Supporting Information

Bioactive Polyethylene by Ring Opening Metathesis Polymerization for Potential Orthopedic Applications

Jiayi Guo,[a] Eun Ju Park,[a] Yew Chin Teo,[a] Asyraf Abbas,[a] Denise Goh,[b] Raymond Alexander Alfred Smith,[c] Yuntong Nie,[d] Hang T L Nguyen,[d] Joe Poh Sheng Yeong,[b] Simon Cool,[b,c] Haruyuki Makio[e] and Peili Teo*[a]

[a]Institute of Materials Research and Engineering (IMRE), Agency for Science, Technology and Research (A*STAR), 2 Fusionopolis Way, Innovis #08-03, Singapore 138634, Republic of Singapore
[b]Institute of Molecular and Cell Biology (IMCB), Agency for Science, Technology and Research (A*STAR), 61 Biopolis Drive, Proteos, Singapore 138673, Republic of Singapore
[c]School of Chemical Engineering, The University of Queensland, Brisbane, QLD 4072 Australia
[d]50 Science Park Road, #06-08 The Kendall, Mitsui Chemicals Singapore R&D Centre, PTE. LTD., Singapore 117406, Republic of Singapore
[e]R&D Center, Mitsui Chemicals, Inc., 580-32 Nagaura, Sodegaura, Chiba 299-0265, Japan

*Email: teo_peili@imre.a-star.edu.sg

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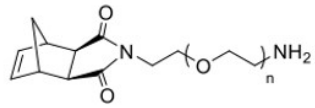
1. General Information

Materials and Methods. Biomacromonomer and polyethylene-peptide copolymer syntheses using ring opening metathesis polymerization (ROMP) were carried out in a glovebox under nitrogen atmosphere.[1-2] Reactions to obtain norbornene-polyethylene glycol (NBPEG), hexamethylenediamine (HMDA)-aminated PE, and to connect the HMDA-aminated PE to a norbornene dicarboxylic anhydride linker (*cis*-5-norbornene-*exo*-2,3-dicarboxylic anhydride) were carried out in a fumehood under atmospheric conditions, following literature procedures.[3-4] Succinic acid-terminated polyethylene (SA-t PE) (MW 1,400 and 5,000) and base polymer ultra-high molecular weight polyethylene (UHMWPE) was supplied by Mitsui Chemicals Inc.[5] Grubbs' catalyst was purchased from Sigma Aldrich and peptides were purchased from Biomatick Inc. *Cis*-norbornene-*exo*-2,3-dicarboxylic anhydride, polyethylene glycol (PEG) diamine (MW1,000 and 3,400), methoxypolyethylene glycol (mPEG) amine (MW1,000 and 5,000) and hexamethylenediamine (HMDA) were purchased from Alfa Aesar. HOBt, HBTU, $^{i}Pr_2EtN$ were purchased from Sigma Aldrich. DMEM low glucose and foetal calf serum (FCS) were purchased from Cytiva. Trypisn, penicillin/streptomycin and pNPP solution were purchased from Thermo Fisher Scientific. Tissue culture plasticware was purchased from Greiner. Recombinant Human BMP-2 was purchased from R&D Systems. C2C12 murine myoblasts were purchased from the American Type Culture Collection (U.S.A.). All purchased reagents were used as received unless otherwise noted.

Characterizations. NMR spectra were recorded on a JEOL 500 MHz NMR spectrometer using *d*-Methanol as solvent for all peptide-based macromonomers. NMR spectra were recorded on Bruker AVANCE III cryo-500 NMR spectrometer in Mitsui Chemical Analysis & Consulting Service, Inc. (MC-ANAC) and Bruker ASCEND cryo-400 NMR spectrometer in Mitsui Chemical Singapore R&D Center (MSRD) using d_4-dichlorobenzene as solvent for PE-based macromonomers and PE-peptide copolymers. ESI-MS was obtained using Agilent 6546 TOF-MS. ICP-MS analysis of Ru residue in the copolymers was recorded by Agilent 7700x. Thermogravimetric analysis (TGA) was performed on a NETZSCH STA 449 from room temperature to 700 °C with a heating rate of 10 °C min^{-1} in an argon atmosphere. Gel permeation chromatography (GPC) of **IIa** were recorded in Mitsui Chemical Analysis & Consulting Service, Inc. (MC-ANAC) by the system PL-GPC220 (Polymer Laboratories)/Cirrus 2.2 Beta 6 (Polymer Laboratories) equipped with column TSKgel GMHHR-H(S) HT/-H(S) HT/-M(S) (Tosoh, 7.8mmID, 300mmL×3). Eluent is 1,2,4-Trichlorobenzene, 0.025%(w/v) BHT with 1.0 ml/min flow rate and column temperature of 160°C. Gel permeation chromatography (GPC) of **IIb, IIIa-d** were recorded in the Institute of Sustainability for Chemicals, Energy and Environment (ISCE^2), Singapore using an Agilent 1260 Infinity II multi-detector system equipped with column Agilent PLgel Olexis 300 X 7.5 mm. Eluent is 1,2,4-Trichlorobenzene, 0.025%(w/v) BHT with 1.0 ml/min flow rate and column temperature of 160°C. Gel permeation chromatography (GPC) of **Ia-d** was recorded in THF on 2 PLgel MIXED-C columns (Agilent) connected in series with an Agilent 1260 Infinity II multi-detector system at a flow rate of 1.0 mL/min and column temperature of 40 °C using polystyrene as calibration standards.

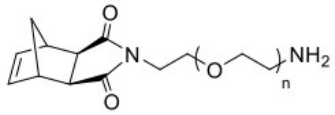
2. Experimental Procedures



PEG 1000

Synthesis of $NBPEG_{1,000}NH_2$

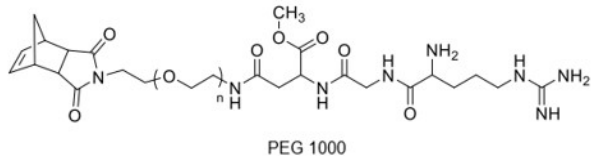
PEG diamine (MW 1,000) (1g, 1mmol) and *cis*-norbornene-*exo*-2,3-dicarboxylic anhydride (1 eq.) were added to a 100 ml rbf, followed by toluene (50 ml). Triethylamine (1 eq.) was added and the mixture stirred under reflux overnight, with a dean stark trap attached for water removal. The resulting solution is evaporated to dryness and dichloromethane (40 ml) was added, followed by 0.1 M HCl (40 ml). The organic layer was extracted and washed with 0.1 M NaOH (50 ml). Another 0.1 M NaOH (50 ml) was added to the aqueous fraction from the acid wash followed by CH_2Cl_2 (30 ml). The organic layer was extracted and combined, washed with saturated NaCl before drying over Na_2SO_4. The material was evaporated to dryness to give a pale orange waxy solid as the product (1.47g, yield 90%). ^{1}H NMR (CD_3OD, 500 MHz, 25^{o}C): δ = 6.33 (t, 2H, *J*=1.75 Hz), 3.64 (m, PEG), 3.56 (s, 4H), 3.18 (t, 2H, *J*=1.75Hz), 2.71 (d, 2H, *J*=1.5Hz), 1.46 (d, 1H, *J*=11.5Hz), 1.40 (d, 2H, *J*=11.5Hz). ESI-MS $(M+H)^+$: calculated: 1131.66; found: 1131.295 (n=21).



PEG 3400

Synthesis of $NBPEG_{3,400}NH_2$

PEG diamine (MW 3,400) (3.4g, 1mmol) and *cis*-norbornene-*exo*-2,3-dicarboxylic anhydride (1 eq.) were added to a 100 ml rbf, followed by toluene (50 ml). Triethylamine (1 eq.) was added and the mixture stirred under reflux overnight, with a dean stark trap attached for water removal. The resulting solution is evaporated to dryness and dichloromethane (40 ml) was added, followed by 0.1 M HCl (40 ml). The organic layer was extracted and washed with 0.1 M NaOH (50 ml). Another 0.1 M NaOH (50 ml) was added to the aqueous fraction from the acid wash followed by CH_2Cl_2 (30 ml). The organic layer was extracted and combined, washed with saturated NaCl before drying over Na_2SO_4. The material was evaporated to dryness to give a yellow waxy solid as the product (3.3g,f yield 93%). ^{1}H NMR (CD_3OD, 500 MHz, 25^{o}C): δ = 6.33 (s, 2H), 3.64 (m, PEG), 3.56 (s, 4H), 3.18 (m, 2H), 2.71 (s, 2H), 1.45 (d, 1H, *J*=7Hz), 1.39 (d, 1H, *J*=10Hz). ESI-MS $(M+H+Na)^{2+}$: calculated: 1788.047; found: 1788.5 (n=76).

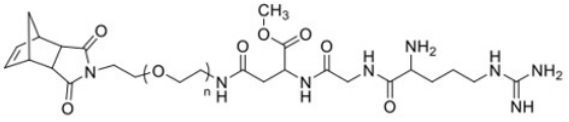


PEG 1000

Synthesis of $NBPEG_{1,000}$RGD Ia

RGD (with 1 carboxylic acid on aspartic acid protected with OMe) (0.0937 g, 0.26 mmol), was dissolved in MeOH (2.5 ml) in a 4 ml vial, in the glovebox. ^{i}Pr$_2$EtN (91 µl, 0.52 mmol) was added to form the solution (A). HOBt (0.0353 g, 0.26 mmol) and HBTU (0.0992 g, 0.26 mmol)

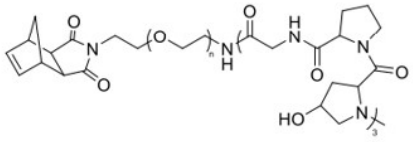
were dissolved in MeOH (12.5 ml) in a20 ml vial at 40°C, followed by addition of the RGD solution (A), to give the mixture (B). The mixture (B) is then added to $NBPEG_{1,000}NH_2$ (0.25 g, 0.218 mmol) in a 40 ml vial and stirred at r.t. for 24h. The resultant mixture was then evaporated to dryness and was added to Et_2O (50 ml). The Et_2O solution was chilled in a freezer for 48 h and decanted. MeOH (5 ml) was added to the residue and filtration was performed. The filtrate was evaporated to dryness followed by dialysis against deionized water and lyophilization to give an orange waxy solid as the product (0.3g, yield 95%). ^1H NMR (D_2O, 500 MHz, 25°C): δ = 6.37 (s, 2H, NB), 4.02 (m, RGD), 3.78 (s, RGD), 3.71 (m, PEG), 3.64 (d, 4H, *J*=5Hz), 3.22 (m, 4H, RGD), 3.01 (d, *J*=5Hz, RGD), 2.86 (s, 2H), 1.96 (m, RGD), 1.70 (m, RGD), 1.51 (d, 1H, *J*=10Hz). ESI-MS $(M+H)^+$: calculated: 1429.80; found: 1430.27 (n=20).



PEG 3400

Synthesis of $NBPEG_{3,400}$RGD Ib

RGD (with 1 carboxylic acid on aspartic acid protected with OMe) (0.0937 g, 0.26 mmol), was dissolved in MeOH (2.5 ml) in a 4 ml vial, in the glovebox. $^iPr_2EtN$ (91 µl, 0.52 mmol) was added to form the solution (A). HOBt (0.0353 g, 0.26 mmol) and HBTU (0.0992 g, 0.26 mmol) were dissolved in MeOH (12.5 ml) in a20 ml vial at 40°C, followed by addition of the RGD solution (A), to give the mixture (B). The mixture (B) is then added to $NBPEG_{3,400}NH_2$ (0.77g, 0.218 mmol) in a 40 ml vial and stirred at r.t. for 24h. The resultant mixture was then evaporated to dryness and was added to Et_2O (50 ml). The Et_2O solution was chilled in a freezer for 48 h and decanted. MeOH (5 ml) was added to the residue and filtration was performed. The filtrate was evaporated to dryness followed by dialysis against deionized water and lyophilization to give pale yellow solid as the product $NBPEG_{3,400}$RGD (0.8g, yield 95%).^1H NMR (D_2O, 500 MHz, 25°C): δ = 6.37 (s, 2H, NB), 4.04 (m, RGD), 3.85 (m, RGD), 3.71 (m, PEG), 3.64 (d, 4H, *J*=2.5Hz, NB), 3.23 (m, RGD), 3.00 (m, RGD), 2.86 (d, 2H, *J*=1.5Hz), 1.92 (m, RGD), 1.70 (m, RGD), 1.52 (d, 1H, *J*=10Hz, NB), 1.32 (d, 1H, *J*=10Hz, NB). ESI-MS $(M+H+Na)^{2+}$: calculated: 1716.99; found: 1716.0 (n=65).

