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A Highly Efficient Asymmetric Synthesis of Vernakalant

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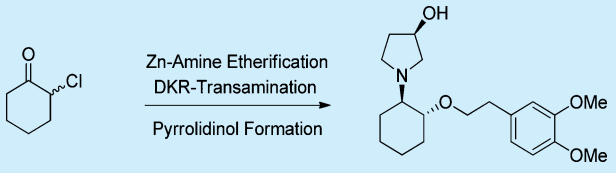
Supporting Information

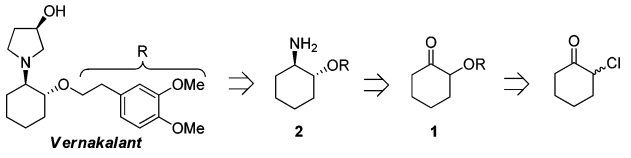
ABSTRACT: A novel synthesis of vernakalant is described. Using inexpensive and readily available reagents, the key transformations involve (1) an efficient zinc-amine-promoted etherification, (2) a highly stereoselective enzyme-catalyzed dynamic asymmetric transamination to set up the two contiguous chiral centers in the cyclohexane ring, and (3) a pyrrolidine ring formation via alkyl-$B(OH)_2$-catalyzed amidation and subsequent imide reduction.



A trial fibrillation (AF), which affects 1−2% of the population, is the most common cardiac arrhythmia leading to 20% of all strokes.[1] While the exact mechanism of AF remains unknown, significant development efforts on antiarrhythmic drugs for AF have focused on achieving high atrial selectivity.[2] Vernakalant, discovered by Cardiome Pharma Corp., was developed as a novel antiarrhythmic agent for the treatment of recent-onset AF via atrial-selective inhibition of the Na- and K-channel currents.[3] The compound has been demonstrated to be effective, safe, and well-tolerated in various clinical studies and has gained approval by the European agency (EMEA) for the intravenous conversion of AF to sinus rhythm under the trade name of Brinavess.

While various routes to the chiral aminocyclohexyl ether core of vernakalant have previously been reported, most employ a classical resolution/separation of a racemic intermediate or utilize an expensive chiral starting material.[4] Our asymmetric approach to vernakalant is illustrated in Scheme 1. We

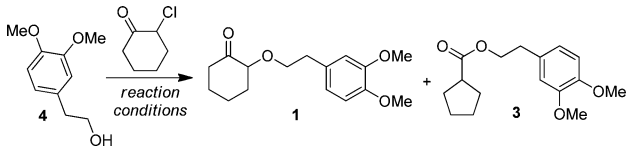
Scheme 1. Retrosynthetic Analysis of Vernakalant



envisioned accessing the active pharmaceutical ingredient (API) through hydroxypyrrolidine ring formation from chiral amine **2**, which could be, in turn, derived from racemic ketone **1** via an enzyme-catalyzed dynamic asymmetric−transamination (DA-TA) process. The ketone **1** could then be prepared from inexpensive and readily available α-chlorocyclohexanone.

The α-etherification of α-chloroketones with an aliphatic alcohol nucleophile has not been reported,[5] largely due to the tendency of such compounds to undergo a Favorskii rearrangement.[6] Not surprisingly, our initial experiment showed that exposure of α-chlorocyclohexanone to alcohol **4** in the presence of Cs_2CO_3 gave mostly Favorskii rearrangement product **3** (Table 1, entry 1).[7]

Table 1. α-Etherification Optimization[a]



entry	base[b]	additives	solvent	temp (°C)	1:3 ratio	yield[c] (%)
1	Cs_2CO_3	none	THF	60	<1:100	45
2	Et_3N	none	THF	60		<1
3	Et_2Zn[d]	none	THF	60	16:1	27
4	DIPEA	$Zn(OTf)_2$[e]	THF	45	>30:1	14
5	none	pyrrolidine[e]	$PhCF_3$	85	>30:1	23
6	Et_3N[e]	pyrrolidine[e]	$PhCF_3$	85	>30:1	46
7	DIPEA	$Zn(OTf)_2$,[e] pyrrolidine[d]	THF	45	>30:1	66
8	DIPEA	$Zn(OTf)_2$,[e] (*S*)-MMP[f,d]	$PhCF_3$	45	>30:1	68[g]
9	DIPEA[d]	$ZnCl_2$,[d] pyrrolidine[h]	$PhCH_3$	100	>30:1	94

[a]All reactions were run for 16−24 h. [b]2 equiv. [c]Estimated assay yields by HPLC. [d]1 equiv. [e]0.4 equiv. [f]MMP = 2-(methoxymethyl)-pyrrolidine. [g]Achieved 53% ee. [h]0.2 equiv.

A more comprehensive screening of inorganic bases such as KOAc, $KHCO_3$, KF, K_2CO_3, and K_3PO_4 and solvents such as THF, toluene, and dichloromethane failed to improve the reaction. Organic bases, such as Et_3N, afforded <1% conversion (Table 1, entry 2). Interestingly, the use of Et_2Zn as a base[8] gave the desired ether **1** as the major product in THF (Table 1, entry 3). Similarly, a combination of $Zn(OTf)_2$ and Hünig's base also afforded the desired product, albeit in low yields

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(Table 1, entry 4). Further attempts to improve the reactivity and selectivity failed with other metal salts, including Li, Mg, and Cu salts with or without Pd, Ir, Ni, or Cu catalysts.

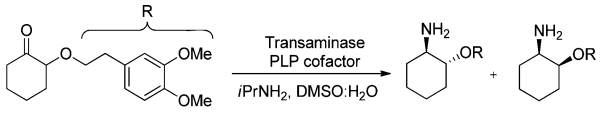
Concurrently, in an attempt to promote this reaction via activation of the ketone with an organoamine catalyst, we screened various primary (1°), secondary (2°), and tertiary (3°) amines and found that pyrrolidine can provide the desired product with little rearrangement (Table 1, entries 5 and 6).[9] Additionally, the combination of Zn and pyrrolidine also provided the product in good yields in THF while preserving high chemoselectivities (Table 1, entry 7). Interestingly, using (*S*)-2-methoxymethylpyrrolidine as a chiral catalyst in $PhCF_3$ gave the desired product in 53% ee (Table 1, entry 8). As the subsequent step does not require an optically pure product, we focused our optimization efforts on nonstereoselective conditions. Our optimal conditions were to perform the reaction in toluene at 100 °C in the presence of 20 mol % of pyrrolidine and stoichiometric amounts of both $ZnCl_2$ and Hünig's base. Under this protocol, compound **1** was obtained in 94% assay yield (Table 1, entry 9) and was used directly in the next step without isolation.

With keto ether **1** in hand, we next evaluated generation of the pyrrolidinol ring. Unfortunately, direct reductive amination of **1** with 3-hydroxypyrrolidine under various reaction conditions afforded poor trans/cis diastereoselectivity.[10] We hypothesized that a dynamic asymmetric-transamination (DA-TA), on the other hand, would allow for a stereoselective introduction of an amino functionality that could be further elaborated. While TA of ketones using selectively engineered transaminases has been well-established,[11] no literature on the dynamic variant,[12] involving *α-substituted ketones*, where two contiguous stereocenters could be simultaneously set, had been reported at the start of our work in 2009.[13]

The initial DA-TA screening was performed with available transaminase variants[14] under known reaction conditions (using pyridoxal 5′-phosphate (PLP) cofactor and *i*-$PrNH_2$ as the nitrogen source), while maintaining basic pH (>9) to promote equilibration of starting enantiomers.[15] Unfortunately, while high enantioselectivities were obtained, the reactions afforded predominantly the cis-diastereomer **5** (entries 1−5, Table 2). A combination of in silico design and directed evolution was subsequently carried out on ATA-013 transaminase.[16] After only one round of evolution, a variant was identified that exhibited a reverse in diastereoselectivity (entry 6). The desired trans-isomer product **2** was obtained with excellent enantioselectivity and a >95:1 trans:cis ratio after three rounds of evolution (entries 6−8). The enzyme was simultaneously evolved to increase its reactivity/selectivity and tolerance to high pH.

Subsequent studies revealed that the enzymatic DA-TA step is sensitive to certain impurities present in the starting material. The presence of either *α*-hydroxy- or *α*-chlorocyclohexanone (>5 wt %) in crude **1** gave lower conversions (50−79%) relative to purer materials (<1 wt %).[17] After screening solvents, pH buffers, and enzyme loadings, the optimal conditions were to treat a solution of the substrate in a 1:9 mixture of DMSO/H_2O with 5 wt % of ATA-303, 1 wt % of PLP cofactor, and 1 M of *i*-$PrNH_2$ at 45 °C, while maintaining a basic pH of 10. Under these conditions, the desired amine **2** was obtained in 85% assay yield, 95:1 dr, and >99.5% ee. While several crystalline salts of the product were identified, isolation of the product as the crystalline D-malate salt is preferred because it offers chemical and physical stability and allows for a purity upgrade to 99.6:0.4 dr in 81% yield from ketone **1** (Scheme 2). Additionally, the D-malate salt could also be conveniently and directly used in subsequent pyrrolidine ring formation.

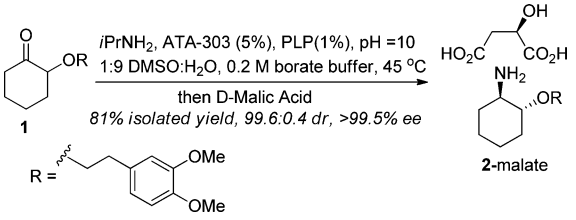
Table 2. Enzyme-Catalyzed Dynamic-TA Optimization[a]



entry	transaminase variant	2:5 ratio[b]
1	ATA-007	1:5
2	ATA-013	1:1.2
3	ATA-018	1:8
4	ATA-024	1:9
5	ATA-032 (CDX-017)	1:12
6	ATA-013 Evolution 1	4:1
7	ATA-013 Evolution 2	14:1
8	ATA-013 Evolution 3 (ATA-303)	>95:1

[a]Screening conditions: 1:9 DMSO/H_2O, 10−100 wt % enzyme loading, pH = 10.5, 45−50 °C, 1 mL scale, 5 g/L ketone, 1 g/L PLP, 1 M *i*-$PrNH_2$ [b]Ratio determined by HPLC; in all cases, >99.5% ee of **2** was obtained.

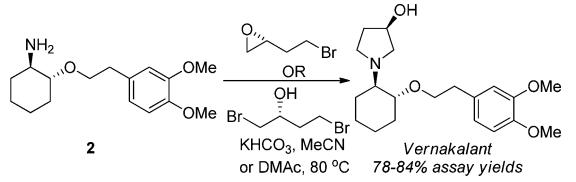
Scheme 2. Optimized DA-TA Conditions[a]



[a]The reaction typically takes 24 h, and partial removal of acetone byproduct might be necessary to drive the conversion further.

Our initial efforts to generate the pyrrolidine ring relied on double alkylation of amine **2** with an appropriate bis-electrophile. Subjection of amine **2** to either (*R*)-4-bromo-1,2-butylene epoxide or (*R*)-1,4-dibromo-2-butanol[18] under basic conditions afforded the API in 78−84% assay yields (Scheme 3). While this approach is convergent, the high risk of potential genotoxic impurity (PGI) contamination from the alkylating agents or reaction intermediates was not deemed amenable for a commercial process.

Scheme 3. Bis-alkylation Approach to API[a]



[a]The reaction typically takes 24 h with 1.1 equiv of bis-electrophiles and 2.0 equiv of base at 80 °C.

We subsequently evaluated a stepwise approach by first generating succinimide intermediate **6** and then performing an exhaustive reduction to access vernakalant. Standard amide

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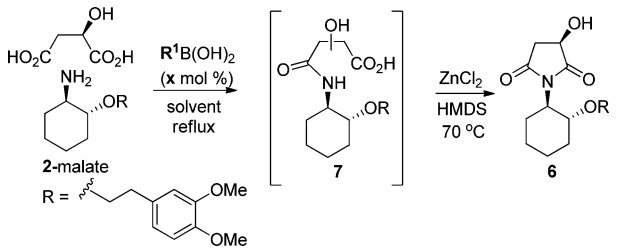
coupling conditions, including the use of catalytic boric acid[19] or aryl boronic acids (Table 3, entry 1),[20] proved ineffective for

Table 3. Formation of Penultimate Imide Intermediate



entry	R^1	solvent[a]	x	% conv to 7[b]	% yield of 6[c]
1	Ar[d]	*i*-PrOAc	20	8−15	n.d.
2	OH	*i*-PrOAc	20	8	n.d.
3	**1°-Alk**[e]	***n*-BuOAc**	**20**	**98−99**	**85**
4	isopropyl	*n*-BuOAc	20	82	n.d.
5	*n*-butyl	*n*-PrOAc	30	82[f]	n.d.
6	*n*-butyl	*n*-PrOAc	20	98[f]	n.d.
7	*n*-butyl	*n*-PrOAc	10	99[f]	n.d.
8	***n*-butyl**	***n*-PrOAc**	**5**	**99**	**85** (77)

[a]Reaction volume = 10−20 mL/g. [b]Conversions after 2 h. [c]Assay yields; the number in parentheses correspond to isolated yield; n.d. = not determined. [d]Ar = Ph, 2-Br-Ph, 2-I-Ph, 3,4,5-F_3-Ph. [e]1°-Alk = methyl, ethyl, *n*-butyl. [f]6 h data point.

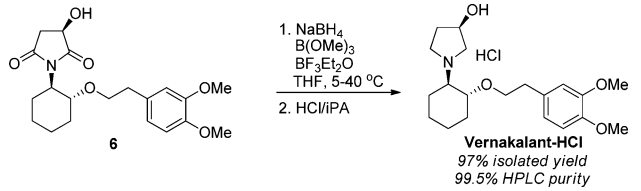
the amidation. However, excellent conversion was observed when 1° alkyl-$B(OH)_2$ catalysts were used (entry 3).[21] Due to its low cost and wide availability, *n*-butyl-$B(OH)_2$ was selected for further development. Interestingly, both turnover number and frequency improved with lower catalyst loadings (entries 5−8), observations that are consistent with a trimeric boroxine catalyst resting state[22] and multiple order catalyst degradation.[23] Further optimization led to the use of 5 mol % of *n*-butyl-$B(OH)_2$ in refluxing *n*-propyl acetate under azeotropic drying, providing a 99% conversion to amides **7** in 2 h.

The cyclization of amides **7** to imide **6** could be effected by using a combination of $ZnCl_2$ and HMDS.[24] Importantly, this transformation was efficient even on the crude amidation reaction mixture and therefore, we developed a one-pot process for the conversion of **2**-malate to **6**. Once *n*-butyl-$B(OH)_2$ catalyzed amidation was completed, the mixture was cooled to 70 °C, treated with HMDS and $ZnCl_2$, and aged for 6 h to afford penultimate **6** in 85% assay yield and 77% isolated yield after crystallization (Table 3, entry 8).

With compound **6** in hand, we next investigated the exhaustive imide reduction step. We found that the optimal transformation involved subjecting imide **6** in THF to $NaBH_4$ (3 equiv) and $B(OMe)_3$ (1 equiv), followed by $BF_3{\cdot}Et_2O$ (4 equiv) to afford the product in high conversions.[25] Upon treatment with HCl in IPA, vernakalant was isolated as a crystalline HCl salt in 97% yield and 99.5% purity (Scheme 4).

In summary, we have identified and developed a highly efficient route to vernakalant starting from readily available and inexpensive starting materials. This approach, which involves three novel transformations, including a $ZnCl_2$ and pyrrolidine-mediated α-etherification, an enzymatic dynamic asymmetric-transamination (DA-TA) of an α-substituted ketone, and an alkyl-$B(OH)_2$-promoted amidation, provides vernakalant in five steps and 56% overall yield. To the best of our knowledge, this work represents the first reported example of an enzymatic DA-TA of an α-substituted ketone (not β-keto esters), affording the corresponding trans-amino product in high enantio- and diastereoselectivities.

Scheme 4. Endgame Chemistry: Exhaustive Reduction of Imide



■ ASSOCIATED CONTENT

Supporting Information

Experimental procedures and characterization of new compounds, including their NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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(14) These were composed of the native transaminase of *Arthrobacter* sp. and its engineered variants, which were used in the synthesis of Sitagliptin, see ref 11.
(15) While rapid epimerization of enantiomerically pure ketone **2** was observed in *i*-$PrNH_2$ (pH = 10) at 45 °C to afford racemic **2**, epimerization of the substrate−PLP imine intermediate might also take place.
(16) The directed evolution work was performed by Codexis, Inc.
(17) Typically <1 wt % of these impurities; if higher level is seen, treatment of crude keto ether **2** with Darco would reduce the level of 2-chlorocyclohexanone.
(18) Both are commercially available at the time of this work.
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