End-functionalized ROMP polymers for Biomedical Applications

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Supporting Information.

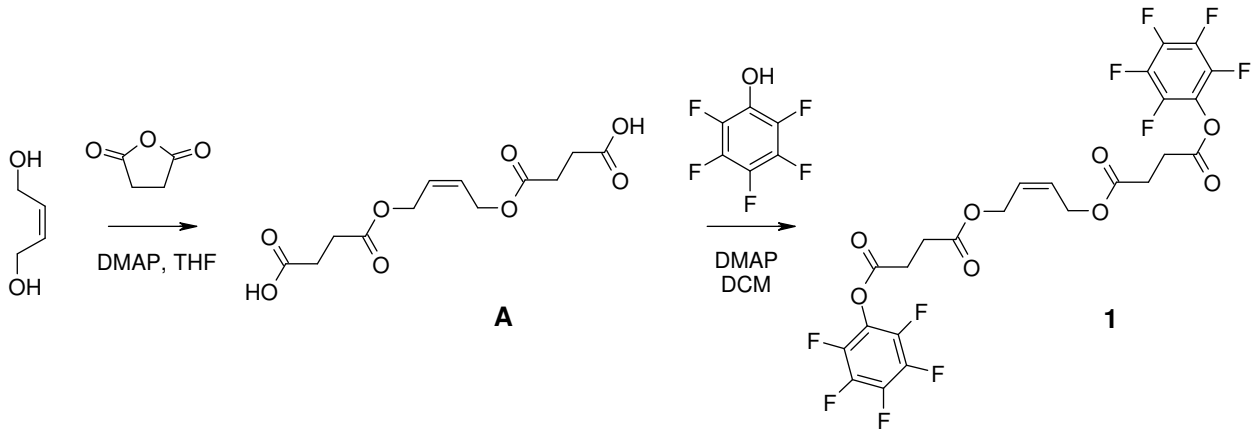
Experimental.

Chemicals (reagent grade) were purchased from Aldrich, VWR, Fluka, Gelest or Fisher and used as received, unless otherwise indicated. 3rd generation Grubbs catalyst (Dichloro-di(3-bromopyridino)-N,N'-Dimesitylenoimidazolino-Ru=CHPh; G3) was synthesized as described previously by Grubbs et al.[31] Dichloromethane and THF (HPLC grade, Fisher Scientific) were distilled from CaH_2 and sodium, respectively, under nitrogen. DMF was purified from amine traces using AldraAmine trapping packets (Aldrich). Gel permeation chromatography ($CHCl_3$, calibrated with polystyrene standards, toluene as flow marker) was measured on a PL50 GPC setup (Polymer Laboratories, Amherst, MA) with a PL Gel 5 µm pre-column and two 10 µm analytical Mixed-D columns (Polymer Laboratories, Amherst, MA). NMR spectra were recorded on a Bruker DPX300 spectrometer (Bruker, Madison, WI). High resolution mass spectra were obtained from a JEOL JMS 700 instrument (JEOL, Peabody, MA); Matrix Assisted Laser Desorption and Ionization Time of Flight Mass Spectra (MALDI-TOF MS) were measured on a Bruker Daltonics Reflex III (Bruker, Madison, WI). Tapping mode AFM images were obtained

on a Veeco Dimension 3100 instrument with a Nanoscope III controller, using Nanoscope software and etched silicon standard AFM tips. Ellipsometric thickness measurements were made with a Rudolph Research model SL-II automatic ellipsometer with an angle of incidence of 70° from the normal. The light source was a He-Ne laser with λ = 632.8 nm. Measurements were performed on 3-5 different locations on each sample, and the thickness was calculated from the Δ and Ψ values using the Rudolph Research Double Absorbing Films Calculations Software. Confocal scanning microscope images were obtained on a Zeiss Axiovert fully automated microscope using a 40x objective in oil immersion.

