Electronic Supplementary Material (ESI) for Chemical Communications.

Supporting Information

A Ring to Rule Them All: A Cyclic Ketene Acetal Comonomer Controls the Nitroxide-Mediated Polymerization of Methacrylates and Confers Tunable Degradability

Vianney Delplace,[1] Elise Guégain,[1] Simon Harrisson,[2] Didier Gigmes,[3] Yohann Guillaneuf,[3] Julien Nicolas[1,]*

[1]Institut Galien Paris-Sud, Université Paris-Sud, UMR CNRS 8612, Faculté de Pharmacie, 5 rue Jean-Baptiste Clément, F-92296 Châtenay-Malabry cedex, France.

[2]Université de Toulouse, CNRS, Laboratoire des Interactions Moléculaires et Réactivité Chimique et Photochimique UMR 5623, 118 route de Narbonne, 31062 Toulouse, France

[3]Aix-Marseille Université, CNRS, Institut de Chimie Radicalaire UMR 7273, Avenue Escadrille Normandie-Niemen, 13397 Marseille Cedex 20, France.

[*] To whom correspondence should be addressed:

Email: julien.nicolas@u-psud.fr Tel.: +33 1 46 83 58 53

1. Materials

Oligo(ethylene glycol) methyl ether methacrylate (MeOEGMA, M_n = 300 g.mol^{-1}), styrene (S, 99%), methyl methacrylate (MMA, 99%), diethyl phosphite (DEP, 98%), chloroacetaldehyde dimethyl acetal (99%), (+/-)-1-phenyl-1,2-ethanediol (97%), Dowex® 50WX8 hydrogen form, potassium *tert*-butoxide (95%), *tert*-butanol anhydrous (≥99.5%) and toluene anhydrous (99.8%) were purchased from Sigma-Aldrich and used as received (except for MMA which was distilled). Deuterated chloroform ($CDCl_3$) was obtained from Eurisotop. All other solvents were purchased from Carlo-Erba. The 2-methyl-2-[*N*-*tert*-butyl-*N*-(1-diethoxyphosphoryl-2,2-dimethylpropyl) aminoxy]propionic acid alkoxyamine (BlocBuilder MA, 99%) and the *N*-tert-butyl-*N*-(1-diethylphosphono-2,2-dimethylpropyl) nitroxide (SG1, 85%) were kindly supplied by Arkema.

2. Analytical methods

2.1 Nuclear magnetic resonance spectroscopy (NMR). ^{1}H NMR spectroscopy was performed in 5 mm diameter tubes in $CDCl_3$ on a Bruker Avance-300 (300 MHz) spectrometer. The chemical shift scale was then calibrated on the basis of the solvent peak (δ = 7.26 ppm). ^{31}P NMR spectroscopy was performed in 5 mm diameter tubes in $CDCl_3$ on a Bruker Avance-400 (400 MHz) spectrometer. Diethylphosphite (DEP) and its characteristic peak (δ = 7,1 ppm) was used as internal reference to calibrate the chemical shift scale.

2.2 Size exclusion chromatography (SEC). SEC was performed at 30 °C with two columns from Polymer Laboratories (PL-gel MIXED-D; 300 × 7.5 mm; bead diameter, 5 μm; linear part, 400−400 000 g.mol^{-1}) and a differential refractive index detector (Spectrasystem RI-150 from Thermo Electron Corp.), using chloroform ($CHCl_3$) as eluent, at a flow rate of 1 mL.min^{-1}, and toluene as a flow-rate marker. The conventional calibration curve was based on poly(methyl methacrylate) (PMMA) standards (peak molar masses, M_p = 625−625 500 g.mol^{-1}) or polystyrene (PS) standards (M_p = 162–523 000

g.mol^{-1}) from Polymer Laboratories. This technique allowed M_n (number-average molar mass), M_w (weight-average molar mass), and M_w/M_n (dispersity, $Đ$) to be determined.

3. Methods

3.1 Synthesis of 2-methylene-4-phenyl-1,3-dioxolane (MPDL). 2-Methylene-4-phenyl-1,3-dioxolane (MPDL) was synthesized in two steps as described by Bailey and co-workers,[1] with some modifications. *Synthesis of 2-chloromethyl-4-phenyl-1,3-dioxolane (Cl-MPDL).* In a 200 mL round-bottom flask fitted with a magnetic bar and equipped with a distillation system (to collect methanol), a mixture of chloroacetaldehyde dimethyl acetal (25 g, 2.01×10^{-1} mol, 1 eq.), styrene glycol (27.75 g, 2.01×10^{-1} mol, 1 eq.) and Dowex 50 (H^+) resin (250 mg) was heated at 120 °C overnight. Note that no methanol was collected by distillation. After the reaction mixture was cooled down to ambient temperature, the resin was then removed by filtration. The residual methanol was then removed under reduce pressure and the crude product was purified by vacuum distillation at 115 °C. Yield: 70 % (28 g, 1.4×10^{-1} mol) of a mixture of two diastereoisomers (a white solid and a colorless liquid).

