Electronic Supplementary Material (ESI) for RSC Advances.

Supporting Information

Enantiopure Polythiophene Nanoparticles. Chirality Dependence of Cellular Uptake, Intracellular Distribution and Antimicrobial Activity

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I. Synthesis of polymers

General considerations. *N*-bromosuccinimide, sodium bicarbonate, potassium tert-butoxide, (R)-(-)-3-Bromo-2-methyl-1-propanol, (S)-(+)-3-Bromo-2-methyl-1-propanol, Methyl Iodide, Buthyl Iodide, NaH, *n*-butyllithium 2.5 M solution in hexane, 5,5'-bis(tributylstannyl) -2,2'-bithiophene, tetrakis(triphenylphosphine)palladium(0), TRITON X-102 were purchased from Sigma-Aldrich Co; sulfur sublimed washed winnowed 99.5% from Carlo Erba. All reagents and solvents were used as received. All ^{1}H NMR and ^{13}C NMR spectra were recorded on a Varian Mercury-400/500 spectrometer equipped with a 5-mm probe. Chemical shifts were calibrated using the internal $CDCl_3$ resonance which was referenced to TMS. Mass spectra were collected on a Thermo Scientific TRACE 1300 gas chromatograph. UV-Vis spectra were recorded using a Agilent Technologies CARY 100 UV-Vis spectrophotometer. Photoluminescence spectra were collected on a Perkin Elmer LS50 spectrofluorometer. Fluorescence measurements were performed using an excitation wavelength corresponding to the maximum absorption lambda.

General synthesis of compounds 2a, 2b. A solution of potassium tert-butoxide (1.5 mmol) in anhydrous ethanol, was added at 0°C to 3-mercapto-thiophene (1 mmol) (prepared according to J. Am. Chem. Soc., 2011, 133, 8654–8661.) under a nitrogen atmosphere and mixture was stirred for 30 min at 0°C. To the solution 1 mmol of (R)-(-)-3-Bromo-2-methyl-1-propanol or (S)-(+)-3-Bromo-2-methyl-1-propanol was slowly added and refluxed for 2 h before quenching with 100 mL of water. The product was extracted with diethyl ether. The combined organic layers were dried over anhydrous sodium sulphate and the solvent was removed under reduced pressure. The residue were isolated by flash chromatography (90:10 cyclohexane:CH_2Cl_2).

2a. →Yield 90 %. Yellow oil; EI-MS 188 *m/z* xx(M^+); ^{1}H NMR (400 MHz, $CDCl_3$, TMS/ppm): δ 7.31 (m, 1H), 7.15 (m, 1H), 7.02 (m, 1H), 3.60 (m, 2H), 3.00 (q, 1H), 2.77 (q, 1H), 1.92 (m, 1H), 1.02 (d, 3H); ^{13}C NMR (400 MHz, $CDCl_3$): δ 132.1, 129.5, 126.2, 122.9, 66.8, 39.0, 35.6, 16.3.

2b. →Yield 80 %. Brown yellow oil; EI-MS 188 *m/z* xx(M^+); ^{1}H NMR (400 MHz, $CDCl_3$, TMS/ppm): δ 7.31 (m, 1H), 7.15 (m, 1H), 7.02 (m, 1H), 3.60 (m, 2H), 3.00 (q, 1H), 2.77 (q, 1H), 1.92 (m, 1H), 1.02 (d, 3H); ^{13}C NMR (400 MHz, $CDCl_3$): δ 132.1, 129.5, 126.2, 122.9, 66.8, 39.0, 35.6, 16.3.

General synthesis of compounds 3a, 3b. To a solution of **2a** or **2b** (1 mmol) in THF at room temperature, 1.2 mmol of NaH was added. After 30 minutes a CH_3I was slowly added and stirred for 15 hours. Then the reaction mixture was quenched by adding 6 mL of MeOH/$N(Et)_3$ (1:1). The product was extracted with $CHCl_3$ and the solvent removed under reduced pressure. The residue was purified by flash chromatography.

3a-Met. →Yield 60 %. Yellow oil; EI-MS 202 *m/z* xx(M^+); ^{1}H NMR (400 MHz, $CDCl_3$, TMS/ppm): δ 7.29 (m, 1H), 7.12 (m, 1H), 7.02 (m, 1H), 3.32 (s, 1H), 3.30 (s, 4H), 3.05 (q, 1H), 2.71 (q, 1H), 2.00 (m, 1H), 1.04 (d, 3H); ^{13}C NMR (400 MHz, $CDCl_3$): δ 132.5, 129.4, 126.0, 122.3, 76.48, 58.8, 39.1, 33.7, 16.6.

3a-But. →Yield 90 %. Uncoloured oil; EI-MS 244 *m/z* xx(M^+); ^{1}H NMR (500 MHz, $CDCl_3$, TMS/ppm): δ 7.31 (dd, J^3=5 Hz, J^4=3 Hz, 1H), 7.13 (d, J=3 Hz, 1H), 7.02 (d, J=5 Hz 1H), 3.38 (m, 2H), 3.34 (d, 2H), 3.08 (q, 1H), 2.71 (q, 1H), 2.02 (m, 1H), 1.56 (m, 2H), 1.39 (m, 2H), 1. 04 (d, 3H), 0.92 (t, 3H); ^{13}C NMR (500 MHz, $CDCl_3$): δ 132.5, 129.4, 126.0, 122.3, 76.48, 58.8, 39.1, 33.7, 16.6.

