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Host–Guest Chemistry of Dendrimer–Drug Complexes: 7. Formation of Stable Inclusions between Acetylated Dendrimers and Drugs Bearing Multiple Charges

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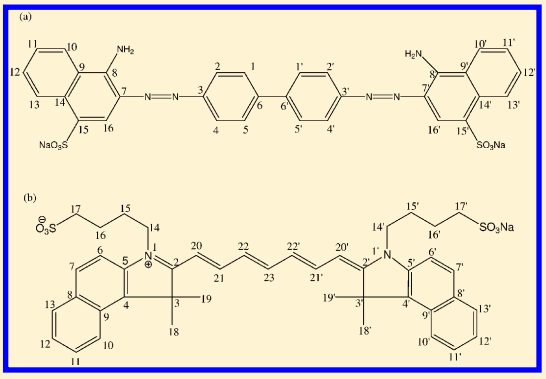
Supporting Information

ABSTRACT: Drug molecules bearing multiple charges usually form precipitates with cationic dendrimers, which presents a challenge during the preparation of dendrimer inclusions for these drugs. In the present study, fully acetylated polyamidoamine (PAMAM) dendrimers were proposed as stable vehicles for drug molecules bearing two negative charges such as Congo red and indocyanine green. NMR techniques including ^{1}H NMR and ^{1}H-^{1}H NOESY were used to characterize the host–guest chemistry of acetylated dendrimer and these guest molecules. The cationic PAMAM dendrimer was found to form a precipitate with Congo red and indocyanine green, but the acetylated one avoided the formation of cross-linking structures in aqueous solutions. NOESY studies revealed the encapsulation of Congo red and indocyanine green within the interior cavities of PAMAM dendrimers at mild acidic conditions and acetylated dendrimers show much stronger ability to encapsulate the guest molecules than cationic ones. Also, UV–vis–NIR studies suggest that acetylated dendrimers significantly improve the photostability of indocyanine green and prevent the formation of indocyanine green J-aggregates in aqueous solutions. The present study provides a new insight into dendrimer-based host–guest systems, especially for those guest molecules bearing multiple charges.



1. INTRODUCTION

Dendrimers are versatile macromolecules with well-defined and hyperbranched structures, nanoscale sizes, low polydispersity, multiple hollow cavities, and high density of surface functionalities.[1−5] These unique features render dendrimers promising alternatives to traditional linear polymers in the design of polymeric drug delivery systems.[6−8] For example, well-defined structures and low polydispersity of dendrimers ensure reproducible pharmacokinetic behavior of the dendrimer-based drug formulations;[7] the nanocharacteristic property of dendrimers allows high drug loading capacity[9] and may generate an enhanced permeability and retention (EPR) effect during cancer therapy;[6] hollow cavities within dendrimers can encapsulate hydrophobic and hydrophilic drugs depending on dendrimer component;[10−12] and the surface functionalities on dendrimer can be conjugated or bound with a list of bioactive moieties such as targeting, imaging, drug, and solubilizing molecules for diagnosis and therapeutic purpose.[13−16] As a result, dendrimer-based drug delivery systems have attracted increasing attention during the past decade.[4,6,7,17]

Previous studies have demonstrated that cationic polyamidoamine (PAMAM) and polypropylenimine (PPI) dendrimers form inclusions or ionic pairs with a list of drugs.[7,18−21] However, these cationic dendrimers have problems delivering drug molecules with multiple charges.[20] For example, amine-terminated PAMAM dendrimers failed to solubilize methotrexate with two carboxyl groups since cationic PAMAM dendrimers and methotrexate form solid precipitates in aqueous solutions during complex formation.[20] On the other hand, though the surface charge on cationic dendrimers plays an important role in drug loading and release processes, high density of cationic groups on dendrimer surface may strongly

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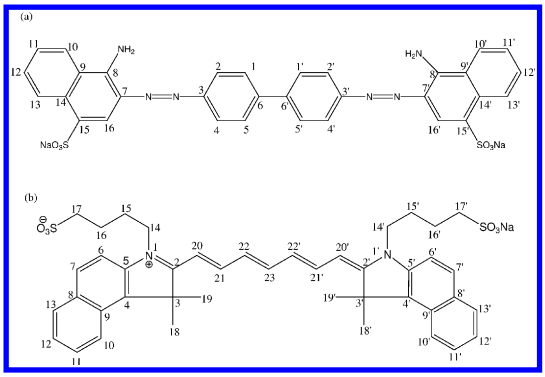
disturb the cell membrane, resulting in a leakage of intracellular components.[7,22] Cationic PAMAM and PPI dendrimers are reported to have serious cytotoxicity and hemolytic activity.[22] Perhaps, the elimination of surface charges on cationic dendrimers is a feasible strategy to prevent the formation of cross-linking supramolecular structures between cationic dendrimers and guest molecules bearing multiple charges and to decrease the cytotoxicity of cationic dendrimers.[23]

