Revisiting Monomer Synthesis and Radical Ring Opening Polymerization of Dimethylated MDO Towards Biodegradable Nanoparticles for Enzymes

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ABSTRACT

Radical ring opening polymerization is a powerful tool to achieve a polyester via radical polymerization. We used it to obtain a dimethylated version of poly(caprolactone) (PdmCL) from dimethylated MDO (DMMDO). First, we revisited monomer synthesis and achieved a milder synthetic protocol by introducing a cobalt-based catalyst. We also developed a new route towards DMMDO via a cyclic carbonate using the Petasis chemistry. Amphiphilic block-copolymers were then generated by free radical polymerization of DMMDO with a PEG-based macroinitiator. The resulting polyesters self-assembled into nanoparticles that were

biodegradable as well as biocompatible. The nanoparticles proved to be an effective protective shell for an entrapped enzyme that was released upon degradation of the polyester by esterase. We are confident that our results will spur further research into block-copolymers resulting from RROP.

INTRODUCTION

Self-assembling materials are a major topic in todays' research.[1] Especially colloidal nanoparticles such as micelles and vesicles (or polymersomes) hold promise as drug delivery systems and nanoreactors for enzymatic reactions.[2-4] Out of the polymers available, several show responsiveness to environmental factors, rendering some biodegradable. Amongst the polymers used to achieve biodegradability are poly(lactic acid) PLA and poly(caprolactone) (PCL)[4-6] Both polymers can be synthesized via ring opening polymerization (ROP) and are FDA approved. However, they are both semi-crystalline at room temperature which limits their applications due to the lack of flexibility.[6, 7] For PCL, this problem can be reduced by the introduction of a methyl substituent, like it is known for Poly(methylcaprolactone) (PmCL).[7] Introducing a second methyl group would soften the material even more but has not been synthesized via ROP so far. Poly(dimethylcaprolactone) (PdmCL compound **3** in Fig. 1a) has been obtained from a cyclic ketene acetal (CKA, compound **2** in Fig. 1a) via radical ring opening polymerization (RROP)).[8-10]

CKAs are the monomers for RROP. In order to polymerize, the CKA is attacked by a radical and the molecule ring-opens and transforms into a polyester (see section 2.7 of the SI).[11-13] Among the monomers applicable for this reaction, 4,7-dimethyl-2-methylene-1,3-dioxepane (DMMDO **2**, Figure 1a) is the one that yields PdmCL. CKA **2** originates from 2,5-hexanediol (compound **1** in Fig. 1a) and transforms into PdmCL (**3**) in the course of RROP (Figure 1).[8, 9, 14] Homopolymers as well as statistical copolymers with acrylates or methacrylates are

known for this CKA and similar ones.[15-17] Due to their polyester backbone, they can be enzymatically degraded.[18-22] This makes DMMDO (**2**) an ideal candidate for self-assembling block-copolymers. Previous works on amphiphilic block-copolymers from CKA **2** utilized a statistical copolymerization with vinylic polymers to create a biodegradable hydrophobic block.[21] This procedure results in longer chain segments with a hydrocarbon backbone, interrupted by degradable ester bonds resulting from RROP.

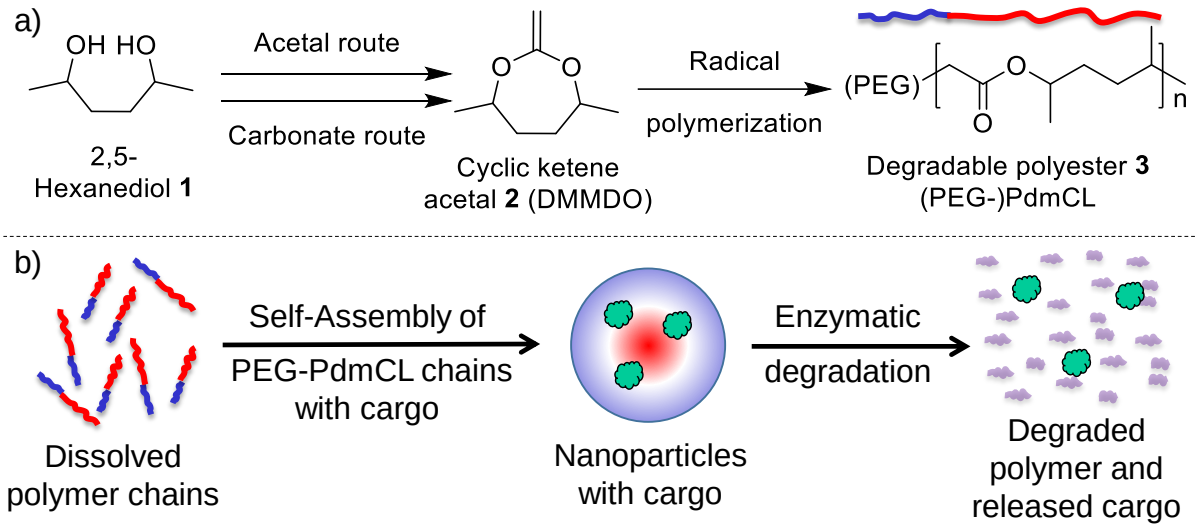

Figure 1: The CKA DMMDO (2) is formed from hexanediol **1**, via the acetal route (established) or the carbonate route (new). The polyester **3** can then be made via RROP that (b) self-assemble into enzymatically degradable nanoparticles, which can serve as removable protective coatings for enzymes (green).

