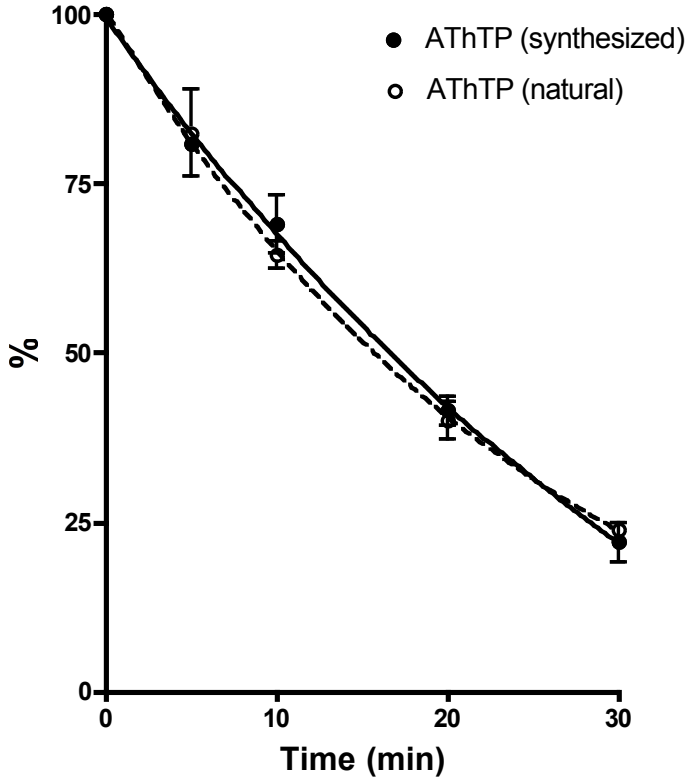
Supplementary Figure 4 Rate of enzymatic breakdown of natural and chemically synthesized AThTP by a bacterial membrane fraction.



E. coli (Mg1655) grown overnight in LB medium were suspended in 50 mM Tris-HCl buffer, pH 7.4, containing 0.15 M KCl and 0.2 mM EDTA and sonicated (100 kHz, 5 x 10 s, on ice). Cell debris were sedimented (1000 x g; 10 min) and discarded. Crude membranes were collected by centrifugation (100,000 x g, 60 min) and suspended in the initial volume of Tris-HCl buffer. AThTP (0.1 mM), either purified from bacteria (○) or chemically synthesized (●) as described in **Supplementary Methods** online, was incubated for 30 min (37 °C) in the presence of 10 µl of the membrane suspension (10 mg of protein/ml), 50 mM MOPS (pH 7.2) and 10 mM $MgSO_4$ in a total volume of 100 µl. After 30 min, the reaction was stopped by addition of 100 µl trichloroacetic acid (20% w/v) and AThTP and other thiamine derivatives was determined by HPLC as described in **Supplementary Methods** online. The results are expressed as mean ± SD for three experiments.

The main products of hydrolysis were ThDP and ThMP (probably formed from ThDP by an endogenous phosphates activity). AMP was formed transiently and rapidly degraded in other products by membrane-associated enzymes.