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Synthesis of a Long Acting HIV Protease Inhibitor *via* Metal or Enzymatic Reduction of the Appropriate Chloro Ketone and Selective Zinc Enolate Condensation with an Amino Epoxide

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Abstract: This paper describes a new convergent approach to the synthesis of an HIV protease inhibitor which was designed to be suitable in long acting formulations. Unique features in the synthesis include an asymmetric hydrogenation as well as enzymatic reduction of a key chloro ketone intermediate, to set the *threo* stereochemistry in the corresponding epoxide and the diastereoselective coupling of the latter with the zinc enolate of a suitable functionalized amide derivative.

Keywords: asymmetric ketone reduction; enzymatic ketone reduction; long acting HIV protease inhibitors; zinc enolate addition to epoxides

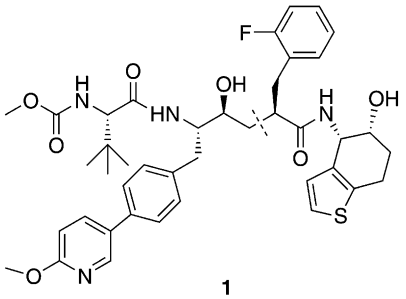
Introduction

In the last few decades, the treatment of HIV has made significant advances. The most recent data indicate that for those patients who comply with the current most successful triple combination therapy, their life expectancy, compared to that in the mid-1980s, has increased by an average of 15 years.[1] However, this is contingent on an early start and adherence to the therapeutic regimen. The latter is a particularly pressing problem and coupled with the significant cost of therapy it continues to be a challenge in controlling viral mutation.

In order to address these issues, long acting HIV protease inhibitors have been considered and are under development in our laboratories in collaboration with Medivir. One promising discovery program[2] has led to a number of potentially successful clinical candidates. However, the significant structural complexity remains a challenge to the initial successful preparation (e.g., for clinical development) and commercialization of such molecules.

Despite the fact that there are a large number of publications[3] describing successful strategies for the synthesis of this class of compounds, the need has increased to produce such molecules at even lower costs, thus making them accessible to a wider number of patients, especially in developing countries. To accomplish this, it has become imperative to devise even better synthetic approaches to these life-saving medicines than the innovative methods in the literature.

In this paper, we describe our efforts towards the convergent synthesis of clinical candidate **1**, a protease inhibitor specifically designed to allow for long acting-controlled release formulations.



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As mentioned above, a number of HIV protease inhibitors have been described in the literature in the last twenty years with several of them possessing the peptidomimetic motifs described in Figure 1 (A, B, C).[4] Access to these motifs was primarily achieved *via* a nucleophilic opening of epoxides I or II. The latter possesses the *erythro* stereochemistry which is the most easily accessible *via* diastereoselective reduction of the corresponding chloro ketone (e.g., **4**).[5] Synthesis of the *threo* analogue is more challenging as we will discuss below.

