Surface Modification of Ferromagnetic *L*1o FePt Nanoparticles Using Biotin–Avidin as Biomolecular Recognition Probes

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Water-dispersible biotin-modified biofunctional ferromagnetic *L*1o FePt nanoparticles (NPs) have been synthesized by using a silica nanoreactor strategy. The coercivity field of the NPs was 16 kOe at room temperature, with a particle size of around 5.19 nm. Biotin-coated *L*1o FePt NPs were biocompatible in an aqueous environment and show site-specific interactions with avidin compounds that could potentially yield a new magnetic material for biological applications, such as for biomolecular recognition.

Magnetic NPs are of interest not only for their unique properties, but also for their possible applications in various fields such as data storage devices,[1] catalyst materials,[2] and biomedical materials.[3,4] In biomedical and biotechnological applications, magnetic NPs have attracted many researchers because they display promising characteristics in terms of handling and manipulation by an external magnetic force. In particular, several magnetic materials have been used in biomedical research, such as magnetic resonance imaging,[5,6] hyperthermia,[7] bacterial detection,[8] toxic decorporation,[9] and drug delivery.[10] However, almost all magnetic materials used in biomedical research are actually superparamagnetic materials. Although higher performance for these applications are expected by using stable ferromagnetic NPs with biomedical functions, so far, it has been difficult to synthesize them. Furthermore, to be useful for applications in biomedical research, the NPs should have water-soluble properties. On the other hand, we have reported the observation of photo-tunable properties for ferromagnetic *L*1o FePt NPs at room temperature by the surface modification of photoresponsive molecules on NPs.[11,12] This opens the possibility of carrying out additional surface modifications using appropriate molecules to make them water-dispersible and hence useful for application to biomedical research.

Our motivation here is to modify the surface of ferromagnetic *L*1o FePt NPs with biotin moieties that will improve the biocompatibility of magnetic NPs in an aqueous environment. One remarkable property of biotin compounds is their strong and site-specific interaction with avidin. Avidin is a protein molecule that binds very tightly to the small water-soluble molecule biotin ($K_a = 10^{15}$).[13] The specificity of biotin binding to avidin provides the basis for developing a bioassay system to detect or quantify analytes. Furthermore, the capability of both avidin and biotin to effectively conjugate to other proteins or various detection reagents without loss of their binding affinity is an important key that can be used in biomolecular recognition.

Results and Discussion

As shown in Figure 1, oleic acid/oleylamine (=(*Z*)-9-octadecenylamine)-coated fcc FePt NPs (**1**) were first synthesized based on the reported literature.[14] Briefly, the synthetic procedure is as follows: platinum acetylacetonate (0.5 mmol), 1,2-hexadecanediol (1.5 mmol), and dioctyl ether (20 mL) were mixed and heated to 100 °C under an Ar atmosphere. Oleic acid (0.5 mmol), oleylamine (0.5 mmol), and [$Fe(CO)_5$] (1 mmol) were added, and the mixture was heated to reflux (297 °C). The refluxing was continued for 30 min. The heat source was then removed, and the reaction mixture was allowed to cool to room temperature. The black product was precipitated by adding ethanol and was separated by centrifugation.

As the next step, silica coating was carried out using Igepal and TEOS.[15] The silica coating was used to protect the NPs from aggregation when annealing was conducted at high temperature. Igepal CO-520 was mixed with 170 mL of cyclohexane in a 250 mL Erlenmeyer flask and stirred. Fcc FePt NPs (**1**) were dispersed in cyclohexane at a concentration of 1 mg mL^{-1} and then injected into the cyclohexane/Igepal solution. Approximately 1.3 mL of 30% NH_4OH aqueous solution (EM Science) was then added dropwise and stirred for 2–3 min, followed by the addition of 1.5 mL of TEOS. The mixture was stirred for 2 days and methanol was added to collect the NPs. The NPs that were obtained, silica-coated fcc FePt NPs (**2**), were precipitated with excess hexane and collected by centrifugation. The silica-coated fcc FePt NPs (**2**) were transformed to the *L*1o structure **3** by heating in a tube furnace at 700 °C for 5 h under an H_2 (4%)/Ar (96%) atmosphere.[16]

