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Published on Web 01/06/2009

Access to Poly-β-Peptides with Functionalized Side Chains and End Groups via Controlled Ring-Opening Polymerization of β-Lactams

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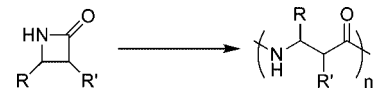
Abstract: Poly-β-peptides are attractive for biomedical applications because the backbone is similar enough to that of proteins for biocompatibility, but the backbone is sufficiently unnatural that these polymers evade proteolytic degradation. Prior investigations of poly-β-peptides have been hindered by two principal limitations: (1) most known examples are insoluble, and (2) the range of accessible side chain functionality has been quite limited (mostly simple hydrocarbon units). The present study describes innovations in poly-β-peptide synthesis that enable the preparation of diversely functionalized examples and provide the basis for broad exploration of the properties and applications of these nylon-3 materials. We describe several β-lactams with a protected amino group in their side chain that readily undergo ring-opening polymerization (ROP). These monomers are available in large quantities via *N*-chlorosulfonylisocyanate (CSI) cycloaddition reactions with functionalized alkenes; previously CSI reactions have been limited to alkenes with hydrocarbon substituents. Postpolymerization deprotection of the amino groups leads to water-soluble poly-β-peptides. In addition, we introduce a simple co-initiation strategy that allows placement of a wide variety of functional groups at the *N*-termini of poly-β-peptide chains. ROP involving the new β-lactams and co-initiation strategy exhibits characteristics of a controlled polymerization and enables the preparation of amphiphilic block copolymers. We have recently shown that cationic copoly-β-peptides made available by these innovations mimic the selective antibacterial activity of host-defense peptides; the results described here provide the foundation for further exploration of this valuable activity and for the pursuit of other biological applications such as DNA/siRNA delivery and tissue engineering.

Introduction

Biologically compatible polymers have attracted widespread interest for biomedical applications, including gene delivery, tissue engineering, and therapeutics.[1] Poly-α-peptides derived from ring-opening polymerization (ROP) of α-amino acid-*N*-carboxyanhydrides (α-NCAs) constitute a prominent class of such materials[2] and have been studied for nearly a century.[3] The recent demonstration that discrete β-peptide oligomers mimic many physical and structural properties of α-peptides and can be engineered to exhibit a variety of biological activities[4] has contributed to a renewed interest in poly-β-peptides.[5] Early interest in these polymers arose from commercial applications of polyamide fibers (nylon materials). Poly-β-peptides (nylon-3 family) have traditionally been prepared via anionic ring-opening polymerization (ROP) of β-lactams (eq 1).[6] Exploration of poly-β-peptide properties has been hampered, however, by the insolubility typically displayed by these polymers.[6a] The very limited side-chain diversity among known poly-β-peptides represents another hindrance to the exploration of their behavior and potential applications: nearly all reported examples have only hydrocarbon units or alkyl esters appended to the backbone. This limitation in functionality is related to constraints on β-lactam synthesis. The most convenient method for multigram-scale preparation of β-lactams involves addition of *N*-chlorosulfonylisocyanate (CSI) to alkenes; however, use of this reaction has been limited to purely hydrocarbon alkenes.



(1)

Here we describe two innovations that should significantly expand the range of available poly-β-peptides and thereby enable broad exploration of biomedical applications of these materials with a uniquely protein-mimetic backbone. First, we show that CSI can be used to prepare β-lactams that contain protected amino groups compatible with anionic ROP. These building blocks provide ready access to cationic poly-β-peptides. Second,

(1) (a) Kiick, K. L. *Science* **2007**, *317*, 1182–1183. (b) Langer, R.; Tirrell, D. A. *Nature* **2004**, *428*, 487–492. (c) Haag, R.; Kratz, F. *Angew. Chem., Int. Ed.* **2006**, *45*, 1198–1215.
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(3) (a) Kricheldorf, H. R. *Models of Biopolymers by Ring-Opening Polymerization*; Penczek, S., Ed.; CRC: Boca Raton, 1990. (b) Deming, T. J. *Adv. Polym. Sci.* **2006**, *202*, 1–18. (c) Kricheldorf, H. R. *Angew. Chem., Int. Ed.* **2006**, *45*, 5752–5784.
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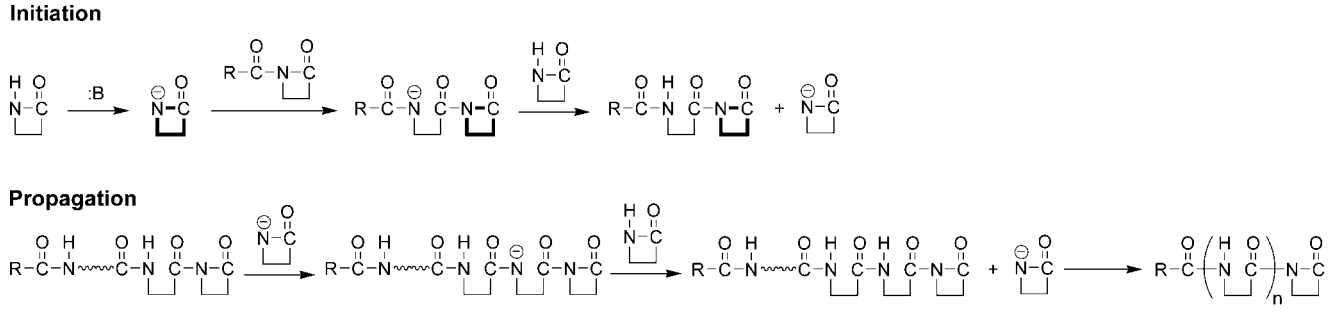
Scheme 1. General Mechanism of Imide-Initiated Anionic Ring-Opening Polymerization (ROP) of β-Lactams



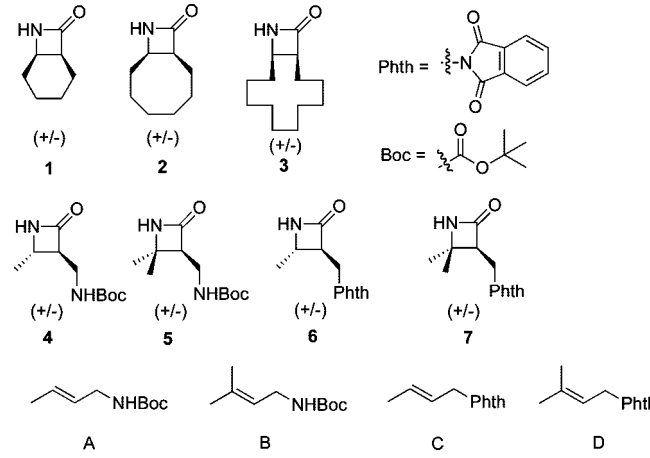
we show that a wide variety of acylating agents can be used for in situ generation of ROP co-initiators. This co-initiator strategy allows convenient placement of diverse functional groups at the *N*-termini of poly-β-peptide chains.

