Self-Assembly of Vesicles by a 2,6-Di(7-benzamidy)quinolin-2-yl)pyridine Derivative Tuned by an Amphiphilic Amide Chain†

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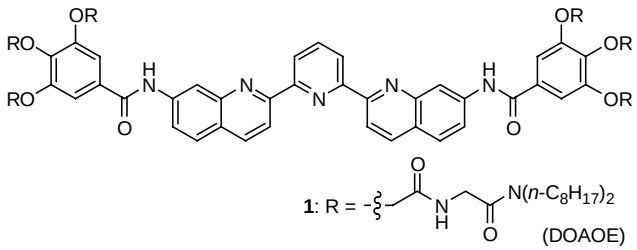
This paper describes 2-(2-(dioctylamino)-2-oxoethyl-amino)-2-oxoethoxyl (DOAOE)-tuned self-assembly of vesicles from a 2,6-di(7-benzamidy)-quinolin-2-yl)pyridine derivative. Scanning electron (SEM), transmission electron (TEM) and optical microscopy and dynamic light scattering (DLS) studies reveal that the molecule self-assembles into vesicular structures in methanol. The influence of the transition metal ions and trifluoroacetic acid on the formation of the vesicles was investigated. The results illustrate that DOAOE is robust in promoting the formation of vesicles for aromatic systems in polar medium.

Keywords vesicle, self-assembly, aromatic heterocycle, amphiphile, amide chain

Introduction

The self-assembly of aromatic systems into vesicular architectures is controlled by the backbones as well as their appended flexible chains.[1-5] We recently reported that hydrogen bonding-induced aromatic hydrazide foldamers form vesicles in methanol or its aqueous solution,[6-8] which are driven by the stacking of the rigid backbone and the hydrogen bonding and van der Waals interactions of the appended aliphatic amide chain, *i.e.*, 2-(2-(dioctylamino)-2-oxoethyl-amino)-2-oxoethoxyl unit (DOAOE).[9,10] Control experiments showed that this new amphiphilic chain is more robust than the conventionally used hydrophilic oligoglycol groups in inducing the formation of vesicles in polar media. To further test if DOAOE is efficient for other kinds of aromatic compounds, we have synthesized aromatic heterocycle **1**, which bears six DOAOE groups. We envisioned that the aromatic backbone would adopt an extended conformation due to the electrostatic repulsion of the neighboring pyrine/quinoline nitrogen atoms and, upon complexing a transition metal ion, a V-shaped folded conformation.[11,12] The stacking tendency of the two different conformations should be very different, which may be utilized to evaluate the capacity of DOAOE in inducing the formation of vesicles. The detailed results are reported in this paper.



Experimental

General methods

All reactions were carried out under a dry nitrogen atmosphere. Melting points were uncorrected. All solvents were dried before use following the standard procedures. Unless otherwise indicated, all starting materials were obtained from commercial suppliers and were used without further purification. Analytical thin-layer chromatography (TLC) was performed on 0.2 mm silica 60 coated on glass plates with F_{254} indicator. The ^{1}H NMR spectra were recorded on 300 or 400 MHz spectrometers in the indicated solvents. Chemical shifts (δ) were referenced to the residual solvent signal (^{1}H: chloroform: δ 7.26; ^{13}C: $CDCl_3$: δ 77.23). Elemental analysis was carried out at the SIOC analytical center.

Compound 3: To a stirred solution of compound **2**[13] (3.0 g, 15.3 mmol) in ethanol (120 mL) and water (30 mL) were added iron powder (17.1 g, 0.31 mol) and ammonium chloride (3.30 g, 62.3 mmol). The suspension was stirred under reflux for 2 h and the solid filtrated off. The filtrate was concentrated with a rotary evaporator and the resulting slurry was suspended in water (100 mL). The suspension was extracted with ethyl acetate (80 mL×4). The organic phases were combined and washed with water (100 mL×2) and brine (100 mL), and dried over sodium sulfate. Upon

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removal of the solvent, the resulting residue was subjected to column chromatography (petroleum ether/AcOEt/NEt_3, $V:V:V=100:100:1$) to give compound **3**[13] as a yellow solid (1.80 g, 87%). ^{1}H NMR (CD_3COCD_3, 300 MHz) δ: 9.47 (s, 1H), 7.16 (d, $J=8.4$ Hz, 1H), 6.68 (br, 1H), 6.05 (dd, $J_1=8.4$ Hz $J_2=2.1$ Hz 1H), 5.91 (d, $J=2.1$ Hz, 1H), 5.43 (br, 1H); MS (ESI) *m*/*z*: 136.9 ($[M]^+$).

