

**Supporting Information for: Transition State Differences in Hydrolysis
Reactions of Alkyl versus Aryl Phosphate Monoester Monoanions**
Piotr K. Grzyska, Przemyslaw G. Czyryca, Jamie Purcell, and Alvan C. Hengge*

Kinetic Isotope Effect Determinations.

The dicyclohexylammonium salt (43 mg) of the appropriate isotopically labeled form of *m*-nitrobenzyl phosphate was dissolved in 10ml of 0.5M acetate buffer at pH 4.0. The solution was placed in thick-walled glass vessels with a pressure cap, which were placed in oil bath maintained at 115 °C. After partial hydrolysis, the reactions were stopped by cooling to room temperature (where the reaction rate is negligible). The extent of reaction was assayed by ³¹P NMR. The reaction mixture was extracted four times (25 mL) with diethyl ether to separate the product *m*-nitrobenzyl alcohol. The ether layers were dried over magnesium sulfate, and the ether removed by rotary evaporation. The aqueous layer, containing the unreacted *m*-nitrobenzyl phosphate was mixed with Tris buffer and brought to pH ~9. About 1 mg of commercial alkaline phosphatase was added to cleave the residual *m*-nitrobenzyl phosphate. After more than 10 half-lives, this mixture was titrated to pH ~4 and treated as described above to recover *m*-nitrobenzyl alcohol. The *m*-nitrobenzyl alcohol was further purified by distillation onto a cold finger apparatus at 105°C under reduced pressure, before isotopic analysis by isotope ratio mass spectrometry using an ANCA-NT combustion system working in tandem with a Europa 20-20 isotope ratio mass spectrometer.

Kinetic Isotope Effect Data Analysis. For each isotope effect at least three reactions were run. The ¹⁵N/¹⁴N ratios were measured for the product (R_p) and of the remaining starting material (R_s) at partial reaction, as well as in the original mixture (R_o). The isotope effects were calculated using equations 1 and 2.¹

$$\text{isotope effect} = \log(1 - f) / \log[(1 - f)(R_s / R_o)] \quad (1)$$

$$\text{isotope effect} = \log(1 - f) / \log(1 - f(R_p / R_o)) \quad (2)$$

For each isotope effect the value calculated from R_o and R_p (equation 1) and from R_o and R_s (equation 2) agreed within experimental error and these were averaged to give the results reported. The ¹⁵N KIE is given directly from these equations. In the ¹⁸O isotope effect experiments the observed KIEs given by the above equations were corrected for the ¹⁵N isotope effect and for incomplete levels of isotopic incorporation.

Calculation of corrected ¹⁸O kinetic isotope effects

In the ¹⁸O isotope effect experiments the observed KIEs were corrected for the ¹⁵N isotope effect and for incomplete levels of isotopic incorporation. The equations used for these corrections and their derivations have been described.² For the labeled substrates and the mixtures used for the ¹⁸k_{ig} experiments, the levels of isotopic incorporation and other quantities used in the correction equation were as follows.

The fraction of ¹⁵N, ¹⁸O-labeled compound **A** in the remote-labeled mixture of **A** and **C** (Figure 1S) = *b* = 0.003657

