JACS
JOURNAL OF THE AMERICAN CHEMICAL SOCIETY
Article
pubs.acs.org/JACS

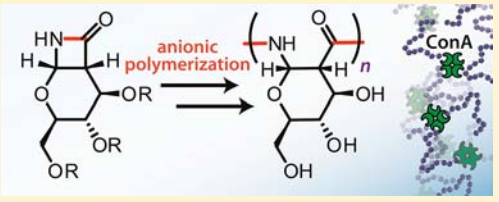
Poly-amido-saccharides: Synthesis via Anionic Polymerization of a β-Lactam Sugar Monomer

Eric L. Dane and Mark W. Grinstaff*

Departments of Chemistry and Biomedical Engineering, Boston University, Boston, Massachusetts 02215, United States

Supporting Information

ABSTRACT: Enantiopure poly-amido-saccharides (PASs) with a defined molecular weight and narrow dispersity are synthesized using an anionic ring-opening polymerization of a β-lactam sugar monomer. The PASs have a previously unreported main chain structure that is composed of pyranose rings linked through the 1- and 2-positions by an amide with α-stereochemistry. The monomer is synthesized in one-step from benzyl-protected D-glucal and polymerized using mild reaction conditions to give degrees of polymerization ranging from 25 to >120 in high yield. Computational modeling reveals how the monomer's structure and steric bulk affect the thermodynamics and kinetics of polymerization. Protected and deprotected polymers and model compounds are characterized using a variety of methods (NMR, GPC, IR, DLS, etc.). On the basis of circular dichroism, the deprotected polymer possesses a regular secondary structure in aqueous solution, which agrees favorably with the prediction of a helical structure using molecular modeling. Furthermore, we provide evidence suggesting that the polymers bind the lectin concanavalin A at the same site as natural carbohydrates, showing the potential of these polymers to mimic natural polysaccharides. PASs offer the advantages associated with synthetic polymers, such as greater control over structure and derivitization. At the same time, they preserve many of the structural features of natural polysaccharides, such as a stereochemically regular, rigid pyranose backbone, that make natural carbohydrate polymers important materials both for their unique properties and useful applications.



INTRODUCTION

Carbohydrate-based polymers that retain the chiral, cyclic main chain structure of natural polysaccharides (Figure 1, top left) and that can be prepared by controlled synthetic methods are of interest for both basic studies and applications. Specifically, novel polymeric structures having a hydrophilic pyranose backbone not joined with ether linkages[1] are fascinating because these materials are not found in nature and provide new molecular architectures to be explored. Amide-linked polysaccharides, which we term poly-amido-saccharides (PASs), are an example of one such polymeric structure. However, access to high molecular weight PASs requires the development of new polymerization methods. Here, we present the first synthesis of a 1,2-linked glucose-based PAS (Figure 1, top right), which is prepared via a robust and controlled anionic ring-opening polymerization of a β-lactam sugar monomer.

Interest in polysaccharides stems from their many varied and essential roles in biological systems, including storing energy (starch), forming rigid structural materials (cellulose), and modulating protein interactions and activity.[2] Examples, such as chitosan[3] and hyaluronic acid,[4] are used clinically in their isolated form and with further functionalization as engineered biomaterials.[5] Polysaccharides isolated from natural sources can be polydisperse and may show batch-to-batch variations.[2b] Additionally, they may require extensive purification and removal of endotoxins prior to use in biomedical applications. The introduction of synthetic methodologies to prepare polymers that mimic natural polysaccharides may give researchers the molecular-level control they are accustomed to with synthetic polymers while taking advantage of the unique chemical and physical properties of natural polysaccharides.

An alternative to polysaccharide synthesis is the conjugation of pendent sugar moieties to synthetic polymers and dendrimers to form glycoconjugates (Chart 1). For example, glycopolymers[6] and glycodendrimers[7] replicate the carbohydrate multivalency commonly found in nature. However, for some applications, the lack of a rigid, stereochemically defined pyranose backbone may be a limitation for these materials. Polymer chemists have prepared synthetic polymers with rigid backbones from both carbohydrate and noncarbohydrate starting materials that show varying levels of structural similarity to polysaccharides,[8] but many of these materials are not stereochemically defined. Others have used the opening of the carbohydrate ring as a strategy to make polymers that can have defined stereochemistry, but at the expense of losing the rigidity imparted by the pyranose ring.[9]

It is a challenge for chemists to synthesize carbohydrate polymers that retain both the cyclic pyranose backbone and the stereochemistry of common natural polysaccharides. Ideally, homopolysaccharides could be accessed using a single polymerization reaction, and such approaches to prepare polymers with a stereochemically defined pyranose backbone are highly desired. However, carbohydrates are challenging synthetic

Received: June 18, 2012
Published: August 31, 2012

dx.doi.org/10.1021/ja305900r | J. Am. Chem. Soc. 2012, 134, 16255−16264

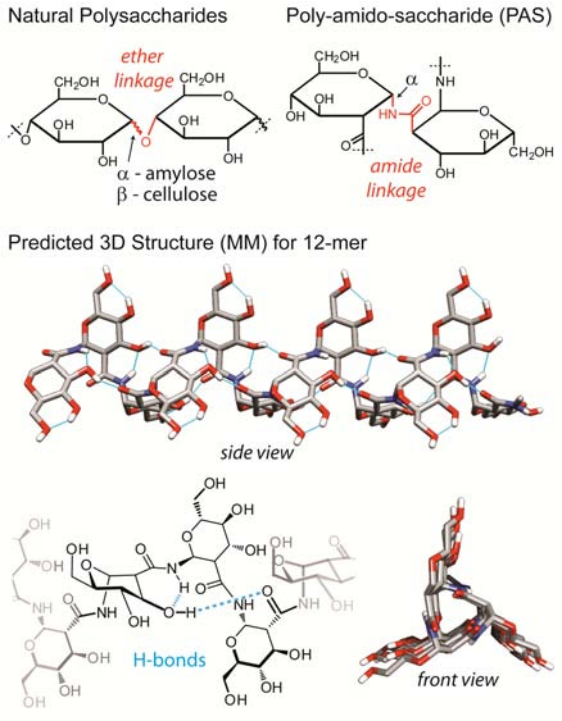
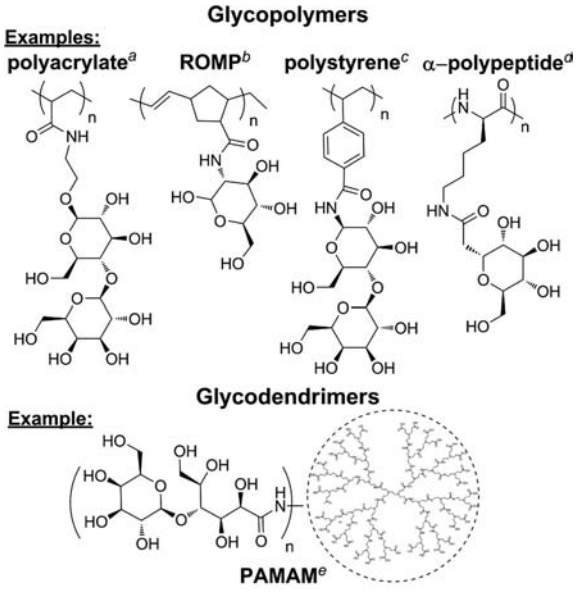


Figure 1. Comparison of natural polysaccharides to PASs and predicted structure of an *α*-*N*-1,2-D-glucose poly-amido-saccharide (*α*-*N*-1,2-D-glc PAS) 12-mer based on gas phase minimization with MMFF94s.

Chart 1. Examples of Glycoconjugates[i]



[i]References for example structures: (*a*) ref 6h, (*b*) ref 6c, (*c*) ref 10, (*d*) ref 6m, (*e*) ref 7b.

targets because they have a high density of similar functional groups and are stereochemically complex.[11] Advances in both solution and solid-phase synthesis provide reliable access to complex oligosaccharides with molecular weights (MW) that are generally less than 2 kDa.[12] However, these stepwise approaches are not amenable to preparing polysaccharides with high degrees of polymerization (DP).

Cationic ring-opening polymerization (ROP) works well to synthesize 1,6-linked polysaccharides with high degrees of polymerization (DPs > 100),[13] but is less effective in preparing polysaccharides with other linkages, such as 1,4-linked cellulose[14] (DP < 20) and chitin[15] (DP < 14)).[6f,16] Furthermore, cationic ROP is not used to make commercially available polysaccharides, but instead these materials are isolated from natural sources or produced using fermentation.[17] The use of isolated enzymes or microorganisms for the controlled synthesis of polysaccharides is an attractive alternative to chemical methods.[18] Enzymatic approaches can avoid the use of protecting groups, but may require expensive activated monomers. Additionally, the synthesis of nonnatural polysaccharides with unique geometries and linkages, such as those described in this report, may be a challenge for natural enzymes.

