Supporting Information

Photoaffinity Labeling of Ras Converting Enzyme 1 (Rce1p) using a Benzophenone-Containing Peptide Substrate

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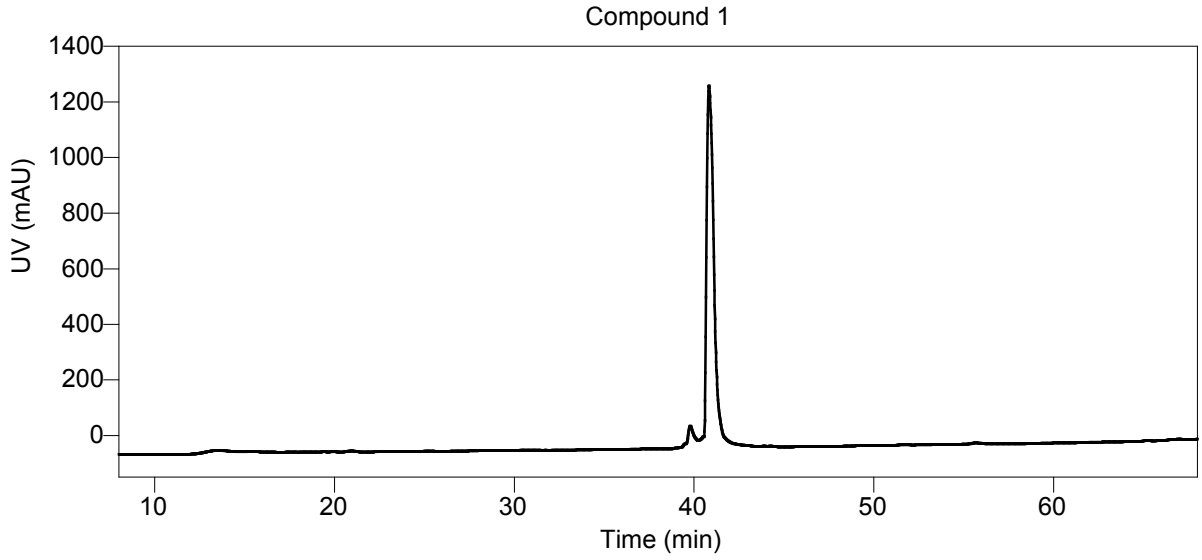
Compound Characterization List:

Retention times (t_R) are based on analytical RP-HPLC using a linear gradient from 100% H_2O to 40% H_2O/60% CH_3CN over 60 min and a flow rate of 1.0 mL/min. Peptide concentrations were determined by UV spectroscopy: ε_{349} = 18,000 $mM^{-1} \bullet cm^{-1}$.

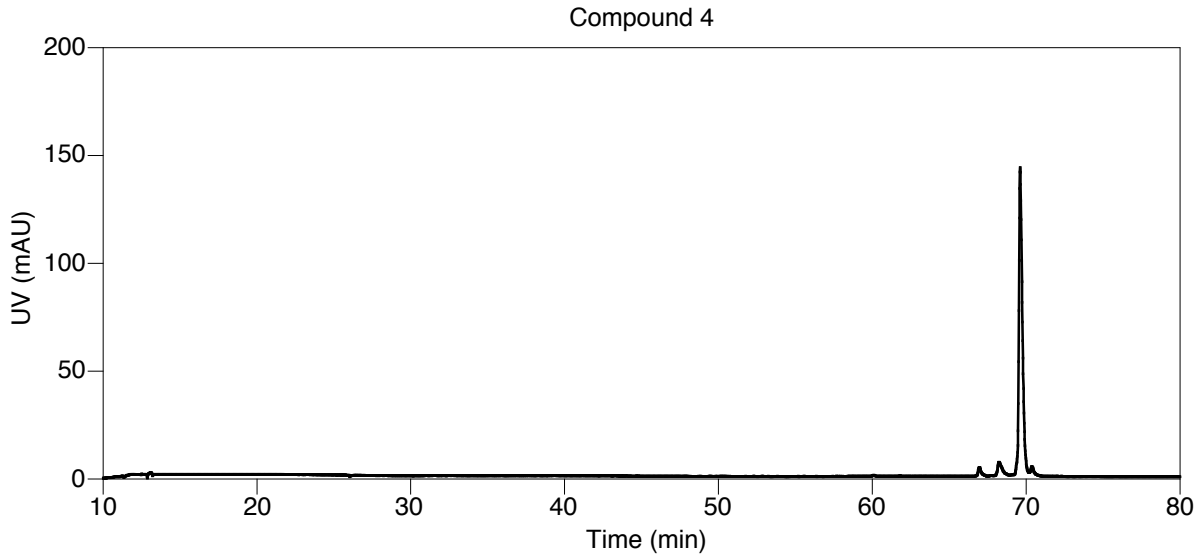
Abz-KSKTKCK(Dnp)IM, 1.

