Experimental Section

General Methods and Materials. Trifluoroacetic acid (TFA), piperidine, thioanisole, ethanedithiol, boric acid, N-bromosuccinimide, thionyl chloride, 33% hydrobromic acid in acetic acid, 2-nitrophenylacetic acid and anisole were purchased from Acros. Appropriately side-chain protected Fmoc-amino acids, 2-(1H-benzotriazole-1-yl)-1,1,3,3 tetramethyluronium hexafluorophosphate (HBTU) and 1-Hydroxybenzotriazole (HOBT) were purchased from Novabiochem. Sodium sulfate and sodium hydroxide were purchased from Fischer Scientific. Carbon tetrachloride and deuterated chloroform were purchased from Aldrich. Elutants for RP-HPLC consisted of solvent A (0.1 % TFA in water) and solvent B (90% acetonitrile, 10% water, 0.1% TFA). A flow rate of 8 mL/min was employed for preparative HPLC.

Synthesis of 2-bromo-2-(2-nitrophenyl)acetic acid (BNPA). BNPA was synthesized using a modified protocol originally reported by Bayley et al.[1] Under a dry nitrogen atmosphere, a 3-neck 50 mL round bottomed flask equipped with a condenser and stir bar was charged with 2-(2-nitrophenyl) acetic acid (1 g, 5.52 mmol). CCl_4 (1 mL) and thionyl chloride (1.60 mL, 21.9 mmol) were added sequentially via syringe. The reaction mixture was stirred for 1.5 hr at 65°C to form the acid chloride. When reaction deemed complete via ^{1}H NMR, N-bromosuccinimide (1.18 g, 6.62 mmol) was added as a solid to the reaction mixture followed by an additional 5 mL of CCl_4 and a catalytic amount of 33% HBr in acetic acid (3 drops). After 5 hr of reflux, the mixture was allowed to cool to room temperature and filtered into a 100 mL round bottomed flask. Then 15 g of ice was added and the mixture vigorously stirred for 1.5 hr to hydrolyze the acyl chloride. The CCl_4 layer was retained and the aqueous phase was extracted with CH_2Cl_2 (3 x 10 mL). The combined organic fractions were washed with 0.1 M HCl/H_2O (1 x 25 mL) and then dried with Na_2SO_4. Rotory evaporation and subsequent drying under vacuum affords 0.73 g of BNPA (2.81 mmol, 51%) as a dark brown oil. This material was used in subsequent reactions without further purification. However, a small fraction was purified for characterization via RP-HPLC (preparative Vydac C4 peptide/protein column) employing a linear gradient from 0-100% B over 102 min. Product elutes at 37 min. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.06 (d, J = 8.1, 1H), 7.99 (d, J = 7.9, 1H), 7.73 (t, J = 8.0, 1H), 7.56 (t, J = 7.8, 1H), 6.12 (s, 1H). ^{13}C NMR ($CDCl_3$, 360 MHz): δ172.2, 134.3, 133.5, 130.4, 130.3, 125.3, 42.6.

Peptide Synthesis of MAX 6 & 7. Peptides were synthesized on PAL amide resin via an automated ABI 433A peptide synthesizer employing standard Fmoc-protocol and HBTU/HOBT activation. The resulting dry resin-bound peptides were cleaved and side-chain deprotected for 2 hr under N_2 atmosphere using a TFA: thioanisole: ethanedithiol: anisole (90:5:3:2) cocktail. Filtration followed by ether precipitation afforded crude peptides which were purified by RP-HPLC (preparative Vydac C18 peptide/protein column). MAX6: Isocratic at 0% B for 2 min then a linear gradient from 0 to 16% B over 10 min, then 16 to 26 % B over 20 min, held isocratically for 2 min at 26% B after which time a linear gradient of 26 to 30% B over 12 min and finally 30 to 100% B over 58 min. Peptide elutes at 32 min. MS (ESI) *m/z*: 1130.9 [$(M+2H)^{2+}$, calcd 1131.0]. MAX7. Isocratic at 0% B for 2 min then a linear gradient from 0 to 18% B over 3 min

then 18 to 100% B over 164 min. The peptide elutes at 26 min. MS (ESI) *m/z*: 1117.6 [$(M+2H)^{2+}$, calcd 1117.9].

Synthesis of MAX7CNB. Under an N_2 atmosphere, an aqueous solution of MAX7 (32.6 mg, 0.01 mmol) was added via syringe to a sealed, foil-covered scintillation vial containing 1 mL of a buffered aqueous solution of BNPA (10.4 mg, 0.04 mmol of BNPA dissolved in 0.2 M Bis-tris-propane, pH 8.5). The reaction was monitored via RP-HPLC (analytical Vydac C18 peptide/protein column) employing a linear gradient from 0 to 100% B over 100 min. Product elutes at 37 min. Due to the slow kinetics of crude BNPA dissolution, the buffered BNPA solution used above is best prepared by the following protocol. Bis-tris-propane (BTP) (56.6 mg, 0.20 mmol) was added to a 1 mL volumetric flask and diluted with 0.5 mL H_2O. Crude BNPA (10.4 mg, 0.04 mmol) was added to an ependorph tube with a small amount of H_2O and sonicated. The solubilized BNPA was transferred to the volumetric flask containing the BTP. An additional small amount of H_2O was added to the remaining unsolubilized BNPA and sonicated. This was then added to the volumetric flask. The pH of this solution was adjusted to pH 8.5 with aqueous NaOH and H_2O added to obtain a final volume of 1 mL. MAX7CNB, MS (ESI) *m/z*: 1207.1 [$(M+2H)^{2+}$, calcd 1207.6].

