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Hyperbranched Polyester Nanorods with Pyrrolo[2,1-a]isoquinoline End-Groups for Fluorescent Recognition of Fe$^{3+}$

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Herein, we reported the synthesis of hyperbranched aromatic-aliphatic co-polyester nanorods HBPE-CICA$_1$ and HBPE-CICA$_2$ by modifying periphery of the second generation hyperbranched polyester (HBPE) with 1-cyano-pyrrolo[2,1-a]isoquinoline-3-carboxylic acid (CICA) groups. Structures of HBPE-CICAs were confirmed by combined studies of fourier transform infrared spectroscopy (FTIR), proton nuclear magnetic resonance ($^1$H NMR), transmission electron microscopy (TEM), atomic force microscopy (AFM) and X-ray diffraction (XRD). The potential application of HBPE-CICAs in ion recognition was investigated, in particular, the HBPE-CICA$_2$, exhibited remarkable selectivity for Fe$^{3+}$. Due to the highly branched structures, multitude of available surface groups and improved solubilities compared to their linear analogues, hyperbranched polymers have attracted extensive interests as an unique class of architectural macromolecules during the past decades.$^{1-4}$ The controlled internal organization of these architectures in the form of fibers, nanotubes, zigzags, and helices has been modified with well-defined and rigid configurations and conformations.$^5$ To date, assemblies of hyperbranched polymers can be engineered to obtain supramolecular assemblies with combined or enhanced properties which remains challenging and has only been addressed in a few studies.$^6-9$

In particular, hyperbranched polyesters (HBPE), one important class of the hyperbranched polymers family, were widely used for academic research$^{10-15}$ and applications in the fields of biology,$^{16}$ medicine,$^{17, 18}$ pharmacy,$^{19, 20}$$^{21}$ and electronics.$^{22}$ Recently, a variety of molecular designs have been proposed for the fabrication of nanostructured HBPE, which would lead to multifunctional macromolecule materials. Santra, Santimukul and coworkers reported the multifunctional hyperbranched polyester-based nanoparticles and nanocomposites with properties ranging from magnetic, fluorescence, antioxidant.$^{10}$ Our group reported novel water-soluble nanoparticles made up of hyperbranched polyester with sulfonic acid end-groups and their anticoagulant effect and cytotoxicity.$^{23}$ Those nanoparticles showed the spherical morphology. However, there are only a handful of reports on the formation of organized nanorod structures from hyperbranched molecules composed of irregular, random branched fragments with the degree of branching well below that observed for the dendrimer architecture.$^{5}$ $^{24-26}$, since hyperbranched molecules are generally not expected to form regular supramolecular nanorod structures owning to their high polydispersities, irregular architectures, and poorly defined shapes. Moreover, the synthesis of hyperbranched polyester nanorods modified with the heterocyclic nitrogen compounds has been sparsely developed, which provoked us to initiate our present study.

Pyrrolo[2,1-a]isoquinolines derivatives are valuable heterocyclic nitrogen compounds, which have been widely utilized in pharmaceutical chemistry,$^{27-29}$ functional materials.$^{30}$ Much attention has been focused on diversity-oriented synthesis$^{31-36}$ to expand the design of architecture, including heterocycles, populating unexplored “chemical space” to aid the discovery of novel lead compounds.$^{37,39}$ Furthermore, N atoms and aromatic nucleus of pyrrolo[2,1-a]isoquinolines derivatives are considered to be efficient hydrogen bond acceptor and π-π stacking units in supramolecular functional polymers with distinctively biological and physical features.

