Supporting Information

Inhibition of the *Yersinia pestis* Methylerythritol Phosphate Pathway of Isoprenoid Biosynthesis by α-Phenyl Substituted Reverse Fosmidomycin Analogs

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Half-maximal inhibition (IC_{50}) determination

Data was collected at 340 nm using an Agilent 8453 UV-Visible Spectrophotometer equipped with a temperature regulated cuvette holder. Half-maximal inhibition (IC_{50}) of enzyme activity was determined using nonlinear regression of a plot of fractional enzyme activity as a function of inhibitor concentration (sigmoidal dose-response curve) using GraphPad Prism 5.0.

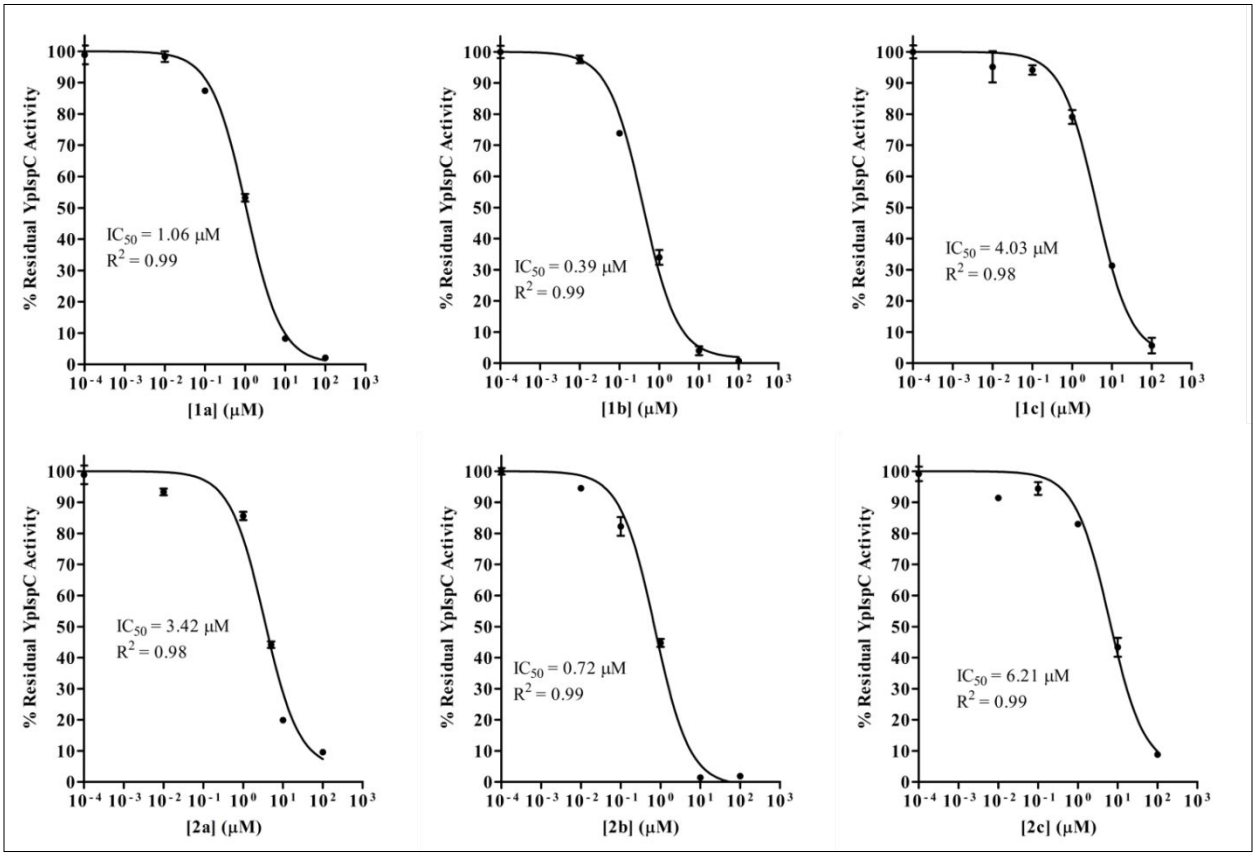


Figure S1. IC_{50} plots indicating dose-dependent inhibition of YpIspC by 1a-2c. The R^2 value for each plot is indicated. IC_{50} values were obtained using GraphPad Prism 5.0. Assays were performed in duplicate. The error bars indicate the standard deviation for each data point.