Because a completely biodegradable backbone would be much more preferred, we set out to generate block-copolymers based purely on the RROP of DMMDO. Starting from a PEG-based macroinitiator, our goal was to synthesize PEG-PdmCL **3** (Figure 1). Due to their entirely degradable hydrophobic segment, self-assembled PEG-PdmCL nanoparticles could serve as temporary protective shells for enzymes (Figure 1). We are confident that degradable nanoparticles from RROP lacking vinylic polymers will be an important step to further establish RROP in the field of polymer science. At the same time we also took the opportunity to more closely explore monomer synthesis. CKAs for RROP are exclusively produced via an

intermediate haloacetal, which is then eliminated towards the CKA.[11] Our aims were to make this process available at lower temperatures and via an intermediate carbonate (Figure 2a). Tebbe et al. reported that a CKA can be formed from the corresponding carbonate using a Titanium alkylidene, but the chemistry has so far not been developed for the CKA **2**.[23]

MATERIALS AND METHODS

MATERIALS: 2,5-Hexanediol was purchased from TCI Chemicals (Belgium). Triphosgene, copper (I) bromide, N,N'-bipyridyl, ethyl-alpha-bromoisobutyrate, 2-cyano-2-propyl benzodithioate, 2-[(ethoxythioxo-methyl)thio]-2-methyl-propionic acid, 2,2' azobis(2-methylpropionitril), cobalt(II)chloride, chloro-acetaldehyde dimethylacetal, bromoacetaldehyde dimethylacetal, chlorotrimethyl-silane (TMSCl), potassium tert-butoxide, tert-butanol, 2,2'-bipyridyl, pyridine, sodium chloride, ammonium chloride, horse raddish peroxidase, esterase from porcine liver and dry acetonitrile were purchased from Sigma-Aldrich (Switzerland). Dimethyltitanocen – 5 % in THF/toluene was purchased from ABCR (Germany). Dichloromethane, ethyl acetate, ethanol, acetone, hexane and diethyl ether were purchased from Brenntag (Germany). All chemicals were used as received.

METHODS:

Gel Permeation Chromatography (GPC) was performed on an Agilent infinity 1200 instrument (Polymer Standard Services, Germany) with chloroform as eluent and two Mixed-C columns (Polymer Standards Services, Germany) were used for separation. The column oven was set to 35°C and a flow rate of 1.0 mL/min applied. All GPC traces shown were recorded using a refractive index (RI) detector.

Gas chromatography with mass spectrometry (GC-MS) was performed on a Shimadzu GCMS-QP2010 SE instrument (Shimadzu, Japan) with hydrogen as carrier gas and a heat flow

detector. The samples were injected at 80°C oven temperature following a linear rise to 280 °C over 20 minutes.

All **NMR** experiments were performed on a Bruker Avance III NMR spectrometer operating at 400 MHz proton frequency and at 100 MHz for spectra of ^{13}C. The instrument was equipped with a direct observe 5-mm BBFO smart probe. The experiments were performed at 295 K and the temperature was calibrated using a methanol standard showing accuracy within +/- 0.2 K.

Dynamic Light Scattering (DLS) was performed on an LS spectrometer from *LS Instrument* (Switzerland) with a HeNe laser (633 nm) with varying scattering angles. A scattering angle of 90° was used for all and the device was set to give the data in intensity-mode. To monitor particle degradation, the laser intensity was set to 3.5 % and the count rate averaged over 3 measurements of 10 seconds each.

Lyophilization was performed on a Christ alpha 2-4 LD plus lyophilizer (Martin Christ, Germany). **Microwave assisted reactions** were performed on a Biotage Initiator+ instrument (Biotage, Sweden) with the power set to 120 W.

Synthetic procedures:

Synthesis of halogenated acetaldehyde cyclic acetals 5a/b

We derived a synthesis from Battisti et al.[24] For **5a**: 2,5-Hexanediol (**1,** 10.0 g, 84.7 mmol, 1.0 equiv.) was dissolved in anhydrous MeCN (840 mL) and $CoCl_2$ (3.62 g, 27.9 mmol, 0.33 equiv.), TMSCl (9.30 g, 85.4 mmol, 1.01 equiv.) and 2-chloro-1,1-dimethoxyethane (**4a**, 10.64 g, 85.4 mmol, 1.01 equiv.) were added sequentially. The mixture was stirred under argon overnight at RT. The mixture was then poured into H_2O (in two batches, 450 mL each), each extracted with EtOAc (3x 400 mL) and the combined organic phases from both batches washed with sat. aq. $NaHCO_3$ (500 mL). The solution was then dried (Na_2SO_4), filtered and the solvent evaporated at reduced pressure. Distillation gave **5a**: 8.63 g, bp 73 °C (6 mBar). Details to

optimization of the experiment (in terms of conversion) can be found in section 2.1. of the SI. Yield: 61 %

The product was a mixture of 3 diastereomers by 1H NMR and GC in a ratio of 58 % / 39 % / 3 %, see details in section 3.2. The analytical data corresponds with literature.[9]

^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 1.08-1.13 (m, 6 H, CH_3 (contains distinct d, J = 6.7 Hz), 1.31 - 1.75 (m, 4 H, CH_2), 3.31/3.32/3.37 (3 x d, J = 5.3 Hz / 5.3 Hz / 5.5 Hz, 2 H, CH_2), 3.60 - 3.97 (m, 2 H, CH), 4.61/4.76/4.87 (3 x t, J=5.2 Hz / 5.3 / 5.3, 1 H, CH).

