

Supporting Information

Enhanced Intrafibrillar Mineralization of Collagen Fibrils Induced by Brush-Like Polymers

Le Yu^{†,#}, Ian J. Martin^{‡,#}, Rajeswari M. Kasi^{‡,§,*}, Mei Wei^{†,§,*}

[†]Department of Materials Science and Engineering, [‡]Department of Chemistry, [§]Polymer Program, [§]Institute of Materials Science, University of Connecticut, Storrs, CT 06269, USA

L Yu and I J Martin contributed equally to this work.

* Authors to whom correspondence should be addressed.

Prof. M Wei: mei.wei@uconn.edu; Tel: +1 (860)-486-5003

Prof. R M Kasi: rajeswari.kasi@uconn.edu; Tel: +1 (860)-486-4713

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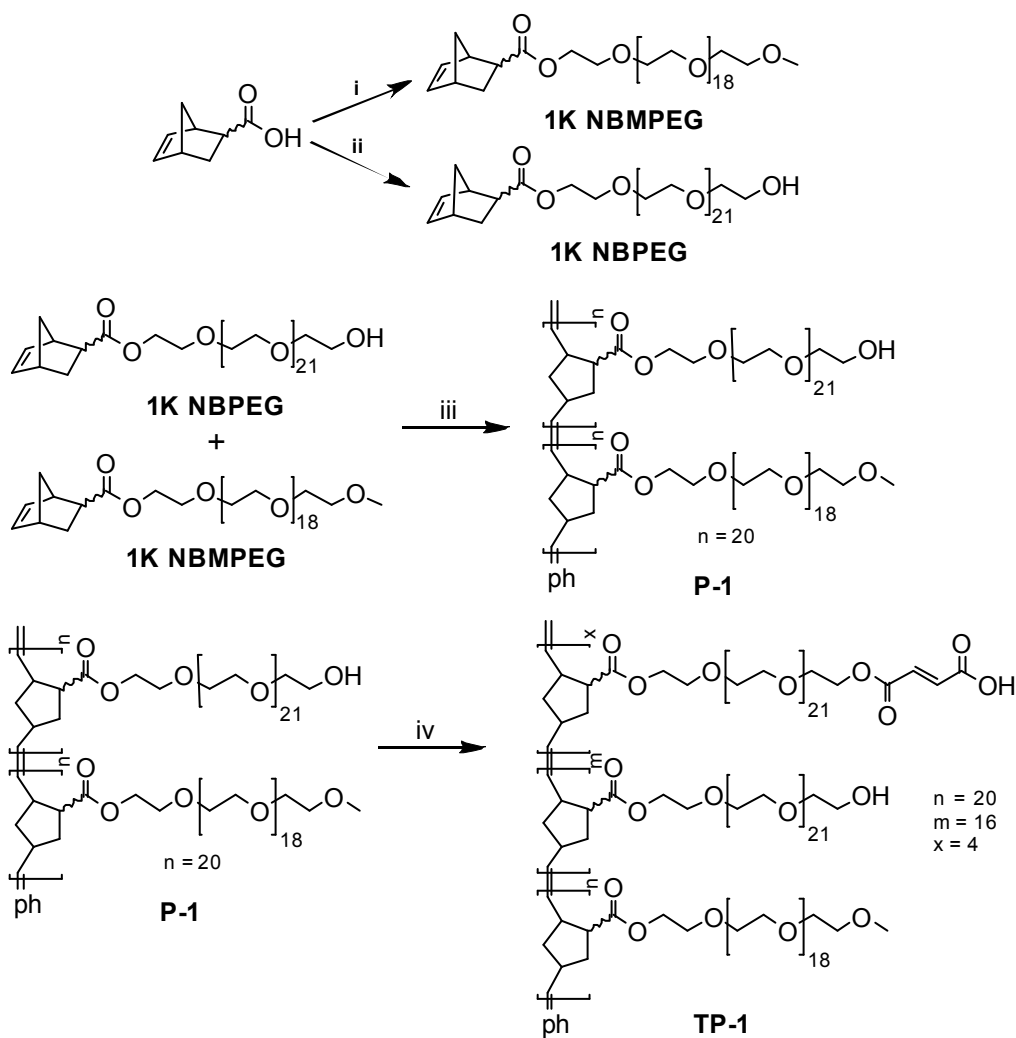
1. Materials for polymer synthesis

Monomers norbornene-poly(ethylene glycol) (NBPEG)¹, norbornene-poly(ethylene glycol) methyl ether (NBMPEG)¹, and macroRAFT agent norbornene trithiocarbonate (NB-TTC)² were synthesized from previously reported procedures. Monomer NB-p(t-BA) was synthesized by a modified procedure². N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC, 99⁺%) was purchased from ProteoChem and used without further purification. Grubbs catalyst second generation, Poly(ethylene glycol) monomethyl ether 1000 (1K MPEG), 4-(Dimethylamino)pyridine (99⁺%) (DMAP), 2,2'-Azobis(2-methylpropionitrile) (recrystallized, 99%) (AIBN), 2-(Dodecylthiocarbonothioylthio)-2-methylpropionic acid (98%), 5-norbornene-2-carboxylic acid (mixture of endo and exo, predominantly endo, 98%) (NB-COOH), 5-norbornene-2-methanol (mixture of endo and exo, 98%), and trifluoroacetic acid (99%) (TFA) were purchased from Sigma-Aldrich and used without further purification. Tert-butyl acrylate (t-BA) was purchased from Sigma-Aldrich and deinhibited by a 60/40 basic/acidic alumina plug. 2-butanone was purchased from Sigma Aldrich and dried using CaH₂. Modified Grubbs catalyst second generation (H₂IMes)(pyr)₂(Cl)₂RuCHPh (mG2)³ was synthesized. Anhydrous CH₂Cl₂ (99.8%), anhydrous DMF (99.8%), anhydrous THF (99.9%), anhydrous pyridine (99.5%), anhydrous pentane (99⁺%), oxalyl chloride (98%), maleic anhydride (99%), and ethyl vinyl ether (EVE, 99%) were purchased from Acros Organics and used without further purification.