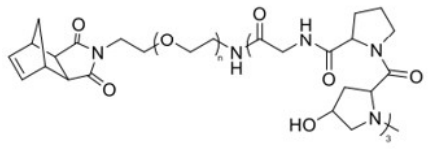


PEG 1000

Synthesis of $NBPEG_{1,000}(GPHyp)_3$ Ic

$(GPHyp)_3$ (0.213 g, 0.26 mmol) was dissolved in MeOH (2.5 ml) in glovebox. $^iPr_2EtN$ (91 µl, 0.52 mmol) was added to solution A. HOBt (0.0353 g, 0.26 mmol) and HBTU (0.0992 g, 0.26 mmol) were dissolved in MeOH (12.5 ml) at 40 °C, followed by addition of solution A to give suspension B. Suspension B was then added to $NBPEG_{1,000}NH_2$ (0.25 g, 0.218 mmol) and stirred at r.t. for 24 h. The resulting pale yellow mixture was then concentrated by solvent evaporation to give a beige mixture. The mixture was dispersed into Et_2O and chilled in freezer for 48h. The Et_2O layer was decanted and MeOH was added to the residue to give a beige suspension. Pale yellow waxy solid (0.4g, yield 95%) was obtained upon filtration, dryness and dialysis against deionized water and lyophilization. ^1H NMR (D_2O, 500 MHz, 25°C): δ = 6.37 (t, 2H, *J*=1.75Hz, NB), 4.64 (s, $(GPHyp)_3$), 4.51 (m, $(GPHyp)_3$), 4.22 (m, $(GPHyp)_3$), 3.97

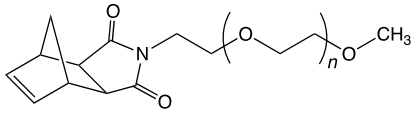
(m,$(GPHyp)_3$), 3.86 (m, $(GPHyp)_3$), 3.71 (s, PEG), 3.57 (m, 4H), 3.45 (m, $(GPHyp)_3$), 3.23 (m, 2H), 2.86 (d, 2H, *J*=1.5Hz), 2.64 (m, $(GPHyp)_3$), 2.42 (m, $(GPHyp)_3$), 2.32 (m, $(GPHyp)_3$), 2.06 (m, $(GPHyp)_3$), 1.52 (d, 1H, *J*=1.75Hz, NB). ESI-MS $(M+Cl)^-$: calculated: 1878.939; found: 1878.072 (n=19).



PEG 3400

Synthesis of $NBPEG_{3,400}(GPHyp)_3$ Id

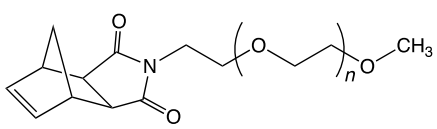
$(GPHyp)_3$ (0.213 g, 0.26 mmol) was dissolved in MeOH (2.5 ml) in glovebox. $^iPr_2EtN$ (91 µl, 0.52 mmol) was added and to form solution A. HOBt (0.0353 g, 0.26 mmol) and HBTU (0.0992 g, 0.26 mmol) were dissolved in MeOH (12.5 ml) at 40 °C, followed by addition of solution A to give suspension B. Suspension B was then added to $NBPEG_{3,400}NH_2$ (0.77 g, 0.218 mmol) and stirred at r.t. for 24 h. The resulting pale yellow mixture was then concentrated by solvent evaporation to give a beige mixture. The mixture was dispersed into Et_2O and chilled in freezer for 48h. The Et_2O layer was decanted and MeOH was added to the residue to give a beige suspension. Beige solid product (0.9 g, yield 95%) was obtained upon filtration, dryness and dialysis against deionized water and lyophilization. ^1H NMR (D_2O, 500 MHz, 25°C): δ = 6.37 (t, 2H, *J*=2Hz, NB), 4.64 (m, $(GPHyp)_3$), 4.51 (m, $(GPHyp)_3$), 4.22 (m, $(GPHyp)_3$), 3.98 (m, $(GPHyp)_3$), 3.85 (m, $(GPHyp)_3$), 3.71 (m, PEG), 3.57 (m, 4H), 3.46 (m, $(GPHyp)_3$), 3.23 (m, 2H), 2.86 (d, 2H, *J*=1.5Hz), 2.56 (m, $(GPHyp)_3$), 2.42 (m, $(GPHyp)_3$), 2.33 (m, $(GPHyp)_3$), 2.06 (m, $(GPHyp)_3$), 1.52 (d, 1H, *J*=10Hz,NB), 1.32 (d, 1H, *J*=10Hz, NB). ESI-MS $(M+H+Na)^{2+}$: calculated: 2076.186; found: 2076.2 (n=71).



mPEG-NH_2 1000

Synthesis of NB-$mPEG_{1,000}$

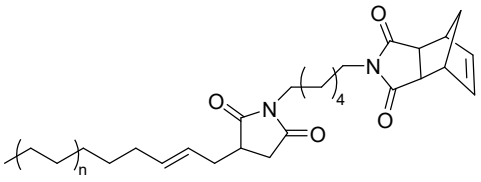
Methoxypolyethylene glycol amine (MW 1,000) (1g, 1mmol) and *cis*-norbornene-*exo*-2,3-dicarboxylic anhydride (1 eq.) were added to a 100 ml rbf, followed by toluene (50 ml). Triethylamine (1 eq.) was added and the mixture stirred under reflux overnight, with a dean stark trap attached for water removal. The solvent was evaporated and resulting mixture was washed with MeOH. The material was evaporated to dryness to give an orange waxy solid as the product (1.03g, yield 90%). ^1H NMR (CD_3OD, 500 MHz, 25°C): δ = 6.33 (t, 2H, *J*=1.75Hz), 3.64 (m, PEG), 3.57 (s, 4H), 3.36 (s, 3H, -OCH_3), 3.18 (t, 2H, *J*=1.75Hz), 2.71 (d, 2H, *J*=1Hz), 1.46 (d, 1H, *J*=9.5Hz), 1.39 (d, 1H, *J*=10Hz). ESI-MS $(M+Na)^+$: calculated: 1064.598; found: 1064.6 (n=19).



mPEG-NH_2 5000

Synthesis of NB-$mPEG_{5,000}$

Methoxypolyethylene glycol amine (MW 5,000) (1g, 0.2 mmol) and *cis*-norbornene-*exo*-2,3-dicarboxylic anhydride (1 eq.) were added to a 100 ml rbf, followed by toluene (20 ml). Triethylamine (1 eq.) was added and the mixture stirred under reflux overnight, with a dean stark trap attached for water removal. The solvent was evaporated and resulting mixture was washed with MeOH. The material was evaporated to dryness to give an orange waxy solid as the product (0.92g, yield 90%). ^{1}H NMR (CD_3OD, 500 MHz, 25^{o}C): δ = 6.33 (t, 2H, *J*=1.75Hz), 3.63 (s, PEG), 3.57 (s, 4H), 3.36 (s, 3H, -OCH_3), 3.18 (t, 2H, *J*=2Hz), 2.71 (d, 2H, *J*=1.5Hz), 1.46 (d, 1H, *J*=9.5Hz), 1.40 (d, 1H, *J*=9.5Hz). ESI-MS $(M+H)^+$: calculated: 3111.84; found: 3111.2 (n=66).

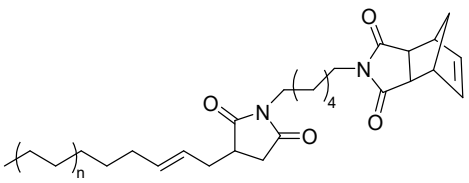


NB-SA-t $PE_{1,400}$

Synthesis of NB-SA-t $PE_{1,400}$ IIa

SA-t $PE_{1,400}$ (10 g, 6.68 mmol) is weighed into a 250 ml rbf followed by addition of toluene (120 ml). Hexamethylenediamine (HMDA) (0.9308 g, 8.01 mmol) and triethylamine (0.93 ml, 6.68 mmol) are then added. The mixture is stirred under reflux overnight with a dean stark trap connected, for water removal. The resultant suspension is then cooled and concentrated, followed by addition of MeOH, to give a beige precipitate. The mixture is filtered and residue is washed with MeOH before dryness, to give SA-t $PE_{1,400}$-HMDA quantitatively.[5]

SA-t $PE_{1,400}$-HMDA (10.6556 g, 6.68 mmol) is added to a 250 ml rbf followed by addition of *cis*-norbornene-*exo*-2,3-dicarboxylic anhydride (1.2202 g, 7.34 mmol), toluene (120 ml) and triethylamine (0.93 ml, 6.68 mmol). The mixture is then refluxed overnight with a dean stark trap connected, for water removal. The resultant suspension is then cooled and concentrated, followed by addition of MeOH, to give a beige precipitate. The mixture is filtered and residue is washed with MeOH before dryness, to give NB-SA-t $PE_{1,400}$ macromonomer (9.9g, yield 90%). ^{1}H NMR (C_7D_8, 500 MHz, 80^{o}C): δ = 5.92 (s, 2H), 5.54 (m, 1H, -C*H*=CH-), 5.46 (m, 1H, -CH=C*H*-), 3.46 (m, 10H, -CH_2 on HMDA), 3.07 (s, 2H, -CH_2 on HMDA), 2.31 (m, 5H), 2.18 (m, PE), 2.05 (m, 4H), 1.39 (m, PE), 0.98 (t, -CH_3).



NB-SA-t $PE_{5,000}$

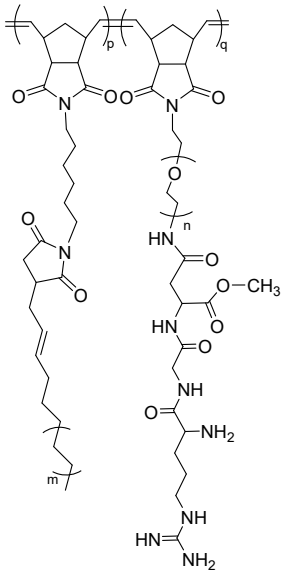
Synthesis of NB-SA-t $PE_{5,000}$ IIb

SA-t $PE_{5,000}$ (10 g, 2 mmol) is weighed into a 250 ml rbf followed by addition of toluene (120 ml). Hexamethylenediamine (HMDA) (0.2784 g, 2.4 mmol) and triethylamine (0.28 ml, 2 mmol) are then added. The mixture is then stirred under reflux overnight with a dean stark trap connected, for water removal. The resultant suspension is then cooled and concentrated, followed by addition of MeOH, to give a beige precipitate. The mixture is filtered and residue is washed with MeOH before dryness, to give SA-t $PE_{5,000}$-HMDA quantitatively.[5]

SA-t $PE_{5,000}$-HMDA (10.2 g, 2 mmol) is added to a 250 ml rbf followed by addition of *cis*-norbornene-*exo*-2,3-dicarboxylic anhydride (0.366 g, 2.2 mmol), toluene (120 ml) and triethylamine (0.28 ml, 2 mmol). The mixture is then refluxed overnight with a dean stark trap connected, for water removal. The resultant suspension is then cooled and concentrated, followed by addition of MeOH, to give a beige precipitate. The mixture is filtered and residue is washed with MeOH before dryness, to give NB-SA-t $PE_{5,000}$ macromonomer (9.4g, yield 90%). ^{1}H NMR (1,2-$C_6D_4Cl_2$, 400 MHz, 120^{o}C): δ = 6.03 (m, 2H, NB), 5.44 (m, 1H, -C*H*=CH-), 5.24 (m, 1H, -CH=C*H*-), 3.39 (m, 6H, -CH_2- on HMDA), 3.05 (s, 3H, -CH_2- on HMDA), 2.41 (m, 5H), 1.95 (m, 3H), 1.22 (m, PE), 0.84 (M, -CH_3).

Typical ROMP procedure of NB-SA-t $PE_{1,400}$ macromonomer and NB-$PEG_{1,000}$-RGD macromonomer as representative ROMP procedure for synthesis of PE-peptide and PE-mPEG copolymers

NB-$PEG_{1,000}$-RGD macromonomer (0.036 g, 0.024 mmol) is weighed into a 20 ml vial followed by addition of NB-SA-t $PE_{1,400}$ (0.2 g, 0.12 mmol). 1,2-diclorobenzene (2.4 ml) is added and the mixture stirred at 75 ^{o}C till a brown solution is obtained. A solution of 2nd generation Grubbs' catalyst in 1,2-diclorobenzene (1.25 mol %, 0.05 M) is added and the mixture is stirred for 24 h at 75 ^{o}C. Ethyl vinyl ether is added to the mixture followed by MeOH (15 ml) to give a beige precipitate. The mixture is filtered and the residue is washed with H_2O/Acetone before dryness. All copolymers were obtained with the isolated yield over 90%. NB-SA-t PE conversion and NB-PEG-peptide incorporation varied.

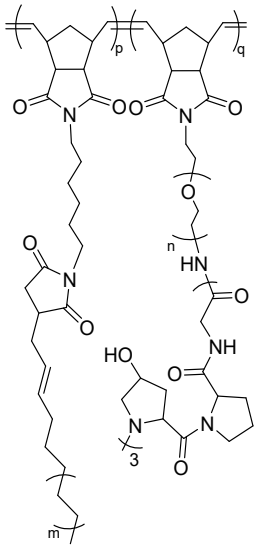


$PE_{1,400}$-RGD copolymer IIIa

^{1}H NMR (1,2-$C_6D_4Cl_2$, 400 MHz, 120^{o}C): δ = 6.06 (bs, unreacted NB-SA-t $PE_{1,400}$), 5.80 (bs), 5.48 (bs, vinyl protons on SA-t $PE_{1,400}$), 5.31 (bs, vinyl protons on SA-t $PE_{1,400}$), 3.54 (s, PEG), 3.43 (bs), 3.26 (m), 2.61 (m), 1.97 (m), 1.29 (s, PE), 0.84 (s, 3H, -CH_3 on SA-t $PE_{1,400}$).

