Supplementary information

Probing riboswitch–ligand interactions using thiamine pyrophosphate analogues

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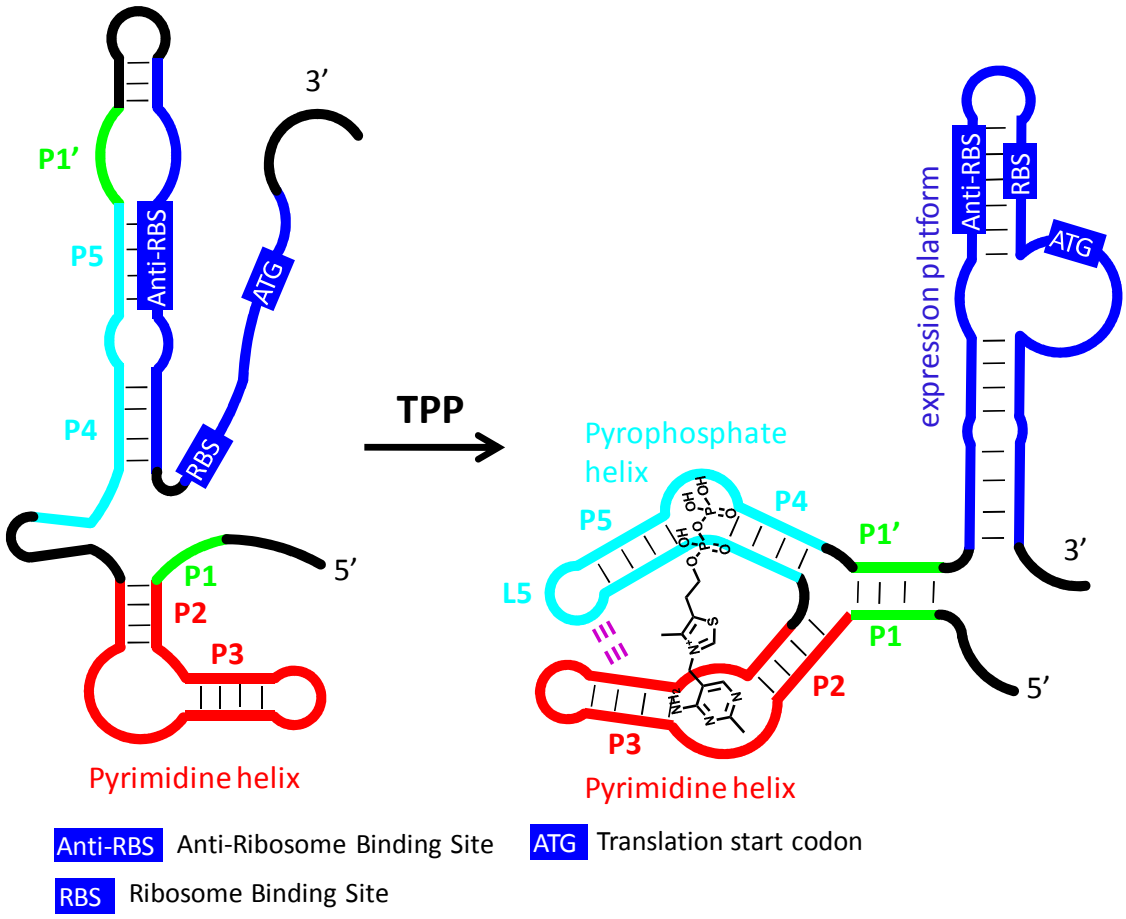


Figure S1. Schematic representation of the two conformational states of the *thiM* riboswitch. The pyrimidine helix is drawn in red, the pyrophosphate binding helix is depicted in cyan, the expression platform in blue and the P1 stem in green. The tertiary contact between the loop L5 and stem P3 is shown as purple dashed lines.[0]

Supplementary Figure S2. Isothermal titration calorimetry

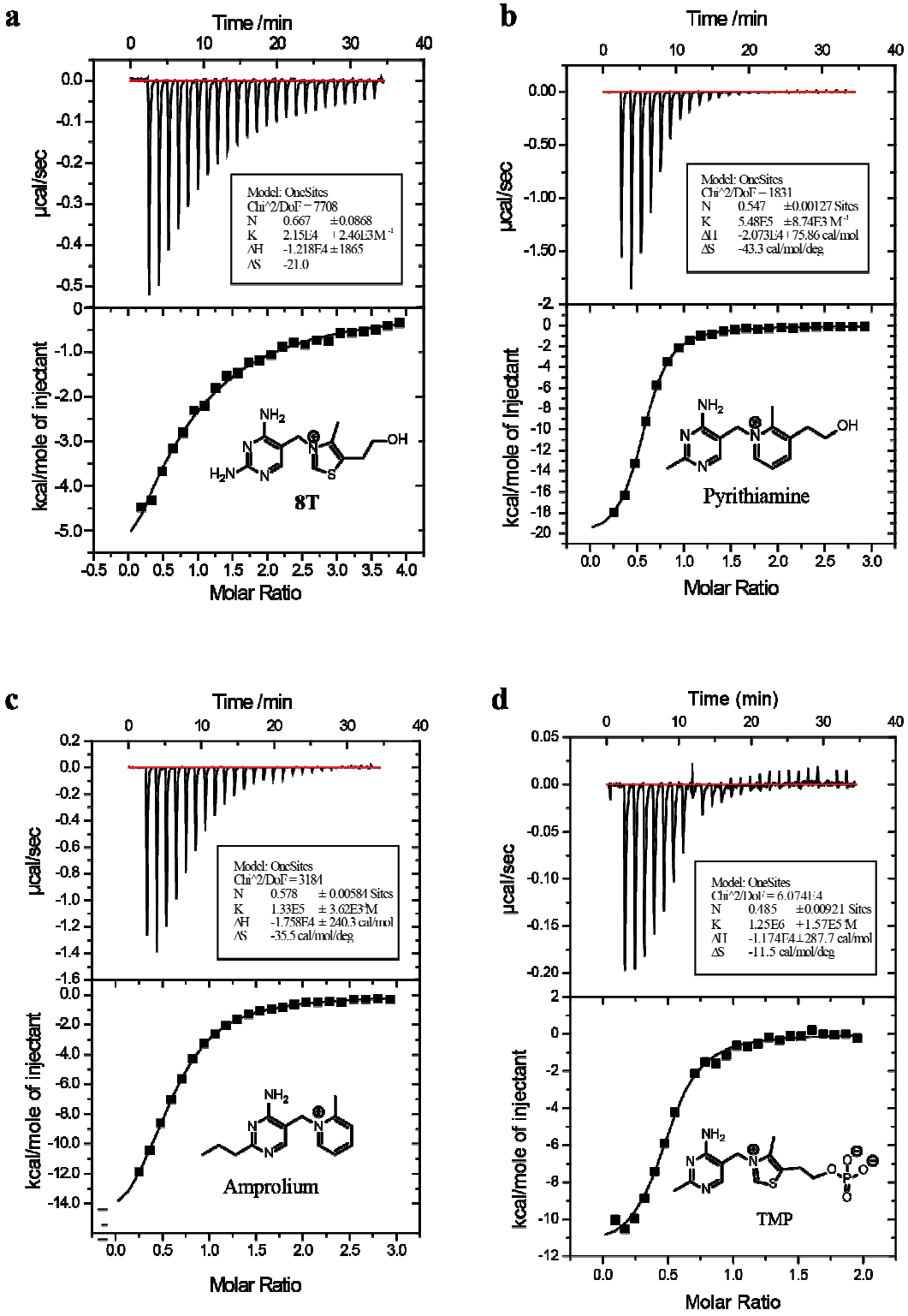
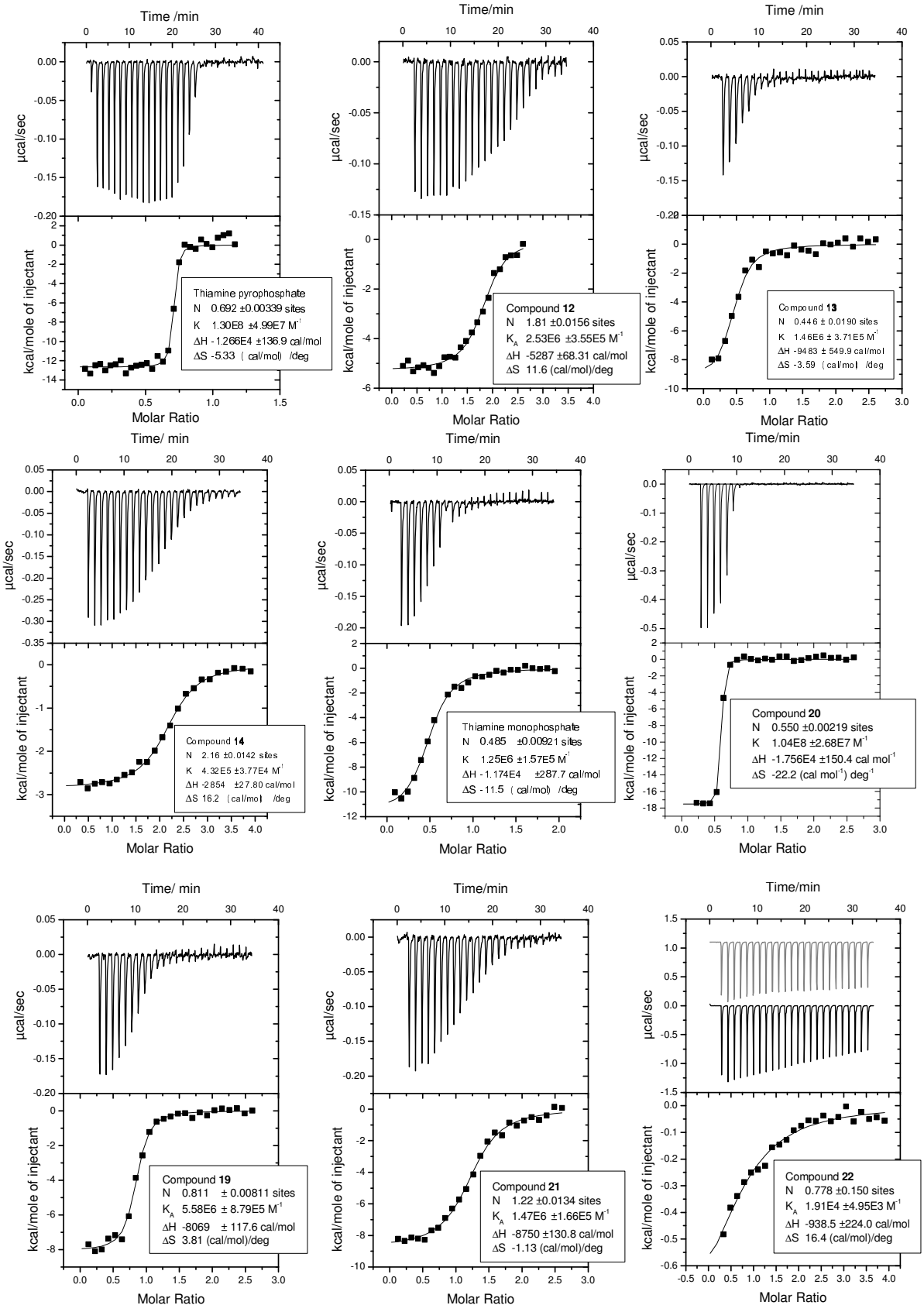


