

## Supporting Information

### Experimental Section

**Materials.** Rink Amide MBHA resins and fluorenyl methoxycarbonyl (Fmoc)-protected amino acids were purchased from Novabiochem. PEGylated ligands were purchased from Aapptec. The chemical reagents and solvents were purchased from Sigma-Aldrich or VWR and used without further purification. Porcine liver esterase (PLE, EC 3.1.1.1, 20 units/mg) was purchased from Sigma-Aldrich and treated per the manufacturer's instructions.<sup>1</sup> (*S*)-(+)-2-(6-Methoxy-2-naphthyl)propionic acid (naproxen) was purchased from Sigma-Aldrich. The solvents were obtained from Mallinckrodt.

**NMR.** Nuclear magnetic resonance (NMR) spectra were obtained at 500 MHz (<sup>1</sup>H) or 125 MHz (<sup>13</sup>C) using CDCl<sub>3</sub> as solvent with a Varian Inova 500 spectrometer equipped with a BroadBand 5 mm probe with Z-gradient.

**Electrospray Ionization Mass Spectroscopy.** ESI-MS for non-water soluble molecules was performed on a Bruker AmaZon X liquid chromatography-MS (LC-MS) quadrupole ion trap spectrometer. ESI-MS of water-soluble molecules was done on a Bruker AmaZon SL LC-MS quadrupole ion trap spectrometer, unless mentioned otherwise.

**PA Synthesis and Purification.** All PAs were synthesized using Fmoc solid phase peptide synthesis, manually or by either Applied Biosystems 433A or CEM Discover System automated peptide synthesizers.<sup>2</sup> Once the peptide was added to the resin, a palmitic acid tail was coupled to the N-terminus. Scheme 2 illustrates the process to add the naproxen derivative **4c** to a PA molecule. The 4-Methyl trityl (Mtt) group was removed from the Lys residue of **5** using 4% TFA, 1% TIPS and 95% DCM. Following, Fmoc-PEG<sub>2</sub>-propionic acid (two couplings, 1 equivalent each time) was coupled to the PA using benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate (PyBop, 1 equivalent per equivalent of linker) to give **6**. The Fmoc protecting group removed and the naproxen derivative **4c** was added using PyBop and *N,N*-diisopropylethylamine (DIPEA) in 24 mL of DCM:DMF, 1:2. The PA was cleaved from the resin and deprotected using a solution of 95% TFA, 2.5% TIPS and 2.5% H<sub>2</sub>O for 4 h.<sup>3</sup> Following, TFA was concentrated on a rotatory evaporator to 1 mL, and PA **7** was precipitated in cold ether. PA **8** was prepared using the same procedure but Fmoc-(2-aminoethoxy)acetic acid was used instead of Fmoc-PEG<sub>2</sub>-propionic acid. PA **9** was synthesized by direct coupling of **4a** to the Lys residue using the PyBop.

For purification, the crude product was dissolved in 0.1% or 1% aqueous ammonium hydroxide to pH 9, and the solution was passed through 0.45 µm and 0.22 µm polypropylene membrane filters. PA was purified by reverse phase-high performance liquid chromatography (RP-HPLC) on a Varian ProStar 210 HPLC system, running a water-acetonitrile gradient through a Phenomenex C<sub>18</sub> Gemini NX basic column. All mobile phase contained 0.1% v/v NH<sub>4</sub>OH. Product-containing HPLC fractions were identified using ESI-MS on an Agilent 6520 Q-TOF LC/MS system. During this step, no hydrolysis of the ester linker was observed by MS. The desired fractions were combined, concentrated by rotary evaporation to remove acetonitrile, and lyophilized using Labconco, FreezeZone6. Purity of the final compound was assessed via HPLC using an Inspire Reverse Phase C<sub>18</sub> 5 µm 250 × 4.6 mm column on an Agilent technologies 1260 HPLC system. PA **7**: ESIMS m/z (rel intensity) 1858 (MH<sup>+</sup>, 100); HRESIMS m/z expected for C<sub>91</sub>H<sub>149</sub>O<sub>27</sub>N<sub>13</sub> 1858.0789 (MH<sup>+</sup>), found 1858.0764 (MNa<sup>+</sup>). PA **8**: ESIMS

m/z (rel intensity) 1696 (MH<sup>+</sup>, 100); HRESIMS m/z expected for C<sub>83</sub>H<sub>134</sub>O<sub>24</sub>N<sub>13</sub> 1696.9659 (MH<sup>+</sup>), found 1696.9659 (MNa<sup>+</sup>). PA **9**: ESIMS m/z (rel intensity) 1579 (MH<sup>+</sup>, 100); HRESIMS m/z expected for C<sub>79</sub>H<sub>126</sub>O<sub>21</sub>N<sub>12</sub> 1579.9239 (MH<sup>+</sup>), found 1579.9221 (MNa<sup>+</sup>). The filler PA (sequence: C<sub>16</sub>-V<sub>3</sub>A<sub>3</sub>E<sub>3</sub>-COOH; **E3**), which was used to dilute the NAP-PA within the supramolecular assembly, was synthesized and purified by similar methods as described above.

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**PA Sample Preparation.** For all studies, naproxen-conjugated PAs **7-9** were diluted with the diluent PA **E3** to either 1:3 or 1:10 (molar ratio). PAs were dissolved separately in hexafluoro-2-propanol (HFIP) at 1 mg/mL, mixed, and lyophilized in a 1.8 mL Eppendorf tube on a Schlenk line. Lyophilized PA was stored at -20 °C until use.

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**Cryogenic Transmission Electron Microscopy and Circular Dichroism.** Lyophilized powder containing PA **7** and PA **E3** (1:3 molar ratio) was dissolved in Milli-Q water (adjusted to pH 7.4 with 0.1 N NaOH) at 10 mg/mL. To image the supramolecular assembly, an aliquot was then diluted to 2.5 mg/mL in Milli-Q water and left to age for 1 day. Cryo-TEM was performed on a JEOL 1230 microscope according to a previously described protocol.<sup>3</sup>

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**pH Dependent Naproxen Release.** Lyophilized powder of PA **7** diluted with PA **E3** (1:3 molar ratio) was dissolved in PBS (1.4 mg in 0.9 mL) and pH adjusted to 7.4, 9.0, or 12.0 using 0.1 N NaOH. The volume was then brought to 1 mL, and an aliquot of 50 µL was taken as a control (time point 0 h) and stored at -20 °C until analysis. The solution was then agitated using a Heidolph Promax 1020 Inkubator at 37 °C and 60 rpm, and 50 µL of Milli-Q water was added to prepare 1 mM of the PA system (0.25 mM of the actual drug-conjugated PA). At each time point, an aliquot of 55 µL was taken, 25 µL of 0.1% TFA-acetonitrile solution was added to quench cleavage, and the sample was stored at -20 °C until analysis. The experiment was performed in triplicate.

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**Enzyme-Activated Naproxen Release.** Lyophilized powder of PA **7** diluted with PA **E3** (1:3 molar ratio) was dissolved in PBS (1.4 mg in 1 mL; pH 7.4). After an aliquot of 50 µL was taken as a control (time point 0 h), 50 µL of B(OH)<sub>3</sub> buffer solution containing 20 units (20 u) of esterase was added to achieve a final concentration of 1 mM of the PA system (0.25 mM drug-conjugated PA). At each time point, an aliquot (50-75 µL) was taken, 25 µL of a 0.1% TFA-acetonitrile solution was added to denature the enzyme, and the sample was stored at -20 °C until analysis. The experiment was performed in triplicate. The same protocol was used to perform release studies of the following PA systems: PA **7** diluted with PA **E3** (1:10 molar ratio), PA **8** diluted with PA **E3** (1:3 molar ratio), and PA **9** diluted with PA **E3** (1:3 molar ratio). Furthermore, an additional study was performed using PA **7** diluted with PA **E3** (1:3 molar ratio) with varied concentrations of the esterase (0.5, 1, 2, and 5 U), following the same protocol. Samples were retrieved after 24 h, and the experiment was performed in quadruplicate. Finally, the effect of adding extra enzyme on the system was studied by quadruplicate. In this case, 20 u of enzyme were added to a sample, after 6 h the sample was split in two equal aliquots. PBS (50 µL) was added to the first aliquot while 10 u of enzyme contained in 50 µL of buffer were added to the second sample. After 24 h, the difference in release between samples was compared.

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**High-Performance Liquid Chromatography.** Stored samples were thawed to room temperature and diluted with 50 µL of 1% v/v NH<sub>4</sub>OH. Quantitative analysis of the hydrolyzed

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products was performed via RP-HPLC, running a water-acetonitrile gradient from 95:5 to 5:95. The solvents contained 0.1% v/v NH<sub>4</sub>OH. Samples (30-50 µL) were run at 25 °C at a flow rate of 1 mL/min and the absorbance was monitored at λ = 260 nm. The change in ratio between the area of the base PA, an internal standard, and the area of the drug-conjugated PA was plotted as % release. The identity of peaks was assigned by MS.

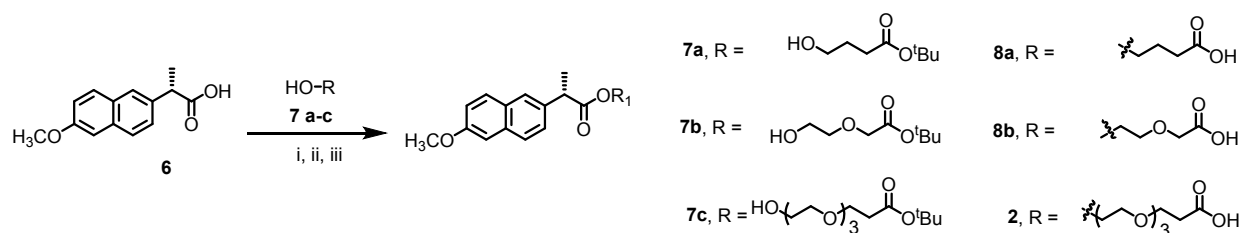
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**COX-2 Inhibition Assay.** Lyophilized powder of PA **7** diluted with PA **E3** (1:3 molar ratio) was dissolved at 1 mM in PBS (1 mL), incubated with 20 units of esterase for 24 h, and lyophilized. The PA/esterase was re-dissolved at 4 mM in 250 µL 1:1 DMSO/water at pH 6.5, horn sonicated, and spun down for 20 min at 13,000 rpm to separate the hydrolyzed naproxen. Following, 150 µL of the supernatant was retrieved. The effect of the hydrolyzed naproxen on recombinant human COX-2 activity was measured using COX Inhibitor Screening Assay Kit per the manufacturer's instructions (Cayman Chemical Co.). Reactions for the assay were performed using 20 µL in quadruplicate. The same procedures were followed with PA **7** diluted with PA **E3** (1:3 molar ratio) without esterase for comparison. In addition, the inhibitory effect of freshly dissolved naproxen in DMSO/water was also measured at a final concentration of 100 µM as a positive control.

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111 Synthesis of Naproxen-ester linkers

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i (COCl)<sub>2</sub>, CH<sub>2</sub>Cl<sub>2</sub> or EDC, Et<sub>3</sub>N, DMF, CH<sub>2</sub>Cl<sub>2</sub>; ii THF, Et<sub>3</sub>N; iii TFA, CH<sub>2</sub>Cl<sub>2</sub>.

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**Synthesis of Naproxen Derivative 8a.** Scheme 1 illustrates the process to synthesize naproxen ester derivatives. (S)-naproxen (**6**, 0.89 g, 3.9 mmol) was dissolved in dry dichloromethane (DCM; 50 mL) and cooled to 0 °C. Oxalyl chloride (2 mL) and a catalytic amount of dimethylformamide (DMF; 2 drops) were sequentially added, and the reaction mixture was stirred for 2 h. Following, more oxalyl chloride (1 mL) and DMF (2 drops) were added and the reaction mixture was stirred for 1 h. The solvent was removed *in vacuo* and the residue was dissolved in dry tetrahydrofuran (THF; 40 mL) at 0 °C. A solution of **7a** (0.55 g, 3.4 mmol, 0.95 equiv), triethylamine (TEA; 1.5 mL), and THF (10 mL) were slowly added, and the reaction mixture was stirred overnight at room temperature. The solvent was removed *in vacuo*, and the residue was dissolved in chloroform. The organic solution was washed successively with saturated sodium bicarbonate, water, and brine (50 mL each), then dried over magnesium sulfate. The solvent was removed and the compound was obtained as a dark yellow oil (0.9 g, 2.4 mmol, 72% yield), which was used without further purification. The compound was dissolved in a mixture of trifluoroacetic acid (TFA; 8 mL) and DCM (5 mL), triisopropylsilane (TIPS; 3 drops)

129 was added, and the mixture was stirred for 8 h at room temperature. The reaction mixture was  
130 diluted with DCM (30 mL) and water (30 mL). The organic phase was extracted and washed  
131 with brine. The solvent was removed *in vacuo* and the residue was purified by silica gel column  
132 chromatography (230–400 mesh), eluting with chloroform to chloroform-methanol, 20:1. The  
133 desired compound **4a** was obtained as a brown oil (0.71 g, 2.2 mmol, 667% yield). <sup>1</sup>H NMR  
134 (CDCl<sub>3</sub>, 500 MHz) δ: 7.71–7.66 (m, 3 H); 7.41 (dd, *J*<sub>1</sub> = 1.5 Hz, *J*<sub>2</sub> = 8.5 Hz, 1 H), 7.15 (dd, *J*<sub>1</sub> =  
135 2.0 Hz, *J*<sub>2</sub> = 9.0 Hz, 1 H), 7.11 (d, *J* = 2.0 Hz, 1 H), 4.13 (d, *J* = 5.5 Hz, 2 H), 3.92 (s, 3 H), 3.84  
136 (d, *J* = 6.5 Hz, 2 H), 2.33 (d, *J* = 6.5 Hz, 2 H), 1.59 (d, *J* = 7.0 Hz, 2 H); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 125  
137 MHz) δ: 178.5, 174.6, 157.6, 135.6, 133.7, 129.3, 128.9, 127.2, 126.2, 125.9, 119.0, 105.6, 63.6,  
138 55.3, 45.4, 30.4, 23.7, 18.4. ESIMS *m/z* (rel intensity) 316 (MH<sup>+</sup>, 100); HRESIMS *m/z* expected  
139 for C<sub>18</sub>H<sub>20</sub>O<sub>5</sub>Na 339.1208 (MNa<sup>+</sup>), found 339.1203 (MNa<sup>+</sup>).

