Table of Contents

1. **Purification of K-Ras G12C and RBD** S3

2. **Electrospray ionization mass spectrometry and proteolytic digestion of peptide** S3

3. **Hydrogen Exchange Mass Spectrometry (HX MS)** S3

4. **Biotin-Raf conjugation and HABA/Avidin assay** S4

5. **Alpha K-Ras:RBD binding assay** S4

6. **Fluorescence Polarization Assay** S5

7. **Docking Method for Covalent docking model of SML-8-73-1 with K-Ras** S5

8. **Synthesis of SML-8-73-1** S6

9 **Synthesis of SML-10-70-1** S9

10. **Cell Proliferation Assays** S11

11. **Ras Signaling Analysis** S11

12. **Desthiobiotin-GTP Pull Down** S11

13. **Supplementary Figures** S12

14. **References** S18

Purification of K-Ras G12C and RBD

Recombinant protein expression constructs for K-Ras and RBD were synthesized by DNA 2.0 (Menlo Park, CA) and contained an N-terminal 6 histidine tag. These were expressed in the E. coli strain BL21(DE3) and purified by immobilized metal affinity chromatography followed by gel filtration. The gel filtration buffer was 20 mM Hepes, 150 mM NaCl, 1 mM DTT, pH 8.0. Samples were concentrated and buffer exchanged using centrifugal filter devices (Millipore, Billerica, MA).

Electrospray ionization mass spectrometry and proteolytic digestion of peptides

To assess covalent addition of SML-8-73-1, purified WT or G12C K-Ras was combined at a concentration of 50 µM with 2.5 mM SML-8-73-1 in PBS with 5% glycerol for 2 h at 37 °C. Analysis of intact proteins by MS was performed essentially as described.[1] For each analysis, ~5 µg protein with or without inhibitor was injected onto a self-packed reverse-phase column (500 µm inner diameter, 5 cm of POROS 10R2 resin). After desalting, protein was eluted with an HPLC gradient (0%–100% B in 1 min, A = 0.2 M acetic acid in water, B = 0.2 M acetic acid in acetonitrile, flow rate = 10 µl min^{-1}) into a linear ion trap mass spectrometer (LTQ, Thermo Fisher Scientific, San Jose, CA). Data were acquired in profile mode scanning *m*/*z* 300-2000. Mass spectra were deconvoluted using MagTran software (version 1.03b2).[2] To identify the site of labeling, protein (~5 µg) was first reduced (DTT) and alkylated (iodoacetamide), then digested with trypsin (6 h). Tryptic fragments were further subjected to chymotrypsin cleavage and products were loaded onto a self-packed pre-column (4 cm POROS10R2). Peptides were resolved on an analytical column (30 µm I.D., packed with 12 cm C18) and eluted into the mass spectrometer (LTQ Orbitrap Velos, Thermo Fisher Scientific) using an HPLC gradient (Waters NanoAcquity, Milford, MA; 0%–35% B in 60 min, A = 0.2 M acetic acid in water, B = 0.2 M acetic acid in acetonitrile, flow rate = ~30 nl min^{-1}). Peptides were subjected to MS2 by CAD (electron multiplier detection, relative collision energy 35%, q = 0.25) as well as HCD (image current detection, resolution at *m*/*z* 400 = 7,500, relative collision energy 35%).

Hydrogen Exchange Mass Spectrometry (HX MS)

HX MS experiments were similar to those described previously.[3] Human K-Ras G12C mutant was bound to GDP, GMPPNP, or SML-8-73-1 by incubating 50 µM protein with 2.5 mM GDP or GTP, or 500 µM SML-8-73-1 at 37 °C for 2 h in either 20 mM Hepes, 150 mM NaCl, 1 mM DTT, pH 8.0 or 10 mM Tris-HCl, 100 mM NaCl, pH 8.0. Each was independently labeled with deuterium using the same experimental conditions to allow comparison between the bound forms. Each deuterium labeling experiment was performed in triplicate, each time with an independent protein preparation. Exchange was initiated by diluting 1.5 µL of ~50 µM protein-bound ligand 15-fold in the identical buffer containing 99% deuterium oxide at 21 °C. At predetermined time points (ranging from 10 s to 4 h) the labeling reaction was quenched to pH 2.5 by the addition of 24.0 µL of 0.8% GdmCl, 0.8% formic acid at pH 2.4 and 0 °C. Quenched samples were immediately flash frozen using dry ice and

were stored for less than one week at –80 °C. For each deuterium labeling time-course, duplicate samples were prepared so that mass determination could be performed twice for each time point and each protein:ligand combination. To measure the deuterium level, each sample was rapidly thawed and injected into a Waters nanoAcquity UPLC equipped with HDX technology.[4] Online pepsin digestion[5] was performed as described previously[3] and peptides were separated with a 5–35% acetonitrile gradient over 6 min at a flow rate of 60 µl min^{-1}. All mobile phases contained 0.1% formic acid. After each sample, a wash cycle was performed to minimize carry-over into the next analytical injection.[6] Mass spectral analyses were performed with a Waters Q-Tof Premier equipped with a standard ESI source. Peptic peptides were identified in undeuterated samples with Waters MSE and Waters ProteinLynx Global Server 2.5 (PLGS) on the same UPLC/QTOF system used for the HX MS experiments.[7] Peptic peptide maps for K-Ras G12C, as shown in Figure S3A, were generated using MS Tools.[8] The deuterium uptake curves (Figure S3B) were created by plotting the relative amount of deuterium in each peptic peptide at each exchange time (the centroid of the isotopic distribution of the undeuterated species subtracted from the centroid of the isotopic distribution for each labeling time point). The data were not corrected for back-exchange and as such, the plots show the relative deuterium level.[9] HX MS analysis on this experimental system has been well characterized previously; the error of each data point generally does not exceed ±0.15 Da.[10] For this work, differences in the relative deuterium level between two protein states which exceeded 0.50 Da were considered to be significant.

Biotin-Raf conjugation and HABA/Avidin assay

Purified Raf-RBD protein (in PBS) was incubated with one molar equivalent of EZ-Link maleimide-PEG2-biotin (Pierce) and incubated at 20 °C for 1 h. A second one molar equivalent of maleimide-biotin was added and incubated for an additonal hour. The reaction was quenched by addition of 50 mM DTT and the labeled protein purified over a 5 mL HiTrap desalting column (GE Healthcare life sciences). To verify biotin conjugation, HABA/Avidin assays were performed following the procedure reccommended by Pierce Scientific. Breifly, a 10 mM HABA (Pierce) dye solution in 0.01 M NaOH was prepared and 30 µL of this dye added to 5 mL of a avidin solution (0.5 mg mL^{-1}, Pierce). In a clear 96-well plate 180 µL of the avidin-dye solution was mixed with 20 µL of the desalted Raf-RBD protein. The concentration of conjugated biotin was determined by comparison to a biotin-alone standard curve, and protein concentration via a bradford -dye based assay (BioRad). The final molar ratio was 0.83 moles biotin per mole of Raf-RBD protein.

