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Water soluble triblock and pentablock poly(methacryloyl nucleosides) from copper-mediated living radical polymerisation using PEG macroinitiators

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ABSTRACT

The monomers methacryloyl uridine and methacryloyl adenosine have been polymerised under copper-mediated living radical conditions using poly(ethylene glycol) (PEG) macroinitiators to give triblock and pentablock copolymers of reasonable dispersity. The triblocks were used as initiators for the successful synthesis of pentablock copolymers. The secondary structures of the triblock and pentablock copolymers have been investigated using circular dichroism (CD) spectroscopy. The adenosine copolymers are found to exhibit more ordered secondary structures in water in comparison to the uridine derived polymers. New supramolecular structure is observed by CD upon mixing adenosine and uridine triblock copolymers in water. The polymers obtained are of interest as potential drug delivery agents through interaction with small molecules and oligonucelotides.

1. Introduction

There have been numerous accounts of biologically active polymers including peptides [1], nucleotides [2] and oligosaccharides [3] being bound to, or synthesised on polymeric support [4,5]. The conjugation of bio-relevant compounds to poly(ethylene glycol) (PEG; 'PEGylation') has attracted particular interest and found many applications [6], including enhancing circulatory lifetime and hence therapeutic potential [7]. Though much debated [8], PEG has remained one of the major polymer components for materials with biological applications [9–12]. This is mainly because of its advantageous properties, including solubility in both aqueous and organic solvents (except diethyl ether or isopropanol) [13], crystallinity of high molecular weight PEGs [14], and a remarkable ability to solubilise even intractable self-assembled structures [15]. It has been shown that PEG-poly(nucleoside) diblocks form supramolecular assemblies in aqueous solution based on interactions between complementary bases [16] and block copolymers containing adenine self-assemble in THF due to the hydrogen-bonding self-complementarity of the adenine units [17].

At the same time as the need for bio-relevant polymers has developed, interest in the preparation of well-defined polymers and copolymers by metal-mediated radical polymerisation has increased enormously since its discovery [18–21]. The synthesis of block copolymers with controlled block length requires efficient controlled/"living" polymerisation [22]. Particular attention has been directed towards water-soluble polymers [23], often using atom transfer radical polymerisation (ATRP) in a biphasic mixture [24], or homogeneous system [25]. In the early development of copper-mediated living radical polymerisations,

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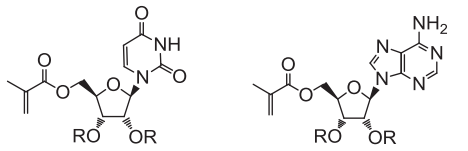


Fig. 1. Methacryloyl nucleoside monomers used for living radical polymerisation.

it was shown that narrow dispersities of water soluble polymers were difficult to obtain from conventional living polymerisation systems [23]. One of the problems is the need for a water miscible co-solvent [26] and although that solvent plays an important role, the nature of the catalytic species in the radical process is not always well understood [27]. Indeed, it has been shown that water can enhance the polymerisation rate significantly and also that ATRP is particularly effective for hydrophilic monomers under mild conditions, either in aqueous [28,29] or alcoholic media [30] leading to the possibility that many solvents or substrates may be expected themselves to produce new catalytically active species. More recently, we have demonstrated how disproportionation of aliphatic copper(I) complexes in water can lead to very effective polymerisation of water soluble acrylates and acrylamides [31,32] even in complex aqueous solvents/media [33].

We previously reported the copper-mediated living radical polymerisation of (un-)protected and methacryloyl uridine and methacryloyl adenosine (Fig. 1) in solution [34], and on a solid support [35]. Recently, there has been renewed interest in these types of polymers in order to use H-bonding to give sequence (and potentially stereo) control, single chain manipulation and controlled polymer folding using different controlled polymerization techniques [36], such as reversible addition-fragmentation chain transfer (RAFT) polymerization [37], nitroxide-mediated polymerization [38] and ATRP [39–41].

In this current work we use copper-mediated living radical polymerization to prepare oligonucleoside PEG copolymers that retain the ability to assemble into supramolecular structures. To this end, two different bromoisobutyroyl PEG macroinitiators were synthesized and examined as initiators for the synthesis of water-soluble triblock and pentablock copolymers. The methacryloyl uridine and adenosine-containing block copolymers were further investigated for their secondary structure in aqueous solution by UV and circular dichroism spectroscopy.[§]

2. Experimental

2.1. Materials

All reactions were carried out under an atmosphere of nitrogen using standard Schlenk techniques. Reagents were purchased from the following sources: 4,4′-azobis(4-cyanovaleric acid) (ACVA) (Acros, 97%), 2-pyridinecarboxaldehyde (Avocado, 99%), 2-bromo-2-methylpropionyl bromide (BIBB) (Aldrich, 98%), mPEG 5,000 and dihydroxy PEG 10,000 (Aldrich, 99%) and pentylamine (Aldrich, 99%). *N*-(*n*-pentyl)-2-pyridylmethanimine (NPMI) and *N*-(*n*-propyl)-2-pyridylmethanimine were prepared as previously reported [42] and stored under anhydrous conditions prior to use. Toluene and distilled water were de-aerated by bubbling nitrogen through them prior to use. Copper(I) bromide (Aldrich, 98%) was purified according to a literature procedure [43]. All other reagents were purchased from Aldrich at the highest purity available and used without further purification unless otherwise stated. All solvents purchased were of the highest grade available from BDH and were used as supplied. Protected (**1**) and unprotected (**2**) methacryloyl uridine and protected (**3**) and unprotected (**4**) methacryloyl adenosine were synthesised according to literature procedures [34].

