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Unusual rate enhancement in the thymine assisted ATRP process of adenine monomers

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Abstract

Thymine and adenine monomers were synthesized and polymerized using ATRP in a controlled fashion. In addition a thymine functionalized block copolymer was prepared using ATRP, starting from a poly(ethylene glycol) macro initiator. Polymerization of adenine monomer in DMSO-d_6 showed a significant increase in polymerization rate when polymeric thymine template was present, while thymine monomer did not show a polymerization rate enhancement. Varying the template to monomer ratio demonstrated that the polymerization rate increased even further if an excess of template was applied. Surprisingly, the addition of monomeric complementary moieties resulted in an even greater rate enhancement. These findings led to our conclusion that non-covalent interaction between adenine and thymine in DMSO-d_6 protects the adenine monomer from interaction with the copper catalyst, thus resulting in a faster polymerization.

Keywords: Nucleobase; Adenine; ATRP

1. Introduction

Template polymerization is crucial to Nature, as it forms the basis for correct DNA replication, transcription and protein synthesis. During these processes single polynucleobase strands are used as polymer template for the production of new, biopolymer chains with an unsurpassed level of control over composition and degree of polymerization. The specific recognition between the two base pairs adenine–thymine and guanine–cytosine, also known as Watson–Crick base pairing, is one of the main elements that enables the occurrence of this template process.

Natural template polymerization has inspired many polymer chemists to investigate this concept for use in synthetic polymer chemistry. Researchers have studied different interactions between polymer chain and monomer, such as electrostatic, dipole–dipole interactions or hydrogen bonding [1,2]. The latter is by far the most versatile and also the most investigated system. Pioneering work was performed by Challa et al. [3–9] and others [10–12] who investigated the use of hydrogen bonding interactions between acrylic acid and vinyl pyrrolidone, and observed, among other things, control over tacticity and polymer chain length by the presence of a template. Takemoto and Inaki [13,14] investigated the effect of the presence of a nucleobase containing polymer on the rate of polymerization of the complementary nucleobase-functional monomer. Although effects could be clearly observed, these early studies on template polymerizations were often hampered by the use of free (uncontrolled) radical polymerization methods.

With the development of controlled radical polymerization [15–18] such as atom transfer radical polymerization (ATRP), researchers are nowadays able to prepare polymers with low polydispersity and with control over architecture, using a large variety of monomers. Haddleton and Gross [19–21] have shown ATRP to be tolerant towards nucleobase functionality in monomers, demonstrating the versatility and robustness of this controlled polymerization technique.

The previous results have inspired us to use ATRP for the detailed investigation of the effects of nucleobases on the polymerization of complementary nucleobase monomers. In this report we describe our studies with respect to the presence of both monomeric and polymeric thymine templates on the ATRP process of adenine methacrylate

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by determining the kinetics of the polymerization via ^{1}H NMR spectroscopy.

2. Experimental

2.1. Materials

All reactions were performed under a nitrogen atmosphere, unless otherwise stated. DMF was dried over anhydrous $MgSO_4$, followed by distillation under reduced pressure and storage under an argon atmosphere. Dichloromethane (DCM), heptane and ethyl acetate (EtOAc) were distilled over calcium hydride (CaH_2) and 1,4-dioxane was freshly distilled over $LiAlH_4$. Copper chloride (CuCl) was purified according to a literature procedure [22]. Silica gel column chromatography (SGCC) was performed using Acros silica gel (0.035–0.070 mm, pore diameter ca. 6 nm). TLC was carried out on Merck pre-coated silica gel 60 F-254 plates. Compounds were visualized using UV and permanganate staining agent. Other chemicals were used as received unless otherwise stated.

2.2. Instrumentation

^{1}H NMR spectra were recorded on a Varian INOVA400 instrument at 400 MHz and ^{13}C NMR spectra were recorded on a Bruker DPX300 instrument at 75 MHz. Chemical shifts (δ) are given in ppm relative to the internal standard (Me_4Si or DMSO-d_6). IR spectra were recorded on an ATI Matson Genesis Series FTIR spectrometer with fitted ATR cell. HRMS spectra were recorded on a VG 7070 or MAT 900 mass spectrometer. GPC measurements were performed on a Shimadzu HPLC system equipped with PL gel 5 μm guard column, a PL gel 5 μm mixed D column and differential refractive index (38 °C) and UV detection (280 nm). The system was operated with a flow of 0.8 mL min^{-1} at 70 °C using dimethylsulfoxide (DMSO, 0.02 M LiCl) as eluent. Polyethylene glycol standards in the range of 1900–124,700 Da were used to calibrate the GPC.

