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Section A. General Considerations

Unless otherwise noted, all reagents were purchased from Sigma Aldrich, Alfa Aesar, or TCI and used as supplied. Degassed, anhydrous tetrahydrofuran and dichloromethane were obtained by filtration through alumina according to the methods described by Grubbs (JC Meyer).[1] Anhydrous 1,4-dioxane was purchased from Sigma Aldrich and was used as received. All moisture sensitive reactions were carried out under an inert atmosphere of nitrogen using standard syringe/septa techniques. Ring opening metathesis polymerization (ROMP) reactions were initiated in a Vacuum Atmospheres glovebox filled with nitrogen. Grubbs 2nd generation catalyst was obtained from Materia, and was converted to Grubbs 3rd generation catalyst (**G3**) following literature protocols.[2] Cyanine7.5-azide was purchased from Lumiprobe. Telmisartan was purchased from AvaChem Scientific. **AXL** was prepared as previously described.[3] Column chromatography was carried out using ZEOprep 60 HYD silica gel (40-63 μm). Automated flash chromatography was conducted using a Biotage Isolera One. Semi-batch processes were conducted using a KD Scientific Legato 100 syringe pump.

GPC-MALLS characterization was performed on an Agilent 1260 LC system equipped with a Wyatt T-rEX refractive index (dRI) detector and Wyatt DAWN HELEOS 18 angle light scattering detector. Samples were run on two Shodex KD-806M (Shodex) or two Agilent PLgel 5 μm MIXED-C (Agilent) GPC columns in series at a temperature of 60 ^{o}C and flow rate of 1 mL/min with dimethyl formamide (DMF) containing 0.025 M LiBr as the eluent.

^{1}H nuclear magnetic resonance (^{1}H-NMR) and ^{13}C nuclear magnetic resonance (^{13}C-NMR) spectra were recorded on a Bruker AVANCE-400 MHz NMR spectrometer, VARIAN Inova-500 MHz NMR spectrometer, or a JEOL 500MHz NMR spectrometer. Spectra were analyzed on MestReNova NMR software. Chemical shifts are expressed in parts per million (ppm); splitting patterns are designated as s (singlet), d (doublet), t (triplet), m (multiplet), and br (broad); and coupling constants, *J*, are reported in hertz (Hz).

High-resolution mass spectra (HRMS) were obtained on a Bruker Daltonics APEXIV 4.7 Tesla Fourier Transform Ion Cyclotron Resonance Mass Spectrometer (FT-ICR-MS) using an electrospray ionization source (ESI) at the MIT Department of Chemistry Instrumentation Facility (DCIF).

Matrix-assisted laser desorption ionization time-of-flight (MALDI-ToF) mass spectra were obtained at the MIT Koch Institute Biopolymers and Proteomics Core Facility using α-cyano-4-hydroxycinnamic acid (CHCA) as the matrix.

Liquid chromatography-mass spectrometry (LC-MS) was performed on an Agilent 1260 LC system equipped with an Agilent 6130 single quadrupole mass spectrometer. Samples were run on an Advanced Materials Technology Halo C18 (HALO) or an Agilent ZORBAX 300SB-C18 (PES) analytical column using a gradient eluent containing 0.1% acetic acid in nanopure water (MilliQ Biocel A10 water purification system) and acetonitrile. Details about specific LC methods are described below as needed.

Dynamic light scattering (DLS) measurements were performed on a Wyatt Technologies Möbiuζ instrument equipped with a 660 nm laser. Samples were prepared by dissolving a BASP sample (pipette

tip of the crude reaction mixture, 0.05M – 0.1M in 1,4-dioxane, or solid lyophilized material) in nanopure water (ca. 0.05 mg/mL) and filtering through a 0.45 μm Nylon syringe filter into a clean disposable polystyrene cuvette. Measurements were made in sets of 20 acquisitions, and the average hydrodynamic diameters (D_H) were calculated using the DLS correlation function via a regularization fitting method (Dynamics 7.3.1.15, Wyatt Technology).

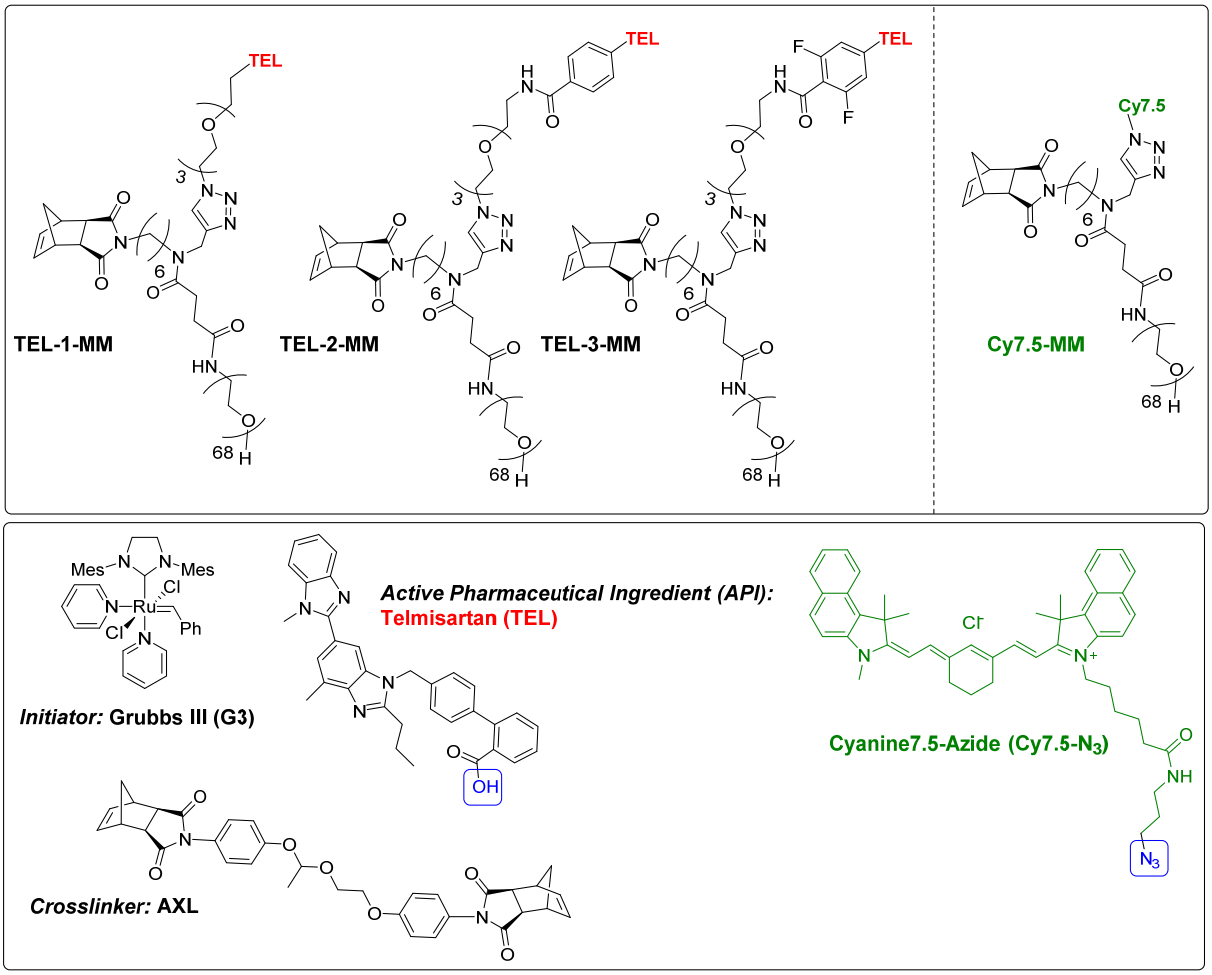
TEM images were acquired using a FEI Tecnai Multipurpose TEM (G2 Spirit TWIN, 120 kV) at the MIT Center for Materials Science and Engineering. A solution (5 μL) of BASP nanoparticle (ca. 1 mg/mL in nanopure H_2O) was dropped onto a carbon film-coated 200-mesh copper grid (Electronic Microscopy Sciences) placed on a piece of parafilm. Excess aqueous solution was carefully wicked away from the TEM grid using the edge of a Kimwipe. The samples were negatively stained by dropping 5 μL of 2 wt% uranyl acetate (Electronic Microscopy Sciences) onto the grid. After 5 minutes, the residual uranyl acetate solution was carefully wicked away using the edge of a Kimwipe. The samples were allowed to fully dry before analysis.

Preparative high-pressure liquid chromatography (prep-HPLC) was performed on an Agilent 1260 LC system equipped with an Agilent ZORBAX 300SB-C18 PrepHT (21.2 x 150 mm) preparative column and using a gradient eluent using a gradient eluent containing 0.1% acetic acid in nanopure water (MilliQ Biocel A10 water purification system) and acetonitrile.

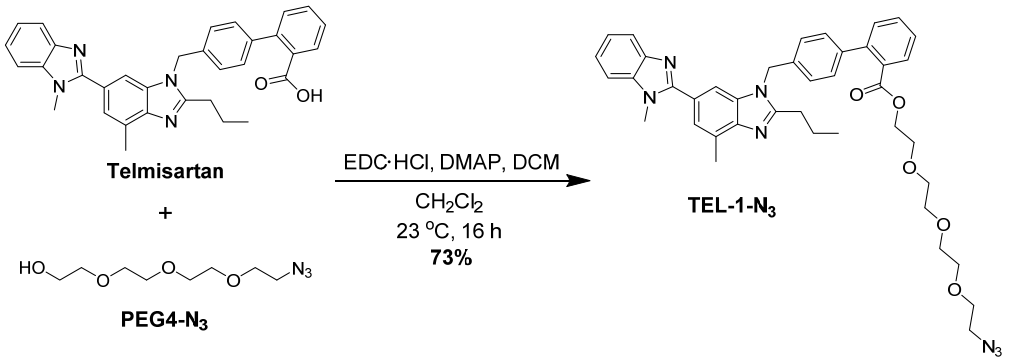
Preparative gel-permeation chromatography (prep-GPC) was performed on a JAI Preparative Recycling HPLC (LaboACE-LC-5060) system equipped with either 2.5HR and 2HR columns in series (20 mm ID x 600 mm length) or 2.5H and 2H columns in series (40 mm ID x 600 mm length) using $CHCl_3$ as the eluent.

Section B. Synthetic Procedures

Overview of chemical structures used in our studies:



1. *Preparation of Telmisartan Analogues:*

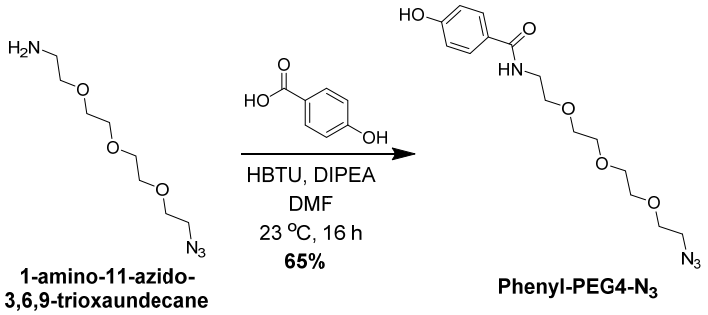


TEL-1-N_3: To a 20-mL scintillation vial charged with a stir bar, **TEL** (180 mg, 0.35 mmol, 1.0 equiv), EDC·HCl (74 mg, 0.39 mmol, 1.1 equiv), and DMAP (8.6 mg, 0.070 mmol, 0.20 equiv) were dissolved in anhydrous CH_2Cl_2 (ca. 0.1 M) under N_2, and stirred until solids were fully dissolved in solution. **PEG4-N_3**[4] (100 mg, 0.46 mmol, 1.3 equiv) was added, and the reaction was stirred at room temperature overnight. The solution was loaded onto a silica cartridge (50 g) and purified by automated flash chromatography (0% MeOH/DCM to 5% MeOH/DCM). The column was repeated again to fully remove **PEG4-N_3** and afford **TEL-1-N_3** (182 mg, 73%) as a viscous oil.

^{1}H NMR (500 MHz, CD_2Cl_2) δ 7.81 (d, *J* = 7.2 Hz, 1H), 7.70 (d, *J* = 8.0 Hz, 1H), 7.54-7.24 (overlap, 10H), 7.13 (d, *J* = 8.0 Hz, 2H), 5.47 (s, 2H), 4.14 (t, *J* = 4.9 Hz, 2H), 3.79 (s, 3H), 3.59-3.40 (overlap, 12H), 3.31 (t, *J* = 4.9 Hz, 2H), 2.93 (t, *J* = 7.8 Hz, 2H), 2.72 (s, 3H), 1.95-1.87 (m, 2H), 1.07 (t, *J* = 7.4 Hz, 3H).

^{13}C-NMR (125 MHz, $CDCl_3$) δ 168.05, 156.58, 154.83, 143.31, 143.06, 142.05, 141.30, 136.82, 135.16, 134.86, 131.57, 130.90, 130.56, 130.19, 129.60, 129.27, 127.50, 126.01, 124.06, 124.00, 122.61, 122.43, 119.70, 109.66, 109.05, 70.77, 70.74, 70.69, 70.60, 70.12, 68.90, 64.12, 50.75, 47.23, 31.99, 30.02, 22.06, 17.05, 14.26.

HRMS calcd. for $C_{41}H_{45}N_7O_5$ $[M+H]^+$, 716.3555; found, 716.3545.

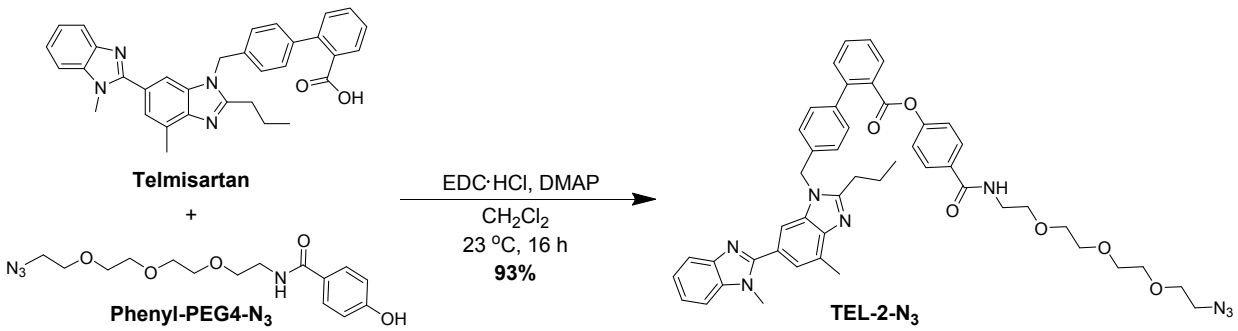


Phenyl-PEG4-N_3: To a 20-mL scintillation vial charged with a stir bar, 1-amino-11-azido-3,6,9-trioxaundecane (400 mg, 1.8 mmol, 2.0 equiv), 4-hydroxybenzoic acid (130 mg, 0.92 mmol, 1.0 equiv), and HBTU (350 mg, 0.92 mmol, 1.0 equiv) were dissolved in anhydrous DMF (10 mL) under N_2, followed by addition of DIPEA (0.080 mL, 0.46 mmol, 0.50 equiv). The reaction was stirred at room temperature overnight; additional HBTU and DIPEA were added if not complete by TLC and the reaction was left stirring for several additional hours. Once the reaction had proceeded to completion, it was diluted with CH_2Cl_2 (100 mL) and was washed with 100 mL of 0.1 M HCl, water, and brine. The organic layer was dried over magnesium sulfate and concentrated by rotary evaporation. The crude material was loaded onto a silica cartridge (50 g) and purified by automated flash chromatography (0% MeOH/DCM to 5% MeOH/DCM) to afford **Phenyl-PEG4-N_3** (203 mg, 65%) as a viscous oil.

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.70 (overlap, 2H), 6.85 (d, *J* = 8.4 Hz, 2H), 6.68 (s, 1 H), 3.69-3.61 (overlap, 14H), 3.34 (t, *J* = 4.7 Hz, 2H).

^{13}C-NMR (100 MHz, $CDCl_3$) δ 168.11, 160.15, 129.14, 125.63, 115.59, 70.68, 70.67, 70.62, 70.25, 70.07, 69.95, 50.72, 39.96.

HRMS calcd. for $C_{15}H_{22}N_4O_5$ $[M+NH_4]^+$, 339.1663; found, 339.1655.

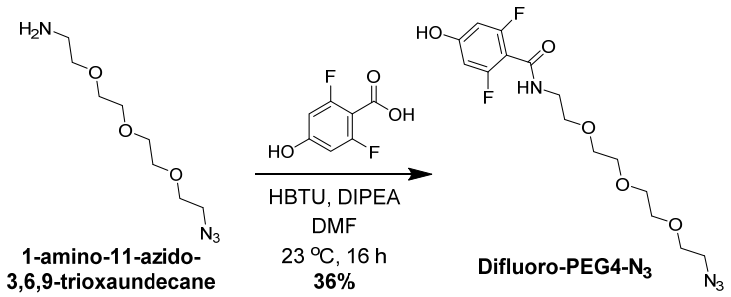


TEL-2-N_3: To a 20-mL scintillation vial charged with a stir bar, **TEL** (300 mg, 0.59 mmol, 1.0 equiv), EDC·HCl (110 mg, 0.59 mmol, 1.0 equiv), and DMAP (7.2 mg, 0.059 mmol, 0.10 equiv) were dissolved in anhydrous CH_2Cl_2 (10 mL) under N_2, and stirred until solids were fully dissolved in solution. **Phenyl-PEG4-N_3** (100 mg, 0.296 mmol, 1 equiv) was added, and the reaction was stirred at room temperature overnight. The reaction mixture was directly loaded onto a silica cartridge (25 g) and purified by automated flash chromatography (0% MeOH/DCM to 5% MeOH/DCM) to afford **TEL-2-N_3** (230 mg, 93%) as a white solid.

^{1}H NMR (500 MHz, CD_2Cl_2) δ 7.95 (d, *J* = 7.6 Hz, 1H), 7.90 (br, 1H), 7.67 (d, *J* = 7.6 Hz, 1H), 7.63-7.21 (overlap, 14H), 6.70 (d, *J* = 8.6 Hz, 2H), 5.45 (s, 2H), 3.77 (s, 3H), 3.70-3.59 (overlap, 14H), 3.32 (t, *J* = 4.9 Hz, 2H), 2.89 (t, *J* = 7.7 Hz, 2H), 2.73 (s, 3H), 1.94-1.87 (m, 2H), 1.06 (t, *J* = 7.5 Hz, 3H).

^{13}C-NMR (125 MHz, $CDCl_3$) δ 166.82, 166.26, 156.54, 154.55, 152.79, 143.23, 142.53, 142.29, 141.09, 136.54, 135.12, 134.91, 132.26, 132.23, 130.98, 130.60, 129.70, 129.60, 129.25, 128.55, 127.71, 126.26, 124.09, 123.75, 122.69, 122.48, 121.12, 119.32, 109.71, 108.70, 70.73, 70.62, 70.55, 70.24, 70.07, 69.99, 69.91, 50.65, 47.08, 39.83, 31.84, 29.81, 21.86, 16.96, 14.13.

HRMS calcd. for $C_{48}H_{50}N_8O_6$ $[M+H]^+$, 835.3926; found, 835.3927.

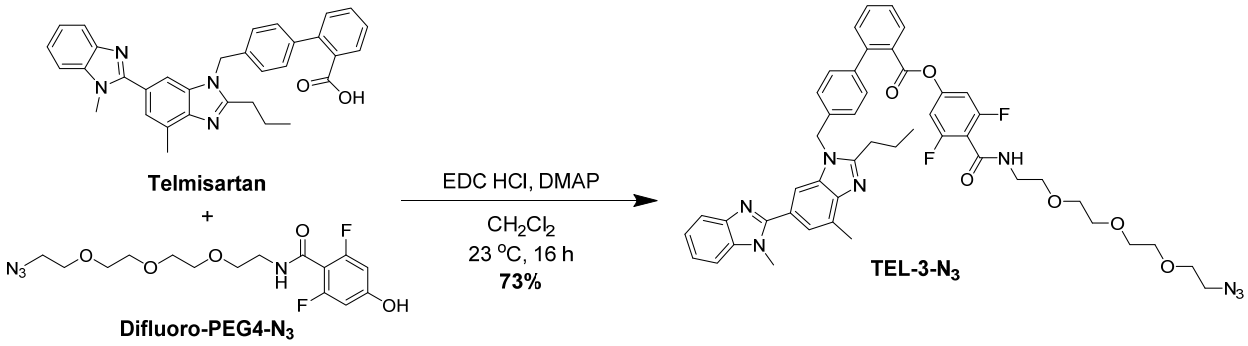


Difluoro-PEG4-N_3: To a 40-mL scintillation vial charged with a stir bar, 1-amino-11-azido-3,6,9-trioxaundecane (2.5 g, 11 mmol, 2.0 equiv), 2,6-Difluoro-4-hydroxybenzoic acid (980 mg, 5.6 mmol, 1.0 equiv), and HBTU (2.1 g, 5.6 mmol, 1.0 equiv) were dissolved in anhydrous DMF (50 mL) under N_2, followed by addition of DIPEA (0.98 mL, 5.6 mmol, 1.0 equiv). The reaction was stirred at room temperature overnight; additional HBTU and DIPEA were added if not complete by TLC and the reaction was left stirring for several additional hours. Once the reaction had proceeded to completion, it was diluted with CH_2Cl_2 (100 mL) and was washed with 100 mL of 0.1 M HCl, water, and brine. The organic layer was dried over magnesium sulfate and concentrated by rotary evaporation. After concentration by rotary evaporation, the crude material was loaded onto a silica cartridge (50 g) and purified by automated flash chromatography (0% MeOH/DCM to 5% MeOH/DCM) to afford **Difluoro-PEG4-N_3** (750 mg, 36% yield) as a viscous oil.

^{1}H NMR (500 MHz, $CDCl_3$) δ 6.87 (s, 1H), 6.34 (d, *J* = 9.8 Hz, 2H), 3.71-3.59 (overlap, 14H), 3.34 (t, *J* = 4.8 Hz, 2H).

^{13}C-NMR (125 MHz, $CDCl_3$) δ 162.05, 161.00 (dd, *J* = 250, 9.9 Hz), 160.60 (t, *J* = 15.1 Hz), 105.11 (t, *J* = 18.7 Hz), 100.14 (d, *J* = 27 Hz), 70.76, 70.58, 70.26, 70.10, 69.68, 50.71, 40.05.

HRMS calcd. for $C_{15}H_{20}F_2N_4O_5$ $[M+H]^+$, 375.1475; found, 375.1468.



TEL-3-N_3: To a 20-mL scintillation vial charged with a stir bar, **TEL** (206 mg, 0.40 mmol, 1.5 equiv), EDC-HCl (76 mg, 0.40 mmol,1.5 equiv), and DMAP (6.5 mg, 0.053 mmol, 0.20 equiv) were dissolved in anhydrous CH_2Cl_2 (10 mL) under N_2, and stirred until solids were fully dissolved in solution. **Difluoro-PEG4-N_3** (100 mg, 0.27 mmol, 1.0 equiv) was added, and the reaction was stirred at room temperature overnight. The reaction mixture was directly loaded onto a silica cartridge (25 g) and purified by automated flash chromatography (0% MeOH/DCM to 5% MeOH/DCM) to afford **TEL-3-N_3** (170 mg, 73%) as a white solid.

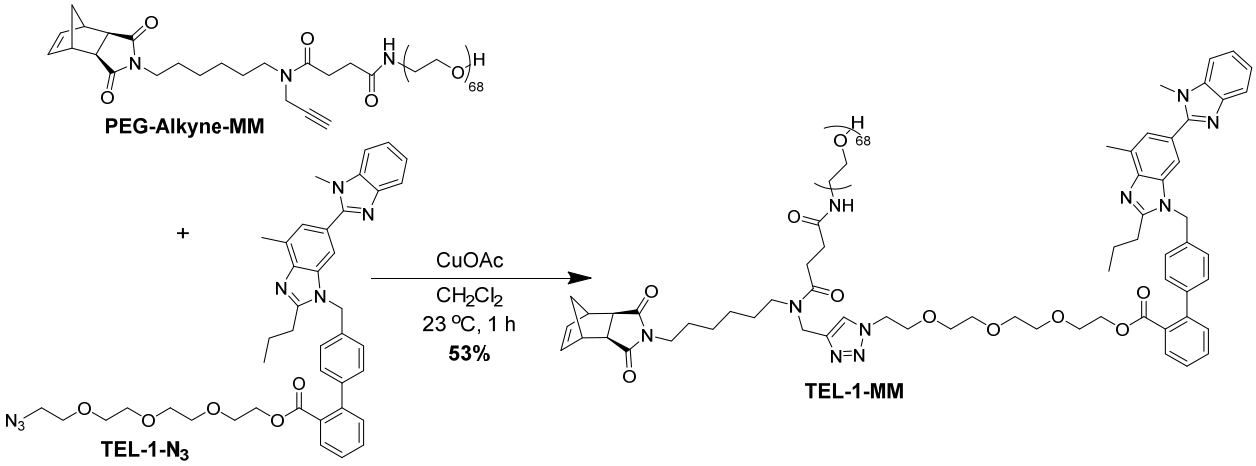
^{1}H NMR (500 MHz, CD_2Cl_2) δ 8.24 (br, 1H), 7.93 (d, *J* = 7.7 Hz, 1H), 7.69 (d, *J* = 7.9 Hz, 1H), 7.63 (t, *J* = 7.5 Hz, 1H), 7.51-7.19 (overlap, 11H), 6.32 (d, *J* = 8.2 Hz, 2H), 5.43 (s, 2H), 3.77 (s, 3H), 3.71-3.54 (overlap, 14H), 3.32 (t, *J* = 5.1 Hz, 2H), 2.81 (t, *J* = 7.7 Hz, 2H), 2.71 (s, 3H), 1.94-1.86 (m, 2H), 1.06 (t, *J* = 7.3 Hz, 3H).

^{13}C-NMR (125 MHz, $CDCl_3$) δ 165.53, 159.98, 159.88 (dd, *J* = 252, 9 Hz), 156.48, 154.66, 152.10 (t, J=13 Hz), 143.20, 142.86, 142.61, 141.00, 136.68, 135.41, 134.98, 132.72, 131.09, 130.87, 129.72, 129.20, 128.84, 127.86, 126.38, 124.15, 123.96, 122.64, 122.46, 119.56, 112.53 (t, *J* = 22.7 Hz), 109.63, 108.68, 105.79 (d, *J* = 28.9 Hz), 70.71, 70.68, 70.63, 70.46, 70.07, 69.99, 50.71, 47.14, 39.91, 31.94, 29.81, 21.92, 17.03, 14.14.

