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Separation of enantiomers by lipase-catalyzed fluorous-phase delabeling

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Dedicated to Professor Dr Hans Schick on the occasion of his 65th birthday

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Abstract—Simultaneous enantiomer-selective fluorous-phase delabeling and kinetic resolution was achieved by lipase-catalyzed alcoholysis of highly fluorinated esters of racemic alcohols. The separation of the fast-reacting delabeled enantiomers and the slow-reacting fluorous-phase labeled enantiomers was performed very efficiently by partition in an organic/fluorous biphasic solvent system.

1. Introduction

Perfluorinated and non-fluorinated organic solvents are very often immiscible at ambient temperature. Based on this fact, in the search for chemical transformations integrating innovative workup procedures fluorous techniques[1,2] are becoming increasingly popular in organic synthesis for the separation of temporarily fluorous labeled from non-labeled compounds. If one or more of the components of a reaction mixture, e.g. homogeneous catalysts,[3–5] reagents or products[6–10] are equipped with a perfluorinated auxiliary, the organic and the fluorous components can be separated easily by either liquid–liquid extraction, solid–liquid extraction or fluorous chromatography.

Due to the increasing demand of enantiomerically pure intermediates enzyme-catalyzed kinetic resolutions particularly lipase-catalyzed acylation, hydrolysis or alcoholysis represent an easy access to enantiomerically pure or enriched alcohols and their carboxylic esters.[11–13] Lipases are relatively inexpensive, highly selective, very robust and easy to handle biocatalysts; many of them are commercially available. Lipase-mediated acylation of a racemic alcohol and alcoholysis or hydrolysis of its corresponding ester is enantioconvergent. It means that always one and the same enantiomer reacts faster. Selection of the conditions affords the certain enantiomer either as the ester or the alcohol. However, the degree of selectivity may be different.

The enantiomer obtained as an alcohol and the other obtained as an ester in lipase-catalyzed resolutions are usually separated from each other by chromatography. This chromatographic step might be regarded as a major drawback for using this type of reactions on large scale in the pharmaceutical industry or in high-throughput kinetic resolutions.

It was our aim to combine fluorous-phase separation with lipase-catalyzed kinetic resolution in order to avoid chromatography. Recently, we were able to demonstrate that it is possible to use a highly fluorinated acyl donor for the kinetic resolution of racemic alcohols in the presence of a lipase.[14,15] We found that the fluorinated ester **1**[16] is a very useful acylating agent that is recognized by a lipase as its substrate and most importantly, the lipase transfers the fluorinated acyl residue with high enantioselectivity onto the fast-reacting enantiomer of a racemic alcohol affording a mixture of a highly fluorinated ester (fast-reacting enantiomer) and an alcohol (slow-reacting enantiomer) (Scheme 1). Indeed, the enantiomer tagged with the fluorinated residue could be separated from the untagged one by a simple liquid–liquid partition between a fluorous and an organic phase with high efficacy demonstrating that the labeled fluorous enantiomer was recognized selectively from the perfluorinated solvent, whereas the unlabeled organic enantiomer remains in the organic solvent. Using this technique the enantiomeric products could be separated without chromatography.[14,15]

In order to prevent side reactions, it is important that the acyl donor requires a spacer consisting of at least two methylene groups between the carbonyl group and the perfluorinated residue.

After having demonstrated that lipase-catalyzed acylation under kinetic resolution simultaneously labels the faster reacting enantiomer with a fluorous tag very efficiently,

Keywords: fluorous phase; kinetic resolution; lipase; perfluorinated solvents.

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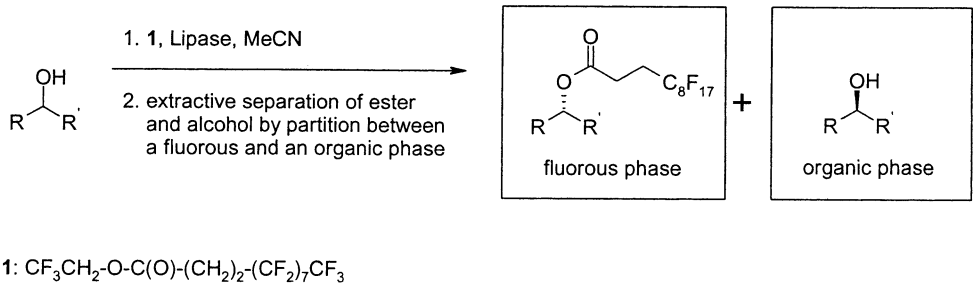


Scheme 1. Lipase-catalyzed enantiomer-selective tagging with a fluorous acyl residue.

