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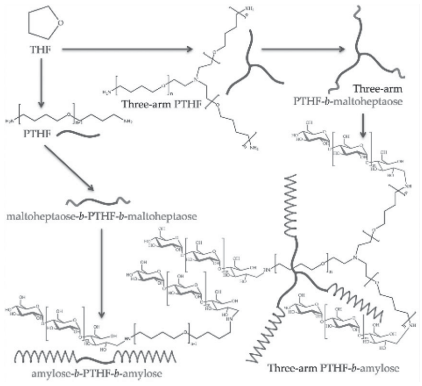
Synthesis of Telechelic and Three-Arm Polytetrahydrofuran-*block*-amylose

Rachmawati Rachmawati, Hilde D. de Gier, Albert J. J. Woortman, Katja Loos*

Telechelic amine terminated polytetrahydrofuran (PTHF) is prepared via cationic ring opening polymerization (CROP) of THF, initiated by trifluoromethanesulphonic anhydride (triflic anhydride). Hexamethylene tetramine (HMTA) is used as a terminating agent. The resulting HMTA terminated PTHF is hydrolyzed to result in an amine terminated PTHF. Reductive amination is carried out by reacting the PTHF with maltoheptaose resulting in maltoheptaose-*b*-PTHF-*b*-maltoheptaose. The product is prepared as a primer for the enzymatic polymerization to synthesize amylose-*b*-PTHF-*b*-amylose. In addition, a three-arm PTHF is prepared via CROP of THF. The initiator is synthesized in situ by the reaction of triflic anhydride and triethanol amine. The resulting amine terminated three-arm PTHF is reacted with maltoheptaose to synthesize a three-arm PTHF-*b*-maltoheptaose which can be used for the enzymatic synthesis of three-arm PTHF-*b*-amylose. Characterization of the products is difficult due to the amphiphilic behavior of both telechelic amylose-*b*-PTHF-*b*-amylose and three-arm PTHF-*b*-amylose. Therefore, the analysis of the products is mainly based on attenuated total reflectance Fourier transform infrared spectroscopy.



1. Introduction

The presence of the hydroxyl groups in amylose chains results in unique helical structures due to intra- and intermolecular hydrogen bonds.[1] The single helical amylose owning a hydrophobic cavity inside and a hydrophilic surface outside, is capable of forming inclusion complexes with various guest molecules such as iodine,[2] lipids,[3–9] and polymers[10–18] via hydrophobic interactions. Molecular recognition between amylose as a host and polytetrahydrofuran (PTHF) as a guest was previously successfully applied to prepare amylose-PTHF complexes.[16–18] The complex formation was favourably employed not only to amylose as a homopolymer but also to diblock copolymers such as PTHF-*b*-amylose which was able to form complexes with guest PTHF.[19] This means that even though the amylose is covalently attached to another polymer, it is still capable of recognizing suitable guest molecules. Moreover, there is an indication that amylose-PTHF complexes are prone to certain solvents[18] which indicates that the complex formation between the attached amylose block in PTHF-*b*-amylose with guest PTHF will also be responsive to solvent changes. This can lead to tailored properties of diblock copolymers.

Block copolymers offer interesting structures, which can be applied extensively in a lot of areas, such as printings, paintings, nano-scale electronic devices, and drug delivery systems.[20–26] Therefore, it becomes a point of interest to study whether the amylose complex formation can also be applied to a wider range of block copolymers, such as stars, dimers, triblock, comb-shaped, or branched polymers. In this case, the host–guest interaction between amylose and PTHF can be used to introduce an additional

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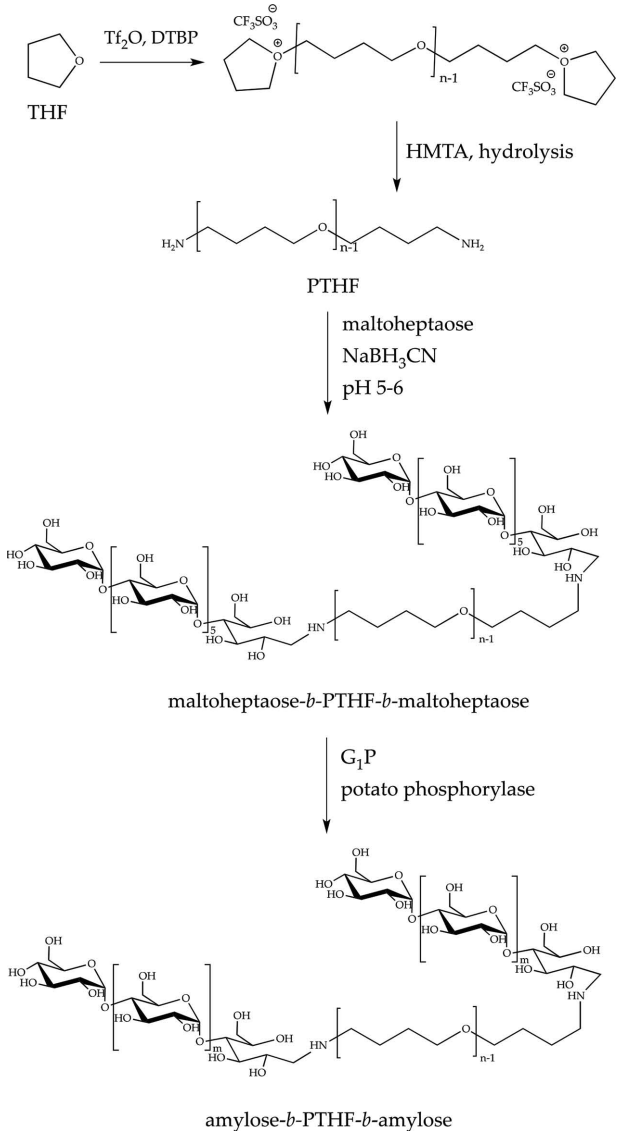
block to the existing block copolymers. This can result in higher ordered structures. Here we discuss the synthesis of amylose-containing block copolymers: amylose-*b*-PTHF-*b*-amylose (an ABA block copolymer) and three-arm PTHF-*b*-amylose (a star block copolymer). As these polymers contain an amylose block, they also can be used as host polymers for complex formation with suitable guest molecules.

Telechelic PTHF can be synthesized via cationic ring opening polymerization (CROP) of THF using triflic anhydride (Tf_2O) as initiator, see Scheme 1. A proton trap, such as 2,6-Di-tert-butylpyridine (DTBP) was added to prevent THF polymerization initiated by triflic acid.[27] Triflic anhydride attacks the oxygen atom of THF, resulting in THF oxonium ions with triflate as ion pairs ($CF_3SO_3^-$). The THF oxonium ions attack other THF monomers and propagate further in both ends of the propagating chains.[27] Terminating agent such as hexamethylenetetramine (HMTA) can be used to terminate the polymerization of THF. The resulting telechelic HMTA-terminated PTHF can be hydrolyzed further to result in telechelic amine terminated PTHF.[28] Reductive amination using $NaBH_3CN$ as a reducing agent can be used to couple maltoheptaose to the synthesized telechelic amine terminated PTHF. The resulting maltoheptaose-*b*-PTHF-*b*-maltoheptaose is expected to be able to act as a primer for the enzymatic polymerization of glucose-1-phosphate (G_1P) to prepare amylose-*b*-PTHF-*b*-amylose.[29–33] In this case, potato phosphorylase is used as a biocatalyst for the enzymatic polymerization (Scheme 1).