2.2. Methods

DSC was carried out on a Perkin Elmer Pyris 1 differential scanning calorimeter and the T_g's quoted refer to inflection midpoints. TGA's were recorded on a Perkin Elmer TGA 7 apparatus, FTIR were recorded on an Avatar 320 FT-IR fitted with a "Golden Gate" attenuated total reflection (ATR) cell attachment. ^{1}H NMR spectra were recorded at 300 MHz on a Bruker DPX 300 instrument, with chemical shifts (δ) quoted in ppm using residual non-deuterated solvents as internal standard [$CDCl_3$ (^{1}H, δ: 7.25 ppm; ^{13}C, δ: 77.0 ppm), d_6-DMSO (^{1}H, δ: 2.50 ppm; ^{13}C, δ: 39.5 ppm)]. Coupling constants (*J*) are quoted in Hz. Absorbance spectra were collected in water in 1 cm pathlength quartz cuvettes using a Jasco V-550 spectrometer. The CD spectra were obtained in water using a Jasco J-715 Circular Dichroism spectropolarimeter with a 1 cm pathlength cuvette. Molar mass distributions were measured using size exclusion chromatography (SEC), on one of two systems. The first, equipped with a guard column, two 30 cm 5 μm PLgel Mixed-D columns (Polymer Laboratories, linear range 200–400,000 MW) and a differential refractive index detector, using tetrahydrofuran at 1 mL min^{-1} as eluent. The second comprised a system equipped with a guard column and on two 30 cm mixed C columns (Agilent) with differential refractive index detection using DMF as eluent (0.5 mL min^{-1}). The SECs were calibrated with 12 poly(methyl methacrylate) standards in the range 6.85×10^5–200 g mol^{-1}. Sample solutions were prepared by adding 2.0 mL of solvent to 4.0 mg of sample; leaving 72 h at 60 °C to dissolve them. Molar mass distributions with PEO/PEG standards were carried out at Rapra Polymer Laboratories on a system equipped with a PLgel 2 × mixed bed B-30 cm and 10 μm columns with differential refractive index detection using DMF with 1% LiBr as eluent (flow rate of 1.0 mL min^{-1}). Sample solutions were prepared by adding 10 mL of solvent to 20 mg of sample, by warming to 80 °C for 20 min then leaving overnight if necessary to dissolve them. In all cases, after thorough mixing, the solutions were filtered through a 0.2 μm PTFE membrane prior to chromatography. Results are expressed as the "PMMA equivalent" or "PEO/PEG equivalent"

[§] Preliminary communication: Garcia, M. Ph D. Thesis, University of Warwick, 2003.

molecular mass. We acknowledge the relative nature of this data and challenge in comparing measurements in different solvents.

2.3. Polymer synthesis

2.3.1. Preparation of bromoisobutyroyl PEG (10,000 g mol^{-1}) initiator (**6**)

Dimethylaminopyridine (293.4 mg, 2.40 mmol) in anhydrous dichloromethane (2.0 mL) was mixed with triethylamine (161.8 mg, 1.60 mmol) and cooled to 0 °C. A solution of BIBB (917.2 mg, 4.00 mmol) in anhydrous dichloromethane (2.0 mL) was added and a yellow suspension was formed. A solution of PEG 10,000 (4.00 g, 0.4 mmol) in anhydrous dichloromethane (40 mL) was added dropwise over 1 h. After addition, the solution was allowed to rise to ambient temperature and was stirred for 18 h. The solution was filtered and half of the solvent was removed under reduced pressure. The PEG initiator **6** was precipitated in cold diethyl ether (120 mL) and filtered. It was then recrystallised overnight from absolute ethanol (75 mL), filtered, washed with cold diethyl ether and dried *in vacuo* to give the *title product* (3.84 g, 93%) as a white solid; m.p. 59 °C by DSC; TGA 379 °C; T_{onset} 335 °C; M_n = 9,160, Đ = 1.2 (GPC-DMF, PEO/PEG standard); M_n = 25,700, Đ = 1.04 (GPC-DMF, PMMA standard); δ_H ($CDCl_3$), 4.28 (4H, t, J = 4.7, CH_2O), 3.60 (PEG methylenes, bs), 1.90 (12H, s, CH_3) ppm; ν_{max} (solid) 2887 (C—H *st*), 1642 (C=O *st*), 1445, 1341 (C—O *st*), 1279, 1240 (C—O *st*), 1147 and 1097 (C—O *as st*), 959 (C—O—C *st*), 841 (γ-CH *st*) cm^{-1}.