Reaction scale: 0.050 mmol, yield: 20.0 mg (30%), purity by RP-HPLC: 94.4%, t_R = 41 min., ESI-MS (m/z): $[M+H]^+$ calcd for $C_{58}H_{94}N_{16}O_{17}S_2$ 1351.6424, found 1351.7236; $[M+2H]^{2+}$ calcd for $C_{58}H_{95}N_{16}O_{17}S_2$ 676.3212, found 676.3596; $[M+3H]^{3+}$ calcd for $C_{58}H_{96}N_{16}O_{17}S_2$ 451.2141, found 451.2424.



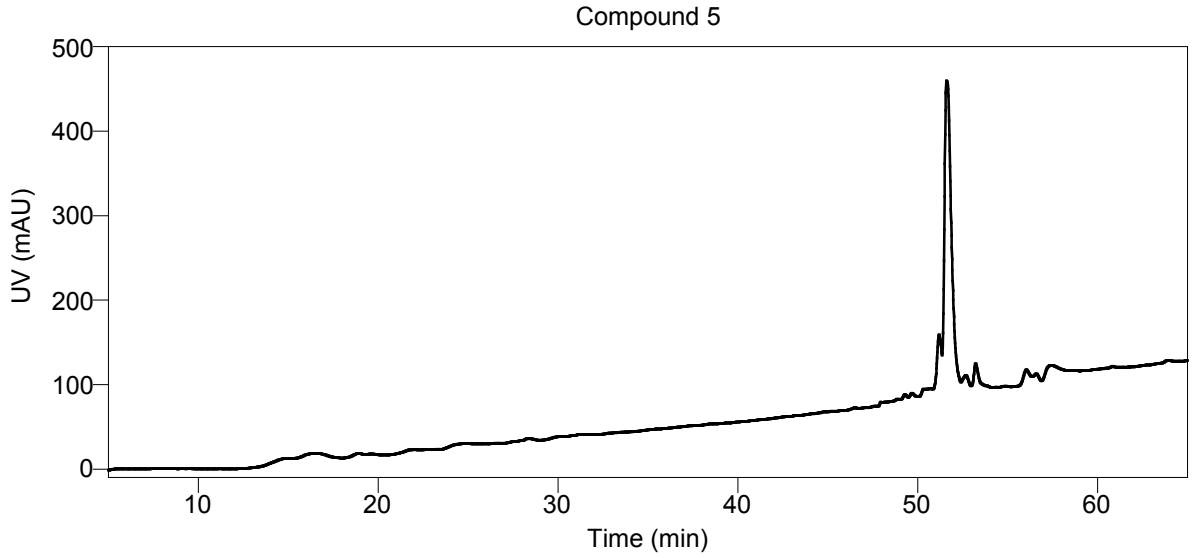
Abz-KSKTKC(C5BP)K(Dnp)IM, 4.

