Polymer Chemistry

COMMUNICATION

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Cite this: *Polym. Chem.*, 2019, **10**, 5285

Received 25th July 2019,
Accepted 19th September 2019

DOI: 10.1039/c9py01103j

rsc.li/polymers

Novel monomers in radical ring-opening polymerisation for biodegradable and pH responsive nanoparticles†

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Responsive and biodegradable nanoparticles are essential for functional drug delivery systems. We herein report the first pH sensitive polyester from radical ring-opening polymerisation of novel amine-bearing cyclic ketene acetals (CKAs). The CKAs were synthesised *via* an intermediate carbonate and the resulting polyesters showed a pK_a around pH 6. Together with an initial application in biodegradable nanoparticles, they open the pathway for a new generation of functional polyesters.

Amphiphilic block-copolymers are the basis of modern research into nanoparticles (NPs) such as micelles or vesicles that can act as nanoreactors and drug delivery systems.[1–4] Materials originating from radical polymerisation (RP) include a number of pH sensitive hydrophobic blocks. For drug delivery systems, pH sensitivity towards protonation in the pH range 4–6 is desired in the final NPs. The range is beneficial, because during endosomal uptake into cells, the pH around the NPs drops from 7.4 in the cytosol to below 4 in lysosomes.[5–7] Such pH sensitivity originates almost exclusively from tertiary amines in the side chain of the hydrophobic block of the amphiphilic block-copolymers. The tertiary amines are protonated upon acidification and turn the hydrophobic block into a hydrophilic one. This causes the NPs to disassemble and release any encapsulated content.[1,4,8,9] Polymers from RP bear an all-carbon main chain, rendering them non-biodegradable, which is a disadvantage. Ring-opening polymerisation (ROP) yields biodegradable polymers such as polyamides or polyesters,[10–13] but does not tolerate amines. A chain-growth polymerisation that delivers both pH sensitivity and biodegradability is required if NPs for drug delivery are to have both these desirable properties. Poly (β-aminoesters) do combine both properties, but are only available from polycondensation, which strongly limits their use in block-copolymers.[14] Radical ring-opening polymerisation (RROP) from cyclic ketene acetals (CKAs)[15–17] is a chain-growth polymerisation with the potential to yield the desired polymers. In RROP, a radical opens the cyclic monomer and the original acetal functionality is transformed into a biodegradable polyester.[18–24] CKAs have been transformed into homopolymers and statistical copolymers with vinyl acetates to introduce biodegradable bonds into the vinylic or methacrylic main chain.[25–27] In earlier work from our group, polyesters from RROP-homopolymerisation were introduced into amphiphilic block-copolymers to yield biodegradable NPs.[28] RROP is currently only known to form non-pH responsive polyesters, giving it no decisive advantage over ROP.[29] However, due to its radical nature, RROP has the potential to tolerate amines and give pH sensitive biodegradable NPs (Fig. 1).

In order to unlock the full potential of RROP, we aimed to synthesise amine-bearing CKAs and to transform them into pH

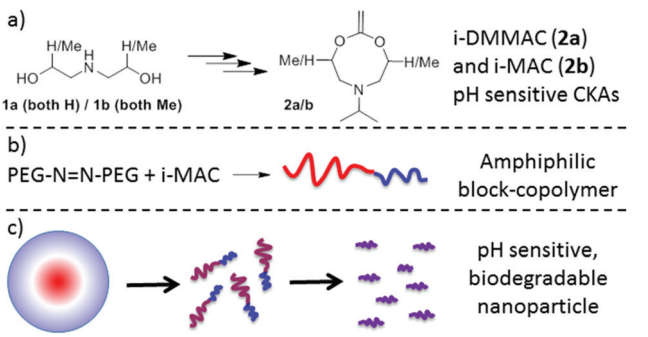

Fig. 1 (a) The diols **1** containing an amine are transformed into pH sensitive CKAs **2** by ring closure and functional group transformations. (b) Polymerising the CKAs with a poly(ethylene glycol) (PEG) based macroinitiator yields amphiphilic block-copolymers, which can then self-assemble into nanoparticles. (c) These nanoparticles can disassemble upon acidification (protonation of the amines) or on adding esterase, triggering biodegradation.