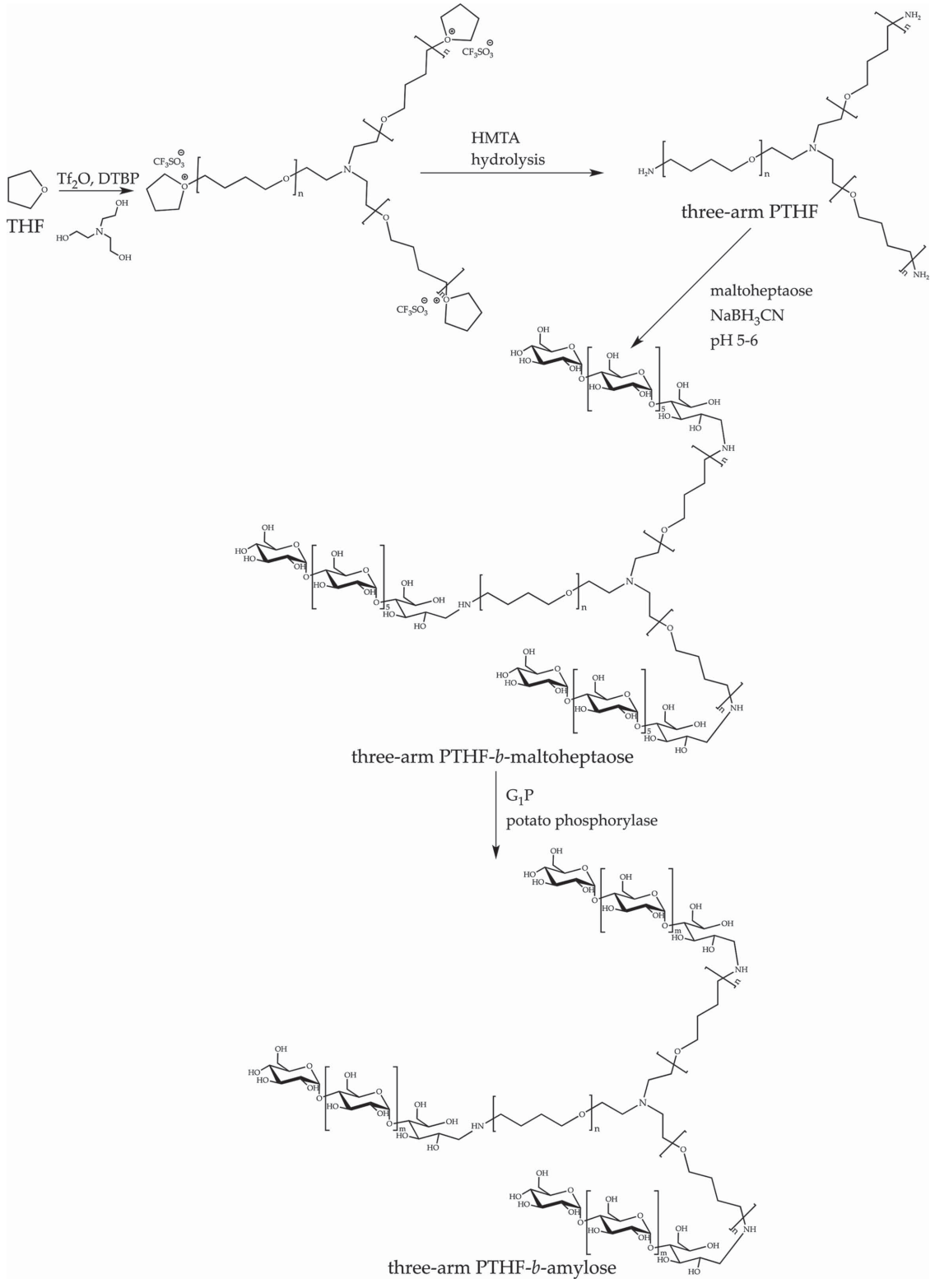


Scheme 1. Synthesis route of amylose-*b*-PTHF-*b*-amylose.

Three-arm PTHF-*b*-amylose can be synthesized based on a reaction route as depicted in Scheme 2. In this route, the synthesis of three-arm PTHF starts form the core of the three-arm and proceeds based on the CROP of THF. A three-arm initiator can be synthesized in situ by reacting a three-arm molecule having hydroxyl groups as terminal groups, such as triethanol amine (TEA), with triflic anhydride (Tf_2O). DTBP as a proton trap was also added to prevent THF polymerization initiated by triflic acid.[27] A terminating agent is then added to result in the desired end groups of the three-arm PTHF.

Another alternative method to prepare three-arm PTHF is by polymerizing THF from one end (not as a telechelic synthesis). A molecule with three arms, such as tris(2-aminoethyl)amine,[34] which is able to terminate up to three propagating chains, is then used to terminate the polymerization. As the three-arm PTHF will be used for reductive amination, this method involving a three-arm terminating agent requires a protected amino initiator. Therefore, the synthesis from the core is preferred. However, for the synthesis of three-arm PTHF without further functionalization, a facile initiator such as methyl triflate can be used in combination with tris(2-aminoethyl)amine as terminating agent.

The amine terminated three-arm PTHF can be used to couple maltoheptaose via reductive amination, which results in three-arm PTHF-*b*-maltoheptaose. Subsequent enzymatic polymerization catalyzed by potato phosphorylase to synthesize PTHF-*b*-amylose can be conducted. In this enzymatic reaction, the three-arm PTHF-*b*-maltoheptaose acts as a primer and glucose-1-phosphate (G_1P) as monomer. The resulting three-arm PTHF-*b*-amylose, which consists of a diblock copolymer, is expected to have morphologies such as lamellar, gyroid, hexagonal, and cubic phases.[35] Additionally, as the three-arm PTHF-*b*-amylose contains an amylose block, it can also be used as a host molecule for complex formation, which can lead to interesting structures. The biodegradable characteristic of amylose also gives additional value to the resulting products.



Scheme 2. Synthesis route of three-arm PTHF-*b*-amylose.

Besides being a biodegradable polymer, the amylose block in amylose-*b*-PTHF-*b*-amylose is presumed to be able to host guest molecules in a similar way to PTHF-*b*-amylose.[19] One of the possible inclusion ways between amylose-*b*-PTHF-*b*-amylose and guest PTHF is depicted in Figure 1. The amylose as the A block is modified into an