2. Materials Synthesis

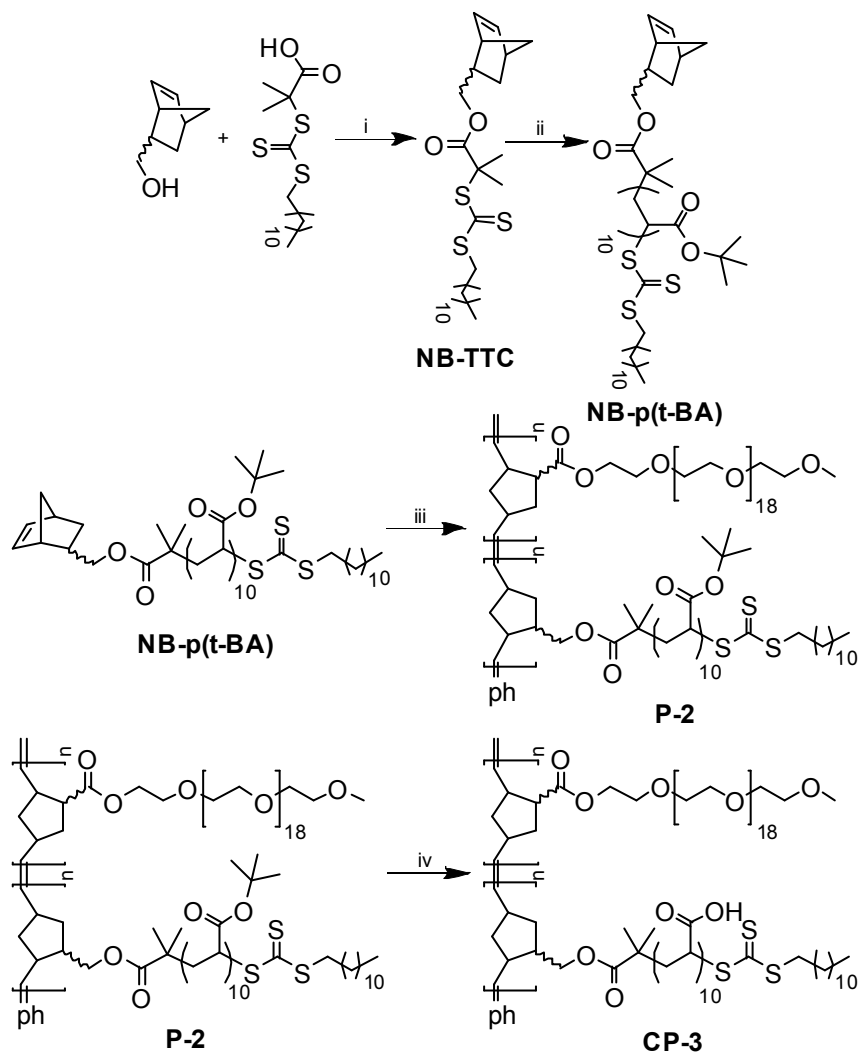
Synthetic Schemes

Scheme S1. Synthesis of random brush-like polynorbornene PEG-COOH terpolymer, TP-1.



- i-1. CH_2Cl_2 , oxalyl chloride, 2 drops of DMF, RT, 4 h.; i-2. 1K MPEG, CH_2Cl_2 , RT, 16 h
ii-1. CH_2Cl_2 , oxalyl chloride, 2 drops of DMF, RT, 4 h.; ii-2. 1K PEG-OH, CH_2Cl_2 , RT, 16 h
iii-1. 1. modified Grubbs 2nd, 20 min, dry CH_2Cl_2 , RT; iii-2. EVE
iv. maleic anhydride, DMAP, dry CH_2Cl_2 , RT, 24 h

Scheme S2. Synthesis of random brush-like polynorbornene PEG-PAA copolymer, CP-3.



i. EDC.HCl, DMAP, dry CH₂Cl₂, RT, 72 h

ii. tert-butyl acrylate, AIBN, 2-butanone, 60°C, 3.5 h

iii-1. 1. modified Grubbs 2nd, 1 h, dry CH₂Cl₂, RT; iii-2. EVE

iv-1. trifluoroacetic acid, dry THF, 60°C, 48 h; iv-2. dialysis in DI H₂O

2.1 Synthesis of MPEG Functionalized Norbornene (1K NBMPEG) Macromonomer

NBMPEG macromonomer is synthesized and purified according to a modified literature procedure¹. NBCOOH (86.4 % endo and 13.6 % exo) (4.0 g, 28.9 mmol) is dissolved in 20 mL of CH₂Cl₂ and purged with nitrogen for 10 min. To this solution, excess oxalyl chloride (9.96 mL, 116 mmol) is injected in a dropwise manner, followed by adding two drops of dimethylformamide (DMF). After 4 h reaction at room temperature, unreacted oxalyl chloride is removed under reduced pressure, resulting in norbornene carbonyl chloride (NBCOCl). To the resultant NBCOCl, a solution of poly(ethylene glycol) monomethyl ether (M_n = 1000 g/mol) (14.5 g, 14.5 mmol) in dry CH₂Cl₂ is added, followed by stirring for 12 h under nitrogen at room temperature. The macromonomer was precipitated from cold diethyl ether twice to give white solid product, Yield 85.0 %. ¹H NMR (500 MHz, CDCl₃) δ 6.18-5.88 (m, 2H, cyclic, -CH=CH-, endo/exo), 4.16 (m, 2H, -COO-CH₂CH₂O-, endo/exo), 3.89-3.43 (m, 100H, -(CH₂CH₂O)-), 3.35 (m, 3H, -OCH₃), 3.19-2.55 (m, 3H, cyclic, -CH-, endo/exo), 1.82, 1.39, 1.23 (m, 2H, cyclic, -CH₂- , endo/exo), 1.42 (m, 2H, cyclic, -CH₂-, endo/exo). ESI-MS: MS data shows mass distribution in **Figure S2** from 450 to 1300 m/z with bimodal distribution. The peak labeled at 1007 Da was chosen as an illustration. This value corresponds to MPEG chains with a repeat unit of 19 (n), the end group molecular weights are 152 Da (one norbornene carbonyl and methyl fragment), and one sodium counter ion. If we sum these values, [(19 × 44 Da) + 152 Da + 23 Da], we obtain an m/z of 1007 Da.

