Hydrogen Bond Template-Directed Polymerization of Protected 5′-Acryloylnucleosides

Afzal Khan, David M. Haddleton, Michael J. Hannon, Dax Kukulj, and Andrew Marsh*

Department of Chemistry, University of Warwick, Coventry, CV4 7AL, UK

Received February 25, 1999; Revised Manuscript Received July 14, 1999

ABSTRACT: Poly(5′-acryloyluridine) has been shown to act as a template in the radical polymerization of the complementary 5′-acryloyladenosine in the presence of the noncomplementary 5′-acryloyluridine. The template was synthesized by polymerizing 5′-acryloyluridine using AIBN as the initiator. The 5′-acryloylnucleosides were synthesized by regiospecific acylation of adenosine and uridine at the 5′-position using the enzyme *Candida antarctica lipase* 435 (CAL 435). The remaining hydroxyl groups were protected as silyl ethers to increase the solubility in nonpolar solvents.

Introduction

Template polymerizations play a pivotal role in nature for the synthesis of biopolymers such as DNA, RNA, and proteins.[1] In the case of nucleic acids, such templated polymerizations occur with utmost precision at the molecular level such that the template and daughter polymer have an exactly complementary sequence and length. In contrast, synthetic polymers are, usually, synthesized in a much less controlled fashion. Control over molecular mass distribution and degree of polymerization is usually achieved with some kind of anionic, cationic, or ring-opening living polymerization. Polymer topology can be altered by an increasing number of strategies to form graft, star, comb, etc., copolymers. One factor that needs to be considered in the chemistry that brings about this selective synthesis is the potential reaction of the active growing species with functionality within the monomer/polymer or solvent. Free radical polymerizations are less susceptible to these types of side reactions than are ionic, coordination polymerizations.[2−6] The concept of polymerizing monomers that have been templated to a preformed oligomer using hydrogen bonding interactions is an elegant route into controlling the sequence of monomer units along a polymer chain.[7−10] Inaki and co-workers have carried out template polymerizations of methacrylamide-substituted nucleoside bases in a mixture of DMSO/ethylene glycol (Figure 1).[7−9] Jones and co-workers have carried out the radical polymerization of 5′-acryloylnucleosides in aqueous solutions.[11,12] They also showed that these polymers interact with short strands of DNA; the polymers were not, however, used for template-directed synthesis. Interactions between complementary bases has also been utilized in HPLC for the separation of oligonucleotides on nucleoside bonded silica gel.[8,13,14]

This current work describes the polymerization of the silyl protected 5′-acryloyluridine (**1**, Figure 2) and 5′-acryloyladenosine (**2**) in the relatively nonpolar solvents ethyl acetate/toluene (2:1). Polymerization in nonpolar solvents allows stronger hydrogen bonding interactions between the template and monomer, hence more faithful transfer of sequence from parent to daughter polymer. Inclusion of the ribose moiety provides a useful handle for derivatization with nonpolar groups to aid solubility.

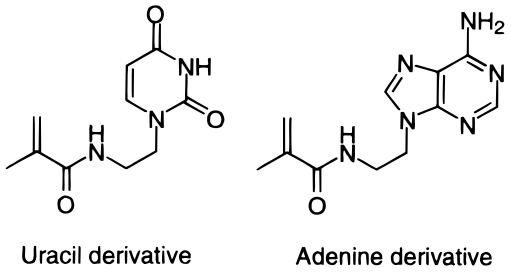


Figure 1. Monomers used in the study by Inaki and co-workers.[7−9]

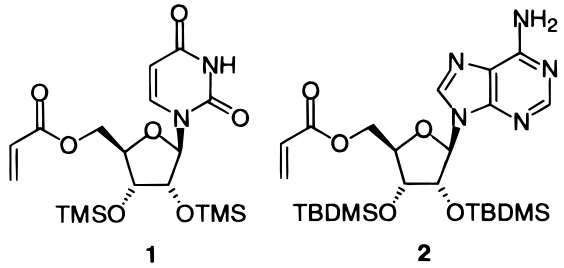


Figure 2. Monomers used in this investigation.

Experimental Section

General Information. All the reactions were carried out under an atmosphere of nitrogen. The reagents were purchased from the following sources: acryloyl chloride (Aldrich, 96%), acetone oxime (Lancaster, 98%), adenosine (Lancaster, 99%), uridine (Lancaster, 99%), pyridine (Fisons, 99.5%), TMS−Cl (Lancaster, 98+%), TBDMS−Cl (Lancaster, 97%), silver nitrate (Lancaster, 99+%), dimethylformamide (Aldrich, anhydrous, 99.8%), AIBN (BDH, 97%). All solvents were purchased from BDH and were used as supplied. Column chromatography refers to flash chromatography on Merck silica gel (Art. No. 109385). TLC was carried out on precoated plates (silica gel 60 F254, Merck 5715), and the products were visualized using UV light or potassium permanganate dip.

