Redox-driven shaving of dendrimers

Winston Ong and Robin L. McCarley*

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A new methodology utilizing a redox-triggered activation for the facile release of dendrimer end groups is reported.

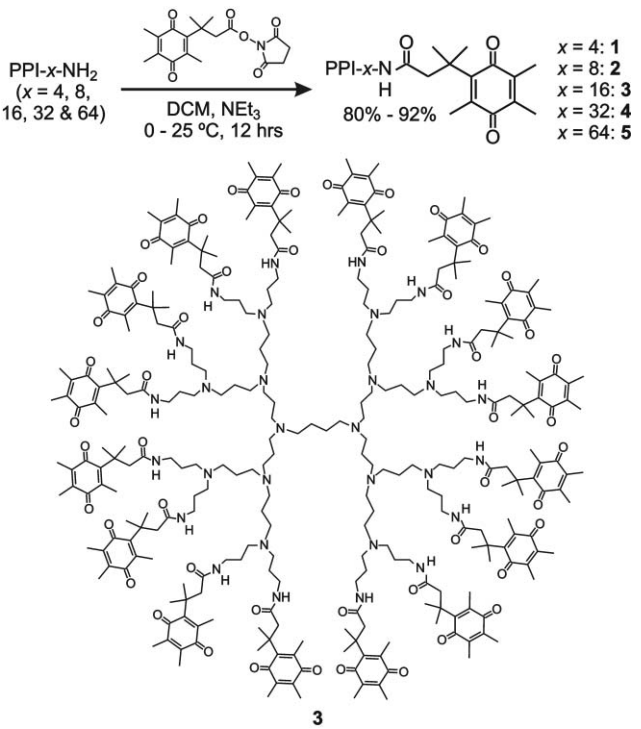
Potential applications for high-generation dendrimers as non-covalent hosts, such as molecule delivery,[1] nanoscopic chemical sensors[2] and catalysis,[3] will benefit from the encapsulating properties imposed by their sterically-demanding structures. Generally, the extent to which dendrimers are able to non-covalently encapsulate the property-active (therapeutic, catalytic, *etc.*) guests strongly depends on the ability of their peripheral crowding groups to physically entrap these guests. Thus, the development of a methodology that can readily regulate the density of end groups on dendrimers bears essential interest.

Our motivation for manipulating the end group densities[4] of symmetric-type dendrimers stems from our earlier work with stimuli-responsive, pyrrole-terminated poly(propylene imine), PPI, dendrimers, where the steric nature of the dendrimer end groups was gated by the redox state of the oligo(pyrrole) terminal units.[4a,4b] Herein, we expand this previous work by reporting a new redox-based methodology to manipulate the steric nature of the redox-labile dendrimer exteriors. This porosity contrast should, in principle, allow us to develop dendrimers whose guest-permeating property is manipulated by an external (electrochemical) stimulus.

Inspired by the redox-induced lactonization of trimethyl-locked quinones developed by Cohen,[5] we synthesized PPI dendrimers decorated with such groups along their peripheries. Generations 1 to 5 of these dendrimers were readily prepared in good yields (80%–92%) by condensing the commercially-available PPI poly-amines with the reactive NHS-activated, trimethyl-locked quinone[6] in dry CH_2Cl_2 overnight at ambient temperature (Scheme 1). ^{1}H NMR spectroscopic and MALDI-TOF MS analyses yielded results expected for these quinone dendrimers.[7] The dendrimer samples used for all of the experiments reported in this communication were handled with minimal exposure to ambient light, as these molecules are light-sensitive.