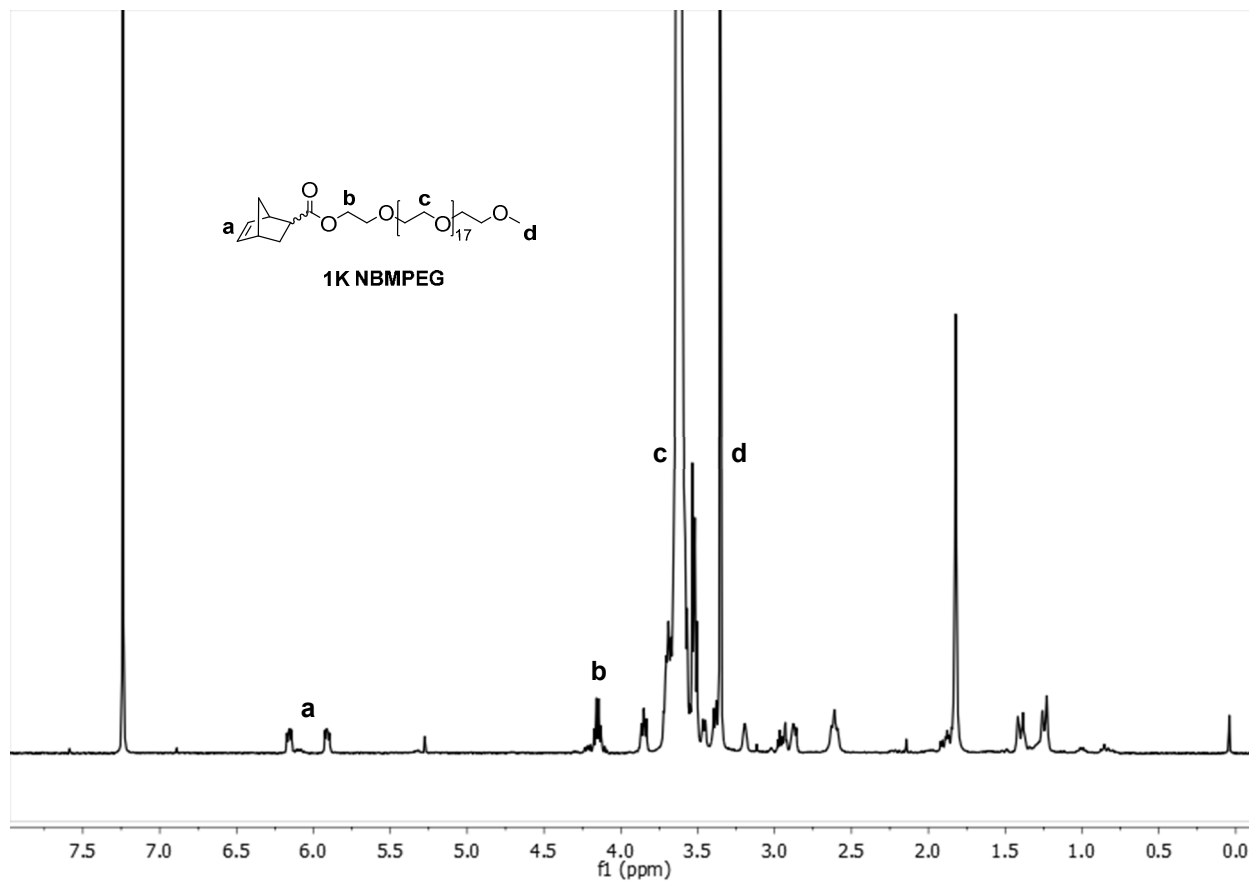


Figure S1. ^1H NMR spectrum of 1K NBMPEG in CDCl_3 collected at room temperature.

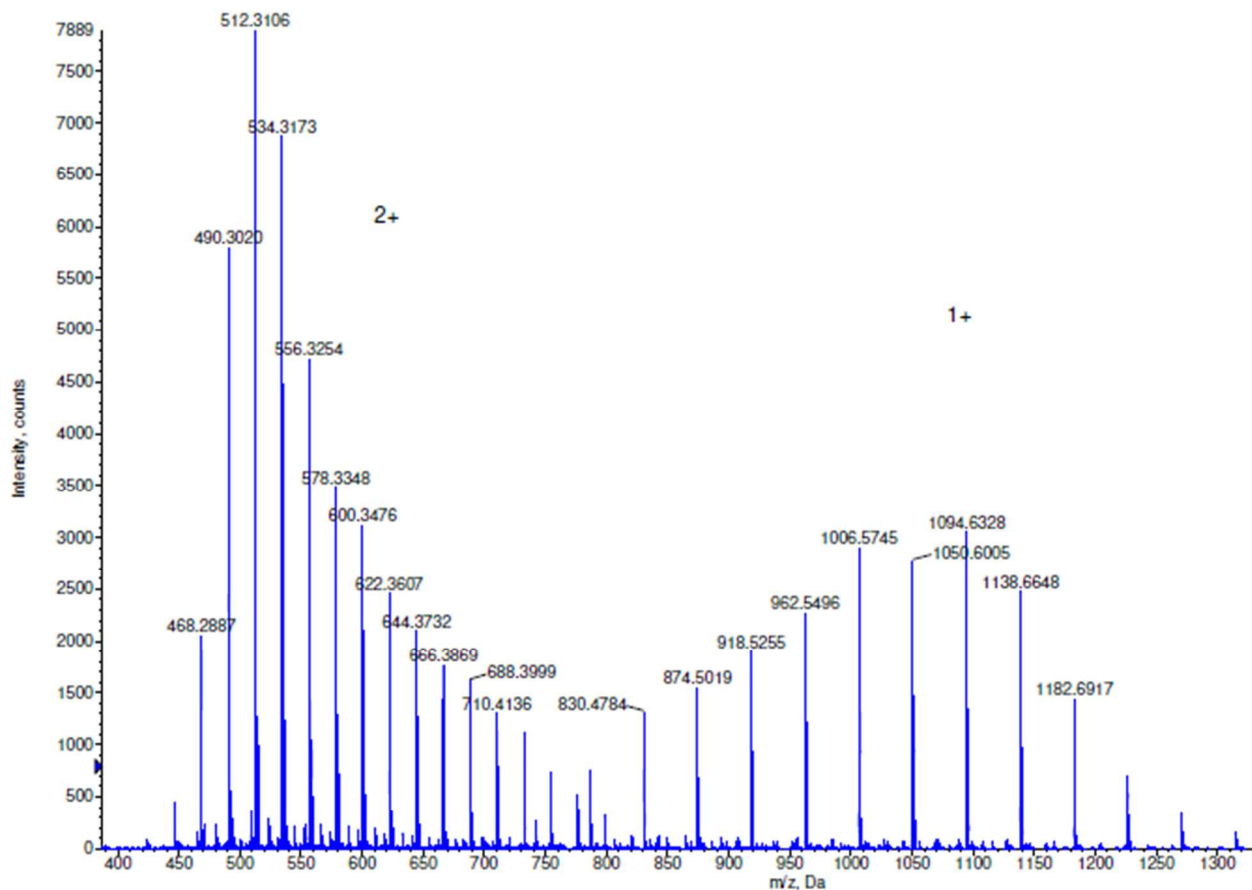


Figure S2. Mass spectrum of 1K NBMPEG with ESI positive ion mode.

2.2 Synthesis of PEG Functionalized Norbornene (1K NBPEG) Macromonomer

NBPEG macromonomer is synthesized and purified according to a modified literature procedure¹. NBCOOH (86.4 % endo and 13.6 % exo) (0.92 g, 6.67 mmol) is dissolved in 20 mL of CH₂Cl₂ and purged with nitrogen for 10 min. To this solution, excess oxalyl chloride (2.29 mL, 26.67 mmol) is injected in a dropwise manner, followed by adding two drops of dimethylformamide (DMF). After 4 h reaction at room temperature, unreacted oxalyl chloride is removed under reduced pressure, resulting in norbornene carbonyl chloride (NBCOCl). To the resultant NBCOCl, a solution of poly(ethylene glycol) (M_n = 1000 g/mol) (20.0 g, 20.0 mmol) in dry CH₂Cl₂ is added, followed by stirring for 12 h under nitrogen at room temperature. The macromonomer was precipitated from cold diethyl ether twice to give white solid product, Yield 20.1 %. ¹H NMR (500 MHz, CDCl₃) δ 6.24-5.92 (m, 2H, cyclic, -CH=CH-, endo/exo), 4.20 (m, 2H, -COO-CH₂CH₂O-, endo/exo), 3.94-3.39 (m, 100H, -(CH₂CH₂O)-), 3.23-2.89 (m, 3H, cyclic, -CH-, endo/exo), 2.70 (br m, 1H, -OH), 1.85, 1.42, 1.24 (m, 2H, cyclic, -CH₂- , endo/exo), 1.45 (m, 2H, cyclic, -CH₂-, endo/exo).