Alpha K-Ras:RBD binding assay

Purified Flag-tagged K-Raas G12C protein was incubated with a 50 fold excess of GMPPNP and 0.1U alkaline phosphatase (Thermo-Fisher) per mg protein at 37 °C for 2 h. Following the incubation the protein was diluted to 37.5 nM in assay buffer (20 mM Tris pH 7.5, 100 mM NaCl, 1 mM $MgCl_2$, 5% glycerol, 0.5% BSA) and mixed with an equal volume of 25 nM biotin-

RBD and 6 µL added to low-volume 384 well plates (Perkin Elmer). 3 µL each of AlphaScreen Streptavidin-donor beads and anti-flag acceptor beads (Perkin Elmer) diluted into assay buffer at 10 µg/mL was then added to each well.

Untagged K-Ras G12C was preloaded with GMPPNP as described then purified over a 5 mL desalting column (GE Healthcare), flash frozen and stored at -80 °C until use. On the day of the assay, an aliquot of the untagged protein was thawed and mixed with a 100 fold excess of GDP, a 10 fold excess of SML-8-73-1, or an equal volume of buffer and incubated at 37 °C for 1 h. A 1:3 dilution series of each was prepared from 3 mM to 0.5 nM and 3 µL of this was added to each well of the 384 well plate. The plate was mixed on a plate shaker briefly and incubated overnight at 4 °C and the plate read using a NEO plate reader (BioTek) with the default AlphaScreen settings. Data analysis and graphs were prepared using GraphPad Prism (Graphpad).

Fluorescence Polarization Assay

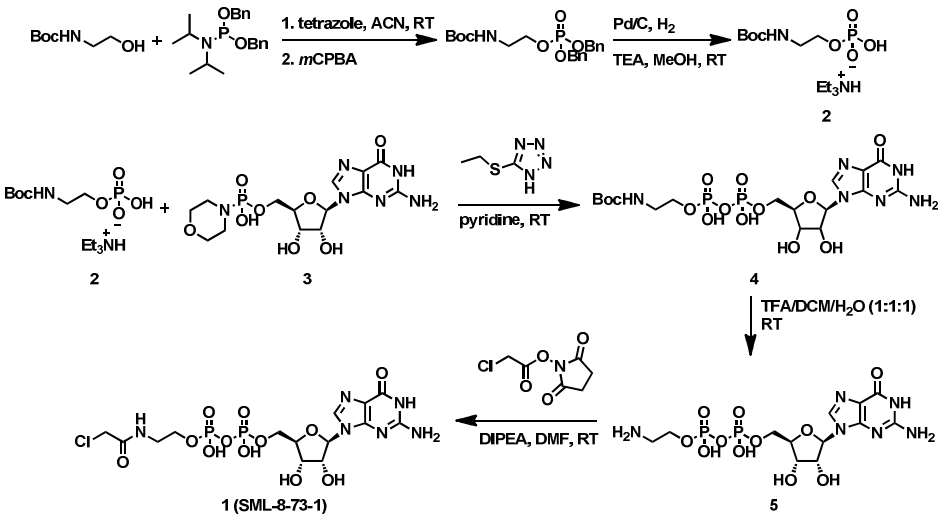
RBD-OG was labeled with Oregon Green® 488 Maleimide (OG, Invitrogen) at a 1:0.5 molar ratio of RBD to OG for 1 h at 37 °C in PBS and quenched with 1 mM glutathione. The mixture was then exchanged into PBS with 5% glycerol using a HiTrap desalting column (GE Healthcare life sciences). Addition of a single OG was verified by mass spectrometry (Figure S4). Fluorescence polarization (FP) was performed in low volume Corning 384 well assay plates. All procedures were done in PBS with 5% glycerol. K-Ras was loaded with nucleotides prior to assay by subjecting mixtures of nucleotides and K-Ras to a 42 °C thermal exchange step for 20 minutes or 37 °C thermal exchange for 2 h. The ratio of K-Ras to GTP or GDP was 1:50 for loading and the ratio of K-Ras to SML-8-73-1 was 1:10. For FP measurements OG-RBD concentration was 0.06 µM. Assay buffer consisted of 20 mM Hepes, 150 mM NaCl, 0.5 mM DTT, pH 8.0. For FP Measurements were made using an Envision plate reader (Perkin Elmer). Excitation was at 400 nm and emission measured at 535 nm.

Docking Method for Covalent docking model of SML-8-73-1 with K-Ras

For the prediction of covalent docking model of SML-8-73-1 with K-Ras, we used the crystal structure of human K-Ras in complex with a GTP analogue from Protein Data Bank (PDB code 3GFT). The protein structure was minimized using the Protein Preparation Wizard in the Schrodinger software graphical user interface Maestro (version 9.3). The SML-8-73-1 was docked into a minimized crystal structure of a Cys 12 in the human K-Ras crystal structure using the covalent docking routine in Prime (Prime, version 3.1, Schrödinger, LLC, New York, NY, 2012). The covalent docking was performed using default settings, keeping all residues fixed except for the connecting Cys 12 in K-Ras. The molecular graphics for the refined covalent docking model of the SML-8-73-1 was generated using PyMol package.

Synthesis of SML-8-73-1

Commercially available reagents and solvents were used without further purification. All reactions were monitored by thin layer chromatography (TLC) with 0.25 mm E. Merck pre-coated silica gel plates (60 F254) and/or Waters LCMS system (Waters 2489 UV/Visible Detector, Waters 3100 Mass, Waters 515 HPLC pump, Waters 2545 Binary Gradient Module, Waters Reagent Manager, Waters 2767 Sample Manager) using SunFireTM C18 column (4.6 x 50 mm, 5 µm particle size): Method A; solvent gradient = 70% A at 0 min, 0% A at 5 min, Method B; solvent gradient = 99% A at 0 min to 50% A at 5 min, solvent A = 0.035% TFA in Water; solvent B = 0.035% TFA in MeOH; flow rate : 2.5 ml min^{-1}. Purification of reaction products was carried out by flash chromatography using CombiFlash®Rf with Teledyne Isco RediSep®Rf High Performance Gold or Silicycle SiliaSepTM High Performance columns (4 g, 12 g or 24 g) and Waters LCMS system using SunFireTM Prep C18 column (19 x 50 mm, 5 µm particle size): solvent gradient = 100% A at 0 min, 50% A at 6 min; solvent A = 0.035% TFA in Water; solvent B = 0.035% TFA in MeOH; flow rate : 25 ml min^{-1}. The purity of all compounds was over 95% and was analyzed with Waters LCMS system. ^{1}H NMR spectra, ^{13}C NMR and ^{31}P NMR were obtained using a Varian Inova-400 (400 MHz for ^{1}H NMR, 100 MHz for ^{13}C NMR and 162 MHz for ^{31}P NMR) and/or Inova-600 (600 MHz for ^{1}H NMR and 150 MHz for ^{13}C) spectrometer. Chemical shifts are reported relative to chloroform (δ = 7.26) for ^{1}H NMR and chloroform (δ = 77.23) for ^{13}C NMR or dimethyl sulfoxide (δ = 2.50) for ^{1}H and dimethyl sulfoxide (δ = 39.51) for ^{13}C NMR. Data are reported as (br = broad, s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet).



Scheme S1. Synthesis of SML-8-73-1.

