



Lipase-catalyzed domino Michael-aldol reaction of 2-methyl-1,3-cycloalkanedione and methyl vinyl ketone for the synthesis of bicyclic compounds

著者	SANO Kaoru, KOHARI Yoshihito, NAKANO Hiroto, SEKI Chigusa, TAKESHITA Mitsuhiro, TOKIWA Michio, HIROSE Yoshihiko, UWAI Koji
journal or publication title	Synthetic Communications
volume	46
number	1
page range	46-54
year	2016
URL	http://hdl.handle.net/10258/00008594

doi: info:doi/10.1080/00397911.2015.1116584

Lipase-catalyzed domino Michael-aldol reaction of 2-methyl-1,3-cycloalkanedione and methyl vinyl ketone for the synthesis of bicyclic compounds

著者	SANO Kaoru, KOHARI Yoshihito, NAKANO Hiroto, SEKI Chigusa, TAKESHITA Mitsuhiro, TOKIWA Michio, HIROSE Yoshihiko, UWAI Koji
journal or publication title	Synthetic Communications
volume	46
number	1
page range	46-54
year	2016
URL	http://hdl.handle.net/10258/00008594

doi: info:doi/10.1080/00397911.2015.1116584

Title

Lipase-catalysed domino Michael-aldol reaction of 2-methyl-1,3-cycloalkanedione and methyl vinyl ketone for the synthesis of bicyclic compounds

Author names and affiliations

Kaoru Sano,^a Yoshihito Kohari,^a Hiroto Nakano,^a Chigusa Seki,^a Mitsuhiro Takeshita,^b Micho Tokiwa,^b Yoshihiko Hirose,^c and Koji Uwai^a

^aDivision of Applied Sciences, Muroran Institute of Technology, 27-1 Mizumoto-cho, Muroran 050-8585, Japan

^bTokiwakai Group, 62 Numajiri, Tsuzura-cho, Uchigo, Iwaki, Fukushima 973-8403, Japan

^cAmano Enzyme Inc. Gifu R&D Center, 1-6 Technoplaza, Kakamigahara-Shi, Gifu 509-0109, Japan

Corresponding author

Koji Uwai

Division of Applied Sciences, Muroran Institute of Technology, 27-1 Mizumoto-cho, Muroran 050-8585, Japan

tel: +81-143-46-5775

fax: +81-143-46-5701

uwai@mmm.muroran-it.ac.jp

Abstract: Synthesis of bicyclic compounds was achieved via a lipase-catalysed, stereoselective, domino Michael-aldol reaction of 2-methyl-1,3-cycloalkanedione and methyl vinyl ketone. Appropriate reaction conditions, including the type of enzyme, solvent, and temperature, were determined. In addition, the effects of solvent polarity and additives were investigated. The reaction proceeded in the presence of lipase AS in a solution of 20% acetone in dimethylsulfoxide (DMSO) at 10°C for 8 days, followed by the addition of *p*-toluenesulfonic acid (TsOH) to afford bicyclic compounds in 51%–83% yields with moderate stereoselectivity. Although this domino Michael-aldol reaction showed only moderate stereoselectivity, even with the acid-supported enhancement of the reaction, these results represent potential new applications for lipase.

Keywords: Biocatalyst, Lipase, Domino reaction, Michael-aldol reaction, Bicyclic compound

1. INTRODUCTION

α -Substitution reactions, including Michael and aldol reactions, are very important in synthetic organic chemistry, because they are convenient for the formation of carbon–carbon bonds. In the past, important compounds have been synthesised using this type of reaction with metal^[1,2] and organo catalysts.^[3,4] For example, the Michael reaction was used for the synthesis of the anti-influenza agent oseltamivir,^[5] whereas an aldol reaction was used for the synthesis of the anti-cancer agent rhizoxin D.^[6] However, most of these catalysts possess various disadvantages, such as expensive syntheses, commercially unavailability, harsh reaction conditions and complicated design. In contrast, biocatalysts have many advantages, including low cost, commercial availability in large quantities, environmental friendliness, mild reaction conditions, and high stereoselectivity even when they are used as received.

