

## Supporting Information

### **Photoaffinity Labeling of Ras Converting Enzyme using Peptide Substrates that Incorporate Benzoylphenylalanine (Bpa) Residues: Improved Labeling and Structural Implications**

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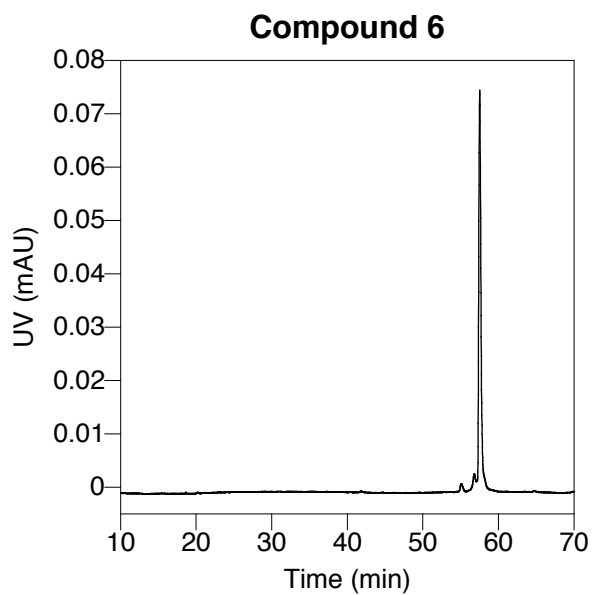
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### Compound Characterization List (6 & 9):

Retention times ( $t_R$ ) are based on analytical RP-HPLC using a linear gradient from 100% H<sub>2</sub>O to 40% H<sub>2</sub>O/60% CH<sub>3</sub>CN over 60 min and a flow rate of 1.0 mL/min. Peptide concentrations were determined by UV spectroscopy:  $\epsilon_{349} = 18,000 \text{ mM}^{-1}\cdot\text{cm}^{-1}$ .

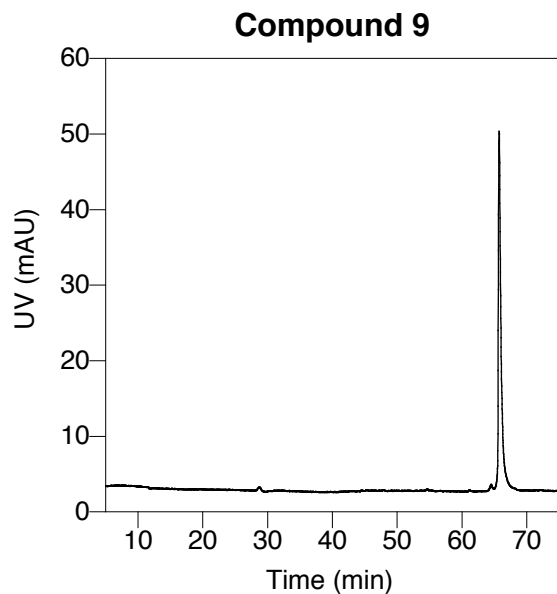
#### Abz-KSKTKC(C10BP)K(Dnp)IM, 6.

Reaction scale: 7.4  $\mu\text{mol}$ , yield: 1.3 mg (10%), purity by RP-HPLC: 92.6%,  $t_R = 57.5 \text{ min.}$ , ESI-MS (m/z):  $[\text{M}+2\text{H}]^{2+}$  calcd for C<sub>82</sub>H<sub>122</sub>N<sub>16</sub>O<sub>19</sub>S<sub>2</sub> 849.4251, found 849.9947;  $[\text{M}+3\text{H}]^{3+}$  calcd for C<sub>82</sub>H<sub>123</sub>N<sub>16</sub>O<sub>19</sub>S<sub>2</sub> 566.6192, found 566.9992.



**Biotin-K(Dde)KSKTKC(C10BP)K(Dnp)IM, 9.**

Reaction scale: 5.0  $\mu\text{mol}$ , yield: 5.3 mg (44%), purity by RP-HPLC: 96.2%,  $t_R =$  66 min., ESI-MS (m/z):  $[\text{M}+2\text{Na}]^{2+}$  calcd for  $\text{C}_{115}\text{H}_{179}\text{N}_{21}\text{O}_{28}\text{S}_3\text{Na}_2$  1222.1096, found 1221.8616;  $[\text{M}+2\text{Na}+\text{H}]^{3+}$  calcd for  $\text{C}_{115}\text{H}_{180}\text{N}_{21}\text{O}_{28}\text{S}_3\text{Na}_2$  815.0730, found 814.9139;  $[\text{M}+2\text{Na}+2\text{H}]^{4+}$  calcd for  $\text{C}_{115}\text{H}_{181}\text{N}_{21}\text{O}_{28}\text{S}_3\text{Na}_2$  611.5548, found 611.4392.

