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

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Abstract

Basic groups from acid hydrolysis: 1,3,5-Triazaadamantanes (TAAs) are shown to degrade in acidic conditions to produce basic by-products. The rate of hydrolysis can be tuned by changing the substituents present on the aromatic rings. TAA containing dendrimers can be synthesized readily as a result of their branched architecture.

Keywords

controlled degradation; dendrimers; macromolecule synthesis; guest binding

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] Poly-phosphazenes[5] are promising alternatives, but there is a need for new monomers that degrade to 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 precursors that are basic. We show that 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 trisamine **1** and benzaldehyde (Figure 1a) was reported by Woulfe.[6a] In our hands, this procedure produced **2a** and **2b** in a 9:1 ratio (^{1}H NMR). 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 minor isomer **2b** to the reaction conditions resulted in a 9:1 ratio of **2a** to **2b**, indicating the reaction to be thermodynamically controlled.

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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 analysis confirmed that the model TAAs degraded to their monomer units upon exposure to aqueous acidic conditions.

Rate constants for TAA hydrolysis at various pH values were measured using UV spectroscopy. A red shift in the absorbance of the λ_{max} was observed upon exposure of the TAA monomer to acidic conditions. The half-life for hydrolysis was calculated by graphing 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 for corresponding substituents, **4c** might reasonably be expected 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 one can envision multiple uses for the TAA unit, its AB_3 functionality suggested use as a dendrimer building block. Thus, dendrimer **10** was synthesized to test its ability to encapsulate small molecules in aqueous environments. Core **6** was prepared from tri-bromide **5**[8] and **2** (Scheme 2). Aldehyde **9** was synthesized by oxidizing **7**,[9] deprotected and alkylated in-situ with PEG-tosylate **8**.[10] Condensation of **6** with ten equivalents of **9** afforded **10** in 49% yield. The product was estimated to be greater than 90% pure based by analytical size exclusion chromatography (SEC), MALDI-MS, and ^{1}H NMR. As a control, dendron **11** was prepared from the condensation of **1** with 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 PBS buffered solution of Rose Bengal[12] resulted in a 19 nm red shift in the λ_{max} of the dyes's absorption spectra.[7] A single isosbestic point was observed consistent with free and complexed dye species in solution.[13] Based on a 1:1 binding isotherm for **10**·Rose Bengal an apparent association constant, $K_{assoc} = 3.1 \pm 0.5 \times 10^5\ M^{-1}$ was determined. No appreciative red shift was observed using **4b**, however, adding three equivalents of dendron **11** to the dye solution, did produce a ~10 nm red shift 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 equivalents) or core **6** to a solution of ANS, a small increase in the intensity of the ANS fluorescence spectrum was observed. 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).

Can **10** degrade in the same manner as **4**?[15] Addition of an excess of 35% (w/w) DCl in D_2O to a THF-d_8 solution of **10** showed by ^{1}H NMR its complete hydrolytic conversion to **6** and **9**. In drug delivery and related applications, it will important that the hydrolysis byproducts be non-toxic. 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 non-toxic under standard physiological conditions. It was calculated that under the conditions of the assay, **4a**, **4b**, and **4c** were hydrolyzed by ca. 98%, 19%, and 98%, respectively, suggesting that both the dendrons and hydrolysis products exhibit a good level of biocompatibility.

