SUPPLEMENTAL DATA

Experimental Procedures

Ninhydrin Assay: A stock solution of leucine was made to be approximately 10 mM in water, using a volumetric flask. A series of more dilute leucine standards were made from this original stock solution to cover the amine concentration range being examined. The products to be tested were dissolved in water at 0.1 mg/mL and pipetted (100 μl per sample) into individual microcentrifuge tubes. Next, 200 μl of ninhydrin reagent was added and the tubes were sealed and placed in boiling water for 10 minutes. The solutions were cooled to room temperature, 600 μl of ethanol was added and the samples were analyzed by looking at the absorbance at 562 nm. A graph of the absorbance intensity versus concentration was linear from mM to nM concentrations and used to determine the concentration of primary amine present in the sample.

(2a,4a,5ß,7ß,10ß,13a)-4,10-bis(acetyloxy)-13-{[(2R,3S)-3-(benzoylamino)-2-oxobutanoic acid-3-phenylpropanoyl]oxy}-1,7-dihydroxy-9-oxo-5,20-epoxytax-11-en-2-yl benzoate (PXL-COOH): Paclitaxel (77.61 mg, 0.091 mmols) was dissolved in methylene chloride (6 mL). Succinic anhydride (13.1 mg, 0.131 mmols) in 0.8 mL of methylene chloride was added followed by pyridine (27 μl). The reaction was stirred for 4 days. TLC (1:1 hexanes: ethyl acetate on silica gel) indicated 4 spots under a UV lamp and with potassium permanganate staining. Staining with bromo-cresol green indicated that the carboxylic acid was the last spot. The reaction was concentrated onto silica gel and purified with the Biotage™ SP1 system using a hexane/ ethyl acetate gradiant followed by flushing with methanol to elute the product. After concentration the last peak eluted was 84.5 mg (97%) of desired product as confirmed by MS. MS $(ESI)^+$ Found: 954.30 Dalton $(M + H)^+$; calculated: 954.99 Da. ($C_{51}H_{56}NO_{17}$).

5-((2S,3S,4S,6R)-3-hydroxy-2-methyl-6-((1S,3S)-3,5,12-trihydroxy-3-(2-hydroxyacetyl)-10-methoxy-6,11-dioxo-1,2,3,4,6,11-hexahydrotetracen-1-yloxy)tetrahydro-2H-pyran-4-ylamino)-5-oxopentanoic acid (DOX-COOH): First, 2.33 mg (0.00402 mmols) of Doxorubicin

((8S,10S)-10-((2R,4S,5S,6S)-4-amino-5-hydroxy-6-methyltetrahydro-2H-pyran-2-yloxy)-6,8,11-trihydroxy-8-(2-hydroxyacetyl)-1-methoxy-7,8,9,10-tetrahydrotetracene-5,12-dione) was dissolved in 0.5 mL of pyridine. Then, glutaric anhydride (0.46 mg, 0.00403 mmols) was added. The reaction was covered with aluminum foil and stirred overnight. Solvent was removed under vacuum and the product was purified on the Biotage SP1™ system with a gradient from 100% methylene chloride to 25% methanol. The product peak was concentrated to give 2.55 mg (96%). ^{1}H NMR 400 MHz; δ 0.9779 (t, 2H), 1.55 (d, 2H), 1.69 (s, 4H), 1.76 (s,2H), 2.72 (s, 2H), 2.98 (s, 6H), 3.41 (bs, 7H), 6.63 (s, 4H), 7.27 (s, 1H), 7.37 (s, 1H), 7.47 (s, 1H), and 8.98 (s, 2H) ppm. ^{13}C NMR 125 MHz. δ 15.7 (CH_3), 21.7, 22.1, 25.2 (CH_2), 32.8 (CH_2), 33.1 (CH_2), 34.1 (CH_2), 36.3 (CH_2), 42.0, 43.5, 54.5 (C-N), 56.1 (CH_3-O), 60.2 (C-O), 65.5 (C-O), 68.3 (C-O), 69.8 (C-O), 72.3 (C-O), 90.3, 94.1 (Acetal), 114.2 (Ar), 116.2 (Ar), 118.2 (Ar), 120.2 (Ar), 121.4 (Ar), 158.3 (Ar), 158.5 (Ar), 158.7 (Ar), 158.8 (Ar), 159.0 (Ar), 171.4 (HNCO), 172.0 (COOH), and 174.7 (C=O) ppm. COSY correlations: 0.98 to 2.98, 1.55 to 1.76, 1.67 to 3.41, 1.67 to 2.98, 2.98 to 8.98 ppm.MADLI-TOF MS (CHCA) Found 680.39 Dalton (M + Na)+ and 696.94 (M+K)+ calculated 680.61 Dalton (C32H35NO14Na) and 696.72 Dalton ($C_{32}H_{35}NO_{14}K$).

2-(benzyloxycarbonyl)-2-methylpropane-1,3-diyl bis[3-(2-tert-butoxycarbonylamino acetate)-2-((2-tert-butoxycarbonylamino acetate)-methyl)-2-methylpropanoate) (Boc-Gly-Dendron, 2): All glassware used in the reaction was flame dried and cooled in a desiccator prior to use. The solvents were anhydrous and all solid materials were dried under vacuum for more than 3 hours prior to use and exposed to anhydrous Ar(g) gas upon breaking the vacuum seal. First, 2-(benzyloxycarbonyl)-2-methylpropane-1,3-diyl bis(3-hydroxy-2-(hydroxymethyl)-2-methylpropanoate) (compound **1**) (1.1849g, 2.60 mmols), 2-(tert-butoxycarbonylamino)acetic acid (Boc-Gly, 2.0277g, 11.6 mmols) and EDCI (2.4257g, 12.7 mmols) were dissolved in DMF individually and then mixed under $Ag_{(g)}$. Next, DIEA (3.6 equivalents) was added via syringe and the reaction was stirred overnight (16 hours). The reaction was concentrated under

reduced pressure, dissolved in methylene chloride (100 mL) and washed with water (3 x 100 mL). The aqueous layers were combined and extracted with methylene chloride (3 x 100 mL). The organic layers were combined and TLC with 1:1 hexanes: ethyl acetate revealed 4 spots (R_f = 0.7, 0.38, 0.2, 0.12) after staining with potassium permanganate. The organic layers were combined and dried with magnesium sulphate, filted and concentrated onto silica gel at reduced pressure. The product was purified on the Biotage SP1 system with a 40i column using a gradiant (100% hexanes to 100% ethyl acetate over 700 mL). The product was isolated as the second peak eluted (R_f = 0.38), and concentrated as a viscous yellow oil to yield 886.6 mg pure product (31%). MS $(ESI)^+$: 1107.5 Dalton $(M + Na)^+$; Calculated: 1107.48 Dalton ($C_{50}H_{76}N_4O_{22}Na$). ^1H NMR 400 MHz (d_6-DMSO) δ 8.00 (s, 2H), 7.35 (s, 5H), 5.18 (s, 2H), 4.13 (m, 11H), 3.77 (dm, 12H), 2.96 (s, 6H), 2.88 (s, 7H), 2.05 (s, 5H), 1.50 (s, 6H), 1.43 (s, 6H), 1.26 (s, 9H), 0.98 (s, 6H) ppm.

2-(benzyloxycarbonyl)-2-methylpropane-1,3-diyl bis[3-(2-amino acetate)-2-((2-amino acetate)-methyl)-2-methylpropanoate) (ND_2, 3): Boc-Gly-Dendron was dissolved in 1 mL of 1:1 methylene chloride to trifluoroacitic acid. The reaction was stirred overnight then concentrated under vacuum to provide an oil with a very slight yellow color. The product was used without further purification. MS $(ESI)_+$: 685.40 Dalton $(M+H)_+$; Calculated: 685.29 Dalton ($C_{30}H_{45}N_4O_{14}$). 1H NMR 400 MHz (d_6-DMSO) δ 8.05 (bs, 4H), 7.36 (s, 5H), 5.14 (s, 2H), 4.25 (bs, 11H), 3.81 (bs, 9H), 1.22 (s, 3H), 1.13 (s, 6H) ppm.