Our approach replaces the ether linkage found in natural polysaccharides with an amide linkage to provide poly-amido-saccharides (PASs) (Figure 1.). Specifically, we report a high-yielding method to synthesize *α*-*N*-1,2-D-glucose (*α*-*N*-1,2-D-glc) PASs of defined molecular weight with low dispersity (Đ) via the anionic ring-opening polymerization of a *β*-lactam sugar monomer, **1** (Scheme 1). Notably, epimerization does not occur at either the 1- or 2-position of the pyranose ring and an enantiopure polymer is obtained using our approach. This is a key advantage as connecting the carbohydrate units by amide bonds eliminates the issue of stereocontrol (*α*/*β*) at the glycosidic linkage. Molecules containing pyranose and furanose rings joined via amide linkages have been previously reported and named in a variety of ways (e.g., saccharide−peptide hybrids, glycosamino acids, and peptidosaccharides).[11b] These compositions differ from the current polymers in that they have been prepared via a stepwise approach from sugar amino acids and are short oligomers (DP < 10).[11b,19] For example, Gervay-Hague and co-workers used the naturally occurring *N*-acetyl neuraminic acid to synthesize amide linked pyranose sugar oligomers.[20] However, oligomers containing the specific *α*-*N*-1,2-linkage reported here have not been previously reported. Because *α*-*N*-1,2-D-glucose PASs contain a 1,2-peptide linkage, they can be considered highly functionalized *β*-polypeptides. *β*-Polypeptides are a class of synthetic polymers known to form defined secondary structures and are of significant interest for a variety of applications.[21] Notably, oligomers of *trans*- and *cis*-2-aminocyclohexanecarboxylic acid (ACHC) show helical secondary structures.[21a,22] On the basis of molecular modeling, *α*-*N*-1,2-D-glc PASs are predicted to have a helical structure that is promoted by extensive internal hydrogen-bonding and by the rigidity of the pyranose-polyamide backbone (Figure 1).

In addition to the synthesis and characterization of *α*-*N*-1,2-D-glucose PASs, we discuss how the *β*-lactam sugar monomer's structure and steric bulk affect the thermodynamics of polymerization by calculating the ring strain of the monomer and comparing it to other *β*-lactam monomers. In addition, we comment on how the steric bulk surrounding the lactam affects the kinetics of the reaction and propose an explanation for why the benzyl-protected monomer polymerizes easily, while the *tert*-butyldimethylsilyl-protected monomer does not polymerize. We investigate the polymer's secondary structure using circular dichroism (CD). The effect of MW on the solid-state morphology is also examined using scanning electron microscopy (SEM). Finally, we report the glucose-dependent binding of higher MW *α*-*N*-1,2-glc PASs to the plant lectin concanavalin A using an established aggregation assay,[23]

showing the potential of PASs to interact with natural carbohydrate receptors.

MATERIALS AND METHODS

Computational Methods. Molecular models were constructed and minimized using the freely available software Avogadro (MMFF94s) and GAMESS 11 (AM1, DFT[B3LYP/6-31G(d)]). X-ray crystal structures (XRCSs) were used as initial geometries when available (2-azetidinone,[24] **CS4**,[25] **CS5**[26]). For AM1 and DFT methods, minimized structures were verified by confirming that no imaginary frequencies were present. Zero point energies calculated using DFT[B3LYP/6-31G(d)] were scaled by 0.977.[27] The 12-mer of α-*N*-1,2-glc PAS was minimized using MMFF94s.

Polymerization. Polymer P1′. In an oven-dried flask under nitrogen, lactam **1** (0.500 g, 1.09 mmol) was dissolved in 9 mL of distilled tetrahydrofuran (THF) dried over 4 Å molecular sieves. The reaction flask was cooled to 0 °C in an ice bath and initiator **2** (0.027 g, 0.044 mmol, 4.0 mol %) was added as a solution in THF (1 mL). Next, 0.090 mL of a 1.0 M solution of LiHMDS in THF (0.088 mmol, 8.0 mol %) was added and the solution was stirred for 1 h, at which time a drop of saturated NH_4Cl solution was added. Reaction progress was monitored by observing the disappearance of the monomer using TLC. After evaporation of THF, the resulting solid was redissolved in diethyl ether (50 mL) and washed with 1 M HCl, saturated $NaHCO_3$, and brine. After drying over sodium sulfate, the crude reaction mixture was isolated and then redissolved in a minimum amount of dichloromethane. The polymer was precipitated by adding dropwise into a flask of stirred, cold pentane (50 mL), and then collected by filtration. The solid was redissolved in a minimum amount of dichloromethane and precipitated by adding dropwise into a flask of stirred, cold methanol (50 mL), and then collected by filtration. After drying under high vacuum, 0.444 g (84%) of an amorphous solid was isolated. $[\alpha]_D$ = 79.1 (7.1 mg/mL in CH_2Cl_2, 26 °C); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.8 (br, 1H), 7.4−6.9 (br, 15H), 5.7 (br, 1H), 4.75−4.25 (br, 5H), 4.2−3.8 (br, 2H), 3.75−3.4 (br, 4H), 2.8 (br, 1H), 1.2 (s, end group, 9H); ^{13}C NMR (126 MHz, $CDCl_3$): δ 170.6, 138.5(2), 138.3, 128.5−127.3, 78.3, 75.0−73.0, 73.1, 68.5, 51.2, 35.0, 31.2; IR (KBr): 1686 (amide I), 1530 (amide II) cm^{-1}; GPC(THF): M_n = 9500; M_w = 10 500; Đ (M_w/M_n) = 1.1.

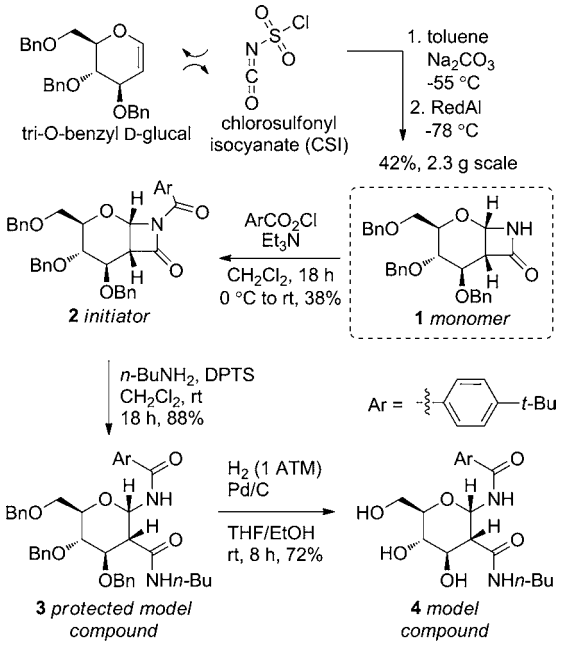
Polymer Debenzylation. Polymer P1. Polymer **P1′** (0.155 g) and 0.045 g (1.2 equiv) of KO*t*-Bu were dissolved in 5.0 mL of THF. The polymer solution was added dropwise to a rapidly stirred solution of sodium in anhydrous liquid ammonia (50 mL) at −78 °C under nitrogen. Sodium was washed in toluene and hexane and cut into small pieces before addition. The solution's deep blue color was maintained by adding additional sodium. After 1 h at −78 °C, sat'd ammonium chloride was added until the blue color disappeared. After evaporation of the ammonia at room temperature, the resulting aqueous layer was washed with diethyl ether twice and then filtered through a 0.22 μm PVDF syringe filter to remove particulates. The solution was dialyzed with 1000 MWCO tubing for 12 h with 3 water changes. After lyophilization, the resulting white solid was washed with methanol (10 mL) and collected by decantation after centrifugation a total of three times. Residual methanol was removed under high vacuum. **P1** (0.063 g, 98%) was obtained as a white amorphous solid. $[\alpha]_D$ = 129 (2.0 mg/mL in H_20, 24 °C); ^{1}H NMR (500 MHz, D_2O): δ 5.75 (d, J = 4.8 Hz, 1H, H_1), 4.12 (pseudo t, J = 10.1, 1H, H_3), 3.75 (br s, 2H, H_6), 3.47 (pseudo t, J = 9.6, 1H, H_5), 3.42 (m, J = 10.2, 1H, H_4), 3.04 (dd, J = 11.2, 4.8, 1H, H_2); ^{13}C NMR (126 MHz, D_2O): δ 170.8 (CO), 75.3, 73.7, 70.6, 69.1, 60.9 (C_6), 51.7 (C_2); IR (ATR): 1682 (amide I), 1545 (amide II) cm^{-1}; GPC(H_2O) M_n = 4100; M_w = 4600; Đ (M_w/M_n) = 1.1; DLS(H_2O, 50 °C) r = 1.6 ± 0.4 nm, M_w = 5000.

Additional experimental details are presented in Supporting Information.

