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Nucleophilic Acyl Substitution of Acyl Diimides

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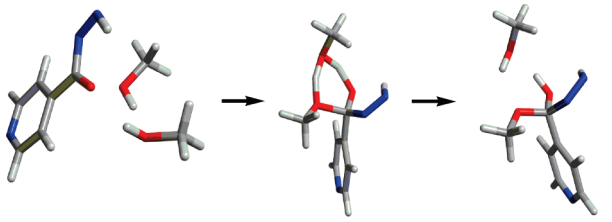


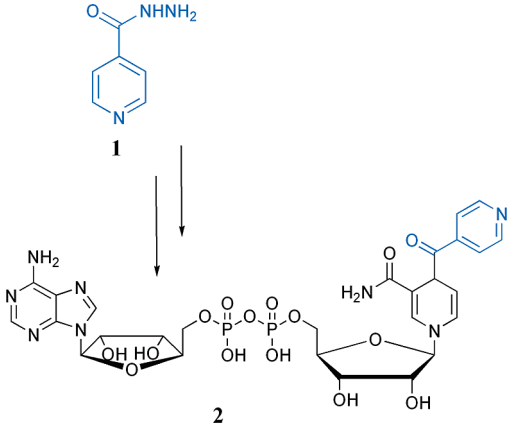
The nucleophilic acyl substitution of the acyl diimide intermediate formed by the oxidation of isoniazid was found to involve two methanol molecules in a six-membered cyclic transition state. Calculations were performed in the gas phase at the B3LYP/6-311 + G(d,p)//B3LYP/6-31G(d) level of theory and solvation effects were included both explicitly and implicitly by using CPCM. The effect of electron withdrawing and donating groups on the aryl ring was also explored. The results obtained are in good agreement with experimental observations for the oxidation of isoniazid.

The hydrazide of isonicotinic acid, isoniazid (**1**, Scheme 1), has been a front line drug against tuberculosis (TB) for almost half a century.[1] Increasingly *Mycobacterium tuberculosis* is becoming resistant to a range of antibiotics including isoniazid. Therefore it is important to investigate the chemistry surrounding the mode of action of this drug as this will help to combat drug resistance.

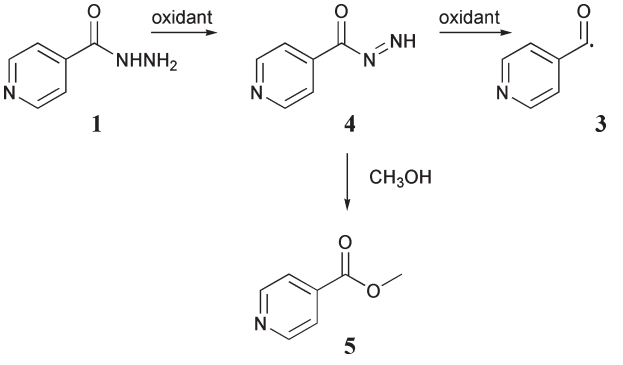
In *Mycobacterium tuberculosis* isoniazid is activated by the catalase-peroxidase enzyme, KatG.[2−8] Once activated, isoniazid forms a reactive intermediate that adds to NAD^+ to form the "true" drug (**2**, Scheme 1).[9] The postulated reactive intermediate is a radical (**3**, Scheme 2)[3,10,11] formed via an acyl diimide intermediate (**4**, Scheme 2).[10,12]

This process of activation is impaired in some isoniazid resistant strains of TB.[6] Experimental investigations have shown that **4** is more prone to nucleophilic acyl substitution under neutral conditions than other aryl hydrazides (Scheme 2).[11] This substitution diverts the chemistry of isoniazid away from the formation of **3** and could therefore, under the right conditions, hinder the formation of **2** (Scheme 1).

SCHEME 1. Activation of Isoniazid 1 To Form the True Drug 2



SCHEME 2. Formation of the Reactive Intermediate 3 via the Acyl Diimide Intermediate 4[a]



[a] The formation of the methyl ester 5 from the nucleophilic substitution of 4 is also shown.

To the best of our knowledge, there have not been any mechanistic studies performed that examine the details of

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nucleophilic acyl substitution on the acyl diimide **4** to form the ester **5** (Scheme 2). Studies on the solvolysis of hydrazides under basic[13] and acidic[14−16] conditions do not involve oxidation to the corresponding acyl diimide and are therefore not directly related to this work.