2.2.1. 3-Bromopropyl methacrylate (*1*)

To a solution of 3-bromopropanol (15.1 mL, 0.174 mol) and triethylamine (25 mL, 0.180 mmol) in 350 mL DCM methacryloyl chloride (16.9 mL, 0.174 mmol) was added drop wise, while cooling the reaction mixture with an ice bath (0 °C). After complete addition the reaction mixture was allowed to warm up to ambient temperature overnight, followed by quenching the excess methacryloyl chloride by addition of 10 mL MeOH. After 30 min the clear solution was poured into 100 mL saturated aqueous $NaHCO_3$, followed by two washing steps with 70 mL water. The organic layer was dried with anhydrous $MgSO_4$, filtered and concentrated in vacuo to give a pink oil. This was further purified by column chromatography (10% EtOAc/heptane) to give 34.82 g (0.168 mol, 96.6%) of the desired product as a colorless oil; R_f=0.36 (10% EtOAc/heptane); ^{1}H NMR ($CDCl_3$) δ 6.11 (s, 1H, O_2C–$C(CH_3)$=CH_B), 5.58 (s, 1H, O_2C–$C(CH_3)$=CH_A), 4.29 (t, J=6.0 Hz, 2H, –O–CH_2–), 3.49 (t, J=6.6 Hz, 2H, Br–CH_2–), 2.24 (quintet, J=6.3 Hz, 2H, –CH_2–CH_2–CH_2–), 1.95 (s, 3H, O_2C–C(CH_3)=CH_2); ^{13}C NMR ($CDCl_3$) δ 167.3, 136.3, 125.8, 62.5, 31.9, 29.5, 18.4; IR (oil) ν 2962, 1718, 1637.

2.2.2. 3-(Thymin-1-yl)propyl methacrylate (*2*)

To a solution of thymine (3.58 g, 28.4 mmol) in 200 mL DMF was added anhydrous potassium carbonate (3.96 g, 28.6 mmol), tetrabutylammonium iodide (TBAI, 0.66 g, 1.80 mmol) and compound **1** (4.04 g, 19.5 mmol). The resulting suspension was stirred at ambient temperature for 24 h. The reaction was quenched by addition of 25 mL water, followed by evaporation to dryness in vacuo. The resulting light yellow solid was two times subjected to column chromatography (2% MeOH/DCM) to yield 1.87 g (7.40 mmol, 37.9%) of **2** as a white solid, mp=119.7±0.07 °C; R_f=0.2 (2% MeOH/DCM); ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.11 (br s, 1H, pyrimidine NH), 6.94 (q, J=1.2 Hz, 1H, pyrimidine H-6), 6.11 (quintet, J=1.0 Hz, 1H, O_2C–$C(CH_3)$=CH_B), 5.58 (quintet, J=1.6 Hz, 1H, O_2C–$C(CH_3)$=CH_A), 4.21 (t, J=6.0 Hz, 2H, –O–CH_2–), 3.80 (t, J=6.9 Hz, 2H, N–CH_2–), 2.09 (quintet, J=6.3 Hz, 2H, –CH_2–CH_2–CH_2–), 1.95 (q, J=1.0 Hz, 3H, O_2C–C(CH_3)=CH_2), 1.91 (d, J=1.2 Hz, 3H, pyrimidine–CH_3); ^{13}C NMR (DMSO-d_6): δ 166.42, 164.26, 150.90, 141.37, 135.76, 125.68, 108.50, 62.06, 44.83, 27.47, 17.90, 11.90. IR (solid) ν 3175, 3044, 2970, 2891, 2813, 1715,1665.

2.2.3. 3-(Adenin-9-yl)propyl methacrylate (*3*)

To a suspension of adenine (2.50 g, 18.5 mmol) in 125 mL DMF was slowly added NaH (0.82 g, 20.6 mmol). The reaction mixture was stirred at ambient temperature for 1 h until no more gas evolved, followed by addition of compound **1** (4.96 g, 24.0 mmol). After stirring at ambient temperature for 17 h the excess NaH was quenched with 10 mL saturated aqueous NH_4Cl solution. The resulting suspension was filtered and concentrated in vacuo. The resulting solid was subjected to column chromatography with eluent 7% MeOH/DCM to yield 3.34 g (12.7 mmol, 69%) of **3** as a white solid, mp=137.0±0.28 °C; R_f=0.12 (5% MeOH/DCM); ^{1}H NMR (DMSO-d_6): δ 8.14 (s, 1H, purine H-2), 8.11 (s, 1H, purine H-8), 7.16 (s, 2H, NH_2), 5.91 (s, 1H, O_2C–$C(CH_3)$=CH_A), 5.62 (s, 1H, O_2C–$C(CH_3)$=CH_B), 4.25 (t, J=6.8 Hz, –O–CH_2–), 4.09 (t, J=6.1 Hz, 2H, –N–CH_2–), 2.20 (quintet, J=6.4 Hz, 2H, –CH_2–CH_2–CH_2–), 1.81 (s, 3H, O_2C–C(CH_3)=CH_2); ^{13}C NMR (DMSO-d_6): δ 166.39, 155.93, 152.36, 149.58, 140.78, 135.66, 125.67, 118.80, 61.86, 40.33, 28.39, 17.86; IR (solid) ν 3372, 3313, 3155, 1710, 1642, 1592.

