Supporting Information

Synthesis, enzymatic peptide incorporation and applications of diazirine-containing isoprenoid diphosphate analogues

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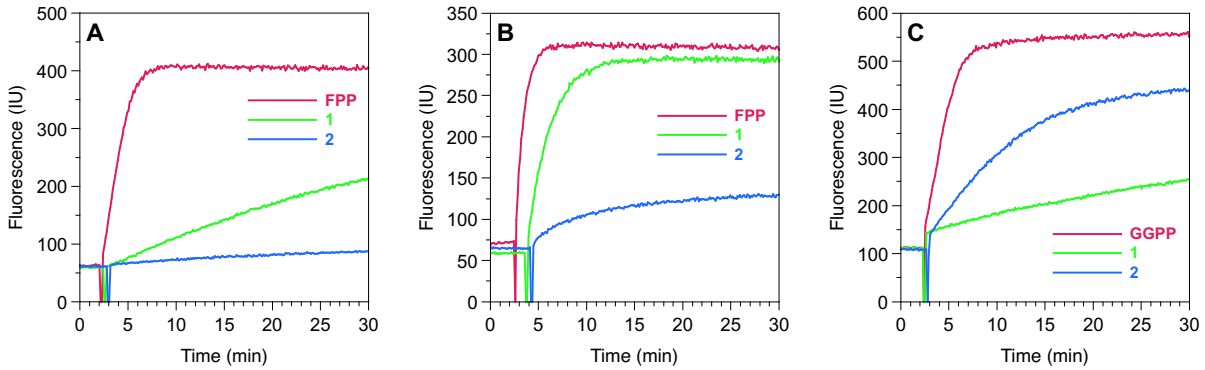


Figure S1. Analysis of prenyltransferase-catalyzed reactions between **1** or **2** and peptide substrates using a spectrofluorimetric assay. A) Analysis of rFTase-catalyzed reactions between **1** or **2** and Dns-GCVLS. Reactions contained FPP, **1** or **2** (10 μM), Dns-GCVLS (2.4 μM), rFTase (500 nM) and were performed at rt. B) Analysis of yFTase-catalyzed reactions between **1** or **2** and Dns-GCVLS. Reactions contained FPP, **1** or **2** (10 μM), Dns-GCVLS (2.4 μM), yFTase (500 nM) and were performed at rt. C) Analysis of rGGTase-catalyzed reactions between **1** or **2** and Dns-GCVLS. Reactions contained GGPP, **1** or **2** (10 μM), Dns-GCVLL (2.4 μM), rGGTase (500 nM) and were performed at rt.

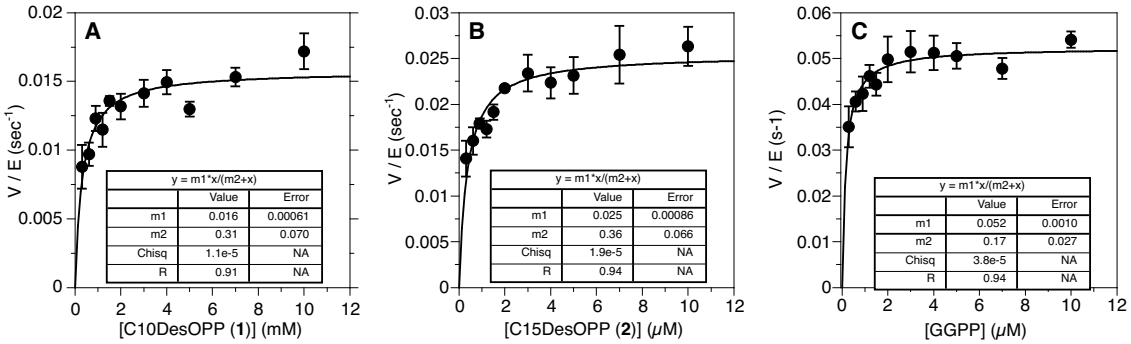


Figure S2. Kinetic analysis of prenylation reactions using diazirine analogues. Kinetic analysis of prenylation reactions of Dns-GCVLS and Dns-GCVLL with rFTase and rGGTase-I using diazirine-containing analogues **1**, **2** and GGPP. A) Kinetic analysis of rFTase-catalyzed reaction between **1** and Dns-GCVLS. B) Kinetic analysis of hGGTase-catalyzed reaction between **2** and Dns-GCVLL. C) Kinetic analysis of rGGTase-catalyzed reaction between GGPP and Dns-GCVLL.

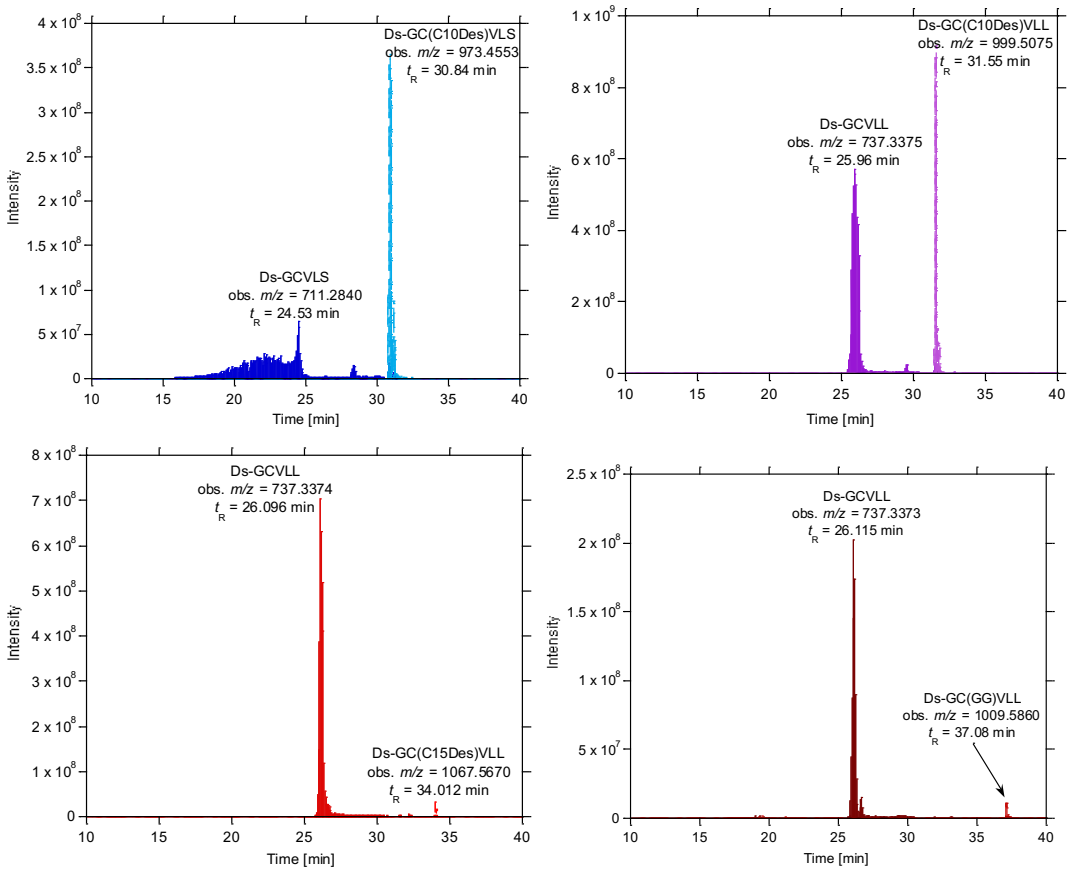


Figure S3. LC-MS analysis to detect SM and product of enzymatic prenylation reactions. (**A**) Dns-GCVLS and Dns-GC(C10Des)VLS using rFTase; (**B**) Dns-GCVLL and Ds-GC(C10Des)VLL using rGGTase; (**C**) Dns-GCVLL and Dns-GC(C15Des)VLL using rGGTase; (**D**) Dns-G-CVLL and Dns-GC(gg)VLL using rGGTase (**D**). Reactions included 10 μM isoprenoid and 2.4 μM peptide and were allowed to react for 1 h followed by analysis using a CID-Fusion HRAM LC-MS/MS instrument and monitoring the $[M+H]^+$, $[M+2H]^{2+}$ and $[M+3H]^{3+}$ ions for both SM and product. Note: all these reactions were essentially complete prior to analysis even though the ion chromatograms show substantial levels of starting peptide.

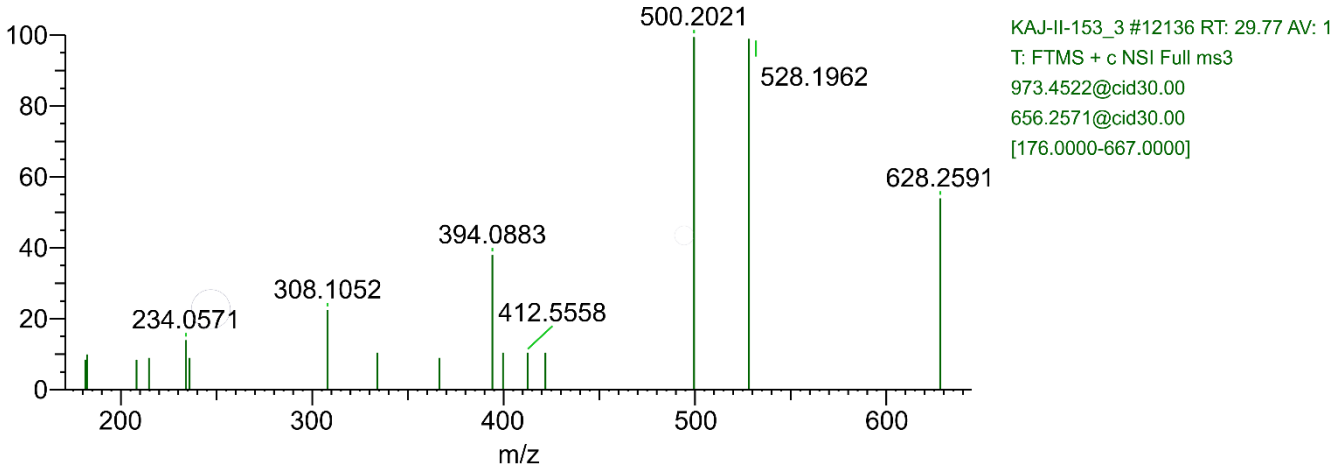


Figure S4. LC-MS^3 analysis of the b3 ion from **14a**. Fragmentation of b3 (m/z = 656) led to the formation of a3 (m/z = 628) and c2 (m/z = 308) ions.

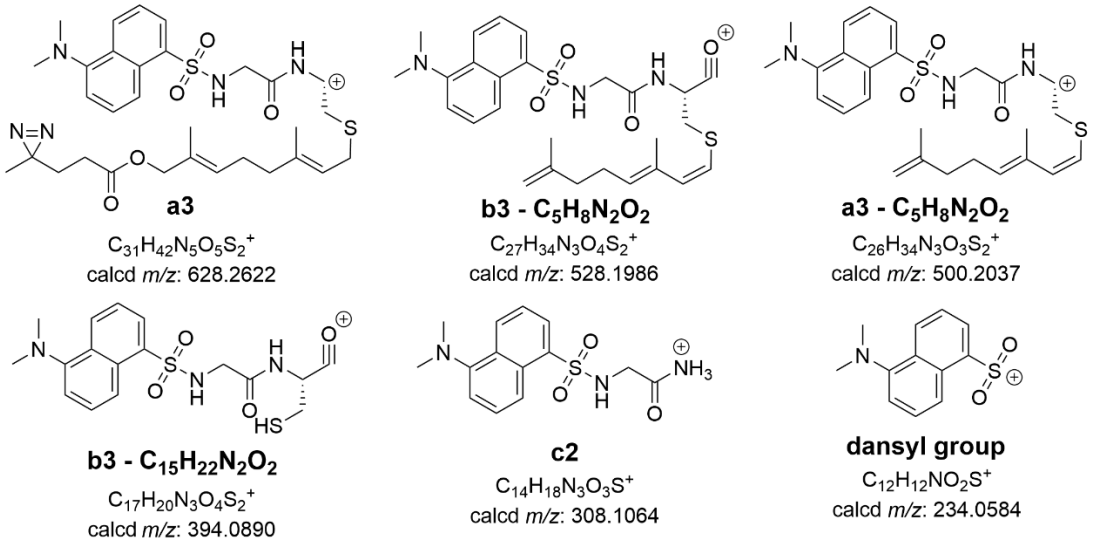


Figure S5. Proposed structures for ions obtained by MS^3 analysis of the b3 ion from **14a.**

H_2N–CKKKKKSKTKCVIM-OH

16

Figure S6. Structure of peptide **16** derived from the C-terminus of K-Ras-4B used for the synthesis of full-length K-Ras4B and as a competitor in photoaffinity labeling experiments.

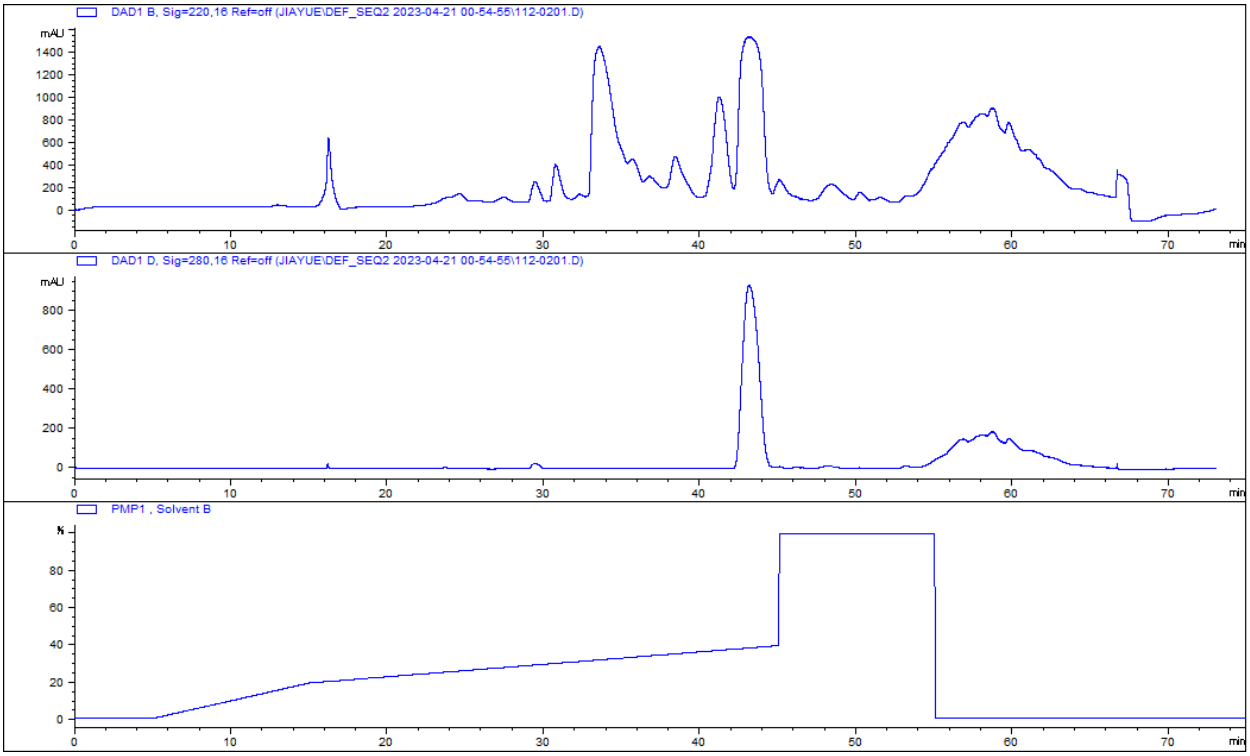


Figure S7: HPLC chromatograms for the purification of crude NH_2-CKKKKKKSKTKCVIM-OH (**16)**. Top: Chromatogram monitored at 220 nm; Middle: Chromatogram monitored at 280 nm; Bottom: Chromatogram showing solvent B gradient profile. The product eluted at 34 min when the gradient profile described above was used on a Pursuit 5C18 250 x 21.2 mm preparative column (PN A3000250x212) using a 5 mL/min flow rate.

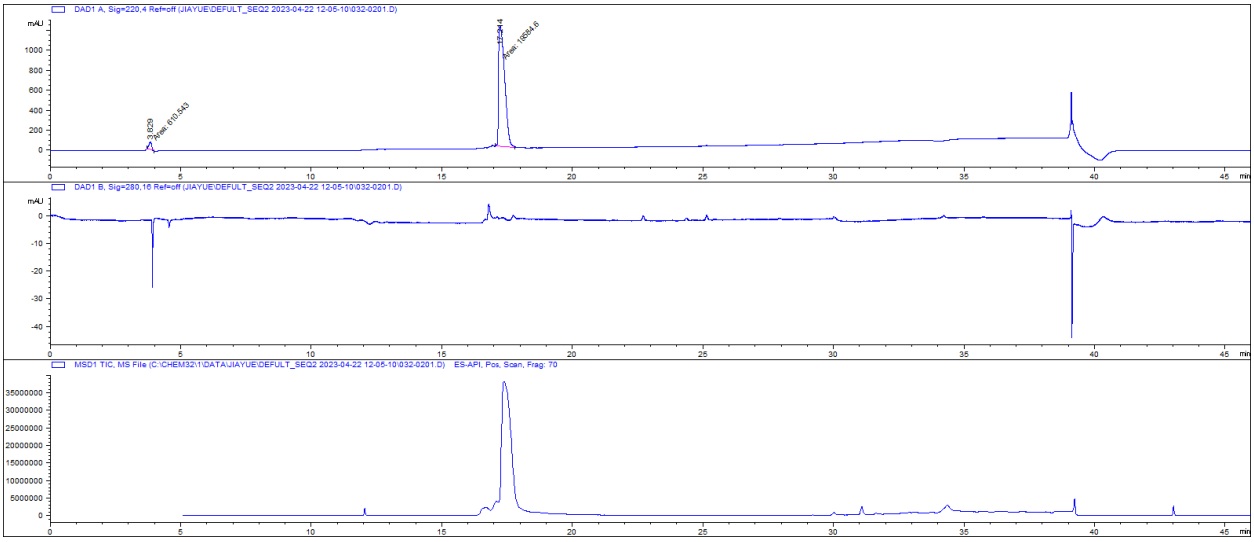


Figure S8: LC-MS chromatogram of purified NH_2-CKKKKKKSKTKCVIM-OH (**16**). A 25 min gradient from 0% B to 100% B was monitored for absorbance using a ZORBAX 300SB-C18 column, with a 5 µm diameter, and 4.6 x 250mm length (PN 880995-902) with a 1 mL/min flow rate. By integrating the peak areas in 220 nm chromatogram, the purity was calculated to be 97%. Top: Chromatogram monitored at 220 nm; Middle: Chromatogram monitored at 280 nm; Bottom: Total Ion Current (TIC) Chromatogram.

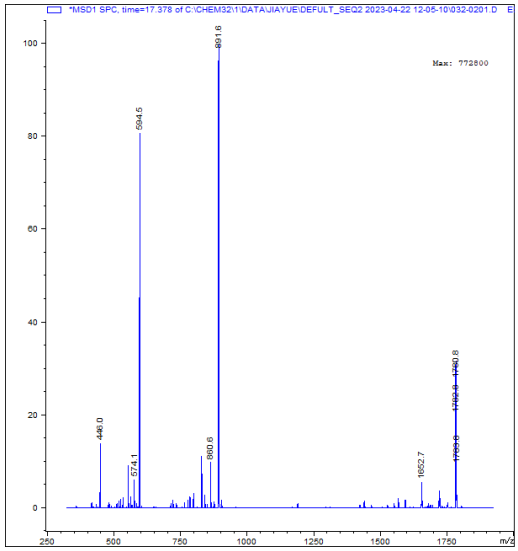


Figure S9: MS of purified NH_2-CKKKKKKSKTKCVIM-OH (**16**). Calcd m/z: $[M+H]^+$ 1781.06, $[M+2H]^{2+}$ 891.03, and $[M+3H]^{3+}$ 594.35. Obs m/z: $[M+H]^+$ 1780.8, $[M+2H]^{2+}$ 891.6, and $[M+3H]^{3+}$ 594.5.

