

Figure S1. The MS analysis for MCLR (A), and its electrophilic addition samples with the β ME (B), AcSH (C), Cys (D), Hcy (E), GSH (F), and Cys-Gly (G).

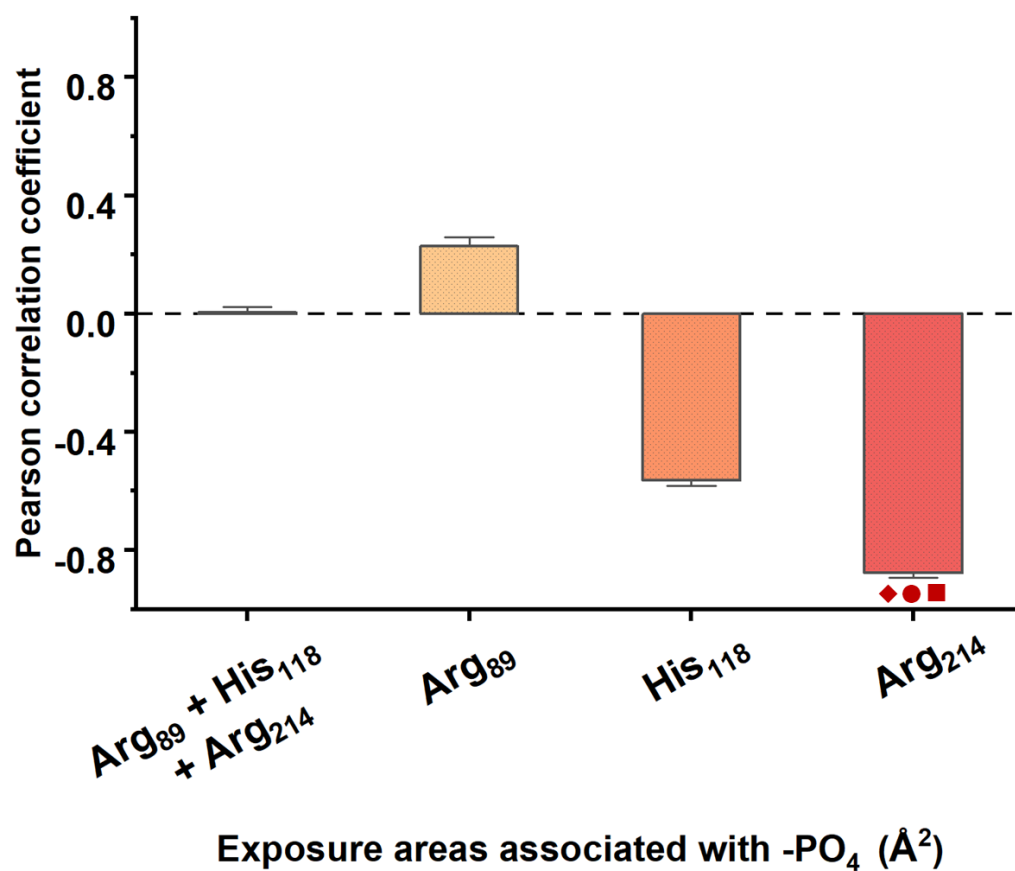


Figure S2. Pearson correlation coefficients between inhibition data and the exposure areas associated with phosphate group.

Table S1. MS/MS identification of MCLR and MCLR-BTPs.

Basic fragment ions	MCLR	MCLR-βME	MCLR-AcSH	MCLR-Cys	MCLR-Hcy	MCLR-GSH	MCLR-Cys-Gly
[M + H] ⁺	995.5557	1073.6902 (+ 78.1345)↑ ^a	1071.6745 (+ 76.1188)↑	1116.7152 (+ 121.1595)↑	1130.7417 (+ 135.1860)↑	1302.8794 (+ 307.3237)↑	1173.7664 (+ 178.2107)↑
[PhCH ₂ CH(OCH ₃)] ⁺	135.0803	135.0803√ ^b	135.0802√	135.0804√	135.0803√	135.0801√	135.0804√
[Glu-Mdha+H] ⁺	213.0832	291.2175↑	289.2017↑	334.2424↑	348.2693↑	520.4070↑	391.2939↑
[MeAsp-Arg+H] ⁺	286.1477	286.1476√	286.1477√	286.1478√	286.1476√	286.1479√	286.1479√
[Mdha-Ala-Leu-MeAsp+H] ⁺ or [Glu-Mdha-Ala+Leu+H] ⁺	397.2079	475.3422↑	473.3265↑	518.3674↑	532.3937↑	704.5315↑	575.4186↑
[Arg-Adda+H] ⁺	470.3124	470.3123√	470.3122√	470.3124√	470.3125√	470.3123√	470.3122√
[Mdha-Ala-Leu-MeAsp-Arg+H] ⁺	553.3072	631.4416↑	629.4258↑	674.4664↑	688.4931↑	860.6307↑	731.5179↑
[MeAsp-Arg-Adda+H] ⁺ or [Arg-Adda-Glu+H] ⁺	599.3542	599.3541√	599.3542√	599.3545√	599.3541√	599.3542√	599.3543√
[Arg-Adda-Glu-Mdha+H] ⁺	682.3957	760.5304↑	758.5143↑	803.5554↑	817.5818↑	989.7195↑	860.6065↑
[Mdha-Ala-Leu-MeAsp-Arg-Adda+H] ⁺ or [Arg-Adda-Glu-Mdha-Ala-Leu+H] ⁺	866.5149	944.6493↑	942.6333↑	987.6744↑	1001.7010↑	1173.8387↑	1044.7254↑
Target residues	---			Mdha			

