COM# Supplementary Material (ESI) for Chemical Communications

Inhibition of pyruvate decarboxylase from *Z. mobilis* by novel analogues of thiamine pyrophosphate: investigating pyrophosphate mimics

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Supporting Information

General methods for assay

All assays were performed on a Cary 100 Bio UV-visible spectrophotometer, using disposable plastic cuvettes (Fisherbrand semi-micro polystyrene). Buffer and assay solutions were prepared on the day of use.

Assay for PDC activity[5]

The catalytic activity of PDC was measured spectrophotometrically (at 340 nm and 25 ºC) by the rate of NADH oxidation in a coupled assay with ADH (alcohol dehydrogenase). The assay solution, used in all experiments, contained ADH (10 units per ml), NADH (0.15 mM), TPP (0.1 mM) and $MgCl_2$ (5 mM) in a MES-KOH buffer (50 mM; pH 6.5). The enzyme activity was tested by first incubation of apoPDC in the buffer system and the assay was then started by addition of the substrate pyruvate (to give 5 mM).

Time-dependent inactivation and reactivation of PDC

ApoPDC (ca. 0.3 mg / ml) was incubated at 25 ºC with $MgCl_2$ (5 mM) and inhibitor (various concentrations, *e.g.* from 2 μM to 10 μM) in MES-KOH buffer (50 mM; pH 6.5). At timed intervals, aliquots (20 μl) were added to the assay mixture (778 μl) and after a further incubation at 25 ºC for 3 min pyruvate (2 μl of a 2 M solution, to give 5 mM) was added and the decrease of absorbance was measured. In order to be able to calculate the activity and to secure the stability of the enzyme, control experiments were carried out without inhibitor present. The percentage activity was calculated from the ratio of the activity with inhibitor to the corresponding activity obtained without inhibitor.

In order to study the rate of unbinding, apoenzyme was first incubated with inhibitor (10-20 μM) until low (or no) activity was observed, an excess of TPP (to give 100 μM to 10 mM) was then added and the recovery of activity was followed for several (7-8) days.

Double exponential curves

Attached below are the double exponential curves produced using a the equation $y= (1-x)*\exp(-k_1t) + x*\exp(-k_2t)$, this gave a better fitting compare to a simple exponential curve, which supports the proposed two-step mechanism. For all the curves in Figs. S1-S4 the value of x was fixed at 0.35. In each case the inset shows the plot of the faster of the two apparent first order rate constants, k_1, plotted versus inhibitor concentration; the slope of the graph gives k_{on}. the second order rate constant for the initial binding of the inhibitor.

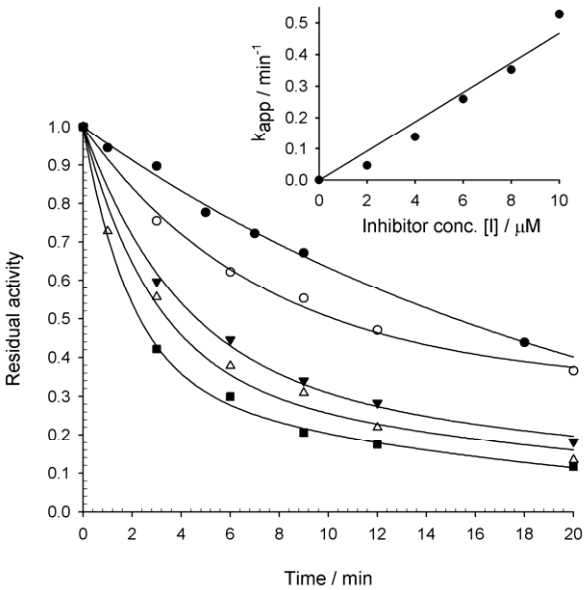

Fig. S1 Inactivation of ZmPDC by various concentrations of TPP analogue **9**; from top to bottom curve:● 2 μM, ○ 4 μM, ▼ 6 μM, Δ 8 μM, ■ 10 μM Inset: Apparent first order rate constants plotted versus inhibitor concentrations, the slope of the graph gives k_{on}.