Circular Dichroism Studies. CD spectra were collected on an AVIV model 215 spectropolarimeter employing 1 mm quartz cells. Peptide samples were prepared by first diluting millimolar aqueous stock solutions with water to 300 μM. Equal volume dilution of this 300 μM stock with stock buffer solution (250 mM Borate, 20 mM NaCl, pH 9.0) directly in the quartz cell affords 150 μM peptide solutions at pH 9.0 (125 mM Borate, 10 mM NaCl). The concentrations of MAX6, MAX7 (ε_{220} = 15750 cm^{-1} M^{-1}) and MAX7CNB (ε_{265} = 7400 cm^{-1} M^{-1}) stock solutions were determined by absorbance after dilution with water. Mean residue ellipticity $[\theta] = (\theta_{obs}/10lc)/r$, where θ_{obs} is the measured ellipticity in millidegrees, l is the length of the cell (cm), c is the concentration (M) and r is the number of residues. Wavelength scans were recorded at indicated temperatures using a 2 nm step size and a 2 sec averaging time. Spectra of de-caged MAX-7CNB were obtained by transferring a CD cell containing caged sample that showed no folding after 13 hr at 37°C to a preheated (37°C) sand bath. A UV lamp (spectroline Model xx-15A, UV 365 nm) was placed 3.5 cm above the sample which was subsequently irradiated for 30 min (quantitative de-caging was verified by RP-HPLC and MS (ESI). CD spectra of the resulting solution indicated β-sheet formation.

Bulk Hydrogel Preparation. MAX7 will undergo gelation at 37°C at concentrations of 0.5 wt% or greater, where MAX7CNB will not. However, gelation of MAX7CNB can be initiated by light using the following procedure that results in 500 μL of a 2 wt% hydrogel: To a solution of 10.0 mg of peptide in 250 μL of water was added 250 μL of a buffer stock solution (250 mM Borate, 20 mM NaCl, pH 9.0) resulting in a 2 wt% solution of peptide (125 mM Borate, 10 mM NaCl, pH 9.0). After minimal pipette mixing this solution is allowed to equilibrate for a few minutes at 37°C, followed by irradiation with the UV-lamp positioned 3.5 cm above the sample for 1 hr affording hydrogel.

Rheology Studies. MAX7 and MAX7CNB solutions at 2 wt% were simultaneously prepared in 1 cm x 3 cm vials. Both vials were positioned horizontally in a preheated (37°C) sand bath, resulting in a solution height of 0.3 cm. Samples were irradiated with a UV-Lamp positioned 3.5 cm above the vials. The vials were rotated every 15 min for 1 h to encourage sample mixing and complete de-caging. The samples were then transferred to the rheometer. Oscillatory rheology experiments were performed on a Rheometrics Ares stress controlled rheometer with a 25 mm diameter parallel plate geometry. Dynamic frequency sweep measurements monitoring G' (storage) and G" (loss) moduli between frequencies 1.0 and 100 rad/s and 1% strain were performed at 37° C.

Dithiodipyridine Assay (DTDP). Four 100 μL solutions of 2 wt% MAX7 were prepared in 1cm x 3 cm vials and transferred to a sand bath preheated to 37^{o}C to initiate gelation for 1 hr. During this time, two of the four samples were irradiated with a UV-Lamp positioned 3.5 cm above the vials. Immediately following, 200 μL of BTP buffer (100 mM BTP, pH 7.5) was added to each vial and incubation for 1 hr resulted in free-flowing solutions. Then, 50 μL from each vial was added to separate 300 μL solutions of buffered DTP (1 mM 2,2'-dithiodipyridine, 50 mM BTP, pH 7.5) and allowed to react for 10 min. 40 μL of each reaction mixture was transferred to a quartz cuvette (1 cm pathlength) containing 200 μL of BTP buffer (50 mM BTP, pH 7.5). Concentration of the free thiolate was determined via absorption[2] at 343 nm (ε_{343} = 7600 cm^{-1} M^{-1}) after correcting for background DTP hydrolysis. The percent of MAX7 oxidized was calculated as 61 % (+/- 5.) in the presence of light and 61 % (+/- 2) in the absence of light.

