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Chemoenzymatic Synthesis of Neuraminic Acid Containing *C*-Glycoside Polymers

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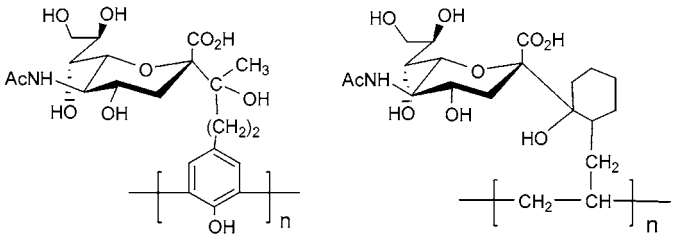
ABSTRACT



Two neuraminic acid-based, *C*-glycoside polymers were synthesized. Preliminary studies on one of these polymers showed potent neuraminidase inhibitory activity, suggesting potential utility as an antipathogenic surface coating for the preparation of antimicrobial biomaterials.

There is growing demand for biomaterials capable of binding and killing harmful microorganisms.[1,2] In nature, glycoproteins, such as mucins, form a biofilm on the surface of epithelial cells, providing a protective barrier by binding microorganisms. The carbohydrate moieties in mucins represent 50−90% of their molecular weight, and consist of a large number of small sialylated glycans, attached through *O*-glycosidic linkages to the serine and threonine residues of their polypeptide backbone.[3] These multiple glycan chains result in tight binding to microbial receptors through multivalent interactions.[4] Studies show that low molecular weight salivary mucin MG2 (MW ∼20 000), which contains a relatively homogeneous collection of oligosaccharides, mainly sialylated disaccharides and trisaccharides,[5] binds to a wide variety of microorganisms. In contrast, high molecular weight (∼400 000) salivary mucin, MG1, which has a divergent collection of glycans, binds to limited sites on microorganisms. The differences between these two natural mucins suggest that synthetic mucin-like polymers with homogeneous neuraminic acid pendants might be useful as protective layers to bind pathogens.

Neuraminic acid (Neu5Ac) is an abundant sugar in glycoproteins and acts as an attachment site for many pathogens. These glycoproteins are susceptible to cleavage by neuraminidase, an exoglycosidase that is found in virus, bacteria, parasites, and mammalian cells. Virus neuraminidase releases progeny virus particles from the surface of erythrocytes and facilitates passage of the virus through the mucin layer covering the epithelial cell surface. In some pathogenic bacteria, neuraminidase also acts as a virulence

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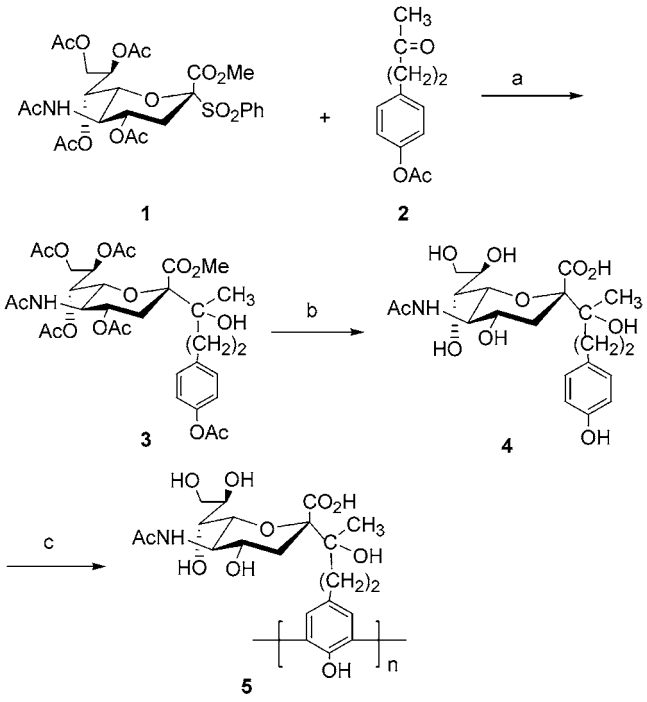
factor by uncovering toxin binding sites.[6] Inhibition of neuraminidase has afforded potential strategies for the development of antiviral and antibacterial agents.[7,8] Two neuraminidase inhibitors, Zanamirvir (Relenza, Glaxo Smith-Kline) and Oseltamivir (Tamiflu, Hoffman-La Roche), are currently used clinically as prophylactic agents against influenza.[9]

