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ARTICLE TYPE

Preparation of 1:1 alternating, nucleobase-containing copolymers for use in sequence-controlled polymerization

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Reversible deactivation radical polymerisation (RDRP) has enabled chemists to generate polymers with precision control over molecular weight and molecular structure. Despite recent advances in the field of template polymerisation, control of the repeat unit sequence requires further development. We describe herein the synthesis of sequence-regulated copolymers of styrene-thymine and maleic anhydride.

DNA, is able to self-replicate with exact control over molecular weight, repeat unit sequence and the resultant helical architecture. During replication, the DNA double helix is unwound to provide two parent templates. DNA polymerase extends a short fragment of DNA by aligning complementary nucleobases along the parent template. Hydrogen bonding interactions between complementary pairs of nucleobases allow recognition of sequence resulting in the templating of a daughter strand, and therefore exact replication. DNA replication is rapid and highly accurate, with only one mistake per 10^7 nucleobases added to the daughter polymer.[1] Such control is essential for the synthesis of biomacromolecules such as DNA, RNA and proteins, which deliver a diverse and complex array of functions fundamental to the living organism. Currently, synthetic processes for polymerisation are unable to emulate nature's precision in the control of repeat unit structure. Overcoming this barrier would enable the synthesis of designed polymers, with varied applications of particular relevance to biomaterials.

Several groups have reported the synthesis of sequence controlled polymers through a variety of methods.[2] Solid phase synthesis of peptides and oligonucleotides, whereby each sequential peptide or nucleotide monomer is added in a step-by-step manner (polycondensation), is the most commonly used technique for the synthesis of sequence controlled polymers.[3] Control of sequence in chain polymerisations (proceeding via chain-initiation and chain-propagation) is far more challenging.[4] Polymers prepared by this mechanism have a statistical distribution of monomers in the polymer chain, which is determined by reactivity ratios and reagent concentrations.[5] One solution is to minimise the distance between a propagating chain and the desired monomer; or between the monomers themselves by taking advantage of covalent or non-covalent interactions. One such approach, inspired by nature's polymerisation of DNA is to use hydrogen-bonding interactions between complementary nucleobases. Lo and Sleiman[6] recently reported a nucleobase templated polymerisation using a parent thymine-containing block copolymer to hydrogen-bond template a daughter adenine-containing polymer via sequential Sonogashira coupling. This approach represents a polycondensation mechanism, however.

Recent advances in radical polymerisations have led to several reversible-deactivation radical polymerisations (RDRP)[7] that enable polymers of defined average molecular weight, molecular structure and 3D architecture to be synthesised. Reversible Addition-Fragmentation chain Transfer (RAFT) polymerisation is one such technique that relies upon the equilibrium between dormant and active polymer species via an activation/deactivation process.[8]

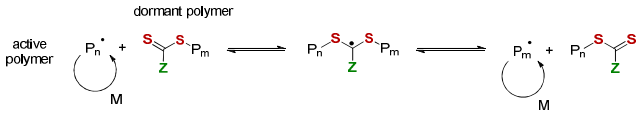


Figure 1: RAFT polymerisation equilibrium between active and dormant polymeric species.

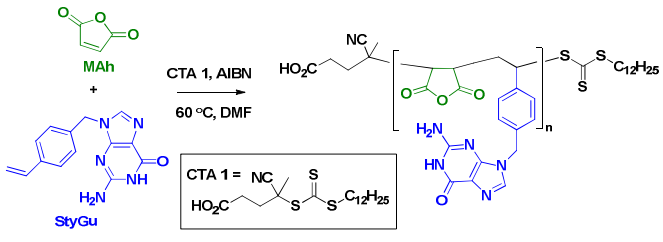
For our research, we required the synthesis of a well-defined nucleobase-containing polymer to act as a parent for templating a daughter polymer comprising the complementary nucleobase via hydrogen-bonding interactions.

The generation of 1:1 alternating copolymers through the adoption of a styrene-*co*-maleic anhydride system has been well studied.[9] The mechanism has been widely debated with evidence from NMR and FTIR spectroscopy showing that the two comonomers form charge transfer complexes (CTCs).[10] However, current opinion is that whilst CTCs are formed, monomers add as a single unit and the mechanism follows the penultimate unit model.[11] In order to further control the polymerisation, several groups have applied the RAFT technique to Sty-MAh copolymerizations to successfully control both the sequence and

the molecular weight distribution.[12] Davies and co-workers investigated the alternating properties of *para*-substituted styrenes with maleic anhydride and demonstrated controlled, alternating polymerisations for all substrates,[13] thus we were encouraged to investigate the copolymerisation of *para*-substituted styrene-nucleobase monomers with maleic anhydride. Herein we report the successful preparation of alternating copolymers of nucleobase-containing styrene monomers with maleic anhydride, analysed by ^{13}C DEPT NMR experiments.