2.2.4. α-(2-Bromo-2-methyl propionate)-ω-methylpoly(ethylene glycol) (*4*)

Poly(ethylene glycol) methyl ether 2000 (5.12 g,

2.56 mmol) and Et_3N (0.5304 g, 5.24 mmol) were dissolved in DCM (100 mL) and cooled to 0 °C. 2-Bromo-isobutyrylbromide (0.8824 g, 3.83 mmol) was added drop wise and the mixture was warmed to ambient temperature. After 18 h an additional amount of 2-bromo-isobutyrylbromide (0.84839 g, 3.69 mmol) was added and the mixture was stirred for another 40 h. The remaining 2-bromo-isobutyrylbromide was quenched with MeOH (10 mL). The mixture was washed with a saturated $NaHCO_3$ solution (50 mL) and water (50 mL). The organic phase was dried over anhydrous $MgSO_4$ and concentrated under reduced pressure to give a waxy yellowish solid which was further purified using column chromatography (MeOH/DCM; 1:9) after which 5.22 g (95%) product **4** was obtained as a white, waxy solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.31 (t, 2H, $J=4.8$ Hz, CH_2–CH_2–O–C(=O)), 3.63 (br m, 180H, {CH_2–CH_2–O}+ endgroups), 3.37 (s, 3H, CH_3–O), 1.93 (s, 6H, C(=O)–C(CH_3)$_2$–Br); IR (solid) ν 1731, 1102$_r$; SEC (THF) $M_n=2.8$ kDa, PDI = 1.06.

2.2.5. 1-Hexyl-thymine (*6*)

To a round bottom flask fitted with a condenser and a $CaCl_2$ tube was added 301.3 mg (2.39 mmol) thymine, 8.0 mg (0.06 mmol) $(NH_4)_2SO_4$ and 4 mL (18.96 mmol) hexamethyldisilazane. The suspension was heated under reflux for 18 h. The resulting clear reaction mixture was concentrated under reduced pressure, yielding a colorless oil. The oil was re-dissolved in dry DCM (4 mL) whereupon tetrabutylammonium iodide (TBAI, 43.9 mg, 0.12 mmol) and 1-bromohexane (335 μL, 2.39 mmol) were added. After refluxing for 4 days the mixture was concentrated under reduced pressure. The crude product was purified with column chromatography (3% MeOH in DCM) yielding a pure white solid (90 mg, 17.9%), mp = 129.2 °C; R_f (3% MeOH in DCM) = 0.16; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.30 (br. s, 1H, H^3 pyrimidine), 6.94 (q, 1H, $J=1.2$ Hz, H^6 pyrimidine), 3.67 (t, 2H, $J=7.2$ Hz, N–CH_2–CH_2), 1.92 (m, 3H, $J=1.2$ Hz, C^5–CH_3 pyrimidine), 1.69 (m, 2H, $J=6.9$ Hz, N–CH_2–CH_2–CH_2), 1.31 (m, 6H, –CH_2–CH_2–CH_2–CH_2–CH_3), 0.88 (m, 3H, 6.60 Hz, CH_2–CH_3); ^{13}C NMR ($CDCl_3$) δ 163.85, 150.57, 140.29, 110.51, 48.82, 31.66, 29.39, 26.43, 22.82, 14.35, 12.72. IR (solid) ν 1687, 1651.

2.2.6. Polymerization of *2* using *4* as initiator (*5*)

Monomer **2** (0.786 g, 3.11 mmol), CuCl (20.5 mg, 0.207 mmol) and 2,2′-bipyridine (bpy, 65.8 mg, 0.421 mmol) were placed in a Schlenk tube. After deoxygenation by three cycles of evacuation, followed by applying a dry nitrogen atmosphere, the Schlenk tube was put under an argon atmosphere. DMSO-d_6 (4.0 mL) was added, followed by addition of an argon purged solution of **4** (0.446 g, 0.207 mmol) in DMSO-d_6 (3.5 mL). The polymerization reaction was performed at ambient temperature. Samples were taken periodically in order to analyze the monomer conversion with ^{1}H NMR spectroscopy. At 540 min 1-phenyl-1-(trimethylsiloxy)-ethylene (0.410 g, 2.13 mmol) was added to quench the ATRP and end cap the polymer. The reaction mixture was precipitated in an aqueous EDTA solution (0.055 M) after which the crude product was extracted with DCM (5×50 mL). The separation required the addition of brine. The organic phase was dried over anhydrous $MgSO_4$ and was concentrated under reduced pressure. The product was further purified using sephadex column chromatography (LH-20, MeOH/DCM; 1:1) resulting after freeze drying in 0.771 g of the desired product as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6): δ 11.14 (br s, NH pyrimidine), 7.55–8.00 (m, CH_2–C(=O)–C_6H_5 aromatic endgroup signals), 7.39 (br s, H^6 pyrimidine), 3.92 (br s, O–CH_2–CH_2), 3.69 (br s, CH_2–CH_2–N_{purine}), 3.50 (br s, {CH_2–CH_2–O} PEG block), 3.24 (s, O–CH_3 endgroup), 2.23 (br s, CH_2–CH_2–CH_2–N_{purine}), 1.6–2.0 (br m, {CH_2–C(CH_3)}), 0.6–1.4 (br m, {CH_2–C(CH_3)}); IR (ATR): 1672, 1105 (ν_{C-O})$_{ether}$; SEC (DMSO): $M_n=4.9$ kDa, $M_w=6.5$ kDa, PDI = 1.30.

