J|A|C|S
COMMUNICATIONS

Published on Web 09/03/2008

N-Trimethylsilyl Amines for Controlled Ring-Opening Polymerization of Amino Acid *N*-Carboxyanhydrides and Facile End Group Functionalization of Polypeptides

Hua Lu and Jianjun Cheng*

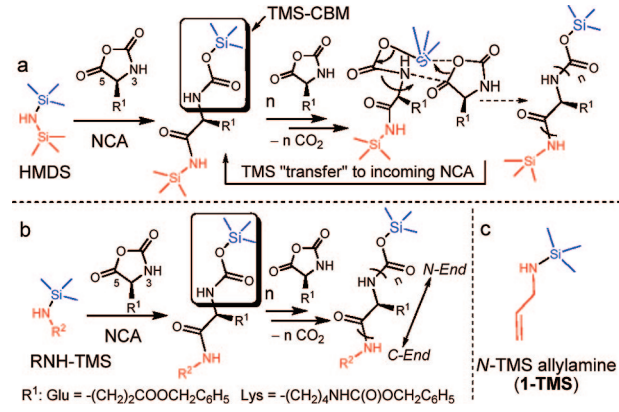
Department of Materials Science and Engineering, University of Illinois at Urbana−Champaign, Urbana, Illinois 61801

Received May 4, 2008; E-mail: jianjunc@uiuc.edu

Polypeptides have been extensively utilized in drug delivery,[1] tissue engineering,[2] sensing,[3] and catalysis.[4] To prepare polypeptides for these applications, it is essential to control their molecular weights (MWs)[5−10] as well as their end groups[8,11−13] during the ring-opening polymerizations (ROPs) of amino acid *N*-carboxyanhydrides (NCAs).[14] We recently reported hexamethyldisilazane (HMDS)-mediated, controlled NCA polymerization.[15] This polymerization proceeds via a unique, trimethylsilyl carbamate (TMS-CBM) propagating group (Scheme 1a), which involves cleavage of the Si−N bond of HMDS during the initiation step. The resulting TMS-amine (red, Scheme 1a) opens the NCA ring at its CO-5 position to form a TMS-amide at the C-end while the TMS group (blue, Scheme 1a) is attached to the N-end to form a TMS-CBM (the propagating chain end). The propagation of polypeptide chains proceeds through the transfer of the TMS group from the terminal TMS-CBM to the incoming monomer and forms a new TMS-CBM propagating chain end (Scheme 1a). We postulate that when a *N*-TMS amine is used as the initiator, cleavage of its Si−N bond will generate an amine and a TMS group (Scheme 1b) that will subsequently form the corresponding amide at the C-end and a TMS-CBM at the N-end after NCA ring opening (Scheme 1b). Thus, chain propagation should proceed in the same manner as HMDS-mediated polymerization (Scheme 1a). Because a large variety of *N*-TMS amines are readily available, this method should allow facile functionalization of the C-termini of polypeptides (Scheme 1b).

To demonstrate this concept, *N*-TMS allylamine (**1-TMS**, **1** = allylamine, Scheme 1c) was utilized as the initiator for the polymerization of γ-benzyl-L-glutamate NCA (Glu-NCA) (Scheme 1b). As shown in Figure 1a, **1-TMS** had remarkable control of Glu-NCA polymerization and gave poly(γ-benzyl-L-glutamate) (PBLG) with the expected MWs and narrow MWDs over a broad range of monomer/initiator (M/I) ratios (M/I = 20−300). The obtained M_n's of PBLG at an M/I ratio of 20 and 300 were 4.6×10^3 and 7.01×10^4 g/mol, respectively, both of which were nearly identical to the expected M_n's (4.4×10^3 and 6.57×10^4 g/mol, respectively). All polymerizations were finished within 12−24 h at room temperature under atmospheric pressure, in contrast to a few recently reported controlled polymerizations that require either reduced temperature[10] or vacuum.[9] The M_n's of PBLG showed a linear correlation with the conversions of Glu-NCA, which were in good agreement with the expected M_n's (Figure 1b). This experiment demonstrated that the propagation of PBLG chains proceeded through a living chain end. **1-TMS** also showed remarkable control of polymerizations of ε-Cbz-L-lysine NCA (Lys-NCA) and resulted in poly(ε-Cbz-L-lysine) (PZLL) with the expected MWs and very narrow MWDs (Figure S1). Block copolypeptides, such as PZLL-*b*-PBLG, can also be readily prepared with the anticipated MWs and narrow MWDs via sequential addition of Lys- and Glu-NCA (Table S1 and Figure S2).