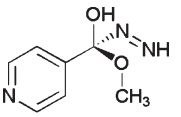
The formation of esters as desired and unwanted products during the oxidation of hydrazides has been documented;[12,17,18] however, these studies did not provide any real insight into the mechanism of their formation. In contrast, the solvolysis of esters has been investigated both experimentally and computationally[19−25] and we expect similar mechanisms to be operating during the solvolysis of acyl diimides.

Solvent molecules play important stabilizing roles during base-catalyzed hydrolysis of esters[20,22,24,25] and experimental[25] and computational[20] studies of acid-catalyzed solvolysis of esters suggest the involvement of solvent molecules in tetrahedral or cyclic intermediates.[20,25] On this basis, we would expect a similar involvement of solvent molecules during the solvolysis of acyl diimide **4**.

We now present the results of a comprehensive study into the mechanism of nucleophilic acyl substitution of the acyl diimide formed by the oxidation of isoniazid, using computational chemistry. This study reveals that solvent molecules also play an important role in this process, providing the means for the cyclic transition state necessary for substitution to occur.

We began this investigation by examining the reaction of acyl diimide **4** with methanol. A range of pathways was explored including various oxidation and protonation states (see the Supporting Information); however, many of these did not give stable intermediates as energy minima on their respective potential energy surfaces.

In exploring the various potential energy surfaces it was found that the tetrahedral species **6** (Figure 1) was a stable intermediate in that it proved to correspond to an energy minimum by frequency analysis. To investigate the mechanism for the formation of **6** a relaxed potential energy surface scan was performed in which the oxygen of a



6

FIGURE 1. The stable intermediate formed by a proton shift during the methanol attack on the acyl diimide.

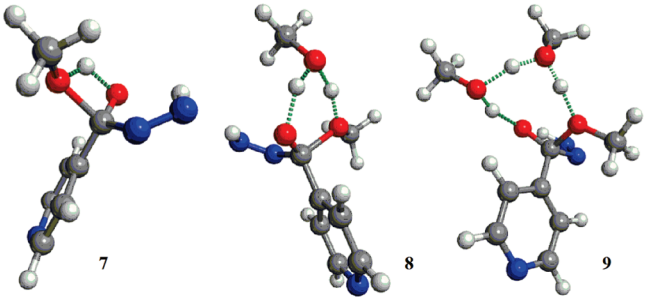


FIGURE 2. Optimized geometries of cyclic transition structures using one (**7**), two (**8**), and three (**9**) methanol molecules.

methanol molecule approached the carbonyl carbon of acyl diimide **4**.[26] During this approach a proton shift from methanol to the carbonyl oxygen was observed that led to the formation of tetrahedral intermediate **6** (Figure 1). We believe that this proton shift is responsible for the absence of any ionic or zwitterionic intermediates. The transition structure **7** (Figures 2 and 3) for this process was located and proved to correspond to a saddle point on the potential energy surface.

The energy of activation (E_a) for the formation of **7** is predicted to be 150.1 kJ mol^{-1}, which under the experimental conditions would be expected to be prohibitive. As experimental studies suggest that this process is highly dependent on the amount of solvent present[11] a second methanol molecule was included to give the six-membered cyclic transition structure **8** (Figures 2 and 3). This proved to have a dramatic effect on the activation energy (E_a), which was calculated to be 84.1 kJ mol^{-1} for the reaction involving **8**, some 66 kJ mol^{-1} lower than that calculated for **7**. Inclusion of a third methanol molecule to give an eight-membered cycle (**9**, Figures 2 and 3) only reduced E_a by a further 2 kJ mol^{-1}. (See Table 1 for reaction barriers, imaginary frequencies, and exothermicity).

It is possible that the proton shift depicted in Scheme 3 is assisted by quantum tunneling. Therefore we chose to use Eckart's[27] method to explore this possibility as it has been reported to give more accurate tunneling correction coefficients than the method of Wigner.[28] The tunneling coefficient is related to the energy of activation by

$$E_a^* = E_a - RT \ln \kappa$$

where E_a^* is the corrected activation energy, E_a is the activation energy in the absence of tunneling, and κ is the Eckart tunneling coefficient.[29] The inclusion of the Eckart coefficient reduced the activation energy for the single

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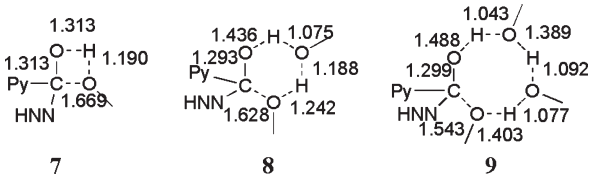


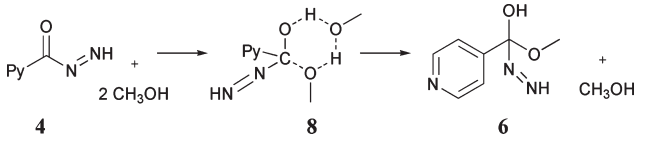
7 8 9

FIGURE 3. Bond lengths (Å) for transition structures **7**, **8**, and **9**.

