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Probing Every Layer in Dendrons

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Dendrimers have been a molecular structure of intense research in recent years.[1] These molecules are being pursued for a wide variety of applications, including targeted drug delivery, controlled drug release, and catalysis.[2] In many cases, the interest in these molecules is motivated by the fact that the globular shape of the dendrimers afford a different microenvironment at the core. Properties of a dendritic core have been studied quite extensively through encapsulations of electro-, photo-, and catalytically active units.[3] However, little if anything is known regarding the properties of functionalities incorporated in the intermediate layers of the dendrimer. In this contribution, we investigate the properties of every layer of dendron by incorporating a single fluorescent probe unit in a specific location. The extent of guest molecule accessibility to each location within a dendrimer is then analyzed using an intermolecular photoinduced electron transfer (PIET)-based fluorescence quenching process. For the first time, we show here that the steepest change in accessibility occurs in the middle layers. We also show that there is a significant difference in generation-dependent accessibility to even the peripheral layers of dendrimer.

Anthracene is used as the fluorescent probe in this study, and benzyl ether dendrimers were used as the scaffold. To incorporate anthracene in specific locations of a dendrimer, we first synthesized an anthracene containing monomer unit **8** (Scheme 1). The combination of the monomer unit **8** and 3,5-dihydroxybenzyl alcohol was used to synthesize 14 different G1−G4 dendrons **1**−**4** (Chart 1) and the control G0 molecule **5** (Scheme 1). Synthetic methodologies, developed by us to sequence dendrimers, were used here to achieve the site-specific incorporation of these fluorescent probes.[4,5] All dendrimers were characterized by ^{1}H, ^{13}C, and mass spectrometry. Size exclusion chromatography was used as an additional test of purity.[5]

Fluorescence intensity of the anthracene moiety decreases with increasing concentration of the quencher, *N*,*N*,*N*′,*N*′,*N*″,*N*″-hexamethyltris(2-aminoethyl)amine (tren-Me$_6$). This intermolecular PIET-based fluorescence quenching is used as the measure of accessibility of tren-Me$_6$ to the anthracene moiety in each dendrimer. Substituents, excited-state energy, and the redox potential of the anthracene derivatives and tren-Me$_6$ suggest that fluorescence quenching due to energy transfer or a heavy atom effect is unlikely; these are, however, appropriate for PIET. Moreover, fluorescence quenching is much less in apolar toluene and more in polar DMF compared to that in the current solvent THF:CH_3CN (6:4), which is a hallmark of PIET.

Extent of this fluorescence quenching is related to the concentration of the quencher through the Stern−Volmer equation: $I_0/I = 1 + K_{SV}[Q]$, where I_0 and I are the fluorescence intensities in the absence and presence of quencher, [*Q*] is the concentration of the quencher, and K_{SV} is the Stern−Volmer quenching constant. The

Scheme 1. Synthesis of the Monomer **8** and G0-Dendron **5**

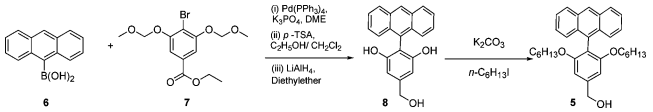
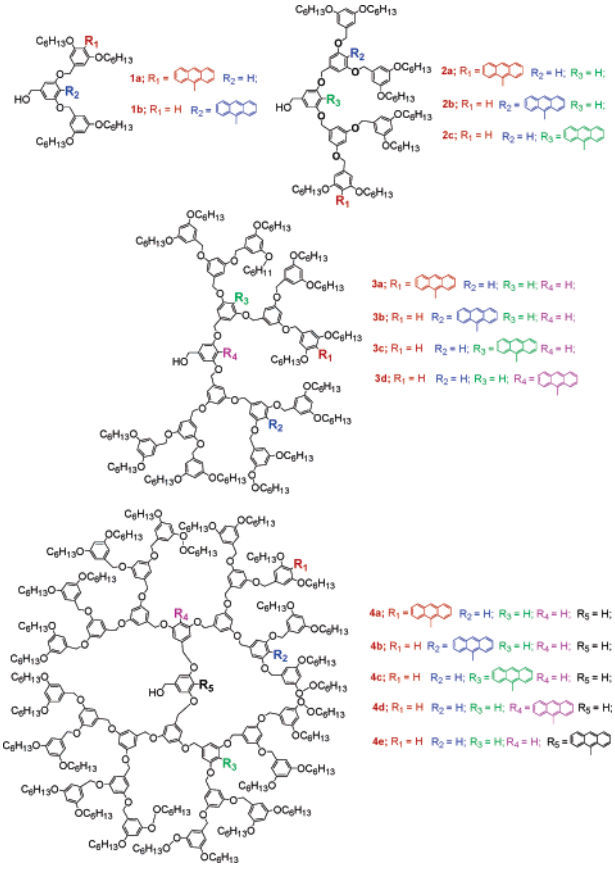


Chart 1. Structures of Dendrimer with Anthracene as the Fluorescent Probe in Specific Locations



constant K_{SV} is a product of the bimolecular quenching rate constant k_q and fluorescence lifetime of anthracene dendrimer in the absence of the quencher (τ_0), i.e., $K_{SV} = k_q \times \tau_0$.[5] The k_q values could be considered as a measure of the accessibility of a quencher to a fluorophore.

