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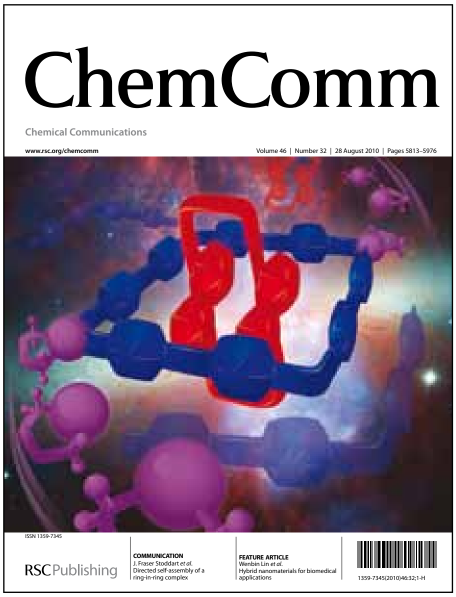

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ARTICLE TYPE

Photostable, Hydrophilic and Functional Near Infrared Quaterrylenediimide-cored Dendrimers for Biomedical Imaging

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We describe a new class of near infrared (NIR) fluorescent dendrimeric quaterrylenediimide dyes with high photostability and hydrophilicity, functionality, as well as low cytotoxicity.

There has been considerable effort in the development of near infrared (NIR, 700-900 nm) fluorescent dyes for in vivo optical imaging, due to the relatively deep tissue penetration and negligible autofluorescence in the NIR region. Ideally, an optimal NIR fluorescent dye used for biomedical imaging has such merits: 1) possessing high molar extinction coefficient (absorbance) and quantum yield (fluorescence); 2) having high chemical and photophysical stability; 3) being biocompatible (low cytotoxicity); 4) showing hydrophilicity (water solubility); and 5) containing functional groups for bioconjugation to targeting moieties such as antibodies, proteins, peptides, receptor ligands and carbohydrates. Unfortunately, few NIR dyes have all the desired properties. Typical NIR dyes are of the polymethine cyanine dye family, comprising benzoxazole, benzothiazole, indolyl, 2-quinoline and 4-quinoline subclasses.[1] These dyes have adjustable optical properties and high extinction coefficients (>150,000 M^{-1} cm^{-1}).[2] In addition, many cyanine dyes can be easily conjugated to targeting moieties, imparting molecular specificity to targeted cells or tissues.[3-6] Unfortunately, conventional NIR cyanine dyes suffer from some limitations. Most of these dyes decompose over time in ambient conditions and are readily photobleached under intense illumination. Therefore, there is a need to develop novel NIR dyes to improve chemical stability and photostability.

Quaterrylene dyes were recently developed for solar cells to conduct solar energy.[7,8] Although these quaterrylene dyes are not suitable for biomedical imaging due to poor hydrophilicity and no functionality, they exhibit extremely high molar extinction coefficients (>150,000 M^{-1} cm^{-1}), outstanding quantum yields (>0.55), as well as excellent chemical and photo stability.[7,9] In addition, this class of dyes has absorption and emission wavelength close to 800 nm,[7] the most preferred wavelength for NIR fluorescence imaging.[10] These properties make quaterrylene dyes stand out as very attractive fluorescent imaging dyes; however, no quaterrylene dyes suitable for biomedical imaging have been developed. One of the largest obstacles is aggregation, which is a common issue of dyes with large and rigid π-electron systems, such as quaterrylene dyes.[7,11,12] To circumvent this challenge and take advantage of the outstanding spectroscopic properties of quaterrylene dyes, we developed a dendrimeric platform with four dendronized polyamides covalently attached to the bay regions of a new quarterrylene dye. Polyamide dendrimers can enhance the biocompatibility of materials and have been used to improve dye properties.[13,14] Such a design will also increase hydrophilicity and introduce functionality. To demonstrate the concept, this communication reports the first two generations of this new quaterrylene-dye based dendrimer family, **QR-G1-COOH** and **QR-G2-COOH** (Fig. 1).