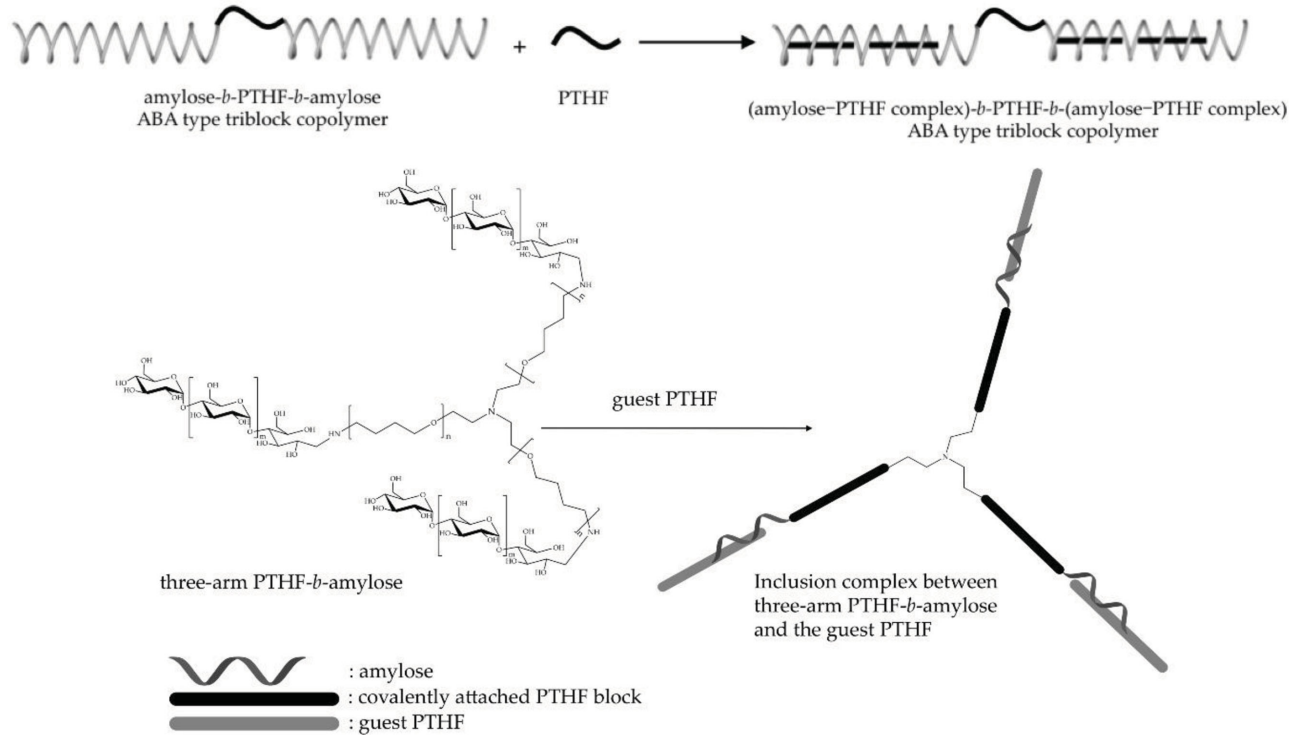


Figure 1. Possible structures of inclusion complex formation between amylose-*b*-PTHF-*b*-amylose and PTHF (top) and between three-arm PTHF-*b*-amylose and PTHF (bottom).

amylose-PTHF complex so that the resulting structure can be written as an ABA triblock copolymer in the form of [amylose–PTHF complex]-*b*-PTHF-*b*-[amylose–PTHF complex]. PTHF-*b*-amylose can also be used as a guest polymer for complex formation with amylose-*b*-PTHF-*b*-amylose. In this case, the possible resulting complex is in the form of a CABAC block copolymer: amylose-*b*-[amylose–PTHF complex]-*b*-PTHF-*b*-[amylose–PTHF complex]-*b*-amylose.

The possible inclusion between the three-arm PTHF-*b*-amylose with the guest PTHF which is depicted in Figure 1 shows that the guest PTHF can be fully included inside the amylose helices. In this case, the resulting structure can be written as the three-arm PTHF-*b*-[amylose–PTHF complex] which consists of diblock copolymers with PTHF as the block A and amylose-PTHF complex as the block B. However, if the guest PTHF is not fully included, the resulting structure is in the form of the three-arm PTHF-*b*-[amylose–PTHF complex]-*b*-PTHF. This means that the arms are in the form of an ABA triblock copolymer. This shows that amylose complex formation can be a versatile approach to modify a three-arm block copolymer.

2. Experimental Section

2.1. Materials

Trifluoromethanesulphonic acid (triflic anhydride, Tf_2O, $(CF_3SO_2)_2O$, ≥99%, from Sigma-Aldrich), 2,6-Di-tert-butylpyridine (DTBP, ≥97%, from Aldrich), triethanolamine (TEA, from Aldrich), hexamethylenetetramine (HMTA, ≥99.5%, from Sigma-Aldrich), sodium amide ($NaNH_2$, 95%, from Aldrich), sodium cyanoborohydride ($NaBH_3CN$, ≥95%, from Fluka), glacial acetic acid (CH_3COOH, ≥99.8%, from Fluka), polytetrahydrofuran bis (3-aminopropil) terminated with molecular weight of 1100 g mol^{-1} (PTHF1100, from Aldrich), deuterated chloroform (CDCl3, 99.8%, from Sigma-Aldrich), sulphuric acid (H_2SO_4, 95–97%, from Merck), α-D-glucose 1-phosphate disodium salt hydrate (G1P, 97%, from Sigma-Aldrich), sodium hydroxide (NaOH, extra pure, from ACROS), methanol (MeOH, 99.8%, from LAB-SCAN), toluene (99.5%, from LAB-SCAN), and potassium carbonate (K_2CO_3, >99%, from Merck) were used as received. Chloroform ($CHCl_3$, 99.5%, from LAB-SCAN) and dichloromethane (DCM, CH_2Cl_2, 99.8%, from LAB-SCAN) were dried over CaH_2 and stored under nitrogen at room temperature. Dimethyl sulfoxide (DMSO, ≥99%, from Aldrich) was dried over molecular sieves. Tetrahydrofuran (THF, >99.5%, from Acros) was distilled over CaH_2 followed by subsequent distillation over sodium and benzophenon and stored under nitrogen at 6 °C. Potato phosphorylase enzyme, Fiske-Subarrow reagents, and maltoheptaose were prepared as described in literature.[31,36]

2.2. Methods

2.2.1. Characterization

Fourier transform infrared (FTIR) Spectroscopy measurements were performed as Attenuated Total Reflectance (ATR-FTIR) on a Bruker IFS88 FTIR spectrometer equipped

with a MCT-A detector. The sample was measured with an average of 50 scans at a resolution of 4 cm^{-1}.

Proton nuclear magnetic resonance (^{1}H-NMR) spectra were obtained using a 400/500 MHz Varian VXR operating at room temperature using deuterated chloroform or DMSO as solvent.

Differential scanning calorimetry (DSC) measurements were performed on a Perkin Elmer Pyris 1 DSC that had been calibrated with indium. An empty pan was taken as a reference. The samples were weighed into DSC large volume cups (LVC) as a suspension in water at a concentration of 10 wt%. The samples were equilibrated overnight before the measurements. The samples were heated and cooled under nitrogen in the range of 1–160 °C with a rate of 10 °C min^{-1}. Air dried samples were measured as 90 wt% dry matter while freeze dried samples were calculated as 97 wt% dry matter.

Gel permeation chromatography (GPC) measurements were performed on a Spectra Physics AS 1000 LC-system equipped with a Viscotek H-502 viscometer and a Shodex RW-71 refractive index detector. Trisec software (Viscotek) was used for the calculation. THF was used as the solvent. The system was calibrated against polystyrene standards from Polymer Laboratories. Universal calibration was used to calculate the molecular weight.

