

SUPPLEMENTARY MATERIALS

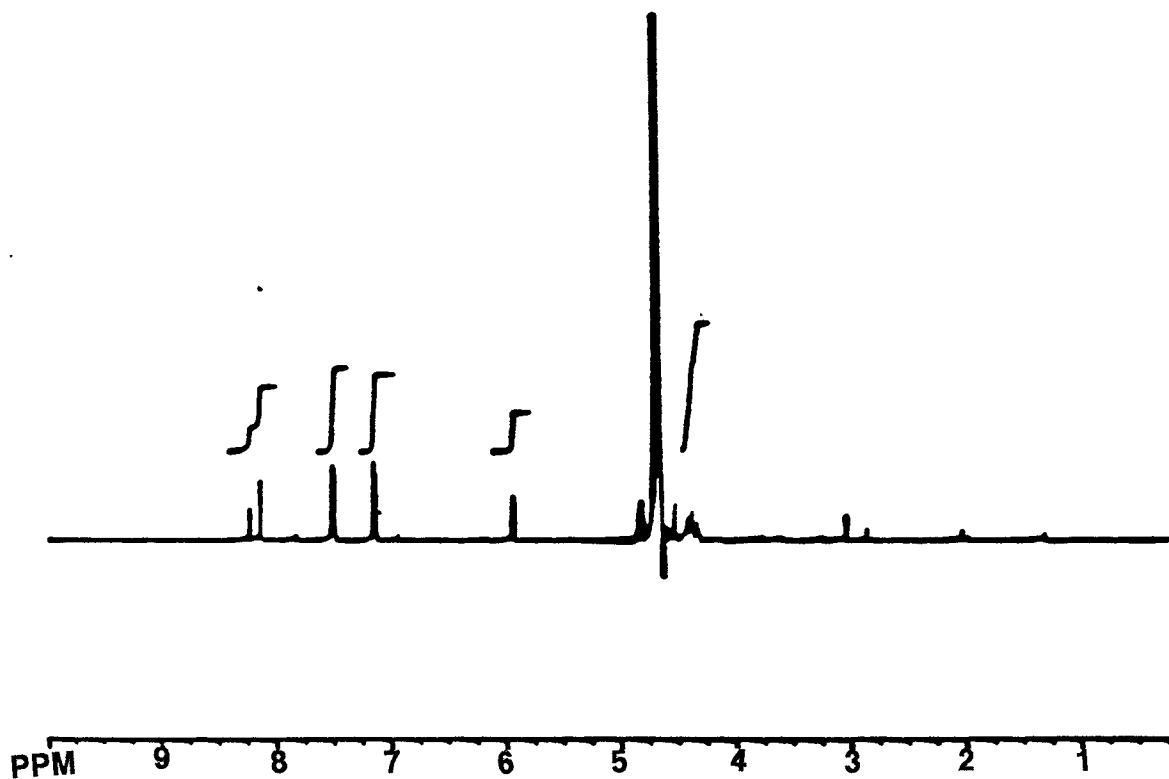


Figure 1. $^1\text{H-NMR}$ spectra of 4-CBA-AMP generated from the reaction of 10 mM 4-CBA, 8 mM ATP, 10 mM MgCl_2 , 30 units of inorganic pyrophosphatase and 25 units of 4-CBA:CoA ligase (17 μM) in 50 mM K^+Hepes (pH 7.5) and purified as described in Methods. The sample was dissolved in D_2O (pH 6.0). The $^1\text{H-NMR}$ spectra were recorded at 25 $^\circ\text{C}$ on a Bruker AMX500 multinuclear spectrometer operating at a frequency of 500 MHz. The chemical shifts (δ) and the assignments for the $^1\text{H-NMR}$, relative to HDO (δ 4.77, 25 $^\circ\text{C}$) and reported in parts per million (ppm), are listed in Table 1.

