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Design and synthesis of trivalent Tn glycoconjugate polymers by nitroxide-mediated polymerization

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ABSTRACT

A new synthetic method for preparing Tn glycoconjugate polymers, containing tumor-associated carbohydrate antigens, by controlled living radical polymerization is reported. To mimic the authentic structures of Tn glycopeptide antigens and to explore the controlled living radical polymerization, three tumor-associated carbohydrate antigens (GalNAc, GalNAcα1-*O*-Ser, and GalNAcα1-*O*-Thr) were attached to a styrene-type monomer through a diethylene glycol spacer. Under nitroxide-mediated polymerization, controlled living radical polymerization proceeded to afford defined glycopeptide polymers with different Tn densities and compositions. The polydispersity index (PDI) and molecular weights were increased and conversions were decreased upon increasing the concentration of Tn glycoconjugate monomers. The resulting Tn glycoconjugate polymers were characterized by NMR and IR. The spectral data indicate that the Tn glycoconjugate moiety did attach to the polymer chain and Tn glycoconjugate density could be adjusted through the nitroxide-mediated polymerization conditions. The number of Tn units containing in the polymer chains could be estimated by NMR integration. This synthetic approach provides a new and efficient tool for constructing novel Tn glycoconjugate polymers.

1. Introduction

The carbohydrate Tn antigen (GalNAcα1-*O*-Ser/Thr), an abnormal mucin-type *O*-glycan, is exclusively found in tumor cells and its expression is associated with various types of cancers and several human disorders [1]. It has a simple core structure, composed of *N*-acetyl-D-galactosamine with a glycosidic linkage to serine/threonine residues in animal glycoproteins (Fig. 1). Because of the association of the Tn antigen with cancers and diseases, development of Tn-based vaccines and other therapeutic approaches based on Tn-related studies were gradually gained attention [2]. Tn antigens, along with other tumor-associated carbohydrate antigens (TACAs), provide ideal targets for the development of anticancer vaccines [3]. However, the isolation of these carbohydrate antigens in sufficient quantities and high purity from natural sources is extremely difficult. Anticancer vaccines with synthetic tumor-associated carbohydrate antigens are emerging as an alternative approach to the treatment of cancers by enhancing the immunogenicity of these carbohydrate antigens upon the production of long-lasting antibodies.

Fully synthetic carbohydrate vaccines with defined compositions of carbohydrate ligands have showed promising results in cancer immunotherapy [4]. Pioneered by Danishefsky, the synthesis of multivalent anticancer vaccines bearing five different tumor-associated carbohydrate antigens (TACAs) through peptide bond formation has been reported [5]. The installation of an appropriate linker, the conjugation to a carrier protein, Keyhole Limpet Hemocyanin (KLH, an immunoenhancer), and the ratio of glycopeptide: KLH (228:1) were determined to be an appropriate chemical structure to serve as an efficient vaccine for the treatment of breast and ovarian cancer (Scheme 1) [6]. These anticancer vaccines are currently in clinical trials by OBI Pharma [7].

Instead of fully synthetic carbohydrate vaccines, several polymeric methods for preparing Tn glycoconjugates were also investigated in attempts to mimic synthetic anticancer vaccines [8–10]. Li and Kunz reported on the synthesis of synthetic polymer glycopeptide conjugates by reversible addition-fragmentation chain-transfer polymerization (RAFT) and click chemistry [8] (see Scheme 2). The polymer-based glycopeptide vaccines were found to induce significant immune reactions and elicit the production of antibodies that were able to recognize breast tumor cells.

Davis and Cameron have also reported on a polymerizable

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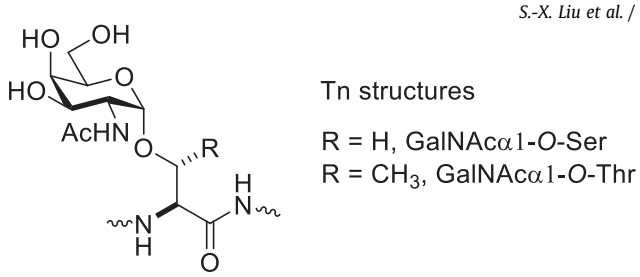


Fig. 1. The structures of Tn carbohydrate antigens.

version of a truncated Tn-antigen glycan (without an amino acid moiety), prepared by reversible addition-fragmentation chain transfer (RAFT) polymerization [9]. The truncated Tn glycopolymer was then conjugated with gold nanoparticles, to produce multicopy-multivalent nanoscale glycoconjugates. Immunological studies indicated that these nanomaterials generated the strong and long-lasting production of antibodies. These results demonstrated a simple approach toward preparing synthetic anticancer vaccines based on nanomaterials without the need for a typical vaccine protein component. Huang developed several ligation methods for enhancing the immunogenicity of these carbohydrate antigens to produce an immune response with a significant amount of antibodies [10], which could be recognized by Tn expressing tumor cells. These methods included (1) azide-alkyne cycloaddition reactions (click chemistry) to produce covalent bonding between Tn moiety and the tobacco mosaic virus (TMV) capsid as a carrier [10a], (2) polymers prepared by cyanoxyl-mediated free radical polymerization, followed by conjugation (amide bond formation) with a Tn antigen [10b], (3) polymers produced by atom transfer radical polymerization (ATRP), followed by ligation with a Tn moiety [10c].

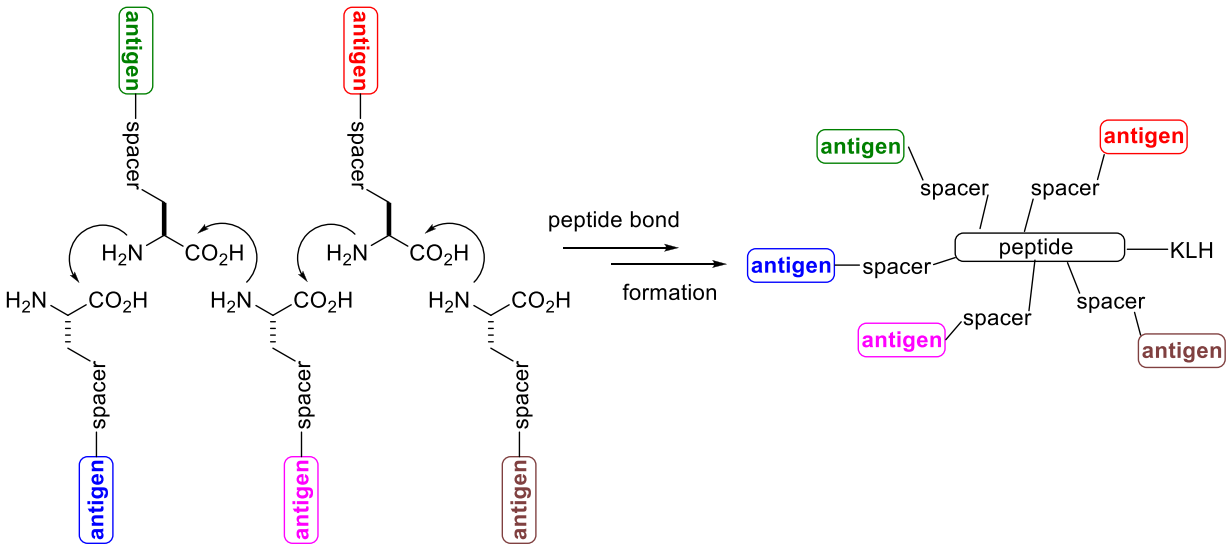
Although the preparation of synthetic glycopeptide conjugates polymer have been previously reported, the main problems with the current synthetic methods are that the polymerization process and conjugation or ligation methods [11] between the polymers and Tn carbohydrate moieties cannot be easily controlled. To prepare strong and longest-lasting antibodies by these tumor-associated carbohydrate antigens, a general method for preparing well-defined and tunable carbohydrate polymers containing Tn carbohydrate antigens with different compositions, desired molecular weights, and defined PDIs continues to be a challenge for biomedical chemists. Several methods have been developed for preparing carbohydrate polymers, including atom transfer radical polymerization (ATRP) [12], reversible addition-fragmentation chain transfer (RAFT) [13], and nitroxide-mediated polymerization (NMP) [14]. Among these polymerization methods, NMP techniques have been attracted a great deal of interest by biomedical chemists, since the NMP method can be used to prepare metal-free or sulfur-free carbohydrate polymers. Hence, carbohydrate polymers prepared by the NMP method are much more compatible with biological and physiological conditions. Furthermore, to be eventually used for the administration to animals or in human testing, an ideal polymerization method that is both metal-free and sulfur-free will be needed.

Herein, we report on a practical and synthetic method for the preparation of Tn glycoconjugate polymers containing tumor-associated carbohydrate antigens. Our approach involved the use of nitroxide-mediated polymerization for preparing well-controlled glycoconjugate polymers with tunable Tn densities, defined molecular weights, and PDIs. The living character of controlled radical polymerization could be further explored to prepare novel glycoconjugates polymers containing trivalent Tn glycoconjugates ligands. With a suitable spacer between the glycosyl amino acid and the polymer backbone, well-defined Tn glycoconjugates polymers bearing Tn tumor-associated carbohydrate antigen were designed. (Scheme 3).

2. Results and discussion

2.1. Synthesis of Tn glycoconjugate monomers

A practical method was designed for the synthesis of Tn glycoconjugate monomers. Tn glycoconjugate monomers are composed of three parts, namely, a Tn carbohydrate moiety, a spacer, and a monomer. Because styryl-TEMPO could only regulate styrene or n-butyl acrylate in a controlled manner, we decided to simply examine the styryl-TEMPO-mediated polymerization of Tn glycoconjugate monomers using polystyrene as the polymer backbone. Diethylene glycol (**1**) and 2-(2-aminoethoxy)ethanol (**4**) were used as the spacers between the styrene and the Tn carbohydrate moiety to avoid possible steric hindrance during the polymerization reactions and also to mimic the glycopeptide bonds on the surface of



Scheme 1. A pentavalent carbohydrate-based anticancer vaccine made by peptide bond formation.

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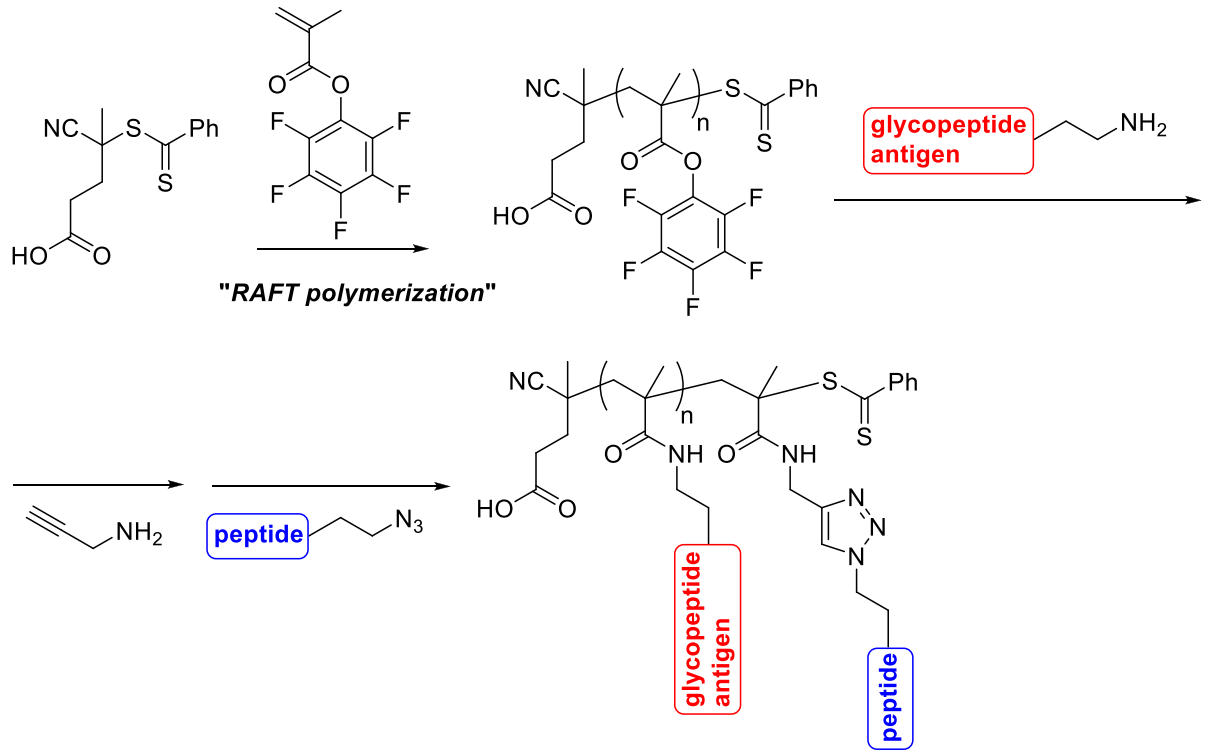
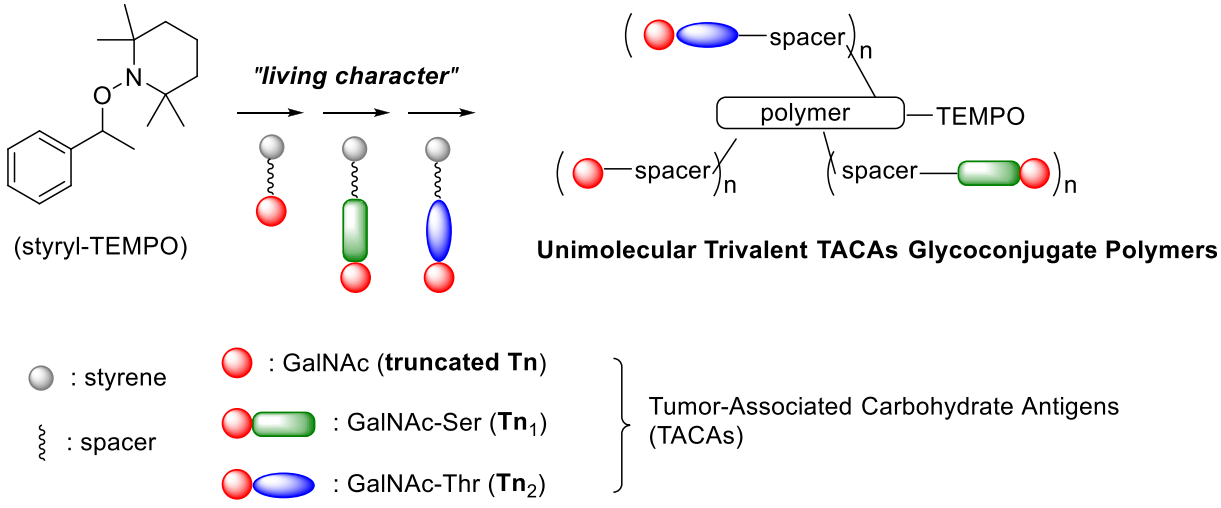


Scheme 2. Polymer-based glycopeptide conjugate prepared by RAFT polymerization and Click Chemistry.



