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# "Amano" lipase DF-catalyzed efficient synthesis of 2,2'-arylmethylene dicyclohexane-1,3-dione derivatives in anhydrous media

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### ABSTRACT

A simple and efficient method was developed for the synthesis of 2,2'-arylmethylene dicyclohexane-1,3dione derivatives *via* the Knoevenagel–Michael cascade reactions of aromatic aldehydes and 1,3-cyclic diketones catalyzed by "Amano" lipase DF, which expands the application field of enzyme catalytic promiscuity. This protocol provides several advantages over the traditional chemical synthesis, such as simple work-up procedure, high yields (up to 94%) and environmental friendliness.

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### 10 **1. Introduction**

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Derivatives of 2,2'-arylmethylene dicyclohexane-1,3-dione, which serve as key intermediates in the synthesis of xanthenes, an important class of biological active compounds, are typically synthesized via the Knoevenagel condensation and Michael addition of aromatic aldehydes with 1,3-cyclohexanedione, or other types of 1,3-cyclic diketones. Jin et al. carried out the condensation by grinding at room temperature [1]. Kantevari and coworkers used HClO<sub>4</sub>-SiO<sub>2</sub> as catalyst to synthesize the title compounds in 44.5-91.0% yields [2]. In the presence of CsF, Nandre et al. synthesized the target product in 84–96% yields [3]. Recently, Li et al. reported that the condensation can be catalyzed by urea under ultrasound [4]. However, these reported chemical synthetic methods suffer from a number of drawbacks, such as low yield, harsh reaction conditions and tedious work-up processes. To fulfill the requirements of green chemistry and to simplify the production process, a simple, efficient and eco-friendly method should be developed for the synthesis of 2,2'-arylmethylene

During the last decade, biocatalysis has emerged as an efficient and powerful tool in modern synthetic chemistry due to its valued features, such as high efficiency, mild reaction conditions and

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dicyclohexane-1,3-dione derivatives.

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lower energy requirements [5]. One current frontier for biocataly-32 sis is enzymatic promiscuity, where enzymes catalyze alternative 33 reactions that differ from their natural physiological reaction. The 34 need for new efficient and eco-friendly catalysts in industry is 35 evident and enzymatic promiscuity can meet these requirements 36 well, which has largely broadened the applicable range of 37 biocatalysis in the synthesis of fine chemicals [6]. Examples of 38 enzyme promiscuity-related reactions include Aldol reaction [7], 39 Baylis-Hillman reaction [8] and Mannich reaction [9], while no 40 enzyme catalyzed Knoevenagel-Michael cascade reactions of 41 aromatic aldehydes and 1,3-cyclic diketones have been reported. 42 Among the promiscuous enzymes, lipases, which naturally 43 hydrolyze triglycerides, have attracted much attention due to 44 their excellent features, such as wide sources, broad substrate 45 spectrum and high stability. In continuation of our work [10] on 46 the enzymatic promiscuity, herein we report a novel method for 47 the synthesis of 2,2'-arylmethylene dicyclohexane-1,3-dione 48 derivatives using "Amano" lipase DF as a catalyst (Scheme 1). 49

### 2. Experimental

Analytical thin layer chromatography (TLC) was performed on Haiyang precoated TLC plates (silica gel GF254), using petroleum ether/ethyl acetate (1/1, v/v) as the elution solution. The <sup>1</sup>H NMR (400 MHz) spectra were recorded on a Bruker Advance 2B 400 instrument. Chemical shifts ( $\delta$ ) are quoted in ppm using CDCl<sub>3</sub> (<sup>1</sup>H SMR  $\delta$  7.26, <sup>13</sup>C NMR  $\delta$  77.16) as the solvent and tetramethylsilane

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**Scheme 1.** "Amano" Lipase DF-catalyzed synthesis of 2,2'-arylmethylene dicyclohexane-1,3-dione derivatives.

57 (TMS) as the internal reference. All reagents were of analytical 58 grade and used as received without any further purification. HPLC 59 was carried out on a Agilent 1100 HPLC system equipped with a 60 Daicel 19423 CHIRALPAK AD-H column, using n-hexane/isopro-61 panol (90:10, v/v) as the mobile phase at a flow rate of 1.0 mL/min 62 and UV detection at 254 nm. All melting points were determined 63 with a X-4 microscope melting-point testing apparatus and were 64 uncorrected. The structures of the products were confirmed by 65 comparing the NMR spectral data with those reported in the 66 literature.