The fraction of ¹⁵N in the ¹⁵N, ¹⁸O-labeled compound **A** = *x* = 0.99,

The fraction of ¹⁸O in the ¹⁵N, ¹⁸O-labeled compound **A** = *y* = 0.93,

The fraction of ¹⁵N in the ¹⁴N-labeled compound **C** = *z* = 0.0002

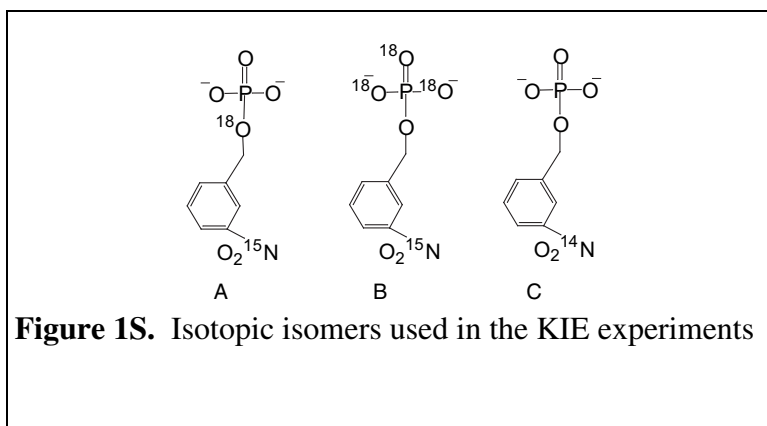
For the labeled substrates and the mixtures used for the $^{18}k_{\text{nonbridge}}$ experiments, the levels of isotopic incorporation and other quantities used in the correction equation were as follows.

The fraction of ^{15}N , ^{18}O -labeled compound **B** in the remote-labeled mixture of **B** and **C** = $b = 0.003674$

The fraction of ^{15}N in the ^{15}N , ^{18}O -labeled compound **B** = $x = 0.99$

The fraction of compound **B** with all three nonbridge oxygen atoms labeled = $y = 0.95$

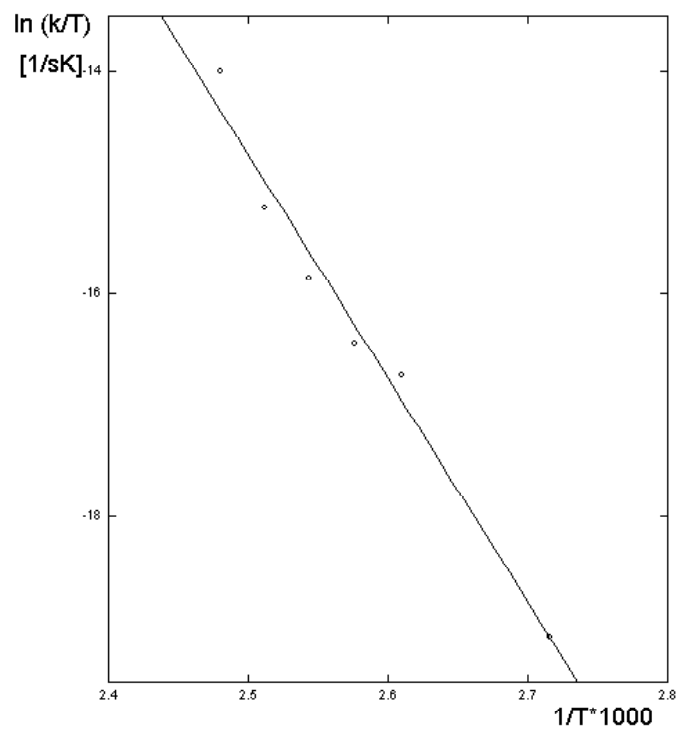
The fraction of ^{15}N in the ^{14}N -labeled compound **C** = $z = 0.0002$



Eyring Plot

The rate constants for hydrolysis of *m*-nitrobenzyl phosphate measured in the temperature range 95 °C to 130 °C are shown in the following table:

Temperature (°C)	k (s ⁻¹)
95	1.89 x 10 ⁻⁶
110	2.07 x 10 ⁻⁵
115	2.8 x 10 ⁻⁵
120	5.09 x 10 ⁻⁵
125	9.71 x 10 ⁻⁵
130	3.00 x 10 ⁻⁴



References

- (1) Bigeleisen, J.; Wolfsberg, M. *Adv. Chem. Phys.* **1958**, *1*, 15-76.
- (2) Hermes, J. D.; Morrical, S. W.; O'Leary, M. H.; Cleland, W. W. *Biochemistry* **1984**, *23*, 5479-5488.