TABLE 1. Transition Structure Energy Barriers (kJ mol^{-1}), Imaginary Frequencies (cm^{-1}), and Energies of the Overall Reaction (kJ mol^{-1})

transition structure	E_a	υ^i	ΔE
7	150.1	1648	12.4
8	84.1	1024	9.7
9	81.9	211	17.5

SCHEME 3. The Formation of Intermediate 6 via a Cyclic Transition State 8



4 8 6

TABLE 2. Eckart Tunneling Coefficients and Their Effect on the Activation Energy of the Hydrogen Shifts

transition structure	κ	E_a* (kJ mol^{-1})
7	94.9	141.0
8	2.3	83.8
9	1.2	81.6

solvent molecule process (**7**) by 9.1 kJ mol^{-1} and did not affect the result for the two and three methanol molecules by any significant amount (Table 2).[30] This strongly suggests that although tunneling may occur it is not predicted to reduce the energy barrier of the process involving **7** enough to allow it to be competitive. In the processes involving **8** and **9** there is very little lowering of the activation energy predicted by hydrogen tunneling calculations.

The final product of this reaction observed experimentally is methyl isonicotinate **5** (Scheme 2). The possibilities for the formation of **5** from the tetrahedral intermediate **6** include the loss of an NNH^- ion, addition of H^+ to the imide portion followed by loss of imide, or loss of H_2 and N_2. All of these options were investigated by using gas phase calculations.

Protonation of the imide portion of **6** was explored and it is predicted that this is the favored pathway, as once protonation takes place at the nitrogen adjacent to the carbonyl, imide formation is a spontaneous process (**C**−**D** Figures 4 and 5). Protonation at the nitrogen further from the carbonyl led to products that were 87 kJ mol^{-1} higher in energy than those in **D**.

The loss of NNH^- was found to correspond to a high energy pathway with a final energy of 1566 kJ mol^{-1} relative to the starting structure **A**. Although the loss of N_2 and H_2 led to a lower energy minimum (**F**), extensive searching of the

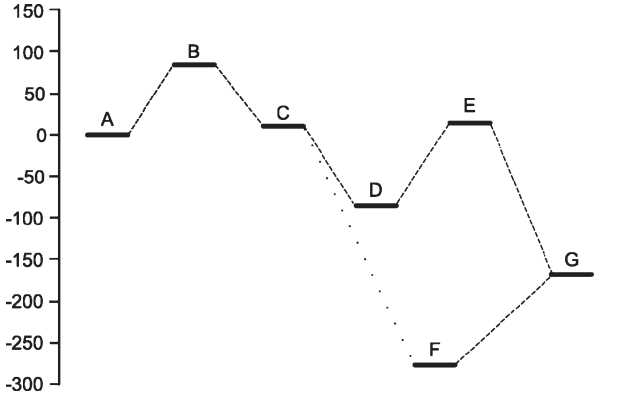


FIGURE 4. Energy diagram for the complete substitution mechanism in the gas phase, finishing with a loss of N_2(g) and H_2(g).

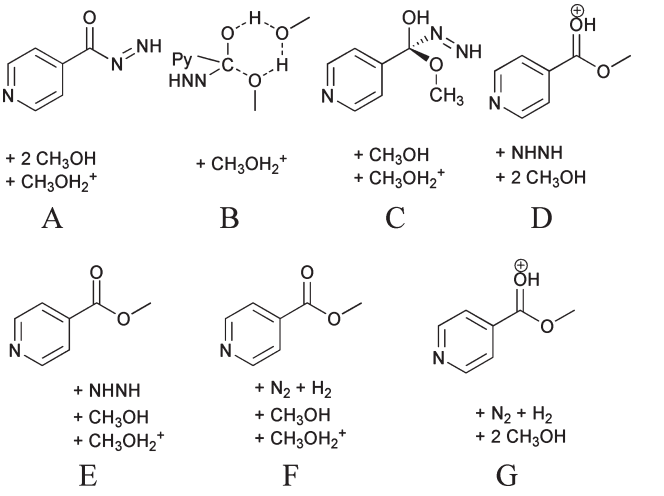


FIGURE 5. Explanatory figures relating to energy diagrams in Figures 4 and 6.

potential energy surface did not lead to a pathway that would form these products from the intermediate structure **6** (**C**).