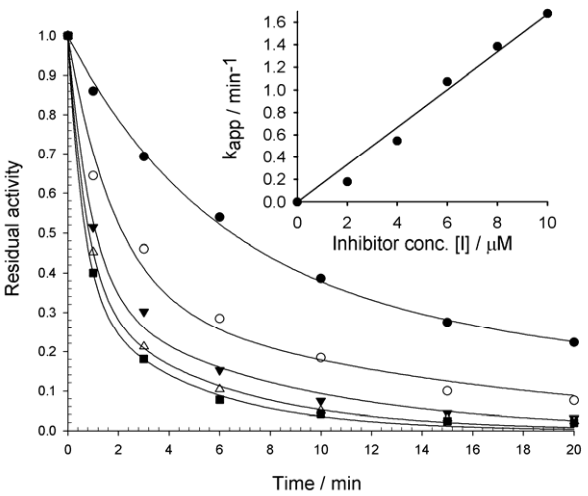

Fig. S2 Inactivation of ZmPDC by various concentrations of TPP analogue **11**; from top to bottom curve:● 2 μM, ○ 4 μM, ▼ 6 μM, Δ 8 μM, ■ 10 μM. Inset: Apparent first order rate constants plotted versus inhibitor concentrations, the slope of the graph gives k_{on}.

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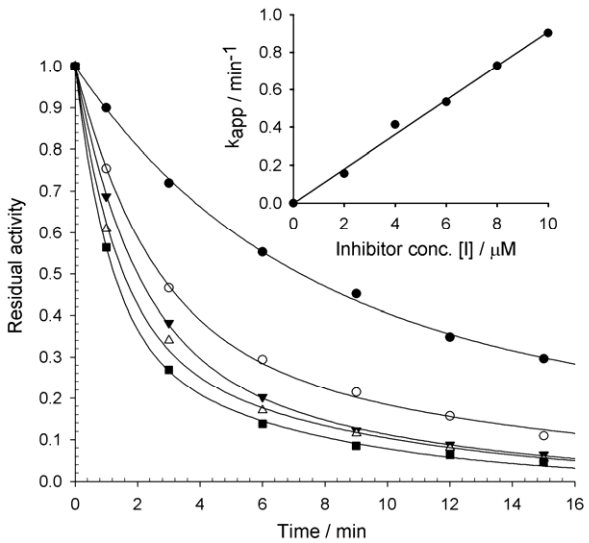

Fig. S3 Inactivation of ZmPDC by various concentrations of TPP analogue **12**; from top to bottom curve:● 2 μM, ○ 4 μM, ▼ 6 μM, Δ 8 μM, ■ 10 μM. Inset: Apparent first order rate constants plotted versus inhibitor concentrations, the slope of the graph gives k_{on}.

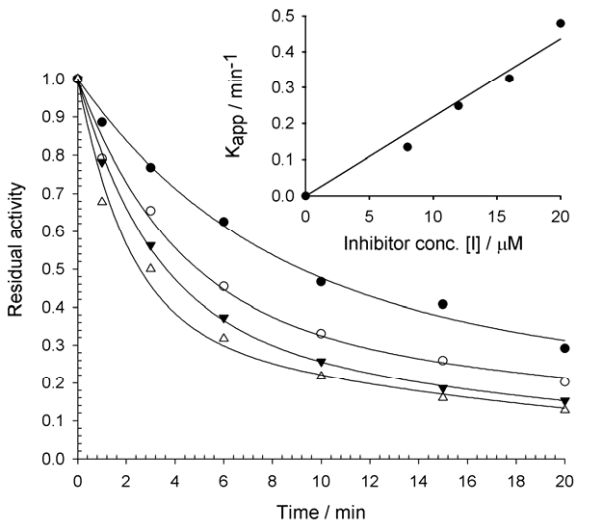

Fig. S4 Inactivation of ZmPDC by various concentrations of TPP analogue **13**; from top to bottom curve: ● 8 μM, ○ 12 μM, ▼ 16 μM, Δ 20 μM. Inset: Apparent first order rate constants plotted versus inhibitor concentrations, the slope of the graph gives k_{on}.

Reactivation graphs

The results from the reactivation studies are shown in the below.