2.2.7. ATRP of *3* (general procedure for polymerization in an NMR tube)

Monomer **3** (17.0 mg, 0.065 mmol) was added to an NMR tube which was placed in a Schlenk tube. Deoxygenation was performed by three cycles of evacuation, and refilling with dry nitrogen. The monomer was dissolved in 0.4 mL DMSO-d_6, followed by addition of 0.1 mL of a catalyst stock solution (4.7 mg (0.047 mmol) CuCl and 15.1 mg (0.096 mmol) bpy dissolved in 0.8 mL DMSO-d_6).

The mixture was purged with argon for 5 min, after which the polymerization was started by addition of ethyl α-bromo isobutyrate (EBIB) (1 μL, 0.007 mmol), followed by immediate recording of the first ^{1}H NMR spectrum at 30 °C. Follow up spectra were recorded at different time intervals by applying steady state scans.

2.2.8. ATRP of *2*

Monomer **2** was polymerized according to the general procedure using **2** (16.6 mg, 0.0658 mmol), 0.4 mL DMSO-d_6, 0.1 mL stock solution (4.6 mg (0.046 mmol) CuCl and 15.0 mg (0.096 mmol) bpy in 0.8 mL DMSO-d_6) and 1.0 μL (6.8 μmol) EBiB.

2.2.9. ATRP of *2* in the presence of *5*

Monomer **2** was polymerized according to the general procedure using **2** (16.8 mg, 0.0666 mmol), **5** (32.0 mg, 0.0072 mmol), 0.4 mL DMSO-d_6, 0.1 mL stock solution (4.6 mg (0.046 mmol) CuCl and 15.1 mg (0.0967 mmol) bpy in 0.8 mL DMSO-d_6) and 1.0 μL (6.8 μmol) EBiB.

2.2.10. ATRP of *3* in the presence of *5*

Monomer **3** was polymerized according to the general procedure using **3** (17.0 mg, 0.0651 mmol), **5** (32.0 mg, 0.0072 mmol), 0.4 mL DMSO-d_6, 0.1 mL stock solution (4.7 mg (0.047 mmol) CuCl and 15.1 mg (0.0967 mmol) bpy in 0.8 mL DMSO-d_6) and 1.0 μL (6.8 μmol) EBiB.

*2.2.11. ATRP of **3** in the presence of **6***

Monomer **3** was polymerized according to the general procedure using **3** (17.2 mg, 0.0658 mmol), **6** (13.8 mg, 0.0657 mmol), 0.4 mL DMSO-d_6, 0.1 mL stock solution (4.6 mg (0.046 mmol) CuCl and 14.7 mg (0.0941 mmol) bpy in 0.8 mL DMSO-d_6) and 1.0 μL (6.8 μmol) EBiB.

*2.2.12. ATRP of **3** in the presence of succinimide*

Monomer **3** was polymerized according to the general procedure using **3** (17.6 mg, 0.0674 mmol), succinimide (6.7 mg, 0.0676 mmol), 0.5 mL DMSO-d_6, 0.1 mL stock solution (4.6 mg (0.046 mmol) CuCl and 15.7 mg (0.0986 mmol) bpy in 0.8 mL DMSO-d_6) and 1.0 μL (6.8 μmol) EBiB.

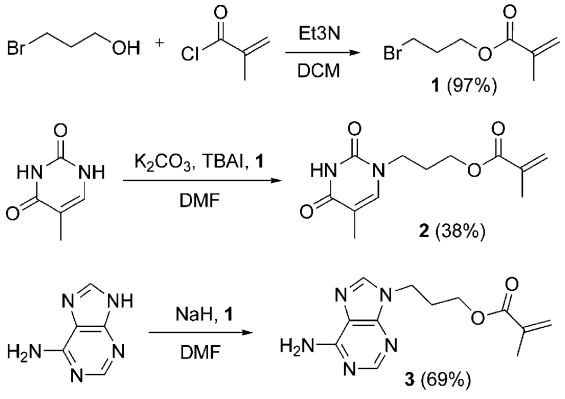
3. Results and discussion

3.1. Monomer synthesis

Thymine monomer **2** was synthesized using 3-bromo propanol as starting material (Scheme 1). Treatment of this alcohol with methacryloyl chloride under basic conditions resulted in 3-bromopropyl methacrylate **1** [23]. Reaction of thymine with K_2CO_3 in DMF, followed by addition of **1** and a catalytic amount (10 mol%) of tetra butyl ammonium iodide (TBAI) gave thymine monomer **2** in 38% yield. Adenine monomer **3** was obtained using a modified procedure of Gokel et al. [24] in which adenine was deprotonated by treatment with sodium hydride (NaH) in DMF. The subsequent addition of **1** resulted in monomer **3** in 69% yield.

