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Organocatalysis by Hydrogen-Bonding: A New Approach to Controlled/Living Polymerization of α-Amino acid *N*-Carboxyanhydrides

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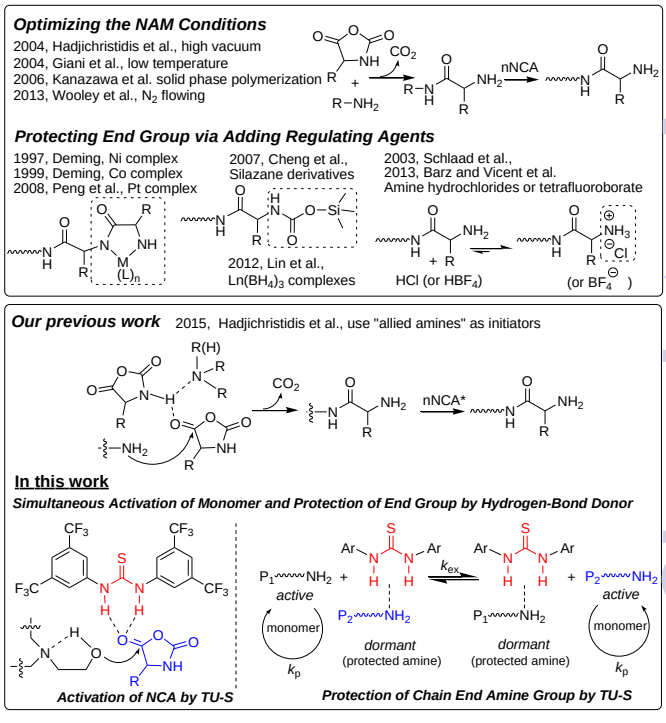
A new method, based on hydrogen-bonding organocatalysis, was developed to achieve living ring-opening polymerization of *N*-carboxyanhydride of α-amino acids using aminoalcohols as initiators in the presence of *N, N'*-bis[3,5-bis(trifluoromethyl)phenyl]thiourea (**TU-S**). The thiourea provides, through hydrogen bonding, simultaneous activation of NCA monomers/reversible deactivation of polymer chain-ends/silencing of the tertiary amine and thus allows the polymerization to proceed in a high controllable mode. For example, using *N,N*-dimethyl ethanolamine (**DMEA**), as initiator in the presence of **TU-S**, a series of well-defined linear polypeptides with different designed MWs (3.01×10^4 – 18.10×10^4) and low PDI values (1.02 - 1.05) were successfully synthesized. This general strategy was also extended to the synthesis of well-defined di- and multi-armed polypeptides by using di-, tri-, or tetra-aminoalcohol initiators (methyldiethanolamine (**MDEA**), triethanolamine (**TEA**) or *N,N,N',N'*-tetrakis(2-hydroxyethyl)ethylenediamine (**THEED**)) in the presence of **TU-S**.

Introduction

Well-defined synthetic polypeptides, as mimics of natural analogues, have found extensive applications in areas such as drug delivery, tissue engineering, sensing and catalysis.[1] Among different methods leading to polypeptides, the ring-opening polymerization (ROP) of α-amino acid *N*-carboxyanhydrides (NCA) mediated by amines is the most efficient one. Although high molecular weight polypeptides can be readily synthesized using this method, typically ill-defined structures (uncontrolled molecular weight and broad molecular weight distribution) are obtained. Therefore, it is necessary and crucial to develop living ROP of NCAs to obtain well-defined polypeptides. In 1997, Deming reported the first example of living ROP of NCAs by using transition metal complexes as initiators.[2] After that, a few controlled NCA polymerizations were reported by employing either new initiators (primary amine hydrochlorides or trifluoroboranes,[3] silazane derivatives[4], Pt based[5a] and rare earth metal complexes[5b,c]) or by optimizing the primary amine initiated polymerizations (high vacuum, low temperature, nitrogen flowing and polymerization in solid phase).[6] Very recently, we developed a new strategy for fast and living polymerization of NCAs at room temperature by employing a series of initiators including both primary and secondary or tertiary amines. In such polymerizations, the secondary or tertiary amines were found to activate the monomer, which resulted in a faster rate of polymerization.[7]



Scheme 1. Reported strategies for the living polymerization of α-amino acid NCAs and the new approach proposed in this work.

Herein, we investigate and report another new approach that also relies on monomer activation but unlike our previous work the catalyst is *N, N'*-bis[3,5-bis(trifluoromethyl)phenyl]thiourea (**TU-S**) and the initiation of the NCA polymerization is triggered by aminoalcohols. The **TU-S** simultaneously activates the NCA

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monomer, reversibly neutralizes the growing $-NH_2$ chain ends and silences the tertiary amine which promotes the undesirable activated monomer mechanism, and thus affords a controlled polymerization as well as the synthesis of well-defined structures. This new strategy not only demonstrates the relevance of **TU-S** in NCA polymerization but also provides a novel approach towards the synthesis of well-defined polypeptide-based architectures.

Experimental Section

General Methods. All reactions were carried out under a dry and oxygen-free argon atmosphere by using Schlenk techniques or under an argon atmosphere in an MBraun glovebox. Solvents were purified using the MBraun SPS system. Anhydrous dimethylformamide (DMF) and dichloromethane (DCM) was further dried by passing through an activated alumina column. THF was dried vigorously with sodium-potassium alloy until a characteristic blue color was evident in the solvent. Anhydrous DCM-d_2 was dried over P_2O_5 at room temperature under Ar overnight followed by distillation under reduced pressure. All other liquids were dried over activated 4 Å molecular sieves for a week and distilled before use, and solid materials were used as received. All purified reagents were stored over 4Å molecular sieves in a glove box. H-Glu(OBn)-OH was purchased from Sigma-Aldrich and used as received. Glu-NCA was prepared and recrystallized four times according to published procedures.[4] N, N'-bis[3,5-bis(trifluoromethyl)phenyl] thiourea (abbreviated as TU-S) was synthesized and purified following literature with slight modifications.[8]

