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

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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

- **Thiamine pyrophosphate**
  - $N = 0.691 \pm 0.003$ sites
  - $K = 1.30 \times 10^{-6}$ M
  - $\Delta H = -1.266 \pm 1.369$ kcal/mol
  - $\Delta S = 11.6$ (cal/mol)/deg

- **Thiamine monophosphate**
  - $N = 0.485 \pm 0.009$ sites
  - $K = 1.25 \times 10^{-6}$ M
  - $\Delta H = -1.174 \pm 2.877$ kcal/mol
  - $\Delta S = 11.5$ (cal/mol)/deg

- **Compound 12**
  - $N = 1.81 \pm 0.016$ sites
  - $K = 2.53 \times 10^{-6}$ M
  - $\Delta H = -5287 \pm 68.3$ kcal/mol
  - $\Delta S = 11.6$ (cal/mol)/deg

- **Compound 13**
  - $N = 2.16 \pm 0.014$ sites
  - $K = 4.32 \times 10^{-6}$ M
  - $\Delta H = -2854 \pm 27.8$ kcal/mol
  - $\Delta S = 16.2$ (cal/mol)/deg

- **Compound 14**
  - $N = 0.550 \pm 0.002$ sites
  - $K = 1.04 \times 10^{-6}$ M
  - $\Delta H = -1.756 \pm 150.4$ kcal/mol
  - $\Delta S = 22.2$ (cal/mol)/deg

- **Compound 19**
  - $N = 0.811 \pm 0.008$ sites
  - $K = 5.86 \times 10^{-6}$ M
  - $\Delta H = -8069 \pm 117.6$ kcal/mol
  - $\Delta S = 3.81$ (cal/mol)/deg

- **Compound 20**
  - $N = 0.811 \pm 0.008$ sites
  - $K = 5.86 \times 10^{-6}$ M
  - $\Delta H = -8069 \pm 117.6$ kcal/mol
  - $\Delta S = 3.81$ (cal/mol)/deg

- **Compound 21**
  - $N = 1.22 \pm 0.013$ sites
  - $K = 1.47 \times 10^{-6}$ M
  - $\Delta H = -8750 \pm 130.8$ kcal/mol
  - $\Delta S = -1.13$ (cal/mol)/deg

- **Compound 22**
  - $N = 0.778 \pm 0.150$ sites
  - $K = 1.91 \times 10^{-6}$ M
  - $\Delta H = -938.5 \pm 224.0$ kcal/mol
  - $\Delta S = -22.2$ (cal/mol)/deg

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 ($\Delta H$) and the entropy of binding ($\Delta S$). In the final isotherm no heat of binding was observed when oxythiamine (1 mM) was titrated into thiM RNA (50 µM).
Supplementary Figure S4.

![Graph showing normalized luminescence (RLU) for different TPP analogues.](image)

**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:

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GGGCGAAUUGGGCCCGACGUCGCAUCUCCCGCCGCCAUUGCCGAGCGGAAUUCGAUUGAUCA
UGAAUUCGCAACAAACGACUCGGGUGCCCUUCUGCGUGAAGGCGAGAAAUACCCGUAUCCUGA
UCUGGAUAAUGCCAGCUGAGGAAGGU
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*In vitro* transcription/translation plasmids were as described previously. 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

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\(_{21}\)N\(_2\)O\(_3\) requires M + H, 267.1743]; \(\nu_{max}/\text{cm}^{-1} 1732\) (C=O), 1584 and 1556 (Ar C=C); \(\delta_H\) (400 MHz, CDCl\(_3\)) 1.04 (3H, d, J 6.3, CH\(_3\)CH), 1.35 (1H, m, CH\(_2\)H\(_6\)(CH\(_2\))\(_2\)OAc), 1.43 (1H, m, CH\(_2\)H\(_6\)(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\(_2\)H\(_6\)NH), 3.70 (1H, d, J 13.2, CH\(_2\)H\(_6\)NH), 3.98 (2H, t, J 6.6, CH\(_2\)OAc), 6.09 (2H, broad s, NH\(_2\)), 7.87 (1H, s, pyrimidine-H); \(\delta_C\) (100 MHz, CDCl\(_3\)) 20.93 and 21.47 (CHCH\(_3\) and CH\(_3\)COO), 23.62 (CH\(_2\)CH\(_2\)OAc), 24.85 (pyrimidine-CH\(_3\)), 34.51 (CH\(_2\)(CH\(_2\))\(_2\)OAc), 45.82 (CH\(_2\)NH), 55.61(CH\(_3\)CH), 64.67 (CH\(_2\)OAc), 110.42 (CCH\(_2\)NH), 157.63 (pyrimidine C-H), 162.43 (CNH\(_2\)), 165.10 (CH\(_3\)), 171.92 (CH\(_3\)COO).