The macromonomer molecular weight was assumed to be the sum of the norbornene carbonyl (121 g/mol) and poly(ethylene glycol) ($M_n = 1000$ g/mol) molecular weight which affords an $M_n = 1121$ g/mol.

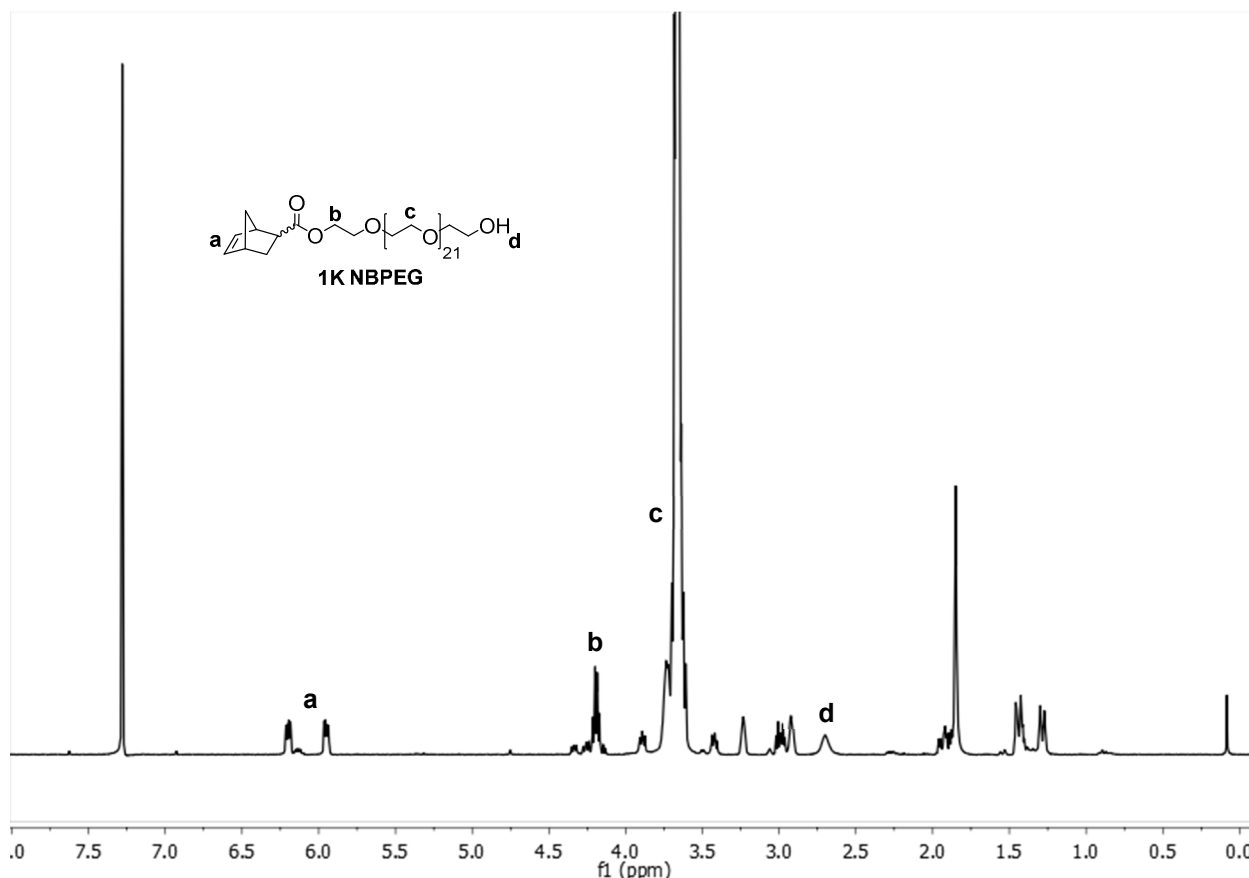


Figure S3. ^1H NMR spectrum of 1K NBPEG in CDCl_3 collected at room temperature.

2.3 Example of copolymer synthesis

A representative synthesis of random copolymer P-1 is described as follows. To a clean, dry 50 round-bottom flask equipped with a magnetic stir bar, monomers 1K NBMPEG (419 mg, .417 mmol) and 1K NBPEG (200 mg, .179 mmol) were added and the flask was capped with a septum and purged under N_2 atmosphere for 5 minutes. Anhydrous CH_2Cl_2 (12 mL) was added to the flask by syringe and the solution was allowed to stir for 10 minutes under N_2 atmosphere. The mG2 catalyst (10.83 mg, .015 mmol) was dissolved in anhydrous CH_2Cl_2 (4 mL) and purged under N_2 atmosphere for 5 minutes. The catalyst solution was added to the 50 mL round-bottom flask all at once under N_2 atmosphere and the reaction mixture was stirred for 20 minutes. The polymerization was terminated with the addition of excess ethyl vinyl ether (1 mL). The resulting polymer was precipitated in hexanes at room temperature, filtered, and dried overnight under vacuum at room temperature.

2.4 P-1 synthesis by ROMP

^1H NMR (500 MHz, CDCl_3) δ 5.28 (br m), 4.21 (br m), 4.05 (br m), 3.62 (br m), 3.35 (br m), 3.11 (br m), 3.00-2.68 (br m), 2.43 (br m), 2.23-1.52 (br m), 1.45-1.20 (br m), 1.06-0.76 (br m). Monomer weight fractions in the final polymer are calculated by integrating the characteristic peak corresponding to each monomer (3.35 and 3.60 ppm corresponding to NBMPEG, and NBPEG, respectively.)

M_n (GPC-ELSD) :- 32 kg/mol, PDI:- 1.14, Molecular weights are determined by using conventional calibrations, which are constructed using linear polystyrene (PS) standards with dimethylacetamide as eluent.