^a: ↑ and ↓ mean mass changes were related to these fragment ions; ^b: √ means ions with the stable m/z were detected by mass spectrograph.

Table S2. Preparation and purification information for the electrophilic addition samples of MCLR.

Conjugation products	Eluted time ^a	Concentration ^b	Total volume	Purity ^c
MCLR-βME	19.7 min	≈1200 μmol/L	5 × 100 μL	98.3%
MCLR-AcSH	20.1 min	≈1000 μmol/L	5 × 100 μL	97.2%
MCLR-Cys	17.4 min	≈1100 μmol/L	5 × 100 μL	98.9%
MCLR-Hcy	17.3 min	≈1100 μmol/L	5 × 100 μL	98.0%
MCLR-GSH	12.5 min	≈1200 μmol/L	5 × 100 μL	98.5%
MCLR-Cys-Gly	15.8 min	≈1200 μmol/L	5 × 100 μL	96.7%

^a Collection time was set for 0.5 min (± 0.25 min around the eluted time).

^b With 200 μmol/L MCLR served as the inner standard for quantification and assumed MCLR and MCLR-BTPs had approximate protonated efficiencies.

^c Purity was directed calculated by the MS signals of MCLR and MCLR-BTPs.

Table S3. The candidate interaction parameters between MCLR/MCLR-BTPs and PP2A.

Molecular simulation parameters		MCLR	MCLR-βME	MCLR-Acsh	MCLR-Cys	MCLR-Hcy	MCLR-GSH	MCLR-Cys-Gly
Combination area (Å ²)	Total	683.0694	716.4927	728.2102	716.6841	703.6324	788.2092	763.7774
	Ala ¹ →PP2A	50.2139	18.2943	11.9711	14.6462	13.6280	12.0700	11.3981
	Leu ² →PP2A	135.0409	125.4982	140.9633	123.6076	119.4272	134.4739	134.4101
	MeAsp ³ →PP2A	59.4977	58.7240	69.3921	67.4127	66.9446	69.8673	68.8829
	Arg ⁴ →PP2A	115.7080	112.9425	110.6440	113.6880	111.2893	108.8118	108.4528
	Adda ⁵ →PP2A	360.9873	359.5962	359.7151	358.5792	358.0810	355.9954	356.1675
	Glu ⁶ →PP2A	171.7180	160.6913	168.2875	160.7650	159.8249	161.6061	165.1835
	Mdha ⁷ →PP2A	120.6914	171.9261	156.0190	183.8890	175.3485	228.8137	208.1651
Positive accessible surface area (Å ²)	Total	438.9368	463.9523	456.8462	456.5521	456.1923	495.0612	496.7734
	Ala ¹ →PP2A	25.0459	17.2165	24.2321	4.6460	3.6280	27.6002	26.9446
	Leu ² →PP2A	90.8749	82.9635	91.6720	79.6961	79.5110	87.1274	87.1095
	MeAsp ³ →PP2A	32.0448	31.4304	38.9354	38.3280	31.1080	38.5582	30.9814
	Arg ⁴ →PP2A	64.9071	64.5195	74.2696	77.0562	78.1798	72.6489	74.0464
	Adda ⁵ →PP2A	232.0883	230.8065	236.1999	235.9119	238.0377	236.7063	236.5531
	Glu ⁶ →PP2A	94.3617	92.7170	92.8100	92.5818	91.9039	90.0932	90.3951
	Mdha ⁷ →PP2A	84.1601	111.4355	117.9418	141.2325	131.8605	195.7521	141.3499
Negative accessible surface area (Å ²)	Total	244.1328	252.5401	271.3640	263.7243	258.1291	293.1485	267.0042
	Ala ¹ →PP2A	15.1677	1.0778	7.7387	3.8035	13.4443	3.4700	4.4534
	Leu ² →PP2A	44.1661	42.5345	49.2910	47.3340	50.6234	47.3470	47.3030
	MeAsp ³ →PP2A	27.4573	27.2935	30.4565	32.6770	39.5256	30.9170	29.9012
	Arg ⁴ →PP2A	37.8009	38.4228	37.3740	41.2243	48.7987	36.4634	36.4063
	Adda ⁵ →PP2A	124.0795	125.1887	121.8808	126.2599	132.3664	122.8900	122.4342
	Glu ⁶ →PP2A	77.3566	67.9743	71.4769	71.7758	78.6100	71.5129	71.7884

