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Stereoselective synthesis of spiro[5.5]undecane derivatives via biocatalytic [5+1] double Michael additions

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ABSTRACT

A novel enzymatic, promiscuous protocol of D-aminoacylase (DA)-catalyzed [5+1] double Michael addition was developed herein, for the synthesis of (hetero)spiro[5.5]undecane derivatives in moderate yields. It is notable that almost only the *cis* isomers were obtained through this biocatalytic methodology in all the cases according to their ^{1}H and ^{13}C NMR spectra. It is the first report on hydrolase-catalyzed double Michael addition in organic solvent.

1. Introduction

The synthesis of spiro[5.5]undecane and heterospiro[5.5]undecane motifs has engrossed substantial attention from organic chemists for many years, not only because of their unique structural properties [1,2], but also because of their presence in several natural products such as elatol (**I**), isoobtusol (**II**) and (−)-sibirine (**III**) (Fig. 1) [3–8]. Of all the construction methods of the spirocyclics, which can be roughly categorized into alkylation, rearrangement, cycloaddition and cleavage of bridged systems, the alkylation on the quaternary carbon, especially 1,4-addition, is one of the most common methods for the preparation for spiro[5.5]undecane derivatives. However, the conventional methods usually involve bases or Lewis acids as the catalyst under homogeneous conditions, which encountered environmental problems [9–12]. Therefore, the invention and introduction of environmentally compatible catalysts have always showed great importance and attracted enormous attention.

Biocatalysis is a powerful tool for organic synthesis due to its high efficiency, good selectivity and great environmental acceptability [13–16]. The recent progress in catalytic promiscuity of enzymes [17–20] has greatly expanded its application scope. Among them, the Michael addition, widely considered to be one of the most basic and powerful methods for the construction of carbon–carbon and carbon-hetero bonds, has been frequently reported and widely used [18–33]. For instance, our group has demonstrated that D-aminoacylase from *Escherichia coli* (DA) could catalyze the C—C bond formations via Michael additions between α,β-unsaturated carbonyl compounds and activated carbon nucleophiles such as acetylacetone and ethyl acetoacetate [29]. Inspired by this promiscuous behavior, we report a novel discovery that the commercially available DA promotes a cascade [5+1] double Michael addition to form *cis*-spiro[5.5]undecane derivatives in the present work (Scheme 1), yet other types of biocatalyzed double Michael addition has never been reported to the best of our knowledge.

2. Experimental

2.1. Materials

Lipase from *Candida antarctica* (**CALB**) immobilized on acrylic resin (≥10,000 U/g, recombinant, expressed in *Aspergillus oryzae*), Lipase from hog pancreas (**HPL**) (2.4 U/mg, 1 U is the amount of immobilized enzyme which forms 1% octyl laurate from 0.5 mmol lauric acid and 1.0 mmol 1-octanol in 10 ml water-saturated isooctane in 1 h at 20 °C) was purchased from Fluka (Switzerland). D-Aminoacylase from *E. coli* (**DA**) (Not less than 5 MU/g, 1 U is defined as enzyme quantity which produces 1 μmol of D-amino acid per 30 min under the condition as below: 0.1 M *N*-acetyl-D-methionine, pH8.0, 37 °C) was purchased from Amano Enzyme Inc

