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Biomimetic iterative method for polyketide synthesis

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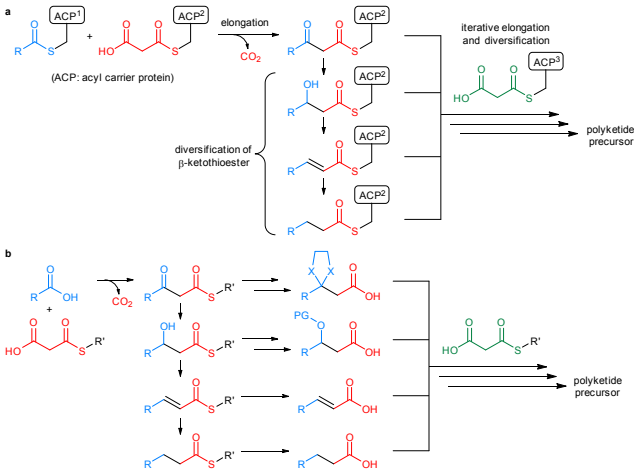
An iterative method for synthesizing polyketides was demonstrated, in which the chain elongation of a carboxylic acid was performed by decarboxylative dehydration condensation with a malonic acid half thioester. After trasforming the resulting β-ketotioester into an appropriate form, the carboxylic acid functionality was regenerated for the next elogation step.

Polyketides constitute an important class of secondary metabolites with a wide variety of molecular structures.[1] A number of polyketide-derived compounds are biologically active, and have been used as potent pharmaceuticals. Despite the diverse structures and oxidation states of polyketides, their frameworks are constructed via the common iterative process in nature (Scheme 1a).[2] First, a starting substrate such as acetyl-CoA and an extender unit, typically malonyl-CoA, are each bound to a separate acyl carrier protein. A ketosynthase then promotes the decarboxylative Claisen condensation of the two thioesters to create a β-ketothioester. Finally, the resulting β-carbonyl group is derivatized into an appropriate form, i.e., the β-ketothioester can be transformed into a β-hydroxythioester, an α,β-unsaturated thioester, or a saturated thioester. Owing to the options for these transformations, the structures and oxidation states of polyketides can be diversified. Through repetitive Claisen condensations and β-carbonyl transformations, linear polyketide precursors are synthesized.

Chemists have utilized the biological system for the production of beneficial polyketides. Recent progress on the characterization and engineering of polyketide synthases has facilitated the use of them for producing natural and non-natural polyketide compounds.[3] The heterologous expressions of polyketide synthases have also been employed for the scale-up synthesis of polyketides.[4] However, there is a drawback in utilizing biocatalysts; the availability of microbes and enzymes necessary for the production of a desired polyketide is limited. By establishing a chemical iterative process without relying on enzymatic synthesis, a scalable production of polyketides with simple manipulations is possible.



Scheme 1 (a) Biosynthesis of polyketides via iterative decarboxylative Claisen condensation and transformation of a β-carbonyl group. (b) Proposed biomimetic scheme based on the decarboxylative condensation of a carboxylic acid and MAHT. PG = protecting group.

In the field of organic synthesis, intensive efforts have been made for the chemical synthesis of complex natural polyketides.[5] Most of the research is target-oriented synthesis, and a synthetic route is optimized for each polyketide compound. For the synthesis of a different type of polyketide, it is necessary to explore a new route and suitable reactions. In addition, elaborate reaction apparatus and well-experienced synthetic skills are sometimes required. Therefore, a simple method that can be used to produce diverse polyketides would be of considerable interest. An iterative method is a promising candidate for such an ideal synthesis, in which polyketides can be obtained by repeating a routine protocol. Although there are notable examples of complex polyketide synthesis via iterative reactions,[6] they are only applicable to

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Electronic Supplementary Information (ESI) available: Scheme S1, experimental procedures, spectral data, HPLC traces and copies of NMR spectra. See DOI: 10.1039/x0xx00000x

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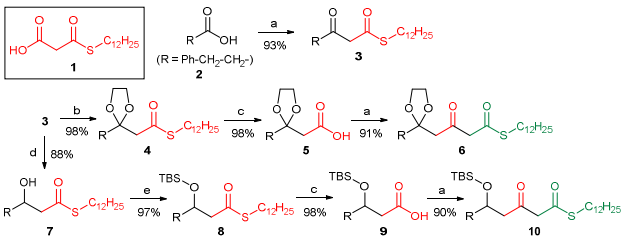
specific types of polyketides such as polyols[7] and deoxy-polypropionates.[8] To establish a more versatile method for the synthesis of various polyketides, it is necessary to include diversification steps in each iterative cycle, as in polyketide biosynthesis.