Compound 5: To a stirred solution of compounds **3** (0.50 g, 3.68 mmol) and **4**[14] (0.25 g, 1.54 mmol) in ethanol (35 mL) was added a saturated solution of potassium hydroxide in ethanol (3 mL). The solution was refluxed for 24 h and cooled to room temperature and then diluted with water (300 mL). The formed precipitate was filtrated and washed with water and dried *in vacuo* to give compound **5** as a yellow solid (0.42 g, 76%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 8.68 (d, $J=7.5$ Hz, 2H), 8.58 (d, $J=8.4$ Hz, 2H), 8.16 (d, $J=8.4$ Hz, 2H), 8.02 (t, $J=7.5$ Hz, 1H), 7.68 (d, $J=8.7$ Hz, 2H), 7.32 (s, 2H), 7.01 (dd, $J_1=8.7$ Hz, $J_2=1.2$ Hz, 2H), 4.08 (br, 4H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 156.6, 155.8, 149.7, 147.7, 137.7, 136.3, 128.7, 122.4, 121.7, 118.8, 115.9, 109.8; MS (ESI) *m*/*z*: 364.1 ($[M+H]^+$); HRMS (ESI) calcd for $C_{23}H_{18}N_5$ ($[M+H]^+$) 364.1557, found 364.1562.

Compound 8: A suspension of compounds **6**[15] (0.40 g, 2.17 mmol), **7**[9] (2.50 g, 6.68 mmol), potassium iodide (0.50 g, 3.01 mmol) and potassium carbonate (3.30 g, 23.9 mmol) in acetone (25 mL) was stirred at 60 ℃ for 12 h and then concentrated with a rotary evaporator. The resulting slurry was triturated with water (20 mL) and dichloromethane (50 mL). The organic phase was separated and washed with water (20 mL) and brine (20 mL), and dried over sodium sulfate. Upon removal of the solvent, the resulting residue was subjected to column chromatography (CH_2Cl_2/acetone, $V:V=6:1$) to give compound **8** as a colorless oil (1.70 g, 75%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 8.41 (s, 1H), 7.69 (s, 2H), 7.33 (s, 2H), 4.82 (s, 2H), 4.69 (s, 4H), 4.18 (d, $J=3.6$ Hz, 2H), 4.09 (d, $J=4.2$ Hz, 4H), 3.88 (s, 3H), 3.29 (t, $J=7.5$ Hz, 6H), 3.16 (t, $J=7.2$ Hz, 6H), 1.54—1.49 (m, 12H), 1.29—1.27 (m, 36H), 0.89—0.87 (m, 18H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 169.0, 167.6, 167.2, 166.6, 165.7, 150.5, 141.7, 126.0, 109.4, 72.7, 68.6, 52.4, 47.0, 46.9, 46.1, 41.0, 40.8, 31.5, 31.5, 31.5, 28.7, 27.5, 26.6, 26.5, 26.5, 22.5, 22.5, 14.0, 13.9; MS (ESI) *m*/*z*: 1053.9 ($[M+Na]^+$); HRMS (ESI) calcd for $C_{56}H_{98}N_6O_{11}Na$ ($[M+Na]^+$) 1053.7186, found 1053.7221.

