RAFT Polymerization of Methyl 6-*O*-Methacryloyl-α-D-glucoside in Homogeneous Aqueous Medium. A Detailed Kinetic Study at the Low Molecular Weight Limit of the Process

Luca Albertin*,† and Neil R. Cameron*

Department of Chemistry and Interdisciplinary Research Centre in Polymer Science and Technology, Durham University, South Road, Durham DH1 3LE, U.K.

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ABSTRACT: We report a detailed kinetic study of the RAFT polymerization of methyl 6-*O*-methacryloyl-α-D-glucoside (a methacrylic ester-type glycomonomer) with the chain transfer agent (CTA) (4-cyanopentanoic acid)-4-dithiobenzoate and initiator 4,4′-azobis(4-cyanopentanoic acid) in homogeneous aqueous media. The influence of temperature, initiator and CTA concentration, molar mass of the CTA radical leaving group, and the presence of residual oxygen on the polymerization kinetics were investigated in comparison with corresponding conventional free radical polymerizations (i.e., with no CTA present). RAFT processes were characterized by an initial non-steady-state period, the length of which depended inversely on the radical flux in the system, and were found to proceed at a significantly slower rate than the corresponding conventional free radical polymerizations. Also, attainment of the steady-state coincided with complete consumption of the initial CTA. The use of a macromolecular CTA reduced the length of the non-steady-state period but, interestingly, did not eliminate it, and the duration of this period was still shown to depend inversely on the initial CTA to initiator ratio. To our knowledge, this is the first time that a non-steady-state period has been observed in a RAFT polymerization initiated by a macromolecular CTA. Finally, the results of this investigation were used as a guide for the preparation of a series of well-defined living glyco-oligomers ($DP_n = 15-66$, PDI = 1.05−1.12) in high yield.

Introduction

Glycopolymers are synthetic polymers possessing a non-carbohydrate backbone but carrying carbohydrate moieties as pendant or terminal groups.[1,2] Numerous examples exist in the literature showing that glycopolymers can engage in recognition and binding events in a similar fashion to natural carbohydrates.[3−6] These interactions are characterized by a high degree of specificity and, depending on the mechanism, can give rise to highly stable aggregates.[7] Consequently, we anticipate that glycosylated macromolecules may be used in nanofabrication[8] and to prepare supramolecular structures capable of interacting with biological molecules (e.g., lectins),[9,10] microorganisms (e.g., viruses or bacteria), and cells.[11] In this prospect, lower molecular weight species (i.e., well-defined glyco-oligomers) might prove to be superior building blocks, since they diffuse more readily in solution and their backbone chain possesses a smaller number of conformational degrees of freedom. At the same time, precise control of the macromolecular architecture will be essential to enable sophisticated functions, hence the need for precise polymerization techniques.

Reversible addition−fragmentation chain transfer (RAFT) polymerization has already established itself as a viable method for the direct synthesis of well-defined glycopolymers in aqueous media, without the need to resort to protective group chemistry.[10,12−17] Nonetheless, detailed kinetic studies on the conventional and living radical polymerization of unprotected vinyl glycomonomers in water and aqueous/organic solutions are scarce, and in the case of RAFT systems there are simply none. Davis et al.[14] recently demonstrated that methyl 6-*O*-methacryloyl-α-D-glucoside (6-*O*-MAMGlc, Scheme 1) can be quantitatively polymerized in water/ethanol solution in the presence of 4,4′-azobis(4-cyanopentanoic acid) (ACPA) and (4-cyanopentanoic acid)-4-dithiobenzoate (CPADB) to afford narrow polydispersity materials with predictable molecular weight. Through the use of different RAFT agent concentrations (all other parameters being equal), poly(6-*O*-MAMGlc) with a number-average degree of polymerization (DP_n) in the range 83−391 was synthesized. Even so, first-order plots indicate that polymerizations achieved steady state only after 20−30 min of reaction, with higher concentrations of chain transfer agent (CTA) resulting in longer non-steady-state periods and slower polymerization rates. Also, when a lower DP species was targeted (and a higher concentration of CTA was used) the polymerization turned out to be strongly retarded, with no significant monomer conversion after nearly 4 h of reaction although oligomeric species were detected by mass spectrometry of the reaction mixture.

Klumperman et al.[18−21] used in situ ^{1}H NMR spectroscopy to study the fate of low molar mass species during the early stages of the styrene/cumyl (or cyanoisopropyl) dithiobenzoate and methyl acrylate/cumyl dithiobenzoate (or phenyl dithioacetate) reversible addition−fragmentation chain transfer polymerizations initiated by 2,2′-azobis(isobutyronitrile) (AIBN). We now report the first detailed kinetic investigation of the conventional radical and RAFT polymerization of an unprotected vinyl glycomonomer in aqueous/organic solution. Thus, the 6-*O*-MAMGlc/CPADB/ACPA system in D_2O/DMSO-d_6 and water/methanol-d_4 was studied by in situ ^{1}H NMR spectroscopy at 60, 70, and 80 °C (Scheme 2). In particular, we focused on the low molecular weight end of the process (which is generally neglected by scientists using RAFT in synthesis), by employing an initial monomer to chain transfer agent ratio ≤70, and

* To whom correspondence should be addressed. E-mail: (L.A.) luca_albertin@yahoo.com; (N.R.C.) n.r.cameron@durham.ac.uk.

† Current address: CNRS-bioMérieux, Ecole Normale Supérieure de Lyon, 46 allée d'Italie, 69364 Lyon Cedex 07, France.

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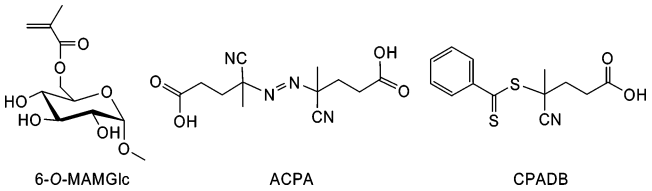
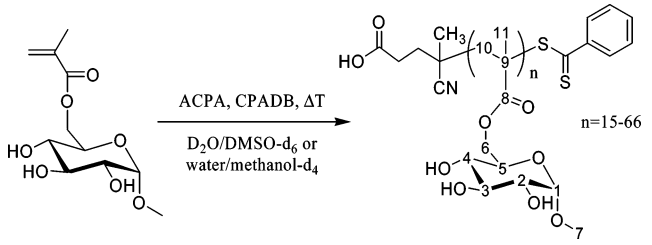
Scheme 1. Structure of the Monomer (6-*O*-MAMGlc), Initiator (ACPA), and Chain Transfer Agent (CPADB) Used in This Study



Scheme 2. Reversible Addition-fragmentation Chain Transfer Polymerization of Methyl 6-*O*-methacryloyl-α-D-glucoside and Position Numbering Used for NMR Peak Assignment



on the influence of several experimental parameters on the occurrence and extent of inhibition and/or retardation phenomena. Finally, we describe how a careful choice of polymerization conditions enables quantitative conversion of 6-*O*-MAMGlc into well-defined living oligomers with DP_n in the range 15−66.

Experimental Section

Materials and Methods. Unless otherwise specified, all chemicals were reagent grade. 4,4′-Azobis(cyanopentanoic acid) (98%, Fluka), deuterium oxide (99.9%, Sigma-Aldrich), dimethyl sulfoxide-d_6 (99.9% d, Goss Scientific), ethanol (spectroscopic grade, Sigma-Aldrich or HPLC grade, Riedel de Haën), ethyl acetate (Fischer), methanol-d_4 (99.9% d, Sigma-Aldrich), *N*,*N*-dimethylformamide (HPLC grade, Fischer), and water (HPLC grade, Riedel de Haën) were used as received. Novozym 435 (used in monomer synthesis) was kindly donated by Novozymes A/S. Flash chromatography was carried out with a 76 mm o.d. glass column loaded with 200 g of silica gel (60 Å, 40−63 μm, Fluorochem) and eluted at a flow rate of 5 cm min^{-1}. TLC analysis was performed on glass-backed silica gel plates (60 Å, 5−17 μm, Macherey-Nagel); following solvent evaporation, the developed plates were immersed in a 20% H_2SO_4/ethanol solution and heated at 110 °C for 15 min for spots detection. Accurate volumes were either measured with automatic pipettes (10−1000 μL; Gilson Pipetman P20, P100 and P1000) or with glass measuring pipettes (1−10 mL). Dialysis purifications were performed at room temperature against purified water (100−150 times the volume of the sample) using Slide-A-Lyzer Dialysis Cassettes (3−13 mL, 3.5 kDa MWCO, Pierce Biotechnology); during the process, the water was changed twice (typically after 4 and 23 h) and the sample was kept in the dark. Methyl 6-*O*-methacryloyl-α-D-glucoside (6-*O*-MAMGlc)[14] and (4-cyanopentanoic acid)-4-dithiobenzoate (CPADB)[22] were prepared according to published methods and their structure was confirmed via ^1H and ^{13}C NMR (see Supporting Information). Unless otherwise specified, degassing of polymerization solutions was performed via four freeze−evacuate−thaw cycles (ultimate pressure 6×10^{-2} mbar).

Analytical Techniques. NMR experiments were conducted on Varian Unity-300 and Varian Inova-500 spectrometers equipped with a variable temperature (VT) module (resonance frequencies of 300.2, 500.1 MHz for ^1H nuclei, and 75.5, 125.8 MHz for ^{13}C nuclei respectively). A 5 mm inverse detection probe was used for kinetic experiments (Inova-500) and the probe temperature was calibrated using an ethylene glycol sample. Spectra were reprocessed using MestReC 4.6 and Origin 7.5 software. For the integration of end-of-chain dithiobenzoate aromatic ortho protons, partially superimposed peaks were deconvoluted via multiple fitting with Lorentzian functions ($r^2 \geq 0.96$).

