

New shells for magnetic nanoparticles based on polypyrrole functionalized with α -amino acids

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Abstract

New functionalized magnetic core-shell nanostructures were developed based on magnetite as magnetic core covered with pyrrole copolymers substituted by α -amino acids. The 3-substituted pyrrole monomers were synthesized by amide formation between an α -amino acid and 4-(1-phenylsulfonyl-(1*H*-pyrrol-3-yl)-4-oxo-butyric acid. Further, these substituted pyrrole monomers were copolymerized with unsubstituted pyrrole by oxidation with ammonium persulfate in the presence of water based magnetic nanofluids yielding functionalized magnetic core-shell nanostructures. The morphology of these magnetic functionalized polypyrrole core-shell nanoparticles was investigated by TEM and HRTEM, while the molecular structures and magnetic properties were investigated by FTIR spectroscopy and magnetization measurements, respectively.

Keywords: Magnetic nanoparticles, polypyrroles, pyrroles, α -amino acids, core-shell nanoparticles

Introduction

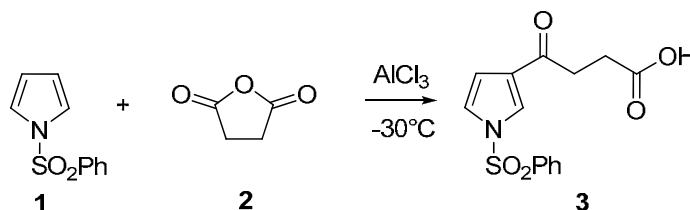
Magnetic nanoparticles (MN) have recently gained broad interest in various fields, such as medicine,¹⁻⁴ biomedicine,⁵⁻¹⁰ in the construction of loudspeakers,^{11, 12} as sealing materials¹³⁻¹⁸ and in sink float separation.¹⁹⁻²² For many medical and biomedical applications it is essential to functionalize their surface. The functions can be directly attached to the magnetic nanoparticles by adsorption or by using polymers, such as polypyrrole (PPy). The latter polymer was found to be biocompatible *in vitro* and *in vivo*^{23, 24} and increasing interest has evolved for biochemical and medical applications. In this field, proteins²⁵⁻³⁰ (glycosidase and other enzymes, collagen,

fibrinogen, heparin), biotin^{31,32} or polysaccharides were immobilized on polypyrrole films. Furthermore DNA chips and arrays were developed on the base of PPy.³³⁻³⁹ Coating with biomolecule-functionalized PPy was recently used to improve the biocompatibility of metallic surfaces in implant material for biomedical application.⁴⁰ Fe₃O₄ nanoparticles coated by biofunctionalized PPy are rare. So far, folic acid⁴¹ and the cancer antibody herceptine⁴² were used to functionalize PPy-Fe₃O₄ nanoparticles. These functions were fixed to the PPy layer of coated nanoparticles by the carbodiimide method. In a preliminary communication we reported PPy-Fe₃O₄ nanoparticles functionalized with *L*-isoleucine without measurement of magnetism.⁴³ Here, we report full experimental details and measuring of magnetism as well as two new examples of 3-substituted PPy-Fe₃O₄ nanoparticles functionalized with *L*-valine and *L*-phenylalanine.

Results and Discussion

Synthesis of 4-oxo-4-[1-(phenylsulfonyl)-1*H*-pyrrol-3-yl]butanoic acid

We tried to introduce the 3-carboxypropionyl group into position 3 of pyrrole by Friedel-Crafts acylation of 1-phenylsulfonylpyrrole with succinic anhydride in the presence of AlCl₃ (Scheme 1). The phenylsulfonyl group is the most commonly used protective group for the pyrrole nitrogen atom in Friedel-Crafts acylation of pyrrole (Py).⁴⁴ However, the formation of mixtures of regioisomeric 2- and 3-substitution products, which are difficult to separate, is a major problem in this chemistry. We tried to overcome this drawback by modifying the reaction conditions performed by M. Kakushima *et al.*,⁴⁵ i.e. by decreasing the reaction temperature to -30 °C and could achieve higher yield (68 %) of the product **3** and eliminate the formation of unwanted 2-acylpyrrole (Scheme 1). In addition, flash column chromatography was used to purify **3**.

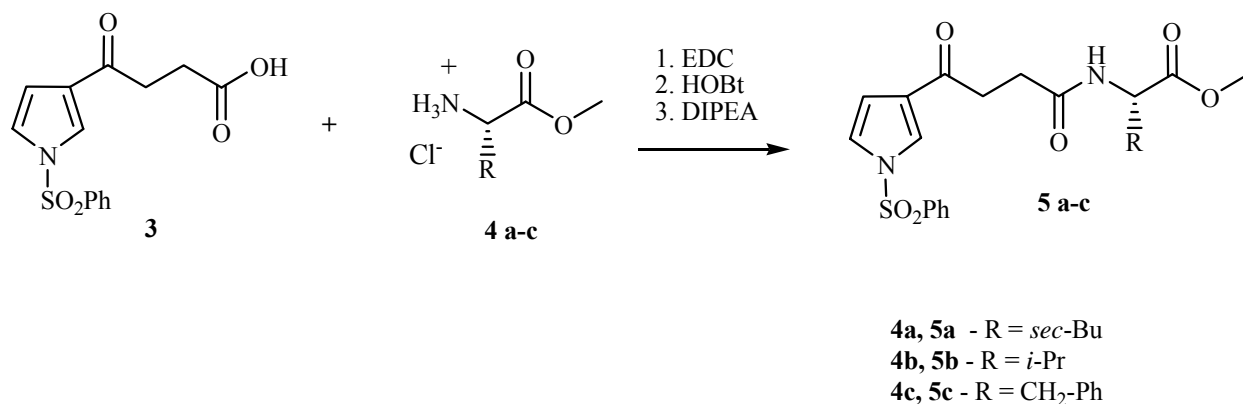


Scheme 1. FC-Acylation of 1-phenylsulfonylpyrrole.