Figure S2. Isotherms obtained with *thiM* RNA and various ligands. a) **8T** (1 mM) titrated into RNA (50 µM), b) pyrithiamine (750 µM) titrated into RNA (50 µM), c) Amprolium (750 µM) titrated into RNA (50 µM), d) TMP (200 µM) titrated into RNA (20 µM)

Supplementary Figure S3. Isothermal titration calorimetry



In grey: the control titration

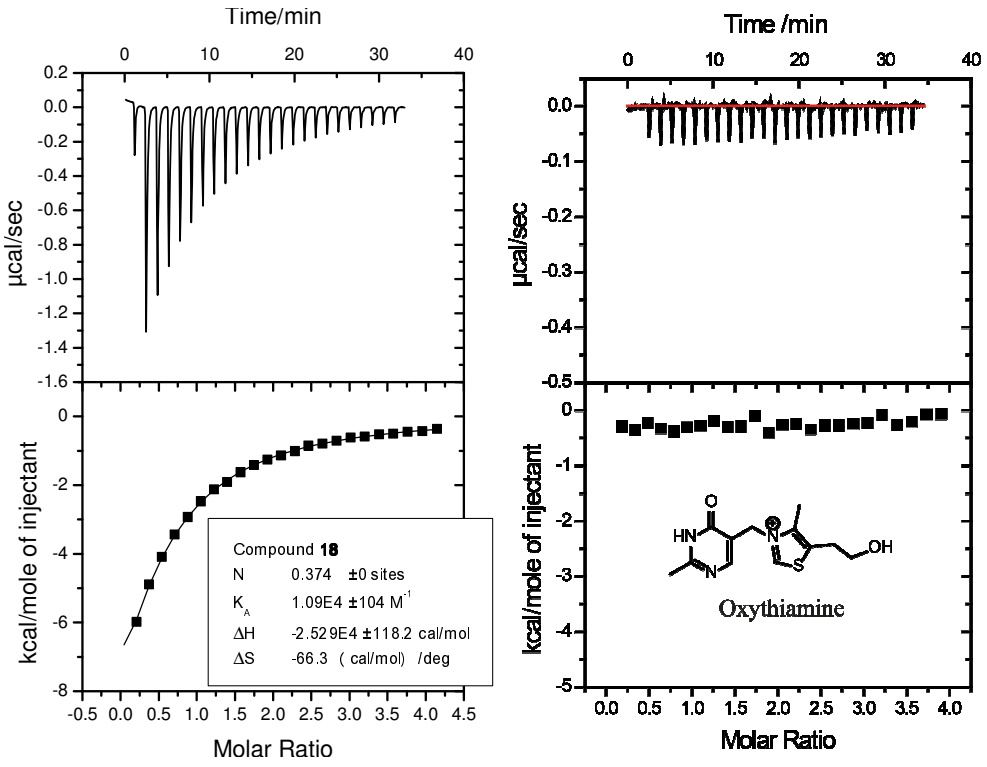


Figure S3. Isothermal titration thermograms of the compounds TPP, **12-14** and **18-22**. The legend shows the values for the binding constant (K_A), the enthalpy of binding (ΔH) and the entropy of binding (ΔS). In the final isotherm no heat of binding was observed when oxythiamine (1 mM) was titrated into *thiM* RNA (50 µM).

Supplementary Figure S4.

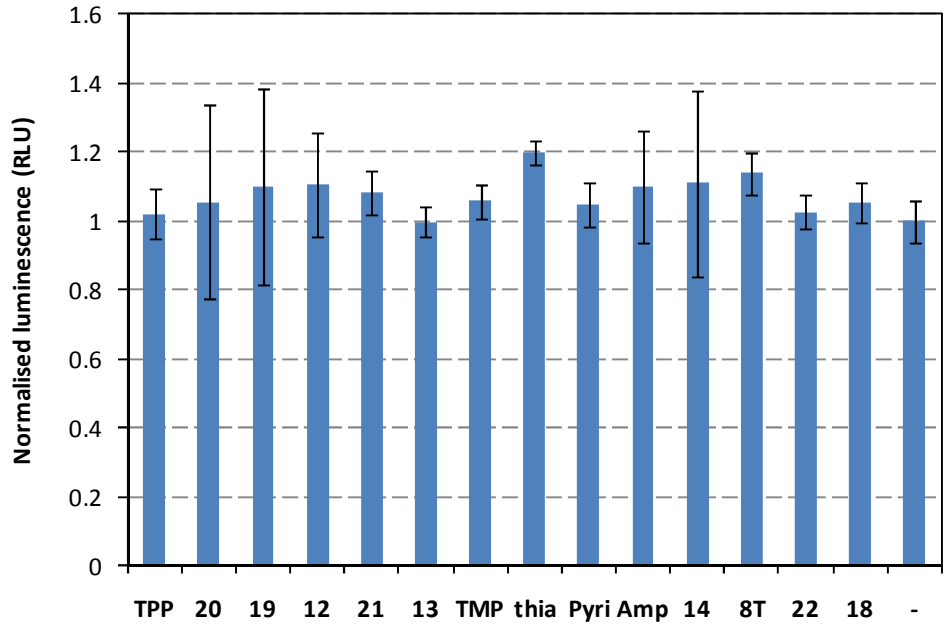


Figure S4. The effect of TPP analogues on luciferase expression using a DNA template without the *thiM* riboswitch. The amount of luciferase produced in IVTT reactions in the presence of 100 µM of the analogues was quantified by measuring the luminescence developed upon addition of the substrate coelenterazine. The graph indicates the luminescence signal normalised to the negative control (–) which contained no analogues. Pyrithiamine is abbreviated as Pyri, thiamine as Thia and amprolium as Amp.

RNA/DNA constructs

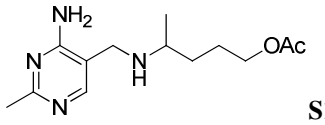
Sequence of *thiM* aptamer RNA used in the biophysical experiments as described previously:[2]

GGGCGAAUUGGGCCCGACGUCGCAUGCUCCCGGCCGCCAUGGCGGCCGCGGGAAUUCGAUUGAUCA
UGAAUUCGCAACCAAACGACUCGGGGUGCCCUUCUGCGUGAAGGCUGAGAAAUACCCGUAUCCUGA
UCUGGAUAAUGCCAGCGUAGGGAAGU

In vitro transcription/translation plasmids were as described previously.[3] In the riboswitch controlled construct, *R. reniformis luc* was fused to the end of the *E. coli thiM* riboswitch sequence and cloned into pBluescript II KS (–) at the *XbaI* and *HindIII* restriction sites. In the control construct, only the last 41 nucleotides of the *thiM* expression platform (including the ribosome binding site and the first few codons of the *thiM* gene) were used. The *luc* gene sequence was codon optimized for *C. reinhardtii.*

Synthesis

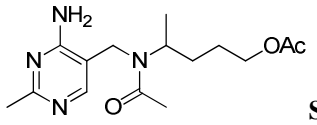
Synthesis of open chain TPP analogues



S1

4-((4-Amino-2-methylpyrimidin-5-yl)methylamino)pentyl acetate S1

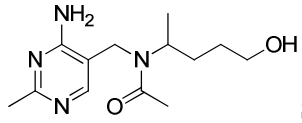
4-Oxopentyl acetate (1.0 g, 6.9 mmol) and 5-(aminomethyl)-2-methylpyrimidin-4-amine (1.5 g, 10.5 mmol) were dissolved in dry methanol (50 ml). Sodium triacetoxyborohydride (3.0 g, 14 mmol) was added and the mixture stirred at room temperature for 18 hours. Saturated sodium bicarbonate was added and the mixture was extracted with ethyl acetate. The organic layer was dried with magnesium sulphate and evaporated under reduced pressure. Purification by column chromatography gave amine **S1** (0.99 g, 54%) as an off-white solid. [Found: M + H^+ (+ESI), 267.1702, $C_{13}H_{22}N_4O_2$ requires M + H, 267.1743]; ν_{max}/cm^{-1} 1732 (C=O), 1584 and 1556 (Ar C=C); δ_H (400 MHz, $CDCl_3$) 1.04 (3H, d, *J* 6.3, *CH*$_3$CH), 1.35 (1H, m, *CH*$_a$H$_b$(CH$_2$)$_2$OAc), 1.43 (1H, m, C*H*$_a$*H*$_b$(CH$_2$)$_2$OAc), 1.58 (2H, m, *CH*$_2$CH$_2$OAc), 1.74 (1H, broad s, CH$_2$*NH*), 1.97 (3H, s, *CH*$_3$COO), 2.41 (3H, s, pyrimidine-CH$_3$), 2.58 (1H, sextet, *J* 6.3, CH$_3$*CH*), 3.60 (1H, d, *J* 13.2, *CH*$_a$H$_b$NH), 3.70 (1H, d, *J* 13.2, C*H*$_a$*H*$_b$NH), 3.98 (2H, t, *J* 6.6, *CH*$_2$OAc), 6.09 (2H, broad s, NH_2), 7.87 (1H, s, pyrimidine-H); δ_C (100 MHz, $CDCl_3$) 20.93 and 21.47 (CH*C*H$_3$ and *C*H$_3$COO), 23.62 (*C*H$_2$CH$_2$OAc), 24.85 (pyrimidine-*C*H$_3$), 34.51 (*C*H$_2$(CH$_2$)$_2$OAc), 45.82 (*C*H$_2$NH), 55.61(CH$_3$*C*H), 64.67 (*C*H$_2$OAc), 110.42 (*C*CH$_2$NH), 157.63 (pyrimidine *C*-H), 162.43 (*C*NH$_2$), 165.10 (*C*CH$_3$), 171.92 (CH$_3$*C*OO).