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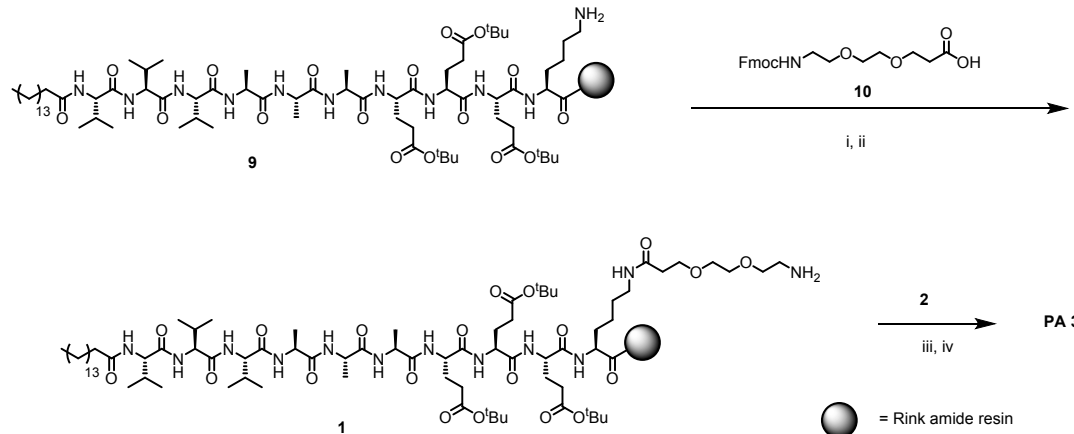
141 **Synthesis of Naproxen Derivative 8b.** (S)-Naproxen (**6**, 1.81 g, 7.84 mmol) was dissolved in a  
142 mixture of dry DCM-DMF, 10:1 (44 mL) at room temperature. 1-Ethyl-3-(3-  
143 dimethylaminopropyl)carbodiimide (EDC; 1.22 g, 7.80 mmol) was added and the reaction  
144 mixture was left to stir for 1 h. Following, **7b** (1.38 g, 7.84 mmol) and TEA (1 mL) were added  
145 and the reaction mixture was left to stir for 24 h. DCM (30 mL) was then added and the organic  
146 solution was successively washed with water, acidified water (1 mL of concentrated HCl), water,  
147 and brine (50 mL each). The organic phase was dried under magnesium sulfate and the solvent  
148 was removed *in vacuo*. The obtained residue was dissolved in a mixture of TFA (6 mL) and  
149 DCM (6 mL), and the reaction mixture was left to stir for 4 h at room temperature. The solvent  
150 was removed *in vacuo* and the residue was purified by silica gel column chromatography, eluting  
151 with DCM to DCM-methanol, 20:1. The desired compound **8b** was obtained as a brown oil (0.9  
152 g, 2.8 mmol, 36% yield). <sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz) δ: 7.71–7.69 (m, 3 H); 7.41 (dd, *J*<sub>1</sub> = 1.5  
153 Hz, *J*<sub>2</sub> = 8.5 Hz, 1 H), 7.14 (dd, *J*<sub>1</sub> = 2.0 Hz, *J*<sub>2</sub> = 9.0 Hz, 1 H), 7.11 (d, *J* = 2.0 Hz, 1 H), 4.31–  
154 4.24 (m, 4 H), 4.03 (d, *J* = 3.0 Hz, 2 H), 3.91–3.89 (m, 4 H), 3.73–3.69 (m, 2 H), 1.59 (d, *J* = 7.0  
155 Hz, 2 H); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 125 MHz) δ: 180.5, 174.8, 157.7, 135.4, 133.8, 129.3, 128.9, 127.3,  
156 126.2, 126.0, 119.0, 105.7, 69.5, 67.9, 63.8, 55.3, 45.4, 18.4. ESI-MS *m/z* (rel intensity) 332  
157 (MH<sup>+</sup>, 100); HRESIMS *m/z* expected for C<sub>18</sub>H<sub>20</sub>O<sub>6</sub>Na 355.1152 (MNa<sup>+</sup>), found 355.1150  
158 (MNa<sup>+</sup>).

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160 **Synthesis of Naproxen Derivative 2.** (S)-Naproxen (**6**, 1.65 g, 7.16 mmol) was dissolved in  
161 benzene (120 mL). Thionyl chloride (5 mL) was added and the reaction mixture was heated at  
162 reflux for 5 hr. The reaction was worked up with oxalyl chloride and DMF, and re-dissolved in  
163 dry THF as described for **8a**. The desired alcohol **7c** (2.00 g, 7.18 mmol) and TEA (1 mL) were  
164 added and the reaction mixture was left to stir overnight at room temperature. The reaction was  
165 continued according to the protocol described for **8a**. The desired compound **2** was obtained as a  
166 brown oil (1.32 g, 3.03 mmol, 42.4% yield). <sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz) δ: 7.70–7.66 (m, 3 H);  
167 7.40 (dd, *J*<sub>1</sub> = 1.5 Hz, *J*<sub>2</sub> = 8.5 Hz, 1 H), 7.13 (dd, *J*<sub>1</sub> = 2.0 Hz, *J*<sub>2</sub> = 9.0 Hz, 1 H), 7.10 (d, *J* = 2.0  
168 Hz, 1 H), 4.23 (d, *J* = 4.5 Hz, 2 H), 3.89–3.80 (m, 4 H), 3.71 (t, *J* = 6.5 Hz, 2 H), 3.62 (d, *J* = 4.5  
169 Hz, 2 H), 3.60–3.48 (m, 8 H), 2.60 (t, *J* = 6.5 Hz, 2 H), 1.57 (d, *J* = 5.0 Hz, 2 H); <sup>13</sup>C NMR  
170 (CDCl<sub>3</sub>, 125 MHz) δ: 176.0, 174.7, 157.6, 133.7, 129.3, 128.9, 127.1, 126.3, 125.9, 119.0, 118.9,  
171 105.6, 70.4 (2 C), 70.3 (2 C), 68.9, 66.3, 64.0, 55.2, 45.3, 34.8, 18.5. ESI-MS *m/z* (rel intensity)  
172 434 (MH<sup>+</sup>, 100); HRESIMS *m/z* expected for C<sub>23</sub>H<sub>30</sub>O<sub>8</sub>Na 457.1838 (MNa<sup>+</sup>), found 457.1837  
173 (MNa<sup>+</sup>).

174 **Scheme S1.** Synthesis of Naproxen-Peptide Amphiphile **3**

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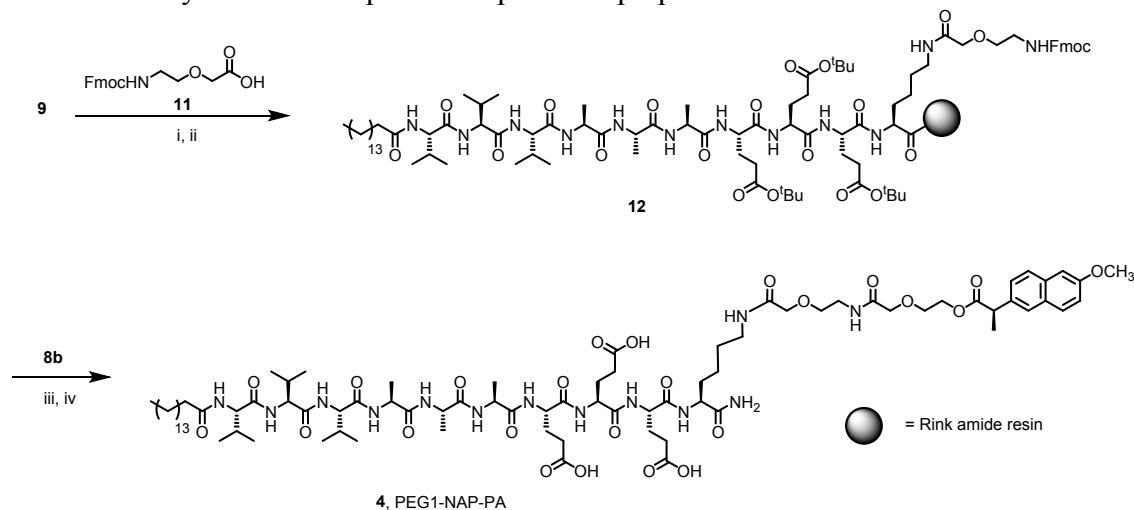
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i. HBTU, DIPEA, DMF, DCM, ii. 4-Me-Pip, DMF; iii. PyBOP, DIPEA, DMF, DCM; iv. TFA, TIPS, H<sub>2</sub>O

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179 **Scheme S2.** Synthesis of Naproxen-Peptide Amphiphile **4**



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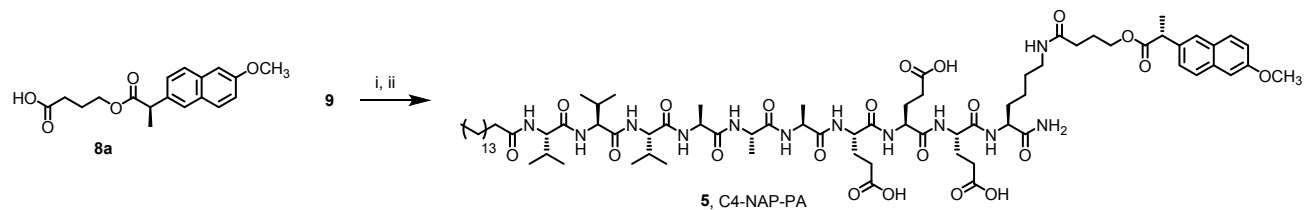
i. HBTU, DIPEA, DMF, DCM, ii. 4-Me-Pip, DMF; iii. PyBOP, DIPEA, DMF, DCM; iv. TFA, TIPS, H<sub>2</sub>O

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183 **Scheme S3.** Synthesis of Naproxen-Peptide Amphiphile **5**

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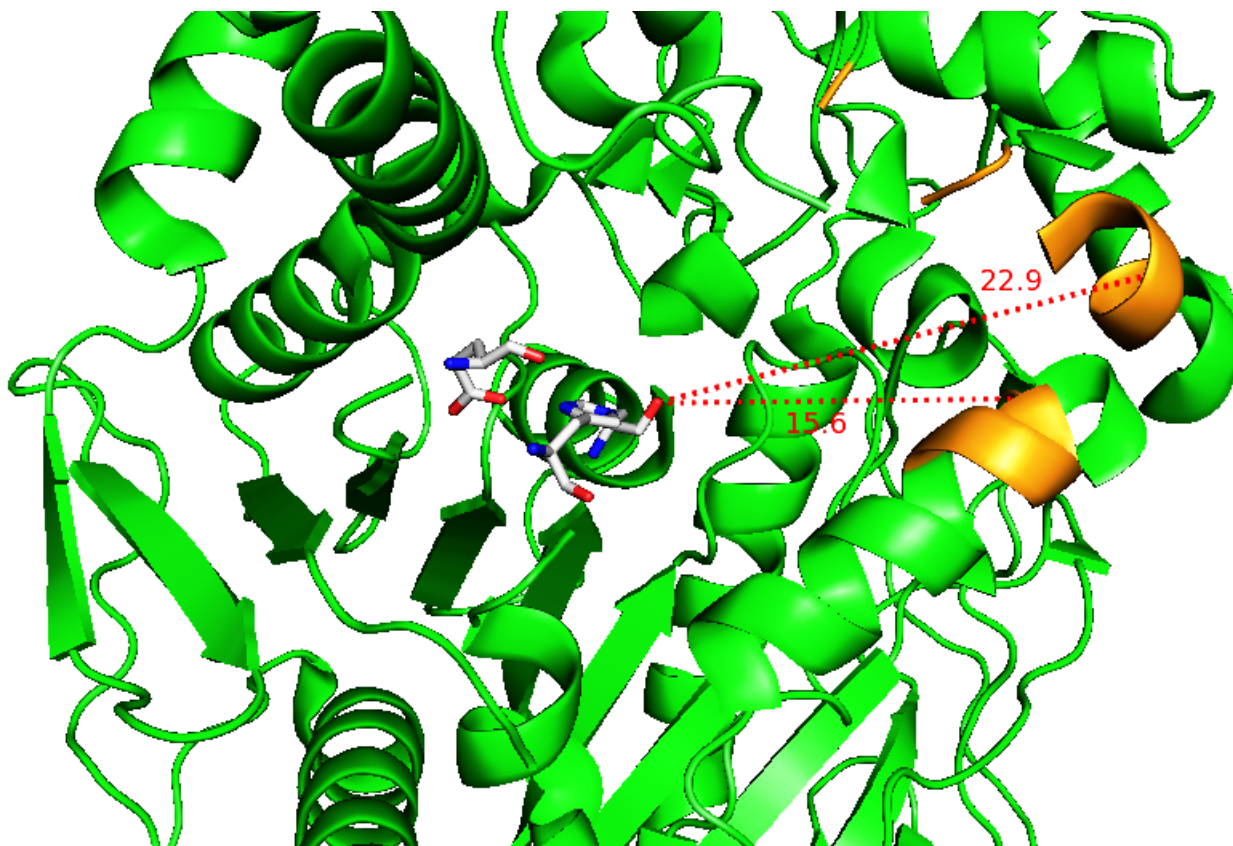