Pyrroles **5** by amino acid coupling to 4-oxo-4-[1-(phenylsulfonyl)-1*H*-pyrrol-3-yl]butanoic acid **3** and their hydrolytic cleavage to unprotected pyrroles **6**

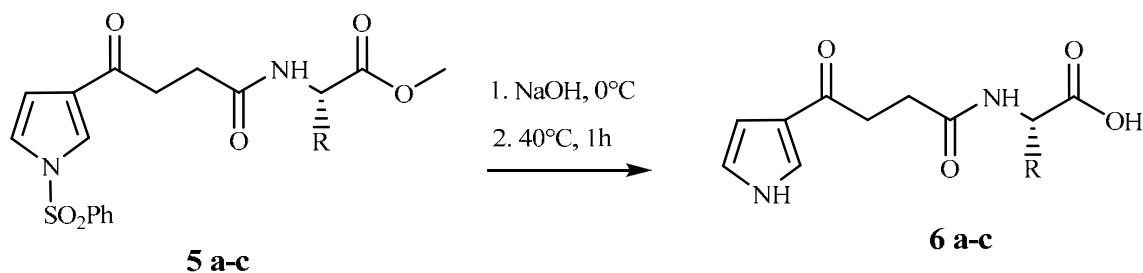
Delabougliose *et al.* reported the synthesis of amino acid-substituted pyrroles and transformed them into PPy polymers by electropolymerisation. They acylated methyl esters of *L*-serine and *L*-valine with 3-pyrrolylacetic acid.⁴⁶ As compared with the 3-pyrrolylacetic acid the pyrrole **3** used

by us as the acid component has a longer linker. Thus the introduced amino acids are far enough from the pyrrole nucleus to avoid possible unwanted change in the conjugation and electronic properties in the resulting polymers by electronic or steric effects. We coupled the methyl esters of *L*-isoleucine **4a**, *L*-valine **4b**, *L*-phenylalanine **4c** with **3** by using an adapted procedure from Abell *et al.*⁴⁷ and obtained **5a-c** in excellent yields (Scheme 2).



Scheme 2. Coupling of amino acid esters **4** with 4-oxo-4-[1-(phenylsulfonyl)-1*H*-pyrrol-3-yl]butanoic acid **3**.

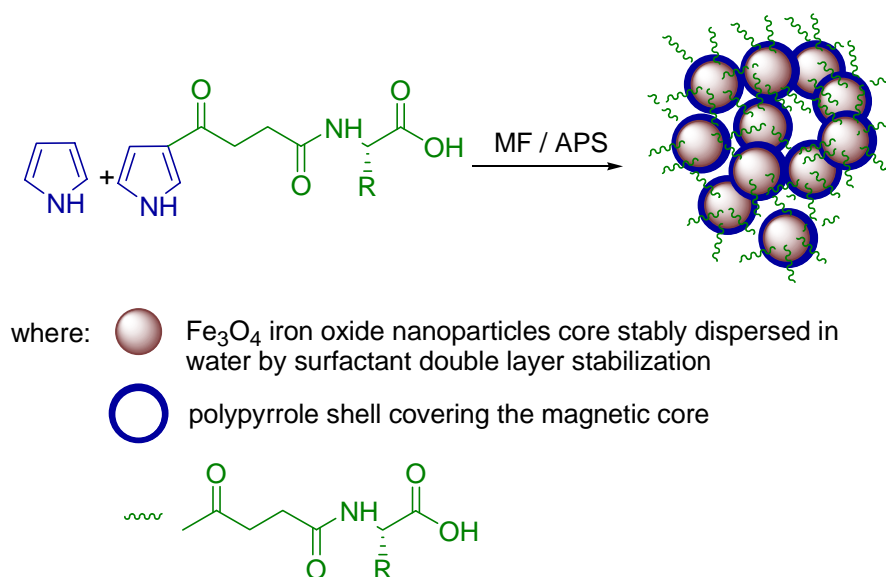
For the envisaged application of these new functionalized pyrroles **5a-c** in copolymerisation reactions with pyrrole it was necessary to remove the 1-phenylsulfonyl protective group. We modified a literature-known method to implement this deprotection under basic conditions (Scheme 3)⁴⁷ and got the free functionalized pyrroles **6a-c** (Table 2). All compounds were purified by flash column chromatography on silica (CH₂Cl₂/MeOH/HCOOH, 90:10:1) affording good yields. Racemization did not occur under these conditions as was checked by chiral HPLC of **6b** and **6c**.



Scheme 3. Deprotection of 3-substituted pyrroles **5**.

Synthesis of functionalized magnetic core-shell nanoparticles based on 3-substituted pyrrole copolymers

The water based magnetic nanofluids (MF) were prepared by a two-stage process, involving first the synthesis of magnetic nanoparticles, followed by their stabilization/dispersion by surfactants. The magnetite nanoparticles (MN) were synthesized through co-precipitation of Fe^{3+} and Fe^{2+} ions in solution with NH_4OH in excess. The temperature was maintained at 80-82 °C, in order to obtain exclusively magnetite nanoparticles and to ensure optimal conditions for chemisorption of the surfactant. Combination of the surfactants lauric acid (LA), dodecylbenzenesulfonate (DBS) and oleic acid (OA), having different chain lengths were used, such as: LA+DBS, LA+LA, DBS+DBS and OA+OA (see also Table 1).^{48, 49} These nanofluids provided the magnetic cores which were covered by copolymer shells obtained from functionalized pyrroles **6** and pyrrole later on. The copolymerisation was carried out in water using ammonium persulfate (APS) as oxidant. The type of substituted pyrrole **6** and the ratio of monomeric pyrroles were varied (Table 1) affording five new core-shell magnetic nanoparticles. These were characterized by FTIR, TEM, HRTEM and measuring of the magnetism.



Scheme 4. Synthesis of functionalized nanoparticles based on 3-substituted pyrrole copolymers.

