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One-Pot Synthesis of Spirooxazino Derivatives *via* Enzyme-Initiated Multicomponent Reactions

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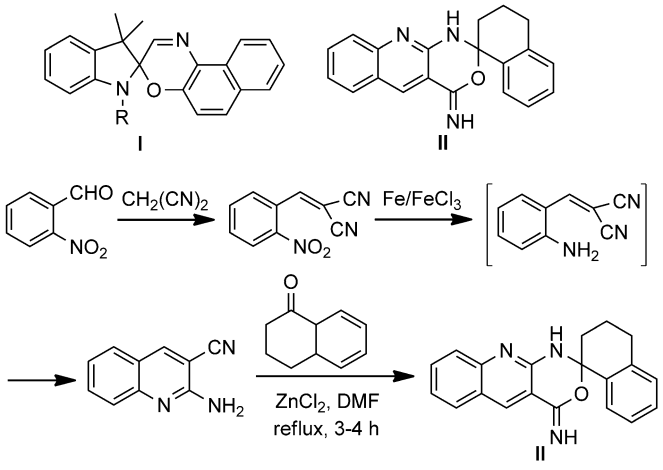
Abstract: A novel enzyme-initiated multicomponent reaction from readily available aldehyde, nitrostyrene, cyclohexanone and acetamide substrates was discovered, enabling the facile construction of six new C–C/–N bonds and two rings in single step, one-pot operation, for the synthesis of spirooxazino derivatives in moderate to high yields. Several methods such as isotope labelling and enzyme mutation were used to probe the possible mechanism of this complex synthesis.

Keywords: enzyme catalysis; multicomponent reactions; one-pot procedure; spirooxazino derivatives

Heterocyclic spiro motifs are found in a wide variety of pharmacological agents and agricultural products, which are used as anticancer agents, anticonvulsant agents, antimicrobial agents.[1] In addition to their medical and agricultural uses, non-natural heterocyclic spiro compounds have also been widely employed in industrial fields as fluorescent probes and electroluminescent devices because of their special rigid structures.[2] The spirooxazine motif (**I**) is the most popular group of photochromic materials and the best representative of heterocyclic spiro compounds with photochromic properties. In this context, efforts to design and develop new reactions that easily access this structural motif have continued to attract much attention. The synthesis of heterocyclic spiro compounds typically relies on the sequential construction of each ring in a stepwise fashion.[3,4] For example, the synthesis of fluorescent spiro-oxazino-quinoline derivatives (**II**) involves a complicated synthetic sequence including Knoevenagel condensation, reduction of nitro to amino group, and cyclocondensation of substituted 2-aminoquinoline-3-carobonitriles and cyclic ketones (Scheme 1).[4] The development of more efficient approaches, which combine two or more ring-forming reactions into a single synthetic operation and allow the concomitant formation of several bonds with a rapid increase in molecular complexity, represents one of the most attractive subjects in synthetic organic chemistry.[5]

In comparison with stepwise processes, multicomponent reactions (MCRs) are more powerful approaches for the preparation of structurally complex heterocyclic spiro compound.[6,7] So far, most reported MCRs were chemically catalyzed, and the enzymatic MCRs have been generally less investigated.[8] However, as more and more new enzymes with new activities, and new functions of old enzymes (enzymatic promiscuities) are being found, the application potential of biocatalysts in synthetic chemistry can be expected to be exciting. Especially enzymatic promiscuities endow one enzyme with catalytic multi-functions, allowing multistep reactions catalyzed by one or two



Scheme 1. Spirooxazine (**I**) and spiro-oxazino-quinoline derivatives (**II**)

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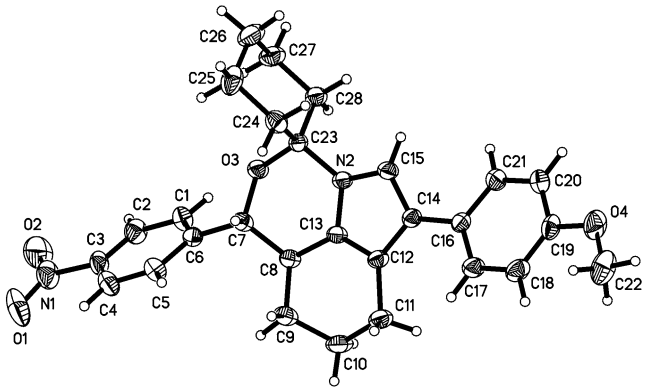
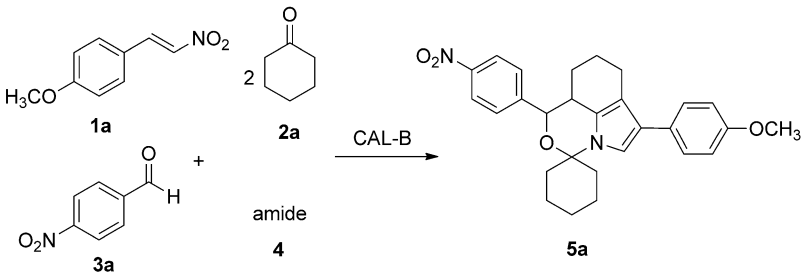