67 General procedure for the synthesis of target compounds (3a-68 3m): A mixture of aromatic aldehydes (0.5 mmol), 1,3-cyclic 69 diketone (0.5 mmol) and "Amano" lipase DF (20 mg/mL) in N,N-70 dimethylformamide (DMF) (5 mL) was shaken at 200 rpm, 40 °C 71 for specified time. Progress of the reaction was monitored by TLC. 72 After completion of the reaction, 10 mL cold distilled water was 73 added to the reaction mixture, then extracted with dichloro-74 methane ( $2 \times 15$  mL). The organic phases were combined, 75 concentrated and purified by silica gel column chromatography 76 (petroleum ether:ethyl acetate = 2:1, v/v) or recrystallization from 77 ethanol to give pure product.

78 Selected data of **3a**: White solid: Mp 210–211 °C: <sup>1</sup>H NMR 79 (400 MHz, CDCl<sub>3</sub>): δ 12.35 (s, 1H, OH), 12.19–11.88 (m, 1H, OH), 80 8.14-8.05 (m, 1H, Ar-H), 7.48 (t, 1H, J = 7.8 Hz, Ar-H), 7.28 (s, 0.3H, 81 Ar-H), 7.24 (s, 0.4H, Ar-H), 7.17 (t, 1H, J = 7.1 Hz, Ar-H), 7.10 (d, 1H, I = 8.1 Hz, Ar–H), 5.47 (s, 1H, CH), 2.75–1.52 (m, 12H, CH<sub>2</sub>); <sup>13</sup>C 82 83 NMR (101 MHz, CDCl<sub>3</sub>): δ 191.82, 189.79, 138.18, 133.70, 130.18, 84 128.50, 128.16, 126.48, 125.85, 116.45, 33.51, 32.99, 32.92, 20.13; 85 EI-MS [M]+*m*/*z* 312.

86 Characterization data of other compounds are shown in87 Supporting information.

### 88 3. Results and discussion

89 Initially, benzaldehyde (1a) and 1,3-cyclohexanedione (2a) 90 were chosen as model substrates to optimize the reaction 91 conditions (Table 1). As expected, no product was observed in the absence of the catalyst (Table 1, entry 1). In the cases of 92 "Amano" lipase AK and "Amano" lipase AS, the target compound 93 3a was obtained in yields of only 12% and 9%, respectively (Table 1, 94 95 entries 2 and 3). CAL-B, Lipozyme RMIM and Lipozyme TLIM also 96 showed low catalytic activities in these cascade reactions, giving 97 the target product in yields of 21%, 18% and 29%, respectively 98 (entries 5–7, Table 1). When the reactants were incubated with 99 "Amano" lipase DF, a yield of 89% was achieved, which was the 100 highest among all enzymes tested (Table 1, entry 4). As a control 101 experiment, the reactants were incubated with the urea-denatured 102 "Amano" lipase DF, compound **3a** was obtained only in 3% yield, 103 indicating the reaction was indeed catalyzed by the biologically 104 active "Amano" lipase DF (Table 1, entry 12).

105 Since enzyme concentration often plays an important role in 106 enzymatic reactions, concentrations of "Amano" lipase DF were 107 tested in the range of 10–40 mg/mL. As shown in Table 1, the yield 108 of the target product was evidently improved when the enzyme 109 concentration increased from 10 mg/mL to 20 mg/mL (Table 1, 110 entries 4, 8 and 9). However, further increase in the enzyme 111 concentration only led to slight change in the yield (Table 1, entries 112 10 and 11), suggesting that 20 mg/mL was a suitable concentration

113 for the catalysis of this cascade reactions.

Table 1

Optimization of the reaction conditions for the synthesis of 3a.ª

Entry	Catalyst	Enzyme concentration (mg/mL)	Yield (%) <sup>b</sup>
1	-	-	0
2	"Amano" lipase AK	20	12
3	"Amano" lipase AS	20	9
4	"Amano" lipase DF	20	89
5	CAL-B	20	21
6	Lipozyme RMIM	20	18
7	Lipozyme TLIM	20	29
8	"Amano" lipase DF	10	58
9	"Amano" lipase DF	15	75
10	"Amano" lipase DF	30	91
11	"Amano" lipase DF	40	90
12	Denatured "Amano" lipase DF <sup>c</sup>	20	3

<sup>a</sup> Experimental conditions: a mixture of benzaldehyde (0.5 mmol), 1,3-cyclohexanedione (0.5 mmol), enzyme and *N*,*N*-dimethylformamide (DMF) (5 mL) was shaken at 200 rpm at 40 °C for 5 h.