Polymer Analysis. Molecular weight distributions were measured using size exclusion chromatography (SEC), on a system equipped with a guard column, two 30 cm mixed D columns (Polymer Laboratories), and a differential refractive index detector, using tetrahydrofuran at 1 mL min^{-1} as eluent.

* Corresponding author. E-mail a.marsh@warwick.ac.uk; fax 44-(0)1203 524112.

10.1021/ma990283j CCC: $18.00
Published on Web 09/18/1999

The SEC was calibrated with 12 poly(MMA) standards in the range 6.85×10^5 to 200 g mol^{-1}. DSC was carried out on a Perkin-Elmer Pyris 1 differential scanning calorimeter, and the T_g's quoted refer to inflection midpoints. The NMR of the polymers were run on a Bruker 400 MHz spectrometer; the remaining compounds were carried out on a Bruker 250 MHz spectrometer.

Synthesis of Acryloylacetone Oxime. Synthesis of acryloylacetone oxime using the reported procedure[15] gave substantial amounts of the 1,4-conjugate addition product. By using a two-phase (dichloromethane−water) solvent system, this side product could be eliminated. To a solution of acryloyl chloride (20.0 g, 0.22 mol) in dichloromethane (130 mL), cooled to 0 °C, was added dropwise a solution of acetone oxime (15.93 g, 0.22 mol) in water (130 mL). When the addition was complete, the reaction mixture was stirred at room temperature for 1 h and the layers were separated. The aqueous phase was extracted with more dichloromethane (2 × 50 mL), and the combined organic extracts were washed with saturated sodium hydrogen carbonate (2 × 50 mL) and then water (50 mL). The solvent was evaporated in vacuo to give the crude product (20.7 g, 74%) which could be purified further by column chromatography (50% ethyl acetate in 40−60 °C petroleum ether), but this was not found to be necessary. CAUTION! Attempted distillation under reduced pressure as per ref 15 resulted in a dangerous explosion. ^{1}H NMR ($CDCl_3$): δ 6.82 (1H, dd, J = 17.1 Hz, J = 1.2 Hz), 6.51 (1H, dd, J = 17.1 Hz, J = 10.4 Hz), 6.22 (1H, dd, J = 1.2 Hz, J = 10.4 Hz), 2.38 (3H, s), 2.34 (3H, s).

Synthesis of 5′-Acryloyladenosine (General Procedure). To a suspension of adenosine (2.0 g, 7.48 mmol) in dioxane (150 mL) was added a catalytic amount of 2,6-di-*tert*-butyl-4-methylphenol (radical inhibitor), acryloylacetone oxime (2.85 g, 22.5 mmol), and CAL 435 (10 g). The reaction mixture was then stirred at 60 °C for 18 h, the enzyme filtered, and silica gel (4.50 g) added to the filtrate. The solvent was evaporated in vacuo (60−70 °C) and the solid residue purified by column chromatography (10% methanol in ethyl acetate) by dry loading to give the product as a white solid (1.04 g, 42%); mp 70−74 °C (decomposition), lit.[11] mp 72−76 °C (decomposition). ^{1}H NMR (d_6-DMSO): δ 8.31 (1H, s), 8.15 (1H, s), 7.32 (2H, NH_2, bs), 6.34 (1H, dd, J = 17.4 Hz, J = 1.8 Hz), 6.18 (1H, dd, J = 17.4 Hz, J = 10.1 Hz), 6.10−5.88 (2H, m), 5.60 (1H, OH, d, J = 5.8 Hz), 5.41 (1H, OH, d, J = 5.50 Hz), 4.54 (1H, q, J = 5.2 Hz), 4.50−4.05 (4H, m); ^{13}C NMR (d_6-DMSO): δ 165.6, 156.4, 153.0, 149.7, 140.1, 132.3, 128.3, 119.5, 88.1, 81.8, 73.2, 70.6, 64.4.