Several groups have developed inhibitors of the influenza virus that are polyvalent in Neu5Ac.[10] The synthetic random copolymers incorporating Neu5Ac *C*-glycoside moieties have been shown to be effective in inhibiting influenza virus-induced agglutination of erythrocytes in vitro.[11] Our laboratory has extensive experience in the chemoenzymatic synthesis of synthetic polymers containing sugar pendents.[12] We have also been particularly interested in the synthesis and biological evaluation of neuraminic acid containing molecules.[13] The current study is focused on developing the highly efficient synthesis of catabolically stable neuraminic acid based *C*-glycoside polymers for investigation as potential biomaterials for application as antimicrobial barriers.

Two such polymers were generated in this study, both based on the initial formation of *C*-glycosides of neuraminic acid. The first route involves the enzymatic polymerization of a phenolic *C*-glycoside with soybean peroxidase. The second route involves the vinyl polymerization of a terminal allyl group on the aglycone.

C-Glycosylation donor, peracetylated neuraminic acid phenyl sulfone (**1**), was synthesized according to a published procedure.[14] Samarium iodide mediated *C*-glycosylation chemistry was utilized to construct a C−C bond connection between the neuraminic acid donor and an acceptor bearing the ketone moiety.[15] Compound **3** represents a Neu5Ac *C*-glycoside linked to a phenolic aglycone (Scheme 1).[16] Carefully designed protecting group chemistry in the donor and the acceptor simplified monomer synthesis. Simultaneous deprotection of the acetyl groups and hydrolysis of the methyl ester afforded the water-soluble phenolic *C*-glycoside monomer (**4**). Both **3** and **4** were racemic (*R*,*S*) mixtures at the newly formed hydroxylmethylene glycosidic carbon. Horseradish peroxidase (HRP) and Soybean peroxidase (SBP) are able to catalyze C−C bond formation, at the ortho-position of the electron donating group-containing phenolic monomers, under mild conditions. Polymerization of *C*-glycoside **4** in aqueous sodium phosphate buffer (pH 7) with soybean peroxidase (SBP) and H_2O_2 afforded a mucin-like polymer **5** having a molecular weight of 20 000.[17] Molecular weight was determined by gel permeation chromatography and calculated based on a standard curve with use of polysaccharide standards. The molecular weight of polymer **5** is similar to that of MG2 mucin (MUC 7).

Scheme 1[a]



[a] Reagents and conditions: (a) THF, SmI_2 10 h. (b) NaOMe, MeOH, 8 h, followed by 0.2 M KOH. (c) Soy Bean peroxidase, H_2O_2, sodium phosphate buffer, pH 7.0, 18 h.