S2

4-(N-((4-Amino-2-methylpyrimidin-5-yl)methyl)acetamido)pentyl acetate S2

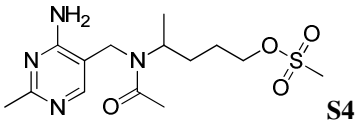
Amine **1** (0.3 g, 1.1 mmol) was dissolved in dry DCM (10 ml) at 0℃. Dimethylaminopyridine (0.007g, 0.06 mmol) and triethylamine (0.18 g, 1.8 mmol) were added to the solution. Acetic anhydride (0.11 ml, 1.2 mmol) was then added with stirring at room temperature. The reaction mixture was diluted with water (10 ml) and extracted with ethyl acetate. The organic layer was dried over magnesium sulphate, filtered and evaporated under reduced pressure. Purification by column chromatography gave acetamide **S2** (0.28 g, 85%) as an off-white solid. [Found: M + H^+ (+ESI), 309.1832, $C_{15}H_{24}N_4O_3$ requires M + H, 309.1848]; ν_{max}/cm^{-1} 1734 (ester C=O), 1663 (amide C=O); δ_H (400 MHz $CDCl_3$) 1.20 (3H, d, *J* 7, *CH*$_3$CH), 1.53 (4H, m, *CH*$_2$*CH*$_2$CH$_2$OAc), 2.01 (3H, s, *CH*$_3$COO), 2.18 (3H, s, *CH*$_3$CON), 2.43 (3H, s, pyrimidine-*CH*$_3$), 3.86 (1H, sextet, *J* 7, *CH*(CH$_2$)$_3$OAc), 3.93 (2H, m, *CH*$_2$OAc), 4.43 (2H, s, *CH*$_2$N), 6.83 (2H, broad s, NH_2), 8.01 (1H, s, pyrimidine-H); δ_C (100 MHz, $CDCl_3$) 20.74, 21.34 and 21.46 (CH*C*H$_3$, *C*H$_3$CON and *C*H$_3$COO), 23.68 (*C*H$_2$CH$_2$OAc), 24.83 (pyrimidine-*C*H$_3$), 34.37 (*C*H$_2$(CH$_2$)$_2$OAc), 45.53 (*C*H$_2$N), 58.94 (CH$_3$*C*H), 65.01(*C*H$_2$OAc), 112.17 (*C*CH$_2$N), 155.89 (pyrimidine *C*-H), 161.78 (*C*NH$_2$), 165.24 (*C*CH$_3$), 172.44 (CH$_3$*C*OO), 174.98 (CH$_3$*C*ON).



S3

N-((4-Amino-2-methylpyrimidin-5-yl)methyl)-N-(5-hydroxypent-2-yl)acetamide S3.

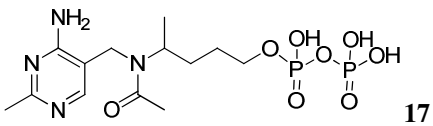
Potassium carbonate (0.67 g, 4.85 mmol) was added to a flask containing dry methanol (10 ml). To this was added acetamide **2** (0.30 g, 0.97 mmol) and the resulting mixture stirred at room temperature for 2 hours. The white solid was filtered and the solution evaporated under reduced pressure to leave a white residue. This residue was washed several times with dry DCM and filtered off. The organic solution was evaporated under reduced pressure to yield the alcohol **S3** (0.25 g, 95%). [Found: M + H^+ (+ESI), 267.1698, $C_{13}H_{22}N_4O_2$ requires M + H, 267.1743]; δ_H (400 MHz $CDCl_3$) 1.22 (3H, d, *J* 6.8, *CH*$_3$CH), 1.39 (2H, m, *CH*$_2$(CH$_2$)$_2$OH), 1.62 (2H, m, *CH*$_2$CH$_2$OH), 2.19 (3H, s, *CH*$_3$CON), 2.45 (3H, s, pyrimidine-CH$_3$), 3.52 (2H, m, *CH*$_2$OH), 3.89 (1H, m, *CH*(CH$_2$)$_3$OH), 4.42 (1H, d, *J* 15.5, *CH*$_a$H$_b$N), 4.49 (1H, d, *J* 15.5, CH$_a$*H*$_b$N), 6.20 (2H, broad s, NH$_2$), 7.98 (1H, s, pyrimidine-H); δ_C 19.89 and 21.24 (CHCH$_3$ and CH$_3$CON), 24.69 (pyrimidine-*C*H$_3$), 32.51 (*C*H$_2$CH$_2$OH), 34.16 (*C*H$_2$(CH$_2$)$_2$OH), 45.48 (CH$_2$N), 59.13 (CH$_3$*C*H), 63.16 (*C*H$_2$OH), 112.08 (*C*CH$_2$N), 155.63 (pyrimidine-*C*H), 161.64 (*C*NH$_2$), 165.09 (*C*CH$_3$), 174.66 (CH$_3$CON).



S4

4-(N-((4-Amino-2-methylpyrimidin-5-yl) methyl)acetamido)pentyl methanesulfonate S4

Alcohol **S3** (0.5 g, 1.9 mmol) was added to a flask containing dry DCM (20 ml) at 0 °C with stirring. To this solution was added triethylamine (0.52 ml, 3.8 mmol) and mesyl chloride (0.22 ml, 2.9 mmol) and stirring continued for 30 minutes. Ice-cold water (20 ml) was added to the solution and the organic layer separated from the aqueous phase. The aqueous phase was extracted with DCM (4 x 10 ml) and the organic layers combined and dried over magnesium sulphate. DCM was removed by evaporating under reduced pressure and the oily residue was purified by column chromatography. The methanesulfonate **S4** (0.39 g, 59%) was isolated as an off-white solid. [Found: M + H^+ (+ESI), 345.1501, $C_{14}H_{24}N_4O_4S$ requires M + H, 345.1518]; δ_H (400 MHz $CDCl_3$) 1.24 (3H, d, *J* 6.8, *CH*$_3$CH), 1.57 (4H, m, *CH*$_2$*CH*$_2$CH$_2$OMs), 2.19 (3H, s, *CH*$_3$CON), 2.45 (3H, s, pyrimidine-*CH*$_3$), 2.98 (3H, s, *CH*$_3$SO$_2$), 3.88 (1H, m, *CH*(CH$_2$)$_3$OMs), 4.05 (2H, m, *CH*$_2$OMs), 4.34 (1H, d, *J* 15.4, *CH*$_a$H$_b$N), 4.54 (1H, d, *J* 15.4, C*H*$_a$*H*$_b$NCO), 6.28 (2H, broad s, NH$_2$), 7.99 (1H, s, pyrimidine-H); δ_C 19.94 and 21.36 (CH*C*H$_3$ and *C*H$_3$CON), 23.42 (*C*H$_2$CH$_2$OMs), 24.73 (pyrimidine-*C*H$_3$), 34.05 (*C*H$_2$(CH$_2$)$_2$OMs), 38.31(CH$_3$SO$_2$), 45.61 (CH$_2$N), 60.11 (CH$_3$*C*H), 70.62 (*C*H$_2$OMs), 112.17 (*C*CH$_2$N), 155.89 (pyrimidine-*C*H), 161.71 (*C*NH$_2$), 165.18 (*C*CH$_3$), 174.83 (CH$_3$CON)

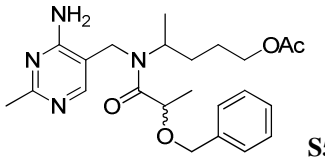


17

4-(N-((4-amino-2-methylpyrimidin-5-yl)methyl)acetamido)pentyl trihydrogen diphosphate 17

Methanesulfonate **S4** (0.04 g, 0.12 mmol) was dissolved in dry acetonitrile (0.4 ml) under argon and the solution cooled to 0 °C. Tris-tetrabutylammonium hydrogen pyrophosphate (0.22 g, 0.24 mmol) was added to this solution

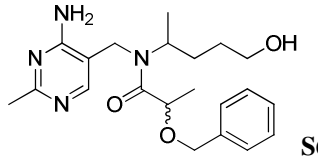
and the temperature allowed to rise to room temperature while stirring. The reaction was followed by LCMS and on completion after 48 hours, was diluted with water to a total volume of 1 ml. The solution was purified by anion exchange chromatography to give the pyrophosphorylated open-chain analogue of thiamine **17** (0.026 g, 51%). [Found: M + H^+ (+ESI), 427.1048, $C_{13}H_{24}N_4O_8P_2$ requires M + H, 427.1069]; δ_H (400 MHz, D_2O) 1.13 (3H, d, *J* 6.7, *CH*$_3$CH), 1.53 (4H, m, *CH*$_2$*CH*$_2$CH$_2$OPP), 2.20 (3H, s, CH_3CON), 2.41 (3H, s, pyrimidine-CH_3), 3.77 (1H, m, *CH*$(CH_2)_3$OPP), 4.09 (2H, q, *J* 6.6, *CH*$_2$OPP), 7.78 (1H, s, pyrimidine-H); δ_C (100 MHz, D_2O) 19.72 and 21.41 (CH*C*H$_3$ and *C*H$_3$CON), 23.57 (*C*H$_2$CH$_2$OPP), 24.89 (pyrimidine-*C*H$_3$), 33.82 (*C*H$_2$(CH$_2$)$_2$OPP), 45.39 (CH_2N), 60.08 (CH_3CH), 67.12 (*C*H$_2$OPP), 112.03 (*C*CH$_2$N), 154.64 (pyrimidine-*C*H), 161.57 (*C*NH_2), 164.85 (*C*CH_3), 174.46 (CH_3CON); δ_P (162 MHz, D_2O) -8.54 (1P, d, *J* 20.4), -10.46 (1P, d, *J* 20.4).



S5

4-(N-((4-amino-2-methylpyrimidin-5-yl)methyl)-2-(benzyloxy)propanamido)pentyl acetate S5

O-Benzyl lactic acid (0.053 g, 0.29 mmol) and dry DCM (10 ml) were treated with DCC (0.061 g, 0.296 mmol) and stirred under argon for 20 minutes at room temperature. The amine **S1** (0.06 g, 0.23 mmol) was added to the mixture and stirring continued overnight. The resulting white precipitate was filtered off and DCM removed by evaporating under reduced pressure. The oily residue was then purified by column chromatography and the amide **S5** (0.071 g, 72%) isolated as an off-white solid. [Found: M + H^+ (+ESI), 429.2409, $C_{23}H_{32}N_4O_4$ requires M + H, 429.2424]; ν_{max}/cm^{-1} 1732 (ester CO), 1663 (amide CO), 1601 and 1565 (aromatic C=C); δ_H (400 MHz, $CDCl_3$) 1.04 (3H, d, *J* 6.8, *CH*$_3$CH$(CH_2)_3$OAc), 1.11 (3H, d, J 6.8, *CH*$_3$CHOBn), 1.28 (2H, m, *CH*$_2$$(CH_2)_2$OAc), 1.42 (2H, m, *CH*$_2CH_2$OAc), 1.94 (3H, s, *CH*$_3$COO), 2.40 (3H, s, pyrimidine-*CH*$_3$), 3.79 (2H, m), 4.00 (1H, m), 4.30-4.60 (5H, m), 6.20 (2H, broad s, *NH*$_2$), 7.25 (5H, m, Ph), 7.93 (1H, s, pyrimidine-*H*); δ_C (100 MHz, $CDCl_3$) 20.43 and 20.65 (2 x CH*C*H$_3$), 21.25 (*C*H$_3$COO) 25.05 (*C*H$_2$CH$_2$OAc), 25.34 (pyrimidine-*C*H$_3$), 32.30 (*C*H$_2$$(CH_2)_2$OAc), 49.47 ($CH_2N$), 60.85 (*C*H$(CH_2)_3$OAc), 63.96 (*C*H$_2$OAc), 71.37 (benzyl-*C*H$_2$), 74.62 (*C*HOBn), 111.12 (*C*CH_2N), 128.14, 128.30 and 128.44 (5 x phenyl *CH*), 137.53 (phenyl *C*), 156.95 (pyrimidine-*C*H), 162.38 (*C*NH_2), 167.85 (*C*CH_3), 171.27 (CH_3CHCON), 174.77 (CH_3COO).

