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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) is an accurate model of the covalent intermediate derived from the condensation of thiamin diphosphate and benzoylformate in benzoylformate decarboxylase. 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.

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 enzymic turnover number (k_{cat}) is about 10^6 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 hydrolysis of ethyl mandelylthiamin (**4**) occurs quantitatively in concentrated hydrochloric acid, with the highly acidic medium facilitating ester cleavage while suppressing decarboxylation. The problematic step in this sequence is the condensation of ethyl benzoylformate with the C2-conjugate base of thiamin in ethanol, which produces very small amounts of ethyl mandelylthiamin (**4**). Our attempts to improve the yield within the established reaction framework were unsuccessful. Extending the duration of the reaction does not affect the composition of the quenched reaction. It is clear that the reaction is reversible and the equilibrium concentrations provide little of the desired product (Scheme 2). As the condensation product is a bulky alkoxide (**4′**), it is likely that both enthalpy and entropy favor the reactants over the products. Furthermore, the basic conditions necessary to generate the conjugate base of thiamin produce the alkoxide of **4′** that eliminates thiamin to produce the benzoylformate ester [12].

In order to improve the synthetic process, it is clear that the condensation equilibrium must be induced to favor the product to a greater extent. Thus, we considered the possibility that an oxyphilic metal ion can form a more stable product with the alkoxide. However, ethoxide and ethanol, used in the existing preparation, would then compete for the metal ion with the alkoxide formed in the addition reaction. Therefore, we conducted the reaction in

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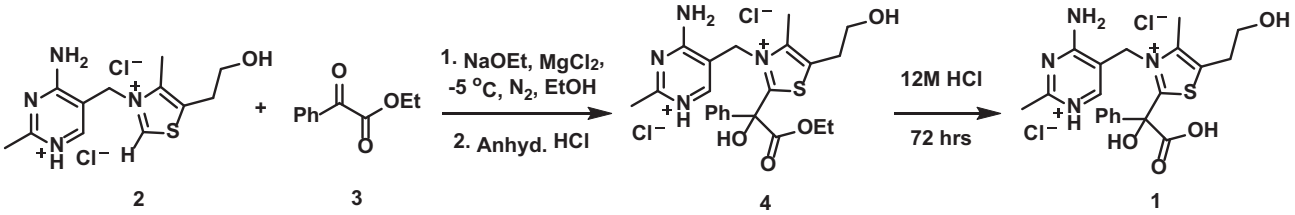
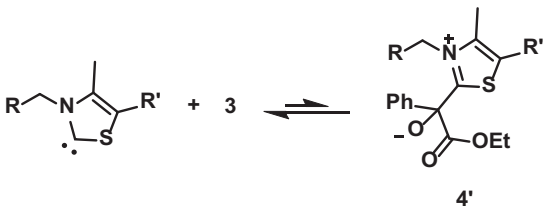


Scheme 1. The previously reported synthesis of mandelylthiamin.



Scheme 2. The addition of the conjugate base of thiamin to benzoylformate ethyl ester in basic ethanol is reversible.

an aprotic solvent using a strong, non-coordinating base (sodium bis(trimethylsilyl)amide (NaHMDS); p*K*a ~ 30) [21] to remove the proton at C-2 of thiamin (p*K*a ~ 17) [22]. This will also remove the proton from thiamin's hydoxyl. 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. Salts 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. ^{1}H NMR (D_2O with a small amount of $KHCO_3$ to prevent hydrolysis) indicated 99% THP-protected product.

^{1}H NMR (600 MHz, DMSO-d_6) [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 $[C17H25N4O2S]^+$, calculated: 349.17, observed: 349.2.

2.3. Synthesis of mandelylthiamin 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 N_2 and cooled to −20 °C in a 35% EtOH:water bath with dry ice. LiHMDS (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 transferred to vigorously stirring trifluroacetic acid (~50 mL) 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 ^{1}H 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). R_f = 0.25 (15% MeOH/DCH; 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 ^{1}H NMR using chloroacetic acid as an internal standard).

^{1}H NMR (600 MHz, D_2O): 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).

^{13}C NMR (100 MHz, D_2O): δ 170.5, 169.9, 161.5, 160.7, 145.4, 137.7, 136.1, 135.1, 129.9, 129.7, 126.1, 107.3, 79.2, 60.1, 55.1, 47.4, 29.5, 20.7, 11.7.

ESI-MS $[C21H25N4O4S]^+$, calculated: 429.16, observed: 429.2.

2.5. Synthesis of mandelylthiamin 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.

^{1}H NMR (300 MHz, D_2O): 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 Hz), 2.47 (s, 3H), 2.38 (s, 3H), 1.28 (t, 3H, J = 7.2).