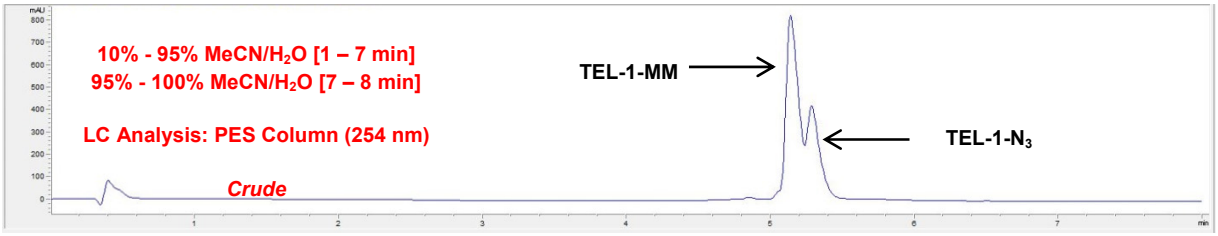
HRMS calcd. for $C_{48}H_{48}F_2N_8O_6$ $[M+H]^+$, 871.3738; found, 871.3740.

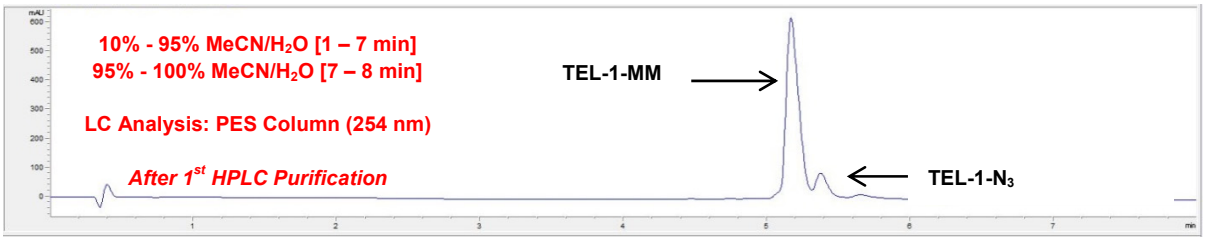
2. *Preparation of Conjugated Macromonomers*

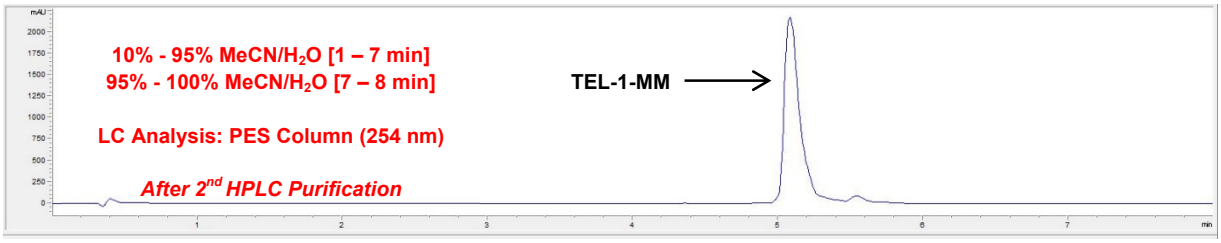
General Procedure: A 20-mL scintillation vial was charged with one of three **TEL-N_3** compounds (**TEL-1-N_3**, **TEL-2-N_3**, or **TEL-3-N_3**) or **Cy7.5-N_3**, **PEG-Alkyne-MM**,[5] and a stir bar. In a nitrogen filled glovebox, the reagents were dissolved in CH_2Cl_2 (1 mL CH_2Cl_2 per 100 mg PEG-alkyne-MM) to which was added approximately 3 equiv of copper (I) acetate. The reaction was stirred until consumption of PEG-alkyne-MM (ca. 1 h) was observed by LC-MS analysis (for a representative example, see **TEL-1-MM** below). As the relative extinction coefficients of the **TEL-N_3** and **PEG-alkyne-MM** are vastly different (the former being much larger), it is important that a small amount of **TEL-N_3** is visible by LC-MS. It is difficult to remove unreacted **PEG-Alkyne-MM** and its presence is detrimental to the subsequent ROMP processes. The crude product was purified by prep-HPLC or prep-GPC. For material purified by prep-HPLC, fractions containing product were concentrated by rotary evaporation and the resulting residue was redissolved in CH_2Cl_2 and dried over sodium sulfate. After concentration by rotary evaporation, the solid polymer was washed with cold diethyl ether by centrifugation of solids and decantation of ether. This wash step was repeated three times to afford the **TEL-MM** or **Cy7.5-MM** as white and green solids, respectively. For material purified by prep-GPC, the crude reaction mixture was first concentrated and taken up in minimal CH_2Cl_2. The solution was passed thru a short column of neutral alumina (~10% MeOH/CH_2Cl_2) and the eluent was concentrated by rotary evaporation. Then after purification by prep-GPC, fractions containing product were concentrated by rotary evaporation. The resulting solid was washed with cold diethyl ether three times (as above) to afford **TEL-MM** as a white powder.

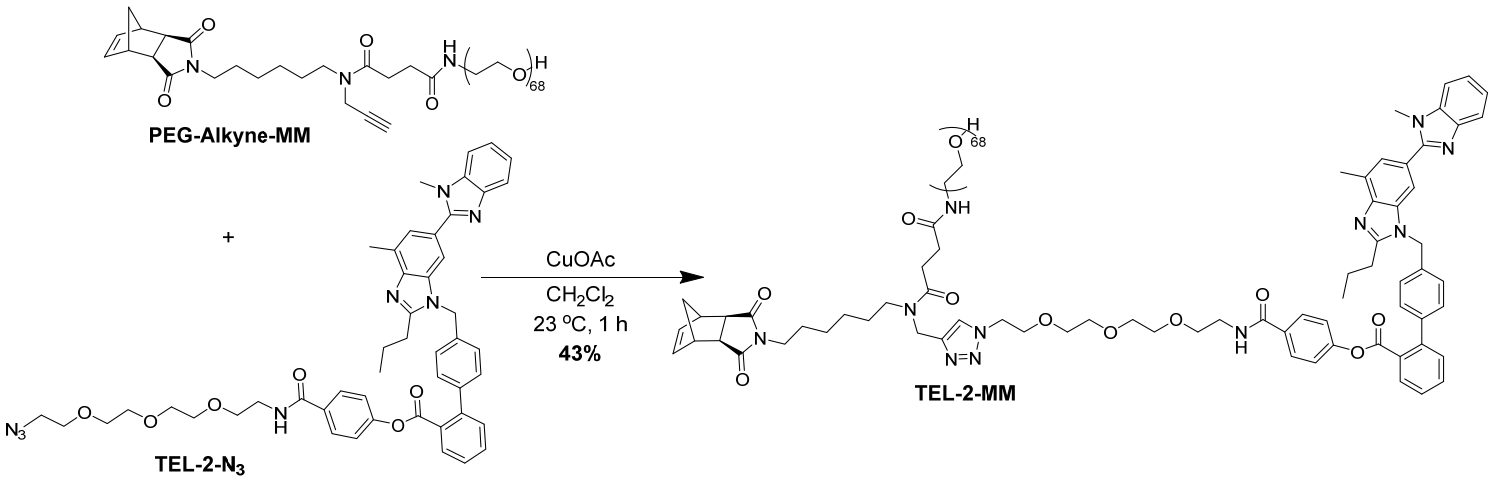


TEL-1-MM: Following the **General Procedure**, **TEL-1-N_3** (120 mg, 0.16 mmol, 1.1 equiv) was reacted with **PEG-Alkyne-MM** (510 mg, 0.15 mmol, 1.0 equiv) to afford **TEL-1-MM** (350 mg, 53%) as an off-white powder after purification by two rounds of prep-HPLC (10% - 60% MeCN/H_2O [0 – 18 min], 60% - 100% MeCN/H_2O [18 – 20 min]) and precipitation with Et_2O. ^{1}H-NMR (500 MHz, $CDCl_3$) and MALDI spectra are provided in **Section C** and **Section D**, respectively.



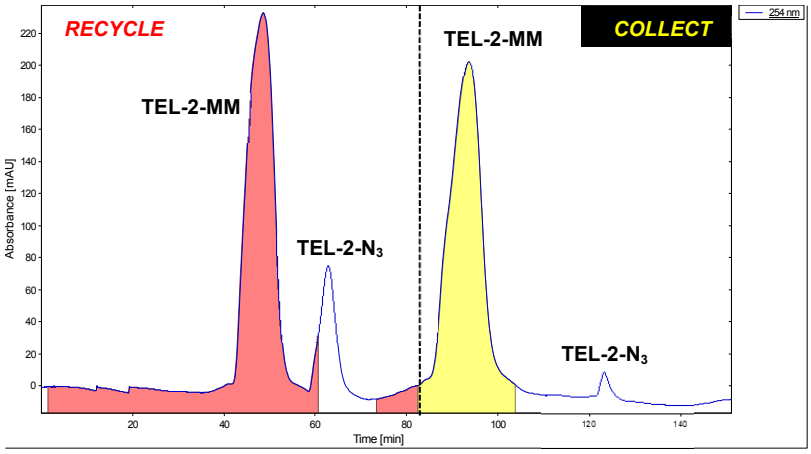




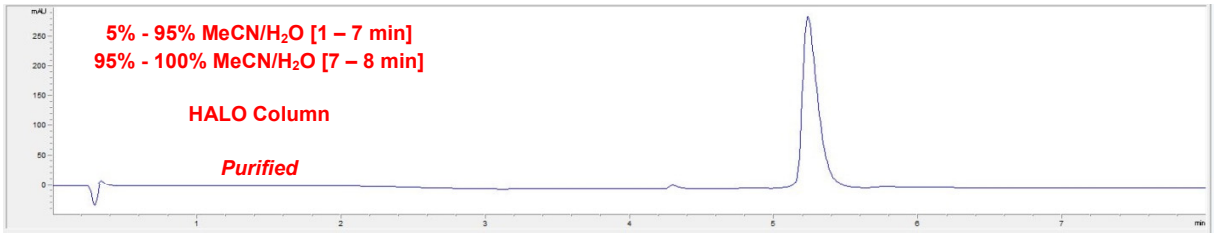


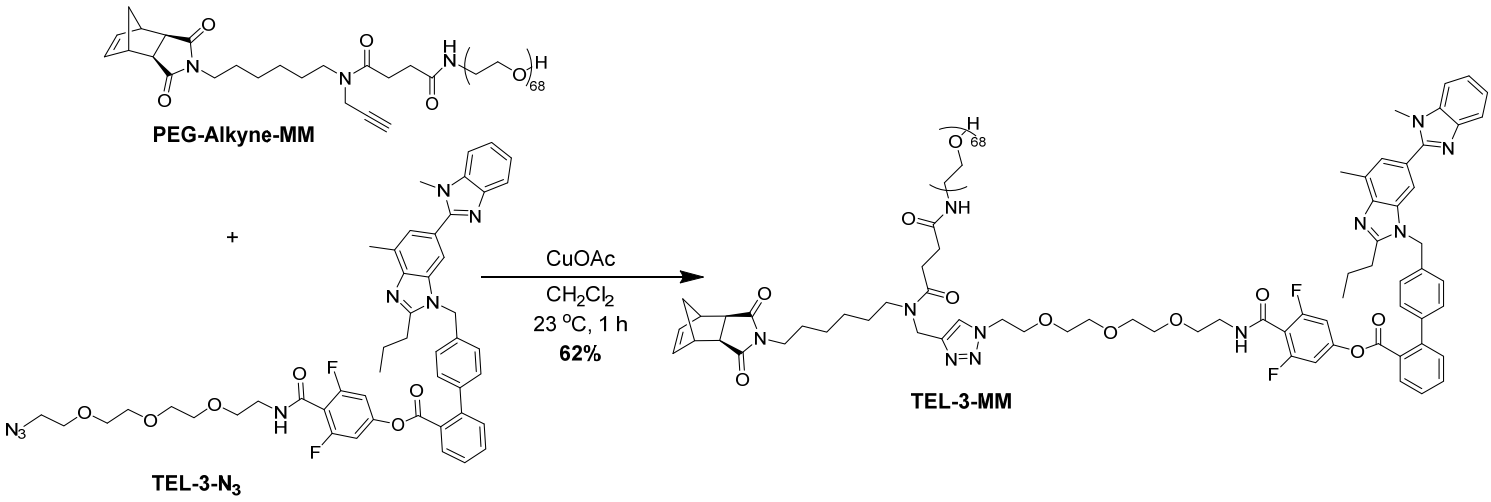
TEL-2-MM: Following the **General Procedure**, **TEL-2-N_3** (88 mg, 0.11 mmol, 1.1 equiv) was reacted with **PEG-Alkyne-MM** (340 mg, 0.10 mmol, 1.0 equiv) to afford **TEL-2-MM** (184 mg, 43% yield) after purification by prep-HPLC (10% - 60% MeCN/H_2O [0 – 18 min], 60% - 100% MeCN/H_2O [18 – 20 min]) and precipitation with Et_2O.^{1}H-NMR (500 MHz, $CDCl_3$) and MALDI spectra are provided in **Section C** and **Section D**, respectively. For larger scale processes (~ 2 g of MM), recycling prep-GPC could be used for MM purification (a representative recycling prep-GPC trace monitored at 254 nm is shown below).

Prep-GPC Trace of **TEL-2-MM***:*



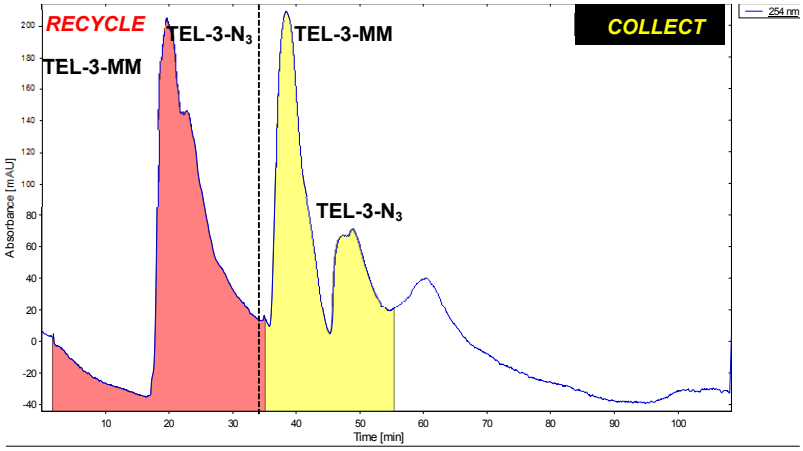
LC Trace of **TEL-2-MM** *After Prep-GPC Purification (280 nm):*



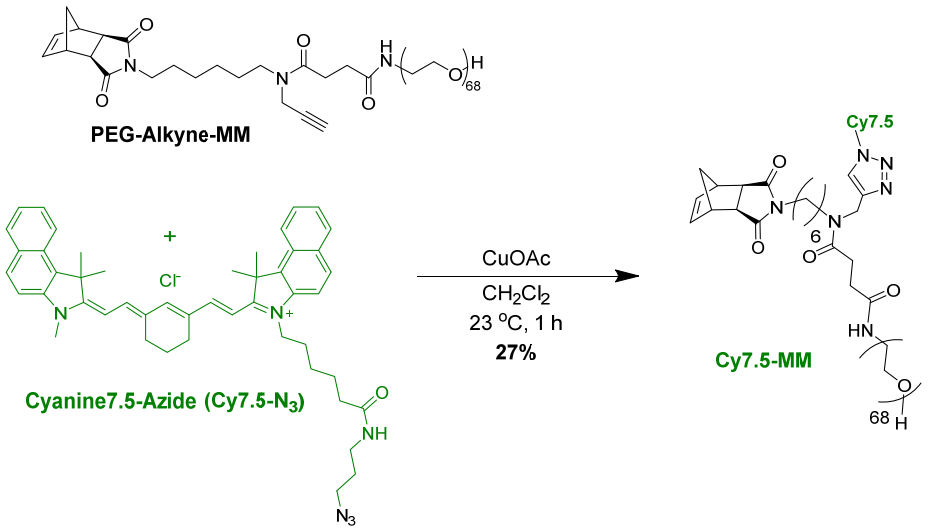


TEL-3-MM: Following the **General Procedure**, **TEL-3-N_3** (410 mg, 0.45 mmol, 1.1 equiv) was reacted with **PEG-Alkyne-MM** (1.5 g, 0.47 mmol, 1.0 equiv) to afford **TEL-3-MM** (1.2 g, 62% yield) after purification by recycling prep-GPC (a representative recycling prep-GPC trace monitored at 254 nm is shown below) and precipitation with Et_2O.^{1}H-NMR (500 MHz, $CDCl_3$) and MALDI spectra are provided in **Section C** and **Section D**, respectively.

Prep-GPC Trace of **TEL-3-MM***:*

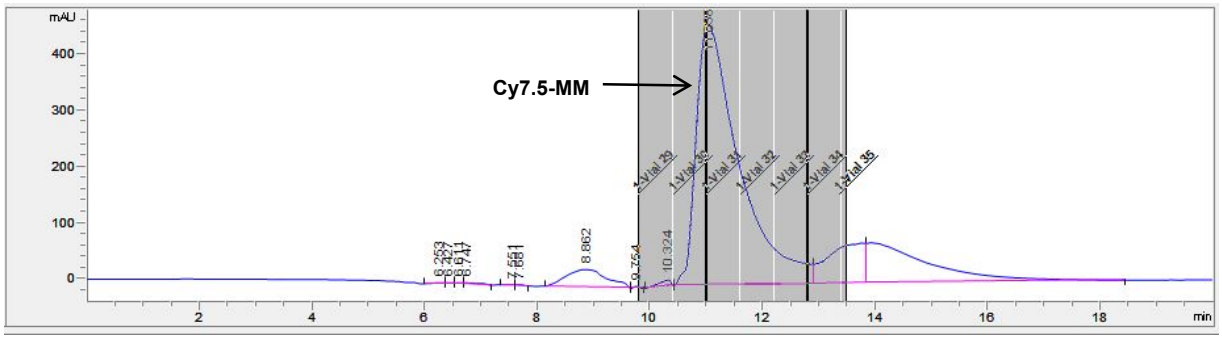


LC Trace of **TEL-3-MM** *After Prep-GPC Purification (280 nm):*



Cy7.5-MM: Following the **General Procedure**, **Cy7.5-N_3** (50 mg, 0.065 mmol, 1.1 equiv) was reacted with **PEG-Alkyne-MM** (210 mg, 0.062 mmol, 1.0 equiv) to afford **Cy7.5-MM** (70 mg, 27% yield) after purification by prep-HPLC (10% - 100% MeCN/H_2O [0 – 17 min], 100% MeCN/H_2O [17 – 20 min]) and precipitation with Et_2O as a dark green solid. ^{1}H-NMR (400 MHz, $CDCl_3$) and MALDI spectra are provided in **Section C** and **Section D**, respectively.

Representative Prep HPLC Trace of **Cy7.5-MM** *During Purification (650 nm):*



3. Preparation of **TEL-2-BASPs** *by ROMP*

Example Procedure (20 mg): TEL-2-MM (20.1 mg, 9.9 equiv) and **Cy7.5-MM** (0.206 mg, 0.1 equiv) were added to a 1 mL vial charged with a Teflon-coated stir bar and septum. In a nitrogen-filled glovebox, the following solutions were prepared: **G3** (2.27 mg in 0.113 mL 1,4-dioxane = 0.02 g/mL) and **AXL** (11.1 mg in 0.191 mL 1,4-dioxane = 0.1 M). To a stirred solution of **MM** (0.079 mL 1,4-dioxane) was added an aliquot of **G3** (0.0176 mL, 1 equiv) as a single stream with a microsyringe. The final volume in the reaction vial (0.079 mL + 0.0176 mL = 0.0966 mL) affords a **MM** concentration of 0.05 M. Upon initiating ROMP, the reaction was allowed to proceed for 15 – 20 minutes before removing from the glovebox and transferring immediately to a Schlenk line under positive N_2 pressure. A 1 mL syringe fitted with a stainless steel needle was charged with the solution of **AXL** (0.191 mL, 0.1 M), and the assembly was placed into a syringe pump (KD Scientific, Legato 100). The tip of the stainless steel needle was carefully placed just under the level of the ROMP reaction medium, and **AXL** solution (0.0436 mL, 9 equiv) was directly infused at a rate of *1 equiv/min*. Upon completion of **AXL** addition, the crosslinking reaction was allowed to proceed for approximately 120 minutes before quenching with ethyl vinyl ether (1 drop). The material was diluted with nanopure H_2O (ca. 0.150 mL, 1:1 dilution) before transferring to dialysis tubing (RC, 8 kDa MWCO) and dialyzing against nanopure H_2O (300 mL, 3 x 2 – 3 h cycles). The contents of the dialysis tubing were then transferred to a clean 20 mL vial and lyophilized (at least 24 h) to afford a dry powder (20 mg, 64%).

Example Procedure (2 g): TEL-2-MM (2.00 g, 9.9 equiv) and **Cy7.5-MM** (20.1 mg, 0.1 equiv) were added to a 40 mL vial charged with a Teflon-coated stir bar and septum. In a nitrogen-filled glovebox, the following solutions were prepared: **G3** (49.8 mg in 2.49 mL 1,4-dioxane = 0.02 g/mL) and **AXL** (304 mg in 5.24 mL 1,4-dioxane = 0.1 M). To a stirred solution of **MM** (3.07 mL 1,4-dioxane) was added an aliquot of **G3** (1.75 mL, 1 equiv) as a single stream. The final volume in the reaction vial (3.07 mL + 1.75 mL = 4.82 mL) affords a **MM** concentration of 0.1 M. Upon initiating ROMP, the reaction was allowed to proceed for 15 – 20 minutes before removing from the glovebox and transferring immediately to a Schlenk line under positive N_2 pressure. A 10 mL syringe fitted with a stainless steel needle was charged with the solution of **AXL** solution (5.24 mL, 0.1 M), and the assembly was placed into a syringe pump (KD Scientific, Legato 100). The tip of the stainless steel needle was carefully placed just under the level of the ROMP reaction medium, and **AXL** solution (4.34 mL, 9 equiv) was directly infused at a rate of *1 equiv/min*. Upon completion of **AXL** addition, the crosslinking reaction was allowed to proceed for approximately 120 minutes before quenching with ethyl vinyl ether (0.300 mL). The material was diluted with nanopure H_2O (ca. 9.5 mL, 1:1 dilution) before transferring to dialysis tubing (RC, 8 kDa MWCO) and dialyzing against nanopure H_2O (15 L, 3 x 2 – 3 h cycles). The contents of the dialysis tubing were then transferred to clean 20 mL vials and lyophilized (at least 24 h) to afford a dry powder (2.1 g, 90%).

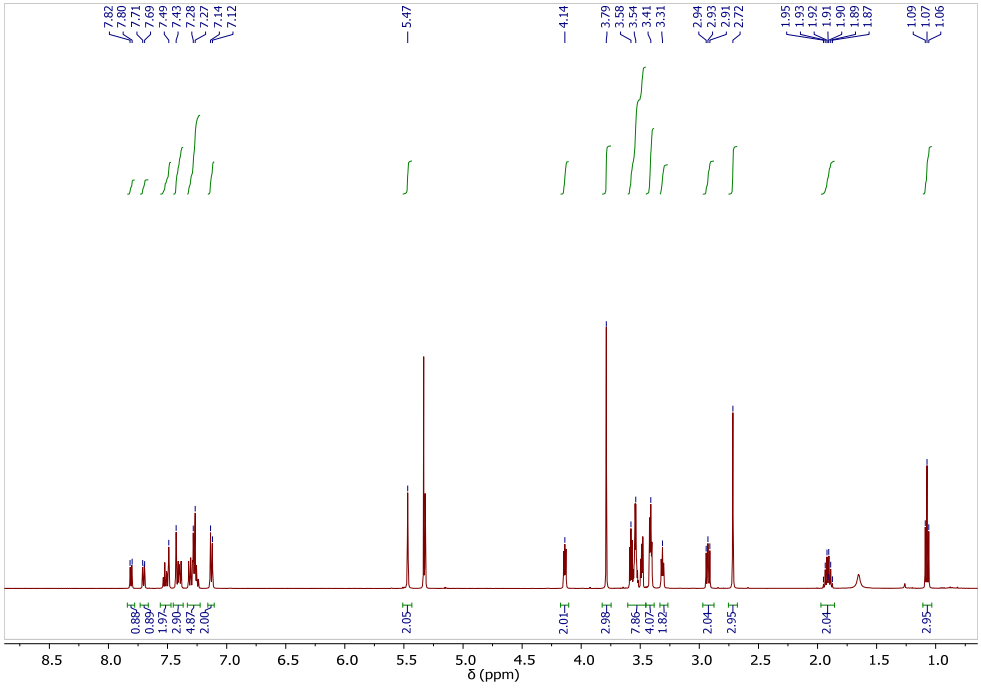
Example Procedure (100 g): Under a nitrogen atmosphere, **TEL-2-MM** (100 g, 10 equiv) was added to a 1 L reactor charged with a mechanical stirrer. To the vessel was added 1,4-dioxane (150 mL, 0.16 M) and the mixture was stirred at 35 °C until complete dissolution of the solid reactant was observed before subsequent cooling to 25 °C. A solution of **G3** (1.72 g, 1 equiv) in addition to a 3 mL solvent rinse were added via syringe. The resulting mixture was stirred for 20 min. **AXL** (12.4 g) was weighed into a 500 mL round-bottom flask and anhydrous 1,4-dioxane (210 ml) was added and stirred at 50 – 55 °C until fully dissolved. The solution of crosslinker (218 mL, 9 equiv) in addition to a 1 mL 1,4-dioxane rinse was added via syringe at ca. 1 equiv/min (the reaction mixture stirring was maintained at 300 rpm). Post injection, the reaction was allowed to proceed for ca. 1 h before adding ethyl vinyl ether (20 mL) to quench the reaction. The solution was further stirred for an additional 15 min. The resultant solution was transferred to a sealed container and frozen at -20 °C before subsequent processing.