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°C. 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\(_{13}\)H\(_{21}\)N\(_2\)O\(_3\) requires M + H, 309.1848]; \(\nu_{max}/\text{cm}^{-1} 1734\) (ester C=O), 1663 (amide C=O); \(\delta_H\) (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\(_2\)CON), 2.43 (3H, s, pyrimidine-CH\(_3\)), 3.86 (1H, sextet, J 7, CH(CH\(_2\))\(_2\)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); \(\delta_C\) (100 MHz, CDCl\(_3\)) 20.74, 21.34 and 21.46 (CHCH\(_3\), CH\(_2\)CON and CH\(_3\)COO), 23.68 (CH\(_2\)CH\(_2\)OAc), 24.83 (pyrimidine-CH\(_3\)), 34.37 (CH\(_2\)(CH\(_2\))\(_2\)OAc), 45.53 (CH\(_2\)N), 58.94 (CH\(_3\)CH), 65.01(CH\(_2\)OAc), 112.17 (CCH\(_2\)N), 155.89 (pyrimidine C-H), 161.78 (CNH\(_2\)), 165.24 (CH\(_2\)), 172.44 (CH\(_3\)COO), 174.98 (CH\(_3\)CON).
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, C₁₀H₁₂N₂O₂ requires M + H⁺, 267.1743]; δ_H (400 MHz CDCl₃) 1.22 (3H, d, J 6.8, CH₂CH₂), 1.39 (2H, m, CH₂(CH₂)₂OH), 1.62 (2H, m, CH₂CH₂OH), 2.19 (3H, s, CH₃CON), 2.45 (3H, s, pyrimidine-CH₃), 3.52 (2H, m, CH₂OH), 3.89 (1H, m, CH(CH₂)₂OH), 4.42 (1H, d, J 15.5, CH₃N), 4.49 (1H, d, J 15.5, CH₂H₃N), 6.20 (2H, broad s, NH₂), 7.98 (1H, s, pyrimidine-H); δ_c 19.89 and 21.24 (CH₂CH₃ and CH₂CON), 24.69 (pyrimidine-CH₂), 32.51 (CH₂CH₂OH), 34.16 (CH₂CH₃₂OH), 45.48 (CH₂N), 59.1 (CH₃CH), 63.16 (CH₂OH), 112.08 (CCH₂N), 155.63 (pyrimidine-CH), 161.6 (CNH₂), 165.09 (CCH₃), 174.66 (CH₂CON).

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, C₁₀H₁₃N₂O₃S requires M + H⁺, 345.1518]; δ_H (400 MHz CDCl₃) 1.24 (3H, d, J 6.8, CH₂CH₂), 1.57 (4H, m, CH₂CH₂CH₂OMs), 2.19 (3H, s, CH₂CON), 2.45 (3H, s, pyrimidine-CH₂), 2.98 (3H, s, CH₂SO₂), 3.88 (1H, m, CH(CH₂)₂OMs), 4.05 (2H, m, CH₂OMs), 4.34 (1H, d, J 15.4, CH₃N), 4.54 (1H, d, J 15.4, CH₂H₂NCO), 6.28 (2H, broad s, NH₂), 7.99 (1H, s, pyrimidine-H); δ_c 19.94 and 21.36 (CH₂CH₃ and CH₂CON), 23.42 (CH₂CH₂OMs), 24.73 (pyrimidine-CH₂), 34.05 (CH₂(CH₂)₂OMs), 38.31(CH₂SO₂), 45.61 (CH₂N), 60.11 (CH₂CH), 70.62 (CH₂OMs), 112.17 (CCH₂N), 155.89 (pyrimidine-CH), 161.71 (CNH₂), 165.18 (CCH₃), 174.83 (CH₂CON).

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-tetraethylammonium 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, C₁₃H₂₃N₄O₄P₂ requires M + H, 427.1069]; δH (400 MHz, D₂O) 1.13 (3H, d, J 6.7, CH(CH₃), 1.53 (4H, m, CH₂CH₂CH₂OHOPP), 2.20 (3H, s, CH₃CON), 2.41 (3H, s, pyrimidine-CH₃), 3.77 (1H, m, CH(CH₃)₂OPP), 4.09 (2H, q, J 6.6, CH₂OPP), 7.78 (1H, s, pyrimidine-H); δC (100 MHz, D₂O) 19.72 and 21.41 (CHCH₃ and CH₂CON), 23.57 (CH₂CH₂OPP), 24.89 (pyrimidine-CH₃), 33.82 (CH₂(CH₂)₂OPP), 45.39 (CH₂N), 60.08 (CH₂CH), 67.12 (CH₂OPP), 112.03 (CCH₂N), 154.64 (pyrimidine-CH), 161.57 (CNH₂), 164.85 (CCH₃), 174.46 (CH₂CON); δP (162 MHz, D₂O) -8.54 (1P, d, J 20.4), -10.46 (1P, d, J 20.4).

