Electronic Supplementary Information

One-Pot Synthesis of Indolizine Functionalized Nanohyperbranched Polyesters with Different Nano Morphologies and their Fluorescent Response to Anthracene

Xiaoxia Wang¹, Fanyang Zeng¹, Can Jin², Yuliang Jiang¹, Qiaorong Han¹*, Bingxiang Wang¹* and Zhenye Ma¹*

¹. Jiangsu Key Laboratory of Biofunctional Materials, Key Laboratory of applied photochemistry, School of Chemistry and Materials Science, Nanjing Normal University, Nanjing 210097 (China)
². Jiangsu Provincial Key Laboratory of Biomass Energy and Materials, National Engineering Laboratory for Biomass Chemical Utilization, Institute of Chemical Industry of Forest Products, CAF, Nanjing 210042 (China).

Table of Contents

1. General Information..................................................................................................................S2
2. Synthesis of 1-cyanoindolizine-3-carboxylic Acid (CIDA).................................................S2
3. Synthesis of HBPE..................................................................................................................S3
4. Synthesis of HBPE-CIDA......................................................................................................S3
5. Characterization of CIDA, HBPE, HBPE-CIDA₁ and HBPE-CIDA₄ ............................S3
6. The Size Distribution of HBPE-CIDA₁ Nanospheres.........................................................S8
7. The Average Height of a single particle of HBPE-CIDA₁ Nanospheres.........................S8
8. The Average Height of a single layer of HBPE-CIDA₄ Nanospindles.........................S8
9. The Fluorescence Properties of HBPE-CIDA₄ after Addition of NA..............................S8
10. The Fluorescence Properties of HBPE-CIDA₄ after Addition of PY...............................S9
11. The Fluorescence Properties of HBPE-CIDA₁ after Addition of NA..............................S9
12. The Fluorescence Properties of HBPE-CIDA₁ after Addition of AN.............................S9
1. General Information.

2, 2-Bis (hydroxymethyl)propionic acid (DMPA) was purchased from Sigma-Aldrich Co. Ltd. and used without purification. Trimethylol propane (TMP) (>99% pure), p-toluene sulfonic acid (P-TSA), dicyclohexylcarbodiimide (DCC) were obtained from Energy Chemical Co. Ltd., China. DMSO (A. R. grade, Shanghai Chemical Reagent Co.) was refluxed with CaH$_2$ and distilled prior to use. All other solvents were AR grade.

The $^1$H NMR and $^{13}$C NMR spectra were recorded in DMSO-$d_6$ on a Brucker Advance III 500 spectrometers or a Bruker Avance 400 spectrometers at ambient temperature. FTIR spectra were recorded on a Nicolet Nexus 670 spectrometers using KBr pellets. The fluorescent spectra were recorded on Varian Cary Eclipse spectrometers. AFM images were recorded on an Agilent PICOPLUS (American). The nanoparticles were examined with transmission electron microscopy (TEM, JEOL JEM 2010F, Japan). Wide-Angle X-ray Diffraction (WAXD) experiments were performed at room temperature on a glass slide using Bruker D8 (Germany) with rotating anode source operated at 40 kV and 20 mA, and the spectra were recorded in the scattering angle ($2\theta$) range of 5°−40° (step size 0.02°). The molecular weights were determined by the matrix-assisted laser desorption ionization time-of flight mass spectrometry (MALDI-TOF MS) and electrospray ionization mass spectrometry (ESI-MS).

Synthesis of 1-Cyanoindolizine-3-Carboxylic Acid (CIDA)

Preparation of compound B. To a solution of pyridine (compound A, 3.96 g, 50 mmol) in ethyl acetate (30 mL), bromoacetonitrile (6.05 g, 50 mmol) was added. The mixture was stirred at room temperature for 24 hours. The resulting precipitate was collected by filtration, washed with ethyl acetate (3 ×10 mL), and dried under vacuum to give the compound B as a white solid (yield: 80%).