As a proof of concept to demonstrate that the end groups of the title dendrimers are readily released in solution upon the application of a redox stimulus, we show in Fig. 1A and 1B that the ^{1}H resonances of dendrimer **1**—particularly those for protons *c*, *d*, *e* and *f*—undergo reduction-induced shifts of up to 0.25 ppm upon the addition of the reducing agent, $Na_2S_2O_4$. In addition to these observed resonance shifts, and similar to the observations of previously reported monomeric systems,[6] the addition of $Na_2S_2O_4$ to the yellow solution of **1** instantly quenches its color. This color disappearance, plus the aforementioned NMR spectroscopic observations, provides clear evidence that the quinone moieties of **1** have been reduced to hydroquinones, HQs. Under the conditions of our present work, the lifetime of the HQ form of **1** is rather short ($t_{1/2} \sim 9$ min), as the release of lactone **6** from the parent dendrimer structure **7** reaches completion in less than 20 min (Fig. 1C). After 20 min, all of the resonances of the $Na_2S_2O_4$–dendrimer solution appear as a collection of the individual resonances of the end products **6** and **7** (Fig. 1D and 1E, respectively). Altogether, these observations support the end group liberation event depicted in Scheme 2. Similar observations were recorded for the higher generation dendrimers **2–5**, proving that end group release occurs regardless of dendrimer growth.

To further ascertain that the quinone units are completely cleaved from the parent dendrimer structures after these end groups are reduced to HQs, we performed Osteryoung square wave voltammetry (OSWV), a technique that has lower detection limits than ^{1}H NMR spectroscopy, on all of the dendrimers before and after the addition of $Na_2S_2O_4$. As a representative voltammogram for all generations, the OSWV trace of **1** in Fig. 2A exhibits the two classical peaks ($E_{1/2}^1 = -0.50$ V and $E_{1/2}^2 = -0.95$ V *vs.* Ag/AgCl) that are known for quinones.[9] When the reverse OSWV sweep was repeated 20 min after the addition of $Na_2S_2O_4$, no oxidative voltammetric signals were observed (Fig. 2B). This observation coincides with the ^{1}H NMR results described previously, where the lifetime of the HQ terminal units was under



Scheme 1 Synthesis of the quinone-terminated PPI dendrimers (**1–5**) and the structure of **3**.