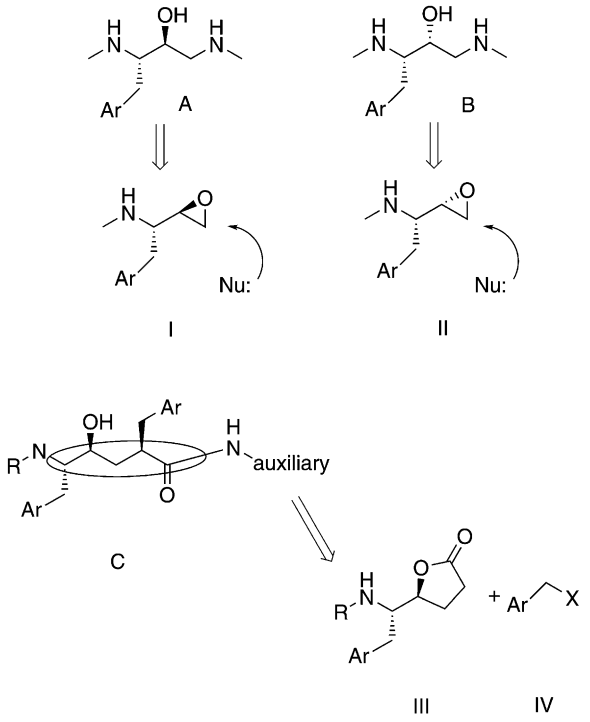
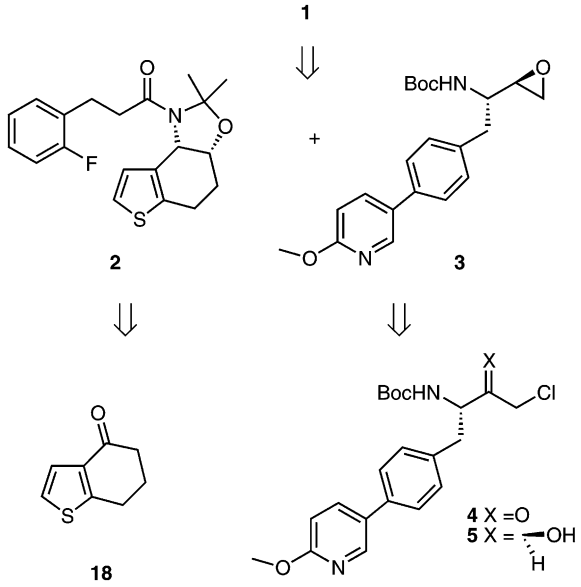


Figure 1. Motifs of HIV protease inhibitors and the preferred approach towards their synthesis.

This epoxide-opening approach is highly successful with heteroatom nucleophiles, such as amines, but it is less so with carbon nucleophiles (motif C, Figure 1), such as enolates, due to the lack of reactivity of the epoxide. To overcome this problem, more circuitous routes have been discovered (C⇒III + IV) where the stereochemistry of the α center can be controlled by employing kinetic or thermodynamic conditions in the alkylation of the lactone enolate depending on the desired stereochemical outcome.[6]

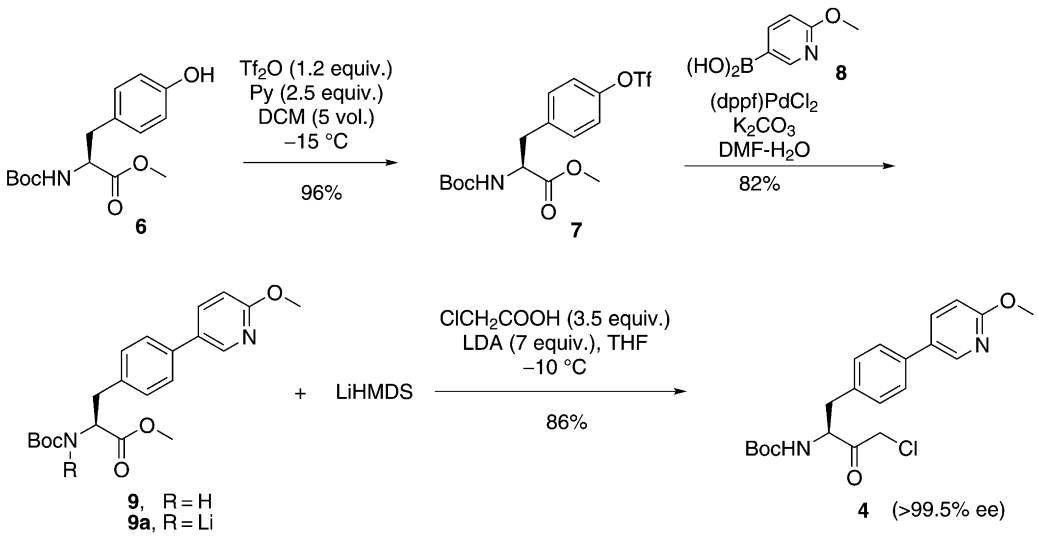
Our potential drug candidate (**1**) combines the most challenging features in this class of molecules, namely the less accessible *threo* stereochemistry of the amino alcohol center and the three stereogenic-center array found in motif C. Nonetheless, instead of employing the more linear alkylation approach, we decided to construct the molecule using a convergent approach *via* an enolate-epoxide opening (Scheme 1). In this synthetic strategy, the key features of the synthesis include the stereocontrolled preparation of the chloro ketone **4** and its challenging reduction to the *threo* alcohol **5**, and corresponding epoxide **3**. As the latter configuration has been such a challenge in the literature[7] we decided early on to devise both enzymatic and asymmetric hydrogenation methodologies to ensure that the most economically efficient synthesis is implemented from the beginning of the project. Finally, the strategic epoxide-enolate opening could be carried out only by use of the Zn-diamine enolate complexes we have reported more than a decade ago[8] and they have allowed us to form the key C–C bond with high efficiency. To the best of our knowledge this is the first time where such a synthetic strategy was employed with the exception of Crixivan[9] where the more reactive glycosidyl tosylate was utilized successfully. It is noteworthy that in our approach we can use the thiophene amino alcohol **22** to control the absolute configuration in a similar fashion to the now well-established aminoindanol in Crixivan