2.3.2. Copper-mediated polymerisation of unprotected methacryloyl uridine **1** using PEG (10,000 g mol^{-1}) initiator (general procedure II, polymer **10**)

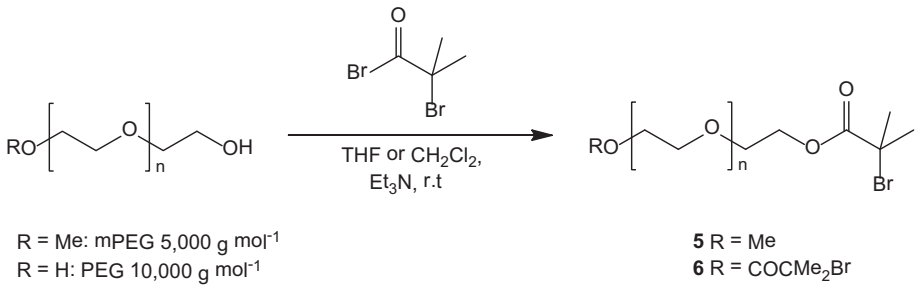
Copper(I) bromide (6.9 mg, 48.1 μmol), copper(II) bromide (10.7 mg, 48.1 μmol), *N*-(n-propyl)-2-pyridylmethanimine ligand (43.1 mg, 291.0 μmol) and distilled water (3.0 mL) were mixed in a Schlenk tube and heated at 40 °C for 5 min to form the catalyst complex. The solution was de-aerated by four freeze–pump-thaw cycles. Methacryloyl uridine **1** (302.9 mg, 970.0 μmol), PEG 10,000 initiator **6** (500 mg, 48.5 μmol) and distilled water (7.0 mL) were mixed in a second Schlenk tube and de-aerated by four freeze–pump-thaw cycles. The catalyst solution was then injected into the initiator solution and stirred at 25 °C for 25 h. The water was removed under reduced pressure. Dichloromethane (3.0 mL) was added to dissolve the product and diethyl ether (30 mL) to precipitate the product. It was stirred for 5 min, filtered, washed with more diethyl ether and *tert*-butanol to remove the copper to give the triblock copolymer **10** (596.7 mg, 99%) as a very pale green-white solid; m.p. 55 °C by DSC; TGA 89.2, 319.2 and 403.0 °C; T_{onset} 275.7 °C; M_n = 16,100 (by ^{1}H NMR) M_n = 11,500, Đ = 1.7 (GPC-DMF, PEO/PEG standard); M_n = 57,300, Đ = 1.05 (GPC-DMF, PMMA standard); δ_H (d_6-DMSO), 11.36 (1H, bs, N*H*), 7.74 [1H, bs, H(6)], 5.78 [1H, bs, H(1′)], 5.71 [1H, bs, H(5)], 5.55 [1H, bs, O*H*(2′)], 5.25 [1H, bs, O*H*(3′)], 4.02 [5H, m, H(2′–5′)], 3.50 (PEG methylenes, bs), 1.50–0.50 (5H, m, CH_3 and CH_2) ppm; ν_{max} (solid): 3500–3300 (N—H *st*), 2883 (C—H *st*), 1678 (C=O *st*), 1446 (N—H δ *ip*), 1342 (O—H *bending*), 1279 (C—O *st*), 1240 (C—N *st*), 1097 (C—O *as st*), 961 (C—O—C *st*), 821 (γ-CH *st*) cm^{-1}.

2.3.3. Copper-mediated polymerisation of unprotected methacryloyl adenosine **3** using PEG (10,000 g mol^{-1}) initiator (polymer **11**)

Following the general procedure II with copper(I) bromide (6.9 mg, 48.1 μmol), copper(II) bromide (10.7 mg, 48.1 μmol), *N*-(*n*-propyl)-2-pyridylmethanimine ligand (43.1 mg, 291.0 μmol) and distilled water (3.0 mL) in the catalyst Schlenk tube and **3** (325.3 mg, 970.0 μmol), **6** (500 mg, 48.5 μmol) and distilled water (7.0 mL) in the second Schlenk tube and heating at 25 °C for 25 h to afford after the usual work-up the copolymer **11** (525 mg, 75%) as a green solid; m.p. 62 °C by DSC; TGA 272, 416 and 623 °C; T_{onset} 225 °C; M_n = 12,600 (by ^{1}H NMR); M_n = 8500, Đ = 1.8 (GPC-DMF, PEO/PEG standard); M_n = 39,900, Đ = 1.31 (GPC-DMF, PMMA standard); δ_H (d_6-DMSO), 8.32 [1H, bs, H(2)], 8.17 [1H, bs, H(8)], 7.37 (2H, bs, NH_2), 5.92 [1H, bs, H(1′)], 5.61 [1H, bs, O*H*(2′)], 5.43 [1H, bs, O*H*(3′)], 4.70 [1H, bs, H(2′)], 4.40–4.00 [4H, m, H(3′–5′)], 3.50 (PEG methylenes, bs), 1.6–0.7 (5H, m, CH_3 and CH_2) ppm; IR, ν_{max} (solid): 3339 (bs, O—H *st*), 2882 (C—H *st*), 1715 (C=O *st*), 1644 (C=O and C=C *st*), 1467 (N—H δ *ip*), 1342 (O—H *bending*), 1279 (C—O *st*), 1241 (C—N *st*), 1096 (C—O *st*), 960 (C—O—C *st*), 841 (γ-CH *st*) cm^{-1}.