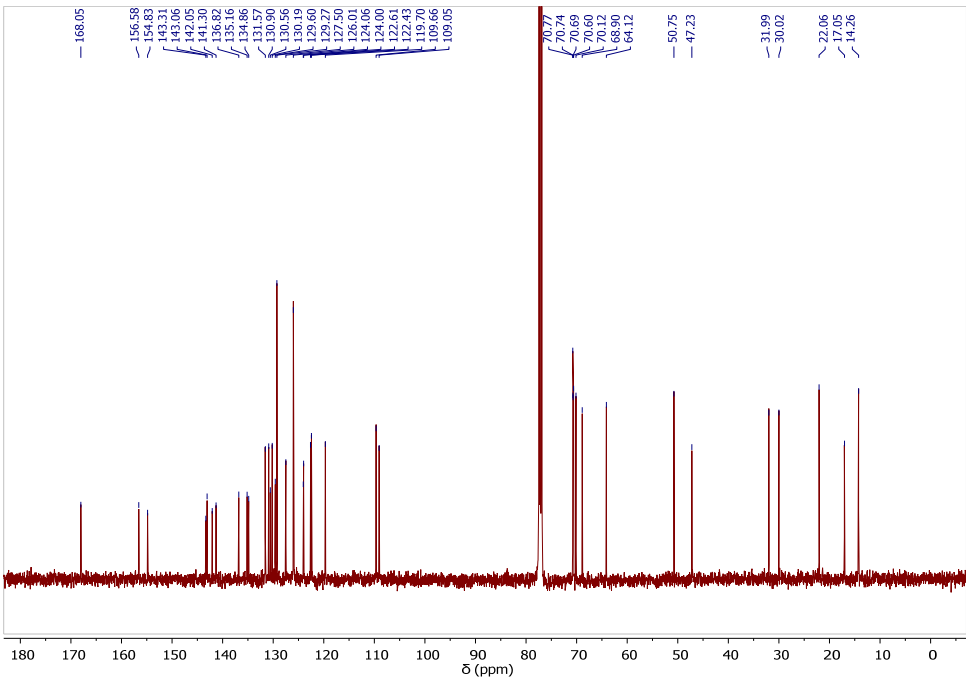
The crude reaction mixture (0.21 g/mL in 1,4-dioxane) was diluted with deionized H_2O (ca. 1:25). The diluted material was charged to a tangential flow filtration system (5400 cm^2, 50 kDa MWCO, mPES) via a peristaltic pump and silicone tubing. The tangential flow filtration process consisted of a 5.3-fold concentration step followed by a 15-diavolume diafiltration step using deionized H_2O. The diafiltration process was run at a constant feed rate of approximately 3.8 L/min, average retentate pressure of 9 psig; an average permeate flow rate of 115 mL/min was observed. Particle size during the tangential flow filtration process, measured via DLS, remained constant at approximately 20 nm.

Using good aseptic technique, the collected retentate was filtered through a 0.2 µm (220 cm^2, PES) sterilizing grade filter. The sterile-filtered material (30 mg/mL **TEL-2-BASP**) was filled into 10 mL tubing lyophilization vials at a target volume of 5 mL via a sterile repeater pipette. The filled vials were partially stoppered and freeze-dried on a shelf lyophilizer (SP Scientific, LyoStar). Following lyophilization, the chamber was backfilled with nitrogen and the vials were stoppered/sealed.

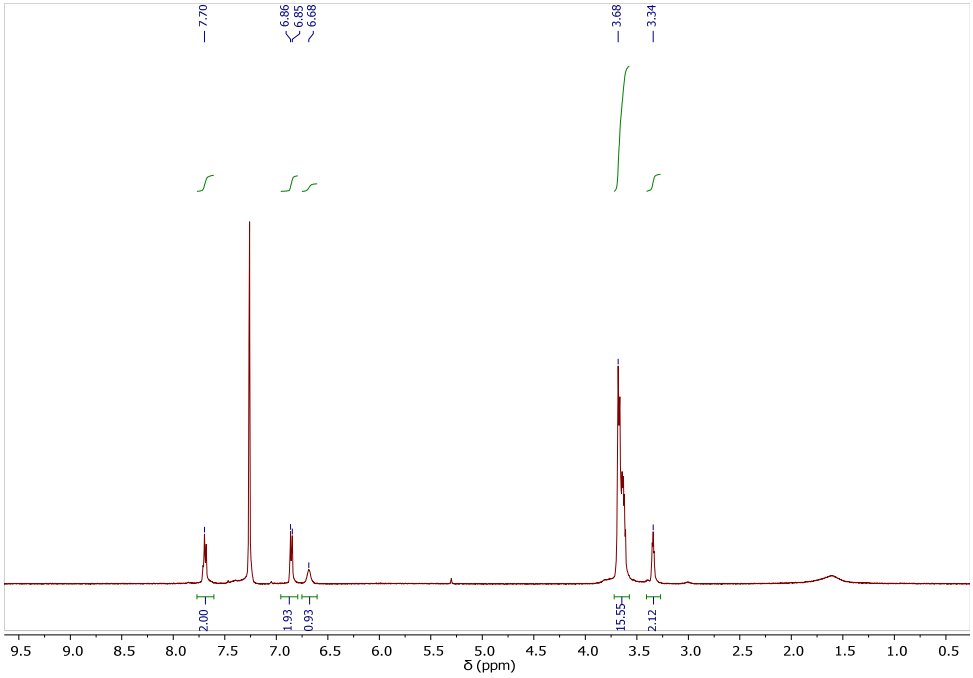
Section C. NMR Spectroscopic Characterization

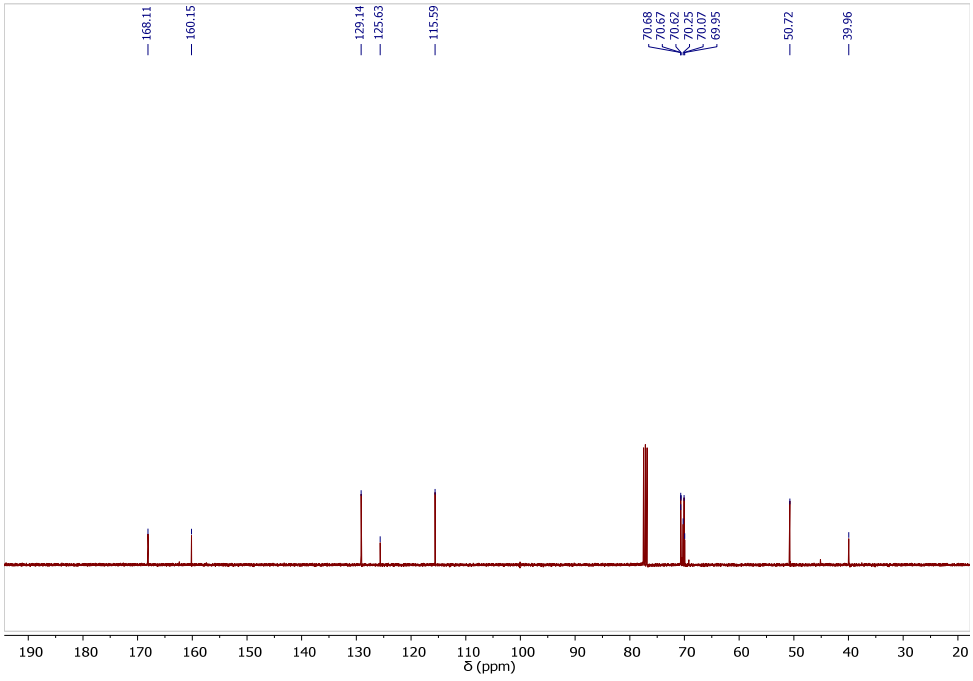
TEL-1-N_3 (^{1}H, ^{13}C):



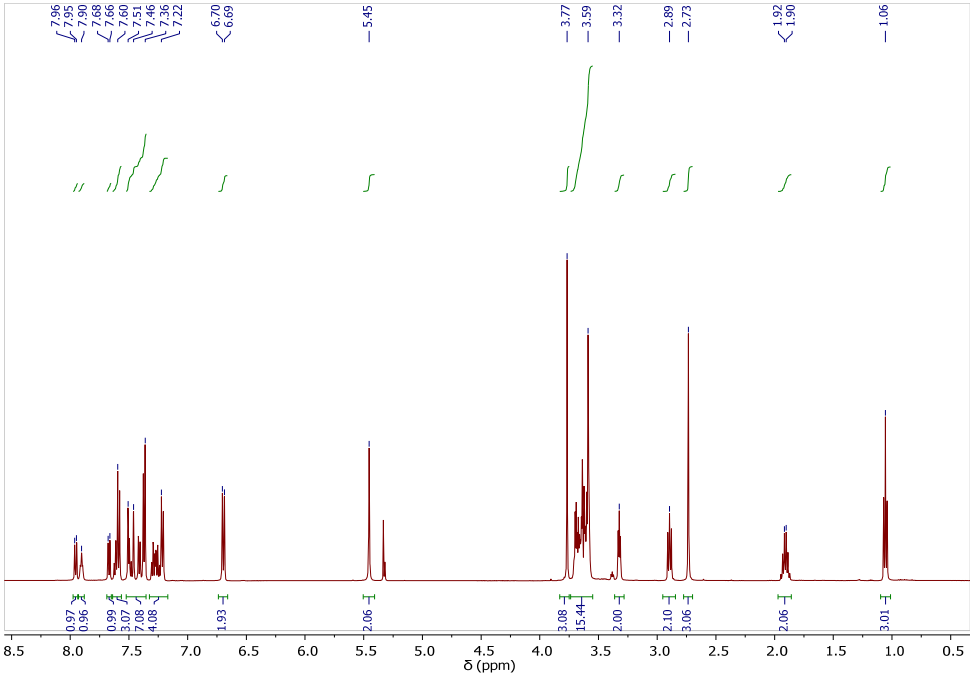


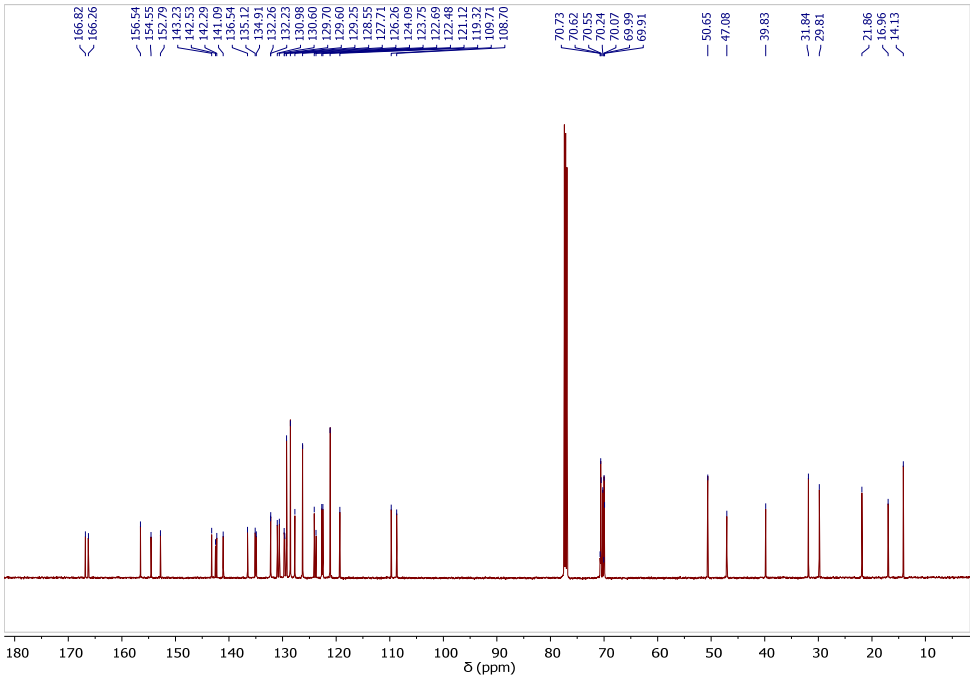
Phenyl-PEG4-N_3 (^{1}H, ^{13}C):



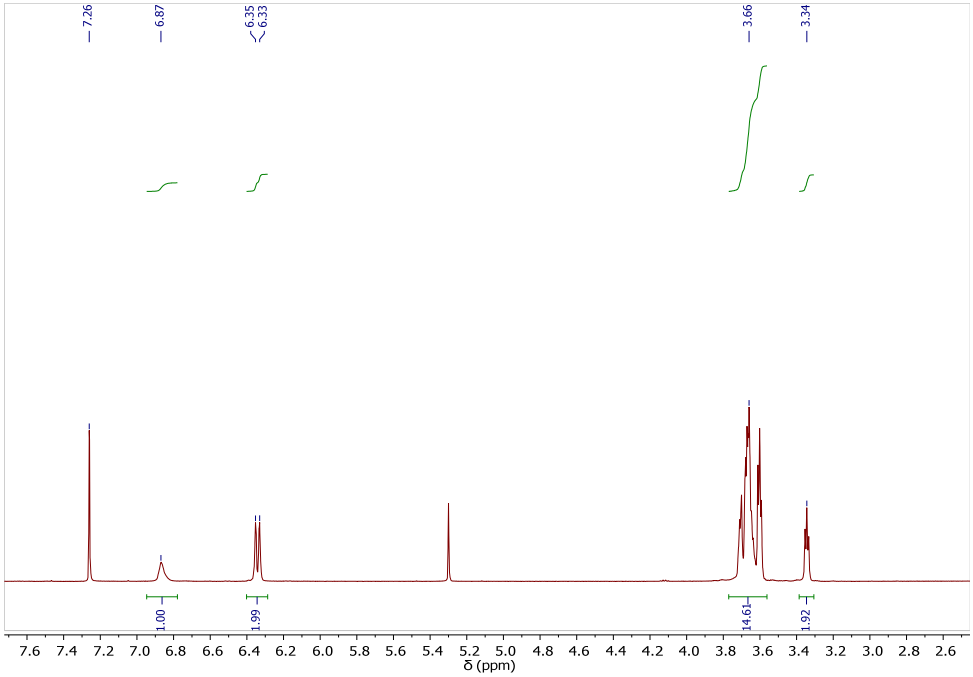


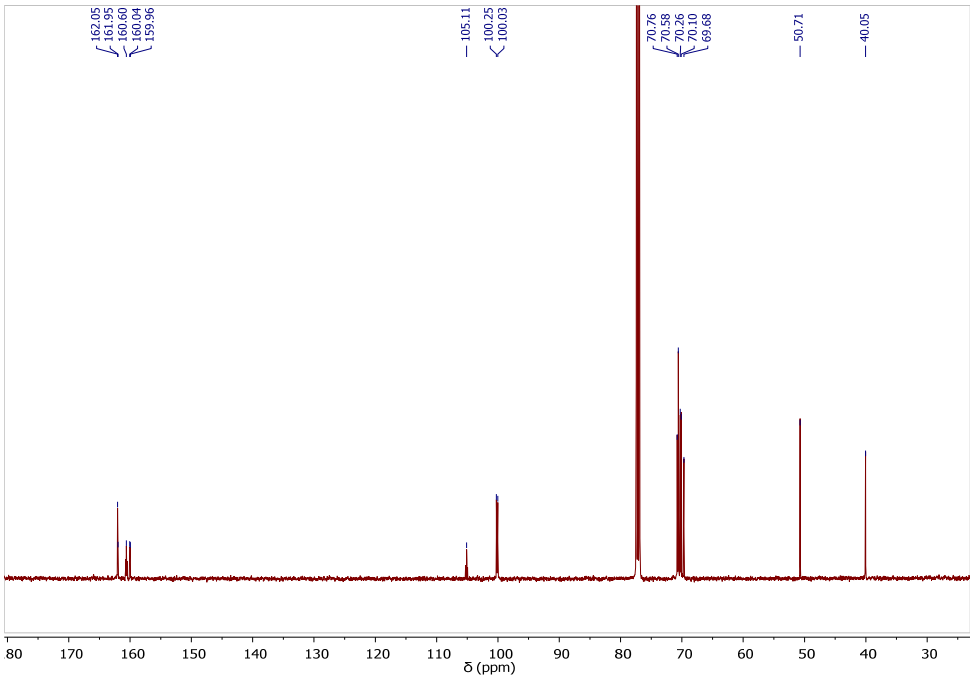
TEL-2-N_3 (^{1}H, ^{13}C):



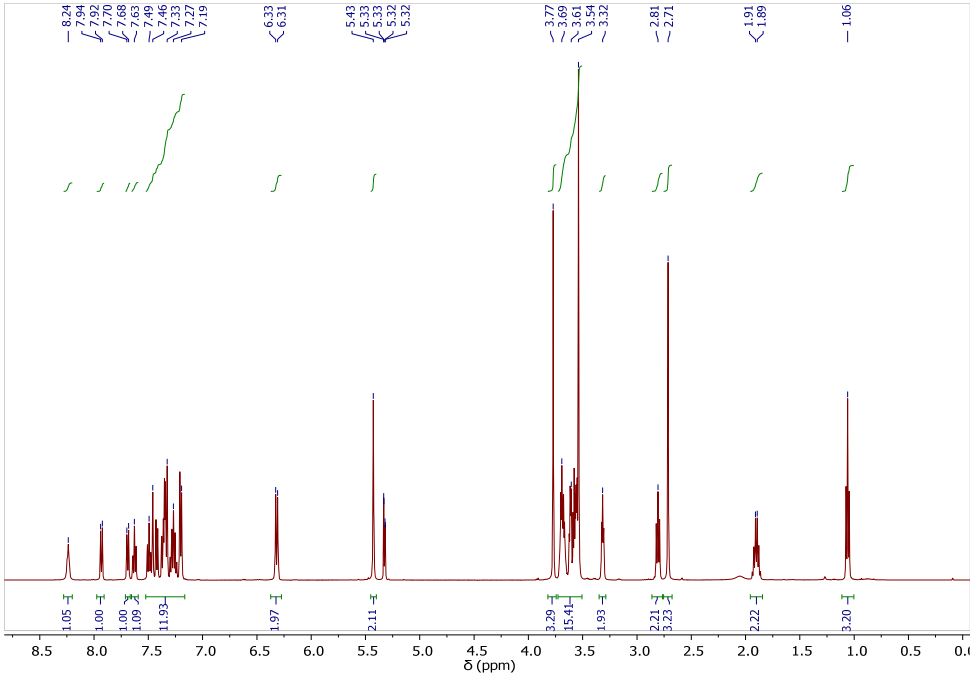


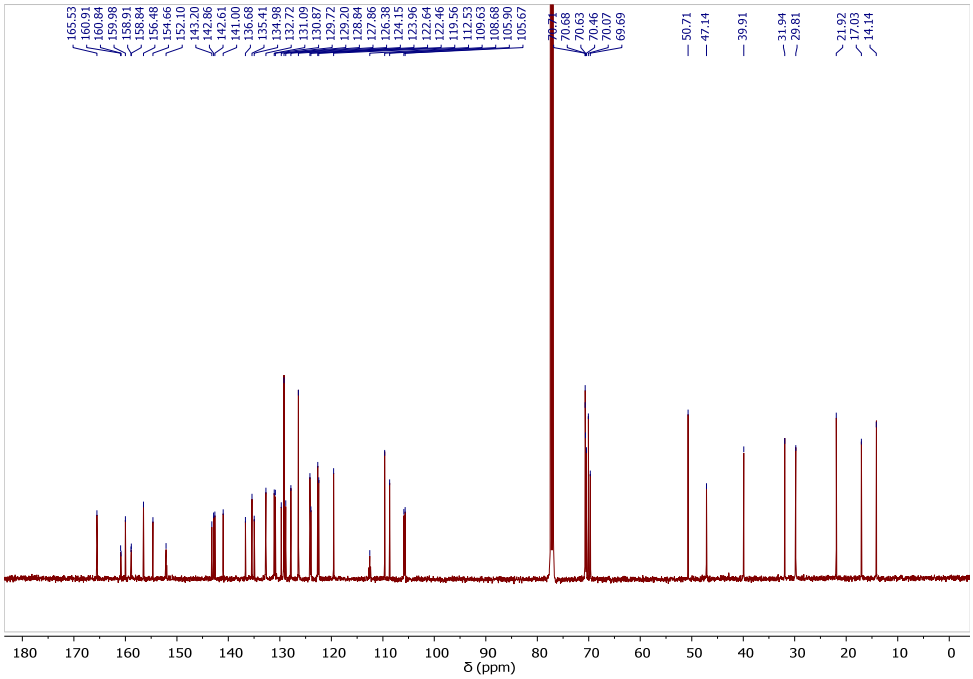
Difluoro-PEG4-N_3 (^{1}H, ^{13}C):



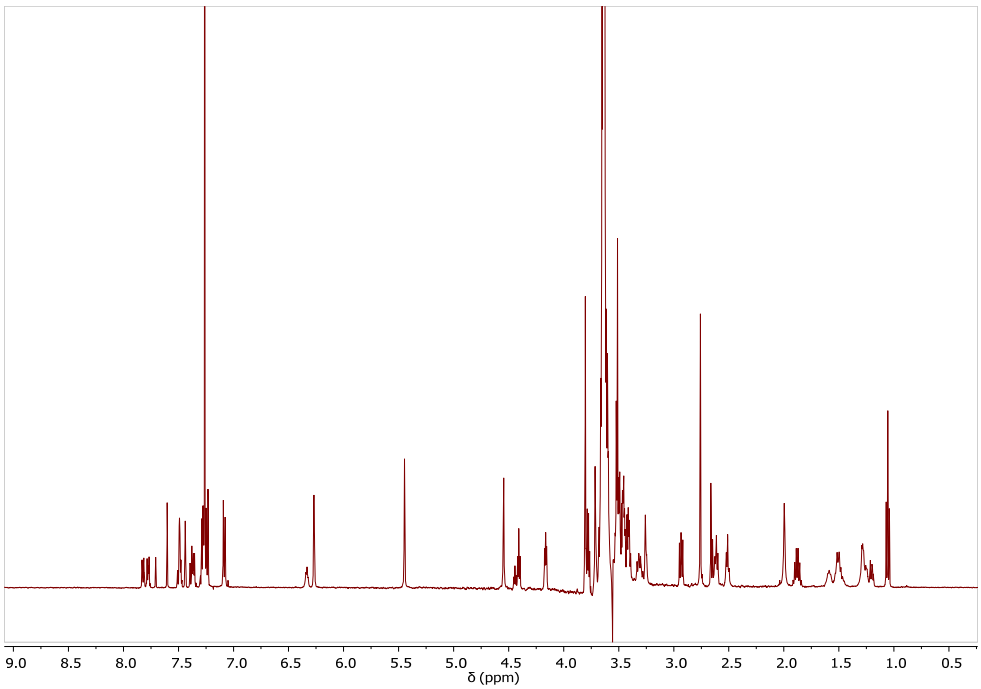


TEL-3-N_3 (^{1}H, ^{13}C):

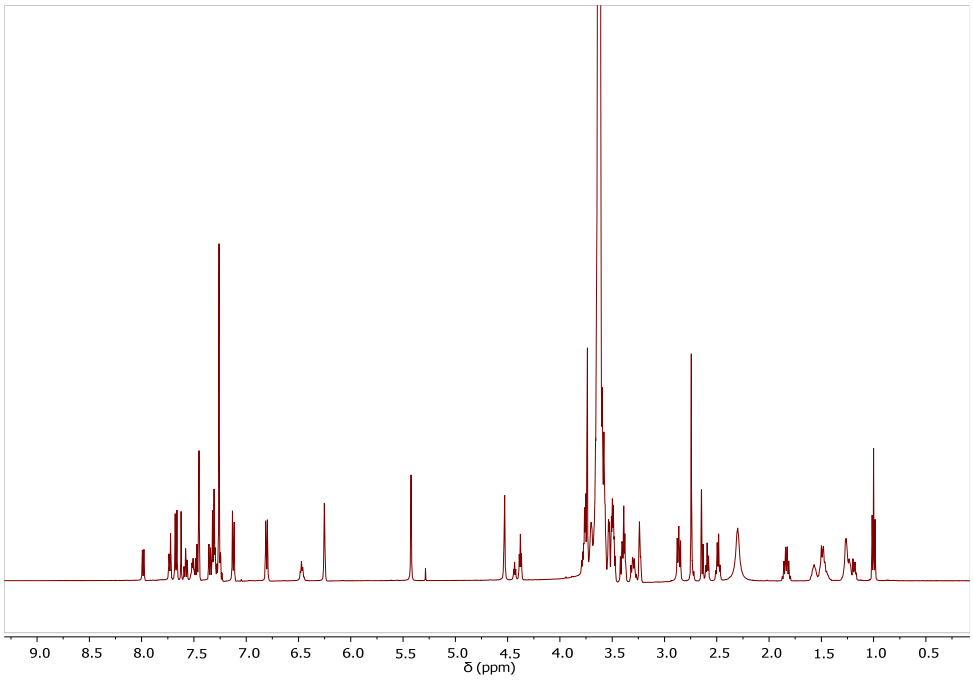




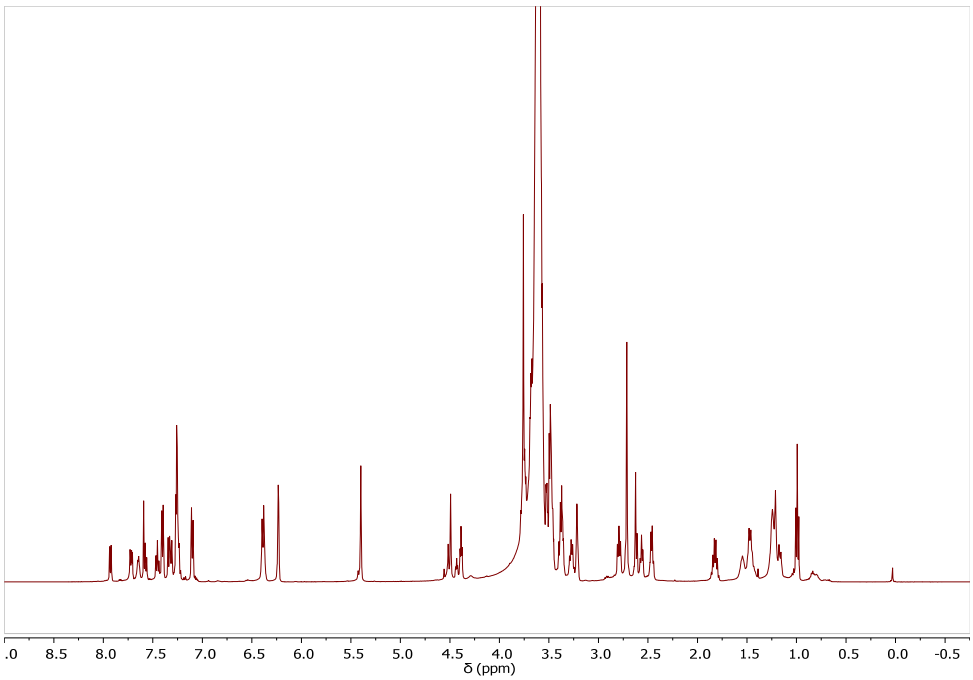
TEL-1-MM (^{1}H):