Synthesis of 2-methylene-4-phenyl-1,3-dioxolane (MPDL). In a 200 mL three-neck round bottom flask, fitted with a magnetic bar and equipped with a Vigreux column, a mixture of potassium *tert*-butoxide (10.1 g, 9.02×10^{-2} mol, 1.2 eq.) in 110 mL dry *tert*-butanol was heated to 80 °C under nitrogen atmosphere. Cl-MPDL (15 g, 7.54×10^{-2} mol, 1 eq.) in 40 mL of dry *tert*-butanol were added dropwise to the mixture using an addition funnel. After the addition was completed, the temperature was raised to 100 °C to maintain a gentle reflux overnight. After the reaction mixture was cooled down to ambient temperature, 200 mL of cold diethyl ether were added and the resulting precipitate was removed by filtration. After the solvents were removed under reduce pressure, the residue was purified by vacuum distillation at 85 °C. No pyridine was further added to stabilize the MPDL. Note that the batch of MPDL must be of high purity and fresh before use to avoid the formation of a small fraction of high molar mass

polymer before and/or during the polymerization. Yield: 37% (4.5 g, 2.8 × 10^{-2} mol) of a colorless liquid (see Figure S1).

3.2 Synthesis of poly[oligo(ethylene glycol) methyl ether methacrylate] (PMeOEGMA, expt. 1). In a 5 mL vial, fitted with a rubber septum and a magnetic bar, a mixture of MeOEGMA (1.50 g, 5.00 × 10^{-3} mol), BlocBuilder MA alkoxyamine initiator (0.019 g, 5.07 × 10^{-5} mol) and anhydrous toluene (1.5 g, 1.73 mL) was deoxygenated under stirring by nitrogen bubbling for 15 min at room temperature. The mixture was then immersed in a preheated oil bath at 90 °C, corresponding to the time zero of the reaction (according to the small volume of solution and its quasi-instantaneous heating). Samples were periodically taken over a period of 6 h and dried to follow the MeOEGMA conversion by ^{1}H NMR spectroscopy (using the methylene protons in α-position to the ester group of OEGMA) and the evolution of the molar mass and the dispersity by SEC from PMMA standards.

3.3 Synthesis of poly[(oligo(ethylene glycol) methyl ether methacrylate)-*co*-(2-methylene-4-phenyl-1,3-dioxolane)] (P(MeOEGMA-*co*-MPDL). A typical solution copolymerization procedure (expt. **3**) is as follows. In a 5 mL vial, fitted with a rubber septum and a magnetic bar, a mixture of MeOEGMA (1.103 g, 3.68 × 10^{-3} mol), MPDL (0.397 g, 2.45 × 10^{-3} mol), the BlocBuilder MA alkoxyamine initiator (0.014 g, 3.73 × 10^{-5} mol) and anhydrous toluene (1.5 g, 1.73 mL) was deoxygenated under stirring by nitrogen bubbling for 15 min at room temperature. The mixture was then immersed in a preheated oil bath at 90 °C, corresponding to the time zero of the reaction (according to the small volume of solution and its quasi-instantaneous heating). Samples were periodically taken and dried to follow the MeOEGMA conversion by ^{1}H NMR spectroscopy (using the methoxy protons of OEG (CH_3), the methylene protons in α-position to the ester group of OEGMA (CH_2) and the proton in α-position to the aromatic group or MPDL (CH), and following Eq. 1) and the evolution of the molar mass evolution and the dispersity by SEC from PMMA standards (note that the theoretical M_n is based on MeOEGMA).

$$\text{Conv.}(\%) = \frac{\left[(I_{CH3} - 2.I_{CH})/3 - I_{CH2}/2\right]}{(I_{CH3} - 2.I_{CH})/3}$$

Eq. 1

The copolymer was then precipitated once in cold diethyl ether and dried under high vacuum until constant weight. ^{1}H NMR spectra of the purified copolymer can be found in Supporting Information (Figure S4). The same procedure was followed by adapting the amount of the reactants for expt. **2** [MeOEGMA (1.322 g, 4.41 × 10^{-3} mol), MPDL (0.178 g, 1.10 × 10^{-3} mol), BlocBuilder MA alkoxyamine initiator (0.017 g, 4.45 × 10^{-5} mol)] and expt. **4** [MeOEGMA (0.664 g, 2.21 × 10^{-3} mol), MPDL (0.836 g, 5.16 × 10^{-3} mol), BlocBuilder MA alkoxyamine initiator (0.0085 g, 2.23 × 10^{-5} mol)]. Note that for the hydrolytic degradation study, expt. **4** has been redone but stopped at 44% conversion to yield a lower molar mass copolymer (M_n = 17200 g.mol^{-1}, $Đ$ = 1.38, F_{MPDL} = 0.248), more comparable to those obtained with expts. **2-3**.

3.4 Synthesis of polystyrene (PS, expt. 5). In a 5 mL vial, fitted with a rubber septum and a magnetic bar, a mixture of S (4.808 g, 4.62 × 10^{-2} mol) and BlocBuilder MA alkoxyamine initiator (0.060 g, 1.56 × 10^{-4} mol) was deoxygenated under stirring by nitrogen bubbling for 15 min at room temperature. The vial was placed in a preheated oil bath at 120 °C, triggering the polymerization (according to the small volume of solution and its quasi-instantaneous heating). After 2 h, the polymerization was stopped by cooling down the reaction medium. The S conversion was determined by ^{1}H NMR spectroscopy (using the vinyl protons) and the molar mass and the dispersity was determined by SEC from PS standards. Conv. (2 h) = 53%, $M_{n,SEC}$ = 15 300 g.mol^{-1}, M_w/M_n = 1.20 (raw copolymer).