Described herein is the synthesis and study of a new class of compounds that can undergo tuneable hydrolytic degradation to well-defined byproducts. The rate at which these molecules decompose can be controlled through substituent effects. TAAs degrade to give products containing basic amine groups. This is in contrast with degradable materials containing esters that provide acidic products. 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 that can exploited such as 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.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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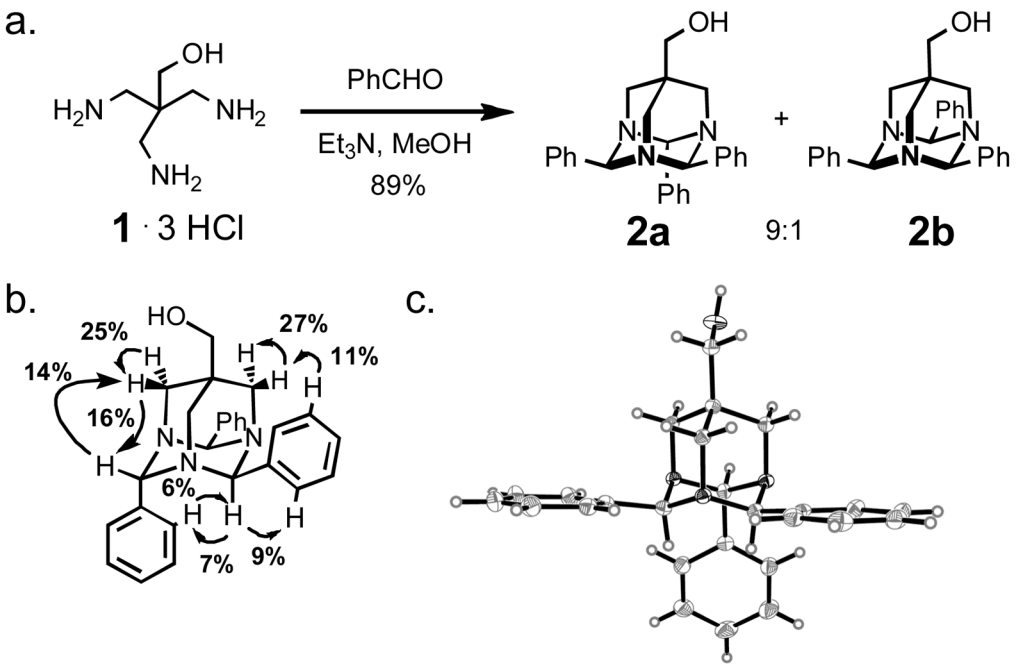


Figure 1.

a) Synthesis of benzaldehyde derived TAA **2**. b) Difference NOEs observed for **2a** in chloroform-*d*. c) ORTEP diagram from X-ray analysis of **2a**. See supporting information for additional details.