2.3.4. Preparation of a pentablock copolymer using unprotected methacryloyl adenosine **3** and the unprotected triblock copoly(methacryloyl uridine) **10** as initiator (polymer **12**)

Following the general procedure II with copper(I) bromide (2.7 mg, 18.8 μmol), copper(II) bromide (4.2 mg,



Scheme 1. Schematic representation of the synthesis of PEG macroinitiators.

Table 1
Analytical data for the block copolymers.

Entry	Initiator	Monomer	Conditions	Yield (%)	Polymer	M_n[a] (g mol^{-1})	M_n (g mol^{-1})	Đ
1	**5**	**4**	A	61	**7**	–	9300[b]	1.09[b]
2	**5**	**3**	B	93	**8**	7800	–	–
3	**5**	**2**	A	77	**9**	–	8300[b]	1.11[b]
4*	**6**	**1**	C	99	**10**	16,100	11,500[c]	1.70[c]
							57,200[d]	1.05[d]
5*	**6**	**3**	C	75	**11**	12,600	8500[c]	1.80[c]
							39,900[d]	1.31[d]
6*	**10**	**3**	C	92	**12**	21,000	9500[c]	2.30[c]
							80,200[d]	1.40[d]
7*	**11**	**1**	C	86	**13**	16,200	11,200[c]	1.50[c]
							62,600[d]	1.30[d]
8	**6**	**2**	A	83	**14**	–	21,900[b]	1.22[b]
9	**6**	**4**	A	57	**15**	–	17,700[b]	1.13[b]

* Water soluble polymers.
[a] Determined by ^{1}H NMR.
[b] GPC-THF, PMMA standard.
[c] GPC-DMF, PEO/PEG standard.
[d] GPC-DMF, PMMA standard; Conditions: A, toluene/CuBr/NPMI; B, H_2O/CuBr/bipy; C, H_2O/Cu(I)Br/Cu(II)Br/*N*-(*n*-propyl)-2-pyridylmethanimine.

18.8 μmol), *N*-(*n*-propyl)-2-pyridylmethanimine ligand (16.5 mg, 111.6 μmol) and distilled water (2.0 mL) in the catalyst Schlenk tube, **3** (124.8 mg, 372.2 μmol), triblock copolymer **10** (300 mg, 18.6 μmol, M_n = 16,100 by ^{1}H NMR) and distilled water (5.0 mL) in the second Schlenk tube and heating at 25 °C for 22 h to afford after the usual work-up the pentablock copolymer **12** (287.5 mg, 92%) as a pale green flake-like solid. The ^{1}H NMR showed a ratio of 57:43 uridine to adenosine; m.p. 59 °C by DSC; TGA 311, 401 and 590 °C; T_{onset} 255 °C; M_n = 21,025 (by ^{1}H NMR); M_n = 9 500, Đ = 2.3 (GPC-DMF, PEO/PEG standard); M_n = 80,200, Đ = 1.40 (GPC-DMF, PMMA standard); δ_H (d_6-DMSO), 11.40 (1H, bs, N*H*), 8.29 [1H, bs, H(2A)], 8.18 [1H, bs, H(8A)], 7.52 (2H, bs, NH_2), 7.30 [1H, bs, H(6U)], 6.03–5.39 [7H, m, H(5U), 2H(1′) and 4O*H*)], 5.27–5.18 [1H, m, H(2′A)], 4.67–4.58 [1H, m, H(2′U)], 4.18–3.80 [8H, m, 2H(3′–5′)], 3.50 (PEG methylenes, bs), 1.70–0.5 (10H, m, 2CH_3 and 2CH_2) ppm; ν_{max} (solid): 3350–3200 (N—H and O—H *st*), 2882 (C—H— *st*), 1682 (C=O *st*), 1467 (N—H δ *ip*), 1341 (O—H *bending*), 1277 (C—N *st*), 1100 (C—O *st*), 991 (C—O—C *st*), 842 (γ-CH *st*) cm^{-1}.