*L*1o FePt NPs (**4**) that were dispersible in nonpolar organic solvents were prepared via an aqueous/organic biphasic reaction using CTAB as a phase-transfer reagent.[16] Finely-ground silica-coated *L*1o FePt NPs (**3**) (0.03 g), aqueous NaOH solution (4 M, 3 g), chloroform (5 g), and CTAB (0.5 g) were mixed and vigorously stirred for 24 h at room temperature. In this process the SiO_2 layer was dissolved off, and bare *L*1o FePt

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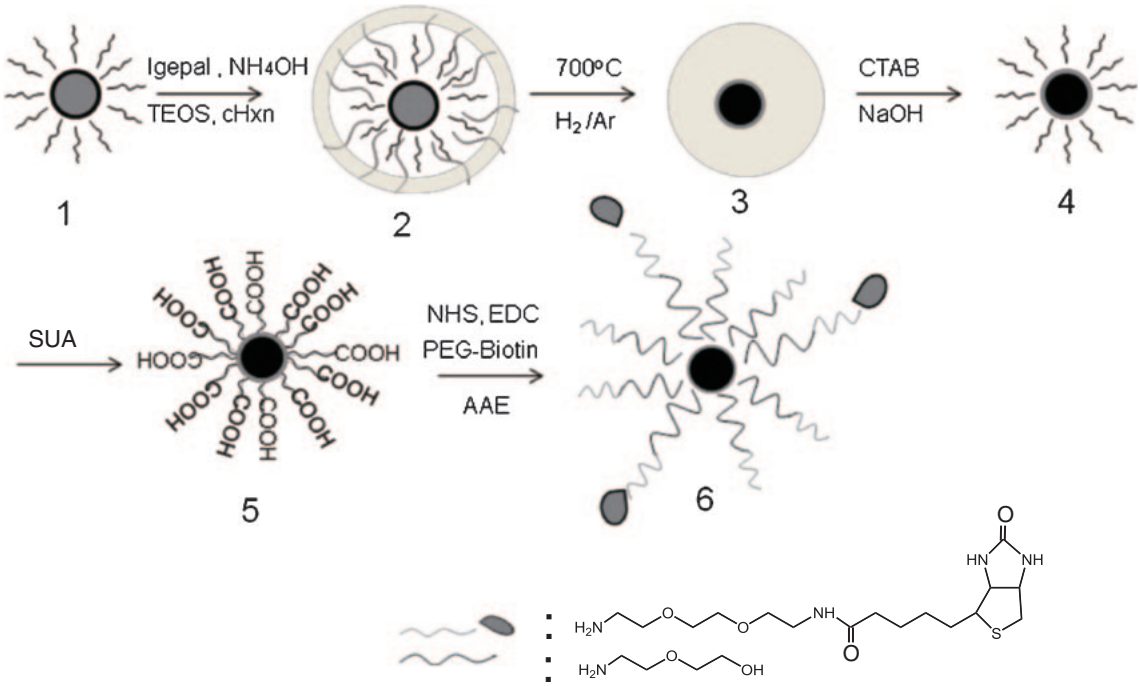

Figure 1. Synthesis scheme of biotin-coated *L*1o FePt NPs (**6**); oleic acid/oleylamine-coated fcc FePt NPs (**1**), silica-coated fcc FePt NPs (**2**), silica-coated *L*1o FePt NPs (**3**), oleylamine/oleic acid-coated *L*1o FePt NPs (**4**), SUA-coated *L*1o FePt NPs (**5**), and biotin-coated *L*1o FePt NPs (**6**).

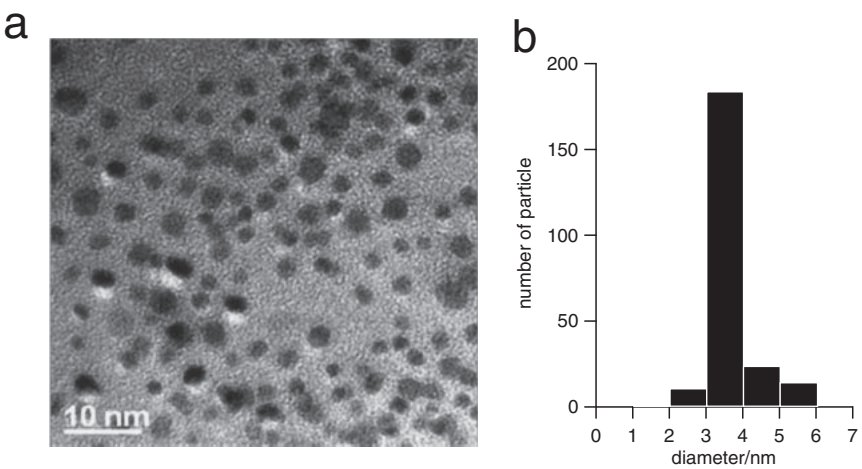

Figure 2. (a) TEM image and (b) the size distribution of fcc FePt NPs (**1**).