3.5 Synthesis of poly[(methyl methacrylate)-*co*-(2-methylene-4-phenyl-1,3-dioxolane)] (P(MMA-*co*-MPDL), expt. 6). In a 5 mL vial, fitted with a rubber septum and a magnetic bar, a mixture of MMA

(0.3138 g, 3.14×10^{-3} mol), MPDL (1.1862 g, 7.32×10^{-3} mol), BlocBuilder MA alkoxyamine initiator (0.006 g, 1.57×10^{-5} mol) and anhydrous toluene (1.5 g, 1.73 mL) was deoxygenated under stirring by nitrogen bubbling for 15 min at room temperature. The mixture was then immersed in a preheated oil bath at 90 °C, corresponding to the time zero of the reaction (according to the small volume of solution and its quasi-instantaneous heating). After 15 h, the copolymerization was stopped. A sample was taken to estimate the MMA conversion by ^{1}H NMR spectroscopy and the molar mass and the dispersity were determined by SEC from PMMA standards. The copolymer was precipitated in cold methanol and dried under high vacuum until constant weight. Conv. MMA (15 h) = 40 %, $M_{n,SEC}$ = 10 200 g.mol^{-1}, M_w/M_n = 1.20 (Figure S5), F_{MPDL} = 0.35.

Table S1. Experimental Conditions and Results of the Different (Co)polymerizations.

Expt.	Main monomer	$f_{MPDL,0}$[(a)]	T (°C)	Toluene (wt.%)	Conv (%) / time (h)	M_n[(b)] (g.mol^{-1})	$Đ$[(b)]	F_{MPDL}[(c)]
1	MeOEGMA	-	90	50	41 / 8	12 500[d]	1.72[d]	-
2	MeOEGMA	0.2	90	50	44 / 22	13 300[d]	1.54[d]	0.036
3	MeOEGMA	0.4	90	50	47 / 24	15 500[d]	1.53[d]	0.113
4	MeOEGMA	0.7	90	50	69 / 24	26 600[d]	1.43[d]	0.248
5	S	-	120	-	53 / 2	15 300	1.20	-
6	MMA	0.7	90	50	40 / 15	10 200	1.20	0.35

[a]Initial molar fraction of MPDL in the monomer feed. [b]Determined by size exclusion chromatography (SEC). [c]Molar fraction of MPDL in the final copolymer. [d]Data for the raw (co)polymers.

3.6 Hydrolytic degradation. In a 5 mL vial, 30 mg of copolymer was dissolved in 3 mL of 5% KOH aqueous solution and stirred at room temperature. Samples of 1 mL were periodically taken, neutralized with 1 M HCl aqueous solution and lyophilized. 2 mL of chloroform was then added, allowing filtration of the salts. Finally, the solvent was removed under reduced pressure and the degradation products were analyzed by SEC.

4. Cytotoxicity study

4.1 Cell lines and cell culture. The embryonic murine fibroblast (NIH/3T3) was cultured in Dulbecco's Modified Eagle Medium (DMEM, Lonza) supplemented with 50 $U.mL^{-1}$ penicillin, 50 $U.mL^{-1}$ streptomycin and 10% fetal bovine serum (FBS, Lonza, Belgium). The J774.A1 murine macrophage-monocyte cell line was cultured in RPMI 1640 medium (Lonza) supplemented with 50 $U.mL^{-1}$ penicillin, 50 $U.mL^{-1}$ streptomycin and 10% heat inactivated FBS. Both cell lines were obtained from ATCC and maintained at 37 °C in a humidified 5% CO_2 atmosphere.

4.2 Cytotoxicity assay. The *in vitro* cytotoxic activity of chosen copolymers and their degradation products was evaluated on both cell lines, using the 3-[4,5-dimethylthiazol-2-yl]-3,5-diphenyltetrazolium bromide (MTT) test. Cells were seeded in 100 µL of growth medium (NIH/3T3, 3×10^4 $cells.mL^{-1}$; J774.A1, 6×10^4 $cells.mL^{-1}$) in 96-well microliter plates (TPP) and preincubated for 24 h. After appropriate dilutions, 100 µL of copolymer solution in cell medium was added over the cells and incubated for 72 h. Initial cell density and incubation time were determined to allow cells to remain in exponential growth and to undergo two cell-doubling times during the assay. At the end of the incubation period, 20 µL of a 5 $mg.mL^{-1}$ MTT (Sigma-Aldrich) solution in phosphate buffered saline (PBS) was added to each well. After 2 h of incubation, the culture medium was removed and replaced by 200 µL of dimethyl sulfoxide (DMSO), in order to dissolve the formazan crystals. The absorbance of the solubilized dye was measured spectrophotometrically with a microplate reader (LAB System Original Multiscan MS) at 570 nm. The percentage of viable cells for each treatment was calculated from the ratio of the absorbance of the well containing the treated cells versus the average absorbance of the control wells (*i.e.*, untreated cells). All experiments were set up in triplicate to determine means and SDs.