Compound 9: A solution of compound **8** (0.94 g, 0.91 mmol) and lithium hydroxide monohydrate (0.10 g, 2.38 mmol) in methanol (3 mL) and water (2 mL) was stirred at room temperature for 7 h and then acidified with diluted hydrochloric acid to pH=2. The mixture was concentrated with a rotavapor and the resulting slurry triturated with dichloromethane (50 mL). The organic phase was washed with water (25 mL×2) and brine (25 mL), and dried over sodium sulfate. The solvent was then removed and the obtained residue subjected to flash chromatography (CH_2Cl_2/MeOH, $V:V=30:1$) to give compound **9** as a sticky solid (0.67 g, 72%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 8.40 (s, 1H), 7.85 (s, 2H), 7.30 (s, 2H), 4.82 (s, 2H), 4.66 (s, 4H), 4.21 (s, 2H), 4.14 (s, 4H), 3.31 (t, $J=7.5$ Hz, 6H), 3.18 (t, $J=6.9$ Hz, 6H), 1.57—1.49 (m, 12H), 1.30—1.28 (m, 36H), 0.89—0.87 (m, 18H); ^{13}CNMR ($CDCl_3$, 125 MHz) δ: 169.3, 167.9, 167.6, 167.3, 167.2, 150.2, 141.4, 126.2, 109.5, 72.5, 68.3, 47.1, 47.1, 46.3, 40.9, 40.8, 31.5, 31.5, 31.5, 28.7, 28.7, 27.6, 27.5, 26.7, 26.6, 26.5, 22.6, 22.5, 14.0, 14.0; MS (ESI) *m*/*z*: 1040.0 ($[M+Na]^+$); HRMS (ESI) calcd for $C_{55}H_{97}N_6O_{11}$ ($[M+H]^+$) 1017.7210, found 1017.7209.

Compound 1: To a solution of compounds **5** (0.02 g, 0.076 mmol), **9** (0.14 g, 0.14 mmol) and lithium chloride (0.05 g, 1.19 mmol) in *N*-methyl pyrrolidone (NMP, 1 mL) and pyridine (0.25 mL) was added triphenyl phosphate (0.13 mL, 0.42 mmol). The mixture was stirred at 90 ℃ for 20 h and diluted with water (20 mL) and acidified with diluted hydrochloric acid (pH=2). The solvent was removed with a rotavapor. The obtained slurry was triturated with water (5 mL) and the mixture was extracted with ethyl acetate (25 mL×2). The organic phases were combined and washed with water (25 mL×2) and brine (25 mL), and dried over sodium sulfate. Upon removal of the solvent with a rotavapor, the resulting residue was subjected to column chromatography (CH_2Cl_2/MeOH, $V:V=20:1$) to give compound **1** as a yellow solid (50 mg, 35%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 9.89 (s, 2H), 8.76—8.72 (m, 4H), 8.53 (s, 2H), 8.45 (s, 2H), 8.24 (d, $J=8.4$ Hz, 2H), 8.04—8.01 (m, 3H), 7.81 (d, $J=8.7$ Hz, 2H), 7.53—7.51 (m, 8H), 4.83—4.77 (m, 12H), 4.18—4.15 (m, 12H), 3.33—3.17 (m, 24H), 1.57—1.36 (m, 24H), 1.29—1.06 (m, 72H), 0.88—0.71 (m, 36H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 168.8, 167.8, 166.6, 166.5, 164.4, 155.8, 155.0, 150.1, 147.9, 140.1, 139.1, 137.2, 135.5, 130.6, 127.2, 125.1, 121.9, 121.4, 119.5, 117.5, 108.2, 72.3, 68.6, 46.6, 45.9, 45.7, 40.5, 40.2, 31.1, 31.0, 31.0, 28.3, 28.2, 27.1, 27.0, 26.2, 26.1, 26.1, 22.1, 22.0, 22.0, 13.5, 13.5, 13.4; MS (MALDI-TOF) *m*/*z*: 2361.6 ($[M+Na]^+$); HRMS (MALDI-TOF) calcd for $C_{133}H_{206}N_{17}O_{20}$ ($[M+H]^+$) 2361.5620, found 2361.5653.

Results and discussion

The synthesis of compound **1** is shown in Scheme 1. Thus, compound **2** was first reduced with iron powder and ammonium chloride in aqueous ethanol to diamine **3**, which was then coupled with **4** in the presence of potassium hydroxide in refluxing ethanol affording diamine **5** in 76% yield. Then, compound **8** was produced in 75% yield from the reaction of **6** and an excess amount of **7** in hot acetone and further hydrolyzed with lithium hydroxide to afford acid **9** in 72% yield. The acid was finally coupled with **5** to produce **1** in 35% yield. Compound **1** was characterized by ^{1}H and ^{13}C NMR and (high resolution) mass spectrometer.