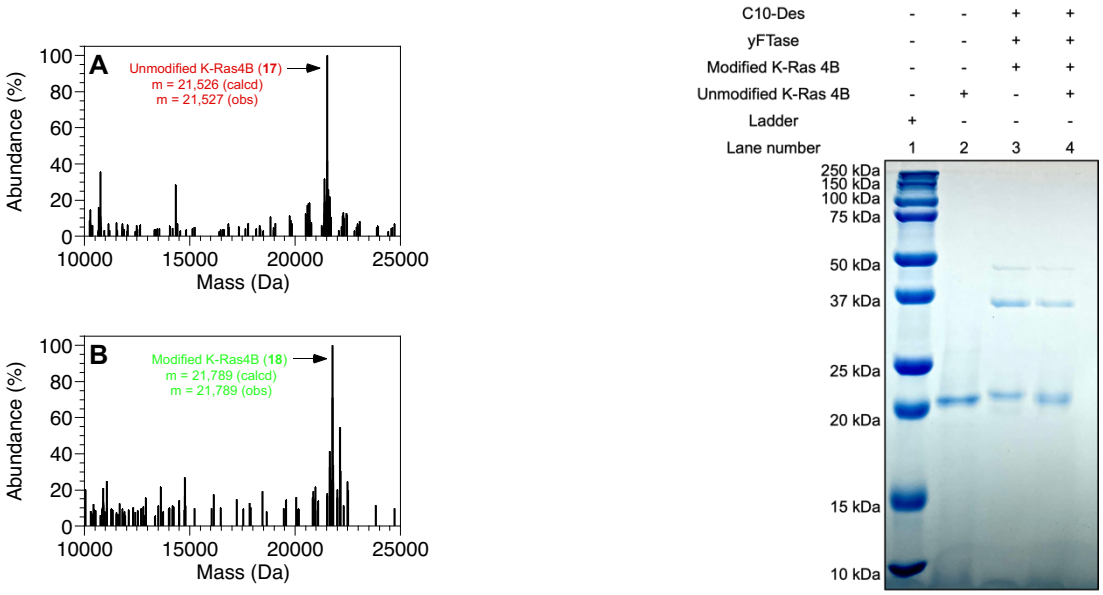


Figure S10. LC-MS and SDS-PAGE analysis of full-length K-Ras4B (**17**) before and after modification with diazirine probe **1**. Left, Panel A) MS of unmodified K-Ras4B (**17**) obtained from LC-MS analysis prior to yFTase addition. Left, Panel B) MS of modified K-Ras4B (**18**) obtained from LC-MS analysis after yFTase addition. Right) SDS-PAGE analysis of full-length K-Ras4B (**17**) before and after modification with diazirine probe **1** using yFTase. Lane 1: MW markers; Lane 2: Sample of full-length K-Ras4B (**17**) prior to modification with **1**; Lane 3: Sample of full-length K-Ras4B after modification with **1** to yield **18**; Lane 4: Mixture of samples from Lanes 2 and 3 to show the small difference in mobility between **17** and **18**.

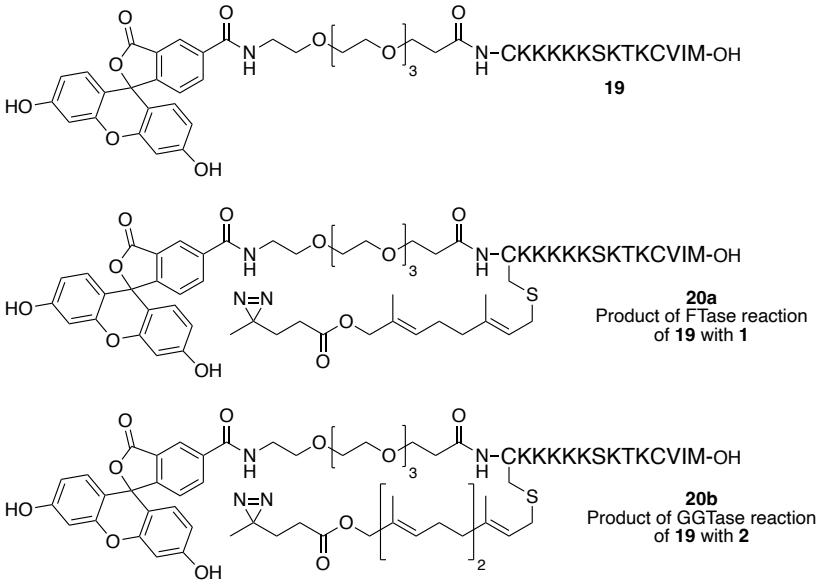


Figure S11. Structure of peptides **19, 20a** and **20b**. Peptide **19** derived from the C-terminus of K-Ras-4B and enzymatically modified with **1** (rFTase) or **2** (rGGTase) to yield **20a** and **20b**, respectively, used in photoaffinity labeling experiments.

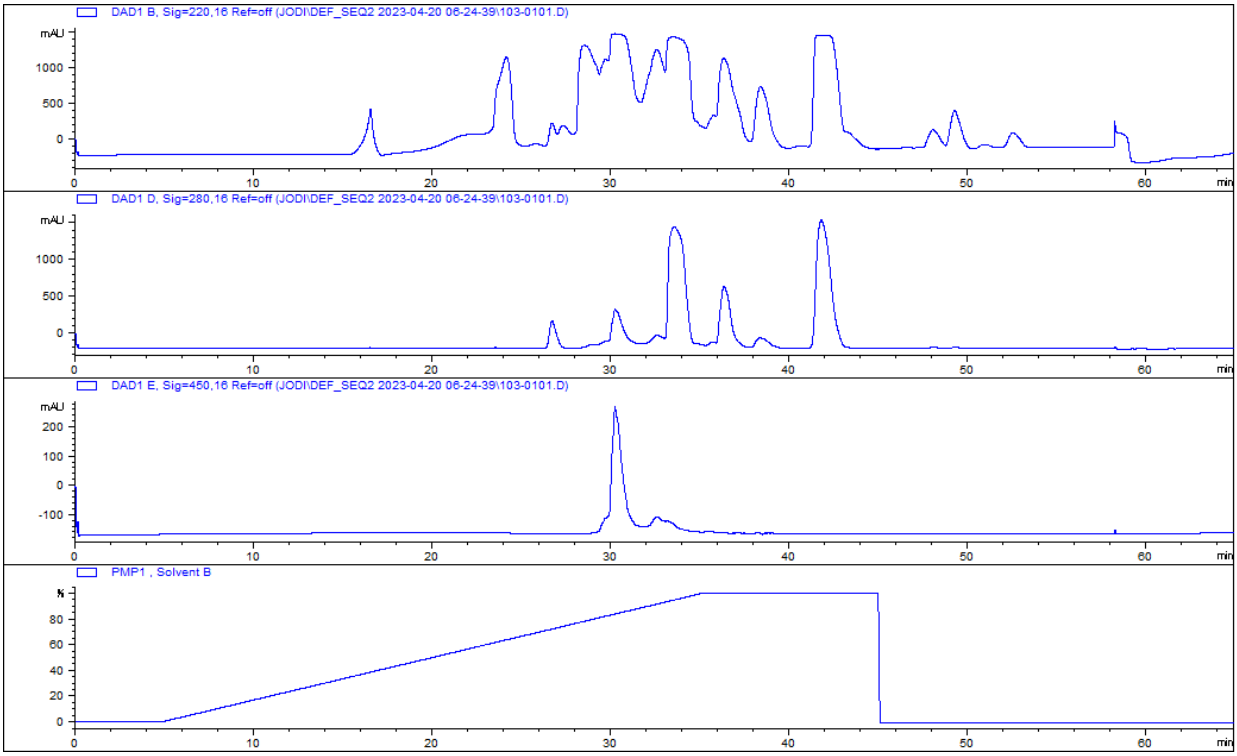


Figure S12. HPLC Chromatograms for the purification of crude 5-Fam-Peg$_4$-CKKKKKKSKTKCVIM-OH (**19**). Top: Chromatogram monitored at 220 nm; Middle, Top: Chromatogram monitored at 280 nm; Middle, Bottom: Chromatogram monitored at 450 nm; Bottom: Chromatogram showing solvent B gradient profile. The product eluted at 31 min when a 30 min gradient profile from 0% B to 100% B was used on a Pursuit 5C18 250 x 21.2 mm preparatory column (PN A3000250x212) using a 5 mL/min flow rate.

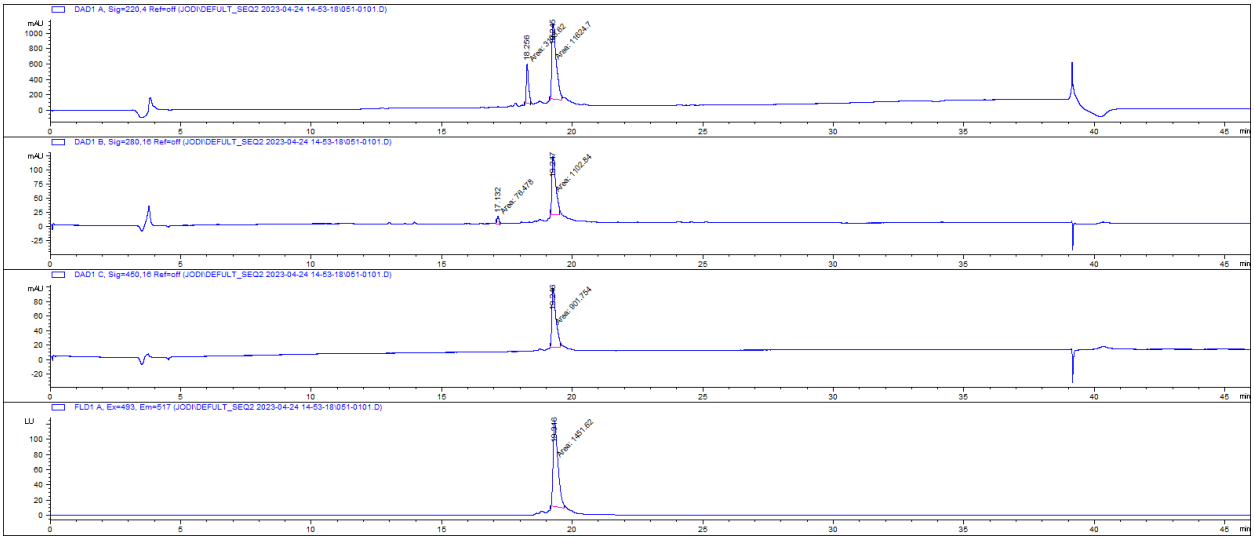


Figure S13: LC-MS chromatogram of purified 5-Fam-Peg$_4$-CKKKKKKSKTKCVIM-OH (**19**). A 25 min gradient from 0% B to 100% B was monitored for absorbance and fluorescence using a ZORBAX 300SB-C18 column, with a 5 µm diameter, and 4.6 x 250 mm length (PN 880995-902) with a 1 mL/min flow rate. By integrating the peak areas in 280 nm chromatogram, the purity was calculated to be 93%. Top: Chromatogram monitored at 220 nm; Middle, Top: Chromatogram monitored at 280 nm; Middle, Bottom: Chromatogram monitored at 450 nm; Bottom: Chromatogram monitored for fluorescence (ex = 417 nm, em = 493 nm). By integrating the peaks in the 280 nm chromatogram, the purity was calculated to be 93%.

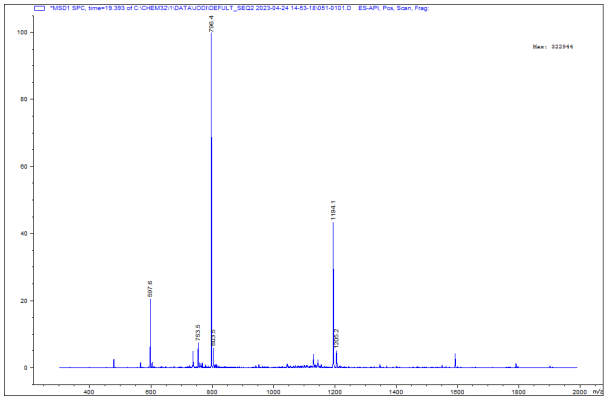


Figure S14. MS of purified 5-Fam-Peg$_4$-CKKKKKKSKTKCVIM-OH (**19**). Calcd m/z: $[M+2H]^{2+}$ 1193.62, $[M+3H]^{3+}$ 796.08, and $[M+4H]^{4+}$ 597.5. Obs m/z: $[M+2H]^{2+}$ 1194.1, $[M+3H]^{3+}$ 796.4, and $[M+4H]^{4+}$ 597.6.

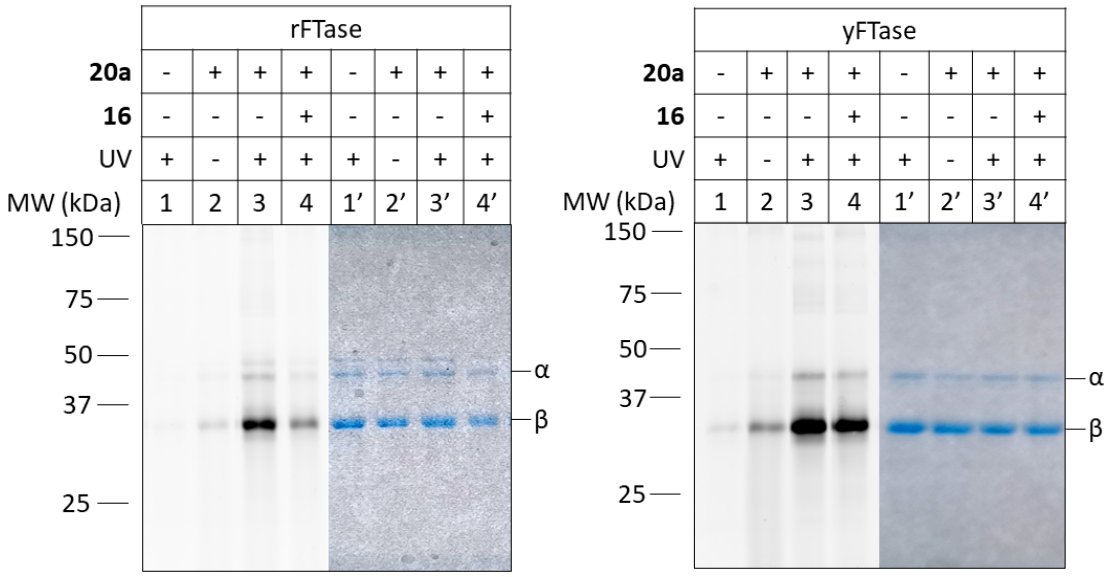


Figure S15. Photoaffinity labeling of FTase enzymes using **20a**. Left Panel) Lane 1: rFTase, No probe (**20a**), + UV; Lane 2: rFTase, Probe (**20a**), - UV; Lane 3: rFTase, Probe (**20a**), + UV; Lane 4: rFTase, Probe (**20a**), + UV, + competitor (**16**). Right Panel) Lane 1: yFTase, No probe (**20a**), + UV; Lane 2: yFTase, Probe (**20a**), - UV; Lane 3: yFTase, Probe (**20a**), + UV; Lane 4: yFTase, Probe (**20a**), + UV, + competitor (**16**). In both panels, Lanes 1-4 were imaged by fluorescence scanning to visualize the crosslinked 5-Fam and Lanes 1'-4' were imaged by Coomassie blue staining to visualize total protein. Compound **20a** was generated in situ with **1** and **19** using FTase prior to photolysis.

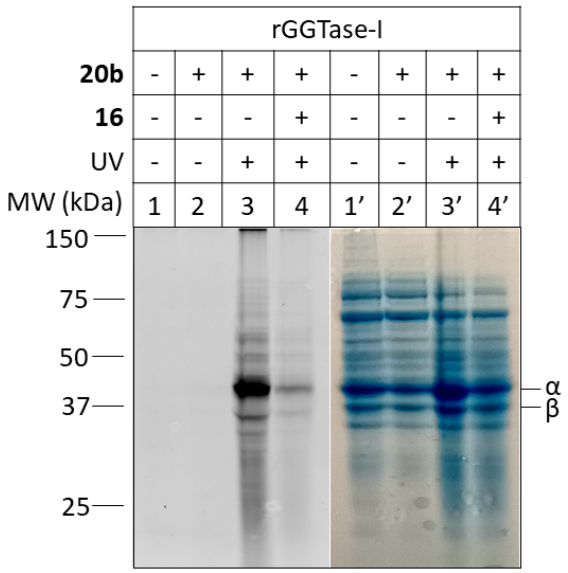


Figure S16. Photoaffinity labeling of rGGTase using **20b**. Lane 1: rGGTase, No probe (**20b**), + UV; Lane 2: rGGTase, Probe (**20b**), - UV; Lane 3: rGGTase, Probe (**20b**), + UV; Lane 4: rGGTase, Probe (**20b**), + UV, + competitor (**16**). Lanes 1-4 were imaged by fluorescence scanning to visualize the crosslinked 5-Fam and Lanes 1'-4' were imaged by Coomassie blue staining to visualize total protein. Compound **20b** was generated in situ with **2** and **19** using rGGTase prior to photolysis.

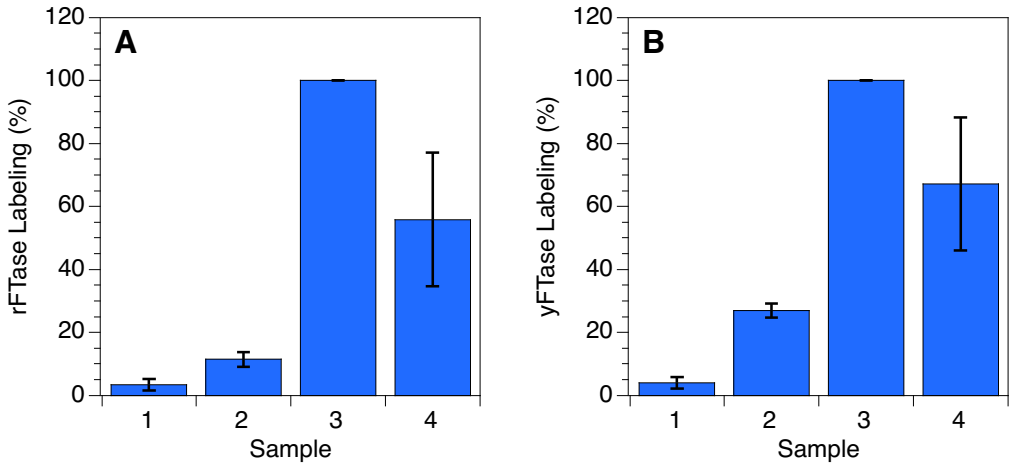


Figure S17. Quantitiative analysis of β-subunit photoaffinity labeling of using **20a**. A) Analysis of labeling of rFTase with **20a**. Sample 1: No probe (**20a**), + UV; Sample 2: Probe (**20a**), - UV; Sample 3: Probe (**20a**), + UV; Sample 4: Probe (**20a**), + UV, + competitior (**16**). B) Analysis of labeling of yFTase with **20a**. Sample 1: No probe (**20a**), + UV; Sample 2: Probe (**20a**), - UV; Sample 3: Probe (**20a**), + UV; Sample 4: Probe (**20a**), + UV, + competitor (**16**).

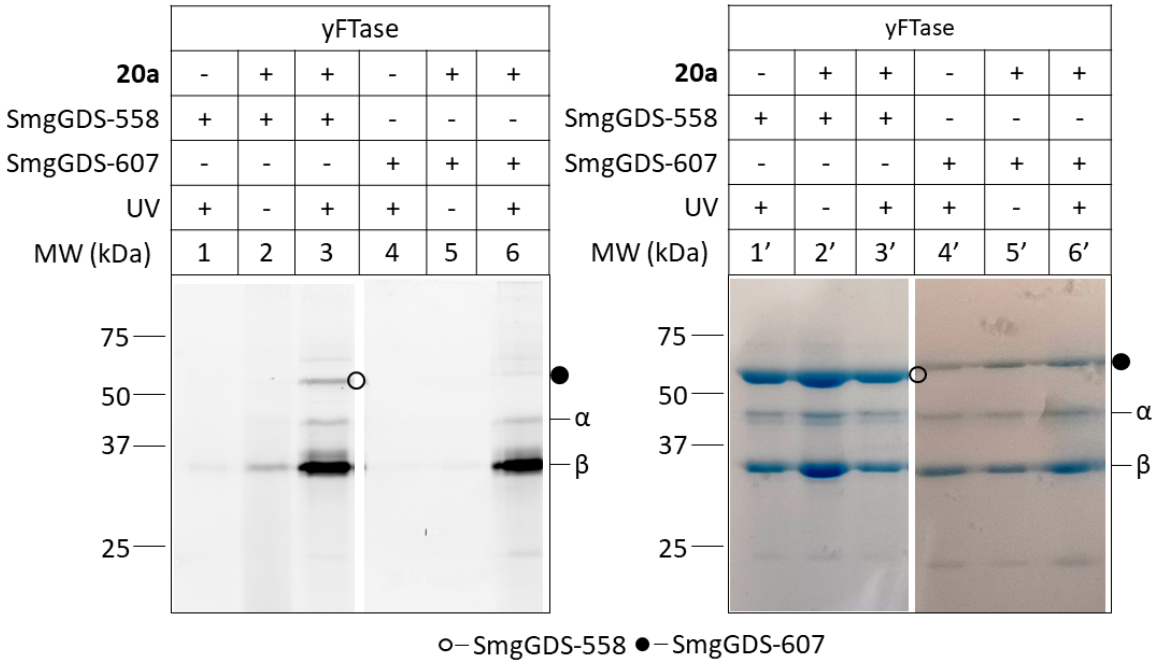


Figure S18. Photoaffinity labeling of proteins using diazirine-functionalized peptides. Panel A) Labeling of rFTase: Lane 1: No diazirine (**19** instead of **20a**), + UV; Lane 2: Probe (**20a**), – UV; Lane 3: Probe (**20a**), + UV; Lane 4: Probe (**20a**) + UV, + competitor (**16**). Panel B) Labeling of SmgGDS isoforms: Lane 1: SmgGDS558, No diazirine (**19** instead of **20a**), + UV; Lane 2: SmgGDS558, Probe (**20a**), – UV; Lane 3: SmgGDS558, Probe (**20a**), + UV; Lane 4: SmgGDS607 No diazirine (**19** instead of **20a**), + UV; Lane 5: SmgGDS607, Probe (**20a**), – UV; Lane 6: SmgGDS607, Probe (**20a**), + UV. Note all reactions with SmgGDS proteins also contained yFTase that was used to enzymatically prepare **20a** from **19** and **1** prior to photolysis. In Panel A, Lane 1, and Panel B, Lanes 1 and 4, diazirine **1** was omitted to prevent the formation of **20a** from **19**. Compound **1** was present in all other samples.La nes 1-6 were imaged by fluorescence scanning to visualize the crosslinked 5-Fam and Lanes 1'-6' were imaged by Coomassie blue staining to visualize total protein.