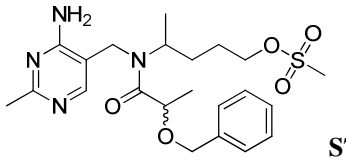


S6

N-((4-Amino-2-methylpyrimidin-5-yl)methyl)-2-(benzyloxy)-N-(5-hydroxypentan-2-yl)propanamide S6

Potassium carbonate (0.097 g, 0.14 mmol) was added to a flask containing dry methanol (5 ml). To this was added **S5** (0.30 g, 0.97 mmol) and the mixture was stirred at room temperature for 2 hours. The white solid was filtered and the solution evaporated under reduced pressure to leave a white residue. This residue was washed several times with dry DCM and filtered off. The organic solution was evaporated under reduced pressure to give the alcohol **S6** (0.34 g, 90%). [Found: M + H^+ (+ESI), 387.2337, $C_{21}H_{30}N_4O_3$ requires M + H, 387.2351]; δ_H (400 MHz, $CDCl_3$) 1.03 (3H, d, *J* 6.7, *CH*$_3$CH$(CH_2)_3$OH), 1.11 (3H, d, *J* 6.7, *CH*$_3$CHOBn), 1.27 (4H, m, *CH*$_2$*CH*$_2$CH$_2$OH), 2.39 (3H, s,

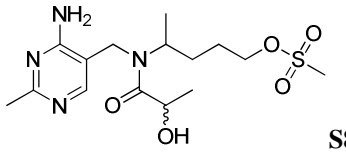
pyrimidine-CH_3), 3.36 (2H, t, *J* 6.2, *CH*$_2$OH), 3.99 (1H, m), 4.30-4.60 (5H, m), 7.25 (5H, m, Ph), 7.92 (1H, s, pyrimidine-*H*); δ_C (100 MHz, $CDCl_3$) 20.49 and 20.73 (2 x CH*C*H$_3$), 25.81 (pyrimidine-*C*H$_3$), 32.36 (CH*C*H$_2$), 49.41 (CH$_2$N), 62.10 (*C*HN), 62.19 (*C*H$_2$OH), 71.39 (benzyl-*C*H$_2$), 73.81 (CH$_3$*C*HOBn), 111.21 (*C*CH$_2$N), 128.15, 128.31 and 128.41 (5 x phenyl *C*), 157.08 (pyrimidine-*C*H), 167.85 (*C*CH$_3$), 171.19 (CH$_3$*C*ON).



S7

4-(N-((4-amino-2-methylpyrimidin-5-yl)methyl)-2-(benzyloxy)propanamido)pentyl methanesulfonate S7

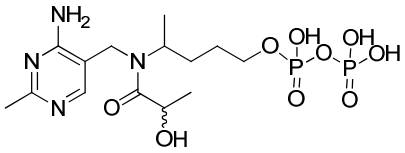
Alcohol **S6** (0.025 g, 0.065 mmol) was added to a flask containing dry DCM (5 ml) at 0 °C with stirring. To this solution was added triethylamine (0.018 ml, 0.13 mmol) and mesyl chloride (0.008 ml, 0.098 mmol) and stirring continued for 30 minutes. Ice-cold water (5 ml) was added to the solution and the organic layer separated from the aqueous phase. The aqueous phase was extracted with DCM (4 x 5 ml) and the organic layers combined and dried over magnesium sulphate. DCM was removed by evaporating under reduced pressure and the oily residue purified by column chromatography. The methanesulfonate **S7** (0.016 g, 53%) was isolated as a white solid. [Found: M + H^+ (+ESI), 465.2074, $C_{22}H_{32}N_4O_5S$ requires M + H, 465.2093]; δ_H (400 MHz, $CDCl_3$) 1.04 (3H, d, *J* 6.7, *CH*$_3$CHN), 1.10 (3H, d, *J* 6.7, *CH*$_3$CHO), 1.26 (4H, m, CH*CH*$_2$*CH*$_2$), 2.39 (3H, s, pyrimidine-CH$_3$), 2.99 (3H, s, *CH*$_3$SO$_2$), 3.72 (2H, t, *J* 6.2, *CH*$_2$OMs), 3.97 (1H, m), 4.30-4.60 (5H, m), 7.23 (5H, m, Ph), 7.93 (1H, s, pyrimidine-*H*); δ_C (100 MHz, $CDCl_3$) 20.49 and 20.73 (2 x CH*C*H$_3$), 25.33 (*C*H$_2$CH$_2$OMs), 25.81 (pyrimidine-*C*H$_3$), 32.41 (CH*C*H$_2$), 49.39 (CH$_2$N), 62.14 (*C*H-N), 69.79 (*C*H$_2$OMs), 71.44 (benzyl-*C*H$_2$), 73.72 (*C*H-O), 111.24 (*C*CH$_2$N), 128.09, 128.33 and 128.41 (5 x phenyl *CH*), 157.12 (pyrimidine-*C*H), 167.83 (*C*CH$_3$), 171.24 (CH$_3$*C*ON)



S8

4-(N-((4-amino-2-methylpyrimidin-5-yl)methyl)-2-hydroxypropanamido)pentyl methanesulfonate S8

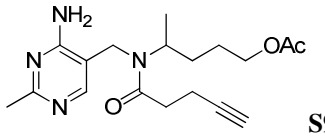
The mesylate **S7** (0.057 g, 0.123 mmol) was dissolved in dry methanol (10 ml) and to this solution was added palladium on charcoal 10% (0.013 g, 0.123 mmol). The flask was flushed with hydrogen 3 times and the mixture stirred under hydrogen at room temperature overnight. The catalyst was filtered off and methanol evaporated under reduced pressure to give the product **S8** (0.046 g, 99%) which did not require further purification. [Found: M + H^+ (+ESI), 375.1589, $C_{15}H_{26}N_4O_5S$ requires M + H, 375.1624]; δ_H (400 MHz, $CDCl_3$) 1.03 (3H, d, *J* 6.7, *CH*$_3$CHN), 1.14 (3H, d, *J* 6.7, *CH*$_3$CHOH), 1.28 (4H, m, CH*CH*$_2$*CH*$_2$), 2.37 (3H, s, pyrimidine-CH$_3$), 2.99 (3H, s, *CH*$_3$SO$_2$), 3.71 (2H, t, *J* 6.2, *CH*$_2$OMs), 3.97 (1H, m), 4.30-4.60 (3H, m), 7.92 (1H, s, pyrimidine-*H*); δ_C (100 MHz, $CDCl_3$) 20.47 and 20.73 (2 x CH*C*H$_3$), 25.35 (*C*H$_2$CH$_2$OMs), 25.84 (pyrimidine-*C*H$_3$), 32.43 (CH*C*H$_2$), 49.41 (CH$_2$N), 62.16 (*C*H-N), 67.72 (*C*HOH), 69.87 (*C*H$_2$OMs), 111.41 (*C*CH$_2$N), 157.15 (pyrimidine-*C*H), 167.73 (*C*CH$_3$), 171.24 (C=O).



15

4-(N-((4-amino-2-methylpyrimidin-5-yl)methyl)-2-hydroxypropanamido)pentyl trihydrogen diphosphate 15

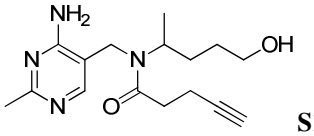
Methanesulfonate **S8** (0.025 g, 0.067 mmol) was dissolved in dry acetonitrile (0.4 ml) under argon and the solution cooled to 0 °C. Tris-tetrabutylammonium hydrogenpyrophosphate (0.18 g, 0.20 mmol) was added to this solution and the temperature allowed to rise to room temperature while stirring. The reaction was followed by LCMS and on completion after 48 hours, was diluted with water to a total volume of 1 ml. The solution was purified by anion exchange chromatography to give the pyrophosphate **15** (0.017 g, 54%). [Found: M + H^+ (+ESI), 457.1163, $C_{14}H_{26}N_4O_9P_2$ requires M + H, 457.1175]; δ_H (400 MHz, $CDCl_3$) 1.04 (3H, d, *J* 6.7, *CH*$_3$CHN), 1.14 (3H, d, *J* 6.7, *CH*$_3$CHOH), 1.28-1.41 (4H, m, CH*CH*$_2$*CH*$_2$), 2.39 (3H, s, pyrimidine-CH_3), 3.87 (1H, m, *CH*-N), 3.99 (1H, q, J 6.7, CH_3CHOH), 4.12 (2H, q, *J* 6.6, *CH*$_2$OPP), 7.92 (1H, s, pyrimidine-*H*); δ_C (100 MHz, $CDCl_3$) 20.47 and 20.73 (2 x CH*C*H$_3$), 25.35 (*C*H$_2$CH$_2$OPP), 25.84 (pyrimidine-*C*H$_3$), 32.43 (CH*C*H$_2$), 49.41 (CH_2N), 62.16 (*C*H-N), 67.72 (*C*HOH), 73.57 (*C*H$_2$OPP), 111.66 (*C*CH$_2$N), 157.29 (pyrimidine-*C*H), 167.74 (*C*CH$_3$), 171.24 (C=O); δ_P (162 MHz, D_2O) -8.30 (1P, d, *J* 20.3), -10.42 (1P, d, *J* 20.3)