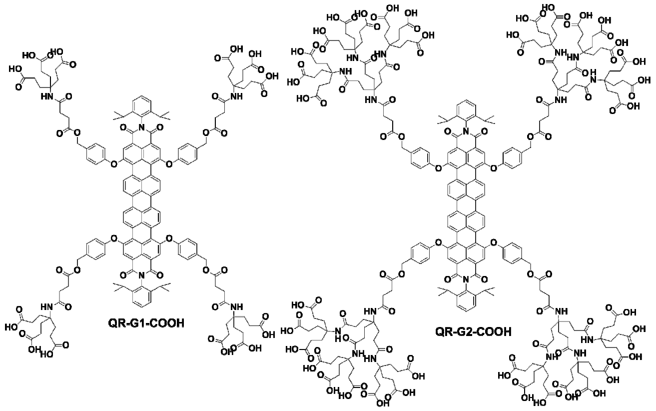
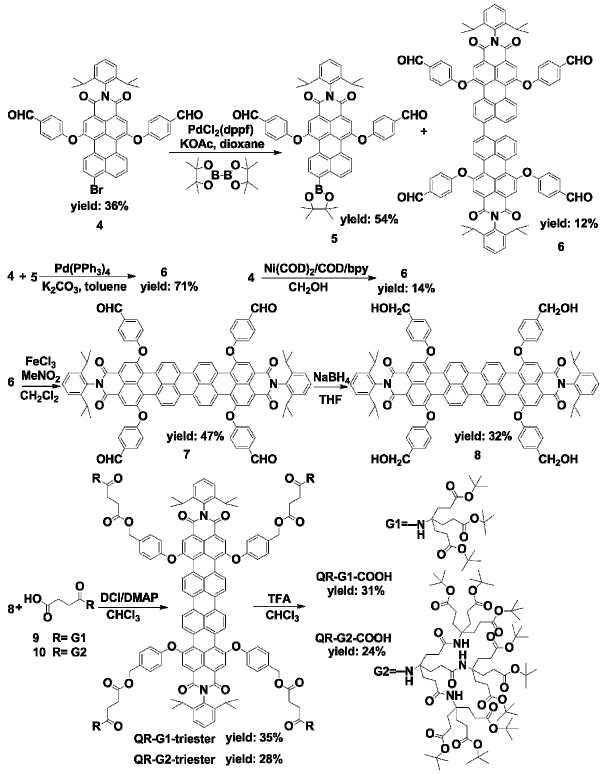


Fig. 1. Structures of **QR-G1-COOH** and **QR-G2-COOH.**

Scheme 1 outlines our synthetic strategy towards developing **QR-G1-COOH** and **QR-G2-COOH**. In order to synthesize the important precursor of quaterrylene, biperylene compound **6**, we used two approaches, Yamamoto coupling and Suzuki coupling. Yamamoto homocoupling with bis(cycloocta-1,5-diene)nickel (0) [$Ni(COD)_2$] has been used to synthesize some biperylenes;[15] unfortunately, it afforded the biperylene 6 with rather low yield (14 %). To improve this reaction yield, we attempted the Suzuki coupling of 9-bromoperylenedicarboximide (4) and its boronic ester (5). Although this approach requires an additional step to synthesize 5, biperylene 6 was found as a by-product during the synthesis of the boronic ester 5, and the overall conversion yield from 4 to 6 was greatly improved (from 14% to 62%). In addition, toxic $Ni(COD)_2$ is avoided by using a Pd catalyst. Therefore, Suzuki coupling appears to be a much better method for the synthesis of **6** than Yamamoto coupling.

The most challenging step encountered during the synthesis of **QR-G1-COOH** and **QR-G2-COOH** was the ring cyclization of biperylene **6.** Many quaterrylenes reported were synthesized through a base-assisted cyclodehydrogenation reaction using molten KOH/glucose, tert-BuOK/DBN in diglyme, or K_2CO_3 in



Scheme 1 Synthesis of **QR-G1-COOH** and **QR-G2-COOH.**

ethanolamine.[7,16-18] However, these reaction systems are not applicable for the synthesis of our quaterrylene **7** because of the high reactivity of aldehyde under the harsh basic conditions. Recently, Müllen's group reported milder conditions for the preparation of quaterrylenedicarboximides and rylenediimides by the Lewis acids $FeCl_3/CH_3NO_2$ and $AlCl_3$/chlorobenzene.[19,20] Although treating **6** with $AlCl_3$/chlorobenzene failed to yield the quaterrylene compound **7**, cyclodehydrogenation of **6** with $FeCl_3/CH_3NO_2$ led to the desired product with a moderate yield (47%). The following reduction of aldehyde groups on **7** by $NaBH_4$ yielded benzyl alcohol **8** with a relatively low yield (32%). Coupling quaterrylene **8** with dendrons **9** and **10** provided the dendrimers **QR-G1-triester** and **QR-G2-triester** in 35% and 28% yields after purification by column chromatography. Dendrimers **QR-G1-triester** and **QR-G2-triester** were highly soluble in organic solvents such as toluene, dichloromethane (DCM), chloroform, ethyl acetate, as well as methanol, and they were characterized by ^{1}H NMR, ^{13}C NMR, and MALDI-TOF mass spectrometry. The next deprotection reaction with TFA/chloroform yielded the target dendrimers **QR-G1-COOH** and **QR-G2-COOH** that possess twelve and thirty-six acid groups, respectively. These carboxylic acid groups provide **QR-G1-COOH** and **QR-G2-COOH** with highly hydrophilicity as sodium salts.

