Tuneable Degradation

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# Substituted 1,3,5-Triazaadamantanes: Biocompatible and Degradable Building Blocks**

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Degradable polymers are important constituents of environmentally benign products and are used in applications that range from tissue engineering[1] to gene[2] and drug delivery.[1,3] Hydrolytically labile polymers, such as polyesters, are particularly useful because of the ubiquitous presence of water in the environment and in living organisms. However, polyesters produce acidic byproducts upon degradation, a limitation for a number of applications.[4] Polyphosphazenes[5] are promising alternatives, but there is a need for new monomers that degrade to form benign byproducts. Herein we report the synthesis and study of 1,3,5-triazaadamantane (TAA) as a water-soluble unit for controlled degradation and aldehyde release. Formed from the condensation of a tris(aminomethyl)methane unit and three aromatic aldehydes,[6] the TAA unit hydrolyzes under physiological conditions reverting to its basic precursors. We demonstrate how the degradation rate of the TAA unit can be tuned with substituents, and further synthesize a hydrophilic TAA-based dendrimer that is capable of binding solvatochromic probes.

The preparation of TAA **2** by the condensation of tris(aminomethyl)ethanol (**1**) and benzaldehyde (Figure 1a) was reported by Woulfe and co-workers.[6a] In our group, this procedure produced **2a** and **2b** in a 9:1 ratio (determined by $^1$H NMR spectroscopy). Difference nuclear Overhauser effect (NOE) studies were performed on **2a** to establish the relative spatial orientation of the methine protons (Figure 1b). To confirm further its identity, an X-ray analysis was performed on crystals of **2a** grown by the slow evaporation of an acetonitrile solution (Figure 1c). Submitting the minor isomer **2b** to the reaction conditions afforded a 9:1 ratio of **2a** to **2b**, indicating the reaction to be thermodynamically controlled.

Although the TAA unit has been used as a protecting group for the tris(aminomethyl)methane unit, little work has focused on controlling its rate of decomposition.[6] We therefore examined the effect of aromatic ring substitutents on the TAA hydrolysis rate. Water-soluble aldehydes **3a–c** were synthesized[7] and condensed with **1** to produce **4a–c** (Scheme 1). $^1$H NMR spectroscopic analysis confirmed that these model TAAs degraded to their monomer units upon exposure to aqueous acidic conditions.

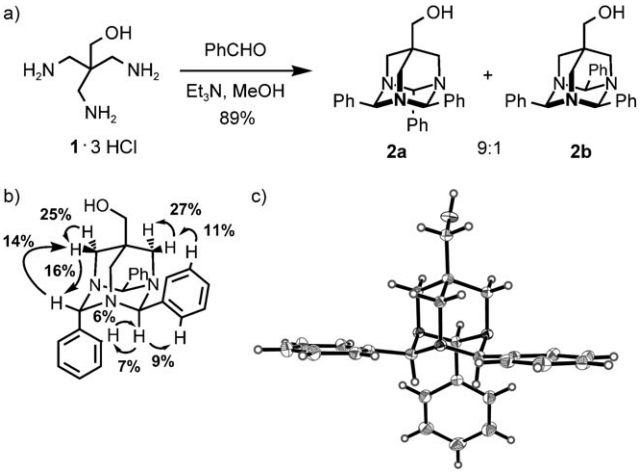
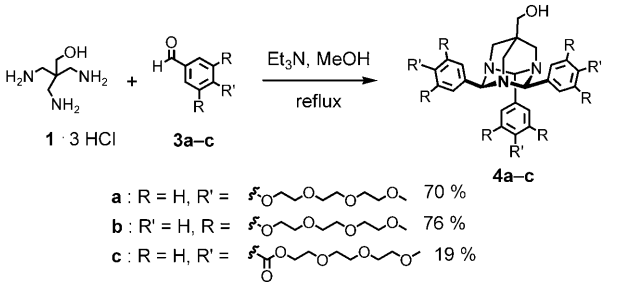



***Figure 1.*** a) Synthesis of benzaldehyde-derived 1,3,5-triazaadamantane (TAA) **2**. b) Difference NOEs detected for **2a** in deuterated chloroform. c) ORTEP representation (from X-ray analysis) of **2a**. Thermal ellipsoids are set at 50% probability. See supporting information for additional details.