Synthesis of 5′-Acryloyluridine. Using the general procedure with uridine (5.0 g, 20.5 mmol), dioxane (350 mL), acryloylacetone oxime, CAL 435 (10.0 g), silica gel (9.50 g), and stirring for 3 days. The product was purified by column chromatography (4% methanol in ethyl acetate) to give the purified 5′-acryloyluridine as a white solid (3.94 g, 65%); mp 129−131 °C, lit.[12] mp 130−132 °C. ^{1}H NMR (d_6-DMSO): δ 11.36 (1H, NH, s), 7.61 (1H, d, J = 8.1 Hz), 6.39 (1H, dd, J = 17.2 Hz, J = 2.0 Hz), 6.21 (1H, dd, J = 17.2 Hz, J = 9.9 Hz), 5.98 (1H, dd, J = 9.9 Hz, J = 2.0 Hz), 5.75 (1H, d, J = 4.7 Hz), 5.65 (1H, d, J = 8.1 Hz), 5.50 (1H, OH, d, J = 5.5 Hz), 5.33 (1H, OH, d, J = 5.5 Hz), 4.45−4.20 (2H, m), 4.15−3.90 (3H, m). ^{13}C NMR (d_6-DMSO): δ 165.6, 163.4, 150.9, 141.1, 132.5, 128.3, 102.4, 89.1, 81.3, 73.0, 70.1, 64.2.

Trimethylsilyl Protection of 5′-Acryloyluridine. To a solution of 5′-acryloyluridine (0.2 g, 0.67 mmol) in dioxane (10 mL) was added pyridine (0.24 g, 2.98 mmol) followed by dropwise addition of trimethylsilyl chloride (0.18 g, 1.56 mmol). The reaction mixture was heated at 60 °C for 1 h and then treated dropwise with more trimethylsilyl chloride (0.18 g, 1.56 mmol) while heating at 60 °C. When the addition was complete, the reaction mixture was heated at 60 °C for a further 3.5 h to give a white precipitate. The dioxane was evaporated in vacuo (∼30 °C) and the residue treated with ether (15 mL) and water (35 mL). The layers were separated, the aqueous phase was extracted with more ether (2 × 10 mL), and the combined organic extracts were dried ($MgSO_4$) and evaporated in vacuo. The resulting orange oil was purified by column chromatography (25% ethyl acetate in 40−60 °C petroleum ether) to give the product as a colorless oil (**1**, 241 mg, 81%) which solidified on standing; mp 92−95 °C. ^{1}H NMR ($CDCl_3$): δ 10.30 (1H, NH, bs), 7.59 (1H, d, J = 8.2 Hz), 6.42 (1H, dd, J = 17.1 Hz, J = 1.5 Hz), 6.08 (1H, dd, J = 17.1 Hz, J = 10.4 Hz), 5.87 (1H, dd, J = 10.4 Hz, J = 1.5 Hz), 5.65 (1H, dd, J = 8.2 Hz, J = 1.5 Hz), 5.61 (1H, d, J = 2.4 Hz), 4.45 (1H, dd, J = 12.2 Hz, J = 2.4 Hz), 4.32−4.10 (3H, m), 3.94 (1H, dd, J = 6.7 Hz, J = 4.3 Hz), 0.10 (9H, s), 0.06 (9H, s). ^{13}C NMR ($CDCl_3$): δ 165.2, 163.9, 150.1, 139.6, 131.9, 127.3, 101.8, 91.1, 80.6, 75.3, 69.9, 62.2, 0.00 (3C), −0.15 (3C). IR, ν_{max} (solid): 3368, 2955, 1683, 1464, 1407, 1250, 1164 cm^{-1}. $[\alpha]_D$ 87.3°, c 1.0 ($CHCl_3$). HRMS (EI) calcd for $C_{18}H_{31}N_2O_7Si_2$ (MH^+): 443.1670. Found: 443.1658.

TBDMS Protection of 5′-Acryloyladenosine. To a solution of 5′-acryloyladenosine (1.04 g, 3.24 mmol) in DMF (4.0 mL) was added silver nitrate (1.65 g, 9.71 mmol), and the solution was stirred until it dissolved (∼5 min). To this clear solution was added TBDMS−Cl (1.66 g, 9.71 mmol), and the reaction mixture was stirred for 40 min to give a gray/blue precipitate. To this was added pyridine (1.04 g, 12.95 mmol), and the stirring continued for 5 min. Water (30 mL) and dichloromethane (100 mL) were then added, and the two-phase mixture was filtered. The organic phase was washed with water (4 × 30 mL), then dried ($MgSO_4$), and evaporated in vacuo to give an orange oil. This was purified by column chromatography (50% ethyl acetate in 40−60 °C petroleum ether) to give the product as a white solid (**2**, 1.23 g, 72%); mp 140−141 °C. ^{1}H NMR ($CDCl_3$): δ 8.29 (1H, s), 7.95 (1H, s), 6.41 (1H, dd, J = 17.1 Hz, J = 1.5 Hz), 6.28 (2H, NH_2, bs), 6.11 (1H, dd, J = 17.1 Hz, J = 10.4 Hz), 5.95−5.75 (2H, m), 4.89 (1H, t, J = 4.0 Hz), 4.58 (1H, dd, J = 12.2 Hz, J = 4.0 Hz), 4.50−4.10 (3H, m), 0.89 (3H, s), 0.81 (9H, s), 0.058 (3H, s), 0.042 (3H, s), −0.020 (3H, s), −0.16 (3H, s). ^{13}C NMR ($CDCl_3$): δ 166.1, 156.2, 153.3, 149.9, 140.1, 132.1, 128.1, 120.8, 90.1, 82.3, 74.9, 72.3, 63.5, 26.17 (3C), 26.09 (3C), 18.4, 18.3, −4.02, −4.35, − 4.50, −4.58. IR, ν_{max} (solid): 3108, 2930, 2857, 1653, 1472, 1250 cm^{-1}. $[\alpha]_D$ −59.2°, c 1.0 ($CHCl_3$). Anal. Calcd for $C_{25}H_{43}N_5O_5Si_2$: C, 54.62; H, 7.89; N, 12.75. Found: C, 54.34; H, 7.90; N, 12.54.