$PE_{5,000}$-RGD copolymer IIIb

^{1}H NMR (1,2-$C_6D_4Cl_2$, 400 MHz, 120^{o}C): δ = 6.06 (s, unreacted NB-SA-t $PE_{5,000}$), 5.48 (m, vinyl proton on SA-t $PE_{5,000}$), 5.30 (m, vinyl protons on SA-t $PE_{5,000}$), 3.55 (s, PEG), 3.41 (m), 3.27 (s), 3.09 (s), 2.44 (m), 1.93 (m), 1.28 (s, PE), 0.84 (m, 3H, -CH_3 on SA-t $PE_{5,000}$).

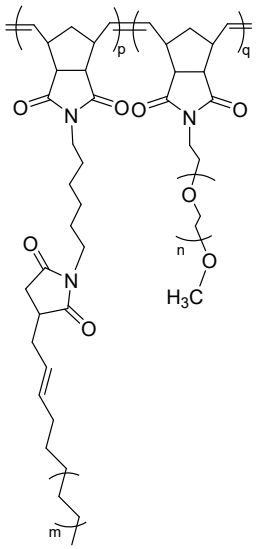


$PE_{1,400}$-$(GPHyp)_3$ copolymer IIIc

^{1}H NMR (1,2-$C_6D_4Cl_2$, 400 MHz, 120°C): δ = 5.80 (bs), 5.46 (m, vinyl proton on SA-t $PE_{1,400}$), 5.30 (bs, vinyl proton on SA-t $PE_{1,400}$), 3.55 (s, PEG), 3.44 (s), 2.70 (m), 1.95 (bs), 1.29 (m, PE), 0.84 (m, 3H, -C*H*$_3$ on SA-t $PE_{1,400}$).

$PE_{5,000}$-$(GPHyp)_3$ copolymer IIId

^{1}H NMR (1,2-$C_6D_4Cl_2$, 400 MHz, 120°C): δ = 6.06 (m, unreacted NB-SA-t $PE_{5,000}$), 5.47 (m, vinyl proton on SA-t $PE_{5,000}$), 5.29 (m, vinyl proton on SA-t $PE_{5,000}$), 3.55 (s, PEG), 3.08 (s), 2.43 (m), 1.95 (m), 1.29 (s, PE), 0.84 (m, 3H, -C*H*$_3$ on SA-t $PE_{5,000}$).



$PE_{1,400}$-$mPEG_{1,000}$ copolymer IVa

^{1}H NMR (1,2-$C_6D_4Cl_2$, 400 MHz, 120°C): δ = 5.81 (bs), 5.41 (m, vinyl proton on SA-t $PE_{1,400}$), 5.36 (s, vinyl proton on SA-t $PE_{1,400}$), 3.55 (s, PEG), 3.45 (bs), 3.24 (m), 1.97 (m), 1.29 (s, PE), 0.84 (m, 3H, -C*H*$_3$ on SA-t $PE_{1,400}$).

$PE_{5,000}$-$mPEG_{5,000}$ copolymer IVb

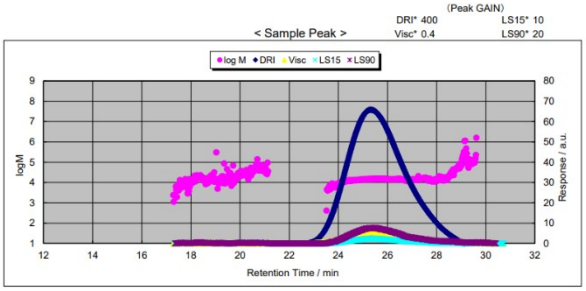
^{1}H NMR (1,2-$C_6D_4Cl_2$, 400 MHz, 120^{o}C): δ = 6.06 (bs, unreacted NB-SA-t $PE_{5,000}$), 5.49 (bs, vinyl proton on SA-t $PE_{5,000}$), 5.31 (bs, vinyl proton on SA-t $PE_{5,000}$), 3.55 (s, PEG), 3.43 (m), 3.23 (d), 2.64 (m), 1.96 (m), 1.29 (s, PE), 0.84 (m, 3H, -C*H*$_3$ on SA-t $PE_{5,000}$).

3. GPC Traces

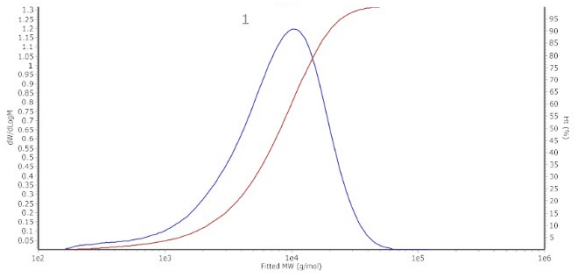
Table S1. Refractive Index Increment [dn/dc (mL/g)] of **IIa-b** and **IIIa-d**

	Polymers	dn/dc (mL/g)[a]
1	NB-SA-t $PE_{1,400}$ **IIa**	0.068
2	NB-SA-t $PE_{5,000}$ **IIb**	0.071
3	$PE_{1,400}$-RGD **IIIa**	0.044
4	$PE_{5,000}$-RGD~~(GPHyp)$_3$~~ **IIIb**	0.061
5	$PE_{1,400}$-~~RGD~~(GPHyp)$_3$ **IIIc**	0.038
6	$PE_{5,000}$-$(GPHyp)_3$ **IIId**	0.036

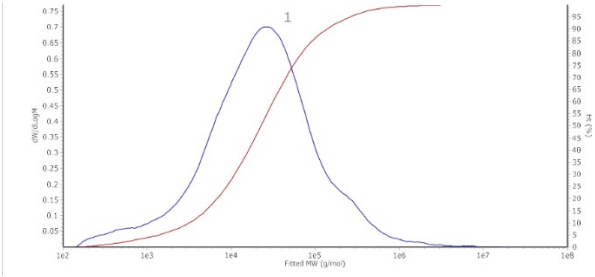
NB-SA-t-$PE_{1,400}$ **IIa**



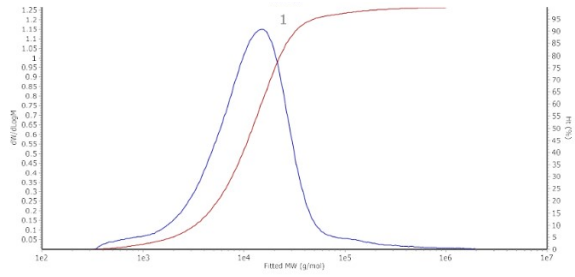
NB-SA-t-$PE_{5,000}$ **IIb**



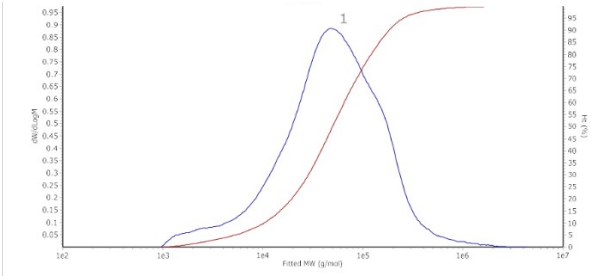
$PE_{1,400}$-RGD **IIIa**



$PE_{5,000}$-RGD **IIIb**



$PE_{1,400}$-$(GPHyp)_3$ **IIIc**



$PE_{5,000}$-$(GPHyp)_3$ **IIId**

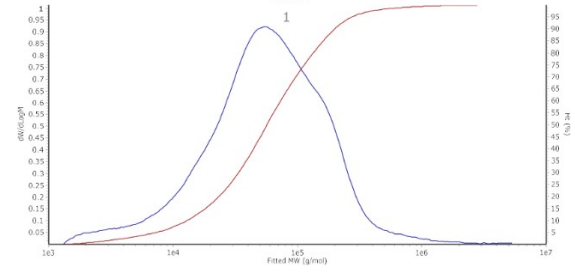


Figure S1. GPC traces of macromonomers **IIa**, **IIb** and brush copolymers **IIIa-d**

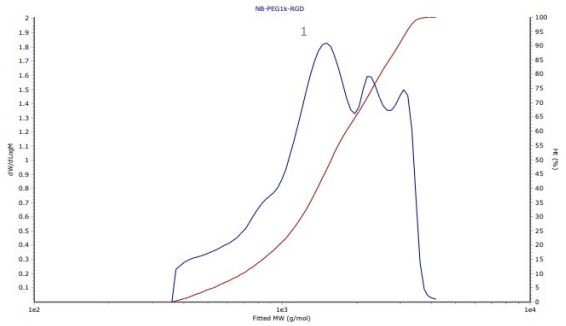
[a]**IIa** was analysed by Mitsui Chemical Analysis & Consulting Service, Inc. (MC-ANAC); **IIb, IIIa-d** were analysed by Institute of Sustainability for Chemicals, Energy and Environment ($ISCE^2$), Agency for Science, Technology and Research (A*STAR), Singapore.

Table S2. GPC data of peptide-based macromonomers **Ia-d**

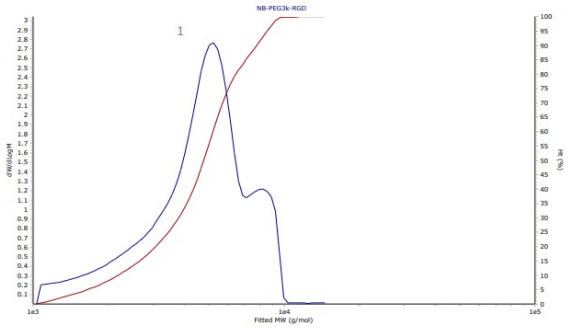
	Polymers	M_n[b] (kDa)	PDI
1	NB-$PEG_{1,000}$-RGD **Ia**	1.3	1.33
2	NB-$PEG_{3,400}$-RGD **Ib**	3.9	1.26
3	NB-$PEG_{1,000}$-$(GPHyp)_3$ **Ic**	1.2	1.34
4	NB-$PEG_{3,400}$-$(GPHyp)_3$ **Id**	4.3	1.28

[b]Determined by GPC in THF using polystyrene standard

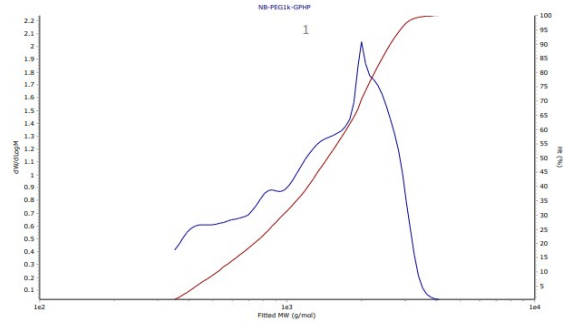
NB-$PEG_{1,000}$-RGD **Ia**



NB-$PEG_{3,400}$-RGD **Ib**



NB-$PEG_{1,000}$-$(GPHyp)_3$ **Ic**



NB-$PEG_{3,400}$-$(GPHyp)_3$ **Id**

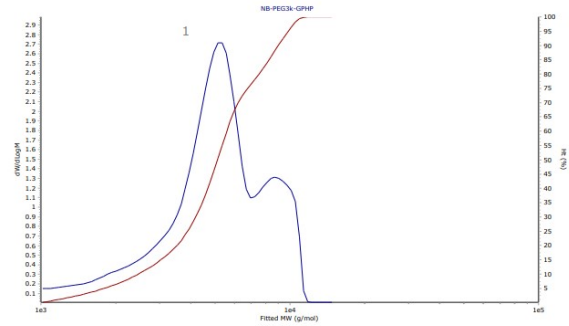


Figure S2. GPC traces of macromonomers **Ia-d**

4. Residual Ru Analysis by ICP-MS

Table S3. ICP-MS analysis of residual Ruin the copolymers

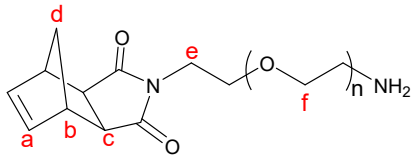
Samples		Concentration (ppm)
$PE_{1,400}$-RGD	**IIIa**	0.432
$PE_{5,000}$-RGD	**IIIb**	0.014
$PE_{1,400}$-$(GPHyp)_3$	**IIIc**	0.013
$PE_{5,000}$-$(GPHyp)_3$	**IIId**	0.009

- The concentration of residual Ru in all copolymers **IIIa-d** are below 1ppm.

