

Short Note

N,N-Bis(hexyl α -D-acetylmannosyl) Acrylamide

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Abstract: Glycosyl monomers for the assembly of multivalent ligands are typically synthesized using carbohydrates with biological functions and polymerizable functional groups such as acrylamide or styrene introduced into the carbohydrate aglycon, and monomers polymerized using a radical initiator. Herein, we report the acryloylation of 6-aminoethyl α -D-mannoside and its conversion into the glycosyl monomer bearing an acrylamide group. The general acryloylation procedure afforded the desired *N*-hexyl α -D-acetylmannosyl acrylamide monomer as well as an unexpected compound with a close R_f value. The compounds were separated and analyzed by nuclear magnetic resonance spectroscopy and mass spectrometry, which revealed the unknown compound to be the bivalent *N,N*-bis(hexyl α -D-acetylmannosyl) acrylamide monomer, which contains two hexyl mannose units and one acrylamide group. To the best of our knowledge, this side reaction has not previously been disclosed, and may be useful for the construction of multivalent sugar ligands.

Keywords: glycosyl monomer; acryloylation; acrylamide; glycopolymer; bivalent ligand



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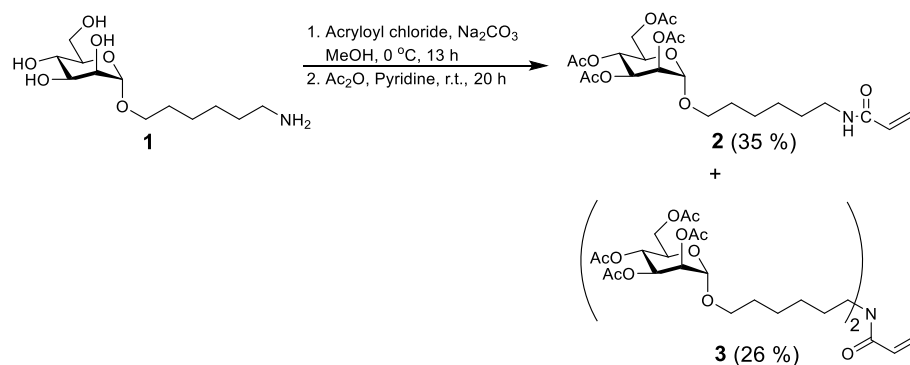
1. Introduction

Many biological events in organisms are associated with carbohydrates on cell surfaces [1–4]. Carbohydrates assemble into clusters in raft domains and on polysaccharides and proteins to acquire high affinities for carbohydrate-binding proteins (CBMs). The clusters, as multivalent ligands, interact with CBMs to induce biological events [5–7]. Multivalency, which is important for carbohydrate–protein interactions, has also been imitated by synthetic polymers that contain carbohydrates; this affinity-enhancing effect of a multivalent ligand is known as the “cluster effect”. The polymer generally contains carbohydrates with pendants on the polymer back bones, which are generally chosen to be acrylamide [8,9], styrene [10,11], or norbornene [12,13]. A polymerizable carbohydrate monomer consists of a carbohydrate, a linker, and a polymerizable functional group. Carbohydrates include monosaccharides as well as oligosaccharides as ligands. The linker controls the flexibility and the binding space in the carbohydrate–protein interaction, while polymerizable functional groups modulate solubility and polymer structure, and these units are chosen to suit the research purpose. Studies into the preparation of glycomonomers and glycopolymers has continued to date with the aim of clarifying the biological mechanism and applying them to devices and biomaterials [14–17].

In this study, a mannosyl monomer was prepared using a general method in which 6-aminoethyl α -D-acetylmannoside was acryloylated to afford *N*-hexyl α -D-acetylmannosyl acrylamide. Surprisingly, a previously undocumented side-reaction was observed during acryloylation, the product of which was separated by silica-gel column chromatography and identified by nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry (MS) to be *N,N*-bis(hexyl α -D-acetylmannosyl) acrylamide, which is a bivalent monomer containing two mannose residues. The discovery of this unusual bivalent monomer is described herein.

2. Results and Discussion

6-Aminohexyl α -D-mannoside (**1**) was prepared from D-mannose in five steps, which included acetylation, 1-deacetylation, introduction of the trichloroacetimidate, glycosylation with 6-aminohexan-1-ol, and deacetylation. Mannoside (**1**) was converted into a mannosyl monomer bearing an acrylamide group by treatment with acryloyl chloride and sodium carbonate in methanol at 0 °C for 13 h, followed by acetylation (Scheme 1). The reaction mixture was evaporated and separated by silica-gel chromatography to provide two compounds that were subjected to NMR spectroscopy (Figures S1 and S2) and matrix assisted laser desorption ionization-time of flight (MALDI-TOF) MS (Figure S3). Their ^1H NMR spectra showed similar peaks; however, integration of the sugar and alkyl peaks of the compound of higher R_f revealed the presence of two 6-aminohexyl α -D-mannoside units per acryloyl group. Furthermore, the amide peak was absent in the NMR spectrum of this compound (Figure 1). In the IR spectrum, the N–H stretching vibration was also not observed (Figure S4). The mass spectrum of this unknown compound is displayed in Figure 2.