3.2. Polymerization

Thymine and adenine monomers **2** and **3** were polymerized using atom transfer radical polymerization (ATRP) with ethyl bromo isobutyrate (EBiB) as initiator and the CuCl/2bpy system as catalyst at 30 °C. To be able to monitor the kinetics of the polymerization DMSO-d_6 was used as a solvent. This allowed us to use ^{1}H NMR spectroscopy to determine the conversion by comparing the ratios of the integral values of the methacrylate proton at 6.00 ppm with the H-6 signal at 7.48 ppm for monomer **2** and the methacrylate proton at 5.91 ppm with the H-2.8 signal at 8.14 ppm for monomer **3**. The polymerizations were performed in an NMR tube under an argon atmosphere. By recording spectra at different time intervals kinetics could be monitored as can be seen in Fig. 1. First order kinetics were obtained, indicating control over the polymerization.



Scheme 1. Synthesis of thymine and adenine monomers **2** and **3**.

To investigate the interaction of adenine with the complementary nucleobase thymine during polymerization a PEG thymine block copolymer was prepared (Scheme 2), to ensure solubility of the complex to be possibly formed. Poly(ethylene glycol) methyl ether was first treated with 2-bromoisobutyryl bromide in the presence of Et_3N to obtain PEG macro initiator **4**. Successively, ATRP of **2** using initiator **4** and the CuCl/2bpy catalyst was performed. At a conversion of 71% the reaction was quenched using a procedure described by Sawamoto [25] by addition of 1-phenyl-1-(trimethylsilyloxy)ethylene, resulting in block copolymer **5**. The quenching step after the polymerization was necessary to remove the bromide end group and to prevent initiation from the thymine block copolymer during the template polymerization experiments of adenine.

As can be seen in Fig. 2, the quenching step proceeded effectively since, monomer conversion stopped after addition of 1-phenyl-1-(trimethylsilyloxy)ethylene **7**. Despite the deviation from first order kinetics the desired PEG-thymine block copolymer was obtained with relatively low PDI (1.30) and the M_n (4.9 kDa) obtained by GPC (Fig. 3) was in agreement with the theoretical molecular weight (4.5 kDa), and with a DP of 10 thymine units, based on NMR calculations.

3.3. ATRP of nucleobase monomers **2** and **3** in the presence of thymine moieties

Under identical conditions as mentioned before both

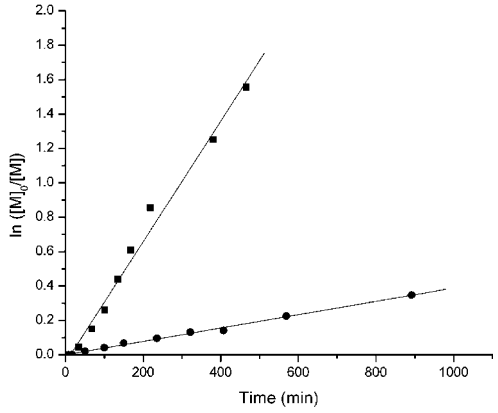
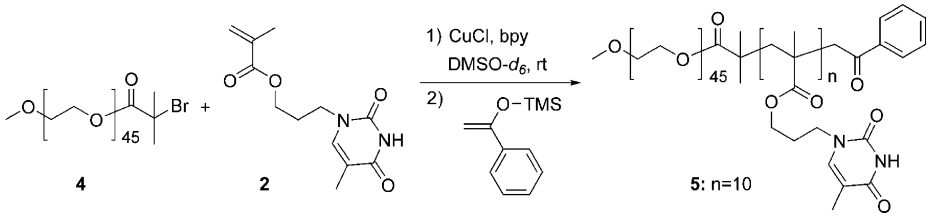


Fig. 1. Plot of ln ($[M]_0/[M]_t$) vs. time of the polymerization of thymine (■, 0.13 M **2**, 0.012 M CuCl, 0.024 M bpy, 0.014 M EBiB) and adenine (●, 0.13 M **3**, 0.012 M CuCl, 0.024 M bpy, 0.014 M EBiB) monomers.



Scheme 2. Synthesis of PEG-*b*-oligoT10 block copolymer **5**.

adenine and thymine monomers were polymerized using ATRP in the presence of the PEG-*b*-oligoT10 block copolymer template **5** in a 1:1 nucleobase ratio. The ratio was determined by the molar ratio between monomer concentration and thymine repeating units present in the template. DMSO-d_6 was chosen as a solvent since, template and monomer were well soluble in this solvent. In addition Inaki [30] already observed a template effect using DMSO/ethylene glycol mixtures.