2.3.5. Preparation of a pentablock copolymer using the unprotected methacryloyl uridine **1** and the unprotected triblock copoly(methacryloyl adenosine) **11** as initiator (polymer **13**)

Following the general procedure II with copper(I) bromide (3.4 mg, 23.8 μmol), copper(II) bromide (5.3 mg, 23.8 μmol), *N*-(*n*-propyl)-2-pyridylmethanimine ligand (21.2 mg, 148.2 μmol) and distilled water (2.0 mL) in the catalyst Schlenk tube, **1** (149.1 mg, 477.3 μmol), triblock initiator **11** (300 mg, 23.8 μmol, M_n = 12,570 by ^{1}H NMR) and distilled water (5.0 mL) in the second Schlenk tube and heating at 25 °C for 22 h to afford the pentablock polymer **13** (412.5 mg, 86%) as a green rock-like solid. The ^{1}H NMR showed a ratio of 39:61 adenosine to uridine; m.p. 53 °C by DSC; TGA 57, 316, 412 and 618 °C; T_{onset} 270 °C; M_n = 16,200 (by ^{1}H NMR); M_n = 11,200, Đ = 1.50 (GPC-DMF, PEO/PEG standard); M_n = 62,600, Đ = 1.30 (GPC-DMF, PMMA standard); δ_H (d_6-DMSO), 11.30 (1H, bs, N*H*), 8.27 [1H, bs, H(2A)], 8.07 [1H, bs, H(8A)], 7.59 (2H, bs, NH_2), 7.25 [1H, bs, H(6U)], 5.90–5.38 [7H, m, H(5U), 2H(1′) and 4 O*H*), 5.30–5.20 [1H, m, H(2′A)], 4.80–4.60 [1H, m, H(2′U)], 4.38–3.82 [8H, m, 2H(3′–5′)], 3.50 (PEG methylenes, bs), 1.75–0.58 (10H, m, 2CH_3 and 2CH_2) ppm; ν_{max} (solid): 2881 (C—H *st*), 1725 (C=O *st*), 1467 (N—H), 1342 (O—H *bending*), 1279 (C—O *st*), 1241 (C—N *st*), 1096 (C—O *st*), 960 (C—O—C *st*), 842 (γ-CH *st*) cm^{-1}.

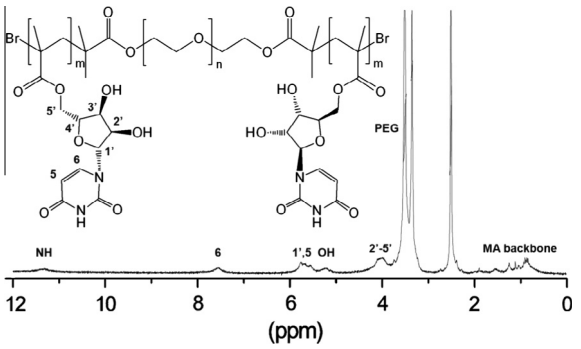
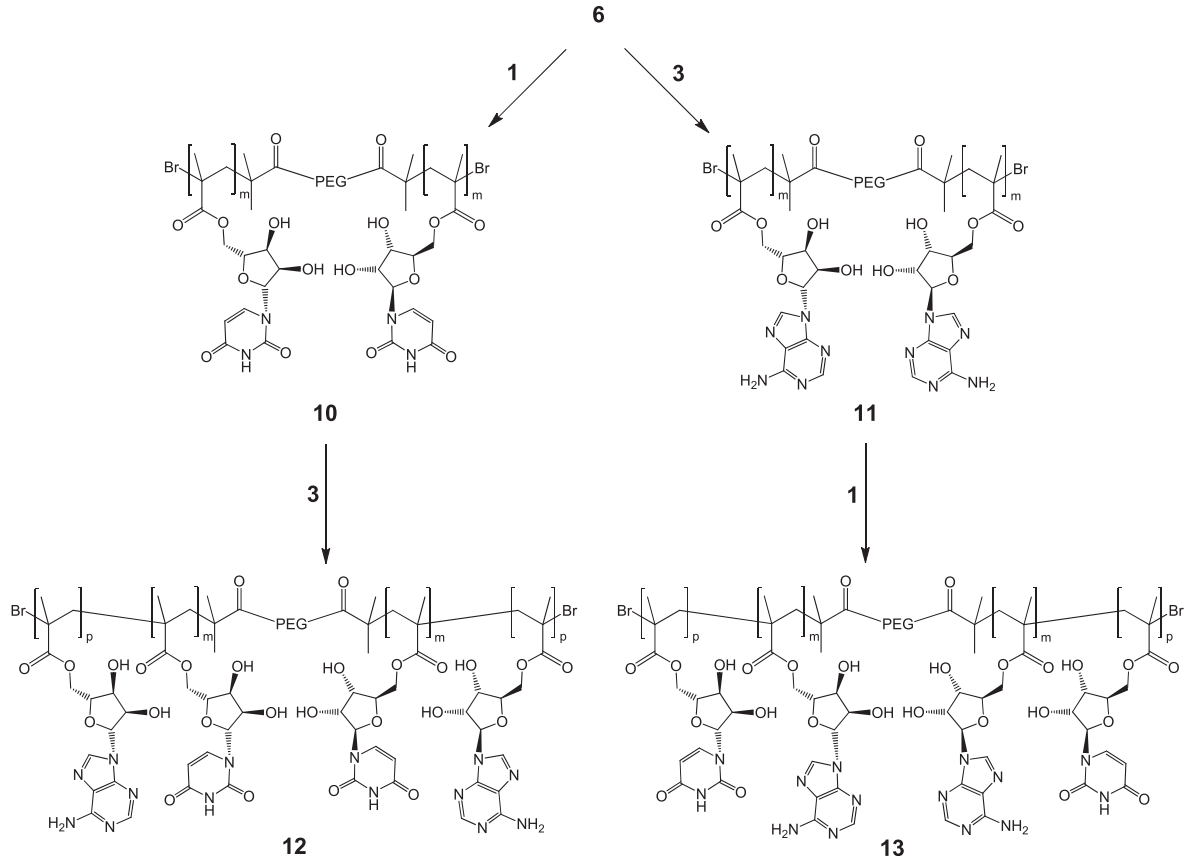


Fig. 2. ^{1}H NMR of poly(methacryloyl uridine) triblock **10** in d_6-DMSO.