Cytotoxicity of NIH3T3 Fibroblasts. Two MAX7CNB solutions (2 wt%, 100μL total volume) were prepared in an 8 well confocal microscope cell culture chamber. The confocal plate was placed in a preheated, 37°C sand bath and irradiated with a UV-Lamp positioned 3.5 cm above the plate for 1 hr after which time hydrogelation was complete. The resultant hydrogels were placed in an incubator at 37^{o}C and 5% CO_2 for 2 hr at which time 400uL of serum free DMEM was added to the top of the hydrogels and allowed to equilibrate overnight. Stock NIH 3T3 cells used in this study were maintained on tissue culture treated polystyrene (TCTP) plates, then trypsinized, and counted using a hemacytometer. The resulting cell suspension was diluted with DMEM (containing 10% calf serum) for addition to the 8 well confocal microscope cell culture plate. Cells were plated (20000 cells / cm^2 in 200 μL) on the control borosilicate culture plate and on the decaged MAX7CNB hydrogel surface. Cells were incubated at 37°C and 5% CO_2 for 24 hr. Cytotoxicity was than assessed via a Live/Dead assay. A stock solution containing both 1 μM calcein AM and 2 μM ethidium homodimer in DMEM was prepared according to the Live/Dead assay (Molecular Probes # L3224) package instructions, and 200 μL of this stock was added to each well. Micrographs of cells were obtained using 10x magnification on a Zeiss 510 LCM confocal microscope. Green cells indicate viability as the cell permeable calcein AM is hydrolyzed to fluorescent calcein. Red fluorescence indicates cell death due to an increase in ethidium homodimer fluorescence upon binding the DNA of cells with compromised membranes.

Actin and Nuclear Staining of NIH3T3 Fibroblasts. MAX7CNB hydrogel preparation and cell seeding were performed as stated above. NIH 3T3 cells were fixed after 8 hr

using a 4% paraformaldehyde solution in Ca/Mg-free PBS (Cellgro 21031CV) for 30 min. Cells were washed three times with PBS and permeabilized for 5 min using 0.5% TRITON-X-100 in water. The cells were again rinsed and stained with 150 μL of a 286 μM solution of 4',6-diamidino-2-phenylindole (Molecular Probes) in Ca/Mg-free PBS. Next, 200 μL of a 22 nM solution of Alexa fluor 488 phalloidin (Molecular Probes A12379) in Ca/Mg-free PBS was added and allowed to incubate for 1 hr. The cells were washed with PBS and stored under refrigeration with 200 μL PBS. Images of cells were obtained using 20x magnification on a Zeiss 510 LCM confocal microscope.

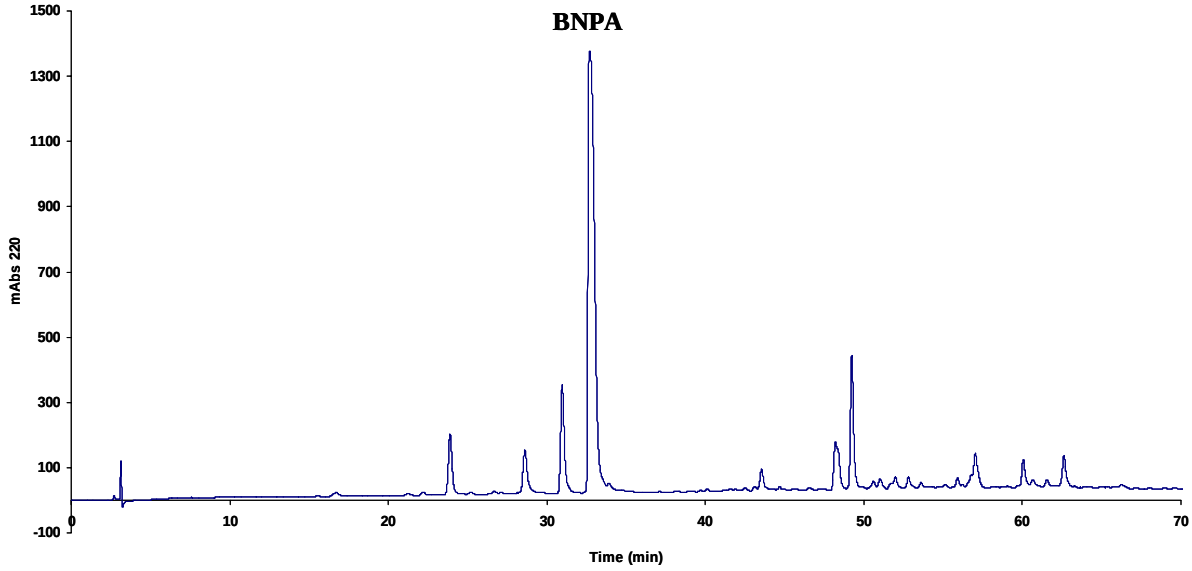
Cell Proliferation Rate Assay. MAX7CNB solutions (2 wt%, 100μL total volume) were prepared in five 48-well tissue culture-treated polystyrene plates. Plates were placed in a preheated, 37°C water bath and irradiated with a UV-Lamp positioned 3.5 cm above the plates for 1 hr after which time hydrogelation was complete. The resultant hydrogels were placed in an incubator at 37^{o}C and 5% CO_2 for 2 hr. After which time, 200μL of serum free DMEM was added to the top of the hydrogels and allowed to equilibrate for 1 hr. This solution was then removed and 200 μL fresh DMEM with 10% calf serum was added and plates were then placed in the incubator overnight. Quiescent NIH 3T3 cells were trypsinized, counted using a hemacytometer, and a stock solution of 200,000 cells/mL was prepared. The DMEM solution above the gels was removed and in tripilicate, to both a control TCTP surface and the MAX7CNB +hν hydrogel, 100 μL of the stock solution was added to each well resulting in 20,000 cells/well. 300 μL of DMEM with 10% calf serum was added resulting in a final volume of 400 μL. The plates were incubated at 37 °C and 5% CO_2 and fresh media was replaced after 24 hours. At each time point (6, 24, 48, and 72 hours) a plate was removed and assayed by ^{3}H-thymidine incorporation. For ^{3}H-thymidine incorporation, media was removed from each well and replaced with 200 μL DMEM (serum free) containing ^{3}H-thymidine (1 μCi/mL). Following incubation for 2 hours, the radioactive media was removed and the cells were washed 6X with DMEM (500 μL, gel samples only) and 2x PBS (200 μL) to remove unincorporated ^{3}H-thymidine. Cellular DNA was then solubilized with 1N NaOH (500 μL) and 450 μL was transferred to a scintillation vial containing 5 mL ScintiSafe cocktail for counting.