2.2.2. Synthesis of Telechelic Amine Terminated PTHF

A clean prebaked 100 mL three-necked flask, which was equipped with a magnetic stir bar, was degassed by applying vacuum and a nitrogen flow for three cycles and kept under nitrogen. Dried THF (20 mL, 2.4×10^{-1} mol) was added via a rubber septum, and stirred at 500 rpm. DTBP (200 µL, 9.0×10^{-4} mol) was added followed by the addition of triflic anhydride (100 µL, 5.9×10^{-4} mol). After 15 min, a clear HMTA solution (0.5 g HMTA in 20 mL $CHCl_3$, 1.2×10^{-2} mol) was added. After 1 h stirring, the white suspension was poured into a 100 mL round flask and concentrated using a rotary evaporator, yielding a white viscous suspension. Methanol (30 mL) and toluene (20 mL) were added to dissolve the suspension. Concentrated H_2SO_4 (0.8 mL) was added, forming a white suspension. The suspension was refluxed at 85 °C in which the suspension turned into a clear solution after 10 min. After3 h, the solution was cooled down to room temperature and neutralized using 1 M NaOH. The salt was filtered off and the filtrate was precipitated in 200 mL cold 1 M NaOH. A white product(1.7 g) was recovered.

^{1}H-NMR (400 MHz, in $CDCl_3$) δ ppm: 1.60 (m, $-C_2\boldsymbol{H}_4-CH_2O-$, polymer backbone), 2.69 (t, $-C\boldsymbol{H}_2-NH_2$), 3.30 (s, $C\boldsymbol{H}_3-O-$, end group), and 3.39 (m, $-C\boldsymbol{H}_2-O-$, polymer backbone).

2.2.3. Synthesis of Three-Arm Amine Terminated PTHF

A clean prebaked 100 mL three-necked flask, which was equipped with a magnetic stir bar, was degassed by applying vacuum and a nitrogen flow for three cycles and kept under nitrogen. Dried dichloromethane (1 mL) was added via a rubber septum, and stirred at 500 rpm. Triflic anhydride (73 µL, 4.3×10^{-4} mol) was added followed by the addition of TEA (19 µL, 1.4×10^{-4} mol). After 1 h, DTBP (196 µL, 8.8×10^{-4} mol) was added. After 10 min, dried THF (5 mL, 6.1×10^{-2} mol) was added. $NaNH_2$ (0.1740 g, 4.2×10^{-3} mol) was added after 30 min. The mixture was stirred overnight and then precipitated in cold 100 mL 1 M NaOH. A white product (0.6 g) was recovered.

^{1}H-NMR (400 MHz, in $CDCl_3$) δ ppm: 1.61 (m, $-C_2\boldsymbol{H}_4-CH_2O-$, polymer backbone), 2.49 (m, $-N-C\boldsymbol{H}_2-$, polymer core), 2.73 (m, $-C\boldsymbol{H}_2-NH_2$), 3.41 (m, $-C\boldsymbol{H}_2-O-$, polymer backbone) and 3.70 (m, $-NCH_2-C\boldsymbol{H}_2-$, polymer core).

2.2.4. Synthesis of Maltoheptaose-*b*-PTHF-*b*-maltoheptaose

Maltoheptaose (0.188 g, 1.6×10^{-4} mol) was added into a clean prebaked 100 mL round flask equipped with a magnetic stir bar. DMSO (5 mL) was added and stirred at 500 rpm. $NaBH_3CN$ (0.052 g, 8.3×10^{-4} mol) and PTHF (0.5 g, $M_{n(NMR)}$ 6200 g mol^{-1}, 8.1×10^{-5} mol) were added followed by the addition of THF (5 mL) and glacial acetic acid (0.6 mL, 1.0×10^{-2} mol). The mixture was stirred at room temperature for 15 min and then refluxed for 18 h at 85 °C. The suspension was cooled down to room temperature and precipitated in cold 200 mL 0.05 M NaOH and stirred under a moderate airflow overnight. The resulting white suspension was centrifuged at 2000 rpm (4 °C, 20 min, three times). The supernatant was decanted and the precipitate was freeze-dried. A white product (0.4 g) was recovered.

2.2.5. Synthesis of Three-Arm PTHF-*b*-maltoheptaose

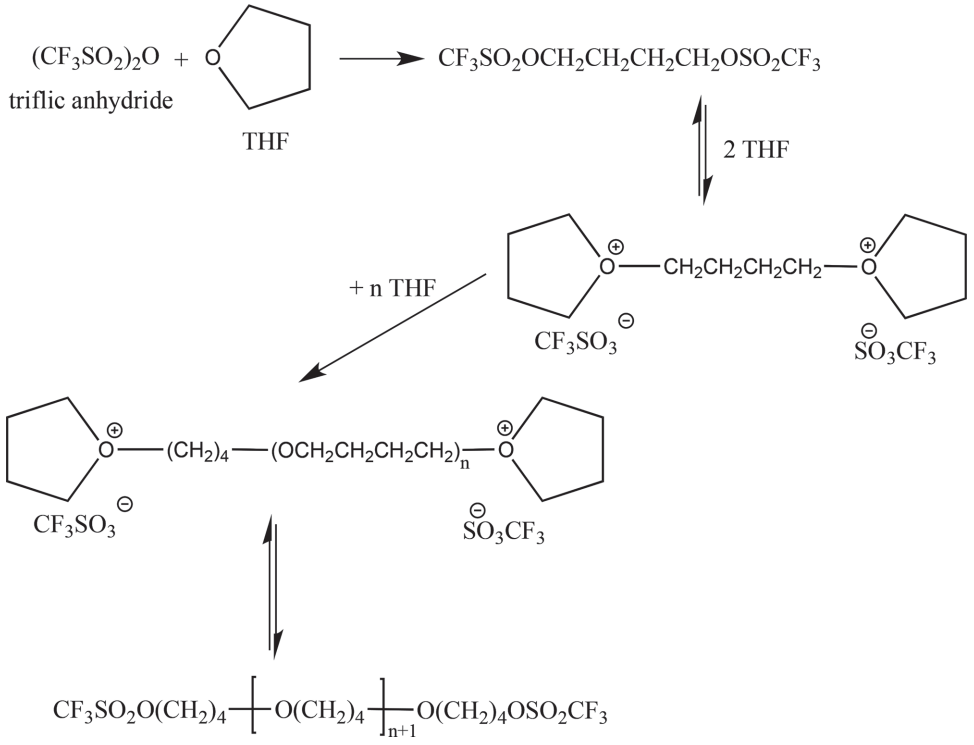
Three-arm PTHF (0.2 g, $M_{n(NMR)}$ 5600 g mol^{-1}, 3.6×10^{-5} mol), $NaBH_3CN$ (0.011 g, 1.8×10^{-4} mol), and maltoheptaose (0.115 g, 1.0×10^{-4} mol) were added into a clean prebaked 100 mL round flask equipped with a magnetic stir bar. DMSO (5 mL) and THF (5 mL) were added and stirred at 500 rpm. Glacial acetic acid (0.6 mL, 1.0×10^{-2} mol) was added. The mixture was stirred at room temperature for 15 min and then refluxed for 5 h at 70 °C. The suspension was cooled down to room temperature, precipitated in cold water, and stirred overnight under a moderate airflow. The resulting white suspension was filtered to yield a white product.

