaniline) were used as initiators for CMFRP with *N*-acryl-glycomonomer and acrylamide. Specifically, sodium nitrate and tetrafluoroboric acid were used to make a diazonium salt from either 4-azidoaniline or 4-ethynylaniline. The subsequent addition of sodium cyanate led to the generation of a cyanoxyl radical which was used for chain elongation initiation. The addition of acrylamide and *N*-acryl monomers allows for chain elongation to occur and afford dual chain-end functionalized glycopolymers with chain-end alkyne or azide on one side and *O*-cyanate on the other side, respectively (Scheme 1).




**Scheme 1**. Facile synthesis of α,ω-end orthogonally functionalizable glycopolymers from native glycans.

The successful amination of free anomeric hydroxy group was confirmed by $^1$H NMR signals of anomeric proton directly adjacent to the introduced amine (4.05 ppm), while the introduction of acrylamide was confirmed by $^1$H NMR signals of protons on the alkene (5.82 and 6.25 ppm) as well as the downfield signal shift of the anomeric proton directly adjacent to the amide (5.05 ppm) as shown in Figure **1A** (**a**, **b**, **c**), **1B** (**a**, **b**, **c**), and **1C** (**a**, **b**, **c**).The dual chain-end functionalized glycopolymers were characterized by $^1$H NMR analysis, which indicated successful polymerization with characteristic peaks including aromatic protons around 7.00 - 7.50 ppm and the polymer backbone peaks above 1.51 - 1.80 and 2.10 - 2.35 ppm (Figure **1A** (**d**, **e**), **1B** (**d**, **e**), and **1C** (**d**, **e**)). In particular, the C3-equatorial proton of Sia (Neu5Ac) near 2.65 ppm, and, the *N*-acetyl protons of Neu5Ac near 2.00 ppm are characteristic peaks for alkyne-α2,6-Sia-Lact glycopolymer and azide-α2,6-Sia-Lact glycopolymer (Figure **1B** (**d**, **e**)) as well as alkyne-α2,3-Sia-Lact glycopolymer and azide-α2,3-Sia-Lact glycopolymer (Figure **1C** (**d**, **e**)). By comparing

the integration of the anomeric proton of Gal with polymer backbone protons (-CHCO- and -$CH_2$-) in alkyne-Lact glycopolymer and azide-Lact glycopolymer (Figure **1A** (**d**, **e**)) and C3-equatorial proton of Neu5Ac with polymer backbone protons in alkyne-α2,6-Sia-Lact glycopolymer and azide-α2,6-Sia-Lact glycopolymer (Figure **1B** (**d**, **e**)) and alkyne-α2,3-Sia-Lact glycopolymer and azide-α2,3-Sia-Lact glycopolymer (Figure **1C** (**d**, **e**)), the molecular weights and the ratios of acrylamide and *N*-acryl-glycans in these glycopolymers were determined, respectively (Figure **1A** (**d**, **e**), **1B** (**d**, **e**), and **1C** (**d**, **e**)).



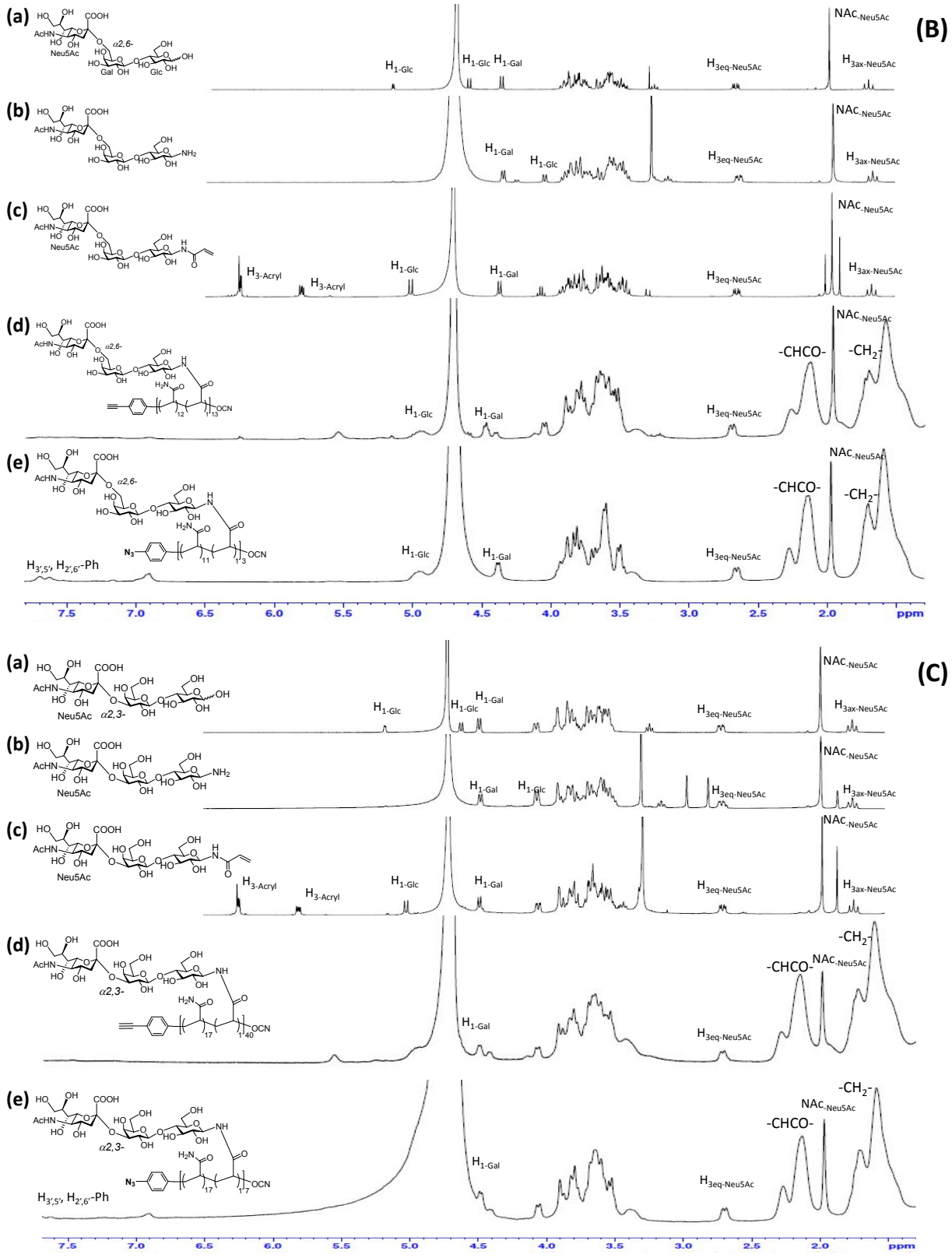
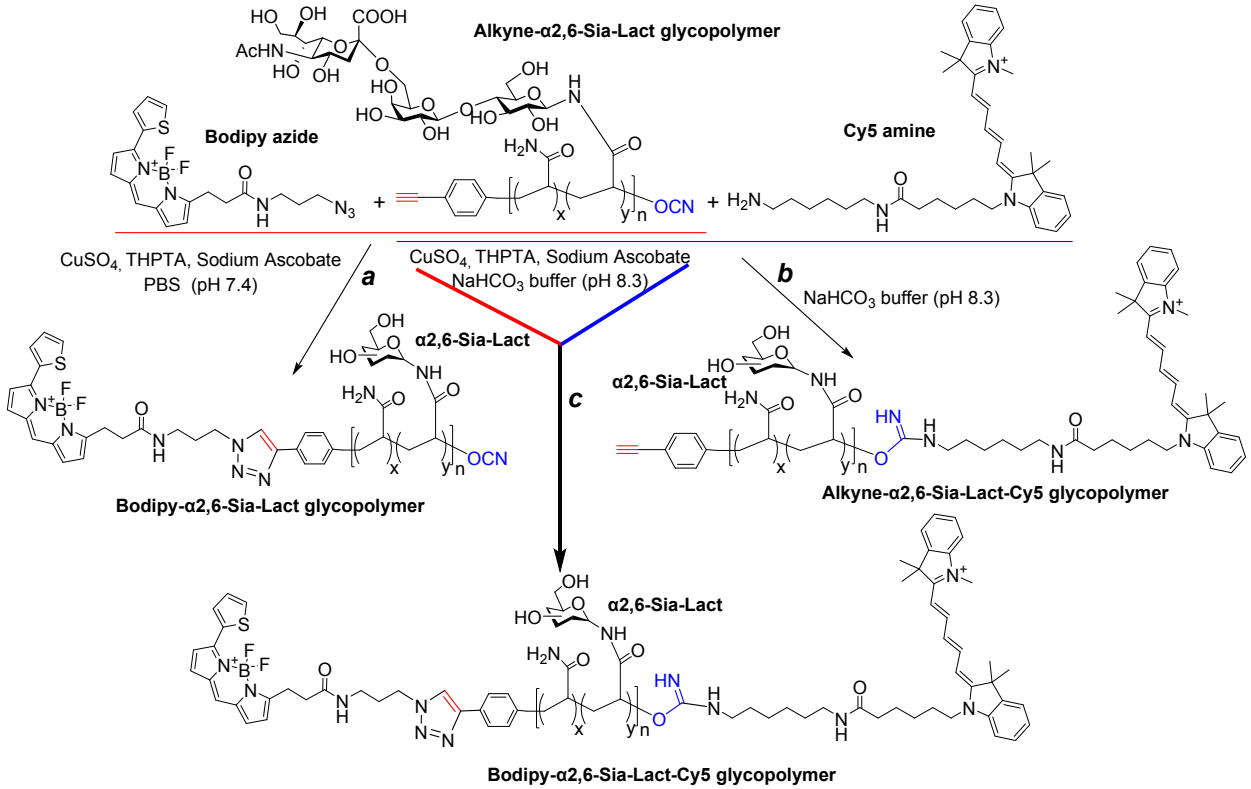