Our findings are significant because they make available a wide array of previously inaccessible poly-β-peptides. This synthetic access will enable broad exploration of biological applications of these materials. We have recently discovered[7] that sequence-random β-peptide copolymers bearing hydrophobic and cationic side chains mimic the activity profile of naturally occurring host-defense peptides[8] by disruption of the membranes of bacteria but not eukaryotic cells. Such findings have important implications because random-copolymer antibacterial agents are much easier to prepare than are sequence-specific antibacterial peptides. Preparation of these antibacterial poly-β-peptides required the developments described here, as will further studies aimed at optimizing this valuable activity or exploring other biological applications of these protein-mimetic polymers, such as DNA or siRNA delivery, antiviral activity, and tissue engineering.

Results and Discussion

***β*-Lactam Synthesis.** The cycloaddition reaction between *N*-chlorosulfonylisocyanate (CSI) and an alkene has long been used for efficient and large-scale preparation of β-lactams.[9] We employed this method, for example, to prepare β-lactams **1**, **2**, and **3** from cyclohexene, cyclooctene, and cyclododecene, respectively. To our knowledge, the use of CSI to prepare β-lactams previously has been limited to purely hydrocarbon alkene starting materials, as in these examples. Poly-β-peptides generated via ROP of β-lactams with hydrocarbon side chains are generally highly insoluble, as is the case for poly-**1**. Poly-**2** and poly-**3**, however, are quite soluble in CH_2Cl_2, THF, and other common solvents, a property that facilitates GPC characterization and systematic study of the polymerization reactions, as discussed below.

Our interest in antibacterial and other biomedical applications required that we be able to introduce cationic side chains into poly-β-peptides. We therefore evaluated the reaction of CSI with alkenes **A** and **B**, which contain Boc-protected amino groups. These reactions did not provide the desired β-lactams, **4** and **5**, perhaps because of Boc group instability under the reaction conditions. Phthalimide-bearing alkenes **C** and **D** are synthetic precursors to **A** and **B**, respectively, and these alkenes turned out to be excellent substrates for the CSI cycloaddition reaction. β-Lactams **6** and **7** could be readily prepared in multigram quantities. The amino groups could be liberated via treatment with hydrazine and then Boc-protected, to generate **4** and **5**. Our initial polymerization efforts focused on these Boc-containing β-lactams, because the Boc group is much easier to deprotect than the phthalimide group.



Polymerization. Anionic ring-opening polymerization (ROP) is the most common method for generating poly-β-peptides from β-lactams,[6a] and we used this technique for our studies.[10] The well-accepted mechanism for anionic β-lactam ROP involves nucleophilic attack of a β-lactamate anion on the carbonyl group of an imide at the polymer chain end (Scheme 1). The β-lactamate anion is formed initially by reaction of the β-lactam and a strong base, and the β-lactamate is then regenerated via proton transfer from β-lactam to the amidate anion formed in the growing polymer backbone. β-Lactam-derived imides such as **I1** (eq 2) are commonly used as "co-initiators," together with a base "initiator," to promote β-lactam ROP.[6a] Use of the imide,

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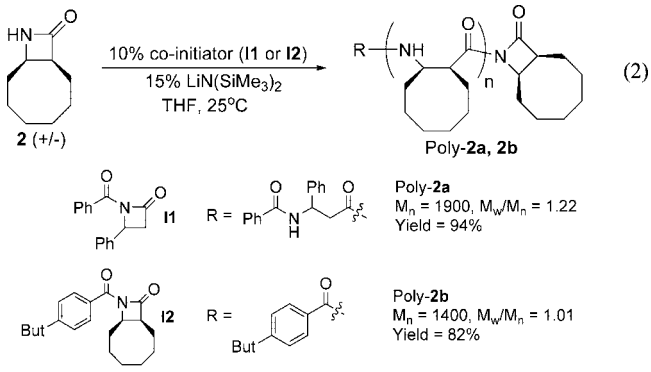
(10) We have examined poly-β-peptides with DP < 20 in some cases. Despite their minimal length, these materials are referred to as "polymers".

which is much more electrophilic than a β-lactam, ensures that chain initiation occurs rapidly, thereby enabling a controlled polymerization process. The optimum conditions we identified involved use of co-initiator **I1** or **I2** with the strong but non-nucleophilic base $LiN(SiMe_3)_2$ in THF (eq 2). Several metal-based initiators for ROP of β-lactams have been previously reported; however, these efforts were constrained by challenges of limited solubility and functionality, and little follow-up work has been reported since the original reports in 2001.[5a] One of the metal-based initiators, $Sc[N(SiMe_3)_2]_3$, was reported to initiate the polymerization of β-lactams bearing a single ester-containing side chain. However, our attempts to polymerize β-lactams **1**−**7** with this initiator were unsuccessful; starting material was recovered quantitatively from the attempted reactions. These results suggest that $Sc[N(SiMe_3)_2]_3$ might be inactive with more highly substituted β-lactams.

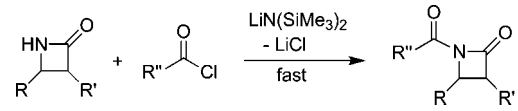


(2)

Consistent with previous reports,[6] we found that many β-lactams bearing aliphatic or aromatic hydrocarbon side chains, such as cyclohexene-derived **1**, produce polymers that do not dissolve in common organic solvents such as THF, CH_2Cl_2, DMF, or DMSO.[11] We discovered, however, that the cyclooctene- and cyclododecene-derived β-lactams **2** and **3** yield homopolymers that are quite soluble in THF or CH_2Cl_2. The origin of the side-chain influence on polymer solubility is not well understood, but we speculate that the size of the large cycloalkyl rings hinders interchain hydrogen bonding, thereby promoting polymer solubility. β-Lactams bearing protected amino groups, **4**−**7**, also gave rise to organic-soluble homopolymers. In some cases (reactions of **2** or **5**), the initial THF reaction solution was slightly cloudy, apparently because of limited solubility of the β-lactamate anions; however, these reaction solutions became clear toward the end of polymerization. When the β-lactamate concentration was <3 mM, the solution remained homogeneous throughout the polymerization. It should be noted that these polymers and all others discussed below are heterochiral, because they are prepared from racemic β-lactams.