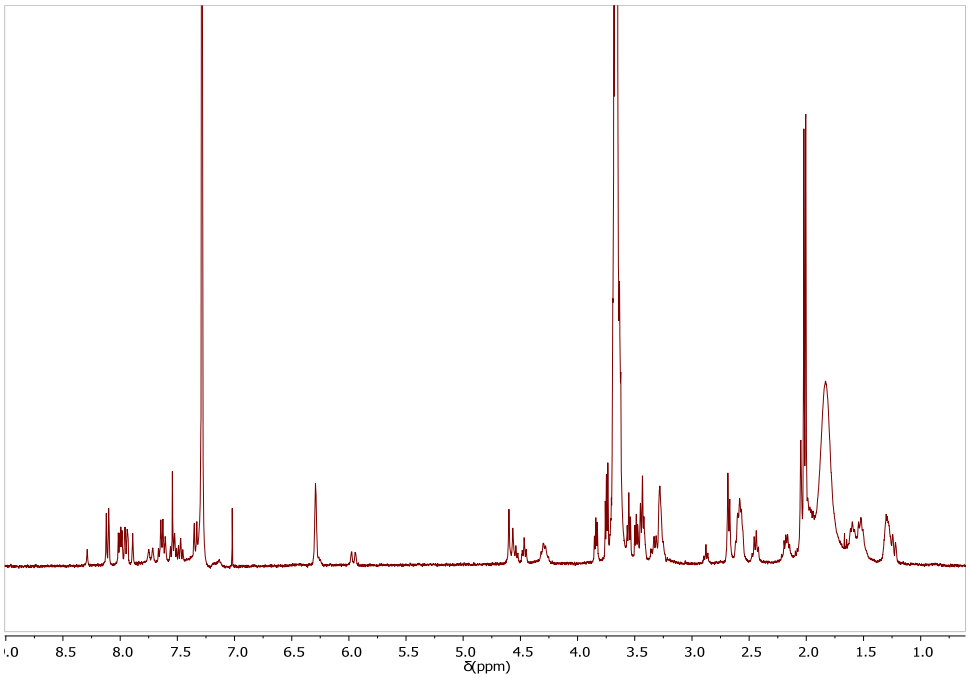
TEL-2-MM (^{1}H):



TEL-3-MM (^{1}H):

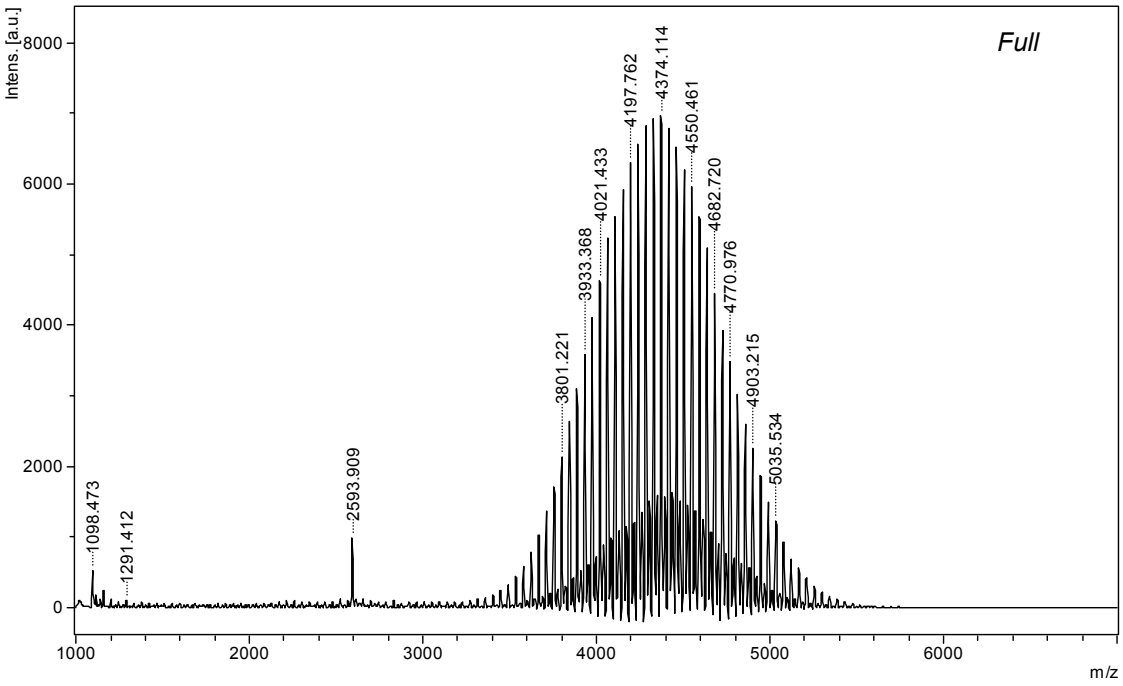


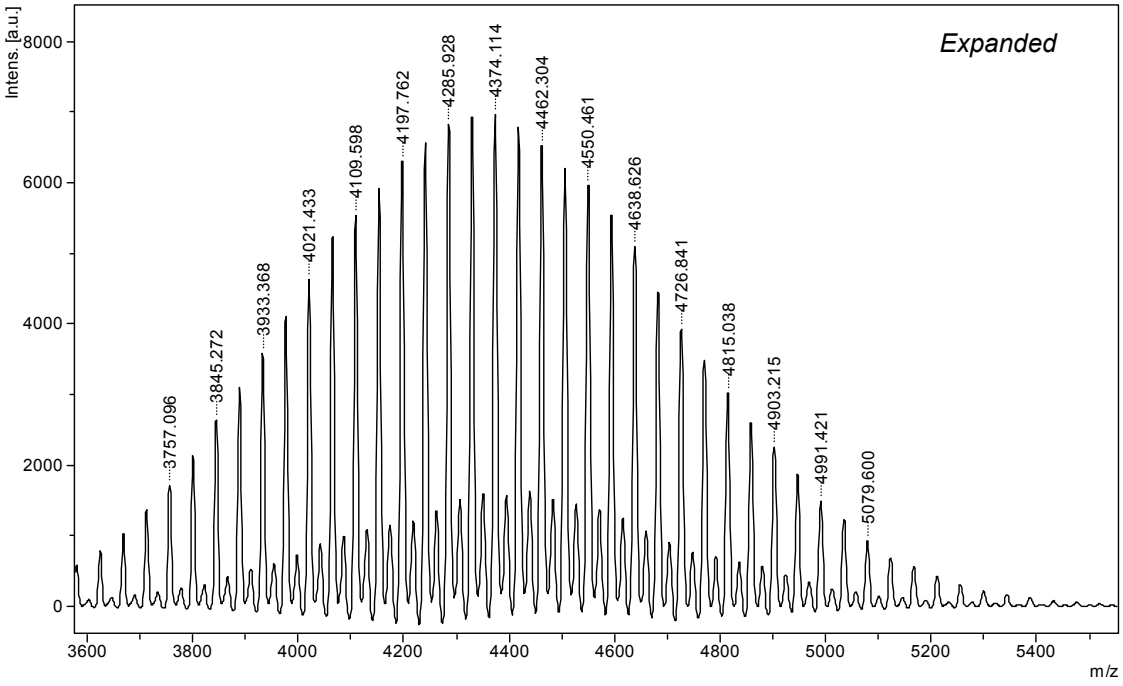
Cy7.5-MM (^{1}H):



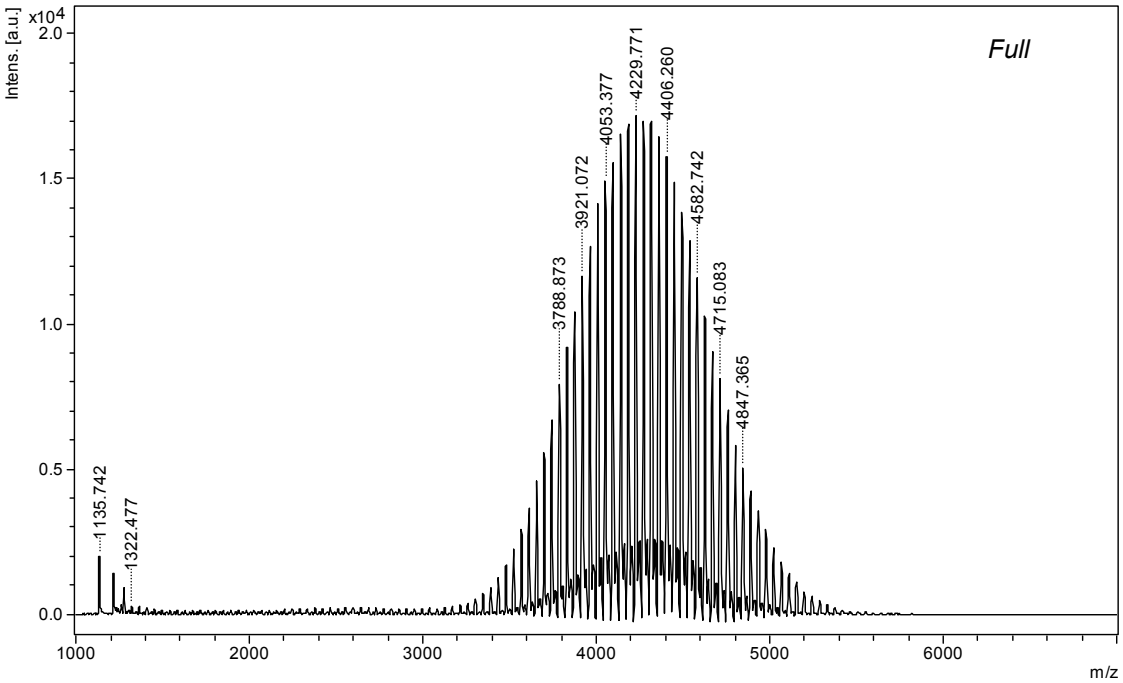
Section D. MALDI-ToF Mass Spectrometric Characterization

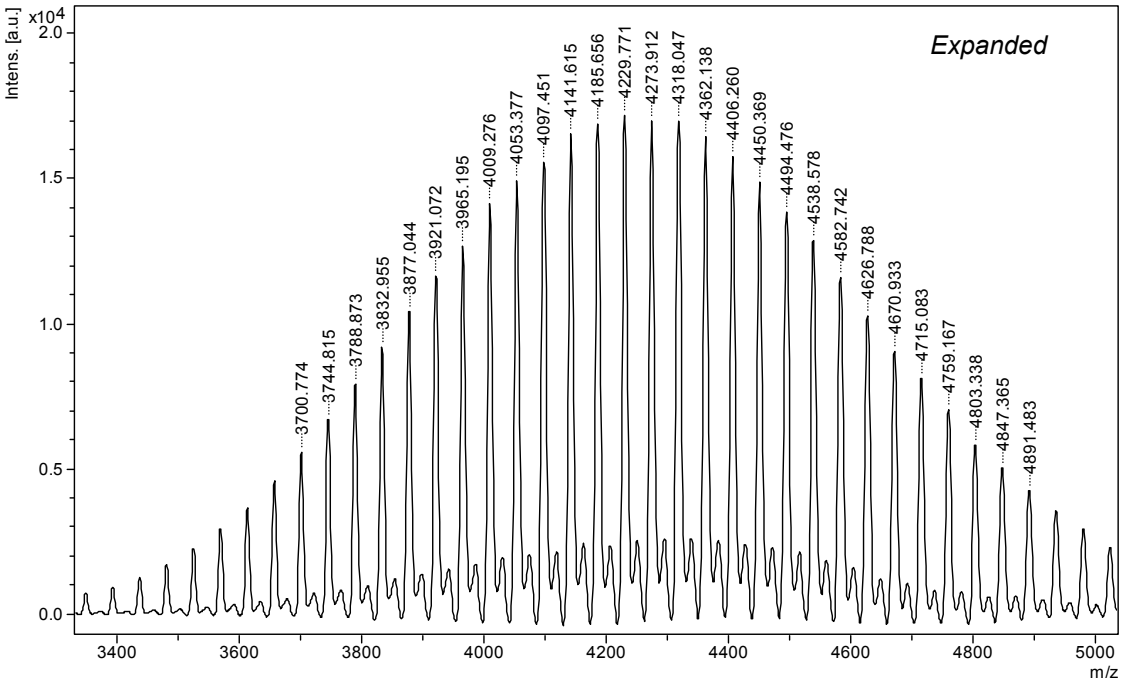
TEL-1-MM: $(M + H)^+$ calcd. for $C_{203}H_{355}N_{10}O_{79}$: 4199.4, observed 4197.8:



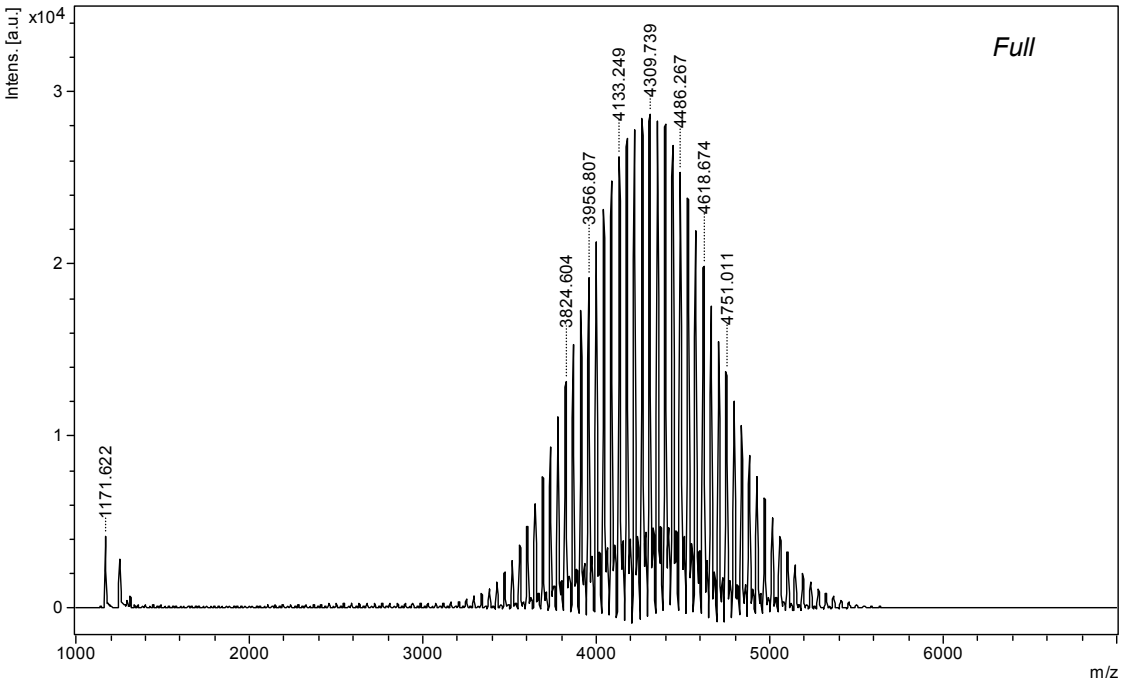


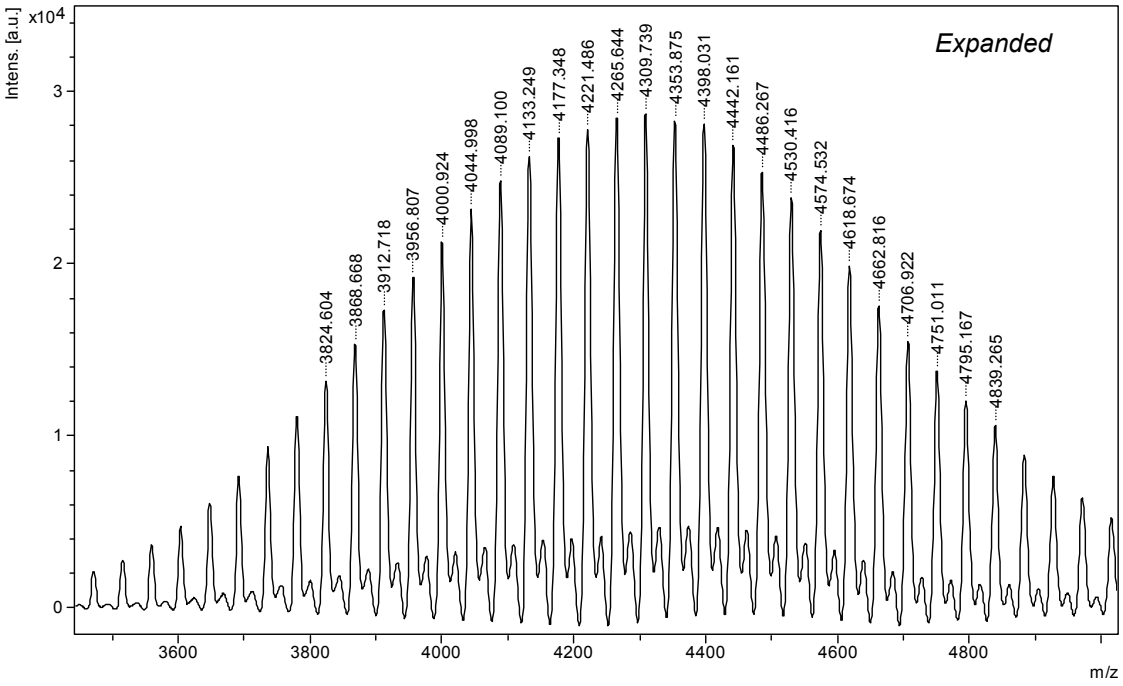
TEL-2-MM: $(M + H)^+$ calcd. for $C_{206}H_{352}N_{11}O_{78}$: 4230.4, observed 4229.8



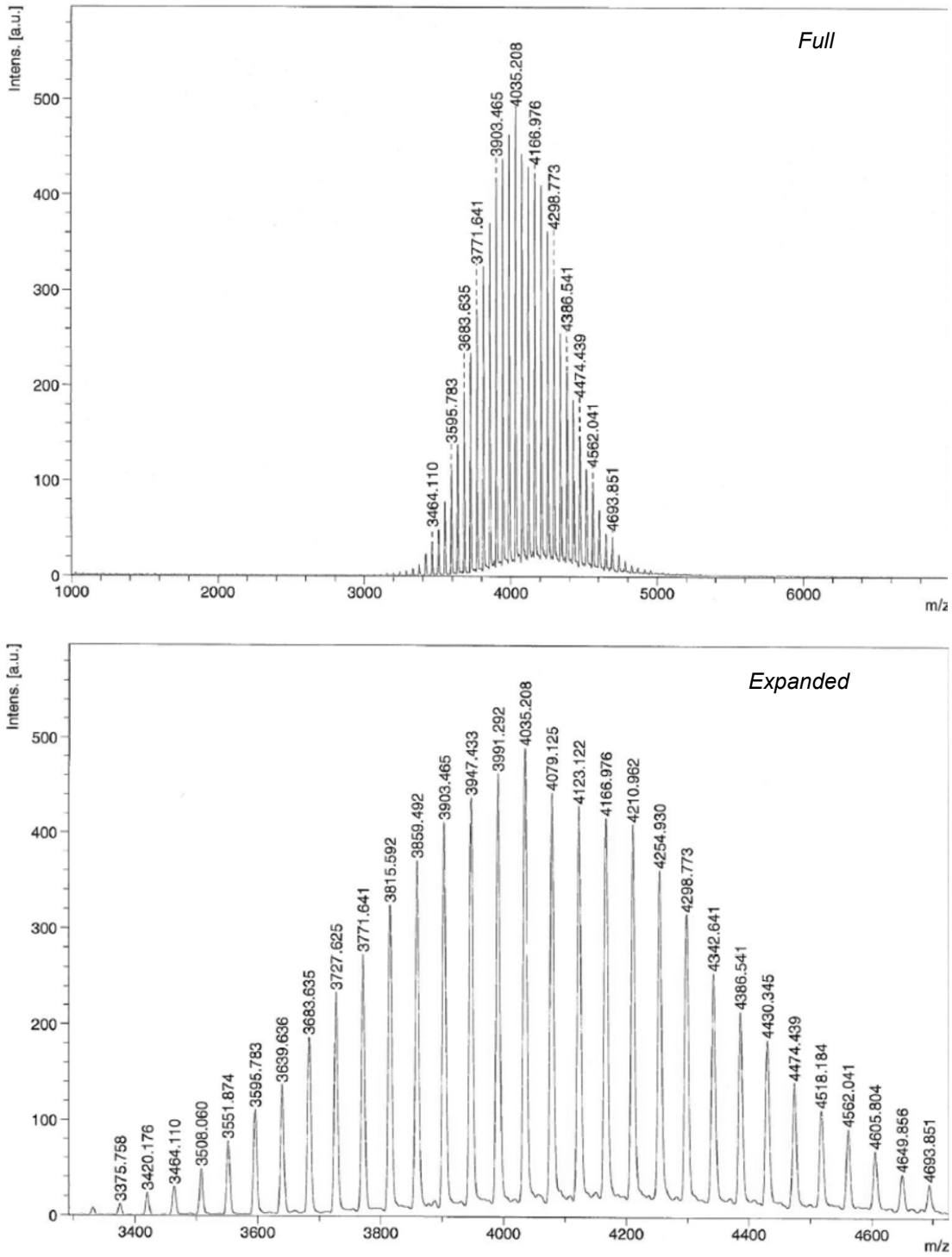


TEL-3-MM: $(M + H)^+$ calcd. for $C_{206}H_{350}N_{11}O_{78}$: 4266.4, observed 4265.6





Cy7.5-MM: $(M - Cl)^+$ calcd. for $C_{206}H_{356}N_9O_{73}$: 4123.5, observed 4123.1



Section E. *In Vitro* Drug Release Assay

Approximately 5 mg of a given MM (**TEL-1-MM**, **TEL-2-MM**, **TEL-3-MM**) was weighed into a clean 4 mL vial. Separately, a stock solution of porcine liver esterase (Sigma E3019-3.5KU, Lot# SLBD9321V, 17 units/mg solid) was made from 24 mg of solid esterase powder and 4 mL of PBS (pH 7.6) such that the final activity of esterase in solution was approximately 100 units/mL. Each vial containing 5 mg of a given MM was dissolved in 1 mL of the esterase stock solution. The polymer solutions were briefly vortexed and immediately portioned into 1 mL vials with Teflon-coated caps. All vials were sealed and placed in a 37 ^{o}C static incubation oven. At each time point, a vial for a given MM was removed from the incubation oven and cooled to room temperature. To each vial was added 200 µL DMSO, and the resulting solution was briefly vortexed before filtering through a 0.45 µm Nylon syringe filter. Analysis by LC (10 – 95% MeCN/H_2O [0 – 10 min], 95% - 100% MeCN/H_2O [10 – 11 min], PES column) provided insight to the amount of **TEL-MM** remaining at a given time point. Quantifications were made by integration of the MM peak at r.t. = 5.4 min.

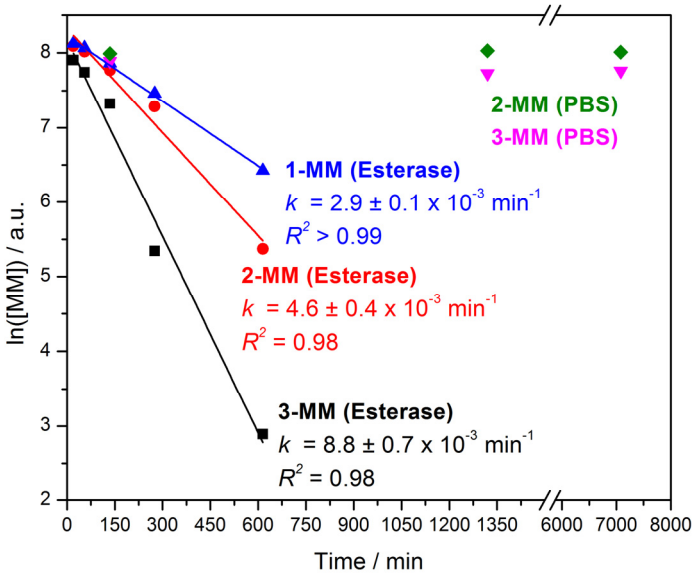


Figure S1. Pseudo-first order rate constants for TEL release from **TEL-1-MM**, **TEL-2-MM**, and **TEL-3-MM** in buffered porcine esterase solution. Based on these data, 16.5% of **TEL-1-MM**, 5.2% of **TEL-2-MM**, and < 0.1% **TEL-3-MM** remain at the final time point (615 minutes). Accordingly, this corresponds to 83.5%, 94.8%, and > 99.9% released telmisartan from **TEL-1-MM**, **TEL-2-MM**, and **TEL-3-MM**, respectively.

Section F. Size Exclusion Chromatography

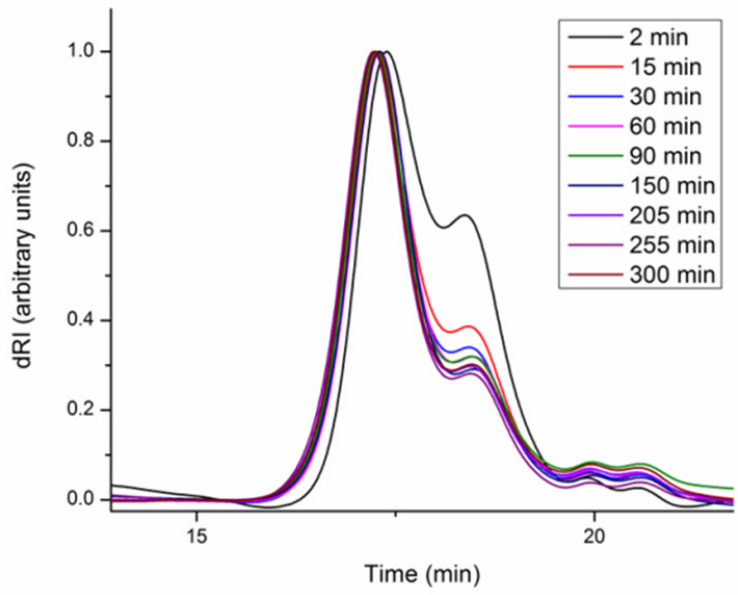
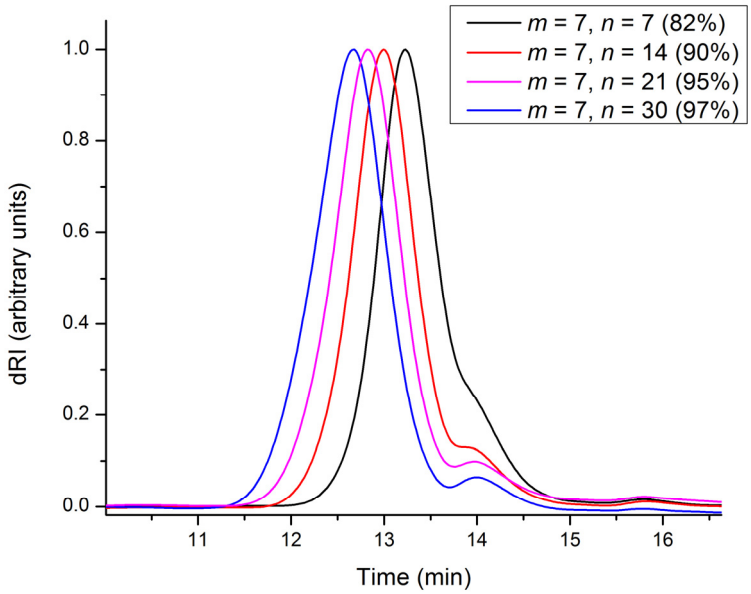
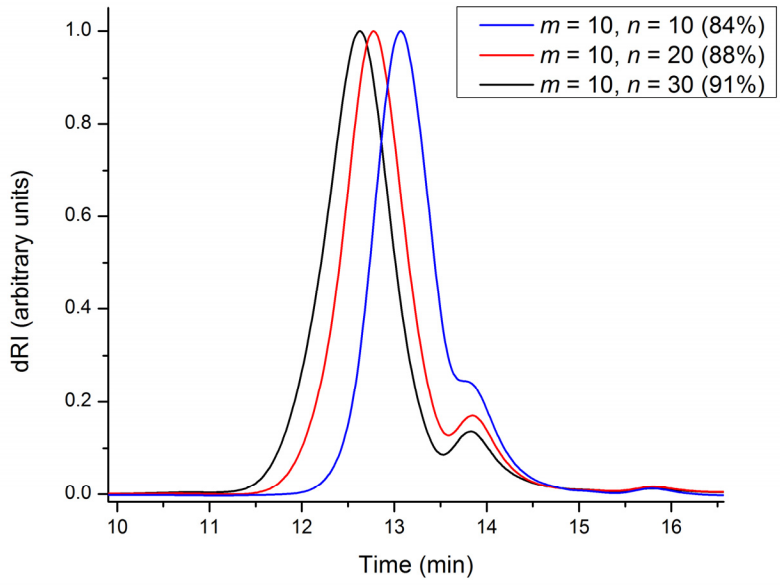


Figure S2. GPC traces (Shodex columns) depicting the kinetics of **TEL-2-BASP** formation on 2 g scale; no significant change in residual brush (lower MW shoulder) was observed after approximately 90 minute.