Homopolymerization of 2 (General Procedure). A solution of AIBN (0.4 mL of a 50 mg in 4.0 mL methanol solution) was placed in a Schlenk tube, and the solvent was removed under vacuum. To this was added **2** (0.50 g, 0.91 mmol), ethyl acetate (2.0 mL), and toluene (1.0 mL). The reaction mixture was degassed three times using the freeze−pump−thaw cycle and then heated at 60 °C for 52.5 h. The solvent was removed in vacuo and the solid washed with dichloromethane/petroleum ether (4:1) to give the pure polymer (**4**, 355 mg, 71%) which was dried at 60 °C under vacuum; T_g 141.4 °C. ^{1}H NMR (d_6-DMSO): δ 8.50−7.80 (2H, m), 7.50−6.90 (2H, NH_2, bs), 6.10−5.70 (1H, bs), 5.20−4.80 (1H, bs), 4.70−3.80 (4H, m), 1.50−0.50) (32H, m). $[\alpha]_D$ 99.4°, c 1.0 (DMSO).

Homopolymerization of 1. Using the general procedure with **1** (0.5 g, 1.13 mmol), AIBN (0.4 mL of a 50 mg in 4.0 mL of methanol solution), ethyl acetate (2.0 mL), toluene (1.0 mL), and heating for 52.5 h gave **3** (450 mg, 90%); T_g 142.0 °C. ^{1}H NMR (d_6-DMSO): δ 11.32 (1H, s), 7.59 (1H, s), 6.00−5.30 (2H, m), 4.60−3.35 (4H, m), 0.40-(−0.40) (20H, m). $[\alpha]_D$ 34.6°, c 1.0 (DMSO).

Statistical Copolymerization of 1 and 2. Using the general procedure with **1** (100 mg, 0.226 mmol), **2** (124 mg, 0.226 mmol), AIBN (0.179 mL of a 25 mg in 2.0 mL of methanol solution), ethyl acetate (1.0 mL), toluene (0.5 mL), and heating for 15.5 h gave the copolymer (67 mg, 67%); T_g 157.7 °C. ^{1}H NMR (d_6-DMSO): δ 11.43 (NH, bs), 8.50−8.00 (2H, m), 8.30−7.40 (1H, bs), 6.10−5.55 (3H, m), 4.70 (10H, m), 1.00-(−0.35) (18H, m), −0.35-(−0.70) (34H, m). $[\alpha]_D$ −58.0°, c 1.0 (DMSO).

Run 1: Templated Polymerization of Monomers 1 and 2 with Template 3. Using the general procedure with **3** (100 mg, 0.226 mmol), **1** (100 mg, 0.226 mmol), **2** (124 mg, 0.226 mmol), AIBN (0.382 mL of a 25 mg, in 2.0 mL of methanol solution), ethyl acetate (4.0 mL), and toluene (2.0 mL). Heated for 21 h, precipitate filtered, and washed with ethyl acetate/

toluene (2:1) to give the pure complex (181 mg, 81%) which was shown by ^{1}H NMR to be a 56:44 ratio of uridine to adenosine; T_g 157.4 °C. ^{1}H NMR (d_6-DMSO): δ 11.43 (NH, bs), 8.50−8.00 (2H, m), 8.30−7.40 (1H, bs), 6.10−5.55 (3H, m), 4.70 (10H, m), 1.00-(−0.35) (18H, m), 0.35-(−0.70) (34H, m). $[\alpha]_D$ −18.0°, c 1.0 (DMSO).

