The Unusual Macrocycle-Forming Thioesterase of Mycolactone

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Online Supporting Information

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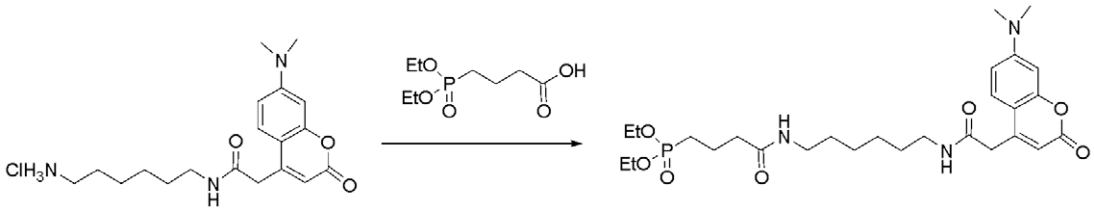
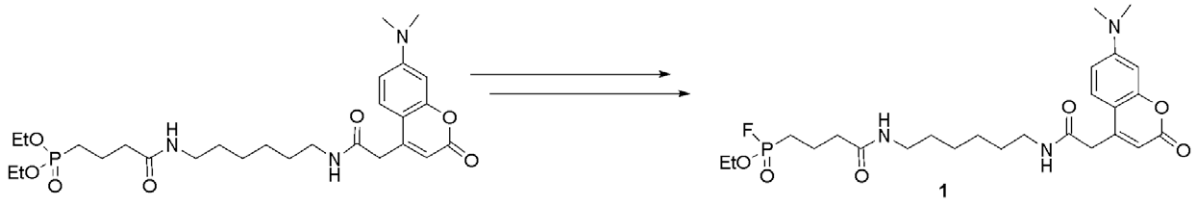
General synthetic procedures and materials

All commercial reagents (Sigma-Aldrich, Spectrum, MP Biomedicals, Alfa Aesar, TCI America, Acros) were used as provided unless otherwise indicated. BODIPY-CoA,[1] 7-dimethylaminocoumarin-hexanediamine HCl,[2] and 4-(diethoxyphosphinyl)-butanoic acid[3] were prepared according to published literature procedures. All reactions were carried out under argon atmosphere in dry solvents with oven-dried glassware and constant magnetic stirring unless otherwise noted. Triethylamine (TEA), N-methyl morpholine (NMM), and ethyl-*N,N*-diisopropylamine (DIPEA) were dried over sodium and freshly distilled. ^{1}H-NMR spectra were taken at 300, 400, or 500 MHz and ^{13}C-NMR spectra were taken at 100.6 or 75.5 MHz on Varian NMR spectrometers and standardized to the NMR solvent signal as reported by Gottlieb.[4] Multiplicities are given as s=singlet, d=doublet, t=triplet, q=quartet, p=pentet, dd=doublet of doublets, bs=broad singlet, bt=broad triplet, m=multiplet using integration and coupling constant in Hertz. TLC analysis was performed using Silica Gel 60 F254 plates (EM Scientific) and visualization was accomplished with UV light (λ=254 nm) and/or the appropriate stain (iodine, 2,4-dinitrophenylhydrazine, cerium molybdate, ninhydrin). Silica gel chromatography was carried out with Silicycle 60 Angstrom 230-400 mesh according to the method of Still.[5] TLC prep plate purification was performed with EMD Silica Gel 60 F_{254} pre-coated plates. Electrospray (ESI) and fast atom bombardment (FAB) mass spectra were obtained at the UCSD Mass Spectrometry Facility by Dr. Yongxuan Su using a Finnigan LCQDECA mass spectrometer and a ThermoFinnigan MAT- 900XL mass spectrometer, respectively.