End-group Synthesis:

Synthesis of terminating agent **1**:



A **1**

Compound **A** was synthesized as described in the literature.[32] Compound **A** (2.0 g, 6.95 mmol), pentafluorphenol (3.2 g, 17.4 mmol) and DMAP (0.21 g, 1.74 mmol) were dissolved in 50 mL dry DCM under N_2. The resulting solution was then cooled to 0 ºC, and EDC (3.33 g, 17.4 mmol) was added to the mixture in portions. The reaction mixture was then allowed to warm to room temperature and stirred for another 12 hours. The mixture was then washed with 10% $KHSO_4$ solution, saturated $NaHCO_3$ solution, and brine. The resulting DCM solution was dried using anhydrous Na_2SO_4, filtered, and the solvent was evaporated. The resulting residue was purified

by filtration through a neutral alumina plug using DCM as eluent, to afford 2.59 g of white solid (yield = 60%).

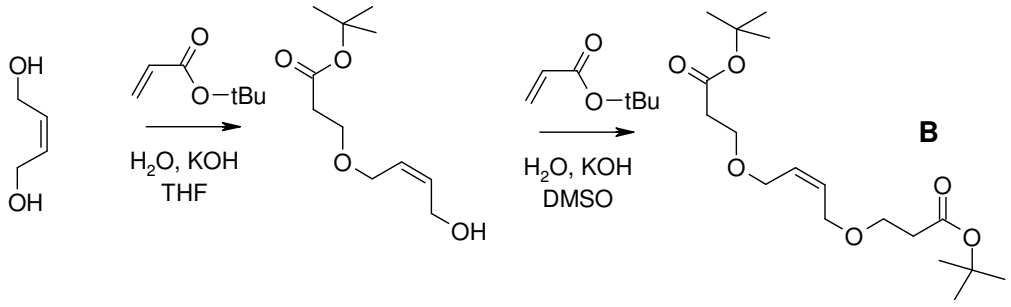
^{1}H-NMR (300 MHz, $CDCl_3$): 5.77 (m, 2H, =C*H*), 4.75 (d, *J* = 5.6 Hz, 4H, =CH-CH_2), 3.05 (t, *J* = 6.2 Hz, 4H, OOC-CH_2-CH_2), 2.95 (t, *J* = 6.2 Hz, 4H, OOC-CH_2-CH_2-COO-C_6F_5).

^{13}C-NMR (75 MHz, $CDCl_3$): 171.1 (*C*=O-O allylic); 168.4 (*C*=O-O-C_6F_5); 142.9, 141.7, 139.5, 137.8 & 136.1 (m, F_5C_6); 127.9 (*C*=*C*), 60.5 (CH_2-C=C), 28.7 & 28.3 (CH_2-CH_2).

MS-FAB: 620.0 (M), 621.0 (M+1), 622 (M+2).

Synthesis of Terminating Agent **2**:

(1)

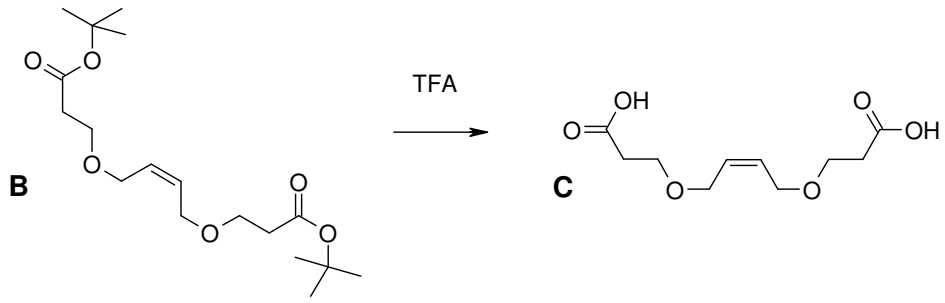


10.0 g cis-2-butene-1,4-diol (114 mmol, 1.0 eq) and 36.4 g (284 mmol, 2.5 eq) tert-butyl acrylate were mixed with 200 mL THF. Catalytic amounts of water and sodium hydroxide were added. The reaction was stirred at room temperature for three days, after which the solvent was evaporated. The product (monoadduct of the Michael addition) and tert-butyl acrylate were dissolved in 100 mL DMSO, adding catalytic amounts of water and sodium hydroxide. After two days, 500 mL of water were added. The mixture was extracted three times with dichloromethane. The organic layers were combined, washed with 10% $KHSO_4$ (3x) and 10% $NaHCO_3$ (3x), and dried over $MgSO_4$. After filtering, the solvent and excess acrylate were removed by evaporation