2.2.6. Synthesis of Amylose-*b*-PTHF-*b*-amylose

Maltoheptaose-*b*-$PTHF_{1100}$-*b*-maltoheptaose (50 mg, 1.47×10^{-5} mol) was suspended in 7 mL buffer citrate in a vial and vibrated for 44 h in a ventilation oven at 85 °C. The suspension was poured into an erlenmeyer flask. 0.2 M G_1P in buffer citrate (58.72 mL, DP_n 400) and 41.72 mL buffer citrate were added followed by incubation at 37 °C for 24 h in a thermostat. Potato phosphorylase enzyme (10 mL) was added. The total volume was maintained to keep the resulted G_1P concentration as 0.1 M. An aliquot of 100 µL was taken right after the addition of the enzyme, and 10–100 µL aliquots were also taken during reaction to check the conversion. After the desired conversion was reached, the mixture was poured into 50 vol% ethanol and stored overnight at 6 °C. The mixture was centrifuged at 4000 rpm for 30 min. The solution was decanted while the suspension was lyophilized to yield a white powder.

2.2.7. Synthesis of Three-Arm PTHF-*b*-amylose

Three-arm PTHF-*b*-maltoheptaose (200 mg, M_n 9500 g mol^{-1}, 2.1×10^{-5} mol) was suspended in 20 mL citrate buffer in an erlenmeyer flask. The suspension was treated for 2 h in an



Scheme 3. Initiation and propagation steps of the CROP of THF by triflic anhydride.[27]

ultrasonic bath. 0.4 M G_1P in buffer citrate (52 mL, 2.1×10^{-2} mol, DP_n 1040) and 25.6 mL citrate buffer were added, followed by 1h incubation at 37 °C in a water bath. Potato phosphorylase enzyme (3 mL) was added. An aliquot (100 µL) was taken right after the addition of the enzyme and also during the reaction (10–100 µL) to check the conversion. After the desired conversion was reached, the mixture was poured into 20 mL ethanol and stirred in an ice bath. Water (80 mL) was added and the suspension was stored overnight at 6 °C. The product was collected by filtration and dried in vacuo to yield a white product (30% conversion of G_1P).

3. Results and Discussion

Telechelic amine terminated PTHF was successfully prepared via the CROP of THF. HMTA was used as terminating agent as it results in a good primary amine functionality to the resulted PTHF as described previously.[19,28] Triflic anhydride was chosen as it can initiate the polymerization of THF which results active sites at both ends of the opened THF.[37] Afterward, the resulted difunctional THF-containing initiator can initiate the propagation of the CROP of THF, as shown in Scheme 3.[27]

In addition, DTBP was used as a proton trap to prevent the initiaton of the CROP of THF by triflic acid (CF_3SO_3H) which can lead to undesired side reactions.[27] The bulk polymerization of THF for the preparation of the telechelic PTHF is shown in Table 1. After 15 min, the bulk polymerization already yielded a high DP_n (M_n 6200 g mol^{-1}). For the synthesis of PTHF with a lower DP_n, the bulk polymerization might proceed too fast and in this case, if it is necessary to slow down the reaction, an alternative CROP of THF in dichloromethane can be conducted.

The telechelic NH_2–PTHF–NH_2 was difficult to analyze by matrix assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-ToF-MS). However, as shown in Figure 2, the ^{1}H-NMR showed the characteristic peak of methylene next to the primary amine group (–C***H***$_2$–NH_2) at 2.69 ppm. This indicates that HMTA can also be used to terminate telechelic PTHF to result in a telechelic amine terminated PTHF.

The synthesized telechelic amine terminated PTHF was reacted with maltoheptaose to synthesize maltoheptaose-*b*-PTHF-*b*-maltoheptaose via reductive

Table 1. CROP of THF at room temperature using triflic anhydride as the initiator and HMTA as the terminating agent.

Entry	[M]/[I]	Time [min]	Yield[a] [%]	M_n[b] [g mol^{-1}]
1	400	15	10	6200
2	200	17	37	8200

[a]Measured gravimetrically based on the weight of the telechelic PTHF:THF; [b]Determined by ^{1}H-NMR. M and I denote monomer and initiator.

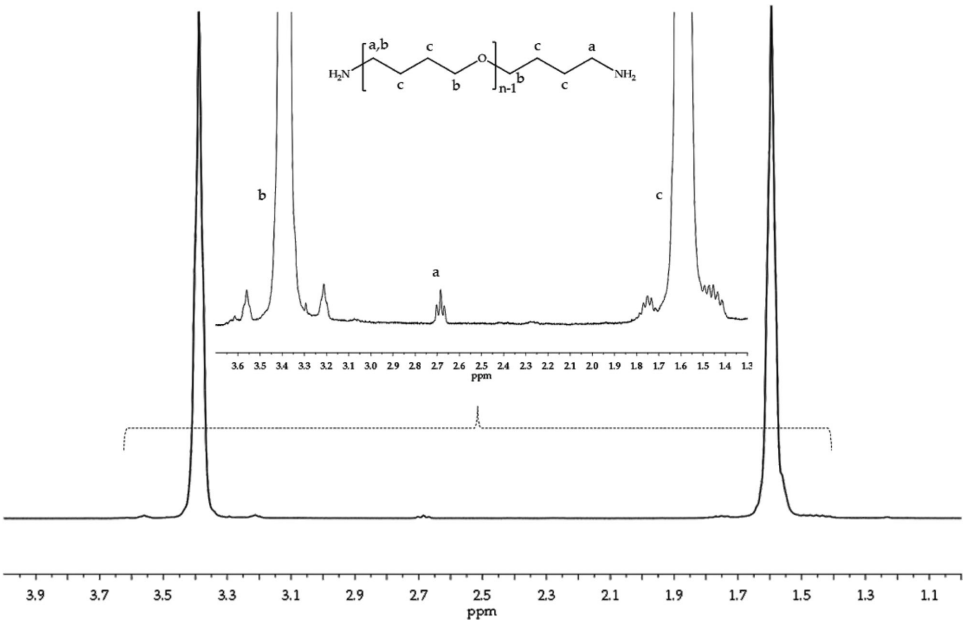


Figure 2. ^{1}H-NMR spectrum of telechelic amine terminated PTHF.

amination. The reaction was more favourable when a mixture of DMSO/THF was used rather than DMSO only. In this case, the THF likely leads to a better solvation of the PTHF thus favouring the reductive amination. Glacial acetic acid was used to adjust the pH of the solution in the range of pH 5–6. In addition, $NaBH_3CN$ was used in fivefold excess compared to maltoheptaose.[19]

As shown in Table 2, the yield of the product from educt PTHF1100 is low (5%–12%), greatly influenced by the purification technique. Water was used to precipitate the product and to wash away unreacted maltoheptaose. However, in the case of maltoheptaose-*b*-$PTHF_{1100}$-*b*-maltoheptaose, the product hardly precipitated even after storage or centrifugation at around 4 °C. This was due to the fact that maltoheptaose-*b*-$PTHF_{1100}$-*b*-maltoheptaose emulsifies well in water and most of the product probably washed away. The products originating from PTHF6200/8200 also emulsified in water. However, after storage or centrifugation at around 4 °C, the maltoheptaose-*b*-$PTHF_{6200/8200}$-*b*-maltoheptaose can be isolated and a higher yield was obtained (58–75 wt% yield).