To a mixture of *tert*-butyl (2-hydroxyethyl)carbamate (1.0 g, 6.2 mmol) and a 0.45 M acetonitrile solution of tetrazole (42 mL, 18.6 mmol) was added dibenzyl *N*,*N*-diisopropylphosphoramidite (4.2 mL, 12.4 mmol) at ambient temperature. The resulting solution was stirred at ambient temperature for 4 h. Then, at ambient temperature, *m*CPBA (70%, 4.59 g, 18.6 mmol) was

added to the reaction mixture that was stirred for further 1 h. The precipitate in the mixture was filtered off through a pad of celite, and the filter cake was further washed with dichloromethane (100 mL). The combined filtrate was diluted with dichloromethane (50 mL) and washed with a saturated aqueous solution of sodium bicarbonate (100 mL, five times) and brine (100 mL). The organic layer was dried with sodium sulfate, filtered, and concentrated in vacuo. The residue was purified by flash column chromatography (4% methanol-dichloromethane) to provide *tert*-butyl (2-((bis(benzyloxy)phosphoryl)oxy)ethyl)carbamate (2.6 g, 68% over two steps) as a white solid. RT = 3.78 (Method A); ^{1}H NMR (400 MHz, [D$_6$]DMSO, 25 °C, TMS): δ=7.31-7.40 (m, 10H), 6.99 (m, 1H), 5.01 (d, J = 7.2 Hz, 4H), 3.94 (q, J = 6.0 Hz, 2H), 3.17 (q, J = 5.4 Hz, 2H), 1.33 (s, 9H); ^{13}C NMR 100 MHz (DMSO-d$_6$) δ 156.07, 136.55, 136.48, 128.91, 128.90, 128.80, 128.78, 128.26, 78.30, 69.07, 68.98, 68.93, 68.85, 66.41, 66.35, 28.60; ^{31}P NMR (162 MHz, [D$_6$]DMSO): δ=-0.59; MS *m*/*z*: 422.20 [M+H]$^+$.

To a solution of *tert*-butyl (2-((bis(benzyloxy)phosphoryl)oxy)ethyl)carbamate (707.7 mg, 1.68 mmol) in methanol (35 mL) were added triethylamine (468.1 µL, 3.36 mmol) and Pd/C (500 mg) at ambient temperature. House vacuum was applied to the flask containing the suspension and hydrogen gas was back-filled to the flask. This process was repeated three times more. Under hydrogen atmosphere, the mixture was stirred at ambient temperature for 3 h. The mixture was diluted with methanol (50 mL) and filtered through a pad of celite. The filter cake was washed with additional methanol (50 mL), and the combined organic solution was concentrated in vacuo. A stock solution of crude **2** was obtained by dissolving crude **2** in anhydrous pyridine (9 mL), and this solution was used in the next step. RT = 1.02 (Method A), 2.77 (Method B); ^{1}H NMR (600 MHz, [D$_6$]DMSO, 25 °C, TMS): δ=7.00 (s, 1H), 3.62 (q, J = 6.0 Hz, 2H), 3.03 (q, J = 6.0 Hz, 2H), 2.90 (q, J = 6.0 Hz, 6H), 1.36 (s, 9H), 1.12 (t, J = 7.8 Hz, 9H); ^{31}P NMR (162 MHz, [D$_6$]DMSO): δ=0.66; MS *m*/*z*: 242.14 [M+H]$^+$.

To a suspension of **3** (98.4 mg, 0.14 mmol, from Sigma G7627-100mg) in anhydrous pyridine (1 mL) were added a solution of **2** (60.1 mg, 0.14 mmol) in pyridine (726 µL) followed by 5-(ethylthio)-1*H*-tetrazole (52.9 mg, 0.41 mmol) at ambient temperature. The resulting mixture was stirred at ambient temperature for 2 h, and concentrated in vacuo. The residue was diluted with water (2 mL), and directly purified by preparative reverse-phase HPLC (methanol/water gradient) to provide a trifluoroacetic acid salt of **4** as a white solid (50.3 mg, 53%). RT = 3.07 (Method B); MS *m*/*z*: 587.29 [M+H]$^+$.

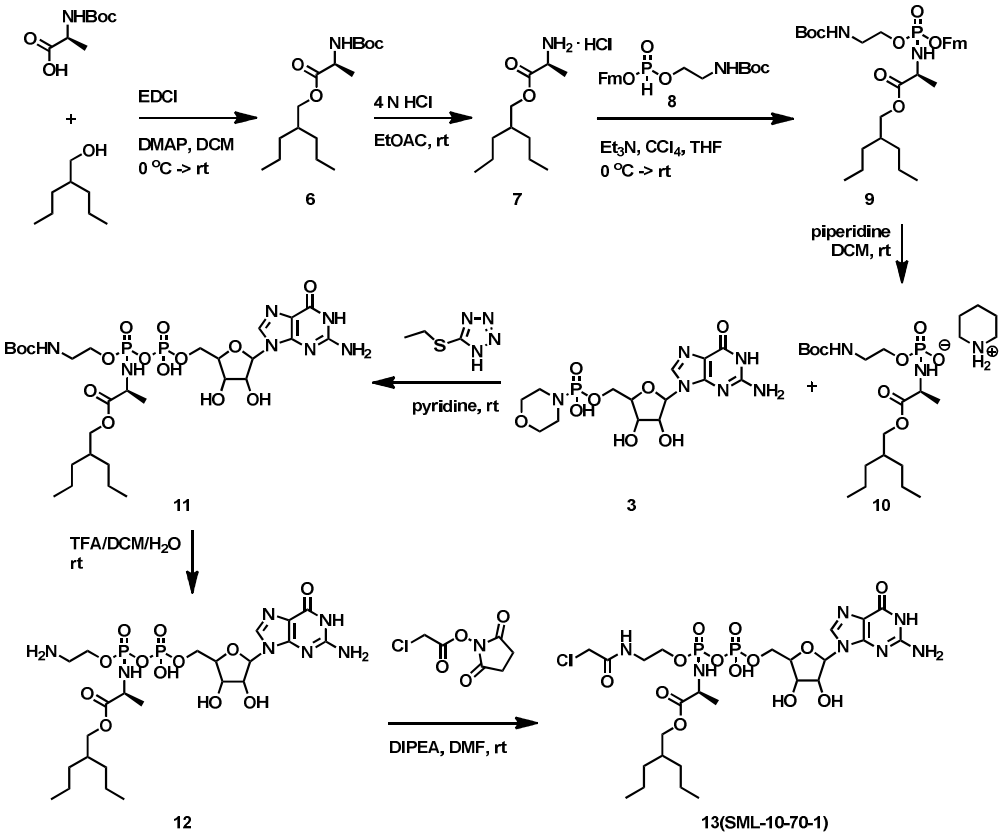
4 (50.3 mg, 0.072 mmol) was suspended in a mixture of dichloromethane (1 mL), water (1 mL), and trifluoroacetic acid (1 mL) at ambient temperature. The reaction mixture was stirred at ambient temperature for 1 h, and concentrated in vacuo. The residue was diluted with water (2 mL), and directly purified by preparative reverse-phase HPLC (methanol/water gradient) to provide a trifluoroacetic acid salt of **5** as a white solid (7.8 mg, 18 %). RT = 0.65 (Method B); ^{1}H NMR (600 MHz, [D$_2$]D$_2$O,

25 °C, TMS): δ=9.04 (m, 1H), 6.00 (m, 1H), 4.62 (m, 1H), 4.44 (m, 1H), 4.35 (m, 1H), 4.29 (m, 1H), 4.19 (m, 1H), 4.16 (m, 2H), 3.24 (m, 2H); ^{13}C NMR 150 MHz (DMSO-d_6) δ 157.94, 157.34, 151.88, 138.26, 138..00, 110.55, 92.48 (d, *J* = 25.8 Hz), 86.38 (d, *J* = 25.8 Hz), 77.16 (d, *J* = 20.1 Hz), 71.77 (d, *J* = 28.7 Hz), 67.00 (t, *J* = 17.2 Hz), 65.00 (t, *J* = 14.3 Hz), 42.41 (d, *J* = 25.8 Hz), TFA peaks were omitted; ^{31}P NMR (162 MHz, [D_6]DMSO): δ=-11.02, -15.11 (d, *J* = 42.1 Hz); MS *m*/*z*: 487.12 $[M+H]^+$.

