Articles

Atom Transfer Radical Polymerization of Adenine, Thymine, Cytosine, and Guanine Nucleobase Monomers

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ABSTRACT: Methacrylate monomers functionalized with thymine, adenine, cytosine, and guanine were prepared by alkylation of the nucleobases with 3-bromopropyl methacrylate. Application of atom transfer radical polymerization in deuterated DMSO allowed controlled polymerization of the adenine-, thymine-, and for the first time, also of the cytosine- and guanine-modified monomers. The guanine and cytosine monomers appeared to form a complex with the copper catalyst, as could be observed with ^{1}H NMR spectroscopy. In the case of guanine, this did not cause any problems for the polymerization process, while in the case of cytosine, a stronger copper binding ligand, PMDETA, needed to be applied to gain control over polymerization.

Introduction

The high fidelity and specificity of the DNA replication and transcription process has been a source of inspiration for materials scientists for many years, resulting in a range of well-defined DNA-based molecular architectures and devices.[1−5] The specific hydrogen bond interaction between the nucleotide pairs adenine−thymine and guanine−cytosine, known as Watson−Crick base pairing, has also been employed in polymer chemistry. Already in the early 1970s, Inaki and co-workers[6−9] conducted free radical polymerization of nucleobase functionalized monomers in DMSO/ethylene glycol mixtures in order to use hydrogen bonding interactions to control the sequence of monomer units in a polymer chain via a templated polymerization mechanism. The DNA recognition concept has also been applied for the construction of supramolecular polymers[11−14] and polymer assemblies[15−17] by introduction of complementary single DNA strands, and even single nucleobase moieties, to the chain ends of synthetic polymers.

Recent developments in controlled metathesis and radical polymerization techniques have enlarged the ability of polymer scientists to create better defined polymers containing nucleobase functionality. Several research groups[18,19] polymerized nucleobase monomers using ring-opening metathesis polymerization (ROMP). (Noncovalent) protection of the adenine and thymine monomers was necessary to obtain high conversions. In the case of cytosine, however, solubility problems hindered further analysis of polymer; guanine-functionalized norbornene derivatives could not be polymerized successfully using ROMP.

Haddleton et al.[20,21] used free radical polymerization and atom transfer radical polymerization (ATRP) to polymerize protected adenosine and uridine monomers and demonstrated it to be possible to polymerize via ATRP methyl methacrylate (MMA) using unprotected adenosine and uridine initiators. In addition, a remarkable template effect was observed when adenosine and uridine monomers were polymerized in the presence of uridine polymer using a free radical polymerization technique. Lutz and co-workers[23] used ATRP to prepare nucleobase functionalized styrene-like copolymers and demonstrated the DNA-like melting behavior in nonpolar solvents. Gross et al.[22] and our group[10,38] polymerized with ATRP unprotected adenine- and thymine-based monomers using polyethylene glycol macroinitiators in order to obtain amphiphilic block copolymers. These diblock copolymers showed an assembly behavior that was affected by the presence of the complementary nucleobases.

As aforementioned, well-defined polymers based on nucleobase monomers adenine, uracil, and thymine have been prepared. However, to the best of our knowledge, the controlled polymerization of the remaining two nucleobases cytosine and guanine has been unreported. The ability to polymerize also these monomers facilitates the use of the stronger triple hydrogen bonds for template polymerizations and polymer assemblies. Moreover, it enables, in combination with the adenine−thymine base pair, the construction of more complex supramolecular systems and fully exploits the complementary nature of Watson−Crick base pairing.

In this paper, we report the synthesis and controlled polymerization of all four nucleobase methacrylate monomers. We have used a convenient two-step synthetic route for the synthesis of thymine, adenine, and cytosine methacrylate monomers. The guanine monomer synthesis was more elaborate and involved several protection and deprotection steps. All four monomers were polymerized in a controlled fashion using ATRP, while kinetics were monitored using ^{1}H NMR spectroscopy.

Experimental Section

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 then stored under an argon atmosphere. Dichloromethane (DCM),

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heptane, and ethyl acetate (EtOAc) were distilled over calcium hydride (CaH_2) prior to use. 1,4-Dioxane was distilled over $LiAlH_4$. Copper bromide (CuBr) and copper chloride (CuCl) were purified according to literature procedures.[24] Triphenylphosphine (Ph_3P) was recrystallized from MeOH. 3-Bromopropyl methacrylate (**1**) and monomers 3-(thymin-1-yl)propyl methacrylate (**2**) and 3-(adenin-9-yl)propyl methacrylate (**3**) were prepared according to a previously published procedure.[10] Other chemicals were used as received unless otherwise stated.

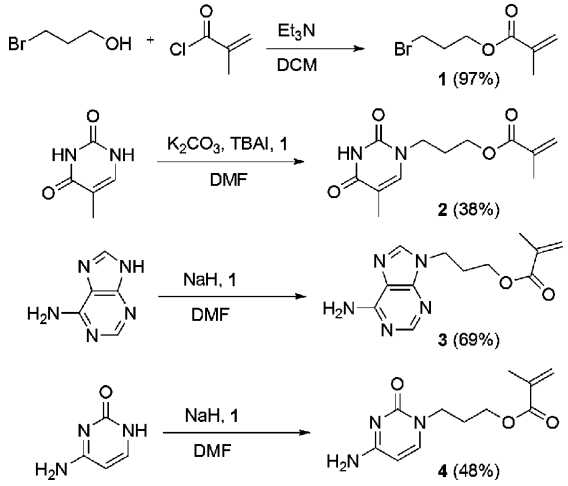
Instrumentation. ^{1}H NMR spectra were recorded on a Varian Inova 400 instrument at 400 MHz, and ^{13}C NMR spectra were recorded on a Bruker DPX300 instrument at 75 MHz. Chemical shifts (δ) are given in ppm relative to the internal standard (Me_4Si or DMSO-d_6). IR spectra were recorded on an ATI Matson Genesis series FTIR spectrometer with fitted ATR cell. GPC measurements were performed using a Shimadzu LC-10ADvp system equipped with a PL gel 5 μm guard column, a PL gel 5 μm mixed D column, differential refractive index detector (Shimadzu RID-10A) at 38 °C, and a UV detector (Shimadzu SPD-10AVvp). The system was operated either using dimethylsulfoxide as an eluent, with a flow of 0.8 mL·min^{-1} at 70 °C (DMSO, 0.02 M LiCl) and calibrated with polyethylene glycol standards in the range of 1900−124 700 Da, or using THF as an eluent with a flow of 1 mL·min^{-1} at 35 °C and polystyrene standards in the range of 580−377 400 Da to calibrate the GPC. High-resolution mass spectroscopy (HRMS) was performed on a VG7070. Silica gel column chromatography was performed using Acros or Merck silica gel (0.035−0.070 mm, pore diameter ca. 6 nm). TLC was carried out on Merck precoated silica gel 60 F-254 plates. Compounds were visualized using UV and permanganate staining agent.