As maltoheptaose-*b*-$PTHF_{1100}$-*b*-maltoheptaose was hardly isolated from water, its dried product was expected to be easily solubilized/emulsified in water. However, the obtained maltoheptaose-*b*-$PTHF_{1100}$-*b*-maltoheptaose was hard to emulsify even after vibration for 24 h or more at 85 °C. This was probably due to the hydrogen bonding between the neighbouring maltoheptaose-*b*-$PTHF_{1100}$-*b*-maltoheptaose. The bonding likely results in a network of copolymers, which make it insoluble in water.

Due to the poor solubility, ATR-FTIR was chosen to characterize the products. As shown in Figure 3, the resulted maltoheptaose-*b*-$PTHF_{1100}$-*b*-maltoheptaose showed an apparent vibration at 3000–3500 cm^{-1}. The peak at 1550 cm^{-1}, which is present in the maltoheptaose but absent in PTHF also appeared in the synthesized maltoheptaose-*b*-$PTHF_{1100}$-*b*-maltoheptaose. The broad vibration at 3000–3500 cm^{-1} results from the OH vibration of the attached maltohepatose.

Table 2. Reductive amination between telechelic PTHF and maltoheptaose in DMSO/THF (1:1).

Entry	M_n PTHF [g mol^{-1}]	*T* [°C]	Time [h]	PTHF:maltoheptaose: acetic acid:$NaBH_3CN$	Yield[a)] [%]
1	1100	70	5	1:2:48:10	12
2[b)]	1100	70	5	1:2:188:10	5
3	6200	85	18	1:2:123:10	58
4	8200	85	18	1:2:170:10	75

[a)]Measured gravimetrically based on the total weight of PTHF and maltoheptaose; [b)]Conducted in DMSO.

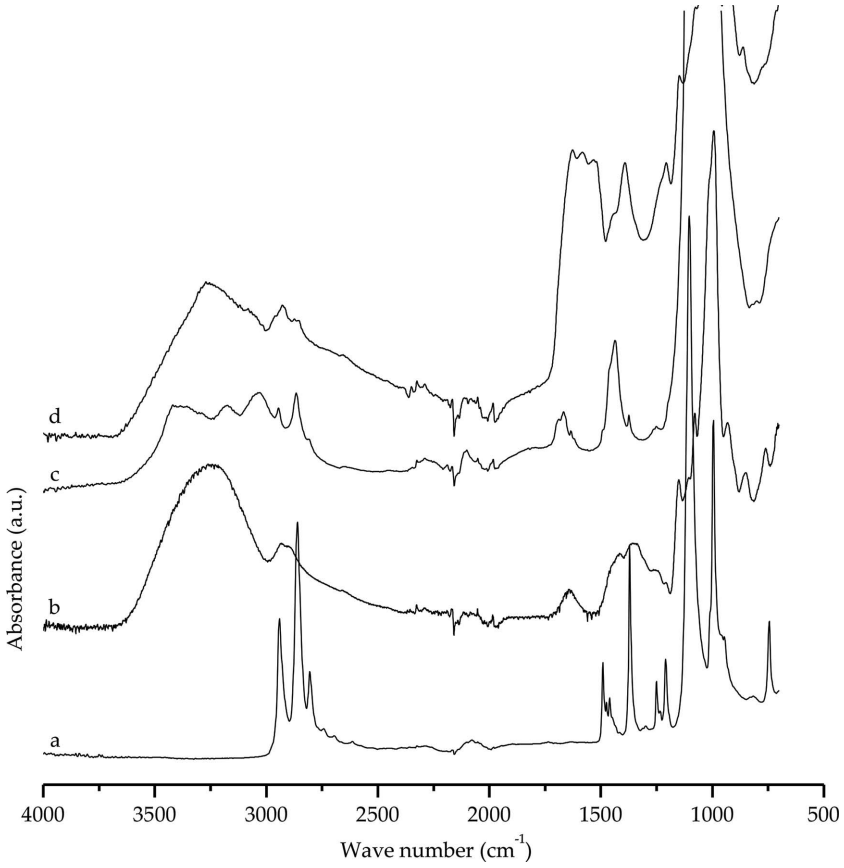


Figure 3. a) ATR-FTIR spectra of telechelic amine terminated PTHF1100, b) maltoheptaose, c) maltoheptaose-*b*-$PTHF_{1100}$-*b*-maltoheptaose, and d) $amylose_{38k}$-*b*-$PTHF_{1100}$-*b*-$amylose_{38k}$.

No apparent carbonyl vibration (only a shoulder peak at 1682 cm^{-1}) and a splitting at 3000–3500 cm^{-1} likely indicate that the resulting maltoheptaose-*b*-$PTHF_{1100}$-*b*-maltoheptaose is a mixture with side products. Nevertheless, this product can be used as a recognition unit for the enzymatic synthesis of the ABA triblock copolymer of amylose-*b*-PTHF-*b*-amylose.

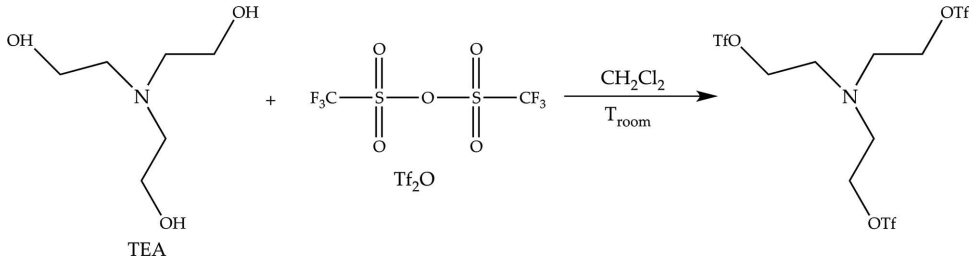
As maltoheptaose-*b*-PTHF-*b*-maltoheptaose is poorly soluble in water, vibration was applied to enhance the emulsification of the polymer. It resulted in a suspension in which the maltoheptaose-*b*-$PTHF_{1100}$-*b*-maltoheptaose was well dispersed. In this case, the enzymatic polymerization to synthesize amylose-*b*-$PTHF_{1100}$-*b*-amylose was conducted as a suspension reaction which resulted in ABA block copolymer with the attached amylose chains having molecular weight of 38 kg mol^{-1}. As shown in Figure 3d, the resulting $amylose_{38k}$-*b*-$PTHF_{1100}$-*b*-$amylose_{38k}$ shows a peak at 3000–3500 cm^{-1}, which correlates with the OH-vibration of the attached amylose. This peak appears sharper compared to maltoheptaose. In addition, the splitting in the region of 3000–3500 cm^{-1}, which was present in maltoheptaose-*b*-$PTHF_{1100}$-*b*-maltoheptaose, disappeared after the enzymatic synthesis of $amylose_{38k}$-*b*-$PTHF_{1100}$-*b*-$amylose_{38k}$. This shows that the possible side products from the synthesis of maltoheptaose-*b*-$PTHF_{1100}$-*b*-maltoheptaose did not affect the enzymatic polymerization. The peak at 2700–3000 cm^{-1} of $amylose_{38k}$-*b*-$PTHF_{1100}$-*b*-$amylose_{38k}$, which is from the $-CH_2$ vibration of the PTHF backbone, appear less sharp compared to maltoheptaose-*b*-$PTHF_{1100}$-*b*-maltoheptaose. This is possibly due to the short length of $PTHF_{1100}$ in comparison with the molecule of $amylose_{38k}$-*b*-$PTHF_{1100}$-*b*-$amylose_{38k}$.