Synthetic Procedures and Spectroscopic Data for Fluorophosphonate 1

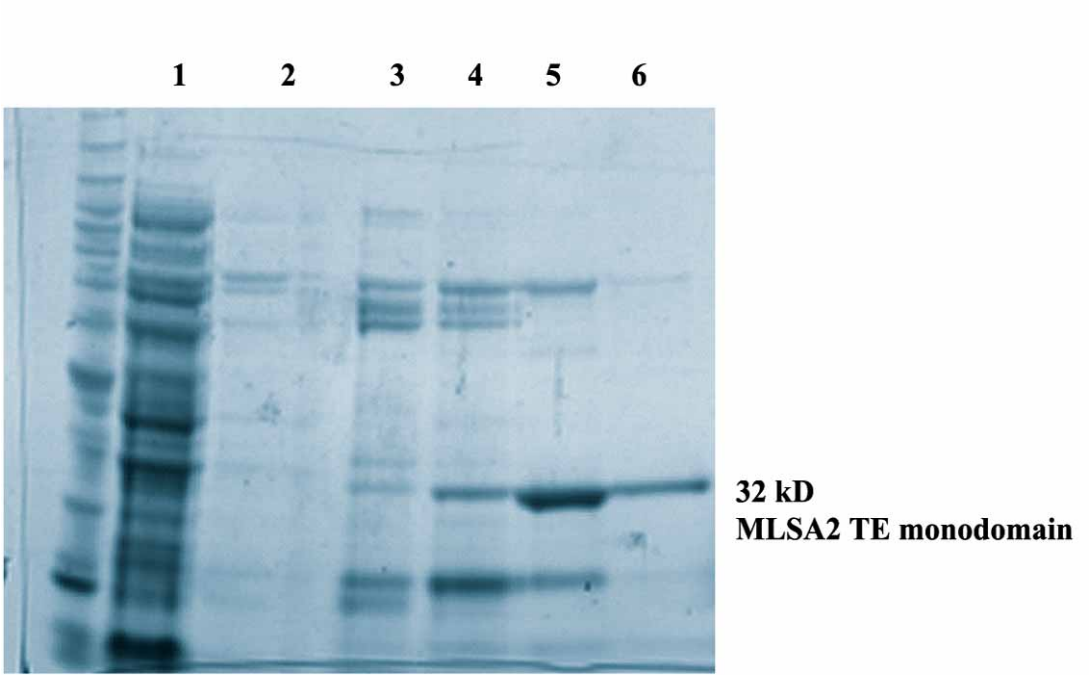


4-(diethoxyphosphinyl)-butanoic acid (220 mg, 0.98 mmol), DIPEA (677 uL, 3.88 mmol), 7-dimethylaminocoumarin-hexanediamine HCl (368 mg, 0.970 mmol), and HOBt (558 mg, 3.71 mmol) were dissolved in DMF (20 mL) with stirring and cooled to 0°C. EDC (558 mg, 2.91 mmol) was added in one portion and the reaction was allowed to slowly warm to RT and stirred overnight. The solvent was removed under reduced pressure to yield a crude oil which was taken up in EtOAc (150 mL) and washed with water (1x100 mL), saturated $NaHCO_3$ (2x125 mL), and brine (1x100 mL). The organic layer was dried over Na_2SO_4, filtered, and evaporated under reduced pressure to yield the diethylphosphonate amide as a fluorescent oil (405 mg, 76%). ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.48 (d, *J*=8.5 Hz, 1H), 6.95 (bt, *J*=6.0 Hz, 1H), 6.55 (dd, *J*=2.5 Hz, 9.0 Hz, 1H), 6.48 (bt, *J*=6.0 Hz, 1H), 6.39 (d, *J*=2.5 Hz, 1H), 6.03 (s, 1H), 4.01 (q, *J*=7.5 Hz, 4H), 3.57 (s, 2H), 3.13 (m, 4H), 2.99 (s, 6H), 2.26 (t, *J*=7.5 Hz, 2H), 1.86 (p, *J*=7.5 Hz, 2H), 1.73 (m, 2H), 1.37 (m, 4H), 1.26 (t, *J*=7.5 Hz, 6H), 1.21 (m, 4H). ^{13}C-NMR (100.6 MHz, $CDCl_3$) δ 172.4, 168.5, 162.3, 156.1, 153.2, 151.0, 126.1, 110.0, 109.3, 108.8, 98.2, 61.8 (d, *J*=6.8 Hz), 40.5, 40.3, 39.6, 39.1, 36.6 (d, *J*=13.8 Hz), 29.6, 29.3, 26.3, 25.5, 24.1, 19.2 (d, *J*=4.6 Hz), 16.6 (d, *J*=6.1 Hz). HRMS (EI) (*m/z*) $[M]^+$ calcd for $C_{21}H_{48}N_3O_7P_1$, 551.2755 found 551.2757.

