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Encapsulation of Neutral Guests by Tri(ethylene oxide)-pyrrole-Terminated Dendrimer Hosts in Water

Amy D. Morara and Robin L. McCarley*

Department of Chemistry, Louisiana State University, Baton Rouge, Louisiana 70803

tunnel@LSU.edu

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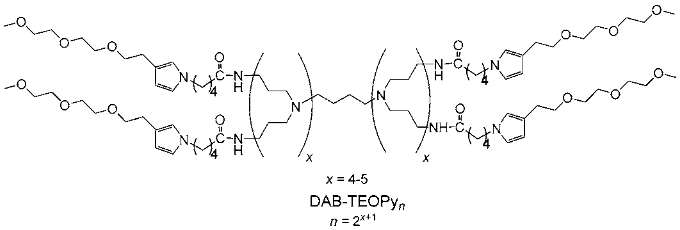
ABSTRACT



This work focuses on the synthesis and guest hosting capabilities of novel, water-soluble tri(ethylene oxide)-pyrrole-functionalized poly(propylene imine), PPI, dendrimers (DAB-TEOPy$_n$). The high-generation DAB-TEOPy$_n$ (n = 32 and 64) possesses the ability to encapsulate hydrophobic guest molecules, such as Nile Red, in aqueous media.

Since Vögtle introduced them in 1978[1] and Newkome[2] and Tomalia[3] proposed their use as small-molecule hosts in 1985, efforts have targeted new synthetic routes to dendrimers,[4,5] with the hopes of readily altering the structure to effect dendrimer hosting capabilities.[6] Although guest entrapment by poly(amido amine), PAMAM, dendrimers was demonstrated in 1989 by Goddard,[7] it was not until 1994 that incarceration of guest molecules was attained by Meijer. In this work, his group succeeded in the "static trapping" of dye molecules (incarcerated guests) inside a poly(propylene imine), PPI, dendrimer modified with bulky, hydrogen-bonding-capable amino acids.[8] Release of the incarcerated guests was demonstrated, but the harsh conditions necessary for the triggered release of the guests from the "dendritic box" are not widely applicable for many host−guest chemistry applications, such as drug delivery,[9] analyte preconcentration,[10] and catalysis.[11] However, this study is a hallmark and provided impetus for the development and study of many dendrimer-based hosting systems.[12,13]

A highly desirable characteristic for an ideal host system is the ability to reversibly switch on or off its hosting capability (guest encapsulation and release) via application of an external stimulus. To that end, a select number of dendrimers have been modified with stimuli-responsive moieties, such as light-responsive azobenzene[14] and temperature-responsive poly(N-isopropylacrylamide),[15] to attempt control over guest retention via steric interactions. Although

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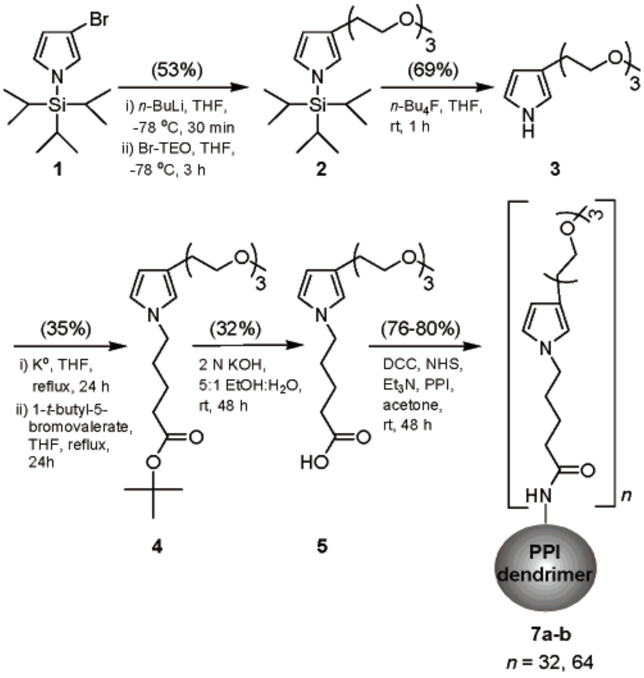
promising, these two dendrimer-based, stimuli-responsive systems have not yet been shown to allow for controlled egress of entrapped guest molecules.

