

Mg²⁺-Imidazole-Catalyzed Self-Condensation of Malonyl Thioesters: Getting Tuned for Biomimetic Polyketide Synthesis?

Naomi Sakai, Nathalie Sordé and Stefan Matile*

Department of Organic Chemistry, University of Geneva, CH-1211 Geneva 4, Switzerland. FAX: (+41) 22 328 7396.

* Author to whom correspondence should be addressed; E-mail: stefan.matile@chiorg.unige.ch

Received: 3 September 2001; in revised form 15 October 2001 / Accepted: 16 October 2001 /

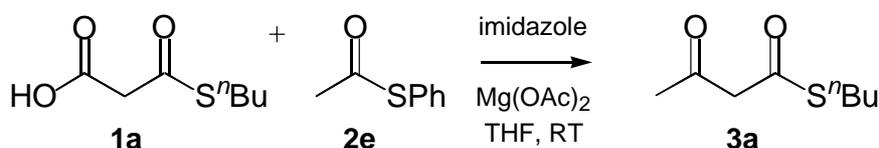
Published: 31 October 2001

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 thioacetoactate **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].

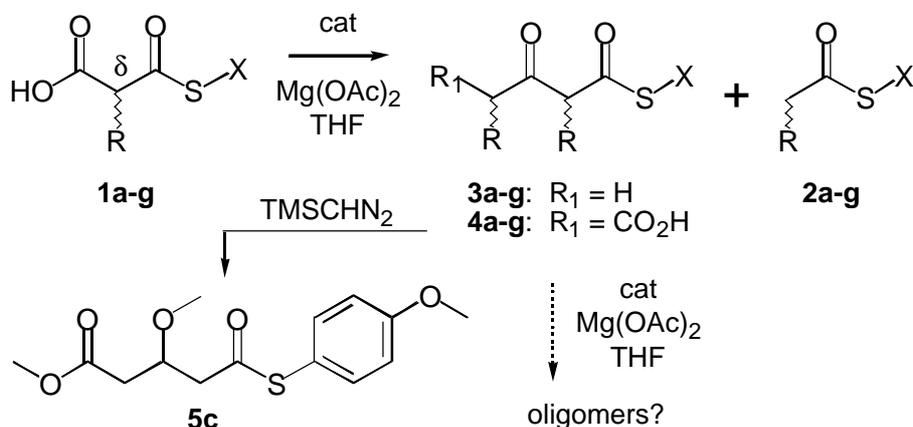
Scheme 1



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.

Scheme 2



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 σ_p [16] (corresponding with one exception to the $\text{p}K_a$ 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 α -hydrogens (Table 1). Indeed the rate of substrate consumption was inversely related to the Hammett σ_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.

Table 1. Claisen self-condensation of thio(methyl)malonates **1a-g** in THF at room temperature in presence of $Mg(OAc)_2$ and imidazole.

entry	Substrate	χ^a	R^a	δ (ppm) ^b	σ_p	pK_a (LG) ^c	t (h)	3 (%) ^d
1	1a		H	3.62	-	~11	-	0
2	1b		H	3.65	-	9.43	96	10
3	1g		CH ₃	3.79	-0.28	7.06	72	0
4	1c		H	3.69	-0.28	7.06	17	37
5	1d		H	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

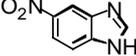
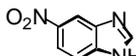
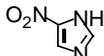
^aCompare Scheme 2. ^bChemical shift δ of the α -proton(s) in the 1H NMR spectra of **1a-g**. ^c pK_a of the conjugate acid of the thiolate leaving groups (LG). ^dYields refer to NMR- and HPLC-pure products isolated after reaction time t needed for substrate consumption.

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 / CH_3CN (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 ($TMSCHN_2$, [19]) 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 1H -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 1H -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 Mg^{2+} by other divalent cations such as Zn^{2+} , Cu^{2+} , Ca^{2+} , or Ba^{2+} under otherwise original KY conditions led to an increase in acetate

(**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		6.95	3	12	36 (22 + 14)
2		5.40	46	18	47 (14 + 33)
3		3.05	48	5	71 (34 + 37)
4 ^d		3.05	72	30	35 (25 + 10)
5		1.50	92	17	51 (18 + 33)

^aImidazole catalysts of different pK_a . ^bYields refer to remaining substrate at time t according to ¹H-NMR-spectra of the reaction mixture. ^cYields 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 β -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 β -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.

Acknowledgments

We thank Prof. J. Yoshida (Kyoto University) for fruitful discussions, A. Pinto, J.-P. Saulnier, the group of Prof. F. Gülaçar, and Dr. H. Eder for NMR, MS, and elemental analyses, respectively, and the Swiss NSF (21-57059.99 and National Research Program “Supramolecular Functional Materials” 4047-057496) for financial support.