5. References

1 W. J. Bailey, S. R. Wu and Z. Ni, *Makromol. Chem.*, 1982, **183**, 1913.

6. Additional figures

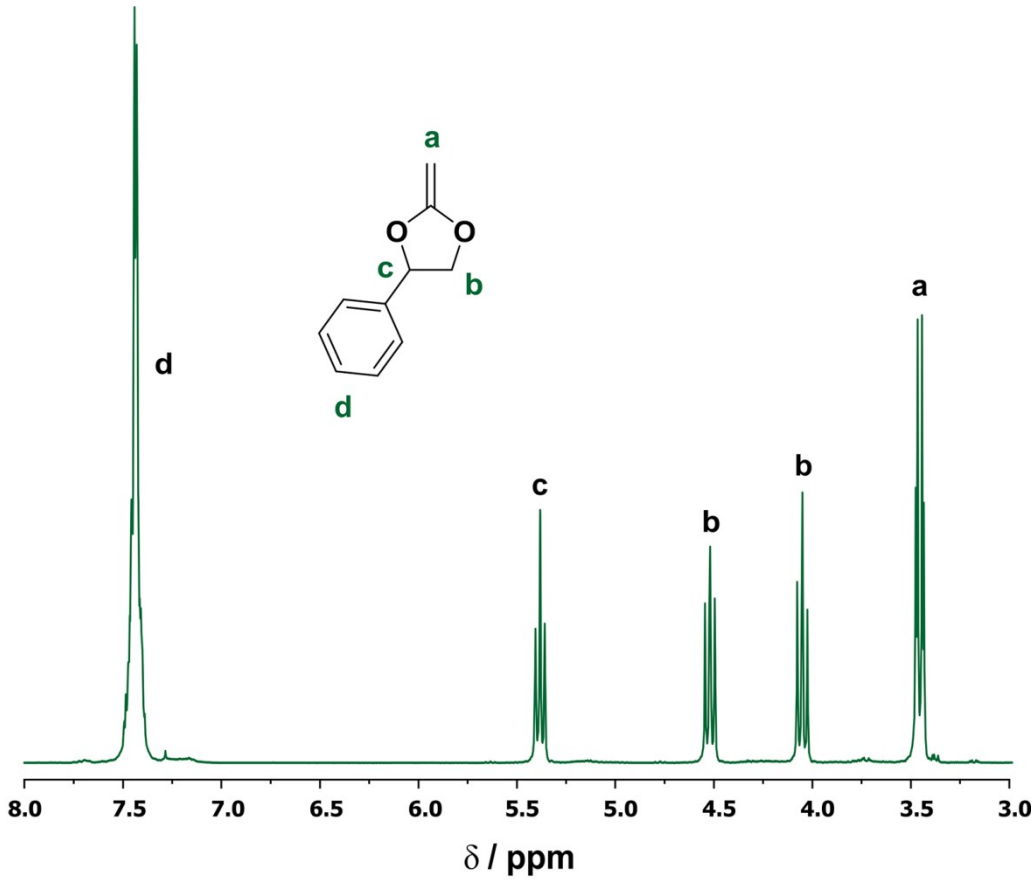

Figure S1. ^{1}H NMR spectrum of 2-methylene-4-phenyl-1,3-dioxolane (MPDL) in the 3.0–8.0 region in $CDCl_3$.