Figure 1. X-ray crystallographic ORTEP drawing of compound **5a**.

enzymes in an MCR manner.[9,10] We have reported several cases of cascade reactions or MCRs in one-pot catalyzed by one enzyme.[11] However, the more complicated cases of enzymatic MCRs, especially for accessing heterocyclic spiro compounds with a rapidly increasing molecular complexity are still highly desirable. Herein, we report an unprecedented lipase-initiated five-component reaction for the one-pot synthesis of complicated spirooxazino derivatives.

Recently, our group reported a lipase/acetamide-catalyzed Michael addition of ketones to nitroolefins,[12] and then we found that this co-catalyst system was also efficient for the aldol reaction (Table S1 in the Supporting Information). In order to extend the application of this system and introduce further structural complexity, olefin (**1a**) with an electron-withdrawing group, cyclohexanone (**2a**) and 4-nitrobenzaldehyde (**3a**) were shaken in one pot catalyzed by lipase/acetamide. However, the designed product of the aldol–Michael cascade reaction was not detected. Unexpectedly, when *Candida antarctica* lipase B (CAL-B) was used as catalyst, an orange crystalline material was obtained, but other enzymes could not catalyze this reaction (Table S2 in the Supporting Information). The structure of this product was confirmed by NMR, HR-MS (please see the Supporting Information) and X-ray single crystal diffraction (Figure 1). Interestingly the product **5a** is a spirooxazino derivative, and the whole reaction process is an apparent five-component reaction as shown in Eq. (1) (Table 1). Six new C–C/C–N bonds and two rings are constructed in a single step, in one pot, leading to a rapid increase in molecular complexity. This serendipitous success inspired us to go forward, and some

Table 1. Optimization of the reaction conditions.[a]



(1)

Entry	Amide	Temperature [°C]	Yield [%]
1	acetamide (**4a**)	50	15/25[g]
2	propanamide (**4b**)	50	3
3	benzamide (**4c**)	50	1
4	acrylamide (**4d**)	50	5
5	acetamide (**4a**)	50	38[b,g]
6	acetamide (**4a**)	50	43[c,g]
7	acetamide (**4a**)	50	55[d,g]
8	acetamide (**4a**)	50	69[d,e,g]
9	acetamide (**4a**)	50	72[c,d,e,f,g]
10	acetamide (**4a**)	25	21[d,e,f,g]
11	acetamide (**4a**)	60	60[d,e,f,g]

[a] Unless otherwise noted, reactions conditions were **1a** (0.5 M), **3a** (0.5 M), enzyme (50 mg), amide (1 M), in **2a** (1 mL) at 50 °C for 72 h.
[b] 1 M **1a** was used.
[c] 1.5 M **1a** was used.
[d] 0.25 M **3a** was used.
[e] 70 mg enzyme were used.
[f] 1.25 M acetamide was used.
[g] The reaction time was 144 h.

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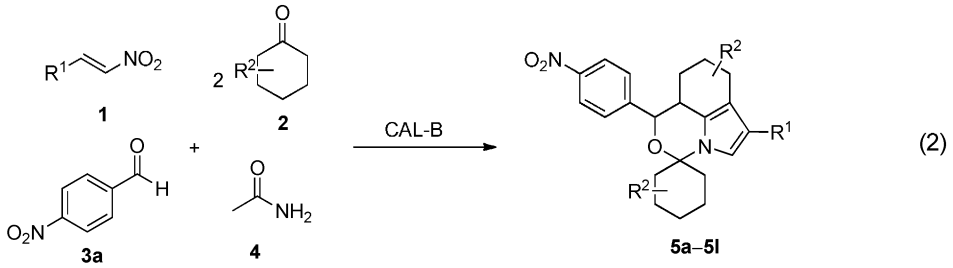
Table 2. Multicomponent reaction catalyzed by CAL-B.[a]



Entry	R^1	R^2	Product	Yield [%]
1	4-CH_3O-C_6H_4	H	**5a**	72
2	4-HO-C_6H_4	H	**5b**	84
3	3-HO-C_6H_4	H	**5c**	85
4	C_6H_5	H	**5d**	30
5	4-CH_3-C_6H_4	H	**5e**	34
6	4-*i*-Pr-C_6H_4	H	**5f**	21
7	4-$N(CH_3)_2$-C_6H_4	H	**5g**	41
8	4-F-C_6H_4	H	**5h**	47
9	C_6H_5	*p*-CH_3	**5i**	25
10	4-F-C_6H_4	*p*-CH_3	**5j**	25
11	4-HO-C_6H_4	*p*-CH_3	**5k**	38
12	4-CH_3O-C_6H_4	*p*-CH_3	**5l**	36

