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Process Development of Tryptophan Hydroxylase Inhibitor LX1031, a Drug Candidate for the Treatment of Irritable Bowel Syndrome

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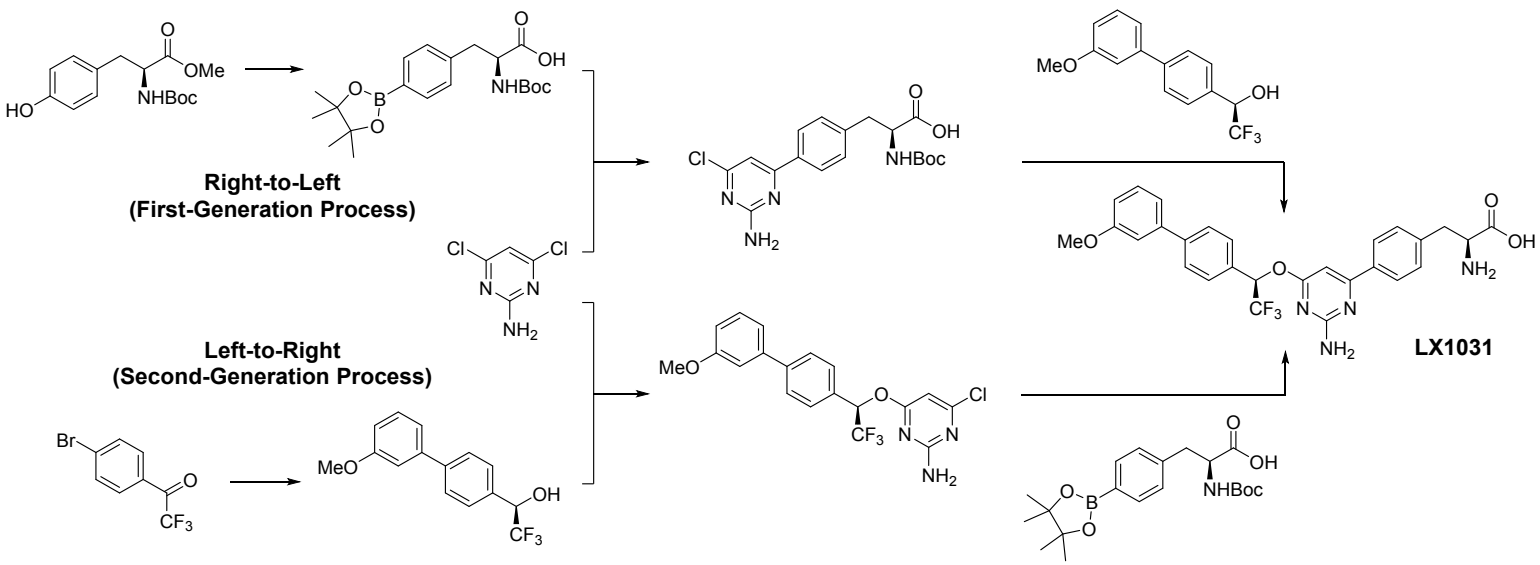
TOC Graphic


Right-to-Left
(First-Generation Process)
Left-to-Right
(Second-Generation Process)
LX1031

ABSTRACT

Two process routes for LX1031, a tryptophan hydroxylase (TPH) inhibitor for the treatment of irritable bowel syndrome (IBS), were developed. They shared the same left-hand and right-hand starting materials as well as the penultimate intermediate. The chiral center in the left-hand moiety was established via a Noyori asymmetric hydrogenation of a trifluoromethyl aryl ketone. The right-hand boronate was prepared via a palladium-catalyzed borylation of L-tyrosine derived aryl triflate. Union of these two fragments to the pyrimidine core, from the right- or left-hand side, constituted the first- and second-generation routes, respectively. Removal of the Boc-protecting group from the penultimate intermediate gave LX1031. The challenges overcome in purification and isolation of the LX1031 zwitterion are also discussed. Both process routes were successfully performed on multi-kilogram scales to supply LX1031 API for the preclinical and clinical studies.

KEYWORDS: Asymmetric hydrogenation, trifluoromethyl carbinol, Suzuki, S_NAr, zwitterion

INTRODUCTION

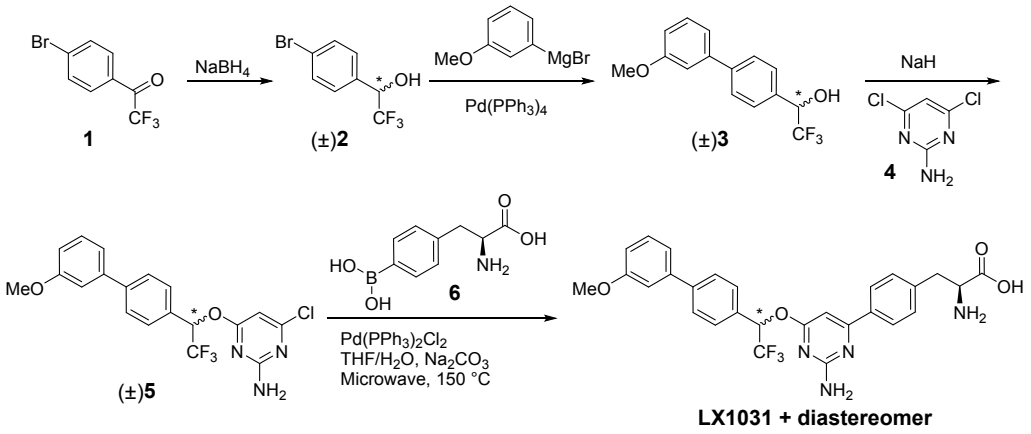
Irritable Bowel Syndrome (IBS) is characterized by abdominal pain, cramping and changes in bowel functions such as constipation, diarrhea, and alternating symptoms. IBS affects approximately 25–45 million Americans who have limited therapeutic options.[1] Overexpression of serotonin (5-hydroxytryptamine or 5-HT) in the GI tract is associated with IBS diarrhea.[2] Serotonin is also involved in important biological functions in the central nervous system (CNS). Existing IBS treatments targeting serotonin receptors often impart undesirable CNS side effects, affecting mood, appetite, and other behavioral functions. Tryptophan hydroxylase (TPH), an enzyme that catalyzes the rate-limiting step in the biosynthesis of serotonin, exists in two distinct isoforms, TPH1 and TPH2. Selective inhibition of TPH1, which is expressed primarily in the GI tract, can potentially provide an effective treatment of IBS without affecting serotonin levels in the brain[3] thereby minimizing potential CNS side effects. In animal models, a locally acting TPH1 inhibitor LX1031, has been shown to reduce 5-HT levels in the GI but not in the brain.[4]

The medicinal chemistry route[4,5] for LX1031 started with reduction of trifluoromethyl aryl ketone **1** with $NaBH_4$ to give racemic alcohol (±)**2** (Scheme 1). Kumada coupling of the latter with *m*-methoxyphenyl magnesium chloride gave racemic biaryl alcohol (±)**3**. Deprotonation of (±)**3** with NaH followed by an S_NAr substitution on 4,6-dichloro-2-aminopyrimidine (DCAP, **4**) gave pyrimidine chloride (±)**5**. Palladium catalyzed Suzuki coupling of (±)**5** with 4-borono-*L*-phenylalanine (L-BPA, **6**) afforded a mixture of LX1031 and a diastereomer.

While this synthesis was relatively concise, several significant challenges had to be overcome before scale-up. First, chiral resolution at the final stage by preparative HPLC or other means was inherently inefficient, so an enantioselective synthesis of chiral alcohol **2** or **3** was desired. Second, NaH must be replaced with a process-friendly base for the S_NAr substitution. Third, L-BPA (**6**) was costly

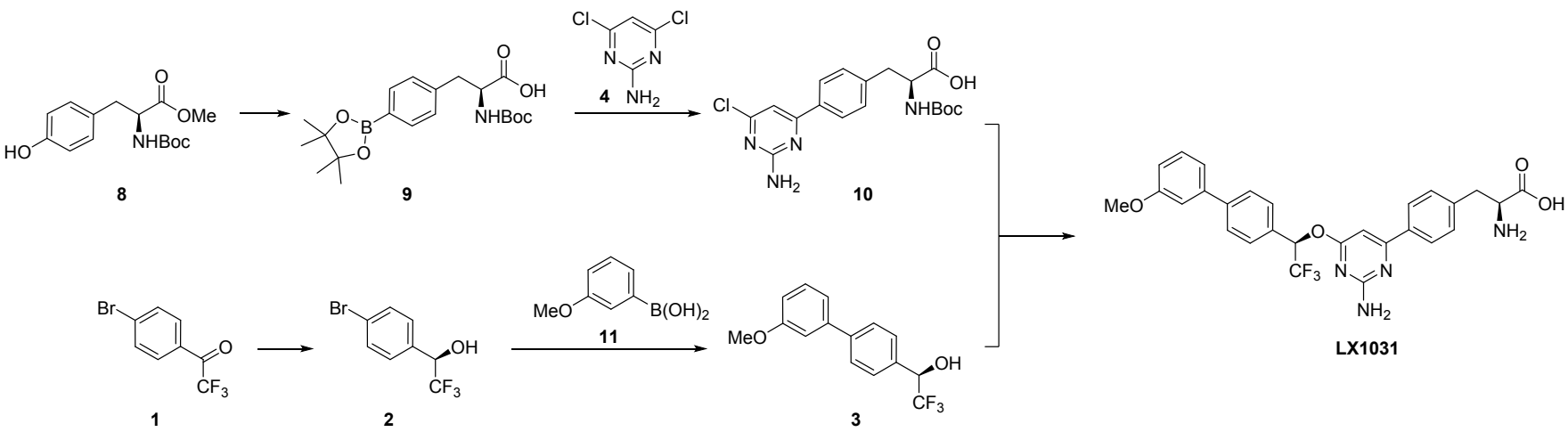
($10,000/100 g) and not readily available in kilogram quantities. A practical synthesis of L-BPA or its surrogate had to be developed. Additionally, the microwave heating (150 °C) used for the Suzuki-coupling of pyrimidine chloride **5** and L-BPA (**6**) was not amenable to scale-up. Finally, it was desirable to identify a crystalline form of LX1031 for isolation and purity upgrade.

Scheme 1 Medicinal Chemistry Route to LX1031



For the enantioselective reduction of the trifluoromethyl aryl ketone (**1**), Noyori asymmetric hydrogenation[6] is one of the most frequently used methods in the literature. Replacing sodium hydride with a more process-friendly base for the S_NAr substitution should also be feasible.[7] However, preliminary screening experiments indicated that it could be challenging for the Suzuki coupling of **5** and **6** to go to completion without microwave heating. Therefore, an alternative synthetic approach (right-to-left) involving Suzuki-coupling with a more active substrate, DCAP (**4**), was explored (Scheme 2). In this case, an L-BPA surrogate, compound **9** was synthesized via borylation of the aryl triflate prepared from commercially available *N*-Boc-tyrosine methyl ester.[8] An added benefit of this approach was that intermediate **10** could be used for synthesizing other potential drug candidates being pursued at the time.

Scheme 2 First Generation Process Approach (Right-to-left)



RESULTS AND DISCUSSION

First-Generation Process Route (Right-to-Left)

*Preparation of boronate **9***: For the synthesis of the right-hand fragment of LX1031, borylation[9] of L-tyrosine triflate **12** appeared the most straightforward (Scheme 3). Triflate **12** was prepared by reaction of Boc-L-tyrosine with triflic anhydride. Attempts to replace pyridine used in the literature[10] with K_2CO_3 was unsuccessful (no reaction). On the other hand, the reaction proceeded cleanly and rapidly with either triethylamine or *N*-methylmorpholine (NMM) as the base, reaching completion immediately after the addition of triflic anhydride at –10 °C in DCM. NMM was selected for easier removal of colored materials by aqueous citric acid washes. The aryl triflate (**12**) was surprisingly stable in the organic layer, showing no appreciable degradation when stirred with aqueous citric acid for three days at rt. Being a low-melting solid, it was not isolated but directly used in the next step after solvent swap to acetonitrile. The acetonitrile solution of **12** also proved to be stable for many months at ambient temperature.

Borylation of **12** by treatment with bis(pinacolato)diboron (pin_2B_2) worked well in acetonitrile with $Pd(dppf)Cl_2$ as the catalyst and two equivalents of KOAc as the base. A small amount of coupling product of dppf with **12** was observed (Figure 1). This issue was resolved by switching to $Pd(OAc)_2/PCy_3$ catalyst. The reaction mixture was cooled and the precipitated byproducts were filtered off. After solvent swap to MTBE, the methyl ester was hydrolyzed in-situ to the carboxylic acid **9** by treatment with aqueous LiOH at 0 °C for 1 hour. This was to eliminate the potential risk of racemization under basic conditions downstream.