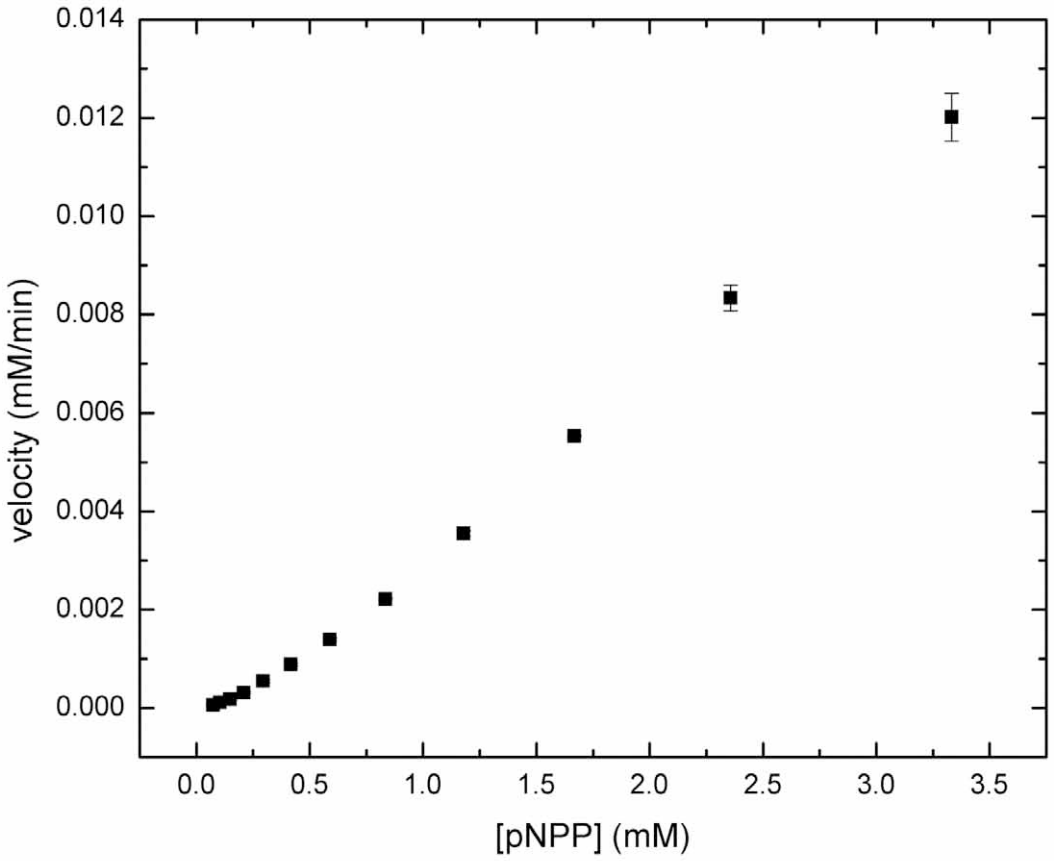


The product of the above reaction (350 mg, 0.635 mmol) was dissolved in DCM (6.5 mL) and bromotrimethylsilane (0.418 mL, 3.24 mmol) was added dropwise at 0°C. The reaction was allowed to slowly attain RT and stir for 2.5 hrs. The reaction was quenched with H_2O and the solvent was removed under reduced pressure via rotovap with an inline base (NaOH) trap. The crude monophosphinic acid was carried forward as a low R_f oil as observed by TLC. This monophosphinic acid residue, was then dissolved in dry DMF (5 mL), transferred to a plastic reaction vial, and cooled to –78°C with stirring. DAST (50 uL, 0.39 mmol) was added dropwise via microsyringe. After 10 minutes the reaction was allowed to slowly attain RT and diluted with EtOAc (250 mL). This solution was washed with H_2O (1x100 mL) and brine (1x100 mL), and the H_2O layer saved and lyophilized to recover the crude monophosphinic acid (282 mg, 85%). The organic layer was then dried over Na_2SO_4, filtered, and evaporated under reduced pressure to yield the crude fluorophosphonate. This residue was redissolved in $CHCl_3$ and purified by flash chromatography ($CHCl_3$ to 1:3 $CHCl_3$:acetone to 1:3:0.1 $CHCl_3$:acetone:MeOH) to give **1** as a