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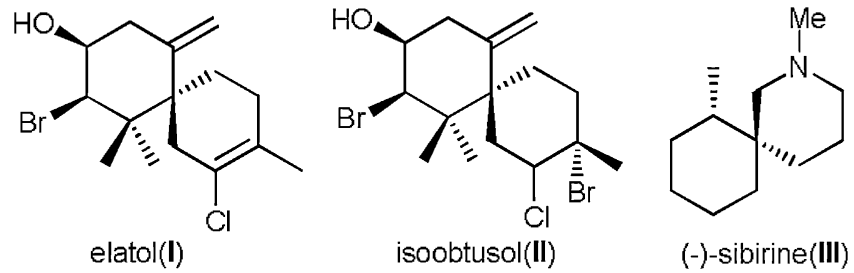
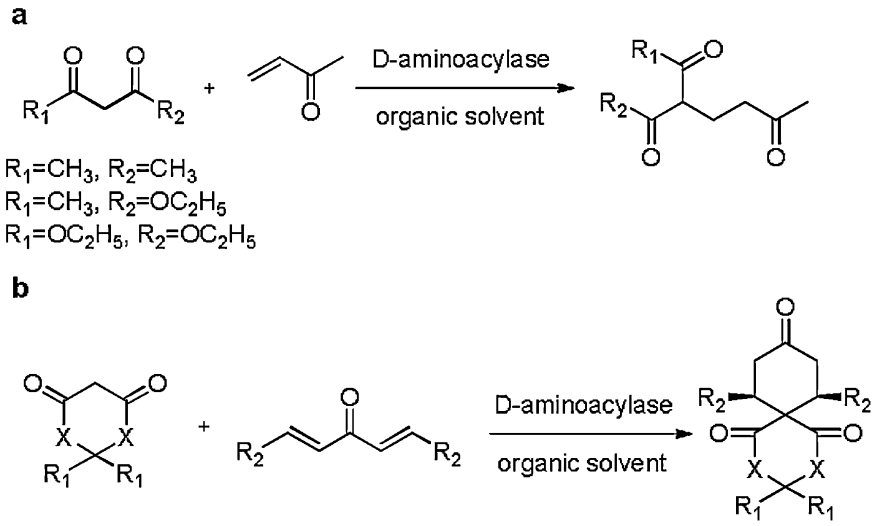


Fig. 1. Some natural products with (hetero)spiro[5.5]undecane.



Scheme 1. (a) DA-catalyzed mono-Michael additions (previous work). (b) DA-catalyzed double Michael additions (this work).

(Japan). All reagents used in the experiments were obtained from commercial sources and used without further purification.

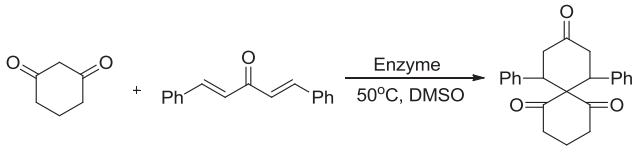
2.2. Analytical methods

The process of reactions was monitored by TLC on silica with Petroleum ether/EtOAc (6/1, v/v) as solvent. The ^{1}H spectra were recorded with TMS as internal standard using a Bruker AMX-400 MHz spectrometer. Chemical shifts were expressed in ppm and coupling constants (*J*) in Hz. Analytical HPLC was performed using a Agilent 1100 series with a reversed-phase Shim-Pack VP-ODS column (150 mm × 4.6 mm) and a UV detector (210 nm). All the known products were characterized by comparing the ^{1}H NMR with those reported in the literature. IR spectra were measured with a Nicolet Nexus FTIR 670 spectrophotometer.

2.3. General procedure for the double Michael additions

1,3-Dione (0.25 mmol), (1E,4E)-1,5-diarylpenta-1,4-dien-3-one (1 mmol), DA (20 mg), DMSO (0.9 ml) and water (0.1 ml) were taken in a flask and the reaction mixture was incubated at 50 °C for 48 or 72 h. Enzyme was filtered off to stop the reaction. CH_2Cl_2 was used to wash the filter paper to assure that products obtained were all dissolved in the filtrate. Then 10 ml of water was added to the filtrate, and the filtrate was extracted with CH_2Cl_2. The organic

Table 1
Double Michael additions catalyzed by various enzymes.[a]



1a 2a 3a .

Entry	Catalyst	Yield (%)
1	Blank	N.R.
2	DA	25 (48[b])
3	Inhibited DA[c]	5
4	BSA	9
5	HPL	8
6	CALB	2

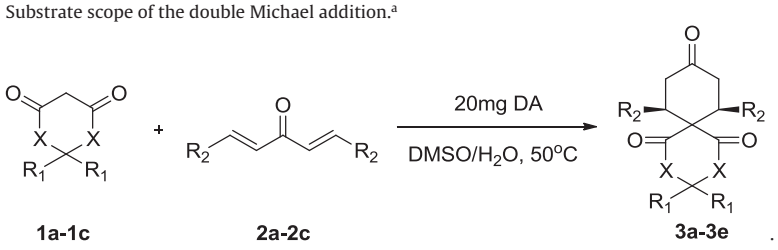
[a] Experimental conditions: 0.25 mmol cyclohexane-1,3-dione (**1a**), 0.25 mmol (1E,4E)-1,5-diphenylpenta-1,4-dien-3-one (**2a**), 20 mg Enzyme, 1 ml DMSO, 50 °C, 48 h. All yields were determined by HPLC. N.R. means no reaction.
[b] 1 mmol (1E,4E)-1,5-diphenylpenta-1,4-dien-3-one (**2a**) was used.
[c] 50 mM $ZnCl_2$ was added to inhibit DA.