Acetylation is one of the most convenient routes to neutralize the surface amine groups on cationic dendrimers.[24] Acetylation reactions occur at mild conditions which will not destroy dendrimer structures and the acetylation degrees on the dendrimer surface can be easily modulated by choosing proper stoichiometry of acetic anhydride and dendrimer. The introduced acetyl groups are hydrophilic and biocompatible, thus can increase the aqueous solubility, stability, and drug loading efficiency of dendrimers, and reduce their nonspecific interactions with cell membranes.[25,26] Also, the size of acetyl groups modified on dendrimer surface is negligible compared to the nanoscale of dendrimers. PAMAM dendrimers are highly pH-sensitive and the interior tertiary amine groups (pK_a ~6.5) of PAMAM dendrimers are quaternized at mild acidic conditions.[1] Quaternization of the interior of the acetylated PAMAM dendrimer by varying pH condition can transfer the surface charge of cationic dendrimers to interior pockets, reducing their cytotoxicity and preventing the formation of cross-linking supramolecular structures with guests bearing multiple charges. These features make acetylated dendrimer a better choice as drug vehicles compared to cationic dendrimers.[27−30]

In the present study, acetylated PAMAM dendrimers were synthesized and used as vehicle candidates of drug molecules bearing multiple charges such as Congo red (CR, Scheme 1a) and indocyanine green (ICG, Scheme 1b). CR is an anionic dye that specifically binds to the amyloid deposits resulting from Alzheimer's, prion, Parkinson's, and Huntington's disease.[31] ICG is a water-soluble and near-infrared fluorescent dye with extremely low cytotoxicity.[32] It has been widely used as a contrast agent to image human vasculature for medical diagnostic applications and has already been approved for clinical use by the food and drug administration (FDA).[33] Both CR and ICG are amphiphilic molecules bearing two negatively charged groups. The inclusion structures of acetylated PAMAM dendrimers with CR and ICG at different pH conditions were revealed by NMR techniques. To the best of our knowledge, this is the first study to investigate the host−guest behaviors of acetylated dendrimers and guest molecules bearing multiple charges, which is an important supplement to dendrimer-based drug delivery systems. Acetylated dendrimers are used to prevent the formation of cross-linking supramolecular complexes of cationic dendrimers with guests bearing multiple charges.

Scheme 1. Chemical Structures of CR (a) and ICG (b) with Proton Labeling



2. EXPERIMENTAL SECTION

2.1. Materials. Generation 3−5 (G3-G5) ethylenediamine (EDA)-cored and primary amine-terminated PAMAM dendrimer was purchased from Dendritech Inc. (Midland, MI). ICG and deuterated dimethyl sulfoxide (d6-DMSO) were obtained from Sigma-Aldrich Co. (St. Louis, MO). CR was obtained from Sangon Biotech. (Shanghai, China). Deuterium oxide (D_2O) was obtained from Beijing Chongxi High-Tech Incubator Co. Ltd. (Beijing, China). Fully acetylated PAMAM dendrimers (Ac-G3, Ac-G4, and Ac-G5) were synthesized according to a well-established method as described elsewhere.[24] Full acetylation of the PAMAM dendrimer surface was confirmed by an ^{1}H NMR spectrum (Figure S1).[29]

2.2. Sample Preparation. G3-G5 PAMAM dendrimers stored in methanol solution were distilled to remove the solvents. G3-G5, Ac-G3, Ac-G4, and Ac-G5 PAMAM dendrimers were dissolved in D_2O at a concentration of 20 mg/mL as stock solutions. Similarly, ICG and CR in d_6-DMSO were prepared at a concentration of 4 mg/mL as guest stock solutions. For the stability studies, 50 μM ICG aqueous solutions were used and the solutions were added with PAMAM or acetylated PAMAM dendrimers. The molar concentration ratio of ICG and dendrimer in the UV−vis−NIR spectroscopy was kept at a constant of 4:1.

2.3. NMR Studies. ^{1}H NMR and ^{1}H-^{1}H nuclear Overhauser effect spectroscopy (NOESY) of acetylated and cationic PAMAM dendrimer/CR or ICG complexes were recorded on a 500.132 MHz Bruker NMR instrument at 298.2 ± 0.1 K. COSY spectra of dendrimer/CR and dendrimer/ICG complexes were obtained by the standard pulse program at Bruker 500.132 MHz NMR spectrometer, with 1024 × 2048 data points. The relaxation delay was 1 s. Sixteen scans were averaged. A sine-bell squared window function and zero filling were applied to both dimensions. The NOESY spectra were obtained with a mixing time of 300 ms and 8.2 μs 1H 90° pulse width. The relaxation delay and acquisition time were set at 2 s and 205 ms, respectively. Eight transients were averaged for each 2048 × 800 complex t_1 increments. The concentrations of cationic (G3, G4, and G5) and acetylated PAMAM (Ac-G3, Ac-G4, and Ac-G5) dendrimers were 0.14 mM. The molar ratios of CR and dendrimer were 12, 24, and 48, respectively, and the molar ratio of ICG and dendrimer was kept constant at 24. A certain amount of ethanol was added into the NMR tubes as an internal standard. All of the data were processed with NMRpipe software on a Linux workstation with standard Lorentz-Gauss window function and zero-filling in both dimensions.

2.4. UV−vis−NIR Studies. UV−vis−NIR spectra were conducted on a Solidspec DUV-3700 spectrophotometer (Shimadzu, Japan) in the range of 200 to 1100 nm. The absorbance of the ICG solutions in the absence and presence of G4 or Ac-G4 PAMAM dendrimers (ICG/dendrimer molar ratio = 4:1) were measured for a period of 356 h.