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i. PyBOP, DIPEA, DMF, DCM; ii. TFA, TIPS, H<sub>2</sub>O

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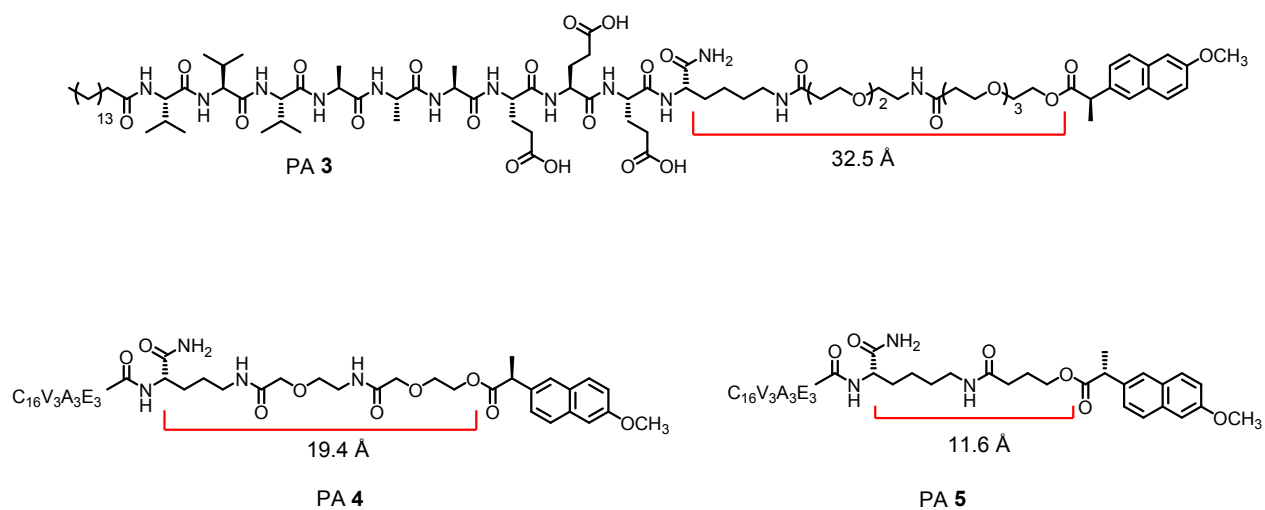


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191 **Figure S1.** Structure of Human Carboxylesterase (Pdb id: 2DR0) and distance from the catalytic  
 192 Ser to the enzyme “gate.” Structure was generated with pymol ([www.pymol.org](http://www.pymol.org)).<sup>4</sup>

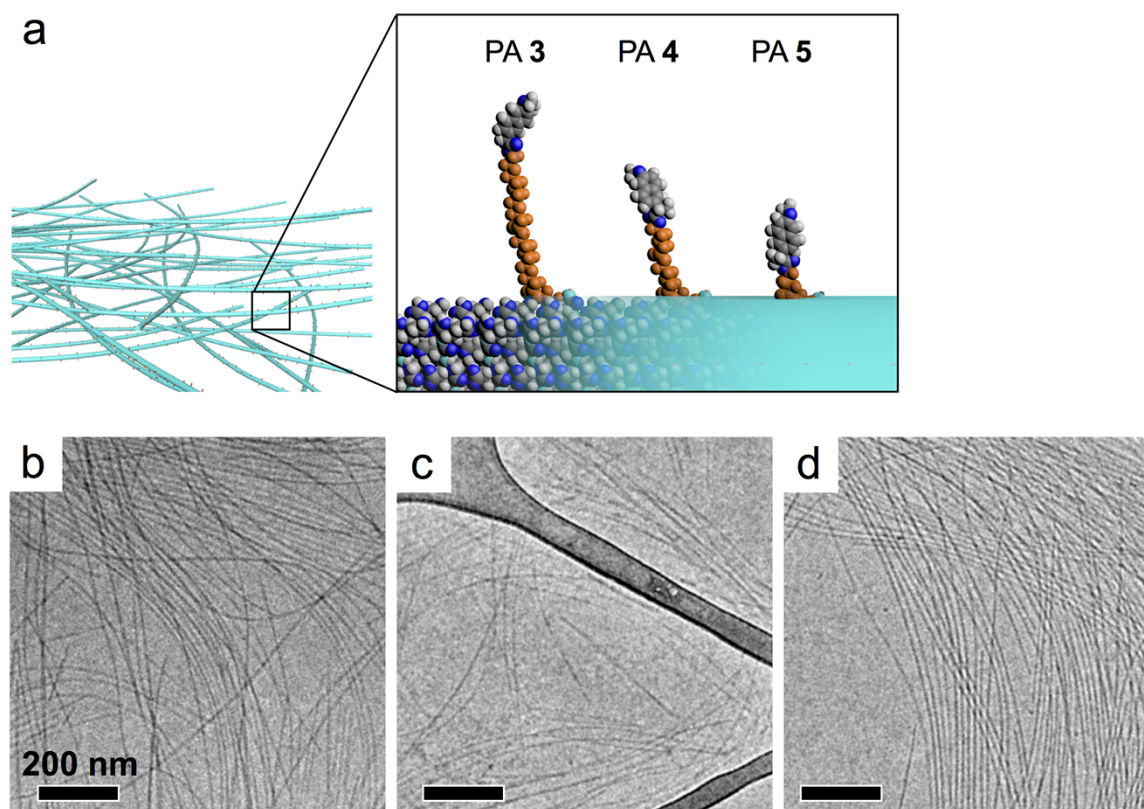
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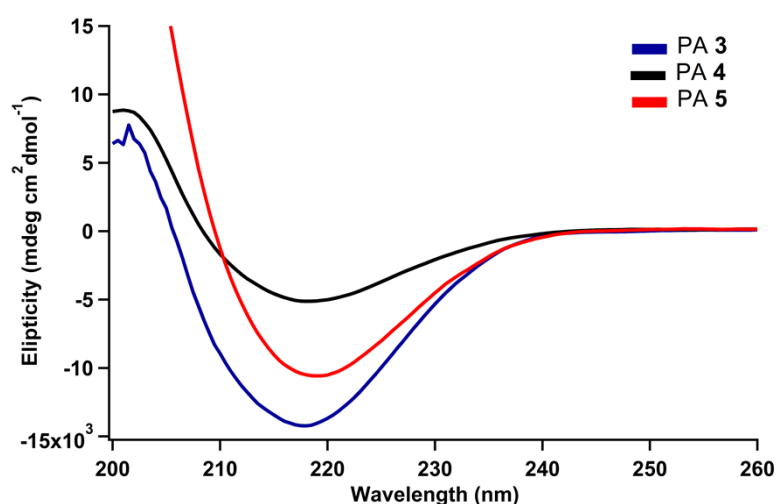
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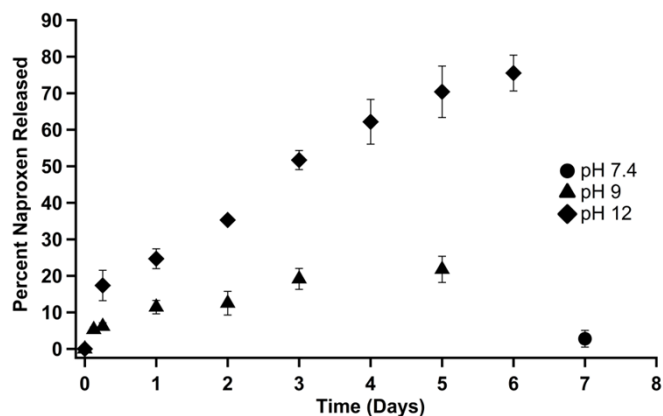
196 **Figure S2.** Distances from Lys residue to ester bond (calculated using HyperChem 7.5).



**Figure S3.** Schematic representation of PA nanofibers and inset comparing the linker length on PAs 3-5 (a). Cryogenic transmission electron micrograph of PA 3, as 1:3 (mole) mixtures with PA E3 (b), PA 4, as 1:3 (mole) mixtures with PA E3 (c); and PA 5, as 1:3 (mole) mixtures with PA E3 (d).

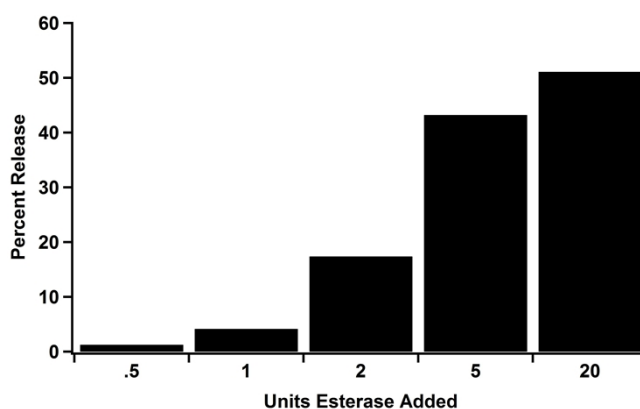


**Figure S4.** Circular dichroism (CD) spectra of (a) PA 3, (b) PA 4, and (c) PA 5 as 1:3 (mole) mixtures with E3 PA. The spectra are red-shifted compared to the canonical  $\beta$ -sheet signature near 195 and 216 nm (maximum and minimum), which is a feature associated with the twisted secondary structure of PA E3.<sup>5</sup>



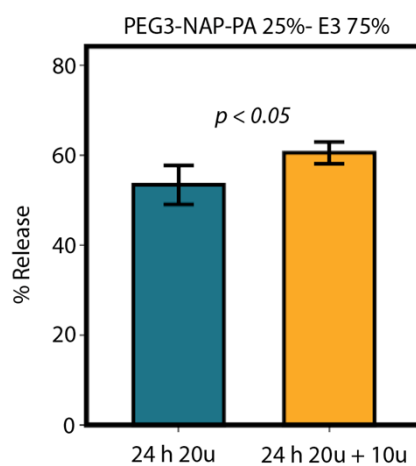
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212 **Figure S5.** pH dependent release of naproxen from PA **3** diluted with PA **E3** (1:3 molar ratio).  
213 The overall PA concentration was 1 mM in PBS. The hydrolysis was evaluated for 7 d. n =3



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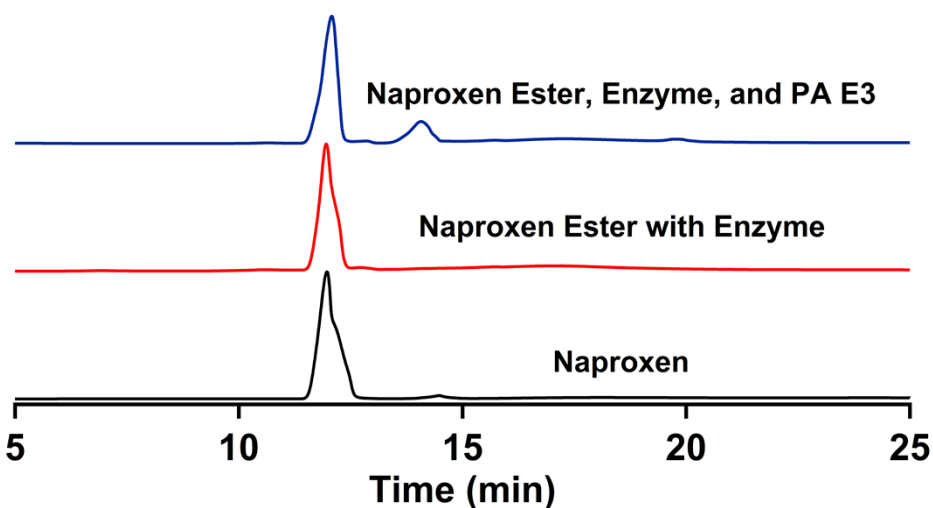
215 **Figure S6.** Naproxen release from PA **3** (1:3 molar ratio with PA **E3**, 1 mM total concentration)  
216 in the presence of varying PLE units.