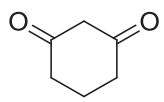
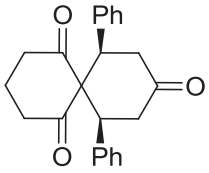
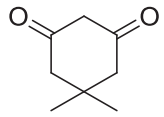
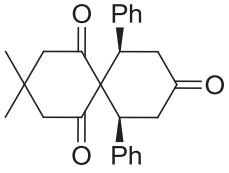
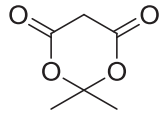
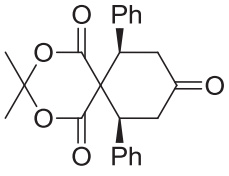
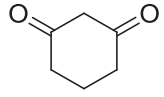
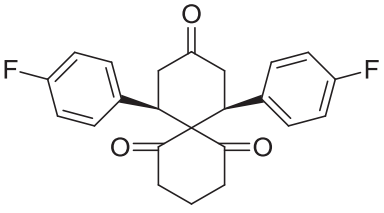
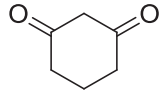
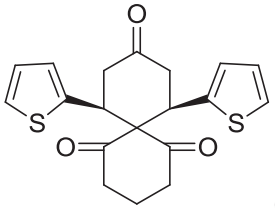
Table 2
Screen of reaction conditions of DA-catalyzed double Michael addition of **1a** and **2a**.[a]

Entry	Solvent	Temp. (°C)	Yield (%)
1	DMSO	50	48
2	DMSO (5% water)	50	57
3	DMSO (10% water)	50	61
4	DMSO (15% water)	50	57
5	DMSO (20% water)	50	56
6	DMSO (30% water)	50	19
7	DMSO (10% water)	40	42
8	DMSO (10% water)	60	57
9	DMSO (10% water)	70	54

[a] Experimental conditions: 0.25 mmol cyclohexane-1,3-dione (**1a**), 1 mmol (1E,4E)-1,5-diphenylpenta-1,4-dien-3-one (**2a**), 20 mg DA, 1 ml solvent, 48 h. All yields were determined by HPLC.

Table 3
Substrate scope of the double Michael addition.[a]



Entry	1	R_2	Product	Yield (%)	*cis*/*trans*[b]
1	 (**1a**)	C_6H_5 (**2a**)	 (**3a**)	61	>100/1
2[c]	 (**1b**)	C_6H_5 (**2a**)	 (**3b**)	36	>100/1
3	 (**1c**)	C_6H_5 (**2a**)	 (**3c**)	41	>100/1
4[c]	 (**1a**)	4-F-C_6H_4 (**2b**)	 (**3d**)	53	20/1
5[c]	 (**1a**)	2-Thienyl (**2c**)	 (**3e**)	30	>100/1

[a] Reaction conditions: 0.25 mmol 1,3-dione (**1a**–**1c**), 1 mmol penta-1,4-dien-3-one (**2a**–**2c**), 20 mg DA, 0.1 ml water, 0.9 ml DMSO, 50 °C, 48 h. All yields were determined by HPLC.
[b] Determined by ^{1}H NMR spectroscopy.
[c] The reaction time was 72 h.

phase was dried over anhydrous Na_2SO_4, and the solvents were removed under reduced pressure. The crude residue was purified by silica gel column chromatography with an eluent consisting of petroleum ether/EtOAc (6/1, v/v). Product-contained fractions were combined, concentrated, and dried to afford the respective product.

3. Results and discussion

First, we started our study with the reaction of cyclohexane-1,3-dione (**1a**) and (1E,4E)-1,5-diphenylpenta-1,4-dien-3-one (**2a**) in the presence of DA in DMSO at 50 °C according to our previous work [29]. The double Michael product

