Supplementary Methods

Determination of thiamine compounds and adenine nucleotides by HPLC. We analyzed thiamine compounds as previously described after transformation into fluorescent thiochrome derivatives using a PRP-1 column (Hamilton)[7]. AThTP and other adenosine derivatives were also quantified using UV detection (254 nm) after separation on a 5 µm Chromsphere C18 column (150 x 4.6 mm, Varian). The mobile phase contained 25 mM tetra-*n*-butylammonium hydrogen sulfate, 50 mM NaH_2PO_4 adjusted to pH 7.0 and 15 % methanol. The flow rate was 1 ml/min.

Growth and processing of bacteria. We grew *E. coli* (BL 21 or MG1655 wild-type K-12) overnight (37 °C, 250 rpm) in 50-100 ml LB medium (tryptone, 10 g/l; yeast extract, 5 g/l; NaCl, 10 g/l at pH 7.0). The bacteria were centrifuged (5 min; 10,000 x g) and suspended in the initial volume of fresh LB medium or M9 minimal medium (Na_2HPO_4, 6 g/l; KH_2PO_4, 3 g/l; NaCl, 0.5 g/l; NH_4Cl, 1 g/l; $CaCl_2$, 3 mg/l; $MgSO_4$, 1 mM, pH 7.0) containing various metabolic substrates. After incubation at 37 °C, the bacteria were sedimented as above, the pellets were suspended in 12% (w/v) trichloroacetic acid, the precipitated proteins spun down (15 min, 15,000 x g) and the pellet dissolved in 0.8 N NaOH for protein determination (Peterson, G.L. *Anal. Biochem.* 83, 346-356, 1977). The supernatant was treated with diethyl ether and analyzed by HPLC[7].

Chemical synthesis of AThTP. We synthesized AThTP by a modification of a previously published method[4] for the synthesis of ThTP and nucleoside triphosphates. Here, ThDP and 5'-AMP (instead of ThDP and H_3PO_4) were used as precursors. Briefly, we mixed 1.35 mmol ThDP, 2.1 mmol 5'-AMP (acid form), 2.1 ml tributylammonium and 1.5 ml H_2O until we obtained a translucent solution. We added this solution to a mixture of 300 ml dimethyl sulfoxide and 267 ml pyridine. The synthesis was initiated by addition of 30 ml dicyclohexylcarbodiimide (0.9 g/ml in pyridine). After 1 h, we added 1800 ml of diethyl ether, and the precipitate was centrifuged (1000 x g, 10 min) and dissolved in 50 ml H_2O. We purified the synthesized compound (0.44 mmol) on the AG 50W-X8 and AG-X1 resins (Bio-Rad) as described for bacterial AThTP (**Supplementary Table 1** online). We further purified approximately 33 µmol (~ 25 mg), by successive 100-µl injections on the Polaris C18 column (Varian) for ^{1}H-NMR and ^{13}C-NMR.