***Scheme 1.*** Synthesis of substituted TAAs **4a–c**.

Rate constants for TAA hydrolysis at various pH values were measured using UV spectroscopy. A red shift in the absorbance at $\lambda_{max}$ was observed upon exposure of the TAA monomer to acidic conditions. The half-life for hydrolysis was calculated by plotting the change of absorbance over time. As seen in Figure 2, **4a–c** rapidly hydrolyzed at pH $< 5$. Under basic conditions, the TAA units degraded at different rates, such that **4a** hydrolyzed the fastest and **4b** degraded the slowest. Based on the σ-values used in Hammett analyses for corresponding substituents, **4c** might reasonably be expected

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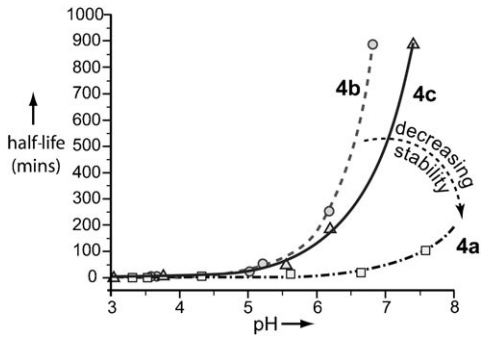



***Figure 2.*** Half-lives of TAAs **4a–c** at 22.5 °C.

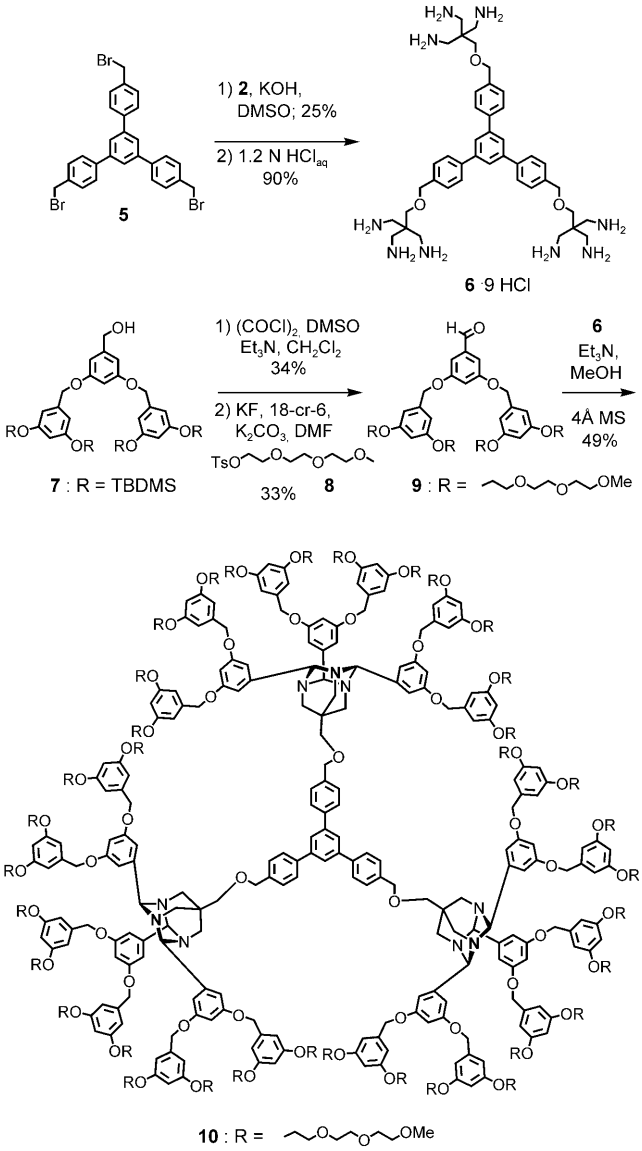
to be least stable. The mechanism of hydrolysis is not known and may change as a function of the substituent. For many applications, **4b** may have the most desirable degradation profile by being stable under neutral conditions but hydrolyzing rapidly upon acidification.

Although multiple uses for the TAA unit can be envisioned, its $AB_3$ functionality suggested use as a building block for dendrimers. Thus, dendrimer **10** was synthesized and its ability tested to encapsulate small molecules in aqueous environments. Core **6** was prepared from tribromide **5**[8] and **2** (Scheme 2). Compound **7** was oxidized, deprotected, and alkylated in situ with tri(ethylene glycol) tosylate **8**, to afford aldehyde **9**.[9,10] Condensation of **6** with ten equivalents of **9** afforded dendrimer **10** in 49% yield. The product was estimated to be greater than 90% pure determined by analytical size exclusion chromatography (SEC), MALDI-MS, and $^1$H NMR spectroscopy. As a control, dendron **11** was prepared from the condensation of **1** with substituted benzaldehyde **9** (Scheme 3).