Scheme 3. Synthesis of Tn glycoconjugate polymers by nitroxide-mediated polymerization.

tumor cells. Diethylene glycol (**1**) was reacted with 4-chloromethylstyrene (**2**) under basic conditions to afford the styrene derivative **3** as the first spacer monomer with a hydroxyl group as its terminal [15] (Scheme 4). For the other spacer, the amino group of 2-(2-aminoethoxy)ethanol (**4**) was first protected by a Boc group, followed by reaction with 4-chloromethylstyrene, and then deprotected to produce the second spacer monomer **7** with an amino group as its terminal.

The Tn carbohydrate moieties were composed of *N*-acetyl galactosamine that was attached to serine/threonine, through a α-glycosidic bond linkage. Starting from D-galactose (**8**), all of the hydroxyl groups were reacted with acetic anhydride in pyridine to give the pentaacetate **9**, which was then converted to the glycosyl bromide **10** by reaction with HBr/HOAc. Reductive elimination by a reaction with zinc power in an aqueous buffer solution afforded the triacetyl galactal **11** [16]. Compound **11** was then converted to **12** via an azidochlorination reaction [17]. Meanwhile, two glycosyl acceptors **14a** and **14b** were prepared by esterifying Fmoc-Ser(OH)–OH (**13a**) and Fmoc-Thr(OH)–OH (**13b**) with the *t*-butyl trichloroimidate reagent in a co-solvent system of EtOAc/cyclohexane [18]. (Scheme 5).

Silver ion-mediated glycosylation reactions [19] between the glycosyl donor **12** and the glycosyl acceptors **14a**/**14b** gave the glycosylation products **15a**/**15b**. However, the α- and β-isomers could not be successfully separated at this stage. To overcome this problem, the reduction of the azido groups to *N*-acetyl groups with

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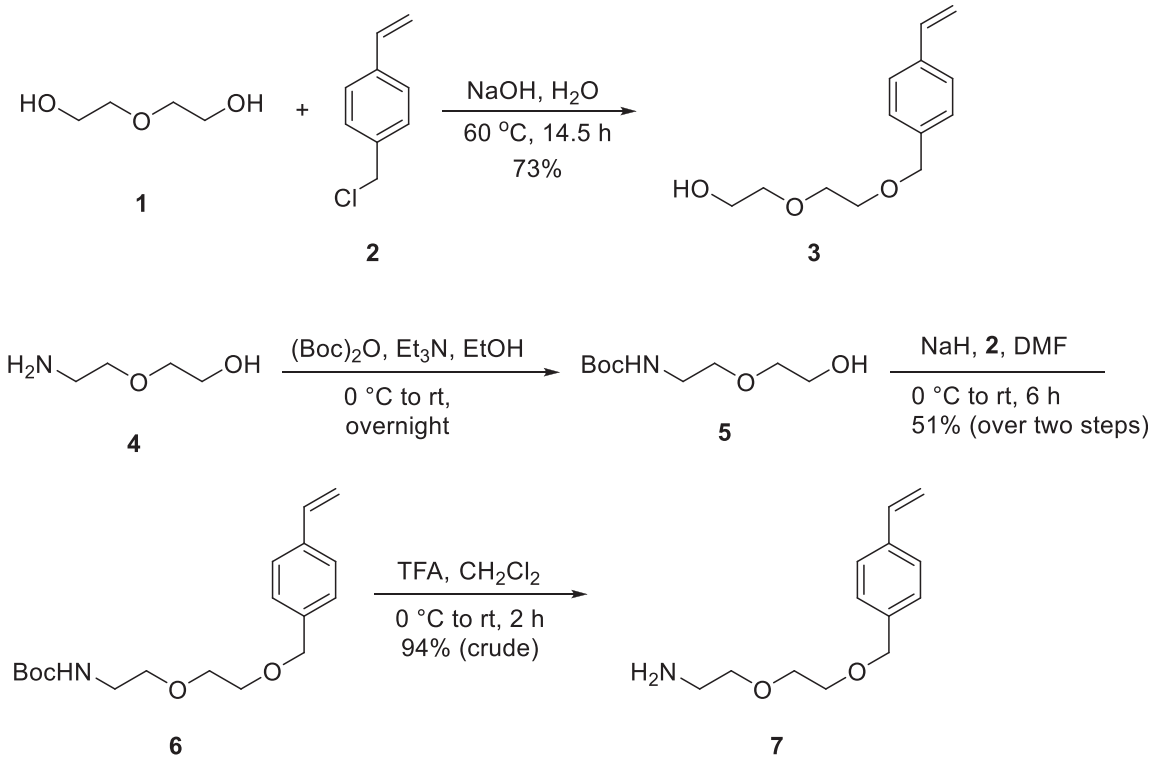
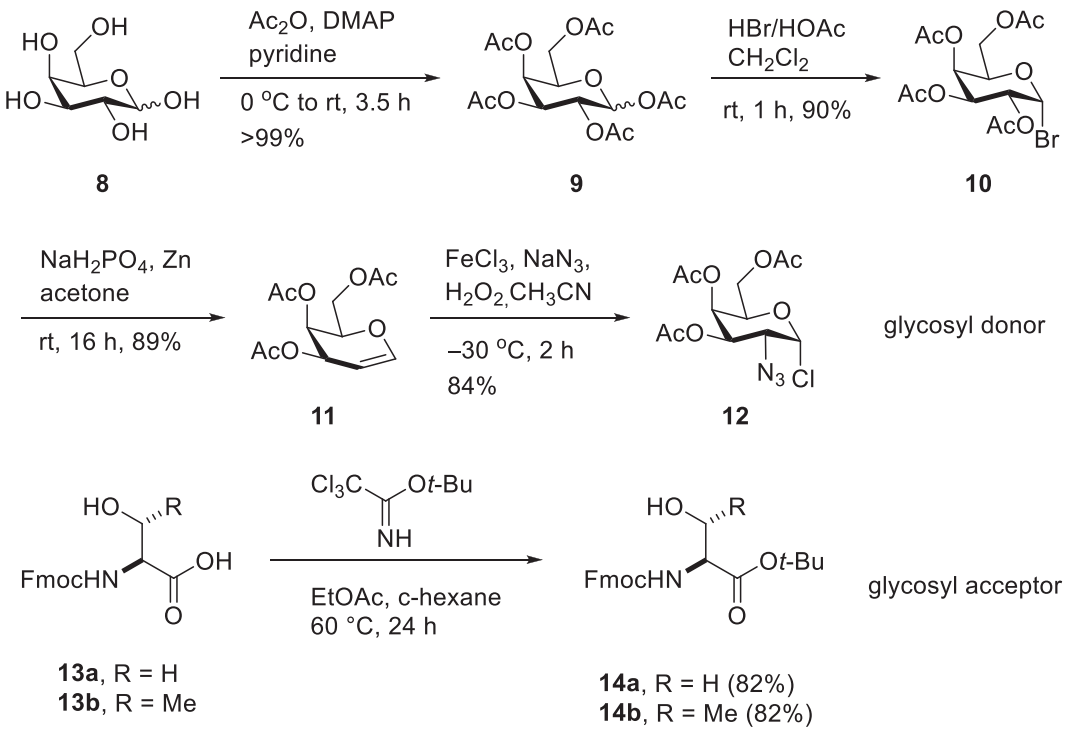


Scheme 4. Synthesis of spacers.



Scheme 5. Synthesis of glycosyl donor and acceptors.

Zn power in presence of acetic anhydride gave the key Tn carbohydrate derivatives **16a**/**16b**, which are versatile synthons for synthesis of a variety of Tn related structures. For example: (1) deprotection of the acetyl groups on **16** gave a structure that could serve as another glycosyl acceptor for the second glycosylation reactions to produce tumor-associated disaccharides STn or TF carbohydrate antigens [20]; (2) deprotection of the Fmoc group on **16** would generated a free amino group, which could be used for the synthesis of an *N*-terminal Tn glycopeptide; (3) deprotection of the *t*-Bu group on **16** would generate a free carboxylic acid group

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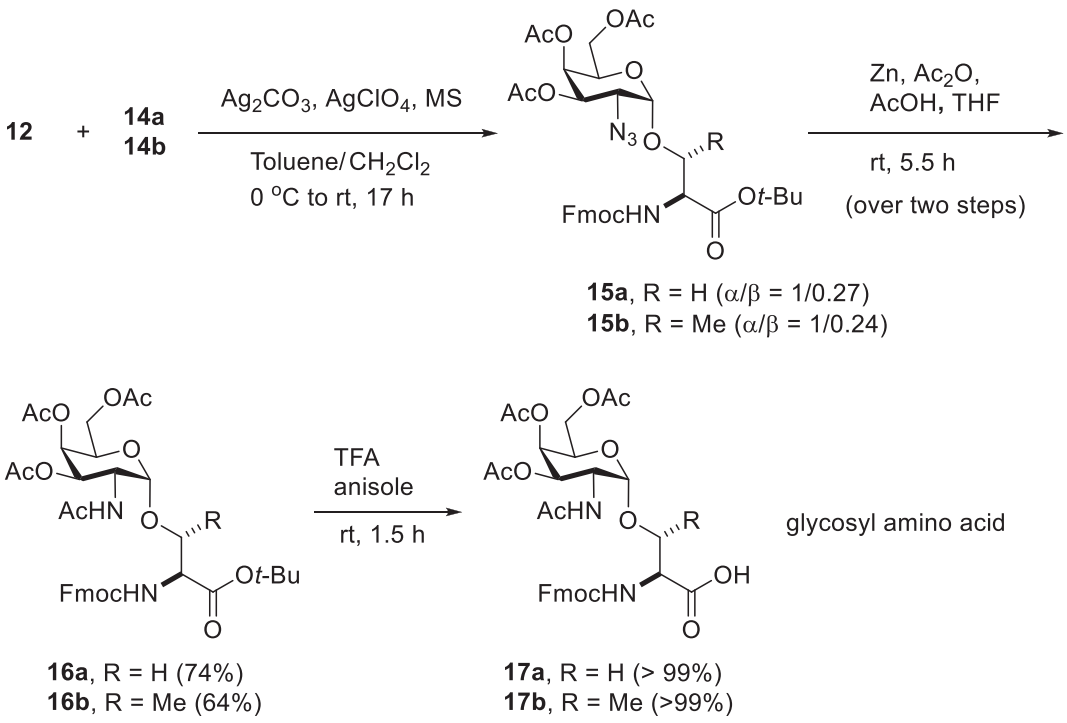
that could be used for the solid-phase synthesis of C-terminal Tn glycoproteins. In this study, deprotection of the *t*-butyl group using TFA in anisole [16] produced Tn glycosyl amino acid **17a**/**17b**, which could then be used in coupling reactions with the spacer monomer **7** to produce Tn glycoconjugate monomers (Scheme 6).

Three different Tn glycoconjugate monomers, truncated Tn (GalNAc), Tn_1 (GalNHAc-Ser), and Tn_2 (GalNAc-Thr), were designed to study their polymerization reactions, shown in Scheme 7. To prepare the truncated Tn glycopolymer (GalNAc, without an amino acid moiety), the silver ion-mediated glycosylation reaction [19] between the glycosyl donor **12** with the spacer **3** generated the glycomonomer **18**. To convert **18** into an *N*-acetyl galactosamine derivative, the azido group was reduced to an amino group with Zinc power in presence of acetic anhydride/acetic acid [16] to directly give the truncated Tn glycomonomer **19**. With Tn glycosyl amino acids (**17a**/**17b**) and the spacer monomer **7** in hand, we are now ready to prepare two Tn glycoconjugate monomers using standard peptide bond formation reactions. After testing a variety of coupling reagents [21], PyBOP/HOBt/DIPEA was found to be the best coupling reagent for this amide bond formation to produce the Tn glycoconjugate monomers **20a**/**20b**. Preliminary studies in the radical polymerization using the Tn glycoconjugate monomers **20a**/**20b** were not successful. NMR and GPC spectra indicated that no polymerization occurred. The unsuccessful results were attributed to the Fmoc protecting group on compounds **20a**/**20b**, which are not a stable functional group under conditions of Styryl-TEMPO polymerization reactions. The higher temperature (125 °C) used in these polymerization reactions caused the degradation of the Fmoc carbamate group, which is a normal process in carbamate synthesis [22]. To cope with this situation and to continue our studies on the Tn glycoconjugate polymerization, alternative approaches were examined. The deprotection of the Fmoc group using 5% piperidine in DMF [23] and gave compounds **21a**/**21b** with a free amino group. The amino group on compounds **21a**/**21b** could be regarded as starting compounds for producing peptide-bonds in Tn glycopeptide polymers. Finally, a simple acetyl group was used to protect the amino group to afford the desired Tn glycoconjugate monomers **22a**/**22b** (Tn_1/Tn_2). Herein, we report on the first synthesis of Tn glycoconjugate monomers, including GalNAc (truncated Tn), GalNAc-Ser (Tn_1), and GalNAc-Thr (Tn_2). With these advanced Tn glycoconjugate monomers, the preparation of Tn glycoconjugate polymers containing tumor-associated carbohydrate antigens will now be easier than before. Meanwhile, with these synthetic methods in hand, the preparation of other tumor-associated glycoconjugate polymers, such as STn and TF will also be feasible.

2.2. Polymerization studies

The polymerization reactions were conducted in sealed tubes using 1 mol% of alkoxyamine and 40 wt % of dimethylformamide (DMF) as the co-solvent with different compositions of Tn glycoconjugate monomers and styrene and the results are presented in Table 1. In some experiments (entries 1–6, and 9), styryl-TEMPO was used as the regulator. In other experiments (entries 7–8), polymeric alkoxyamines were used as regulators in the living radical polymerizations. The Tn glycoconjugate polymers were collected by centrifugation and dried at 60 °C for 12 h in a vacuum-drying cabinet. Conversions were evaluated gravimetrically. Molecular weights and polydispersity index (PDI) were determined by size exclusion chromatography (SEC). Because the synthesis of the Tn glycoconjugate monomer **22a** and **22b** are laborious [24], at this stage, only a ratio (10/90; ratio to styrene) of monomer compositions in the polymerizations were studied (entries 5–8). In one experiment (entry 9), the co-polymerization of three different Tn glycoconjugate monomers was also studied.

The copolymerization reactions of the truncated glycoconjugate monomer **19** and styrene using different ratios were studied first (entry 1–4) at 125 °C and an optimized reaction time (24 h) was used. A conversion of 70% and a narrow PDI of 1.13 were obtained when a ratio of 10/90 (monomer **19**/styrene) was used in the styrene polymerization (entry 1). When the concentration of



Scheme 6. Synthesis of Tn and glycosyl amino acids.