$ND_2^{(Fmoc\text{-}Pep)}$ (4): All glassware used in the reaction was flame dried and cooled in a desiccator prior to use. The solvents were anhydrous and all solid materials were dried under vacuum for more than 3 hours prior to use and exposed to anhydrous Ar(g) gas upon breaking the vacuum seal. The peptide of sequence Fmoc-[Ahx]-AVRWLLTA-[Ahx]-COOH (35.12 mg, 0.026 mmols) was dissolved in 3.5 mL DMF with 4□ molecular sieves). Solutions of EDCI (20 mg/mL, 5.87 mg, 0.0306 mmols) and BOP (20 mg/mL, 13.5 mg, 0.0305 mmols) were made separately and

added to the peptide solution followed by DIEA (10 μl. 0.109 mmols). The peptide solution was stirred for 30 minutes at room temperature before ND_2 (3.5 mg, 0.00511 mmols) was added to the reaction. The reaction was stirred under $Ar_{(g)}$ for 69 hours with SEC being completed after 0.3, 1.5, 21, 24, 44, 49 and 69 hours. The reaction was filtered and the filter cake with molecular sieves was washed with DMF. The filtrate was concentrated under reduced pressure to give a brown oil. The oil was dissolved in actetonitrile/water and purified by SEC to elute 6 fractions, which were concentrated and characterized individually. The first peak eluted provided 22.5 mg (72%) of product **4**. MADLI-TOF MS (CHCA): Found 6,167 Dalton $(M+K)^+$; calculated 6,162 Dalton ($C_{314}H_{453}N_{60}O_{66}K$). SEC (55% 0.05% TFA in Acetonitrile/ 45% 0.065% TFA in water) elution time 14 minutes. Ninhydrin assay (see procedure above): no primary amines present.

ND_2^{Pep} (5): $ND_2^{(Fmoc\text{-}Pep)}$ (**4**) (4.01 mg) was dissolved in 1 mL of DMF and injected onto the SEC column. Then 1 mL of piperidine was added followed by a second SEC injection (within 60 seconds). The reaction was stirred overnight, injected onto the SEC column, concentrated under vacuum. The oily product was dissolved in water and lyophilized overnight to give an extremely viscous yellow product. MADLI-TOF MS (CHCA) Found 1,256 Dalton (Fragment: [Ahx]-AVRWLLTA-[Ahx]-Gly + $K)^+$; calculated 1,251 Dalton ($C_{58}H_{97}N_{15}O_{13}K$). SEC (55% 0.05% TFA in Acetonitrile/ 45% 0.065% TFA in water) elution time 21.5 minutes. Ninhydrin assay (see procedure above): 4 primary amines present.

ND_2^{DOX} (6): All glassware used in the reaction was flame dried and cooled in a desiccator prior to use. The solvents were anhydrous and all solid materials were dried under vacuum for more than 3 hours prior to use and exposed to anhydrous Ar(g) gas upon breaking the vacuum seal. DOX-COOH (1.37 mg, 0.00209 mmols) was dissolve in anhydrous DMF and EDCI (0.7 mg, 0.00365 mmols) and DIEA (0.7 μl, 0.00765 mmols) were added. The reaction was stirred for 30 minutes prior to the addition of ND_2 (1.37 mg, 0.000519 mmols) in 0.5 mL DMF. The reaction

was stirred under $Ar_{(g)}$ until SEC indicated completion. SEC was completed after 5 minutes, 2, 6, 24, 27 and 48 hours. After 48 hours, not change had occurred since the 27 minute chromatogram. The reaction was concentrated under vacuum and purified with SEC chromatography to give 25 mg (79%) of ND_2^{DOX}. MADLI-TOF MS (CHCA) Found 1981 Dalton $(M+4K)^{+4}$; calculated 1,984 $(C_{81}H_{543}N_{64}O_{110}K_4)/4$.

ND_2^{PXL} (7): All glassware used in the reaction was flame dried and cooled in a desiccator prior to use. The solvents were anhydrous and all solid materials were dried under vacuum for more than 3 hours prior to use and exposed to anhydrous Ar(g) gas upon breaking the vacuum seal. First, PXL-COOH (8.51 mg, 0.0091 mmols) was dissolved in 0.5 mL DMF. Next, EDCI (5.00 mg, 0.026 mmols) and BOP (5 mg, 0.011 mmols) were added to the PXL-COOH solution followed by DIEA (180 μl). The reaction was stirred for 30 minutes prior to adding ND_2 (3.56 mg, 0.00068 mmols), dissolved in 0.5 mL DMF. The reaction was stirred under $Ar_{(g)}$ overnight with SEC spectra taken 5 minutes and 15 hours after ND_2^{Pep} addition. A notable change in the elution time of the first peak indicated coupling occurred. The reaction was concentrated under vacuum, and dissolved in DMSO. The product was purified by first using Amicon Centrifugation Diafiltration tubes (3,000 MWCO) to concentrate the high molecular weight compounds followed by three washings with DMSO to remove all the low molecular weight compounds. The remaining high molecular weight compounds were separated using SEC to provide 5.13 mg (85%) of pure ND_2^{PXL}. MADLI-TOF MS (CHCA) Found 2,121 Dalton (Fragment: PXL-[Ahx]-AVRWLLTA-[Ahx]-Gly+H)$^+$; calculated 2,119 Dalton ($C_{108}H_{148}N_{16}O_{28}$); Found 4,409 Dalton (Fragment: (PXL-[Ahx]-AVRWLLTA-[Ahx]-Gly)4-Dendron/4+H)$^+$; calculated 4,408 Dalton ($C_{447}H_{609}N_{64}O_{121}$). SEC (55% 0.05% TFA in Acetonitrile/ 45% 0.065% TFA in water) elution time 7.7 minutes.

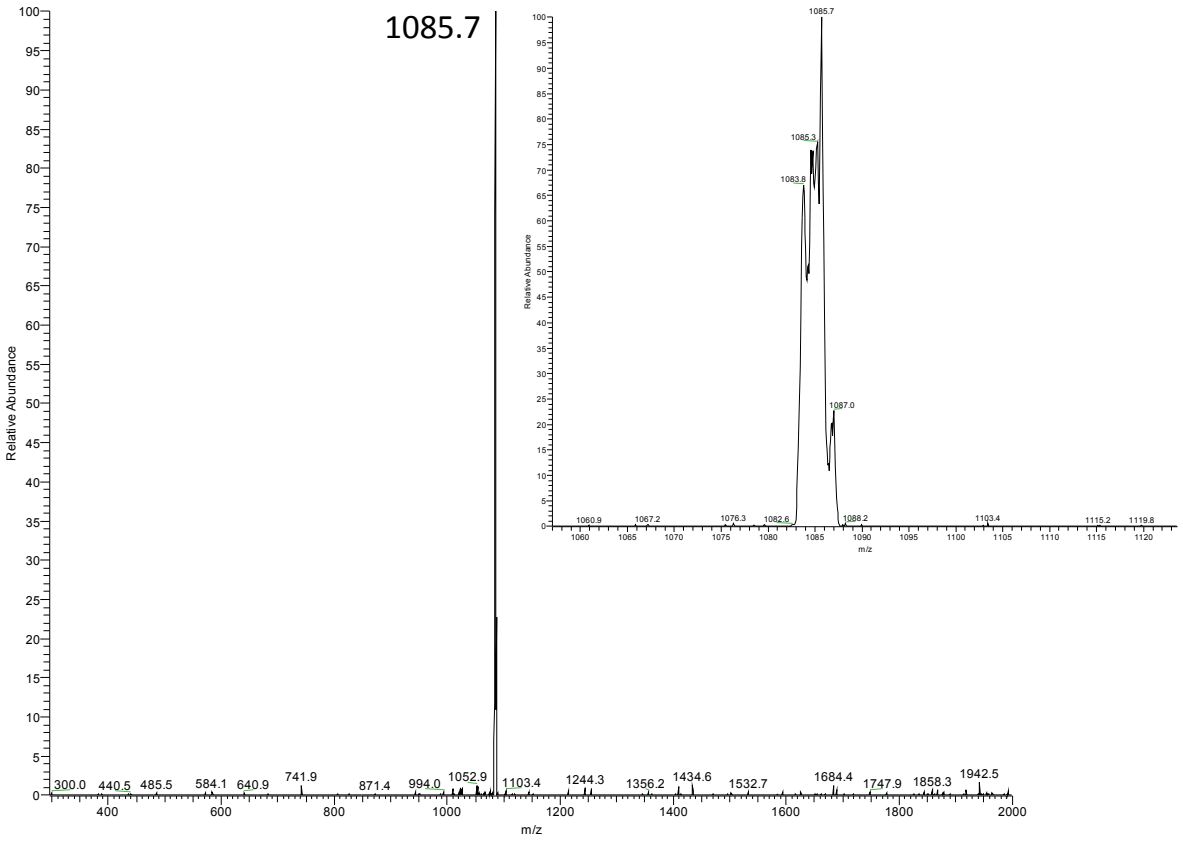


Figure S1. ESI MS of ND_2^{Boc} (**2**).