3-(Cytosin-1-yl)propyl Methacrylate (4). To a suspension of cytosine (5.00 g, 45.0 mmol) in 125 mL of DMF was slowly added NaH (2.41 g, 60.3 mmol). The suspension was stirred at room temperature for 1 h until no more gas evolved. The resulting thick slurry was diluted with 40 mL of DMF, followed by addition of 3-bromopropyl methacrylate **1** (9.71 g, 46.9 mmol). After 24 h, the excess NaH was quenched with 50 mL of saturated aqueous NH_4Cl solution. The reaction mixture was concentrated in vacuo and subjected to column chromatography (eluent 10% MeOH/DCM) to yield 5.10 g (21.5 mmol, 47.7%) of **4** as a white solid. R_f = 0.20 (10% MeOH/DCM), mp = 146.8 ± 0.42 °C. HRMS (EI+) calcd for $C_{11}H_{15}N_3O_3$ 261.1113, found 261.1113. ^{1}H NMR (DMSO-d_6): δ 7.57 (d, J = 7.2 Hz, 1H, pyrimidine-H6), 7.07 (br d, NH_2), 6.02 (s, 1H, $O_2C-C(CH_3)=CH_A$), 5.66 (s, 1H, $O_2C-C(CH_3)=CH_B$), 5.64 (d, J = 7.2 Hz, 1H, pyrimidine-H5), 4.09 (t, J = 6.3 Hz, 2H, $O-CH_2-$), 3.73 (t, J = 6.7 Hz, 2H, $N-CH_2-$), 1.97−1.90 (m, 2H, $-CH_2-CH_2-CH_2-$), 1.87 (s, 3H, $O_2C-C(CH_3)=CH_2$). ^{13}C NMR (DMSO-d_6): δ 165.98, 165.05, 154.90, 145.86, 135.42, 125.42, 93.10, 61.88, 46.16, 27.74, 18.03. FTIR (solid): ν 3348, 3104, 1888, 1713, 1659, 1608, 1527.

Phenylacetic Anhydride (11). Phenylacetic acid (14.22 g, 0.1045 mol) was suspended in acetic anhydride (25 mL) and heated to reflux for 18 h. The resulting clear-yellow solution was concentrated in vacuo, after which it was precipitated into water. The product was then filtered off and washed two more times, followed by three washing steps with petroleum ether (80−100), after which 11.96 g (0.04598 mol, 88.0%) of product was obtained as a white powder after drying in vacuum; mp = 68.9 ± 0.1 °C. HRMS (EI+) calcd for $C_{16}H_{14}O_3$ 254.0943, found 254.0951. ^{1}H NMR ($CDCl_3$): δ 7.32−7.18 (m, 10H, Ar*H*), 3.71 (s, 4H, CH_2). ^{13}C NMR ($CDCl_3$): δ 166.68, 131.89, 129.30, 128.69, 127.52, 42.29. FTIR (solid): ν 3030, 1812, 1739, 1497, 1454.

***N*-(9-Acetyl-6-oxo-6,9-dihydro-1*H*-purin-2-yl)-2-phenylacetamide (6).** To a suspension of 5.02 g (33.2 mmol) guanine in 150 mL of *N*,*N*-dimethyl acetamide (DMA) was added 22.72 g (89.3 mmol) phenylacetic anhydride. The reaction mixture was heated to reflux until a clear solution was obtained, after which heating was continued for 30 min. After cooling to room temperature, the reaction mixture was concentrated in vacuo, suspended in EtOAc, filtered, and dried to obtain crude *N*-(2-phenylacetyl)guanine. To a suspension of crude *N*-(2-phenylacetyl)guanine in 41 mL of DMF was added 6.77 g (66.4 mmol) acetic anhydride. The reaction mixture was heated to 105 °C until a clear solution was obtained (30 min). Solvent was removed in vacuo, followed by washing of the solid on a filter with abs EtOH and subsequent drying to yield 8.64 g, (27.7 mmol, 83.4%) **6** as an off-white powder. R_f = 0.22 (10% MeOH/DCM). HRMS (EI+) calcd for $C_{15}H_{13}N_5O_3$ 311.1018, found 311.10185. ^{1}H NMR (300 MHz, DMSO-d_6): δ 12.96 (br s, 2H, purine-NH, NH−C=O), 8.46 (s, 1H, purine-H8), 7.27−7.35 (m, 5H, ArH), 3.85 (s, 2H, O=C−CH_2-), 2.83 (s, 3H, $-CH_3$). ^{13}C NMR (DMSO-d_6): δ 173.79, 167.55, 154.18, 147.96, 147.40, 137.24, 133.74, 129.06, 128.11, 126.68, 121.35, 42.50, 24.79. FTIR (solid): ν 3133, 1749, 1697, 1672, 1597, 1541.

