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Catalytic Asymmetric Total Synthesis of Tangutorine

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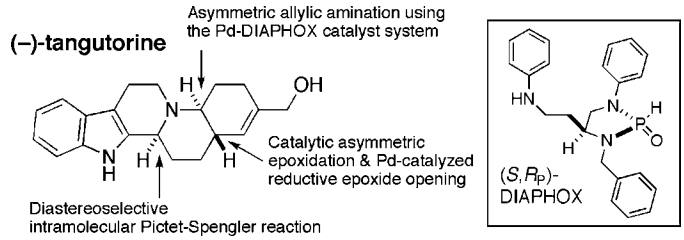
ABSTRACT



The first enantioselective total synthesis of tangutorine has been achieved, wherein a Pd-catalyzed asymmetric allylic amination using a chiral diaminophosphine oxide (DIAPHOX) preligand was the key step.

In 1999, Che and co-workers reported the isolation of a novel β-carboline alkaloid, tangutorine (**1**), from the leaves of the Chinese medicinal plant *Nitraria tangutorum*.[1] Tangutorine is the only known natural product that possesses a benz[*f*]indolo[2,3-a]quinolizidine skeleton. It is noteworthy that this alkaloid naturally occurs as a racemate, despite the presence of three chiral centers in the quinolizidine moiety. Recent studies revealed that tangutorine exhibits cytotoxic activity against human colon cancer HT-29 cells.[2] This bioactivity originates from the induction of cyclin kinase inhibitor p21 and the inhibition of topoisomerase II expression. These structural and biological profiles make tangutorine an attractive target for synthetic organic chemists. So far, total syntheses of racemic tangutorine have been reported by Jokela et al.,[3] Hsung et al.,[4] Ho et al.,[5] and Poupon et al.[6] A formal synthesis[7] and synthetic approaches[8] have also been reported. There are no reports, however, of an asymmetric total synthesis of tangutorine.[9] This background led us to focus our attention on enantioselective synthesis of tangutorine and evaluation of its cytotoxic activity using optically pure tangutorine. Herein, we report the first enantioselective total synthesis of tangutorine, wherein a Pd-catalyzed asymmetric allylic amination using a chiral diaminophosphine oxide (DIAPHOX) preligand was the key step. The cytotoxic activity of each enantiomer was also evaluated.

The plan for the enantioselective synthesis of tangutorine is shown in Scheme 1. Our retrosynthetic disconnection began with compound **2**, which was utilized as the key intermediate in previous racemic syntheses.[4,5] Control of the stereochemical arrangement on the piperidine ring is a challenging task in this synthesis. We envisioned that an

(1) Duan, J.-A.; Williams, I. D.; Che, C.-T.; Zhou, R.-H.; Zhao, S.-X. *Tetrahedron Lett.* **1999**, *40*, 2593–2596.
(2) Liu, B. P. L.; Chong, E. Y. Y.; Cheung, F. W. K.; Duan, J.-A.; Che, C.-T.; Liu, W. K. *Biochem. Pharmacol.* **2005**, *70*, 287–299.
(3) (a) Putkonen, T.; Tolvanen, A.; Jokela, R. *Tetrahedron Lett.* **2001**, *42*, 6593–6594. (b) Putkonen, T.; Tolvanen, A.; Jokela, R.; Caccamese, S.; Parrinello, N. *Tetrahedron* **2003**, *59*, 8589–8595.
(4) Luo, S.; Zificsak, C. A.; Hsung, R. P. *Org. Lett.* **2003**, *5*, 4709–4712.
(5) Ho, T.-L.; Chen, C.-K. *Helv. Chim. Acta* **2006**, *89*, 122–126.
(6) Salame, R.; Gravel, E.; Leblanc, K.; Poupon, E. *Org. Lett.* **2009**, *11*, 1891–1894.
(7) Luo, S.; Zhao, J.; Zhai, H. *J. Org. Chem.* **2004**, *69*, 4548–4550.
(8) Wilkinson, J. A.; Ardes-Guisot, N.; Ducki, S.; Leonard, J. *Tetrahedron Lett.* **2005**, *46*, 8053–8056.
(9) Optical resolution studies of racemic tangutorine using chiral HPLC have been reported (ref 3b). The reported optical rotations of each enantiomer, however, imply the difficulty of effective resolution.