Instruments and Measurements. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AV600 (FT, 600 MHz for ^{1}H; 150 MHz for ^{13}C) spectrometer. NMR assignments were confirmed by ^{1}H-^{1}H (COSY), ^{1}H-^{13}C (HMQC), and ^{13}C NMR (DEPT) experiments when necessary. *In situ* IR study of NCA polymerization was carried out by using ReactIR 45m with MCT Detector from METTLER TOLEDO. AutoChem. DiComp probe (Diamond) was connected via AgX 9.5mm x 1.5m Fiber (Silver Halide). Spectra were taken from 2800 cm^{-1} to 650 cm^{-1} at 8 wavenumber resolution and the automatic sampling interval was 10 seconds. The real-time concentration of NCA was quantified by measuring the intensity of NCA's anhydride peak at 1792 cm^{-1} by FT-IR. The conversion of NCA was determined by comparing the NCA concentration during polymerization with the NCA concentration at t = 0. Polymer characterization was carried out by the Agilent 1260 infinity SEC instrument equipped with a 1200 HPLC pump, an Optilab T-rEX RI detector, a ViscoStar-II viscometer and a DAWN HELEOS-II multiangle laser-light scattering (MALLS) detector at a wavelength of 690 nm (from Wyatt Technology). One guard column and three 7.8×300 mm columns (Styragel® HT 2 DMF, Styragel® HT 3 DMF and Styragel® HT 4 DMF) were used. HPLC-grade DMF (containing 0.1 M LiBr) was used as the mobile phase at a flow rate of 1.0 mL/min. The whole system, including columns and detectors, was maintained at 60 °C. Polymer solutions with concentrations between 5.0 and 10.0 mg/mL were injected at an injection volume of 200 µL. ASTRA software from Wyatt Technology was used to collect and analyze the data from the detectors.

Synthesis of DMEA-TMS'. A solution of *tert*-butyldimethylchlorosilane (7.61g, 0.05 mol) in *N,N*-dimethylformamide (DMF; 20 mL) was added dropwise to a mixture of *N,N*-dimethyl ethanolamine DMEA (5g, 0.06 mol), imidazole (7.64 g, 0.11mol), and DMF (20 mL) at 0 °C under nitrogen. The mixture was stirred for 6 h at room temperature, then washed with water, extracted with DCM and dried over anhydrous $MgSO_4$. The solvents were removed in vacuum to afford DMEA-TMS'. The crude product was purified by a silica gel column using hexane-dichloromethane (1:1, v/v) as eluent. Isolated yield: 90%. Purity: 99% (by gas chromatography). ^{1}H NMR (CD_2Cl_2, 500M, 25 °C): δ_H=0.05 (s, 6H, -Si(C*H*$_3$)$_2$C(CH$_3$)$_3$), 0.88 (s, 9H, -Si(CH$_3$)$_2$C(C*H*$_3$)$_3$), 2.20 (s, 6H, -N(C*H*$_3$)$_2$), 2.38 (t, 2H, -NC*H*$_2$CH$_2$O-), 3.68 (t, 2H, -NCH$_2$C*H*$_2$O-). ^{13}C NMR (CD_2Cl_2, 125M, 25°C): δ_C=-5.28 (2C, -Si(*C*H$_3$)$_2$C(CH$_3$)$_3$), 18.55 (1C, -Si(CH$_3$)$_2$*C*(CH$_3$)$_3$), 26.05 (3C, -Si(CH$_3$)$_2$C(*C*H$_3$)$_3$), 46.25 (2C, -N(*C*H$_3$)$_2$), 62.03 (1C, -N*C*H$_2$CH$_2$O-), 62.11 (4C, -NCH$_2$*C*H$_2$O-). ^{1}H NMR and ^{13}C NMR see supporting information.

Synthesis of MDEA-TMS'. This compound was synthesized using the same procedure as for DMEA-TMS' from *tert*-butyldimethylchlorosilane (18.97g, 0.13 mol), imidazole (11.43 g, 0.17mol), and methyldiethanolamine MDEA (5g, 0.04 mol). Isolated yield: 90%. Purity: 99% (by gas chromatography). ^{1}H NMR (CD_2Cl_2, 500M, 25 °C): δ_H=0.04 (s, 12H, -Si(C*H*$_3$)$_2$C(CH$_3$)$_3$), 0.88 (s, 18H, -Si(CH$_3$)$_2$C(C*H*$_3$)$_3$), 2.28 (s, 3H, -N(C*H*$_3$)-), 2.52 (t, 4H, -NC*H*$_2$CH$_2$O-), 3.66 (t, 4H, -NCH$_2$C*H*$_2$O-). ^{13}C NMR (CD_2Cl_2, 150M, 25°C): δ_C=-5.25 (4C, -Si(*C*H$_3$)$_2$C(CH$_3$)$_3$), 18.54 (2C, -Si(CH$_3$)$_2$*C*(CH$_3$)$_3$), 26.06(6C, -Si(CH$_3$)$_2$C(*C*H$_3$)$_3$), 43.85 (1C, -N(*C*H$_3$)-), 60.64(2C, -NC*H*$_2$CH$_2$O-), 62.10 (4C, -NCH$_2$*C*H$_2$O-). ^{1}H NMR and ^{13}C NMR see supporting information.

Synthesis of TEA-TMS'. This compound was synthesized using the same procedure as for DMEA-TMS' from *tert*-butyldimethylchlorosilane (20.20g, 0.13 mol), imidazole (13.69 g, 0.20mol), and triethanolamine TEA (5g, 0.03 mol). Isolated yield: 92%. Purity: 99% (by gas chromatography). ^{1}H NMR (CD_2Cl_2, 500M, 25 °C): δ_H=0.04 (s, 18H, -Si(C*H*$_3$)$_2$C(CH$_3$)$_3$), 0.88 (s, 27H, -Si(CH$_3$)$_2$C(C*H*$_3$)$_3$), 2.67 (t, 6H, -NC*H*$_2$CH$_2$O-), 3.62 (t, 6H, -NCH$_2$C*H*$_2$O-). ^{13}C NMR (CD_2Cl_2, 150M, 25°C): δ_C=-5.24 (6C, -Si(*C*H$_3$)$_2$C(CH$_3$)$_3$), 18.54 (3C, -Si(CH$_3$)$_2$*C*(CH$_3$)$_3$), 26.08 (9C, -Si(CH$_3$)$_2$C(*C*H$_3$)$_3$), 58.32 (3C, -N*C*H$_2$CH$_2$O-), 62.58 (3C, -NCH$_2$*C*H$_2$O-). ^{1}H NMR and ^{13}C NMR see supporting information.