Preparation of compound C. Compound B (1.99 g, 10 mmol) was dissolved in DMF (30 mL), then triethylamine (10 mL, TEA), methyl acrylate (4.3 g, 50 mmol) and CrO$_3$ (4.4 g, 44 mmol) were added stepwise. The reaction mixture was heated at 90 °C for 5 hours. The resulting solution was cooled, and then poured into a 5% HCl aqueous solution (100 mL) with stirring. The mixture was kept standing for an hour. The yellow precipitate was collected by filtration, and then purified.
by column chromatography (silica gel, eluent: ethyl acetate/petroleum ether = 1:3) to give compound C as a yellow solid (yield: 85%).

**Preparation of CIDA.** NaOH (10% in water, 20 mL) was added to a solution of compound C (1.0 g, 5 mmol) in 40 mL of ethanol. The mixture was heated at 80 °C for 2 hours, then cooled down to room temperature. The resulting solution was treated with 5% HCl solution to adjust pH to 2. A precipitate was formed and collected by filtration. The crude product was recrystallized in ethanol, and dried under vacuum to give CIDA as a blue solid (yield: 80%).

**Synthesis of HBPE**

All reactions were carried out under nitrogen atmosphere. The second generation hyperbranched polyester with trimethylol propane as cores was prepared according to published procedures. Accordingly, as shown in Scheme 2, 2, 2-Bis(hydroxymethyl)propionic acid (DMPA) and Trimethylol propane (TMP) were mixed in a ratio of 9:1, then heated at 140 °C in presence of catalytic amount of p-toluenesulfonic acid (0.3 mol%) for 2 hours. The resulting solution was distilled under vacuum for 2 hours, then the crude product was dissolved in acetone and precipitated by n-hexane, the precipitate was dried under vacuum, to give the desired product with 12 terminal hydroxyl groups and a molecule weight of 1179 g/mol (as describe in the literature S1).

**Synthesis of HBPE-CIDA**

To a solution of HBPE (0.12 g, 0.1 mmol) and CIDA (0.24 g, 1.3 mmol) in DMSO (60 mL), N,N'-dicyclohexyl carbodiimide (DCC, 0.29 g, 1.39 mmol) was added. The mixture was heated at 85 °C under nitrogen for 20 hours, which was monitored with TLC. The resulting solution was evaporated, and the residue was poured into ethyl acetate (60 mL), then filtered. The filtrate was concentrated and subjected to column chromatography (silica gel, eluent: ethyl acetate/petroleum ether = 1/2). Two yellow products of HBPE-CIDA$_1$ and HBPE-CIDA$_4$ were obtained with yields of 50% and 30%, respectively.

**Characterization of CIDA, HBPE, HBPE-CIDA$_1$ and HBPE-CIDA$_4$ nanoparticles**
The Fourier transform infrared spectroscopy (FTIR) spectra were obtained with KBr pellets on a Bruker Tensor 27. The proton nuclear magnetic resonance (^1H NMR, ^13C NMR) spectra were obtained by operating on a Bruker Advance DMX 300. ^1H NMR (400 MHz) spectra were recorded on a Bruker Avance 400 spectrometers (Bruker, Germany) at room temperature. The measurements were carried out in DMSO solution at ambient temperature. The chemical shifts were referenced to a tetramethylsilane (TMS) standard. The nano morphologies were examined with transmission electron microscopy (TEM, JEOL JEM 2010F, Japan) and scanning electron microscope (SEM, JSM-6300). For TEM measurement, in a typical experiment, one drop of ethyl acetate dispersion of HBPE-CICA nanospheres and nanospindles was introduced onto a copper grid with a microgrid carbon film. The droplet was allowed to dry under reduced pressure and then observed under TEM operating at an acceleration voltage of 100 kV. The electrospray ionization mass spectrometry (ESI-MS) was obtained from mass spectrometer (LCQ/M/Z = 50–1850, Finnigan, U.S.) and micromass GCT. The MALDI-TOF-MS were obtained from mass spectrometer (Micro Flex), 2, 5-dihydroxybenzoic acid (DHB), 1, 8, 9-triacetoxyanthracene (THA) and NaI as the matrix. Wide-Angle X-ray Diffraction (WAXD) experiments were performed at room temperature on a glass slide using Bruker D8 (Germany). The absolutely fluorescence quantum yields of HBPE-CIDA₁ and HBPE-CIDA₄ were measured on the FluoroMax-4 spectrofluorometer, and the excitation wavelength was 350 nm.