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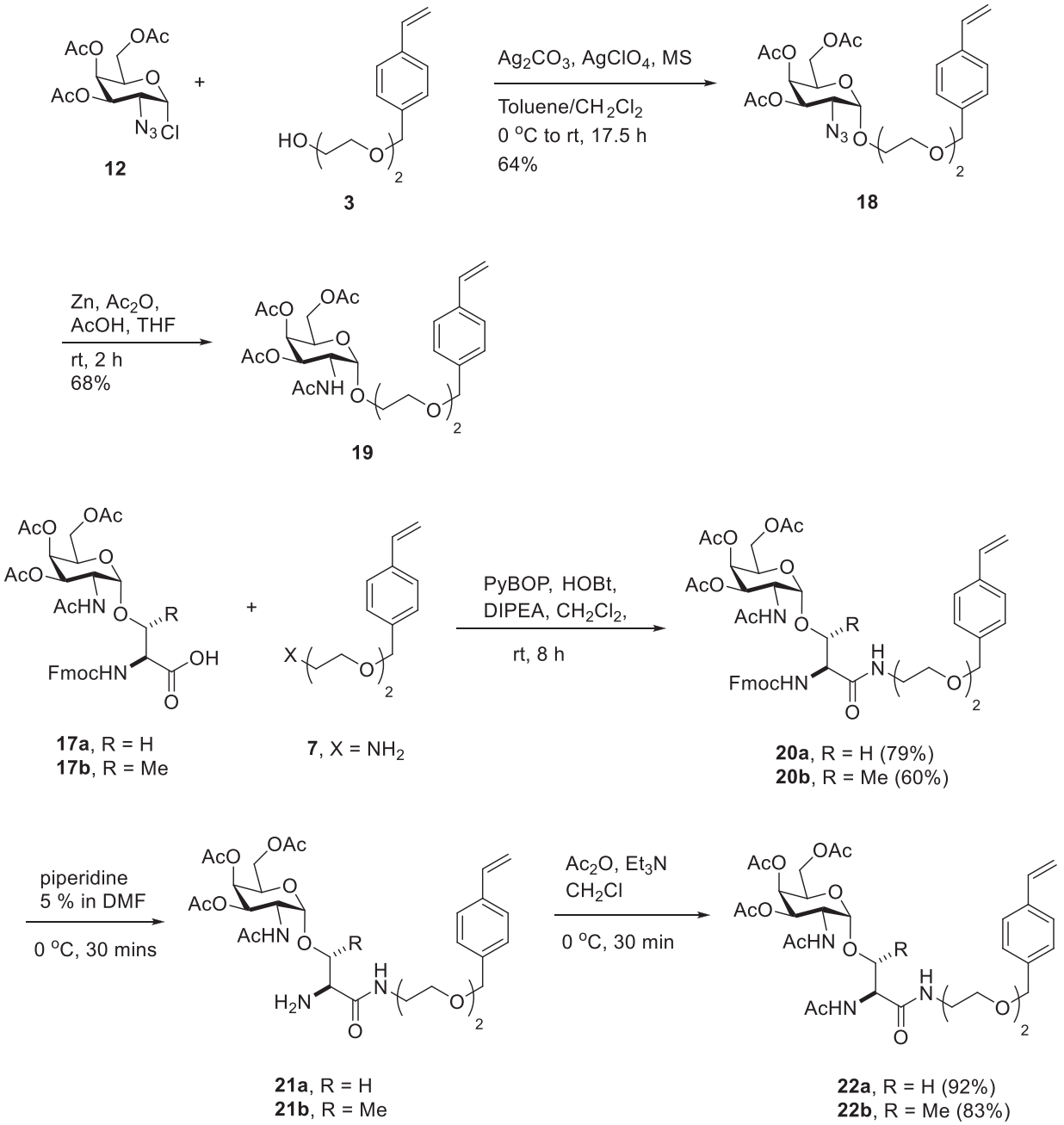

Scheme 7. Synthesis of Tn glycoconjugate monomers.

truncated glycoconjugate monomer **19** in the styrene polymerization reaction was increased, fluctuations in conversions between 45 and 70% were observed and narrow PDIs between 1.09 and 1.16 were detected. When the concentration of glycoconjugate monomer **19** was increased to 75%, a broader PDI of 1.40 was observed (entry 4). In general, the results showed that increasing the concentration of the Tn glycoconjugate monomer in the reactions resulted in a decrease in conversion, a decrease in molecular weight (Mn), and an increase in PDIs.

We then continued our studies on the polymerization of the Tn glycoconjugate monomers **22a** and **22 b** at 125 °C. As expected that when Tn glycoconjugate monomers **22a** and **22b** were attached on the styrene, the reactivity was decreased. The results could be referred to the steric effect. Bulky Tn molecule caused inefficient polymerization, compared to the monomer **19**. Longer reaction time was needed. To obtain reasonable conversions, the reaction time was increased from 24 h to 30 h. Because the synthesis of the monomers **22a** and **22b** were laborious and highly dense Tn glycoconjugate polymers are not suitable for synthetic mimics of potential anticancer vaccines [25]. Therefore, only a ratio of 10/90 in the polymerization reactions was studied. Similar results for the polymerization of the Tn glycoconjugate monomers **22a** and **22b** were obtained (entry 5, conversion = 89%; PDI = 1.08) and (entry 6, conversion = 79%; PDI = 1.27). At this stage, commercially available styryl-TEMPO could be used to regulate controlled radical polymerizations and Tn glycoconjugate polymers could be prepared with reasonable conversions (~80%) and narrow PDIs (~1.2).

We next turned our attention to achieve our synthetic goal, i.e.,

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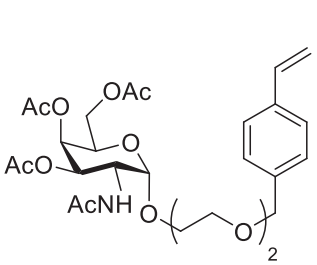
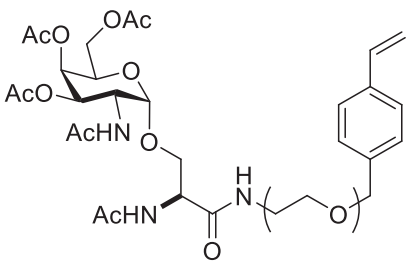
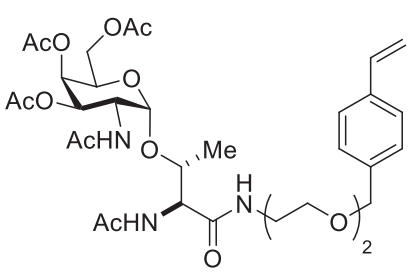
Table 1
Copolymerization of Tn Glycoconjugate Monomers and Styrene[a]



19 (GalNAc)



22a (GalNAc-Ser)



22b (GalNAc-Thr)

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Entry	Glycoconjugate monomer	Ratio of glycoconjugate monomer/styrene	Time (h)	Conversion (%)	M_n (g/mol)	PDI
1	**19**	10/90	24	70	7651	1.13
2	**19**	25/75	24	45	8031	1.09
3	**19**	50/50	24	57	5868	1.16
4	**19**	75/25	24	41	3280	1.40
5	**22a**	10/90	30	89	14163	1.08
6	**22b**	10/90	30	79	13171	1.28
7[b]	**22a**	10/90	30	95	19947	1.46
8[c]	**19**	10/90	30	90	35347	1.61
9	**19**+**22a**+**22b**	10/9/11/70	30	59	31806	1.09

[a] The ratio of styryl-TEMPO to the total monomers is 1/100, ratios of different monomers in polymerization are indicated in the table, and DMF was used as co-solvent (40 wt %).
[b] The polymerization was initiated by the polymeric alkoxyamine **a**, obtained from entry 6 under radical living conditions.
[c] The polymerization was initiated by the polymeric alkoxyamine **b**, obtained from entry 7 under radical living conditions.

to produce three different Tn glycoconjugate carbohydrate moieties attached on one single polymer chain to produce a mimic for tumor-associated carbohydrate anticancer vaccines bearing unimolecular trivalent Tn ligands. Two polymerization methods using these three Tn glycoconjugate monomers (**19**, **22a**, and **22b**) were examined, as shown in Scheme 8. One is a radical living polymerization using polymeric alkoxyamines (entry 6 → 7 → 8) and the other is a one-pot random polymerization reaction using three Tn glycoconjugate monomers (entry 9). In general, when % Tn glycomonomer was increased, both conversion and Mn decreased, it indicated that polymerization reaction rates were slowed down when Tn glycomonomer was added. It also indicated that polymerization rates of Tn glycoonomer is slow than simple styrene.

In subsequent radical living polymerization reactions, once the Tn glycoconjugate polymer was prepared, this polymer chain (a polymeric alkoxyamine) contained a TEMPO unit as its terminal, which could be used as a living radical site. When another potion of monomer was added, this polymer chain would be expected to grow again, as shown in Scheme 8. This is the advantage of nitroxide-mediated polymerization (NMP).

To achieve a living, controlled radical polymerization, the polymerizations of the Tn glycoconjugate monomer **22a** and styrene were initiated and regulated by the polymeric alkoxyamine **A** (obtained from entry 6) to delivery a second polymeric alkoxyamine **B** bearing an unimolecular divalent Tn ligand (entry 7). The polymer molecular weight was indeed increased (Mn = 13K → 20K) and the PDI became broader (1.28 → 1.46). This glycoconjugate polymeric alkoxyamine **B** could be further polymerized in the presence of the glycoconjugate monomer **19** and styrene to provide a novel polymeric alkoxyamine **C** bearing an unimolecular trivalent Tn ligand with a molecular weight of 35K and a PDI of 1.61 (entry 8). In one-pot random reaction, the polymerization of three Tn glycoconjugate monomers (**19**, **22a** and **22b**) and styrene in a ratio of (10/9/11/70) were initiated and regulated by styryl-TEMPO. The result was successful, with a conversion of 59%, Mn = 32K, and a PDI of 1.09 being obtained. This is the first report of the application of a TEMPO-mediated polymerization to produce novel Tn glycoconjugate polymers bearing unimolecular trivalent tumor-associated carbohydrate ligands with the desired molecular weights and defined PDIs. This synthetic method provides an alternative approach for the rapid synthesis of Tn glycoconjugate polymers with multivalent tumor-associated carbohydrate ligands in one single polymerization sequence. Furthermore, these Tn glycoconjugate polymers, carrying a TEMPO unit as its terminal, constitute a living radical site for further polymerization reactions. Block copolymerization in the presence of three Tn glycoconjugate monomers in a one-pot polymerization reaction could be used to produce a variety of novel Tn glycoconjugate polymers with the desired sugar compositions. Since multivalent tumor-associated carbohydrate anticancer vaccines were recently used in clinical trials for immunocancer therapy, these results provide an alternative and practical approach for the preparation of tumor-associated Tn glycoconjugate polymers with the desired molecular weights and controlled PDIs. This appears to be the first report of the use of nitroxide-mediated polymerization reactions to produce a polymeric mimic of tumor-associated Tn glycoconjugate glycopolymers. NMP provides a powerful method for preparing these novel tumor-associated Tn glycoconjugate polymers.

2.3. Characterization of Tn glycopolymers by NMR

^{1}H NMR measurements were also carried out to confirm that the Tn carbohydrate moieties were, in fact, attached to the polystyrene chain, as shown in Fig. 2. In spectrum 2a, the characteristic peaks of the Tn glycoconjugate monomer **22b** appear at around 5.3, 5.1, and 4.8 ppm, and are assigned to the H_4, H_3, and H_1 protons of the GalNAc carbohydrate moieties, respectively. The characteristic singlet peak around 4.5 ppm is assigned to the benzyl protons

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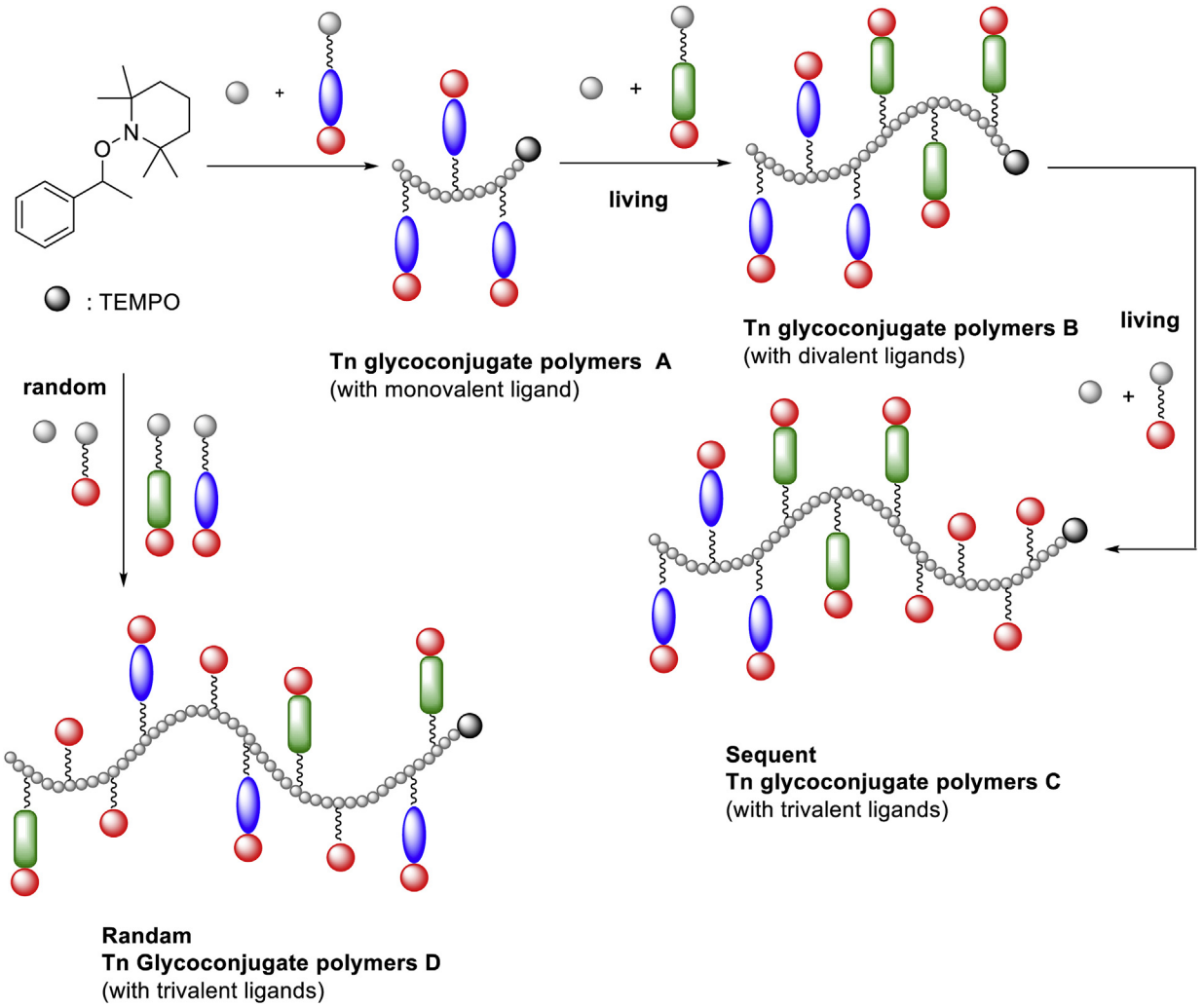

Scheme 8. Preparation of Novel Glycoconjugate Polymers with Trivalent Ligands Bearing a Tumor-Associated Carbohydrate Antigen (Tn).