In relation to the enzymatic chain elongation in natural polyketide synthesis, increasing attention has been paid to the reaction with malonic acid half thioester (MAHT). MAHT is regarded as a mimic of malonyl-CoA, and used for carbon–carbon bond-forming reactions. In 2003, Shair and co-workers reported a copper-catalyzed decarboxylative aldol reaction of aldehydes using MAHT as an aldol donor.[9] Since then, a number of the studies using MAHT for decarboxylative aldol, Mannich, and Michael reactions have been reported.[10] The reactions with MAHT proceed under mild reaction conditions in the presence of various catalysts, and afford thioester products that can be readily transformed into different functionalities. In spite of such attractive features, none of these reactions have been employed to the iterative synthesis of polyketides. In our attempt to apply Shair's aldol reaction for an iterative polyketide synthesis, it was revealed that the reaction efficiency significantly decreased with each repetition[9] (Scheme S1, ESI). Additionally, the thioester needs to be reduced to an aldehyde for elongation, which is a disadvantage in terms of chemical yield and redox economy.[11]

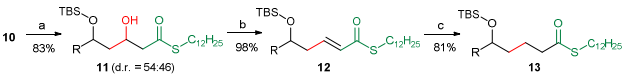
In this paper, we propose an iterative polyketide synthesis based on decarboxylative dehydration condensation with MAHT (Scheme 1b). Following the chain elongation of a carboxylic acid, the resulting β-ketothioester could be transformed into an appropriate form and hydrolyzed to a carboxylic acid for subsequent elongation. Notably, during condensation and hydrolysis, the oxidation state of the functional groups at the growing terminus did not change, i.e., from carboxylic acid to thioester. A high chemical yield for the chain elongation was achieved with the aid of a condensation reagent, and the high hydrolytic reactivity of the thioester group enabled the facile regeneration of the carboxylic acid under mild conditions. By combining the chain elongation with MAHT and the transformation of the β-ketothioester, linear polyketide precursors with the desired structure and the degree of oxidation were produced. Herein, we demonstrate the viability of this iterative scheme.

The chain elongation by the decarboxylative condensation using MAHT is the pivotal reaction for the proposed iterative scheme. First, reaction conditions were optimized. MAHT **1** prepared from malonic acid and odorless dodecanethiol was converted into a magnesium salt,[12] which was subsequently added to carboxylic acid **2** preactivated by a coupling reagent (Scheme 2). By employing COMU and DIEA, β-Ketothioester **3** was efficiently produced as a mixture of the keto and enol forms (only the keto form is displayed in the schemes for clarity). Notably, the chromatographic isolation of β-ketothioester **3** was facile because it is far less polar than the starting materials and condensation reagents.

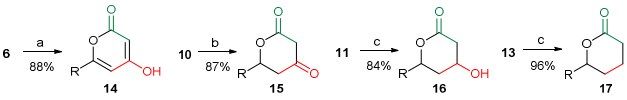
To examine the second chain elongation, β-ketothioester **3** was transformed. After the β-carbonyl group of **3** was protected as a 1,3-dioxolane, thioester **4** was hydrolyzed to carboxylic acid **5**. The hydrolysis readily occurred in the presence of cesium hydroxide and hydrogen peroxide, and the product could be isolated by extractions. The second elongation of carboxylic acid **5** with MAHT **1** under the optimized conditions afforded β-ketothioester **6**. Meanwhile, the reduction of β-ketothioester **3** followed by silyl protection of the resulting alcohol produced compound **8**. This was hydrolyzed and subjected to chain elongation, giving β-ketothioester **10**. The yields in the second elongations were comparative to that in the first elongation, indicating that the condensation strategy with MAHT is suitable for the iterative synthesis.