RESULTS AND DISCUSSION

Monomer Design and Synthesis. Anionic ROP of certain β-lactam monomers can yield polymers of low dispersity and controlled length when an appropriate initiator and base are used.[28] The proposed polymerization mechanism[28] involves cleavage and reformation of an achiral amide bond, and therefore is expected to produce an enantiopure polymer if a chiral monomer is used.[28f,29] The amount of initiator added determines how many polymer chains are grown and hence the polymer length. However, the polymerization of cyclic sugar-derived β-lactam monomers, such as benzyl-protected monomer **1** (Scheme 1), has not been studied. Benzyl ethers are

Scheme 1. Monomer Synthesis[a]



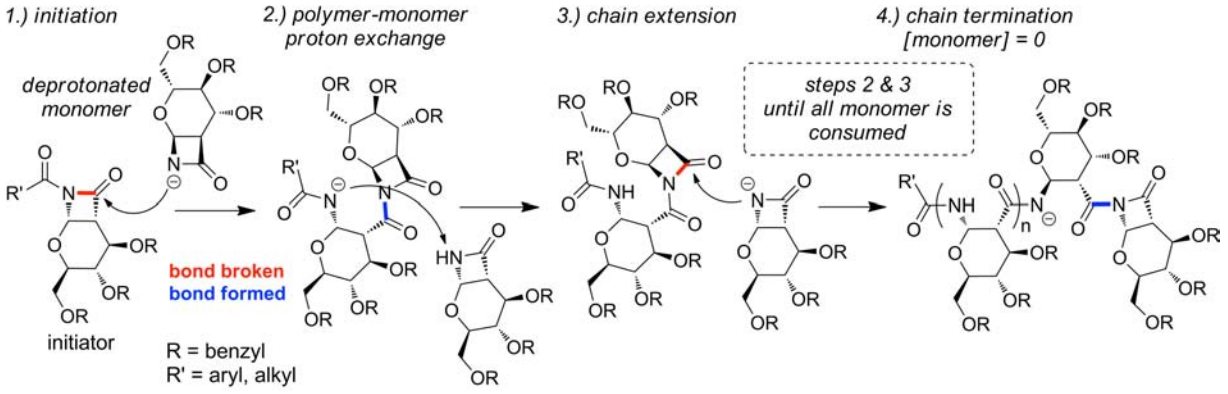
[a]DPTS = 4-(dimethylamino)pyridinium *p*-toluenesulfonate.

attractive protecting groups for polysaccharides because they can be removed efficiently from large molecules via either Pd-catalyzed hydrogenation[15] or metal-ammonia reduction.[13] Additionally, the use of **1** as a monomer is novel because the polymerization of β-lactams in which the lactam is part of a hemiamidal (a hemiaminal where the amine is replace with an amide) has not been explored. The previously reported β-lactam **1**[30] was accessed on multigram scales in moderate yield via the stereoselective cycloaddition of tri-O-benzyl-D-glucal and chlorosulfonyl isocyanate (CSI) followed by *in situ* reduction[31] to remove the sulfonyl group (Scheme 1). Monomer **1** was reacted with 4-*tert*-butylbenzoyl chloride to provide initiator **2**. In anticipation of characterizing the polymers by NMR and IR, a protected model compound (**3**) was synthesized by opening the β-lactam of **2** with excess *n*-butylamine and deprotected to form **4**. The proposed mechanism for the anionic ROP of monomer **1** is shown in Scheme 2.

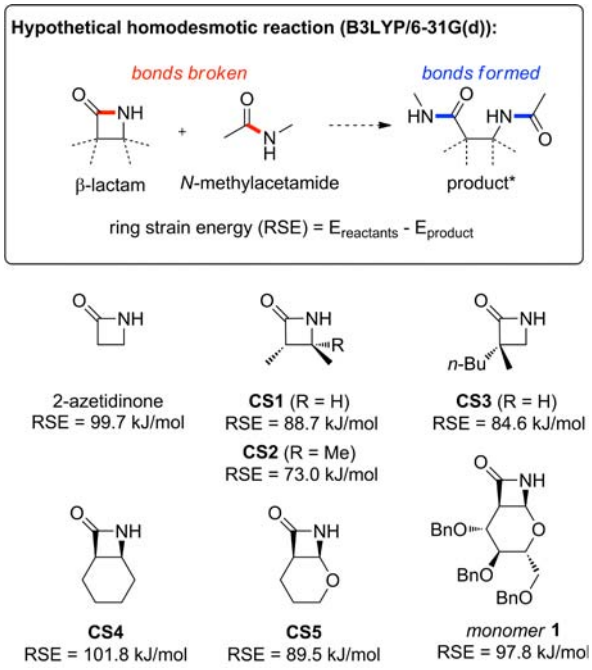
The ring strains of a series of β-lactam monomers were estimated using a homodesmotic reaction as shown in Scheme 3. DFT geometry minimization and energy calculations were performed to determine the energy difference between the reactants and the product for the hypothetical ring-opening reaction.[32] All of the ring-opened structures contained an intramolecular hydrogen bond (see Figure S1 for structures). The presence of this H-bond in the product but not in the reactants inflates the ring strain energy calculated by this

dx.doi.org/10.1021/ja305900r | J. Am. Chem. Soc. 2012, 134, 16255−16264

Scheme 2. Proposed Mechanism of Anionic Ring-Opening Polymerization



Scheme 3. β-Lactam Ring Strain Computation[a]



[a]Intramolecular hydrogen bond is not shown in product, see Figure S1.

method, but because it is present in all of the products it should not significantly affect the trends.

For the simplest β-lactam, 2-azetidinone, the ring strain energy (RSE) was calculated to be 99.7 kJ/mol. For comparison, the experimentally determined value for the ring strain of 2-azetidinone is 119.4 ± 5.7 kJ/mol.[33] Additionally, an experimentally determined ring strain of 116 kJ/mol has been reported for the β-lactam in the antibiotic penicillin G.[34] Next, we analyzed two computational structures (**CS1** and **CS2**), which are simplified structures based on monomers reported to readily polymerize.[28g−i] As would be expected, the addition of substituents to the ring decreases the ring strain by stabilizing the ring-closed form in relation to the ring-open form. The lowering of ring strain energy by the replacement of hydrogen atoms with alkyl groups has been noted for a range of lactam monomers.[35] This effect is observed for **CS1** (88.7 kJ/mol) relative to 2-azetidinone, and to a larger extent for **CS2** (73.0 kJ/mol), which has an additional methyl group. For **CS3**, the experimentally determined heat of polymerization (ΔH_p) for anionic ROP in toluene at 25 °C is reported as 80 kJ/mol.[28a] The computational method estimates a RSE of 84.6 kJ/mol, which is in good agreement with the experimental value.

For the β-lactam cis-fused with a cyclohexane ring (**CS4**), an increased ring strain (101.8 kJ/mol) was calculated as compared to 2-azetidinone. In this case, the fused bicyclic system induces additional ring strain. This conclusion is supported by the fact that the cyclohexane ring of **CS4** is forced to adopt a boat rather than a chair conformation in both the XRCS and the DFT-minimized structure. **CS4** readily polymerizes but forms very insoluble homopolymers.[28g,h] In contrast to **CS4**, the calculated ring strain in **CS5** (89.5 kJ/mol) is lower than that of 2-azetidinone. The decrease in ring strain in **CS5** may be a consequence of stabilization due to a larger anomeric interaction between the ethereal oxygen and the nitrogen in the lactam as compared to the ring-opened form. The polymerization of **CS5** has not been reported. The calculated RSE of monomer **1** (97.8 kJ/mol) is less than those of 2-azetidinone and **CS4**, but more than those of **CS1**−**CS3** and **CS5**. We attribute the increased RSE of **1** in comparison to **CS5** to steric interactions that are relieved upon ring-opening as the pyranose ring relaxes from a twisted-chair to a chair conformation. In general, these results suggest that the polymerization of monomer **1** is highly thermodynamically favored due to the strained β-lactam ring, and that the greater steric bulk of the benzyl ethers increases this strain. These results do not comment on the effect of the monomer's increased steric bulk on the kinetics of the polymerization. However, the anionic ROP of less strained β-lactams (derivatives of **CS1** and **CS2**, and **CS3**) is rapid at mild temperatures.[28h]

Next, we investigated the conformation adopted by the pyranose ring in **1** by X-ray crystallography and NMR spectroscopy in order to confirm predictions made by modeling. We attempted to grow single crystals of **1** large enough for X-ray analysis, but were unsuccessful. In contrast, the *tert*-butyldimethylsilyl protected derivative **5**[30] easily formed crystals and an X-ray crystal structure (XRCS) revealed that in the solid state the six-member ring adopts a boat conformation (Figure 2, ORTEP in Supporting Information). This is in contrast to a previously reported XRCS of **6**,[25,36] the deprotected form of **1**, in which the ring adopts a half-chair conformation. Geometry minimization (B3LYP/6-31G(d)) suggests that **1** adopts a conformation closer to that of a half-chair rather than a boat (Figure 2D). For cyclic sugar derivatives, the *J*-couplings between adjacent protons provide information about the ring's conformation because the strength

dx.doi.org/10.1021/ja305900r | J. Am. Chem. Soc. 2012, 134, 16255−16264

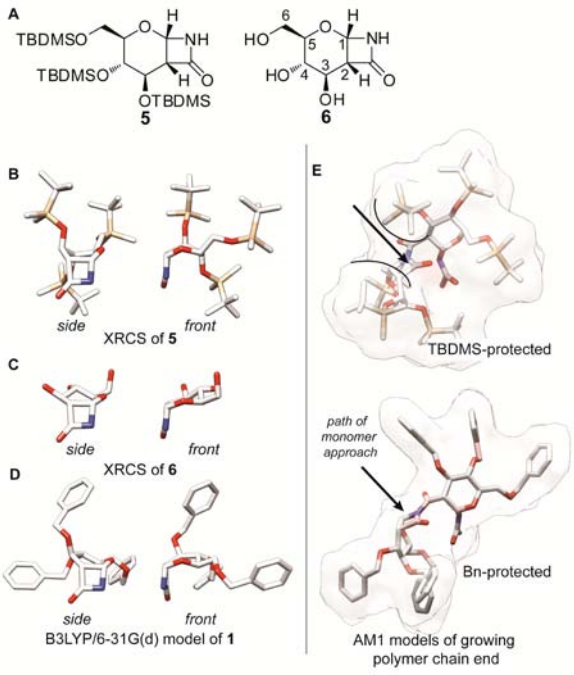


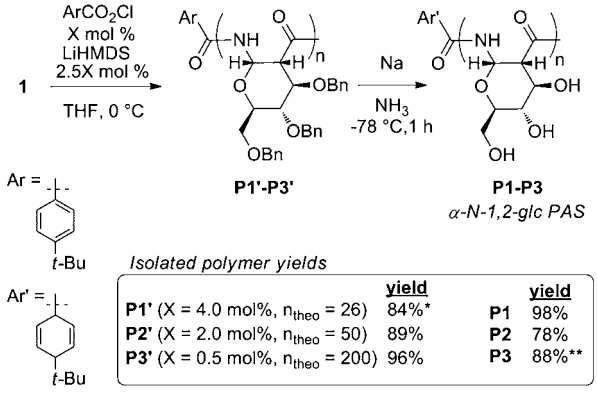
Figure 2. Monomer structure. (A) Structures of **5** and **6**; (B) XRCS of **5**; (C) XRCS of **6**; (D) structure of **1** geometry minimized using B3LYP/6-31G(d); (E) models of benzyl and TBDMS protected chain ends minimized using AM1 showing the effect of protecting group size on steric barriers to polymerization.