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218 **Figure S7.** Naproxen release from PA **3** –PA **E3** (1:3 molar ratio) 1 mM concentration with 20 u  
219 of PLE at pH 7.4, 60 rpm and 37 °C, before and after addition of extra PLE (10u, after 6 h), n =  
220 4, after 1 day.

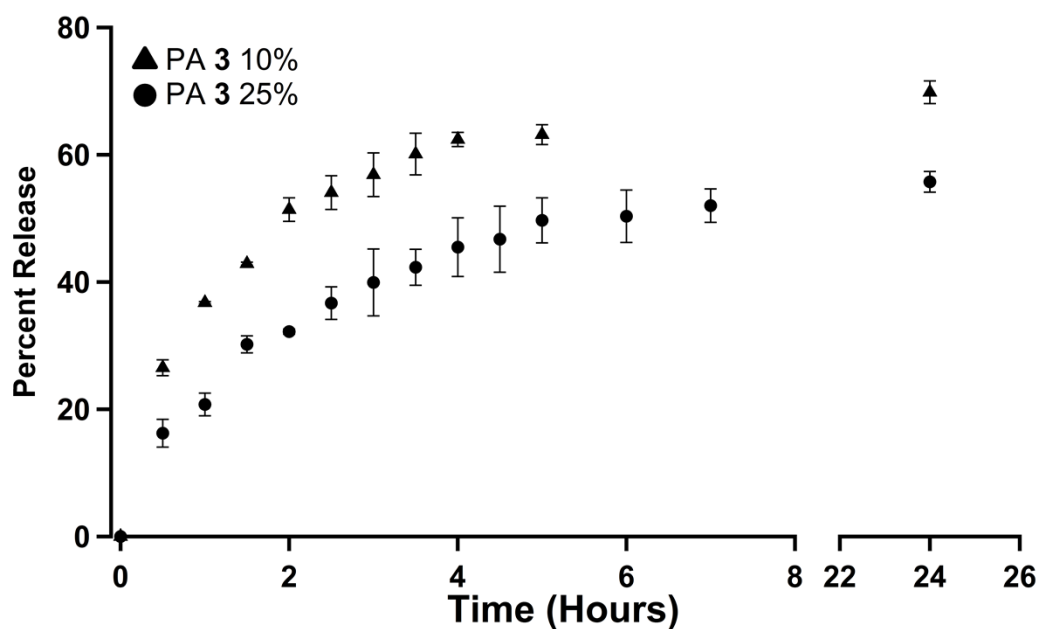




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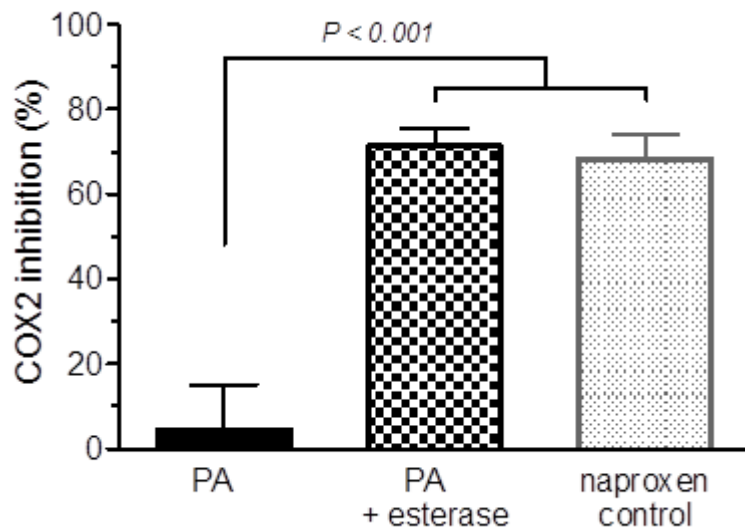
222 **Figure S8.** Naproxen hydrolysis from linker **2** in presence of esterase enzyme. All done at 0.25  
 223 mM of linker **2** in presence PLE (20 U/ mL) at pH 7.4, 60 rpm and 37 °C. Top: Naproxen linker  
 224 **2** (0.25 mM)+ 0.75 PA **E3** (0.75 mM)+ PLE (20 U/ mL), first peak is naproxen, peak at 14.8  
 225 min corresponds to PA **E3**. Middle: Naproxen linker **2** (0.25 mM)+ PLE (20 U/ mL). Bottom:  
 226 Naproxen control (0.25 mM).

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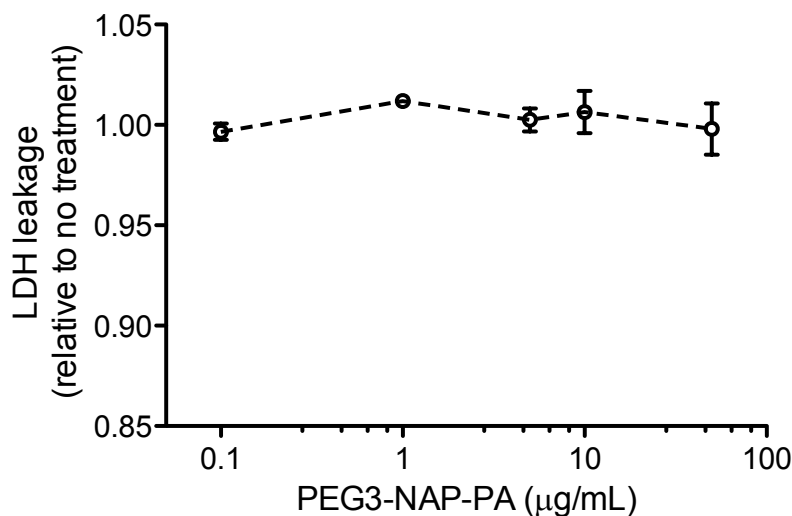
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229 **Figure S9.** Enzymatic release of naproxen from PA **3** diluted with PA **E3** to either 25% or 10%  
 230 (molar ratio). The overall PA concentration was kept constant at 1mM in PBS, and hydrolysis by  
 231 porcine liver esterase (20 U/mL) was evaluated after 24 h. n =3



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233 **Figure S10.** Inhibitory effect of hydrolyzed naproxen on COX-2 activity. PA **3** was diluted with  
 234 PA **E3** (1:3 molar ratio) and treated with or without porcine liver esterase. Free naproxen (100  
 235  $\mu\text{M}$ ) was used as a positive control for the assay.  $n = 3$



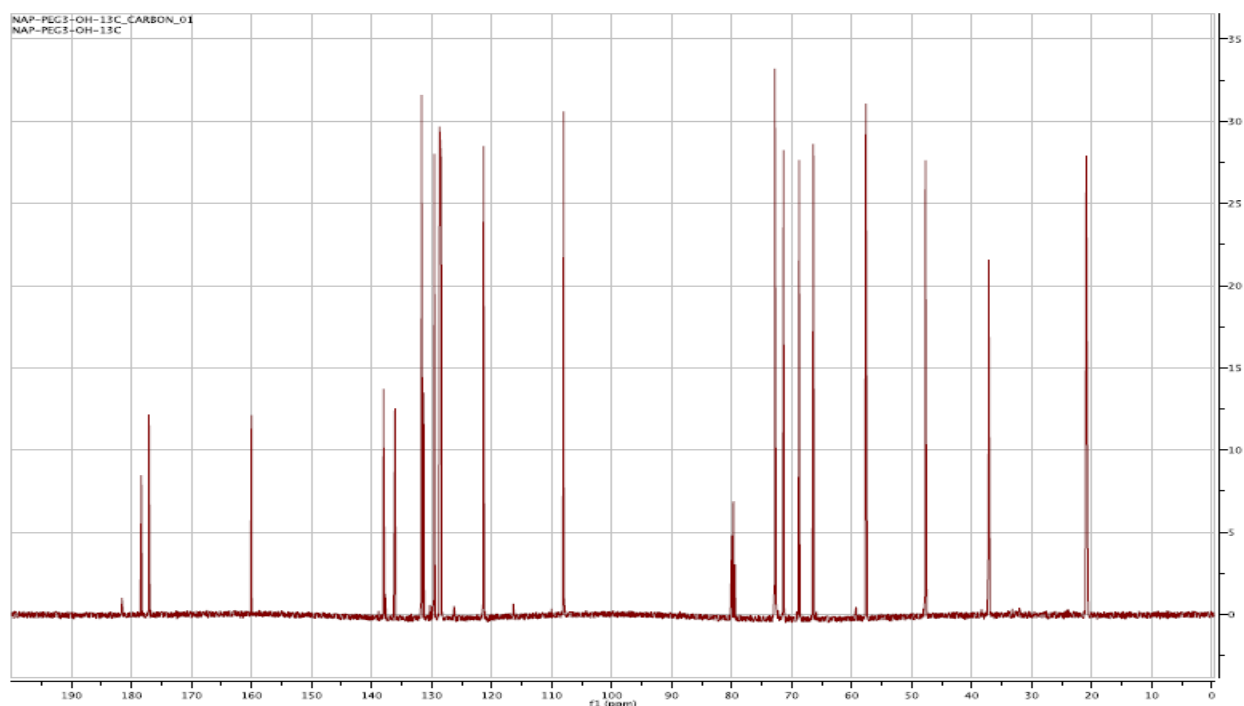
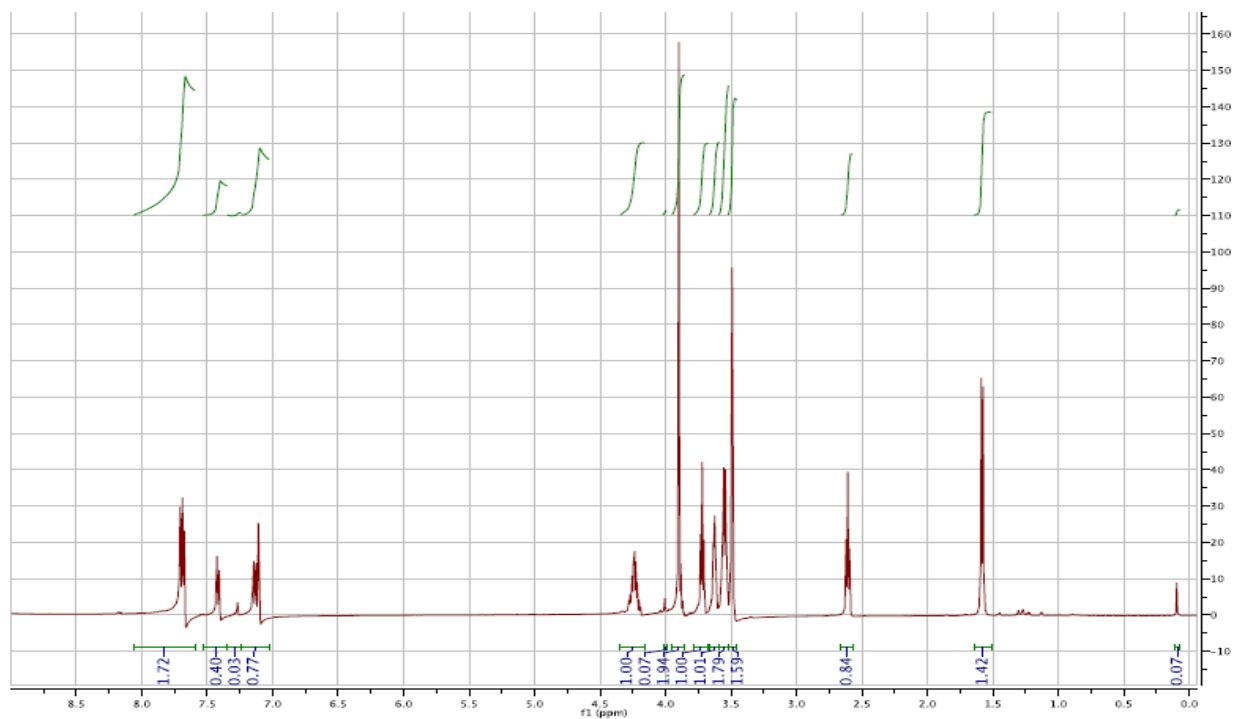
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237 **Figure S11.** Biocompatibility assay. Mouse mesenchymal stem cells (mMSC) were treated with  
 238 PA at varied concentrations for 5 h, and the presence of lactate dehydrogenase (LDH), an  
 239 indicator for cell lysis, in the cell culture media was measured. PA **3** was diluted with PA **E3** (1:3  
 240 mole %) for the assay.  $n = 3$