Scheme 1. Acryloylation of 6-aminohexyl α -D-mannoside (**1**).

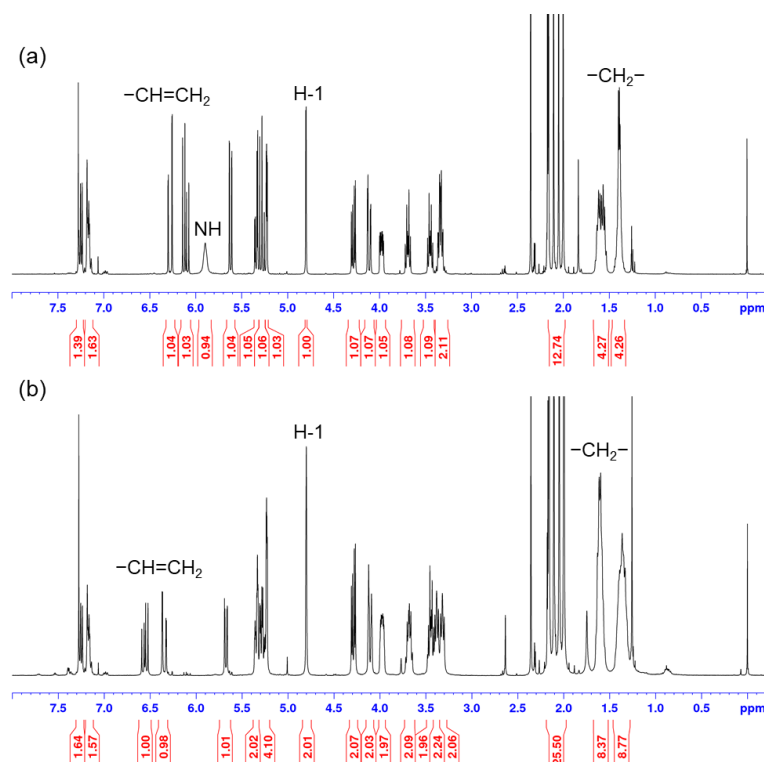


Figure 1. ^1H NMR spectra of (a) *N*-hexyl α -D-acetylmannosyl acrylamide (**2**) and (b) *N,N*-bis(hexyl α -D-acetylmannosyl) acrylamide (**3**).

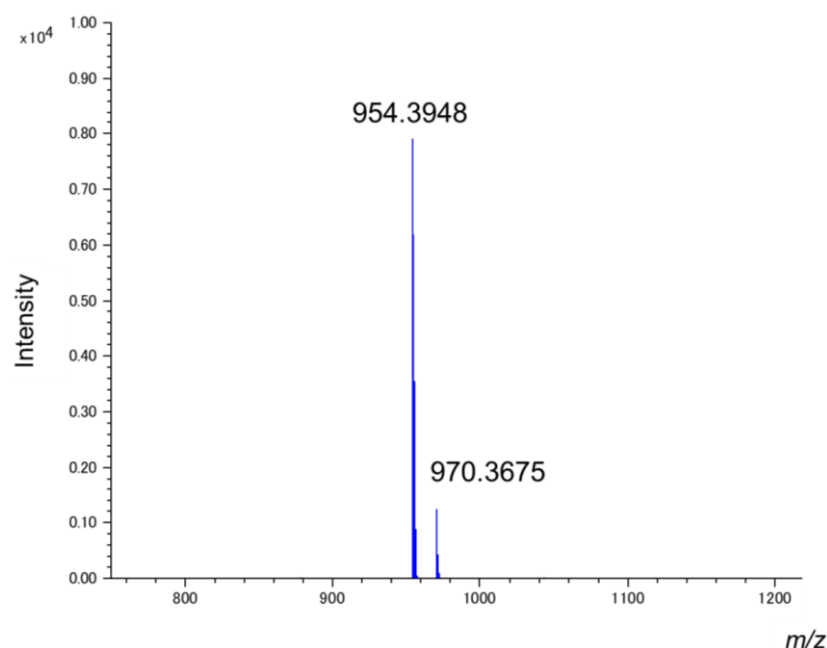


Figure 2. MALDI-TOF mass spectrum of *N,N*-bis(hexyl α -D-acetylmannosyl) acrylamide (**3**).