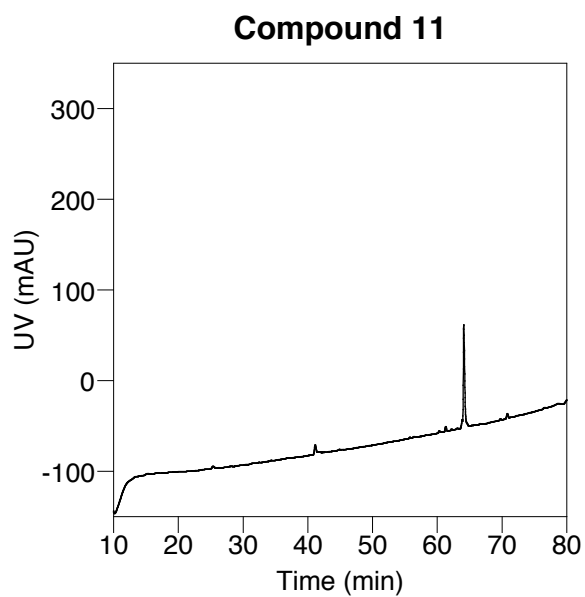


### Compound Characterization List (11-14):

Retention times ( $t_R$ ) are based on analytical RP-HPLC using a linear gradient from 100% H<sub>2</sub>O to 30% H<sub>2</sub>O/70% CH<sub>3</sub>CN over 70 min and a flow rate of 1.0 mL/min. Peptide concentrations of **11-14** were determined by mass.

**Biotin-PEG<sub>3</sub>-Bpa-SKTKC(Fr)VIM, 11.** Purity by RP-HPLC: 91.0%,  $t_R = 64.5$  min.,

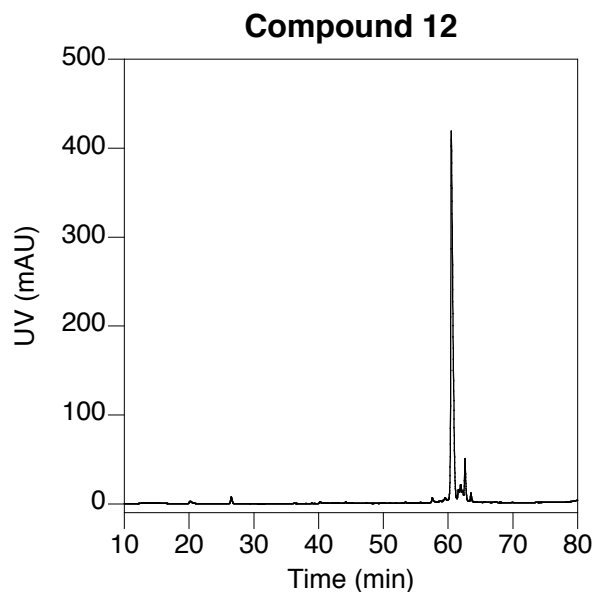
ESI-MS (m/z):  $[M+2H]^{2+}$  calcd for C<sub>89</sub>H<sub>146</sub>N<sub>14</sub>O<sub>18</sub>S<sub>3</sub> 897.5045, found 897.6320.



**Biotin-PEG<sub>3</sub>-K-Bpa-KTKC(Fr)VIM, 12.** Purity by RP-HPLC: 81.3%,  $t_R = 61.5$  min.,

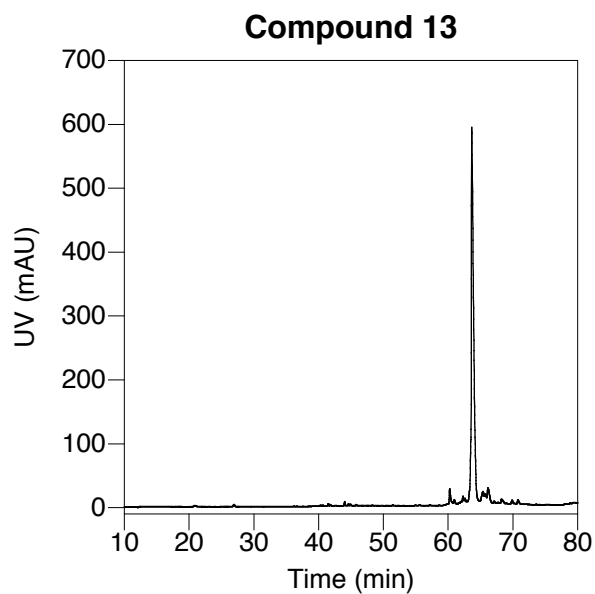
ESI-MS (m/z):  $[M+2H]^{2+}$  calcd for C<sub>92</sub>H<sub>153</sub>N<sub>15</sub>O<sub>17</sub>S<sub>3</sub> 918.7622, found 918.6713;

$[M+3H]^{3+}$  calcd for C<sub>92</sub>H<sub>154</sub>N<sub>15</sub>O<sub>17</sub>S<sub>3</sub> 612.8440, found 612.7869.