of the coupling varies with the dihedral angle. Therefore, we compared the solution ^{1}H NMR *J*-couplings among H_3, H_4, and H_5 for compounds **1**, **5**, and **6** (Figure S2). The coupling constants for **1** and **5** were measured in $CDCl_3$ and the coupling constants for **6** were measured in D_2O. The couplings for monomer **1** are approximately the same as those of **6**[36] and differ significantly from those of **5**, supporting the hypothesis that **1** has a conformation closer to a half chair rather than a boat, if we assume that the solid-state structures of **5** and **6** are representative of their solution conformations.

In addition to studying the monomer's ring strain, we used modeling to predict how the steric bulk of the benzyl protecting groups would affect the kinetics of polymerization. Zhang and co-workers attributed a slower reaction rate for a **CS2** derivative as compared to a **CS1** derivative to the additional steric bulk of the added methyl group in **CS2**.[28g,h] In this case, the proximity of the methyl group to the lactam clearly suggests a steric influence. However, the model of **1** reveals that the steric effects of the benzyl groups may be relatively modest because they are positioned so that one face of the monomer is open for reaction, even in a model of the growing polymer chain (Figure 2E, bottom). In contrast, the *tert*-butyldimethylsilyl groups pose a steric impediment to polymerization of **5** in a model of the growing polymer chain (Figure 2E, top). As predicted, our attempts to polymerize **5** under the conditions used for **1** (and with heating to 40 °C) were unsuccessful. On the basis of our calculations of RSE in related structures, such as **CS5**, the polymerization of **5** is likely very thermodynamically favorable. Therefore, the observation that **5** does not polymerize suggests that additional steric bulk beyond that of the benzyl group can reduce the reactivity of a *β*-lactam sugar monomer in anionic ROP by increasing kinetic barriers to reaction.

Polymer Synthesis and Characterization. Monomer **1** was polymerized with 4 mol % initiator to obtain polymer **P1′** (DP_{theo} = 26) (Scheme 4). For polymers **P2′** (DP_{theo} = 50) and

Scheme 4. Polymer Synthesis[a]



Isolated polymer yields

	yield		yield
P1′ (X = 4.0 mol%, n_{theo} = 26)	84%*	**P1**	98%
P2′ (X = 2.0 mol%, n_{theo} = 50)	89%	**P2**	78%
P3′ (X = 0.5 mol%, n_{theo} = 200)	96%	**P3**	88%**

[a]Asterisk(s) indicate (*) 4 mol % initiator **2** was used with 8.0 mol % LiHMDS; (**) reaction was warmed to −42 °C.

P3′ (DP_{theo} = 200), the initiator was formed *in situ* by adding the appropriate amount of 4-*tert*-butylbenzoyl chloride. Polymerization conditions were based on those reported by Zhang et al.[28g−i] The average polymer yield over a variety of theoretical molecular weight targets was 89 ± 5% based on eight runs. Polymers **P1′**−**P3′** were characterized with ^{1}H NMR (Figure S3) and ^{13}C NMR, GPC, polarimetry, and IR. The DP was estimated by comparing the integration of the initiator's *tert*-butyl signal at 1.2 ppm to the polymer integration. In the ^{13}C NMR spectra, signals at 170 ppm (amide), 73 ppm (C_1), and 51 ppm (C_2) are clearly present. Characterization of **P1′**−**P3′** with GPC(THF) and polystyrene standards indicated that **P1′**−**P3′** had low levels of dispersity (Đ = 1.1). For **P2′** and **P3′**, the DP as measured by GPC is lower than the theoretical value and the value measured by NMR (Table 1). The specific rotations of **P1′**−**P3′** measured in CH_2Cl_2 increased slightly with polymer length ($[\alpha]_D$ = 79.1 (**P1′**), 82.1 (**P2′**), 83.3 (**P3′**)). The IR spectra of **P1′**−**P3′** show strong amide stretches (amide I ≈ 1690 cm^{-1} and amide II ≈ 1530 cm^{-1}) and are in good agreement with the spectrum of the protected model compound **3** (Figure S4).

On the basis of TLC, the monomer was completely consumed in less than 30 min at either 0 or 25 °C for all of the polymerizations. To better observe the reaction progress, we performed the polymerization of **P2′** while monitoring the IR signal with an *in situ* probe (Figure 3). The monomer was consumed in less than 5 min at 25 °C, based on the decrease in the signal from the carbonyl stretch of the *β*-lactam (1784 cm^{-1}, red). We also observed an increase in the amide carbonyl stretch of the polymer (1693 cm^{-1}, blue) within the first 5 min. Future kinetic studies of the polymerization will be performed in order to make quantitative comparisons between the rate of polymerization of monomer **1** and the rates reported for other monomers.[28h] Our observations confirm that the polymerization of **1** is rapid and that the steric bulk of the benzyl protecting groups does not significantly hinder polymerization.

Attempts at polymer debenzylation using Pd-catalyzed hydrogenation at room temperature and elevated pressure (3 atm) failed. At elevated temperature (70 °C) in dimethylacetamide, the hydrogenation was successful for shorter polymers, but resulted in low yields. Hence, sodium metal in ammonia

dx.doi.org/10.1021/ja305900r | J. Am. Chem. Soc. 2012, 134, 16255−16264

Table 1. Polymer Characterization

	$M_{n(theo)}$ (kDa)	$M_{n(NMR)}$ [a] (kDa)	$M_{n(GPC)}$ [b] (kDa)	$M_{w(GPC)}$ [b] (kDa)	Đ [c]	DP_{theo}	DP_{NMR} [a]	DP_{GPC} [b]	$[\alpha]_D$ (CH_2Cl_2)
P1′	11.9	11.0	9.5	10.5	1.1	26	24	21	79.1
P2′	23.0	27.5	16.7	19.0	1.1	50	60	36	82.1
P3′	91.8	82.6	56.2	64.5	1.1	200	180	122	83.3
	$M_{n(theo)}$ (kDa)	$M_{n(NMR)}$ [d] (kDa)	$M_{n(GPC)}$ [e] (kDa)	$M_{w(GPC)}$ [e] (kDa)	Đ [c]	DP_{theo}	DP_{NMR} [d]	DP_{GPC} [e]	$[\alpha]_D$ (H_2O)
P1	4.7	5.7	4.1	4.6	1.1	26	30	22	129
P2	9.5	8.9	7.8	8.4	1.1	50	47	41	n.d.
P3	37.8	n.d.	n.d.	n.d.	n.d.	200	n.d.	n.d.	n.d.

[a]Determined by integration of the ^{1}H NMR signal from the *t*-butyl group on the initiator. [b]THF GPC with polystyrene standards. [c]Đ = M_w/M_n. [d]Determined by integrating *t*-butyl groups of reduced *t*-butyl benzamide end group. [e]Aqueous GPC using 0.2 M $NaNO_3$, 0.01 M phosphate buffer, pH 7 as eluent at 40 °C with dextran standards. n.d. = not determined.

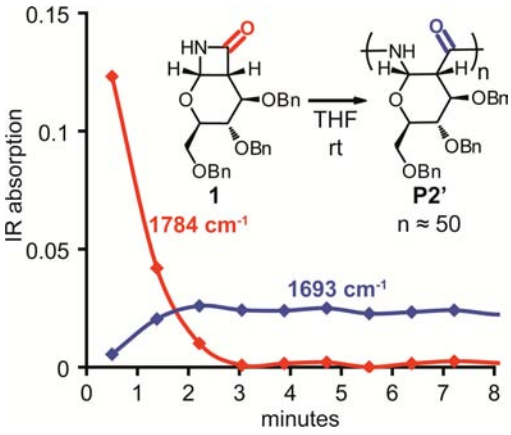


Figure 3. The progress of the polymerization was monitored by observing the decrease in the IR absorbance of the monomer *β*-lactam carbonyl stretch (1784 cm^{-1}, red) and the increase in the polymer amide carbonyl stretch (1693 cm^{-1}, blue). Reaction conditions: THF, rt, [**1**] = 0.15 M, 2 mol % of 4-*tert*-butylbenzoyl chloride, and 5 mol % LiHMDS. Data recording began 30 s after addition of the base.