^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 22.0 / 22.2 (CH_3), 32.2 / 32.8 (CH_2) 44.0 / 45.1 (CH_2), 68.9 / 75.6 (CH), 98.1 / 100.7 (CH)

5b. Following an identical procedure to above, but with 2-bromo-1,1-dimethoxyethane (**4b**, 84.5 mmol) gave **5b**: 9.52, bp 75 °C (6 mBar). Yield: 51%

^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 1.05-1,13 (m, 6 H, CH_3 (contains distinct d J = 6.7 Hz), 1.31 - 1.75 (m, 4 H, CH_2), 3.17/3.18/3.22 (3 x d, J = 5.2 Hz / 5.0 Hz / 5.5 Hz, 2 H, CH_2), 3.60 - 3.97 (m, 2 H, CH), 4.61/4.76/4.87 (3 x t, J=5.2 Hz / 5.3 / 5.3 , 1 H, CH).

^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 22.2 / 22.3 (CH_3), 32.5 / 33.1 (CH_2) 35.7 / 36.0 (CH_2), 75.9 / 76.0 (CH), 98.3 / 100.9 (CH)

Synthesis of CKA 2 from halogenated Acetal 5

We derived a method from Bailey et al.[10] The acetal **5a** (4.10 g 23.0 mmol, 1.0 equiv.) was dissolved in ^{t}BuOH (5 mL) and KO^{t}Bu (3.10 g, 27.6 mmol, 1.2 equiv.) was added which gave a thick slurry. The mixture was stirred for 16 h in a sealed tube at 120 °C. During the reaction, the solution became much less viscous. The reaction was cooled to RT and the addition of Et_2O (50 mL) lead to the formation of a precipitate. The solution was centrifuged (2000 rpm, 5

minutes) and the supernatant decanted and evaporated. The residue was distilled to give CKA **2:** 1.24 g, bp 51 °C (12 mBar). Yield: 32 %

Please note: Running the reaction in a microwave vessel at 130 °C gave no conversion after 1 h and the reaction vessel broke soon after due to a sudden increase in pressure.

According to ^{1}H NMR, a mixture of two diastereomers was obtained. Ratio: 65% / 35%. The analytical data corresponds to literature.[9, 25]

^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 1.28/1.30 (2 x d, J = 6.4 / 6.7 Hz, 6 H, CH_3), 1.40 - 1.87 (m, 4 H, CH_2), 3.44 / 3.49 (2 x s, 2 H, CH_2), 3.97 - 4.07 / 4.19 - 4.32 (2 x m, 2 H, CH)

^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 19.1 / 22.3 (CH_3), 32.2 / 35.5 (CH_2), 67.1 / 70.0 (CH_2), 72.3 / 76.9 (CH), 160.3 / 162.9 (C)

Synthesis of cyclic carbonate 6

This step is based on a method by Hicklin et al.[26] 2,5-Hexanediol (**1**, 1.20 g, 10.2 mmol, 1.0 eq.) was dissolved in CH_2Cl_2 (170 mL) and pyridine (7.50 mL, 91.5 mmol, 9.0 eq.) and the solution was purged with argon at -20 °C. A solution of triphosgene (4.55 g, 15.2 mmol, 1.5 eq.) in CH_2Cl_2 (90 mL) was slowly added (over 10 minutes) to the solution through a dropping funnel. The cooling bath was removed and the reaction mixture stirred for 20 minutes before being quenched with sat. aq. NH_4Cl (100 mL). The solution was extracted with CH_2Cl_2 (3x 150 mL), the combined organic layers washed with brine (2x 100 mL), dried (Na_2SO_4), filtered and evaporated. Distillation gave 4,7-dimethyl-1,3-dioxepan-2-one (**6**): 0.96 g, bp 95°C (1 mbar). Yield: 66%

According to ^{1}H NMR and GC, a mixture of two diastereomers was obtained. Ratio: 55% / 45%, see section 3.3 for details. The compound was already reported.[27]

^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 1.39/1.42 (2 x d, J = 6.4 / 6.5 Hz, 6 H, CH_3), 1.66 - 1.99 (m, 4 H, CH_2), 4.33 - 4.43 / 4.55 - 4.66 (2 x m, 2 H, CH)

^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 20.6 / 22.3 (CH_3), 37.6 / 36.9 (CH_2) 77.2 / 78.8 (CH), 155.5 / 153.1 (C)

Synthesis of CKA 2 from Carbonate 6

We adopted a method from Petasis et al.[23] A 5 wt-% solution of Cp_2TiMe_2 (2.00 mL, 0.50 mmol, 3.0 eq.) in THF/toluene (50/50 (Vol/Vol)) was mixed with the cyclic carbonate **6** (0.023 g, 0.16 mmol, 1.0 eq.) under an argon atmosphere in the dark at 60-65 °C for 20 h. Addition of hexane (10 mL) led to the formation of a yellow precipitate, which was filtered off. The filtrate was concentrated under reduced pressure to give the CKA **2** as a pale yellow oil (0.015 g). Yield: 65 %

Spectroscopic data was identical to that reported in section 2.2 of the supporting information.