Synthesis of THEED-TMS'. This compound was synthesized using the same procedure as for DMEA-TMS' from *tert*-butyldimethylchlorosilane (15.94g, 0.10 mol), imidazole (11.52 g, 0.17mol), and *N,N,N',N'*-tetrakis(2-hydroxyethyl)ethylenediamine THEED (5g, 0.02 mol). Isolated yield: 95%. Purity: 99% (by gas chromatography). ^{1}H NMR (CD_2Cl_2, 500M, 25 °C): δ_H=0.05 (s, 24H, -Si(C*H*$_3$)$_2$C(CH$_3$)$_3$), 0.88 (s, 36H, -Si(CH$_3$)$_2$C(C*H*$_3$)$_3$), 2.59 (s, 4H, -NC*H*$_2$C*H*$_2$N-), 2.62 (t, 8H, -NC*H*$_2$CH$_2$O-), 3.62 (t, 8H, -NCH$_2$C*H*$_2$O-). ^{13}C NMR (CD_2Cl_2, 150M, 25°C): δ_C=-5.09 (8C, -Si(*C*H$_3$)$_2$C(CH$_3$)$_3$), 18.65 (4C, -Si(CH$_3$)$_2$*C*(CH$_3$)$_3$), 26.21 (12C, -Si(CH$_3$)$_2$C(*C*H$_3$)$_3$), 54.98 (2C, -N*C*H$_2$C*H*$_2$N-), 58.18 (4C, -N*C*H$_2$CH$_2$O-), 62.64 (4C, -NCH$_2$*C*H$_2$O-). ^{1}H NMR and ^{13}C NMR see supporting information.

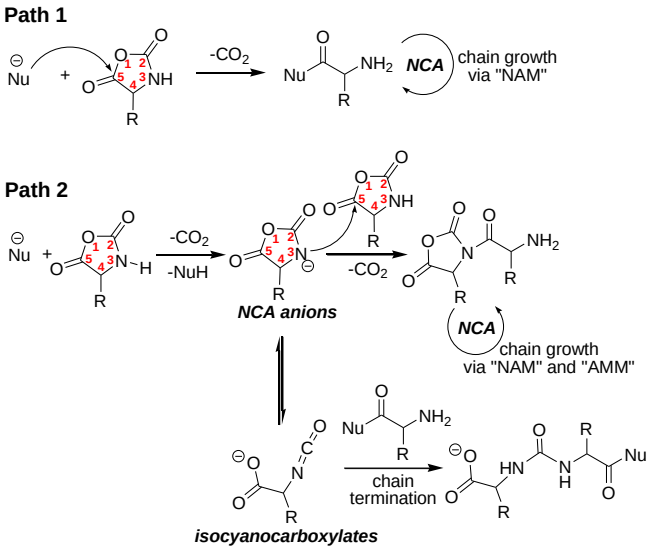
Polymerization procedure. A typical procedure for polymerization of NCA was performed in a 25 mL flask in a Braun Labmaster glovebox. To a vigorously stirred solution of DMEA and **TU-S** in 4 mL of solvent (DMF, THF or DCM) was added 0.4g NCA monomer in 4 mL of solvent (DMF, THF or DCM). AutoChem. DiComp probe (Diamond) of ReactIR 45m (METTLER TOLEDO) was connected

to reaction flask via AgX 9.5mm x 1.5m Fiber (Silver Halide) to monitor the conversion of monomer. After specific time, 0.2 ml of the reaction mixture was taken out from the system and diluted to 10 mg (PBLG or PZLL) /mL using DMF (containing 0.1 M LiBr). The solution was then analyzed by SEC to determine the molecular weight and PDI of obtained polypeptides. The remaining final reaction mixture was precipitated with methanol, sonicated and centrifuged to remove the solvent. The obtained polymer was collected and dried under vacuum overnight after two repetitions of sonication-centrifugation procedure.

Results and Discussion

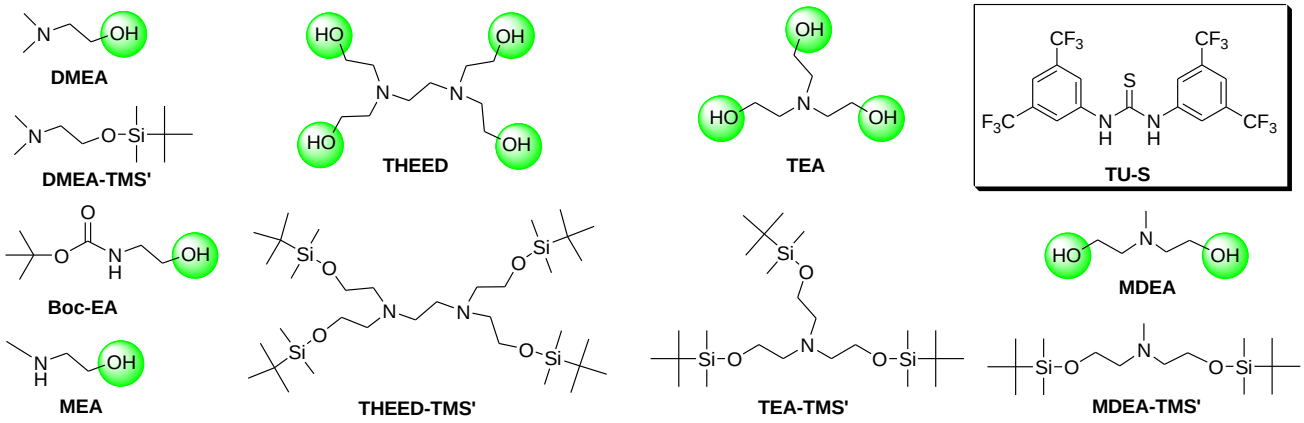
In α-amino acid NCAs, the carbonyl group (C5) is highly electrophilic and can easily be attacked by various nucleophiles, inducing ROP initiation (Scheme 2, path 1). In addition, the imine (N3) of NCA possesses a very active acidic N–H proton which can easily be abstracted under basic conditions. The resulting NCA anion can thus nucleophilically attack the carbonyl group (C5) of another NCA molecule and trigger the uncontrolled ROP of NCA in "activated monomer mechanism" (AMM), or can undergo rearrangement to isocyanocarboxylates and terminate the growing NH_2-end active chains (Scheme 2, path 2). Highly nucleophilic initiators can thus induce fast initiation that is required for living polymerization of NCA, but simultaneously abstract the N–H proton of NCA, resulting in the unwanted formation of NCA anions in the system. Consequently, it is challenging to find appropriate initiators for the living α-amino acid NCAs polymerization.

Inspired by the activation of C=O bond in organic reactions by hydrogen bonding[9] and the pioneer work of Waymouth group in ROP of cyclic esters,[10] we selected a weak nucleophile, *N,N*-dimethyl ethanolamine (**DMEA**), as initiator and a hydrogen-bonding donor compound, **TU-S**, as activator of NCA monomers. Since **DMEA** is not a base, the possibility of abstracting the acidic N-H proton of NCA is very low and thus the formation of unwanted NCA anions can be excluded. On the other hand, **TU-S** is a widely used hydrogen-bonding donor that can dramatically increase the electrophilicity of carbon in carbonyls. Consequently, the **TU-S**/aminoalcohol combination should be an ideal and efficient catalytic system for NCA polymerization.