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† Electronic supplementary information (ESI) available: Experimental procedures and additional graphs and calculations. See DOI: 10.1039/c9py01103j

sensitive polyesters that are inaccessible by RP or ROP. Starting from two diols (**1a** and **1b**), we synthesised two previously unknown CKAs with tertiary amines, 6-iso-propyl-4,8-dimethyl-2-methylene-1,3,6-dioxazocane (i-DMMAC, **2a**, Fig. 1) and 6-iso-propyl-2-methylene-1,3,6-dioxazocane (i-MAC, **2b**, Fig. 1). Both were polymerised into pH sensitive biodegradable polyesters for an initial study of biodegradable and pH-sensitive NPs (Fig. 1).

To ensure that the amine could not induce instability, or be separated from the polymer by elimination or substitution, we decided that the tertiary amine should be in the main chain of the polyester. This required a CKA bearing a tertiary amine within its ring and in turn required the starting diols **1a** and **1b** to have a secondary amine in their main chain. Protonated poly(diisopropylaminoethyl methacrylate) (PDPA) has a pK_a of about 6–6.5 in water, and is widely used in NPs for drug delivery.[30–32] To mimic this physical property, we synthesised the *N*-isopropyl derivatives **3a** and **3b**.

Alkylation of bis(2-hydroxypropyl)amine (**1a**) with 2-iodo-propane gave the tertiary amine **3a** in 85% yield. Multiple attempts at the traditional ring-closure to form CKAs *via* an intermediate haloacetal[17,23,28] failed. Our recently published route *via* an intermediate cyclic carbonate[28] proved to be successful. Formation of the cyclic carbonate with ethyl chloroformate gave **4a** as a crude mixture of *cis*- and *trans*-diastereomers. To investigate any difference in reactivity of the corresponding diastereomeric CKAs in the polymerisation, the individual major *cis*-(52%) and minor *trans*-(29%) diastereomers were separated by standard silica gel chromatography. Olefination[28,33] of the major *cis*-**4a** with freshly prepared Petasis reagent gave CKA **2a** as a liquid in 24% yield (i-DMMAC, Fig. 2). Attempted olefination of *trans*-**4a** under identical conditions gave an undefined complex mixture, in which the CKA could not be detected by NMR. The non-methylated analogue amine-bearing CKA **2b** was synthesised to investigate the influence of the methyl groups on the polymerisation (see mechanism in section 3b of the ESI†). The synthesis of **2b** followed a similar pathway to that for **2a**. The reaction conditions for the *N*-alkylation had to be optimised and used 2-bromopropane in water, giving the tertiary amine **3b** in 64% yield. Conversion of **3b** into the achiral cyclic carbonate **4b** (68%) and subsequent treatment with freshly prepared Petasis reagent (**5**) gave i-MAC **2b**, as a liquid in 12% yield (Fig. 2). The low yields for both CKAs reflected their lower stability compared to CKAs without amines, which also resulted in a lower shelf-life (typically 2 weeks at −20 °C under argon).

