Lessons Learned from the Reactivity of Mandelylthiamin and Derivatives

by

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Abstract

Mandelylthiamin (MTh) is an accurate model of the pre-decarboxylation intermediate formed on benzoylformate decarboxylase (BFDC). In solution, it decarboxylates to the Breslow intermediate $10^8$ times slower than on the enzyme. Earlier studies proposed that the C–C bond-breaking step in the decarboxylation of MTh is highly reversible, and thus possibly impeding the overall reaction rate. More recently, it was observed that the decarboxylation of MTh could be more effective through formation of HCO$_3^-$, suggesting the enzyme could be applying a similar strategy to maximize its catalytic efficiency. This thesis investigates in detail the decarboxylation of MTh and related analogs in solution phase, and proposes possible enzymic strategies for catalysis.

Research on MTh had suffered from a low yield synthesis and ineffective purification. The work in this thesis was made possible because lithium ions were discovered to greatly promote formation of the MTh ester and its analogs. Using the new synthetic methodology I could synthesize previously unattainable MTh variants.

This thesis can be divided into three main sections: 1) the study of MTh analogs and other compounds to understand molecular features responsible for decarboxylation by formation of HCO$_3^-$, This was probed through observation of base catalysis and a solvent kinetic isotope effect...
(SKIE), which are indications of a decarboxylation by formation of HCO$_3^-$.

Based on the collected results, it was concluded that a base catalyzed decarboxylation is a unique phenomenon. A coincidental outcome of this study was the discovery of intramolecular cyclization reactions of the Breslow intermediate. 2) the study of substituent effects on the decarboxylation of MTh to probe the nature of the BF-derived Breslow intermediate in solution and comparison of results to the enzymic decarboxylation. This revealed that certain enzymes likely enforce an unnatural geometry of the Breslow intermediate to achieve catalytic perfection. 3) the study of the fragmentation of the BF-derived Breslow intermediate from a radicals’ formation perspective. This was done after recent reports that radicals are involved in related transformations. My results lead to a conclusion that radicals are unlikely to be involved, and instead a simple polar alternative that is consistent with experimental observations was proposed.
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Authorship, Contribution, and Permission Statements

Item 1

Location in thesis: Chapter 2 and Appendix B

Content attached: whole paper and supporting information

Title: Lithium-stabilized nucleophilic addition of thiamin to a ketone provides an efficient route to mandelylthiamin, a critical pre-decarboxylation intermediate

Authors: Michael Bielecki, Graeme W. Howe, Ronald Kluger


DOI: 10.1016/j.bioorg.2015.08.004

Contribution: All experimental work and data interpretation was done by Michael Bielecki. All authors contributed equally in writing this manuscript

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Location in thesis: Chapter 8 and Appendix C

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Authors: Michael Bielecki, Ronald Kluger


DOI: 10.1002/anie.201702240
Contribution: All experimental work and data interpretation was done by Michael Bielecki. The manuscript was written by Michael Bielecki and Ronald Kluger.

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Location in thesis: Chapter 3, Figure 3.6

Content attached: Figure 7 from page 2329

Title: Studies on structure-function relationships of indolepyruvate decarboxylase from Enterobacter cloacae, a key enzyme of the indole acetic acid pathway

Authors: Tittmann, Kai and co-workers


DOI: 10.1046/j.1432-1033.2003.03602.x

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Item 4

Location in thesis: Chapter 5, Figure 5.1

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Title: An Integrated Path Integral and Free-Energy Perturbation-Umbrella Sampling Method for Computing Kinetic Isotope Effects of Chemical Reactions in Solution and in Enzymes

Authors: Major, D.T; Gao, Jiali.

DOI: 10.1021/ct600371k

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Item 5

Location in thesis: Chapter 5, Figure 5.2

Content attached: Figure 3 from page 10976

Title: 
Decarboxylation without CO$_2$: Why Bicarbonate Forms Directly as Trichloroacetate Is Converted to Chloroform.

Authors: Howe, Graeme, Kluger, Ronald.


DOI: 10.1021/jo501990u

Permission: attached with permission from the American Chemical Society (no license required)

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Base-Catalyzed Decarboxylation of Mandelylthiamin: Direct Formation of Bicarbonate as an Alternative to Formation of CO$_2$

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Published: Journal of American Chemical Society, 2012, 134, pp 20621-20623.

Contribution: Michael Bielecki provide mandelylthiamin. Data collection, data interpretation and writing of the manuscript was done by Howe, G and Kluger, R.

DOI: 10.1021/ja310952a
Permission: attached with permission from the American Chemical Society (no license required)
### List of Abbreviations

<table>
<thead>
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<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>AP</td>
<td>2,5-dimethyl-4-aminopyrimidin-3-yl group</td>
</tr>
<tr>
<td>APH⁺</td>
<td>$N'\text{I}$ protonated AP</td>
</tr>
<tr>
<td>APyr</td>
<td>4-aminopyridin-3-yl group</td>
</tr>
<tr>
<td>APyrH⁺</td>
<td>$N'\text{I}$ protonated APyr</td>
</tr>
<tr>
<td>BF</td>
<td>benzoylformic acid</td>
</tr>
<tr>
<td>BFDC</td>
<td>benzoylformate decarboxylase</td>
</tr>
<tr>
<td>Bn</td>
<td>benzene group</td>
</tr>
<tr>
<td>CA</td>
<td>carbonic anhydrase</td>
</tr>
<tr>
<td>DMAP</td>
<td>2,5-dimethyl-4-aminopyrimidine</td>
</tr>
<tr>
<td>EPR</td>
<td>electron paramagnetic resonance</td>
</tr>
<tr>
<td>ESI</td>
<td>electron spray ionization</td>
</tr>
<tr>
<td>GDS</td>
<td>ground state destabilization</td>
</tr>
<tr>
<td>gCOSy</td>
<td>homonuclear correlation spectroscopy</td>
</tr>
<tr>
<td>HBNTh</td>
<td>2-(1-hydroxybenzyl)thiamin</td>
</tr>
<tr>
<td>HMBC</td>
<td>heteronuclear multiple bond correlation spectroscopy</td>
</tr>
<tr>
<td>HSQC</td>
<td>heteronuclear single quantum coherence spectroscopy</td>
</tr>
<tr>
<td>IPDC</td>
<td>indolepyruvate decarboxylase</td>
</tr>
<tr>
<td>isoHBNTh</td>
<td>constitutional isomer of HBNTh</td>
</tr>
<tr>
<td>LiHMDS</td>
<td>bis(trimethylsilyl)amide lithium salt</td>
</tr>
<tr>
<td>LTh</td>
<td>lactylthiamin</td>
</tr>
<tr>
<td>LThH⁺</td>
<td>$N'\text{I}$ protonated LTh</td>
</tr>
<tr>
<td>LThDP</td>
<td>lactylthiamin diphosphate</td>
</tr>
<tr>
<td>MTh</td>
<td>mandelylthiamin</td>
</tr>
<tr>
<td>MThH⁺</td>
<td>$N'\text{I}$ protonated MTh</td>
</tr>
<tr>
<td>MThDP</td>
<td>mandelylthiamin diphosphate</td>
</tr>
<tr>
<td>NADH</td>
<td>nicotinamide adenine dinucleotide</td>
</tr>
<tr>
<td>NAH⁺</td>
<td>oxidized NADH</td>
</tr>
<tr>
<td>NMPA</td>
<td>$N$-methylpicolinic acid or $N$-methylpicolinate</td>
</tr>
<tr>
<td>NMR</td>
<td>nuclear magnetic resonance spectroscopy</td>
</tr>
<tr>
<td>PTK</td>
<td>phenylthiazole ketone</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Definition</td>
</tr>
<tr>
<td>--------------</td>
<td>----------------------------------</td>
</tr>
<tr>
<td>SKIE</td>
<td>solvent kinetic isotope effect</td>
</tr>
<tr>
<td>TBDPS</td>
<td>tert-butylidiphenylsilyl group</td>
</tr>
<tr>
<td>TCA</td>
<td>trichloroacetic acid</td>
</tr>
<tr>
<td>ThDP</td>
<td>thiamin diphosphate</td>
</tr>
<tr>
<td>THP</td>
<td>tetrahydropyran</td>
</tr>
<tr>
<td>VDW</td>
<td>Van der Waals</td>
</tr>
</tbody>
</table>
Chapter 1
Introduction

1.1 Decarboxylation reactions

Decarboxylation reactions typically replace a carboxyl group with a proton. The net stoichiometry is consistent with the formation of CO$_2$ from the atoms of the carboxyl group (Scheme 1.1).

$$\begin{align*}
\text{R} - \text{COOH} & \rightarrow \text{R}^- + \text{O} = \text{C} = \text{O} \\
\text{Scheme 1.1.} \text{ A decarboxylation reaction.}
\end{align*}$$

We expect that the C–C bond-breaking step to occur from the ionized carboxyl, which leads directly to the release of CO$_2$. Without loss of the proton, bond-breaking would lead to formation of protonated carbon dioxide (CO$_2$H$^+$), which has a $pK_a$ of $\sim 39$,[1] and hence is energetically inaccessible (Scheme 1.2).

$$\begin{align*}
\text{A.} & \text{ R} - \text{COOH} \rightarrow \text{R}^- + \text{O} = \text{C} = \text{O} + \text{H}^+ \rightarrow \text{RH} \\
\text{B.} & \text{ R} - \text{COOH} \rightarrow \text{R}^- + \text{O} = \text{C} = \text{OH} \\
\text{Scheme 1.2.} \text{ Decarboxylation from the carboxylate (A) and an unlikely alternative in which CO}_2\text{H}^+ \text{ would form directly from the unionized carboxyl (B).}
\end{align*}$$

The negative charge from the carboxylate group is transferred to the residual carbon group, R, with the immediate product being a carbanion or a carbanion equivalent. The energy of the R$^-$ intermediate is normally higher than that of the substrate. Hence, the rate of decarboxylation will
be affected by the energetics of the C–C bond-breaking step. Since protonation of the resulting carbanion is usually irreversible, the overall decarboxylation reaction is also irreversible.

1.2 Stability of the R− group

Formation of a stable carbanionic intermediate is crucial for decarboxylation to occur. The stability that is needed can be provided by electronegative substituents (inductive effect) or by an “electron sink” (resonance effect) that is conjugated to the site of where the negative charge develops (Scheme 1.3).[2]

A.

\[
\begin{align*}
\text{Cl} & \quad \text{Cl} \\
\text{Cl} & \quad \text{Cl}
\end{align*}
\rightarrow
\begin{align*}
\text{Cl} & \quad \text{Cl} \\
\text{O} & \quad \text{C} = \text{O}
\end{align*}
\]

B.

\[
\begin{align*}
\text{O} & \quad \text{N}^+ \\
\text{O} & \quad \text{O}
\end{align*}
\rightarrow
\begin{align*}
\text{O} & \quad \text{N}^+ \\
\text{O} & \quad \text{C} = \text{O}
\end{align*}
\]

Scheme 1.3. Decarboxylation of trichloroacetate (A) and nitroacetate (B) leads to formation of a stable carbanion and a carbanion equivalent, respectively.

In the absence of stabilizing factors, the R group must undergo some chemical transformation to allow the reaction to proceed. For example, decarboxylation of cinnamic acids is promoted by protonation of the double (Scheme 1.4).[3]

\[
\begin{align*}
\text{R}^1 & \quad \text{R}^2 \\
\text{Ph} & \quad \text{O}
\end{align*}
\rightarrow
\begin{align*}
\text{R}^1 & \quad \text{R}^2 \\
\text{Ph} & \quad \text{O}
\end{align*}
\rightarrow
\begin{align*}
\text{R}^1 & \quad \text{R}^2 \\
\text{Ph} & \quad \text{Ph}
\end{align*}
\]

Scheme 1.4. Proposed mechanism for the decarboxylation of cinnamic acids.

1.3 Alternatives to the loss of CO₂

1.3.1 Loss of protonated carbonic acid

So far, we have focused on the nature of the residual group, R−, as the sole factor affecting the decarboxylation. However, a decarboxylation does not need to occur by loss of CO₂, providing a second dimension to this reaction. Dunn and Lee observed that the decarboxylation of pyrrole-2-
carboxylic acid is accelerated in concentrated mineral acids.\cite{4} They proposed that under the strongly acidic conditions, the heteroaromatic ring is protonated, turning into an excellent leaving group (Scheme 1.5). However, under the same conditions the ionized carboxylate group is not accessible, and the decarboxylation from $R^+\text{COOH}$ is not feasible because it would lead to formation of an energetically impossible $\text{CO}_2\text{H}^+$ intermediate. Mundle et al. proposed that water adds to the carboxyl group to form an orthoacid intermediate that can decompose directly to the more accessible\cite{5} protonated carbonic acid ($\text{H}_3\text{CO}_3^+$, Scheme 1.5).\cite{6}

![Scheme 1.5](image)

**Scheme 1.5.** Mechanism for the hydrolytic decarboxylation of pyrrole-2-carboxylic acid in strongly acidic solutions.

### 1.3.2 Loss of carbonic acid or bicarbonate

Formation of an orthoacid ($\text{RC(OH)}_3$) by addition of water to a carboxylic acid ($\text{RCOOH}$) is evidenced from observation of $^{18}\text{O}$ isotope exchange in the carboxyl group. The covalent hydration of carboxylic acids was studied by Irving and Urey, Redington, and O’Connor and co-workers.\cite{7} In neutral and alkaline solutions, the orthoacid must be in equilibrium with its deprotonated forms ($\text{R(OH)}_2\text{O}^-$ or $\text{R(OH)}\text{O}_2^{2-}$), and these could, in principle, decompose with loss of carbonic acid ($\text{H}_2\text{CO}_3$) and $\text{HCO}_3^-$, respectively (Scheme 1.6). Both $\text{H}_2\text{CO}_3$ ($\text{pK}_a \sim 3.6$) and $\text{HCO}_3^-$ ($\text{pK}_a \sim 10$) are accessible intermediates in aqueous solutions. Therefore, they should be considered as potential products of a hydrolytic decarboxylation in neutral or alkaline solutions.
Scheme 1.6. Decarboxylation through formation of H$_2$CO$_3$ (top) and HCO$_3^-$ (bottom).

There are only few reports of such reactivity. Smith and co-workers reported a decarboxylation reaction through formation of HCO$_3^-$ on an enzyme (β-ketoacyl synthase) and proposed that it proceeds from an orthoacid dianion intermediate.[8] Kluger and co-workers proposed that MTh decarboxylates with formation of HCO$_3^-$ from a base assisted decomposition of an orthoacid anion in acetate buffers (Scheme 1.7).[9] The decarboxylation of MTh is general base catalyzed (β ~ 0.28) with a solvent isotope effect (SKIE) of ~ 2. The proposed mechanism would also be consistent with a rate limiting C–C bond breaking step, consistent with a large carbon isotope effect for this reaction ($^{13}$C/$^{12}$C = 1.058).[10]

Scheme 1.7. The proposed mechanism for the general base catalyzed decarboxylation of MTh in water.

If a decarboxylation reaction occurs through loss of H$_2$CO$_3$ or HCO$_3^-$, then formation of CO$_2$ is either not accessible or slower. Under what circumstances is loss of CO$_2$ problematic (assuming RCOO$^-$ is accessible)? It has been proposed that carbon dioxide may readily recombine with the nascent carbanion, reducing the overall rate of product release.[11]
1.3.3 How to differentiate between formation of CO$_2$ and HCO$_3^-$?

Krebs and Roughton developed methods to distinguish if a decarboxylation reaction occurs through formation of CO$_2$ or HCO$_3^-$.[12] Their method is based on the relatively slow hydration of CO$_2$ to HCO$_3^-$ in water ($\sim 4 \times 10^{-2}$ s$^{-1}$).[13] If a sufficiently fast decarboxylation reaction occurs through loss of CO$_2$ in water, there will be an initial spike in CO$_2$ concentration, hence also pressure, because its equilibration to HCO$_3^-$ will be slower than its formation. This can be followed with a pressure gauge. The reference reaction contains carbonic anhydrase (CA), resulting in a “instant” equilibration of CO$_2$ and an initial spike in pressure will not be observed. If the decarboxylation occurs through formation of HCO$_3^-$, the presence of CA will not affect the generated pressure. Jordan later extended this approach, by monitoring formation of HCO$_3^-$ instead.[14] This was achieved by using a coupled enzyme assay, where phosphor(enol)pyruvate carboxylase converts HCO$_3^-$ (but not CO$_2$) to oxaloacetate, which is reduced by malate dehydrogenase to malate which in turn reduces NADH, and depletion of the latter is followed spectroscopically. Thus, a decarboxylation through loss of CO$_2$ will result in an initial lag in the formation of NAD$^+$. Unfortunately, most non-enzymic decarboxylation reactions are slower than the hydration rate of CO$_2$ to HCO$_3^-$, and hence the above described methods are not applicable. This limits us to indirect methods, such as observation of general base catalysis, as was demonstrated for the decarboxylation of MTh. If the decarboxylation from the orthoacid dianion (RC(OH)O$_2^{2-}$) is competitive, specific base catalysis could also be observed. Hydrolysis of various ketones generally depicted as RC(O)X (where X is a carbon leaving group) have been demonstrated to, at least partially, occur from the dianion hydrate species (RCXO$_2^{2-}$).[15]

1.4 Reversible decarboxylation

It was proposed earlier that decarboxylation of MTh through the loss of CO$_2$ is slowed by recombination of CO$_2$ with the nascent carbanion (Scheme 1.8.A). This would be significant if separation, which requires desolvation, has a barrier that is as high or higher than that for the recombination. The idea of a reversible C–C bond breaking step in the decarboxylation of MTh stems from the observation that pyridinium ions catalyze the decarboxylation of MTh.[11] It was proposed that the pyridinium cation pre-associates (presumably through $\pi$-stacking and/or H-bonding) to MTh, allowing for a rapid protonation (entropic advantage) of the BF-derived
carbanion (Scheme 1.8.B). This would decrease the extent of reformation of MTh, resulting in an increase of the overall rate of decarboxylation.

A.

\[
\begin{align*}
\text{R}^- + \text{O} = \text{C} = \text{O} & \rightleftharpoons \text{R}^- + \text{O} = \text{C} = \text{O} \\
\end{align*}
\]

B.

\[
\begin{align*}
\left(\begin{array}{c}
\text{N} \\
\text{H} \\
\text{R} \\
\end{array}\right) + \text{O} = \text{C} = \text{O} & \rightarrow \left(\begin{array}{c}
\text{N} \\
\text{H} \\
\text{R} \\
\end{array}\right) + \text{O} = \text{C} = \text{O} \\
\end{align*}
\]

C.

\[
\begin{align*}
\text{H}_2\text{O} + \text{R}^- + \text{O} = \text{C} = \text{O} & \rightarrow \text{R}^- + \text{H} + \text{O} = \text{C} = \text{O} \\
\end{align*}
\]

**Scheme 1.8.** Decarboxylation of MTh by formation of CO\(_2\) (A), via a pyridinium complex (B), and by loss of HCO\(_3^-\) (C).

Rapid recombination of CO\(_2\):R\(^-\) pairs is implied in the energy computations by Major and Gao.\(^{[16]}\) Their studies showed minimal barriers to recombination (\(\sim 2\) kcal/mol) in the decarboxylation of orotic acid and N-methylpicolinic acid (NMPA) in water. Similarly, Howe et al. calculated a tiny barrier to recombination of CO\(_2\) in the decarboxylation of trichloroacetic acid (TCA).\(^{[17]}\) The low barrier to reversal after formation of CO\(_2\), competitive with separation, may be a common characteristic for decarboxylation reactions that proceed through formation of a localized carbanion and CO\(_2\). In an important contrast, Howe reports a substantial energy barrier (\(\sim 15\) kcal/mol) for the addition of the trichloromethyl carbanion to H\(_2\)CO\(_3\), the intermediates that would form in the hydrolytic decarboxylation of TCA.\(^{[17]}\) This parallels the basis of the proposition that MTh decarboxylates by loss of HCO\(_3^-\) (Scheme 1.8C).

### 1.5 Thiamin, carbenes and Breslow intermediates

Thiamin (Th), also known as vitamin B\(_1\), is a precursor to the bioactive thiamin diphosphate (ThDP, Figure 1.1). ThDP is an essential cofactor for various enzymes, for example, decarboxylases of various \(\alpha\)-ketoacids.
Figure 1.1. Thiamin (left) and thiamin diphosphate (right).

Though the structure of thiamin was known since mid-1930s,[18] it was only until 1957 that Ronald Breslow elucidated thiamin’s role in enzyme catalysis.[19] Observation of deuterium incorporation at the C2 position in Th lead to the proposal of formation of a nucleophilic carbene/carbanion intermediate (the “first” Breslow intermediate) (Scheme 1.9). A year later Breslow proposed formation of a thiamin:aldehyde adduct, that deprotonates at C2α to form a second carbanionic intermediate (Scheme 1.10).[20] Such an intermediate can react as an acyl carbanion equivalent (umpolung chemistry) and thus thiamin parallels cyanide catalysis in benzoin condensation.[21]

Scheme 1.9. Formation of the thiamin-derived carbene as proposed by Breslow.

In 1967 Sable synthesized the aforementioned thiamin:adduct (2-(1-hydroxyethylthiamin, HBnTh), and observed deuterium exchange at the C2α position, thus, providing evidence for existence of the “second Breslow intermediate”. The Breslow intermediate can be considered as a 1,2-enaminol derivative. It is this intermediate and its synthetic derivatives that we now commonly refer to as Breslow intermediates.
Scheme 1.10. Loss of a proton at the C2α in a thiamin:aldehyde adduct yields the classic Breslow intermediate.

In the last few decades there has been a renewed interest in Breslow intermediates because of the growing importance of \(N\)-heterocyclic carbene (NHC) catalysis in organic synthesis.\textsuperscript{[22]} In 2012 Berkessel for the first time isolated the Breslow intermediate and fully characterized it by X-ray crystallography, \(^1\)H NMR and \(^{13}\)C NMR.\textsuperscript{[23]}

### 1.6 Decarboxylation of \(\alpha\)-ketoacids

The direct decarboxylation of an \(\alpha\)-ketoacid is not observed since it would require formation of an energetically inaccessible acyl carbanion (Scheme 1.11).

\[ \text{R-C=O} \quad \text{x} \quad \text{R} + \text{O=C=O} \quad \text{R-H} \]

Scheme 1.11. Direct decarboxylation of an \(\alpha\)-ketoacid would lead to an energetically unattainable acyl carbanion.

For this reason, even nature’s best catalysts – enzymes, require ThDP as a cofactor to decarboxylate \(\alpha\)-ketoacids. In solution thiamin or its derivatives form adducts with \(\alpha\)-ketoacids that undergo a spontaneous decarboxylation to the neutral Breslow intermediate. A subsequent protonation and elimination step yields the decarboxylation product (aldehyde) and regenerates the catalyst (Scheme 1.12). Though this type of catalysis for the decarboxylation of \(\alpha\)-ketoacids is most common, other pathways exist, for example, through formation of radicals or involvement of transition metals.\textsuperscript{[24]} However, unlike thiamin catalyzed reactions, they require elevated temperatures and are not biologically relevant.
Scheme 1.12. Catalytic cycle of a Th catalyzed decarboxylation of an α-ketoacid.

1.7 Mandelythiamin and benzoylformate decarboxylase

On the enzyme ThDP is anchored to the protein through an electrostatic interaction between the diphosphate and magnesium ions in the binding site. All ThDP-decarboxylases follow the general thiamin catalysis mechanism as outlined in Scheme 1.12. MTh, is a functional analogue of a thiamin diphosphate (ThDP)-derived intermediate that is formed in catalysis by benzoylformate decarboxylase (BFDC). Detailed analysis of the enzyme’s kinetics were done by Cook and co-workers. More recent research on BFDC was conducted by McLeish and Tittmann. BFDC is highly specific for BF and its para-substituted derivatives. BFDC converts BF to benzaldehyde and carbon dioxide with a $k_{cat} \sim 450 \text{ s}^{-1}$ and $K_m \sim 0.23 \text{ mM}$ at 30 °C. It has the fastest reported decarboxylation step amongst all ThDP-dependent decarboxylases ($k = 1.6 \times 10^4 \text{ s}^{-1}$). Previous studies on BFDC suggested neither a reversible decarboxylation nor loss of HCO$_3^-$

1.8 Enzymic vs. non-enzymic reactivity of intermediates

1.8.1 How do ThDP-dependent enzymes accelerate decarboxylation?

Determining the reactivity of an enzymic intermediate when it is not bound to the protein (solution phase) can provide useful information about the enzyme’s mode of action. For example, by using such an approach, intrinsically fast transformations, which do not require catalysis, can be distinguished from slow transformations that require the assistance of the enzyme. Moreover, observation of solution phase catalysis may be a source of insight on the enzymic mechanism. We
have observed that the covalent intermediates that are like those derived from ThDP on enzymes can be synthesized in the laboratory and their reactivity studied. The Breslow mechanism accounts for likely steps but does not account for the high rates of the enzymic reactions. An important intermediate that forms on ThDP-enzyme is the pre-decarboxylation intermediate. This undergoes decarboxylation to the Breslow intermediate, followed by protonation (Scheme 1.13). Measurements of the rate of decarboxylation of related compounds off the enzyme, indicate that the enzyme accelerates the decarboxylation typically by a factor $10^6$.

Scheme 1.13. The pre-decarboxylation intermediate on a ThDP-dependent enzyme undergoes decarboxylation to the Breslow intermediate followed by protonation to form an aldehyde:ThDP adduct.

Although the nonenzymic synthesis of a pre-decarboxylation intermediate that includes the diphosphate of the cofactor has been accomplished,[29] the diphosphate is usually omitted as it complicates the synthesis. The remote location of this group makes it unlikely to have a significant effect on decarboxylation. Compounds with a general structure shown in Figure 1.2B are used as accurate models for the study of the reactivity of the pre-decarboxylation intermediate, including MTh (Figure 1.2B, R = Ph).

Figure 1.2. A general structure of an enzymic intermediates (A) and its accurate model (B).
A good example of utilizing accurate models of enzymic intermediates was reported by Lienhard and coworkers.[30] They prepared a very simplified model compound of lactylthiamin diphosphate (LThDP, Figure 1.2A, R = CH₃) and measured the rate of decarboxylation, which was orders of magnitude smaller in solution than on the enzyme (pyruvate decarboxylase). This result showed that the thiazolium electron sink alone cannot account for the enzymic catalysis. How does then the enzyme further accelerate the decarboxylation? Lienhard observed that the decarboxylation was $10^4$-fold faster in ethanol than in water and suggested that this was a case of ground state destabilization (GSD). Jordan and co-workers reported an enzyme-like rate for decarboxylation of LTh (Figure 1.2B, R = CH₃) in tetrahydrofuran.[31] He also proposed that the enzyme accelerates the reaction through GSD.[32] The low dielectric constant of the enzyme's active site, as determined with a fluorescent probe, supported this.[33] However, enzymes bind intermediates through a highly structured network of dipoles and the interactions with an unreactive fluorescent probe will differ from the interactions of the polar intermediates.

As with other thiamin derivatives, the rate constant for decarboxylation of MTh is $10^8$ times smaller than the enzymic process ($\sim10^{-4}$ s⁻¹ vs. $10^4$ s⁻¹). Both Qingyan Hu’s observation of pyridinium catalysis and Graeme Howe’s observation of general base catalysis of the decarboxylation of MTh required a more complex mechanism than simply C–C bond-breaking. It was proposed (based on the specific catalytic effects of pyridinium salts) that the fast decarboxylation on BFDC could utilize the enzyme’s ability to minimize recombination of the Breslow intermediate with CO₂.[28] Interestingly, it has also been suggested that the enzyme could be trapping CO₂ with the active site Ser26 as a carbonate ester. This can then decompose to CO₂ at a more remote location, and thus avoid recombination with the Breslow intermediate (Scheme 1.15A).[34] This idea emerged from observation of an irreversible inhibition of BFDC by a phosphonate analogue of BF (benzoylphosphonate salt).[35] The cause of the irreversible inhibition is phosphorylation of Ser26 by a reaction with the conjugate of the inhibitor and ThDP. This demonstrates that Ser26 is both nucleophilic and properly oriented for CO₂ interception. Alternatively, Ser26 could add to the carboxyl group to form an orthoacid ester intermediate, paralleling Howe’s mechanism for the general base catalyzed decarboxylation of MTh (Scheme 1.15B). In either case, this would provide a kinetic advantage by having the loss of CO₂ occurring at a location that is protected from recombination. However, mutation of Ser26 to a hydrophobic
amino acid resulted in only a relatively modest reduction (10-fold) of catalytic efficiency,\textsuperscript{[36]} indicating reversibility is not the only factor that the enzyme must overcome.


Scheme 1.15. Possible mechanisms for a Ser26-assisted decarboxylation of MThDP on BFDC.

1.9 Oka fragmentation of 2-(1-hydroxybenzyl)thiamin

In 1970 Oka attempted to extend the synthesis of conjugates of thiamin and aliphatic aldehydes to produce 2-(1-hydroxybenzyl)thiamin (HBnTh) from benzaldehyde. Instead, he reported that the reaction of thiamin with benzaldehyde in the presence of triethylamine in refluxing methanol, yields trace amounts of HBnTh along with benzoin, and substantial amounts of products, resulting from disruption of thiamin’s core structure.\textsuperscript{[37]} Oka’s skill in product separation and structural analysis, led him to isolation and identification of 2,5-dimethyl-4-aminopyrimidine (DMAP), phenylthiazole ketone (PTK) and smaller amounts of an isomer of HBnTh (isoHBnTh) (Figure 1.3)
Figure 1.3. Products from the reaction of thiamin with benzaldehyde in the presence of triethylamine in refluxing methanol.

Kluger and co-workers identified PTK and DMAP as the major products of general base catalyzed decomposition of HBnTh in neutral buffers.\textsuperscript{[38]} This was an important development because Washabaugh had reported, incorrectly, that general base catalyzed decomposition of HBnTh produces benzaldehyde and thiamin from HBnTh.\textsuperscript{[39]} There would be no likely role for the catalysts in that case. Unlike benzaldehyde, PTK has a characteristic red-shifted band at 328 nm, as reported by Oka, and with this the products were readily identified.\textsuperscript{[37]} It was later determined that PTK and DMAP form from a very rapid (\(10^4\) s\(^{-1}\) at 40 °C)\textsuperscript{[40]} and irreversible fragmentation of the BF-derived Breslow intermediate, which results from C2α deprotonation of HBnTh or decarboxylation of MTh (Scheme 1.16)
Scheme 1.16. Decarboxylation of MTh or deprotonation of HBnTh yields a Breslow intermediate that undergoes a rapid fragmentation.

It was proposed that the Oka fragmentation could be either a stepwise process (Scheme 1.17),\cite{41} or a pericyclic reaction (Scheme 1.18).\cite{42} With reports of radicals in reactions involving the Breslow intermediate,\cite{43} McIntosh and co-workers proposed a radical based mechanism for reactions similar to the Oka fragmentation (Scheme 1.21).\cite{44} Other mechanisms were considered to be unlikely. For example, a stepwise carbocation mechanism, which is based on Zoltekwicz’s mechanism for thiamin fragmentation by sulfite ion,\cite{45} was ruled out based on lack of trapping of the carbocation intermediate by azide ions (Scheme 1.19).\cite{46} An intramolecular hydride shift in HBnTh was excluded by observation of an external (from solvent) proton abstraction as evidenced from a single deuterium incorporation in DMAPs methyl group (Scheme 1.20).\cite{47}

None of the proposed mechanisms seem to be correct in detail, for example, breaking of the C–N bond (Scheme 1.17) is unlikely to be concerted with a proton transfer due to electronic and structural constraints. The Breslow intermediate is also short of a π-orbital needed for a pericyclic reaction (Scheme 1.18). The renewed interest in the properties of Breslow intermediates makes the mechanism of the Oka fragmentation an interesting contemporary challenge despite recent claims.\cite{22-23}

Fragmentation of the BF-derived Breslow intermediate is two orders of magnitude faster than BFDCs $k_{cat}$. Yet, the equivalent intermediate on BFDC does not undergo any fragmentation. The enzyme could be avoiding fragmentation because of a fast protonation with a rate constant of likely
at least $10^6$ s$^{-1}$. It is also possible that the enzyme enforces a conformation of the Breslow intermediate from which the cofactor cannot fragment. However, without being able to consider the likely mechanism for the fragmentation, it is impossible to know how it can be resisted.

Scheme 1.17. A previously proposed mechanism for fragmentation of the Breslow intermediate where a H$^+$ addition to C is concerted with C–N cleavage.


Scheme 1.19. Hypothetical mechanism for fragmentation of the Breslow intermediate leading to a carbocation intermediate.
Scheme 1.20. In D$_2$O, the Breslow intermediate fragments to yield DMAP which has a single deuterium in its 5-methyl group.

Scheme 1.21. Radical decomposition of the Breslow intermediate proposed by McIntosh and co-workers.

1.10 Inhibition by bromide elimination

Kenyon and co-workers discovered that $p$-(bromomethyl)benzoylformate ($p$-BrCH$_2$BF) is a potent inhibitor of BFDC.$^{[48]}$ On the enzyme, $p$-BrCH$_2$BF forms the expected adduct with ThDP which decarboxylates to the Breslow intermediate. Instead of protonation, the Breslow intermediate
eliminates bromide to form a xylylene derivative that tautomerizes to 2-(p-toluyl)ThDP, which is unreactive until it becomes hydrolyzed to p-toluic acid and ThDP (Scheme 1.22).

Scheme 1.22. The p-BrCH₂BF-derived Breslow intermediate eliminates bromide to form a xylylene species that tautomerizes to a ketone. Interestingly, p-(chloromethyl)benzoylformate (p-ClCH₂BF) behaves mostly as a normal substrate, with only a minimal amount of chloride elimination. The fluoro analogue (p-FCH₂BF) is processed by BFDC without any elimination. This is consistent with conventional trends for halides as nucleofuges. The bromide elimination step must be faster than the protonation of the Breslow intermediate because the protonation product (p-BrCH₂HBnTh) is not formed. The proposed mechanism for this inhibition with its intermediates has never been validated.

1.11 Localized or delocalized charge?

The neutralization of the C2α negative charge by the thiazolium acting as an electron sink is the logical consequence of avoiding a localized and thus a high energy carbanion. X-ray diffraction images of several ThDP-dependent enzymes show that the protein-bound Breslow intermediates have a trigonal planar geometry at C2α.[49] However, enzyme bound Breslow intermediates which have a tetrahedral C2α have been recently reported based on high resolution X-ray crystallography.[50] Large substituent effects on $k_{cat}$ for the decarboxylation of various para-substituted BFs by indolepyruvate decarboxylase (IPDC) have been also reported. This is consistent with formation of a localized carbanion-like Breslow intermediate.[51] Distorted bond
angles and bond lengths in intermediates bound to ThDP-dependent transketolases have been observed, suggesting GSD. The carbanion-like Breslow intermediates seem to be an extension of this.\textsuperscript{[52]} Ground state destabilization of the Breslow intermediate can provide a kinetic advantage if this leads to an optimized energy profile. The details of such a process are outlined by Albery and Knowles.\textsuperscript{[53]}

1.12 Purpose and scope

Research that I present in this thesis was inspired by Howe’s unexpected observation of general base catalyzed decarboxylation of MTh. It suggested that a simple mechanism required a proton transfer with C–C bond-cleavage in a rate-determining step. The proposed hydrolytic decarboxylation through formation of HCO\textsuperscript{3–} accounts for this. This was a unique case and I sought to understand the structure-reactivity relation behind it.