Molecular weights and molecular weight distributions were measured using a size exclusion chromatography (SEC) instrument consisting of a GBC LC1110 HPLC pump, a Viscotek VE5111 manual injector port, and a Viscotek TriSEC model 302 triple detector array comprising a 90° angle laser light scattering detector and a differential refractometer operating at the same wavelength (λ = 670 nm). The system was equipped with a 50 × 7.5 mm guard column and three 300 × 7.5 mm linear columns (PLgel 500, 10^3 and 10^4 Å pore size; 5 μm particle size; Polymer Laboratories). *N*,*N*-Dimethylformamide (0.1% w/v LiBr, 0.05% w/v 2,6-di-*tert*-butyl-4-methylphenol) was used as eluant at a flow rate of 1 mL min^{-1} while the column's temperature was maintained at 60 °C. Polymer solutions (2−3 mg mL^{-1} in DMF eluant) were injected in 100 μL volumes. SEC traces were analyzed with OmniSEC 4.0 software (Viscotek). A d*n*/d*c* value of 0.090 was used for molecular weight calculations that was determined from repeated injections of pure poly(methyl 6-*O*-methacryloyl-α-D-glucoside) dithiobenzoate solutions of known concentration (M_n (SEC) 20,400, PDI (SEC) 1.05).

Mass spectrometry analyses were performed with a Thermo-Finnigan LTQ FT ion-trap mass spectrometer (Thermo Finnigan, San José, CA) equipped with an atmospheric pressure-ionization source operated in nebulizer-assisted electro-spray mode (ESI). The instrument was calibrated with caffeine (Aldrich), MRFA (tetrapeptide, Thermo Finnigan), and Ultramark 1621 (Lancaster) in the mass range of 195−2000 amu. All spectra were acquired in positive ion mode; nitrogen was used as the sheath gas and helium as the dumping gas. Samples (∼1 mg mL^{-1}) were dissolved in acetonitrile/1 mM aqueous $NH_4^+HCOO^-$ 4:6 and infused to the ESI interface at a constant flow rate.

Polymerization Kinetics. All experiments were carried out starting from the same stock solution of methyl 6-*O*-methacryloyl-α-D-glucoside in D_2O (1.00 M). The solution was stored in a freezer (−18 °C) until needed and thawed at room temperature just in time for sampling out the required volume. Polymerizations were performed in NMR tubes (5 mm diameter) fitted with a Young's valve, and conversions were monitored via in situ ^1H nuclear magnetic resonance spectroscopy at 500.1 MHz (Varian Inova-500 with variable temperature module). Prior to acquisition, the cavity of the magnet was heated at the required temperature and allowed to stabilize for at least 30 min. Meanwhile, an NMR tube containing a dummy sample consisting of methyl-α-D-glucoside (0.862 M) and 4,4′-azobis(4-cyanopentanoic acid) (12.1 mM) in D_2O/DMSO-d_6 86:14 was removed from the refrigerator and degassed. The tube was then lowered into the cavity of the magnet and allowed to equilibrate for at least 5 min, after which the magnet was fully shimmed on it.

In a typical run, 1.00 mL of monomer solution (c = 0.863 M) were mixed with a calculated amount of radical initiator (4,4′-azobis(4-cyanopentanoic acid), ACPA in Scheme 1) and RAFT agent ((4-cyanopentanoic acid)-4-dithiobenzoate, CPADB), both as DMSO-d_6 solutions (see Table 1 for quantities of initiator and CTA used in each experiment). About 0.6 mL of the resulting mixture was transferred to an NMR tube, degassed, and lowered into the magnet cavity. The sample was allowed to equilibrate for about 1 min, after which additional shimming was carried out to optimize the system fully. Data acquisition was started 3−5 min after the sample had been inserted into the magnet, the exact time being noted. A pulse program lasting 36 s (2.3 μs (40°) pulse width, 2 s acquisition time) was repeated at 144 s intervals for the duration of the experiment. The beginning of each pulse program was taken as time point for conversion vs time plots. The progress of the reaction was monitored by the disappearance of the vinyl protons' signals. Conversions (x) were estimated from the ratio between the area (A) of the vinyl proton peak H_E-10[14] and that of the glucoside

Table 1. Summary of Reversible Addition Fragmentation Chain Transfer (RAFT) Polymerization Kinetic Experiments [a]

run no.	monomer$_0$ (M)	initiator$_0$ (mM)	CTA$_0$ (mM)	CTA$_0$/initiator$_0$	monomer$_0$/CTA$_0$	temp (°C)	$R^{\bullet}_{cumul}$/CTA$_0$ (%) [d]	parameter studied
i	0.863	5.82				70		temp
ii	0.863	5.82	12.3	2.1	70	70	2.5	
iii	0.863	5.63				80		
iv	0.863	5.63	12.3	2.2	70	80	4.3	
v	0.863	12.1				60		[initiator]$_0$
vi	0.863	12.1	12.3	1.0	70	60	1.2	
vii	0.863	6.03				60		
viii	0.863	6.05	12.3	2.0	70	60	0.94	
ix	0.864	3.13				60		
x	0.864	3.13	12.4	4.0	70	60	0.75	
xi	0.864	2.00				60		[CTA]$_0$
xii	0.864	2.00	4.00	2.0	216	60	1.1	
xiii	0.864	1.02				60		
xiv	0.864	1.02	2.03	2.0	426	60	1.1	
xv	0.863	6.03	12.3 [b]	2.0	70	60	0.34	macroCTA
xvi	0.863	3.13	12.4 [b]	3.9	70	60	0.31	
xvii [c]	0.863	6.04				60		residual oxygen
xviii [c]	0.863	6.04	12.4	2.1	70	60		

[a] Monomer = methyl 6-*O*-methacryloyl-α-D-glucoside; initiator = 4,4′-azobis(4-cyanopentanoic acid); CTA (chain transfer agent) = 4-cyanopentanoic acid-4-dithiobenzoate; solvent = D_2O/DMSO-d_6 86:14; degassing via 4 freeze−pump−thaw cycles (ultimate pressure 6×10^{-2} mbar). [b] Oligo(methyl 6-*O*-methacryloyl-α-D-glucoside) dithiobenzoate M_n (NMR) 8400; M_n (SEC) 9900; PDI (SEC) 1.09. [c] No degassing. [d] Ratio between moles of primary radicals injected into the system by the time the reaction achieved steady state and the moles of initial chain transfer agent; we assumed a cumulative value of $f = 0.6$ and $\log (k_d/s^{-1}) = 17.0 - \{142\ kJ\}/\{(RT \ln 10)\}$.[24]

Table 2. Summary of Experiments on the Synthesis of Well-Defined Glyco-Oligomers and Macro-Chain Transfer Agents via RAFT [a]

run no.	monomer$_0$ (M)	initiator$_0$ (mM)	CTA$_0$ (mM)	reaction time (min)	convn (%) [b]	M_n/Da (theory)[c]	M_n/Da (SEC) [d]	M_w/M_n (SEC) [d]	DP$_n$ (SEC) [c]	L (%) [e]
xix	0.867	6.19	12.4	75	97	17 200	17 500	1.06	66	95
xx	0.865	10.9	21.5	90	100	10 100	11 400	1.05	42	94
xxi	0.796	20.3	39.9	90	100	5200	7400	1.05	27	94
xxii	0.531	25.7	52.9	125	93	2500	4300	1.12	15	92
xxiii	0.857	10.7	21.4	50	-	-	9900	1.09	37	96

[a] Monomer = methyl 6-*O*-methacryloyl-α-D-glucoside; initiator = 4,4′-azobis(4-cyanopentanoic acid); CTA (chain transfer agent) = 4-cyanopentanoic acid-4-dithiobenzoate; temperature 70 °C; $[CPADB]_0/[ACPA]_0 = 2.0$. Solvent: water/methanol-d_4, % alcohol (v/v) = xix-xx, xxiii 14; xxi 21; xxii 47. [b] From ^{1}H NMR, calculated from the ratio between the area of the vinyl proton peak at 6.5 ppm (H_E-10)[14] and that of the glucoside anomeric proton peak. [c] Calculated according to eq 7. [d] From SEC−RALS. [e] Fraction of living chains estimated according to eq 8 with a cumulative value of $f = 0.6$ and $\delta = 1$.

anomeric proton peak (H-1, internal standard) according to the formula:

$$x = 1 - \frac{A_{H_E10}}{A_{H1}} \quad (1)$$

where A_{H_E10}/A_{H1} for the monomer (zero conversion) was 1.0.

Synthesis of Well-Defined Glyco-Oligomers. The modus operandi was similar to that of kinetics experiments, with a few modifications. All experiments were carried out starting from the same stock solution of methyl 6-*O*-methacryloyl-α-D-glucoside (1.00 M) in water (HPLC grade). The radical initiator and RAFT agent were added as methanol-d_4 solutions. A dummy sample consisting of distilled water/methanol-d_4 87:13 was used for preshimming the magnet, and a water signal suppression sequence was used during ^{1}H NMR acquisition. The pulse program was repeated at 264 s intervals for the duration of the experiment. At the end of the polymerization, samples were stored in a refrigerator before being freeze-dried overnight. Part of each dried sample was then redissolved in DMF eluant and injected to the size exclusion chromatograph for molecular mass determination.