Radical polymerisation of CKA 2

The radical polymerisation techniques have been adopted from methods published earlier, details can be found below in the specific reaction conditions.[28-30]

Free radical polymerisation of CKA 2

A solution of CKA **2** (300 mg, 2.1 mmol) and 2,2' azobis(2-methylpropionitril) (**8,** 9.0 mg, 55 µmol) in 0.05 mL of toluene was purged with argon for 15 minutes at RT. The reaction was then stirred at 85 °C for 66 h. The reaction mixture was cooled, opened to air and diluted with CH_2Cl_2 (50 mL). The solution was dialysed (MWCO 1000 Da) against CH_2Cl_2 (300 mL), exchanging the solvent three times, leaving each batch of solvent at least 3 h. The solvent was removed under reduced pressure to yield the polymer (120 mg) (Dispersity from GPC: 1.3-1.5, see Table 1 and Figure 3 in the main paper for sample elugram).

^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 4.87 (m, 1H, CH), 4.42 (m, 2H, CH_2), 4.27 (m, 2H, CH_2), 4.04 (m, 2H, CH_2), 3.83 (m, 2H, CH_2), 3.45 (s, 2H, CH_2), 2.10 (m, 2H, CH_2), 1.60 (m), 0.94 (m, 3H, CH_3).

Controlled Radical Polymerisation of CKA 2

We used standard procedures for controlled radical polymerisation (ATRP, RAFT, MADIX-RAFT). None gave a polymer, as comprized in figure 3. Details can be found in the supporting information (SI)

Synthesis of the PEG-Macroinitiator

We prepared the initiator using the Steglich-esterification. Polyethylene glycol monomethyl ether (Mn 550 g/mol, 1.10 g, 2.00 mmol, 5.00 eq., PEG) and DMAP (6, 24.4 mg, 200 µmol, 0.50 eq.) were dried by adding toluene (5.00 mL) and evaporating the solvent under reduced pressure. EDCI (7, 169 mg, 880 mol, 2.20 eq.) was dissolved in DCM (4.00 mL). In a second round bottom ask, PEG, DMAP 6 and 4,4'-(diazene- 1,2-diyl)bis(4-cyanopentanoic acid) (5, 112 mg, 400 µmol, 1.00 eq.) were dissolved in DCM (4.00 mL). Both solutions were stored in the freezer at 20 °C for 1 h. The EDCI solution was added to the second solution drop by drop. The reaction mixture was allowed to warm up to RT and stirred for 48 h in an argon atmosphere. Then the reaction mixture was added to Et_2O (100 mL) to precipitate unreacted acid and longer polymers. The supernatant was skimmed oand the solvent evaporated. The residue was redissolved in DCM (2.00 mL) and added to hexane (100 mL) to precipitate the polymer. The precipitation in hexane was repeated twice.

Synthesis of the Block-Copolymers PEG-PdmCL

The PEG macroinitiator (4.58 mg, 3.36 µmol, 0.03 eq.) was dissolved in toluene (5 drops). CKA **2** (200 mg, 1.41 mmol, 1.00 eq.) was added. The mixture was purged with argon for 15 min. Then the mixture was heated to 130 °C and stirred for 4 days in an argon atmosphere. The reaction mixture was dissolved in EtOH (10.0 mL) and dialyzed (MWCO 1000 Da) against

EtOH, exchanging the solvent three times and leaving each batch of solvent for 2 h. The solvent was then removed under reduced pressure to yield PEG-PdmCL block-copolymer (114 mg).

^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 4.87 (m, 1H, CH, PdmCL), 4.42 (m, 2H, CH_2, PdmCL), 4.27 (m, 2H, CH_2, PdmCL), 4.04 (m, 2H, CH_2, PdmCL), 3.83 (m, 2H, CH_2, PdmCL), 3.63 (s, 4H, PEG) 3.45 (s, 2H, CH_2, PdmCL), 2.10 (m, 2H, CH_2, PdmCL), 1.60 (m, PdmCL), 0.94 (m, 3H, CH_3, PdmCL).

Formation of the Nanoparticles

PEG-PdmCL block copolymer (3.00 mg) was dissolved in DCM (1.00 mL) inside a 2.5 mL glass vial. The vial was placed in a vacuum oven and the solvent evaporated by setting the temperature to 45 °C and the pressure to 400 mbar for 1 h, supplying a constant flow of air. Then the pressure was set to 0 mbar for 3 h. Pure water (1.00 mL, filtered with a hydrophilic PTFE filter with 0.45 μm pores) was added and the mixture was stirred at RT for at least 2 days. The hydrodynamic radius of the formed nanoparticles was measured by DLS, conducted in "2D Pseudocross" mode with a HeNe laser (λ=633 nm). The correlation function was measured at 25 °C and at scattering angles of 45°, 90° and 135°, with an acquisition time of 20 s, measuring three times per angle. The intensity size distribution of the samples was obtained by estimating the translational diffusion coefficient from the correlation function using Contin analysis and convert it to the hydrodynamic radius using the Stokes-Einstein equation.

Degradation of the Nanoparticles

The degradation of PEG-PdmCL nanoparticles was observed by measuring the intensity of the scattered light of a nanoparticle suspension (count rate at the detector). The apparatus described in section was used for this experiment. The laser intensity was set to 296 ± μW, the temperature to 25 °C and the scattering intensity was measured at an angle of 90°. After the

first measurement, an aqueous solution of porcine liver esterase (40 µL, 1.20 mg/mL, 2 mass-% of the polymer) was added. The scattering intensity was then measured at different points in time over two days, using the previously described settings.