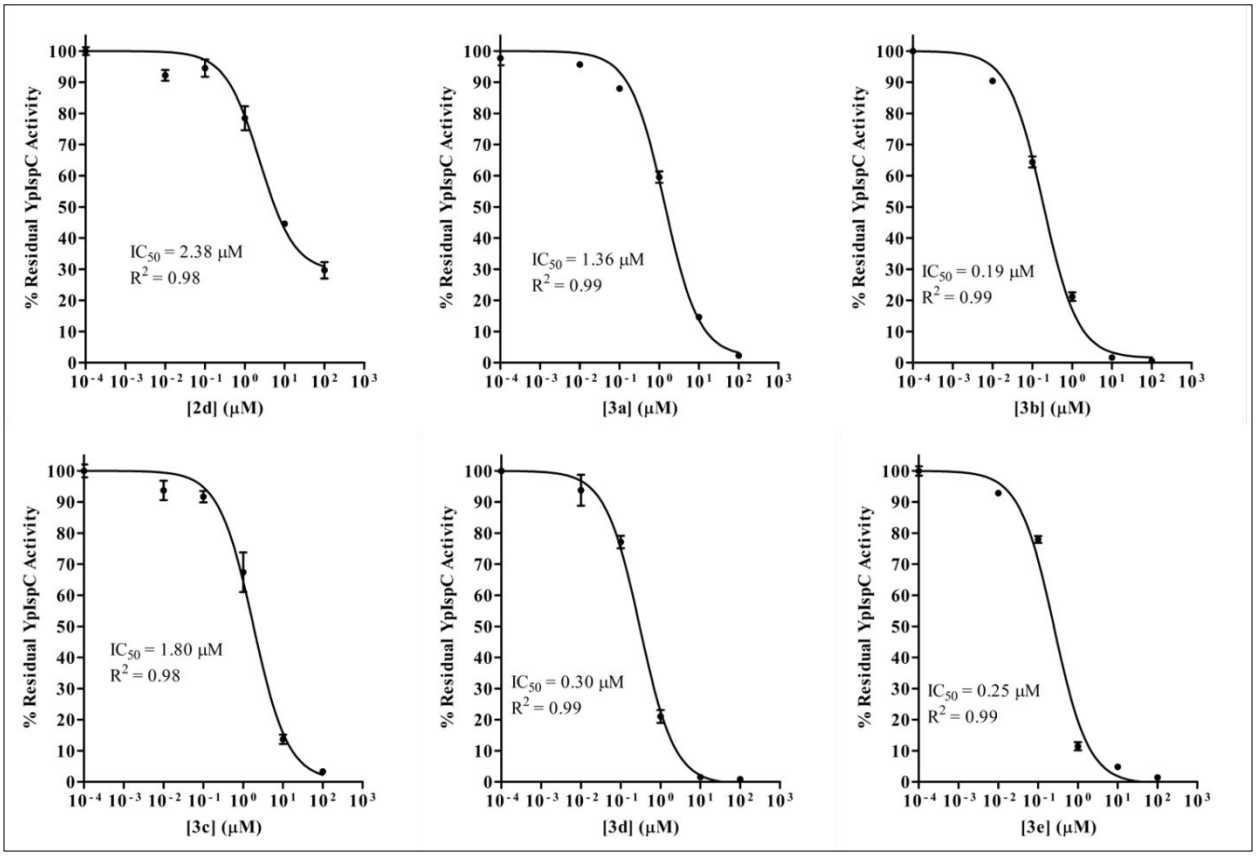


Figure S2. IC_{50} plots indicating dose-dependent inhibition of YpIspC by 2d-3c. The R^2 value for each plot is indicated. IC_{50} values were obtained using GraphPad Prism 5.0. Assays were performed in duplicate. The error bars indicate the standard deviation for each data point.

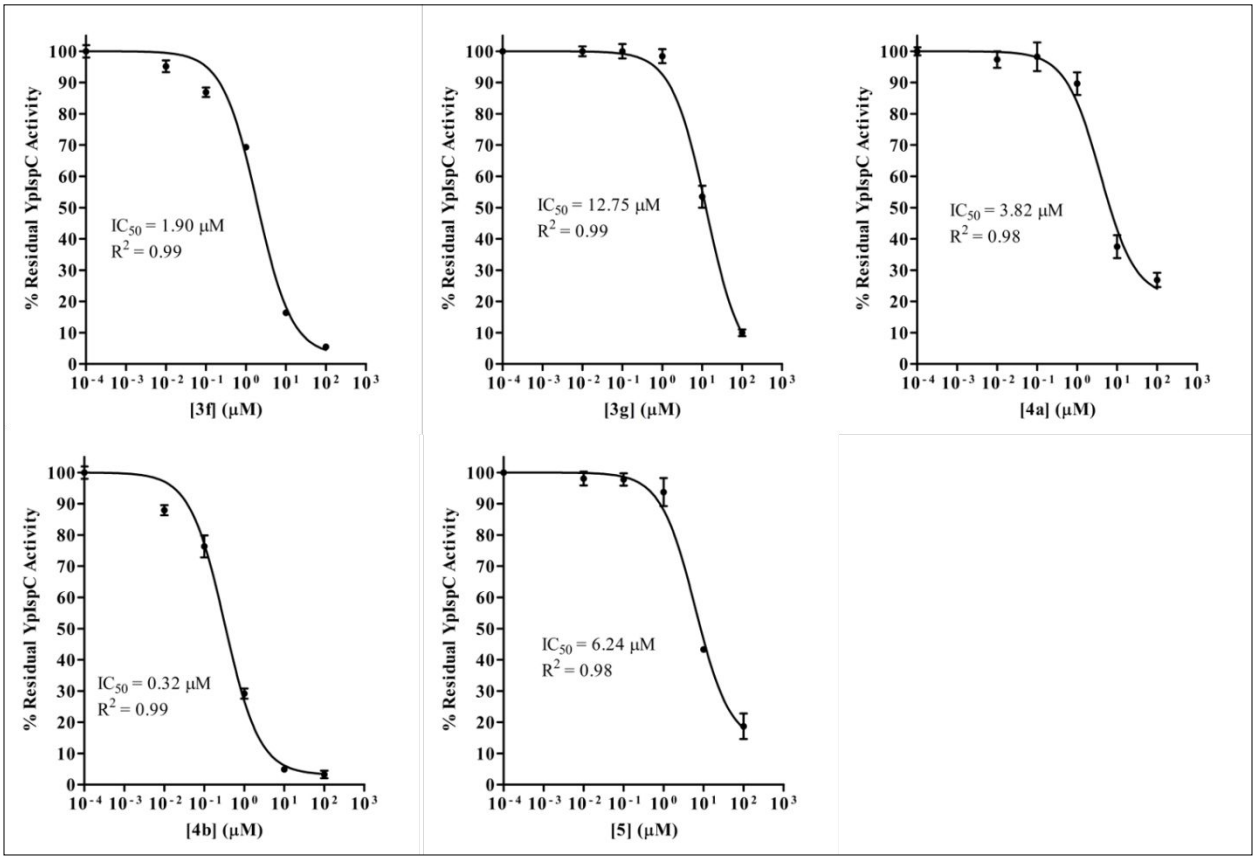
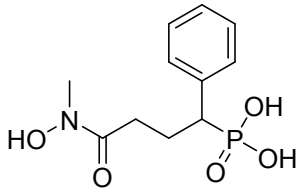


Figure S3. IC_{50} plots indicating dose-dependent inhibition of YpIspC by 3f-5. The R^2 value for each plot is indicated. IC_{50} values were obtained using GraphPad Prism 5.0. Assays were performed in duplicate. The error bars indicate the standard deviation for each data point.