Table 1. Synthesis of functionalized magnetic nanoparticles

Sample name	Py / substituted Py	Substituted Py monomer	Type of nanofluid
MN-Py-ile	1 / 1	6a	Fe_3O_4 / LA+DBS
MN-Py-val	1 / 1	6b	Fe_3O_4 / LA+LA
MN-Py-phe 1	1 / 1	6c	Fe_3O_4 / DBS+DBS
MN-Py-phe 2	1 / 1	6c	Fe_3O_4 / OA+OA
MN-Py-phe 3	1 / 3	6c	Fe_3O_4 (DBS+DBS)

Morphologic characterization

The functionalized magnetic nanoparticles based on PPy, obtained by the copolymerization of different pyrrole monomers **6** in the presence of Fe₃O₄ nanofluids (Table 1) have a core-shell structure where Fe₃O₄ is the magnetic core and PPy forms a conducting shell. The TEM image of Fe₃O₄ magnetic nanoparticles from the ferrofluid Fe₃O₄ (DBS+DBS) (Figure 1a) shows almost spherical shapes with an average diameter in the range of 5-8 nm. The magnetic nanoparticles **MN-Py-phe 3** covered by copolymer (Figure 1b) have average diameters of 15-25 nm, some of them form aggregates.

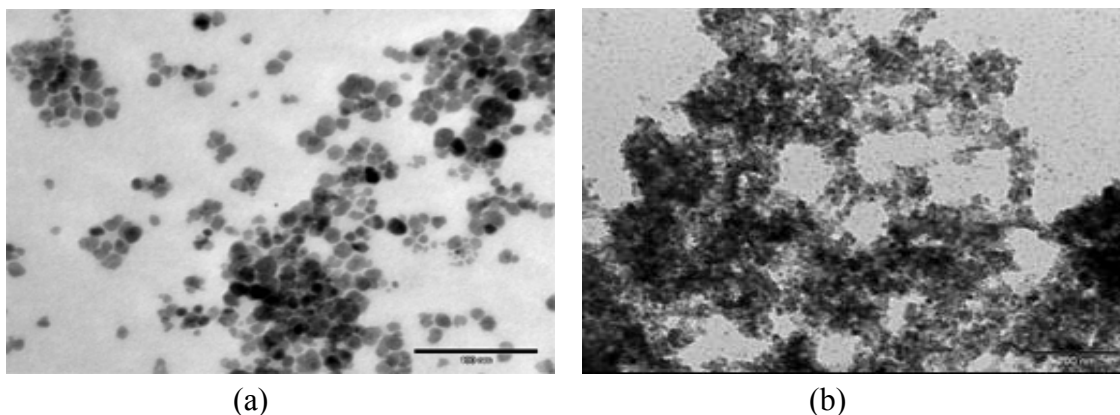


Figure 1. TEM image of (a) magnetic Fe₃O₄-nanoparticles Fe₃O₄ (DBS+DBS) (bar-size 100 nm), (b) functionalized magnetic core-shell nanoparticles **MN-Py-phe 3** (bar-size 200 nm).

The HRTEM images given in Figure 2 show core-shell structures of functionalized magnetic copolymer nanostructures, with a crystalline magnetic core covered by a thin (1.5 - 3 nm) copolymer shell, which is similar to those found in magnetic core-shell nanoparticles formed with unsubstituted pyrrole.

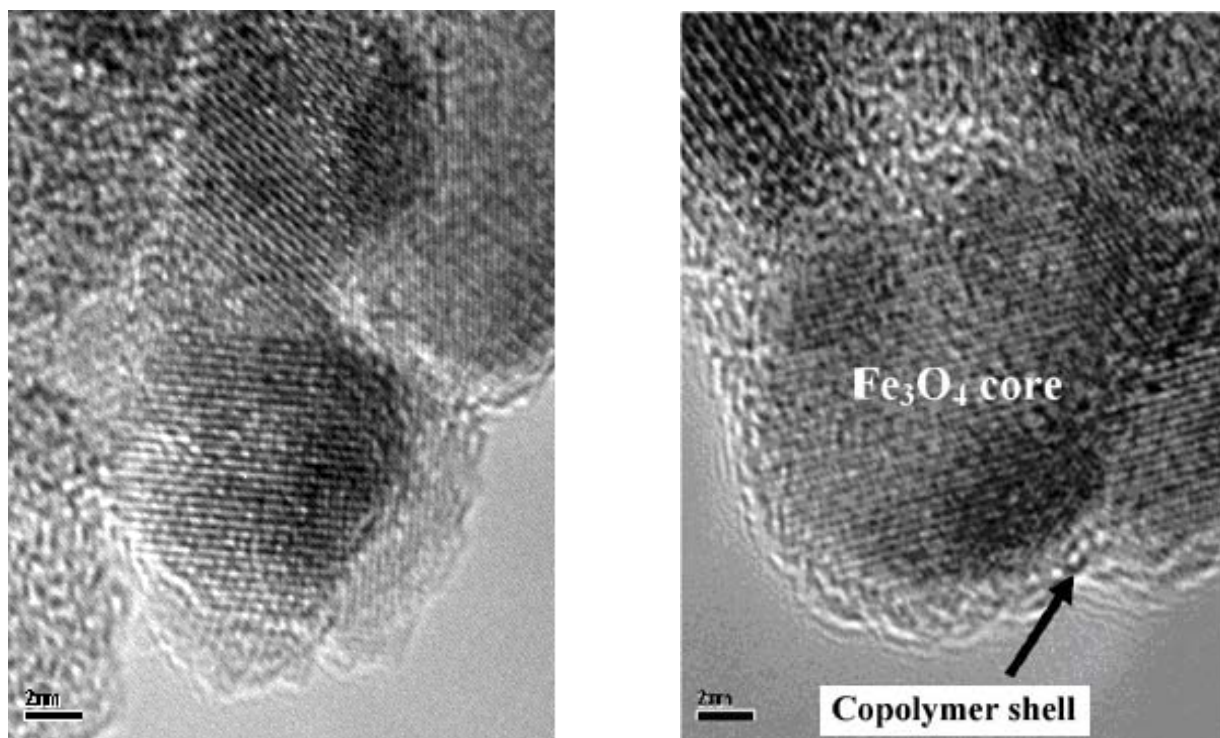


Figure 2. HRTEM images of functionalized magnetic nanoparticles **MN-Py-phe 1**.