Our initial investigations of the copolymerisation of 5-methyl-1-(4-vinylbenzyl)pyrimidine-2,4(1H,3H)-dione (Styrene-Thymine; StyThy) and maleic anhydride followed conditions reported by You and co-workers[14] for the RAFT copolymerisation of styrene and maleic anhydride at 22 °C in THF without initiator. It is known that the higher the polymerisation temperature, the more random the polymer sequence, thus employing these low temperatures should facilitate truly alternating behaviour. Due to the poor solubility of the StyThy monomer, the polymerisation was carried out in DMF at a 0.4 M concentration of StyThy with a targeted degree of polymerisation (DP) of 50. Under these low temperature conditions no polymer could be seen by ^{1}H NMR or GPC analysis after 4 hours. This might be attributed to slow initiation due to the high dilution conditions necessitated by low monomer solubility. However, subsequent addition of 2,2'-azobis(4-methoxy-2.4-dimethyl valeronitrile) initiator (V70) and heating to 35 °C for a further 24 hours failed to furnish desired polymer.

Attempts to copolymerise StyThy with MAh in presence of CTA **1** at 60 °C with AIBN in DMF also failed to provide any polymer. We attribute this to the incompatibility of DMF solvent to polymerisation of the StyThy monomer. Styrene guanine monomer (StyGu) gave only very low conversions (19%), but excellent dispersity for the polymer formed by copolymerisation with MAh under these conditions at 0.4 M monomer. (**Scheme 1; Table 1**).



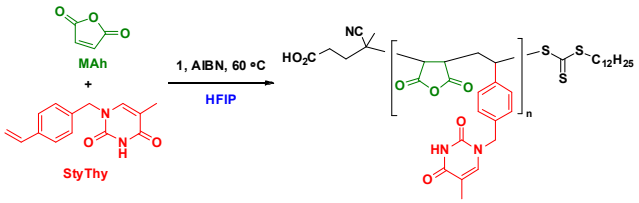
Scheme 1: Alternating copolymerisation of StyGu and MAh at 60 °C in DMF using thiocarbonylthio CTA **1**.

Table 1: Copolymerisation of StyGu and MAh in DMF

[M]/[CTA]	Conv. StyGu/%[a]	Calc. DP[b]	M_n[b] x10^{-3}	M_n[c] x10^{-3}	M_w[c] x10^{-3}	$Đ$
50	19	10	3.7	3.8	3.9	1.03

[a] Conversion based upon ^{1}H NMR data
[b] Calc. DP/M_n calculated from ^{1}H NMR conversions of StyGu monomer where M_n = M(RAFT) + (conv.*M(monomer)). All M_n and M_w values are kg/mol.
[c] M_n values from DMAc GPC data and are based upon PMMA calibration curves.

Hexafluoroisopropanol was subsequently chosen as a solvent for our styrene-nucleobase/MAh copolymer system, based on the success of Kamigaito et al. in the preparation of Limonene-alt-N-phenylmaleimide AAB sequence regulated copolymers.[15] Attempts to copolymerise StyGu and MAh in HFIP were thwarted by the insolubility of the guanine functionalised styrene in the fluorinated solvent, thus efforts focussed on the more soluble StyThy/MAh system.



Scheme 2: Copolymerisation of StyThy and MAh in HFIP with CTA **1** and AIBN at 60 °C.

A DP of 50 was targeted to provide a M_n of 17,000 at 100% monomer conversion. An initial monomer feed ratio of 1:1 was employed with [StyThy]/[MAh]/[CTA]/[AIBN] = 25/25/1/0.2. StyThy displayed much greater solubility in HFIP than in DMF and enabled a higher reaction concentration of 1.4 M StyThy – more than three times the concentration of StyThy employed in DMF solvent. After 2 h 15 minutes, the solution had become highly viscous. The reaction was stopped after 3 h at almost full monomer conversion (**Table 2**).

Table 2: Copolymerisation of StyThy and MAh in HFIP

[M]/[CTA]	Conv. StyThy/%[a]	Calc. DP[b]	M_n[b] x10^{-3}	M_n[c] x10^{-3}	M_w[c] x10^{-3}	$Đ$
50	91	46	15.7	22.3	27.5	1.23

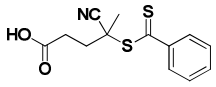
[a] Conversion based upon ^{1}H NMR data
[b] DP/M_n calculated from ^{1}H NMR conversions of StyThy monomer where M_n = M(RAFT) + (conv.*M(monomer)). All M_n and M_w values are kg/mol.
[c] M_n values from DMAc GPC data and are based upon PMMA calibration curves.