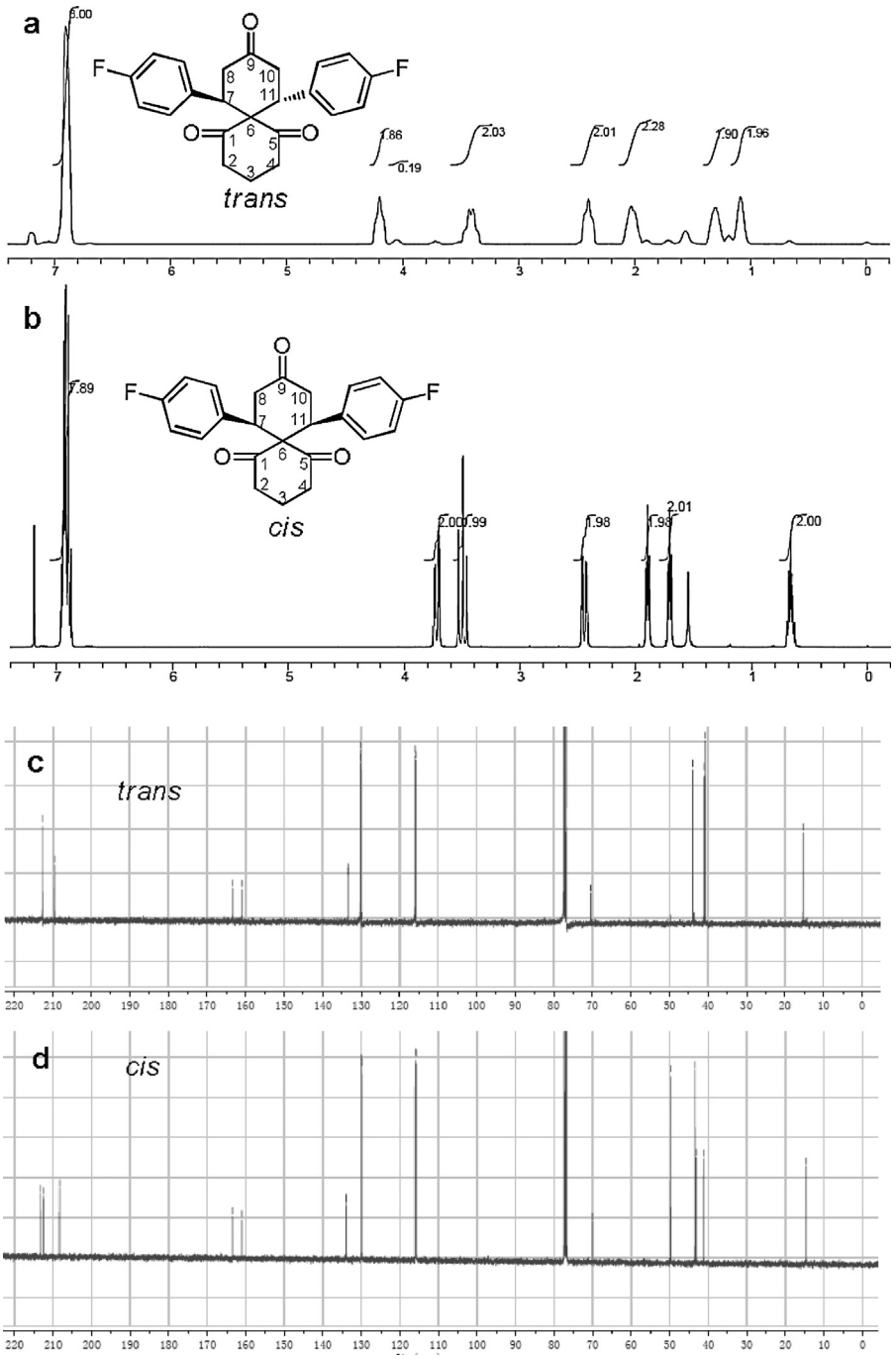


Fig. 2. (a) ^{1}H NMR spectrum of *trans*-**3d**; (b) ^{1}H NMR spectrum of *cis*-**3d**; (c) ^{13}C NMR spectrum of *trans*-**3d**; (d) ^{13}C NMR spectrum of *cis*-**3d**.