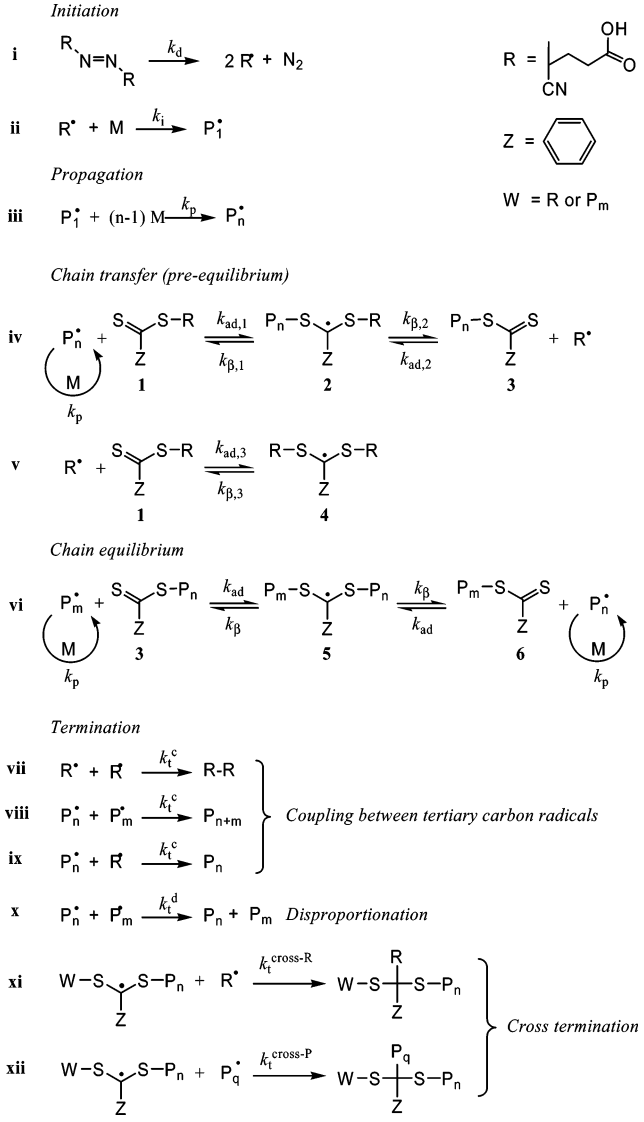
Synthesis of Oligo(methyl 6-*O*-methacryloyl-α-D-glucoside) Dithiobenzoate Macrochain Transfer Agent. Run xxiii in Table 2. The monomer solution (12.5 mL, 1.25×10^{-2} mol, HPLC water) was introduced in a Schlenk tube and mixed with ethanol solutions of 4,4′-azobis(4-cyanopentanoic acid) (9.71×10^{-2} M, 1.60 mL, 1.55×10^{-4} mol) and (4-cyanopentanoic acid)-4-dithiobenzoate (6.56×10^{-1} M, 0.475 mL, 3.11×10^{-4} mol). The tube was sealed with a greased glass stopper, degassed with three freeze−evacuate−thaw cycles and transferred to an oil bath preheated to 70 °C. After 50 min, the reaction was stopped by cooling in ice−water (5 min), and the tube was stored in a refrigerator for 1 h. Dialysis of the reaction mixture against purified water (24 h) followed by freeze-drying (5 days, dark) afforded the macroCTA in its pure form. Yield: 1.86 g, 55%. M_n (NMR) 8400; M_n (SEC) 9900; PDI (SEC) 1.09. ^{1}H NMR (300 MHz, D_2O, 50 °C) δ (ppm): 0.97 and 1.13 (H-11), 1.91 (CH_2 chain), 3.40 (4-H), 3.46 (*H*-7), 3.60 (2-H), 3.70 (3-H), 3.82 (5-H), 4.10 and 4.37 (6-H), 4.82 (*H*-1), 7.56 (H_{meta} arom), 7.73 (H_{para} arom), 7.97 and 8.00 (H_{ortho} arom) (see Scheme 2 for position numbering). After characterization, the compound was stored in a freezer (−18 °C) protected from moisture until needed.[23]

Results and Discussion

The monomer employed in this study (Scheme 1) was synthesized and purified in two batches according to the published method.[14] From each batch, a stock solution (1.00 M) in D_2O or water (HPLC grade) was prepared and stored in a freezer until needed. In order to maximize the consistency of results, kinetic experiments and glyco-oligomer syntheses were carried out starting from the same stock solution of monomer in D_2O and water, respectively.

The RAFT polymerization kinetics of methyl 6-*O*-methacryloyl-α-D-glucoside with 4-cyanopentanoic acid-4-dithiobenzoate in homogeneous aqueous medium (Scheme 2) was investigated via in situ ^{1}H NMR spectroscopy. 4,4′-Azobis(4-cyanopentanoic acid) was chosen as the initiator since upon decomposition it generates primary radicals identical to the leaving group of the RAFT agent, thus simplifying the mechanistic picture of preequilibrium (Scheme 3). Experimental parameters were varied

Scheme 3. Accepted Mechanism of Raft Polymerization with an Azo-Initiator [a]



[a] M = monomer, P_n = polymer chain with degree of polymerization "*n*".

so to elucidate the influence of temperature, initiator and chain transfer agent (CTA) concentration, molecular mass of the leaving group of the CTA, as well as the presence of residual oxygen on the occurrence (and extent) of inhibition and/or retardation phenomena (Table 1). In order to facilitate direct appreciation of the effect of CPADB on polymerization kinetics, most acquisition runs were conducted in couples of conventional radical (no CTA) and RAFT polymerizations identical in all respects except the presence of the chain transfer agent.

Effect of the Temperature. Figure 1a shows the first-order plot for the conventional radical and RAFT polymerization of 6-*O*-MAMGlc conducted at different temperatures (60, 70 and 80 °C) while keeping all other parameters constant. (Slightly smaller amounts of initiator were actually used for runs at higher temperature, but the difference was 6.7% at most; see entry nos. i−iv and vii−viii in Table 1.) In the absence of the CTA, polymerizations were very fast and reached completion in 30 to 60 min depending on temperature. Pseudo-first-order kinetics were obeyed up to high conversion, as confirmed by the excellent linearity of all plots (squared correlation coefficient $r^2 \geq 0.999$ for linear fit of the first four to five points), and no inhibition was observed (the first point was taken after 3−5 min of reaction). The corresponding RAFT experiments presented instead a more complex behavior: after a very slow start, the processes gradually evolved toward faster polymerization rates and eventually attained steady-state kinetics, which was then followed throughout. At 60 °C no monomer conversion was observed during the first 7 min of reaction. Higher polymerization temperatures clearly resulted in a shorter initial non-steady-state regime, as can be better appreciated by a plot of $\langle k_p \rangle [P^\bullet]$ vs time (Figure 1b): at 60 and 70 °C $\langle k_p \rangle [P^\bullet]$ increased monotonically over 55 and 35 min, respectively, before reaching a constant value (steady-state), whereas at 80 °C the same rose rapidly during the first 15 min, reached a maximum and then decreased to slightly lower values, possibly because of fast initiator consumption. Here the following formulas were used:

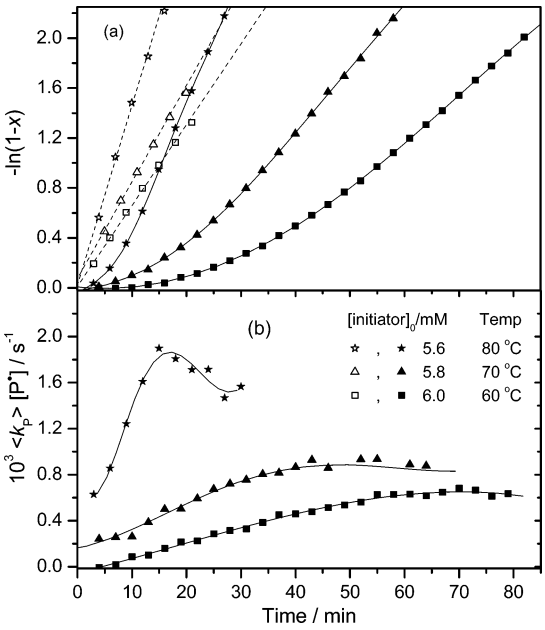


Figure 1. First-order plot (a) and evolution of $k_p[P^\bullet]$ with time (b) for the polymerization of methyl 6-*O*-methacryloyl-α-D-glucoside at different temperatures (see runs i−iv and vii−viii in Table 1; x = fractional conversion). Conditions: $[6\text{-}O\text{-MAMGlc}]_0 = 0.86$ M, $[CPADB]_0 = 12$ mM, D_2O/DMSO-d_6, 86:14 (refer to Scheme 1 for definition of abbreviations). Open symbols refer to conventional radical polymerization (RAFT agent-free) under identical conditions. Dashed and solid lines indicate linear and polynomial regression of experimental data, respectively.

$$R_p = -\frac{d[M]}{dt} = [M]_0 \frac{dx}{dt} \quad (2)$$

$$\frac{R_p}{[M]} = \langle k_p \rangle [P^\bullet] \quad (3)$$

where x is conversion, [M] and $[P^\bullet]$ are the monomer and propagating radical concentration respectively, and $\langle k_p \rangle$ is the chain-length-averaged propagation rate coefficient (see below). It is worth noting that eq 2 and 3 imply that monomer consumption is only due to chain propagation and not to initiation and chain transfer.[25] This assumption is reasonable for all reactions summarized in Table 1, since no more than 1.6% of the monomer was consumed in initiation reactions,[26,24] and methacrylates have a very low chain transfer coefficient toward both water[27] and DMSO[28] (e.g., $C_s < 10^{-5}$ for methyl methacrylate).

In theory, from $\langle k_p \rangle [P^\bullet]$ the concentration of propagating radicals could be directly estimated simply dividing by $\langle k_p \rangle$.[29] Unfortunately, not only has the chain-length-averaged propaga-

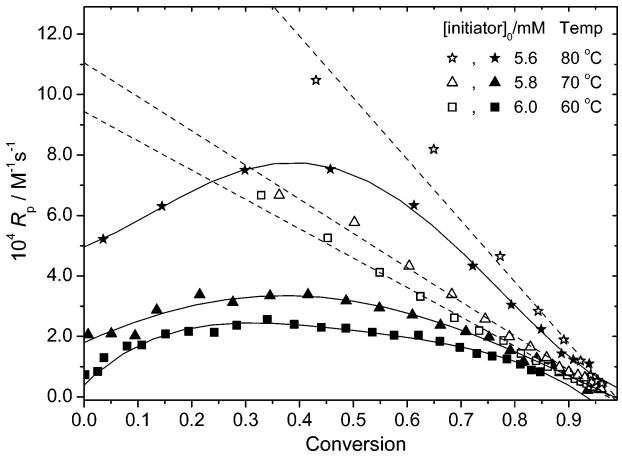


Figure 2. Plot of the rate of polymerization (R_P) vs conversion for the polymerization of methyl 6-*O*-methacryloyl-α-D-glucoside at different temperatures (see runs i−iv and vii−viii in Table 1). Conditions: [6-*O*-MAMGlc]$_0$ = 0.86 M, [CPADB]$_0$ = 12 mM, D_2O/DMSO-d_6, 86:14 (refer to Scheme 1 for definition of abbreviations). Open symbols refer to conventional radical polymerization (RAFT agent-free) under identical conditions. Dashed and solid lines indicate linear and polynomial regression of experimental data, respectively.