The initiator for the synthesis of three-arm PTHF was synthesized in situ by reacting triflic anhydride with TEA, as shown in Scheme 4. The resulting OTf group is a good leaving group and therefore effective to initiate the CROP of THF.[27,34] Similar to the synthesis of telechelic THF, DTBP was also added as a proton trap.[27]

The synthesis of three-arm PTHF was carried out at 0 °C and at room temperature to optimize the reaction conditions for the desired molecular weight and polydispersity. The resulting polymer was difficult to characterize by MALDI-ToF-MS. However, from the MALDI analysis of the product with a molecular weight around 1500 and 3000 g mol^{-1}, there was an indication that the product was a mixture between linear and three-arm PTHF.

As shown in Table 3, the determined molecular weights of the products by GPC were mostly larger than the calculated molecular weights based on ^{1}H-NMR. In addition, the polydispersity (D) is quite large: $1.47 < D_{Troom} < 2.35$ and $1.84 < D_0$ °C < 2.68 probably due to inhomogeneous propagation steps from the three-arm core. This likely resulted in a mixture of products (linear and three-arm PTHF) as observed from the MALDI analysis, which led to the difference of the determined molecular weights between GPC and ^{1}H-NMR. In addition, for a similar reaction condition (entry 2 and 3), longer termination time resulted in higher polydispersities due to side reactions such as chain transfers. Accordingly, termination with HMTA can be an alternative to obtain better results.

For a similar condition ([M]/[I] = 622), the CROP of THF at 0 °C reached similar molecular weight as the one at room temperature ($M_{n\text{-}^1\text{H-NMR}}$ around 9 kg mol^{-1}) after a two times longer reaction time (Table 3). However, the yield was lower (12 wt%) compared to the one at room temperature (30 wt%). In addition, for the reaction



Scheme 4. In situ formation of the initiator for the CROP of THF.

condition of [M]/[I] = 425, the GPC results demonstrated that the polydispersity of the polymer synthesized at room temperature was more narrow (D = 1.47) compared to the one that was synthesized at 0 °C (D = 2.11). This shows that the CROP of THF at room temperature to prepare a three-arm PTHF is preferable. Apparently room temperature provides enough energy to dissolve the reactants and to accelerate the bulk polymerization kinetics without facilitating other side reactions.

The ^{1}H-NMR spectrum of the product as depicted in Figure 4 showed that the peak related to the methylene next to the primary amine at 2.73 ppm was mostly observed as a bump rather than a triplet as observed in the corresponding methylene in CH_3–PTHF–NH_2 or NH_2–PTHF–NH_2. This is likely due to a methylene conformation in the three-arm PTHF. Since it is not a planar structure, the methylene peak has a high mobility in solution. The conformation of the methylene peak changes quickly in time; therefore it is detected as a bump. This structure, in which the methylene peak was detected as a bump was also reported for a three-arm PTHF terminated by tris(2-aminoethyl)amine.[34]

As summarized in Table 4, the reductive amination between maltoheptaose and the three-arm PTHF (synthesized at room temperature with overnight termination, Table 3 entry 3, 7, and 8) proceeded readily. The reductive amination was conducted in DMSO as well as in a mixture of DMSO/THF. Another alternative solvent mixture that can be used for this reaction is THF/methanol. $NaBH_3CN$ was used as the reducing agent and acetic acid was used to adjust the pH of the solution into pH 5–6. No product was obtained for the reaction using the three-arm PTHF

Table 3. CROP of THF using triflic anhydride and TEA as the in situ initiator and $NaNH_2$ as the terminating agent.

Entry	[M]/[I]	Time [min]	Yield[a)] [wt%]	M_n[b)] [g mol^{-1}]	M_n[c)] [g mol^{-1}]	D[c)] [M_w/M_n]
At T_{room}						
1	207	20	3	3900	8.3	2.15
2	425	30	22	6400	17.9	1.47
3[d)]	425	30	13	5600	13.6	1.93
4	622	45	30	8800	25.5	1.62
5	808	60	22	12 000	26.0	1.67
6	105	60	98	3000	33.5	2.35
7[d)]	155	85	34	13 300	...	...
8[d)]	243	180	14	17 500	...	...
At T_0 °C						
1	105	20	9	2900	...	...
2	105	30	68	8200	13.9	2.11
3	207	35	3	5500	9.9	2.25
4	425	60	2	7200	13.5	2.11
5	622	90	12	9200	31.3	1.84
6	622	120	8	12 300	19.7	2.68

[a)]Determined gravimetrically based on the weight of the telechelic PTHF:THF; [b)]Determined based on ^{1}H-NMR; [c)]Determined based on GPC in THF using universal calibration; [d)]Synthesized with overnight termination, while the rest is with 1 h termination. M and I denote monomer and initiator.

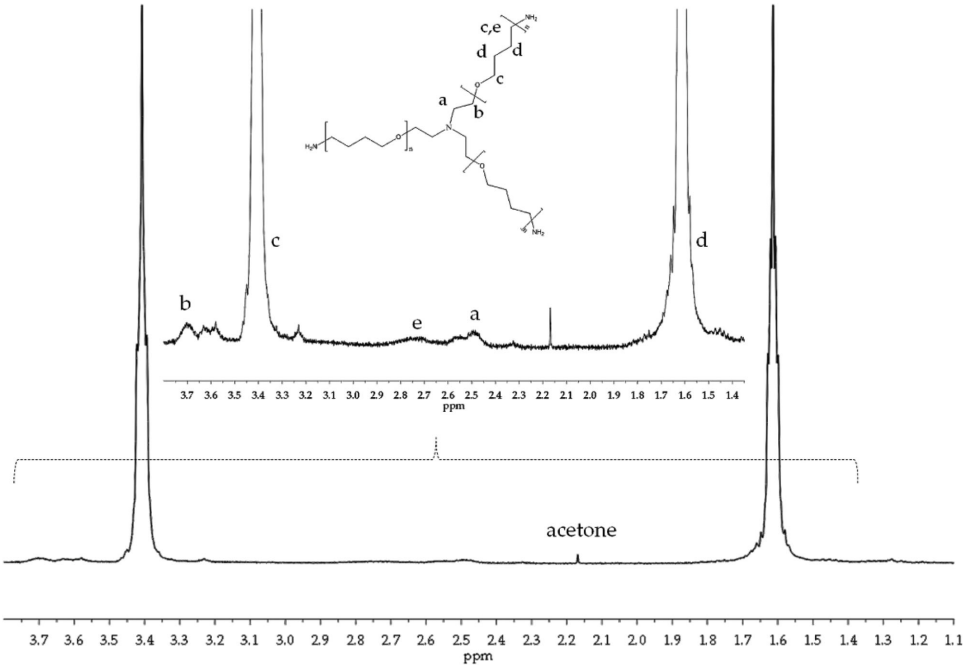


Figure 4. ^{1}H-NMR spectrum of three-arm amine terminated PTHF.

that was synthesized with 1h termination, even though the reaction conditions of the reductive amination had been varied. In this case, there is the possibility that the complete termination of the three-arm PTHF takes longer than 1 h. This led to incomplete functionalization of the synthesized three-arm PTHF which results in an unsuccessful reductive amination reaction.