A


m = 7, n = 7 (82%)
m = 7, n = 14 (90%)
m = 7, n = 21 (95%)
m = 7, n = 30 (97%)
dRI (arbitrary units)
Time (min)

B


m = 10, n = 10 (84%)
m = 10, n = 20 (88%)
m = 10, n = 30 (91%)
dRI (arbitrary units)
Time (min)

C

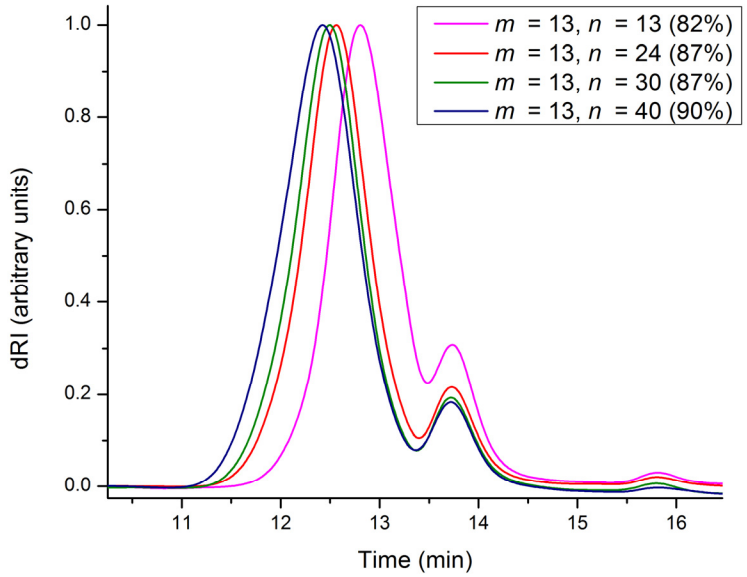


Figure S3: GPC traces (Agilent columns) of **TEL-2-BASP** formation screening various values of *m* and *n*: *m* = 7 (**A**), *m* = 10 (**B**), m = *13* (**C**). Conversions of brush to BASP are given in parentheses. *m* = equivalents of MM relative to **G3**; *n* = equivalents of **AXL** relative to **G3**.

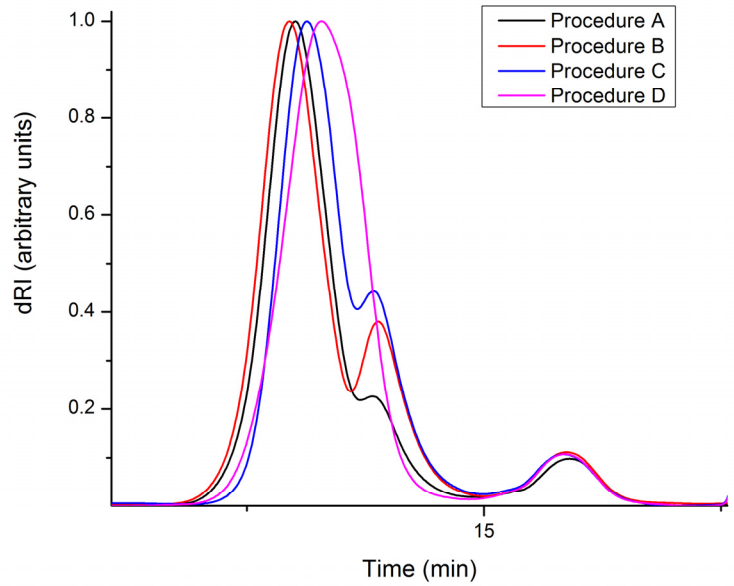


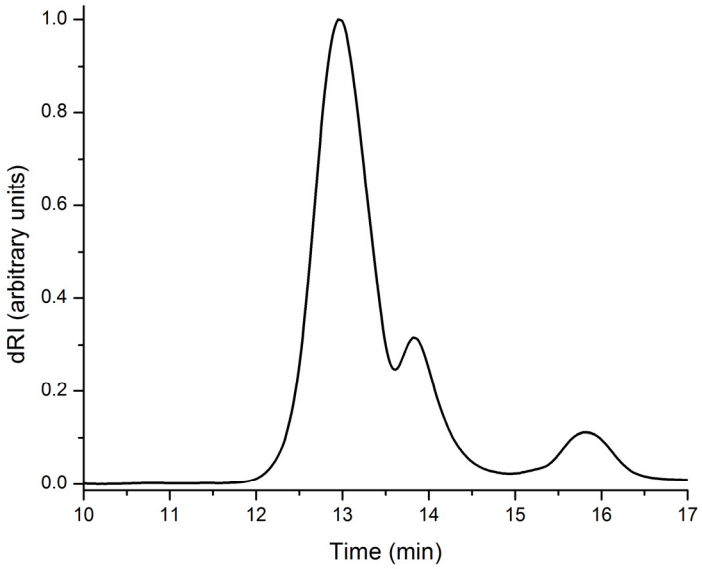
Figure S4: GPC traces (Agilent columns) of alternative **TEL-2-BASP** formation methods (procedures described below; *m* = 10, *n* = 9).

Procedure A: Standard Procedure (XL added slowly to living brush via syringe; 30 mg MM) **Conv = 84%**

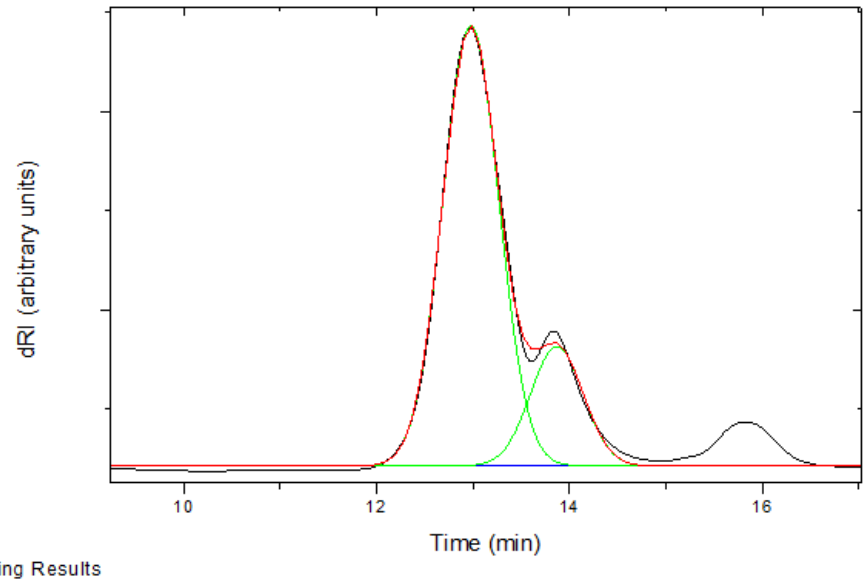
Procedure B: Living brush added quickly (steady stream; 100 µL) to solid XL (30 mg MM) **Conv = 77%**

Procedure C: XL added quickly (steady stream; 110 µL) to living brush solution (90 mg MM) **Conv = 73%**

Procedure D: Grubbs III added to solution of MM/XL mixture (30 mg MM) **Conv = N/A**



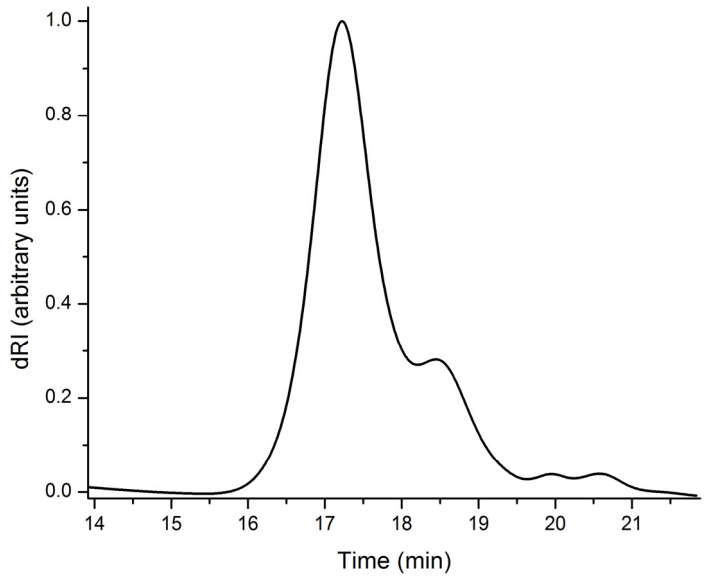
Chi^2=4.38675E-014 Adj. R-Square=9.88161E-001 # of Data Points=1107.
SS=4.82981E-011 Degree of Freedom=1101.



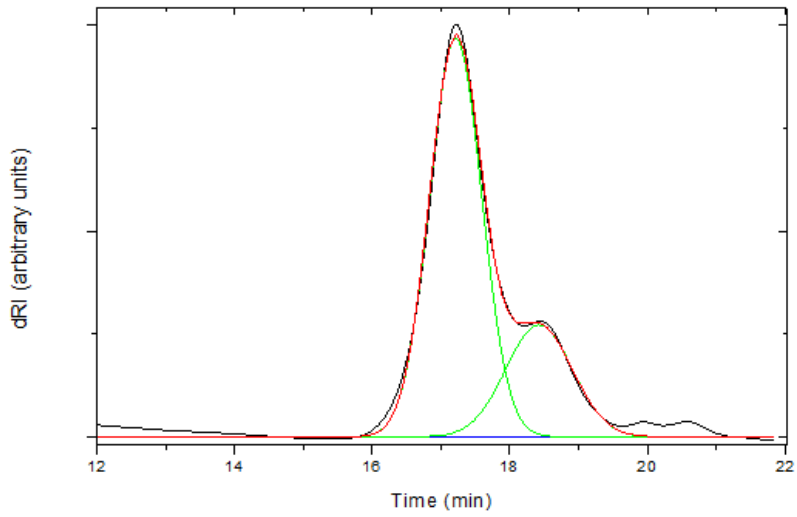
Fitting Results

Peak Index	Peak Type	Area Intg	FWHM	Max Height	Center Grvty	Area IntgP
1.	Gaussian	1E-5	0.68229	1E-5	12.97633	79.63939
2.	Gaussian	0	0.64429	0	13.86851	20.36061

Figure S5. GPC trace (Agilent columns) and curve fitting of **TEL-2-BASP** (20 mg scale).



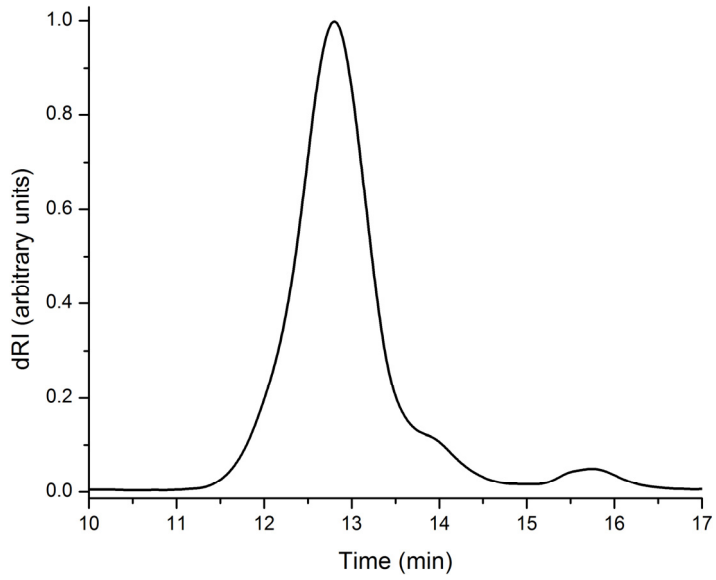
Chi^2= 4.80978E-003 Adj. R-Square= 8.22750E-001 # of Data Points= 1384.
SS= 6.62788E+000 Degree of Freedom= 1378.



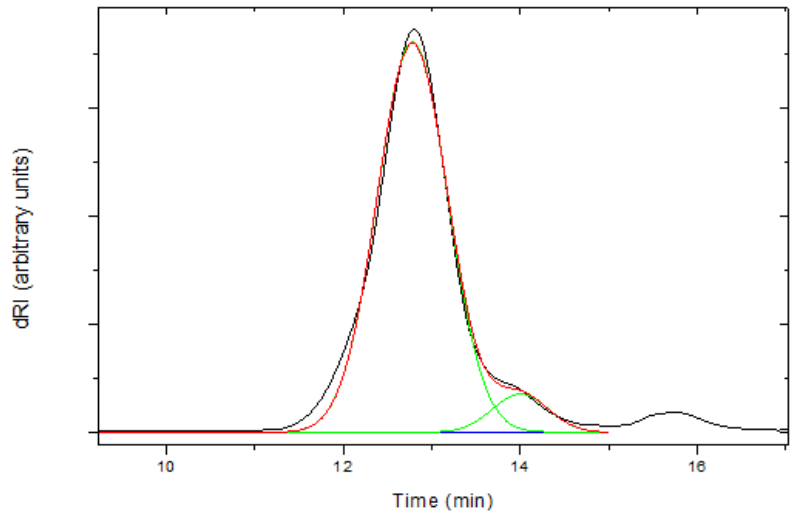
Fitting Results

Peak Index	Peak Type	Area Intg	FWHM	Max Height	Center Grvty	Area Intg P
1.	Gaussian	0.91505	0.89077	0.96504	17.21516	73.66095
2.	Gaussian	0.3272	1.13664	0.27043	18.42686	26.33905

Figure S6. GPC trace (Shodex columns) and curve fitting of **TEL-2-BASP** (2 g scale).



Chi^2=1.01214E+004 Adj. R-Square=9.95419E-001 # of Data Points=2085.
SS=2.10424E+007 Degree of Freedom=2079.



Fitting Results

Peak Index	Peak Type	Area Intg	FWHM	Max Height	Center Grvty	Area Intg P
1.	Gaussian	7196.99088	0.93679	7217.30735	12.78385	92.76731
2.	Gaussian	561.12013	0.7444	708.69809	14.00171	7.23269

Figure S7. GPC trace (Agilent columns) and curve fitting of **TEL-2-BASP** (100 g scale).

Section G. Aqueous Dynamic Light Scattering Measurements

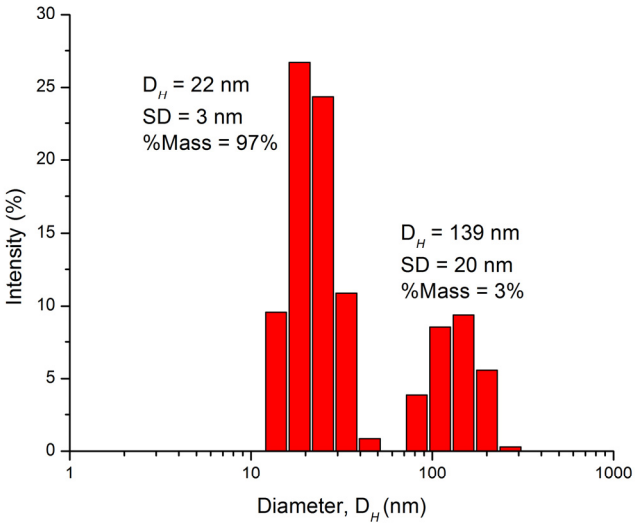


Figure S8. DLS histogram of **TEL-2-BASP** (m = 7, n = 7).

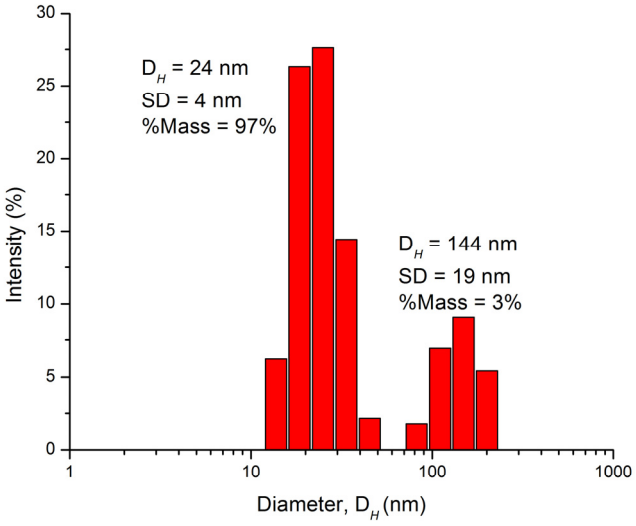


Figure S9. DLS histogram of **TEL-2-BASP** (m = 7, n = 14).

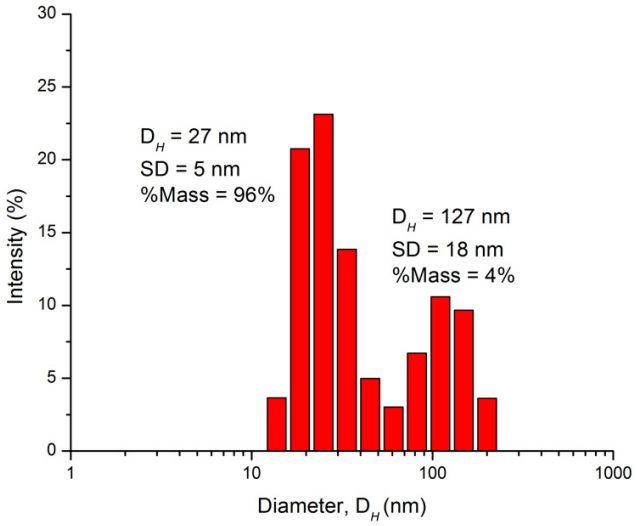


Figure S10. DLS histogram of **TEL-2-BASP** (m = 7, n = 21).

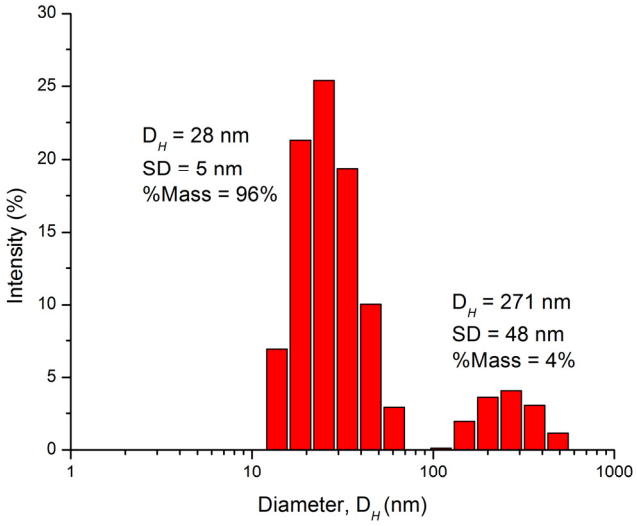


Figure S11. DLS histogram of **TEL-2-BASP** (m = 10, n = 20).

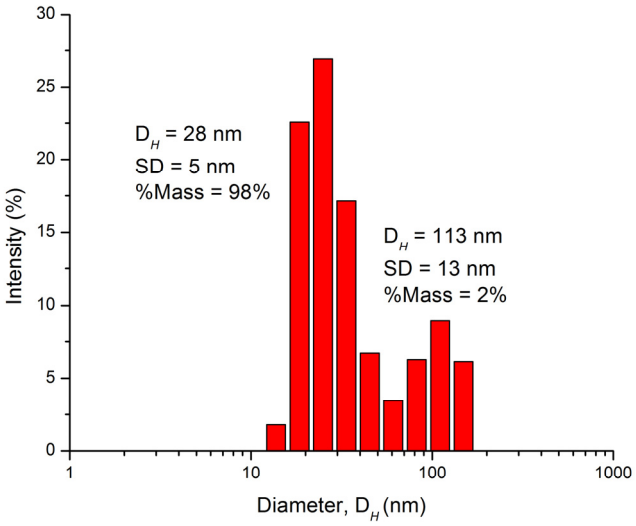


Figure S12. DLS histogram of **TEL-2-BASP** (m = 10, n = 30).

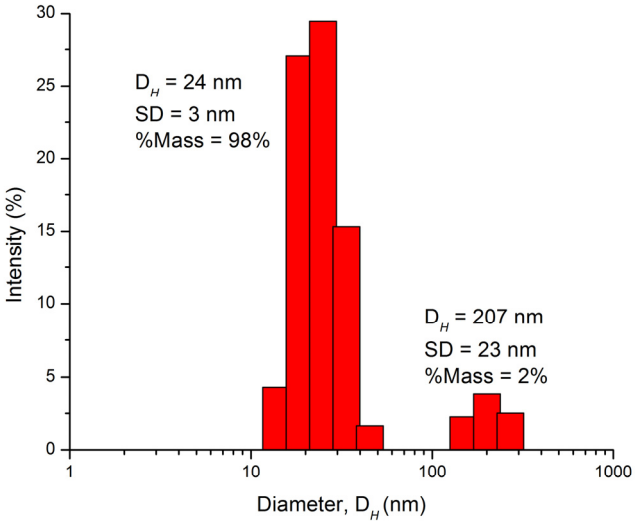


Figure S13. DLS histogram of **TEL-2-BASP** (m = 13, n = 13).

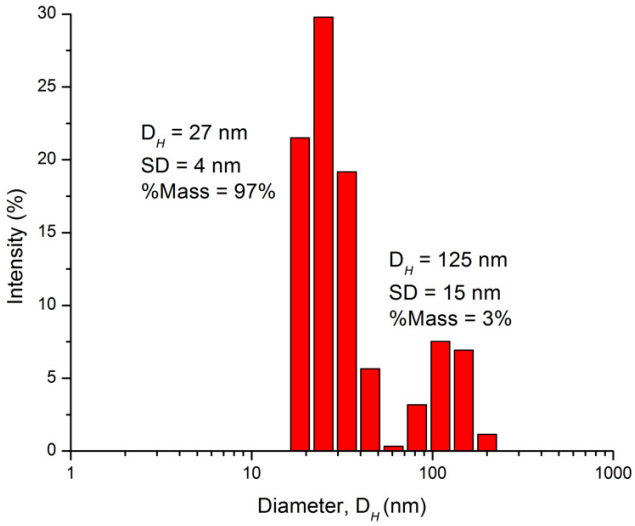


Figure S14. DLS histogram of **TEL-2-BASP** (m = 13, n = 24).

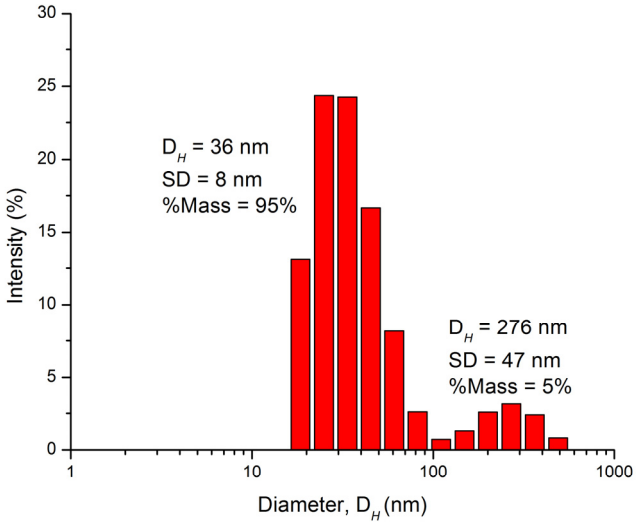


Figure S15. DLS histogram of **TEL-2-BASP** (m = 13, n = 30).

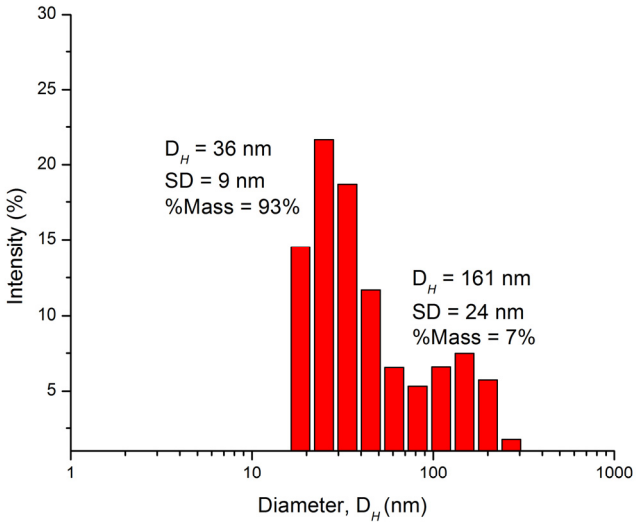


Figure S16. DLS histogram of **TEL-2-BASP** (m = 13, n = 40).