3. Results and discussion

3.1. Block copolymer synthesis

The preparation of the unprotected and silyl protected methacryloyl uridine (**1** and **2**, Fig. 1) and methacryloyl adenosine (**3** and **4**) has been described previously [34]. PEG macroinitiators (**5** and **6**, Scheme 1) were synthesised using a modified published procedure [22]. Solutions of mPEG (5000 g mol^{-1}), or dihydroxy PEG (10,000 g mol^{-1}), and triethylamine in anhydrous THF or CH_2Cl_2 were treated dropwise with a solution of 2-bromo-2-methylpropionyl bromide (BIBB) to form the initiator mPEG$_{5k}$ (**5**) and PEG$_{10k}$ (**6**).



Scheme 2. Schematic representation of the synthesis of water soluble triblock (**10**, **11**) and pentablock (**12**, **13**) copoly(methacryloyl nucleosides) using PEG 10,000 (**6**) as initiator.

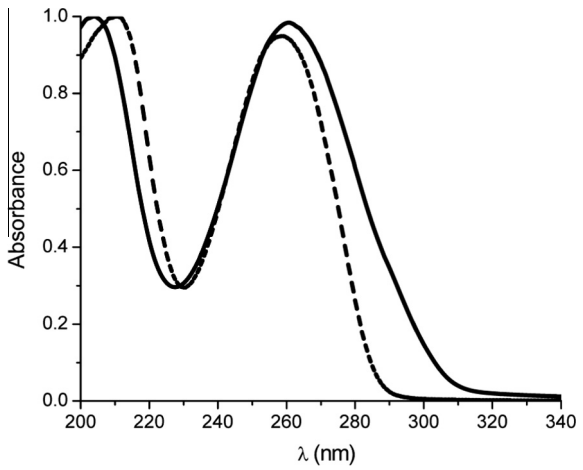


Fig. 3. UV spectra of methacryloyl uridine **1** (---) and triblock copolymer **10** (–).

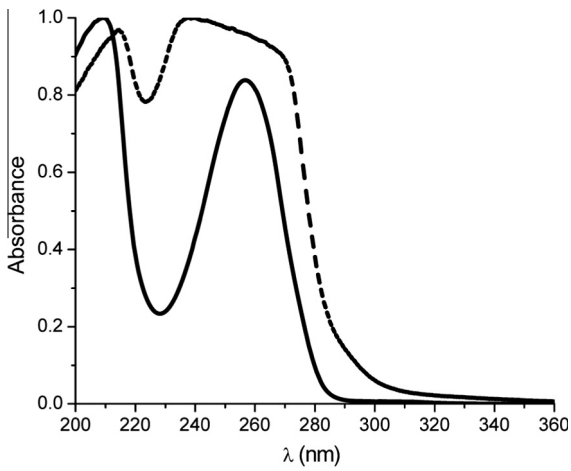


Fig. 4. UV spectra of methacryloyl adenosine **3** (---) and triblock copolymers **11** (–).

Firstly, $mPEG_{5k}$ **5** was used as an initiator for the polymerization of the protected uridine **2** and adenosine monomers **4** and NPMI as ligand with toluene as solvent to give the block copolymers (**9** and **7**) of relatively narrow dispersity (Đ) as determined by SEC (Table 1, entries 1 and 2).

The adenosine monomer **3** gave a lower yield (as observed previously with a related polymerisation) possibly due to the complexation of the copper by this monomer [34]. However, both copolymers were found to be insoluble in water, presumably because the hydrophobic

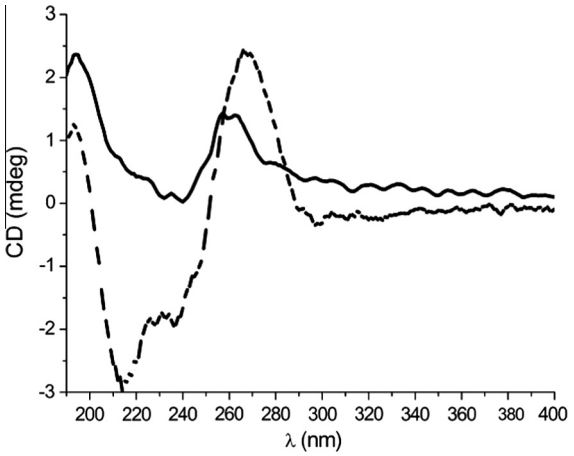


Fig. 5. CD spectra of methacryloyl uridine monomer **1** (---) and triblock copolymer **10** (—).