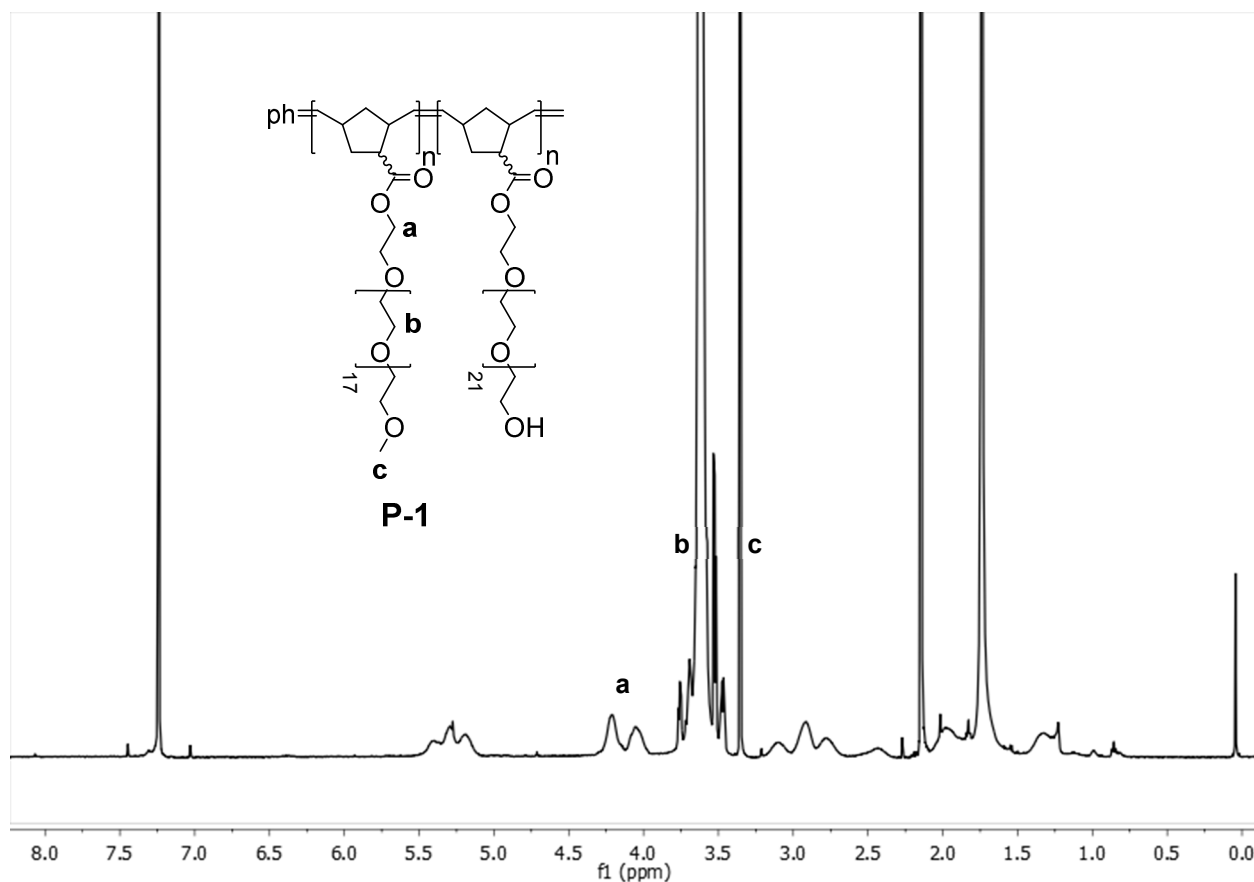


Figure S4. ^1H NMR spectrum of P-1 in CDCl_3 collected at room temperature.

2.5 TP-1 synthesis

To a clean, dry 50 mL round-bottom flask equipped with a magnetic stir bar, maleic anhydride (35 mg, .0597 mmol) and DMAP (15 mg, .0072 mmol) were dissolved in anhydrous CH_2Cl_2 (12 mL) and purged under N_2 atmosphere for 5 minutes while being stirred and cooled to 0°C . CP-1

(480 mg, .072 mmol) was dissolved in anhydrous CH₂Cl₂ (8 mL) in a scintillation vial and added dropwise to the cold reaction mixture, followed by purging under N₂ atmosphere for 10 minutes. The ice bath was removed and the reaction mixture was allowed to stir for 24 hours at room temperature under N₂ atmosphere. The reaction mixture was concentrated by rotary evaporation, dialyzed in deionized H₂O (4x2000 mL), and freeze dried. ¹H NMR (500 MHz, CDCl₃) δ 5.28 (br m), 4.22 (br m), 4.06 (br m), 3.62 (br m), 3.35 (br m), 3.10 (br m), 2.98-2.69 (br m), 2.44 (br m), 2.34-1.57 (br m), 1.42-1.19 (br m), 1.06-0.76 (br m).

Monomer weight fractions in the final polymer are calculated by integrating the characteristic peak corresponding to each monomer (3.35, 3.60, and 6.19/6.36 ppm corresponding to NBMPEG, NBPEG, and NBPEG-COOH respectively.)

Extent of COOH functionalization was calculated by comparing the sum of the integrations of the olefinic peaks at 6.19 and 6.36 ppm of the reacted maleic anhydride to the integration of the norbornene olefinic backbone at 5.29 ppm:

$$\frac{(\text{maleic olefin peaks integration})/(2 \text{ H})}{(\text{NB olefinic peak integration})/(4 \text{ H})} \times 100 = \% \text{ COOH functionalized NB repeat units}$$

$$\frac{0.955}{8.785} \times 100 = 11\% \text{ COOH functionalized NB repeat units}$$

$$11\% \text{ COOH functionalized NB repeat units} \times 40 = 4 \text{ COOH functionalized NB repeat units}$$