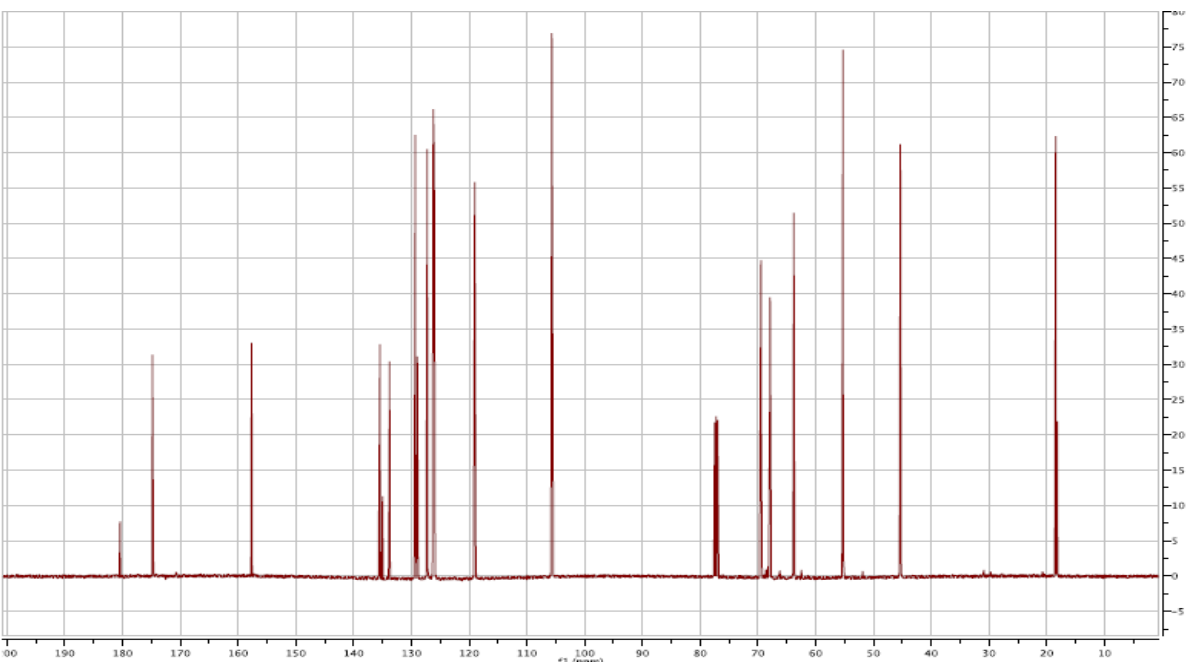
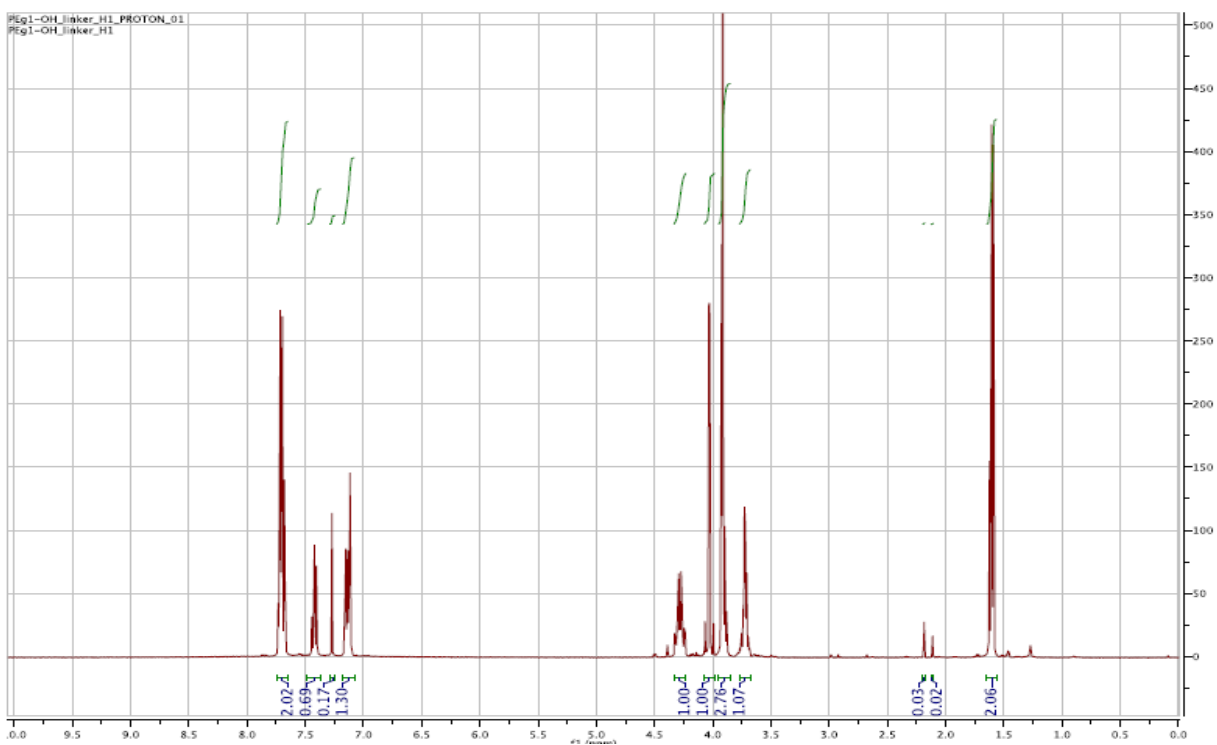
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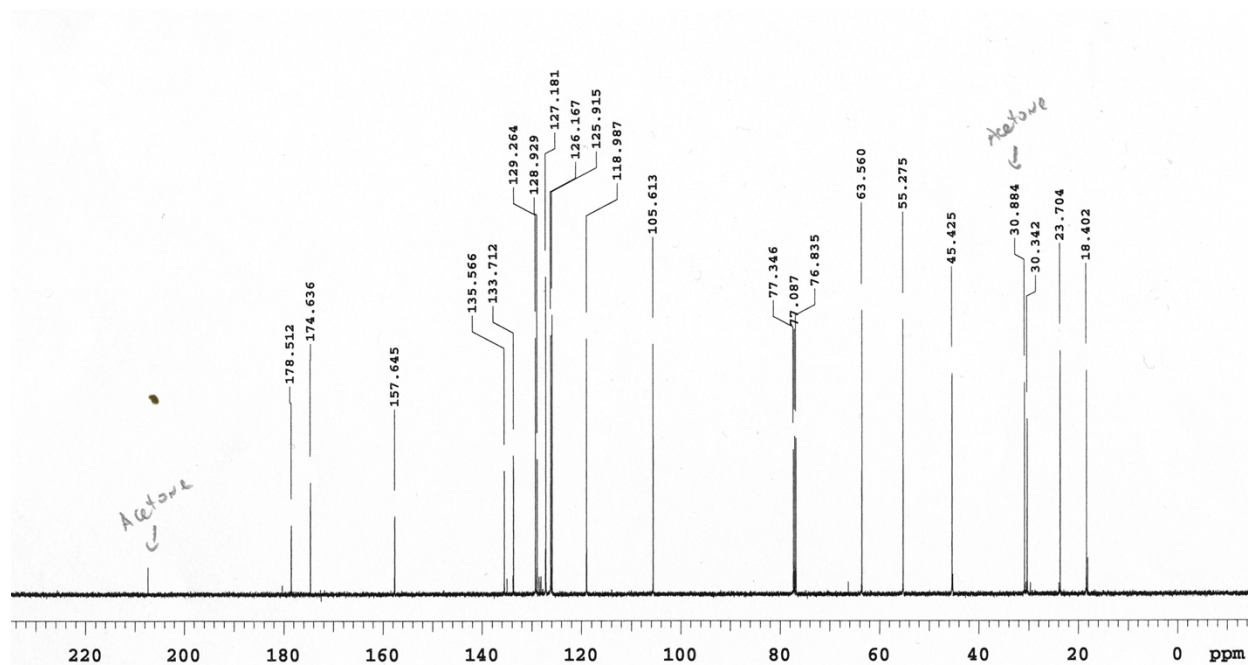
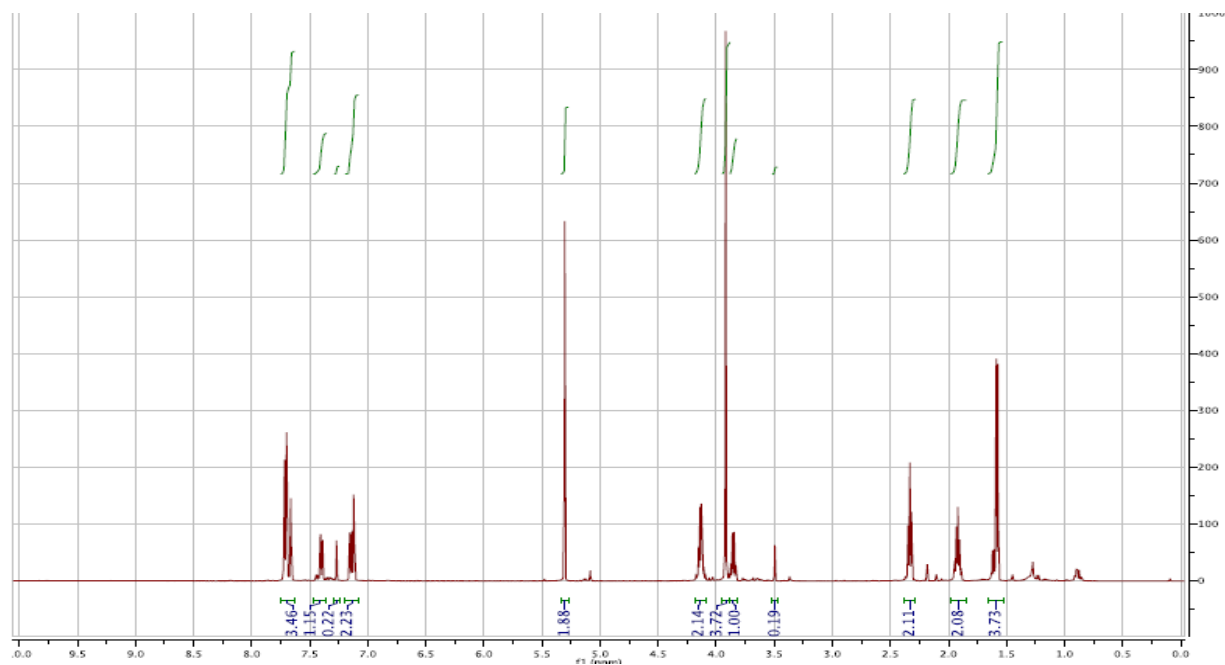
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**Figure S12.** Top; proton NMR of ligand **2**. Bottom; carbon NMR of ligand **2**.