**Figure 1. $^{1}$H NMR spectra of α,ω-end orthogonally functionalizable glycopolymers.** (**A**) $^{1}$H NMR spectrum of *β*-lactose (**a**), *β*-D-lactopyranosylamine (**b**), *N*-(prop-2-enoyl)-β-D-

lactopyranosylamine (**c**), *p*-ethynyl-phenyl β-D-Gal(1-4)-*β*-D-Glc-*N*-glycopolymer (**d**), *p*-azido-phenyl-*β*-D-Gal(1-4)-β-D-Glc-*N*-glycopolymer (**e**); (**B**) $^1$H NMR spectrum of *a*2,6-siallyllactose (**a**), *a*2,6-siallyllactamine (**b**), *N*-(prop-2-enoyl)-*a*2,6-siallyllactamine (**c**), *p*-ethynyl-phenyl *a*2,6-Neu5Ac-*β*-D-Gal(1-4)-*β*-D-Glc-*N*-glycopolymer (**d**), *p*-azido-phenyl-*a*2,6-Neu5Ac-*β*-D-Gal(1-4)-*β*-D-Glc-*N*-glycopolymer (**e**); (**C**) $^1$H NMR spectrum of *a*2,3-siallyllactose (**a**), *a*2,3-siallyllactamine (**b**), *N*-(prop-2-enoyl)-*α*2,3-siallyllactamine (**c**), and *p*-ethynyl-phenyl-*a*2,3-Neu5Ac-*β*-D-Gal(1-4)-*β*-D-Glc-*N*-glycopolymer (**d**), *p*-azido-phenyl-*a*2,3-Neu5Ac-*β*-D-Gal(1-4)-*β*-D-Glc-*N*-glycopolymer (**e**). (400 MHz, $D_2O$).

**Orthogonal functionalization of alkyne and *O*-cyanate chain-end group of glycopolymer.** First, we investigated sequential and one-pot orthogonal functionalization of both chain-end groups of alkyne-α2,6-Sia-Lact glycopolymer using Bodipy-azide and Cy5-amine *via* click chemistry and isourea bond formation, respectively (Scheme 2). For functionalization with Bodipy azide, the glycopolymer was dissolved in PBS (pH 7.4) and Bodipy azide, which was solubilized in DMSO, was added dropwise to the reaction mixture. $CuSO_4$ (0.1 mM), THPTA (0.5 mM), and sodium ascorbate (0.5 mM) were also added to the reaction mixture as they are needed for the copper catalyzed azide-alkyne cycloaddition (CuAAC) reaction. The reaction was left for 4 h at rt and then the reaction mixture was purified using dialysis (MWCO 3 kDa). The remaining solution was then dried using lyophilization to afford Bodipy-α2,6-Sia-Lact glycopolymer as a light brown powder (76% yield) (Scheme 2**a**). For functionalization with Cy5-amine, the glycopolymer was dissolved in 0.1M $NaHCO_3$ (pH 8.3) buffer, then, Cy5-amine solubilized in 0.1M $NaHCO_3$ (pH 8.3) buffer was added dropwise, and the reaction was left for 4 h at rt. The reaction mixture was purified using dialysis (MWCO 3 kDa) and the remaining solution was lyophilized to yield alkyne-α2,6-Sia-Lact-Cy5 glycopolymer as a green powder (78% yield) (Scheme **2b**). For dual functionalization of alkyne-α2,6-Sia-Lact polymer, a one-pot reaction was used. $NaHCO_3$ (pH 8.3) buffer was used as isourea bond formation needs basic condition. Briefly, alkyne-α2,6-Sia-Lact polymer was added into 0.1 M $NaHCO_3$ buffer (pH 8.3) which contained $CuSO_4$ (0.1 mM),

THPTA (0.5 mM), and sodium ascorbate (0.5m M), then, Cy5 amine and Bodipy azide were added dropwise into the reaction mixture. The reaction was left for 4 h at rt and then purified using dialysis (MWCO 3 kDa) and the resulting solution was lyophilized to yield Bodipy-α2,6-Sia-Lact-Cy5 glycopolymer as a brown powder (73% yield) (Scheme **2c**).




**Scheme 2.** Sequential (***a*** and ***b***) and one-pot orthogonal functionalization (***c***) of the dual chain-end of glycopolymers *via* click chemistry and isourea bond formation.

Characterization of the fluorescent dye-modified glycopolymers was done by using $^{1}$H NMR spectra analysis and color changes of the dissolved glycopolymers under UV light (Figure 2), as well as fluorescent spectroscopy of glycopolymers at 502 nm (Bodipy's excitement wavelength) and 646 nm (Cy5's excitement wavelength) (Figure 3). The $^{1}$H NMR spectra of each of the chain-end functionalized alkyne-α2,6-Sia-Lact glycopolymer looks mostly similar, but there are some