Scheme 1



We next compared the polypeptides obtained through **1**-initiated and **1-TMS**-initiated Glu-NCA polymerizations. The PBLG resulting from **1**-initiated polymerization at an M/I ratio of 20 had a broad MWD (M_w/M_n = 1.52) based on the MALDI-TOF MS analysis (red, Figure 1c). In contrast, a similar polymerization mediated by **1-TMS** resulted in PBLG with a much narrower MWD (M_w/M_n = 1.17). The MALDI-TOF MS spectrum of the latter polymer showed a Poisson distribution centered at the exact

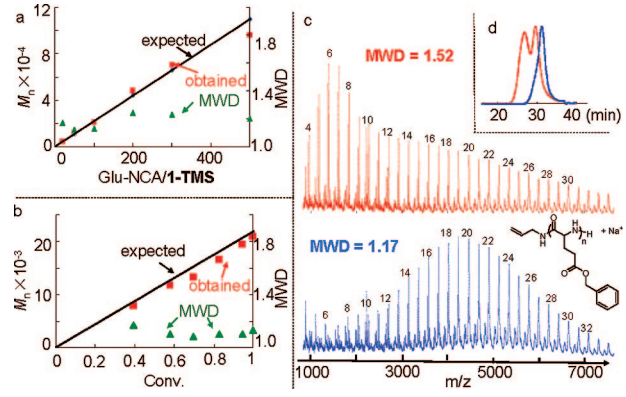


Figure 1. (a) MW and MWD of the PBLG prepared via **1-TMS**-mediated ROP of Glu-NCA at various M/I ratios. (b) MW and MWD of the PBLG prepared via **1-TMS**-mediated ROP of Glu-NCA at an M/I ratio of 100 relative to the conversion of Glu-NCA. (c) MALDI-TOF MS spectra of the PBLG prepared via **1-TMS** (blue)- and **1** (red)-mediated Glu-NCA polymerization at an M/I ratio of 20. The number on the MS spectra denotes the number of repeating units of PBLG (*n*). (d) GPC curve of the PBLG prepared via **1-TMS** (blue)- and **1** (red)-mediated Glu-NCA polymerization at an M/I ratio of 50, respectively.

10.1021/ja803304x CCC: $40.75

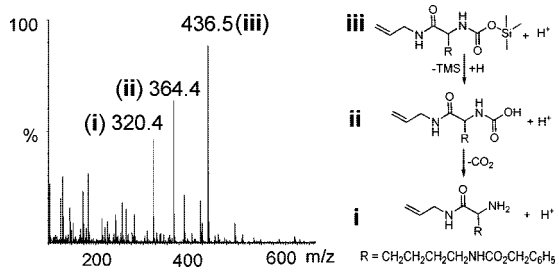


Figure 2. ESI-MS of equal molar mixture of **1-TMS** and Lys-NCA.

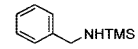
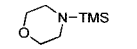
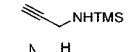
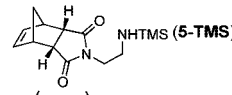
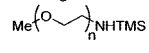
theoretical MW of the PBLG containing the anticipated allyl amide end group (MW = allylamine + $(Glu)_{20}$) (blue, Figure 1c). This MS distribution pattern differed dramatically from that of the former PBLG (red, Figure 1c). The bimodal GPC curve of the former PBLG (red, Figure 1d) compared to the monomodal curve of the latter PBLG (blue, Figure 1d) further demonstrated the improved control of polymerization through subtle modification of the amine initiator.

To confirm the formation of TMS-CBM during **1-TMS**-mediated NCA polymerization, we mixed equal molar Lys-NCA and **1-TMS** in DMSO-d_6 and then analyzed the mixture using ESI-MS. As expected, **1**-Lys-TMS-CBM (**iii**, *m*/*z* 436.5) was successfully identified as the dominating component in the mixture (Figure 2). This experiment demonstrated cleavage of the Si−N bond of **1-TMS** and subsequent NCA ring opening by **1** at CO-5 and formation of TMS-CBM (Scheme 1b). When the MS experiment was performed under anhydrous conditions, **iii** and its decomposed derivatives **ii** and **i** were detected (Figure 2). However, when the reaction mixture was exposed to air or when D_2O was added, only **i** was detected (data not shown). These observations were in agreement with the expected moisture sensitivity of TMS-CBM.[15] Formation of TMS-CBM during the initiation step was further confirmed by ^{13}C NMR (Figure S3).

To evaluate whether this **1-TMS**-mediated, controlled NCA polymerization can be extended to other *N*-TMS amines, we selected benzylamine (**2**), morpholine (**3**), propargylamine (**4**), *N*-(aminoethylene)-5-norbornene-endo-2,3-dicarboximide (**5**), and $mPEG_{2000}$ amine (**6**) to represent primary (**2**) and secondary amines (**3**), amines containing functional groups that can be used for further reactions such as click chemistry (**4**)[16] and ring-opening metathesis polymerization (**5**),[17] and terminal amines of polymers (**6**).[12] *N*-TMS's of **2**−**6** were prepared (see Supporting Information) and used to initiate Glu-NCA polymerization. As expected, all initiators gave excellent control of PBLG MWs. At an M/I ratio of 100, the M_n's of PBLG were 2.35×10^4, 2.18×10^4, 2.19×10^4, 2.38×10^4, and 2.85×10^4 g/mol for PBLG derived from **2-TMS** through **6-TMS** mediated Glu-NCA polymerizations, respectively, which were in nearly perfect agreement with the expected M_n's (Table 1). Polymerizations of Glu- and Lys-NCAs with these initiators over a broad range of M/I ratios all gave corresponding polypeptides with controlled MWs and narrow MWDs (Table S2).