In the initial scale-up of LX1031 (1.4 kg), the MTBE solution of compound **9** was used directly in the next step after solvent swap to ethanol. In subsequent campaigns, boronate **9** was crystallized from heptane/MTBE mixture in ~90% yield and with > 99% purity.

Scheme 3 Synthesis of pyrimidine chloride **10**

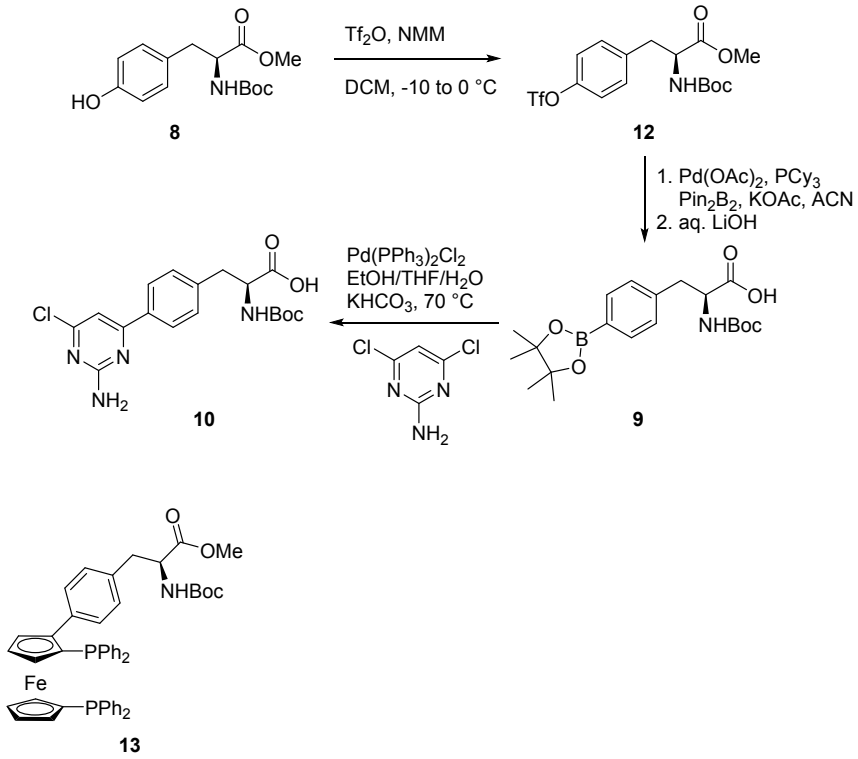


Figure 1 DPPF adduct

*Synthesis of pyrimidine chloride (**10**)* As expected, the Suzuki-coupling of aryl boronate **9** with DCAP was indeed facile. With 1 mol% of $Pd(PPh_3)_2Cl_2$ as the catalyst and $KHCO_3$ as the base, the reaction proceeded smoothly at 70 °C in 5/1 EtOH/water (Scheme 3). However, due to the high reactivity of DCAP, several impurities such as bis-coupling product **14**, amination impurity **15**, and hydrolysis/solvolysis of DCAP, **16a**/**16b**, were generated at significant levels (Figure 2). For example, with 1.2 equivalents of DCAP, bis-coupling product **14** was about 20%. The formation of this impurity was suppressed to ~ 6% by increasing DCAP to 1.8 equivalents, albeit requiring a high solvent volume (20 vol) to fully dissolve DCAP. Unfortunately, the increased charge of DCAP led to increased formation of amination impurity **15** (~ 4%) particularly at the end of the reaction. In fact, it increased to 10% in 15 h in a stress experiment. Therefore, the reaction mixture should be cooled promptly at the end of the reaction. DCAP solvolysis product **16b**, typically 2% at the end of the reaction, also

increased to 10% during the stress test. This impurity was less of a concern since it was readily purged in the workup and isolation.

Table 1 Impurities vs DCAP stoichiometry and age time

Entry	DCAP (equiv)	Time (h)	**14** (A%)	**15** (A%)
1	1.2	5	20	< 1
2	1.5	5	5.5	2.5
3	1.8	5	6	4
4	1.8	20	6	10

*1 mol% $Pd(PPh_3)_2Cl_2$, 3 equiv $KHCO_3$, 5/1 EtOH/H_2O (~20 vol), 75–80 °C.

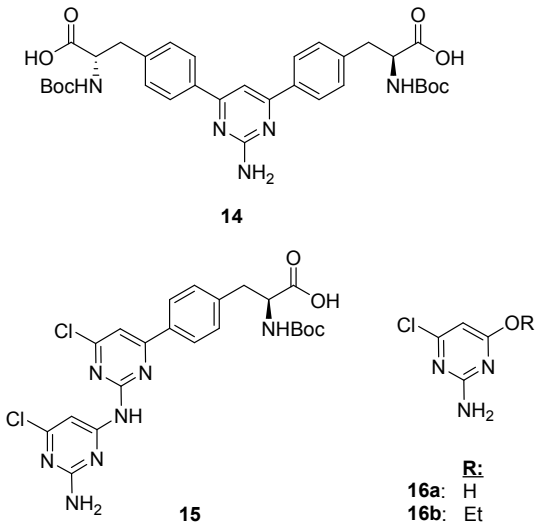


Figure 2 Impurities in Suzuki coupling

Early in development, isolation of **10** was a challenge due to lack of any known crystalline forms. After completion of the reaction, the mixture was concentrated to precipitate most of the excess DCAP for removal by filtration. The filtrate was extracted with EtOAc to remove residual DCAP and then treated with Darco-G60 to remove residual palladium. The product was then precipitated by acidification with aqueous citric acid. However, this operation proved problematic in the initial 600 g scale-up due to formation of sticky mass at pH 5.5–6.0 which blocked the stirring. Although the sticky mass eventually hardened at pH 4 allowing it to be crushed, filtered, washed, and dried, the purity of

the isolated **10** was only 76% and residual palladium level was >1000 ppm. This problem was remedied by slowly adding the crude aqueous solution into a citrate buffer maintained at pH 4 by concomitant addition of aqueous citric acid to give a free-flowing suspension. Additionally, improved residual palladium control was achieved by adding (n-Bu)$_3$P in the EtOAc wash. These improvements were implemented in the next scale-up batch affording 4.65 kg of **10** (~60% yield corrected). The main impurities were diacid **14** (5.6%) and amination impurity **15** (8.9%). The higher than typical level of **15** was attributed to the longer reaction time (12 h vs < 5 h).

While the diacid (**14**) was inert and mostly purged in the next step in the workup, **15** was expected to propagate to new impurities, increasing impurity load in the purification and isolation of LX1031. Thus, additional catalyst screening focused on minimizing the formation of **15**. Among the catalysts screened (PEPPSI and CombiPhos's POPd, PXPd catalyst families), POPd6 was the most promising, controlling **15** to 0.5–1%. However, diacid **14** was significantly higher than with $Pd(PPh_3)_2Cl_2$ (10% vs 5%). By increasing DCAP charge to 3 equivalents, **14** was controlled to ~8%. Meanwhile, a crystalline toluene solvate of **10** was discovered and utilized for its isolation in two scale-up batches (25 kg and 40 kg scale) affording **10** with ~88% purity in 50–60% yield (corrected for ~75% w/w assay, 10–15% w/w toluene). Amination impurity **15** was ~0.5%, but the diacid **14** remained relatively high at ~8%.

While POPd6 was successful in minimizing amination impurity **15**, the level of diacid **14** was rather high, wasting significant amounts of valuable boronate **9**. Therefore, other catalyst systems including $Pd(OAc)_2/P(Cy)_3$, $Pd(OAc)_2/PPh_3$, and $Pd(dppf)Cl_2$ were evaluated (Table 2). Even higher level of bis-coupling product **14** (18% vs 8.8%) was observed with $Pd(OAc)_2/P(Cy)_3$ as the catalyst (entry 2). $Pd(dppf)Cl_2$ was less active, reaching only 41% conversion after aging for 15 h at 75–80 °C (entry 3). $Pd(OAc)_2/PPh_3$ gave the most promising results and was selected for further optimization (entry 4).

Table 2 Alternative catalysts to control diacid impurity **14**

Entry	Catalyst	Reaction Time	Conversion	**14** (%)	**15** (%)
1	POPd6	15 h	100	8.8	0.55
2	$Pd(OAc)_2/P(Cy)_3$ (1 mol%/2 mol%)	10 h	100	17.8	1.1
3	$Pd(dppf)Cl_2$	15 h	41	--	--
4	$Pd(OAc)_2/PPh_3$ (1 mol%/2 mol%)	10 h	100	4.3	2.5

*1 mol% Pd, 5/1 EtOH/H_2O (20–35 vol), 75–80 °C

It was observed that stronger base such as K_3PO_4 significantly increased the formation of amination impurity **15** (Table 3, entry 1). The Suzuki-coupling reaction was completely shut down in acetonitrile (entry 3). The reaction was also very slow in THF or acetone perhaps due to the lower refluxing temperatures (entry 4, 5). The reaction proceeded well in DME, dioxane, or a dioxane/EtOH mixture (entry 6–8). But DME and dioxane are not desirable solvents due to safety and environmental concerns. The reaction performed better in THF/EtOH mixtures than EtOH alone, controlling the diacid **14** and amination impurity **15** to < 4% and < 2.5%, respectively (entry 9, 10). The reaction mixture contained ~90% area **10** (excluding DCAP and DCAP-derived impurities **16a/16b**) under the optimized reaction conditions ($Pd(OAc)_2/PPh_3$, 3.5 equiv $KHCO_3$, THF/EtOH/water, 75 °C).

Table 3 Optimization of Suzuki-coupling with $Pd(OAc)_2/PPh_3$ catalyst

Entry	Solvent	Base	Temp, time	Conv (%)	**14** (%)	**15** (%)
1	EtOH	K_3PO_4	75–80 °C, 15 h	100	3.8	8.2
2	EtOH	$KHCO_3$	75–80 °C, 5 h	99	3.8	3.5
3	Acetonitrile	$KHCO_3$	75–80 °C 20 h	0	--	--
4	THF	$KHCO_3$	Reflux, 8 h	26	--	--
5	Acetone	$KHCO_3$	Reflux, 15 h	35	--	--
6	DME	$KHCO_3$	75–80 °C, 20 h	93	3.1	2.1
7	Dioxane	$KHCO_3$	75–80 °C, 15 h	98	3.7	1.9
8	dioxane : EtOH	$KHCO_3$	75–80 °C 6 h	100	3.8	3.2

	(1 : 1)					
9	THF : EtOH (7 : 3)	$KHCO_3$	67–70 °C, 9 h	99	3.2	2.3
10	THF : EtOH (3 : 7)	$KHCO_3$	70–73 °C, 14 h	95	3.5	2.1

* ~20 vol total solvent, 0.75 mol% $Pd(OAc)_2$, 1.5 mol% PPh_3, 75–80 °C or reflux

The reaction mixture was concentrated to a low volume, diluted with water to precipitate most of the excess DCAP for removal by filtration. The aqueous filtrate was washed with EtOAc twice to remove residual DCAP. After adjusting the pH to 2.5–3.5 with 6 *N* HCl, the product was extracted into THF/toluene. The organic layer was treated with activated carbon and solvent swapped to toluene to crystallize **10** as a toluene solvate in ~65% yield with ~95 A% purity (~2% **14**, ~0.5% **15**). On multiple 50-kg batches, residual palladium levels in most batches were higher (> 100 ppm) than lab batches perhaps due to more efficient removal of THF during solvent swap. Recrystallization from THF/toluene (3.2 : 23 vol) reduced residual palladium to < 100 ppm and improved its purity to ~97% with ~90% recovery.