**Biotin-PEG<sub>3</sub>-KS-Bpa-TKC(Fr)VIM, 13.** Purity by RP-HPLC: 74.4%,  $t_R = 64$  min.,

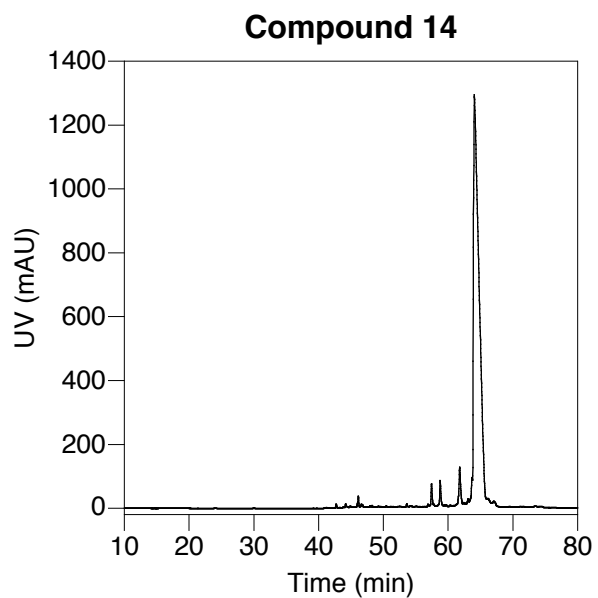
ESI-MS (m/z):  $[M+2H]^{2+}$  calcd for C<sub>89</sub>H<sub>146</sub>N<sub>14</sub>O<sub>18</sub>S<sub>3</sub> 897.5045, found 897.6381.

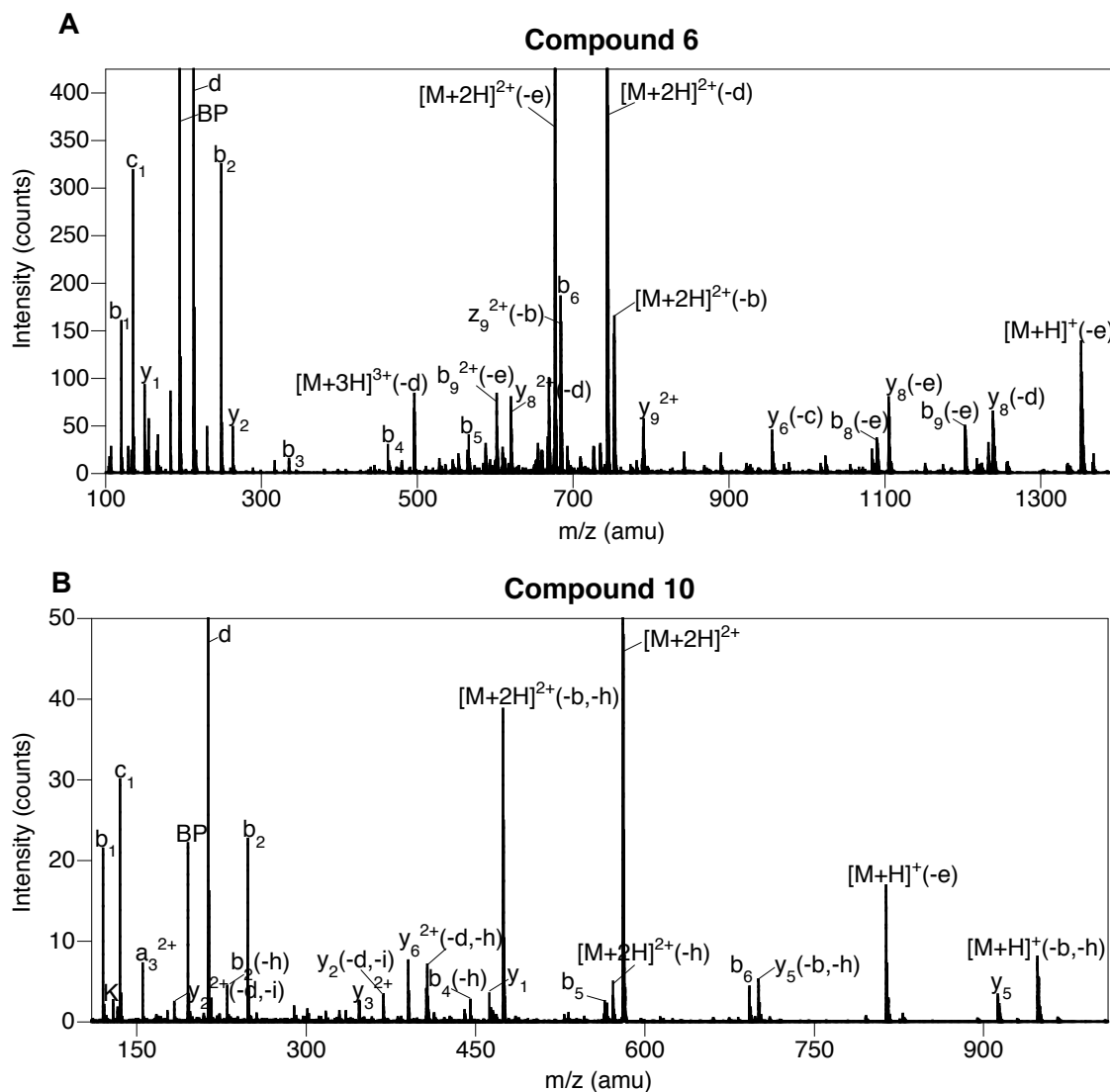


**Biotin-PEG<sub>3</sub>-KSK-Bpa-KC(Fr)VIM, 14.** Purity by RP-HPLC: 91.4%,  $t_R = 64$  min.,

ESI-MS (m/z):  $[M+2H]^{2+}$  calcd for C<sub>91</sub>H<sub>151</sub>N<sub>15</sub>O<sub>17</sub>S<sub>3</sub> 911.0282, found 911.6836;

$[M+3H]^{3+}$  calcd for C<sub>91</sub>H<sub>152</sub>N<sub>15</sub>O<sub>17</sub>S<sub>3</sub> 608.1682, found 608.1268.