Scheme 1. Retrosynthetic analysis for the synthesis of **1**.

Results and Discussion

The preparation of the epoxide **3** started from protected tyrosine **6**, followed by activation of the phenol function as the triflate **7** with triflic anhydride in the presence of pyridine in high yield (Scheme 2). The activated intermediate was used without further purification in the Suzuki reaction. Unfortunately the published procedure[10] that worked well with simple arylboronic acids failed to give any product with our pyri-



Scheme 2. Preparation of the chloro ketone **4**.

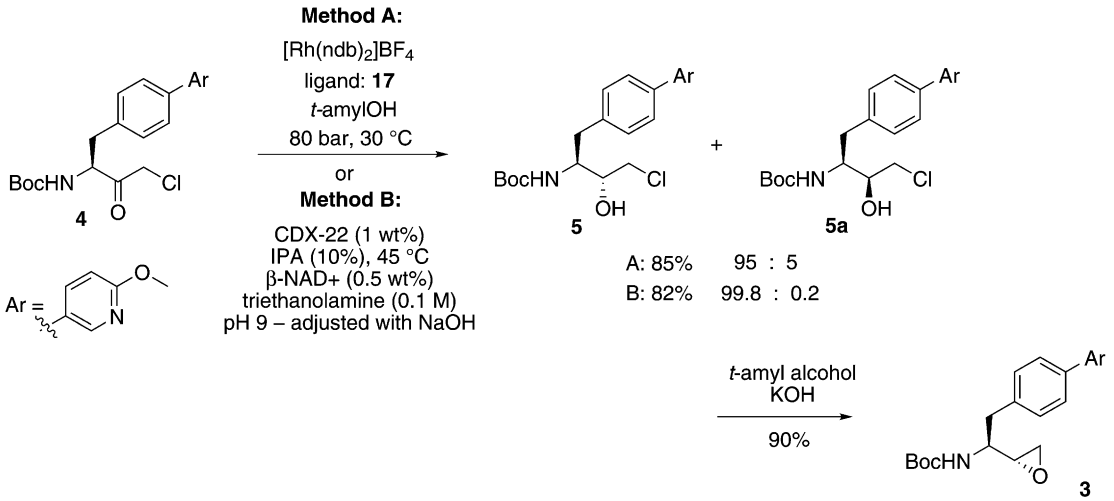
dylboronic acid derivative **8** and thus an investigation was undertaken to define the best ligand, solvent, base and catalyst precursors for this transformation. The study revealed that non-aqueous systems alone (MeOH, DME, toluene, DMF, dioxane) were not suitable for the reaction, but upon addition of water, a number of these same solvents resulted in useful conversions to the coupled product **9**. The commercially available, ferrocenyl based catalyst Cl_2Pd(dppf)-DCM and $PdCl_2$-BINAP proved to be the most successful and cost-effective catalysts.