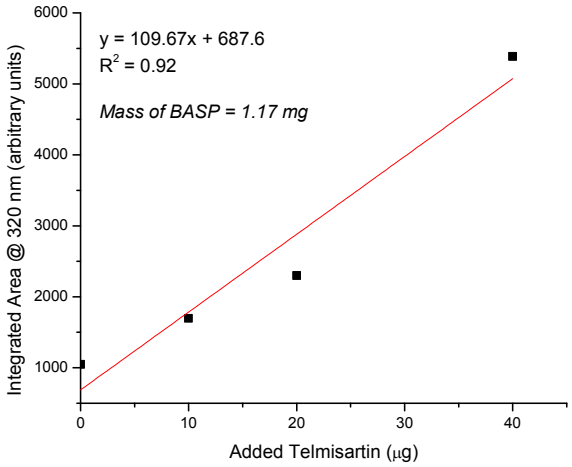
Section H. Drug Loading Assay

Standard Addition Curve Setup and Analysis

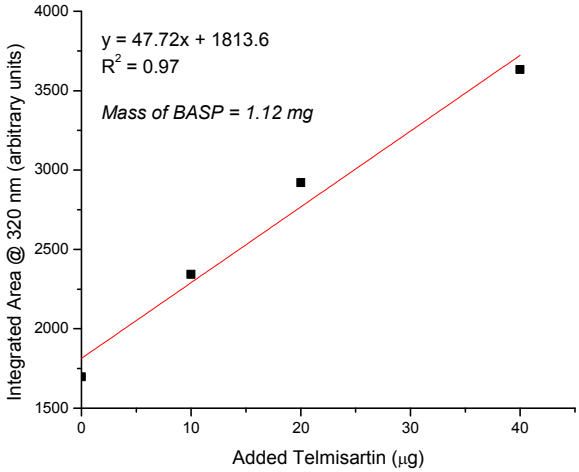
1. A certain mass of BASP (recorded, ca. 1 - 2 mg) was added to a 4 mL vial with 250 µL of 1 M LiOH (aq). The sample was mixed well with vortexing.
2. The vial was sealed and stored in the dark for 24 h under ambient conditions to allow full base-mediated hydrolysis of the telmisartan-bound ester.
3. For each sample (run in triplicate), four solutions were made for a standard addition curve as follows (a 1 mg/mL stock solution of **TEL** in THF was used for **TEL-Stock**:
    a. 40 µL **TEL-Stock**, 205 µL THF, 40 µL sample, 5 µL TFA
    b. 20 µL **TEL-Stock**, 225 µL THF, 40 µL sample, 5 µL TFA
    c. 10 µL **TEL-Stock**, 235 µL THF, 40 µL sample, 5 µL TFA
    d. 0 µL **TEL-Stock**, 245 µL THF, 40 µL sample, 5 µL TFA
4. Note that for each trial (a – d), 16% of the degradation sample was used (40 µL / 250 µL = 16%).
5. Each solution was analyzed by LC (10 – 95% MeCN/H_2O [0 – 7 min], 95% - 100% MeCN/H_2O [7 – 8 min], 320 nm, r.t. = 5.5 min, PES column).
6. A plot was constructed of µg of telmisartan "spiked" into the sample versus integration. A line of best fit was determined with a linear regression analysis. The x-intercept was recorded.
7. The negative x-intercept was divided by the percentage of BASP sample used for analysis (in this case, 16%).
8. The value from ***7*** was divided by the weight of BASP sample to obtain the final drug loading percentage.

Example (from 2 g BASP synthesis)

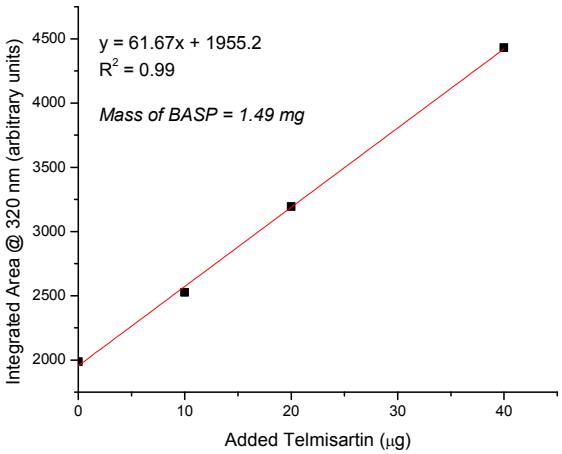
1. Determine negative X-intercept
2. Divide by percentage of BASP sample used for analysis (in this case, 16%)
3. Divide by weight of BASP sample to obtain drug loading percentage
4. Mean drug loading = **10.4 (± 1.1)%**



18.6	X-Intercept
116	Divide by 0.16
0.0994	Weight of Drug in BASP
9.94%	%Weight of Drug in BASP



20.9	X-Intercept
130	Divide by 0.16
0.116	Weight of Drug in BASP
11.7%	%Weight of Drug in BASP



23.0	X-Intercept
144	Divide by 0.16
0.0965	Weight of Drug in BASP
9.65%	%Weight of Drug in BASP

Section I. *In Vivo* Methods

1. Biodistribution (BD) and Pharmacokinetics (PK) Analysis

The lyophilized BASP product was reconstituted in PBS (40 mg/mL), sterile filtered, and administered intravenously (i.v., 200 mg/kg) to BALB/c mice (*n* = 15). All animal procedures were performed in accordance with Charles River Laboratories Animal Welfare and Humane Treatment of Animals Policy and were approved by Charles River Laboratories IACUC. Blood and liver tissue samples were collected for longitudinal LC-MS/MS (see Section **2** below) measurements of both free ("unconjugated") and polymer conjugated **TEL**. The presence of polymer could be quantified in liver homogenate by Cy7.5 imaging after resolution on an SDS-PAGE gel following a higher dose of **TEL-2-BASP** (300 mg/kg) (Fig. 4d and Fig. S18). Similarly, PEG clearance was measured by anti-PEG ELISA (Fig. 4e and Fig. S17). For the ELISA assay, liver tissues were homogenized and lysates prepared in T-PER reagent (Tissue protein extraction reagent, ThermoFisher Scientific) and normalized for protein concentration. Anti-PEG ELISA was performed on the liver homogenates following standard procedures (PEGylated protein ELISA kit, ab133065, Abcam). Briefly, test samples were added to 96-well plate coated with monoclonal antibody specific to PEGylated protein. This is followed by addition of biotinylated PEG protein and incubation at room temperature for 30 min. The wells are washed to remove excess unbound protein and a solution of streptavidin-HRP conjugate is then added. Following incubation, excess reagents are washed and TMB substrate is added and further incubated at room temperature. Stop solution is then added and O.D. absorbance was measured at 450 nm using a SpectraMax plate reader.

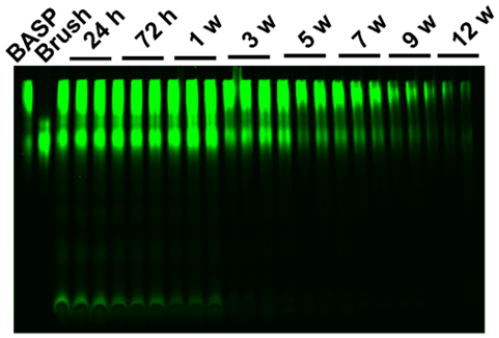


Figure S17. SDS-PAGE gel of liver homogenates following **TEL-2-BASP** dosing at different time points (24 h – 12 w) in mice (single dose of 300 mg/kg BASP, *n* = 3 animals per time point). See Fig. 4d in the main text for Cy7.5 quantification values.

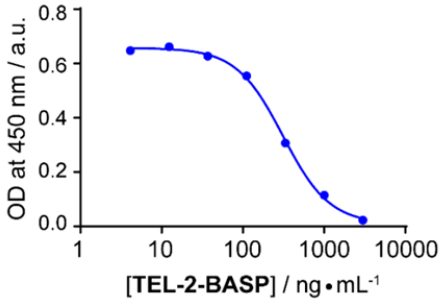


Figure S18. Calibration curve for anti-PEG ELISA using **TEL-2-BASP** as the standard. See Fig. 4e in the main text for ELISA data.

2. Quantification of TEL in Plasma and Tissue Samples by UPLC/MS/MS

Blood samples were divided into two aliquots to measure the concentration of both unconjugated **TEL** (Aliquot 1) and total **TEL** (Aliquot 2) measured hydrolysis (*vide infra*) to release all **TEL** from the drug polymer conjugate. The amount of conjugated **TEL** was then estimated using the formula: Conjugated **TEL** = Total **TEL** (measured after sample hydrolysis, Aliquot 2) – unconjugated **TEL** (measured without sample hydrolysis, Aliquot 1).

UPLC/MS/MS Analysis Methods and Conditions: Quantitation of **TEL** was carried out on a Waters Acquity UPLC system coupled with a Waters Xevo TQ-S tandem quadrupole mass spectrometer. A Waters CORTECS UPLC, C18, 1.6 µm, 2.1 X 50 mm analytical column (column temperature = 50 °C) by a reverse phase gradient elution method with *mobile phase A* as 0.1% formic acid/water and *mobile phase B* as 0.1% formic acid/acetonitrile at a flow rate of 0.6 mL/min. The MS/MS analysis was carried out in electrospray positive ion mode at a capillary voltage of 0.75 kV, desolvation temperature of 500 °C with a desolvation flow rate of 900 L/h, and cone gas flow rate of 150 L/h. The MRM transitions monitored for released **TEL** were 515.2 → 76.2 (used for quantitation) and 515.3 → 305.2 (used for confirmation). The MRM transition for the stable isotope labeled internal standard ($^{13}CD_3$-**TEL**) is 515.3 → 280.2. Calibration standards for the UPLC/MS/MS analysis were prepared by spiking an analyte working solution in the respective biological matrix. These standards were extracted using the same protocol as for the tissue preparations described below. The standards were subjected to UPLC/MS/MS analysis and a calibration curve was constructed to quantify **TEL** levels in unknown samples.

Whole Blood Sample Preparation Procedure for Measuring Unconjugated **TEL** (i.e. No Hydrolysis, Aliquot 1): 27 µL of whole blood sample was mixed with 3 µL of 0.5 µg/mL stable isotope labeled internal standard ($^{13}CD_3$-**TEL**) and 120 µL of ice cold acetonitrile. Vials were vortexed, spun down at 16000 rpm, and stored at 4 °C for 15 minutes in an Eppendorf 5417R centrifuge. The supernatant was removed and subjected to the UPLC/MS/MS analysis for the quantitation of **TEL**.

Whole Blood Sample Preparation for Total Conjugated **TEL** (i.e. After Hydrolysis – Aliquot 2): 27 µL of blood was mixed with 3 µL of 100 µg/mL stable isotope labeled internal standard ($^{13}CD_3$-**TEL**) and 270 µL of 4M KOH (MeOH). Vials were vortexed, spun down at 16000 rpm, and stored at 4 °C for 35 minutes in an Eppendorf 5417R centrifuge. The supernatant was removed and stored at room temperature for 21 h, whereupon a 10 µL aliquot was and diluted 90x with MeOH. The final solution was vortexed, syringe filtered into a UPLC vial and subjected to UPLC/MS/MS analysis for the quantitation of **TEL**.

Tissue Homogenization: For homogenization, about 50 – 100 mg of sample tissue was diluted 5x with DPBS buffer (Dulbecco's Phosphate Buffered Saline, 1x). Two 3.5 mm UFO stainless steel beads were added, and the sample was homogenized twice for two minutes at "Speed 16" in the Next Advance Bullet Blender Gold. The homogenate was left for 10 minutes to settle and then two 90 µL aliquots were transferred to Eppendorf vials for unconjugated **TEL** (no hydrolysis) and conjugated **TEL** (after hydrolysis) sample preparation.

Tissue Sample Preparation Procedure for Released **TEL** (No Hydrolysis) Analysis: To one of the 90 µL homogenate aliquots, 5 µL of DMSO and 5 µL of 3 µg/mL stable isotope labeled internal standard ($^{13}CD_3$-**TEL**) were added and vortexed. 200 µL of ice cold acetonitrile was added to the sample. Samples were vortexed, spun down at 16000 rpm, and stored at 4 °C for 15 minutes in an Eppendorf 5417R centrifuge. The supernatant was removed, syringe filtered, and subjected to UPLC/MS/MS analysis for **TEL** quantification. The tissue sample preparation involves a dilution factor

of 16.7. So for the unknown samples, the detected **TEL** concentration at the instrument should be multiplied by the dilution factor in order to determine the **TEL** levels present in the original tissue.

Tissue Sample Preparation Procedure for Total Released **TEL** (After Hydrolysis) Analysis: To one of the 90 µL of tissue homogenate, 5 µL of DMSO and 5 µL of 60 µg/mL stable isotope labeled internal standard ($^{13}CD_3$-**TEL**) were added and vortexed. 200 µL of 4M KOH (MeOH) was added to the sample. Samples were vortexed, spun down at 16000 rpm, and stored at 4 °C for 35 minutes in an Eppendorf 5417R centrifuge. The supernatant was removed and incubated for 21 hours. A 50 µL aliquot was removed from the sample and diluted 10x with methanol. This solution was vortexed, syringe filtered into a UPLC vial, and subjected to the UPLC/MS/MS analysis for **TEL** quantification. The tissue sample preparation involves a dilution factor of 16.7. So for the unknown samples, the detected **TEL** concentration at the instrument should be multiplied by the dilution factor in order to determine the **TEL** levels present in the original tissue.

3. **Ex vivo Biodistribution Imaging**
   Formalin fixed tissues were placed directly on an Odessy Licor imager and scanned at 700 nm and 800 nm to quantitatively detect the Cy7.5-conjugated **TEL-2-BASP**.

4. **Blood Pressure Measurement: CODA Non-Invasive Blood Pressure Monitoring System**
   Blood pressure measurements were recorded using tail cuffs on a CODA Non-Invasive Blood Pressure Monitoring System (Kent Scientific) according to manufactures instructions. In brief, four mice were loaded into their individual constraints and placed on the warming table covered with a warming blanket. Cuffs were placed appropriately and when the tail temperature of each animal reached at least 32 ^{o}C the systolic and diastolic pressures were recorded based on a total of 20 total cycles in each 20 minute session. Once the session has completed, the mice are returned to their cages and the data exported to Excel for analysis.

5. **Histology, Immunohistochemistry, and Biomarker Quantification**
   Five-micron thick paraffin sections cut from each of the blocks were deparaffinized in xylene and hydrated in a series of ethanol. Standard procedures were followed for hematoxylin and eosin (H&E) staining. For immunohistochemistry, after heat-induced antigen retrieval in TRIS-EDTA buffer (pH 9), the samples were blocked with 5% goat serum/PBS and stained with antibodies for cleaved caspase-3 (Cell Signaling Cat#: 9661) or SSEA1 (Biolegend Cat#: 12560). Procedures for secondary antibody were performed by standard immunohistochemistry protocols. To quantitate positive staining areas of cleaved caspase-3 or SSEA1, an algorithm learning tool was performed utilizing the inForm Cell Analysis^{TM} software (PerkinElmer) package to train for selecting only the appropriate tissue regions and subsequently completed segmentation of stained areas.

6. **Rat Safety Toxicology**
   Study Design: To evaluate the potential toxicity and toxicokinetic profile of **TEL-2-BASP** when administered as a single intravenous (bolus) injection to animals, as well as to evaluate the recovery, persistence or progression of any potential lesions following a 7-day recovery period, a non-GLP safety/toxicology study was conducted at Charles River Laboratories in Sprague Dawley rats. All animal procedures were performed in accordance with Charles River Laboratories Animal Welfare and Humane Treatment of Animals Policy and were approved by Charles River Laboratories IACUC. **TEL-2-BASP** in the vehicle (0.9% Sodium chloride for injection, USP [sterile saline]) was administered once by intravenous (slow push 1 – 2 minutes) injection to 3 groups (Groups 2, 3, and 4) of Crl:CD(SD) rats. Dose levels were 50, 150, and 500 mg/kg for Groups 2, 3, and 4, respectively. A concurrent control group (Group 1) received the vehicle on a comparable regimen. The dose

volume was 5 mL/kg for all groups. Groups 1–4 each consisted of 6 animals/sex. Following a single dose administration and a 7-day non-dosing (recovery) period, all animals were euthanized.

For toxicology assessment, all animals were observed twice daily for mortality and moribundity. Clinical examinations were performed daily at the time of dosing, 1 to 2 hours post-dosing, and once daily on non-dosing days. Detailed physical examinations were performed within 4 days of receipt, on the day of randomization, Study Days 0, 1, 4, and 6, and on the day of the scheduled necropsy. Individual body weights were recorded within 4 days of receipt, on the day of randomization, on Study Days 0, 1, 4, and 6, and on the day of the scheduled necropsy. Cage food weights were recorded once weekly (± 2 days) beginning following randomization. Clinical pathology parameters (hematology and serum chemistry) were analyzed for all animals on Study Day 2 (approximately 48 hours post-dose), and for all animals assigned to the scheduled necropsy (Study Day 7). Blood samples (whole and plasma) were collected from 3 animals/sex/group/time point at approximately 5 minutes, and 0.5, 2, 24, 72, and 168 hours after dose administration on Study Day 0. In addition, at necropsy blood samples (whole and plasma) were collected from all animals following euthanasia. Complete necropsies were conducted on all animals, and selected organs were weighed at the scheduled necropsy. Selected tissues (liver, kidney, spleen, brain, choroid plexus, lung and heart) were examined microscopically from all animals in the control and 500 mg/kg dose group.

Results: In preparation for IND-enabling studies, non-GLP dose-escalation and safety toxicology studies have been conducted in rat (Sprague Dawley) at Charles River Laboratories. Following the M3(R2) regulatory guidance, animals were dosed up to the maximum feasible dose (MFD) for an i.v. slow-push bolus injection (5 mL/kg). Findings from these studies show no test article-related clinical observations or effects on body weight, food consumption, hematology, or organ weights in any of the dose groups. At necropsy (Day 7 post dosing), there were no test article-related macroscopic or microscopic findings and there were no statistically significant differences in serum chemistry parameters between the control group and the test article-treated groups. Likewise, the differential blood cell counts were normal showing no evidence of immune activation. In addition, 12 week safety/toxicology and PK studies with repeat dosing in mice (100 mg/kg; once weekly, three repeats) also confirm the lack of any test article-related microscopic lesions or changes. This includes lack of any PEG-induced cellular vacuolization that has been reported for 6 of the 12 FDA approved PEGylated drug products. Hence, it can be concluded that the BASP formulation is very well tolerated in proposed toxicology species at all dosages up to the maximum feasible dose. See Tables S1 and S2 below for a summary of blood biochemistry values.

Male	2 Days After Dosing				7 Days After Dosing			
Dose Level	**ALP (U/L)**	**ALT (U/L)**	**AST (U/L)**	**GGT (U/L)**	**ALP (U/L)**	**ALT (U/L)**	**AST (U/L)**	**GGT (U/L)**
0 mg/kg	277 (20)	49 (3)	130 (7)	0 (0)	268 (27)	52 (4)	119 (9)	0 (0)
50 mg/kg	235 (15)	51 (3)	102 (7)	0 (0)	232 (15)	47 (3)	115 (10)	0 (0)
150 mg/kg	285 (18)	46 (3)	112 (5)	0 (0)	276 (23)	44 (2)	108 (12)	0 (0)
500 mg/kg	222 (17)	46 (4)	86 (11)	0 (0)	231 (16)	48 (3)	116 (12)	0 (0)

Table S1. Serum biochemistry measurements for **TEL-2-BASP** toxicity study in male Sprague Dawley rats. Injections were made at 0 mg/kg (control), 50 mg/kg, 150 mg/kg, or 500 mg/kg (MFD) to groups of animals (*n* = 6 animals per group). Enzyme levels were collected 2 and 7 days after dosing. The following parameters are reported: alkaline phosphatase (ALP), alanine transaminase (ALT), aspartate transaminase (AST), and gamma-glutamyl transferase (GGT). All enzyme activity data is expressed in international units per liter (U/L). Data is reported as mean ± SEM.

Female	2 Days After Dosing				7 Days After Dosing			
Dose Level	**ALP (U/L)**	**ALT (U/L)**	**AST (U/L)**	**GGT (U/L)**	**ALP (U/L)**	**ALT (U/L)**	**AST (U/L)**	**GGT (U/L)**
0 mg/kg	153 (12)	38 (2)	98 (4)	0 (0)	133 (12)	38 (2)	127 (15)	0 (0)
50 mg/kg	167 (24)	40 (2)	96 (12)	0 (0)	155 (24)	32 (3)	93 (5)	0 (0)
150 mg/kg	156 (16)	34 (2)	98 (3)	0 (0)	131 (14)	35 (3)	89 (6)	0 (0)
500 mg/kg	148 (11)	33 (2)	87 (7)	0 (0)	139 (9)	30 (2)	92 (6)	0 (0)

Table S2. Serum biochemistry measurements for **TEL-2-BASP** toxicity study in female Sprague Dawley rats. Injections were made at 0 mg/kg (control), 50 mg/kg, 150 mg/kg, or 500 mg/kg (MFD) to groups of animals (*n* = 6 animals per group). Enzyme levels were collected 2 and 7 days after dosing. The following parameters are reported: alkaline phosphatase (ALP), alanine transaminase (ALT), aspartate transaminase (AST), and gamma-glutamyl transferase (GGT). All enzyme activity data is expressed in international units per liter (U/L). Data is reported as mean ± SEM.

7. Dog Safety Toxicology

Study Design: To evaluate the potential tolerability, toxicity and toxicokinetic profile of **TEL-2-BASP** when administered as an escalating single dose intravenous (bolus) injection, as well as the effects following repeat dosing (2 doses, approximately 1 week apart), a non-GLP safety/toxicology study was conducted at Charles River Laboratories in Beagle dogs. All animal procedures were performed in accordance with Charles River Laboratories Animal Welfare and Humane Treatment of Animals Policy and were approved by Charles River Laboratories IACUC. This species and breed of animal is recognized to be appropriate for repeat-dose toxicity studies. The Beagle dog was chosen because it is a widely used species and breed for which significant historical control data are available. The number of animals used on study is the minimum number required to yield scientifically meaningful data to accomplish the goals of this toxicity study and is consistent with our expectations. A single male and female animal was used for dose escalation with 2 animals per sex used for the confirmation (repeat) dosing. **TEL-2-BASP** in the vehicle (0.9% Sodium chloride for injection, USP [sterile saline]) was administered as a single escalating intravenous (slow push 1 – 2 minutes) injection to a single male and female animal. Dose levels were 36, 100, and 500 mg/kg. Dose escalation occurred after approximately seven days following each dose. An additional two groups of animals (one group with 2 males and one group with 2 females) received the MFD (500 mg/kg) followed by an additional dosing at 500 mg/kg 1 week later. The dose volume was 5 mL/kg for all groups. The three dosing levels of 36 mg/kg, 100 mg/kg, and 500 mg/kg correspond to levels approximately 3x, 10x and 40x above the human equivalent dose of generic telmisartan. Following a 7-day non-dosing (recovery) period after the last injection, all animals were euthanized.

All animals were observed twice daily for mortality and moribundity. Clinical examinations were performed 7 and 5 days before dosing, immediately following dosing (within 5 minutes), approximately 1 hour postdose, and once daily on non-dosing days. On non-dosing days, the animals were observed once daily. The absence or presence of findings was recorded for individual animals at the scheduled intervals. Detailed physical examinations were performed on the day of transfer to the study, weekly (± 2 days) during the acclimation period, on the day of randomization, on each day of dosing, and on the days of the scheduled necropsies. Individual body weights were recorded within on the day of transfer to the study, weekly (± 2 days) during the acclimation period, on the day of randomization, on Study Days 0 (prior to dosing), 1, 2, and 3 for each dose, on Study Days 7 (prior to second dose), 8, 9, and 10 (repeat dose animals only), on the day prior to the scheduled necropsies (nonfasted). Final body weights (fasted) were recorded on the day of the scheduled necropsies. Individual food weights were recorded daily during acclimation, beginning at least 6 days prior to

randomization, and daily throughout the study period until the day prior to the scheduled necropsies. Clinical pathology parameters (hematology and serum chemistry) were analyzed for all animals during acclimation (Study Day -7) and approximately 24 hours following each dose. The animals were fasted overnight prior to blood collection. Blood samples (whole and plasma) were collected from all animals/sex/group/time prior to dose administration, and at approximately 5 and 30 minutes and 2, 6, 24, and 72 hours after dose administration on Study Days 0 and 7, and prior to the schedule necropsies. Heart rates, arterial blood pressures, pulse temperatures, and body temperatures were collected continuously for at least 1 hour in the animals' home cage and for at least 15 minutes in the sling restraint prior to injection, and continuously for approximately 1 hour after each dose while the dogs were restrained. After 1 hour post-dose, the dogs were returned to their home cage and data were collected for a minimum of 20 hours post-dose. Complete necropsies were conducted on all animals, and selected organs were weighed at the scheduled necropsy.

Results: In preparation for IND-enabling studies, non-GLP dose-escalation and safety toxicology studies have been conducted in Beagle dogs at Charles River Laboratories. Following the M3(R2) regulatory guidance, animals were dosed up to the maximum feasible dose (MFD) for an i.v. slow-push bolus injection (5 mL/kg). Based on the results of this study, intravenous (bolus) administration of **TEL-2-BASP** to Beagle dogs at dose levels of 36, 100, and 500 mg/kg was well tolerated at all doses. Findings from these studies show no test article-related clinical observations or effects on body weight, food consumption, hematology, or organ weights in any of the dose groups. At necropsy (Day 7 post dosing), there were no test article-related macroscopic or microscopic findings and there were no statistically significant differences in serum chemistry parameters between the control group and the test article-treated groups. In the 500 mg/kg repeat dose animals, observations of salivation, pale and reddened gums, reddened ears, reddened and swollen facial area, and a slightly elevated heart rate were noted. Observations were generally noted shortly after completion of the injection with findings generally resolved by the 1 hour post-dose observation. Heart rates remained slightly elevated for ca. 18 hours post-dose. See Tables S3 and S4 for a summary of blood biochemistry values.

Male		1 Week Before Injection				1 Day After Dosing				1 Day After Second Dosing (if applicable)			
Dose Level	Dose Type	ALP (U/L)	ALT (U/L)	AST (U/L)	GGT (U/L)	ALP (U/L)	ALT (U/L)	AST (U/L)	GGT (U/L)	ALP (U/L)	ALT (U/L)	AST (U/L)	GGT (U/L)
36 mg/kg	Escalation					44	49	31	0				
100 mg/kg	Escalation					47	41	40	0				
500 mg/kg	Escalation					39	43	44	0				
500 mg/kg	Single Injection	77	31	34	0	60	33	26	0.5	52	32	26	0

Table S3. Serum biochemistry measurements for **TEL-2-BASP** toxicity study in male Beagle dogs with either dose escalation or repeat single injection dosing regimens. For the former, a single animal was used for the 36 mg/kg, 100 mg/kg, and 500 mg/kg injections; there was a one week period between injections. For the repeat dosing study, two animals were used and the mean is reported. The following parameters are reported: alkaline phosphatase (ALP), alanine transaminase (ALT), aspartate transaminase (AST), and gamma-glutamyl transferase (GGT). All enzyme activity data is expressed in international units per liter (U/L).