**Figure 1.** ESI-MS-MS of full unprocessed substrate **6** and the Rce1p-catalyzed proteolysis product **10**. (A) Benzophenone-modified substrate **6**. (B) Benzophenone-modified proteolysis product **10**. (K, lysine fragment; BP, benzophenone fragment,  $(C_{14}H_{11}O^+)$ ; -b, loss of benzophenone fragment; -c, loss of C<sub>5</sub>-prenyl benzophenone,  $(C_{19}H_{20}O_2)$ ; d, benzophenone-H<sub>2</sub>O,  $(C_{14}H_{13}O_2)$ ; -d, loss of benzophenone-H<sub>2</sub>O; -e, loss of C<sub>10</sub>-prenyl benzophenone,  $(C_{24}H_{28}O_2)$ ; -h, loss of H<sub>2</sub>O; -i, loss of NH<sub>3</sub>).

**Table 1**

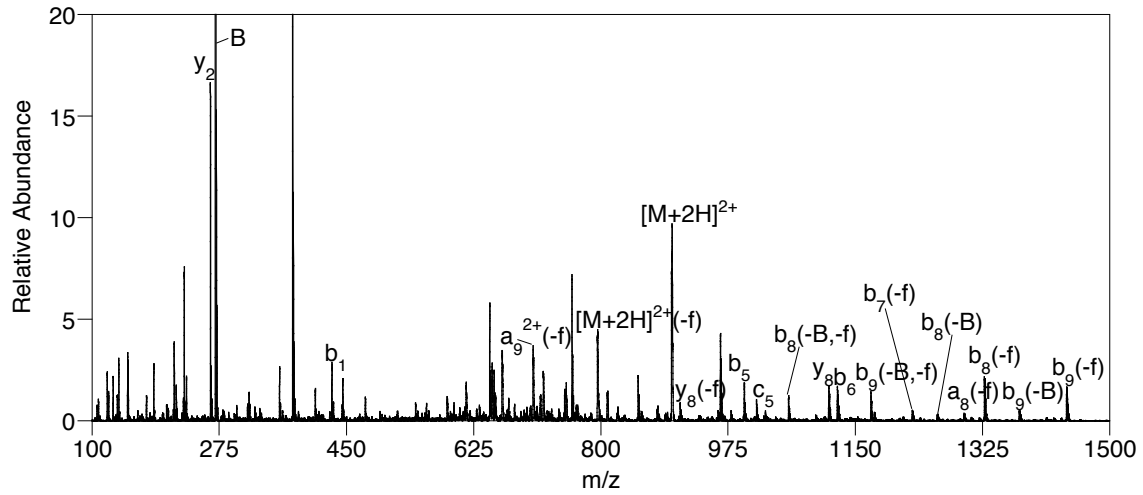
Summary of ESI-MS-MS for unprocessed substrate **6** and the Rce1p-catalyzed proteolysis product **10** (K, lysine fragment; BP, benzophenone fragment, (3-benzoylphenyl)methylium (C<sub>14</sub>H<sub>11</sub>O<sup>+</sup>); -b, loss of benzophenone fragment; -c, loss of (*E*)-(3-(((2-methylbut-2-en-1-yl)oxy)methyl)phenyl)(phenyl)methanone (C<sub>19</sub>H<sub>20</sub>O<sub>2</sub>); d, benzophenone-H<sub>2</sub>O, (3-(hydroxymethyl)phenyl)(phenyl)methanone (C<sub>14</sub>H<sub>13</sub>O<sub>2</sub>); -d, loss of benzophenone-H<sub>2</sub>O; -e, loss of (3-(((2*E*,6*E*)-2,6-dimethylocta-2,6-dien-1-yl)oxy)methyl)phenyl)(phenyl)methanone (C<sub>24</sub>H<sub>28</sub>O<sub>2</sub>); -h, loss of H<sub>2</sub>O; -i, loss of NH<sub>3</sub>).

