Mg$^{2+}$-Imidazole-Catalyzed Self-Condensation of Malonyl Thioesters: Getting Tuned for Biomimetic Polyketide Synthesis?

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Abstract: We report that a subtle balance of carbanion reactivity, leaving group activation, and pK$_a$ of the catalyst is required for efficient self-condensation of thiomalonates to thioacetoacetates in up to 71% yield under “biomimetic” conditions originally proposed by Kobuke and Yoshida (Tetrahedron Lett. 1978, 19, 367).

Keywords: Bioorganic chemistry, Claisen condensation, enzyme mimics, polyketide synthesis.

The Claisen condensation of malonyl thioesters is one of the central processes in the biosynthesis of polyketide natural products [1]. Although the diverse enzymes that catalyze this remarkable biooligomerization are increasingly well understood and extensively exploited in modern biotechnology [2-4], synthetic catalysts for similarly controlled oligomerization of malonyl thioesters in enzyme-free systems do not exist. Kobuke and Yoshida have, however, demonstrated more than twenty years ago that Claisen condensation of n-butyl thiomalonate 1a and phenyl thioacetate 2e can be catalyzed by imidazole and magnesium cations in THF at room temperature to give n-butyl thioacetoacetate 3a in 87 h and 60% yield (Scheme 1) [5]. These Kobuke-Yoshida (KY) conditions [5,6] contrast sharply with the harsher conditions required in other model systems for polyketide synthesis [7-9] but not polyketide cyclization [10-12].
Results and Discussion

The original KY-conditions are of potential significance for the construction of artificial polyketide synthases because many histidine-rich organic architectonics with esterase and/or phosphatase activity have been elegantly devised over the past two decades [13-15]. Original KY-conditions are, however, incompatible with the Claisen self-condensation required for oligomerizations leading to polyketides (Scheme 2). Specifically, self-condensation of \( n \)-butyl thiomalonate 1a or phenyl thioacetate 2e was impossible because of poor leaving group (LG) ability of \( n \)-butylthiolate or lack of access to activated carbanion intermediates via decarboxylation, respectively [5]. Here we report that Mg\(^{2+}\)-imidazole-catalyzed thiomalonate dimerization is possible in up to 71% yield under refined conditions by precise fine tuning of the properties of carbanion intermediate, thiolate leaving group, and catalyst, and show that future applicability of this approach toward biomimetic polyketide synthesis is nevertheless problematic.

To elucidate the subtle balance between leaving group and carbanion activation needed for thiomalonate dimerization under KY-conditions, we prepared substrates 1a-f with systematically varied leaving groups (LG) (Scheme 2, Table 1). Reduced Hammett \( \sigma_p \) [16] (corresponding with one exception to the p\( K_a \) of the employed thiophenols [17,18]) should reduce LG-activation and increase the reactivity of carbanion intermediates, which can be seen as up-field shifts of the \( \alpha \)-hydrogens (Table 1). Indeed the rate of substrate consumption was inversely related to the Hammett \( \sigma_p \).
However, highly stabilized carbanions did not further react with the electrophiles, thus dominant formation of decarboxylation products 2e-2g resulted. Thiomalonate self-condensation giving rise to thioacetoacetates 3b-3f in up to 37% yield for 3c occurred only at intermediate activation of both carbanion and LG.

The most promising thiomalonate 1c was studied in more detail. Separation of the product mixture by reverse-phase HPLC was possible on analytical YMC-Pro-C8-columns applying a linear solvent gradient of water / CH₃CN (0.1% TFA) = 20% - 100% over 20 min. Only one additional product with a retention time $R_t = 5.22$ min similar to that of substrate 1c ($R_t = 4.94$ min) was observed besides the expected acetoacetate 3c ($R_t = 5.90$ min), 4-thioanisole ($R_t = 6.31$ min) and 4-methoxyphenyl thioacetate 2c ($R_t = 6.31$ min). This new product decomposed easily and could not be fully purified. However, in-situ methylation with trimethylsilyl diazomethane (TMSCHN₂) in toluene-methanol gave a stable product with $m/z = 319$ in the ESI-MS that was consistent with $[M + Na]^+$ expected for methyl ester 5c. The $^1$H-NMR spectrum of 5c revealed about 50%-conjugation of the enol ether with both carbonyl groups. This derivatization demonstrated that the new unstable product formed from 1c under KY-conditions is carboxylate 4c. Identification of 4c allowed us to assign all new resonances appearing in $^1$H-NMR spectra recorded during the course of a reaction in THF-$d_8$ to either 3c, 4c, 2c or acetylimidazole.