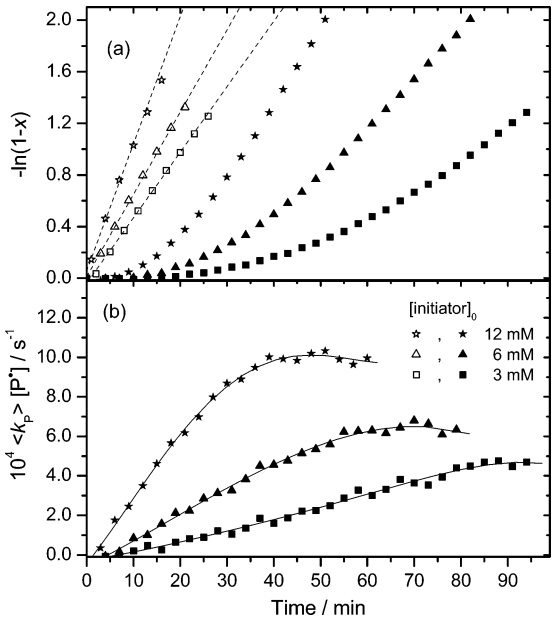


Figure 3. First-order plot (a) and evolution of $k_p[P^\bullet]$ with time (b) for the polymerization of methyl 6-*O*-methacryloyl-α-D-glucoside with different initiator concentrations (see runs v−x in Table 1; x = fractional conversion). Conditions: [6-*O*-MAMGlc]$_0$ = 0.86 M, [CPADB]$_0$ = 12 mM, 60 °C, D_2O/DMSO-d_6, 86:14 (refer to Scheme 1 for definition of abbreviations). Open symbols refer to conventional radical polymerization (RAFT agent-free) under identical conditions. Dashed and solid lines indicate linear and polynomial regression of experimental data, respectively.

tion rate coefficient for 6-*O*-MAMGlc (in any solvent) never been measured, but in our case its chain-length dependence (CLD) should be taken into account explicitly. In fact, in a well-behaved RAFT polymerization (Scheme 3) the average chain length of propagating radicals increases linearly with conversion according to the formula:[30]

$$DP_n \approx x \frac{[M]_0}{[CTA]_0} \quad (4)$$

where x is conversion and $[M]_0$ and $[CTA]_0$ are the initial concentrations of monomer and chain transfer agent, respectively. It follows that at low conversion only short radicals are present in the reaction medium, and the rate coefficients (k_p, k_t, k_β, etc.) governing the process are positively chain length dependent.[31,32] In particular, in the case of k_p a body of evidence[33] indicates that the long-chain value is attained only after ∼4 propagation steps (excluding initiation), and that $k_p^1 > k_p^2 > k_p^3 > k_p^4 \approx k_p$. Thus, for an initial monomer to chain transfer agent ratio of 70, $\langle k_p \rangle$ will keep decreasing up to ∼6% conversion, after which it can be assumed to be constant. It follows that for all kinetic experiments reported in this part of our study (Table 1), above ∼6% conversion any variation in the value of $\langle k_p \rangle [P^\bullet]$ can be safely ascribed to a variation in the concentration of propagating radicals, and that a constant value for $\langle k_p \rangle [P^\bullet]$ indicates the steady-state.

Figure 2 shows the evolution of the rate of polymerization (R_p) with conversion for the same set of experiments (runs i−iv and vii−viii), and allows immediate appreciation of the rate retardation accompanying the RAFT process. In the case of conventional radical polymerization (RP), R_p decreases linearly with conversion as expected for pseudo-first-order kinetics ($r^2 \geq 0.974$ for linear fit); whereas in the corresponding (same initial concentration of initiator) RAFT processes at 60 and 70 °C R_p increases up to ∼20% conversion, remains approximately constant for a protracted conversion range (up to ∼55%), and then decreases almost linearly. The same general trend is observed for RAFT polymerization at 80 °C but in this case R_p stays constant for a significantly shorter span. At all temperatures tested and for any given conversion, RAFT polymerization is slower than the corresponding RP process, the difference being macroscopic for the first half of the process and narrowing to less than ∼1/4 above 80% conversion. By the end of the polymerization (30−100 min) about 2.6, 7.6, and 16% of the starting initiator is consumed at 60, 70, and 80 °C respectively.[34]

Influence of the Initiator Concentration. In the previous set of experiments, by changing the polymerization temperature while keeping the initial concentration of initiator constant, the flux of primary radicals injected into the system was varied. Charreyre et al.[35] already reported that for the RAFT polymerization of *N*-acryloyl morpholine with *tert*-butyl dithiobenzoate at 90 °C a high CTA$_0$/initiator$_0$ ratio can result in a long induction period. In order to verify whether the flux of primary radicals plays a role in the length of the initial non-steady-state period for the 6-*O*-MAMGlc/CPADB system, three experiments were carried out identical in all respects other than the initial amount of initiator (60 °C, 6-*O*-MAMGlc$_0$/CPADB$_0$ = 70; see runs v−x in Table 1). The corresponding first-order plots are shown in Figure 3a. As observed previously, conventional radical polymerizations were very fast, followed pseudo-first-order kinetics up to high conversion ($r^2 \geq 0.998$ for linear fit of the first 4−5 points), and displayed no induction period (the first point was taken after 1−3 min of reaction). By contrast, RAFT polymerizations started slowly, gradually accelerated, and attained steady-state kinetics only late in the process. In run x, no monomer consumption was actually observed for the first 10 min of reaction. Clearly, the length of the non-steady-state period was inversely proportional to the initial concentration of initiator, and consequently to the initial CTA/initiator ratio. This aspect is best appreciated from the $\langle k_p \rangle [P^\bullet]$ vs time plot (Figure 3b), which shows how the non-steady-state regime was shortened from 85 to 55 and 35 min as the CTA$_0$/initiator$_0$ ratio decreased from 4.0, to 2.0, to 1.0 respectively. As expected, the steady-state value of $\langle k_p \rangle [P^\bullet]$ scales as $[\text{initiator}]_0^{1/2}$.

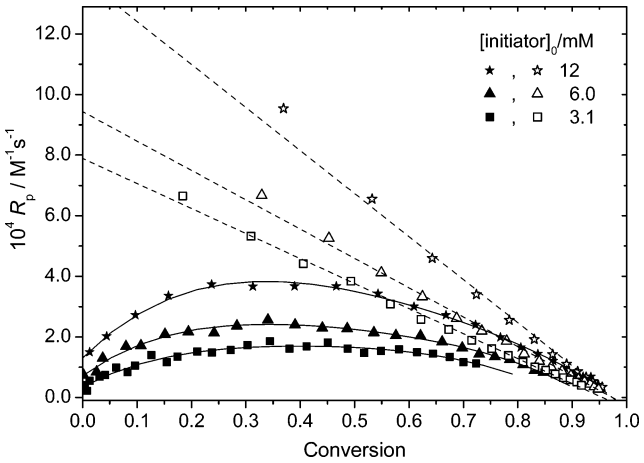


Figure 4. Plot of the rate of polymerization (R_P) vs conversion for the polymerization of methyl 6-*O*-methacryloyl-α-D-glucoside with different initiator concentrations (see runs v−x in Table 1). Conditions: [6-*O*-MAMGlc]$_0$ = 0.86 M, [CPADB]$_0$ = 12 mM, 60 °C, D_2O/DMSO-d_6, 86:14 (refer to Scheme 1 for definition of abbreviations). Open symbols refer to conventional radical polymerization (RAFT agent-free) under identical conditions. Dashed and solid lines indicate linear and polynomial regression of experimental data, respectively.

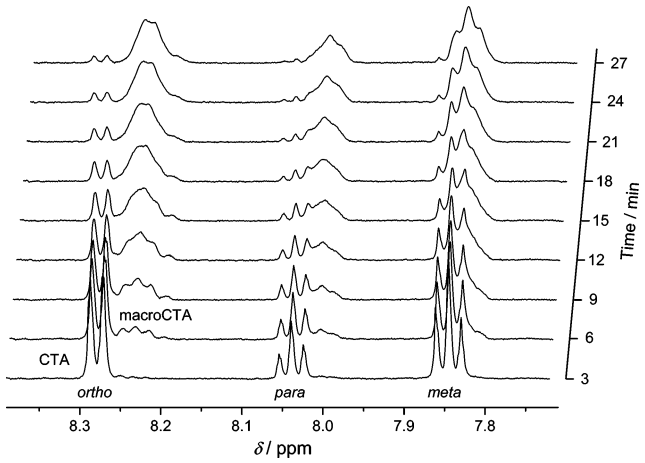


Figure 5. Evolution of the NMR peaks of the dithiobenzoyl aromatic protons with time for the polymerization of methyl 6-*O*-methacryloyl-α-D-glucoside with (4-cyanopentanoic acid)-4-dithiobenzoate (see run vi in Table 1). Conditions: [6-*O*-MAMGlc]$_0$ = 0.86 M, [CPADB]$_0$ = 12 mM, 60 °C, D_2O/DMSO-d_6, 86:14; 500 MHz (refer to Scheme 1 for definition of abbreviations).