3b-Met. → Yield 70 %. Yellow oil; EI-MS 202 *m/z* xx(M^+); ^{1}H NMR (400 MHz, $CDCl_3$, TMS/ppm): δ 7.29 (m, 1H), 7.12 (m, 1H), 7.02 (m, 1H), 3.32 (s, 1H), 3.30 (s, 4H), 3.05 (q, 1H), 2.71 (q, 1H), 2.00 (m, 1H), 1.04 (d, 3H); ^{13}C NMR (400 MHz, $CDCl_3$): δ 132.5, 129.4, 126.0, 122.3, 76.48, 58.8, 39.1, 33.7, 16.6.

3b-But. →Yield 89 %. Uncoloured oil; EI-MS 244 *m/z* xx(M^+); ^{1}H NMR (500 MHz, $CDCl_3$, TMS/ppm): δ 7.31 (dd, J^3=5 Hz, J^4=3 Hz, 1H), 7.13 (d, J=3 Hz, 1H), 7.02 (d, J=5 Hz 1H), 3.38 (m, 2H), 3.34 (d, 2H), 3.08 (q, 1H), 2.71 (q, 1H), 2.02 (m, 1H), 1.56 (m, 2H), 1.39 (m, 2H), 1. 04 (d, 3H), 0.92 (t, 3H); ^{13}C NMR (500 MHz, $CDCl_3$): δ 132.5, 129.4, 126.0, 122.3, 76.48, 58.8, 39.1, 33.7, 16.6.

General synthesis of compounds 4a, 4b: To a solution of **3a** or **3b** (Met or But) (1 mmol) in $CHCl_3$, 2.2 mmol of NBS were added. The reaction mixture was reacted overnight. The solvent was removed and the product purified by flash chromatography.

4a-Met. →Yield 99 %. Colorless oil; EI-MS 358 *m/z* xx(M^+); ^{1}H NMR (400 MHz, $CDCl_3$, TMS/ppm): δ 6.93 (s, 1H), 3.29 (m, 5H), 3.00 (q, 1H), 2.70 (q, 1H), 1.92 (m, 1H), 1.03 (d, 3H); ^{13}C NMR (400 MHz, $CDCl_3$): δ 134.3, 132.1, 112.1, 110.9, 76.1, 58.8, 39.0, 33.9, 16.4.

4a-But. →Yield 99 %. Brown yellow oil; EI-MS 400 *m/z* xx(M^+); ^{1}H NMR (500 MHz, $CDCl_3$, TMS/ppm): δ 6.96 (s, 1H), 3.31 (m, 4H), 3.05 (q, 1H), 2.70 (q, 1H), 1.94 (m, 1H), 1.54 (m, 2H), 1.37 (m, 2H), 1. 03 (d, 3H), 0.94 (t, 3H); ^{13}C NMR (500 MHz, $CDCl_3$): δ 134.6, 132.0, 111.6, 110.9, 74.1, 70.9, 39.1, 34.2, 31.8, 19.4, 16.4, 13.9.

4b-Met. → Yield 96 %. Colorless oil; EI-MS 358 *m/z* xx(M^+); ^{1}H NMR (400 MHz, $CDCl_3$, TMS/ppm): δ 6.93 (s, 1H), 3.29 (m, 5H), 3.00 (q, 1H), 2.70 (q, 1H), 1.92 (m, 1H), 1.03 (d, 3H); ^{13}C NMR (400 MHz, $CDCl_3$): δ 134.3, 132.1, 112.1, 110.9, 76.1, 58.8, 39.0, 33.9, 16.4.

4b-But. →Yield 98 %. Brown yellow oil; EI-MS 400 *m/z* xx(M^+); ^{1}H NMR (500 MHz, $CDCl_3$, TMS/ppm): δ 6.96 (s, 1H), 3.31 (m, 4H), 3.05 (q, 1H), 2.70 (q, 1H), 1.94 (m, 1H), 1.54 (m, 2H), 1.37 (m, 2H), 1. 03 (d, 3H), 0.94 (t, 3H); ^{13}C NMR (500 MHz, $CDCl_3$): δ 134.6, 132.0, 111.6, 110.9, 74.1, 70.9, 39.1, 34.2, 31.8, 19.4, 16.4, 13.9.

General synthesis of polymers 5a,b and 6a,b: A mixture of **4a** or **4b** (Met or But) (1 mmol), 5,5'-bis(tributylstannyl) -2,2'-bithiophene (1 mmol), $Pd(PPh_3)_4$ (0.05 mmol) in Toluene was refluxed for 12 h. The reaction mixture was brought to room temperature and the solvent was evaporated under reduced pressure. The crude product was extracted three times in $CHCl_3/H_2O$, filtered to remove the insoluble fraction and then precipitated for three times by addition of methanol to a solution of the polymers in chloroform.