Lipases are biocatalysts known to catalyse the hydrolysis of triglycerides *in vivo* and are used as catalysts for kinetic resolution in synthetic organic chemistry by utilising the difference in reactivity of both enantiomers in hydrolysis.^[7] In addition, lipases have been found to be promiscuous during the catalysis of Michael^[8,9] and aldol reactions.^[10,11] We also reported the Michael reaction of 4-hydroxycoumarin and benzylideneacetone, which was catalysed by lipase AS in dimethylsulfoxide (DMSO) at 20°C for 8 days. This afforded the well-known anti-coagulant agent warfarin in 85% and 45% enantiomeric excess (ee).^[13] This is the best stereoselectivity reported for the synthesis of warfarin catalysed by lipases.^[14]

Domino reactions are very effective in forming more than one bond in a single step via several consecutive reactions. For example, the bicyclic compounds Hajos–Parrish ketone (**1a**), Wieland–Miescher ketone (**1b**) and compound **1c** were synthesised via a domino Michael–aldol reaction of the corresponding diketones (**2a–c**) and methyl vinyl ketone (**3**) (**Scheme 1**). These bicyclic compounds are useful synthetic intermediates for many bioactive compounds such as the anti-angiogenic agent cortistatin A,^[15] anti-cancer agent paclitaxel,^[16] and anti methicillin resistant staphylococcus aureus (MRSA) and anti vancomycin-resistant enterococcus faecium (VREF) antibiotic guanacastepene A.^[17] Bicyclic compounds **1a–c** were previously synthesised using organo catalysts such as proline derivatives,^[18,19] but their catalytic efficiencies and complicated synthetic methods remain an issue. In contrast, only a few reports on promiscuous, single enzyme-catalysed, multi-step or domino reactions have been described.^[20–22] While a lipase-catalysed domino reaction of Wieland–Miescher ketone was recently reported, no stereoselectivity was observed.^[23]

(Scheme 1)

We proposed that the Michael reaction used in the synthesis of warfarin^[13] could be applied to the domino reaction, affording stereoselective products from the novel lipase-catalyzed reaction that

require less catalyst. Thus, we developed a lipase-catalysed, stereoselective, domino Michael-aldol reaction for synthesis of bicyclic compounds (**1a–c**) from 1,3-diketones (**2a–c**) and methyl vinyl ketone (**3**).

2. RESULTS AND DISCUSSION

To determine the appropriate reaction conditions, we investigated the synthesis of Wieland–Miescher ketone (**1b**) via a lipase-catalyzed domino Michael-aldol reaction of 2-methyl-1,3-cyclohexanedione (**2b**) and methyl vinyl ketone (**3**). The initial reaction conditions were **2b** (0.14 mmol), **3** (0.42 mmol), lipase (28.4 mg) and anhydrous DMSO (0.7 mL), in accordance with our previous work.^[13]

First, a series of commercially available lipases were screened to determine a suitable catalyst for the reaction (**Table 1**). **Focusing simply on the compound 1b**, Lipase AYS, PS, AK, PL, Novozyme 435, Lipozyme RM IM, Lipazyme CAL-B, Lipozyme TL 100L, Palatase 20000L, Stick Away, and Lipex 100L afforded low conversions with no stereoselectivity (entries 2–5 and 11–17). In contrast, lipase AS, QLM, OF, SL, TL, MY-30, PPL, F-AP-15 and immobilised lipase showed slight stereoselectivity (entries 1, 6–10 and 18–20). Lipase AS was determined to be the best catalyst for this domino Michael-aldol reaction (entry 1, 28% yield, 9% ee). In the presence of BSA and the absence of lipase, the product was not observed (entries 21 and 22). Thus, the domino Michael-aldol reaction seemed to be catalysed by lipase. The stereochemistry of **1b** obtained under these reaction conditions was entirely *S*-form. **Unfortunately, in these reactions, the intermediate Michael adduct (4b) in addition to desired 1b were obtained. The results suggested that the equilibrium of the second step in scheme 1 (between 4b and 5b) of the aldol reaction was in favour of the Michael adduct (4b) as we discuss in detail below (around Table 4 and 5).**

(Table 1)

In general, the solvent for the enzyme-catalysed reaction affected not only chemical yield but also stereoselectivity;^[12] therefore, the choice of optimal reaction solvent is very important. In the past, some conventional organic solvents were surveyed. The results indicated that varying the solvent had significant effects on the activity and stereoselectivity of the lipase AS-catalysed domino Michael-aldol reaction (**Table 2**). While no product was observed with acetone (entry 10), cyclohexane, *n*-hexane, toluene, 1,4-dioxane, tetrahydrofuran (THF), EtOAc, CHCl₃, EtOH, H₂O and methyl vinyl ketone showed moderate chemical yields but no stereoselectivity (entries 1–3, 5–7, 9, 14, 16 and 17). In contrast, Et₂O, CH₂Cl₂, dimethylformamide (DMF), DMSO, 2-PrOH and MeOH demonstrated slight stereoselectivity (entries 4, 8, 11–13 and 15). The best result was obtained in DMSO with product yield of 34% and 9% ee (entry 12).