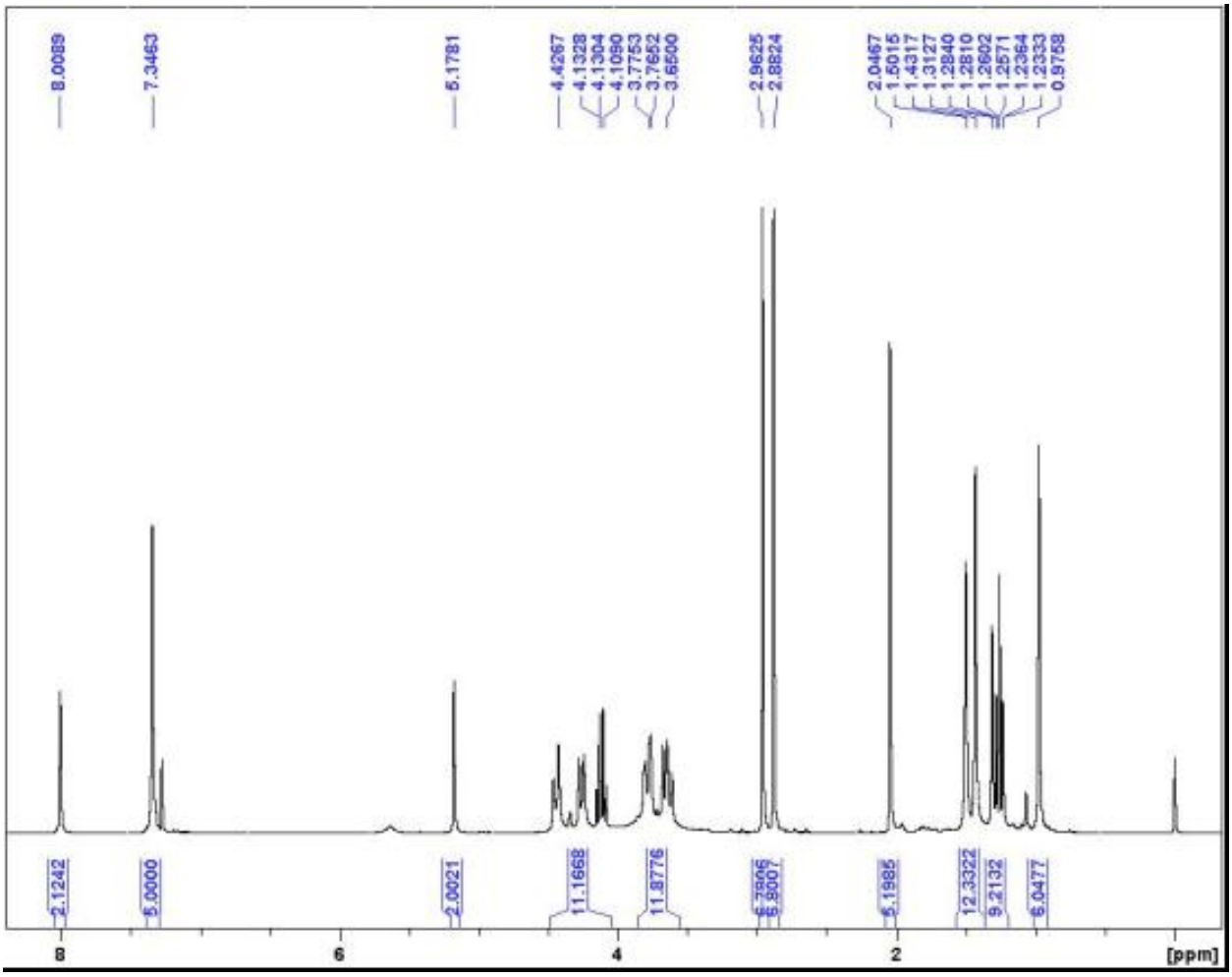


Figure S2. NMR of ND_2^{Boc} (**2**).

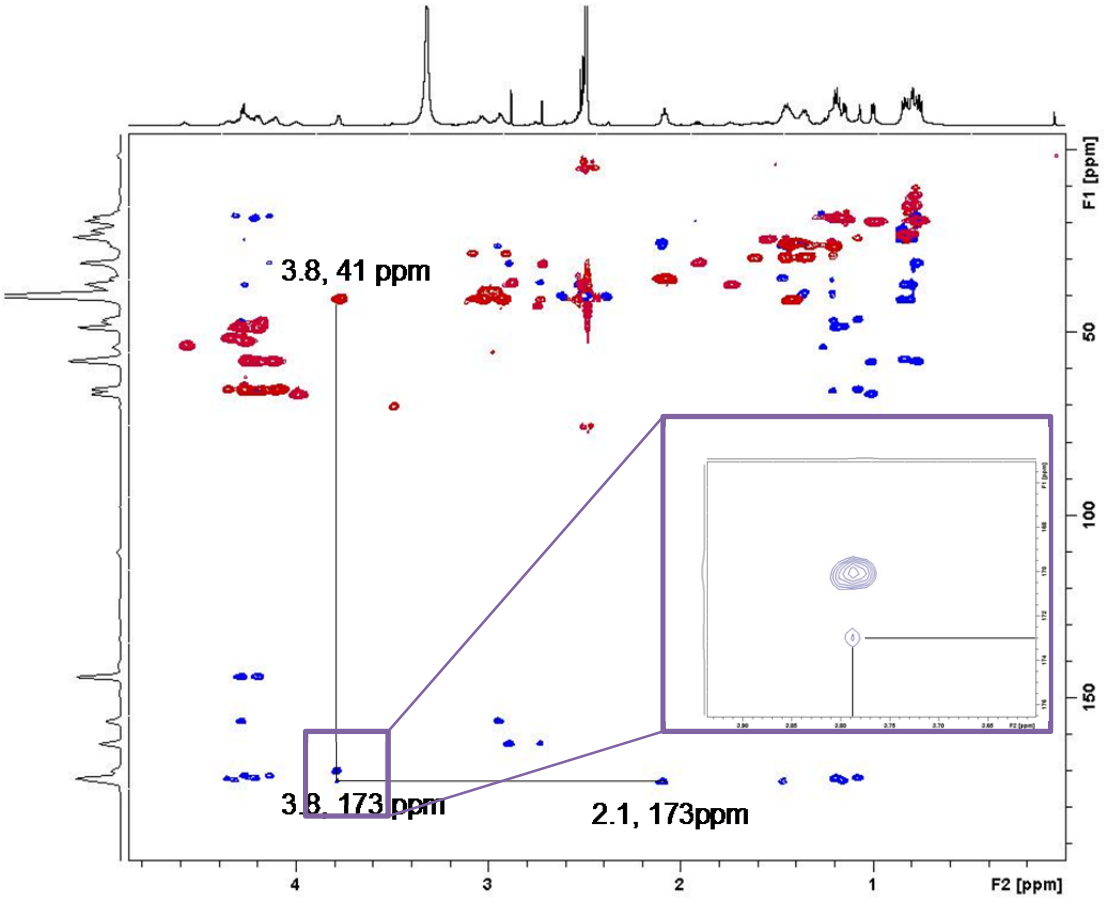


Figure S3. An overlay of the HMBC (blue) and HSQC (red) NMR spectra of $ND_2^{(Fmoc\text{-}pep)}$ (**4**). Expanded image of the peak at 3.8 and 173 ppm which indicates coupling between the peptide carboxy terminus and the Gly methylene of the dendron.

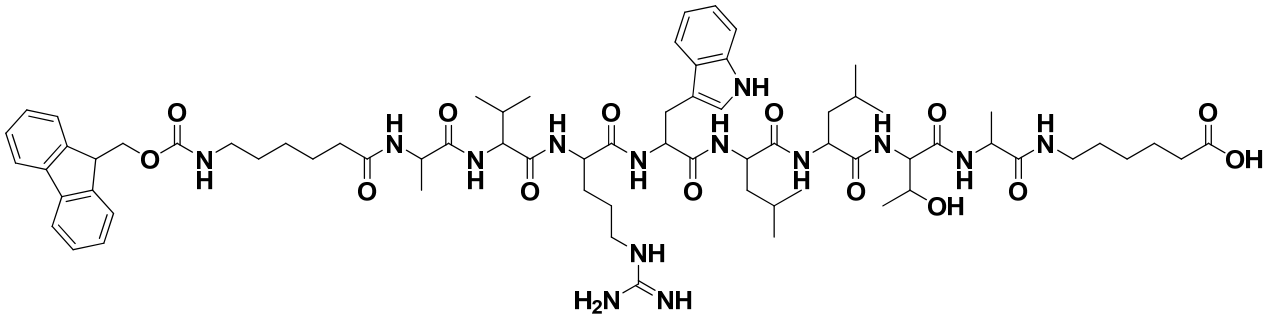


Figure S4. The structure of the peptide linker used to make MMP9-activated prodrugs.