![Image of compound S5](image-url)

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 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, C₁₃H₃₂N₄O₄ requires M + H, 429.2424]; νmax/cm⁻¹ 1732 (ester CO), 1663 (amide CO), 1601 and 1656 (aromatic C=C); δH (400 MHz, CDCl₃) 1.04 (3H, d, J 6.8, CH₃CH(CH₂)₂OAc), 1.11 (3H, d, J 6.8, CH₃CHOHCO₂), 1.28 (2H, m, CH₂(CH₂)₂OAc), 1.42 (2H, m, CH₂CH₂OAc), 1.94 (3H, s, CH₃COO), 2.40 (3H, s, pyrimidine-CH₃), 3.79 (2H, m), 4.00 (1H, m), 4.30-4.60 (5H, m), 6.20 (2H, broad s, NH₂), 7.25 (5H, m, Ph), 7.93 (1H, s, pyrimidine-H); δC (100 MHz, CDCl₃) 20.43 and 20.65 (2 x CHCH₃), 21.25 (CH₂COO) 25.05 (CH₃CH₂OAc), 25.34 (pyrimidine-CH₃), 32.30 (CH₂(CH₂)₂OAc), 49.47 (CH₂N), 60.85 (CH(CH₂)₃OAc), 63.96 (CH₂OAc), 71.37 (benzyl-CH₂), 74.62 (CHOBn), 111.12 (CCH₃), 128.14, 128.30 and 128.44 (5 x phenyl CH), 137.53 (phenyl C), 156.95 (pyrimidine-CH), 162.38 (CNH₂), 167.85 (CCH₂), 171.27 (CH₂CHCON), 174.77 (CH₂COO).

![Image of compound S6](image-url)

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, C₂₁H₂₉N₄O₄ requires M + H, 387.2351]; δH (400 MHz, CDCl₃) 1.03 (3H, d, J 6.7, CH₃CH(CH₂)₂OH), 1.11 (3H, d, J 6.7, CH₃CHOHCO₂), 1.27 (4H, m, CH₂CH₂CH₂OH), 2.39 (3H, s,
pyrimidine-CH₃), 3.36 (2H, t, J 6.2, CH₂OH), 3.99 (1H, m), 4.30-4.60 (5H, m), 7.25 (5H, m, Ph), 7.92 (1H, s, pyrimidine-H); δC (100 MHz, CDCl₃) 20.49 and 20.73 (2 x CH, CH₂N), 62.10 (CHN), 62.19 (CH₂OH), 71.39 (benzyl-CH₂), 73.81 (CH₂CHOBn), 111.21 (CCH₂N), 128.15, 128.31 and 128.41 (5 x phenyl C), 157.08 (pyrimidine-CH), 167.85 (CCH₃), 171.19 (CH₃CON).

4-(N-(4-amino-2-methylpyrimidin-5-yl)methyl)-2-(benzoyloxy)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, C₂H₃₂N₄O₃S requires M + H, 465.2093]; δH (400 MHz, CDCl₃) 1.04 (3H, d, J 6.7, CH₃CHN), 1.10 (3H, d, J 6.7, CH₂CHO), 1.26 (4H, m, CHCH₂CH₃), 2.39 (3H, s, pyrimidine-CH₃), 2.99 (3H, s, CH₂SO₂), 3.72 (2H, t, J 6.2, CH₂OMs), 3.97 (1H, m), 4.30-4.60 (5H, m), 7.23 (5H, m, Ph), 7.93 (1H, s, pyrimidine-H); δC (100 MHz, CDCl₃) 20.49 and 20.73 (2 x CH, CH₂N), 62.10 (CHN), 62.19 (CH₂OH), 71.39 (benzyl-CH₂), 73.72 (CH-O), 111.24 (CCH₂N), 128.09, 128.33 and 128.41 (5 x phenyl CH), 157.12 (pyrimidine-CH), 167.83 (CCH₃), 171.24 (CH₃CON).

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, C₁₅H₂₆N₄O₃S requires M + H, 375.1624]; δH (400 MHz, CDCl₃) 1.03 (3H, d, J 6.7, CH₃CHN), 1.14 (3H, d, J 6.7, CH₂CHOH), 1.28 (4H, m, CHCH₂CH₃), 2.37 (3H, s, pyrimidine-CH₃), 2.99 (3H, s, CH₂SO₂), 3.71 (2H, t, J 6.2, CH₂OMs), 3.97 (1H, m), 4.30-4.60 (3H, m), 7.92 (1H, s, pyrimidine-H); δC (100 MHz, CDCl₃) 20.47 and 20.73 (2 x CH, CH₂N), 25.35 (CH₂CH₂OMs), 25.84 (pyrimidine-CH₃), 32.43 (CHCH₂), 49.41 (CH₂N), 67.72 (CHOH), 69.87 (CH₂OMs), 111.41 (CCH₂N), 157.15 (pyrimidine-CH), 167.73 (CCH₃), 171.24 (C=O).
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_{4}O_{4}P_{2} requires M + H, 457.1175]; δ_H (400 MHz, CDCl_3) 1.04 (3H, d, J 6.7, CH,CHN), 1.14 (3H, d, J 6.7, CH,CHOH), 1.28-1.41 (4H, m, CHCH_2CH_3), 2.39 (3H, s, pyrimidine-CH_3), 3.87 (1H, m, CH-H), 3.99 (1H, q, J 6.7, CH,CHOH), 4.12 (2H, q, J 6.6, CH,OPP), 7.92 (1H, s, pyrimidine-H); δ_C (100 MHz, CDCl_3) 20.47 and 20.73 (2x CHCH_3), 25.35 (CH_2CH_2OPP), 25.84 (pyrimidine-CH_3), 32.43 (CHCH_2), 49.41 (CH_2N), 62.16 (CH-N), 67.72 (CHOH), 73.57 (CHOPP), 111.66 (CCH_2N), 157.29 (pyrimidine-CH), 167.74 (CCH_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)