Scheme 2 Decarboxylative condensation with MAHT **1**, and second elongation after the modification of a β-carbonyl group. (a) COMU, DIEA, then **1**, *i*PrMgBr, DME, rt; (b) $TMSO(CH_2)_2OTMS$, TMSOTf, CH_2Cl_2, 0 °C to rt; (c) CsOH, H_2O_2, 1,4-dioxane/H_2O, rt; (d) $NaBH_3CN$, $CeCl_3{\cdot}7H_2O$, THF/AcOH, rt; (e) TBS-Cl, imidazole, DMF, rt. COMU = 1-[(1-(cyano-2-ethoxy-2-oxoethylideneaminooxy)-dimethylamino-morpholinomethylene)]methanaminium hexafluorophosphate, DIEA = *N*,*N*-diisopropylethylamine, DME = 1,2-dimethoxyethane, TMS = trimethylsilyl, Tf = trifluoromethane sulfonyl, TBS = *t*-butyldimethylsilyl.



Scheme 3 Transformation of compound **10**. (a) $NaBH_3CN$, $CeCl_3{\cdot}7H_2O$, THF/AcOH, rt; (b) Martin sulfurane, CH_2Cl_2, rt; (c) polymethylhydrosiloxane, $Cu(OAc)_2$, 1,2-bis(diphenylphosphino)benzene, toluene/THF, rt.



Scheme 4 Formation of triketide lactones with different degrees of oxidation. (a) $FeCl_3{\cdot}6H_2O$, CH_2Cl_2, rt, then DBU, CH_2Cl_2, rt; (b) TsOH, 1,4-dioxane/H_2O, rt, then KOH aq., rt; (c) $TsOH{\cdot}H_2O$, CH_3CN, rt. DBU = 1,8-diazabicyclo[5.4.0]undec-7-ene, TsOH = *p*-toluenesulfonic acid.

The derivatization of β-ketothioesters after the chain elongation is the key for the diversification of polyketide products. To imitate the biological diversifying strategy, the stepwise transformations of the β-carbonyl group of compound **10** were carried out (Scheme 3). The reduction of **10** with sodium cyanoborohydride and subsequent dehydration with Martin sulfurane smoothly proceeded at room temperature to give α,β-unsaturated thioester **12**. The copper-catalyzed hydrogenation[13] of **12** produced saturated thioester **13**. These reactions could be performed under ambient conditions without affecting the labile silyl ether. Accordingly, it is expected that desired linear polyketide precursors can be prepared via iterating the chain elongation with MAHT and the subsequent β-carbonyl transformation.

A number of polyketides have lactone substructures constructed via intramolecular transesterification catalyzed by a thioesterase. A linear triketide precursor is the minimal unit

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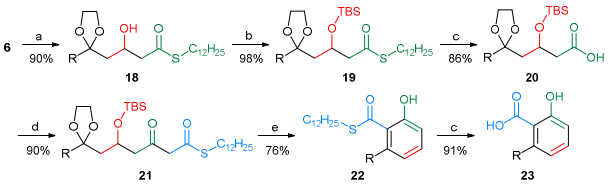
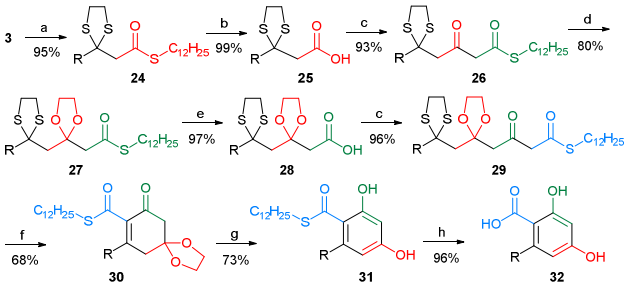
for lactonization. The utility of compounds **6**, **10**, **11**, and **13** was examined for the formation of triketide lactones with different oxidation states (Scheme 4). The dioxolane-protecting group of **6** was removed with iron(III) chloride hexahydrate,[14] and the resulting intermediate was treated with DBU. Cyclization rapidly occurred to form a pyrone ring. Compounds **10**, **11**, and **13** were deprotected, and the resulting intermediates were cyclized to afford lactones **15**, **16**, and **17**, respectively. The oxidation states of the δ-lactone skeleton reflected those of the linear precursors.



Scheme 5 Synthesis of 6-Substituted salicylic acid. (a) $NaBH_3CN$, $CeCl_3{\cdot}7H_2O$, THF/AcOH, rt; (b) TBS-Cl, imidazole, DMF, rt; (c) CsOH, H_2O_2, 1,4-dioxane/H_2O, rt; (d) COMU, DIEA, then **1**, *i*PrMgBr, DME, rt; (e) $FeCl_3{\cdot}6H_2O/SiO_2$, CH_2Cl_2, rt, then DBU, CH_2Cl_2, rt.