Both novel CKAs **2a** and **2b** were then subject to RROP polymerisation. Instead of the intended ring opening, CKAs can remain closed and the acetal-radical can propagate the polymerisation. The percentage of ring-opening in all polymers was assessed by ^{13}C-NMR spectroscopy (reported to the nearest 5%; see section 3b of the ESI†) and despite investigating several conditions (Fig. 3), i-DMMAC **2a** did not polymerise in the presence of different radical initiators and solvents. With azo-bis-isobutyronitrile (AIBN, **6**) as the initiator, small amounts of solvent (50 μL for 200 μL of CKA) had to be added to dissolve AIBN. Ethyl acetate, toluene and chlorobenzene as solvents gave no polymerisation at various temperatures. All ^{1}H-NMR spectra of the post-reaction mixtures contained the prominent methylene peak of the CKA (section 2i of the ESI†). Changing the reaction conditions to photo-initiated RROP[34] with benzoin methyl ether (**7**, BME, Fig. 3) as initiator, yielded a solid material and GPC verified the formation of an oligomer

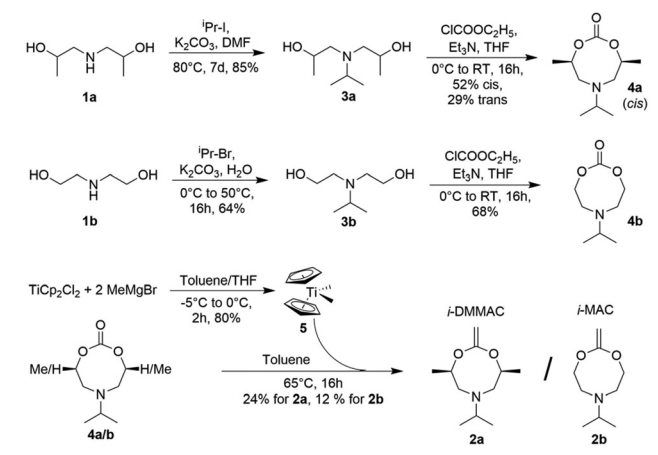

Fig. 2 Amino-diols were iso-propylated to give the intermediate diols **3a** and **3b**. Using ethyl chloroformate, the rings were closed to yield the respective carbonates **4a** and **4b**. Using freshly prepared Petasis reagent (**5**), the carbonates were transformed into the CKAs **2a** and **2b**.

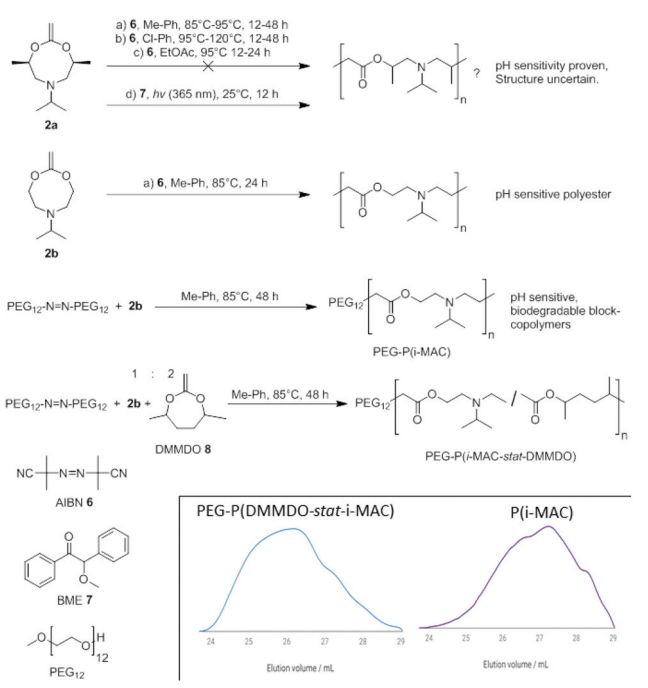

Fig. 3 i-DMMAC **2a** did not polymerise under various conditions, using different solvents, temperatures and AIBN **6** as an initiator. A polymerisation in bulk using UV-activated polymerisation with BME **7** led to a pH sensitive material. i-MAC **2b** yielded a polymer using classic free radical polymerisation with AIBN **6** and was then transformed into a block-copolymer with a PEG-based macroinitiator by itself and also in a 1 : 2 mix with DMMDO.