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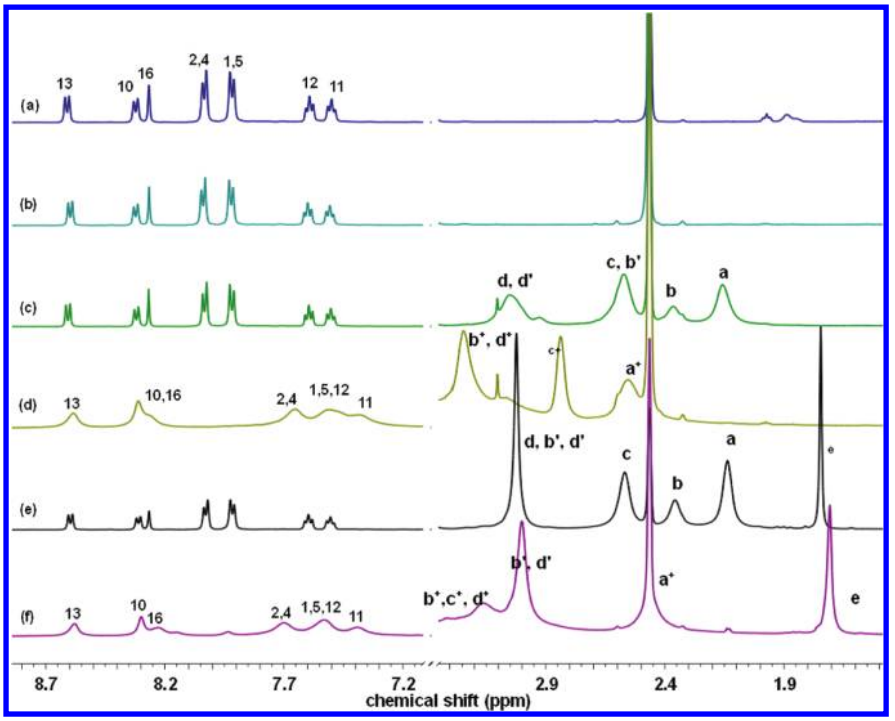

Figure 1. Expanded region of the ^{1}H NMR spectra for (a) CR at neutral pH condition, (b) CR at pH 5.0, (c) G4/CR complex without pH adjustment, (d) G4/CR complex at pH 5.0, (e) Ac-G4/CR complex without pH adjustment, and (f) Ac-G4/CR complex at pH 5.0 in d6-DMSO/D2O (80/20, V/V). The molar ratio of CR and G4 or Ac-G4 dendrimer is 24. The CR concentration is kept at a constant of 3.36 mM.

3. RESULTS AND DISCUSSION

3.1. NMR Studies. CR is an amphiphilic molecule that has a strong tendency to self-aggregate in aqueous solutions (Scheme 1a).[31] CR molecules bear two sulfonate groups with pK_a values around 1.0, and the lowest pH value of the dendrimer/CR complexes is 5.0 in this study. As a result, the two sulfonate groups are negatively charged in the complexes. When G4 PAMAM dendrimer was added into the CR aqueous solution, precipitate was observed in the mixture solution, suggesting the formation of previously mentioned cross-linking supramolecular structures between the cationic G4 dendrimer surface and the two sulfonate groups of CR through ionic interactions. However, the addition of Ac-G4 dendrimer into the CR solution did not cause obvious changes. This result proved that acetylation can prevent the formation of cross-linking structures between cationic dendrimers and CR molecules.

CR molecules dissolved in D_2O showed broad ^{1}H NMR peaks in the range of 6.0−8.5 ppm (Figure S2). The broad resonances of CR peaks are attributed to the formation of CR aggregates in D_2O. It is reported that CR molecules form rod-like or ribbon-like micellar entities in aqueous solution through $\pi-\pi$ stacking and hydrophobic interactions.[34] To make it easier for us to analyze the interactions between dendrimers and CR by NMR techniques, we used a d_6-DMSO/D_2O (80/20, V/V) solvent for the dendrimer/CR system. The protons in CR structure are labeled as 1−16. The structures of G4 and Ac-G4 PAMAM dendrimers with proton labeling are shown in Scheme S1. As shown in Figure 1, the ^{1}H NMR spectrum of CR in d_6-DMSO/D_2O (80/20, V/V) has seven peaks corresponding to the protons on benzidine ($H_{1,5}$, 7.98 ppm, doublet; and $H_{2,4}$, 8.11 ppm, doublet) and the protons on naphthalene rings (H_{10}, 8.42 ppm, doublet; H_{11}, 7.54 ppm, triplet; H_{12}, 7.63 ppm, triplet; H_{13}, 8.71 ppm, doublet; H_{16}, 8.32 ppm, singlet).[35] In the presence of G4 or Ac-G4 PAMAM dendrimers, the ^{1}H NMR spectrum of G4/CR or Ac-G4/CR are scarcely changed compared to that of free CR molecules, suggesting that only weak interactions occur between G4 or Ac-G4 dendrimers and CR molecules. However, the peaks for CR significantly broaden when the mixture solutions of G4 or Ac-G4 and CR are adjusted to pH 5.0. Since the ^{1}H NMR spectra of CR are similar before and after pH adjustment, the spectra change should be attributed to the formation of large and stable dendrimer/CR complexes with inherent broader lines and/or the existence of chemical exchanges between free- and bound-state of CR molecules in the dendrimer/CR complexes.[36] To help with the chemical shift assignment of CR protons in the Ac-G4/CR and G4/CR complexes, ^{1}H-^{1}H COSY spectra of the complexes were conducted (Figure S3) and the assignments of broad CR peaks are shown in Figure 1. The broadening of the ^{1}H NMR signals in Figure 1 can be recognized as the evidence of interactions between G4 or Ac-G4 dendrimers and CR molecules at acidic conditions. PAMAM dendrimers are pH-sensitive because of the surface primary amine groups (pK_a ~10.5) and/or the interior tertiary amine groups (pK_a ~6.5).[37] The charge property of G4 or Ac-G4 dendrimer can be modulated by varying pH values of the dendrimer solution. At pH 5.0, most of the tertiary amine groups are protonated, and the interior pockets of G4 or Ac-G4 dendrimer are positively charged and have strong tendency to encapsulate CR molecules bearing two negative charges through ionic interactions.[29]