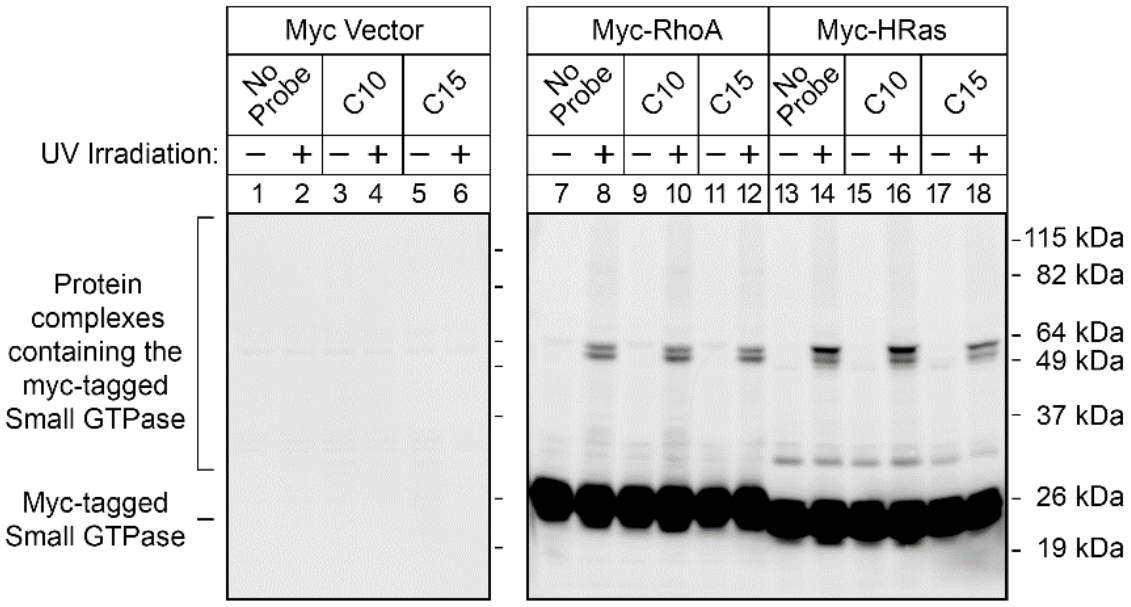


Figure S19. Western blot analysis of photocrosslinking reactions performed in live HEK293T cells transfected with cDNA constructs encoding myc-tagged small GTPases, or transfected with the control myc-vector cDNA. The transfected cells were treated with vehicle or 10 μM of the indicated probes and cultured for 24 h, followed by treatment with or without UV irradiation for 40 min. The cells were lysed and the total cell lysates were subjected to SDS-PAGE and western blotting using myc antibody. Results are shown for cells transfected with empty myc-vector (lanes 1-6: Myc Vector), myc-tagged RhoA (lanes 7-12: Myc-RhoA) or myc-tagged HRas (lanes 13-18: Myc-HRas). The results are representative of three independent experiments. Initially, these experiments appeared quite promising with new myc-containing crosslinked bands appearing in the 49-64 kDa region of the gel. Importantly, those bands were only observed upon UV irradiation (e.g., compare Lanes 9 and 10 or 11 and 12). However, similar bands were observed in control samples where no probe was added (e.g., compare Lanes 7 and 8) indicating that the observed crosslinking was occurring by a process that was unrelated to the presence of the diazirine. Those crosslinks may be forming via thiol-ene reactions between the natural prenyl groups on the proteins and thiol groups positioned on proximal interacting proteins. It is noteworthy that Waldmann and coworkers have used such chemistry to immobilize farnesylated proteins on thiol-containing surfaces.[1] Nevertheless, it does not change the fact that no diazirine-mediated crosslinking was detected in these live cell experiments. Given that it was possible to detect crosslinking to FTase and GGTase-I in the *in vitro* experiments (see Fig. 5), it appears that enrichment of the crosslinked products will be necessary in order to detect them in the *in cellulo* photolabeling experiments due to the limited efficiency of photoaffinity labeling coupled with the low concentration of prenyltransferases and their cognate substrate proteins.

Tables

Table S1. Ions observed in the MS^2 analysis of **14a**

Fragment	Type of fragment ion	calcd *m/z*	obs *m/z*
$C_{46}H_{67}N_8O_{10}S_2$	MH – H_20	955.4416	955.4409
$C_{43}H_{62}N_7O_8S_2$	b5	868.4096	868.4091
$C_{41}H_{61}N_6O_9S_2$	$[M+H]^+$ – $C_5H_8N_2O_2$ loss of diazirine ester	845.3936	845.3907
$C_{37}H_{51}N_6O_7S_2$	b4	755.3255	755.3251
$C_{34}H_{58}N_7O_9S_1$ $C_{38}H_{54}N_5O_6S_2$	y5 or b5 – $C_5H_8N_2O_2$ (loss of diazirine ester)	740.4011 740.3510	740.3508
$C_{31}H_{47}N_6O_9S_2$	$[M+H]^+$ – $C_{15}H_{22}N_2O_2$ loss of prenyl group	711.2840	711.2814
$C_{31}H_{46}N_6O_9S_1$	$[M+H]^+$ – $C_{15}H_{24}N_2O_2S$ loss of prenyl thiol group	677.2963	677.2924
$C_{32}H_{42}N_5O_6S_2$	b3	656.2571	656.2571
$C_{31}H_{42}N_5O_5S_2$	a3	628.2622	628.2614
$C_{32}H_{43}N_4O_5S_2$	b4 – $C_5H_8N_2O_2$ loss of diazirine ester	627.2669	627.2644
$C_{28}H_{40}N_5O_6S_2$	b5 – $C_{15}H_{22}N_2O_2$ loss of prenyl group	606.2415	606.2393
$C_{27}H_{34}N_3O_4S_2$	b3 – $C_5H_8N_2O_2$ loss of diazirine ester	528.1985	528.1969
$C_{22}H_{29}N_4O_5S_2$	b4 – $C_{15}H_{22}N_2O_2$ loss of prenyl group	493.1574	493.1555
$C_{22}H_{28}N_4O_5S_1$	b4 – $C_{15}H_{24}N_2O_2S$ loss of prenyl thiol group	459.1996	459.1696
$C_{17}H_{20}N_3O_4S_2$	b3 – $C_{15}H_{22}N_2O_2$ loss of prenyl group	394.0890	394.0871
$C_{17}H_{19}N_3O_4S_1$	b3 – $C_{15}H_{24}N_2O_2S$ loss of prenyl thiol group	360.1012	360.1011
$C_{14}H_{28}N_3O_5$	y3	318.2023	318.2012

Table S2. Ions observed in the MS^2 analysis of **15a**

Fragment	Type of fragment ion	calcd *m/z*	obs *m/z*
$C_{44}H_{67}N_6O_8S_2$	$[M+H]^+ - C_5H_8N_2O_2$ loss of diazirine ester	871.4456	871.4464
$C_{43}H_{62}N_7O_8S_2$	b5	868.4096	868.4107
$C_{38}H_{54}N_5O_6S_2$	b5 – $C_5H_8N_2O_2$ loss of diazirine ester	740.3510	740.3521
$C_{37}H_{51}N_6O_7S_2$	b4	755.3255	755.3257
$C_{34}H_{53}N_6O_8S_2$	$[M+H]^+ - C_{15}H_{22}N_2O_2$ loss of prenyl group	737.3361	737.3366
$C_{34}H_{51}N_6O_8S$	$[M+H]^+ - C_{15}H_{24}N_2O_2S$ loss of prenyl thiol group	703.3484	703.3494
$C_{32}H_{42}N_5O_6S_2$	b3	656.2571	656.2578
$C_{32}H_{43}N_4O_5S_2$	b4 – $C_5H_8N_2O_2$ loss of diazirine ester	627.2669	627.2673
$C_{28}H_{40}N_5O_6S_2$	b5 – $C_{15}H_{22}N_2O_2$ loss of prenyl group	606.2415	606.2421
$C_{31}H_{43}N_4O_4S_2$	a4 – $C_5H_8N_2O_2$ loss of diazirine ester	599.2720	599.2727
$C_{27}H_{34}N_3O_4S_2$	b3 – $C_5H_8N_2O_2$ loss of diazirine ester	528.1985	528.1994
$C_{22}H_{29}N_4O_5S_2$	b4 – $C_{15}H_{22}N_2O_2$ loss of prenyl group	493.1574	493.1577
$C_{22}H_{28}N_4O_5S_1$	b4 – $C_{15}H_{24}N_2O_2S$ loss of prenyl thiol group	459.1996	459.1702
$C_{17}H_{20}N_3O_4S_2$	b3 – $C_{15}H_{22}N_2O_2$ loss of prenyl group	394.0890	394.0892
$C_{17}H_{19}N_3O_4S_1$	b3 – $C_{15}H_{24}N_2O_2S$ loss of prenyl thiol group	360.1012	360.1015
$C_{17}H_{34}N_3O_4$	y3	344.2544	344.2560

Table S3. Ions observed in the MS^2 analysis of **15b**

Fragment	Type of fragment ion	calcd *m/z*	obs *m/z*
$C_{54}H_{83}N_8O_{10}S_2^{+1}$	$[M+H]^+$	1067.5668	1067.5687
$C_{49}H_{75}N_6O_8S_2$	$[M+H]^+ - C_5H_8N_2O_2$ loss of diazirine ester	939.5082	939.5109
$C_{48}H_{70}N_7O_8S_2$	b5	936.4722	936.4748
$C_{47}H_{70}N_7O_7S_2$	a5	908.4773	908.4644
$C_{42}H_{59}N_6O_7S_2$	b4	823.3881	823.3892
$C_{43}H_{62}N_5O_6S_2$	b5 – $C_5H_8N_2O_2$ loss of diazirine ester	808.4136	808.4162
$C_{41}H_{59}N_6O_6S_2$	a4	795.3932	795.3844
$C_{42}H_{62}N_5O_5S_2$	a5 – $C_5H_8N_2O_2$ loss of diazirine ester	780.4187	780.4208
$C_{34}H_{53}N_6O_8S_2$	$[M+H]^+ - C_{20}H_{30}N_2O_2$ loss of prenyl group	737.3361	737.3370
$C_{34}H_{51}N_6O_8S$	$[M+H]^+ - C_{20}H_{32}N_2O_2S$ loss of prenyl thiol group	703.3484	703.3484
$C_{36}H_{50}N_5O_5S_2$	a3	696.3248	696.3275
$C_{37}H_{51}N_4O_5S_2$	b4 – $C_5H_8N_2O_2$ loss of diazirine ester	695.3295	695.3309
$C_{28}H_{40}N_5O_6S_2$	b5 – $C_{20}H_{30}N_2O_2$ loss of prenyl group	606.2415	606.2426
$C_{32}H_{42}N_3O_4S_2$	b3 – $C_5H_8N_2O_2$ loss of diazirine ester	596.2611	596.2623
$C_{28}H_{39}N_5O_6S_1$	b5 – $C_{20}H_{32}N_2O_2S$ loss of prenyl thiol group	572.2574	572.2536
$C_{22}H_{29}N_4O_5S_2$	b4 – $C_{20}H_{30}N_2O_2$ loss of prenyl group	493.1574	493.1582
$C_{22}H_{28}N_4O_5S_1$	b4 – $C_{20}H_{32}N_2O_2S$ loss of prenyl thiol group	459.1996	459.1706
$C_{17}H_{20}N_3O_4S_2$	b3 – $C_{20}H_{22}N_2O_2$ loss of prenyl group	394.0890	394.0891
$C_{17}H_{19}N_3O_4S_1$	b3 – $C_{20}H_{32}N_2O_2S$ loss of prenyl thiol group	360.1012	360.1016
$C_{12}H_{25}N_2O_3$	y2	245.1860	245.1857

Table S4. Ions observed in the MS^2 analysis of geranylgeranylated **13**

Fragment	Type of fragment ion	calcd *m/z*	obs *m/z*
$C_{54}H_{83}N_6O_7S_2$	$[M+H]^+ - H_2O$	991.5759	991.5742
$C_{48}H_{72}N_5O_6S_2$	b5	878.4919	878.4903
$C_{47}H_{72}N_5O_5S_2$	a5	850.4969	850.4951
$C_{42}H_{61}N_4O_5S_2$	b4	765.4078	765.4064
$C_{41}H_{61}N_4O_4S_2$	a4	737.4129	737.4120
$C_{40}H_{71}N_4O_5S_1$	y4	719.5140	719.3292
$C_{37}H_{52}N_3O_4S_2$	b3	666.3394	666.3378
$C_{36}H_{52}N_3O_3S_2$	a3	638.3445	638.3433
$C_{28}H_{40}N_5O_6S_2$	b5 – $C_{20}H_{32}$ loss of geranylgeranyl group	606.2415	606.2410
$C_{22}H_{29}N_4O_5S_2$	b4 – $C_{20}H_{32}$ loss of geranylgeranyl group	493.1574	493.1568
$C_{22}H_{28}N_4O_5S_1$	b4 – $C_{20}H_{34}S$ loss of geranylgeranyl thiol group	459.1996	459.1691
$C_{17}H_{19}N_3O_4S_1$	b3 – $C_{20}H_{34}S$ loss of geranylgeranyl thiol group	360.1012	360.1008

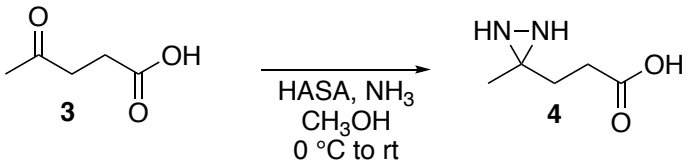
Table S5. Ions observed in the MS^3 analysis of the b3 ion from **14a**

Fragment from MS^2 subjected to MS^3	Fragment	Type of fragment ion on MS^3	calcd *m/z*	obs *m/z*
b3 (656.2571)	$C_{31}H_{42}N_5O_5S_2$	a3	628.2622	628.2587
	$C_{27}H_{34}N_3O_4S_2$	b3 – $C_5H_8N_2O_2$ loss of diazirine ester	528.1985	528.1973
	$C_{26}H_{34}N_3O_3S_2$	a3 – $C_5H_8N_2O_2$ loss of diazirine ester	500.2036	500.2023
	$C_{17}H_{20}N_3O_4S_2$	b3 – $C_{15}H_{22}N_2O_2$ loss of prenyl group	394.0890	394.0883
	$C_{14}H_{18}N_3O_3S$	c2	308.1063	308.1052
	$C_{12}H_{12}NO_2S$	dansyl group	234.0589	234.0571

General Materials

All synthetic reactions were carried out at rt and stirred magnetically unless otherwise noted. Thin layer chromatography (TLC) was performed on pre-coated (250 μm) silica gel 60 F-254 plates from Merck. Plates were visualized by staining with $KMnO_4$. Flash chromatography silica gel (60-200 mesh, 75-250 μm) was obtained from Mallinckrodt Inc. Dry CH_2Cl_2, CH_3CN, and THF were obtained from Sigma-Aldrich. PPh_3 (polymer-supported beads) was purchased from Sigma-Aldrich. Deuterated NMR solvents were purchased from Cambridge Isotope Laboratories, Inc. ^{1}H-NMR spectra were obtained at 400 MHz. ^{13}C-NMR spectra were obtained at 100 MHz. ^{31}P-NMR spectra were obtained at 162 MHz. All NMR spectra were acquired on Varian instruments at 25 ^{o}C. Chemical shifts are reported in ppm and *J* values are in Hz. MS spectra for synthetic compounds were obtained with a Bruker BioTOF II instrument. HPLC separations for peptide analysis and purification were performed using an Agilent 1100 instrument, equipped with a UV detector and a Phenomenex C_{18} column (Luna, 10 μm, 10 × 250 mm) with a 5 cm guard column. Fluorescence assay data were obtained using a Varian model Cary Eclipse Fluorescence Spectrophotometer. LC-MS was performed using an Agilent 1200 instrument, equipped with an Agilent G6130 MS using an Agilent C_{18} column (ZORBAX 300SB-C18, 5 μm, 4 × 250 mm). LC-MS2 and LC-MS3 experiments were performed using a Thermo Scientific Orbitrap Fusion Tribrid mass spectrometer with CID ionization method. Recombinant rat FTase (rFTase),[2] yeast FTase (yFTase)[3] and rat GGTase-I (rGGTase)[4,5] were overexpressed in *E.coli* BL21(DE3)/pRFT529 and *E.coli* DH5α/ and purified as previously described. The concentrations of the purified enzymes were determined via Bradford assay using BSA standards. Dansylated peptides were prepared as previously described.[6]

General Synthetic Procedure I

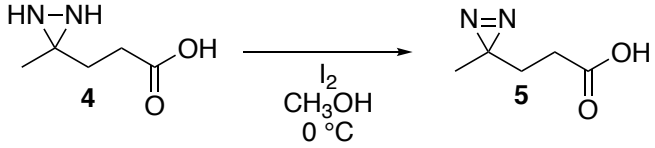


3-(3-methyldiaziridin-3-yl)propanoic acid (4)

Levulinic acid **3** (2 g, 17.2 mmol, 1 equiv) was dissolved in 7 N NH_3 in CH_3OH (17.2 mL, 121 mmol, 7 equiv). The resulting solution was stirred under N_2 in an ice-bath for 3 h. A solution of hydrolxylamine-O-sulfonic acid (2.30 g, 20.7 mmol, 1.2 equiv) in CH_3OH (12 mL) was added dropwise at a rate of 1 drop/s. The reaction mixture was stirred for 20 h and allowed to warm to rt. N_2 was bubbled through the solution for 1 h to remove NH_3 gas. Vacuum filtration and concentration resulted in a yellow oil that was used in the next step without purification.[1]

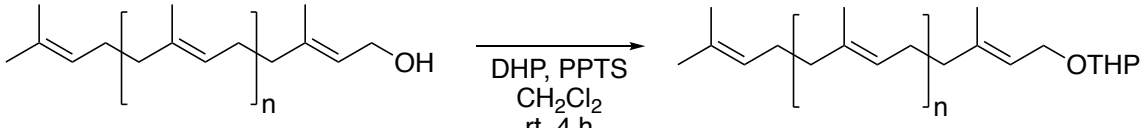
General Synthetic Procedure II

3-(3-methyl-3H-diazirin-3-yl)propanoic acid (5)



Diaziridine **4** was dissolved in CH_3OH (15 mL) and stirred on ice for 5 min in a foil-covered flask. Triethylamine (4.6 mL, 33.5 mmol) was added and allowed to stir for 5 min. Slowly, chips of I_2 were added until the solution remained a brown-red color for longer than 5 min after the last addition. The reaction solution was diluted with EtOAc and washed with 1 M HCl and aqueous 10% $Na_2S_2O_3$ until the organic layer was colorless. The aqueous layer was further extracted with EtOAc (2 × 20 mL). The organic layers were combined, dried over $MgSO_4$, and concentrated to afford diazirine acid **5** as a brown residue (1.07 g, 50%). Spectral data obtained were in good agreement with those reported in the literature.[1]

General Synthetic Procedure III: Protection of Isoprenoid alcohol



6a (n = 0) Geraniol
6b (n = 1) Farnesol

7a (n = 0)
7b (n = 1)

(*E*)-2-((3,7-dimethylocta-2,6-dien-1-yl)oxy)tetrahydro-2H-pyran (7a)

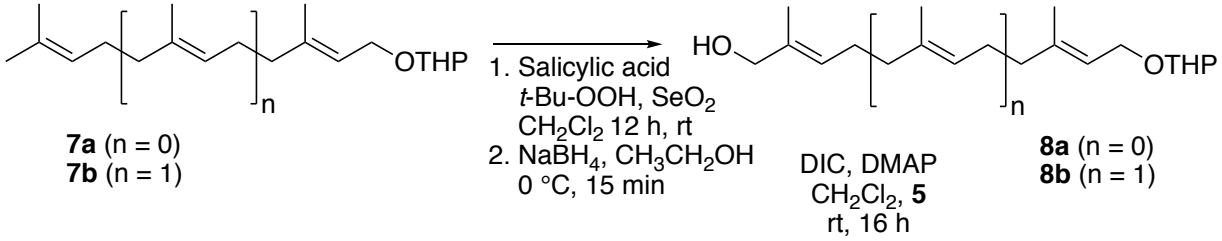
To a solution of geraniol (**6a**) (5.00 g, 32.5 mmol, 1 equiv) in CH_2Cl_2 (20 mL) were added DHP (11.0 g, 65.0 mmol, 2 equiv) and PPTS (0.800 g, 3.20 mmol. 0.1 equiv), and the resulting solution was stirred

for 4 h at rt. The reaction mixture was quenched with saturated aqueous $NaHCO_3$ and extracted with CH_2Cl_2. The organic layer was dried over $MgSO_4$ and concentrated to afford **7a** as a clear oil (7.0 g, 91%). Spectral data obtained were in good agreement with those reported in the literature.[1]

2-(((2*E*,6*E*)-3,7,11-trimethyldodeca-2,6,10-trien-1-yl)oxy)tetrahydro-2H-pyran (7b)

Compound **7b** was prepared using general synthetic procedure III using farnesol **6b** (3.00 g, 13.5 mmol, 1 equiv) and **7b** was isolated as a clear oil (3.7 g, 90 %). Spectral data obtained were in good agreement with those reported in the literature.[2]

General Synthetic Procedure IV: Riley Oxidation



(2*E*,6*E*)-2,6-dimethyl-8-((tetrahydro-2H-pyran-2-yl)oxy)octa-2,6-dien-1-ol (8a)

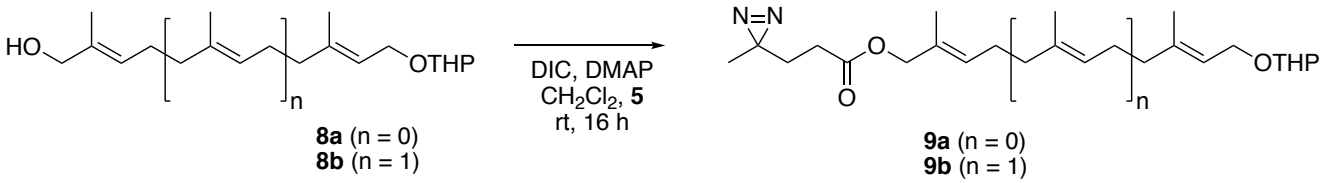
Protected geraniol (**7a**) was dissolved in CH_2Cl_2 (25 mL). In turn, 70% t-Bu-OOH in H_2O (7.70 mL, 55.5 mmol, 3 equiv), salicylic acid (0.255 g, 1.85 mmol, 0.1 equiv), and SeO_2 (0.205 g, 1.85 mmol, 0.1 equiv) were added, and the resulting solution was stirred overnight at rt. The reaction mixture was quenched with saturated aqueous $NaHCO_3$, extracted with CH_2Cl_2, dried over $MgSO_4$ and concentrated under reduced pressure.