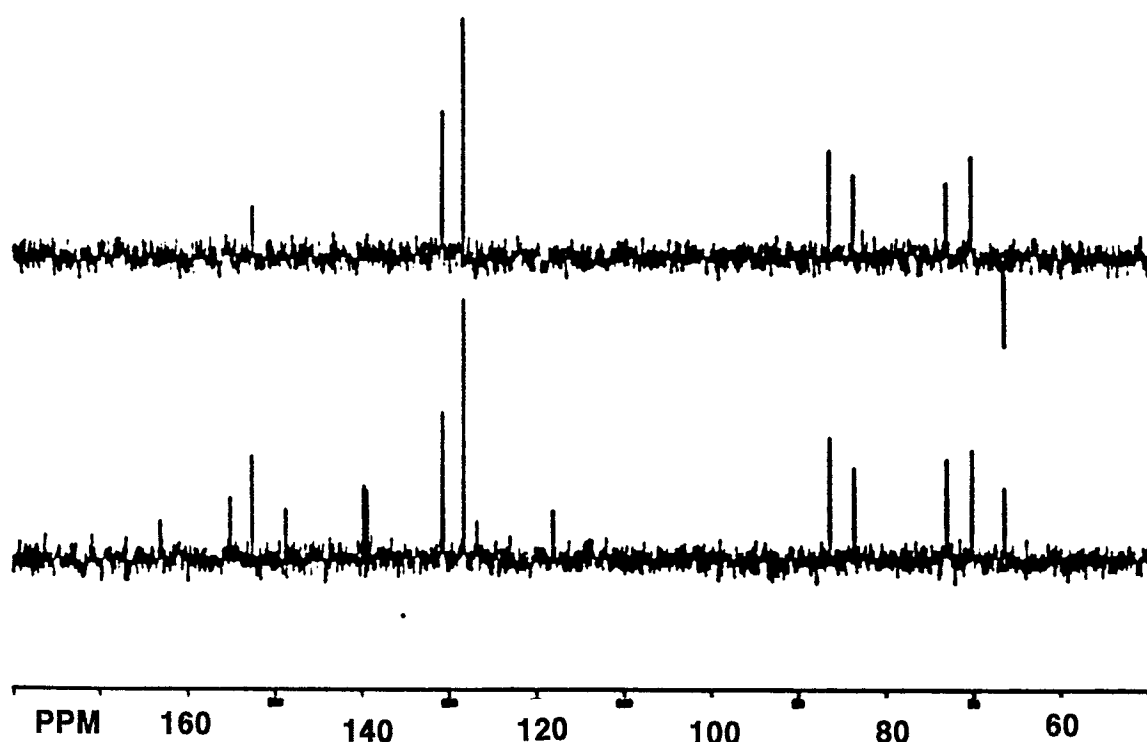


Figure 2. ^{13}C -NMR spectra of 4-CBA-AMP generated from the reaction of 10 mM 4-CBA, 8 mM ATP, 10 mM MgCl_2 , 30 units of inorganic pyrophosphatase and 25 units of 4-CBA:CoA ligase ($17 \mu\text{M}$) in 50 mM K^+ Hepes (pH 7.5) and purified as described in Methods. The sample was dissolved in D_2O (pH 6.0). The ^{13}C -NMR spectra were recorded at 25°C on a Bruker AMX500 multinuclear spectrometer operating at a frequency of 125 MHz. The ^{13}C -NMR resonance assignments were aided by use of the DEPT technique to determine the numbers of attached hydrogens. The chemical shifts (δ) and the assignments for the ^{13}C -NMR, relative to CDCl_3 (δ 77.00, 25°C) and reported in parts per million (ppm), are listed in Table 1.

