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Synthesis of Cationic Water-Soluble Light-Harvesting Dendrimers

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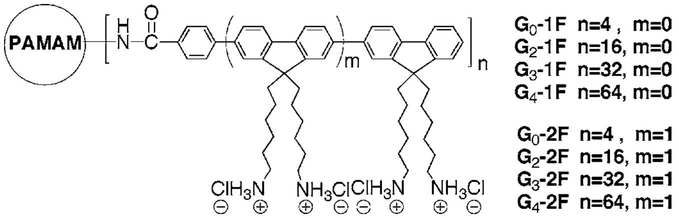
ABSTRACT



Four generations of phenylenefluorene (1F)- and phenylenebis(fluorene) (2F)-terminated polyamidoamine (PAMAM) dendrimers were synthesized by coupling activated esters with commercially available PAMAM precursors. Treatment of Boc-terminated pendant groups on the optically active units with 3 M HCl in dioxane yields cationic water-soluble dendrimers. Fluorescence resonance energy transfer (FRET) experiments with the cationic dendrimers as the donor and double stranded DNA containing a fluorescein label as the acceptor reveal cooperative optical behavior.

Dendrimers are hyperbranched organic macromolecules with well-defined three-dimensional architectures and a large number of terminal groups that can be varied to control properties such as size, molecular weight, topology, and surface reactivity.[1] They can be used to generate a highly dense collection of chromophores with overall properties similar to those of natural photosynthetic systems, which absorb light and very efficiently transfer the resulting excitations to lower energy sites.[2] Recent interest has grown in utilizing the light-harvesting (or antenna-like) properties of dendrimers to achieve sensory signal amplification in the presence of suitable energy or electron acceptors.[3]

Light-harvesting properties and efficient fluorescence energy transfer (FRET) can also be attained with water-soluble cationic conjugated polymers and oligomers.[4] The resulting optical amplification of emission intensities from reporter dyes has been used for devising strand-specific DNA or RNA detection methods.[4,5] The positive charges in these conjugated polyelectrolytes are important for water solubility and for orchestrating electrostatic interactions with the negatively charged DNA (or RNA).[6] However, structural uncertainties in these polymers (i.e. molecular weight distribution, variations in average molecular weight, and

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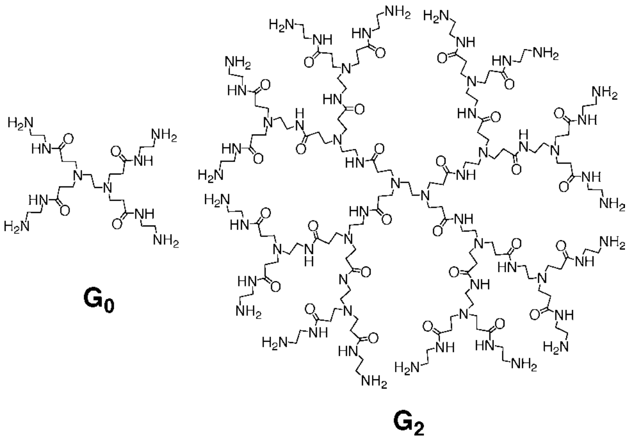
structural defects) limit the determination of detailed relationships between molecular structure and optical properties.[7]