The problematic synthesis of MTh (very low yield and unreliable purification) led me to develop an improved approach. I discovered that lithium ions promote product formation. Together with improved reaction conditions (including a different solvent, base and temperature), and standard functional group protecting chemistry resulted in efficient formation of MTh. The product was successfully purified by flash chromatography on a sodium bromide treated silica stationary phase. The obtained product was pure by \textsuperscript{1}H NMR and available on a gram scale. Both the synthesis and purification were well-suited for preparation of various derivatives of MTh that were crucial to my research. If it was not for discovering the unique effect of lithium ion on MTh formation, most of the work presented in this thesis would not have been possible.

One of the first things I did, was to probe the decarboxylation reaction of MTh by conducting a Hammett study with various phenyl-substituted MTh derivatives. This provided an insight into the nature of the transition state for decarboxylation and negative charge distribution in the resulting Breslow intermediate. Interestingly, an analogous enzymic Hammett study was previously reported, providing me with a unique opportunity to compare the nature of the enzyme-bound Breslow intermediate and its accurate model in solution phase. Similarly, the halide elimination reaction observed on BFDC was performed off the enzyme by using accurate models.

I later designed and synthesized variants of MTh that possess structural features that were believed to be responsible for the base catalyzed decarboxylation of MTh. I also investigated decarboxylation of NMPA in aqueous buffers to test for evidence for a hydrolytic mechanism.
(SKIE, base catalysis). The decarboxylation of NMPA by departure of CO$_2$ was previously calculated to be highly reversible, making NMPA a logical candidate for a decarboxylation through formation of HCO$_3^-$ or H$_2$CO$_3$.

During my investigations, I noticed that MTh does not cleanly decarboxylate to HBnTh, even under acidic conditions. Careful analysis of the product mixture, revealed a rather complex pattern of reactivity of the BF-derived Breslow intermediate. I was able to begin to elucidate structures and propose mechanisms for formation of the different products.

Finally, I investigated the fragmentation of the BF-derived Breslow intermediate and tested for the potential involvement of radicals. I examined the proposal from McIntosh and co-workers that Breslow intermediates undergo spontaneous decomposition to a radical pair that can disproportionate to the fragmentation products. Based on uncertainties that arose from that analysis, I investigated the experimental evidence for radical formation and then considered alternatives.

### 1.13 References


Chapter 2
Lithium-Promoted Synthesis of Mandelylthiamin and Derivatives


*(see Appendix B for supporting information)*

2.1 Overview

Mandelylthiamin is an accurate model for the pre-decarboxylation intermediate in catalysis by BFDC (Figure 2.1).\(^1\) Comparison of reactivity of enzyme-bound with enzyme-free intermediates defines the acceleration provided by the enzyme.

![Mandelylthiamin and enzymic intermediate](image)

**Figure 2.1.** The pre-decarboxylation intermediate found on BFDC (A) and MTh (B).

The procedure\(^{1a}\) that was used to successfully make lactylthiamin (LTh), a related thiamin:α-ketoacid adduct, failed to yield MTh. In that method, sodium ethoxide deprotonates thiamin to the corresponding carbene nucleophile, which then adds to the carbonyl group of ethyl pyruvate. The alkoxide intermediate is quenched with acid to provide the ethyl ester of LTh. After purification by recrystallization it is hydrolyzed to LTh in concentrated hydrochloric acid (Scheme 2.1). Substitution of ethyl pyruvate with ethyl benzoylformate into this scheme does not produce the corresponding thiamin conjugate.
Qingyan Hu discovered that addition of a magnesium salt to the condensation reaction produces a very small (few % conversion) but isolable amount of the MTh ester. She assumed that Mg$^{2+}$ acts as a Lewis acid catalyst that activates the carbonyl group of ethyl benzoylformate toward condensation with the thiamin carbene. The product required a problematic purification on a cellulose column. From my experience, this purification method was very unreliable, rarely providing pure product. Nevertheless, MTh from that route was sufficient for studies on the reactivity of MTh.

I developed a procedure to make MTh in high yield (70-90% conversion based on thiamin). The purification of MTh, however, remained a problem. Fortunately, I came across a paper devoted to purification of pyridinium salts by silica chromatography that revealed the value of treating silica with sodium bromide. This dramatically increases retention factors and minimizes streaking of cationic organic molecules on silica. Such column purified MTh has > 98% purity by $^1$H NMR. Importantly, both the synthesis and column purification turned out to be versatile in producing adducts from the condensation of thiazolium derivatives with various α-ketoacid esters. This became a standard procedure for making such compounds in our laboratory.

The success of the new synthesis is based on the presence of lithium ions. I determined that the addition of the thiamin-derived carbene to ethyl benzoylformate is an energetically uphill process and the low yield is a result of an unfavorable equilibrium. This became evident by observing that the amount of product does not change after few minutes from the moment the reaction is initiated, even though both starting materials were present. I proposed that the alkoxide intermediate forms a complex with Li$^+$ that is more stable than the complex with Na$^+$, shifting the equilibrium towards
the product (Scheme 2.2). The small radius and large charge density of the lithium ion (hard Lewis acid) is likely responsible for a strong bidentate complexation to the α-ketoalkoxide group (hard Lewis base). The lack of product formation when the same reaction was performed in the presence of a lithium-complexing crown ether, shows that this is a specific effect of lithium ions. Substitution of the 2,5-dimethyl-4-aminopyrimidine (AP) ring in thiamin did not alter the reactivity pattern, indicating that lithium is involved with the mandelylthiazolium moiety. This is consistent with lithium-alkoxide complexing. It is important to stress that the success of this method is also a result of applying protecting-group chemistry (tetrahydropyran, THP), using an aprotic solvent (dichloromethane) and a strong, non-nucleophilic base (lithium bis(trimethylsilyl)amid, LiHMDS).

![Scheme 2.2](image)

**Scheme 2.2.** The thiamin carbene in equilibrium with C2α hydroxy conjugate base of MTh (alkoxide).

### 2.2 Further developments

Protecting the hydroxyl with a *tert*-butyldiphenylsilyl (TBDPS) group instead of the THP, provides additional advantages. The large size and low polarity of TBDPS results in a protected MTh product that is not soluble in water but was soluble in organic solvents, such as dichloromethane. The MTh product that comes out of the column, although free of organic contaminants, may contain some inorganic salts and bromine, which forms on the column. With a TBDPS-protected MTh ester product, both inorganic salts and bromine can be completely removed by washing the dichloromethane layer with brine that contains a small amount of sodium thiosulphate. This reduces bromine to bromide. The resulting product is then free of inorganic salts and bromine. TBDPS also makes the product move faster on silica and with less streaking.
2.2.1 Experimental

All reagents were obtained from commercial sources and used without further purification. $^1$H NMR spectra are reference to the residual solvent peaks, except for MTh in 20% DCl which was referenced to 3-(trimethylsilyl)propionic-2,2,3,3-d$_4$ acid as standard (0 ppm).

2.2.1.1 O-TBDPS-protected thiamin hydrochloride

Thiamin hydrochloride hydrate was dehydrated by heating under vacuum at 135 °C for 4–5 h. Anhydrous thiamin hydrochloride (10.0 g, 29.6 mmol) and tert-butyl(chloro)diphenylsilane (9.8 g, 35.6 mmol) were combined in dichloromethane. The mixture was cooled in an ice bath, and imidazole (13.7 g, 201 mmol) was slowly added with stirring. The reaction mixture was warmed to room temperature and stirred for 1 h. The solvent was removed by roto-evaporation and the residue was treated with a 1:3 water:methanol mixture containing 3.00 mL of 36% hydrochloric acid. This was gently extracted three times with hexanes. The aqueous/methanol layer was separated and reduced by roto-evaporation to obtain a white thick slurry. The slurry was treated with brine and gently extracted three times with dichloromethane. The organic layer was dried with anhydrous magnesium sulfate, filtered and roto-evaporated to obtain an off-white solid (15 g, 96% yield). $^1$H NMR (600 MHz, MeOD) δ 8.14 (s, 1H), 7.64 (d, J = 6.7 Hz, 4H), 7.49 – 7.44 (m, 2H), 7.42 (t, J = 7.2 Hz, 4H), 5.43 (s, 2H), 3.97 (t, J = 5.5 Hz, 2H), 3.21 (t, J = 5.5 Hz, 2H), 2.55 (s, 3H), 2.49 (s, 3H), 1.07 (s, 9H). MS(ESI+) m/z: M$^+$ Calcd for C$_{27}$H$_{33}$N$_4$OSSi$^+$ 503.2 Found: 503.2

2.2.1.2 O-TBDPS-protected MTh methyl ester
**O-TBDS-protected thiamin hydrochloride** (500 mg, 0.92 mmol), and methyl benzoylformate (1.18 g, 5.56 mmol) were combined in some dichloromethane. The reaction flask was cooled in a 35% ethanol/dry ice bath and purged with argon. LiHMDS (1.0 M toluene sol, 1.86 mL, 2.2 eq.) was slowly added. After 10 min, the reaction was quenched with acetic acid, followed by trifluoroacetic acid (532 µL). The reaction mixture was roto-evaporated, and the crude material purified by chromatography on silica which was pre-soaked in a concentrated sodium bromide methanol solution. A gradient elution was performed (2 to 15 v/v% methanol in ethyl acetate, with 0.02 v/v% trifluoroacetic acid). The product elutes before the O-TBDPS-protected thiamin starting material. The product fractions are combined and roto-evaporated. The orange residue was dissolved in dichloromethane and gently stirred with brine, containing a small amount of sodium thiosulfate. The organic layer gradually becomes almost colorless. In this step residual bromine, which inherently forms during elution, is reduced to bromide. The organic layer was separated, washed twice with brine, dried with anhydrous magnesium sulfate, filtered and roto-evaporated to obtain a light-yellow residue. $^1$H NMR (600 MHz, MeOD) δ 7.65 (d, J = 7.8 Hz, 4H), 7.57 (d, J = 9.2 Hz, 2H), 7.51 – 7.38 (m, 9H), 7.20 (s, 1H), 5.70 (d, J = 17.6 Hz, 1H), 5.50 (d, J = 17.4 Hz, 1H), 3.97 (t, J = 5.4 Hz, 2H), 3.91 (s, 3H), 3.21 (t, J = 5.4 Hz, 2H), 2.52 (s, 3H), 2.34 (s, 3H), 1.06 (s, 9H). MS(ESI+) m/z: M$^+$ Calcd for C$_{37}$H$_{43}$N$_4$O$_4$SSi$^+$ 667.3 Found: 667.3

### 2.2.1.3 MTh

*O-TBDS-protected MTh ester* was combined with 37% hydrochloric acid and stirred at room temperature for three days. The acidic mixture was washed three times with dichloromethane and the aqueous layer was concentrated to a syrup-like consistency by roto-evaporation and kept at -80 °C. $^1$H NMR (400 MHz, 20% DCl, TMSP-d$_4$) δ 7.59 – 7.54 (m, 2H), 7.35 – 7.26 (m, 3H), 6.86 (s, 1H), 5.82 (d, J = 19.5 Hz, 1H), 5.40 (d, J = 19.5 Hz, 1H), 3.98 (t, J = 5.7 Hz, 2H), 3.26 (t, J = 5.7 Hz, 2H), 2.54 (s, 3H), 2.41 (s3H). MS(ESI+) m/z: M$^+$ Calcd for C$_{20}$H$_{23}$N$_4$O$_4$S$^+$ 415.1 Found: 371.1 [M - CO$_2$]
2.3 References


Lithium-stabilized nucleophilic addition of thiamin to a ketone provides an efficient route to mandelylthiamin, a critical pre-decarboxylation intermediate

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ABSTRACT

Mandelylthiamin (MTH), an accurate model of the covalent intermediate derived from the condensation of thiamin diphosphate and benzoylformate in benzoylformate decarboxylation. The properties and catalytic susceptibilities of mandelylthiamin are the subjects of considerable interest. However, the existing synthesis gives only trace amounts of the precursor to MTH as it is conducted under reversible conditions. An improved approach derives from the unique ability of lithium ions to drive to completion the otherwise unfavorable condensation of the conjugate base of thiamin and methyl benzoylformate. The unique efficiency of the condensation reaction in the presence of lithium ions is established in contrast to the effects of other Lewis acids. Interpretation of the pattern of the results indicates that the condensation of the ketone and thiamin is thermodynamically controlled. It is proposed that the addition of lithium ions displaces the equilibrium toward the product through formation of a stable lithium-alkoxide.

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1. Introduction

Mandelylthiamin (1) accurately models the intrinsic nonenzymic reactivity of mandelylthiamin diphosphate, the protein-bound intermediate derived from thiamin diphosphate in reactions catalyzed by benzoylformate decarboxylase (BFD) [1–5]. The enzymatic turnover number (kcat) is about 104 times larger than the unimolecular rate constant for decarboxylation of 1 and the ratio for the decarboxylation step alone is probably considerably higher [6]. The mechanisms of catalysis of these reactions have generated considerable interest and have significant implications for our understanding of how decarboxylation can be dramatically accelerated [7–20]. However, the previous synthesis of 1 is extremely low-yielding (~7% yield before purification) and isolation of the pure ester precursor is difficult [12]. That procedure involves condensation of thiamin chloride hydrochloride (2) with ethyl benzoylformate (3) and magnesium chloride using sodium ethoxide to deprotonate 2 at C2 (Scheme 1; the acidic proton is shown explicitly on the thiazolium ring in 2) [6]. The subsequent

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an aprotic solvent using a strong, non-coordinating base (sodium bis(trimethylsilyl)amide (NaHMDS); pKa ~ 30) [21] to remove the proton at C-2 of thiamin (pKa ~ 17) [22]. This will also remove the proton from thiamin’s hydroxyl. Converting the hydroxyl to the tetrahydropyranyl (THP) ether (2a) removes this competition. After condensation, the THP group is readily cleaved with trifluoroacetic acid (TFA), producing the condensation product to a very large extent. Finally, the ester is readily hydrolyzed in a highly acidic solution, giving 1 in an overall high yield [6].

2. Experimental section

2.1. Materials

All materials were obtained from commercial suppliers and used without further purification. Solvents were dried overnight at 210 °C prior to use. All reactions were conducted under dry nitrogen.

2.2. Synthesis of O-THP-thiamin (2a)

2 (20 g, 47.5 mmol) and 3,4-dihydropyran (40 mL) were suspended in DMF (300 mL). The addition of p-toluenesulfonic acid monohydrate (4.8 g, 25.8 mmol) was followed by 6 h of stirring at room temperature. An additional portion of 3,4-dihydropyran (20 mL) was then added and the mixture was stirred overnight. The product was collected by filtration and thoroughly washed with diethyl ether. 18.5 g (72%) [23] of a white powder was obtained. 1H NMR (D2O with a small amount of KHCO3 to prevent hydrolysis) indicated 99% THP-protected product.

1H NMR (600 MHz, DMSO-d6) [24]: δ 10.09 (s, 1H), 9.23 (m, 1H), 8.38 (s, 1H), 5.73 (s, 2H), 4.65 (t, 1H, J = 3.3 Hz), 3.86–3.82 (m, 1H), 3.68–3.64 (m, 1H), 3.60–3.57 (m, 1H), 3.46–3.42 (m, 1H), 3.26–3.17 (m, 2H), 2.57 (s, 3H), 2.55 (s, 3H), 1.75–1.69 (m, 1H), 1.67–1.62 (m, 1H), 1.51–1.47 (m, 3H), 1.47–1.39 (m, 1H).

ESI-MS [C17H25N4O4S]⁺, calculated: 429.16, observed: 429.2.

2.3. Synthesis of mandelythiamin methyl ester (4a)

2a (2 g, 4.7 mmol) and benzoylformate methyl ester (3a; 5.46 g, 33.3 mmol) were suspended in 20 mL of dry dichloromethane (DCM). The reaction flask was purged with N2 and cooled to –20 °C in a 35% EtOH/water bath with dry ice. LHMDS (10.8 mL of a 1 M solution [25], 10.8 mmol) was added drop wise to the stirring mixture. After 20 min, the reaction was transferred to vigorously stirring trifluoroacetic acid (–50 °C) through a cannula. The acid and solvent was removed on a rotary evaporator to obtain a yellow oil. This material was dissolved in water and excess 3a was extracted with ether (3 × 50 mL). The aqueous phase was lyophilized to dryness. Approximately 70–80% of the starting material is converted to 4a (based on 1H NMR of crude material).

2.4. Purification of 4a

Purification of quaternary ammonium salts on silica is difficult because these compounds streak along the stationary phase. This problem does not occur with sodium bromide-impregnated silica [26]. A saturated solution of sodium bromide in methanol was passed through a column of silica gel. The silica was thoroughly dried with continued air flow. The column is then loaded with 5% methanol in DCM. The crude material was dry loaded by dissolving it in a small amount of NaBr-saturated methanol and adding silica. A mobile phase gradient (5–15% MeOH/DCM) was used to facilitate separation. The product elutes between two yellow colored bands with a slight overlap with the first yellow band (minor impurity). Rp = 0.25 (15% MeOH/DCM; NaBr-impregnated silica). The fractions containing the product were combined and acidified with a small amount of TFA. The solvent was removed by rotary evaporation to obtain an oily residue. This material contains NaBr which is removed by passing the product through a short silica column with 10% MeOH in DCM (0.1% TFA) [27]. The combined product fractions are treated with carbon until the product is pure by TLC [28]. The product is obtained as a mixed salt–bromide/trifluoroacetate. The counterions can be replaced with chloride by treating the product with 1.0 M HCl and subsequent evaporation to dryness. The isolated yield was approximately 50% (determined by 1H NMR using chloroacetic acid as an internal standard).

1H NMR (600 MHz, D2O): δ 7.53 (d, 2H, J = 12.0 Hz), 7.31 (t, 2H, J = 6.0 Hz), 7.25 (t, 1H, J = 6.0 Hz), 6.85 (s, 1H), 5.87 (b, 1H), 5.39 (d, 1H, J = 18.0 Hz), 3.98 (t, 2H, J = 6.0 Hz), 3.94 (s, 3H), 3.25 (t, 2H, J = 6.0 Hz), 2.52 (s, 3H), 2.43 (s, 3H).

13C NMR (100 MHz, D2O): δ 170.5, 169.9, 161.5, 160.7, 145.4, 137.7, 136.1, 135.1, 129.9, 129.7, 126.1, 117.3, 79.2, 60.1, 55.1, 47.4, 29.5, 20.7, 11.7.

ESI-MS [C17H25N4O4S]⁺, calculated: 429.16, observed: 429.2.

2.5. Synthesis of mandelythiamin ethyl ester (4b)

The synthetic procedure is analogous to those reported for 4a, substituting the ethyl ester of benzoylformate (3b) for 3a. Yields and purification protocol are unchanged by this substitution.

1H NMR (300 MHz, D2O): δ 7.51 (d, 2H, J = 12.0 Hz), 7.34–7.23 (m, 3H), 6.73 (s, 1H), 5.83 (d, 1H, J = 18.0 Hz), 5.36 (d, 1H, J = 18.0 Hz), 4.41 (q, 2H, J = 7.2 Hz), 3.93 (t, 2H, J = 6.0 Hz), 3.22 (t, 2H, J = 6.0 Hz), 2.47 (s, 3H), 2.38 (s, 3H), 1.28 (t, 3H, J = 7.2 Hz).
13C NMR (400 MHz, D2O): δ 170.3, 169.5, 161.7, 160.7, 145.3, 138.3, 136.0, 135.0, 129.8, 129.6, 125.9, 107.3, 78.9, 65.3, 60.0, 47.3, 29.3, 20.6, 13.00, 11.3.
ESI-MS [C22H27N4O4S]+ calculated: 443.17; observed: 443.2.

2.6. Synthesis of mandelythlamin tert-butylic ester (4c)

t-Butyl benzoylformate (3c) was prepared by the method of Enders [29]. The synthetic procedure is analogous to those reported for 4a, substituting 3c for 3a. The reaction is quenched in acetic acid to avoid cleavage of the t-butylic group. This quench leaves the THP group intact as well. Also, excess ester is extracted with hexanes. Approximately 60–70% of the starting material is converted to 4c (based on 1H NMR of the crude material).

2.7. Purification of 4c

The protocol for purification is essentially unchanged with the following exceptions. Poor separation was observed when the THP group was not cleaved. To improve separation, the THP group was cleaved by stirring the crude material in 10% (v/v) TFA in methanol for 1 h. Potassium acetate was then added carefully to neutralize the excess TFA. The material was then dry loaded and purified as with 4a and 4b. The isolated yield is approximately 40%.

1H NMR (600 MHz, MeOD): δ 7.50 (d, 2H, J = 9.0 Hz), 7.37 (t, 2H, J = 7.3 Hz), 7.32 (t, 1H, J = 7.3 Hz), 7.20 (s, 1H), 5.84 (d, 1H, J = 17.4 Hz), 5.40 (d, 1H, J = 17.4 Hz), 3.93 (m, 2H), 3.22 (d, 2H, J = 6.3 Hz), 2.51 (s, 3H), 2.44 (s, 3H), 1.51 (s, 9H).

13C NMR (150 MHz, MeOD): δ 171.2, 168.2, 161.5, 160.9, 144.4, 138.8, 136.6, 129.3, 129.1, 126.0, 107.3, 86.2, 79.0, 59.7, 47.5, 29.8, 26.5, 20.1, 11.3.
ESI-MS [C24H31N4O4S]+ calculated: 471.21; observed: 471.2.

2.8. Synthesis of N-methyl-3-(bromomethyl)pyridinium triflate (5)

3-(bromomethyl)pyridine was prepared from 3-pyrdlycarbinol and concentrated hydrobromic acid as previously described [30]. This material was directly taken forward to the methylation reaction. This involved treating 3-(bromomethyl)pyridine (3.44 g, 20 mmol) with one equivalent of methyl triflate in ether. After stirring for 30 min at room temperature, the product was extracted into water and washed with additional ether. The water is removed by lyophilization to give the product as a yellow oil (6.05 g, 90%).

1H NMR (400 MHz, D2O): δ 8.96 (s, 1H), 8.75 (d, J = 8.0 Hz, 1H), 8.62 (d, J = 8.0 Hz, 1H), 8.05 (app t, J = 6.0 Hz, 2H), 4.76 (s, 2H), 4.42 (s, 3H).

2.9. Synthesis of N-methyl pyridythiazolium bromide triflate (6)

5 (6.02 g, 17.9 mmol) and 4-methyl-5-thiazoleethanol (3.84 g, 26.9 mmol) were dissolved in acetonitrile (10 mL). After refluxing this mixture overnight, the solvent was removed by rotary evaporation. The product was dissolved in water (20 mL) and excess thiazole was removed by extraction into ether (3 × 50 mL). The aqueous layer was lyophilized to give a yellow semi-solid (6.63 g, 5%).

1H NMR (400 MHz, D2O): δ 9.98 (s, 1H), 8.93 (s, 1H), 8.89 (d, J = 6.0 Hz, 1H), 8.44 (d, J = 8.0 Hz, 1H), 8.13 (dd, J = 8.0 Hz, 6.0 Hz, 1H), 6.00 (s, 2H), 4.43 (s, 3H), 3.67 (t, J = 6.0 Hz, 2H), 3.17 (t, J = 6.0 Hz, 2H), 2.44 (s, 3H).

2.10. Synthesis of THP-protected N-methyl pyridythiazolium bromide triflate (7)

6 (4.40 g, 9.2 mmol) and 3,4-dihydropyran (8.35 mL, 91.5 mmol) were dissolved in acetonitrile (20 mL). p-toluenesulfonic acid monohydrate (17 mg, 0.1 mol%) was then added and the mixture was stirred for 24 h. After neutralizing with sodium bicarbonate, the solvent was removed by rotary evaporation. The crude material was dissolved in water (20 mL) and excess dihydropyran was extracted with diethyl ether (3 × 30 mL). Lyophilization of the aqueous layer gave an orange solid (~4.9 g, ~95%) [31].

1H NMR (400 MHz, D2O): δ 8.97 (s, 1H), 8.93 (d, J = 6.0 Hz, 1H), 8.45 (d, J = 8.4 Hz, 8.17 (app t, J = 7.2 Hz, 1H), 6.03 (s, 2H), 4.47–4.42 (m, 4H), 4.09–4.02 (m, 1H), 3.87–3.79 (m, 2H), 3.30–3.26 (m, 2H), 2.48 (s, 3H), 1.83–1.56 (m, 6H).

2.11. Preparation of N-methyl pyrdylmandelythlamin methyl ester (8)

The synthesis is analogous to that reported for 4a. Use of 7 in place of 2a does not significantly alter the reaction yield (95% conversion and approximately 70% isolated yield) or purification protocol.

1H NMR (300 MHz, D2O): δ 8.59 (d, J = 6 Hz, 1H), 8.20 (s, 1H), 7.85–7.77 (m, 2H), 7.43 (app d, J = 6 Hz, 7.27–7.19 (m, 3H), 6.05 (d, J = 18 Hz, 1H), 5.81 (d, J = 18 Hz, 1H), 4.21 (s, 3H), 3.86–3.83 (m, 5H), 3.14 (t, J = 5.7 Hz, 2H).

3. Results

As a control, we carried out the condensation of 2a with 3 using NaNHMS in aprotic solvents without added counter-ions. As expected, there is only a low yield of the condensation product (Fig. 1A). The condensation of 2a with 3 was then carried out with added Lewis acids. Aside from the identity of the salts, all reactions
were run under identical conditions. The addition of AlCl₃, BF₃-THF, ZnCl₂, TiCl₄, SnCl₄ and Mg(OiPr)₂ had no effect on the yield. However, a clearly positive result was obtained only with the addition of lithium chloride, where the yield increases to 30%. We then performed the condensation using potassium, sodium and lithium HMDS bases with no additional salts in the reaction mixture. While only minimal conversion to the desired product was observed when NaHMDS or KHMDS were employed (∼4–7%), approximately 70% conversion occurs when LiHMDS is used (Fig. 1B). These results indicate that lithium ions uniquely drive the condensation.

We also tested if this result is from a specific interaction of lithium ions with a component of the reaction or if it is a nonspecific medium effect by testing LiHMDS in the presence of crown ethers that ligate the cation. When the condensation is carried out in the presence of 15-crown-5 or 12-crown-4 ether, there is almost no formation of 4a (∼2% conversion). This suggests a specific stabilizing interaction between the lithium ion and the alkoxy of the condensed product.

In order to understand the basis of the effect of lithium, the condensation of 2a with methyl benzoylformate (3a) [32] was carried out using NaHMDS in the presence and absence of lithium salts. The yields were determined as a function of time. Similar time-course studies were performed using magnesium triflate. The yield does not change over the course of the measurements (Fig. 2). We have also examined the condensation with increasing concentrations of lithium triflate and observed an approximately linear relation between the yield and the equivalents of LiOTf (Fig. 3). Increasing concentrations of 3a also give higher yields. However, the yield is lower where more than 10 equivalents of 3a are added (Figure S1, S1).

Compound 7 was synthesized to test if the result is specific for thiamin. Negligible product formation was observed when a mixture of 7 and 3a was treated with NaHMDS (Fig. 4A). Utilizing four equivalents of lithium triflate gave an impressive 98% conversion (Fig. 4B). Thus, the effect of lithium ions is not limited to the condensation of 2a with 1,2-ketoesters.

4. Discussion

Addition of metal-ion Lewis acids in general, including AlCl₃, BF₃-THF, ZnCl₂, TiCl₄, SnCl₄ and Mg(OiPr)₂ does not increase the yield of the condensation to produce an ester precursor of mandel-lithiamin. The unique ability of a lithium salt to drive the equilibrium toward the product is notable as lithium is not a particularly powerful Lewis acid [33,34]. This is as expected if it is not involved in lowering the energy of the transition state of the addition process itself but is involved in stabilizing the product. To rule out a non-specific medium effect caused by addition of the lithium salt, we performed the condensation reaction with the lithium salt in the presence of 15-crown-5 and 12-crown-4 ethers. Both have high
affinity for Li⁺. The results illustrate that product formation is highly suppressed upon addition of the crown ethers under conditions that are otherwise effective. Therefore, the increased yield is caused specifically by interaction of lithium with the tertiary alkoxide.

The time course experiments show that equilibrium with the product is established within minutes whether or not lithium is added. This establishes that the amount of product that is available is thermodynamically controlled. Consistent with a system at equilibrium, increasing the amounts of lithium and 3a increases the amount of available product. This levels off at the greatest concentrations of lithium triflate, limited only by its solubility and eventual competing complexation from weaker complexes. The combination of 7, 2a and NaHMDMS does not lead to formation of the product. However, addition of lithium triflate to the reaction mixture results in almost complete conversion. These observations lead us to propose that the lithium promoted addition of a carbene to a 1,2-ketoester is a generalizable phenomenon.

We have shown that in the absence of lithium ions, the condensation proceeds through reversible formation of 4 and that the equilibrium is significantly shifted toward products in the presence of those ions. While lithium stabilizes the desired addition product, this cannot be the result of ion-pair formation as stronger Lewis acids have no effect on the position of the equilibrium. It is possible that this reflects the ability of lithium ions to form stable aggregates with alkoxides (Scheme 3). We have also confirmed that this effect is not limited to the stabilization of 4 using the pyridine-derived analogue of thiamin (8).

As such, the resulting gain in translational entropy upon elimination of thiamin diphosphate is much less significant than for the corresponding nonenzymic process. Furthermore, protonation of the alkoxide on the enzyme will be endergonic, contributing to the stability of the conjugate.

5. Conclusions

The effective synthesis of the key compound, mandelithiamin (1), is based on our improved strategy for the addition of a heterocyclic carbene to a ketone, utilizing the effect of lithium ion in stabilizing the alkoxide from the addition of thiamin to ethyl benzoylformate. Our new procedure has improved the product yield 10-fold in comparison to the previous method. Moreover, we have provided a simple and reliable purification protocol leading to very pure products and good isolated yields. This method should extend beyond the synthesis of esters of 1. The availability of the highly crowded conjugates of thiamin permits extended studies of rates and the further development of bio-organic catalytic systems. The unusual reactivity patterns of 1 has also attracted applications of computational chemistry [8,10,15,16]. The availability of sufficient quantities of the material can lead to tests of predictions of its reactivity patterns.

Acknowledgment

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bioorg.2015.08.004.

References

[23] More product can be precipitated from the filtrate with the addition of ether. However, this precipitate is the p-toluene sulfonate salt of 2a.
[24] 1H NMR spectra can be obtained in D2O if a small amount of potassium bicarbonate is added to prevent hydrolysis of the protecting group.
[25] This base is available as a solution in THF and toluene. We obtained no significant differences in yield with either solution.
[27] An alternative is to treat the product with a small amount of isopropanol which will dissolve the product and leave behind most of the sodium bromide as a precipitate.
[28] TLC plates must also be treated with sodium bromide to prevent streaking.
[31] This material is extremely hygroscopic. As such, the reported yield of this reaction is less accurate than the other syntheses.
[32] The methyl and ethyl esters of benzoylformate are interchangeable. In all cases examined, results with both esters were identical. The methyl ester was eventually employed due to the relative cost and availability of these compounds.
Chapter 3
Enzymes Likely Distort the Breslow Intermediate:
A Hammett Study

3.1 Introduction

The function of ThDP as a cofactor in enzyme-catalyzed decarboxylation of α-ketoacids was an unsolved but tantalizing problem until 1956 when Breslow demonstrated the relatively rapid exchange of the C2 proton in the thiazolium moiety.[1] This led Breslow to conclude that addition of the thiazolium’s conjugate base, a nucleophilic carbene, to the carbonyl of the α-ketoacid would readily promote departure of CO$_2$.[2] The carbanion that develops upon loss of CO$_2$ would be internally neutralized directly by thiazolium in the decarboxylation process to form a neutral 1,2-enaminol. This species is now known as the Breslow intermediate, although the demonstration of formation of the thiamin-derived carbene was the original evidence that led to the proposed mechanism (Scheme 3.1).

![Scheme 3.1](image)

Scheme 3.1. The thiazolium of ThDP dissociates a proton at C2 followed by addition to the α-ketoacid and decarboxylation.

The neutralization of the negative charge through the thiazolium ring allows the C–C bond to break to release CO$_2$. Studies of compounds related to the Breslow intermediate, including UV-VIS spectroscopy, $^{13}$C NMR and X-ray crystallography, are consistent with the expected 1,2-enaminol resulting from the shift of electron density into the thiazolium ring.[3] [4] [5] [6] [7] Reports of X-ray crystal structures of ThDP-dependent intermediates show the expected trigonal planar geometry at the C2α position of the Breslow intermediate.[8] However, important contrasting X-ray crystallography images of enzyme-bound Breslow intermediates revealed a carbanion-like Breslow intermediate with a tetrahedral C2α.[9] Large substituent effects on $k_{cat}$ for the decarboxylation of various para-substituted benzoylformates by IPDC are also consistent with formation of a carbanion-like Breslow intermediate and not the 1,2-enaminol.[10]
Hu et al. studied the nonenzymic decarboxylation of MTh. They observed that the acid component of pyridine buffers catalyze the decarboxylation of MTh.\textsuperscript{[11]} The authors concluded that to have catalysis by protonated pyridine, proton transfer must be involved in the decarboxylation process. In order to accommodate the catalysis, the authors had to consider a reversible C–C bond-breaking step. In such a scenario, the Brønsted acid could intercept the incipient carbanion and trap it, preventing recombination of the CO$_2$:R$^-$ pair. Pyridinium would form a complex with MTh prior to C–C bond cleavage, functioning as a pre-associated proton source. The interaction of π systems would facilitate the pre-association of the protonated pyridine and reduce the extent of the combination of CO$_2$ with the carbanion, resulting in a greater reaction flux.

For the C–C bond-cleavage step to be reversible, the rate of addition of the Breslow intermediate to CO$_2$ must be competitive with that for diffusional separation ($k_{\text{diff}} \sim 10^{10-11} \text{ s}^{-1}$). Such a fast recombination would occur if a localized carbanion is produced. This was shown in computational analyses of the decarboxylations of orotic acid, NMPA\textsuperscript{[12]} and TCA.\textsuperscript{[13]} Therefore, the observation of catalysis by pyridinium ion suggested formation of localized carbanion-like Breslow intermediate.\textsuperscript{[14]}

Although there is overwhelming evidence that some synthetic analogues of Breslow intermediates are properly depicted as neutral 1,2-enaminols, this does not seem to apply to actual enzymic intermediates as seen in the high-resolution X-ray structures. It is, however, not clear if the enzyme enforces such a conformation or if it is a natural state of thiamin-derived Breslow intermediates. Based on the powerful electron-withdrawing effect of a thiazolium substituent, it is possible the localized carbanion-like form of the Breslow intermediate might be particularly stable.