small peak differences in the aromatic region (6.50 - 8.00 ppm) for each of the compounds, which indicates there are changes in the aromatic protons by either CuAAC with the alkyne group, or introduction of aromatic protons from either of the fluorescent functional groups. Also, the aromatic region of the Bodipy-α2,6-Sia-Lact-Cy5 glycopolymer looks like a combination of the aromatic regions of both the single functionalized fluorescent glycopolymers. In addition, both Bodipy-α2,6-Sia-Lact glycopolymer and Bodipy-α2,6-Sia-Lact-Cy5 glycopolymer showed fluorescence when excited under UV light (365 nm), while the alkyne-α2,6-Sia-Lact glycopolymer and alkyne-α2,6-Sia-Lact-Cy5 glycopolymer did not show fluorescence under UV light (Figure 2). Further, the fluorescence spectra of each glycopolymer at both 502 nm and 646 nm indicate the presence or absence of Bodipy or Cy5 (Figure 3). For alkyne-α2,6-Sia-Lact glycopolymer, there is just a small amount of background signal for each wavelength, indicating that there is no fluorescent light being emitted by this glycopolymer. Bodipy-α2,6-Sia-Lact glycopolymer showed a strong emission peak around 511 nm when excited with 502 nm, which indicates the presence of Bodipy within the molecule. Alkyne-α2,6-Sia-Lact-Cy5 showed a strong emission peak around 660 nm when excited with 646 nm wavelength light, which indicates the Cy5 group has been successfully introduced into the molecule. For Bodipy-α2,6-Sia-Lact-Cy5, there is an emission peak around 511 nm when excited with 502 nm wavelength light and also an emission at 660 nm when excited with 646 nm wavelength light. These results indicate that the dual functionalized glycopolymer was formed, and also alkyne-α2,6-Sia-Lact glycopolymer is capable of performing separate orthogonal reactions on each chain-end in a one-pot reaction.

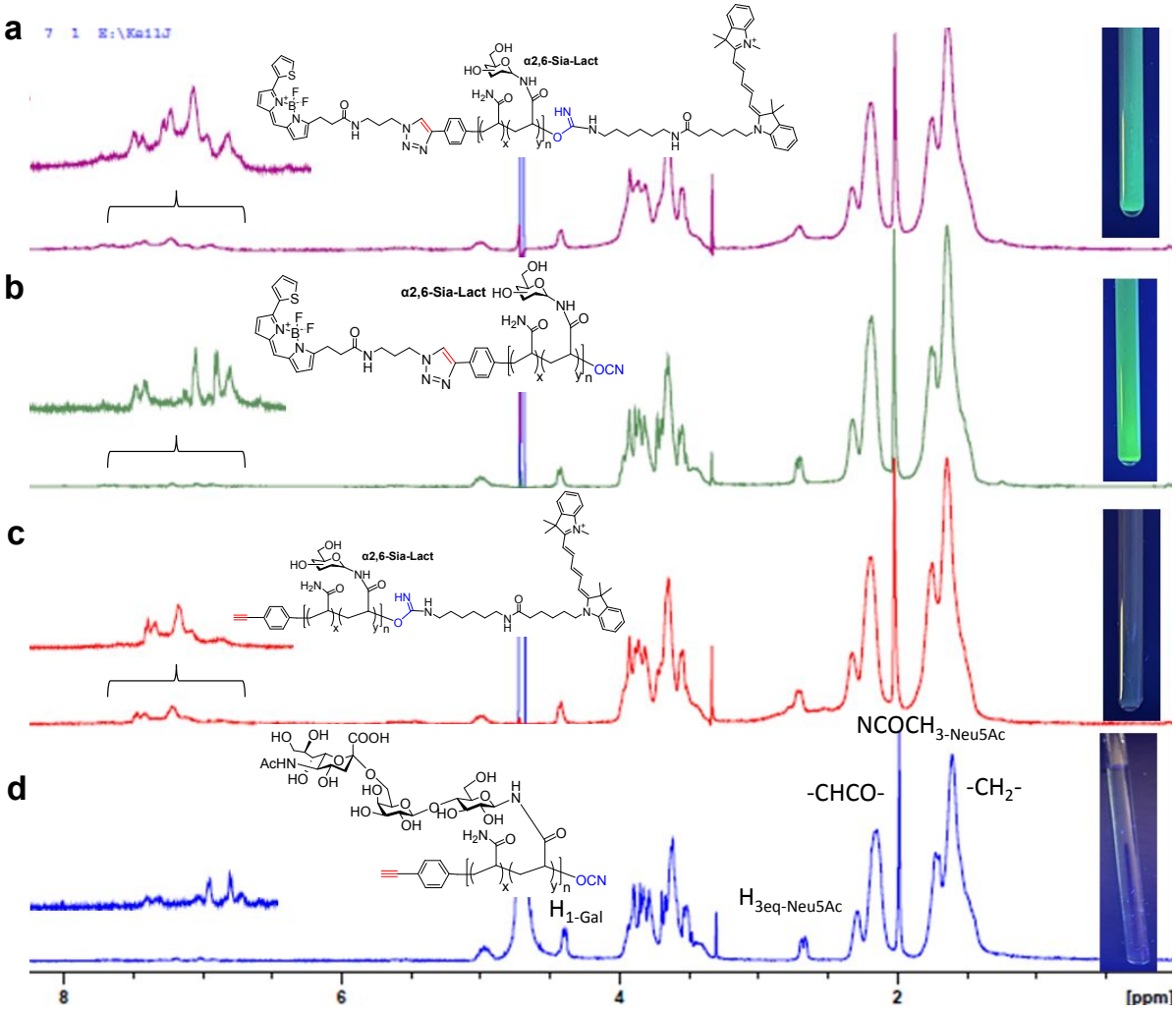



**Figure 2. $^1$H NMR Spectra and photo images under UV light of chain-end functionalized alkyne-α2,6-Sia-Lact glycopolymers. a**) Bodipy-α2,6-Sia-Lact-Cy5 glycopolymer, **b**) Bodipy-α2,6-Sia-Lact glycopolymer, **c**) Alkyne-α2,6-Sia-Lact-Cy5 glycopolymer, **d**) Alkyne-α2,6-Sia-Lact glycopolymer (400 MHz, $D_2O$).