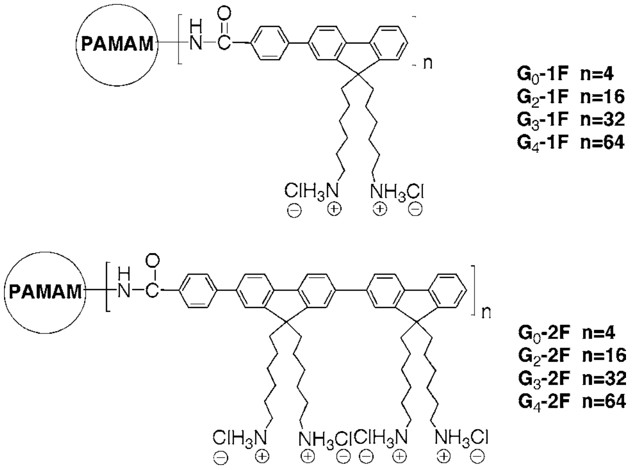
It occurred to us that cationic dendrimers with precise structures could be used in similar sensing schemes. In this contribution, we report the synthesis of a series of cationic polyamidoamine (PAMAM) dendrimer derivatives with chromophores on the macromolecular surface. The positive charges, located primarily on the exterior of the dendrimer, yield water-soluble materials. For reference, the structures of the zero- and second-generation PAMAM cores are shown in Scheme 1.

Scheme 1. Molecular Structures of the Zero (G_0) and Second (G_2) Generations of PAMAM Dendrimers

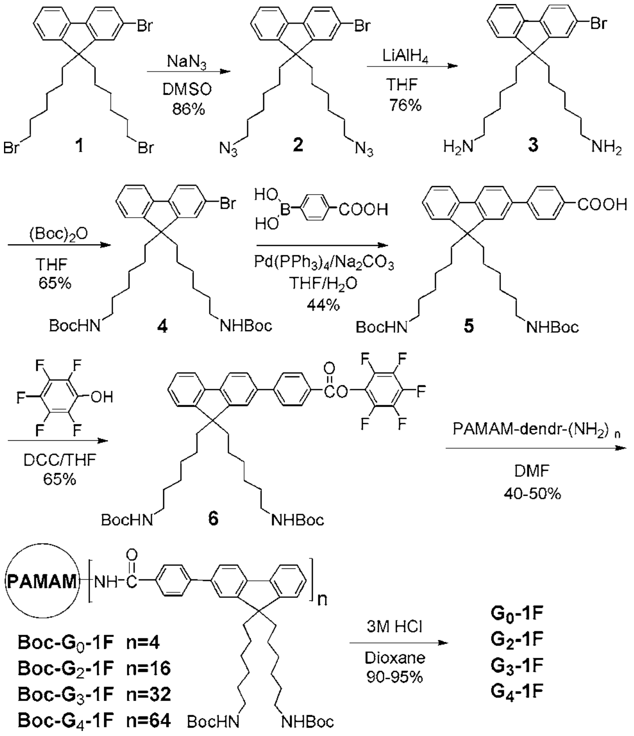


We chose as synthetic targets four generations of PAMAM dendrimers with either phenylenefluorene (**G$_{0-4}$-1F**, where the subscript refers to the generation number and **1F** is phenylenefluorene, see Scheme 2) or phenylenebis(fluorene) (**G$_{0-4}$-2F**, where **2F** is phenylenebis(fluorene)) at the surface of the macromolecules. At near neutral pH only the amine groups on the terminal chromophores are protonated; the PAMAM interior is neutral.[8]

Scheme 2. Molecular Structures of **G$_{0-4}$-1F** and **G$_{0-4}$-2F**



Scheme 3. Synthesis of **G$_{0-4}$-1F**



Scheme 3 shows the synthetic entry into the **G$_{0-4}$-1F** series. Reaction of 2-bromo-9,9-bis(6′-bromohexyl)fluorene (**1**)[5d] with sodium azide in DMSO provides 2-bromo-9,9-bis(6′-azidohexyl)fluorene (**2**) in 86% yield. Reduction by using lithium aluminum hydride and subsequent amine protection with di-*tert*-butyldicarbonate (Boc_2O) yields 2-bromo-9,9-bis(6′-*tert*-butoxycarbonylaminohexyl)fluorene (**4**) in 65% yield. Suzuki cross-coupling[9] of **4** with 4-carboxyphenylboronic acid using $Pd(PPh_3)_4$ in 2 M aqueous sodium carbonate gives 2-(4′-carboxyphenyl)-9,9-bis(6′-*tert*-butoxycarbonylaminohexyl)fluorene (**5**) in 44% yield. After reaction with pentafluorophenol in the presence of dicyclohexylcarbodiimide (DCC), the activated pentafluorophenol 4-(9′,9′-bis(6″-*tert*-butoxycarbonylaminohexyl)-2′-fluorenyl)benzoate (**6**) was obtained in 65% yield. Four different generations of Boc-protected phenylene-fluorene terminated dendrimers (**Boc-G$_{0-4}$-1F**) were obtained by coupling the activated ester **6** with commercially available amine terminated PAMAM cores. Treatment with 3 M HCl in dioxane yields the cationic, water-soluble dendrimers **G$_{0-4}$-1F**.