(OCH_2Ar), *para*-to the styrene group. The multiplets around 3.7–3.4 ppm are assigned to the methylene protons of the spacer on the 2(2-aminoethoxy)ethanol group. Peaks corresponding to five acetyl groups (OAc) appear at around 2.0–2.2 ppm. The characteristic peaks of the Tn glycoconjugate **A** in spectrum 2b appear at around 5.3, 5.1, and 4.8 ppm, and are assigned to the H_4, H_3, and H_1 protons of the GalNAc carbohydrate moieties. The peaks at 4.5 ppm of benzyl protons (OCH_2Ar), the peaks at 3.7–3.4 ppm of the 2-aminoethoxy group, and the peaks at around 2.0 ppm of the acetyl groups are all present in the spectrum. These peaks serve to confirm polymers containing a Tn glycoconjugate were, in fact, prepared. The Tn glycoconjugate polymer **B** containing a divalent ligand and polymer **C** (trivalent ligands) are also shown in spectra 2c and 2d, respectively. As shown in Fig. 2e, random polymerization in the presence of three Tn glycoconjugate monomers gave the Tn glycoconjugate polymer **D** containing trivalent ligands. During the living polymerization sequence (**A** → **B** → **C**), characteristic peaks with a relatively low intensity corresponding to the Tn glycoconjugate, compared to the characteristic beaks around 7.3–6.3 ppm of polystyrene, were observed. This indicates that the polymerization efficiency indeed decreased as the polymers grew in size.

Regarding the numbers of Tn containing in polymer chains, we could also estimate by NMR integration. Integration of the carbohydrate specific peak (~5.35 ppm, 1H, H_4) and the aromatic protons (6.3–7.3 ppm, 5H, Ph), we could determine the Tn units per 100 units of styrene. (Please see the Supporting Information for NMR integration and detail calculation).

When the ratio of glycomonomer/styrene is 10/90 in polymerization reactions (entry 6, 7, and 8), it supposed to have 10 Tn units per 100 units of styrene. However, by NMR integration, there are about 3.37 units in Tn glycopolymer **A** (entry 6); 3.14 Tn units in Tn glycopolymer **B** (entry 7), 2.29 units in Tn glycopolymer **C** (entry 8). In general, it only has around 2–3 Tn units per 100 units of styrene in the real products (entry 6–8).

When the ratio of glycomonomer/styrene is 30/70 in polymerization reactions (entry 9), it supposed to have 30 Tn units per 100 units of styrene. However, there are around 22–23 Tn units per 100 units of styrene in Tn glycopolymer **D** (entry 9). Another example is, when the ratio of glycomonomer/styrene is 50/50 in polymerization reactions (entry 3), it supposed to have 50 Tn units per 100 units of styrene. However, by NMR integration, there are about 45 units per 100 units of styrene in glycopolymers (entry 3). The more concentrated Tn glycomonomers in polymerization reactions, the more accurate is the NMR integration used to determine Tn numbers in the real products.

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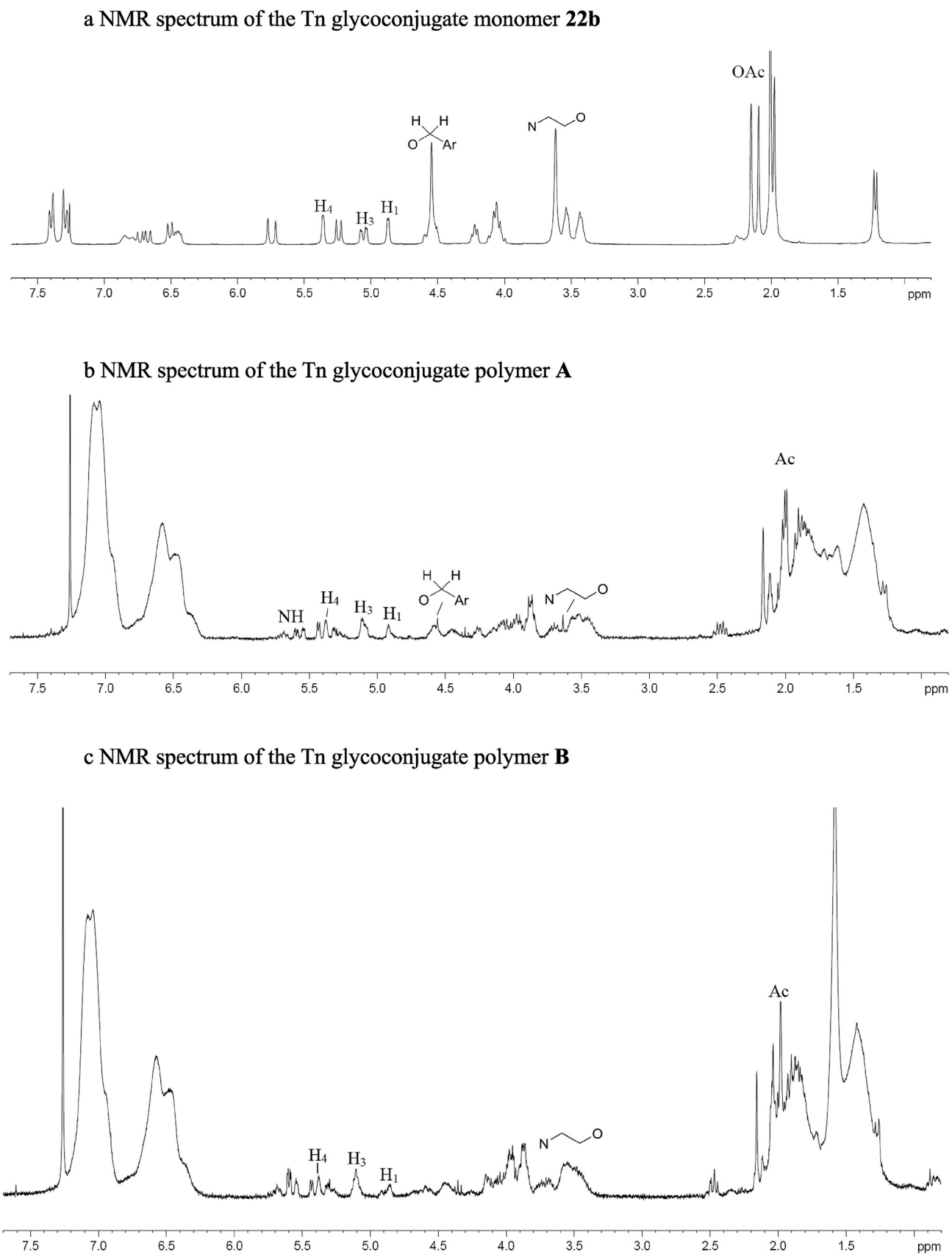

Fig. 2. **a** NMR spectrum of the Tn glycoconjugate monomer **22b**, **b** NMR spectrum of the Tn glycoconjugate polymer **A**, **c** NMR spectrum of the Tn glycoconjugate polymer **B**, **d** NMR spectrum of the Tn glycoconjugate polymer **C**, **e** NMR spectrum of the Tn glycoconjugate polymer **D**.

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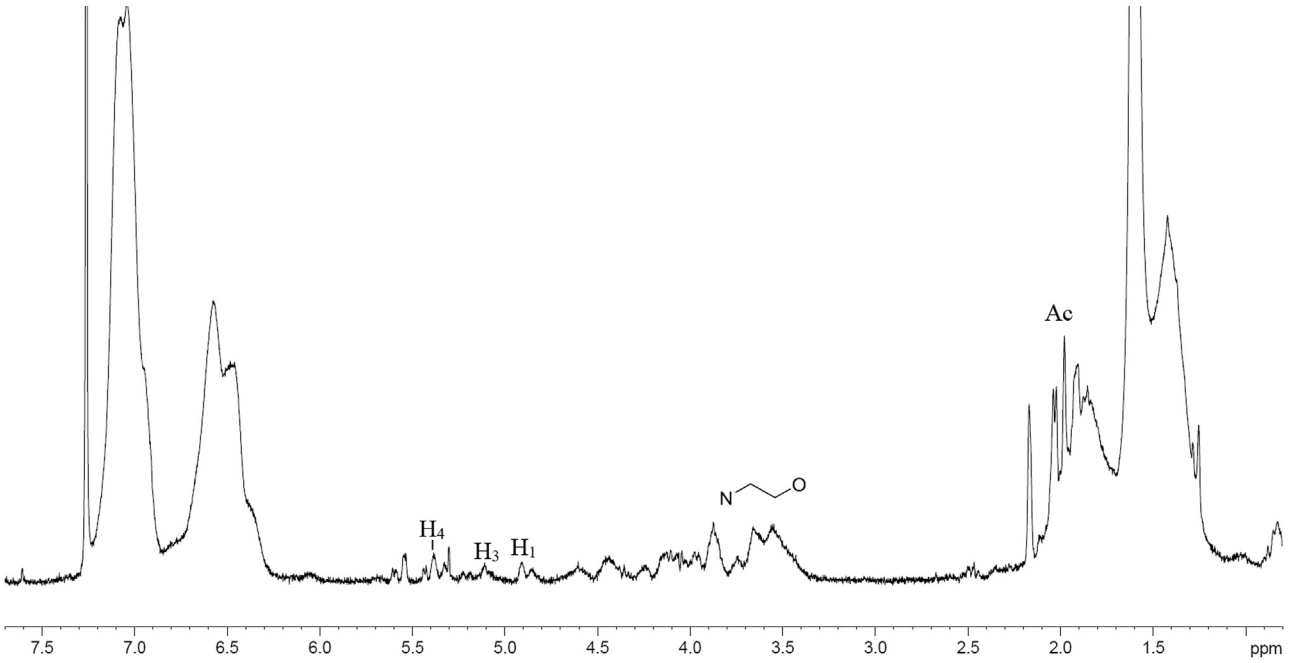
d NMR spectrum of the Tn glycoconjugate polymer **C**



e NMR spectrum of the Tn glycoconjugate polymer **D**

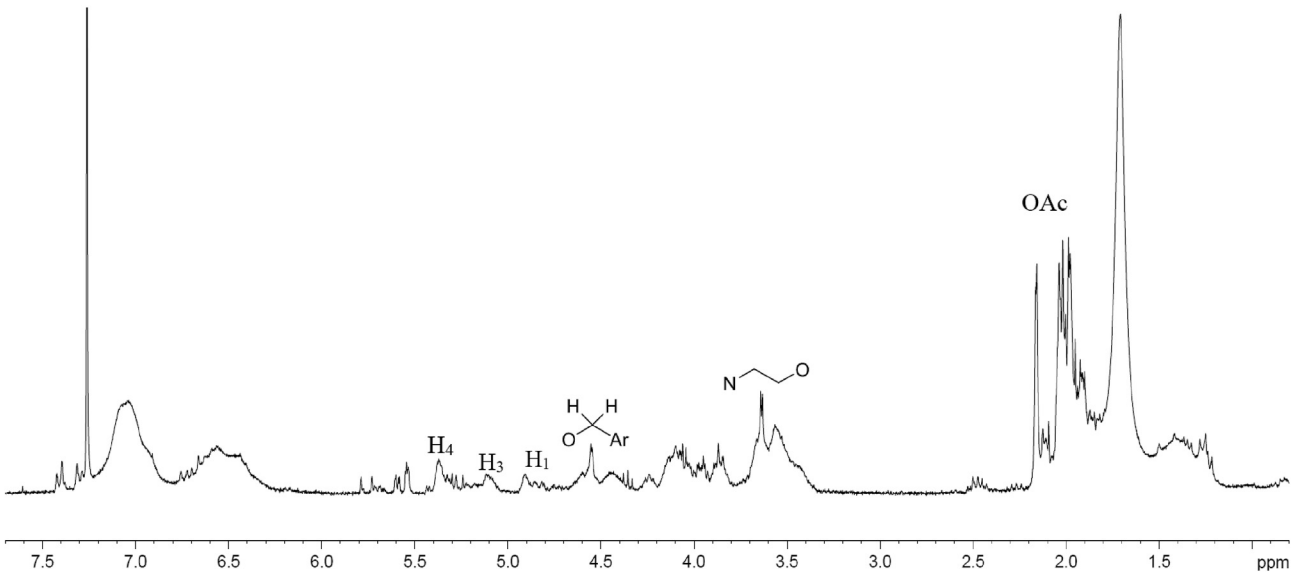


Fig. 2. (*continued*).

2.4. Characterization of Tn glycopolymers by IR

IR experiments were also performed to confirm that the polymers prepared from NMP indeed contain Tn glycoconjugate moieties. (Fig. 3) The characteristic bands [26] for the carbonyl absorption bands appear at 1744 cm^{-1} (ester) and 1680 cm^{-1} (amide), indicating that the Tn conjugate monomer **22b** contained OAc and NHAc functional groups (red line), respectively. Similarly, characteristic bands for the carbonyl absorption were also observed in the spectra of the Tn glycoconjugate polymer (entry 6/black line, entry 7/blue line, entry 8/brown line, entry 9/peach red line). However, in the case of the Tn glycoconjugate polymer, the characteristic bands of the polystyrene backbone and aromatic carbon-carbon double bonds appeared at 2928 cm^{-1} ($C_{(sp3)}$–H bond) and 1600 cm^{-1} (C═C double bond), respectively. These IR spectra further confirm that these polymers indeed contain Tn glycoconjugate units.

