

## Supporting Information

### General Procedures for Peptide Synthesis, Purification and Characterization:

Monomeric peptide synthesis was performed on a Symphony™ Synthesizer (Rainin Instruments, Protein Technologies, Inc. Woburn, MA) by Fmoc solid-phase peptide synthesis on a Rink Amide AM resin (substitution level 0.71 mmol/g) using standard protocols. Fmoc-protected amino acids were coupled at a 5-fold excess using HBTU, HOBT and NMM coupling (45 min). Piperidine in DMF (20%) was employed to remove N-terminal Fmoc protecting groups. Fmoc-NH-(PEG)<sub>11</sub>-COOH (C<sub>42</sub>H<sub>65</sub>NO<sub>16</sub>, Polypure, Oslo, Norway) was coupled in the same fashion. Upon completion of the synthesis, peptides were cleaved from the resin using a TFA: triisopropylsilane: H<sub>2</sub>O cocktail (95%:2.5%:2.5%) and precipitated in cold diethyl ether.

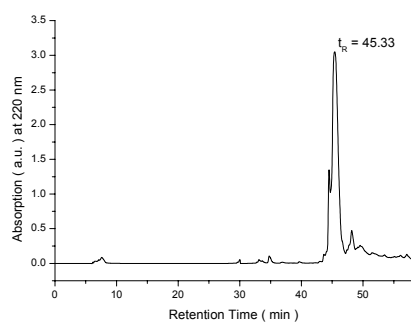
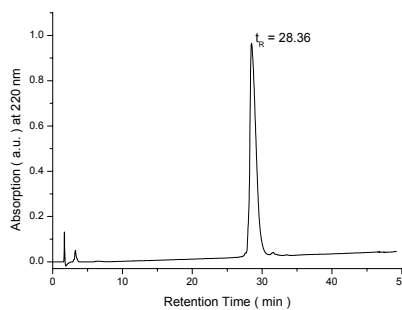
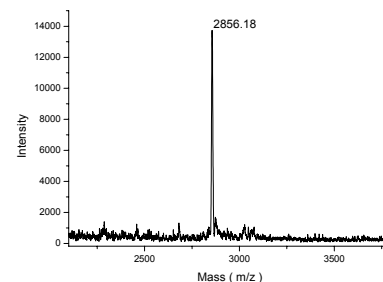
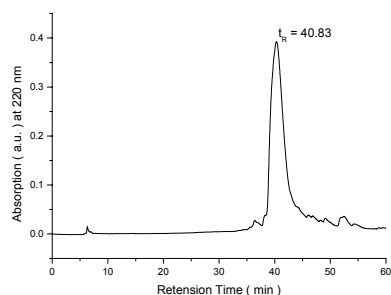
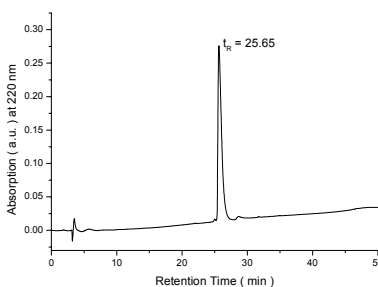
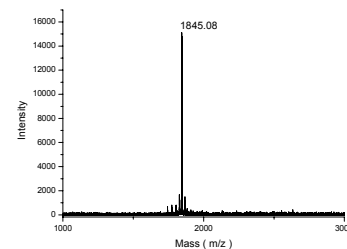
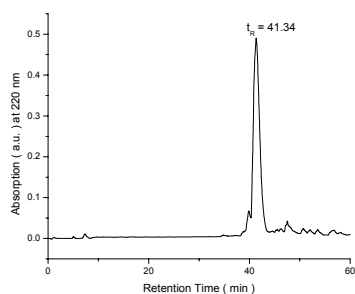
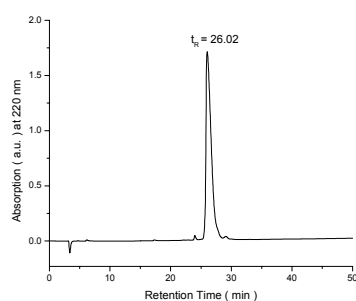
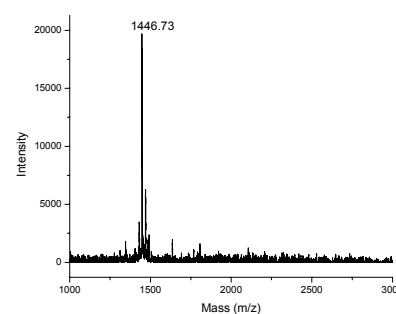
Crude peptides were purified by reverse phase HPLC using a Vydac PR-C18 column (250mm ×22mm, 10 μm) on a Breeze™ HPLC (Water Inc.) with eluents of H<sub>2</sub>O/0.1% TFA (eluent A) and acetonitrile/0.1%TFA (eluent B). The following elution profile (referred to as Method A) was utilized: 0-1 minute, 90%A,10%B; 1-61 minutes, eluent B was increased from 10-40% at a flow rate of 10 mL /minute. Elution of the peptides was monitored by UV absorbance at 220 nM.

The purified peptides were analyzed by RP-HPLC (Varian, PR-C18 column, 250mm ×4.6mm, 5 μm) using the following elution profile (Method B): 0-1 minute, 90%A, 10%B; 1-51 minutes, eluent B was increased from 10-60% at a flow rate of 1 mL /minute. The purity of the peptides was determined to be greater than 95% by HPLC (Figures 1SB-3SB). The peptide mass was confirmed by matrix assisted laser desorption ionization time of flight mass spectrometry (MALDI MS) in reflector mode using a Voyager DE™ Pro instrument (Applied Biosystems Inc.) using α-cyano-4-hydroxycinnamic acid as the matrix.

**Table 1S. H2009 Peptides Synthesized for Cell Binding Experiments**

Compound Name	Peptide Sequence <sup>a</sup>	Elemental Composition	Expected Monoisotopic Mass	MH <sup>+</sup>	Purity <sup>b</sup>
Compound 1	RGDLATLRQLAQEDGVVGVR(PEG) <sub>11</sub> C	C <sub>120</sub> H <sub>216</sub> N <sub>34</sub> O <sub>43</sub> S <sub>1</sub>	2853.55	2854.23	95%
Compound 2	RGDLATLRQL(PEG) <sub>11</sub> C	C <sub>78</sub> H <sub>146</sub> N <sub>20</sub> O <sub>28</sub> S <sub>1</sub>	1843.02	1844.18	98%
Compound 3	RGDLATL(PEG) <sub>11</sub> C	C <sub>61</sub> H <sub>115</sub> N <sub>13</sub> O <sub>24</sub> S <sub>1</sub>	1445.78	1446.73	96%

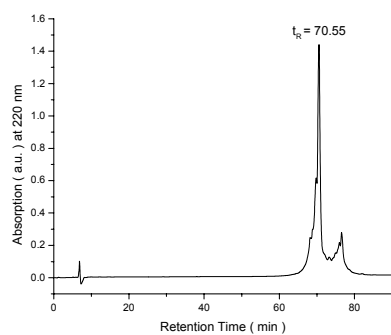
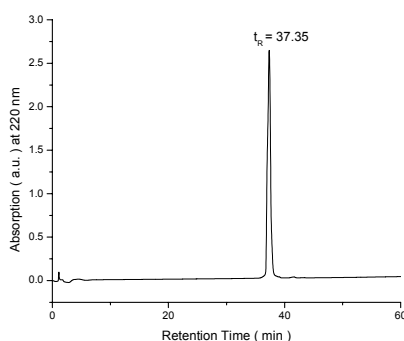
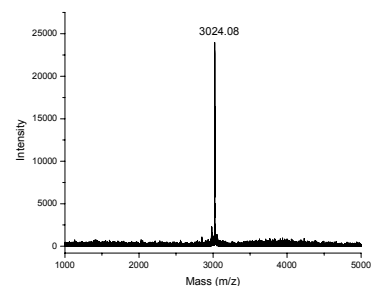
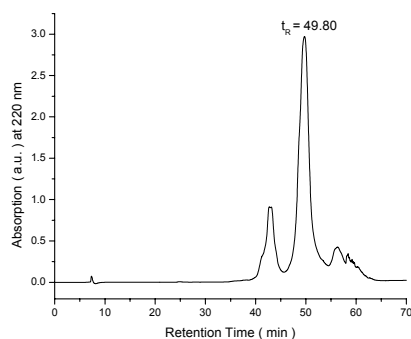
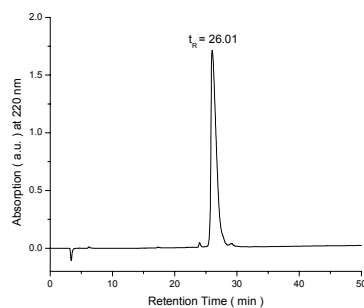
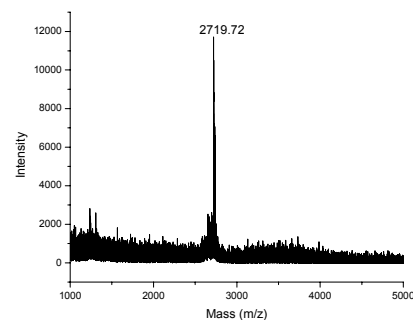
<sup>a</sup>Peptide sequences are given in single letter code in standard N-terminal to C-terminal notation. The Fmoc-NH-(PEG)<sub>11</sub>-COOH (C<sub>42</sub>H<sub>65</sub>NO<sub>16</sub>) was purchased from Polypure (Oslo, Norway). All peptides were synthesized as C-terminal amides. <sup>b</sup>As determined by analytical RP HPLC monitoring at 220 nM.