To a solution of **5** (7.8 mg, 0.013 mmol) in DMF (1 mL) were added DIPEA (11.3 µL, 0.065 mmol) and 2,5-dioxopyrrolidin-1-yl 2-chloroacetate (5.0 mg, 0.026 mmol) at ambient temperature. The reaction mixture was stirred at ambient temperature for 1 h. It was diluted with water (10 mL) and washed with dichloromethane (10 mL). The aqueous layer was concentrated in vacuo, diluted with water (1 mL), and directly purified by preparative reverse-phase HPLC (methanol/water gradient) to provide a trifluoroacetic acid salt of SML-8-73-1 (**1**) as a white solid (4.3 mg, 49%). RT = 1.82 (Method B); ^{1}H NMR (600 MHz, [D_2]D_2O, 25 °C, TMS): δ=9.07 (s, 1H), 6.03 (d, *J* = 1.8 Hz, 1H), 4.65 (m, 1H), 4.45 (m, 1H), 4.36 (m, 1H), 4.31 (m, 1H), 4.19 (m, 1H), 4.10 (s, 2H), 4.00 (m, 2H), 3.44 (m, 2H); ^{13}C NMR 150 MHz (DMSO-d_6) δ 172.40, 158.02, 157.67, 152.15, 138.40, 110.98, 92.32, 86.51 (d, *J* = 6.6 Hz), 77.21, 71.81, 67.02 (m), 44.86, 42.89, TFA peaks were omitted; ^{31}P NMR (162 MHz, [D_6]DMSO): δ=-11.130, -14.903; MS *m*/*z*: 563.07 $[M+H]^+$.

To a solution of *N*-hydroxysuccinimide (640.3 mg, 5.56 mmol) in chloroform (8.5 mL) was added triethylamine (861.6 µL, 6.18 mmol) 0 °C. Then, α-chloroacetyl chloride was added dropwise over a 5 minute period and stirred for an additional 20 minutes at 0 °C. The reaction mixture was washed with ice-cold water (15 mL) and brine (15 mL), concentrated to a volume of 1.7 mL in vacuo, then dried with sodium sulfate and filtered. To the resulting solution were added ethyl acetate (170 µL) and hexanes (1.2 mL), and the mixture was cooled down to 0 °C stirred for 2 h, and a white solid was precipitated. It was filtered and washed first with ice-cold 10 mL portion of hexanes/ethyl acetate (4:1), then with 10 mL hexanes/ethyl acetate (9:1), and finally with hexanes (10 mL, twice). The resulting white solid was dried under house vacuum to yield 2,5-dioxopyrrolidin-1-yl 2-chloroacetate (563.9 mg, 53%). ^{1}H NMR (400 MHz, [D_1]$CDCl_3$, 25 °C, TMS): δ=4.37 (s, 2H), 2.87 (s, 4H).

Synthesis of SML-10-70-1



Scheme S2. Synthesis of SML-10-70-1.

To a solution of (*S*)-2-((*tert*-butoxycarbonyl)amino)propanoic acid (1.14 g, 6.0 mmol) in dichloromethane (14.2 mL) were added 2-propylpentan-1-ol (941.4 µL, 6.0 mmol), DMAP (73.3 mg, 0.6 mmol), and EDCI (1.27 g, 6.6 mmol) at ambient temperature. The resulting dark yellow solution was stirred at ambient temperature for 14 h. It was diluted with ethyl acetate (100 mL), and washed with a saturated aqueous solution of sodium bicarbonate (100 mL, three times) and brine (100 mL, twice). The organic layer was dried with sodium sulfate, filtered, and concentrated *in vacuo*. The residue containing crude **6** was used in the next step without further purification. MS *m*/*z*: 302.00 (M+1).

To a solution of crude **6** in ethyl acetate (10 mL) was added a 4 N dioxane solution of hydrochloric acid (10.5 mL, 42 mmol) at ambient temperature. The yellow solution was stirred at ambient temperature for 2 h, and concentrated *in vacuo*. The residue containing crude **7** was used in the next step without further purification. MS *m*/*z*: 201.94 (M).

To a solution of diphenyl phosphite (1.15 mL, 6.0 mmol) in pyridine (13 mL) was added a solution of 9-fluorenemethanol (981.2 mg, 5.0 mmol) in pyridine (13 mL) at –5 °C. The reaction mixture was stirred at –5 °C for 30 min. Then, a solution of

N-Boc-ethanolamine (1.08 mL, 7.0 mmol) in pyridine (13 mL) was added to the mixture that was heated to 40 °C and stirred for 1 h. The mixture was concentrated *in vacuo*. The residue containing crude **8** was used in the next step without further purification. MS *m*/*z*: 403.83 (M).

To a solution of crude (*S*)-2-propylpentyl 2-aminopropanoate in THF (25 mL) were added triethylamine (1.39 mL, 10 mmol) and carbon tetrachloride (2.5 mL) at –5 °C. To this mixture was added crude compound **8** in THF (10 mL) at –5 °C, and the resulting mixture was warmed up to ambient temperature and stirred for 3 h. It was concentrated *in vacuo*, and the residue was diluted with dichloromethane (200 mL), and washed with 0.1 N hydrochloric acid (150 mL, three times). The organic layer was dried with sodium sulfate, filtered, and concentrated *in vacuo*. The residue was purified by flash column chromatography (5% methanol-dichloromethane) to provide the compound **9**. MS *m*/*z*: 603.21 (M+1).

To a solution of **9** (1.0 mmol) in dichloromethane (5 mL) was added piperidine (1 mL) at ambient temperature. The reaction mixture was stirred at ambient temperature for 30 min, and concentrated *in vacuo*. The residue was diluted with water (30 mL) and white precipitate was formed. The precipitate was filtered through a filter paper by gravity. Dichloromethane (30 mL) was added to the filtrate, and after a few shake two layers were formed and separated. The organic layer was concentrated *in vacuo*. The residue containing crude compound **10** was used directly in the next step without further purification. MS *m*/*z*: 425.40 (M+1).

To a suspension of **3** (93.6 mg, 0.13 mmol) in pyridine (1 mL) were added a solution of **10** (85.4 mg, 0.17 mmol) in pyridine (1 mL) followed by 5-(ethylthio)-1*H*-tetrazole (50.4 mg, 0.39 mmol) at ambient temperature. The resulting mixture was stirred at ambient temperature for 3 h, and concentrated *in vacuo*. The residue containing crude **11** was suspended in a mixture of dichloromethane (1 mL), water (1 mL), and trifluoroacetic acid (2 mL) at ambient temperature. The reaction mixture was stirred at ambient temperature for 1 h, and concentrated *in vacuo*. The residue was diluted with water (1 mL) and purified by preparative reverse-phase HPLC (methanol/water gradient) to provide **12.** MS *m*/*z*: 670.46 (M+1).