In this chapter, I present a study of the rates of spontaneous decarboxylation of phenyl-substituted derivatives of MTh. The results provide the basis for an important Hammett plot. The $\rho$ value indicates the nature of the transition state for decarboxylation in terms of carbanionic character and contrast sharply with the enzyme catalyzed reaction.\textsuperscript{[15]} Comparison of the Breslow intermediate in solution with its enzyme-bound counterpart provides a basis for understanding how the enzyme has evolved to become more efficient through raising the local energy of the intermediate.
3.2 Experimental

3.2.1 Synthesis

3.2.1.1 General considerations

All reagents and starting compounds were purchased from commercial sources. The substituted derivatives of MTh were synthesized using the method I developed for MTh via O-TBDS-protected thiamin and para- and meta-substituted benzoylformate esters (see Chapter 2).\footnote{16} Commercially available acetophenones were oxidized to the corresponding benzoylformic acids based on a reported procedure.\footnote{17} Methyl benzoylformates were prepared by esterification with dimethyl sulfate. The acid catalyzed reaction of carboxylic acids with isobutylene was used to produce tert-butyl esters. The various synthetic steps are shown in Scheme 2. $^1$H NMR spectra were referenced to the residual solvent peak (CH$_3$OH) or in case of samples in 20% DCl, the spectra were referenced to the AP C1' proton peak, which shift was assumed to be identical to the one in MTh (6.86 ppm). The ester MTh products are obtained likely as mixed salts (bromide/CF$_3$COO$^-$). The final MTh products are mostly chloride salts after reducing the hydrochloric acid solutions.

Scheme 3.2. Synthetic steps leading to formation of MTh and its $p$- and $m$-derivatives. Reagents/Conditions: (a) i. SeO$_2$ 2 eq, pyridine, reflux, overnight; ii. extraction (b) i. Me$_2$SO$_4$ 1.5 eq, K$_2$CO$_3$ 2 eq, DMSO, 25 °C, 20 min; ii. extraction (c) i. isobutylene in DCM, H$_2$SO$_4$ cat, 25 °C, 24 h ii. NH$_3$ quench iii. extraction (d) i. O-TBDP-thiamin chloride 0.20 eq, 1.0 M LiHMDS in
toluene 0.3 eq, DCM, -20 °C, 5 min; ii. AcOH+TFA quench; iii. NaBr-impregnated silica flash chromatography (d) i. 36% HCl, 25 °C, 1-4 days; ii. DCM wash.

3.2.1.2 General procedure for preparation of methyl benzoylformates
Benzoylfomic acid (5.00 mmol) was combined with potassium carbonate (1.00 g, 1.70 mmol) in 2.0 mL of dimethylsulphoxide. Dimethyl sulfate (0.57 mL, 6.00 mmol) was added drop-wise. After stirring at room temperature for 20 min, the mixture was transferred to a separatory funnel, combined with ether, and washed three times with dilute potassium carbonate solution and once with brine. The organic layer was dried with anhydrous magnesium sulfate, filtered, and roto-evaporated to dryness. The process gives the various products in 40–90% yield.

3.2.1.3 General procedure for preparation tert-butyl benzoylformates
Benzoylfomic acid (5.00 mmol) was combined with dichloromethane (3 mL) in a glass pressure bottle equipped with a magnetic stirring bar, and cooled in an acetone/dry ice bath. Sulfuric acid was added (28 µL) followed by liquid isobutylene (2-3 mL) and the bottle was sealed off. The reaction mixture was stirred at room temperature for 12 h. An excess of concentrated ammonia was added to neutralize the acid and the mixture was roto-evaporated to remove dichloromethane and unreacted isobutylene. Ether was added and washed three times with brine containing potassium carbonate. The ether layer was dried with anhydrous magnesium sulfate, filtered and roto-evaporated to obtain the tert-butyl ester (40–60% yield).

3.2.1.4 p-CH₃O-MTh tert-butyl ester

\[ \begin{align*}
\text{\textsuperscript{1}H NMR (300 MHz, CD}_3\text{OD)} & \delta 7.65 (d, J = 7.7 \text{ Hz}, 4H), 7.56 – 7.34 (m, 8H), 7.10 (s, 1H), 6.86 (d, J = 9.0 \text{ Hz}, 0H), 5.72 (d, J = 17.7 \text{ Hz}, 1H), 5.47 (d, J = 17.7 \text{ Hz}, 1H), 3.97 (t, J = 5.4 \text{ Hz}, 2H),
\end{align*} \]
3.77 (s, 3H), 3.20 (t, \( J = 5.3 \) Hz, 2H), 2.51 (s, 3H), 2.32 (s, 3H), 1.48 (s, 9H), 1.07 (s, 9H). MS(ESI+) m/z: M⁺ Calcd for C_{41}H_{51}N_{4}O_{5}SSi⁺ 739.3 Found: 739.3

3.2.1.5 \( p \)-CH₃O-MTh

![Molecule Structure]

\(^1\)H NMR (300 MHz, 20% DCl) δ 7.52 (d, \( J = 8.9 \) Hz, 2H), 6.88 (apparent d, \( J = 8.9 \) Hz, 3H), 5.78 (d, \( J = 17.9 \) Hz, 1H), 5.43 (d, \( J = 17.9 \) Hz, 1H), 4.1 (t, \( J = 6.0 \) Hz, 2H), 3.81 (s, 3H), 3.29 (t, \( J = 6.0 \) Hz, 2H), 2.58 (s, 3H), 2.45 (s, 3H), MS(ESI+) m/z: M⁺ Calcd for C_{21}H_{25}N_{4}O_{5}S⁺ 445.15 Found: 401.2 [M - CO₂]

3.2.1.6 \( m \)-CH₃O-MTh methyl ester

![Molecule Structure]

\(^1\)H NMR (400 MHz, CD_{3}OD) δ 7.68 – 7.63 (m, 4H), 7.52 – 7.41 (m, 6H), 7.26 (t, \( J = 8.1 \) Hz, 1H), 7.13 (d, \( J = 2.0 \) Hz, 1H), 7.12 (s, 1H), 7.01 (ddd, \( J = 7.8, 1.8, 0.8 \) Hz, 1H), 6.87 (ddd, \( J = 8.3, 2.5, 0.8 \) Hz, 1H), 5.74 (d, \( J = 17.8 \) Hz, 1H), 5.46 (d, \( J = 17.8 \) Hz, 1H), 3.97 (t, \( J = 5.5 \) Hz, 2H), 3.91 (s, 3H), 3.78 (s, 3H), 3.21 (t, \( J = 5.4 \) Hz, 2H), 2.52 (s, 3H), 2.33 (s, 3H), 1.07 (s, 9H). MS(ESI+) m/z: M⁺ Calcd for C_{38}H_{45}N_{4}O_{5}SSi⁺ 697.3 Found: 679.3
3.2.1.7 \textit{m-CH}_3\text{O-MTh}

\begin{center}
\includegraphics[width=0.2\textwidth]{m-CH3O-MTh.png}
\end{center}

\[^1\text{H}\text{ NMR}\ (400\text{ MHz, 20}\%\text{ DCl})\ \delta\ 6.97\ (t,\ J = 8.0\text{ Hz, 1H}),\ 6.88\ (s,\ 1H),\ 6.86\ (s,\ 1H),\ 6.77\ (d,\ J = 7.4\text{ Hz, 1H}),\ 6.54\ (d,\ J = 10.2\text{ Hz, 1H}),\ 5.57\ (d,\ J = 17.1\text{ Hz, 1H}),\ 5.16\ (d,\ J = 17.1\text{ Hz, 1H}),\ 3.70\ (t,\ J = 5.4\text{ Hz, 2H}),\ 3.49\ (s,\ 3H),\ 2.96\ (t,\ J = 5.4\text{ Hz, 2H}),\ 2.43\ (s,\ 3H),\ 2.12\ (s,\ 3H).\ \text{MS(ESI+)}\ m/z: M^+\ \text{Calcd for C}_{21}H_{25}N_4O_5S^+\ 445.15\ \text{Found:}\ 401.2\ [M - \text{CO}_2]\]

3.2.1.8 \textit{p-CH}_3\text{-MTh methyl ester}

\begin{center}
\includegraphics[width=0.2\textwidth]{p-CH3-MTh.png}
\end{center}

\[^1\text{H}\text{ NMR}\ (300\text{ MHz, CD}_3\text{OD})\ \delta\ 7.69 - 7.61\ (m,\ 4H),\ 7.51 - 7.38\ (m,\ 8H),\ 7.23 - 7.15\ (m,\ 3H),\ 5.68\ (d,\ J = 17.4\text{ Hz, 1H}),\ 5.50\ (d,\ J = 17.4\text{ Hz, 1H}),\ 3.97\ (t,\ J = 5.4\text{ Hz, 2H}),\ 3.91\ (s,\ 3H),\ 3.21\ (t,\ J = 5.4\text{ Hz, 2H}),\ 2.54\ (s,\ 3H),\ 2.34\ (s,\ 3H),\ 2.28\ (s,\ 3H),\ 1.05\ (s,\ 9H).\ \text{MS(ESI+)}\ m/z: M^+\ \text{Calcd for C}_{38}H_{45}N_4O_4S\text{Si}^+\ 681.3\ \text{Found:}\ 681.3\]

3.2.1.9 $p$-CH$_3$-MTh

![Chemical Structure of $p$-CH$_3$-MTh](image)

$^1$H NMR (300 MHz, 20% DCl) $\delta$ 7.47 (d, $J = 8.2$ Hz, overlap with H$_2$O peak), 7.18 (d, 2H), 6.86 (s, 1H), 5.83 (d, $J = 18.4$ Hz, 1H), 5.44 (d, $J = 18.4$ Hz, 1H), 4.03 (t, $J = 5.7$ Hz, 2H), 3.31 (t, $J = 5.7$ Hz, 2H), 2.62 (s, 1H), 2.46 (s, 1H), 2.25 (s, 1H). MS(ESI+) m/z: M$^+$ Calcd for C$_{21}$H$_{25}$N$_4$O$_4$S$^+$ 429.2 Found: 385.2 [M - CO$_2$]

3.2.1.10 MTh methyl ester

![Chemical Structure of MTh Methyl Ester](image)

$^1$H NMR (600 MHz, CD$_3$OD) $\delta$ 7.65 (d, $J = 7.8$ Hz, 4H), 7.57 (d, $J = 9.2$ Hz, 2H), 7.51 – 7.38 (m, 9H), 7.20 (s, 1H), 5.70 (d, $J = 17.6$ Hz, 1H), 5.50 (d, $J = 17.4$ Hz, 1H), 3.97 (t, $J = 5.4$ Hz, 2H), 3.91 (s, 3H), 3.21 (t, $J = 5.4$ Hz, 2H), 2.52 (s, 3H), 2.34 (s, 3H), 1.06 (s, 9H). MS(ESI+) m/z: M$^+$ Calcd for C$_{37}$H$_{43}$N$_4$O$_4$SSi$^+$ 667.3 Found: 667.3

3.2.1.11 MTh

![Chemical Structure of MTh](image)
$^1$H NMR (400 MHz, 20% DCl, TMSP-$d_4$) $\delta$ 7.59 – 7.54 (m, 2H), 7.35 – 7.26 (m, 3H), 6.86 (s, 1H), 5.82 (d, $J = 19.5$ Hz, 1H), 5.40 (d, $J = 19.5$ Hz, 1H), 3.98 (t, $J = 5.7$ Hz, 2H), 3.26 (t, $J = 5.7$ Hz, 2H), 2.54 (s, 3H), 2.41 (s, 3H). MS(ESI+) m/z: M$^+$/Calcd for C$_{20}$H$_{23}$N$_2$O$_4$S$^+$ 415.1 Found: 371.1 [M - CO$_2$]

3.2.1.12 $p$-F-MTh methyl ester

![Molecule](image)

$^1$H NMR (300 MHz, CD$_3$OD) $\delta$ 7.74 – 7.58 (m, 6H), 7.49 – 7.37 (m, 7H), 7.13 (t, $J = 8.7$ Hz, 2H), 5.69 (d, $J = 17.5$ Hz, 1H), 5.53 (d, $J = 17.5$ Hz, 1H), 3.98 (t, $J = 5.4$ Hz, 2H), 3.91 (s, 3H), 3.22 (t, $J = 5.4$ Hz, 2H), 2.55 (s, 3H), 2.37 (s, 3H), 1.05 (s, 9H). MS(ESI+) m/z: M$^+$/Calcd for C$_{37}$H$_{43}$FNO$_4$Si$^+$ 685.3 Found: 685.3

3.2.1.13 $p$-F-MTh

![Molecule](image)

$^1$H NMR (300 MHz, 20% DCl) $\delta$ 7.46 (dd, $J = 9.0, 5.1$ Hz, 2H), 6.96 (t, $J = 8.8$ Hz, 2H), 6.86 (s, 1H), 5.63 (d, $J = 18.0$ Hz, 1H), 5.27 (d, $J = 18.0$ Hz, 1H), 3.81 (t, $J = 5.8$ Hz, 2H), 3.10 (t, $J = 5.7$ Hz, 2H), 2.42 (s, 3H), 2.28 (s, 3H). MS(ESI+) m/z: M$^+$/Calcd for C$_{20}$H$_{22}$FNO$_4$S$^+$ 433.1 Found: 389.1 [M - CO$_2$]
3.2.1.14 p-Cl-MTh methyl ester

\[
\text{\textsuperscript{1}H NMR (300 MHz, CD}_3\text{OD) } \delta 7.68-7.65- (m, 4H), 7.58 - 7.35 (m, 11H), 7.26 (s, 1H), 5.73 (d, } J = 18.0 \text{ Hz, 1H), 5.47 (d, } J = 18.0 \text{ Hz, 1H), 3.98 (t, } J = 5.4 \text{ Hz, 2H), 3.91 (s, 3H), 3.22 (t, } J = 5.4 \text{ Hz, 2H), 2.56 (s, 3H), 2.35 (s, 3H), 1.07 (s, 9H). MS(ESI\textsuperscript{+}) m/z: M\textsuperscript{+} Calcd for C\textsubscript{37}H\textsubscript{42}ClN\textsubscript{4}O\textsubscript{4}SSi\textsuperscript{+} 701.2 Found: 701.2}
\]

3.2.1.15 p-Cl-MTh

\[
\text{\textsuperscript{1}H NMR (300 MHz, 20% DCl) } \delta 7.51 (d, } J = 8.8 \text{ Hz, 2H), 7.29 (d, } J = 8.8 \text{ Hz, 2H), 6.86 (s, 1H), 5.79 (d, } J = 18.4 \text{ Hz, 1H), 5.33 (d, } J = 18.4 \text{ Hz, 1H), 3.94 (t, } J = 5.8 \text{ Hz, 2H), 3.22 (t, } J = 5.8 \text{ Hz, 2H), 2.57 (s, 3H), 2.38 (s, 3H). MS(ESI\textsuperscript{+}) m/z: M\textsuperscript{+} Calcd for C\textsubscript{20}H\textsubscript{22}ClN\textsubscript{4}O\textsubscript{4}S\textsuperscript{+} 449.1 Found: 405.1 [M - CO\textsubscript{2}]}
\]
3.2.1.16 m-Br-MTh methyl ester

\[
\text{H NMR (600 MHz, CD}_{3}\text{OD)} \delta 7.76 (t, J = 1.8 \text{ Hz}, 1\text{H}), 7.69 - 7.62 (m, 4\text{H}), 7.53 (ddd, J = 8.0, 1.9, 0.9 \text{ Hz}, 1\text{H}), 7.52 - 7.47 (m, 3\text{H}), 7.47 - 7.42 (m, 4\text{H}), 7.29 (t, J = 8.0 \text{ Hz}, 1\text{H}), 7.25 (s, 1\text{H}), 5.72 (d, J = 17.5 \text{ Hz}, 1\text{H}), 5.47 (d, J = 17.5 \text{ Hz}, 1\text{H}), 3.98 (t, J = 5.4 \text{ Hz}, 2\text{H}), 3.91 (s, 3\text{H}), 3.22 (t, J = 5.4 \text{ Hz}, 2\text{H}), 2.54 (s, 3\text{H}), 2.35 (s, 3\text{H}), 1.07 (s, 9\text{H}). \text{ MS(ESI+)} m/z: \text{ M}^+ \text{ Calcd for C}_{37}\text{H}_{42}\text{BrN}_{4}\text{O}_{4}\text{Si}^+ 745.2 \text{ Found: 745.2}
\]

3.2.1.17 m-Br-MTh

\[
\text{H NMR (400 MHz, 20% DCl) } \delta 7.63 (s, 1\text{H}), 7.41 (d, J = 8.0 \text{ Hz}, 1\text{H}), 7.31 (d, J = 8.0 \text{ Hz}, 1\text{H}), 7.12 \ 7.07 (t, J = 8.0 \text{ Hz}, 1\text{H}), 6.86 (s, 1\text{H}), 5.73 (d, J = 19.2 \text{ Hz}, 1\text{H}), 5.27 (d, J = 18.0 \text{ Hz}, 1\text{H}), 3.86 (t, J = 5.6 \text{ Hz}, 4\text{H}), 3.15 (t, J = 5.6 \text{ Hz}, 2\text{H}), 2.44 (s, 2\text{H}), 2.32 (s, 2\text{H}). \text{ MS(ESI+)} m/z: \text{ M}^+ \text{ Calcd for C}_{20}\text{H}_{22}\text{BrN}_{4}\text{O}_{4}\text{S}^+ 493.1 \text{ Found: 449.1 [M - CO} _2\text{]}
\]

3.2.2 Kinetics

The decarboxylation of MTh derivatives was studied at 25 °C in pH 7.0 HEPES buffers (0.10, 0.200, 0.30 and 0.40 M) at 1.00 M (KCl) ionic strength. At this pH the AP ring is completely deprotonated (see Appendix A). Progress of the reaction was followed by UV-VIS spectroscopy
with the sample maintained at 25.0(±0.1) °C. The data for the reactions follow the rate law for first order kinetics. Repetitive scans produced two isosbestic points and accurate fits to the integrated law. The resulting rate constants were derived from exponential fits (using GraFit program) at 330 nm, where the change in absorbance was the largest. These values were essentially identical to the ones obtained at 290 nm. The absorbance at 330 nm comes from the PTK derivative which forms from a fast (10^4 s^{-1})\cite{18} fragmentation of the Breslow intermediate. The decarboxylation step is relatively slow (∼ 10^{-4} s^{-1}), and thus the rate of formation of the PTK derivative was equal to the rate of decarboxylation. All measurements were done in triplicates. All reported rate constants are for zero buffer concentration and were obtained by a linear extrapolation from the data in buffer concentration plots. A product study using ^1\text{H} NMR confirmed the expected formation of both protonation and fragmentation products.

3.3 Results

3.3.1 UV-VIS spectroscopy

![Graph showing UV-VIS spectroscopy](image)

**Figure 3.1.** A typical UV-VIS spectroscopy time-study of the decarboxylation of a MTh derivative in pH 7.0 buffer. The increase at 330 nm is the result of formation of a PTK derivative.
**Figure 3.2.** Absorbance change at 330 nm as the decarboxylation of a MTh derivative progresses with time.

**Figure 3.3.** Absorbance change at 290 nm as the decarboxylation of a MTh derivative progresses with time.
3.3.2 Buffer dependence

![Buffer concentration plot](image)

**Figure 3.4.** A representation of a typical buffer concentration plot for the decarboxylation of a MTh derivative (error bars are not visible)

3.3.3 Hammett plot

![Hammett plot](image)

**Figure 3.5.** The Hammett plot for the decarboxylation of phenyl-substituted derivatives of MTh (p-MeO, p-Me, H, p-F, m-MeO, p-Cl, m-Br) in neutral buffers. The $\rho$ value is 0.6 (±0.1) and $R^2 = 0.86$. 
Table 3.1. Rate constants for decarboxylation of phenyl-substituted MTh derivatives at pH 7.0 (zero buffer concentration), \( I = 1.00 \text{ M and } 25 \, ^\circ\text{C} \). The corresponding Hammett \( \sigma \) values are also listed.

<table>
<thead>
<tr>
<th>Substituent</th>
<th>( \sigma ) value</th>
<th>( 10^4 \times k_{co_2} ) s(^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>( p)-MeO</td>
<td>-0.27</td>
<td>1.53(±0.05)</td>
</tr>
<tr>
<td>( p)-Me</td>
<td>-0.17</td>
<td>1.52(±0.05)</td>
</tr>
<tr>
<td>H</td>
<td>0.00</td>
<td>2.15(±0.05)</td>
</tr>
<tr>
<td>( p)-Fl</td>
<td>0.06</td>
<td>2.80(±0.06)</td>
</tr>
<tr>
<td>( m)-MeO</td>
<td>0.12</td>
<td>2.26(±0.05)</td>
</tr>
<tr>
<td>( p)-Cl</td>
<td>0.23</td>
<td>2.30(±0.07)</td>
</tr>
<tr>
<td>( m)-Br</td>
<td>0.39</td>
<td>4.30(±0.05)</td>
</tr>
</tbody>
</table>

3.4 Discussion

3.4.1 Correlation between \( \rho \) values and degree of charge localization in product

All the MTh derivatives lose CO\(_2\) rapidly at pH 7.0, producing the corresponding HBnTh derivative and fragmentation products (Scheme 3.3). The resulting Hammett plot has \( \rho = 0.6(±0.1) \).

![Scheme 3.3](image)

**Scheme 3.3.** Decarboxylation of substituted MTh derivatives results in formation of the classic Breslow intermediate followed by a rapid protonation and fragmentation reaction.

To correlate the \( \rho \) value with the degree of negative charge development at the transition state, my results were compared to literature \( \rho \) values for reactions that generate carbanionic intermediates. The anionic polymerization of styrene, deprotonation of a benzylic proton or E\(_1\)CB reactions (Scheme 3.4A) result in \( \rho \) values > 4.0.\(^{19}\) On the other hand, reactions leading to dispersal of
electron density have significantly smaller $\rho$ values. Transfer of the $\alpha$ proton of 1-phenylnitromethane produced $\rho = 1.2$ (Scheme 3.4B).\textsuperscript{[20]} This suggests that the transition state for decarboxylation of MTh has the residual electron pair significantly transferred into the thiazolium ring. Plotting the log($k_\alpha/k_H$) values against $\sigma^-$ values resulted in a somewhat worst fit, again consistent with lack of delocalization into the phenyl ring.

\textbf{Scheme 3.4.} Examples of reactions leading to a localized carbanion intermediate (A) and a highly delocalized carbanion intermediate (B). The corresponding $\rho$ values are also shown.

The resulting loss of aromaticity of the thiazolium upon loss of CO$_2$ would make the Breslow intermediate high in energy and thus closer in structure to the transition state (Hammond’s postulate).\textsuperscript{[15]} Therefore, the small $\rho$ value indicates the enzyme-free Breslow intermediate is best represented as a neutral 1,2-enaminol as depicted for other Breslow intermediates.

3.4.2 How would recombination affect the observable $\rho$ value?

The potential importance of recombination of CO$_2$ and the carbanion residue would require that the observed first order rate constant is the result of the net throughput of several steps, each uniquely affected by the phenyl substituents. Consequently, the $\rho$ value is an overall outcome since the Hammett plot was constructed from observable rate constants and not the intrinsic rate constant for the C–C bond breaking step. How does the potentially reversible C–C bond-breaking step affect my interpretation of the $\rho$ value? We can determine this by considering the decarboxylation process as in Scheme 3.5.
Scheme 3.5. A decarboxylation mechanism that includes reversibility for departure of CO$_2$

If $k_1$, $k_{-1}$ and $k_2$ are the rate constants for C–C bond-breaking, recombination, and separation, respectively, the observable rate constant $k_{obs}$ for such a reaction scheme is expressed by Eq. 3.1 (steady state approximation).

$$k_{obs} = \frac{k_1 k_2}{k_1 + k_2}$$

Eq. 3.1

In the Hammett relation, the $\rho$ value is the slope of the linear correlation (Eq. 3.1)

$$\rho = \frac{\Delta \log k_{obs}}{\Delta \sigma}$$

Eq. 3.2

Therefore, from Eq. 3.1 and Eq. 3.2 we arrive at equation Eq. 3.3.

$$\Delta \sigma \rho_{obs} = \Delta \log k_{obs} = \Delta \log k_1 + \Delta \log k_2 - \Delta \log (k_{-1} + k_2)$$

Eq. 3.3

As we can see, the $\rho$ value depends on a combination of rate constants for bond-breaking, recombination, and diffusion. Diffusion is not expected to be affected by substituents ($\Delta \log k_2 \sim 0$), thus the observed $\rho$ value is proportional to the net of the forward and reverse processes: $\rho_{obs} \propto \rho_1 - \rho_{-1}$. The value of $\rho_{-1}$ must be negative because at the transition state for the recombination step, the negative charge at the C2α position on the Breslow intermediate will be partially transferred to the carboxylate oxygens, resulting in more stabilization of the ground state than the transition state. Therefore, the observable $\rho$ value will not be diminished by a reversible C–C bond-breaking step. This can also be seen by considering the decarboxylation step as a pre-equilibrium. Electron withdrawing substituents will greatly favor formation of a localized carbanion-like product. With formation of a neutral 1,2-enaminol the equilibrium will not be significantly affected by the phenyl substituents.
3.4.3 Comparison to the enzymic results

Data from kinetic studies on IPDC with substituted BFs produces a nonlinear Hammett plot (Figure 3.6).[10] The concave down curvature indicates a change in the rate limiting step with variation of the substituents.[21] The two linear regions have ρ values of 4.4 and -2.5. The large positive slope is of particular interest because it is characteristic for reactions that produce localized carbanions. The only enzymic step that could account for this is the formation of CO2 and a localize carbanion-like Breslow intermediate. Electron donating groups would slow down the decarboxylation, making it rate limiting. On the other hand, electron withdrawing groups accelerate the decarboxylation, and another step becomes rate limiting. Other work has shown that the decarboxylation of p-CH3BF and p-CH3OBF on BFDC is mostly rate limiting as evidenced from a 13C isotope kinetic effect.[22] It has been also shown that the decarboxylation step on BFDC is the fastest from all other ThDP-dependent decarboxylases.[23] Therefore, a nearly completely rate limiting decarboxylation step on IPDC for decarboxylation of a non-native substrate seems reasonable.

![Figure 3.6](image)

Figure 3.6. The Hammett plot reported by Tittmann and co-workers, for the decarboxylation para-substituted BFs on IPDC. Reproduced from: Eur. J. Biochem. 2003, 270, 2322-2331, with permission.
The large positive $\rho$ value could also be a result of remote catalysis-disruptive interactions between the para-substituents and enzyme residues at the active site. However, the significantly larger para-ethyl group has less effect on $k_{cat}$ than the smaller para-methyl group, indicating that the substituent effect in this case are mostly electronic. If we assume electronic effects only, such a large $\rho$ value (4.4) can only be explained with formation of a localized carbanion and in this catalytic cycle only formation of the Breslow intermediate could account for this.

3.4.4 Why do enzymes maintain a localized Breslow intermediate?

As mentioned earlier, MTh is an accurate model for the enzymic-intermediate found on BFDC. My work shows that that this Breslow intermediate does not have a significant negative charge at the C2α. Thus, the localized carbanion-like Breslow intermediates on certain ThDP-dependent enzymes must be the result of the enzymic surroundings. Why would an enzyme evolve to prevent the Breslow intermediate’s internal neutralization?

3.4.4.1 Intermediate ground state delocalization

According to Albery and Knowles, enzymes evolve toward having all intermediates in similar Gibbs free energy states.[24] In other words, in addition to stabilizing transition states, enzymes prevent intermediates from relaxing too much, which would have added the released energy to subsequent kinetic barriers (Figure 3.7).

![Figure 3.7](image)

**Figure 3.7.** Hypothetical energy profiles for the enzymic reaction involving a 1,2-enaminol-like Breslow intermediate (broken line) and an improved profile (solid line) with a carbanion-like Breslow intermediate.
3.4.4.2 Avoiding intrinsic barriers associated with multiple atom re-hybridizations

Retaining the carbanion-like structure minimizes barriers associated with structural changes (Figure 3.9). A decarboxylation reaction leading to an enamine-like Breslow intermediate requires multiple atoms to rehybridize, resulting in significant changes in bond angles and in bond lengths (Figure 3.8A). The delocalization also causes a large change in charge distribution by going from a zwitterionic substrate (localized carbanion) to a neutral product (1,2-enaminol) that would require significant changes in solvation, further contributing to the energy barrier. This can be seen in Kresge’s work on proton transfers, where charge delocalization is responsible for large intrinsic barriers for proton transfers.[25]

![Diagram A](image1)

**Figure 3.8.** Delocalization of charge into the thiazolium ring requires rehybridization of several atoms (A). This is avoided if a localized carbanion-like Breslow intermediate is formed (B).

If enzymes maintain a localized carbanion-like Breslow intermediate they avoid large structural changes during the decarboxylation step but also in the subsequent protonation step (Figure 3.8). Formation of an 1,2-enaminol-like Breslow intermediate in solution indicates that localization of charge on the 2α carbon would be higher in energy. However, on the enzyme, strong electrostatic interactions[26] can provide adequate stabilization of the Breslow intermediate without delocalization of the negative charge into the thiazolium ring. Finally, the two enzymic strategies could operate together to maximize throughput (Figure 3.10).
Figure 3.9. Hypothetical energy profiles for the enzymic reaction involving a 1,2-enaminol-like Breslow intermediate (broken line) and an alternative profile (solid line) with a carbanion-like Breslow intermediate.

Figure 3.10. Hypothetical energy profiles for the enzymic reaction involving a 1,2-enaminol-like Breslow intermediate (broken line) and an alternative profile (solid line) with a carbanion-like Breslow intermediate.

3.4.5 Localization of charge and avoidance of fragmentation

The localization of charge could also have a coincidental outcome of blocking the intrinsically rapid and irreversible fragmentation of the thiamin cofactor. The fragmentation reaction occurs at a rate constant of $10^4 \text{ s}^{-1}$ (40 °C, neutral buffer), which is two orders of magnitude faster than the
enzymic turnover. Yet, the fragmentation reaction does not happen on enzymes. The remarkable ability to avoid fragmentation is likely an outcome of localization of the Breslow intermediate. As mentioned earlier, protonation of a localized carbanion would be a barrier-free process. Such an ultrafast protonation would quench the Breslow intermediate before it can fragment.

3.5 References


[13] G. W. Howe, University of Toronto (Toronto, Canada), **2016**.


Chapter 4
Halide Elimination on BFDC

4.1 Introduction

Benzoylformate decarboxylase catalyzes the conversion of benzoylformate to benzaldehyde. The mechanism of decarboxylation parallels that of the well-known ThDP dependent enzymes that promote the decarboxylation of α-ketoacids. The key process in BFDC converts the conjugate of ThDP and BF to the C2α conjugate base of 2-(1-hydroxybenzyl)ThDP (HBnThDP), known as the Breslow Intermediate, and CO2. Protonation completes the catalytic cycle to produce HBnThDP, followed by formation of benzaldehyde and ThDP. In addition to BF, BFDC catalyzes the decarboxylation of para-substituted derivatives of BF.[1] Kenyon, Kozarich and co-workers reported that (4-bromomethyl)benzoylformate (p-BrCH2BF) is a strong inhibitor of BFDC.[2] The inhibition occurs as p-BrCH2BF is converted to p-toluic acid, bromide ion, and carbon dioxide. It was proposed that after the loss of CO2 from the conjugate with ThDP, the Breslow intermediate, undergoes bromide elimination, producing a xylylene derivative species that tautomerizes to 2-(p-tolyl)ThDP. This will hydrolyze to p-toluic acid and ThDP, eventually restoring the enzyme’s activity (Scheme 4.1).

![Scheme 4.1. Sequence of chemical steps that lead to inhibition of BFDC by p-BrCH2BF](image)

In contrast to the bromo derivative, p-ClCH2BF undergoes only 0.6% halide elimination on BFDC and p-FCH2BF proceeds to form the aldehyde without elimination of fluoride ion.[3] This follows the relative ability of halides to function as nucleofuges: Br− > Cl− >> F−.[4]
High-resolution studies show that certain ThDP-dependent enzymes enforce localized carbanion-like structures (see Chapter 3). Comparison of Hammett results between the enzymic and non-enzymic decarboxylation of MTh/MThDP suggested that a localized carbanion-like Breslow intermediate could also form on BFDC. By maintaining the carbanion form, the enzyme could have decreased the barrier to elimination through an E1CB mechanism.\(^\text{[5]}\)\(^\text{[6]}\) In solution, were the Breslow intermediate acquires 1,2-enaminol form, the elimination would less likely to occur.\(^\text{[7]}\) Thus, halide elimination could potentially serve as a test for formation of the localized Breslow intermediate on BFDC.

In this chapter, I describe the synthesis, product analysis and reaction kinetics of accurate models of the pre-decarboxylation intermediates in BFDC derived from \(p\)-BrCH\(_2\)BF and \(p\)-ClCH\(_2\)BF. Their spontaneous decarboxylation lead directly to the key Breslow intermediate. My results also suggest that the Breslow intermediates on and off the enzyme are different in their reactivity patterns. This is consistent, with BFDC enforcing a localized carbanion-like Breslow intermediate that would otherwise be internally neutralized to the 1,2-enaminol form. Finally, the formation of the 2-(\(p\)-toluyl)Th intermediate and its tautomerization was verified spectrscopically and through a deuterium isotope incorporation experiment.

