Supporting Information

Selective Labeling of Polypeptides Using Protein Farnesyltransferase via Rapid Oxime Ligation

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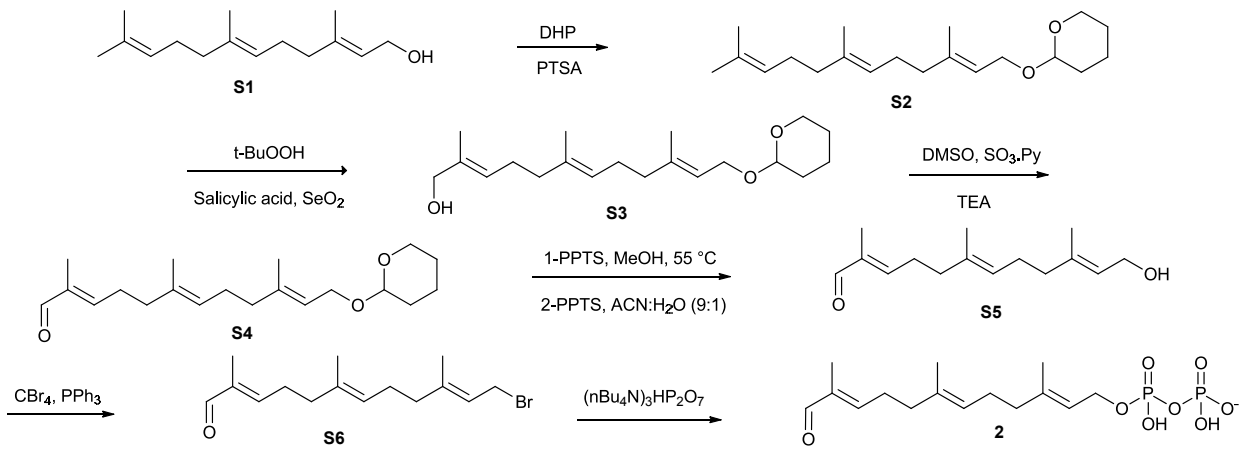
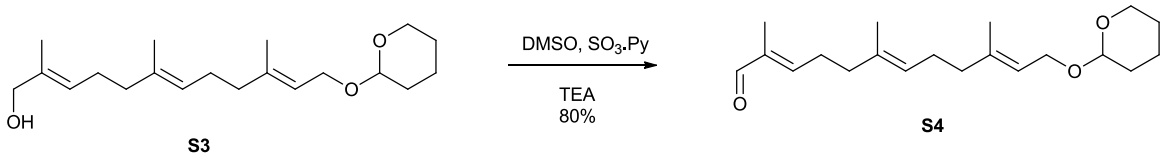
General: All synthetic reactions were carried out at 25 °C and stirred magnetically unless otherwise noted. TLC was performed on precoated (250 mm) silica gel 60 F-254 plates (Merck). Plates were visualized by staining with $KMnO_4$ or hand-held UV lamp. Flash chromatography silica gel (60–200 mesh, 75–250 μm) was obtained from Mallinckrodt Inc. CH_2Cl_2, CH_3CN, and THF were dried by using a Mbraun solvent purification system. Deuterated NMR solvents were purchased from Cambridge Isotope Laboratories, Inc. ^1H NMR spectra were obtained at 300 or 500 MHz; ^{13}C NMR spectra were obtained at 125 MHz; ^{31}P NMR spectra were obtained at 121 MHz. All NMR spectra were acquired on Varian instruments at 25°C. Chemical shifts are reported in ppm and *J* values are in Hz. Fluorescence assay data were obtained by using a Varian Cary Eclipse Fluorescence Spectrophotometer. Analytical HPLC was performed on a Beckman model 125/166 instrument, equipped with a diode array UV detector, ABI Analytical Spectroflow 980 fluorescence detector, and a Varian C_{18} column (Microsorb-MV, 5 μm, 4.6x250 mm). Preparative HPLC separations were performed by using a Beckman model 127/166 instrument, equipped with a UV detector and a Phenomenex C18 column (Luna, 10 μm, 10x250 mm). MS spectra for synthetic reactions were obtained on a Bruker BioTOF II instrument. MS and LC/MS spectra of modified peptides were obtained with an Applied Biosystems/MDS SCIEX QSTAR® Elite Hybrid LC-MS system. Sep-Pak cartridges were purchased from Waters (Milford, MA). Yeast PFTase was prepared as previously described.[1]

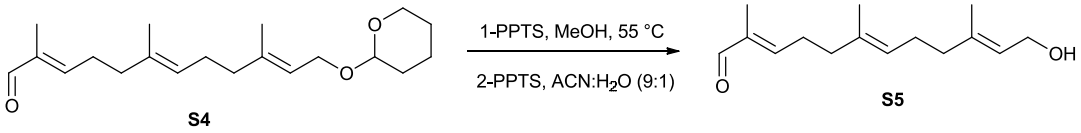


Scheme S1. Syntheses of Farnesyl Aldehyde Pyrophosphate (FAPP, **2**).



(2E,6E,10E)-2,6,10-trimethyl-12-*O*-THP-dodeca-2,6,10-trienal (S4): Compound **S2** and **S3** were prepared as previously described.[2] Alcohol **S3** (1.00 g, 3.94 mmol) was dissolved in 20 mL anhydrous CH_2Cl_2 in a 50 mL flask and the solution was cooled to 0 °C in ice bath. DMSO (3.1 mL, 43 mmol) was added dropwise to the solution mixture followed by addition of 2.75 mL triethylamine (197 mmol). Sulfur trioxide pyridine complex (SO_3·Py, 2.52 g, 15.75 mmol) was added slowly over 10 min to the reaction mixture. The reaction was stirred at 0 °C for an additional h where TLC analysis showed almost complete conversion to the product. The reaction was stopped by addition of 100 mL CH_2Cl_2 and washed with 5 M HCl (2 x 10mL) until the aqueous phase remained acidic (via pH paper) which shows there is no more base left the in reaction mixture. Next solution was washed with 15 mL $NaHCO_3$ followed by 2 x 10 mL brine. The solution was dried over Na_2SO_4 and solvent was removed *in vacuo*. Compound **S4** was

