Electronic Supplementary Material (ESI) for Polymer Chemistry.

Supporting information for the manuscript entitled precise synthesis of thermoresponsive polyvinylphosphonate-biomolecule conjugates *via* thiol–ene click chemistry†

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1. Material and methods

General Information

All reactions were carried out under argon atmosphere using standard Schlenk or glovebox techniques. All glassware was heat dried under vacuum prior to use. Unless otherwise stated, all chemicals were purchased from Sigma-Aldrich, ABCR, Acros Organics or TCI Europe and used as received. Toluene, THF and diethyl ether were dried using a MBraun SPS-800 solvent purification system. HPLC grade acetonitrile was purchased from VWR Chemicals and dried prior to use. The precursor complexes $Y(CH_2Si(CH_3)_3)_3(THF)_2$ and $LiCH_2TMS$ and the catalyst $Cp_2Y(CH_2TMS)(THF)$ (**6**) are prepared according to literature procedures.[1-4] Diethyl vinyl phosphonate (DEVP) is synthesised according to literature procedures and dried over calcium hydride and distilled prior to use.[5]

Nuclear Magnetic Resonance Spectroscopy

NMR spectra were recorded on a Bruker AVIII-300, AV-500HD and AVIII-500 Cryo spectrometer. ^{1}H- and ^{13}C-NMR spectroscopic chemical shifts δ are reported in ppm relative to the residual proton signal of the solvent. δ (^{1}H) is calibrated to the residual proton signal, δ (^{13}C) to the carbon signal of the solvent. Unless otherwise stated, coupling constants *J* are averaged values and refer to couplings between two protons. Deuterated solvents were obtained from Sigma-Aldrich and dried over 3 Å molecular sieves.

Mass Spectrometry (ESI-MS)

ESI mass spectra were measured on a Varian 500-MS spectrometer in acetonitrile and methanol.

Elemental Analysis (EA)

Elemental analyses were measured on a Vario EL (Elementar) at the Laboratory for Microanalysis at the Institute of Inorganic Chemistry at the Technische Universität München.

Thin-Layer Chromatography (TLC)

Thin-layer chromatography was performed on either silica coated aluminium plates (0.2 mm, F254) from Macherey-Nagel or aluminium oxide coated aluminium plates (0.2 mm, F254) from Macherey-Nagel. The compounds were detected by UV-light (λ = 254 nm, 366 nm) and by staining with a potassium permanganate solution or ninhydrin solution followed by heat treatment (100-150 °C).

$KMnO_4$-staining solution: 0.50 wt% $KMnO_4$ in 1 M sodium hydroxide solution.

Column Chromatography

Purification *via* column chromatography was performed on silica gel (grain size: 60-200 µm) from Acros Organics or aluminium oxide (activated, neutral; grain size: 50-150 µm) from Sigma-Aldrich. The eluent ratios are given for the corresponding procedures.

Flash Chromatography

Flash chromatography was performed on a chromatography system IntelliFlash 310 from *Varian* with PuriFlash cartridges from Interchim filled with silica gel (grain size: 50 µm). Prior to purification the crude product was dry loaded on silica

gel. Gradients of the eluents hexane and ethyl acetate are given for the corresponding procedures. The compounds were detected by UV-light at a wavelength of 254 nm.

Gel Permeation Chromatography

Gel Permeation Chromatography was performed on a *Varian* LC-920 equipped with two PL Polargel M columns with samples of 5 mg/mL. A mixture of 50% THF, 50% water, 9 g/L tetrabutylammonium bromide (TBAB) and 340 mg/L 3,5-Di-*tert*-butyl-4-hydroxytoluene (BHT) as stabilising agent was used as eluent. Absolute molecular weights have been determined by multiangle light scattering (MALS) analysis using a Wyatt Dawn Heleos II in combination with a Wyatt Optilab rEX as concentration source.

Additionally, GPC measurements were carried out on a PL-GPC 50 System (Agilent Technologies) equipped with two PLgel columns with samples of 5 mg/mL. A mixture of 50% THF, 50% water, 9 g/L TBAB and 340 mg/L BHT as stabilising agent was used as eluent. Absolute molecular weights have been determined by a dual-angle light scattering detector in combination with an integrated RI detection unit as concentration source.

Turbidity Measurements

Turbidity measurements were performed on a Cary 50 UV-vis spectrophotometer (Varian). The cloud point of the aqueous polymer solutions was determined by spectrophotometric detection of the changes in transmittance at $\lambda = 500$ nm. The samples were heated/cooled at a rate of 1.0 K/min in steps of 1 K followed by a five minutes long period of constant temperature to ensure equilibration. The cloud point was defined as the temperature corresponding to a 10% decrease in optical transmittance.

Centrifugation

Separation of solids *via* centrifugation was carried out with the ultracentrifuge Sorvall MX Plus (Thermo Fisher Scientific) as well as the centrifuges Sorvall RC 6 Plus and Heraeus Megafuge 40 centrifuge series from Thermo Fisher Scientific.

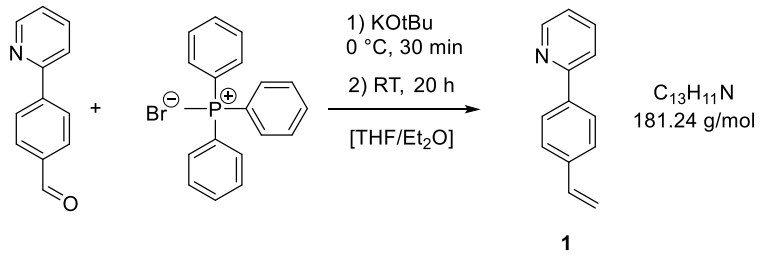
Dialysis

Purification *via* dialysis was performed with a Spectra/Por 1 dialysis tubing (regenerated cellulose) with a molecular weight cut-off (MWCO) of 6-8 kDa (Spectrumlabs). Before use the membranes were treated with deionised water over night and then rinsed with deionised water. A 100:1 ratio of dialysis fluid to sample volume was applied. Specific solvents used as dialysis fluid are given for the corresponding procedures.

2. Syntheses

2.1 Initiator synthesis

2-(4-Vinylphenyl)pyridine (1)[6]



A solution of 4.00 equivalents KO^{t}Bu (6.12 g, 54.5 mmol) in tetrahydrofuran (55.0 mL) was added dropwise to a suspension of methyl triphenylphosphonium bromide (9.72 g, 27.2 mmol, 2.00 eq.) in diethyl ether (250 mL) giving a yellow colored solution and indicating the ylide formation. After stirring for 30 minutes at 0 °C a solution of 4-(pyridin-2-yl)benzaldehyde (2.50 g, 13.6 mmol, 1.00 eq.) in tetrahydrofuran (38.0 mL) was added dropwise. The reaction mixture was stirred over night in the absence of light and then mixed with deionised water (125 mL). After extraction with diethyl ether (three times) the combined organic layers were dried over $MgSO_4$, filtrated and the solvent was removed *in vacuo*. The residue was dissolved in toluene (100 mL), warmed to 40 °C and $MgCl_2$ was added to remove triphenylphosphine oxide. After six hours of stirring the precipitate was removed *via* filtration and the solvent was removed *in vacuo*. The crude product was purified *via* column chromatography (SiO_2, H/EtOAc = 20/1 → 6/1) and lyophilised yielding in a light-yellow oil (1.36 g, 7.53 mmol, 55%).

TLC: R_f = 0.40 (H/Et_2O = 20/1) [UV].

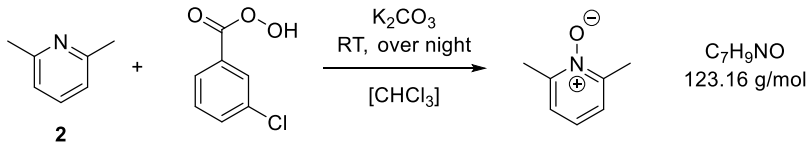
^{1}H-NMR (300 MHz, $CDCl_3$, 300 K): δ (ppm) = 8.70 (d, J_3 = 4.7 Hz, 1H, H_{arom}), 7.98 (d, J_3 = 8.3 Hz, 2H, H_{arom}), 7.83 – 7.69 (m, 2H, H_{arom}), 7.52 (d, J_3 = 8.3 Hz, 2H, H_{arom}), 7.23 (ddd, J = 5.7, 4.8, 2.6 Hz, 1H, H_{arom}), 6.77 (dd, J_3 = 17.9, 10.9 Hz, 1H, H_{vinyl}), 5.83 (d, J_3 = 17.9 Hz, 1H, H_{vinyl}), 5.31 (d, J_3 = 10.9 Hz, 1H, H_{vinyl}).

^{13}C-NMR (76 MHz, $CDCl_3$, 300 K): δ (ppm) = 156.8 (s), 149.5 (s), 138.4 (s), 138.2 (s), 136.9 (s), 136.3 (s), 127.0 (s), 126.6 (s), 122.1 (s), 120.4 (s), 114.5 (s).

ESI-MS: calculated: 182.10 $[M-H]^+$, found: 182.06 $[M-H]^+$.

EA:	calculated:	C 86.15	H 6.12	N 7.73
	found:	C 86.03	H 6.23	N 7.87

2,6-Dimethylpyridine-*N*-oxide[7]

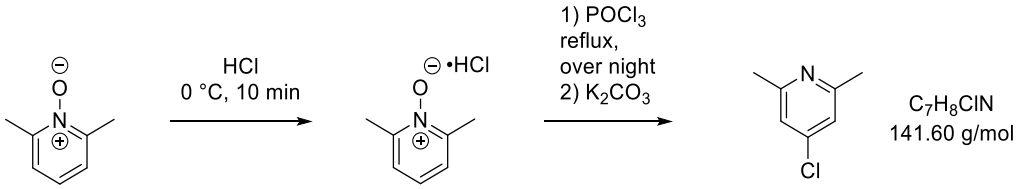


2,6-Dimethylpyridine (**2**) (73.7 mL, 636 mmol, 1.00 eq.) was dissolved in chloroform (250 mL) and then cooled to 0 °C. At this temperature 3-chloroperbenzoic acid (143 g, 636 mmol, 1.00 eq.) was added to the solution and stirred over night at room temperature. The mixture was diluted in chloroform (2000 mL) and K_2CO_3 (352 g, 2.55 mol, 4.00 eq.) was added under vigorous stirring. After 10 minutes, a white solid was separated *via* filtration and washed with 500 mL chloroform. The filtrate was dried over Na_2SO_4, filtrated and the solvent was removed *in vacuo*. The product was obtained as a colorless solid (68.9 g, 559 mmol, 88%).