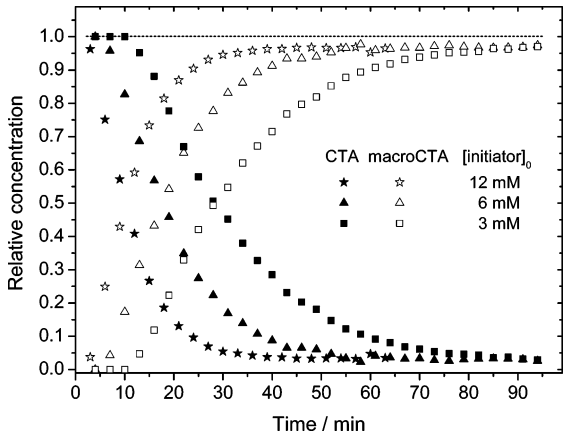


Figure 6. Relative concentrations of the aromatic *ortho*-proton of dithiobenzoate species vs time in the RAFT polymerization of methyl 6-*O*-methacryloyl-α-D-glucoside with different initiator concentrations (see runs vi, viii, and x in Table 1). Conditions: [6-*O*-MAMGlc]$_0$ = 0.86 M, [CPADB]$_0$ = 12 mM, 60 °C, D_2O/DMSO-d_6, 86:14 (refer to Scheme 1 for definition of abbreviations).

Figure 4 shows the evolution of the rate of polymerization with conversion for the same set of experiments (runs v−x). In the case of conventional radical polymerizations R_p decreases linearly with conversion ($r^2 \geq 0.994$ for a linear fit) as expected for pseudo-first-order kinetics, while in the analogous RAFT processes R_p follows an almost parabolic trend in which it remains roughly constant for an extended range between ∼25 and ∼60% conversion. For all starting initiator concentrations tested and for any given conversion, the RAFT process was slower than the corresponding RP, the difference becoming quite small (less than $\sim^1/_3$) only above 80% conversion. The linear fits of the R_p vs conversion data for conventional radical polymerization were extrapolated to zero conversion and afforded R_p^0 values for different starting initiator concentrations. Assuming $\langle k_t \rangle \cong 7 \times 10^7$ M^{-1} s^{-1}, $k_d \cong 5 \times 10^{-6}$ s^{-1} and $f \cong 0.6$, a rough estimate of the propagation rate constant at 60 °C was calculated to be $\langle k_p \rangle \cong 7 \times 10^4$ M^{-1} s^{-1}.[24,31] This value is of the same order of magnitude of $\langle k_p \rangle$ of nonionized methacrylic acid at 60 °C in aqueous solution ($\langle k_p \rangle = 1.2 \times 10^4$ M^{-1} s^{-1} for [MAA]$_0 \cong 0.6$ M).[36]

Overall, these results are quite similar to what was observed by changing the polymerization temperature while keeping the initial concentration of initiator constant (Figures 1 and 2), and they seem to indicate that the ratio between the starting amount of CTA present in solution and the flux of primary radicals injected into it plays a key role in determining the onset of steady-state. We believe that the same could be said of temperature alone (with higher temperatures resulting in shorter non-steady-state periods in the presence a constant flux of primary radicals), but our experiments cannot prove that. Concerning the effect of temperature, Barner-Kowollik et al. recently found for the RAFT polymerization of styrene with cumyl dithiobenzoate in bulk and under a constant source of γ radiation (i.e., a low and constant radical flux),[37] the duration of the non-steady-state period is greatly reduced by raising the temperature from 30 to 70 °C.[38] They also found that at elevated temperatures the rate of polymerization for the RAFT and non-RAFT processes are identical, as predicted by the slow fragmentation model.[39]

The fact that the ratio between the initial amount of CTA and the flux of primary radicals determines the length of the initial non-steady-state period, suggests that transformation of chain transfer agent **1** into macrochain transfer agent **3** (Scheme 3) is a prerequisite for the establishment of a steady-state regime. When a sufficiently high concentration of (4-cyanopentanoic acid)-4-dithiobenzoate was used, this transformation could be monitored through the change with time of the dithiobenzoyl aromatic ortho proton NMR peaks. As shown in Figure 5, the initial double-doublet at 8.28 ppm gradually disappears in favor of a new multiplet centered at 8.24 ppm. Deconvolution of the two peaks and integration allowed us to estimate the relative concentration of CTA and macroCTA throughout the course of runs ii, vi, viii, and x (Table 1). We thus found that the initial chain transfer agent did not disappear at the beginning of the reaction but was gradually consumed over a period of time that was not small in the time scale of the experiment (Figure 6). In particular, at 60 °C it took 85, 55, and 40 min for the concentration of CTA to reach its minimum value when a $CPADB_0/ACPA_0$ ratio of 4, 2, and 1 was used, respectively. These values match exactly the duration of the non-steady-state period as estimated by a $\langle k_p \rangle [P^\bullet]$ vs time plot (Figure 3b). Also, in run x, no chain transfer agent was consumed during the first 10 min of reaction; a period coinciding with complete inhibition

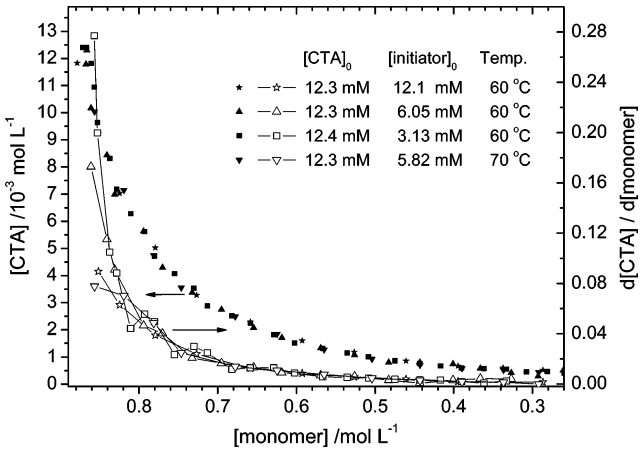


Figure 7. Plot of the chain transfer agent vs monomer concentration (solid symbols) and rate of consumption of the CTA (open symbols) for the RAFT polymerization of methyl 6-*O*-methacryloyl-α-D-glucoside at different temperatures and with different initiator concentrations (see runs ii, vi, viii, and x in Table 1). Conditions: $[6\text{-}O\text{-MAMGlc}]_0 = 0.86$ M, D_2O/DMSO-d_6, 86:14 (refer to Scheme 1 for definition of abbreviations).

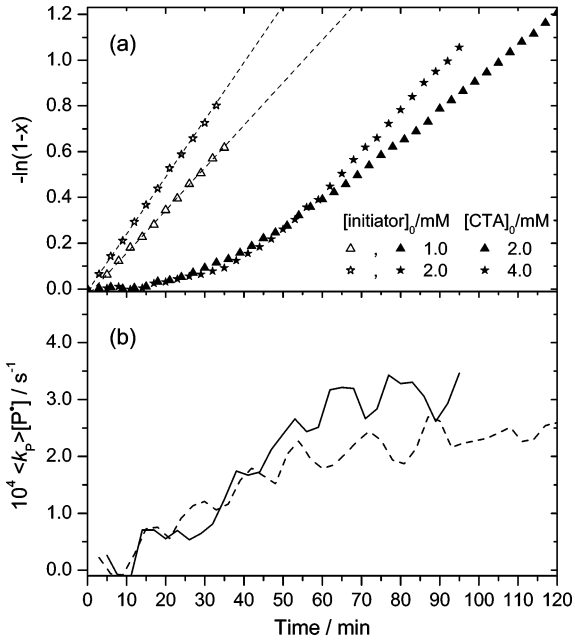


Figure 8. First-order kinetic plot (a) and evolution of $k_p[P^•]$ with time (b) for the polymerization of methyl 6-*O*-methacryloyl-α-D-glucoside with different chain transfer agent concentrations and a constant chain transfer agent to initiator ratio (see runs xi−xiv in Table 1; x = fractional conversion). Conditions: $[6\text{-}O\text{-MAMGlc}]_0 = 0.86$ M, $CPADB_0/ACPA_0 = 2.0$, 60 °C, D_2O/DMSO-d_6, 86:14 (refer to Scheme 1 for definition of abbreviations). Open symbols refer to conventional radical polymerization (RAFT agent-free) under identical conditions. Dashed lines in graph a indicate linear regression of experimental data.

of the polymerization process (see Figure 3a). In all cases the concentration of unreacted dithiobenzoyl groups dropped monotonically down to ∼3% of its initial value, after which it remained constant for the rest of reaction. This might well indicate unused (4-cyanopentanoic acid)-4-dithiobenzoate, or most probably a degradation product or the product of a side-reaction.

Interestingly, a plot of the CTA vs monomer concentration (Figure 7) reveals that complete consumption of the initial chain transfer agent is only attained around 60% monomer conversion, irrespective of the $CPADB_0/ACPA_0$ ratio and of temperature (here we ignore the residual NMR signal). Indeed the rate of consumption of CPADB is almost identical in the four cases examined, with a higher flux of primary radicals resulting in a lower rate of utilization at low conversion. Rizzardo et al.[40,41] suggested that "in the case of reversible chain transfer, the rate of consumption of the transfer agent depends on two transfer coefficients, C_{tr} ($=k_{tr}/k_p$) and C_{-tr} ($=k_{-tr}/k_i$), which describe the reactivity of the propagating radical ($P_n^•$) and of the expelled radical ($R^•$) respectively", and that the same can be estimated by solving numerically the equations (Scheme 3).

$$\frac{d[\mathbf{1}]}{d[M]} \approx C_{tr}\frac{[\mathbf{1}]}{[M] + C_{tr}[\mathbf{1}] + C_{-tr}[\mathbf{3}]} \qquad (5)$$

Unfortunately, this analysis is based on a steady-state approximation for the concentration of primary and propagating radicals which does not clearly hold for the system under study. For this reason, although all necessary data are available, eq 5 was not used to derive chain transfer coefficients for reaction iv (pre-equilibrium in Scheme 3).

The most noticeable consequence of slow consumption of the chain transfer agent is that at low conversion the molecular weight of the obtained polymer is higher than the theoretical value calculated from the formula[30]

$$M_n^{\text{theory}} \approx M_M x\frac{[M]_0}{[CTA]_0} + M_{CTA} \qquad (6)$$

where M_M and M_{CTA} are the molecular weights of monomer and CTA respectively, x is conversion, and $[M]_0$ and $[CTA]_0$ are the initial concentrations of monomer and CTA. This phenomenon was in fact observed by Davis et al.[14] for the system under study, although part of the discrepancy in that work might have arisen from the use of polystyrene calibration to calculate molecular weights.

