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COMMUNICATION

Damages and Repair in Informational Poly(*N*-substituted urethane)s

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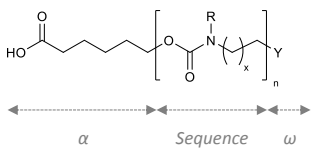
Abstract: The degradation and repair of uniform sequence-defined poly(*N*-substituted urethanes) was studied. Polymers containing an ω-OH end-group and only ethyl carbamate main-chain repeat units rapidly degrade in NaOH solution via an ω→α depolymerization mechanism with no apparent sign of random chain cleavage. The degradation mechanism is not notably affected by the nature of the side-chain *N*-substituents and took place for all studied sequences. On the other hand, depolymerization is significantly influenced by the molecular structure of the main-chain repeat units. For instance, hexyl carbamate main-chain motifs block unzipping and can therefore be used to control the degradation of specific sequence sections. Interestingly, the partially-degraded polymers can also be repaired; for example using a combination of *N*,*N*'-disuccinimidyl carbonate with a secondary amine building-block. Overall, these findings open up interesting new avenues for chain-healing and sequence editing.

Informational polymers are macromolecules that store information in a controlled comonomer sequence.[1] A typical example of that is DNA, which is a biological storage medium. In recent years, it has also been reported that data (*e.g.* in a binary format) can be stored in synthetic macromolecules, for example in polyamides, polyesters and polyurethanes, just to name a few.[2] This new class of functional polymers has opened up interesting applications in areas such as data storage, cryptography, anti-counterfeiting, traceability and plastic recycling.[3] Yet, there are still pronounced differences between natural and non-natural informational polymers. Man-made polymers mainly enable passive information storage, while the information sequences of DNA can be copied, mutated, damaged, modified (*i.e.* through epigenetics) and repaired.[4] There are currently only a couple of examples of synthetic information sequences that can be degraded or transformed;[2c, 5] although such behaviors could be useful for the aforementioned applications as well as for more distant goals such as the development of artificial Life.[6]

Here, we report the controllable degradation and possible repair of informational poly(*N*-substituted urethane)s. Sequence-defined polyurethanes can be synthesized via different solid-phase chemistry approaches.[2d, 7] Different types of functional sequences can be prepared, including digitally-encoded ones.[2d, 7e] It is also well-known that some linear or dendritic polyurethanes undergo controllable depolymerization (sometimes referred to as self-immolative behavior).[8] In these macromolecules, chain-degradation is triggered by a nucleophile (*e.g.* a primary amine) and proceeds via an unzipping mechanism. This interesting mechanism has been explored for biomedical applications, for instance for triggered drug release.[9] Very recently, Anslyn and coworkers[10] have reported that the depolymerization of sequence-defined polyurethanes can be kinetically-controlled to enable sequencing.[11] In this case, chain unzipping was triggered by a terminal OH-group and required basic conditions and elevated temperatures (70°C, microwave) to proceed. In the present work, we investigated the depolymerization in basic medium of sequence-coded poly(*N*-substituted urethane)s, a promising new family of digital polymers.[7e] As described in the following paragraphs, depolymerization occurs at room temperature and can be finely tuned by macromolecular design.

The poly(*N*-substituted urethane)s studied in this work were all synthesized using a recently-reported orthogonal solid-phase synthesis protocol.[7e] Figure 1 shows their general structure. These informational polymers are directional and contains therefore two distinct chain ends denoted as α and ω. The former one is a caproic acid residue, whereas the latter one is either an OH or a methyl group. The coded sequences were constructed using a set of nine different informational monomers **A**-**I**. Figure S1 shows the molecular structure of the polymers **P1**-**P13** studied in this work. The molecular uniformity of the polymers was assessed by high-resolution electrospray mass spectrometry (ESI-HRMS) and size exclusion chromatography (SEC), while their coded sequences were characterized by tandem mass spectrometry (MS/MS) (Table S1 and Figures S2-S14).[12]



(**A**) R = Me, x = 1
(**B**) R = Et, x = 1
(**C**) R = Pr, x = 1
(**D**) R = Bu, x = 1
(**E**) R = Bz, x = 1
(**F**) R = Bz, x = 2
(**G**) R = Bz, x = 3
(**H**) R = Bz, x = 4
(**I**) R = Bz, x = 5

Y = OH or CH_3

Figure 1. General structure of the informational poly(*N*-substituted urethane)s.