^{1}H-NMR (500 MHz, $CDCl_3$, 300 K): δ (ppm) = 7.12 (d, J_3 = 7.6 Hz, 2H, H_{arom}), 7.08 – 7.01 (m, 1H, H_{arom}), 2.51 (s, 6H, CH_3).

^{13}C-NMR (126 MHz, $CDCl_3$, 300 K): δ (ppm) = 149.1 (s), 124.8 (s), 124.0 (s), 18.4 (s).

4-Chloro-2,6-dimethylpyridine[8]



At 0 °C 12 M hydrochloric acid (51.1 mL, 614 mmol, 1.10 eq.) was added dropwise to 2,6-dimethyl-pyridine-*N*-oxide (68.7 g, 558 mmol, 1.00 eq.) and stirred for ten minutes. The solid was separated *via* filtration and washed with *iso*-propanol. The solvent of the yellowish filtrate was removed *in vacuo* and the resulting solid was again washed with *iso*-propanol. Drying of the combined salts yielded in a colorless solid (78.1 g, 489 mmol, 88%), which was used without further purification for the chlorination reaction.

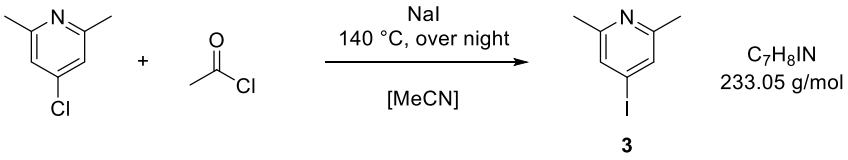
A suspension of 2,6-dimethylpyridine-*N*-oxide hydrochloride (78.0 g, 489 mmol, 1.00 eq.) in 2.50 equivalents of phosphoryl chloride (116 mL, 1.22 mol) was refluxed for 16 hours. Removal of the excess of phosphoryl chloride *in vacuo* resulted in a brown, viscous residue. This residue was slowly added to a mixture of ice and K_2CO_3 at 0 °C under vigorous stirring. By addition of further K_2CO_3 and ice the temperature and an alkaline pH was kept up. The resulting liquid was extracted five times with chloroform and the solvent removed *in vacuo* resulting in a brown oil. The residue was dissolved in ethanol (375 mL), triethylamine (57 mL) was added and the mixture was refluxed for 24 hours. Solvents were removed

in vacuo resulting in a brown oil again. Diethyl ether (250 mL) and water (250 mL) were added to the residue and the aqueous layer was extracted two times with diethyl ether. The combined organic layers were dried over Na_2SO_4, filtrated and the solvent was removed *in vacuo*. Purification of the crude product was performed *via* vacuum distillation (86-88 °C, 54 mbar) yielding in a colorless liquid (41.6 g, 294 mmol, 60%).

^{1}H-NMR (500 MHz, $CDCl_3$, 300 K): δ (ppm) = 6.96 (s, 2H, H_{arom}), 2.47 (s, 6H, CH_3).

^{13}C-NMR (126 MHz, $CDCl_3$, 300 K): δ (ppm) = 159.3 (s), 144.3 (s), 120.6 (s), 24.4 (s).

4-Iodo-2,6-dimethylpyridine (3)[9]



3

Sodium iodide (19.1 g, 127 mmol, 6.00 eq.) was suspended in a solution of 4-chloro-2,6-dimethylpyridine (3.00 g, 21.2 mmol, 1.00 eq.) in acetonitrile (50.0 mL) in an autoclave (stainless steel). To this suspension 1.50 equivalents of acetyl chloride (2.27 mL, 31.8 mmol) were added dropwise and the mixture was heated to 140 °C and stirred over night. After cooling to room temperature aqueous solutions of K_2CO_3 (25.0 mL, 10.0 wt%), K_2SO_3 (25.0 mL, 5.00 wt%) and $K_2S_2O_3$ (20.0 mL, concentrated solution) were added and ethyl acetate was added until phase separation could be observed. The aqueous phase was extracted with ethyl acetate and the combined organic phase was dried over Na_2SO_4. After filtration, the solvent was removed *in vacuo* giving a brown solid. The combined crude products of four experiments were purified *via* flash chromatography (H → H/EtOAc = 10/1) and yielded in light green crystals (7.82 g, 33.6 mmol, 40%).

TLC: R_f = 0.42 (H/EtOAc = 5/1) [UV].

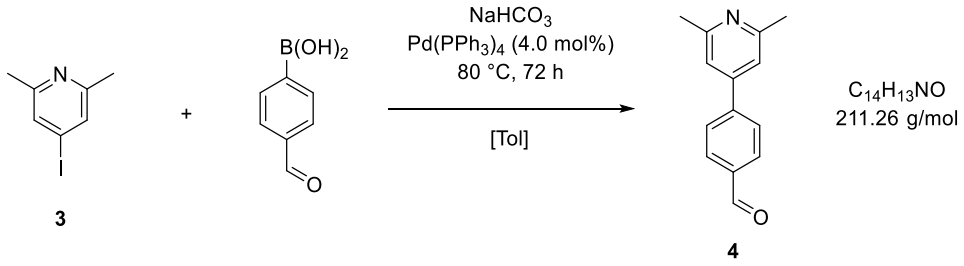
^{1}H-NMR (500 MHz, $CDCl_3$, 300 K): δ (ppm) = 7.37 (s, 2H, H_{arom}), 2.46 (s, 6H, CH_3).

^{13}C-NMR (126 MHz, $CDCl_3$, 300 K): δ (ppm) = 158.7 (s), 129.6 (s), 106.4 (s), 24.1 (s).

ESI-MS: calculated: 233.98 $[M-H]^+$, found: 233.95 $[M-H]^+$.

EA:	calculated:	C 36.08	H 3.46	N 5.74	I 54.45
	found:	C 36.62	H 3.43	N 6.01	I 52.60

4-(2,6-Dimethylpyridin-4-yl)benzaldehyde (4)



3 **4**

A solution of 4-iodo-2,6-dimethylpyridine (**3**) (3.70 g, 15.9 mmol, 1.00 eq.) in toluene (130 mL) was added to a solution of 4-formylphenylboronic acid (2.62 g, 17.5 mmol, 1.10 eq.) in ethanol (30.0 mL) The solution was degassed *via* drawing vacuum and filling with argon (15 iterations). Afterwards catalytic amounts of $Pd(PPh_3)_4$ (730 mg, 640 µmol, 4.00 mol%) were added and the suspension was heated to 80 °C and stirred for 72 hours. After cooling to room temperature decomposed catalyst residues were removed *via* filtration, the mixture was extracted three times against ethyl acetate, the combined organic layers were dried over $MgSO_4$, filtrated and the solvent was removed *in vacuo*. The crude product was purified *via* column chromatography (Alox, H/EtOAc = 10/1 → H/EtOAc = 2/1) yielding in a colorless solid (2.58 g, 12.2 mmol, 77%).

TLC: R_f = 0.34 (H/EtOAc = 5/1) [UV].

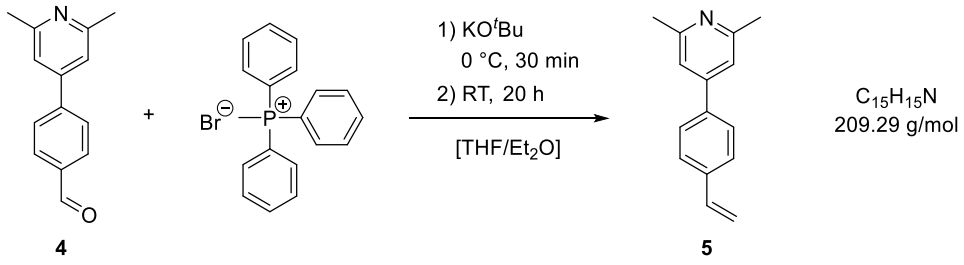
^{1}H-NMR (500 MHz, $CDCl_3$, 300 K): δ (ppm) = 10.08 (s, 1H, $H_{aldehyde}$), 8.07 – 7.90 (m, 2H, H_{arom}), 7.80 – 7.71 (m, 2H, H_{arom}), 7.21 (s, 2H, H_{arom}), 2.61 (s, 6H, CH_3).

^{13}C-NMR (126 MHz, $CDCl_3$, 300 K): δ (ppm) = 191.9 (s), 158.7 (s), 144.8 (s), 136.7 (s), 130.5 (s), 127.9 (s), 118.6 (s), 24.8 (s).

ESI-MS: calculated: 212.11 $[M-H]^+$, found: 212.07 $[M-H]^+$.

EA:	calculated:	C 79.59	H 6.20	N 6.63
	found:	C 79.42	H 6.25	N 6.49

2,6-Dimethyl-4-(4-vinylphenyl)pyridine (5)



4 **5**

A solution of 4.00 equivalents KO^{t}Bu (11.5 g, 102 mmol) in tetrahydrofuran (103 mL) was added dropwise to a suspension of methyl triphenylphosphonium bromide (18.3 g, 51.2 mmol, 2.00 eq.) in diethyl ether (470 mL) giving a yellow colored

solution and indicating the ylide formation. After stirring for 30 minutes at 0 °C a solution of 4-(2,6-dimethylpyridin-4-yl)benzaldehyde (**4**) (5.40 g, 25.6 mmol, 1.00 eq.) in tetrahydrofuran (70.0 mL) was added dropwise. The reaction mixture was stirred over night in the absence of light and mixed with deionised water (235 mL). After extraction with diethyl ether (three times), the combined organic layers were dried over $MgSO_4$, filtrated and the solvent was removed *in vacuo*. The crude product was purified *via* column chromatography (Alox, H/EtOAc = 10/1) yielding in a colorless solid (3.76 g, 18.0 mmol, 70%).

TLC: R_f = 0.57 (H/EtOAc =5/1) [UV].

^{1}H-NMR (300 MHz, C_6D_6, 300 K): δ (ppm) = 7.38 – 7.25 (m, 4H, H_{arom}), 6.94 (s, 2H, H_{arom}), 6.63 (dd, J_3 = 17.6 Hz, 10.9 Hz, 1H, H_{vinyl}), 5.68 (dd, J_3 = 17.6 Hz, J_2 = 0.9 Hz, 1H, H_{vinyl}), 5.14 (dd, J_3 = 10.9 Hz, J_2 = 0.9 Hz, 1H, H_{vinyl}), 2.52 (s, 6H, CH_3).