The above-mentioned results suggest that the unexpected compound contains two hexyl mannose units and one acryloyl group, from which we concluded that the acryloylation of **1** gave *N*-hexyl α -D-acetylmannosyl acrylamide (**2**) and *N,N*-bis(hexyl α -D-acetylmannosyl) acrylamide (**3**), which were isolated as pure syrups in yields of 35% and 26%, respectively, along with a considerable amount of a mixture of the two. It is interesting to note that the analogous side reaction that forms the bisamide was not observed in the case of 2-aminoethyl α -D-mannoside and the acryloylation for *N*-hexyl α -D-acetylmannosyl acrylamide (**2**). Therefore, *N,N*-bis(hexyl α -D-acetylmannosyl) acrylamide (**3**) was given only in the acryloylation for 6-aminoethyl α -D-mannoside (**1**). To the best of our knowledge, this is the first report of such a side reaction using the general acryloylation procedure. While the reaction mechanism is not clear, the bivalent monomer bearing the two sugar units may be useful for the preparation of multivalent glycopolymers.

3. Materials and Methods

3.1. General

All reagents were purchased from FUJIFILM Wako Pure Chemical Corporation (Osaka, Japan). Methanol was prepared by storage over molecular sieves (3Å) that were activated under vacuum at 200 °C. Analytical thin-layer chromatography (TLC) was performed using Merck silica gel 60 F₂₅₄ plates (layer thickness: 0.25 mm, Darmstadt, Germany). TLC plates were dipped in 85:10:5 (*v/v/v*) methanol/resorcinol/concentrated sulfuric acid and/or 0.7% ninhydrin/ethanol, followed by heating for a few minutes for visualization purposes. Column chromatography was performed using silica gel (Silica gel 60 N, spherical neutral, particle size 63–210 μ m, Kanto Chemical, Tokyo, Japan). ¹H and ¹³C NMR spectra were acquired on an AVANCE 400 Plus spectrometer (Bruker, Rheinstetten, Germany) in chloroform-*d* and reported in δ relative to tetramethylsilane (0.00 ppm for ¹H and 77.0 ppm for ¹³C). MALDI-TOF mass spectra were recorded on a Jeol JMS-S3000 spectrometer (Tokyo, Japan) using 2,5-dihydroxybenzoic acid as the matrix. The Fourier transform infrared (FTIR) spectra were measured on a Jasco FT-IR 4100 spectrometer (Tokyo, Japan) and reported as wavenumber (cm^{−1}). Pellet samples for FTIR were fabricated using KBr.

3.2. Acryloylation of 6-Aminoethyl α -D-Mannside (**1**)

6-Aminoethyl α -D-mannside **1** (976 mg, 3.13 mmol) was dissolved in methanol (21.0 mL). Sodium carbonate was added to the solution and the mixture was stirred

at 0 °C, after which acryloyl chloride (380 µL, 4.70 mmol) was slowly added dropwise over 10 min and then stirred at 0 °C for 13 h. The mixture was evaporated and acetic anhydride (11.0 mL, 117 mmol) and pyridine (11.0 mL, 136 mmol) were added at room temperature, stirred for 20 h, and then evaporated. The residue was extracted with chloroform and washed successively with aqueous 1 M hydrochloric acid, aqueous sodium hydrogen carbonate, and brine, dried over anhydrous sodium sulfate, filtered, and then evaporated. The residue was purified by silica gel chromatography three times using 5:1 (v/v) toluene/acetone as the eluent to give **2** (553 mg, 35%), **3** (391 mg, 26%), and a mixture of **2** and **3** (231 mg; **2**:**3** = 2.7:1).