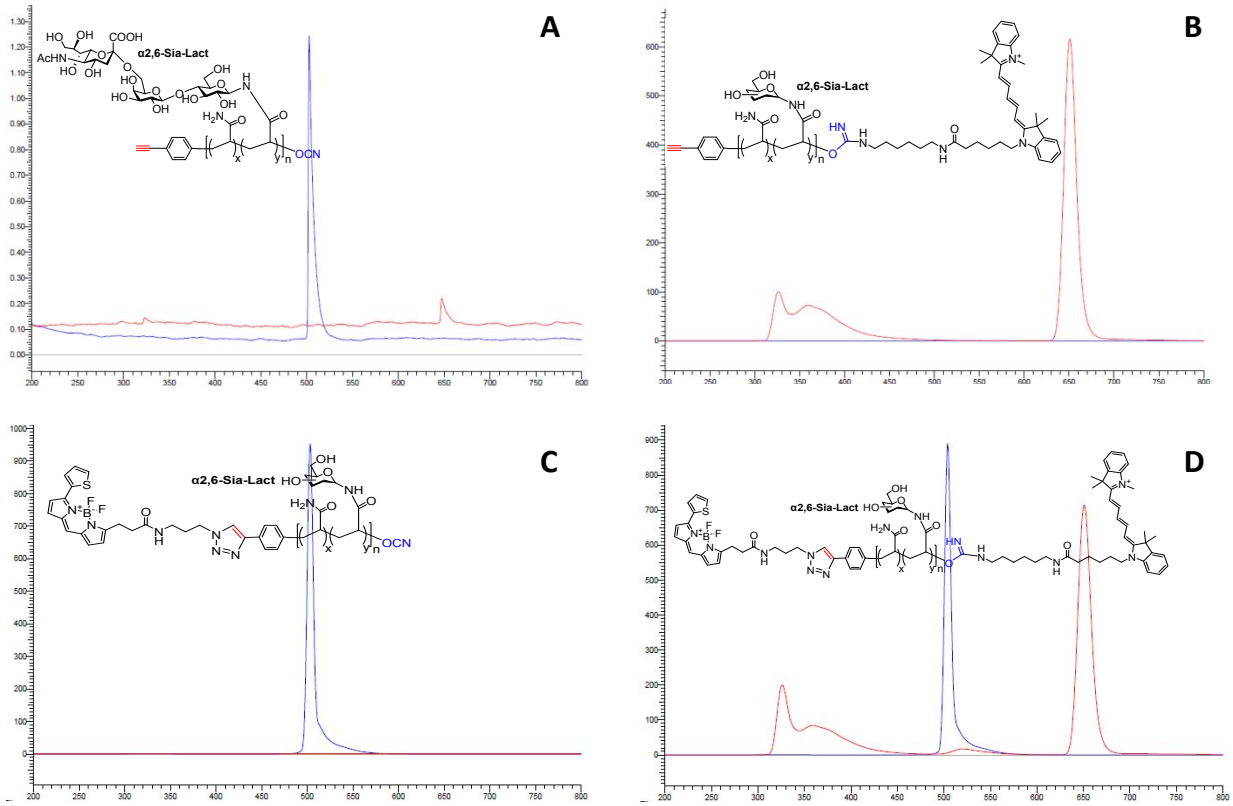
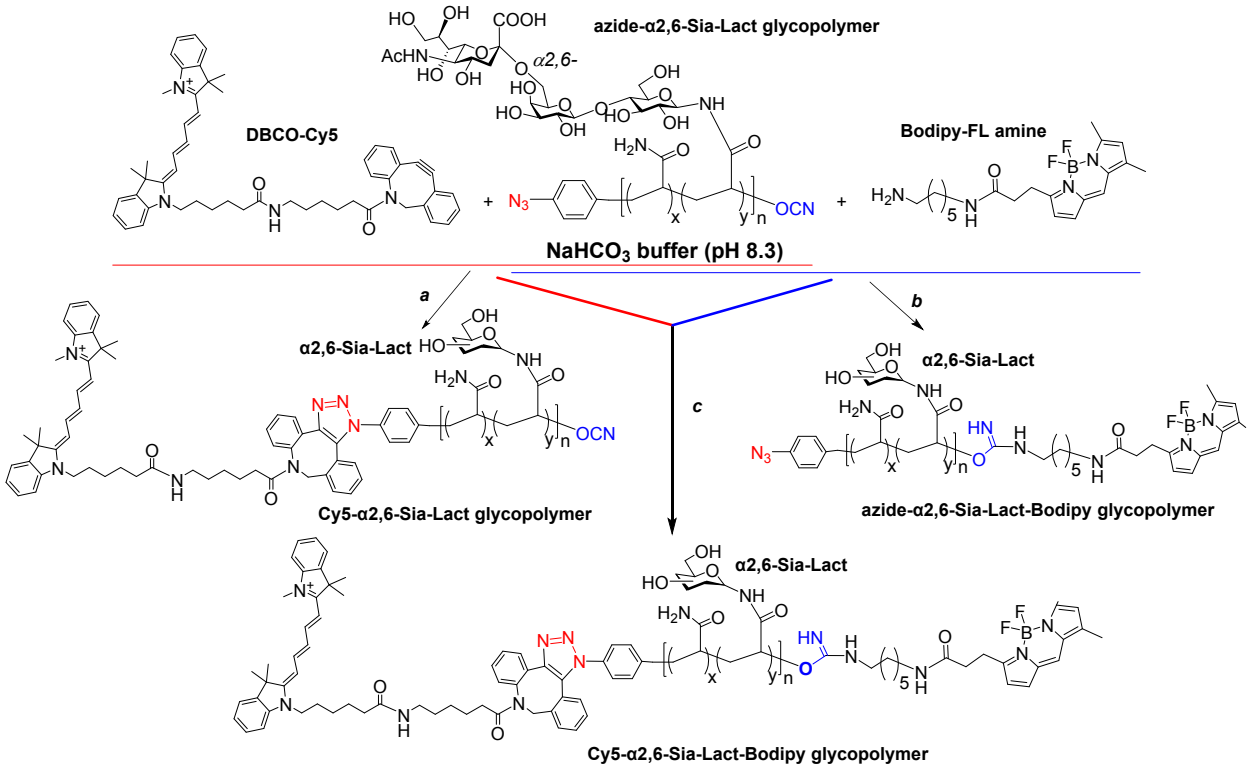



**Figure 3. Fluorescent emission spectra of chain-end functionalized alkyne-α2,6-Sia-Lact glycopolymer**. (**A**) Alkyne-α2,6-Sia-Lact glycopolymer, (**B**) Alkyne-α2,6-Sia-Lact-Cy5 glycopolymer, (**C**) Bodipy-α2,6-Sia-Lact glycopolymer, (**D**) Bodipy-α2,6-Sia-Lact-Cy5 glycopolymer (Red excitation wavelength 646 nm, blue excitation wavelength 502 nm in $H_2O$).

**Orthogonal functionalization of azide and *O*-cyanate chain-end group of glycopolymer.** Next, we investigated sequential and one-pot orthogonal functionalization of azide-α2,6-Sia-Lact polymer with DBCO-Cy5 *via* SPAAC and Bodipy-amine *via* isourea bond formation, respectively (Scheme 3). Briefly, functionalization with DBCO-Cy5 was done by first dissolving azide-α2,6-Sia-Lact glycopolymer in 0.1 M $NaHCO_3$ (pH 8.3) buffer, then, DBCO-Cy5 in a small amount of DMSO was added dropwise and the reaction was left to react for 4h at rt. The reaction mixture was then purified using dialysis (MWCO 3 kDa) and the remaining solution was dried using lyophilization to afford Cy5-α2,6-Sia-Lact glycopolymer (Scheme **3a**). Functionalization with Bodipy-FL amine was done by dissolving azide-α2,6-Sia-Lact polymer in 0.1 M $NaHCO_3$ (pH

8.3) buffer, then, Bodipy-FL amine solubilized in a small amount of DMSO was added dropwise to the reaction solution as well. The reaction was left for 4 h at rt and then the reaction mixture was purified with dialysis (MWCO 3 kDa). The remaining solution was dried by lyophilization to yield azide-α2,6-Sia-Lact-Bodipy glycopolymer (Scheme **3b**). Dual functionalization of azide-α2,6-Sia-Lact glycopolymer was done in a one-pot reaction in 0.1 M $NaHCO_3$ (pH 8.3) buffer with DBCO-Cy5 and Bodipy-FL amine for 4 h at rt. The reaction mixture was purified using dialysis (MWCO 3 kDa) and the remaining solution was dried by lyophilization to afford Cy5-α2,6-Sia-Lact-Bodipy glycopolymer as a blue powder (Scheme **3c**).




**Scheme 3.** Sequential (***a*** and ***b***) and one-pot orthogonal functionalization (***c***) of the dual chain-end of glycopolymers *via* SPAAC and isourea bond formation.

Characterization of the chain-end fluorescent dye-functionalized glycopolymers was also done by using $^1$H NMR analysis and color changes of dissolved glycopolymers under UV light (Figure

4), as well as fluorescent spectroscopy of glycopolymer (10 μM) at 502 nm (Bodipy's excitement wavelength) and 646 nm (Cy5's excitement wavelength) (Figure 5). As shown in Figure 4, each fluorescent glycopolymer showed different the aromatic region (6.50 - 8.00 ppm). Also, under UV light (365 nm), azide-α2,6-Sia-Lact-Bodipy and Cy5-α2,6-Sia-Lact-Bodipy showed fluorescence, however, the azide-α2,6-Sia-Lact polymer and Cy5-azide-α2,6-Sia-Lact did not show fluorescence at all (Figure 4). Further, the fluorescence spectra of each glycopolymer at both 502 nm and 646 nm confirmed the presence or absence of Bodipy or Cy5 (Figure 5). For azide-α2,6-Sia-Lact polymer, there is no fluorescent light being emitted by this glycopolymer. Azide-α2,6-Sia-Lact-Bodipy showed a strong emission peak around 511 nm when excited with 502 nm. Cy5-α2,6-Sia-Lact polymer showed a strong emission peak around 660 nm when excited with 646 nm wavelength light. For Cy5-α2,6-Sia-Lact-Bodipy, there is an emission peak around 511 nm when excited with 502 nm wavelength light and also an emission at 660 nm when excited with 646 nm wavelength light. These results indicated that the dual end functionalization of the glycopolymer was successfully conducted by performing separate orthogonal reactions on each chain-end in a one-pot reaction, which could be useful for many future applications such as immobilization and probing biological systems.