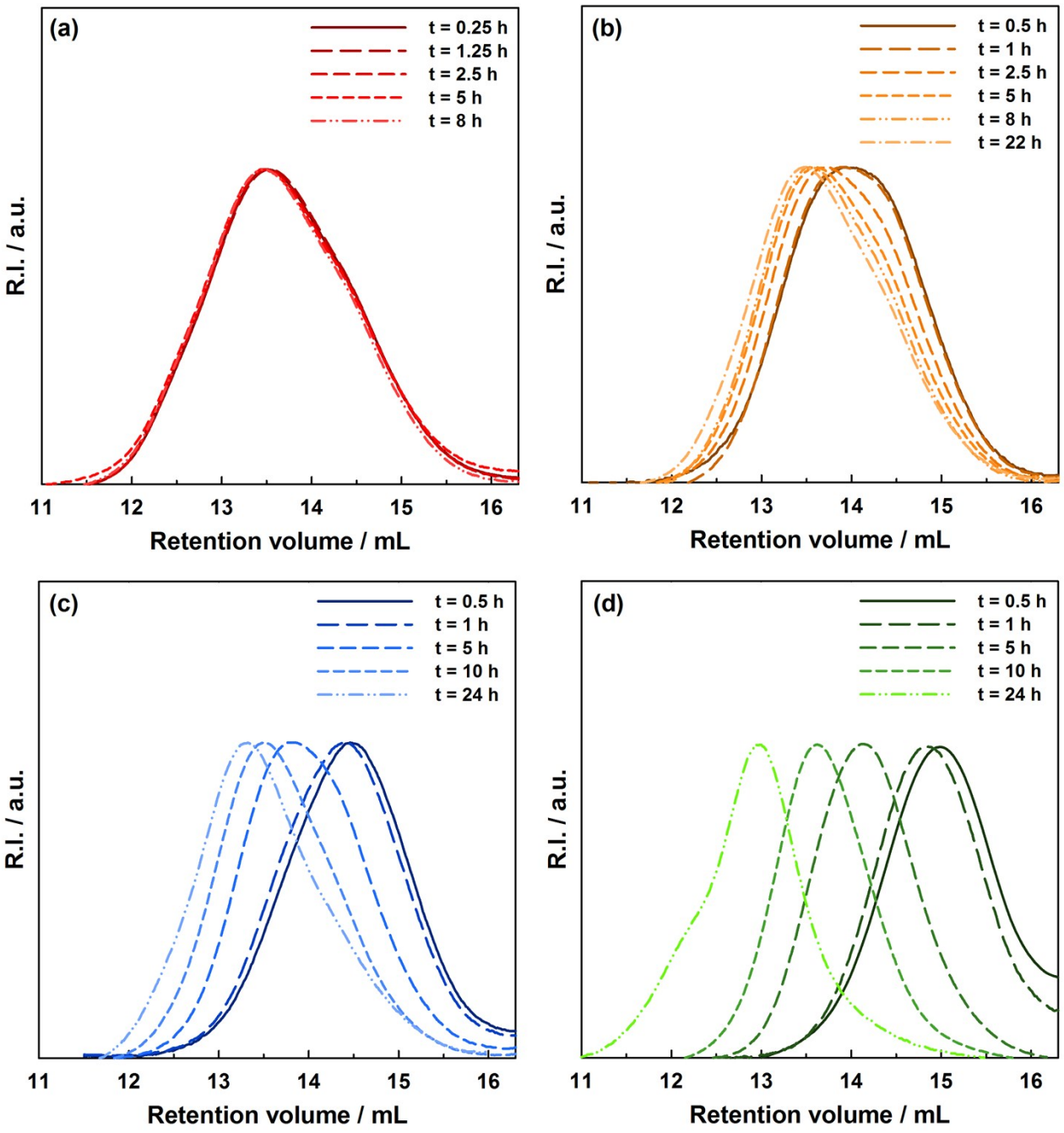

Figure S2. SEC traces taken at different time intervals during the NMP of oligo(ethylene glycol) methyl ether methacrylate (MeOEGMA) and 2-methylene-4-phenyl-1,3-dioxolane (MPDL) in toluene initiated by the BlocBuilder alkoxyamine at 90 °C, as a function of the initial amount of MPDL: (a) expt. **1** ($f_{MPDL,0} = 0$); (b) expt. **2** ($f_{MPDL,0} = 0.2$); (c) expt. **3** ($f_{MPDL,0} = 0.4$); (d) expt. **4** ($f_{MPDL,0} = 0.7$).

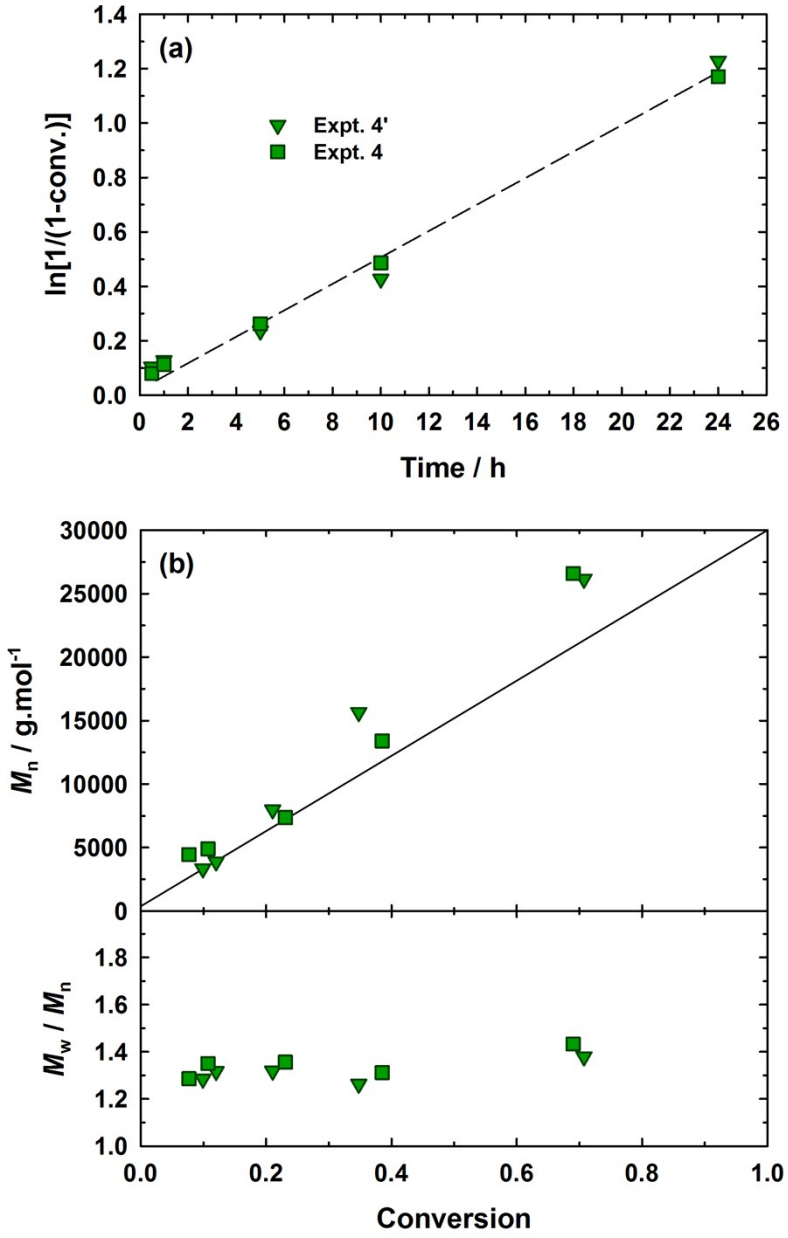

Figure S3. Reproducibility of the NMP of oligo(ethylene glycol) methyl ether methacrylate (MeOEGMA) and 2-methylene-4-phenyl-1,3-dioxolane (MPDL) in toluene initiated by the BlocBuilder alkoxyamine at 90 °C for $f_{MPDL,0}$ = 0.7. (a) Ln[1/(1-conv.)] vs time (conv. = MeOEGMA conversion). (b) Number-average molar mass M_n, and dispersity, M_w/M_n, vs conversion. The full line represents the theoretical M_n and the dashed ones are guides for the eye only.