(Table 2)

In previous reports, the chemical yield and stereoselectivity of lipase-catalysed, asymmetric aldol reactions were improved when a mixed solvent was used.^[24] Therefore, we investigated the use of a DMSO-based mixed solvent system. As a result (**Table 3**), 1 : 1 ratio of solvent/DMSO did not improve the results obtained with pure DMSO (entries 1–5). However, the reaction in a mixed solvent system of 20% acetone in DMSO afforded the best result with a 44% yield and 11% ee (entry 7). This indicated that an aprotic polar solvent was preferred for the lipase-catalyzed domino Michael-aldol reaction with the polarity of the solvent being important factor.

(Table 3)

The obtained results (**Table 3**, entry 7) suggested that the equilibrium of the second step (between **4b** and **5b**) of the aldol reaction was in favour of the Michael adduct (**4b**). Thus, to shift the equilibrium to the terminal product (**1b**), dehydration of the aldol adduct (**5b**) was promoted through the addition of an acid to the reaction system. Various acids (0.5 mol%) were added after reacting for 3 days. When HCl, camphorsulfonic acid (CSA), trifluoroacetic acid (TFA) and pyridinium *p*-toluenesulfonate (PPTS) were added, chemical yield and stereoselectivity were nearly equal to those without additives. However, the addition of *p*-toluenesulfonic acid (TsOH) improved the chemical yield and stereoselectivity to 64% and 12% ee, respectively (**Table 4**, entry 2). Any products were observed for the reaction in the presence of with TsOH and the absence of lipase (**entry 4**). These results indicated that the addition of acid enhanced the dehydration reaction. Following this, we investigated the amount of added TsOH (1.0–10.0 mol%) and reaction time (0.5–12 h). The results indicated that the optimal amount of TsOH was 5.0 mol% (entry 2). Furthermore, the chemical yield was increased upon extending the reaction time to 2 h, resulting in 64% chemical yield and 12% ee (**entry 6**). From these results, we established a lipase catalysed domino Michael-aldol reaction with good chemical yield.

(Table 4)

The optimal conditions for the formation of **1b** involved a mixed solvent system of 20% acetone in DMSO in the presence of TsOH (64% yield, 12% ee, **Table 5**, entry 5). However, as shown in Table 2, entry 10, no product was obtained when pure acetone was used as the solvent. Thus, we proposed that the polarity of the solvent is important for the reactivity of the lipase; therefore, the relationship between polarity values [$E_T(30)$] and reactivity was investigated (**Figure 1**). The examined solvent systems were acetone/DMSO and DMF/DMSO. **The more the ratio of DMSO increased (ratio**

of acetone (or DMSO) decreased), the more $E_T(30)$ values increased. The reactivity in both system were had a maximal value around $E_T(30) = 44.5$ at which the components of the solvent system were 20% acetone in DMSO (44% yield, 11% ee) and 30% DMF in DMSO (40% yield, 10% ee). This suggested that the solvent polarity was appropriate at least for this type of reaction.

(Figure 1)

Next, we applied this method to the synthesis of other bicyclic compounds (**1a** and **1c**). Diketone **2c** was synthesized according to the procedure reported by Piekut et al.^[25] As a result (**Table 6**), bicyclic compounds (**1a–c**) were obtained from all diketones with chemical yields being increased upon the expansion of the ring size, resulting in poor stereoselectivities of 3% ee (**1a**), 4% ee (**1c**) and 12% ee (**1b**) (entries 1-3).

(Table 5)

(Figure 2)

In general, chemical yield and stereoselectivity were affected by reaction temperature. When the reaction temperature sets lower, the reaction rate becomes lower but the stereoselectivity getting higher in the enzymatic reaction.^[26] Therefore, the reaction temperature was investigated to improve the stereoselectivity of the domino Michael-aldol reaction (**Figure 2a**). The best chemical yields [66% (**1a**), 82% (**1b**) and 83% (**1c**)] with low stereoselectivity were obtained at 50°C, while the best stereoselectivities [5% ee (**1a**), 18% ee (**1b**) and 8% ee (**1c**)] were obtained at 10°C. The chemical yield increased upon increasing reaction temperature, while stereoselectivity increased with decreasing reaction temperature. Next, the reaction time was investigated to improve the chemical yield at 10°C. As a result (**Figure 2b**), chemical yields were increased upon increasing reaction time, while maintaining the stereoselectivity. The best results were 51% yield 5% ee (**1a**), 79% yield 18% ee (**1b**) and 83% yield 8% ee (**1c**) at 10°C for 8 days. No improvement in chemical yield was observed after this time.