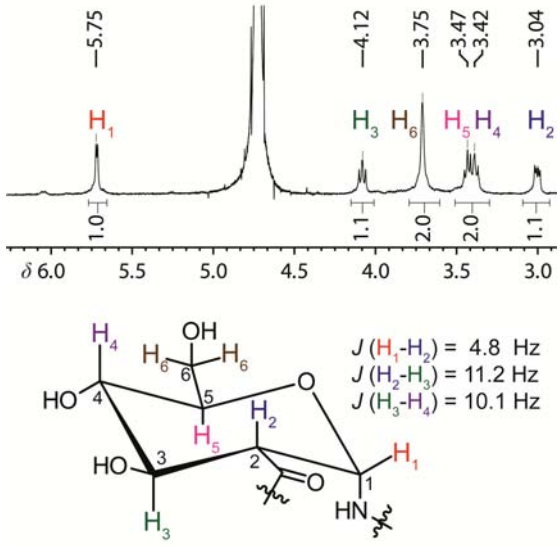


Figure 4. Proton NMR spectrum of **P1** (D_2O, rt, 500 MHz). The coupling constant (*J*) of 4.8 Hz between protons H_1 and H_2 indicates that they are in an equatorial−axial relationship. The larger coupling constants between H_2−H_3 (11.2 Hz) and H_3−H_4 (10.1 Hz) indicate that they are in an axial−axial relationship. These relationships suggest that the polymer adopts a chair conformation with the C_1−N bond axial and the other ring substituents equatorial.

(Birch reduction) was used for deprotection. Prior to reduction, the polymers were treated with potassium *tert*-butoxide to deprotonate and therefore protect the amide groups from reduction. At −78 °C, the debenzylation of **P3** was incomplete. Repeating the procedure with warming to −42 °C provided complete removal of the protecting groups based on ^{1}H NMR and IR. Deprotected polymers (**P1**−**P3**) were purified by dialysis and then lyophilized. Further washing with methanol, in which the polymers are insoluble, removed trace salts.

Polymers **P1**−**P3** were characterized with aqueous GPC (Table 1), ^{1}H NMR (Figure 4 and Figure S5), and IR (Figure S4). On the basis of aqueous GPC against dextran standards, **P1** and **P2** have M_n's of 4.1 kDa and 7.8 kDa, respectively, and a Đ of 1.1. The results from GPC for **P1** and **P2** show no evidence of a decrease in chain length or of an increase in dispersity. Because of issues of solubility and aggregation, **P3** could not be analyzed using GPC and therefore we cannot directly measure the molecular weight and dispersity for this polymer. The M_w of **P1** was also measured using dynamic light scattering (DLS). For **P1**, a radius of 1.6 ± 0.4 nm and M_w of 5 kDa were determined in water at 50 °C (average of 30 measurements, based on model for linear polysaccharides). Under the same conditions, **P2** and **P3** were aggregated (radii >50 nm). In general, the solubility of the deprotected polymers decreased with increasing chain length. When first isolated, **P1** (DP_{theo} = 26) had a solubility of >5.0 mg/mL in pure water, which decreased over time as a precipitate formed within 1−2 h. Polymers **P2** and **P3** had lower initial solubilities, 2.0 mg/mL and 1.0 mg/mL, which also decreased over time. Whistler has previously noted that natural, linear polysaccharides show decreasing solubility with increasing chain length, but it is unclear whether this will be a general property of PASs.[37]

The proton NMR spectra of **P1**-**P3** were well-resolved (Figure 4) and a gCOSY spectrum of **P1** was collected to confirm coupling assignments. In regards to the structure of the end group after debenzylation, aromatic amides with a hydrogen on the nitrogen do not undergo amide cleavage under Birch reduction conditions, but rather the aromatic ring is partially reduced to several products with a preference for the 1,4-dihydro product (Ar′ in Scheme 4).[38] Integration of the *tert*-butyl signals of the reduced aromatic end-group suggests a DP of 30 as compared to a DP of 22 measured by GPC. In the ^{1}H NMR spectrum of **P1**, the H_1 signal of the N-terminus repeat unit is visible as an undefined multiplet at 6.08 ppm and the H_2 signal of the C-terminus repeat unit is visible as a doublet of doublets centered at 2.83 ppm (see Supporting

dx.doi.org/10.1021/ja305900r | J. Am. Chem. Soc. 2012, 134, 16255−16264

Information, page S26). A ^{13}C NMR spectrum of freshly dissolved **P1** showed the expected 7 signals. Polymers **P2** and **P3** gave ^{1}H NMR spectra similar to **P1** with attenuated signals due to the lower solubility of the higher molecular weight polymers. For **P2**, a DP of 47 was estimated by integration of the *tert*-butyl signals as compared to a DP of 41 estimated by GPC. For **P3**, the *tert*-butyl signals could not be distinguished from the baseline.

Beyond estimating the DP, the ^{1}H NMR spectra (Figure 4, Figure S5) of **P1**−**P3** suggest that in aqueous solution at room temperature the polymer backbone has a chair conformation. This analysis is based on the smaller *J*-coupling between protons H_1 and H_2 ($J(H_1-H_2)$ = 4.8 Hz) being indicative of an equatorial-axial relationship between H_1 and H_2 and the larger couplings between protons H_2 and H_3 and between protons H_3 and H_4 being indicative of an axial−axial relationship (Figure 4). Consequently, this chair conformation would position the bond between C_1 and the nitrogen of the amide linkage in an axial position as shown in Figure 4. For comparison, the monosaccharide methyl α-D-glucopyranoside, which has a chair conformation in solution, has reported values in D_2O of $J(H_1-H_2)$ = 3.8 Hz, $J(H_2-H_3)$ = 9.8 Hz, and $J(H_3-H_4)$ = 9.1 Hz.[39] In addition to the dihedral angle between the C−H bonds, coupling constants are affected by other factors such as the electronegativity of substituents, bond lengths, and bond angles, and differences in these variables may account for the larger couplings for **P1** versus methyl α-D-glucopyranoside.[40] The spectra of all the polymers show that the stereochemistry of the polymer is not affected by the reductive debenzylation.

The specific rotation of **P1** measured in H_2O was positive ($[\alpha]_D$ = 129), but the specific rotations of **P2** and **P3** were not measured due to their aggregated structures. IR spectra of **P1**−**P3** show a single peak in the amide I region and the complete removal of the benzyl groups as demonstrated by the disappearance of the aromatic C−H signals between 3000 to 3100 cm^{-1} (see Figure S4).

The formation of helical secondary structures by oligo- and poly-β-peptides has been previously reported and studied with CD.[41] Therefore, we recorded the CD spectrum of **P1** in water at room temperature (Figure 5). **P1** has a minimum at 221 nm (mean residue ellipticity (MRE) = −16 000 deg cm^2/dmol) and a maximum at 190 nm (MRE = 54 000 deg cm^2/dmol), where recording was stopped. The spectrum is similar to those reported by Seebach et al. for oligo-β-peptides with a left-handed 3_1 helical conformation.[41a,42] Molecular modeling (MMFF94s) of a 12-mer also suggests that the polymer structure has a left-handed 3_1 helical conformation (Figure 1). At this time, the evidence supporting a 3_1 helix is suggestive but not conclusive, and therefore additional studies are underway to further characterize the polymer's secondary structure.

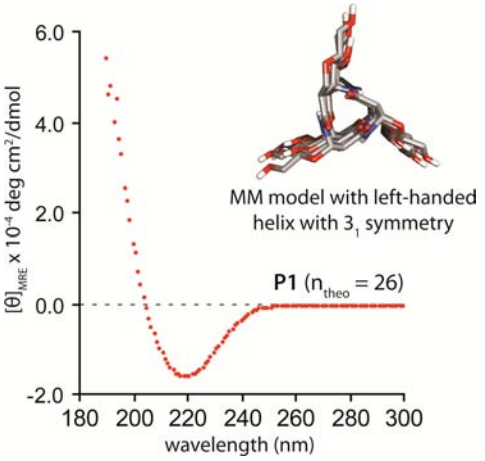


Figure 5. CD spectrum of **P1** and model of an α-*N*-1,2-D-glc PAS 12-mer based on minimization with MMFF94s. [**P1**] = 0.030 mg/mL in H_20, rt.