The satisfactory outcome with substrate 1c encouraged us to study the influence of additional parameters. Thiomethylmalonate 1g gave decarboxylation product 2g only, probably due to the steric effect of the methyl group on the α-position (Table 1). Replacement of Mg²⁺ by other divalent cations such as Zn²⁺, Cu²⁺, Ca²⁺, or Ba²⁺ under otherwise original KY conditions led to an increase in acetate

| entry | Substrate | $\chi^a$ | $R^a$ | $\delta$ (ppm)$^b$ | $\sigma_p$ | $pK_a$ (LG)$^c$ | $t$ (h) | 3 (%)$^d$ |
|-------|-----------|---------|-------|-----------------|----------|-----------------|--------|--------|
| 1     | 1a        |         | H     | 3.62            | -        | -               | 0      | 0      |
| 2     | 1b        |         | H     | 3.65            | -        | 9.43            | 96     | 10     |
| 3     | 1g        |         | CH₃   | 3.79            | -0.28    | 7.06            | 72     | 0      |
| 4     | 1c        |         | CH₃   | 3.69            | -0.28    | 7.06            | 17     | 37     |
| 5     | 1d        |         | CH₃   | 3.70            | -0.14    | 7.08            | 2      | 30     |
| 6     | 1e        |         | H     | 3.72            | 0.00     | 6.81            | 1      | 14     |
| 7     | 1f        |         | H     | 3.73            | 0.24     | 6.53            | 1      | 5      |

$^a$Compare Scheme 2. $^b$Chemical shift $\delta$ of the α-proton(s) in the $^1$H NMR spectra of 1a-g. $^c$p$K_a$ of the conjugate acid of the thiolate leaving groups (LG). $^d$Yields refer to NMR- and HPLC-pure products isolated after reaction time $t$ needed for substrate consumption.

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(2a-2f) and/or hydrolyzed products rather than Claisen self-condensation. The rate of substrate consumption decreased with decreasing $pK_a$ of the imidazole catalyst [20] (Table 2). The yield of Claisen products 3c and 4c, however, increased from imidazole (36%) over benzimidazole (47%) to 71% with 8-nitrobenzimidazole. Further decrease in catalyst $pK_a$ gave 51% for 4(5)-nitroimidazole. This identified $pK_a \approx 3$ as optimum catalyst $pK_a$ for Claisen self-condensation under these conditions (Table 2).

**Table 2.** Claisen self-condensation of thiomalonate 3 in THF$^d$ at room temperature in presence of Mg(OAc)$_2$ and the indicated imidazole catalyst.

| entry | cat$^a$ | $pK_a$ $^a$ | $t$ (h) | 3 (%)$^b$ | 3a+3b (%)$^c$ |
|-------|--------|-------------|--------|----------|--------------|
| 1     | ![imidazole catalyst](image) | 6.95        | 3      | 12       | 36 (22 + 14) |
| 2     | ![imidazole catalyst](image) | 5.40        | 46     | 18       | 47 (14 + 33) |
| 3     | ![imidazole catalyst](image) | 3.05        | 48     | 5        | 71 (34 + 37) |
| 4$^d$ | ![imidazole catalyst](image) | 3.05        | 72     | 30       | 35 (25 + 10) |
| 5     | ![imidazole catalyst](image) | 1.50        | 92     | 17       | 51 (18 + 33) |

$^a$Imidazole catalysts of different $pK_a$. $^b$Yields refer to remaining substrate at time $t$ according to 1H-NMR-spectra of the reaction mixture. $^c$Yields refer to NMR- and HPLC-pure products isolated, yields in parenthesis give the relative distribution between Claisen products 3a and 3b in RP-HPLC of product mixtures after reaction time $t$ (Scheme 2). $^d$CHCl$_3$/18-crown-6 9:1 instead of THF.

Replacement of THF with other solvents except dioxane and addition of more than 10% water inhibited self-condensation. Kobuke and Yoshida’s original implication that ether-coordination to Mg$^{2+}$ is essential for catalysis was further partially supported by slow appearance of up to 35% Claisen products 3c and 4c in CHCl$_3$ containing 10% 18-crown-6 with 8-nitrobenzimidazole as catalyst (Table 2, entry 4). The reaction did, however, not proceed in the “ether-rich” micelles formed by 10% Triton X-100 in water, also when N-acetylhistidine was used instead of imidazole or 8-nitrobenzimidazole.

No indications for further reaction of thiomalonate 1c with Claisen products 3c or 4c were found. This suggests that Aldol and Claisen reaction with $\beta$-ketone and thioester for formal continuation along the terpenoid and polyketide pathway [1], respectively, are not possible under these conditions. Novel approaches toward transient deactivation of the $\beta$-ketone in acetoacetates required for polyketide synthesis under these conditions are under investigation. Preliminary studies with “additives” such as TMSCHN$_2$, TES-Cl, n-butylamine, $p$-methoxyaniline and phenylhydrazine [21] were not successful.
Conclusions

In summary, we have found that Claisen self-condensation of thiomalonates to thioacetoacetate under “biomimetic” conditions is dependent on a subtle balance of carbanion and leaving group activation and the $pK_a$ of the imidazole catalyst. These findings demonstrate that future development of more refined artificial “metallo-Claisenases” is possible along the route originally proposed by Kobuke and Yoshida [5]. The possibility to expand Mg$^{2+}$-imidazole-catalyzed Claisen self-condensation under these conditions toward polyketide synthesis remains, however, questionable.