Run 2: Repeated Templated Polymerization of Monomers 1 and 2 in the Presence of Template 3. Using the general procedure with **3** (100 mg, 0.226 mmol), **1** (100 mg, 0.226 mmol), **2** (124 mg, 0.226 mmol), AIBN (0.382 mL of a 25 mg in 2.0 mL methanol solution), ethyl acetate (4.0 mL), and toluene (2.0 mL). Heated for 16 h, precipitate filtered, and washed with ethyl acetate/toluene (2:1) to give the pure complex as a white solid (180 mg, 80%). ^{1}H NMR of this complex showed a 53:47 ratio of uridine to adenosine. ^{1}H NMR (d_6-DMSO): δ 11.43 (NH, bs), 8.50−8.00 (2H, m), 8.30−7.40 (1H, bs), 6.10−5.55 (3H, m), 4.70 (10H, m), 1.00-(−0.35) (18H, m), 0.35-(−0.70) (34H, m); T_g 165.7 °C. The filtrate was evaporated, dissolved in dichloromethane, and then precipitated by pouring into petroleum ether, 40−60 °C. The precipitate was filtered and washed with dichloromethane/petroleum ether (1:1) to give a white solid (92 mg, 92%). ^{1}H NMR of this polymer showed a 93:7 ratio of uridine to adenosine, as expected the major polymer was due to the nontemplated polymerization of the uridine monomer **1**.

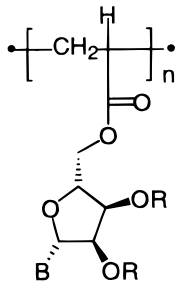
Run 3: Templated Polymerization of 1.2 equiv of Monomers 1 and 2 in the Presence of 1 equiv of the Template 3. Using the general procedure with **3** (50 mg, 0.113 mmol), **1** (60 mg, 0.136 mmol), **2** (74.5 mg, 0.136 mmol), AIBN (0.229 mL of a 25 mg in 2.0 mL of methanol solution), ethyl acetate (2.0 mL), and toluene (1.0 mL). Heated for 18.5 h, precipitate filtered, and washed with ethyl acetate/toluene (2:1) to give the pure complex (102 mg, 91%) as a white solid. ^{1}H NMR of this complex showed a 52:48 ratio of uridine to adenosine. ^{1}H NMR (d_6-DMSO): δ 11.43 (NH, bs), 8.50−8.00 (2H, m), 8.30−7.40 (1H, bs), 6.10−5.55 (3H, m), 4.70 (10H, m), 1.00-(−0.35) (18H, m), 0.35-(−0.70) (34H, m); T_g 161.7 °C.

Separation of Poly(5′-acryloyluridine)−Poly(5′-acryloyladenosine) Complex. To the polymer−polymer complex (50 mg, 53:47 ratio of uridine:adenosine) from the templated polymerization reaction (run 2) was added 0.5 M sodium hydroxide (8.0 mL), and the suspension was stirred for 70 h. The white solid was filtered, washed with water, and dried in a vacuum desiccator to give a white solid (24 mg, 87%). ^{1}H NMR of this solid showed a 94:6 ratio of adenosine to uridine. Neutralization of the filtrate with 2 M hydrochloric acid gave only a small amount of precipitate (∼2.0 mg).

TMS Deprotection of Poly(5′-acryloyluridine) 3. Homopolymer **3** (120 mg) was dissolved in 0.5 M sodium hydroxide (7 mL), and the clear solution was stirred for 10 min. It was then neutralized by dropwise addition of 2 M hydrochloric acid to give a white precipitate which was then filtered and washed with water. The product was dried in a vacuum desiccator to give the deprotected homopolymer **5** as a white solid (80 mg, 99%); ^{1}H NMR (d_6-DMSO): d 11.26 (NH, s), 7.54 (1H, s), 5.74 (1H, s), 5.66 (1H, s), 5.46 (OH, s), 5.14 (OH, s), 3.50−4.50 (5H, m), 2.70−2.00 (1H, bs), 2.00−1.00 (2H, bs); T_g 170.7 °C.

Results and Discussion

It was possible to carry out acrylation of adenosine and uridine regiospecifically using a modified procedure of Moris and Gotor (Scheme 1).[15] This involved using a large excess of the readily available enzyme *Candida antarctica lipase* 435 (CAL 435) in dilute solutions of dioxane. Using this method, we have also been able to carry out regiospecific acrylations of thymidine and cytidine, without prior protection of the amine group, using acryloylacetone oxime. The acryloylacetone oxime was prepared using a modification of a literature procedure.[15] Considerable difficulty was encountered when we followed the literature method, the major product being 1,4-conjugate addition of acetone oxime



3: R = $SiMe_3$, B = Uracil
4: R = $SiMe_2{}^tBu$, B = Adenine
5: R = H, B = Uracil

Figure 3.