The aim of this study was to design and synthesize a new class of HBPE-CICA nanorods by modifying 1-cyano-pyrrolo[2,1-a]isoquinoline-3-carboxylic acid (CICA) to the second generation of HBPE structure. Because the modified molecules with the presence of CICA tails and residual hydroxyl groups in the flexible core, might exhibit multiple intermolecular interactions among highly branched molecules, and facilitate their assembly into supramolecular nanorod structures. It is worth to understand the role of amphiphilic balances and the combination of the functional terminal groups during nanorod structures formation in one-pot synthesis. In addition, we expect to investigate the fluorescence diversification of these functionalized HBPE-CICA nanorods in presence of various metal cations to look into their potentials as fluorescence sensors in chemical or biological applications.

The synthetic route of 1-cyano-pyrrolo[2,1-a]isoquinoline-3-carboxylic acid (CICA) in three steps were described in scheme S1 and the synthesis methods were described in scheme S1. The target compound was characterized by IR (Fig. 1), $^1$H NMR(Fig.
2) and ESI-MS spectra (Fig. S1 in Supporting Information).

Scheme 1 Synthetic route of CICA

The synthetic routes of HBPE and HBPE-CICA were described as scheme 2 and the synthesis methods were described in scheme S2. The structures of modified hyperbranched polyester HBPE-CICA	extsubscript{a} and HBPE-CICA	extsubscript{b} were confirmed by FT-IR, ¹H NMR and ESI-MS. As shown in Fig. 1, the FT-IR spectra of HBPE-CICA	extsubscript{a} and HBPE-CICA	extsubscript{b} were found to be quite similar to those of CICA and HBPE. All of them showed similar characteristic peaks of benzene ring at 1617, 1550, 1499 and 1455 cm\(^{-1}\); signals of C=O at 1695 cm\(^{-1}\) and 1638 cm\(^{-1}\); signals of -C-O-C- at 1125 cm\(^{-1}\). The characteristic peaks of -CN at 2217 cm\(^{-1}\) were obviously observed only for CICA, HBPE-CICA	extsubscript{a} and HBPE-CICA	extsubscript{b} (blue, black and red curves, respectively), while not exist in unmodified HBPE (green curve).

Scheme 2 Synthetic route of HBPE, HBPE-CICA	extsubscript{a} and HBPE-CICA	extsubscript{b}

Fig. 1 FTIR spectra of CICA (blue line), HBPE (green line), HBPE-CICA	extsubscript{a} (black line) and HBPE-CICA	extsubscript{b} (red line)

³¹H NMR (Fig. 2) spectra was further carried out to confirm the chemical composition of the modified samples. Signals at 7.33-8.73 ppm were attributed to the modified end-groups (CICA). Protons of R₂COOCH₃ and ArCOOCH₃ could be observed at 3.42 and 4.10 ppm, respectively, while those of methyl groups appear at 0.83–1.75 ppm. Moreover, the CICA grafting of HBPE-CICA	extsubscript{a} and HBPE-CICA	extsubscript{b} could be calculated by integration ratio of the aromatic protons (b) to aliphatic protons (a) (CH\(_{17}\) and CH\(_{17}\)CH\(_{2}\)- groups) with the formula (8S\(_{b}/21S\(_{b}\)) were about 50% and 13%, respectively. The ESI-MS(m/z) of HBPE-CICA	extsubscript{a} (Fig. S1) was 1614.15 (calculated, 1614.10). The ESI-MS (m/z) of CICA and HBPE, the ³¹H NMR of HBPE and ¹³C NMR characterization of HBPE-CICA	extsubscript{a} and HBPE-CICA	extsubscript{b} polymers were shown in ESI part (Fig. S2-S5).