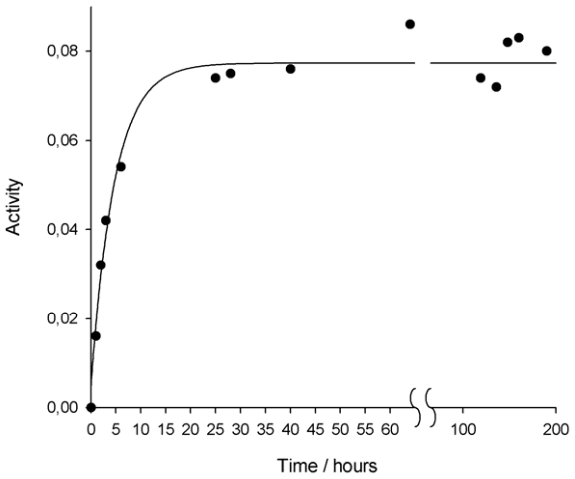

Fig. S5 Recovery of activity for ZmPDC fully inhibited by TPP analogue **9** (10 μM) and then incubated with TPP (10 mM).

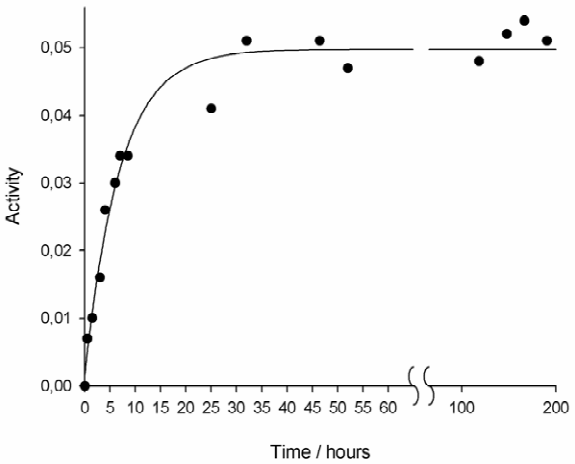

Fig. S6 Recovery of activity for ZmPDC fully inhibited by TPP analogue **10** (10 μM) and then incubated with TPP (10 mM).

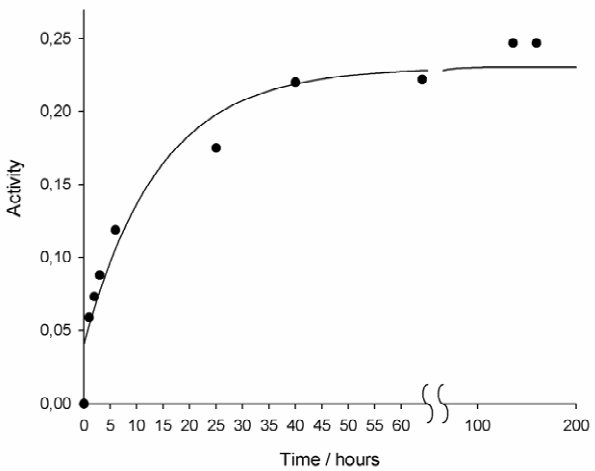

Fig. S7 Recovery of activity for ZmPDC fully inhibited by TPP analogue **11** (10 μM) and then incubated with TPP (1 mM).

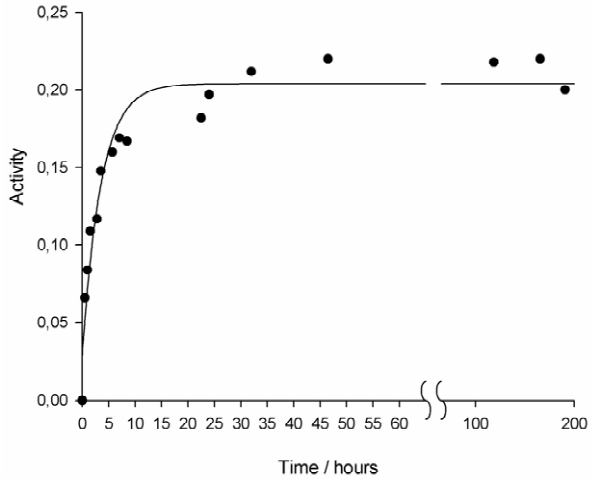

Fig. S8 Recovery of activity for ZmPDC fully inhibited by TPP analogue **12** (10 μM) and then incubated with TPP (1 mM).