Compound characterization

Melting points (M.p.) were taken in open capillaries on a Stuart melting point apparatus SMP11 and are uncorrected. Proton (^{1}H), carbon (^{13}C) and phosphorus (^{31}P) NMR spectra were recorded on a Bruker Avance 300, 500 and 600MHz using $CDCl_3$ and DMSO-d_6 as solvent. Chemical shifts are given in parts per million (ppm), (δ relative to residual solvent peak for ^{1}H and ^{13}C). High-resolution mass spectrometry (HRMS) analysis was performed using a UHR-TOF maXis 4G instrument (Bruker Daltonics, Bremen, Germany). The purity of compounds was determined by Elemental analysis using a Perkin-Elmer PE 2400 CHN elemental analyzer or by high-performance liquid chromatography (HPLC). Instrument: Elite LaChrom system [Hitachi L-2130 (pump) and L-2400 (UV-detector)]; column: Phenomenex Luna C-18(2) 5 µm particle size (250 mm × 4.6 mm), supported by Phenomenex Security Guard Cartridge Kit C18 (4.0 mm × 3.0 mm).

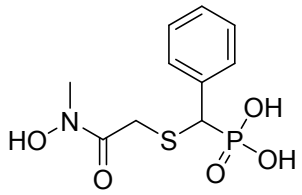


1a

Chemical Formula: $C_{11}H_{16}NO_5P$

Figure S4. [3-[Hydroxy(methyl)amino]-3-oxo-1-phenyl-propyl] phosphonic acid (1a).[1] Yellow oil (238 mg, 92%); ^{1}H NMR (500 MHz, DMSO-d_6): δ=7.54-6.68 (m, 5H), 3.40 (ddd, J = 21.7, 11.0, 2.6 Hz, 1H), 3.22-3.08 (m, 3H), 2.98 (s, 1H), 2.94-2.82 (m, 1H) ppm; ^{13}C NMR (125 MHz, DMSO-d_6): δ=170.6 (d, $^{3}J_{C\text{-}P}$=17.7 Hz), 138.4 (d, $^{2}J_{C\text{-}P}$ = 6.7 Hz), 128.9 (d, $^{3}J_{C\text{-}P}$ = 6.0 Hz), 127.6, 125.9 (d, $^{4}J_{C\text{-}P}$ = 1.7 Hz),

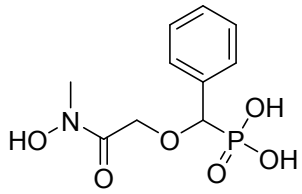
40.5 (d, $^{1}J_{C\text{-}P}$ = 131.4 Hz), 35.7, 32.5 ppm. Anal. calcd. for $C_{10}H_{14}NO_5P$: C 46.34, H 5.44, N 5.40, found: C 46.18, H 6.23, N 4.64. HPLC analysis: retention time = 1.880 min; peak area: 97.83%. Eluent A: 5 mM NH_4OAc solution; eluent B: CH_3CN, isocratic (50:50) over 20 min at a flow rate of 1 mL/min.



1b

Chemical Formula: $C_{10}H_{14}NO_5PS$

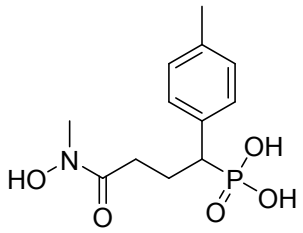
Figure S5. (((2-(Hydroxy(methyl)amino)-2-oxoethyl) thio) (phenyl)methyl) phosphonic acid (1b).[2] White solid; yield 38% (0.111 g, 0.38 mmol). M.p. 112 °C; ^{1}H NMR (500.13 MHz, DMSO-d_6): δ=9.99 (br. s, OH), 7.47–7.35 (m, 2H), 7.34–7.26 (m, 2H), 7.26–7.18 (m, 1H), 4.25 (d, ^{2}J(H,P)=19.0 Hz, 1H, PCH), 3.50 (d, ^{2}J(H,H)=14.6 Hz, 1H, SCH_2), 3.23 (d, ^{2}J(H,H)=14.6 Hz, 1H, SCH_2), 3.06 (s, 3H, NCH_3); ^{13}C NMR (125.76 MHz, DMSO-d_6): δ=168.93 (C=O), 137.36 (d, ^{2}J(C,P)=3.8 Hz), 129.23 (d, ^{3}J(C,P)=5.8 Hz), 127.86, 126.78, 45.72 (d, ^{1}J(C,P)=139.2 Hz, PC), 35.71 (NCH_3), 31.85 (d, ^{3}J(C,P)=6.2 Hz, SCH_2). HRMS (ESI): m/z calculated for $C_{10}H_{14}NO_5PS+H^+$ [M+H^+]: 292.04031; found: 292.03987; HPLC analysis: retention time=1.870 min, peak area: 99.08%, eluent A: NH_4OAc solution (5mM), eluent B: CH_3CN, isocratic (1:1) over 20 min with a flow rate of 1 mL min^{-1}.