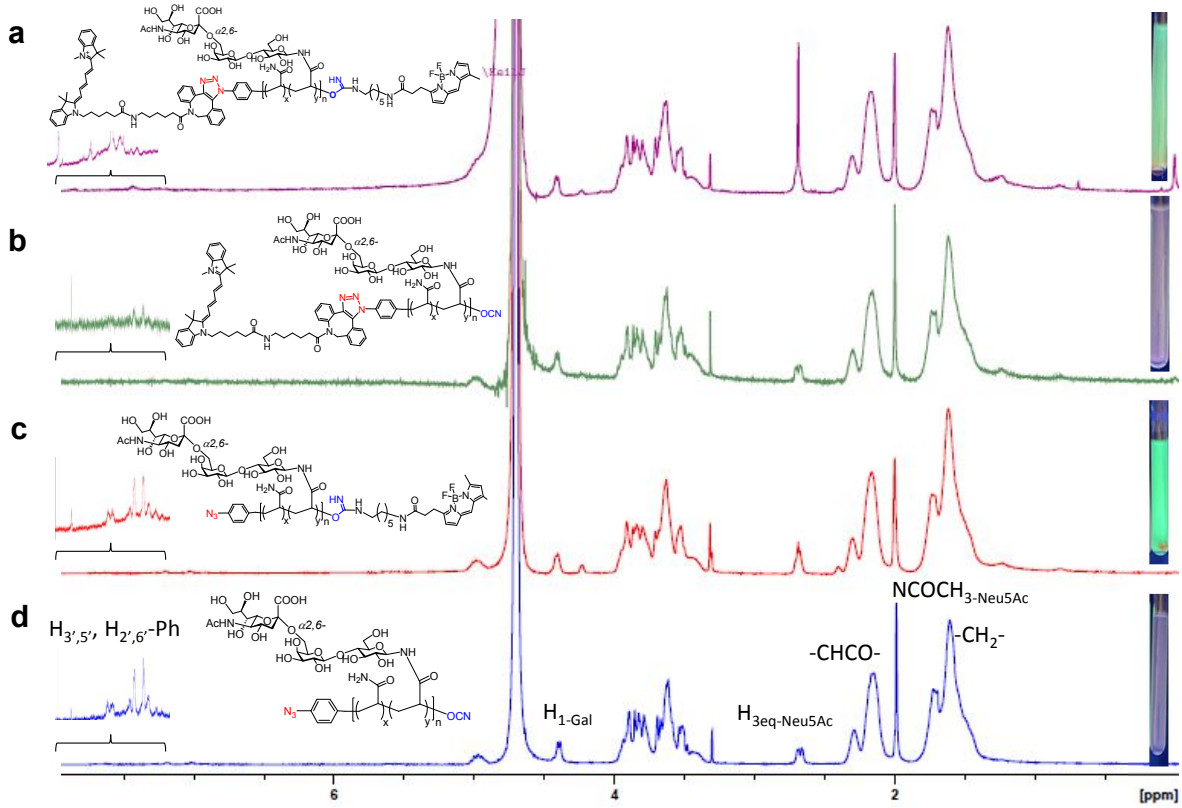



**Figure 4. $^1$H NMR spectra and photo images under UV light of chain-end functionalized α2,6-Sia-Lact glycopolymers**. **a**) Cy5-α2,6-Sia-Lact-Bodipy glycopolymer, **b**) Cy5-α2,6-Sia-Lact glycopolymer, **c**) Azide-α2,6-Sia-Lact-Bodipy glycopolymer, **d**) Azide-α2,6-Sia-Lact glycopolymer (400 MHz, $D_2O$).

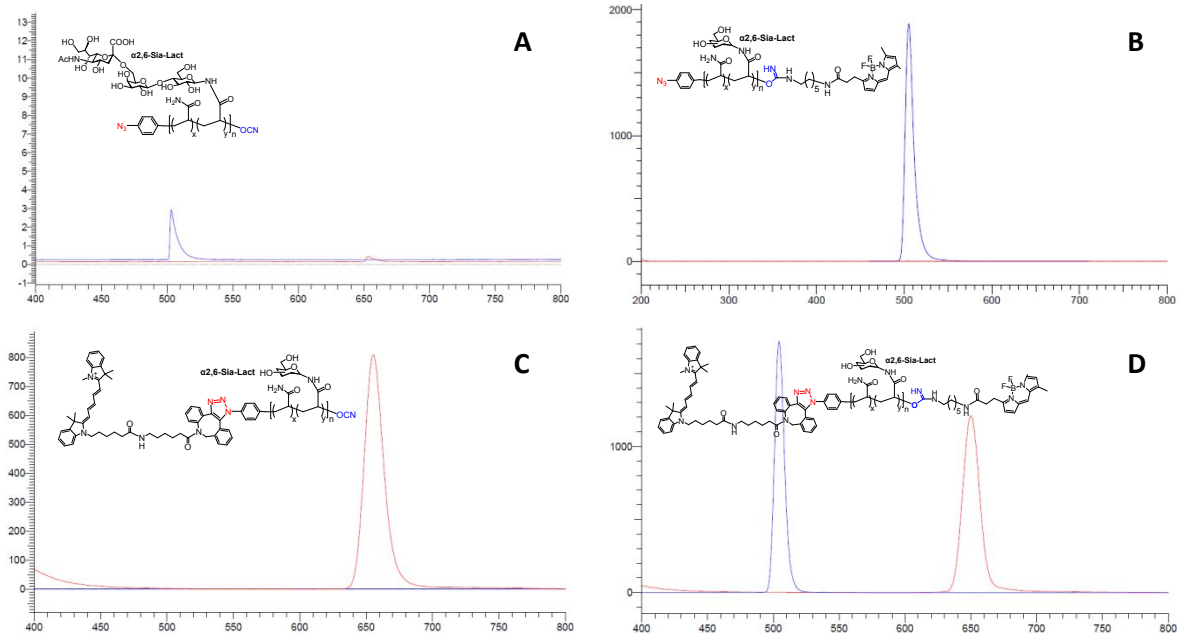
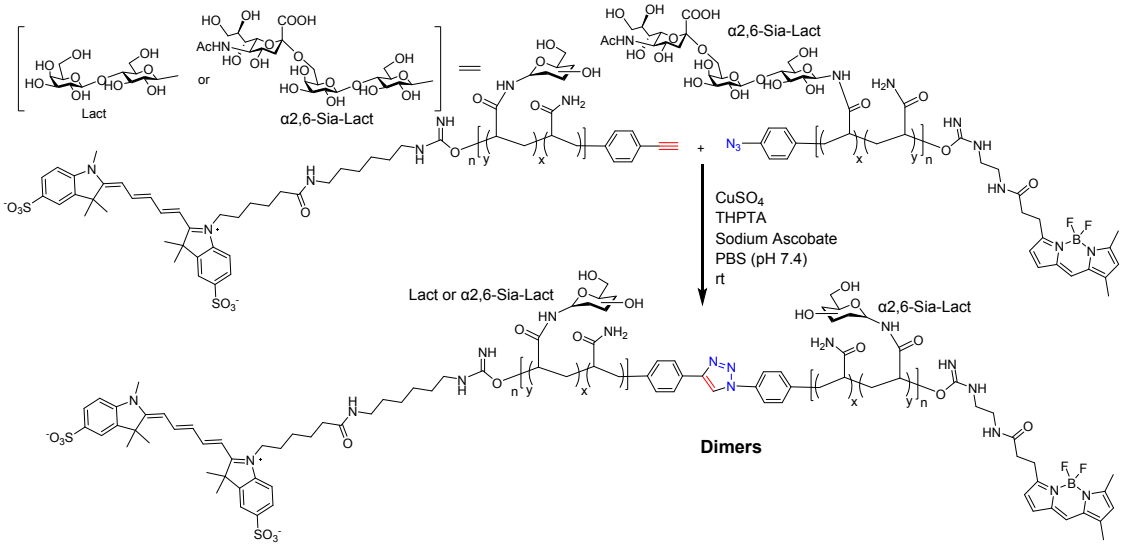