To better understand how the molecular weights of **P1**−**P3** affect their aggregated structures, we used both light and electron microscopy to visualize the solid-state structure of the polymers. The samples for observation were prepared by allowing a saturated aqueous solution of the polymers to precipitate over 24 h. The lowest MW polymer, **P1**, gave an amorphous structure with high surface roughness that showed little tendency to form films when viewed by SEM (Figure 6A,B). In contrast to **P1**, polymer **P2** formed smooth films that,

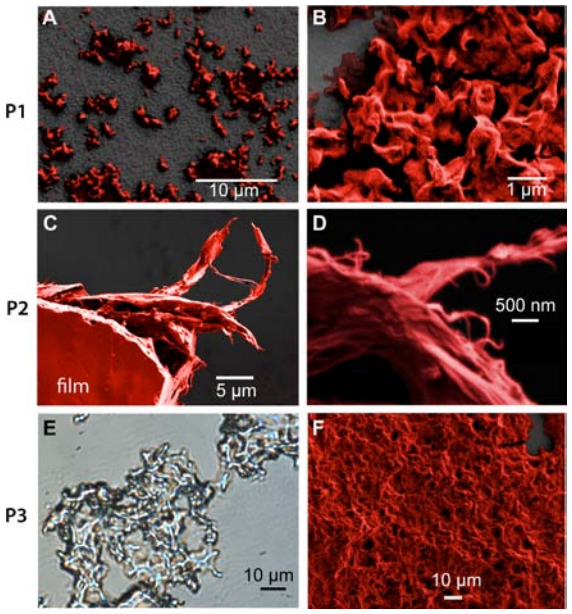


Figure 6. Electron and optical microscopy. (A and B) SEM micrographs of **P1** showing absence of film formation. (C and D.) SEM micrograph of **P2** showing a piece of polymer film that tore apart during sample preparation leaving behind fibrous shreds at two magnifications. (E) phase contrast light micrograph of **P3** showing complex morphology. (F) SEM micrograph of **P3** showing an amorphous morphology. SEM images have been colorized and the dark gray background is the substrate.

when cracked during SEM sample preparation, revealed fibrillar alignment of the polymers (Figure 6C,D). For **P3**, what appeared to be fibrils of approximately 1 μm in diameter were observed using phase contrast light microscopy (Figure 6E). In SEM, **P3**'s surface appeared amorphous and rough, but well-defined fibrils could not be observed (Figure 6F).

Biological Activity. Because PASs **P1**−**P3** are based on a unique structure not found in nature, we next determined whether these PASs are recognized by a carbohydrate binding protein. Concanavalin A (Con A), a readily available lectin, is known to bind glucose or mannose groups with an α-orientation at C_1 by interacting predominantly with the C_3, C_4, and C_6 alcohols.[23a] We reasoned that the polymers reported here may bind in the same pocket as natural glucose derivatives because **P1**−**P3** are joined via a linkage of α-stereochemistry

and the 1,2-linkage does not disturb the alcohols at C_3, C_4, and C_6. Using an established assay, the binding of Con A to polysaccharides can be measured by the increased turbidity of a solution due to the aggregated structures (Figure 7A) formed

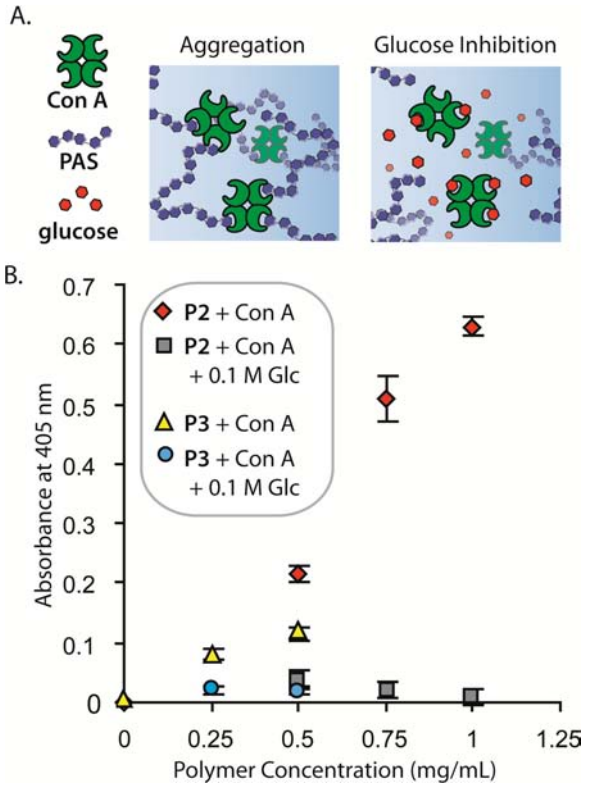


Figure 7. Concanavalin A binding. (A) Schematic representation of the binding of the lectin concanavalin A with PASs. (B) The turbidity was measured based on the scattering at 405 nm for samples of **P2** and **P3** in the presence of Con A and with Con A and 0.1 M glucose. [Con A] = 1 mg/mL; Tris buffer, pH = 7.2; each data point is the average of three samples and error bars show 1 standard deviation.

by multivalent polysaccharides and the tetravalent lectin.[23b,c] On a mass concentration basis, **P2** showed the largest increase in turbidity in the presence of Con A (Figure 7B, red diamonds). No significant response was observed for **P1**. It has previously been noted that polymers that cannot span the distance of approximately 6.5 nm between adjacent carbohydrate binding sites in a Con A tetramer show lower binding affinities because they cannot readily engage in multivalent interactions.[43] On the basis of measurements made from modeling, **P1** would be between 6 and 7 nm long if fully extended and therefore may not be able to benefit from significant multivalent interactions when it binds. **P2** showed more intense scattering when bound as compared to **P3** (yellow triangles), when evaluated on a mass concentration basis. To confirm that **P2** and **P3** bind Con A at the same site as natural carbohydrates, 0.1 M glucose was shown to inhibit aggregation (gray squares, blue circles, respectively).[23b] Glucose inhibition of binding was nearly complete for both **P2** and **P3**. A minimal amount of scattering remains in the presence of glucose which is also observed when the binding of glycogen to Con A is inhibited with 0.1 M glucose using these assay conditions.

We suggest two explanations for the higher response from **P2** as compared to **P3**. First, Con A may have a preference for binding at the end of polymer chains rather than at inner residues due to steric issues.[7c] At a given mass concentration (mg/mL), a solution of **P2** will contain more polymer ends for binding because **P2** is significantly shorter and weighs less than **P3**. Second, the solution aggregation state of **P3** may differ significantly from that of **P2**. **P3** may be aggregated in a manner than makes its sugar residues less available for binding. We infer this difference in aggregation behavior from the lower solubility of **P3** as compared to **P2**.

CONCLUSION

In summary, *α*-*N*-1,2-D-glucose poly-amido-saccharides (PASs) are novel carbohydrate-derived polymers in which the ether linkage found in natural polysaccharides is replaced with an amide linkage. They are synthesized using the anionic ring-opening polymerization of a chiral *β*-lactam monomer derived from benzyl-protected D-glucal. The mild and high-yielding polymerization method provides materials of controlled molecular weight and narrow dispersity. After debenzylation, the resulting hydrophilic *β*-polypeptides contain a rigid pyranose ring in the main chain with a 1,2-linkage of defined stereochemistry that molecular modeling suggests may promote a left-handed helical structure. Computational modeling results suggest that the monomer is highly reactive in part because of additional ring strain induced by the half-chair conformation of the pyranose ring. In addition, modeling reveals that negative steric effects due to the benzyl groups appear to be minimized because of the monomer's geometry and the flexibility of the ether bond. Characterization of model compounds and polymers using NMR, GPC, DLS, and IR confirms that the polymers have the desired molecular structure. Finally, glucose-dependent binding of higher molecular weight *α*-*N*-1,2-D-glc PASs to the plant lectin concanavalin A demonstrates the potential of PASs to interact with natural carbohydrate receptors. Future studies will focus on expanding the range of structures that can be synthesized, further understanding the solution and solid-state aggregation structures, and identifying biomedical applications where *α*-*N*-1,2-D-glc PASs may offer unique advantages.

ASSOCIATED CONTENT

Supporting Information

Experimental details, supplemental figures, IR spectra, ^{1}HNMR, gCOSY, ^{13}C spectra, XRCS details, and xyz coordinates and energies for computational models. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author

mgrin@bu.edu

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

E.L.D. acknowledges receipt of an NIH/NIGMS Postdoctoral Fellowship (1F32GM097781). The authors thank Aaron Colby for help obtaining SEM images, Andrew Lynch and Prof. Karen Allen for help obtaining DLS data, and Dr. Jeffrey Bacon for collecting X-ray crystal diffraction data and solving the structure.

REFERENCES

(1) Galbis, J. A.; García-Martín, M. G. In *Monomers, Polymers and Composites from Renewable Resources*; Mohamed Naceur, B., Alessandro, G., Eds.; Elsevier: Amsterdam, 2008; p 89.

(2) (a) Dwek, R. A. *Chem. Rev.* **1996**, *96*, 683. (b) Dumitriu, S. *Polysaccharides: Structural Diversity and Functional Versatility*; 2nd ed.; Marcel Dekker, Inc.: New York, 2005; (c) Stern, R.; Jedrzejas, M. J. *Chem. Rev.* **2008**, *108*, 5061. (d) Ernst, B.; Magnani, J. L. *Nat. Rev. Drug Discovery* **2009**, *8*, 661.

(3) Kumar, M. N. V. R.; Muzzarelli, R. A. A.; Muzzarelli, C.; Sashiwa, H.; Domb, A. J. *Chem. Rev.* **2004**, *104*, 6017.

(4) (a) Lapčík, L., Jr.; De Smedt, S.; Demeester, J.; Chabreček, P. *Chem. Rev.* **1998**, *98*, 2663. (b) Burdick, J. A.; Prestwich, G. D. *Adv. Mater.* **2011**, *23*, H41.

(5) (a) Marchessault, R. H.; Ravenelle, F.; Zhu, X. X. *Polysaccharides for Drug Delivery and Pharmaceutical Applications*; Oxford University Press: New York, NY, 2006; (b) Rinaudo, M. *Polym. Int.* **2008**, *57*, 397. (c) Burdick, J. A.; Mauck, R. L. *Biomaterials for Tissue Engineering Applications*; Springer-Verlag/Wien: New York, NY, 2011.