In amine-initiated NCA polymerization, an amine can function in two ways. It can be a nucleophile that attacks the CO-5 of the NCA by following the so-called "amine mechanism". It can also function as a base to deprotonate the NH-3 of NCA by following the so-called "activated monomer mechanism".[18,19] These complex and concurrent mechanisms make it very difficult to achieve controlled NCA polymerization using amine initiators. When *N*-TMS amine is used as an initiator, polypeptide chain transfer via the "activated monomer mechanism" is eliminated since TMS-CBM is unable to deprotonate the NH-3. Chain propagation thus can only proceed through the ring opening at the CO-5 position of the NCA, resembling ammonium-mediated NCA polymerization, but occurring much faster.[7]

Table 1. N-TMS Amines for Glu-NCA Polymerization (M/I = 100)

initiator	expected M_n (g/mol)	obtained M_n (g/mol)	M_w/M_n	conv.of NCA (%)
 (**2-TMS**)	21,900	23,500	1.26	>99
 (**3-TMS**)	21,900	21,800	1.21	>99
 (**4-TMS**)	21,900	21,900	1.18	>99
 (**5-TMS**)	22,000	23,800	1.17	>99
 (**6-TMS**)	23,900	28,500	1.1	>99

In conclusion, *N*-TMS amines can initiate controlled NCA polymerizations and allow facile functionalization at the C-termini of polypeptides. Polymerizations initiated by *N*-TMS amines are fast, give quantitative monomer conversion, and do not require excessive monomer purification.[9] This methodology is useful for controlled synthesis of functional polypeptides, polypeptide macromonomers, and polypeptide copolymers.

Acknowledgment. This work was supported by the National Science Foundation No. CHE-0809420, the American Chemical Society Petroleum Research Fund, and partially by the Siteman Center for Cancer Nanotechnology Excellence (SCCNE)-Center for Nanoscale Science and Technology (CNST, University of Illinois at Urbana−Champaign). We thank Professors Martin Burke and Jeffrey Moore for providing anhydrous solvents.

Supporting Information Available: Experimental procedure, GPC and NMR data. This materials is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Deming, T. J. *Adv. Drug Delivery Rev.* **2002**, *54*, 1145–1155.
(2) Wang, X. Y.; Kim, H. J.; Wong, C.; Vepari, C.; Matsumoto, A.; Kaplan, D. L. *Mater. Today* **2006**, *9*, 44–53.
(3) Dos Santos, S.; Chandravarkar, A.; Mandal, B.; Mimna, R.; Murat, K.; Saucede, L.; Tella, P.; Tuchscherer, G.; Mutter, M. *J. Am. Chem. Soc.* **2005**, *127*, 11888–11889.
(4) Mart, R. J.; Osborne, R. D.; Stevens, M. M.; Ulijn, R. V. *Soft Matter* **2006**, *2*, 822–835.
(5) Deming, T. J. *Nature* **1997**, *390*, 386–389.
(6) Deming, T. J. *J. Am. Chem. Soc.* **1998**, *120*, 4240–4241.
(7) Dimitrov, I.; Schlaad, H. *Chem. Commun.* **2003**, 2944–2945.
(8) Lutz, J. F.; Schutt, D.; Kubowicz, S. *Macromol. Rapid Commun.* **2005**, *26*, 23–28.
(9) Aliferis, T.; Iatrou, H.; Hadjichristidis, N. *Biomacromolecules* **2004**, *5*, 1653–1656.
(10) Vayaboury, W.; Giani, O.; Cottet, H.; Deratani, A.; Schue, F. *Macromol. Rapid Commun.* **2004**, *25*, 1221–1224.
(11) Curtin, S. A.; Deming, T. J. *J. Am. Chem. Soc.* **1999**, *121*, 7427–7428.
(12) Osada, K.; Kataoka, K. *Adv. Polym. Sci.* **2006**, 113–153.
(13) Deming, T. J. *Adv. Polym. Sci.* **2006**, *202*, 1–18.
(14) Kricheldorf, H. R. *Angew. Chem., Int. Ed.* **2006**, *45*, 5752–5784.
(15) Lu, H.; Cheng, J. J. *J. Am. Chem. Soc.* **2007**, *129*, 14114–14115.
(16) Lutz, J. F. *Angew. Chem., Int. Ed.* **2007**, *46*, 1018–1025.
(17) Nguyen, S. T.; Johnson, L. K.; Grubbs, R. H.; Ziller, J. W. *J. Am. Chem. Soc.* **1992**, *114*, 3974–3975.
(18) Deming, T. J. *Adv. Polym. Sci.* **2006**, *202*, 1–18.
(19) Kricheldorf, H. In *Models of Biopolymers by Ring Opening Polymerization*; Penczek, S., Ed.; CRC Press: Boca Raton, FL, 1990; pp 1−132.

JA803304X