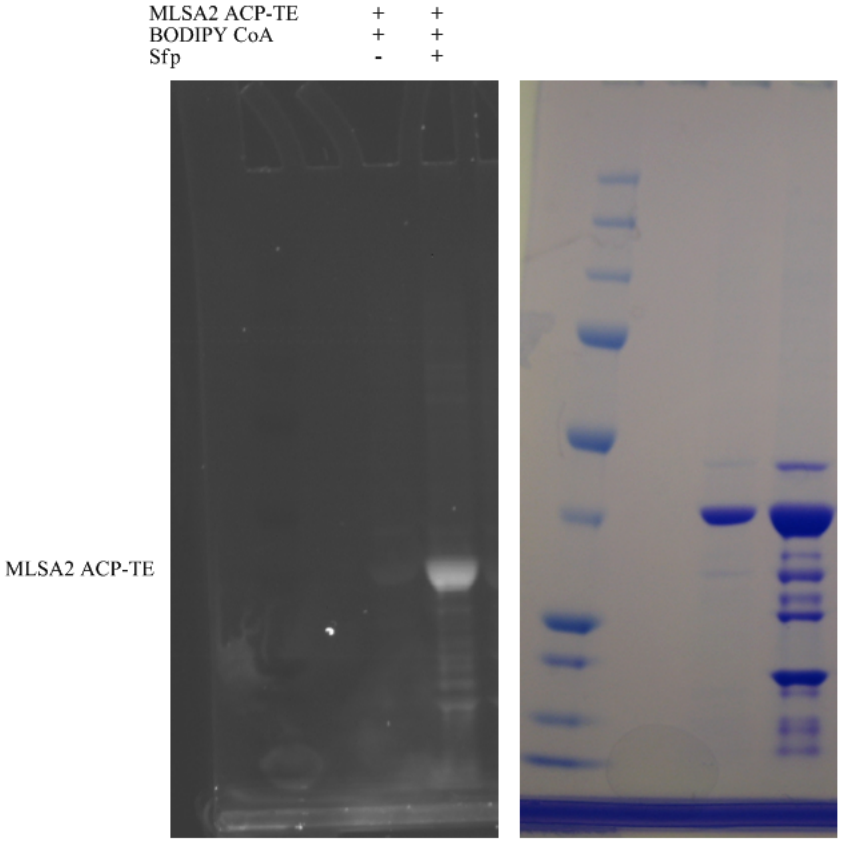
fluorescent oil. This procedure was repeated on the recovered monophosphinic acid an additional time to provide a total of 22.5 mg (6.5 % yield for two steps) of fluorophosphonate **1** (estimated 90% purity by ^{1}H-NMR). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.47 (m, 1H), 6.60 (m, 2H), 6.48 (m, 1H), 6.23 (bs, 1H), 6.04 (s, 1H), 3.60 (m, 2H), 3.18 (m, 4H), 3.03 (s, 6H), 2.31 (t, *J*=7.0 Hz, 2H), 1.99 (m, 4H), 1.40 (m, 4H), 1.25 (m, 7H). ^{13}C-NMR (100.6 MHz, $CDCl_3$) δ 171.9, 168.1, 160.7, 155.1, 153.3, 153.2, 150.9, 126.1, 113.8, 110.2, 109.5, 98.3, 53.4 (d, *J*=6.9 Hz), 43.5, 41.7, 40.4, 39.5, 36.0 (d, *J*=15.7 Hz), 29.5, 29.2, 26.1, 24.0, 22.6, 18.5 (d, *J*=4.6 Hz), 14.5. ^{19}F-NMR (282 Hz, $CDCl_3$) δ -66.9 (d, J_p=1071 Hz, F-P). ^{31}P-NMR (162 Hz, $CDCl_3$) δ 33.3 (d, J_F=1071 Hz, P-F). HRMS (EI) (*m/z*) $[M]^+$ calcd for $C_{25}H_{37}N_3O_6F_1P_1$, 525.2399, found 525.2398.