5a. → Yield 58 %. Dark purple solid. ^{1}H NMR (400 MHz, $CDCl_3$, TMS/ppm): δ 7.10 (m), 3.33 (m), 3.07 (m), 2.78 (m), 2.05 (m), 1.59 (m), 1.31 (m), 1.06 (m), 0.91 (m). GPC: Mw= 4510, Mn= 3110, PD= 1.4.

5b. → Yield 50 %. Dark purple solid. ^{1}H NMR (400 MHz, $CDCl_3$, TMS/ppm): δ 7.08 (m), 3.31 (m), 3.07 (m), 2.78 (m), 2.05 (m), 1.57 (m), 1.33 (m), 1.04 (m), 0.89 (m). GPC: Mw= 3670, Mn= 2740, PD= 1.4.

6a. →Yield 55 %. Dark red solid. ^{1}H NMR (400 MHz, $CDCl_3$, TMS/ppm): δ 7.06 (m), 3.34 (m), 3.08 (m), 2.76 (m), 2.02 (m), 1.54 (m), 1.35 (m), 1. 10 (m), 0.89 (m). GPC: Mw= 4660, Mn= 2100, PD= 2.2.

6b. → Yield 60 %. Dark red solid. ^{1}H NMR (400 MHz, $CDCl_3$, TMS/ppm): δ 7.06 (m), 3.34 (m), 3.08 (m), 2.76 (m), 2.02 (m), 1.54 (m), 1.35 (m), 1. 10 (m), 0.89 (m). GPC: Mw= 3400, Mn= 1660, PD=2.0.

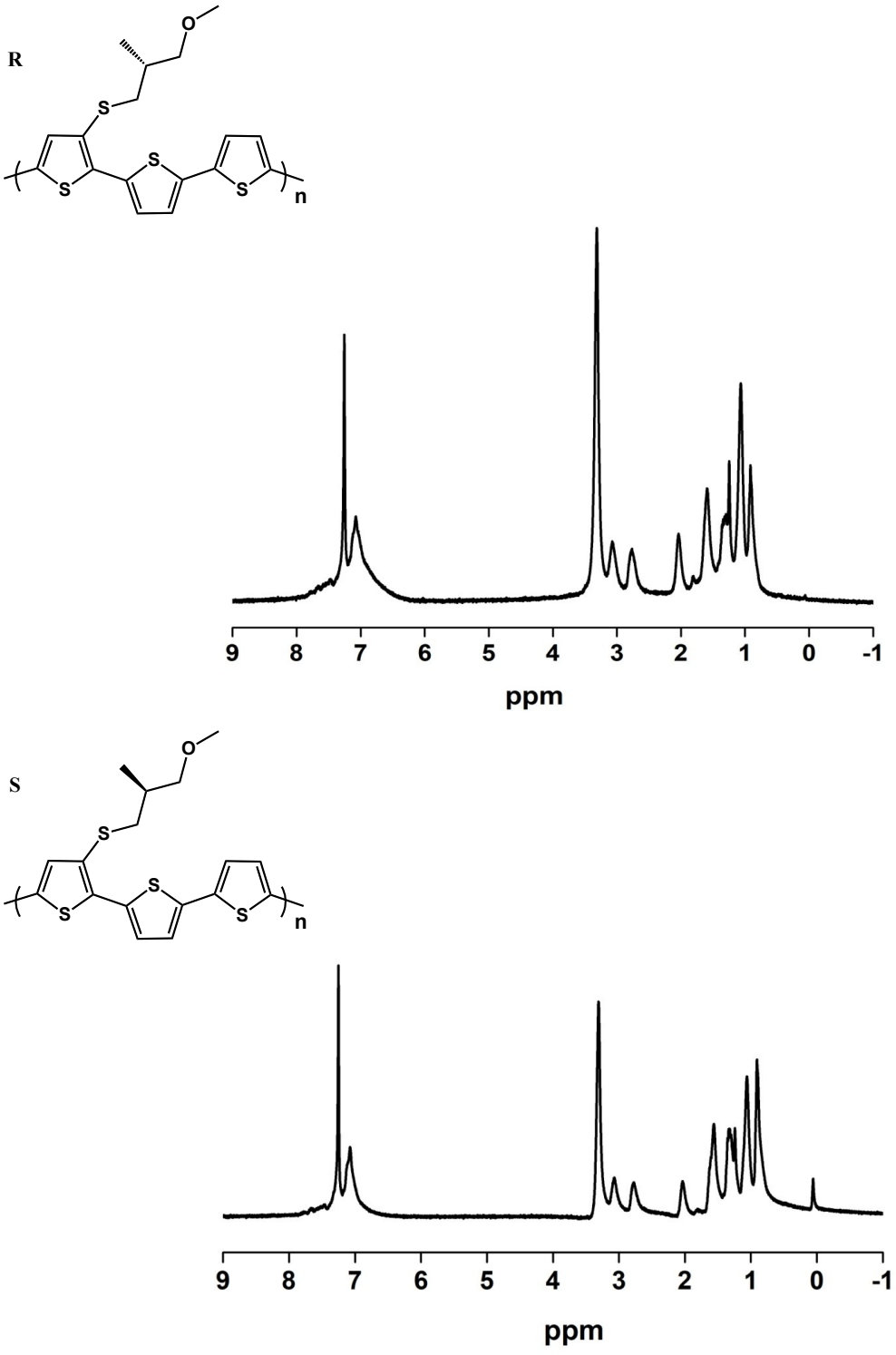


Figure S1. ^{1}H spectra of compounds **5a** and **5b**.