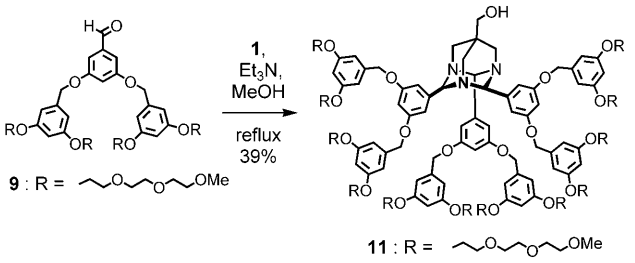
Binding studies were performed using solvatochromic dyes to assess the binding of small molecules by dendrimer **10**.[11] Titration of **10** into a phosphate-buffered saline (PBS) solution of Rose Bengal[12] resulted in a red shift of 19 nm in the $\lambda_{max}$ of the dyes's absorption spectrum.[7] A single isosbestic point was detected, consistent with free and complexed dye species in solution.[13] Based on a 1:1 binding isotherm for **10** with Rose Bengal, an apparent association constant, $K_{assoc} = 3.1 \pm 0.5 \times 10^5\,\text{M}^{-1}$, was determined. No appreciable red shift occurred on using **4b** in place of **10**. However, adding three equivalents of dendron **11** to the dye solution, did produce a red shift of approximately 10 nm for $\lambda_{max}$ in the absorption spectrum, indicating that **11** can also interact with the dye.

Additional insight into the interaction of small molecules with **10** was obtained through fluorescence studies with 1-anilino-8-sulfonic acid (ANS), a dye with low emission in aqueous environments.[14] Upon addition of dendron **11** (3 equiv) or core **6** to a solution of ANS, a small increase in the intensity of the ANS fluorescence spectrum was detected. However introduction of dendrimer **10** to a similar ANS solution produced a much larger increase in fluorescence intensity, indicating that the solvatochromic dye was within a more hydrophobic environment (Figure 3).

To test whether **10** degrades in the same manner as **4**,[15] an excess of 35% (w/w) DCl in $D_2O$ was added to a [$D_8$]THF




***Scheme 2.*** Synthesis of dendrimer **10**.




***Scheme 3.*** Synthesis of dendron **11**.

solution of **10**, effecting (as determined by $^1$H NMR spectroscopy) its complete hydrolytic conversion into **6** and **9**. In drug delivery and related applications, it is important that the hydrolysis byproducts are nontoxic. Toward this end, compounds **9**, **10**, and **4a–c** were tested in a standard XTT cell viability assay.[7] All five compounds were found to be nontoxic under standard physiological conditions. It was

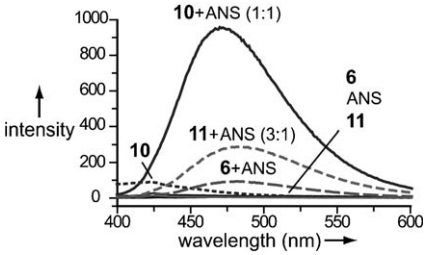



***Figure 3.*** Fluorescence spectra for encapsulation studies of **6**, **10**, and **11** with 1-anilino-8-sulfonic acid (ANS). Spectra of ANS, **6**, and **11** alone appear near the baseline.

calculated that under the conditions of the assay, **4a**, **4b**, and **4c** were hydrolyzed by approximately 98%, 19%, and 98%, respectively, suggesting that both the dendrons and hydrolysis products exhibit a good level of biocompatibility.

In summary, described herein is the synthesis and study of a new class of compounds that can undergo tunable hydrolytic degradation to well-defined byproducts. The rate at which these molecules decompose can be controlled through substituent effects. In contrast with degradable materials containing esters, that provide acidic products, TAAs degrade to give products containing basic amine groups. The TAA unit possessed a branched architecture that was utilized for the synthesis of a water-soluble dendrimer that binds Rose Bengal and ANS and degrades in acidic conditions. TAAs have considerable potential in biological applications, especially in cases where relevant pH gradients can exploited, such as in the extracellular space of tumor tissue and cellular endosomes.[2] The ability to buffer endosomes has also been shown to be important for certain applications.[16] Current efforts are directed toward developing TAA-based materials for gene and drug delivery, as well as tissue engineering.

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