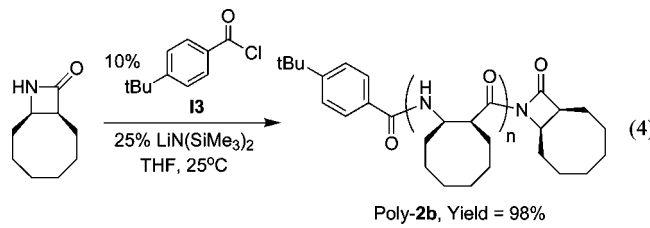
Anionic ROP of β-lactams **2**−**7** co-initiated by **I1** or **I2** generated the corresponding polymers in high yield (>96%). Gel permeation chromatographic (GPC) analysis of these materials suggested that the molecular weight distributions were generally narrow and unimodal, consistent with rapid chain initiation and the absence of side reactions that contribute to branched polymer chains. Neither additives nor elevated temperatures were required to characterize these polymers in organic solvents. In contrast, GPC analysis of other poly-β-peptides and related polyamides often is not possible or, in some favorable cases, can be achieved through the use of polar solvents such as dimethylformamide (DMF) or dimethylacetamide (DMAc) containing salts such as LiCl and LiBr to prevent polymer aggregation.[12] Poly-β-peptides generated from **2**−**7** were readily characterized by GPC using THF as eluent. Light scattering data indicated that there is no noticeable aggregation of these polymers in THF.

Acylating Agents as Co-initiators for β-Lactam Polymerization. When an imide is used to initiate β-lactam ROP, a fragment derived from the imide remains at the *N*-terminus of the polymer (Scheme 1). Therefore, the *N*-terminal functionality of poly-β-peptides can be controlled via choice of the imide co-initiator. β-Lactam-derived imides must be synthesized and purified prior to their use as ROP co-initiators, and we wondered whether *N*-terminal functionalization could be streamlined by generating the desired imide in situ. Specifically, we hypothesized that the imide could be formed by adding a reactive acylating agent, such as an acid chloride or an anhydride, together with a strong base to the solution of β-lactam monomer (eq 3). The imide formed in this way would then function as the co-initiator for ROP. Because a wide range of acid chlorides and anhydrides is readily available, this strategy constitutes a simple and versatile method for preparing poly-β-peptides with diverse *N*-terminal groups.



(3)

To test our hypothesis, we combined β-lactam **2** with 10 mol% 4-*tert*-butylbenzoyl chloride (**I3**) in THF and then added 25 mol% $LiN(SiMe_3)_2$ (eq 4). The formation of polymer was confirmed by GPC (M_n = 1350, M_w/M_n = 1.08) and MALDI-TOF MS (M_n = 1210, M_w/M_n = 1.05). ^{1}H NMR analysis of the isolated polymer, which was obtained by adding pentane to the solution and collecting the precipitate, revealed resonances in the aromatic region, suggesting incorporation of the *t*Bu-phenyl group. Integration of the terminal aromatic protons relative to the polymer backbone protons suggested that there are on average 10 β-amino acid residues in a single polymer chain (degree of polymerization (DP) = 10), consistent with expectations for a 1:10 co-initiator/monomer ratio. MALDI-TOF MS analysis of the polymer provided evidence for an end group molecular weight of 161, as expected for the *t*-Bu-benzoyl fragment.[13] This polymer had an identical chain end to that formed with imide co-initiator **I2**.



(4)

We compared the kinetics of β-lactam ROP co-initiated with imide **I1** and β-lactam ROP co-initiated with acid chloride **I3** by using GC to monitor the remaining β-lactam precursor over time. In both cases β-lactam **2** was completely depleted within ~10 min (Figure 1). This kinetic similarity suggests that in situ generation of **I1** from the acid chloride and β-lactamate occurs very rapidly relative to initiation of polymerization. It should

(11) See Supporting Information (Chart S1) for examples of monomers tested.

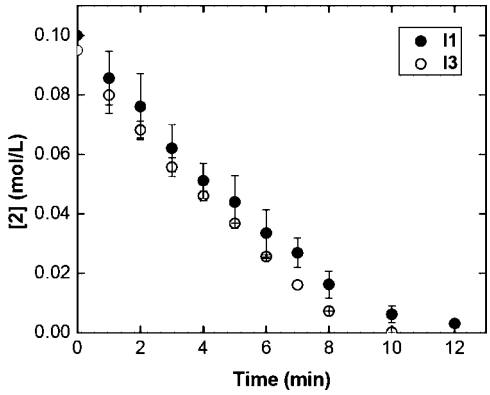


Figure 1. Polymerization of **2** with co-initiator **I1** or **I3** as a function of time, monitored by using GC to quantify remaining β-lactam. Conditions: 0.10 M **2** in THF; 5 mol% co-initiator; 7.5 mol% LiN(SiMe$_3$)$_2$ for **I1**, and 12.5 mol% LiN(SiMe$_3$)$_2$ for **I3**; 25 °C. GC internal standard: triphenylmethane. Note that 1 equiv of monomer is consumed during in situ imide formation when **I3** is used as the co-initiator; thus the data points are offset by approximately 1 equiv of monomer relative to co-initiator.

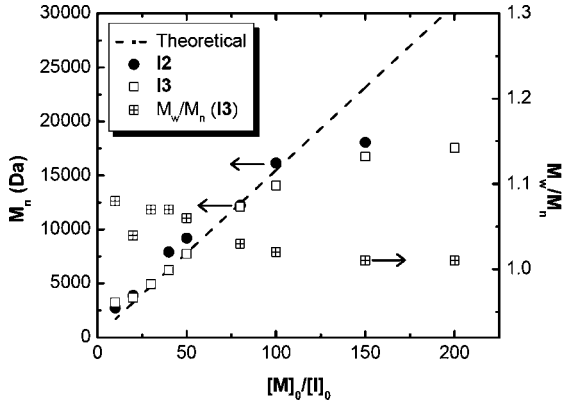


Figure 2. Molecular weight, M_n, versus initial monomer to co-initiator ratio, $[M]_0/[I]_0$ for poly-**2b**. Theoretical M_n, M_n of polymers obtained with co-initiator **I2** and **I3**, and molecular weight distribution (M_w/M_n) of polymers obtained with **I3** are shown. M_n and M_w/M_n values were determined by GPC-MALS in THF with dn/dc = 0.138. Conditions: $[M]_0$ = 0.1 M, 2.0 equiv of LiN(SiMe$_3$)$_2$ (relative to **I3**), THF, 25 °C.

be noted that when an imide co-initiator is employed, 1 equiv of base per equiv of co-initiator is used, but when an acid chloride is employed, 2 equiv of base are required relative to the co-initiator because 1 equiv of base is consumed to generate the imide in situ. Since β-lactams such as **2** can undergo base-induced polymerization in the absence of imide co-initiator (unactivated polymerization), large excesses of base relative to the acid chloride (>2.5 equiv) should be avoided.