NPs (**4**) were extracted to the chloroform phase. After washing and collecting by centrifugation, the NPs were dispersed in chloroform containing oleic acid and oleylamine.

A ligand-exchange procedure of oleic acid/oleylamine-coated *L*1o FePt NPs with 11-sulfanylundecanoic acid (SUA) was carried out as follows:[17] 50 mg of *L*1o FePt NPs (**4**) dispersed in 0.5 mL of hexane was added to a solution of SUA (2.5 g of SUA in 5 mL of cyclohexanone). The mixture was shaken well and the solution was centrifuged to separate the precipitate. After washing with cyclohexanone, ethanol, and acetone respectively, a quantity of water-dispersible SUA-coated FePt NPs (**5**) was obtained.

To obtain biotin-coated *L*1o FePt NPs (**6**),[18] the SUA-coated FePt NPs (**5**) were reacted with a mixture of freshly prepared 50 mM NHS and 200 mM EDC solution in PBS for 30 min, and continued by reacting with AEE (22 mM) and BA (2.4 mM) for 3 h (see Experimental section for abbreviations). Excess BA and AEE were removed by centrifugation. The retentate that contained biotin-coated *L*1o FePt NPs (**6**) was resuspended in PBS.

Figure 2a shows a high-resolution transmission electron microscope (TEM) image of fcc FePt NPs (**1**). The fcc FePt NPs show good dispersibility in chloroform and have a uniform particle shape. Their size is relevantly small, with average diameters of around 3.3 nm and a narrow size distribution (Figure 2b) with a standard deviation of 9%. The spaces between the particles were occupied by ligands of oleic acid, and oleylamine capped the NPs. The composition of fcc

FePt NPs (**1**), as calculated by energy-dispersive X-ray (EDX) analysis, is found to be $Fe_{57}Pt_{43}$ (Figure S1).

The magnetic properties of the fcc FePt NPs (**1**) were examined by using a SQUID magnetometer. The magnetization curve shows the superparamagnetic behavior at room temperature (300 K) with magnetization at 50 kOe around 20 emu g^{-1} (Figure 3).

Figure S2 shows a TEM image of the oleic acid/oleylamine-coated FePt NPs that were coated with silica (**2**). The core FePt NPs were size-monodisperse with an average diameter that was the same as their fcc FePt precursor. These FePt NPs were coated with silica using nonionic surfactant-stabilized water-in-oil microemulsions as the reaction media. The decomposition of TEOS to silica was catalyzed using NH_4OH. By adjusting the TEOS concentration and the ratio of TEOS to FePt NPs that was used during the coating step, the SiO_2 shell thickness could be controlled from 7 to 23 nm.[15] Because of the smaller size of the fcc FePt NPs precursor (**1**) at 3 nm in diameter, the silica coating resulted in multiple FePt NPs that were encapsulated into silica shells. If a larger diameter of NPs had been used, then the NPs could have been capped individually into silica shells.[15]

As shown in Figure S3b, fcc FePt NPs (**1**) exhibit a disordered fcc structure, with (111) observed as the main peak. After annealing at 700 °C for 5 h, the fcc structure of FePt NPs was transformed to an ordered tetragonal $L1_0$ structure (Figure S3a). The weak peaks for the (001), (110), and (002) Bragg peaks in $L1_0$ FePt are typical superlattice reflections that cannot be observed in the disordered fcc phase. These superlattice reflections suggest the existence of the partially ordered $L1_0$ phase in the synthesized FePt NPs. The (111) diffraction peak also shifts to a slightly higher angle, confirming the fcc-to-$L1_0$ phase transition. To demonstrate the transformation process quantitatively, an order parameter S is estimated. Generally, quantitative values of S are obtained from comparisons between the integrated intensities of the superlattice and the fundamental reflections of the XRD spectra. An approximate relationship between S and c/a can be defined as

$$S^2 = \frac{1 - S_a}{1 - S_f} \quad (1)$$

where S_a and S_f were the a/c axis ratios of the target samples and perfectly ordered samples (known as 0.956), respectively.[19] The S value for the obtained $L1_0$ FePt was 0.78.