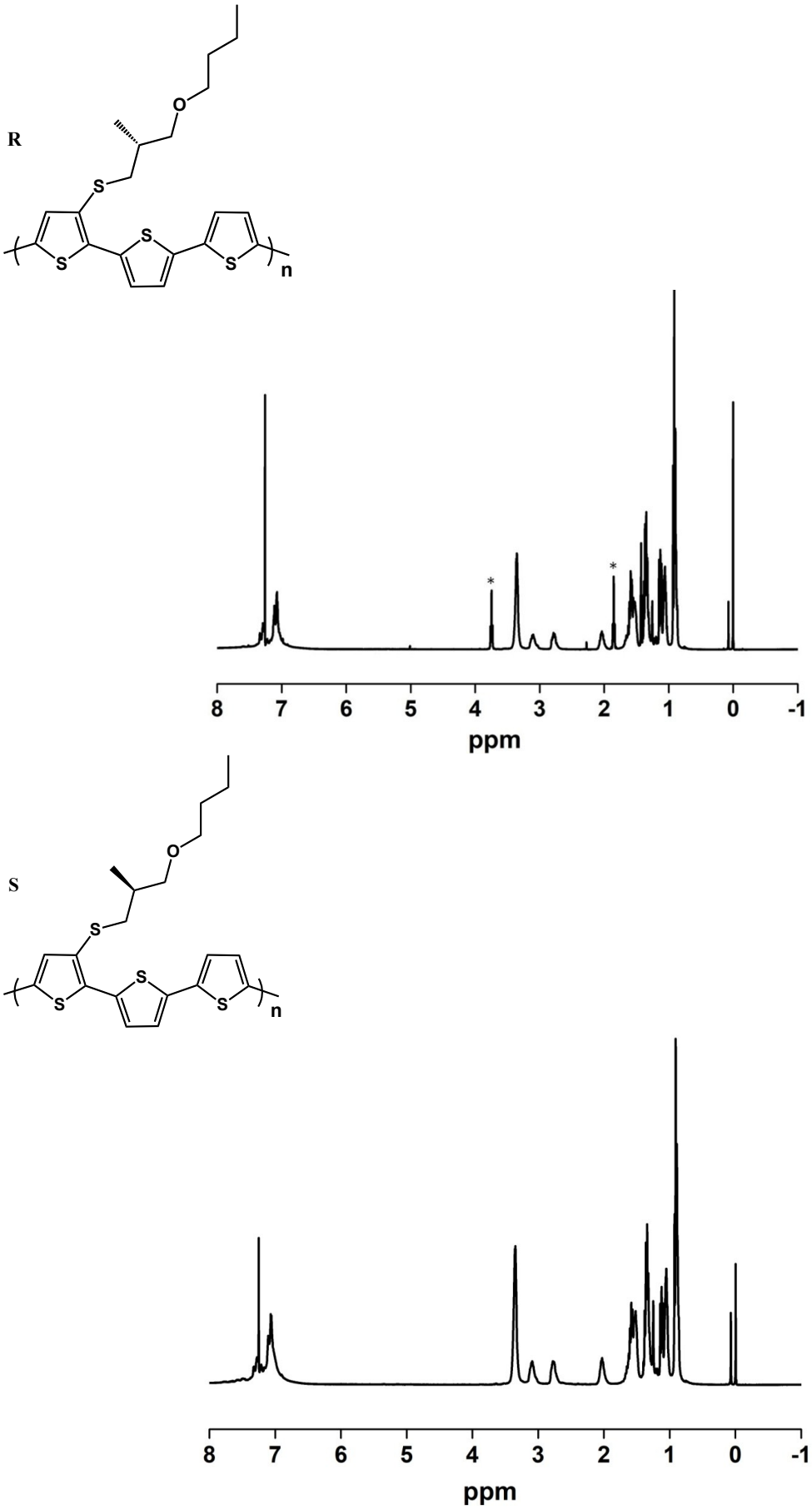


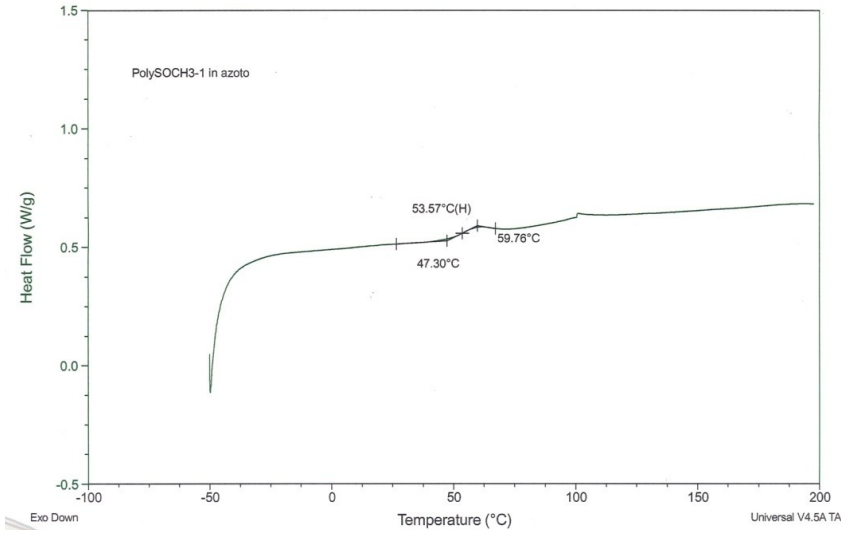
Figure S2. ^{1}H spectra of compounds **6a** and **6b**.

II. Thermal analysis

Table S1. Molecular weights and Thermal Properties of Polymers **5a,6a** and **5b,6b**.

Sample	$\mathbf{M_w}^a$ (g/mol)	$\mathbf{M_n}^a$ (g/mol)	$\mathbf{M_w}^a/\mathbf{M_n}$	$\mathbf{DP_n}^b$	$\mathbf{T_d}^d$ (°C)	$\mathbf{T_g}^d$ (°C)
5a (R)	4510	3110	1.4	8.5	359.2	53.6
5b (S)	3760	2740	1.4	7.5	358.0	69.8
6a (R)	4660	2100	2.2	5.1	371.4	46.5
6b (S)	3400	1660	2.0	4.1	381.6	45.5

[a]By GPC in THF and polystyrene standard. [b]Average polymerization degree relative to repeating units. [c]By TGA. [d] By DSC.