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_{4}O_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, CHCH_3), 1.46 (4H, m, (CH_2)CH_2O), 2.16 (3H, s, CH_2CO_2), 2.27 (2H, t, J 6.8, CH_2CH_2C=CH), 2.33 (3H, s, pyrimidine-CH_3), 2.39 (2H, t, J 6.8, CH_2CH_2=CH), 3.74 (1H, m, CHCH_3), 4.13 (2H, m, CH_2OAc), 7.91 (1H, s, pyrimidine-H); δ_C (100 MHz, CDCl_3) 18.55 (CH_2CH_2C=CH), 19.69 (CH_2CH), 23.54 (CH_2CH_2OAc), 24.83 (pyrimidine-CH_3), 33.52 (CH_2CH_2O), 45.17 (CH_2N), 60.10 (CH_2CH), 66.47 (CH_2OAc), 70.01 (CH_2CH_2C=CH), 83.32 (CH_2CH_2C=CH), 112.07 (CCH_2N), 154.87 (pyrimidine-CH), 161.38 (CNH_2), 164.91 (CCH_3), 171.48 (CON), 174.35 (CH_2COO).
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 MgSO₄ and evaporated under reduced pressure to give the alcohol S10 (607 mg, 96%) as a colourless gum. [Found: M + H⁺ (+ESI), 305.1857, C₁₂H₁₄N₂O₂ requires M + H, 305.1899]; δ₁H (400 MHz, CDCl₃) 1.05 (3H, d, J 6.6, CH₂CH), 1.44 (4H, m, (CH₂)₃CH₂OH), 2.26 (2H, t, J 6.8, CH₂CH₂C=CH), 2.33 (3H, s, pyrimidine-CH₃), 2.37 (2H, t, J 6.8, CH₂CH₂C=CH), 3.46 (2H, m, CH₂OH), 3.72 (1H, m, CH₂OH), 7.91 (1H, s, pyrimidine-H); δ₁C (100 MHz, CDCl₃) 18.55 (CH₂CH₂CH₂OH), 33.53 (CHCH₂), 45.19 (CH₂N), 60.10 (CH₂CH), 66.51 (CH₂OH), 70.03 (CH₂CH₂C=CH), 83.29 (CH₂CH₂C=CH), 112.14 (C=N), 154.87 (pyrimidine-CH), 161.39 (CNH₂), 164.94 (CH₃), 171.42 (C=O).

![S11](image)

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 MgSO₄ 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, C₁₂H₂₃N₄O₄S requires M + H, 383.1675]; δ₁H (400 MHz, CDCl₃) 1.06 (3H, d, J 6.6, CH₂CH), 1.43 (4H, m, CH(CH₂)₃), 2.28 (2H, t, J 6.8, CH₂CH₂C=CH), 2.33 (3H, s, pyrimidine-CH₃), 2.39 (2H, t, J 6.8, CH₂CH₂C=CH), 3.70 (2H, m, CH₂OMs), 3.72 (1H, m, CH₂CH), 7.89 (1H, s, pyrimidine-H); δ₁C (100 MHz, CDCl₃) 18.55 (CH₂CH₂C=CH), 19.63 (CH₂CH), 24.81 (pyrimidine-CH₂), 25.84 (CH₂CH₂OMs), 33.51 (CH₂CH₂), 45.21 (CH₂N), 60.10 (CH₂CH), 66.59 (CH₂OMs), 70.01 (CH₂CH₂C=CH), 83.27 (CH₂CH₂C=CH), 112.14 (C=N), 154.88 (pyrimidine-CH), 161.39 (CNH₂), 164.92 (CH₃), 171.42 (C=O).

![16](image)

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 the was 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\(_{15}\)N\(_{5}\)O\(_3\)P\(_2\) requires M + H, 465.1226]; \(\delta\)_\(\text{H}(400 \text{ MHz, D}_2\text{O})\) 1.06 (3H, d, J 6.6, CH\(_3\)CH), 1.46 (4H, m, CH(CH\(_2\))\(_2\)). 2.25 (2H, t, J 6.8, CH\(_2\)C=CH), 2.33 (3H, s, pyrimidine-CH\(_3\)), 2.38 (2H, t, J 6.8, CH\(_2\)CH\(_2\)C=CH), 3.73 (1H, m, CHCH\(_3\)), 4.11 (2H, m, CH\(_2\)OPP), 7.72 (1H, s, pyrimidine-H); \(\delta\)_\(\text{C}\) (100 MHz, CDCl\(_3\)) 18.58 (CH\(_2\)CH\(_2\)C=CH), 19.72 (CH\(_3\)CH), 23.61 (CH\(_2\)CH\(_2\)OPP), 24.83 (pyrimidine-CH\(_3\)), 33.57 (CHCH\(_2\)), 45.21 (CH\(_2\)N), 60.14 (CH\(_3\)CH), 67.07 (CH\(_2\)OPP), 70.13 (CH\(_2\)CH\(_2\)C=CH), 83.34 (CH\(_2\)CH\(_2\)C=CH) 112.07 (CHCH\(_2\)N), 154.89 (pyrimidine-CH), 161.43 (CH\(_2\)N), 164.91 (CH\(_2\)CH), 174.40 (C=O); \(\delta\)_\(\text{P}\) (162 MHz, D\(_2\)O) -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) (Chloromethyl)-3-methyl-4-nitropyridine 1-oxide, 116418-98-5, Specs. 5) (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) (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:

\[ \text{2,4-Diaminopyrimidin-5-yl} \text{methanol I} \]