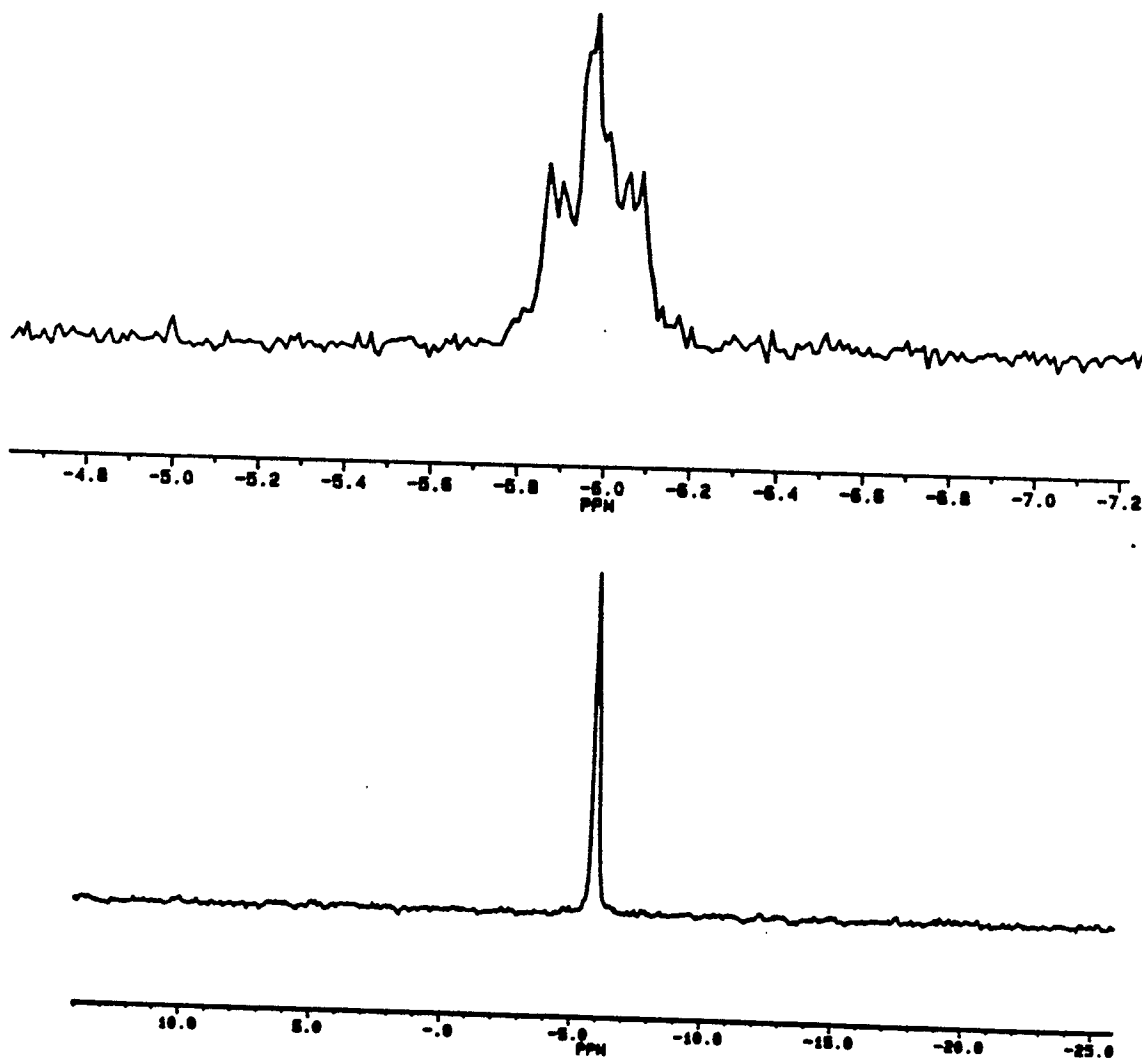


Figure 3. ^{31}P -NMR spectra of 4-CBA-AMP generated from the reaction of 10 mM 4-CBA, 8 mM ATP, 10 mM MgCl_2 , 30 units of inorganic pyrophosphatase and 25 units of 4-CBA:CoA ligase ($17 \mu\text{M}$) in 50 mM K^+ Hepes (pH 7.5) and purified as described in Methods. The sample was dissolved in D_2O (pH 6.0). The ^{31}P -NMR spectra were recorded at 25°C on a Bruker WP-200 multinuclear spectrometer operating at 84 MHz. The chemical shifts (δ) and the assignments for the ^{31}P -NMR, referenced to 85% H_3PO_4 (δ 0.00, 25°C) and reported in parts per million (ppm), are listed in Table 1.