To further characterize the complexes of G4 and Ac-G4 PAMAM dendrimers with CR, NOESY was used to predict the inclusion structures. NOE is a manifestation of cross-relaxation between two nuclear spins which are close to each other in space.[19] Protons within a spatial distance of 5 Å give rise to NOE cross-peaks in ^{1}H-^{1}H NOESY spectrum and the intensity of the cross-peak decreases with distance.[38,39] Relative distances between drug molecules and dendrimer scaffolds and

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orientations of drugs within the dendrimer pockets can be analyzed through the cross-peaks in a NOESY spectrum.[19] G4 PAMAM/CR in D_2O failed to give a NOESY spectrum due to the formation of precipitates between G4 and CR in aqueous solution. However, NOESY spectrum of Ac-G4 and CR in D_2O in Figure S4 clearly shows NOE cross-peaks between CR protons and dendrimer scaffolds, suggesting the encapsulation of partial CR molecules within Ac-G4 dendrimer pockets.[20,36,40] Since the surface of Ac-G4 dendrimer is neutral and the interior tertiary amine groups are not protonated at this pH condition (pH ~9.0), the formation of inclusion structure is mainly due to the hydrophobic interactions between the lipophilic polyaromatic moiety of CR molecules and the relatively nonpolar dendrimer pockets.

The ^{1}H-^{1}H NOESY spectra of Ac-G4/CR and G4/CR in d_6-DMSO/D_2O (80/20, V/V) are shown in Figure 2, panels a and b, respectively. No NOE cross-peaks between G4 or Ac-G4 dendrimer and CR molecules were observed in Figure 2. Previous NOE NMR studies have revealed that G3-G6 PAMAM dendrimers can encapsulate amphiphilic molecules such as sodium dodecyl sulfate (SDS) and sodium deoxycholate,[40] and hydrophobic drugs such as phenylbutazone and mycophenolic acid in aqueous solutions.[19] Here, the strong polarity of solvent (d_6-DMSO/D_2O) is responsible for the absence of NOE peaks between G4 or Ac-G4 and CR in Figure 2. The interior pockets of G4 or Ac-G4 dendrimers are filled with d_6-DMSO and D_2O molecules before the addition of CR molecules. The inclusion of CR within the dendrimer pockets should first require breaking of the strong hydrogen-bond interactions between d_6-DMSO/D_2O and the dendrimer scaffolds (−CONH−) and removing these molecules out of the dendrimer pockets. Also, the solvent of d_6-DMSO/D_2O can well-disperse the CR molecules; thus, no NOE cross-peaks were observed between CR molecules and dendrimer pockets (Figure 2 and Figure S5).

To improve the formation of inclusion complexes between Ac-G4 PAMAM dendrimer and CR molecules, the pH value of the Ac-G4/CR in d6-DMSO/D_2O was adjusted to 5.0. At mild acidic conditions, the interior tertiary amine groups are partially or fully quaternized, thus the interior pockets of Ac-G4 dendrimer are positively charged and can be encapsulated with CR molecules through ionic interactions. As shown in Figure 3,