In general, lipase has an active site for histidine, aspartic acid and serine for hydrolysis. Although the amino acid sequence of lipase AS has no yet been determined, we assumed that these histidine, aspartic acid and oxyanion holes contributed to the mechanism of this lipase-catalyzed domino Michael-aldol reaction similar to hydrolysis, leading to the proposed aldol reaction^[12] (**Scheme 2**). The negatively charged carboxylate anion of aspartic acid was able to abstract the imidazole proton of histidine. The imidazole anion of histidine acted as a base to abstract the carbonyl α -proton of diketone **2b**.

Meanwhile, the carbonyl oxygen of **3** can become trapped in the oxyanion hole activating the α,β -unsaturated ketone. Diketone **2b** acted as a Michael donor for **3**, affording **4b**. The carbonyl oxygen of **4b** may then be trapped by the oxyanion hole, abstracting its carbonyl α -proton as before. Negatively charged **4b** underwent intramolecular nucleophilic attack at the trapped carbonyl carbon in the oxyanion hole. The hydroxy group of the aldol adduct (**5b**) was then activated for protonation by TsOH, affording **1b** via dehydration. Stereoselectivity may be affected by the conformation of the amino acid side chains of lipase; however, the detailed conformation of lipase AS is still unclear.

(Scheme 2)

3. CONCLUSION

In summary, we developed a stereoselective domino Michael-aldol reaction catalyzed by lipase. The syntheses of bicyclic compounds (**1a–c**) using this lipase-catalyzed domino Michael-aldol reaction were accomplished using lipase AS in a mixed solvent system of 20% acetone in DMSO at 10°C for 8 days, followed by the addition of TsOH at 10°C, resulting in 51%–83% chemical yields and 5%–18% ee. However, for the practical use, this reaction temperature and reaction time are might be slightly unuseful and the reaction at 30°C may be more applicable even if they have some very low selectivity.

(Scheme 3)

In the past, lipase-catalyzed domino Michael-aldol reactions were found to have no stereoselectivity. Although this domino Michael-aldol reaction showed only moderate stereoselectivity, even with the acid-supported enhancement of the reaction, these results represent potential new applications for lipase. Further studies are now underway in our laboratory, including the investigation of superior reaction conditions using mutant lipases, as well as studies exploring the reaction mechanism.

4. EXPERIMENTAL

Typical reaction procedure

A mixture of 2-methyl-1,3-cycloalkanedione (**2a–c**) (0.14 mmol) and lipase AS (28.4 mg, 0.81 μ mol, 0.6 mol%) in a solution of 20% acetone in DMSO (0.7 mL) was incubated under an Ar atmosphere at 10°C for 5 min. Methyl vinyl ketone (**3**) (35 μ L, 0.42 mmol, 3.0 equiv.) was then added to the reaction mixture and incubated under Ar at 10°C for 8 days, at which point TsOH was added and the reaction proceeded at 10°C for 2 h. The progress of the reaction was monitored by TLC. The enzyme was filtered and partitioned between EtOAc and H₂O. The organic layer was dried over anhydrous MgSO₄ and concentrated *in vacuo*. Purification of the residual mixture by column chromatography on

aluminum oxide using hexane: EtOAc (3:1 then 1:1, v/v) as the eluent afforded (*S*)-bicyclic compounds (**1a–c**).

ACKNOWLEDGEMENT

Financial support from an MuIT Grant for Selected Research is gratefully acknowledged. We thank Meito Sangyo Co, Novozymes A/S and Amano Enzyme Inc for their generous donation of lipase.

SUPPORTING INFORMATION

Materials, Experimental Section Characterisation Data for Compounds. This material can be found via the “Supplementary Content” section of this article’s webpage.