Encapsulation of HRP and Assay with Amplex-Red

A PEG-PdmCL film was formed as indicated previously. The film was rehydrated with an aqueous solution of horse raddish peroxidas (HRP) (1.00 mL, 20 ng/mL) and the mixture was stirred at RT for 1 week. Separation of the nanoparticles from free HRP was achieved using SEC, which was performed on a column packed with Sepharose 2B and monitored with an Aecta Prime UV-Detector (Amersham Pharmacia Biotech). Pure water was used as eluent. See section 3.2 of the SI for more details on the used amounts.

Cell Viability Test

A PEG-PdmCL polymer film was prepared as mentioned in section 0 and rehydrated with 0,1M PBS. The mixture was stirred at RT for 1 week.

The effect of PEG-PdmCL particles on cell viability was tested by a standard MTS assay (CellTiter 96® AQueous one solution cell proliferation assay, Promega). Briefly, 3x103 HeLa cells per well were seeded in 96-well microtiter plates and cultured for 24 h at 37°C in a 5% CO_2-95% air incubator. Particles were added at a maximum concentration of 0.4 µg/mL and incubation continued for 24 h. 20 µl of MTS reagent were directly added to the culture medium of each well and incubated for 2h at 37 °C. The absorbance of treated and untreated cells was measured at OD 490 nm with a microtiter plate reader (SpectraMax M5, Molecular Devices). All experimental conditions were tested in quadruplicate.

RESULTS AND DISCUSSION

We realized that the predominantly used acetal route is rather harsh. Producing the intermediate acetal **5** using the dimethylated haloacetal **4** (Figure 2a) requires high temperatures to constantly distill off methanol.[10] Recent research has shown that $CoCl_2$ together with chlorotrimethylsilane (TMSCl) can be an effective catalytic system for the formation of cyclic acetals.[24] Applying this protocol to our system led to the closed ring acetal **5** with 61 % yield. Key properties of the $CoCl_2$/TMSCl induced formation of acetal **5** are its high reproducibility and ambient reaction conditions (see section 2.2 of the SI for details). The subsequent elimination reaction with potassium tert-butoxide yielded the CKA **2** in 32%, which amounts to an overall yield of 20 %. This relatively low yield prompted us to investigate an alternative route towards DMMDO *via* the intermediate carbonate **6** and consecutive olefination. Carbonate **6** was formed from diol **1** by treatment with triphosgene in 66% yield (Figure 2a). Because it proved to be an effective carbonylation agent, triphosgene is useful despite its toxicity provided that extra care is taken during the reaction. Timing proved to be critical for this reaction. Kinetic studies *via* gas chromatography (GC) showed that the product formation had reached its peak after 20 minutes, and side-products started to appear afterwards. Over the following hours, the fraction of carbonate product decreased considerably (see section 2.4 of the SI for details). Therefore, the optimal reaction conditions were found to be 20 minutes at room temperature. The olefination reaction was then performed with commercially available Petasis reagent[23, 31]. The Petasis reagent (**7**, Figure 2a) is a titanium based methylenation reagent similar to the Tebbe reagent,[31, 32] but is free from Lewis acids.[23, 31, 33] GC and NMR confirmed the formation of the same CKA **2** as with the acetal pathway, now with a yield of 65% yield (see sections 2.5 and 2.6 of the SI). These results proved that the titanium compound **7** had successfully transferred one methylene unit onto the carbonate **6** (Figure 2a). The overall

yield of this procedure was 39%. Because this yield is a significant improvement over the acetal pathway, we plan to explore its use for other CKAs.