The crude product was dissolved in CH_3CH_2OH (25 mL) and kept in an ice bath under an N_2 atmosphere. $NaBH_4$ (0.763 g, 1.85 mmol, 0.1 equiv) was added portion-wise and the reaction mixture was kept in an ice bath for 15 min. The reaction mixture was quenched with ice, and the CH_3CH_2OH was removed by rotory evaporation. The resulting thick oil was diluted with CH_2Cl_2, washed with saturated aqueous $NaHCO_3$, brine, and extracted with CH_2Cl_2. The organic layer was dried over $MgSO_4$, concentrated under reduced pressure and the crude product purified by silica gel flash column chromatography using 20% EtOAc/hexanes (v/v) to yield **8a** (2.3 g, 50 %) as colorless oil. Spectral data obtained were in good agreement with those reported in the literature.[1]

(2*E*,6*E*,10*E*)-2,6,10-trimethyl-12-((tetrahydro-2H-pyran-2-yl)oxy)dodeca-2,6,10-trien-1-ol (8b)

Compound **8b** was prepared using general synthetic procedure IV using protected farnesol (**7b**) as the starting material. Compound **8b** was isolated (460 mg, 15 %) as colorless oil. Spectral data obtained were in good agreement with those reported in the literature.[2]

General Synthetic Procedure V: Esterification



8a (n = 0)
8b (n = 1)

9a (n = 0)
9b (n = 1)

(2*E*,6*E*)-2,6-dimethyl-8-((tetrahydro-2H-pyran-2-yl)oxy)octa-2,6-dien-1-yl 3-(3-methyl-3H-diazirin-3-yl)propanoate (9a)

To a solution of protected alcohol **8a** (0.575 g, 4.49 mmol, 1 equiv) and DMAP (55.0 mg, 0.450 mmol, 0.1 equiv) in CH_2Cl_2 (6 mL) was added a solution of the diazirine acid **5** (1.26 g, 4.94 mmol, 1.1 equiv) in CH_2Cl_2 (6 mL). DIC (0.679 g, 5.39 mmol, 1.2 equiv) was added, and the resulting solution was stirred at rt for 16 h. The reaction mixture was filtered and concentrated. Purification by flash chromatography (hexanes/EtOAc, 4:1, v/v) afforded 848 mg (53%) of diazirine **9a** as a clear oil. Spectral data obtained were in good agreement with those reported in the literature.[1]

(2*E*,6*E*,10*E*)-2,6,10-trimethyl-12-((tetrahydro-2H-pyran-2-yl)oxy)dodeca-2,6,10-trien-1-yl 3-(3-methyl-3H-diazirin-3-yl)propanoate (9b):

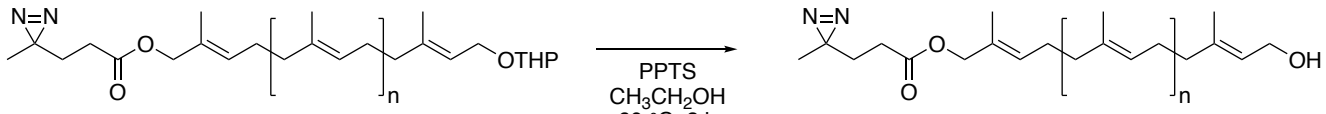
Compound **9b** was prepared using general synthetic procedure V using protected alcohol **8b** (0.436 g, 1.72 mmol, 1.1 equiv). Diazirine **9b** was isolated (370 mg, 55 %) as a clear oil.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.50-5.54 (m, 1H), 5.42-5.35 (m, 1H), 5.17-5.10 (m, 1H), 4.67-4.60 (m, 1H), 4.48 (s, 2H), 4.26 (dd, *J* = 11.9, 6.3 Hz, 1H), 4.05 (dd, *J* = 11.9, 7.4 Hz, 1H), 3.96-3.86 (m, 1H), 3.65-3.45 (m, 1H), 2.27-1.99 (m, 10H), 1.77-1.69 (m, 6H), 1.67 (bs, 2H), 1.62 (bs, 3H), 1.59 (bs, 3H), 1.25 (d, *J* = 6.4 Hz, 3H), 1.05 (s, 3H).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 172.2, 140.3, 134.7, 129.9, 129.8, 124.3, 120.7, 97.8, 70.6, 70.6, 63.7, 62.3, 39.6, 39.0, 30.7, 29.7, 28.8, 26.4, 26.3, 25.5, 24.6, 19.6, 16.4, 16.0, 14.0.

HRMS (ESI) m/z: $[M+Na]^+$ Calcd for $C_{25}H_{40}N_2O_4Na$ 455.2880; Found 455.2875.

General Synthetic Procedure VI: Deprotection



9a (n = 0)
9b (n = 1)

10a (n = 0)
10b (n = 1)

(2*E*,6*E*)-8-hydroxy-2,6-dimethylocta-2,6-dien-1-yl 3-(3-methyl-3H-diazirin-3-yl)propanoate (10a)

To a solution of **9a** (800 mg, 2.2 mmol, 1 equiv) in EtOH (12 mL) was added PPTS (55.2 mg, 0.22 mmol, 0.1 equiv). The reaction flask stirred at 60 °C (oil bath) for 2 h. The reaction mixture was concentrated in vacuo and purified by flash chromatography (hexanes/ EtOAc, 7:3, v/v) to afford 570 mg (93 %) of diazirine alcohol **10a** as a clear oil. Spectral data obtained were in good agreement with those reported in the literature.[1]

(2*E*,6*E*,10*E*)-12-hydroxy-2,6,10-trimethyldodeca-2,6,10-trien-1-yl 3-(3-methyl-3H-diazirin-3-yl)propanoate (10b)

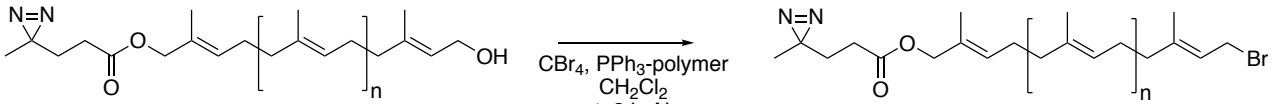
Compound **10b** was prepared using general synthetic procedure VI using ester **9b** (370 mg, 0.86 mmol, 1 equiv) and diazirine alcohol **10b** was isolated (180 mg, 60 %) as a clear oil.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.52-5.5.38 (m, 2H), 5.13 (t, *J* = 6.4 Hz, 1H), 4.48 (s, 2H), 4.17 (d, *J* = 6.9 Hz, 2H), 2.31-1.99 (m, 10H), 1.78-1.59 (m, 11H), 1.05 (s, 3H).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 172.2, 139.6, 134.7, 129.8, 129.8, 124.3, 123.5, 70.6, 59.4, 39.5, 38.98, 29.7, 28.8, 26.3, 26.3, 19.7, 16.3, 16.0, 14.0.

HRMS (ESI) m/z: $[M+Na]^+$ Calcd for $C_{20}H_{32}N_2O_3Na$ 371.2305; Found 371.2307.

General Synthetic Procedure VII: Bromination



10a (n = 0)
10b (n = 1)

11a (n = 0)
11b (n = 1)

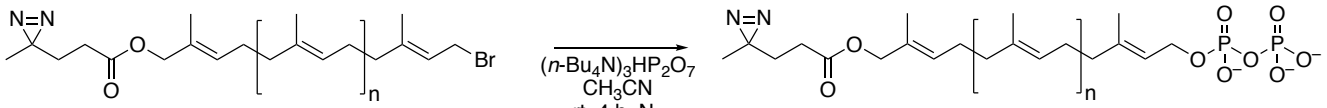
(2*E*,6*E*)-8-bromo-2,6-dimethylocta-2,6-dien-1-yl 3-(3-methyl-3H-diazirin-3-yl)propanoate (11a)

Diazirine alcohol **10a** (100 mg, 0.357 mmol, 1 equiv) was converted to the corresponding bromide in the presence of resin-bound PPh_3 (374 mg, 1.43 mmol, 4 equiv) and CBr_4 (474 mg, 1.43 mmol, 4 equiv) dissolved in CH_2Cl_2 (8 mL). The resulting solution was allowed to stir for 2 h at rt. After the reaction was complete, excess CBr_4 and resin-bound PPh_3 were removed from the mixture by filtration. Removal of the solvent afforded 105 mg (60%) of diazirine bromide **11a** as a clear oil, which was unstable due to allylic bromide and used for for the next step without purification.

(2*E*,6*E*,10*E*)-12-bromo-2,6,10-trimethyldodeca-2,6,10-trien-1-yl 3-(3-methyl-3H-diazirin-3-yl)propanoate (11b)

Compound **11b** was prepared using general synthetic procedure VII using diazirine alcohol **10b** (70 mg, 0.201 mmol, 1 equiv). Assuming quantitative conversion, the crude bromide **11b** was used for the next step without purification.

General Procedure VIII: Diphosphate Preparation



11a (n = 0)
11b (n = 1)

1 (n = 0)
2 (n = 1)

(2*E*,6*E*)-3,7-dimethyl-8-((3-(3-methyl-3H-diazirin-3-yl)propanoyl)oxy)octa-2,6-dien-1-yl diphosphate (1):

The crude bromide **11a** described above (122 mg, 0.357 mmol) was dissolved in dry CH_3CN and a solution of (n-$Bu_4N)_3HP_2O_7$ (644 mg, 0.714 mmol) in dry CH_3CN was added into the reaction mixture at rt. After 4 h, the reaction mixture was passed through Dowex Resin (ion-exchange column, Amberchrome® 50WX8 100-200H, hydrogen form, 50-100 mesh). For that procedure, the resin was packed into a column, washed with 3 bed volumes of H_2O/conc. NH_4OH (3/1, v/v), and equilibrated with 4 bed volumes of 25 mM NH_4HCO_3/i-PrOH (98/2, v/v) denoted as solvent C. The crude product was loaded on the ion-exchange column and eluted with 60 mL solvent C. The compound-containing fractions were collected and lyophilized. Next, the crude material was dissolved in 3 mL 0.1 M NH_4HCO_3 and 12 mL 1:1 CH_3CN:IPA (v/v) was added to the mixture. The mixture was vortexed and then centrifuged for 3 min. The solvent was then decanted and the extraction was repeated again. The combined solvent fractions were evaporated, and the crude residue was purified by cellulose column chromatography (Cellulose fibers, (medium), Sigma-Aldrich C6288). The column (2.0 x 14 cm) was packed with cellulose resin and equilibrated with 90:10 THF: 0.1 M NH_4HCO_3 (v/v) and the sample applied. First, 200 mL of 90:10 THF:0.1M NH_4HCO_3 was used, followed by 200 mL of 80:20 THF:0.1 M NH_4HCO_3 (v/v) and then 100 mL 70:30 THF:0.1 M NH4HCO$_3$ (v/v). During elution, a flow rate of 5.0-6.0 mL/min was used throughout. Fractions were collected in 13 x 100 mm test tubes and checked by ESI mass spectroscopy. The pure fractions containing the desired diphosphate were pooled, the THF evaporated and then lyophilized. The yield of the reaction was determined by the ^{31}P NMR spectroscopy using an internal standard (Na_2HPO_4) as 13 mg (17 %).

^{1}H NMR (400 MHz, D_2O): δ = 5.45 (d, J = 5.9 Hz, 1H), 5.36 (s, 1H), 4.43 (s, 2H), 4.36 (t, J = 6.0 Hz, 2H), 2.22 (t, J = 7.0 Hz, 2H), 2.15-2.08 (m, 2H), 2.06-1.99 (m, 2H), 1.66-1.50 (m, 8H), 0.93 (s, 4H).

^{31}P NMR (162 MHz, D_2O): δ = -6.54 (d, J = 21.8 Hz), -10.31 (d, J = 21.8 Hz).

HRMS (ESI) m/z: $[M-H]^-$ Calcd for $C_{15}H_{25}N_2O_9P_2$ 439.1041; Found 439.1062.

(2*E*,6*E*,10*E*)-3,7,11-trimethyl-12-((3-(3-methyl-3H-diazirin-3-yl)propanoyl)oxy)dodeca-2,6,10-trien-1-yl diphosphate (2):

Compound 2 was prepared using general procedure VIII using crude bromide **11b** (82 mg, 0.201 mmol). The yield of the reaction was determined by the ^{31}P NMR spectroscopy using an internal standard (Na_2HPO_4) as 6 mg (5 %).

^{1}H NMR (400 MHz, D_2O): δ = 5.34-5.27 (m, 2H), 5.04-4.97 (m, 1H), 4.61 (d, J = 11.1 Hz, 2H), 4.41-4.15 (m, 2H), 3.15-2.97 (m, 2H), 2.35-1.72 (m, 8H), 1.65-1.36 (m, 9H), 1.28-1.18 (m, 3H), 0.84-0.71 (m, 3H).

^{31}P NMR (162 MHz, D_2O): δ = -6.30 (d, J = 21.9 Hz), -10.27 (d, J = 21.9 Hz).

HRMS (ESI) m/z: $[M-H]^-$ Calcd for $C_{20}H_{33}N_2O_9P_2$ 507.1667; Found 507.1637.

Synthesis of K-Ras peptide 16.

Peptide **16** was synthesized using an automated solid-phase peptide synthesizer (PurePep Chorus, Protein Technologies Inc., Uppsala Sweden) employing Fmoc/HCTU-based chemistry. Fmoc-Met-Wang resin (0.2 mmol) was placed in the reaction vessel and swelled in 10 mL DMF for 10 min 3x then deprotected twice using 10 mL of 20% piperidine in DMF for 5 min each time. Amino acid couplings were performed by adding 3 mL of a 333 mM amino acid (4.0 eq) solution in DMF, 3 mL of a 333 mM HCTU (4.0 eq) solution in DMF, and 3 mL of a 1.2M DIPEA (12.0 eq) solution in DMF to the reaction vessel. Standard incubation time for each coupling was 20 min. After each coupling, the peptide was capped using 3mL of Ac_2O and 3 mL of 1.2M DIPEA, dissolved in DMF with an incubation time of 15 min. The resin was washed with 5 mL of CH_2Cl_2 3x. Global deprotection and resin cleavage was accomplished via treatment with 10 mL of reagent K (82.5% TFA, 5% phenol, 5% water, 5% thioanisole, and 2.5% ethanedithiol) for 2 h. Cleaved peptides were precipitated with 50 mL Et_2O and centrifuged before decanting the Et_2O layer Then, the peptide pellet was re-dissolved in 2 mL TFA and precipitated in 50 mL Et_2O followed by centrifugation (2x). The resulting crude peptide was dried via a stream of dry N_2, and then dissolved in 8 mL of a mixture of H_2O/CH_3CN (7:3, v/v) containing 0.1% TFA. The solution was filtered using a 0.2 µm PTFE filter and then purified using preparative reverse-phase (RP)-HPLC using a sequence consisting of the following: 0-5 min, 1% B, 5-15 min, gradient of 1% B to 20% B, 15-45 min, 20% B to 30% B, then 45-55 min at 100% B using a flow rate of 5 mL/min. The peak at 34 min was collected and then HPLC fractions were pooled and lyophilized, then dissolved in 8 mL of 500 mM Tris-HCl, pH 7.5.

MS (ESI) m/z: $[M+H]^+$ Calcd for $C_{77}H_{150}N_{23}O_{18}S_3$ 1781.1; Found 1780.8; $[M+2H]^{2+}$ Calcd for $C_{77}H_{151}N_{23}O_{18}S_3$ 891.0; Found 891.6; $[M+3H]^{3+}$ Calcd for $C_{77}H_{152}N_{23}O_{18}S_3$ 594.4; Found 594.5.

Preparation of full-length K-Ras4B and modification with 1.

Expression and purification of K-Ras 4B $^{1-174}$- MESNA thioester. This procedure is based on one previously reported by Chen et al. A plasmid encoding the truncated K-Ras 4B $^{1-174}$ DNA sequence was transformed into *E.coli* BL21 (DE3) (Thermo Scientific, cat: EC0114). The resulting bacteria were grown in LB media, induced using 1 mM isopropyl-β-D-1-thiogalactopyranoside (IPTG) followed by incubation for 20 h at 25 °C. The cells were harvested by centrifugation, resuspended in lysis buffer (50 mM HEPES-Na, 500 mM NaCl, 1 mM $MgCl_2$, pH 7.5) which contained protease inhibitor tablets (2 tablets, Roche, cat: 04693159001) and 0.1% Triton-X 100 (v/v). The resulting cell lysate was clarified by centrifugation 5000×g, 30 min, 4 °C) and the supernatant mixed with chitin affinity resin (New England Biolabs, Cat: S6651S) and incubated at 4 °C for 40 min (Lysate from 2 L bacterial culture mixed with 20 mL resin slurry.) After incubation, the lysate and resin mixture was packed into a gravity column. The undesired cytosolic proteins were removed by washing (50 mM HEPES-Na, 500 mM NaCl, 1 mM $MgCl_2$, pH 7.5). at a flow rate of 1 mL/min. Next, the chitin column was given a quick wash with with 3 column

volumes of intein-splicing buffer (50 mM HEPES-Na, 500 mM NaCl, 1 mM $MgCl_2$, 250 mM MESNA, pH 7.5, flow rate: 5 mL/min) and then incubated with intein-splicing buffer for 16 h at 4 °C. Finally, K-Ras 4B $^{1\text{-}174}$-MESNA thioester was eluted out using washing buffer (5 column volumes, flow rate 2 mL/min). The protein thioester was exchanged into expressed protein ligation (EPL) buffer (50 mM HEPES-Na, 500 mM NaCl, 1 mM $MgCl_2$, pH 7.5) via ultrafiltration (Amicon Ultra-15 centrifugal filters, cat: UCF901024, 5400×g), and the concentration was estimated via BCA assay using BSA as a standard. The purified K-Ras 4B $^{1\text{-}174}$-MESNA thioester was analyzed by SDS-PAGE and LC-ESI-MS analysis (Orbitrap Elite Hybrid Mass Spectrometer, Column: Agilent, ZORBAX 300 SB-C3, 2.1×100 mm, 1.8-µm, Cat: 858750-909). LC-ESI-MS was performed at a flow rate of 300 μL/min. The buffer system used included solvent A: H_2O and solvent B: CH_3CN containing 0.1% (v/v) TFA (CF_3COOH). The gradient was as follows: 1 min at 20% solvent B, 2 min ramp to 100% B, 2 min hold at 100% B and then 1 min ramp down to 20% B. The retention time for K-Ras 4B $^{1\text{-}174}$-MESNA thioester is 3.23 min.
ESI-MS: MW ($[M+H]^+$) calcd for K-Ras 4B $^{1\text{-}174}$-MESNA thioester: 19888 Da, found 19887 Da.