REFERENCES

- [1] Yang, H. M.; Li, L.; Jiang, K. Z.; Jiang, J. X.; Lai, G. Q.; Xu, L. W. *Tetrahedron* **2010**, 66, 9708–9713.
- [2] Taylor, S. J.; Duffey, M. O.; Morken, J. P. *J. Am. Chem. Soc.* **2000**, 122, 2000, 4528–4529.
- [3] Torre, O.; Alfonso, I.; Gotor, V. *Chem. Commun.* **2004**, 15, 1724–1725.
- [4] Sakthivel, K.; Notz, W.; Bui, T.; Barbas, C. F. *J. Am. Chem. Soc.* **2001**, 123, 5260–5267.
- [5] Ishikawa, H.; Suzuki, T.; Hayashi, Y. *Angew. Chem. Int. Ed.* **2009**, 48, 1304–1307.
- [6] White, J. D.; Blakemore, P. R.; Green, N. J.; Hauser, E. B.; Holoboski, M. A.; Keown, L. E.; Kolz, C. S. N.; Phillips, B. W. *J. Org. Chem.* **2002**, 67, 7750–7760.
- [7] Sugai, T.; Kakeya, H.; Ohta, H. *J. Org. Chem.* **1990**, 55, 4643–4647.
- [8] Ramírez, J. D. R.; Escalantea, J.; Munguía, A. L.; Marty, A.; Castillo, E. *J. Mol. Cat. B: Enzymatic* **2015**, 112, 76–82.
- [9] Cai, J. F.; Guan, Z.; He, Y. H. *J. Mol. Cat. B: Enzymatic* **2011**, 68, 240–244.
- [10] Zhang, Y.; Wang, N.; Xie, Z. B.; Zhou, L. H.; Yu, X. Q. *J. Mol. Cat. B: Enzymatic* **2014**, 110, 100–110.
- [11] Zheng, J.; Xie, B. H.; Chen, Y. L.; Cao, J. F.; Yang, Y.; Guan, Z.; He, Y. H. *Z. Naturforsch C* **2014**, 69, 170–180.
- [12] Li, C.; Feng, X. W.; Wang, N.; Zhou, Y. J.; Yu, X. Q. *Green Chem.* **2008**, 10, 616–618.
- [13] Sano, K.; Saito, S.; Hirose, Y.; Kohari, Y.; Nakano, H.; Seki, C.; Tokiwa, M.; Takeshita, M.; Uwai, K. *Heterocycles* **2013**, 87, 1269–1278.
- [14] Xie, B. H.; Guan, Z.; He, Y. H. *J. Chem. Tech. Biotech.* **2012**, 87, 1709–1714.
- [15] Aoki, S.; Watanabe, Y.; Sanagawa, M.; Setiawan, A.; Kotoku, N.; Kobayashi, M. *J. Am. Chem. Soc.* **2006**, 128, 3148–3149.
- [16] Danishefsky, S. J.; Masters, J. J.; Young, W. B.; Link, J. T.; Snyder, L. B.; Magee, T. V.; Jung, D. K.; Isaacs, R. C. A.; Bornmann, W. G.; Alaono, C. A.; Coburn, C. A.; Grandi, M. J. D. *J. Am.*

- Chem. Soc.* **1996**, 118, 2843–2859.
- [17] Brady, S. F.; Singh, M. P.; Janso, J. E.; Clardy, J. *J. Am. Chem. Soc.* **2000**, 122, 2116–2117.
- [18] Bui, T.; Baras, C. F. *Tetrahedron Lett.* **2000**, 41, 6951–6954.
- [19] Bradshaw, B.; Jardi, G. E.; Bonjoch, J. *J. Am. Chem. Soc.* **2010**, 132, 5966–5967.
- [20] Zhou, L. H.; Wang, N.; Zhang, W.; Xie, Z. B.; Yu, X. Q. *J. Mol. Cat. B. Enzymatic* **2013**, 91, 37–43.
- [21] Xu, J. C.; Li, W. M.; Zheng, H.; Lai, Y. F.; Zhang, P. F. *Tetrahedron* **2011**, 67, 9582–9587.
- [22] Wu, Q.; Xu, J. M.; Xia, L.; Wang, J. L.; Lin, X. F. *Adv. Synth. Catal.* **2009**, 351, 1833–1841.
- [23] Lai, Y. F.; Zhang, P. F. *Res. Chem. Intermed.* **2015**, 41, 4077–4082.
- [24] Yoshida, M.; Ohno, Y.; Hara, S. *Tetrahedron Lett.* **2010**, 51, 5134–5136.
- [25] Piekut, S. L.; Okamura, W. H. *Synth. Commun.* **1980**, 10, 415–420.
- [26] Sakai, T. *Tetrahedron Asymmetry* **2004**, 15, 2749–2756.

Table 1. Domino Michael-aldol reaction of 2-methyl-1,3-cyclohexanedione (**2b**) and methyl vinyl ketone (**3**) catalysed by various lipases^{a)}

Entry	Enzyme	4b yield ^{b)} (%)	1b	
			yield ^{b)} (%)	ee ^{c)} (%)
1	AS	7	34	9
2	AYS	11	12	-
3	PS	11	13	-
4	AK	5	7	-
5	PL	5	5	-
6	QLM	8	9	4
7	OF	15	7	2
8	SL	8	13	4
9	TL	13	5	4
10	MY-30	8	4	2
11	Novozyme 435	1	23	-
12	Lipozyme RM IM	n.d. ^{d)}	10	-
13	Lipozyme CAL-B	4	18	-
14	Lipozyme TL 100L	11	7	-
15	Palatase 20000L	3	5	-
16	Stick Away	15	13	-
17	Lipex 100L	4	9	-
18	PPL	12	20	3
19	F-AP-15	13	5	5
20	Immobilised lipase	4	2	3
21	BSA	n.d. ^{d)}	n.d. ^{d)}	-
22	Blank	n.d. ^{d)}	n.d. ^{d)}	-

a) Experimental conditions: 2-methyl-1,3-cyclohexanedione (0.14 mmol), methyl vinyl ketone (0.42 mmol) and lipase (28.4 mg) in anhydrous DMSO (0.7 mL) were incubated at 30°C for 3 days in an Ar atmosphere. b) Isolated yield. c) Determined by chiral HPLC analysis. d) Not detected.