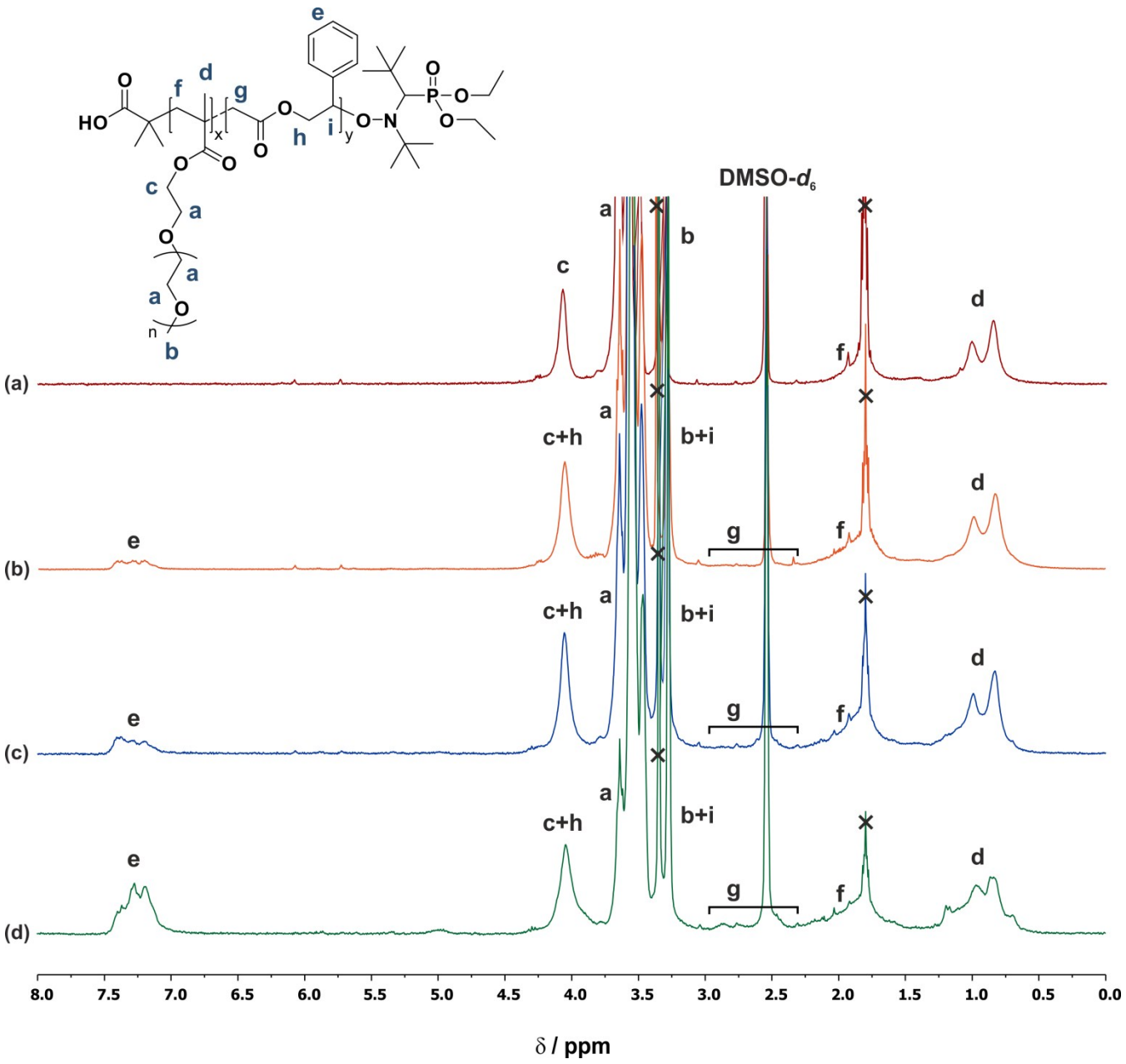

Figure S4. ^{1}H NMR spectra in DMSO-d_6 of the purified (co)polymers resulting from the NMP of oligo(ethylene glycol) methyl ether methacrylate (MeOEGMA) and 2-methylene-4-phenyl-1,3-dioxolane (MPDL) in toluene initiated by the BlocBuilder alkoxyamine at 90 °C, as a function of the initial amount of MPDL: (a) expt. **1** ($f_{MPDL,0} = 0$); (b) expt. **2** ($f_{MPDL,0} = 0.2$); (c) expt. **3** ($f_{MPDL,0} = 0.4$); (d) expt. **4** ($f_{MPDL,0} = 0.7$).