	Mdha ⁷ →PP2A	36.5312	42.2267	38.8030	39.9037	41.1499	41.3724	41.4820
Hydrophobic surface area (Å ²)	Total	404.7893	408.4791	408.6372	401.2150	408.7348	405.9594	405.5256
	Ala ¹ →PP2A	8.6175	2.2335	3.6345	1.2400	0.3619	4.8209	4.6412
	Leu ² →PP2A	59.2876	52.8599	62.9961	49.6412	46.6786	56.7017	56.2078
	MeAsp ³ →PP2A	6.6383	4.8506	8.0488	8.3986	8.2832	8.0563	8.9237
	Arg ⁴ →PP2A	39.8851	41.3816	38.9211	41.0544	41.8457	36.9658	37.8200
	Adda ⁵ →PP2A	245.7937	242.2990	242.9248	244.8030	240.0145	238.1998	238.0276
	Glu ⁶ →PP2A	49.8923	45.4832	48.0880	46.1070	42.9830	40.8250	43.5837
	Mdha ⁷ →PP2A	87.8027	101.4919	93.2629	95.9085	98.9036	91.4386	90.7020
Polar surface area (Å ²)	Total	278.2801	308.0134	319.5731	315.4689	294.8981	377.2498	351.2517
	Ala ¹ →PP2A	41.5964	16.0608	28.3363	3.4061	3.2663	26.2488	26.7570
	Leu ² →PP2A	75.7535	72.6383	77.9671	73.9661	72.7487	77.7724	78.2022
	MeAsp ³ →PP2A	52.8644	53.8734	61.3445	59.0138	58.6617	61.4187	59.9589
	Arg ⁴ →PP2A	62.8229	61.5608	72.7224	73.6337	74.4438	72.1461	72.6327
	Adda ⁵ →PP2A	118.1400	117.7955	115.0663	138.7763	113.7906	64.2971	113.1936
	Glu ⁶ →PP2A	121.8257	115.2084	120.1989	114.6581	116.8472	120.7811	121.5998
	Mdha ⁷ →PP2A	32.8887	70.4345	73.7561	87.9808	67.4454	136.3751	115.4629
Active center exposure (Å ²)	Mn ₁ ²⁺ +Asp ₅₇ +Asp ₈₅ +Asn ₁₁₇ +His ₁₆₇ +His ₂₄₁	819.0067	819.8222	821.4860	823.0130	822.2631	824.9606	822.1924
	Mn ₁ ²⁺ +Asp ₅₇	314.8653	317.4326	317.4961	317.9027	318.2437	317.3058	317.6216
	Mn ₁ ²⁺ +Asp ₈₅	310.2860	306.5590	307.7299	307.0732	307.6104	309.5630	307.9066
	Mn ₁ ²⁺ +Asn ₁₁₇	320.5904	321.9788	321.9153	321.3859	321.5473	321.5898	321.8677
	Mn ₁ ²⁺ +His ₂₄₁	356.1933	354.9973	355.6072	354.4759	356.6136	354.5811	356.0070
	Mn ₂ ²⁺ +Asp ₅₇ + His ₅₉ +Asp ₈₅	562.7151	558.7158	558.6845	562.9078	570.1757	568.3002	562.5989
	Mn ₂ ²⁺ +His ₅₉	347.0402	342.9848	338.8241	346.8755	347.2521	348.9503	347.8009