FTIR investigations

FTIR spectra of **PPy** and several functionalized core shell PPy MN were recorded (Figure 3). The strong absorption band located at around 570 cm^{-1} in all spectra of functionalized magnetic nanoparticles samples is attributed to Fe_3O_4 . A weak band found at 1702 cm^{-1} in **MN-Py-ile**, 1707 cm^{-1} in **MN-Py-val** and 1711 cm^{-1} in **MN-Py-phe 2** is ascribed to the amide $\text{C}=\text{O}$ bond, demonstrating that the functionalized pyrroles **6** were incorporated. This analytic tool was also used by Lam et al. for the characterisation of copolymers composed of biofunctionalized pyrroles.⁵⁰ However, the ratio of unsubstituted to substituted pyrrole in the copolymer can not be determined in this way. It can be assumed that the substituted pyrroles **6** are less reactive in the copolymerisation than unsubstituted pyrrole and thus the ratio in the copolymer will not be equivalent to the ratio of reactants used in the polymerisation.

The spectra of magnetic nanoparticles **MN-Py-ile**, **MN-Py-val** and **MN-Py-phe 2** containing functionalized polypyrroles show significant changes of the intensities and peak positions of the typical pyrrole ring vibration bands as compared with polypyrrole **PPy**. The peaks located at 914 , 1190 , 1465 cm^{-1} for **PPy** are shifted to higher wave numbers in the functionalized cases. Furthermore, the adsorption bands ascribed to the collective vibration mode of intra-ring and inter-ring $\text{C}=\text{C}/\text{C}-\text{C}$ were shifted from 1548 cm^{-1} in **PPy** to 1553 cm^{-1} in magnetic nanoparticles **MN-Py-phe 2**, to 1557 cm^{-1} in magnetic nanoparticles **MN-Py-val** and to 1561 cm^{-1} in magnetic nanoparticles **MN-Py-ile**. The position of this band is correlated with the conjugation length of

the polymer chain,⁵¹ the shift to higher frequencies indicates a decrease of the conjugation length of the copolymer in the functionalized magnetic nanoparticles as compared to **PPy**.

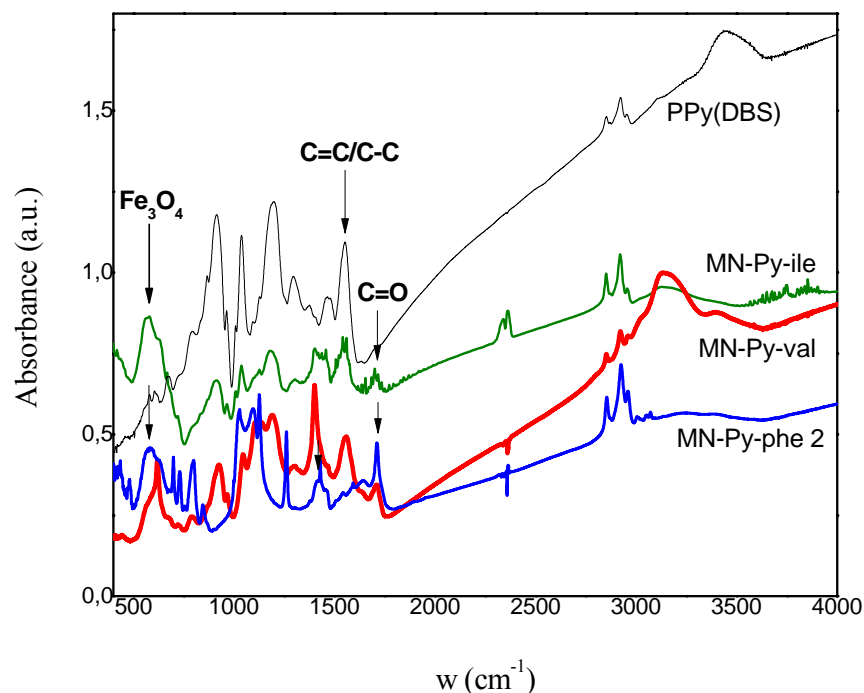


Figure 3. FTIR spectra of functionalized magnetic nanoparticles **MN-Py-ile**, **MN-Py-val**, **MN-Py-phe 2** and mere **PPy** doped with dodecylbenzenesulfonic acid.

Magnetic properties

The typical behaviour of the magnetization *vs.* applied magnetic field of the functionalized magnetic nanocomposites **MN-Py-val**, **MN-Py-phe 1** and **MN-Py-phe2** at room temperature is shown in Figures 4a and 4b, respectively. The values of the magnetization are calculated related to the Fe_3O_4 content of each nanocomposite. For all the reported functionalized magnetic nanocomposites, the magnetization does not show a hysteresis loop, being consistent with superparamagnetic behaviour. The saturation magnetization, M_S for the nanocomposites is found at around 66 emu/g Fe_3O_4 , which is in agreement with that usually reported for nanometric size magnetite.⁵²