The addition of a proton followed by loss of imide is not predicted to occur in the gas phase due to thermodynamic considerations (**E**, Figures 4 and 5). This reaction is, however, solvent dependent and therefore bulk solvation calculations were performed to see if there was a stabilizing effect throughout the reaction. The resulting potential energy diagram showing the lowest energy pathway is found in Figure 6.[31] It can be seen that the inclusion of the bulk solvent stabilizes all of the stationary points in the reaction path and that loss of imide is now predicted to be a thermodynamically favorable mechanism. The transition structure (**8**, **B**) in particular is stabilized relative to the reactants and products by the inclusion of the solvent and the energy barrier for this reaction is predicted to be reduced to 23.3 kJ mol^{-1} (Table 3).

In the experimental setting, different aromatic groups show differing susceptibility to nucleophilic acyl substitution. Gas-phase calculations for the two-solvent-molecule nucleophilic acyl substitution process were repeated to investigate the effect of electron withdrawing and donating

(30) Many thanks to Dr. Mark Taylor for helpful discussions and a spreadsheet to enable the calculation of Eckart coefficients.

(31) The relative energy for *G* is not shown as it is off the scale for this graph at −205 kJ mol^{-1}.

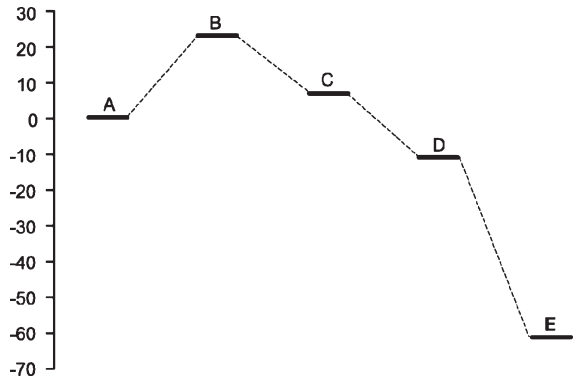
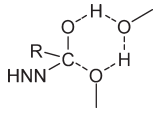


FIGURE 6. Energy diagram (kJ mol^{-1}) including CPCM calculations of solvent stabilizing effects, for the nucleophilic substitution by methanol of the acyl diimide formed by oxidation of isoniazid.

TABLE 3. Calculated Energy Barriers for the Formation of Transition Structures with Various R Groups (kJ mol^{-1}), and Related Imaginary Frequencies (cm^{-1}) without and with CPCM Solvation



				with solvent	
	E_a	ΔE	v^i	E_a	ΔE
R = phenyl	94.1	20.1	1102	30.9	14.6
R = *p*-methoxyphenyl	102.5	29.4	1137	42.0	26.3
R = *p*-nitrophenyl	84.1	11.1	993	24.7	7.4
R = 4-pyridyl (**8**)	84.1	9.7	1024	23.3	6.9

effects. The results (found in Table 3) showed a typical Hammett susceptibility with electron withdrawing groups enabling, and electron donating groups hindering, the process of substitution. While this is consistent with experimental results[11] to a point, it does not explain why a greater amount of substitution occurred with the pyridyl hydrazide (isoniazid, **1**) experimentally.[11] However, when the bulk solvent effect is added it can be seen that the energy barrier for the transition state is less for isoniazid (**8**) than for all of the other groups. This finding is supported by experimental results which show that isoniazid is more prone to solvolysis by polar solvents than other hydrazides studied.[11]

These results may provide some insight into why some strains of *Mycobacterium tuberculosis* are resistant to isoniazid. It might be possible that as isoniazid undergoes oxidation in the bacterium, drug resistant strains are able to promote the solvolysis pathway, effectively interfering with the formation of the "true drug" **2**.

In conclusion, the nucleophilic acyl substitution of hydrazides under oxidative conditions was found to proceed via a six-membered cyclic transition structure. This is a solvent-dependent process requiring at least two solvent molecules in the transition state. The inclusion of various functional groups showed that, in comparison, isoniazid is predicted to undergo this reaction in a facile manner. This computational outcome is supported by experimental results and has implications in the understanding of resistance to isoniazid found in resistant strains of TB. As oxidized (or activated) isoniazid is prone to undergo nucleophilic acyl substitution it may be that resistant strains of TB promote this process in competition with the further oxidation to the free radical and therefore the formation of the true drug. An understanding of the reactivity of isoniazid could play a role in future drug design.

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Supporting Information Available: Various intermediates tested to find a possibility for the formation of **6**; geometries of transition structures, associated products, and reactants for structures **7**−**9** and those in Table 3; and all computational details. This material is available free of charge via the Internet at http://pubs.acs.org.