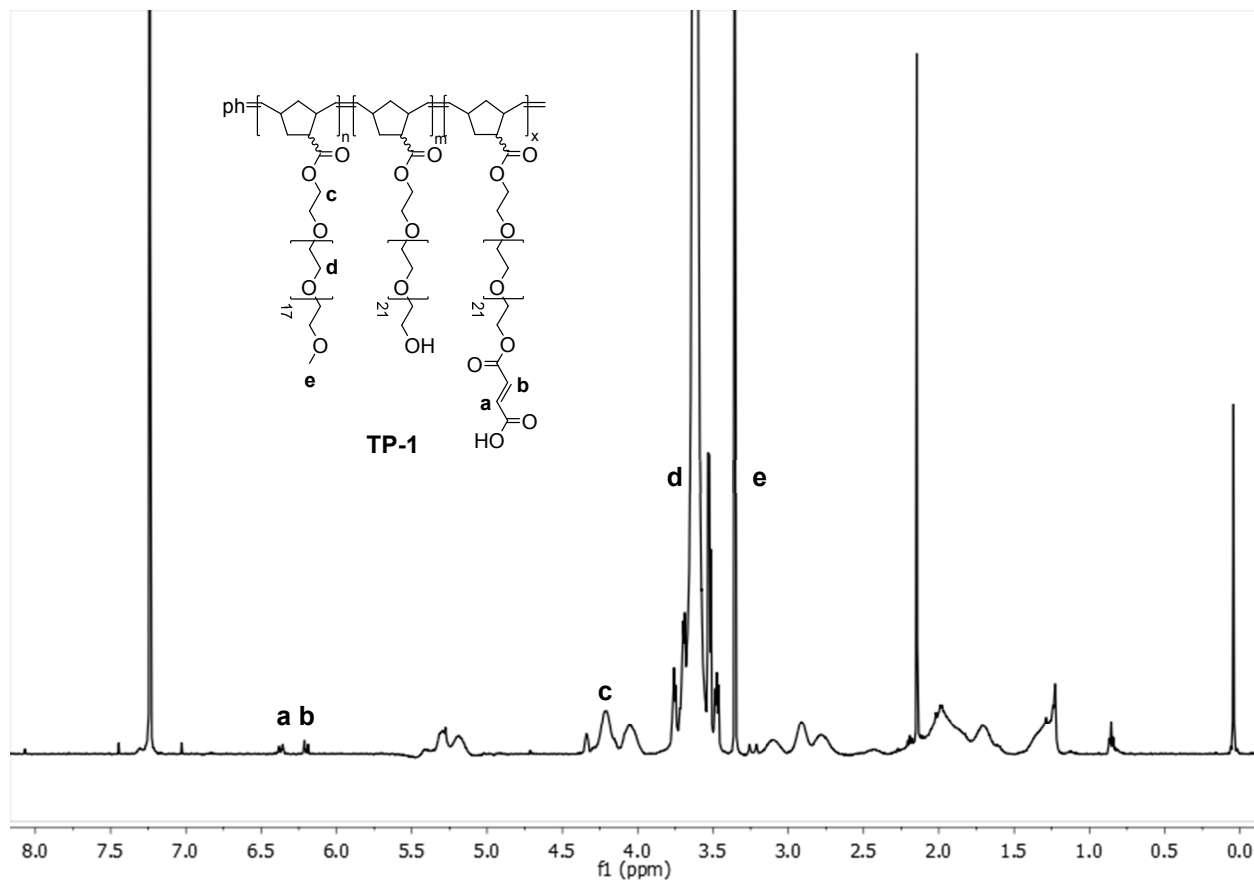


Figure S5. ¹H NMR spectrum of TP-1 in CDCl₃ collected at room temperature.

2.6 RAFT-based synthesis of NB-p(t-BA)²

To a clean, dry 25 mL schlenk tube equipped with a magnetic stir bar, NB-TTC (800 mg, 1.64 mmol), tert-butyl acrylate (7.60 mL, 52.37 mmol), and 2-butanone (8.36 mL) were added and allowed to stir for 2 minutes at room temperature. AIBN stock solution in 2-butanone (1 mg/mL, 5.38 mL, 2 mol %) was added and the reaction mixture underwent 5 cycles of freeze-pump-thaw. The reaction mixture was allowed to stir at 60°C for 3.5 hours. The polymerization was quenched at 30% monomer conversion by freezing in a liquid nitrogen bath. The reaction mixture was precipitated four times in 20% H₂O-methanol at -78°C and dried overnight by vacuum at room temperature to afford a yellow solid. Yield: 1.49 g, 20%. $M_n^{NMR} = 1,769$ g/mol. ¹H NMR (500 MHz, CDCl₃) δ 6.13-5.90 (m, 2H), 4.64 (m), 4.09 (m), 3.93 (m), 3.79 (m), 3.59 (m), 3.31 (m), 2.84-2.78 (m), 2.66 (m), 2.37 (m), 2.20 (20 H, br m), 2.00-1.80 (br m), 1.65 (m), 1.57-1.23 (br m), 1.13 (m), 0.86 (m).

M_n (GPC-ELSD) :- 2.4 kg/mol, PDI:- 1.07, Molecular weights are determined by using conventional calibrations, which are constructed using linear polystyrene (PS) standards with dimethylacetamide as eluent.

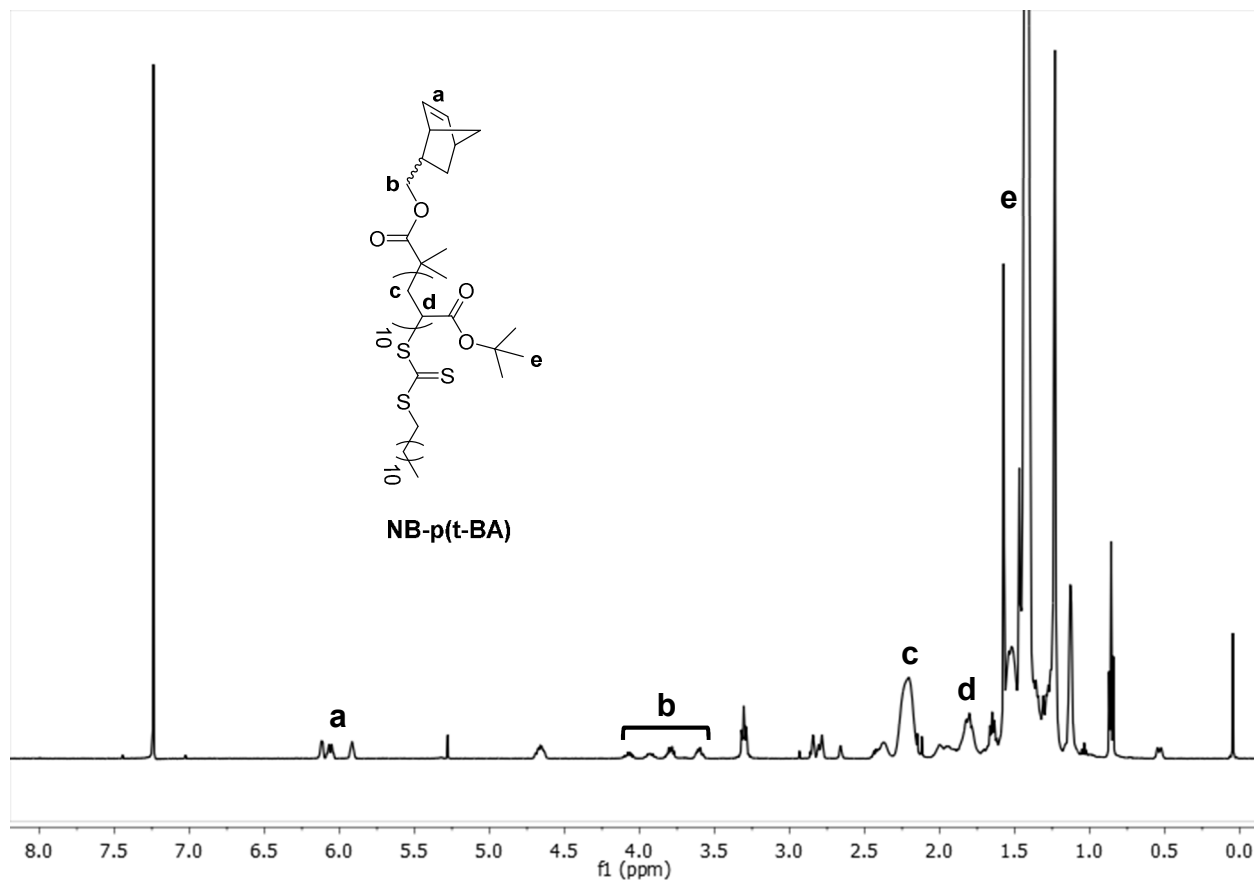


Figure S6. ^1H NMR spectrum of NB-p(t-BA) in CDCl_3 collected at room temperature.