Expressed protein ligation for unmodified K-Ras4B. Peptide **16**, designed based on hypervariable region (HVR) of K-Ras4B, was dissolved into EPL buffer (50 mM HEPES-Na, 500 mM NaCl, 1 mM $MgCl_2$, pH 7.5) to generate a stock solution (8.9 mM). 1M NaOH was added dropwise to completely dissolve the thiol reagent, 4-mercaptophenylacetic acid (MPAA), in EPL buffer to generate a 2 M stock solution which was made freshly before ligation. A fresh 50 mM TCEP stock solution was also prepared. The pH of each of the above-mentioned stock solutions was adjusted to 7 (estimated using pH paper). To initiate the ligation reaction, K-Ras4B-$^{1\text{-}174}$-MESNA thioester was pre-incubated with TECP (final concentration of 5 mM) for 15 min on ice. K-Ras 4B-$^{1\text{-}174}$-MESNA (final concentration 100 μM) was ligated with HVR peptide **16** (final concentration 1 mM) in ligation buffer (50 mM HEPES-Na, 500 mM NaCl, 1 mM $MgCl_2$, 5 mM TCEP, 120 mM MPAA, pH 7.2) under Ar (g) for 3 h at rt. The ligation was monitored by SDS-PAGE. After the ligation, the reaction mixture was diluted 10-fold into desalting buffer (Solvent B: 25 mM NaAc, 1mM $MgCl_2$, pH 6.3). The aqueous was applied to the Hi-Trap SP FF column (GE Healthcare, cat: 17-5157-01) for cation exchange (100%B, flow rate: 0.5 mL/min). The column was attached to the Knauer Azura FPLC system, equilibrated and washed with desalting buffer (100% B, flow rate: 1mL/min). Unmodified K-Ras 4B was eluted using high salt buffer (Solvent C: 25 mM NaAc, 1 M NaCl, 1 mM $MgCl_2$, pH 6.3, gradient: 100%B to 100%C in 5 min, flow rate: 1mL/min). After collecting the full-length K-Ras4B (**17**), the fractions were concentrated and exchanged into protein stock buffer (10 mM HEPES-Na, 150 mM NaCl, 5 mM $MgCl_2$, pH 7.4) by ultrafiltration (Amicon Ultra-15 centrifugal filters, cat: UCF901024, 5400×g). Unmodified K-Ras4B (**17**) protein was characterized using SDS-PAGE and LC-ESI-MS (Orbitrap Elite Hybrid Mass Spectrometer, Column: Agilent, ZORBAX 300 SB-C3, 2.1×100 mm, 1.8-µm, Cat: 858750-909). In each run, the column was washed with 20% solvent B

and 80% solvent A for the first 1 min followed by a gradient from 20% solvent B to 100% solvent B in 3 min. The column was then washed with 100% solvent B for 2 min. The retention time for unmodified K-Ras 4B was 3.20 min. The protein concentration was determined by BCA assay and stored at -80 °C
ESI-MS: MW ($[M+H]^+$) calcd for unmodified K-Ras 4B (**17**): 21527 Da, found 21526 Da.

Farnesylation of unmodified K-Ras 4B. For a 100 μL scale reaction, 12 μL full length K-Ras4B **17**, (final concentration 5 μM) was pre-incubated in 10 μL 10X prenylation buffer (500 mM Tris-HCl, 100 mM $MgCl_2$, 300 mM KCl, 100μM $ZnCl_2$, 50 mM DTT) for 15 min on ice. After incubation, 12 μL C10-Des-OPP probe (**1**, final concentration 30 μM), 7.3 μL yeast FTase (final concentration 400 nM) and 58.7 μL H_2O were added to the tube. The reaction was allowed to proceed at 33 °C for 6 h. The modified K-Ras 4B was characterized by SDS-PAGE and LC-ESI-MS (Orbitrap Elite Hybrid Mass Spectrometer, Column: Agilent, ZORBAX 300 SB-C3, 2.1×100 mm, 1.8-µm, Cat: 858750-909). The gradient profile consisted of isocratic elution with 20% solvent B and 80% solvent A for 1 min followed by gradient elution from 20% solvent B to 100% solvent B over 3 min and isocratic elution with 100% solvent B for 2 min (6 min total time). The retention time for the prenylated form of K-Ras4B (**18**) was 3.22 min.
ESI-MS: MW ($[M+H]^+$) calcd for prenylated K-Ras 4B (**18**): 21789 Da, found 21789 Da.

Synthesis of K-Ras peptide 19 for photolysis studies.

Peptide **19** was synthesized using an automated solid-phase peptide synthesizer (PurePep Chorus, Protein Technologies Inc., Uppsala Sweden) employing Fmoc/HCTU-based chemistry. Fmoc-Met-Wang resin (0.2 mmol) was placed in the reaction vessel and swelled in 10 mL DMF for 10 min 3x then deprotected twice using 10 mL of 20% piperidine in DMF for 5 min each time. Amino acid couplings were performed by adding 3 mL of a 333 mM amino acid (4.0 equiv) solution in DMF, 3 mL of a 333 mM HCTU (4.0 equiv) solution in DMF, and 3 mL of a 1.2 M DIPEA (12.0 equiv) solution in DMF to the reaction vessel. Standard incubation time for each coupling was 20 min. After each coupling, the peptide was capped using 3 mL of Ac_2O and 3mL of 1.2 M DIPEA, dissolved in DMF with an incubation time of 15 min.

Manual couplings of Fmoc-Peg_4-COOH and 5-carboxyfluorescein were performed in a polypropylene filter syringe equipped with a polyethylene/nylon stopcock. Four equiv of Fmoc-Peg_4-COOH was activated with 4 equiv of HCTU and HOBt-Cl, 12 equiv of DIPEA in 5 mL of DMF for 5 min before coupling to resin for 2 h. Reaction completion was tested every h using a ninhydrin test. After 2 h, the coupling was complete, and Fmoc was deprotected with 2 x 5 mL 20% piperidine in DMF for 5 min each time before completing the 5-carboxyfluorescein coupling. Six equiv of 5-carboxyfluorescein was activated with 6 equiv of PyAOP and 12 equiv of DIPEA in 4 mL of a 1:1 solution (v/v) of DMF and NMP (N-methylpyrrolidone) for 10 min before coupling with the resin-bound peptide overnight. The resin was

washed with 5 mL of CH_2Cl_2 3x. Global deprotection and resin cleavage was accomplished via treatment with 10 mL of reagent K (82.5% TFA, 5% phenol, 5% water, 5% thioanisole, and 2.5% ethanedithiol) for 2 h. Cleaved peptides were precipitated with 50 mL Et_2O and centrifuged before decanting the Et_2O layer Then, the peptide pellet was re-dissolved in 2 mL TFA and precipitated in 50 mL Et_2O followed by centrifugation (2x). The resulting crude peptide was dried via a stream of dry N_2, and then dissolved in 8 mL of a mixture of H_2O/CH_3CN (7:3, v/v) containing 0.1% TFA. The solution was filtered using a 0.2 μm PTFE filter and then purified using preparative reverse-phase (RP)-HPLC using a 30 min gradient from 0% B to 100% B. HPLC fractions were pooled and lyophilized, then dissolved in 8 mL of 500 mM Tris-HCl, pH 7.5 and the concentration was quantified by taking 20 uL of the peptide stock and diluting it in 280 uL of 20 mM Tris buffer, pH 9.0. The absorbance of the solution was used to determine a stock concentration of 330 uM using the ε_{492} (79,000 $M^{-1}cm^{-1}$, pH 9.0) value for 5-carboxyfluorescein.

MS (ESI) m/z: $[M+H]^{2+}$ Calcd for $C_{109}H_{182}N_{25}O_{29}S_3$ 1193.6; Found 1194.1; $[M+3H]^{3+}$ Calcd for $C_{109}H_{182}N_{25}O_{29}S_3$ 796.1; Found 796.4; $[M+3H]^{3+}$ Calcd for $C_{109}H_{183}N_{25}O_{29}S_3$ 597.5; Found 597.6.

Substrate studies with 1 and 2 monitored by fluorescence

To study whether **1** and 2 were alternative substrate of rPFTase and rPGGTase, the rates of reaction were tested using a fluorescence-based enzyme assay. First, dithiothreitol (DTT, 20 μL of 0.5 M stock solution) and Dns-GCVLS (7.2 μL of 668 μM stock solution) or Dns-GCVLL (1.7 μL of 2.8 mM stock solution) were mixed by rotation at rt for 1 h to reduce any disulfide bonds. Then this mixture was added to the other buffer components to yield solutions with the following final concentrations: Peptide (2.4 μM), DTT (5 mM), Tris•HCl, pH 7.5 (50 mM), $MgCl_2$ (10 mM), $ZnCl_2$ (10 mM) and DDM (0.04% (w/v)) For reactions containing Dns-GCVLL, KCl (20 mM, final concn) was also included. The total volume for each reaction was 500 μL. The fluorimeter was zeroed on buffer and after addition of 2 or 10 μM (final concn) of FPP, GGPP, **1** or **2**. The fluorescence (λ_{ex}=340 nm, λ_{em}=505 nm) was monitored for approximately 2 min and then 0.5 μM of rFTase or rGGTase-I (final concn) was added to initiate the reaction and the fluorescence was measured for 30 min.

Steady-state kinetic characterization of 1 and 2

The reactivity of analogues **1** and **2** were determined with rFTase and rGGTase-I using dansylated peptide Dns-GCVLS and Dns-GCVLL, respectively, in a previously reported fluorescence-based prenylation assay in 96-well black low adhesion plates.[7] Briefly, reactions containing 20 nM enzyme were incubated with diazirine analogues (0.3 - 10 μM) in 1X reaction buffer (50 mM HEPPSO-NaOH pH 7.8, 5 mM TCEP, and 5 mM $MgCl_2$ in FTase reactions) for twenty minutes. Reactions were initiated by addition of 3 μM peptide substrate that was preincubated in 1X reaction buffer at rt in the dark for 20

min. Fluorescence was measured using a BioTek H1 Synergy plate reader (λ_{ex}= 340nm and λ_{em}= 520nm) over time courses from 30 min to 3 h as appropriate. The fluorescence signal was converted to concentration (Fl/ μM product) using a correction factor determined by total change in fluorescence upon reaction completion. Initial velocities (μM /sec) were determined in the initial linear range of each reaction, and initial velocities were divided by enzyme concentration and plotted against analogue concentration to generate V/E vs S plots. These data were fitted to a Michaelis-Menten model to determine k_{cat} and K_M values with KaleidaGraph (Synergy Software, Reading, PA) used for curve fitting.

LC-MS and MS-MS analysis of enzymatic reactions between Dns-GCVLS or Dns-GCVLL and 1 or 2 using rFTase or rGGTase-I.

First, dithiothreitol (DTT, 200 μL of 0.5 M stock solution) and Dns-GCVLS (72 μL of 668 μM stock solution) or Dns-GCVLL (17 μL of 2.8 mM stock solution) were mixed by rotation at rt for 1 h to reduce any disulfide bonds. Then this mixture was added to the other buffer components to yield solutions with the following final concentrations: Peptide (2.4 μM), DTT (5 mM), Tris•HCl, pH 7.5 (50 mM), $MgCl_2$ (10 mM), $ZnCl_2$ (10 mM) and DDM (0.04% (w/v)) For reactions containing Dns-GCVLL, KCl (20 mM, final concn) was also included. The total volume of the reactions was 5 mL. To the Dns–GCVLS-containing reaction, **1** (10 μM final concn) and rFTase (0.5 μM final concn) were added and to the Dns–GCVLL-containing reaction, **1** or **2** (10 μM final concn) and rGGTase-I (0.5 μM final concn) were added. The reactions were equilibrated to 37°C and allowed to react for approximately 1 h. After this time, the reaction was frozen using liquid N_2 to stop the reaction, thawed and applied onto a Waters Sep-Pak Plus reverse-phase C18 Environmental Cartridge. Before sample loading, the cartridge was washed with CH_3CN + 0.1% TFA (5 mL), and H_2O + 0.1% TFA (5 mL). Then the reaction mixture was applied, washed with H_2O + 0.1% TFA (5 mL) and the product was eluted with CH_3CN + 0.1% TFA (3 mL). The green fluorescent product in these fractions was clearly visible using a handheld UV lamp. After this desalting step, the CH_3CN was evaporated, the sample was lyophilized and redissolved in 500 μL H_2O + 0.1% TFA:CH_3CN + 0.1% TFA (7:3, v/v) with one drop of DMF to assist in solubilizing the peptides. This solution was subjected either to LC-MS or CID-Fusion HRAM LC-MS/MS.

Enzymatic modification of 19 with 1 or 2 and subsequent photolysis

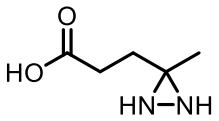
First, dithiothreitol (DTT, 24 μL of 0.5 M stock solution) and 5-Fam-PEG4-KRas-CVIM (17.4 μL of 330 μM stock solution) were mixed by rotation at 0 ^oC overnight to reduce any disulfide bonds. Then this mixture was added to the other buffer compartments (final concn): Peptide (2.4 μM), DTT (5 mM), Tris•HCl, pH 7.5 (50 mM), $MgCl_2$ (10 mM), $ZnCl_2$ (10 mM) and DDM (0.04% (w/v)). For rGGTase-I reactions KCl (20 mM, final concn) was added. The total volume of each solution for each enzyme was 0.8 mL. Each of these solutions was prepared in triplicate (3 x 800 μL [rFTase], 3 x 800 μL [yFTase] or 3 x 800 μL [rGGTase-I]). To each of the above Eppendorf tubes, 0.5 μM rFTase, yFTase or hGGTas-I

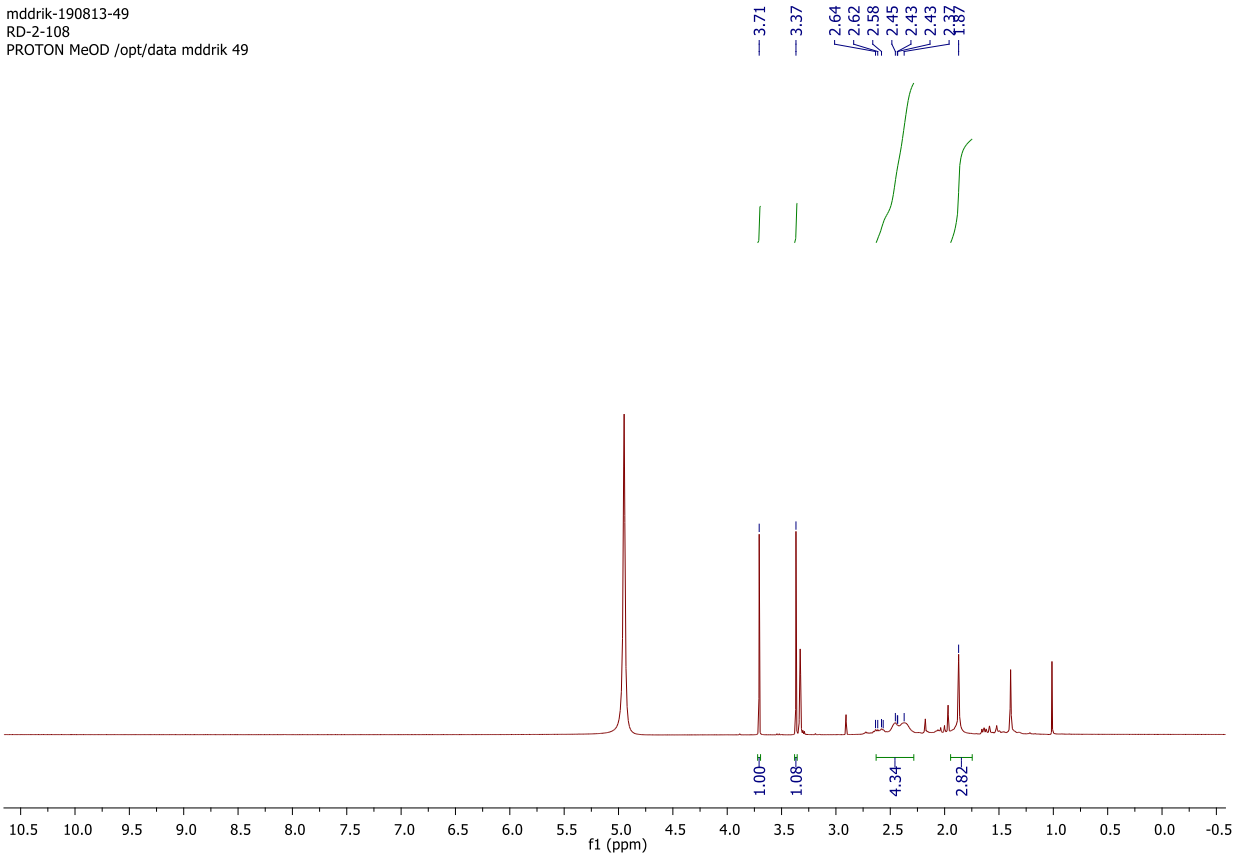
was added respectively. Each 800 µL replicate was divided into four reactions (200 µL each). Reaction 1 contained no diazirine probe, while reactions 2-4 contained **1** (2.4 µM for rFTase and yFTase) or **2** (2.4 µM for rGGTase-I). The reactions were equilibrated to 37°C and allowed to react for about 2 h to generate the prenylated peptide *in situ*. After this time, peptide **16** (competitor) was added where appropriate (Reaction 4) and samples were the irradiated for 5 min (except for control experiments where no irradiation was performed. Protein precipitation was then performed using a ProteoExtract® Protein Precipitation Kit (VWR 53-918-01KIT). Protein pellets were then dissolved in 1X Laemmli SDS PAGE loading buffer (60 µL) and 20 µL samples were resolved on a 12% SDS-PAGE gel. Detection for FAM fluorescence was performed using a Typhoon FLA 9500 (GE Healthcare) followed by staining with Coomassie blue. Gel images were processed in ImageJ.