A reviewer suggested that cross termination of intermediate radicals (reactions xi and xii in Scheme 3) might explain the observed kinetics. Yet, results from a previous paper[42] suggest that in the case of poly(methyl 6-*O*-methacryloyl-α-D-glucoside)dithiobenzoate coupling reactions between macromolecular species do not take place easily. Also, if irreversible termination of the intermediate radical were the main cause of retardation, R_p would be reduced by a constant factor,[43] and the extended non-stationary-state period observed in the first part of the reaction could only be explained by assuming that the rate coefficients governing the process (k_{ad}, k_β, k_t^{cross}) are chain-length-dependent up to $DP_n \approx 40$; which seems unlikely.

Influence of the Chain Transfer Agent Concentration. To further corroborate our hypothesis, two experiments were conducted at different (and lower) CPADB concentrations (2.0 and 4.0 mM) while holding the CPADB to initiator ratio constant ($CTA_0/initiator_0 = 2.0$, temperature 60 °C).[44] As shown in Figure 8, during the first ∼50 min of reaction both the resulting first-order plots and the concentration of propagating radicals are virtually identical, and both reactions achieved steady state after ca. 65 min. As expected, the steady-state value of $\langle k_p\rangle[P^•]$ scales with $[initiator]_0^{1/2}$. Clearly, for the system under study it is consumption of the initial CTA (rather than its absolute value) that dominates reaction kinetics during the non-steady-state regime. Nevertheless, a lower concentration of CTA resulted in lesser rate retardation: for instance, comparison of Figure 9 and Figure 4 shows that above 60% conversion no rate retardation is observed for $[CPADB]_0 = 1.0$ mM, whereas for $[CPADB]_0 = 12$ mM the RAFT process is ∼30−45% slower than the corresponding radical polymerization (runs xiii−xiv and vii−viii in Table 1).

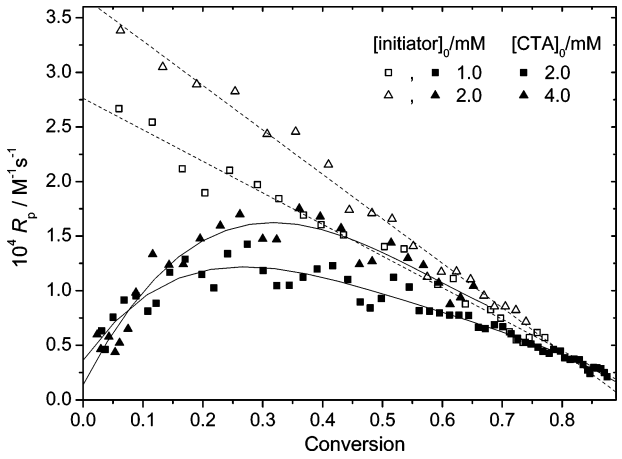


Figure 9. Plot of the rate of polymerization (R_p) vs conversion for the polymerization of methyl 6-*O*-methacryloyl-α-D-glucoside with different chain transfer agent concentrations and a constant chain transfer agent to initiator ratio (see runs xi−xiv in Table 1). Conditions: [6-*O*-MAMGlc]$_0$ = 0.86 M, CPADB$_0$/ACPA$_0$ = 2.0, 60 °C, D_2O/DMSO-d_6, 86:14 (refer to Scheme 1 for definition of abbreviations). Open symbols refer to conventional radical polymerization (RAFT agent-free) under identical conditions. Dashed and solid lines indicate linear and polynomial regression of experimental data, respectively.

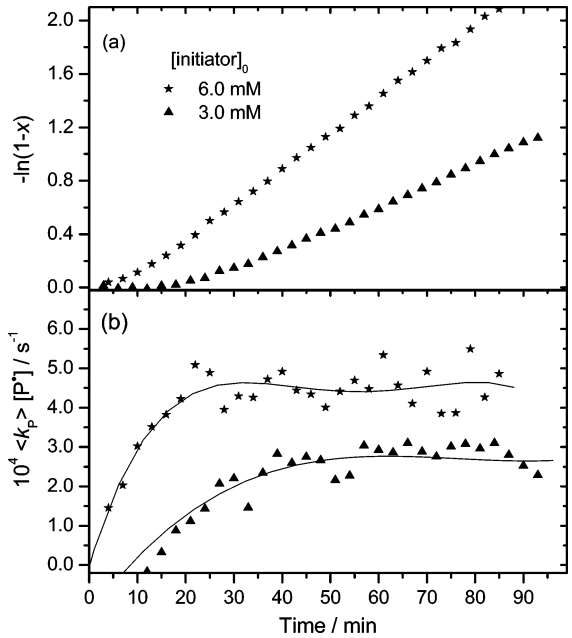


Figure 10. First-order kinetic plot (a) and evolution of $\langle k_p \rangle$[P$^\bullet$] with time (b) for the RAFT polymerization of methyl 6-*O*-methacryloyl-α-D-glucoside with a constant macrochain transfer agent concentration and different initiator concentrations (x = fractional conversion). Conditions: [6-*O*-MAMGlc]$_0$ = 0.86 M, [macroCTA]$_0$ = 12 mM, 60 °C, D_2O/DMSO-d_6, 86:14 (refer to Scheme 1 for definition of abbreviations). Solid lines indicate polynomial regression of experimental data.

Use of a Macrochain Transfer Agent. For the RAFT polymerization of methyl 6-*O*-methacryloyl-α-D-glucoside with (4-cyanopentanoic acid)-4-dithiobenzoate, the initial non-steady-state period appears to coincide with time needed to transform the initial chain transfer agent **1** into a polymeric CTA **3** (Scheme 3). If this were the only prerequisite for steady-state kinetics, the use of a macroCTA would eliminate the non-steady-state period. Consequently, we repeated two of the polymerizations previously described (runs viii and x in Table 1) replacing the initial CPADB with poly(6-*O*-MAMGlc)dithiobenzoate (M_n = 9900; PDI = 1.09) used at the same concentration ([macroCTA]$_0$ = 12 mM; runs xv−xvi). The results are shown in Figure 10: When a macroCTA$_0$/initiator$_0$ ratio of 2.0 was used, it took ∼20 min for the system to attain steady state; when a lower concentration of initiator was used (macroCTA$_0$/initiator$_0$ = 3.9), an inhibition period of ∼10 min resulted which was followed by a non-steady-state period of ∼25 min. As for previous runs, once a steady state was achieved, it was retained for the rest of the process. By comparison, equivalent experiments with CPADB took a total of 55 and 85 min to achieve steady state, and the same apparent inhibition period of ∼10 min was observed for CTA$_0$/initiator$_0$ = 4.0.

Hence, replacing CPADB with a polymeric chain transfer agent did abbreviate the time needed to reach steady state, but did not eliminate it completely. Moreover, its duration proved to still depend on the CTA$_0$/initiator$_0$ ratio. It is worth noting that there is no obvious relationship between the moles of primary radicals injected into the system by the time the reaction achieved steady state and the moles of initial CTA (Table 1). In fact, at 60 °C the ratio between the two was 0.75−1.2% when CPADB was the chain transfer agent and ∼0.3% when a macroRAFT agent was used. In the case of CPABD, the same ratio was 2.6% and 4.3% for reaction conducted at 70 and 80 °C, respectively. Consequently, contamination of the CTA with a radical scavenger can be safely ruled out as possible cause for the observed kinetics (^{1}H and ^{13}C NMR spectra of the CPADB used in this study can be found in the Supporting Information).

To the best of our knowledge, this is the first report describing a non-steady-state period for a RAFT polymerization mediated by a polymeric chain transfer agent. Fukuda et al.[45] reported that both the styrene/PS-dithioacetate and the methyl methacrylate/PMMA-dithiobenzoate systems display no induction period and follow first-order kinetics from the start. Davis et al.[46] observed the same for the methyl acrylate/PMA-dithiobenzoate system while Charreyre et al.[35] reported a ∼2−3 min induction period for the *N*-acryloyl morpholine/PNAM−dithiobenzoate system (although the latter value was within experimental error).[47] Interestingly, in their paper on the synthesis of diblock glycopolymers via RAFT,[15] Davis et al. reported that during chain extension of poly(methyl 6-*O*-methacryloyl-α-D-glucoside) dithiobenzoate and poly(2-methacryloxyethyl glucoside) dithiobenzoate with methacrylate-type glycomonomers, a ∼10 min induction period was followed by pseudo-first-order kinetics; a CTA$_0$/initiator$_0$ ratio of 1.7 and 1.2 was used in the two cases.

Effect of Residual Oxygen. In its most stable form, oxygen exists as a diradical in which two unpaired electrons occupy two degenerate molecular orbitals of the molecule (triplet oxygen). As a result, oxygen interferes with radical polymerizations in many different ways,[48] and is well-known for inducing inhibition periods and retarded polymerization kinetics.[49] The rate constant for reaction between a primary or propagating radical and oxygen (∼10^9 M^{-1} s^{-1})[50,51] is much higher than that for addition of the same radical to a monomer molecule (k_i, k_p ∼10^1−10^5),[52] and is relatively insensitive to substituents. It follows that between 0.01 and 100 ppm of O_2 are sufficient to compete with monomer molecules for radical addition and to alter polymerization kinetics.[53,54] Favier et al.[55] already published a spectroscopic investigation into the effect of oxygen in the xanthate-mediated polymerization of vinyl acetate in bulk and found that not degassing the reaction mixture induces an inhibition period varying from 1.5 to over 5 h.