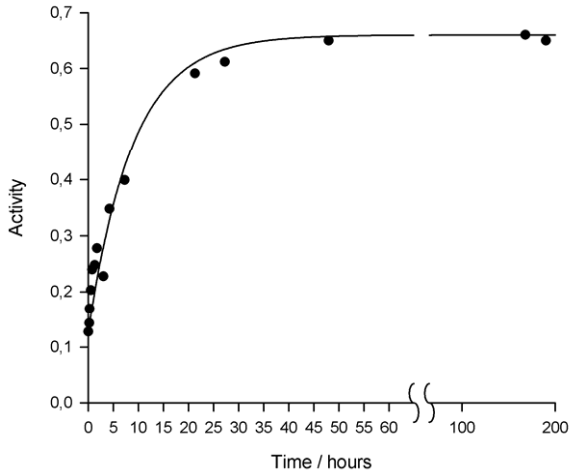

Fig. S9 Recovery of activity for ZmPDC inhibited by TPP analogue **13** (20 μM) and then incubated with TPP (100 μM).

General methods for synthesis

Proton NMR spectra were recorded on a Bruker AM/DPX 400 (400 MHz) or a Bruker DPX 500 (500 MHz) spectrometers. The chemical shifts (δ) and coupling constants (*J*) are given in ppm (reported downfield TMS as internal standard) and Hz, respectively. Carbon NMR spectra were recorded on either a Bruker AC/DPX 400 (100 MHz) or a Bruker DPX 500 (125 MHz) spectrometer. Mass spectra were run on a Bruker BioApex II 4.7e FTICR or Waters LCT Premier using electron-spray ionization (ESI).

Characterisation data

Reported below are some analysis data of the final products **9** to **16**.

2-[1-(4-Amino-2-methyl-pyrimidin-5-ylmethyl)-1*H*-[1,2,3]triazol-4-yl]ethyl pyrophosphate (9)

[Found: M + H^+ (+ESI), 395.0645 $C_{10}H_{16}N_6O_7$ requires M + H, 395.0628]; δ_H (400 MHz, D_2O) 2.31 (3H, s, CH_3), 2.94 (2H, t, *J* 6.6 CH_2CH$_2$O), 4.02 (2H, dt, *J* 6.6 and *J* 6.7 CH$_2$CH_2OP), 5.37 (2H, s, CH_2 bridge), 7.85 (1 H, s, triazoleC*H*), 7.97 (1 H, s, pyrimidineC*H*); δ_C (126 MHz, D_2O) 23.5 (*C*H$_3$), 26.3 (*C*H$_2$CH$_2$O), 47.5 (*C*H$_2$ bridge), 64.3 (CH$_2$*C*H$_2$O), 108.4 (*C*CNH$_2$), 124.2 (triazole*C*H), 145.3 (triazole*C*), 154.6 (pyrimidine*C*H), 161.8 and 167.3 (*C*N*C*NH$_2$).

2-[1-(4-Amino-2-methyl-pyrimidin-5-ylmethyl)-5-methyl-1*H*-[1,2,3]triazol-4-yl]ethyl pyrophosphate (10)

[Found: M + H^+ (+ESI), 409.0794 $C_{10}H_{16}N_6O_7$ requires M + H, 409.0785]; δ_H (400 MHz, D_2O) 2.22 (3H, s, CH_3), 2.34 (3H, s, CH_3), 2.92 (2H, t, *J* 6.5 CH_2CH$_2$O), 4.03 (2H, dt, *J* 6.5 and *J* 6.9 CH$_2$CH_2OP), 5.30 (2H, s, CH_2 bridge), 7.78 (1 H, s, pyrimidineC*H*); δ_C (126 MHz, D_2O) 7.07 (triazole-*C*H$_3$), 22.3 (pyrimidine-*C*H$_3$), 25.6 (*C*H$_2$CH$_2$O), 45.2 (*C*H$_2$-bridge), 65.0 (CH$_2$*C*H$_2$O), 108.6 (*C*CNH$_2$), 133.7 and 142.7 (triazole*C*), 150.4 (pyrimidine*C*H), 162.1 and 165.1 (*C*N*C*NH$_2$).