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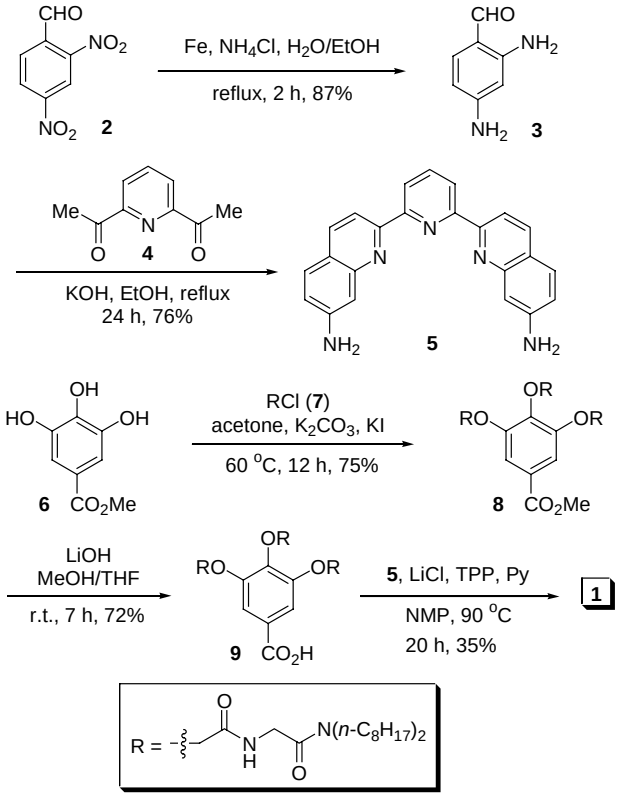
Scheme 1 Synthesis of compound **1**



SEM and optical microscopic images showed that compound **1** formed vesicles in methanol (Figures 1a and 1b). The size of the vesicles ranged from hundreds of nanometers to several micrometers. Dynamic light scattering (DLS) experiment in methanol revealed that the mean hydrodynamic diameter of the vesicles was 531 nm at 0.5 mmol•L^{-1} (Figure 2a).[16] The TEM image of the vesicles evidenced their hollow feature, because the global structures exhibited discernible rings around the periphery, which should be produced through the evaporation of the entrapped solvent on the surface (Figure 1c).[9] These results indicate that the six DOAOE groups in **1** were efficient in promoting the stacking of its central aromatic backbone to form ordered membranes.

The aromatic heterocyclic backbone is a typical tridentate liagnd for transition metal ions.[17,18] Therefore, the influence of transition metal ions, including Cu^{2+}, Fe^{2+}, Zn^{2+}, and Pt^{2+}, on the formation of vesicles was investigated. The metal ions were expected to impede the formation of vesicles because their complexes with **1** should exhibit a smaller stacking tendency due to the electrostatic repulsion of the complexes. Both SEM and TEM images showed that, even in the presence of 1 equiv. of $Cu(NO_3)_2$, $FeCl_2$, $Pt(COD)Cl_2$, $ZnOTf_2$, vesicular structures were still observed, although the sizes of the vesicles were generally smaller than those formed by the pure sample of **1**. As examples, the SEM and TEM images in the presence of cupric nitrate are provided in Figure 3. The formation of vesicles was