1c

Chemical Formula: $C_{10}H_{14}NO_6P$

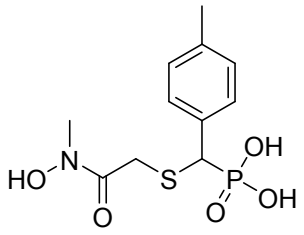
Figure S6. ((2-(hydroxy(methyl)amino)-2-oxoethoxy) (phenyl)methyl) phosphonic acid (1c).[3] White crystals (330 mg, 85%); M.p. 169 °C. ^{1}H-NMR(500 MHz, DMSO-d_6) = δ (ppm) 3.07 (s, 3H, NC*H*$_3$), 4.20 (d, *J*=16.1 Hz, 1H, OC*H*$_2$C=O), 4.34 (d, *J*=16.1 Hz, 1H, OC*H*$_2$C=O), 4.68 (d, $_2$*J*H,P=14.4 Hz, 1H, PC*H*), 7.25–7.40 (m, 5H, arom.), 9.86 (s, 1H, O*H*). 13C-NMR (200 MHz, DMSO-d_6) = δ (ppm) 35.63 (N*C*H$_3$) 66.75 (d, $_3$*J*C, P=10.8 Hz, O*C*H2C=O), 78.65 (d, $_1$*J*C, P=159.0 Hz, P*C*H), 127.35, 127.68 (2C), 127.97 (d, *J*C,P=5.4 Hz, 2C), 136.17, 169.49 (*C*=O); Anal. calcd. for $C_{10}H_{14}NO_6P$: C 43.65, H 5.13, N 5.09, found: C 43.40, H 5.05, N 4.91.



2a

Chemical Formula: $C_{12}H_{18}NO_5P$

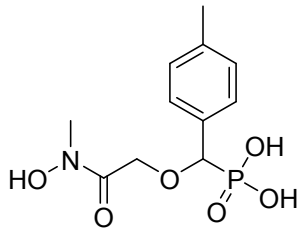
Figure S7. (4-(hydroxy(methyl)amino)-4-oxo-1-(p-tolyl) butyl) phosphonic acid (2a).[4] Colorless oil (430 mg, 93%), ^{1}H-NMR: (500 MHz, DMSO-d_6), (ppm):1.86-2.00 (m, 1H, CHC*H*$_2$), 2.11- 2.23 (m, 3H, CH_2), 2.26 (s, 3H, $PhCH_3$), 2.73-2.89 (m, 1H, PCH), 3.02 (s, 3H, NCH_3), 6.95-7.10 (m, 2H), 7.18 (dd, *J* = 8.01, 1.76 Hz, 2H, aromat.), 10.16 (OH). ^{13}C-NMR: (125 MHz, DMSO-d_6), (ppm): 20.51 ($PhCH_3$), 25.14 (CH_2CH_2).29.98 (d, $_2$*J*C-P = 15.25 Hz, CH*C*H$_2$), 35.54 (NCH_3), 44.26 (d,$_1$*J*C-P = 133.13 Hz, PCH), 128.43, 128.86 (d, $_3$*J*C-P = 6 Hz), 134.85 (d, $_5$*J*C-P = 2.86 Hz), 135.34 (d, $_2$*J*C-P = 6.24 Hz, aromat.), 172.54 (C=O) ^{31}P-NMR: (202,5 MHz, DMSO-d_6), (ppm): 25,07. HPLC analysis: retention time=1.887 min, peak area: 99.6%, eluent A: NH_4OAc solution (5mM), eluent B: CH_3CN, isocratic (1:1) over 20 min with a flow rate of 1 mL min^{-1}.



2b

Chemical Formula: $C_{11}H_{16}NO_5PS$

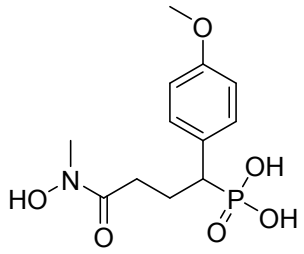
Figure S8. ((3,4-Difluorophenyl) ((2-(hydroxy(methyl)amino)-2-oxoethyl) thio) methyl) phosphonic acid (2b).[5] White solid; yield 45% (0.15 g, 0.46 mmol). M.p. 137 °C; ^{1}H NMR (500.13 MHz, DMSO-d_6): δ=10.04 (br. s, OH), 7.51-7.31 (m, 2H), 7.28–7.14 (m, 1H), 4.28 (d, ^2J(H,P)=19.6 Hz, 0.83H, PCH), 4.21 (d, ^2J(H,P)=19.2 Hz, 0.17H, PCH), 3.46 (d, ^2J(H,H)=14.6 Hz, 1H, SCH_2), 3.23 (d, ^2J(H,H)=14.6 Hz, 1H, SCH_2), 3.09 (s, 0.21H, NCH_3), 3.05 (s, 2.54H, NCH_3), 3.01 ppm (s, 0.25H, NCH_3); ^{13}C NMR (125.76 MHz, DMSO-d_6): δ=168.66 (C=O), 148.79 (dd, ^1J(C,F)=244.7 Hz, ^2J(C,F)=11.7 Hz), 148.34 (dd, ^1J(C,F)=244.8 Hz, ^2J(C,F)=12.5 Hz), 135.58, 126.12 (m), 117.80 (dd, ^2J(C,F)=17.7 Hz, ^3J(C,F)=^3J(C,P)=5.6 Hz), 116.77 (d, ^2J(C,F)=17.0 Hz), 45.01 (d, ^1J(C,P)=137.8 Hz, PC), 35.70 (NCH_3), 31.88 ppm (d, ^3J(C,P)=7.4 Hz, SCH_2); Elemental analysis calculated (%) for $C_{10}H_{12}F_2NO_5PS$: C 36.70, H 3.70, N 4.28, found: C 36.97, H 4.00, N 3.92.



2c

Chemical Formula: $C_{11}H_{16}NO_6P$

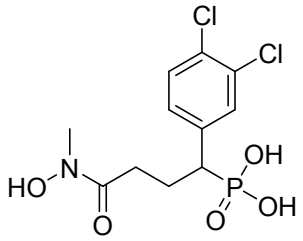
Figure S9. ((2-(hydroxy(methyl)amino)-2-oxoethoxy) (p-tolyl) methyl) phosphonic acid (2c).[3] White crystals (410mg, 65%). M. p. 164 °C. ^{1}H-NMR(500 MHz, DMSO-d_6) = δ (ppm) 2.29 (s, 1H, CH_3), 3.06 (s, 3H, NCH_3), 4.16 (d, *J*=16.1 Hz, 1H, OCH_2C=O), 4.30 (d, *J*=16.1 Hz, 1H, OCH_2C=O), 4.62 (d, ^{2}JH,P=14.3 Hz, 1H, PC*H*), 7.14 (d, *J*=7.8 , 2H, arom.), 7.26 (d, *J*=7.9 Hz, 2H, arom.), 9.84 (s, 1H, O*H*) ^{13}C-NMR(125 MHz, DMSO-d_6) = δ (ppm) 20.64 (*C*H_3), 35.63 (N*C*H_3), 66.55 (d, ^{3}JC,P=11.1), 78.47 (d, ^{1}JC,P=160.0 Hz, P*C*H), 128.02 (d, 3*J*C,P=5.4 Hz, 2C), 128.19 (2C), 133.03, 136.59 (d, ^{5}JP,C=1.3 Hz), 169.53 (*C*=O). Anal. calcd. for $C_{11}H_{16}NO_6P$: C 45.68, H 5.58, N 4.84, found: C 45.46, H 5.72, N 4.93.