5. References

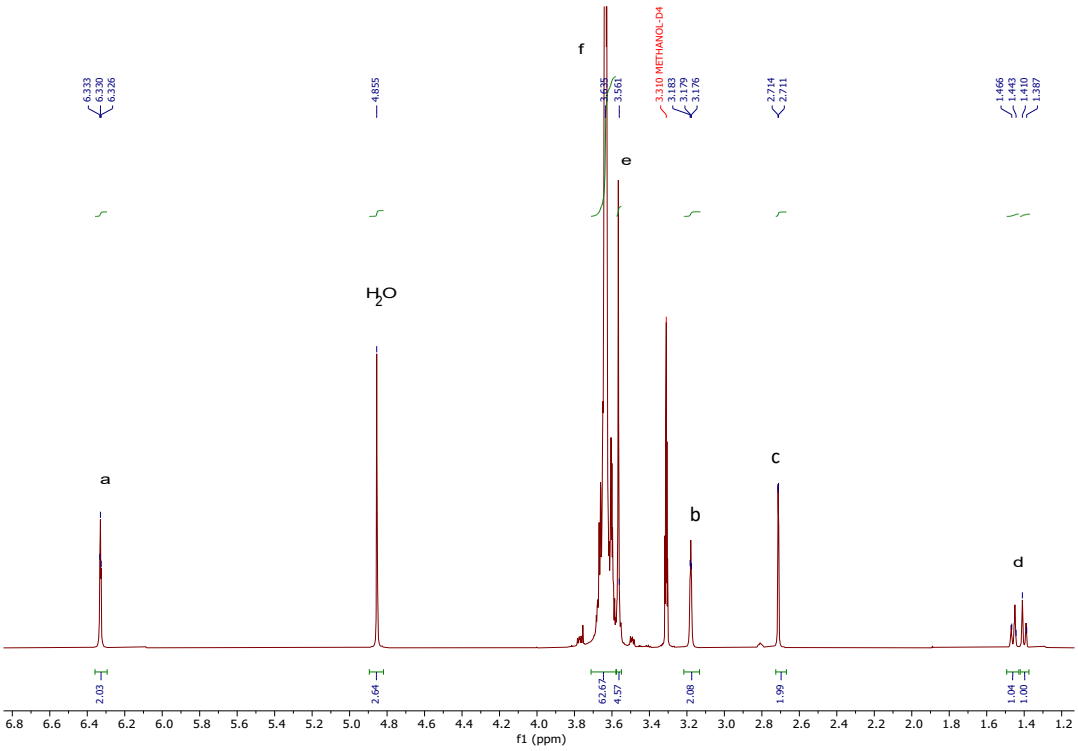
1. Héroguez, V.; Chemtob, A.; Quemener, D., ROMP in Dispersed Media. In *Handbook of Metathesis*, 2015; pp 25-44.
2. Slugovc, C., Synthesis of Homopolymers and Copolymers. In *Handbook of Metathesis*, 2015; pp 1-23.
3. Matson, J. B.; Grubbs, Robert. H., Synthesis of Fluorine-18 Functionalized Nanoparticles for use as *in vivo* Molecular Imaging Agents. *J. Am. Chem. Soc.* **2008**, *130*, 6731–6733.
4. Maynard, H. D.; Okada, S. Y.; Grubbs, R. H., Synthesis of Norbornenyl Polymers with Bioactive Oligopeptides by Ring-Opening Metathesis Polymerization. *Macromolecules* **2000**, *33*, 6239-6248.
5. Sainath, A. V. S.; Isokawa, M.; Suzuki, M.; Ishii, S.; Matsuura,S.; Nagai, N.; Fujita, T, Synthesis and Characteristics of Succinic Anhydride and Disodium Succinate-Terminated Low Molecular Weight Polyethylenes. *Macromolecules* **2009**, *42*, 4356.

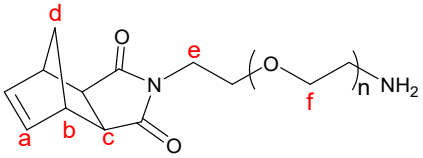
6. NMR Spectra



(PEG 1,000)

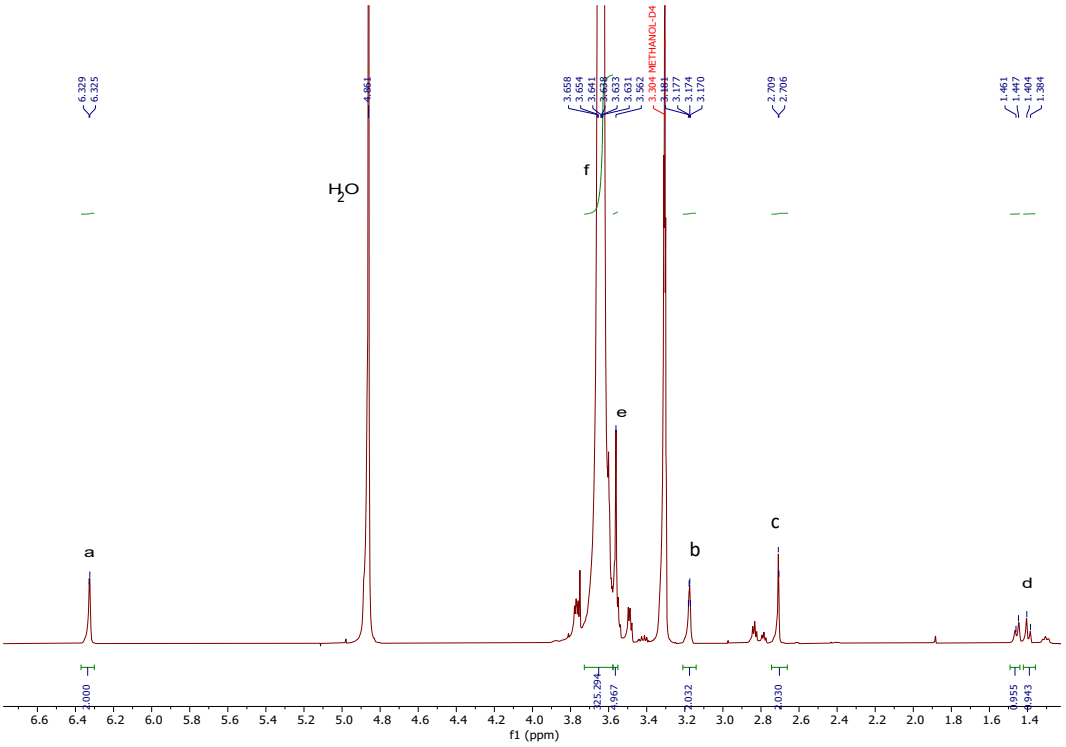
(^{1}H NMR, CD_3OD, 25°C, 500MHz)

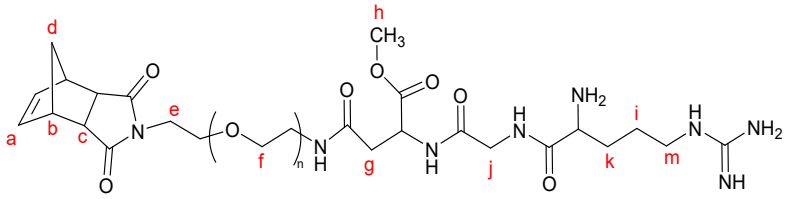




(PEG 3,400)

(^{1}H NMR, CD_3OD, 25°C, 500MHz)

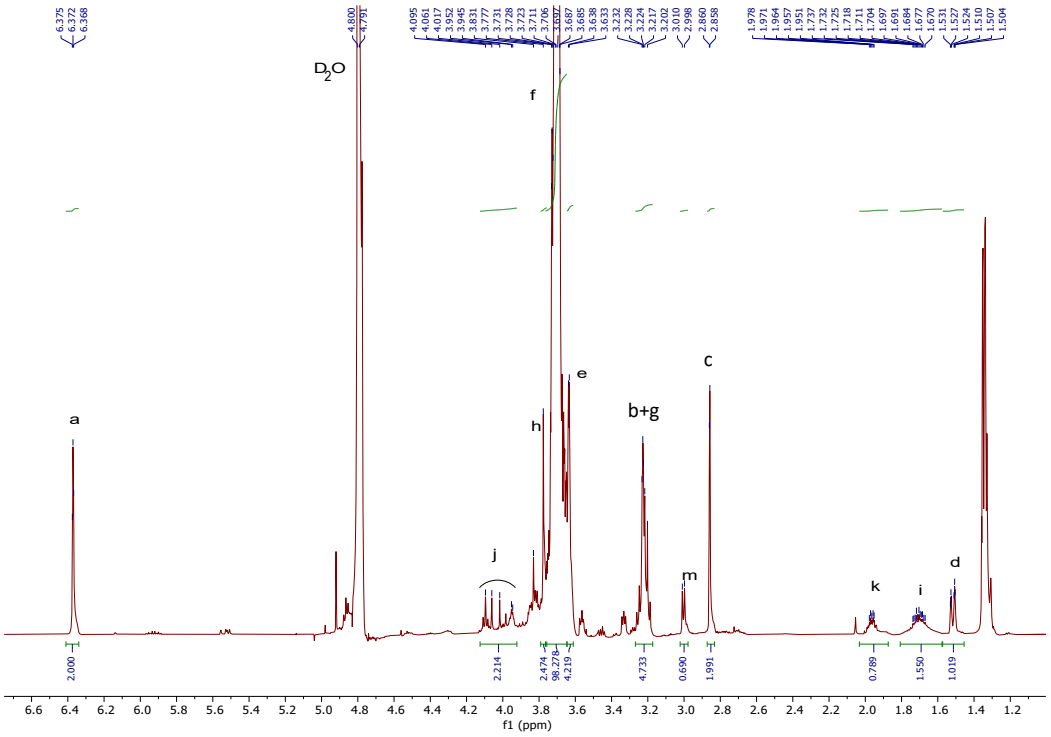


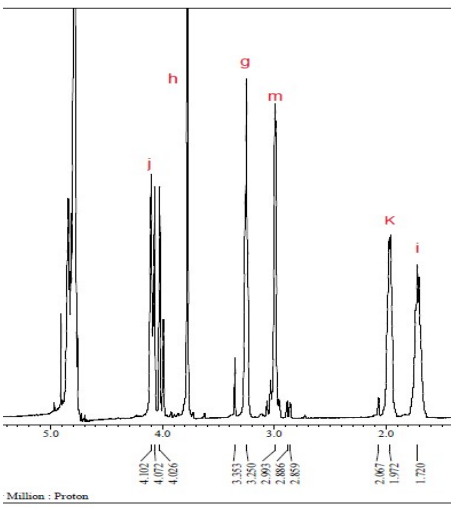


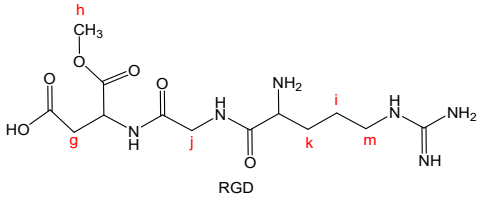
PEG(1,000)

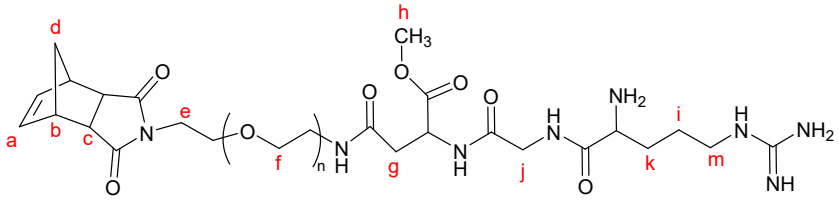
Ia

(^{1}H NMR, D_2O, 25°C, 500MHz)