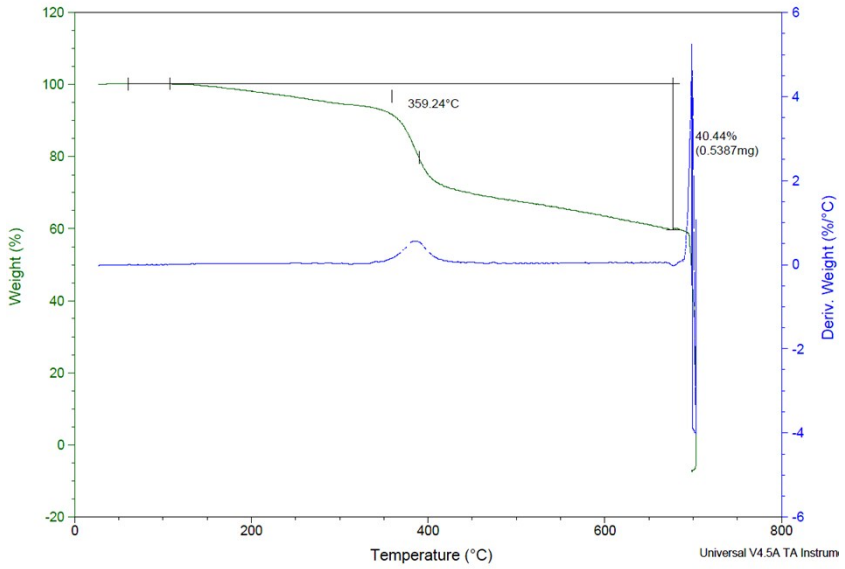


Figure S3. DSC and TGA of **5a**

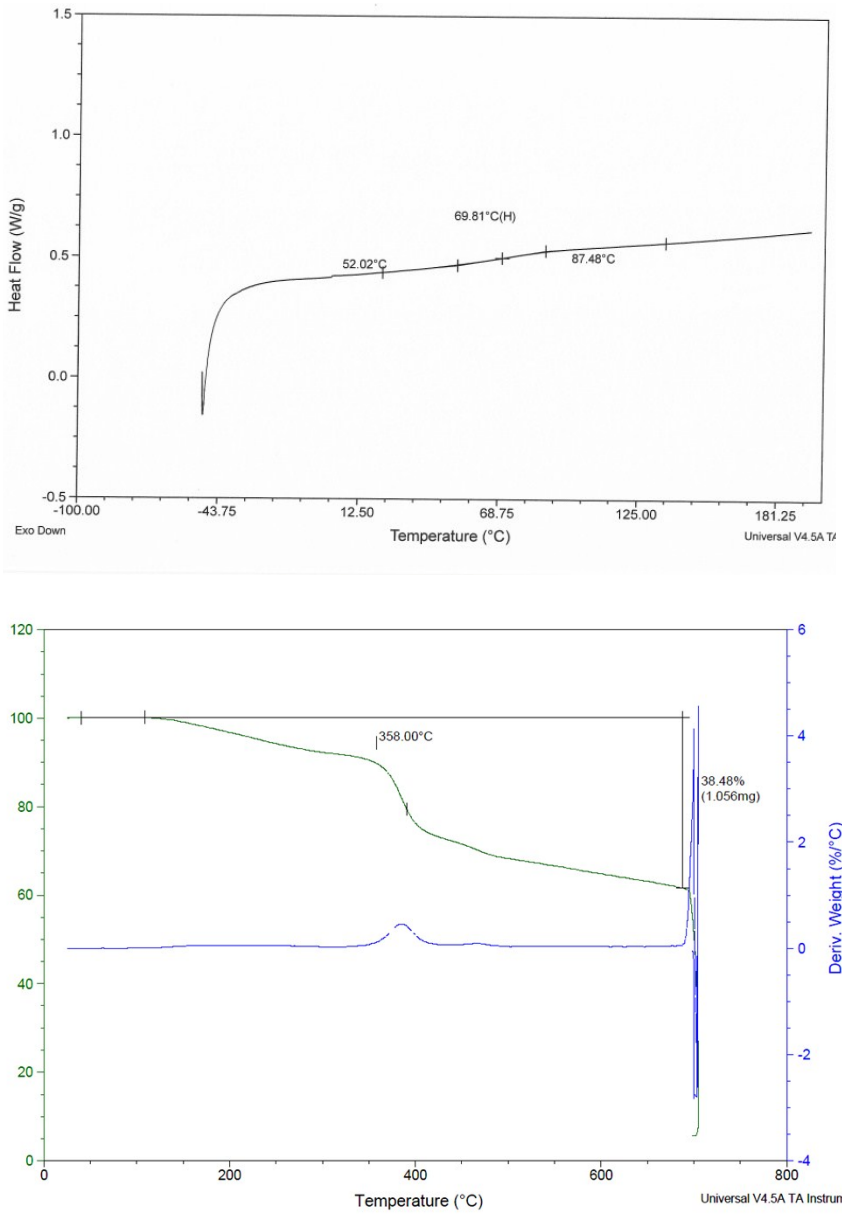
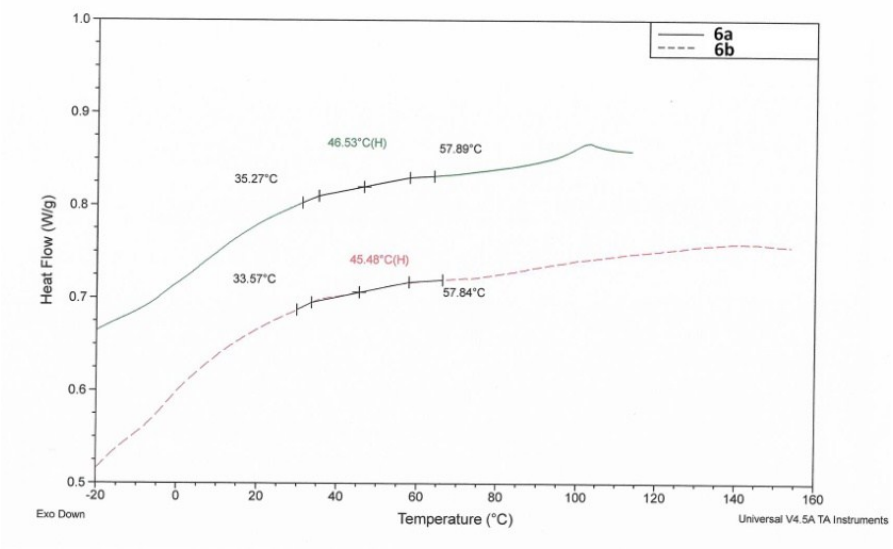