The reaction that was conducted in DMSO/THF showed a high yield (92%), which indicates that better solubilization leads to a better reductive amination. For the reaction in DMSO, the yield was greatly improved by prolonging the reaction time (from 5 to 6 h). In this case, due to the solubility aspect, the reaction between three-arm PTHF and maltoheptaose in DMSO possibly takes longer time to complete.

The resulting three-arm PTHF-*b*-maltoheptaose was difficult to analyze in solution. Therefore the solid characterization was performed by ATR-FTIR as shown in Figure 5. The OH vibration of maltoheptaose appeared at around 3000–3700 cm^{-1} and the peak at 790 cm^{-1} became more apparent in the three-arm PTHF-*b*-maltoheptaose compared to the three-arm PTHF.

The three-arm $PTHF_{5600}$-*b*-maltoheptaose was used as a primer for the enzymatic synthesis of $PTHF_{5600}$-*b*-amylose. As the three-arm $PTHF_{5600}$-*b*-maltoheptaose is not soluble in water, its suspension was treated for 2 h in an ultrasonic bath before the enzymatic polymerization started. Glucose-1-phosphate (G_1P) as a monomer for the amylose synthesis was added in excess and potato phosphorylase was used as a biocatalyst. The enzymatic polymerization proceeded as a suspension reaction.

As shown in Figure 5, the OH vibration at 3000–3700 cm^{-1} and the vibration peak at 1635 cm^{-1} became more apparent in the $PTHF_{5600}$-*b*-$amylose_{23k}$ compared to the corresponding $PTHF_{5600}$-*b*-maltoheptaose. An additional peak appeared at 1550 cm^{-1} and the vibrations at 790 and 1255 cm^{-1} were significantly high compared to the original products. It indicates that despite the poor solubility in the citrate buffer, the three-arm $PTHF_{5600}$-

Table 4. Reductive amination between three-arm PTHF and maltoheptaose at 70 °C in DMSO.

Entry	M_n PTHF[a)] [kg mol^{-1}]	Time [h]	PTHF:maltoheptaose: acetic acid:$NaBH_3CN$	Yield[b)] [%]
1[c)]	5.6	5	1:2.8:300:5	92
2	13.3	5	1:3.5:361:3	26
3	17.5	6	1:3:290:2	82

[a)]Determined by ^{1}H-NMR; [b)]Measured gravimetrically based on the total weight of PTHF and maltoheptaose; [c)]Conducted in DMSO/THF (1:1).

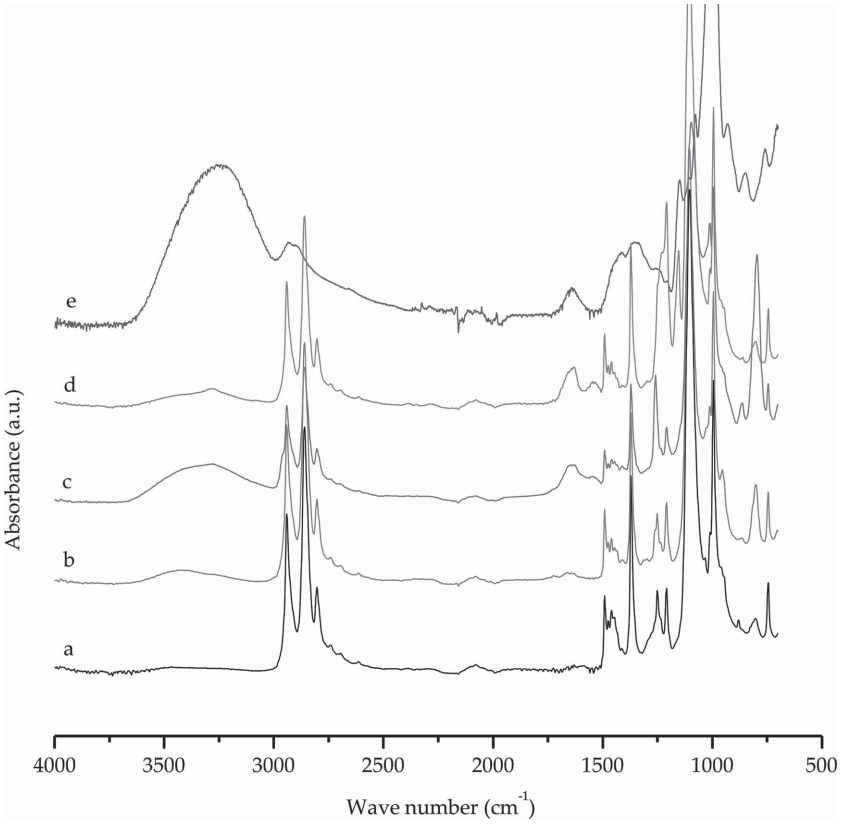


Figure 5. a) ATR-FTIR of three-arm $PTHF_{5600}$, b) three-arm $PTHF_{5600}$-*b*-maltoheptaose, c) three-arm $PTHF_{5600}$-*b*-$amylose_{23k}$, d) amylose synthesized in the presence of three-arm $PTHF_{8800}$, and e) maltoheptaose.

b-maltoheptaose is indeed able to act as the recognition unit of the potato phosphorylase enzyme.

To investigate the possibility of in situ complex formation between amylose and three-arm PTHF, amylose was synthesized enzymatically in the presence of the three-arm $PTHF_{8800}$. Amylose with a DP_n of 90 was formed, which indicated that the three-arm $PTHF_{8800}$ did not inactivate the potato phosphorylase. As shown in Figure 5, the OH vibration of the amylose appeared at 3000–3700 cm^{-1}. Compared to the three-arm $PTHF_{5600}$-*b*-$amylose_{23k}$ (Figure 5c), the peak at 1550 cm^{-1} became more distinctive. In addition, the peaks at 1205 cm^{-1} and 1255 cm^{-1} are shifted to 1151 cm^{-1} and 1211 cm^{-1}. The peak at 790 cm^{-1} is comparable to the three-arm PTHF, which probably indicates that the synthesized amylose did not form inclusion complexes with the three-arm $PTHF_{8800}$ via "vine twinning polymerization." The three-arm $PTHF_{8800}$ was likely too big to be included by the growing amylose chain.

4. Conclusions

Amine terminated telechelic PTHF can be synthesized via the CROP of THF using triflic anhydride as initiator and HMTA as terminating agent. As for the synthesis of amine terminated three-arm PTHF, triflic anhydride and an OH-containing starting material can be used as initiators. $NaBH_3CN$ can reduce the corresponding amine terminated polymers in the presence of maltoheptaose to result in maltoheptaose-*b*-PTHF-*b*-maltoheptaose and three-arm PTHF-*b*-maltoheptaose. The resulted products can be used as the recognition units for the enzymatic synthesis of amylose-*b*-PTHF-*b*-amylose and three-arm PTHF-*b*-amylose.

These amylose-containing polymers can be used as host molecules for inclusion complex formation with guest molecules such as PTHF but also other compounds to result in supramolecular structures. The possibility of attaching a noncovalent additional block can lead to more structures with tuneable and responsive characteristics that attract a lot of applications, such as delivery system, thermo responsive materials, and solvent responsive materials. The range of AB-, ABC-, or ABA-type block copolymers containing PTHF can also be broadened by introducing additional blocks that do not form an inclusion complex with amylose. The added block can be either a bulky block such as polystyrene[38] or a hydrophilic block such as polyethylene oxide (PEO).[39]

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