Table 1. Photophysical properties of **QR-G1-COOH** and **QR-G2-COOH**

	QR-G1-COOH			QR-G2-COOH		
	λ_{abs}/ nm	$\varepsilon/10^5$ $M^{-1}cm^{-1}$	λ_{em}/nm (Φ/%)^a	λ_{abs}/ nm	$\varepsilon/10^5$ M^{-1} cm^{-1}	λ_{em}/nm (Φ/%)^a
Methanol	771	1.46	813	781	1.21	815
	728	1.43				
DMSO	788	1.53	816	788	1.19	816
	720	0.76	(0.11)	723	0.55	(0.32)
water	739	0.74	---	743	0.56	---
Water, Pluronic^b	782	1.22	807	791	1.31	809
	712	0.57		723	0.6	

a) Fluorescence quantum yield; ICG in methanol was used as standard (Φ=7.8%, ref 6). b) The Pluronic concentration is ~0.4 wt %/wt for QR-G1-COOH and ~10 wt %/wt for QR-G2-COOH.

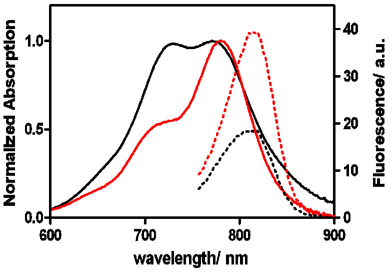


Fig. 2. Emission (dash lines) and normalized absorption (solid lines) spectra of **QR-G1-COOH** (black) and **QR-G2-COOH** (red) at concentration of 1×10^{-6} M in MeOH. λ_{ex}=720 nm.

The UV-vis absorption and emission spectra of **QR-G1-COOH** and **QR-G2-COOH** in methanol are displayed in Fig. 2 and the spectroscopic data in various solvents are summarized in Table 1. **QR-G1-COOH** has maximum absorption at 771 nm (ε=1.46×10^5 M^{-1} cm^{-1}), with a comparable absorption band at 728 nm (ε=1.43×10^5 M^{-1} cm^{-1}). As expected, when exited at 720 nm, **QR-G1-COOH** showed relatively weak emission at 813 nm in methanol and nearly no fluorescence in water. Similar observations have been reported on extended perylenediimides.[9,21,22] The large absorption band at 728 nm and poor fluorescence indicate the presence of strong H-aggregates for **QR-G1-COOH**, which was further evidenced by the large fluorescence enhancement when the molecule was dissolved in 0.4% wt/wt of pluronic (Fig. S1). This fluorescence enhancement in pluronic compared to water can be explained by the disruption of the non-fluorescent dye aggregates by Pluronic, which formed micelles with dye monomers inside. [9,21,22] The strong absorption in the NIR region and weak emission may render **QR-G1-COOH** to a promising photoscoustic imaging agent.

QR-G2-COOH has a similar maximum absorption wavelength (781 nm, ε=1.21×10^5 M^{-1} cm^{-1}) as **QR-G1-COOH**, but with a lower absorption shoulder at around 720 nm (Fig. 2). In addition, **QR-G2-COOH** has significantly higher fluorescence than **QR-G1-COOH**, with a quantum yield roughly two times higher than that of **QR-G1-COOH** (Table 1). These data indicate that **QR-G2-COOH** has less H-aggregates than **QR-G1-COOH**, and enlargement of the dendrimer size appears to be an effective approach to improve the fluorescence of quaterrylene dendrimers. Similarly, Heek and Zimmerman reported that bulky groups can reduce the intermolecular aggregation and improve the emission of perylene dyes.[23,24]

To evaluate the photostability of **QR-G1-COOH** and **QR-G2-COOH**, we monitored the NIR absorbance of these molecules exposed to ambient light and compared the results with that of the FDA approved NIR dye, indocyanine green (ICG). As shown in Fig. 3, after being exposed to ambient light for only 2 hours, the absorbance of ICG solution decreased by 48%, whereas solutions

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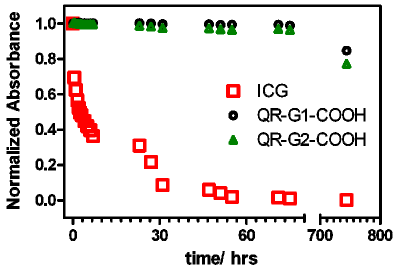

Fig. 3. The photostability of **QR-G1-COOH**, **QR-G2-COOH** and ICG in DMSO. Samples were exposed to the ambient light and the change of optical density at absorption maximum wavelength was monitored over time. All samples have the initial absorbance of about 1.2 at the absorption maximum wavelength. The signals were normalized against the values at 0 hour.