References:

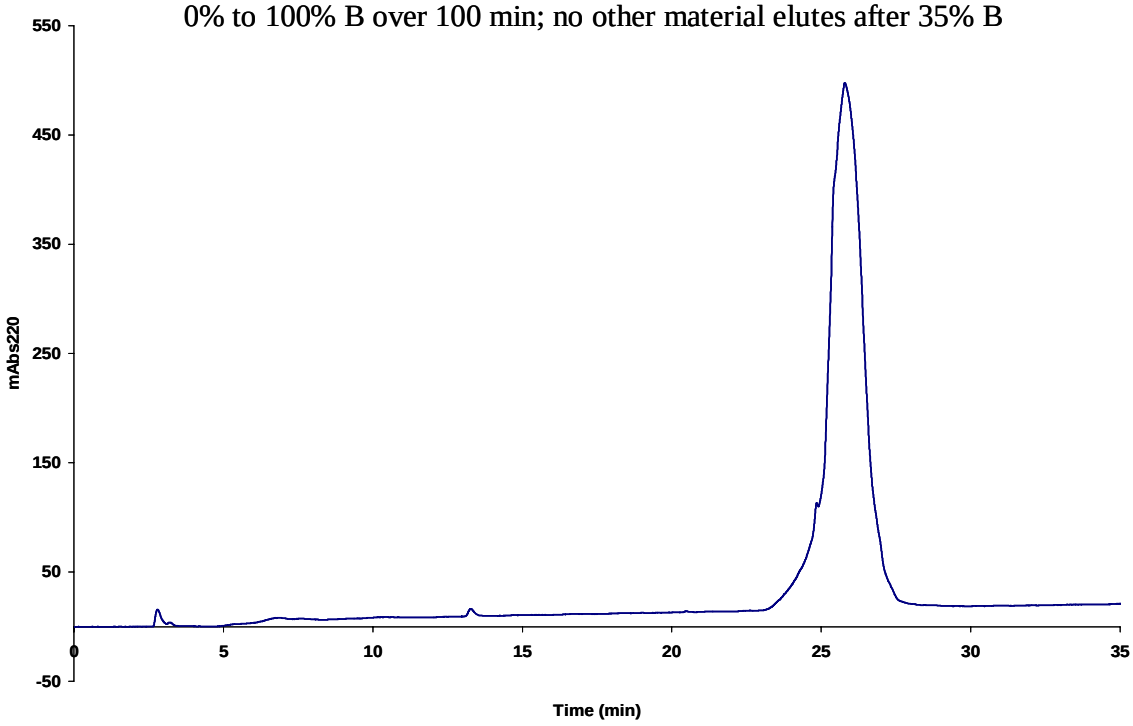
(1) Chang, C. Y.; Niblack, B.; Walker, B.; Bayley, H., *Chemistry & Biology* **1995,** *2,* 391-400.
(2) Pedersen, A. O.; Jacobsen, J., *European Journal of Biochemistry* **1980,** *106,* 291-295.