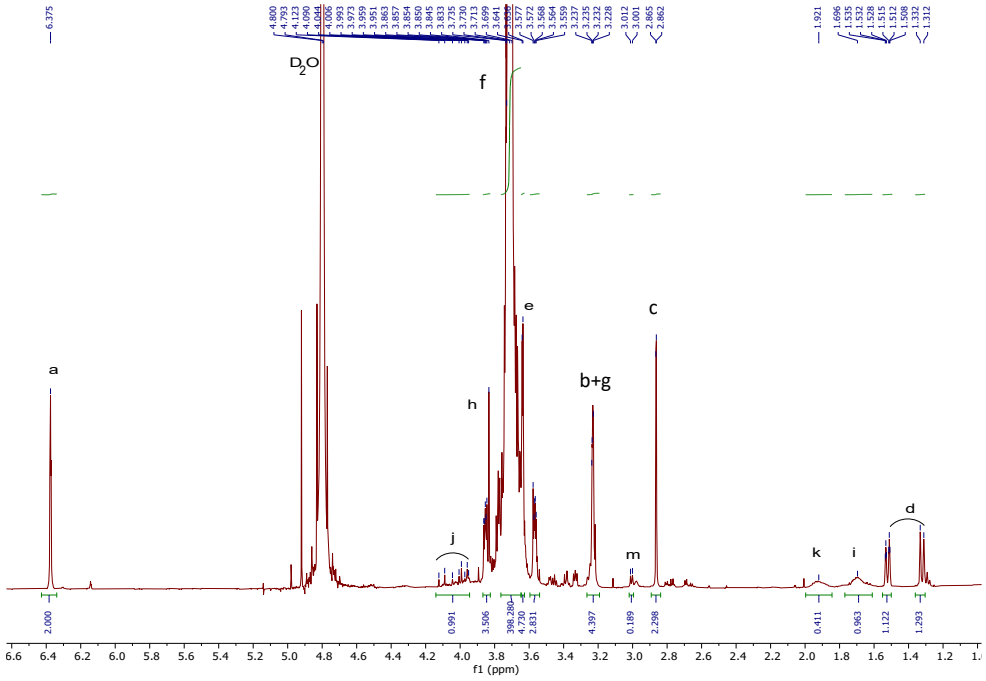


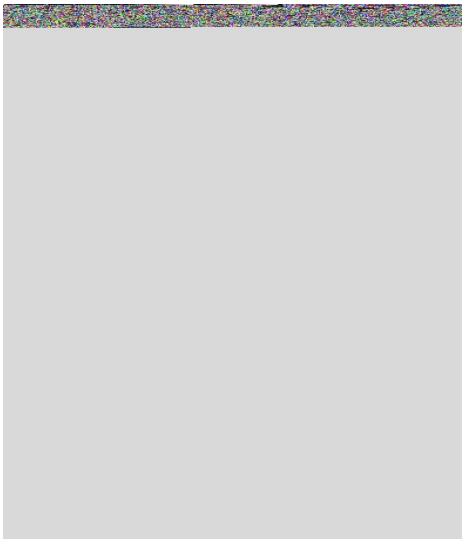


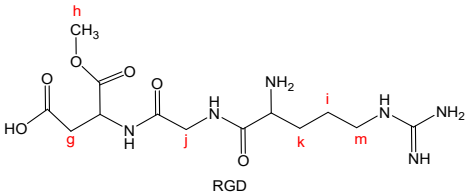
PEG(3,400)

Ib

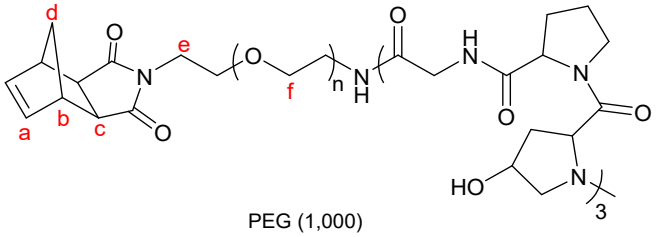
(^{1}H NMR, D_2O, 25^{o}C, 500MHz)







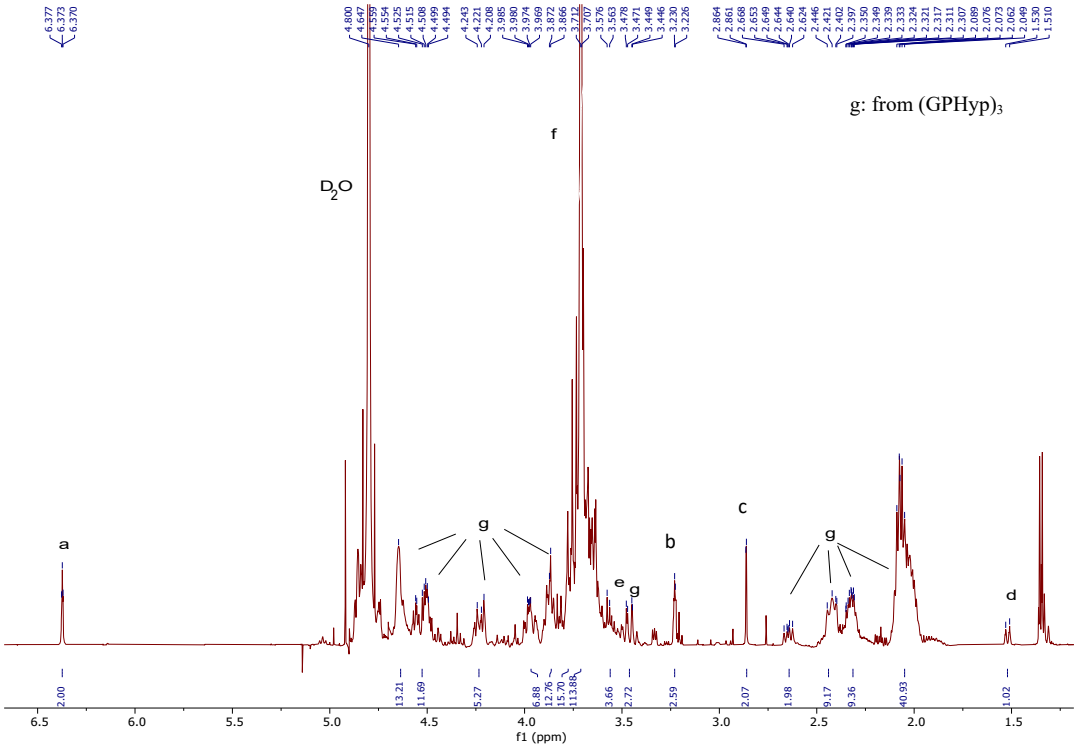
RGD

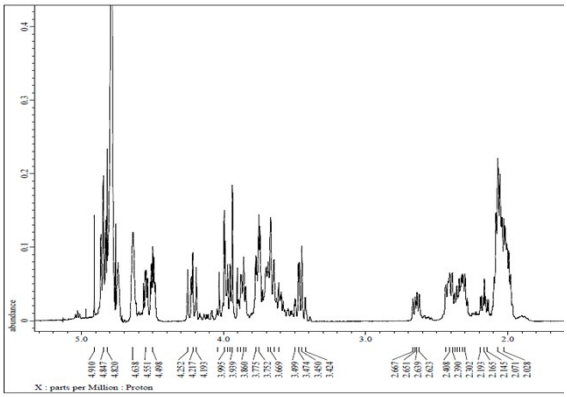


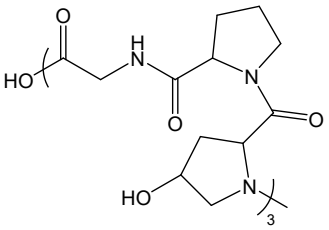
PEG (1,000)

Ic

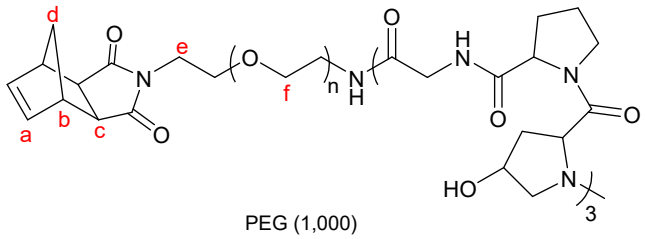
(^{1}H NMR, D_2O, 25°C, 500MHz)







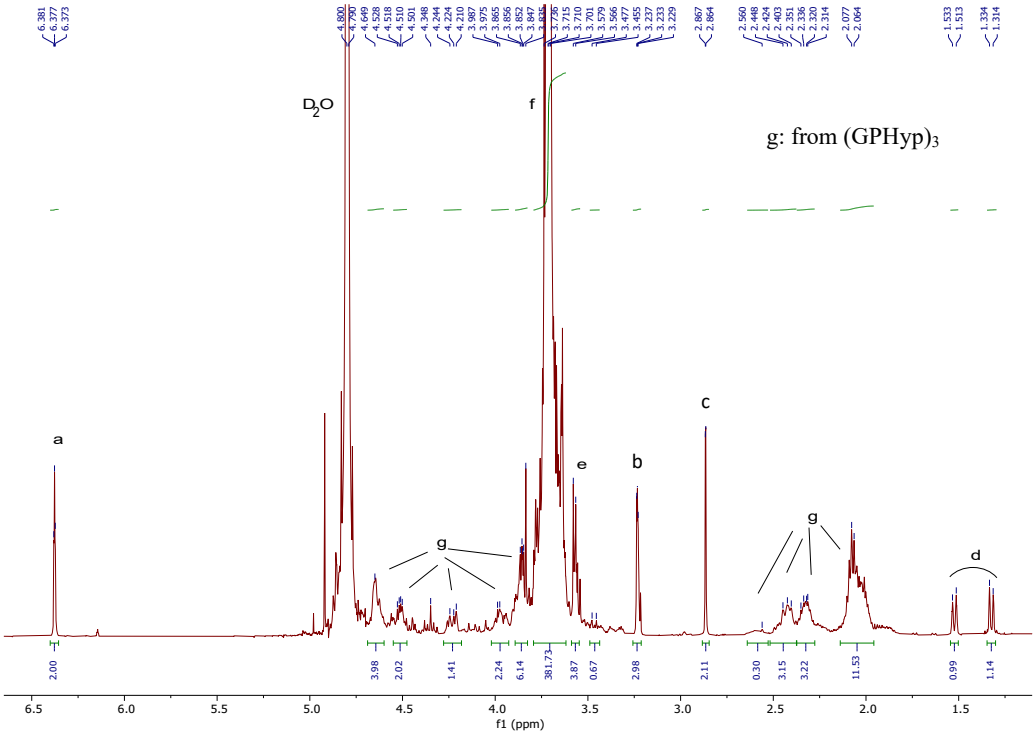
$(GPHyp)_3$

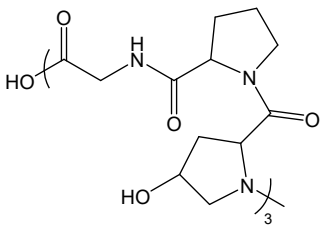


PEG (1,000)

Id

(^{1}H NMR, D_2O, 25°C, 500MHz)





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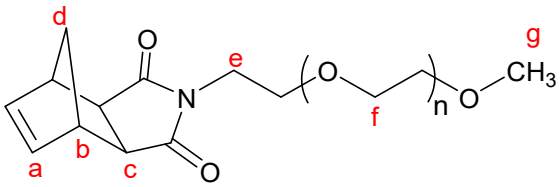

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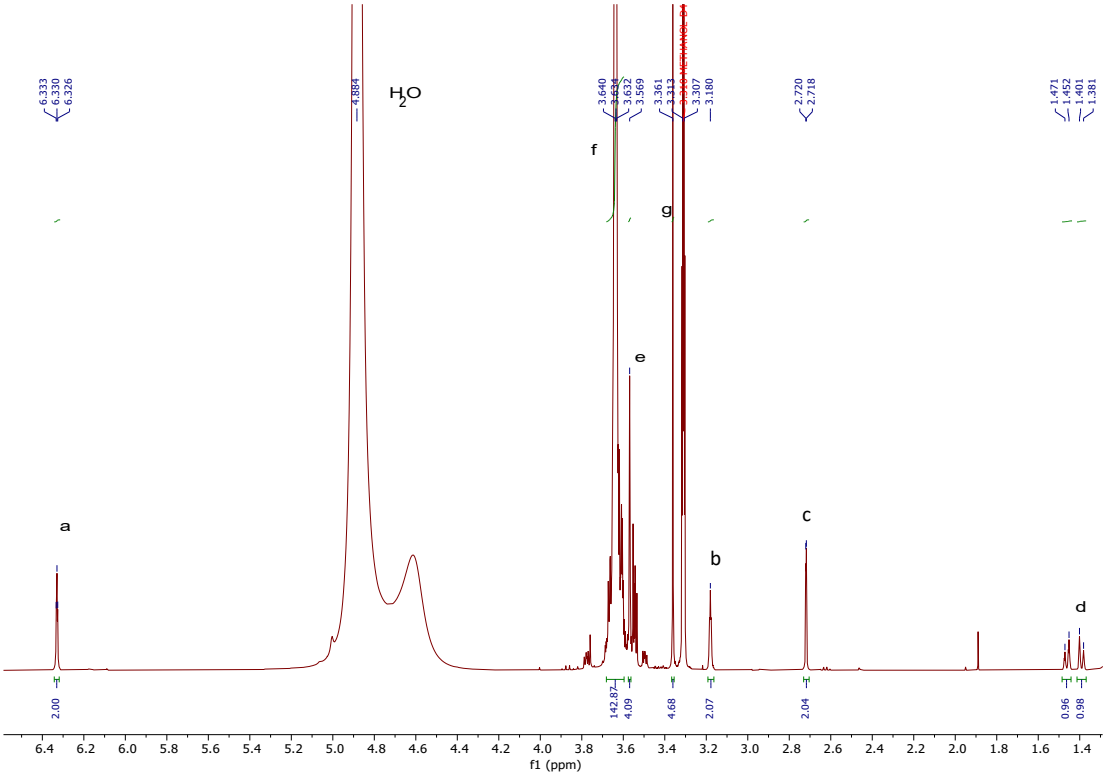
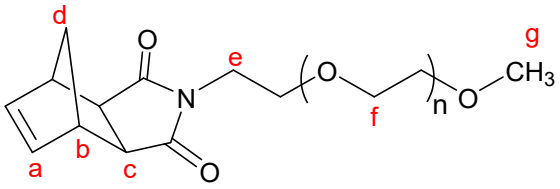
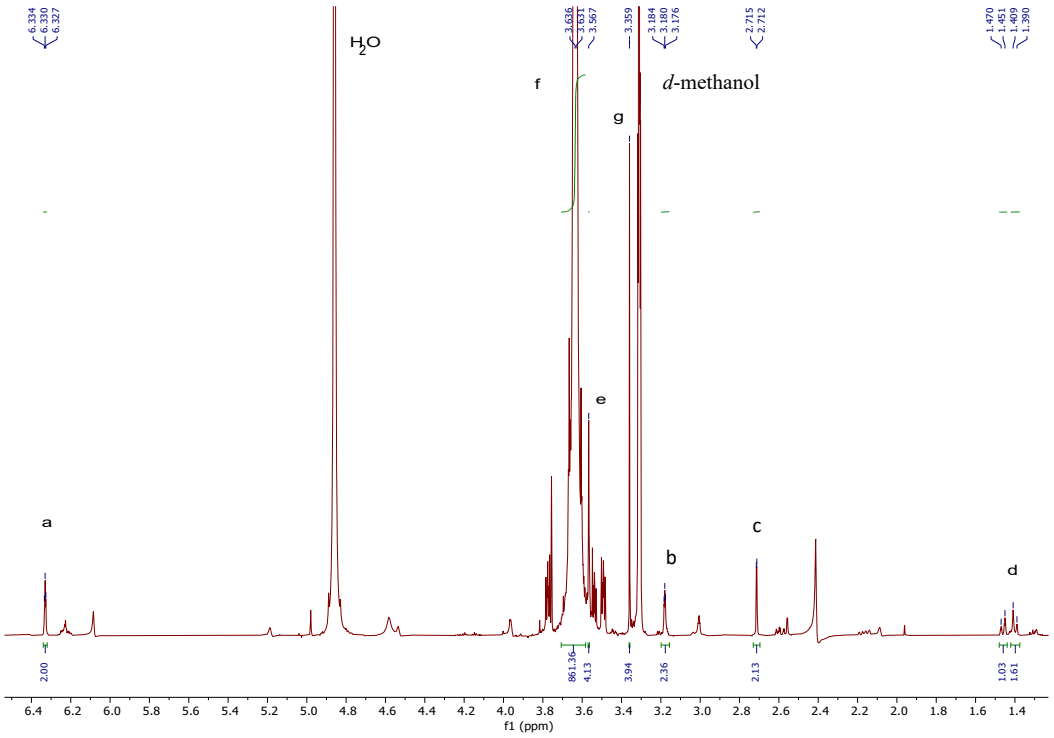
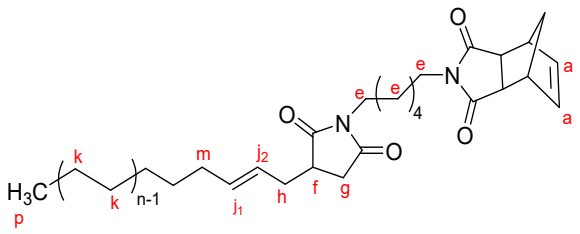