2.7 P-2 synthesis by ROMP

P-2 was synthesized following the same procedure as P-1. The crude polymer was dried overnight under vacuum at room temperature and was used in the next step without further purification. ^1H NMR (500 MHz CDCl_3): ^1H NMR (500 MHz, CDCl_3) δ 5.51-5.15 (br m), 4.73 (br m), 4.27 (br m), 4.09 (br m), 3.86-3.52 (br m), 3.42-3.34 (br m), 3.17 (br m), 2.98 (br m), 2.84 (br m), 2.48 (br m), 2.29 (br m), 2.02 (br m), 1.75 (br m), 1.60-1.12 (br m), 0.92 (br m).

Monomer weight fractions in the final polymer are assumed as 50 mol % each of NBMPEG and NB-p(t-BA) and M_n is assumed to be equal to $M_n^{\text{Theo}} = 55.5$ kDa due to the ^1H NMR of the crude polymer mixture showing no unreacted monomer.

M_n (GPC-ELSD): 20.6 kg/mol, PDI: 1.08, Molecular weights are determined by using conventional calibrations, which are constructed using linear polystyrene (PS) standards with dimethylacetamide as eluent.

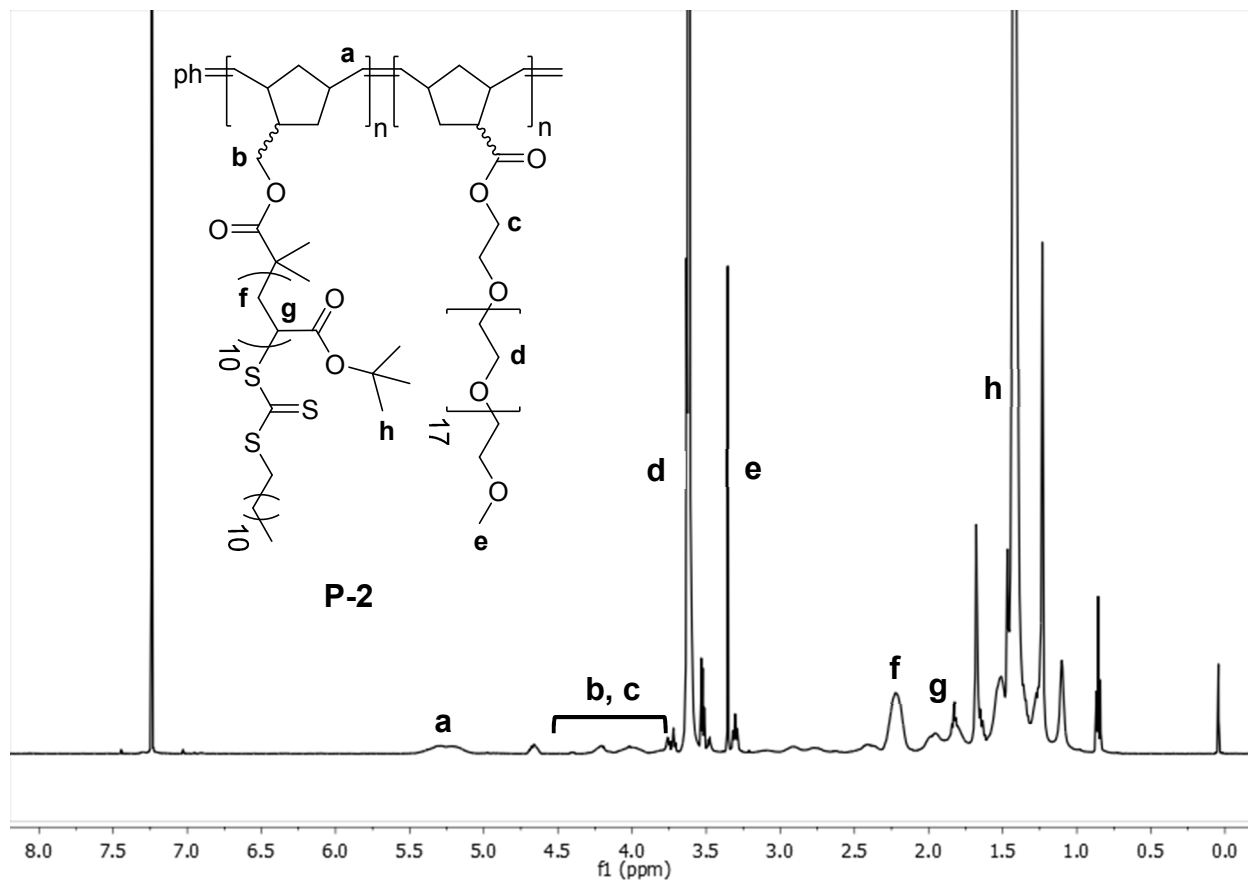


Figure S7. ^1H NMR spectrum of P-2 in CDCl_3 collected at room temperature.

2.8 CP-1 synthesis

To a clean, dry 25 mL round-bottom flask equipped with a small magnetic stir bar, poly(NBMPEG-r-NB-p(t-BA)) (300 mg, .005 mmol) was dissolved in THF (4.14 mL) followed by dropwise addition of trifluoroacetic acid (50 equiv. to 1 equiv. tert-butyl group, 4.14 mL, 54.0 mmol). The reaction mixture was capped with a glass stopper, sealed with teflon tape, and allowed to stir at 60°C for 72 hours. The resulting black reaction mixture was cooled to room temperature and dialyzed with deionized H_2O (4x2000 mL), followed by freeze drying. ^1H NMR (500 MHz, DMSO-d_6) δ 12.51-11.92 (br s), 5.35- 4.95 (br m), 4.43 (br m), 3.61 (br m), 3.38 (br m), 2.32-1.79 (br m), 1.63-0.47 (br m).

Monomer weight fractions in the final polymer are assumed as 50 mol % each of NBMPEG and NB-PAA and $M_n^{\text{Theo}} = 44.3$ kDa due to the assumption of complete hydrolysis of tert-butyl groups as seen in the **Figure S8** inset which shows the loss of the tert-butyl protons from **P-2** to **CP-1**.