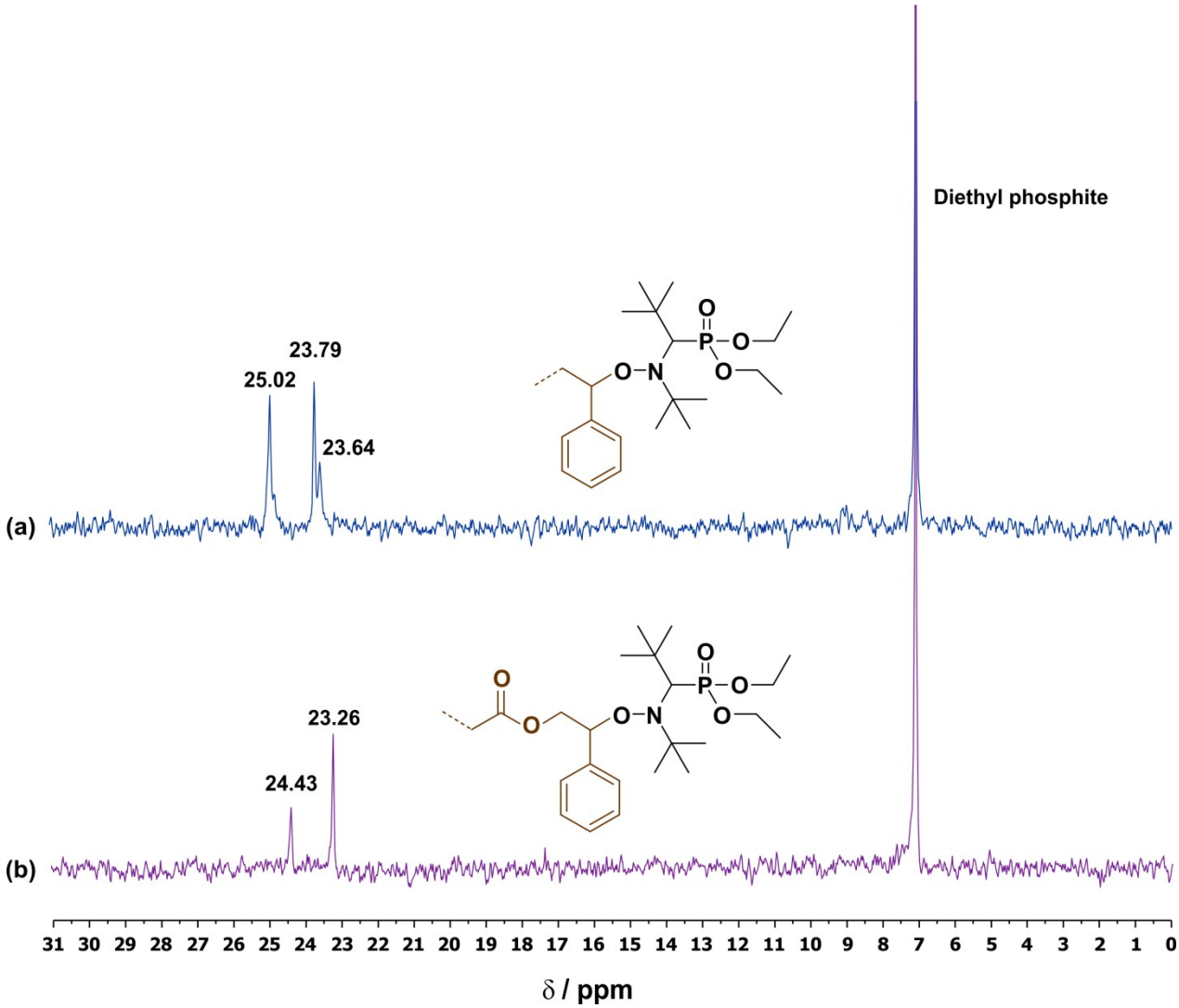

Figure S5. ^{31}P NMR spectra in $CDCl_3$ of: (a) PS-SG1 (expt. **5**) and (b) P(MeOEGMA-*co*-MPDL)-SG1 ($f_{MPDL,0}$ = 0.4, expt. **3**).

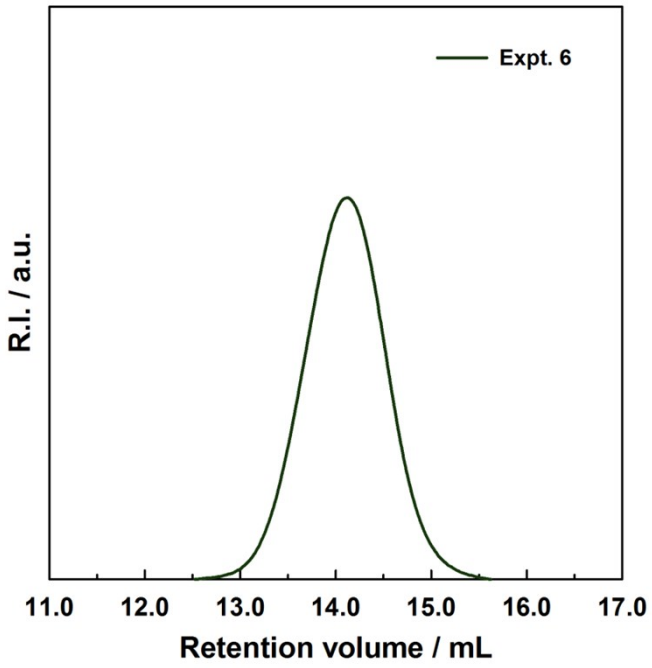

Figure S6. SEC traces of the purified P(MMA-*co*-MPDL) copolymer (expt. **6**).