*Synthesis of biaryl chiral alcohol **3***. For the left-hand fragment, the biaryl chiral alcohol (**3**), the most straightforward synthesis was via asymmetric reduction of trifluoromethyl ketone **1** followed by Suzuki-coupling with 3-methyoxyphenylboronic acid (**11**) (Scheme 4). Trifluoromethyl ketone **1** was prepared from 1,4-dibromobenzene according to literature procedures.[11] Several methods for asymmetric reduction of ketone **1** were evaluated and the results summarized in Table 5. (*S*)-Me-CBS[12] catalyzed reduction with catecholborane gave chiral alcohol **2** with only 56% ee (entry 1). Reduction with (+)-DIP-Cl[13] afforded significantly higher enantioselectivity (86% ee) (entry 2). However, the reaction was sluggish (3 days) even with three equivalents of DIP-Cl. Increasing the reaction temperature to 60 °C decreased the stereoselectivity to 65% and 68% ee in toluene and heptane, respectively. Noyori asymmetric hydrogenation in IPA using (*R*)-Xyl-P-Phos $RuCl_2$/(*R*)-

DAIPEN catalyst in the presence of a small amount of *t*-BuOK afforded chiral alcohol **2** in 86% ee (entry 6). Similarly, Noyori transfer-hydrogenation with Me_5Cp^*IrCl(*R*,*R*-TsDPEN) catalyst gave **2** in 82% ee (entry 7). Though not ideal, the enantioselectivity of the Noyori asymmetric hydrogenation was satisfactory as the chiral purity of **2** could be readily upgraded to > 99% ee by crystallization from heptane. The reaction conditions were further optimized and implemented on hundred-kilogram scale, delivering chiral alcohol **2** in ~56% yield with > 99% ee.

Scheme 4 Synthesis of biaryl chiral alcohol **3**

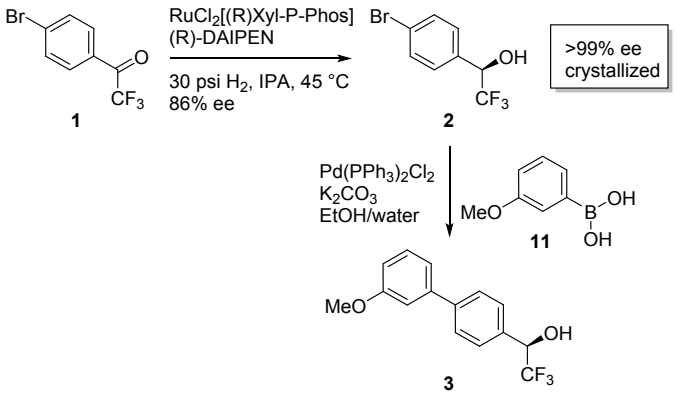


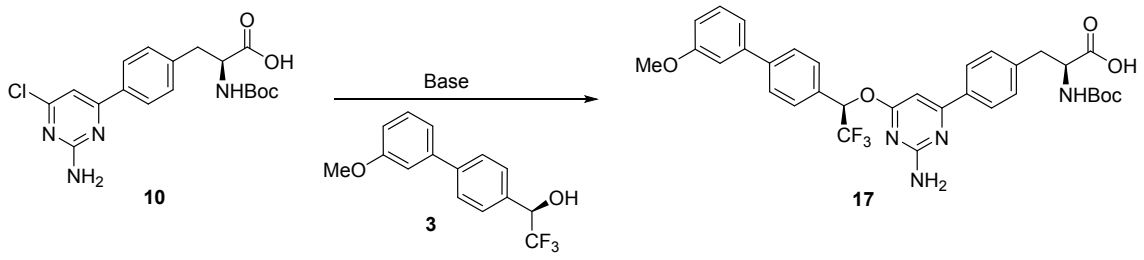
Table 4 Asymmetric reduction of ketone **1**

Entry	Conditions	**2** (e.e.%)
1	(S)-Me-CBS, catecholborane, THF, -78 to -30 °C	56%
2	3 equiv (+)-DIP-Cl, heptane, 3 days, RT	86%
3	2.5 equiv (+)-DIP-Cl, heptane, 60 °C, 24 h	68%
4	2.5 equiv (+)-DIP-Cl, toluene, 60 °C, 24 h	65%
5	(R,R)-TsDPEN Ru(cymene)Cl	41%
6	(R)-Xyl-P-Phos $RuCl_2$ (R)-DAIPEN (0.01 mol%); KO^tBu (0.4 mol%), 30 psi H_2, IPA, 45 °C	86%
7	Et_3N-HCO_2H/$Me_5CpIrCl$ (R,R-TsDPEN)	82%

For the aryl-aryl bond formation, Kumada-coupling used in the medicinal chemistry route required excess Grignard reagent (> 2 equiv) due to the presence of an acidic proton on the chiral alcohol (**2**). Therefore, Suzuki-coupling with commercially available 3-methyoxyphenylboronic acid (**11**) was explored. With 1.2 equivalents of **11**, 3 equivalents of K_2CO_3 and 0.1 mol% $Pd(PPh_3)_2Cl_2$ catalyst, the reaction was facile in EtOH/water (9/1 vol), achieving complete conversion in 2–5 h at 75–80 °C. It should be noted that the boronic acid (**11**) must be charged within 1–2 h after the catalyst charge to minimize catalyst deactivation and de-bromination of **2**. The workup and isolation of biaryl chiral alcohol **3** entailed dilution with water, concentration, extraction with MTBE, washing with 1 N NaOH (to remove excess boronic acid **11**), treatment with Darco-G60, and crystallization by solvent swap to *n*-heptane. On a 120-kg batch, the biaryl chiral alcohol (**3**) was isolated in 93% yield with > 99% purity and > 99% ee.

*S_NAr reaction of biaryl chiral alcohol **3** with pyrimidine chloride **10**.* With both pyrimidine chloride **10** and biaryl chiral alcohol **3** in hand, their coupling via an S_NAr reaction[14] without using NaH was investigated (Scheme 5).

Scheme 5 S_NAr reaction of pyrimidine chloride **10** with chiral biaryl alcohol **3**



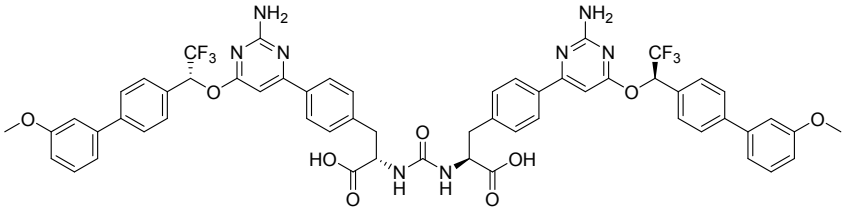
Organic bases such as DBU and TMG were ineffective (Table 6, entry 1, 2). Potassium carbonate was also ineffective even in the presence of 20 mol% 18-crown-6 (entry 3). Strong bases such as sodium *t*-butoxide (3 equiv) or sodium *t*-pentoxide (3 equiv) gave complex reaction mixtures partially

due to cleavage of the Boc-protecting group (entry 4, 5). The most promising result was obtained with Cs_2CO_3 in dioxane at 90–100 °C (entries 6–10). However, the reaction was rather slow (15 h) even with 5 equivalents of Cs_2CO_3 (entry 6). Adding 20 mol% 18-crown-6 seemed to accelerate the reaction but with substantially higher cost (entry 7). Decreasing the Cs_2CO_3 charge to 3 equivalents to maintain a similar cost led to a much slower reaction (entry 8). Inexpensive phase-transfer agent Bu_4NHSO_4 (10 mol%) also seemed to accelerate the reaction, achieving complete conversion in 16 h with 3.5 equivalents of Cs_2CO_3 (entry 9). Milled Cs_2CO_3 performed substantially better than granular material (entry 10). Even under the optimized conditions (1.2 equiv **3**, 3.5 equiv. milled Cs_2CO_3, 5 vol dioxane) the reaction generally required 15–24 h at 90–100 °C. Dimeric urea impurity **18** (Figure 3)[15] and LX1031 (premature cleavage of the Boc-protecting group), were typically observed at 2–5% each at the end of the reaction. These impurities continued to increase with age time mandating prompt quenching of the reaction.

Table 5 S_NAr reaction of chiral alcohol **3** with pyrimidine chloride **10**

Entry	Base (equiv)	Additive (mol%)	**3**	Dioxane	Time (h)	Con (%)
1	DBU (5)	--	1.2	10	20	0
2	TMG (5)	--	1.2	10	20	0
3	K_2CO_3 (5)	18-crown-6 (20 mol%)	1.2	10	20	0
4	NaO^{t}Bu (3)	--	1.2	10	2	NA*
5	NaO^{t}Pent (3)	--	1.2	10	2	NA*
6	Cs_2CO_3 (5)	--	1.2	10	15	> 98%
7	Cs_2CO_3 (5)	18-crown-6 (20 mol%)	1.2	10	8	> 98%
8	Cs_2CO_3 (3)	18-crown-6 (20 mol%)	1.2	10	30	> 98%
9	Cs_2CO_3 (3.5)	Bu_4NHSO_4 (10%)	1.2	5	16	> 98%
10	Cs_2CO_3 (3.5) (milled)	--	1.2	5	16	> 98%

*complex reaction mixture due to cleavage of Boc-protecting group.



18

Figure 3 Dimeric urea impurity **18**.

A notable operational challenge was the severe gelling of the cesium salts of starting material **10** and product **17** below 70 °C which could completely immobilize the reaction mixture and damage the agitator. To mitigate this problem, starting material **10** was charged into a suspension of Cs_2CO_3 and the biaryl chiral alcohol (**3**) in dioxane at 80–90 °C. The reaction mixture was then aged at 100 °C until reaction completion. The reaction mixture was then quenched with water at 80–90 °C before further cooling and continuing the workup.

Serendipitously, it was found that by adding an appropriate amount of water, a clean phase split was achieved where the cesium salt of **17** was forced into the organic layer while most of the inorganic salts remained in the aqueous layer. This phase split obviated the need for neutralizing the large amounts of Cs_2CO_3 in the reaction mixture. Diacid impurity **14** was also mostly removed in the aqueous layer.

Since no crystalline form of **17** was identified at the time, the organic layer was directly treated with aqueous HCl to remove the Boc-protecting group to give LX1031. Excess biaryl chiral alcohol **3** and other neutral impurities were removed by extraction with IPAc.

Isolation and purification of LX1031 via dihydrochloride salt. The isolation of LX1031 was particularly challenging due to its unusual physicochemical properties. Being a zwitterion with a large hydrophobic aromatic moiety, it behaved like a surfactant. Attempted isolation by neutralization to its

isoelectric point (pH 6.5) gave a gel that was difficult to filter and dry. Subsequently, a manageable suspension of LX1031 zwitterion was obtained by slowly adding a solution of LX1031 in aqueous HCl into a sodium phosphate buffer maintained at pH 6.5 by concomitant addition of aqueous NaOH.

In the first scale-up batch, starting from 4.57 kg of pyrimidine chloride **10** (78.7% purity), 3.23 kg of LX1031 zwitterion was obtained as a dark yellow solid with 87% purity. Initial salt screening suggested that crystallization of LX1031 dihydrochloride could provide significant purity upgrade. Thus, this batch of LX1031 zwitterion was converted into the dihydrochloride salt in a mixture of THF/IPA/IPAc, improving its purity to 95.4% (2.99 kg, 82% yield as-is). Recrystallization (86% recovery) in a similar solvent system (THF/IPA/IPAc) only slightly improved its purity to 96.3%. To further reduce the color and residual palladium level, it was dissolved in 1 *N* NaOH and treated with activated carbon again (0.3X). Unfortunately, the filtration through a pad of cellulose powder was excruciatingly slow. Switching to a pad of diatomaceous earth led to significant breakthrough of activated carbon. Meanwhile, nickel contamination was detected for this batch (~600 ppm), which was traced to the use of HCl in a new Hastelloy reactor. Fortunately, adding chelating agent EDTA to the filtrate was effective in keeping the nickel in the mother liquor during free basing in phosphate buffer (3 ppm in the isolated LX1031, 1.57 kg). To remove the trace amounts of activated carbon, it was dissolved in 1 *N* HCl, filtered through a pad of diatomaceous earth followed by a cartridge-filter and then precipitated from pH 6.5 phosphate buffer to give 1.45 kg of LX1031 zwitterion (97.7% purity) as a pale-yellow solid (45% overall yield from **10**).

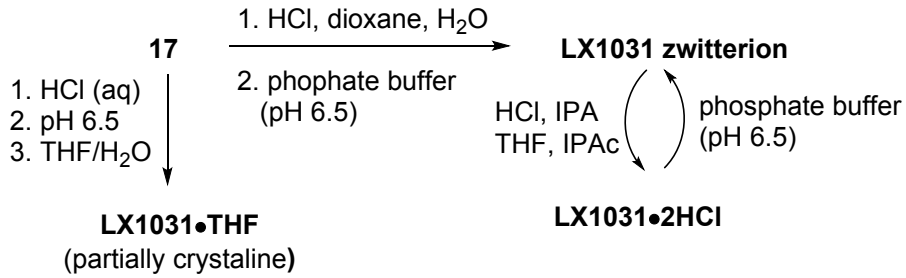
For the 2nd scale-up batch (28.3 kg of **10**), the S_NAr reaction and deprotection went smoothly. However, the isolation of LX1031 API was still problematic. The filtration/wash of the precipitated LX1031 zwitterion from pH 6.5 sodium phosphate buffer was slow (23 h). The drying was even more time consuming (4.5 days at 50–60 °C) affording 23.8 kg (61% yield) crude LX1031 (94.0% purity).