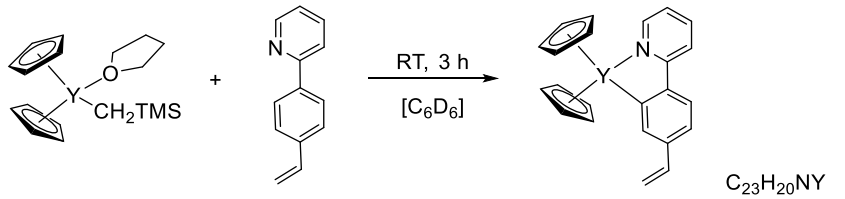
^{13}C-NMR (76 MHz, C_6D_6, 300 K): δ (ppm) = 158.6 (s), 148.3 (s), 138.7 (s), 138.2 (s), 136.7 (s), 127.5 (s), 127.1 (s), 118.1 (s), 114.5 (s), 24.7 (s).

ESI-MS: calculated: 210.13 $[M-H]^+$, found: 210.13 $[M-H]^+$.

EA:	calculated:	C 86.08	H 7.22	N 6.69
	found:	C 86.22	H 7.42	N 6.58

2.2 Complex synthesis

$Cp_2YC_{13}H_{10}N$ (7)



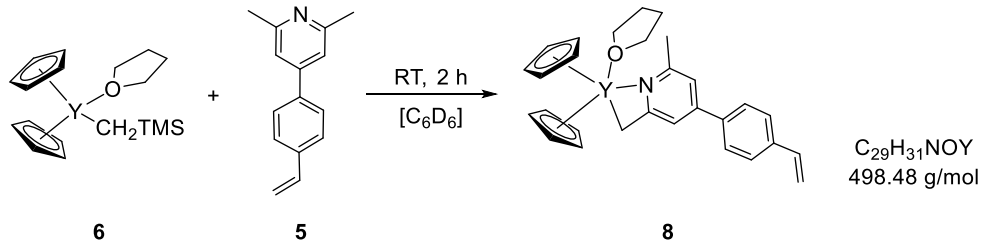
6 **1** **7**

2-(4-Vinylphenyl)pyridine (**1**) (4.78 mg, 26.4 µmol, 1.00 eq.) was dissolved in C_6D_6 (0.50 mL) and added to $Cp_2YCH_2TMS(THF)$ (**6**) (10.0 mg, 26.4 µmol, 1.00 eq.) at room temperature. The solution showed an instant orange coloring. After three hours ^{1}H-NMR spectroscopy showed quantitative conversion. The pure compound (100%) was received after removal of the solvent *in vacuo* as an orange solid.

^{1}H-NMR (500 MHz, C_6D_6, 300 K): δ (ppm) = 8.58 – 8.50 (m, 1H, H_{arom}), 8.05 (d, J_3 = 8.3 Hz, 2H, H_{arom}), 7.30 (dd, J_3 = 8.3, 3.7 Hz, 3H, H_{arom}), 6.83 – 6.50 (m, 2H, H_{arom}, H_{vinyl}), 6.18 (s, 10H, Cp-H), 5.63 (m, J_3 = 18.5 Hz, 1H, H_{vinyl}), 5.10 (d, J_3 = 11.8 Hz, 1H, H_{vinyl}).

EA:	calculated:	C 69.18	H 5.05	N 3.51
	found:	C 68.31	H 5.22	N 3.37

$Cp_2YC_{15}H_{14}N(THF)$ (8)



6 **5** **8**

2,6-Dimethyl-4-(4-vinylphenyl)pyridine (**5**) (5.53 mg, 26.4 µmol, 1.00 eq.) was dissolved in C_6D_6 (0.50 mL) and added to $Cp_2YCH_2TMS(THF)$ (**6**) (10.0 mg, 26.4 µmol, 1.00 eq) at room temperature. The solution showed an instant orange coloring. After two hours ^{1}H-NMR spectroscopy showed quantitative conversion. The pure compound (100%) was received after removal of the solvent *in vacuo* as an orange solid.

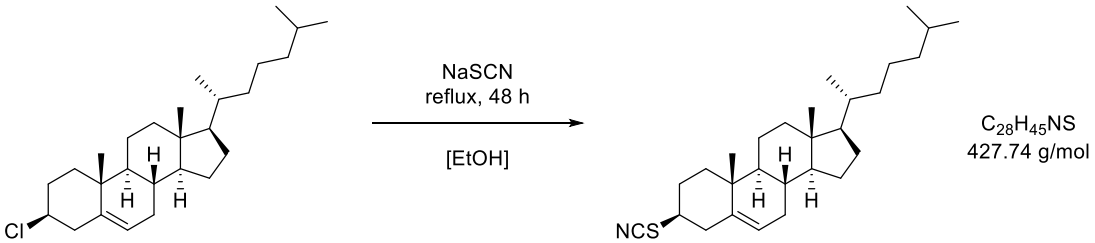
^{1}H NMR (500 MHz, C_6D_6, 300 K): δ (ppm) = 7.46 (d, J_3 = 8.3 Hz, 2H, H_{arom}), 7.27 (d, J_3 = 8.3 Hz, 2H, H_{arom}), 6.87 (s, 1H, H_{arom}), 6.62 (dd, J_3 = 17.6, 10.9 Hz, 1H, H_{vinyl}), 6.34 (s, 1H, H_{arom}), 6.06 (s, 10H, Cp-H), 5.65 (dd, J_3 = 17.6 Hz, J_2 = 1.0 Hz, 1H, H_{vinyl}), 5.12 (dd, J_3 = 10.9 Hz, J_2 = 1.0 Hz, 1H, H_{vinyl}), 3.48 – 3.37 (m, 4H, THF), 2.41 (s, 2H, CH_2), 2.14 (s, 2H, CH_3), 1.37 – 1.16 (m, 4H, THF).

^{13}C-NMR (126 MHz, C_6D_6, 300 K) δ (ppm) =167.4 (s, C_{arom}), 157.5 (s, C_{arom}), 148.5 (s, C_{arom}), 139.4 (s, C_{arom}), 138.1 (s, C_{arom}), 136.8 (s, C_{vinyl}), 127.4 (s, C_{arom}), 127.0 (s, C_{arom}), 114.0 (s, C_{vinyl}), 110.8 (s, C_{arom}), 110.3 (s, Cp-C), 108.1 (s, C_{arom}), 70.2 (s, THF), 42.7 (d, J_{CY} = 10.7 Hz. CH_2), 25.6 (s, THF), 24.1 (s, CH_3).

EA:	calculated:	C 69.88	H 6.27	N 2.81
	found:	C 69.83	H 6.36	N 2.82

2.3 Synthesis of functionalised biomolecules

Cholesteryl thiocyanate[10]



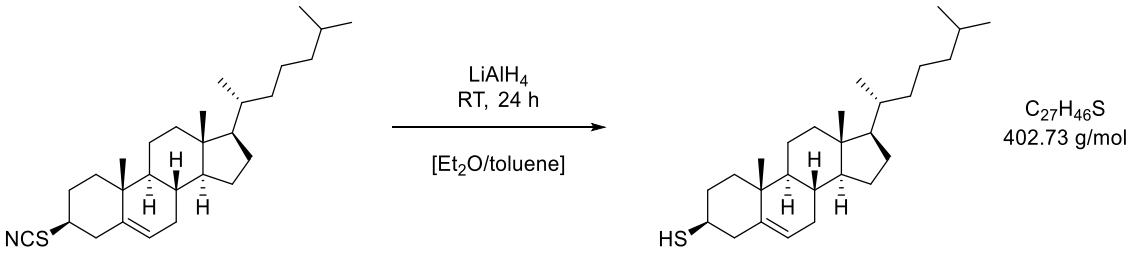
Cholesteryl chloride (12.5 g, 31.0 mmol, 1.00 eq.) was added to a solution of NaSCN (9.40 g, 1.16 mol, 37.4 eq.) in ethanol (600 mL) and was refluxed at 100 °C for 48 hours. Solids were removed *via* hot filtration and washed with warm ethanol. After the product had precipitated from the filtrate it was recrystallised from a 1:1 mixture of ethyl acetate and ethanol and was obtained as light-yellow solid (11.3 g, 26.4 mmol, 85%).

^{1}H-NMR (300 MHz, $CDCl_3$, 300 K): δ (ppm) = 5.41 (d, J_3 = 5.1 Hz, 1H, H_{vinyl}), 3.08 (tt, J_3 = 12.3, 4.2 Hz, 1H, C*H*SCN), 2.61 – 2.36 (m, 2H, chol), 2.09 – 1.74 (m, 6H, chol), 1.62 – 0.98 (m, 23H, chol, CH_3), 0.91 (d, J_3 = 6.5 Hz, 3H, CH_3), 0.87 (s, 3H, CH_3), 0.85 (s, 3H, CH_3), 0.68 (s, 3H, CH_3).

^{13}C-NMR (75 MHz, $CDCl_3$, 300 K): δ (ppm) = 140.1 (s), 123.3 (s), 111.4 (s), 56.8 (s), 56.3 (s), 50.2 (s), 48.2 (s), 42.4 (s), 39.9 (s), 39.8 (s), 39.7 (s), 39.5 (s), 36.6 (s), 36.3 (s), 35.9 (s), 31.9 (s), 31.8 (s), 30.1 (s), 28.4 (s), 28.2 (s), 24.4 (s), 23.9 (s), 22.9 (s), 22.7 (s), 21.0 (s), 19.3 (s), 18.9 (s), 12.0 (s).

EA:	calculated:	C 78.63	H 10.60	N 3.27	S 7.50
	found:	C 78.73	H 10.86	N 3.17	S 7.29

Thiocholesterol[10]



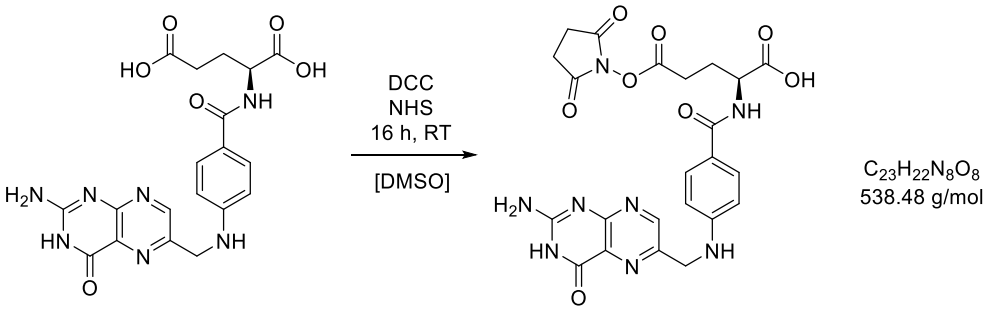
Over a period of two hours a solution of cholesteryl thiocyanate (5.00 g, 11.7 mmol, 1.00 eq.) in toluene (50.0 mL) was added dropwise to a suspension of $LiAlH_4$ (1.00 g, 26.4 mmol, 2.30 eq.) in 100 mL diethyl ether. The suspension was stirred for 24 hours at room temperature and the reaction quenched by slow addition of 50.0 mL 6 N HCl. The organic phase was washed three times with water (150 mL), dried over $MgSO_4$ and filtrated. The solvent was removed *in vacuo*, the crude product was recrystallised from a 3:1 mixture of ethanol and ethyl acetate and thiocholesterol (3.47 g, 8.60 mmol, 74%) was obtained as colorless solid.