Figure S4. DSC and TGA of **5b**



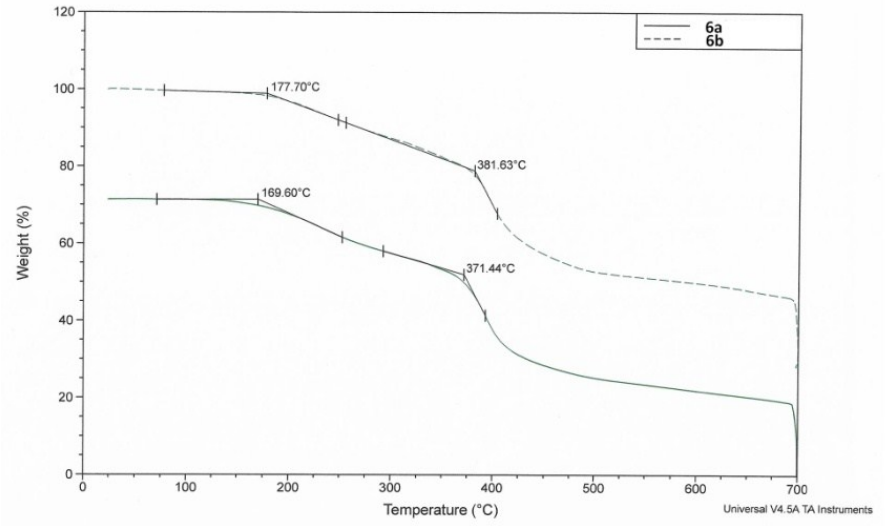
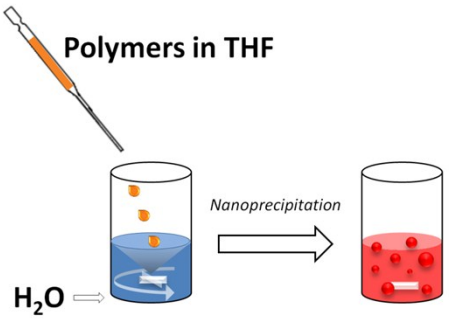