Analytical data for **2**. R_f 0.54 (toluene/acetone, 1:1); ^1H NMR (400 MHz, CDCl_3) δ 6.28 (dd, $J_{\text{CH=Htrans}} = 16.4$ Hz, $J_{\text{Htrans,Hcis}} = 1.6$ Hz, 1 H, $=\text{CH}_{\text{trans}}\text{H}$), 6.11 (dd, $J_{\text{CH=Hcis}} = 10.4$ Hz, 1 H, $=\text{CH}-$), 5.90 (s, 1 H, NH), 5.62 (dd, 1 H, $=\text{CHH}_{\text{cis}}$), 5.34 (dd, $J_{2,3} = 3.6$ Hz, $J_{3,4} = 10.0$ Hz, 1 H, H-3), 5.28 (dd, $J_{4,5} = 19.8$ Hz, 1 H, H-4), 5.22 (dt, $J_{1,2} = 1.6$ Hz, 1 H, H-2), 4.80 (d, 1 H, H-1), 4.28 (dd, $J_{5,6a} = 5.6$ Hz, $J_{6a,6b} = 12.2$ Hz, 1 H, H-6a), 4.11 (dd, $J_{5,6b} = 2.4$ Hz, 1 H, H-6b), 3.98 (dt, 1 H, H-5), 3.69 (dt, $J_{\text{OCHa},2\text{CH}_2} = 9.6$ Hz, $J_{\text{OCHa},\text{OCHb}} = 6.4$ Hz, 1 H, $-\text{OCH}_a\text{H}_b-$), 3.45 (dt, $J_{\text{OCHa},2\text{CH}_2} = 6.4$ Hz, 1 H, $-\text{OCH}_a\text{H}_b-$), 3.33 (dq, $J_{\text{CHaNH},\text{CHbNH}} = 3.2$ Hz, $J_{\text{CH}_2\text{NH},5\text{CH}_2} = 7.2$ Hz, 2 H, $-\text{CH}_2\text{NH}-$), 2.16 (s, 3 H, COCH_3), 2.11 (s, 3 H, COCH_3), 2.05 (s, 3 H, COCH_3), 2.00 (s, 3 H, COCH_3), 1.65–1.53 (m, 4 H, $^2\text{CH}_2$, $^5\text{CH}_2$), 1.43–1.38 (m, 4 H, $^3\text{CH}_2$, $^4\text{CH}_2$); ^{13}C NMR (101 MHz, CDCl_3) δ 170.6, 170.1, 170.0, 169.7, 165.5, 130.9, 129.0 (toluene), 128.2 (toluene), 125.2 (toluene), 126.0, 97.5, 69.6, 69.1, 68.4, 66.2, 62.5, 39.4, 30.9 (toluene), 29.4, 29.0, 26.6, 25.9, 20.8, 20.7, 20.7.

Analytical data for **3**. R_f 0.64 (toluene/acetone, 1:1); ^1H NMR (400 MHz, CDCl_3) δ 6.55 (dd, $J_{\text{CH=Htrans}} = 16.8$ Hz, $J_{\text{Htrans,Hcis}} = 2.4$ Hz, 1 H, $=\text{CH}_{\text{trans}}\text{H}$), 6.35 (dd, $J_{\text{CH=Hcis}} = 10.4$ Hz, 1 H, $=\text{CH}-$), 5.68 (dd, 1 H, $=\text{CHH}_{\text{cis}}$), 5.36–5.22 (m, 6 H, H-2, H-3, H-4), 4.80 (s, 2 H, H-1), 4.30 (dd, $J_{5,6a} = 5.2$ Hz, $J_{6a,6b} = 12.0$ Hz, 2 H, H-6a), 4.11 (d, 2 H, H-6b), 3.97 (dt, 2 H, H-5), 3.68 (dt, $J_{\text{OCHa},2\text{CH}_2} = 9.6$ Hz, $J_{\text{OCHa},\text{OCHb}} = 6.8$ Hz, 2 H, $-\text{OCH}_a\text{H}_b-$), 3.48–3.30 (m, 6 H, $-\text{OCH}_a\text{H}_b-$, $-\text{CH}_2\text{NH}-$), 2.16 (s, 6 H, COCH_3), 2.11 (s, 6 H, COCH_3), 2.05 (s, 6 H, COCH_3), 2.00 (s, 6 H, COCH_3), 1.63–1.60 (m, 8 H, $^2\text{CH}_2$, $^5\text{CH}_2$), 1.39–1.33 (m, 8 H, $^3\text{CH}_2$, $^4\text{CH}_2$); ^{13}C NMR (101 MHz, CDCl_3) δ 170.6, 170.6, 170.1, 169.9, 169.8, 169.7, 169.7, 165.9, 127.8, 129.0 (toluene), 128.2 (toluene), 127.6, 97.5, 69.7, 69.1, 68.4, 68.1, 66.2, 62.5, 48.0, 46.5, 29.6, 29.2, 27.7, 26.8, 26.7, 25.9, 25.9, 20.9, 20.7, 20.7, 20.7; IR (KBr, cm^{-1}): 2937 (C–H), 2862 (C–H), 1751 (C=O), 1647 (C=C), 1611 (C=O), 1433 (C–H), 1373 (C–H); MALDI-TOF-MS m/z : $[\text{M}+\text{Na}]^+$ Calcd for $\text{C}_{43}\text{H}_{65}\text{N}_1\text{Na}_1\text{O}_{21}^+$: 954.3941, found: 954.3948; $[\text{M}+\text{K}]^+$ Calcd for $\text{C}_{43}\text{H}_{65}\text{K}_1\text{N}_1\text{O}_{21}^+$: 970.3681, found: 970.3675.

Supplementary Materials: The following are available online. ^1H and ^{13}C NMR spectra of **2** and **3** (Figures S1 and S2), and MALDI-TOF-mass and FTIR spectra of **3** (Figures S3 and S4).

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