({2-[1-(4-Amino-2-methyl-pyrimidin-5-ylmethyl)-1*H*-[1,2,3]triazol-4-yl]-ethoxy}hydroxyphosphorylmethyl) phosphonic acid (11)

[Found: M - H^+ (-ESI), 391.0685 $C_{11}H_{18}N_6O_6P_2$ requires M - H, 391.0685]; δ_H (400 MHz, D_2O) 2.00 (2H, t, *J* 19.8, PCH_2P), 2.36 (3H, s, CH_3), 2.95 (2H, t, *J* 6.4, CH_2CH$_2$O), 4.00 (2H, dt, *J* 6.5 and *J* 6.7 CH$_2$CH_2OP), 5.41 (2H, s, CH_2 bridge), 7.88 (1 H, s, triazoleC*H*), 8.01 (1 H, s, pyrimidineC*H*); δ_C (126 MHz, D_2O) 12.7 (P*C*H$_2$P), 23.4 (*C*H$_3$), 26.6 (*C*H$_2$CH$_2$O), 47.4 (*C*H$_2$ bridge), 63.1 (CH$_2$*C*H$_2$O), 108.5 (*C*CNH$_2$), 124.1 (triazole*C*H), 145.3 (triazole*C*), 153.9 (pyrimidine*C*H), 161.8 and 167.0 (*C*N*C*NH$_2$).

({2-[1-(4-Amino-2-methyl-pyrimidin-5-ylmethyl)-1*H*-[1,2,3]triazol-4-yl]-ethoxy}hydroxyphosphoryldifluoromethyl) phosphonic acid (12)

[Found: M + H^+ (+ESI), 429.0645 $C_{11}H_{18}N_6O_6P_2$ requires M + H, 429.0647]; δ_H (400 MHz, D_2O) 2.34 (3H, s, CH_3), 2.96 (2H, t, *J* 6.5, CH_2CH$_2$O), 4.14 (2H, dt, *J* 6.6 and *J* 7.0 CH$_2$CH_2OP), 5.40 (2H, s, CH_2 bridge), 7.90 (1 H, s, triazoleC*H*), 8.02 (1 H, s, pyrimidineC*H*); δ_C (126 MHz, D_2O) 22.8 (*C*H$_3$), 27.5 (*C*H$_2$CH$_2$O), 49.8 (*C*H$_2$-bridge), 66.1 (CH$_2$*C*H$_2$O), 110.1 (*C*CNH$_2$), 128.4 (triazole*C*H), 141.3 (triazole*C*), 145.2 (pyrimidine*C*H), 164.7 and 165.2 (*C*N*C*NH$_2$).

N-(O-{2-[1-(4-Amino-2-methyl-pyrimidin-5-ylmethyl)-1*H*-[1,2,3]triazol-4-yl]ethoxy}sulfonyl)phosphoramidic acid (13)

[Found: M + H^+ (+ESI), 394.0702 $C_{10}H_{16}N_7O_6PS$ requires M + H, 394.0699]; δ_H (400 MHz, D_2O) 2.32 (3H, s, CH_3), 3.04 (2H, t, *J* 6.2 CH_2CH$_2$O), 4.28 (2H, t, *J* 6.2, CH$_2$CH_2OP), 5.38 (2H, s, CH_2 bridge), 7.85 (1 H, s, triazoleC*H*), 7.98 (1 H, s, pyrimidineC*H*); δ_C (126 MHz, D_2O) 23.3 (*C*H$_3$), 24.4 (*C*H$_2$CH$_2$O), 47.1 (*C*H$_2$ bridge), 67.7 (CH$_2$*C*H$_2$O), 107.9 (*C*CNH$_2$), 123.7 (triazole*C*H), 144.1 (triazole*C*), 155.0 (pyrimidine*C*H), 161.2 and 167.3 (*C*N*C*NH$_2$).