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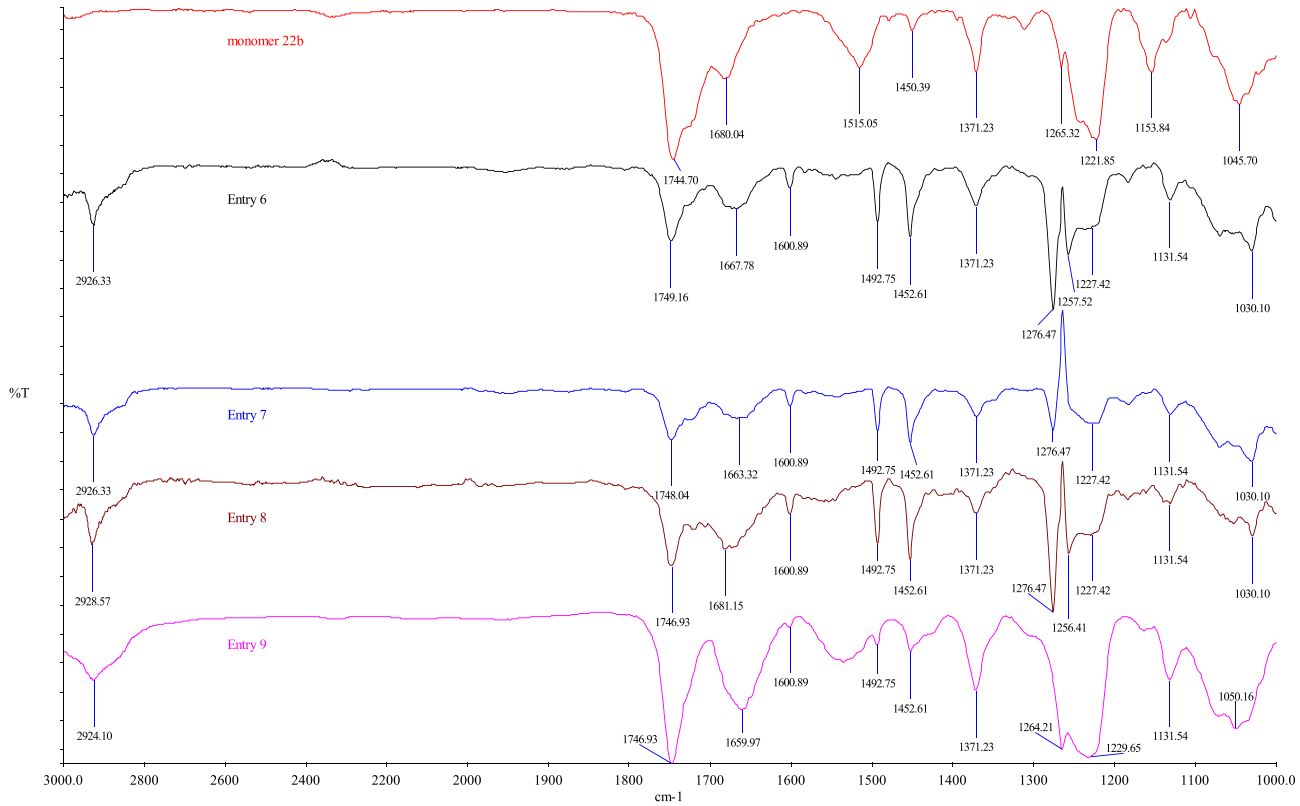

Fig. 3. IR spectrum of the Tn glycoconjugate monomer **22b** (red line), polymer **A** (entry 6/black line), polymer **B** (entry 7, blue line), polymer **C** (entry 8, brown line), and polymer **D** (entry 9, peach red line).

3. Conclusions

To simply examine the synthesis of Tn glycoconjugate polymers could be prepared by nitroxide-mediated polymerization, the first synthetic methods for preparing Tn glycoconjugate monomers containing different Tn glycopeptide antigens were successfully developed. Three tumor-associated Tn antigens (GalNAc, GalNAcα1-*O*-Ser, and GalNAcα1-*O*-Thr) were attached to a styrene-type monomer through a diethylene glycol spacer unit. The nitroxide-mediate polymerization of these three monomers resulted in the production of some defined Tn glycoconjugates polymers that could simply mimic the authentic structures of Tn glycoconjugate carbohydrate antigens. Controlled living radical polymerization afforded defined glycoconjugate polymers with different Tn densities and different compositions. The PDIs and molecular weights were increased and conversions were decreased with increasing the concentration of the Tn glycoconjugate monomers. The resulting Tn glycoconjugate polymers were characterized by NMR and IR. The spectra indicate that the Tn glycoconjugate moiety was attached to the polymer chain and that the density and composition of the Tn glycoconjugate could be adjusted though a controlled-living radical polymerization. The number of Tn units containing in the polymer chains could be estimated by NMR integration. This is the first report of the production of Tn glycoconjugate polymers by NMP, which provide an alternative method to afford Tn glycoconjugate polymers. These preliminary results provide an alternative synthetic approach for the rapid synthesis of multiple tumor-associated carbohydrates antigens in one single living polymerization sequence. To the best of our knowledge, this is the first report of the preparation of tumor-associated carbohydrate polymers bearing unimolecular trivalent Tn ligands with defined molecular weights and PDIs. The approach presented here provides a demonstration to show that nitroxide-mediated polymerization is a powerful method for preparing novel Tn glycoconjugate polymers with specific tumor-associated carbohydrate ligands. The preparation of carbohydrate polymers containing other tumor-associated carbohydrate antigens, such as TF and STn, could also be prepared using presented methods. However, there is an issue that the current Tn glycoconjugate polymers could not be used for cell-based assays or human clinical trials. Because these Tn glycoconjugate polymers contained polystyrene, which may become the major epitope to induce a strong immune response. Hence, to produce Tn glycoconjugate polymers containing water soluble monomers or biocompatible monomers may be another choice for the next generation of developing synthetic anticancer vaccines. To produce water soluble monomers or bio-compatible monomers should rely on using low temperature nitroxide-mediated polymerization techniques [27], which is currently underway. The whole synthetic approaches may provide an alternative method that can be applied to research, related to cancer immunotherapy.

4. Experimental section

4.1. General

^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz) spectra were recorded on a Bruker AVIII -300 MHz. The NMR spectra were recorded in $CDCl_3$ or CD_3OD. Chloroform ($\delta = 7.26$ ppm in ^{1}H NMR; $\delta = 77.0$ ppm in ^{13}C NMR) and methanol ($\delta = 3.31$ ppm in ^{1}H NMR; $\delta = 49.00$ ppm in ^{13}C NMR) were used as internal standard, respectively. Splitting patterns were reported as following: s: singlet, d: doublet, t: triplet, q: quartet, m: multiplet. Coupling constant (J) was reported in Hz. IR were recorded on a Perkin Elmer

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Spectrum 100 FT-IR spectrometer and reported in cm^{-1}. High resolution mass spectrometry (HRMS) were recorded on a Shimadzu LCMS-IT-TOF spectrometer (ESI-MS). Optical rotations were measured on a Horiba SEPA-300 Digital polarimeter. TLC (Merck Art. 60 F_{254}, 0.25 mm) precoated sheet was used. The reaction products were isolated by flash chromatography performed on Merck Art. Geduran Si 60 (0.040–0.063 mm) silica gel. Yields of products refer to chromatographically purified products unless otherwise stated. The benzene used for radical cyclizations was deoxygenated by passing a gentle stream of argon through for 30 min before use. All reactions were performed under a blanket of N_2 or Ar. The carbohydrate polymers were collected by centrifugal sedimentation on Eppendorf Centrifuge 5810 R as a rotation rate of 8000 rpm for 10 min and further dried in a vacuum-drying cabinet at 60 °C for 12 h. Size exclusion chromatography (SEC) was carried out with THF as eluent at a flow rate of 1.0 mL/min at room temperature on a system consisting of a PU-1580 isocratic pump (Jasco), a KF-804L column (Shodex), and a RI-71 refractometer detector (Shodex). Data were analyzed with Elite EC2000 software based upon calibration curves built upon polystyrene standards (Polymer Standards Service) with peak molecular weights ranging from 1800 to 56000 g/mol.

4.2. N-[2-(2-Hydroxyethoxy)ethyl]carbamic acid tert-butyl ester (**5**)

To a stirred solution of 2-(2-aminoethoxy)-ethanol (2 g, 19.02 mmol) in anhydrous CH_2Cl_2 (32 mL) was added di-*tert*-butyl dicarbonate (4.94 g mL, 22.63 mmol, 1.2 equiv) and trimethylamine (2.47 g, 24.39 mmol) at 0 °C. The reaction mixture was stirred for 3.5 h at room temperature. The solution was then diluted with CH_2Cl_2 (100 mL) and washed with brine (20 mL). The organic layer was dried over anhydrous sodium sulfate, and concentrated under reduced pressure to give the crude product **5** (>99%) as a pale yellow oil, which was directly used for the next step.

IR (neat) 3443, 3056, 2981, 2938, 2872, 2306, 1706 (C═O), 1507, 1456, 1423, 1393, 1368, 1265, 1171, 1125, 1065, 896, 863, 732, 730 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.03 (br s, 1H, NHBoc), 3.76–3.69 (m, 2H), 3.59–3.51 (m, 4H), 3.32 (q, J = 5.3 Hz, 2H), 2.46 (t, J = 5.4 Hz, 1H, OH), 1.43 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 156.1 (C), 79.4 (C), 72.2 (CH_2) 70.3 (CH_2), 61.7 (CH_2), 40.3 (CH_2), 28.4 ($CH_3 \times 3$).

4.3. tert-Butyl (2-(2-((4-vinylbenzyl)oxy)ethoxy)ethyl)carbamate (**6**)

To a solution of compound **5** (0.98 g, 4.76 mmol) in DMF (47.6 mL) was slowly added sodium hydride (0.23 g, 5.71 mmol) at 0 °C. The resulting white suspension was stirred magnetically at the same temperature for 30 min, followed by addition of 4-vinylbenzyl chloride (1.34 mL, 9.51 mmol). The reaction mixture was stirred at room temperature for overnight. The reaction was quenched by addition of 1 mL of MeOH, and then concentrated under reduced pressure to give a crude residue. The residue was diluted with CH_2Cl_2 (180 mL) and then washed with water (36 mL × 2) and brine (36 mL), dried over anhydrous sodium sulfate, filtered, and concentrated to give a crude product, which was purified by flash chromatography with the eluent of EtOAc/hexanes = 3/7 to give the desired product **6** (0.77 g, 51% over two steps) as a yellow oil.

IR (neat) 3442, 2980, 2868, 1708 (C═O), 1630, 1506, 1456, 1392, 1366, 1352, 1276, 1248, 1171, 1135, 1095, 1039, 1016, 992, 911, 845, 829, 779, 734 cm^{-1};^{1}H NMR (300 MHz, $CDCl_3$) δ 7.38 (d, J = 8.1 Hz, 2H, *para*), 7.27 (d, J = 8.1 Hz, 2H, *para*), 6.70 (dd, J = 17.6, 11.0 Hz, 1H, ArCH═CH_2), 5.73 (dd, J = 17.7, 0.6 Hz, 1H, ArCH═CH_2), 5.23 (dd, J = 11.1, 0.6 Hz, 1H, ArCH═CH_2), 5.02 (br s, 1H, NHBoc), 4.54 (s, 2H, $ArCH_2O$), 3.65–3.56 (m, 4H, $CH_2 \times 2$), 3.53 (t, J = 5.1 Hz, 2H, CH_2), 3.31 (q, J = 5.3 Hz, 2H, CH_2), 1.43 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 155.9 (C), 137.6 (C), 137.0 (C), 136.4 (CH), 127.9 ($CH \times 2$), 126.2 ($CH \times 2$), 113.7 (CH_2), 79.1 (C), 72.9 (CH_2), 70.3 (CH_2), 70.2 (CH_2), 69.2 (CH_2), 40.3 (CH_2), 28.3 ($CH_3 \times 3$); HRMS (ESI$^+$): m/z calcd for $C_{18}H_{28}NO_4$ $[M+H]^+$: 322.2013; found: 322.2008.

4.4. 2-[2-(4-Vinylbenzyloxy)ethoxy] ethanamine (**7**)

To a stirred solution of compound **6** (0.33 g, 1.03 mmol) in anhydrous CH_2Cl_2 (10.3 mL) was slowly added trifluoroacetic acid (0.95 mL) at 0 °C. The reaction mixture was stirred at room temperature for 1.5 h, then quenched by addition of 10 mL of 15% NaOH solution at 0 °C. The resulting mixture was diluted with CH_2Cl_2 (120 mL). The organic layer was washed, first with water (24 mL × 2) and brine (24 mL), dried over anhydrous sodium sulfate, and concentrated under reduced pressure to give the crude product **7** (0.22 g, 94%) as a pale yellow oil.

IR (neat) 3017, 2971, 2947, 2866, 1739, 1630, 1571, 1512, 1443, 1407, 1366, 1353, 1265, 1229, 1217, 1134, 1095, 1041, 1016, 992, 946, 913, 844, 829, 731, 702; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.38 (d, J = 8.1 Hz, 2H, *para*-), 7.30 (d, J = 7.8 Hz, 2H, *para*-), 6.70 (dd, J = 17.7, 10.8 Hz, 1H, ArCH═CH_2), 5.74 (d, J = 17.7 Hz, 1H, ArCH═CH_2), 5.23 (d, J = 10.8 Hz, 1H, ArCH═CH_2), 4.55 (s, 2H, $ArCH_2O$), 3.63 (s, 4H, $CH_2 \times 2$), 3.50 (t, J = 5.3 Hz, 2H, CH_2), 2.86 (t, J = 4.8 Hz, 2H, CH_2), 1.60 (br s, 2H, NH_2); ^{13}C NMR (75 MHz, $CDCl_3$) δ 137.7 (C), 136.9 (C), 136.5 (CH), 127.9 ($CH \times 2$), 126.2 ($CH \times 2$), 113.8 (CH_2), 73.4 (CH_2), 72.9 (CH_2), 70.3 (CH_2), 69.3 (CH_2), 41.7 (CH_2); HRMS (ESI$^+$): m/z calcd for $C_{13}H_{20}NO_2$ $[M+H]^+$: 222.1489; found: 222.1486.

4.5. 1,2,3,4,6-Penta-O-acetyl-D-galactopyranose (**9**)

To a solution of D-galactose (5.00 g, 27.75 mmol) and DMAP (0.34 g, 2.78 mmol) in dry pyridine (35 mL) was added dropwise acetic anhydride (26.24 mL, 277.50 mmol) at 0 °C. The reaction mixture was stirred at the room temperature for 3.5 h and then worked up by addition of cold water, then concentrated to dryness in vacuum. The residue was diluted with EtOAc (500 mL), and washed with 1 M HCl (100 mL × 2), saturated $NaHCO_{3(aq)}$ (100 mL × 3), water (100 mL), and brine (100 mL). The organic layer was dried over $MgSO_4$ and concentrated to give a crude product, which was purified by flash chromatography with the eluent of EtOAc/hexanes = 4/6 to give the desired product **9** (11 g, > 99%) as a white solid.