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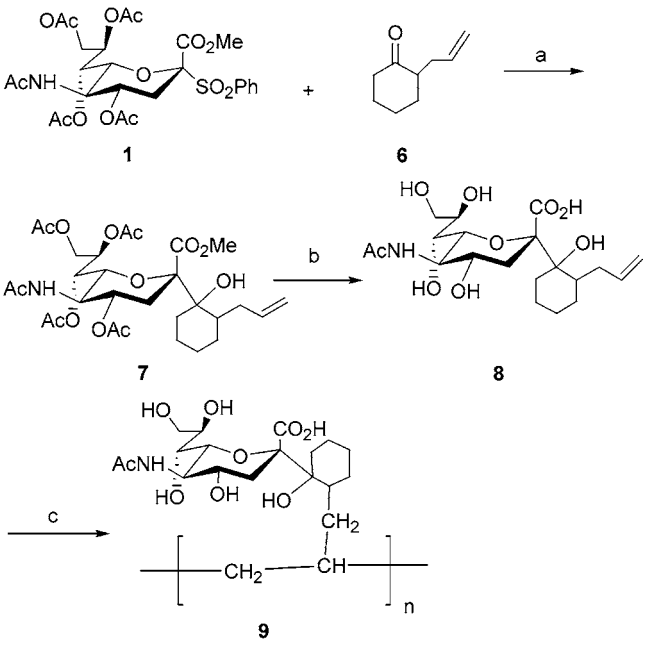
(16) To prepare methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-2,6-anhydro-3,5-dideoxy-2-*C*-[(2-hydroxy-butylphenyl-acetate) methyl]-D-*erythro*-L-manno-nononate **3**, Neu5Ac phenyl sulfone **1** (100 mg) and 5 equiv of 4-(3-oxobutyl)phenyl acetate **2** were dried together under high vacuum for 4 h. SmI_2 (4 equiv, freshly prepared from Sm and ICH_2CH_2I, 0.1 M in THF) was added under argon in one portion at room temperature with vigorous stirring. The dark blue solution was stirred overnight. The reaction mixture was diluted with ether and extracted with 1 N HCl, saturated $Na_2S_2O_3$, saturated $NaHCO_3$, and brine solution. The organic layer was dried over anhydrous Na_2SO_4. The filtrate was concentrated under reduced pressure and purified on a silica gel column with EtOAc as eluent. The *C*-glycoside was obtained as oil in 82% yield. ^{1}H NMR ($CDCl_3$) δ 1.80−2.20 (19H, $6COCH_3$, H-3ax, CH_2CH_2Ph), 2.45 (m, 1 H, H-3$_{eq}$), 2.63−2.67 (ddd, 1H, CH_2Ph), 2.78−2.85 (ddd, 1H, CH_2Ph), 3.76 (d, 3H, $COOCH_3$), 3.95−4.10 (m, 3 H, H-5, H-6, H-9a), 4.34 (m, 1 H, H-9b), 4.74 (m, H-4), 5.22 (m, 1 H, NH), 5.8 (m, 1 H, H-7), 5.40 (m, 1 H, H-8). HRFABMS: calcd for $C_{32}H_{43}NO_{15}Na$ [M + Na]$^+$ 704.2556, found *m*/*z* 704.2530 [M + Na]$^+$
(17) To prepare phenolic mucin analogue **5**, fully deprotected monomer **4** (10 mg, 0.022 mmol) was dissolved in sodium phosphate buffer (50 mM, pH 7, 200 μL) 80 vol % in CH_3CN. Soybean peroxidase (1 mg) was added to the solution, followed by the dropwise (10 μL/4 min) addition of H_2O_2 (100 μL 0.2 M). After 12 h, the reaction mixture was freeze-dried and subjected to BioGel P-10 column to determine molecular weight. ^{1}H NMR of resulting polymer shows broad peaks in the phenolic and carbohydrate regions. The intrinstic viscosity is 2.5 × 10^{-3} m^3/kg.

Polymer **5** was investigated as a neuraminidase inhibitor with use of an assay commonly applied to simple Neu5Ac-*C*-glycosides.[18] Specifically, K_i values were determined by incubation of the fluorescent substrate, 2′-(4-methylumbelliferyl)-α-D-*N*-acetylneuraminic acid, *C. perfringens* neuraminidase, and sodium acetate buffer at pH 5 in the presence of various concentrations of **5**. The results were fit to different inhibition models, with the best fit obtained for a competitive inhibition model giving a K_i of 900 nM based on the concentration of polymer. The observed K_i was over 10-fold lower than simple Neu5Ac-*C*-glycosides with hydrophobic aglycones.[13b] This enhanced inhibitory activity is ascribed to the multivalency of the polymeric inhibitor, which likely results from an increase of the local concentration of the inhibitor. The synthesis of this neuraminic acid-based polymer occurs under mild conditions, using an enzymatic reaction, and may be feasible under physiological conditions. Thus, a stable mucin-like polymer might be synthesized in situ (at the surface of a wound) by application of **4** or a suitable derivative.

Next, we examined the potential utility of such synthetic materials as protective layers against pathogen infection. Preliminary studies showed that mucin analogue **5** could be incorporated on biomaterials through simple coating techniques. Treatment of polystyrene beads (20−50 mesh) with polymer **5** resulted in its binding at a high loading capacity (2.9 mg of **5**/mL of polystyrene resin), presumably through hydrophobic interactions. The resulting activated surface is currently being evaluated for its ability to inhibit pathogen infection. Modification of polymer **5** was next undertaken in an attempt to prepare higher molecular weight glycoproteins that resemble most natural mucins.[19] The use of multiple monomers or cross-linkers can often facilitate the enzymatic preparation of higher molecular weight polymers. However, the copolymerization of **4** with arbutin (4-hydroxyphenyl-β-D-glucopyranoside) or bisphenol A as comonomers, while affording copolymers, did not result in higher molecular weight mucin analogues.