Since the depolymerization of sequence-defined poly(*N*-substituted urethane)s was never investigated, the degradation mechanism and kinetics were first analyzed in details. For polymers having an ω-hydroxyl end-group and ethyl carbamate repeat units (x = 1 in Figure 1), complete depolymerization occurs at room temperature in the presence of sodium hydroxide. For example, Figure S2 shows the ESI-HRMS spectrum and SEC chromatogram of **P1** having the sequence α-DCEC-OH after 16

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hours in basic medium. Both analytical methods evidence the disappearance of **P1** and the formation of three low molecular weight species of mass 129.1, 142.1 and 177.1 Da, which correspond to the oxazolidinone rings resulting from the cyclization of repeat units **C**, **D** and **E**, respectively. As proposed in Scheme S1, these compounds are likely formed through a backbiting reaction leading to complete chain depolymerization. To validate this mechanism, **P2** having the same primary structure as **P1** but no terminal OH group (i.e. α-DCEC-CH_3) was studied in the same basic conditions (Figure S3). This experiment indicated no sign of degradation, thus suggesting that depolymerization solely occurs through an ω→α unzipping mechanism, *i.e.* without contribution from other mechanisms such as random chain cleavage.[13] Furthermore, this behavior is fully orthogonal as shown in Figure 2, which compares the ESI-HRMS spectra of a mixture of **P1** and **P2** before and after basic treatment. The selective depolymerization of **P1** is observed in these conditions.

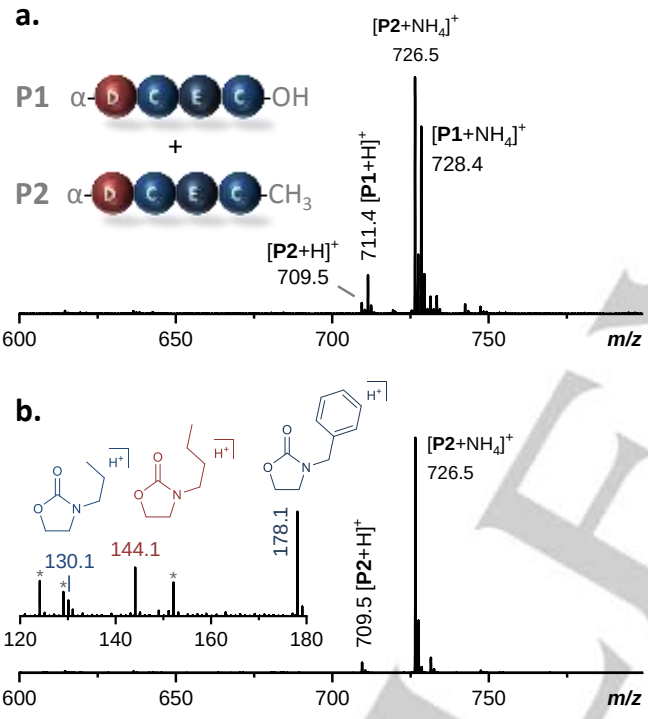

Figure 2. Positive mode ESI-HRMS spectra of a 1:1 w/w mixture of polymers **P1** and **P2**: (**a**) before basic treatment and (**b**) after 16h at RT in NaOH solution in MeOH/H_2O 2:1 v/v. The inset of panel **b** shows a zoom of the 120-180 *m/z* region of the obtained spectrum. As compared to the main spectrum, the intensity scale of the inset has been increased by a factor of about 18. The asterisks indicate background noise peaks.

Figures 3 and S15 show the kinetics of depolymerization of polymer **P1**, as monitored by SEC. In Figure 3, the evolution of the ratio of the peak areas of the partially-degraded products (α-DCE-OH, α-DC-OH and α-D-OH) over the peak area of the remaining initial polymer **P1** are displayed as a function of time. These results suggest that α-DCE-OH is first predominantly formed, followed by the gradual accumulation of α-DC-OH and α-D-OH. Comparable data were although recorded by ESI-MS (Figure S16), even though peak intensities should be interpreted with caution in mass spectrometry. Altogether, these results suggest a gradual unzipping degradation, as already reported for regular sequence-defined polyurethanes.[10]