The purity was upgraded to 98.9% via crystallization of its dihydrochloride salt (18.1 kg, 67% yield). Again, the filtration (6 days) and drying (3 days) were extremely slow. The dihydrochloride salt was converted to LX1031 zwitterion (10.2 kg, 68% yield) as described previously. The low overall yield (27.3%) from **10** was partially attributed to physical loss due to a missing dust collector in the filter-dryer.

To alleviate the challenges of purity upgrade at the API stage, identifying a crystalline form of penultimate intermediate **17** was focused on. An extensive solvent screen led to the discovery a crystalline form of **17** in acetonitrile. The crystallization was incorporated into the process stream, allowing the isolation of **17** with 95.3% purity in a scale-up batch (85.9 kg, 81% yield as-is). The urea impurity **18** was reduced from ~5% to ~2%.

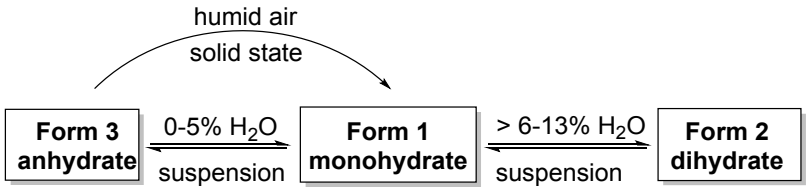
Meanwhile, a semi-crystalline THF solvate of LX1031 zwitterion was discovered, which could provide significantly improved filtration rate. The crystallization was sensitive to temperature (< 40 °C), THF/water ratio (> 1/3) and levels of residual inorganic salt (the lower the better). It also required heavy seeding for a more consistent performance. The solvated THF was readily removed during drying to give amorphous LX1031. In a scale-up batch (85 kg of **17**), it was found that the filtration and wash were still slow (19 h/32 h), suggesting that the purportedly THF solvate might not have formed. The wet-cake also contained large amounts of water requiring prolonged drying (39 h) at 45 °C under reduced pressure to give 75.3 kg of LX1031 zwitterion (91.4 % yield corrected) with 95.3% purity.

Scheme 6 Initial processes for deprotection and isolation of LX1031



Purification of LX1031 via tosylate salt. To achieve a more robust purity upgrade, impurity purging via isolation of a crystalline salt of LX1031 was investigated. Salt screening showed that the tosylate salt was promising. Extensive experimentation revealed that LX1031 tosylate exhibited rather complex polymorphism. It could be isolated as an anhydrate, monohydrate or dihydrate, depending on the water contents of crystallization medium (THF/ACN/water) (Scheme 7). Below 5 vol% water, the anhydrate was the preferred form. However, it readily converted to the monohydrate upon exposure to humid air even in solid state. The monohydrate was stable in solid-state regardless of humidity levels. But suspension equilibration experiments showed that it was stable only within a narrow range of water contents (5 ± 1%). On the other hand, the dihydrate was proved stable in a much wider range of water contents (6–13%), and selected for development. The optimal composition for the crystallization medium was 5–8% water, 10–25% THF, with the remainder being acetonitrile. This crystallization provided robust purity upgrade as demonstrated on a scale-up batch. Starting from 95.3% purity LX1031 zwitterion (64 kg), 99.5% purity LX1031•TsOH•2H_2O was obtained in 82.3% yield. A portion of the batch (27.2 kg) was converted to LX1031 zwitterion in 87.4% yield (17.2 kg) in THF/water. Unfortunately, the filtration/wash and drying operations were still slow.

Scheme 7 Polymorphism of LX1031 tosylate



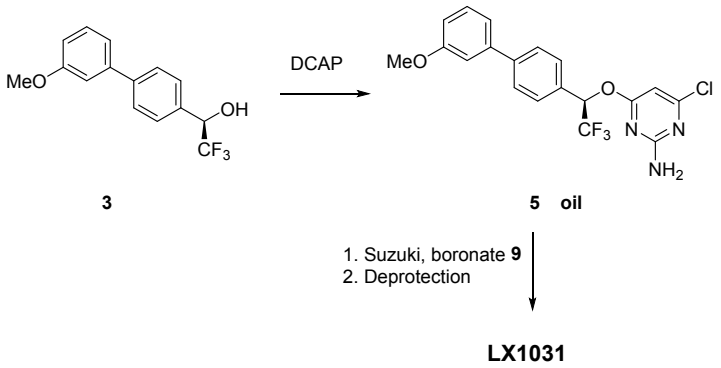
Crystallization of LX1031 acetonitrile solvate. As amorphous LX1031 zwitterion was used in early clinical studies, it was desirable to continue using this form in mid-stage trials. Therefore, a better process for the isolation of the zwitterion, particularly with improved filtration and drying performance was sought. Attempted crystallization from 2-MeTHF gave a gel even after repeated heating/cooling cycles with periodic sonication. Crystallization from IPA afforded a slow-filtering suspension. Crystallization in ethanol gave a LX1031 EtOH solvate that filtered reasonably well. But the residual EtOH was difficult to remove. After drying at 60 °C for 16 h *in vacuo*, 26 mol% EtOH remained. Fortunately, it was discovered that LX1031 acetonitrile solvate monohydrate was highly crystalline and filtered easily. Residual acetonitrile and water were also readily removed during drying at 50 °C under reduced pressure. This discovery was utilized for the free-basing of the next portion of LX1031 tosylate dihydrate (25.5 kg). Thus, after neutralization (pH 6.5) with 8% aq. NaOH, the THF layer was washed with 15% aqueous NaCl, concentrated and solvent swapped to acetonitrile to crystallized LX1031 acetonitrile solvate monohydrate. The filtration, washes (water, acetonitrile) and drying were expedient delivering amorphous LX1031 zwitterion in 91% yield (16.7 kg). Residual acetonitrile was at undetectable level (< 100 ppm).

SECOND-GENERATION PROCESS ROUTE TO LX1031

While the first-generation process route was amenable for production after implementing the many process improvements, the projected high dose of the drug (>2 g/day) prompted us to further reduce the cost of the drug substance. The main cost driver of the process was the production of pyrimidine

chloride **10**. This was attributed to the tedious and volume-intensive process as well as a modest yield (60–65%). Therefore, an alternative "left-to-right" approach like the medicinal chemistry route was reevaluated (Scheme 8). In this approach, the main challenge was to overcome the sluggishness of the Suzuki-coupling of pyrimidine chloride **5** with L-BPA.

Scheme 8 Left-to-right Approach



3 **5 oil**

LX1031

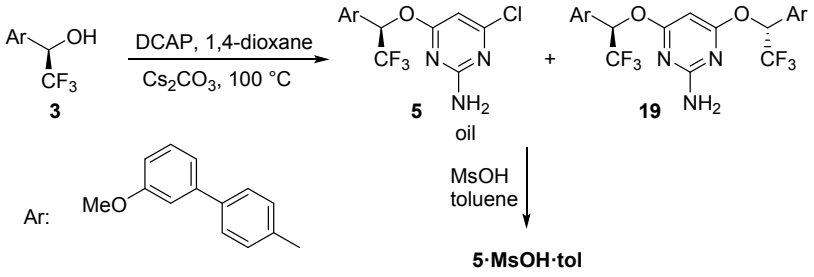
*S_NAr reaction of chiral alcohol (**3**) with DCAP.* The S_NAr substitution reaction of biaryl chiral alcohol **3** with DCAP was facile in 1,4-dioxane, achieving complete conversion in 5 h at 100 °C with 1.5 equivalents of Cs_2CO_3. Although less expensive K_2CO_3 could be used, a highly polar solvent such as DMF, DMSO or DMAc was required, complicating the workup. Interestingly, in *t*-amyl alcohol, the reaction proceeded even with K_2CO_3 as the base, albeit requiring 20 h at refluxing temperature. As expected, less hindered alcohol EtOH gave substantial amounts (~10%) of ethoxy adduct of DCAP **16b**. In comparison, IPA and *i*-BuOH gave < 1% and ~3% of corresponding adducts. The cost saving of using K_2CO_3 vs. Cs_2CO_3 did not justify the longer cycle-time, however.

DCAP charge was reduced from 2.0 to 1.5 equivalents while still controlling bis-substitution impurity **19** to ~3% (Scheme 9). Further cut (1.3 equivalents) resulted in a much slower reaction and noticeably increased **19** (4%). Reducing solvent from 10 to 3 volumes not only improved volume productivity but also the reaction rate. Under the optimized reaction conditions (1.5 equiv Cs_2CO_3, 1.5

equiv DCAP, 3 vol dioxane, 100 °C), the reaction mixture was typically composed of 95% of **5**, 3% of **19**, and two unidentified impurities at 1% and 0.6% (normalized area% excluding DCAP peak).

To minimize consumption of valuable boronate **9** in the subsequent Suzuki-coupling step, removal of residual DCAP was crucial. Since acid or base extractions was deemed unfeasible, the reaction mixture was solvent swapped to toluene and dilution with *n*-heptane to precipitate most of the DCAP for removal by filtration. Unfortunately, ~6 mol% DCAP still remained in the filtrate. Attempted crystallization of **5** in various organic solvents or aqueous systems was unsuccessful. Therefore, crystallization of **5** as a salt was explored. Only strong acids such as HCl, MsOH and *p*-TsOH were evaluated considering the weak basicity of **5** (predicted pKa = 0.98). Neither HCl nor *p*-TsOH was effective in forming a crystalline salt with **5**. In contrast, adding MsOH to a solution of **5** in toluene/dioxane produced a white precipitate nearly instantly. XRPD data of the filtered and dried solid confirmed its crystallinity. The ^{1}HNMR spectrum showed 1 : 1 : 1 molar ratio of **5** : MsOH : toluene, suggesting that toluene was incorporated into the crystal lattice. Indeed, no appreciable toluene loss was observed when the solid was dried *in vacuo* at 45 °C. Under more forceful drying conditions (85 °C *in vacuo*), the powdery solid shrunk, melted and turned into a glassy solid upon cooling. Similarly, suspending the solid in warm heptane (60 °C) to remove toluene also gave a gummy solid. Thermal Gravimetric Analysis (TGA) showed 16% weight-loss between 70–180 °C, consistent with the calculated value of 15.4% for 1.0 equivalent toluene.

Scheme 9 S_NAr substitution of **3** and DCAP



The optimal toluene/dioxane ratio for the crystallization was determined to be 5/1 based on solubility data and impurity purging capacity. Thus, after completion of the reaction, the reaction mixture was concentrated, flushed with toluene and filtered to remove the precipitated DCAP as well as inorganic salts. After adjusting the toluene/dioxane ratio to 5/1, a portion of MsOH (0.38 equiv) was charged at 50 °C to give a thin suspension. The remaining MsOH (0.76 equiv) was then slowly added and the mixture was cooled and aged to complete the crystallization. This isolation efficiently purged both DCAP and bis-substitution impurity **19**, furnishing **5** in 87% yield (35.8 kg) with 99.7% purity.

*Suzuki-coupling to penultimate intermediate (**17**).* As mentioned earlier, developing a scalable Suzuki-coupling of pyrimidine chloride **5** with boronate **9** was crucial for the success of the second-generation process route (Scheme 10). Initial catalyst screening reactions were carried out in refluxing EtOH/H_2O using crude pyrimidine chloride **5** with $KHCO_3$ as the base (Table 6). While the reactions were still sluggish, they were more promising than when L-BPA (**5**) was used in medicinal chemistry route. Perhaps this was due to decreased substrate inhibition when the amino group was protected by a Boc-group. With 2 mol% POPd6 or PXPd4, after refluxing for 16 h, the conversions were 21% and 16%, respectively (entries 1, 2). Catalyst Pd(dppf)Cl_2 was slightly more active, giving 28% conversion in 16 h (entry 3). However, a dramatically higher level of amination impurity **20** was observed (20% vs ~0.2%). Relatively inexpensive Pd(PPh_3)$_2Cl_2$ performed the best, giving 34% conversion in 16 h and 62% in 40 h (entry 4). After additional 1.0 equivalent of boronate **9**, the reaction reached completion in 65 h. With 1.5 equivalents of **9**, the conversion reached 70% after 40 h (entry 5).

Scheme 10 Suzuki coupling of pyrimidine chloride **5** and boronate **9**

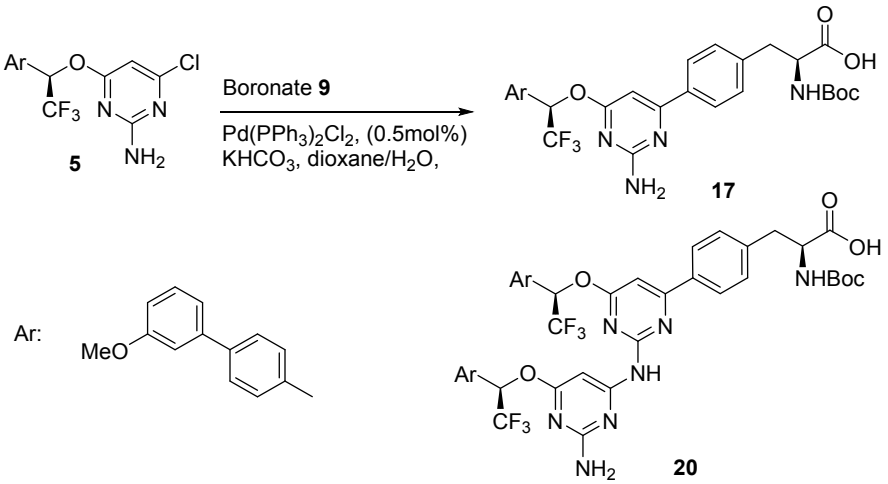


Table 6 Catalyst Screening in EtOH/H_2O/$KHCO_3$

Entry	Catalyst	Boronate **9** (equiv)	Time (h)	Conv (%)
1	POPd6	1.0	16	21
2	PXPd4	1.0	16	16
3	$Pd(dppf)Cl_2$	1.0	16 40	28 49
4	$Pd(PPh_3)_2Cl_2$	1.0 +1.0	40 +65	62 100
5	$Pd(PPh_3)_2Cl_2$	1.5	40	70

*2 mol% catalyst, 3.5 equiv $KHCO_3$, EtOH/H_2O, reflux (75 °C)

To achieve acceptable reaction rate for production, higher boiling solvent systems were evaluated (Table 7). Reaction in refluxing *i*-BuOH/H_2O for 24 h gave 31% conversion (entry 1). In contrast, the reaction in refluxing 5 : 1 dioxane : water reached completion in 16 h (entry 2). Decreasing charge of boronate **9** to 1.3 equivalents led to much longer reaction time (48 h) (entry 3).

The reaction condition was further optimized to enhance the reaction rate. Decreasing the dioxane : water ratio from 5 : 1 to 2 : 1 (total solvent volume = 9 vol) markedly increased the reaction rate, reducing the reaction time from 12 h to 3–4 h (**Figure 4**). Interestingly, further decreasing dioxane : water ratio to 1 : 1 led to a slower reaction. This was likely due to oiling of substrate **5** at the outset.

Another surprising observation was that the reaction was faster under more dilute conditions. For example, increasing total solvent volume from 7.5 to 12 volumes (2 : 1 dioxane : water) shorten the reaction time from 10 h to 6 h (**Figure 5**). Not surprisingly, with slow agitation, the reaction required ~15 h to reach completion compared to 8 h with vigorous stirring (**Figure 6**).

Table 7 Screening of higher boiling solvent systems

Entry	Solvent (15/3 vol)	Boronate **9** (equiv)	Time (h)	Conv (%)
1	*i*-BuOH/H_2O	1.5	24	31
2	dioxane/H_2O	1.5	16	100
3	dioxane/H_2O	1.3	48	98

Conditions: 2 mol% $Pd(PPh_3)_2Cl_2$, 3.5 equiv $KHCO_3$, reflux.

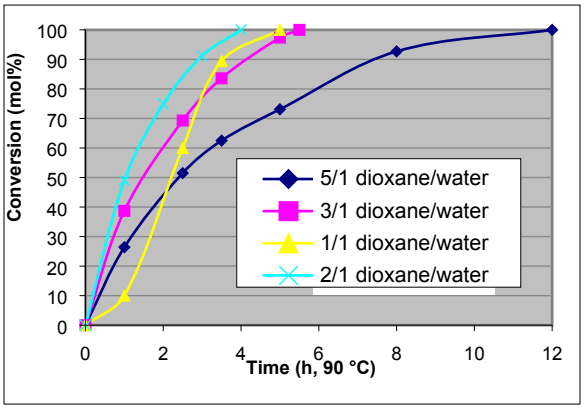


Figure 4. Effects of dioxane : water ratio

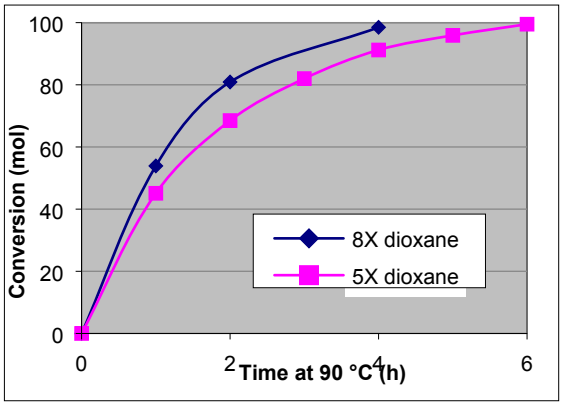


Figure 5. Effect of solvent volume

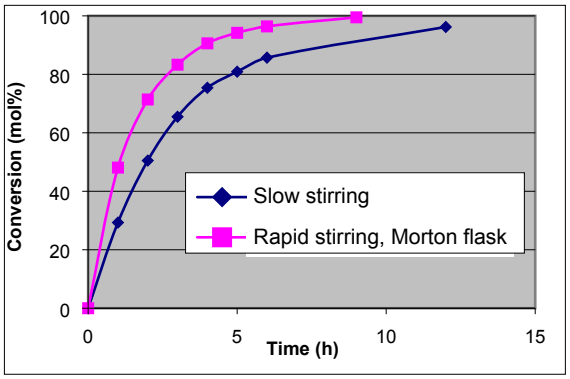


Figure 6. Effect of agitation

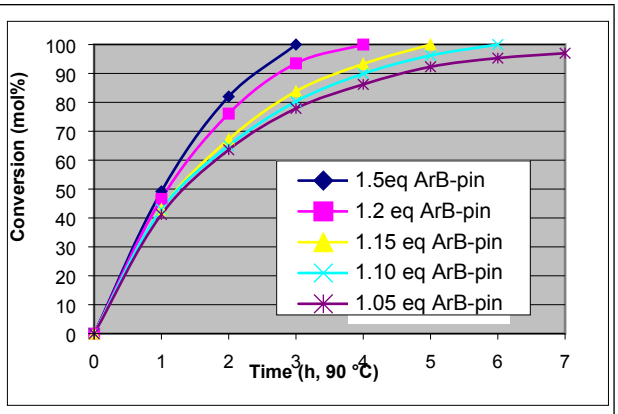


Figure 7. Effect of boronate **9** equivalents

These observations could be rationalized by the efficiency of bring the two coupling partners into contact in the biphasic reaction mixture. It was observed that boronate **9** partitioned mostly in the aqueous phase while pyrimidine chloride **5** in the organic phase. Lower concentration or lower dioxane : water ratio decreased the ionic strength of the aqueous layer which should lead to better miscibility of the two phases. On the other hand, at a higher concentration, pyrimidine chloride **5** became a significant portion of the organic phase and acted like a hydrophobic solvent, thereby decreasing miscibility of the two phases.

Another operational issue was the aerosolization of boronate **9** while being charged into $KHCO_3$ by the CO_2 generated. This risk was mitigated by replacing $KHCO_3$ (3.5 equiv) with K_2CO_3 (1.75 equiv).[16] Under the optimized reaction conditions (0.5 mol% $Pd(PPh_3)_2Cl_2$, 1.75 equiv. K_2CO_3, 9 vol 2 : 1 dioxane : water, reflux), boronate charge could be reduced from 1.5 to 1.1 equivalents while still achieving complete conversion in 6–8 h (**Figure 7**). This constituted a substantial cost saving for the second-generation process.

With higher purity of the crystalline **5**·MsOH, the Suzuki reaction was expected to proceed more rapidly. To the contrary, the reaction was noticeably slower, requiring ~10 h (vs ~4 h) even after

adjusting the K_2CO_3 charge to compensate for the neutralization of MsOH. This was attributed to less effective mixing of the biphasic mixture due to higher ionic strength of the aqueous layer (potassium mesylate salt). Indeed, by performing an in-situ free-basing of the mesylate salt with 0.6 equivalents of K_2CO_3 in dioxane : water and removing the aqueous layer, the typical reaction rate was restored.

As for the workup and isolation, the reaction mixture was cooled, acidified with 5 *N* HCl to pH 2–3, and saturated with NaCl to aid the phase separation. The organic layer was treated with activated carbon and solvent swapped to acetonitrile to crystallize product **17**. The main drawbacks of this process included a slow phase separation and tedious solvent swap from higher boiling 1,4-dioxane to lower boiling acetonitrile. Therefore, a more streamlined process was developed where the reaction mixture was treated with activated carbon, and then acidified with aq. HCl to pH 2–3 to directly crystallize **17** in 83% yield with ~98% purity. Residual Pd levels were somewhat higher and more variable (100–1000 ppm) than the previous workup procedure (< 100 ppm). Retrospective data analysis revealed an inverse correlation between residual palladium and reaction rates, i.e. lower residual palladium levels were correlated to faster reactions. In these cases, the reaction mixtures also turned darker more rapidly after reaction completion. This phenomenon suggested that once pyrimidine chloride **5** was completely consumed, the catalyst became unstable and began to precipitate as palladium black. Indeed, by extending the aging time by additional 3–5 h after reaction completion, residual palladium levels were consistently below 100 ppm in the isolated product **17**.

Deprotection and isolation of LX1031. Previously, the deprotection was carried out in THF and aqueous HCl. As THF might not be completely stable to the strongly acidic conditions, it was advantageous to conduct the deprotection without it. The reaction was sluggish in 2 *N* aqueous HCl at 50 °C but much more expeditious at 60 °C (2–3 h). For safety and practicality in the plant, penultimate **17** was charged in portions over a period of 2 h to avoid excessive foaming of the reaction mixture

and aerosolization of powdery **17** due to vigorous CO_2 evolution. With higher purity penultimate **17** (~98%) from the second-generation process route, high quality LX1031 zwitterion could be isolated directly, obviating the need for purity upgrade via tosylate salt formation. Thus, upon reaction completion, the reaction mixture was cooled, partially (~60%) neutralized with 50% aqueous NaOH, and then further neutralized to pH 6.5 after adding THF (5 vol). The organic layer was separated and flushed with acetonitrile to crystallize LX1031 acetonitrile solvate monohydrate which upon drying furnished amorphous LX1031 zwitterion in 86% yield (20 kg scale) and 98.7% purity.

CONCLUSIONS

In summary, two process routes were developed to produce LX1031, a drug candidate for the treatment of irritable bowel syndrome. They shared the key intermediates biaryl chiral alcohol (**3**) and L-tyrosine derived boronate (**9**), as well as the penultimate intermediate (**17**). Compound **3** was synthesized via asymmetric hydrogenation of trifluoromethyl ketone **1** followed by Suzuki coupling with 3-methoxyphenylboronic acid (**11**). The boronate intermediate (**9**) was synthesized via borylation of triflate of Boc-L-tyrosine methyl ester with pin_2B_2. The first-generation process route assembled the target molecule from right-hand to left-hand side while the second-generation process from left-hand to right-hand side to give the same penultimate intermediate (**17**). For the first-generation route, the challenges in the isolation and purification of LX1031 zwitterion were overcome by isolating LX1031 tosylate dihydrate followed by free basing and crystallization of LX1031 acetonitrile solvate. With higher quality penultimate intermediate **17** in the second-generation synthesis, high purity LX1031 zwitterion was isolated directly. The second-generation route not only employed cleaner reactions but was also more productive (62% vs 36% overall yield). Both process routes had been carried out on multi-kg scales to provide LX1031 drug substance for pre-clinical and early clinical programs.

EXPERIMENTAL SECTION

General. All reagents and materials were used as received, unless otherwise noted. Reactions were monitored by reverse-phase HPLC using a C18 or phenyl-hexyl column with water/MeCN or water/MeOH mobile phase and TFA as a modifier. Melting points were determined by using a melting point apparatus or based on the peak temperatures of the endotherm on a differential scanning calorimeter (DSC). NMR spectra were acquired in deuterated solvents with a 300 or 400 MHz (^{1}H) NMR spectrometer. Mass spectrometry data were obtained during LC-MS analysis. Compound purity data were determined by reverse phase HPLC analysis.