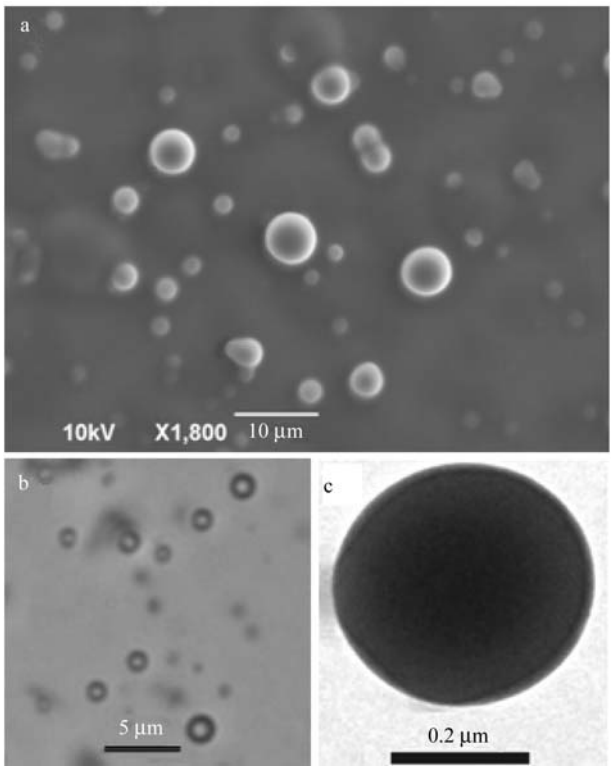


Figure 1 (a) SEM image (0.5 mmol•L^{-1}), (b) optical microscopic image (0.5 mmol•L^{-1}), and (c) TEM image (0.2 mmol•L^{-1}) of the samples of **1**, obtained by evaporating the solution of methanol.

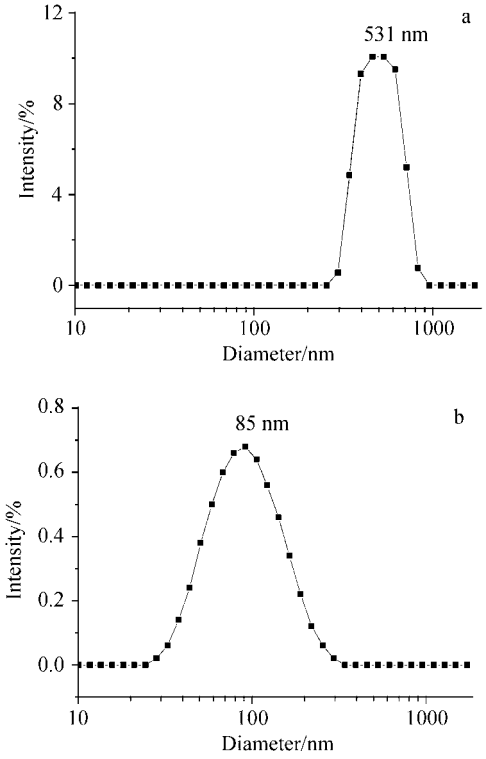


Figure 2 The intensity-weighted distribution of the vesicles obtained from the DLS measurements of **1** from methanol at 0.5 mmol•L^{-1} in the absence (a) and in the presence (b) of 1 equiv. of cupric nitrate.

further confirmed by the DLS study (Figure 2b). The decrease of the size suggested that the aromatic stacking was weakened and the stacking ordering was reduced. As a result, the curving of the membranes became easier to lead to the formation of smaller vesicles. The size of vesicles was recovered after 1 equiv. of ethylenediaminetetraacetic acid (EDTA) (Figure 3c), suggesting that the reduction of size was caused by the complexation of the tridentate of **1** toward the ions.

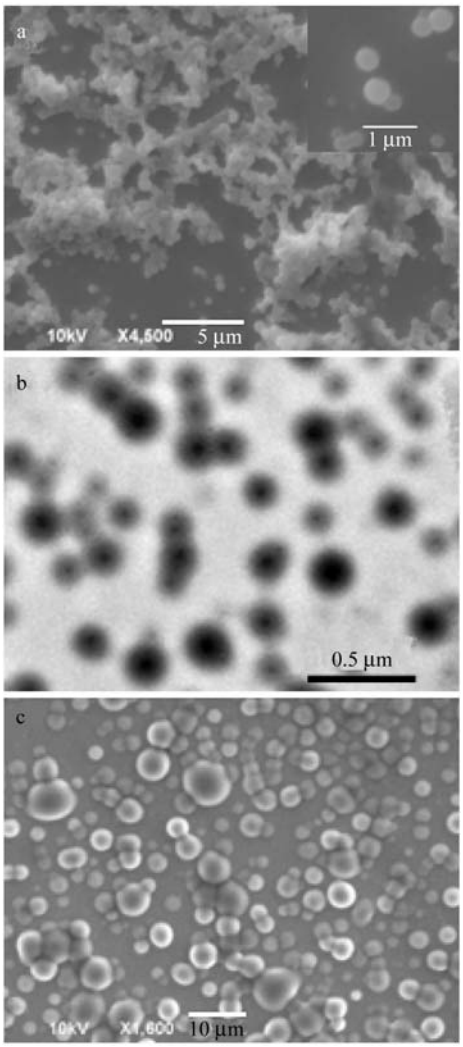


Figure 3 (a) SEM (0.5 mmol•L^{-1}) and (b) TEM (0.2 mmol•L^{-1}) images of the samples of **1** in methanol on mica surface upon evaporation of the solvent in the presence of 1 equiv. of cupric nitrate; (c) SEM image of the first sample with the addition of 1 equiv. of EDTA.