2-[1-(4-Amino-2-methyl-pyrimidin-5-ylmethyl)-1*H*-[1,2,3]triazol-4-yl]ethyl N-(sulfo)carbamate (14)

[Found: M + H^+ (+ESI), 358.0920 $C_{11}H_{15}N_7O_5S$ requires M + H, 358.0934]; ν_{max}/cm^{-1} 3137 (NH$_2$), 1714 (carbonyl), 1651 and 1601 (pyrimidine ring); δ_H (400 MHz, D_2O) 2.34 (3H, s, CH_3), 2.98 (2H,

t, *J* 6.1 CH_2CH$_2$O), 4.27 (2H, t, *J* 6.1, CH$_2$CH_2O), 5.39 (2H, s, CH_2 bridge), 7.79 (1 H, s, triazoleC*H*), 8.00 (1 H, s, pyrimidineC*H*); δ_C (100 MHz, D$_2$O) 22.3 (*C*H$_3$), 23.8 (*C*H$_2$CH$_2$O), 46.4 (*C*H$_2$ bridge), 63.5 (CH$_2$*C*H$_2$O), 107.6 (*C*CNH$_2$), 123.1 (triazole*C*H), 143.8 (triazole*C*), 152.2 (pyrimidine*C*H), 160.9 and 165.8 (*C*N*C*NH$_2$).

Mono-{2-[1-(4-amino-2-methyl-pyrimidin-5-ylmethyl)-1*H*-[1,2,3]triazol-4-yl]ethyl} malonate (15)

[Found: M + H$^+$ (+ESI), 321.1297 $C_{13}H_{16}N_6O_4$ requires M + H, 321.1311]; δ_H (400 MHz, MeOD) 2.54 (3 H, s, C*H*$_3$), 3.07 (2H, t, *J* 6.4 CH_2CH$_2$O), 3.35 (2H, s, CH_2), 4.38 (2H, t, *J* 6.4 CH$_2$CH_2O), 5.53 (2H, s, CH_2 bridge), 7.92 (1 H, s, triazoleC*H*), 8.01 (1 H, s, pyrimidineC*H*); δ_C (100 MHz, MeOD) 24.4 (*C*H$_3$), 28.8 (*C*H$_2$CH$_2$O) 50.2 (*C*H$_2$ bridge), 67.6 (CH$_2$*C*H$_2$O), 113.8 (*C*CNH$_2$), 127.6 (triazole*C*H), 147.4 (pyrimidine*C*H), 148.6 (triazole*C*), 166.1, 167.8, 171.2 and 172.9 (2x carbonyl and *C*N*C*NH$_2$).

({2-[1-(4-Amino-2-methyl-pyrimidin-5-ylmethyl)-1H-[1,2,3]triazol-4-yl]ethoxycarbonylmethyl}amino)acetic acid (16)

[Found: M + H$^+$ (+ESI), 350.1582 $C_{14}H_{19}N_7O_4$ requires M + H, 350.1571]; δ_H (400 MHz, D$_2$O) 2.33 (3H, s, C*H*$_3$), 3.00 (2H, t, *J* 6.2 CH_2CH$_2$O), 3.49 (2H, s, CH_2), 3.83 (2H, s, CH_2), 4.40 (2H, t, *J* 6.2, CH$_2$CH_2O), 5.39 (2H, s, CH_2 bridge), 7.78 (1 H, s, triazoleC*H*), 7.95 (1 H, s, pyrimidineC*H*); δ_C (100 MHz, D$_2$O) 23.9 (*C*H$_3$), 24.7 (*C*H$_2$CH$_2$O), 47.6 and 47.9 (2xCH_2), 49.7 (*C*H$_2$ bridge), 65.5 (CH$_2$*C*H$_2$O), 108.9 (*C*CNH$_2$), 124.2 (triazole*C*H), 145.0 (triazole*C*), 154.8 (pyrimidine*C*H), 162.2 and 167.8 (*C*N*C*NH$_2$), 168.2 and 172.0 (2 x *C*=O).

References

1 J.A. Zoltewicz, G.M. Kauffman, *J. Am. Chem. Soc*., 1977, **99**, 3134.
2 G. Uray, I. Kriessmann, J.A. Zoltewicz, *Bioorg. Chem*., 1993, **21**, 294.
3 G. Eglinton, M. C. Whiting, *J. Chem. Soc.*, 1950, 3650
4 F. Himo, T. Lovell, R. Hilgraf, V.V. Rostovtsev, L. Noodleman, K.B. Sharpless, V.V. Fokin. *J. Am. Chem. Soc*., 2005, **127**, 210
5 S. Mann, C. Perez Melero, D. Hawksley, F.J. Leeper, *Org. Biomol. Chem*, 2004, **2**, 1732.