Scheme 2. Possible reactions in ROP of α-amino acid NCAs by nucleophilic initiators ("NAM" and "AMM" refer to "normal amine mechanism" and "activated monomer mechanism" respectively)

Table 1. Living polymerization of NCAs using alcohol/thiourea hydrogen-bonding organocatalytic system.



run [a]	initiator	solvent	$[M]_0/[I]_0$ /[**TU-S**]	[**TU-S**] (mol %)	time (min)	conv. (%)[b]	$M_{n,calcd}$ $\times10^{-4}$ [c]	$M_{n,SEC-LS}$ $\times10^{-4}$ [d]	PDI [d]
1[e]	**DMEA**	DMF	120/1/-	0	240	100	2.63	5.73	1.58
2	**DMEA**	THF	120/1/-	0	180	100	2.63	29.67	1.28
3	**DMEA**	DCM	120/1/-	0	6	100	2.63	5.27	1.08
4	**-**	DCM	120/-/5	4	360	0	-	-	-
5[e]	**DMEA**	DMF	120/1/1	0.8	1440	100	2.63	7.28	1.59
6[e]	**DMEA**	THF	120/1/1	0.8	130	100	2.63	14.86	1.22
7	**DMEA**	DCM	120/1/1	0.8	12	100	2.63	3.01	1.05
8	**DMEA**	DCM	240/1/1	0.8	60	100	5.26	5.56	1.02
9	**DMEA**	DCM	480/1/1	0.8	300	91	9.57	9.53	1.04
10	**DMEA**	DCM	800/1/1	0.8	480	100	17.54	18.10	1.05
11[e]	**DMEA-TMS'**	DCM	120/1/-	0	1440	90	2.37	26.80	1.61
12	**DMEA-TMS'**	DCM	120/1/1	0.8	30	-	-	-	-
13	**DMEA**	DCM	120/1/2	1.6	30	100	2.63	2.79	1.02
14	**DMEA**	DCM	120/1/3	2.4	90	100	2.63	2.61	1.02
15	**DMEA**	DCM	120/1/5	4	240	90	2.63	2.60	1.04
16	**DMEA**	DCM	120/1/10	8	240	57	1.50	1.58	1.05
17[e]	**THEED**	DMF	120/1/-	0	60	73	1.92	3.62	1.29
18	**THEED**	THF	120/1/-	0	60	91	2.39	19.3	1.12
19	**THEED**	DCM	120/1/-	0	5.5	100	2.63	6.58	1.06
20	**-**	DCM	120/-/5	4	360	0	-	-	-
21[e]	**THEED**	DMF	120/1/1	0.8	270	81	2.13	1.63	1.18
22[e]	**THEED**	THF	120/1/1	0.8	60	95	2.50	5.51	1.29
23	**THEED**	DCM	120/1/1	0.8	10	100	2.63	3.22	1.04
24	**THEED**	DCM	240/1/1	0.8	26	100	5.26	5.60	1.04
25	**THEED**	DCM	480/1/1	0.8	120	100	10.52	10.69	1.05
26	**THEED**	DCM	800/1/1	0.8	300	100	17.54	17.80	1.08
27	**THEED-TMS'**	DCM	120/1/-	0	240	89	2.34	39.18	1.80
28	**THEED-TMS'**	DCM	120/1/1	0.8	20	0	-	-	-
29	**THEED**	DCM	120/1/2	1.6	20	100	2.63	2.90	1.05
30	**THEED**	DCM	120/1/3	2.4	50	100	2.63	2.92	1.05
31	**THEED**	DCM	120/1/5	4	60	75	1.97	1.99	1.05
32	**TEA**	DCM	120/1/2	1.6	25	100	2.63	2.80	1.06
33	**TEA**	DCM	240/1/1	0.8	30	100	5.26	5.40	1.05
34	**TEA**	DCM	480/1/1	0.8	135	100	10.52	10.75	1.04
35[e]	**TEA-TMS'**	DCM	120/1/-	0	1440	85	2.24	30.10	1.73
36	**TEA-TMS'**	DCM	120/1/2	1.6	60	-	-	-	-
37	**MDEA**	DCM	120/1/2	1.6	27	100	2.63	2.67	1.04
38	**MDEA**	DCM	240/1/1	0.8	40	100	5.26	5.32	1.04
39	**MDEA**	DCM	480/1/1	0.8	260	100	10.52	10.68	1.05
40[e]	**MDEA-TMS'**	DCM	120/1/-	0	1440	91	2.39	29.6	1.68
41	**MDEA-TMS'**	DCM	120/1/2	1.6	60	-	-	-	-
42	**Boc-EA**	DCM	120/1/1	0.8	1440	0	-	-	-
43	**MEA**	DCM	120/1/1	0.8	135	100	2.63	3.23	1.20

[a] Polymerization was performed at 25 °C with $[NCA]_0$ = 0.19M. [b] *In situ* IR was used to determine the conversion of NCA by analysing the intensity of the NCA anhydride absorption band at 1792 cm^{-1}. [c] Calculated by [NCA]/[I] × (M_{NCA}-44) × X (X = Conv.). [d] Determined by size-exclusion chromatography (SEC) combined with multi-angle light scattering (MALS), viscometry (VISC), and differential refractive index (DRI) triple detection in 0.1 M LiBr in DMF at 60 °C. [e] The SEC curves are bimodal.

Polymerization of *Glu*-NCA by DMEA or TU-S

The polymerization of Glu-NCA initiated by DMEA was first evaluated in three different solvents *N, N*-dimethylformamide (DMF), tetrahydrofuran (THF) and dichloromethane (DCM) in the absence of TU-S (Table 1, runs 1-3). Under identical conditions (25°C, [Glu-NCA]=0.19M and M/I=120) the SEC trace of polypeptide obtained in DMF was bimodal (PDI=1.58), indicating that the two mechanisms (NAM and AMM) occurred simultaneously during polymerization. When the polymerization was carried out in THF, a higher activity (100% monomer conversion in 3h) compared to that in DMF was achieved but the molecular weight (MW) (29.67×10^4) was much higher than the targeted one. The PDI of the obtained polypeptide was relatively high (1.28) and the SEC trace was asymmetric. When the solvent was changed to DCM, the polymerization was completed in 6 minutes and the MW of the produced polypeptide (5.27×10^4) was two times that of the targeted MW (2.59×10^4). The PDI of the final polymer was low (1.08), but the SEC chromatogram was still asymmetric (Figure 1). These polymerization results indicate that, in a non-hydrogen bond forming solvent like DCM, **DMEA** promotes a faster ROP of Glu-NCA (vs. that in DMF or THF) and simultaneously exhibits a higher initiating efficiency (indicated by the value of $M_{n,calcd}/M_{n,SEC\text{-}LS}$). Therefore, most of the polymerizations of Glu-NCA initiated by **DMEA** were conducted in DCM.