(rotary evaporator, followed by high vacuum). The crude product **B** (yield 95%) was taken to the next reaction step.

^{1}H-NMR (300 MHz, $CDCl_3$): 5.79 (m, 2H, =C*H*), 4.04 (d, *J* = 4.7 Hz, 4H, =CH-CH_2), 3.64 (t, *J* = 6.4 Hz, 4H, O-CH_2-CH_2), 2.48 (t, *J* = 6.4 Hz, 4H, O-CH_2-CH_2), 1.44 (s, 18 H, t-butyl).

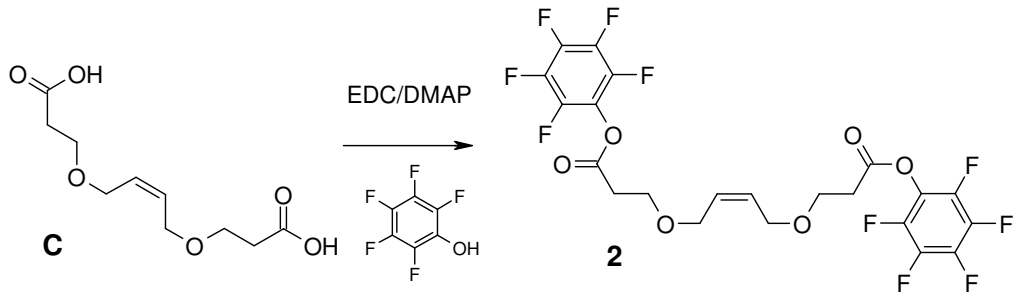
(2)



5.00 g (14.5 mmol) of **B** were dissolved in a mixture of 15 mL trifluoroacetic acid and 15 mL dichloromethane. After stirring over night at room temperature, the solvent was removed at the rotovap. 50 mL dichloromethane was added and evaporated three times (azeotropic removal of excess acid). The crude product **C**, obtained with quantitative conversion according to NMR, was dried in high vacuum. The solid was recrystallized from hexane/ethylacetate.

^{1}H-NMR (300 MHz, $CDCl_3$): 8.04 (br s, 2H, COO*H*), 5.73 (m, 2H, =C*H*), 4.08 (d, *J* = 6.1 Hz, 4H, =CH-CH_2), 3.71 (t, *J* = 6.1 Hz, 4H, O-CH_2-CH_2), 2.63 (t, *J* = 6.1 Hz, 4H, O-CH_2-CH_2).

(3)



2.69 g (11.6 mmol, 1 eq) **C** were dissolved in 50 mL anhydrous dichloromethane under nitrogen. Catalytic amounts of 4-dimethylaminopyridine and 6.40 g (34.8 mmol, 3 eq) pentafluorophenol were added. The reaction mixture was cooled to 0°C, and 6.68 g (34.8 mmol, 3 eq) of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimid was added. The reaction was stirred over night. It was then washed with 10% $KHSO_4$ (2x), water (1x) and 10% $NaHCO_3$ (2x), and dried over $MgSO_4$. After filtering, the solvent was evaporated and the product was vacuum dried.

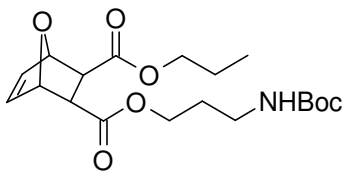
^{1}H-NMR (300 MHz, $CDCl_3$): 5.77 (m, 2H, =C*H*), 4.14 (d, *J* = 5.4 Hz, 4H, =CH-CH_2), 3.84 (t, *J* = 6.2 Hz, 4H, O-CH_2-CH_2), 2.95 (t, *J* = 6.2 Hz, 4H, O-CH_2-CH_2).