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Experimental

General

Synthetic reagents were purchased from Fluka or Acros. Column chromatography was carried out on silica gel 60 (Fluka, 40-63 mm). Analytical thin layer chromatography (TLC) and preparative thin layer chromatography were performed on silica gel 60 (Fluka, 0.2 mm) and silica gel GF-2 (Aldrich, 1 mm), respectively. Reverse Phase column chromatography was performed with Silicagel 100 C18-Reverse Phase. $^1$H- and $^{13}$C-NMR spectra were recorded on Bruker 400 MHz Spectrometer. ESI-MS were performed on a Finnigan MAT SSQ 7000. HPLC was carried out using a YMC Pro-C8 (4 x 50 mm) prepacked column. Anhydrous THF and ethyl ether were distilled over Na and benzophenone.

Thiomalonate 1c: General procedure for preparation of thiomalonate esters

Thiomalonate esters were prepared following the procedure in reference [5]. Namely, to a solution of malonylchloride (1.6 mL, 16 mmol) in dry ether (20 mL) 4-methoxybenzenethiol (2.0 mL, 16 mmol) was added at room temperature (rt) under nitrogen atmosphere. After stirring for 3h, saturated aqueous NaHCO$_3$ solution was added to the reaction mixture. The ether layer was discarded, and the aqueous layer was further washed with EtOAc. After acidification of the aqueous layer, the product was obtained by extraction (EtOAc), washing (brine), drying (Na$_2$SO$_4$) and evaporation under reduced pressure. The crude product was purified by silica gel column chromatography (acetone) then by recrystalization (dichloromethane) to give pure title compound as colorless crystals (1.7 g, 45 %): mp
89-90 °C; \(^1\)H-NMR (CDCl\(_3\)) \(\delta\) 9.80 (br.s, 1 H), 7.36 (d, \(J = 6.7\) Hz, 2 H), 6.95 (d, \(J = 6.7\) Hz, 2 H), 3.83 (s, 3 H), 3.69 (s, 2 H); \(^{13}\)C-NMR (CDCl\(_3\)) \(\delta\) 191.0 (s), 171.0 (s), 161.0 (s), 136.1 (2 x d), 117.0 (s), 115.1 (2 x d), 55.4 (q), 48.0 (t).

**Acetoacetate 3c: General procedure for Claisen condensation**

To a solution of 4-methoxyphenyl thiomalonate (1c, 20 mg, 0.088 mmol) in THF (1 mL), Mg(OAc)$_2$·4H$_2$O (10 mg, 0.047 mmol) and imidazole (6 mg, 0.088 mmol) were successively added. The mixture was stirred for 17 h at rt, and then it was diluted with CH$_2$Cl$_2$, washed with 1 M HCl and brine, dried over Na$_2$SO$_4$ and concentrated under reduced pressure. The crude product was purified by column chromatography (1:1 to 0:1 petroleum-ether/CH$_2$Cl$_2$) to give pure acetoacetate 3c as a colorless oil (3.7 mg, 37 %): \(^1\)H-NMR (CDCl$_3$) \(\delta\) 7.37 (d, \(J = 8.8\) Hz, 0.6 H), 7.32 (d, \(J = 8.8\) Hz, 1.4 H), 6.94 (d, \(J = 8.8\) Hz, 2 H), 5.47 (s, 0.3 H), 3.82 (s, 3 H), 3.73 (s, 1.4 H), 2.27 (s, 2.1 H), 1.94 (s, 0.9 H).

**Methyl ester 5c**

The crude product obtained following the general procedure for Claisen condensation starting from 1c (213 mg) was fractionated by column chromatography (ODS, 40 to 100 % CH$_3$CN in 0.1 % aqueous TFA). The fractions containing 4c were concentrated briefly and then extracted with EtOAc. The organic layer was washed with brine, dried over Na$_2$SO$_4$ and concentrated under reduced pressure to give crude product (32 mg) consisting of 1c and 4c. The product mixture obtained was dissolved in toluene (0.5 mL) and methanol (0.5 mL), and TMSCHN$_2$ (2 M in hexane, 0.3 mL) was added. After stirring for 30 min, the mixture was concentrated under reduced pressure and purified by preparative TLC (CH$_2$Cl$_2$, \(R_f\) 0.26) to give pure 5c (3.5 mg, 1.2%): \(^1\)H-NMR (CDCl$_3$) \(\delta\) 7.36 (d, \(J = 8.9\) Hz, 2 H), 6.94 (d, \(J = 8.9\) Hz, 0.8 H), 6.93 (d, \(J = 8.9\) Hz, 1.2 H), 5.62 (s, 0.4 H), 5.23 (s, 0.6 H), 4.18 (s, 1.2 H), 3.83 (s, 1.2 H), 3.82 (s, 1.8 H), 3.80 (s, 0.8 H), 3.75 (s, 1.2 H), 3.71 (s, 1.8 H), 3.70 (s, 1.8 H), 3.67 (s, 1.2 H); ESI-MS (CH$_2$Cl$_2$ + TFA) \(m/z\) 334.9 (8.25) [M + K]$^+$, 319.0 (100) [M + Na]$^+$, 157.4 (31.9) [M - MeOC$_6$H$_4$S]$^+$.

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*Sample Availability:* Available from the authors.