Reaction scale: 0.007 mmol, yield: 3.3 mg (27%), purity by RP-HPLC: 91.5%, t_R = 60 min., ESI-MS (m/z): $[M+2H]^{2+}$ calcd for $C_{77}H_{113}N_{16}O_{19}S_2$ 815.3866, found 815.3873; $[M+3H]^{3+}$ calcd for $C_{77}H_{114}N_{16}O_{19}S_2$ 543.9244, found 543.9283.



Biotin-K(Dde)KSKTKCK(Dnp)IM, 5.

Reaction scale: 0.040 mmol, yield: 20.0 mg (28%), purity by RP-HPLC: 75.6%, t_R = 52 min., ESI-MS (m/z): $[M+2Na]^{2+}$ calcd for $C_{91}H_{153}N_{21}O_{26}S_3Na_2$ 1049.0127, found 1048.6907; $[M+2Na+H]^{3+}$ calcd for $C_{91}H_{154}N_{21}O_{26}S_3Na_2$ 699.6751, found 699.4679; $[M+2Na+2H]^{4+}$ calcd for $C_{91}H_{155}N_{21}O_{26}S_3Na_2$ 525.0063, found 524.5923.



Biotin-K(Dde)KSKTKC(C5BP)K(Dnp)IM, 6.

Reaction scale: 0.007 mmol, yield: 12.0 mg (74%), purity by RP-HPLC: 97.0%, t_R = 61 min., ESI-MS (m/z): $[M+2Na]^{2+}$ calcd for $C_{110}H_{171}N_{21}O_{28}S_3Na_2$ 1188.0780, found 1187.8760; $[M+2Na+H]^{3+}$ calcd for $C_{110}H_{172}N_{21}O_{28}S_3Na_2$ 792.3853, found 792.2635; $[M+2Na+2H]^{4+}$ calcd for $C_{110}H_{173}N_{21}O_{28}S_3Na_2$ 594.5390, found 594.4493.

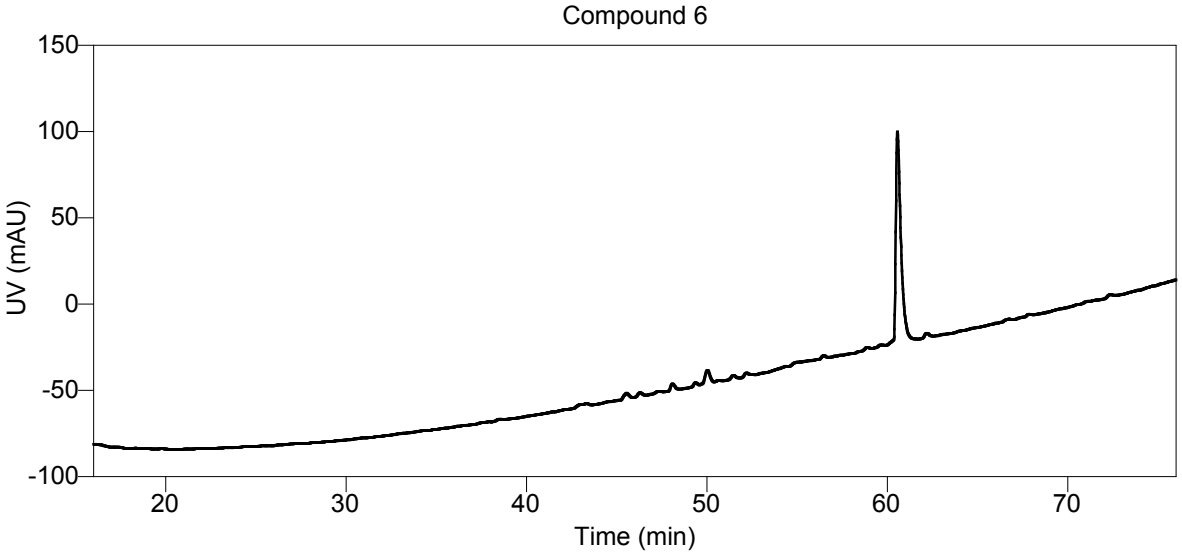


Table 2

Summary of ESI-MS-MS for unprocessed peptide, **2**, and cleavage product, **7** (-f, loss of farnesyl ($C_{15}H_{25}$); -g, loss of farnesyl-H_2O ($C_{15}H_{27}O$); -h, loss of H_2O; -i, loss of NH_3).