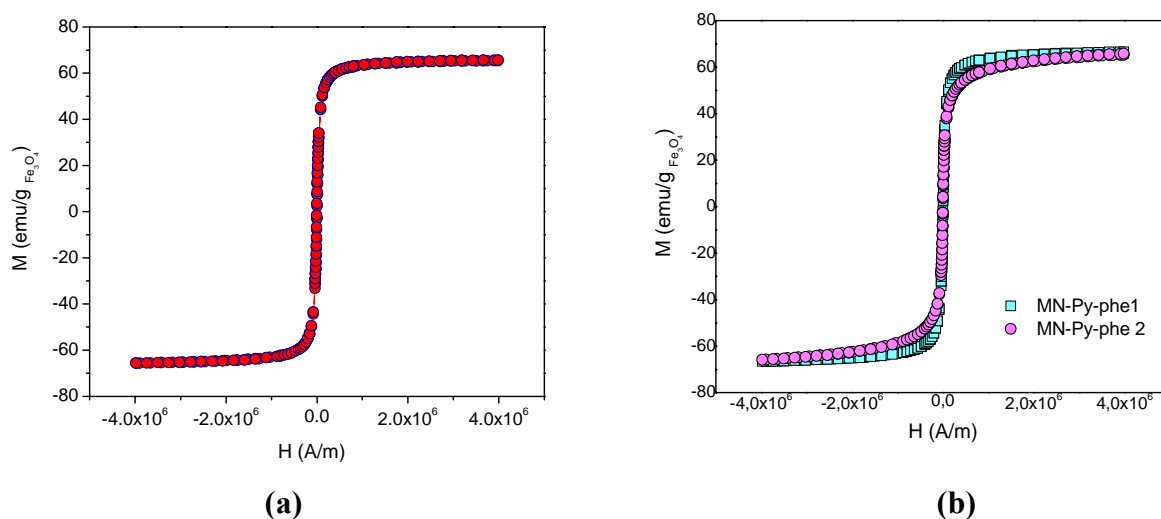


Figure 4. Magnetization vs. applied magnetic field of functionalized magnetic nanocomposite (a) MN-Py-val and (b) MN-Py-phe 1 and MN-Py-phe2 at room temperature.

Conclusions

This study reports a convenient and effective synthetic route to new functionalized pyrrole monomers which are substituted by amino acids via a linker in position 3. Novel functionalized magnetic nanostructures with a magnetite core and different pyrrole copolymer shells were obtained by chemical oxidative polymerization in aqueous solutions in the presence of Fe_3O_4 nanofluid. Formation of the functionalized core-shell magnetic nanoparticles was shown by HRTEM. FTIR spectra exhibited characteristic adsorption bands of both component materials, namely the functionalized polypyrrole and Fe_3O_4 .

The missing hysteresis loop in the magnetization vs. applied magnetic field proved the superparamagnetic behaviour for the functionalized magnetic nanostructures based on functionalized polypyrrole.

Because the synthesis is very flexible, it could also be applied to other amino acids or peptides, in particular to those exhibiting biological recognition functions. Such investigations as well as the application of the magnetic core-shell nanoparticles for the separation of enantiomers are currently underway in our laboratories.

Experimental Section

General Procedures. All reagents were purchased from Aldrich Chemical Company and were used as received without further purification. Solvents were dried by distillation under an inert

atmosphere. All reactions were performed under ambient conditions. $^1\text{H-NMR}$ and $^{13}\text{C-NMR}$ solution spectra were recorded at 300 MHz and 75 MHz, respectively; with a Bruker AC 300 in CDCl_3 as the solvent in 5mm NMR tubes with TMS as internal standard. The morphology of functionalized magnetic nanoparticles based on PPy was determined by TEM using 1010 JEOL microscope and HRTEM using Hitachi H9000NAR transmission electron microscope. FT-IR spectra were obtained with a JASCO FTIR 610 spectrophotometer. The magnetic measurements were performed at room temperature using a Vibrating Sample Magnetometer Cryogenics. The purity of all products was checked by HPLC.

Synthesis of 4-oxo-4-[1-(phenylsulfonyl)-1H-pyrrol-3-yl]butanoic acid (3)

To a suspension of AlCl_3 (4.80 g, 36.0 mmol, 3.0 eq.) in dry dichloromethane (20 ml) succinic anhydride **2** (1.80 g, 18.0 mmol, 1.5 eq.) was added at $-30\text{ }^\circ\text{C}$ and the mixture was stirred at $-30\text{ }^\circ\text{C}$ for 15 min. Subsequently, a solution of 1-(phenylsulfonyl)-1H-pyrrole **1** (2.49 g, 12.0 mmol, 1.0 eq.) in dry dichloromethane (20 ml) was added by an infusion pump over a period of 120 min. After completion of addition, the reaction was allowed to warm to room temperature and the progress of the reaction was followed up by TLC (about 30 minutes). The reaction was quenched with ice and the organic layer was separated. The aqueous layer was extracted with dichloromethane (3 x 20 ml), the combined organic layers were washed with water (2 x 50 ml) and dried over MgSO_4 . Removal of the solvent under reduced pressure resulted in the crude product as a brown oil, which was then purified by flash column chromatography $R_f = 0.11$ ($\text{CH}_2\text{Cl}_2/\text{MeOH}/\text{HCOOH}$, 98:2:0.5) to yield 68 % **3** as a colorless solid (mp = $124\text{--}127\text{ }^\circ\text{C}$). $^1\text{H-NMR}$ (CDCl_3): $\delta = 2.72$ (t, 2H, $J = 6.6$ Hz), 3.07 (t, 2H, $J = 6.6$ Hz), 6.69 (dd, 1H, $J_1 = 1.6$, $J_2 = 3.3$ Hz), 7.15 (dd, 1H, $J_1 = 2.2$, $J_2 = 3.3$ Hz), 7.56 (m, 2H), 7.65 (m, 1H), 7.79 (m, 1H), 7.91 (m, 2H). $^{13}\text{C-NMR}$ (CDCl_3): $\delta = 27.8$ ($\text{CH}_2\text{-CH}_2\text{-COOH}$), 34.1 ($\text{CO-CH}_2\text{-CH}_2$), 112.5 ($\text{CH}_{\text{ar, Py}}$), 121.8 ($\text{CH}_{\text{ar, Py}}$), 124.5 ($\text{CH}_{\text{ar, Py}}$), 127.3 ($2\times\text{CH}_{\text{ar}}$), 128.5 ($\text{C}_{\text{q, ar}}$), 129.9 ($2\times\text{CH}_{\text{ar}}$), 134.8 (CH_{ar}), 138.1 ($\text{C}_{\text{q, ar}}$), 178.6 (C=O), 193.0 (C=O).