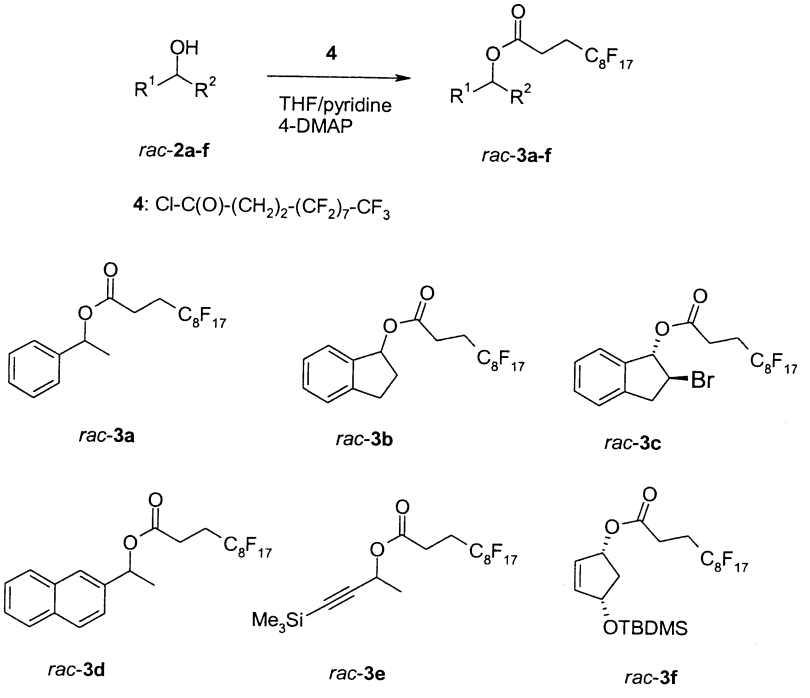
our next aim was to investigate the possibility of an enantiomer-selective detagging procedure by subjecting highly fluorinated esters of racemic alcohols to a lipase-catalyzed alcoholysis under deacylation of the fast-reacting enantiomer and leaving the slow-reacting enantiomer attached to the fluorous tag.

2. Results and discussion

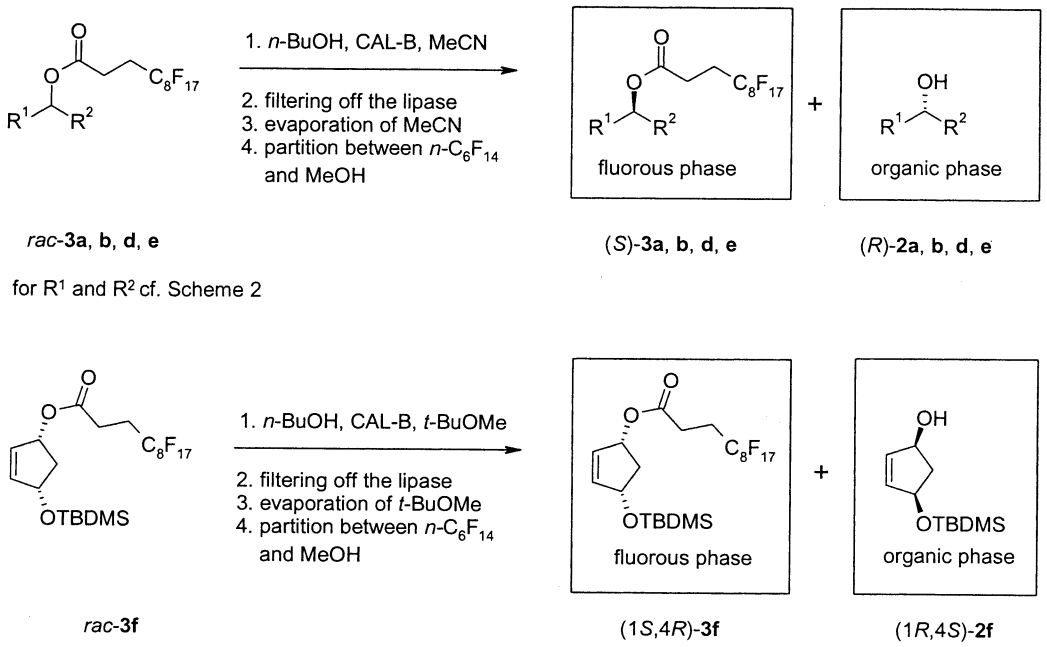
The racemic fluorinated esters *rac*-**3a**–**f** were prepared by acylation of the corresponding alcohols *rac*-**2a**–**f** with the acid chloride **4**[16] (Scheme 2) which already has been used for the synthesis of the fluorous acyl donor **1** depicted in Scheme 1.[14,15]

The racemic esters *rac*-**3a**–**e** were subjected to a lipase-catalyzed alcoholysis in acetonitrile or *tert*-butyl methyl ether for *rac*-**3f** in the presence of *Candida antarctica* B lipase (CAL-B) and four equivalents of *n*-butanol yielding except for the bromoindanol derivative *rac*-**3c** the fluorinated esters (*S*)-**3a**, **b**, **d**, **e** or (1*S*,4*R*)-**3f** and the non-fluorinated alcohols (*R*)-**2a**, **b**, **d**, **e** or (1*R*,4*S*)-**2f**, respectively (Scheme 3). For *rac*-**3f** due to the higher rate of conversion *tert*-butyl methyl ether was used instead of acetonitrile as the solvent. After filtering off the lipase and evaporation of the solvent under reduced pressure the ester and the alcohol were separated by partition between perfluoro-*n*-hexane and methanol. This biphasic fluorous/organic solvent system was found to be very useful in the separation of the highly fluorinated esters from non-fluorinated alcohols.[14,15]

The results of the kinetic resolutions are summarized in Table 1. The outcomes from these reactions confirm that apart from *rac*-**3c** the other fluorinated esters were accepted as substrates for CAL-B and resolved with excellent enantiomer-selectivity. For all conversions, the *E*-value[17] of the reaction was >200. The extractive separation by partition between methanol and perfluoro-*n*-hexane as expected works very well, because for the resolution of *rac*-**2a**, **e**, **f** by lipase-catalyzed acylation the separation of the products in the biphasic system was already achieved.[14,15] In a control experiment (*S*)-**3d** and (*R*)-**2d** were separated conventionally by flash chromatography showing almost identical results compared with the



Scheme 2. Synthesis of the racemic esters.



Scheme 3. Lipase-catalyzed enantiomer-selective detagging.

extractive separation. Remarkably, the alcoholysis of *rac*-**3f** shows a much higher selectivity than the acylation of *rac*-**2f** where the *E*-value was only 7.6 as demonstrated recently.[15]

The reason for the resistance of *rac*-**3c** towards alcoholysis in the presence of CAL-B is very likely the sterical hindrance at the reacting stereogenic center preventing the formation of the necessary transition state leading to the formation of the acyl–enzyme intermediate which finally must be attacked by *n*-butanol. Changing of the standard conditions (acetonitrile, room temperature) by using other solvents such as chloroform, toluene, diisopropyl ether and *tert*-butyl methyl ether at room temperature or in some cases at 50°C did not convert *rac*-**3c**.

The fluorous phases contain besides the esters (*S*)-**3a**, **b**, **d**, **e** or (1*S*,4*R*)-**3f** butyl 2*H*,2*H*,3*H*,3*H*-perfluoroundecanoate which was removed in the cases of (*S*)-**3a**, **b**, **d** or (1*S*,4*R*)-**3f** by saponification with lithium hydroxide and subsequent partition of the reaction mixture in an organic/aqueous biphasic system affording the alcohols (*S*)-**2a**, **b**, **d** or (1*S*,4*R*)-**2f** in the organic and lithium 2*H*,2*H*,3*H*,3*H*-perfluoroundecanoate in the aqueous phase, respectively. Due to instability of silylalkynes under alkaline conditions cleavage of the ester (*S*)-**3e** was performed by acid-catalyzed methanolysis and subsequent partition between methanol containing (*S*)-**2e** and perfluoro-*n*-hexane containing the fluorinated species.

The fluorine content of the slow-reacting enantiomers in all cases was sufficiently high for an efficient extractive separation from the non-fluorinated enantiomer as already demonstrated previously.[15]

3. Conclusion

We were able to demonstrate that highly fluorinated carboxylic esters of racemic alcohols are substrates for lipase from *C. antarctica* B. Enantiomer-selective alcoholysis of this type of esters selectively cleaves the fluorous label from the fast-reacting enantiomer affording a mixture of an alcohol and the corresponding enantiomeric fluorinated ester. The products can be separated very

Table 1. Lipase-catalyzed kinetic resolution of the esters *rac*-**3a**–**f** by alcoholysis with *n*-butanol

Substrate	Time (d)	Ester			Alcohol			*E*-value	Conversion (%)
		Configuration	% ee	% Yield[a]	Configuration	% ee	% Yield		
rac-**3a**	0.8	*S*[b]	97	43	*R*[b]	99	48	>200	49
rac-**3b**	8	*S*[b]	90	41	*R*[b]	99	44	>200	48
rac-**3c**	No conversion								
rac-**3d**	7	*S*[b]	94 (96[c])	43	*R*[b]	99 (99[c])	44	>200	49
rac-**3e**	8	*S*[d]	68	25[e]	*R*[d]	99	17[e]	>200	41
rac-**3f**	4	1*S*,4*R*[d]	74	42	1*R*,4*S*[d]	99	41	>200	43

[a] Determined after saponification or transesterification (cf. Section 4).
[b] Assigned by comparison with one of the commercially available enantiomers.
[c] The numbers in brackets correspond to the ee values determined after separation by flash chromatography.
[d] Assigned on the basis of the known $[\alpha]_D$-values of the free alcohols, cf. Ref. 18 for **2e** and Ref. 19 for **2f**.
[e] The yields are low due to the high volatility of the alkynol **2**.

efficiently by partition in the biphasic solvent system methanol/perfluoro-*n*-hexane avoiding chromatography. These results represent another example for the successful combination of fluorous techniques with lipase-catalyzed kinetic resolutions where the final separation procedure is already integrated in the initial chemical transformation.

4. Experimental

Starting materials, reagents and solvents were of commercial grade. *C. antarctica* B lipase (Chirazyme L-2, c.-f., lyo.) was purchased from Roche Diagnostics, Mannheim. The acid chloride **4** was prepared according to Refs. 14,15. The alcohols *rac*-**2e** and *rac*-**2f** were prepared according to Refs. 18,19, respectively. The ^{1}H NMR spectra were recorded on a Varian Gemini 300 at 300 MHz. HPLC was carried out on a Merck-Hitachi system consisting of L-6200A pump, L-4000 UV detector or Differential Refractometer RI-71, and Chromato-Integrator D-2500.

4.1. General procedure for the synthesis of the racemic esters *rac*-3a–f

To an ice-cold solution of the acid chloride **4** (2.55 g, 5.0 mmol) and the alcohols *rac*-**2a**–**f** (5.25 mmol) in anhydrous THF (5 mL) containing a catalytic amount of 4-DMAP (20 mg) was added drop-wise anhydrous pyridine (415 mg, 5.25 mmol) over 5 min. The cooling bath was removed and the reaction mixture was allowed to reach room temperature. After stirring for 2 h at room temperature, the precipitate was filtered, the filtrate was concentrated under reduced pressure and the residue was partitioned between *tert*-butyl methyl ether (10 mL) and 2N HCl (2.5 mL). The organic layer was washed with water (2.5 mL), dried with Na_2SO_4 and concentrated. Flash chromatography (cyclohexane/ethyl acetate=10:1) of the residue on silica gel yielded the pure esters *rac*-**3a**–**f**.

4.1.1. *rac*-3a. Yield 78%; pale yellow oil; ^{1}H NMR ($CDCl_3$): 1.56 (d, J=6.6 Hz, 3H); 2.47 (m, 2H); 2.65 (m, 2H); 5.96 (q, J=6.6 Hz, 1H); 7.26–7.39 (m, 5H).

4.1.2. *rac*-3b. Yield 78%; white wax; mp 31–32°C; ^{1}H NMR ($CDCl_3$): 2.10 (m, 1H); 2.37–2.68 (m, 5H); 2.90 (m, 1H); 3.13 (m, 1H); 6.25 (dd, J=6.9 and 3.6 Hz, 1H); 7.20–7.45 (m, 4H).

4.1.3. *rac*-3c. Yield 78%; white powder; mp 63–65°C; ^{1}H NMR ($CDCl_3$): 2.40–2.70 (m, 4H); 3.29 (dd, J=16.6 and 3.9 Hz, 1H); 3.72 (dd, J=16.6 and 6.6 Hz, 1H); 4.50 (m, 1H); 6.37 (d, J=3.6 Hz, 1H); 7.25–7.42 (m, 4H).

4.1.4. *rac*-3d. Yield 82%; white powder; mp 66–67°C; ^{1}H NMR ($CDCl_3$): 1.65 (d, J=6.6 Hz, 3H); 2.50 (m, 2H); 2.68 (m, 2H); 6.10 (q, J=6.6 Hz, 1H); 7.40–7.92 (m, 7H).

4.1.5. *rac*-3e. Yield 80%; pale yellow wax; mp 26–27°C; ^{1}H NMR ($CDCl_3$): 0.17 (s, 9H); 1.48 (d, J=6.6 Hz, 3H); 2.50 (m, 2H); 2.68 (m, 2H); 5.53 (q, J=6.6 Hz, 1H).

4.1.6. *rac*-3f. Yield 72%; pale yellow oil; ^{1}H NMR ($CDCl_3$): 0.08 and 0.09 (2 s, 6H); 0.89 (s, 9H); 1.60 (m, 1H); 2.38–2.65 (m, 4H,); 2.75 (m, ^{1}H); 4.72 (m, 1H); 5.48 (m, 1H); 5.98 (m, 1H); 6.02 (m, 1H).

4.2. General procedure for the kinetic resolution

Solutions of the esters *rac*-**3a**–**e** (5 mmol) in acetonitrile (120 mL) or *tert*-butyl methyl ether for *rac*-**3f** (120 mL) were treated with *n*-butanol (1.48 g, 1.83 mL, 20 mmol) and CAL-B (Chirazyme L-2, c.-f., lyo. from Roche Diagnostics, Mannheim) (8.0 g). The reaction mixture was stirred at ambient temperature until the conversion reached ca. 50% (estimated by TLC). The lipase was removed by filtration and washed with acetone (2×40 mL). The combined filtrates were evaporated under reduced pressure. The residue was dissolved in MeOH (25 mL) and the resulting solution was extracted with *n*-C_6F_{14} (6×25 mL). The organic phase was concentrated to dryness yielding the pure (*R*)-alcohols **2a**, **b**, **d**, **e** and (1*R*,4*S*)-**2f**.

The enantiomeric excesses of the alcohols were determined by chiral HPLC (see conditions below).

The fluorous phase was concentrated yielding a mixture of butyl 2*H*,2*H*,3*H*,3*H*-perfluoro undecanoate and the corresponding (*S*)-esters **3a**, **b**, **d**, **e** or (1*S*,4*R*)-**3f**, respectively.

The esters (*S*)-**3a**, **b**, **d** and (1*S*,4*R*)-**3f** were cleaved by saponification as follows. The residue from the fluorous phases were dissolved in a 1:1 mixture of THF/H_2O (40 mL) containing LiOH (0.36 g, 15 mmol) and refluxed for 3 h. Subsequently, the reaction mixture was diluted with a mixture of cyclohexane (70 mL) and *tert*-butyl methyl ether (30 mL), and the two distinct phases separated. The aqueous phase was washed with a mixture of cyclohexane (7 mL) and *tert*-butyl methyl ether (3 mL). The combined organic phases were concentrated to dryness to yield the alcohols (*S*)-**2a**, **b**, **d** or (1*S*,4*R*)-**2f**, respectively.

The ester (*S*)-**3e** was cleaved as follows. The fluorous phase was concentrated under reduced pressure. The residue was dissolved in MeOH (200 mL) containing a catalytic amount of *p*-toluenesulfonic acid (500 mg) and refluxed for 68 h. The solvent was removed under reduced pressure and the residue was dissolved in cyclohexane/*tert*-butyl methyl ether (200:50 mL). The resulting solution was filtered and concentrated to dryness yielding a mixture of (*S*)-**2e** and methyl 2*H*,2*H*,3*H*,3*H*-perfluoro undecanoate which were separated after dissolution in MeOH (25 mL) and extraction with *n*-C_6F_{14} (6×25 mL). From the organic phase (*S*)-**2e** was isolated.