mPEG (5,000)

(^{1}H NMR, CD_3OD, 25°C, 500MHz)



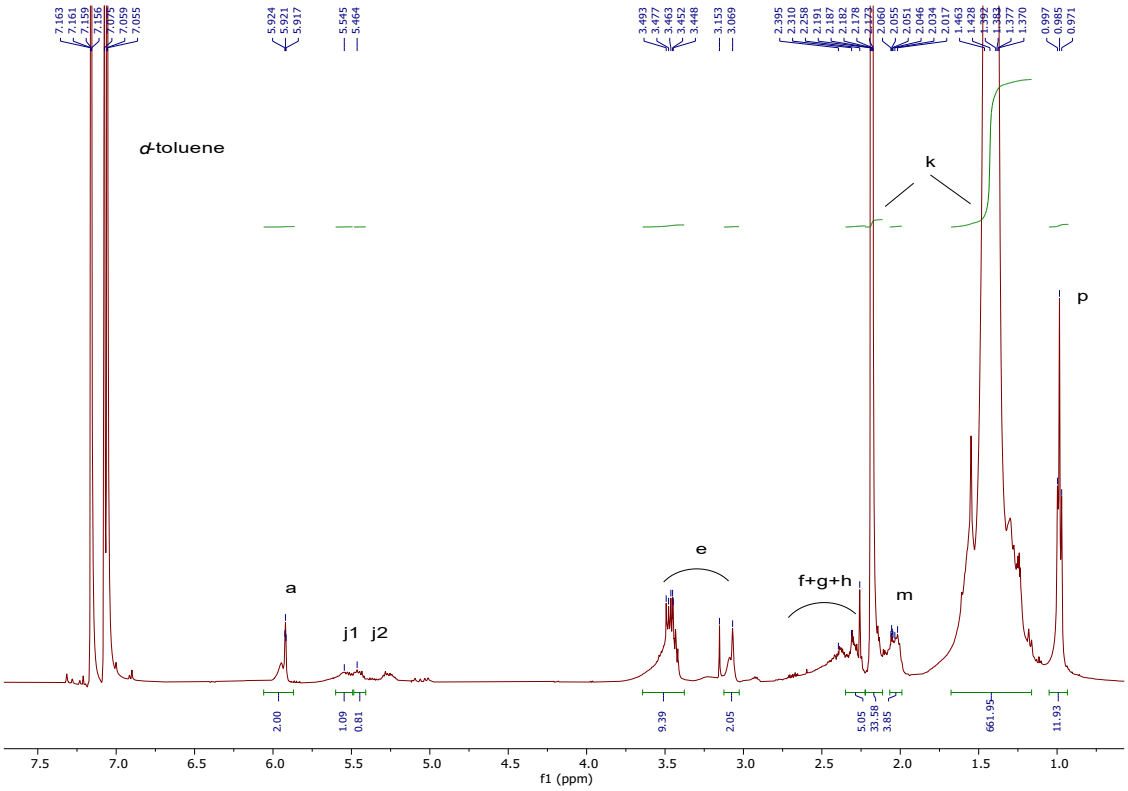


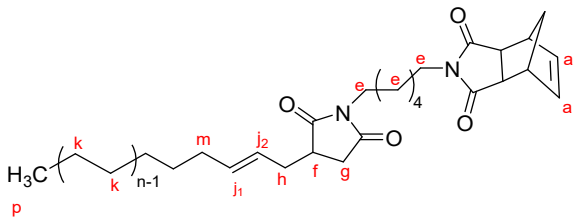
NB-SA-t $PE_{1,400}$

IIa

Conversion[5]=(a/2)/j_1=2.00/2/1.09=92%

(^{1}H NMR, *d*-toluene, 80^{o}C, 500MHz)



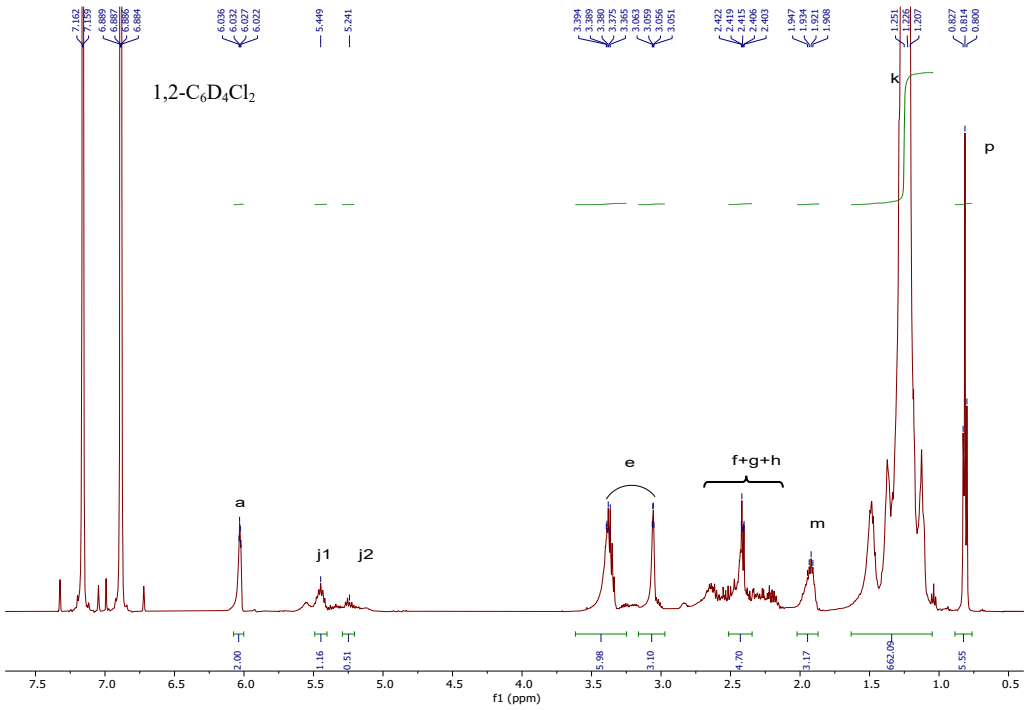


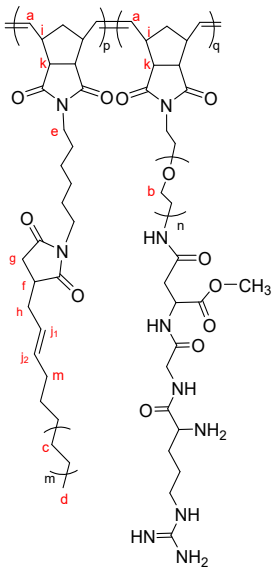
NB-SA-t $PE_{5,000}$

IIb

Conversion[5]=(a/2)/j_1=2.00/2/1.16=86%

(^{1}H NMR, 1,2-$C_6D_4Cl_2$, 120°C, 400MHz)



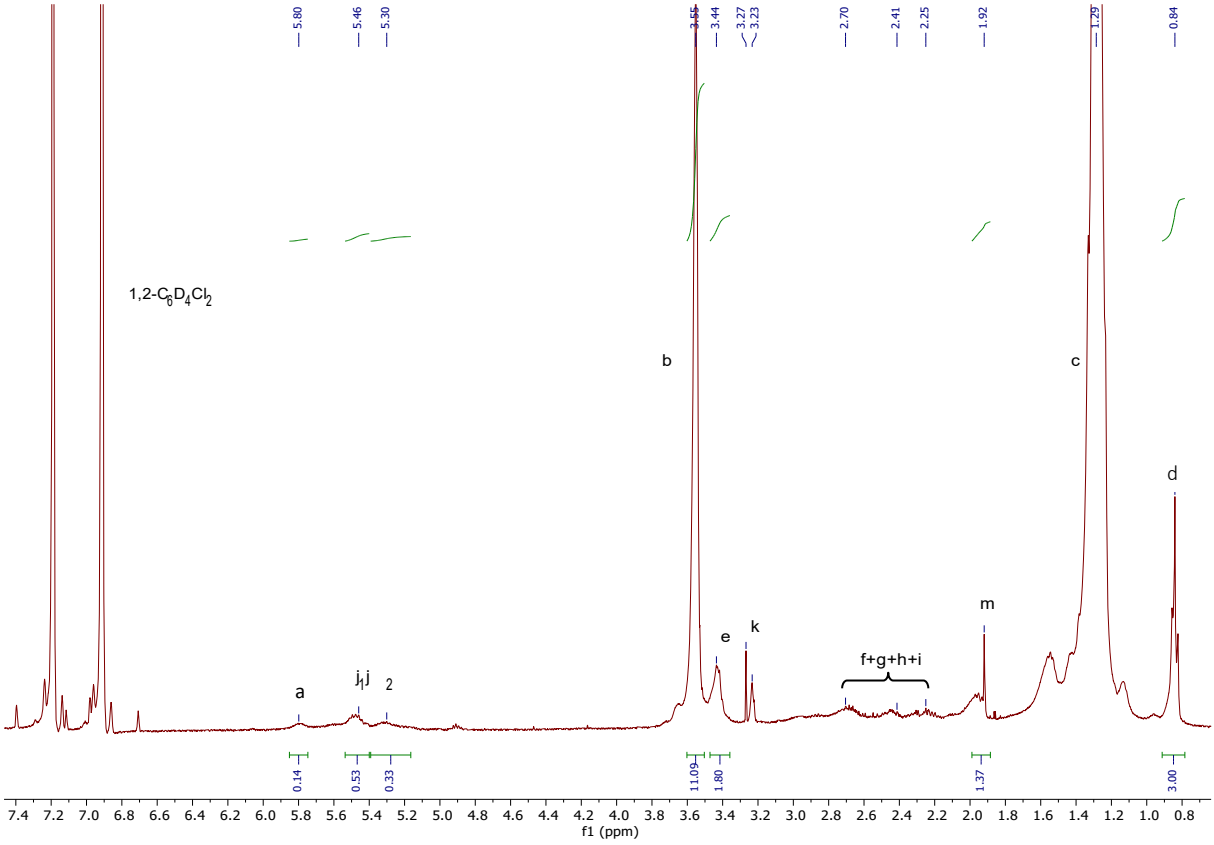


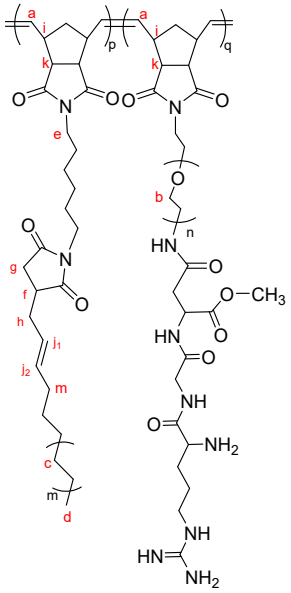
PE$_{1,400}$-RGD **IIIa**

NB-SA-t PE conversion=(1-unreacted NB-PE$_{1,400}$/2)%>99%

NB-PEG-peptide incorporation=(PEG protons)/(total PEG$_{1,000}$ protons per monomer)=b/21x4=11.09/84=13%