purified by silica gel column chromatography using a step gradient of solvent (Hexane:EtOAc) starting from 1:0 (v/v) going to 3:1 (v/v) to afford 0.5 g of compound **S4** as a pale yellow oil (50% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.55 (m, 5H), 1.63 (s, 3H), 1.67 (s, 3H), 1.74 (s, 3H), 1.82 (m, 1H), 2.0-2.25 (m, 6H), 2.46 (m, 2H), 3.51 (m, 1H), 3.89 (m, 1H), 4.02 (dd, J = 7.3 Hz, J = 12 Hz, 1H), 4.24 (dd, J = 6.3 Hz, J = 12 Hz, 1H), 4.62 (dd, J = 3.0 Hz, J = 4.5 Hz, 1H), 5.16 (t, J = 6.2 Hz, 3 H), 5.36 (dd, J) 6.3 Hz, J = 7.3 Hz, 1H), 6.46 (t, J) 7.2 Hz, 1H), 9.37 (s, 1H). ^{13}C NMR (75 MHz, CDCl3) δ 195.38, 154.53, 139.95, 133.71, 125.33, 121.75, 120.86, 97.91, 63.70, 62.35, 39.51, 38.01, 30.77, 27.45, 26.25, 25.55, 19.69, 16.47, 15.97, 9.28. HR-ESI-MS calcd for $C_{20}H_{32}O_3Na$ $[M+Na]^+$ 343.2249, found 343.2246.

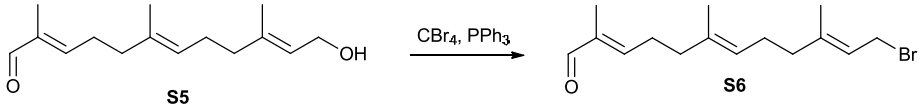


S4 S5

(2E,6E,10E)-12-hydroxy-2,6,10-trimethyldodeca-2,6,10-trienal (S5): Protected aldehyde **S4** (0.66 g, 2.6 mmol) was dissolved in 15 mL CH_3OH in a 25 mL flask. PPTS (40 mg) was added as catalyst. The reaction was then refluxed at 55 °C for 3 h. It was then quenched by adding 10 mL sat. $NaHCO_3$ and 100 mL EtOAc. The organic layer was separated and dried over Na_2SO_4. Early ^{1}H NMR analysis revealed deprotection of THP group in high yield but also protection of the aldehyde to a acetal with two methoxy groups as was expected. The product was dissolved in 15 mL of CH_3CN and 1.5 mL of H_2O. PPTS (25 mg) was then added and the reaction was stirred overnight at rt. 100 mL EtOAc was added to the reaction which was then washed with 10 mL sat. $NaHCO_3$ followed by 2 x 10 mL brine. The organic phase was collected and dried over Na_2SO_4. Solvent was evaporated *in vacuo* and the product was further purified by silica gel column chromatography with gradient elution (hexane:EtOAc) from 10:1 (v/v) going to 2:1 (v/v) to

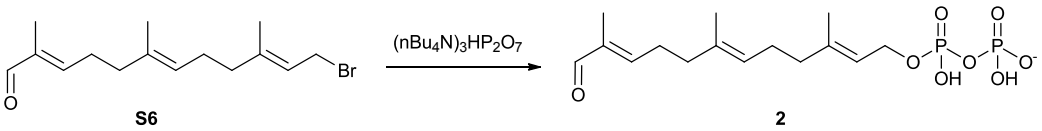
afford 0.22 g of compound **S5** as a pale yellow oil (38% yield). It should be mentioned that aldehyde deprotection was only 50% achieved when THF was used instead of CH_3CN as solvent.

^{1}H NMR (500 MHz, CDCl3) δ 1.64 (s, 3H), 1.68 (s, 3H), 1.74 (s, 3H), 2.0-2.25 (m, 6H), 2.45 (t, J = 7.5, 2H), 4.143 (t, J = 7 Hz, 1H), 5.16 (t, J = 7 Hz, 3 H), 5.41 (t, J = 4.5), 6.48 (t, J = 7.5 Hz, 1H), 9.37 (s, 1H). ^{13}C-NMR (75 MHz, CDCl3) δ 195.352, 154.618, 139.262, 133.602, 125.160, 123.718, 117.303, 63.569, 39.334, 38.084, 27.336, 26.174, 16.203, 15.851, 9.163. HR-ESI-MS calcd for $C_{15}H_{24}O_2Na$ $[M+Na]^+$ 259.1674, found 259.1669.



(2E,6E,10E)-12-bromo-2,6,10-trimethyldodeca-2,6,10-trienal (S6): PPh_3 (polymer-supported beads, 167 mg, 0.5 mmol) was added to a solution of **S5** (107 mg, 0.454 mmol) in CH_2Cl_2 (15 mL), and the reaction was stirred for 20 min at room temperature to let the beads swell in the solution. CBr_4 (165 mg, 0.5 mmol) was added slowly to the reaction mixture and the reaction was stirred for 15 min when TLC analysis showed most of starting material was converted to product. The reaction mixture was filtered to separate beads, and solvent was evaporated *in vacuo*. The product was further purified by silica gel flash chromatography to yield 120 mg of **S6** as a pale yellow liquid. (86%).

^{1}H NMR (500 MHz, CDCl3) δ 1.628 (s, 3H), 1.723 (s, 3H), 1.738 (s, 3H), 2.0-2.25 (m, 6H), 2.45 (t, J = 7.5, 2H), 4.013 (t, J = 8.5 Hz, 2H), 5.125 (t, J = 7 Hz, 1 H), 5.520 (t, J = 4.5, 1H), 6.462 (t, J = 7.5 Hz, 1H), 9.382 (s, 1H). ^{13}C-NMR (75 MHz, CDCl3) δ 195.288, 154.450, 141.242, 134.066, 124.679, 121.644, 120.763, 39.329, 38.076, 37.940, 27.392, 26.951, 15.995, 15.947, 9.259.