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), \(\delta\)_\(\text{H}(400 \text{ MHz, CD}_3\text{OD})\) 7.75 (1 H, s, H\(_6\)), 4.40 (2 H, s, CH\(_2\)), \(\delta\)_\(\text{C}\) (75 MHz; CD\(_3\)OD) 165 (C\(_3\)), 156 (C\(_2\)), 138 (C\(_6\)). 130 (C\(_4\)), 57 (CH\(_2\)); \(m/z\) (ES) 141 [M+H]\.\(^+\)

\[ \text{5-(Bromomethyl)pyrimidine-2,4-diamine II} \]

12
2,4-Diamo-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

![Chemical structure of compounds](image)

**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 Na2CO₃, and extracted with diethyl ether (6 x 100 mL), the combined organic layers (dried with MgSO₄) were concentrated in vacuo, and the residue was purified by flash column chromatography to afford the target ketone (III) (6 g, 27%). Rf 0.32 (hexane:EtOAc, 1:1); δH (400 MHz; CDCl₃) 8.51 (1 H, dd., J 6.5 and 1.5, H₆), 7.90 (1 H, dd, J 10.5 and 1.5, H₄), 7.17 (1 H, dd, J 10.5 and 6.5, H₃), 2.67 (3 H, s, CH₃), 2.52 (3 H, s, CH₃); δC (75 MHz; CDCl₃) 200.7 (C=O), 158.4 (C₂), 154.6 (C₆), 137.1 (C₄), 133.1 (C₃), 121.2 (C₅), 29.7 (CH₃), 25.0 (CH₃); 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 (MgSO₄) 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%). Rf 0.28 (EtOAc, 10% MeOH); δH (400 MHz; CDCl₃) 8.43 (1 H, d, J 4.4, H₆), 7.51 (1 H, d, J 7.6, H₄), 7.16 (1 H, dd, J 7.6 and 4.4, H₃), 4.40 (2 H, m, H₄'), 4.20 (2 H, s, CH₂), 3.80 (2 H, m, H₄'), 3.60 (4 H, m, H₄' and H₄''), 2.55 (3 H, s, CH₃), δC (75 MHz; CDCl₃) 199.4 (C=S), 159.2 (C₂), 148.0 (C₆), 135.3 (C₄), 130.3 (C₃), 122.1 (C₅), 66.9 (CH₂), 66.6 (CH₂), 51.0 (CH₂), 50.4 (CH₂), 47.1 (CH₂), 22.9 (CH₃); 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 H₂SO₄ (7.5 mL) in methanol (15 mL) and heated at reflux for 16 h; the mixture was neutralized with Na₂CO₃ and extracted with DCM (3 x 15 mL) to give the ester (V) (6.1 g, 98%), which was used without further purification. Rᵣ 0.31 (EtOAc); δᵣ (400 MHz; CDCl₃) 8.42 (1 H, d, H₆), 7.55 (1 H, dd, H₄), 7.18 (1 H, dd, H₅), 3.65 (3 H, CH₃), 2.45 (3 H, s, CH₃).

**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. NH₄Cl and filtered through Celite, washing with DCM then CHCl₃. The filtrate was evaporated under reduced pressure to afford the alcohol (VI) (2.3 g, 84%) as a solid. Rᵣ 0.37 (9:1, EtOAc:MeOH); δᵣ (300 MHz; CDCl₃) 8.22 (1 H, d, J 4.9, H₆), 7.46 (1 H, d, J 7.4, H₄), 7.04 (1 H, dd, J 7.4 and 4.9, H₅), 3.84 (2 H, t, J 6.7, CH₂OH), 2.86 (2 H, t, J 6.7, ArCH₂), 2.49 (3 H, s, CH₃) δₑ (75 MHz; CDCl₃) 157.2 (C₂), 147.0 (C₆), 137.9 (C₁), 132.9 (C₃), 121.7 (C₁₂), 62.0 (CH₂OH) 36.3 (ArCH₂) 22.4 (CH₃); 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 (10 x 2 mL) to give the thiamine analogue as its halide salt.

**Analogue Characterization**

**1T**

Found: C, 43.44; H, 4.69; N, 13.87; C₁₁H₁₃N₂O₂SCl requires C, 43.49; H, 4.65; N, 13.83); δᵣ (400 MHz; MeOD) 9.80 (1 H, s, H₅), 7.78 (1 H, s, H₄), 5.32 (2 H, s, CH₂), 3.82, (2 H, t, J 5.2, CH₂OH), 3.13 (2 H, t, J 5.2, ArCH₂), 2.63 (3 H, s, CH₃), δₑ (100 MHz; CD₃OD) 166.1 (C₄), 158.1 (C₅'), 153.4 (C₂), 146.4 (C₆), 144.1 (C₁'), 137.3 (C₃'), 105.9 (C₃), 61.8 (CH₂OH), 51.7 (CH₂), 31.2 (ArCH₂), 12.6 (CH₃); m/z (ES) 268 [M]⁺.
2T