In our study, we compared the polymerization kinetics of two couples of experiments identical in all respects other than the deoxygenation protocol (runs vii−viii and xvii−xviii in

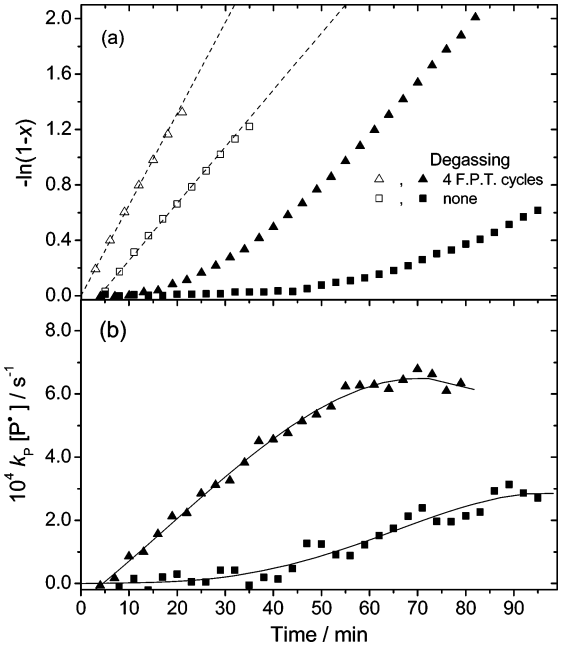


Figure 11. First-order kinetic plot (a) and evolution of $k_p[P^\bullet]$ with time (b) for the polymerization of methyl 6-*O*-methacryloyl-α-D-glucoside following either 4 freeze−pump−thaw (F.P.T.) cycles or no degassing at all (see runs vii−viii and xvii−xviii in Table 1; x = fractional conversion). Conditions: $[6\text{-}O\text{-MAMGlc}]_0 = 0.86$ M, $[CPADB]_0 = 12$ mM, $[ACPA]_0 = 6.0$ mM, 60 °C, D_2O/DMSO-d_6, 86:14 (refer to Scheme 1 for definition of abbreviations). Open symbols refer to conventional radical (RAFT agent-free) polymerization under identical conditions (see Table 1). Dashed and solid lines indicate linear and polynomial regression of experimental data, respectively.

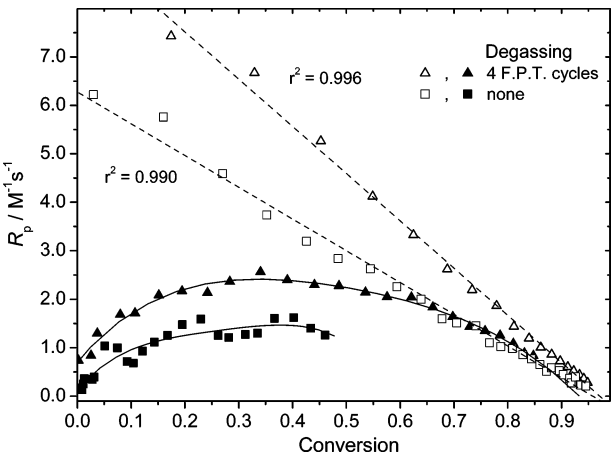


Figure 12. Plot of the rate of polymerization (R_p) vs conversion for the polymerization of methyl 6-*O*-methacryloyl-α-D-glucoside following a different number of freeze−pump−thaw (F.P.T.) cycles (see runs vii−viii and xvii−xviii in Table 1). Conditions: $[6\text{-}O\text{-MAMGlc}]_0 = 0.86$ M, $[CPADB]_0 = 12$ mM, $[ACPA]_0 = 6.0$ mM, 60 °C, D_2O/DMSO-d_6, 86:14 (refer to Scheme 1 for definition of abbreviations). Open symbols refer to conventional radical (RAFT agent-free) polymerization under identical conditions (see Table 1). Dashed and solid lines indicate linear and polynomial regression of experimental data, respectively.

Table 1), which consisted either of four freeze−pump−thaw cycles or of no degassing at all. As shown in Figure 11 and Figure 12, not degassing the reaction mixture resulted both in a short induction period (∼4 min) and in a 37% reduction in the rate of conventional radical polymerization (as judged by the slope of first-order plots). After the initial inhibition period, both polymerizations followed first-order kinetics up to high conversion ($r^2 \geq 0.998$ for linear fit of the first five points). The effect of oxygen was more pronounced in the corresponding

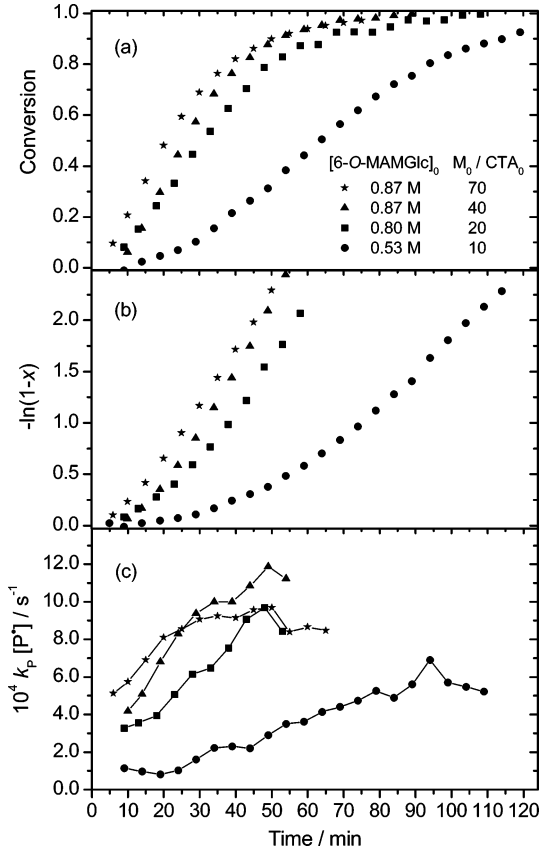


Figure 13. Conversion vs time (a), first-order (b), and $k_p[P^\bullet]$ vs time plot (c) for the synthesis of oligo(methyl 6-*O*-methacryloyl-α-D-glucoside) with a number-average degree of polymerization in the range 15−66 (runs xix−xxii in Table 2; x = fractional conversion). Conditions: 70 °C, water/methanol-d_4, $CPADB_0/ACPA_0 = 2.0$ (refer to Scheme 1 for definition of abbreviations).

RAFT experiments. When the reaction mixture was not degassed (the tube was nevertheless sealed), an inhibition period of 40 min resulted which was followed by a much retarded polymerization (40−50% reduction in R_p at equal conversion). The latter result is consistent with the accepted mechanism of RAFT polymerization (Scheme 2), and suggests the probable termination of intermediate radicals **2**, **4**, and **5** with oxygen.

Synthesis of Well-Defined Glyco-Oligomers via RAFT. Findings from the above-reported kinetic study were applied to the synthesis of well-defined oligo(methyl 6-*O*-methacryloyl-α-D-glucoside) in high yield (Table 2). In particular, 6-*O*-MAMGlc was directly polymerized in water/methanol-d_4 mixtures at relatively high-temperature (70 °C) and using a fairly high chain transfer agent to initiator ratio ($CPADB_0/ACPA_0 = 2.0$ in all cases). These conditions were chosen so as to favor rapid fragmentation of intermediate radicals **2**, **4**, and **5** (Scheme 3)[39] and to balance rapid monomer consumption with a final content of living chains >90% (see below).[56]

Four experiments were conducted in which the target DP_n at 100% conversion varied from 10, to 20, to 40 and 70 (runs xix−xxiii in Table 2). Reactions were monitored via in situ ^1H NMR using a water signal suppression sequence and reached completion after 75−125 min depending on the reagents concentration (Figure 13a; run xxii was actually stopped at 93% conversion). As can be seen in Figure 13, parts b and c, runs xx and xxi achieved steady state very late in the process (after ∼45 min), when 90% of the initial monomer had already been consumed; whereas it took 30 and 80 min in runs xix and xxii respectively, which corresponds to ∼75% conversion. It is

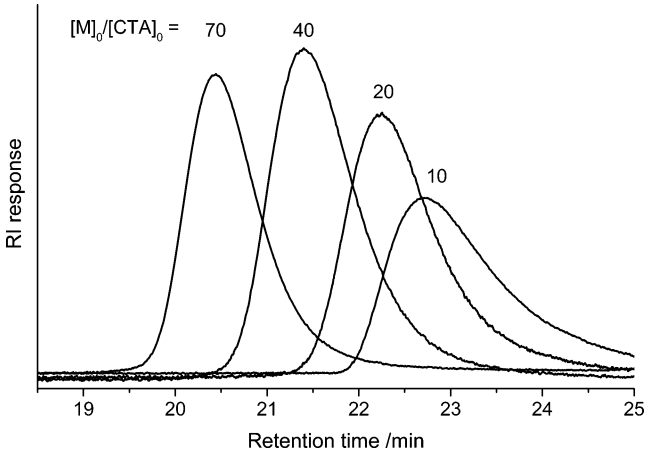


Figure 14. Size exclusion chromatography traces of the glyco-oligomers obtained from the RAFT polymerization of methyl 6-*O*-methacryloyl-α-D-glucoside with different monomer to chain transfer agent ratios (normalized areas; conversion >93% in all cases; see runs xix−xxii in Table 2). Chromatographic conditions: *N*,*N*-Dimethylformamide /LiBr 0.1% w/v /2,6-di-*tert*-butyl-4-methylphenol 0.05% w/v, 1 mL min^{-1}, 60 °C.

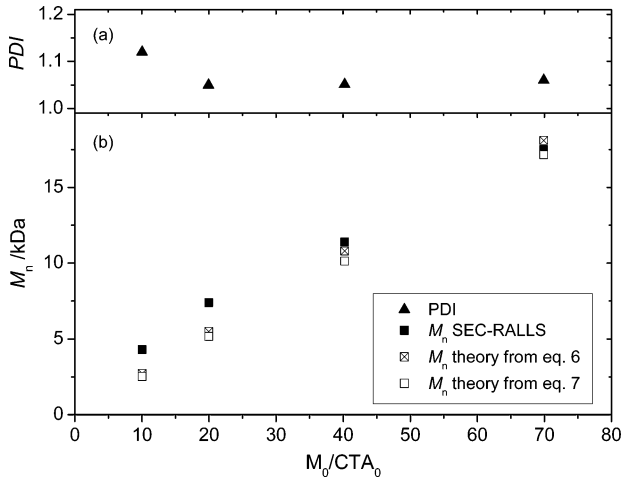


Figure 15. Relationship between the experimental polydispersity index (a), the experimental and theoretical molecular weight (b), and the initial monomer to CTA ratio for the synthesis of well-defined oligo(methyl 6-*O*-methacryloyl-α-D-glucoside) via RAFT.

gratifying to note that the extent of the non-steady-state period for run xix (30 min) is comparable to that of the analogous reaction conducted in D_2O/DMSO-d_6, 86:14 (35 min), especially if the slightly smaller amount of initiator is taken into account (see run ii in Table 1). Also, both reactions have a steady-state value of $k_p[P^\bullet] \approx 9 \times 10^{-4}$ s^{-1}.