^{1}H-NMR (300 MHz, $CDCl_3$, 300 K): δ (ppm) = 5.32 (dd, J_3 = 5.2, 1.6 Hz, 1H, H_{vinyl}), 2.76 – 2.60 (m, 1H, C*H*SH), 2.35 – 2.23 (m, 2H, CH_2CHSH), 2.08 – 1.74 (m, 5H, chol), 1.64 – 1.02 (m, 22H, chol), 1.00 (s, 3H, CH_3), 0.91 (d, J = 6.5 Hz, 3H, CH_3), 0.86 (d, J_3 = 6.6 Hz, 3H, CH_3), 0.86 (d, J_3 = 6.6 Hz, 3H, CH_3), 0.67 (s, 3H, CH_3).

^{13}C-NMR (75 MHz, $CDCl_3$, 300 K): δ (ppm) = 142.1 (s), 121.2 (s), 56.9 (s), 56.3 (s), 50.4 (s), 44.3 (s), 42.5 (s), 40.1 (s), 39.9 (s), 39.7 (s), 39.6 (s), 36.5 (s), 36.3 (s), 35.9 (s), 34.2 (s), 31.9 (s), 28.4 (s), 28.2 (s), 24.4 (s), 24.0 (s), 22.9 (s), 22.7 (s), 21.0 (s), 19.4 (s), 18.9 (s), 12.0 (s).

EA:	calculated:	C 80.53	H 11.51	S 7.96
	found:	C 80.44	H 11.74	S 7.63

Folate-(*γ*)-NHS[11, 12]



Folic acid (3.00 g, 6.80 mmol,1.00 eq.) was dissolved in dimethyl sulfoxide (50.0 mL) by light heating. After complete dissolution 2.10 g dicyclohexylcarbodiimide (10.2 mmol, 1.50 eq.) and 1.17 g *N*-hydroxysuccinimide (10.2 mmol, 1.50 eq.) were added successively and the mixture was stirred for 16 hours at room temperature. The resulting urea was removed by filtration, the folate derivative was obtained *via* precipitation with excess acetone under vigorous stirring and washed with diethyl ether four times. After drying folate-NHS (3.15 g, 5.85 mmol, 86%) was obtained as a yellow solid.

^{1}H-NMR (400 MHz, DMSO-d_6, 300 K): δ (ppm) = 8.65 (s, 1H, C*H*$_{pteridine}$), 7.79 – 7.53 (m, 2H, H$_{arom}$), 7.04 – 6.83 (br s, 3H, CH_2N*H*), 6.71 – 6.53 (m, 2H, H$_{arom}$), 4.88 (dd, J_3 = 7.3 Hz, 1H, C*H*NH(C=O)), 4.49 (d, J_3 = 6.2 Hz, 2H, C*H*$_2$NH), 3.11 – 2.66 (m, 2H, C*H*$_2$CH$_2$CHNH), 2.81 (s, 4H, C*H*$_2$C*H*$_2$), 2.36 – 1.85 (m, 2H, CH$_2$C*H*$_2$CHNH).

^{13}C-NMR (126 MHz, DMSO-d_6, 300 K): δ (ppm) = 172.8 (s, C=O), 170.2 (s, C =O), 170.1 (s, C =O), 166.8 (s, *C*=O), 160.9 (s, C=O$_{pteridine}$), 153.8 (s), 150.9 (s), 148.7(s, *C*H$_{pteridine}$), 129.1 (s), 128.0 (s), 121.2 (s), 111.2 (s), 51.5 (s, CH$_2$CH$_2$*C*H), 45.0 (s, *C*H$_2$NH), 30.5 (s, *C*H$_2$CH$_2$CH), 27.5 (s, CH$_2$*C*H$_2$CH), 25.8 (s, *C*.

3. Polymerisation investigations

3.1 Kinetic measurements of DEVP polymerisations

A solution of the corresponding initiator (21.7 µmol, 1.00 eq.) in 5.00 mL toluene was added to a solution of 21.7 µmol catalyst (1.00 eq.) in 5.00 mL toluene at room temperature and showed an instant orange coloring. The mixture was stirred over night and quantitative conversion was confirmed by ^{1}H-NMR spectroscopy. DEVP (13.0 mmol, 600 eq.) was added in one portion and aliquots were taken from the reaction solution at regular time intervals and quenched by pouring the sample into MeOH. The conversion of DEVP of each aliquot was determined by ^{31}P-NMR spectroscopy. The molecular weight of the polymer samples was determined by GPC-MALS analysis after removal of the solvent *via* drying at ambient temperature.

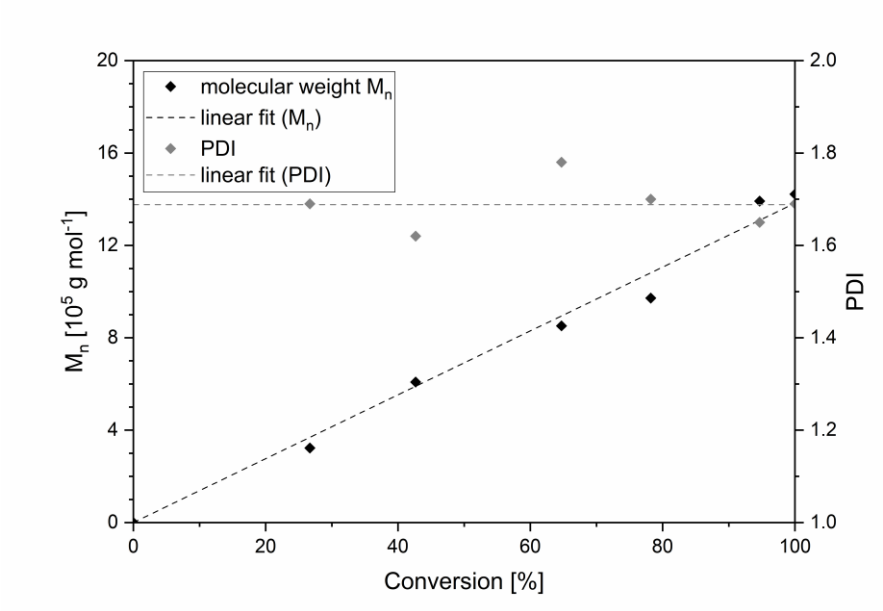


Figure S1: Conversion-dependent plot of M_n and the respective PDI of the polymer aliquots generated during kinetic investigations using *in situ* generated $Cp_2YC_{13}H_{10}N$, (21.7 µmol catalyst, 600 eq. DEVP in 10.0 mL toluene, 30 °C).

3.2 Polymerisation procedure and analysis

A solution of 2,6-dimethyl-4-(4-vinylphenyl)pyridine (65.1 µmol, 1.00 eq.) in 5.00 mL toluene was added to a solution of 65.1 µmol $Cp_2Y(C_2TMS)(THF)$ (1.00 eq.) in toluene (5.00 mL). After quantitative conversion was shown by ^{1}H-NMR spectroscopy, DEVP (6.51 mmol, 100 eq.) was added in one portion and the conversion of DEVP was determined by ^{31}P-NMR spectroscopy after three hours. The reaction was quenched by addition of methanol (0.50 mL) and the polymer was precipitated by pouring the reaction mixture into pentane (150 mL). The clear solution was decanted of, residual solvent was removed by drying at ambient temperature and the polymer was dissolved in water and lyophilised. Molecular weights of the obtained polymers were determined by GPC-MALS and the determination of the cloud points was carried out *via* turbidity measurements.

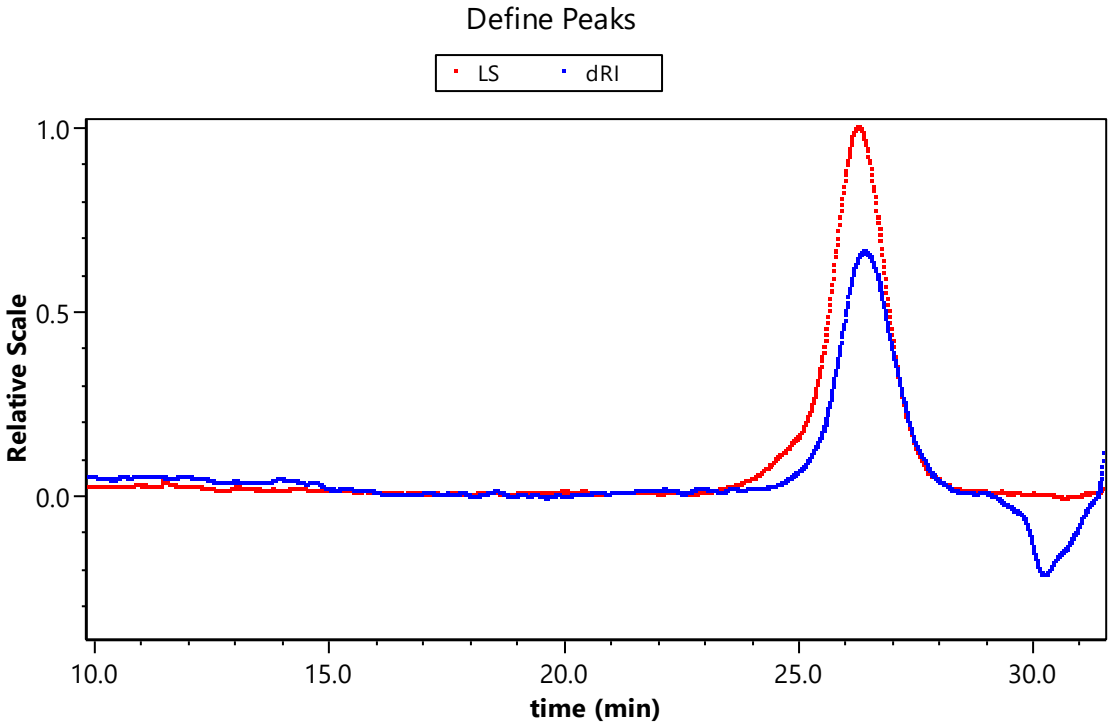


Figure S2: GPC-traces of PDEVP (100 eq. DEVP; entry 1, table 1).