[a] *Experimental conditions:* 0.25 M aldehyde, 1.5 M nitroalkene, 1 mL monocarbonyl compound, 1.25 M acetamide, 70 mg CAL-B, 50 °C, 144 h.

reaction conditions were screened based on the model reaction of aldehyde (**3a**), olefin (**1a**) and cyclohexanone (**2a**) to see whether it was possible to improve the enzymatic MCR. Firstly, control reactions under the catalysis of BSA or denatured CAL-B confirmed the essentially catalytic roles of active lipase in the MCR (entries 11 and 12, Table S2 in the Supporting Information). Further screening of structurally different amides indicated that acetamide was the ideal choice for this transformation, while three other amides including propanamide, benzamide and acrylamide displayed almost no effects (entries 1–4, Table 1). The molar ratio of substrates, the amounts of enzyme and amide, and the temperature were then examined carefully. The reaction was found to proceed with 1.5 M nitroalkene, 0.25 M 4-nitrobenzaldehyde, 1.25 M acetamide, and 70 mg CAL-B in cyclohexanone at 50 °C giving spirocompound **5a** in the highest (72%) yield (entries 5–11, Table 1).

Under the above optimized conditions, the substrate scope and generality of this new MCR process were examined, typical results are summarized in Table 2. Various nitrostyrenes were subjected to the reaction, and the corresponding products were obtained in moderate to high yields. Substituted nitrostyrenes bearing oxy-groups afforded the spiro compounds in better yields, compared with other nitrostyrenes (entries 1–3, Table 2). Concerning the structure of the cyclohexanone, substituted cyclohexanones such as 4-methylcyclohexanone could also be applied in the transformation, with acceptable yields (entries 9-12, Table 2). Concerning other aldehyde substrates, the spiro compound was formed most efficiently in the presence of 4-nitrobenzaldehyde (**3a**). Unfortunately, when other aldehydes were used, although the corresponding products could be detected, the reaction yields were very low.

In order to probe the possible mechanism of this multicomponent reaction, several important questions should be considered. (i) When starting from **1a**, **2a**, and **3a**, is the reaction process proceeding *via* a Michael addition or an aldol reaction? (ii) Is the N atom of the spiro product from the acetamide molecule **4** or the NO_2 group in **1a**? (iii) What are the roles of acetamide and CAL-B in the MCR. Regarding item 1, we synthesized the intermediate of the Michael addition or aldol reaction, respectively, from **1a**+**2a** and **3a**+**2a**, and used them independently as the new starting molecules with the addition of other necessary materials, and checked the final spiro-product of the MCR catalyzed by CAL-B. It was discovered that the spiro compound could also be formed from the MCR using aldol intermediate as the starting material [Eq. (3) in Scheme 2]. However, no reaction was observed when using the Michael addition intermediate as the substrate [Eq. (4) in Scheme 2]. Thus it is clear that the first step is the aldol reaction, supported by the observation that a small amount of this intermediate was also detected in the MCR.

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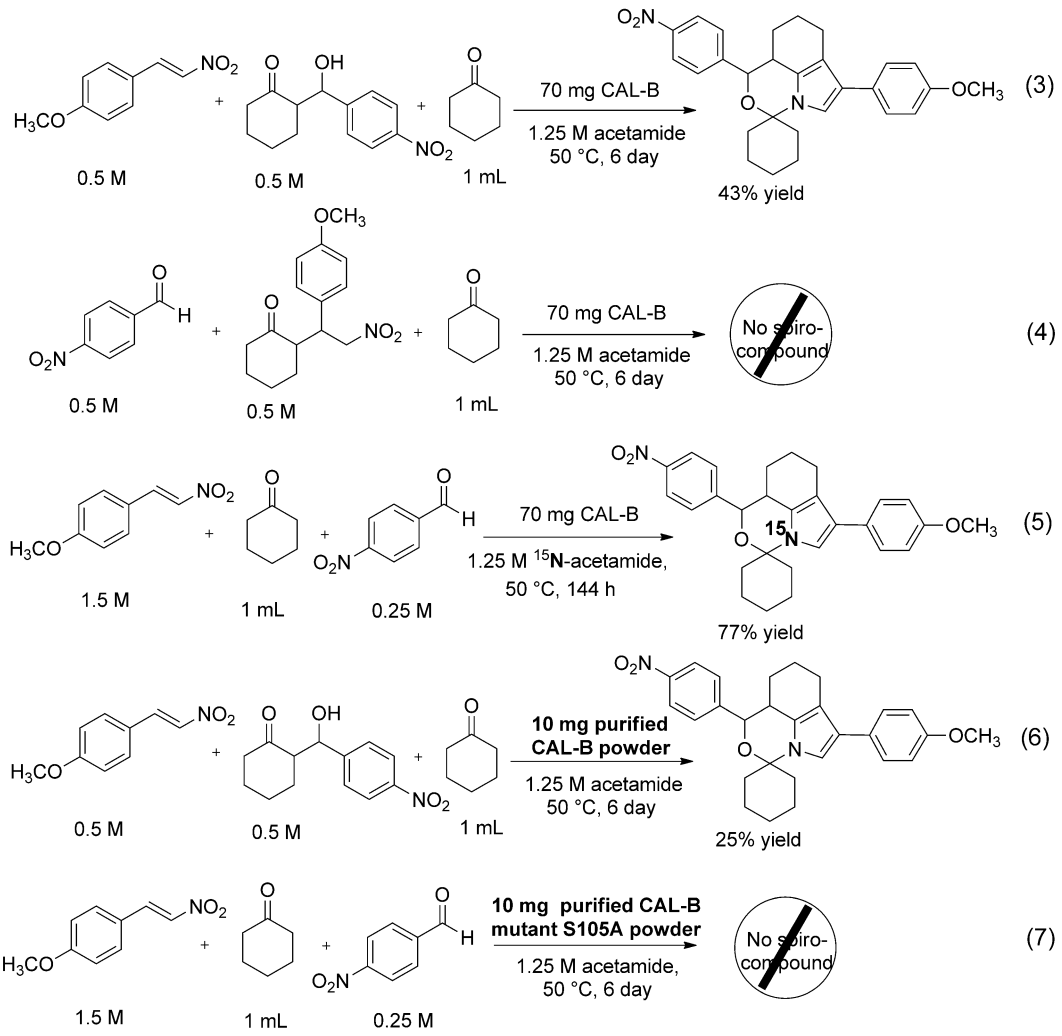