HPLC solvent A: (0.1 % TFA in water)
HPLC solvent B: (90% acetonitrile, 10% water, 0.1% TFA)

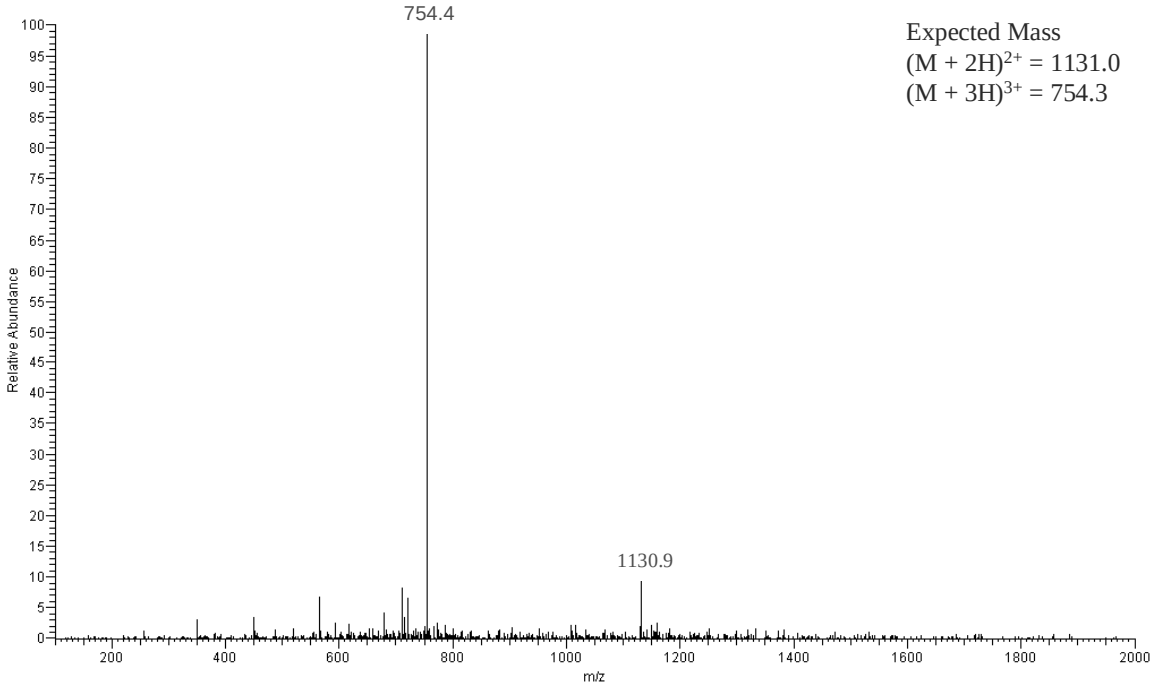
Analytical HPLC (Vydac C18) of Crude BNPA
0% to 100% B over 100 min



Analytical HPLC (Vydac C18) of Purified MAX-6
0% to 100% B over 100 min; no other material elutes after 35% B