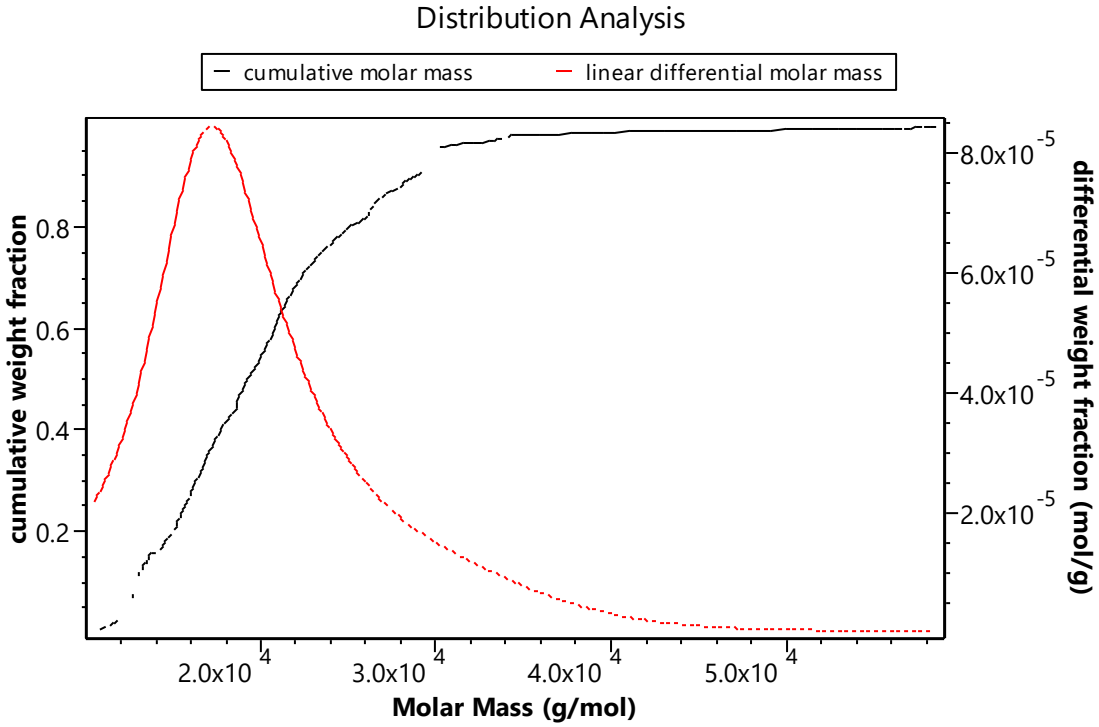


Figure S3: Distribution plot of PDEVP (100 eq. DEVP).

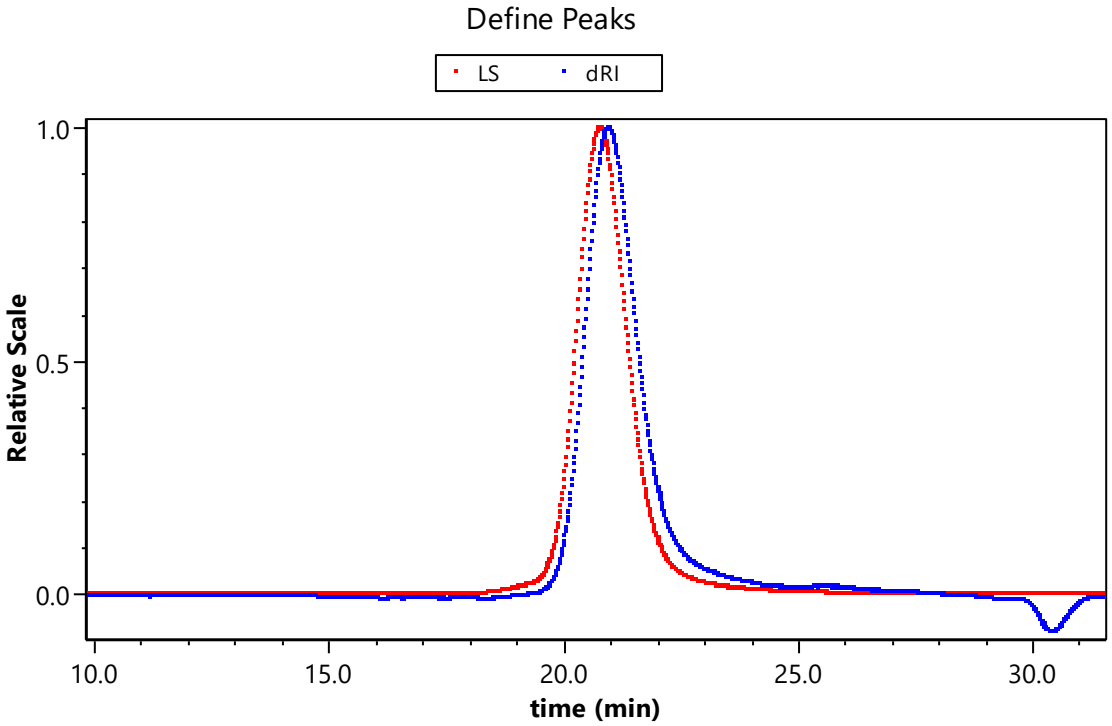


Figure S4: GPC-traces of PDEVP (600 eq. DEVP; entry 2, table 1).

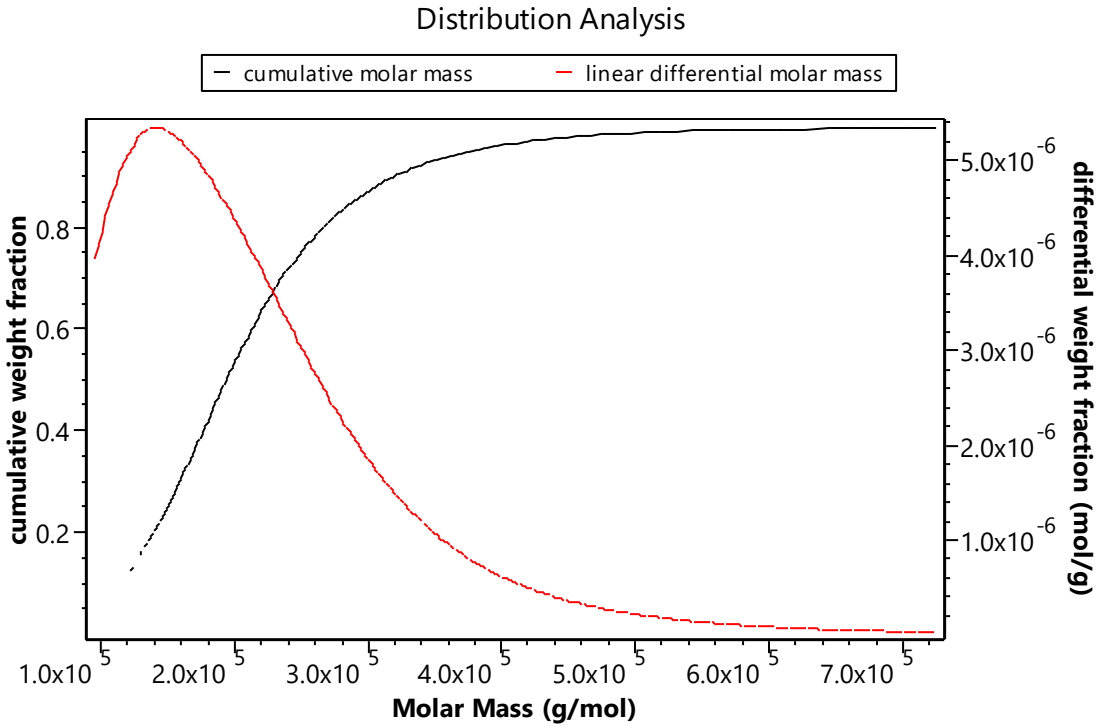


Figure S5: Distribution plot of PDEVP (600 eq. DEVP).

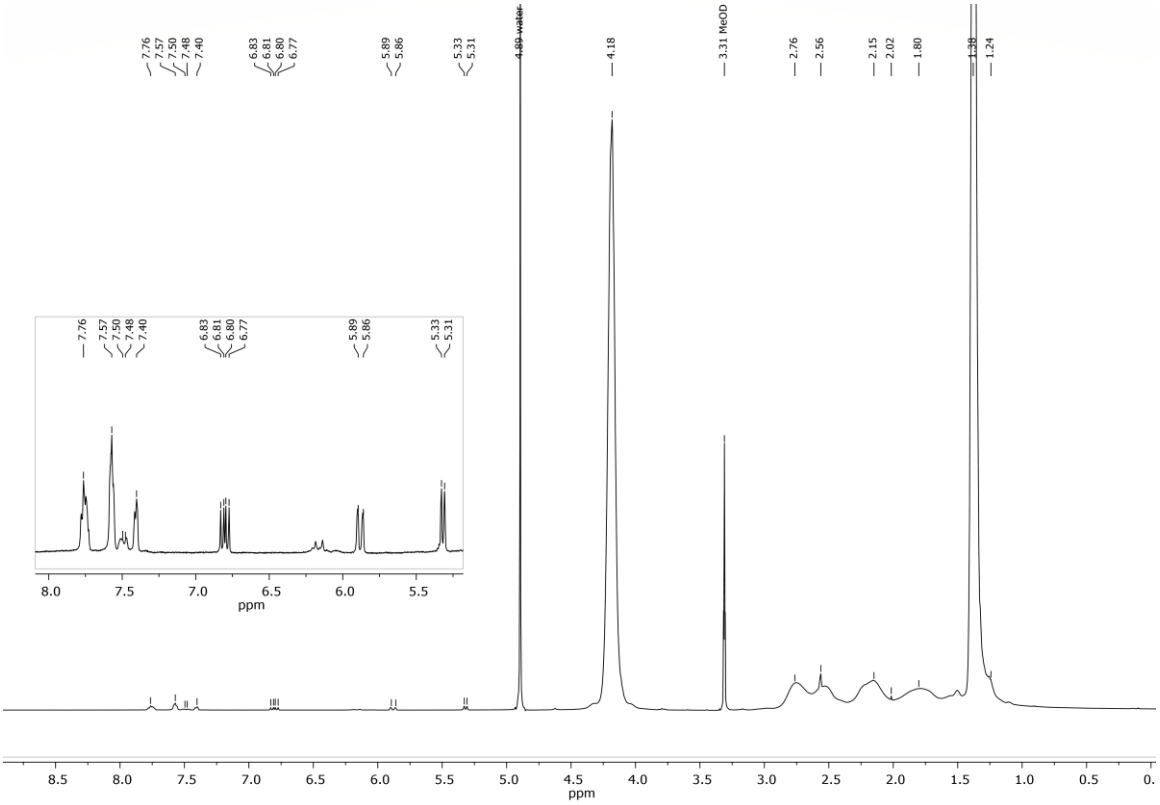


Figure S6: ^{1}H-NMR spectrum of PDEVP (100 eq. DEVP; entry 1, table 1) in MeOD.