Transmission electron microscope (TEM) experiments were performed to estimate the size and morphology of HBPE-CICA	extsubscript{a} and HBPE-CICA	extsubscript{b}. As shown in Fig. 3A, HBPE-CICA	extsubscript{a} exhibited a nanorod morphology with an average diameter of 100 nm and length of 1 μm. The slight agglomeration between the nanorods could also be observed, which might be related to its higher grafting rate. As a comparison, Fig. 3B illustrated the TEM image of HBPE-CICA	extsubscript{b} with a nanorod morphology as well, while an average diameter of 200 nm and length of 1 μm. Additionally, HBPE-CICA	extsubscript{b} nanoparticles could self-assemble to form short nanorods with an average diameter of around 100 nm (Fig. 3C), and further assemble into long HBPE-CICA	extsubscript{b} nanorods (Fig. 3D). We suggest that the synergistic effect by hydrogen bonding in the flexible cores and π-π stacking interactions of peripheral CICA groups were responsible for the 1D molecular designs demonstrated here.\textsuperscript{26,40,41} The 1D structures from intermolecular hydrogen bonds among hydroxy groups of the core and the π-π
stacking interactions of peripheral CICA groups stacked in a face-to-face manner can be considered the primary cause for the formation of straight microrods (Fig. 3E). For the HBPE-CICA₂ showed the better nanorod morphology than that of the HBPE-CICA₆, its solution was stored at 4 °C for one month and it was observed that there was no change in size and morphology (Fig. S6). The results indicated that the HBPE-CICA₂ nanorods were very stable in ethyl acetate solution.

For the π-π conjugate structure of the modified CICA groups, we examined the fluorescence of CICA, HBPE-CICA₆ and HBPE-CICA₂. Fluorescent spectra, digital camera image and fluorescent images of the CICA, HBPE-CICA₆ and HBPE-CICA₂ in DMSO solution were showed in Fig. 6, when the concentrations of the CICA, HBPE-CICA₆ and HBPE-CICA₂ solutions were same, the HBPE-CICA₂ nanorods showed the strongest fluorescent than HBPE-CICA₆ and CICA (order: HBPE-CICA₂ > HBPE-CICA₆ ≈ CICA), which was shown in digital camera images of Fig. 6.
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Iron is an ubiquitous metal in cells and plays a crucial role in a variety of vital cell functions. However, both excess and deficiency from the normal permissible limit can induce serious disorders. Thus, there is an urgent need to develop chemical sensors that are capable of detecting the presence of iron ions in environmental and biological samples.

The influences of Fe$^{3+}$ on the fluorescence intensity of the CICA, HBPE-CICA$_6$ and HBPE-CICA$_2$ have been evaluated by a fluorescence decrease (FD) which is calculated by the ratio of the reduced fluorescence intensity in the presence of metal cations (I) and the fluorescence intensity without metal cations (I$_0$). The fluorescence responses of CICA, HBPE-CICA$_6$ and HBPE-CICA$_2$ to Fe$^{3+}$ and other metal ions were shown in fluorescent part (Fig. 7-S11). The results indicated that they all showed better selectivities to Fe$^{3+}$ than other metal ions. The fluorescent response of CICA and HBPE-CICA$_6$ to Fe$^{3+}$ were shown in fluorescent part (Fig. 7-S10).

To further study the influence of grafting ratio of CICA on its sensitivity to the recognition of Fe$^{3+}$, the change of the fluorescent intensity of the HBPE-CICA$_6$ and HBPE-CICA$_2$ nanorods upon gradual titration with Fe$^{3+}$ was carried out. As shown in Fig. 8, when the concentration of the Fe$^{3+}$ was maintained, the fluorescent decrease factor (FD) of HBPE-CICA$_2$ was lower than that of HBPE-CICA$_6$. The relationship between the concentration of Fe$^{3+}$ and FD was FD = 0.81-0.014×[Fe$^{3+}$] for HBPE-CICA$_6$ and the fitting constant R = 9734, and for the HBPE-CICA$_2$, FD = 0.65-0.014×[Fe$^{3+}$], the fitting constant R = 9571, which showed that HBPE-CICA$_2$ with the lower grafting rate exhibited better sensitivity to Fe$^{3+}$, this might be the intermolecular hydrogen bonds among hydroxy groups of the flexible core and the π-π stacking interactions of peripheral CICA groups with the lower grafting rate could be easily destroyed by Fe$^{3+}$. As a result, in the process of design and synthesis, it’s not need to graft more CICA binding units to the nanorods.