Metabolic labeling and UV crosslinking studies

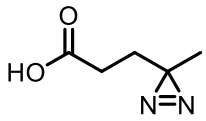
Human HEK293T cells were obtained from the American Type Culture Collection. The HEK293T cells were plated in duplicate 24-well plates at a concentration of 1.2 x 10^5 cells/well in Dulbecco's modified Eagle's medium (DMEM) containing 10% heat-inactivated fetal bovine serum and antibiotics. The cells in the duplicate plates were cultured overnight at 37°C, 5% CO_2 and then transfected with cDNA constructs using Lipofectamine 2000 (Life Technologies) according to the manufacturer's protocol. The cDNA constructs consisted of the pcDNA3.1 vector encoding either human RhoA with an N-terminal myc epitope tag (myc-RhoA), human HRas with an N-terminal myc epitope (myc-HRas), or only the myc epitope (myc vector). Within 90 minutes after transfection of these cDNAs, the media in each well was supplemented with either 10 µM C10DesOPP (**1**), 10 µM C15DesOPP (**2**), or vehicle alone. After culturing the cells in the duplicate plates overnight, the cells in one of the plates were exposed to UV irradiation by putting the plate on ice in a Boekel Scientific UV Crosslinker (Model #234100, with 368 nm UV light bulbs), and irradiating the plate at a distance of 3 cm for 40 min. The cells in the other 24-well plate were protected from UV irradiation by wrapping the plate in tinfoil and keeping it on ice for 40 min. The cells in both plates were then lysed in SDS lysis buffer and the cell lysates were subjected to SDS-PAGE and immunoblotting using myc antibody (Protein Tech catalog number 60003-2-Ig). Bound primary antibodies were visualized using horseradish peroxidase-linked secondary antibodies (GE Healthcare), and immunoblot images were obtained using an ImageQuant LAS4000 Biomolecular Imager, as previously described.[8]

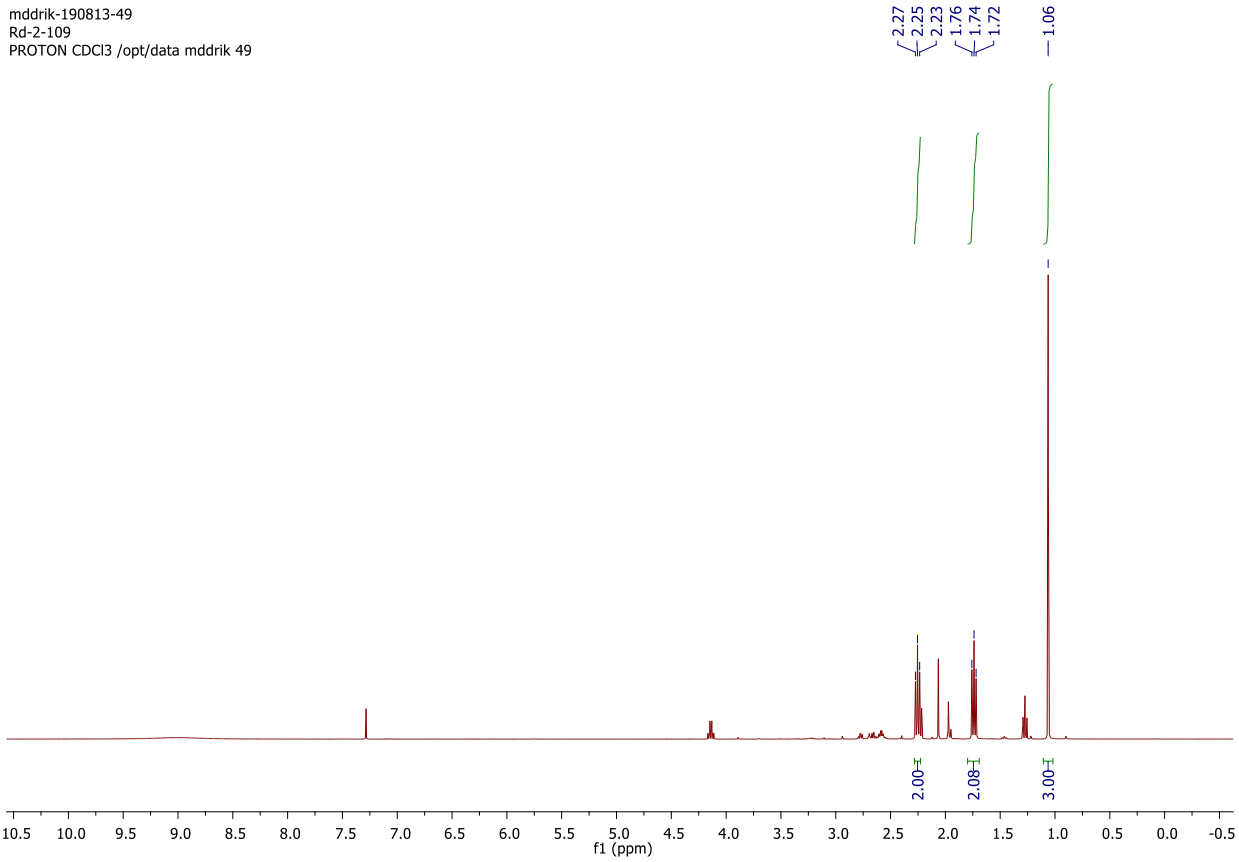
^{1}H NMR (400 MHz, $CDCl_3$) for compound 4 (Known compound)



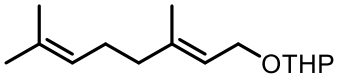


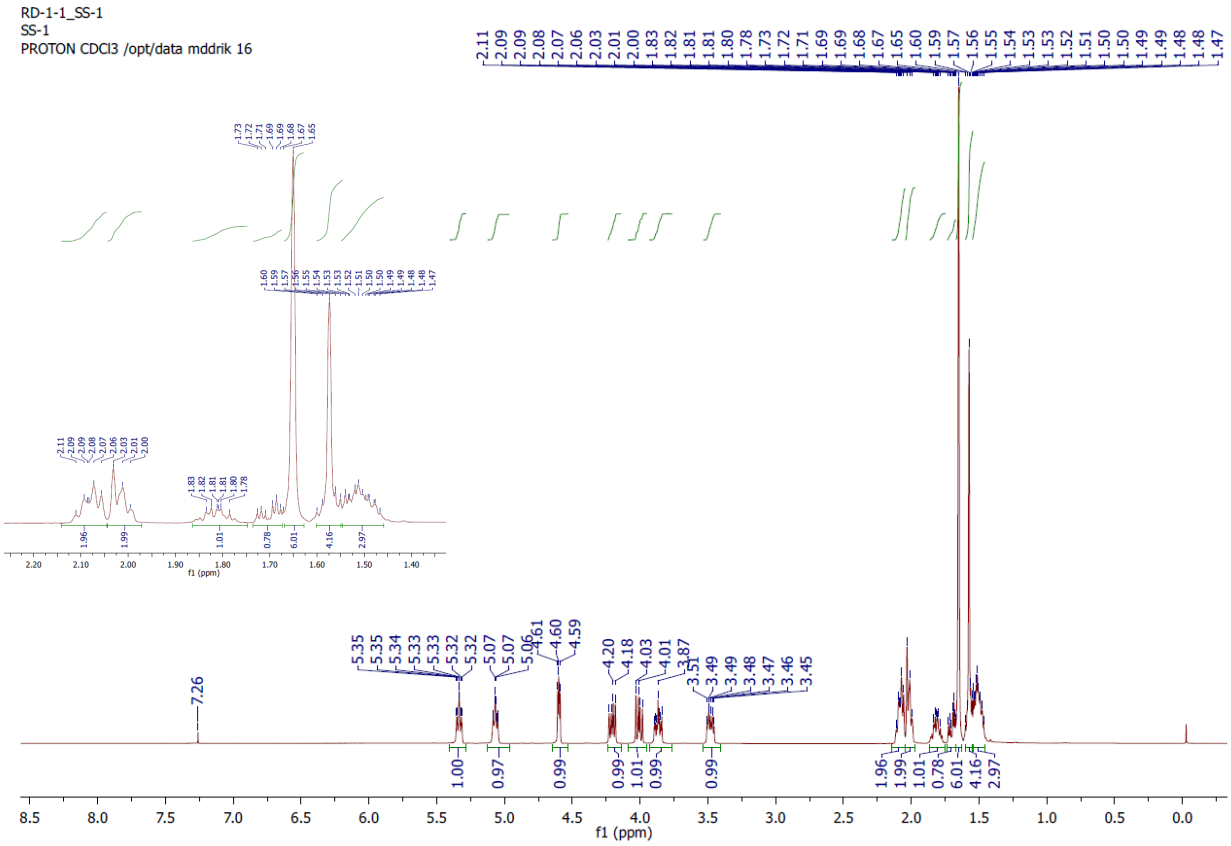
^{1}H NMR (400 MHz, $CDCl_3$) for compound 5 (Known compound)



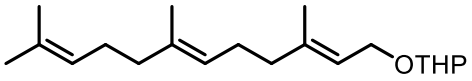


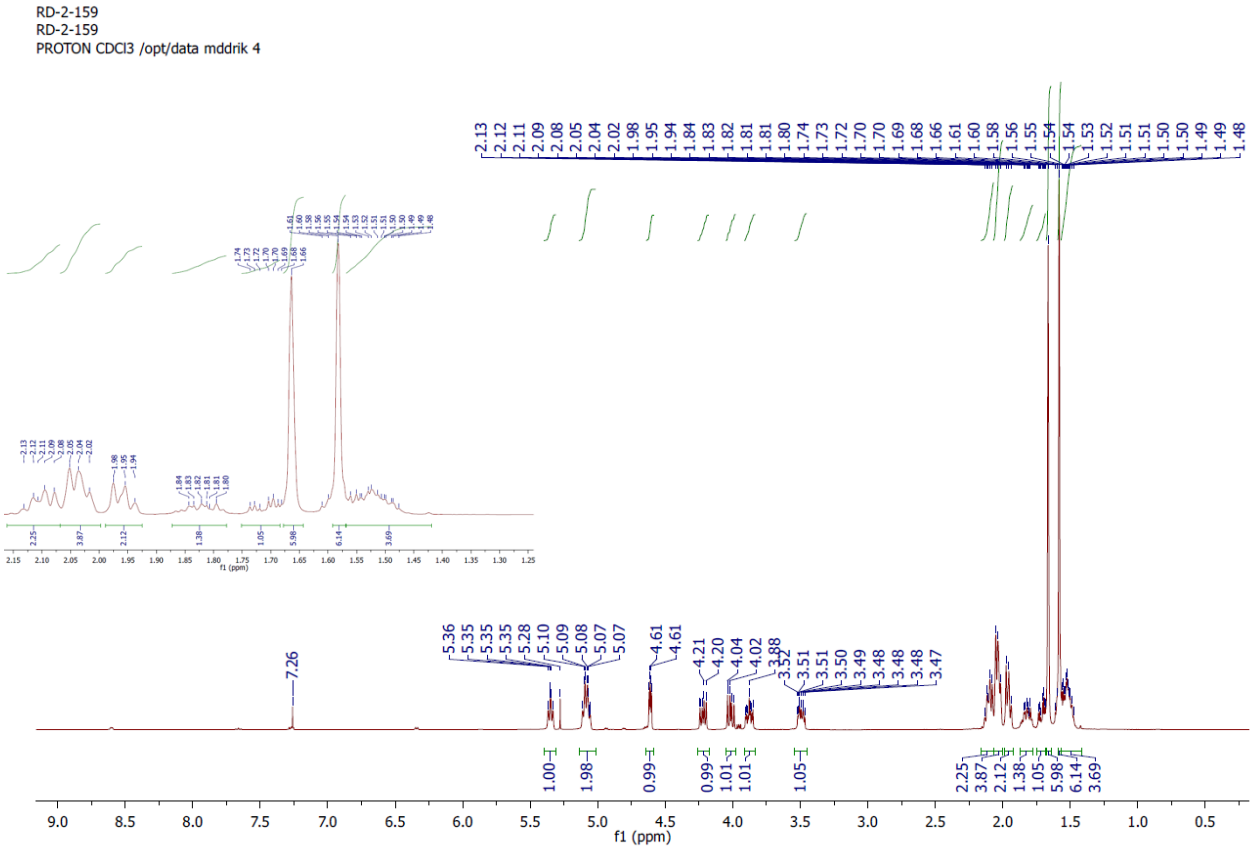
^{1}H NMR (400 MHz, $CDCl_3$) for compound 7a (Known compound)



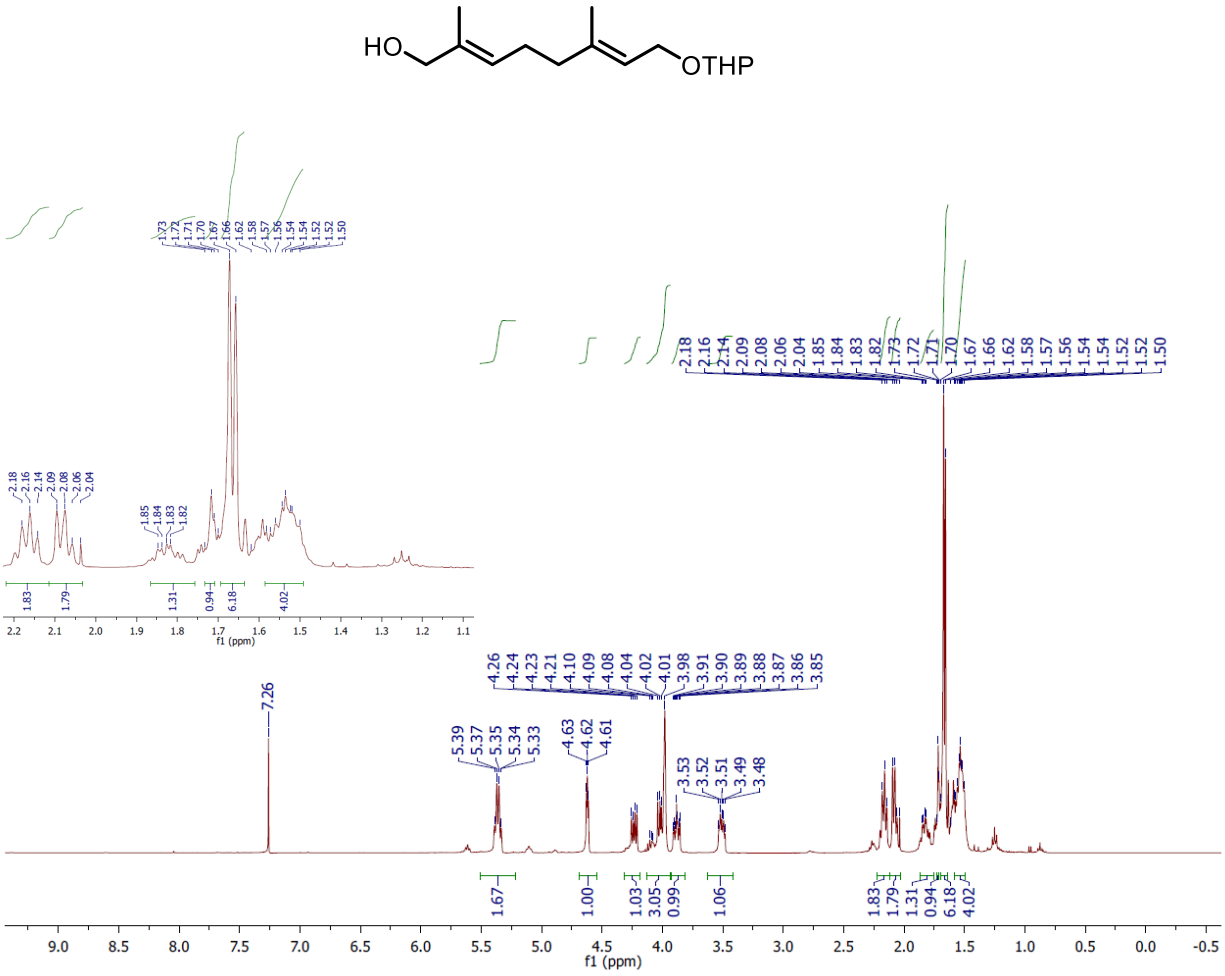


^{1}H NMR (400 MHz, $CDCl_3$) for compound 7b (Known compound)

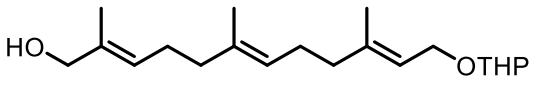


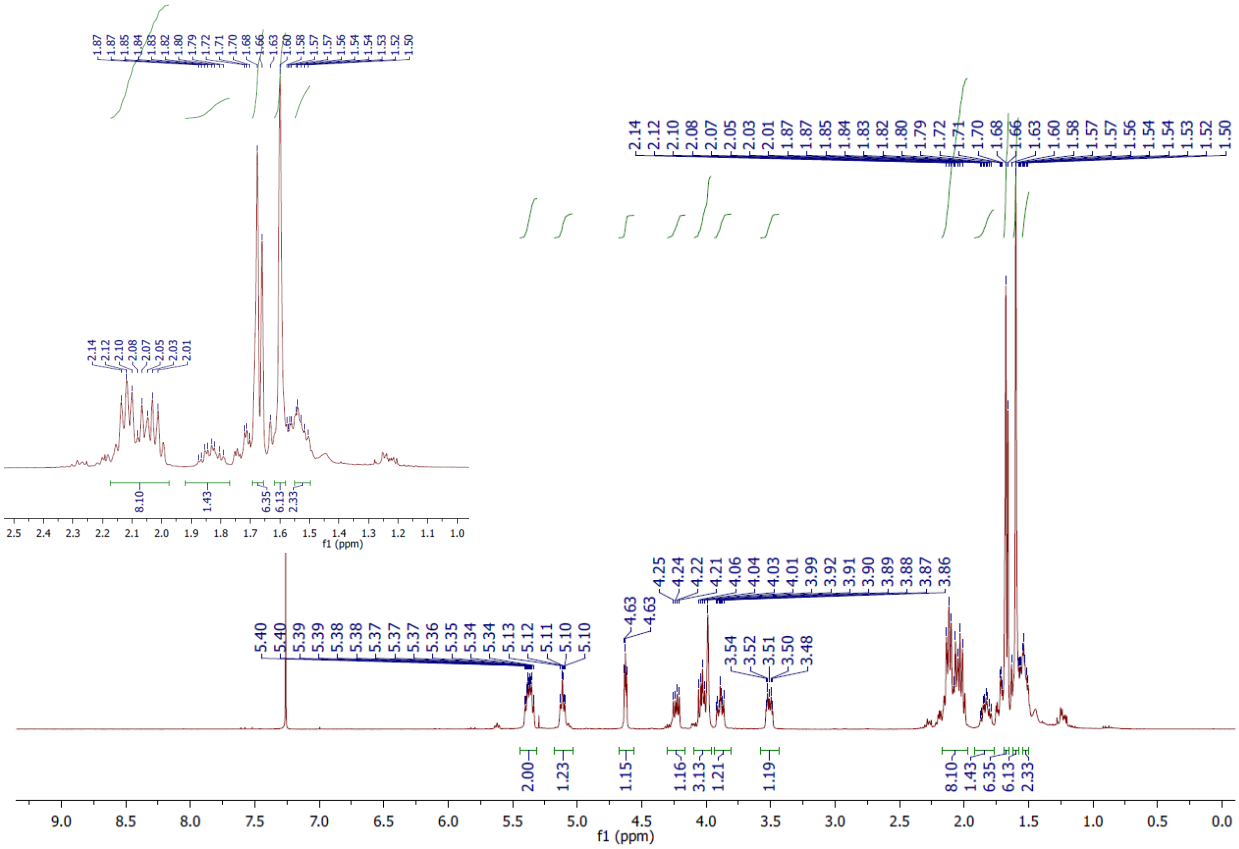


^{1}H NMR (400 MHz, $CDCl_3$) for compound 8a (Known compound)

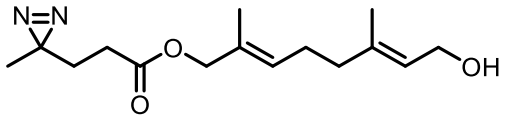


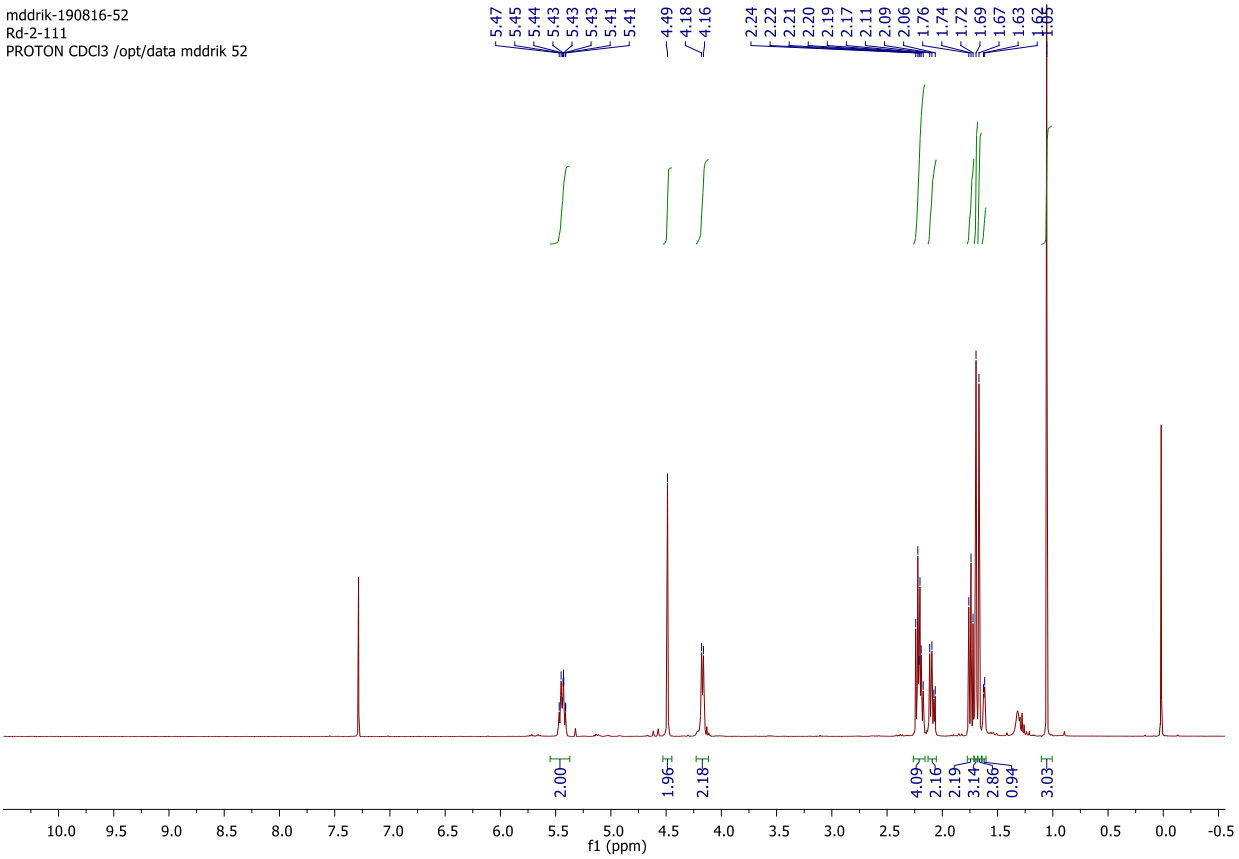
^{1}H NMR (400 MHz, $CDCl_3$) for compound 8b (Known compound)