silyl groups on the poly(methacryloyl nucleoside) segment prevented the block copolymer from dissolving. The unprotected adenosine monomer **3** was therefore polymerised in water using bipy as the ligand, but this gave the polymer **8** as an insoluble precipitate in 93% yield (Table 1, entry 2). In order to assess the solubility of the homopolymers of the unprotected monomers **1** and **3**, free radical polymerisation was carried out in water using the water soluble radical initiator 4,4′-azobis(4-cyanovaleric acid) (ACVA) (see Supplementary Information). This gave poly(methacryloyl uridine) **16** and poly(methacryloyl adenosine) **17** in 96% and 86% yields, respectively, as almost insoluble precipitates. Hence the PEG_{10k} macroinitiator **6** was prepared to overcome this insolubility of the poly(methacryloyl nucleosides).

The unprotected methacryloyl uridine monomer **1** was polymerized using PEG_{10k} **6** as initiator, a mixture of copper(I) and copper(II) bromide as catalyst and *N*-(*n*-propyl)-2-pyridylmethanimine as ligand (the propyl ligand forms a water-soluble complex with the catalyst leading to homogeneous solutions) [27]. The signals for the NH and H-6 protons can be seen quite clearly at 11.36 and 7.74 ppm, respectively in the ^{1}H NMR, Fig. 2. The two signals at 5.78 and 5.71 ppm are due to the H-1′ and H-5 protons, the signals at 5.55 and 5.25 ppm are the two hydroxyl group protons. The remaining protons attached to the sugar ring are seen at 4 ppm close to the large PEG signal at 3.50 ppm. Integration of the uridine protons relative to the PEG protons gave a molar mass of 6100 g mol^{-1} for the poly(methacryloyl uridine) blocks, thereby giving an overall molar mass of 15,300 g mol^{-1} for the triblock **10** (Table 1, entry 4).

The unprotected adenosine monomer **3** polymerised in 75% yield using the PEG_{10k} **6** initiator. From ^{1}H NMR the overall molar mass of polymer **11** was calculated to be 12,600 g mol^{-1} (Table 1, entry 5), which is considerably lower than the molar mass of the triblock **10**, which is possibly due to the lower solubility of monomer **3** in aqueous solutions at ambient temperature, or interaction of the adenosine moieties with the Cu complex, changing the nature of the catalytic species.

In the next step, the unprotected uridine triblock **10** was used as initiator with the unprotected adenosine monomer **3** to give the water soluble pentablock copolymer **12** (Scheme 2; Table 1, entry 6). The ^{1}H NMR showed a ratio of 57:43 uridine to adenosine, by comparison of the integrations of the protons H_2 and H_8 of the adenosine with the H_6 of the uridine. The unprotected adenosine triblock **11** was used as the initiator with unprotected methacryloyl uridine **1** to give the water soluble pentablock copolymer **13** (Scheme 2; Table 1, entry 7). ^{1}H NMR showed a ratio of 39:61 adenosine to uridine, again by comparing the integration of the protons H_2 and H_8 of the adenosine with the H_6 of the uridine. Somewhat narrower molar mass distributions were found for the pentablock **13** compared to pentablock **12**. Furthermore, the solubility of **13** in water was higher than of **12** and the triblocks **10** and **11** under the conditions used for the analysis of the polymers. Interestingly, the addition of the second monomer **1** to form the pentablock **13** gave a polymer of higher thermal

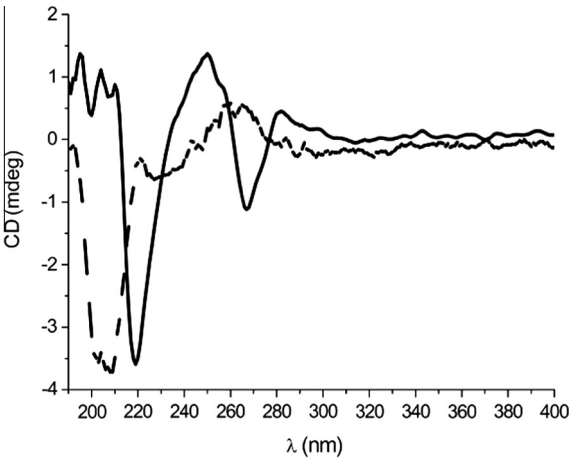


Fig. 6. CD spectra of methacryloyl adenosine monomer **3** (---) and triblock copolymer **11** (—).

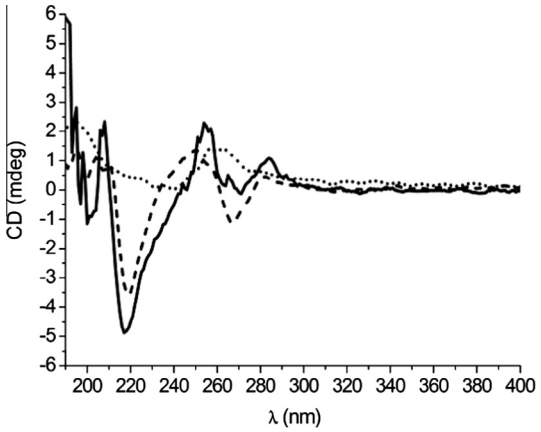


Fig. 7. CD spectra of uridine triblock **10** (···), adenosine triblock **11** (---) and the mixture of triblocks **10** and **11** (—).