Pyrroles **5** by amino acid coupling to 4-oxo-4-[1-(phenylsulfonyl)-1H-pyrrol-3-yl]butanoic acid (3). General procedure A

To a stirred (0.1 M) solution of pyrrole carboxylic acid **3** (1.0 eq. mol) and the *L*-amino acid ester hydrochloride **4a-c** (1.1 eq. mol) in dry dichloromethane 1-ethyl-3-[3-dimethylaminopropyl]carbodiimide (EDC) (1.3 eq. mol), *N*-hydroxybenzotriazole (HOBT) (1.5 eq. mol) and diisopropylamine (1.1 eq. mol) were added under argon at room temperature. The mixture was stirred for 16-18 h and then diluted with the same amount of dichloromethane. The organic layer was separated and was washed with 3 M HCl (2 x 20ml), water (2 x 20 ml), dried with MgSO_4 and evaporated under reduced pressure. The resultant oil was purified by flash column chromatography on silica (cyclohexane/EtOAc, 3:7).

Methyl (2S,3S)-3-methyl-2-((4-oxo-4-[1-(phenylsulfonyl)-1H-pyrrol-3-yl]butanoyl)amino)-pentanoate (5a). According to general procedure A carboxylic acid **3** (1.23 g, 4.0 mmol, 1.0 eq.), *L*-isoleucine methylester hydrochloride **4a** (799 mg, 4.4 mmol, 1.1 eq.), EDC, 807 mg

(5.2 mmol, 1.3 eq.), HOBt (811 mg, 6.0 mmol, 1.5 eq.) and DIPEA (569 mg, 4.4 mmol, 1.1 eq.) in dichloromethane (40 ml) gave 1.78 g (4.0 mmol, quant.) product **5a** as brown oil after washing (2x20 ml 3 M HCl, 2x25 ml water). $[\alpha]_D^{20} = + 8.2^\circ$ (c = 1.01 CH₂Cl₂), R_f = 0.40 (cyclohexane/EtOAc, 3:7). ¹H-NMR (CDCl₃): δ = 0.83 (t, 6H, J = 7.4 Hz), 1.13 (m, 1H), 1.37 (m, 1H), 1.81 (m, 1H), 2.59 (m, 2H), 3.08 (m, 2H), 3.65 (s, 3H), 4.51 (dd, 1H, J₁ = 5.1, J₂ = 8.6 Hz), 6.48 (d, 1H, J = 8.6 Hz), 6.65 (dd, 1H, J₁ = 1.6, J₂ = 3.3 Hz), 7.10 (dd, 1H, J₁ = 2.2, J₂ = 3.3 Hz), 7.50 (m, 2H), 7.61 (m, 1H), 7.77 (m, 1H), 7.87 (m, 2H). ¹³C-NMR (CDCl₃): δ = 11.5 (CH-CH₂-CH₃), 15.4 (CH₂-CH-CH₃), 25.1 (CH-CH₂-CH₃), 29.7 (CH₂-CH₂-CO-NH), 34.7 (CO-CH₂-CH₂), 37.8 (CH-CH₃), 52.0 (OCH₃), 56.5 (C_αH), 112.3 (CH_{ar}, Py), 121.6 (CH_{ar}, Py), 124.5 (CH_{ar}, Py), 127.2 (2xCH_{ar}), 128.6 (C_q, ar), 129.7 (2xCH_{ar}), 134.6 (CH_{ar}), 138.0 (C_q, ar), 171.8 (C=O), 172.5 (C=O), 193.8 (C=O). HRMS (ESI) calculated for C₂₁H₂₇N₂O₆S [M+H]⁺: 435.1584, found: 435.1587.

Methyl (2S)-3-methyl-2-((4-oxo-4-[1-(phenylsulfonyl)-1H-pyrrol-3-yl]butanoyl)amino)-butanoate (5b). According to general procedure A carboxylic acid **3** (1.23 g, 4.0 mmol, 1.0 eq.), L-valine methylester hydrochloride **4b** (738 mg, 4.4 mmol, 1.1 eq.), EDC (807 mg, 5.2 mmol, 1.3 eq.), HOBt (811 mg, 6.0 mmol, 1.5 eq.) and DIPEA (569 mg, 4.4 mmol, 1.1 eq.) in dichloromethane (40 ml) gave 1.68 g (4.0 mmol, quant.) product **5b** as a brown oil after washing (2x20 ml 3 M HCl, 2x25 ml water). Clear highly viscous brown oil, $[\alpha]_D^{20} = + 2.9^\circ$ (c = 1.23 CH₂Cl₂), R_f = 0.40 (cyclohexane/EtOAc, 3:7). ¹H-NMR (CDCl₃): δ = 0.88 (dd, 6H, J₁ = 4.7, J₂ = 6.9 Hz), 2.11 (m, 1H), 2.62 (m, 2H), 3.11 (m, 2H), 3.69 (s, 3H), 4.50 (dd, 1H, J₁ = 5.0, J₂ = 8.7 Hz), 6.34 (m, 1H), 6.68 (dd, 1H, J₁ = 1.6, J₂ = 3.3 Hz), 7.13 (dd, 1H, J₁ = 2.2, J₂ = 3.3 Hz), 7.54 (m, 2H), 7.64 (m, 1H), 7.78 (m, 1H), 7.90 (m, 2H). ¹³C-NMR (CDCl₃): δ = 17.9 (CH₃-CH-CH₃), 19.0 (CH₃-CH-CH₃), 29.8 (CH₂-CH₂-CO-NH), 31.3 (CH₃-CH-CH₃), 34.8 (CO-CH₂-CH₂), 52.2 (OCH₃), 57.2 (C_αH), 112.4 (CH_{ar}, Py), 121.7 (CH_{ar}, Py), 124.5 (CH_{ar}, Py), 127.3 (2xCH_{ar}), 128.7 (C_q, ar), 129.8 (2xCH_{ar}), 134.7 (CH_{ar}), 138.1 (C_q, ar), 172.0 (C=O), 172.6 (C=O), 193.9 (C=O). HRMS (ESI) calculated for C₂₀H₂₅N₂O₆S [M+H]⁺: 421.1428, found: 421.1427.