S6 2

(2E,6E,10E)-3,7,11-trimethyl-12-oxododeca-2,6,10-trien-1-yl dihydrogen diphosphate (2):

Compound **S6** (100 mg, 0.317 mmol) and $[(n\text{Bu})_4\text{N})]_3\text{HP}_2\text{O}_7$ (902 mg, 1.0 mmol) were mixed in dry CH_3CN (1 mL) and stirred at rt for 3 h, after which the solvent was removed *in vacuo*. Bio-Rad AG 50W-X8 ion-exchange resin (100–200 mesh, H^+ form) was used to convert the product to its ammonium form. The resin was packed and washed with three column volumes of H_2O/conc. NH_4OH (2:1, v/v) until the elution solvent became basic. The resin was then equilibrated with four volumes of NH_4HCO_3 (25 mm)/*i*PrOH (49:1, *v/v*; solvent A). The crude product was dissolved in 1 mL of solvent A and applied to the column and eluted with additional solvent A (20 mL), lyophilized, and purified by RP-HPLC with a semipreparative column using the following conditions: detection: 214 nm; flow rate: 5.0 mL min^{-1}; 2 mL injection loop; gradient 0–30% solvent B in 30 min, 60–100% in 5 min; solvent A: 25 mm NH_4HCO_3, solvent B: CH_3CN. Compound **2** eluted from 20–25% solvent B. Fractions containing pure **2** were collected and lyophilized to yield 0.7 mg (0.54%) of a white powder.

^{1}H NMR: (500 MHz, D_2O) δ 1.488 (s, 3H), 1.545 (s, 6H), 1.917 (t, J = 7 Hz, 2H), 1.996 (t, J = 7 Hz, 2H), 2.067 (t, J = 7 Hz, 2H), 2.377 (t, J = 7 Hz, 2H), 4.302 (t, J = 6 Hz, 2H), 5.081 (t, J = 6 Hz, 1H), 5.293 (t, J = 7 Hz, 1H), 6.613 (t, J = 7.5 Hz, 1H), 9.108 (s, 1H). ^{31}P NMR: (121 MHz, D_2O) δ -5.971 (d, J = 22.6, 1P), -10.013 (d, J = 22.6, 1P). HR-ESI-MS calcd for $C_{15}H_{26}O_8P_2$ $[M-H]^-$ 395.1025, found 395.0907.

Enzymatic studies of FAPP (2) using a continuous fluorescence assay: Enzymatic reaction mixtures contained 50 mM Tris·HCl, pH 7.5, 10 mM $MgCl_2$, 10 μM $ZnCl_2$, 5.0 mM DTT, 2.4 μM *N*-dansylGCVIA, 0.040 % (*w/v*) *n*-dodecyl-ß-D-maltoside, 80 nM PFTase, and varying concentrations of **2** (0-10 μM), in a final volume of 450 μL. The reaction mixtures were equilibrated at 30 °C for 5 min, initiated by the addition of PFTase, and monitored for an increase in fluorescence (λ_{ex} = 340 nm, λ_{em} = 505 nm, slit widths = 10 nm for both) for approximately 10 min. The initial rates of formation of products were obtained as slopes in IU/min using least squares analysis. Correction was applied to all rate calculation based on the difference of intensity of fluorescence of product and starting peptide. The difference corresponds only to the fluorescence of total amount of the product. The slope was then divided to the difference followed by multiplying to the total concentration of peptide which then gives the relative rate of formation of product. It should be noted that the K_M values reported here are actually apparent K_M values since the measurements were performed at only a single peptide concentration. The data were fit to a Michaelis-Menten model using KaleidaGraph, a nonlinear regression program, to determine K_M.

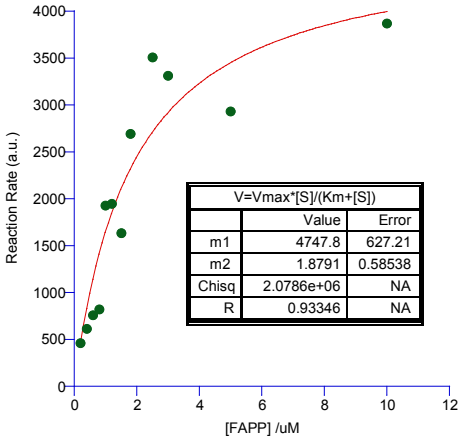


Fig. S1. Fluorescence-based PFTase enzyme assay for prenylation of **3** using varying concentrations of **2**.

To compare the catalytic efficiency of incorporation of FPP and **2**, enzymatic reactions were performed as described above with the exception of the isoprenoid substrates, FPP or **2**, which were included at 10 or 30 μM, respectively. Reaction mixtures were monitored spectrofluorometrically for 1 h to insure the endpoint was reached. The rate of reaction was then determined from the initial slope.

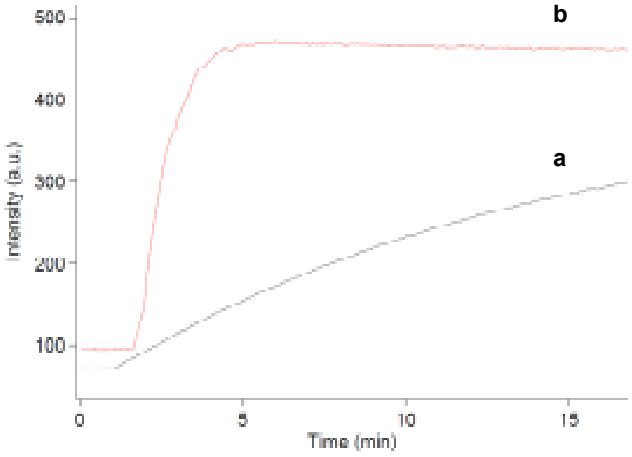
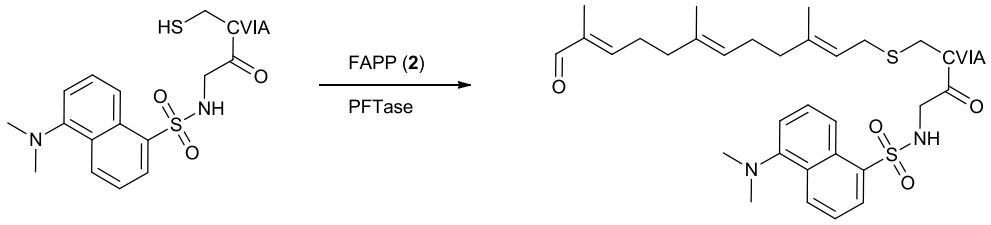