232 Choppin Hall, Baton Rouge, LA 70803, USA.
E-mail: tunnel@lsu.edu; Fax: +1 225 578 3458; Tel: +1 225 578 3239

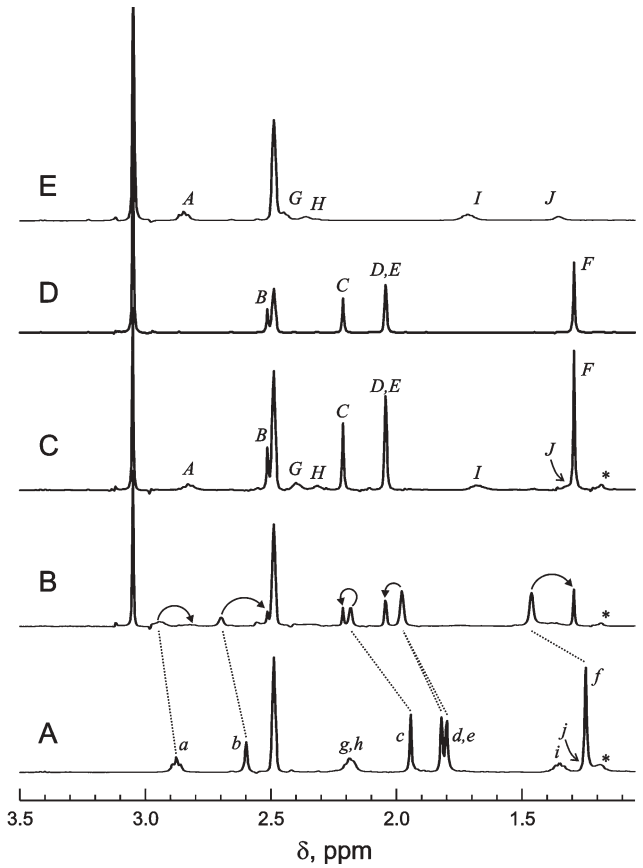


Fig. 1 ^{1}H NMR (400 MHz) spectra of **1** {end group [quinone] = 7.2 × 10^{-3} M, in DMSO-d_6–D_2O (85:15, v/v)} before (A) and after the addition of $Na_2S_2O_4$ (B–C): B, 4 min after the addition of $Na_2S_2O_4$–D_2O (final [$Na_2S_2O_4$] = 2.8 × 10^{-2} M) to a DMSO-d_6 solution of **1**, and C, same solution as in spectrum 1B after 20 total min. The spectra of lactone **6** and commercial PPI-4-NH_2 (2 × 10^{-3} M), **7**, in DMSO-d_6–D_2O (85:15, v/v), both also containing $Na_2S_2O_4$ (2.8 × 10^{-2} M), are shown for comparison (spectra 1D and 1E,[8] respectively). The resonance assignments denoted in all of the spectra correspond to the structures depicted in Scheme 2. The resonance at δ 3.05 ppm is from the internal standard, tetramethylammonium bromide (Me_4NBr, 6 × 10^{-3} M), that was added together with the $Na_2S_2O_4$–D_2O aliquot. All chemical shifts were referenced to the residual protic DMSO resonance, which was arbitrarily set to δ 2.49 ppm. For spectra 1B–1E, $Na_2S_2O_4$–D_2O was added to the neat DMSO solutions of **1**, **6** or PPI-4-NH_2 until the solvent composition reached 85:15 DMSO-d_6–D_2O (v/v). The resonances at around δ 1.28 ppm, denoted with asterisks, are from the residual hexanes that remain from the purification of the dendrimer.

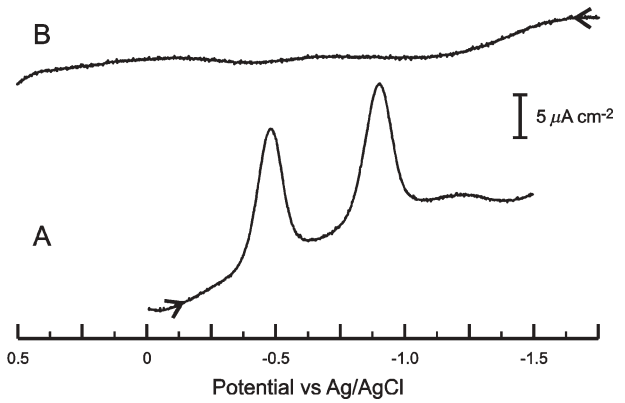
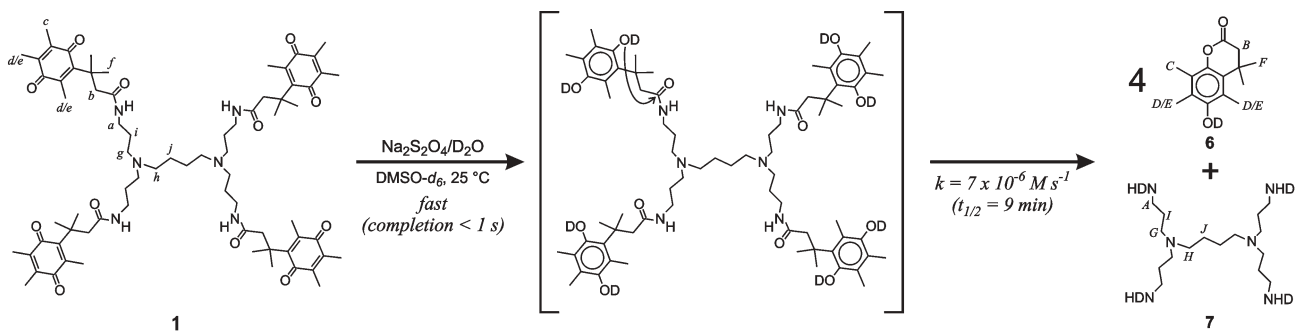


Fig. 2 OSWV traces (ΔE_p = 0.025 V, ΔE_s = 0.002 V, t_p = 0.010 s) of **1** using a glassy carbon electrode and 0.1 M n-Bu_4NPF_6 as supporting electrolyte: (A) freshly-prepared solution and (B) 20 min after the addition of aqueous $Na_2S_2O_4$ (2.5 × 10^{-3} M) to a DMSO solution of **1**. In both cases, the final concentration of **1**, reported in terms of quinone end groups, was 7.0 × 10^{-4} M, and the solvent composition for the solutions was 85:15 DMSO–H_2O. The arrows indicate the direction of the sweeps.