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. 1H - 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_2SO_4) and evaporation under reduced pressure. The crude product was purified by silica gel column chromatography (acetone) then by recrystallization (dichloromethane) to give pure title compound as colorless crystals (1.7 g, 45 %): mp

89-90 °C; $^1\text{H-NMR}$ (CDCl_3) δ 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); $^{13}\text{C-NMR}$ (CDCl_3) δ 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), $\text{Mg}(\text{OAc})_2 \cdot 4\text{H}_2\text{O}$ (10 mg, 0.047 mmol) and imidazole (6 mg, 0.088 mmol) were successively added. The mixture was stirred for 17h at rt, and then it was diluted with CH_2Cl_2 , washed with 1M HCl and brine, dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by column chromatography (1:1 to 0:1 petroleum-ether/ CH_2Cl_2) to give pure acetoacetate **3c** as a colorless oil (3.7 mg, 37 %): $^1\text{H-NMR}$ (CDCl_3) δ 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_3CN 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_2SO_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_2Cl_2 , R_f 0.26) to give pure **5c** (3.5 mg, 1.2%): $^1\text{H-NMR}$ (CDCl_3) δ 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 ($\text{CH}_2\text{Cl}_2 + \text{TFA}$) m/z 334.9 (8.25) $[\text{M} + \text{K}]^+$, 319.0 (100) $[\text{M} + \text{Na}]^+$, 157.4 (31.9) $[\text{M} - \text{MeOC}_6\text{H}_4\text{S}]^+$.

References

1. Herbert, R. B. *The Biosynthesis of Secondary Metabolites*, Chapman & Hall: London, **1994**.
2. Rawlings, B. J. *Nat. Prod. Rep.* **1999**, *16*, 425.
3. Khosla, C.; Harbury, P. B. *Nature* **2001**, *409*, 247.
4. Abe, I.; Morita, H.; Nomura, A.; Noguchi, H. *J. Am. Chem. Soc.* **2000**, *122*, 11242.
5. Kobuke, Y.; Yoshida, J. *Tetrahedron Lett.* **1978**, *19*, 367.
6. Brooks, D. W.; Lu, L. D.-L.; Masamune, S. *Angew. Chem., Int. Ed. Engl.* **1979**, *18*, 72.
7. Scott, A. I.; Wiesner, C. J.; Yoo, S.; Chung, S.-K. *J. Am. Chem. Soc.* **1975**, *97*, 11242.
8. Sun, S.; Edwards, L.; Harrison, P. *J. Chem. Soc., Perkin Trans. I* **1998**, 437.

9. Talapatra, S. K.; Pal, P.; Biswas, K.; Shaw, A.; Chakrabarti, R.; Talapatra, B. *J. Ind. Chem. Soc.* **1998**, *75*, 590.
10. Harris, T. M.; Harris, C. M. *Pure Appl. Chem.* **1986**, *58*, 283.
11. Crombie, L.; Games, D. E.; James, A. W. G. *J. Chem. Soc., Perkin Trans. I* **1996**, 2715.
12. Yamaguchi, M.; Shibato, K.; Nakashima, H.; Minami, T. *Tetrahedron* **1988**, *44*, 4767.
13. A. J. Kirby, A. J. *Enzyme Mimics*, in *Stimulating Concepts in Chemistry*, 341-353, M. Shibasaki, M.; Stoddart, J. F.; Vögtle, F.; Eds., VCH: New York, **2000**.
14. Dugas, H. *Bioorganic Chemistry*, Springer: New York, **1996**.
15. Matile, S. *Chem. Soc. Rev.* **2001**, *30*, 158.
16. Hansch, C.; Leo, A.; Taft, R. W. *Chem. Rev.* **1991**, *91* 185.
17. Douglas, K. T.; Alborz, M.; Rullo, G. R.; Yaggi, N. F. *J. Chem. Soc., Perkin Trans. II* **1982**, 1675.
18. Chuchani, G.; Frohlich, A. *J. Chem. Soc. (B)* **1971**, 1417.
19. Hashimoto, N.; Aoyama, T.; Shioiri, T. *Chem. Pharm. Bull.* **1981**, *29*, 1475.
20. Bruice, T. C.; Schmir, G. L. *J. Am. Chem. Soc.* **1958**, *80*, 148.
21. D'Angeli, F.; Filira, F.; Scoffone, E. *Tetrahedron Lett.* **1965**, *6*, 605.

Sample Availability: Available from the authors.