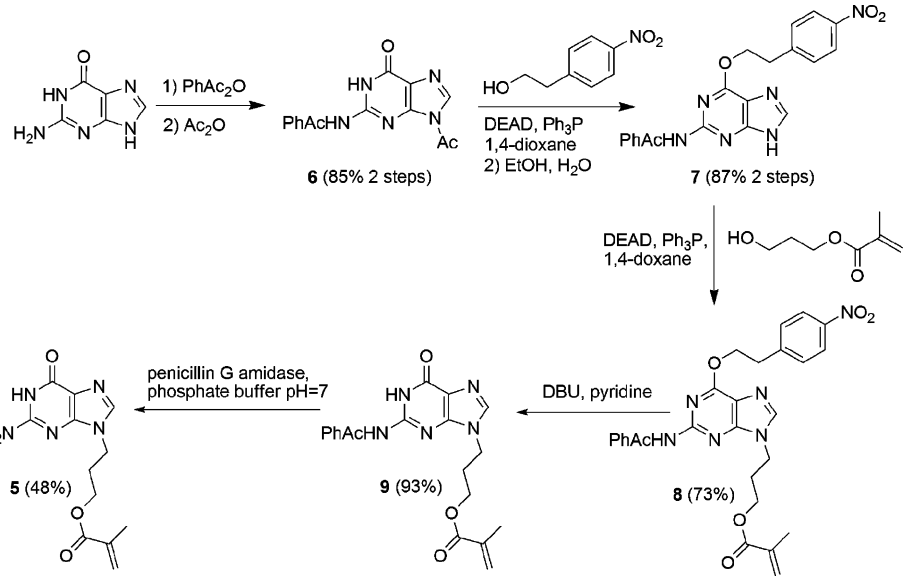
Scheme 1. Synthesis of Thymine, Adenine, and Cytosine Methacrylate Monomers 2−4



***N*-[6-(4-Nitrophenethoxy)-9*H*-purin-2-yl]-2-phenylacetamide (7).** To a suspension of 1.96 g (6.29 mmol) **6** in 70 mL of 1,4-dioxane was added 3.30 g (12.58 mmol) Ph_3P and 2.11 g (12.60 mmol) of 2-(*p*-nitrophenyl)ethanol. The reaction mixture was cooled with a cold-water bath before adding 2.37 g (13.63 mmol) of diethyl azodicarboxylate (DEAD) dropwise. After addition of DEAD, the mixture was allowed to stir overnight at room temperature. TLC indicated incomplete conversion, therefore additional Ph_3P (0.359, 1.4 mmol) and DEAD (0.293, 1.7 mmol) were added, resulting in a clear solution. After 7 h, the reaction mixture was concentrated in vacuo to $^1/_2$ of its volume and added to 750 mL of EtOH/H_2O (1:1, v/v) mixture, followed by heating to reflux until a clear solution was obtained. Upon cooling to room temperature and then to −18 °C for 18 h, product was obtained as crystals. Filtration and drying yielded 2.28 g (5.45 mmol, 86.7%) of **7**. R_f = 0.43 (10% MeOH/DCM). HRMS (EI+) calcd for $C_{21}H_{19}N_6O_4$ 419.1468, found 419.14758. ^{1}H NMR (DMSO-d_6): δ 13.19 (s, 1H, purine-NH), 10.56 (s, 1H, amide-NH), 8.16 (s, 1H, purine-H8), 8.15 (d, J = 8.4 Hz, 2H, Ar*H*−NO_2), 7.63 (d, J = 8.4 Hz, 2H, Ar*H*−NO_2), 7.35−7.21 (m, 5H, $-CH_2-$Ar*H*), 4.76 (t, J = 6.7 Hz, 2H, $O-CH_2-$), 3.81 (s, 2H, $-CH_2-$Ar), 3.30 (t, J = 6.7 Hz, 2H, $-CH_2-$Ar−NO_2). ^{13}C NMR (DMSO-d_6): δ 168.56, 159.11, 153.42, 151.27, 146.14, 145.88, 140.75, 135.49, 130.02, 128.98, 127.90, 126.16, 123.08, 116.56, 66.18, 43.13, 34.28. FTIR (solid): ν 3315, 3059, 1678, 1589, 1517, 1429, 1409, 1344.

3-Hydroxypropyl Methacrylate (10). To a cooled solution (0 °C) of 1,3-propanediol (14.62 g, 0.192 mol) and triethylamine (10 mL, 0.072 mol) in 100 mL of DCM was added dropwise a solution of methacryloyl chloride (3.0 mL, 0.174 mmol) in 30 mL of DCM while stirring vigorously. After complete addition, the reaction mixture was allowed to heat up to room temperature. The reaction mixture was poured into 110 mL saturated aqueous $NaHCO_3$, followed by three washing steps with 100 mL of water. The organic layer was dried with anhydrous $MgSO_4$, filtered, and concentrated in vacuo to give a light-yellow oil. This was further purified by column chromatography (33% EtOAc/heptane) to give 2.519 g (0.017 mol, 50.9%) of **10** as colorless oil. R_f = 0.19 (33% EtOAc/heptane). ^{1}H NMR (DMSO-d_6): δ 6.02−6.00 (m, 1H, O_2C-

Scheme 2. Guanine Monomer 5 Synthesis



C(CH_3)=CH_A), 5.67−5.65 (m, 1H, O_2C−C(CH_3)=CH_B), 4.53 (t, J = 5.2 Hz, 1H, HO−CH_2−), 4.15 (t, J = 6.5 Hz, 2H, −CH_2−O_2C−), 3.48 (dt, 2H, J = 5.2 Hz, J = 6.3 Hz, HO−CH_2−), 1.88 (dd, 3H, J = 1.0 Hz, J = 1.6 Hz, O_2C−C(CH_3)=CH_2), 1.79−1.72 (m, 2H, CH_2−CH_2−CH_2−). ^{13}C NMR ($CDCl_3$): δ 167.60, 136.14, 125.65, 61.75, 59.20, 31.97, 18.67. FTIR (oil): ν 3503, 3173, 3037, 2987, 2886, 2827, 1891, 1759, 1716, 1608.

3-[6-(4-Nitrophenethoxy)-2-(2-phenylacetamido)-9*H*-purin-9-yl]propyl Methacrylate (8). DEAD (1.18 mL) was added to a stirred suspension of **7** (2.10 g, 5.01 mmol), Ph_3P (1.97 g, 7.51 mmol), and **10** (0.86 g, 5.97 mmol) in 50 mL of dry dioxane. The solution was allowed to stir at room temperature for 18 h. The resulting orange−yellow clear reaction mixture was concentrated in vacuo, after which the crude product was further purified with column chromatography. (EtOAc/heptane 4:1) to yield 2.20 g of pure **8**. R_f = 0.48 (5% MeOH/DCM); mp = 230.4 ± 0.78 °C. ^{1}H NMR (DMSO-d_6): δ 10.61 (s, 1H, NH), 8.23 (s, 1H, purine-H8), 8.15 (d, 2H, ArH−NO_2), 7.63 (d, 2H, CH_2−ArH−NO_2), 7.29 (m, 5H, ArH−CH_2), 5.85 (s, 1H, O_2C−C(CH_3)=CH_A), 5.54 (s, 1H, O_2C−C(CH_3)=CH_B), 4.76 (t, J = 6.8 Hz, 2H, O−CH_2−), 4.25 (t, J = 6.8 Hz, 2H, O−CH_2−), 4.09 (t, J = 6.0 Hz, 2H, N−CH_2−), 3.85 (s, 2H CH_2−Ar), 3.30 (t, J = 6.8 Hz, 2H, Ar−CH_2), 2.21 (m, 2H, CH_2−CH_2−CH_2), 1.76 (s, 3H,). ^{13}C NMR ($CDCl_3$): δ 168.63, 165.79, 162,06, 155.47, 151,24, 146.26, 145.86, 135.57, 135.15, 129.89, 129.03, 127.89, 126.14, 125.40, 123.11, 108.67, 66.46, 61.62, 44.26, 43.11, 34.07, 29.39, 17.90.