Mechanistic Studies of the Polymerization Reaction. The polymerization mechanism shown in Scheme 1 predicts that β-lactam ROP should exhibit characteristics associated with a "living" process. We tested this hypothesis in several ways. We first examined the relationship between polymer molecular weight and initial monomer-to-co-initiator ratio ($[M]_0/[I]_0$), using either preformed imide **I2** or acid chloride **I3** as co-initiator. A linear relationship was found when $[M]_0/[I]_0 < 100$ (Figure 2). For the resulting polymers, the measured molecular weight, M_n, was very close to the theoretical molecular weight, $M_{n(theo)}$, as calculated by eq 5, which assumes complete conversion of monomer to polymer. Moreover, these polymers exhibited narrow molecular weight distributions (MWD), indicated by the low polydispersities ($M_w/M_n < 1.1$). These results show that

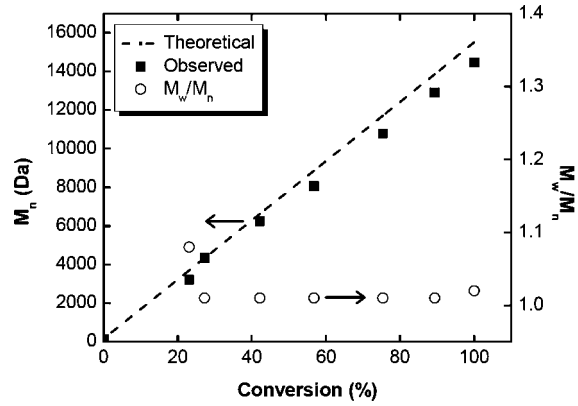


Figure 3. Dependence of molecular weight of poly-**2b** on monomer conversion. Polymerization was carried out in THF at 25 °C using **I3** with $[M]_0$ = 0.10 M, $[M]_0/[I]_0$ = 100 and 2 mol% LiN(SiMe$_3$)$_2$. Conversion was determined by monitoring monomer concentration *via* GC using triphenylmethane as internal standard. M_n and M_w/M_n values were obtained by GPC-MALS in THF with dn/dc = 0.138.

the molecular weight of poly-**2** can be precisely controlled up to ~15000, consistent with a controlled polymerization process. That polymer samples obtained with either of the two co-initiators, imide **I2** or acid chloride **I3**, displayed almost identical molecular weights suggests that conversion of the acid chloride to the imide in situ is nearly quantitative.

$$M_{n(theo)} = M_{(monomer)} \times \frac{[M]_0}{[I]_0} \times \text{Conv.} + M_{(initiator)} \qquad (5)$$

For $[M]_0/[I]_0 > 100$, a negative deviation from the theoretical molecular weight was observed with both co-initiators. These deviations arise from incomplete conversion of the monomer; unreacted β-lactam was detected after the polymerization. Extending the reaction time did not increase the extent of β-lactam conversion, suggesting that β-lactam ROP suffers from chain termination for $[M]_0/[I]_0 > 100$. The highest molecular weight we have achieved for poly-**2b** is ~20000. Although this molecular weight restriction represents a potential limitation of this polymerization method, the accessible molecular weight range is well-suited for biological applications of current interest, as shown by our recent results with antibacterial poly-β-peptides.[7]

The temporal evolution of poly-**2b** in the reaction co-initiated with acid chloride **I3** ($[M]_0/[I]_0$ = 100) was examined by quenching the polymerization solution at different times with methanol. The crude product was then directly analyzed by GC to determine the extent of monomer conversion and by GPC to determine polymer molecular weight. Figure 3 shows that polymer molecular weight (M_n) increases linearly as the extent of monomer conversion increases up to 100%, consistent with a controlled polymerization process that exhibits negligible chain termination or transfer. This conclusion is supported by the consistently low polydispersities observed throughout the polymerization process. The near-perfect agreement of the measured M_n with the theoretical M_n supports our previous conclusion that high initiation efficiency is achieved with acid chloride **I3**.

Acid chloride-initiated polymerization was successfully implemented with other β-lactams (**3**−**7**), and the resulting homopolymers were obtained in >93% yield (Table 1, entries 1−4). As observed for β-lactam **2**, good correlation between polymer molecular weight and $[M]_0/[I]_0$ was observed for **3**−**7**

Table 1. Homopolymerization and Random Copolymerization of β-Lactams Using Acid Chloride **I3** as Co-initiator[a]

entry	monomer(s)	$[M]_0/[I]_0$	time (h)	conv. (%)[b]	yield (%)[c]	$M_{n(theo)}$	M_n[d]	M_w/M_n[e]	DP[f]
1	**3**	10	3	>99	98	2254	1870[e]	1.10	8
2	**4**	10	1	>99	98	2303	1700[e]	1.16	7
3	**5**	20	3	>99	93	4725	4660	1.06	20
4	**7**	20	3	>99	98	5327	5610	1.03	21
5	**1** + **4**[g]	20[h]	2	>99	99	3555	4600	1.07	26
6	**2** + **5**[g]	20[h]	6	>99	96	3975	4460	1.05	22
7	**2** + **7**[g]	20[h]	6	>99	98	4273	5560	1.05	26

[a] Conditions: $[M]_0 = 0.1$ M, 2.0 equiv of $LiN(SiMe_3)_2$ (relative to **I3**) as base, THF as solvent, 25 °C. [b] Monomer conversion measured by GC. [c] Isolated yield. [d] Determined by GPC-MALS. dn/dc in THF (mL/g): poly-**5**, 0.074; poly-**7**, 0.133; poly-(**1**+**5**), 0.098; poly-(**2**+**5**), 0.136; poly-(**2**+**7**), 0.107. [e] Estimated by RI signal using PMMA as standard. [f] DP = degree of polymerization. [g] Random copolymerization with equal molar ratio of two monomers. [h] $[M]_0$ is the total concentration of the two monomers.

up to a limiting value of $[M]_0/[I]_0$. For the cyclododecyl- and phthalimide-containing monomers, **3**, **6**, and **7**, this limiting value was ~100, as found for **2**. For Boc-containing β-lactams **4** and **5**, however, controlled polymerization was observed only for $[M]_0/[I]_0 \leq 50$. In addition, the poly-β-peptides derived from **4** and **5** displayed a slightly broader molecular weight distribution ($M_w/M_n \approx 1.2$) than did the other poly-β-peptides. These observations suggest that the Boc-protected amino group in **4** and **5** may diminish polymerization performance, perhaps because of the moderately acidic proton of the urethane group or the incompatibility of the Boc group under the polymerization conditions. The phthalimide group, on the other hand, did not seem to interfere with polymerization relative to pure hydrocarbon substituents on the β-lactam. Binary random copolymerization of β-lactams proceeded smoothly (Table 1, entries 5−7). The resulting random copolymers showed the expected molecular weights and low polydispersities.