4.2 Experimental

4.2.1 Synthesis

4.2.1.1 General considerations

All chemicals were obtained from commercial sources and used without further purification. All synthesis has been performed according to a previously described methodology, using an appropriate benzoylformate ester as starting material. \(^1\)H NMR spectra are referenced to the residual solvent peak (CH\(_3\)OH) or in case of samples dissolved in 20% DCl, the AP ring C1’ proton peak was referenced as 6.86 ppm (based on the previous assignment in MTh).
4.2.1.2 O-TBDS-protected \( p \)-BrCH\(_2\)MTh

This was synthesized by condensation of \( O \)-TBDS-protected thiamin with \textit{tert}-butyl \( p \)-(bromomethyl)benzoylformate (also see Chapter 2).\(^8\) \textit{Tert}-butyl \( p \)-(bromomethyl)benzoylformate was prepared from \( p \)-(bromomethyl)benzoylformic acid\(^3\) and isobutylene in the presence of catalytic amounts of sulfuric acid. \(^1\)H NMR (MeOD, 300 MHz): \( \delta \)\(_{\text{H}} \) 7.67–7.41 (m, 14H), 7.19 (s, 1H), 5.79 (d, 1H, \( J \) = 18.0 Hz), 5.50 (d, 1H, \( J \) = 18.0 Hz), 4.50 (s, 2H), 4.09 (t, 2H, \( J \) = 5.0 Hz), 3.21 (t, 2H, \( J \) = 5.0 Hz), 2.51 (s, 3H), 2.31 (s, 3H), 1.46 (s, 10H), 1.08 (s, 10H). MS(ESI+) m/z: M\(^+\) Calcd for C\(_{38}\)H\(_{44}\)BrN\(_4\)O\(_4\)S\(_2\) 801.3 Found: 801.3

4.2.1.3 \( p \)-BrCH\(_2\)MTh

\( O \)-TBDS-protected \( p \)-BrCH\(_2\)MTh was stirred in concentrated hydrobromic acid overnight at 25 °C. The mixture was washed three times with DCM and the acid was roto-evaporated to an oily residue. This was stored at -80 °C. \(^1\)H NMR (20% DCl, 400 MHz): \( \delta \)\(_{\text{H}} \) 7.55 (d, \( J \) = 8.0 Hz, 2H), 7.03 (d, \( J \) = 8.0 Hz, 2H), 6.86 (s, 1H), 5.21 (d, \( J \) = 16.0 Hz, 1H), 5.11 (d, \( J \) = 16.0 Hz, 1H), 4.21 (s, 2H), 3.63 (t, \( J \) = 6.0 Hz, 2H), 2.88 (t, \( J \) = 6.0 Hz, 2H), 2.38 (s, 3H), 2.03 (s, 3H). MS(ESI+) m/z: M\(^+\) Calcd for C\(_{21}\)H\(_{24}\)BrN\(_4\)O\(_4\)S\(_2\) 507.1 Found: 463.1 (M\(^+\) - CO\(_2\))
4.2.1.4 O-TBDS-protected $p$-ClCH$_2$MTh

This was prepared in an analogous way as O-TBDS-protected $p$-BrCH$_2$MTh, with ethyl $p$-(chloromethyl)benzoylformate as starting material.$^{[3, 8]}$ This was synthesized from ethyl $p$-methylbenzoylformate$^{[9]}$ by free radical bromination$^{[10]}$ followed by bromide to chloride exchange with bismuth(III) chloride.$^{[11]}$ MS (ESI+) was used to verify that that there was less than ~1% of O-TBDS-protected $p$-BrCH$_2$MTh. $^1$H NMR (400 MHz, CD$_3$OD) $\delta$ 7.65 (d, $J = 7.9$ Hz, 4H), 7.58 (d, $J = 8.3$ Hz, 4H), 7.49 (t, $J = 7.0$ Hz, 4H), 7.44 (t, $J = 7.5$ Hz, 6H), 7.17 (s, 1H), 5.76 (d, $J = 17.8$ Hz, 1H), 5.49 (d, $J = 17.8$ Hz, 1H), 4.59 (s, 2H), 4.36 (qd, $J = 10.8$, 7.1 Hz, 2H), 3.97 (t, $J = 5.2$ Hz, 2H), 3.21 (t, $J = 5.2$ Hz, 2H), 2.50 (s, 3H), 2.32 (s, 3H), 1.27 (t, $J = 7.1$ Hz, 3H), 1.07 (s, 9H). MS(ESI+) m/z: $^{+}$ Calcd for C$_{39}$H$_{46}$ClN$_4$O$_4$SSi $^{+}$ 729.3 Found: 729.3

4.2.1.5 p-ClCH$_2$MTh

$O$-TBDS-protected $p$-ClCH$_2$MTh was stirred in concentrated hydrochloric acid, overnight at 25 °C. The mixture was washed three times with DCM and the acid was rotary evaporated to an oily residue. This was stored at -80 °C. $^1$H NMR (400 MHz, 20% DCl) $\delta$ 7.56 (d, $J = 7.9$ Hz, 2H), 6.86 (s, 1H), 5.83 (d, $J = 16.5$ Hz, 1H), 5.36 (d, $J = 16.5$ Hz, 1H), 4.54 (s, 2H), 3.94 (t, $J = 4.5$ Hz,
2H), 3.22 (t, J = 4.5 Hz, 2H), 2.50 (s, 3H), 2.36 (s, 3H). MS(ESI+) m/z: M⁺ Calcd for C₂₁H₂₄ClN₄O₄S⁺ 463.1 Found: 419.1 (M⁺ - CO₂)

4.2.2 Kinetics

4.2.2.1 Rate constants

All kinetic data were obtained in triplicates at 25 °C an ionic strength 1.00 M (KCl). The decarboxylation reaction of p-BrCH₂MTh in buffered solutions was followed by UV-VIS spectroscopy and ¹H NMR. The observable rate constants were obtained from exponential fits from plots of absorbance at 290 nm versus time. All rate constants are extrapolated to zero buffer concentration. At 0.1 M and higher acidity, where 2-benozylthiamin is stable, rate data was collected at 330 nm. In pH 2.4 buffer, the rate constant was obtained from an ¹H NMR array experiment because the decarboxylation and hydrolysis steps are both rate limiting. The C6’ proton peak (6.86 ppm) of the substrate was recorded to monitor the progress of the decarboxylation reaction. The decarboxylation of p-ClCH₂MTh was investigated at pH 7.0. A standard titration curve was fitted to the pH profile. Buffer preparation

Buffers at various pH values were prepared at 25 °C and ionic strength 1.00 M (KCl) by titrating an appropriate amount of the base component of the buffer (K₂HPO₄, AcOK, MES) with hydrochloric acid solutions and monitoring the pH.

4.2.2.3 Product study

The products of the decarboxylation reaction were identified by spiking the ¹H NMR sample with commercially available thiamin and p-toluic acid. The reaction mixtures were acidified and extracted with dichloromethane and analyzed by MS(ESI⁺). Product ratios were obtained from integrations of ¹H NMR spectra that were obtained with a 10 s relaxation delay.

4.2.2.4 Deuterium incorporation

The decarboxylation reaction was performed in a pD 7.0 phosphate buffer. The products of this were studied by ¹H NMR and MS(ESI⁺), as described above.
4.3 Results

4.3.1 UV-VIS traces

**Figure 4.1.** UV-VIS traces of the decarboxylation of $p$-BrCH$_2$MTh at pH 7.0 (A.) and in 1.0 M HCl (B.).

**Figure 4.2.** Changes in absorbance at 280 nm in pH 7.0 buffer (A), 330 nm in 1.0 M HCl solution (B), and at 330 nm pH 2.4 (C) as the decarboxylation of $p$-BrCH$_2$MTh progresses.
4.3.2 pH profile

Figure 4.3. The pH-rate profile for decarboxylation of $p$-BrCH$_2$MTh. The profile is consistent with the decarboxylation reaction being faster when the $N1'$ position of the AP ring is protonated. The rate constant at pH 2.4 was obtained from an $^1$H NMR array experiment.

4.3.3 Solvent isotope effect

Figure 4.4. The $p$-toluic acid methyl group signal when the decarboxylation reaction is carried out in neutral deuterated buffer. Both the integration and splitting pattern are consistent with incorporation of a single deuterium into the resulting methyl group. Residual CH$_3$ peak is also seen.
**Table 4.1.** Decarboxylation rate constants at 25 °C and *I* = 1.0 M (KCl) (*a* – AP, *b* – APH*+*)

<table>
<thead>
<tr>
<th>compound</th>
<th><em>k</em>₁</th>
<th><em>k</em>₂</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>p</em>-BrCH₂MTh</td>
<td>3.1(±0.05)×10⁻⁴</td>
<td>1.3×10⁻³</td>
</tr>
<tr>
<td><em>p</em>-ClCH₂MTh</td>
<td>2.1(±0.05)×10⁻⁴</td>
<td>n/a</td>
</tr>
</tbody>
</table>

4.3.4 Product study

![NMR spectra](image)

**Figure 4.5.** An ¹H NMR array experiment for the decarboxylation of *p*-BrCH₂MTh in a pD 7.0 phosphate buffer. This shows formation of thiamin and *p*-toluic acid as the only products. The lack of transient peaks is consistent with a slow, rate limiting decarboxylation step.

4.4 Discussion

4.4.1 Bromide elimination

The decarboxylation of *p*-BrCH₂MTh in neutral buffers occurs with a rate constant of 3.1×10⁻⁴ s⁻¹. Unlike the decarboxylation of many other *para*-substituted MTh derivatives in neutral buffers, the decarboxylation of *p*-BrCH₂MTh proceeds without fragmentation or intramolecular cyclization reactions (see Chapter 7). This was concluded from the lack of formation of the
characteristic red-shifted absorbance at 328 nm from the PTK derivative (Figure 4.1A). An $^1$H NMR study of the product mixture revealed formation of only two compounds. These were later identified as thiamin and $p$-toluic acid. An $^1$H NMR array experiment in pD 7.0 phosphate buffer did not show transient peaks, indicating that the decarboxylation step at this pH is rate limiting (Figure 4.5). Thus, the reactivity in solution parallels the enzymic one, where bromide elimination completely outcompetes protonation of the Breslow intermediate.

4.4.2 Observation of 2-($p$-toluyl)thiamin

2-acylthiazolium salts rapidly hydrolyze in neutral solutions ($k \sim 10 \text{s}^{-1}$), thus 2-($p$-toluyl)Th is not observed during the decarboxylation reaction at pH > 4.0 (Scheme 4.2). However, the hydrolysis is much slower at lower pH. Indeed, when $p$-BrCH$_2$MTh reacts at pH 2.4 or lower, a transient red shifted band appears at 320 nm (Figure 4.1, Figure 4.2C). The absorbance shows an initial increase followed by a decrease to 0.0, consistent with the reactivity of 2-acylthiazolium ions. Once the reaction is completed, thiamin and $p$-toluic acid are the sole products. In 1.0 M HCl, the 330 nm band remains unchanged for many hours due to its higher stability at higher acidities (Figure 4.2B).

![Scheme 4.2. A general mechanism for hydrolysis of 2-acylthiazolium salts.](image)

4.4.3 Deuterium incorporation

Performing the decarboxylation of $p$-BrCH$_2$MTh in a pD 7.0 phosphate buffer leads to incorporation of a single deuterium into the methyl group of the $p$-toluic acid product. This is seen in the decrease of the relative integrations of the methyl group signals from three (reaction in H$_2$O) to two (reaction in D$_2$O). The signals for the two protons in the deuterated methyl group appear as a 1:1:1 triplet, as expected for a splitting from a single deuterium. MS (ESI+) gave a [M+1]$^+$ signal (Figure 4.4). This result is consistent with the tautomerization of the xylylene species to 2-($p$-toluyl)Th (Scheme 4.3).
Scheme 4.3. Tautomerization of the xylylene species in a deuterated buffer must result in a single deuterium incorporation into the methyl group of 2-(p-tolyl)Th.

4.4.4 Rate of bromide elimination

As mentioned earlier, both the fragmentation products (PTK derivative and DMAP) and protonation product (HBnTh) was not detected by $^1$H NMR in the product mixture from decarboxylation of $p$-BrCH$_2$MTh in a pH 7.0 phosphate buffer. Estimation of the rate of protonation of the MTh-derived Breslow intermediate was reported to be $\sim 7 \times 10^6$ M$^{-1}$s$^{-1}$ (in H$_2$PO$_4$/$HPO_4^{2-}$) at pH 7.0 and 25 $^\circ$C.$^{[13]}$ If we assume a 1% limit of detection by $^1$H NMR and a 0.5 M phosphate buffer concentration, then the bromide elimination step must be happening at a rate constant greater than $10^8$ s$^{-1}$ as the protonation product is not observed.

4.4.5 Rate of chloride elimination

Interestingly, $p$-ClCH$_2$MTh decarboxylates in a 0.50 M pH 7.0 buffer to give approximately 50-60 mol% chloride elimination, with the remainder being the corresponding protonation product. This was established from the $^1$H NMR spectrum by integrating the bridging CH$_2$ peak areas in thiamin and $p$-(ClCH$_2$)HBnTh. The elimination of chloride thus occurs with a rate constant of $\sim 10^6$ s$^{-1}$ (0.5 M pH 7 phosphate buffer, 25 $^\circ$C).

4.4.6 Concerted vs. step-wise mechanism for halide elimination

Points for rate constants for the decarboxylation of $p$-BrCH$_2$MTh and $p$-ClCH$_2$MTh fall on the data line on the Hammett plot obtained from rates of decarboxylation of other para- and meta-substituted MTh derivatives (see Chapter 3). This indicates that the differences in the rates of decarboxylation of $p$-BrCH$_2$MTh and $p$-ClCH$_2$MTh are the result of inductive effects of the $p$-BrCH$_2$ and $p$-ClCH$_2$ groups only. This makes the concerted mechanism (Scheme 4.4B) unlikely.
Scheme 4.4. Two possible mechanisms for the decarboxylation of $p$-XCH$_2$MTh, where X is the leaving halide ion.

4.4.7 Enzymic vs. non-enzymic Breslow intermediate

Against what I predicted, the results rule out the possibility that the enzyme is facilitating halide elimination by localization of the Breslow intermediate. The halide elimination reaction is an intrinsically fast process. However, the tiny amount (0.6%) of chloride elimination on BFDC, indicates that the protein is in fact suppressing the elimination. In this case, the localization of charge may in fact be preventing inhibition because localization of charge makes protonation a barrierless process.$^{[14]}$ Though such a fast proton transfer in not enough to compete with bromide elimination, it outcompetes chloride elimination, and blocks fluoride elimination entirely. In solution, a 1,2-enaminol Breslow intermediate would have an intrinsically slow protonation transfer and elimination would be predominant in all cases.

4.4.8 Estimating the rate of protonation of the Breslow intermediate on BFDC

The rate of fragmentation of the MTh-derived Breslow intermediate in solution phase is $10^4$ s$^{-1}$ (40 °C). From this, we can determine that the enzymic rate constant for protonation has to be at least $10^6$ s$^{-1}$, since the fragmentation of the thiamin co-factor is not observed. If the rate of chloride
elimination is $\sim 10^6$ s$^{-1}$ in solution, and less than 1% of Cl$^-$ elimination occurs on the enzyme, then protonation of the Breslow intermediate must be $\sim 10^8$ s$^{-1}$. This was calculated assuming that the enzyme does not alter to a significant extent the rates of fragmentation and chloride elimination. Such a large rate of protonation is consistent with Tittmann’s proposal that BFDC evolved to efficiently protonate the localized carbanion-like Breslow intermediate.$^{[15]}$

4.4.9 The “actual” mechanism of inhibition

It is noteworthy that Kenyon, Kozarich and co-workers proposed that a slow hydrolysis of the 2-(p-toluyl)Th is responsible for enzyme’s recovery from inhibition. This could only be possible if the enzyme’s active site is shielded from water because 2-(p-toluyl)Th, as in other 2-acetylthiamin derivatives, would be rapidly hydrolyzed in neutral solution. The fleeting nature of 2-(p-toluyl)Th has been verified by not being able to observe (via UV-VIS spectroscopy and $^1$H NMR) its buildup in neutral buffers at 25 °C. Interestingly, Kenyon and Kozarich also determined that in the absence of thiamin diphosphate in the buffer, the enzyme fails to recover from inhibition, even after 4 h.$^{[3]}$ Based on that description, it is more likely that the bromide elimination “opens up” the active site, which results in expulsion of the cofactor. In that case, $p$-BrCH$_2$MTh inhibits the enzyme by a chemically induced release of the cofactor! We can consider that 2-p-toluylThDP can function as a benzoylating agent for nucleophilic residues at the active site. This could result in disruption of key cofactor-binding interactions. However, these questions remain to be addressed.

4.5 References


Chapter 5
How General is Decarboxylation by Formation of Bicarbonate? Part I.

5.1 Introduction

Graeme Howe observed that the decarboxylation of MTh is subject to general base catalysis in neutral solution.\textsuperscript{[1]} The reaction generates a Breslow intermediate upon replacement of the carboxyl group by a proton. The Brønsted plot for the general base with substituted acetates gives $\beta = 0.28$. The reported SKIE of approximately 2 indicates a partial proton transfer in the rate-determining step. Direct loss of CO$_2$ should not be subject to general base catalysis. However, the results are consistent with a fast addition of water to the carboxyl of MTh to form an orthoacid intermediate, followed by a rate-determining base assisted decomposition to HCO$_3^-$ and the Breslow intermediate (Scheme 5.1).

Scheme 5.1. Base catalyzed decarboxylation of MTh.

The proton transfer that is concerted with C–C bond breaking is consistent with the SKIE. The hydrolytic decarboxylation is also consistent with the previously reported carbon kinetic isotope effect for this reaction.\textsuperscript{[2]} The value of $\beta$ is about the same as that for H-bond formation.

It was proposed that MTh decarboxylates through formation of HCO$_3^-$ to avoid the fast recombination of CO$_2$ with the nascent carbanion.\textsuperscript{[1, 3]} Bicarbonate is more readily solvated and less electrophilic than CO$_2$, providing an effective alternative in this case.\textsuperscript{[3a, 3b]}

The reversion problem in decarboxylation reactions can be seen in the theoretical work on orotic and N-methylpicolinic acid by Major and Gao,\textsuperscript{[4]} and in Howe’s study of the decarboxylation of trichloroacetic acid.\textsuperscript{[5]} The computations show minimal barriers for the recombination of the
resulting CO$_2$:R$^-$ pairs (Figure 5.1 and Figure 5.2). Moreover, Howe’s study also shows that departure of the less electrophilic carbonic acid provides a significant barrier (~ 15 kcal/mol) to recombination. This result can be extended to loss of HCO$_3^-$.

**Figure 5.1.** The energy profile reported by Major and Gao for the decarboxylation of NMPA through loss of CO$_2$, (*J. Chem. Theory Comput.* 2007, 3, 949-960). Reproduced with permission from the publisher (American Chemical Society)

**Figure 5.2** The energy profile reported by Howe et al. for the decarboxylation of TCA through loss of CO$_2$ (*J.Org. Chem.* 2014, 79, 10972-10980). Reproduced with permission from the publisher (American Chemical Society).

The decarboxylation of MTh is the first case of a decarboxylation reaction that has been shown to be subject to general base catalysis. Although organic bases have been implicated in the decarboxylation of trichloroacetic acid,$^{[6]}$ the limited data does not allow to determine if general/specific catalysis occurs.$^{[7]}$ Investigation of the decarboxylation of trichloroacetate is complicated by a side reaction (formation of dichlorocarbene),$^{[8]}$ and nucleophilic substitutions at
the α-position\textsuperscript{[7]} and as well experimental challenges associated with following the progress of the reaction.

The compound that was studied in the computations of Major and Gao, NMPA, has an absorption maximum at 275 nm. Its decarboxylation forms a stable $N$-methylpyridinium ion.\textsuperscript{[9]} Since the results of computations indicate that the NMPA-derived CO$_2$:R$^-$ pair has a minimal barrier for combination, the hydrolytic decarboxylation could be an faster alternative (Scheme 5.2). The decarboxylation of NMPA is well behaved (no side reactions) and can be followed by UV-VIS spectroscopy.

\begin{center}
\begin{tabular}{c}
A. \\
\begin{tabular}{c}
$\text{N}^+\text{H}^-\text{O}^-\text{C}^\text{O}$ \\
$\leftrightarrow$ \\
$\text{N}^+\text{H}^-\text{O}_2\text{C}^\text{O}$
\end{tabular} \\
$\text{O}_2\text{C}^\text{O}$ + $\text{O}_2\text{C}^\text{O}$ + $\text{H}^+$ \\
\begin{tabular}{c}
$\text{N}^+\text{H}^-\text{O}^-\text{C}^\text{O}$ \\
$\rightarrow$ \\
$\text{N}^+\text{H}^-\text{O}_2\text{C}^\text{O}$
\end{tabular}
\end{tabular}

B. \\
\begin{tabular}{c}
$\text{N}^+\text{H}^-\text{O}^-\text{C}^\text{O}$ \\
$\rightarrow$ \\
$\text{N}^+\text{H}^-\text{O}_2\text{C}^\text{O}$
\end{tabular} \\
\begin{tabular}{c}
$\text{O}_2\text{C}^\text{O}$ + $\text{O}_2\text{C}^\text{O}$ + $\text{H}^+$ \\
$\text{N}^+\text{H}^-\text{O}_2\text{C}^\text{O}$
\end{tabular}

C. \\
\begin{tabular}{c}
$\text{N}^+\text{H}^-\text{O}^-\text{C}^\text{O}$ \\
$\rightarrow$ \\
$\text{N}^+\text{H}^-\text{O}_2\text{C}^\text{O}$
\end{tabular} \\
\begin{tabular}{c}
$\text{O}_2\text{C}^\text{O}$ + $\text{O}_2\text{C}^\text{O}$ + $\text{BH}$ + $\text{H}^+$ \\
$\text{N}^+\text{H}^-\text{O}_2\text{C}^\text{O}$
\end{tabular}

D. \\
\begin{tabular}{c}
$\text{N}^+\text{H}^-\text{O}^-\text{C}^\text{O}$ \\
$\rightarrow$ \\
$\text{N}^+\text{H}^-\text{O}_2\text{C}^\text{O}$
\end{tabular} \\
\begin{tabular}{c}
$\text{O}_2\text{C}^\text{O}$ + $\text{H}^+$ \\
$\text{N}^+\text{H}^-\text{O}_2\text{C}^\text{O}$
\end{tabular}
\end{tabular}
\end{center}

Scheme 5.2. Decarboxylation of NMPA through formation CO$_2$ (A) and hypothetical hydrolytic pathways (B, C, and D)

For these reasons, I decided to study the decarboxylation of NMPA to determine if it is subject to a hydrolytic pathway. Direct identification of HCO$_3^-$ as the leaving group by the methods described by Krebs and Roughton\textsuperscript{[10]} or Jordan and co-workers\textsuperscript{[11]} is not possible because the decarboxylation reaction is slower than hydration of HCO$_3^-$ (See Chapter 1.3.3). I was thus limited to observation of general base catalysis and a solvent kinetic isotope effect as indications of such a mechanism (Scheme 5.2C). Moreover, the decomposition of the orthoacid dianion species to HCO$_3^-$ could also result in specific base catalyzed decarboxylation (Scheme 5.2D).

The relative stability of NMPA and the product of decarboxylation, allowed me to determine the reaction rates in alkaline solutions where any base catalysis could become more discernible.
Besides my kinetic studies on the decarboxylation of NMPA, I evaluated thermodynamic and kinetic properties of NMPA-derived orthoacids and considered the overall prospects for the hydrolytic pathways and their detection.

5.2 Experimental

5.2.1 Synthesis

5.2.1.1 General considerations

All reagents were purchased from commercial sources and used without further purification. Direct methylation of picolinic acid with methyl triflate and di-tert-butyl-4-methylpyridine as base in benzene gave a by-product. For this reason, picolinic acid was first esterified, followed by methylation and hydrolysis to yield the final product.

5.2.1.2 Methyl picolinate

\[
\text{\begin{figure}[h]
\centering
\includegraphics[width=0.2\textwidth]{methyl_picolinate.png}
\caption{Methyl picolinate molecule.}
\end{figure}}
\]

Picolinic acid (5.00 g, 40.6 mmol) was dissolved in methanol (50 mL) and 98% sulfuric acid was added. The mixture was stirred under reflux for 5 h. The reaction mixture was cooled and neutralized with sodium bicarbonate (some water was added to assist dissolution of the base). Methanol was removed by roto-evaporation. Water was added to the residue, and extracted three times with dichloromethane. The organic layer was dried with anhydrous magnesium sulphate, filtered, and roto-evaporated to dryness to obtain a slightly yellow oil (2.00 g, 36% yield).

\(^1\)H NMR (300 MHz, CDCl\(_3\)) \(\delta\) 8.71 (d, \(J = 4.7\) Hz, 1H), 8.10 (d, \(J = 8.9\) Hz, 1H), 7.81 (t, \(J = 7.7\) Hz, 1H), 7.44 (m, 1H), 3.97 (s, 3H). MS(ESI+) m/z: [M + H]^+ Calcd for C\(_7\)H\(_8\)NO\(_2\) 138.1; Found: 138.1.

5.2.1.3 \(N\)-methyl methylpicolinate triflate

\[
\text{\begin{figure}[h]
\centering
\includegraphics[width=0.2\textwidth]{N_methyl_methylpicolinate_triflate.png}
\caption{\(N\)-methyl methylpicolinate triflate molecule.}
\end{figure}}
\]
Methyl picolinate (0.50 g, 3.65 mmol) was dissolved in a small amount of benzene, and methyl triflate (0.467 mL, 3.83 mmol) was added drop-wise with stirring. Two layers formed, and after 4 h the bottom layer was solidified upon scratching with a glass rod. The solid was separated and triturated with benzene followed by ether, and dried under vacuum to obtain a white powder (0.95 g, 81% yield). $^1$H NMR (300 MHz, D$_2$O) δ 8.97 (d, $J = 6.7$ Hz, 1H), 8.71 (t, $J = 8.1$ Hz, 1H), 8.59 (d, $J = 9.5$ Hz, 1H), 8.22 (t, $J = 7.0$ Hz, 1H), 4.58 (s, 3H), 4.10 (s, 3H). MS(ESI+) m/z: M$^+$ Calcd for C$_8$H$_{10}$NO$_2$ 152.1; Found: 152.1.

5.2.1.4 $N$-methylpicolinic acid (homarine)

$N$-methyl methylpicolinate triflate was dissolved in conc. hydrochloric acid and stirred overnight at 60 °C. The acid mixture was roto-evaporated to dryness to yield an off-white solid (100% yield). $^1$H NMR (500 MHz, D$_2$O) δ 8.68 (d, $J = 6.0$ Hz, 1H), 8.51 (t, $J = 8.2$ Hz, 1H), 8.00 (d, $J = 7.9$ Hz, 1H), 7.95 (t, $J = 7.6$ Hz, 1H), 4.33 (s, 3H). MS(ESI+) m/z: M$^+$ Calcd for C$_7$H$_8$NO$_2$ $^+138.1$; Found: 138.1.

5.2.2 Kinetics

5.2.2.1 General considerations

The decarboxylation of $N$-picolinic acid was performed in aqueous buffers at 150(±1) °C and ionic strength 1.00 M (KCl). These are the same conditions that were previously used for related kinetic studies.$^{[9a]}$ The combination of high temperature and water as solvent required that the reaction be pressurized. Therefore, the reactions were conducted in sealed glass pressure tubes. Stock solutions were prepared by dissolving an appropriate amount of NMPA in a buffer so that after a 5-fold dilution, the absorbance at 275 nm would be below 1.5 AU. Such a stock is quite stable at room temperature but was kept frozen between uses. The pressure tube, containing 1.00 mL of the stock solution, was submerged into an oil bath maintained at 150 °C. To avoid condensation on the inside of the tube, the surface of the hot oil was at the level of the front seal. The decarboxylation reaction was quenched by placing the tube into room temperature water. The contents of the reaction mixture were transferred to a 5.00 mL volumetric flask and diluted with...
distilled water. After vigorous mixing, the spectrum of the solution from 220 to 300 nm was recorded. An endpoint spectrum was obtained after ~ 24 h. The recordings established that at 280 nm the change was the largest and the absorbance decreases essentially to zero when the reaction is completed. The collected absorption values at 280 nm were plotted as a function of time and rate constants were calculated by fitting exponential functions in the program GraFit. For samples with a pH above and equal to 9, the rate constants were obtained from initial rates because of the noticeable corrosiveness of the alkaline solutions towards the glass pressure tube and to avoid potential hydroxide mediated side reactions. All measurements were done in duplicates.

5.2.2.2 Buffer Preparation

All buffers were prepared at room temperature. Since the reaction is conducted at an elevated temperature, the pH of the buffer will change. However, the change is small enough that the overall trends will prevail, and this approach has been used before in studies on the decarboxylation of N-methylpicolinic acid. Acetate buffers were prepared at different concentrations (0.10, 0.20, 0.40, and 0.80 M) and pH values (3.80, 4.6 and pH 5.6). A single concentration of 0.10 M was used to prepare two phosphate buffers (pH 7.5 and 11) and one borate buffer (pH 9.0) because of their negligible effect on the rate of decarboxylation. For pH 13 and 14, a 0.10 M and 1.00 M potassium hydroxide solutions were used, respectively. The ionic strength was adjusted to 1.00 M with potassium chloride. For the solvent isotope effect experiment, water was replaced with deuterium oxide.

5.2.2.3 Product study

The products of decarboxylation of NMPA have been determined by $^1$H NMR analysis by spiking the reaction mixture with authentic samples of N-methylpyridinium iodide and NMPA.
5.2.3 Estimation of equilibrium and rate constants through linear free-energy relationships

5.2.3.1 Dissociation constants for orthoacids

\[ \text{Scheme 5.3.} \] The NMPA-derived orthoacid will be in equilibrium with its deprotonated forms.

I estimated the pK\(_a\) values of the orthoacid intermediates based on a method described by Hine and Koser.\(^{[12]}\) Accordingly, the pK\(_a\) of species having the general structure RR’C(OH)\(_2\) can be predicted from linear free-energy relationship (Eq. 5.1)

\[
pK_a = 14.9 - 1.3(\sigma_R^* + \sigma_{R'}^*)
\]

**Eq. 5.1**

where \(\sigma^*\) is the Taft’s polar substituent constant. The \(\sigma^*\) value for \(-\text{OH}\) is +1.55,\(^{[13]}\) but the value for \(N\)-methylpiryd-2-yl group \((-\text{C}_6\text{H}_5\text{N}^+)\) is unknown. It can be, however, determined from a linear free-energy correlation between the pK\(_a\) of carboxylic acids and \(\sigma^*\) values. This can be achieved because the ionization of RCOOH is practically independent of steric interaction.\(^{[14]}\) Therefore, a Taft acidity correlation Eq. 5.2 was obtained from a linear regression line from a pK\(_a\)\(^{[15]}\) versus \(\sigma^*\)\(^{[14]}\) plot for seven carboxylic acids (Eq. 5.2)

\[
pK_a = -1.59\sigma^* + 4.72; \quad R^2 = 0.981
\]

**Eq. 5.2**
Table 5.1. A list of seven carboxylic acids (RCOOH) with their corresponding pKₐ values, and Taft’s σ* values for the R group.

<table>
<thead>
<tr>
<th>R:</th>
<th>pKₐ</th>
<th>σ*</th>
</tr>
</thead>
<tbody>
<tr>
<td>(CH₃)₃C</td>
<td>5.03</td>
<td>-0.30</td>
</tr>
<tr>
<td>CH₃CH₂</td>
<td>4.88</td>
<td>-0.10</td>
</tr>
<tr>
<td>CH₃</td>
<td>4.76</td>
<td>0.00</td>
</tr>
<tr>
<td>C₆H₆</td>
<td>4.20</td>
<td>0.60</td>
</tr>
<tr>
<td>CClH₂</td>
<td>2.86</td>
<td>1.05</td>
</tr>
<tr>
<td>CCl₂H</td>
<td>1.35</td>
<td>1.94</td>
</tr>
<tr>
<td>CCl₃</td>
<td>0.66</td>
<td>2.65</td>
</tr>
</tbody>
</table>

From Eq. 5.2 and Eq. 5.2 and the known pKₐ value for N-methylpicolinic acid (~1 at 25 °C)⁹,¹¹, the σ* value for −C₆H₅N⁺ group was estimated to be +2.3. Inserting this into Eq. 5.1 provided a pKₐ value of 9.8. According to Hine and Koser the acidity of the second hydroxy group will decrease by a factor of 10^{4.4} because of the destabilizing effect of the negatively charged oxygen atom. Thus, pKₐ₂ was estimated to be approximately 14.2.

5.2.3.2 Hydration equilibrium constants

\[ \Delta G_H^0 = -3.8\sigma^* - 10.9; \quad R^2 = 1.000 \]

Eq. 5.3

Scheme 5.4. In water, NMPA is partially hydrated to an orthoacid.

Guthrie determined that unlike hydration equilibria for esters, the hydration of carboxylic acids (RCOOH) is not affected by size of the R group.¹⁶ Based on values for HCOOH, CF₃COOH and CH₃COOH which are reported in his study (Table 5.2), a linear free-energy correlation for the hydration was obtained (Eq. 5.3)
Table 5.2. Reported $\Delta G_H$ for hydration of carboxylic acids (RCOOH) and corresponding Taft’s polar substituent constant.

<table>
<thead>
<tr>
<th>R</th>
<th>$\Delta G_H^0$ kcal/mol</th>
<th>$\sigma^*$</th>
</tr>
</thead>
<tbody>
<tr>
<td>CH₃</td>
<td>11</td>
<td>0.00</td>
</tr>
<tr>
<td>H</td>
<td>9</td>
<td>0.49</td>
</tr>
<tr>
<td>CF₃</td>
<td>1</td>
<td>2.60</td>
</tr>
</tbody>
</table>

Inserting +2.3 for $\sigma^*$ in Eq. 5.3, I obtained $\Delta G_H^0$ equal to 2.3 kcal/mol, which corresponds to $K_H = 2.0 \times 10^{-2}$ for the hydration of NMPA (Scheme 5.4). However, since NMPA has a $pK_a$ value of ~1, and our experiments are performed at $pH > 3$, we should consider the hydration of the NMPA carboxylate to the orthoacid anion (Scheme 5.5)

\[
\text{Scheme 5.5. Addition of water to NMPA leads to formation of an orthoacid anion intermediate.}
\]

The $\Delta G^0$ for this process can be calculated by considering the following Bordwell thermodynamic cycle:

\[
\text{Scheme 5.6. A Bordwell cycle for the hydration reaction of NMPA.}
\]
Thus $\Delta G'_{H2} = \Delta G'_{H1} + \Delta G'_{a2} - \Delta G'_{a} = 2.3 + 13.4 - 1.4 = 14.3 \text{ kcal/mol}$. This value must be temperature corrected because it was obtained for 25 °C whereas the decarboxylation reaction was performed at 150 °C. Assuming that both $H$ and $S$ are constant with temperature and pressure, and assuming a rather generous values for $\Delta S$ of +30 e.u (0.03 kcal K$^{-1}$M$^{-1}$) for the reverse of hydration,$^{17}$ the formation of the orthoacid will be disfavored by no more than ~ 4 kcal/mol by going from 25 °C to 150 °C. Therefore, at $\Delta G_{H2}(150 \degree C) = 18.3 \text{ kcal/mol}$.

### 5.2.3.3 Hydration rate constants

#### 5.2.3.3.1 Water addition to a carboxylate

![Scheme 5.7.](image)

The hydration equilibria for carboxylic acids $\text{RCOOH} \rightleftharpoons \text{RC(OH)}_3$ are practically independent of steric effects.$^{16}$ Therefore, it is likely that the rate of addition of water to a carboxylate is only dependent on the polar effect of the R group as well. Rate constants for hydration of carboxylates had been previously reported for $\text{CF}_3\text{COO}^-$,$^{18}$ $\text{CCl}_3\text{COO}^-$,$^{18}$ $\text{CH}_3\text{COO}^-$,$^{19}$ $\text{CH}_3\text{CH}_2\text{COO}^-$,$^{20}$ and $\text{(CH}_3)_3\text{CCOO}^-$,$^{19}$ but not at the same temperature. Arrhenius equations (Eq. 5.4) for hydration of $\text{CH}_3\text{COO}^-$ and $\text{(CH}_3)_2\text{CCOO}^-$ are available and both have an identical pre-exponential parameter ($\log A = 11$).$^{19}$ Having in mind the very different R groups ($\text{CH}_3$ vs $\text{(CH}_3)_3\text{C}$), it is likely that $\log A = 11$ also holds for the remaining carboxylates. Assuming $\log A = 11$ for all cases and using the reported rate constants, the corresponding rate constants at 150 °C were estimated. From this a linear free-energy relationship equation was obtained (Eq. 5.5).

$$k = A e^{-\frac{E_a}{RT}}$$

**Eq. 5.4**

$$\log(k) = 1.4\sigma^* - 4.4; R^2 = 0.999$$

**Eq. 5.5**
The perfect correlation validates my earlier assumption that steric interactions do not affect rates of hydration of carboxylates. From this I estimated that the rate constant for addition of H2O to the NMPA zwitterion at 150 °C is 7 × 10^2 s⁻¹ (G‡ ~ 24 kcal/mol).