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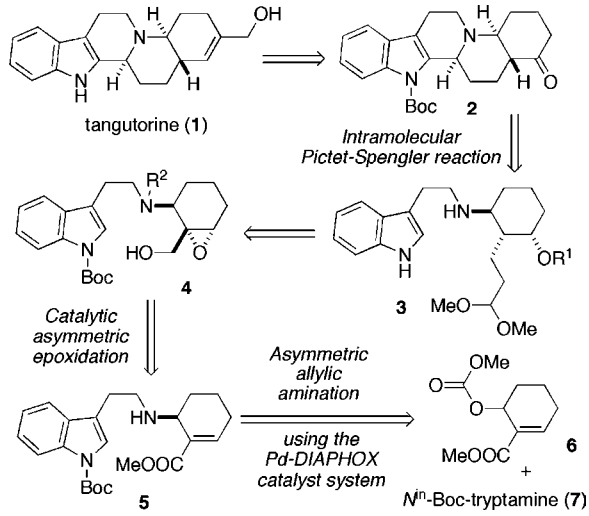
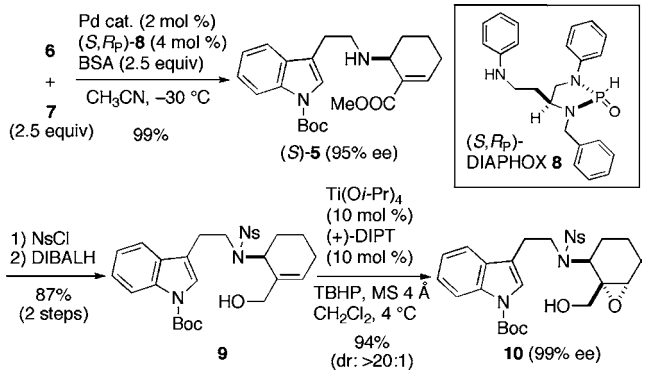
Scheme 1. Synthetic Plan for the Asymmetric Synthesis of **1**



intramolecular Pictet−Spengler reaction of amino acetal **3** would be applicable to construct the quinolizidine moiety. The present reaction would preferentially provide the configurationally more stable diastereomer, in which all substituents on the piperidine ring are in the equatorial positions. Compound **3**, bearing three contiguous chiral centers on the cyclohexane ring, would be prepared from epoxy alcohol **4** using an epoxide opening reaction with a hydride nucleophile, which, in turn, would be obtained by catalytic asymmetric epoxidation.[10] Finally, compound **5**, a reasonable precursor of the chiral epoxy alcohol, would be prepared via asymmetric allylic amination of **6** with N^{in}-Boc-tryptamine (**7**) using the Pd−DIAPHOX catalyst system that was recently developed in our laboratory.[11,12]

Our synthesis began with a Pd-catalyzed asymmetric allylic amination of readily available cyclic allylic carbonate **6**[13] with **7** (Scheme 2).[11a,14,15] Using 1 mol % of [η^3-$C_3H_5PdCl]_2$, 4 mol % of (S,R_P)-DIAPHOX **8**, and *N*,*O*-bis(trimethylsilyl)acetamide (BSA), asymmetric allylic amination proceeded smoothly at −30 °C, providing the corresponding chiral amine **5** in 99% yield with 95% ee. The absolute configuration of **5** was determined to be *S* by comparing the optical rotation with that of an authentic sample prepared from the known *N*-benzyl derivative.[16] Protection of the secondary amine with a 2-nitrobenzenesulfonyl (Ns) group, followed by reduction of the α,β-unsaturated ester with diisobutylaluminum hydride (DIBALH), proceeded smoothly to provide the corresponding allylic alcohol **9** in 87% yield (two steps). The following Sharpless asymmetric epoxidation of enantiomerically enriched **9** (95% ee) was performed in the presence of 10 mol % of Ti(O*i*-Pr)$_4$, 10 mol % of (+)-diisopropyl tartrate, 2 equiv of *tert*-butyl hydroperoxide, and MS 4 Å at 4 °C, giving the corresponding epoxy alcohol **10** with the α-epoxide in 94% yield with a high diastereomeric ratio. Enantiomeric excess of the major isomer was determined by chiral HPLC analysis (99% ee).