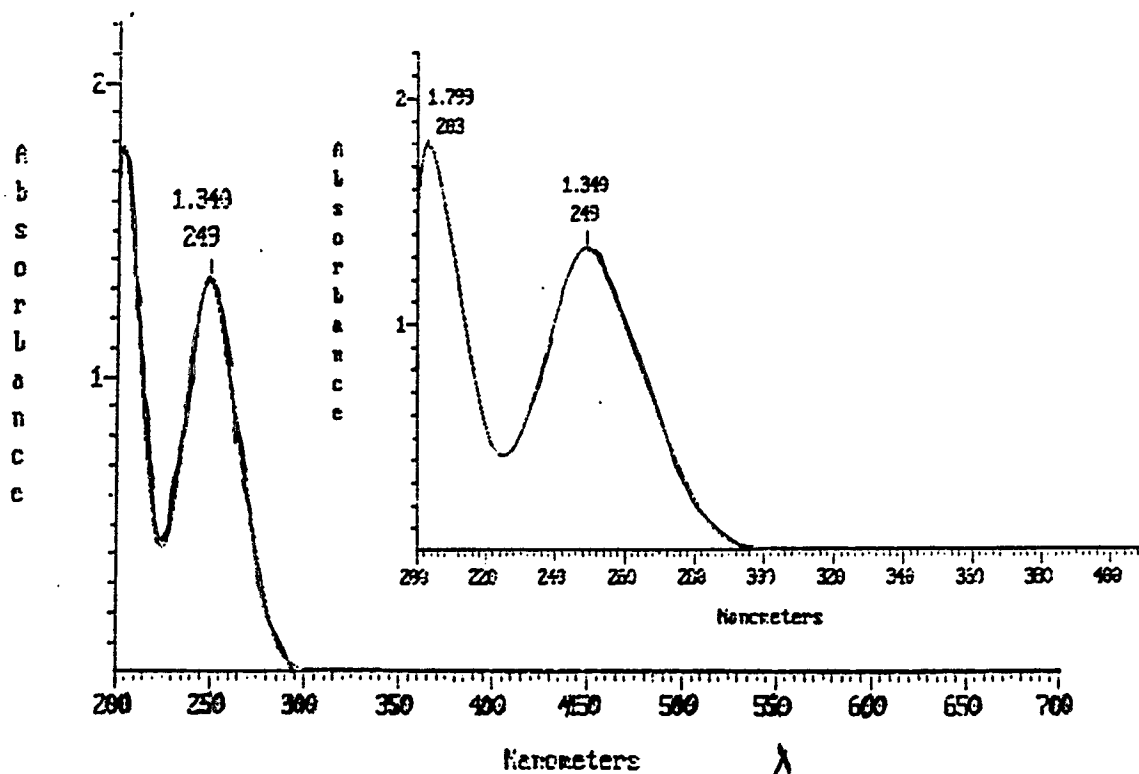


Figure 4. Absorbance spectrum of 4-CBA-AMP generated from the reaction of 10 mM 4-CBA, 8 mM ATP, 10 mM MgCl₂, 30 units of inorganic pyrophosphatase and 25 units of 4-CBA:CoA ligase (17 μM) in 50 mM K⁺Hepes (pH 7.5) and purified as described in Methods. The sample was dissolved in H₂O (pH 6.0). The spectrum, recorded at 25 °C on a Milton Roy Spectronic® 3000 spectrophotometer, shows maximal absorbance of 4-CBA-AMP at 203 nm ($\epsilon = 20 \text{ mM}^{-1}\text{cm}^{-1}$) and 249 nm ($\epsilon = 15 \text{ mM}^{-1}\text{cm}^{-1}$).