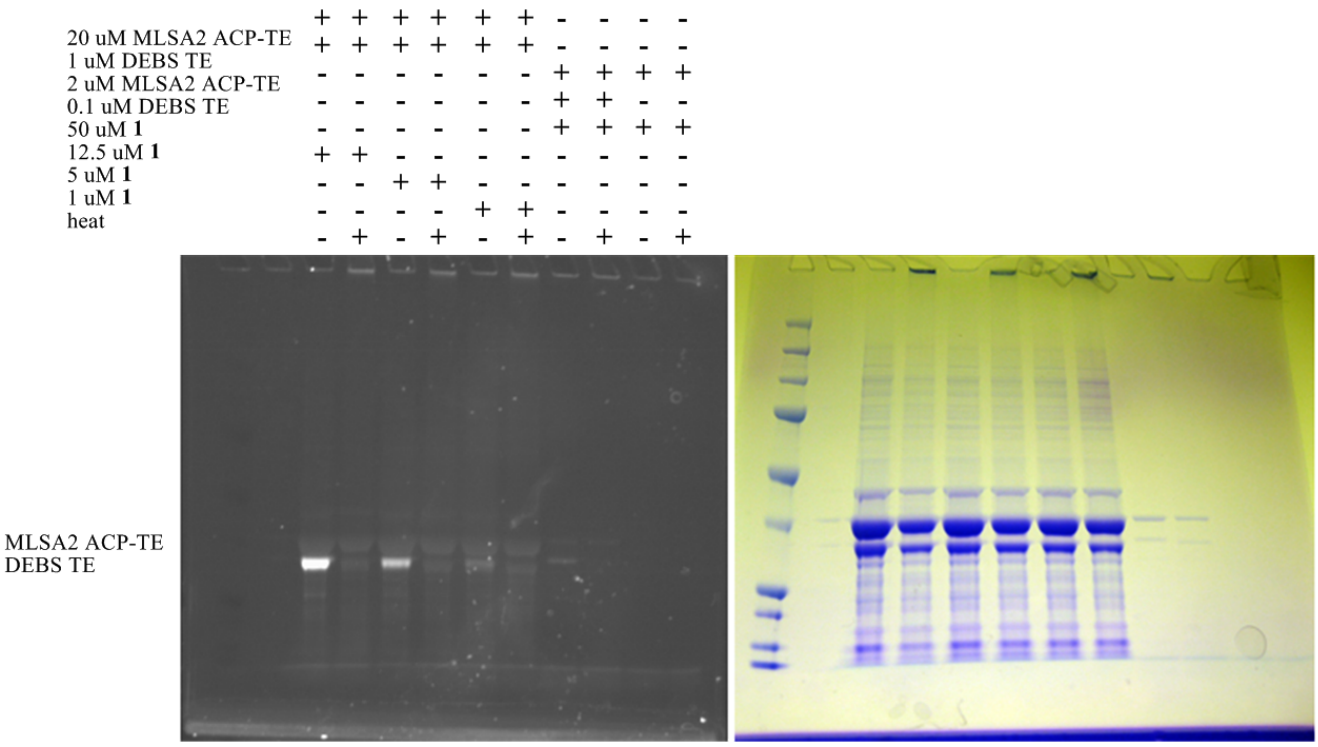
Complete Gel Image for Purification of MLSA2 TE Monodomain. MLSA2 TE was cloned and expressed as detailed in the main text. Gel lanes depict fractions taken during Ni-NTA chromatography and are as follows: 1) flow through, 2) wash, 3) 10 mM imidazole wash, 4) 50 mM imidazole, 5) 100 mM imidazole, 6) 200 mM imidazole.