(see section 3a of the ESI†). Although the material exhibited pH sensitive behaviour around pH 5 (section 4b of the ESI†), the suggested structure of the polyester could not be proven by ^{1}H-NMR and ^{13}C-NMR. Furthermore, BME-initiated photo-polymerisation did not allow the use of a PEG macroinitiator, hindering the production of amphiphilic block-copolymers for NPs. The second amine-bearing CKA, i-MAC **2b**, did polymerise using AIBN **6** as an initiator with small amounts of toluene as solvent (50 μL, Fig. 3). Classic pH titration of poly(i-MAC) proved the pH sensitivity of the polyester and showed a pK_a of 6.0 (inflection point in Fig. 4a), which is similar to the pK_a of PDPA.[7] The titration also confirmed the precipitation of the polymer upon deprotonation as it switched from hydrophilic (blue in Fig. 4a) to hydrophobic (red in Fig. 4a). A ring-opening content of 70% (by ^{13}C NMR; see sections 2l and 3b of the ESI† for structure and calculations) now allowed for the synthesis of amphiphilic block-copolymers for biodegradable and pH-sensitive NPs.

Poly(ethylene glycol) (PEG_{12}) was the set hydrophilic block as it could be transformed into a macroinitiator based on AIBN for free radical polymerisation.[28] It was combined with two different hydrophobic blocks; one was a pure polymerised i-MAC (P(i-MAC)) and one was a 1 : 2 mixture of i-MAC **2b** with 4,7-dimethyl-2-methylene-1,3-dioxepane (**8**) (DMMDO, Fig. 3). DMMDO was already a known CKA for RROP and was added to compare the degradation behaviour of pure P(i-MAC) in the hydrophobic block to a statistical copolymer with P(DMMDO). GPC of both block co-polymers (PEG-P(i-MAC), 70% ring-opening) and (PEG-P(DMMDO-*stat*-i-MAC), 100% ring-opening) revealed a short hydrophobic block, confirming a successful reaction (Fig. 3). Short hydrophobic blocks were expected due to the high termination rate, which is typical for RROP.[15,28] Impurities of homopolymers, especially from PEG, were still present. This was not necessarily detrimental as PEG homopolymers are known to help in nanoparticle formation in some cases.[35,36] The two amphiphilic block-copolymers allowed for an initial study of NP formation and their biodegradation.

The NPs were formed by basifying (with 0.1 M NaOH) a homogeneous acidic (pH 5.0) solution of each polymer. The addition of acid (0.1 M HCl) triggered a pH dependent disassembly of the NPs, proving their pH-sensitivity. The disassembly process was monitored by the count rate of dynamic light scattering (DLS) as a function of pH. PEG-P(i-MAC) showed a continuous decay starting from pH 7, indicating that the disassembly started around the pK_a value of P(i-MAC) (see section 4c of the ESI†). NPs from PEG-P(DMMDO-*stat*-i-MAC) disassembled slightly below pH 4 (Fig. 4). Since only a third of the hydrophobic block is composed of the pH sensitive P(i-MAC), a greater acidity was needed to achieve the same overall charge required to trigger a disassembly of the NPs. DLS traces, especially of intermediate stages, were multimodal (Table S1 in the ESI†), suggesting a diverse degradation process, which will be addressed in detail in future studies.

With pH sensitivity demonstrated, biodegradability was investigated by adding 2 wt% esterase (porcine liver) to the NPs in phosphate buffer saline (PBS). DLS of NPs of both block-copolymers showed a brief increase in scattering intensity before decaying rapidly (Fig. 4). This has been reported for a similar system.[28] When the esterase started digesting the block-copolymers, we postulate that the first ester bond attacked was the one between PEG and the polyester. The exposed hydrophobic polymers then agglomerated and led to a brief period where larger NPs scattered the light with a greater intensity. NPs from PEG-P(i-MAC) degraded considerably more slowly than ones from the copolymer with DMMDO (10 h instead of 90 minutes; see section 4d of the ESI†). Adding DMMDO was thus beneficial for the esterase-based degradation process. The partially multimodal DLS traces (Table S1 of the ESI†) suggested that the NPs had large size distribution, which was consistent with the broadly dispersed polymer from the free radical polymerisation. A more controlled polymerisation needs to be developed in order to obtain NPs with a narrower size distribution, which are required for a biomedical or pharmaceutical application as a drug-delivery system. These initial results show the formation of the first pH sensitive and biodegradable NPs from a polymer formed from RROP.