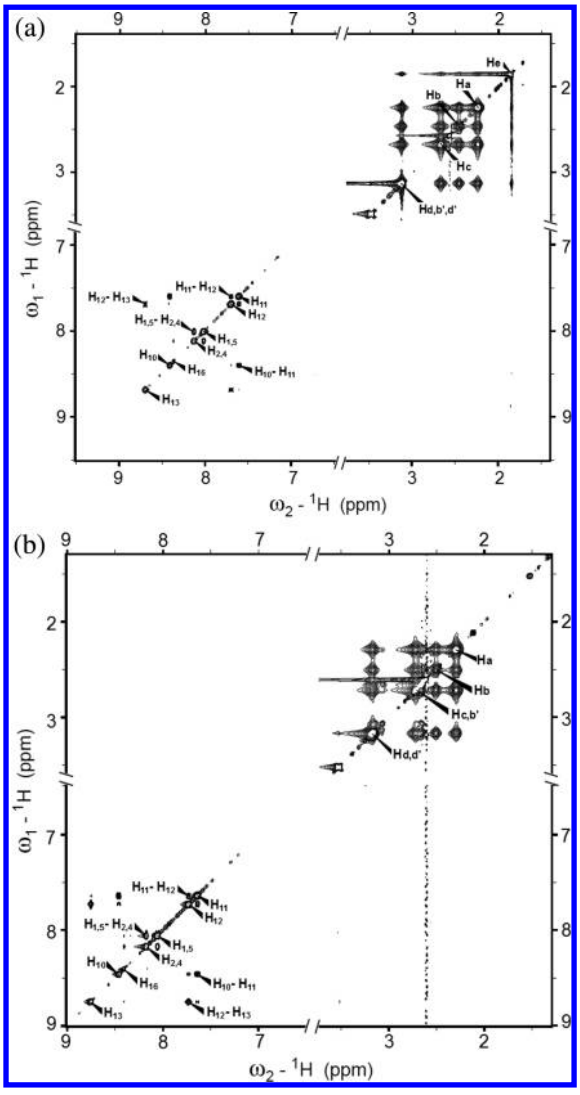

Figure 2. ^{1}H-^{1}H NOESY spectra of Ac-G4/CR (a) and G4/CR (b) complexes in d_6-DMSO/D_2O (80/20, V/V) without pH adjustment at a mixing time of 300 ms. The molar ratio of CR and G4 or Ac-G4 dendrimer is 24. The dendrimer concentration is kept at a constant of 0.14 mM.

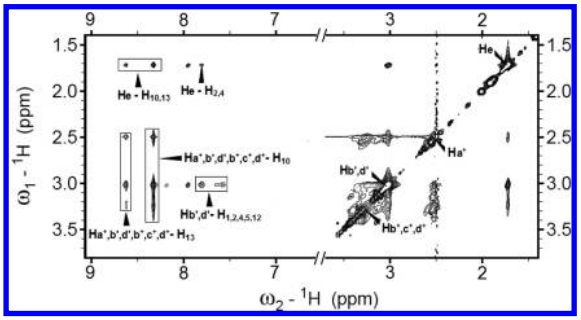

Figure 3. ^{1}H-^{1}H NOESY spectrum of Ac-G4/CR complex in d_6-DMSO/D_2O (80/20, V/V) at pH 5.0 and a mixing time of 300 ms. The molar ratio of CR and Ac-G4 dendrimer is 24. The dendrimer concentration is kept at a constant of 0.14 mM.

strong NOE cross-peaks between nearly all the protons ($H_{1,2,4,5,10,12,13}$) of CR and protons of G4 dendrimer scaffold, suggesting CR molecules are encapsulated within the Ac-G4 pockets and these encapsulated CR are in close proximity with the dendrimer scaffold.[19] No obvious change in the NOESY spectrum of Ac-G4/CR complex solution was observed during a period of two months. These results confirmed that Ac-G4 PAMAM dendrimer forms stable inclusions with CR molecules at pH 5.0. Besides ionic interactions, hydrophobic interactions between the aliphatic scaffold of G4 PAMAM and the aromatic region of CR molecules and hydrogen-bond interactions between the sulfate groups of CR and the amide groups of G4 PAMAM may together contribute to the formation of Ac-G4/CR inclusion complex.[36] It is worth noticing that strong NOE interactions are also observed between nearly all the CR protons and the acetyl protons located on the surface of Ac-G4 dendrimer. This phenomenon is much different from the NOESY spectra of previously reported PAMAM dendrimer/drug complexes, in which few drug molecules are located in the outmost layer of dendrimer pockets.[29,36] This result indicates that CR molecules with a molecular weight of 697 Da cannot be fully encapsulated within the cavities due to the space limit of Ac-G4 dendrimer interior.

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In the case of G4 PAMAM/CR at pH 5.0, the NOESY spectrum in Figure 4 shows weak NOE interactions between

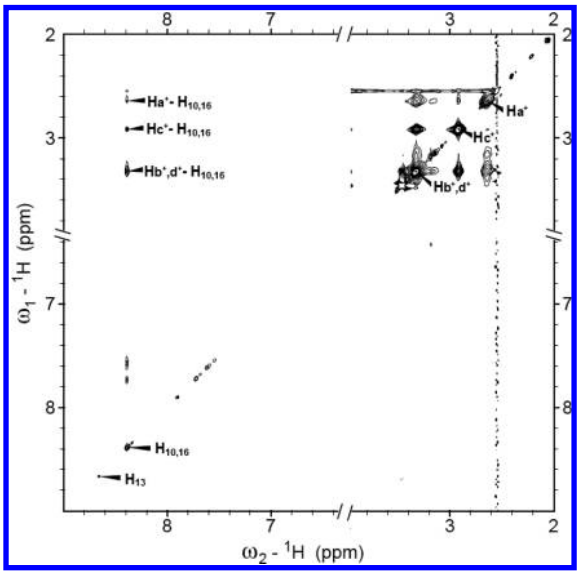

Figure 4. ^{1}H-^{1}H NOESY spectrum of G4/CR complex in d_6-DMSO/D_2O (80/20, V/V) at pH 5.0 and a mixing time of 300 ms. The molar ratio of CR and G4 dendrimer is 24. The dendrimer concentration is kept at a constant of 0.14 mM.