2a

Chemical Formula: $C_{12}H_{18}NO_6P$

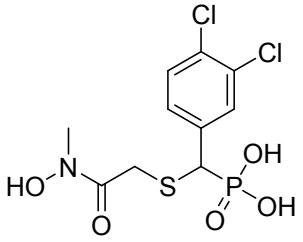
Figure S10. 4-(Hydroxyamino)-1-(4-methoxyphenyl)-4-oxobutylphosphonic acid (2d).[4] White solid (246 mg, 85 %), Mp: 168 °C; 1H NMR (600.22 MHz, DMSO-d_6): δ = 1.77-1.80 (m, 2H, CH2C*H2*), 1.86-1.92 (m, 1H, CHCH_2), 2.19-2.25 (m, 1H, CHCH_2), 2.74 (ddd, *J* = 21.7, 11.1, 3.5 Hz, 1H, PC*H*), 3.72 (s, 3H, OCH_3), 6.85 (d, *J* = 8.5 Hz), 7.15 (d, *J* = 7.1 Hz), 10.28 (s, 1H) ppm; ^{13}C NMR (150.93 MHz, DMSO-d_6): δ = 26.3 (CH_2CH_2), 30.6 (d, $_{2}J$C-P = 14.9 Hz, CH*C*H2), 43.9 (d, ^{1}JC-P = 134.8 Hz, P*C*H), 54.9 (O*C*H_3), 113.4, 129.9 (d, ^{3}JC-P = 5.8 Hz), 157.6, 168.8 (*C*=O) ppm; HPLC analysis: retention time = 1,87 min; peak area: 95.8%. Eluent A, 5 mM NH4OAc solution; eluent B, CH3CN/isocratic (1:1) over 20 min at a flow rate of 1 mL min-1.



3a

Chemical Formula: $C_{11}H_{14}Cl_2NO_5P$

Figure S11. (1-(3,4-Dichlorophenyl)-4-[hydroxy(methyl)amino]-4-oxo-butyl) phosphonic acid (3a).[2] Orange oil (430mg, 93%). ^{1}H-NMR: (500 MHz, DMSO-d_6), (ppm): 1.83-2.00 (m, 1H, CHCH_2), 2.08-2.34 (m, 3H, CH_2), 2.92-3.01 (m, 1H, PCH), 3.03 (s, 3H, NCH_3), 7.15-7.39 (m, 1H), 7.49 (t, J = 1.9 Hz, 1H), 7.57 (d, J = 8.3 Hz, 1H, aromat.), 9.68 (s, 1H) 13C-NMR: (125 MHz, DMSO-d_6), (ppm): 25.34 (CH_2CH_2), 30.02 (d, $_2J$C-P = 14.6 Hz, CHCH$_2$), 36.01 (NCH_3), 44.12 (d, ^1JC-P = 132.6 Hz, PCH), 129.19 (d, 4JC-P = 3.6 Hz), 129.92 (d, 3JC-P = 6.2 Hz), 130.50, 130.90 (d, 4JC-P = 2.5 Hz), 131.21 (d, 3JC-P = 6 Hz), 140.25 (d, 2JC-P = 6.4 Hz, aromat.), 172.66 (C=O) 31P-NMR: (202,5 MHz, DMSO-*d6*), (ppm): 23,13 HPLC analysis: retention time = 1,967 min; peak area: 99.0%. Eluent A, 5 mM NH4OAc solution; eluent B, CH3CN/isocratic (1:1) over 20 min at a flow rate of 1 mL min-1.

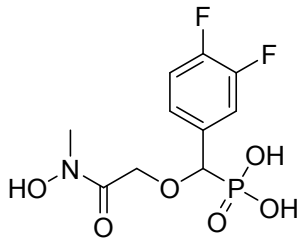


3b

Chemical Formula: $C_{10}H_{12}Cl_2NO_5PS$

Figure S12. (((2-(Hydroxy(methyl)amino-2-oxoethyl) thio)(naphthalen-1-yl)methyl)phosphonic acid (3b).[6] White solid; yield 55% (0.188 g, 0.55 mmol). M.p. 137 °C;

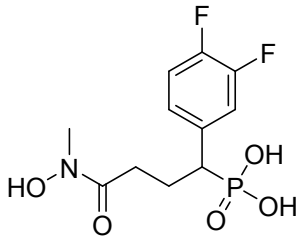
^{1}H NMR (500.13 MHz, [D_6]DMSO): δ=9.99 (br. s, OH), 8.20 (d, *J*=6.9 Hz, 1H), 7.92 (d, *J*=7.9 Hz, 1H), 7.88 (s, 1H), 7.83 (d, *J*=8.1 Hz, 1H), 7.59–7.44 (m, 3H), 5.19 (d, ^{2}J(H,P)=18.3 Hz, 1H, PCH), 3.74 (d, ^{2}J(H,H)=14.2 Hz, 1H, SCH_2), 3.34 (d, ^{2}J(H,H)=14.5 Hz, 1H, SCH_2), 3.05 ppm (s, 3H, NCH_3); ^{13}C NMR (125.76 MHz DMSO-d_6): δ=169.11 (C=O), 133.55 (m), 133.26 (m), 131.28 (m), 128.46, 127.36, 126.95 (m), 126.00 (m), 125.47, 125.22, 123.61, 38.61 (m, PC), 35.72 (NCH_3), 32.34 ppm (m, SCH_2); HRMS (ESI): m/z calculated for $C_{14}H_{16}NO_5PS+H^+$ [M+H^+]: 342.05596; found: 342.05537; HPLC analysis: retention time=1.860 min, peak area: 99.39%, eluent A: NH_4OAc solution (5mM), eluent B: CH_3CN, isocratic (1:1) over 20 min with a flow rate of 1 mL min^{-1} and detection at 254 nm; column temperature: RT.