Scheme 2. Catalytic Asymmetric Approach to Optically Pure **10**



With nearly optically pure epoxy alcohol in hand, we next tried to establish an efficient synthetic route to a substrate for the intramolecular Pictet−Spengler reaction (Scheme 3). Although the regioselective epoxide opening reaction of epoxy alcohol **10** was first examined under several reaction conditions, the desired product with three contiguous chiral centers could not be obtained, probably due to the severe steric hindrance around the quaternary carbon. We thus attempted to perform this pivotal transformation at a more advanced stage. After detailed examination, the following three-step sequence was found to be an efficient method of synthesizing the functionalized cyclohexane ring with the requisite stereochemistry. Dess-Martin oxidation of the alcohol, followed by treatment of the resulting aldehyde with a Wittig reagent, afforded α,β-unsaturated ester **11** in 94% yield over two steps. Subsequent reductive epoxide opening

(10) For reviews on catalytic asymmetric epoxidation, see: (a) Katsuki, T.; Martin, V. S. *Org. React.* **1996**, *48*, 1–299. (b) Johnson, R. A.; Sharpless, K. B. *Catalytic Asymmetric Synthesis*, 2nd ed.; Ojima, I., Ed.; Wiley-VCH: Weinheim, 2000; pp 231−280.

(11) (a) Nemoto, T.; Fukuyama, T.; Yamamoto, E.; Tamura, S.; Fukuda, T.; Matsumoto, T.; Akimoto, Y.; Hamada, Y. *Org. Lett.* **2007**, *9*, 927–930. See also: (b) Nemoto, T.; Masuda, T.; Akimoto, Y.; Fukuyama, T.; Hamada, Y. *Org. Lett.* **2005**, *7*, 4447–4450. (c) Nemoto, T.; Tamura, S.; Sakamoto, T.; Hamada, Y. *Tetrahedron: Asymmetry* **2008**, *19*, 1751–1759.

(12) For reviews on transition-metal-catalyzed asymmetric reactions using chiral diaminophosphine oxides and related pentavalent phosphorus compounds, see: (a) Dubrovina, N. V.; Börner, A. *Angew. Chem., Int. Ed.* **2004**, *43*, 5883–5886. (b) Ackermann, L. *Synthesis* **2006**, 1557–1571. (c) Nemoto, T.; Hamada, Y. *Chem. Rec.* **2007**, *7*, 150–158. (d) Nemoto, T. *Chem. Pharm. Bull.* **2008**, *56*, 1213–1228.

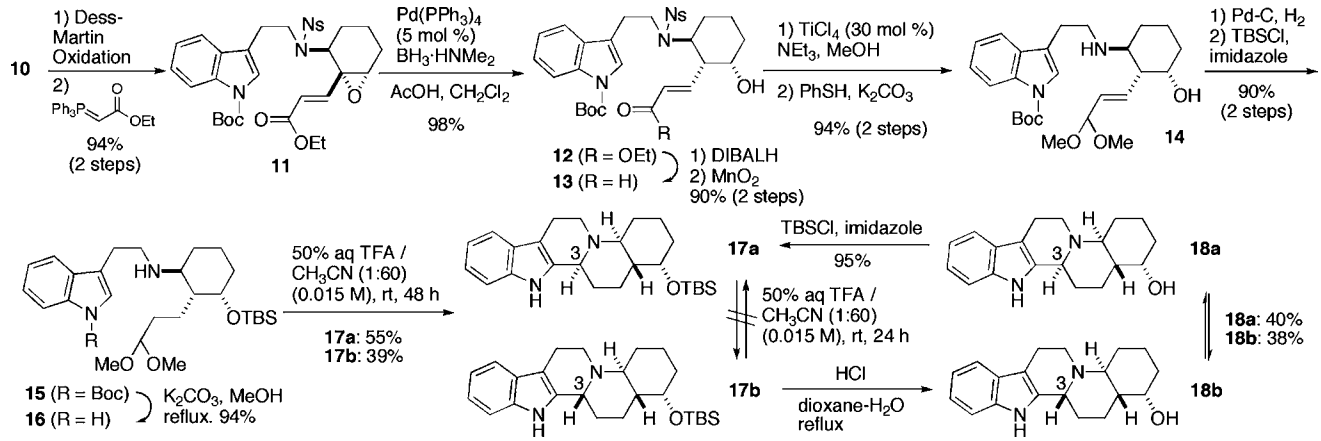
(13) The corresponding allylic alcohol can be prepared from glutaraldehyde and trimethyl phosphonoacetate in a single-step reaction. See: Graff, M.; Al Dilaimi, A.; Seguineau, P.; Rambaud, M.; Villieras, J. *Tetrahedron Lett.* **1986**, *27*, 1577–1578.