3-(6-Oxo-2-(2-phenylacetamido)-6,9-dihydro-1*H*-purin-9-yl)-propyl Methacrylate (9). Compound **8** (520 mg, 0.95 mmol) containing triphenylphosphine oxide was dissolved in a 0.5 M DBU solution in pyridine and stirred at ambient temperature for 18 h. The solvent was removed in vacuo and crude product was further purified by means of column chromatography (2−5% MeOH/DCM) to yield 200 mg (0.506 mmol, 53%) of pure **9** as a white solid. R_f = 0.46 (10% MeOH/DCM); mp = 165.0 ± 1.0 °C. HRMS (EI+) calcd for $C_{20}H_{21}N_5O_4$ 395.1594, found 395.15793. ^{1}H NMR (DMSO-d_6): δ 11.91 (br s, 2H, 2× NH), 8.00 (s, 1H, purine-H8), 7.30 (m, 5H, ArH), 5.90 (s, 1H, O_2C(CH_3)=CH_A), 5.62 (s, 1H, O_2C(CH_3)=CH_B), 4.18 (t, J = 6.7 Hz, 2H, O−CH_2−), 4.12 (t, J = 6.7 Hz, 2H, N−CH_2−), 3.81 (s, 2H, Ar−CH_2−) 2.22−2.15 (m, 2H, CH_2−CH_2−CH_2−), 1.81 (s, 3H, O_2C(CH_3)=CH_2). ^{13}C NMR ($CDCl_3$): δ 173.51, 165.88, 154.37, 148.16, 147.08, 139.47, 135.22, 133.91, 129.00, 128.09, 126.64, 125.45, 120.02, 61.81, 42.48, 40.83, 28.35, 17.94. FTIR (solid): ν 3223, 3084, 2955, 1714, 1665, 1598, 1557.

3-(2-Amino-6-oxo-purin-9-yl)propyl Methacrylate (5). To a suspension of 0.5 g of penicillin amidase on Eupergit C in 20 mL of phosphate buffer (0.07 M, pH 7.0) was added a solution of compound **9** (87 mg, 0.152 mmol) in 2 mL of MeOH. The suspension was shaken at 250 rpm at 28 °C for 24 h until TLC indicated complete conversion. The Eupergit was filtered off, after which the filtrate was freeze-dried. The resulting crude product was purified by column chromatography (eluent 10% MeOH/DCM) to yield 20 mg (0.072 mmol, 48%) of product **5** as a white solid. R_f = 0.19 (10% MeOH/DCM). ^{1}H NMR (DMSO-d_6): δ 10.52 (s, 1H, purine-NH), 7.73 (s, 1H, purine-H8), 6.37 (s, 2H, purine-NH_2), 5.96 (s, 1H, O_2C−C(CH_3)=CH_A), 5.63 (s, 1H, O_2C−C(CH_3)=CH_B), 4.09 (t, J = 6.3 Hz, 2H, O−CH_2−), 4.05 (t, J = 6.8 Hz, 2H, N−CH_2−), 2.15−2.09 (m, 2H, CH_2−CH_2−CH_2), 1.84 (s, 3H, O_2C(CH_3)=CH_2). ^{13}C NMR (DMSO-d_6): δ 167.12, 156.43, 146.72, 145.12, 139.54, 134.49, 125.33, 120.02, 61.78, 41.04, 28.39, 18.02.

Poly[3-(thymin-1-yl)propyl Methacrylate] (General Procedure) (12). Compound **2** (436 mg, 1.73 mmol), CuCl (6 mg, 0.06 mmol), and 2,2′-bipyridine (bpy) (17 mg, 0.11 mmol) were added to a Schlenk tube, followed by three cycles of evacuation and nitrogen refilling. Deoxygenated DMSO-d_6 (3 mL) was added and the polymerization was started by addition of 8 μL (0.05 mmol) of ethyl bromoisobutyrate (EBiB). During the reaction, samples were taken and conversion was determined using ^{1}H NMR spectroscopy by comparing the acrylate signal with the thymine H6 signal. After polymerization, the reaction mixture was precipitated in an aqueous solution of EDTA (0.07 M), the precipitate was redissolved in DMSO, and precipitated again to yield 345 mg of polymer. $M_{n,theo}$ = 6.4 kg/mol. SEC (DMSO): M_n = 6.8 kg/mol, M_w/M_n = 1.21. ^{1}H NMR (DMSO-d_6): δ 11.15 (br s, pyrimidine-NH), 7.45 (br s, pyrimidine-H6), 3.92 (br s, O−CH_2−CH_2), 3.71 (br s, CH_2−CH_2−N), 1.95−1.60 (br m, {CH_2−C(CH_3)}, pyrimidine−CH_3, CH_2−CH_2−CH_2−N), 1.20−0.65 (br m, {CH_2−C(CH_3)}).

Poly[3-(adenin-9-yl)propyl Methacrylate] (13). Using the procedure described for polymer **12**, a reaction mixture composed of **3** (347 mg, 1.33 mmol), CuCl (5 mg, 0.05 mmol), bpy (15 mg, 0.10 mmol) DMSO-d_6 (3.7 mL), and EBiB (6 μL, 0.04 mmol) gave 147 mg of polymer. $M_{n,theo}$ = 5.7 kg/mol. SEC (DMSO): M_n = 6.9 kg/mol, M_w/M_n = 1.19. ^{1}H NMR (DMSO-d_6): δ 8.15 (br s, purine-H2,8), 7.25 (br s, purine-NH_2), 4.23 (br s, O−CH_2−CH_2), 3.90 (br s, CH_2−CH_2−N), 2.16 (br s, O−CH_2−CH_2−CH_2−N), 1.89−1.55 (br s, {CH_2−C(CH_3)}), 1.10−0.55 (br m, {CH_2−C(CH_3)}).