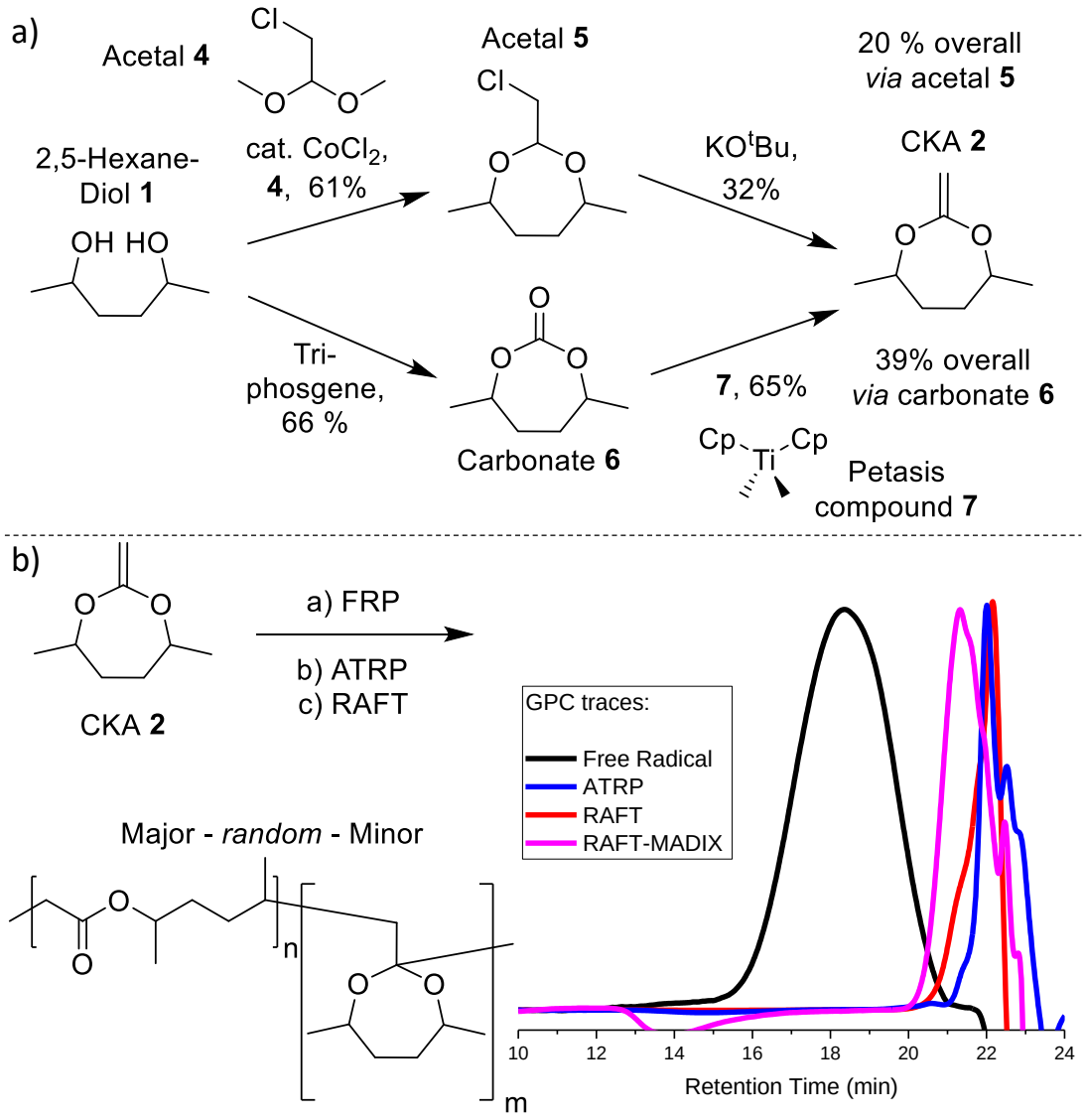

Figure 2: a) The CKA **2** can be produced via the acetal pathway (top) or the carbonate pathway (bottom). GC confirmed that both routes lead to the same product (section 2.6 of the SI). b) CKA 2 was then polymerized using different polymerization protocols and the results analyzed with GPC. A polymer was only formed using FRP.

The homopolymerization of CKA **2** with free and controlled radical polymerization techniques (approaches known to work in RROP) was investigated next (Figure 2b).[13, 19, 34-36] We observed that neither atom transfer radical polymerization (ATRP), nor reversible addition-fragmentation chain transfer polymerization (RAFT) led to polymerization. While xanthate-

based RAFT[18, 19] (RAFT-MADIX, see section 1.1 of the SI for details) led to oligomers, it was also not suitable to generate polymers. Free radical polymerization (FRP), however, led to short polymers of about 5000 g/mol (determined via GPC), which could be purified towards a low dispersity (Figure 2b). Further optimization of the FRP reaction conditions showed that a polymerization at 90 °C produced larger polymers at 110 °C or 130 °C, whereas polymers obtained at 130 °C exhibited a lower dispersity. As expected, lower amounts of initiator yielded longer polymers, although this trend was not linear (see Table 1-SI of the SI for details). The results suggest a low reactivity of DMMDO, making it react slowly in FRP and since CRP protocols lower the reaction rate, it then becomes unreactive. Evidently, more research on controlled homopolymerization of DMMDO is required. Nevertheless, the synthesized polyester proved to be completely degradable in basic acetonitrile. Due to a lack of solubility, degradation, basic or enzymatic, in aqueous media showed only limited success (see section 1.2 of the SI for details).

Since FRP yielded polymers, a PEG-modified derivative of AIBN allowed for the production of PEG-PdmCL, an amphiphilic block-copolymer (Figure 3a, details in section 1.3 of the SI). Like all amphiphilic block-copolymers, PEG-PdmCL self-assembled in aqueous media into nanoparticles. DLS and TEM analysis of the corresponding solution revealed a particle radius of 40 nm and 20-30 nm, respectively (Figure 3b). The larger size obtained from DLS is due to the water shell present around the nanoparticles. Due to their block-length ratio, the block-copolymer could self-assemble vesicles.[37] However, the particles do not collapse on a TEM grid, as one would expect for vesicles, and are too big to be simple micelles. They are thus likely to be multi-compartment micelles. [38]

Since the polymer was in a colloidal suspension, aqueous degradation ought to be possible. Adding esterase to the micelles led to a quick degradation of the polyester and thus also of the micelles.[39] At the same laser intensity, the count rate in DLS showed a short increase, but

then decreased rapidly over time (Figure 3c). Because the esterase can attack the nanoparticles only from the outside, one of the first bonds cleaved is the linkage between the PEG and PdmCL. Exposed hydrophobic PdmCL segments would then agglomerate and form undefined clusters of nanoparticles before further degradation. This explains the initial spike in the DLS count rate after esterase addition as well as the slow increase in size (see section 3.1 of the SI for details). With ongoing degradation, the number of agglomerates and thus the count rate decreases.