Fig. S2 Continuous fluorescence assay for PFTase catalyzed peptide prenylation of peptide 3 with 4 μM FPP or 10 μM FAPP at same concentration of enzyme (80 nM). **a**: Reaction of **2** (30 μM). **b**: Reaction of FPP (10 μM).



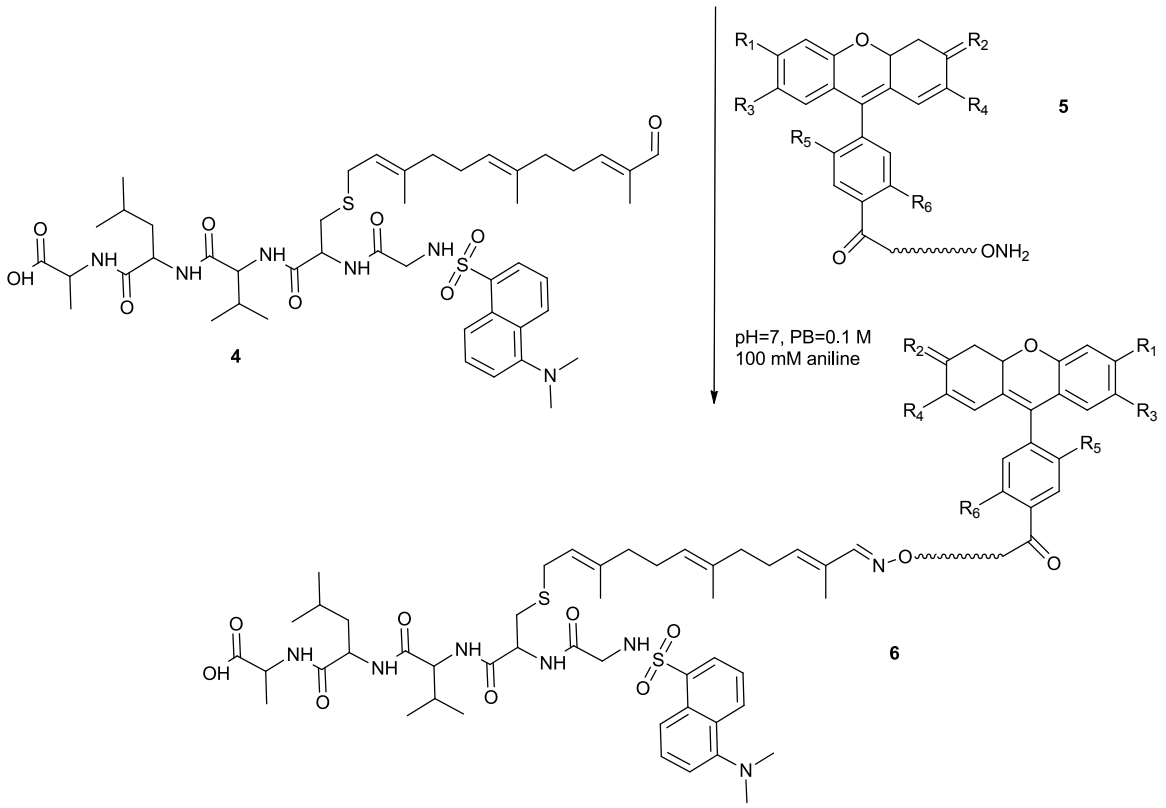
3 4

Enzymatic synthesis of 4: Enzymatic reactions (26 mL) contained Tris-HCl (50 mM, pH 7.5), $MgCl_2$ (10 mM), $ZnCl_2$ (10 mM), DTT (5.0 mM), **3** (2.4 μM), PFTase (80 nM), and **2** (30 μM). To ensure complete disulfide reduction of the peptide, all reagents except FAPP and enzyme were premixed and incubated, for 2 h at 4 °C. With all reagents mixed, the reaction was initiated by the addition of enzyme and the resulting mixture was incubated at 30°C for 1 h. The reaction progress was monitored by fluorescence detection (λ_{ex}=340 nm, λ_{em}=504 nm) using analytical RP-HPLC. The following conditions were employed: flow rate: 1 mL min^{-1}; 100 mL injection

loop; gradient 0–100% B in 40 min; solvent A: NH_4HCO_3 25 mM in H2O; solvent B: CH3CN. After 1 h, the reaction was purified by using a Waters Sep-Pak Plus reversed-phase C_{18} Environmental Cartridge. The cartridge was first washed with solvent B (10 mL) followed by equilibration with solvent A (20 mL). The crude enzymatic reaction mixture was applied to the cartridge and a gradient elution was performed in the following sequence: 10 mL solvent A, 10 mL solvent C (20% solvent B, 80% solvent A), 10 mL solvent D (40% solvent B, 60% solvent A), 10 mL solvent E (60% solvent B, 40% solvent A). Fractions (1 mL) were collected and product elution was monitored by using a handheld UV lamp. The green-fluorescent product was clearly visible and the brightest fraction was selected and its purity was confirmed by HPLC.

LC-MS analysis of the purified product gave an ion of 913.5 as the predominant species, which is consistent with $[M+H]^+$ for **4** (Fig. S3A).