Scheme 2. MCRs under the different reaction conditions.

For the item 2, the source of N atom in the final product is very important. Initially, we suspected that the N(NH) fragment was the reduction product of NO_2 from substrate **1a**. However, this is almost impossible because lipases have never been reported to be able to catalyze a reduction reaction. Another possible source is the acetamide. In a previous report of an acetamide-based enzymatic Hantzsch reaction, the acetamide was involved as a novel ammonia source.[11c] It seemed useful to us to use ^{15}N-acetamide in the MCR and to see whether the acetamide is also an ammonia source. Interestingly the ^{15}N labelled product was obtained in good yield [Eq. (5) in Scheme 2].

The presence of acetamide was critical to the MCR not only as the source of the N(NH) fragment, but also as a synergistic catalytic molecule. It is clear that acetamide cannot catalyze any reaction process in this MCR, after checking some control reactions. But we found that acetamide had an important synergistic effect on the CAL-B-catalyzed aldol reaction of 4-nitrobenzaldehyde (**3a**) and cyclohexanone (**2a**). Accordingly, a 62% yield was obtained under the catalysis of CAL-B with acetamide, while CAL-B almost could not catalyze this aldol reaction in the absence of acetamide (Table S1 in the Supporting Information). A similar synergistic effect of acetamide on CAL-B was also found in our previous work,[12] and we speculated that acetamide played a role as co-catalyst to activate the substrates in this aldol reaction. Such effects of small organic molecules on the synthetic performance of enzymes need more studies for the insight into the inherent mechanisms.

In this complicated MCR, the catalytic role of CAL-B should be strictly confirmed. Considering that the used commercial CAL-B are crude and immobilized enzyme preparations, we excluded the possible catalytic activity of the immobilization carrier, the acidic or basic amino acids on the protein surface and also the impurity of the preparation using some control experiments. The first one is that the completely

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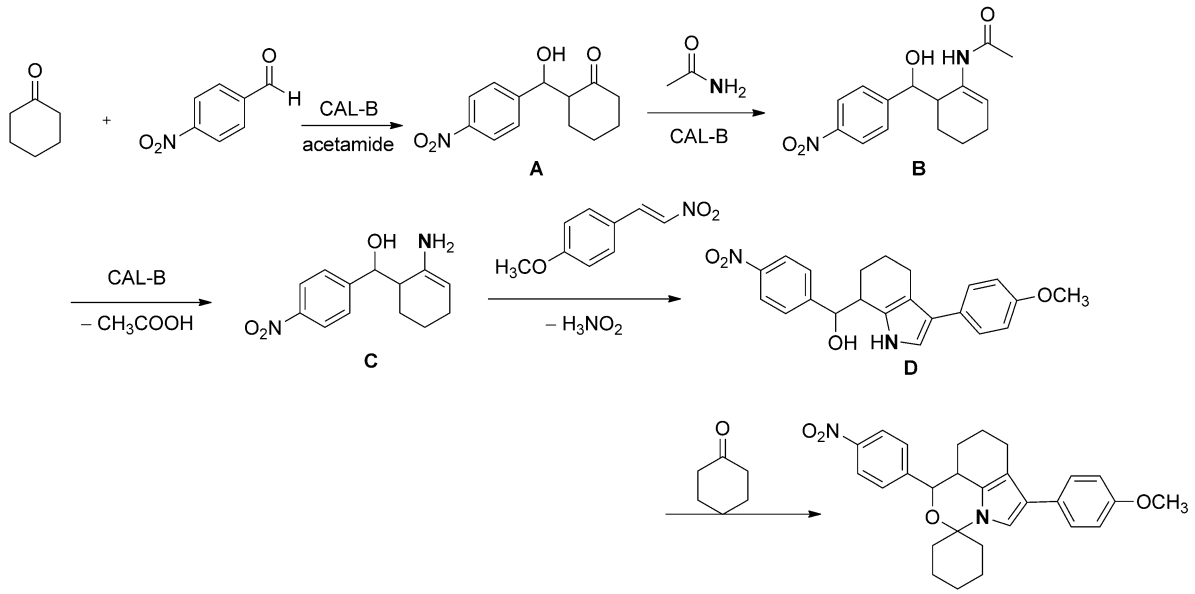