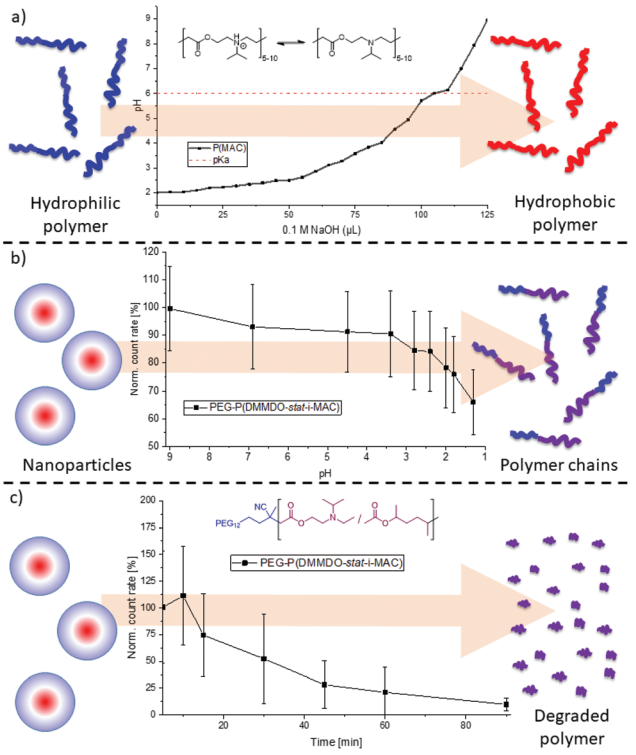

Fig. 4 (a) P(i-MAC) showed pH sensitive behaviour at a pH of 6.0. At this pH the hydrophilic protonated version (blue) becomes hydrophobic (red). (b) The nanoparticles from PEG-P(DMMDO-*stat*-i-MAC) disassembled into single polymer chains upon acidification from a pH of 4 and below. (c) The same nanoparticles were degraded upon the addition of esterase (porcine liver) as shown by the decay of the count rate in DLS.

Conclusions

Two new CKAs **2a,b** containing tertiary amines were synthesised *via* our recently reported carbonate route.[28] The inter-

mediate carbonates were transformed into the CKAs using the Petasis reagent. Both CKAs mark a major addition to the library of CKAs since they unlock pH responsive behaviour for polyesters from CKAs. RROP proved to be feasible with these monomers, transforming them into pH sensitive materials using photo-initiated radical polymerisation or free radical polymerisation. The consecutive formation of amphiphilic block-copolymers enabled the formation of NPs with a new combination of properties. All NPs formed showed the intended pH responsiveness originating from the tertiary amines as well as biodegradability resulting from the ester units. This combination of properties was previously unknown in polymers from RROP and from other chain-growth polymerisations. The results represent an important milestone for the development of biodegradable polymers from RROP as it can now produce polymers inaccessible by any other polymerisation. The combination of new monomers and new functional polymers form the basis for a large new class of materials to be used in nanoparticle research.

Conflicts of interest

There are no conflicts to declare for all authors.

Acknowledgements

Financial support of the Novartis–University of Basel Fellowship for Excellence in Life Sciences for J. G. and the Swiss National Science Foundation (SNSF) National Centre for Competence in Research for Molecular Systems Engineering (NCCR-MSE) is gratefully acknowledged.

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