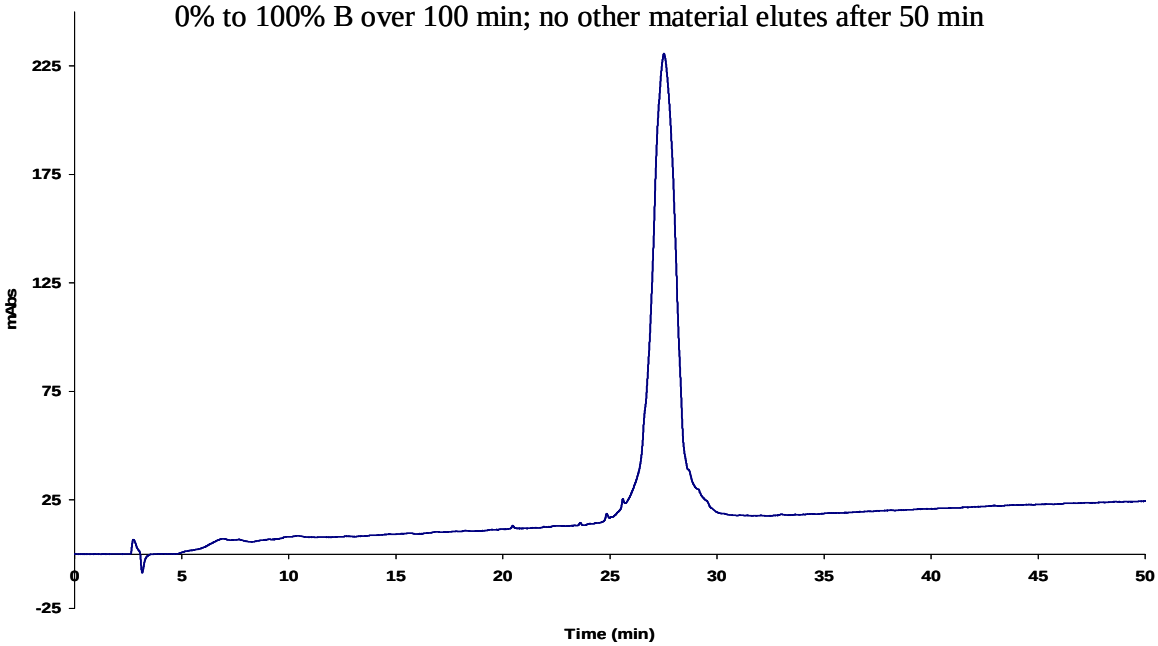
ESI Mass Spectrum of Purified MAX-6



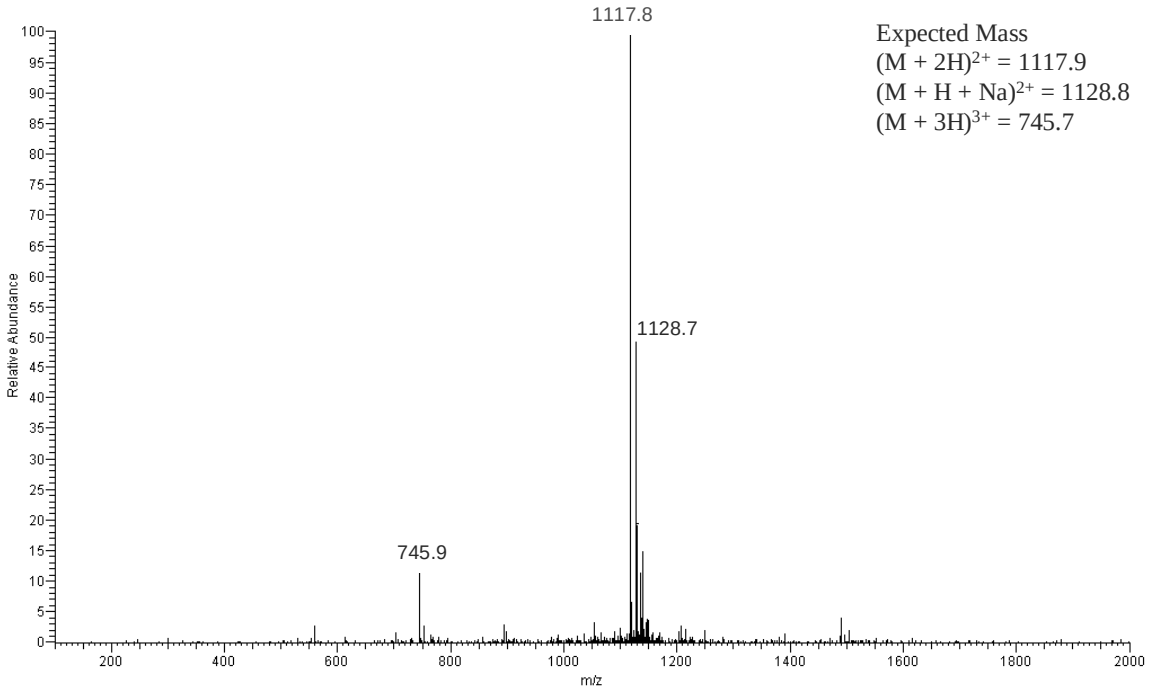
Expected Mass
$(M + 2H)^{2+}$ = 1131.0
$(M + 3H)^{3+}$ = 754.3