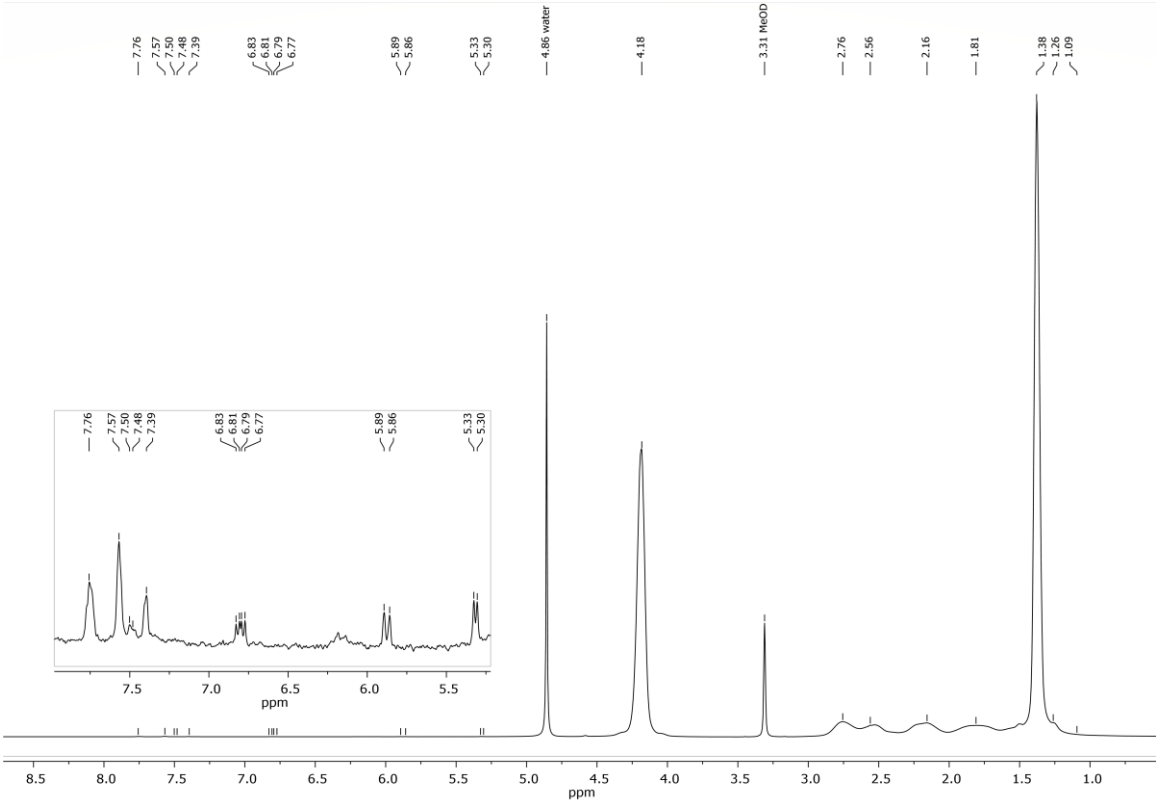


Figure S7: ^{1}H-NMR spectrum of PDEVP (600 eq. DEVP; entry 2, table 1) in MeOD.

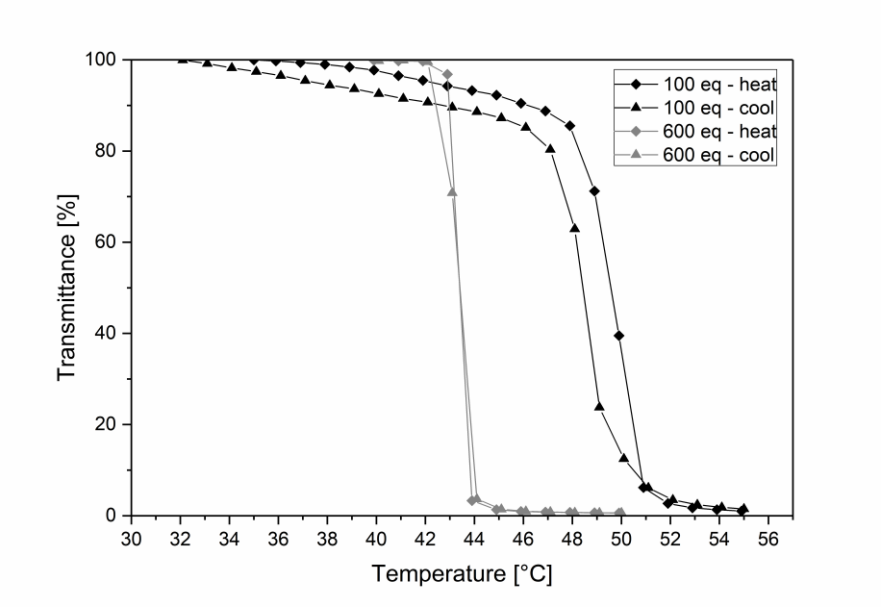


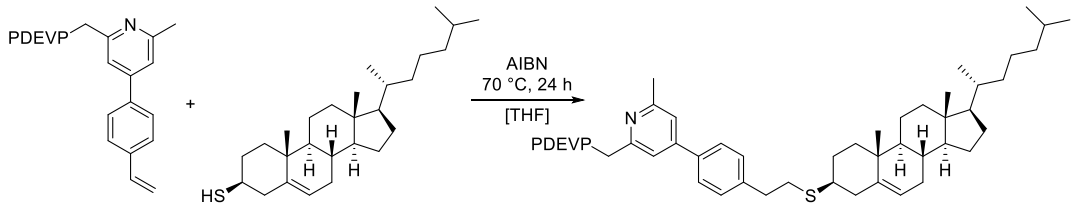
Figure S8: Determination of the LCST of short-chain PDEVP (100 eq.) and long-chain PDEVP (600 eq.). The cloud point was determined at 10% decrease of transmittance for aqueous solutions of PDEVP (2.50 mg/mL).

4. End-group analysis *via* ESI-MS and NMR

For the elucidation of the polymerisation mechanism *via* end-group analysis oligomeric PDEVP was generated: 122 µmol of $Cp_2Y(CH_2TMS)(THF)$ was dissolved in 2.50 mL toluene and was mixed with a solution of the corresponding initiator (122 µmol). After quantitative conversion was confirmed by ^{1}H-NMR spectroscopy 5.00 equivalents of DEVP were added and conversion was determined after two hours *via* ^{31}P-NMR spectroscopy. The signals found by ESI-MS analysis can be attributed to $M_{Initiator} + n \times M_{DEVP}$ with either H^+ or Na^+ as charge carrier.

5. Thiol-ene click reactions

General procedure: Thiol-ene coupling of thiocholesterol to poly(diethyl vinylphosphonates)



Thiocholesterol (5.00 eq.) and catalytic amounts of azobisisobutyronitrile (0.33 eq.) were added to a solution of 1.00 equivalent of poly(diethyl vinylphosphonate) in tetrahydrofuran (10.0 mL per 1.00 g polymer) in a pressuriseable schlenk flask. The mixture was degassed *via* evacuation and filling with argon (20 iterations) and stirred for 24 hours at

70 °C. After this time period ^{1}H-NMR spectroscopy showed quantitative conversion of the vinyl group. The polymer was purified by precipitation from the reaction solution with excess pentane. The polymeric residue was dissolved in toluene, precipitated with pentane two more times, was dried to remove pentane, dissolved in water and lyophilised.

Table S1: Chemical shifts in ^{1}H- und ^{31}P-NMR of cholesterol-functionalised PDEVP

	Substrate	**^{1}H-NMR (500 MHz, CD_3OD, 300 K) δ [ppm]**	**^{31}P-NMR (203 MHz, CD_3OD, 300 K) δ [ppm]**
1	PDEVP (100 eq.)	7.79 – 7.66 (m, H_{arom}), 7.62 – 7.44 (m, H_{arom}), 7.39 (d, J = 6.3 Hz, H_{arom}), 5.34 (s, chol), 4.20 (s, POCH_2), 2.89 – 2.85 (m, chol), 2.89 – 1.18 (m, PDEVP), 1.40 (m, $POCH_2CH_3$), 1.02 (s, $CH_{3,Chol}$), 0.96 (d, J_3 = 6.5 Hz, $CH_{3,Chol}$), 0.91 (d, J = 6.6 Hz, $CH_{3,Chol}$), 0.90 (d, J_3 = 6.6 Hz, $CH_{3,Chol}$), 0.73 (s, $CH_{3,Chol}$).	33.1
2	PDEVP (600 eq.)	8.09 – 7.21 (m, H_{arom}), 5.36 (s, chol), 4.21 (m, POCH_2), 3.60 (d, J_3 = 7.4 Hz, chol), 2.89 – 1.13 (m, PDEVP), 1.40 (m, $POCH_2CH_3$), 1.05 (s, $CH_{3,chol}$), 0.94 (s, $CH_{3,chol}$), 0.91 (s, $CH_{3,chol}$), 0.90 (s, $CH_{3,chol}$), 0.74 (s, $CH_{3,chol}$).	33.2

