Supplementary Table 1 Purification of AThTP from *E. coli*

Purification step	Concentration (μM)	Volume (ml)	Quantity (nmol)
Supernatant	5.9	37	218 (100)
AG 50W-X8	1.36	140	190 (87)
After lyophilization	18.8	10	188 (86)
AG-X1	0.34	280	95 (44)
After lyophilization	22	3	66 (30)
Prevail C18	21	1	21 (9,6)
Polaris C18	130	0.1	13 (6)

During purification, AThTP was estimated after oxidation, followed by HPLC analysis (**Supplementary Methods** online). The percentage yield is indicated under parentheses.

E. coli (BL 21 or MG1655) were grown overnight in 2 x 500 ml LB medium. The bacteria were centrifuged (5,500 x g, 10 min), suspended in 2 x 500 ml M9 medium without any carbon source and incubated for 3 h (37 °C, 250 rpm) for synthesis of AThTP. The bacteria were then centrifuged (5,500 x g, 10 min) and the pellets were combined and resuspended in 40 ml of 12% trichloroacetic acid. After 30 min on ice, the proteins were sedimented (10,000 x g, 15 min) and the trichloroacetic acid was extracted from the supernatant by 3 x 150 ml diethyl ether. The solution (37 ml) was then layered on a column (9 x 2.5 cm) filled with AG 50W-X8 cation exchange resin (H^+ form, Bio-Rad) in water. The column was washed with 260 ml of ice-cold water at a flow rate of 2 ml/min and 8 ml fractions were collected. AThTP was eluted with ice-cold ammonium acetate (0.2 M, pH 7.0). The fractions containing AThTP (400-540 ml) were pooled and lyophilized. The residue was dissolved in 10 ml of water and applied to a column (9 x 2.5 cm) filled with AG-X1 resin (Cl^- form, Bio-Rad) conditioned with water. The flow rate was 2 ml/min and 10 ml fractions were collected. The column was washed with 160 ml of ice-cold water, followed by 160 ml of ammonium acetate (0.25 M, pH 5.0) and then by 300 ml of ammonium acetate (0.5 M, pH 5.0). Nearly half of the AThTP was lost during this step, which is nevertheless essential to separate AThTP from the other thiamine derivatives (essentially ThMP and ThDP). The relevant fractions were pooled (280 ml) and lyophilized. The residue was dissolved in 3 ml of water and filtered on a Millex-GP filter unit (0.22 μM, ∅ 25 mm, Millipore). Aliquots of 100 μl of the pool were further purified on a Prevail C18 column (Grace) using gradient elution (0 - 20% methanol in water in 20 min) at a flow rate of 1 ml/min. All the fractions containing AThTP (retention time of

16 min) from the different runs were pooled, lyophilized and the residue dissolved in 1 ml of water. Aliquots of 100 µl of the pool were then purified on a Polaris C18 column (4.6 x 150 mm, 5 µm, Varian). The mobile phase consisted of 50 mM ammonium acetate and 5% methanol in water and the flow rate was 1 ml/min. AThTP was eluted as a symmetrical peak with a retention time of 7.5 min (**Supplementary Fig. 1** online). The peak was collected, lyophilized and used for mass spectrometry and ^{1}H-NMR.