Table 2. Domino Michael-aldol reaction of 2-methyl-1,3-cyclohexanedione (**2b**) and methyl vinyl ketone (**3**) in various solvents^{a)}

Entry	Solvent	4b yield ^{b)} (%)	1b	
			yield ^{b)} (%)	ee ^{c)} (%)
1	Cyclohexane	2	3	-
2	<i>n</i> -Hexane	3	4	-
3	Toluene	12	5	-
4	Et ₂ O	3	10	2
5	1,4-Dioxane	3	3	-
6	THF	5	11	-
7	EtOAc	4	3	-
8	CH ₂ Cl ₂	10	17	3
9	CHCl ₃	7	18	-
10	Acetone	n.d. ^{d)}	n.d. ^{d)}	-
11	DMF	9	23	5
12	DMSO	7	34	9
13	2-PrOH	5	13	2
14	EtOH	14	4	-
15	MeOH	8	7	4
16	H ₂ O	15	13	-
17 ^{e)}	Methyl vinyl ketone	24	27	-

a) Experimental conditions: 2-methyl-1,3-cyclohexanedione (0.14 mmol), methyl vinyl ketone (0.42 mmol) and lipase AS (28.4 mg) in solvent (0.7 mL) were incubated at 30°C for 3 days in an Ar atmosphere. b) Isolated yield. c) Determined by chiral HPLC analysis. d) Not detected. e) No solvent.

Table 3. Domino Michael-aldol reaction of 2-methyl-1,3-cyclohexanedione (**2b**) and methyl vinyl ketone (**3**) in various DMSO-based various mixed solvent systems^{a)}

Entry	Solvent/DMSO	4b yield ^{b)} (%)	1b	
			yield ^{b)} (%)	ee ^{c)} (%)
1	50% 2-PrOH	5	21	5
2	50% MVK	17	29	3
3	50% CH ₂ Cl ₂	10	21	5
4	50% DMF	10	20	7
5	50% Acetone	4	5	3
6	30% Acetone	13	25	4
7	20% Acetone	15	44	11
8	10% Acetone	8	35	9

a) Experimental conditions: 2-methyl-1,3-cyclohexanedione (0.14 mmol), methyl vinyl ketone (0.42 mmol) and lipase AS (28.4 mg) in a mixed solvent DMSO system (0.7 mL) were incubated at 30°C for 3 days in an Ar atmosphere. b) Isolated yield. c) Determined by chiral HPLC analysis.

Table 4. Effect of the amount of TsOH (1–10 mol%) and reaction time (0.5–12 h) on the reaction of 2-methyl-1,3-cyclohexanedione (**2b**) and methyl vinyl ketone (**3**)^a

Entry	TsOH (mol%)	Time (h)	4b yield ^b (%)	1b	
				yield ^b (%)	ee ^c (%)
1	1.0	1.0	2	55	12
2	5.0	1.0	3	64	12
3	10.0	1.0	2	58	11
4	0 (no lipase)	1.0	n.d. ^d	n.d. ^d	-
5	5.0	0.5	9	52	11
6	5.0	2.0	3	64	12
7	5.0	6.0	2	62	11
8	5.0	12.0	2	63	13

a) Experimental conditions: 2-methyl-1,3-cyclohexanedione (0.14 mmol), methyl vinyl ketone (0.42 mmol) and lipase AS (28.4 mg) in a solution of 20% acetone in DMSO (0.7 mL) were incubated at 30°C for 3 days in an Ar atmosphere, followed by the addition of TsOH (1.0–10.0 mol%) and incubation for additional 0.5–12.0 h. b) Isolated yield. c) Determined by chiral HPLC analysis.

Table 5. Syntheses of bicyclic compounds (**1a–c**) *via* the domino Michael-aldol reaction of diketones (**2a–c**) and methyl vinyl ketone (**3**)^{a)}

Entry	n	4a–c yield ^{b)} (%)	1a–c	
			yield ^{b)} (%)	ee ^{c)} (%)
1	1	5	45	3
2	2	3	64	12
3	3	4	67	4

a) Experimental conditions: 2-methyl-1,3-cyclohexanedione (0.14 mmol), methyl vinyl ketone (0.42 mmol) and lipase AS (28.4 mg) in a solution of 20% acetone in DMSO (0.7 mL) were incubated at 30°C for 3 days in an Ar atmosphere, followed by the addition of TsOH (0.5 mol%) and incubation for additional 2 h. b) Isolated yield. c) Determined by chiral HPLC analysis.