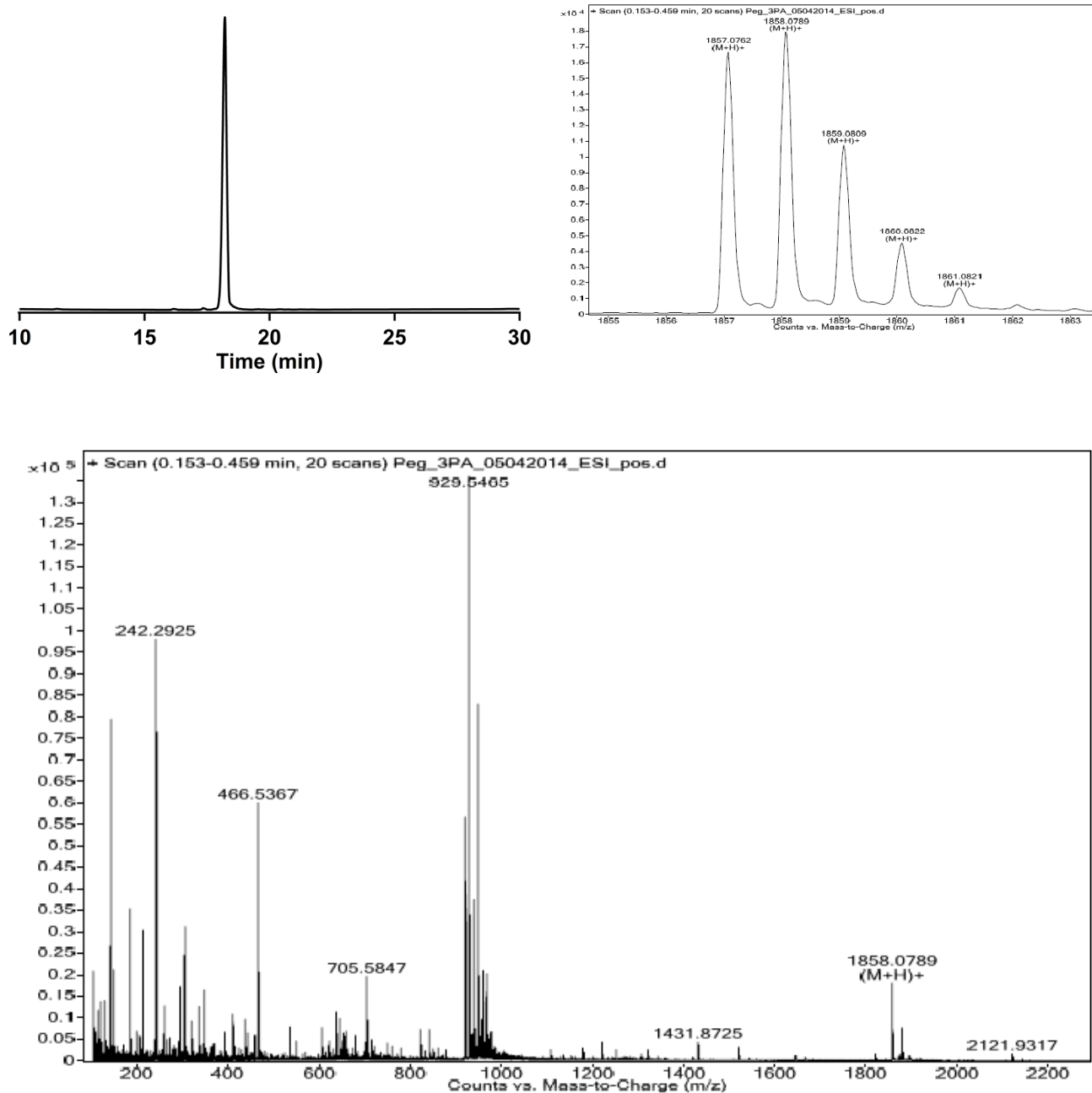


**Figure S13.** Top; proton NMR of ligand **8b**. Bottom; carbon NMR of ligand **8b**.



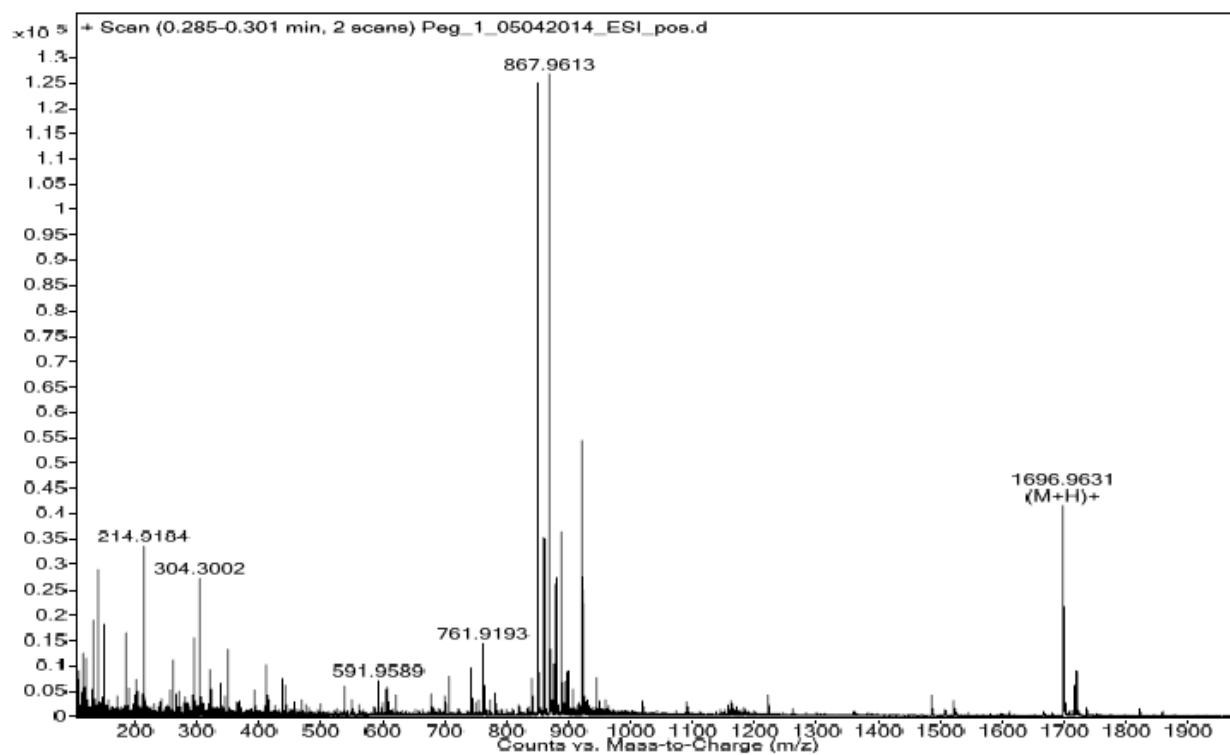
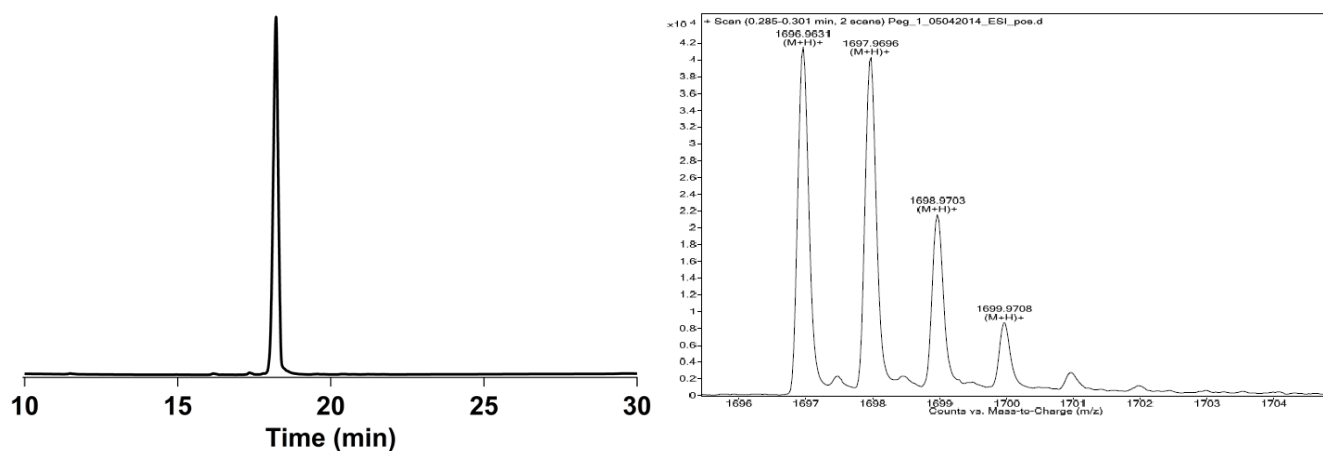
**Figure S14.** Top; proton NMR of ligand **8a**. Bottom; carbon NMR of ligand **8a**.

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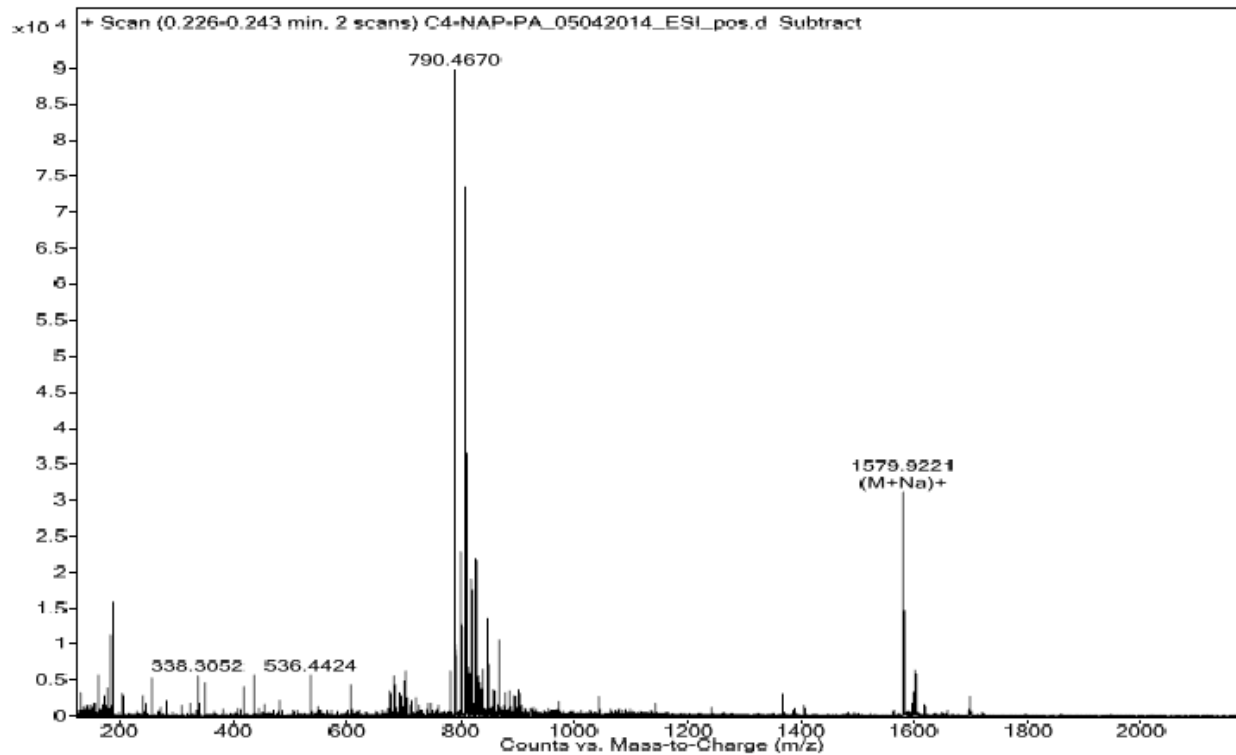
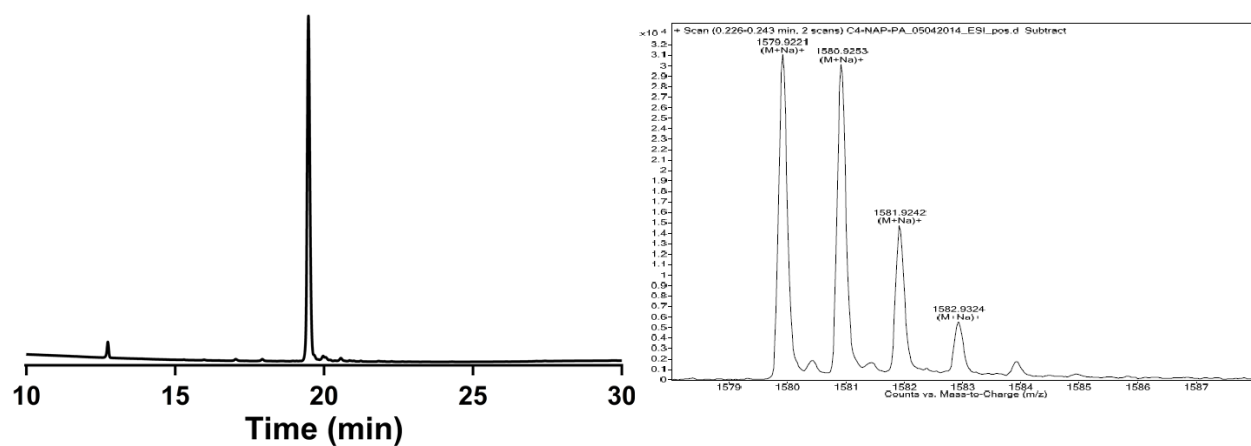


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**Figure S15.** PA 3 HPLC trace, purity > 95% (top left), PA 3 high resolution MS isotope pattern, (top right) and high resolution MS (bottom).

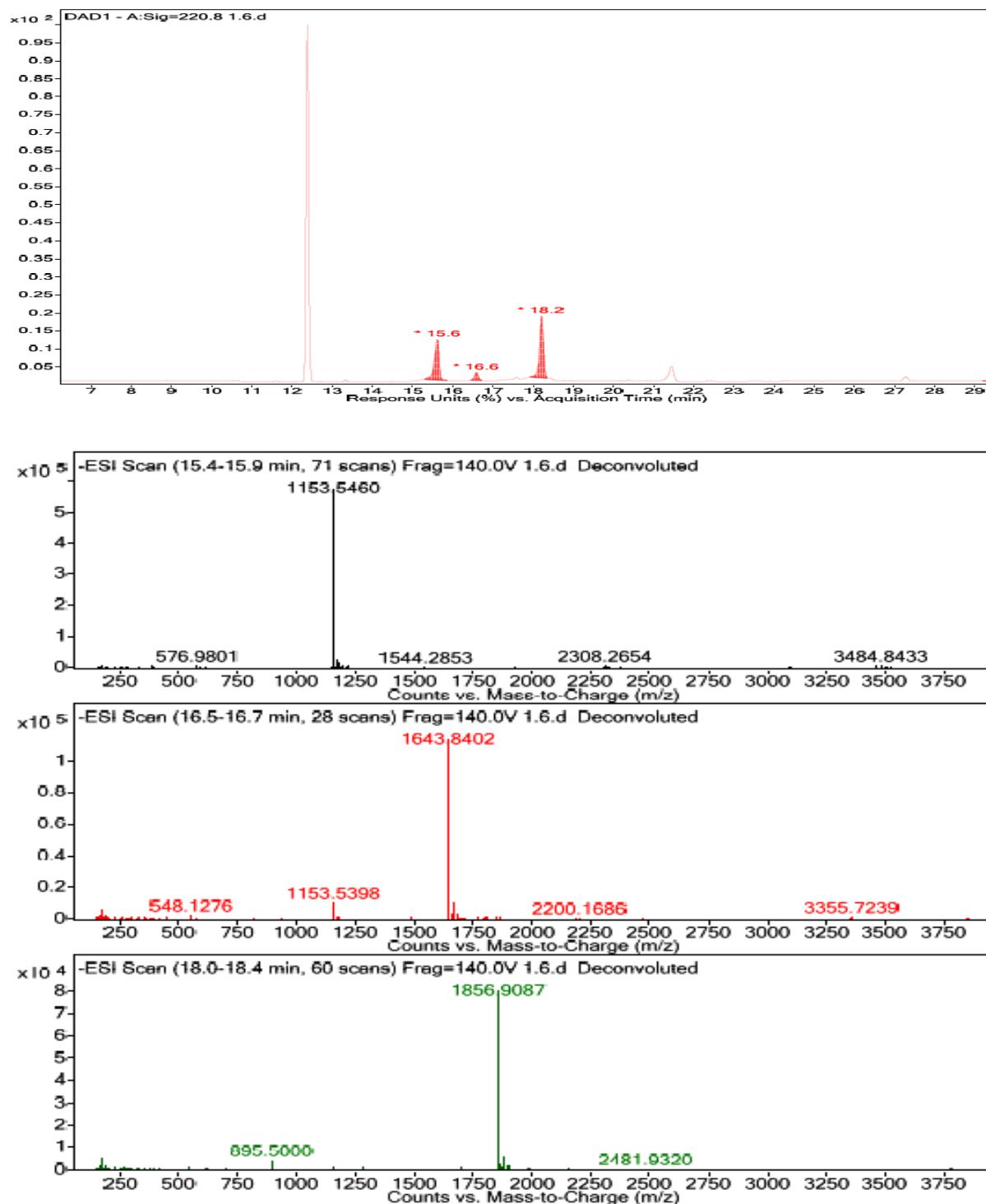


**Figure S16.** PA 4 HPLC trace, purity > 95% (top left) PA 5 high resolution MS isotope pattern (top right) and high resolution MS (bottom).