<sup>b</sup> Determined by HPLC using the external standard method.

<sup>c</sup> Pre-treated with urea at 100 °C for 8 h.

With the optimal reaction conditions in hand, a series of 114 aromatic aldehydes and 1,3-cyclic diketones were investigated to 115 test the generality and scope of this "Amano" lipase DF-catalyzed 116 Knoevenagel–Michael cascade reactions. The results are shown in 117 Table 2. Aromatic aldehydes bearing both electron-donating and 118 electron-withdrawing groups formed corresponding 2,2'-aryl-119 methylene dicyclohexane-1,3-dione derivatives in good to excel-120 lent yields (Table 2, entries 1–13). Among the factors influencing 121 the reaction rate, the substituent position on the benzene ring of 122 aromatic aldehydes played an important role. The substituent in 123 the ortho-position led to a longer reaction time compared to that in 124 the para-position, which was most likely due to steric hinderance 125 (entry 12 vs. entry 13, Table 2). In addition, longer reaction times 126 were required with 5,5-dimethyl-1,3-cyclohexanedione in com-127 parison to 1,3-cyclohexanedione, which can also be attributed to 128 steric hinderance (entries 1–5 vs. entries 6–13, Table 2). 129

Based on these experimental results and the widely accepted viewpoint that the hydrolysis active site in lipase is responsible for its promiscuous performance [11], a mechanism is proposed and depicted in Scheme 2. Firstly, 1,3-cyclic diketone was activated by the Asp-His dyad to form an enolate anion, which was stabilized by the oxyanion hole of the lipase. Then the enolate anion attacked the aromatic aldehyde to form the intermediate I. Subsequently, dehydration took place to generate the intermediate II. Finally,

### Table 2

Synthesis of compound 3a-3m by lipase DF in DMF.

Ar-CHO +	R R O	Lipase D	$F \xrightarrow{R} R$	OH OH	R
Entry	Ar	R	Time (h)	Product	Yield (%) <sup>a</sup>
1	C <sub>6</sub> H <sub>5</sub>	Н	5	3a	89
2	$4-CH_3C_6H_4$	Н	5	3b	91
3	4-CH <sub>3</sub> OC <sub>6</sub> H <sub>4</sub>	Н	5	3c	90
4	2-Furanyl	Н	5	3d	84
5	2-Thienyl	Н	5	3e	88
6	C <sub>6</sub> H <sub>5</sub>	CH <sub>3</sub>	6	3f	83
7	2-FC <sub>6</sub> H <sub>4</sub>	$CH_3$	8	3g	85
8	4-ClC <sub>6</sub> H <sub>4</sub>	CH <sub>3</sub>	6	3h	92
9	3,4-Cl <sub>2</sub> C <sub>6</sub> H <sub>3</sub>	$CH_3$	8	3i	81
10	$4-CH_3C_6H_4$	$CH_3$	6	3j	87
11	4-CH <sub>3</sub> OC <sub>6</sub> H <sub>4</sub>	$CH_3$	6	3k	86
12	2-02NC6H4	$CH_3$	8	31	93
13	$4-O_2NC_6H_4$	CH <sub>3</sub>	6	3m	94

<sup>a</sup> Isolated yields.

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Scheme 2. Proposed mechanism for "Amano" lipase DF-catalyzed synthesis of 2,2'-arylmethylene dicyclohexane-1,3-dione derivatives.

- 138 Michael addition occurred when another enolate anion attacked
- 139 the intermediate II and gave the product III.

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140 **4. Conclusion** 

141 In summary, we have developed a novel method for the 142 enzymatic synthesis of 2,2'-arylmethylene dicyclohexane-1,3-143 dione derivatives *via* Knoevenagel–Michael cascade reactions 144 catalyzed by "Amano" lipase DF in anhydrous media. High yields, 145 mild reaction conditions, simple work-up procedure and eco-146 friendliness are the features of this new protocol with great 147 potential in industrial applications.

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### 155 Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.cclet.2014.04.007.

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