Both polymerizations of **3** and **2** (Figs. 4 and 5, respectively) proceeded via first order kinetics, indicating control over the polymerization while block copolymer template was present. As can be seen in Fig. 4 the polymerization of adenine monomer was considerably accelerated by the presence of complementary thymine block copolymer **5**, whereas the polymerization rate of thymine monomer (Fig. 5) was not significantly affected by addition of **5**. These results suggest the occurrence of a specific adenine–thymine interaction, and even could point in the direction of a template polymerization process.

In order to obtain more information about this acceleration phenomenon, the effect of the molar ratio between adenine monomer **3** and thymine functionalized polymer **5** on the polymerization rate was subsequently investigated. The concentration of **5** and initiator was kept constant while the amount of adenine monomer **3** was varied. The initial apparent rate constants for the polymerization of the adenine monomer are depicted in Fig. 6 and compiled in Table 1.

As can be seen in Table 1 the rate constant increased upon addition of template polymer with a factor of 3 when a 1:1 ratio (entry 1 and 3) was used. Compared to this value the rate decreased (entry 2) when an adenine/thymine ratio of 2/1 was used and markedly increased by a factor of 100 when an excess of template was added (entry 5 and 6).

To further investigate this phenomenon and to elucidate whether this rate enhancement was due to template polymerization we studied the effect of addition of single thymine containing molecules on the ATRP of **3**. In order to maintain solubility upon polymerization 1-hexyl-thymine instead of thymine was used, which was readily synthesized via the route depicted in Scheme 3 [31].

When monomer **3** was polymerized in the presence of 1-hexyl-thymine **6** first order kinetics were observed as can be seen in Fig. 7. Also the polymerization rate was much faster ($k_{app}=35.8\times10^{-3}$) compared to the polymerization without thymine units present ($k_{app}=2.96\times10^{-3}$), or even in the presence of polymer template.

Besides thymine derivative **6** succinimide was used in a template polymerization experiment since, this molecule should also be able to form hydrogen bonds with the adenine monomer In this case too a higher polymerization rate was observed ($k_{app}=19.5\times10^{-3}$). An overview of the template polymerization results is depicted in Fig. 7.

3.4. Discussion

All ATRP experiments were performed in DMSO for several reasons. Nucleobase monomers and polymers were well soluble in this solvent, which therefore, enabled kinetics studies that were not hampered by precipitation or

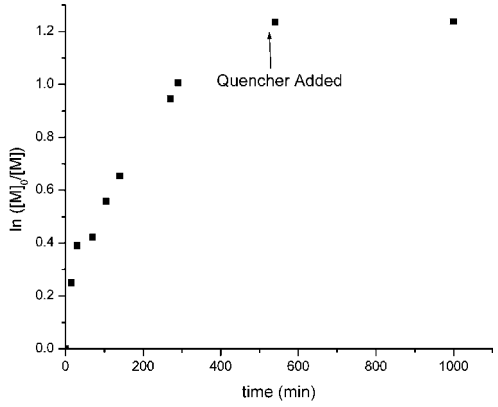


Fig. 2. Plot of ln ($[M]_0/[M]_t$) vs. time of the polymerization of **2** (0.415 M) with PEG initiator **4** (0.028 M), CuCl (0.028 M), bpy (0.056 M) and, after 540 min **7** (0.284 M).

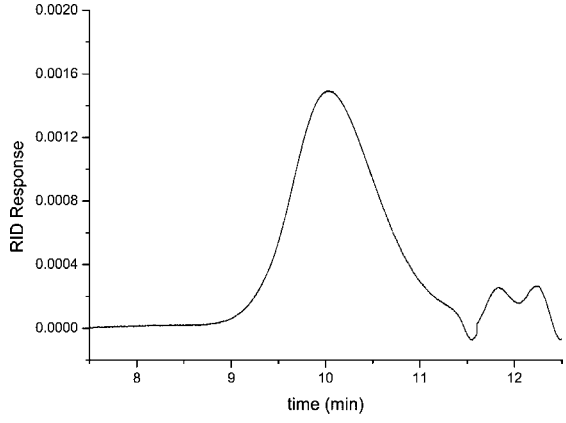


Fig. 3. GPC trace of PEG-*b*-oligoT10 (**5**) in DMSO at 70 °C after work up.