**Table 5.3.** Experimental and calculated rate constants for water addition to RCOO⁻.  

<table>
<thead>
<tr>
<th>R</th>
<th>σ*</th>
<th>10^6 k s⁻¹ (exp.)</th>
<th>10⁻¹ k s⁻¹ 150 °C (calc.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CF₃</td>
<td>2.58</td>
<td>1.1b</td>
<td>1.1</td>
</tr>
<tr>
<td>CCl₃</td>
<td>2.65</td>
<td>3.9b</td>
<td>2.8</td>
</tr>
<tr>
<td>CH₃</td>
<td>0.00</td>
<td>0.4c</td>
<td>4.0×10⁻⁴</td>
</tr>
<tr>
<td>t-C₄H₉</td>
<td>-0.30</td>
<td>0.1c</td>
<td>1.2 ×10⁻⁴</td>
</tr>
<tr>
<td>C₂H₅</td>
<td>-0.10</td>
<td>0.3c</td>
<td>3.6 ×10⁻⁴</td>
</tr>
</tbody>
</table>

5.2.3.4 Hydroxide addition to carboxylates

![Scheme 5.8](image)

**Scheme 5.8.** Hydroxide addition to a carboxylate to form an orthoacid dianion intermediate.

With a strongly electron withdrawing R group, for example CCl₃, addition of hydroxide to the carboxylate group becomes significant.[21] By using the Arrhenius equation and reported rate constant at 25 °C (E_a = 14 kcal/mol, logA=7) [21] the rate constants for hydroxide addition to trichloroacetate at 150 °C was estimated to be 0.7 M⁻¹s⁻¹. Based on relatively similar σ* values for –C₆H₅N⁺ and –CCl₃ (2.3 vs 2.6, respectively) the hydroxide addition to NMPA should be significantly contributing above pH 12.
5.3 Results

5.3.1 UV-VIS spectroscopy

Figure 5.3. Overlapped UV-VIS spectra (recorded at 25 °C) of a NMPA aqueous solution as the decarboxylation reaction progresses with time.

Figure 5.4. Absorbance change at 280 nm as the decarboxylation of NMPA progresses with time.

5.3.2 Acetate effect

The rate constants in buffer (represented as the slope in the buffer concentration plot) are approximately $10^{-5}$ M$^{-1}$s$^{-1}$. 
Figure 5.5. The effect of acetate buffers on the rate of decarboxylation of NMPA; pH 3.8 (square), pH 4.6 (filled circle), pH 5.6 (empty circle). The slopes of the linear fits are approximately $10^{-5} \text{M}^{-1}\text{s}^{-1}$.

Figure 5.6. Acetate buffer ratio profile for the decarboxylation of NMPA.
5.3.3 pH profile

![pH profile diagram](image)

**Figure 5.7.** The pH profile for the decarboxylation of NMPA in various aqueous buffers (full circles) and deuterated buffers (empty triangles).

5.3.4 Solvent isotope effect

The SKIE was approximately one at pH 3.8, 7.5 and 13.

5.3.5 Product study

*N*-methylpyridinium was the sole product formed during the decarboxylation of NMPA at the various conditions.
Figure 5.8. $^1$HNMR (400 MHz, 10% D$_2$O) spectra of an authentic NMPA and N-methylpyridinium iodide, stacked over a spectrum of the reaction mixture at pH 7.0 after 1 h and 24 h at 150 °C.

5.4 Discussion

5.4.1 Rate of decarboxylation and its pH dependence

The pK$_a$ of NMPA is ~ 0.7 at 150 °C[9a] and under our experimental conditions (pH > 3.8) it will be completely ionized to the zwitterionic form.[9a] The UV-VIS kinetic data gave excellent computer calculated fits to the integrated first order rate law. The observed first order rate constant for decarboxylation of the NMPA zwitterion is $1.5(\pm 0.1) \times 10^{-4}$ s$^{-1}$ (150 °C, $I = 1.00$ M), which is close to the value of $1.3 \times 10^{-4}$ s$^{-1}$ (150 °C) reported by Dunn and co-workers.[9a] The rate constant remains the same over a wide range of solution acidity, from pH 3.8 to 1.00 M hydroxide (Figure 5.7).

5.4.2 Acetate effect

Acetate buffers have a small effect on the rate of decarboxylation of NMPA (Figure 5.6). The rate in buffer is 10-fold smaller than the observable rate of decarboxylation. Importantly, there is no significant distinction between the effect of acetic acid and acetate, and thus this is unlikely to be due to acid/base catalysis and is probably a result of the change of the reaction medium. Therefore, this behavior is very different from the one observed for decarboxylation of MTh were the rate
constant in AcO⁻ was much larger than the overall reaction rate and AcOH had negligible effect on the reaction kinetics.⁴¹

5.4.3 Solvent kinetic isotope effect
The rate of decarboxylation at pH/pD 4, 7.5 and 13 was not affected to any significant extent when the reaction was carried out in deuterium oxide buffers/solutions. Based on this it was concluded that the decarboxylation of NMPA does not have a proton transfer concerted with C–C bond cleavage.

5.4.4 Accessibility of the orthoacid intermediates
From the linear free-energy relationship analysis, I estimate that the addition of water to the NMPA zwitterion is endothermic by ~ 18 kcal/mol. Using the Arrhenius equation and the experimental rate constant for decarboxylation \( k = 1.5 \times 10^{-4} \text{s}^{-1} \), I calculated the activation energy for the decarboxylation of NMPA to be approximately 32 kcal/mol. It is noteworthy that Major and Gao computed an almost identical value of 32.9 kcal/mol.⁴⁴ The formation of the orthoacid anion from NMPA is below the reaction’s activation barrier, and thus, it is a reasonable intermediate. These result parallel Howe’s computed thermodynamic values for TCA,⁴² which is not surprising owing to the similar electron withdrawing ability of the trichloromethyl and N-methypirydin-2-yl groups, with \( \sigma^* \) values of 2.6 and 2.3, respectively. Moreover, the estimated first order rate constant of 7 \( \times 10^{-2} \text{s}^{-1} \) for addition of water under pseudo first order conditions to the NMAP zwitterion at 150 °C is about 500 times greater than the observed first order rate constant for decarboxylation. Thus, the hydration reaction is also kinetically competent.

5.4.5 Decarboxylation through formation of \( \text{H}_2\text{CO}_3 \)

\[
\begin{align*}
\text{R}^\text{–} + \text{H}_2\text{O} & \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \text{HO}^\text{–}\text{OH} \\
\text{HO}^\text{–}\text{OH} & \overset{k_2}{\rightarrow} \text{R}^\text{–} + \text{HO}^\text{–}\text{CO}_2^\text{–}
\end{align*}
\]

**Scheme 5.9.** Non-base assisted hydrolytic decarboxylation.

The presence of general base catalysis and an SKIE > 1 would not be observed if the decomposition step of the orthoacid anion occurs spontaneously (Scheme 5.9).⁴⁵ Assuming pre-equilibrium conditions, we can consider the decarboxylation through loss of carbonic acid to happen according
to Scheme 5.10. From this we can derive a rate law (Eq. 5.6), where [R] is the total amount of the NMPA-derived species.

\[
\frac{d [P]}{dt} = \frac{kK_{H2}}{1 + K_{H2} + \frac{K_{H2}[H^+]}{K_{a1}}}[R]
\]

Eq. 5.6

With \( K_{H2} \sim 10^{-10} \) and \( 1/K_{a1} \sim 10^{10} \) the denominator in equation (Eq. 5.6) will reduce to 1, simplifying the expression to equation (Eq. 5.7).

\[
\frac{d [P]}{dt} = kK_{H2}[R]
\]

Eq. 5.7

Therefore, decarboxylation through the loss of carbonic acid will be independent of \( H^+ \) concentration (or \( OH^- \))

![Scheme 5.10. Reaction steps and equilibria expected for a decarboxylation through the loss of carbonic acid.](image)

5.4.6 Decarboxylation from the orthoacid dianion to form HCO_3^-

Scheme

![Scheme 5.11. A hypothetical decarboxylation reaction from the orthoacid anion species.](image)
Again, assuming pre-equilibrium conditions, and $[R]$ to be the total concentration of the NMPA-derived species, the rate law for the decarboxylation through the loss of $\text{HCO}_3^-$ from the orthoacid anion form can be represented with Eq. 5.8. This can be simplified to Eq. 5.9 because $K_{\text{H}_2}[\text{H}^+] \ll [\text{H}^+]$ and $K_{\text{H}_2}K_{a2} \ll [\text{H}^+]$.

\[
\frac{d\, [\text{P}]}{dt} = k_2 \frac{K_{\text{H}_2}K_{a2}}{[\text{H}^+] + K_{\text{H}_2}[\text{H}^+] + K_{\text{H}_2}K_{a2}} [R]
\]

Eq. 5.8

\[
\frac{d\, [\text{P}]}{dt} = k_2 \frac{K_{\text{H}_2}K_{a2}}{[\text{H}^+]} [R]
\]

Eq. 5.9

Therefore, the hydrolytic decarboxylation of NMPA, in principle, could be specific base catalyzed. Such behavior has been demonstrated for hydrolysis of ketones with the general structure of $\text{RC(O)}\text{X}$, where X is a carbon leaving group. However, this would only happen if the decomposition of the orthoacid dianion occurs at a rate that is competitive with a decarboxylation through loss of $\text{H}_2\text{CO}_3$.

5.4.7 NMPA does not undergo a hydrolytic decarboxylation?

Observation of general/specific base catalyzed decarboxylation of NMPA in water would provide irrefutable evidence for formation of $\text{HCO}_3^-$ or $\text{H}_2\text{CO}_3$ rather than $\text{CO}_2$. However, lack of such reactivity does not exclude a hydrolytic mechanism because the decarboxylation could be primarily occurring by decomposition of the orthoacid anion to $\text{H}_2\text{CO}_3$, which does not require participation of base (Scheme 5.9).

5.4.8 Can NMPA decarboxylate through $\text{CO}_2$?

Although the recombination of $\text{CO}_2$ with the nascent carbanion in the decarboxylation of NMPA, TCA or orotic acid is often considered as being “barrierless”, this is not accurate. In all three cases a minimal barrier exists of roughly 2 kcal/mol.\cite{4-5,24} The energy barrier for desolvation is not known for the NMPA-derived $\text{CO}_2:R^-$ pair, but I can approximate it to be roughly 1 kcal/mol, which is the barrier for desolvation of a pair of methane molecules in water.\cite{25} This seems like a reasonable assumption because like methane, carbon dioxide is non-polar and poorly solvated by water (no H-bonding). The attractive force between $\text{CO}_2$ and the nascent carbanion is not expected
to be large because quadrupole-dipole interactions are very weak, hence it is unlikely that the barrier for desolvation of the CO$_2$-carbanion pair will be larger than 2 kcal/mol. Therefore, the decarboxylation of NMPA by formation of CO$_2$ can thus proceed relatively unimpeded.

5.4.9 Potential limitation for the hydrolytic decarboxylation

There is no doubt that the hydrolytic decarboxylation can in theory provide a faster alternative for an otherwise highly reversible decarboxylation by formation of CO$_2$. This, however, can only be achieved if the activation energy for the hydrolytic decarboxylation remains close to that for loss of CO$_2$. Computational studies on TCA show that the activation energy for a decarboxylation through formation of H$_2$CO$_3$ to be higher than that for loss of CO$_2$, regardless of the calculation method.$^{[5]}$ This makes me believe that the loss of HCO$_3^-$ in the decarboxylation of MTh is a unique case. The following chapter describes my attempt to determine what structural features in MTh are responsible for its unusual behavior.

5.5 References


[24] The ~2 kcal/mol trichloroacetic acid decarboxylation barrier is based on the reported CCSD basis set calculation which is considered to be more accurate for determining activation barriers than other calculations (see *J. Org. Chem.* 2014, 79, 10972-10980)

Chapter 6
How General is Decarboxylation by Formation of Bicarbonate? Part II.

6.1 Introduction

Graeme Howe made the unexpected observation that decarboxylation of MTh is general base catalyzed with an SKIE of roughly 2. Being more specific, this was demonstrated on the N1'-protonated form of MTh (MThH⁺). It was proposed that the carboxyl group in MThH⁺ becomes hydrated to the orthoacid anion intermediate, which with the assistance of base decomposes to the Breslow intermediate and HCO₃⁻ and not CO₂ (Scheme 6.1). The more complex hydrolytic pathway would be kinetically advantageous, because the less electrophilic HCO₃⁻ will show less recombination with the Breslow intermediate than does CO₂. This was discussed in more detail in the previous chapter.

![Scheme 6.1. Decarboxylation of MThH⁺ via HCO₃⁻.](image)

The extent to which other decarboxylation reactions follow hydrolytic pathways is unknown and their detection would require circumstances that would allow for base catalysis. In the previous chapter, I reported that decarboxylation of NMPA in water is not base catalyzed. Yet, I also estimated that the orthoacid intermediate is both energetically and kinetically accessible. The decarboxylation of NMPA has been studied by high level theory where it was shown that the loss of CO₂ is highly reversible. Therefore, a hydrolytic alternative seemed likely,[1] but base catalysis was not observed. However, the lack of base catalysis in a decarboxylation reaction does not exclude the possibility of a hydrolytic mechanism because the spontaneous decomposition of the orthoacid ion to H₂CO₃ rather than HCO₃⁻ can occur without a proton transfer. Nevertheless, observation of such catalysis combined with an SKIE has been the only experimental evidence cited for the hydrolytic pathway in solution. Therefore, understanding conditions under which a decarboxylation reaction can proceed through formation of an orthoacid is an important goal.
As there are no other examples of general base catalyzed decarboxylation, I decided to focus on MThH⁺ and investigate what structural features are responsible for its unusual reactivity. Experimental and computational work by Howe provides some important clues.² In the optimized structure of MThH⁺, Howe observed an intramolecular hydrogen bond between the amino group of the 2,4-dimethyl-4-aminopyrimidinium (APH⁺) and the carboxylate. Such an interaction would make the carboxylate acquire a carboxyl-like character, assisting addition of water (Scheme 6.2).³

As expected, calculations also demonstrated that the intramolecular hydrogen bond stabilizes the orthoacid intermediate, making it energetically accessible (Scheme 6.2).²

![Scheme 6.2](image)

**Scheme 6.2.** An iminium-like APH⁺ ring will form a stronger hydrogen bond both with the carboxylate group in the substrate and in the orthoacid intermediate.

Interestingly, the decarboxylation of MThH⁺ is general base catalyzed only if the APH⁺ ring remains protonated.² The change in reactivity going from MThH⁺ to MTh can be considered in terms of resonance contributors. Both APH⁺ and AP groups have a non-aromatic iminium form in which the electron pair of the amino group is transferred to the N1' position of the ring. In AP the iminium form has a negative charge on the ring nitrogen, making it a less significant contributor (Figure 6.1). In contrast, in APH⁺ the negative charge is avoided and the stable iminium form becomes a significant resonance contributor. Therefore, the amino group in APH⁺ is unusually acidic thus permitting formation of a strong hydrogen bond with the carboxyl group.⁴

![Figure 6.1](image)

**Figure 6.1.** Iminium contributors in APH⁺ and AP.
In an important contrast, the decarboxylation of $N1^\prime$-protonated lactylthiamin (LThH$^+$) is not promoted by bases.$^{[2]}$ Why does replacing the phenyl ring in MThH$^+$ with a methyl group change the susceptibility to catalysis of decarboxylation? This requires that there is an additional factor. One possibility is that the bulk of the phenyl group is necessary at the C2α position to force a conformation in which the intramolecular hydrogen bond is possible. Consistent with this, the optimized structure of LThH$^+$ does not have the intramolecular hydrogen bond.$^{[2]}$

Summarizing, general base catalyzed decarboxylation of MThH$^+$ is believed to be the result of two structural features: 1) an acidic iminium-like amino group on APH$^+$, 2) a bulky substituent at C2α. Both are believed to facilitate an intramolecular bond to the carboxyl group, that in turn promotes formation of the orthoacid intermediate.

I tested this hypothesis with two analogues of MThH$^+$, each separately presenting the two contributing effects. In the first model (1), the APH$^+$ ring was replaced with a 4-aminopyridinium group (APyrH$^+$) that mimics the iminium-like amino group in MThH$^+$ (Figure 6.2). In the second model (2), a cyclohexyl ring was installed at C2α to mimic the steric bulk of the Bn group (Figure 6.2). Decarboxylation of both models was probed for general base catalysis with acetate buffers and observation of SKIE.

![Figure 6.2. Structures of compounds 1 and 2.](image-url)
6.2 Experimental

6.2.1 Synthesis

6.2.1.1 Synthesis of 1

6.2.1.1.1 $N$-(pyridin-4-yl)pivalamide

\[
\begin{center}
\text{HN}
\end{center}
\]

4-aminopyridine (20 g, 0.21 mol) and triethylamine (26.9 g, 37.1 mL, 0.27 mol) was dissolved in 300 mL of dichloromethane and cooled in ice/water bath. To this a solution of pivaloyl chloride (28.2 g, 28.8 mL, 0.23 mol, in 43 mL of dichloromethane) was slowly added. The formed mixture was stirred in ice for 15 min and then at r.t. for 2 h. The organic layer was washed 3 times with water, dried with anhydrous magnesium sulfate, filtered and roto-evaporated to obtain 28 g of an off-white solid (73% yield). The product was recrystallized from an ethyl acetate/hexane mixture to obtain 18 g of white crystals (48% yield). $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 8.43 (d, $J = 6.4$ Hz, 2H), 7.93 (s, 1H), 7.54 (d, $J = 6.4$ Hz, 2H), 1.29 (s, 9H).

6.2.1.1.2 $N$-(3-formylpyridin-4-yl)pivalamide

\[
\begin{center}
\text{HN}
\end{center}
\]

$N$-(pyridin-4-yl)pivalamide (10 g, 56.1 mmol) was transferred to a flame dried round-bottom flask and purged with nitrogen. Followed by addition 170 mL of tetrahydrofuran freshly distilled over sodium. The mixture was cooled in a dry ice/isopropanol bath, and 11.0 M $n$-butyllithium in hexanes (12.8 mL, 140.25 mmol) was slowly added. The reaction flask was transferred to an ice-bath. After 4 h the mixture turned from a clear yellow solution to a thick light-yellow mixture with a very fine solid formed. To this, dry dimethylformamide (0.168 mmol, 13 mL, dried over freshly activate molecular sieves) was added. After warming to room temperature, the reaction mixture
was quenched by pouring it onto ice mixed with some concentrated hydrochloric acid. The mixture was stirred for 5 min followed by basification with potassium carbonate which resulted in forming a vibrant yellow organic layer. The aqueous layer was washed 3 times with ether and the organic layers where combined, washed twice with brine, dried with anhydrous magnesium sulfate, filtered and roto-evaporated to yield a dark yellow oily residue. This was treated with hot hexanes, and the yellow solution was decanted over the residue. The hexanes solutions were cooled to room temperature and an oil precipitates. The next day the oil had solidified. This gave 10.5 g of product (90% yield). $^1$H NMR (400 MHz, CDCl$_3$) δ 11.43 (s, 1H), 10.00 (s, 1H), 8.80 (s, 1H), 8.62 (s, 2H), 1.33 (s, 9H).

### 6.2.1.1.3 4-aminonicotinaldehyde

![4-aminonicotinaldehyde](image)

$N$-(3-formylpyridin-4-yl)pivalamide (2 g, 9.70 mmol) was added to 3.0 M hydrochloric acid (50 mL) and heated for 8 h under reflux. The dark brown solution was extracted 5 times with dichloromethane and basified with potassium carbonate until strongly alkaline. The mixture was filtered to obtain a bright orange solution which was rotor evaporated almost dryness. The residue was vigorously shaken with dichloromethane. The organic layer was dried with anhydrous magnesium sulfate, filtered and roto-evaporated to obtain 1.0 g (85% yield) of a yellow powder. $^1$H NMR (399 MHz, CDCl$_3$) δ 9.91 (s, 1H), 8.55 (s, 1H), 8.20 (d, $J = 6.0$ Hz, 1H), 6.52 (d, $J = 6.0$ Hz, 1H).

### 6.2.1.1.4 (4-aminopyridin-3-yl)methanol

![4-aminopyridin-3-yl)methanol](image)

4-aminonicotinaldehyde (5.0 g, 41 mmol) was dissolved in methanol. Sodium borohydride (3.1 g, 2 eq.) was slowly added with stirring. The reaction was quenched by slowly adding concentrated hydrochloric acid. The solution was then basified with potassium carbonate and roto-evaporated to dryness. The residue was treated with isopropanol. The organic layer was dried with anhydrous
magnesium sulfate, filtered, and roto-evaporated to dryness to obtain 2.4 g (47% yield). $^1$H NMR (300 MHz, D$_2$O) $\delta$ 7.99 (m, 2H), 6.67 (d, $J = 5.7$ Hz, 1H), 4.55 (s, 2H).

6.2.1.1.5 3-(bromomethyl)pyridin-4-amine hydrobromide

$\text{Br} \begin{array}{c} \text{NH}_2 \\ \text{Br}^- \end{array}$

4-aminopyridin-3-yl)methanol (2.0 g, 16.1 mmol) and 31–33% HBr/AcOH (20 mL) where mixed in a round-bottom glass pressure flask. The reaction mixture was heated to 100 °C, resulting in complete dissolution of the solid. After 4 h of stirring the mixture is cooled, and the formed crystal were filtered off and washed with ethyl acetate. The crystals were dried under vacuum at 100 °C to obtain 3.6 g of light orange crystals (84% yield). The $^1$H NMR spectrum was practically indistinguishable from that of the starting material, but MS(ESI+) revealed complete conversion to product. $^1$H NMR (300 MHz, D$_2$O) $\delta$ 8.25 (s, 1H), 8.00 (d, $J = 7.0$ Hz, 1H), 6.97 (d, $J = 7.0$ Hz, 1H), 4.60 (s, 2H). MS(ESI+) m/z: M$^+$ Calcd for C$_6$H$_8$BrN$_2$+ 187.0 Found: 187.0.

6.2.1.1.6 O-TBDS-protected 5-hydroxyethyl-4-methylthiazole

$\begin{array}{c} \text{Si} \\ \text{O} \\ \text{N} \end{array}$

2-(4-methylthiazol-5-yl)ethan-1-ol (15.00 g, 105 mmol), tert-butyl(chloro)diphenylsilane 27.36 g, 99.5 mmol) were combined in some dichloromethane, and the mixture was cooled in an ice/water bath. Imidazole (16.94 g, 249 mmol) was slowly added, and the reaction mixture was stirred for 10-15 min and left stirring at room temperature overnight. Solvent was removed by rotary evaporation and the crude product was treated with ether followed by extensive washings of the organic layer with water until the washings no longer were basic (pH ~ 6). The ether layer was then washed once with brine, dried with anhydrous magnesium sulfate, and roto-evaporated to obtain 36 g of an off-white oil (94% yield). $^1$H NMR (200 MHz, CDCl$_3$) $\delta$ 8.59 (s, 1H), 7.62 (m, 4H), 7.39 (m, 6H), 3.81 (t, $J = 6.3$ Hz, 3H), 2.99 (t, $J = 6.3$ Hz, 3H), 2.33 (s, 3H), 1.06 (s, 9H).
6.2.1.1.7 O-TBDP-protected thiazolium precursor to 1

3-(bromomethyl)pyridin-4-amine hydrobromide (1.0 g, 3.7 mmol) and O-TBDS-protected 5-hydroxyethyl-4-methylthiazole (2.1 g, 5.6 mmol, 1.5 eq.) were combined in 14 mL of acetonitrile. The suspension was heated overnight under reflux. The mixture was cooled to room temperature and the solid product filtered off and washed with acetonitrile to obtain 1.58 g of an off-white powder (63% yield). $^1$H NMR (300 MHz, CD$_3$OD) $\delta$ 8.39 (s, 1H), 8.22 (d, $J = 7.7$ Hz, 1H), 7.67 (d, $J = 9.1$ Hz, 4H), 7.45 (m, 6H), 7.11 (d, $J = 7.1$ Hz, 1H), 5.61 (s, 2H), 3.98 (t, $J = 5.6$ Hz, 2H), 3.23 (t, $J = 5.5$ Hz, 2H), 2.59 (s, 3H), 1.09 (s, 9H); NOTE: the C2 proton has exchanged with D$^+$ and was not present in the spectrum.

6.2.1.1.8 O-TBDS-protected 1

O-TBDS-protected thiazolium precursor to 1 (1.00 g, 1.54 mmol), methyl benzoylformate (1.61 mL, 6 eq.) were combined in dichloromethane (15 mL), the mixture was purged with argon and cooled in a 35 v/v% ethanol/dry ice cooling bath. LiHMDS (1.0 M toluene sol, 3.33 mL, 2.2 eq.) was slowly added followed by 0.46 mL of dimethylformamide (to facilitate dissolution of the thiazolium derivative). The reaction was quenched by injecting an excess of acetic acid followed by trifluoroacetic acid (0.6 mL) and roto-evaporated. The conversion to product was estimated to be 60% by $^1$H NMR. The product was purified on a silica column pre-soaked in a saturated sodium bromide in methanol solution. The product was eluted with 10% dichloromethane in methanol, containing 0.02 v/v% of trifluoroacetic acid. The product eluted before the thiazolium starting
material fraction, and contained some dimethylformamide but this was not a complication for the next synthetic step. H NMR (300 MHz, CD$_3$OD) δ 7.97 (d, J = 6.9 Hz, 2H), 7.64 (d, J = 7.2 Hz, 4H), 7.61 – 7.55 (m, 2H), 7.53 – 7.30 (m, 8H), 7.28 (s, 1H), 6.90 (d, J = 9 Hz, 1H), 5.74 (d, J = 16 Hz, 1H), 5.60 (d, J = 16 Hz, 1H), 5.60 (d, J = 16 Hz, 1H), 3.98 (t, J = 5.4 Hz, 2H), 3.89 (s, 3H), 3.22 (t, J = 5.4 Hz, 2H), 2.32 (s, 3H), 1.04 (s, 9H). MS(ESI+) m/z: M$^+$ Calcd for C$_{37}$H$_{42}$N$_3$O$_4$SSi$^+$ 652.3 Found: 652.3

6.2.1.1.9 Compound 1

O-TBDS-protected 1 was combined with concentrated hydrochloric acid and stirred at room temperature for one week. The acidic mixture was washed three times with dichloromethane and the aqueous layer was concentrated to a syrup-like consistency by roto-evaporation and kept at -80 °C. H NMR (400 MHz, D$_2$O) δ 7.90 (d, J = 7.0 Hz, 1H), 7.55 (d, J = 7.6 Hz, 2H), 7.28 (t, J = 7.5 Hz, 2H), 7.21 (t, J = 7.3 Hz, 1H), 6.88 (d, J = 7.0 Hz, 1H), 6.83 (s, 1H), 5.79 (d, J = 17.5 Hz, 1H), 5.44 (d, J = 17.6 Hz, 1H), 4.00 (t, J = 5.5 Hz, 2H), 3.29 (t, J = 5.5 Hz, 2H), 2.41 (s, 3H). MS(ESI+) m/z: M$^+$ Calcd for C$_{20}$H$_{22}$N$_3$O$_4$S$^+$ 400.1 Found: 356.1 [M - CO$_2$]

6.2.1.2 Synthesis of 2

6.2.1.2.1 2-cyclohexyl-2-oxoacetic acid

Bromocyclohexane (30 g, 0.18 mmol), Mg (4.47 g, 0.18 mmol) were combined in tetrahydrofuran (freshly distilled over sodium) followed by tiny amount of 1,2-dibromoethane as an initiator. The mixture was reflux for 1 h with stirring. The reaction mixture was cooled in acetone/dry ice and two equivalents of diethyl oxalate (dried over molecular sieves) were added. The reaction was stirred for 15 min. and then left to warm to room temperature. The reaction mixture was quenched
with dilute hydrochloric acid and extracted with dichloromethane. The organic layer was dried with magnesium sulfate, filtered and roto-evaporated. The product was isolated by vacuum distillation at 85–90 °C. The product distills off after the unreacted ester and before some unknowns. Some overlap of fractions occurred but the product was considered pure enough for further the second step. Ethyl 2-cyclohexyl-2-oxoacetate (5.5 g, 30 mmol) was added to an excess of 1.0 M potassium hydroxide solution and stirred for 1 h at room temperature. The strongly basic solution was washed twice with dichloromethane to remove any non-polar contaminants from the previous step. The aqueous layer was acidified with concentrated hydrochloric acid and extracted 4 times with dichloromethane. The organic layer was dried with anhydrous magnesium sulfate, filtered and roto-evaporated to obtain 4.1 g (88% yield) of an off-white solid. \(^1\)H NMR (300 MHz, CDCl\(_3\)) \(\delta\) 3.29-3.19 (m, 1H), 2.00-1.65 (m, 5H), 1.44-1.12 (m, 5H). MS(DART+) m/z: M\(^+\) Calcd for C\(_{10}\)H\(_{16}\)O\(_3\) 184.1 Found: 202.2 [M+NH\(_4\)]\(^+\)

6.2.1.2.2 Tert-butyl 2-cyclohexyl-2-oxoacetate

2-cyclohexyl-2-oxoacetic acid (4.1 g, 25 mmol) was combined in dichloromethane (20 mL) and conc. sulfuric acid (350 µL) was added in a pressure bottle equipped with magnetic bar. The mixture was cooled in dry ice/acetone bath and liquid isobutylene (25 mL) was added. The bottle was sealed off and the reaction mixture was stirred at room temperature overnight. The bottle was carefully opened and an excess of 30% ammonia was added. The organic solvent was removed by roto-evaporation and the product extracted with ether. The organic layer was dried with anhydrous magnesium sulfate, filtered and roto-evaporated to obtain 3 g (36% yield) of a yellow solid. \(^1\)H NMR (300 MHz, CDCl\(_3\)) \(\delta\) 2.97–2.90 (m, 1H), 1.95-1.63 (m, 6H), 1.540 (s, 9H), 1.38–1.28 (m, 4H).
6.2.1.2.3 O-TBDP-protected 2

*O*-TBDS-protected thiamin hydrochloride (see Chapter 2 for synthesis, 500 mg, 0.92 mmol), and *tert*-butyl 2-cyclohexyl-2-oxoacetate (1.18 g, 5.56 mmol) were combined in some dichloromethane. The reaction flask was cooled in 35% ethanol/dry ice and purged with argon. LiHMDS (1.0 M toluene sol, 1.86 mL, 2.2 eq.) was added slowly. After 20 min, the reaction was quenched with acidic acid, followed by trifluoroacetic acid (532 µL). The reaction mixture was roto-evaporated, and the crude material purified by chromatography on silica which was pre-soaked in a concentrated sodium bromide methanol solution. A gradient elution is performed (2 to 15% methanol in ethyl acetate, with 0.02% trifluoroacetic acid). The product elutes before the *O*-THP-protected thiamin hydrochloride starting material. The product fractions are combined and roto-evaporated. The orange residue was dissolved in dichloromethane and gently stirred with brine, containing a small amount of sodium thiosulfate. The organic layer gradually becomes almost colorless. In this step residual bromine, which inherently forms during elution, is reduced to bromide. The organic layer was separated, washed twice with brine, dried with anhydrous magnesium sulfate, filtered and roto-evaporated to obtain a light-yellow residue. $^1$H NMR (300 MHz, CD$_3$OD) δ 7.74 – 7.62 (m, 4H), 7.56 – 7.36 (m, 6H), 5.88 (d, $J = 16.5$ Hz, 1H), 5.71 (d, $J = 16.5$ Hz, 2H), 4.14 – 3.94 (m, 2H), 3.25 (t, $J = 4.5$ Hz, 2H), 2.59 (s, 3H), 2.41 (s, 3H), 2.25 (t, $J = 10.5$ Hz, 1H), 1.91 – 1.62 (m, 4H), 1.50 (s, 9H), 1.47 – 1.14 (m, 6H), 1.14 (s, 9H).

6.2.1.2.4 Compound 2
*O-TBDS*-protected 2 was combined with conc. hydrochloric acid and stirred at room temperature for one week. The acidic mixture was washed three times with dichloromethane and the aqueous layer was concentrated to a syrup-like consistency by rotary evaporation and kept at -80 °C. ¹H NMR (399 MHz, 20% DCl) δ 5.94 (d, J = 17.2 Hz, 1H), 5.68 (d, J = 17.2 Hz, 1H), 3.94 (t, J = 5.8 Hz, 2H), 3.23 (t, J = 5.7 Hz, 2H), 2.65 (s, 3H), 2.46 (s, 3H), 2.43 – 2.32 (m, 1H), 1.86 – 1.47 (m, 5H), 1.43 – 1.06 (m, 4H).

6.2.2 Buffers

Stock buffer solutions (1.00 M) were prepared by dissolving an appropriate amount of either the base or acid buffer component in an amount of water that is 60% of the final buffer volume. This was followed by addition of an appropriate amount of potassium chloride so that the final buffer solution had an ionic strength of 1.00 M. The solutions were titrated with either hydrochloric acid or potassium hydroxide to the desired pH, transferred to a volumetric flask, and diluted with water. The buffer stocks were diluted with 1.00 M KCl solution to the desired concentrations.

6.2.3 Product study

Both compounds reacted to completion at pH 4.0 and pH 7.0 – 8.0 at 25 °C. The samples were acidified with HCl, and concentrated. The residues were dissolved in CD₃OD or DMSO-d₆, filtered and analyzed by ¹H NMR.

6.2.4 Kinetics

The progress of the decarboxylation reaction was followed by UV-VIS spectroscopy at 25 °C in 0.05 M buffers and ionic strength 1.00 M (KCl). Rate constants were obtained from exponential fits (GraFit) from the spectroscopic traces at 290 nm. There was a very small buffer dependence and I used rate constants from 0.05 M buffers as a good approximation for the rate at zero buffer concentration. The second order rate constants in buffer was obtained as the slope of the linear correlations between k_{obs} and buffer concentration. Finally, second order rate constants in acetate and acetic acid were extrapolated from the linear buffer ratio plots. All relevant to general base catalysis rates were obtained when either the APH⁺ or APyrH⁺ rings remained completely protonated. The pKₐ of the N/I' position of APH⁺ in MThH⁺ has been determined to be approximately 5.7 (see Appendix A). There are no reasons to believe that the pKₐ of APH⁺ in 2 would differ significantly from this, hence performing tests at pH below 4.7 assured complete
protonation. The $pK_a$ of APyrH$^+$ is $\sim 9^{[5]}$ and thus it will remain completely protonated in acetate buffers.