^{1}H NMR (300 MHz, $CDCl_3$) δ 6.35 (d, J = 1.2 Hz, 1H), 5.48 (d, J = 0.9 Hz, 1H), 5.31 (d, J = 1.2 Hz, 2H), 4.32 (t, J = 6.6 Hz, 1H), 4.01–4.14 (m, 2H), 2.14 (s, 6H), 2.02 (s, 3H), 2.00 (s, 3H), 1.98 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.3 (C), 170.1 (C × 2), 169.8 (C), 168.9 (C), 89.7 (CH), 68.7 (CH), 67.4 (CH), 67.3 (CH), 66.4 (CH), 61.2 (CH), 20.9 (CH_3), 20.6 ($CH_3 \times 3$), 20.5 (CH_3).

4.6. 2,3,4,6-Tetra-O-acetyl-α-D-galactopyranosyl bromide (**10**)

To a solution of compound **9** (8.14 g, 20.86 mmol) in dry CH_2Cl_2 (41.72 mL) was added HBr (33% in acetic acid, 24.4 mL). The reaction mixture was stirred at the room temperature for 5 h. The resulting mixture was diluted with CH_2Cl_2 (250 mL), washed with saturated $NaHCO_{3\ (aq)}$ (60 mL × 3), and brine (60 mL). The organic layer was dried over $MgSO_4$ and concentrated to give the crude product **3** (7.72 g, 90%) as a pale yellow oil, which was directly used for the next step.

^{1}H NMR (300 MHz, $CDCl_3$) δ 6.67 (d, J = 3.9 Hz, 1H, H_1), 5.49 (dd, J = 3.0, 0.6 Hz, 1H), 5.37 (dd, J = 10.5, 3.3 Hz, 1H), 5.02 (dd, J = 10.8, 4.1 Hz, 1H), 4.46 (t, J = 6.6 Hz, 1H, H_3), 4.16 (dd, J = 11.4, 6.0 Hz, 1H,

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H_6), 4.08 (dd, $J = 11.4$, 6.9 Hz, 1H, H_6'), 2.13 (s, 3H, Ac), 2.09 (s, 3H, Ac), 2.03 (s, 3H, Ac), 1.99 (s, 3H, Ac); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.2 (C), 170.0 (C), 169.8 (C), 169.7 (C), 88.1 (CH, anomeric carbon), 71.0 (CH), 68.0 (CH), 67.7 (CH), 66.7 (CH), 60.7 (CH_2), 2.7 (CH_3), 20.5 ($CH_3 \times 3$).

4.7. 3,4,6-Tri-O-acetyl-D-galactal (**11**)

To a solution of compound **10** (9.20 g, 22.37 mmol) in acetone (44.4 mL) was added saturated NaH_2PO_4 solution (89.5 mL) and zinc dust (18.29 g, 279.63 mmol). The reaction mixture was stirred at the room temperature for overnight. The mixture was then diluted with EtOAc and filtered through a pad of celite, then concentrated to dryness in vacuum. The residue was diluted with EtOAc (500 mL), washed with saturated $NaHCO_{3(aq)}$ (100 mL × 2), water (100 mL), and brine (100 mL). The organic layer was dried over $MgSO_4$ and concentrated to give a crude product, which was purified by flash chromatography with the eluent of EtOAc/hexanes = 3/7 to give the desired product **11** (5.40 g, 89%) as a colorless oil.

^{1}H NMR (300 MHz, $CDCl_3$) δ 6.46 (dd, $J = 6.3$, 1.5 Hz, 1H), 5.58–5.53 (m, 1H), 5.42 (dt, $J = 4.8$, 1.7 Hz, 1H), 4.73 (ddd, $J = 6.3$, 2.9, 1.7 Hz, 1H), 4.35–4.17 (m, 3H), 2.13 (s, 3H), 2.08 (s, 3H), 2.03 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.6 (C), 170.1 (C), 170.3 (C), 170.1 (C), 145.4 (CH), 98.8 (CH), 72.8 (CH), 63.8 (CH), 63.7 (CH), 61.9 (CH_2), 20.8 (CH_3), 20.7 (CH_3), 20.6 (CH_3).

4.8. (S)–N-(Fluoren-9-ylmethoxycarbonyl)-L-serine tert-butyl ester (**14a**)

To a solution of Fmoc-L-Ser(OH)–OH (1.5 g, 4.58 mmol) in dry EtOAc (34.0 mL) was added another solution of *tert*-butyl 2,2,2-trichloroacetimidate (1.475 mL, 6.412 mmol) in cyclohexane (4.5 mL). The reaction mixture was stirred at the 60 °C for 24.5 h. The reaction mixture was then concentrated to give a crude product, which was first purified by recrystallization with DCM and hexane. The collected solid was purified again by flash chromatography with the eluent EtOAc/hexanes = 4/6 to give the product **14a** (1.438 g, 82%) as a white solid.

IR (neat) 3408 (O–H), 1729 (C=O), 1264 (C–O), 1157, 1081, 833, 729, 703 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.77 (d, $J = 7.5$ Hz, 2H, Fmoc), 7.61 (d, $J = 7.2$ Hz, 2H, Fmoc), 7.40 (t, $J = 7.5$ Hz, 2H, Fmoc), 7.32 (t, $J = 7.2$ Hz, 2H), 5.72 (br d, $J = 5.7$ Hz, 1H, NH), 4.42 (d, $J = 6.9$ Hz, 2H, $CO_2C\underline{H}_2$), 4.33 (br s, 1H, α-H), 4.23 (t, $J = 6.9$ Hz, 1H), 3.93 (br d, $J = 1.2$ Hz, 2H, β-H), 1.90 (br s, 1H, OH), 1.49 (s, 9H, *t*-Bu); ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.4 (C), 156.3 (C), 143.8 (C), 143.7 (C), 141.3 (C), 141.3 (C), 127.7 (CH × 2), 127.1 (CH × 2), 127.0 (CH × 2), 125.0 (CH × 2), 120.0 (CH), 83.0 (C), 67.1 (CH_2), 63.7 (CH_2), 56.6 (CH), 47.1 ($CH_3 \times 3$).

4.9. (S)–N-(Fluoren-9-ylmethoxycarbonyl)-L-threonine tert-butyl ester (**14b**)

To a solution of Fmoc-L-Thr(OH)–OH (3.00 g, 8.63 mmol) in dry EtOAc (64 mL) was added another solution of *tert*-butyl 2,2,2-trichloroacetimidate (2.16 mL, 12.08 mmol) in cyclohexane (9.0 mL). The reaction was stirred at the room temperature. The reaction progress was monitored by TLC analysis. Another part of *tert*-butyl 2,2,2-trichloroacetimidate (2.00 mL, 11.16 mmol) was added every 24 h until the starting material was consumed completed at 94 h. The reaction mixture was concentrated to give a crude product, which was first purified by recrystallization with DCM and hexane. The collected solid was purified again by flash chromatography with the eluent EtOAc/hexanes = 35/65 to give the product compound **14b** (2.820 g, 82%) as a white solid.

IR (neat) 3368 (O–H), 2980, 1721 (C=O), 1515, 1450, 1370, 1310, 1223 (C–O), 1154, 1057, 839, 731 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.76 (d, $J = 7.2$ Hz, 2H, Fmoc), 7.62 (d, $J = 7.5$ Hz, 2H, Fmoc), 7.40 (t, $J = 7.5$ Hz, 2H, Fmoc), 7.31 (t, $J = 7.5$ Hz, 2H, Fmoc), 5.66 (br d, $J = 9.0$ Hz, 1H, N$\underline{H}$Fmoc), 4.41 (d, $J = 7.2$ Hz, 2H, Fmoc-C$\underline{H}_2$), 4.36–4.19 (m, overlapped with one t at 4.23, $J = 6.8$ Hz, included Fmoc-C$\underline{H}$, H_α, and H_β), 2.25 (br s, 1H, OH), 1.49 (s, 9H, *t*-Bu), 1.25 (d, $J = 6.3$ Hz, 3H, H_γ); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.3 (C), 156.8 (C), 143.9 (C), 143.8 (C), 141.3 (C × 2), 127.7 (CH × 2), 127.1 (CH × 2), 125.2 (CH), 120.0 (CH × 2), 82.7 (C), 68.3 (CH), 67.2 (CH_2), 59.9 (CH), 47.2 (CH × 2), 28.0 ($CH_3 \times 3$), 20.0(CH_3); HRMS: *m*/*z* calcd for [M+H]+: 398.1962; found: 398.1959.

4.10. O-(2-Acetamido-3,4,6-tri-O-acetyl-2-deoxy-α-D-galactopyranosyl)-N-(9-fuorenylmethoxycarbonyl)-L-serine tert-butyl ester (**16a**)

To a two-neck round-bottom flask containing iron trichloride (3.30 g, 20.00 mml) and sodium azide (0.86 g, 13.35 mmol) was added a solution of the galcal **11** (1.82 g, 6.67 mmol) in acetonitrile (56 mL) at −30 °C, followed by dropwise addition of 35% hydrogen peroxide (2.63 mL, 30.03 mmol) at the same temperature. The reaction mixture was stirred at the same temperature for 3.5 h. After TLC analysis indicated that the starting material was consumed completed, the reaction mixture was allowed to warm to room temperature. The mixture was concentrated to give a residue, and diluted with DCM (250 mL). The ether solution was washed with water (50 mL × 4), saturated $NaHCO_3$ (50 mL × 3), brine (50 mL × 2), dried over MgSO4, filtered, and concentrated to give a crude residue, which was purified by column chromatography to give the glycosyl chloride **12** (1.792 g, 77%), as a yellow oil.

To a three-neck round-bottom flask containing silver perchlorate (80.8 mg, 0.390 mmol) and 4 Å molecular sieves (1.71 g) was dried by heating the flask to 100–110 °C under reduced pressure for overnight. This flask was then cooled down to the room temperature, and was added solid silver carbonate (0.726 g, 2.660 mmol). The whole system was added a solution of compound **14a** (0.487 g, 1.270 mmol) in CH_2Cl_2/toluene (3.9 mL/3.9 mL) at 0 °C and stirred for 30 min. Followed by addition of another solution of previous glycosyl chloride **12** (1.772 g, 5.067 mmol) in CH_2Cl_2/toluene (3.9 mL/3.9 mL) at 0 °C. The reaction mixture was stirred at room temperature and protected from light for 15.8 h. After TLC analysis indicated that the reaction progress was completed. The reaction mixture was then filtered through a pad of celite, and washed with CH_2Cl_2. The filtrate was concentrated and then diluted again with CH_2Cl_2 (200 mL). The solution was washed with water (50 mL × 2), saturated $NaHCO_{3(aq)}$ (65 mL × 2), and brine (65 mL). The organic layer was dried over $MgSO_4$ and concentrated to give a crude product, which was preliminarily purified by flash chromatography with the eluent of EtOAc/hexanes = 3/7 to 4/6 to give the cure product (0.84 g, crude yield, 95%) as a colorless oil.

To a solution of this cure product (0.840 g, 1.206 mmol) in THF (24.1 mL) was added zinc dust (1.621 g, 24.119 mmol), acetic anhydride (1.14 mL, 12.06 mmol), and acetic acid (0.69 mL, 12.06 mmol). The reaction was stirred at room temperature for 7.1 h. After TLC analysis indicated that the starting material was consumed completely. The reaction mixture was then filtrated through a pad of celite, and washed with EtOAc. The filtrate was concentrated and then diluted again with EtOAc (180 mL). The solution was washed with saturated $NaHCO_{3(aq)}$ (45 mL × 2), and brine (45 mL). The organic layer was dried over $MgSO_4$ and concentrated to give a crude product, which was purified by flash chromatography with the eluent of EtOAc/hexanes = 7/3 to 8/2 to give the product **16a** (0.860 g, 74%) as a colorless oil.

IR (neat) 3341, 2936, 1744 (C=O), 1673 (C=O), 1523, 1450, 1369,

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1223 (C–O), 1154, 1133, 1048, 834 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.77 (d, $J = 7.5$ Hz, 2H), 7.62 (d, $J = 7.2$ Hz, 2H), 7.41 (t, $J = 7.2$ Hz, 2H), 7.33 (t, $J = 7.5$ Hz, 2H), 5.82 (br d, $J = 8.4$ Hz, 2H, NHAc, NHFmoc), 5.37 (d, $J = 3.0$ Hz, 1H, H_4), 5.12 (dd, $J = 11.1$, 3.3 Hz, 1H, H_3), 4.87–4.80 (m, 1H, H_1), 4.64–4.54 (td, $J = 10.5$, 3.6 Hz, 1H, H_2), 4.43 (d, $J = 7.2$ Hz, 2H, Fmoc-CH_2), 4.25 (t, $J = 6.9$ Hz, 1H, Fmoc-CH), 4.15–4.01 (m, 3H), 4.01–3.81 (m, 2H, $H_6 \times 2$), 2.16 (s, 3H, NHAc), 2.00 (s, 3H, OAc), 1.99 (s, 3H, OAc), 1.93 (s, 3H, OAc), 1.49 (s, 9H, *t*-Bu); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.9 (C), 170.4 (C), 170.3 (C), 170.1 (C), 169.0 (C), 155.8 (C), 143.7 (C × 2), 141.3 (C × 2), 127.8 (CH × 2), 127.1 (CH × 2), 125.0 (CH × 2), 120.0 (CH × 2), 98.8 (CH, anomeric carbon), 83.1 (C), 69.2 (CH_2), 68.4 (CH), 67.2 (CH × 2), 61.9 (CH_2 × 2), 54.8 (CH), 47.5 (CH), 47.1 (CH), 28.0 (CH_3 × 3, *t*-Bu), 23.2 (CH_3), 20.7 (CH_3), 20.7 (CH_3), 20.6 (CH_3).

4.11. O-(2-Acetamido-3,4,6-tri-O-acetyl-2-deoxy-α-D-galactopyranosyl)-N-(9-fuorenylmethoxycarbonyl)-L-threonine tert-butyl ester (**16b**)

To a two-neck round-bottom flask containing iron trichloride (5.38 g, 33.15 mml) and sodium azide (1.44 g, 22.16 mmol) was added a solution of the galcal **11** (3.02 g, 11.05 mmol) in acetonitrile (92 mL) at −30 °C, followed by dropwise addition of 35% hydrogen peroxide (3.88 mL, 44.21 mmol). The reaction mixture was stirred at the same temperature for 4 h. After TLC analysis indicated that the starting material was consumed completed, the reaction mixture was allowed to warm to room temperature. The mixture was concentrated to give a residue, and diluted with ether (300 mL). The ether solution was washed with water (80 mL × 4), saturated $NaHCO_3$ (80 mL × 2), brine (80 mL × 2), dried over $MgSO_4$, filtered, and concentrated to give a crude residue, which was purified by column chromatography to give the glycosyl chloride **12** (1.749 g, 45%), as a yellow oil.