**A. Preparative HPLC of peptide****B. Analytical HPLC of peptide****C. MALDI MS of peptide.****Figure 1S: Purification and Characterization of Peptide 1, RGDLATLRQLAQEDGVVGVR(PEG)<sub>11</sub>C****A. Preparative HPLC of peptide****B. Analytical HPLC of peptide****C. MALDI MS of peptide.****Figure 2S: Purification and Characterization of Peptide 2, RGDLATLRQL (PEG)<sub>11</sub>C****A. Preparative HPLC of peptide****B. Analytical HPLC of peptide****C. MALDI MS of peptide.****Figure 3S: Purification and Characterization of Peptide 3, RGDLATL (PEG)<sub>11</sub>C**

**Table 2S. H1299 peptides synthesized for cell binding experiments**

Compound Name	Peptide Sequence <sup>a</sup>	Elemental Composition	Expected Monoisotopic Mass	MH+	Purity <sup>b</sup>
Compound 4	VSQTMRQTAVPLLWFWTGS(PEG) <sub>11</sub> C	C <sub>78</sub> H <sub>146</sub> N <sub>20</sub> O <sub>28</sub> S <sub>1</sub>	3021.56	3022.10	98%
Compound 5	YAAWPASGAWTGTAPC(Acm)SAGT(PEG) <sub>11</sub> C	C <sub>119</sub> H <sub>183</sub> N <sub>26</sub> O <sub>41</sub> S <sub>2</sub>	2696.23 <sup>c</sup>	2719.72	98%

<sup>a</sup>All peptides were synthesized as C-terminal amides. <sup>b</sup>As determined by analytical RP HPLC monitoring at 220 nm. <sup>c</sup>M Na<sup>+</sup> adduct was observed.

**A. Preparative HPLC of peptide****B. Analytical HPLC of peptide****C. MALDI MS of peptide.****Figure 4S :Purification and Characterization of Peptide H1299.1, compound 4****A. Preparative HPLC of peptide****B. Analytical HPLC of peptide****C. MALDI MS of peptide.****Figure 5S :Purification and Characterization of Peptide H1299.2(Acm), compound 5**

The deletion peptides used to map the critical binding domain were synthesized as above. The PEG linker was not included and all peptides were synthesized as C-terminal amides. The characteristics of these peptides are listed in Table 3S.

**Table 3S. H2009 Monomeric Deletion Peptide Synthesized for Cell Binding Experiments**

Peptide Name	Peptide Sequence	Elemental Composition	Expected Monoisotopic Mass	MH+	Purity
H2009.1 10-mer	RGDLATLRQL-NH <sub>2</sub>	C <sub>48</sub> H <sub>88</sub> N <sub>18</sub> O <sub>14</sub>	1140.67	1141.35	98%
H2009.1 9-mer	RGDLATLRQ-NH <sub>2</sub>	C <sub>42</sub> H <sub>77</sub> N <sub>17</sub> O <sub>13</sub>	1027.59	1028.96	98%
H2009.1 8-mer	RGDLATLR-NH <sub>2</sub>	C <sub>37</sub> H <sub>69</sub> N <sub>15</sub> O <sub>11</sub>	899.53	900.65	98%
H2009.1 7-mer	RGDLATL-NH <sub>2</sub>	C <sub>31</sub> H <sub>57</sub> N <sub>11</sub> O <sub>10</sub>	743.43	745.02	98%
H2009.1 6-mer	RGDLAT-NH <sub>2</sub>	C <sub>25</sub> H <sub>46</sub> N <sub>10</sub> O <sub>9</sub>	630.34	631.48	98%

**Synthesis, Purification, and Characterization of Maleimido Activated Cores (compounds 6-10):**

The maleimido tetrameric core was synthesized on Fmoc<sub>4</sub>-Lys<sub>2</sub>-Lys-β-Ala-CLEAR™ Acid Resin, Fmoc<sub>4</sub>-Lys<sub>2</sub>-Lys-Lys(Biotin-PEG)-β-Ala-CLEAR™ Acid Resin and Fmoc<sub>4</sub>-Lys<sub>2</sub>-Lys-Cys(Acm)-β-Ala-CLEAR™ Acid Resin respectively as described in the text. The compounds were purified by reverse phase HPLC using method A and characterized by analytical HPLC (method B). MALDI MS were obtained using α-cyano-4-hydroxycinnamic acid as the matrix. The structure and characterization of the compounds are shown in Figure 6S and Table 4S.

The maleimido dimeric core was obtained by Fmoc solid-phase peptide synthesis on a Rink Amide AM resin (substitution level 0.71 mmol/g) using standard protocols. Fmoc-β-Ala-OH was coupled at a 5-fold excess using HBTU, HOBt and NMM coupling (45 min). Piperidine in DMF (20%) was employed to remove N-terminal Fmoc protecting groups. Fmoc-Lys(Fmoc)-OH and 3-maleimidopropionic acid was coupled in the same fashion. Upon completion of the synthesis, the maleimido dimeric core was cleaved from the resin, purified by reverse phase HPLC using method A and characterized by analytical HPLC (method B). MALDI MS were obtained using α-cyano-4-hydroxycinnamic acid as the matrix.

The maleimido trimeric core was synthesized on Fmoc-β-Ala-CLEAR™ Acid Resin, (substitution level 0.52 mmol/g) using standard protocols. Fmoc-Lys(Mtt)-OH was coupled at a 5-fold excess using HBTU, HOBt and NMM coupling (45 min). Piperidine in DMF (20%) was employed to remove N-terminal Fmoc protecting groups. Fmoc-Lys(Fmoc)-OH was coupled in the same fashion. The Mtt protecting group was then removed by treating

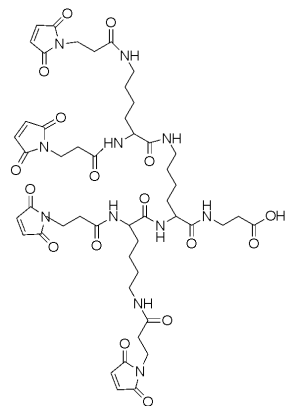
the resin with 1%TFA in DCM for 2 min, 5 times<sup>1</sup>. After thoroughly washing the resin with DCM and DMF, 3-maleimidopropionic acid was coupled using standard protocols. Upon completion of the synthesis, the maleimido trimeric core was cleaved from the resin, purified by reverse phase HPLC using method A and characterized by analytical HPLC (method B). MALDI MS were obtained using  $\alpha$ -cyano-4-hydroxycinnamic acid as the matrix.