To understand the level of control imparted by CTA **1** in HFIP, DPs of 10, 25 and 100 were targeted. **Table 3** (entries A-C) details the excellent control of the RAFT agent in accessing the targeted M_n for each DP. Similar conditions were applied to these copolymerisations, with a lower StyThy concentration of 0.6 M to facilitate reaction sampling, and an initial monomer ratio of 1:1.

Table 3: Copolymerisation of StyThy and MAh, targeting DPs of 10, 25, 50 and 100 ([AIBN]$_0$/[CTA]$_0$ = 1:5)

	Time / h	CTA	[M]/[CTA]	Conv. StyThy/%[a]	Calc. DP[b]	M_n[b] x10^{-3}	M_n[c] x10^{-3}	M_w[c] x10^{-3}	$Đ$
A	2.5	1	10	73	7	2.4	5.4	6.2	1.15
B	16	1	25	100	25	8.5	13.5	14.8	1.10
C	16	1	100	88	88	30.0	33.8	40.0	1.18
D	16	1	50	97	49	16.7	21.9	24.5	1.12
E	16	2	50	72	36	12.3	11.5	12.5	1.09

[a] Conversion based upon ^{1}H NMR data
[b] Calc. DP/M_n calculated from ^{1}H NMR conversions of StyThy monomer where M_n = M(RAFT) + (conv.*M(monomer)). All M_n and M_w values are kg/mol.
[c] M_n values from DMAc GPC data and are based upon PMMA calibration curves.



CTA 2

The effectiveness of dithiobenzoate CTA **2** (4-cyano-4-(phenylcarbonothioylthio)pentanoic acid) was studied at a DP of 50, for comparison with CTA **1** which had proven very effective at promoting a living copolymerisation of thymine functionalised styrene with MAh. A concentration of 0.6 M StyThy was applied to the copolymerisation (**Table 3**, entry **E**). Both CTAs displayed end group fidelity, based on retention of colour within purified polymers and block extension with OEGMA (See ESI for experimental data), with good control over the copolymerisation at high conversion.[16]

The effect of concentration on the rate of conversion of monomer to polymer was also studied for the StyThy/MAh system. A targeted DP of 50 was applied at two different concentrations of StyThy (**Table 4**). At both concentrations, similar conversions of StyThy with time were noted, with the final copolymers displaying almost identical M_n values, therefore monomer conversion is independent of monomer concentration (See ESI for GPC data).

Table 4: Effect of concentration on copolymerisation of StyThy and MAh with CTA **1** in HFIP solvent: 1.2 M (**A**) and 0.6 M (**B**)

	Time / h	[M]/ [CTA]	Conv. StyThy/%[a]	Calc. DP[b]	M_n.[b] x10^{-3}	M_n[c] x10^{-3}	M_w[c] x10^{-3}	$Đ$
A		50						
	1		28	14	-	8.3	12.3	1.24
	6		97	49	16.7	23.9	32.8	1.38
B		50						
	1		21	11	-	7.8	8.7	1.12
	6		97	49	16.7	21.9	24.5	1.12

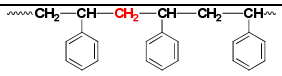
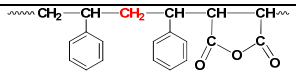
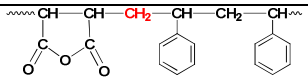
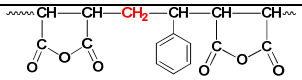
[a] Conversion based upon ^{1}H NMR data

[b] DP/M_n calculated from ^{1}H NMR conversions of StyThy monomer where M_n = M(RAFT) + (conv.*M(monomer)). All M_n and M_w values are kg/mol.

[c] M_n values from DMAc GPC data and are based upon PMMA calibration curves

In order to ascertain the sequence of these copolymers, NMR studies were conducted using ^{13}C DEPT NMR and HSQC experiments to assign styrene methylene signals in the polymer backbone, which would identify the nature of the triad sequence (See Supporting Information for experimental protocol and data). Barron and co-workers reported the use of ^{13}C DEPT NMR experiments to provide simplified subspectra of polymer CH and CH_2 groups to identify the triad environment of the methine and methylene groups in the polymer backbone of 1:1 alternating Sty/MAh copolymers.[17] The styrene methylene peak has definitive chemical shifts in a ^{13}C NMR spectrum, depending on the triad environment it is contained within. Barron assigned the triads SSS, SSM, MSS and MSM of copolymers of different compositions to characteristic chemical shifts in acetone d^6 spectra (**Table 5**).