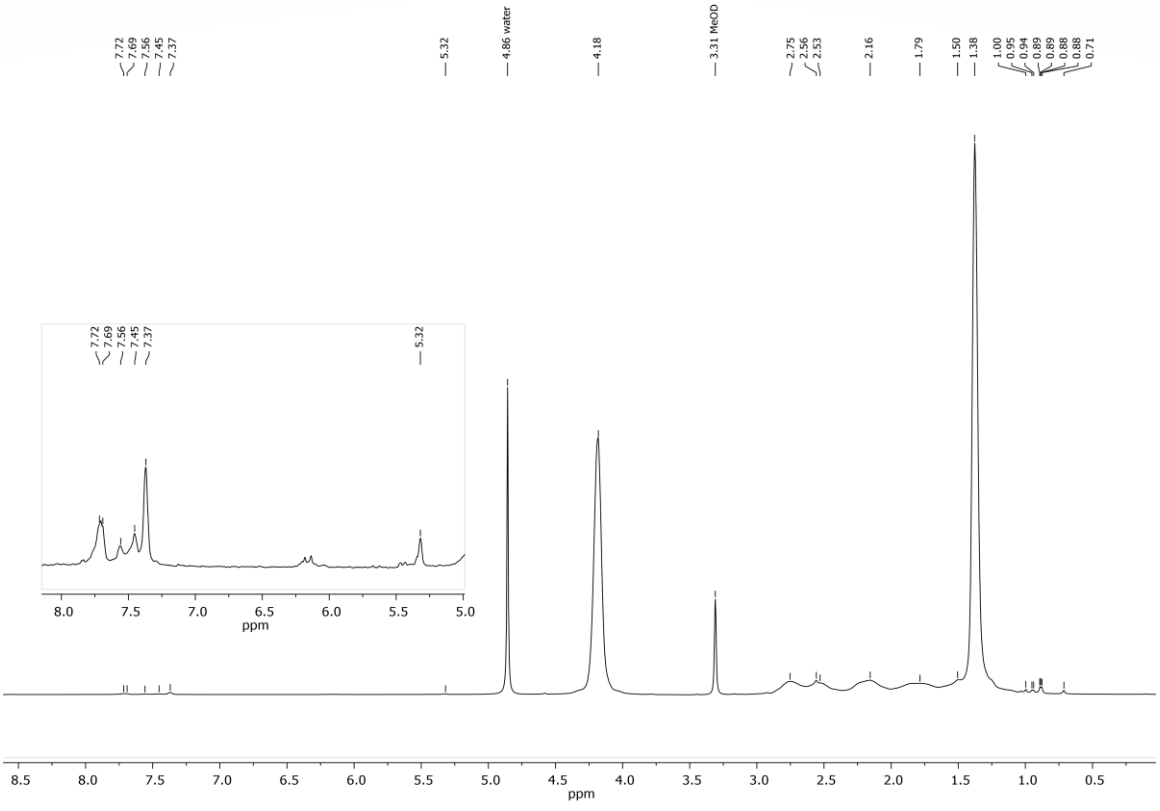


Figure S9: ^{1}H-NMR of PDEVP (100 eq.) after functionalisation with thiocholesterol in MeOD.

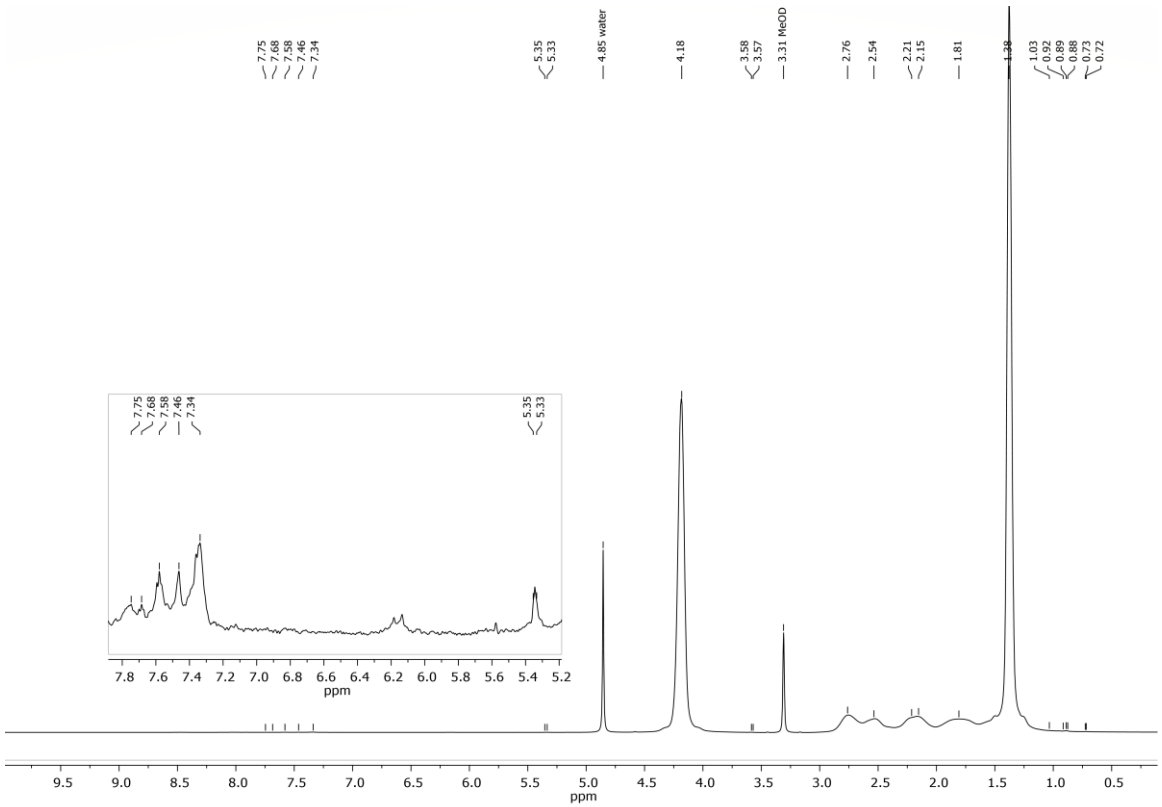


Figure S10: ^{1}H-NMR of PDEVP (600 eq.) after functionalisation with thiocholesterol in MeOD.

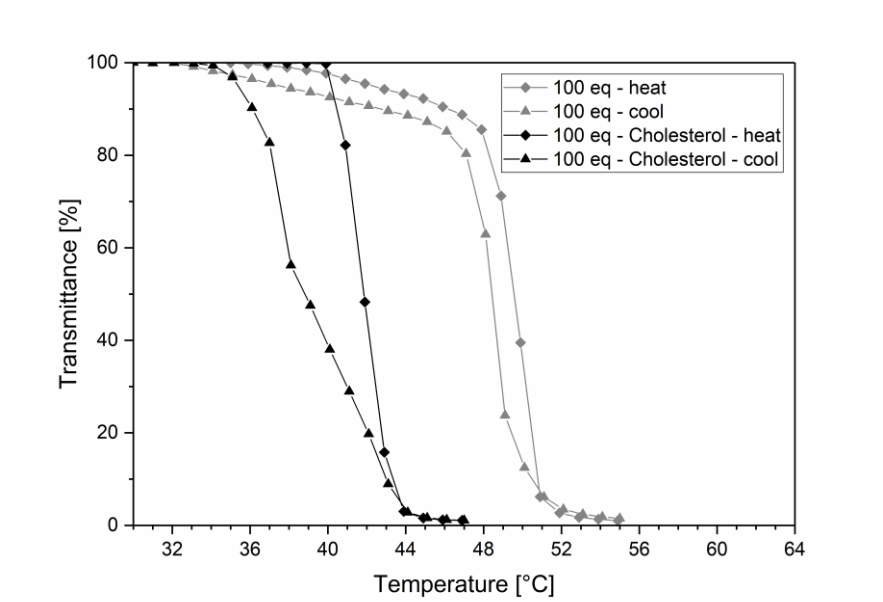


Figure S11: Determination of the LCST of cholesterol-functionalised PDEVP (100 eq.) and the corresponding short-chain PDEVP (100 eq.). The cloud point was determined at 10% decrease of transmittance for aqueous solutions of the polymer substrates (2.50 mg/mL).

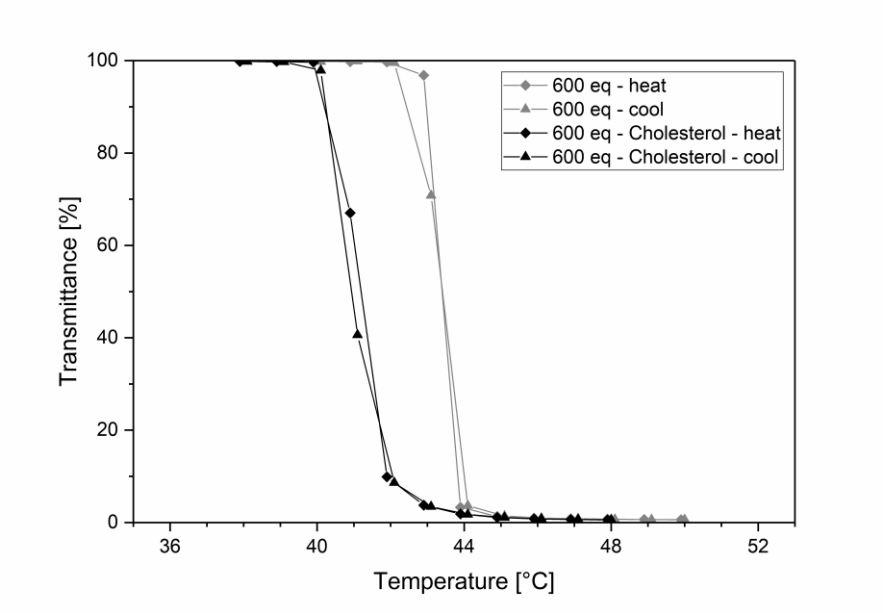
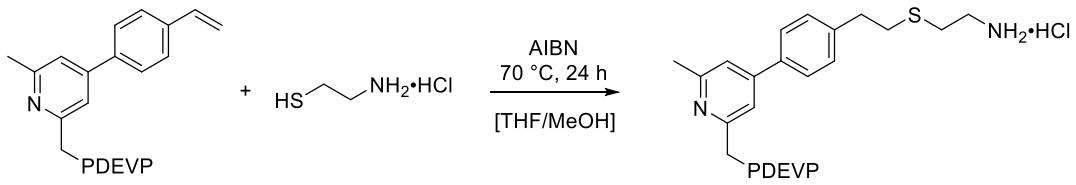


Figure S12: Determination of the LCST of cholesterol-functionalised PDEVP (600 eq.) and the corresponding short-chain PDEVP (600 eq.). The cloud point was determined at 10% decrease of transmittance for aqueous solutions of the polymer substrates (2.50 mg/mL).