**Figure 5. Fluorescent emission spectra of chain-end functionalized α2,6-Sia-Lact glycopolymers.** (**A**) Azide-α2,6-Sia-Lact glycopolymer, (**B**) Azide-α2,6-Sia-Lact-Bodipy glycopolymer, (**C**) Cy5-α2,6-Sia-Lact glycopolymer, (**D**) Cy5-α2,6-Sia-Lact-Bodipy glycopolymer (Red excitation wavelength 646 nm, blue excitation wavelength 502 nm in $H_2O$).

**End-to-end dimerization of glycopolymer *via* Click Chemistry.** Finally, end-to-end dimerization was investigated with azide and alkyne chain-end functionalized glycopolymer *via* CuAAC (Scheme 4). Specifically, azide-α2,6-Sia-Lact-Bodipy glycopolymer was conjugated to either alkyne-Lact-Cy5 glycopolymer or alkyne-α2,6-Sia-Lact-Cy5 glycopolymer *via* CuAAC. Briefly, azide-α2,6-Sia-Lact-Bodipy glycopolymer and alkyne-Lact-Cy5 glycopolymer were dissolved in PBS (7.4) buffer with $CuSO_4$ (0.1 mM), THPTA (0.5 mM), and sodium ascorbate (0.5 mM) and left to react for 4 h at rt. The reaction mixture was purified by dialysis (MWCO 12-14 kDa) and the resulting solution was lyophilized to yield the heterodimer as a brown powder. For dimerization of azide-α2,6-Sia-Lact-Bodipy glycopolymer and alkyne-α2,6-Sia-Lact-Cy5 polymer, each polymer was added to PBS (7.4) buffer with $CuSO_4$ (0.1 mM), THPTA (0.5 mM), and sodium ascorbate (0.5 mM) and left to react for 4 h at rt. The reaction mixture was purified with dialysis (MWCO 12-14 kDa) and the resulting solution was lyophilized to yield a homodimer as a brown powder.




**Scheme 4.** Synthesis of glycopolymer dimers *via* CuAAC.

Characterization of the fluorescent glycopolymer dimers was done using $^{1}$H NMR and photo imaging of the glycopolymers under UV light (Figure 6 and 7), as well as fluorescent spectra of the glycopolymer at 502 nm (Bodipy's excitement wavelength) and 646 nm (Cy5's excitement wavelength) (Figure 8). The $^{1}$H NMR for azide-α2,6-Sia-Lact-Bodipy and alkyne-Lact -Cy5 dimer (Figure 6) show the peak at around 2.00 ppm for *N*-acetyl peaks of α2,6-Sia-Lac lower its intensity, which is consistent with the conjugation of the two glycopolymers because only azide-α2,6-Sia-Lact-Bodipy glycopolymer contains Sia. The photo imaging under the UV light also shows fluorescence consistent with Bodipy for both the dimer and the Bodipy containing glycopolymer (Figure 6). The $^{1}$H NMR of azide-α2,6-Sia-Lact-Bodipy polymer and alkyne-α2,6-Sia-Lact-Cy5 polymer dimer (Figure 7) show differences in the aromatic region of the spectra (6.50 - 8.00 ppm) which would occur with the two different fluorescent functional groups and also because of the formation of the triazole ring in the dimer. The images under the UV light show that the Bodipy containing glycopolymers and the synthesized heterodimer shows the fluorescence expected for a Bodipy containing molecule, but the synthesized homodimer does not have any fluorescence under

the UV light (Figure 7). This lack of visible fluorescent light could be due to some intermolecular and intramolecular quenching due to sugar interactions between the dimers in solution. The fluorescent spectra of both dimers (Figure 8) show an emission peak around 511 nm when excited with 502 nm wavelength and an emission peak around 660 nm when excited with 646 nm wavelength light. This shows that both Bodipy and Cy5 are present in each of the dimers, indicating a successful dimerization of azide and alkyne chain-end functionalized glycopolymer *via* CuAAC.

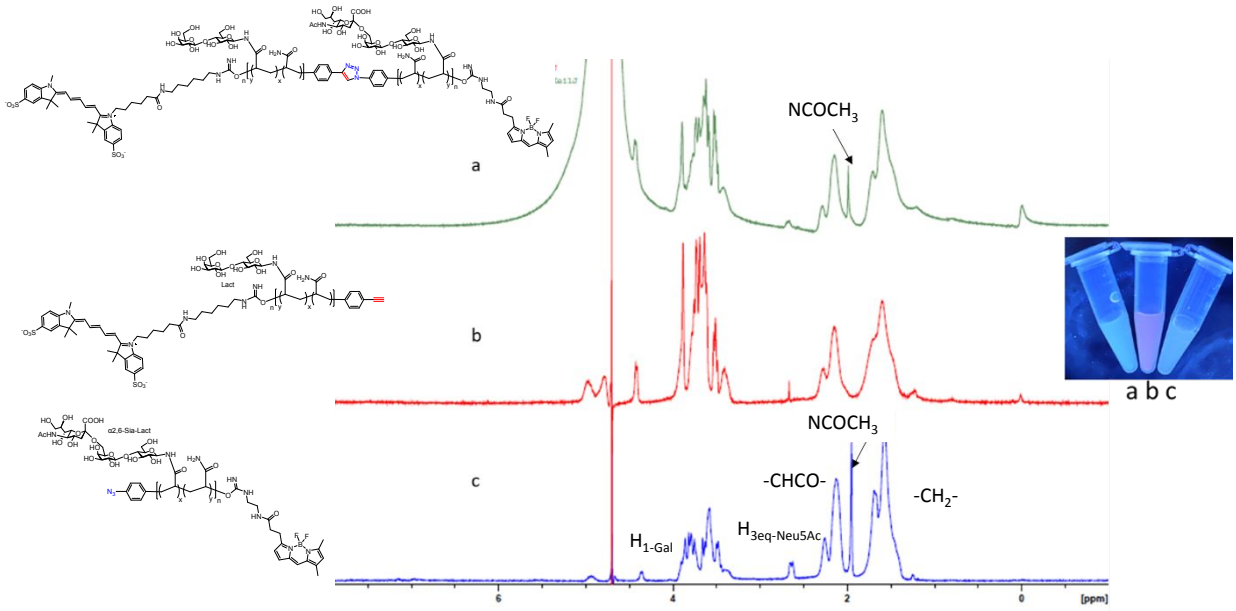



**Figure 6.** $^1$H NMR spectra and photo images under UV light of **a**) α2,6-Sia-Lact-Bodipy and Lact glycopolymer-Cy5 dimer, **b**) Alkyne-Lact glycopolymer-Cy5, **c**) Azide-α2,6-Sia-Lact-Bodipy glycopolymer (400 MHz, $D_2O$).