**CIDA**: IR νmax/cm⁻¹: 3642–2460 broad peaks, 3144, 2949, 2743, 2597, 2342, 1668, 1521, 1218, 1130, 1032, 758, 661. ^1H NMR (DMSO-d₆, 500 MHz), δ (ppm): 9.52 (d, 1H), 7.95 (s, 1H), 7.85 (d, 1H), 7.22 (t, 3H), 7.25 (t, 3H), 12.5–14.01 (1H). ESI-MS (m/z): 187.1 [M+H]⁺ (C₁₀H₆N₂O₂, calculated, 186.1).

**HBPE**: IR νmax/cm⁻¹: 3426, 2946, 2890, 1635, 1523, 1431, 1342, 1234, 850, 743. ^1H NMR (DMSO-d₆, 400 MHz), δ (ppm): 0.81 (m, 3H, CH₃CH₂–), 1.08 (m, 27H, CH₃–CR₃), 1.33 (m, 2H, CH₂CH₂–), 3.42 (m, 24H, CH₂OH), 4.10 (m, 18H, R₃C–CH₂–OOC), 4.62 (6H, CH₂OH), 4.94 (6H, CH₂OH). ESI-MS (m/z), 1201.7 [M+Na]⁺, calculated, 1179.2.

**HBPE-CIDA₁**: IR νmax/cm⁻¹: 3499, 3303, 3147, 2931, 2853, 2354, 1699, 1631, 1533, 1445, 1367, 1120, 1083, 897, 641. ^1H NMR (DMSO-d₆, 500 MHz), δ (ppm): 0.85 (m, 3H, CH₃CH₂–), 1.15 (m, 27H, CH₃–CR₃), 1.56 (m, 2H, CH₂CH₂–), 3.75 (m, 22H, –CH₂OH), 4.13 (m, 18H, R₃C–CH₂–OOC), 4.23 (2H, Ar-COOCCH₂–), 6.99-9.12 (5H, Ar-H). ESI-MS (m/z):1348.4 [M+H⁺] (calculated, 1347.7). ^13C NMR (DMSO-d₆, 75 MHz), 163.4, 160.8(Ar-COOCH₂–, R₃C–COO–CH₂), 156.9,
154.8, 128.9, 137.6, 128.2, 124.4, 119.3, 113.5, 107.9 (Ar-C), 117.6 (-CN), 54.1 (R_3C–CH₂–OOC), 49.7 (R_3C–CH₂–OH), 47.2 (CR_3–COO–), 33.8 (CR_3–CH₃), 25.5 (CR_3–CH₃).

HBPE-CIDA$_4$: IR $\nu_{\text{max}}$/cm$^{-1}$: 3489, 3332, 3166, 2922, 2843, 2354, 1670, 1621, 1572, 1533, 1445, 1386, 1318, 1240, 1073, 897, 643. $^1$H NMR(DMSO-d$_6$, 500 MHz), 0.87(m, 3H, CH$_3$CH$_2$), 1.16(m, 27H, CH$_3$–CR$_3$), 1.63(m, 2H, CH$_3$CH$_2$–), 3.77(m, 16H, CH$_2$OH), 4.04 (m, 18H, R$_3$C–CH$_2$–OOC), 4.26(8H, Ar-COOCH$_2$–), 6.97-9.63 (m, 20H, Ar-H) . $^{13}$C NMR (75 MHz, DMSO-d$_6$), 163.8, 163.3 (Ar-COOCH$_2$–, R$_3$C–COO–CH$_2$), 157.1, 154.8, 137.4, 127.5, 123.7, 119.4, 113.5, 107.9 (Ar-C), 117.6 (-CN), 53.8 (R$_3$C–CH$_2$–OOC), 49.7 (R$_3$C–CH$_2$–OH), 47.7 (CR_3–COO–), 33.6 (CR_3–CH$_3$), 25.2 (CR_3–CH$_3$). MALDI-MS (m/z):1851.7 (calculated, 1852.3). The CIDA grafting of HBPE-CIDA$_4$ and HBPE-CIDA$_1$ could be calculated by integration ratio of the aromatic protons (b) and alphatic protons (a) (CH$_3$- of the CH$_3$CH$_2$- groups) with the formula ($S_b/(20S_a)$) were about 30% and 5.2%, respectively.