We also checked whether **TU-S** can itself initiate the polymerization of NCA and found that **TU-S** alone is unable to promote NCA polymerization. Under similar conditions to the previous case (25°C, DCM, [Glu-NCA]=0.19 M and M/I=120), the monomer conversion was zero in 6 h (Table 1, run 4). The ^{1}H NMR study of **TU-S**, Glu-NCA and **TU-S**/Glu-NCA mixture shows that **TU-S** cannot induce the ROP of NCA and consequently is only a hydrogen bonding donor to Glu-NCA. The signal of imide in **TU-S** is indeed desheilded from 7.90 to 8.13 ppm in the presence of Glu-NCA due to the hydrogen bonding between Glu-NCA and **TU-S** (Figure 2 and Figure S1-S2). According to the literatures, hydrogen bonding between **TU-S** and the oxygen atom of carbonyl can dramatically enhance the activity of carbonyl.[8]

Polymerization of *Glu*-NCA by DMEA in the Presence of TU-S

We then studied the polymerization promoted by **DMEA** in the presence of **TU-S**, expecting that the hydrogen-bonding donor **TU-S** can play a positive role in improving the performance of Glu-NCA polymerization. As expected, with a 0.8 mol% loading of **TU-S** relative to Glu-NCA (**TU-S**/**DMEA**=1, M/I=120), the polymerization went very fast in DCM at room temperature and all NCA monomer was converted into polypeptides in 12 min. Moreover, the obtained polymer exhibits a very narrow symmetric SEC trace (PDI=1.05) and the MW is close to the targeted one (Table 1, run 7). When the M/I ratio ([Glu-NCA]/[**DMEA**]) was gradually increased from 120 to 800 by decreasing the [**DMEA**], the MW of the obtained polypeptide increased linearly as a function of M/I and the PDI: 1.02 to 1.05, suggesting the absence of chain-breaking reactions during polymerization (Figure S3).[11] The livingness of polymerization was further demonstrated by the linearity of M_n vs conversion and the very low PDI values (Figure S4). Remarkably, when M/I ratio was increased to 800, 100% monomer conversion was obtained in 8h, resulting in polymer with expected MW and low PDI (18.10×10^4, 1.05). By varying the M/I ratios between 100 and 800, a series of polypeptides with different targeted MWs (from 3.01×10^4 to 18.10×10^4) and low PDI values (between 1.02 and 1.05) was successfully synthesized. Additionally, we compared the behavior of the **TU-S**/**DMEA** system in different polymerization solvents (DMF, THF and DCM). Well-defined polypeptides could only be obtained in the non-hydrogen bond forming solvent, DCM (Table 1, runs 5-7 and Figure S5). This indicates that hydrogen bonding is crucial for monomer activation and its subsequent polymerization, and that DMF and THF by their polar nature disrupt the interaction between **TU-S** and monomer.

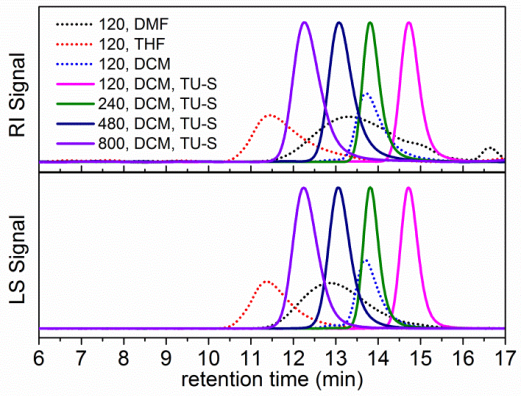


Figure 1. SEC traces of polypeptides obtained in Table 1(runs 1-3, and 7-10).

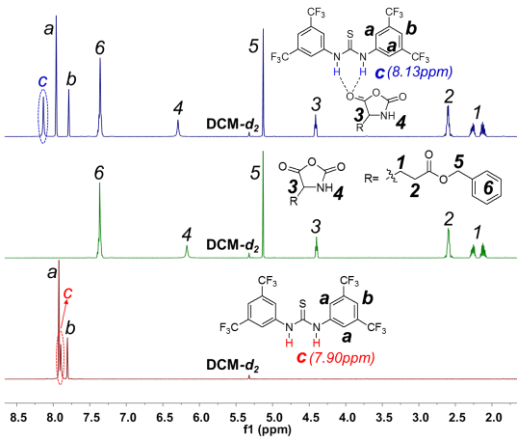


Figure 2. Expanded ^{1}H NMR spectra of **TU-S**, Glu-NCA and **TU-S**/Glu-NCA (1:1) mixture (500M, 25°C, CD_2Cl_2, Full spectra see Figure S1-S2).

Investigation of the Initiation Step

As **DMEA** contains both a primary alcohol and a tertiary amine, initiation may also occur through the latter group. In order to clarify this question, we protected the hydroxyl of **DMEA** by reacting with *tert*-butyldimethylsilane (resulted in **DMEA-TMS'**) and then used **DMEA-TMS'** to initiate polymerization. We found that **DMEA-TMS'** initiates the polymerization in the absence of **TU-S** but exhibits very low activity (24h, 90%) and afforded a polymer sample of very high MW and PDI (M_n=26.80×10^4, PDI=1.61). In the presence of **TU-S**, **DMEA-TMS'** showed no activity towards the ROP of Glu-NCA under the same conditions. ^{1}H NMR indicates that **TU-S** is a hydrogen-bonding donor to the tertiary amine of **DMEA-TMS'** and thus neutralizes the initiation capability of tertiary amine. The signal of imide in TU-S shifts "downfield" from 7.90 to 9.23 ppm and the signals of **DMEA-TMS'** (H_d, H_e, and H_f) also shift "downfield" (Figure 3 and Figure S6-S7). These results suggest that the tertiary amine of **DMEA** cannot trigger the polymerization of Glu-NCA in the presence of **TU-S** and the only initiating site is the hydroxyl group of **DMEA**. A further proof of the -OH initiating sites is coming from the NMR spectra of low-MW polypeptides where the initiator residuals can clearly be observed (see Figure S29-S32).