S9

4-(N-((4-amino-2-methylpyrimidin-5-yl)methyl)pent-4-ynamido)pentyl acetate S9

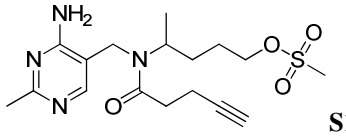
Pent-4-ynoic acid (194 mg, 1.98 mmol, 1.4 equiv.), 4-dimethylaminopyridine (DMAP) (64 mg, 0.52 mmol, 0.4 equiv.) and EDCI (437 mg, 2.28 mmol, 1.6 equiv.) were dissolved in THF (20 mL) and stirred under an atmosphere of nitrogen for 10 min. The solution was cooled on ice and then amine **S1** (380 mg, 1.43 mmol, 1 equiv.) was added. The mixture was allowed to warm to room temperature and stirred for a further 12 h. Saturated aqueous sodium bicarbonate (20 mL) was added and the mixture was extracted with DCM (3x 40 mL). The combined organic phases were dried (washed with brine then $MgSO_4$ added) and evaporated under reduced pressure. Purification by preparative thin layer chromatography (7:93 v/v MeOH:DCM) gave the amide **S9** (418 mg, 79%) as a white solid. [Found: M + H^+ (+ESI), 347.1986, $C_{18}H_{26}N_4O_3$ requires M + H, 347.2005]; ν_{max}/cm^{-1} 1732 (ester C=O), 1665 (amide C=O); δ_H (400 MHz, $CDCl_3$) 1.05 (3H, d, *J* 6.6, CH*CH*$_3$), 1.46 (4H, m, (*CH*$_2$)$_2$CH$_2$O), 2.16 (3H, s, *CH*$_3$CO$_2$), 2.27 (2H, t, *J* 6.8, CH$_2$*CH*$_2$C≡CH), 2.33 (3H, s, pyrimidine-CH_3), 2.39 (2H, t, J 6.8, *CH*$_2$CH$_2$C≡CH), 3.74 (1H, m, *CH*CH$_3$), 4.13 (2H, m, *CH*$_2$OAc), 7.91 (1H, s, pyrimidine-H); δ_C (100 MHz, $CDCl_3$) 18.55 (CH$_2$*C*H$_2$C≡CH), 19.69 (*C*H$_3$CH), 23.54 (*C*H$_2$CH$_2$OAc), 24.83 (pyrimidine-*C*H$_3$), 33.52 (*C*H$_2$(CH$_2$)$_2$O), 45.17 (CH_2N), 60.10 (CH$_3$*C*H), 66.47 (*C*H$_2$OAc), 70.01 (CH$_2$CH$_2$C≡*C*H), 83.32 (CH$_2$CH$_2$*C*≡CH), 112.07 (*C*CH$_2$N), 154.87 (pyrimidine-*C*H), 161.38 (*C*NH$_2$), 164.91 (*C*CH$_3$), 171.48 (*C*ON), 174.35 (CH$_3$*C*OO).



S10

N-((4-amino-2-methylpyrimidin-5-yl)methyl)-N-(5-hydroxypentan-2-yl)pent-4-ynamide S10

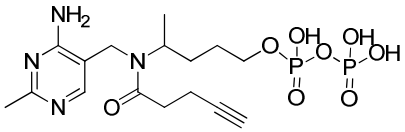
Acetate ester **S9** (722 mg, 2.08 mmol) and sodium carbonate (287 mg, 2.71 mmol, 1.3 equiv.) were stirred in methanol (25 mL) at room temperature until all starting material had been consumed (as observed by TLC). The methanol was removed under reduced pressure, the residue was finely ground and DCM (20 mL) was added. The solution was filtered and the remaining solid washed on the filter with more DCM (3x 20 mL). The filtrate and washings were dried with $MgSO_4$ and evaporated under reduced pressure to give the alcohol **S10** (607 mg, 96%) as a colourless gum. [Found: M + H^+ (+ESI), 305.1857, $C_{16}H_{24}N_4O_2$ requires M + H, 305.1899]; δ_H (400 MHz, $CDCl_3$) 1.05 (3H, d, *J* 6.6, *CH*$_3$CH), 1.44 (4H, m, (*CH*$_2$)$_2$CH$_2$OH), 2.26 (2H, t, *J* 6.8, CH$_2$*CH*$_2$C≡CH), 2.33 (3H, s, pyrimidine-CH$_3$), 2.37 (2H, t, J 6.8, *CH*$_2$CH$_2$C≡CH), 3.46 (2H, m, *CH*$_2$OH), 3.72 (1H, m, CH$_3$*CH*), 7.91 (1H, s, pyrimidine-H); δ_C (100 MHz, $CDCl_3$) 18.57 (CH$_2$*C*H$_2$C≡CH), 19.65 (*C*H$_3$CH), 24.83 (pyrimidine-*C*H$_3$), 31.85 (*C*H$_2$CH$_2$OH), 33.53 (CH*C*H$_2$), 45.19 (CH$_2$N), 60.10 (CH$_3$*C*H), 66.51 (*C*H$_2$OH), 70.03 (CH$_2$CH$_2$C≡*C*H), 83.29 (CH$_2$CH$_2$*C*≡CH), 112.10 (*C*CH$_2$N), 154.87 (pyrimidine-*C*H), 161.39 (*C*NH$_2$), 164.94 (*C*CH$_3$), 171.42 (C=O).



S11

4-(N-((4-amino-2-methylpyrimidin-5-yl)methyl)pent-4-ynamido)pentyl methanesulfonate S11

Alcohol **S10** (500 mg, 1.64 mmol) was dissolved in DCM (15 mL) and the solution was cooled to 0 °C under an atmosphere of nitrogen. A solution of triethylamine (0.34 mL, 2.46 mmol, 1.5 equiv.) and methanesulfonyl chloride (0.25 mL, 3.28 mmol, 2 equiv.) in DCM (5 mL) was then added dropwise. The mixture was then allowed to warm to room temperature, stirred until all of the alcohol had been converted into product (approx. 20 min), quenched with ice water (50 mL) and extracted with DCM (2x 50 mL). The organic phase was washed with water (100 mL) then brine (100 mL), dried with $MgSO_4$ and evaporated under reduced pressure. Purification by silica chromatography (eluting with 7:93 v/v MeOH:DCM) gave the mesylate **S11** (489 mg, 78%) as a white solid. [Found: M + H^+ (+ESI), 383.1659, $C_{17}H_{26}N_4O_4S$ requires M + H, 383.1675]; δ_H (400 MHz, $CDCl_3$) 1.06 (3H, d, *J* 6.6, *CH*$_3$CH), 1.43 (4H, m, CH(*CH*$_2$)$_2$), 2.28 (2H, t, *J* 6.8, CH$_2$*CH*$_2$C≡CH), 2.33 (3H, s, pyrimidine-CH$_3$), 2.39 (2H, t, J 6.8, *CH*$_2$CH$_2$C≡CH), 3.70 (2H, m, *CH*$_2$OMs), 3.72 (1H, m, CH$_3$*CH*), 7.89 (1H, s, pyrimidine-H); δ_C (100 MHz, $CDCl_3$) 18.55 (CH$_2$*C*H$_2$C≡CH), 19.65 (*C*H$_3$CH), 24.81 (pyrimidine-*C*H$_3$), 25.84 (*C*H$_2$CH$_2$OMs), 33.51 (CH*C*H$_2$), 45.21 (CH$_2$N), 60.10 (CH$_3$*C*H), 66.59 (*C*H$_2$OMs), 70.01 (CH$_2$CH$_2$C≡*C*H), 83.27 (CH$_2$CH$_2$*C*≡CH), 112.14 (*C*CH$_2$N), 154.88 (pyrimidine-*C*H), 161.39 (*C*NH$_2$), 164.92 (*C*CH$_3$), 171.42 (C=O).



16

4-(N-((4-amino-2-methylpyrimidin-5-yl)methyl)pent-4-ynamido)pentyl trihydrogen diphosphate 16

Mesylate **S11** (485 mg, 1.27 mmol) was dissolved in acetonitrile (5 mL) and cooled to 0 °C under an atmosphere of nitrogen. A solution of tris-tetrabutylammonium hydrogen pyrophosphate (3.43 g, 3.8 mol, 3 equiv.) in acetonitrile (5 mL) was added and the reaction mixture was allowed to warm to room temperature. The reaction mixture was stirred at room temperature for 24 h and then concentrated under reduced pressure (to approx. 1 mL). Water (to

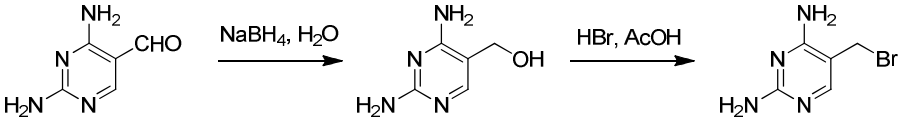
approx. 5 mL) was added and thewas solution purified by anion exchange chromatography (on diethylaminoethyl cellulose resin, eluting with 0–250 mM ammonium bicarbonate solution). After lyophilisation, the ammonium salt of the pyrophosphate **16** (290 mg, 44%) was obtained as a white powder. [Found: M + H^+ (+ESI), 465.1196, $C_{16}H_{26}N_4O_8P_2$ requires M + H, 465.1226]; δ_H (400 MHz, D_2O) 1.06 (3H,d, *J* 6.6, CH_3CH), 1.46 (4H, m, $CH(CH_2)_2$), 2.25 (2H, t, J 6.8, $CH_2C{\equiv}CH$), 2.33 (3H, s, pyrimidine-CH_3), 2.38 (2H, t, J 6.8, $CH_2CH_2C{\equiv}CH$), 3.73 (1H, m, $CHCH_3$), 4.11 (2H, m, CH_2OPP), 7.72 (1H, s, pyrimidine-H); δ_C (100 MHz, $CDCl_3$) 18.58 ($CH_2CH_2C{\equiv}CH$), 19.72 (CH_3CH), 23.61 (CH_2CH_2OPP), 24.83 (pyrimidine-CH_3), 33.57 ($CHCH_2$), 45.21 (CH_2N), 60.14 (CH_3CH), 67.07 (CH_2OPP), 70.13 ($CH_2CH_2C{\equiv}CH$), 83.34 ($CH_2CH_2C{\equiv}CH$) 112.07 (CCH_2N), 154.89 (pyrimidine-*C*H), 161.43 (CNH_2), 164.91 (CCH_3), 174.40 (C=O); δ_P (162 MHz, D_2O) -8.60 (1P, d, *J* 20.2), -10.72 (1P, d, *J* 20.2)

Procedures for the preparation of thiamine analogues (1-11, T, M, P)

Ring A derivatives

Ring A building blocks were purchased from the following companies or prepared as detailed below:

1) 5-Chloromethyluracil, 3590-48-5, Sigma-Aldrich. **2**) 6-(Chloromethyl)uracil, 18592-13-7, Sigma-Aldrich. **3**) 6-(Chloromethyl)-2-methyl-4-pyrimidinol, 35252-96-1, Bionet Research.**4**) 2-(Chloromethyl)-3-methyl-4-nitropyridine 1-oxide, 116418-98-5, Specs. **5**) 2-(Chloromethyl)-5,6-dimethylthieno[2,3-d]pyrimidin-4(3H)-one, 89567-05-5, Specs. **6**) 2-Chloromethyl-4-nitrophenol, 2973-19-5, Sigma-Aldrich. **7**) 3,5-Dinitrobenzyl chloride, 74367-78-5, Sigma-Aldrich. **9**) 2-(Chloromethyl)-6-methyl-4-pyrimidinol, 23862-02-4, Bionet Research. **10**) 2-(Iodomethyl)-6-methyl-4-pyrimidinylamine, 108260-15-7, Specs. **11**) 1,3,5-Triazine-2,4-diamine, 6-(chloromethyl), 10581-62-1, Enamine **8**) 5-(Bromomethyl)pyrimidine-2,4-diamine, was prepared as follows:



I ***II***

(2,4-Diaminopyrimidin-5-yl)methanol *I*

2,4-Diamino-5-(hydroxymethyl)pyrimidine was prepared from the commercially available 2,4-diamino-5-carbaldehyde-pyrimidine (Specs) using the method reported by Tieckelmann *et al.*.[4] The aldehyde (690.7 mg, 5 mmol) dissolved in water (25 mL) was treated with $NaBH_4$ (190 mg, 5 mmol) dissolved in water (19 mL), the suspension was heated at 50 °C for 1 h, and then allowed to stand at ambient temperature for 16 h. Solvent was removed *in vacuo*, the resultant white solid was extracted with hot ethanol to afford the target alcohol (***I***) (380 mg, 55%), m.p. 280 °C (decomp) R_f 0.21 (4:1 EtOAc:Hex), δ_H (400 MHz; CD_3OD) 7.75 (1 H, s, H_6), 4.40 (2 H, s, CH_2), δ_C (75 MHz; CD_3OD) 165 (C_5), 156 (C_2), 138 (C_6). 130 (C_4), 57 (CH_2); *m/z* (ES) 141 $[M+H]^+$.

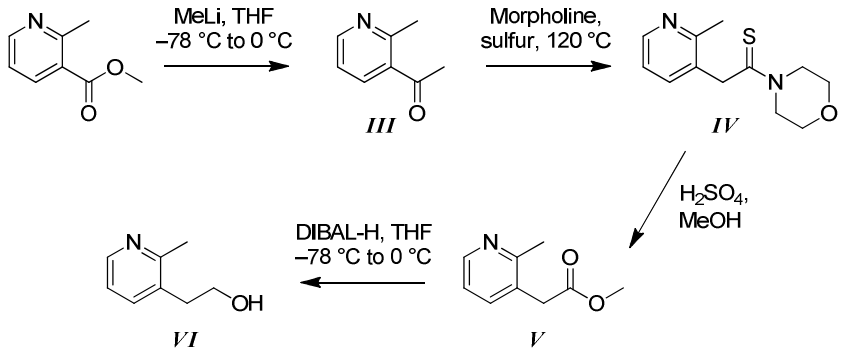
5-(Bromomethyl)pyrimidine-2,4-diamine *II*

2,4-Diamino-5-(hydroxymethyl)pyrimidine (70 mg, 0.5 mmol) was dissolved in acetic acid (600 µL) and treated with a mixture of HBr in acetic acid (30%, 0.7 mL, 2.5 eq.). The suspension was heated at reflux for 3 h, cooled and solvents carefully removed *in vacuo*. The crude product was used for subsequent steps without further purification.

Ring B derivatives

Ring B building blocks were purchased from the following companies or prepared as detailed below:

T) 5-(2-Hydroxyethyl)-4-methylthiazole, 137-00-8, Sigma-Aldrich. **P**) 3-(2-Hydroxyethyl)pyridine, 6293-56-7, Maybridge. **M)** 2-(2-Methylpyridin-3-yl)ethanol was prepared as follows using the previously reported method of Ivanova *et al.*:[5]



1-(2-Methylpyridin-3-yl)ethanone *III*

Methyl 2-methylnicotinate (24.19 g, 160 mmol) dissolved in dry THF (100 mL) was cooled to -78 °C, and treated with 1.6 M MeLi in THF (100 mL, 160 mmol) via drop-wise canula addition. The reaction was allowed to warm to room temperature with stirring over 2 h, then re-cooled to 0 °C, quenched by careful addition of 2 M HCl and allowed to warm to ambient temperature, the mixture was adjusted to pH 8 by the addition of Na_2CO_3, and extracted with diethyl ether (6 x 100 mL), the combined organic layers (dried with $MgSO_4$) were concentrated *in vacuo*, and the residue was purified by flash column chromatography to afford the target ketone (***III***) (6 g, 27%). R_f 0.32 (hexane:EtOAc, 1:1); δ_H (400 MHz; $CDCl_3$) 8.51 (1 H, dd, , *J* 6.5 and 1.5, H_6), 7.90 (1 H, dd, *J* 10.5 and 1.5, H_4), 7.17 (1 H, dd, *J* 10.5 and 6.5, H_5), 2.67, (3 H, s, CH_3), 2.52 (3 H, s, CH_3); δ_C (75 MHz; $CDCl_3$) 200.7 (C=O), 158.4 (C_2), 154.6 (C_6), 137.1 (C_4), 133.1 (C_3), 121.2 (C_5), 29.7 (CH_3), 25.0 (CH_3); *m/z* (ES) 135 (MH^+).

2-(2-Methylpyridin-3-yl)-1-morpholinoethanethione *IV*

The ketone ***III*** (6.0 g, 43.74 mmol) was treated with sulfur (5.14 g, 1 eq.), and morpholine (4 mL, 1.05 eq.) and heated at 120 °C for 14 h. The mixture was then treated with water and extracted with DCM and then EtOAc. The combined organic layers were dried ($MgSO_4$) and evaporated under reduced pressure. The residue was purified by flash column chromatography (gradient elution, 1:1, Hex:EtOAc to 9:1 EtOAc:MeOH), to afford the thioamide (***IV***) (8.9 g, 86%). R_f 0.28 (EtOAc, 10% MeOH); δ_H (400 MHz; $CDCl_3$) 8.43 (1 H, d, *J* 4.4, H_6), 7.51 (1 H, d, *J* 7.6, H_4), 7.16 (1 H, dd, *J* 7.6 and 4.4, H_5), 4.40 (2 H, m, H_1'), 4.20 (2 H, s, CH_2), 3.80 (2 H, m, H_2'), 3.60 (4 H, m, H_3' and H_4'), 2.55 (3 H, s, CH_3), δ_C (75 MHz; $CDCl_3$) 199.4 (C=S), 159.2 (C_2), 148.0 (C_6), 135.3 (C_4), 130.3 (C_3), 122.1 (C_5), 66.9 (CH_2), 66.6 (CH_2), 51.0 (CH_2), 50.4 (CH_2), 47.1 (CH_2), 22.9 (CH_3); *m/z* (ES) 237 (MH^+).

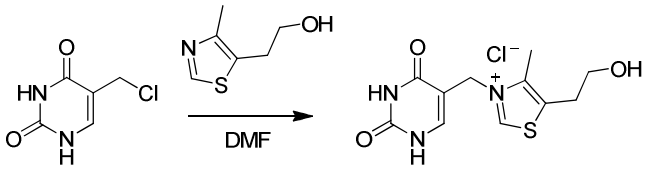
Methyl (2-methylpyridin-3-yl)acetate *V*

The thioamide ***IV*** (8.85 g, 37.5 mmol) was treated with 2 M H_2SO_4 (7.5 mL) in methanol (15 mL) and heated at reflux for 16 h; the mixture was neutralized with Na_2CO_3 and extracted with DCM (3x15 mL) to give the ester (***V***) (6.1 g, 98%), which was used without further purification. R_f 0.31 (EtOAc); δ_H (400 MHz; $CDCl_3$) 8.42 (1 H, d, H_6), 7.55 (1 H, dd, H_4), 7.18 (1 H, dd, H_5), 3.65 (3 H, CH_3), 2.45 (3 H, s, CH_3).

2-(2-Methylpyridin-3-yl)ethanol *VI*

The ester ***V*** (3.3 g, 20 mmol) was dissolved in dry DCM (100 mL), cooled to –78 °C, treated with 1.0 M DIBAL-H (50 mL, 50 mmol, 2.5 eq.), and then stirred and allowed to warm to ambient temperature over 3 h. The reaction mixture was then quenched with sat. aq. NH_4Cl and filtered through Celite, washing with DCM then $CHCl_3$. The filtrate was evaporated under reduced pressure to afford the alcohol (***VI***) (2.3 g, 84%) as a solid. R_f 0.37 (9:1, EtOAc:MeOH); δ_H (300 MHz; $CDCl_3$) 8.22 (1 H, d, *J* 4.9, H_6), 7.46 (1 H, d, *J* 7.4, H_4), 7.04 (1 H, dd, *J* 7.4 and 4.9, H_5), 3.84 (2 H, t, *J* 6.7, CH_2OH), 2.86 (2 H, t, *J* 6.7, $ArCH_2$), 2.49 (3 H, s, CH_3) δ_C (75 MHz; $CDCl_3$) 157.2 (C_2), 147.0 (C_6), 137.9 (C_4), 132.9 (C_3), 121.7 (C_5) 62.0 (CH_2OH) 36.3 ($ArCH_2$) 22.4 (CH_3); *m/z* (ES) 138 (MH^+).

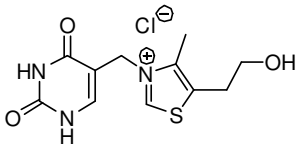
General Procedure to for thiamine analogue synthesis (1-11, T, M, P)



Benzyl halide A (**1-11**) (125 µmol), and the appropriate hetero-aryl B ring (**T, M, P**) (1.5 eq.) in DMF (250 µL) were added to a micro reaction tube fitted with a 2 µm filter, and mixed for 16 h at room temperature, after which time the product had precipitated out of solution. The reaction mixture was then cooled at –20 °C for 6 h, to induce further precipitation of the product. The mixture was then filtered, washing with ice-cold diethyl ether (10x2 mL) to give the thiamine analogue as its halide salt.