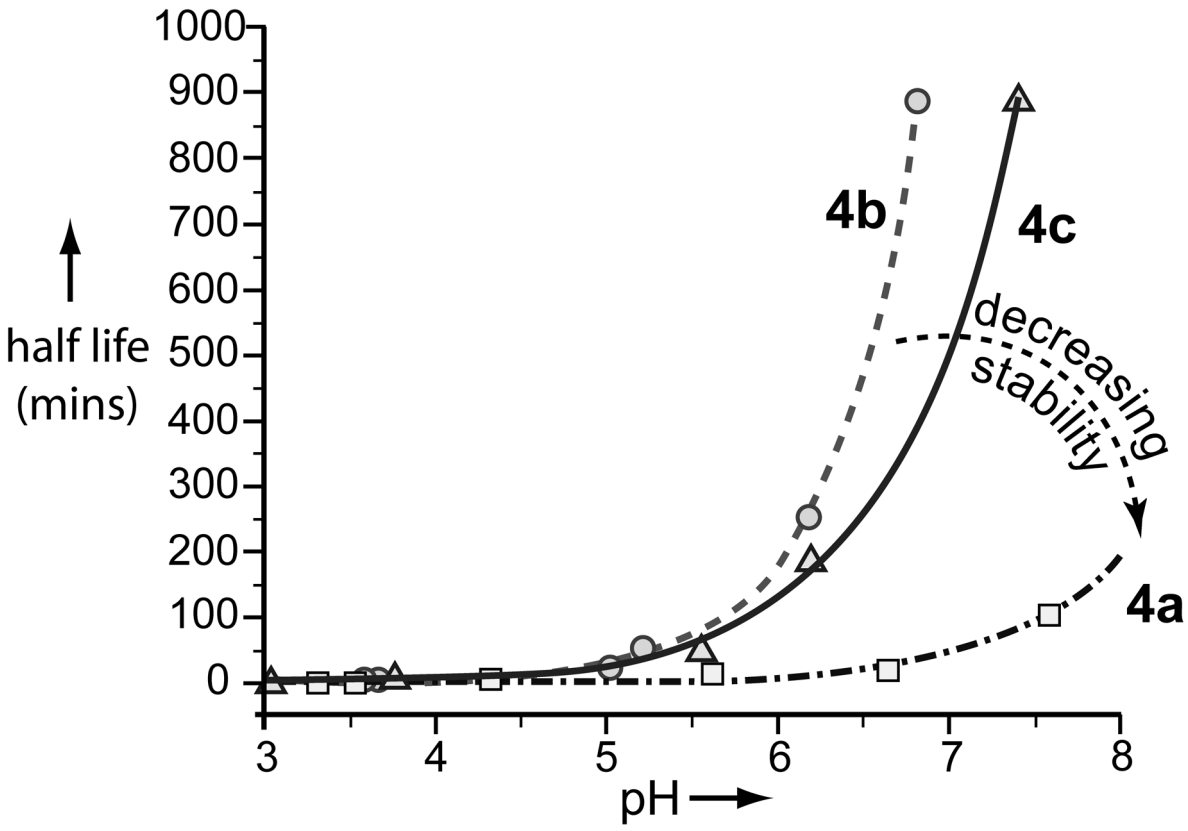


Figure 2.
TAA half lives at 22.5 °C.