(^{1}H NMR, 1,2-$C_6D_4Cl_2$, 120^{o}C, 400MHz)





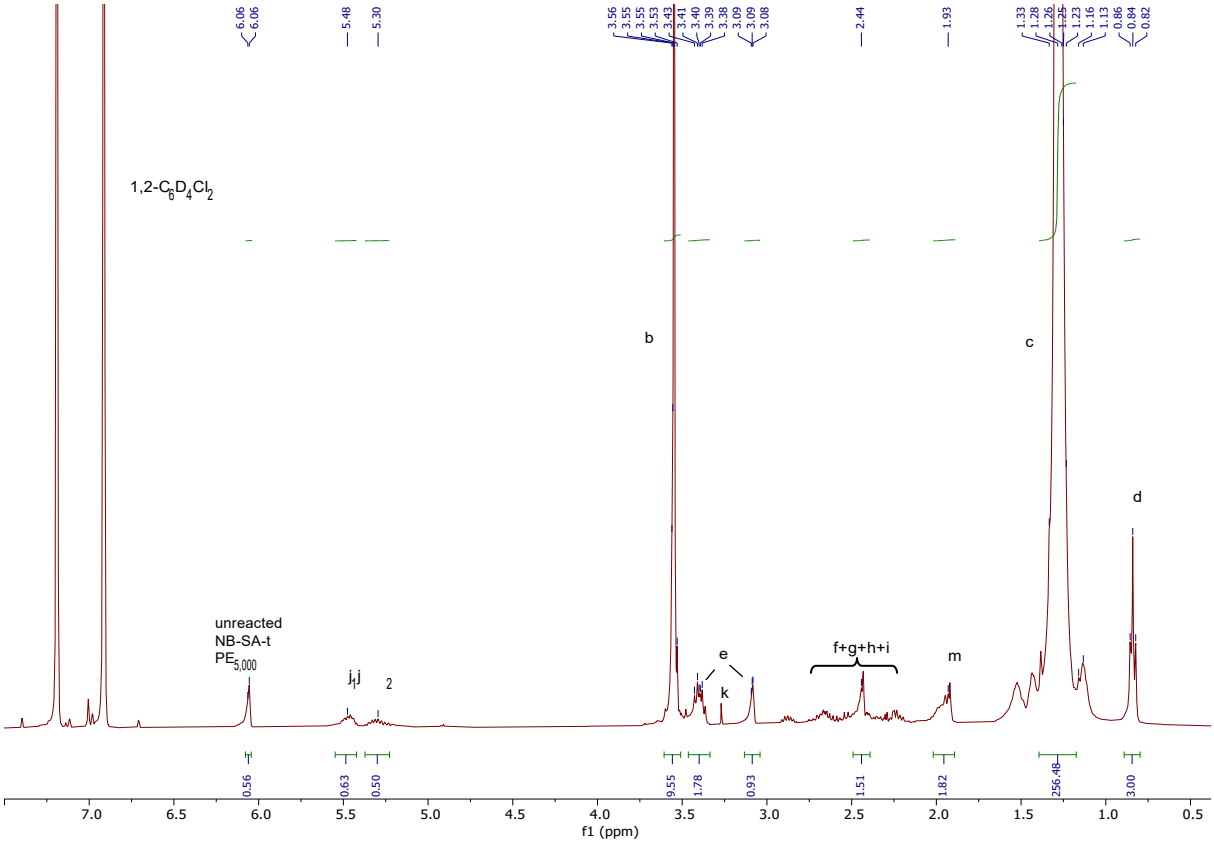
PE$_{5,000}$-RGD **IIIb**

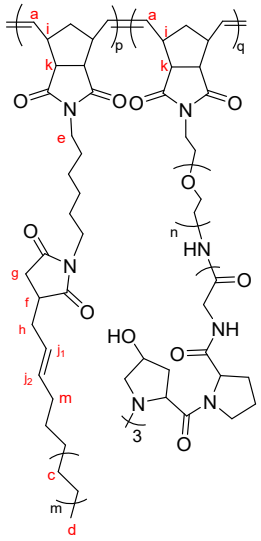
NB-SA-t PE conversion=(1-unreacted NB-PE$_{5,000}$/2)%=(1-0.56/2)=72%

NB-PEG-peptide incorporation=

(PEG protons)/(total PEG$_{3,400}$ protons per monomer)=b/(76x4)=9.55/304=3%

(^{1}H NMR, 1,2-$C_6D_4Cl_2$, 120^{o}C, 400MHz)



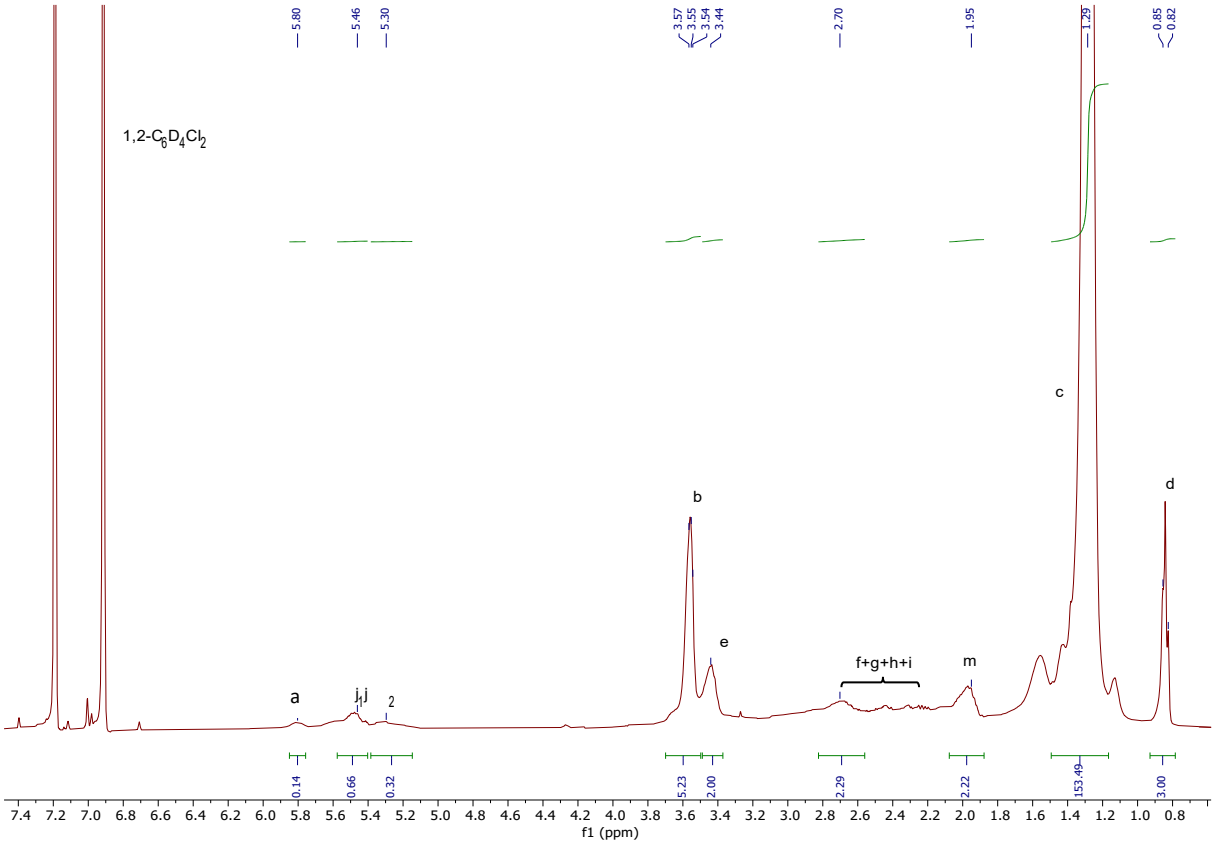


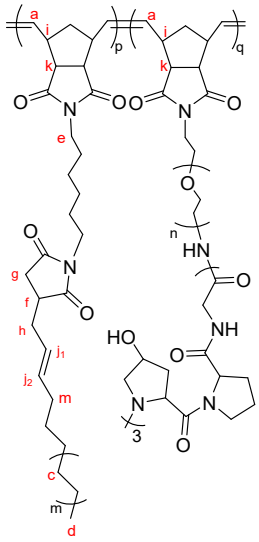
$PE_{1,400}$-$(GPHyp)_3$ **IIIc**

NB-SA-t PE conversion=(1-unreacted NB-$PE_{1,400}$/2)%>99%

NB-PEG-peptide incorporation=(PEG protons)/(total $PEG_{1,000}$ protons per monomer)=b/(21x4)=5.23/84=6%

(^{1}H NMR, 1,2-$C_6D_4Cl_2$, 120^{o}C, 400MHz)





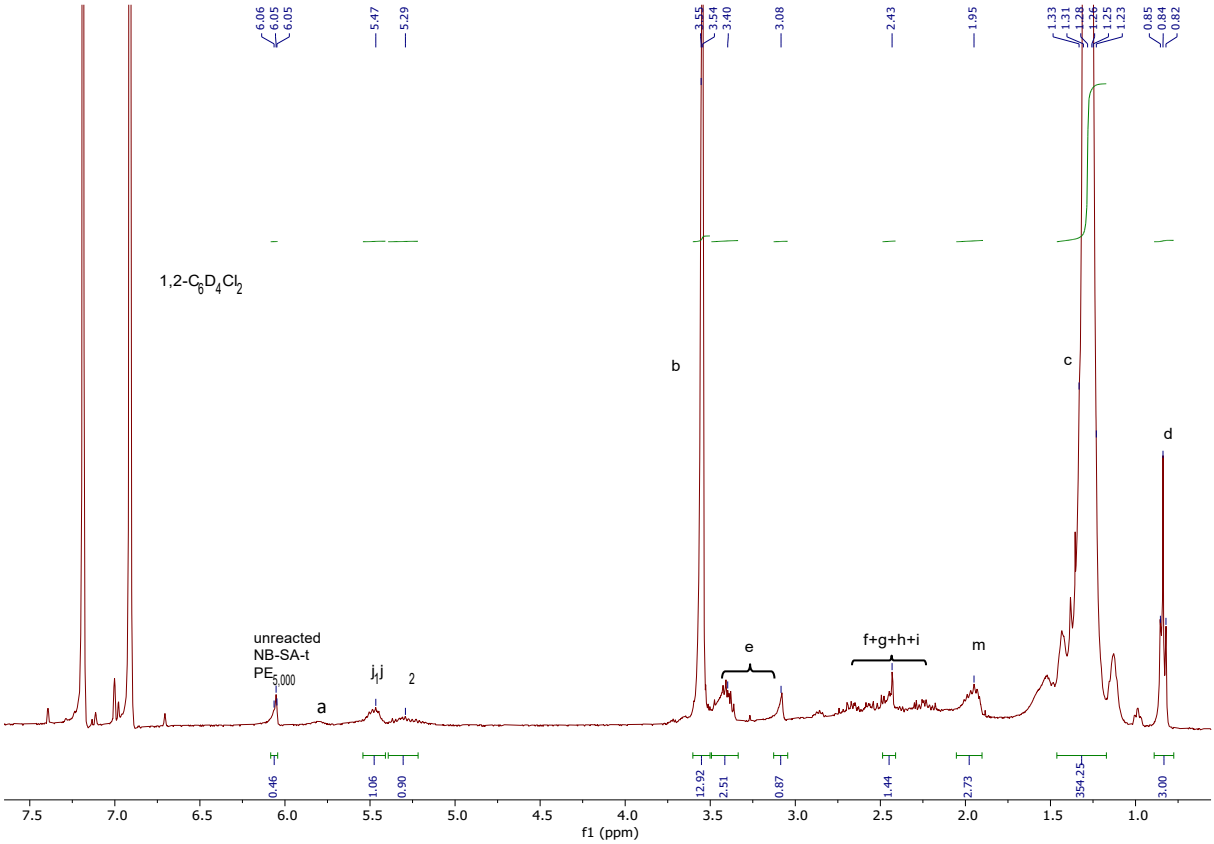
$PE_{5,000}$-$(GPHyp)_3$ **IIId**

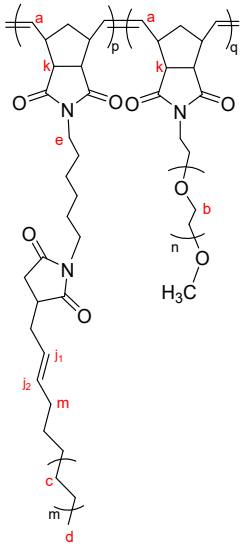
NB-SA-t PE Conversion=(1-unreacted NB-$PE_{5,000}$/2)%=(1-0.46/2)=77%