The proposed mechanism of β-lactam ROP (Scheme 1) reveals that the *C*-terminus of the growing polymer chain should contain a β-lactam-derived imide. The presence of an imide was confirmed by a ^{13}C labeling experiment: addition of $^{13}C(=O)$-labeled β-lactam **2** to the reaction mixture immediately following preparation of poly-**2b** from unlabeled **2** resulted in an average of one $^{13}C(=O)$ labeled monomer unit at the *C*-terminus of each polymer chain. The ^1H NMR spectrum of the resulting polymer was identical to that of unlabeled poly-**2b**; however, the ^{13}C NMR revealed a ^{13}C-enriched resonance at 173.0 ppm (Figure 4), which is attributed to the imide end group.

The presence of a *C*-terminal imide together with the evidence for a controlled (quasi-living) polymerization process suggests that it should be possible to prepare block copolymers. Chain extension experiments in the polymerization of **2** co-initiated by **I3** provided preliminary support for this prospect. An initial reaction was conducted with $[M]_0/[I]_0 = 20$, and a portion of the polymer product was separated from the reaction solution. This material had a molecular weight ($M_n = 4010$) close to the expected value ($M_{n(theo)} = 3225$) and a low polydispersity ($M_w/M_n = 1.02$) (Figure 5, trace A). More monomer was then added to the original reaction solution together with another equiv of $LiN(SiMe_3)_2$ relative to the initial co-initiator.[14] Chain extension was evident from the GPC trace (Figure 5, trace B), which revealed the presence of a polymer having a molecular weight consistent with the expected value ($M_n = 12990$, $M_{n(theo)} = 12417$) and low polydispersity ($M_w/M_n = 1.01$). No residue from the first stage of polymerization was observed by either GPC or MALDI-TOF MS. These results indicate that all of the polymer chain ends were active after the first round of polymerization and that the final polymer solely arose from extension of the first stage polymer. A similar protocol was employed to prepare heterodiblock copolymers from monomers with different hydrocarbon side chains, β-lactams **2** and **3**. Polymerization of **2** with $LiN(SiMe_3)_2$ (10 mol %) and **I2** (5 mol %) led to a well-defined homopolymer ($M_n = 3330$; $M_{n(theo)} = 3225$; $M_w/M_n = 1.07$). Subsequent addition of **3** together with more $LiN(SiMe_3)_2$ resulted in formation of poly(**2**)-*b*-poly(**3**), for which the GPC trace revealed that no residual poly-**2b** remained ($M_n = 10110$; $M_{n(theo)} = 9609$; $M_w/M_n = 1.05$).[15]

Diblock copolymers were prepared from monomers containing a mixture of hydrocarbon and amino-functionalized side chains: cyclooctyl β-lactam **2** with each of the Boc-containing β-lactams **4** and **5**. These reactions were somewhat less well

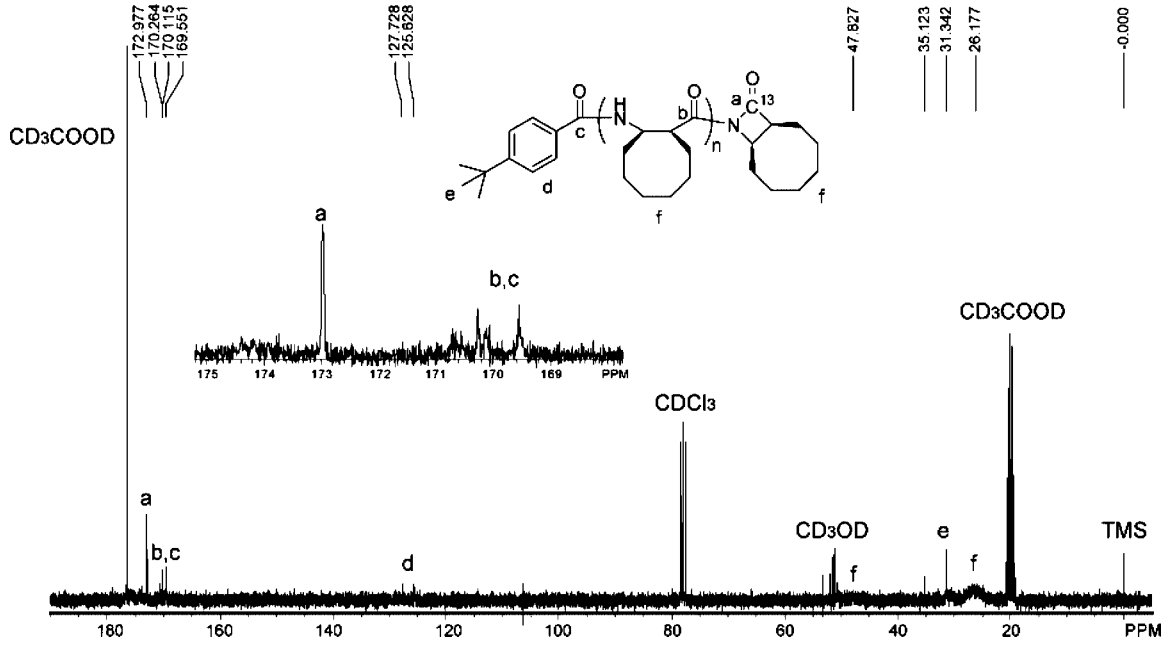


Figure 4. 300 MHz ^{13}C NMR spectrum of poly-**2b** with a $^{13}C(=O)$ labeled *C*-terminus. Solvent: $CDCl_3/CD_3CO_2D/CD_3OD$ (20:15:3).

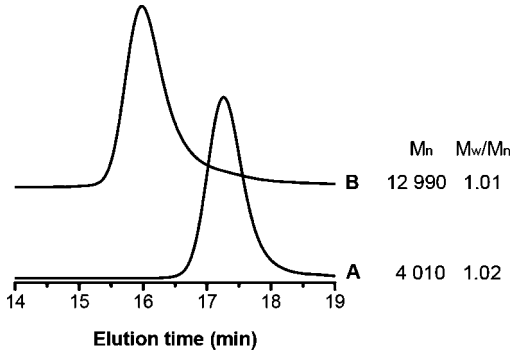


Figure 5. GPC analysis (RI detection) of poly-**2b** obtained in a chain extension experiment. (A) First stage polymer. (B) Chain-extended polymer. The first stage polymerization was carried out in THF at 25 °C for 1 h with $[M]_0 = 0.10$ M, 5.0 mol% **I3**, and 12.5 mol% $LiN(SiMe_3)_2$. Then 60 equiv of the monomer and 1 equiv of $LiN(SiMe_3)_2$ relative to initial **I3** were added to extend the chain. M_n and M_w/M_n were determined by light scattering in THF, dn/dc = 0.138.