Scheme 1. Synthesis of Monomers

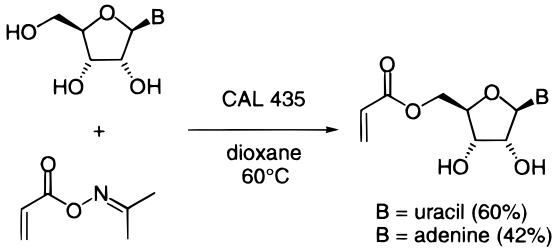


Table 1. Polymerization Data for the Homo- and Copolymerizations of 1 and 2

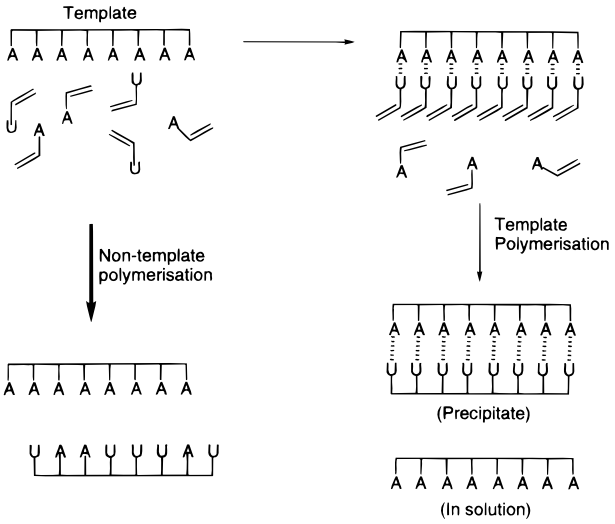
monomer	AIBN (wt %)	yield (%)	M_w	M_n	PDI
1	0.16	82	65 400	18 800	3.50
1	1.00	90	33 700	10 700	3.14
2	1.00	71	15 300	5 200	2.94
2	10.0	65	7 400	3 400	2.13
1/2[a]	1.00	67	41 900	26 100	1.60
1/2[a]	2.13	66	15 200	6 700	2.26

[a] 50 mol % **1** and **2**.

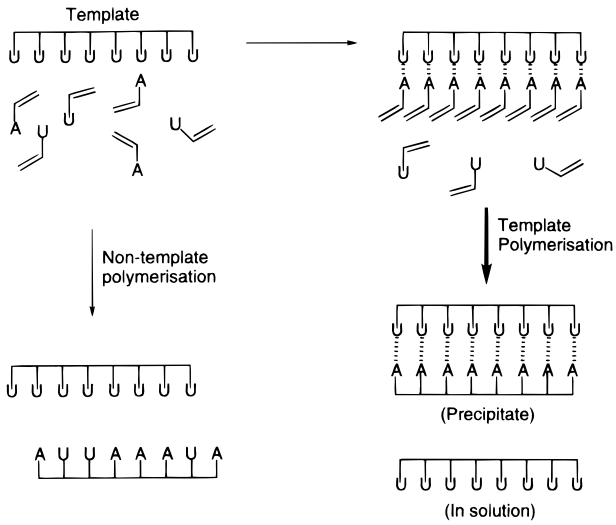
to the double bond of the acrylate. This problem was solved by using a two-phase dichloromethane−water solvent system, thus keeping the excess acetone oxime away from the organic phase where reaction takes place. The 5′-acryloyluridine was protected with trimethylsilyl chloride in dioxane in the presence of pyridine base (**1**, 81%). Protection of the 5′-acryloyladenosine proved problematic; the reaction failed with both trimethylsilyl chloride and *tert*-butyldimethylsilyl chloride (TBDMS−Cl). However, it was discovered that the protection could be achieved using silver nitrate[16,17] with TBDMS−Cl (**2**, 72%). ^{1}H NMR of a mixture of the protected nucleosides (**1:2** = 1.24:1) in $CDCl_3$ showed a change of chemical shift of the uridine NH proton from δ 8.84 to δ 10.83. This shift of about 2 ppm clearly shows hydrogen-bonding interactions between these complementary nucleosides. It is known from infrared studies of model nucleosides[18] that these interactions can persist at temperatures of up to 58 °C. We thus felt optimistic about seeing some kind of interaction for the templated polymerizations.

The homo- and statistical copolymerizations of **1** and **2** were carried out at 60 °C in a mixture of ethyl acetate/toluene (2:1) using azobis(isobutyronitrile) (AIBN) as the initiator (Figure 3). Prior to heating, the reaction mixture was deoxygenated by three freeze−pump−thaw cycles. Table 1 shows results from these polymerization reactions. The molecular masses were determined by size-exclusion chromatography (SEC) with THF eluent with toluene as a flow rate marker. As expected, the

Scheme 2



Scheme 3



molecular masses of the polymers was dependent on the amount of initiator used; the higher the amount of initiator, the lower the molecular mass. The polydispersities are relatively broad as would be expected under these reaction conditions. Because of the lack of solubility in appropriate solvents, the circular dichroism (CD) spectra, and hence the secondary structure, of the polymer chains could not be determined.