Scheme 3. Mechanistic proposal and possible reaction process.

denatured CAL-B with the immobilization carrier could not catalyze the aldol reaction of 4-nitrobenzaldehyde with cyclohexanone (entry 5 of Table S1 in the Supporting Information), as well as the whole MCR of route 1 (entry 11 of Table S2 in the Supporting Information), ruling out the catalytic possibility of both denatured protein and the immobilization carrier. Actually, the catalytic promiscuity of CAL-B for the aldol reaction or the Michael addition has been confirmed by many publications to some extent.[13] The control experiment of BSA catalysis further excluded the catalytic role of some amino acids on the protein surface (entry 12 of Table S2 in the Supporting Information). Another important control experiment is using the purified CAL-B protein as the catalyst to rule out the catalytic role of any impurity in commercial CAL-B preparation and confirm what is really the catalyst. The purification of CAL-B is shown in the Supporting Information. Purified CAL-B provided the final product with 25% yield [Eq. (6) in Scheme 2], which was a bit lower than that from the catalysis with commercial CAL-B, possibly ascribable to the beneficial effect of immobilization. In the pioneering works on the mechanism of CAL-B promiscuity by Berglund and co-workers, the S105A mutant of CAL-B showed higher promiscuous activity than WT CAL-B because the S105A mutation breaks the hydrogen bond between S105–H224 and thus strengthens the deprotonation ability of His 224, supporting the catalytic role of the H224–D187 diad for promiscuous reactions.[13a,b,14] Herein, the S105A mutant of CAL-B was prepared and purified according to the previous literature[15] (details are shown in the Supporting Information), and used for the whole MCR process, however no spiro compound was detected except for some intermediate of the aldol reaction [Eq. (7) in Scheme 2]. This implies that some natural reaction activity of CAL-B such as hydrolysis or esterification was also involved in the MCR process, because CAL-B S105A cannot catalyze a natural hydrolysis or esterification reaction due to the lack of the essential residue 105Ser. All these results suggest that the tertiary structure and the specific active site of CAL-B are responsible for the MCR process.

At this stage, based on these data and some previous references, we could propose a tentative mechanism illustrating the internal reaction sequence (Scheme 3). In this mechanism, CAL-B is responsible for the early steps forming the possible intermediates **A**–**C**. The aldol reaction catalyzed by lipase was well investigated recently and also confirmed by some data in the reaction sequence of this MCR, thus β-hydroxy ketone **A** was produced. In the presence of CAL-B, β-hydroxy ketone **A** could react with activated acetamide through a tandem condensation/hydrolysis reaction to afford the enamine intermediate **C**.[16] Similar activation of acetamide by CAL-B was also observed in another report of an acetamide-attending CAL-B-catalyzed Hantzsch reaction.[11c] The hydrolysis process was also partially confirmed by the presence of a small amount acetic acid and the above control reaction catalyzed by CAL-B S105A mutant [Eq. (7) in Scheme 2]. Next, the reaction of nitroolefins with enamine derivatives **C** at room temperature can afford the intermediate **D** efficiently *via* a sequential Michael addition/intramolecular Nef reaction.[17] The final spiro compound product was obtained from the condensation of intermediate **D** and cyclohexanone.[18]

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As can be seen in Table 2, all spiro products have at least two stereocenters (formed from reactions of cyclohexanone, while >2 when using substituted cyclohexanones). We tried to examine the *ee* value of these products by chiral HPLC. To our disappointment, no enantioselectivity could be observed. Actually, we found that the first step (aldol reaction) where the stereocenters of the final spiro-products were produced, failed to provide any enantioselectivity, thus it would be not expected to have good stereoselectivity in the whole MCR process. To the best of our knowledge, a CAL-B-catalyzed asymmetric aldol reaction has never been reported. Similarly for the CAL-B-catalyzed Michael addition, a possible reason is that the active site of CAL-B is too voluminous to have clearly distinguished binding modes for formed enantiomers.[19] In order to improve the stereoselectivity of this MCR process and overcome the structure defect of the active site of CAL-B, directed evolution and site-mutagenesis of CAL-B for the asymmetric aldol reaction are in progress in our lab.