Fig. S1. The ESI-MS spectra of the CIDA

Fig. S2. The ESI-MS spectra of the HBPE
Fig. S3. $^1$H NMR spectra of HBPE in DMSO-$d_6$

Fig. S4. $^{13}$C NMR spectra of HBPE-CIDA$_{1}$ (A) and HBPE-CIDA$_{4}$ (B) in DMSO-$d_6$
Fig. S5. The MALDI-TOF spectra of the HBPE-CIDA₁ (A: DHB as the matrix, B: THA and NaI as the matrix)

Fig. S5 (A) showed the real molecular weight of HBPE-CIDA₁ is 1370.3 [M+Na⁺], which is consistent with the theory molecular weight (1347.7), DHB as the matrix. The peaks 1347.7 and 1206.3 possess a distance of 141, which corresponds to the part of the CIDA unit, as the green section as the illustration, this further proved that the calculation of the grafting rate of HBPE-CIDA₁ is accurate. Fig. S5 (B) also clearly showed the real molecular molecular weight (1348.7 [M+H⁺]), THA and NaI as the co-matrix.

Fig. S6. The MALDI-TOF spectra of the HBPE-CIDA₄, DHB as the matrix (A), THA and NaI as the matrix (B)

No obvious target product peak of HBPE-CIDA₄ could be observed in Fig. S6 (A), but it showed some of the important fragment ion peaks at 1175.3, 1362.4, 1515.1. The peak at 1175.3 represent the lost of four modified peripheral CIDA groups (the green section as the illustration), while the peak at 1515.1 represent the lost of two modified CIDA groups (the green section as the illustration). The peaks of 1515.1 and 1362.4 possess a distance of 153, which corresponds to part of the CIDA units as the blue section as the illustration. Fig. S6 (B) clearly showed the real molecular molecular weight 1853.3 [M+H⁺], THA and NaI as the matrix, which is consistent with
the calculated molecular weight (1851.7). All the data proved that the calculation of the grafting rate of HBPE-CIDA₄ is accurate.

**Fig. S7.** The size distribution of HBPE-CIDA₁ nanospheres

**Fig. S8.** The average height of a single particle of HBPE-CIDA₁ nanospheres

**Fig. S9.** The average height of a single layer of HBPE-CIDA₁ nanospindles

**Fig. S10.** Fluorescence spectra of the HBPE-CIDA₄ in ethanol solution (c = 2.0 × 10⁻⁵ M) in the presence of NA at a concentration from 0 to 1.3× 10⁻⁴ M, λex =290 nm, the excitation and emission slits are 5nm/2.5nm. The absolutely fluorescence quantum yield of HBPE-CIDA₄ was 12.14%.

S8
**Fig. S11.** Fluorescence spectra of the HBPE-CIDA$_4$ in ethanol solution (c = $2.0 \times 10^{-5}$ M) in the presence of PY at a concentration from 0 to $1.3 \times 10^{-4}$ M, $\lambda_{ex} = 290$ nm, the excitation and emission slits are 5nm/2.5nm.

**Fig. S12.** Fluorescence spectra of the HBPE-CIDA$_1$ in ethanol solution (c = $2.0 \times 10^{-5}$ M) in the presence of NA at a concentration from 0 to $1.3 \times 10^{-4}$ M, $\lambda_{ex} = 290$ nm, the excitation and emission slits are 2.5nm/2.5nm. The absolutely fluorescence quantum yield of HBPE-CIDA$_1$ was 10.77%.

**Fig. S13.** Fluorescence spectra of the HBPE-CIDA$_1$ in ethanol solution (c = $2.0 \times 10^{-5}$ M) in the presence of AN at a concentration from 0 to $1.3 \times 10^{-4}$ M, $\lambda_{ex} = 290$ nm, the excitation and emission slits are 2.5nm/2.5nm.
**Fig. S14.** Fluorescence spectra of the HBPE-CIDA$_1$ in ethanol solution ($c = 2.0 \times 10^{-5}$ M) in the presence of PY at a concentration from 0 to $1.3 \times 10^{-4}$ M, $\lambda_{ex} = 290$ nm, the excitation and emission slits are 2.5nm/2.5nm.