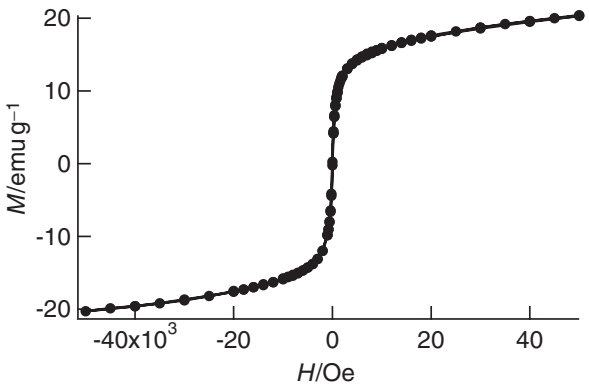

Figure 3. *M–H* plot of fcc FePt NPs (**1**) at 300 K.

The hard magnetic $L1_0$ FePt (**3**) phase was obtained by annealing silica-coated FePt NPs (**2**) in a tube furnace under 4%/96% H_2/Ar. After dissolving the silica shell using NaOH as a base, the ligands on the NPs were exchanged using oleic acid and oleylamine. Then, the NPs **4** could be dispersed again in chloroform. The next step was to react with SUA in order to obtain carboxy-terminated FePt NPs **5** dissolved in water. Biotin-coated $L1_0$ FePt NPs (**6**) were achieved by reacting with amine–PEG–biotin using EDC as a water-soluble crosslinker and AEE as a spacer ligand.

TEM images of the biotin-coated $L1_0$ FePt NPs (**6**) showed good dispersibility with an average diameter of 5.19 ± 1.30 nm (Figure 4). A small increase in particle size was observed, from 3 nm for the fcc FePt NPs (**1**), possibly due to the annealing process at high temperature. However, aggregation could be prevented by employing a nanoreactor silica-coating strategy.

The magnetic properties were examined by using a SQUID magnetometer with an applied field from −50 to 50 kOe. As shown in Figure 5, the biotin-coated $L1_0$ FePt NPs (**6**) behaved as a ferromagnetic material at room temperature (300 K). The coercivity of the NPs was observed as 16 kOe, confirming that Fe and Pt atoms form a well-ordered $L1_0$ structure and may cause high uniaxial magnetocrystalline anisotropy, and this is also responsible for the high coercivity. However, the hysteresis loop shows a kink at low magnetic field. This indicates that

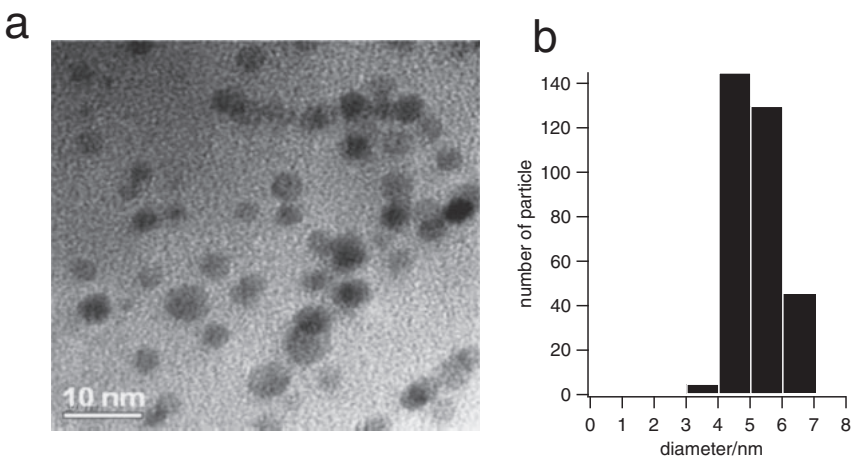

Figure 4. (a) TEM image and (b) the size distribution of biotin-coated $L1_0$ FePt NPs (**6**).