Female		1 Week Before Injection				1 Day After Dosing				1 Day After Second Dosing (if applicable)			
Dose Level	**Dose Type**	**ALP (U/L)**	**ALT (U/L)**	**AST (U/L)**	**GGT (U/L)**	**ALP (U/L)**	**ALT (U/L)**	**AST (U/L)**	**GGT (U/L)**	**ALP (U/L)**	**ALT (U/L)**	**AST (U/L)**	**GGT (U/L)**
36 mg/kg	Escalation					34	24	19	0				
100 mg/kg	Escalation					36	25	21	0				
500 mg/kg	Escalation					33	23	21	0				
500 mg/kg	Single Injection	44	33	32	0	42	33	27	0	34	30	24	0

Table S4. Serum biochemistry measurements for **TEL-2-BASP** toxicity study in female Beagle dogs with either dose escalation or repeat single injection dosing regimens. For the former, a single animal was used for the 36 mg/kg, 100 mg/kg, and 500 mg/kg injections; there was a one week period between injections. For the repeat dosing study, two animals were used and the mean is reported. The following parameters are reported: alkaline phosphatase (ALP), alanine transaminase (ALT), aspartate transaminase (AST), and gamma-glutamyl transferase (GGT). All enzyme activity data is expressed in international units per liter (U/L).

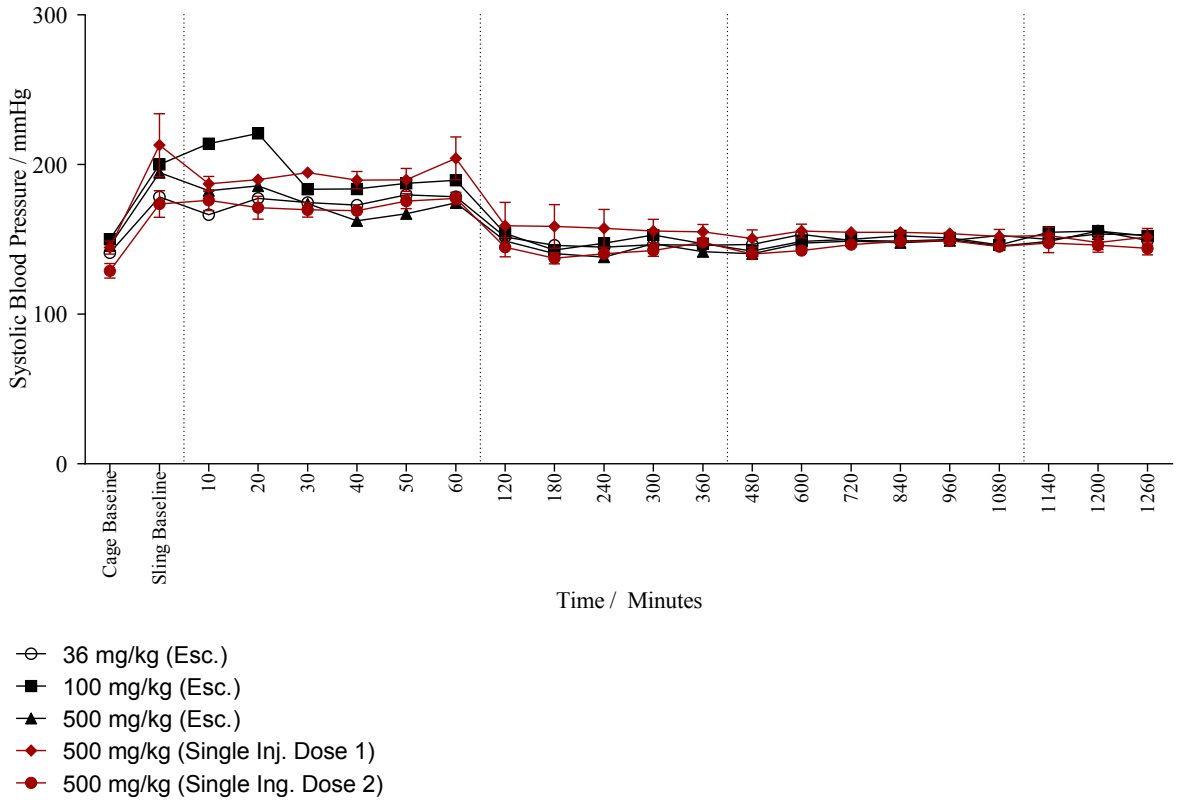


Figure S19. Average systolic blood pressure measurements over time in male and female Beagle dogs upon dosing **TEL-2-BASP**. For dose escalation study, *n* = 2 dogs; for repeat dosing, *n* = 4 dogs. Data for the repeat dosing study is reported as mean ± SEM.

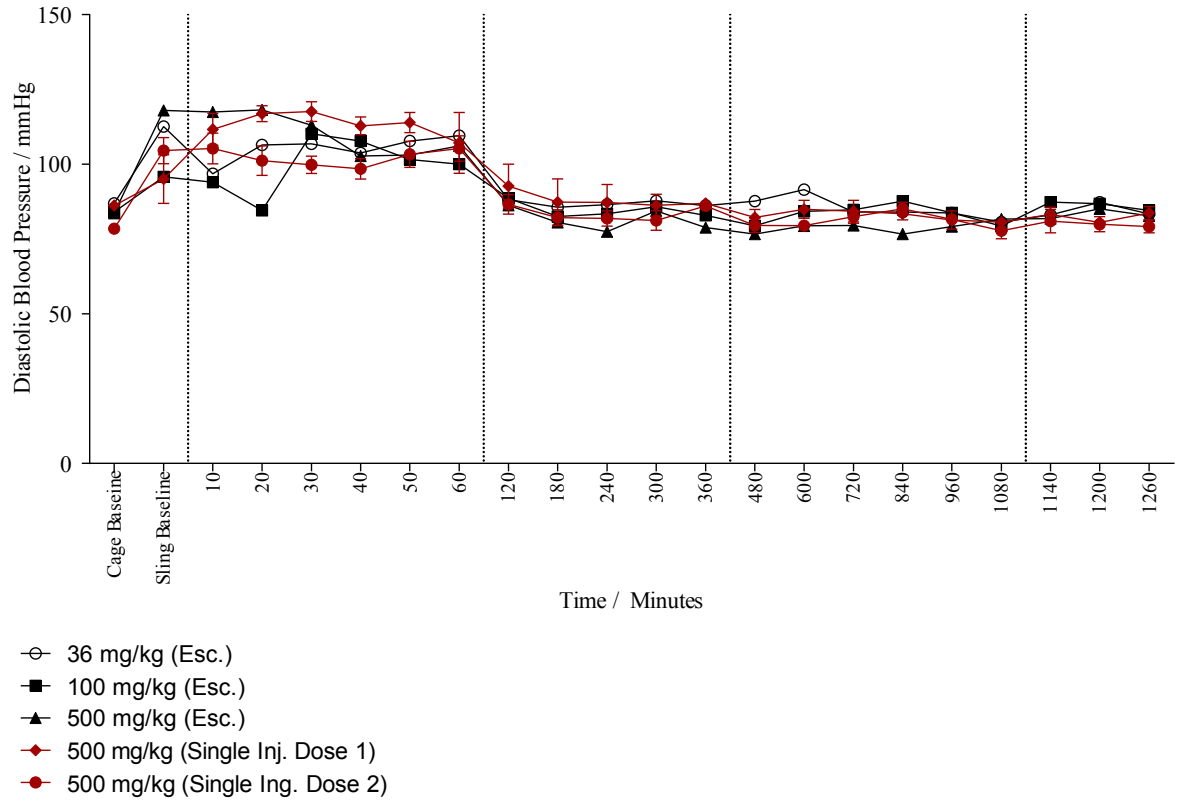


Figure S20. Average diastolic blood pressure measurements over time in male and female Beagle dogs upon dosing **TEL-2-BASP**. For dose escalation study, *n* = 2 dogs; for repeat dosing, *n* = 4 dogs. Data for the repeat dosing study is reported as mean ± SEM.

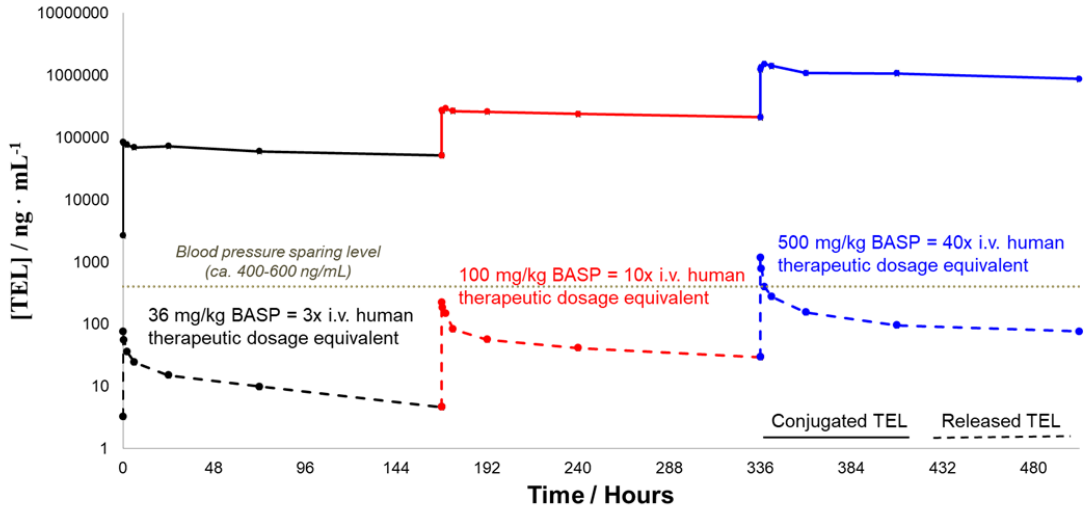


Figure S21. Mean released and conjugated TEL pharmacokinetic data from ascending dose escalation study in dogs (*n* = 1 animal per sex per dosing group).

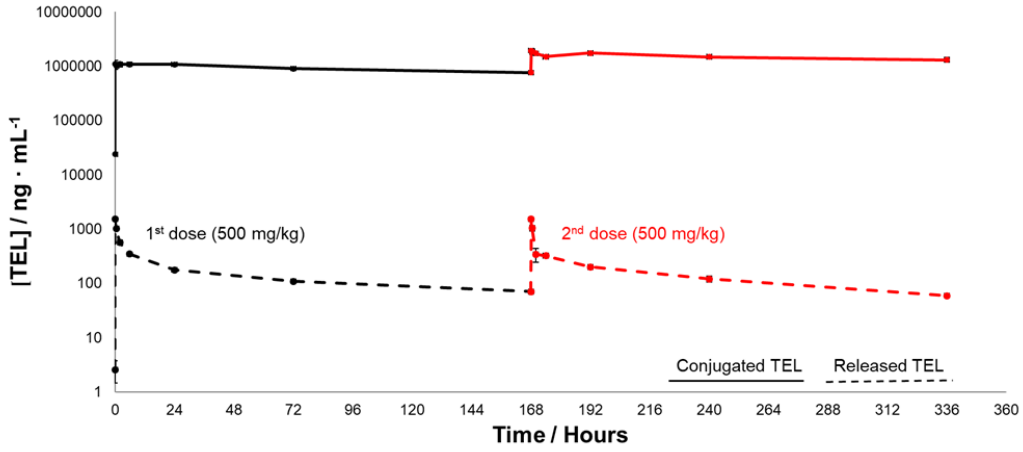


Figure S22. Released and conjugated TEL pharmacokinetic data from maximum feasible dose confirmation study in dogs (*n* = 2 animal per sex). 500 mg/kg (i.v.) of **TEL-2-BASP** was administered for both injections; this dose corresponds to 40x the human therapeutic dosage equivalent for generic TEL. Data is reported as mean ± SEM.

8. STAM Mouse Model

The STAM model of non-alcoholic steatohepatitis in rodents is metabolically-induced such that the disease phenotype is driven by diabetes and a high fat diet.

Animals: Female mice (C57BL/6, 14-day-pregnant) were ordered from Japan SLC, Inc. and were hosted at Stelic and Co., Inc. (Japan). The animals were housed in TPX cages (CLEA Japan) with a maximum of 4 mice per cage. Sterilized Paper-Clean (Japan SLC) was used for bedding and replaced once per week. All procedures were performed in accordance with the Japanese Pharmacological Society Guidelines for Animal Use and were approved by Stelic IACUC. Animals had access to food *ad libitum*. Pure water was provided *ad libitum* from a water bottle equipped with a robber stopper and a sipper tube. Water bottles were changed once per week, cleaned, sterilized in an autoclave and reused. The animals were housed in a SPF facility under controlled conditions of temperature (23 ± 2 ^{o}C), humidity (45 ± 10%), lighting (12-hour artificial light and dark cycles, light from 08:00 to 20:00) and air exchange. A high pressure was maintained in the experiment room to prevent contamination of the facility.

NASH induction and allocation of treatment groups: Control male mice (*n* = 7) were fed with a normal diet without any treatment until 9 weeks of age. “Vehicle-only” male mice (*n* = 7) were intravenously administered vehicle (saline) in a volume of 5 mL/kg at the beginning of week 6 and at the beginning of week 7 (total of two doses). For the study group, NASH was induced in male mice by a single subcutaneous injection of 200 µg streptozotocin (STZ, Sigma-Aldrich, USA) solution 2 days after birth and feeding with a high fat diet (HFD, 57 kcal% fat, Cat# HFD32, CLEA Japan Inc., Japan) after 4 weeks of age.

Dosing: Groups receiving treatment got two doses of **TEL-2-BASP** (300 mg/kg reconstituted in sterile saline) were administered as a single i.v. injection (bolus) in a volume of 5 mL/kg at 6 weeks of age and 7 weeks of age. Mice were sacrificed at 9 weeks of age (*n* = 7 animals per group) and assessed

for fibrotic area by PicroSirius Red (PSR) staining of harvested liver tissue and subsequent digital image quantification.

Liver tissue collection: The whole liver tissue was flushed quickly with ice-cold PBS, blotted briefly on paper towel, and weighed the liver weight. Liver tissue was dissected into pieces: the right lobe was fixed in 10% neutral formalin; the left lobe was shock frozen in liquid nitrogen for tissue PK analysis.

Tissue fixation protocol: Tissues were placed into 10% neutral buffered formalin (NBF; 20X volume) and incubated overnight (at least 12 hours) at 4 °C. The following day the tissue was moved to room temperature for 4-5 hours, before being washed twice with PBS and store at 4 °C for subsequent paraffin embedding and IHC processing.

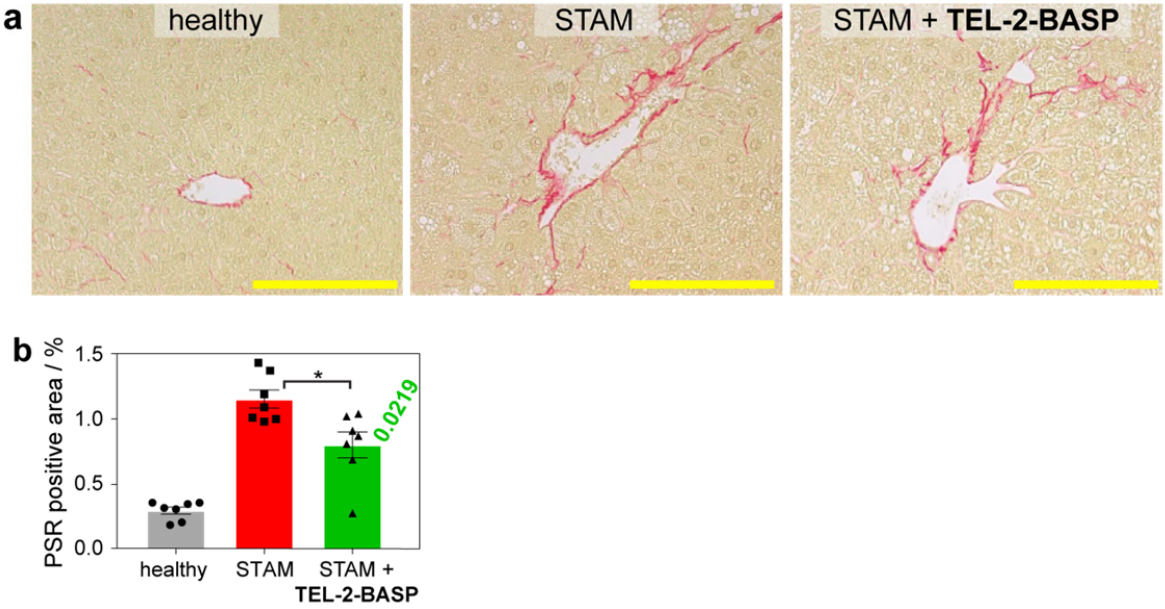


Figure S23. (**a**) PicroSirius Red (PSR) stained images of healthy, diseased, and treated livers from STAM model. Scale bar = 200 µm. (**b**) Percent PSR positive area showing efficacy of **TEL-2-BASP**. Data reported as mean ± SEM; statistical analysis performed with two-tailed Student's *t* test (* = $P < 0.05$).

9. CCl_4 Mouse Model

Carbon tetrachloride (CCl_4) induced hepatic fibrosis and cirrhosis in rodents is a widely accepted experimental model for the study of liver fibrosis and cirrhosis. In many aspects, this model mirrors the pattern of human disease progression associated with toxic damages such as viral hepatitis, alcohol abuse, metabolic diseases due to overload of iron or copper, etc. The study was to evaluate the efficacy of test compound on liver fibrosis induced by CCl_4 administration in BALB/c mice – 6 week study with dosing of experimental test agents during the last two weeks of study.

Animals: Female mice (BALB/c) were ordered from Beijing Vital River Laboratory Animal Co. Ltd and hosted at PharmaLegacy Laboratories in a separate room required due to the toxicity of CCl_4. The animals were specific pathogen free and approximately 6 - 7 weeks old upon arrival at

PharmaLegacy Laboratories vivarium where they were housed in clear polycarbonate plastic cages (260 mm x 160 mm x 127 mm); 5 animals per cage. All procedures were performed in accordance with PharmaLegacy regulations and were approved by PharmaLegacy Laboratories IACUC. A health inspection was performed on each animal to include evaluation of the coat, extremities and orifices. Each animal was also examined for any abnormal signs in posture or movement. The period of acclimatization was 7 days. Animals had *ad libitum* access to rodent food (irradiated, Shanghai SLAC Laboratory Animal Co. Ltd., China). The bedding material was autoclaved corn-cob bedding (Shanghai MaoSheng Biologic Science & Technology Development Co., Ltd., China) that was changed twice per week. The room was supplied with HEPA filtered air at the rate of 15 - 25 air changes per hour. The temperature was maintained at 20 – 26 °C (68 – 79 °F) with a relative humidity of 40 - 70%. Temperature and humidity were continuously monitored and recorded. Illumination was fluorescent light for 12-hour light (08:00 - 20:00) and 12-hour dark (Cat# MO1-F).

CCl_4 induction and allocation of treatment groups: Animals were assigned to treatment groups (n = 10 – 12 animals per group) by randomization in BioBook software to achieve similar group mean weight, which provided for control of bias. Additionally, disease animals (CCl_4 group) were assigned to the different groups according to their ALT/AST levels first and body weight second after 4 weeks of CCl_4 treatment. Animals were injected intraperitoneally with CCl_4 (i.p.) 1 mL/kg (4 mL/kg of 25% CCl_4 in olive oil), twice per week for a total period of 6 weeks with test agents administrated during the last two weeks of study. Induction of fibrosis was assessed first in both male and female BALB/c mice by PicroSirius Red staining of harvested liver tissue. The aggressive, bi-weekly, intraperitoneal CCl_4 dosing regimen led to a more consistent level of fibrosis in female mice compared to male mice. The male mice displayed higher variability and mortality rates during the 6 week continued CCl_4 induction. Hence, female mice were chosen so there would be consistency across treatment groups when comparing effect sizes.

Dosing: Telmisartan was formulated from dry powder in 0.5% hydroxyl propyl methyl cellulose (HPMC) with 0.2% Tween-80 solution and the test agent was administrated by daily oral gavage. **TEL-2-BASP** (lyophilized material) was reconstituted in PBS on the day of dosing (70 mg/mL solution), sterile filter (0.22 μM) and dosed intravenously according to body weight (i.e. 200 μL per 20 g animal; total of two doses).

Liver tissue collection: The whole liver tissue was flushed quickly with ice-cold PBS, blotted briefly on paper towel, and weighed. Liver tissue was dissected into pieces: the right lobe was fixed in 10% neutral formalin; the left lobe was shock frozen in liquid nitrogen for tissue PK analysis.

Tissue fixation protocol: Tissues were placed into 10% neutral buffered formalin (NBF; 20X volume) and incubated overnight (at least 12 hours) at 4 °C. The following day the tissue was moved to room temperature for 4-5 hours, before being washed twice with PBS and stored at 4 °C for subsequent paraffin embedding and IHC processing.

Blood biochemistry and PK: Animal blood samples (non-fasting) were collected at sacrifice for terminal PK analysis (whole blood in EDTA tubes) and plasma was processed prepared by refrigerated centrifugation within 30 min of collection (2000 x g for 10 minutes at 4 ^{o}C) and the plasma levels of alanine transferase (ALT), aspartate transaminase (AST) and total bilirubin (TB) were measured using an automatic biochemistry analyzer (HITACHI 7020).

Histology: At the conclusion of the study, liver sections from *n* = 6 random animals per group were analyzed by H&E staining and PSR staining (representative images from three animals per group are shown below in Figs. S24 – S29). Scores were generated by a blinded pathologist; these data are presented in Fig. S34 (H&E) and Fig. 6b (PSR).

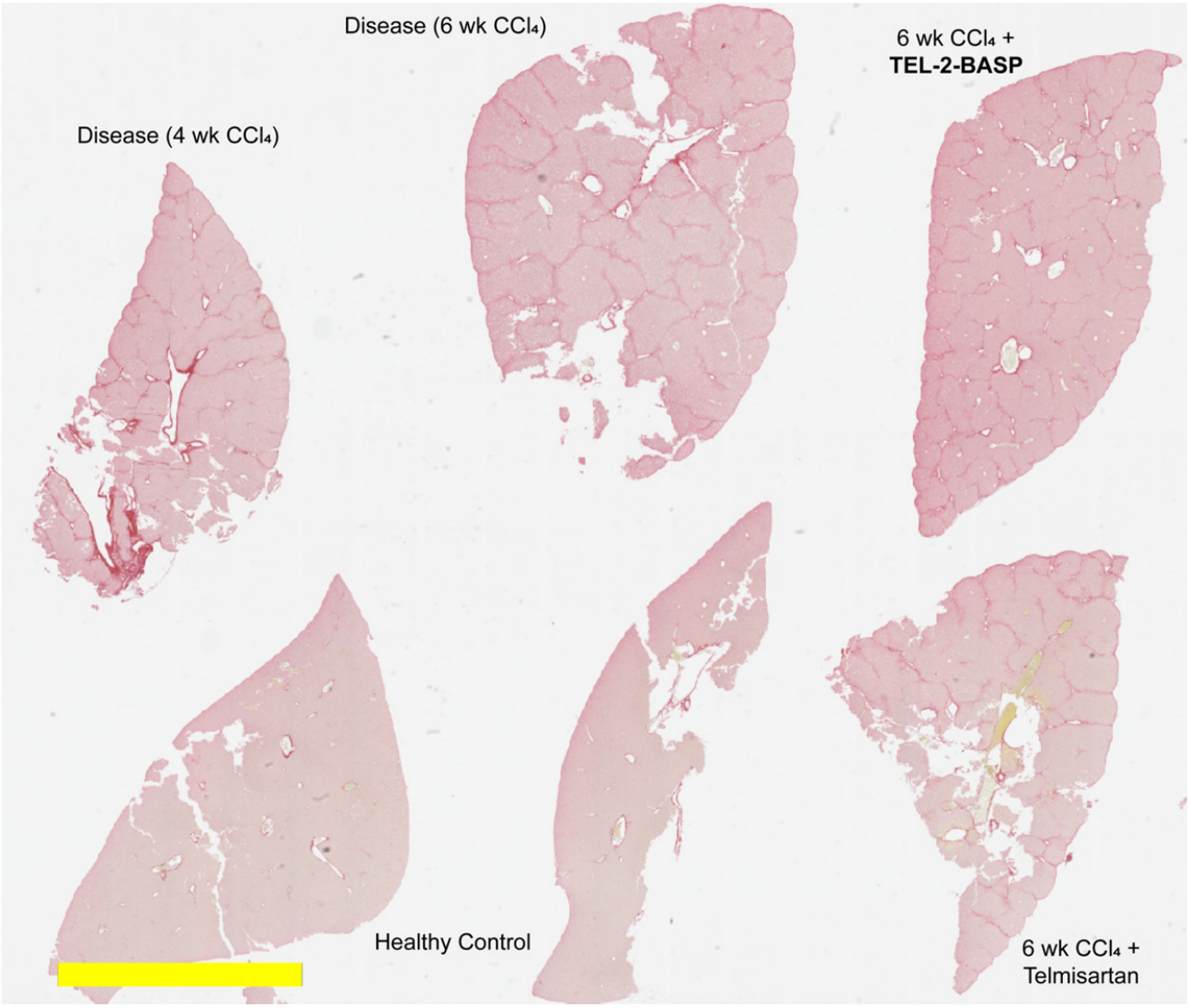


Figure S24. Histological PicroSirius Red (PSR) stained full liver images from healthy, diseased (4 or 6 weeks CCl_4), and treated (6 weeks CCl_4 + Telmisartan or **TEL-2-BASP**) mice (*Representative group 1/6*). Scale bar = 4 mm.