We are interested in constructing stimuli-responsive dendrimer systems possessing hosting capabilities that are controlled by electron-transfer reactions, which lead to changes in the steric and electronic nature of groups appended at the periphery of the dendrimer host.[16] In one approach, dendrimer end groups will be electroactive, conjugated oligomers based on pyrrole that experience conformational changes caused by alterations in the oligomer oxidation state.[17] To form the oligo(pyrrole)-terminated responsive dendrimer hosts, pyrrole monomer-terminated dendrimers must first be constructed and characterized, and their dynamic hosting capabilities evaluated in aqueous media. To achieve water solubility and address biocompatibility issues for future biological applications, the pyrrole monomer-terminated dendrimers that we discuss here possess tri(ethylene oxide) functionalities at the 3-position of the pyrrole ring, to allow for oligomerization of pyrrole at the 2- and 5-positions.[17] Here, we describe the synthesis of three unreported 3-substituted pyrrole compounds (**2**, **4**, **5**) and a new dendrimer series (**7a**,**b**) possessing dynamic hosting capabilities.

To produce 3-substituted pyrroles, a protecting group on the pyrrole nitrogen was needed to block the α-positions of the pyrrole ring to prevent the favored substitution at the 2- and 5-positions of pyrrole.[18,19] The tri(ethylene oxide), TEO, chain has been previously placed at the 3-position of pyrrole using a tosyl protecting group at the N-position.[20,21] We chose to use the tri(isopropyl)silyl protecting group at the N-position because previous molecular modeling[22] and experimental studies have shown it to be a bulky enough protecting group to substantially hinder electrophilic attack at the α-positions, and it is easily deprotected with fluoride salts.[18,19] Three substitution of the silylated pyrrole **1** was achieved in a manner similar to that of Bray[19] but with some modifications, due to the unique solubility/polarity of the TEO group. Selective bromination of *N*-(triisopropylsilyl)pyrrole was achieved at the β-position ($<10\%$ α, $>90\%$ β by ^{1}H NMR), after which a halogen–metal exchange reaction was executed to produce the lithiated pyrrole species.[19] An S$_N$2 reaction was then carried out with the lithiated species using the Br(CH$_2$CH$_2$O)$_3$CH$_3$, Br-TEO,[23] substrate to generate 3-(3,6,9-trioxadecanyl)-*N*-(triisopropylsilyl)pyrrole **2**. Desilylation with tetra-*n*-butylammonium fluoride[19] was confirmed to be a gentle deprotecting route to generate 3-(3,6,9-trioxadecanyl) pyrrole **3** (Scheme 1).

Scheme 1



After deprotection of **2**, addition of a linker arm to the 3-substituted pyrrole was required. The linker chosen was ω-bromo-*n*-pentanoic acid, protected with a *tert*-butyl group.[24] Potassium metal was used to generate the pyrrole anion species, which subsequently underwent an S$_N$2 reaction with the protected linker chain to give **4**. Removal of the *tert*-butyl group was achieved with potassium hydroxide[25] to generate the carboxylic acid **5**.

The activated ester **6** (not isolated) was then formed in the presence of the amine-terminated PPI dendrimers to give the pyrrole-terminated dendrimers **7a**,**b** (DAB-TEOPy$_n$; n = 32, 64) in reasonable yields (80% and 76%). On the basis of ^{1}H NMR and MALDI-MS data, the degree of PPI derivatization by the activated pyrrole ester **6** ranged between 84% and 100%.[26]

The hydrodynamic radius (R_h) values of each dendrimer, as determined from dynamic light scattering (DLS) experiments in acetone, are shown in Table 1. The R_h values are in good agreement with those of other oligo(ethylene oxide)-modified PPI dendrimers,[27] qualitatively pointing to a relatively high degree of pyrrole group derivatization. In

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Table 1. Hydrodynamic Radius of Dendrimers as Determined by Dynamic Light Scattering at 25 °C[a]

dendrimer[b]	R_h, nm
DAB-TEOPy$_{32}$	2.0−2.1
DAB-TEOPy$_{64}$	2.3−2.9

[a] Measurements taken at 30° and 45° angles. [b] [DAB-TEOPy$_n$] was 9 mg mL^{-1} for $n = 32$ and 2 mg mL^{-1} for $n = 64$ both in acetone.

addition, preliminary light scattering studies of **7a**,**b** in aqueous milieu demonstrate that they do not aggregate under the conditions reported here.

One of the desired characteristics of the system described here is the ability to host hydrophobic guests in an aqueous environment. The interest in this capability is due to potential applications for the targeted delivery of hydrophobic drugs in mammals or for reagent storage and distribution in "lab-on-a-chip" technologies. To investigate the ability of the DAB-TEOPy$_n$ to host hydrophobic guests in water, aqueous solutions of the neutral, hydrophobic dye Nile Red in the presence of **7a** and **7b** were investigated by optical absorption spectroscopy. Nile Red is a well-known environmentally sensitive dye[28,29] that is chemically stable and charge neutral under the conditions used here (pK_a is near 1).[30]

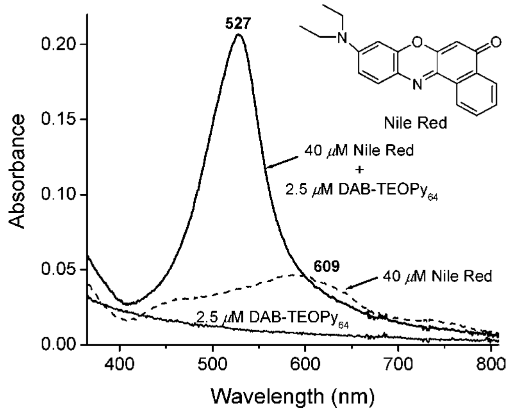

Figure 1. Absorption spectra of aqueous 40 μM Nile Red with and without 2.5 μM DAB-TEOPy$_{64}$.