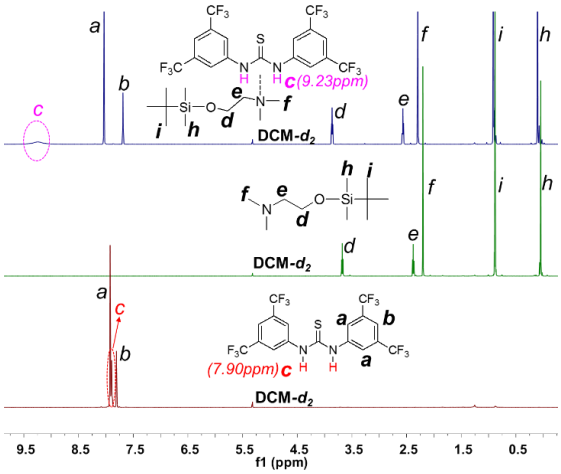


Figure 3. Expanded ^{1}H NMR spectra of **TU-S**, **DMEA-TMS'** and **TU-S/DMEA-TMS'** (1:1) mixture (500M, 25°C, CD_2Cl_2, Full spectra see Figure S6-S7).

For purpose of investigating the kinetics of polymerization, Glu-NCA in the presence of **TU-S** ([M]$_0$= 0.19 M, [M]$_0$/[**DMEA**]$_0$/[**TU-S**]$_0$= 120:1:1) was polymerized at 25 °C in DCM. The progress of the polymerization reaction was monitored *in situ* by IR at fixed time intervals (10 seconds) for a minimum of four half-live times. The polymerization exhibits a first-order dependence on the monomer concentration (Figure 4, black line). The plot of ln([M]$_0$/[M]$_t$) vs time gives two successive straight lines with different slops. The difference in the slop is due to the secondary structure transition of PBGL from β-sheets (non-helical, DP < about12) to α-helices, DP> about12). The linearity of the two lines suggests that the initiation is faster or comparable to chain propagation under the experimental conditions.[12] After initiation, the active chain ends are primary amines and the whole polymerization exclusively follows normal amine mechanism.

TU-S Effect on the Polymerization Rate

We then increased the amount of **TU-S** in the reaction medium from 0.8 mol% (**TU-S**/**DMEA**=1, M/I=120, Table 1, run 7) to 1.6 mol% (**TU-S**/**DMEA**=2, M/I=120, Table 1, run 13) with a view of increasing the overall rate of polymerization. Instead of an increase, the rate of polymerization underwent a decrease and longer time was required to complete polymerization (30 minutes). Increasing further the **TU-S** loading to 2.4 mol% (**TU-S**/**DMEA**=3, M/I=120, Table 1, run 14), the polymerization needed 90 minutes to achieve 100% conversion. A further increase to 8.0 mol% (**TU-S**/**DMEA**=10, M/I=120, Table 1, run 16) yielded only 57% monomer consumption after 4h. The kinetic studies using the *in situ* IR showed that the observed polymerization rate k_{obs} decreased from 75.1×10^{-4} to 0.5 ×10^{-4}s^{-1} upon increasing [**TU-S**] from 0.8 to 8.0 mol% in the system (Figure 4). It is worth noticing that the incremental addition of **TU-S** in the medium did not affect the integrity of the samples. Good correlation between molecular weight values and initial [Glu-NCA]/[**DMEA**] value and narrow dispersity of polymers could still be obtained in all cases.

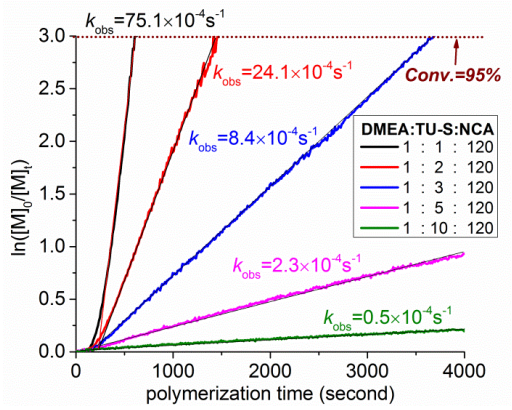


Figure 4. Kinetics of the ROP of Glu-NCA promoted by **DMEA/TU-S** ([M]=0.19M, [Glu-NCA]/[**DMEA**]=120, [**TU-S**]/[**DMEA**]=1, 2, 3, 5, and 10, 25°C, CH_2Cl_2, the automatic sampling interval of *in situ* IR is 10 seconds).

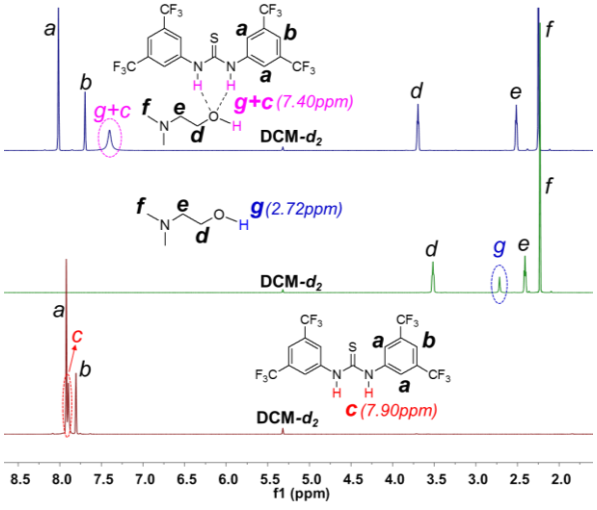


Figure 5. ^{1}H NMR spectra of **TU-S**, **DMEA** and **TU-S/DMEA** (1:1) mixture (500M, 25°C, CD_2Cl_2, Full spectra see Figure S8).