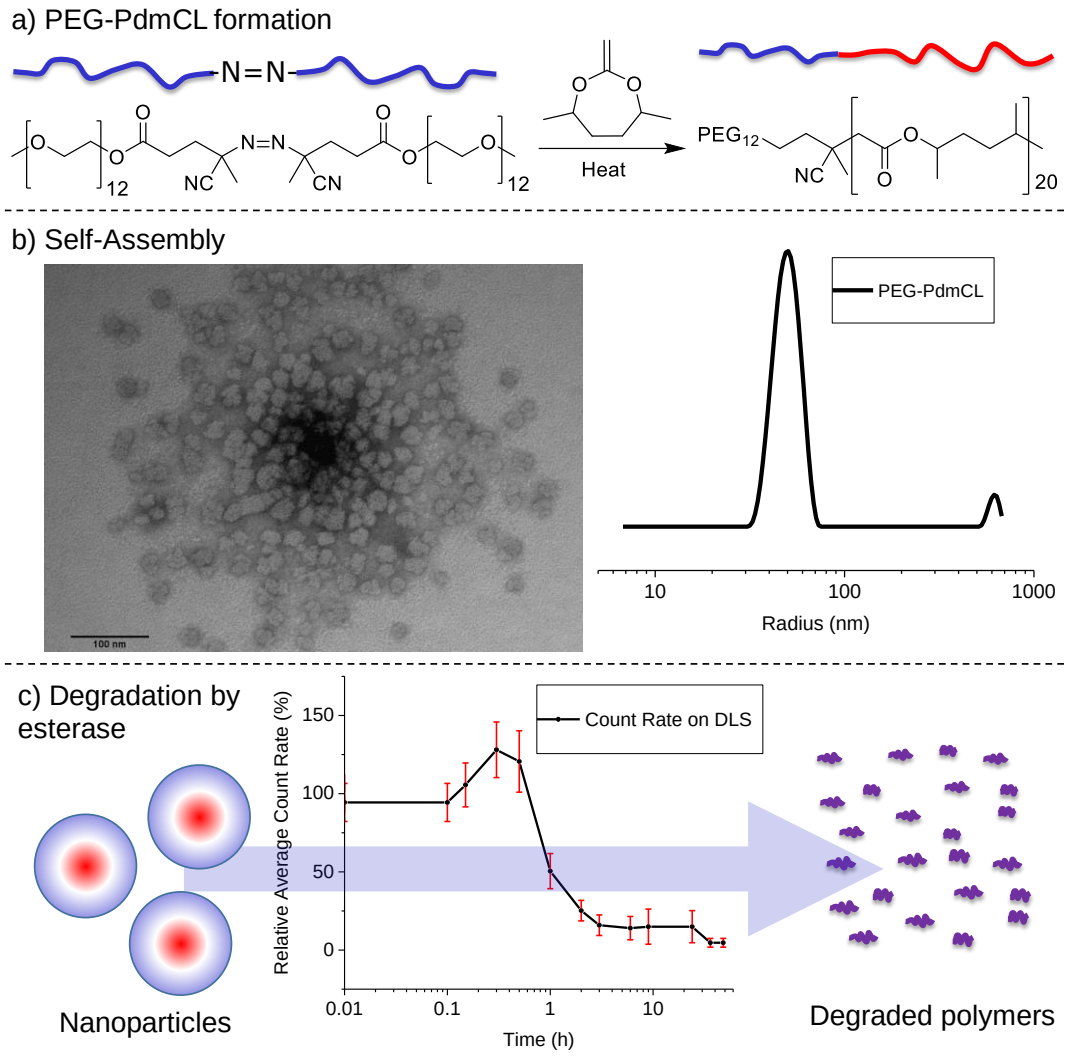

Figure 3: a) A PEG-modified derivative of AIBN leads to amphiphilic block-copolymers (b) which can then self-assemble into nanoparticles of a defined size as illustrated by TEM and the DLS intensity plot. c) Adding esterase leads to a disassembly of the nanoparticles as shown by the time-course of the DLS count rate.

Biodegradable PEG-PdmCL nanoparticles are promising candidates for drug delivery, which is why we tested their biocompatibility on cells in vitro. An MTS cell proliferation assay with HeLa cells showed that the particles did not affect cell viability up to a concentration 400 µg/mL of polymer. These results encourage us to further explore the use of PEG-PdmCL based micelles for drug delivery (Figure 4a).

