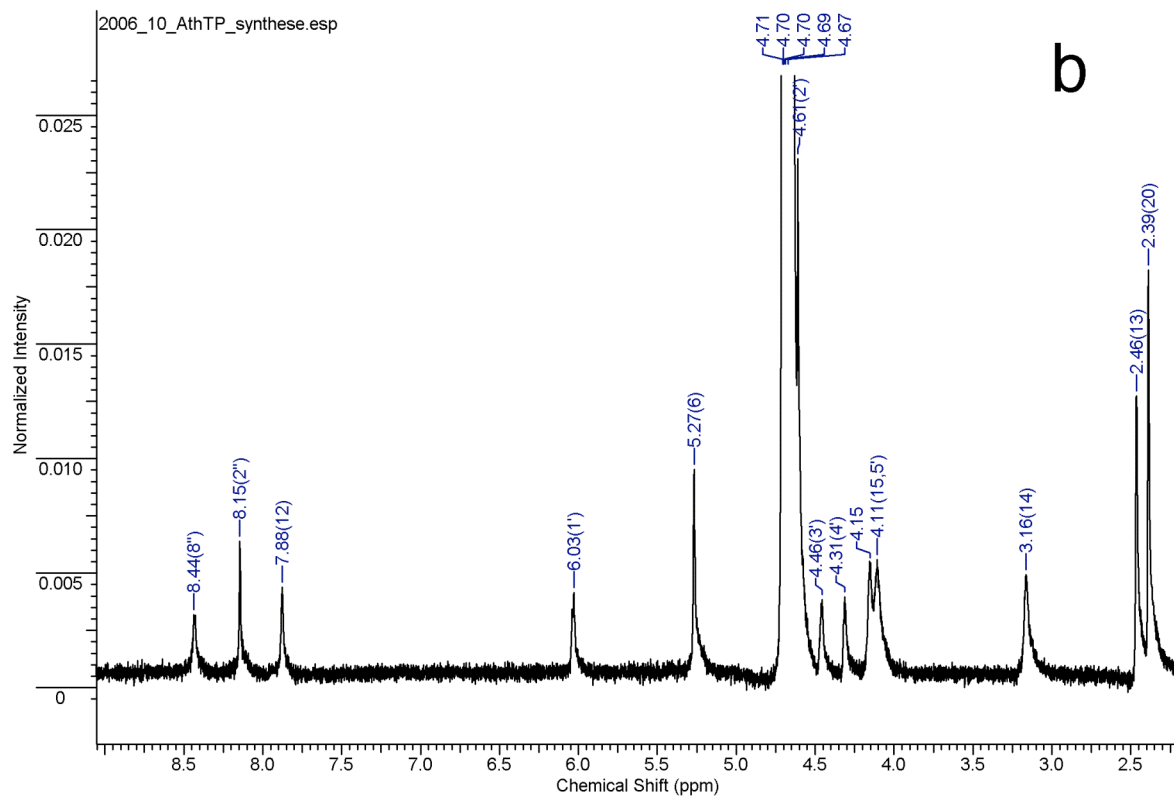
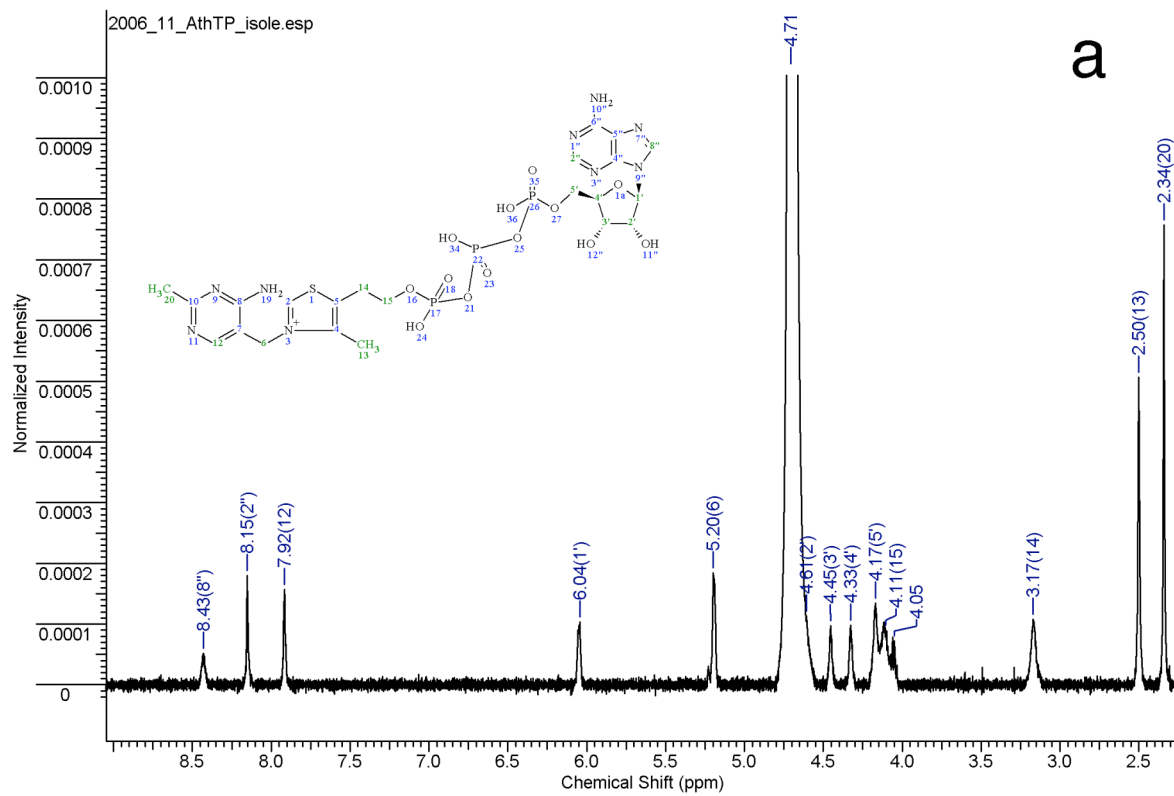