When the concentration of CICA, HBPE-CICA$_6$ and HBPE-CICA$_2$ were same, HBPE-CICA$_2$ showed much stronger fluorescent, and in the case of the same concentration of Fe$^{3+}$, HBPE-CICA$_2$ also showed much the less data of FD, which can be observed from Fig. 7. So we focused on the study of the fluorescent response of HBPE-CICA$_2$ to Fe$^{3+}$.

![Fluorescence spectra of the HBPE-CICA$_2$ (a), HBPE-CICA$_6$ (b) nanorods and CICA (c) in DMSO solution (c = 5.0 × 10$^{-3}$ M, $\lambda_{ex}$ = 260 nm, the excitation and emission slits are 5nm/2.5nm). (B)The digital camera image of HBPE-CICA$_2$ (left) and HBPE-CICA$_6$ (middle) and CICA(right) in DMSO solution under sunlight. (C) The fluorescent image of HBPE-CICA$_2$ (left) and HBPE-CICA$_6$ (middle) and CICA (right) under UV light ($\lambda$= 260 nm), c = 5.0 × 10$^{-3}$ M.](image)

![Fluorescence decrease factors (FD) of the HBPE-CICA$_2$ nanorods in DMSO solution (C = 5.0 × 10$^{-3}$ M) and HBPE-CICA$_6$ (c = 5.0 × 10$^{-3}$ M) nanorods in DMSO solution in the presence of Fe$^{3+}$ at a concentration of 6.6× 10$^{-3}$ M ~4.7 × 10$^{-4}$ M, $\lambda_{em}$ = 380 nm.](image)

![Fluorescence spectra of the HBPE-CICA$_2$ nanorods in DMSO solution (C = 5.0 × 10$^{-3}$ M, $\lambda_{ex}$= 260 nm, the excitation and emission slit are 5nm/2.5nm) in the presence of Fe$^{3+}$ at a concentration of 0~5.3 × 10$^{-4}$ M.](image)
In summary, herein we reported a novel class of functionalized hyperbranched polymers with CICA unit which were synthesized by a facile method under mild reaction condition. We found that the amplification of directional supramolecular interactions facilitated by the presence of multiple peripheral branches of even irregular, flexible molecules could lead to efficient self-assembly and form remarkably stable nanorods. The results demonstrated that one-dimensional supramolecular assembling could be achieved by highly branched but irregular molecules without a tedious, multistep synthesis of the well-defined, shape-persistent molecules. On the other hand, such hyperbranched aromatic-aliphatic polymers exhibited strong fluorescent intensity, different grafting rate, nanorod morphology and good solubilities. Furthermore, HBPE-CICA and HBPE-CICA$_2$ were established to be selective fluorescent sensor for Fe$^{3+}$ ion detection since fluorescent responses of the functionalized HBPE were disparate to tested metal ions, in particular, lower grafted HBPE-CICA nanorods showed better sensitivity to Fe$^{3+}$ ion. The chelation formed between Fe$^{3+}$ and the HBPE-CICA nanorods could be potentially applied for the design of new organic-inorganic hybrid materials.

**Notes and references**

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**References**

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The novel functionalized hyperbranched aromatic-aliphatic co-polyester nanorods HBPE-CICA$_6$ and HBPE-CICA$_2$ were synthesized by a facile method under mild reaction conditions by modifying periphery of the second generation hyperbranched polyester (HBPE) with 1-cyano-pyrrolo[2,1-a]isoquinoline-3-carboxylic acid (CICA) groups. Such hyperbranched polyesters nanorods exhibited strong fluorescent intensity, different grafting rate, nanorod morphology and good solubilities. Interestingly, lower grafted HBPE-CICA$_2$ nanorods was established to be a highly sensitive fluorescent sensor for Fe$^{3+}$ ion.