δ\textsubscript{H} (400 MHz; D\textsubscript{2}O) 5.44 (2 H, s, CH\textsubscript{2}), 5.15 (1 H, s, H\textsubscript{3}), 3.75 (2 H, t, J 5.7, CH\textsubscript{2}OH), 3.11 (2 H, t, J 5.7, ArCH\textsubscript{2}), 2.41 (3 H, s, CH\textsubscript{3}), δ\textsubscript{C} (100 MHz; CDCl\textsubscript{3}) 166.4 (C\textsubscript{4}), 157.9 (C\textsubscript{3′}), 157.2 (C\textsubscript{2}), 149.3 (C\textsubscript{6}), 143.1 (C\textsubscript{2′}), 136.8 (C\textsubscript{3}), 100.0 (C\textsubscript{3}), 60.5 (CH\textsubscript{2}OH), 52.1 (CH\textsubscript{2}), 29.6 (ArCH\textsubscript{2}), 11.8 (CH\textsubscript{3}); m/z (ES) 268 [M]+.

3T

δ\textsubscript{H} (400 MHz; CD\textsubscript{3}OD) 10.1 (1 H, s, H\textsubscript{3′}), 6.40 (1 H, s, H\textsubscript{3}), 5.76 (2 H, s, CH\textsubscript{2}), 3.86 (2 H, t, J 5.4, CH\textsubscript{2}), 3.16 (2 H, t, J 5.4, CH\textsubscript{2}), 2.47 (3 H, s, CH\textsubscript{3}), 2.25 (3 H, s, CH\textsubscript{3}); m/z (ES) 266 [M]+.

4T

δ\textsubscript{H} (400 MHz; CD\textsubscript{3}OD) 8.45 (1 H, d, J 7.2, ArH), 8.22 (1 H, d, J 7.2, ArH), 5.95 (2 H, s, CH\textsubscript{2}), 3.85 (2 H, t, J 5.5, CH\textsubscript{2}), 3.18 (2 H, t, J 5.5, CH\textsubscript{2}), 2.77 (3 H, s, CH\textsubscript{3}), 2.71 (3 H, s, CH\textsubscript{3}); m/z (ES) 310 [M]+.

5T

δ\textsubscript{H} (400 MHz; CD\textsubscript{3}OD) 5.78 (2 H, s, CH\textsubscript{2}), 3.87 (2 H, t, J 5.4, CH\textsubscript{2}), 3.18 (2 H, t, J 5.4, CH\textsubscript{2}), 2.49 (3 H, s, CH\textsubscript{3}), 2.47 (3 H, s, CH\textsubscript{3}), 2.40 (3 H, s, CH\textsubscript{3}); m/z (ES) 337.5 [M]+.

6T

δ\textsubscript{H} (400 MHz; CD\textsubscript{3}OD) 9.85 (1 H, s, H\textsubscript{3′}), 8.38 (1 H, s, H\textsubscript{3}), 8.29 (1 H, d, J 9.0, H\textsubscript{3}), 7.08 (1 H, d, J 9.0, H\textsubscript{3}), 5.73 (2 H, s, CH\textsubscript{2}), 3.83 (2 H, t, J 5.4, CH\textsubscript{2}OH), 3.10 (2 H, d, J 5.4, ArCH\textsubscript{2}), 2.60 (3 H, s, CH\textsubscript{3}); m/z (ES) 296.4 [M]+.
**7T**

![Chemical Structure](image)

\[ \delta_{H} \text{ (400 MHz; CD$_2$OD) } 9.10 \text{ (1 H, t, J 2.0, } p\text{ArH)}, 8.68 \text{ (2 H, d, J 2.0, 2 x } o\text{ArH)}, 6.00 \text{ (2 H, s, CH$_2$)}, 3.85 \text{ (2 H, t, J 5.6, CH$_2$OH)}, 3.15 \text{ (2 H, t, J 5.6, ArCH$_2$)}, 2.52 \text{ (3 H, s, CH$_3$)}; \text{ m/z (ES) 323.9 [M$^+$].} \]

**8T**

Found: C, 24.69; H, 3.72; N, 13.20; C$_{11}$H$_9$BrNO$_5$SBr$_3$ requires C, 24.37; H, 3.72; N, 12.92); m.p. 180-181°C (decomp); \( \delta_{H} \text{ (400 MHz; CD$_2$OD) } 9.76 \text{ (1 H, s, H$_5^-$)}, 8.08 \text{ (1 H, s, H$_6$)}, 5.44 \text{ (2 H, s, CH$_2$)}, 3.86 \text{ (2 H, t, J 5.5, CH$_2$OH)}, 3.18 \text{ (2 H, t, J 5.5, ArCH$_2$)}, 2.66 \text{ (3 H, s, CH$_3$)}, \delta_{C} \text{ (100 MHz; CD$_2$OD) } 166.0 \text{ (C$_4$)}, 157.2 \text{ (C$_2$)}, 155.6 \text{ (C$_5^+$)}, 148.0 \text{ (C$_6$)}, 144.5 \text{ (C$_4^+$)}, 138.4 \text{ (C$_5^+$), 100.9 (C$_3$), 61.7 (CH$_2$OH), 52.3 (CH$_2$), 31.2 (ArCH$_2$), 12.6 (CH$_3$); m/z (ES) 266 (100, M$^+$-Br).}