Table 5: Triad sequences and their characteristic chemical shifts for the methylene carbon (red) of the styrene residue in Sty/MAh copolymers in acetone-d_6[17]

Triad	Structure	Chemical Shift /ppm
SSS		42-47
SSM		37-42
MSS		37-42
MSM		33-37

The StyThy/MAh copolymers were soluble in DMSO-d_6 rather than acetone-d_6, thus Sty and MAh were copolymerised in HFIP at 60 °C for 2.5 h to act as a guide for NMR experiments (M_n = 9,600, $Đ$ = 1.17, [StyThy]/[MAh]/[CTA]/[AIBN] = 25/25/1/0.2). HSQC experiments were conducted to assign polymer backbone carbons and identify signals due to the styrene methylene carbon of Sty/MAh copolymer in acetone and DMSO. The signals within the Sty/MAh copolymer in DMSO did not differ substantially from those recorded in acetone, neither did those from the StyThy/MAh copolymer, thus the characteristic methylene styrene chemical shifts reported by Barron could be applied to the StyThy/MAh copolymers in DMSO. As shown in **Figure 3**, the styrene methylene carbon shifts for Sty/MA and StyThy/MAh show a major broad resonance between 36.2 – 36.8 ppm in the DEPT CH_2-subspectrum (CH_3 and CH resonances are subtracted from this spectrum to clearly display only CH_2 resonances). This signifies the presence of the MSM triad within the polymer. For the StyThy/MAh copolymer, this is the only visible peak within the triad regions, representing a 1:1 alternating copolymer. The Sty/MAh copolymer, however, shows broad peaks between 42-44 ppm, signifying the presence of SSS triads, and 40-42 ppm for MSS/SSM triads. Further, when initial monomer feed ratios of StyThy:MAh at 1:1, 1:3 and 1:7 were used, the resulting polymers displayed the same MSM triads at 36-38 ppm in the CH_2 ^{13}C NMR DEPT spectrum (See ESI for experimental protocol and DEPT analysis).

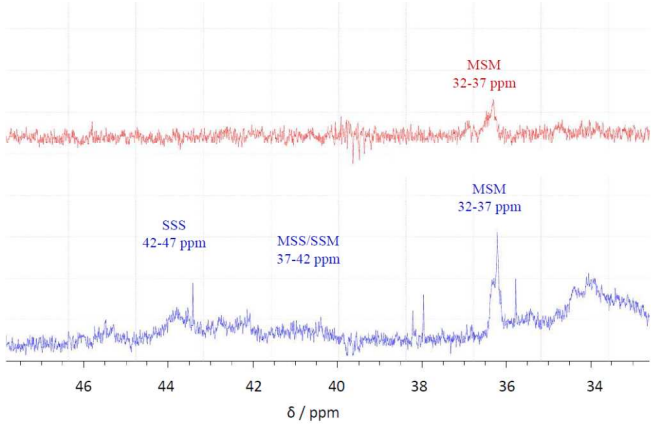


Figure 2: StyThy/MAh (red) and Sty/MAh (blue) copolymer DEPT CH_2 subspectra in DMSO showing characteristic sequence resonances for CH_2 methylene carbons of the polymer backbone.[14,17]

In conclusion, fully controlled, 1:1 alternating, nucleobase-containing polymers have been synthesised through the use of fluorinated solvent in the RAFT polymerisation process. These polymers exhibit low dispersities, with high conversion of the constituent monomers. Such sequence-controlled, nucleobase-containing polymers have potential in bioapplications, particularly when forming second order architectures. Current investigations into their use as templates for the synthesis of daughter polymers of the corresponding nucleobase are underway.

Notes and references

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† Electronic Supplementary Information (ESI) available: Experimental details for the synthesis of monomers, polymerisation conditions and NMR experimental details. See DOI: 10.1039/b000000x/

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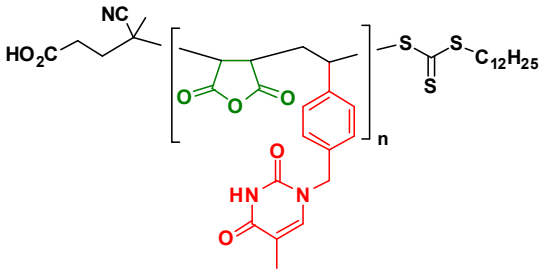
Graphical Abstract



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