To a solution of **12** (15.0 mg, 0.019 mmol) in DMF (1 mL) were added DIPEA (16.7 µL, 0.096 mmol) and 2,5-dioxopyrrolidin-1-yl-2-chloroacetate (7.3 mg, 0.038 mmol) at ambient temperature. The reaction mixture was stirred at ambient temperature for 30 min. It was diluted with water (10 mL) and washed with dichloromethane (10 mL). The aqueous layer was concentrated *in vacuo*, diluted with water (1 mL), and directly purified by preparative reverse-phase HPLC (methanol/water gradient) to provide **13** (**SML-10-70-1).** MS *m*/*z*: 746.41 (M+1).

Cell Proliferation Assays

Anti-proliferation assays were carried out in 96 well, white bottom plates. 2000-4000 cells were seeded per well 100 µl of culture media with 10% serum. Compounds were added and cells were incubated for 3 days. Cell viability was assessed by the addition CellTiter-Glo® Luminescent Assay reagent. After addition of 10ul CellTiter-Glo® reagent per well, plates were agitated for 2 minutes to induce cell lysis at room temperature. Luminescent signal was detected with a Perkin Elmer EnVision plate reader. The cell numbers were normalized by the DMSO control. EC50s were calculated using GraphPad Prism.

Ras Signaling Analysis

H358 cells at 80% confluence were treated with compounds at the indicated concentration for 6 h and then lysed with lysis buffer containing 25 mM Tris-HCl pH 7.4, 150 mM NaCl, 1 mM EDTA, 1% NP-40, 5% glycerol, Roche PhosSTOP phosphatase inhibitor cocktail tablets and Roche Complete Protease inhibitor cocktail tablets. The cell lysate was rotated end-to-end for approximately 30 min and then centrifuged at 10,000g for 15 minutes at 4 °C. The supernatant was transferred to new tubes and the total protein concentration was measured by Pierce BCA protein assay, after which the protein concentration for each sample was adjusted to 1.0 mg ml^{-1} with lysis buffer. To each sample, an equal part of loading buffer was added and samples were heated to 95 °C for 10 min. SDS-PAGE was done and proteins were transferred to a membrane and immunoblotted with the following antibodies: Phospho-Akt (Ser308) (Cell Signaling 4056); Akt (Cell Signaling 9272); Phospho-p44/42 MAPK (Erk1/2) (Thr202/Thr204), (Cell Signaling 9101); and p44/42 MAPK (Erk1/2), (Cell Signaling 9102).

Desthiobiotin-GTP Pull Down

H358 cells were treated with compounds for 6 h at 100 µM. Cells were lysed in lysis buffer as described above and the lysate buffer exchanged using DG10 columns to PBS. 5 µM ActiveX GTP-desthiobiotin (Thermo Scientific) and MgCl2 were added. After 5 min incubation the lysate was again buffer exchanged to PBS using another DG10 column, then subjected to 8 M urea to denature all protein. A streptavidin bead suspention was added and the mixture was rotated end-to-end for 2 h. Beads were collected by centrifugation at 7000 rpm for 2 min, washed with PBS for 3 times, then heated 95 °C for 10 min. Total Ras was detected after SDS-PAGE by western blot (antibody: Santa Cruz SC-30).

SUPPLEMENTAL FIGURES

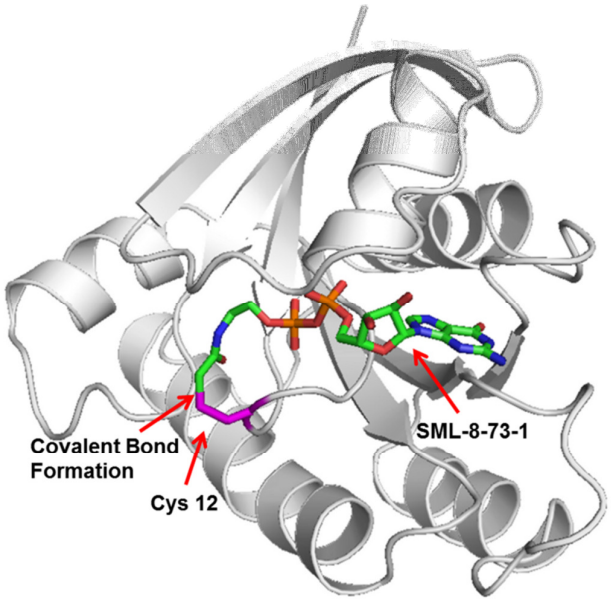


Figure S1. Molecular docking studies of SML-8-73-1 using a homology model of K-Ras G12C. This study suggested that SML-8-73-1 appeared to have favorable geometric properties for reaction with the cysteine in position 12 and had the potential to form a covalent bond with the cysteine 12.

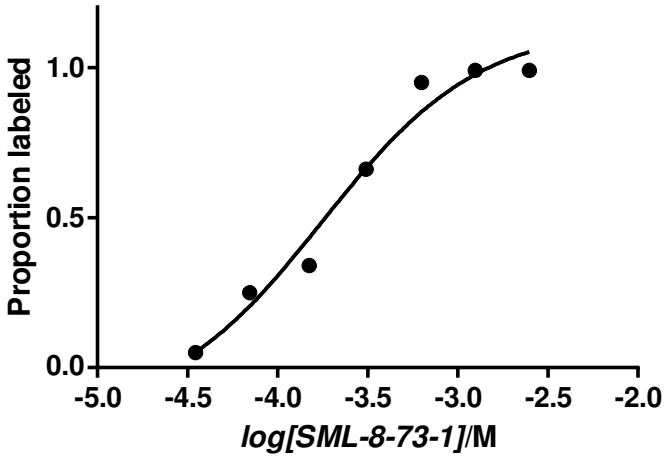


Figure S2. Labeling of K-Ras G12C by SML-8-73-1. Purified K-Ras G12C at a concentration of 50 μM was incubated with SML-8-73-1 at the concentrations indicated for 2 h at 37 °C as described above. Proportion of K-Ras G12C covalently modified by addition of SML-8-73-1 to K-Ras was assessed by electrospray ionization mass spectrometry of the intact protein.