G4 dendrimer and CR molecules. Only protons ($H_{10,16}$) of CR are associated with the dendrimer scaffold, and the intensities of these cross-peaks are much weaker than those observed in Figure 3. Also, the intermolecular NOE interactions between CR molecules are weak, suggesting few CR molecules are localized in the G4 pockets. Though the interior pockets of G4 dendrimer are positively charged as that of Ac-G4 dendrimer at pH 5.0, the cationic surface of G4 PAMAM has a strong tendency to interact with CR molecules through ionic interactions. Therefore, G4 PAMAM dendrimer forms inclusion structures with only a small part of the CR molecules in the solution.

To investigate the effect of dendrimer generation and guest/dendrimer molar ratio on the host behavior of acetylated PAMAM dendrimers, we conducted the NOESY spectra of Ac-G3/CR and Ac-G5/CR complexes. As shown in Figure 5, Ac-G5/CR complex shows stronger NOE cross-peaks than Ac-G3/CR and Ac-G4/CR complexes, suggesting that higher generation acetylated dendrimers have strong host ability toward CR. This result is in accordance with our previous results on dendrimer/SDS inclusion structures.[21b] Figure S6 shows the NOESY spectra of Ac-G4/CR complexes at CR/dendrimer molar ratios of 12, 24, and 48, respectively. Surprisingly, Ac-G4/CR complex at CR/dendrimer molar ratio of 12 shows much stronger NOE cross-peaks than that at molar ratio of 24 and 48. This is probably due to the fact that CR molecules have a higher tendency to form inclusion complexes with acetylated PAMAM dendrimer at higher dendrimer concentrations.

To confirm the conclusions obtained from acetylated PAMAM/CR complexes, ICG was used as another model drug bearing multiple negative charges. The COSY spectrum of Ac-G4/ICG complex is shown in Figure 6. G4 PAMAM

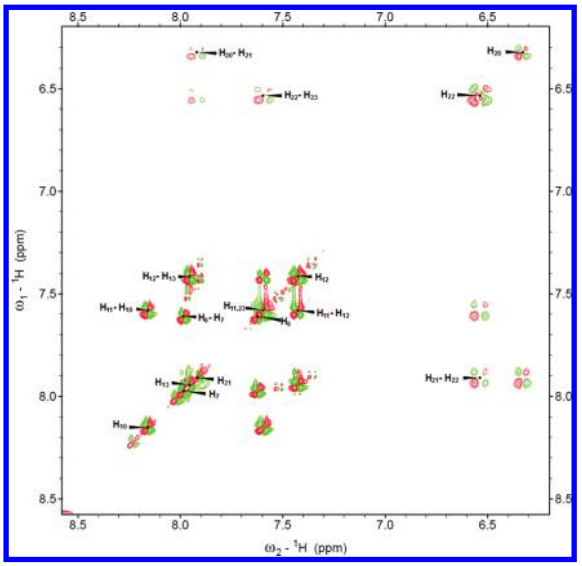

Figure 6. ^{1}H-^{1}H COSY spectrum of Ac-G4/ICG in d_6-DMSO/D_2O (80/20, V/V) at pH 5.0.

dendrimer forms cross-linking supramolecular structure with ICG and the resulting G4/ICG complexes are precipitates in aqueous solutions. However, Ac-G4/ICG solutions are clear at pH 5.0 and pH 7.4 (Figure S7). As shown in Figure 7, panels a and b, Ac-G4 dendrimer failed to encapsulate large amounts of ICG molecules in d6-DMSO/D_2O (80/20, V/V) at pH ~8.1. However, strong NOE interactions are observed between ICG protons ($H_{6,7,10,11,13,21,23}$) and dendrimer protons and between the ICG protons (left-down part of Figure 7b) at pH 5.0. These results suggested that Ac-G4 PAMAM dendrimers are able to prevent the cross-linking supramolecular structures between

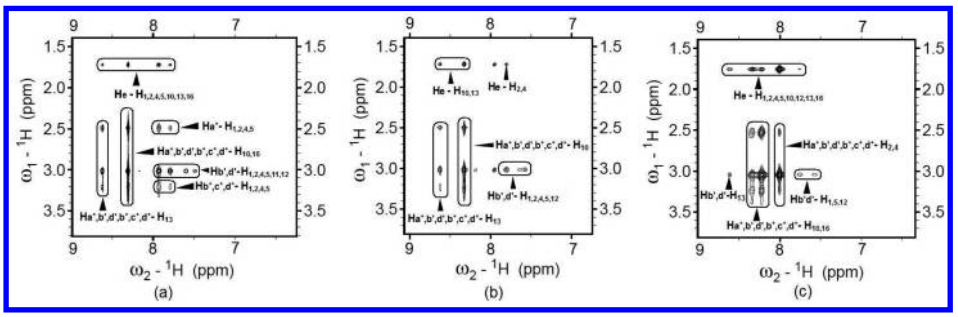

Figure 5. Regional ^{1}H-^{1}H NOESY spectra of (a) Ac-G3/CR, (b) Ac-G4/CR, and (c) Ac-G5/CR complexes in d_6-DMSO/D_2O (80/20, V/V) at pH 5.0 and a mixing time of 300 ms. The molar ratio of CR and acetylated dendrimer is 24. The dendrimer concentration is kept at a constant of 0.14 mM.