^{13}C-NMR (75 MHz, $CDCl_3$): 167.5 (*C*=O-O); 143.2, 141.2, 139.8, 139.8 & 136.3 (m, F_5C_6); 128.2 (*C*=*C*), 67.0 (O-*C*H_2-C=C), 64.5 (CH_2-*C*H_2-O); 34.5 (*C*H_2-COO-C_6F_5).

MS-FAB: 562 (M-2), 563 (M-1), 564 (M), 565 (M+1), 566 (M+2).

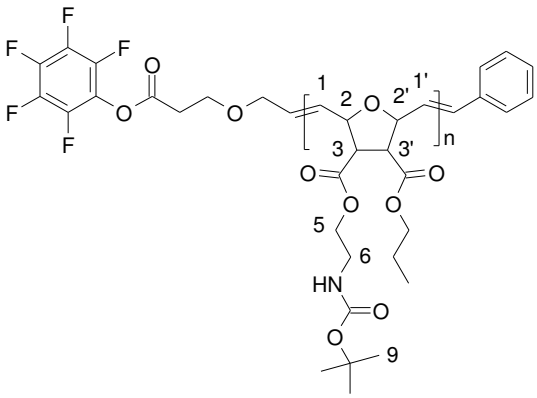
Example of reaction conditions for polymer end-functionalization:

Monomer **D** (e.g. 100 mg/0.27 mmol) was dissolved in 1 mL of anhydrous dichloromethane. 26.6 mg (0.03 mmol, 1 eq) Grubbs 3rd generation catalyst was dissolved in 0.5 mL anhydrous dichloromethane and added quickly to the monomer solution. The reaction mixture was allowed to stir at room temperature for 10 min. A sample was taken via syringe for analysis. 170 mg (0.30 mmol, 10 eq) of the end group in 1 mL dichloromethane was added to the remainder of the solution, and stirred over night. The excess of the end group was removed by filtration over a short silica gel column (7 cm length, 3 cm diameter). The unreacted end-group and any side products were washed from the column with dichloromethane, while the polymer remained on the column and was recovered with ethyl acetate, which was evaporated to yield the pure product.



D

^{1}H-NMR (300 MHz, $CDCl_3$): 0.91 (m, 3H, CH_2-CH_3), 1.41 (s, 9H, H9), 1.62 (m, 2H, β-CH_2), 2.92 (br t, 0.1 H, O-C$H_2$$CH_2$ of endgroup), 3.10 (br m, 2H, H3 & H3'), 3.34 (br m, 2H, H6), 3.80 (br t, 0.1 H, O-CH_2CH_2 of endgroup), 4.09 (m, 4H, α-CH_2 and H5), 4.69 (br m, 1H, H2 & H2' trans), 5.10 (br m, 1H, H2 cis & H2'), 5.40 (br m, 1H, H1 & H1' cis), 5.59 (br s, 1H, NH), 5.88 (br m, 1H, H1 & H1' trans), 7.31-7.43 (m, Phenyl endgroup).



Sample 1 (for dye-labeling):

GPC (DMF, LiCl, PS standards): M_n = 4000 g mol^{-1}, M_w/M_n = 1.08

MALDI-TOF MS (with endgroup): m/z = (372 + 23 + n · 369) g mol^{-1}; M_{peak} = 4823 g mol^{-1} (calc.), 4821.9 g mol^{-1} (measured).

MALDI-TOF MS (CH_2 endgroup): m/z = (104 + 23 + n · 369) g mol^{-1}; M_{peak} = 4555 g mol^{-1} (calc.), 4560 g mol^{-1} (measured).