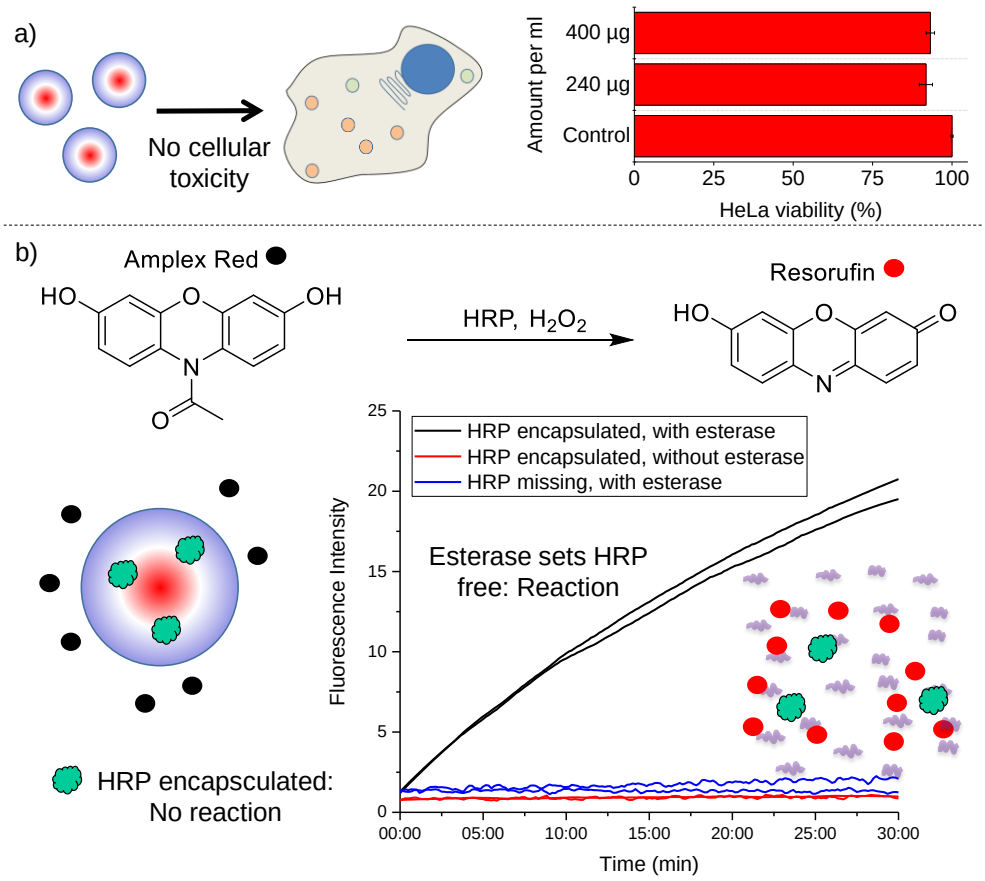

Figure 4: (a) PEG-PdmCL nanoparticles did not affect HeLa viability at a concentration of 400 µg/mL, (b) Encapsulated HRP in the presence of hydrogen peroxide did not convert Amplex Red (black dots) into resorufin (red dots). After adding esterase, the conversion took place. No reaction was observed when empty nanoparticles were treated with esterase. The graph represents duplicate data sets for all experiments.

Biodegradable nanoparticles can be used as a temporary shell for protecting enzymes.[3, 40] To test the suitability of our PEG-PdmCL nanoparticles for this application, we encapsulated horseradish peroxidase (HRP). The enzyme is known to oxidize Amplex Red into resorufin in the presence of hydrogen peroxide.[41, 42] Based on its fluorescence, resorufin can easily be

detected. Addition of Amplex Red and hydrogen peroxide to PEG-PdmCL nanoparticles with entrapped HRP did not yield fluorescence for 30 minutes (Figure 4b). This indicates that the Amplex Red was unable to penetrate the PEG-PdmCL shell and the entrapped HRP was unable to diffuse out of the nanoparticles. Thus, the nanoparticles appear diffusion-proof for at least 30 minutes. It is also noteworthy that hydrogen peroxide did not induce degradation of the polyester. Just like for the empty nanoparticles, adding esterase induced the degradation of the nanoparticles with entrapped HRP. The process creates unprotected HRP, which catalyzed the conversion of Amplex Red to resorufin (Figure 4b). Control micelles without HRP did not promote resorufin formation when treated with esterase and hydrogen peroxide. This finding underlines that the release of HRP from the nanoparticles initiated the conversion of Amplex Red. We conclude that PEG-PdmCL based nanoparticles act as protection for enzymes that are set free and activated upon cleavage of the polyester. This is of particular interest in light of our previous report showing that free enzymes tend to become inactive if left in an aqueous solution.[43] In contrast, PEG-PdmCL encapsulated HRP retained activity for at least one week.

CONCLUSION

In conclusion, we found that the introduction of the $CoCl_2$/TMSCl catalyst decreased the reaction temperature of the conventional monomer synthesis to room temperature. Moreover, we established a new carbonate route involving the Petasis compound to produce the DMMDO broadening this approach for CKA synthesis. Larger synthetic diversity to gain CKAs may eventually extend the overall scope of RROP if more monomers become available. We achieved homopolymers from DMMDO using free radical polymerization, but also amphiphilic block-copolymers using a PEG-based macroinitiator. Empty and enzyme encapsulating nanoparticles readily self-assembled from the PEG-PdmCL block copolymers. The sensitivity of the nanoparticles to esterase degradation offers a release mechanism to

control the activity of entrapped enzymes. Our results motivate us to use the polymer in a more general context as a temporary protective cover for enzymes. Moreover, the nanoparticles did not affect cell viability, making them interesting candidates for drug delivery. Our data shows that polyesters from RROP are already readily available and are especially promising in applications involving self-assembled nanoparticles.

ASSOCIATED CONTENT

Supporting Information. Details on the applied chemicals, synthetic protocols, NMR data, GC data on monomer synthesis, GPC traces protocols for the enzymatic reactions and cell tests are available in the supporting information. This material is available free of charge via the Internet at http://pubs.acs.org.

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ABBREVIATIONS

AIBN, azobisobutyronitril; ATRP, atom transfer radical polymerisation; CKA, cyclic ketene acetal; DLS, dynamic light scattering; DMMDO, 4,7-dimethyl-2-methylene-1,3-dioxepane; HRP, horse raddish peroxidase; MADIX, macromolecular design via interchange of xanthates; PCL, poly(caprolactone); PdmCL, poly(dimethylcaprolactone); RAFT, reversible addition and chain fragment transfer polymerisation; RROP, radical ring opening polymerization; TEM, transmission electron microscopy

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