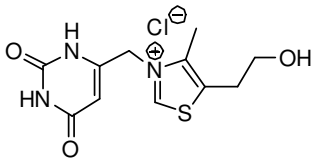
Analogue Characterization

1T



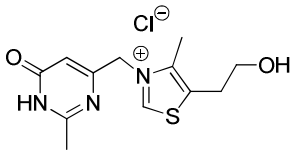
Found: C, 43.44; H, 4.69; N, 13.87; $C_{11}H_{14}N_3O_3SCl$ requires C, 43.49; H, 4.65; N, 13.83); δ_H (400 MHz; MeOD) 9.80 (1 H, s, $H_{5'}$), 7.78 (1 H, s, H_6), 5.32 (2 H, s, CH_2), 3.82, (2 H, t, *J* 5.2, CH_2OH), 3.13 (2 H, t, *J* 5.2, $ArCH_2$), 2.63 (3 H, s, CH_3), δ_C (100 MHz; CD_3OD) 166.1 (C_4), 158.1 (C_5'), 153.4 (C_2), 146.4 (C_6), 144.1 (C_2'), 137.3 (C_3'), 105.9 (C_5), 61.8 (CH_2OH), 51.7 (CH_2), 31.2 ($ArCH_2$), 12.6 (CH_3); *m/z* (ES) 268 $[M]^+$.

2T



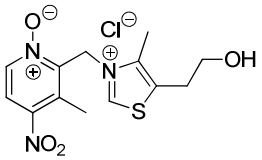
δ_H (400 MHz; D_2O) 5.44 (2 H, s, CH_2), 5.15 (1 H, s, H_5), 3.75 (2 H, t, *J* 5.7, CH_2OH), 3.11 (2 H, t, *J* 5.7, $ArCH_2$), 2.41 (3 H, s, CH_3), δ_C (100 MHz; $CDCl_3$) 166.4 (C_4), 157.9 (C_5'), 157.2 (C_2), 149.3 (C_6), 143.1 (C_2'), 136.8 (C_3'), 100.0 (C_5), 60.5 (CH_2OH), 52.1 (CH_2), 29.6 ($ArCH_2$), 11.8 (CH_3); *m/z* (ES) 268 $[M]^+$.

3T



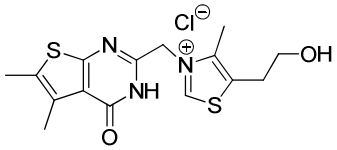
δ_H (400 MHz; CD_3OD) 10.1 (1 H, s, H_5'), 6.40 (1 H, s, H_5), 5.76 (2 H, s, CH_2), 3.86 (2 H, t, *J* 5.4, CH_2), 3.16 (2 H, t, *J* 5.4, CH_2), 2.47 (3 H, s, CH_3), 2.25 (3 H, s, CH_3); *m/z* (ES) 266 $[M]^+$.

4T



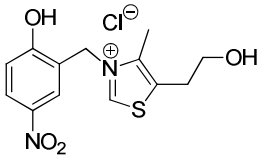
δ_H (400 MHz; CD_3OD) 8.45 (1 H, d, *J* 7.2, ArH), 8.22 (1 H, d, *J* 7.2, ArH), 5.95 (2 H, s, CH_2), 3.85 (2 H, t, *J* 5.5, CH_2), 3.18 (2 H, t, *J* 5.5, CH_2), 2.77 (3 H, s, CH_3), 2.71 (3 H, s, CH_3); *m/z* (ES) 310 $[M]^+$.

5T



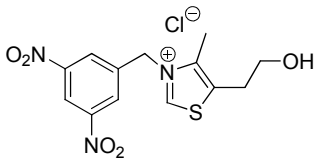
δ_H (400 MHz; CD_3OD) 5.78 (2 H, s, CH_2), 3.87 (2 H, t, *J* 5.4, CH_2), 3.18 (2 H, t, *J* 5.4, CH_2), 2.49 (3 H, s, CH_3), 2.47 (3 H, s, CH_3), 2.40 (3 H, s, CH_3); *m/z* (ES) 337.5 $[M]^+$.

6T



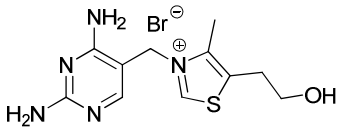
δ_H (400 MHz; CD_3OD) 9.85 (1 H, s, H_5'), 8.38 (1 H, s, H_3), 8.29 (1 H, d, *J* 9.0, H_5), 7.08 (1 H, d, *J* 9.0, H_6), 5.73 (2 H, s, CH_2), 3.83 (2 H, t, *J* 5.4, CH_2OH), 3.10 (2 H, d, *J* 5.4, $ArCH_2$), 2.60 (3 H, s, CH_3); *m/z* (ES) 296.4 $[M]^+$.

7T



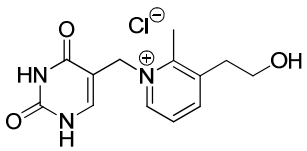
δ_H (400 MHz; CD_3OD) 9.10 (1 H, t, *J* 2.0, *p*ArH), 8.68 (2 H, d, *J* 2.0, 2 x *o*ArH), 6.00 (2 H, s, CH_2), 3.85 (2 H, t, *J* 5.6, CH_2OH), 3.15 (2 H, t, *J* 5.6, $ArCH_2$), 2.52 (3 H, s, CH_3); *m/z* (ES) 323.9 $[M]^+$.

8T



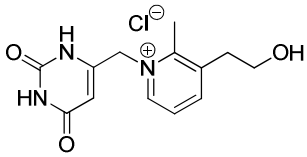
Found: C, 24.69; H, 3.72; N, 13.20; $C_{11}H_{20}N_5O_3SBr_3$ requires C, 24.37; H, 3.72; N, 12.92); m.p. 180-181°C (decomp); δ_H (400 MHz; CD_3OD) 9.76 (1 H, s, H_5'), 8.08 (1 H, s, H_6), 5.44 (2 H, s, CH_2), 3.86 (2 H, t, *J* 5.5, CH_2OH), 3.18 (2 H, t, *J* 5.5, $ArCH_2$), 2.66 (3 H, s, CH_3), δ_C (100 MHz; CD_3OD) 166.0 (C_4), 157.2 (C_2), 155.6 (C_5'), 148.0 (C_6), 144.5 (C_2'), 138.4 (C_3'), 100.9 (C_5), 61.7 (CH_2OH), 52.3 (CH_2), 31.2 ($ArCH_2$), 12.6 (CH_3); *m/z* (ES) 266 (100, M^+-Br).

1M



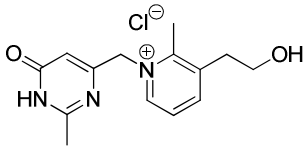
δ_H (400 MHz; CD_3OD) 8.89 (1 H, d, *J* 6.3, H_6'), 8.39 (1 H, d, *J* 8.8, H_4'), 7.95 (1 H, s, H_6), 7.95 (1 H (1 H, dd, *J* 6.3 and 8.8, H_5'), 5.59 (2 H, s, CH_2), 3.88 (2 H, t, *J* 6.2 CH_2OH), 3.13 (2 H, t, *J* 6.2, $ArCH_2$), 3.02 (3 H, s, CH_3), δ_C (100 MHz; CD_3OD) 166.0 (C_4), 157.1 (C_2'), 152.3 (C_2), 148.1 (C_4'), 146.4 (C_6), 145.4 (C_6'), 142.0 (C_3'), 125.6 (C_5'), 106.5 (C_5), 62.1 (CH_2OH), 56.8 (CH_2), 37.3 ($ArCH_2$), 17.7 (CH_3); *m/z* (ES) 262.1 $[M]^+$.

2M



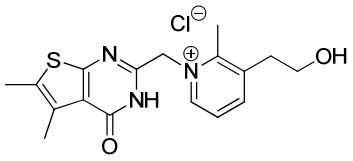
δ_H (400 MHz; CD_3OD) 8.89 (1 H, d, 6.0 H_6'), 8.57 (1 H, m, H_4'), 8.00 (1 H, dd, *J* 8.2 and 6.0, H_5'), 5.67 (2 H, s, CH_2), 5.52 (1 H, s, H_5), 3.92 (2 H, t, *J* 6.0 CH_2OH), 3.17 (2 H, t, *J* 6.0, $ArCH_2$), 2.86 (3 H, s, CH_3), δ_C (100 MHz; CD_3OD) 166.5, 158.3, 157.9, 157.5, 153.2, 149.6, 146.3, 143.3, 126.9, 100.3, 62.0, 37.1, 17.7; *m/z* (ES) 262.1 $[M]^+$.

3M



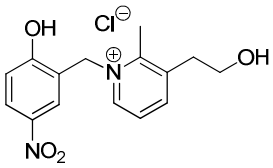
δ_H (400 MHz; CD_3OD) 8.86 (1 H, d, 6.1 H_6'), 8.49 (1 H, d, *J* 7.9 H_4'), 7.93 (1 H, dd, *J* 6.1 and 7.9, H_5'), 6.35 (1 H, s, H_5), 5.78 (2 H, s, CH_2), 3.90 (2 H, t, *J* 5.9 CH_2OH), 3.14 (2 H, t, *J* 5.9, $ArCH_2$), 2.84 (3 H, s, CH_3), 2.29 (3 H, s, CH_3); *m/z* (ES) 260.1 $[M]^+$.

5M



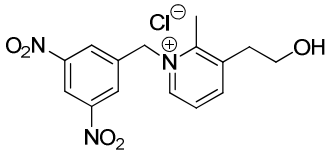
δ_H (400 MHz; CD_3OD) 8.87 (1 H, d, *J* 6.1 H_6'), 8.55 (1 H, d, *J* 7.9 H_4'), 7.97 (1 H, dd, *J* 6.1 and 7.9, H_5'), 5.92 (2 H, s, CH_2), 3.92 (2 H, t, *J* 6.1, CH_2OH), 3.17 (2 H, t, *J* 6.1, $ArCH_2$), 2.80 (3 H, s, CH_3), 2.47 (3 H, s, CH_3), 2.39 (3 H, s, CH_3); *m/z* (ES) 330.1 $[M]^+$.

6M



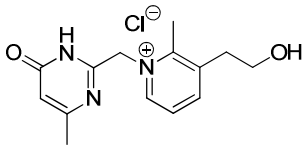
δ_H (400 MHz; CD_3OD) 8.84 (1 H, d, *J* 5.7 H_6'), 8.46 (1 H, d, *J* 7.9 H_4'), 8.28 (1 H, dd, *J* 9.0 and 2.8, H_5), 8.22 (1 H, d, *J* 2.8, H_3), 7.89 (1 H, dd, *J* 5.7 and 7.9 H_5'), 7.07 (1 H, d, *J* 9.0 H_6), 5.91 (2 H, s, CH_2), 3.89 (2 H, t, *J* 6.1 CH_2OH), 3.14 (2 H, t, *J* 6.1, $ArCH_2$), 2.91 (3 H, s, CH_3); *m/z* (ES) 289.1 $[M]^+$.