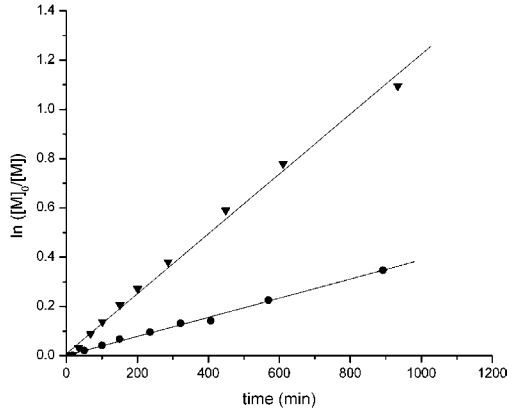


Fig. 4. Kinetic plots for ATRP of adenine monomer **3** (●, 0.13 M, 0.012 M CuCl, 0.024 M bpy, 0.014 M EBiB) and **3** in the presence of **5** (▼, 0.13 M, 0.012 M CuCl, 0.024 M bpy, 0.014 M EBiB, 0.014 M **5** with a ratio thymine/adenine units = 1.04/1).

aggregation. Because these experiments were performed in DMSO-d_6, ^{1}H NMR spectroscopy could be used for the determination of the kinetics. A major disadvantage of the use of a polar solvent such as DMSO is that no strong hydrogen bonding interactions between adenine and thymine were expected. In addition it has been shown that polar solvents in general have a rate enhancing effect on ATRP [26,27]. Those rate enhancements were explained by the existence of a monomeric copper species. Data from literature [28,29] also shows that DMSO can coordinate to copper, changing the nature of the catalyst. Because Takemoto and Inaki [30,32] showed that a template effect still could occur in polar solvents as a result of complementary nucleobase interactions, we decided to start our investigations with DMSO solutions.

Also in our case the presence of a specific interaction could be interpreted from the fact that thymine functionalized polymer accelerates the polymerization of adenine monomer, while thymine monomer is not accelerated by the same polymer template (Figs. 4 and 5).

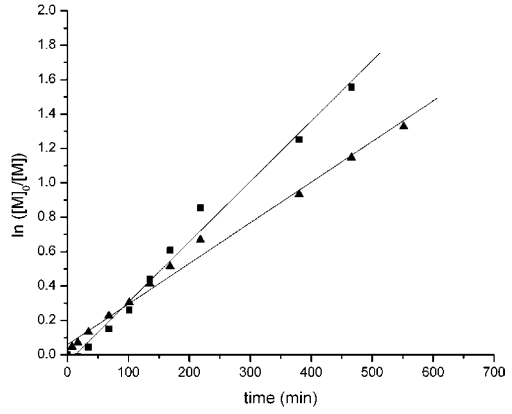


Fig. 5. Kinetic plots for the ATRP of thymine monomer **2** (■, 0.13 M **2**, 0.012 M CuCl, 0.024 M bpy, 0.014 M EBiB) and **2** in the presence of **5** (▲, 0.13 M **2**, 0.012 M CuCl, 0.024 M bpy, 0.014 M EBiB, 0.014 M **5**) with a ratio of thymine/thymine = 0.98/1.

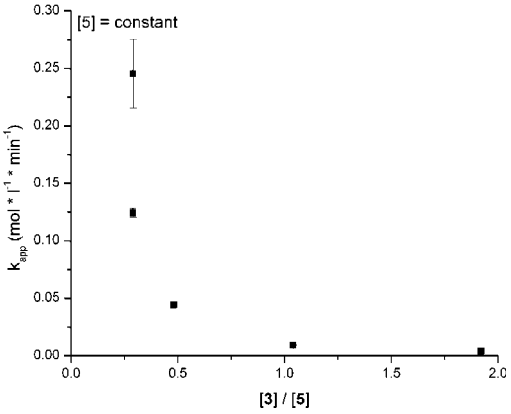


Fig. 6. Apparent rate constants of the ATRP experiments at different monomer (**3**) to template (**5**) ratios.

To further investigate these results the ratio between monomer and polymer template was changed. In general, during template polymerizations the rate of polymerization depends on the presence of template polymer and on the strength of the interaction between template and monomer. The $k_{p,app}$ should have an optimum around a 1 to 1 ratio of monomer to templating moiety [1,2,5,6]

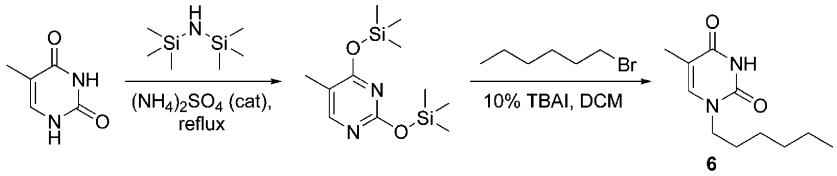
However, in our case, when we increased the amount of template, the polymerization rate increased even more. Surprisingly, the polymerization speed of adenine monomer increased by a factor of 100 when a large excess of thymine functionalized polymer was used (Table 1, entry 5 and 6). Therefore, a different mechanism must be in effect during the polymerization of adenine monomer with complementary thymine polymer present.

To investigate further what effect was responsible for the polymerization rate increase adenine monomer was polymerized in the presence of 1-hexyl-thymine moiety **6**. Also in this case a rate enhancement, even greater than with polymer **5** was observed. In addition, succinimide, which has the same hydrogen bonding capabilities as thymine, but is less specific for adenine, shows also an increase in the polymerization rate of adenine, albeit smaller than for thymine. Succinimide has been used before to enable the polymerization of adenine monomer via ROMP [33].