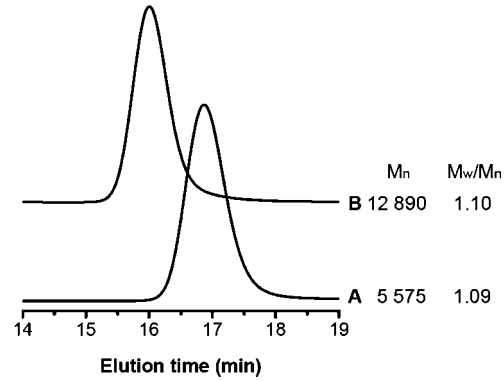
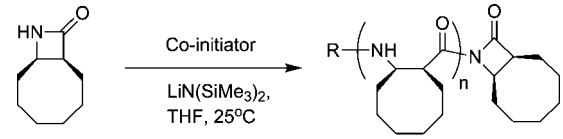


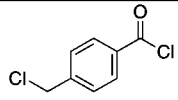
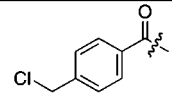
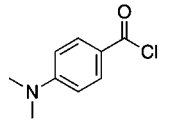
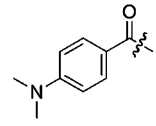
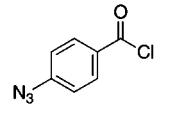
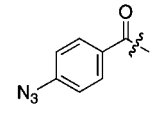
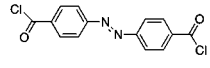
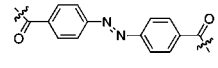
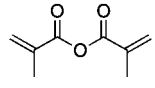
Figure 6. GPC analysis (RI) of first stage polymer poly(**7**) (A) and diblock copolymer poly(**7**)-*b*-poly(**2**) (B). The first stage polymerization of **7** was carried out in THF at 25 °C for 12 h with $[M]_0 = 0.10$ M, 5.0 mol% **I3**, and 12.5 mol% $LiN(SiMe_3)_2$. Then 50 equiv of **2** relative to initial **I3** were added to prepare the diblock copolymer. M_n and M_w/M_n were determined by light scattering in THF, dn/dc (poly(**7**)) = 0.133, dn/dc (copolymer) = 0.136.

controlled than the block copolymerization involving monomers with purely hydrocarbon side chains described above. For example, formation of the diblock copolymer poly(**2**)-*b*-poly(**5**) was partially accomplished by adding β-lactam **5** and additional $LiN(SiMe_3)_2$ to a preformed block of poly-**2b**; however, a tail was evident in the GPC trace of the final product corresponding to a residual amount of the first block of poly-**2b**. This result revealed that not all of the initial polymer chains underwent further reaction during the second stage. When **5** was used as the first block, only a small amount of block copolymerization of the second β-lactam, **2**, occurred. These results are consistent with conclusions drawn above regarding imperfect reactivity of Boc-containing β-lactams. These complications can be addressed by employing the phthalimide-protected β-lactam **7**, which does not have an N−H group in the protected amine side chain. β-Lactam **7** was polymerized with **I3** as co-initiator and $LiN(SiMe_3)_2$ ($[M]_0/[I]_0 = 20$). When this reaction was complete, β-lactam **2** and more $LiN(SiMe_3)_2$ (5 mol%) were added to the reaction solution. As shown in Figure 6, the GPC traces for the polymers obtained after the first and second stages of polymerization are narrow, symmetrical, and unimodal, suggesting good control over the polymer structure during both

Table 2. Polymerization of β-Lactam **2** with Different Co-initiators[a]



2 (+/-)

entry		co-initiator	polymer (R =)	M_n[b]	M_w/M_n[b]	yield[c]
1	**I4**		Poly-**2c** 	1 310	1.07	93
2	**I5**		Poly-**2d** 	1 480	1.22	95
3	**I6**		Poly-**2e** 	1 390	1.10	93
4	**I7**		Poly-**2f** 	1 800	1.08	97
5	**I8**		Poly-**2g** 	1 080	1.05	95

[a] Conditions: $[M]_0 = 0.25$ M, $[M]_0/[I]_0 = 10$, 25 mol% $LiN(SiMe_3)_2$ in entries 1−3 and 5, 50 mol% $LiN(SiMe_3)_2$ in entry 4. THF as solvent, 25 °C. [b] Determined by GPC-RI using PMMA as standard. [c] Isolated yield in percentage.

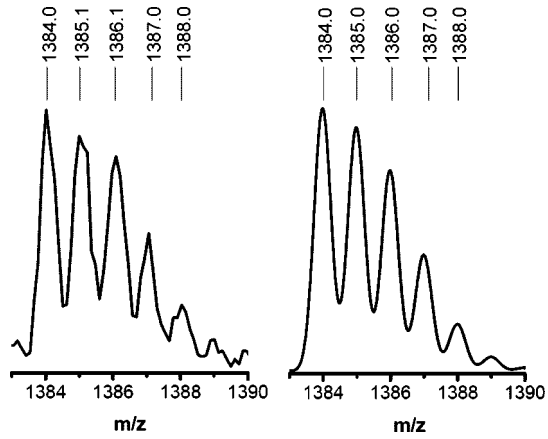


Figure 7. MALDI-TOF MS spectra of the 1384.0 Da oligomer ([M + Li]$^+$, 8 monomer units) of poly-**2c**. Left: measured spectrum; right: theoretical spectrum ([$C_{80}H_{124}ClN_8O_9$ + Li]$^+$, created using IDCalc by Michael J. MacCoss, University of Washington).