Poly[3-(cytosin-1-yl)propyl Methacrylate] (14). CuCl (16 mg, 0.16 mmol) and $CuCl_2$ (2 mg, 0.02 mmol) were premixed, after which 4 mg (0.037 mmol CuCl, 0.003 mmol $CuCl_2$) of copper mixture was added to a Schlenk tube containing compound **4** (325 mg, 1.37 mmol). This was followed by three cycles of evacuation and nitrogen refilling. Deoxygenated DMSO-d_6 (6.086 g, 5.12 mL), 5 μL (0.023 mmol) of *N*,*N*,*N*′,*N*″,*N*″-pentamethyldiethylenetriamine

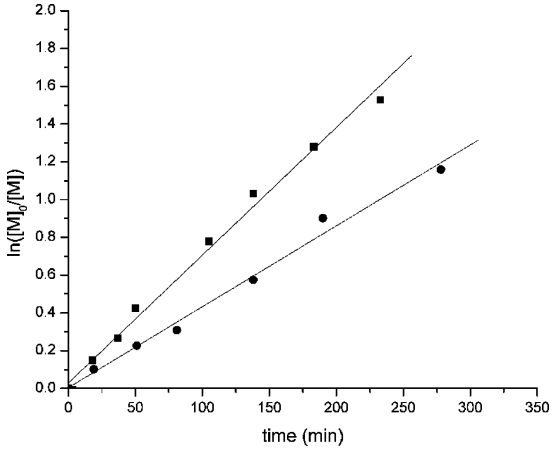


Figure 1. Kinetic plot of ATRP of thymine monomer (■, **2** 0.58 M, CuCl 0.020 M, bpy 0.037 M, EBiB 0.017 M) and adenine monomer (●, **3** 0.36 M, CuCl 0.013 M, bpy 0.027 M, EBiB 0.011 M).

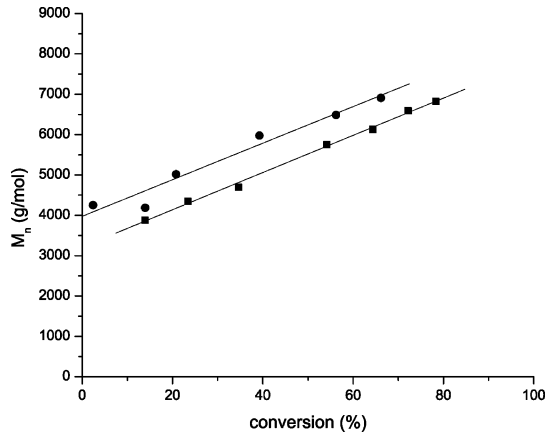


Figure 2. Evolution of M_n (GPC) with conversion for the polymerization of thymine (■) and adenine monomer (●).

(PMDETA) were added, after which the polymerization was started by addition of 9 μL (0.06 mmol) of EBiB. Precipitation of polymer in aqueous EDTA solution (0.07 M) gave 147 mg of polymer. $M_{n,theo}$ = 3.5 kg/mol. SEC (DMSO): M_n = 4.5, M_w/M_n = 1.15. ^{1}H NMR (DMSO-d_6): δ 7.65 (br s, pyrimidine-*H*6), 7.30 (br s, NH_2), 5.75 (br s, pyrimidine-*H*5), 3.91 (br s, O−CH_2−CH$_2$), 3.71 (br s, CH$_2$−CH_2−N), 2.0−1.7 (br m, {CH_2−C(CH$_3$)}, CH$_2$−CH_2−CH$_2$−N), 1.2−0.6 (br m, {CH$_2$−C(CH_3)}).

Poly[3-(guanin-9-yl)propyl Methacrylate] (15). A stock solution (22 μL) containing 0.3 M CuBr (6.6 μmol) and 0.6 M bpy (13 μmol) in DMSO-d_6 was added to an NMR tube charged with a solution of monomer **5** (26.8 mg, 96.7 μmol) in 0.7 mL of DMSO-d_6. After deoxygenating by purging with argon for 20 min, the polymerization was started by addition of 12 μL of 0.5 M EBiB stock solution (6 μmol) in DMSO-d_6, followed by immediate recording of the first ^{1}H NMR spectrum at 40 °C. Follow-up spectra were recorded at different time intervals by applying steady-state scans. The polymerization was quenched by precipitation of the reaction mixture in an aqueous EDTA solution (0.07 M). Centrifuging the precipitated product yielded 10 mg of polymer. $M_{n,theo}$ = 3.2 kg/mol, SEC (DMSO): M_n = 6.5 kg/mol, M_w/M_n = 1.15. ^{1}H NMR (DMSO-d_6): δ 10.6 (br s, purine-N*H*), 8.15 (br s, purine-*H*8), 6.45 (br s, purine-NH_2), 4.05−3.86 (br s, O−CH_2−CH$_2$ + CH$_2$−CH_2−N$_{purine}$), 2.09 (br s, O−CH$_2$−CH_2−CH$_2$−N$_{purine}$), 1.89−1.55 (br s, CH_2−C(CH$_3$)}), 1.10−0.55 (br m, CH$_2$−C(CH_3)}).

Results and Discussion

Monomer Synthesis. The synthesis of nucleobase containing monomers **2**, **3**, and **4** was performed using 3-bromopropyl methacrylate **1** as alkylating agent according to Scheme 1 and analogous to earlier described procedures.[10,26] The yield for monomer **2** was rather low due to side reactions like dialkylation at the N1 and N3 position. Several attempts to increase the yield using a literature procedure that involved protection of thymine with trimethylsilyl groups prior to alkylation[25,26] in order to prevent dialkylation did not increase the yield because the bis-(trimethylsilyl)thymine is readily hydrolyzed back to its starting compound. Adenine and cytosine monomers (**3** and **4**) were obtained by direct alkylation in reasonable yields of 69% and 48%, respectively.