(6) (a) Nishimura, S.; Matsuoka, K.; Furuike, T.; Ishii, S.; Kurita, K.; Nishimura, K. M. *Macromolecules* **1991**, *24*, 4236. (b) Mortell, K. H.; Gingras, M.; Kiessling, L. L. *J. Am. Chem. Soc.* **1994**, *116*, 12053. (c) Fraser, C.; Grubbs, R. H. *Macromolecules* **1995**, *28*, 7248. (d) Havard, J. M.; Vladimirov, N.; Fréchet, J. M. J.; Yamada, S.; Willson, C. G.; Byers, J. D. *Macromolecules* **1998**, *32*, 86. (e) Grande, D.; Baskaran, S.; Chaikof, E. L. *Macromolecules* **2001**, *34*, 1640. (f) Wang, Q.; Dordick, J. S.; Linhardt, R. J. *Chem. Mater.* **2002**, *14*, 3232. (g) Baskaran, S.; Grande, D.; Sun, X.-L.; Yayon, A.; Chaikof, E. L. *Bioconjugate Chem.* **2002**, *13*, 1309. (h) Sun, X.-L.; Grande, D.; Baskaran, S.; Hanson, S. R.; Chaikof, E. L. *Biomacromolecules* **2002**, *3*, 1065. (i) Ladmiral, V.; Melia, E.; Haddleton, D. M. *Eur. Polym. J.* **2004**, *40*, 431. (j) Papp, I.; Dernedde, J.; Enders, S.; Haag, R. *Chem. Commun.* **2008**, 5851. (k) Becer, C. R.; Gibson, M. I.; Geng, J.; Ilyas, R.; Wallis, R.; Mitchell, D. A.; Haddleton, D. M. *J. Am. Chem. Soc.* **2010**, *132*, 15130. (l) Lee, S.-G.; Brown, J. M.; Rogers, C. J.; Matson, J. B.; Krishnamurthy, C.; Rawat, M.; Hsieh-Wilson, L. C. *Chem. Sci.* **2010**, *1*, 322. (m) Kramer, J. R.; Deming, T. J. *J. Am. Chem. Soc.* **2010**, *132*, 15068. (n) Kramer, J. R.; Deming, T. J. *J. Am. Chem. Soc.* **2012**, *134*, 4112.

(7) (a) Roy, R.; Zanini, D.; Meunier, S. J.; Romanowska, A. *J. Chem. Soc., Chem. Commun.* **1993**, 1869. (b) Aoi, K.; Itoh, K.; Okada, M. *Macromolecules* **1995**, *28*, 5391. (c) Woller, E. K.; Walter, E. D.; Morgan, J. R.; Singel, D. J.; Cloninger, M. J. *J. Am. Chem. Soc.* **2003**, *125*, 8820. (d) Wolfenden, M. L.; Cloninger, M. J. *J. Am. Chem. Soc.* **2005**, *127*, 12168. (e) Chabre, Y. M.; Roy, R. *Adv. Carbohydr. Chem. Biochem.* **2010**, *63*, 165.

(8) (a) Kurita, K.; Masuda, N.; Aibe, S.; Murakami, K.; Ishii, S.; Nishimura, S.-I. *Macromolecules* **1994**, *27*, 7544. (b) Chen, X.; Gross, R. A. *Macromolecules* **1998**, *32*, 308. (c) Shen, Y.; Chen, X.; Gross, R. A. *Macromolecules* **1999**, *32*, 2799. (d) Shen, Y.; Chen, X.; Gross, R. A. *Macromolecules* **1999**, *32*, 3891. (e) Meier, S.; Reisinger, H.; Haag, R.; Mecking, S.; Mulhaupt, R.; Stelzer, F. *Chem. Commun.* **2001**, 855. (f) Kumar, R.; Gao, W.; Gross, R. A. *Macromolecules* **2002**, *35*, 6835. (g) Haba, O.; Tomizuka, H.; Endo, T. *Macromolecules* **2005**, *38*, 3562. (h) Gao, W.; Hagver, R.; Shah, V.; Xie, W.; Gross, R. A.; Ilker, M. F.; Bell, C.; Burke, K. A.; Coughlin, E. B. *Macromolecules* **2006**, *40*, 145. (i) Lienkamp, K.; Kins, C. F.; Alfred, S. F.; Madkour, A. E.; Tew, G. N. *J. Polym. Sci., Part A: Polym. Chem.* **2009**, *47*, 1266. (j) Wathier, M.; Stoddart, S. S.; Sheehy, M. J.; Grinstaff, M. W. *J. Am. Chem. Soc.* **2010**, *132*, 15887.

(9) (a) García-Martín, M. d. G.; de Paz Báñez, M. V.; Galbis, J. A. *J. Carbohydr. Chem.* **2000**, *19*, 805. (b) García-Martín, M. d. G.; Báñez, M. V. d. P.; Galbis, J. A. *J. Carbohydr. Chem.* **2001**, *20*, 145. (c) Metzke, M.; Bai, J. Z.; Guan, Z. *J. Am. Chem. Soc.* **2003**, *125*, 7760. (d) Metzke, M.; Guan, Z. *Biomacromolecules* **2007**, *9*, 208. (e) Urakami, H.; Guan, Z. *Biomacromolecules* **2008**, *9*, 592. (f) Liao, S. W.; Yu, T.-B.; Guan, Z. *J. Am. Chem. Soc.* **2009**, *131*, 17638.

(10) Kobayashi, K.; Tsuchida, A.; Usui, T.; Akaike, T. *Macromolecules* **1997**, *30*, 2016.

(11) (a) Seeberger, P. H.; Haase, W.-C. *Chem. Rev.* **2000**, *100*, 4349. (b) Gruner, S. A. W.; Locardi, E.; Lohof, E.; Kessler, H. *Chem. Rev.* **2002**, *102*, 491.

(12) Stick, R. V.; Williams, S. J. In *Carbohydrates The Essential Molecules of Life*; 2nd ed.; Elsevier: Oxford, 2009; p 203.

(13) (a) Frechet, J.; Schuerch, C. *J. Am. Chem. Soc.* **1969**, *91*, 1161. (b) Zachoval, J.; Schuerch, C. *J. Am. Chem. Soc.* **1969**, *91*, 1165. (c) Schuerch, C. *Acc. Chem. Res.* **1973**, *6*, 184.

(14) Nakatsubo, F.; Kamitakahara, H.; Hori, M. *J. Am. Chem. Soc.* **1996**, *118*, 1677.

(15) Kadokawa, J.-i.; Kasai, S.; Watanabe, Y.; Karasu, M.; Tagaya, H.; Chiba, K. *Macromolecules* **1997**, *30*, 8212.

(16) (a) Penczek, S. *Models of Biopolymers By Ring-Opening Polymerization*; CRC Press: Boca Raton, Fl, 1989; (b) Klemm, D.; Heublein, B.; Fink, H.-P.; Bohn, A. *Angew. Chem., Int. Ed.* **2005**, *44*, 3358.

(17) Lichtenthaler, F. W. In *Ullmann's Encyclopedia of Industrial Chemistry*; Wiley-VCH Verlag: New York, 2000.

(18) (a) Weigel, P. H.; DeAngelis, P. L. *J. Biol. Chem.* **2007**, *282*, 36777. (b) Kadokawa, J.-i. *Chem. Rev.* **2011**, *111*, 4308.

(19) (a) Risseeuw, M. D. P.; Overhand, M.; Fleet, G. W. J.; Simone, M. I. *Tetrahedron: Asymmetry* **2007**, *18*, 2001. (b) Fuchs, E.-F.; Lehmann, J. *Carbohydr. Res.* **1976**, *49*, 267. (c) Mostowicz, D.; Chmielewski, M. *Carbohydr. Res.* **1994**, *257*, 137. (d) Suhara, Y.; Hildreth, J. E. K.; Ichikawa, Y. *Tetrahedron Lett.* **1996**, *37*, 1575. (e) Graf von Roedern, E.; Lohof, E.; Hessler, G.; Hoffmann, M.; Kessler, H. *J. Am. Chem. Soc.* **1996**, *118*, 10156. (f) Suhara, Y.; Ichikawa, M.; Hildreth, J. E. K.; Ichikawa, Y. *Tetrahedron Lett.* **1996**, *37*, 2549. (g) Timmers, C. M.; Turner, J. J.; Ward, C. M.; van der Marel, G. A.; Kouwijzer, M. L. C. E.; Grootenhuis, P. D. J.; van Boom, J. H. *Chem.—Eur. J.* **1997**, *3*, 920. (h) Sabesan, S. *Tetrahedron Lett.* **1997**, *38*, 3127. (i) Nishimura, S.-I. *Chem. Commun.* **1998**, 617. (j) Suhara, Y.; Yamaguchi, Y.; Collins, B.; Schnaar, R. L.; Yanagishita, M.; Hildreth, J. E. K.; Shimada, I.; Ichikawa, Y. *Bioorg. Med. Chem.* **2002**, *10*, 1999. (k) Gervay-Hague, J.; Weathers, J. T. M. *J. Carbohydr. Chem.* **2002**, *21*, 867. (l) Gruner, S. A. W.; Truffault, V.; Voll, G.; Locardi, E.; Stöckle, M.; Kessler, H. *Chem.—Eur. J.* **2002**, *8*, 4365. (m) Sharma, G. V. M; Reddy, K. R.; Krishna, P. R.; Sankar, A. R.; Narsimulu, K.; Kumar, S. K.; Jayaprakash, P.; Jagannadh, B.; Kunwar, A. C. *J. Am. Chem. Soc.* **2003**, *125*, 13670. (n) Fujimura, F.; Hirata, T.; Morita, T.; Kimura, S.; Horikawa, Y.; Sugiyama, J. *Biomacromolecules* **2006**, *7*, 2394. (o) Saludes, J. P.; Ames, J. B.; Gervay-Hague, J. *J. Am. Chem. Soc.* **2009**, *131*, 5495. (p) Soengas, R. G.; Estévez, A. M.; Estévez, J. C.; Estévez, R. J. *C. R. Chim.* **2011**, *14*, 313.