Peptide Ion	<b>6</b>		<b>10</b>	
	Calc.	Obs.	Calc.	Obs.
[M+H] <sup>+</sup> (-b, -h)			947.54	947.67
[M+H] <sup>+</sup> (-e)	1351.65	1351.66	813.43	813.58
[M+2H] <sup>2+</sup>			580.31	580.39
[M+2H] <sup>2+</sup> (-h)			571.31	571.39
[M+2H] <sup>2+</sup> (-b)	752.39	752.40		
[M+2H] <sup>2+</sup> (-d)	743.88	743.38		
[M+2H] <sup>2+</sup> (-e)	676.33	676.33		
[M+2H] <sup>2+</sup> (-b, -h)			474.27	474.35
[M+3H] <sup>3+</sup> (-d)	496.26	496.26		
z <sub>9</sub> <sup>2+</sup> (-b)	684.86	684.87		
y <sub>5</sub>			912.49	912.66
y <sub>8</sub> (-d)	1238.64	1238.64		
y <sub>8</sub> (-e)	1104.52	1104.54		
y <sub>6</sub> (-c)	956.44	956.47		
y <sub>9</sub> <sup>2+</sup>	789.91	790.41		
y <sub>5</sub> (-b, -h)			700.41	700.53
y <sub>8</sub> <sup>2+</sup> (-d)	620.32	619.82		
y <sub>2</sub>	263.14	263.15		
y <sub>1</sub>	150.06	150.06	468.22	468.12
y <sub>6</sub> <sup>2+</sup> (-d, -h)			406.25	406.29
y <sub>2</sub> (-d, -i)			368.21	368.30
y <sub>3</sub> <sup>2+</sup>			349.19	349.47
y <sub>2</sub> <sup>2+</sup> (-d, -i)			184.61	184.12
c <sub>1</sub>	135.06	135.12	135.06	135.14
b <sub>9</sub> (-e)	1202.60	1202.62		
b <sub>8</sub> (-e)	1089.51	1089.53		
b <sub>6</sub>	692.41	692.43	692.41	692.79
b <sub>9</sub> <sup>2+</sup> (-e)	601.80	601.80		
b <sub>5</sub>	564.31	564.32	564.31	564.21
b <sub>4</sub>	463.27	463.29		
b <sub>4</sub> (-h)			445.26	445.36
b <sub>3</sub>	335.17	335.18		
b <sub>2</sub>	248.14	248.14	248.14	248.18
b <sub>2</sub> (-h)			230.15	230.18
b <sub>1</sub>	120.10	120.05	120.10	120.07
a <sub>3</sub> <sup>2+</sup>			154.10	154.11
d	213.09	213.10	213.09	213.14
BP	195.12	195.08	195.12	195.12
K			129.10	129.13

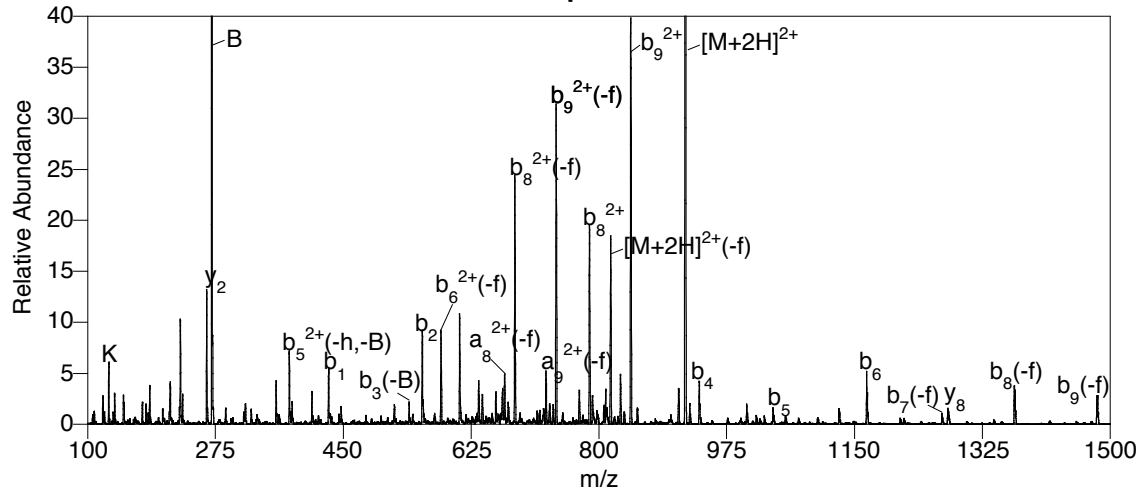


ESI-MS-MS Spectra of Compounds 11-13.