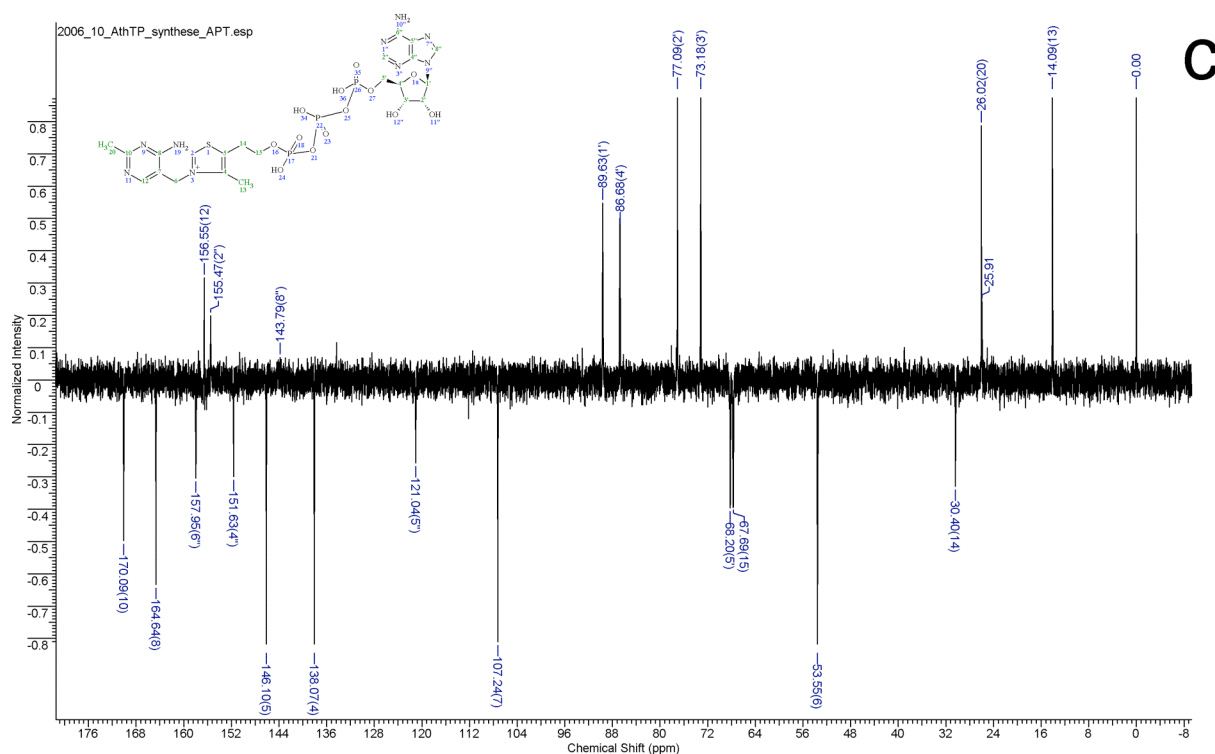