Methyl (2S)-2-((4-oxo-4-[1-(phenylsulfonyl)-1H-pyrrol-3-yl]butanoyl)amino)-3-phenylpropanoate (5c). According to general procedure A carboxylic acid **3** (1.08 g, 3.50 mmol, 1.0 eq.), L-phenylalanine methylester hydrochloride **4c** (830 mg, 3.85 mmol, 1.1 eq.), EDC (706 mg, 4.55 mmol, 1.3 eq.), HOBt (709 mg, 5.25 mmol, 1.5 eq.) and DIPEA (498 mg, 3.85 mmol, 1.1 eq.) in dichloromethane (35 ml) gave 1.59 g (4.0 mmol, 97 %) product **5c** as brown oil after washing (2x18 ml 3 M HCl, 2x25 ml water) and purification by flash column chromatography. Clear highly viscous brown oil, $[\alpha]_D^{20} = + 48.6^\circ$ (c = 1.00 CH₂Cl₂), R_f = 0.38 (cyclohexane/EtOAc, 3:7). ¹H-NMR (CDCl₃): δ = 2.54 (m, 2H), 3.05 (m, 4H), 3.66 (s, 3H), 4.83 (td, 1H, J₁ = 6.0, J₂ = 7.8 Hz), 6.38 (d, 1H, J = 7.9 Hz), 6.67 (dd, 1H, J₁ = 1.6, J₂ = 3.3 Hz), 7.15 (m, 6H), 7.52 (m, 2H), 7.63 (m, 1H), 7.78 (m, 1H), 7.90 (m, 2H). ¹³C-NMR (CDCl₃): δ = 29.5 (CH₂-CH₂-CO-NH), 34.6 (CO-CH₂-CH₂), 37.9 (CH₂-Ph), 52.3 (OCH₃), 53.2 (C_αH), 112.4 (CH_{ar}, Py), 121.6 (CH_{ar}, Py), 124.5 (CH_{ar}, Py), 127.0 (CH_{ar}), 127.2 (2xCH_{ar}), 128.5 (2xCH_{ar}), 128.6 (C_q, ar), 129.3 (2xCH_{ar}), 129.8 (2xCH_{ar}), 134.7 (CH_{ar}), 135.9 (C_q, ar), 138.0 (C_q, ar), 171.6 (C=O),

172.0 ($\underline{\text{C}}=\text{O}$), 193.7 ($\underline{\text{C}}=\text{O}$). HRMS (ESI) calculated for $\text{C}_{24}\text{H}_{25}\text{N}_2\text{O}_6\text{S}$ $[\text{M}+\text{H}]^+$: 469.1428, found: 469.1429.

Unprotected pyrroles **6** by basic hydrolysis. General procedure B

To a solution of the pyrrole ester **5** (1.0 eq.) in methanol 2 M NaOH (2.5 eq.) was added at 0 °C resulting in a 1:1 mixture (v:v). The mixture was stirred at 40 °C for 1 h, diluted with fivefold amount of water, and the methanol was removed under reduced pressure. The aqueous solution was cooled to 0 °C and acidified with 3 M HCl to pH 2-3 and thoroughly extracted with ethyl acetate. The combined extracts were washed with water (2x), with brine (2x), dried over Na_2SO_4 and evaporated under reduced pressure. The crude product was purified by flash column chromatography.

(2S,3S)-3-Methyl-2-([4-oxo-4-(1H-pyrrol-3-yl)butanoyl]amino)pentanoic acid (6a). The ester **5a** (1.74 g, 4.0 mmol, 1.0 eq.) dissolved in 5 ml methanol was hydrolyzed by general procedure B. Extracting with ethyl acetate (3x20 ml), washing with water (2x25 ml) and brine (2x20 ml), purification by chromatography gave 834 mg (2.98 mmol, 74 %) **6a** as a slightly hygroscopic foam. Pink foam, mp = 47–49 °C, $[\alpha]_D^{25} = + 7.8^\circ$ (c = 1.01 MeOH), $R_f = 0.49$ ($\text{CH}_2\text{Cl}_2/\text{MeOH}/\text{HCOOH}$, 90:10:1). $^1\text{H-NMR}$ (CD_3OD): $\delta = 0.93$ (m, 6H), 1.25 (m, 1H), 1.52 (m, 1H), 1.88 (m, 1H), 2.63 (m, 2H), 3.12 (m, 2H), 4.37 (d, 1H, $J = 5.6$ Hz), 6.58 (dd, 1H, $J_1 = 1.6$, $J_2 = 2.9$ Hz), 6.78 (dd, 1H, $J_1 = 1.8$, $J_2 = 2.9$ Hz), 7.55 (m, 1H). $^{13}\text{C-NMR}$ (CD_3OD): $\delta = 11.9$ ($\text{CH}-\text{CH}_2-\underline{\text{C}}\text{H}_3$), 16.0 ($\text{CH}_2-\text{CH}-\underline{\text{C}}\text{H}_3$), 26.2 ($\text{CH}-\underline{\text{C}}\text{H}_2-\text{CH}_3$), 31.1 ($\text{CH}_2-\underline{\text{C}}\text{H}_2-\text{CO}-\text{NH}$), 35.5 ($\text{CO}-\underline{\text{C}}\text{H}_2-\text{CH}_2$), 38.4 ($\underline{\text{C}}\text{H}-\text{CH}_3$), 58.2 ($\underline{\text{C}}_\alpha\text{H}$), 109.0 ($\underline{\text{C}}\text{H}_{\text{ar, Py}}$), 121.0 ($\underline{\text{C}}\text{H}_{\text{ar, Py}}$), 125.7 ($\underline{\text{C}}\text{H}_{\text{ar, Py}}$), 126.0 ($\underline{\text{C}}_{\text{q, ar}}$), 175.1 ($\underline{\text{C}}=\text{O}$), 175.3 ($\underline{\text{C}}=\text{O}$), 197.3 ($\underline{\text{C}}=\text{O}$). HRMS (ESI) calculated for $\text{C}_{14}\text{H}_{21}\text{N}_2\text{O}_4$ $[\text{M}+\text{H}]^+$: 281.1496, found: 281.1499.