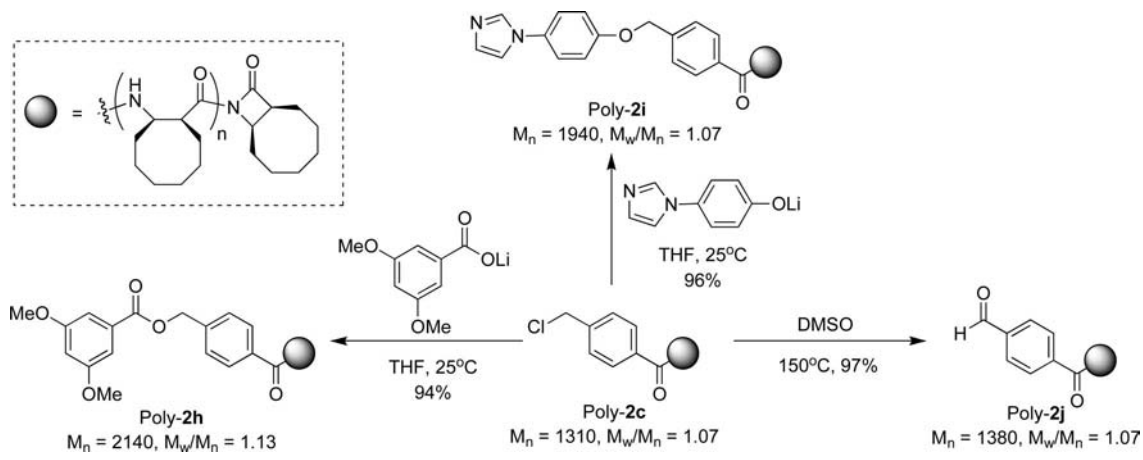
stages and quantitative conversion of the first-stage polymer into the final block copolymer.

End Group Functionalization of Poly-β-peptides. The success of acid chloride **I3** as a co-initiator for β-lactam ROP raises the possibility that a wide variety of commercially available acid chlorides or anhydrides could be used to generate polymers with diverse functionality at the *N*-terminus. Further, it might be possible to expand the variety of terminal functionalization if reactive units could be installed during polymerization and then transformed afterward. We have begun to explore these possibilities by examining four acid chlorides and one anhydride (**I4**−**I8**; Table 2) as co-initiators for polymerization of β-lactam **2**. Each of these co-initiators led to formation of polymer in ≥93% isolated yield. These polymers displayed the expected molecular weights, based on $[M]_0/[I]_0$, and low polydispersities. The incorporation of the intended *N*-terminal functional groups was indicated by NMR and MALDI-TOF MS data.[16] For example, the ^{1}H NMR spectrum of the polymer (poly-**2c**) co-initiated from 4-(chloromethyl)benzoyl chloride (**I4**) (sample dissolved in a mixture of $CDCl_3$, CD_3OD, and CD_3COOD) shows a singlet at 4.65 ppm, corresponding to the benzylic protons adjacent to chlorine. The presence of chlorine in poly-**2c** was further supported by MALDI-TOF MS data. For instance, the 1384.0 Da oligomer ([M + Li]$^+$, 8 monomer units) produced a mass spectral isotope pattern consistent with the expected composition (Figure 7). It is noteworthy that the benzylic chloride group of poly-**2c** is intact after the anionic ROP reaction, because this group might have been reactive toward the β-lactamate that is an intermediate in the polymerization.[17] When a bifunctional diazobenzene co-initiator (**I7**) was used, the resulting polymer (poly-**2f**) exhibited UV absorption maxima at 245 and 331 nm, which indicates the presence of the diazyl functional group since the poly-β-peptide itself does not absorb in this region of the UV spectrum. Poly-**2f** is bright orange, in contrast to the other poly-β-peptides mentioned above, which are white.

The results obtained with co-initiators **I4**, **I6**, and **I8** (benzylic chloride, aryl azide, and enoate, respectively) show that anionic ROP allows installation of reactive *N*-terminal groups that could allow further manipulation of *N*-terminal functionality via postpolymerization reactions. We briefly explored this strategy with poly-**2c**, which contains a benzylic chloride at the *N*-terminus. This polymer reacts cleanly with nucleophiles to incorporate a variety of other functional groups, including ester (poly-**2h**) and phenol ether (poly-**2i**) groups (Scheme 2). The benzylic chloride can be oxidized to an aldehyde upon heating in DMSO (poly-**2j**). The yields of these reactions are generally >85%. Successful chain end transformations were supported by NMR spectroscopic and MALDI-TOF MS data.[18] GPC analysis of these functionalized polymers showed similar molecular weights and polydispersities to those of the sample of poly-**2c** from which they were derived, indicating that the polymer chain was intact after functionalization. Numerous possible applications of the various chain-end functionalizations can be envisioned. For example, poly-**2g** could be used as a macromonomer for acrylate-type polymerization to prepare poly-β-peptide-grafted polyacrylates. The azide and aldehyde moieties of poly-**2e** and poly-**2j** should enable conjugation of poly-β-peptides to other polymers, biomolecules, or functionalized surfaces via "click" chemistry[19] or hydrazone or oxime formation.

Water-Soluble Poly-β-peptides. Most biological applications of interest require access to water-soluble polymers or oligomers. The Boc groups can be easily and quantitatively removed from poly-**4** or poly-**5** via acid treatment to yield polycationic materials, poly-**8** and poly-**9**, respectively (eq 6). These poly-β-peptides, as trifluoroacetic acid (TFA) or HCl salts, are readily soluble in aqueous solution but not in organic solvents. ^{1}H NMR spectroscopic analysis of poly-**9** in D_2O shows the expected set of resonances.[20] Figure 8 shows aqueous GPC traces of poly-**9** and a random copolymer (poly-**1/4**) derived from β-lactams **1** and **4**. The measured molecular weights of the deprotected

Scheme 2. Chemical Transformations of the Benzyl Chloride End Group in Poly-**2c**



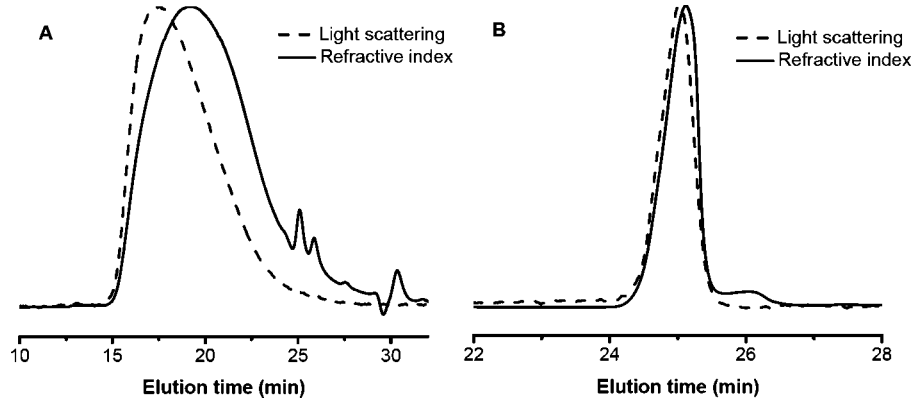
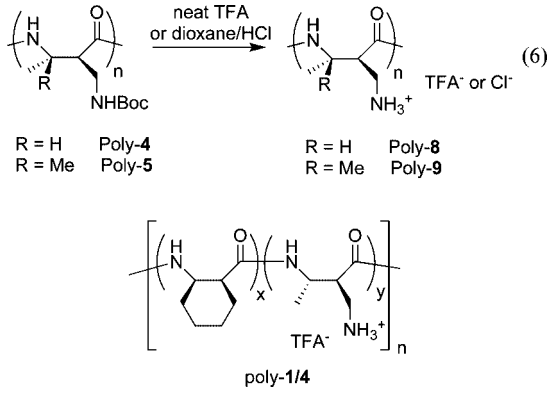
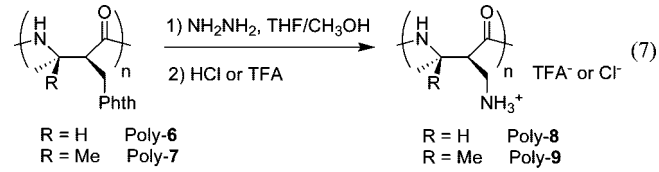