When using k_q as a measure for estimating the relative accessibility of dendrimers **1**−**4**, two factors need to be taken in to account: (i) Is the fluorescence quenching static or dynamic? and (ii) Is the excited-state energy for the anthracene species in all dendrimers the same to allow a comparison? We have addressed both these issues. Stern−Volmer quenching constants of anthracene-based dendrimers were measured through both steady-state and

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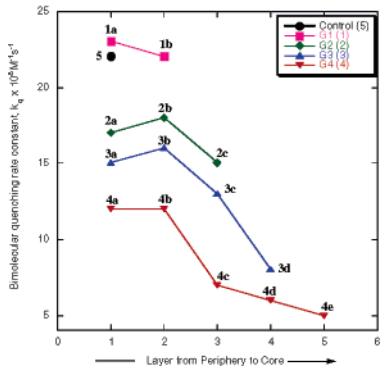

	k_q x 10^8, $M^{-1}s^{-1}$
1a	23.6
1b	22.8
2a	17.7
2b	18.0
2c	15.5
3a	15.5
3b	16.6
3c	13.1
3d	8.96
4a	12.4
4b	12.5
4c	7.44
4d	6.60
4e	5.01
5	22.7

Figure 1. Plot of k_q for different layers in dendrons.

time-resolved spectroscopy. The obtained K_{SV}'s were similar in both techniques for a most encapsulated anthracene (fourth generation) as well as a least encapsulated anthracene (first generation). These results confirm that the observed fluorescence quenching is the result of a dynamic process. As an additional evidence, we did not observe any saturation in fluorescence quenching or change in absorption or fluorescence spectra upon increasing the concentration of quencher. We are also confident that the excited-state energy of the anthracene moiety for all the compounds **1**−**5** is the same, because neither the absorption nor the emission spectra exhibited any shift. Note that the intersection of absorption and emission spectra is considered to be an estimate of ΔE_{0-0}. This confirms that the change in k_q values observed in various compounds is not due to the inherent change in the electronic property of the anthracene moiety in different dendrimers.

The observed bimolecular quenching rate constants and the trends for the compounds **1**−**5** are shown in Figure 1. Three interesting trends are noteworthy: (i) Comparison of the peripheral moieties in **1**−**4**, (ii) comparison of a position in one generation with another position in a different generation, and (iii) a trend within different layers of a dendrimer.

Encapsulation of the anthracene moiety in the periphery varies significantly with generation (compare **1a**−**4a**). There is essentially no difference in accessibility between **1a** and the control molecule **5**, while there is about a 26% change in accessibility between **1a** and **2a**. The difference between **1a** and **4a** is about 48%. In fact, the accessibility of the peripheral anthracene in **4a** is less than that in the core anthracene in **2c** and is about the same as that in the anthracene moiety in **3c**. The fact that there is a generation dependence in accessibility to periphery is not that surprising, since the backfolding of these dendrimers will result in burying of some of the branches.[6] Since only one of the branches has the anthracene unit, the k_q value is a true measure of the average accessibility of the peripheral moieties. It is surprising to us, however, that the difference is significant even at the second generation.

Another interesting observation in this study is that beyond the second layer of a dendron, the accessibility of a layer within a dendron seems to be similar to the next layer of the previous generation. For example, the second-layer compound **3b** exhibits a similar k_q value as the third-layer compound **2c**. Similar trends can also be noted by comparing **3c** with **4b** and **3d** with **4c**. The reasons for this trend are not obvious to us at this time and are under investigation.

The difference between the periphery and the next layer of the dendrons is negligible in all dendrons. From the second to third layer, there is a significant difference in accessibility. In the third generation, a gradual decrease in accessibility from the second to the fourth layer (**3b**−**3d)** is observed. However, in the fourth-generation **4**, the steepest difference seems to be between the second and third layer, where there is about 42% change in the k_q value (compare **4b** and **4c**). From the third to fourth to the focal point (**4c**−**4e**), there is only about 15% change with each layer. It is interesting that the first significant change in accessibility occurs at the third layer from the periphery in these dendrons. In the case of the fourth generation, the third layer exhibits the steepest change in accessibility (compare **4a**−**4e**). In fact, it is in this layer that there is maximum difference between the generations (compare **2c**−**4c**). Therefore, it is the difference at this layer that is translated to varied levels of encapsulation at the focal point.

In summary, we have incorporated a probe in each layer of dendron and investigated the accessibility of these locations using an intermolecular PIET process. The trends reported here should have implications in areas such as catalysis and drug delivery. The study is relevant to catalysis because PIET is considered to be based on a bimolecular collision process, an event that is necessary between a catalytic site and the substrate. In drug delivery, approaches using cleavable dendrimer−drug conjugates have been suggested.[7] It is necessary in these cases that the linkers are not cleaved until they reach the target and therefore should be encapsulated. It is also advantageous to load several drug molecules in a single dendrimer. Note, however, that loadability decreases as one moves toward the core and the encapsulation increases toward the core. The current study suggests that the intermediate layers with significant loading capacity and encapsulation could be useful (e.g., third layer of the G4 in this case). While these observations clearly have implications, further understanding of the reasons for the observed behavior is still needed, which is the focus of current work in our laboratories.

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Supporting Information Available: Synthetic and other experimental details. This material is available free of charge via the Internet at http://pubs.acs.org.

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(5) See Supporting Information for details.

(6) An alternate explanation is the possible differences caused by aggregation of these dendrons. The size of G2−G4 dendrons (measured by DLS) ranged from 2 to 4 nm depending on the generation in the solvent under study (THF:CH_3CN, 6:4), indicating a monomeric state. Therefore, this possibility is unlikely. We were unable to study G1 by DLS because of the small size. We thank a reviewer for suggesting this possibility.

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