3c

Chemical Formula: $C_{10}H_{12}F_2NO_6P$

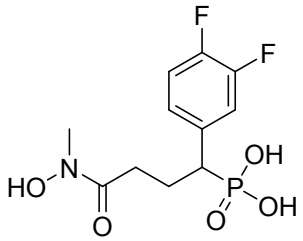
Figure S13. ((3,4-Dichlorphenyl) (2-(hydroxy(methyl)amino)-2-oxoethoxy) methyl) phosphonic acid (3c).[3] Colorless crystals (400 mg, 21%). M. p. 154 °C. 1H-NMR(500 MHz, DMSO-d6) = δ (ppm) 3.07 (s, 3H, NC*H*3), 4.20 (d, *J*=16.0 Hz, 1H, OC*H*2C=O), 4.37 (d, *J*=16.2 Hz, 1H, OC*H*2C=O), 4.74 (d, 2*J*H,P=14.2 Hz, 1H, PC*H*), 7.36 (dt, *J*=8.2, 1.7 Hz, 1H, arom.), 7.59 (t, *J*=1.9 Hz, 1H, arom.), 7.62 (d, *J*=8.3 Hz, 1H, arom.), 9.87 (br. s, 1H, O*H*). ^{13}C-NMR(125 MHz, DMSO-d6) = δ (ppm) 35.63 (N*C*H3), 67.30 (d, 3*J*C,P=9.3 Hz, O*C*H2C=O), 77.45 (d, 1*J*C,P=157.4 Hz, P*C*H), 128.10 (d, 3*J*C,P=5.1 Hz, *C*6H3Cl2: C6), 129.48 (d, 3*J*C,P=5.1 Hz, *C*6H3Cl2: C2), 129.85 (m, 2C), 130.36 (d, *J*C,P=1.9 Hz), 138.01, 169.19 (*C*=O). Anal. calcd. for $C_{11}H_{16}NO_6P$: C 45.68, H 5.58, N 4.84, found: C 45.46, H 5.72, N 4.93.



3d

Chemical Formula: $C_{11}H_{14}F_2NO_5P$

Figure S14. (1-(3,4-Difluorophenyl)-4-[hydroxy(methyl)amino]-4-oxo-butyl) phosphonic acid (3d).[2] Colorless solid (400 mg, 84%). M.p. 116 °C. ^{1}H-NMR: (500 MHz, DMSO-d_6), (ppm): 1.82-1.98 (m, 1H, CHCH_2), 2.20 (s, 3H, CH_2), 2.97 (dd, *J* = 21.7, 9.6 Hz, 1H, PCH), 3.02 (s, 3H, NCH_3), 6.86-7.18 (m, 1H), 7.22-7.30 (m, 1H), 7.35 (dd, *J* = 19.0, 8.91 Hz, 1H, aromat.), 9.67 (s, 1H, OH) ^{13}C-NMR: (125 MHz, DMSO-d_6), (ppm): 25.00 (CH2*C*H2), 29.69 (d, 2*J*C-P = 15.1 Hz, CH*C*H_2), 35.54 (NCH_3), 43.61 (d, ^{1}*J*C-P = 133.4 Hz, PCH), 116.77 (d, ^{2}*J*C-F = 16.6 Hz, ^{3}*J*C-F = 1.5 Hz), 117.54 (dd, ^{2}*J*C-F = 16.9 Hz, 3*J*C-F = 5.8 Hz), 125.82, 136.13, 148.96 (dd, ^{1}*J*C-F = 250.6 Hz, 2*J*C-F = 13.9 Hz), 149.76 (dd, ^{1}*J*C-F = 242.9 Hz, ^{2}*J*C-F = 10.8 Hz, aromat.), 172.20 (C=O) ^{31}P-NMR: (202,5 MHz, DMSO-*d6*), (ppm): 23,84. Anal. calcd. for $C_{11}H_{14}NF_2O_5P$: C 42.73, H 4.56, N 4.53, found: C 43.00, H 4.77, N 4.50.

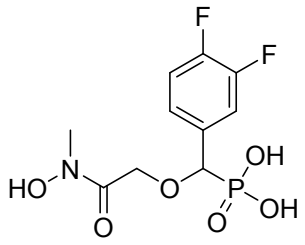


3e

Chemical Formula: $C_{11}H_{14}F_2NO_5P$

Figure S15. ((3,4-Difluorophenyl) ((2-(hydroxy(methyl)amino)-2-oxoethyl) thio) methyl) phosphonic acid (3e): White solid; yield 45% (0.15 g, 0.46 mmol). M.p. 137 °C; ^{1}H NMR