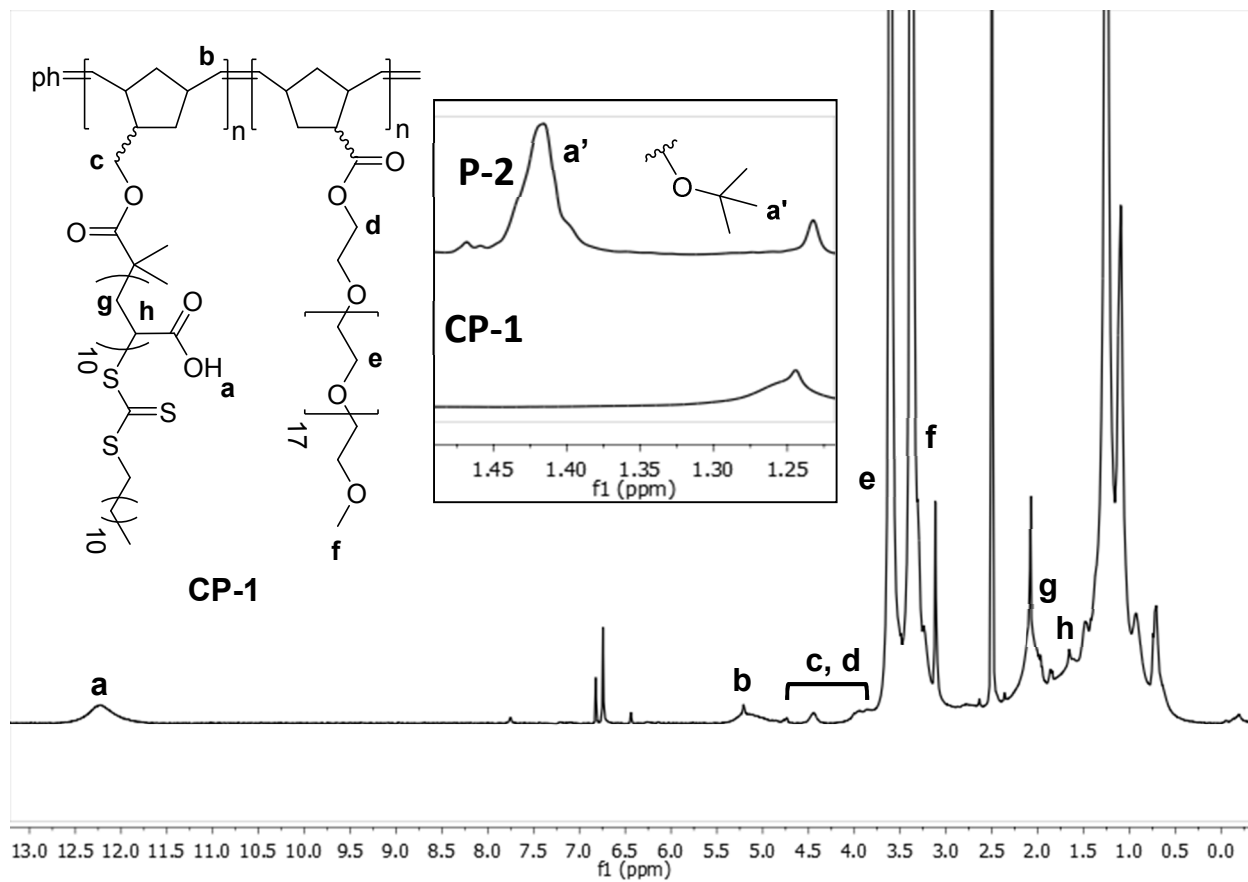


Figure S8. ^1H NMR spectrum of CP-1 in DMSO-d_6 collected at room temperature.



Figure S9. TEM image of collagen fibrils formed in the absence of sequestration analog (PAA or brush-like polymers), showing that no D-banding can be observed in the presence of templating analog itself (TPP).

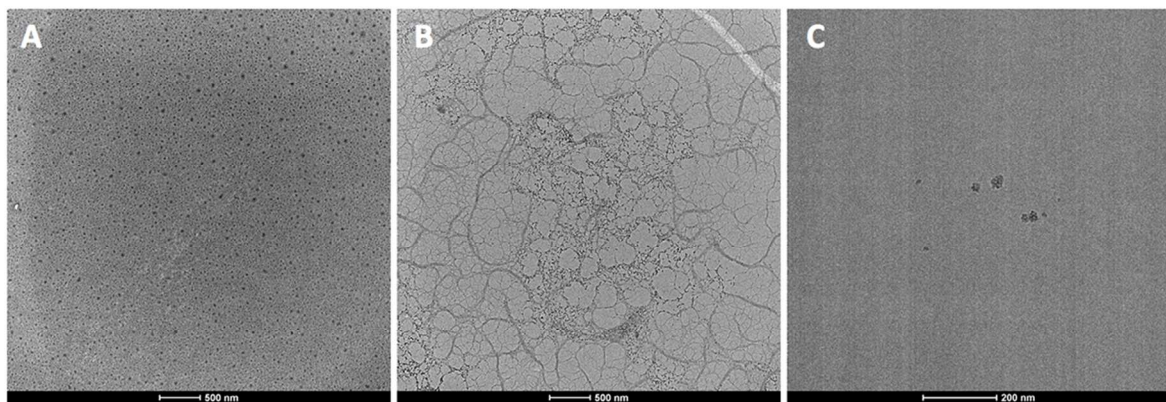


Figure S10. TEM images of PEG-COOH brush polymer dispersed in DIW at pH 6.4 showing round-like morphology with a diameter of 10.3 ± 3.3 nm ($n=273$) (A), and in the collagen-free m-SBF solution at pH 7.2 showing network structure (B); and PAA dispersed in the collagen-free m-SBF solution at pH 7.2 showing no network formation (C).

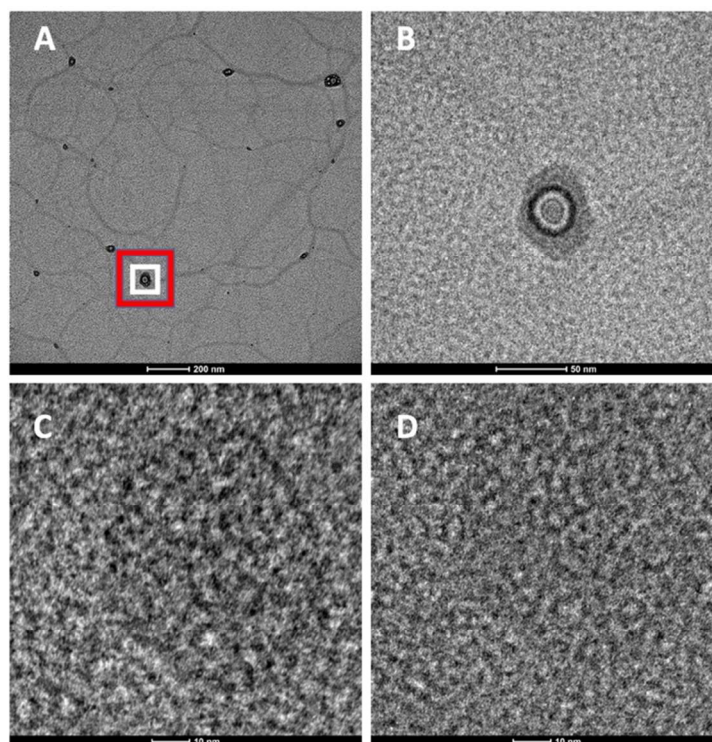


Figure S11. TEM (A/B) and HRTEM under defocus state (C) showing the profile of the particle and focus state (D) showing the amorphous state of the calcium phosphate (ACP) nanoprecursor formed in the presence of PEG-COOH terpolymer (TP-1).

Table S1. Ion concentration (mM) of human blood plasma and m-SBF.

Ion	Human blood plasma (mM)	m-SBF (mM)
Na ⁺	142.0	109.9
K ⁺	5.0	6.2
Mg ²⁺	1.5	1.5
Ca ²⁺	2.5	7.9
Cl ⁻	103.0	111.2
HCO ₃ ³⁻	27.0	17.5
HPO ₄ ²⁻	1.0	3.1
SO ₄ ²⁻	0.5	0

References

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