The series **G$_{0-4}$-2F** was prepared by the sequence of reactions in Scheme 4. In a procedure similar to that for **2**,

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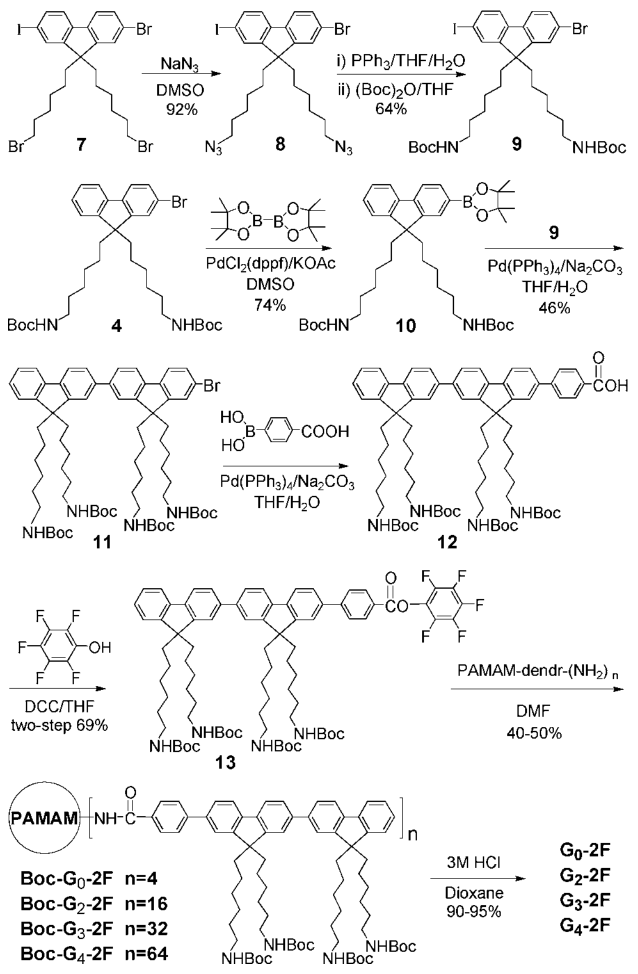
Scheme 4. Synthesis of $\mathbf{G_{0-4}}$**-2F**



2-bromo-7-iodo-9,9-bis(6′-bromohexyl)fluorene (**7**)[5d] was converted to 2-bromo-7-iodo-9,9-bis(6′-azidohexyl)fluorene (**8**) by reaction with sodium azide in DMSO in 92% yield. Reduction by using triphenylphosphine in THF/water (v/v 7:1), followed by protection of the amino groups with Boc_2O yields 2-bromo-7-iodo-9,9-bis(6′-*tert*-butoxycarbonylamino-hexyl)fluorene (**9**). Selective Suzuki cross-coupling of compound **10** with the iodide site in **9** gives 4-[9′,9′;9″,9″-tetra(6‴-*tert*-butoxycarbonylamino-hexyl)-7′,2″-bisfluoren-2′-yl]benzoic acid (**12**).[5d] The activated pentafluorophenyl ester **13** was obtained starting with **12** by a similar method for the preparation of **6**. The $\mathbf{G_{0-4}}$**-2F** series was synthesized by coupling the activated ester **13** with the appropriate PAMAM dendrimer, followed by HCl treatment.