Scheme 6 Synthesis of orsellinic acid analogue. (a) $HS(CH_2)_2SH$, TMSOTf, 1,2-dichloroethane, rt; (b) CsOH, 1,4-dioxane/H_2O, rt; (c) COMU, DIEA, then **1**, *i*PrMgBr, DME, rt; (d) $TMSO(CH_2)_2OTMS$, TMSOTf, CH_2Cl_2, 0 °C; (e) CsOH, 1,4-dioxane/H_2O, 30 °C; (f) H_5IO_6, DME/CH_2Cl_2, 0 °C to rt, then Et_3N, CH_2Cl_2, rt; (g) HCO_2H/H_2O, rt; (h) mCPBA, 1,4-dioxane/H_2O, rt. mCPBA = *m*-chloroperoxybenzoic acid.

Next, the synthesis of tetraketide compounds was examined by conducting further chain elongation. 6-Methylsalicylic acid is a representative aromatic tetraketide that is constructed via the assembly of acetyl-CoA and three malonyl-CoAs in the enzymatic system.[15] After the second elongation with malonyl-CoA, a ketoreductase reduces the β-carbonyl group; thus, the linear tetraketide bears a δ-hydroxy group. This precursor cyclizes through an intramolecular aldol condensation and aromatizes via dehydration and enolization. As a mimic of this biosynthesis, 6-substituted salicylic acid was synthesized (Scheme 5). Compound **6** was reduced to β-hydroxythioester **18**, which was then derivatized to silyl ether **19**. The hydrolysis of **19** to carboxylic acid **20** and subsequent elongation afforded linear precursor **21**. This compounds was obtained in 55% overall yield starting from carboxylic acid **2**. Following the removal of the dioxolane protection, the intermediate was treated with DBU. Intramolecular aldol condensation and elimination of a silanol produced aromatic compound **22**. Substituted salicylic acid **23** was obtained upon hydrolysis of the thioester group.

The iterative synthesis was applied to another aromatic tetraketide with a different oxidation state. Orsellinic acid is a tetraketide compound formed by the cyclization of a non-reduced tetraketide precursor. Harris and co-workers intensively studied on the synthesis of such polyketides derived from poly-β-carbonyl compounds.[16] In their method, linear poly-β-carbonyl compounds are prepared under strongly basic conditions. For example, a triketone is converted into the corresponding trianion, and it is used for chain elongation to afford a tetraketone by adding a carbonyl source. Because of the harsh reaction conditions, the compounds with base sensitive functionalities are not applicable. As a demonstration of our approach, the iterative method with MAHT was applied to the synthesis of an orsellinic acid analogue (Scheme 6). During the cyclization of the linear precursor with four carbonyl functionalities, it is necessary to control the cyclization mode, i.e., aldol condensation, Claisen-type cyclization, or lactonization. Thus, two kinds of protecting groups were employed for the selective masking and unmasking of carbonyl groups. The β-carbonyl group of compound **3** was protected as a 1,3-dithiolane. The hydrolysis of thioester **24** to carboxylic acid **25** was performed in the absence of hydrogen peroxide to avoid the concomitant reaction of the dithiolane. After the second chain elongation, the resulting β-carbonyl group was protected as a 1,3-dioxolane. The hydrolysis of thioester **27** and the third chain elongation afforded linear tetraketide precursor **29**. The total yield for preparing the linear precursor from carboxylic acid **2** was 61%. Cyclic product **30** was obtained through the selective removal of the dithiolane protecting group and intramolecular aldol condensation. The deprotection of the dioxolane led to the formation of aromatic compound **31**. Although the final hydrolysis of the thioester failed under both basic and acidic conditions, the oxidation of the thioester with mCPBA in aqueous media afforded orsellinic acid analogue **32**. The capability for oxidative hydrolysis under mild conditions is a merit of the use of the MAHT monomer.