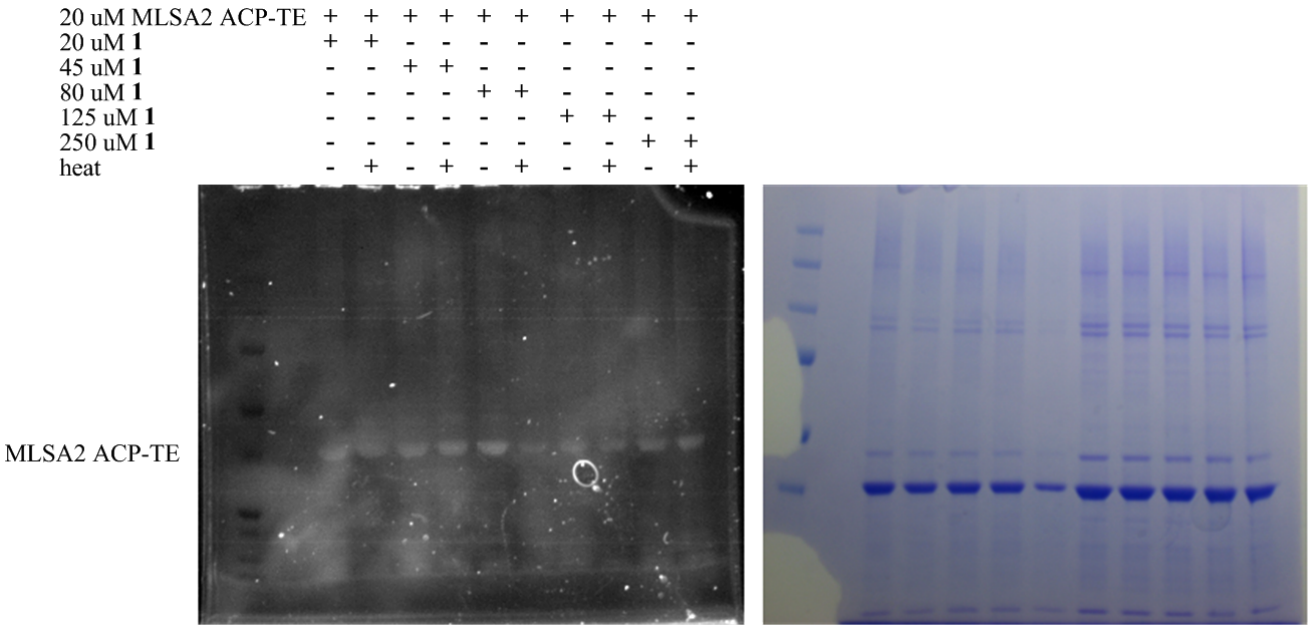


Kinetic Plot of MLSA2 TE Monodomain: Kinetics of recombinant MLSA2 TE monodomain with pNPP. Initial values allowe estimation of K_{cat}/K_m for the monodomain at 0.16 ± 0.00041 (compared to 0.20 for the didomain).



Complete Gel Data from Figure 4. Sfp dependent labeling of MLSA2 ACP-TE by BODIPY CoA and the PPTase Sfp.