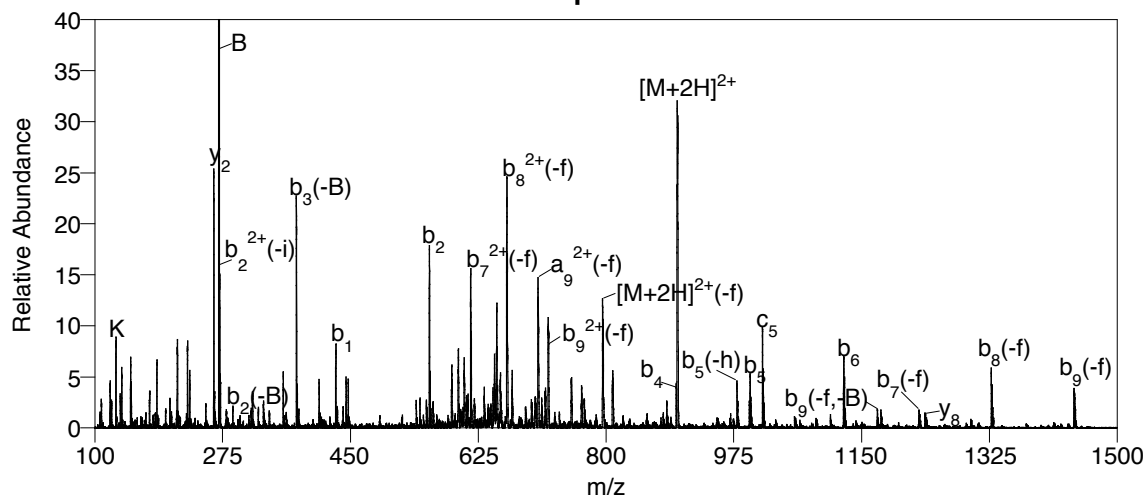
**Compound 11**



**Compound 12**



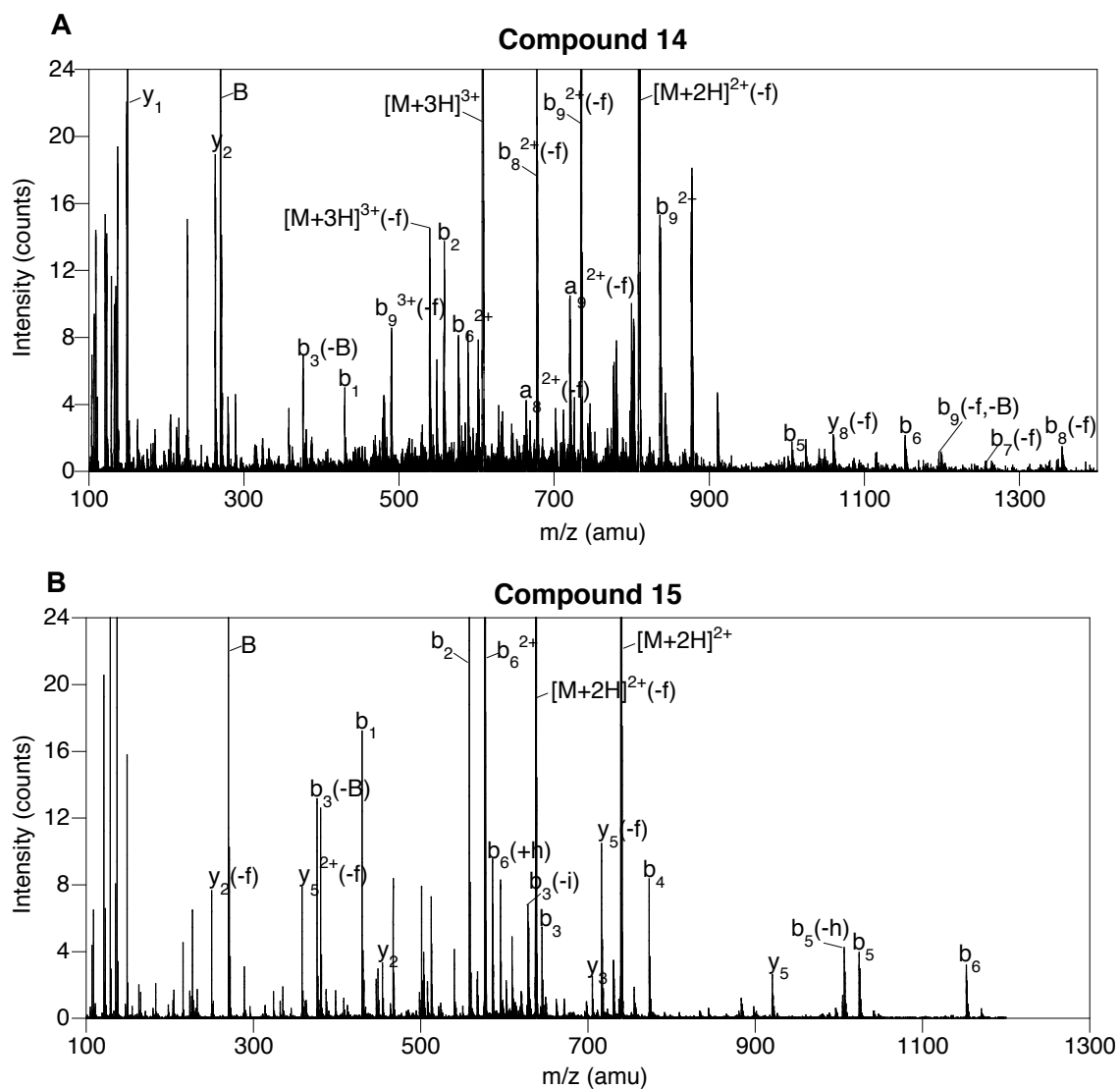
### Compound 13



**Table 2**

Summary of ESI-MS-MS for unprocessed Bpa-containing peptides, **11-14** (-f, loss of farnesyl (C<sub>15</sub>H<sub>25</sub>); -h, loss of H<sub>2</sub>O; B, biotin fragment, 2-(5-((3a*R*,4*S*,6a*S*)-2-oxohexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl)pentanamido)ethan-1-ylum (C<sub>12</sub>H<sub>20</sub>N<sub>3</sub>O<sub>2</sub>S<sup>+</sup>), -B, loss of biotin fragment; -i, loss of NH<sub>3</sub>; K, lysine fragment).