Figure 1

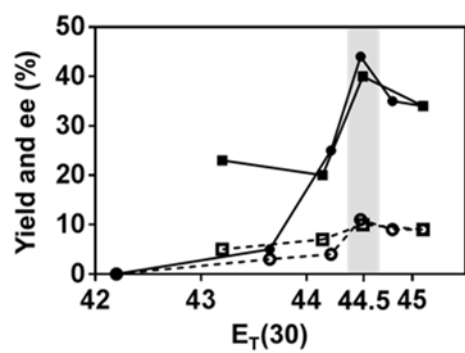


Figure 2

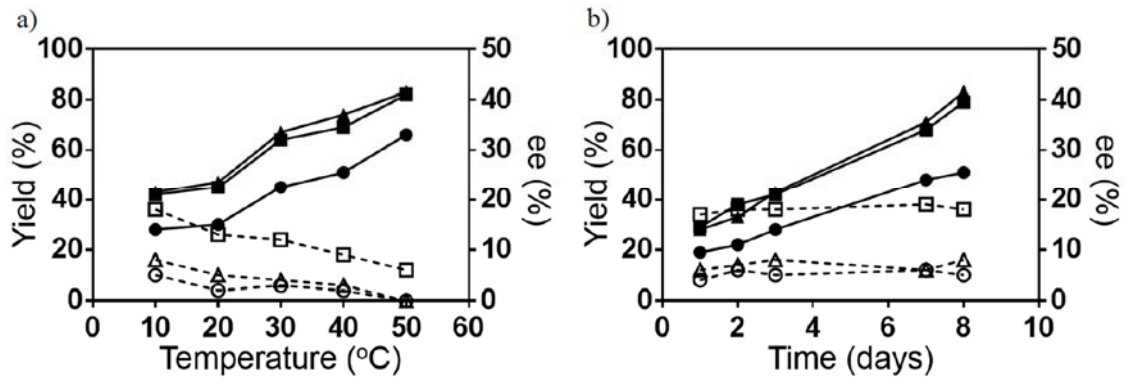


Figure captions

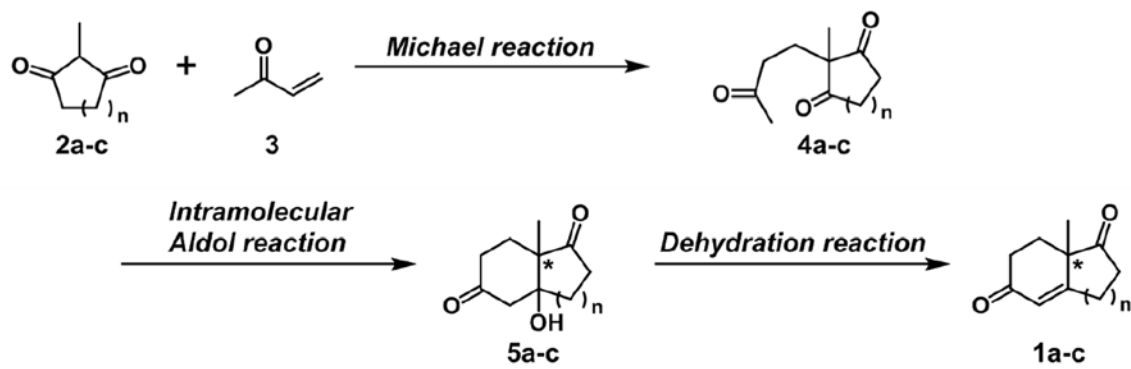
Figure 1. Effect of the polarity value [$E_T(30)$] of the mixed solvent system on the domino Michael-aldol reaction (acetone/DMSO yield: ●, ee: ■, DMF/DMSO yield: ○, ee: □).

Reaction conditions: 2-methyl-1,3-cyclohexanedione (**2b**, 0.14 mmol), methyl vinyl ketone (**3**, 0.42 mmol), and lipase AS (28.4 mg) in a mixed solvent (0.7 mL) at 30°C for 3 days, followed by the addition of TsOH (0.5 mol%) and incubation at 30°C for 2 h.