**Table 4S. Characteristics of Maleimido Activated Cores**

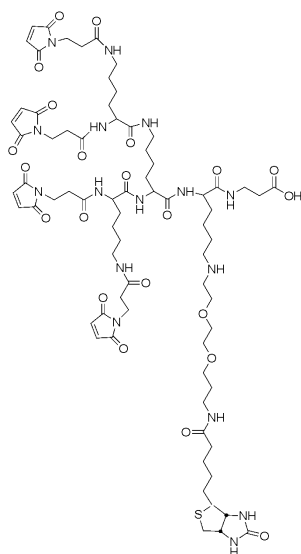
Compound Name	Elemental Composition	Expected Mass (monoisotopic)	MNa <sup>+</sup> <sup>a</sup>	Purity <sup>b</sup>
Compound 6	C <sub>49</sub> H <sub>63</sub> N <sub>11</sub> O <sub>17</sub>	1077.44	1099.83	96%
Compound 7	C <sub>71</sub> H <sub>100</sub> N <sub>16</sub> O <sub>23</sub> S <sub>1</sub>	1576.69	1599.79	95%
Compound 8	C <sub>55</sub> H <sub>73</sub> N <sub>13</sub> O <sub>19</sub> S <sub>1</sub>	1251.49	1274.27	95%
Compound 9	C <sub>23</sub> H <sub>30</sub> N <sub>6</sub> O <sub>8</sub>	518.21	541.28	98%
Compound 10	C <sub>36</sub> H <sub>46</sub> N <sub>8</sub> O <sub>13</sub>	798.32	821.30	96%

<sup>a</sup>The sodium adduct, not the MH<sup>+</sup>, was the observed parent ion for compounds 3-6. <sup>b</sup>As determined by analytical RP HPLC monitoring at 220 nM.

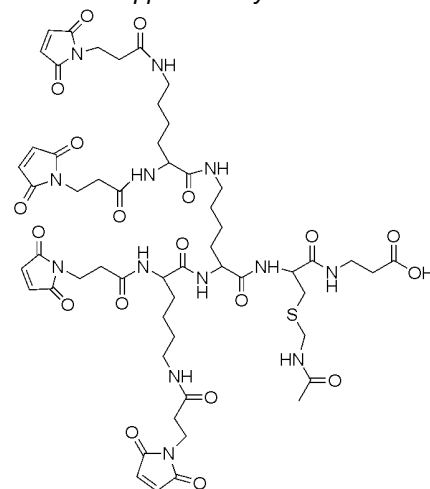
**Synthesis of Compound 11:** 8 $\mu$ mol of compound **1** and 1 $\mu$ mol of compound **6** was dissolved in 1.5 mL of Ar-purged 1  $\times$  PBS/0.01M EDTA. The reaction mixture was stirred at RT for 2 hrs (Scheme I). The products were then purified with PR-HPLC using the Method A. The purified peptide was analyzed by analytical HPLC using the Method B. MALDI Mass Spectra was obtained in linear mode using sinapinic acid as matrix. HPLC and MALDI MS data are shown in (Table 5S and Figure 7S). The purities are based on analytical HPLC data monitoring elution at 220nm. Surprisingly, while only tetrameric peptide is observed by analytical HPLC, some monomeric peptide is observed by MALDI MS. This may be trace amounts of material that is not observed by HPLC but is detected by MS. However, it should be noted that MALDI MS is not quantitative, and the intensity ratios of tetrameric product and monomeric peptide cannot be used as a measure of peptide purity. This is especially true as the monomeric peptide is expected to ionize better than the branched tetrameric version. Alternatively, we cannot rule out the possibility that the peptide conjugate is unstable under the MS conditions.



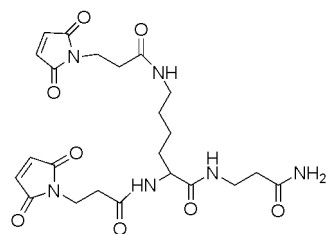
**Compound 6**



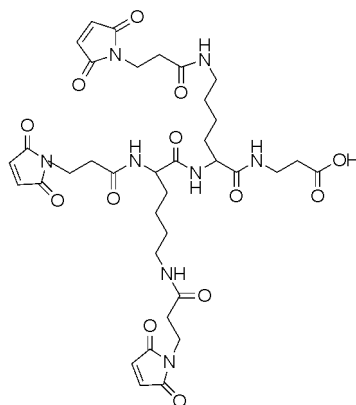
**Compound 7**



**Compound 8**

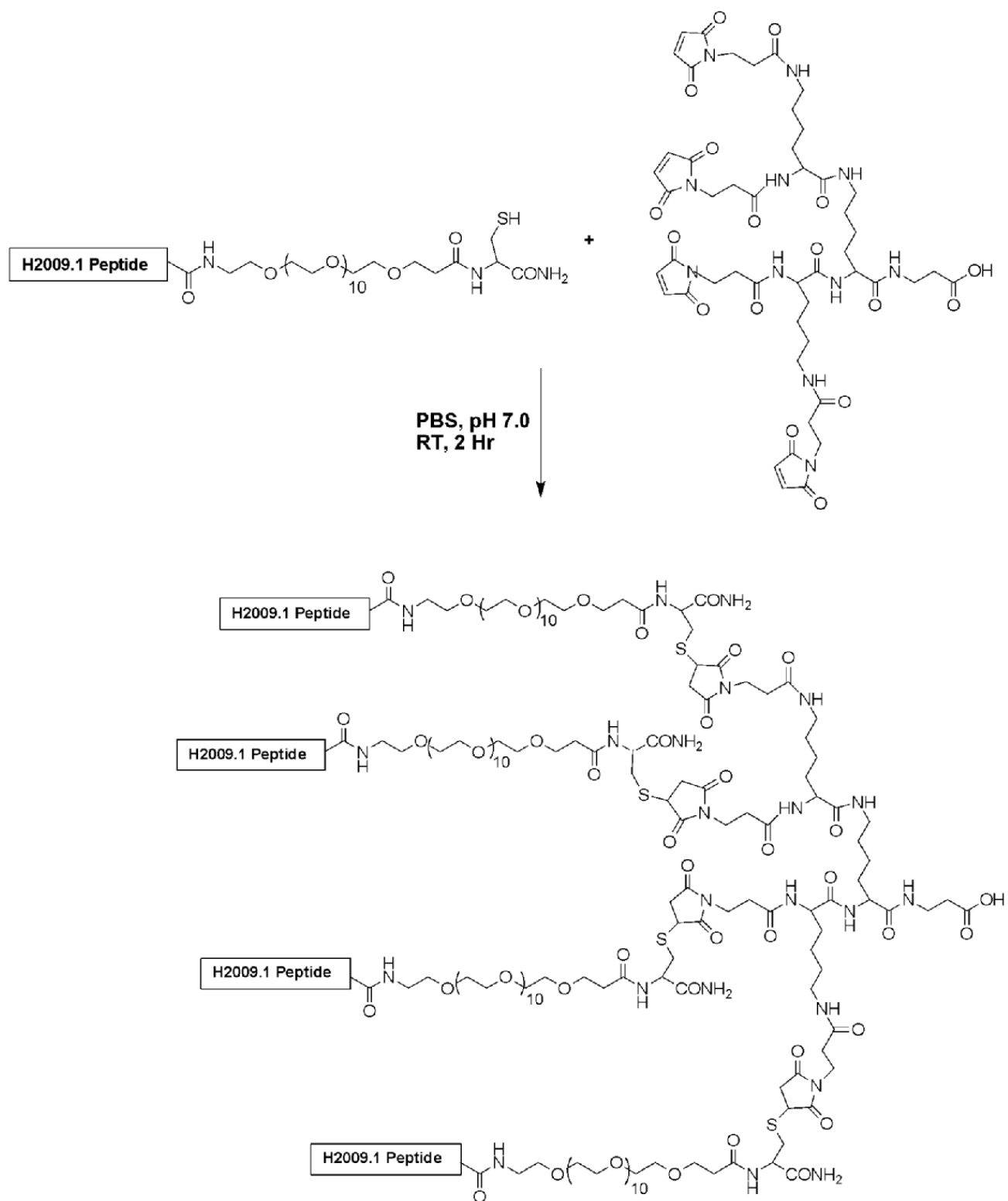


**Compound 9**



**Compound 10**

**Figure 6S: Structure of Activated Maleimido Cores, Compounds 6-10**

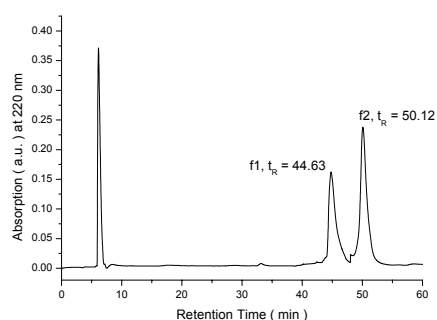


**Scheme 1. Convergent Synthesis of Tetrameric Peptides**

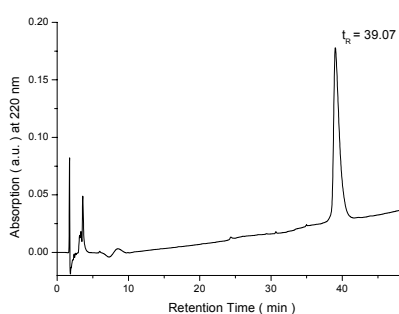
**Table 5S: Characteristics of Multimeric H2009 Peptides**