**Figure S17.** PA 5 HPLC trace, purity > 95% (top left) PA 5 high resolution MS isotope pattern (top right) and high Resolution MS (bottom).

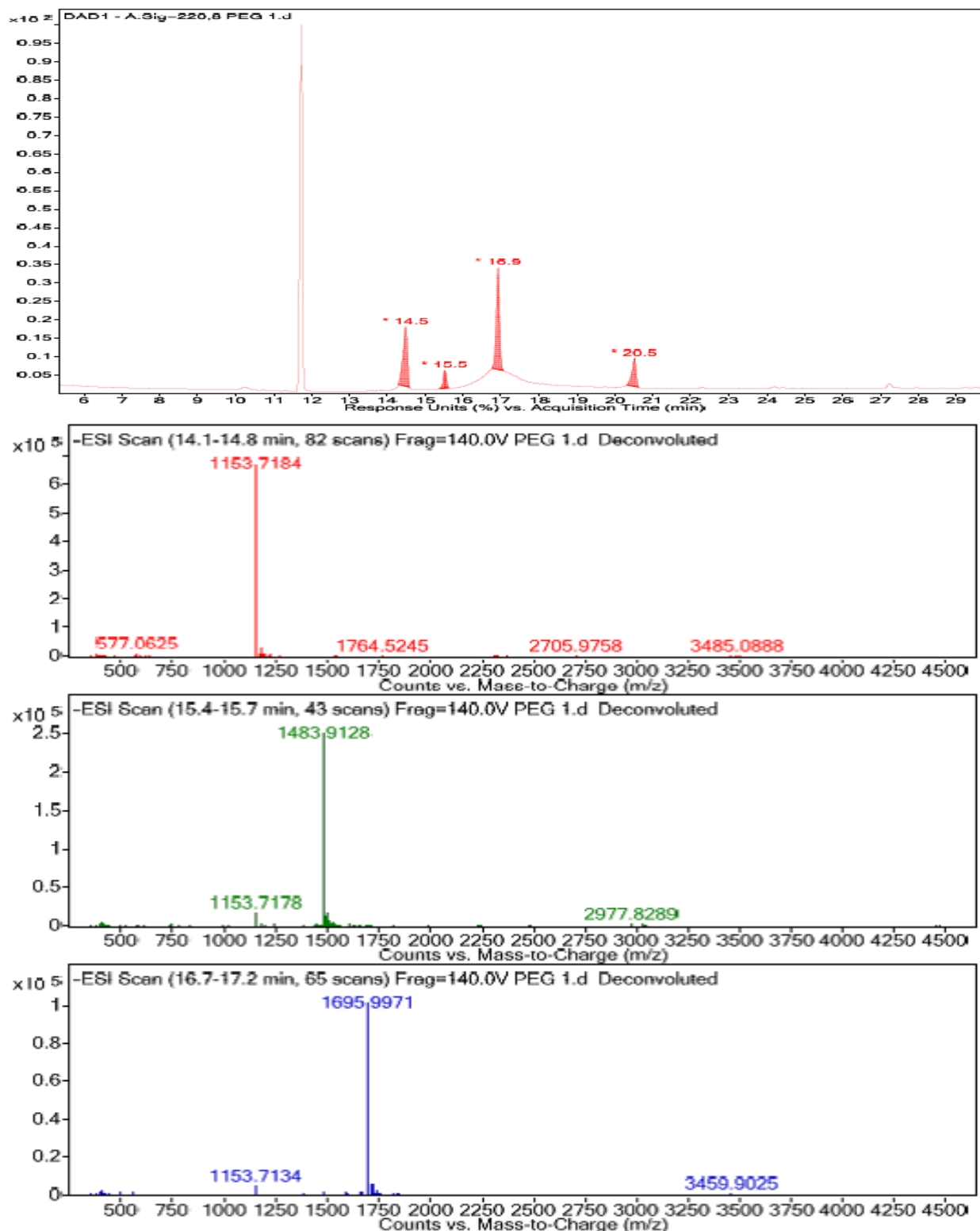




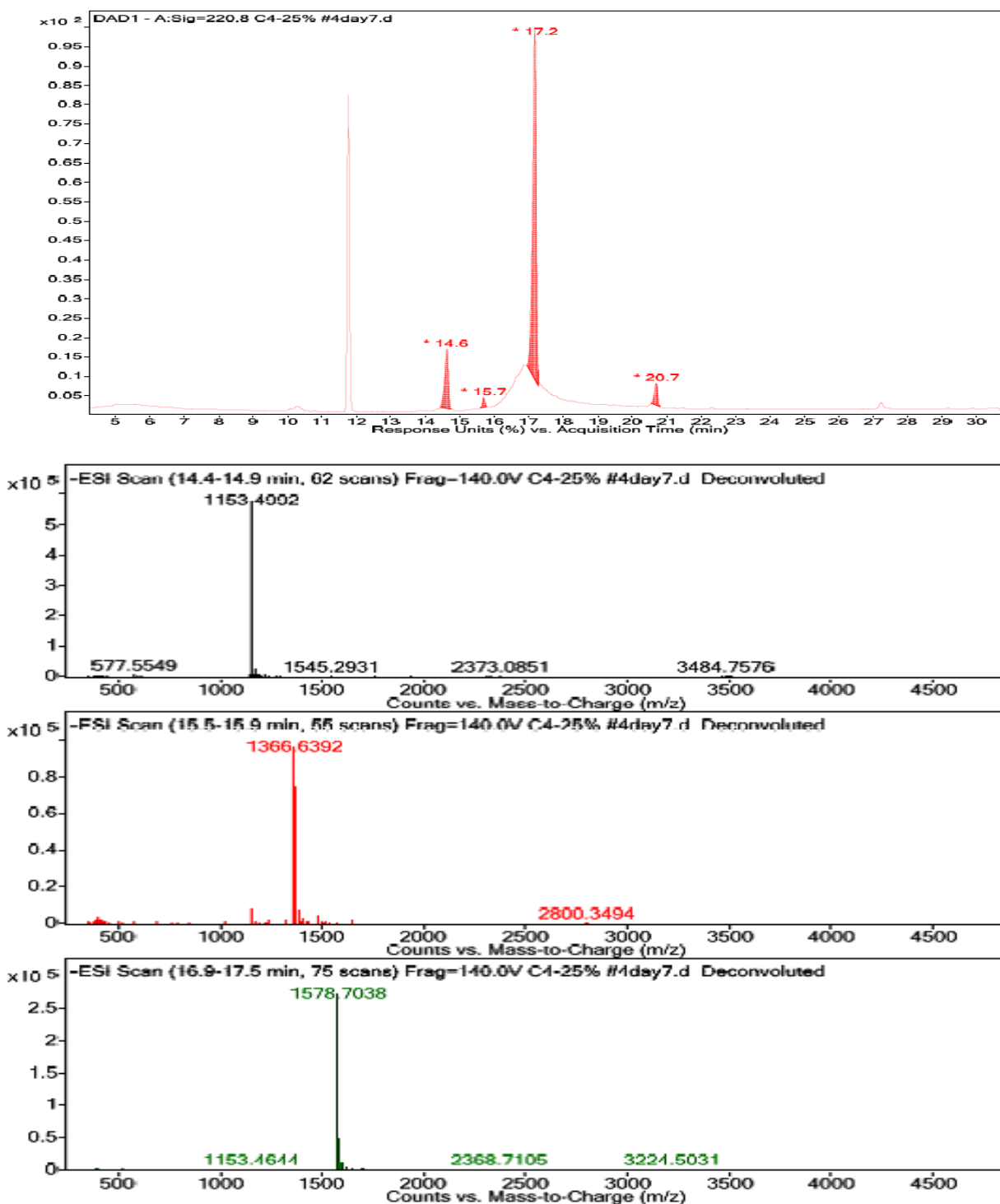
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311 **Figure S18.** Top panel; LC-MS trace of PA 3- PA E3 (1:3 molar ratio) treated with PLE. Bottom  
 312 three panels; MS of PA E3, hydrolyzed product, and PA 3.



**Figure S19.** Top panel; LC-MS trace of PA 4- PA E3 (1:3 molar ratio) treated with PLE. Bottom three panels; MS of PA E3, hydrolyzed product, and PA 4.

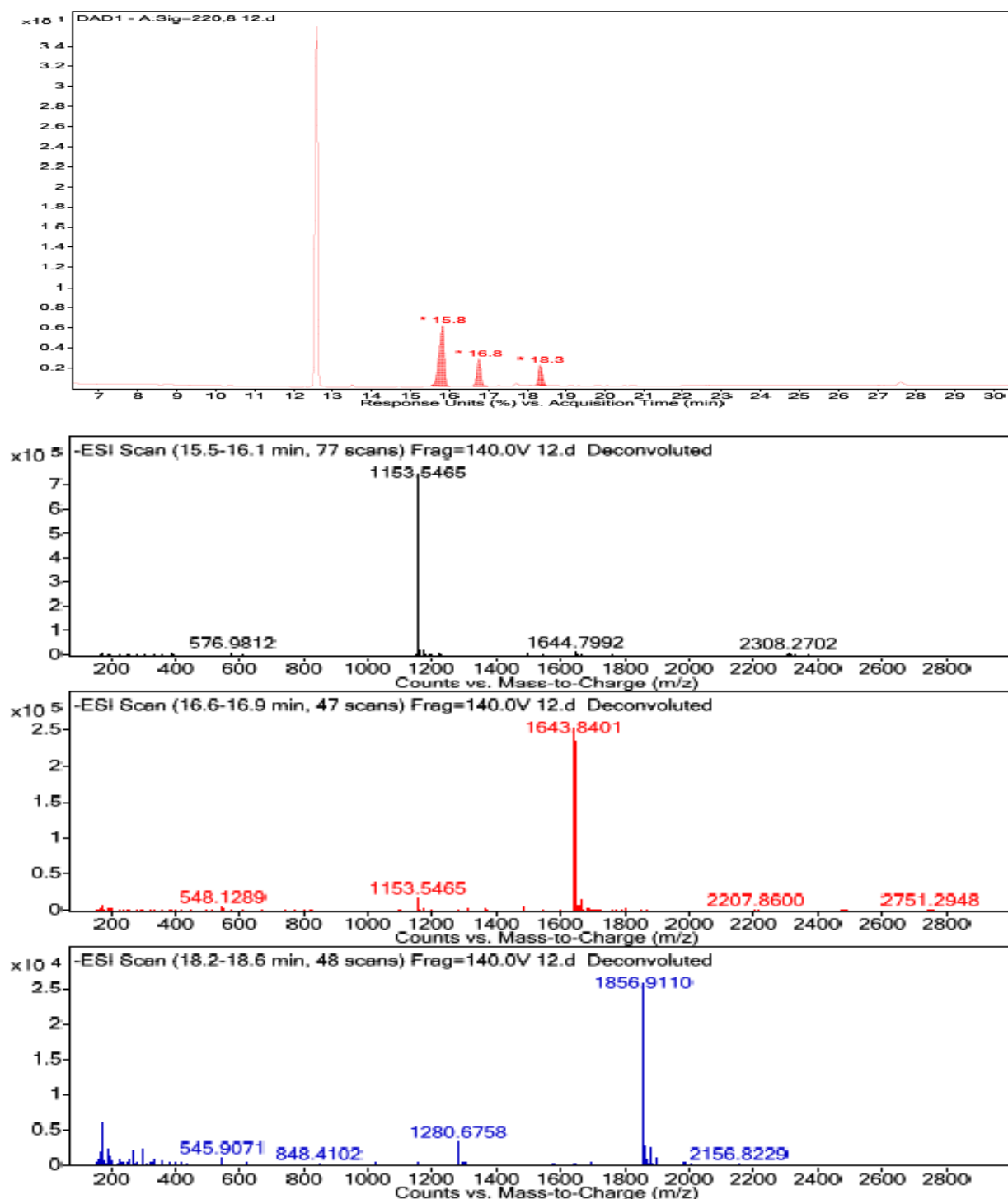


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320 **Figure S20.** Top panel; LC-MS trace of PA 5- PA E3 (1:3 molar ratio) treated with PLE. Bottom  
 321 three panels; MS of PA E3, hydrolyzed product, and PA 5.