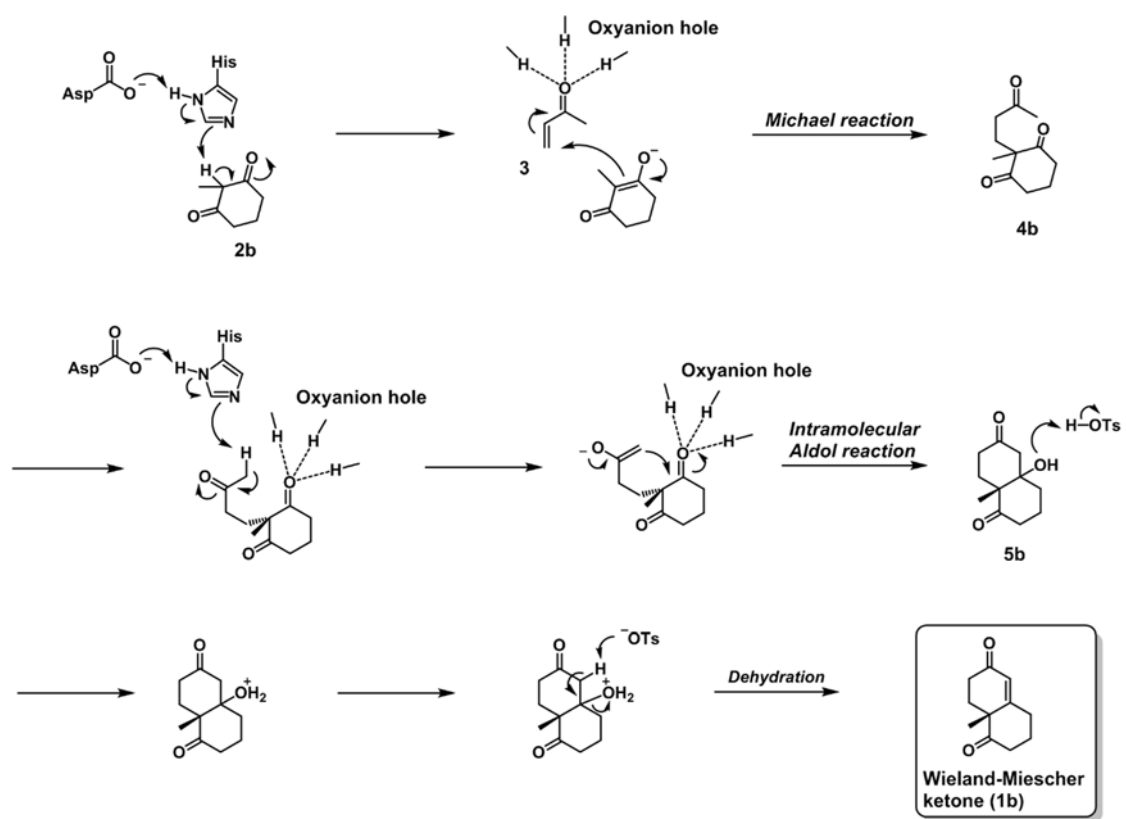
Figure 2. Effect of (a) reaction temperature and (b) reaction time on the domino Michael-aldol reaction (yield: **1a** ●, **1b** ■, **1c** ▲, ee: **1a** ○, **1b** □, **1c** △).

Reaction conditions: 2-methyl-1,3-cycloalkanedione (**2a-c**, 0.14 mmol), methyl vinyl ketone (**3**, 0.42 mmol), lipase AS (28.4 mg) in a solution of 20% acetone in DMSO (0.7 mL).

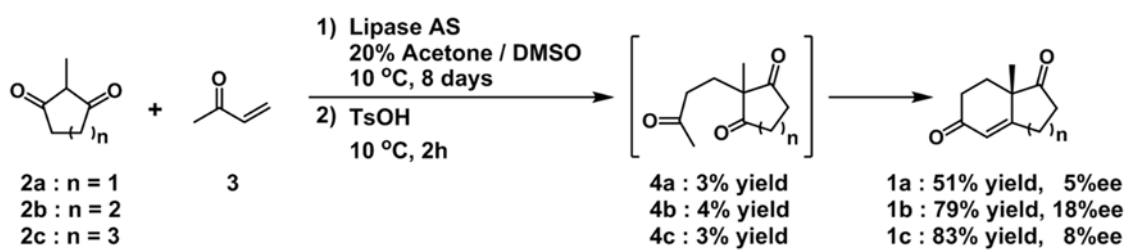
Scheme 1



Scheme 2



Scheme 3



Scheme Captions

Scheme 1. Syntheses of various bicyclic compounds (a: $n = 1$, b: $n = 2$, c: $n = 3$)

Scheme 2. Proposed mechanism of lipase AS-catalysed domino Michael-aldol reaction of 2-methyl-1,3-cyclohexanedione (**2b**) with methyl vinyl ketone (**3**)

Scheme 3. Optimal conditions for the lipase catalysed domino Michael-aldol reaction