Analytical HPLC (Vydac C18) of Purified MAX-7

0% to 100% B over 100 min; no other material elutes after 50 min

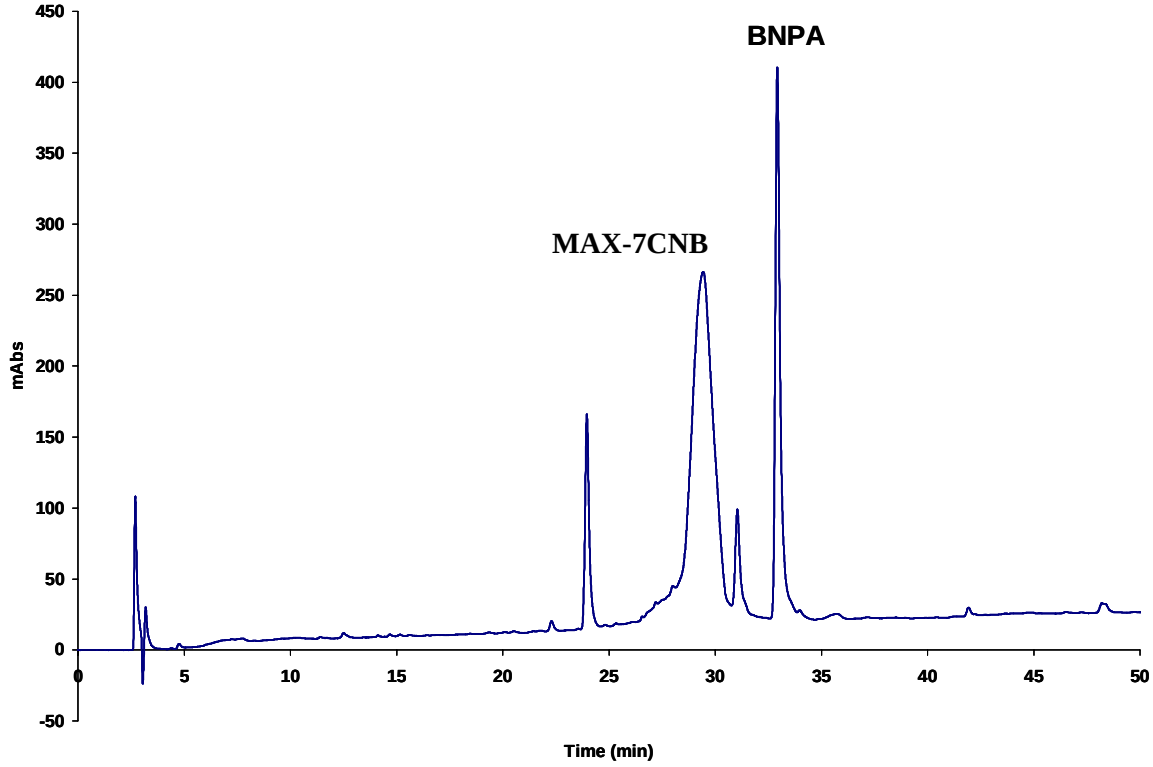


ESI Mass Spectrum of Purified MAX-7



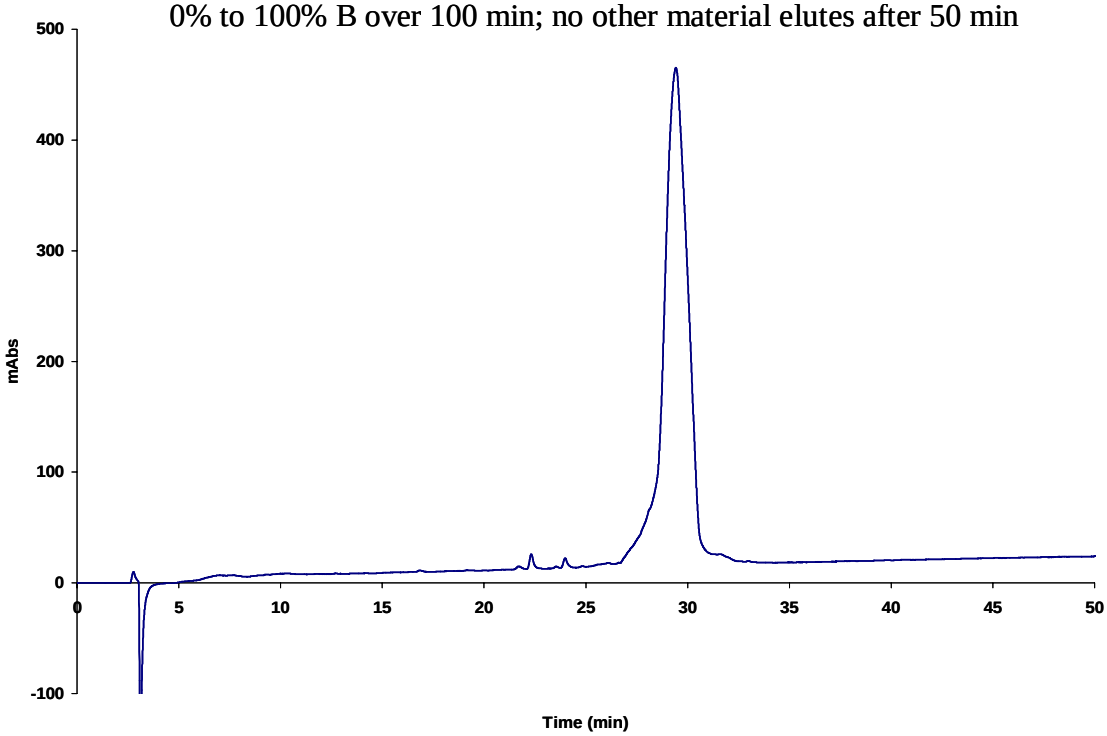
Analytical HPLC (Vydac C18) of Purified MAX-7 / Crude BNPA Reaction Mixture