(500.13 MHz, DMSO-d_6): δ=10.04 (br. s, OH), 7.51-7.31 (m, 2H), 7.28–7.14 (m, 1H), 4.28 (d, ^2J(H,P)=19.6 Hz, 0.83H, PCH), 4.21 (d, ^2J(H,P)=19.2 Hz, 0.17H, PCH), 3.46 (d, ^2J(H,H)=14.6 Hz, 1H, SCH_2), 3.23 (d, ^2J(H,H)=14.6 Hz, 1H, SCH_2), 3.09 (s, 0.21H, NCH_3), 3.05 (s, 2.54H, NCH_3), 3.01 ppm (s, 0.25H, NCH_3); ^{13}C NMR (125.76 MHz, DMSO-d_6): δ=168.66 (C=O), 148.79 (dd, ^1J(C,F)=244.7 Hz, ^2J(C,F)=11.7 Hz), 148.34 (dd, ^1J(C,F)=244.8 Hz, ^2J(C,F)=12.5 Hz), 135.58, 126.12 (m), 117.80 (dd, ^2J(C,F)=17.7 Hz, ^3J(C,F)=^3J(C,P)=5.6 Hz), 116.77 (d, ^2J(C,F)=17.0 Hz), 45.01 (d, ^1J(C,P)=137.8 Hz, PC), 35.70 (NCH_3), 31.88 ppm (d, ^3J(C,P)=7.4 Hz, SCH_2); Analysis calculated (%) for $C_{10}H_{12}F_2NO_5PS$: C 36.70, H 3.70, N 4.28, found: C 36.97, H 4.00, N 3.92.

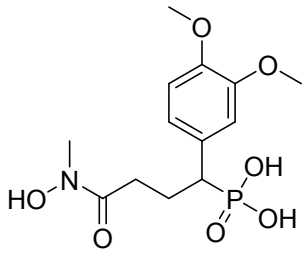


3f

Chemical Formula: $C_{10}H_{12}F_2NO_6P$

Figure S16. (3,4-Difluorphenyl) (2-(hydroxy(methyl)amino)-2-oxoethoxy) methyl) phosphonic acid (3f).[6] Colorless crystal (230mg, 55%). M. p. 160°C. ^{1}H-NMR(500 MHz, DMSO-d_6) = δ (ppm) 3.07 (s, 3H, NCH_3), 4.26 (d, *J*=16.1 Hz, 1H, OCH_2C=O), 4.36 (d, *J*=16.1 Hz, 1H, OCH_2C=O), 4.72 (d, ^2JH,P=14.1 Hz, 1H, PC*H*), 7.19–7.25 (m, 1H, $C_6H_3F_2$), 7.34–7.46 (m, 2H, $C_6H_3F_2$), 9.88 (br. s, 1H, O*H*) ^{13}C-NMR(125 MHz, DMSO-d_6) = δ (ppm) 35.65 (N*C*H_3), 67.14 (d, $_3J$C,P=8.5 Hz, O*C*H_2C=O), 77.51 (d, ^1JC,P=158.7 Hz, P*C*H), 116.63 (m, $C_6H_3F_2$: C2, C5), 124.68 (m, $C_6H_3F_2$: C6), 134.42 (m, $C_6H_3F_2$: C1), 148.73 (ddd, ^1JC,F=244.2 Hz, ^2JC,F=12.6 Hz, 5JC,P=2.6 Hz, $C_6H_3F_2$: C3), 148.89 (ddd, ^1JC,F=244.9 Hz, ^2JC,F=12.6 Hz,

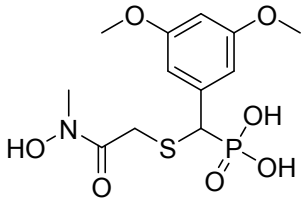
^{4}JC,P=2.1 Hz, $C_6H_3F_2$: C4), 169.27 (C=O) Anal. calcd. for $C_{10}H_{12}NF_2NO_5P$: C 38.36, H 3.89, N 4.50, found: C 38.85, H 4.08, N 4.58.



3g

Chemical Formula: $C_{13}H_{20}NO_7P$

Figure S17. 1-(3,4-Dimethoxyphenyl)-4-[hydroxy(methyl)amino]-4-oxobutylphosphonic acid (3g).[4] White solid (290 mg, 87 %) mp: 70 °C; ^{1}H NMR (500.13 MHz, DMSO-d_6): δ = 1.89-1.99 (m, 1H, CH_2), 2.19 (s, 3H, CH_2), 2.77-2.84 (m, 1H, PC*H*), 3.03 (s, 3H, NCH_3), 3.71 (s, 3H, OCH_3), 3.72 (s, 3H, OCH_3), 6.74-6.76 (m, 1H), 6.85-6.87 (m, 2H), 9.68 (br s, 1H, O*H*) ppm; ^{13}C NMR (125.76 MHz, DMSO-d_6): δ = 25.3 (CH_2CH_2), 30.1 (d, $^{2}J_{C\text{-}P}$ = 15.2 Hz, $CHCH_2$), 35.6 (NCH_3), 44.1 (d, $^{1}J_{C\text{-}P}$ = 136.8 Hz, P*C*H), 55.3 (OCH_3), 55.45 (OCH_3), 111.6, 112.8 (d, $^{3}J_{C\text{-}P}$ = 5.6 Hz), 121.3 (d, $^{2}J_{C\text{-}P}$ = 7.2 Hz), 130.5 (d, $^{3}J_{C\text{-}P}$ = 6.5 Hz), 147.3, 148.3, 172.7 (*C*=O) ppm; Anal. calcd. for $C_{13}H_{20}NO_7P$: C 46.85, H 6.05, N 4.20, found: C 46.61, H 6.05, N 4.06.

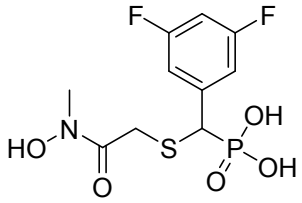


4a

Chemical Formula: $C_{12}H_{18}NO_7PS$

Figure S18. (((2-(Hydroxy(methyl)amino)-2-oxoethyl)thio)(4-(methylthio)phenyl)methyl)phosphonic acid (4a).[5] white solid; yield: 80%. M. p. 134 °C; ^{1}H