NB-PEG-peptide incorporation=

(PEG protons)/(total $PEG_{3,400}$ protons per monomer)=b/(76x4)=12.92/304=4%

(^{1}H NMR, 1,2-$C_6D_4Cl_2$, 120°C, 400MHz)



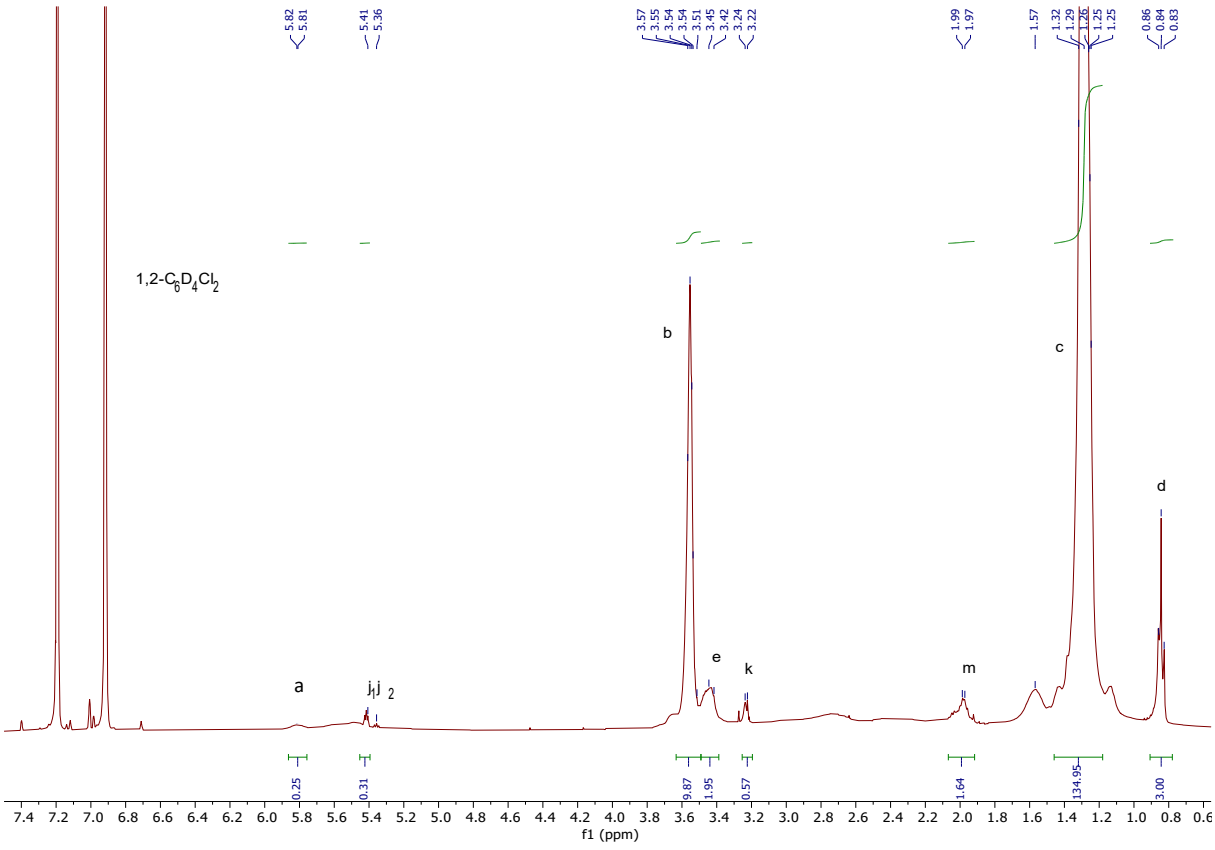


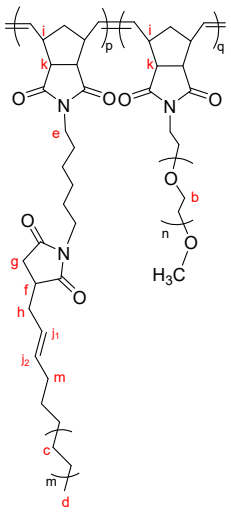
$PE_{1,400}$-$mPEG_{1,000}$ **IVa**

NB-SA-t PE conversion=(1-unreacted NB-$PE_{1,400}$/2)%>99%

NB-mPEG incorporation=(PEG protons)/(total $PEG_{1,000}$ protons per monomer)=b/(21x4)=9.87/84=12%

(^{1}H NMR, 1,2-$C_6D_4Cl_2$, 120°C, 400MHz)



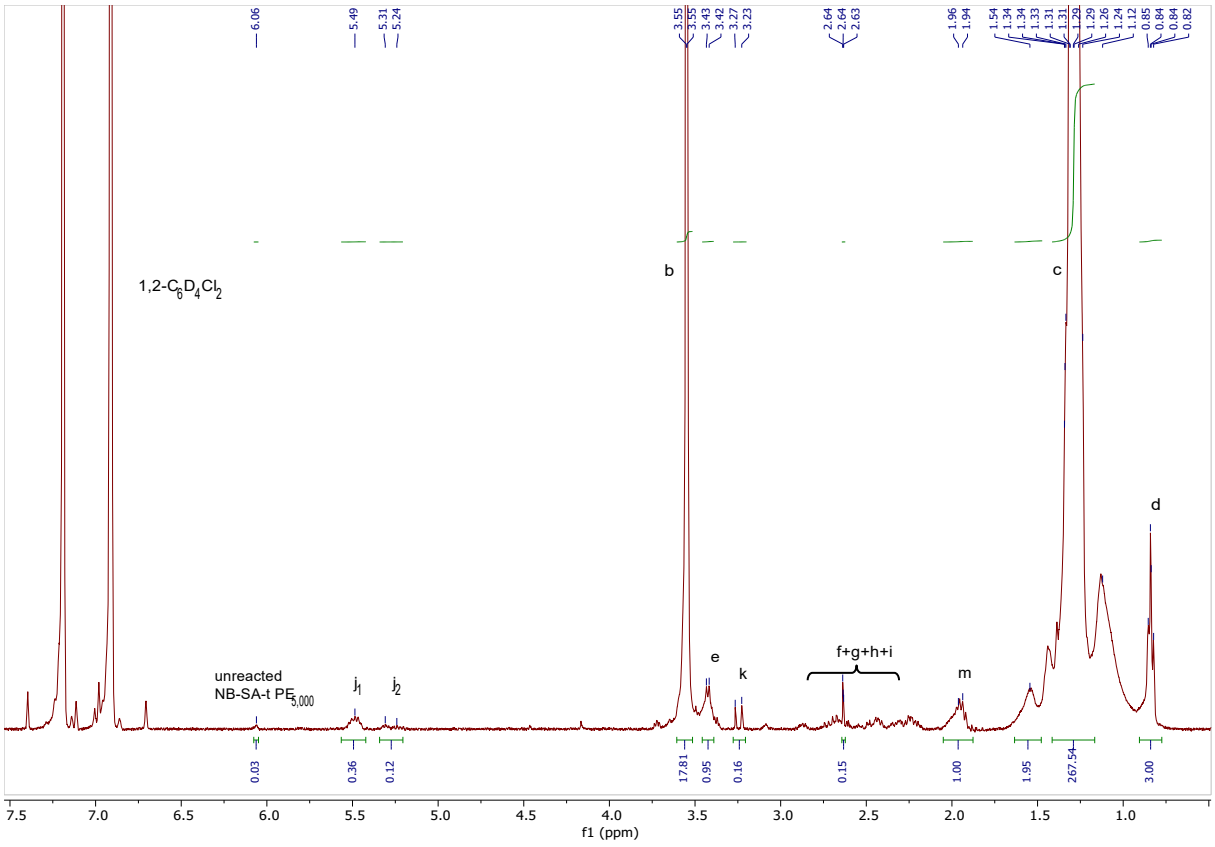


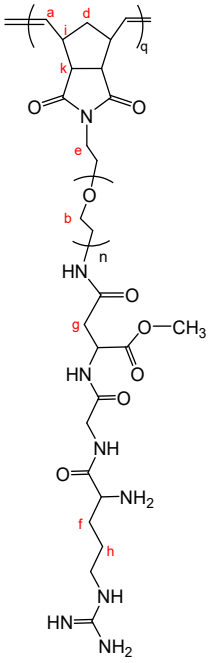
$PE_{5,000}$-$mPEG_{3,400}$ **IVb**

NB-SA-t PE conversion=(1-unreacted NB-$PE_{5,000}$/2)%=(1-0.03/2)=99%

NB-mPEG incorporation=(PEG protons)/(total $PEG_{5,000}$ protons per monomer)=b/(102x4)=17.81/408=4%

(^{1}H NMR, 1,2-$C_6D_4Cl_2$, 120^{o}C, 400MHz)

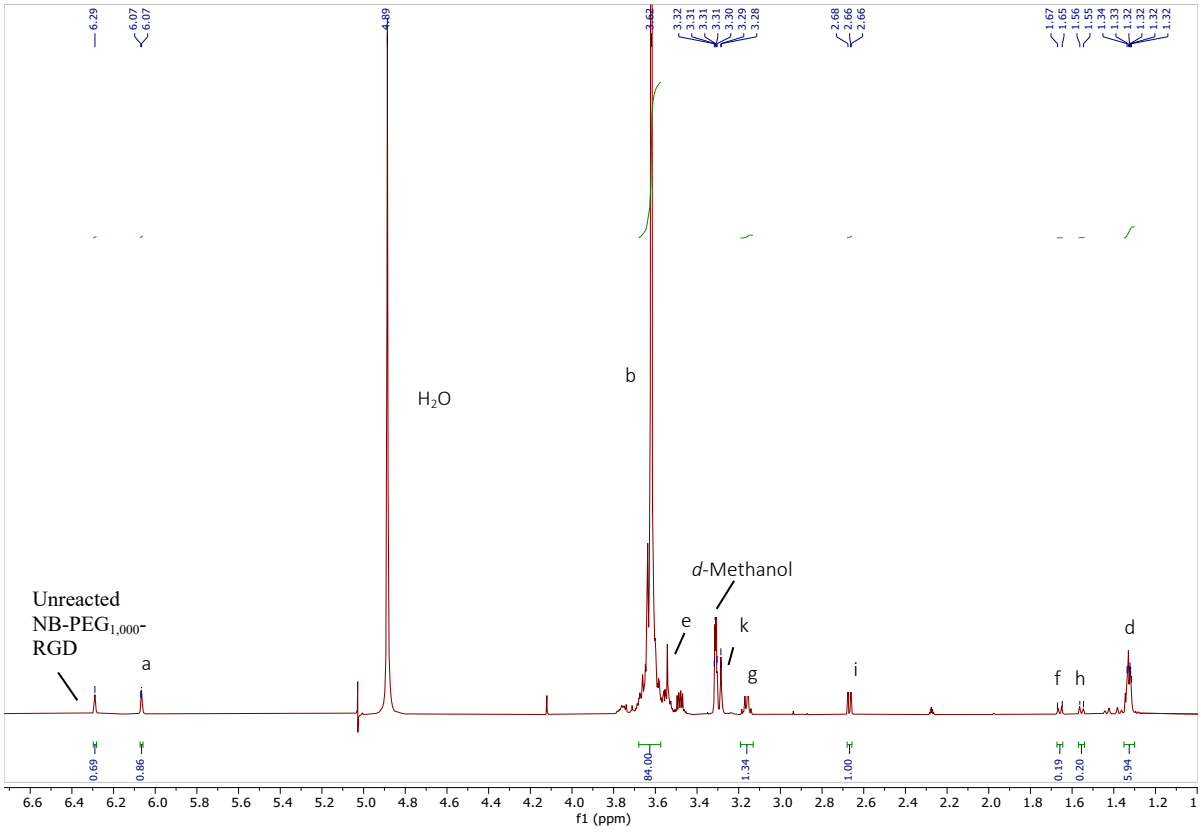


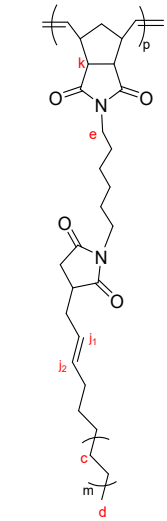


NB-PEG$_{1,400}$-RGD Homopolymer

NB-PEG$_{1,000}$-RGD conversion=(1-unreacted NB-PEG$_{1,000}$-RGD/2)%=(1-0.69/2)=66%

(^{1}H NMR, CD_3OD, 25°C, 500MHz)





NB-SA-t $PE_{1,400}$ Homopolymer

NB-SA-t $PE_{1,400}$ conversion=(1-unreacted NB-$PE_{1,400}$/2)%=(1-0.16/2)=92%

(^{1}H NMR, *d*-Toluene, 80^{o}C, 500MHz)