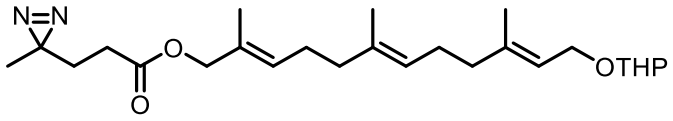


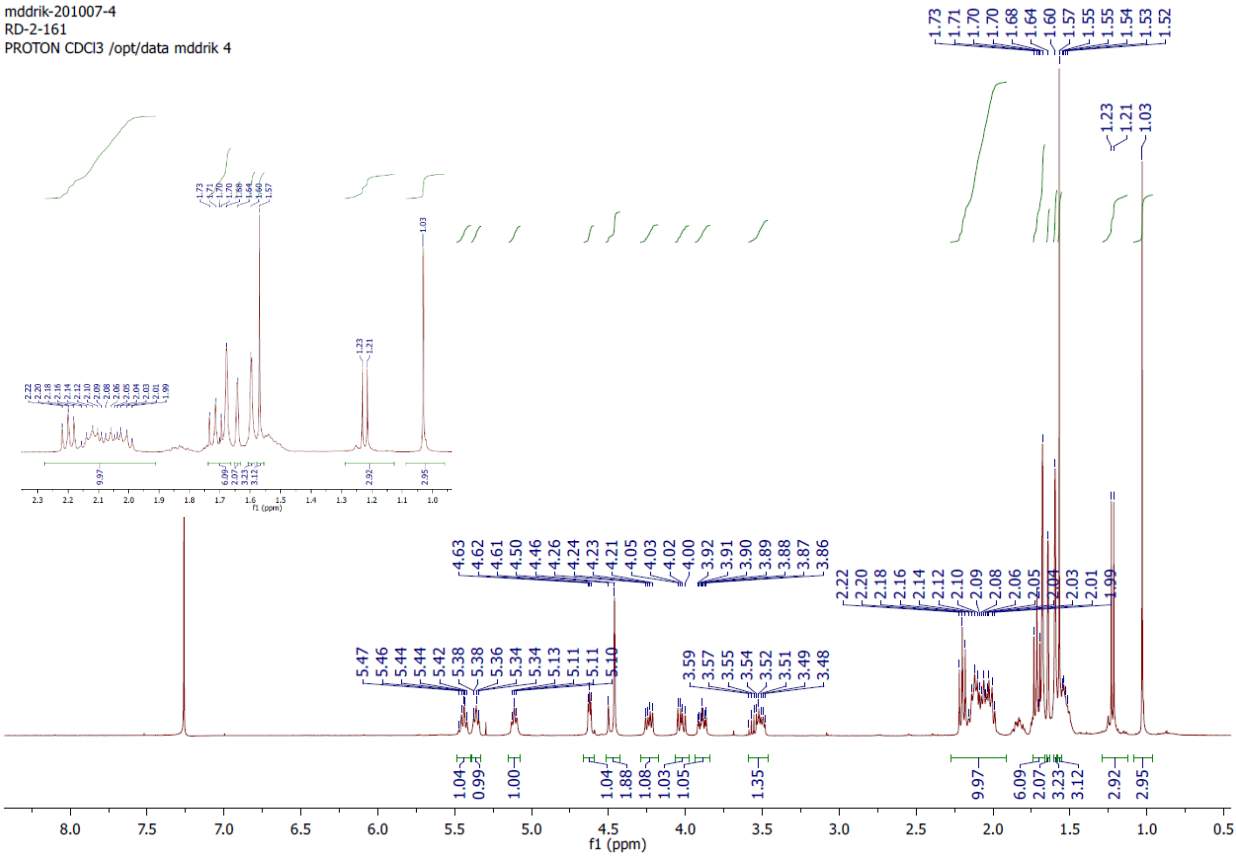
^{1}H NMR (400 MHz, $CDCl_3$) for compound 9a (Known compound)



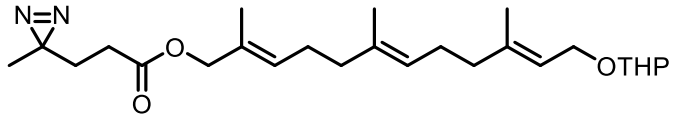


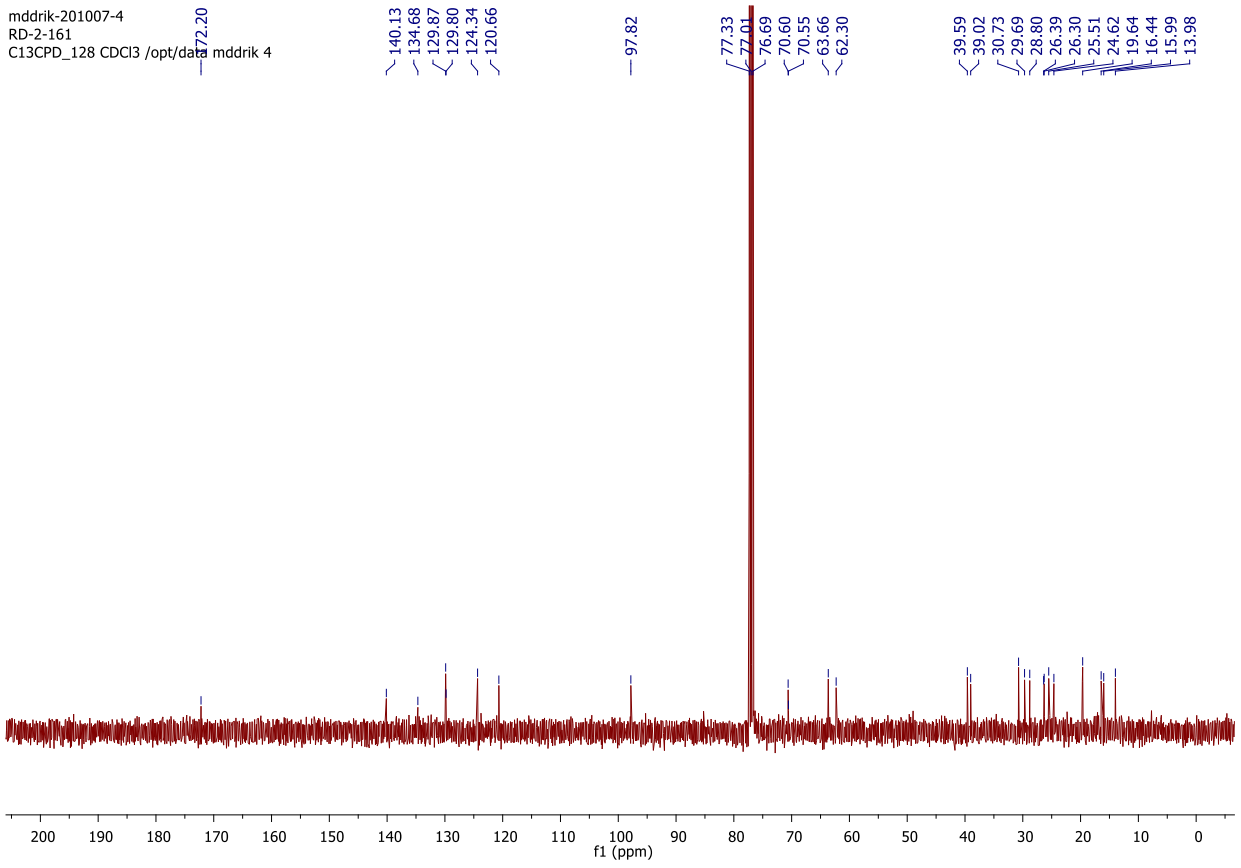
^{1}H NMR (400 MHz, $CDCl_3$) for compound 9b (New compound)



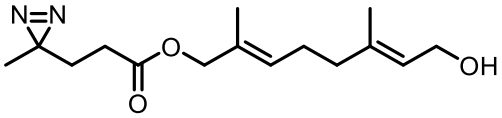


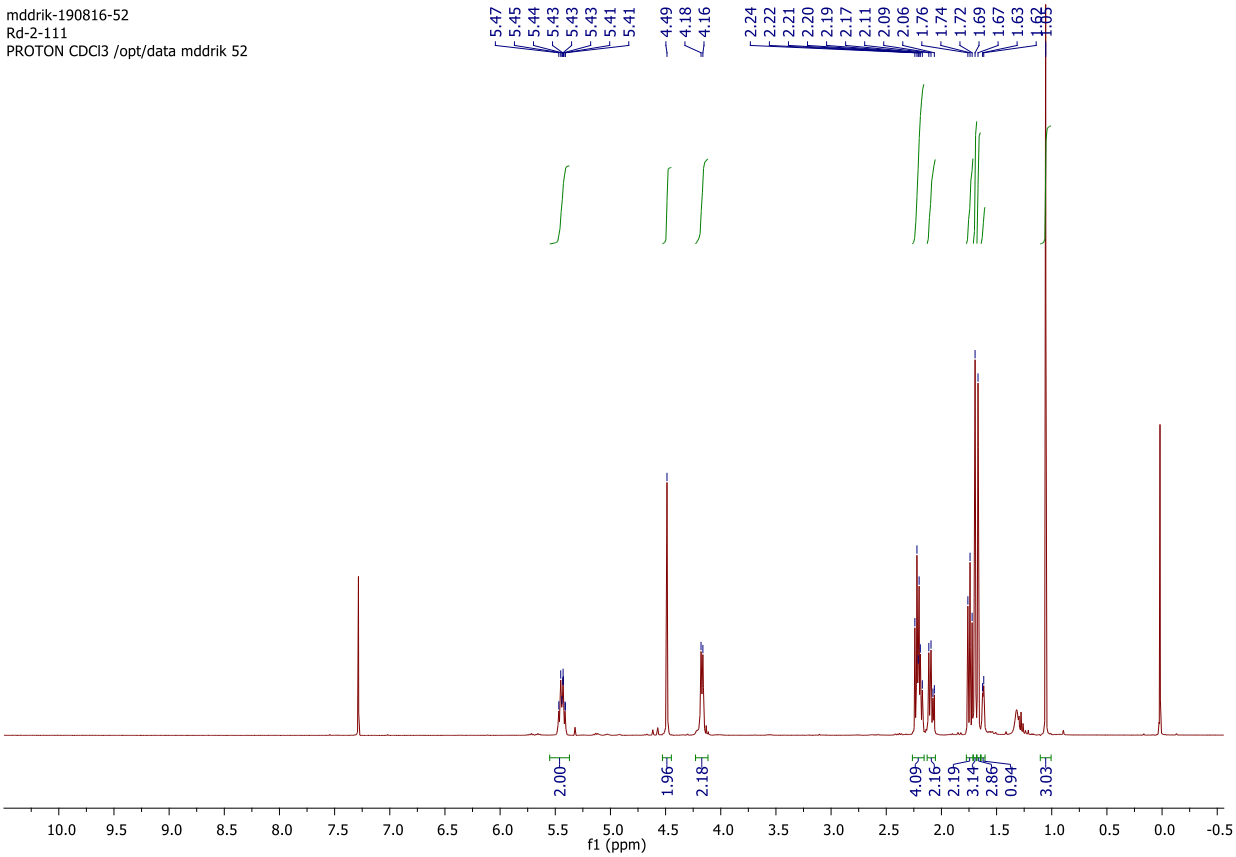
^{13}C NMR (100 MHz, $CDCl_3$) for compound 9b (New compound)



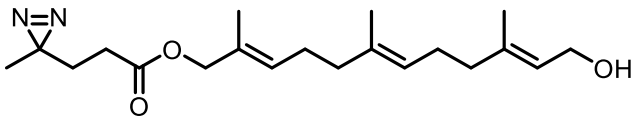


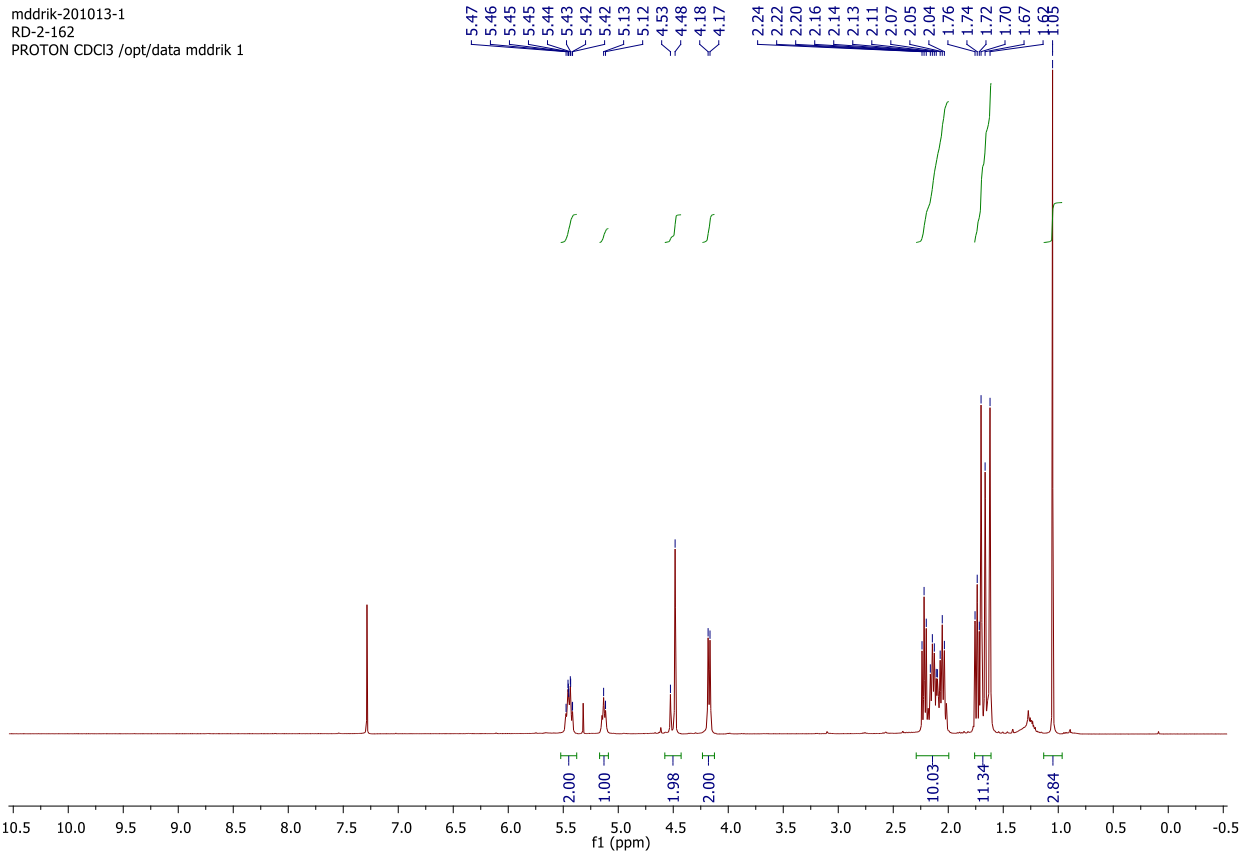
^{1}H NMR (400 MHz, $CDCl_3$) for compound 10a (Known compound)



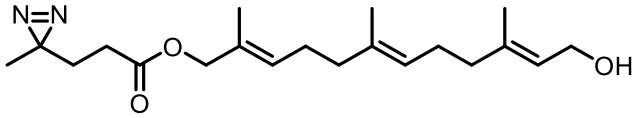


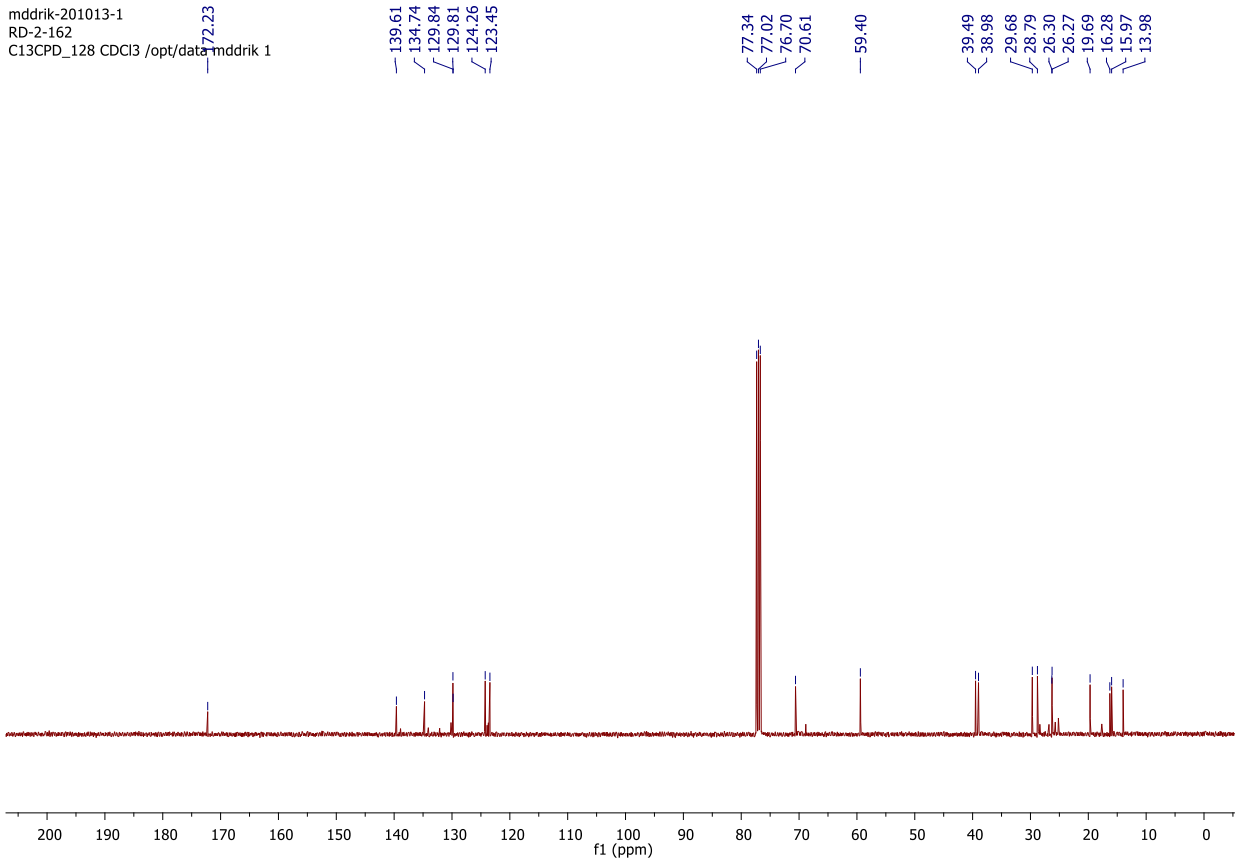
^{1}H NMR (400 MHz, $CDCl_3$) for compound 10b (New compound)