Peptide Ion	11		12		13		14	
	Calc.	Obs.	Calc.	Obs.	Calc.	Obs.	Calc.	Obs.
[M+2H] <sup>2+</sup>	897.50	897.66	918.04	918.25	897.50	897.56		
[M+2H] <sup>2+</sup> (-f)	795.41	795.45	815.94	816.13	795.41	795.44	808.93	808.93
[M+3H] <sup>3+</sup>							607.68	607.83
[M+3H] <sup>3+</sup> (-f)							539.62	539.64
y <sub>8</sub>	1113.68	1113.69	1277.74	1277.79	1236.68	1236.72		
y <sub>8</sub> (-h)	1095.66	1095.72						
y <sub>8</sub> (-B,-f)					1032.48	1032.44		
y <sub>8</sub> (-f)	909.48	909.45					1059.54	1059.51
y <sub>2</sub>	263.14	262.98	263.14	263.07	263.14	263.12	263.14	263.09
y <sub>1</sub>							150.06	150.03
c <sub>5</sub>	1014.53	1014.44			1014.53	1014.48		
b <sub>9</sub> (-f)	1440.76	1440.80	1481.82	1482.02	1440.76	1440.80		
b <sub>9</sub> (-B)	1375.83	1376.01						
b <sub>8</sub> (-f)	1327.68	1327.71	1368.74	1368.85	1327.68	1327.72	1354.72	1354.81
b <sub>8</sub> (-B)	1262.74	1262.82						
b <sub>7</sub> (-f)	1228.61	1228.68	1269.67	1269.67	1228.61	1228.60	1255.66	1255.67
b <sub>9</sub> (-B,-f)	1171.64	1171.62			1171.64	1171.64	1198.69	1198.63
b <sub>6</sub>	1125.60	1125.57	1166.66	1166.57	1125.60	1125.60	1152.65	1152.63
b <sub>6</sub> (-h)					1107.59	1107.52		
b <sub>6</sub> (-B,-f)	1058.56	1058.52			1058.56	1058.56		
b <sub>5</sub>	997.50	997.47	1038.56	1038.42	997.50	997.48	1024.55	1024.64
b <sub>5</sub> (-h)					979.50	979.48		
b <sub>4</sub>			937.52	937.50	896.46	896.44		
b <sub>9</sub> <sup>2+</sup>			843.51	843.64				
b <sub>3</sub>								
b <sub>9</sub> <sup>2+</sup>							836.50	836.50
b <sub>8</sub> <sup>2+</sup>			786.96	787.09				
b <sub>9</sub> <sup>2+</sup> (-f)			741.42	741.52	720.88	720.88	734.40	734.36
b <sub>8</sub> <sup>2+</sup> (-f)			684.87	684.97	664.34	664.36	667.86	667.84
b <sub>7</sub> <sup>2+</sup> (-f)					614.80	614.84		
b <sub>6</sub> <sup>2+</sup> (-f)			583.84	583.90				
b <sub>6</sub> <sup>2+</sup>							576.82	576.82
b <sub>2</sub>			558.33	558.22	558.33	558.28	558.33	558.23
b <sub>9</sub> <sup>3+</sup> (-f)							489.94	490.00
b <sub>1</sub>	430.20	430.14	430.20	430.13	430.20	430.16	430.20	430.14
b <sub>3</sub> (-B)			540.30	540.23	376.24	376.24	376.24	376.08
b <sub>5</sub> <sup>2+</sup> (-B,-h)			376.22	376.10				
b <sub>2</sub> (-B)					289.21	289.16		
b <sub>2</sub> <sup>2+</sup> (-i)					271.16	271.12		
a <sub>8</sub> (-f)	1299.68	1299.69						
a <sub>9</sub> <sup>2+</sup> (-f)	706.88	706.92	727.42	727.59	706.88	706.92	720.41	720.38
a <sub>8</sub> <sup>2+</sup> (-f)			670.88	670.90			663.86	663.87
B	270.13	270.09	270.13	270.14	270.13	270.12	270.13	270.04
K			129.10	129.11	129.10	129.08		