7M



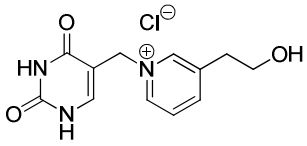
δ_H (400 MHz; CD_3OD) 9.08 (1H, t, *J* 2.0, H_4) 8.99 (1 H, d, *J* 6.2, H_6'), 8.57 (1 H, d, *J* 9.2 H_4') 8.55 (2 H, d, *J* 2.0, H_2 and H_6), 8.03 (1 H, m, H_5'), 6.20 (2 H, s, CH_2), 3.91 (2 H, t, *J* 6.0 CH_2OH), 3.15 (2 H, t, *J* 6.0, $ArCH_2$), 2.89 (3 H, s, CH_3); *m/z* (ES) 318.0 $[M]^+$.

9M



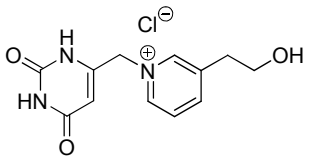
δ_H (400 MHz; CD_3OD) 8.84 (1 H, d, 6.1 H_6'), 8.52 (1 H, d, 7.9 H_4'), 7.95 (1 H, dd, *J* 6.1 and 7.9, H_5'), 6.33 (1 H, bs, H_5), 5.89 (2 H, s, CH_2), 3.91 (2 H, t, *J* 5.9 CH_2OH), 3.16 (2 H, t, *J* 5.9, $ArCH_2$), 2.78 (3 H, s, CH_3), 2.21 (3 H, s, CH_3), *m/z* (ES) 260.1 $[M]^+$.

1P



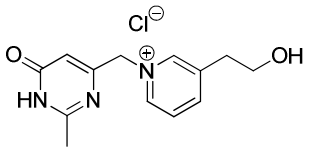
δ_H (400 MHz; CD_3OD) 9.00 (1 H, s, H_2'), 8.96 (1 H, d, *J* 6.1 H_6'), 8.51 (1 H, d, *J* 8.0 H_4'), 8.04-8.00 (2 H, m, H_5' and H_6), 5.48 (2 H, s, CH_2), 3.85 (2 H, t, J 6.0, CH_2OH), 3.06 (2 H, t, *J* 6.0, $ArCH_2$); *m/z* (ES) 248.2 $[M]^+$.

2P



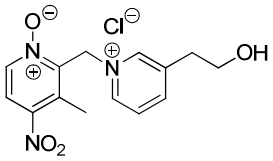
δ_H (400 MHz; CD_3OD) 9.01 (1 H, s, H_1'), 8.96 (1 H, d, *J* 6.1 H_6'), 8.66 (1 H, d, *J* 8.1 H_4'), 8.16 (1 H, dd, *J* 6.1 and 8.1, H_5'), 5.66 (2 H, s, CH_2), 5.48 (1 H, s, H_5) 3.92 (2 H, t, *J* 5.9 CH_2OH), 3.12 (2 H, t, *J* 5.9, $ArCH_2$), δ_C (100 MHz; MeOD) 166.5, 150.1, 149.5, 147.2, 144.9, 144.1, 129.6, 102.6, 85.2, 62.1, 61.2, 36.9; *m/z* (ES) 248.2 $[M]^+$.

3P



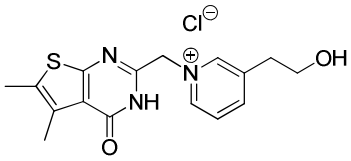
δ_H (400 MHz; CD_3OD) 9.01 (1 H, s, H_2') 8.95 (1 H, d, *J* 6.1 H_6'), 8.59 (1 H, d, *J* 8.1, H_4'), 8.10 (1 H, dd, *J* 6.1 and 8.1, H_5'), 6.46 (1H, s, H_5), 5.67 (2 H, s, CH_2), 3.90 (2 H, t, *J* 6.0 CH_2OH), 3.11 (2 H, t, *J* 6.0, $ArCH_2$), 2.37 (3 H, s, CH_3); *m/z* (ES) 246.2 $[M]^+$.

4P



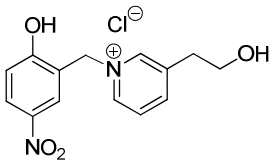
δ_H (400 MHz; CD_3OD) 8.85 (1 H, s, H_2'), 8.82 (1 H, d, *J* 8.0 H_6'), 8.43 (1 H, d, *J* 8.0, H_4'), 8.30 (1 H, d, *J* 6.0, H_5), 8.07 (1 H, d, *J* 6.0, H_6), 7.93 (1 H, dd, *J* 8.0 and 8.0, H_5'), 6.06 (2 H, bs, CH_2), 3.76 (2 H, t, *J* 6.0, CH_2OH), 3.04 (2 H, t, *J* 6.0, $ArCH_2$), 2.72 (3 H, s, CH_3), δ_C (100 MHz; CD_3OD) 148.5 (C_4'), 147.4 (C_4), 146.9 (C_2'), 145.5 (C_2), 144.7 (C_6'), 143.2 (C_3'), 140.1 (C_5), 135.3 (C_3), 128.8 (C_5'), 124.5 (C_6) 62.3 (CH_2OH), 56.0 (CH_2), 36.9 ($ArCH_2$), 16.4 (CH_3); *m/z* (ES) 290.2 $[M]^+$.

5P



δ_H (400 MHz; CD_3OD) 9.02 (1 H, s, H_2'), 8.96 (1 H, d, *J* 6.1 H_6'), 8.66 (1 H, d, *J* 8.1, H_4'), 8.15 (1 H, dd, *J* 8.1 and 6.1, H_5'), 5.92 (2 H, s, CH_2), 3.92 (2 H, t, *J* 5.9, CH_2OH), 3.12 (2 H, t, *J* 5.9 $ArCH_2OH$), 2.38 (3 H, s, CH_3), 2.29 (3 H, s, CH_3); *m/z* (ES) 316.2 $[M]^+$.

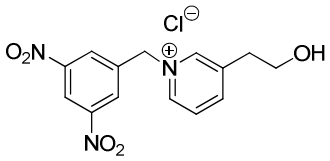
6P



δ_H (400 MHz; CD_3OD) 9.04 (1 H, s, H_2'), 8.97 (1 H, d, *J* 5.8 H_6'), 8.58 (1 H, s, H_3), 8.52 (1 H, d, *J* 7.9, H_4'), 8.28 (1 H, d, *J* 9.0, H_5), 8.04 (1 H, dd, *J* 5.8 and 7.9 H_5'), 7.05 (1 H, d, *J* 9.0, H_6), 5.87 (2 H, s, CH_2), 3.86 (2 H, t, *J* 5.8

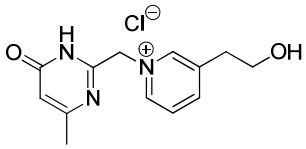
CH_2OH), 3.06 (2 H, t, *J* 5.8, $ArCH_2$); *m/z* (ES) 275.2 $[M]^+$.

7P



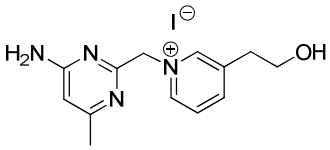
δ_H (400 MHz; CD_3OD) 9.14 (1 H, s, H_2'), 9.10-9.07 (2 H, m, H_6' and H_4), 8.89 (2 H, s, H_2 and H_6), 8.63 (1 H, d, 8.1, H_4'), 8.13 (1 H, dd, *J* 6.2 and 8.1, H_5'), 6.14 (2 H, s, CH_2), 3.91 (2 H, t, *J* 5.9, CH_2OH), 3.11 (2 H, t, *J* 5.9, $ArCH_2$), δ_C (100 MHz; CD_3OD) 150.9, 148.9 (2xC), 146.8, 144.4, 144.2, 138.7, 131.2 (2xC), 129.8 (2xC), 121.2, 63.9, 62.1, 36.9; *m/z* (ES) 304.1 $[M]^+$.

9P



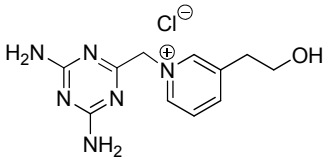
δ_H (400 MHz; CD_3OD) 8.98 (1 H, s, H_1'), 8.96 (1 H, d, *J* 6.1 H_6'), 8.64 (1 H, d, *J* 8.1 H_4'), 8.13 (1 H, dd, *J* 6.1 and 8.1, H_5'), 6.30 (1 H, s, H_5), 5.87 (2 H, s, CH_2), 3.92 (2 H, t, *J* 6.0 CH_2OH), 3.12 (2 H, t, *J* 6.0, $ArCH_2$), 2.17 (3H, s, CH_3); *m/z* (ES) 246.2 $[M]^+$.

10P



δ_H (400 MHz; CD_3OD) 8.97 (1 H, s, H_2), 8.90 (1 H, d, *J* 6.1, H_6'), 8.59 (1 H, d, *J* 7.9 H_4'), 8.09 (1 H, m, H_5'), 6.31 (1 H, s, H_5), 5.72 (2 H, s, CH_2), 3.91 (2 H, t, *J* 6.0, CH_2OH), 3.12 (2 H, t, *J* 6.0, $ArCH_2$), 2.28 (3 H, s, CH_3); *m/z* (ES) 245.2 $[M]^+$.

11P



δ_H (400 MHz; CD_3OD) 8.93 (1 H, s, H_2), 8.87 (1 H, d, 6.0 H_6'), 8.59 (1 H, d, 8.0 H_4'), 8.08 (1 H, dd, *J* 8.0 and 6.0, H_5'), 3.91 (2 H, t, *J* 5.9 CH_2OH), 3.09 (2 H, t, *J* 5.9, $ArCH_2$); *m/z* (ES) 247.1 $[M]^+$.

References:

1. K. Lang, R. Rieder, R. Micura, *Nucl. Acids Res.* **2007**, 35, 5370–5378; A. Rentmeister, G. Mayer, N. Kuhn, M. Famulok, *Nucleic Acids Res.* **2007**, 35, 3713–3722.
2. Chen, L., Cressina, E., Leeper, F.J., Smith, A.G. & Abell, C. A fragment-based approach to identifying ligands for riboswitches. *ACS Chemical Biology* **5**, 355-358 (2010).
3. Cressina, E., Chen, L., Abell, C., Leeper, F.J. & Smith, A.G. Fragment screening against the thiamine pyrophosphate riboswitch thiM. *Chemical Science* **2**, 157-165 (2011).
4. Tieckelmann, H., Guthrie and R., Nairn. J, *J. Org. Chem.*, 1960, **25** (7), 1257–1259
5. Ivanova, M. E., Kurteva V. B. and Lyapova, M. J., *Org. Prep. Proced. Int.*, 1994, **26** (5), 549–551