(14) For examples of Pd-catalyzed asymmetric allylic substitutions of ester-conjugated cycloalkenyl alcohol derivatives, see: (a) Trost, B. M.; Oslob, J. D. *J. Am. Chem. Soc.* **1999**, *121*, 3057–3064. (b) Mori, M.; Nakanishi, D.; Kajishima, D.; Sato, Y. *J. Am. Chem. Soc.* **2003**, *125*, 9801–9807. (c) Trost, B. M.; Machacek, M. R.; Tsui, H. C. *J. Am. Chem. Soc.* **2005**, *127*, 7014–7024. (d) Trost, B. M.; Tang, W.; Toste, F. D. *J. Am. Chem. Soc.* **2005**, *127*, 14875–14803. (e) Trost, B. M.; Malhotra, S.; Olson, D. E.; Maruniak, A.; Du Bois, J. *J. Am. Chem. Soc.* **2009**, *131*, 4190–4191.

(15) For reviews on Pd-catalyzed asymmetric allylic substitution, see: (a) Trost, B. M. *Chem. Pharm. Bull.* **2002**, *50*, 1–14. (b) Trost, B. M.; Crawley, M. L. *Chem. Rev.* **2003**, *103*, 2921–2943. (c) Lu, Z.; Ma, S. *Angew. Chem., Int. Ed.* **2008**, *47*, 258–297.

(16) For the determination of the absolute configuration of **5** and other discussions, see the Supporting Information.

Scheme 3. Construction of the Pentacyclic Skeleton



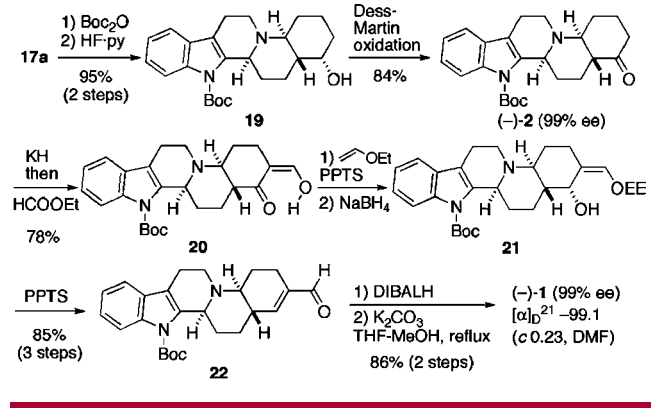
reaction via a π-allyl palladium intermediate proceeded in the presence of 5 mol % of $Pd(PPh_3)_4$, providing alcohol **12** in 98% yield in a highly diastereoselective manner.[17] Conversion of α,β-unsaturated ester **12** to aldehyde **13** was achieved by a two-step process involving DIBALH reduction, followed by MnO_2 oxidation (90% yield, two steps). After transformation of the aldehyde moiety into dimethyl acetal, the Ns group was deprotected with thiophenol to give compound **14** in 94% yield over two steps. The following hydrogenation of an olefin, as well as protection of the secondary alcohol with a TBS group, proceeded smoothly to provide compound **15** in 90% yield over two steps. Finally, deprotection of the Boc group was performed under basic conditions, affording amino acetal **16** in 94% yield.