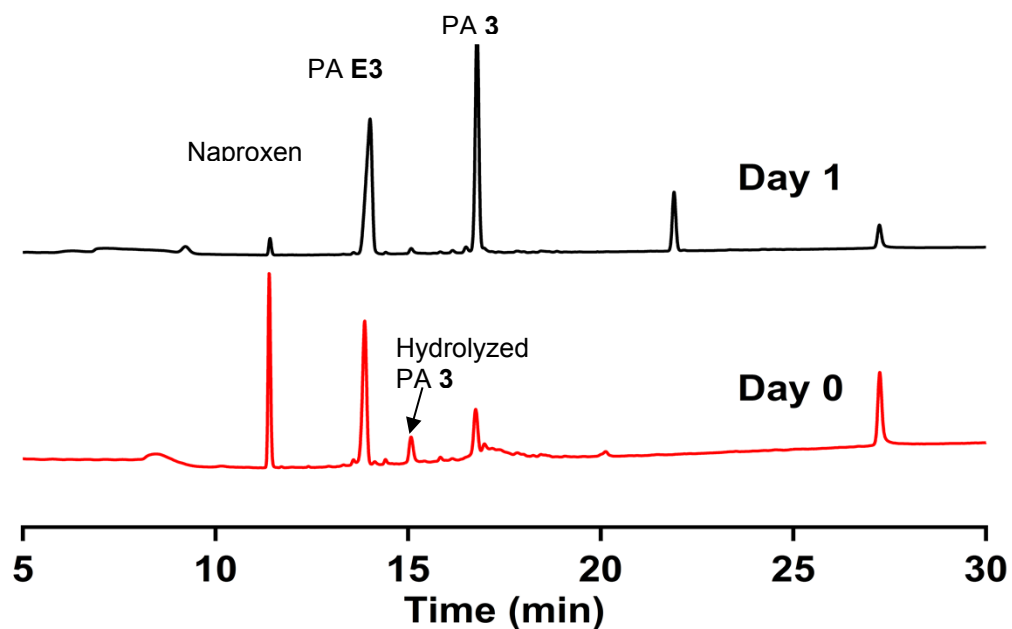


323 **Figure S21.** Top panel; LC-MS trace of PA 3- PA E3 (1:3 molar ratio) at pH 12. Bottom three  
 324 panels; MS of PA E3, hydrolyzed product, and PA 3.

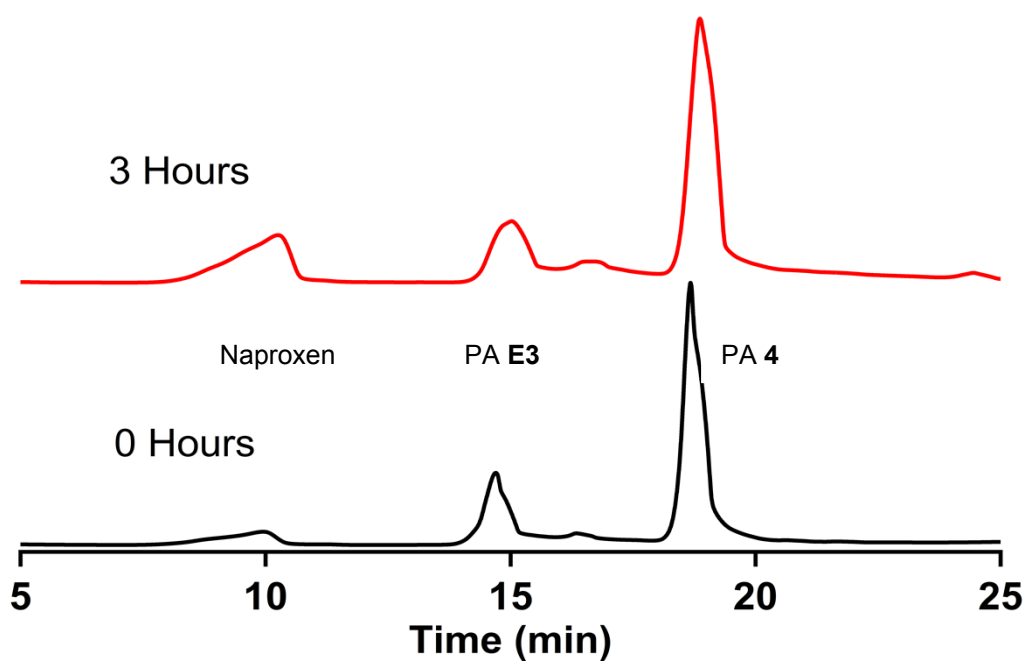
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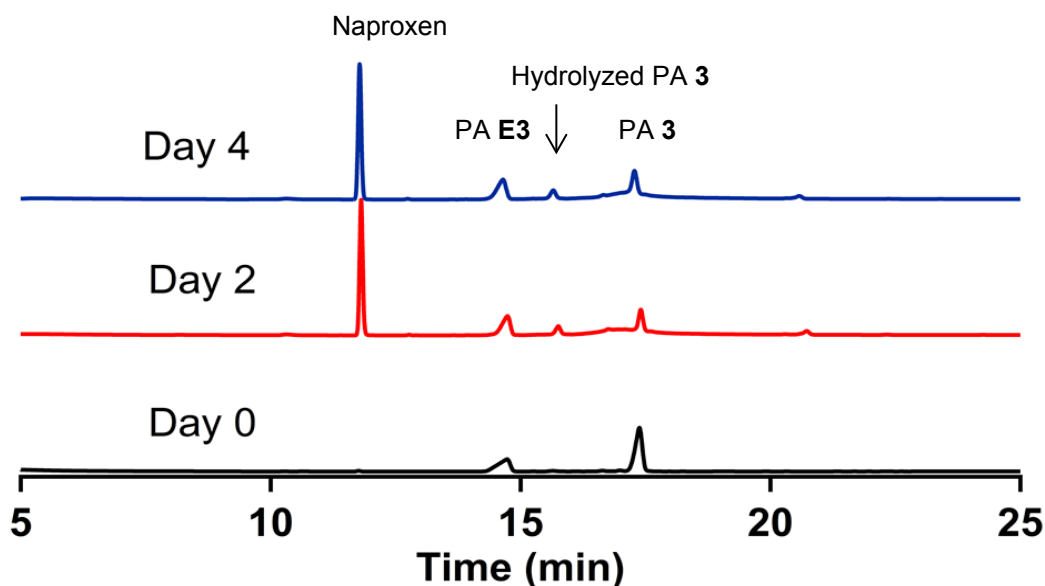
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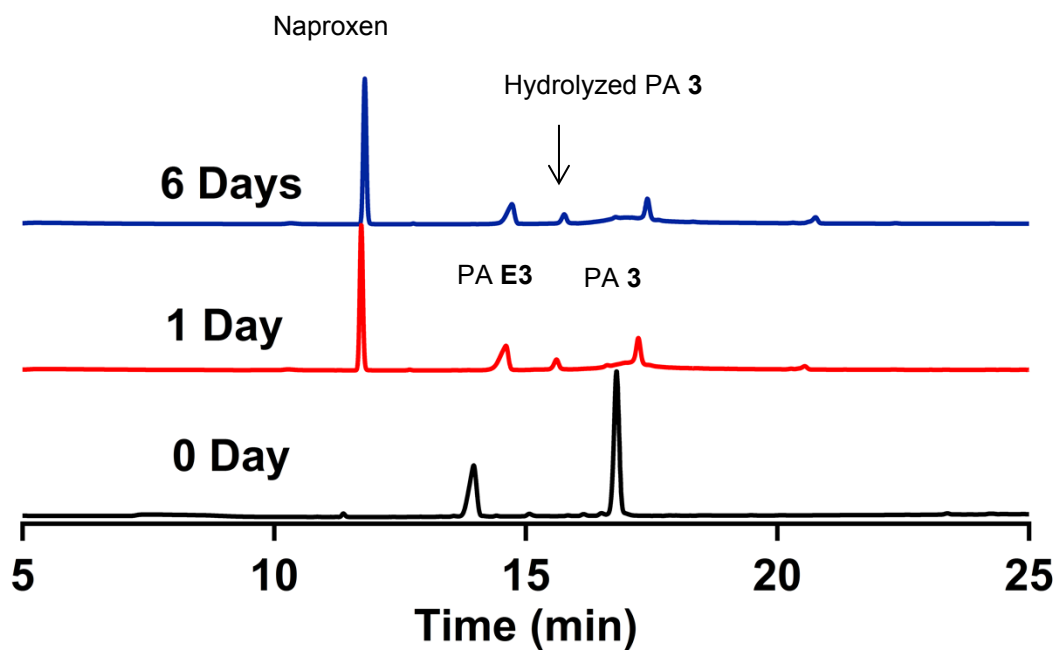
**Figure S22.** Representative HPLC trace for PA E3-PA 3 (9: 1 molar ratio)



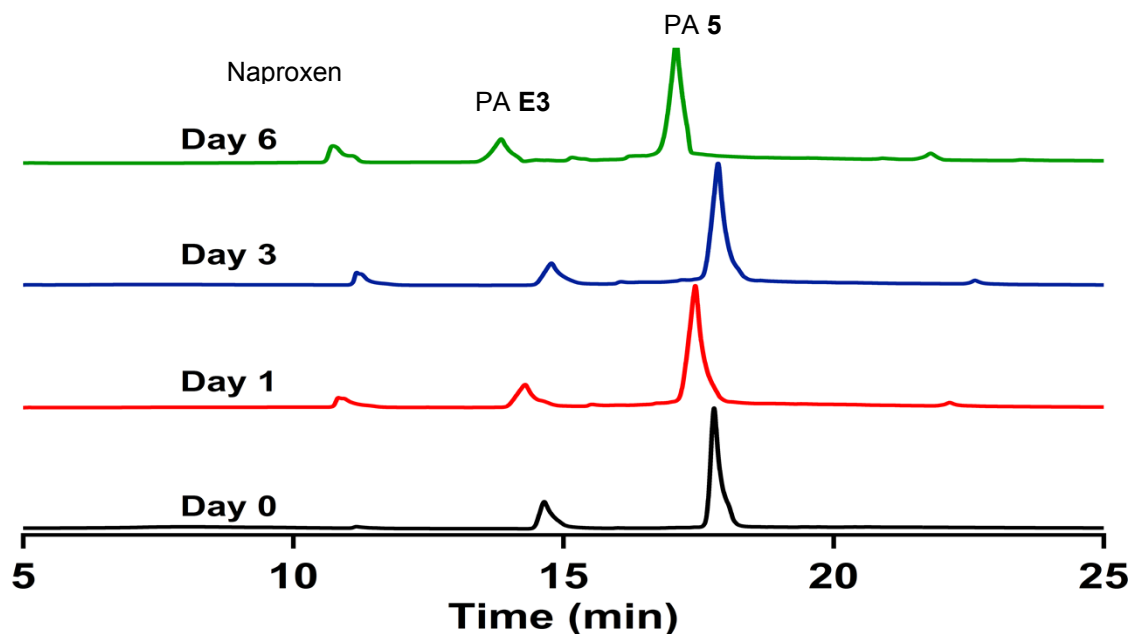
**Figure S23.** Representative HPLC trace for PA 4- PA E3 (1:3 molar ratio).



**Figure S24.** Representative HPLC trace for PA 3- PA E3 (1:3 molar ratio) at different time points.



**Figure S25.** Representative HPLC trace for PA 3- PA E3 (1:3 molar ratio) at different time points.



**Figure S26.** Representative HPLC trace for PA 5- PA E3 (1:3 molar ratio) at different time points.

#### References:

- (1) Adler, A. J.; Kistiakowsky, G. B. *J. Am. Chem. Soc.* **1962**, *84*, 695.
- (2) Guler, M. O.; Hsu, L.; Soukasene, S.; Harrington, D. A.; Hulvat, J. F.; Stupp, S. I. *Biomacromolecules* **2006**, *7*, 1855.
- (3) Matson, J. B.; Stupp, S. I. *Chem. Commun.* **2011**, *47*, 7962.
- (4) The PyMol Molecular Graphics System, V. e., Schrodinger, LLC.
- (5) Pashuck, E. T.; Cui, H. G.; Stupp, S. I. *J Am Chem Soc* **2010**, *132*, 6041.