Figure S5. DSC and TGA of **6a** and **6b**

III. Scheme of NPs preparation by reprecipitation method



Scheme S1

Table S2 . Properties of the nanoparticles obtained from polymers **5a** and **5b**

Item	***Particles diameter (nm)***	***Z-potential (mV)***
5a (R)	150±21	-45.0
	200±18	-46.2
	280±19	-38.2
5b (S)	150±15	-38.7
	180±22	-40.1
	250±18	-39.1

IV Circular Dichroism

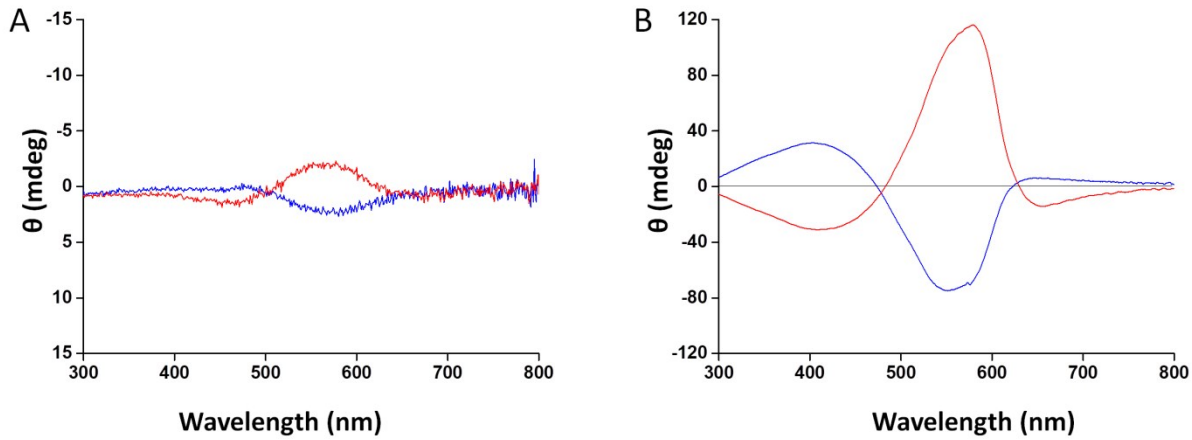
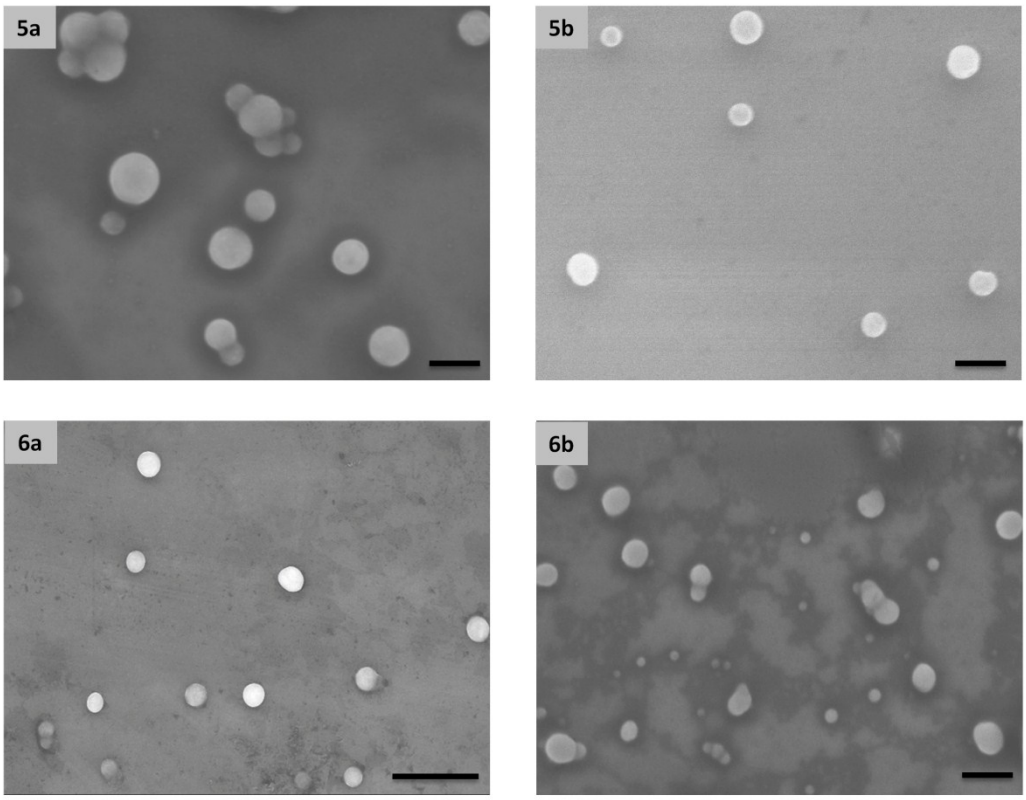