Complete Gel Data from Figure 5. Top: Labeling of DEBS-TE and MLSA2 ACP-TE in one-pot with fluorophosphonate **1**. Heat dependent labeling is observed with DEBS-TE but not MLSA2. Bottom: Concentration dependence of MLSA2 labeling by fluorophosphonate **1**. In contrast to the DEBS labeling in the top gel no clear ± labeling is observed.

Compiled MS Data from fluorophosphonate treated MLSA2 ACP-TE

MLSA2 ACP-TE Didomain – Fluorophosphonate Treatment and Chymotrypsin Digest

1 MKHHHHHHHH HGGLESTSLY KKAGSTLATL **VAAATATVLG HHTPESISPA**
51 **TAFKDLGIDS LTALELRNTL THNTGLDLPP TLIFDHPTPT ALTQHLHTRL**
101 **TQSHTPVGPI ASLLSHAIDE GKFRAGADLL MAASNLNQSF** SNMAEL**NQLP**
151 **AVTDIADASP DGLLTLICIS TSENEYARLA AANIHSLTFA** EIAAPGFYDA
201 QLPNSIETSA EALATAITGA YANTSIVL**VA HSIVCELAQA TMTRLQDADI**
251 **DLVGL**VLLDP LEGTNSTEDY VET**VLTRIEH INAPRVGVDG YLAALGRYLQ**
301 **FHEDRRIPIP ETRHMTLHSD TKIDRAQTPM NL**LQDEAALT AL**KIGNWMND**
351 **TGSIAVTLRD GPVFL**GRARS VNMR

Purple = peptide sequence coverage received for chymotrypsin digest of MLSA2 ACP-TE treated with **1**
Green = peptide sequence coverage received for chymotrypsin digest of untreated MLSA2 ACP-TE
Red = overlapping peptide sequence coverage received from both proteome treatments

MLSA2 ACP-TE Didomain – Fluorophosphonate Treatment and Pepsin Digest

1 MKHHHHHHHH HGGLESTSLY KKAGSTL**ATL VAAATATVLG HHTPESISPA**
51 **TAFKDLGIDS LTALELRNTL THNTGLDLPP TLIFDHPTPT ALTQHLHTRL**
101 **TQSHTPVGPI ASLLSHAIDE GKFRAGADLL MAASNLNQSF SNMAELNQLP**
151 **AVTDIADASP DGLLTLICIS TSENEYARLA AANIHSLTFA EIAAPGFYDA**
201 **QLPNSIETSA EALATAITGA Y**ANTSIVL**VA HSIVCELAQA TMTRLQDADI**
251 **DLVGLVLLDP LEGTNSTEDY VETVLTRIEH INAPRVGVDG YLAALGRYLQ**
301 **FHEDRRIPIP ETRHMTLHSD TKIDRAQTPM NL**LQDEAALT AL**KIGNWMND**
351 **TGSIAVTLRD GPVFL**GRARS VNMR

Purple = peptide sequence coverage received for pepsin digest of MLSA2 ACP-TE pretreated with **1**
Green = peptide sequence coverage received for pepsin digest of untreated MLSA2 ACP-TE
Red = overlapping peptide sequence coverage received from both proteome treatments

Peptide sequence coverage received upon chymotyrpsin or pepsin digest of MLSA2 ACP-TE treated with fluorophosphonate 1. In both digests the proposed catalytic serine (residue 232 following the convention of the report above) is observed unmodified after treatment with 50x fluorophosphonate. No evidence of the fluorophosphonate modified or dehydroalanine eliminated Ser232 was observed. Note: the above report differs in numbering from that of the sequence analysis (in which the above S232 is referred to as S126) due to incorporation of the CP domain in the above analysis.

References:

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4. Gottlieb HE, Kotlyar V, Nudelman A (1997) NMR Chemical Shifts of Common Laboratory Solvents As Trace Impurities. J. Org. Chem. 62: 7512-7515.

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