Finally, in order to illustrate the utility of the developed method, we applied it to the synthesis of natural products. Kavain (**40**) is a kavalactone, which is produced from cinnamic acid with the aid of polyketide synthesis machinery in plants.[17] Likewise, we synthesized kavain by assembling cinnamic acid and two malonic acid molecules (Scheme 7). Following the chain elongation of cinnamic acid, the reduction of β-ketothioester **34** was conducted. Under the conditions with cerium(III) chloride,[18] 1,2-selective reduction proceeded to give β-hydroxythioester **35**. After the silyl protection of the hydroxy group, hydrolysis and elongation produced linear precursor **38**. Through deprotection, lactonization, and methylation, kavain was obtained in 52% total yield. In the reduction of β-ketothioester **34** to **35**, a stereogenic center is generated at the β-position. Notably, the reduction could be performed in an enantioselective manner via the use of a ruthenium catalyst with a chiral bisphosphine ligand[19] (Scheme 8). Enantioenriched β-hydroxythioester **35** was converted to kavain without affecting the enantiopurity. By employing asymmetric hydrogenation in the iterative cycle, the present method can be expanded to the stereoselective synthesis of polyketides.

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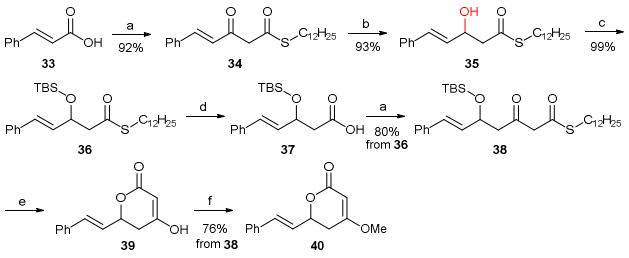
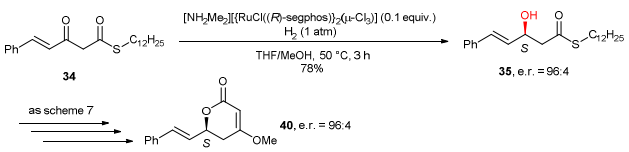
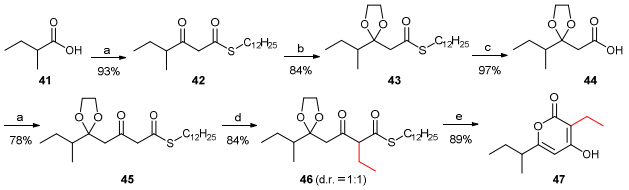
Germicidin A (**47**) is a polyketide with an alkyl-substituted 4-hydroxy-2-pyrone ring.[20] Ethylmalonyl-CoA is employed during the second chain elongation in the biosynthesis of this compound;[21] therefore, the final product has a 3-ethyl group. We synthesized germicidin A to demonstrate the applicability of the iterative method for such an alkyl-substituted compound (Scheme 9). The chain elongation of carboxylic acid **41** was performed twice to obtain protected triketide **45**. Alkylation with ethyl iodide and one equivalent of DBU afforded α-monoethylated compound **46**. Removal of the dioxolane protecting group and subsequent cyclization afforded germicidin A in 44% total yield.



Scheme 7 Synthesis of kavain. (a) COMU, DIEA, then **1**, *i*PrMgBr, DME, rt; (b) $NaBH_3CN$, $CeCl_3{\cdot}7H_2O$, THF/AcOH, rt; (c) TBS-Cl, imidazole, DMF, rt; (d) CsOH, H_2O_2, 1,4-dioxane/H_2O, rt; (e) TsOH, 1,4-dioxane/H_2O, rt, then KOH aq., rt; (f) $Me_3O{\cdot}BF_4$, DIEA, CH_2Cl_2, –78 °C to rt.



Scheme 8 Enantioselective reduction of a β-ketoester.



Scheme 9 Synthesis of germicidin A. (a) COMU, DIEA, then **1**, *i*PrMgBr, DME, rt; (b) $TMSO(CH_2)_2OTMS$, TMSOTf, CH_2Cl_2, 0 °C; (c) CsOH, H_2O_2, 1,4-dioxane/H_2O, rt; (d) EtI, DBU, DMF, rt; (e) $FeCl_3{\cdot}6H_2O$, CH_2Cl_2, rt, then DBU, CH_2Cl_2, rt.

In conclusion, we have demonstrated the biomimetic iterative synthesis of polyketides based on decarboxylative dehydration condensation with MAHT. The iterative cycle includes four options for the transformation of the β-ketothioester after the chain elongation, which enables the preparation of diverse linear precursors. Thus, the method is a comprehensive approach for synthesizing different types of polyketides with a few kinds of reactions.

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