Figure S6. Circular dichroism spectra of polymers **6a** (R, red line) and **6b** (S, blue line) in $CHCl_3$ (A) and as NPs (B).

IV. Scanning Electron Microscopy (SEM) of chiral NPs

SEM characterization has been performed using a ZEISS Leo 1530 scanning electron microscope, equipped with ETH and In-lens detectors for secondary electrons, OXFORD X-Rays EDS spectrometer and CENTAURUS BSE scintillator detector.



Scale bar 200nm

Figure S7. SEM images of the NPs from **5a**,**5b**, **6a** and **6b**, acquired using InLens detector

V. LSCM images of cellular uptake

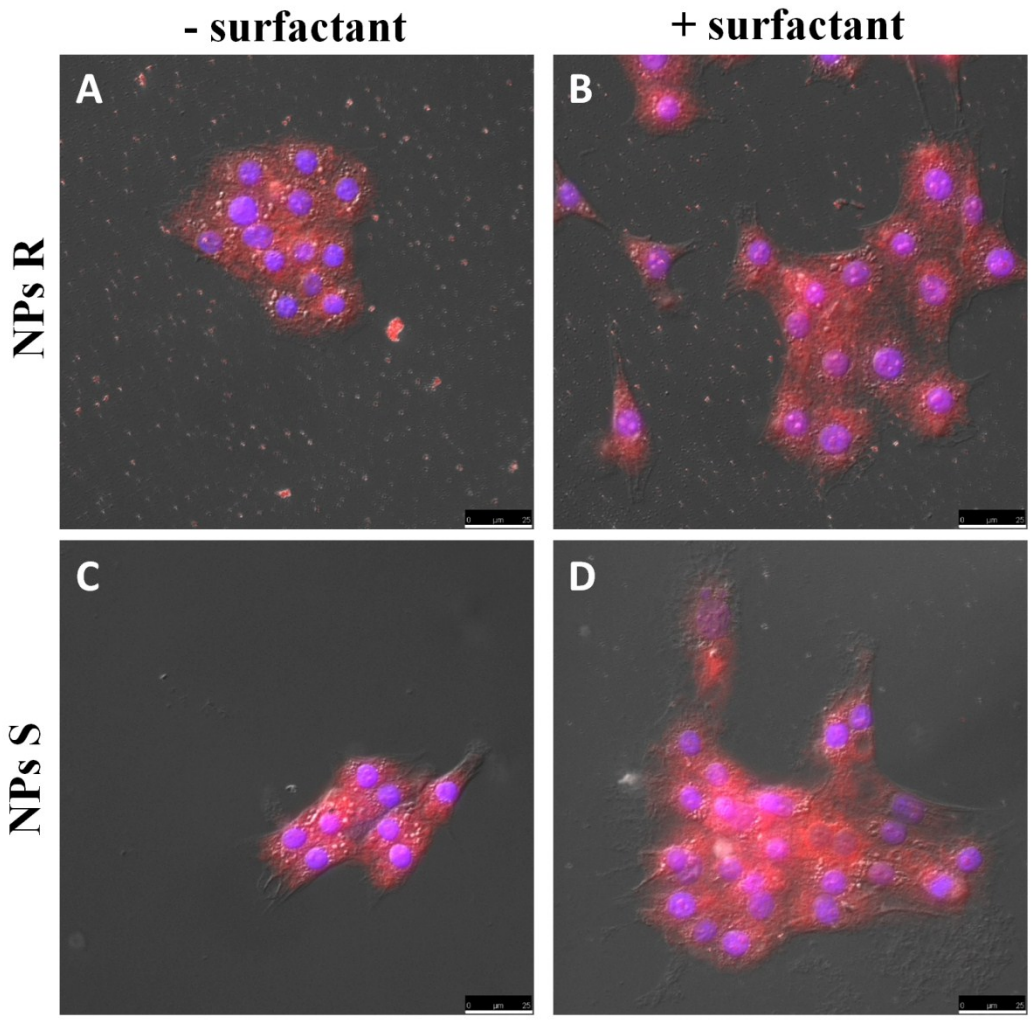


Figure S8. LSCM merge images of cellular uptake of S and R NPs from polymers **5a**,**5b** and without surfactant (A,B) with (B,D) deposited on glass coverslips and after 24 h of incubation with fibroblasts. Cell nuclei were labelled with DAPI (blue). *Scale bars*: 25 µm.