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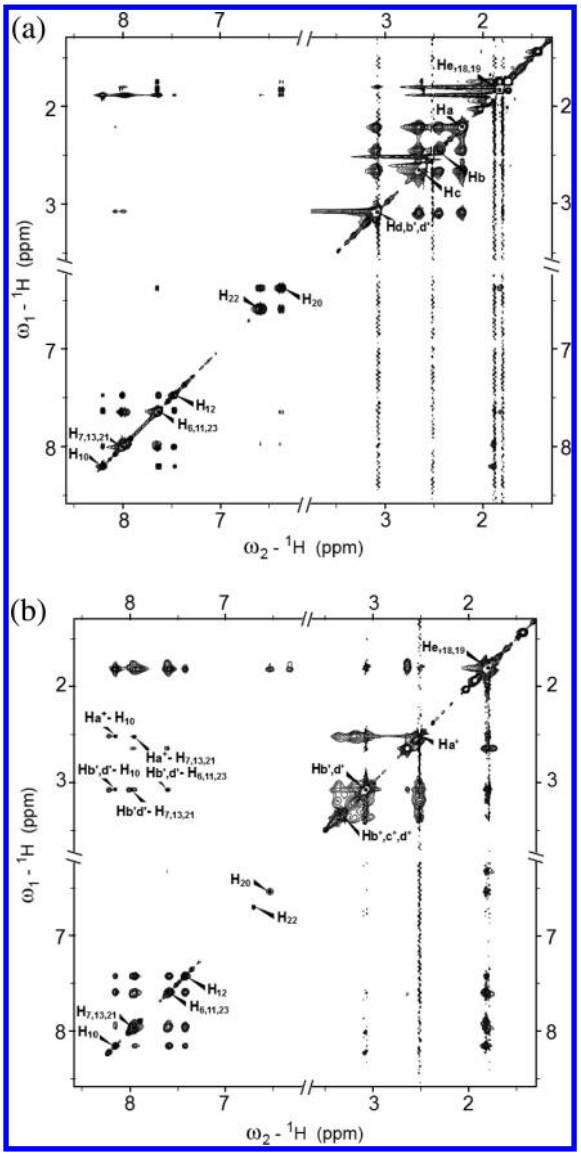

Figure 7. ^{1}H-^{1}H NOESY spectra of Ac-G4/ICG complex in d_6-DMSO/D_2O (80/20, V/V) without pH adjustment (a) and at pH 5.0 (b). The molar ratio of ICG and Ac-G4 dendrimer is 24. The dendrimer concentration is kept at a constant of 0.14 mM.

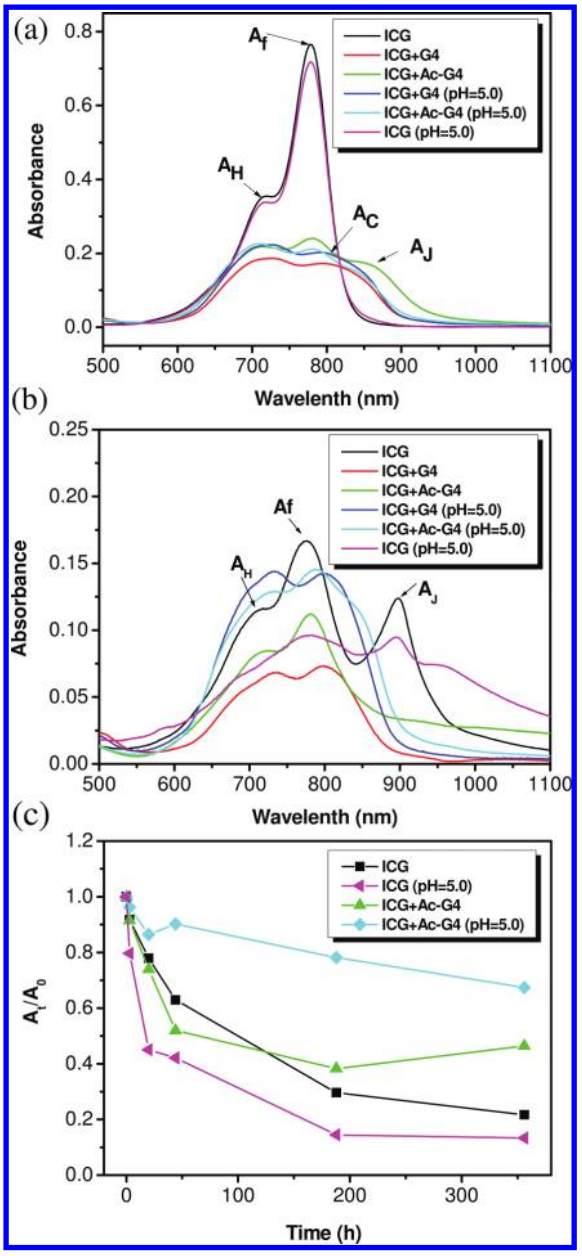

Figure 8. Absorption spectra of ICG solutions in the absence and presence of G4 or Ac-G4 PAMAM dendrimers stored at room temperature for a period of 0 h (a) and 356 h (b). The concentration of ICG is kept at 50 μM and the molar ratio of ICG/dendrimer is 4. The variations of ICG absorbance at 778 nm in the absence and presence of Ac-G4 dendrimer as a function of storage time are shown in (c).

cationic dendrimers and drug molecules bearing multiple charges such as CR and ICG, and to form stable inclusion complexes with these drug molecules.