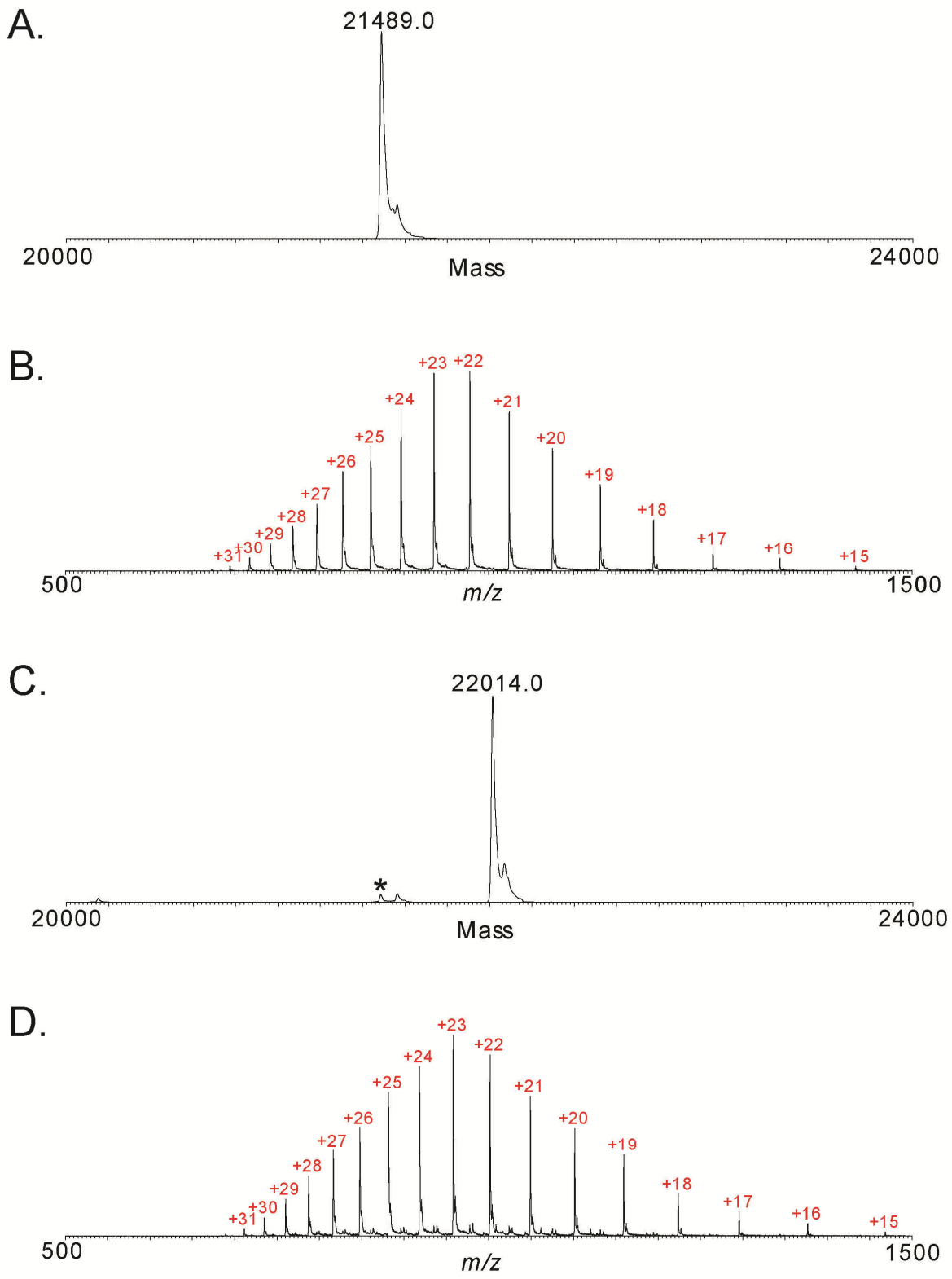
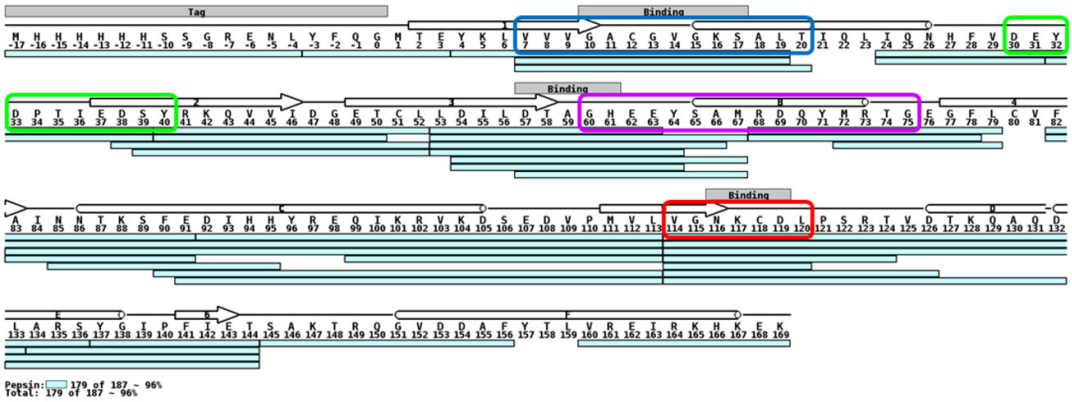
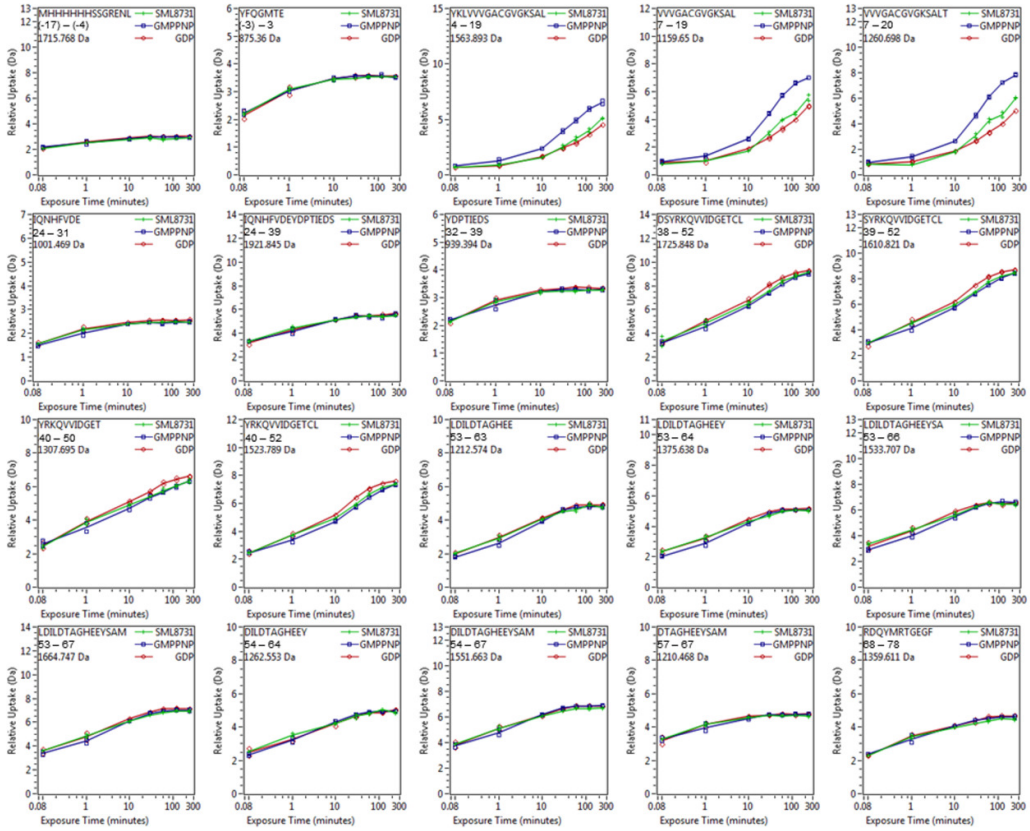


Figure S3. Labeling of K-Ras G12C by SML-8-7-31 in the presence of GTP and GDP. (A, C) Deconvoluted and (B, D) raw mass spectra obtained for 10 µM K-Ras G12C with 1 mM GTP and 1 mM GDP (A, B) before and (C, D) after incubation with 100 µM SML-8-7-31 for 2 h. *, <5% unmodified protein observed after labeling.