**1M**

![Chemical Structure](image)

\[ \delta_{H} \text{ (400 MHz; CD$_2$OD) } 8.89 \text{ (1 H, d, J 6.3, H$_5^-$)}, 8.39 \text{ (1 H, d, J 8.8, H$_4^+$)}, 7.95 \text{ (1 H, s, H$_6$)}, 7.95 \text{ (1 H, dd, J 6.3 and 8.8, H$_5^-$)}, 5.59 \text{ (2 H, s, CH$_2$)}, 3.88 \text{ (2 H, t, J 6.2 CH$_2$OH)}, 3.13 \text{ (2 H, t, J 6.2, ArCH$_2$)}, 3.02 \text{ (3 H, s, CH$_3$)}, \delta_{C} \text{ (100 MHz; CD$_2$OD) } 166.0 \text{ (C$_4$)}, 157.1 \text{ (C$_4^+$)}, 152.3 \text{ (C$_2$)}, 148.1 \text{ (C$_5^+$)}, 146.4 \text{ (C$_6$)}, 145.4 \text{ (C$_5^+$)}, 142.0 \text{ (C$_4^+$)}, 125.6 \text{ (C$_5^+$)}, 106.5 \text{ (C$_3$)}, 62.1 \text{ (CH$_2$OH), 56.8 (CH$_2$), 37.3 (ArCH$_2$), 17.7 (CH$_3$); m/z (ES) 262.1 [M$^+$].}

**2M**

![Chemical Structure](image)

\[ \delta_{H} \text{ (400 MHz; CD$_2$OD) } 8.89 \text{ (1 H, d, 6.0 H$_5^-$)}, 8.57 \text{ (1 H, m, H$_4^+$)}, 8.00 \text{ (1 H, dd, J 8.2 and 6.0, H$_5^-$)}, 5.67 \text{ (2 H, s, CH$_2$)}, 5.52 \text{ (1 H, s, H$_6$)}, 3.92 \text{ (2 H, t, J 6.0 CH$_2$OH)}, 3.17 \text{ (2 H, t, J 6.0, ArCH$_2$)}, 2.86 \text{ (3 H, s, CH$_3$)}, \delta_{C} \text{ (100 MHz; CD$_2$OD) } 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**

![Chemical Structure](image)

\[ \delta_{H} \text{ (400 MHz; CD$_2$OD) } 8.86 \text{ (1 H, d, 6.1 H$_5^-$)}, 8.49 \text{ (1 H, d, J 7.9 H$_5^-$)}, 7.93 \text{ (1 H, dd, J 6.1 and 7.9, H$_5^-$)}, 6.35 \text{ (1 H, s, H$_5$)}, 5.78 \text{ (2 H, s, CH$_2$)}, 3.90 \text{ (2 H, t, J 5.9 CH$_2$OH)}, 3.14 \text{ (2 H, t, J 5.9, ArCH$_2$)}, 2.84 \text{ (3 H, s, CH$_3$)}, 2.29 \text{ (3 H, s, CH$_3$); m/z (ES ) 260.1 [M$^+$].} \]
5M

\[ \delta_{H} (400 \text{ MHz; CD}_{2} \text{OD}) \]

\[ 8.87 \ (1 \ H, d, J \ 6.1 \ H_{6}'), \ 8.55 \ (1 \ H, d, J \ 7.9 \ H_{1}'), \ 7.97 \ (1 \ H, dd, J \ 6.1 \ and \ 7.9, \ H_{3}'), \ 5.92 \ (2 \ H, s, \ CH_{2}), \ 3.92 \ (2 \ H, t, J \ 6.1, \ CH_{2}OH), \ 3.17 \ (2 \ H, t, J \ 6.1, \ ArCH_{2}), \ 2.80 \ (3 \ H, s, \ CH_{3}), \ 2.47 \ (3 \ H, s, \ CH_{3}), \ 2.39 \ (3 \ H, s, \ CH_{3}); \ m/z \ (ES) \ 330.1 [M]^+ \]

6M

\[ \delta_{H} (400 \text{ MHz; CD}_{2} \text{OD}) \]

\[ 8.46 \ (1 \ H, d, J \ 5.7 \ H_{6}'), \ 8.28 \ (1 \ H, dd, J \ 9.0 \ and \ 2.8, \ H_{3}), \ 8.22 \ (1 \ H, d, J \ 2.8, \ H_{3}), \ 7.89 \ (1 \ H, dd, J \ 5.7 \ and \ 7.9, \ H_{5}'), \ 7.07 \ (1 \ H, d, J \ 9.0 \ H_{6}), \ 5.91 \ (2 \ H, s, \ CH_{2}), \ 3.89 \ (2 \ H, t, J \ 6.1 \ CH_{2}OH), \ 3.14 \ (2 \ H, t, J \ 6.1, \ ArCH_{2}), \ 2.91 \ (3 \ H, s, \ CH_{3}); \ m/z \ (ES) \ 289.1 [M]^+ \]

