Tetrahedron 67 (2011) 2689–2692

Contents lists available at ScienceDirect

Tetrahedron

journal homepage: www.elsevier.com/locate/tet

Candida antarctica lipase B-catalyzed the unprecedented three-component Hantzsch-type reaction of aldehyde with acetamide and 1,3-dicarbonyl compounds in non-aqueous solvent

Jun-Liang Wang, Bo-Kai Liu, Cui Yin, Qi Wu, Xian-Fu Lin *

Department of Chemistry, Zhejiang University, Hangzhou 310027, People's Republic of China

ARTICLE INFO

Article history:
Received 1 September 2010
Received in revised form 13 January 2011
Accepted 18 January 2011
Available online 22 January 2011

Keywords:
CAL-B
Hantzsch-type reaction
Acetamide
1,4-dihydropyridines
Non-aqueous solvent
Enzyme-catalyzed

ABSTRACT

A direct approach to 1,4-dihydropyridines by lipase-catalyzed unprecedented three-component Hantzsch-type reaction of aldehyde with 1,3-dicarbonyl compounds and acetamide in non-aqueous solvent has been developed. Some control experiments have been performed to demonstrate the specific catalytic effect of CAL-B. Acetamide was utilized as a novel ammonia source in the Hantzsch-type reaction for the first time. An array of 1,4-dihydropyridines was successfully synthesized through this methodology.

1. Introduction

Enzyme catalysts are efficient biotransformation tools in both organic and bioorganic synthesis.[1] The seminal work by Klibanov in the early 1980s has initiated the non-aqueous enzymology.[2] Recently a growing number of enzymes have been found to be capable of catalyzing secondary reactions besides their primary function.[3] This kind of catalytic promiscuity largely enriches the application of biocatalysts in organic synthesis. For example, lipase and acylase can catalyze the formation of C–C, C–N, C–O, and C–S through Michael addition and Markovnikov addition,[4] racemase can catalyze PLP-dependent aldol additions, [5a] and arylmalonate decarboxylase can catalyze aldol additions.[5b] In spite of the great efforts dedicated to this field, the catalytic promiscuity of biocatalysts was mainly focused on two-component reactions by now.

Multicomponent reactions (MCRs), which can form complex bioactive molecules from simple starting materials have attracted increasing attention in recent years.[6] Efforts to explore the efficient catalysts have continued to increase in popularity. Amino acid derivatives have exhibited promising catalytic ability in multicomponent reactions. Lately, Wang et al. have reported a novel lipase-catalyzed direct three-component reactions.[7] Exploiting the promiscuous activity of biocatalyst to achieve multicomponent reactions becomes particularly fascinating and remains a great challenge.

1,4-dihydropyridines (1,4-DHPs), which possess important pharmacological activity and chemical activity in medicinal and organic chemistry, were usually obtained from the classic examples of the MCRs Hantzsch reaction.[8] In the development of Hantzsch reaction, 1,4-dihydropyridines were commonly prepared by using ammonium acetate and liquid ammonia as an ammonia source. More recently, Bridgwood et al. reported magnesium nitride could also be used as a new source of ammonia.[9] In this paper, we wish to report that under the catalysis of CAL-B, acetamide was firstly employed as a novel source of ammonia undergoing with aldehydes and 1,3-dicarbonyl compounds to furnish 1,4-DHPs in non-aqueous solvent.

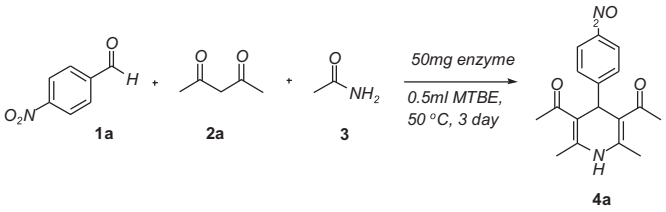
2. Results and discussion

At the outset, the reaction of 4-nitrobenzaldehyde **1a**, acetylacetone **2a**, and acetamide **3** was carried out using the mixture of acetylacetone/methyl *tert*-butyl ether (MTBE) as the reaction media. Several hydrolases were chosen as the catalysts and the results were summarized in Table 1. However, five candidates, such as alkaline protease from *Bacillus subtilis* (Subtilis), lipase from hog pancreas (HPL), acylase 'Amano' from *Aspergillus oryzae* (AA), immobilized penicillin G acylase from *Escherichia coli* (PGA) and

* Corresponding author. Fax: +86 571 87952618; e-mail address: llc123@zju.edu.cn (X.-F. Lin).

0040-4020/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2011.01.045

Table 1
Screening of different catalysts



1a 2a 3 4a

Entry	Catalyst	Yield[a](%)
1	Subtilis	<1
2	HPL	<1
3	AA	5
4	DA	<1
5	PGA	<1
6	CAL-B	25
7	CAL-B[b]	0
8	BSA	0
9	Blank	0

[a] Experimental conditions: 0.5 M 4-nitrobenzaldehyde **1a**, 0.5 ml acetylacetone **2a**, 0.5 M acetamide **3**, 0.5 ml methyl *tert*-butyl ether (MTBE), 50 mg enzyme, 50 °C, 3day. All yields were detected by HPLC.
[b] Enzyme predenatured with urea at 100 °C for 24 h.

D-aminoacylase from *E. coli* (DA) (entries 1–5, Table 1) showed no or low activity towards this reaction. *Candida antarctica* lipase B (CAL-B) seemed slightly superior to other hydrolases, and the corresponding product was obtained in 25% yield (entry 6, Table 1). When the reactants were incubated with denatured CAL-B or the bovine serum albumin (BSA) (entries 7 and 8, Table 1), no product was formed, ruling out the possibility that the polymeric support or the similar amino acid distribution on the protein surface promoted the process. The control experiments (entries 7–9, Table 1) suggested that the specific active sites and the tertiary structure of CAL-B were responsible for the three-component reaction.

In order to improve the activity of enzyme, reaction conditions including solvents, temperature, molar ratio, and enzyme concentration were investigated. Examination of the results from different solvents revealed that solvent played an important role in governing the activity of CAL-B. Among the solvents tested in Table 2, MTBE was found to be the most appropriate reaction medium (entry 4 vs entries 1–3 and 5–6, Table 2). Further screening on the ratio of the mixed solvent led us to find that the best ratio between MTBE and acetylacetone was 6:4 (entries 4 vs entries 9 and 10, Table 2). Next, the influence of reaction temperature on the enzymatic three-component reaction was also considered. As decreasing the reaction temperature from 50 °C to 25 °C, a noticeable decrease in the yield of the product was observed (entry 4 vs entries 7 and 8, Table 2). Then, the effect of the molar ratio between two substrates was also tested (entries 4 and 11–13, Table 2). The best result was obtained by using 4-nitrobenzaldehyde and acetamide in a molar ratio of 1:4. Finally, the enzyme concentration was optimized. As the data shown in Table 2, the yield of the product was improved greatly by increasing the enzyme concentration from 50 to 100 mg/ml and reached a plateau after 100 mg/ml (entries 14–16, Table 2).

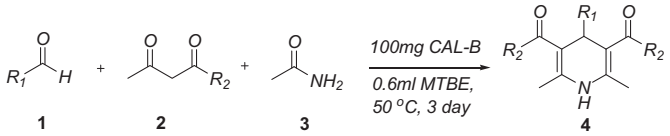
Table 2
Optimization for CAL-B-catalyzed three-component reaction[a]

Entry	Solvent	T/°C	Catalyst/mg	Yield(%)[b]
1	DMSO	50	50	0
2	Acetonitrile	50	50	4
3	THF	50	50	4
4	MTBE	50	50	25
5	Acetylacetone	50	50	8
6	*n*-Hexane	50	50	0
7	MTBE	37	50	5
8	MTBE	25	50	0
9	MTBE	50	50	28[c]
10	MTBE	50	50	27[d]
11	MTBE	50	50	55[c,e]
12	MTBE	50	50	69[c,f]
13	MTBE	50	50	60[c,g]
14	MTBE	50	75	85[c,f]
15	MTBE	50	100	91[c,f]
16	MTBE	50	125	92[c,f]

[a] Unless otherwise noted, the reaction was performed by employing 4-nitrobenzaldehyde **1a** (0.5 mmol), acetamide **3** (0.5 mmol), and CAL-B (50 mg) in 1 ml mixed solvent (acetylacetone/organic solvent=1:1 by vol) at 50 °C for 72 h.
[b] Determined by HPLC analysis.
[c] The volume ratio of MTBE and acetylacetone in 1 ml mixed solvent was 6:4.
[d] The volume ratio of MTBE and acetylacetone in 1 ml mixed solvent was 7:3.
[e] Compound **1a** (0.25 mmol) was used.
[f] Compound **1a** (0.125 mmol) was used.
[g] Compound **1a** (0.0625 mmol) was used.

On the basis of the previously optimized reaction conditions, the scope of this three-component reaction was evaluated. As seen from Table 3, 1,4-dihydropyridines were obtained as the major product in all cases. For aryl aldehydes, substitution with electron-withdrawing groups could enhance the reactivity of the substrate, compared with benzaldehyde (entries 1, 6, 7 and 8, Table 3). Conversely, electron-donating substituents, such as *p*-methyl and *p*-methoxyl substitutions caused a significant loss in the yield (entries 11–12, Table 3). On the other hand, the position of the nitro group on the aromatic ring of aryl aldehydes was tolerated with yield ranging from 85 to 90% (entries 1, 4 and 5, Table 3). Employing methyl acetoacetate and ethyl acetoacetate in place of acetylacetone, the reaction also proceeded smoothly (entries 2 and 3, Table 3). In addition, satisfactory results were also observed from heterocyclic aldehydes and aliphatic aldehydes (entries 13–15, Table 3).

Table 3
Substrate scope[a]



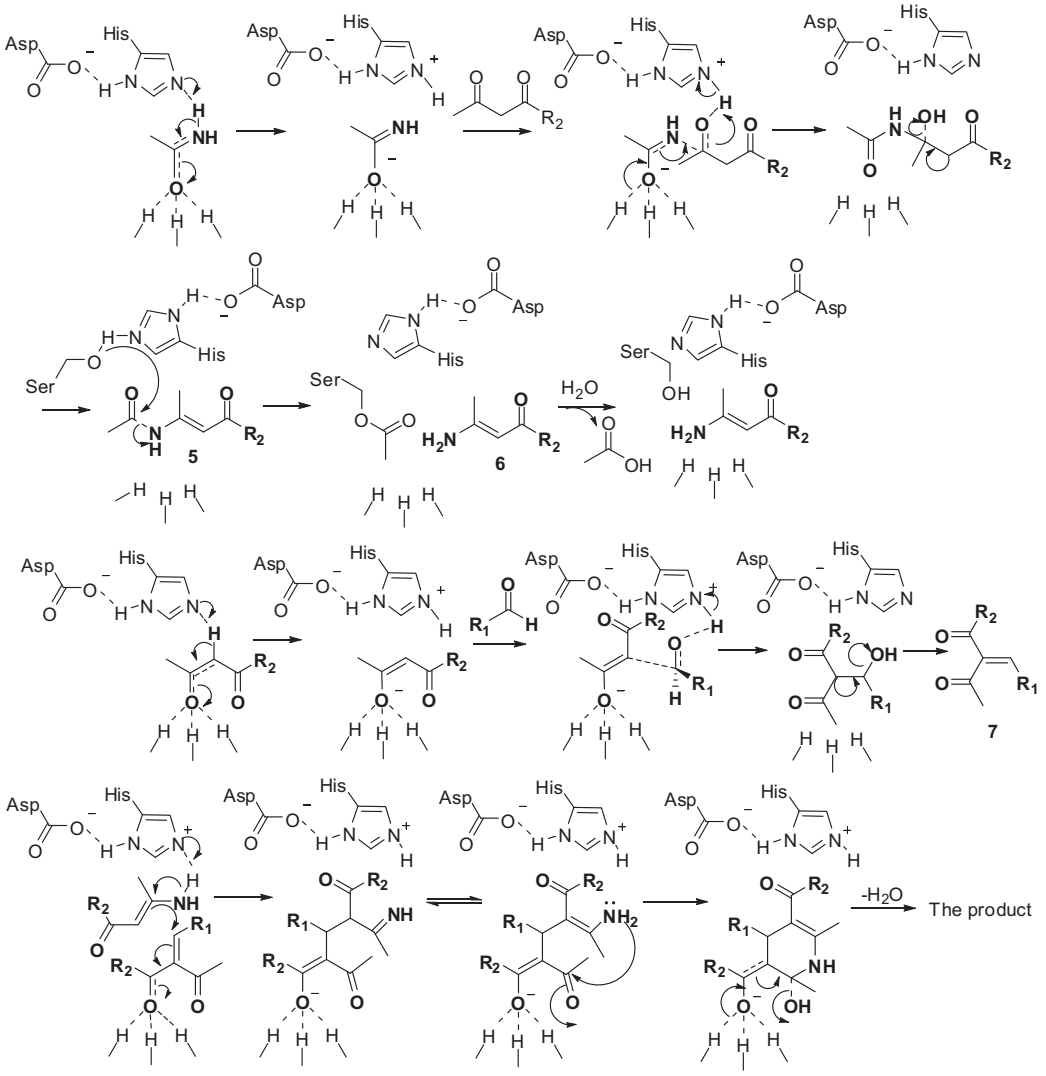
1 2 3 4

Entry	R_1	R_2	Product	Yield(%)[b]
1	*p*-$NO_2C_6H_4$	CH_3	**4a**	90
2	*p*-$NO_2C_6H_4$	OCH_3[c]	**4b**	82
3	*p*-$NO_2C_6H_4$	OC_2H_5[d]	**4c**	76
4	*o*-$NO_2C_6H_4$	CH_3	**4d**	85
5	*m*-$NO_2C_6H_4$	CH_3	**4e**	90
6	*p*-ClC_6H_4	CH_3	**4f**	82
7	*p*-FC_6H_4	CH_3	**4g**	86
8	*p*-$CF_3C_6H_4$	CH_3	**4h**	89
9	*p*-OHC_6H_4	CH_3	**4i**	39
10	C_6H_5	CH_3	**4j**	44
11	*p*-$CH_3C_6H_4$	CH_3	**4k**	22[e]
12	*p*-$OCH_3C_6H_4$	CH_3	**4l**	17[e]
13	3-Pyridyl	CH_3	**4m**	93
14	2-Furyl	CH_3	**4n**	92
15	$(CH_3)_2CH$	CH_3	**4o**	86

[a] All the reactions were performed with aldehyde 1 (0.125 mmol), acetamide (0.5 mmol), and 100 mg CAL-B in 1 ml mixed solvent (acetylacetone/organic solvent=0.4:0.6) at 50 °C for 72 h.
[b] Yield of isolated products except entries 11 and 12.
[c] Using methyl acetoacetate instead of acetylacetone as the solvent.
[d] Using ethyl acetoacetate instead of acetylacetone as the solvent.
[e] Determined by HPLC analysis.

In order to further understand the reaction mechanism, an additional control reaction to determine the role of CAL-B was carried out. We investigated the hydrolysis ability of CAL-B on acetamide in MTBE. The results showed that acetamide couldn't be hydrolyzed

by CAL-B under the specified conditions and no free ammonia generated in our reaction system. It is well accepted that the catalytic triad Asp-His-Ser in the active site of CAL-B can contribute to the promiscuous activity.[4b,10] Based on the common mechanistic pathway of the Hantzsch reaction, we proposed a tentative mechanism of lipase-catalyzed Hantzsch-type reaction of aldehyde with acetamide and 1,3-dicarbonyl compounds and showed them in Scheme 1. CAL-B was hypothesized to play different roles during the catalytic process. Firstly, the Asp-His dyad and the oxyanion hole in the active site stabilized the substrate acetamide. 1,3-dicarbonyl compounds reacted with activated acetamide through a condensation reaction to afford the intermediate **5**. Then, the intermediate **5** was hydrolyzed by the nature activity of CAL-B and the enamine intermediate **6** was formed. Meanwhile, a CAL-B-catalyzed Knoevenagel condensation reaction of 1,3-dicarbonyl compound with the aldehyde generated another intermediate α,β-unsaturated carbonyl compound **7**. Subsequently, the intermediate **7** was stabilized by the Asp-His dyad and the oxyanion hole and underwent Michael addition and an intramolecular condensation with the intermediate **6** to give the final product 1,4-dihydropyridine **4**.



Scheme 1. Proposed mechanism of lipase-catalyzed Hantzsch-type reaction of aldehyde with acetamide and 1,3-dicarbonyl compounds.

3. Conclusion

In conclusion, a novel three-component reaction yielding 1,4-dihydropyridines in a one-pot procedure catalyzed by CAL-B in non-aqueous solvent has been developed. This approach allowed acetamide as an ammonia source for Hantzsch-type reaction. The catalytic promiscuity of CAL-B was demonstrated by the combination of different control experiments. The influence of reaction conditions on the three-component reaction, including solvents, temperature, and molar ratio of substrate was systematically investigated. A series of 1,4-dihydropyridines were obtained in moderate to excellent yields. Further study on the synthetic applications as well as scope and limitations of the present reaction are currently being conducted in our laboratory.

4. Experimental

4.1. Materials

Alkaline protease from *B. subtilis* (10 U/mg, 1 U corresponds to the amount of enzyme, which liberates 1 μmol folin-positive amino

acids and peptides per minute at pH 7.5 and 37 °C) was obtained from Wuxi Enzyme Co. Ltd. (Wuxi, PR China). Lipase from hog pancreas (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). Immobilized penicillin G acylase from *E. coli* (EC 3.5.1.11, immobilized on acrylic beads) was purchased from Hunan Flag Biotech Co. Lipase immobilized on acrylic resin from *C. antarctica* (≥10 000 U/g, recombinant, expressed in *A. oryzae*) was purchased from Sigma (Steinheim, Germany). D-aminoacylase from *E. coli* (10 000 U/mg, 1 U is defined as enzyme quantity, which produces 1 μmol of D-Amino acid per 30 min) and Acylase 'Amano' (AA) from *A. oryzae* (≥30 000 U/g, 1 U is defined as enzyme quantity, which produces 1 μmol of L-Amino acid per 30 min) were purchased from Amano Enzyme Inc (Japan). All solvents were analytical grade and were dried by storing over activated 3 Å molecular sieves for 24 h prior to use. All other reagents were used as received.

4.2. Analytical methods

The ^{1}H and ^{13}C NMR spectra were recorded with TMS as internal standard using a Bruker AMX-400 MHz spectrometer. Chemical shifts were expressed in parts per million and coupling constants (*J*) in hertz. Analytical HPLC was performed using an Agilent 1100 series with a reversed-phase Shim-Pack VP-ODS column (150×4.6 mm) and a UV detector (210 nm). IR spectra were measured with a Nicolet Nexus FTIR 670 spectrophotometer. Melting points were determined using XT-4 apparatus and were not corrected. All the known products were characterized by comparing the ^{1}H NMR data with those reported in the literature. The structures of new compounds were confirmed by ^{1}H, ^{13}C NMR, and HRMS.

4.3. General procedure for the synthesis of 1,4-dihydropyridines derivatives

Aldehyde (0.125 mmol), acetamide (0.5 mmol), and CAL-B (100 mg) in the mixed solvent (1 ml, 1,3-dicarbonyl compounds/methyl *tert*-butyl ether=4:6, by vol) at 50 °C for 72 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 petroleum ether/ethyl acetate (1/1 v/v). Product-contained fractions were combined, concentrated, and dried to give the product 1,4-dihydropyridine.

Acknowledgements

The financial support from the National Natural Science Foundation of China (No. 20704037, 20872130, 20874086) and the Zhejiang Provincial Natural Science Foundation (Project no. 2009-Y4080198, 2010-Z4090225) is gratefully acknowledged.

Supplementary data

Supplementary data related to this article can be found online at doi:10.1016/j.tet.2011.01.045. These data include MOL files and InChiKeys of the most important compounds described in this article.

References and notes

1. (a) Hult, K.; Berglund, P. *Curr. Opin. Biotechnol.* **2003**, *14*, 395; (b) Panke, S.; Held, M.; Wubbolts, M. *Curr. Opin. Biotechnol.* **2004**, *15*, 272; (c) Kopp, F.; Marahiel, M. A. *Curr. Opin. Biotechnol.* **2007**, *18*, 513; (d) Woodley, J. M. *Trends Biotechnol.* **2008**, *26*, 321.
2. (a) Zaks, A.; Klibanov, A. M. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 3192; (b) Klibanov, A. M. *CHEMTECH* **1986**, *16*, 354.
3. (a) O'Brien, P. J.; Herschlag, D. *Chem. Biol.* **1999**, *6*, 91; (b) Copley, S. D. *Curr. Opin. Chem. Biol.* **2003**, *7*, 265; (c) Bornscheuer, U. T.; Kazlauskas, R. J. *Angew. Chem., Int. Ed.* **2004**, *43*, 6032; (d) Khersonsky, O.; Roodveldt, C.; Tawfik, D. S. *Curr. Opin. Chem. Biol.* **2006**, *10*, 498; (e) Hult, K.; Berglund, P. *Trends Biotechnol.* **2007**, *25*, 231; (f) Taglieber, A.; Hobenreich, H.; Carballeira, J. D.; Mondiere, RJ. G.; Reetz, M. T. *Angew. Chem., Int. Ed.* **2007**, *46*, 8597.
4. (a) Kitazume, T.; Ikeya, T.; Murata, K. *J. Chem. Soc., Chem. Commun.* **1986**, 1331; (b) Torre, O.; Alfonso, I.; Gotor, V. *Chem. Commun.* **2004**, 1724; (c) Svedendahl, M.; Hult, K.; Berglund, P. *J. Am. Chem. Soc.* **2005**, *127*, 17988; (d) Carlqvist, P.; Svedendahl, M.; Branneby, C.; Hult, K.; Brinck, T.; Berglund, P. *ChemBioChem* **2005**, *6*, 331; (e) Wu, W. B.; Wang, N.; Xu, J. M.; Wu, Q.; Lin, X. F. *Chem. Commun.* **2005**, *18*, 2348; (f) Wu, W. B.; Xu, J. M.; Wu, Q.; Lv, D. S.; Lin, X. F. *Adv. Synth. Catal.* **2006**, *348*, 487; (g) Xu, J. M.; Zhang, F.; Liu, B. K.; Wu, Q.; Lin, X. F. *Chem. Commun.* **2007**, *20*, 2078; (h) Lou, F. W.; Liu, B. K.; Wu, Q.; Lv, D. S.; Lin, X. F. *Adv. Synth. Catal.* **2008**, *350*, 1959.
5. (a) Seebeck, F. P.; Hilvert, D. *J. Am. Chem. Soc.* **2003**, *125*, 10158; (b) Terao, Y.; Miyamoto, K.; Ohta, H. *Chem. Lett.* **2007**, *36*, 420.
6. For recent reviews, see: (a) Dömling, A.; Ugi, I. *Angew. Chem., Int. Ed.* **2000**, *39*, 3168; (b) Zhu, J. *Eur. J. Org. Chem.* **2003**, 1133; (c) Ulaczyk-Lesanko, A.; Hall, D. G. *Curr. Opin. Chem. Biol.* **2005**, *9*, 266; (d) Dömling, A. *Chem. Rev.* **2006**, *106*, 17; (e) Tejedor, D.; García-Tellado, F. *Chem. Soc. Rev.* **2007**, *36*, 484; (f) D'Souza, D. M.; Müller, T. J. J. *Chem. Soc. Rev.* **2007**, *36*, 1095; (g) Isambert, N.; Lavilla, R. *Chem.—Eur. J.* **2008**, *14*, 8444; (h) Hulme, C.; Dietrich, J. *Mol. Diversity* **2009**, *13*, 195; (i) Ganem, B. *Acc. Chem. Res.* **2009**, *42*, 463.
7. Li, K.; He, T.; Li, C.; Feng, X. W.; Wang, N.; Yu, X. Q. *Green Chem.* **2009**, *11*, 777.
8. For recent reviews on Hantszch 1,4-DHP synthesis, see: (a) Langer, P. *Chem.—Eur. J.* **2001**, *7*, 3858; (b) Langer, P. *Synthesis* **2002**, 441; (c) Simon, C.; Constantieux, T.; Rodriguez, J. *Eur. J. Org. Chem.* **2004**, 4957.
9. Bridgwood, K. L.; Veitch, G. E.; Ley, S. V. *Org. Lett.* **2008**, *10*, 3627.
10. Branneby, C.; Carlqvist, P.; Magnusson, A.; Hult, K.; Brinck, T.; Berglund, P. *J. Am. Chem. Soc.* **2003**, *125*, 874.