20 min. Because the liberated lactone **6** is redox silent over the potential range studied, the absence of any voltammetric peaks in Fig. 2B suggests the complete removal of the redox-active quinone and HQ units that previously resided at the dendrimer termini.

Fig. 3 illustrates the time-dependent liberation of the terminal HQ units from the central dendrimer structures (after their quinone precursors were reduced with $Na_2S_2O_4$–D_2O) as monitored by ^{1}H NMR spectroscopy at 25 °C,† with two key observations. First, despite the complexity of the dendrimer structures (up to 64 HQs for **5**), all of the HQ units are released following simple zero-order kinetics. The apparent zero-order decay is unclear at this point considering that other trimethyl-locked quinone systems exclusively lactonize following first-order kinetics.[5,6] Second, there is no clear trend showing any generation-dependent rates for the release of the HQ end groups. That is to say, all dendrimers release the HQ end groups at rates (k ~ 7 × 10^{-6} M s^{-1}, $t_{1/2}$ ~ 9 min) within the margin of error for the experiment. It is worth mentioning that when the lactonization experiments are performed without deoxygenating the deuterated solvents, the end group removal rates are decreased by approximately three fold (zero order plot not shown). Thus, any possible uncertainties in the rates shown in Fig. 3 can be attributed to the varying amounts of trace oxygen that may have been



Scheme 2 Reduction of **1** with $Na_2S_2O_4$–D_2O to produce a dendrimer intermediate containing unstable HQ end groups, which are subsequently released from the parent dendrimer structure (**7**) as lactone **6**.

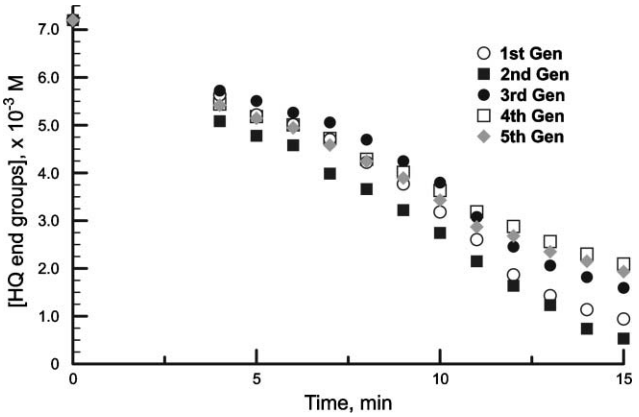


Fig. 3 Time-dependent release of the HQ end groups of dendrimers **1–5** (after the reduction of their precursor quinones) at 25 °C showing zero-order liberation. The missing data points during the first three minutes represent the lapse between the sample preparation and data acquisition.† Linear fitting of the data points (not shown for clarity) to the zeroth-order model produced an average k value of 7×10^{-6} M s^{-1} for all five generations, with correlation coefficients greater than 0.99 in all cases. For all measurements, the end-group concentration of quinones was 7.2×10^{-3} M.

present in the deoxygenated solutions of the quinone dendrimers. However, in all cases, we did not observe the re-oxidation of the reduced dendrimer HQs back to **1–5**, even when using non-deoxygenated solvents.[10] Preliminary cyclic voltammetric studies of **1–5** in DMSO reveal that the half-wave potentials for the two redox couples of the quinone units in these dendrimers do not vary substantially with generation (data not shown).[11] Because all of the quinone units undergo reduction at similar redox potentials regardless of dendrimer size, and assuming that the end group quinones in all generations are equally accessible to any diffusing $Na_2S_2O_4$, the end group liberation profiles in Fig. 3 are less likely to contain errors resulting from the quinones being reduced to HQs at vastly fluctuating rates.[12]

The work described in this communication illustrates the first example of end group liberation in dendrimers by using a redox stimulus as the activating source. Such a liberation method is attractive to dendrimers relying on their void interiors (and dense end groups) for hosting properties, such as the PPI family described in this communication, because the removal of end groups is fast ($\leqslant$ 20 min) and takes place under relatively mild conditions[13] (room temperature). In light of the recent emergence of self-immolative dendrimers, SIDs, as potential therapeutic carriers for drug-delivery applications,[14] the work described here opens an alternative approach for high-generation PPI dendrimers to be used as container molecules that can potentially release non-covalently incarcerated guests *via* redox-gating conditions.