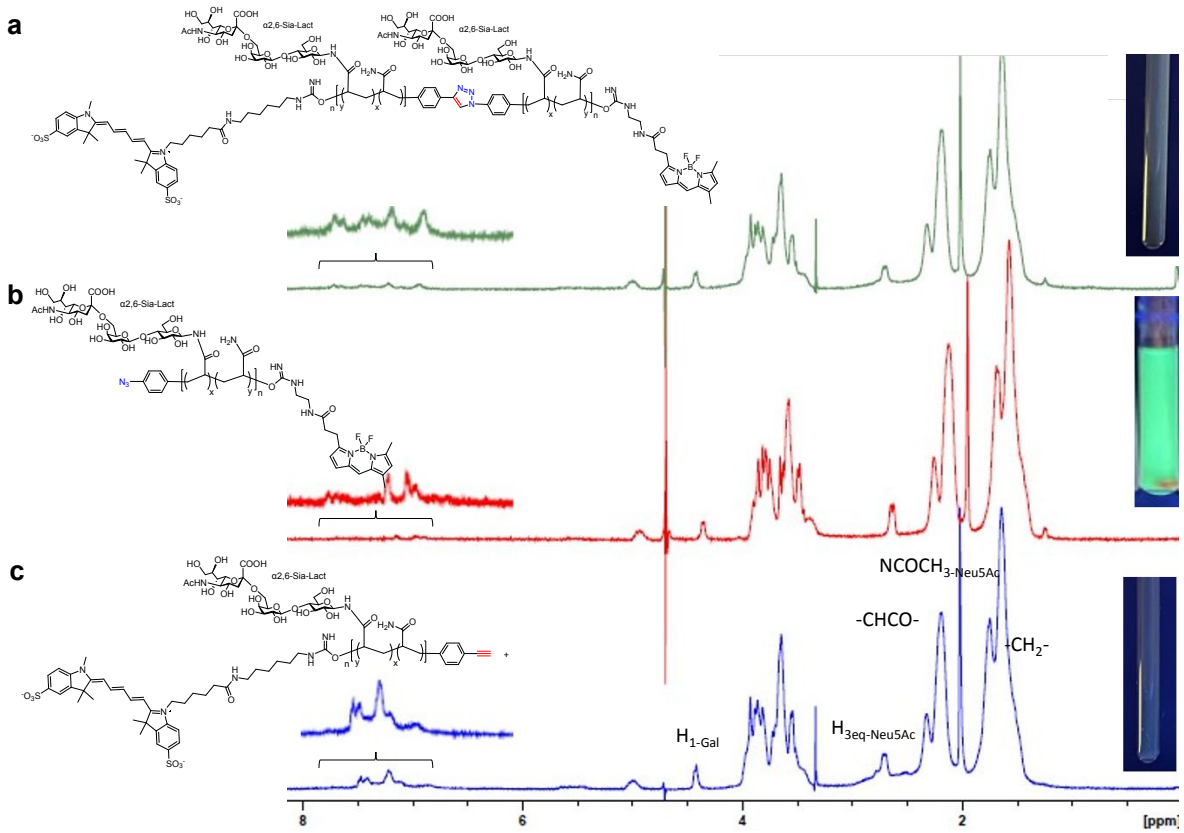



**Figure 7.** $^{1}$H NMR spectra and photo images under UV light of **a**) α2,6-Sia-Lact-Bodipy and α2,6-Sia-Lact-Cy5 dimer, **b**) Azide-α2,6-Sia-Lact-Bodipy glycopolymer, **c**) Alkyne-α2,6-Sia-Lact-Cy5 glycopolymer (400 MHz, $D_2O$).

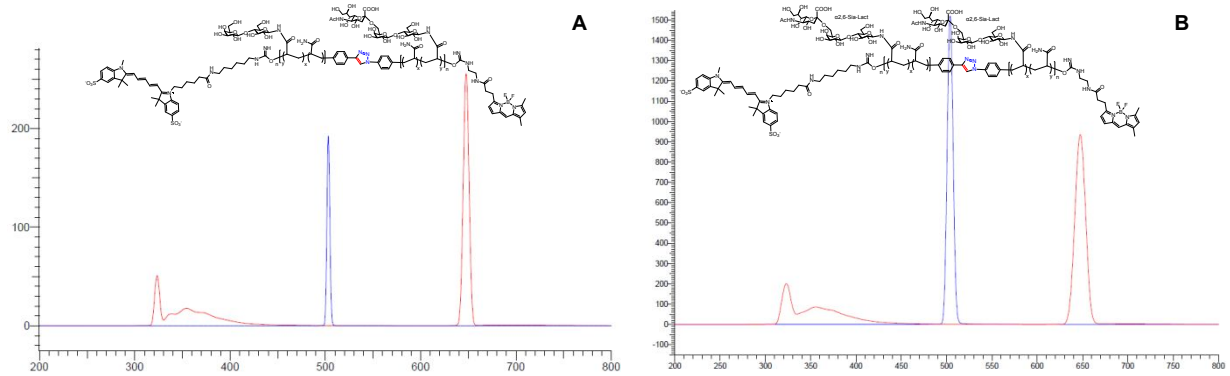



**Figure 8. Fluorescent emission spectra of glycopolymer dimers.** (**A**) Cy5-Lact glycopolymer and α2,6-Sia-Lact-Bodipy glycopolymer dimer and **(B)** Cy5-α2,6-Sia-Lact glycopolymer and α2,6-Sia-Lact-Bodipy glycopolymer dimer (Red excitation wavelength 646 nm, blue excitation wavelength 502 nm in $H_2O$).

## Conclusions

Glycopolymers with an alkyne or azide group at one end and an *O*-cyanate on the other end were successfully synthesized with native glycans without protection and deprotection. The chain-end azide or alkyne and *O*-cyanate could be modified using orthogonal reactions in one-pot *via* CuAAC or SPAAC and isourea bond formation with different fluorescent dyes (Bodipy and Cy5). The fluorescent glycopolymers were characterized using $^{1}$H NMR, photo imaging under UV light, and fluorescent spectra at 502 nm and 646 nm, respectively. The glycopolymers described herein are able to be functionalized at each chain-end in sequential or one-pot as needed, which could be useful in the future to use in a number of studies involving fluorescent probing, immobilization, or functionalization for biomolecules which can react specifically at one chain-end. Due chain-end functionalization of glycopolymers is an important technique that allows for studying, visualizing, and modulating biological processes through important sugar interactions.

**ASSOCIATE CONTENT**

**Supporting Information**

The Supporting Information is available free of charge at xxx

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## Author Contributions

The manuscript was written through the contributions of both authors and both have given their approval to the final version of the manuscript JM. Keil: conceptualization, data curation, investigation, methodology, writing-original draft, writing-review & editing. KK Chan: conceptualization, data curation, investigation, methodology, writing-review & editing, X-L. Sun: conceptualization, funding acquisition, methodology, writing-review & editing.

## Conflict of Interest

The authors declare no competing financial interests.

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## References

(1) M. R. Thalji,; A. Amin, I. Kwok, F. Chong, *Glycopolymer - Based Materials : Synthesis , Properties ,*; Springer International Publishing, 2022; Vol. 380.

(2) Y. Miura, Design and Synthesis of Well-Defined Glycopolymers for the Control of Biological Functionalities. *Polym J* 2012, **44**, 679-689.

(3) S.-G. Lee, J. M. Brown, C. J. Rogers, J. B. Matson, C. Krishnamurthy, M. Rawat, L. C. Hsieh-Wilson, End-Functionalized Glycopolymers as Mimetics of Chondroitin Sulfate Proteoglycans. *Chem Sci* 2010, **1**, 322.