Oxime ligation between peptide-aldehyde 4 and aminooxy alexafluor-488 (5):



Coupling reactions contained 7.5 μM **4**, 200 μM alexafluor-488 (**5**), 0.1 M PB, pH 7.0, and 100 mM aniline in final volume of 500 μL. Reactions were performed at rt and were initiated by addition of aniline (100 mM). To monitor the reactions, aliquots were withdrawn at 40 sec intervals and flash frozen by liquid nitrogen. LC-MS analysis of the reaction mixture gave an ion of 698.2 as the predominant species, which is consistent with $[M+2H]^{2+}$ for **6** (Fig. S3B).

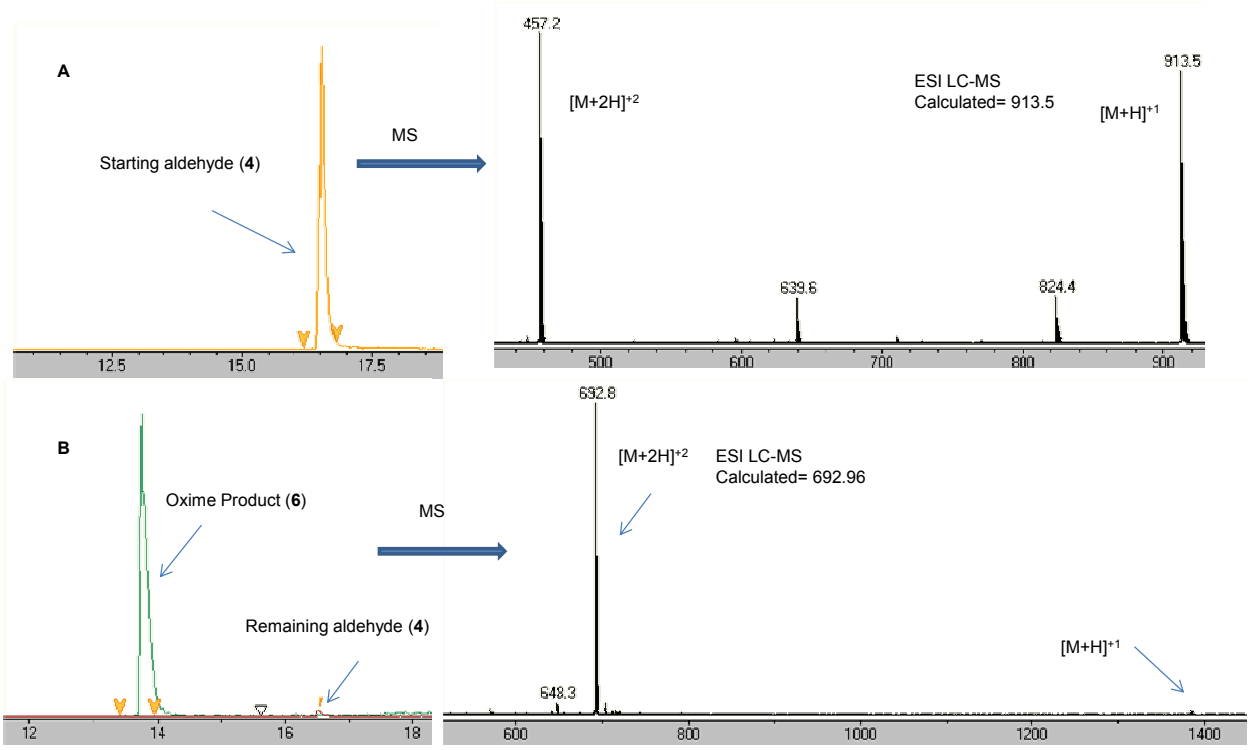


Fig. S3 ESI/LC-MS analysis of oxime ligation reaction between peptide aldehyde **4**, and alexaflour-488 (**5**). Left panels are extracted ion chromatograms and right panels are corresponding MS spectra. **A**) Extracted ion chromatogram of starting peptide-aldehyde **4** and corresponding MS spectrum. **B**) Extracted ion chromatogram of coupled product (**6**) superimposed on extracted ion chromatogram of starting aldehyde (**4**). Both chromatograms are extracted from the total ion chromatogram of the reaction mixture after 1 h of reaction. Relative intensity of peaks shows completion of oxime ligation to 99% conversion. Corresponding MS spectrum of the oxime product (**6**) confirms the formation of oxime bond.

eGFP-CVIA: Protein was prepared as previously described with one modification.[3] In the final phenyl sepharose chromatography step, after the protein was loaded onto the column and washed with buffer as explained in the original work, the protein was eluted from column by adding

water (no buffer). Protein could not be eluted from the column by adding buffer only (as it was described in the original reference).

Enzymatic incorporation of FAPP moiety into eGFP-CVIA: Enzymatic reactions (10 mL) contained Tris-HCl (50 mM, pH 7.0), $MgCl_2$ (10 mM), $ZnCl_2$ (10 μM), DTT (5.0 mM), eGFP–CVIA (2.0 μM), **2** (30 μM), and PFTase (80 nM). After incubation at 30°C for 3 h, the reaction mixture was concentrated using an Amicon Centriprep centrifugation device (10,000 MW cutoff), and excess **2** was removed by using a NAP-5 column (Amersham). The subsequent protein concentration was calculated based on volume change assuming 100% recovery.

Thiol titration of eGFP for determining efficiency of prenylation: $KHPO_3$ (0.1 M, pH 7.4, KPB) was used as buffer; 5 mM DTNB solution was prepared by dissolving 99 mg of DTNB in 50 mL of KPB. Dissolution of DTNB is slow and requires vortexing for a few minutes to become completely clear. Protein free of exogenous thiols (15 μL, prepared by NAP-5 desalting) was added to a solution of 75 μL of 6 M Guanidine•HCl and 10 μL of above DTNB solution. The final mixture was vortexed and the absorbance at 412 nm was measured after 5 min. Each measurement was repeated 3 times. A standard curve was prepared using cysteine as a standard. All measurements were repeated in triplicate.