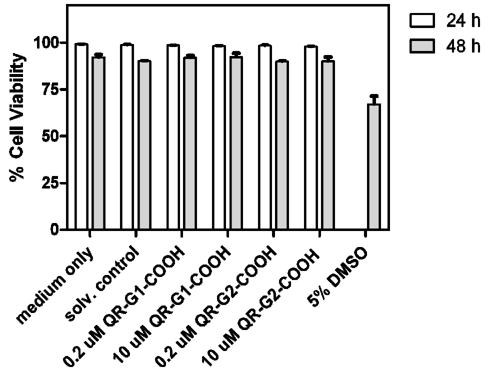

Fig. 4. Cell viability tests of **QR-G1-COOH** and **QR-G2-COOH** on the wt-DBT cell line. Values are presented as means ± standard deviations.

of **QR-G1-COOH** and **QR-G2-COOH** exhibited no significant absorbance change. After 3 days of illumination, no significant absorbance from ICG sample was observed, while **QR-G1-COOH** and **QR-G2-COOH** showed an absorbance change of only 1% and 3%, respectively. Even after being exposed to the ambient light for 31 days, **QR-G1-COOH** and **QR-G2-COOH** remained strong NIR absorbance of 85% and 77%, respectively. These data demonstrate that **QR-G1-COOH** and **QR-G2-COOH** are dramatically more photostable than ICG. The remarkable photostability of these dendrimers provides opportunities in long-term imaging and accurate signal quantification.

The in vitro cytotoxicity of **QR-G1-COOH** and **QR-G2-COOH** against wild-type mouse malignant astrocytoma DBT (wt-DBT) cells was investigated using a Hemocytometer-based trypan blue dye exclusion method.[25] Fig. 4 shows the cell viability after incubation with **QR-G1-COOH** and **QR-G2-COOH** at concentrations of 2×10^{-7} and 1×10^{-5} M for 24 and 48 hours. Cell viabilities of more than 97% were observed for both quaterrylene dendrimers at the tested concentrations in 24 h; after 48 h, their cell viabilities were about 90% and the values were comparable to those of the solvent and cell medium control groups, while only 66.9% live cells were found in the positive control group (5% DMSO). The cytotoxicity results indicate that **QR-G1-COOH** and **QR-G2-COOH** have low cytotoxicity to DBT cells.

In conclusion, we have developed two polyamide-based quaterrylenediimide dendrimers with twelve and thirty-six carboxylic acid groups. With strong NIR absorption, remarkable photostability, low toxicity to DBT cells, high hydrophilicity and functionality ready for bioconjugation, these NIR dendrimers appear to be promising for biomedical imaging applications. In addition, **QR-G2-COOH** appears to be more emissive than **QR-G1-COOH,** indicating that increasing dendrimer size is an effective strategy to improve the emission of the quaterrylenediimide dendrimers. As such, the development of quaterrylenediimide dendrimers with higher generations is currently in process.

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Notes and references

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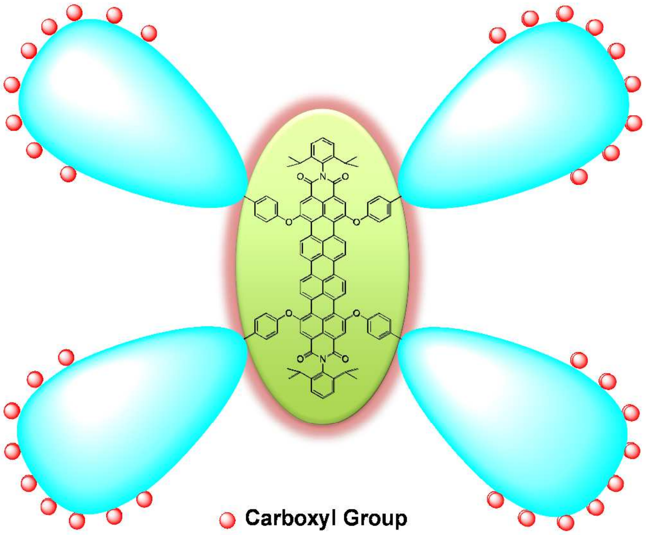

Novel near infrared (NIR) fluorescent dendrimeric quaterrylenediimide dyes were synthesized. Both dendrimers showed high photostability and hydrophilicity, functionality, as well as low cytotoxicity.