We then examined the construction of the pentacyclic skeleton through the intramolecular Pictet−Spengler reaction.[18] Initial screening revealed that the reaction proceeded smoothly using trifluoroacetic acid (TFA) as a promoter in a CH_3CN-H_2O solvent system (50% aq TFA/CH_3CN = 1/5), although undesired isomer **17b** was obtained as a major product (24 h, 100% conversion, **17a**/**17b** = 9/91). There was a gradual increase in the product ratio until the proportion of 50% aq TFA to CH_3CN decreased to 1:60 (24 h, 89% conversion, **17a**/**17b** = 52/37). Encouraged by this result, we investigated the effect of an acid promoter in detail. All trials, however, gave less satisfactory results. After all, the intramolecular Pictet−Spengler reaction of **16** proceeded to completion in 48 h under the aforementioned conditions (50% aq TFA/CH_3CN = 1/60) to afford **17a** and **17b** in 55% and 39% isolated yield, respectively.[19]

Thermodynamically controlled C-3 epimerization of indolo[2,3-a]quinolizidines proceeds under acidic conditions.[20] There was no interconversion, however, between **17a** and **17b** in the presence of TFA, indicating that the product ratio of the intramolecular Pictet−Spengler reaction was kinetically controlled. On the other hand, we were pleased to find that the C-3 epimerization of **17b** proceeded under harsher reaction conditions. When **17b** was refluxed in a 4 N HCl/dioxane−H_2O solvent system for 18 h, both deprotection of the TBS group and epimerization of the C-3 stereocenter occurred simultaneously, affording **18b** and **18a** in 38% yield and 40% yield, respectively.[21] Compound **18a** was transformed into **17a** in 95% yield. In addition, C-3 epimerization of isolated **18b** proceeded under the same conditions to provide a mixture of **18a** and **18b** (**18a**:**18b** = 45:55, determined by ^1H NMR analysis of the crude sample), revealing an efficient recycling process for **17b**.

The final stage of the asymmetric synthesis is outlined in Scheme 4. Protection of the nitrogen on the indole ring of

Scheme 4. Enantioselective Total Synthesis of **1**



17a with a Boc group, followed by deprotection of the TBS group using the HF•pyridine complex, afforded compound **19** in 95% yield over two steps. Subsequent oxidation of the resulting hydroxyl group with Dess-Martin reagent gave the known synthetic intermediate (−)-**2** in 84% yield. The enantiomeric excess of (−)-**2**, determined by chiral HPLC

(17) (a) Oshima, M.; Yamazaki, H.; Shimizu, I.; Nisar, M.; Tsuji, J. *J. Am. Chem. Soc.* **1989**, *111*, 6280–6287. (b) David, H.; Dupuis, L.; Guillerez, M.-G.; Guibé, F. *Tetrahedron Lett.* **2000**, *41*, 3335–3338.

(18) For a review on the Pictet−Spengler reaction, see: Cox, E. D.; Cook, J. M. *Chem. Rev.* **1995**, *95*, 1797–1842.

(19) For detailed data of the intramolecular Pictet−Spengler reaction, see the Supporting Information.

analysis (99% ee), indicated that no erosion of enantiomeric purity had occurred over the course of the transformations.

Transformation of the known intermediate **2** into tangutorine was performed using the mixed enol acetalization protocol.[22] Introduction of a C1 unit on the α-position to the ketone was achieved by the formation of an enolate with KH at 0 °C, followed by the addition of ethyl formate. As a result, enol **20** with a *Z* geometry was obtained in 78% yield. After protection of the enol with an ethoxyethyl (EE) group (89% yield), the resulting product was reduced with $NaBH_4$ to give alcohol **21** in a highly stereoselective manner. The coupling constant of the newly introduced hydrogen in the ^{1}H NMR spectrum (J = 7.2 Hz) revealed that the hydroxyl group was in the axial position. The obtained crude residue was treated with PPTS without purification, affording the α,*β*-unsaturated aldehyde **22** in 95% yield over two steps.[23] Finally, reduction of the α,*β*-unsaturated aldehyde with DIBALH, followed by deprotection of the Boc group on the indole, afforded tangutorine (−)-**1** in 86% yield over two steps ($[\alpha]_D^{21}$ −99.1 [*c* 0.23, DMF]) (57% overall yield in six steps from **2**).[24] The NMR data (^{1}H NMR and ^{13}C NMR) were identical to the reported data for **1**, and enantiomeric excess of (−)-**1** was unequivocally determined by chiral HPLC analysis (99% ee). On the basis of the present synthetic method, (+)-**1** (99% ee) was also prepared using the Pd-(R,S_P)-DIAPHOX catalyst system.