In conclusion, we have shown that trimethyl-locked quinone end groups can be removed from poly(propylene imine) dendrimers under mild conditions by using a chemical redox stimulus. The end group removal process, which does not show any generation-dependent rates, reaches completion in approximately 20 min for all generations and proceeds *via* zero-order kinetics. Efforts toward elucidating the electrochemical properties of these quinone-terminated dendrimers, both in their freely-diffusing (in solution) and chemisorbed (in redox-controllable surfaces) forms, are currently underway.

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Notes and references

† Experimental procedure for the kinetic studies: To a deoxygenated DMSO-d_6 solution of the corresponding dendrimer generation (0.51 mL, end-group quinone = 4.3 mmol) in an NMR tube was added a deoxygenated, freshly-prepared aliquot of $Na_2S_2O_4$–D_2O (0.090 mL, 17 mmol). Upon mixing of the $Na_2S_2O_4$–D_2O and the DMSO-d_6 dendrimer solutions inside the NMR tube, the yellow color of the solution was quenched instantly (completion <1 s). After the sample tube was loaded in an NMR spectrometer (Bruker DPX-400) for spectral acquisition, a macro was used to automatically acquire successive spectra at exactly 1 min intervals. Each spectrum was set to acquire 2 scans with pre-scan delay, acquisition time and line broadening values set to 2 s, 2 s and 1 Hz, respectively. The integrals for the overlapping resonances of protons *d* and *e* (see Scheme 2) were measured relative to the internal standard Me_4NBr to yield the zero-order plots depicted in Fig. 3.

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7 Full synthetic and characterization details will be reported in a future full paper.
8 All of the ^{1}H resonances for PPI-4-NH_2, **7**, in Fig. 1 are slightly shifted downfield by the same magnitude ($\sim$0.03 ppm) relative to those of PPI-4-NHD in Fig. 1C. In addition to the resonances of **7** in this solvent mixture being very concentration dependent, we believe that the sulfate- and/or sulfite-containing by-products of $Na_2S_2O_4$ produced in the unbuffered medium may have slightly affected the apparent pD of the entire solution, thereby causing the minimal, yet observable, shifts in the resonances of PPI-4-NHD relative to **7**. As suggested by a referee, we verified this media effect by titrating the solution shown in Fig. 1C with increasing amounts of pristine PPI-4-NH_2. The titration did not show any new peak evolution but rather caused the resonances of the PPI-4-NHD by-product to shift slightly and increase in intensity. Thus, this observation clearly concludes that the slight difference between the resonances of spectra 1D and 1E is due to a media effect.
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10 It has been reported that in some cases, the presence of O_2 from ambient air can re-oxidize the trimethyl-locked hydroquinones back to their quinone forms (see ref. 6 for details).
11 The $E_{1/2}$ values in DMSO–0.1 M $TBAPF_6$ shift by less than 10 mV in going from **1** to **5**. Results of the detailed electrochemical studies on these dendrimers will be reported in a future publication.
12 In addition to the instant visual disappearance of the yellow color of **1–5** upon the addition of $Na_2S_2O_4$, it is known that the reduction of trimethyl-lock quinones to their HQ forms by $Na_2S_2O_4$ occurs instantly. See ref. 5 and 6 for details.
13 For instance, the classic "Meijer dendritic box" (J. F. G. A. Jansen and E. W. Meijer, *J. Am. Chem. Soc.*, 1995, **117**, 4417) requires harsh conditions (reflux for 2 h in 12 M HCl) to hydrolyze the amino acid end groups of the dendrimers.
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