(4) R. J. Mancini, J. Lee, H. D. Maynard, Trehalose Glycopolymers for Stabilization of Protein Conjugates to Environmental Stressors. *J Am Chem Soc* 2012, **134**, 8474–8479.

(5) H. Zhang, J. Weingart, V. Gruzdys, X. L. Sun, Synthesis of an End-to-End Protein-Glycopolymer Conjugate via Bio-Orthogonal Chemistry. *ACS Macro Lett* 2016, **5**, 73–77.

(6) X. L. Sun, D. Grande, S. Baskaran, S. R. Hanson, E. L. Chaikof, Glycosaminoglycan Mimetic Biomaterials. Synthesis of Sulfated Lactose-Based Glycopolymers That Exhibit Anticoagulant Activity. *Biomacromolecules* 2002, **3**, 1065–1070.

(7) J. Geng, G. Mantovani, L. Tao, J. Nicolas, G. Chen, R. Wallis, D. A. Mitchell, B. R. G. Johnson, S. D. Evans, D. M. Haddleton, Site-Directed Conjugation of "Clicked" Glycopolymers To Form Glycoprotein Mimics: Binding to Mammalian Lectin and Induction of Immunological Function. *J Am Chem Soc* 2007, **129**, 15156–15163.

(8) K. K. Chan, Q. Lei, J. Tang, X. L. Sun, Synthesis of Aryl Azide Chain-End Functionalized: N -Linked Glycan Polymers and Their Photo-Labelling of Specific Protein. *RSC Adv* 2020, **10**, 38561–38565.

(9) A. Wibowo, E. C. Peters, L. C. Hsieh-Wilson, Photoactivatable Glycopolymers for the Proteome-Wide Identification of Fucose-α(1-2)-Galactose Binding Proteins. *J Am Chem Soc* 2014, **136**, 9528–9531.

(10) H. Wu, J. Kohler, Photocrosslinking Probes for Capture of Carbohydrate Interactions. *Curr Opin Chem Biol* 2019, **53**, 173–182.

(11) A. Fujita, J. J. Kohler, Photocrosslinking Sugars for Capturing Glycan-Dependent Interactions. *Trends in Glycoscience and Glycotechnology* 2015, **27**, E1–E7.

(12) S. N. Narla, X.-L. Sun, Orientated Glyco-Macroligand Formation Based on Site-Specific Immobilization of O-Cyanate Chain-End Functionalized Glycopolymer. *Org. Biomol. Chem.* 2011, **9**, 845–850.

(13) E. J. Gordon, J. E. Gestwicki, L. E. Strong, L. L. Kiessling, Synthesis of End-Labeled Multivalent Ligands for Exploring Cell-Surface-Receptor–Ligand Interactions. *Chem Biol* 2000, **7**, 9–16.

(14) J. E. Gestwicki, C. W. Cairo, L. E. Strong, K. A. Oetjen, L. L. Kiessling, Influencing Receptor−Ligand Binding Mechanisms with Multivalent Ligand Architecture. *J Am Chem Soc* 2002, **124**, 14922–14933.

(15) X. L. Sun, D. Grande, S. Baskaran, S. R. Hanson, E. L. Chaikof, Glycosaminoglycan Mimetic Biomaterials. Synthesis of Sulfated Lactose-Based Glycopolymers That Exhibit Anticoagulant Activity. *Biomacromolecules* 2002, **3**, 1065–1070.

(16) S. Hou, X. L. Sun, C. M. Dong, E. L. Chaikof, Facile Synthesis of Chain-End Functionalized Glycopolymers for Site-Specific Bioconjugation. *Bioconjug Chem* 2004, **15**, 954–959.

(17) S. S. Gupta,; K. S. Raja, E. Kaltgrad, E. Strable, M. G. Finn, Virus–Glycopolymer Conjugates by Copper(i) Catalysis of Atom Transfer Radical Polymerization and Azide–Alkyne Cycloaddition. *Chemical Communications* 2005, **34**, 4315.

(18) V. Vázquez-Dorbatt, H. D. Maynard,Biotinylated Glycopolymers Synthesized by Atom Transfer Radical Polymerization. *Biomacromolecules* 2006, **7**, 2297–2302.

(19) V. Vázquez-Dorbatt, Z. P. Tolstyka, C.-W. Chang, H. D. Maynard, Synthesis of a Pyridyl Disulfide End-Functionalized Glycopolymer for Conjugation to Biomolecules and Patterning on Gold Surfaces. *Biomacromolecules* 2009, **10**, 2207–2212.

(20) M. Bathfield, F. D'Agosto, R. Spitz, M.-T. Charreyre, T. Delair, Versatile Precursors of Functional RAFT Agents. Application to the Synthesis of Bio-Related End-Functionalized Polymers. *J Am Chem Soc* 2006, **128**, 2546–2547.

(21) A. Housni, H. Cai, S. Liu, S. H. Pun, R. Narain, Facile Preparation of Glyconanoparticles and Their Bioconjugation to Streptavidin. *Langmuir* 2007, ***23***, 5056–5061.

(22) K. Godula, D. Rabuka, K. T. Nam, C. R. Bertozzi, Synthesis and Microcontact Printing of Dual End-Functionalized Mucin-like Glycopolymers for Microarray Applications. *Angewandte Chemie* 2009, **121**, 5073–5076.

(23) X. Jiang, A. Housni, G. Gody, P. Boullanger, M.-T. Charreyre, T. Delair, R. Narain, Synthesis of Biotinylated R-D-Mannoside or N-Acetyl -D-Glucosaminoside Decorated Gold Nanoparticles: Study of Their Biomolecular Recognition with Con A and WGA Lectins. *Bioconjug Chem* 2010, **21**, 521–530.

(24) K. Godula, C. R. Bertozzi, Synthesis of Glycopolymers for Microarray Applications via Ligation of Reducing Sugars to a Poly(Acryloyl Hydrazide) Scaffold. *J Am Chem Soc* 2010, **132**, 9963–9965.

(25) J. Tang, E. Ozhegov, Y. Liu, D. Wang, X. Yao, X.-L. Sun, Straightforward Synthesis of N -Glycan Polymers from Free Glycans via Cyanoxyl Free Radical-Mediated Polymerization. *ACS Macro Lett* 2017, ***6***, 107-111.

(26) S.N. Narla, X.-L. Sun, Glyco-Macroligand Microarray with Controlled Orientation and Glycan Density. *Lab Chip* 2012, **12**, 1656.

(27) V. Gruzdys, J. Tang, E. Chaikof, X.-L. Sun, Synthesis of Chain-End Functionalized Glycopolymers via Cyanoxyl-Mediated Free Radical Polymerization (CMFRP); In: Sun, XL. (eds) Macro-Glycoligands. Methods in Molecular Biology, Humana Press, New York, NY. 2016, **1367**, 3-12.

(28) R. E. Bird, S. A. Lemmel, X. Yu, Q. A. Zhou, Bioorthogonal Chemistry and Its Applications. *Bioconjug Chem* 2021, **32**, 2457–2479.

(29). C. Fasting, C. A. Schalley, M. Weber, O. Seitz, S. Hecht, B. Koksch, J. Dernedde, C. Graf, E. W. Knapp, R. Haag. Multivalency as a chemical organization and action principle. *Angew Chem Int Ed Engl.* 2012, **51**, 10472-98.

(30) C. H. Wong, S. C. Zimmerman, Orthogonality in organic, polymer, and supramolecular chemistry: from Merrifield to click chemistry. *Chem. Commun.***,** 2013,**49**, 1679-1695

(31) N. E. Mbua, J. Guo, M. A. Wolfert, R. Steet, G. J. Boons. Strain-promoted alkyne-azide cycloadditions (SPAAC) reveal new features of glycoconjugate biosynthesis. *Chembiochem*. 2011, **12**, 1912-1921.