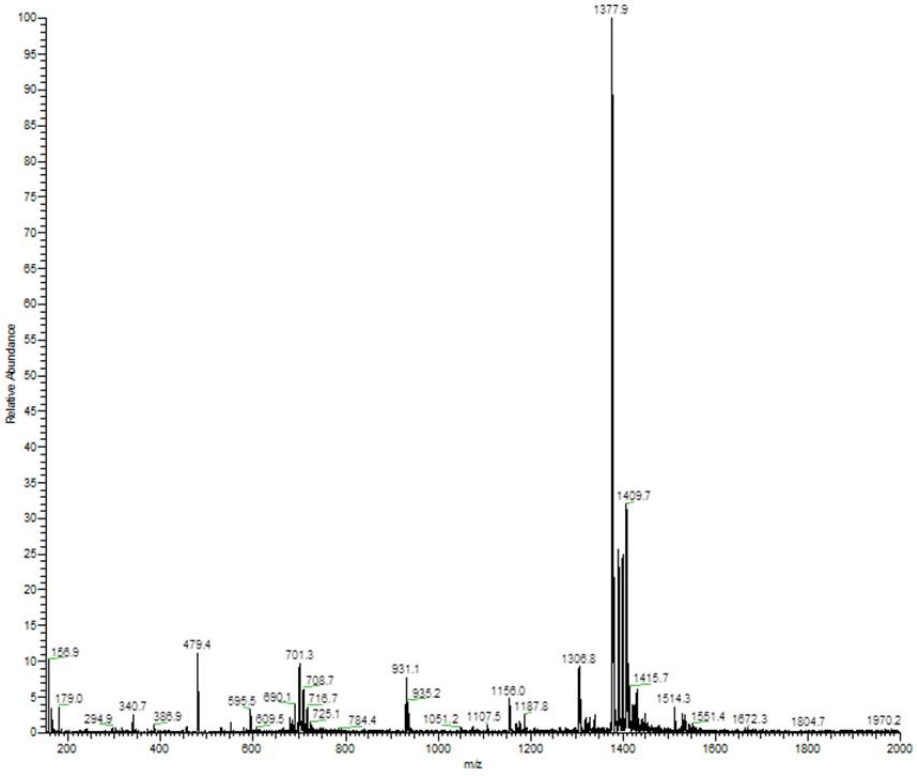


Figure S5. The ESI+ MS of the Fmoc-[Ahx]-AVRWLLTA-[Ahx] (see Figure S4 for structure).

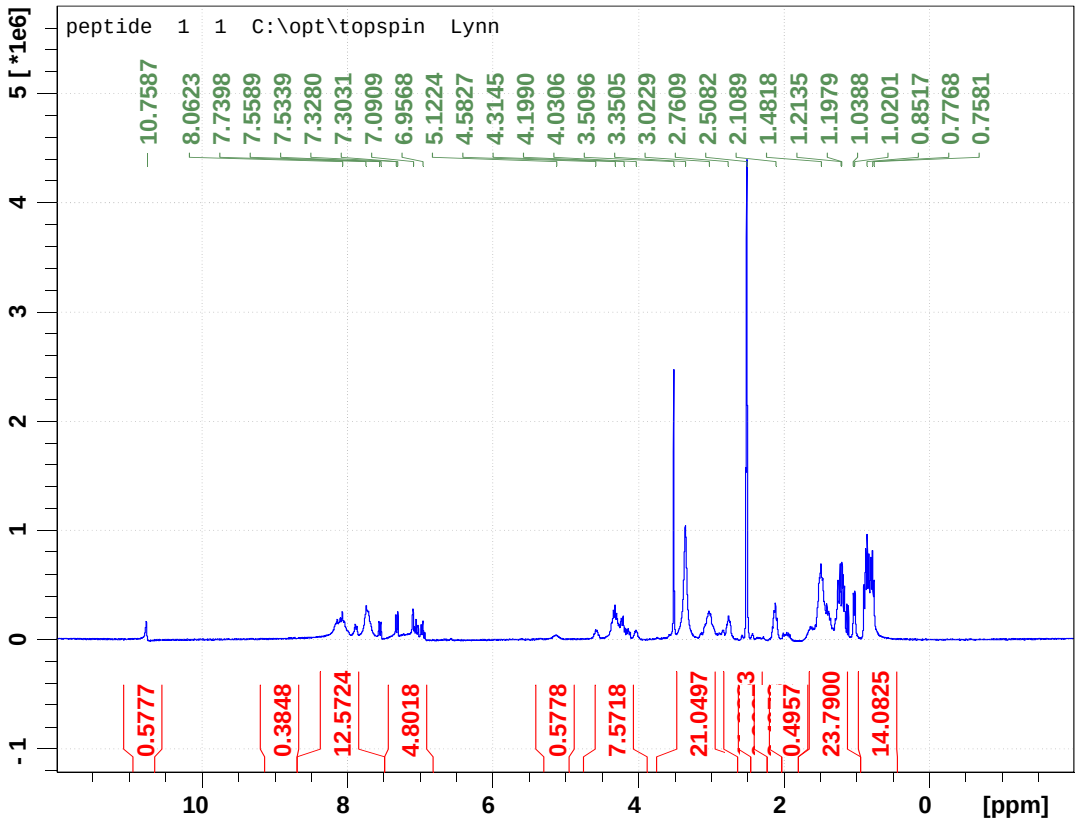


Figure S6. ^{1}H NMR Spectra of Fmoc-[Ahx]-AVRWLLTA-[Ahx] (see Figure S4 for structure).

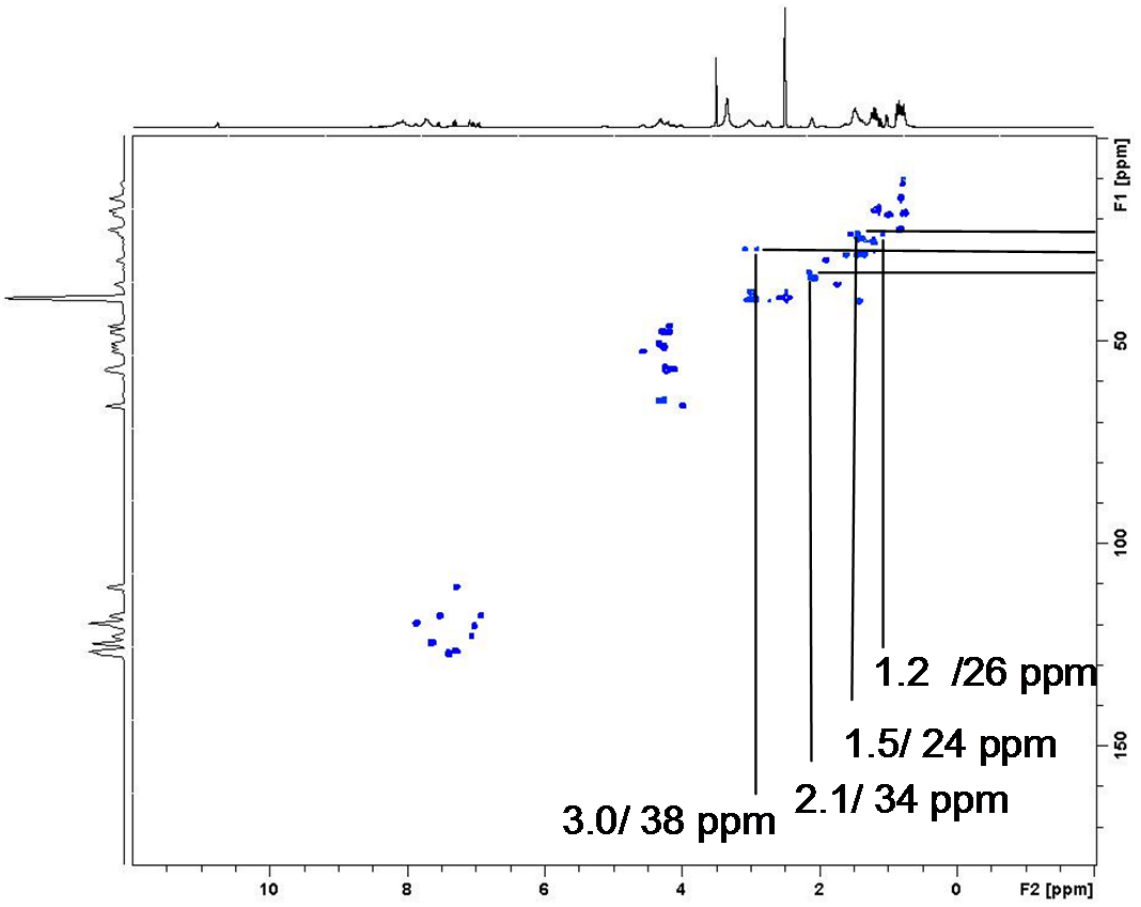


Figure S7. HMBC NMR spectra of the Fmoc-[Ahx]-AVRWLLTA-[Ahx] (see Figure S4 for structure).

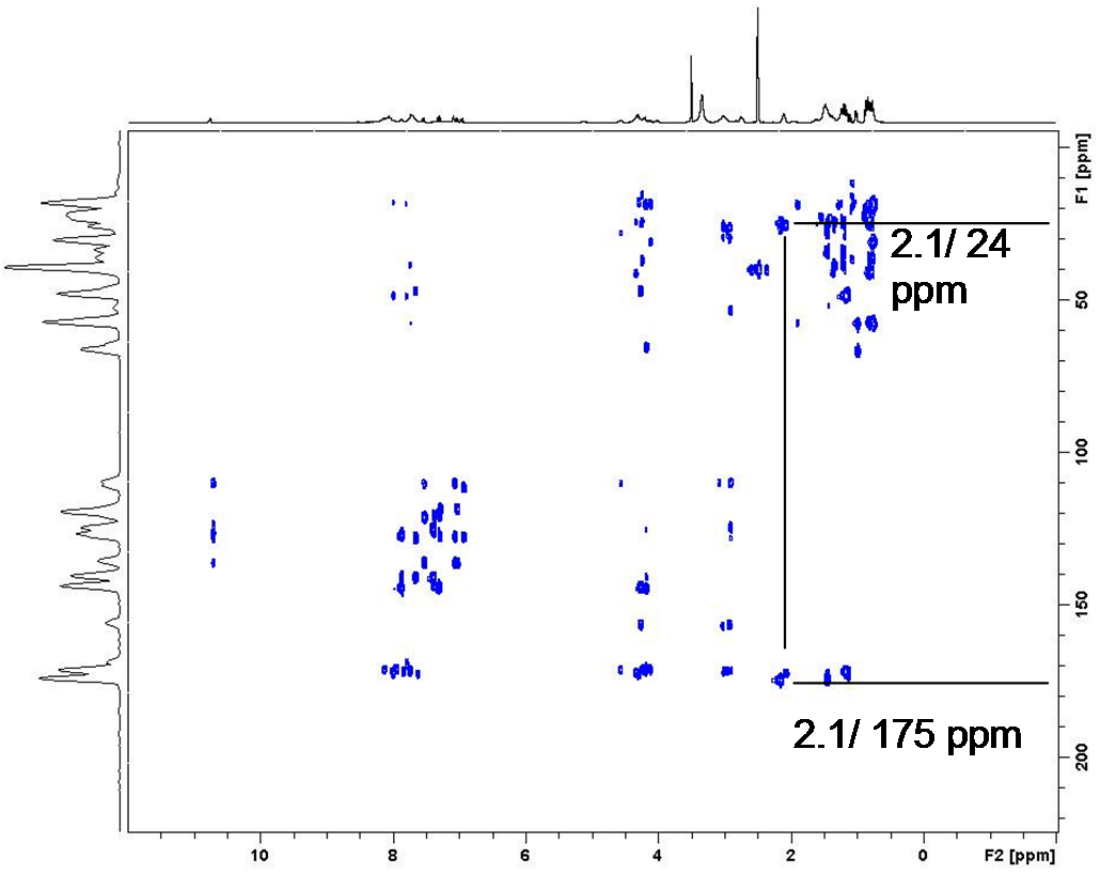


Figure S8. HSQC NMR spectra of the Fmoc-[Ahx]-AVRWLLTA-[Ahx] (see Figure S4 for the structure).

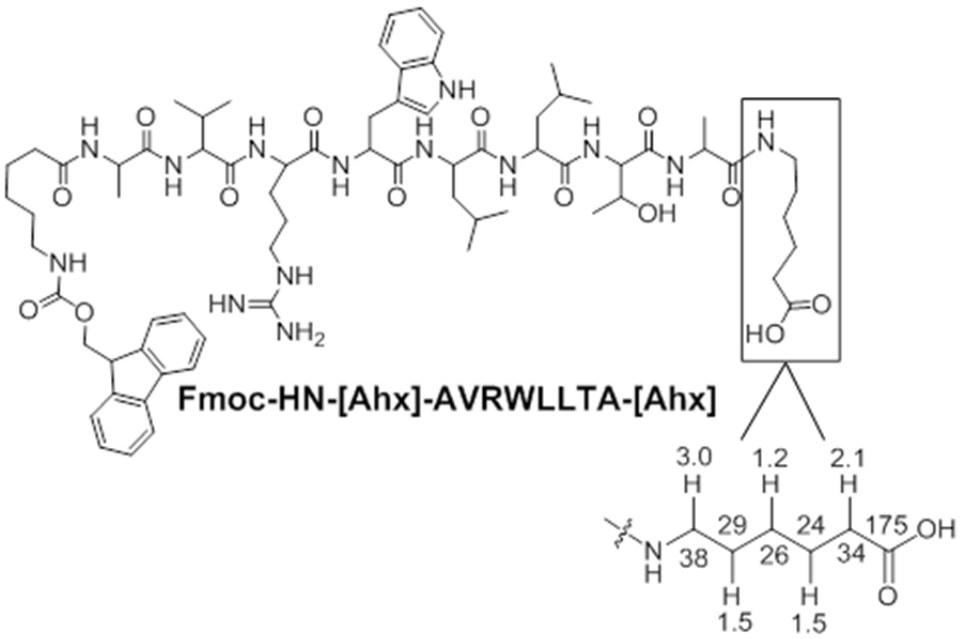


Figure S9. The proton assignments for the Fmoc-[Ahx]-AVRWLLTA-[Ahx].

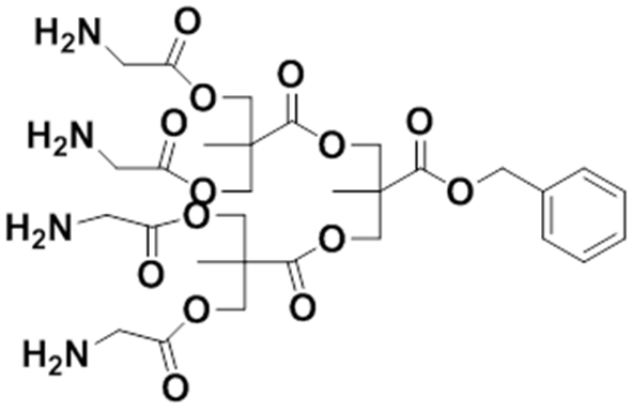


Figure S10. Structure of ND_2 (**3**).

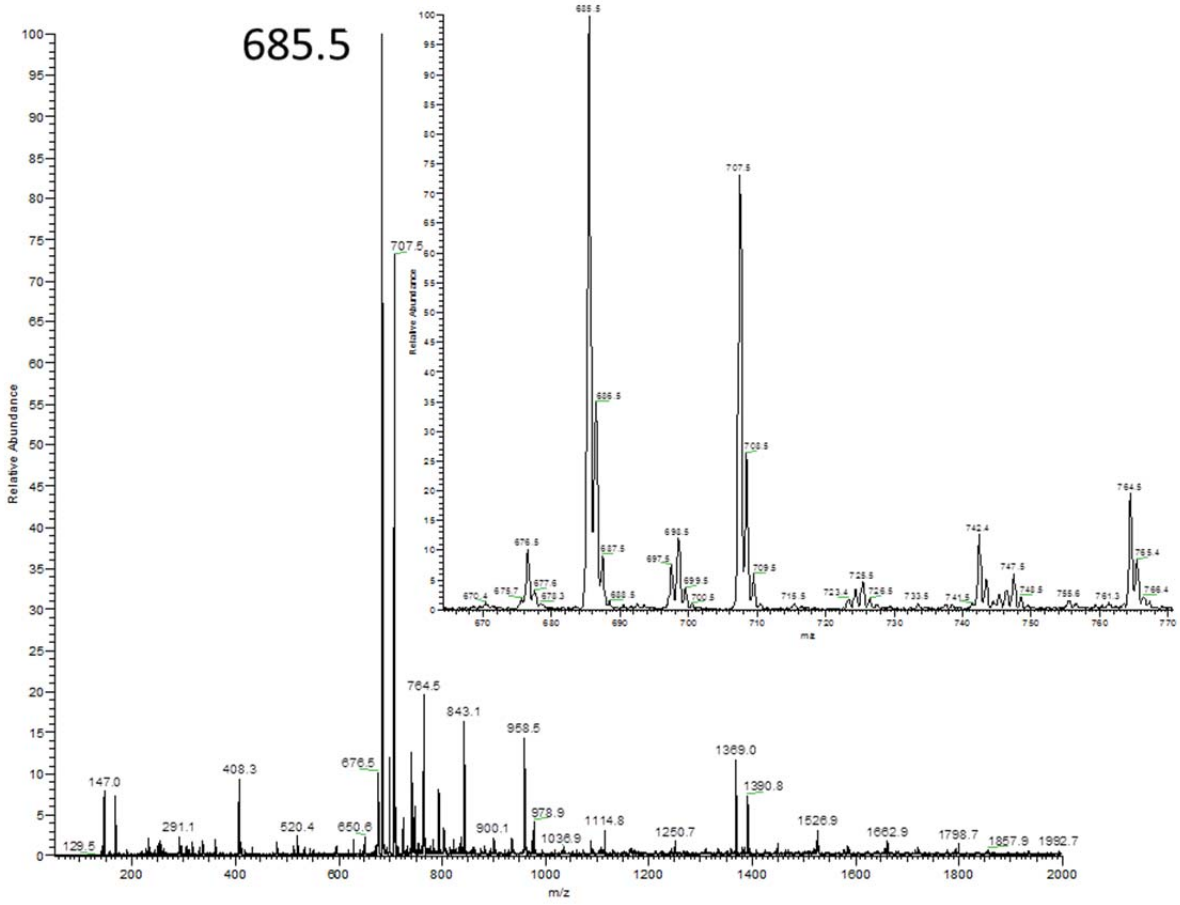


Figure S11. ESI$^+$ MS of ND_2 (**3**).

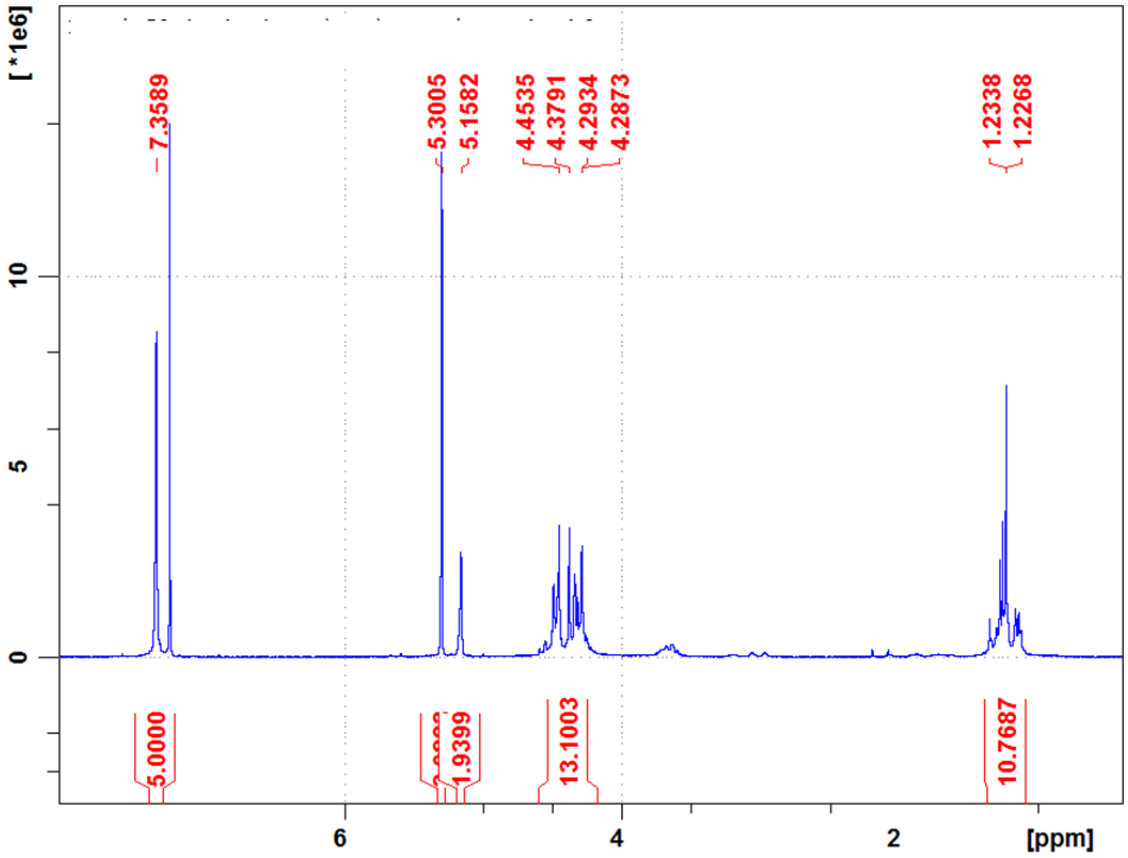


Figure S12. ^{1}H NMR spectra of ND_2 (**3**).

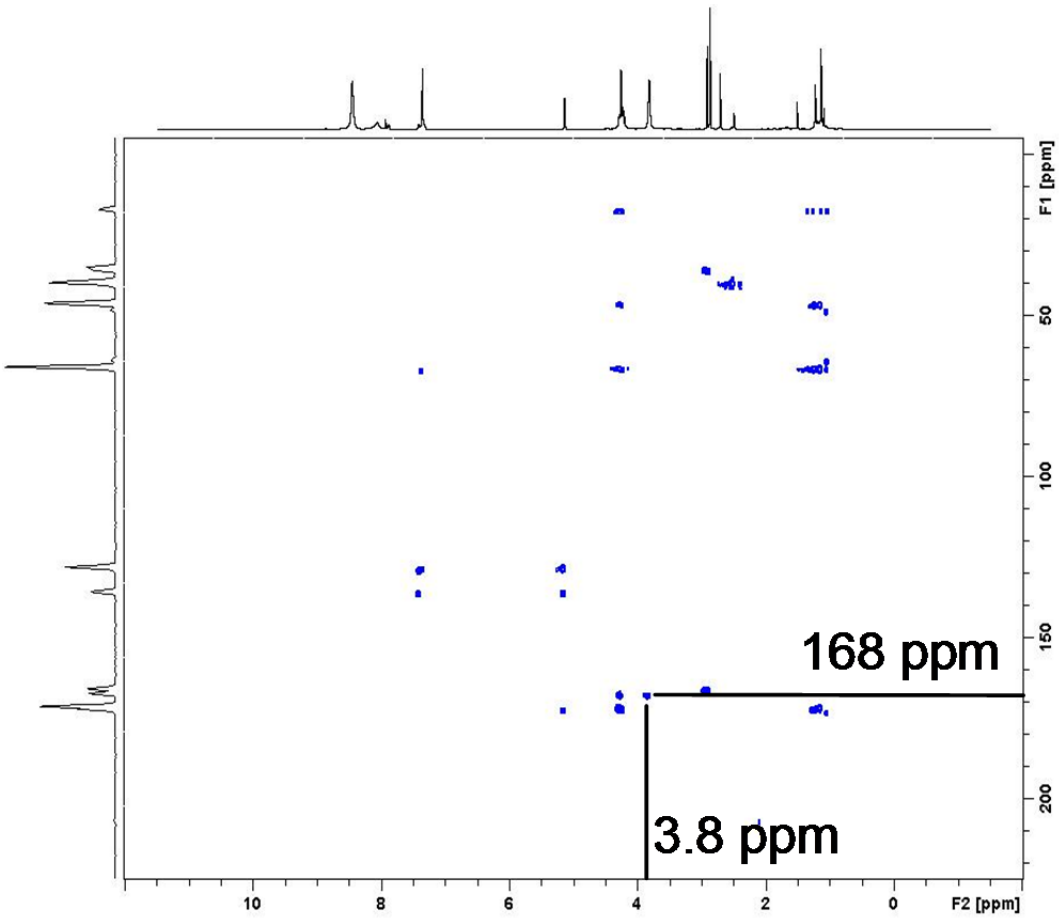


Figure S13. The HMBC spectra of ND_2 (**2**). The point between 3.8 ppm and 168 ppm is from a two bond coupling between the CH_2 on the Gly and the carbonyl carbon.

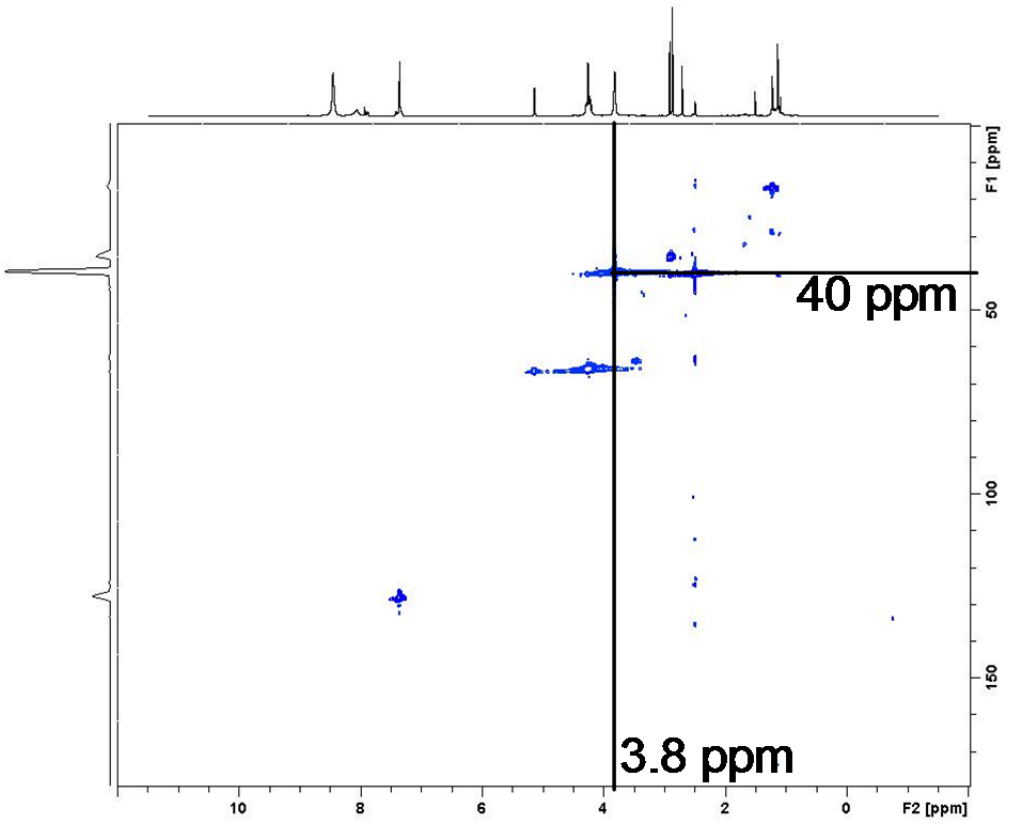
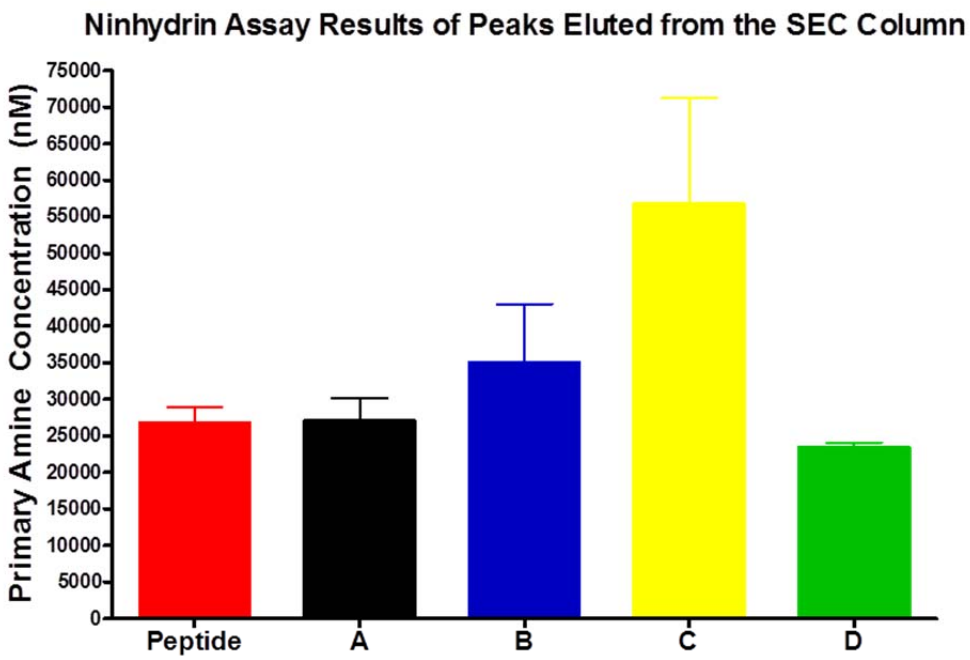


Figure S14. The HSQC spectra of the ND_2 (**3**). The point at 3.8 and 40 ppm is from coupling of the hydrogen on the Gly CH_2 and the corresponding carbon.



Compound	Calculated Concentration	Peak	Concentration
Fmoc-[Ahx]-AVRWLLTA-[Ahx]	0 uM		
4 peptides attached	0 uM	A	0 uM
3 Peptides Attached	21 uM	B	21 uM
1 & 2 Peptides Attached (mix)	59 uM	C	77 uM
Peptide (as eluted from SEC)	147 uM	D	0 uM

Figure S15. Concentration of amines in peaks A-D eluted from the SEC column as determined by the ninhydrin assay. The table depicts the assigned product, the concentration of primary amine in the coupled product and the calculated concentration for each theoretical product.

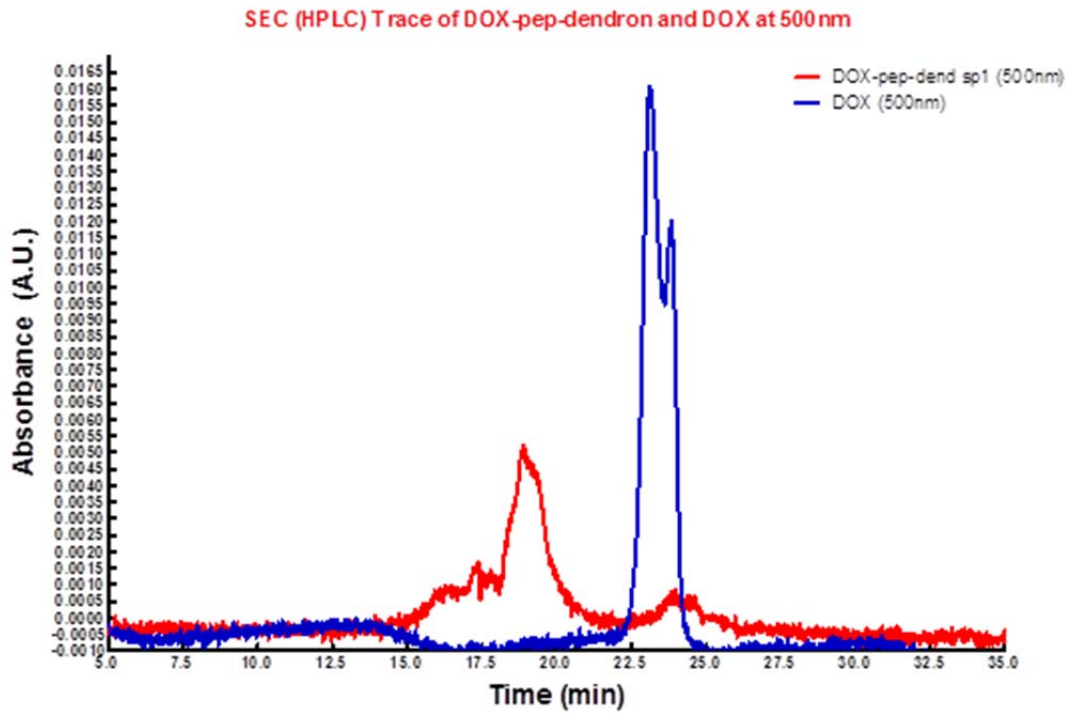


Figure S16. SEC traces of ND_2^{DOX} (**6**) and DOX-COOH.

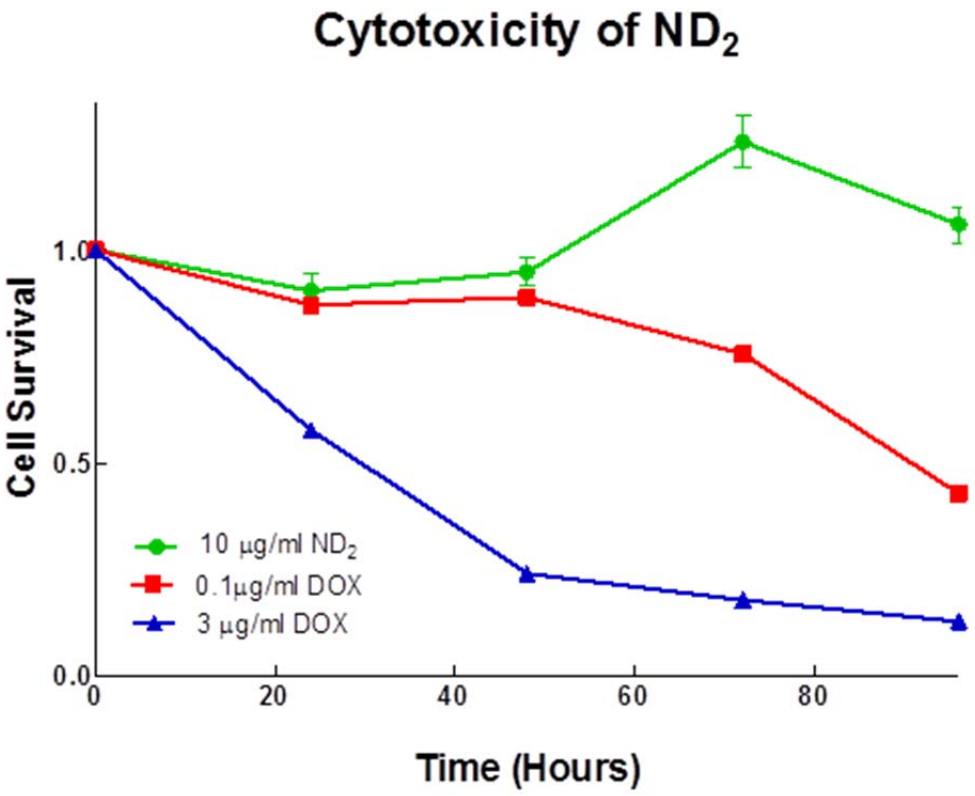


Figure S17. Cytotoxicity of ND_2 and ND_2^{DOX} compared to DOX as test by an MTS assay (following the product protocol).

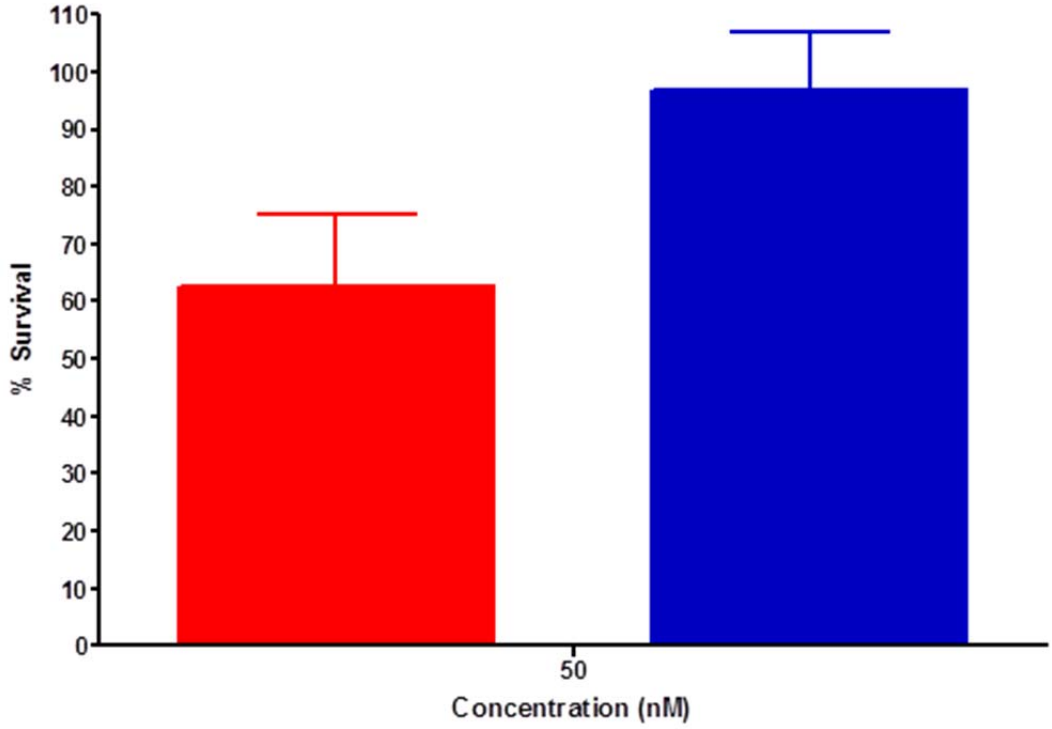


Figure S18. Bar graph of cellular cytotoxicity with and without an MMP inhibitor, GM6001, in R221A-luc cells. Red is ND_2^{PXL} and Blue is ND_2^{PXL}+GM6001.

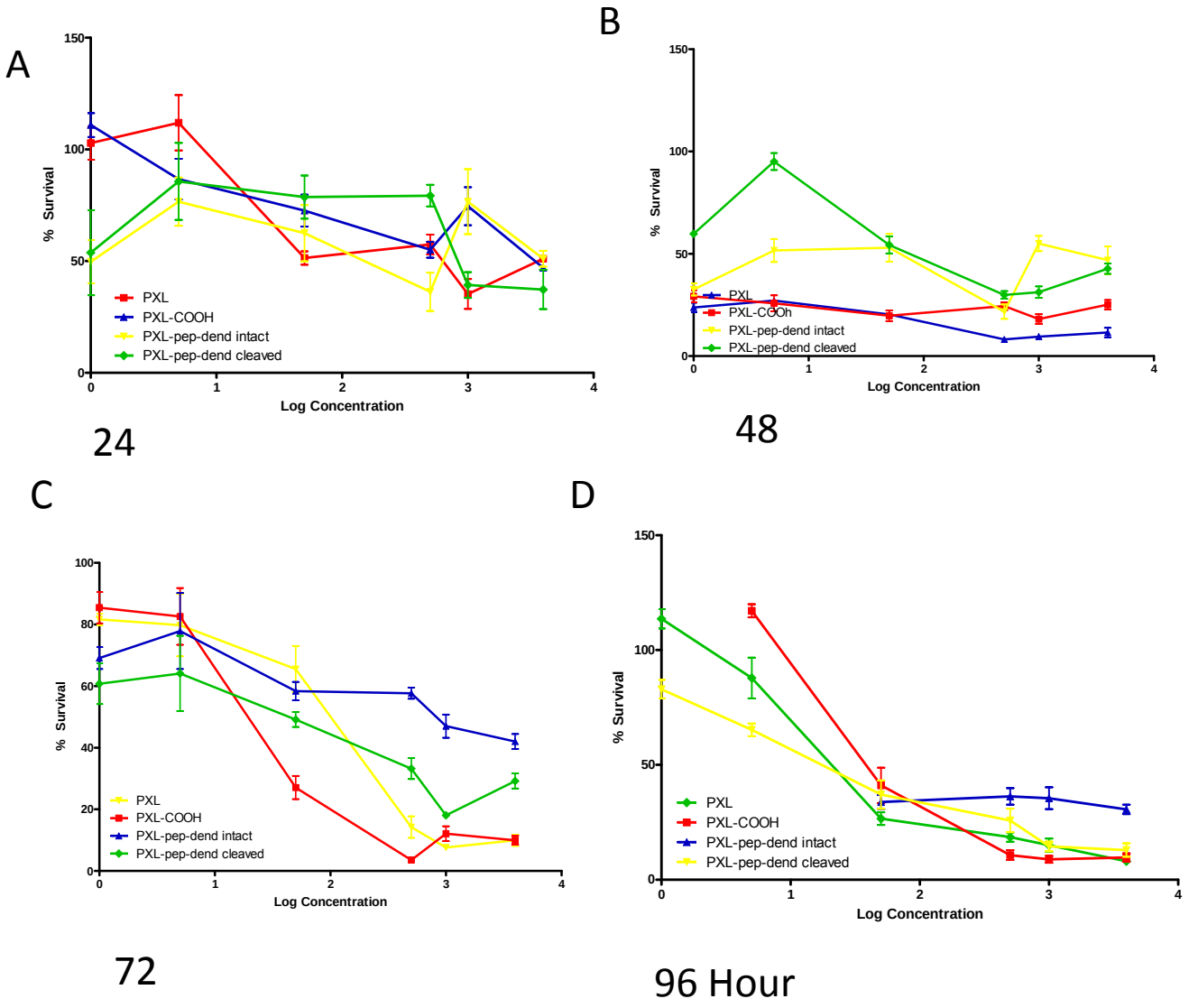


Figure S19. Cytotoxicity of PXL compounds determined using the trypan blue exclusion assay at increasing concentrations in R221A-luc cells at 24 hours (**A**), 48 hours (**B**), 72 hours (**C**) and 96 hours (**D**).

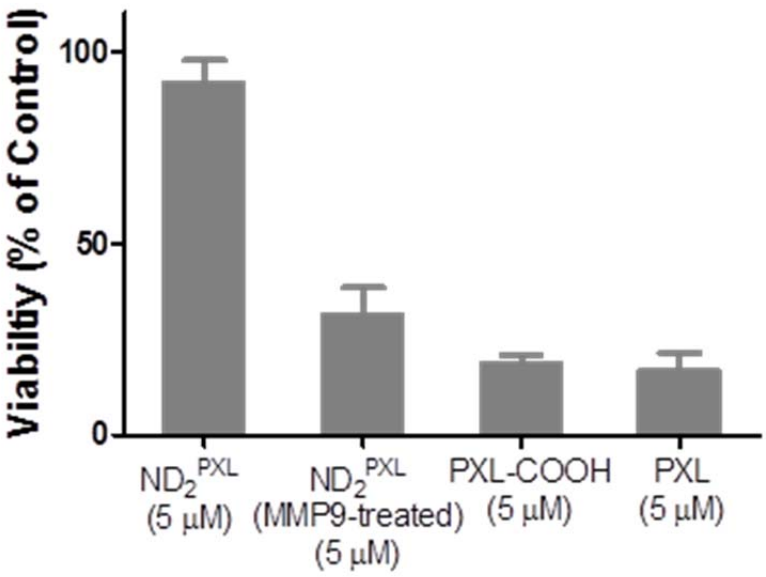


Figure S20. Cytotoxicity of ND_2^{PXL} in LLC^{RSV} cells upon treatment with 5 μM after 48 hours.