A


Tag
Binding
Binding
Binding
Pepsin: 179 of 187 ~ 96%
Total: 179 of 187 ~ 96%

B


MHHHHHHSSGRENL
-17) – (-4)
1715.768 Da
YFQGMTE
-3) – 3
875.36 Da
YKLVVGACGVGKSAL
4 – 19
1563.893 Da
VVVGACGVGKSAL
7 – 19
1159.65 Da
VVVGACGVGKSALT
7 – 20
1260.698 Da
IQNHFVDE
24 – 31
1001.469 Da
IQNHFVDEYDPTIEDS
24 – 39
1921.845 Da
YDPTIEDS
32 – 39
939.394 Da
DSYRKQVVIDGETCL
38 – 52
1725.848 Da
SYRKQVVIDGETCL
39 – 52
1610.821 Da
YRKQVVIDGET
40 – 50
1307.695 Da
YRKQVVIDGETCL
40 – 52
1523.789 Da
LDILDTAGHEE
53 – 63
1212.574 Da
LDILDTAGHEEY
53 – 64
1375.638 Da
LDILDTAGHEEYSA
53 – 66
1533.707 Da
LDILDTAGHEEYSAM
53 – 67
1664.747 Da
DILDTAGHEEY
54 – 64
1262.553 Da
DILDTAGHEEYSAM
54 – 67
1551.663 Da
DTAGHEEYSAM
57 – 67
1210.468 Da
RDQYMRTGEGF
68 – 78
1359.611 Da
SML8731
GMPPNP
GDP
Relative Uptake (Da)
Exposure Time (minutes)

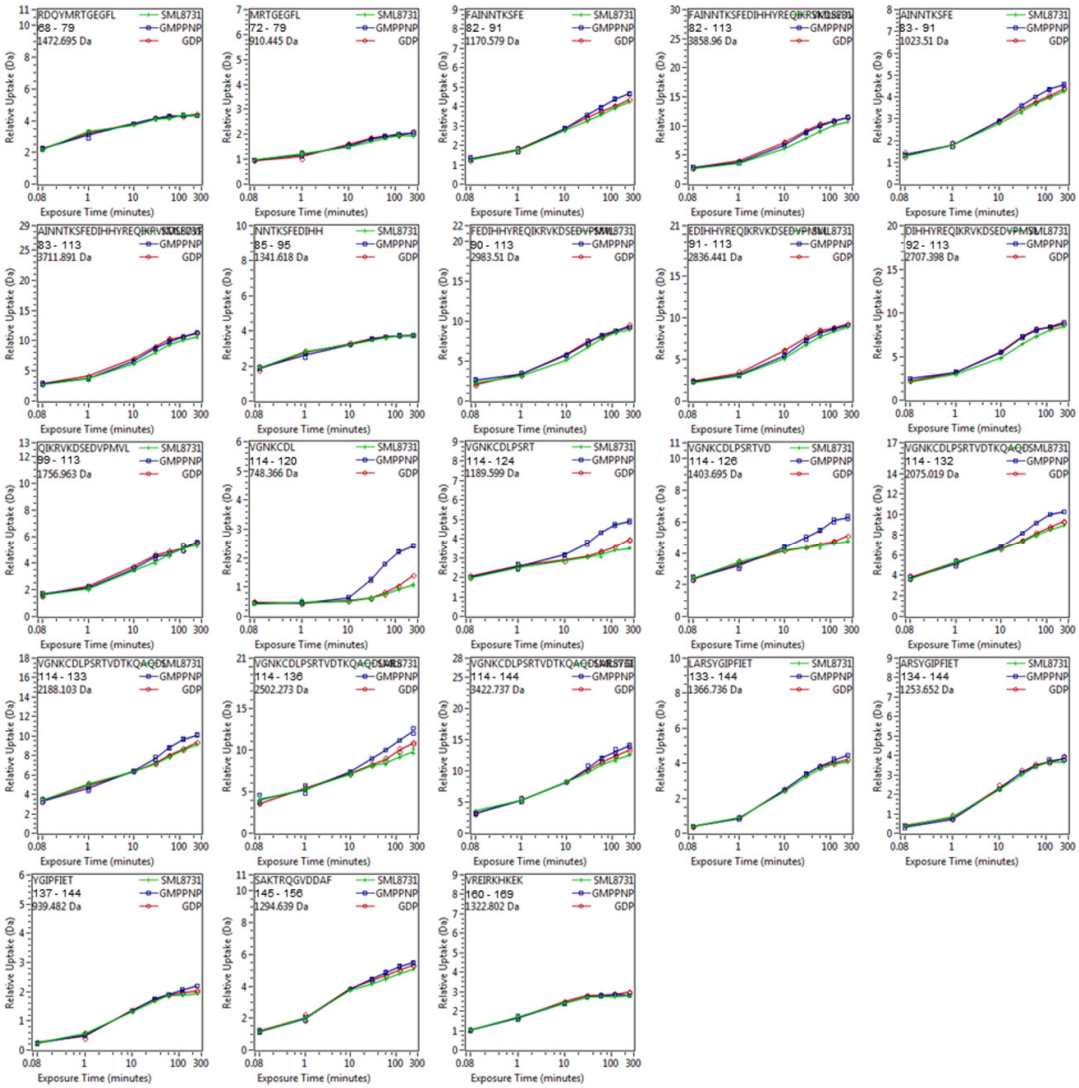
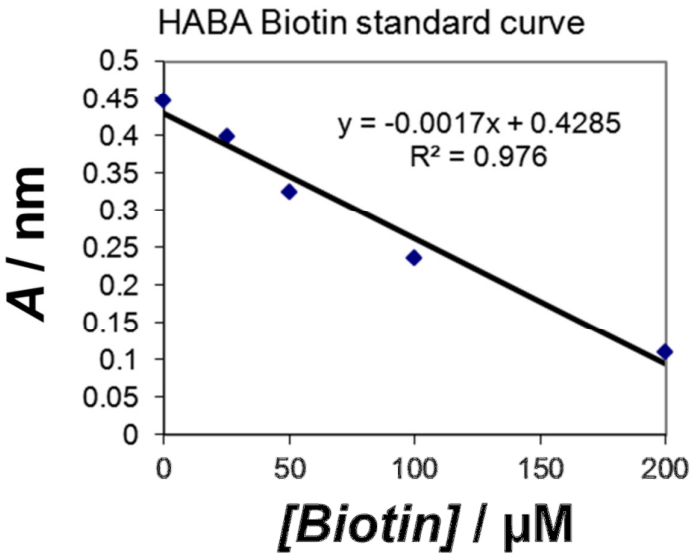


Figure S4. HX MS of K-Ras G12C bound to various compounds. (A) Peptic peptides produced by online pepsin digestion of K-Ras G12C. Each cyan bar under the sequence represents a peptic peptide where deuterium exchange was monitored. Highlighted portions include the region of largest difference in deuterium incorporation between the different protein states (blue box), the region of slight difference in deuterium incorporation (red box), the Switch I region (green box), and the Switch II region (magenta box). This figure was generated using MS Tools.[8] (B) Relative deuterium uptake plots for each peptic peptide from panel A. Each labeling reaction was performed in triplicate (i.e., experimental replicates) using three different preparations of protein. All three experimental replicates produced similar results but only one representative dataset is shown in this figure. Within each experimental replicate, two samples (i.e., mass measurement replicates) were prepared per time point; therefore, duplicate mass measurements were obtained and each time point has two data point markers per protein:ligand combination. These data were not corrected for back-exchange and as such, the plots show the relative deuterium level.[9] The error of each data point generally does not exceed ± 0.15 Da; therefore, differences in excess of 0.50 Da were considered significant.[10]



Labeled RBD AU	0.36
[Biotin] uM	38.9
[Protein] uM	46.7
Ratio Biotin:Protein	0.83

Figure S5. HABA/Avidin assay was perfomed on Raf-RBD to verify biotin conjugation. The ratio of biotin to RBD is 0.83.