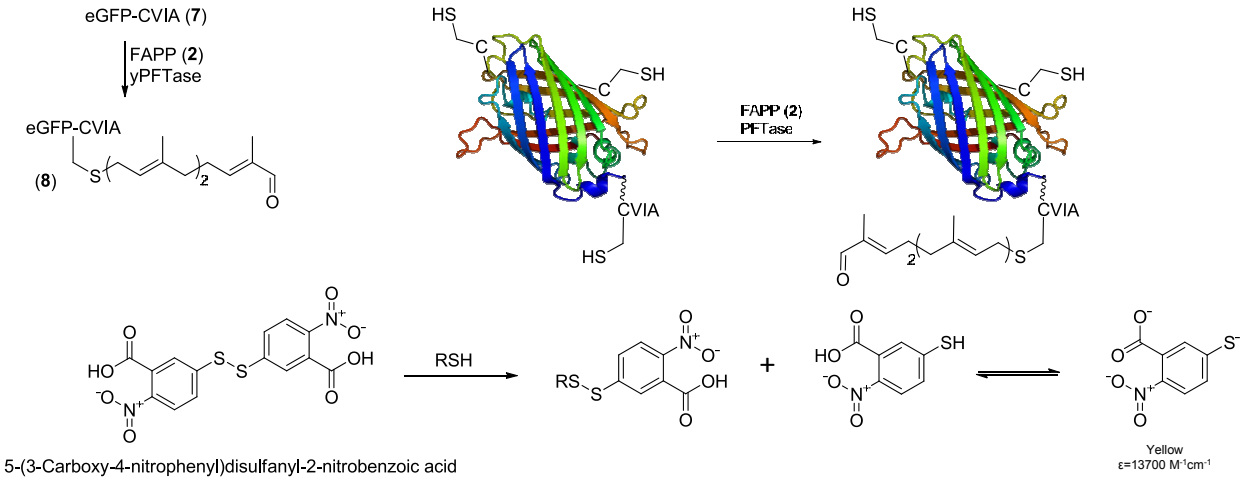
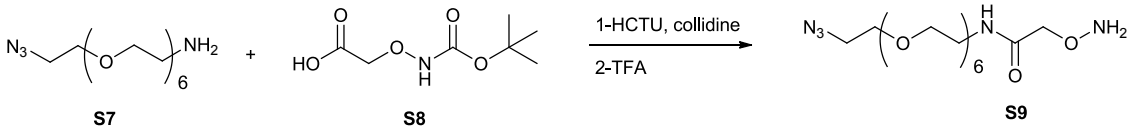


Fig S4. Thiol titration of prenylated and unprenylated eGFP-CVIA by DTNB.

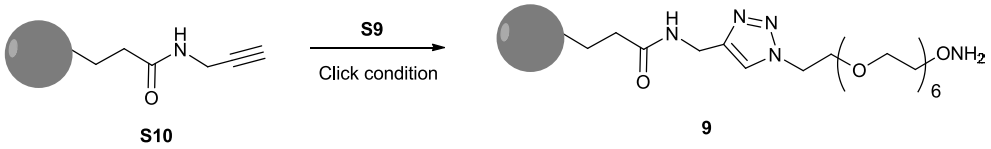
Aminooxy agarose beads (11): Alkyne beads were prepared as previously described.[3] Aminooxy beads were prepared by treating aminooxy-PEG azide (**S9**) with alkyne agarose resin under click chemistry conditions. Aminooxy-PEG azide was synthesized from amine-PEG azide (**S7**) by coupling to N-Boc-aminooxy-acetic acid (**S8**) followed by Boc removal with TFA.



S7 **S8** **S9**

PEG **S7** (100 mg, 0.29 mmol) was added to a solution of 360 mg HCTU (0.87 mmol), 268 µL of collidine[4] (2.03 mmol) and 166 mg **S8** (0.87 mmol) in 1 mL of DMF. The reaction was allowed to proceed for 2 h. TFA (3 mL) dissolved in CH_2Cl_2 (4 mL) was added to mixture and the reaction was allowed to proceed for an additional h. Solvent was removed *in vacuo* to give **S9** which was used without further purification. ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.177 (m, 4H), 3.381 (b, 24H), 4.425 (s, 2H), 8.090 (s, 1H). HR-ESI-MS calcd for $C_{16}H_{34}O_8N_5$ $[M+H]^+$ 424.2407,

found 424.2435. ^{1}H NMR and MS analysis show ~complete conversion of starting amino-PEG **S7** to final aminooxy-PEG **S9**.



S10 **9**

One tenth of the product solution of **S9** (0.03 mmol PEG) was removed and the pH was adjusted with a mixture of PB and NaOH to neutrality. Alkyne beads **S10** (1 mL) in PB, 0.1 M, pH 7 was added to this solution. $CuSO_4$ (1 mM), TCEP (1 mM), TBTA (100 μM) were added and the reaction was allowed to proceed for 2 h. Beads were centrifuged down and the overlaying solution was exchanged for PB. The beads were centrifuged and the buffer exchanged again. This was repeated 5 times. Beads were stored in PB solution at 4 °C for future use.

Immobilization of eGFP onto aminooxy agarose beads: To immobilize the protein onto the agarose beads, aldehyde-functionalized eGFP–CVIA (**8**, 50 μL, 40 μM stock in PB) was added to beads (**9**) in PB (20 μL). The ligation reaction was initiated by the addition of 100 mM aniline. For control reactions, eGFP-CVIA (**7**) was added instead. GFP fluorescence of the supernatant was measured as a function of time. Beads were centrifuged and 5 μL of supernatant was removed, diluted into 445 μL of PB and the fluorescence was measured (λ_{ex}=488 nm, λ_{em}=510 nm).