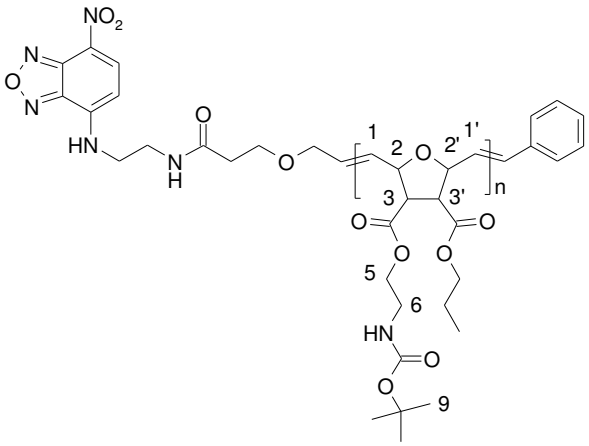
Sample 2 (for surface functionalization):

GPC (DMF, LiCl, PS standards): M_n = 12500 g mol^{-1}, M_w/M_n = 1.05

Example of reaction conditions for polymer modification:

125.2 mg (0.03 mmol reactive endgroups, 1 eq) end-functionalized polymer were dissolved in 1 mL amine-free N,N-dimethyl formamide. 12.17 mg (0.036 mmol, 1.2 eq) amine-functionalized NBD-dye was added. The reaction mixture was allowed to stir at room temperature for three days. The solvent was evaporated, and the reaction product was purified by column chromatography using ethyl acetate.

^{1}H-NMR (300 MHz, $CDCl_3$): 0.93 (m, 3H, CH_2-CH_3), 1.45 (s, 9H, H9), 1.64 (m, 2H, β-CH_2), 2.51 (t, J = 6.5 Hz, 0.1 H, N-CH_2CH_2-NH-Ar from endgroup), 2.95 (br t, 0.1 H, O-C$H_2$$CH_2$ from endgroup), 3.14 (br m, 2H, H3 & H3'), 3.38 (br m, 2H, H6), 3.67 (t, J = 6.4 Hz, 0.1 H, N-C$H_2$$CH_2$-NH-



Ar from endgroup), 3.84 (t, 0.1 H, O-CH_2CH_2 of endgroup), 4.12 (m, 4H, α-CH_2 and H5), 4.72 (br m, 1H, H2 & H2' trans), 5.13 (br m, 1H, H2 cis & H2'), 5.42 (br m, 1H, H1 & H1' cis), 5.61 (br s, 1H, NH), 5.90 (br m, 1H, H1 & H1' trans), 7.31-7.43 (m, Phenyl endgroup).

MALDI-TOF MS: no peak observed.

Example of reaction conditions for grafting onto surfaces:

A standard silicon wafer (1.1 x 1.1 cm) was plasma cleaned and functionalized with amino groups by reaction with 3-aminopropyl dimethyl ethoxysilane as described in the literature.[33] After washing off excess reagent, it was dried under a flow of N_2 and placed into a flat bottom vial with 12 mm diameter. 10 mg polymer with pentafluorophenol endgroup ($2 \cdot 10^{-4}$ mmol, M = 50 000 g/mol) was dissolved in 0.5 mL anhydrous dichloromethane, and the wafer was covered with that solution. 0.025 mg ($2 \cdot 10^{-4}$ mmol) 4-dimethylamino pyridine were added. After 3 hours, 0.04 mg ($2 \cdot 10^{-4}$ mmol) N,N'-dicyclohexylcarbodiimid was added, and the mixture was allowed to react for three days. The wafer was then washed successively with hexane (3x), dichloromethane (2x), ethanol (2x), water (2x) and ethanol (2x) to remove excess polymer, base and reaction side products. The wafers were dried under a stream of N_2 over night and then examined by AFM and ellipsometry (Δ = 157.00, Ψ = 11.00; polymer layer thickness: 6.1 nm).

Conditions for Confocal Microscopy Image

To a 0.5 mL suspension of *E. coli* (10^8 cells/cm^3 in PBS buffer), 1.25 µL dye-labelled SMAMP solution ($c = 4$ g L^{-1}) was added. The mixture was incubated for 15 min prior to imaging.