SEM images also showed that, in the presence of 1 equiv. of trifluoroacetic acid (TFA), vesicular structures could also be observed. It was expected that protonation of the pyridine/quinoline units also decreased the stacking of the aromatic backbone due to the electrostatic repulsion. This result thus again demonstrated the robust capacity of DOAOE in promoting the formation of vesicles. Adding 6 equiv. of TFA completely inhibited the formation of vesicles. It was expected that, under this condition, all the pyridine/quinoline units were protonated and the stacking became very weak or even impossible. However, the vesicles could be formed again when the acid was neutralized by sodium hydroxide. Previously, we have established that the cooperative interactions of the stacking of the central aromatic units, the intermolecular hydrogen bonding of the amide units of the side chain and the hydrophobic interaction of the appended octyl chains induce the formation of vesicles for other molecules.[9,10] The formation of the vesicles by compound **1** should follow the similar mechanism. That is, the one-layer stacking of the molecule forms column-styled architectures, which further aggregate to generate one-layered membrane.

The maximum absorbance wavelength of compound **1** in chloroform in the UV-Vis spectrum appeared at 274 nm (10 μmol•L^{-1}). In methanol, this absorbance peak became weaker and was shifted to 279 nm, indicating that its aromatic backbone underwent J-aggregation in polar methanol (Figure 4).[19] Keeping the same concentration, adding 1 equiv. of cupric nitrate caused the solution to turn to yellow. The maximum absorbance was weakened and shifted to 284 nm. In addition, a new peak at about 393 nm was produced as a result of the formation of the complex. Adding more amount of cupric nitrate did not significantly cause the peak to increase, implying that the conversion was completed. This result also indicated that the vesicles observed in Figure 3 were formed exclusively by the metal complex, because the concentration used for their formation was remarkably higher.

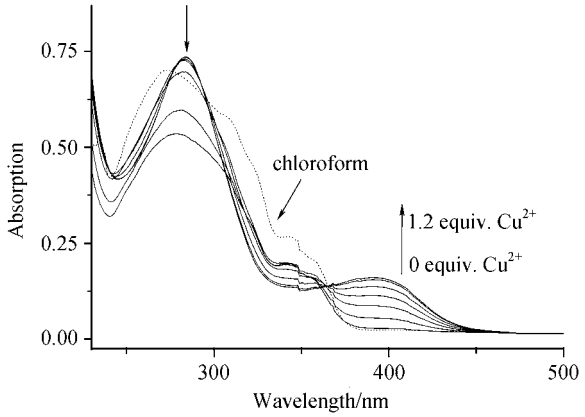


Figure 4 UV-Vis spectra of **1** (10 μmol•L^{-1}) in chloroform (dotted) and methanol in the presence of varying amount of cupric nitrate (0—1.2 equiv.) at 25 ℃.

The X-ray diffraction (XRD) diagram of compound **1** (Figure 5a), obtained by evaporating its solution in methanol (0.5 mmol•L^{-1}), exhibited the strongest peak at 1.75 nm, which may be assigned to that of the rigid backbone because molecular modeling showed that the backbone had a length of about 1.80 nm. The peak at 3.45 nm should correspond to the whole molecule, which had a length of about 3.5 nm when adopting an

extended conformation. The weak and broad peak at about 0.40 nm was consistent with the loose stacking of the aromatic backbone.[20] The IR spectrum of the sample exhibited a peak at 3354 cm^{-1} for the amide N—H stretching, indicating that it formed intermolecular hydrogen bonding.[21] In polar methanol, this hydrogen bonding might also be formed if the amide units were screened by the peripheral octyl groups in the stacked structures.[9]