Table 1. Polymerization Characteristics of Thymine (2), Adenine (3), and Guanine (5) Monomers

polymer	monomer	temp (°C)	[M]	$M_{n,theo}$[a] kg/mol	M_n[b] kg/mol	M_w/M_n[b]
12	**2**	ambient	0.58	6.4	6.8	1.21
13	**3**	ambient	0.36	5.8	6.9	1.19
15	**5**	40	0.13	3.2	6.5	1.15

[a] Determined from conversion. [b] Determined by GPC with PEG calibration and RID detection.

The synthesis of guanine monomer **5** was more elaborate because direct alkylation leads to a virtually inseparable mixture of N7 and N9 alkylated products.[27,28] In order to obtain the desired guanine monomer, we therefore adapted a procedure described by Benner et al.[29] for selective N9 alkylation of guanine using Mitsunobu-type conditions, as depicted in Scheme 2. For selective N9 alkylation, an O6 and N2 protected guanine precursor was needed. Especially, the appropriate choice of the protective groups for the N2 position was crucial because most protective groups described in literature[30] require deprotection conditions that would also hydrolyze the methacrylate ester function in the final monomer. To prevent this unwanted side reaction, a protective group that could be removed under mild conditions was necessary. Since enzymatic protecting group techniques have proven their efficiency with complete selective removal under mild conditions, we chose the enzyme-labile phenylacetyl as a protective group,[31−33] which could be removed efficiently using penicillin acylase.

Therefore, phenylacetic anhydride **11**, freshly prepared by treatment of phenylacetic acid with acetic anhydride, was condensed with guanine in refluxing DMF, followed by acylation at N9 to obtain 9-acetyl-*N*-phenylacetylguanine **6** in 83% yield for the two steps. Purine ether derivative **7** was obtained from **6** by Mitsunobu coupling with 2-(*p*-nitrophenyl)ethanol, followed by N7 deacetylation upon refluxing in a water/ethanol mixture (87% yield overall). For the next step, 3-hydroxypropylmethacrylate **10** was prepared by esterification of 1,3-propanediol with methacryloyl chloride under basic conditions. The second Mitsunobu condensation under strictly anhydrous conditions with intermediate **7**, performed immediately after purification of alcohol **10** due to its rather low stability, alkylated exclusively at the 9 position, resulting in the protected guanine methacrylate ester **8**. In the final two steps of the sequence, smooth deprotection of the 2-(*p*-nitrophenyl)ethyl group with DBU, followed by enzymatic removal of the phenylacetyl protective group using penicillin acylase, resulted in the unprotected 3-(guanin-9-yl)-propyl methacrylate **5** in an overall yield of 24%.

Polymerization. Because of the relatively low solubility of the nucleobase monomers in organic solvents commonly used for ATRP and the anticipated solubility problems of the nucleobase polymers, polymerizations were performed in DMSO. Previous research in our group with peptide-based monomers[34] has already established DMSO as an adequate solvent for ATRP. Furthermore, the use of deuterated DMSO-d_6 offers the pos-

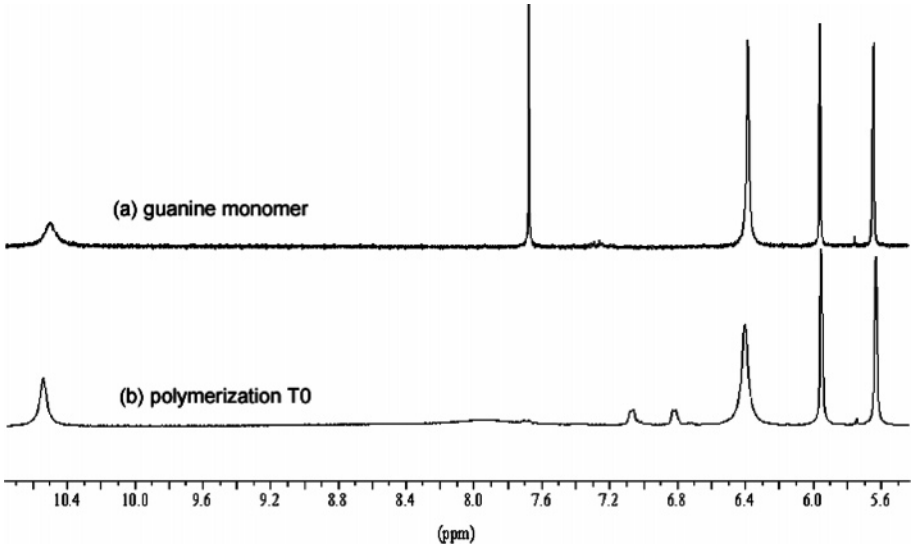


Figure 3. Part of the ^{1}H NMR spectrum of guanine monomer **5** (upper trace) and T0 of the polymerization of **5** with CuBr/(bpy)$_2$ in DMSO-d_6 (lower trace).

sibility to monitor kinetics directly by following the reaction with NMR spectroscopy. Thus, ATRP of monomer **2** and **3** was performed under the action of cuprous chloride (CuCl) and 2,2′-bipyridine (bpy) as a catalyst and ethyl 2-bromoisobutyrate (EBiB) as an initiator.

The polymerizations were monitored using NMR spectroscopy by comparison of the integrals of the methacrylate proton at 6.11 ppm with the thymine H6 proton signal at 6.94 ppm for monomer **2** and the methacrylate proton at 5.91 ppm with the adenine H2 and H8 signals at 8.14 ppm in the case of monomer **3**. Initially, the thymine monomer polymerization was carried out at 80 °C, but due to the fact that at this temperature polymerization proceeded extremely fast (reaching 92% conversion in 60 min), the polydispersity of the polymer was rather high (PDI > 1.3). Therefore, reaction temperature was lowered to ambient temperature, and initial monomer concentration lowered to 0.5 mol/L to obtain a controlled polymerization rate. It was found that, under these conditions, the polymerization of thymine monomer **2** proceeded smoothly with first-order kinetics and a linear evolution of molecular weight (Figures 1 and 2). Under identical conditions, 3-(adenine-9-yl)propyl methacrylate **3** also polymerized in a controlled fashion, supported by the observation of first-order kinetics and linear evolution of the molecular weight, indicating control over polymerization and a narrow molecular weight distribution (PDI = 1.19) of the polymer, as was found by GPC.