To a three-neck round-bottom flask containing silver perchlorate (0.125 mg, 0.600 mmol) and 4 Å molecular sieves (0.80 g) was dried by heating the flask to 100–110 °C under reduced pressure for overnight. The flask was then cooled down to the room temperature, and was added solid silver carbonate (1.059 g, 4.083 mmol). The whole system was added a solution of compound **14b** (0.955 g, 2.402 mmol) in CH_2Cl_2/toluene (3.0 mL/3.0 mL) at 0 °C and stirred for 30 min. Followed by addition of another solution of compound **12** (1.750 g, 3.003 mmol) in CH_2Cl_2/toluene (3.0 mL/3.0 mL) at 0 °C. The reaction mixture was stirred at room temperature and protected from light for 48 h. After TLC analysis indicated that the reaction progress was completed. The reaction mixture was then filtered through a pad of celite, and washed with CH_2Cl_2. The filtrate was concentrated and then diluted again with CH_2Cl_2 (300 mL). The solution was washed with water (60 mL × 1), saturated $NaHCO_{3(aq)}$ (60 mL × 3), and brine (60 mL). The organic layer was dried over $MgSO_4$ and concentrated to give a crude product, which was preliminarily purified by flash chromatography with the eluent of EtOAc/hexanes = 4/6 to give the crude product (1.237 g, crude yield, 74%), as a colorless oil.

To a solution of this cure product (1.237 g, 1.740 mmol) in THF (34.8 mL) was added zinc dust (2.29 g, 34.80 mmol), acetic anhydride (1.64 mL, 17.40 mmol), and acetic acid (1.00 mL, 17.40 mmol). The reaction was stirred at room temperature for 2.4 h. After TLC analysis indicated that the starting material was consumed completely. The reaction mixture was then filtrated through a pad of celite, and washed with EtOAc. The filtrate was concentrated and then diluted again with EtOAc (300 mL). The solution was washed with saturated $NaHCO_{3(aq)}$ (80 mL × 2), and brine (80 mL). The organic layer was dried with $MgSO_4$ and concentrated to give a crude product, which was purified by flash chromatography with the eluent of EtOAc/hexanes = 7/3 to give the product **16b** (0.794 g, 63%), as a colorless oil.

IR (neat) 3413, 3038, 2973, 2936, 1745 (C=O), 1682 (C=O), 1514, 1450, 1370, 1311, 1266, 1222 (C–O), 1154, 1046, 909, 729 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.78 (d, $J = 7.5$ Hz, 2H), 7.64 (d, $J = 7.5$ Hz, 2H), 7.41 (t, $J = 7.2$ Hz, 2H), 7.33 (t, $J = 7.4$ Hz, 2H), 6.04 (d, $J = 9.9$ Hz, 1H, NHAc), 5.65 (d, $J = 9.6$ Hz, 1H, NHFmoc), 5.38 (d, $J = 2.1$ Hz, 1H, H_4), 5.09 (dd, $J = 11.4$, 3.0 Hz, 1H, H_3), 4.88 (d, $J = 3.3$ Hz, 1H, H_1), 4.61 (td, $J = 10.7$, 3.1 Hz, 1H, H_2), 4.54–4.37 (m, 2H, Fmoc-CH_2), 4.32–4.13 (m, 4H, included H_5, H_α, H_β, and Fmoc-CH), 4.13–4.00 (m, 2H, H_6), 2.17 (s, 3H, NHAc), 2.04 (s, 3H, OAc), 2.00 (s, 3H, OAc), 1.99 (s, 3H, OAc), 1.45 (s, 9H, *t*-Bu), 1.32 (d, $J = 6.3$ Hz, 3H, $H_\gamma \times 3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.9 (C), 170.3 (C × 4), 170.0 (C), 156.4 (C), 143.7 (C), 141.3 (C × 2), 127.7 (CH × 2), 127.1 (CH × 2), 125.1 (CH), 125.0 (CH), 120.0 (CH × 2), 99.9 (CH, anomeric carbon), 83.2 (C), 77.0 (CH), 68.7 (CH), 67.3 (CH, CH_2), 62.1 (CH_2), 58.9 (CH), 47.3 (CH), 47.1 (CH × 2), 28.1 (CH_3 × 3, *t*-Bu), 23.2 (CH_3), 20.7 (CH_3 × 2), 20.6 (CH_3), 18.5 (CH_3); HRMS (ESI^+): *m/z* calcd for $C_{37}H_{47}O_{13}N_2$ [M+H]: 727.3073; found: 727.3057.

4.12. 2-[2-(4-Vinylbenzyloxy)ethoxy]ethyl-O-2-azido-2-deoxy-3,4,6-triacetate-α-D-galactopyranoside (**18**)

To a two-neck round-bottom flask containing iron trichloride (1.50 g, 6.17 mml) and sodium azide (0.80 g, 12.34 mmol) was added a solution of the galcal **11** (1.68 g, 6.17 mmol) in acetonitrile (21 mL) at −30 °C, followed by dropwise addition of 35% hydrogen peroxide (5.76 mL, 61.70 mmol). The reaction mixture was stirred at the same temperature for 12 h. After TLC analysis indicated that the starting material was consumed completed, the reaction mixture was allowed to warm to room temperature. The mixture was concentrated to give a residue, and diluted with ether (210 mL). The ether solution was washed with water (40 mL × 3), saturated $NaHCO_3$ (40 mL), brine (40 mL), dried over $MgSO_4$, filtered, and concentrated to give a crude residue, which was purified by column chromatography to give the glycosyl chloride **12** (1.51 g, 70%), as a yellow oil.

To a three-neck round-bottom flask containing silver perchlorate (0.20 g, 0.95 mmol) and 4 Å molecular sieves (2.00 g) was dried by heating the flask to 100–110 °C under reduced pressure for overnight. The flask was then cooled down to the room temperature, and was added solid silver carbonate (1.04 g, 3.80 mmol). The whole system was added a solution of compound **12** (0.42 g, 1.90 mmol) in toluene (7.0 mL) at 0 °C and stirred for 30 min. Followed by addition of another solution of compound **3** (1.26 g, 5.69 mmol) in CH_2Cl_2 (6.5 mL) at 0 °C. The reaction mixture was stirred at room temperature and protected from light for 21 h. After TLC analysis indicated that the reaction progress was completed. The reaction mixture was then filtered through a pad of celite, and washed with CH_2Cl_2. The filtrate was concentrated and then diluted again with CH_2Cl_2 (240 mL). The solution was washed with water (45 mL), saturated $NaHCO_{3(aq)}$ (45 mL × 3), and brine (50 mL). The organic layer was dried over $MgSO_4$ and concentrated to give a crude product, which was preliminarily purified by flash chromatography with the eluent of EtOAc/hexanes = 3/7 to 45/55 to give the product **18** (0.64 g, 64%), as a colorless oil.

IR (neat) 3409, 3056, 2983, 2924, 1747 (C=O), 1644, 1512, 1424, 1371, 1321, 1226 (C–O), 1130, 1067, 1043, 1017, 971, 943, 915, 846, 822, 732, 702, 624, 612, 603, 591, 575, 551 cm^{-1}; HRMS (ESI^+): *m/z* calcd for $C_{25}H_{33}O_{10}N_3$ $[M+Na]^+$: 558.2058; found: 558.2055.

α-form: $[\alpha]^{[17]}_D$ + 90.45 (c = 0.37, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ 7.39 (d, $J = 8.1$ Hz, 2H), 7.30 (d, $J = 8.1$ Hz, 2H), 6.71 (dd, $J = 17.4$, 10.8 Hz, 1H, ArCH=CH_2), 5.74 (dd, $J = 17.7$, 0.6 Hz, 1H, ArCH=CH_2, cis to Ph), 5.45–5.35 (m, 2H, H_4, H_3), 5.23 (dd, $J = 10.8$, 0.6 Hz, 1H, ArCH=CH_2, trans to Ph), 5.08 (d, $J = 3.3$ Hz, 1H, H_1), 4.55 (s, 2H, ArCH_2O), 4.30 (t, $J = 6.9$ Hz, 1H, H_5), 4.09 (dd, $J = 11.4$, 6.5 Hz,

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1H, H_{6a} or H_{6b}), 4.05 (dd, $J = 11.4$, 6.9 Hz, 1H, H_{6a} or H_{6b}), 3.90–3.58 (m, 9H, $(OCH_2CH_2)_2O$, and H_2), 2.14 (s, 3H), 2.05 (s, 3H), 2.02 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.4 (C), 170.0 (C), 169.8 (C), 137.8 (C), 136.9 (C), 136.5 (CH), 127.9 (CH × 2), 126.2 (CH × 2), 113.7 (CH_2), 98.2 (CH), 72.9 (CH_2), 70.7 (CH_2), 70.1 (CH_2), 69.4 (CH_2), 68.1 (CH), 67.7 (CH_2), 67.6 (CH), 66.5 (CH), 61.6 (CH_2), 57.4 (CH), 20.6 (CH_3 × 3).

β-form was reported: $[\alpha]^{[26]}_D$ − 12.68 (c = 0.32, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ 7.39 (d, $J = 8.1$ Hz, 2H), 7.30 (d, $J = 8.1$ Hz, 2H), 6.71 (dd, $J = 17.7$, 10.8 Hz, 1H, ArCH=CH_2), 5.74 (d, $J = 17.7$ Hz, 1H, ArCH=CH_2, cis to Ph), 5.29 (dd, $J = 3.3$, 0.9 Hz, 1H, H_4), 5.23 (d, $J = 11.1$, 0.9 Hz, 1H, ArCH=CH_2, trans to Ph), 4.74 (dd, $J = 10.8$, 3.3 Hz, 1H, H_3), 4.55 (s, 2H, $ArCH_2O$), 4.48 (d, $J = 8.1$ Hz, 1H, H_1), 4.15–4.00 (m, 2H, H_{6a}, H_{6b}), 3.87–3.51 (m, 10H, $(OCH_2CH_2)_2O$, H_2, and H_5), 2.13 (s, 3H), 2.05 (s, 3H), 2.04 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.4 (C), 170.0 (C), 169.8 (C), 137.8 (C), 137.0 (C), 136.5 (CH), 128.0 (CH × 2), 126.2 (CH × 2), 113.8 (CH_2), 102.5 (CH), 73.0 (CH_2), 71.0 (CH), 70.7 (CH_2), 70.6 (CH), 70.4 (CH_2), 69.4 (CH_2 × 2), 66.4 (CH), 61.3 (CH_2), 60.7 (CH), 20.7 (CH × 2), 20.6 (CH_3).

4.13. 2-[2-(4-Vinylbenzyloxy)ethoxy]ethyl-O-2-acetamido-2-deoxy-3,4,6-triacetate-α-D-galactopyranoside (**19**)

To a solution of compound **18** (0.24 g, 0.45 mmol) in THF (9.00 mL) was added zinc dust (0.59 g, 9.00 mmol), acetic anhydride (0.43 mL, 4.50 mmol), and acetic acid (0.26 mL, 4.50 mmol). The reaction was stirred at room temperature for 3 h. After TLC analysis indicated that the starting material was consumed completely. The reaction mixture was then filtrated through a pad of celite, and washed with EtOAc. The filtrate was concentrated and then diluted again with EtOAc (100 mL). The solution was washed with saturated $NaHCO_{3(aq)}$ (20 mL × 2), and brine (20 mL). The organic layer was dried with $MgSO_4$ and concentrated to give a crude product, which was purified by flash chromatography with the eluent of EtOAc to give the product **19** (0.17 g, 68%), as a colorless oil.

Due to difficulty of products separation, only major product (α-form) was reported: $[\alpha]^{[26]}_D$ + 2.11 (c = 2.97, $CHCl_3$); IR (neat) 3762, 3594, 2983, 3436, 3302, 3056, 2871, 1745 (C=O), 1667 (C=O) amide, 1513, 1429, 1408, 1371, 1221 (C–O), 1161, 1130, 1045, 1018, 993, 949, 918, 845, 828, 732, 702, 622, 590, 557 cm^{-1}; found: 553.2436; ^1H NMR (300 MHz, $CDCl_3$) δ 7.38 (d, $J = 8.1$ Hz, 2H), 7.28 (d, $J = 8.1$ Hz, 2H), 6.70 (dd, $J = 17.4$, 10.8 Hz, 1H, ArCH=CH_2), 5,87 (br d, $J = 9.6$ Hz,1H, NHAc), 5.74 (d, $J = 17.4$ Hz, 1H, ArCH=CH_2, cis to Ph), 5.35 (d, $J = 3.0$ Hz 1H, H_4), 5.23 (dd, $J = 10.7$, 0.3 Hz, 1H, ArCH=CH_2, trans to Ph), 5.17 (dd, $J = 11.4$, 3.3 Hz, 1H, H_3), 4.89 (d, $J = 3.6$ Hz, 1H, H_1), 4.63–4.54 (m, 1H, H_2) 4.53 (s, 2H, $ArCH_2O$), 4.23 (t, $J = 6.3$ Hz, 1H, H_5), 4.14–4.01 (m, 2H, H_{6a}, H_{6b}), 3.89–3.58 (m, 8H, $(OCH_2CH_2)_2O$), 2.15 (s, 3H, $NHCOCH_3$), 2.02 (s, 3H), 1.98 (s, 3H), 1.90 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.9 (C), 170.4 (C), 170.3 (C), 170.1 (C), 137.5 (C), 137.1 (C), 136.4 (CH), 127.9 (CH × 2), 126.2 (CH × 2), 113.9 (CH_2), 98.0 (CH), 72.9 (CH_2), 70.75 (CH_2), 69.8 (CH_2), 69.2 (CH_2), 68.5 (CH), 67.6 (CH_2), 67.3 (CH), 66.7 (CH), 61.9 (CH_2), 47.6 (CH), 23.2 (CH_3), 20.7 (CH_3 × 3); HRMS (ESI^+): *m/z* calcd for $C_{27}H_{38}O_{11}N$ $[M+Na]^+$: 552.2439.

4.14. N-(9H-Fluoren-9-yl)-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-α-D-galactopyranosyl)-L-serine 2-[2-(4-vinylbenzyloxy) ethoxy]ethyl ester (**20a**)

To a stirred solution of compound **16a** (0.480 g, 0.674 mmol) in anhydrous CH_2Cl_2 (6.74 mL) was slowly added trifluoroacetic acid (4.84 mL) at 0 °C. The reaction mixture was stirred at room temperature for 5.0 h. The reaction solution was then concentrated under reduce pressure. To this flash containing free acid **17a** was co-evaporated with benzene for three times to efficiently remove moisture, as well as the compound **7**.