General procedure: Thiol-ene coupling of cysteamine hydrochloride to poly(diethyl vinylphosphonates)

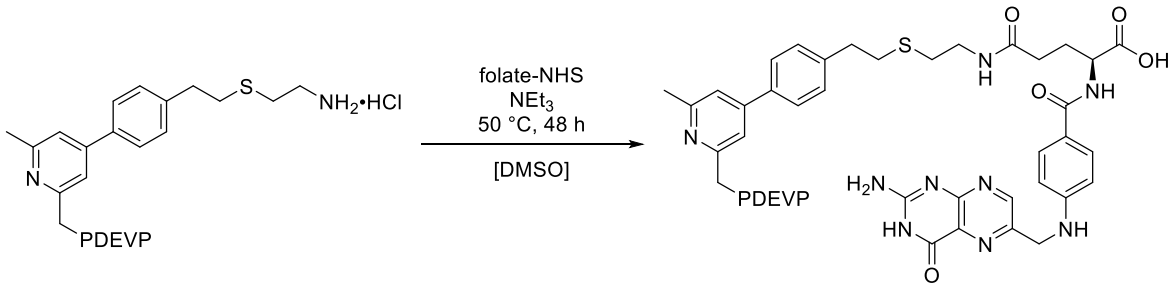


Cysteamine hydrochloride (5.00 eq.) and catalytic amounts of azobisisobutyronitrile (0.33 eq.) were added to a solution of 1.00 equivalent of poly(diethyl vinylphosphonate) in tetrahydrofuran and methanol (10:1, 10.0 mL per 1.00 g polymer) in a pressuriseable schlenk flask. The mixture was degassed *via* drawing vacuum and filling with argon (20 iterations) and stirred for 24 hours at 70 °C. After this time period ^{1}H-NMR spectroscopy showed quantitative conversion of the vinyl group. The polymer was purified *via* dialysis against deionised water. After replacing the dialysate after two and four hours the mixture was dialysed over night and the resulting solution lyophilised.

Table S2: Chemical shifts in ^{1}H- und ^{31}P-NMR of PDEVP after reaction with cysteamine hydrochloride

	Substrate	^{1}H-NMR (500 MHz, CD_3OD, 300K) δ [ppm]	^{31}P-NMR (121 MHz, CD_3OD, 300 K) δ [ppm]	Yield [%]
1	PDEVP (100 eq.)	7.76 – 7.37 (m, H_{arom}), 7.31 – 7.17 (m, H_{arom}), 4.18 (s, POCH_2), 2.98 (t, J_3 = 7.5 Hz, $CH_{2,aliphatic}$), 2.88 (t, J_3 = 5.4 Hz, $CH_{2,aliphatic}$), 2.83 – 1.20 (m, PDEVP), 1.38 (s, $POCH_2CH_3$).	33.2	100
2	PDEVP (600 eq.)	7.86 – 7.43 (m, H_{arom}), 7.30 – 7.12 (m, H_{arom}), 4.18 (s, POCH_2), 2.98 (t, J_3 = 7.1 Hz, $CH_{2,aliphatic}$), 2.93 – 2.87 (m, $CH_{2,aliphatic}$), 2.85 – 1.20 (m, PDEVP), 1.38 (s, $POCH_2CH_3$).	33.2	100

General procedure: Conversion of polymer-bound cysteamine linker with activated folate species



5.00 equivalents of folate-NHS and 6.00 equivalents of triethylamine were added to a solution of 1.00 equivalent of the stated cysteamine-containing poly(diethyl vinylphosphonate) in dimethyl sulfoxide (20.0 mL solvent per 1.00 g polymer). The mixture was stirred for 48 hours at 50 °C and after this period purified *via* dialysis against deionised water. After replacing the dialysate after two and four hours the mixture was dialysed over night and the resulting solution lyophilised.

Table S3: Chemical shifts of folate-containing PDEVP in ^{1}H- und ^{31}P-NMR

	Substrate	^{1}H-NMR (500 MHz, CD_3OD, 300K) δ [ppm]	^{31}P-NMR (203 MHz, CD_3OD, 300 K) δ [ppm]
1	PDEVP (100 eq.)	8.68 (s, pteridine), 7.84 – 6.99 (m, folate, H_{arom}), 6.80 – 6.56 (m, H_{arom}), 4.67 (s, CH_2NH_2), 4.54 (s, CH_2HN), 4.18 (s, POCH_2), 3.14 – 1.06 (m, PDEVP), 1.38 (s, $POCH_2CH_3$).	33.2
2	PDEVP (600 eq.)	8.71 (s, pteridine), 7.77 – 7.51 (m, folate, H_{arom}), 7.55 – 6.98 (m, H_{arom}), 6.79 – 6.60 (m, H_{arom}), 4.60 (s, CH_2NH_2), 4.49 (s, CH_2NH), 4.18 (s, POCH_2), 2.86 – 1.17 (m, PDEVP), 1.38 (s, $POCH_2CH_3$).	33.2

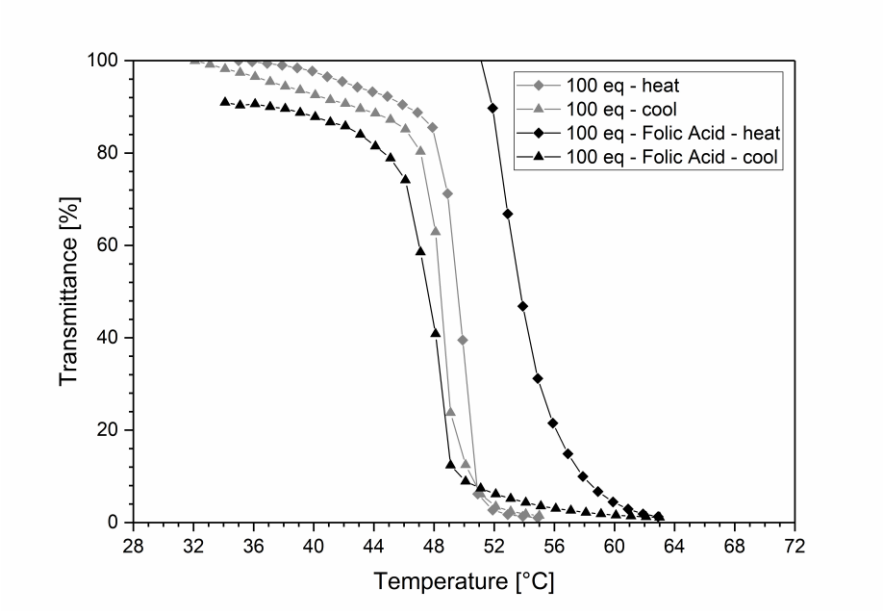


Figure S13: Determination of the LCST of folate-functionalised PDEVP (100 eq.) and the corresponding short-chain PDEVP (100 eq.). The cloud point was determined at 10% decrease of transmittance for aqueous solutions of the polymer substrates (2.50 mg/mL).

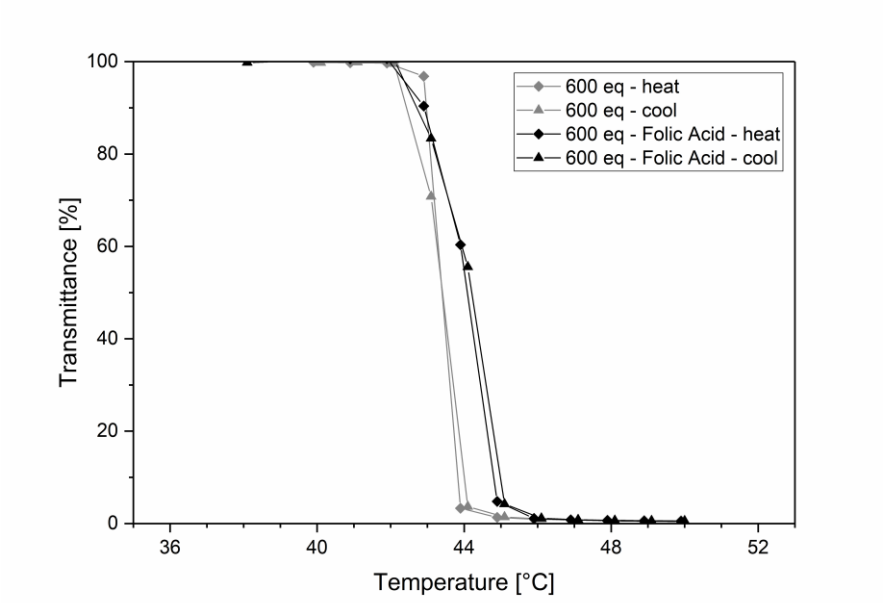


Figure S14: Determination of the LCST of folate-functionalised PDEVP (600 eq.) and the corresponding long-chain PDEVP (600 eq.). The cloud point was determined at 10% decrease of transmittance for aqueous solutions of the polymer substrates (2.50 mg/mL).

6. Cell viability assay

6.1 Cell culture

In vitro studies on polymer samples were performed in HEK and HMEC cells. Cells were cultured in Dulbecco's Modified Eagle Medium (Life Technologies) equipped with 10% (v/v) Fetal Bovine Serum (Biochrom) and 1%

Penicillin/Streptomycin 10000 U/mL /10000 µg/mL (Biochrom) at 37 °C in a humidified atmosphere containing 5% CO_2. For splitting and sub-culturing of cells Trypsin 0.05%/EDTA 0.02% in PBS (PAN Biotech) was used.

6.2 Cell viability studies

The growth inhibition of the polymer samples on HEK and HMEC cells, was determined by analysing their cell viability of in presence of increasing polymer concentrations (0.078 mg/mL to 5.00 mg/mL). Prior to the addition of the polymers the cells were cultured for 24 h in 96 well flat bottom plates (TPP) with a density of 20000 cells/well or 10000 cells/well (for 48 h). After 24 or 48 hours of incubation at 37 °C in a humidified atmosphere with 5% CO_2, the cell viability of the treated cells was determined using the MTT reagent (Sigma Aldrich). Therefore, MTT was dissolved at a concentration of 5 mg/mL in RPMI-1640 without phenol red (Life Technologies). PBS treated cells were used as positive control (100% viability). DMSO treated cells were used as negative control (0% viability). After 3 h of incubation with 50 µL of MTT per well at 37 °C, the blue formazan crystals were dissolved for 15 min on a plate shaker at 550 min^{-1} with 100 µL 0.04 N HCl in isopropanol and exclusion of light. Following the absorbance of each well was measured at 570 nm with 690 nm as background wavelength at a Tecan Genios Plus plate reader.

Shown are mean values of at least three independent biological replicates and the respective standard deviations are indicated.

7. Literature

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