For the polymerization of the guanine monomer, similar ATRP conditions were applied. Because the guanine monomer **5** has a relative low solubility even in DMSO-d_6, a maximum monomer concentration of 0.13 M could be obtained. Furthermore, the reaction was performed in an NMR tube in order to scale down the reaction and still be able to monitor the polymerization kinetics in more detail. In an NMR tube, a solution of monomer **5** and the CuBr/bpy catalyst in DMSO-d_6 was deoxygenated by purging with argon for a few minutes. Surprisingly, the ^{1}H NMR spectrum of guanine monomer showed disappearance of the H8 (8.67 ppm) signal and broadening of the other peaks upon addition of the catalyst stock solution, as can be seen in Figure 3. Also, bipyridine signals (6.83 and 7.34 ppm) could be observed, indicating a chelating effect of guanine monomer with copper replacing the 2,2′-bipyridine as ligand. Interestingly, complex formation had no significant negative effect on the polymerization of **5**.

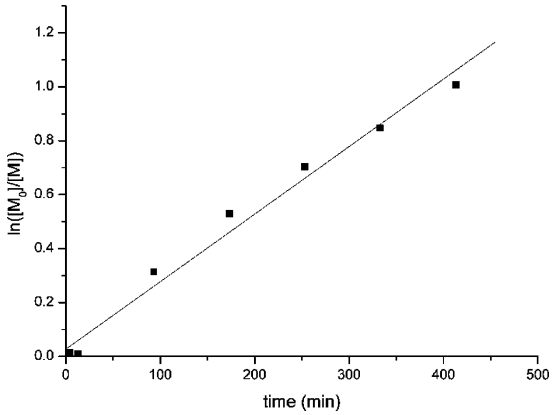


Figure 4. Kinetic plot of ATRP of guanine monomer (**5** 0.13 M, CuBr 9.4 mM, bpy 18.8 mM, EBiB 8.5 mM).

Polymerization was started by addition of initiator followed by immediate recording of the first ^{1}H NMR spectrum (T0), after which at different time intervals more ^{1}H NMR spectra were recorded. Relative monomer concentrations could be determined from these spectra by comparing the methacrylate proton at 5.96 ppm of the monomer with the NH_2 protons at 6.37 ppm of guanine in monomer and polymer. Because the reaction was performed in an NMR tube, no evaluation of molecular weight development with conversion could be made. The kinetic plot depicted in Figure 4 shows that polymerization proceeded with minor deviation from first-order kinetics. However, GPC analysis revealed a discrepancy between the theoretical and observed molecular weight, which is indicative for a low initiation efficiency. Nevertheless a good polydispersity, indicating control over the polymerization, was observed (Table 1).

When the same ATRP-conditions used for monomer **2** and **3** were applied to cytosine monomer **4**, no polymerization occurred even after the temperature was increased from 25 to 80 °C. Detailed analysis of the ^{1}H NMR spectrum showed also in this case broadening, i.e., the NH_2 signal at 7.0−7.2 ppm changed from a broad doublet to a broad singlet. In contrast, the H6 proton at 7.59 ppm remained a sharp doublet, whereas the H5 proton at 5.65 ppm of cytosine disappeared (Figure 5). These findings indicate chelation of the cytosine monomer to copper and are in line with literature, where addition of only a small