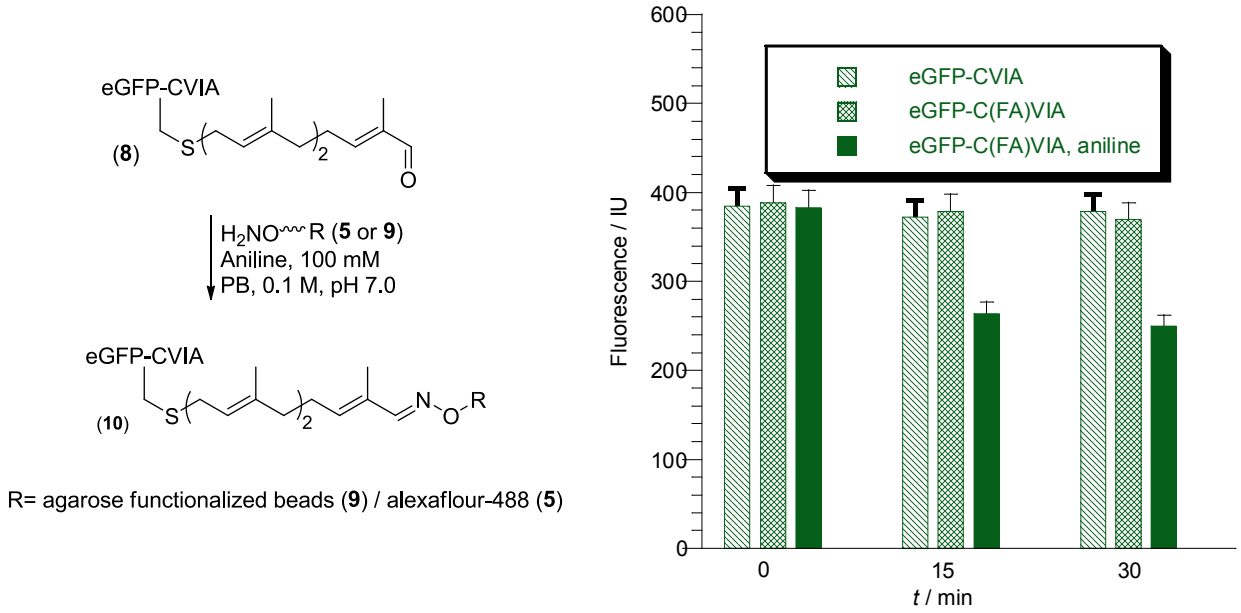


Fig. S5. Immobilization efficiencies of aldehyde-labeled eGFP **8** onto aminooxy agarose beads (**9**). To determine the immobilization efficiencies and background labeling, eGFP-CVIA and aldehyde functionalized eGFP–CVIA (30 μM) were incubated with or without 100 mM aniline. At the appropriate times, beads were centrifuged and the fluorescence remaining in the supernatant was determined by spectrofluorimetry.

Coupling reaction between aldehyde-labeled eGFP-CVIA and alexafluor-488:

Alexafluor-488 (**5**) (7 μL of 1.9 mM solution in DMSO was added to 100 μL of eGFP-C(FA)VIA (**8**) (stock solution of ~30 μM in PB). The reaction was initiated by adding 100 mM aniline and was allowed to proceed for 30 min at rt. The mixture was then transferred to a micro dialysis cassette (10,000 MWCO, Pierce) and dialyzed against PB (15 mL) at 4°C for 3 days. The control reaction contained 7 μL of DMSO instead of the **5**. After dialysis, the final volume of sample was measured to be 165 μL. The control reaction solution was also diluted to 165 μL

and UV spectra were obtained. The concentrations of eGFP in both samples were determined by Bradford assay to be 17.1 μM for the control and 17.9 μM for the coupling reaction.

References:

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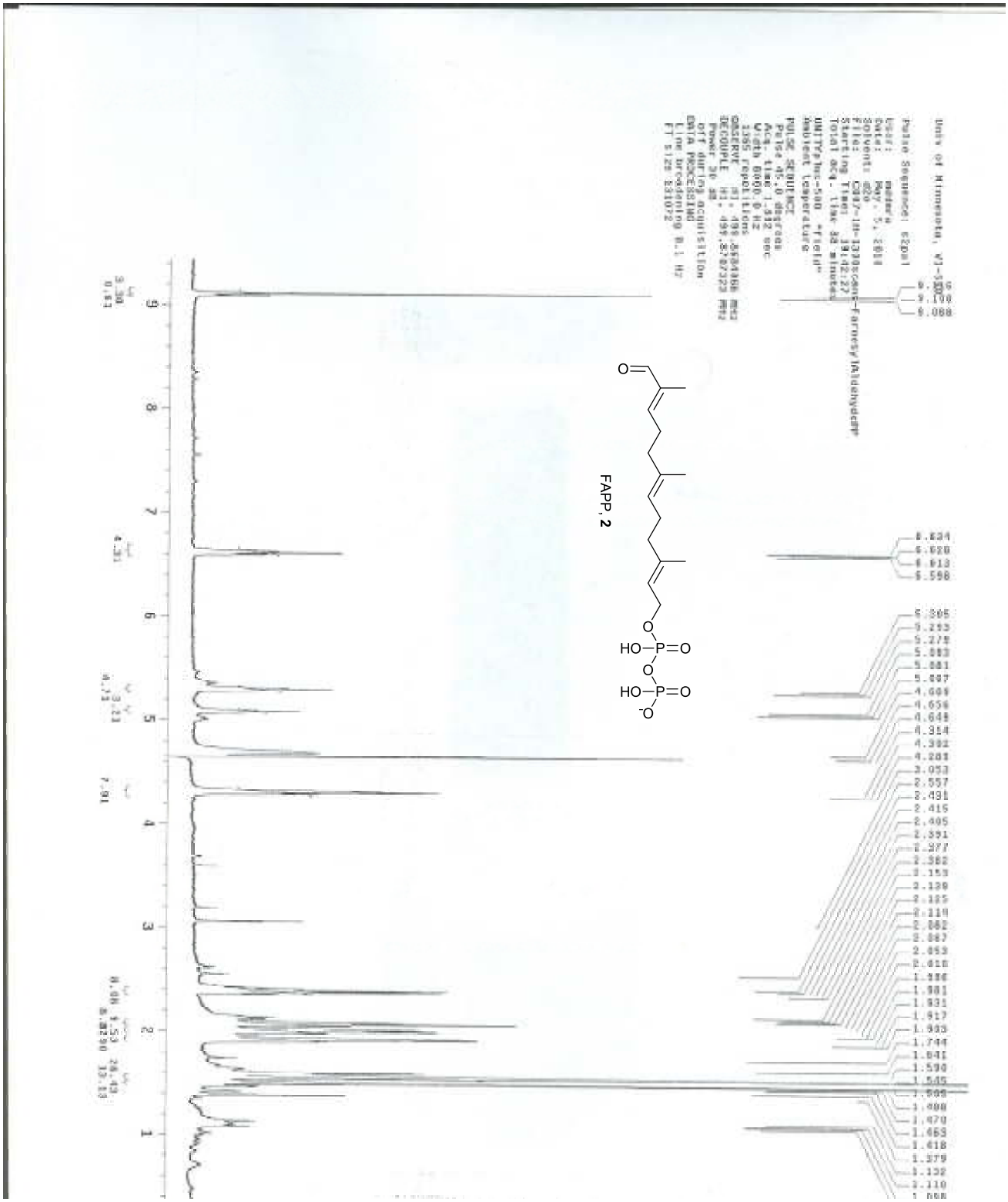
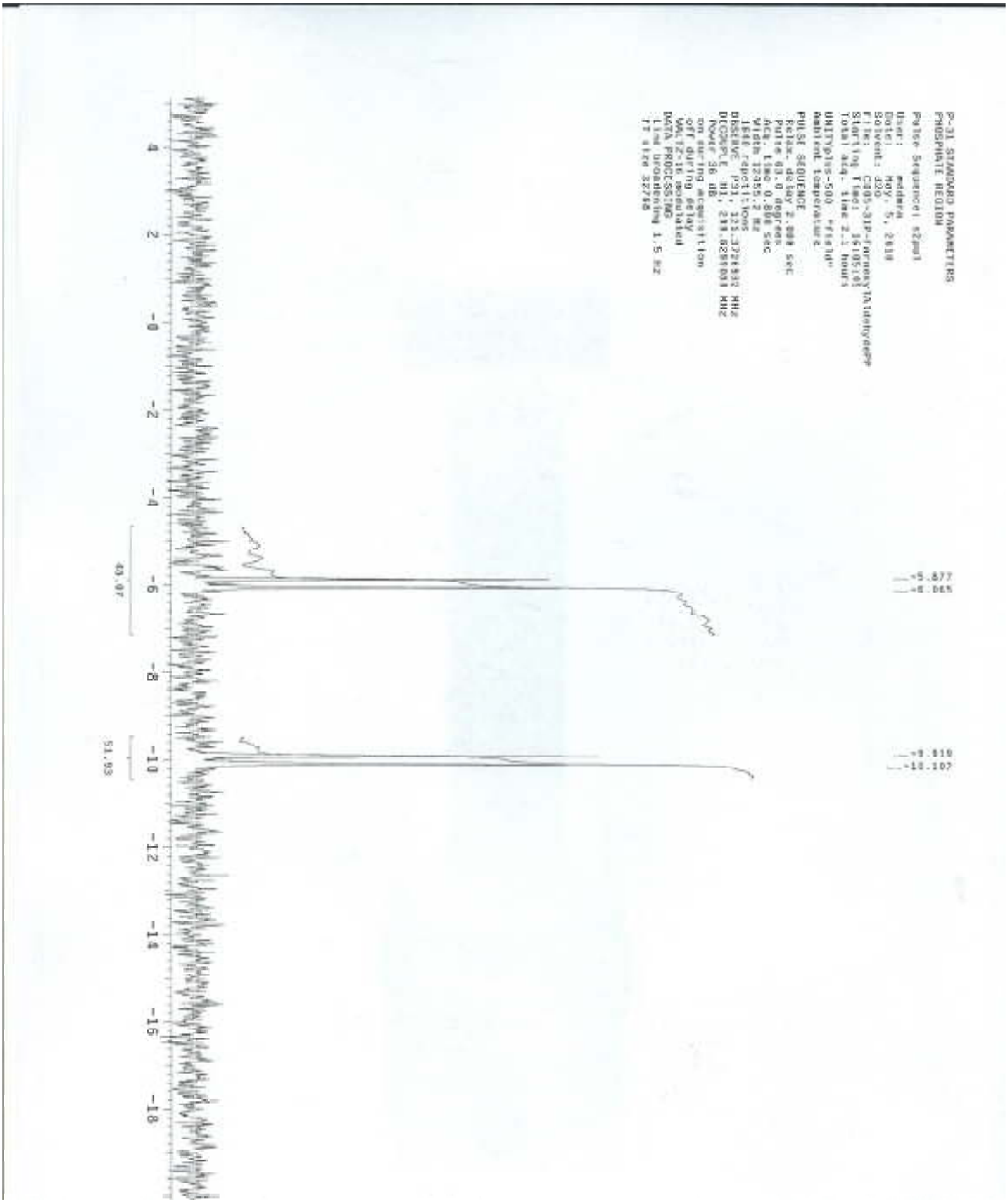
3. B. P. Duckworth, J. Xu, T. A. Taton, A. Guo, and M. D. Distefano, *Bioconj. Chem.*, 2006, **17**, 967-974.
4. I. P. Decostaire, D. Lelièvre, H. Zhang, and A. F. Delmas, *Tetrahedron Letters*, 2006, **47**, 7057-7060.


FAPP, 2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DECOUPLE H1, 218.8259968 MHz
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off during delay
WALTZ-16 modulated
DATA PROCESSING
line broadening 1.5 Hz
FT size 32768
43.97
51.93
5.877
4.065
-9.819
-10.102
4
2
0
-2
-4
-6
-8
-10
-12
-14
-16
-18