6.2.5 Solvent kinetic isotope effect

The SKIE was determined at pH 4.6 in 0.50 M acetate buffer. Deuterium oxide was used instead of water for buffer preparation.

6.3 Results

6.3.1 General

The rate of decarboxylation of 1 and 2 was unaffected by pH change when the acetate buffer effect was probed (pH 3.8–5.6 for 1, and pH 3.8–4.6 for 2). However, at pH 8, both 1 and 2 decarboxylated significantly slower, which is consistent with the effect of deprotonation of APH$^+$ to AP, and APyrH$^+$ to APyr, respectively. This confirmed that both 1 and 2 had their heteroaromatic rings completely protonated when the buffer effect was investigated.

6.3.2 Acetate effect

![Figure 6.3. An acetate buffer ratio plot for the decarboxylation of 1 at 25 °C, where $k_2$ is the second order rate constant in buffer.](image)

Figure 6.3. An acetate buffer ratio plot for the decarboxylation of 1 at 25 °C, where $k_2$ is the second order rate constant in buffer.
Figure 6.4. An acetate buffer ratio plot for the decarboxylation of 2 at 25 °C, where $k_2$ is the second order rate constant in buffer.

Table 6.1. Compiled kinetic results for 1 and 2. The previously reported results\cite{6} for MThH$^+$ are also shown for comparison.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>$10^{-4}k_{\text{obs}}$ s$^{-1}$</th>
<th>$10^{-4}k_2$ in AcO$^-$ M$^{-1}$s$^{-1}$</th>
<th>$10^{-4}k_2$ in AcOH M$^{-1}$s$^{-1}$</th>
<th>SKIE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5.7(±0.1)</td>
<td>0.7(±0.03)</td>
<td>1.4(±0.4)</td>
<td>1.0(±0.1)</td>
</tr>
<tr>
<td>2</td>
<td>2.9(±0.1)</td>
<td>0.8(±0.04)</td>
<td>9.7(±0.2)</td>
<td>1.0(±0.1)</td>
</tr>
<tr>
<td>MTh</td>
<td>4.2(±0.1)</td>
<td>24</td>
<td>≈0</td>
<td>1.8</td>
</tr>
</tbody>
</table>

6.3.3 Solvent kinetic isotope effect

Both the decarboxylation of 1 and 2 have a SKIE equal to approximately unity.

6.3.4 Product Study

$^1$H NMR spectra indicated that 1 decarboxylates to a single product (at pH 4.6 and 7.0) shown in Figure 6.5. Both integration and splitting patterns in the $^1$H-NMR spectrum (Figure 6.6) were consistent with the expected protonation product ($^1$H NMR (400 MHz, CD$_3$OD) δ 7.87 (d, $J$ = 6.9 Hz, 1H), 7.47 (d, $J$ = 7.0 Hz, 2H), 7.24 (t, $J$ = 7.3 Hz, 2H), 7.18 (t, $J$ = 7.3 Hz, 1H), 6.89 (s, 1H), 6.85 (d, $J$ = 6.9 Hz, 1H), 6.46 (s, 1H), 5.52 (d, $J$ = 18.0 Hz, 1H), 5.45 (d, $J$ = 18.0 Hz, 1H), 3.97 – 3.83 (m, 2H), 3.17 (t, $J$ = 5.5 Hz, 1H), 2.37 (s, 1H)). MS(ESI+) spectrum showed a strong peak at the expected m/z value of 356.1 (m/z: 356.1 calculated for C$_{19}$H$_{22}$N$_3$O$_2$S$^+$)
Figure 6.5. Structure of the product of decarboxylation of 1 in an aqueous buffer.

Figure 6.6. An $^1$H NMR (400 MHz, CD$_3$OD) of the reaction mixture after the decarboxylation of 1 was completed at 25 °C in a pH 4.6 or 7.0 buffer.

The decarboxylation of 2 also shows a clean conversion to a single protonation which structure is shown in Figure 6.7. The $^1$H NMR spectrum (Figure 6.8) was obtained in DMSO-d$_6$ because in CD$_3$OD or D$_2$O the residual solvent peak overlaps with that of the C2α proton peak ($^1$H NMR (400 MHz, DMSO-d$_6$) δ 8.50 (s, 1H), 6.61 (d, $J = 17.7$ Hz, 1H), 6.41 (d, $J = 17.8$ Hz, 1H), 5.96 (b, 1H), 4.50 (t, $J = 5.6$ Hz, 2H), 3.86 (t, $J = 5.6$ Hz, 2H), 3.36 (s, 3H), 3.14 (s, 3H), 2.59 – 2.37 (m, 6H), 2.17 – 1.81 (m, 4H)). The C2α proton peak appears as a broad singlet (5.96 ppm) and not as the expected doublet. This indicates that the dihedral angle between the C2α hydrogen and the adjacent cyclohexane hydrogen is approximately 0°, resulting in a small coupling constant (see Karplus
plot\textsuperscript{[7]}. MS(ESI+) has a large peak at m/z 377.2 (m/z: 377.2 calculated for C\textsubscript{19}H\textsubscript{29}N\textsubscript{4}O\textsubscript{2}S\textsuperscript{+}), confirming formation of the expected decarboxylation product.

![Structure of the product of decarboxylation of 2 in aqueous buffers.](image)

**Figure 6.7.** Structure of the product of decarboxylation of 2 in aqueous buffers.

![NMR spectrum](image)

**Figure 6.8.** \textsuperscript{1}H NMR (400 MHz, DMSO-d\textsubscript{6}) spectrum of the reaction mixture when the decarboxylation of 2 was completed at pH 4.6 and 7.0 (25 °C).

### 6.4 Discussion

### 6.4.1 Lack of general base catalysis

The rate constant for the decarboxylation of 1 and 2 in acetate buffers at 25 °C is approximately $5.7 \times 10^{-4}$ s\textsuperscript{-1} and $2.9 \times 10^{-4}$ s\textsuperscript{-1}, respectively. This is similar to the decarboxylation rate of MThH\textsuperscript{+} ($\sim 4.2 \times 10^{-4}$ s\textsuperscript{-1}).\textsuperscript{[6]} The decarboxylation of 1 and 2 leads to formation of a single product through a formal replacement of the carboxyl group by a proton. This was confirmed by \textsuperscript{1}H NMR and MS.
It was also verified that the heteroaromatic rings in 1 and 2, were completely protonated when the acetate effect or SKIE was probed. The second order rate constants in AcO$^-$ are several times smaller than the corresponding 1$^{st}$ order rate constants, which is the opposite to what was observed in the decarboxylation of MThH$^+$ (Table 6.1). Moreover, the effect of AcO$^-$ on the decarboxylation of 1 and 2 is smaller than that of the AcOH. This is likely a result of a non-specific medium effect. Consistent with the lack of general base catalysis, there is no SKIE. Based on this, I cannot conclude that the decarboxylation of 1 and 2 involves loss of HCO$_3^-$ or H$_2$CO$_3$. These results led me to consider whether the models adequately mimic the factors which are believed to be necessary for observing the proposed catalysis.

6.4.2 Strength of the intramolecular hydrogen bond in 1

In MThH$^+$, the intramolecular bond between the amino group and the carboxylate group was proposed to be crucial for the decarboxylation to occur via formation of HCO$_3^-$ from the orthoacid intermediate. The strength of this interaction will depend on the pK$_a$ difference between the hydrogen bond donor and the conjugate acid of the acceptor.$^{[4]}$ The acidity of the amino group in MThH$^+$ is enhanced because of its iminium-like character, and its pK$_a$ is estimated to be 12.1 based on the pK$_a$ of N1'-methyl-4-aminopyrimidine.$^{[8]}$ This is fifteen orders of magnitude more acidic than aniline (pK$_a$ of ~28)$^{[9]}$ The acidity of the amino group in 1 was similarly approximated to be 12.6$^{[10]}$ Therefore, based on the acidity of the amino groups, APyrH$^+$ is expected to form an equally strong hydrogen bond.

6.4.3 Steric crowding at the C2α position in 2

Decarboxylation of LThH$^+$ is not base catalyzed. It was hypothesized that the bulkiness of the Bn group is needed to ‘lock’ the intramolecular hydrogen bond. Consistent with this, the optimized structure of LThH$^+$ does not show an internal hydrogen bond. We can use van der Waals (VDW) volumes of substituents as an indicator of their overall steric effect. The VDW volumes of a methane, benzene and cyclohexane molecule were calculated by the method of Abraham and co-workers,$^{[11]}$ and are 26 Å$^3$, 96 Å$^3$ and 104 Å$^3$, respectively. As we can see, a cyclohexyl ring should accurately mimic the overall steric crowding at the 2α position in MThH$^+$. 
6.4.4 Further criteria for general base catalyzed decarboxylation

The absence of base catalysis in the decarboxylation of 1 and 2 contrast with the observations for MThH\(^+\). The basis of this remains to be established as the criteria I tested with the analogues did not lead to observable catalysis. If the intramolecular hydrogen bond between the amino group and the carboxylate group is necessary for the base promoted decarboxylation, there must be further requirements for formation of the intramolecular hydrogen bond. It is also possible that the internal hydrogen bond observed by Howe in the optimized structure of MThH\(^+\) and its general base catalyzed decarboxylation are simply a coincidence. It is also possible that the phenyl group and the APH\(^+\) group participate in a \(\pi-\pi\) or \(\pi\)-cation interaction that forces NH\(_2\) to H-bond to the carboxyl. Regardless of what is responsible for general base catalyzed decarboxylation, small changes to the structure of MThH\(^+\) eliminate this mode of catalysis. Further studies are needed to understand why decarboxylation of MThH\(^+\) is general base catalyzed.

6.5 References


Chapter 7
Complex Reactivity of the Benzyolformate-derived Breslow Intermediate

7.1 Introduction

My improved synthesis of MTh provided sufficient amounts of pure material for reliable analysis of product distributions in various buffer solutions. It is well established that MTh decarboxylates to the Breslow intermediate. This is protonated at C2α to form HBnTh. In addition, the Breslow intermediate spontaneously fragments to PTK and DMAP (Scheme 7.1). Ian Moore determined that the rate constant for the fragmentation is approximately $10^4$ s$^{-1}$ at 40 °C, which is much faster than the decarboxylation step.$^{[1]}$

![Scheme 7.1. Decarboxylation of MTh in neutral buffers leads to formation of the Breslow intermediate, which undergoes protonation, fragmentation and rearrangement.](image)

PTK and DMAP were reported by Oka as unexpected products in the condensation of thiamin with benzaldehyde.$^{[2]}$ Oka also reported formation of small amounts of an isomer of HBnTh (isoHBnTh, Scheme 7.2).

The extent of fragmentation of the Breslow intermediate rapidly diminishes with increasing hydronium and buffer concentration from competing protonation to give HBnTh.$^{[3]}$ I found that formation of PTK and DMAP is insignificant at pH below 5.4 and buffer concentrations above 0.10 M. For this reason, it had been probably assumed that the product of decarboxylation of MTh
under acidic and strongly acidic conditions results only in the formation of HBnTh. However, my detailed study of this reaction in aqueous buffers and acid solutions by $^1$H NMR revealed that the product distribution is more complex. HBnTh is never the sole product, even in highly acidic solutions. Some products, such as thiamin and benzoic acid, are readily apparent but their origin turns out to be unexpected. In addition, two previously unknown products (1, 2) form in significant amounts below pH 6.0. Compound 1 forms in solutions whose pH ranges from 2.0 to 6.0 while 2 forms in solutions with acidities greater than 0.1 M HCl. With the aid of mass spectrometry, 2D NMR, deuterium incorporation, and analysis of proton splitting patterns, I assigned logical structures for those species. Reasonable mechanisms for formation of the various products of decarboxylation of MTh are proposed.

Scheme 7.2. The fate of the BF-derived Breslow intermediate is far more complex than previously assumed.

7.2 Experimental

7.2.1 Materials

Commercially available reagents were used without further purification. Other compounds such as MTh, HBnTh, PTk, DMAP or isoHBnTh were prepared as described before.\textsuperscript{[4]} MTh was used as an 1–3 M solution in concentrated hydrochloric acid, which was stored at -80 °C. All MTh
samples were ~99% pure by $^1$H NMR, and lacked peaks associated with HBnTh, DMAP, PTK, isoHBnTh thiamin and benzoic acid.

### 7.2.2 Instrumentation

$^1$H NMR spectra were typically recorded using 600 MHz or 500 MHz instruments. Reactions that were conducted in the absence of air were prepared using five cycles of freeze-pump-thaw, and transfers were done using Schlenk line techniques under argon. Collected data were analyzed to produce $^1$H NMR, gCOSY, and C–H gc2HSQCse and gc2HMBC spectra.

A technician obtained mass spectra, using either electron spray ionization (ESI) or direct analysis in real time (DART), both in positive mode.

### 7.2.3 Decarboxylation in buffers

Decarboxylation reactions of MTh that were run in aqueous buffers were acidified with concentrated hydrochloric acid when completed and roto-evaporated. The residues were dissolved in deuterated solvents for NMR analysis. Acidification was necessary to prevent base promoted decomposition as the buffer was evaporated to dryness. Reactions in deuterated buffers were analyzed directly. A 1.0 M concentrated buffer was sufficient to maintain the desired pH after addition of the MTh solution (1-5 µL per 600-1000 µL of buffer). For lower concentrations, the buffer was prepared in situ by mixing the highly acidic MTh sample with an appropriate amount of a 0.5-1 M solution of the base component of the buffer. Whatman® Panphea™ pH indicator strips were used for pH determinations. Reactions conducted in deuterated buffers were set up in the same way as in water so that pD = pH. The pH was tested both at the start and end of the reaction. Most product determinations were done at pH 7.0, 4.6 and in 1.0 M acid. Reactivity at intermediate acidities was also checked (pH 2.0 and 5.4).

### 7.2.4 Product identification

Product identifications are based on $^1$H NMR spectral comparisons, spiking with authentic reagents, and by mass spectrometry. Unfortunately, I could not isolate compounds 1 and 2 because both have retention factors practically identical to HBnTh on silica and with various elution mixtures. Fortunately, compounds 1 and 2 form under different conditions, and their peaks show
little overlap with remaining known products. Therefore, by elimination, $^1$H NMR peaks were assigned to 1 and 2.

When studying 1, the reaction buffer was acidified with concentrated hydrochloric acid and washed with dichloromethane to remove PTK and other nonpolar products. The aqueous solution was roto-evaporated and triturated with isopropanol. The alcohol layer was filtered and evaporated to dryness to obtain a mixture containing mostly HBnTh and 1. It was verified that these steps did not affect formation 1. This was done because it provides a purer product for $^1$H NMR analysis. Nonetheless, 1 and 2 are always formed in a mixture with HBnTh. Products that were obtained in reactions performed in deuterated buffers were treated with water to replace exchangeable deuterium atoms, avoiding complications in mass spectral analysis.

7.3 Results

7.3.1 General

The relative amounts of various products of decarboxylation are not dependent on the initial concentration of MTh. The decarboxylation reaction in deuterated buffers resulted in more products than are not HBnTh. As the pH is decreased from 7.0 to 5.0, the fragmentation products diminish and the amount of 1 increases. Further lowering of pH decreases the amount of 1. In solutions more acidic than 0.10 M $\text{H}^+$, 2 forms in an increasingly greater proportion relative to HBnTh. Thiamin and benzoic acid are formed at pH as low as 2.0.

7.3.2 pH 6–7

7.3.2.1 Formation of isoHBnTh

At pH between 6.0 and 7.0, the main products of decarboxylation of MTh are HBnTh, PTK and DMAP, consistent with previous reports (Figure 7.1). However, a careful spectral comparison and spiking with an authentic sample revealed that the rearrangement product (isoHBnTh) forms in very small amounts (Figure 7.2). Thiamin and benzoic acid are also formed.
Figure 7.1. $^1$H NMR spectrum of the product mixture from the decarboxylation of MTh in a pD 7.0 buffer.
Figure 7.2. The $^1$H NMR spectrum of a genuine sample of isoHzBTh (top) stacked over the $^1$H NMR spectrum of the reaction mixture of the decarboxylation of MTh at pH 7.0 buffer. All isoHBnTh peaks have a match in the reaction product mixture as indicated with arrows.

7.3.3 pH 2–5

The major products of decarboxylation of MTh in solutions between pH 2.0 and pH 5.0 are HBnTh and 1, which forms at ~ 30 mol% (Figure 7.3). Fragmentation products are still observed but they decrease with increasing acidity. In addition, thiamin and benzoic acid form in a 1:1 molar ratio.
Figure 7.3. A typical $^1$H NMR spectrum of the product mixture obtained from the decarboxylation of MTh in a pD = 4.6, 1.0 M acetate buffer.

7.3.3.1 Formation of thiamin

$^1$H NMR spectral comparison and spiking with a genuine thiamin sample indicates that thiamin is a product in the decarboxylation of MTh at pH 4.6 (Figure 7.4). Performing the reaction under strict oxygen-free conditions does not affect the formation of thiamin. Thiamin also forms during the decarboxylation of MTh solution with pH as low as 2.0.
Figure 7.4. $^1$H NMR (600 MH, D$_2$O) spectrum of thiamin hydrochloride (top) and $^1$H-NMR spectrum (600 MHz, D$_2$O) of the decarboxylation of MTh in a pH 4.6 buffer (bottom). All thiamin peaks show a match in the reaction product mixture as indicated with arrows.

7.3.3.2 Formation of benzoic acid

MTh was left to decarboxylate at pH 4.6. The buffer was acidified and extracted four times with dichloromethane. After evaporation of the organic solvent, the residue was dissolved in CDCl$_3$ and a $^1$H NMR spectrum was obtained. Spectral comparison and spiking with genuine benzoic acid and PTK results in an exact match for both compounds (Figure 7.5). MS(DART+) of the dichloromethane extract showed a peak expected for benzoic acid. Therefore, I concluded that benzoic acid is a product of decarboxylation of MTh at pH 4.6. The same reaction conducted under strict oxygen-free conditions did not produce benzoic acid. Instead, benzaldehyde is produced. As with the formation of thiamin, benzoic acid forms between pH 2.0 and pH 7.0.
**Figure 7.5.** $^1$H NMR (600 MHz, CDCl$_3$) spectrum (top) of a PTK:benzoic acid mixture in a 1:5 molar ratio, respectively, stacked over an $^1$H NMR (600 MHz, CDCl$_3$) spectrum (bottom) of the ether extract from the product mixture of the decarboxylation of MTh in a pH 4.6 buffer. Both benzoic acid and PTK show a match.

### 7.3.3.3 Formation of compound 1

Critical peaks that I needed to propose the structure of 1 are overlap partially with the residual water peak (Figure 7.6). Therefore, I used DMSO-d$_6$ as the solvent for $^1$H NMR. Mass spectra (ESI +) show a significant peak at m/z = 390.1 and a smaller peak at m/z = 371.2 (HBnTh). In deuterated buffers, 1 produced to peaks at m/z = 391.1 and 372.2.
Figure 7.6. $^1$H NMR (500 MHz, D$_2$O) of the iPrOH extract from decarboxylation of MTh in a pH 4.6 buffer. In addition to the expected product (HBnTh), an unknown product is formed in ~30 mol% (peaks marked as !)

Figure 7.7. The 3–6 ppm region in the $^1$H NMR (500 MHz) spectrum of 1/HBnTh mixture in DMSO-d$_6$. 
Figure 7.8. gCOSY (600 MHz, DMSO-d$_6$) of the 3–6 ppm region of compound 1. The four diagnostic proton peaks (peaks marked as !) are coupling to each other.
Figure 7.9. $^1$H NMR (500 MHz, DMSO-d$_6$) of the 3–6 ppm region of compound 1 obtained from decarboxylation in a deuterated buffer solution. Only three of the four diagnostic proton peaks are seen (marked as !) but with changed splitting patterns. The expected position of the 4th peak is marked with an x.

Table 7.1. Splitting patterns and coupling constants of the "diagnostic" $^1$H NMR (500 MHz, DMSO-d$_6$) peaks from compound 1.

<table>
<thead>
<tr>
<th>shift [ppm]</th>
<th>splitting pattern</th>
<th>coupling constant $J$ [Hz]</th>
<th>relative integration</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.31</td>
<td>d</td>
<td>10.2</td>
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<td>5.09</td>
<td>dd</td>
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<td>1</td>
</tr>
<tr>
<td>4.66</td>
<td>dd</td>
<td>14.5; 9.3</td>
<td>1</td>
</tr>
<tr>
<td>3.34</td>
<td>td</td>
<td>9.4; 6.7</td>
<td>1</td>
</tr>
</tbody>
</table>
Table 7.2. Splitting patterns and coupling constants of the "diagnostic" $^1$H NMR (500 MHz, DMSO-$d_6$) peaks (!) from compound 1 that was produced in a deuterated buffer.

<table>
<thead>
<tr>
<th>shift [ppm]</th>
<th>splitting pattern</th>
<th>coupling constant $J$ [Hz]</th>
<th>relative integration</th>
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<tr>
<td>5.31</td>
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<tr>
<td>5.09</td>
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</tr>
<tr>
<td>4.67</td>
<td>d</td>
<td>14.4</td>
<td>1</td>
</tr>
</tbody>
</table>

7.3.4 pH 0

MTh decarboxylates in 1.0 M hydrochloric acid to form HBnTh, and an unknown product (2) which forms in ~ 50 mol% or ~ 75 mol% depending on whether the reaction is in HCl or DC1, respectively. There are no spectral differences between products from a reaction run in HCl or DC1, apart from a larger amount of 2 and lack of the C2α proton peak in HBnTh as expected for the D$_2$O reaction.

The peaks from compound 2 overlap partially with that of residual water and HBnTh in D$_2$O (Figure 7.10). Therefore, the products were analyzed in DMSO-$d_6$ which was acidified with concentrated DC1 for sample stability. Addition of 1/HBnTh to 2/HBnTh results in three sets of peaks in the $^1$H NMR spectrum.

The mass spectrum (ESI+) of the 2/HBnTh mixture shows a major peak at m/z = 372.3 and a minor peak at m/z = 371.3 (HBnTh). The 2/HBnTh mixture obtained from a reaction in 1.0 M DC1 has two major peaks at m/z 372 and 373.3.
7.3.4.1 Formation of compound 2

In addition to the expected product (HBnTh), an unknown 2 is formed in ~50 mol% (peaks marked as #)

**Figure 7.10.** $^1$H NMR (500 MHz, D$_2$O) of the product mixture from decarboxylation of MTh 1.0 M HCl.
**Figure 7.11.** gCOSY (500 MHz, DMSO-<i>d</i><sub>6</sub>) of the 3 to 6 ppm region of compound 2/HBnTh mixture obtained from the decarboxylation of MTh in a 1.0 M HCl solution. There are three diagnostic proton peaks, which are marked as #.

**Table 7.3.** Splitting patterns and coupling constants of the “diagnostic” <sup>1</sup>H NMR (500 MHz, DMSO-<i>d</i><sub>6</sub>) peaks from compound 2.

<table>
<thead>
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<td>d</td>
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</table>
7.4 Discussion

7.4.1 IsoHBnTh

The rearrangement product (isoHBnTh) of HBnTh is present to an extent that is \(\sim 10\) mol\% of the fragmentation products (PTK and DMAP), which are always present in equal concentrations. The extent of formation isoHBnTh relative to the fragmentation products is independent of the initial concentration of MTh, indicating that its formation is not the outcome of a second order reaction. Thus, it forms from the direct decomposition of the Breslow intermediate. A simple step-wise mechanism via formation of a DMAP-derived carbanion has been proposed to account for both the fragmentation and rearrangement of the Breslow intermediate (Scheme 7.3).\[^4\] This and other
potential mechanisms for the rearrangement and fragmentation reaction are discussed in more detail in the following chapter.

![Diagram](image)

**Scheme 7.3.** Proposed mechanism for formation of the rearrangement product.

### 7.4.2 The origin of thiamin and benzoic acid

The formation of thiamin and benzoic acid in a pH 7.0 buffer can result from a slow elimination reaction from the conjugate base of HBnTh. Benzaldehyde would then be rapidly oxidized by air to benzoic acid (Scheme 7.4).

![Diagram](image)

**Scheme 7.4.** A possible route of benzoic acid and thiamin, both which are observed to form during the decarboxylation of MTh in aqueous buffers.

Thiamin and benzoic acid were also formed during the decarboxylation of MTh at pH 4.0 and as low as pH 2.0. I verified that under these conditions, HBnTh is completely stable and the reaction presented in Scheme 7.4 does not occur to any extent. It is likely that the Breslow intermediate is oxidized to a 2-benzoylthiamin intermediate that rapidly hydrolyzes to thiamin and benzoic acid (Scheme 7.5). Oxidation of Breslow-like intermediates by oxygen is well known and exploited in synthesis.
Scheme 7.5. The Breslow intermediate can be oxidized by oxygen to form a 2-benzoylthiamin intermediate that undergoes rapid hydrolysis to thiamin and benzoic acid.

However, the decarboxylation of MTh under strict oxygen-free conditions still produces thiamin but not benzoic acid. Appearance of certain $^1$H NMR peaks in the aromatic region was consistent with formation of benzaldehyde. The results led me to consider that the Breslow intermediate undergoes a water-assisted intramolecular proton transfer that generates the C2α–OH conjugate base of HBnTh. This decomposes rapidly to thiamin and benzaldehyde (Scheme 7.6).

Scheme 7.6. Mechanism for formation of thiamin and benzoic acid from the BF-derived Breslow intermediate.

He et al. used high level computations to demonstrated that a Breslow intermediate can be efficiently generated from the aldehyde adduct by a solvent assisted intramolecular proton transfer which competes with the intermolecular process (Scheme 7.7).[8] This process is the reverse of what I proposed above.
Scheme 7.7. He et al. proposed that the Breslow intermediate can form from the conjugate alkoxide base of a triazolium-aldehyde conjugate.

The computational work is therefore consistent with my proposal. A question now arises if the decomposition of the tetrahedral intermediate to thiamin can compete with a diffusion controlled protonation of the C2α alkoxide intermediate. Indeed, decomposition of tetrahedral intermediates can compete with a fast protonation. This has been determined, for example, from the study of hydroxide catalyzed hemiortho ester hydrolysis (Scheme 7.8).

Scheme 7.8. Proposed mechanism for base catalyzed hydrolysis of hemiortho esters.

With an observable rate constant in hydroxide in the range of $10^{10} - 10^{11} \text{ M}^{-1} \text{ s}^{-1}$ and a protonation rate constant ($k_{-1}$) of $10^7 - 10^8 \text{ s}^{-1}$, the decomposition of the tetrahedral intermediate has to be close to a diffusion controlled process.$^9$ Further support comes from Bender’s measurements of $^{18}$O isotope exchange during saponification of benzoate esters.$^{10}$ Observation of a small incorporation of $^{18}$O isotope into the ester carbonyl group during base-catalyzed hydrolysis is consistent with a very low barrier to decomposition of the tetrahedral intermediate. For the ester carbonyl oxygen to exchange, the intermediate must be first protonated (Scheme 7.9).

Scheme 7.9. Mechanism for $^{18}$O isotope incorporation during hydroxide catalyzed hydrolysis of benzoate esters.
Therefore, both computational results and rates of decomposition of tetrahedral intermediates support my explanation of how thiamin and benzoic acid are formed during the decarboxylation of MTh in acidic buffers. This shows that in principle the elimination of the product of a thiamin catalyzed decarboxylation of benzoylformic acid can occur without formation of HBnTh. This could have an important implication in understanding the related enzymic mechanism. By not forming the stable HBnTh, the enzyme avoids having the intermediate fall into an “energetic well” that would lower the rate of the catalytic turnover.[11]

7.4.2 Compound 1

As mentioned before, I could not isolate compound 1 in pure form but only as a mixture with HBnTh. Fortunately, both products show distinct peaks in the $^1$H NMR and spectral analysis were successfully performed on the mixture.

The starting point in elucidating the structure of 1 came from the disappearance of the proton signal from the C6’ (aromatic) and C2α protons in HBnTh. Both peaks appear as singlets (> 6 ppm) in $^1$H NMR spectra of HBnTh. The characteristic two doublets associated with the diastereotopic bridging protons in the CH$_2$ group of HBnTh are also absent (Figure 7.6). Instead, there are two doublets of doublets with a common $J$-value of 14.5 Hz. It was this large $J$-value that allowed me to identify them as the geminal hydrogens. gCOSY also showed that both protons are coupled to a single proton at 3.4 ppm (Figure 7.8), which is not a pre-existing proton because its signal is absent when the decarboxylation of MTh is carried out in a deuterated buffer (Figure 7.9). Finally, the signal at 3.4 ppm couples to a signal at 5.3 ppm, which most likely comes from the former aromatic C6’ hydrogen. MS shows a significant peak at m/z = 390.2, suggesting that 1 has a molecular formula of C$_{19}$H$_{24}$N$_3$O$_4$S$^+$. Thus the –NH$_2$ has been replaced by an –OH. The proposed structure for 1 and deuterated 1 are depicted in Figure 7.13 and Figure 7.14.
Figure 7.13. Proposed structure of 1.

Figure 7.14. Proposed structure of monodeuterated 1.

Splitting constant from a 3-bond proton-deuterium coupling is very small and not observed in our Fourier transformed \(^1\)H NMR spectra. Consistent with this, the deuterated form of 1 has the CH\(_2\) and C6’ proton signals appear as two doublets and a singlet, respectively. As expected, MS gives a peak at m/z 391.2 [M+1].

I proposed that the sequence of steps leading to 1 starts with an intramolecular addition of the C2\(\alpha\) carbon to the C6’ position in the Breslow intermediate. Such a transformation occurs in the known reactivity of pyrimidines towards nucleophilic additions. For example, Zoltewicz\(^{12}\) demonstrated that decomposition of thiamin in aqueous sulfite solutions is initiated by a nucleophilic addition of the SO\(_3^2\)- anion at the C6’ position of the AP ring. The intermediate that follows the intramolecular addition step tautomerizes to an amidine-like intermediate. Subsequent hydration steps would lead to the final product (Scheme 7.10)
According to works of Zoltewicz, the addition of sulfite to the C6’ position induces homolysis of the C–N\(^+\) bond to form a carbocation intermediate. Following this, I have also considered an alternative structure for the unknown (1’) and a mechanism for its formation (Scheme 7.11 and Figure 7.15).

**Scheme 7.11.** An alternative structure for 1 and a mechanism for its formation based on Zoltewicz’s carbocation intermediate.

However, \(^1\)H NMR simulations in MestReNova show that the two geminal protons in 1’ will be significantly more up-field (3.7–4.1 ppm) than in structure 1 (4.3–4.8 ppm). The experimental
shifts are at 4.7 and 5.1 ppm, and thus consistent with the simulated shifts for structure 1. Therefore, it seems more likely that the CH₂–N⁺ connectivity is preserved and the product is 1 and not 1’.

![Structure 1'](image)

**Figure 7.15.** An alternative structure of the unknown product.

### 7.4.3 Compound 2

Determination of the structure of 2 was a more challenging task and its identity remains less certain. MS(ESI+) gives a peak at 372.1 which would correspond to a C₁₉H₂₂N₃O₃S⁺ molecular formula. Thus, it differs from HBnTh by replacement of an amino group with a hydroxyl. It was, however, excluded that 2 is 2-(1-hydroxybenzoyl)oxythiamin (Figure 7.16). MS(ESI+) of the product mixture of decarboxylation of MTh in 1.0 M DCl provides a large peak at 373.1, consistent with incorporation of a single deuterium. However, the same sample also shows a significant peak at 372.1, suggesting that this deuterium is slowly exchanging when the sample is treated with water before submitting to MS (this is done to replace exchangeable deuteriums). ¹H NMR results support the exchange hypothesis because there are no obvious peaks missing in the ¹H NMR of the product mixture when the decarboxylation of MTh is performed in 1.0 M DCl (Figure 7.10).

![Structure 1’](image)

**Figure 7.16.** Structure of 2-(1-hydroxybenzoyl)oxythiamin.

Like compound 1, the spectrum of 2 shows a set of two doublets with a $J$-value of 15.4 Hz, characteristic for a 2-bond splitting, hence they were assigned to the bridging methylene group.
Like compound 1, 2 did not show the characteristic aromatic C6’ peak, suggesting disruption of the APH+ aromaticity by an intramolecular addition. Consistent with this, there is a singlet at 5.17 ppm, most likely the former aromatic C6’ hydrogen. This was confirmed by gc2HSQCse (Figure 7.12) were the proton at 5.17 ppm is coupled to a non-aromatic carbon, as evidenced from the carbon signal’s upfield position of ~ 55 ppm.

The formation of 1 decreases from pH ~ 4.6 to ~ 2 without significant amounts of 2 being formed. Compound 2, however, is produced in 50 mol% in 1.0 M acid. This is an important observation because it suggests that the intramolecular addition diminishes with increasing acidity only to reappear at very high acidities. Such behavior is consistent with a faster protonation of the Breslow intermediate to HBnTh as a result of a greater H3O+ concentration. The formation of 2 at very high acidities, however, suggests a rate enhancement of the intramolecular addition process to a point where it again competes with protonation. This is further supported by a higher yield of 2 if the decarboxylation reaction is carried in a deuterium chloride solution because of a slower D+ transfer due to the kinetic isotope effect. HBnTh did not show any signs of decomposition in 1.0 M acid, even after several days at room temperature. Therefore, 2 must be forming from the Breslow intermediate and not from HBnTh. The enhancement of electrophilicity of the APH+ ring is probably a result of a secondary protonation to APH23+.

The signal for the bridging CH2 protons in 2 appears as two doublets, demonstrating that they have no neighboring protons. This and the replacement of the NH2 group by an OH, as suggested by MS results, led me to believe that the structure of 2 is as depicted in Figure 7.17.

![Figure 7.17. Proposed structure of 2.](image)

As in the proposed mechanism for the formation of 1, it seems likely that the formation of 2 starts with an intramolecular addition of the 2α carbon to the APH+ ring. However, rather than adding to the C6’ position of the heteroaromatic ring, the carbon nucleophile adds to the C4’ position (Scheme 7.12). The change in the site of the nucleophilic addition is probably a result of
protonation of the second nitrogen at very high acidities. This follows a loss of ammonia and aromatization to a pyrimidine ring. Thus, a formal intramolecular nucleophilic aromatic substitution has occurred. An addition of water to the pyrimidine ring yields 2. Although addition of a water nucleophile to a heteroaromatic ring is not expected, examples of water additions across aromatic C=N bonds are well known. Many cations derived from related heterocyclic compounds exist predominantly as covalent hydrates in acidic aqueous solutions.\textsuperscript{[13]}

![Diagram of proposed mechanism](image)

**Scheme 7.12.** Proposed mechanism for formation of 2 from the MTh-derived Breslow intermediate.

### 7.4.4 Why side-products are not observed in decarboxylation of LThH\textsuperscript{+}?

Lactylthiamin (LThH\textsuperscript{+}) differs from MTh only by substitution of the benzene ring with a methyl group. Interestingly, LTh decarboxylates in aqueous buffers only to form the protonation product (HBnTh). Lack of side products, is consistent with a diffusion controlled \((k = 10^{10-11} \text{ s}^{-1})\) protonation of the LTh-derived Breslow intermediate, as reported by Washabaugh.\textsuperscript{[14]} In contrast, protonation of the BF-derived Breslow intermediate is relatively slow \((k \sim 10^{4-5} \text{ s}^{-1})\).\textsuperscript{[15]} Such a fast quench will effectively “protect” the Breslow intermediate from various side reactions that I described in this chapter.
7.5 References


Chapter 8
Are radicals involved in the fragmentation of the Breslow intermediate?