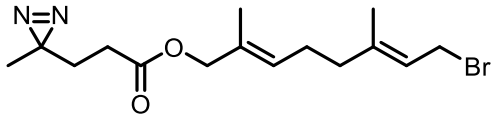


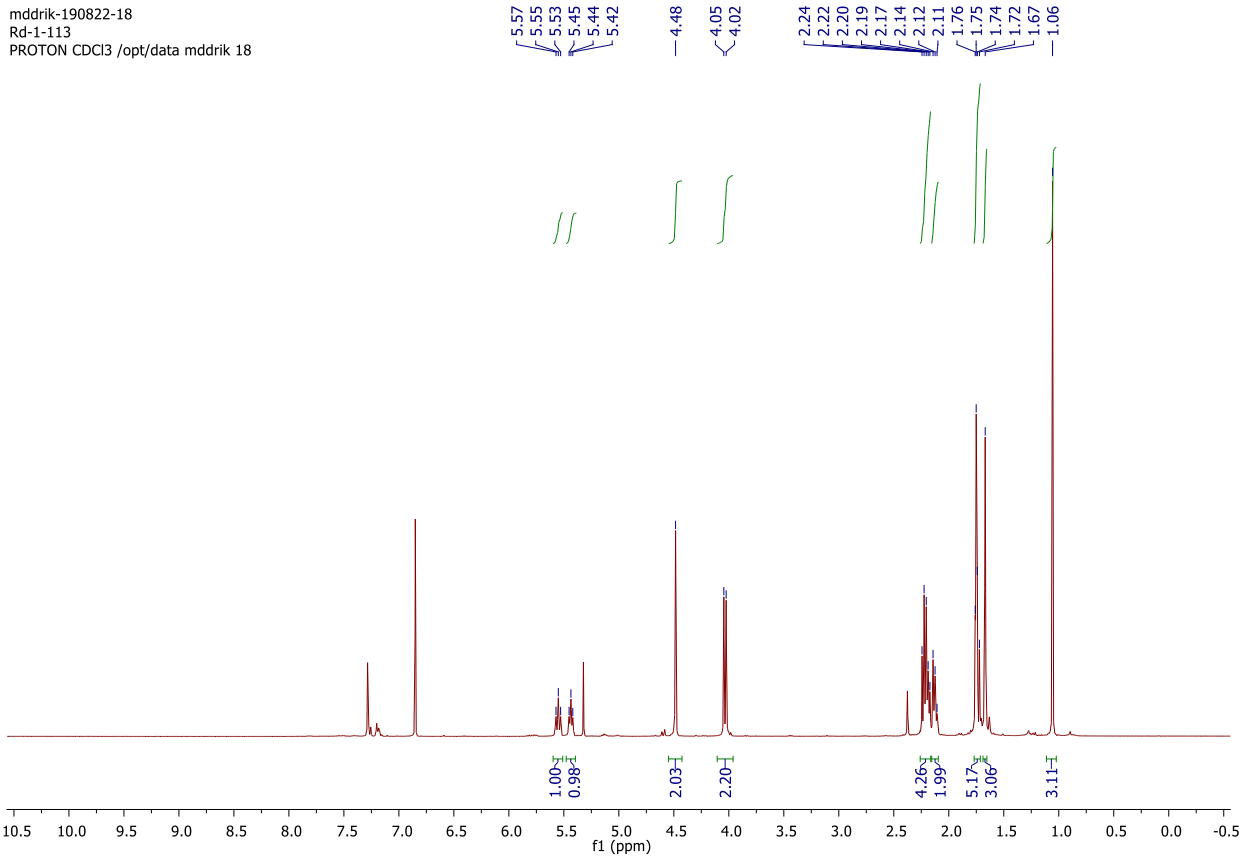
^{13}C NMR (100 MHz, $CDCl_3$) for compound 10b (New compound)



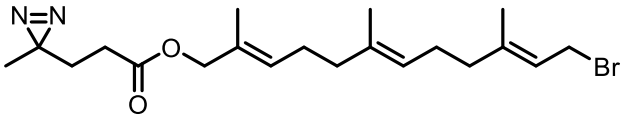


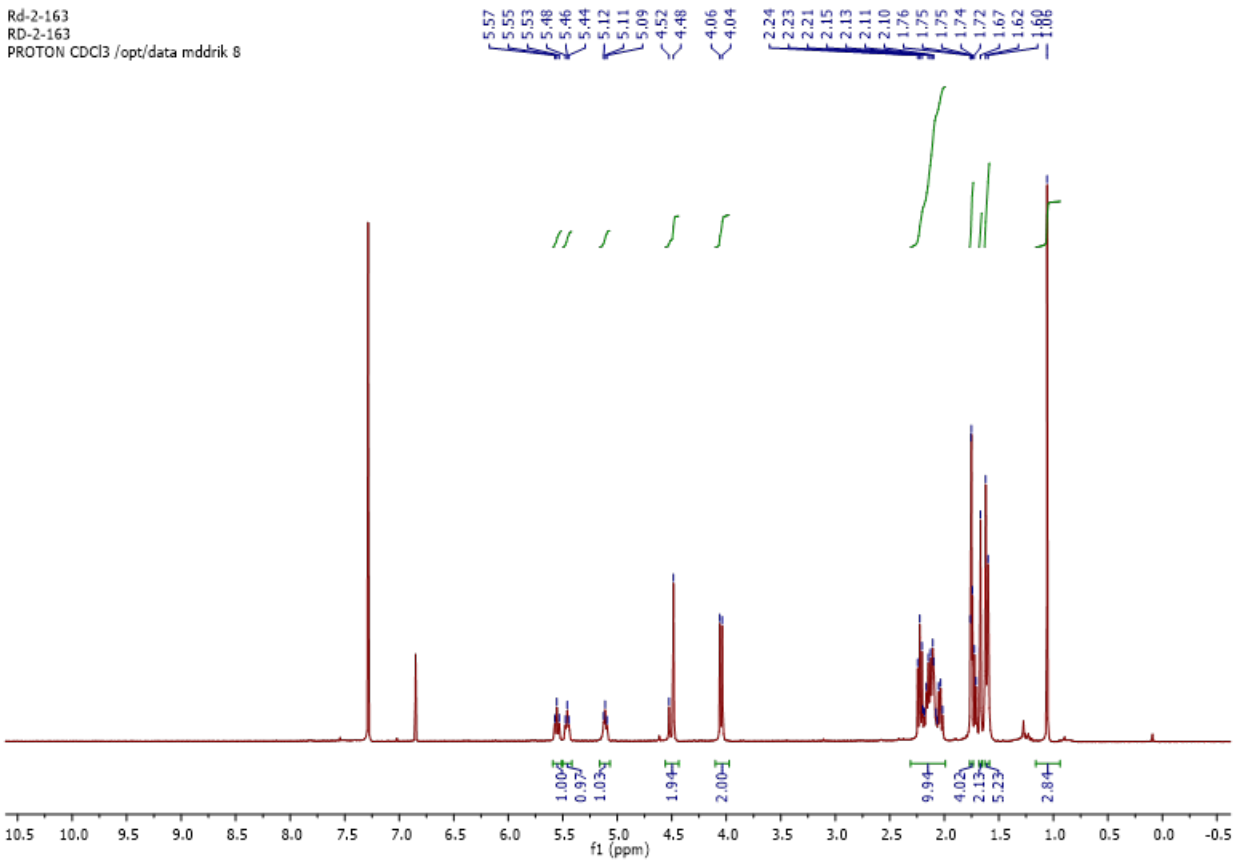
^{1}H NMR (400 MHz, $CDCl_3$) for intermediate 11a (Unstable known compound)



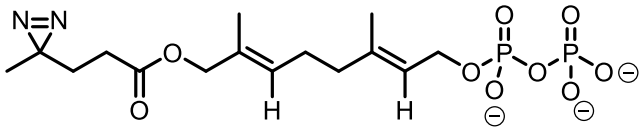


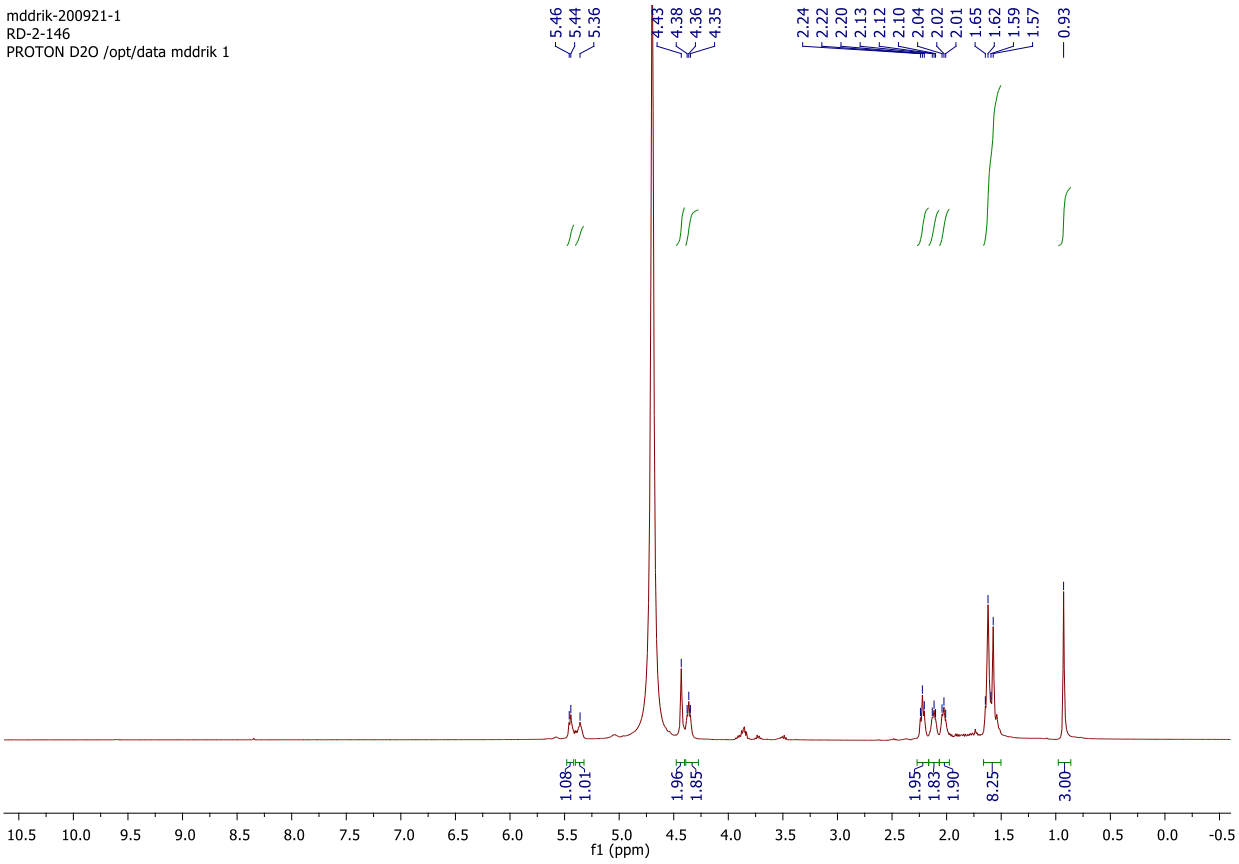
^{1}H NMR (400 MHz, $CDCl_3$) for intermediate 11b (New compound)



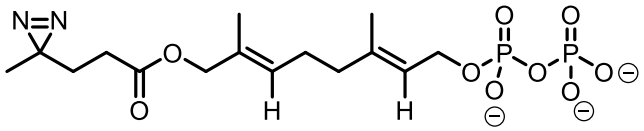


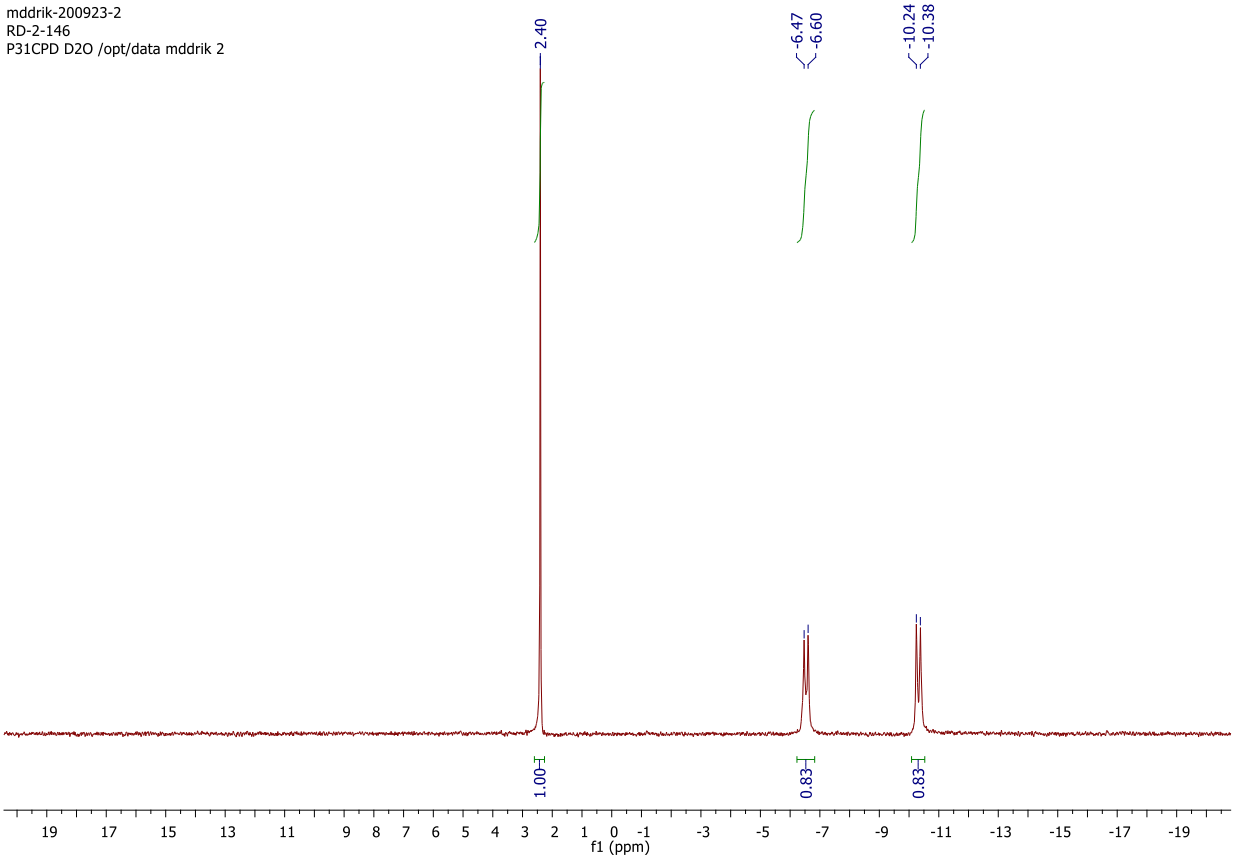
^{1}H NMR (400 MHz, D_2O) for compound 1 (New compound)



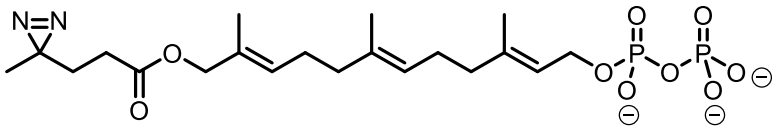


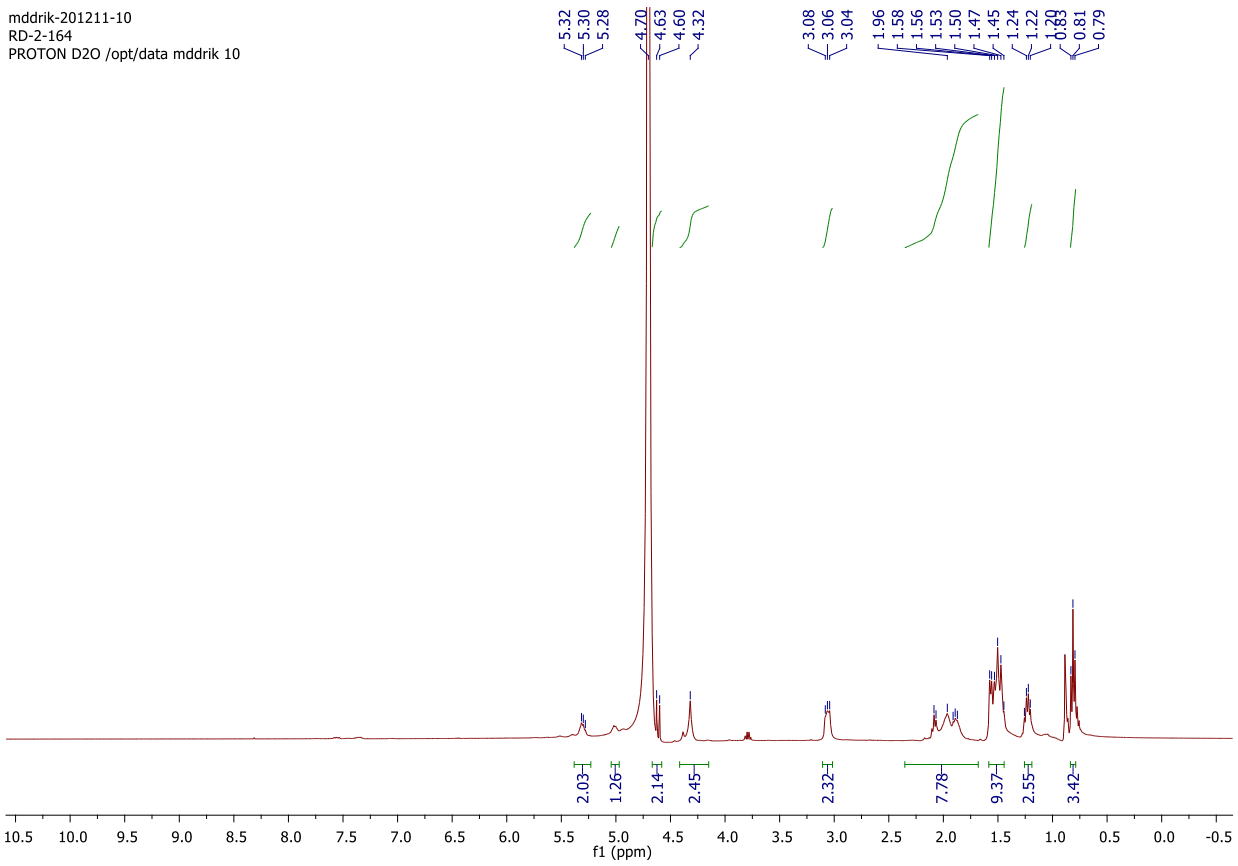
^{31}P NMR (162 MHz, D_2O) for compound 1 (New compound)



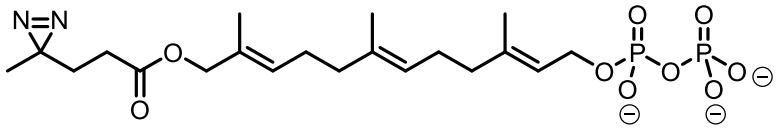


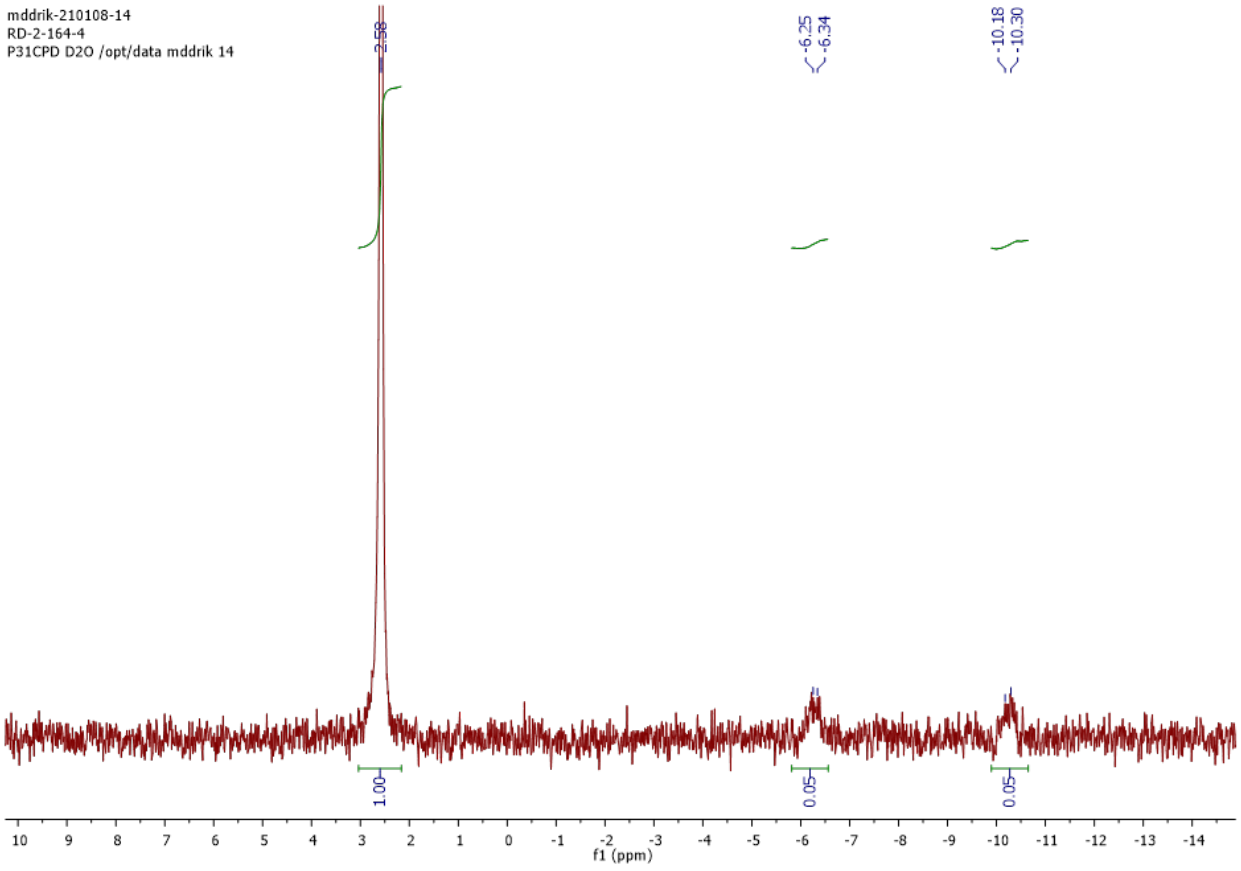
^{1}H NMR (400 MHz, D_2O) for compound 2 (New compound)





^{31}P NMR (162 MHz, D_2O) for compound 2 (New compound)





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