Cytotoxic activity against HT-29 cells was evaluated using optically pure (−)-**1**, (+)-**1**, and racemic **1** (Figure 1). The

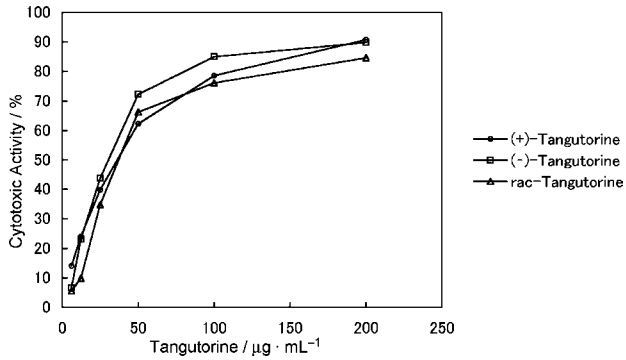


Figure 1. Cytotoxic activity of (−)-**1**, (+)-**1**, and (±)-**1**. HT-29 cells were incubated with (−)-**1**, (+)-**1**, or (±)-**1** for 48 h at the concentrations of 6.25, 12.5, 25, 50, 100, and 200 μg/mL. The absorbance without cell was taken as 100%. Means for three independent measurements are represented.

cells were incubated with serial dilutions of tangutorine (6.25 to 200 μg/mL) for 48 h and then subjected to WST assay.[25] Although a dose-dependent increase in cell death was observed in each sample, cytotoxic activity did not significantly differ between the enantiomers.

In conclusion, we achieved the first enantioselective total synthesis of tangutorine with a 15.8% overall yield in 24 steps.[26] The three chiral centers were constructed by asymmetric allylic amination using the Pd−DIAPHOX catalyst system, catalytic asymmetric epoxidation, and a diastereoselective intramolecular Pictet−Spengler reaction. This synthetic method will provide access to a variety of compounds with the rare pentacyclic skeleton in an optically active form, which should be an attractive resource for medicinal chemistry. Moreover, the present enantioselective synthesis clarified the cytotoxic activity of optically pure tangutorine. This novel information will provide a useful guideline for studies of the structure−activity relationship of tangutorine. Further investigations into the biological activity of tangutorine, as well as its structural analogues, are in progress.

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Supporting Information Available: Experimental procedures, supplementary data, compound characterization, and NMR charts. This material is available free of charge via the Internet at http://pubs.acs.org.

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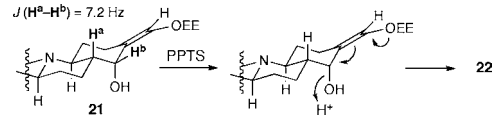
(20) (a) Lounasmaa, M.; Berner, M.; Tolvanen, A. *Heterocycles* **1998**, *48*, 1275–1290. (b) Berner, M.; Tolvanen, A.; Jokela, R. *Tetrahedron Lett.* **1999**, *40*, 7119–7122.

(21) The product ratio of **18a** and **18b**, determined by ^{1}H NMR analysis of the crude sample, was 45:55. Prolonging the reaction time (30 h) did not affect the product ratio. Enantiomeric excess of **18a** was determined by chiral HPLC analysis (99% ee) after converting into compound **2**.

(22) (a) Kawanobe, T.; Iwamoto, M.; Kogami, K.; Matsui, M. *Agric. Biol. Chem.* **1987**, *51*, 791–796. (b) Zhang, F.; Danishefsky, S. J. *Angew. Chem., Int. Ed.* **2002**, *41*, 1434–1437. (c) Watanabe, K.; Iwasaki, K.; Abe, T.; Inoue, M.; Ohkubo, K.; Suzuki, T.; Katoh, T. *Org. Lett.* **2005**, *7*, 3745–3748.

(23) It is assumed that transformation of **21** into **22** was initiated by protonation of the hydroxyl group with PPTS. The molecular conformation, in which the hydroxyl group is in the axial position, likely facilitates this reaction.



(24) This overall yield is superior to those of the previous synthesis (refs 4 and 5). Hsung's method: 46% overall yield. Ho's method: 23% overall yield.

(25) See Supporting Information for details. The racemic sample was prepared using Ho's method.

(26) The effect of recycling **17b** into **17a** is not taken into account to calculate the overall yield.