7M

\[ \delta_{H} (400 \text{ MHz; CD}_{2} \text{OD}) \]

\[ 9.08 \ (1H, t, J \ 2.0, \ H_{2}), \ 8.99 \ (1 \ H, d, J \ 6.2, \ H_{6}'), \ 8.57 \ (1 \ H, d, J \ 9.2 \ H_{5}'), \ 8.55 \ (2 \ H, d, J \ 2.0, \ H_{2} \ and \ H_{6}), \ 8.03 \ (1 \ H, m, H_{5}), \ 6.20 \ (2 \ H, s, \ CH_{2}), \ 3.91 \ (2 \ H, t, J \ 6.0 \ CH_{2}OH), \ 3.15 \ (2 \ H, t, J \ 6.0, \ ArCH_{2}), \ 2.89 \ (3 \ H, s, \ CH_{3}); \ m/z \ (ES) \ 318.0 [M]^+ \]

9M

\[ \delta_{H} (400 \text{ MHz; CD}_{2} \text{OD}) \]

\[ 8.84 \ (1 \ H, d, J \ 6.1 \ H_{6}'), \ 8.52 \ (1 \ H, d, J \ 7.9 \ H_{1}'), \ 7.95 \ (1 \ H, dd, J \ 6.1 \ and \ 7.9, \ H_{3}'), \ 6.33 \ (1 \ H, bs, \ H_{3}), \ 5.89 \ (2 \ H, s, \ CH_{2}), \ 3.91 \ (2 \ H, t, J \ 5.9 \ CH_{2}OH), \ 3.16 \ (2 \ H, t, J \ 5.9, \ ArCH_{2}), \ 2.78 \ (3 \ H, s, \ CH_{3}), \ 2.21 \ (3 \ H, s, \ CH_{3}); \ m/z \ (ES) \ 260.1 [M]^+ \]

1P

\[ \delta_{H} (400 \text{ MHz; CD}_{2} \text{OD}) \]

\[ 9.00 \ (1 \ H, s, H_{5}'), \ 8.96 \ (1 \ H, d, J \ 6.1 \ H_{6}'), \ 8.51 \ (1 \ H, d, J \ 8.0 \ H_{4}'), \ 8.04-8.00 \ (2 \ H, m, H_{5}' \ and \ H_{6}), \ 5.48 \ (2 \ H, s, \ CH_{2}), \ 3.85 \ (2 \ H, t, J \ 6.0, \ CH_{2}OH), \ 3.06 \ (2 \ H, t, J \ 6.0, \ ArCH_{2}); \ m/z \ (ES) \ 248.2 [M]^+ \]

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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_3) 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]^+.

δ_H (400 MHz; CD_3OD) 9.01 (1 H, s, H_1')
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_3), 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]^+.

δ_H (400 MHz; CD_3OD) 8.85 (1 H, s, H_1'), 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_3), 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), 148.5 (C_4'), 144.7 (C_6'), 143.2 (C_5'), 140.1 (C_4), 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]^+.

δ_H (400 MHz; CD_3OD) 9.02 (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'), 8.15 (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]^+.

δ_H (400 MHz; CD_3OD) 9.04 (1 H, s, H_1'), 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_3), 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
CH₂OH), 3.06 (2 H, t, J 5.8, ArCH₂); m/z (ES) 275.2 [M]+.

7P

δH (400 MHz; CD₂OD) 9.14 (1 H, s, H2'), 9.10–9.07 (2 H, m, H4' and H5), 8.89 (2 H, s, H2 and H6), 8.63 (1 H, d, 8.1, H1'), 8.13 (1 H, dd, J 6.2 and 8.1, H5'), 6.14 (2 H, s, CH₃), 3.91 (2 H, t, J 5.9, CH₂OH), 3.11 (2 H, t, J 5.9, ArCH₂), δC (100 MHz; CD₂OD) 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

δH (400 MHz; CD₂OD) 8.98 (1 H, s, H1'), 8.96 (1 H, d, J 6.1 H4'), 8.64 (1 H, d, J 8.1 H5'), 8.13 (1 H, dd, J 6.1 and 8.1, H1'), 6.30 (1 H, s, H6), 5.87 (2 H, s, CH₃), 3.92 (2 H, t, J 6.0 CH₂OH), 3.12 (2 H, t, J 6.0, ArCH₂), 2.17 (3H, s, CH₃); m/z (ES) 246.2 [M]+.

10P

δH (400 MHz; CD₂OD) 8.97 (1 H, s, H2), 8.90 (1 H, d, J 6.1, H6'), 8.59 (1 H, d, J 7.9 H5'), 8.09 (1 H, m, H3'), 6.31 (1 H, s, H3), 5.72 (2 H, s, CH₂), 3.91 (2 H, t, J 6.0, CH₂OH), 3.12 (2 H, t, J 6.0, ArCH₂), 2.28 (3 H, s, CH₃); m/z (ES) 245.2 [M]+.

11P

δH (400 MHz; CD₂OD) 8.93 (1 H, s, H2), 8.87 (1 H, d, 6.0 H6'), 8.59 (1 H, d, 8.0 H4'), 8.08 (1 H, dd, J 8.0 and 6.0, H3'), 3.91 (2 H, t, J 5.9 CH₂OH), 3.09 (2 H, t, J 5.9, ArCH₂); m/z (ES) 247.1 [M]+.

References: