JOC The Journal of Organic Chemistry

Article

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Enzyme-Catalyzed Direct Three-Component Aza-Diels–Alder Reaction Using Hen Egg White Lysozyme

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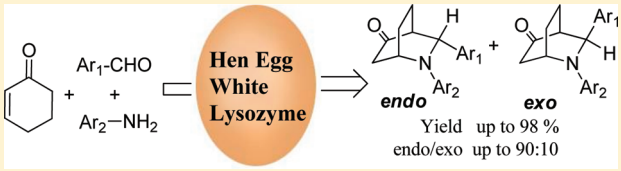
Supporting Information

ABSTRACT: The direct three-component aza-Diels–Alder reaction of aromatic aldehyde, aromatic amine, and 2-cyclohexen-1-one was catalyzed by hen egg white lysozyme for the first time. Under the optimized conditions investigated in this paper, the enzyme-catalyzed aza-Diels–Alder reaction gave yields up to 98% and stereoselectivity of *endo*/*exo* ratios up to 90:10.



INTRODUCTION

Since the first example of imine participating as a hetero-dienophile was reported in 1943,[1] the aza-Diels–Alder reaction has gained substantial synthetic and industrial significance as one of the most powerful tools to construct nitrogen-containing six-membered heterocycles which are commonly found in modern medicines and lots of natural products.[2] In most cases, the performed diene such as Danishefsky's diene[3] were used in the cycloaddition. Recently, there has been increasing interest in substituting the performed diene with cyclohexenone derivatives. Because of the lower reactivity of these derivatives, great efforts have been devoted to exploring effective catalysts for the reaction, and positive progress has been made. Generally, these catalysts include some Lewis and Brønsted acids[4] and proline derivatives.[5] Although several successful organocatalysts for asymmetric aza-Diels–Alder reaction have been described with high efficiency and enantioselectivity, most catalysts suffer disadvantages such as expensiveness, moisture-sensitivity, multistep synthesis, and toxicity to the environment and humans. Therefore, the development of sustainable, environmentally-benign, and cost-efficient catalysts for the aza-Diels–Alder reaction still remains a significant challenge.

Enzyme catalysts, as efficient and green biotransformation tools in organic synthesis, show immense advantages such as mild reaction conditions, simple separation, good selectivity, high yields, etc. Nowadays, a growing number of enzymes have been found to be capable to catalyze synthetic reactions which vary from their natural roles.[6] It is widely believed that exploiting enzyme catalytic promiscuity has great potential of expanding the repertoire of synthetic methodologies. Hydrolases are the main promiscuous enzymes which have been utilized to catalyze the formation of C–C and C–heteroatom bonds through aldol, Markovnikov, Michael, and Mannich additions.[7] Herein we wish to report a novel discovery that the readily available hen egg white lysozyme (HEWL) (EC 3.2.1.17) efficiently promotes the one-pot, three-component aza-Diels–Alder reaction of aromatic aldehyde, aromatic amine,and 2-cyclohexen-1-one resulting in moderate to excellent yields. It is the first example of enzyme-catalyzed direct aza-Diels–Alder reaction.

HEWL is a powerful hydrolytic enzyme belonging to the glycosylase family. Its enzymatic and physiological properties have been extensively studied. It is well documented that the HEWL damages the bacterial cell walls by splitting 1,4-β-linkages between *N*-acetylmuramic acid and *N*-acetylglucosamine of peptidoglycan. In this study, HEWL was first used to catalyze direct the three-component aza-Diels–Alder reaction. It provided a novel case of enzyme catalytic promiscuity and might be a potential synthetic method for organic chemistry.

RESULTS AND DISCUSSION

The Catalytic Activity of Different Hydrolases in Aza-Diels–Alder Reaction. Initial studies were undertaken using 4-chlorobenzaldehyde, 4-anisidine, and 2-cyclohexen-1-one as a model reaction (Scheme 1).

We chose water/MeCN (v/v = 0.3) as the medium, and the reaction was performed at 25 °C. Several commercially available hydrolases were screened using the model reaction, and the results were summarized in Table 1. It was found that the product in yield of 23% with 58:42 (*endo*/*exo*) was achieved by using HEWL as the catalyst (Table 1, entry 8). However, seven other candidates including immobilized lipase from *Aspergillus oryzae*, lipase PS "amano" SD, neutral protease from *Bacillus subtilis* A.S.1.398, alkaline proteinase from *Bacillus licheniformis* No. 2709, bromelain from pineapple peduncle, cellulase from *Trichoderma*, and chymopapain from *Carica papaya* showed no activity toward the reaction (Table 1, entries 1–7). It indicated that not all the enzymes can catalyze this reaction, which on the other hand excluded the amino acid catalysis. Moreover, nonenzyme protein egg albumin was also used in the reaction, which gave no product (Table 1, entry 9). This further confirmed that the catalysis was not simply arisen by the amino acid residues on the surface of the protein. In addition, to demonstrate the specific catalytic effect of HEWL,

Received: August 14, 2011
Published: November 22, 2011

Scheme 1. Enzyme-Catalyzed Aza-Diels–Alder Reaction

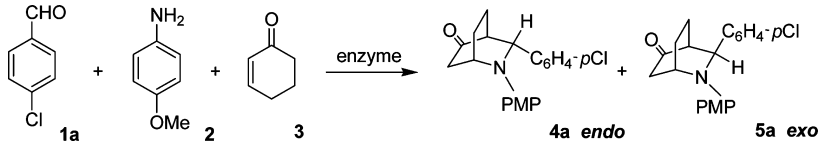


Table 1. Catalytic Activity of Different Hydrolases and Amino Acids in the Direct Aza-Diels–Alder Reaction[a]

entry	catalyst	yield[b] (%)	4a/5a
1	immobilized lipase from *Aspergillus oryzae*	NR	
2	lipase PS "Amano" SD	NR	
3	neutral proteinase from *Bacillus subtilis* A.S.1.398	NR	
4	alkaline proteinase from *Bacillus licheniformis* No 2709	NR	
5	bromelain from pineapple peduncle	NR	
6	cellulase from *Trichoderma*	NR	
7	chymopapain from *Carica papaya*	NR	
8	HEWL	23	58:42
9	egg albumin	NR	
10	none	NR	
11	HEWL denatured with urea[c]	NR	
12	HEWL denatured with high temperature[d]	NR	
13	proline[e]	27 (11)[f]	59:41
14	glycine[e]	NR	

[a]Unless otherwise noted, reaction conditions: 4-chlorobenzaldehyde **1a** (1 mmol), 4-anisidine **2** (0.5 mmol), cyclohexenone **3** (2 mmol), and enzyme (100 mg) in MeCN (1 mL) and deionized water (0.3 mL) at 25 °C for 96 h. [b]Yield of the isolated products (**4a** + **5a**). [c]The mixture of HEWL (100 mg), MeCN (1 mL), deionized water (0.3 mL), and urea (100 mg) was stirred at 25 °C for 48 h before use. [d]HEWL was predenatured at 100 °C for 24 h. [e]A mixture of **1a** (1 mmol), **2** (0.5 mmol), **3** (2 mmol), and amino acid (20 mol %) in MeCN (1 mL) was stirred at 25 °C for 96 h. [f]The value given in parentheses refers to the yield in the presence of proline (10 mol %).

some control experiments were performed. In the absence of HEWL, no product was observed (Table 1, entry 10). When the urea-denatured HEWL and high temperature-denatured HEWL were used, no product was detected for the reaction (Table 1, entries 11 and 12). These results indicated that the specific natural fold of HEWL was responsible for its activity in the reaction. Besides, it has been reported that proline could catalyze aza-Diels–Alder reaction of aqueous formaldehyde, 4-anisidine, and cyclohexenone giving the product in moderate yield with high enantioselectivity.[5a] For comparison purposes, proline and glycine were also used to catalyze the model reaction (Table 1, entries 13 and 14). When 20 mol % of proline (12 mg, 0.1 mmol, 100 mM) was used, it gave almost the same yield and diastereoselectivity with HEWL (100 mg, 0.007 mmol, 5.4 mM). However, glycine did not show any catalytic activity for the reaction. To explore the novel enzymatic activity of HEWL and expand the repertoire of synthetic methodologies, we further investigated HEWL-catalyzed aza-Diels–Alder reaction.

Effect of Substrate Concentration on the Rate of the Reaction. We first investigated the effect of substrate concentration on the rate of the HEWL-catalyzed aza-Diels–Alder reaction using the model reaction. We systematically changed one substrate concentration at a time and measured the average rate of the reaction within the yield of 16.2% (no side reaction was observed). Since the total volume of the reaction system varied with the addition of different amount of substrates, we used the amount (mmol) of substrates in MeCN (2 mL) and deionized water (0.6 mL) to express the concentration. The results are shown in Table 2. It could be seen that doubling the concentration of 4-chlorobenzaldehyde **1a** only led to a slight increase of the reaction rate (Table 2, entries 1 and 2). However, higher concentration of the aldehyde slowed the reaction obviously and even completely inhibited the reaction (Table 2, entries 3 and 4). Furthermore, increasing the concentration of 4-anisidine **2** could improve the reaction rate remarkably (Table 2, entries 1, 5, and 6), but a large excess of amine caused a slight decrease of the reaction rate (Table 2, entry 7). In addition, increasing the concentration of cyclohexenone **3** only led to a slight enhancement of the reaction rate (Table 2, entries 1 and 8–10). The above results indicated that high concentration of aldehyde might cause the deactivation of HEWL while amine and enone appeared to exhibit saturation kinetics.

Table 2. Effect of Substrate Concentration on the Rate of HEWL-Catalyzed Direct Aza-Diels–Alder Reaction[a]

entry	1a/2/3	1a (mmol)	2 (mmol)	3 (mmol)	rate (yield %/h)	time (h)	yield[b] (%)
1	1:1:1	1.0	1.0	1.0	0.22	48	10.5
2	2:1:1	2.0	1.0	1.0	0.24	48	11.4
3	4:1:1	4.0	1.0	1.0	0.10	48	5.0
4	6:1:1	6.0	1.0	1.0	0	48	no product
5	1:2:1	1.0	2.0	1.0	0.90	12	10.8
6	1:4:1	1.0	4.0	1.0	1.22	12	14.7
7	1:6:1	1.0	6.0	1.0	1.07	12	12.9
8	1:1:2	1.0	1.0	2.0	0.23	48	11.2
9	1:1:4	1.0	1.0	4.0	0.30	48	14.6
10	1:1:6	1.0	1.0	6.0	0.33	48	16.2

[a]Reaction conditions: HEWL (200 mg), MeCN (2 mL), deionized water (0.6 mL), and substrates at 25 °C. [b]Yield of the isolated products (**4a** + **5a**).

Effect of Mole Ratio of Substrates. In order to further improve the yield of HEWL-catalyzed aza-Diels–Alder reaction, the effect of mole ratio of substrates on the model reaction was investigated. The results were summarized in Table 3. It could be seen that the mole ratio of substrates had a remarkable influence on the yield and selectivity of the reaction. When the ratio of **1a**/**2**/**3** was 1:3:3 (Table 3, entry 10), the product was obtained in the best yield of 73% with 74:26 (*endo*/*exo*) after 96 h. Further increasing the ratio of **1a**/**2**/**3** to 1:3:6 led to the best selectivity of 81:19 (*endo*/*exo*), but lower yield of 66% (Table 3, entry 12). Thus, we chose the mole ratio (**1a**/**2**/**3** = 1:3:3) for the HEWL-catalyzed aza-Diels–Alder reaction.

Effect of Solvents. Since enzymes can work in organic media to acquire remarkable properties such as enhanced

dx.doi.org/10.1021/jo2016696 | J. Org. Chem. 2012, 77, 200–207

Table 3. Effect of Mole Ratio of Substrates on the Yield of HEWL-Catalyzed Direct Aza-Diels–Alder Reaction[a]

entry	1a/2/3	1a (mmol)	2 (mmol)	3 (mmol)	yield[b] (%)	4a/5a
1	3:1:4	1.5	0.5	2.0	18	54:46
2	2:1:4	1.0	0.5	2.0	23	58:42
3	1:1:4	0.5	0.5	2.0	24	65:35
4	1:2:4	0.5	1.0	2.0	55	69:31
5	1:3:4	0.5	1.5	2.0	73	76:24
6	1:4:4	0.5	2.0	2.0	39	66:34
7	1:5:4	0.5	2.5	2.0	44	58:42
8	1:3:1	0.5	1.5	0.5	42	74:26
9	1:3:2	0.5	1.5	1.0	56	66:34
10	1:3:3	0.5	1.5	1.5	73	74:26
11	1:3:5	0.5	1.5	2.5	69	73:27
12	1:3:6	0.5	1.5	3.0	66	81:19
13	1:3:7	0.5	1.5	3.5	51	76:24

[a]Reaction conditions: HEWL (100 mg), MeCN (1 mL), deionized water (0.3 mL), and substrates with specified mole ratio at 25 °C for 96 h. [b]Yield of the isolated products (**4a** + **5a**).

stability, altered substrate specificity, and the ability to catalyze unusual reactions which was impossible in aqueous media,[8] we investigated the effect of different solvents on the model reaction (Table 4). The results revealed that solvent played an

Table 4. Effect of Solvents on the HEWL-Catalyzed Direct Aza-Diels–Alder Reaction[a]

entry	solvent	yield[b] (%)	4a/5a
1	MeCN	73	74:26
2	xylene	56	55:45
3	ethyl acetate	47	64:36
4	DMF	43	67:33
5	toluene	40	73:27
6	DMSO	15	75:25
7	hexane	13	54:46
8	water	9	0:100

[a]Reaction conditions: 4-chlorobenzaldehyde **1a** (0.5 mmol), 4-anisidine **2** (1.5 mmol), cyclohexenone **3** (1.5 mmol), and HEWL (100 mg) in organic solvent (1 mL) and deionized water (0.3 mL) at 25 °C for 96 h. [b]Yield of the isolated products (**4a** + **5a**).

important role in HEWL-catalyzed aza-Diels–Alder reaction. The reaction in MeCN gave the best yield of 73% after 96 h (Table 4, entry1) while the reactions in xylene, ethyl acetate, DMF, and toluene provided the product in yields 40–56% (Table 4, entries 2–5). The other tested solvents including DMSO, hexane, and water gave the low yields (Table 4, entries 6–8). Interestingly, the *endo* product **4a** was received as the major product in organic media (Table 4, entries 1–7); however, the *exo* product **5a** was obtained exclusively in water (Table 4, entry 8). Furthermore, no clear correlation between the solvent polarity and the enzyme activity was observed. This results may be attributed to the specific interactions between the solvent and HEWL. On the basis of the results of solvent screen, MeCN was chosen as the optimum solvent for the HEWL-catalyzed aza-Diels –Alder reaction.

Effect of Water Content. Water plays a major role as a "molecular lubricant" in enzymes, resulting in conformational flexibility, and the increased hydration leads to enhanced activity in nonaqueous solvents.[9] Thus, it was significant to confirm the optimal percentage of water in reaction systems. The results are summarized in Table 5. It was found that the catalytic activity of HEWL in the aza-Diels–Alder reaction

Table 5. Effect of Water Contents on the HEWL-Catalyzed Direct Aza-Diels–Alder Reaction[a]

entry	water content (%)	yield[b] (%)	4a/5a
1	0	53	92:8
2	10	77	85:15
3	20	71	73:27
4	30	66	68:12
5	40	56	57:43
6	50	48	55:45
7	60	34	55:45
8	70	23	47:53
9	80	16	44:56

[a]Reaction conditions: 4-chlorobenzaldehyde **1a** (0.5 mmol), 4-anisidine **2** (1.5 mmol), cyclohexenone **3** (1.5 mmol), HEWL (100 mg), and deionized water from 0% to 80% [water/(water + MeCN), v/v] at 25 °C for 96 h. [b]Yield of the isolated products (**4a** + **5a**).

could be evidently affected by the water content in MeCN. The reaction reached a high yield of 77% with 85:15 (*endo/exo*) at 10% water content [water/(water + MeCN), v/v] after 96 h (Table 5, entry 2). However, once the water content surpassed 10%, the yield dropped obviously (Table 5, entries 3–9). Notably, the water contents also had a clear effect on the stereoselectivity for the HEWL-catalyzed aza-Diels–Alder reaction. Increasing the water content favored the formation of *exo* product **5a**. On the contrary, decreasing the water content favored the formation of *endo* product **4a**. When the water content was 0, the best selectivity of 92:8 (*endo/exo*) was obtained with 53% yield (Table 5, entry 1). All of the results indicated that water is obviously essential in the HEWL-catalyzed direct aza-Diels–Alder reaction. In consideration of the yield of the reaction, we chose 10% water content for the aza-Diels–Alder reaction.

The Effect of Temperature. Temperature also plays an important role in enzyme-catalyzed reaction because of its effects on enzyme stability and reaction rate. Thus, a temperature screening was performed. As shown in Table 6,

Table 6. Effect of Temperature on the HEWL-Catalyzed Direct Aza-Diels–Alder Reaction[a]

entry	temp (°C)	yield[b] (%)	4a/5a
1	10	24	75:25
2	25	60	68:32
3	35	93	82:18
4	45	69	80:20
5	55	32	73:27

[a]Reaction conditions: 4-chlorobenzaldehyde **1a** (0.5 mmol), 4-anisidine **2** (1.5 mmol), cyclohexenone **3** (1.5 mmol), and HEWL (100 mg) in MeCN (0.9 mL) and deionized water (0.1 mL) at specified temperature for 53 h. [b]Yield of the isolated products (**4a** + **5a**).

the activity and selectivity of HEWL in the model reaction could be influenced significantly by the temperature and reached the best yield of 93% with the best selectivity of 82:18 (*endo/exo*) at 35 °C after 53 h (Table 6, entry 3). However, once the temperature surpassed 35 °C, the yield of the product decreased (Table 6, entries 4 and 5), probably due to the

dx.doi.org/10.1021/jo2016696 | J. Org. Chem. 2012, 77, 200–207

denaturation of HEWL caused by the high temperature. This phenomenon, on the other hand, confirmed once more that the catalytic behavior of HEWL was not simply caused by the amino acid distribution on the protein surface. The specific natural fold of HEWL was required for its ability to catalyze the aza-Diels–Alder reaction. Based on the temperature screening, we chose 35 °C as the optimum temperature for the reaction.

Effect of Enzyme Concentration. Next, we investigated the effect of enzyme concentration on the average rate of the model reaction at a relatively early stage (Figure 1). It was found that there was a near linear correlation between the reaction rate and HEWL concentration. The results add up to convincing evidence for HEWL-catalyzed aza-Diels–Alder reaction.

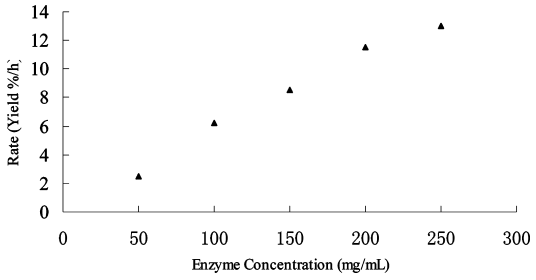


Figure 1. Conditions: For enzyme concentration of 50 or 100 mg/mL: 4-chlorobenzaldehyde **1a** (1 mmol), 4-anisidine **2** (3 mmol), cyclohexenone **3** (3 mmol), and HEWL (100 or 200 mg) in MeCN (1.8 mL) and deionized water (0.2 mL) at 35 °C for 2 h. For enzyme concentrations of 150, 200, or 250 mg/mL: 4-chlorobenzaldehyde **1a** (0.5 mmol), 4-anisidine **2** (1.5 mmol), cyclohexenone **3** (1.5 mmol), and HEWL (150, 200, or 250 mg) in MeCN (0.9 mL) and deionized water (0.1 mL) at 35 °C for 2 h. The reaction rate was equal to the reaction yield divided by reaction time. The yield refers to the isolated products (**4a** + **5a**).

Effect of HEWL Concentration on the Yield and Selectivity of the Model Reaction. Then, in order to find out a suitable enzyme concentration for the reaction, we investigated the effect of HEWL concentration on the yield of the model reaction between 0.5 mmol of 4-chlorobenzaldehyde, 1.5 mmol of 4-anisidine and 1.5 mmol of cyclohexenone in 1 mL of MeCN/H_2O at 35 °C for 53 h (Table 7). When the HEWL concentration of 50 mg/mL was used, the reaction only gave a moderate yield of 77% (Table 7, entry 1). Increasing the HEWL concentration to 100 mg/mL led to a good yield of 93% (Table 7, entry 2). However, higher HEWL concentration did not give better yields, and the selectivity of *endo*/*exo* ratios almost kept constant, which indicated that the reaction had reached the equilibrium at 53 h time point under the reaction conditions. In consideration of both the effective and economical aspects of the reaction, we chose 100 mg/mL of HEWL as the optimum enzyme concentration for the model reaction.

Table 7. Effect of HEWL Concentration on the Yield and Selectivity of the Model Reaction[a]

entry	HEWL conc (mg/mL)	yield[b] (%)	**4a**/**5a**
1	50	77	84:16
2	100	93	82:18
3	150	93	86:14
4	200	91	86:14
5	300	90	84:16

[a]Reaction conditions: 4-chlorobenzaldehyde **1a** (0.5 mmol), 4-anisidine **2** (1.5 mmol), cyclohexenone **3** (1.5 mmol), and specified amount of HEWL in MeCN (0.9 mL) and deionized water (0.1 mL) at 35 °C for 53 h. [b]Yield of the isolated products (**4a** + **5a**).

Time Course of the Reaction. Next, the time course of the HEWL-catalyzed direct aza-Diels–Alder reaction was investigated (Table 8). Interestingly, in the initial stages of the reaction, the *exo* isomer (**5a**) was obtained as the major product (Table 8, entry 1). However, as the reaction progressed, the ratio of *endo* to *exo* increased immediately, and the *endo* isomer (**4a**) became the major product (Table 8, entries 2–11). A possible explanation for the phenomena was that the *exo* isomer (**5a**) was the product of kinetic control while the *endo* isomer (**4a**) was the product of thermodynamic control. The best yield of 93% was obtained after 53 h (Table 8, entry 10), and prolonging the reaction time could not increase the yield. The best ratio of *endo* to *exo* was obtained in 85:15 (Table 8, entry 11).

Table 8. Time Course of the HEWL-Catalyzed Direct Aza-Diels–Alder Reaction[a]

entry	time (h)	yield[b] (%)	**4a**/**5a**
1	2	12	36:64
2	4	18	51:49
3	6	25	57:43
4	8	32	66:34
5	16	43	71:29
6	24	54	74:26
7	32	61	75:25
8	40	72	80:20
9	48	80	84:16
10	53	93	82:18
11	60	91	85:15

[a]Reaction conditions: 4-chlorobenzaldehyde **1a** (0.5 mmol), 4-anisidine **2** (1.5 mmol), cyclohexenone **3** (1.5 mmol), and HEWL (100 mg) in MeCN (0.9 mL) and deionized water (0.1 mL) at 35 °C for specified time. [b]Yield of the isolated products (**4a**+**5a**).

Effect of pH on the Enzyme Activity. It is widely accepted that pH could change enzyme catalytic activity significantly. Thus, in the present study, the effect of pH on the yield and selectivity of the reaction was investigated using phosphate buffer (pH from 3.08 to 8.98) to replace the optimized water content in the reaction system [buffer/(buffer + MeCN) = 10%, v/v], and the results are summarized in Table

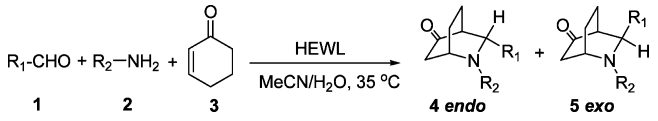
Table 9. Effect of pH on the HEWL-Catalyzed Direct Aza-Diels–Alder Reaction[a]

entry	pH	yield[b] (%)	**4a**/**5a**
1	3.08	91	70:30
2	4.16	91	77:23
3	5.10	94	81:19
4	5.90	90	83:17
5	6.92	88	80:20
6	7.91	77	72:28
7	8.98	65	65:35

[a]Reaction conditions: 4-chlorobenzaldehyde **1a** (0.5 mmol), 4-anisidine **2** (1.5 mmol), cyclohexenone **3** (1.5 mmol), and HEWL (100 mg) in MeCN (0.9 mL) and phosphate buffer solution (0.1 mL) at 35 °C for 53 h. [b]Yield of the isolated products (**4a**+**5a**).

dx.doi.org/10.1021/jo2016696 | J. Org. Chem. 2012, 77, 200–207

Table 10. Scope of the HEWL-Catalyzed Direct Aza-Diels–Alder Reactions[a]



entry	R_1	R_2	time (h)	yield (%)[b]	4/5
1	4-ClC_6H_4 (**1a**)	4-$MeOC_6H_4$	53	93	82:18
2	3-ClC_6H_4 (**1b**)	4-$MeOC_6H_4$	48	96	56:44
3	2-ClC_6H_4 (**1c**)	4-$MeOC_6H_4$	53	72	81:19
4	4-FC_6H_4 (**1d**)	4-$MeOC_6H_4$	48	98	90:10
5	3-FC_6H_4 (**1e**)	4-$MeOC_6H_4$	48	96	86:14
6	4-BrC_6H_4 (**1f**)	4-$MeOC_6H_4$	48	80	83:17
7	3- BrC_6H_4 (**1g**)	4-$MeOC_6H_4$	48	88	90:10
8	C_6H_5 (**1h**)	4-$MeOC_6H_4$	96	78	80:20
9	4-MeC_6H_4 (**1i**)	4-$MeOC_6H_4$	96	73	81:19
10	4-$MeOC_6H_4$ (**1j**)	4-$MeOC_6H_4$	96	69	76:24
11	4-FC_6H_4 (**1k**)	C_6H_5	96	97	80:20

[a]Reaction conditions: aldehyde **1** (0.5 mmol), aromatic amine **2** (1.5 mmol), cyclohexenone **3** (1.5 mmol), and HEWL (100 mg) in MeCN (0.9 mL) and deionized water (0.1 mL) at 35 °C. [b]Yield of the isolated products (**4** + **5**).

Table 11. Comparison Experiments of Direct Aza-Diels–Alder Reaction[a]

Entry	imine (mmol)	aldehyde (mmol)	amine (mmol)	enone (mmol)	catalyst	yield[b] (%)	4a/5a
1	0.5	0	0	1.5	HEWL	31	54:46
2	0	0.5	0.5	1.5	HEWL	27	64:36
3	0.5	0	1.0	1.5	HEWL	95	77:23
4	0	0.5	1.5	1.5	HEWL	93	82:18
5	0	0.5	1.5	1.5	Asp	39	55:45
6	0	0.5	1.5	1.5	Glu	21	60:40

[a]Reaction conditions: the substrates were 4-chlorobenzaldehyde **1a**, 4-anisidine **2**, cyclohexenone **3**, and imine prepared from 4-chlorobenzaldehyde **1a** and 4-anisidine **2**. For entries 1–4: HEWL (100 mg) in MeCN (0.9 mL), deionized water (0.1 mL), and substrates at 35 °C for 53 h. For entries 5 and 6: Asp or Glu (10 mol %), MeCN (1.0 mL), and substrates at 35 °C for 53 h. [b]Yield of the isolated products (**4a** + **5a**).

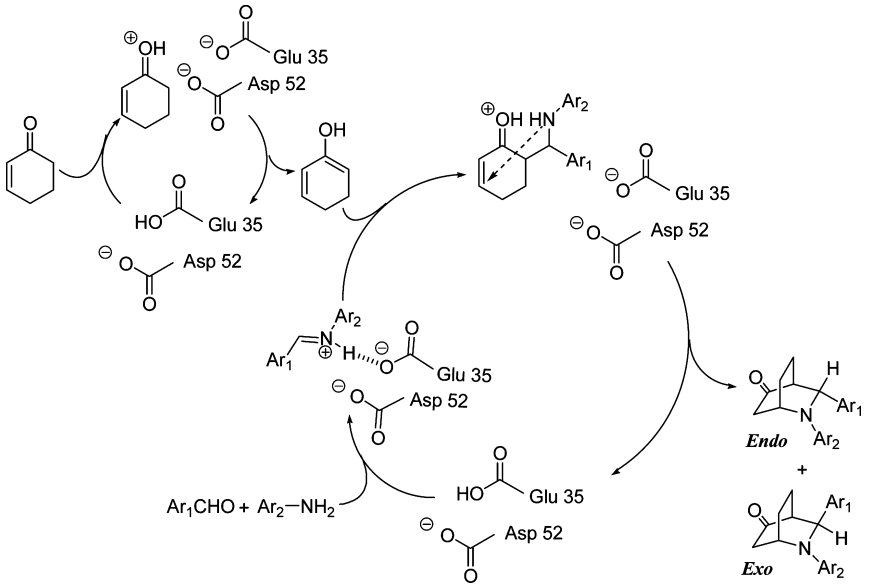
9. Generally, lower pH (3.08 to 6.92) favored the reaction giving the product in good yields (Table 9, entries 1–5). It could be due to the following reasons: On one hand, acidic condition favors the formation of imine and the enolization of cyclohexenone (in some reports on aza-Diels–Alder reaction, acid was used as a necessary additive[11]); on the other hand, HEWL is more stable under acidic to neutral conditions than under basic conditions. When the phosphate buffer solution with higher pH (7.91 or 8.98) was used, the yield dropped sharply (Table 9, entries 6 and 7). Moreover, it could be seen that pH also had effects on the selectivity of the reaction, and the best ratio of *endo* to *exo* was obtained in 83:17 using pH 5.90 buffer (Table 9, entry 4). Although different pH phosphate buffers could obviously affect the yield and selectivity of HEWL, the best results obtained in the presence of buffer were almost equal to the reaction without buffer. Therefore, we still chose MeCN/water as the reaction medium for the HEWL-catalyzed direct aza-Diels–Alder reaction.

Scope of the HEWL-Catalyzed Aza-Diels–Alder Reactions. With the optimized conditions in hand, some other aromatic aldehydes including electron-withdrawing, electron-donating, and neutral groups on the aromatic rings were used to expand upon the HEWL-catalyzed aza-Diels–Alder reaction to test the generality and scope of the new enzymatic promiscuity. As shown in Table 10, all reactions afforded the *endo* isomers as the major products. The yields of reaction greatly depended on the substituents of aryl ring of aldehyde. In general, aromatic aldehydes with electron-withdrawing substituents gave better yields than aromatic aldehydes with electron-donating substituents (Table 10, entries 4, 8, 9, and 10). On the contrary, substituents on aromatic amines did not affect the yields greatly. Moreover, the effect of steric hindrance of substituents on benzaldehydes had a great impact on the yield. 3-Chloro- and 4-chlorobenzaldehyde gave good yields of 96% and 93%, respectively (Table 10, entries 2 and 1); however, 2-chlorobenzaldehyde only gave a low yield of 72% (Table 10, entry 3) due to the steric hindrance of the substituent. Furthermore, the diasterselectivities appeared not to be greatly affected by the electronic nature of substituents. The *endo*/*exo* ratio ranged from 76:24 to 90:10 (Table 10, entries 1 and 3–11), except for 3-chlorobenzaldehyde, which gave an *endo*/*exo* ratio of 56:44 (Table 10, entry 2). Unfortunately, there was no obvious enantiomeric excess of the products observed by the chiral-phase HPLC analysis.

Proposed Catalytic Mechanism for HEWL-Catalyzed Direct Three-Component Aza-Diels–Alder Reaction. Generally, it is believed that the aza-Diels–Alder reaction proceeds via a Mannich–Michael process rather than a concerted manner, and the in situ generated imine participates in the mannich reaction as an electrophile.[10] In the present study, all of the reactions involved in situ formation of the imine from aldehyde and amine. In order to know whether the imine could be used directly in this enzymatic reaction, the experiments using imine in place of aldehyde and amine were performed (Table 11). The reaction of imine (0.5 mmol) and cyclohexenone (1.5 mmol) gave the yield of 31% with 54:46 (*endo*/*exo*) (Table 11, entry 1). As a comparison the direct aza-Diels–Alder reaction of 4-chlorobenzaldehyde (0.5 mmol), 4-

dx.doi.org/10.1021/jo2016696 | J. Org. Chem. 2012, 77, 200–207

Scheme 2. Hypothesized Mechanism of HEWL-Catalyzed Direct Three-Component Aza-Diels–Alder Reaction



anisidine (0.5 mmol), and cyclohexenone (1.5 mmol) was also conducted, which gave a yield of 27% with 64:36 (*endo*/*exo*) (Table 11, entry 2). It could be seen that the reaction using imine gave slightly higher yield but lower selectivity than the direct aza-Diels–Alder reaction. Moreover, to further verify this observation, the reactions with optimized molar ratio of substrates were carried out. The reaction of imine (0.5 mmol), 4-anisidine (1.0 mmol), and cyclohexenone (1.5 mmol) gave a yield of 95% with 77:23 (*endo*/*exo*) (Table 11, entry 3), while the reaction of 4-chlorobenzaldehyde (0.5 mmol), 4-anisidine (1.5 mmol), and cyclohexenone (1.5 mmol) gave 93% yield with 82:18 (*endo*/*exo*) (Table 11, entry 4). The results confirmed that imine could be used directly in this enzymatic reaction, but the three-component reaction with in situ formation of imine from aldehyde and amine gave better selectivity. Therefore, it could be deduced that the enzyme might be involved in the formation of imine.

HEWL's active site is composed of glutamate residue (Glu 35) and aspartate residue (Asp 52). We questioned whether the Glu or Asp could be used as catalyst for the direct three-component aza-Diels–Alder reaction. Thus, the reaction of 4-chlorobenzaldehyde (0.5 mmol), 4-anisidine (1.5 mmol), cyclohexenone (1.5 mmol), and Asp (10 mol %) was carried out, which only gave a yield of 39% with 55:45 (*endo*/*exo*) (Table 11, entry 5). When Glu was used in place of Asp, the reaction gave a lower yield of 21% with 60:40 (*endo*/*exo*) (Table 11, entry 6). In comparison with HEWL, which gave an excellent yield of 93% with 82:18 (*endo*/*exo*) (Table 11, entry 4), Glu and Asp could catalyze the direct aza-Diels–Alder reaction, but reactivity and selectivity were much lower.

Finally, based on Vocadlo and co-worker's confirmation of the catalytic mechanism for HEWL,[11] we hypothesized the mechanism of HEWL-catalyzed direct three-component aza-Diels–Alder reaction (Scheme 2). First, HEWL catalyzes the formation of the enol. In this step, Glu 35 protonates the carboxyl functional group of cyclohexenone while Asp 52 acts as a nucleophile to attack the acidic proton. Next, the enol cyclize with the in situ generated imine via a Mannich–Michael process to give the final products with the aid of HEWL.

CONCLUSION

We describe here the first enzyme-catalyzed aza-Diels–Alder reaction. The HEWL as a safe, economical, environmentally benign, and sustainable catalyst from inexpensive regenerable resources can catalyze a direct three-component aza-Diels–Alder reaction with a wide range of substrates resulting in moderate to good yields. The influence of reaction conditions including mole ratio of substrates, solvents, water content, temperature, enzyme concentration, pH, and reaction time was also investigated. This HEWL-catalyzed aza-Diels–Alder reaction provides a novel case of catalytic promiscuity which widens the applicability of HEWL in organic synthesis and might be a useful synthetic method for application.

EXPERIMENT SECTION

General Information for the Reagents. HEWL (hen egg white lysozyme, 20000 U/mg), bromelain from pineapple peduncle (500 U/mg), cellulase from *Aspergillus niger* (10 U/mg), and chymopapain from the latex of the unripe fruits of *Carica papaya* (20 U/mg) were purchased from Guangxi Nanning Pangbo Biological Engineering Co. Ltd. Lipozyme TLIM (immobilized lipase from *Thermomyces lanuginosus*, 0.25 U/mg) was purchased from Novozymes Biotechnology Co., Ltd. Lipase PS "Amano" SD from *Burkholderia cepacia* (≥23 U/mg) was a gift from Amano Enzyme, Inc. Neutral proteinase from *Bacillus subtilis* A.S.1.398 (130 U/mg) and alkaline proteinase from *Bacillus licheniformis* No 2709 (200 U/mg) were purchased from Wuxi Xuemei Enzyme Co. Ltd.

Typical Procedure for the HEWL-Catalyzed Aza-Diels–Alder Reaction. HEWL (100 mg) was added to a 25 mL round-bottom flask containing aldehyde (0.5 mmol), aromatic amine (1.5 mmol), cyclohexenone (1.5 mmol), MeCN (0.9 mL), and deionized water (0.1 mL). The resulting mixture was stirred at 35 °C for the specified reaction time and monitored by thin-layer chromatography (TLC). The reaction was terminated by filtering the enzyme. The filtrate was diluted with ethyl acetate (10 mL) and washed with water (5 mL × 2). The aqueous phase was back-extracted with ethyl acetate (10 mL × 2). Combined organic phase was washed with water and concentrated in vacuo. The crude product was purified by flash column chromatography (ethyl acetate/petroleum ether).

*3-endo-(4-Chlorophenyl)-2-(4-methoxyphenyl)-2-azabicyclo-[2.2.2]octan-5-one (**4a**) (Table 10, Entry 1).* ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.75 (m, 1 H), 2.06 (m, 1 H), 2.13 (m, 1 H), 2.23 (m, 1 H), 2.47 (m, 1H), 2.74 (m, 2 H), 3.70 (s, 3 H), 4.42 (s, 1 H), 4.55 (s,

dx.doi.org/10.1021/jo2016696 | J. Org. Chem. 2012, 77, 200–207

1 H), 6.59 (m, 2 H), 6.77 (m, 2 H), 7.25 −7.27 (m, 4 H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 22.2, 22.4, 48.1, 49.5, 52.1, 55.6, 65.5, 114.7 (2C), 114.7 (2C), 127.1, 129.1, 133.1, 140.7, 142.1, 152.5, 211.5.

*3-exo-(4-Chlorophenyl)-2-(4-methoxyphenyl)-2-azabicyclo-[2.2.2]octan-5-one (**5a**) (Table 10, Entry 1).* ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.67 (m, 2 H), 1.87 (m, 1 H), 2.23 (m, 1 H), 2.36 (m, 1 H), 2.62 (s, 1 H), 2.75 (m, 1 H), 3.71 (s, 3 H), 4.43 (s, 1 H), 4.67 (s, 1 H), 6.53 (m, 2 H), 6.75 (m, 2 H), 7.35 (m, 4 H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 16.2, 26.3, 41.9, 48.9, 50.8, 55.7, 62.1, 114.3 (2C), 114.9 (2C), 127.7, 129.0, 133.1, 139.0, 142.3, 152.2, 213.5.

*3-endo-(3-Chlorophenyl)-2-(4-methoxyphenyl)-2-azabicyclo-[2.2.2]octan-5-one (**4b**) (Table 10, entry 2).* ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.74 (m, 1 H), 2.03 (m, 1 H), 2.13 (m, 1 H), 2.26 (m, 1 H), 2.45 (m, 1 H), 2.76 (m, 2 H), 3.73 (s, 3 H), 4.43 (s, 1 H), 4.55 (s, 1 H), 6.60 (m, 2 H), 6.78 (m, 2 H), 7.20−7.29 (m, 4 H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 22.2, 22.6, 46.1, 49.4, 52.0, 55.6, 65.7, 114.6, 114.7 (2C), 114.9, 123.9, 125.9, 127.8, 130.2, 134.9, 142.2, 144.5, 152.3, 211.4.

*3-exo-(3-Chlorophenyl)-2-(4-methoxyphenyl)-2-azabicyclo-[2.2.2]octan-5-one (**5b**) (Table 10, Entry 2).* ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.66−1.71 (m, 2 H), 1.89 (m, 1 H), 2.25 (m, 1 H), 2.37 (m, 1 H), 2.64 (m, 1 H), 2.75 (m, 1 H), 3.71 (s, 3 H), 4.42 (s, 1 H), 4.67 (s, 1 H), 6.54 (m, 2 H), 6.75 (m, 2 H), 7.26 −7.43 (m, 4 H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 16.2, 26.3, 41.8, 48.9, 50.7, 62.3, 114.2, 114.4, 114.5, 114.6, 124.4, 126.4, 127.7, 130.1, 134.9, 142.3, 142.9, 152.2, 213.3.

*3-endo-(2-Chlorophenyl)-2-(4-methoxyphenyl)-2-azabicyclo-[2.2.2]octan-5-one (**4c**) (Table 10, entry 3).* ^{1}H NMR (300 MHz, $CDCl_3$):δ = 1.82 (m, 1 H), 2.01 (m, 1 H), 2.22 (m, 2 H), 2.48 (m, 1H), 2.81 (m, 2 H), 3.71 (s, 3 H), 4.47 (s, 1 H), 4.96 (s, 1 H), 6.56 (m, 2 H), 6.75 (m, 2 H), 7.17 (m, 2 H), 7.37 (m, 2 H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 22.2, 22.5, 45.9, 49.1, 50.2, 55.6, 62.9, 114.7 (2 C), 155.0, 155.1, 127.4, 127.5, 128.8, 130.1, 131.7, 138.8, 141.8, 152.5, 212.1.

*3-exo-(2-Chlorophenyl)-2-(4-methoxyphenyl)-2-aza-bicyclo-[2.2.2]octan-5-one (**5c**) (Table 10, Entry 3).* ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.72 (m, 2 H), 1.91 (m, 1 H), 2.33 (m, 1 H), 2.39 (m, 1 H), 2.78 (m, 1 H), 2.90 (m, 1 H), 3.70 (s, 3 H), 4.47 (s, 1 H), 5.06 (s, 1 H), 6.52 (m, 2 H), 6.75 (m, 2 H), 7.26 −7.67 (m, 4 H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 16.6, 26.2, 42.3, 46.9, 49.5, 55.6, 60.0, 114.5, 114.7, 114.9, 115.1, 127.1, 128.6, 128.8, 130.4, 132.4, 136.8, 142.1, 152.3, 213.2.

*3-endo-(4-Fluorophenyl)-2-(4-methoxyphenyl)-2-azabicyclo-[2.2.2]octan-5-one (**4d**) (Table 10, entry 4).* ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.75 (m, 1H), 2.03 (m, 1H), 2.14 (m, 1H), 2.26 (m, 1H), 2.44 (m, 1H), 2.71 (d, J=18.8 Hz, 1H), 3.73 (s,3H), 4.44 (s, 1 H), 4.58 (s, 1 H), 6.61 (m, 2H), 6.77 (m, 2H), 7.00 (m, 2 H), 7.26 (m, 2 H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 22.3, 22.5, 46.1, 49.5, 52.3, 55.6, 65.5, 114.3, 114.6, 114.7, 114.9, 115.6, 115.9, 127.3, 127.4, 137.9, 142.2, 152.2, 162.0 (d, J = 246.75 Hz), 211.9.

*3-exo-(4-Fluorophenyl)-2-(4-methoxyphenyl)-2-azabicyclo-[2.2.2]octan-5-one (**5d**) (Table 10, Entry 4).* ^{1}H NMR (300 MHz, $CDCl_3$):δ = 1.64 (m, 2 H), 1.86 (m, 1 H), 2.25 (m, 1 H), 2.35 (m, 1 H), 2.63 (m, 1 H), 2.73 (d, J = 18.8 Hz, 1 H), 3.71 (s, 3 H), 4.43 (s, 1 H), 4.69 (s, 1 H), 6.54 (m, 2H), 6.75 (m, 2H), 7.07 (m, 2 H), 7.40 (m, 2 H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 16.2, 26.3, 41.9, 48.9, 51.0, 55.6, 62.0, 114.3 (2C), 114.8 (2C), 115.5, 115.8, 127.7, 127.8, 136.0, 142.4, 152.1, 162.1 (d, J = 249 Hz), 213.7.

*3-endo-(3-Fluorophenyl)-2-(4-methoxyphenyl)-2-aza-bicyclo-[2.2.2]octan-5-one (**4e**) (Table 10, Entry 5).* ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.71 (m, 1H), 2.03 (m, 2H), 2.13 (m, 1H), 2.44 (dd, J=2.54, 1H), 2.74 (m, 2H), 3.73 (s, 3H), 4.44 (s, 1H), 4.58 (s, 1H), 6.61 (m, 2H), 6.78 (m, 2H), 7.02−7.31 (m, 4H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 22.2, 22.6, 48.1, 49.5, 52.1, 55.7, 65.6, 112.7, 112.9, 114.3, 114.6, 114.7, 114.8, 121.3, 130.5, 130.6, 142.2, 145.2, 145.1, 152.3, 161.7, 164.9, 211.6.

*3-exo-(3-Fluorophenyl)-2-(4-methoxyphenyl)-2-azabicyclo-[2.2.2]octan-5-one (**5e**) (Table 10, Entry 5).* ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.70 (m, 2H), 1.91 (m, 1H), 2.26 (m, 1H), 1.86 (m, 1H), 2.26 (m, 1H), 2.73 (m, 1H), 2.73 (m, 2H), 3.72 (s, 3H), 4.44 (s, 1H), 4.70 (s, 1H), 6.54 (m, 2H), 6.76 (m, 2H), 6.99 (m, 1H), 7.17 (m, 2H), 7.35 (m, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 16.3, 26.3, 41.8, 48.9, 50.8, 55.6, 62.3, 113.2, 113.5, 114.2, 114.4, 114.5, 114.9, 121.8, 121.8,130.3, 130.4, 142.4, 143.6, 143.7, 152.2, 161.9, 165.1, 213.2.

*3-endo-(4-Bromophenyl)-2-(4-methoxyphenyl)-2-aza-bicyclo-[2.2.2]octan-5-one (**4f**) (Table 10, Entry 6).* ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.75 (m, 1 H), 2.05 (m, 1 H), 2.12 (m, 1 H), 2.25 (m, 1 H), 2.44 (m, 1 H), 2.70 (m, 2 H), 3.72 (s, 3 H), 4.42 (s, 1 H), 4.53 (s, 1 H), 6.58 (m, 2 H), 6.76 (m, 2 H), 7.17 (m, 2 H), 7.42 (m, 2 H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 22.3, 22.5, 46.1, 49.5, 52.1, 55.6, 65.6, 114.7 (2C), 114.8 (2C), 121.3, 127.5, 127.5, 132.0, 132.1, 141.3, 142.0, 152.3, 211.7.

*3-exo-(4-Bromophenyl)-2-(4-methoxyphenyl)-2-azabicyclo-[2.2.2]octan-5-one (**5f**) (Table 10, Entry 6).* ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.69 (m, 2 H), 1.89 (m, 1 H), 2.23 (m, 1 H), 2.36 (d, J = 18.7 Hz, 1 H), 2.63 (m, 1 H), 2.75 (d, J = 18.8 Hz, 1 H), 3.71 (s, 3 H), 4.43 (s, 1 H), 4.66 (s, 1 H), 6.52 (m, 2 H), 6.74 (m, 2 H), 7.30 (m, 2 H), 7.50 (m, 2 H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 16.2, 26.3, 41.9, 48.9, 50.8, 55.6, 62.1, 114.3, 114.7, 114.8, 114.8, 121.2, 128.0, 128.2, 131.9, 132.0, 139.6, 142.3, 152.2, 213.5.

*3-endo-(3-Bromophenyl)-2-(4-methoxyphenyl)-2-azabicyclo-[2.2.2]octan-5-one (**4g**) (Table 10, Entry 7).* ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.75 (m, 1H), 2.03 (m, 1H), 2.13 (m, 1H), 2.26 (m, 1H), 2.45 (m, 1H), 2.72 (m, 2H), 4.43 (s, 1H), 4.55 (s, 1H), 6.59 (m, 2H), 6.77 (m, 2H), 7.18 (m, 2H), 7.35 (m, 1H), 7.48 (m, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 22.2, 22.6, 48.1, 49.4, 52.1, 55.6, 65.7, 114.7 (4C), 123.2, 124.4, 128.9, 130.5, 130.7, 142.1, 144.8, 152.3, 211.5.

*3-exo-(3-Bromophenyl)-2-(4-methoxyphenyl)-2-azabicyclo-[2.2.2]octan-5-one (**5g**) (Table 10, entry 7).* ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.66−1.69 (m, 2H), 1.90 (m, 1H), 2.22 (m, 1H), 2.36 (m, 1H), 2.65 (m, 1H), 2.73 (m, 1H), 3.72 (s, 3H), 4.43 (s, 1H), 4.67 (s, 1H), 6.53 (m, 2H), 6.75 (m, 2H), 7.23−7.59 (m, 4H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 16.3, 26.3, 41.8, 48.8, 50.7, 55.6, 62.3, 114.4 (2C), 114.9 (2C), 123.2, 124.9, 129.3, 130.4, 130.6, 142.3, 143.2, 152.2, 213.3.

*3-endo-Phenyl-2-(4-methoxyphenyl)-2-azabicyclo[2.2.2]octan-5-one (**4h**) (Table 10, entry 8).* ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.74 (m, 1H), 2.04 (m, 1H), 2.15 (m, 1H), 2.28 (m, 1H), 2.45 (m, 1H), 2.77 (m, 2H), 3.73 (s, 3H), 4.45 (s, 1H), 4.61 (s, 1H), 6.64 (m, 2H), 6.78 (m, 2H), 7.25−7.34 (m, 4H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 22.3, 22.7, 46.1, 49.3, 52.2, 55.6, 66.2, 114.4, 114.6, 114.7 (4C), 125.7, 127.5, 128.9, 142.2, 142.5, 152.0, 212.1.

*3-exo-Phenyl-2-(4-methoxyphenyl)-2-azabicyclo[2.2.2]octan-5-one (**5h**) (Table 10, Entry 8).* ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.65 (m, 1 H), 1.75 (m, 1 H), 1.90 (m, 1 H), 2.28 (m, 1 H), 2.42 (d, J = 18.8 Hz, 1 H), 2.66 (d, J = 2.7 Hz, 1 H), 2.76 (d, J = 18.8 Hz, 1 H), 3.70 (s, 3 H), 4.44 (s, 1 H), 4.70 (s, 1 H), 6.51 (m, 2 H), 6.74 (m, 2 H), 7.29−7.43 (m, 5 H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 16.3, 26.3, 41.9, 48.8, 51.0, 55.6, 62.6, 114.2, 114.6, 114.8, 115.0, 126.2, 127.4, 128.8, 140.4, 142.6, 151.9, 214.0.

*3-endo-(4-Methylphenyl)-2-(4-methoxyphenyl)-2-azabicyclo-[2.2.2]octan-5-one (**4i**) (Table 10, Entry 9).* ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.74 (m, 1 H), 2.00 (m, 1 H), 2.13 (m, 1 H), 2.27 (m, 1 H), 2.32 (s, 3H), 2.43 (m, 1 H), 2.73 (m, 2 H), 3.70 (s, 3 H), 4.44 (s, 1 H), 4.57 (s, 1 H), 6.62 (d, J = 9.1 Hz, 2 H), 6.78 (m, 2 H), 7.11- 7.21 (m, 4 H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 21.1, 22.6, 46.1, 49.2, 52.3, 55.6, 66.0, 114.3, 114.5, 114.7, 114.9, 125.5, 129.6, 129.8, 137.0, 139.2, 142.6, 151.9, 212.3.

*3-exo-(4-Methylphenyl)-2-(4-methoxyphenyl)-2-azabicyclo-[2.2.2]octan-5-one (**5i**) (Table 10, Entry 9).* ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.63 (m, 1 H), 1.76 (m, 1 H), 1.89 (m, 1 H), 2.27 (m, 1 H), 2.37 (s, 3 H), 2.41 (m, 1 H), 2.64 (m, 1 H), 2.73 (m, 1 H), 3.70 (s, 3 H), 4.44 (s, 1 H), 4.68 (s, 1 H), 6.54 (m, 2 H), 6.73 (m, 2 H), 7.18 (m, 2 H), 7.31 (m, 2 H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 16.1, 21.1, 26.3, 41.9, 48.8, 51.1, 62.4, 114.2, 114.4, 114.8, 114.9, 125.5, 126.1, 129.5, 137.0, 142.6, 151.9, 214.3.

*3-endo-(4-Methoxyphenyl)-2-(4-methoxyphenyl)-2-azabicyclo-[2.2.2]octan-5-one (**4j**) (Table 10, Entry 10).* ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.72 (m, 1 H), 2.01 (m, 1 H), 2.12 (m, 1 H), 2.26 (m, 1 H), 2.43 (m, 1 H), 2.72 (m, 2 H), 3.73 (s, 3 H), 3.77 (s, 3 H), 4.43 (s, 1 H), 4.55 (s, 1 H), 6.62 (m, 2 H), 6.76 (m, 2 H), 6.83 (m, 2 H), 7.20 (m, 2 H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 22.3, 22.5, 46.1, 49.3,

dx.doi.org/10.1021/jo2016696 | J. Org. Chem. 2012, 77, 200−207

52.4, 55.2, 55.6, 65.7, 114.2, 114.4, 114.5 (2C), 114.7 (2C), 126.7, 126.9, 134.2, 142.5, 152.0, 158.8, 212.3.

*3-exo-(4-Methoxyphenyl)-2-(4-methoxyphenyl)-2-azabicyclo-[2.2.2]octan-5-one (**5j**) (Table 10, Entry 10).* ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.62 (m, 1 H), 1.75 (m, 1 H), 1.88 (m, 1 H), 2.25 (m, 1 H), 2.34 (m, 1 H), 2.61 (m, 1 H), 2.72 (m, 1 H), 3.71 (s, 3 H), 3.82 (s, 3 H), 4.42 (s, 1 H), 4.66 (s, 1 H), 6.55 (m, 2 H), 6.73 (m, 2 H), 6.90 (m, 2 H), 7.32 (m, 2 H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 16.2, 26.3, 41.9, 48.8, 51.2, 55.3, 55.5, 62.1, 114.1 (2C), 114.2 (2C), 114.8 (2C), 127.3 (2C), 132.2, 142.7, 152.9, 158.9, 214.5.

*3-endo-(4-Fluorophenyl)-2-phenyl-2-aza-bicyclo[2.2.2]octan-5-one (**4k**) (Table 10, Entry 11).* ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.72−1.81 (m, 1H), 2.06−2.14 (m, 2H), 2.25−2.33 (m, 1H), 2.52 (m, 1H), 2.78 (m, 2H), 4.57 (s, 1H), 4.67 (s, 1H), 6.67−6.79 (m, 2H), 6.81 (m, 1H), 7.00−7.05 (m, 2H), 7.19−7.31 (m, 4H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 22.4, 22.7, 45.9, 48.7, 52.2, 65.2, 113.4, 115.7, 116.0, 118.0, 127.2 (2C), 129.3, 137.6, 147.9, 160.6, 163.8, 211.5.

■ ASSOCIATED CONTENT

Supporting Information

^{1}H and ^{13}C NMR spectra for all products in Table 10. This material is available free of charge via the Internet at http://pubs.acs.org.

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■ ACKNOWLEDGMENTS

Financial support from the Natural Science Foundation Project of CQ CSTC (2009BA5051) is gratefully acknowledged.

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