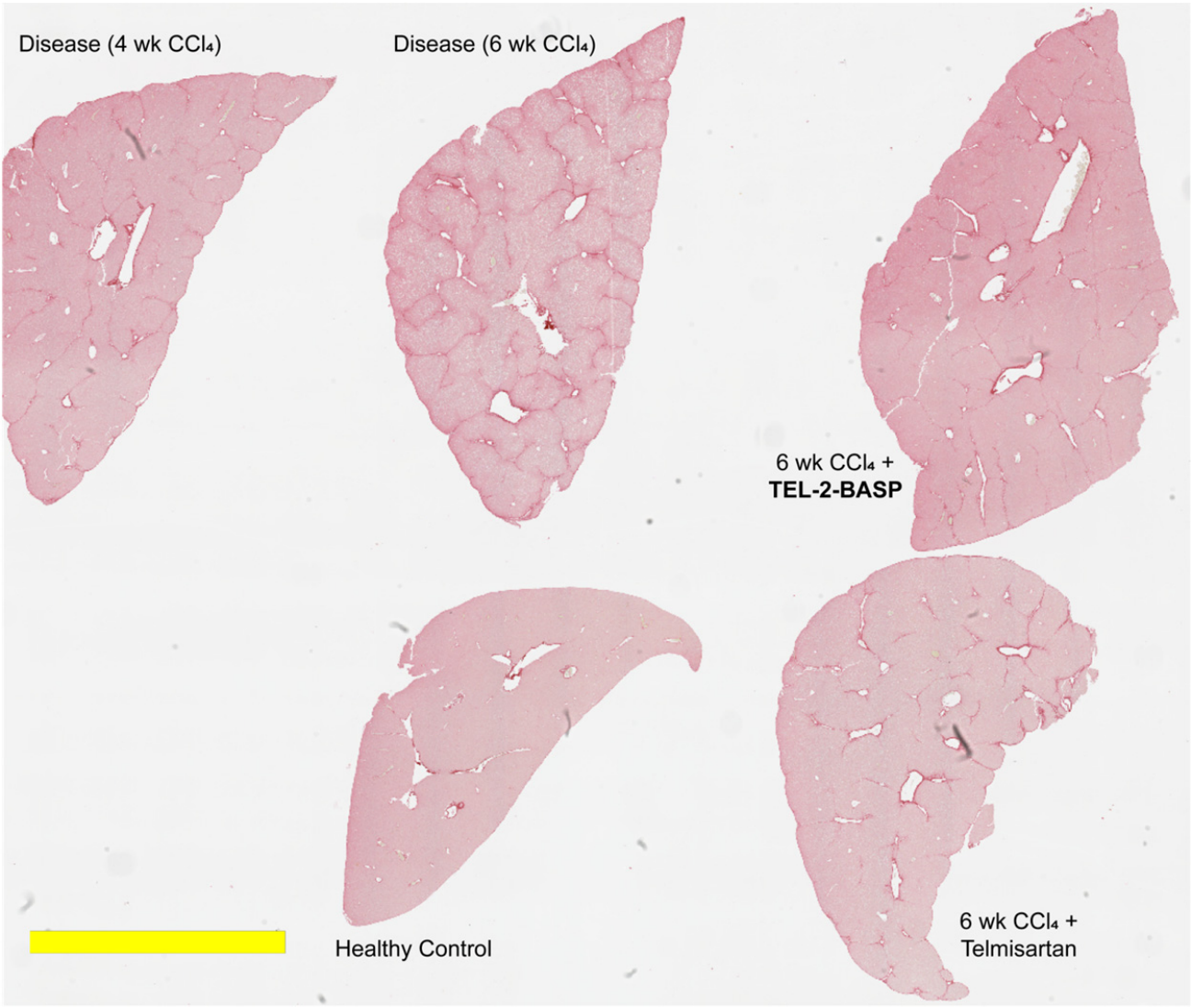


Figure S25. Histological PicroSirius Red (PSR) stained full liver images from healthy, diseased (4 or 6 weeks CCl_4), and treated (6 weeks CCl_4 + Telmisartan or **TEL-2-BASP**) mice (*Representative group 2/6*). Scale bar = 4 mm.

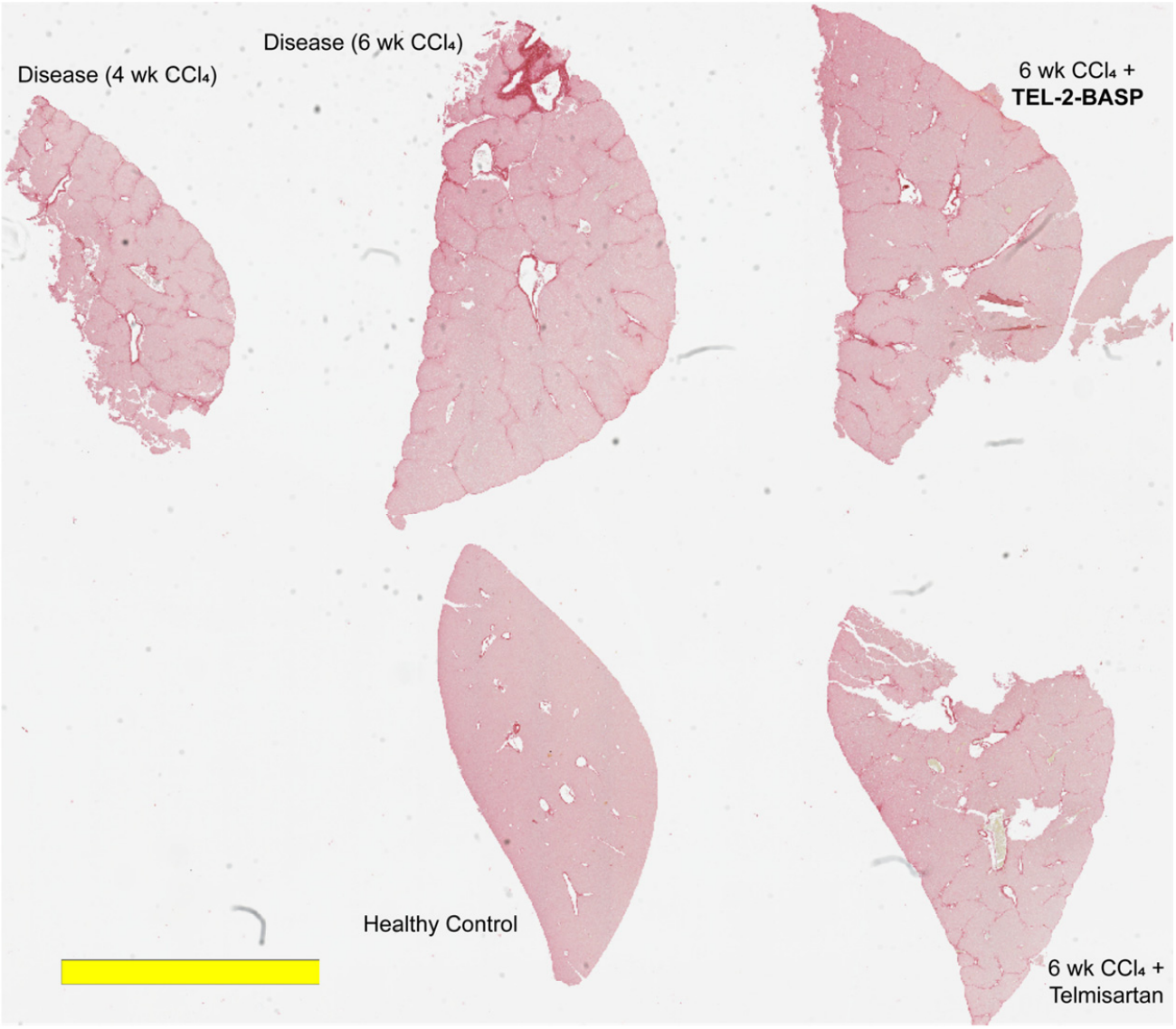


Figure S26. Histological PicroSirius Red (PSR) stained full liver images from healthy, diseased (4 or 6 weeks CCl_4), and treated (6 weeks CCl_4 + Telmisartan or **TEL-2-BASP**) mice (*Representative group 3/6*). Scale bar = 4 mm.

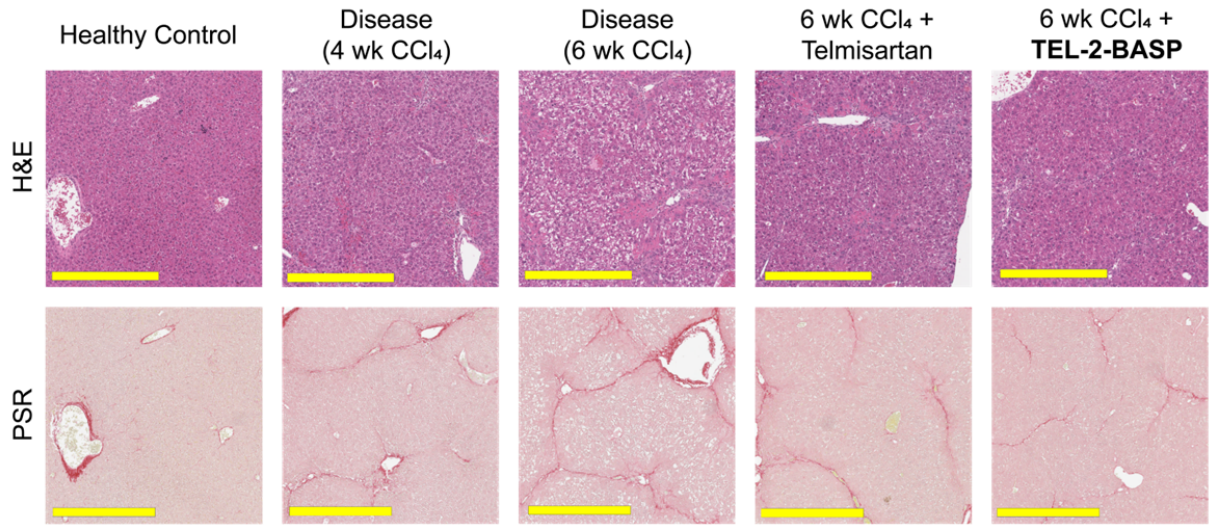


Figure S27. Histological H&E and PicroSirius Red (PSR) stained liver images from healthy, diseased (4 or 6 weeks CCl_4), and treated (6 weeks CCl_4 + Telmisartan or **TEL-2-BASP**) mice (*Representative group 1/6*). Scale bar = 500 µm.

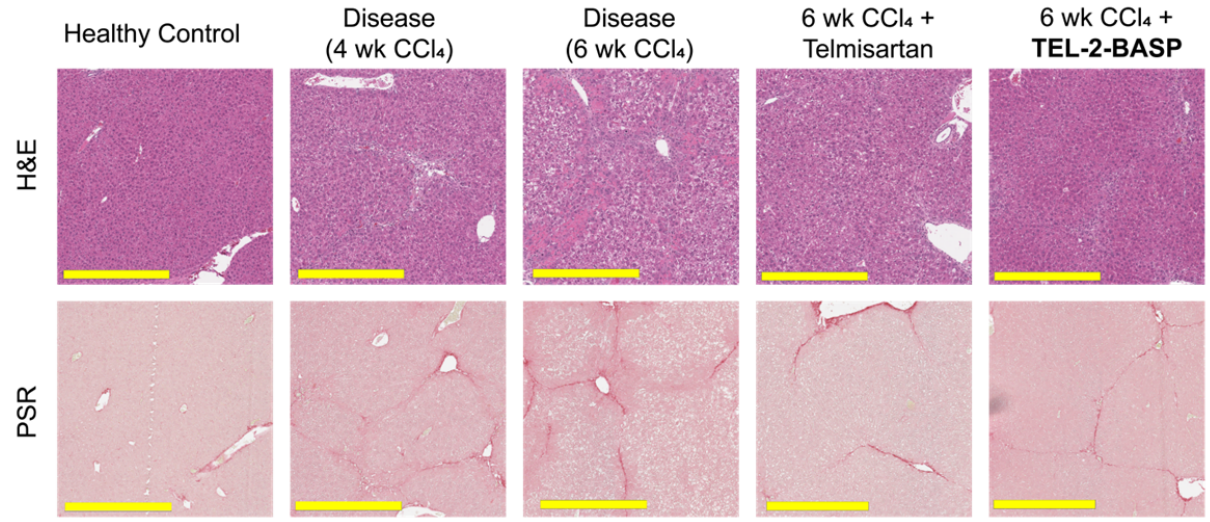


Figure S28. Histological H&E and PicroSirius Red (PSR) stained liver images from healthy, diseased (4 or 6 weeks CCl_4), and treated (6 weeks CCl_4 + Telmisartan or **TEL-2-BASP**) mice (*Representative group 2/6*). Scale bar = 500 µm.

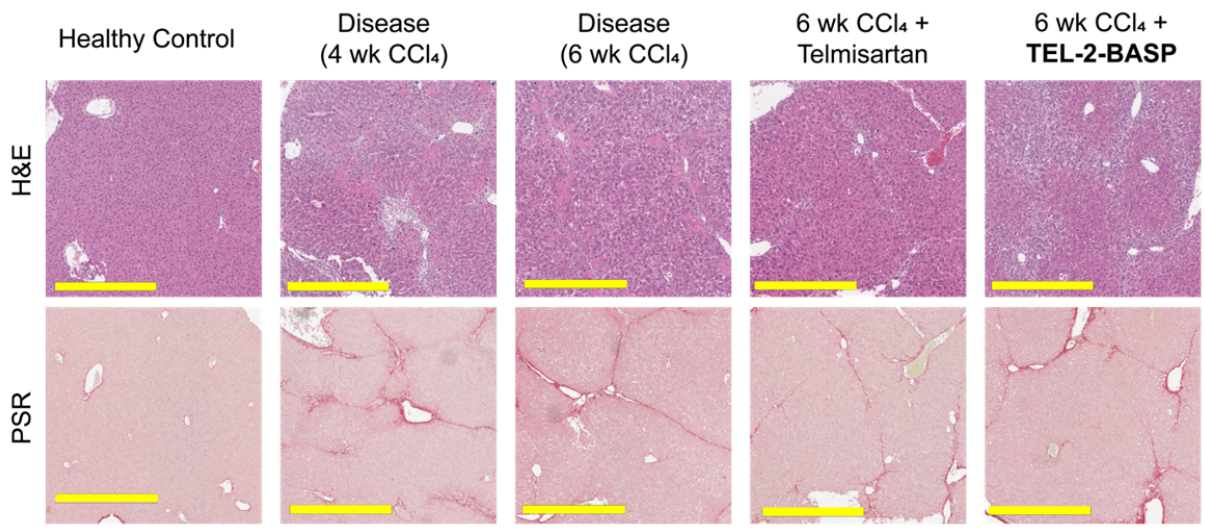


Figure S29. Histological H&E and PicroSirius Red (PSR) stained liver images from healthy, diseased (4 or 6 weeks CCl_4), and treated (6 weeks CCl_4 + Telmisartan or **TEL-2-BASP**) mice (*Representative group 3/6*). Scale bar = 500 µm.

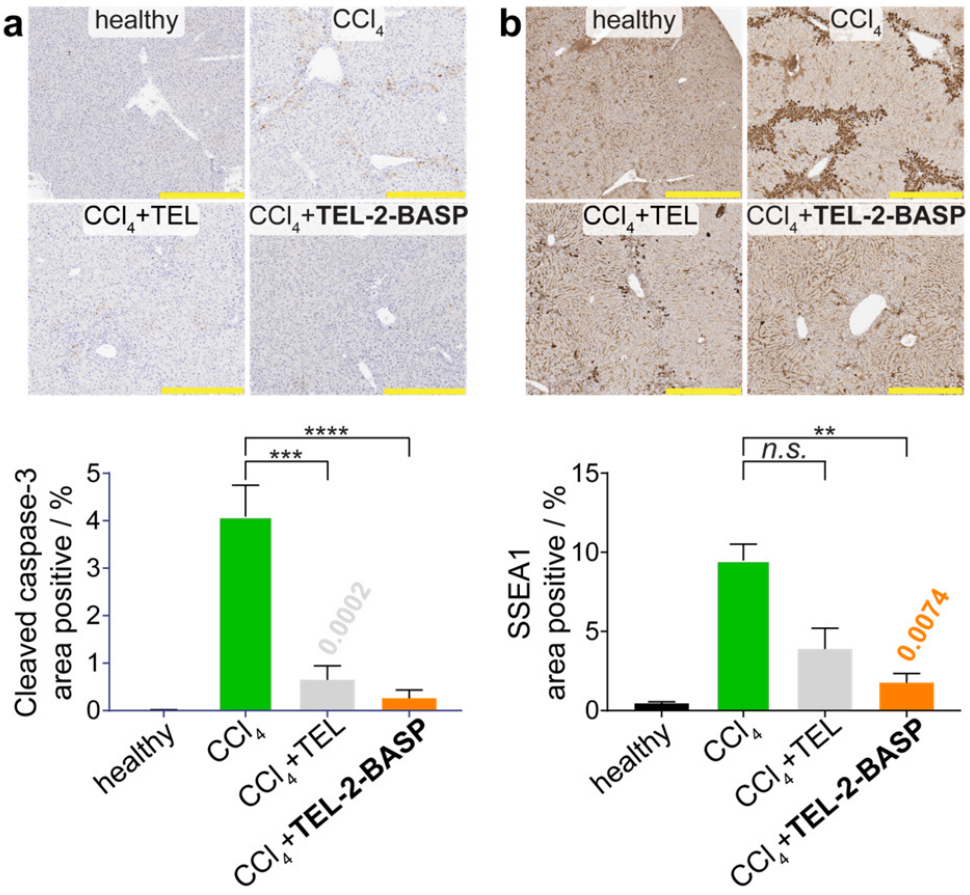


Figure S30. Representative stained immunohistological images for (**a**) cleaved Caspase-3 and (**b**) SSEA1 antigen from CCl_4-induced mouse liver fibrosis model for all treatment groups- healthy, untreated, and test agents: TEL (10 mg/kg, p.o. q.d.; total of 14 doses) and **TEL-2-BASP** (700 mg/kg i.v. q.w.; total of two doses) during 6 weeks of CCl_4 intoxication. Scale bars = 500 µm. Image quantifications (directly below each set of images) show hepatocycte-protective effects of **TEL-2-BASP** (**a**) and a decrease in SSEA1 antigen in assayed liver tissue after treatment with **TEL-2-BASP** compared to generic TEL (**b**). Data reported as mean ± SD, statistical analyses performed with two-tailed Student's *t* test (* = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$). Individual *P* values are noted above each bar when statistical differences are present.

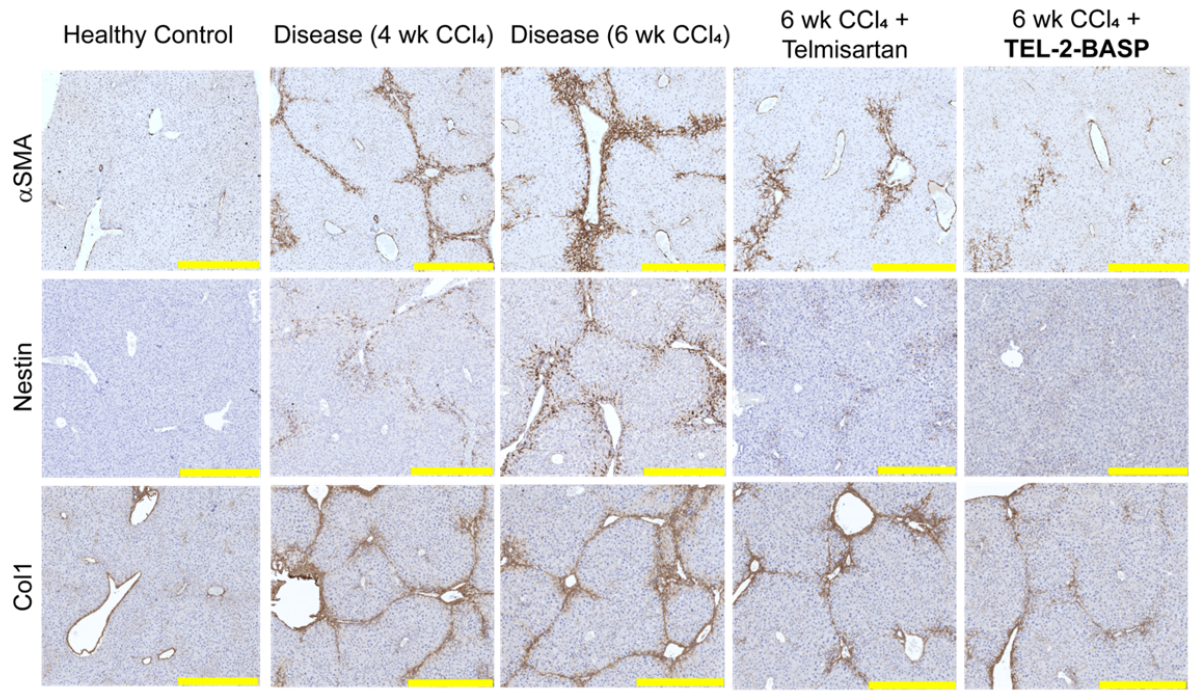


Figure S31. Representative immunofluorescent images for α-smooth muscle actin (α-SMR), Nestin, and collagen-1 (Col1) from healthy, diseased (4 or 6 weeks CCl_4), and treated (6 weeks CCl_4 + Telmisartan or **TEL-2-BASP**) mice. Scale bars = 500 μm.

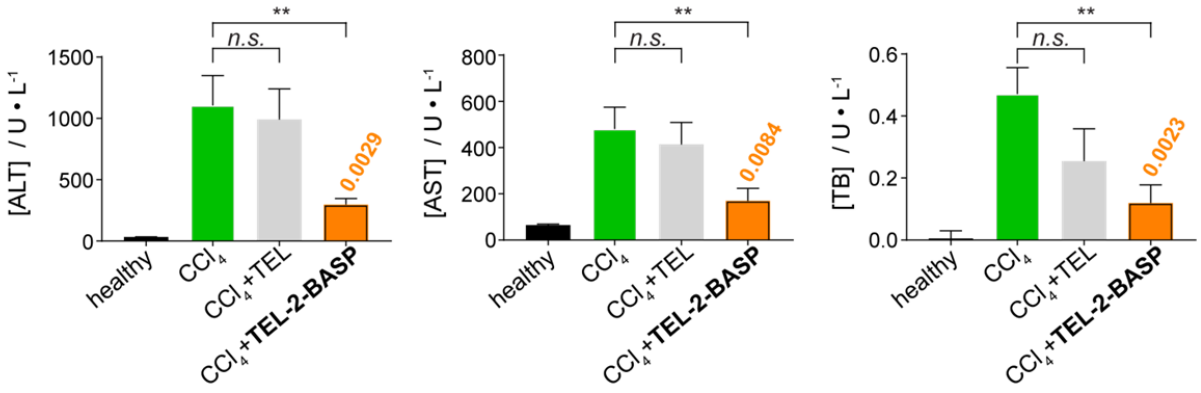


Figure S32. Alanine aminotransferase (ALT), aspartate aminotransferase (AST), and total bilirubin (TB) concentrations in healthy, diseased, diseased + generic TEL, and diseased + **TEL-2-BASP** mice. Data reported as mean ± SD, statistical analyses performed with two-tailed Student's *t* test (* = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$). Individual *P* values are noted above each bar when statistical differences are present.

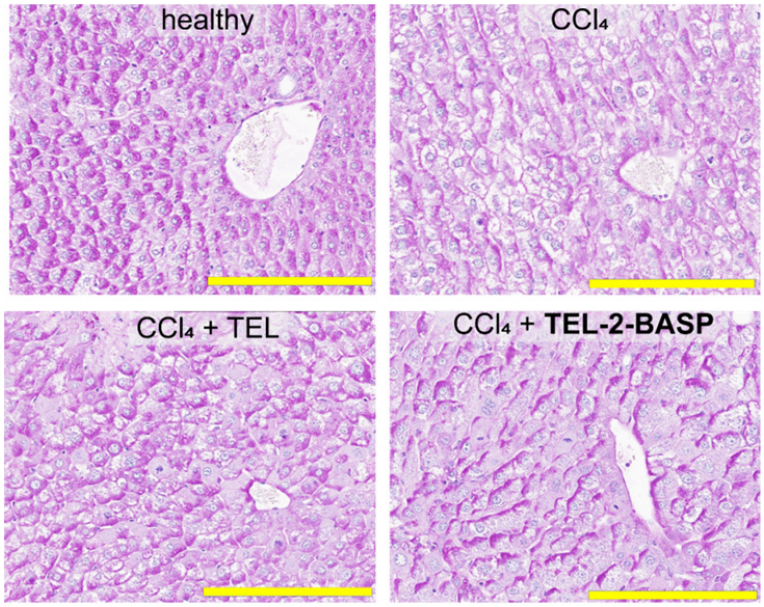


Figure S33. Periodic Acid-Schiff (PAS) stain for glycogen confirming restoration of liver function and metabolism. Scale bar = 200 μm.

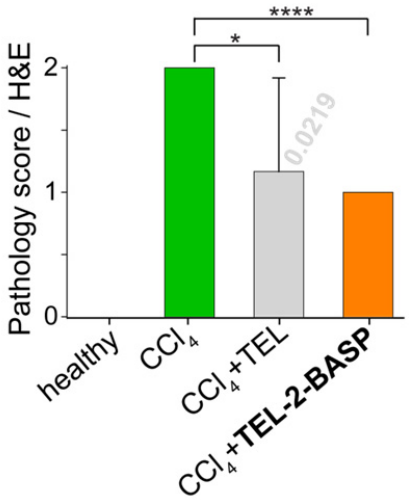


Figure S34. Visualization of pathology scores for necrosis and inflammation (H&E) from Figs. S24 – S26 (*vide supra*). Data reported as mean ± SD, statistical analyses performed with two-tailed Student's *t* test (* = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$, **** = $P < 0.00001$). Individual *P* values are noted above each bar when statistical differences are present.

10. Gene Expression Analysis

Total RNA was isolated from liver tissues using RNAeasy kit (Qiagen) and subjected to oligo (dT) capture and mRNA enrichment. Library preparations were performed following standard procedures. Briefly, mRNA was fragmented and first strand of cDNA was synthesized by using random hexamer-primer. Buffer, dNTPs, RNase H and DNA polymerase I were added to synthesize the second strand, and the resulting double stranded cDNA was purified with magnetic beads. End reparation and 3'-end single nucleotide A (adenine) addition was performed and sequencing adaptors were ligated to the fragments followed by enrichment by PCR amplification. Library quality control (QC) was performed using an Agilent 2100 Bioanaylzer and ABI StepOnePlus Real-Time PCR System. Sequencing was performed on the Illumina Highseq 4000 instrument following standard procedures. After filtering, clean reads were mapped against the mouse genome and gene expression analysis was performed using the software package: RSEM (RNASeq by Expectation Maximization). The FPKM method was used to calculate gene expression level using the formula:

$$FPKM= 10^6C/(NL/10^3)$$

where C = number of fragments that are uniquely aligned to gene A, N= total number of fragments that are uniquely aligned to all genes, and L = number of bases on gene A and the relative change in gene expression (% mRNA) was calculated based on the FPKM values (i.e. ratio between the CCl_4 disease group and the drug treatment group).

Section J. References

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