In conclusion, we have developed a novel enzyme-initiated multicomponent reaction for the one-pot synthesis of spirooxazino derivatives starting from readily available aldehydes, activated olefins, cyclohexanone and acetamide. 12 various spiro compounds with different substitutions could be prepared in moderate to high yields. The ability of the MCR to construct six new C–C/C–N bonds and two rings in a single step strongly proves its important application potential in complicated organic synthesis. The preliminary investigation of the reaction mechanism revealed the source of the N atom in the final spiro products and the important catalytic roles of the active site of *Candida antarctica* lipase B in the domino process.

Experimental Section

General Procedure for the Multicomponent Reactions

4-Nitrobenzaldehyde (0.25 M), nitroalkene (1.5 M), acetamide (1.25 M), and CAL-B (Novozym435) (70 mg) in 1 mL monocarbonyl compound were allowed to react at 50 °C for 144 h. The reaction was terminated by filtering off the enzyme. The crude residue was purified by silica gel column chromatography with an eluent consisting of petrol ether/acetone (20/1 v/v). Product-contained fractions were combined, concentrated, and dried to give **5a–5l**.

Acknowledgements

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References

[1] a) C. V. Galliford, K. A. Scheidt, *Angew. Chem.* **2007**, *119*, 8902–8912; *Angew. Chem. Int. Ed.* **2007**, *46*, 8748–8758; b) K. Ding, Y. P. Lu, N. Coleska, *J. Med. Chem.* **2006**, *49*, 3432–3435; c) P. D. O'Connor, M. A. Brimble, *Nat. Prod. Rep.* **2007**, *24*, 869–885; d) K. S. Yeung, I. Paterson, *Chem. Rev.* **2005**, *105*, 4237–4313; e) Z. G. Ding, J. Y. Zhao, M. G. Li, R. Huang, Q. M. Li, X. L. Cui, H. J. Zhu, M. L. Wen, *J. Nat. Prod.* **2012**, *75*, 1994–1998; f) K. Kaminski, J. Obniska, M. Dybala, *Eur. J. Med. Chem.* **2008**, *43*, 53–61; g) N. V. Borrero, A. Aponick, *J. Org. Chem.* **2012**, *77*, 8410–8416.

[2] a) T. P. I. Saragi, T. Spehr, A. Siebert, T. Fuhrmann-Lieker, J. Salbeck, *Chem. Rev.* **2007**, *107*, 1011–1065; b) G. Berkovic, V. Krongauz, V. Weiss, *Chem. Rev.* **2000**, *100*, 1741–1753; c) L. Yuan, W. Y. Lin, K. B. Zheng, S. S. Zhu, *Acc. Chem. Res.* **2013**, *46*, 1462–1473.

[3] a) M. Singh, N. P. Argade, *Synthesis* **2011**, 1137–1141; b) B. K. Sarma, D. Manna, M. Minoura, G. Mugesh, *J. Am. Chem. Soc.* **2010**, *132*, 5364–5374; c) L. N. Aldrich, C. B. Berry, B. S. Bates, L. C. Konkol, M. So, C. W. Lindsley, *Eur. J. Org. Chem.* **2013**, *20*, 4215–4218; d) A. K. Arya, M. Kumar, *Mol. Diversity* **2011**, *15*, 781–789; e) Y. A. Zhang, Q. Liu, C. Wang, Y. X. Jia, *Org. Lett.* **2013**, *15*, 3662–3665; f) H. Bienaymé, L. Chêne, S. Grisoni, A. Grondin, E. B. Kaloun, S. Poigny, H. Rahali, E. Tam, *Bioorg. Med. Chem. Lett.* **2006**, *16*, 4830–4833.

[4] B. S. Rane, M. A. Kazi, S. M. Bagul, D. P. Shelar, R. B. Toche, M. N. Jachak, *J. Fluoresc.* **2010**, *20*, 415–420.

[5] a) J. C. Wasilke, S. J. Obrey, R. T. Baker, G. C. Bazan, *Chem. Rev.* **2005**, *105*, 1001–1020; b) L. F. Tietze, N. Rackelmann, *Pure Appl. Chem.* **2004**, *76*, 1967–1983; c) M. Z. Wang, C. Y. Zhou, C. M. Che, *Chem. Commun.* **2011**, *47*, 1312–1314; d) N. Majumdar, K. A. Korthals, W. D. Wulff, *J. Am. Chem. Soc.* **2012**, *134*, 1357–1362; e) M. M. Hussain, P. J. Walsh, *Acc. Chem. Res.* **2008**, *41*, 883–893; f) H. Shi, L. Fang, C. Tan, L. Shi, W. Zhang, C. C. Li, T. Luo, Z. Yang, *J. Am. Chem. Soc.* **2011**, *133*, 14944–14947; g) B. J. Naysmith, M. A. Rrimble, *Org. Lett.* **2013**, *15*, 2006–2009; h) D. Enders, C. Joie, K. Deckers, *Chem. Eur. J.* **2013**, *19*, 10818–10821.