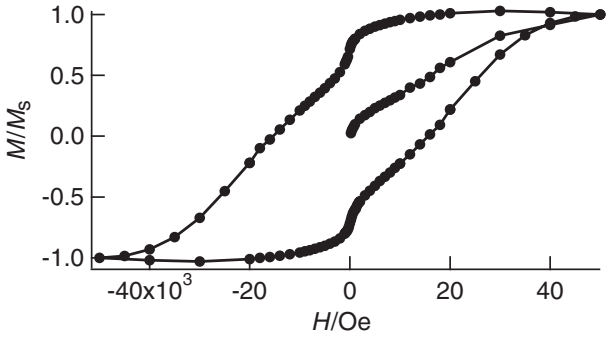

Figure 5. *M–H* plot of biotin-coated *L*1o FePt NPs (**6**) at 300 K.

Table 1. Binding Energies (eV) for Core-Level Electrons of Biotin-Coated *L*1o FePt NPs (**6**)

	C1s			
	N–(C=O)–N	N–C=O	C–O, C–N	CHx
Binding energy/eV	287.1	286.2	285.4	285

the sample includes a magnetically soft phase, i.e., the fcc-phase, in addition to the *L*1o-phase, as confirmed by the order parameter calculation, $S = 0.78$.

XPS was investigated to confirm the existence of coordination bonds between the coating ligands, the biotin molecules and the surface atoms of the core FePt NPs (**6**) (Figure S4, Table 1). The binding energies were found to be in agreement with the values reported in the literature.[18,20] These results confirm that biotin successfully reacted with the terminal carboxy group and that stable FePt NPs were obtained with biotin.

FTIR measurements to further confirm the conformation of the NPs were carried out for the SUA-coated *L*1o FePt NPs (**5**) and biotin-coated *L*1o FePt NPs (**6**), respectively. The differences in the environments of SUA-coated *L*1o FePt NPs (**5**) and biotin-coated *L*1o FePt NPs (**6**) can be deduced from the different peaks observed for the CHx biotin mode around 2960 cm^{-1} in the spectra of biotin-coated *L*1o FePt NPs (**6**) (Figure S5a). A different –OH environment was observed when the peak of the biotin-coated *L*1o FePt NPs (**6**) was shifted a little to the right. A broad –OH mode derived from the carboxy groups was observed around 3420 cm^{-1} in the case of SUA-coated *L*1o FePt NPs (**5**), while that from the spacing ligand AEE was observed around 3440 cm^{-1} (Figure S5b). Furthermore, the peak around 1734 cm^{-1} was only observed in SUA-coated *L*1o FePt NPs (**5**), and is assigned to the C=O of the carboxy groups (Figure S5c). On the other hand, the peak around 1697 cm^{-1} was only observed in biotin-coated *L*1o FePt NPs (**6**), and is assigned to the C=O group from biotin (Figure S5d). These results confirmed that the reaction of SUA-coated *L*1o FePt NPs (**5**) with biotin compounds was successful, and that biotin-coated *L*1o FePt NPs were obtained. Table S1 shows a list of the frequencies of the peaks in the spectra of the SUA-coated *L*1o FePt NPs (**5**) and biotin-coated *L*1o FePt NPs (**6**).

The amount and bioavailability of biotin presented from the biotin-coated *L*1o FePt NPs (**6**) was quantified using the

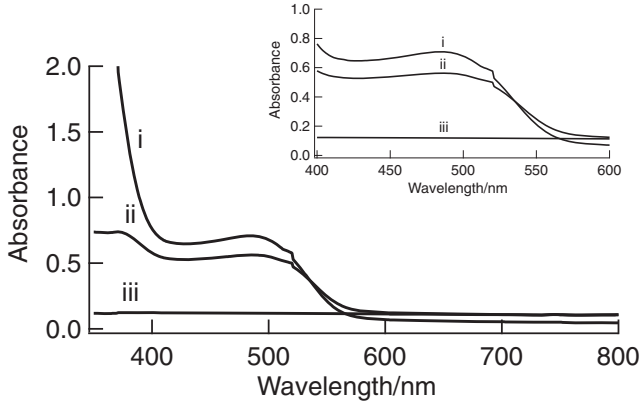

Figure 6. UV–vis spectra of HABA/avidin reagent before and after addition of biotin-coated *L*1o FePt NPs (**6**): (i) HABA/avidin (HA) reagent, (ii) biotin-coated *L*1o FePt–HA, and (iii) biotin-coated *L*1o FePt NPs. Inset is magnification of the spectra.