Peptide	**2**		**7**	
Ion	Calc.	Obs.	Calc.	Obs.
$[M+H]^+$(-f)	1352.66	1352.68	813.44	813.49
$[M+2H]^{2+}$			509.31	509.38
$[M+2H]^{2+}$(-f)	676.83	676.33	406.72	407.27
$[M+2H]^{2+}$(-g)	667.83	667.33		
y_9(-f)	1232.61	1232.61		
y_8(-f)	1104.52	1104.52		
y_7(-f)	1017.49	1017.51		
y_6(-f)	889.39	889.42		
y_5			769.48	770.55
y_9^{2+}	718.90	719.40		
y_7^{2+}(-h)	602.33	601.80		
y_5(-f)			566.30	566.34
y_8^{2+}(-f)	552.76	552.76		
y_2	263.14	263.15		
y_1	150.06	150.06		
b_9(-f)	1202.61	1202.60		
b_8^{2+}(-f)	545.76	545.26		
b_6			692.42	692.46
b_4	463.27	463.26	463.27	463.34
b_3	335.18	335.17		
b_2	248.15	248.14	248.15	248.19
b_2(-h)	230.15	230.14	230.15	230.19
b_1	120.10	120.05	120.10	120.08
a_7(-i)	955.59	955.47		
a_9^{2+}	690.40	690.88		
a_9^{2+}(-f)	588.31	587.81		
a_3^{2+}	154.60	155.08	154.60	155.12

Table 3

Summary of ESI-MS-MS for unprocessed peptide, **4**, and cleavage product, **8** (BP, benzophenone fragment, (3-benzoylphenyl)methylium ($C_{14}H_{11}O^+$); -b, loss of benzophenone fragment; -c, loss of (*E*)-(3-(((2-methylbut-2-en-1-yl)oxy)methyl)phenyl)(phenyl)methanone ($C_{19}H_{20}O_2$); -d, loss of benzophenone-H_2O, (3-(hydroxymethyl)phenyl)(phenyl)methanone ($C_{14}H_{13}O_2$); -h, loss of H_2O).

Peptide	**4**		**8**	
Ion	Calc.	Obs.	Calc.	Obs.
$[M+H]^+$(-b)			897.49	897.52
$[M+2H]^{2+}$(-b)	718.86	718.51		
$[M+2H]^{2+}$(-d)	709.86	709.51	440.56	440.33
$[M+2H]^{2+}$			546.28	546.36
$[M+3H]^{3+}$	544.27	543.96		
$[M+3H]^{3+}$(-d)	473.24	473.26		
$[M+2H]^{2+}$(-c)			406.72	406.29
y_8(-b)	1188.56	1188.71		
y_6			972.51	972.53
y_5			844.42	844.59
y_4			757.39	757.52
y_9^{2+}	755.88	756.04		
y_8^{2+}	691.83	691.97		
y_7^{2+}	649.31	649.95		
y_5(-d)			632.35	632.44
y_3			629.29	629.40
y_6^{2+}	585.26	585.86		
y_2	263.14	263.16	528.24	528.31
y_5^{2+}			423.21	422.80
y_1	150.06	150.09	400.15	400.23
b_9^{2+}	741.37	741.05		
b_6			692.42	692.51
b_9^{2+}(-d)	635.33	634.94		
b_5			564.32	564.37
b_4(-h)			445.26	445.36
b_6^{2+}			347.21	346.77
b_3	335.18	335.24	335.18	335.22
b_3(-h)			317.17	317.23
b_2	248.15	248.16	248.15	248.18
b_2(-h)	230.15	230.17	230.15	230.17
b_3^{2+}	167.59	167.12		
b_1	120.10	120.05	120.10	120.07
a_5			537.33	537.35
BP	195.12	195.12	195.12	195.12