Afterward, to a solution of PyBOP (0.526 g, 1.011 mmol), HOBt (0.137 g, 1.011 mmol) in CH_2Cl_2 (1.4 mL), was added a solution of previous free acid **17a** in CH_2Cl_2 (4.0 mL), another solution of compound **7** (0.217 g, 0.981 mmol) in CH_2Cl_2 (4.0 mL), and then *N*, *N*-diisopropylethylamine (0.233 mL, 1.348 mmol) at the room temperature. The reaction mixture was stirred for 15 h, and diluted the result with CH_2Cl_2 (150 mL). The solution was washed with water (30 mL), saturated $NaHCO_{3(aq)}$ (40 mL × 2), and brine (40 mL), dried with $MgSO_4$, and concentrated to give a crude product, which was purified by flash chromatography with the eluent of $MeOH/CH_2Cl_2 = 1/16$ to give the desired product compound **20a** (0.456 g, 79%) as a colorless oil.

IR (neat) 1746 (C=O), 1723 (C=O), 1681 (C=O), 1514, 1450, 1370, 1266, 1245, 1225, 1154, 1048, 730 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.76 (d, $J = 7.2$ Hz, 2H), 7.59 (d, $J = 7.2$ Hz, 2H), 7.45–7.21 (m, 8H, included H_f), 6.76–6.61 (m, 2H, H_g and NH–speacer), 6.07 (d, $J = 9.6$ Hz, 1H, NHAc), 5.80 (d, $J = 7.5$ Hz, 1H, NHFmoc), 5.73 (d, $J = 17.4$ Hz, 1H, H_h), 5.36 (d, $J = 3.0$ Hz, 1H, H_4), 5.23 (d, $J = 11.3$ Hz, 1H, H_h), 5.07 (dd, $J = 11.3$, 2.9 Hz, 1H, H_3), 4.81 (d, $J = 3.3$, 1H, H_1), 4.63–4.47 (m, overlapped with 1 s at 4.50, 3H, H_2 and H_e), 4.44 (d, $J = 6.6$, 2H, Fmoc-CH_2), 4.32 (br s, 1H, H_α), 4.22 (t, $J = 6.9$ Hz, 1H, Fmoc-CH), 4.15–3.97 (m, 3H, H_6 and H_5), 3.92–3.69 (m, 2H, H_β), 3.67–3.37 (m, 8H, H_{a-d}), 2.16 (s, 3H, NHAc), 1.99 (s, 3H, OAc), 1.98 (s, 3H, OAc), 1.95 (s, 3H, OAc); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.8 (C), 170.4 (C), 170.3 (C × 2), 169.1 (C), 155.9 (C), 143.6 (C × 2), 141.3 (C × 2), 137.2 (C), 136.3 (CH), 128.0 (CH × 2), 127.8 (CH × 2), 127.1 (CH × 2), 126.3 (CH × 2), 125.0 (CH), 120.0 (CH × 2), 114.1 (CH_2), 99.1 (CH), 72.9 (CH_2), 70.1 (CH_2), 69.5 (CH_2), 69.2 (CH_2 × 2), 68.5 (CH), 67.2 (CH_2), 67.2 (CH × 2), 61.8 (CH_2), 54.5 (CH), 47.4 (CH), 47.1 (CH), 39.4 (CH_2), 23.1 (CH_3), 20.7 (CH_3 × 2), 20.6 (CH_3); HRMS (ESI^+): *m/z* calcd for $C_{45}H_{54}N_3O_{14}$ $[M+H]^+$:860.3600; found: 860.3591.

4.15. N-(9H-Fluoren-9-yl)-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-α-D-galactopyranosyl)-L-threonine 2-[2-(4-vinylbenzyloxy) ethoxy]ethyl ester (**20b**)

To a stirred solution of compound **16b** (0.120 g, 0.166 mmol) in anisole (0.436 mL) was slowly added trifluoroacetic acid (4.36 mL) at 0 °C. The reaction mixture was stirred for 1.2 h at room temperature. The reaction solution was then concentrated under reduce pressure. To this flash containing free acid **17b** was co-evaporated with toluene/benzene for three times to efficiently remove moisture, as well as the compound **7**.

Afterward, to a solution of PyBOP (0.103 g, 0.199 mmol), HOBt (0.027 g, 0.199 mmol) in CH_2Cl_2 (0.26 mL), was added a solution of previous free acid **17b** in CH_2Cl_2 (0.7 mL), another solution of compound **7** (0.609 g, 0.275 mmol) in CH_2Cl_2 (0.7 mL), and then *N*,*N*-Diisopropylethylamine (0.060 mL, 0.347 mmol) at the room temperature. The reaction mixture was stirred for 14.8 h, and diluted the result with CH_2Cl_2 (60 mL). The solution was washed with water (30 mL), saturated $NaHCO_{3(aq)}$ (30 mL × 2), and brine (30 mL), dried with $MgSO_4$, and concentrated to give a crude product, which was purified by flash chromatography with the eluent of EtOAc to give the desired product compound **20b** (0.107 g, 60%) as a colorless oil.

IR (neat) 3340, 2980, 1740 (C=O), 1721 (C=O), 1683 (C=O), 1515. 1450, 1370, 1311, 1272, 1224 (C–O), 1154, 839, 731 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.77 (d, $J = 7.2$ Hz, 2H), 7.63 (d, $J = 6.9$ Hz, 2H), 7.46–7.23 (m, 8H), 6.74–6.60 (m, 2H, H_g and NH–speacer), 6.43 (d, $J = 9.0$ Hz, 1H, NHAc), 5.73 (d, $J = 17.4$ Hz, 1H, H_h), 5.64 (d, $J = 8.7$ Hz, 1H, NHFmoc), 5.38 (s, 1H, H_4), 5.23 (d, $J = 10.8$ Hz, 1H, H_h), 5.05 (dd, $J = 11.3$, 3.0 Hz, 1H, H_3), 4.89 (s, 1H, H_1), 4.64–4.36 (m, 5H, included H_2 and H_e), 4.31–4.15 (m, 3H, H_5 and H_α), 4.15–3.97 (m, 3H, H_6 and H_β), 3.68–3.37 (m, 8H, H_{a-d}), 2.16 (s, 3H, NHAc), 2.01 (s,

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6H, OAc × 2), 1.98 (s, 3H, OAc), 1.23 (d, $J = 6.3$ Hz, H_γ); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.8 (C), 170.7 (C), 170.3 (C × 2), 169.9 (C), 156.6 (C), 143.7 (C × 2), 141.3 (C × 2), 137.3 (C), 137.2 (C), 136.3 (CH), 127.9 (CH × 2), 127.8 (CH × 2), 127.1 (CH × 2), 126.3 (CH × 2), 126.2 (CH × 2), 125.1 (CH), 120.0 (CH × 2), 114.0 (CH_2), 100.0 (CH), 77.4 (CH), 72.9 (CH_2), 70.3 (CH_2), 69.3 (CH_2), 69.2 (CH), 68.8 (CH), 67.4 (CH_2), 67.3 (CH × 2), 62.0 (CH_2), 58.5 (CH), 47.5 (CH), 47.1 (CH × 2), 39.4 (CH_2), 23.0 (CH_3), 20.7 (CH_3 × 2), 20.6 (CH_3), 17.7 (CH_3); HRMS (ESI$^+$): m/z calcd for $C_{46}H_{55}N_3O_{14}$ [M+H]: 874.3757; found: 874.3753.

4.16. N-(9-Acetyl)-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-α-D-galactopyranosyl)-L-serine 2-[2-(4-vinylbenzyloxy)ethoxy]ethyl ester (**22a**)

To a two neck round-bottom flask containing compound **20a** (0.125 g, 0.146 mmol) was treated with 5% piperidine/DMF (0.073/1.5 mL) to remove the *N*-Fmoc protecting group. The reaction mixture was stirred at room temperature for 2.3 h, and then concentrated under reduce pressure to give a crude product as a pale yellow oil. To this flash containing free amine **21a** was co-evaporated with benzene for three times to efficiently remove moisture.

To a solution of this free amine **21a** in CH_2Cl_2 (1.66 mL) was added triethylamine (0.030 mL, 0.219 mmol) and acetic anhydride (0.021 mL, 0.219 mmol). The reaction mixture was stirred at room temperature for 30 min, and concentrated to give a crude product, which was purified by flash chromatography with the eluent of $MeOH/CH_2Cl_2 = 1/20–1/15$ to give the compound **22a** (0.092 g, 92%) as a pale yellow oil.

$[\alpha]^{[23]}_D$ +81.88 (c = 1.66, $CHCl_3$); IR (neat) 3085, 2935, 2875, 1748 (C═O), 1661 (C═O), 1539, 1434, 1407, 1373, 1235, 1133, 1080, 1050, 949, 912, 848, 830, 720 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.40 (d, $J = 8.1$ Hz, 2H, *para*), 7.29 (d, $J = 8.1$ Hz, 2H, *para*), 6.81 (br s, 1H, C(O)NHCH$_2$), 6.70 (dd, $J = 17.7$, 10.8 Hz, 1H, ArCH═CH$_2$), 6.59 (d, $J = 7.8$ Hz, 1H, NHAc), 6.20 (d, $J = 9.9$ Hz, 1H, NHAc), 5.75 (d, $J = 17.3$ Hz, 1H, ArCH═CH$_2$), 5.35 (d, $J = 2.7$ Hz, 1H, H_4), 5.25 (d, $J = 11.1$ Hz, 1H, ArCH═CH$_2$), 5.07 (dd, $J = 11.1$, 3.3 Hz, 1H, H_3), 4.80 (d, $J = 3.6$ Hz, 1H, H_1), 4.65–4.50 (m, 4H, overlapped 1 s at 4.56, H_2, H_α and ArCH$_2$O), 4.15–3.98 (m, 3H, H_5 and H_6 × 2), 3.84 (dd, $J = 10.2$, 4.2 Hz, 1H, H_β), 3.71–3.52 (m, 7H, $H_{\beta'}$ and CH_2 × 3), 3.51–3.36 (m, 2H, NHCH$_2$), 2.15 (s, 3H), 2.04 (s, 6H), 1.98 (s, 3H), 1.96 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.9 (C), 170.5 (C), 170.4 (C), 173.3 (C), 170.2 (C), 169.3 (C), 137.3 (C), 137.2 (C), 136.3 (CH), 128.1 (CH × 2), 126.3 (CH × 2), 114.1 (CH_2), 99.1 (CH, anomeric carbon), 73.0 (CH_2), 70.2 (CH_2), 69.3 (CH_2 × 3), 68.6 (CH), 67.1 (CH × 2), 61.8 (CH_2), 52.6 (CH), 47.3 (CH), 39.4 (CH_2), 23.2 (CH_3), 23.1 (CH_3), 20.8 (CH_3), 20.7 (CH_3 × 2); HRMS (ESI$^+$): m/z calcd for $C_{32}H_{46}N_3O_{13}$ [M+H]$^+$:680.3025; found: 680.3020.

4.17. N-(9-Acetyl)-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-α-D-galactopyranosyl)-L-threonine 2-[2-(4-vinylbenzyloxy)ethoxy]ethyl ester (**22b**)

To a two neck round-bottom flask containing compound **20b** (0.152 g, 0.174 mmol) was treated with 5% piperidine/DMF (0.09/1.74 mL) to remove the *N*-Fmoc protecting group. The reaction mixture was stirred at room temperature for 5.6 h, and then concentrated under reduce pressure to give a crude product as a pale yellow oil. To this flash containing free amine **21b** was co-evaporated with benzene for three times to efficiently remove moisture.

To a solution of this free amine **21b** in CH_2Cl_2 (1.74 mL) was added *N*,*N*-diisopropylethylamine (0.039 mL, 0.278 mmol) and acetic anhydride (0.026 mL, 0.278 mmol). The reaction mixture was stirred at room temperature for 2.8 h, and concentrated to give a crude product, which was purified by flash chromatography with the eluent of $MeOH/CH_2Cl_2 = 1/15$ to give the compound **22b** (0.10 g, 83%) as a pale yellow oil.

IR (neat) 2986, 1745 (C═O), 1723(C═O), 1681 (C═O), 1515, 1450, 1370, 1310, 1266, 1245, 1154, 1135, 1046, 909, 842, 730 cm-^{1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.40 (d, $J = 8.1$ Hz, 2H, H_f), 7.29 (d, $J = 8.1$ Hz, 2H, H_f), 6.96 (d, $J = 4.8$ Hz, 1H), 6.70 (dd, $J = 4.8$ Hz, 1H, H_g), 6.61–6.47 (m, 2H, NHAc × 2), 5.75 (d, $J = 17.7$ Hz, 1H, H_h), 5.35 (s, 1H, H_4), 5.24 (d, $J = 11.1$ Hz, 1H, H_h), 5.07 (dd, $J = 11.4$, 3.0 Hz, 1H, H_3), 4.88 (d, $J = 3.3$ Hz, 1H, H_1), 4.66–4.45 (m, 4H, included H_2 and H_α), 4.22 (t, $J = 6.3$ Hz, 1H, H_5), 4.15–3.96 (m, 3H, H_6 and H_β), 3.70–3.33 (m, 8H), 2.15 (s, 3H, NHAc), 2.09 (s, 3H, NHAc), 2.01 (s, 6H, OAc × 2), 1.98 (s, 3H, OAc), 1.22 (d, $J = 6.3$ Hz, H_γ); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.0 (C), 170.7 (C × 2), 170.4 (C × 2), 170.4 (C), 170.1 (C), 137.2 (C × 2), 136.3 (CH), 128.0 (CH × 2), 126.3 (CH × 2), 114.1 (CH_2), 100.4 (CH), 78.1 (CH), 73.0(CH_2), 70.3 (CH_2), 69.3 (CH_2 × 2), 69.0 (CH), 67.3 (CH_2), 62.2 (CH_2), 56.5 (CH), 47.4 (CH), 40.2 (CH_2), 23.2 (CH_3), 23.0 (CH_3), 20.8 (CH_3), 20.7 (CH_3 × 2), 18.0 (CH_3); HRMS (ESI$^+$): m/z calcd for $C_{33}H_{47}N_3O_{13}$ [M+H]: 694.3182; found: 694.3177.

4.18. Typical procedure for polymerization of carbohydrate polymers

A Schlenk-tube (thick = 1.6 mm) was charged with styryl-TEMPO (1%), carbohydrate monomer, styrene, and *N*,*N*-dimethylformamide (40 wt%). The tube was subjected to three freeze-thaw cycles and sealed off under argon. The polymerization was carried out under argon at 125 °C for an indicated period (please see Table 1). The resulting mixtures were cooled to room temperature and precipitated in methanol, diethyl ether, or hexane. The carbohydrate polymers were collected by centrifugal sedimentation and dried in a vacuum-drying cabinet at 60 °C for 12 h. Conversion was evaluated gravimetrically. Molecular weight and polydispersity index (PDI) were determined by size exclusion chromatography (SEC).

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Appendix A. Supplementary data

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