[6] For recent reviews, see: a) V. Estévez, M. Villacampa, J. C. Menéndez, *Chem. Soc. Rev.* **2010**, *39*, 4402–4421; b) A. Dömling, *Chem. Rev.* **2006**, *106*, 17–89; c) E. Ruijter, R. Scheffelaar, R. V. A. Orru, *Angew. Chem.* **2011**, *123*, 6358–6371; *Angew. Chem. Int. Ed.* **2011**, *50*, 6234–6246; d) C. D. Graaff, E. Ruijter, R. V. A. Orru, *Chem. Soc. Rev.* **2012**, *41*, 3969–4009; e) B. Ganem, *Acc. Chem. Res.* **2009**, *42*, 463–472; f) J. Zhu, H. Bienayme, in: *Multicomponent Reactions*, Wiley-VCH, Weinheim, **2005**; g) L. Banfi, R. Riva, *Synlett* **2010**, 23–41; h) A. Dömling, I. Ugi, *Angew. Chem.* **2000**, *112*, 3300–3344; *Angew. Chem. Int. Ed.* **2000**, *39*, 3168–3210; i) J. E. Biggs-Houck, A. Younai, J. T. Shaw, *Curr. Opin. Chem. Biol.* **2010**, *14*, 371–382.

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[7] For selected recent examples, see: a) K. Jiang, Z. J. Jia, X. Yin, L. Wu, Y. C. Chen, *Org. Lett.* **2010**, *12*, 2766–2769; b) M. Presset, Y. Coquerel, J. Rodriguez, *Org. Lett.* **2010**, *12*, 4212–4215; c) X. H. Chen, Q. Wei, S. W. Luo, L. Z. Gong, *J. Am. Chem. Soc.* **2009**, *131*, 13819–13825; d) C. Cheng, B. Jiang, S. J. Tu, G. G. Li, *Green Chem.* **2011**, *13*, 2107–2115.

[8] a) K. Li, T. He, C. Li, X. W. Feng, N. Wang, X. Q. Yu, *Green Chem.* **2009**, *11*, 777–779; b) J. C. Xu, W. M. Li, H. Zheng, Y. F. Lai, P. F. Zhang, *Tetrahedron* **2011**, *67*, 9582–9587; c) S. J. Chai, Y. F. Lai, J. C. Xu, H. Zheng, Q. Zhu, P. F. Zhang, *Adv. Synth. Catal.* **2011**, *353*, 371–375; d) Z. Q. Liu, B. K. Liu, Q. Wu, X. F. Lin, *Tetrahedron* **2011**, *67*, 9736–9740; e) Y. Xue, L. P. Li, Y. H. He, Z. Guan, *Sci. Rep.* **2012**, *2*, 761; f) S. Kłossowski, B. Wiraszka, S. Berłożecki, R. Ostaszewski, *Org. Lett.* **2013**, *15*, 566–569; g) P. P. Bora, M. Bihani, G. Bez, *J. Mol. Catal. B: Enzym.* **2013**, *92*, 24–33; h) H. Zheng, Y. J. Mei, K. Du, Q. Y. Shi, P. F. Zhang, *Catal. Lett.* **2013**, *143*, 298–301.

[9] For recent reviews, see: a) U. T. Bornscheuer, R. J. Kazlauskas, *Angew. Chem.* **2004**, *116*, 6156–6165; *Angew. Chem. Int. Ed.* **2004**, *43*, 6032–6040; b) E. Busto, V. Gotor-Fernández, V. Gotor, *Chem. Soc. Rev.* **2010**, *39*, 4504–4523; c) M. D. Toscano, K. J. Woycechowsky, D. Hilvert, *Angew. Chem.* **2007**, *119*, 3274–3300; *Angew. Chem. Int. Ed.* **2007**, *46*, 3212–3236; d) Q. Wu, B. K. Liu, X. F. Lin, *Curr. Org. Chem.* **2010**, *14*, 1966–1988; e) A. Babtie, N. Tokuriki, F. Hollfelder, *Curr. Opin. Chem. Biol.* **2010**, *14*, 200–207; f) O. Khersonsky, D. S. Tawfik, *Annu. Rev. Biochem.* **2010**, *79*, 471–505; g) M. S. Humble, P. Berglund, *Eur. J. Org. Chem.* **2011**, 3391–3401.