The best results were obtained when the reaction was run in DMF:H_2O (2:1) at mild temperatures (60–65 °C) in the presence of 3 mol% of the catalyst and 1.5 equivalents of K_2CO_3. It is noteworthy that higher temperatures and larger amounts of base resulted in significant racemization and/or methyl ester hydrolysis. Interestingly, under these conditions homocoupling of neither of the reaction partners was observed. Similarly, no reduction products were identified (Ar−OTf→Ar−H). After aqueous work up, and removal of the residual Pd by extraction with *N*-acetylcysteine, the product was isolated in 82% yield by crystallization from heptanes with 97% chemical purity and >99.5% enantiomeric purity.

The synthesis of analogues of chloro ketone **4**, with simpler aromatic substituents, has been performed in a variety of ways in the literature,[11] most involving activation of the carboxylic acid corresponding to ester **9** followed by addition of a variety of chloromethylating reagents. When these methods were attempted, significant racemization was observed. Therefore, we decided to employ the methods developed by Wang and co-workers[12] that utilized the chloroacetic acid dianion directly on the methyl ester **9**. Unfortunately, preliminary experiments showed significant racemization (>15%) when the dianion (3.5 equivalents) was added to **9** under a variety of conditions. Control experiments showed that the chloroacetic acid dianion itself was sufficient to cause racemization in both the starting material and the product. This problem was resolved by utilizing a milder base such as lithium hexamethyldisilazide to deprotonate the carbamate to produce **9a**. This deprotonation significantly slowed the epimerization of **9a** (and product **4**); with this modification, addition of the chloroacetic acid dianion caused essentially no racemization and, after work up, the product was obtained in 86–89% solution yield with >99.5% *ee*.

The reduction of similar systems to **4** has been described extensively (Scheme 3, where Ar=H).[13] Thus, reduction of these unsubstituted derivatives with $NaBH_4$ and other hydride reagents proceeds readily, except that the undesired *erythro* isomer is invariably produced. So, not surprisingly, hydride reductions of **4** under the conditions described in the literature[13] resulted in good yields of the reduction products with ratios of **5**:**5a** varying from 1:1 to 1:8 in favour of the undesired isomer. Although the *threo* isomer has been prepared before with a variety of methods,[13] we desired a more direct route than the ones described thus far. In order to accomplish this, we undertook one study employing asymmetric hydrogenation methodology and a parallel effort studying the enzymatic reduction of **4**.

In the asymmetric hydrogenation approach,[14] an extensive screening program was initiated that evaluated dozens of metal-ligand combinations that are normally useful in asymmetric ketone reductions such as the Josiphos, Taniaphos Walphos, and MeoBiphep class of ligands in combination with Rh, Ru and Ir catalyst precursors.[15] Representative results for a few of these diverse classes of ligands (see Figure 2) are shown in Table 1.



Scheme 3. Preparation of the epoxide **3**.

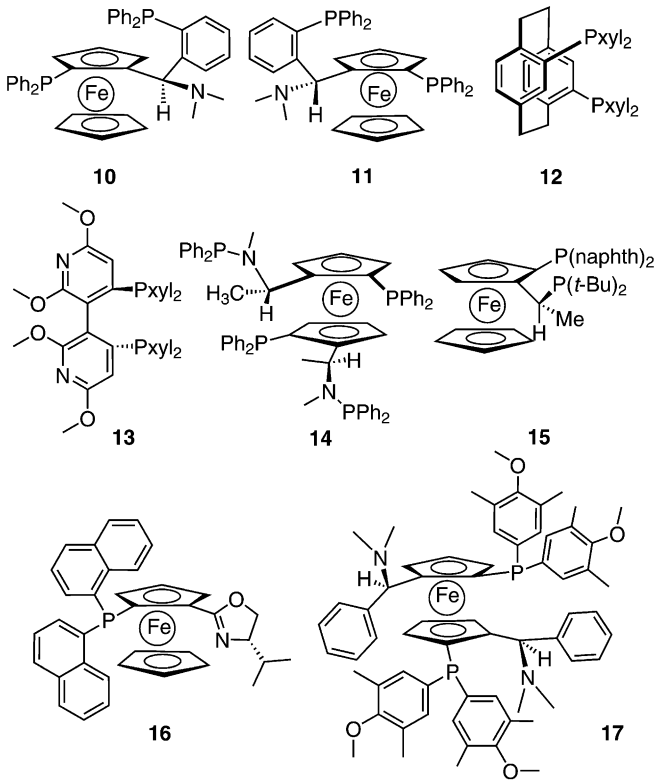


Figure 2. Ligands used in the screening

Initial results rapidly showed that both ruthenium and iridium catalysts were not useful for this transformation. The former gave predominantly the methyl ketone by-product from reduction of the C–Cl bond. Moreover, even in cases where useful amounts of **5** were formed (Table 1, entry 1) the selectivity was rather low. Most iridium catalysts examined gave low conversions (Table 1, entries 3 and 4) with one notable exception (Table 1, entry 2). However this system was not optimized further as the substrate to catalyst (S/C) ratio could never be improved beyond S/C = 50.

Consequently we focused on the more promising Rh-based catalysts in combination with the Mandyphos ligand **17** (Table 1, entries 7 and 8) which showed higher reactivity and generated fewer by-products in the initial screens.

An investigation into the factors affecting the reactivity and selectivity of the catalyst (Table 2) revealed that the steric demand of the solvent had the most significant effect on the selectivity of the reduction. Thus, *tert*-amyl alcohol gave the highest selectivity (Table 2, entry 5) compared to smaller alcohols (Table 2, entries 1 and 2). Non-protic solvents were detrimental to the reaction while acid or amine additives showed no effect. The reaction could be scaled up successfully on a multikilogram scale, but higher pressures (*ca.* 100 bar) were required to achieve commercially useful substrate to catalyst ratios (Table 2, entry 7).

Not surprisingly, the reaction is air sensitive and at the desired high S/C ratios the quality of the starting chloro ketone was of paramount importance (Table 2, entries 8 and 9). Even small amounts of contaminants (e.g., chloride ion) caused incomplete conversions. Moreover, relatively high pressures (80–100 bar) were required to achieve good selectivity limiting the choice of equipment that could be used for this transformation.

For these reasons the enzymatic reduction using the ketoreductase family of enzymes, which employ NADH or NADPH as a cofactor, was studied.[16] Ordinarily, the high cost of the cofactor would lead to prohibitive costs for this transformation; however, the cofactor can be regenerated *in situ* by the same enzyme using isopropyl alcohol as the stoichiometric reductant (Figure 3).

Table 1. Homogeneous hydrogenation of ketone **4**.

Entry	Metal/L[a]	Ligand/Solvent	Temperature/Pressure[b]	Conversion/A [%] **5**+**5a**[c]	Ratio **5**:**5a**
1	$[RuI_2(p\text{-cymene})]_2$	**10**/*i*-PrOH	40/40	97%/54%[d]	73:27
2	$[Ir(cod)Cl]_2$	**12**/EtOH	50/20	98%/98%	80:20
3	$[Ir(cod)]_2$ BARF	**16**/EtOH	50/20	22%/22%	84:16
4	$[Ir(cod)]_2$ BARF	**13**/MeOH	50/20	18%/10%	54:46
5	$[Rh(cod)_2]$ OTf	**15**/TFE	50/20	100%/90%	20:80
6	$[Rh(ndb)_2]$ BF_4	**14**/MeOH	40/40	72%/70%	52:48
7	$[Rh(ndb)_2]$ BF_4	**17**/*i*PrOH	45/30	99%/88%	88:12
8	$[Rh(ndb)_2]$ BF_4	**17**/MeOH	40/30	79%/72%	82:17
9	$[Rh(ndb)_2]$ BF_4	**11**/MeOH	40/40	98%/90%	60:40

[a] For screening experiments an S/C ratio of 50 was used. M:L molar ratio was 1:1.
[b] Temperature in °C and pressure in bar.
[c] Conversion expressed as total of products/starting material. A [%] indicates the relative abundance of **5**+**5a** in the screening and is expressed as their total HPLC area % minus the by-products.
[d] 45% of the dechlorinated product was formed.

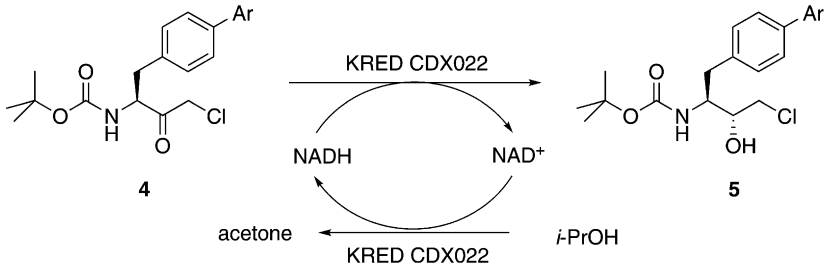


Figure 3. Enzymatic reduction.

Table 2. Optimization of the reduction of ketone **4** with **17** and $[Rh(ndb)_2]BF_4$ catalyst system.

Entry	Solvent/Additives[a]	Temperature [°C]/Time [h]	Pressure [bar]	S/C ratio.	Ratio **5**:**5a**
1	EtOH	40/18	30	50	85:15
2	*i*-PrOH	45/29	30	50	88:12
3	*i*-PrOH/AcOH	40/20	40	50	89:11
4	*i*-PrOH/toluene	40/20	40	50	NR[b]
5	*tert*-amyl alcohol	40/20	40	50	95:5
6	*tert*-amyl alcohol	30/20	80	200	94:6
7	*tert*-amyl alcohol	30/20	100	300	95:5
8	*tert*-amyl alcohol	30/20	80[c]	300	95:5
9	*tert*-amyl alcohol	30/20	80[d]	200	90:10
10	*tert*-amyl alcohol/AcOH	30/20	80	200	95:5
11	*tert*-amyl alcohol/$(i\text{-Pr})_2$NEt	30/20	80	200	94:6

[a] 10 equiv. of AcOH or $(i\text{-Pr})_2$NEt *vs.* Rh were added. The *i*-PrOH:toluene ratio was 1:1.
[b] None of the desired products were produced although **4** was completely consumed.
[c] The reaction was run in the presence of air. Conversion was 69%.
[d] Starting material contained 2–3% of chloride ion. Conversion was 79%.

The initial evaluation tested 288 NADPH-dependent enzyme mutants (3 sets of 96)[17] and was performed by dissolving the substrate in isopropyl alcohol-THF and exposing it to the enzyme and cofactor in pH 7 buffer. Some representative results are shown in Table 3. At first the results seemed rather disappointing as the majority of enzymes tested (>280) gave either low reactivity or high selectivity for the *undesired* diastereomer **5a** (Table 3 entries 1–10). Only two enzymes gave the desired **5** in excellent selectivity and although the conversion was not high, it was sufficient to warrant further investigation (Table 3 entries 11 and 12).

Table 3. Enzymatic reduction of ketone **4** with Codexis ketoreductases.