The homopolymers thus produced were subsequently used as templates for the polymerization of the complementary monomers. Polymerization of **1** in the presence of polymer **4** resulted in the formation of a white precipitate; ^{1}H NMR showed a 1:1 ratio of adenosine to uridine units. The formation of this complex may be due to either a template effect; alternatively, the uridine monomer may have polymerized in solution *prior* to complexation with the template to produce this precipitate. To demonstrate that a template effect is occurring, polymer **4** (1 mol equiv) was used as a template for both monomer **1** (1 mol equiv) and monomer **2** (1 mol equiv, Scheme 2).[7] If **4** acts as a template for the complementary monomer **2**, then polymerization should give a polymer−polymer complex containing a 1:1 ratio of adenosine to uridine. If no template effect occurs, then ^{1}H NMR should show a 2:1 ratio of adenosine to uridine. On polymerization a precipitate was again formed; ^{1}H NMR showed an adenosine-to-uridine ratio of approximately 2:1. Thus, the precipitate is the complex formed between the template and the statistical copolymer indicating a *non*templated polymerization.

It has been previously shown that polymers containing the adenine base are prone to intramolecular hydrogen-bonding interactions.[9] This would make intermolecular interactions with the complementary monomer difficult and thus **4** a poor template. Inaki and coworkers resorted to acidic conditions to extend polymers of adenine by electrostatic repulsions.[19] To overcome this detrimental effect, polymer **3** was used as the template, as it is less prone to intramolecular association. Equimolar amounts of **1** and **2** were polymerized in the presence of the template **3** (1 mol equiv). ^{1}H NMR of the precipitated product showed a 56:44 ratio of uridine to adenosine side groups. Thus, **3** is indeed acting as a template for polymerization of **2** (Scheme 3). Repeating this experiment under identical conditions gave a precipitated product with a 53:47 ratio of uridine to adenosine side groups. The remaining filtrate was evaporated, dissolved in dichloromethane, and then precipitated by pouring into petroleum ether and after filtration and washing gave material that was demonstrated by ^{1}H NMR to be a 93:7 ratio of uridine to adenosine side chains. As expected, the major polymer from this fraction was due to off-template polymerization of the uridine monomer **1**. The presence of a small amount of adenosine could be due to either some nontemplated copolymerization occurring between **1** and **2** in solution or some low molecular weight complex being washed into the filtrate.

Treatment of the initially precipitated polymer complex with 0.5 M sodium hydroxide solution resulted in dissolution of ca. half the material. Filtration gave a white solid that was shown by NMR to be a 94:6 ratio of adenosine to uridine. This is excellent confirmation that the polymerization in the presence of **3** is indeed a templated process with a high fidelity of transcription from parent to daughter polymer. A nontemplated polymerization would have given a 1:1 ratio of adenosine to uridine in the separated polymer. Neutralization of the filtrate with 2 M hydrochloric acid gave only a small amount of precipitate. In a separate experiment it was found that exposure of homopolymer **3** to 0.5 M sodium hydroxide solution for 10 min followed by neutralization with 2 M hydrochloric acid gave a white precipitate which was found to be the TMS-deprotected polymer **5** (Figure 3). Since this precipitate was not observed in the polymer decomplexation experiment above, it is assumed that prolonged exposure of **3** to base leads to saponification of the ester side groups. This was confirmed by stirring homopolymer **3** overnight with sodium hydroxide solution which lead to only a small amount of precipitate upon neutralization with hydrochloric acid. This separation of the polymer chains is a clear demonstration of the formation of a discrete **3**:**4** polymer complex rather than the formation of a statistical copolymer from **1** and **2**.

The templated polymerization was repeated in the presence of 1.2 equiv of each acryloylnucleoside **1** and **2**. This gave a 52:48 ratio of uridine to adenosine, indicating that a slight excess of monomer might enable the complementary acryloylnucleoside to compete for places on the template more efficiently. Repeating the polymerization using 2 mol equiv of the noncomplementary **1** gave a product with a 3:2 ratio of uridine to adenosine functionality. The template effect still oper-

ates, but if more than 1 equiv of the noncomplementary monomer is present, competition with the complementary monomer for the template sites occurs.