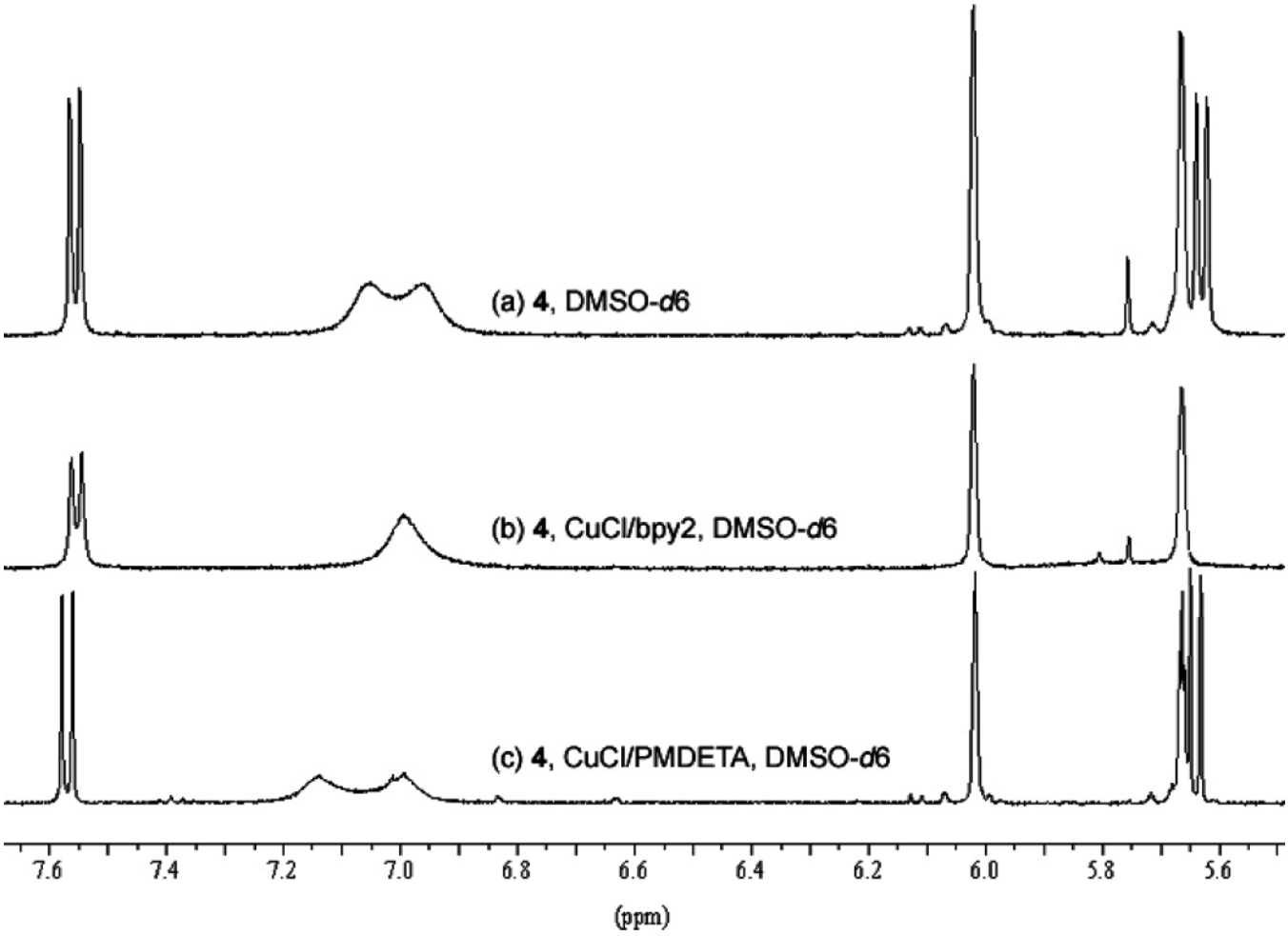


Figure 5. Part of ^{1}H NMR spectra of (a) cytosine monomer **4**, (b) **4** with CuCl/(bpy)2 as catalyst, and (c) **4** with CuCl/PMDETA as catalyst.

Table 2. Polymerization Conditions for Cytosine Monomer 4

		ratio								
entry	ligand	[EBiB]/	[Cu(I)Cl]/	[Cu(II)Cl_2]/	[ligand]	temp (°C)	conversion (%)	M_n	M_w	M_w/M_n
1	bpy	1.00	1.13	0	2.25	rt−80	0			
2	PMDETA	1.00	1.08	0	2.02	rt	74	7.2	9.7	1.35
3	PMDETA	1.00	0.50	0	0.50	rt	83	6.8	8.8	1.29
4	PMDETA	1.00	0.62	0.05	0.40	30	64	4.5	5.2	1.15

amount of copper(I) (6×10^{-5} M) already completely broadens the H5 proton in the ^{1}H NMR spectrum.[35,36]

Unfortunately, complex formation of cytosine with copper renders the catalyst inactive and, consequently, no polymerization occurs. We therefore decided to use the tridentate ligand *N*,*N*,*N*′,*N*″,*N*″-pentamethyldiethylenetriamine (PMDETA) instead of bipyridine, because PMDETA is known to bind more strongly to copper.[37] However, due to the high activation constant of PMDETA in polar solvents, the polymerization rate at room temperature was already very high and the kinetics did not show first-order behavior (Figures 6 and 7, Table 2). Consequently, to lower the polymerization rate and improve control over the polymerization, the amount of catalyst (CuCl/PMDETA) was lowered. Although the polymerization rate was effectively lowered, still no good first-order kinetics were observed and the measured polydispersities remained relatively high (PDI = 1.29). Better kinetics were observed, established by NMR, when 7.5 mol % cupric chloride ($CuCl_2$) was added to the catalyst. GPC analysis gave a linear increase in molecular weight with some deviation toward the end of the polymerization, indicating some termination had occurred. Additionally, a molecular weight distribution (PDI) of 1.17, DP = 8, confirmed the observations of the kinetics experiment.

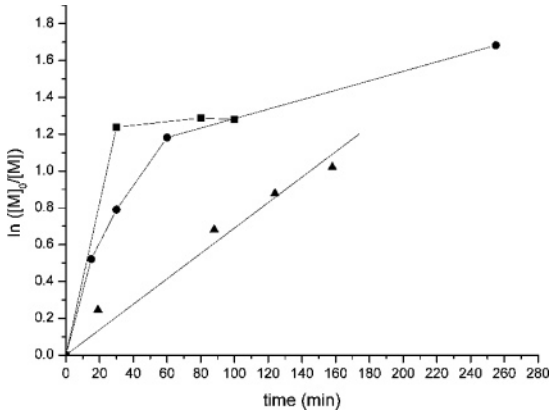


Figure 6. Kinetic plot for the polymerization of cytosine monomer **4** with different CuCl/PMDETA ratios and weight % $CuCl_2$: (■, 1:2, 0%), (●, 1:1, 0%), (▲, 10.6, 10%).

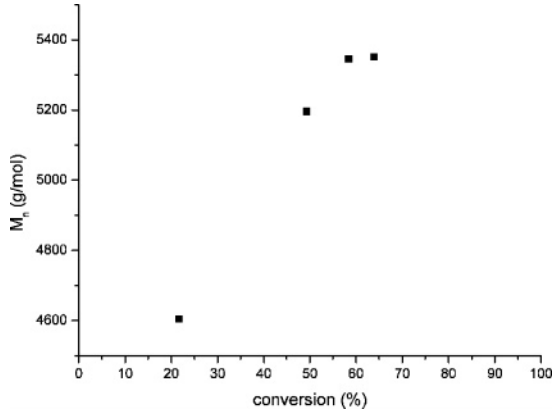


Figure 7. Evolution of M_n (GPC) with conversion for the polymerization of cytosine monomer (Table 2, entry 4).

Studies toward the effects of copper binding to guanine and cytosine nucleobase monomers during polymerization, similar to previously published work on nucleobase interactions during ATRP,[10] are currently being performed. In conclusion, all four unprotected methacrylate nucleobase monomers were successfully synthesized. Thymine, adenine, and cytosine monomers could be obtained via direct alkylation of the unprotected free nucleobase with 3-bromopropyl methacrylate **1**. Guanine monomer **5** was obtained after several protection and deprotection steps in an overall yield of 24%. Conveniently, DMSO-d_6 was used as a solvent to enable monitoring of kinetics using ^{1}H NMR spectroscopy. Complex formation of guanine and cytosine monomer with copper(II) could be observed by NMR analysis, and in the case of cytosine, the use of PMDETA as a ligand and addition of 10 mol % copper(II) was necessary to obtain a sufficiently active catalytic system that allowed control over the polymerization. Although all four unprotected nucleobase monomers were polymerized in a controlled fashion using ATRP, there still is room for some improvement to increase the level of control that could possibly be achieved by performing polymerizations in the presence of the complementary nucleobase in order to prevent interactions with the Cu catalyst. Because all four nucleobase monomers can now be polymerized, exploitation of the specific and complementary nature of base pairing for template polymerizations or supramolecular polymers comes within reach.

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