HABA/avidin binding assay. HABA (4′-hydroxyazobenzene-2-carboxylic acid) is a dye that binds to avidin, and when HABA is reacted with avidin in water, the aqueous solution has a maximum absorbance at 500 nm. Upon the addition of biotin or biotinylated reagents to an HABA/avidin solution, HABA is displaced quantitatively by the available biotin, since the affinity of avidin for biotin ($K_d = 10^{-15}$ M) is much higher than that of HABA ($K_d = 10^{-6}$ M).[18] By monitoring the decrease in UV absorbance at a wavelength of 500 nm, the amount of biotin attached to the FePt NPs could be calculated quantitatively.

After the addition of the biotin-coated *L*1o FePt NPs (**6**), the absorbance value of HABA at 500 nm decreases, indicating that HABA is displaced from the HABA/avidin complex by the available biotin that is present on the surface of the NPs. The results of this analysis are shown in the overlaid UV–vis spectra (Figure 6). The amount of biotin in each biotinylated NPs solution could be calculated by using the following formula:

$$\Delta A_{500} = 0.9A_{\text{HABA/avidin}} + A_{\text{NPs}} - A_{\text{HABA/avidin+NPs}} \quad (2)$$

The amount is calculated as 10 ($\Delta A_{500}/34$) (μmol biotin mL^{-1}), where the dilution factor of HABA/avidin is 0.9, the mM extinction coefficient of biotin at 500 nm is 34, and the dilution factor of NPs is 10. Here, the quantity of available biotin from biotin-coated *L*1o FePt NPs (**6**) could be calculated to be 154.2 μmol g^{-1} of FePt NPs.

Biotin-coated biofunctional *L*1o FePt NPs had been synthesized. The obtained NPs show ferromagnetic properties with coercivity of 16 kOe at room temperature. Biotin-coated *L*1o FePt NPs were biocompatible in an aqueous environment and exhibited site-specific interaction with avidin compounds that have the potential to provide new magnetic materials for biological applications.

Experimental

Materials. The chemicals that were used are products from Sigma-Aldrich, except where stated otherwise. Platinum acetylacetonate, 1,2-hexadecanediol, dioctyl ether, oleic acid, oleylamine, and iron pentacarbonyl ([$Fe(CO)_5$]), tetraethyl

orthosilicate (TEOS), Igepal CO-520, cyclohexane, 4′-hydroxyazobenzene-2-carboxylic acid (HABA)–avidin reagent, 11-sulfanylundecanoic acid (SUA), phosphate buffer solution (PBS), aminoethoxyethanol (AEE), *N*-3-(dimethylaminopropyl)-*N*′-ethylcarbodiimide (EDC), *N*-hydroxysuccinimide (NHS), cetyltrimethylammonium bromide (CTAB), methanol, ethanol, acetone, NH_4OH aqueous solution (EM Science), and EZ-Link Amine–PEG2–Biotin (BA) (Thermo Fischer).

HABA Avidin Binding Assay. The 4′-hydroxyazobenzene-2-carboxylic acid (HABA)/avidin binding assay was performed as described by Green's group.[21,22] The absorbance values of the UV–visible spectra at 500 nm for the HABA/avidin reagent and for the solution after the addition of biotin-coated *L*1o FePt NPs (**6**) were monitored. The change in absorbance (ΔA) at 500 nm of HABA/avidin and after HABA/avidin was added to the surface functionalized NPs should be at 0.1–0.4.

Characterization. The size and morphology and composition of the FePt NPs were characterized using field emission transmission electron microscopy (FETEM) and energy-dispersive X-ray spectrometry (EDX) with a TECNAI F20 (200 kV). X-ray powder diffraction (XRD) patterns were recorded using a Bruker AXS D8-Advanced diffractometer with Cu Kα radiation ($\lambda = 1.5418$ Å). Magnetic studies were performed with an MPMS-XL using a Quantum Design Superconducting Quantum Interface Device (SQUID) with a field up to 50 kOe. X-ray photoelectron spectroscopy (XPS) measurements were performed on a JPS-9000MC (Shimadzu). Fourier transform infrared spectroscopy (FT-IR) measurements were performed using a JASCO-660 Plus. The UV–visible measurements were performed using a JASCO V-560.

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Supporting Information

Additional information regarding characterization are provided. This material is available free of charge on the web at http://www.csj.jp/journals/bcsj/.

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