	Mn ²⁺ +Asp ⁸⁵	316.8060	316.4693	315.4002	316.2285	316.6199	316.9627	315.1873
Exposure areas associated with -PO ₄ (Å ²)	Arg ⁸⁹ +His ¹¹⁸ +Arg ²¹⁴	1001.8087	1005.6117	927.2913	924.2706	922.9355	1000.8712	1000.4306
	Arg ⁸⁹	374.1155	375.9034	375.4064	375.9305	376.6839	372.9275	375.3104
	His ¹¹⁸	319.8264	318.1479	320.9894	319.5455	319.8376	319.6681	321.7038
	Arg ²¹⁴	322.0467	375.2818	374.9326	373.0033	371.4669	372.916	373.6877
Hydrogen bond (KJ/Mol)	Total	-44.3	-37	-42	-42.2	-35.4	-50.9	-49.9
	Pro ²¹³ ←Arg ⁴	-7	-6.7	-6.5	-6.5	-6.5	-6.5	-6.7
	Arg ²⁶⁸ →Ala ¹	-2.6	---	---	---	---	---	---
	Arg ⁸⁹ →Leu ²	-2.2	-2.6	-2.3	-4.3	-4.1	-1.9	-2.6
	Arg ⁸⁹ →MeAsp ³	-6.8	-5.8	-6.8	-4.1	-3.9	-7.2	-6.3
	Arg ²¹⁴ →Arg ⁴	-6.6	-5.2	-5.1	-4.9	-4.9	-5.3	-5.3
	Asn ¹¹⁷ →Adda ⁵	-0.9	-2.6	-1	-2.3	-2.5	---	---
	His ¹¹⁸ →Adda ⁵	-1.7	-1.5	-1.5	-1.5	-1.5	-1.5	-1.4
	Arg ⁸⁹ →Glu ⁶	-8.2	-1.6	-8.1	-0.5	---	-7.5	-7.4
	Arg ²⁶⁸ ←Mdha ⁷	-6.7	-6.8	-9.2	-8.8	-8.9	-13.4	-13.7
	Arg ²⁶⁸ →Mdha ⁷	---	-1.9	---	-6.6	-1.5	-2.2	-4.1
	Tyr ¹²⁷ →MeAsp ³	-1.6	---	-1.5	-1.5	-1.6	-1.5	-1.6
	Cys ²⁶⁹ →Mdha ⁷	---	---	---	-1.2	---	-1.1	-0.8
	Gly ²⁷⁰ →Mdha ⁷	---	---	---	---	---	-2.8	---
	Cys ²⁶⁶ →Mdha ⁷	---	-2.3	---	---	---	---	---
Ionic bond (KJ/Mol)	Total	-110.4	-106.6	-109.5	-104.7	-104	-109.7	-109
	Arg ⁸⁹ -MeAsp ³	-7.5	-7.7	-7.7	-6.8	-6.9	-7.9	-7.6
	Arg ⁸⁹ -Glu ⁶	-6.9	-3.2	-5.9	-2.3	-1.7	-6.5	-6.3
	Mn ¹²⁺ -Glu ⁶	-12.2	-12.2	-12.1	-12.1	-12.1	-11.9	-11.8
	Mn ²²⁺ -Glu ⁶	-22.5	-22.4	-22.8	-22.4	-22.3	-22.6	-22.8

	Asp ₅₇ -Mn ₁ ²⁺	-12.2	-12.1	-11.9	-12.1	-11.9	-11.9	-11.9
	Asp ₅₇ -Mn ₂ ²⁺	-15.3	-15.5	-15.4	-15.5	-15.6	-15.4	-15.2
	Asp ₈₅ -Mn ₁ ²⁺	-21.6	-21.5	-21.5	-21.5	-21.5	-21.3	-21.2
	Asp ₈₅ -Mn ₂ ²⁺	-12.2	-12	-12.2	-12	-12	-12.2	-12.2
Metal bond (KJ/Mol)	Total	-42.8	-42.6	-42.1	-42.5	-42.1	-42.5	-42.6
	Mn ₁ ²⁺ -Glu ⁶	-4.6	-4.7	-4.5	-4.7	-4.5	-4.5	-4.3
	Mn ₂ ²⁺ -Glu ⁶	-9.2	-9	-9	-8.9	-8.7	-9.1	-9.1
	Asp ₅₇ -Mn ₁ ²⁺	-5.3	-5.2	-5.1	-5.2	-5.1	-5	-5.1
	Asp ₅₇ -Mn ₂ ²⁺	-5.7	-5.8	-5.8	-5.8	-5.8	-5.7	-5.7
	Asp ₈₅ -Mn ₁ ²⁺	-6.8	-6.5	-6.3	-6.6	-6.8	-6.7	-6.8
	Asp ₈₅ -Mn ₂ ²⁺	-4.8	-4.8	-4.9	-4.9	-4.8	-4.8	-4.8
	His ₂₄₁ -Mn ₁ ²⁺	-2	-2.2	-2.2	-2.1	-2.1	-2.4	-2.6
	Asn ₁₁₇ -Mn ₁ ²⁺	-4.4	-4.4	-4.3	-4.3	-4.3	-4.3	-4.2

^a: --- no related parameter was detected