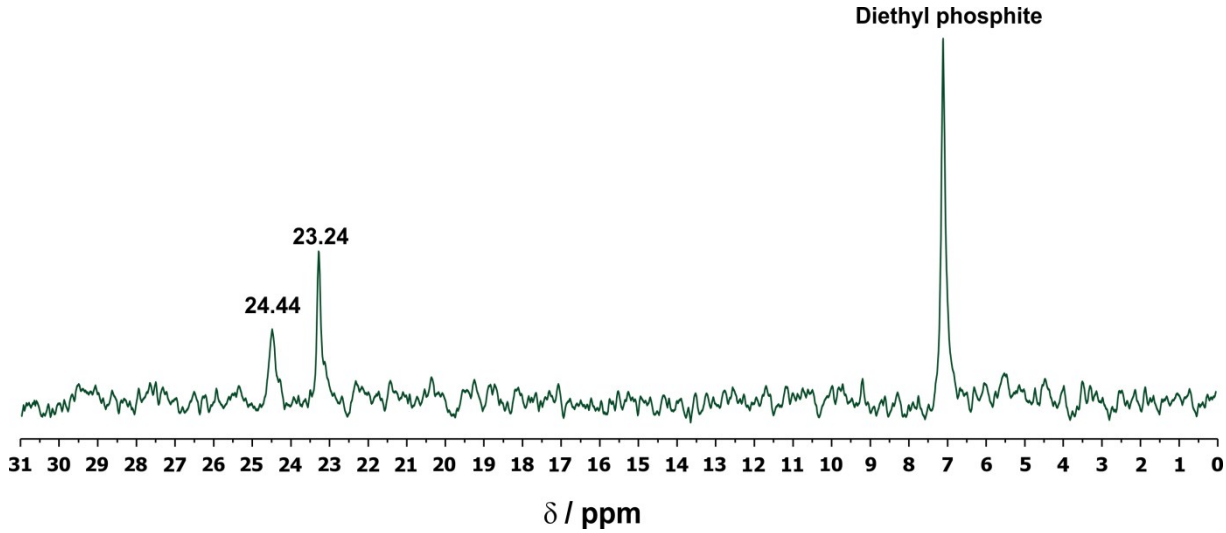

Figure S7. ^{1}H NMR spectra in DMSO-d_6 of the purified copolymer resulting from the NMP of methyl methacrylate (MMA) and 2-methylene-4-phenyl-1,3-dioxolane (MPDL) in toluene initiated by the BlocBuilder alkoxyamine at 90 °C (expt. **6**).

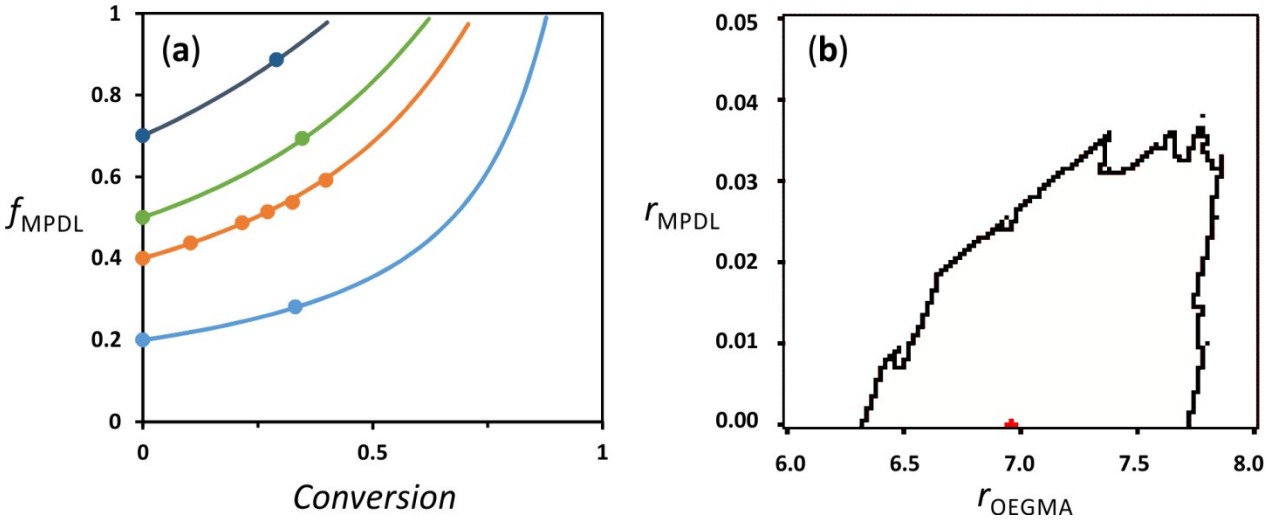

Figure S8. (a) Mole fraction of MPDL in the monomer feed as a function of conversion. Circles represent experimental data and lines are the predictions of the copolymer composition using the copolymer composition equation with reactivity ratios of r_{MPDL} = 0, $r_{MeOEGMA}$ = 6.95. (b) 95% joint confidence region (JCR) for reactivity ratios of MeOEGMA and MPDL calculated by the 'visualisation of the sum of least squares space' method of van den Brink et al. (M. van den Brink, A. M. van Herk and A. L. German, *J. Polym. Sci., Part A: Polym. Chem.*, 1999, **37**, 3793).