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(see Appendix C for supporting information)

8.1 Overview

8.1.1 Radicals

Interest in Breslow intermediates has increased in recent years due to their many applications and unique reactivity, culminating in spectroscopic and X-ray crystallographic observations by Berkessel in 2012.[1] We know that the BF-derived Breslow intermediate undergoes a rapid fragmentation to PTK and DMAP.[2] While our group noted that the products had been reported by Oka in 1970, there had been no effort directed at the mechanism of the fragmentation itself as it was beyond the focus of catalytic reactions related to thiamin. The increased interest in the structure and reactivity of Breslow intermediates reawakened interest in discovering the mechanism of the Oka fragmentation and related transformations. In 2016, McIntosh and co-workers proposed a clever radical-based mechanism for the rearrangement and fragmentation of Breslow intermediates derived from thiazolium (Scheme 8.1, X = C, Y = S) or triazolium (Scheme 8.1, X, Y = N).[3] McIntosh proposed that this class of intermediates fragment and rearrange by the homolytic production of a pair of radicals whose separation leads to products that include those that were first reported by Oka.

In support of the idea of radical pathways, McIntosh et al. observed EPR signals when the reactions occur in the presence of spin traps. In addition, they computed low enthalpies for homolysis of the investigated Breslow intermediates.

\[
\text{Scheme 8.1. A radical rearrangement of Breslow intermediates.}
\]
In addition to recombination, the radical pair could undergo disproportionation, leading to the observed Oka fragmentation. Thus, the authors suggested that this is likely also the mechanism for fragmentation of the BF-derived Breslow intermediate to PTK and DMAP (Scheme 8.2)

![Scheme 8.2. A radical mechanism for fragmentation of the MTh-derived Breslow intermediate.](image)

However, the reported EPR experiment was conducted under conditions with other potential sources of radicals, including reactions with oxygen since the experiment was performed under air.[4] Moreover, lack of quantification of the EPR signal precludes the EPR results as evidence for a radical mechanism. Nevertheless, McIntosh’s clever idea of using radicals to explain the fragmentation and rearrangement reaction of the Breslow intermediate remained a possibility. The publication that follows is the result of my independent study that focused on evaluation of evidence for radical mechanisms proposed by McIntosh for the Oka fragmentation.

From my spin trapping experiments, I found that there is only a negligible EPR signal from the Breslow intermediate. This means that either radicals are not formed or that they combine/disproportionate more rapidly than they can separate. The rate of combination of resonance-stabilized radicals is faster than disproportionation, with the disproportionation/combination ratio usually being much smaller than unity.[5] Therefore, the observed 10-fold excess of disproportionation products (PTK and DMAP) over the combination product (isoHBnTh) is inconsistent with the reactivity of typical resonance-stabilized radical pairs.

8.1.2 An enolate alternative

While my work indicated that the radical mechanism is unlikely, it was also important to provide a positive alternative for consideration. It was noticed that the enol derived from mandelic acid is structurally similar to the Breslow intermediate in terms of substitution with electronegative elements (Scheme 8.3). In the mandelic acid enol, the hydroxyl group has a measured $pK_a$ of ~ 6.

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**Scheme 8.3.** The BF-derived Breslow intermediate has similar structure to the mandelic acid enol.

Based on this, it is likely that 1,2-enaminol spontaneously ionizes in neutral buffers to the 1,2-enaminolate and this in turn can decompose directly to produce PTK and a DMAP-derived carbanion. The process is probably promoted by energy gained by formation of the aromatic thiazolium substituent. The carbanion that results can add to the carbonyl group of PTK to form isoHBnTh or be rapidly protonated to DMAP (Scheme 8.5). The low concentration of the rearrangement product relative to that of both PTK and DMAP is consistent with a fast protonation of carbanions in water. Incorporation of a single deuterium into the methyl group of the resulting DMAP when the Breslow intermediate is generated in deuterated buffers is consistent with this mechanism. The DMAP-derived carbanion is stabilized through resonance, and thus is a reasonable intermediate (Scheme 8.5).

**Scheme 8.4.** Proposed ionic mechanism for the fragmentation and rearrangement of the BF-derived Breslow intermediate.
Scheme 8.5. Resonance structures of the DMAP-derived carbanion.

8.1.3 Lack of fragmentation on the enzyme

On the BFDC the fragmentation reaction is not detected, and it was suggested that BFDC rapidly protonates the Breslow intermediate before it can fragment.\[6\] Reports that certain ThDP-dependent enzyme are enforcing a localized carbanion-like Breslow intermediates would be consistent with this since protonation of localized carbanions will be a barrierless process.\[7\] In solution, protonation of the BF-derived Breslow intermediate is relatively slow (10^{-6} s^{-1}), but protonation of the pyruvate-derived Breslow intermediate is diffusion controlled.\[8\] Consistent with this, decarboxylation of LTh in aqueous buffer does not produce side-products (see Chapter 7.5).

On the other hand, if there is no delocalization on the enzyme, the 1,2-enaminol intermediate never forms and the fragmentation does not occur. Therefore, the fragmentation is avoided not because of a fast protonation but because the intermediate leading to it does not form.

8.2 References


The Need for an Alternative to Radicals as the Cause of Fragmentation of a Thiamin-Derived Breslow Intermediate

Michael Bielecki and Ronald Kluger* 

Abstract: Mandelythiamin (1) is a conjugate of benzoylformate and thiamin that forms CO, to form the classic Breslow intermediate (2), whose expected fate is formation of the thiamin conjugate of benzaldehyde (3). Surprisingly, it was observed that 2 decomposes to 4 and 5 and rearranges to 6 in competition with the expected protonation to give 3. Recent reports propose that the alternatives to protonation arise from homolysis followed by radical-centered processes. It is now found, instead, that the spectroscopic observations cited in support of the proposed radical pathways are likely to be the result of other events. An alternative explanation is that ionization of the enolic hydroxy group of 2 and resultant electronic reorganization leads to C–C bond cleavage and non-radical intermediates that readily form 4, 5, and 6.

Breslow’s insightful studies showed that the formation of acyl carbanion equivalents in decarboxylases result from addition of a nucleophilic carbene derived from enzyme-bound thiamin diphosphate to 2-ketoesters. This led to generalizations in which the addition of thiamin-related N-heterocyclic carbones (NHC) to aldehydes lead to synthetically useful acyl carbanion synthons. Mandelythiamin (1, Scheme 1), the conjugate of thiamin and benzoylformate, loses CO, to produce the expected Breslow intermediate 2. Protonation gives isobutyl-1-(1-hydroxybenzyl)thiamin (3). An attempt by Oka to achieve a thiamin-catalyzed benzoin condensation of benzaldehyde via formation of 2 unexpectedly gave 4, 5, and 6 (Scheme 2). Studies of the reactions of 2 revealed its spontaneous fragmentation to Oka’s products 4 and 5 where the absorbance of 5 at 328 nm is characteristic, providing a spectroscopic handle for kinetic studies.

McIntosh and co-workers recently reported that N-heterocyclic carbones react with benzaldehyde to give products that parallel the outcomes in Scheme 2. They ascribe the results to reactions of radicals from homolysis of the Breslow intermediate observed in EPR spectra and computations. They propose that radicals lead to the products from 1 in Scheme 2, both the rearrangement and fragmentation forming via a radical pair from homolysis of the Breslow intermediate (Scheme 3). Recombination and disproportionation lead to both sets of products. Simulated EPR spectra of expected radicals are consistent with observed spectra and computations indicate that the proposed radicals are energetically accessible. Nonetheless, it is surprising to encounter formation of radicals under the conditions in which we see similar products from 2.

McIntosh and co-workers generate their Breslow intermediate in methanol by combining benzaldehyde with the NHC precursor and DBU in the presence of oxygen. Under these conditions, other reactions can produce radicals: 1) air-oxidation of benzaldehyde, 2) reaction of benzaldehyde with the Breslow intermediate, 3) reaction of benzaldehyde with the conjugate acid of the intermediate, 4) air-oxidation of the intermediate, and 5) air-oxidation of benzoin. These potential side reactions and lack of controls permit neither confident assignment of a signal to the proposed radical pair nor a basis for quantitation. Attempts to detect or quantify these side reactions prove fruitless.

Scheme 1. Decarboxylation of 1 in neutral aqueous solutions produces Breslow intermediate 2 followed by protonation to the thiamin–benzaldehyde adduct 3.

Scheme 2. Combining thiamin with benzaldehyde and triethylamine as base in refluxing methanol results in products 4, 5, and 6 via a fragmentation and rearrangement of 2, respectively.

Scheme 3. A radical mechanism, based on McIntosh and co-workers’ proposal, for the formation of 4, 5, and 6. The Breslow intermediate 2 undergoes a spontaneous decomposition to a radical pair. The pair undergoes disproportionation to form 4 and 5 and recombination to give 6.
isolate TEMPO adducts that would be expected from radicals also gave negative results.\(^7\)

It is well-established that 1 decarboxylates in neutral aqueous buffers to form 2, which rapidly undergoes fragmentation to 4 and 5 (and potentially other products).\(^7\) Thus, we prepared 1\(^{12}\) and analyzed the products of its decarboxylation in phosphate buffers in D$_2$O by $^1$H NMR spectroscopy. We added genuine samples of potential products and compared their NMR signals with those from the reaction in solution.\(^{13}\) We confirmed the formation of 3, 4, 5, and 6 in relative molar concentrations of 100:30:30:3. We had not previously noted formation of 6, as previous studies were concerned with the rate of the decarboxylation process. We also note the formation of small amounts (2-3 mol%) of thiamin and benzoic acid, which could result from hydrolysis of 2-benzoylthiamin upon oxidation of 2.\(^{14}\) However, in the absence of oxygen, the products do not include benzoic acid. In the presence of oxygen, benzaldehyde, produced from 3, reacts with oxygen to form benzoic acid.

We attempted to trap intermediate 7a, which would form from a radical pathway, by adding the water-soluble radical trap 4-hydroxy-2,6,6-tetramethylpiperidine 1-oxyl (4-hydroxy-TEMPO) to the reaction mixture. UV/Vis spectroscopy revealed formation of none of the characteristic band at 328 nm from 5.\(^{16}\) Lower concentrations of 4-hydroxy-TEMPO significantly decrease that band. Analogous reactions were conducted in D$_2$O and studied by $^1$H NMR spectroscopy. These reveal only two major products: thiamin and benzoic acid. This is consistent with oxidation of 2 by 4-hydroxy-TEMPO as reported by Studer and co-workers, who propose a single-electron transfer (SET): TEMPO or 4-hydroxy-TEMPO oxidizes the Breslow intermediate to an acyl derivative in 2:1 stoichiometry, respectively.\(^7\)

The aminoxy radical\(^{17}\) itself is reduced to the corresponding hydroxylamine. In the present case, oxidation of the intermediate produces 2-benzoylthiamin, which reacts rapidly with water to produce thiamin and benzoic acid. Both the UV and $^1$H NMR spectra indicate that fragmentation products do not form when the decarboxylation of 1 occurs in the presence of mm amounts of 4-hydroxy-TEMPO. The rate constant for the fragmentation reaction of 2 at 25°C is about 10$^4$ s$^{-1}$.\(^{18}\) Since we can detect the presence of fragmentation products at 1 mol%, the rate constant for reaction of 4-hydroxy-TEMPO with 2 must be at least 10$^6$ s$^{-1}$. This suggests why McIntosh and co-workers do not isolate TEMPO-radical conjugates.\(^7\)

We also investigated the fragmentation of 2 using nitrore spin traps and EPR. With N-tert-butyl-o-(2-sulophenyl)nitrorene sodium salt (2-SPBN-Na) as a water-soluble spin trap, EPR established a limit of detection of 100 nm with 4-hydroxy-TEMPO standards. The samples contained 10 mM 1 and 20 mM 2-SPBN-Na in pH 7.0 phosphate buffer. All of the EPR experiments where conducted in the absence of oxygen and light. UV spectroscopy reveals that 10 mol% 1 undergoes fragmentation. We recorded the initial EPR spectrum within 20 min and another after 5 h and a reference spectrum in the absence of 1. There is no EPR signal over 5 h. However, exposure of the reaction mixture after 5 h to oxygen produces a signal. The control did not produce a signal when exposed to oxygen.

We conducted analogous studies with 5,5-dimethyl-1-pyrroline N-oxide (DMPO). As with 2-SPBN-Na, there is no EPR signal, while exposure to oxygen produces a signal. Given the oxidative reactivity of 4-hydroxy-TEMPO towards 2, this results from reduction of aminoxy radicals by 2 to the EPR-silent hydroxylamines. Exposure to oxygen regenerates the radicals.\(^{17}\) By integrating the EPR signal with 1.0 µM 4-hydroxy-TEMPO solution as a standard, we estimate that 0.8 nmol of a radical species is trapped. Assuming fragmentation to be a radical process occurring at 10 mol%, this corresponds to only 0.05 mol% interception by the spin trap. This very low extent of trapping may arise from radical pair recombination being faster than desolvation or from minor side reactions. Nucleophilic additions to both nitroso and nitrene spin traps may lead to false-positive results.\(^{17}\) Hydroxylamines that form from addition react with atmospheric oxygen to give EPR-active aminoxy radicals. Based on the nucleophilic properties of Breslow intermediates, such an event can account for a signal, which is not relevant to the fragmentation process.\(^{17}\) Finally, the small amount of benzaldehyde that forms would be air-oxidized and then produce radicals.

If a radical pair were responsible for the EPR signal, our spin-trapping results indicate that radical disproportionation and recombination would have to be faster than desolvation. From our product studies, the radical disproportionation/recombination ratio ($k_{sp}/k_{rad}$), from the relative amounts of 4 and 5 to 6, would have to be about 10:1. Studies on resonance-stabilized radical pair recombination and disproportionation reactions show that spin delocalization greatly favors recombination over disproportionation. Typically, $k_{sp}/k_{rad}$ is below 0.1.\(^{20,21}\) Although steric factors may affect recombination, values of $k_{sp}/k_{rad}>1$ are not accessible.\(^{20,21}\) Both 7a and 7b exhibit delocalization of electron spin into aromatic rings. Moreover, the fragmentation, which is a β-elimination, would require homolysis of an RO–H bond, a process that is not normally accessible.\(^{20,21}\) Therefore, in a radical process more of the rearrangement product (6) would form than would 4 and 5. Under these circumstances, $k_{sp}/k_{rad}$≈10 is inconsistent with reactivity patterns of radical pairs.\(^{20,21}\)

Thus, decarboxylation of 1 in aqueous solutions produces 2 from which the rearrangement product 6 and fragmentation products 4 and 5 are clearly formed. Therefore, the reactivity of 2 parallels the reactivity of Breslow intermediates described by McIntosh and co-workers. Unlike generation of a Breslow intermediate from a mixture containing a thiamin-like carbene precursor, benzaldehyde, and base, decarboxylation of 1 avoids side reactions that lead to radicals. Our spin-trapping experiments produce only a weak EPR signal, corresponding to 0.05 mol% of the total amount of radicals that would form if fragmentation were a radical process. The rapid reduction of aminoxy radicals by the Breslow intermediate poses an experimental challenge where oxygen would rescue the reduced spin-trap adducts. However, this will also lead to oxidation of hydroxylamines that would have been formed by nucleophilic additions to the spin trap, giving an irrelevant EPR signal. The product distribution is also inconsistent with a radical pair mechanism, where the recombination product (6) would exceed the fragmentation products (4 and 5).
Scheme 4. An anionic mechanism for the fragmentation and rearrangement reactions of the Breslow intermediate.

A more likely mechanism for fragmentation and rearrangement involves a reactive carbocation (4'; Scheme 4). We propose that enol 2 ionizes to form a zwitterion (2') by transfer of the proton from its hydroxyl group substituent, followed by a C=N bond-breaking. The product (5) derives stability from re-aromatization of the thiazole ring and drives elimination of a charge-stabilized carbocation. The ionization of the OH group of 2 closely resembles that of the enol form of mandelic acid with its trio of electronegative atom substituents acidifying the hydroxyl group with pK_a = 6.66. The incorporation of a single deuterium into the CH_3 group of 4 upon fragmentation in D_2O is also consistent with the formation of the carbocation precursor and with the formation of 6 from the addition of 4' to 5. D_2O cannot serve as a source of deuterium atoms in a radical mechanism, owing to the very high energy of hydroxyl radicals.

In conclusion, our results indicate that the hypothetical formation of radicals does not account for the products of fragmentation from Breslow intermediate 2. It is also important to note that enzymes produce Breslow intermediates that do not undergo fragmentation or rearrangement. Yet, the potential fragmentation rate constant is larger than the enzymic k_cat. This is consistent with enzymes avoiding decomplexation of the C2s carbocation as an incidental consequence in their evolved efficiency.

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Conflict of interest

The authors declare no conflict of interest.

Keywords: Breslow intermediates · C=C bond cleavage · fragmentation · reaction mechanisms · thiamin

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[13] A slight turbidity was observed as the reaction approached completion, which is due to the low solubility of 5 and 6. To assure complete dissolution of all products, a few drops of MeOD were added before acquiring 1H NMR spectra.
[16] IUPAC recommends using “aminoxy radical(s)” rather than “nitrone(s)”, “nitrone radical(s)” or “nitrone(s)”. Pure Appl. Chem. 1995, 67, 1307–1375, see p. 1352.

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Chapter 9
Conclusions and Future Scope

9.1 Conclusions

9.1.1 Lithium promoted synthesis

The lack of an efficient and reproducible synthesis of MTh had been limiting progress in our effort to understand its unusual reactivity, most notably its general base catalyzed decarboxylation. Development of an improved synthesis of MTh proved to be a critical step in my research but also for the whole “thiamin project” team. By using the new synthetic methodology I could synthesize a variety of MTh analogs that allowed me to probe its reactivity in detail.

9.1.2 General base catalyzed decarboxylation is not so general

9.1.3 Decarboxylation of NMPA in water

The decarboxylation of MThH⁺ is the only described case were a decarboxylation reaction is general base catalyzed. This effect was explained by hydration of the carboxylate group to an orthoacid intermediate and its decarboxylation to form HCO₃⁻ and not CO₂. This hydrolytic decarboxylation pathway provides room for participation of bases. It was theorized that decarboxylation reactions that lead to a rapid recombination of the CO₂:R⁻ pair, will decompose preferentially by the loss HCO₃⁻ or H₂CO₃. A classic example of such a reaction is the decarboxylation of NMPA, were high theory computations showed a minimal barrier to recombination of such a pair. However, I did not observe any base catalysis for its decarboxylation even in strongly alkaline solutions. This does not necessarily exclude a hydrolytic decarboxylation; however, it does show that manifestation of such a pathway in the form of base catalysis could be a rare occurrence. Based on the very fast rate of separating a pair of methane molecules in water, I concluded that NMPA can decarboxylate by formation CO₂ relatively unimpeded.

9.1.4 Decarboxylation of MThH⁺ analogs

It was suggested before that the general base catalyzed decarboxylation of MThH⁺ is a result of a strong internal hydrogen (NH₂---OOC) which facilitates hydration of the carboxylate group to the orthoacid anion specie. Howe’s computational and experimental results suggested that two features are required for such a hydrogen bond. First, an acidic iminium-like amino group to form
a strong hydrogen bond with the carboxyl group. Two, the bulk of the benzene group to "lock on" the conformation that has the aforementioned intramolecular H-bond. I carefully designed and then synthesized two analogs of MTh, each accurately mimicking one of the two factors described above. However, neither showed general base catalyzed decarboxylation. This further reinforced my suspicion that the base catalysis is a very rare phenomenon, one which yet needs to be understood.

9.1.5 Loss of HCO$_3^-$ or CO$_2$?

It is not clear why decarboxylation MThH$^+$ is general base catalyzed and show SKIE. The Hammett result (see Chapter 3) indicates formation of a neutral 1,2-enaminol Breslow intermediate. The neutralization of the negative charge by the thiazolium makes a fast recombination of the Breslow intermediate with CO$_2$ unlikely. An irreversible decarboxylation of MTh in water is also supported by computational results from Tuñón and co-workers.$^{[1]}$ If the recombination of CO$_2$-Breslow intermediate pair is not reversible why would HCO$_3^-$ form? A logical answer to this, is that the loss of HCO$_3$ has a lower activation energy than the loss of CO$_2$. It is not clear why this would be the case though.

9.1.6 Enzymes likely enforce and unnatural conformation of the Breslow intermediate

The Hammett study for the decarboxylation of MTh in neutral buffers provided an insight into the electron “movement” during the decarboxylation. The $\rho$ value of 0.6 indicated little negative charge build up the transition state, and hence advanced delocalization into the thiazolium ring. This extensive delocalization can be further extended to the intermediate since by being a high energy species it will resemble the TS (Hammond postulate). In other words, the decarboxylation of MTh in solution leads to formation of a neutral 1,2-enaminol like Breslow intermediate. Interestingly, this is in contrasts with the previously reported Hammett results obtained on IPDC with BFs as substrates. The large substituent effect ($\rho = 4.4$) on the enzyme suggests that the Breslow intermediate on the enzyme has a localized carbanion-like structure. Therefore, IPDC is enforcing an unnatural state of the Breslow intermediate!

Is the localization of the Breslow intermediate also occurring on BFDC? Different reactivity patterns of accurate models in solution and enzyme-bound intermediates indirectly suggest that this is the case. For example, an almost complete suppression of chloride elimination from the $\rho$-
ClCH₂BF-derived Breslow intermediate on the enzyme suggests a much more rapid protonation of the Breslow intermediate than in solution where 60 mol% of chloride elimination is observed. Localization of the negative charge at the C2α position will be consistent with a diffusion controlled protonation. Furthermore, the rapid (10⁴ s⁻¹) and destructive fragmentation of the BF-derived Breslow intermediate is not detected on the enzyme. This is also consistent with a barrierless protonation. However, the lack of fragmentation could be a coincidental result of the enzyme enforcing a localized-carbanion like intermediate. In Chapter 8, I have proposed that the fragmentation occurs from a 1,2-enaminolate species. Therefore, the enzyme by forming a localized carbanion-like Breslow avoids fragmentation, regardless if protonation is fast or slow.

The prevention of delocalization of the negative charge on the Breslow intermediate, is likely an enzymic strategy to avoid formation of a too stable intermediate (energy well) and/or avoiding intrinsic barriers from multiple rehybridizations. As a result, the catalytic efficiency of the enzyme is maximized.

### 9.1.7 Fragmentation is not so radical

Inspired by McIntosh’s clever proposal that fragmentation of a Breslow intermediate is a radical process, I put a considerable amount of effort to demonstrate this experimentally. However, my results argued that radicals were not involved. A negligible EPR signal in spin trapping experiments and an unusual for a resonance-stabilized radical pair combination to disproportionation product ratio, made me believe that radicals are unlikely involved in the fragmentation reaction. I proposed a polar mechanism which requires formation of a neutral 1,2-enaminol Breslow intermediate. This mechanism can account for the observed deuterium incorporation, formation of the fragmentation and rearrangement products. Moreover, it also consistent with a small amount of the rearrangement product since its formation will be competing with a very rapid protonation of the DMAP-derived carbanion.

### 9.1.8 Cyclization of the BF-derived Breslow intermediate

I observed that under acidic conditions MTh decarboxylates to two previously unreported compounds. Analysis of ¹H NMR and MS data lead to proposal that both compounds form as a consequence of a fast, nucleophilic addition of the C2α position to the APH⁺ ring, followed by tautomerization and hydration reactions. This further expanded the already complex fate of the
BF-derived Breslow intermediate in aqueous buffers. As with the other side reactions involving this intermediate, the enzyme completely avoids them most likely because of a very fast protonation of the Breslow intermediate to HBnTh.

9.2 Future Scope

9.2.1 Testing the 1,2-enaminolate mechanism for the fragmentation reaction

Efforts are underway to test the proposed fragmentation mechanism via a 1,2-enaminolate intermediate. I had proposed that the fragmentation occurs by re-aromatization of the thiazole ring which requires simultaneous breaking of the C–N bond. This results in formation of PTK and a DMAP-derived carbanion (Scheme 9.1). Thus, at the transition state of this process there will be considerable C–N bond breaking.

Scheme 9.1. Proposed mechanism for the fragmentation reaction via a 1,2-enaminolate intermediate.

If the AP group in MTh is replaced with a typical carbanion stabilizing functionality, for example benzoyl, we should observe significant fragmentation (Scheme 9.2). This would be a critical test for this mechanism.

Scheme 9.2. Fragmentation of hypothetical Breslow intermediate to PTK and an enolate.
9.2.2 An alternative mechanism for fragmentation

The above proposed mechanism for fragmentation of the BF-derived Breslow intermediate is elegant because of its simplicity and because it can also account for formation of isoHBNTh. Based on observations of intramolecular cyclization reactions of the Breslow intermediate at pH < 6, one should consider that this could also be happening in neutral buffers. If it does, could it be responsible for the fragmentation reaction? A hypothetical mechanism is shown in Scheme 9.3. This pathway does not form a carbanion because the proton transfer can be concerted with C–C bond-breaking. This mechanism would also be consistent with the observed single deuterium incorporation into the methyl group in DMAP. However, it does not explain formation of isoHBNTh. Nevertheless, such an alternative could be considered if the critical test for the carbanionic mechanism fails.

Scheme 9.3. An alternative mechanism for fragmentation of the BF-derived Breslow intermediate via a tricyclic intermediate.

9.2.3 $^{18}$O isotope exchange in NMPA and MTh.

I did my best, by using linear free energy relationships, to show that the NMPA-derived orthoacid intermediate is both thermodynamically and kinetically accessible under the decarboxylation conditions. However, as with any extrapolation, error is possible. Therefore, the accessibility of
the orthoacid intermediate should be verified experimentally. The rate of orthoacid formation could be determined from the rate of $^{18}$O isotope exchange in the carboxyl group. The rate of hydration should also be evaluated for MTh and its derivatives which were described in Chapter 6.

9.3 References

Appendix A

The pK\textsubscript{a} values associated with protonation of the N1' position in MTh, HBnTh and thiamin were determined by UV-VIS-spectroscopy. Aqueous stock solutions were prepared (for MTh conc. HCl was used instead) so that when 2 μL of the stock was added to 2.500 mL of buffer in a 10 mm quartz cuvettes, the absorbance at 250 nm was below 1.0 AU. A series of 0.60 M and buffers (ionic strength 1.00 M) were prepared to cover a pH range from 2.0 to 8.0. It took no more than 15 s from the moment the stock was added to the buffer and acquisition of the spectrum. A short time-study at pH 4.0 and pH 8.0, indicated that the progress of decarboxylation of MTh within the first 15 s is negligible. It was verified with a potentiometer that addition of 2 μL of the acidic MTh stock did not affect the pH to any significant extent. All scans were acquired at 25 °C and in triplicates. For all three compounds, a significant increase in absorbance at 250 nm was observed at pH < 7. N1'-methylated MTh did not show any significant change in absorbance, confirming that the change in absorbance was the result of changing the protonation state of the DMAP ring. The absorbance at 250 nm was plotted against pH and a standard titration curve was fitted (see figure below). The obtained pK\textsubscript{a} values for MTh, HBnTh and thiamin were 5.7, 5.6 and 5.6, respectively.

![Figure A1](image)

Figure A1: Absorbance change at 250 nm as function of pH; circle - MTh, triangle - N1'-CH\textsubscript{3} - MTh.

The pK\textsubscript{a} of 5.7 for MTh matches closely the kinetic pK\textsubscript{a} of 5.5 obtained from the rate of decarboxylation of p-BrCH\textsubscript{2}MTh (see Chapter 4). Interestingly, this value is far off from the
kinetic $pK_a$ value of 3.7 from the rate of decarboxylation of MTh as reported by Qingyan Hu (PhD Thesis, *The Surprising Reactivity Patterns of Intermediates Derived From Thiamin and Benzoylformate*, University of Toronto, 2006). It is not clear what is the source if this discrepancy.

Appendix B

Supporting information for:
Lithium-stabilized nucleophilic addition of thiamin to a ketone provides an efficient route to mandelylthiamin, a critical pre-decarboxylation intermediate

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2a Spectra

$^1$H-NMR (600 MHz, DMSO-$d_6$): $\delta$ 10.09 (s, 1H, H$_a$), 9.23 (m, 1H, H$_{6b}$), 8.38 (s, 1H, H$_d$), 5.73 (s, 2H, H$_b$), 4.65 (t, 1H, J = 3.3 Hz, H$_c$), 3.86-3.82 (m, 1H, H$_{c'}$), 3.68-3.64 (m, 1H, H$_{6c}$), 3.60-3.57 (m, 1H, H$_{c''}$), 3.46-3.42 (m, 1H, H$_{a}$), 3.26-3.17 (m, 2H, H$_a$), 2.97 (s, 3H, H$_e$), 2.85 (s, 3H, H$_f$), 1.75-1.69 (m, 1H, H$_{6a'}$), 1.67-1.62 (m, 1H, H$_{1a'}$), 1.51-1.47 (m, 3H, H$_{1a''}$, H$_{1a'''}$), 1.47-1.39 (m, 1H, H$_{1a'}$).

ESI-MS [C$_7$H$_{25}$N$_4$O$_2$S]$^+$, calculated: 349.17, observed: 349.2.
\textbf{\textsuperscript{1}H-NMR (600 MHz, DMSO-\textit{d}_6):}
gCOSY (600 MHz, DMSO-d6):
4a Spectra

$^1$H NMR (600 MHz, D$_2$O): $\delta$ 7.53 (d, $J = 12.0$ Hz, H$_a$), 7.31 (t, $2H, J = 6.0$ Hz, H$_b$), 7.25 (t, $tH, J = 6.0$ Hz, H$_c$), 6.85 (s, 2H, H$_d$), 5.87 (b, tH, H$_{e\alpha}$), 5.39 (d, tH, $J = 18.0$ Hz, H$_{e\alpha}$), 3.98 (t, 2H, $J = 6.0$ Hz, H$_f$), 3.94 (s, 3H, H$_g$), 3.25 (t, 2H, $J = 6.0$ Hz, H$_h$), 2.52 (s, 3H, H$_i$), 2.43 (s, 3H, H$_j$).

$^{13}$C NMR (100 MHz, D$_2$O): $\delta$ 170.5 (C$_1$), 169.9 (C$_2$), 161.5 (C$_3$), 160.7 (C$_4$), 145.4 (C$_5$), 137.7 (C$_6$), 136.1 (C$_7$), 135.1 (C$_8$), 129.9 (C$_9$), 129.7 (C$_{10}$), 126.1 (C$_{11}$), 107.3 (C$_{12}$), 79.2 (C$_{13}$), 60.1 (C$_{14}$), 55.4 (C$_{15}$), 47.4 (C$_{16}$), 29.5 (C$_{17}$), 20.7 (C$_{18}$), 11.7 (C$_{19}$).

ESI-MS [C$_{21}$H$_{25}$N$_4$O$_4$S]$^+$, calculated: 429.16, observed: 429.2.
$^1$H NMR (300 MHz, D$_2$O):
$^{13}$C NMR (100 MHz, D$_2$O):
gCOSY (300 MHz, D₂O):
HSQC (600 MHz, D$_2$O):
$^1$H NMR (300 MHz, D$_2$O): $\delta$ 7.51 (d, 2H, J = 12.0 Hz, H$_2$), 7.34–7.33 (m, 3H, H$_{10}$), 6.73 (s, 1H, H$_6$), 5.83 (d, 1H, J = 18.0 Hz, H$_{16}$), 5.36 (d, 1H, J = 18.0 Hz, H$_{15}$), 4.41 (q, 2H, J = 7.2 Hz, H$_7$), 3.90 (t, 2H, J = 6.0 Hz, H$_8$), 2.59 (s, 3H, H$_9$), 2.47 (s, 3H, H$_1$), 2.18 (t, 3H, J = 7.2, H$_3$).

$^{13}$C NMR (400 MHz, D$_2$O): $\delta$ 170.3 (C$_1$), 169.5 (C$_2$), 161.7 (C$_3$), 160.7 (C$_4$), 145.3 (C$_5$), 138.3 (C$_6$), 136.0 (C$_7$), 135.0 (C$_8$), 129.8 (C$_9$), 129.6 (C$_{10}$), 125.9 (C$_{11}$), 107.3 (C$_{12}$), 78.0 (C$_{13}$), 65.3 (C$_{14}$), 60.0 (C$_{15}$), 47.3 (C$_{16}$), 29.3 (C$_{17}$), 20.6 (C$_{18}$), 13.00 (C$_{19}$).

ESI-MS [C$_{22}$H$_{27}$N$_4$O$_4$S]$^+$, calculated: 443.17, observed: 443.2.
$^1$H NMR (400 MHz, D$_2$O):
$^1$H NMR (600 MHz, MeOD): 5.76 (d, 2H, J = 9.0 Hz, H$_a$); 7.35 (t, 2H, J = 7.3 Hz, H$_b$); 7.30 (t, 1H, J = 7.3 Hz, H$_c$); 7.19 (s, 1H, H$_d$); 5.82 (d, 1H, J = 17.4 Hz, H$_e$); 5.38 (d, 1H, J = 17.4 Hz, H$_f$); 3.92 (m, 2H, H$_g$); 3.20 (t, 2H, J = 6.3 Hz, H$_h$); 2.90 (s, 3H, H$_i$); 2.43 (s, 3H, H$_j$); 1.49 (s, 9H, H$_k$).

$^{13}$C NMR (150 MHz, MeOD): 81.712 (H$_a$), 168.2 (H$_b$), 160.5 (H$_c$), 160.0 (H$_d$), 144.4 (H$_e$), 138.8 (H$_f$), 136.6 (H$_g$), 136.6 (H$_h$), 129.3 (H$_i$), 129.1 (H$_j$), 126.0 (H$_k$), 107.3 (H$_l$), 56.2 (H$_m$), 58.0 (H$_n$), 47.5 (H$_o$), 29.8 (H$_p$), 26.5 (H$_q$), 20.1 (H$_r$), 11.3 (H$_s$).