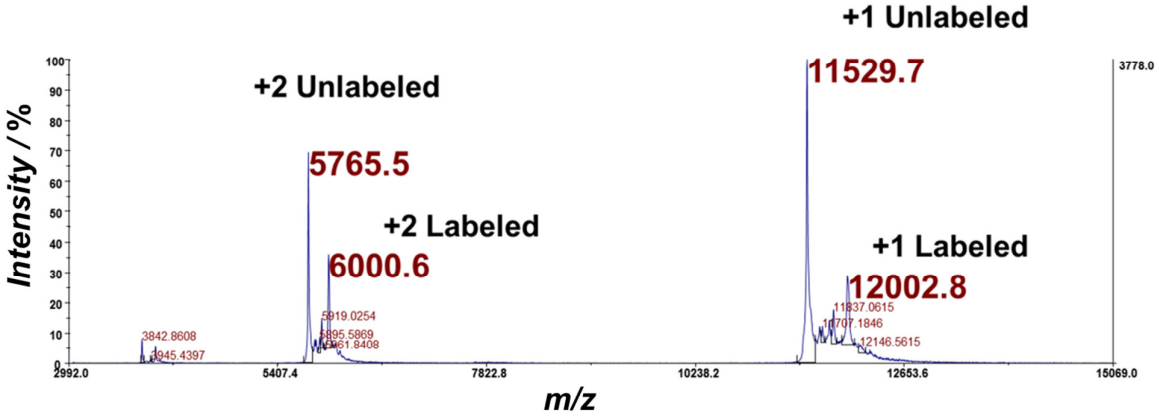


Figure S6. RBD protein labeling by Oregon Green® 488 Maleimide. Deconvoluted mass spectrum obtained for recombinant RBD protein after incubation with Oregon Green 488 maleimide (OG). Singly and doubly charged species are indicated on the spectrum. Masses for "labeled" species correspond to the predicted mass of a single OG adduct to RBD. Labeling efficiency was 30%.

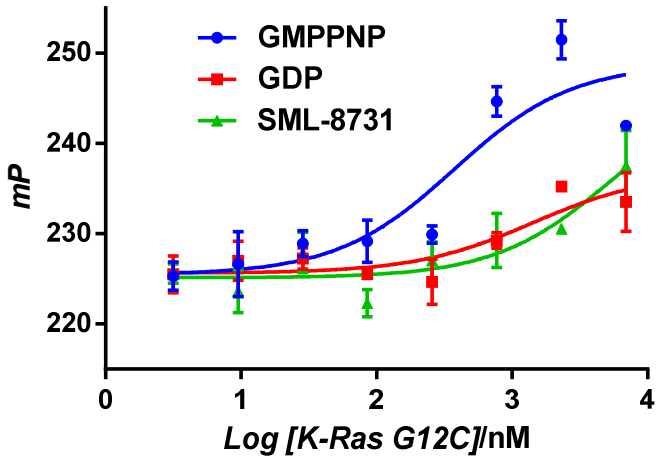


Figure S7. Flourescence polarization assay confirms that SML-8-73-1 addition renders K-Ras G12C biochemically inactive. GTP-bound K-Ras G12C has a higher affinity for RBD (blue curve) than the GDP-bound form (red curve). SML-8-73-1-bound K-Ras G12C does not productively interact with RBD (green curve).

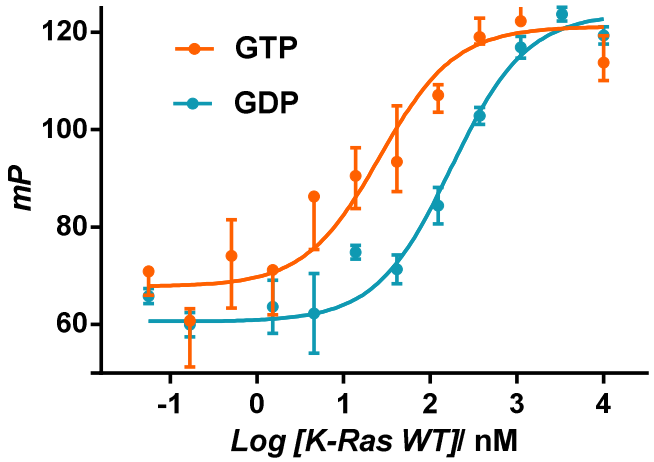


Figure S8. Flourescence polarization assay with WT K-Ras. G12C mutation does not significantly affect the binding affinities of both GTP and GDP to K-Ras (see Figure 4 in text). GTP-bound WT K-Ras has a higher affinity for RBD (orange curve) than the GDP-bound form (sky-blue curve).

References

[1] T. Zhang, F. Inesta-Vaquera, M. Niepel, J. Zhang, S. B. Ficarro, T. Machleidt, T. Xie, J. A. Marto, N. Kim, T. Sim, J. D. Laughlin, H. Park, P. V. LoGrasso, M. Patricelli, T. K. Nomanbhoy, P. K. Sorger, D. R. Alessi, N. S. Gray, *Chem. Biol.* **2012**, *19*, 140-154.

[2] Z. Zhang, A. G. Marshall, *J. Am. Soc. Mass Spectrom.* **1998**, *9*, 225-233.

[3] R. E. Iacob, J. Zhang, N. S. Gray, J. R. Engen, *PLoS One* **2011**, *6*, e15929.

[4] T. E. Wales, K. E. Fadgen, G. C. Gerhardt, J. R. Engen, *Anal. Chem.* **2008**, *80*, 6815-6820.

[5] L. Wang, H. Pan, D. L. Smith, *Mol. Cell. Proteomics* **2002**, *1*, 132-138.

[6] J. Fang, K. D. Rand, P. J. Beuning, J. R. Engen, *Int. J. Mass Spectrom.* **2011**, *302*, 19-25.

[7] R. S. Plumb, K. A. Johnson, P. Rainville, B. W. Smith, I. D. Wilson, J. M. Castro-Perez, J. K. Nicholson, *Rapid Commun. Mass Spectrom.* **2006**, *20*, 1989-1994.

[8] D. Kavan, P. Man, *Int. J. Mass Spectrom.* **2011**, *302*, 53-58.

[9] T. E. Wales, J. R. Engen, *Mass Spectrom. Rev.* **2006**, *25*, 158-170.

[10] D. Houde, S. A. Berkowitz, J. R. Engen, *J. Pharm. Sci.* **2011**, *100*, 2071-2086.