The ^{1}H NMR spectra in $CDCl_3$ of Boc-protected dendrimers can be used to calculate the degree of surface functionalization. For the Boc-protected dendrimers, the internal dendrimer methylene protons are observed from 3.2 to 3.6 ppm and from 2.3 to 2.7 ppm. The peaks from 7.2 to 8.0 ppm are due to proton signals on the aromatic terminal groups. The ratio of the integrations of *aromatic*/CH_2 proton signals provides the degree of surface functionalization. In **Boc-**$\mathbf{G_0}$**-1F**, with 44 *aromatic* and 36 CH_2 protons, the theoretical ratio is 1.22. The observed ratio is 1.23 (>99% functionalization). In **Boc-**$\mathbf{G_2}$**-1F**, the theoretical ratio is 0.77 and the observed ratio is 0.77 (>99% functionalization). For higher generation dendrimers ($\mathbf{G_3}$ and $\mathbf{G_4}$), the NMR data were consistent with 90−95% surface functionalization. Incomplete coverage is attributed to the nonquantitative yield of the coupling steps and known imperfections of the commercial PAMAM dendrimers, together with difficulties in the purification of the larger structures.

The optical properties of the $\mathbf{G_{0-4}}$**-1F** and $\mathbf{G_{0-4}}$**-2F** are summarized in Table 1. Measurements were performed in a 50 mM potassium phosphate buffer at pH 6 to ensure protonation of only the surface basic sites. The absorption maxima are observed at 320 and 345 nm for $\mathbf{G_{0-4}}$**-1F** and $\mathbf{G_{0-4}}$**-2F**, respectively, corresponding to the $\pi-\pi^*$ transition of the conjugated units. For both $\mathbf{G_{0-4}}$**-1F** and $\mathbf{G_{0-4}}$**-2F**, the extinction coefficient (ϵ) of the molecules increases as a function of generation, while the ϵ value contributed by each optical unit (OU) decreases. This drop in ϵ/OU ratio is consistent with interchromophore contacts that become more pronounced as the number of surface OUs increases. Slight hypsochromic shifts and reductions in fluorescence quantum efficiencies (QEs) are also observed for the larger generations, consistent with the increase in chromophore−chromophore interactions.[10] For a given generation, the absorption and fluorescence maxima and the extinction coefficient for $\mathbf{G_{0-4}}$**-2F** are larger than those of $\mathbf{G_{0-4}}$**-1F**, which reflect the more extended conjugation length in the $\mathbf{G_{0-4}}$**-2F** series.

Table 1. Summary of Optical Properties of $\mathbf{G_{0-4}}$**-1F** and $\mathbf{G_{0-4}}$**-2F** (pH 6.0), Where PL is Photoluminescence

	UV (λ_{max}, nm)	PL (λ_{max}, nm)	ϵ ($M^{-1}\cdot cm^{-1}$) $\times 10^5$	ϵ/OU ($M^{-1}\cdot cm^{-1}$) $\times 10^4$	QE (%)
$\mathbf{G_0}$**-1F**	320	403	0.9	2.3	35
$\mathbf{G_2}$**-1F**	320	400	3.7	2.3	29
$\mathbf{G_3}$**-1F**	320	400	7.0	2.2	24
$\mathbf{G_4}$**-1F**	322	395	13	2.0	20
$\mathbf{G_0}$**-2F**	345	426	1.6	3.9	40
$\mathbf{G_2}$**-2F**	346	426	5.6	3.5	35
$\mathbf{G_3}$**-2F**	345	423	9.1	2.8	33
$\mathbf{G_4}$**-2F**	344	423	18	2.8	26