As seen in Figure 1, the absorption spectrum of Nile Red in aqueous solution possesses a very broad, low-intensity band centered near 609 nm, indicating that the Nile Red is present in an aqueous (polar) microenvironment.[28,29] Upon addition of Nile Red to aqueous DAB-TEOPy$_n$ solutions, a distinct blue shift in the absorption energy is observed, as well as a significant absorption intensity increase. This shift to a distinctly shorter wavelength (527 nm for $n = 64$ and 522 nm for $n = 32$ (data not shown)) is characteristic of a less polar microenvironment for Nile Red, pointing to Nile Red uptake by the dendrimer host into its relatively nonpolar[13a] interior. The absorption maximum for Nile Red in neat Et_3N (506 nm), acetone (532 nm), and tri(ethylene glycol) monomethyl ether (545 nm) obtained in our laboratories strongly suggests the dye is located in the interior PPI region of both DAB-TEOPy$_n$ dendrimers.

The microenvironment polarity difference of the two dendrimers presents the question of the precise location of the Nile Red guest within the DAB-TEOPy$_n$ dendrimer interior. At this time, it is unclear why the fourth-generation material appears to provide a slightly less polar environment than its fifth-generation counterpart. Preliminary fluorescence quenching studies of Nile Red encapsulated by the two dendrimers indicate that the dye is located near the tertiary amines in the case of the fifth-generation material and not the fourth-generation host, an observation that is in accord with the Nile Red absorption data. A similar observation has been noted for the fifth-generation amine-terminated PPI with the solvatochromic probe phenol blue in water, wherein the dye was found to reside near the tertiary amines.[13a] We speculate that the depth to which the Nile Red is able to penetrate into the dendrimer is greater for $n = 32$ because of its more accessible interior than $n = 64$, thereby resulting in the dye residing closer to the aliphatic core. Also, it is possible that the larger interior volume (vide infra) of the fifth-generation dendrimer results in the presence of more water molecules, in turn causing the polarity index to be higher in this case.

The maximum number of Nile Red molecules encapsulated per DAB-TEOPy$_{64}$ host was estimated to be 2,[31] and for DAB-TEOPy$_{32}$, it was 1. It is important to note that this is the first study to quantify the number of *neutral* guest molecules inside a PPI host in *aqueous solution*. A previous study indicates that anionic dye guests (4,5,6,7-tetrachlorofluorescein and Rose Bengal) can be hosted in an oligo-(ethylene oxide)-aryl-functionalized PPI dendrimer at dye-to-host ratios of 20:1 and 40:1; however, this enhanced level of loading is greatly impacted by acid−base interactions that result between the cationic, tertiary amine interior of the PPI interior and the carboxylate dyes.[27] The modest level of dye encapsulation found here is therefore expected based on the fact that the guest is neutral. Preliminary studies of Nile Red retention upon size-based membrane separation indicate that the dye is retained in DAB-TEOPy$_{64}$ for up to 3 days, whereas it is completely released after only 1 day with DAB-TEOPy$_{32}$. These dynamic trapping outcomes are encouraging for our future work involving static trapping of guests through oligomerization of the pyrrole periphery.[16a]

In summary, the synthesis of a new water-soluble host and three new 3-substituted pyrrole intermediates has been reported. The hosts have great potential as a molecule delivery system because of their demonstrated ability to

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encapsulate hydrophobic *neutral* guest molecules in aqueous media. Future work in our laboratory will focus on more elaborate characterization of this new class of hosts through dynamic light scattering, fluorescence, and nuclear magnetic resonance relaxation studies. Furthermore, oligomerization of the pyrrole-terminated periphery[16a] will also be studied using IR spectroscopy and mass spectrometry to learn how to effect the stimuli-responsive hosting nature of the DAB-TEOPy$_n$.

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Supporting Information Available: Experimental procedures and characterization of compounds **2**, **4**, **5**, and **7a**,**b** are included. Characterization includes ^{1}H NMR and ^{13}C NMR for all molecules, ESI-HRMS for **2**, **4**, and **5**, and MALDI-MS for **7a**,**b**. Details regarding encapsulation and DLS are also described. This material is available free of charge via the Internet at http://pubs.acs.org.

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