A second mucin-like polymer was prepared by chemical polymerization (Scheme 2). Monomer **7**, containing a cyclohexylallyl functionality, was synthesized from the *C*-glycosylation of Neu5Ac peracetyl-sulfone **1** with 2-allyl

Scheme 2[a]



[a] Reagents and conditions: (a) SmI_2, THF. (b) NaOMe, MeOH, 1 N NaOH. (c) 2,2′-azobis-(2-amidinopropane) dihydrochloride at 70 °C.

cyclohexanone (**6**).[20] Deprotection of **7** afforded monomer **8**,[21] which was polymerized with 2,2′-azobis(2-amidinopropane) dihydrochloride.[22] The resulting polymer **9** was precipitated from 80% aqueous methanol to yield a highly disperse material with a MW >20 000 making it potentially more suitable as a synthetic mucous barrier for anti-infective applications.

These two synthetic polymers prepared bear *C*-linked Neu5Ac, a specific, lectin-binding carbohydrate unit. These polymers are, therefore, likely capable of tight multivalent interactions with proteins and may find use as mimics of the natural glycans found in mucins. By applying such polymers to surfaces, such as glass, plastics, woven and nonwoven fibers, medical instruments, etc., it may be possible to establish a protective layer against pathogens.

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(20) To prepare protected allyl *C*-glycoside monomer **7**, *C*-glycosylation between Neu5Ac phenyl sulfone donor **1** and 2-allylcyclohexone **6** acceptor was carried out according to a similar procedure described for **3**. The reaction mixture was purified on silica gel chromatography with ethyl acetate as eluent to obtain **7** as a racemic compound (yield 45%). The ratio of two diastereomers was closed to 1:1. ^{1}H NMR ($CDCl_3$) δ 1.25−1.70 (m, 8H, CH_2 × 4 in cyclohexane), 1.89 (3H, CH_3CONH), 2.03−2.20 (15H, 4COCH_3, H-3ax, CH_2CH═), 2.45−2.56 (m, 2 H, H-3$_{eq}$, CH in cyclohexane), 2.63−2.67 (ddd, 1H, CH_2Ph), 2.78−2.85 (ddd, 1H, CH_2Ph), 3.80 (d, 3 H, COOCH_3), 3.95−4.13 (m, 3 H, H-5, H-6, H-9a), 4.30−4.40 (m, 1 H, H-9b), 4.91 (m, 2 H, H-4, H-7), 5.31 (m, 1 H, NH), 5.23−5.31 (m, 3 H, ═CH_2, H-8), 5.65−5.85 (m, 1 H, CH═).

(21) To deprotect poly(vinyl) mucin analogue monomer **8**, a solution of *C*-glycoside **8** (33 mg) was added to MeOH (1 mL) and NaOMe in MeOH (0.5 M, 1 mL). The mixture was stirred overnight at room temperature, then neutralized with of Amberlite IR-120 (H^+) exchange resin, filtered, and concentrated to dryness. The residue was purified by silica gel chromatography eluting with 100% methylene chloride, 90% and 80% methylene chloride in methanol. ^{1}H NMR (D_2O) (*R*:*S* 1:1) δ 1.20−1.79 (m, H-3eq, CH_2 × 4 in cyclohexane), 1.94 (d, 3 H, NHCOCH_3), 2.39 (m, 1 H, H-3$_{ax}$), 3.35−3.80 (m, 9 H).

(22) To prepare poly(vinyl) mucin analogue **9**, monomer **8** (30 mg) was dissolved in bidistilled water (2 mL). The mixture was purged with argon for 10 min and warmed in an oil bath up to 70 °C with continuous stirring. The polymerization was started by addition of the initiator 2,2′-azobis-(2-amidinopropane) dihydrochloride dissolved in bidistilled water (0.1 mL). The reaction time was 24 h. The reaction mixture was freeze-dried and the product was precipitated in 80% methanol. The precipitate was centrifuged and white powder was obtained after complete drying. GPC analysis showed that the molecular weight of **9** was greater than 20 000.