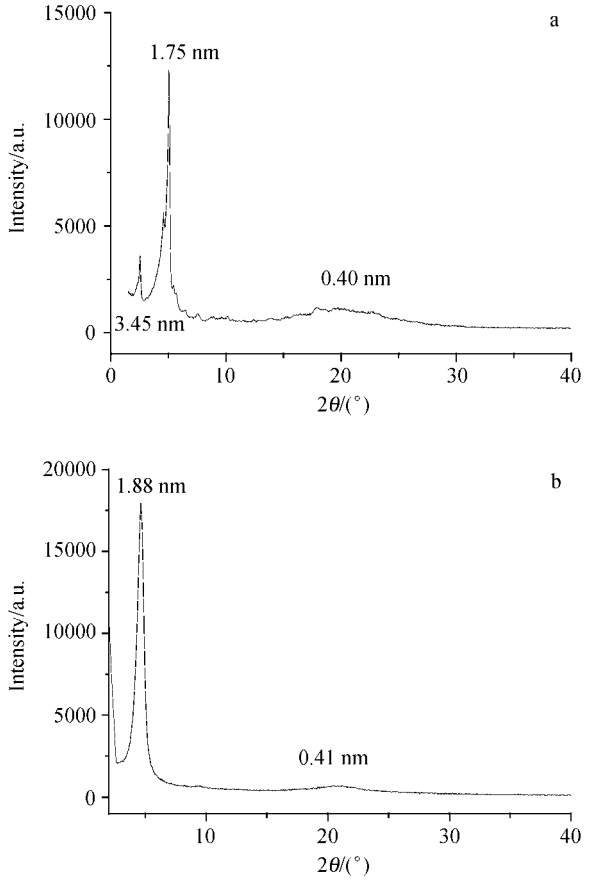


Figure 5 The XRD diagrams of **1**. The samples were prepared by evaporating (a) the solution of methanol (0.5 mmol•L^{-1}) and (b) its organogel with decalin (10 mmol•L^{-1}).

Because several aromatic hydrazide-based foldamers that are appended with DOAOE chains had been revealed to gelate aliphatic hydrocarbons,[9,10,22] we further investigated the self-assembling property of **1** in nonpolar decalin. Compound **1** did gelate decalin and the lowest gelation concentration was estimated to be 10 mmol•L^{-1}. Both SEM and fluorescent microscopy images showed that the xerogel consisted of the cotton-like fibers. The gel could be broken by addition of TFA. Although cupric nitrate is insoluble in decalin, when the 1∶1 mixture of **1** and cupric nitrate (10 mL) was cooled from 100 ℃ to room temperature, the salt was dissolved completely to give a red solution. Clearly, a complex was formed, which had a good solubility in decalin. Interestingly, when 6 equiv. of cupric nitrate was added, the solution could be immobilized, upon cooling from 100 ℃, to form a yellow-green gel, presumably due to the complexation of the metal ion by the amide units in the side chains. SEM image of the xerogel of this mixture showed that short cotton-like fibers were formed.

The XRD diagram of the xerogel of pure **1** exhibited a strong peak at 1.88 nm and a broad peak at 0.41 nm, which corresponded to the aromatic backbone and its loose stacking, respectively (Figure 5b), while the xerogel generated by the mixture did not exhibit similar peaks, indicating that no ordered stacking existed in the mixture. The IR spectra of the two xerogels exhibited peaks at 3348 and 3314 cm^{-1} for their amide stretching, respectively, indicating that they were involved with the hydrogen bonding. The former also displayed two C=O stretching frequencies at 1679 and 1646 cm^{-1}, while the latter displayed only a strong C=O stretching frequency at 1632 cm^{-1}, supporting that both the aromatic and aliphatic amide C=O groups were involved with complexation with Cu^{2+} ion. We proposed that this complexation, together with the intermolecular hydrogen bonding of the amide uinits, induced the formation of the organogel.

Conclusion

As an extension of our previous works, in this paper we demonstrate the robust capacity of DOAOE as an amphiphilic side chain in promoting aromatic structures to form vesicles. The aromatic structures may be linear or two-dimensional. Thus, DOAOE may be developed as a new general amphiphilic side chain for the formation of vesicles from aromatic systems,[23] although more aromatic derivatives have to be prepared for exploiting the scope. Therefore, it opens many opportunities in the future for designing new functionalized vesicular structures by introducing the chain to photo- or electro-active, electron-rich and deficient conjugated systems.

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