(20) (a) Gervay, J.; Flaherty, T. M.; Nguyen, C. *Tetrahedron Lett.* **1997**, *38*, 1493. (b) Szabo, L.; Smith, B. L.; McReynolds, K. D.; Parrill, A. L.; Morris, E. R.; Gervay, J. *J. Org. Chem.* **1998**, *63*, 1074. (c) Gregar, T. Q.; Gervay-Hague, J. *J. Org. Chem.* **2003**, *69*, 1001.

(21) (a) Appella, D. H.; Christianson, L. A.; Klein, D. A.; Powell, D. R.; Huang, X.; Barchi, J. J.; Gellman, S. H. *Nature* **1997**, *387*, 381. (b) Gellman, S. H. *Acc. Chem. Res.* **1998**, *31*, 173. (c) Cheng, R. P.; Gellman, S. H.; DeGrado, W. F. *Chem. Rev.* **2001**, *101*, 3219. (d) García-Martín, M. d. G.; de Paz Báñez, M. V.; García-Alvarez, M.; Muñoz-Guerra, S.; Galbis, J. A. *Macromolecules* **2001**, *34*, 5042. (e) Hill, D. J.; Mio, M. J.; Prince, R. B.; Hughes, T. S.; Moore, J. S. *Chem. Rev.* **2001**, *101*, 3893. (f) Horne, W. S.; Gellman, S. H. *Acc. Chem. Res.* **2008**, *41*, 1399. (g) Seebach, D.; Gardiner, J. *Acc. Chem. Res.* **2008**, *41*, 1366. (h) Lee, M.-R.; Stahl, S. S.; Gellman, S. H.; Masters, K. S. *J. Am. Chem. Soc.* **2009**, *131*, 16779. (i) Mowery, B. P.; Lindner, A. H.; Weisblum, B.; Stahl, S. S.; Gellman, S. H. *J. Am. Chem. Soc.* **2009**, *131*, 9735. (j) Dohm, M. T.; Mowery, B. P.; Czyzewski, A. M.; Stahl, S. S.; Gellman, S. H.; Barron, A. E. *J. Am. Chem. Soc.* **2010**, *132*, 7957. (k) Zhang, J.; Markiewicz, M. J.; Mowery, B. P.; Weisblum, B.; Stahl, S. S.; Gellman, S. H. *Biomacromolecules* **2011**, *13*, 323. (l) Liu, R.; Masters, K. S.; Gellman, S. H. *Biomacromolecules* **2012**, *13*, 1100.

(22) (a) Appella, D.; Christianson, L. A.; Karle, I. L.; Powell, D. R.; Gellman, S. *J. Am. Chem. Soc.* **1996**, *118*, 13071. (b) Mándity, I. n. M.;

Fülöp, L.; Vass, E. r.; Tóth, G. b. K.; Martinek, T. s. A.; Fülöp, F. *Org. Lett.* **2010**, *12*, 5584.
(23) (a) Goldstein, I. J.; Hollerman, C. E.; Smith, E. E. *Biochemistry* **1965**, *4*, 876. (b) Doyle, R. J.; Woodside, E. E.; Fishel, C. W. *Biochem. J.* **1968**, *106*, 35. (c) Colonna, P.; Biton, V.; Mercier, C. *Carbohydr. Res.* **1985**, *137*, 151.
(24) Yang, Q.-C.; Seiler, P.; Dunitz, J. D. *Acta Crystallogr., Sect. C: Cryst. Struct. Commun.* **1987**, *43*, 565.
(25) Urbancyzk-Lipkowska, Z.; Suwinska, K.; Luboradzki, R.; Mostowicz, D.; Kaluza, Z.; Grodner, J.; Chmielewski, M. *Carbohydr. Res.* **1994**, *256*, 1.
(26) Argay, G.; Fabian, L.; Kalman, A.; Bernath, G.; Gyarmati, Z. C. *Acta Crystallogr., Sect. E: Struct. Rep. Online* **2004**, *60*, o170.
(27) Alecu, I. M.; Zheng, J.; Zhao, Y.; Truhlar, D. G. *J. Chem. Theory Comput.* **2010**, *6*, 2872.
(28) (a) Šebenda, J.; Hauer, J.; Biroš, J. *J. Polym. Sci., Polym. Chem. Ed.* **1976**, *14*, 2357. (b) Šebenda, J.; Hauer, J. *Polym. Bull.* **1981**, *5*, 529. (c) Hashimoto, K.; Oi, T.; Yasuda, J.; Hotta, K.; Okada, M. *J. Polym. Sci., Part A: Polym. Chem.* **1997**, *35*, 1831. (d) Hashimoto, K.; Yasuda, J.; Kobayashi, M. *J. Polym. Sci., Part A: Polym. Chem.* **1999**, *37*, 909. (e) Hashimoto, K. *Prog. Polym. Sci.* **2000**, *25*, 1411. (f) Cheng, J.; Deming, T. J. *J. Am. Chem. Soc.* **2001**, *123*, 9457. (g) Zhang, J.; Kissounko, D. A.; Lee, S. E.; Gellman, S. H.; Stahl, S. S. *J. Am. Chem. Soc.* **2009**, *131*, 1589. (h) Zhang, J.; Gellman, S. H.; Stahl, S. S. *Macromolecules* **2010**, *43*, 5618. (i) Zhang, J.; Markiewicz, M. J.; Weisblum, B.; Stahl, S. S.; Gellman, S. H. *ACS Macro Lett.* **2012**, 714.
(29) Hashimoto, K.; Mori, K.; Okada, M. *Macromolecules* **1992**, *25*, 2592.
(30) Chmielewski, M.; Kaluza, Z. *Carbohydr. Res.* **1987**, *167*, 143.
(31) Kałuża, Z.; Abramski, W.; Bełżecki, C.; Grodner, J.; Mostowicz, D.; Urbański, R.; Chmielewski, M. *Synlett* **1994**, 539.
(32) Wheeler, S. E.; Houk, K. N.; Schleyer, P. v. R.; Allen, W. D. *J. Am. Chem. Soc.* **2009**, *131*, 2547.
(33) Roux, M. V.; Jiménez, P.; Dávalos, J. Z.; Castaño, O.; Molina, M. T.; Notario, R.; Herreros, M.; Abboud, J. L. M. *J. Am. Chem. Soc.* **1996**, *118*, 12735.
(34) Kishore, N.; Tewari, Y. B.; Yap, W. T.; Goldberg, R. N. *Biophys. Chem.* **1994**, *49*, 163.
(35) (a) Cubbon, R. C. P. *Makromol. Chem.* **1964**, *80*, 44. (b) Šebenda, J. *Pure Appl. Chem.* **1976**, *48*, 329.
(36) Chmielewski, M.; Kaluza, Z.; Dodziuk, H.; Suwinska, K.; Rosenbaum, D.; Duddeck, H.; Magnus, P. D.; Huffman, J. C. *Carbohydr. Res.* **1990**, *203*, 183.
(37) Whistler, R. L. In *Carbohydrates in Solution*; American Chemical Society: Washington, DC, 1973; Vol. *117*, p 242.
(38) Dickson, L.; Matuszak, C. A.; Qazi, A. H. *J. Org. Chem.* **1978**, *43*, 1007.
(39) Podlasek, C. A.; Wu, J.; Stripe, W. A.; Bondo, P. B.; Serianni, A. S. *J. Am. Chem. Soc.* **1995**, *117*, 8635.
(40) Forrest, T. P. *Can. J. Chem.* **1974**, *52*, 4095.
(41) (a) Seebach, D.; Overhand, M.; Kühnle, F. N. M.; Martinoni, B.; Oberer, L.; Hommel, U.; Widmer, H. *Helv. Chim. Acta* **1996**, *79*, 913. (b) Cheng, J.; Deming, T. J. *Macromolecules* **2001**, *34*, 5169.
(42) Abele, S.; Guichard, G.; Seebach, D. *Helv. Chim. Acta* **1998**, *81*, 2141.
(43) (a) Mortell, K. H.; Weatherman, R. V.; Kiessling, L. L. *J. Am. Chem. Soc.* **1996**, *118*, 2297. (b) Kanai, M.; Mortell, K. H.; Kiessling, L. L. *J. Am. Chem. Soc.* **1997**, *119*, 9931. (c) Mann, D. A.; Kanai, M.; Maly, D. J.; Kiessling, L. L. *J. Am. Chem. Soc.* **1998**, *120*, 10575.