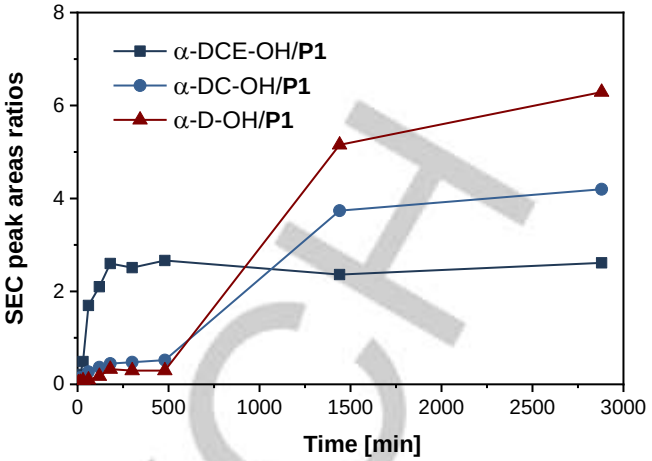

Figure 3. Evolution of SEC peak areas ratios as a function of time for the degradation of **P1** in NaOH solution in MeOH/H_2O 2:1 v/v. Peak areas were estimated by deconvoluting the chromatograms of Figure S15b using the Origin software. For a given time, the ratios α-DCE-OH/**P1**, α-DC-OH/**P1** and α-D-OH/**P1** are calculated from the corresponding chromatogram.

Possible sequence effects were also investigated. Polymers **P3** and **P4** having ω-hydroxyl end-group and ethyl carbamate repeat units but a different sequence and a different terminal monomer unit from **P1** were examined (Figures S4-S5). **P3** and **P4** have a similar terminal unit but a different penultimate unit. Depolymerization proceeded in all cases and appeared to be sequence-independent and side-group-independent (for both penultimate and terminal positions). Yet, minor deviations in depolymerization kinetics cannot be ruled out but could not be evidenced in the performed experiments.

Furthermore, the influence of the number of the methylene groups in the repeat units was investigated. A series of polymers **P4**-**P8** having all the same sequence but different terminal monomer units (R = Bz, x ranging from 1 to 5, Figure 1) was studied (Figures S5-S9). After 16h of basic treatment in methanol/water, the polymer having an ethyl terminal (**P4**) unit was entirely degraded. The polymer having a propyl-based (**P5**) terminal units was significantly degraded but a residual peak of the initial polymer was still observed. For polymers having butyl- (**P6**) and pentyl-based (**P7**) terminal units, the pristine polymer was still observed as a dominant species, although traces of degradation were detected. The polymer having a hexyl-based terminal unit (**P8**) obviously did not degrade in the studied conditions and only exhibited marginal oxazolidinone peaks. Behaviors comparable to those described for **P5**-**P8** were obtained with polymers with alternative monomer sequences **P9**-**P12**. Altogether, these results imply that the depolymerization mechanism proceeds well when 5- and 6-membered rings are formed but is slowed down or hindered by repeat units leading to the formation of larger rings. To confirm this hypothesis, a mixture of polymers **P4**-**P8** was treated in basic medium for 16h. ESI-HRMS analysis (Figure S17) indicated complete disappearance of **P4**, significant disappearance of **P5**, partial disappearance of **P6** and **P7**, whereas **P8** remained the most abundant species. Although these results should be interpreted with caution due to potential suppression effects during the ionization process, this experiment confirms that main-chain alkyls strongly influence the depolymerization mechanism.