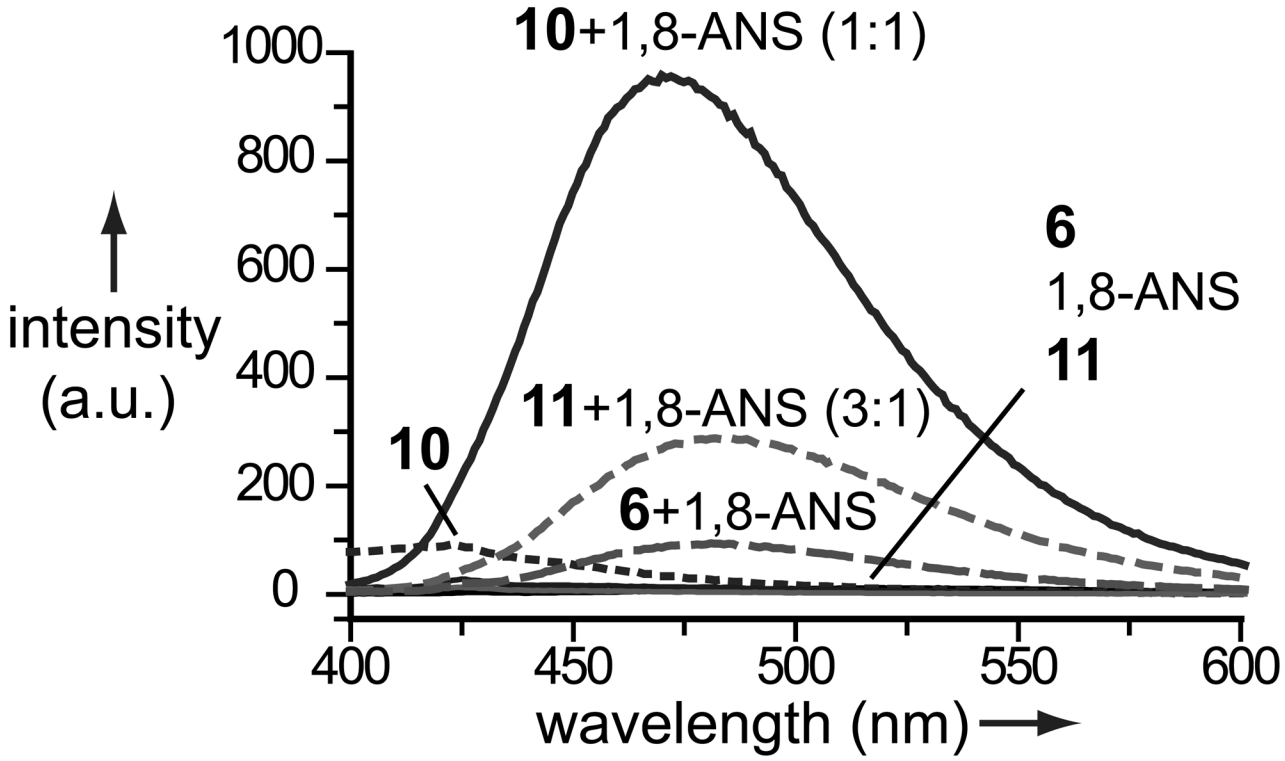
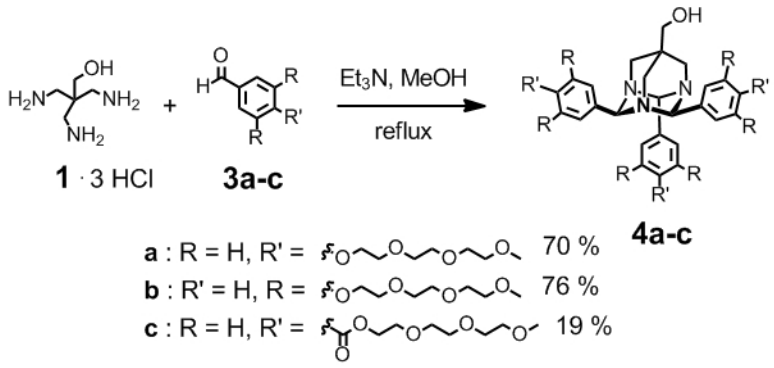
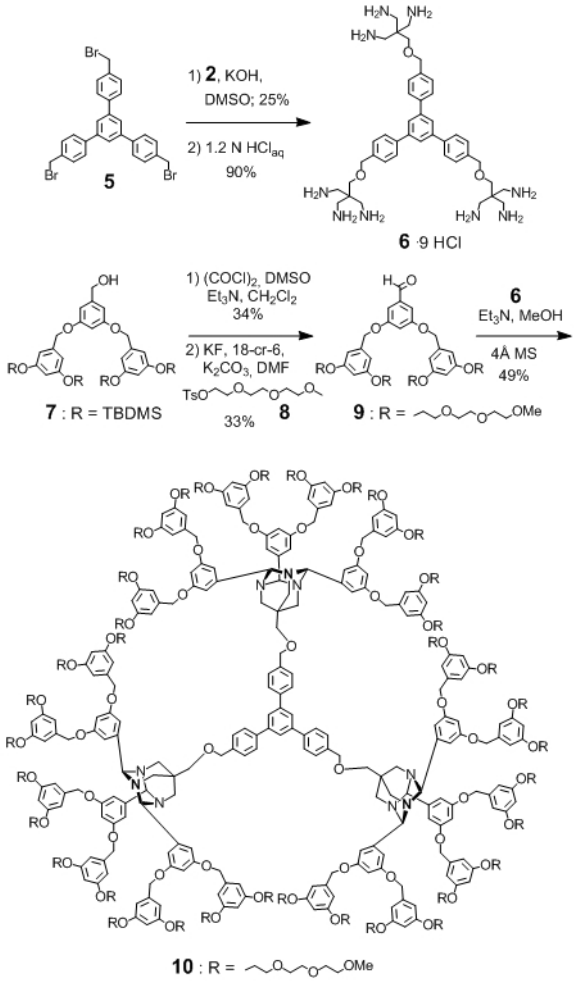


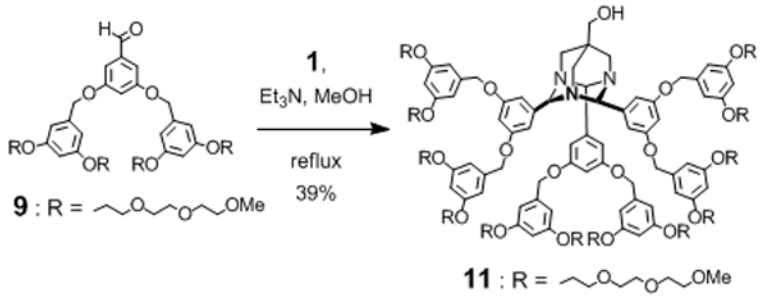
Figure 3.
Fluorescence spectra of 1-anilino-8-sulfonic acid (ANS) encapsulation studies. Spectra of ANS, **6**, and **11** alone are near baseline.



Scheme 1.



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



Scheme 3.