The enantiomeric excesses of the esters from the fluorous phases were determined after saponification or transesterification, respectively, to the corresponding alcohols by chiral HPLC.

Conditions for the determination of the enantiomeric excess by HPLC on chiral phases:

(R)- and (S)-2a. Chiralpak AD®, *n*-heptane/*n*-propanol=95:5, UV-detection at λ=254 nm, flow rate=1.00 mL/min.

*(R)- and (S)-**2b***. Chiralcel OD®, *n*-heptane/*n*-propanol=90:10, UV-detection at λ=254 nm, flow rate=1.00 mL/min.

*(R)- and (S)-**2d***. Chiralcel OJ®, *n*-hexane/2-propanol=90:10, UV-detection at λ=254 nm, flow rate=1.50 mL/min.

*(R)- and (S)-**2e***. Chiralpak AD®, *n*-heptane/*n*-propanol=99:1, RI-detection, flow rate=0.60 mL/min.

*(1S,4R)- and (1R,4S)-**2f***. Chiralcel OD®, *n*-heptane/*n*-propanol=99:1, RI-detection, flow rate=1.00 mL/min.

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References

1. Curran, D. P. *Green Chem.* **2001**, G3–G7.
2. Curran, D. P. *Angew. Chem.* **1998**, *110*, 1231–1255 *Angew. Chem. Int. Ed. Engl.* **1998**, *37*, 1174–1196.
3. Horváth, I. T.; Rábai, J. *Science* **1994**, *266*, 72–75.
4. Richter, B.; Spek, A. L.; van Koten, G.; Deelman, B.-J. *J. Am. Chem. Soc.* **2000**, *122*, 3945–3951.
5. Soós, T.; Bennett, B. L.; Rutherford, D.; Barthel-Rosa, L. P.; Gladysz, J. A. *Organometallics* **2001**, *20*, 3079–3086.
6. Curran, D. P.; Hoshino, M. *J. Org. Chem.* **1996**, *61*, 6480–6481.
7. Wipf, P.; Reeves, J. T. *Tetrahedron Lett.* **1999**, *40*, 4649–4652.
8. Röver, S.; Wipf, P. *Tetrahedron Lett.* **1999**, *40*, 5667–5670.
9. Luo, Z.; Williams, J.; Read, R. W.; Curran, D. P. *J. Org. Chem.* **2001**, *66*, 4261–4266.
10. Crich, D.; Neelamkavil, S. *J. Am. Chem. Soc.* **2001**, *123*, 7449–7450.
11. Theil, F. *Chem. Rev.* **1995**, *95*, 2203–2227.
12. Bornscheuer, U. T.; Kazlauskas, R. J. *Hydrolases in Organic Synthesis*; Wiley/VCH: Weinheim, 1999.
13. Carrea, G.; Riva, S. *Angew. Chem.* **2000**, *112*, 2312–2341 *Angew. Chem. Int. Ed. Engl.* **2000**, *39*, 2226–2254.
14. Hungerhoff, B.; Sonnenschein, H.; Theil, F. *Angew. Chem.* **2001**, *113*, 2550–2552 *Angew. Chem. Int. Ed. Engl.* **2001**, *40*, 2492–2494.
15. Hungerhoff, B.; Sonnenschein, H.; Theil, F. *J. Org. Chem.* **2002**, *67*, 1781–1785.
16. Synthesized from the commercially available 1*H*,1*H*,2*H*,2*H*-heptafluorodecanol according to Refs. 14,15.
17. Chen, C.-S.; Fujimoto, Y.; Girdaukas, G.; Sih, C.-J. *J. Am. Chem. Soc.* **1982**, *104*, 7294–7299.
18. Burgess, K.; Jennings, L. D. *J. Am. Chem. Soc.* **1991**, *113*, 6129–6139.
19. Curran, T. T.; Hay, D. A.; Koegel, C. P.; Evans, J. C. *Tetrahedron* **1997**, *53*, 1983–2004.