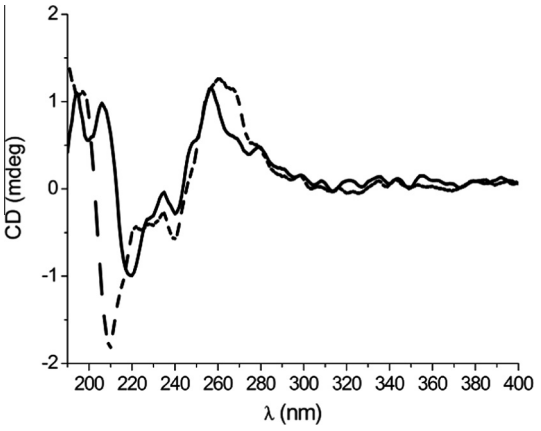


Fig. 8. CD spectrum of pentablock **12** (---) and **13** (—).

stability evidenced by TGA revealing T_{onset} of 225.2 °C for the triblock **11** and 270.4 °C for the pentablock **13**.

Polymerisation of the protected methacryloyl uridine **2** and adenosine **4** monomers with PEG_{10k} **6** initiator gave triblock copolymers **14** and **15** respectively, with narrow dispersity (Table 1, entries 11 and 12). However, both these polymers were found to be insoluble in water (see Supplementary Information).

3.2. Secondary structure

The UV absorbance and circular dichroism (CD) spectra of the monomers and polymers were investigated to better understand how these molecules behave in solution. The UV spectra of triblock copolymers **10** and **11** and their respective monomers **1** and **3** are very similar, although the absorption maximum (peak at 270 nm) is shifted to slightly higher wavelength and extends above 300 nm, indicative of π–π interactions between the nucleobases in the polymer (Figs. 3 and 4).

CD measures the difference in absorbance of left and right circularly polarised light and is sensitive to the chirality of a system, indicating the formation of secondary structural motifs [44]. The uridine monomer **1** shows peaks due to the chirality in the sugar ring and upon polymerisation the spectrum changes giving increased intensity in the long wavelength region, a small change in intensity of the negative CD peak at 240 nm, and a larger change at 210 nm (Fig. 5). The adenosine-substituted polymer **11** shows an even larger change from the monomer CD spectrum, with apparent sign inversion or large wavelength shifts of all peaks (Fig. 6).

The 260 nm and 210 nm monomer bands become excitonic in appearance in the polymer, suggesting there may be stacking of the planar chromophores. This is indicative of a more organised secondary structure achieved by the adenosine polymer due to its ability to hydrogen bond to itself [17], probably by Hoogsteen-type interactions, more readily than uridine.

Polymers **10** and **11** were mixed and their CD spectra were compared with the spectra of the individual polymers (Fig. 7). The CD spectrum of the new complex is similar to the adenosine copolymer **11** although clearly not the result of simple additive effects of the two individual spectra of **10** and **11**. This result indicates the formation of a new structure, more organised than the uridine triblock **10** and with similar features to the adenosine triblock **11**. This may be due to the formation of hydrogen bonds between those two polymers, although stacking contributions are probably playing a part in the new supramolecular structure. Indeed, a hyperchromic shift is observed, which is indicative of new π–π interactions in the solution structure.

The CD spectra of the pentablocks **12** and **13** are less well defined (Fig. 8). In the case of the pentablock **12**, the CD spectrum is similar to the triblock **11** (Fig. 6) and the complex formed by mixing the two triblocks (Fig. 7). In the case of pentablock **13**, a shift towards lower wavelength was observed. The weak band below 200 nm (exhibited by other secondary structures of nucleic acids) may indicate base stacking.

4. Conclusions

It has been demonstrated that bromoisobutyroyl PEG can act as an initiator for copper-mediated polymerisation of the multifunctional methacryloyl uridine and methacryloyl adenosine monomers possessing unprotected and silyl protected hydroxyl groups. The dispersities of the resulting block copolymers were narrow in certain cases indicating the controlled nature of those polymerisations. The unprotected uridine and adenosine homopolymers, which alone are totally insoluble in water, were made soluble by attaching them to PEG (10,000 g mol^{-1}). Initial studies into the secondary structure of the polymers indicate that whilst the methacryloyl uridine polymer is largely unstructured, the poly(methacryloyl adenosine) possesses a degree of higher structural order in aqueous solution. The two polymers interact in solution, probably through a mixture of hydrogen bonding and stacking interactions. Further synthetic developments using the monomers herein, studies into template directed polymerisations [41,45–48] and interactions with single and double stranded nucleic acids of nucleoside containing copolymers in aqueous solutions are the subject of ongoing work.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.eurpolymj.2015.03.014.

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