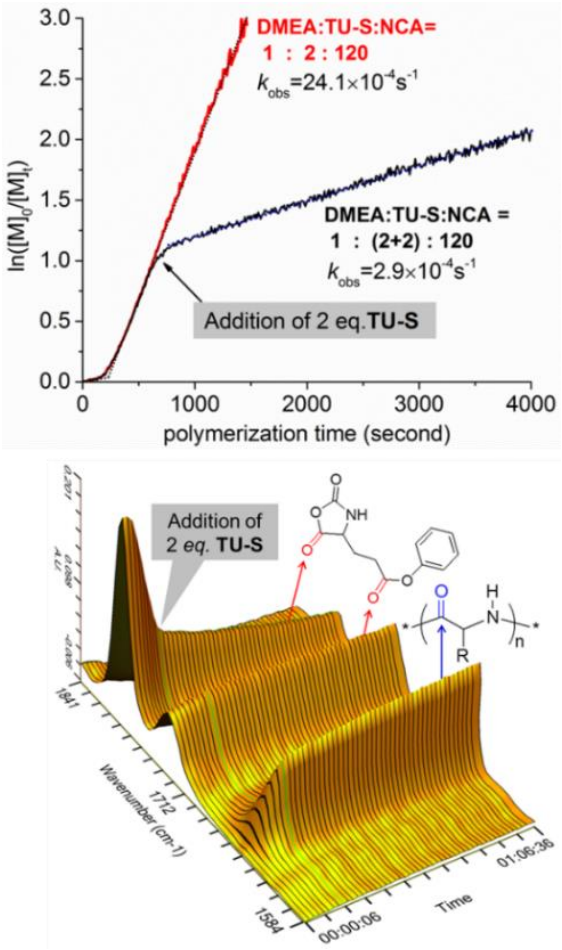


Figure 6. ln([NCA]$_0$/([NCA]$_t$) vs time for the ROP of Glu-NCA initiated by **DMEA/TU-S** ([M]=0.19M,[Glu-NCA]/[**TU-S**]/[**DMEA**]=120/(2+2)/1, 25°C, CH_2Cl_2) and corresponding 3D kinetic behavior profile from *in situ* IR (the sampling interval of *in situ* IR is 10 seconds).

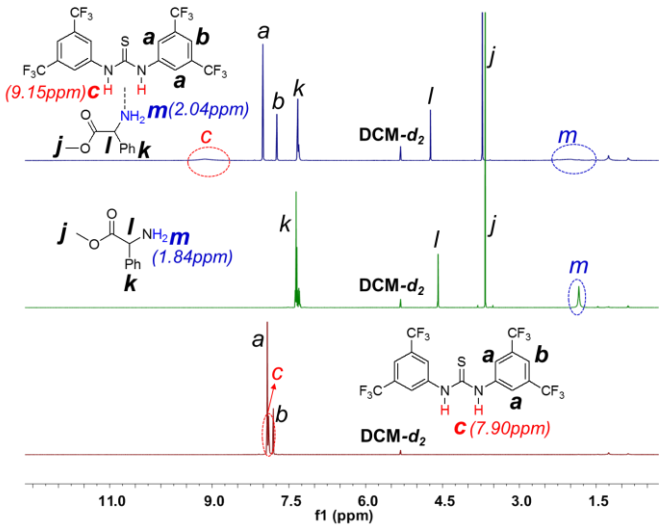


Figure 7. ^{1}H NMR spectra of **TU-S**, methyl 2-amino-2-phenylacetate (**MAP**) and **TU-S**/**MAP** mixture (500M, 25°C, CD_2Cl_2, Full spectrum of the mixture see Figure S9).

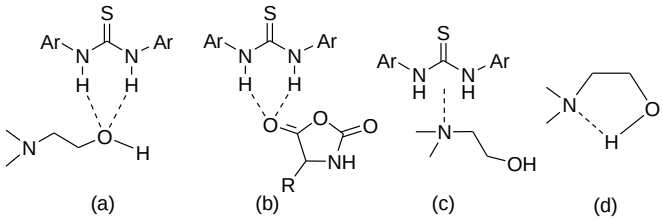
^{1}H NMR investigation of **TU-S**, **DMEA** and **TU-S**/**DMEA** mixture in DCM-d_2 revealed that hydrogen bonding was formed between **TU-S** and **DMEA**. In the presence of **TU-S**, the signal of the hydroxyl group in **DMEA** shifts "downfield" from 2.72 to 7.40 ppm and the signal of the imide protons in **TU-S** shifts "upfield" from 7.90 to 7.40 ppm (Figure 5 and Figure S8), meaning that the nucleophilic character of the hydroxyl group in **DMEA** decreases upon formation of hydrogen-bonding with **TU-S**. Therefore, the presence of **TU-S** in system resulted in a relative deactivation of the hydroxyl group. With the increase of **TU-S**, the concentration of **TU-S**-deactivated –OH increased, decreasing the rate of polymerization. However, all polymers from polymerization experiments with different amounts of **TU-S** possess the same targeted MW and very low PDI, suggesting that all hydroxyl groups of **DMEA** (**TU-S** deactivated -OH and free -OH) trigger the polymerization of Glu-NCA through rapid exchange between **TU-S** deactivated -OH and free –OH. Since the rate of exchange is faster than that of the propagation, all hydroxyl groups have an equal chance to trigger the polymerization. After initiation, this exchange between dormant and active species still occurs between the amine end groups, which was confirmed by the results from *in situ* IR monitoring showing that the rate of polymerization slowed down after adding excess **TU-S** into system during the polymerization (Figure 6). This dormant/active exchange was further supported by the ^{1}H NMR investigation of **TU-S**/2-amino-2-phenylacetate (**MAP**), a small model molecule with similar structure to that of polymer chain end (Figure 7 and Figure S9).

TU-S has thus a triple effect through its ability to donate hydrogen-bonding: (1) to activate the monomer; (2) to reversibly deactivate the growing amines, which results in a slower rate of polymerization; (3) to totally silence the tertiary amine in DMEA thus preventing proton abstraction of the monomers and the occurrence of AMM.

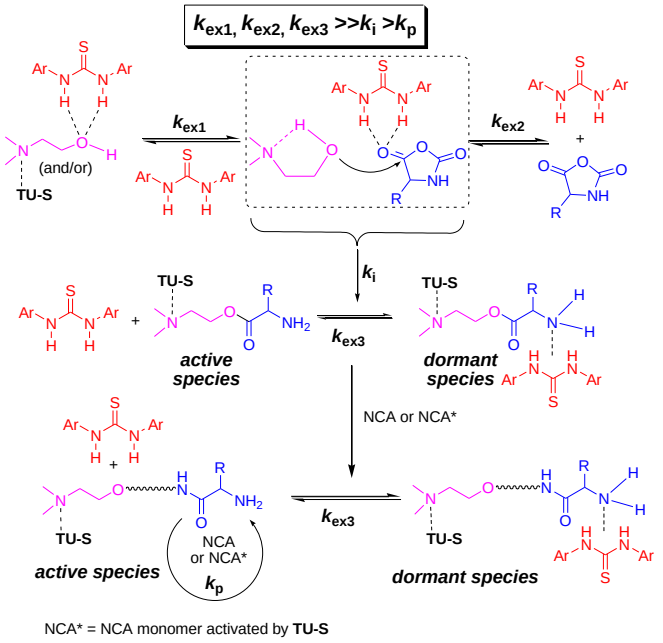
Proposed mechanism and Extension of initiators to tri-, di- or mono- alcohols