7,11-diphenylspiro[5.5]undecane-1,5,9-trione (**3a**) was obtained in a 25% yield after 48 h (Table 1, entry 2). No product was detected in the absence of enzyme (Table 1, entry 1). The DA is presumed to be zinc-dependent and our previous work [29] has proposed that the zinc atom is involved in DA-catalyzed additions. To demonstrate the specific catalytic effect of DA in this reaction, control reactions were run by adding 50 mM non-competitive inhibitor $ZnCl_2$ to inhibit DA. As the ligation of the inhibitory zinc ion by the highly conserved residues Asp366, His67 and His69 in the active site would lower their pKa values and/or hold the nucleophile to perturb the proton shuttle and intermediate stabilization [34], product **3a** was only obtained in a low yield of 5% (Table 1, entry 3). The experiments using bovine serum albumin (BSA) and Lipase from hog pancreas (HPL) gave the results similar to the inhibited DA (Table 1, entries 4 and 5), implying that the active site of DA and protein surface were both responsible for the double Michael addition, while the active site played a dominant role. However, surprisingly, CALB, which was widely applied in other Michael type additions [24,27,28], did not catalyze this reaction smoothly (Table 1, entry 6), which might be caused by the difficulty for the bulky substrates entering into the active site of CALB. Several recent publications demonstrated that earlier claimed promiscuous enzyme reactions turned out not to be promiscuous, such as Markovnikov addition [35], decarboxylative aldol reaction [36] and Henry reaction [37], but doubt about the enzymatic Michael reaction have never been raised to the best of our knowledge.

The biocatalyst's concentration and the molar ratio of substrates were examined first (Table S1, S2 in Support Information). Then the

water content of the reaction media was investigated in detail with the optimized catalyst. It was known that all enzymes need essentially bound water, and some enzymatic activity in organic solvent depends on water content [38]. Solvent mixtures containing 0–30% water were screened in the DA-catalyzed reaction (Table 2, entries 1–6). The data showed that a water content of 10% attained a highest yield up to 61% after 48 h. The yield decreased evidently once the water content surpassed 20%, maybe due to the low solubility of substrates. Next, the influence of reaction temperature on this enzymatic double Michael addition reaction was also considered (Table 2 entries 3, 7–9). The highest yield was obtained at 50 °C. Finally, we investigated the time course of the double Michael reaction under the optimal conditions and the best yield was obtained after 2 days (Fig. S1 in Support Information).In order to extend the scope of this methodology, Meldrum's acid and some other (1E,4E)-1,5-diarylpenta-1,4-dien-3-ones were examined under the optimized conditions (Table 3). When 5,5-dimethylcyclohexane-1,3-dione or Meldrum's acid were used as Michael donors, the products were obtained in a lower yield (Table 3, entries 1 and 2) due to the steric hindrance. (1E,4E)-1,5-Diphenylpenta-1,4-dien-3-one with an electron-withdrawing group (4-F) furnished the spirotrione **4d** in 53% yield (Table 3 entry 4), but electron-donating functionalities were not compatible (4-MeO and 4-Me were tested with trace yields). In addition, (1E,4E)-1,5-di(thiophen-2-yl)penta-1,4-dien-3-one (**2c**) could also be reactive, giving the spiro product **3e** in 30% yields (Table 3, entry 5). Based on these results, this DA-catalyzed double Michael reaction could be of great utility in the generation of libraries of spirotriones **3** with moderate yields in a stereospecific manner from simple substrates.

To confirm the structure of the spiro products, both ^{1}H NMR and ^{13}C NMR spectra of the obtained compounds were compared with the literature reports [10]. It is notable that almost only the *cis* isomers were obtained through this biocatalytic methodology in all the cases. In addition, the double Michael additions could proceed in toluene under catalyst-free condition with lower yield and/or stereoselectivity (Table S3 in Support Information, and no blank reaction in DMSO/H_2O system). Take the adduct of compound **1a** and **2b** as an example, several differences could be found after comparing the NMR spectra of *trans* and *cis* **3d** (Fig. 2). For example the *cis* isomer had three distinct carbonyl groups (C-1, C-5, C-9), while the *trans* isomer had two equivalent carbonyl groups (C-1≡C-5) due to its C_2 symmetry. These results clearly showed that the active site was responsible for this double Michael addition.

4. Conclusion

In conclusion, the synthesis of (hetero)spiro[5.5]undecane derivatives has been achieved via DA-catalyzed [5+1] double Michael additions under mild conditions. The specific catalytic effect of DA was demonstrated by a series of control experiments. The presented enzymatic reactions gave the products in moderate yields with *cis*-stereoselectivity according to their ^{1}H NMR and ^{13}C NMR spectra. We believe that these results can not only highlight the biocatalytic promiscuity but also afford a facile access to functionalized spiro[5.5]undecane derivatives.

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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.molcatb.2013.07.012.

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