First-generation synthesis:

*(R)-1-(4-bromophenyl)-2,2,2-trifluoroethan-1-ol (**2**).* A solution of 1-(4-bromophenyl)-2,2,2-trifluoroethan-1-one (**1**) (290 kg, 1146 mol, 1.00 equiv) in IPA (341 kg) was hydrogenated at 45 °C under 30 psi hydrogen pressure in the presence of (*R*)-Xyl-p-Phos $RuCl_2$ (*R*)-Daipen catalyst (178 g, 0.013 mol%) and *t*-BuOK (498 g, 0.4 mol%) until reaction completion (~20 h). The reaction mixture was concentrated to an oil, diluted with *n*-heptane (595 kg), washed with water (358 kg) followed by 20% aq. NaCl (174 kg). The organic layer was treated with activated carbon (58.0 kg) and diatomaceous earth (58 kg) at 65–70 °C for 1 h and then filtered at 60–70 °C. The filter-cake was washed with *n*-heptane and the combined filtrate concentrated to an oil. The latter was diluted with *n*-heptane (595 kg) and heated to 65–70 °C to dissolve the solid. The resulting solution was slowly cooled to 5–10 °C and aged for 5 h. The crystallized product was filtered, washed with cold *n*-heptane (48 kg, ~0 °C), and dried to give 164.8 kg of the chiral alcohol (**2**) as a white solid, 56.4% yield, 99.9% purity (HPLC area), 99.9% ee (chiral HPLC); mp 58 °C; ^{1}H NMR (300 MHz, $CDCl_3$) 2.80 (d, J = 4.4 Hz, 1H), 5.00 (qd, J = 6.5, 4.7 Hz, 1H), 7.35 (d, J = 8.2 Hz, 2H), 7.55 (ddd, J = 8.4, 2.5, 2.0 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) 72.2 (q, $J_{C–F}$ = 32 Hz), 123.8, 123.9 (q, $J_{C–F}$ = 281 Hz), 129.0, 132.8, 132.7.

*(R)-2,2,2-trifluoro-1-(3'-methoxy-[1,1'-biphenyl]-4-yl)ethan-1-ol (**3**)*. A suspension of $Pd(PPh_3)_2Cl_2$ (0.33 kg, 0.1 mol%) in EtOH (1 kg) was added to a mixture of K_2CO_3 (195.2 kg, 3.1 equiv), water (241 kg), EtOH (435 kg), and the chiral alcohol (**2**) (117.3 kg, 1.0 equiv). A solution of 3-methoxyphenylboronic acid (**11**) (86.0 kg, 1.2 equiv) in EtOH (433 kg) was then added at 75 °C and the reaction mixture was aged until reaction completion. Water (433 kg) was added and the mixture concentrated to ~425 L at 60–70 °C. The residue was cooled to 20–25 °C and extracted with MTBE (360 kg). The organic layer was washed sequentially with aq. NaOH (18.5 kg NaOH in 180 kg water),

water (120 kg), 20% aq NaCl (141 kg), and then treated with activated carbon (12 kg) at 50 °C for 2 h. The mixture was filtered at 35–40 °C and the filter-cake washed with MTBE (2 × 266 kg). The combined filtrate was concentrated to ~375 L and flushed with *n*-heptane (2 × 258 kg) with a final volume of ~475 L. It was cooled 20 °C, aged for 6 h and filtered. The solid was dried at 45–50 °C under reduced pressure to give 120.9 kg of the biaryl chiral alcohol (**3**) as a white solid, 93.1% yield, 99.9% purity (HPLC); mp 107.6 °C; [α] = -31.85 (c 1.067, ethanol); LC-MS (ESI): $[M+H]^+$ = 283.1; ^{1}H NMR (400 MHz, $CDCl_3$) 3.88 (s, 3H), 5.08 (q, *J* = 6.6 Hz, 1H), 6.96 (ddd, *J* = 8.2, 2.5, 1.2 Hz, 1H), 7.15 (t, *J* = 2 Hz, 1H), 7.20 (dt, *J* = 7.9, 1.2 Hz, 1H), 7.39 (t, *J* = 7.6 Hz, 1H), 7.55 (d, *J* = 8.1 Hz, 2H), 7.64 (d, *J* = 8.4 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) 55.3, 72.7 (q, J_{C-F} = 32 Hz), 113.0, 113.1, 119.7, 124.3 (q, J_{C-F} = 281 Hz), 127.4, 127.8, 129.9, 133.0, 141.9, 142.4, 160.0; ^{19}F NMR ($CDCl_3$) −78.3 (d, *J* = 6.4 Hz); Elemental Anal. calcd for $C_{15}H_{13}F_3O_2$: C, 63.83; H, 4.64. Found: C, 63.78; H, 4.60.

*Methyl (S)-2-((tert-butoxycarbonyl)amino)-3-(4-(((trifluoromethyl)sulfonyl)oxy)phenyl)propanoate (**12**).* Triflate anhydride (156 kg, 1.09 equiv) was slowly added to a mixture of Boc-L-tyrosine methyl ester (150 kg, 1.0 equiv) and *N*-methylmorpholine (81.0 kg, 1.58 equiv) in DCM (1500 kg) at −10 to 0 °C. The reaction mixture was quenched into 10% aq. citric acid (825 kg) at 5–10 °C. The aqueous layer was extracted with DCM (362 kg) and the combined organic layer washed sequentially with 10% aq. citric acid (825 kg), water (3 × 800 L), and then dried over Na_2SO_4 (50 kg) and filtered. The filter-cake was washed with acetonitrile (127 kg). The combined filtrate was concentrated to ~200 L, and flushed with acetonitrile (321 kg) to give 263 kg of a solution of triflate **12**, 95.5% yield corrected for 78.9% w/w assay, 99.2% purity (HPLC area). An analytical sample was obtained by concentrating the solution to a solid. mp 50.6 °C; LC-MS calcd $[M+NH_4]^+$ 445.1; found m/z 445.0; ^{1}H NMR (400 MHz, $CDCl_3$) 1.41 (s, 9H), 3.04 (dd, *J* = 13.7, 6.3 Hz, 1H), 3.16 (dd, *J* = 13.7, 5.7 Hz, 1H), 3.71

(s, 3H), 4.42 (br, 0.13H, minor rotamer), 4.60 (br d, J = 6.3 Hz, 1H), 4.87 (br, 0.12H, minor rotamer), 5.05 (br d, J = 7.1 Hz, 1H), 7.17–7.28 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) 28.2, 37.9, 52.3, 54.2, 80.2, 118.7 (q, J_{C-F} = 321 Hz), 121.3, 131.1, 136.9, 148.6, 154.9, 171.9.

(S)-2-((tert-butoxycarbonyl)amino)-3-(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)-propanoic acid (**9**). *(a) borylation*: Tricyclohexylphosphine (2.65 kg, 1.9 mol%), palladium acetate (0.99 kg, 0.90 mol%) and triflate **12** (262.6 kg, 78.9 wt% in acetonitrile, 207.2 kg active) were added to a mixture of bis(pinacolato)diboron (136.0 kg, 1.10 equiv) and potassium acetate (95.8 kg, 2.0 equiv) in acetonitrile (653 kg) under nitrogen. The mixture was stirred at refluxing temperature until reaction completion (5 h), cooled to 30–40 °C and filtered through a pad of cellulose powder (21.5 kg). The filter-cake was washed with acetonitrile. The combined filtrate was concentrated to ~260 L, and flushed with MTBE (2 × 449 kg). The distillation residue was diluted with MTBE (658 kg) and water (300 L) with stirring. The aqueous layer was separated and extracted with MTBE twice (348 kg; 120 kg). The combined organic layer was washed with water (2 × 295 kg), filtered through a pad of Na_2SO_4 (31 kg) and concentrated to ~600 L below 45 °C to give solution of methyl ester of **9** in MTBE (405.6 kg 48.8 wt%, yield 100%, 96.7% purity HPLC area). *(b) Hydrolysis of the methyl ester*: An aqueous solution of LiOH (41.4 kg LiOH monohydrate, 5.3 equiv; 960 kg water) was added to a solution of the methyl ester in MTBE (132.5 kg active, 467.4 kg solution) at 0 °C. The mixture was aged for 0.5 h and the aqueous layer was separated, washed with MTBE (2 × 198 kg), then filtered through a pad of cellulose powder. The combined organic layer was extracted with water (200 kg) and the aqueous layer washed with MTBE (193 kg) then filtered through the pad of cellulose powder above and combined with the main batch. MTBE (370 kg) was added to the combined aqueous layer and the mixture acidified with 3% aq. HCl until pH 2.5–3.5 at 0 °C. The organic layer was separated and aqueous layer extracted with MTBE (2 × 370 kg). The combined organic layer was washed with water

(352 kg), filtered through a pad of anhydrous Na_2SO_4 (26.4 kg), concentrated to ~460 L, and flushed with heptane (2 × 400 kg). Additional heptane (547 kg) was added and the mixture cooled to 5 °C and aged to crystallize the product. The suspension was filtered; the filter-cake was washed with heptane and then dried under reduced pressure at 40–45 °C to give 119.1 kg of boronate **9**, 89% yield corrected for 96 wt% assay; 99.9% purity (HPLC area); 100% ee; mp 132 °C; ^{1}H NMR (300 MHz, $CDCl_3$, mixture of rotamers) 1.34 (s, 12H), 1.42 (br s, 9H), 3.06–3.26 (m, 2H), 4.40 (br, 0.15H), 4.60 (br q *J* = 5.7 Hz, 0.7H), 4.91 (br d, *J* = 7.2 Hz, 0.7H), 5.91 (br s, 0.15H), 9.20 (d, *J* = 7.4 Hz, 2H), 7.76 (br d, *J* = 7.8 Hz, 2H); ^{13}C NMR (75.5 MHz, $CDCl_3$) 24.8, 28.3, 37.7, 54.2, 80.4, 83.9, 128.8, 135.1, 139.0, 155.4, 175.6.

(S)-3-(4-(2-amino-6-chloropyrimidin-4-yl)phenyl)-2-((tert-butoxycarbonyl)amino)propanoic acid (**10**). An aqueous solution of $KHCO_3$ (55.0 kg, 550 mol, 3.6 equiv in 350 kg water) was slowly added to a mixture of boronate **9** (60.0 kg, 153 mol, 1.0 equiv), 2-amino-4,6-dichloropyrimidine (DCAP) (75.3 kg, 3.0 equiv), PPh_3 (0.60 kg, 1.5 mol%), $Pd(OAc)_2$ (0.258 kg, 0.75 mol%), EtOH (350 kg), and THF (794 kg). The mixture was degassed, heated to 70 °C and aged until reaction completion (20 h). It was concentrated to 460 L, flushed with water (401 kg + 363 kg), then cooled to 20 °C, aged and filtered to remove precipitated DCAP. The filter-cake was washed with water (230 kg), and the combined filtrate washed twice with EtOAc (471 kg, 319 kg). Toluene (560 kg) and THF (720 kg) were added to the aqueous layer and the mixture was slowly acidified with 6 *N* HCl (~85 kg) to pH ~3 at ~20 °C. Solid NaCl (550 kg) was added and the mixture stirred to dissolve the solid. The organic layer was separated, and the aqueous layer extracted twice with EtOAc (2 × 470 kg). The combined organic layer was treated with activated carbon twice (2 × 30 kg) at 45 °C for 10 h, filtered through a pad of cellulose powder (10 kg) and Na_2SO_4 (15 kg). The filtrate was concentrated to ~180 L, flushed with toluene (186 kg), cooled to 0 °C, aged and filtered. The filter-cake was washed with toluene (120

kg) and dried at 40–45 °C under reduced pressure to give 51.5 kg of pyrimidine chloride **10** as a toluene solvate, 66% yield corrected for 77.7% assay (15.8 wt% toluene); 95.8% purity (HPLC area); mp 185 °C (decomposition); LC-MS calcd $[M+H]^+$ 393.1; found m/z 393.0; ^{1}H NMR (400 MHz, DMSO-d_6, mixture of rotamers) 1.25 (br s, 1.3H), 1.31 (br s, 7.7H), 2.89 (dd, J = 13.8, 10.6 Hz, 1H), 3.08 (dd, J = 13.8, 4.3 Hz, 1H), 4.08–4.18 (m, 1H), 7.13–7.18 (m, 3 H), 7.24 (s, 1H), 7.37 (d, J = 8.1 Hz, 2H), 8.01 (d, J = 8.3 Hz, 2H), 12.66 (br s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) 28.1, 36.2, 54.8, 78.1, 104.4, 126.8, 129.5, 133.9, 141.5, 155.4, 161.0, 163.5, 165.8, 173.4.