The most plausible explanation for all the phenomena

Table 1
Apparent rate constants for the polymerization of monomer **3** with different additives at different ratios

	Additive	Ratio (adenine/additive)	$k_{app} \times 10^{-3}$ (mol L^{-1} min^{-1})
1		1.0:0	2.96
2	PEG-*b*-T10 (**5**)	1.9:1	3.99
3	PEG-*b*-T10 (**5**)	1.0:1	9.30
4	PEG-*b*-T10 (**5**)	0.5:1	44.3
5	PEG-*b*-T10 (**5**)	0.3:1	124
6	PEG-*b*-T10 (**5**)	0.3:1	246
7	Succinimide	1.0:1	19.5
8	Thy-C6 (**6**)	1.0:1	35.8



Scheme 3. Synthesis of 1-hexyl-thymine **6**.

observed is that the rate acceleration is a result of a specific interaction between adenine and thymine moieties. The exact type of interaction is until now rather elusive. Although hydrogen bonding between the complementary nucleobases, or between succinimide and adenine would be a logical assumption, the use of DMSO-d_6 as a solvent hampers the identification in the ^{1}H NMR and FTIR spectra of the occurrence of hydrogen bonding interaction between adenine and thymine. A possible reason why single nucleobase interactions result in acceleration of ATRP of adenine methacrylate **3** can be found when one compares the rates of polymerization of **2** and **3** in absence of any polymeric template. From these experiments it is clear that thymine polymerization proceeds much faster than adenine polymerization. The adenine moiety, therefore, slows down the ATRP process. Adenine seems to interact with the ATRP copper complex, thereby partly deactivating the catalyst or creating a different, less active catalytic copper species. If on the other hand thymine would influence or activate the copper catalyst, deviations from first order kinetics would be expected during the polymerization of thymine monomer, because monomeric thymine is much better at increasing the polymerization rate compared to polymeric thymine. Therefore, during ATRP of thymine the acceleration effect should diminish. Since, this is not the case the explanation that adenine affects the catalyst is more plausible.

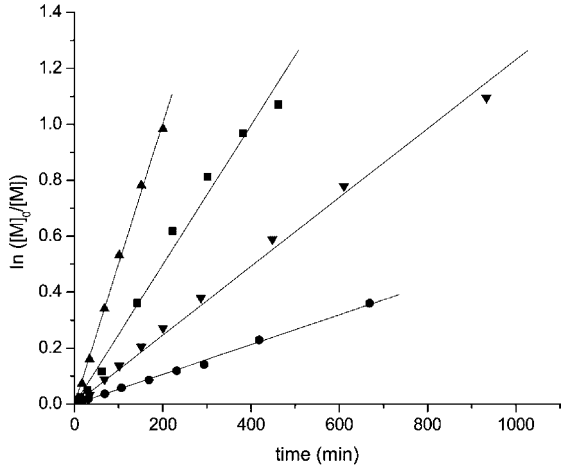


Fig. 7. Kinetic plot of adenine polymerization in the presence of different additives ● (0.13 M **3**, 0.012 M CuCl, 0.024 M bpy, 0.014 M EBiB), ▼ (0.13 M **3**, 0.012 M CuCl, 0.024 M bpy, 0.014 M EBiB, 0.014 M **5**), ■ (0.11 M **3**, 0.0096 M CuCl, 0.021 M bpy, 0.011 M EBiB, 0.11 M succinimide), ▲ (0.13 M **3**, 0.012 M CuCl, 0.024 M bpy, 0.014 M EBiB, 0.13 M **6**).

By adding thymine either in polymeric or monomeric form, or by adding succinimide, which is complementary to adenine, to the polymerization, adenine interacts with these complementary species and, therefore, less with the copper catalyst, generating a more active copper catalyst species. As a result the rate of polymerization is increased. This explanation is currently investigated in more detail in our laboratories.

4. Conclusions

Adenine and thymine methacrylate monomers can be synthesized and polymerized in a controlled fashion using ATRP in DMSO-d_6 as a solvent. When a thymine functionalized block copolymer was added as a template to the polymerization of adenine monomer a significant rate enhancement was observed. In addition this rate enhancement proved to be specific for the adenine–thymine base pair because the polymerization rate of thymine monomer was not amplified. Further elucidation of the polymerization rate enhancement by changing the adenine to thymine ratios showed that if an excess of thymine block copolymer was added the rate enhancement was increased up to 100 times. This interesting phenomenon was not in agreement with any of the known template polymerization studies or other polymerizations of functional monomers in DMSO found in literature. Therefore, complementary monomeric moieties were also included during polymerization and surprisingly they showed an even greater rate enhancement compared to the polymeric thymine. These findings showed that in DMSO different catalytic species were formed that influence the rate of polymerization significantly of this system.

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