Entry	Enzyme[a]	Temperature [°C]	pH	Conversion	**5**:**5a**
1	P1A1	25	7	99.1%	0.7:99.3
2	P1A2	25	7	94%	1:99
3	P1B1	25	7	32.3%	3.6:96.4
4	P1B2	25	7	92.9%	1:99
5	P1F4	25	7	65.2%	1:99
6	P2D1	25	7	33.1%	3:97
7	P2G9	25	7	40%	2:98
8	P2H9	25	7	18.8%	4:96
9	P3 A2	25	7	2%	83:17
10	P3B3	25	7	4%	94:6
11	P3C12	25	7	20%	96.6:3.4
12	P3G6	25	7	22%	99:1
13	CDX-022	25	7	99.7%	100:0
14[b]	CDX-022	45	8.5	>97%	>99.5:0.5
15[b]	CDX-022	45	9	> 97%	>99.5:0.5
16[b]	CDX-022	45	9.5	>97%	> 99.5:0.5

[a] Enzyme kits available from Codexis. Enzyme codes represent specific ketoreductase mutants. *Conditions:* 2.4 mg **4**, 0.01 mg cofactor ($NADP^+$ for entries 1–12, NAD^+ for entry 13), 100 mg enzyme lysate, *i*-PrOH-THF-0.1 M phosphate buffer pH 7 (50 μL + 15 μL + 100 μL), overnight at 25 °C.

[b] 2 wt/wt% CDX-022, 0.5 wt/wt% NAD^+, *i*-PrOH-0.1 M triethanolamine buffer (10/90), overnight at 45 °C.

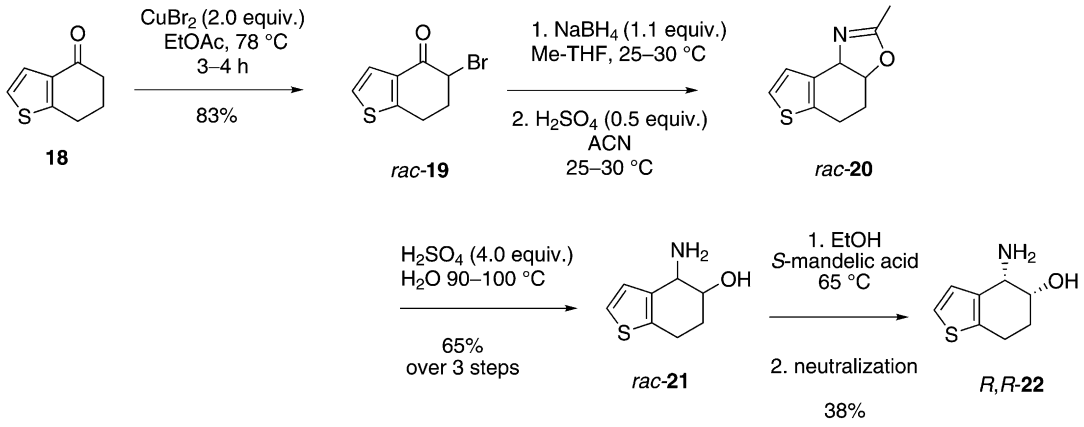
Finally, after careful investigation of mutants related to the initial leads, enzyme CDX-022 was selected for further development (Table 3, entry 13). The optimization studies evaluated the substrate concentration, enzyme and cofactor loads, temperature, pH, co-solvent amount and stirring rate.[18]

These studies revealed the following trends. Optimal substrate concentration was approximately 160 g per L in a mixture of pH 9 buffer and isopropyl alcohol (5: 1). There was little variability in the rate of the reaction when 10–20 volume % of isopropyl alcohol was used but incomplete reactions were observed with 5 volume % isopropyl alcohol. Interestingly, at this concentration the reaction mixture becomes more viscous as the reaction nears completion necessitating an increase in the agitation rate (from 150 to 250 rpm) to achieve complete conversions. The reaction performs well with enzyme loads between 0.5 and 5 weight% with no differences in the rate or conversion. To ensure robustness 1 weight % of the enzyme was used along with 0.5 weight % of NAD^+. Higher temperatures were tolerated well by the enzyme and resulted in increased reaction rates with the reaction requiring 6 h for completion at 50 °C. As the enzyme denatured above that temperature, the reaction was performed at 40–45 °C. The pH was expected to be a significant factor in the reaction; however, the enzyme proved to be quite stable within a reasonable pH range (Table 3, entries 14–16). For example, during scale-up, the initial value of pH 9, upon mixing of the components of the reaction, quickly fell to pH 8.4 as the reaction proceeded without any meaningful drop in enzyme activity. No further adjustment of the pH was needed apart from buffer additions. Indeed the enzyme performs well between pH 8 and 9.5 affording reproducible rates and high yields of the desired compound in good chemical yield (82%) and excellent diastereoselectivity (>99.8:0.2).