As mentioned before, the ^{1}H NMR study indicates that **TU-S** not only interacts with the carbonyl of NCA monomer but also with the tertiary amine and hydroxyl group of **DMEA** (Figures 2, 3 and 5 and Scheme 3). To investigate the possible generality of the above observation, we used benzyl alcohol (**BnOH**) and *tert*-butyl 2-hydroxyethylcarbamate (**Boc-EA**) as initiating system to promote the ROP of Glu-NCA in the presence of TU-S but failed to get polymer. This indicates that the tertiary amine in **DMEA** plays an important role in the initiating step. The polymerizations respectively initiated by **Boc-EA**, **MEA**, or **DMEA** further supported this conclusion. In the case of **MEA**, the polymerization could be successfully triggered, but the polypeptide formed exhibits a high MW and PDI due to the poor capability of secondary amine to activate the hydroxyl group (vs. that of tertiary amine)(Table 1, run 43). So, a plausible polymerization pathway is shown in scheme 4, in which multiple hydrogen-bonding equilibria involving initiator (**DMEA**) and catalyst (**TU-S**) are existed in the ROP of NCA monomer. By using tetra- tri-, or di- alcohols with tertiary amines (**THEED**, **TEA**, or **MDEA**), we successfully extended this strategy to multi-armed and well-defined PBLG with designed MW and low PDI (Table 1, runs 23-26, 29-34 and 37-39). In addition, NMR and polymerization experiments as that for **DMEA** were carried out by using **THEED** as initiator. Similar effects of **TU-S** on the

polymerization rate and multiple hydrogen-bonding involving **THEED**, **TU-S** and NCA monomer were also observed (the detailed results see SI Figure S10-S17). The lower viscosities in the Mark-Houwink-Sakurada plots (Figure S33) of PBLG prepared by **THEED** (four -OH initiator) than those prepared by **DMEA** (one -OH) and hexamethylene diamine **HMDA** (two -NH_2) is giving another proof that the -OH groups are the initiating species of the ROP of NCAs.



Scheme 3. Multiple hydrogen-bonding involving initiator (**DMEA**), catalyst (**TU-S**) and NCA monomer.



Scheme 4. Multiple hydrogen-bonding equilibria involving initiator (**DMEA**), catalyst (**TU-S**) and monomer during the ROP of NCA.

Polymerization of Lys-NCA and Copolymerization of Glu-and Lys-NCA

Beside the homopolymerization of Glu-NCA, we also tried the homopolymerization of Lys-NCA and the copolymerization of Glu- and Lys-NCA. As shown in Table 2, all initiators (**DMEA**, **MDEA**, **TEA**, and **THEED**) could trigger controlled ROP of Lys-NCA in the presence of **TU-S** and give polypeptides with targeted MW and very low PDI (1.02-1.05). Especially, by using **THEED** as initiator, multi-armed PBLG-*block*-PZLL and PBLG-*random*-PZLL with targeted MW and narrow PDI were easily prepared.

Conclusions

In summary, we have demonstrated, for the first time, that aminoalcohols in the presence of *N,N'*-bis[3,5-bis(trifluoromethyl)phenyl]thiourea (**TU-S**) catalyst promotes the fast controlled/living ROP of Glu-NCA under mild conditions. It has been proven that **TU-S** has a triple effect through its ability to donate hydrogen-bonding: (1) to activate the monomer; (2) to reversibly deactivate the growing amines, which results in a protection of active polymer chain ends; (3) to totally silence the tertiary amine in **DMEA** thus preventing proton abstraction of the monomers and the occurrence of AMM. This general strategy was successfully extended to the synthesis of well-defined di- and multi-armed polypeptides by using di-, tri-, or tetra-aminoalcohol initiators. Further study will be carried out to extend this strategy to other NCA monomers and to the synthesis of functionalized polypeptides.

Table 2. Homo- and co-polymerization of Lys-NCA and Glu-NCA

run [a]	initiator	$[M]_0/[I]_0$	Time (min) [b]	$M_{n,calcd}$ ×10^{-4} [c]	$M_{n,exp}$ ×10^{-4} [d]	PDI [d]
1	**DMEA**	120/1	53	3.15	2.90	1.02
2	**DMEA**	240/1	160	6.30	6.42	1.04
3	**MDEA**	120/1	45	3.15	2.64	1.04
4	**MDEA**	240/1	130	6.30	6.51	1.03
5	**TEA**	120/1	39	3.15	3.02	1.05
6	**TEA**	240/1	120	6.30	6.43	1.04
7	**THEED**	120/1	35	3.15	3.05	1.05
8	**THEED**	240/1	100	6.30	6.30	1.04
9	**THEED**	480/1	300	12.60	13.03	1.05
10	**THEED (block)**	(120+120)/1	155+25	5.78	5.91	1.07
11	**THEED (random)**	(120+120)/1	200	5.78	5.83	1.08

[a] Polymerization was performed in DCM at 25 °C with [TU-S]=7.6mM and $[NCA]_0$ = 0.19M. [b] *In situ* IR was used to determine the time when >99% monomer conversion was achieved. [c] Calculated by [NCA]/[I] × (M_{NCA}-44) × X (X = Conv.). [d] Determined by SEC combined with MALS-VISC-DRI triple detection in 0.1 M LiBr in DMF at 60 °C. dn/dc(PBLG-co-PZLL)=0.114mL·g^{-1}, dn/dc(PBLG)= 0.104mL·g^{-1} and dn/dc(PZLL)= 0.123 mL·g^{-1}.

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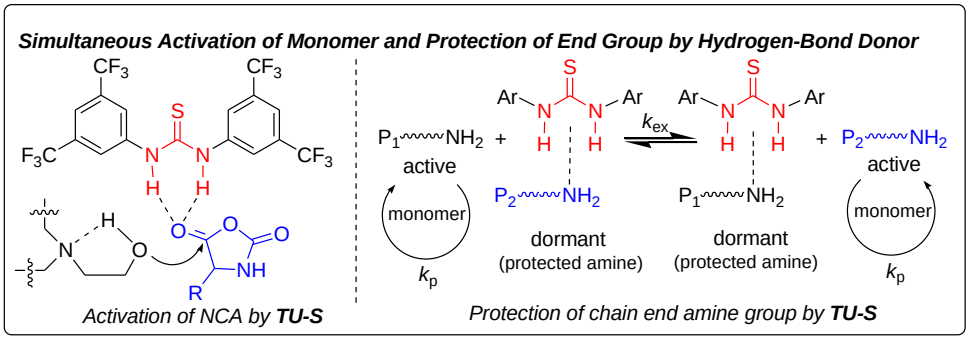

Hydrogen-Bonding Organocatalytic Controlled/Living ROP of α-Amino acid *N*-Carboxyanhydrides