NMR (600.22 MHz, DMSO-d_6): δ=10.00 (br. s, OH), 7.33 (dd, ^3J(H,H)=8.3 Hz, ^4J(H,H)=1.6 Hz, 2H, $H_{arom.}$), 7.20 (d, ^3J(H,H)=8.3 Hz, 2H, $H_{arom.}$), 4.23 (d, ^2J(H,P)=19.2 Hz, 1H, PCH), 3.48 (d, ^2J(H,H)=14.6 Hz, 1H, SCH_2), 3.21 (d, ^2J(H,H)=14.6 Hz, 1H, SCH_2), 3.06 (s, 3H, NCH_3), 2.46 ppm (s, 3H, SCH_3); ^{13}C NMR (150.93 MHz, DMSO-d_6): δ=168.90 (C=O), 136.49 ($C_{arom.}$), 133.93 (d, ^2J(C,P)=4.1 Hz, $C_{arom.}$), 129.79 (d, ^3J(C,P)=5.8 Hz, $C_{arom.}$), 125.49 ($C_{arom.}$), 45.32 (d, ^1J(C,P)=139.5 Hz, PC), 35.73 (NCH_3), 31.76 (d, ^3J(C,P)=6.5 Hz, SCH_2), 14.65 ppm (SCH_3); HPLC analysis: retention time=2.011 min, peak area: 98.90%, eluent A: NH_4OAc solution (5 mM), eluent B: CH_3CN, isocratic (1:1) over 30 min with a flow rate of 1 mL min^{-1} and detection at 254 nm.

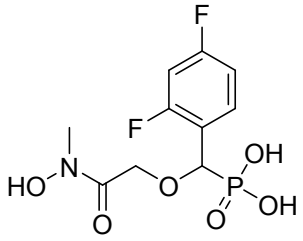


4b

Chemical Formula: $C_{10}H_{12}F_2NO_5PS$

Figure S19. ((3,5-Difluorophenyl) ((2-(hydroxy(methyl)amino)-2-oxoethyl) thio) methyl) phosphonic acid (4b).[5] White solid; yield: 60%. M. p. 135 °C; ^{1}H NMR (500.13 MHz, DMSO-d_6): δ=10.01 (br. s, OH), 7.17-7.12 (m, 1H, $H_{arom.}$), 7.09 (d, ^3J(H,H)=8.3 Hz, 2H, $H_{arom.}$), 4.33 (d, ^2J(H,P)=19.8 Hz, 0.87H, PCH), 4.25 (d, ^2J(H,P)=19.8 Hz, 0.13H, PCH), 3.48 (d, ^2J(H,H)=14.6 Hz, 1H, SCH_2), 3.28 (d, ^2J(H,H)=14.7 Hz, 1H, SCH_2), 3.09 (s, 0.31H, NCH_3), 3.05 (s, 2.53H, NCH_3), 3.01 ppm (s, 0.16H, NCH_3); ^{13}C NMR (125.76 MHz, DMSO-d_6): δ=168.54 (C=O), 161.78 (dd, ^1J(C,F)=246.8 Hz, ^3J(C,F)=13.0 Hz, $C_{arom.}$), 142.47-142.26 (m, $C_{arom.}$), 112.27 (dd, ^2J(C,F)=25.7 Hz, ^4J(C,F)=^3J(C,P)=5.7 Hz, $C_{arom.}$), 102.69-102.16 (m, $C_{arom.}$), 45.39 (d, ^1J(C,P)=137.5 Hz, PC), 35.69 (NCH_3), 32.04 ppm (d, ^3J(C,P)=7.8 Hz, SCH_2); HPLC

analysis: retention time=1.870 min, peak area: 99.41%, eluent A: NH_4OAc solution (5 mM), eluent B: CH_3CN, isocratic (1:1) over 20 min with a flow rate of 1 mL min^{-1} and detection at 254 nm.



4b

Chemical Formula: $C_{10}H_{12}F_2NO_6P$

Figure S20. ((2,4-Difluorphenyl) (2-(hydroxy(methyl)amino)-2-oxoethoxy) methyl) phosphonic acid (5).[3] Colorless crystals (740mg, 47%). M. p. 155°C. 1H-NMR(500 MHz, DMSO-d6) = δ (ppm) 3.06 (s, 3H, NC*H*3), 4.22 (d, *J*=16.1 Hz, 1H, OC*H*2C=O), 4.35 (d, *J*=16.0 Hz, 1H, OC*H*2C=O), 4.93 (d, 2*J*H,P=13.5 Hz, 1H, PC*H*), 7.12 (dt, 3*J*H,F=8.6 Hz, 5*J*H,P=2.2 Hz, 1H, C6*H*3F2: H3), 7.19 (dt, 3*J*H,H=3*J*H,F=9.8 Hz, 5*J*H,P=2.1 Hz, 1H, C6*H*3F2: H5), 7.54 (ddt, 3*J*H,H=4*J*H,F=8.4 Hz, 4*J*H,F'=6.8 Hz, 4*J*H,P=1.6 Hz, 1H, C6*H*3F2: H6), 9.82 (s, 1H, O*H*) 13C-NMR(125 MHz, DMSO-d6) = δ (ppm) 35.58 (N*C*H3), 66.77 (d, 3*J*C,P=8.8 Hz, O*C*H2C=O), 70.93 (d, 1*J*C,P=162.9 Hz, P*C*H), 103.18 (t, 2*J*C,F=2*J*C,F'=26.5 Hz, *C*6H3F2: C3), 111.20 (d, 2*J*C,F=21.2 Hz, *C*6H3F2: C5) 120.23 (dd, 2*J*C,F=14.8 Hz, 2*J*C,P=2.5 Hz, *C*6H3F2: C1), 130.76 (m, *C*6H3F2: C6), 159.94 (ddd, 1*J*C,F=248.2 Hz, 3*J*C,F=12.5 Hz, 3*J*C,P=6.5 Hz, *C*6H3F2: C2), 161.14 (ddd, 1*J*C,F=245.3 Hz, 3*J*C,F=12.4 Hz, 5*J*C,P=2.7 Hz, *C*6H3F2: C4), 169.02 (C=O)Anal. calcd. for $C_{10}H_{12}F_2NO_6P$: C 38.60, H 3.89, N 4.50, found: C 38.83, H 3.79, N 4.40

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