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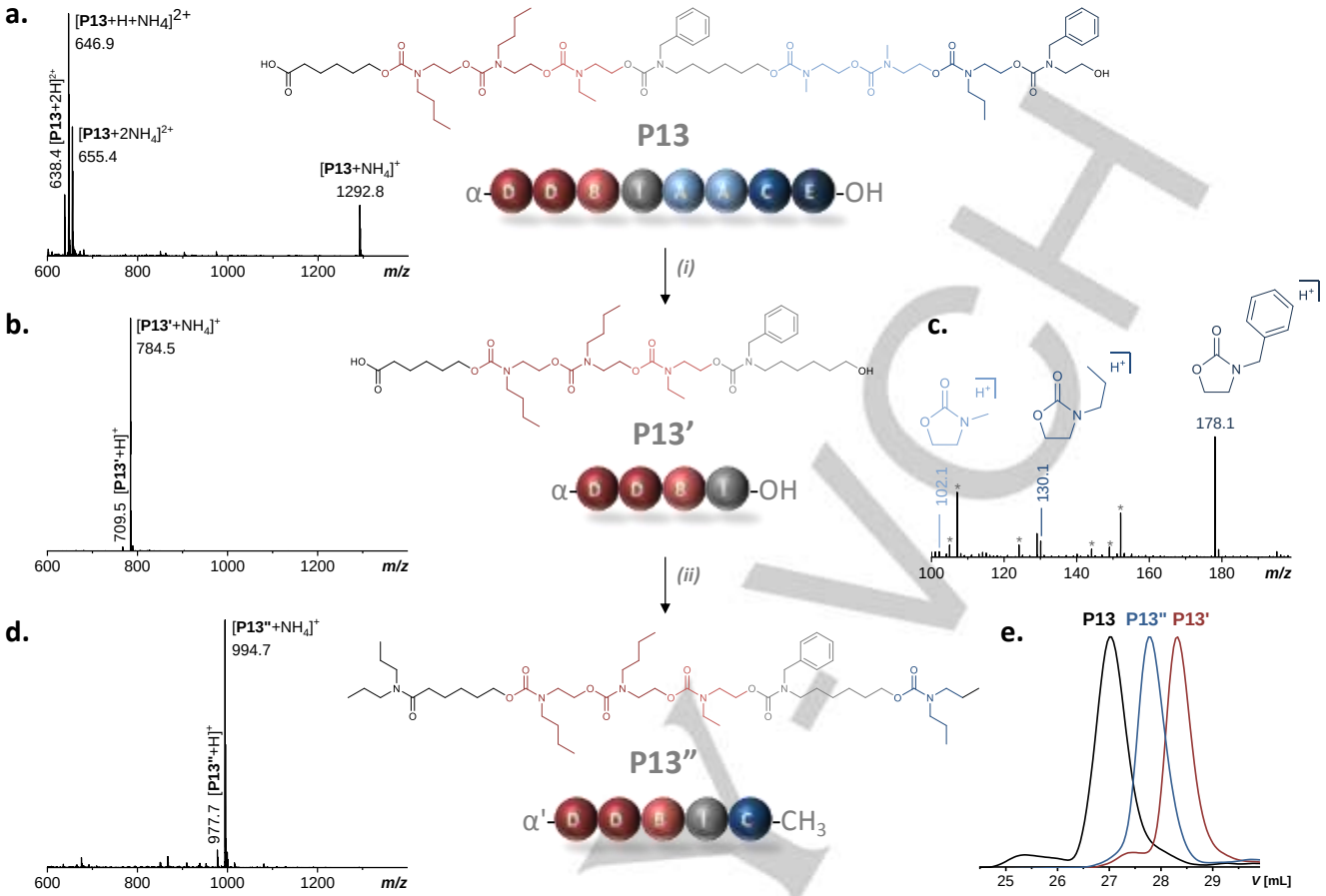

Figure 4. Partial degradation and repair of sequence-coded poly(*N*-substituted urethane) **P13**. Positive mode ESI-HRMS spectra of (**a**) the original polymer **P13** before basic treatment, (**b**, **c**) the degraded polymer **P13'** after NaOH treatment, and (**d**) the repaired polymer **P13"** after molecular healing. Panel **c** shows a zoom of the 100-200 *m/z* range for **P13'**. As compared to the spectrum shown in panel **b**, the intensity scale of the inset in panel **c** has been increased by a factor of about 11. The asterisks indicate background noise peaks. Experimental conditions: *(i)* NaOH, MeOH/H_2O, RT *(ii)* DSC, pyridine, ACN, 60°C, microwave then di-*n*-propyl-amine, pyridine, DMF, RT. (**e**) SEC chromatograms recorded in THF for **P13**, **P13'** and **P13"**. The high molecular weight shoulders are most likely due to the formation of polyesters, as discussed in previous publications.[7e, 14]