[10] For selected recent examples of enzymatic promiscuity: a) E. Zandvoort, E. M. Geertsema, B. Baas, W. J. Quax, G. J. Poelarends, *Angew. Chem.* **2012**, *124*, 1266–1269; *Angew. Chem. Int. Ed.* **2012**, *51*, 1240–1243; b) P. S. Coelho, E. M. Brustad, A. Kannan, F. H. Arnold, *Science* **2013**, *339*, 307–310; c) V. López-Canut, M. Roca, J. Bertrán, V. Moliner, I. Tuñón, *J. Am. Chem. Soc.* **2011**, *133*, 12050–12062; d) M. Lopez, H. Vu, C. K. Wang, M. G. Wolf, G. Groenhof, A. Innocenti, C. T. Supuran, S. Poulsen, *J. Am. Chem. Soc.* **2011**, *133*, 18452–18462; e) C. H. Wang, Z. Guan, Y. H. He, *Green Chem.* **2011**, *13*, 2048–2054; f) M. López-Iglesias, E. Busto, V. Gotor, V. Gotor-Fernández, *Adv. Synth. Catal.* **2011**, *353*, 2345–2353; g) B. K. Liu, Q. Wu, D. S. Lv, X. F. Lin, *J. Biotechnol.* **2011**, *153*, 111–115; h) B. Baas, E. Zandvoort, E. M. Geertsema, G. J. Poelarends, *ChemBioChem* **2013**, *14*, 917–926.

[11] a) S. P. Yao, D. S. Lu, Q. Wu, Y. Cai, S. H. Xu, X. F. Lin, *Chem. Commun.* **2004**, 2006–2007; b) Q. Wu, J. M. Xu, L. Xia, J. L. Wang, X. F. Lin, *Adv. Synth. Catal.* **2009**, *351*, 1833–1841; c) J. L. Wang, B. K. Liu, C. Yin, Q. Wu, X. F. Lin, *Tetrahedron* **2011**, *67*, 2689–2692.

[12] X. Y. Chen, G. J. Chen, J. L. Wang, Q. Wu, X. F. Lin, *Adv. Synth. Catal.* **2013**, *355*, 864–868.

[13] a) C. Branneby, P. Carlqvist, A. Magnusson, K. Hult, T. Brinck, P. Berglund, *J. Am. Chem. Soc.* **2003**, *125*, 874–875; b) C. Branneby, P. Carlqvist, K. Hult, T. Brinck, P. Berglund, *J. Mol. Catal. B: Enzym.* **2004**, *31*, 123–128; c) A. B. Majumder, N. G. Ramesh, M. N. Gupta, *Tetrahedron Lett.* **2009**, *50*, 5190–5193; d) C. Li, X. W. Feng, N. Wang, Y. J. Zhou, X. Q. Yu, *Green Chem.* **2008**, *10*, 616–618; e) Y. H. He, H. H. Li, Y. L. Chen, Y. Xue, Y. Yuan, Z. Guan, *Adv. Synth. Catal.* **2012**, *354*, 712–719; f) E. Busto, V. Gotor-Fernández, V. Gotor, *Org. Process Res. Dev.* **2011**, *15*, 236–240.

[14] a) M. Svedendahl, K. Hult, P. Berglund, *J. Am. Chem. Soc.* **2005**, *127*, 17988–17989; b) P. Carlqvist, M. Svedendahl, C. Branneby, K. Hult, T. Brinck, P. Berglund, *ChemBioChem* **2005**, *6*, 331–336.

[15] Q. Wu, P. Soni, M. T. Reetz, *J. Am. Chem. Soc.* **2013**, *135*, 1872–1881.

[16] a) E. Forró, F. Fülöp, *Chem. Eur. J.* **2006**, *12*, 2587–2592; b) T. Wagegg, M. M. Enzelberger, U. T. Bornscheuer, R. D. Schmid, *J. Biotechnol.* **1998**, *61*, 75–78.

[17] a) A. W. Trautwein, G. Jung, *Tetrahedron Lett.* **1998**, *39*, 8263–8266; b) Z. H. Guan, L. Li, Z. H. Ren, J. L. Li, M. N. Zhao, *Green Chem.* **2011**, *13*, 1664–1668; c) A. Palmieri, S. Gabrielli, C. Cimarelli, R. Ballini, *Green Chem.* **2011**, *13*, 3333–3336.

[18] a) M. Darabantu, G. Plé, C. Maiereanu, I. Silaghi-Dumitrescu, Y. Ramondenc, S. Mager, *Tetrahedron* **2000**, *56*, 3799–3816; b) M. Senkus, *J. Am. Chem. Soc.* **1945**, *67*, 1515–1519.

[19] G. A. Strohmeier, T. Sović, G. Steinkellner, F. S. Hartner, A. Andryushkova, T. Purkarthofer, A. Glieder, K. Gruber, H. Griengl, *Tetrahedron* **2009**, *65*, 5663–5668.

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