(2S)-3-Methyl-2-([4-oxo-4-(1H-pyrrol-3-yl)butanoyl]amino)butanoic acid (6b). General procedure B was carried out using the ester **5b** (1.539 g, 3.66 mmol, 1.0 eq.) and 2M aqu. NaOH (4.6 ml, 9.2 mmol, 368 mg, 2.5 eq.) to give 686 mg (2.58 mmol, 70 %) **6b** as a slightly hygroscopic foam after purification. White foam, mp = 47–51 °C, $[\alpha]_D^{20} = - 4.9^\circ$ (c = 1.00 AcOH), $R_f = 0.26$ ($\text{CH}_2\text{Cl}_2/\text{MeOH}/\text{HCOOH}$, 90:10:0.5). $^1\text{H-NMR}$ (CD_3OD): $\delta = 0.97$ (d, 6H, $J = 6.9$ Hz), 2.16 (m, 1H), 2.64 (m, 2H), 3.13 (m, 2H), 4.33 (d, 1H, $J = 5.6$ Hz), 6.58 (dd, 1H, $J_1 = 1.6$, $J_2 = 3.0$ Hz), 6.77 (dd, 1H, $J_1 = 1.8$, $J_2 = 3.0$ Hz), 7.55 (t, 1H, $J = 1.7$ Hz). $^{13}\text{C-NMR}$ (CD_3OD): $\delta = 18.3$ ($\text{CH}_3-\text{CH}-\underline{\text{C}}\text{H}_3$), 19.6 ($\underline{\text{C}}\text{H}_3-\text{CH}-\text{CH}_3$), 31.0 ($\text{CH}_2-\underline{\text{C}}\text{H}_2-\text{CO}-\text{NH}$), 31.7 ($\text{CH}_3-\underline{\text{C}}\text{H}-\text{CH}_3$), 35.4 ($\text{CO}-\underline{\text{C}}\text{H}_2-\text{CH}_2$), 59.0 ($\underline{\text{C}}_\alpha\text{H}$), 109.0 ($\underline{\text{C}}\text{H}_{\text{ar, Py}}$), 119.8 ($\underline{\text{C}}\text{H}_{\text{ar, Py}}$), 124.1 ($\underline{\text{C}}\text{H}_{\text{ar, Py}}$), 126.0 ($\underline{\text{C}}_{\text{q, ar}}$), 175.0 ($\underline{\text{C}}=\text{O}$), 175.4 ($\underline{\text{C}}=\text{O}$), 197.2 ($\underline{\text{C}}=\text{O}$). HRMS (ESI) calculated for $\text{C}_{13}\text{H}_{19}\text{N}_2\text{O}_4$ $[\text{M}+\text{H}]^+$: 267.1339, found: 267.1340.

(2S)-2-([4-Oxo-4-(1H-pyrrol-3-yl)butanoyl]amino)-3-phenylpropanoic acid (6c). According to general procedure B the ester **5c** (776 mg, 1.67 mmol, 1.0 eq.) in methanol (2.5 ml), 2 M NaOH (2.5 ml, 4.14 mmol, 166 mg, 2.5 eq.) gave 398 mg (1.27 mmol, 76 %) product **6c** as a slightly hygroscopic foam after extracting with ethyl acetate (3x 15 ml), washing (2x20 ml water, 2x20 ml brine) and purification by flash column chromatography. Yellow foam, mp = 151–152 °C, $[\alpha]_D^{20} = + 23.4^\circ$ (c = 1.00 MeOH), $R_f = 0.29$ ($\text{CH}_2\text{Cl}_2/\text{MeOH}/\text{HCOOH}$, 90:10:0.5). $^1\text{H-NMR}$

(CD₃OD): δ = 2.52 (m, 2H), 3.00 (m, 3H), 3.19 (dd, 1H, J_1 = 5.2, J_2 = 13.9 Hz), 4.67 (dd, 1H, J_1 = 5.2, J_2 = 8.7 Hz), 6.56 (dd, 1H, J_1 = 1.6, J_2 = 3.0 Hz), 6.77 (dd, 1H, J_1 = 1.8, J_2 = 3.0 Hz), 7.22 (m, 5H), 7.50 (t, 1H, J = 1.6 Hz). ¹³C-NMR (CD₃OD): δ = 31.1 (CH₂-CH₂-CO-NH), 35.5 (CO-CH₂-CH₂), 38.5 (CH₂-Ph), 55.1 (C_αH), 109.0 (CH_{ar}, Py), 120.9 (CH_{ar}, Py), 125.6 (CH_{ar}, Py), 125.9 (C_q, ar), 127.7 (CH_{ar}), 129.4 (2xCH_{ar}), 130.3 (2xCH_{ar}), 138.4 (C_q, ar), 174.7 (C=O), 175.0 (C=O), 197.1 (C=O). HRMS (ESI) calculated for C₁₇H₁₉N₂O₄ [M+H]⁺: 315.1339, found:315.1341.

Synthesis of functionalize magnetic core shell nanoparticles (see Table 1). General Procedure. A suspension of pyrrole (1 mmol), 3-substituted pyrrole **6a-c** (1 or 3 mmol) and Fe₃O₄ magnetic nanofluid (0.788 g, 16.07 ml) in distilled water (30 ml) was stirred for 30 minutes. Ammonium persulfate (APS) (1 mmol) was dissolved in distilled water (85 ml) and added drop wise to this preformed mixture (Scheme 4). The reaction proceeded at room temperature under mechanical stirring for 20 h. The resulting black precipitate was separated by magnetic separation, washed with water and ethanol and dried at 60 °C for 24 h.

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