0% to 100% B over 100 min; no other material elutes after 50 min

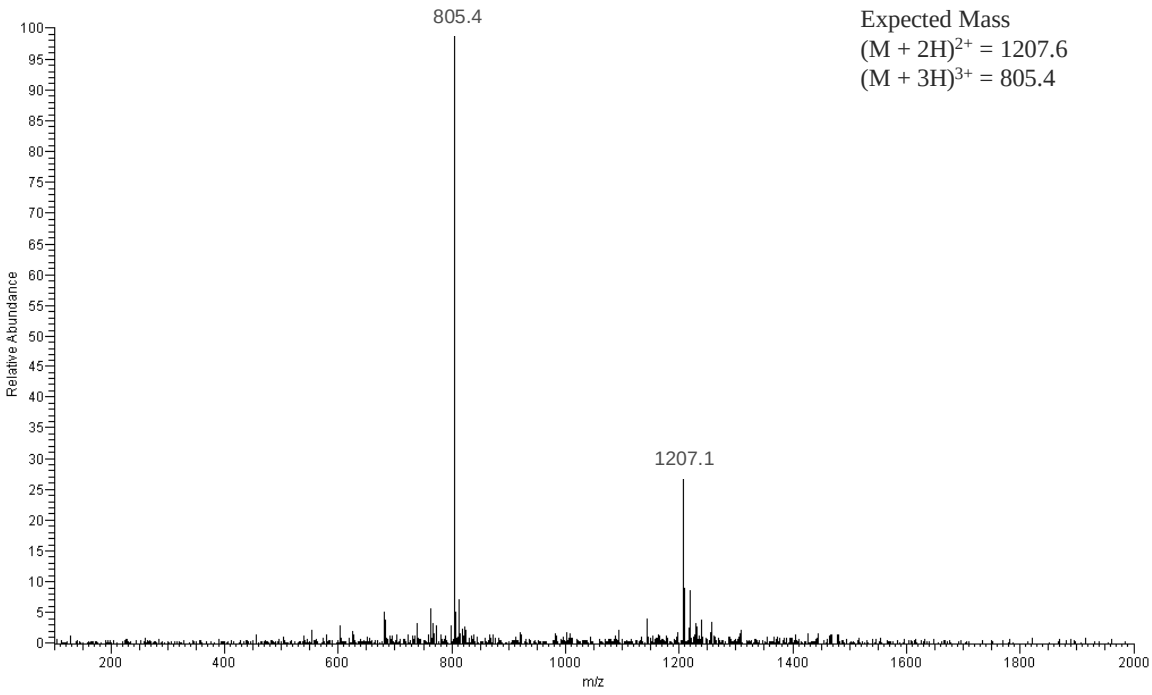


Analytical HPLC (Vydac C18) of Purified MAX-7CNB

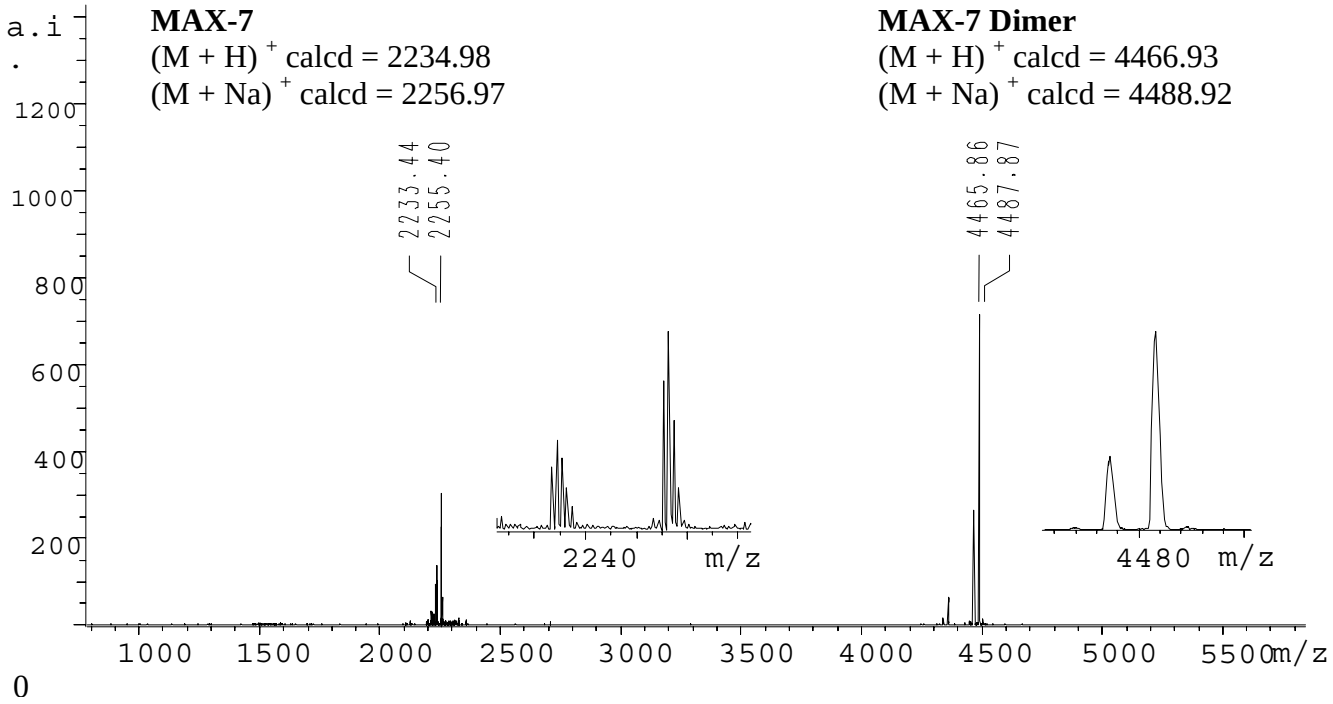
0% to 100% B over 100 min; no other material elutes after 50 min



ESI Mass Spectrum of Purified MAX-7CNB



MALDI-TOF of MAX-7 after Gelation



MAX-7
$(M + H)^+$ calcd = 2234.98
$(M + Na)^+$ calcd = 2256.97

MAX-7 Dimer
$(M + H)^+$ calcd = 4466.93
$(M + Na)^+$ calcd = 4488.92