The fact that chain depolymerization depends on the number of methylene groups in a repeat unit is appealing because it suggests that the content of an informational sequence can be tuned by monomer design. Indeed, in informational polymers, molecular bits are usually set by using side-chain motifs of different molar mass but can also be obtained using main-chain motifs of different length or composition.[15] For instance, in the present case, informational units containing hexyl spacers, such as monomer **I**, can be used as a stopper to interrupt depolymerization at a precise chain-location. As a proof-of-concept, polymer **P13** with sequence α-DDBIAACE-OH was investigated (Figure 4a). It contains a single **I** unit placed in between two blocks that are composed of ethyl carbamate repeat units. In NaOH medium, this polymer depolymerizes only until the stopping point, as evidenced by the ESI-MS spectrum (Figure 4b and Figure S18) and SEC chromatogram (Figure 4e) of the resulting polymer **P13'**. In ESI-MS, the transformation **P13**→**P13'** leads to the expected mass decrease of 508 Da, whereas in SEC an apparent diminution of 466 Da is measured between peak maxima M_p. Partial depolymerization is also supported by the appearance of the oxazolidinone rings, expelled from the decomposed AACE sequence, in the ESI-MS spectrum of **P13'** (Figure 4d). These results confirm that preset sequence sections can be selectively degraded using appropriate molecular design. Furthermore, the degraded chain still exhibits reactive chain ends (*i.e.* α-COOH and ω-OH) and can therefore be repaired. Hence, the same sequence (complete or partial) or a different one can be reconstructed on the partially-degraded polymer. Here, **P13'** was partially-repaired by reacting it first with *N*,*N*'-disuccinimidyl carbonate (DSC) and then with di-*n*-propyl-amine. This modification leads to modification of both chain-ends, as shown in Figure 4c. Yet, it should be noted that the amidification of the α-chain-end can be avoided if needed; for instance by modifying the terminal acid group during or after cleavage. Here, the targeted modification was successfully performed, as evidenced by the ESI-MS spectrum (Figure 4c and Figure S19) and SEC chromatogram (Figure 4e) of the repaired polymer **P13"**. Indeed, the transformation **P13'**→**P13"** led to the expected mass increase of 210 Da in ESI-MS and to an apparent M_p increase of 168 Da in SEC.

In summary, OH-terminated poly(*N*-substituted urethane)s degrade via an ω→α unzipping mechanism in basic conditions at room temperature. If the polymer chain is only composed of ethyl carbamate repeat units, full decomposition can be attained. Yet, if some repeat units of the chain contain longer alkyl spacers, degradation can be slowed down or even suppressed. For instance, polymers containing a hexyl carbamate terminal unit do not degrade in basic conditions; at least not notably during the investigated periods of times. Furthermore, if the hexyl carbamate unit is placed inside an otherwise ethyl carbamate-based

sequence, partial depolymerization proceeds from the ω-terminus to the stopping hexyl carbamate unit. Therefore, specific information sections can be selectively erased from an informational sequence. This property is particularly interesting for anti-counterfeiting applications,[3b] because molecular barcodes could be purposely altered when exposed to specific conditions. Perhaps even more importantly, the partial degradation mechanism found in this work opens up possibilities of repairing informational polymers. For instance, it was demonstrated herein that intentionally-damaged sequences can be re-engineered using simple modification conditions. This proof-of-concept of molecular healing paves the way for the development of more complex deconstruction/reconstruction mechanisms, including chain rewriting and editing.

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Keywords: Sequence-controlled polymers • Digital polymers • Polymer degradation • Molecular healing • Sequencing

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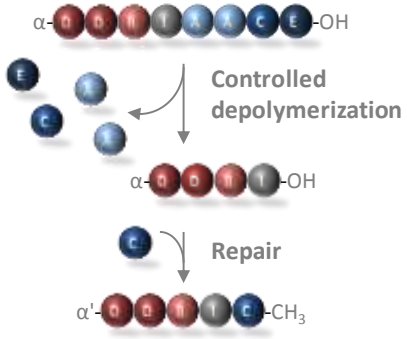

Insert text for Table of Contents here. In nature, the monomer sequences of informational biopolymers undergo damages, mutations and repair. In the present work, we show that comparable modifications can be obtained in non-natural sequence-defined poly(*N*-substituted urethane)s. Polymers having ethyl carbamate main-chain repeat units and an ω-OH end-group depolymerize in basic conditions. Yet, through careful monomer design, this behavior can be controlled and repair can be performed.

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