3.2. UV−vis−NIR Studies. ICG is an amphiphilic molecule which self-assembles into larger aggregates in aqueous solutions.[33,41] Therefore, ICG is instable in water and a progressive loss of color is observed for ICG aqueous solution.[32] The inclusion of CR and ICG within Ac-G4 pockets revealed by NMR techniques provides a new strategy in preventing the self-assembly of this amphiphilic dye. Here, the photostability of ICG molecules in the absence and presence of G4 or Ac-G4 PAMAM dendrimer is investigated by UV−vis-NIR spectroscopy.

As shown in Figure 8a, ICG in aqueous solution at 50 μM shows two peaks: monomeric form (A_f) of ICG at 778 nm and H-aggregate form (dimers and oligomers, A_H) of ICG at 718 nm.[32] In the presence of G4 PAMAM dendrimer at pH 8.9 and pH 5.0, the absorbance peak of ICG at 778 nm significantly decreased and obvious broadening and red-shift (to A_C) of the ICG peaks were observed in Figure 8a. Also, the appearance of an isosbestic point is observed. The broadening and red-shift of ICG absorbance peaks are attributed to the formation of G4/ICG complexes through ionic interactions as revealed by NMR techniques. Compared to the spectra of G4/ICG solution, the absorbance spectra of Ac-G4/ICG solution show triplet bands which is characterized as A_H, A_f, and A_J.[32] The adjustment of pH value of the Ac-G4/ICG solution to mild acidic conditions causes the decrease of this J-aggregate absorbance band, suggesting that the presence of Ac-G4 PAMAM dendrimer will not promote the formation of ICG J-aggregates in aqueous solutions. The decrease of J-aggregation absorbance band at mild acidic condition is attributed to the fact that ICG

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molecules are partially encapsulated within the interior cavities of Ac-G4 dendrimer. This pH-dependent encapsulation behavior has already been proved by NMR studies in section 3.1. Though the appearance of J-aggregate absorbance band for ICG is observed in the presence of Ac-G4, the absorbance band for J-aggregate is not observed after 356 h (Figure 8b). In contrast, strong J-aggregate absorbance band at 898 nm appeared in the absorbance spectra of free ICG aqueous solutions after their storage for 356 h. Also, a significant decrease of ICG A_f absorbance band is observed, suggesting that more and more ICG molecules self-assemble into J-aggregates.[32,41]

To compare the photostability of ICG molecules in the absence and presence of Ac-G4 PAMAM dendrimer at different pH conditions, the absorbance of ICG and Ac-G4/ICG solutions at 778 nm during the storage period of 356 h were shown in Figure 8c. It is clearly observed that the absorbance of ICG molecules in the absence of Ac-G4 decreases rapidly both at mild acidic and basic conditions. However, Ac-G4 PAMAM dendrimer improved the photostability of ICG molecules in aqueous solutions at both pH conditions. The prevention of ICG self-assembly is especially effective at mild acidic conditions due to the formation of stable inclusion complexes between quaternized Ac-G4 and ICG molecules, which is in accordance with NMR results in section 3.1. Based on the UV–vis–NIR studies, ICG molecules incorporated into Ac-G4 nanoparticles can significantly improve their half-life in aqueous solutions compared to free ICG molecules, which is essential for the diagnostic applications of ICG in clinical trials.[32,33]

4. CONCLUSIONS

The present study used fully acetylated PAMAM dendrimer to solve the precipitation problems of cationic PAMAM dendrimer and drugs bearing multiple negative charges. Acetylated PAMAM dendrimer can effectively prevent the formation of cross-linking supramolecular structures between cationic PAMAM and CR or ICG molecules. NMR studies confirmed that acetylated dendrimers form stable inclusion complexes with CR and ICG molecules at mild acid conditions, while cationic PAMAM dendrimers only encapsulate a small fraction of drug molecules at the same condition. Also, acetylated dendrimers can significantly improve the photostability of ICG molecules in aqueous solution and effectively reduce the formation of ICG J-aggregates, which are not beneficial for the diagnostic applications of ICG in clinical trials. As demonstrated by previous studies, acetylated PAMAM dendrimers showed much reduced cytotoxicity on several cell lines and maintained the penetration ability of cationic dendrimers across cell membranes.[29,30] These biocompatible dendrimers are promising nanovehicles in the delivery of therapeutic agents bearing multiple charges.[19]

■ ASSOCIATED CONTENT

Supporting Information

Further information on ^{1}H NMR, COSY, and ^{1}H-^{1}H NOESY spectra of the complexes of cationic or acetylated PAMAM dendrimers with CR or ICG molecules. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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