Figure 8. Aqueous GPC analysis of deprotected cationic poly-β-peptides. (A) Poly-**9**, $M_n = 29510$, $M_w/M_n = 1.46$. (B) Poly-**1/4**, a random copolymer prepared from 40% **1** and 60% **4**, $M_n = 2710$, $M_w/M_n = 1.06$. M_n and M_w/M_n values were determined by light scattering with dn/dc = 0.128 (A) and 0.120 (B), respectively. The solid and dashed curves are RI and LS detection, respectively.

polymers ($M_n = 29510$ and 2710, respectively) are reasonably close to the expected molecular weights ($M_n = 23300$ and 2030, respectively) based on the corresponding protected polymers, indicating complete side chain deprotection. The GPC mobile phase is a crucial consideration for the analysis of water-soluble poly-β-peptides. Acidic mobile phases such as 0.1% TFA in aqueous solution exhibit a much better performance than do neutral or basic mobile phases, presumably because an acidic mobile phase keeps the polymer in a polycationic state. In addition, an acidic mobile phase may minimize ionic interaction between the polymer and the stationary phase, which is slightly anionic. Significant broadening of the polymer peak was observed for the highly charged poly-β-peptide (poly-**9**, Figure 8a), possibly due to electrostatic interaction between the sample and the column materials. On the other hand, copolymer poly-**1/4**, containing both hydrophobic and cationic subunits, displays a narrower peak (Figure 8b).



Poly-**8** and poly-**9** have been prepared from the corresponding phthalimide-bearing poly-β-peptides, poly-**6** and poly-**7** (eq 7), although stronger reagents and a longer time are required for phthalimide deprotection than for Boc deprotection. Hydrazine treatment of poly-**6** or poly-**7** followed by acid workup and dialysis against water gives poly-**8** or poly-**9**, respectively, in $\sim$ 80% yield. The lack of phthalimide resonances in the ^{1}H NMR spectrum of poly-**8** and poly-**9** suggests complete deprotection of side chains.[21]



Conclusions

This work documents two significant advances in the preparation of poly-β-peptide materials. One innovation involves facile large-scale preparation of β-lactams bearing protected amino groups in a side chain, which ultimately provide water-soluble cationic polymers. The other innovation is a novel chain-initiation strategy, employing readily available acylating agents such as acid chlorides and anhydrides, that allows diverse functionalization of the polymer *N*-terminus. These developments enable the synthesis of a broad range of previously inaccessible poly-β-peptides and provide approaches to poly-β-peptide-containing block copolymers. We recently showed that poly-β-peptides made available by the new techniques mimic the biological activity profile of natural host-defense peptides.[7] Success in that case depended upon our ability to tune the lipophilic/cationic balance in copolymers and to identify *N*-terminal groups with optimal properties.[7,22] The preparative advances documented here will enable further refinement of antibacterial activity as well as evaluation of poly-β-peptides in the context of other biomedical applications. For example, discrete cationic β-peptide oligomers have recently been shown to display antifungal[23] and antiviral[24] properties; the methods

(12) Hashimoto, K.; Hotta, K.; Okada, M.; Nagata, S. *J. Polym. Sci., Part A: Polym. Chem.* **1995**, *33*, 1995–1999.
(13) See Supporting Information (S2−7) for the MALDI spectrum.
(14) In principle, additional base should not be needed to restart chain elongation. In practice, however, we find that re-initiation of the chain ends is incomplete if additional base is not included.
(15) See Supporting Information (S2−54) for the GPC chromatograph.
(16) See Supporting Information (S2−10 to S2−29) for the NMR, MALDI, and GPC data.
(17) A model compound containing a benzylic chloride was found to be unreactive with β-lactamate under conditions identical to polymerization conditions, but reactive with other nucleophiles. See Supporting Information for these experiments.
(18) See Supporting Information (S2−30 to S2−41) for the NMR, MALDI, and GPC data.
(19) Kolb, H. C.; Finn, M. G.; Sharpless, K. B. *Angew. Chem., Int. Ed.* **2001**, *40*, 2004–2021.
(20) See Supporting Information (S2−50) for the NMR spectrum.
(21) See Supporting Information (S2−53) for the NMR spectrum.
(22) Mowery, B. P.; Dillon, A. H.; Lee, S. E.; Weisblum, B.; Stahl, S. S.; Gellman. S. H., manuscript in preparation.

described above will allow one to determine whether comparable behavior can be achieved with cationic poly-β-peptides, which would be much easier to prepare than discrete oligomers. More broadly, cationic polymers have been employed as nucleic acid packaging agents for DNA or siRNA delivery[25] and as scaffolds for tissue engineering.[26] It is now possible to explore poly-β-peptides for these applications.

Acknowledgment. This research was supported by the NSF CRC Program (CHE-0404704) and the UW-Madison NSEC (DMR-0425880). We thank Dr. M. F. Ilker for performing some initial studies.

(23) Karlsson, A. J.; Pomerantz, W. C.; Weisblum, B.; Gellman, S. H.; Palecek, S. P. *J. Am. Chem. Soc.* **2006**, *128*, 12630–12631.
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Note Added after ASAP Publication. The uncorrected proof version of this article was published ASAP in error on January 6, 2009. Corrections made in the text and graphics on January 9, 2009, are reflected in the current Web and print versions of the article.

Supporting Information Available: Experimental details and characterization data including GPC, MALDI-TOF MS, and NMR data for all polymers. This material is available free of charge via the Internet at http://pubs.acs.org.

JA8069192

(25) Dykxhoorn, D. M.; Lieberman, J. *Annu. Rev. Biomed. Eng.* **2006**, *8*, 377–402.
(26) Lee, K. Y.; Mooney, D. J. *Chem. Rev.* **2001**, *101*, 1869–1879.