(S)-3-(4-(2-amino-6-((R)-2,2,2-trifluoro-1-(3'-methoxy-[1,1'-biphenyl]-4-yl)ethoxy)pyrimidin-4-yl)phenyl)-2-((tert-butoxycarbonyl)amino)propanoic acid (**17**). A suspension of pyrimidine chloride **10** (82.0 kg, assay corrected, 209 mol, 1.0 equiv) in 1,4-dioxane (248 kg) was added to a mixture of biaryl chiral alcohol **3** (70.9 kg, 252 mol, 1.20 equiv), Cs_2CO_3 (272 kg, 835 mol, 4.0 equiv) and 1,4-dioxane (485 kg) at 90–100 °C. The mixture was aged until reaction completion (15–20 h) and then cooled to 90 °C. Water (1008 kg) was added and the mixture cooled to 30 °C. Di-*tert*-butyl dicarbonate (6.8 kg, 31.2 mol, 0.15 equiv) was added and the mixture aged for 2 h. Toluene (403 kg) was added and the mixture stirred and then settled. The aqueous layer was removed and the organic layer was slowly acidified with 2 *N* HCl (120 L) to pH 3–4. The organic layer was washed with water (103 kg), distilled to ~550 L, and flushed with acetonitrile (620 kg + 1150 kg) with a final volume of ~1160 L. It was cooled to 20–25 °C, aged and filtered. The filter-cake was washed with acetonitrile (2 × 240 kg), and dried to give 85.9 kg of **17** as a white solid, 81% yield, 95.3% purity (HPLC area); LC-MS calcd $[M+H]^+$ 639.2; found m/z 639.3; mp 213 °C; ^{1}H NMR (400 MHz, DMSO-d_6, mixture of rotamers) 1.26 (br s, 1.3H), 1.32 (s, 7.7H), 2.90 (dd, J = 13.5, 10.7 Hz, 1H), 3.09 (dd, J = 13.6, 4.4 Hz, 1H), 3.82 (s, 3H), 4.16 (ddd, J = 10.0, 9.0, 4.2 Hz, 1H), 6.79 (br, 2H), 6.8–6.9 (m, 2H), 6.97 (dd, J = 8.3, 2.0 Hz, 1H), 7.15–7.27 (m, 3H), 7.32–7.42 (m, 3H), 7.67 (d, J = 8.3 Hz, 2H), 7.77 (d, J = 8.3

Hz, 2H), 8.01 (d, J = 8.1 Hz, 2H), 12.67 (br s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) 28.1, 36.2, 55.1, 66.4, 71.7, 78.1, 91.3, 112.4, 113.5, 119.2, 123.8 (q, $J_{C–F}$ = 282 Hz), 126.7, 127.3, 128.5, 129.3, 130.1, 130.7, 134.8, 140.9, 141.5, 155.5, 159.8, 163.0, 166.1, 168.5, 173.5; ^{19}F NMR (DMSO-d_6) −75.04 (d, $J_{H–F}$ = 6.9 Hz).

(S)-2-amino-3-(4-(2-amino-6-((R)-2,2,2-trifluoro-1-(3'-methoxy-[1,1'-biphenyl]-4-yl)ethoxy)pyrimidin-4-yl)phenyl)propanoic acid (LX1031 zwitterion THF solvate): Aq. HCl (45.5 kg, 32%, 399 mol, 3.0 equiv) was added to a mixture of Boc-acid **17** (84.7 kg, 132.6 mol, 1.0 equiv) in THF (226 kg) and water (27.3 kg) at 50–55 °C over 3 h with slow nitrogen sweep. The reaction mixture was aged until reaction completion (~4 h) then cooled to 30 °C. The pH of the reaction mixture was adjusted to 6.5–7.0 with 50% NaOH (51.5 kg, 386 mol, 2.9 equiv). The organic layer was separated, diluted with water (181 kg), seeded with a suspension of LX1031 THF solvate (0.34 kg) in water (2 kg), and aged for 12 h at 30 °C. Additional water (467 kg) was charged over ~3 h and the mixture aged for 2 h at 30 °C then 12 h at 20 °C. The resulting suspension was filtered (19 h) and the filter-cake washed with a mixture of THF/water (75 kg/212 kg) (32 h). The wet-cake was dried at 45 °C under reduced pressure (39 h) until loss on drying was < 10% to give 75.32 kg of LX1031 zwitterion, 91.4% yield corrected for 87.8% assay (KF = 8.5%, 95.3% HPLC area).

(S)-2-amonium-3-(4-(2-amino-6-((R)-2,2,2-trifluoro-1-(3'-methoxy-[1,1'-biphenyl]-4-yl)ethoxy)pyrimidin-4-yl)phenyl)propanoic acid toluenesulfonate dihydrate (LX1031·TsOH·2H_2O): LX1031 zwitterion (73 kg, 64.1 kg active, 119 mol) and TsOH·H_2O (24.2 kg, 127 mol, 1.07 equiv) were dissolved in a mixture of THF (233 kg) and water (29 kg) at 50–55 °C. Acetonitrile (57 kg) and water (4 kg) were then added and the mixture was cooled to 40–45 °C. LX1031·TsOH·2H_2O seeds (0.38 kg) and water (2 kg) were added and the mixture was aged at 40–45 °C for 1 h. A mixture of acetonitrile (513 kg) and water (31 kg) was added over 3 h. After aging for 2 h, the mixture was cooled

to 15–20 °C and aged for 12 h. The suspension was filtered, and the filter-cake washed with a mixture of acetonitrile (190 kg), THF (43 kg), and water (15 kg). The wet-cake was dried at 30 °C under reduced pressure to give 73.75 kg of LX1031·TsOH·2H_2O, 82.3% yield (99.5% HPLC area). LC-MS calcd $[M+H]^+$ 539.2, observed m/z 539.2; XRD crystalline; mp 239 °C (DSC peak); ^{1}H NMR (400 MHz, DMSO-d_6) 2.28 (s, 3H), 3.10–3.21 (m, 2H), 3.81 (s, 3H), 4.19 (t, J = 6.4 Hz, 1H), 6.77 (br s, 2H), 6.82–6.89 (m, 2H), 6.97 (ddd, J = 8.0, 2.5, 0.9 Hz, 1H), 7.11 (d, J = 7.7 Hz, 2H), 7.20 (t, J = 2 Hz, 1H), 7.24 (d, J = 7.5 Hz, 1H), 7.35–7.41 (m, 3H), 7.49 (d, J = 7.6 Hz, 2H), 7.66 (d, J = 8.1 Hz, 2H), 7.76 (d, J = 7.8 Hz, 2H), 8.05 (d, J = 8.3 Hz, 2H), 8–9 (br, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) 20.8, 35.7, 53.0, 55.1, 71.5 (q, $J_{C–F}$ = 32 Hz), 91.4, 112.4, 113.5, 119.2, 123.8 (q, $J_{C–F}$ = 280 Hz), 125.5, 127.0, 127.3, 128.1, 128.5, 129.8, 130.1, 130.7, 135.7, 137.5, 137.6, 140.8, 141.6, 145.7, 159.8, 163.0, 165.9, 168.5, 170.4.

*(S)-2-amino-3-(4-(2-amino-6-((R)-2,2,2-trifluoro-1-(3'-methoxy-[1,1'-biphenyl]-4-yl)ethoxy)pyrimidin-4-yl)phenyl)propanoic acid (**LX1031 zwitterion**)* from LX1031 tosylate:

Aq. NaOH (8%, ~17 kg) was slowly added to a mixture of LX1031·TsOH·H_2O (25.5 kg) in THF (456 kg) at 35 °C with stirring until pH reached 6.5. Aqueous 15% NaCl (280 kg) was added, and the organic layer was separated, washed with 15% NaCl (5 × 280 kg), flushed with acetonitrile (94 kg) with a final volume of 120 L. The distillation residue was diluted with acetonitrile (94 kg), aged at 60–65 °C for 1 h, then slowly cooled to 10–15 °C and aged for 15 h. The product was filtered, washed sequentially with acetonitrile (28 kg), water (190 kg + 36 kg) and acetonitrile (36 kg). It was then dried at 60–65 °C to give 16.7 kg of LX1031 zwitterion as an off-white solid, 90.7% yield, 99.8% purity (HPLC area). LC-MS, calcd $[M+H]^+$ 539.2, found m/z 539.2; XRD, amorphous; mp 255 °C (DSC peak, dec); ^{1}H NMR (400 MHz, DMSO-d_6) 2.93 (dd, J = 14.4, 8.1 Hz, 1H), 3.21 (dd, J = 14.3, 4.7 Hz, 1H), 3.48 (dd, J = 8.1, 4.8 Hz, 1H), 3.81 (s, 3H), 6.63 (s, 2H), 6.80 (s, 1H), 6.86 (q, J = 7.2 Hz, 1H),

6.96 (dd, J = 8.2, 2.4 Hz, 1H), 7.21 (t, J = 2.0 Hz, 1H), 7.24 (d, J = 7.5 Hz, 1H), 7.34–7.43 (m, 3H), 7.67 (d, J = 8.3 Hz, 2H), 7.76 (d, J = 8.3 Hz, 2H), 7.99 (d, J = 8.1 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) 36.6, 55.1, 55.2, 71.6 (q, $J_{C–F}$ = 32 Hz), 91.3, 112.4, 113.5, 119.2, 123.7 (q, $J_{C–F}$ = 278 Hz), 126.7, 127.3, 128.5, 129.8, 130.1, 130.8, 135.0, 140.1, 140.9, 141.6, 159.8, 163.1, 166.3, 168.5, 170.1; ^{19}F NMR (376 MHz, DMSO-d_6) −75.0.

2nd Generation synthesis:

*(R)-4-chloro-6-(2,2,2-trifluoro-1-(3'-methoxy-[1,1'-biphenyl]-4-yl)ethoxy)pyrimidin-2-amine mesylate (**5**)*: A mixture of biaryl chiral alcohol **3** (20.0 kg, 70.9 mol, 1.00 equiv), 4,6-dichloro-2-aminopyrimidine (**4**, 17.6 kg, 107 mol, 1.50 equiv) and cesium carbonate (34.8 kg, 107 mol, 1.50 equiv) in 1,4-dioxane (83 kg) was stirred at 100 °C until reaction completion (~5 h). The reaction mixture was cooled to 50 °C, concentrated to ~50 L, and flushed with toluene (52 kg × 2). Toluene (53 kg) and cellulose powder (7.4 kg) were added and the mixture was aged, filtered through a pad of cellulose powder (5.3 kg). The filter-cake was washed with toluene (142 kg), and dioxane (35 kg) was added to the combined filtrate (toluene/dioxane ~5:1 v/v). Methanesulfonic acid (2.6 kg, 27 mol, 0.38 equiv) was added and the mixture aged for 1 h at 50 °C to give a thin suspension. The remaining MsOH (5.2 kg, 54 mol, 0.76 equiv) was added over 2 h. After aging at 50 °C for 2 h, the mixture was slowly cooled to rt and aged for 3 h. The crystallized product was filtered, washed sequentially with 5:1 toluene/dioxane (55 kg) and toluene (91 kg), and then dried under reduced pressure at 40–50 °C to give 35.8 kg of **5**·MsOH·toluene as an off-white solid; 87% yield; 99.7% purity (HPLC area); LC-MS calcd $[M+H]^+$ 410.1, found m/z 410.1; mp 109 °C (DSC peak); ^{1}H NMR showed 1.0 equivalent of MsOH and toluene. ^{1}H NMR (400 MHz, $CDCl_3$) 2.37 (s, 3H, tol), 2.89 (s, 3H, MsOH), 3.86 (s, 3H), 6.40 (s, 1H), 6.68 (q, J = 6.5 Hz, 1H), 6.94 (d, J = 8.4 Hz, 1H), 7.10–7.22 (m, 5H), 7.25–7.30 (m, 1H), 7.36 (t, J = 7.8 Hz, 1H), 7.63 (s, 4H); Free base of **5**: ^{1}H NMR (400 MHz,

$CDCl_3$) 3.87 (s, 3H), 5.32 (br s, 2H), 6.33 (s, 1H), 6.57 (q, J = 6.82 Hz, 1H), 6.93 (dd, J = 8.4, 2.5 Hz, 1H), 7.12 (t, J = 2 Hz, 1H), 7.18 (d, J = 7.4 Hz, 1H), 7.37 (t, J = 7.9 Hz, 1H), 7.57 (d, J = 8.2 Hz, 2H), 7.62 (d, J = 8.2 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) 55.3, 73.3 (q, J_{C-F} = 34 Hz), 97.4, 113.0, 113.1, 119.7, 123.3 (q, J_{C-F} = 280 Hz), 127.4, 128.4, 129.9, 130.4, 141.6, 142.6, 160.0, 161.77, 161.79, 169.0.