In spite of the high concentration of azo-initiator used and of the nearly quantitative monomer conversion, the size exclusion chromatography traces of the reaction mixture (Figure 14) are all monomodal and symmetrical, with only run xxii (6-*O*-MAMGlc$_0$/CPADB$_0$ = 10) displaying significant tailing. No high molecular weight shoulder is detectable. Narrow polydispersity materials were obtained in all cases (PDI = 1.05−1.12), while a good correlation between theoretical and experimental molecular weights was only observed for 6-*O*-MAMGlc$_0$/CPADB$_0$ = 40 and 70 (Figure 15b). When shorter oligomers were targeted (runs xxi and xxii), the molecular weight measured by SEC was in fact 40−70% higher than predicted by eq 7, thus confirming the idea of nonimmediate chain transfer to the CTA. Concerning theoretical molecular weights, it should be noted that since under the experimental conditions used the

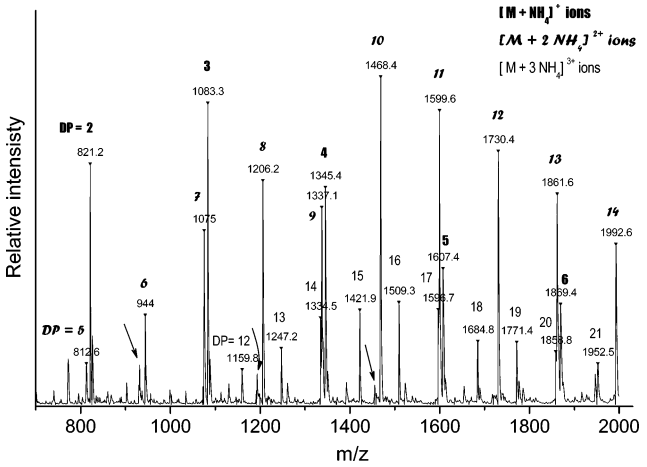


Figure 16. Electrospray ionization-ion trap mass spectrum of oligo-(methyl 6-*O*-methacryloyl-α-D-glucoside) obtained in run xxii (Table 2). The sample mostly consists of oligomers with degree of polymerization ranging from 2 to 21 and still bearing the end-of-chain dithiobenzoyl group (labeled peaks). The complex peak pattern is due to multiple ionization of part of the chains. Arrows indicate peaks corresponding to chains terminated via disproportionation. Conditions: acetonitrile/1 mM aqueous $NH_4^+HCOO^-$ 4:6.

fraction of initiator-derived chains is not negligible, the following formula should be used for calculations:[30]

$$M_n^{\text{theory}} = M_M x \frac{[M]_0}{[CTA]_0 + f([I]_0 - [I])(1+\delta)} + M_{CTA} \quad (7)$$

where $[I]_0$ and $[I]$ are initial and current initiator concentrations, f is the initiator efficiency, and δ is the contribution of disproportionation to the overall termination process ($0 \leq \delta \leq 1$).

Also, of the total number of polymer chains produced by the RAFT process, a number equal to those initiated by CTA derived radicals will possess a thiocarbonylthio end group and hence remain living, whereas a number equal to those initiated by primary radicals will terminate irreversibly.[57] It follows that the fraction of living chains in a sample will be given by the ratio between the number of CTA molecules and the number of chains produced by primary radicals *plus* the number of CTA molecules (eq 8).

$$L = \frac{[CTA]_0}{[CTA]_0 + f([I]_0 - [I])(1+\delta)} \quad (8)$$

For the synthesis of oligo(6-*O*-MAMGlc) via RAFT, the relatively fast polymerization rates in aqueous solution allowed complete conversion in 75−125 min. By assuming a cumulative value of $f = 0.6$ and that termination occurred exclusively by disproportionation ($\delta = 1$), we estimate that 92−95% of chains in the final glyco-oligomer samples were still living (Table 2) and that the same ratio was 96% in the case of the macroCTA used in the kinetic study (run xxiii).

At the end of the reaction, the polymerization mixture from run xxii (6-*O*-MAMGlc$_0$/CPADB$_0$ = 10) was redissolved in acetonitrile/1 mM aqueous $NH_4^+HCOO^-$ and analyzed via electrospray ionization-ion trap mass spectrometry. As shown in Figure 16, under the conditions used part of the oligomer chains form multiply ionized species (i.e., species containing more than one ammonium ion) and give rise to a complex peak pattern. All labeled peaks could be assigned to oligomers still bearing the dithiobenzoyl end group (Scheme 2) and containing between 2 and 21 repeating units. These figures are consistent

with the DP_n value of 15 estimated by size exclusion chromatography and confirm that chain transfer to CPADB is not immediate.

No residual "monomeric" species was detected ($n = 1$ in Scheme 2; $m/z = 559.2$ calcd for $M + NH_4^+$), but an intense peak at 572.4 was observed that could not be assigned. Also, among lower intensity peaks we could clearly identify masses corresponding to chains terminated via disproportionation (reaction x in Scheme 3; $m/z = 667.3$, 669.4, 929.4, 931.4, 1193.5, 1455.6), but not to chains terminated via coupling (reactions viii and ix). This observation confirms the marked preference of poly(methyl 6-*O*-methacryloyl-α-D-glucoside) to terminate via disproportionation.[42]

Conclusion

A detailed kinetic study of the RAFT polymerization of methyl 6-*O*-methacryloyl-α-D-glucoside with (4-cyanopentanoic acid)-4-dithiobenzoate and 4,4′-azobis(4-cyanopentanoic acid) in D_2O/DMSO-d_6 and water/methanol-d_4 was realized via in situ ^1H NMR spectroscopy. In particular, we focused on the influence of temperature, initiator and chain transfer agent concentration, molecular mass of the CTA leaving group, as well as the presence of residual oxygen on polymerization kinetics. In order to facilitate direct appreciation of the effect of CPADB on polymerization kinetics, CTA-free polymerizations under identical conditions were also conducted.

Conventional radical polymerization of 6-*O*-MAMGlc is very fast and obeys pseudo-first-order kinetics up to high conversion. No inhibition period was observed and an approximate value of $\langle k_p \rangle \cong 7 \times 10^4\ M^{-1}\ s^{-1}$ was estimated (D_2O/DMSO-d_6 86:14, 60 °C). In general, RAFT processes were slower than the corresponding conventional radical polymerizations, the difference being macroscopic up to ~80% conversion and quite small (less than $\sim 1/3$) afterward. For a given CTA_0/$initiator_0$ ratio, a lower initial concentration of chain transfer agent resulted in lesser rate retardation.

Under all tested conditions, an initial non-steady-state period was observed for RAFT polymerization whose duration was inversely proportional to the ratio between the initial amount of CTA and the flux of primary radicals. For polymerizations mediated by CPADB, attainment of steady-state coincided with complete consumption of the initial CTA. Nevertheless when CPADB was replaced by a polymeric chain transfer agent, the time needed to reach steady state was shortened but not eliminated, and its duration proved to still depend on the CTA_0/$initiator_0$ ratio. To the best of our knowledge, this is the first time a non-steady-state period for a RAFT polymerization mediated by a polymeric chain transfer agent is reported. Our findings suggest that transformation of the initial chain transfer agent into a macrochain transfer agent is a prerequisite for the establishment of a steady-state regime in the studied system.

The presence of residual oxygen induced a short induction period (~4 min) and 37% rate retardation in conventional radical polymerization, whereas it resulted in a 40 min inhibition period followed by much retarded polymerization in the analogous RAFT experiment.

Finally, findings from our kinetic study were applied to the synthesis of well-defined oligo(methyl 6-*O*-methacryloyl-α-D-glucoside) in high yield. By conducting polymerizations at relatively high-temperature (70 °C) and with a fairly high chain transfer agent to initiator ratio ($CPADB_0/ACPA_0 = 2.0$), 6-*O*-MAMGlc was quantitatively converted into well-defined living oligomers having 15−66 repeating units and PDI = 1.05−1.12.

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Supporting Information Available: Figures of the ^1H NMR spectrum of run x at zero conversion and of the macroCTA used in the kinetic investigation, ^1H and ^{13}C NMR spectra of the (4-cyanopentanoic acid)-4-dithiobenzoate used in this study, and a plot of the rate of polymerization vs conversion for the radical polymerization of methyl 6-*O*-methacryloyl-α-D-glucoside with different initiator concentrations (runs v, vii, and ix) and extrapolation to zero conversion. This material is available free of charge via the Internet at http://pubs.acs.org.

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(23) The fact that M_n of the macroCTA as measured by NMR (8400) is ~15% lower than the value measured by SEC-RALS (9900) is most probably the result of a slight overestimation of the molecular mass by the latter technique. The intrinsic difficulty in obtaining strong light scattering signals from oligomer species combines with a less than ideal baseline resolution of their chromatographic peak from the injection "trash" peak. In its turn, the latter may lead to a slight underestimation of the peak area and overestimation of the molecular mass. Although this systematic error cannot explain the deviations observed in runs xxi and xxii (40−70%), the M_n value measured by NMR was used in stoichiometric calculations performed for chain extension experiments (runs xv−xvi), since it accounts only for the living proportion of oligomer chains (i.e. those still bearing an end-of-chain dithiobenzoate moiety).

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