The function of $\mathbf{G_{0-4}}$**-1F** and $\mathbf{G_{0-4}}$**-2F** as donors in FRET processes was examined.[11] Fluorescein (Fl) was chosen as the acceptor, since its absorption spectrum overlaps with the emission spectra of **1F** and **2F**. Fluorescein was attached to (negatively charged) double stranded DNA (dsDNA-Fl) to ensure association with the cationic $\mathbf{G_{0-4}}$**-1F** and $\mathbf{G_{0-4}}$**-2F** by electrostatic forces. Double stranded DNA (dsDNA-Fl) was obtained by hybridization of ssDNA-Fl (Fl-5′-ATCT-TGACTATGTGGGTGCT-3′) with a complementary strand of equal length.

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[10] Although the ϵ/OU was not evaluated for the Boc-protected dendrimers, previous work has shown that interchromophoric coupling exists with other organic-soluble dendrimers. See ref 3b.

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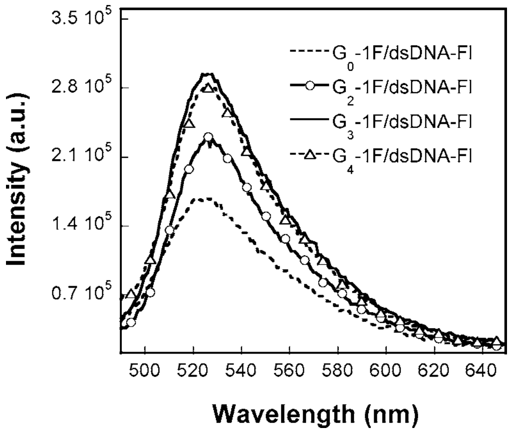

Figure 1. The emission spectra of dsDNA-Fl in the presence of **G_{0-4}-1F** in potassium phosphate buffer solution (50 mM, pH 6.0); [OU] = 6.4×10^{-7} M, [dsDNA-Fl] = 1.0×10^{-8} M, excitation wavelength is 330 nm.

As shown in Figure 1, excitation of **G_{0-4}-1F** at 330 nm results in successful FRET to the dsDNA-Fl ([dsDNA-Fl] = 1.0×10^{-8} M, [OU] = 6.4×10^{-7} M). The spectra also reveal a more effective "antenna effect" for the higher generation dendrimers.[12] FRET efficiency reaches a limiting value for the third and fourth generation structures. This limit is consistent with the lower extinction coefficient (per optical unit) and QE, compared to the third generation dendrimers. For **G_3-1F**, a 6-fold increase in Fl emission is observed by the sensitized excitation of the dendrimer at 330 nm, relative to the direct excitation of Fl at its absorption maximum (480 nm). As a control, no FRET takes place when Fl unattached to DNA is added. These results demonstrate that higher Fl emission intensities are attained by the overall larger optical cross section of the dendrimer structure and efficient FRET to the Fl, than by direct excitation of the Fl. Furthermore, the negative charge of the DNA is responsible for complexation to the cationic dendrimer and satisfying the distance requirement for FRET. It should be noted that the sensitized Fl emission intensity by **G_{0-4}-2F** is approximately twice that obtained with **G_{0-4}-1F**, when the same generation is compared. Although the number of chromophores in this comparison is equal, the **G_{0-4}-2F** OUs have better spectral overlap with Fl, a larger extinction coefficient, and a larger quantum yield, relative to the **G_{0-4}-1F** series.

In summary, new cationic light-harvesting dendrimers were designed, synthesized, and characterized. FRET experiments demonstrate that higher generation dendrimers exhibit better signal amplification of DNA-bound fluorescent reporters. These water-soluble dendrimers may prove useful in biosensor applications, due to their collective behavior and well-defined structures.

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Supporting Information Available: Detailed experimental procedures and characterization data of all intermediates and dendrimer products. This material is available free of charge via the Internet at http://pubs.acs.org.

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[12] The "antenna effect" arises from a larger ϵ and a higher binding constant due to multivalency of the higher generation dendrimers.