(S)-3-(4-(2-amino-6-((R)-2,2,2-trifluoro-1-(3'-methoxy-[1,1'-biphenyl]-4-yl)ethoxy)pyrimidin-4-yl)phenyl)-2-((tert-butoxycarbonyl)amino)propanoic acid (**17**). To a mixture of 1,4-dioxane (165 kg), water (30 kg) and K_2CO_3 (4.5 kg, 32.6 mol, 0.60 equiv), was added **5**·MsOH·toluene (30.0 kg, 51.4 mol, 1.0 equiv). The mixture was stirred until all solid dissolved. The aqueous layer was removed, K_2CO_3 (10.5 kg, 76.0 mol, 1.50 equiv), water (78.0 kg), and boronate **9** (24 kg, 55.7 mol, 1.08 equiv) were added and the mixture was degassed by vacuum-nitrogen fill cycles. $PdCl_2(PPh_3)_2$ (190 g, 0.27 mol, 0.5 mol%) was added and the mixture was degassed again. The mixture was heated to reflux (85–90 °C) and aged with vigorous agitation (~5 h) until reaction completion. It was aged for additional 3 h, cooled to 40 °C, treated with activated carbon (6.8 kg) at 45 °C for 4 h, cooled to 20–25 °C, and filtered through a pad of cellulose powder. The filter-cake was washed with 1/1 ACN/water (90 kg). The combined filtrate was heated to 40 °C, acidified to pH 5–6 with 5 *N* HCl (10 kg), seeded (0.18 kg) and aged for 0.5 h. It was then slowly acidified with 5 *N* HCl (~8 kg) to pH 2.0–3.0, aged for 3 h, cooled to 10–15 °C, aged for 4 h and then filtered. The filter-cake was washed sequentially with 1/1 ACN/water (90 kg), ACN (72 kg), and then dried to give 28.0 kg of Boc-acid **17** (83% yield, 98% purity by HPLC area).

(S)-2-amino-3-(4-(2-amino-6-((R)-2,2,2-trifluoro-1-(3'-methoxy-[1,1'-biphenyl]-4-yl)ethoxy)pyrimidin-4-yl)phenyl)propanoic acid (LX1031 zwitterion). Boc-acid **17** (27.0 kg active, 42.3 mol, 1.0 equiv) was added in portions to 2 *N* HCl (134 kg, 255 mol, 6.0 equiv) at 60 °C over 2 h. The mixture was aged at 60 °C until reaction completion (1–2 h) then cooled to 30 °C. With vigorous

stirring, NaOH (50% aq., 12.6 kg, 154 mol, 3.6 equiv) was added at 30–45 °C followed by THF (120 kg) and additional NaOH (50% aq., ~10 kg, 122 mol, 2.9 equiv) until pH 6.0–6.5. The organic layer was separated, concentrated to ~120 L and flushed with acetonitrile (2 × 110 kg). The distillation residue (~120 L) was heated to 60 °C, then slowly cooled to 10–15 °C and aged for 12 h. The suspension was filtered, washed sequentially with acetonitrile (42 kg), water (100 kg + 35 kg) and acetonitrile (41 kg). The filter-cake was dried under reduced pressure at 60 °C to give 20.4 kg of LX1031 zwitterion as a white solid, 86% yield corrected for 96.2 w% assay (98.7% purity by HPLC area).

ASSOCIATED CONTENT

Supporting Information. Copies of ^{1}H and ^{13}C NMR spectra for new compounds. Chiral HPLC data for chiral intermediates and final product.

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REFERENCES

[1] Tegaserod (Zelnorm™, by Novartis) for IBS-C, withdrawn in 2007; Alosetron (Lotronex™, by GSK) for IBS-D, withdrawn in 2000; reintroduced in 2002 with restriction.

[2] Gershon, MD. Serotonin and its implication for the management of irritable bowel syndrome. *Rev. Gastroenterol Disord.* **2003**, *3*, Suppl. 2: S25–34.

[3] Walther, D. J.; Peter, J-U; Bashammakh, S.; Hörtnagl, H.; Voits, M.; Fink, H.; Bader, M. Synthesis of serotonin by a second tryptophan hydroxylase isoform. *Science* **2003**, *299*, 76.

[4] Shi, Z-C; Devasagayaraj, A.; Gu, K.; Jin, H.; Marinelli, B.; Samala, L.; Scott, S.; Stouch, T.; Tunoori, A.; Wang, Y.; Zang, Y.; Zhang, C.; Kimball, S. D.; Main, A. J.; Sun, W.; Yang, Q.; Nouraldeen, A.; Yu, X-Q.; Buxton, E.; Patel, S.; Nguyen, N.; Swaffield, J.; Powell, D. R.; Wilson, A.;

Liu, Q. Modulation of peripheral serotonin levels by novel tryptophan hydroxylase inhibitors for the potential treatment of functional gastrointestinal disorders. *J. Med. Chem*. **2008**, *51*, 3684–3687.

[5] Burgoon, H. A.; Kanamarlapudi, R.; Pickersgill, I. F.; Shi, Z-C; Wu, W.; Zhang, H.; Zhao, M. M. Compounds useful in the preparation of tryptophan hydroxylase inhibitors. US patent 8,450,532 B2, **2013**.

[6] (a) McGarrity J. F.; Zanotti-Gerosa, A. A feasibility study on the synthesis of phenylephrine via ruthenium-catalyzed homogeneous asymmetric hydrogenation. *Tetrahedron: Asymmetry* **2010**, *21*, 2479-2486. (b) Palmer, A.; Webel, M.; Scheufler, C.; Haag, D.; Müller, B. Large-scale asymmetric synthesis of the 3,6,7,8-tetrahydrochromeno[7,8-d]imidazole BYK 405879: a promising candidate for the treatment of acid-related diseases. *Org. Process. Res. Dev*. **2008**, *12*, 1170–1182. (c) Ohkuma, T.; Koizumi, M.; Doucet, H.; Pham, T.; Kozawa, M.; Murata, K.; Katayama, E.; Yokozawa, T.; Ikariya, T.; Noyori, R. Asymmetric hydrogenation of alkenyl, cyclopropyl, and aryl ketones. $RuCl_2$(xylbinap)(1,2-diamine) as a precatalyst exhibiting a wide scope. *J. Am. Chem. Soc*. **1998**, *120*, 13529–13530. (d) Kuroki, Y.; Sakamaki, Y.; Iseki, K. Enantioselective rhodium(I)-catalyzed hydrogenation of trifluoromethyl ketones. *Org. Lett.* **2001**, *3*, 457–459. (e) Jeulin, S.; de Paule, S. D.; Ratovelomanana-Vidal, V.; Geˆnet, J.-P.; Champion, N.; Dellis, P. Difluorphos, an electron-poor diphosphane: a good match between electronic and steric features. *Angew. Chem., Int. Ed*. **2004**, *43*, 320–325. (f) Sterk, D.; Stephan, M.; Mohar, B. Highly enantioselective transfer hydrogenation of fluoroalkyl ketones. *Org. Lett*. **2006**, *8*, 5935–5938.

[7] (a) Burger, M. T.; Pecchi, S.; Wagman, A.; Ni, Z. J.; Knapp, M.; Hendrickson, T.; Atallah, G.; Pfister, K.; Zhang, Y.; Bartulis, S.; Frazier, K.; Ng, S.; Smith, A.; Verhagen, J.; Haznedar, J.; Huh, K.;

Iwanowicz, E.; Xin, X.; Menezes, D.; Merritt, H.; Lee, I.; Wiesmann, M.; Kaufman, S.; Crawford, K.; Chin, M.; Bussiere, D.; Shoemaker, K.; Zaror, I.; Maira, S. M.; Voliva, C. F. Identification of NVP-BKM120 as a potent, selective, orally bioavailable class I PI3 kinase inhibitor for treating cancer. *ACS Med. Chem. Lett.*, **2011**, *2*, 774–779. (b) Goldberg, D. R.; Lombaert, S. D.; Aiello, R.; Bourassa, P.; Barucci, N.; Zhang. Q.; Paralkar. V.; Valentine, J.; Zavadoski, W. Discovery of spirocyclic proline tryptophan hydroxylase-1 inhibitors. *Biorg. Med. Chem. Lett*., **2016**, *26*, 1124–1129.

[8] (a) Nakamura, H.; Fujiwara, M.; Yamamoto, Y. A concise synthesis of enantiomerically pure L-(4-boronophenyl)alanine from L-tyrosine. *J. Org. Chem*. **1998**, *63*, 7529–7530. (b) Malan, C.; Morin, C. A concise preparation of 4-borono-L-phenylalanine (L-BPA) from L-phenylalanine. *J. Org. Chem*. **1998**, *63*, 8019–8020.

[9] (a) See ref 6a. (b) Iimura, S.; Wu, W. Palladium-catalyzed borylation of L-tyrosine triflate derivative with pinacolborane: practical route to 4-borono-l-phenylalanine (L-BPA) derivatives. *Tetrahedron Lett*. **2010**, *51*, 1353–1355.

[10] Shieh, W. C.; Carlson, J. A. A simple asymmetric synthesis of 4-arylphenylalanines via palladium-catalyzed cross coupling reaction of arylboronic acids with tyrosine triflate. *J. Org. Chem*. **1992**, *57*, 379–381.

[11] (a) Chen, L. S.; Chen, G. J.; Tamborski, C. Regiospecific synthesis of aromatic compounds via organometallic intermediates. I. para-Substituted benzenes. *J. Organomet. Chem.* **1983**, *251*, 139–148. (b) Xavier, C. Reaction of organometallic reagents with ethyl trifluoroacetate and diethyl oxalate. Formation of trifluoromethyl ketones and α-keto esters via stable tetrahedral adducts. *J. Org. Chem.* **1987***, 52*, 5026–5030.

[12] (a) Corey, E. J.; Bakshi, R. K.; Shibata, S. Highly enantioselective borane reduction of ketones catalyzed by chiral oxazaborolidines. Mechanism and synthetic implications. *J. Am. Chem. Soc*., **1987**, *109*, 5551–5553; (b) Corey, E. J.; Bakshi, R. K.; Shibata, S.; Chen, C. P.; Singh, V. K. A Stable and easily prepared catalyst for the enantioselective reduction of ketones. Applications to multistep syntheses. *J. Am. Chem. Soc*. **1987**, *109*, 7925–7926; (c) O'Shea, P. D.; Chen, C-Y; Gauvreau, D.; Gosselin, F.; Hughes, G.; Nadeau, C.; Volante, R. P. A practical enantioselective synthesis of odanacatib, a potent cathepsin K inhibitor, via triflate displacement of an α-trifluoromethylbenzyl triflate. *J. Org. Chem*. **2009**, *74*, 1605–1610.

[13] (a) Ramachandran, P. V.; Teodorovic´, A. V.; Brown, H. C. Chiral synthesis via organoboranes. 38. Selective reductions. 48. Asymmetric reduction of trifluoromethyl ketones by *B*-chlorodiisopinocampheylborane in high enantiomeric purity. *Tetrahedron* **1993**, *49*, 1725–1738. (b) Brown, H. C.; Ramachandran, P. V. Asymmetric reduction with chiral organoboranes based on α-pinene. *Acc. Chem. Res.* **1992**, *25*, 16–24. (c) Brown, H. C.; Ramachandran, P. V. Recent advances in the boron route to asymmetric synthesis. *Pure Appl. Chem.* **1994**, *66*, 201–212. (d) Brown, H. C.; Ramachandran, P. V. Versatile α-pinene-based borane reagents for asymmetric syntheses. *J. Organometal. Chem*. **1995**, *500*, 1–19. (e) Zhao, M.; King, A. O.; Larsen, R.; Verhoeven, T. R.; Reider, P. J. A convenient and economical method for the preparation of DIP-chloride and its application in the asymmetric reduction of aralkyl ketones. *Tetrahedron Lett.* **1997**, *38*, 2641–2644.

[14] B-Rao, C; Kulkarni-Almeida, A.; Katkar, K. V.; Khanna. S.; Ghosh, U.; Keche, A.; Shah, P.; Srivastava, A.; Korde, V.; Nemmani, K. V. S.; Deshmukh, N. J.; Dixit, A.; Brahma, M. K.; Bahirat, U; Doshi. L.; Sharma. R.; Sivaramakrishnan, H. Identification of novel isocytosine derivatives as

xanthine oxidase inhibitors from a set of virtual screening hits. *Bioorg. Med. Chem*, **2012**, *20*, 2930–2939.

[15] The mechanism for the formation of **18** is not well understood

[16] Adding boronate **9** to excess K_2CO_3 resulting in $KHCO_3$ with minimal CO_2 evolution.