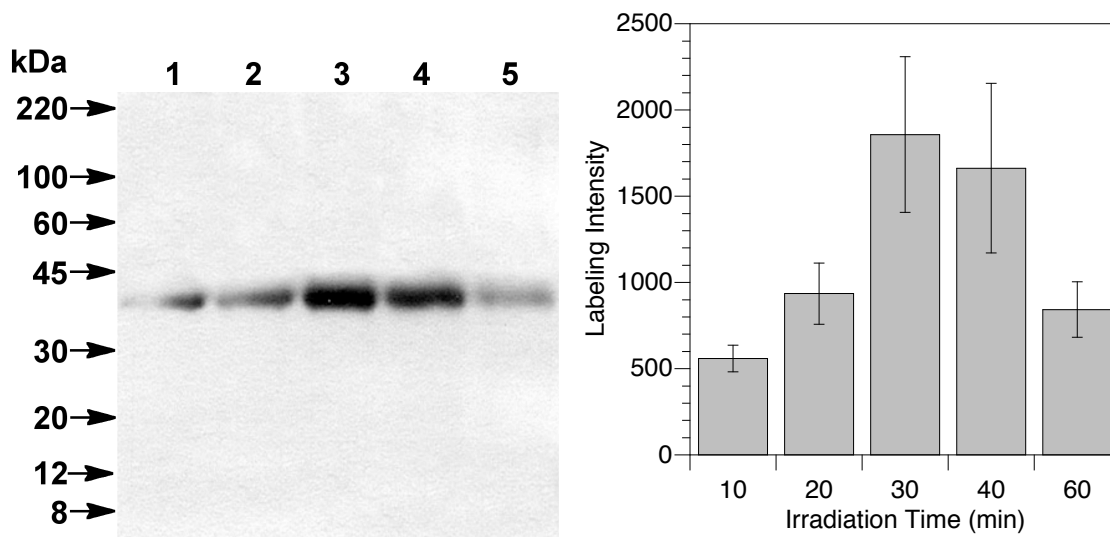


**Figure 2.** ESI-MS-MS of full unprocessed Substrate **14** and the Rce1p-catalyzed proteolysis product **15**. (A) BPA-containing substrate **14**. (B) Bpa-containing proteolysis product **15**. -f, loss of farnesyl ( $C_{15}H_{25}$ ); -h, loss of  $H_2O$ ; +h, gain of  $H_2O$ ; B, biotin fragment, ( $C_{12}H_{20}N_3O_2S^+$ ), -B, loss of biotin fragment; -i, loss of  $NH_3$ .

**Table 3**

Summary of ESI-MS-MS for unprocessed substrate **14** and the Rce1p-catalyzed proteolysis product **15** (-f, loss of farnesyl (C<sub>15</sub>H<sub>25</sub>); -h, loss of H<sub>2</sub>O; +h, gain of H<sub>2</sub>O; B, biotin fragment, 2-(5-((3*aR*,4*S*,6*aS*)-2-oxohexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl)pentanamido)ethan-1-ylum (C<sub>12</sub>H<sub>20</sub>N<sub>3</sub>O<sub>2</sub>S<sup>+</sup>), -B, loss of biotin fragment; -i, loss of NH<sub>3</sub>).

Peptide	14		15	
	Calc.	Obs.	Calc.	Obs.
Ion				
[M+2H] <sup>2+</sup>			739.43	739.42
[M+2H] <sup>2+</sup> (-f)	808.93	808.93	637.34	637.32
[M+3H] <sup>3+</sup>	607.68	607.83		
[M+3H] <sup>3+</sup> (-f)	539.62	539.64		
y <sub>8</sub> (-f)	1059.54	1059.51		
y <sub>5</sub>			920.53	920.52
y <sub>5</sub> (-f)			716.34	716.34
y <sub>3</sub>			705.40	705.39
y <sub>2</sub>	263.14	263.09	454.31	454.31
y <sub>5</sub> <sup>2+</sup> (-f)			358.67	358.74
y <sub>2</sub> (-f)			250.12	250.12
y <sub>1</sub>	150.06	150.03		
b <sub>8</sub> (-f)	1354.72	1354.81		
b <sub>7</sub> (-f)	1255.66	1255.67		
b <sub>9</sub> (-B,-f)	1198.69	1198.63		
b <sub>6</sub>	1152.65	1152.63	1152.64	1152.61
b <sub>5</sub>	1024.55	1024.64	1024.55	1024.50
b <sub>5</sub> (-h)			1006.54	1006.51
b <sub>4</sub>			773.46	773.42
b <sub>3</sub>			645.36	645.33
b <sub>9</sub> <sup>2+</sup>	836.50	836.50		
b <sub>9</sub> <sup>2+</sup> (-f)	734.40	734.36		
b <sub>8</sub> <sup>2+</sup> (-f)	667.86	667.84		
b <sub>3</sub> (-i)			628.34	628.32
b <sub>6</sub> <sup>2+</sup> (+h)			585.83	585.82
b <sub>6</sub> <sup>2+</sup>	576.82	576.82	576.82	576.81
b <sub>2</sub>	558.33	558.23	558.33	558.30
b <sub>9</sub> <sup>3+</sup> (-f)	489.94	490.00		
b <sub>1</sub>	430.20	430.14	430.20	430.20
b <sub>3</sub> (-B)	376.24	376.08	376.24	376.20
a <sub>9</sub> <sup>2+</sup> (-f)	720.41	720.38		
a <sub>8</sub> <sup>2+</sup> (-f)	663.86	663.87		
B	270.13	270.04	270.13	270.13



**Figure 3.** Western blot analysis (left) and densitometric quantification (right) of time course photolabeling of Rce1p using probe **14** detected with anti-HA following SPD and SDS-PAGE separation. Lanes 1-5 correspond to Rce1p-containing membranes (*RCE1-HA*) photolyzed with probe **14** (15  $\mu$ M) for 10, 20, 30, 40, and 60 min, respectively. Columns represent densitometric data obtained from three replicate experiments using probe **14** for the indicated amounts of time.