Differential scanning calorimetry (DSC) of the two homopolymers and the polymer−polymer complex shows a marked difference in the glass transition temperatures (T_g). Polymers **3** and **4** showed T_g's of 142 and 141 °C, respectively, whereas the complex of these polymers from the templated polymerization (run 1) shows a T_g of 157 °C. An equimolar mixture of polymers **3** and **4** was heated at 60 °C in ethyl acetate/toluene (2:1) for 18 h; the DSC of the resulting polymer had a T_g of 153.0 °C. The slightly higher T_g in the former complex is due to better matching of the base pairs than is achieved by simply mixing the polymers.[7] The difference in T_g's between runs 1−3 can be attributed to the differences in batches of template polymer **3** used for the polymerization experiments.

Polymerizations were initiated by UV irradiation and AIBN initiation. It was envisaged that templated polymerization should be more favorable with UV initiation as elevated temperatures can disrupt weak monomer−polymer hydrogen bonds. However, it was found that temperature had very little effect on the product. Equimolar amounts of **1** and **2** were polymerized in the presence of template **3** (1 mol equiv) using UV initiation (30−35 °C). This resulted in a near identical 55:45 ratio of adenosine to uridine.

Polymerizations were also carried out in polar solvents for comparison with the above nonpolar solvent system. The unprotected 5′-acryloyluridine and 5′-acryloyladenosine were polymerized in aqueous solution using a water-soluble initiator, azobis(isocyanovaleric acid) at 60 °C. The resulting polymers were found to precipitate from solution and were insoluble in almost all solvents, except DMSO in which they were only sparingly soluble. The templated polymerizations, carried out in DMSO, gave precipitates that could not be analyzed due to their insolubility in normal solvents.

In summary, it has been shown that **3** can act as a template for the polymerization of the complementary monomer **2** in the presence of the noncomplementary monomer **1**. Because of intramolecular association, **4** did not template polymerization of **1**. The polymerizations could be initiated using either heat or UV radiation. Rate studies in the presence and absence of the templates are being investigated.

Acknowledgment. We are grateful to the University of Warwick for a postdoctoral fellowship (A.K.) and EPSRC (D.K., GR/L10314) for funding and Novo Nordisk for a generous donation of CAL 435. We thank the referees for helpful comments and suggestions.

References and Notes

(1) Rothwell, N. V. In *Understanding Genetics: A Molecular Approach*; Wiley-Liss: New York, 1993; Chapters 11 and 12.
(2) Matyjaszewski, K. *Curr. Trends Solid State Mater. Sci.* **1996**, *1*, 769.
(3) Sawamoto, M.; Kamigaito, M. *Trends Polym. Sci.* **1996**, *4*, 371.
(4) Hawker, C. J. *Trends Polym. Sci.* **1996**, *4*, 183.
(5) Georges, M. K.; Veregin, R. P. N.; Kazmaier, P. M.; Hamer, G. K. *Trends Polym. Sci.* **1994**, *4*, 66.
(6) Davis, T. P.; Kukulj, D.; Haddleton, D. M.; Maloney, D. R. *Trends Polym. Sci.* **1995**, *3*, 365.
(7) Inaki, Y.; Ebisutani, K.; Takemoto, K. *J. Polym. Sci., Polym. Ed.* **1986**, *24*, 3249.
(8) Inaki, Y. *Prog. Polym. Sci.* **1992**, *17*, 515.
(9) Inaki, Y.; Takemoto, K. *Adv. Polym. Sci.* **1981**, *41*, 1.
(10) Challa, G.; Tan, Y. Y. *Pure Appl. Chem.* **1981**, *53*, 627.
(11) Cooper, M. J.; Goody, R. S.; Jones, A. S.; Tittensor, J. R.; Walker, R. T. *J. Chem. Soc. (C)* **1971**, 3183.
(12) Boulton, M. J.; Jones, A. S.; Walker, R. T. *J. Chem. Soc. (C)* **1968**, 1216.
(13) Inaki, Y.; Mutsukawa, K.; Wang, H.; Mochizuki, E.; Wada, T.; Takemoto, K. *Bunseki Kugaku* **1993**, *42*, 99.
(14) Inaki, Y.; Kawano, H.; Matsukawa, K.; Takemoto, K. *Abstr. Pap.—Am. Chem. Soc.* **1992**, *203*, 222.
(15) Moris, F.; Gotor, V. *J. Org. Chem.* **1993**, *58*, 653.
(16) Lalonde, M.; Chan, T. H. *Synthesis* **1985**, 817.
(17) Gundlach, C. W.; Glick, G. D. *Tetrahedron Lett.* **1997**, *38*, 4039.
(18) Kyogoku, Y.; Lord, R. C.; Rich, A. *J. Am. Chem. Soc.* **1967**, *89*, 496.
(19) Inaki, Y.; Kamo, S.; Miyata, M. *React. Funct. Polym.* **1998**, *37*, 189.

MA990283J