Supplementary Figure 3 ^1H -NMR spectrum of natural AThTP (**a**) and ^1H -NMR (**b**) and ^{13}C -NMR (**c**) spectra of chemically synthesized AThTP. Couplings between protons 14, 15 and 1', 2', 3', 4', 5' were also observed in the COSY spectrum. NMR data are in agreement with data recorded under the same conditions for thiamine and adenosine reference samples. All data are consistent with the structural formula given in **figure 1c**. Bacterial AThTP was produced from 10 liter of culture and purified as described in **Supplementary Table 1**. A total amount of 0.4 mg of AThTP was obtained from the bacterial extract and, after purification, 50 μg remained that were used for ^1H -NMR.

Identification of AThTP by ^1H -NMR. One-dimensional ^1H -NMR spectra of synthetic and natural AThTP were recorded at 15 $^\circ\text{C}$ on a Bruker Avance 500 spectrometer operating at a proton NMR frequency of 500.13 MHz, using a 5 mm probe, 1.7 mm inserts (Bruker-Biospin) and a simple pulse-acquire sequence. Acquisition parameters consisted of a spectral width of 10330.6 Hz, a pulse width of 2.541 μs , an acquisition time of 3.17 s and a relaxation delay of 1 s. 2048 scans were recorded. FIDs were Fourier transformed with $\text{LB} = 0.3$ Hz and $\text{GB} = 0$. The resonance at 4.750 ppm due to residual solvent (HOD) was used as internal reference. A COSY spectrum was also recorded using standard Bruker parameters (cosygpqf pulse program) and 32 scans. AThTP NMR data: ^1H -NMR (500 MHz, deuterium oxide) δ ppm 2.34 (s, CH₃-20), 2.50 (s, CH₃-13), 3.17 (br. s., CH₂-14), 4.11 (m, CH₂-15), 4.17 (br. s., CH₂-5'), 4.33 (br. s., CH-4'), 4.45 (br. s., CH-3'), 4.62 (m, CH-2'), 5.20 (br. s., CH₂-6), 6.04 (br. s., CH-1'), 7.92 (s, CH-12), 8.15 (s, CH-2''), 8.43 (s, CH-8'').

Identification of AThTP by ^{13}C -NMR. One and two-dimensional ^{13}C -NMR spectra (APT, HSQC, HMBC) of synthetic AThTP were also recorded at 15 $^\circ\text{C}$ on a Bruker Avance spectrometer operating at a carbon frequency of 125.03 MHz, using a 5 mm probe and standard Bruker parameters ($n_s = 10\text{k}$ for APT, $n_s = 128$ for 2D experiments). TMS was used as internal reference. AThTP NMR data: ^{13}C -NMR (125 MHz, deuterium oxide) δ ppm 14.1 (CH₃-13), 26.0 (CH₃-20), 30.4 (CH₂-14), 53.6 (CH₂-6), 67.7 (CH₂-15), 68.2 (CH₂-5'), 73.2 (CH-3'), 77.1 (CH-2'), 86.7 (CH-4'), 89.6 (CH-1'), 107.2 (C-7), 121.1 (C-5''), 138.1 (C-4), 142.4 (CH-8''), 146.1 (C-5), 151.6 (C-4''), 155.5 (CH-2''), 156.6 (CH-12), 158.0 (C-6''), 164.6 (C-8), 170.1 (C-10).





Comparison of the $^1\text{H-NMR}$ and $^{13}\text{C-NMR}$ chemical shifts (ppm) of the 2'-, 3'- and 5'-hydroxyl groups of natural (only $^1\text{H-NMR}$) and chemically synthesized AThTP with commercially available 5'-AMP and 3'AMP.

| | AThTP (natural) | AThTP (chemical) | 5'-AMP | 3'-AMP |
|---------------------------------------|--------------------|---------------------|-------------------|-------------------|
| $^1\text{H-NMR}$ | | | | |
| 2'-H | 4.61 ¹ | 4.61 ¹ | 4.71 ¹ | 4.86 |
| 3'-H | 4.45 | 4.46 | 4.47 | 4.71 ¹ |
| 5'-H ₂ | 4.17 | 4.15 | 4.10 | 3.87 |
| $^{13}\text{C-NMR}$ | | | | |
| 2'-CH | - | 77.09 | 77.18 | 75.92 |
| 3'-CH | - | 73.18 | 73.25 | 76.95 |
| 5'-CH ₂ | - | 68.20 | 67.22 | 64.17 |

¹ Interference with HOD peak.