Compound Name	Elemental Composition	Expected Mass (average)	MH <sup>+</sup> (average)	Purity <sup>a</sup>
Compound 11	C <sub>529</sub> H <sub>927</sub> N <sub>147</sub> O <sub>189</sub> S <sub>4</sub>	12499.15	12497.91	95%
Compound 12	C <sub>361</sub> H <sub>647</sub> N <sub>91</sub> O <sub>129</sub> S <sub>4</sub>	8454.79	8451.98	98%
Compound 13	C <sub>179</sub> H <sub>322</sub> N <sub>46</sub> O <sub>64</sub> S <sub>2</sub>	4206.87	4205.42	98%
Compound 14	C <sub>383</sub> H <sub>684</sub> N <sub>96</sub> O <sub>135</sub> S <sub>5</sub>	8954.42	8952.21	95%
Compound 15	C <sub>367</sub> H <sub>657</sub> N <sub>93</sub> O <sub>131</sub> S <sub>5</sub>	8629.01	8626.77	95%
Compound 16	C <sub>364</sub> H <sub>652</sub> N <sub>92</sub> O <sub>130</sub> S <sub>5</sub>	8557.94	8556.88	98%
Compound 17	C <sub>270</sub> H <sub>484</sub> N <sub>68</sub> O <sub>97</sub> S <sub>3</sub>	6331.32	6334.04	95%
Compound 18	C <sub>299</sub> H <sub>533</sub> N <sub>65</sub> O <sub>115</sub> S <sub>5</sub>	7039.12	7040.81	95%

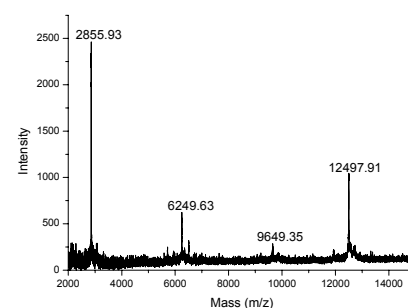
<sup>a</sup>As determined by analytical RP HPLC monitoring at 220 nM.



**A. Preparative HPLC of peptide f1: monomer; f2: tetramer**



**B. Analytical HPLC of peptide**



**C. MALDI MS of peptide**  
Note: both singly charged and doubly charged parent ions are observed.

**Figure 7S: Purification and Characterization of Compound 11**

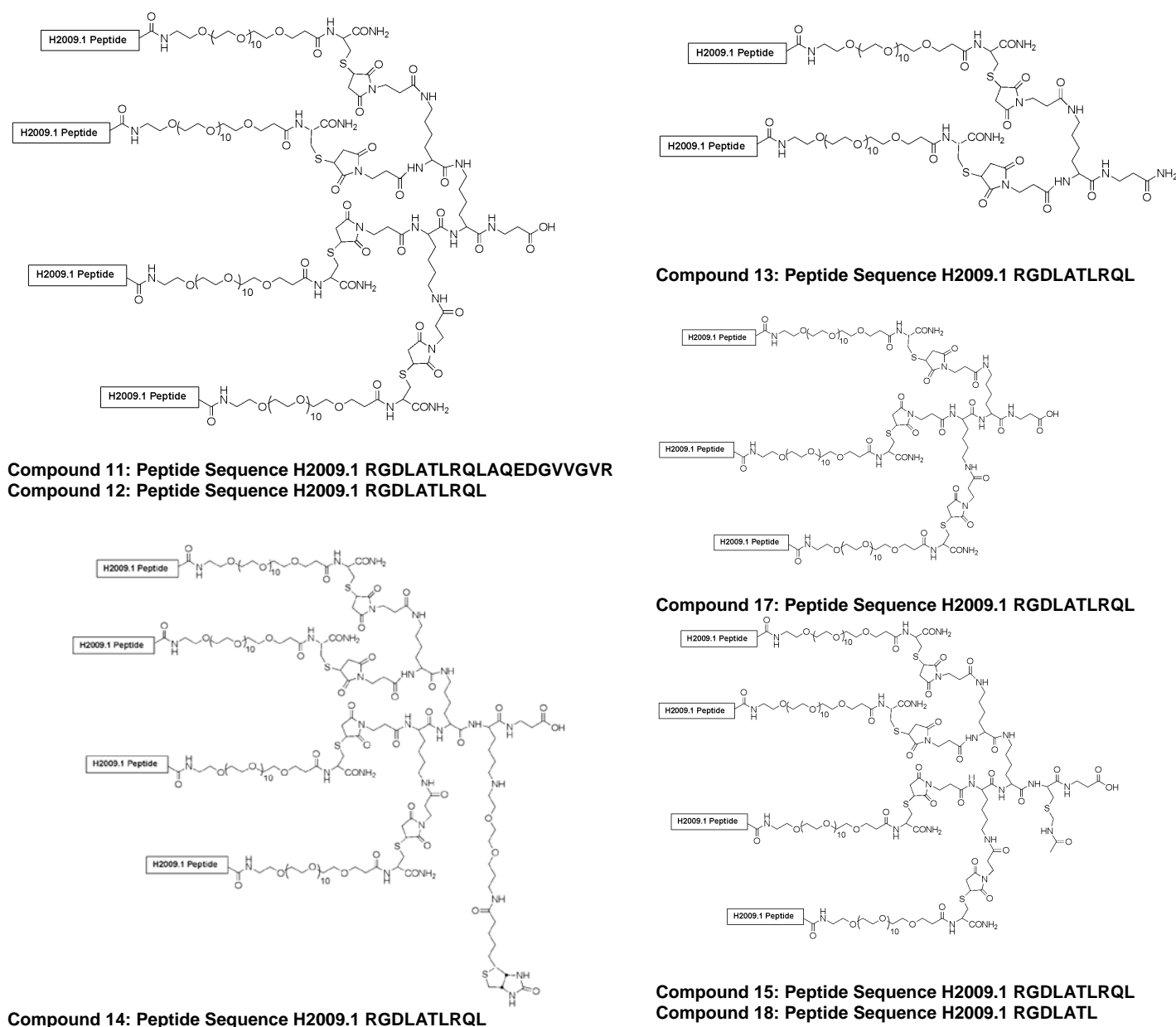
**Synthesis of compounds 12-15 and 17-18:** The synthesis of compounds **12-15** and **17-18** is similar to that of compound **11**, but that the following combinations of activated lysine cores and peptides were used:

**Compound 2 + Compound 6 = Compound 12**  
**Compound 2 + Compound 9 = Compound 13**  
**Compound 2 + Compound 7 = Compound 14**  
**Compound 2 + Compound 8 = Compound 15**  
**Compound 2 + Compound 10 = Compound 17**  
**Compound 3 + Compound 8 = Compound 18**

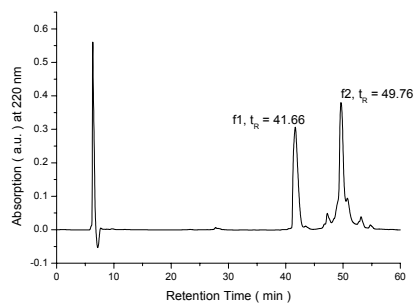
The structures of the compounds **11-18** are shown in Figure 8S. Characteristics of the peptides are given in Table 5S and Figures 7S and 9S-15S.



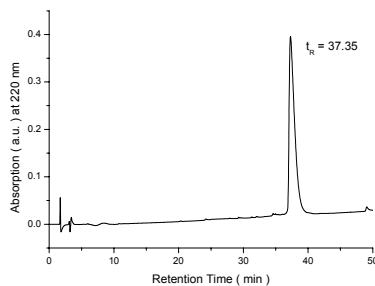
**Synthesis of Compound 16:** The acetamidomethyl group of the cysteine was removed according to published procedure<sup>2</sup>. Specifically, 1  $\mu\text{mol}$  of compound **15** was dissolved in 1 mL of TFA/anisole (99:1) to which 28 mg (167  $\mu\text{mol}$ ) of silver acetate was added. The solution was stirred for 2 hrs at 4°C and then concentrated under Argon to 0.5 mL. The peptide was precipitated by the addition of 5 mL of cold ether and the precipitate was collected by centrifugation. The precipitate was then treated with 1 mL of dithiothreitol (0.2 M) in 1 M acetic acid at RT for 3 hrs. The solution was filtered and purified by HPLC (Method A). HPLC traces and MALDI MS are shown in figure 13S.



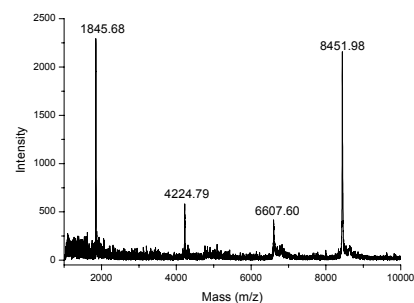
**Figure 8S: Chemical Structures of Compounds 11-15 and 17-18.**



**A. Preparative HPLC of peptide f1: monomer; f2: tetramer**

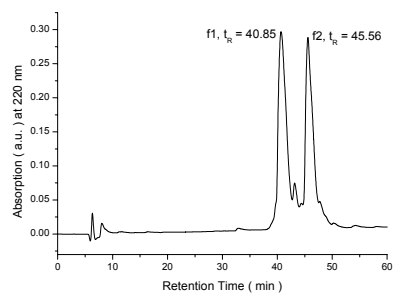


**B. Analytical HPLC of peptide**

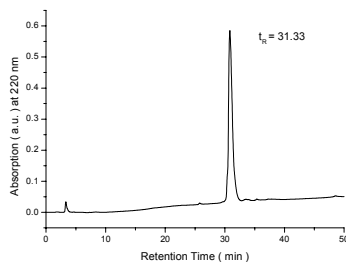


**C. MALDI MS of peptide**  
Note: both singly charged and doubly charged parent ions are observed.

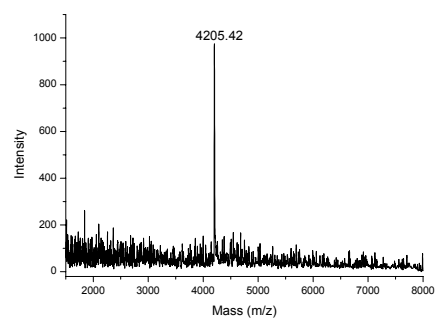
**Figure 9S: Purification and Characterization of Compound 12**



**A. Preparative HPLC of peptide f1: monomer; f2: dimer**

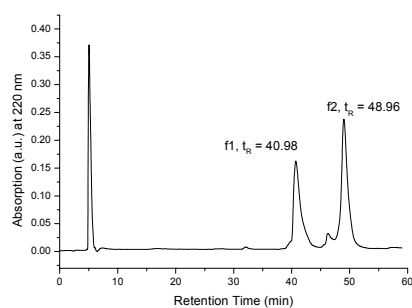


**B. Analytical HPLC of peptide**

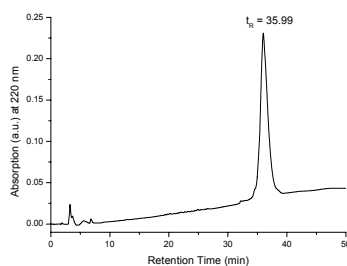


**C. MALDI MS of peptide**

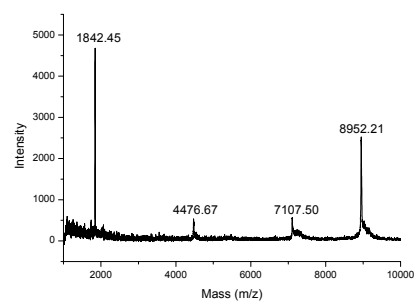
**Figure 10S: Purification and Characterization of Compound 13**



**A. Preparative HPLC of peptide f1: monomer; f2: tetramer**

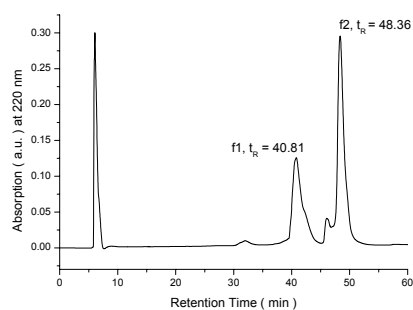


**B. Analytical HPLC of peptide**

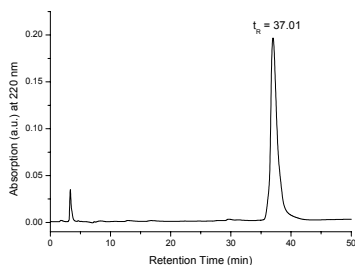


**C. MALDI MS of peptide**  
Note: both singly charged and doubly charged parent ions are observed.

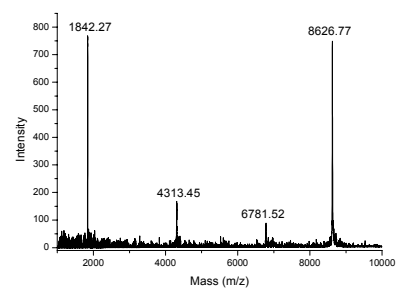
**Figure 11S: Purification and Characterization of Compound 14**



**A. Preparative HPLC of peptide f1: monomer; f2: tetramer**

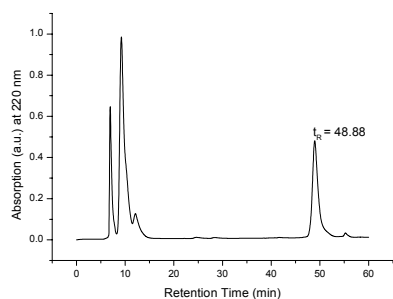


**B. Analytical HPLC of peptide**

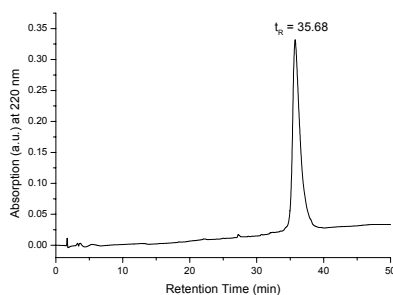


**C. MALDI MS of peptide**  
Note: both singly charged and doubly charged parent ions are observed.

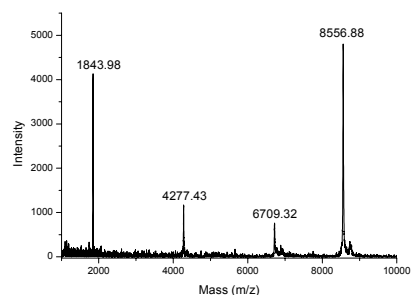
**Figure 12S: Purification and Characterization of Compound 15**



**A. Preparative HPLC of peptide**

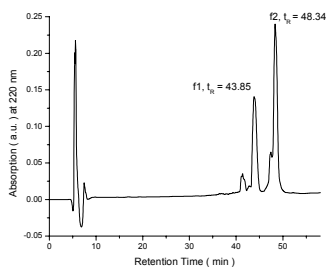


**B. Analytical HPLC of peptide**

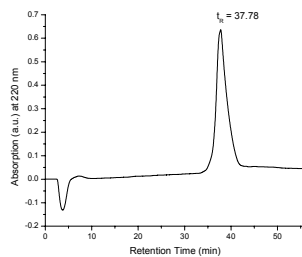


**C. MALDI MS of peptide**  
Note: both singly charged and doubly charged parent ions are observed.

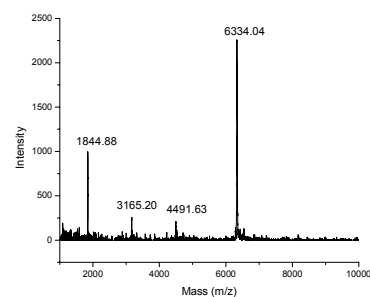
**Figure 13S: Purification and Characterization of Compound 16**



**A. Preparative HPLC of peptide f1: monomer; f2: trimer**

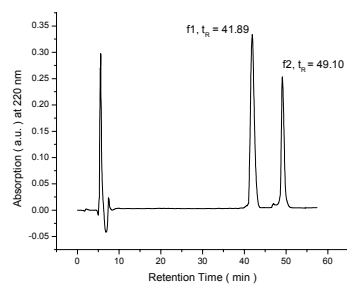


**B. Analytical HPLC of peptide**

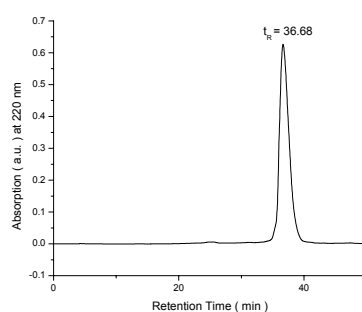


**C. MALDI MS of peptide**

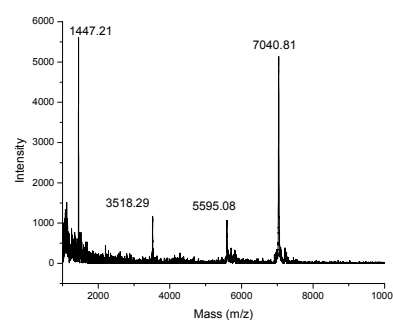
**Figure 14S: Purification and Characterization of Compound 17**



**A. Preparative HPLC of peptide  
f1: monomer; f2: tetramer**



**B. Analytical HPLC of peptide**



**C. MALDI MS of peptide**

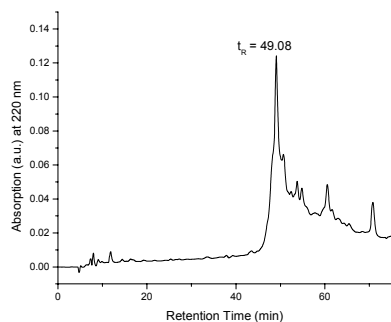
**Figure 15S :Purification and Characterization of Compound 18**

### Synthesis of the Tetrameric 10-mer H2009.1 Peptide by Linear SPPS, Compounds 19-20

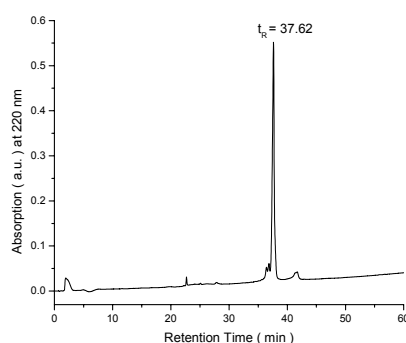
Synthesis of the tetrameric 10-mer H2009.1 peptide (compound **19**) by linear synthesis was performed on a Symphony™ Synthesizer (Rainin Instruments, Protein Technologies, Inc. Woburn, MA) by Fmoc solid-phase peptide synthesis on a Fmoc<sub>4</sub>-Lys<sub>2</sub>-Lys-Cys(Acm)-β-Ala-CLEAR™ Acid Resin (substitution level 0.21 mmol/g) using standard protocols. Fmoc-protected amino acids were doubly coupled at a 5-fold excess using HBTU, HOBT and NMM coupling (60 min). Piperidine in DMF (20%) was employed to remove N-terminal Fmoc protecting groups. A single coupling of Fmoc-NH-(PEG)<sub>11</sub>-COOH was performed overnight. Upon completion of the synthesis, peptide was cleaved from the resin, purified by reverse phase HPLC using method A and characterized by analytical HPLC (method B). MALDI MS were obtained in linear mode using sinapinic acid as matrix. Removal of the Acm protecting group from the cysteine to reveal a free thiol (compound **20**) was performed in the same manner as described above for the synthesis of compound **16**.

**Table 6S: Characteristics of Tetrameric H2009 Peptides Synthesized by Linear Fmoc SPPS**

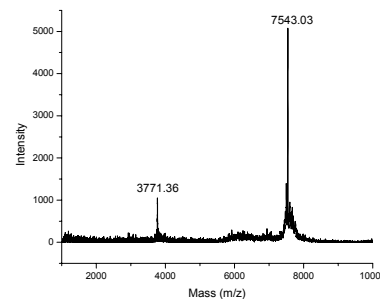
Compound Name	Elemental Composition	Expected Mass (average)	MH <sup>+</sup> (average)	Purity
Compound 19	C <sub>327</sub> H <sub>606</sub> N <sub>82</sub> O <sub>114</sub> S <sub>1</sub>	7542.86	7543.03	95%
Compound 20	C <sub>324</sub> H <sub>601</sub> N <sub>81</sub> O <sub>113</sub> S <sub>1</sub>	7471.78	7473.63	95%



A. Preparative HPLC of peptide

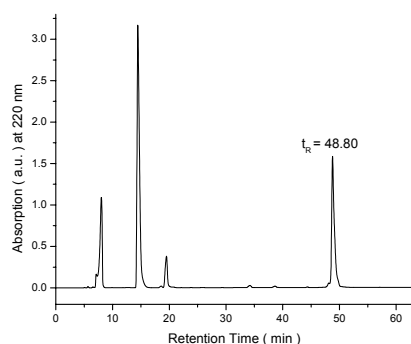


B. Analytical HPLC of peptide

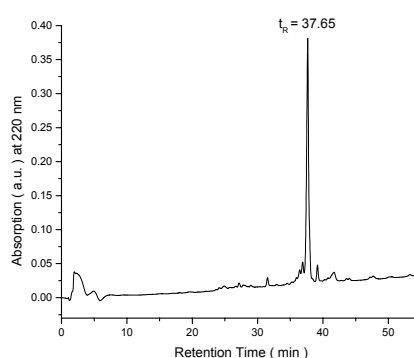


C. MALDI MS of peptide.

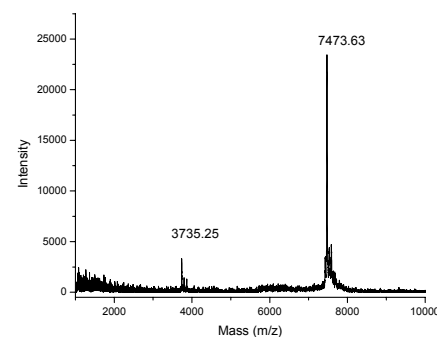
**Figure 16S :Purification and Characterization of H2009.1 10-mer tetramer linear synthesis(Acm), compound 19**



A. Preparative HPLC of peptide

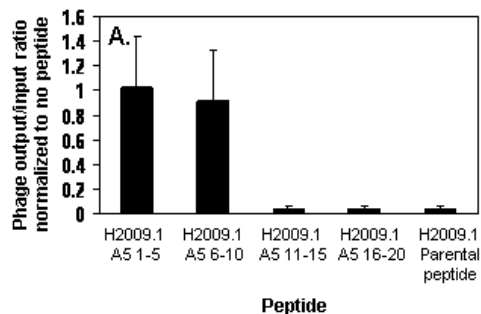


B. Analytical HPLC of peptide



C. MALDI MS of peptide.

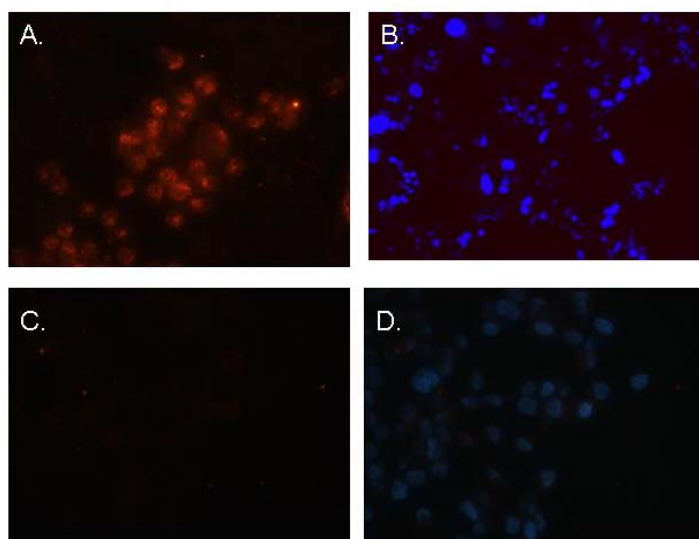
**Figure 17S :Purification and Characterization of H2009.1 10-mer tetramer linear synthesis after removal of the Acm protecting group, Compound 20**



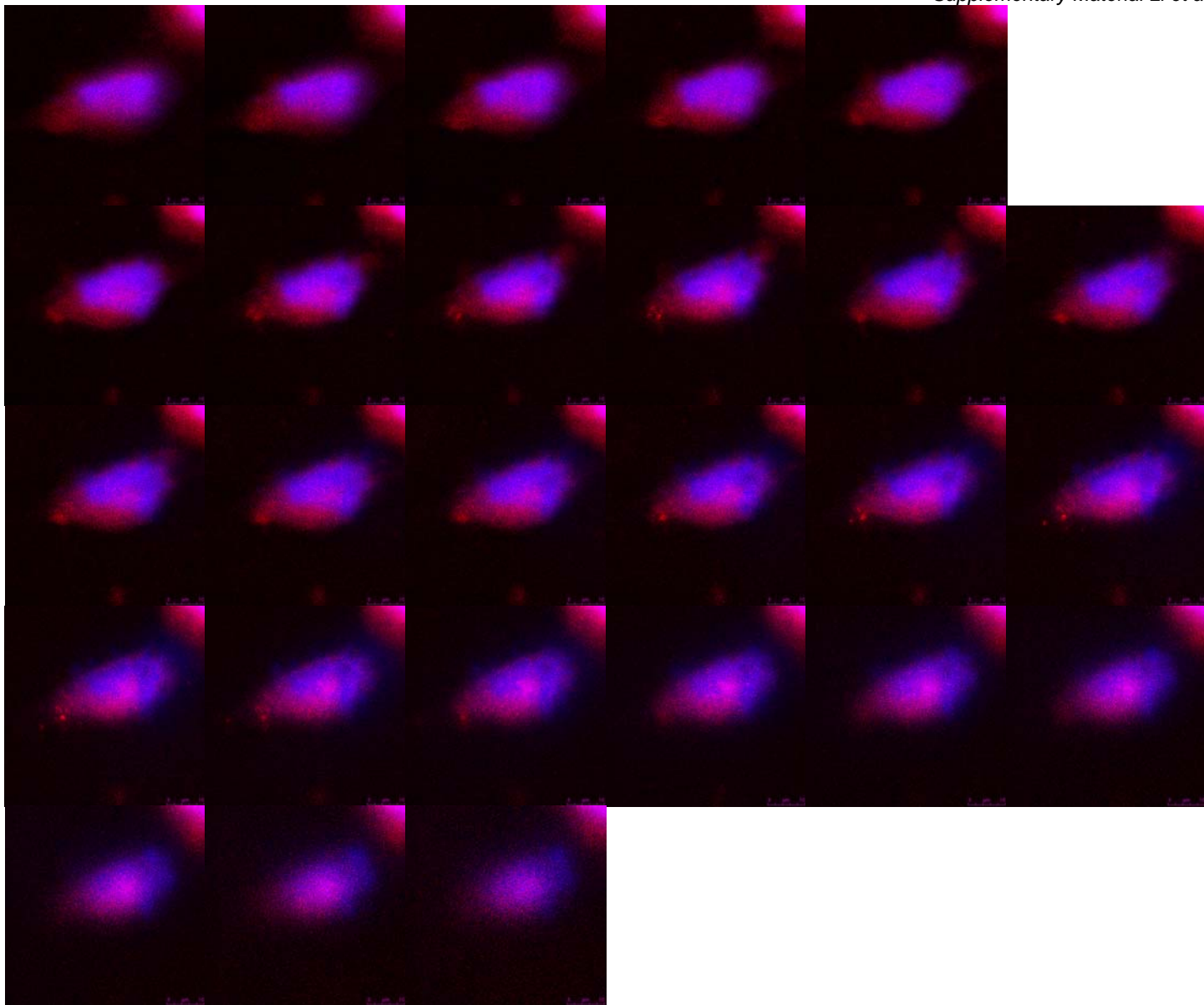
**Figure 18S. The H2009.1 Peptide can be Truncated.** Four alanine scanning derivatives of the H2009.1 peptide were synthesized. The ability of the peptides to block uptake of the H2009.1 phage on H2009 cells was determined. Values are normalized to the output/input ratio with no peptide included.

**Peptide Blocking Experiments:** Peptide solutions were prepared in phosphate buffered saline, pH7.4 and concentrations were confirmed by Edman sequencing. The ability of the peptides to block their cognate phage was determined as previously described.<sup>3,4</sup> Peptide and phage clones were added to the cells simultaneously. No prior incubation of the peptide with the target cells was performed. Phage uptake was determined by standard bacterial titering.

**Cellular uptake of the peptide-Qdot conjugate:** The biotin labeled tetrameric peptide (compound 14, 4000 nM) was incubated with Streptavidin-Qdot 605 made in house (SAQdot605, 200 nM) for 2 hrs at RT. The peptide-SAQdot605 conjugate solution were diluted with PBS<sup>+</sup>/0.1%BSA to a final Qdot concentration of 20 nM before overlaying onto cells as previously described.<sup>3</sup> A sequence scrambled control peptide was used to assure that the uptake was specific (sequence: DALRLQGTLR). H2009.1 scrambled-10-mer tetrameric peptide-on biotin containing trilycine core (C<sub>383</sub>H<sub>684</sub>N<sub>96</sub>O<sub>135</sub>S<sub>5</sub>): expected mass (average): 8954.42; MH+(average): 9021; Purity: 95%. Fluorescent microscopy images are shown in Figure 19S and 20S.



**Figure 19S: The Tetrameric 10-mer H2009.1 Peptide Mediates Cellular Uptake of a Qdot in the Human Lung Adenocarcinoma Cell Line, H2009.** **A:** The peptide was conjugated to the Qdot via a streptavidin-biotin interaction. Cells were incubated with 20 nM SAQdot605 conjugated to the tetrameric H2009.1 10-mer peptide and visualized with 400-fold magnification using a fluorescent microscope. **B:** A scrambled H2009.1 peptide was conjugated to the Qdots as described above. No uptake of the fluorescent Qdots was observed. Overlay of the DAPI nuclear stain is shown so that cells can be observed. **C:** Cells were incubated with 20 nM SAQdot605 that had been quenched with a 20X excess of free biotin prior to incubating with H2009 cells. No accumulation of Qdots is observed confirming that the uptake of the fluorescent nanoparticle is mediated by the cell binding peptide. **D:** The corresponding nuclear staining with Hoechst 33342 confirms the presence of cells in the microscopic field shown in panel C.



**Figure 20S: Cellular uptake of the peptide-Qdot conjugate:** The biotin labeled tetrameric peptide (4000 nM) was incubated with Streptavidin-Qdot 605 made in house (SAQdot605, 200 nM) for 2 h at RT. The peptide-SAQdot605 conjugate solution were diluted with PBS<sup>+</sup>/0.1%BSA to a final Qdot concentration of 20 nM before overlaying onto cells as previously described.<sup>3</sup> Cells were incubated with peptide-labeled Qdots for 10 minutes at 37°C before washing 4 times with PBS<sup>+</sup>/0.1%BSA and once with pH2.2 glycine buffer at room temperature. Cells were washed 2 times with PBS<sup>+</sup>/0.1%BSA to remove acid. Vectashield containing nuclear stain was added before sealing the coverslip and visualization on the microscope. A sequence scrambled peptide-labeled Qdot was used to assure that the uptake was specific. Images were captured on a Leica TCS SP5 confocal microscope at 400x magnification with 7x optical zoom. Images are presented as an overlay series of 26 frames from the Z-stack acquisition as well as an individual slice from the middle of the stack. Similar data was collected from distinct areas of the slide.

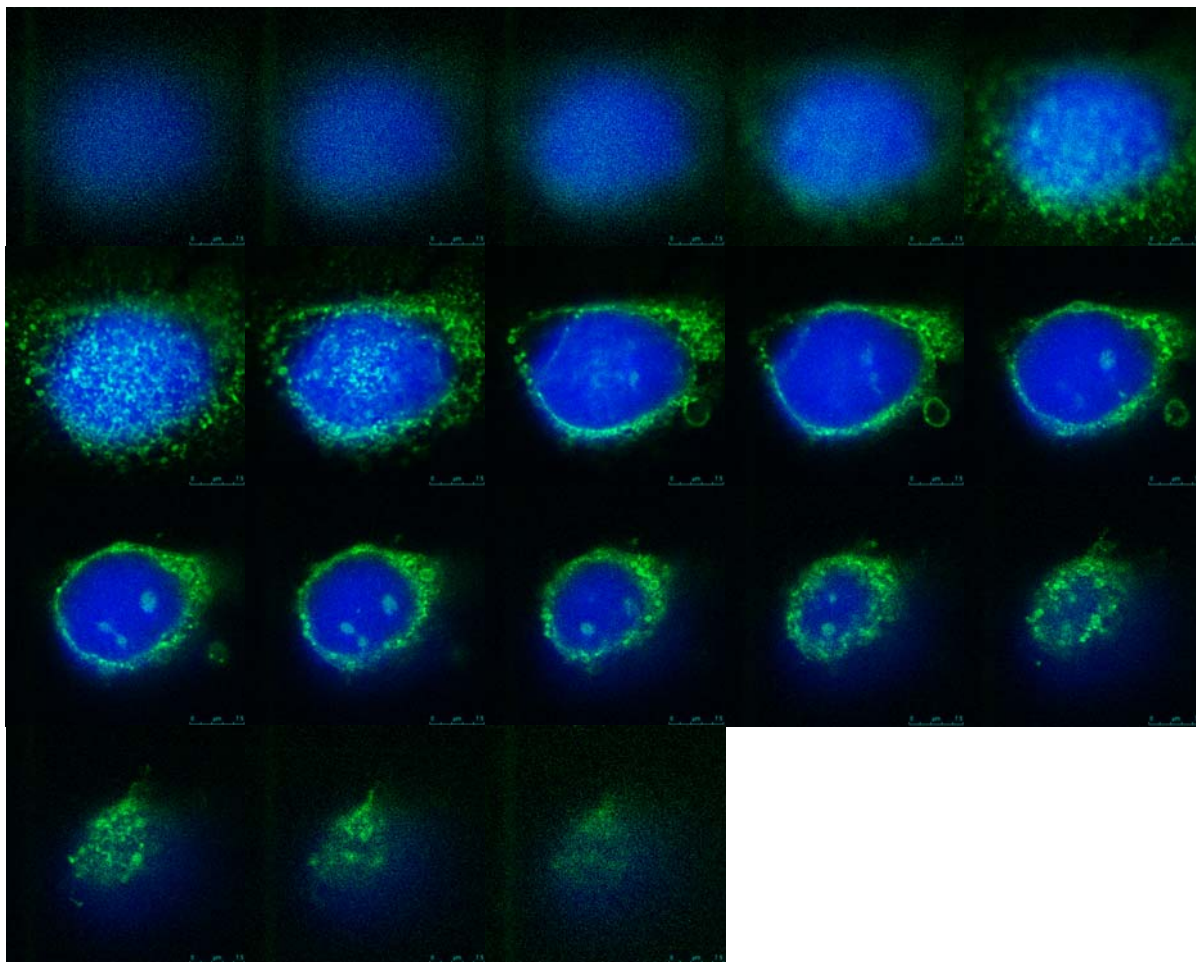
### Synthesis of Tetrameric Peptide- Alexa Fluor 488 C<sub>5</sub>-Maleimide Dye Conjugates, H2009.1 10-mer

#### tetrameric peptide-dye and H2009.1 10-mer scrambled peptide-dye:

A solution of 0.015  $\mu\text{mol}$  H2009.1 tetrameric-SH peptide (compound 16) was prepared in 500  $\mu\text{L}$  of Ar-purged

1× PBS/0.01 M EDTA. A solution of 0.15  $\mu\text{mol}$  Alexa Fluor 488 C<sub>5</sub>-maleimide dye (C<sub>30</sub>H<sub>25</sub>N<sub>4</sub>NaO<sub>12</sub>S<sub>2</sub>, Invitrogen Corporation, Carlsbad, CA) in 50  $\mu\text{L}$  1× PBS was added to the peptide. The reaction mixture was stirred at RT in dark for 12 hrs before adding 0.75  $\mu\text{mol}$  mercaptoethanol to consume excess thiol-reactive dye for 10 min. The products were then purified by reverse phase HPLC using Method B. The purified peptide was analyzed by analytical HPLC using the Method B. MALDI Mass Spectra was obtained in linear mode using sinapinic acid as matrix. H2009.1 10-mer tetrameric peptide-dye (C<sub>394</sub>H<sub>677</sub>N<sub>96</sub>NaO<sub>142</sub>S<sub>7</sub>): expected mass(average): 9278.59 ; MH<sup>+</sup>(average): 9272.69; Purity: 96%.

*Confocal Microscopy Analysis:* H2009 cells were incubated for 10 minutes at 37°C with 1  $\mu\text{M}$  peptide-dye conjugate prior to washing and microscopic observation as detailed for Qdots. The scrambled sequence peptide-dye conjugate was used as a control. Z-stack images are shown in Figure 21S.



**Figure 21S: Cellular uptake of H2009.1 10mer tetramer-Alexa Fluor 488 conjugate.** H2009 cells were incubated for 10 minutes at 37°C with 1  $\mu\text{M}$  peptide-dye conjugate prior to washing and microscopic observation as detailed for Qdots. Images were captured on a Leica TCS SP5 confocal microscope at 400x magnification with 7x optical zoom. Images are presented as an overlay series of 18 frames from the Z-stack acquisition. Similar data was collected from distinct areas of the slide.

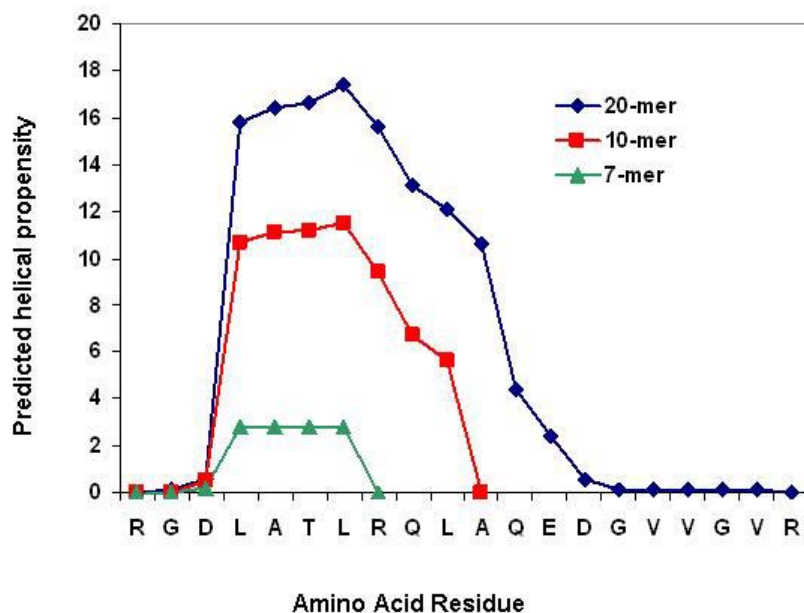


### Preparation of $^{111}\text{In}$ labeled peptides

The peptide solutions were prepared by dissolving 100  $\mu\text{g}$  of each lyophilized DOTA-peptide conjugate in 100  $\mu\text{L}$  of 0.1 M sodium acetate (pH 6.5). To 200  $\mu\text{L}$  of each DOTA-peptide solution (20  $\mu\text{g}$ ), 4  $\mu\text{L}$  of  $^{111}\text{InCl}_3$  solution (2-2.3 mCi, in 0.05 N HCl from Trace Life Sciences, Denton, TX) was added. Reaction mixtures were incubated at 37  $^\circ\text{C}$  for 1 hr with a constant shaking (600 rpm). After adding 5  $\mu\text{L}$  of 5 mM DTPA to challenge the free  $^{111}\text{In}$ , the radiochemical yield was determined by radio-TLC (Raytest, VA; static phase: ITLC; mobile phase: 10 mM PBS). The  $^{111}\text{In}$ -labeled H2009.1 was purified by a desalting column (MWCO 5,000 Da, Pierce, Rockford, IL). The radiochemical purity (RCP) was determined by a radio-HPLC system with an XTera C<sub>18</sub> column as static phase and a mobile phase of a linear gradient from water (0.1% TFA) to acetonitrile (0.1% TFA) within 50 min.

### Prediction of Helical Content of the Monomeric H2009.1 peptides

The helical propensity of the H2009.1 20-mer (compound 1) 10-mer (compound 2) and 7-mer (compound 3) was determined using AGADIR, a freely available web-based program from to predict helical propensity of peptides at the amino acid level (software update August 6th 2003, <http://www.embl-heidelberg.de/Services/serrano/agadir/agadir-start.html>). The helical propensity of the peptides is shown in Figure 22S.



**Figure 22S: Helical propensity of the H2009.1 monomeric peptide increases with length.**

**References:**

- (1) Bourel, L.; Carion, O.; Gras-Masse, H.; Melnyk, O. J. *J. Pep. Sci.* **2000**, *6*, 264-270.
- (2) Kawakami, T.; Aimoto, S.; Nishimura, T.; Nakatsuji, T.; Sonobe, H. *Peptide Science* **1999**, *35*, 149-152.
- (3) Oyama, T.; Rombel, I. T.; Samli, K. N.; Zhou, X.; Brown, K. C. *Biosensors Bioelect.* **2006**, *21*, 1867-1875.
- (4) Oyama, T.; Sykes, K. F.; Samli, K. N.; Minna, J. D.; Johnston, S. A.; Brown, K. C. *Cancer Lett.* **2003**, *202*, 219-230.
- (5) Zhou, X.; Chang, Y.; Oyama, T.; McGuire, M. J.; Brown, K. C. *J. Am. Chem. Soc.* **2004**, *129*, 15656-15657.