ESI-MS [C$_{24}$H$_{31}$N$_4$O$_4$S]$^+$, calculated: 471.21, observed: 471.2.
$^1$H NMR (600 MHz, MeOD):
Experimental protocol for time vs. yield study

2a (0.77 g, 1.8 mmol) was suspended in dry THF (8 mL) and 3a (3.00 g, 18.2 mmol) was added. The reaction mixture was purged with N₂ and cooled to -20 °C (35% ethanol/dry ice). NaHMDS (4.2 mL of a 1M solution in THF, 4.20 mmol, 2.3 eq.) was added. 1.00 mL aliquots were taken at different time intervals over a period of 1.5 h. The aliquots were quenched immediately by injecting them into a vigorously stirred trifluoroacetic acid (4 mL). The acid and solvents were removed by rotary evaporation. The oily residue was dissolved in 0.70 mL of D₂O containing a maleic acid standard (166 mg/25.00 mL). Excess ester was extracted by the addition of 2.0 mL of diethyl ether and vigorous shaking. The whole mixture was then transferred to a 15 mL Falcon tube and diethyl ether was added to the 9 mL mark. The tube was shaken and 0.6 mL of the aqueous layer was removed and submitted to ¹H NMR. The yields were calculated from the relative integration values of the diastereotopic methylene hydrogens, the maleic acid reference peak, and the initial concentration of O-THP-thiamine. To test the effect of lithium and magnesium ions, the same procedure was followed with anhydrous lithium chloride (200 mg, 4.68 mmol) and magnesium triflate (1.33 g, 4.14 mmol) added prior to addition of the NaHMDS.
Experimental protocol for the lithium ion/3a concentration vs yield study

The effect of increasing amounts of lithium ion was also determined. Lithium triflate (LiOTf) was employed in this study because of its higher solubility in THF relative to LiCl. Reactions were carried out in the same manner as in the above procedure. However, the volume of THF was decreased with increasing amount of added LiOTf or 3a so that the volume of the reaction mixture remained constant (volumes ranged from 1.5 mL to 1.05 mL of THF). All reactions were quenched after 20 minutes.

Figure S1. The effect of 3a concentration on the yield of the condensation. Equivalents is given relative to 2a.

Experimental protocol for determining the effect of various Lewis acid additives

2a (51 mg, 0.12 mmol) and 3a (0.19 g, 1.16 mmol) were suspended in 0.5 mL of dry THF. Following the addition of two equivalents of the tested Lewis acid, the reaction mixture was purged with N2 and cooled to -20 °C. NaHMDS (0.28 mL of a 1 M solution in THF, 0.28 mmol) was added gradually to the stirring mixture. After 10 minutes, the mixture was injected into 2 mL of stirring acetic acid. The yields were calculated by the same procedure reported for the time vs. yield study.

Experimental protocol for determining the effect of crown ethers on the yield of the reaction

Reactions were carried out in the same manner as in the time vs. yield study. Two equivalents of 15-crown-5 and 12-crown-4 were added to the reaction mixture prior to the addition of LiHMDS. The yields were determined as mentioned above.

Experimental protocol for determining the effect of lithium on the condensation of 7 with methylbenzoylformate.

The condensation reaction was carried in a 2:3 v/v THF:DCM solution. This solvent mixture provides complete dissolution of both 7 and LiOTf. All reactions are conducted under nitrogen.

7 (62 mg, 0.11 mmol), methyl benzoylformate (3a) (90 mg, 0.55 mmol) are dissolved in 1 mL of THF:DCM solvent mixture in a glass vial equipped with a magnetic bar. The mixture is stirred and cooled in a 35% ethanol/solid CO3 bath (−20 °C). 1 M NaHMDS in THF solution (0.150 mL, 0.150 mmol) is added dropwise. The reaction is quenched after 10 min by injecting 0.5 mL of TFA. The quenched reaction mixture is rotor evaporated under high vacuum. The crude material is shaken with 1 mL of D2O and 10 mL of ether. The D2O layer is separated and submitted to 1H-NMR, which show no indication of product formation (see figure S3b). The same reaction was repeated but LiOTf (68 mg, 0.44 mmol) was added before the addition of base. 1H-NMR indicates almost complete conversion (see figure S3a).

Figure S3. The bridging CH2 proton peaks in the 1H-NMR spectrum of the quenched condensation reaction of 7 with 3a. The CH2 peaks appear as two doublets in the condensation product because of formation of a chiral center: A - with LiOTf, B - without LiOTf. Without Li+ there is no product formation.
Effect of different solvents on the yield of the reaction

The effect of different solvents on reaction yields has been determined and the results are summarized in Table S1. The reaction yield is clearly higher in dichloromethane; DCM is relatively a good solvent for O-THP thiamine chloride hydrochloride, and does not coordinate Li⁺ unlike THF. Ethanol as solvent shows minimal product formation, whereas t-butanol gives yields comparable to THF. Ethoxide as a strong nucleophile can participate in undesirable reactions, and ethanol will be complexing Li⁺. On the contrary, tert-butoxide is a rather poor nucleophile and tert-butanol is expected to poorly solvate Li⁺. Non-polar solvents, such as diethyl ether or toluene show minimal product formation most likely because of lack of solubility of O-THP-thiamine chloride hydrochloride in them.

<table>
<thead>
<tr>
<th>Solvent</th>
<th>% yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>dichloromethane</td>
<td>78</td>
</tr>
<tr>
<td>tetrahydrofuran</td>
<td>28</td>
</tr>
<tr>
<td>tert-butanol</td>
<td>2.1</td>
</tr>
<tr>
<td>ethanol</td>
<td>2.5</td>
</tr>
<tr>
<td>toluene</td>
<td>0.8</td>
</tr>
<tr>
<td>ether</td>
<td>0.4</td>
</tr>
</tbody>
</table>

Hydrolysis and storage of the esters

4a and 4b were hydrolyzed by stirring in concentrated HCl at room temperature for about three days. The 4c was hydrolyzed by stirring in TFA for 6 hours. The hydrolyzed products were kept dissolved in a small amount of concentrated HCl at 4 °C. Under these conditions, there was no noticeable degradation over three months (evaluated by 1H NMR in 35% DCl in D2O).

(1) More product can be precipitated from the filtrate with the addition of ether. However, this precipitate is the p-toluenesulfonate salt of 2a.
(2) 1H NMR can be obtained in D2O if a small amount of potassium bicarbonate must be added to prevent hydrolysis of the protecting group.
(3) This base is available as a solution in THF and toluene. We obtained no significant differences in yield using either solution.
(5) An alternative is to treat the product with a small amount of isopropanol which will dissolve the product and leave behind most of the sodium bromide as precipitate.
(6) TLC plates must also be treated with sodium bromide to prevent streaking.
(9) This material is extremely hygroscopic. As such, the determined yield of this reaction is less accurate than the other reported syntheses.
Appendix C

Supporting Information

The Need for an Alternative to Radicals as the Cause of Fragmentation of a Thiamin-Derived Breslow Intermediate

Michael Bielecki and Ronald Kluger*

anie_201702240_sm_misellaneous_information.pdf
Supporting Information

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General considerations

All experiments were conducted using reagent grade chemicals which were obtained from commercial sources. Methyl benzoylformate was de-acidified and dried by mixing with K₂CO₃ and anhydrous MgSO₄, followed by filtration. Thiamin hydrochloride was purchased as anhydrate and was dehydrated by heating at 135 °C for 4-5 h under vacuum. The remaining reagents were used without further purification. All ¹H NMR spectra were obtained on a 600 MHz instrument, except for samples in 20% DCl, which were run on a 300 MHz instrument because of better tuning and shimming capabilities of the instrument for high ionic strength samples. ¹H NMR spectra were referenced to the residual solvent peak or in case of 20% DCl samples, to 3-(trimethylsilyl)-propionic 2,2,3,3-d₄ acid (TMSP-d₄). Oxygen-free samples were prepared using six cycles of freeze-pump-thaw with argon as the inert gas. Oxygen-free reactions were carried out under a positive argon pressure and then transferred to NMR or EPR tubes under a flow of argon, sealed off with Teflon tape and an appropriate cap.

Synthesis

O-TBDP-thiamin chloride

Anhydrous thiamin hydrochloride (20.0 g, 59.2 mmol) and tert-butyl chloro)diphenylsilane (19.5 g, 71.1 mmol) were added to dichloromethane (DCM) in a round-bottom flask equipped with a magnetic bar. The mixture was stirred in an ice bath, and imidazole (13.7 g, 201 mmol) was slowly added. The reaction mixture was further stirred for 1 h at room temperature. The solvent was removed by roto-evaporation and 70% methanol (MeOH) in water, containing 6.00 mL of 36% HCl, was added, and the mixture was gently extracted three times with hexanes. The aqueous/MeOH layer was separated and roto-evaporated. The residue was treated with brine and extracted three times with DCM. The organic layer was dried with anhydrous MgSO₄ and roto-evaporated to obtain an off-white solid (30.5 g, 96% yield). ¹H NMR (600 MHz, MeOD) δ 8.14 (s, 1H), 7.64 (d, J = 6.7 Hz, 4H), 7.49 – 7.44 (m, 2H), 7.42 (t, J = 7.2 Hz, 4H), 5.43 (s, 2H), 3.97 (t, J = 5.5 Hz, 2H), 3.21 (t, J = 5.5 Hz, 2H), 2.55 (s, 3H), 2.49 (s, 3H), 1.07 (s, 9H). MS(ESI⁺): m/z 503.2

O-TBDP-mandelylthiamin methyl ester (O-TBDP-1 Me ester)

This procedure is based on our previously published method.[1] O-TBDP-thiamin chloride (3.00 g, 5.57 mmol) was dissolved in DCM followed by addition of methyl benzoylformate (4.57 g, 27.8 mmol) in a round-bottom flask equipped with a magnetic bar. The mixture was cooled to -20°C in a 35% ethanol/solid CO₂ bath and purged with argon. 1 M lithium bis(trimethylsilyl)amide in toluene solution (8.36 mL, 8.36 mmol) was added dropwise. The reaction mixture was quenched after 5 min by addition of an excess of acetic acid, followed by addition trifluoroacetic acid (TFA, 3.81 g, 25.6 mL, 334 mmol). The mixture was roto-evaporated until a thick yellow residue was obtained. ¹H NMR of the crude material (MeOD) indicated
>90% conversion. The crude product was purified via silica gel flash chromatography; the silica was impregnated with NaBr by soaking in a saturated NaBr solution in MeOH and dried in a stream of air. The product was dry-loaded, and a gradient elution was performed from 2 to 25 v/v % MeOH in ethyl acetate (EtAcO) and 0.02 v/v % TFA. The product eluted before a yellow colored by-product. The product fractions were further acidified with few drops of TFA as they were collected. The product fractions were combined and roto-evaporated to dryness. The residue was treated with DCM and gently extracted with DCM and brine which contained a small amount sodium thiosulfate (to remove bromine that inherently forms during the elution). The organic layer was washed two times with brine, dried with anhydrous MgSO₄, and evaporated to dryness to give an off-white solid (3.4 g, 41% yield, calculated as the HBr salt). ¹H NMR (600 MHz, MeOD) δ 7.65 (d, J = 7.8 Hz, 4H), 7.57 (d, J = 9.2 Hz, 2H), 7.51 – 7.38 (m, 9H), 7.20 (s, 1H), 5.70 (d, J = 17.6 Hz, 1H), 5.50 (d, J = 17.4 Hz, 1H), 3.97 (t, J = 5.4 Hz, 2H), 3.91 (s, 3H), 3.21 (t, J = 5.4 Hz, 2H), 2.52 (s, 3H), 2.34 (s, 3H), 1.06 (s, 9H). MS(ESI+): m/z 667.3

Mandelythiamin (1)

The O-TBDP·1 Me ester was quantitatively deprotected by stirring for 4 days in 36% HCl at room temperature. The reaction mixture was extracted three times with DCM, and the aqueous layer was concentrated under vacuum to obtain a thick liquid. This solution is stable for many weeks at -20 °C. For long term storage the sample should be kept at -80 °C and further acidified with 36% HCl. ¹H NMR (300 MHz, 20% DCl, TMSP-d₄) δ 7.57 (d, J = 9.0, 2H), 6.86 (s, 1H), 5.81 (d, J = 18.4 Hz, 1H), 5.40 (d, J = 18.4 Hz, 1H), 3.97 (t, J = 5.5 Hz, 2H), 3.25 (t, J = 5.5 Hz, 2H), 2.54 (s, 3H), 2.41 (s, 3H). MS(ESI+): m/z 415.1

2α-(1-hydroxybenzyl)thiamin (3)

3 was prepared based on a previously described procedure.¹ Thiamin hydrochloride (5.00 g, 14.8 mmol) was suspended in 100 mL of absolute ethanol (abs. EtOH) in a round-bottom flask equipped with a magnetic bar. The mixture was cooled in an ice bath and purged with argon. To the stirred suspension, a 21 wt. % sodium ethoxide solution (11.2 mL, 30.0 mmol) was slowly added, maintaining the temperature at 0 °C. Benzaldehyde (3.00 mL, 30 mmol) was added in one portion, and after 5 min of stirring, the reaction mixture was evaporated to obtain a thick oil, which was dissolved in water and extracted four times with DCM. The aqueous layer was roto-evaporated, and the semi-solid was treated with warm abs. EtOH and filtered. The filtrate was roto-evaporated and the obtained residue was redissolved in warm abs. EtOH, the volume was reduced by roto-evaporation to ~20 mL and chilled in the fridge. The crystals were filtered, washed with ice-cold abs. EtOH and dried to yield 3.00 g of 3. ¹H NMR (600 MHz, D2O, pD = 7.0, KD2PO4/K2DPO4) δ 7.33 (d, J = 9.6 Hz, 2H), 7.18 (d, J = 7.1 Hz, 3H), 6.41 (s, 1H), 6.40 (s, 1H), 5.27 (d, J = 17.3 Hz, 1H), 5.18 (d, J = 17.3 Hz, 1H), 3.94 (t, J = 6.0 Hz, 2H), 3.20 (t, J = 6.0 Hz, 2H), 2.36 (s, 3H), 2.26 (s, 3H). MS(ESI+): m/z 371.2
Fragmentation and rearrangement products (4, 5, and 6)

4, 5, and 6 were prepared based on procedures described in literature.[1] The three products were isolated from the same crude material which was obtained by refluxing for 5 h a mixture containing thiamin hydrochloride (20 g, 59.3 mmol), triethylamine (12.5 g, 123 mmol), and benzaldehyde (12.5 g, 11.8 mmol) in 250 mL of MeOH. The solvent was removed by roto-evaporation. 200 mL of EtAcO was added to the residue and extracted with 10% HCl. The aqueous acid layer was kept for isolation of 4. The organic layer was filtered, dried with anhydrous MgSO4, and roto-evaporated. The obtained residue was triturated with ether (Et2O) and used later to obtain 5. Finally, the Et2O layer was saved to isolate 6.

2,5-dimethylpyrimidin-4-amine (4)

\[
\begin{align*}
\text{NH}_2 & \\
\text{N} & \\
\text{N} & \\
4
\end{align*}
\]

The acidic aqueous layer was neutralized with NaHCO3 and extracted three times with EtAcO. The organic layer was dried with anhydrous MgSO4 and evaporated to dryness. The product was purified on silica using a gradient elution from 1 to 25 v/v % MeOH in DCM. The product fractions were combined and evaporated to dryness to yield 2.00 g of 4 as a white solid. [H NMR (600 MHz, D2O, pD = 7.0, K2PO4/K2HPO4) δ 7.87 (s, 1H), 2.45 (s, 3H), 2.06 (s, 3H), MS(ESI+): m/z 124.1.

2-benzoyl-4-(2-hydroxyethyl)-5-methylthiazole (5)

\[
\begin{align*}
\text{O} & \\
\text{N} & \\
\text{S} & \\
\text{Ph} & \\
\text{OH} & \\
5
\end{align*}
\]

The Et2O-washed residue was dissolved in toluene, filtered, and the filtrate was roto-evaporated to give ~20 g of a thick oil. The product was purified on silica using a gradient elution from 10 to 0 v/v % hexanes in DCM, yielding 3.50 g of an off-white solid. [H NMR (600 MHz, D2O, pD = 7.0, K2PO4/K2HPO4) δ 7.57 (s, 1H), 7.50 (d, J = 7.7 Hz, 2H), 7.43 - 7.39 (m, 3H), 3.77 - 3.68 (m, 2H), 3.54 (s, 2H), 2.96 (t, J = 6.3 Hz, 2H), 2.37 (s, 3H), 2.32 (s, 3H), MS(ESI+): m/z 248.1.

2α-(2,5-dimethylpyrimidine-4-amine-5-yl)-2α-(1-hydroxybenzyl)-4-(2-hydroxyethyl)-5-methylthiazole (6)

\[
\begin{align*}
\text{NH}_2 & \\
\text{N} & \\
\text{N} & \\
\text{S} & \\
\text{Ph} & \\
\text{OH} & \\
6
\end{align*}
\]

The Et2O solution was roto-evaporated to dryness, and the crude product was purified by silica gel chromatography with a gradient elution from 5 to 20 v/v % MeOH in DCM. The product came off after 4, which was determined to be the main contaminant. [H NMR (600 MHz, D2O, pD = 7.0, K2PO4/K2HPO4) δ
$7.57$ (s, 1H), 7.50 (d, $J = 7.7$ Hz, 2H), 7.43 – 7.39 (m, 3H), 3.77 – 3.68 (m, 2H), 3.54 (s, 2H), 2.96 (t, $J = 6.3$ Hz, 2H), 2.37 (s, 3H), 2.32 (s, 3H). MS(ESI+): $m/z$ 371.1

$^1$H NMR product studies

$^1$H NMR peaks were assigned by spectral comparisons and spiking with genuine samples.

pD 7.0(±0.5), phosphate buffer

The concentration of 1 in the stock HCl acid solution was determined to be 1.0 M by quantitative $^1$H NMR, with maleic acid or thiamin as references. $6 \mu$L of the 1 stock solution were added to 594 $\mu$L of a 0.35 M K$_2$HPO$_4$ solution to give a 10 mM final concentration of 1. The amount of K$_2$HPO$_4$ was calibrated so that upon addition of 1 a pD 7.0(±0.5) buffer was obtained. Whatman® PanpH®TM pH indicator strips (±0.5) and deuterated reference buffers were used to determine the pD of our samples. After 5 h the reaction was completed and submitted for $^1$H NMR analysis. A 10 s relaxation time was used for the $^1$H NMR spectrum acquisition to obtain accurate peak ratios.

![NMR spectrum](image)

Figure 1. The $^1$H NMR (600 MHz) spectrum of the product mixture formed from the decarboxylation of 1 in a pH 7.0(±0.5) phosphate buffer.
Figure 2. A close-up of the $^1$H NMR spectrum from Figure 1 in the 7 to 8 ppm range. Peaks are labeled using the numerical assignment. In addition, thiamin and benzoic acid peaks are labeled as Th and Bz, respectively.
Figure 3. A close-up of the $^1$H NMR spectrum from Figure 1 in the 4 to 7 ppm range. Peaks are labeled using the numerical assignment, and thiamin is labeled as Th.
Figure 4. A close-up of the $^1$H NMR spectrum from Figure 1 in the 2 to 4 ppm range. Peaks are labeled using the numerical assignment. Thiamin is labeled as Th.

pD 7.0(±0.5), phosphate buffer, containing 4-hydroxyTEMPO

The 10 mM, pD 7.0(±0.5) buffered solution of 1 was prepared as described earlier but in the presence of 25 mM of 4-hydroxyTEMPO. The reaction mixture was prepared under oxygen-free conditions. After 5 hours when the reaction was completed the sample was transferred to an NMR tube under argon and submitted to $^1$H NMR spectroscopy (600 MHz, 10 s relaxation time). As expected, the ratio of thiamin to benzoic acid and to 4-hydroxyTEMPO-derived hydroxylamine is 1:1:2 (Figure 5).
Figure 5. $^1$H NMR spectrum of the decarboxylation of 1 (10 mM) in a pH 7.0 ($\pm$0.5) phosphate buffer containing 25 mM of 4-hydroxyTEMPO. Under these conditions the major products are thiamin (Th), benzoic acid (Bz), and the 4-hydroxyTEMPO-derived hydroxylamine (NOH).

UV-VIS spectroscopy experiment

Decarboxylation of 1 (~100 $\mu$M) in a 0.05 M, pH 7.0 phosphate buffer was followed by UV-VIS spectroscopy in the absence and presence of 1 mM of 4-hydroxyTEMPO. The negligible absorbance at 328 nm in the 1 mM 4-hydroxyTEMPO buffer indicates that 5 does not form to any significant extent (Figure 6).
Figure 6. UV-VIS traces of the decarboxylation product of 1 at pH 7.0 after 5 h, in the absence (—) and presence (—- -) of 1 mM of 4-hydroxyTEMPO.

EPR spectroscopy experiments

4 μl of the 1 stock solution was added to 380 μL of a 0.37 M K₂HPO₄ solution, immediately followed by addition of 16 μL of a 0.50 M spin trap solution (S-PBN-Na or DMPO). The resulting mixture contained 10 mM of 1, 20 mM of the spin trap, and 350 mM of phosphates. All solution were deoxygenated by six freeze-pump-shaw cycles using argon. Every operation, including transfer to EPR tubes, was conducted with the exclusion of oxygen. The reaction itself was run in the dark. The reaction mixture’s pH was tested with an indicator paper (Whatman® Panphea™) and remained constant at 7.0(±0.5) for the duration of the reaction. Samples were oxygenated by bubbling O₂ for few minutes. The extent of fragmentation under the EPR conditions was established by ¹H NMR analysis, as well UV-VIS spectroscopy by measuring the intensity at 328 nm and comparing it to a reference solution of 5. It was determined that 10 mol % of 1 underwent fragmentation. 30 μL of the reaction mixture was transferred to an EPR tube (1 mm inner diameter). Spectra were obtained at a 9.35 GHz microwave frequency, 8.9 mW power, 1.0 G amplitude modulation, 80 G sweep width, 80 s sweep time, and 4 scans. A 1 μM 4-hydroxyTEMPO solution was used for each experiment for signal strength quantification. An approximately 100 nM limit of detection was achieved.
Figure 7. EPR results for the decarboxylation of 10 mM of 1 at pH 7.0±0.5 in the presence of 20 mM of S-PBN-Na. A – 20 min and B – 5 h from the start of the reaction, C – reaction sample exposed to O2 after 5 h, D – 1 μM 4-hydroxyTEMPO solution used a reference for signal intensity.
Figure 8. EPR results for the decarboxylation of 10 mM of 1 at pH 7(±0.5) in the presence of 20 mM of DMPO. A – 20 min and B – 5 h from the start of the reaction, C – reaction sample exposed to O₂ after 5 h, D – 1 μM 4-hydroxyTEMPO solution used as a reference for signal intensity.
$^1$H-NMR spectra

**O-TBDP-thiamin chloride**

$^1$H NMR (600 MHz, MeOD) $\delta$ 8.14 (s, 1H), 7.64 (d, $J = 6.7$ Hz, 4H), 7.49 – 7.44 (m, 2H), 7.42 (t, $J = 7.2$ Hz, 4H), 5.43 (s, 2H), 3.97 (t, $J = 5.5$ Hz, 2H), 3.21 (t, $J = 5.5$ Hz, 2H), 2.55 (s, 3H), 2.49 (s, 3H), 1.07 (s, 9H).
$^1$H NMR (600 MHz, MeOD) δ 7.65 (d, J = 7.8 Hz, 4H), 7.57 (d, J = 9.2 Hz, 2H), 7.51 – 7.38 (m, 9H), 7.20 (s, 1H), 5.70 (d, J = 17.6 Hz, 1H), 5.50 (d, J = 17.4 Hz, 1H), 3.97 (t, J = 5.4 Hz, 2H), 3.91 (s, 3H), 3.21 (t, J = 5.4 Hz, 2H), 2.52 (s, 3H), 2.34 (s, 3H), 1.06 (s, 9H).
Mandelythiamin (1):

$^1$H NMR (300 MHz, 20% DCl, TMS-d$_4$) $\delta$ 7.57 (d, $J = 9.0$, 2H), 6.86 (s, 1H), 5.81 (d, $J = 18.4$ Hz, 1H), 5.40 (d, $J = 18.4$ Hz, 1H), 3.97 (t, $J = 5.5$ Hz, 2H), 3.25 (t, $J = 5.5$ Hz, 2H), 2.54 (s, 3H), 2.41 (s, 3H).
2α-(1-hydroxybenzyl)thiamin (3)

$^1$H NMR (600 MHz, D$_2$O, pD = 7.0±0.5), K$_2$PO$_4$/K$_3$PO$_4$ δ 7.33 (d, $J$ = 9.6 Hz, 2H), 7.18 (d, $J$ = 7.1 Hz, 3H), 6.41 (s, 1H), 6.40 (s, 1H), 5.27 (d, $J$ = 17.3 Hz, 1H), 5.18 (d, $J$ = 17.3 Hz, 1H), 3.94 (t, $J$ = 6.0 Hz, 2H), 3.20 (t, $J$ = 6.0 Hz, 2H), 2.36 (s, 3H), 2.26 (s, 3H).
Fragmentation products (4 and 5)

2,5-dimethylpyrimidin-4-amine (4)

\[ \text{\textsuperscript{1}H NMR (600 MHz, D}_2\text{O, pD = 7.0(±0.5), KCl:PO}_4/K_2\text{PO}_4) \delta 7.87 (s, 1H), 2.45 (s, 3H), 2.06 (s, 3H).} \]
2-benzoyl-(5-(2-hydroxyethyl))-4-methylthiazole (5)

$^1$H NMR (600 MHz, D$_2$O, pD = 7.0±0.5, KD$_2$PO$_4$/K$_2$HPO$_4$) $\delta$ 8.07 (d, $J$ = 8.4 Hz, 2H), 7.75 (t, $J$ = 8.0 Hz, 1H), 7.60 (t, $J$ = 8.0 Hz, 2H), 3.88 (t, $J$ = 6.0 Hz, 2H), 3.14 (t, $J$ = 6.0 Hz, 2H), 2.44 (s, 3H).
$^1$H NMR (600 MHz, D$_2$O, pD = 7.0±0.5), K$_2$PO$_4$/KPO$_4$) δ 7.57 (s, 1H), 7.50 (d, $J = 7.7$ Hz, 2H), 7.43 – 7.39 (m, 3H), 3.77 – 3.68 (m, 2H), 3.54 (s, 2H), 2.96 (t, $J = 6.3$ Hz, 2H), 2.37 (s, 3H), 2.32 (s, 3H).
Thiamin hydrochloride

$^1$H NMR (600 MHz, D$_2$O, $\rho$D = 7.0(±0.5), KD$_2$PO$_4$/K$_2$HPO$_4$) $\delta$ 8.03 (s, 1H), 5.45 (s, 2H), 3.87 (t, $J = 5.8$ Hz, 2H), 3.17 (t, $J = 5.8$ Hz, 2H), 2.55 (s, 3H), 2.48 (s, 3H).
Benzoic acid

$^{1}H$ NMR (600 MHz, D$_2$O, pD = 7.0(±0.5), KD$_2$PO$_4$/K$_2$HPO$_4$) δ 7.87 (d, $J = 9.7$ Hz, 2H), 7.56 – 7.54 (m, 1H), 7.48 (t, $J = 7.5$ Hz, 2H).

References

Appendix D

Base-Catalyzed Decarboxylation of Mandelylthiamin: Direct Formation of Bicarbonate as an Alternative to Formation of CO$_2$

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Supporting Information

ABSTRACT: The decarboxylation of mandelylthiamin is subject to general base catalysis ($\beta = 0.26$), an outcome that is inconsistent with the expected dissociative transition state in which CO$_2$ forms along with a residual carbanion. The results implicate a previously unrecognized associative route in which addition of water to a carboxylate followed by base-catalyzed proton transfer and C–C cleavage produces bicarbonate directly. Various reports of the presence or absence of base catalysis in decarboxylation reactions are consistent with the associative route’s occurrence in cases where nucleophiles would be generated along with CO$_2$ in the usual dissociative route.

Decarboxylation reactions of carboxylic acids in water nominally involve the replacement of a carboxyl group by a proton.$^1$ Reactivity patterns suggest that the deprotonated form, the carboxylate, either directly or indirectly, is the reactive species.$^2$ Carbon–carbon bond cleavage from the carboxylate produces carbon dioxide and a residual electron pair in a stabilized carbanion, a carbanion equivalent, or an anionic leaving group. Evidence from catalytic patterns,$^3$ stereochemical outcomes,$^4$ changes in isotope effects,$^5$ and QM/MM calculations$^8$ suggests that in some cases the reaction may be slowed by recombination of CO$_2$ and the nascent nucleophile. It has been proposed that in a reaction that is slowed by recombination, a presociated acid that quenches the carbanion (Scheme 1) increases the overall rate.$^7$ Recent reports of computational analyses dispute the possibility that the catalysis occurs by protonation in competition with recombination of CO$_2$.$^{8,9}$ One of those reports concludes that the catalysis arises from stabilization of the transition state through complexation rather than proton transfer.$^{9}$

A contrasting specific acid-catalyzed route is consistent with initial addition of water and a proton to a neutral carboxyl group in an associative process. A proton shift and departure of protonated carbonic acid (rather than protonated CO$_2$) eventually produces CO$_2$.$^{10-13}$ In that process, C–C cleavage is facilitated as the residual lone pair is absorbed by the conjugated cationic center (Scheme 2). Recent computational results support this mechanism.$^{14,15}$

Scheme 2

Mandelylthiamin (MTh)$^{16}$ is an accurate functional model of the key covalent intermediate (MTh diprophosphate, MThDP) in catalysis by benzoylformate decarboxylase.$^{17}$ However, the rate constant for decarboxylation of MThDP in the enzymatic reaction is at least 10$^5$ times larger than that of MTh in water.$^{18,19}$ The source of the enzyme’s rate enhancement is unknown. An analogy to the proposed addition of an active-site serine hydroxyl to the carboxyl of enzyme-bound MThDP proposed for BAL A285$^{18}$ (Scheme 3) would be a reasonable prospect.

Scheme 3

In the decarboxylation of MTh, a neutral associative pathway could occur by initial addition of water to the carboxyl. In that case, removal of a proton and C–C cleavage would produce a carbanion and bicarbonate, a weaker electrophile than CO$_2$ that would be less susceptible to internal return.$^{19}$ We found that consistent with such an expectation, general base catalysis facilitates the decarboxylation of MTh. Increasing concentrations of acetic acid/acetate buffers gave a linear increase in

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the observed first-order rate constant for conversion of MTh to 2-(1-hydroxybenzyl)thiamin (HBnTh) (Figure 1). We con-

Figure 1. The observed first-order rate constant increased linearly with acetate buffer concentration at 25 °C, as observed by UV spectroscopic analysis of the product and reactant concentrations.

firmed that the reaction gave the expected products of decarboxylation and that there were no inhibitory materials in solution or effects originating from ionic strength variation.

A plot of the observed rate constant for the buffer-dependent process as a function of the buffer component ratio indicated that only the Bronsted base is catalytic (Figure 2). The slope of

Figure 2. The observed catalysis arises from the action of the base component of the acetate buffer on protonated MTh.

the plot for a series of substituted acetates as catalysts in the decarboxylation of MTh gave $\beta = 0.26$ (Figure 3). This implicates a rate-determining transition state in which there is a very small extent of proton transfer from water to the Bronsted base. This also rules out a process in which simple C–C bond cleavage alone is rate-determining with these catalysts. We also observed a solvent kinetic isotope effect of $k_{H_2O}/k_{D_2O} = 1.8$ in 0.4 M 1:1 acetate/acetic acid buffer. This is consistent with the conclusion that proton transfer is a component of the transition state of the rate-determining step in an associative mechanism.

Since transfer of a proton from MTh cannot accelerate the unimolecular dissociative decarboxylation process, the catalytic effect of the base must be achieved by its interaction with water and MTh in combination. Base-catalyzed addition of water to the carboxyl of MTh could produce a dianionic intermediate (similar to those postulated by Hine and Koser in the reactions of aldehydes$^{21}$) in a rate-determining process. That intermediate could release bicarbonate and the conjugate base of HBnTh (Scheme 4). However, given the high basicity of such

Scheme 4

$$
\begin{align*}
H_2O + & \quad HOCOCH_3 \quad k_B \quad R-HOCOCH_3 + B^- \quad k_d
\end{align*}
$$

an intermediate, it is more likely that transfer of the proton would be concerted with cleavage of the C–C bond, producing bicarbonate in the same step (Scheme 5). Extrapolation of the

Scheme 5

$$
\begin{align*}
H_2O + & \quad HOCOCH_3 \quad k_B \quad R-HOCOCH_3 + B^- \quad k_d
\end{align*}
$$

base-catalyzed rate to that for the reaction in the absence of buffer suggests a variant on the same general mechanism in which bicarbonate is the initial product (Scheme 6).

Scheme 6

The reaction pattern suggests that when the formation of CO$_2$ occurs in concert with annihilation of the negative charge (i.e., where there is no residual nucleophile), there is no route for recombination with CO$_2$. Therefore, base catalysis following addition of water will not be observed. The decarboxylation of carboxyisoxazoles is consistent with this hypothesis (Figure 4). Kemp and Paul reported that "a discrete carbonan [upon loss of CO$_2$] is excluded as a significant reactive intermediate." Fragmentation of the carbonan is concerted with its formation along with CO$_2$, leading to stabilized products that cannot recombine. Those authors also reported that the reaction is not subject to buffer catalysis, which is consistent with the concerted loss of the leaving group and CO$_2$. Theoretical
analysis also led to the conclusion that the reaction has a high barrier to reversal that accounts for the lack of catalysis.\textsuperscript{23}

In a related reaction, the decarboxylation of trichloroacetate (TCA) produces the conjugate base of chloroform. The rates of deprotonation of chloroform\textsuperscript{27} and of HBrO\textsubscript{2}\textsuperscript{7} by hydroxide are similar. Therefore, according to our proposed mechanistic criteria, decarboxylation of TCA should also be accelerated in a base-catalyzed associative route. This is consistent with otherwise inexplicable observations that have stood for more than a century. Silberrstein reported in 1884 that aniline promotes the decarboxylation of TCA.\textsuperscript{26} This was confirmed and investigated in greater detail by Goldschmidt and Bräuer in 1906.\textsuperscript{27} The production of deuterochloroform from hexachloroacetone involves decarboxylation of TCA and is catalyzed by pyridine\textsuperscript{28} and by sodium deuterioxide.\textsuperscript{29} The carbon kinetic isotope effect in the spontaneous decarboxylation of TCA is consistent with rate-determining C–C bond cleavage.\textsuperscript{30}

However, in connection with the observation of the products of decomposition of the resulting conjugate base of chloroform, Bigeleisen and Allen noted that "alkali increases the rate of chloride production," attributing this to factors that may be interpreted as a consequence of acceleration of the decarboxylation process.\textsuperscript{31} As we have noted, this is required in the associative route that produces bicarbonate (which then produces CO\textsubscript{2}) but is not consistent with a dissociative process that forms CO\textsubscript{2} initially.

In conclusion, our results provide a basis for the development of a comprehensive view of decarboxylation that specifies a role for base catalysis. The addition of water to the carbonyl is followed by base-catalyzed loss of bicarbonate. The usual dissociative route that forms CO\textsubscript{2} may be slowed by reversion, while addition of water to the carbonyl group provides an opportunity for initial formation of the less reactive bicarbonate ion along with the residual carbonyl. This also supports the notion that enzymes may provide active-site nucleophiles to overcome internal return of CO\textsubscript{2} accounting for the apparent acceleration of what is usually presented as a unimolecular dissociative process.\textsuperscript{31,32}

**REFERENCES**