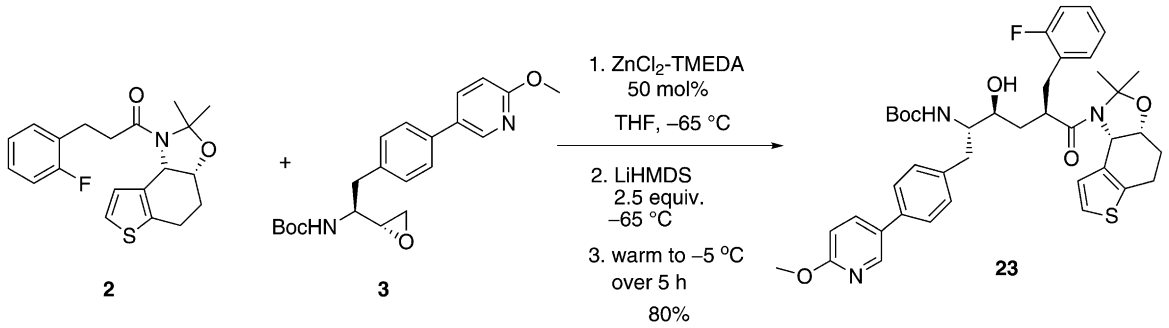
The key intermediate, epoxide **3**, can be obtained from either the metal-catalyzed or enzymatic process in high yield by exposing **5** to aqueous potassium hydroxide in *tert*-amyl alcohol followed by crystallization from heptanes (Scheme 3).

The optically pure amino alcohol (*R,R*-**22**), needed for the convergent construction of **1**, was prepared using the route shown in Scheme 4. Apart from being essential for the biological activity of **1**, this amino alcohol functions as an effective chiral auxiliary, when it is incorporated in **2**, determining the selectivity of the addition of the enolate of **2** to the epoxide **3** (Scheme 5) in a similar fashion as the aminoindanol moiety in the synthesis of Crixivan. Thus the ketone **18** was brominated selectively at the alpha aliphatic position, avoiding any aromatic bromination by-products, by using $CuBr_2$ in refluxing EtOAc to give *rac*-**19**. An $NaBH_4$ reduction and Ritter reaction sequence afforded the racemic *cis*-amino alcohol **21**. The optically active derivative (*R,R*-**22**) was obtained in good yield *via* a resolution with *S*-mandelic acid. The optically active amide **2** was prepared *via* well established methods.[19]

The results for the critical coupling reaction that established the last stereocenter of the molecule are shown in Scheme 5 and Table 4. Attempts to repeat the original literature procedure[20] of deprotonating **2**



Scheme 4. Preparation of the chiral amino alcohol **22**.



Scheme 5. End-game coupling.

with *n*-BuLi followed by addition of **3** at low temperature failed to afford any product, confirming our suspicion that **3** was too unreactive to be opened by an enolate at −78 °C. Warming up of the reaction mixture did result in the formation of the desired product in about 40% yield. Even this modest yield was not

Table 4. Strategic coupling to produce **23**.

Entry	Base[a]	Solvent	Temperature [°C]	Additive	Conversion/Yield [%][e]
1	*n*-BuLi	THF	−78	–	0/0
2	*n*-BuLi	THF	−25	–	80/40
3	KHMDS	THF	−25	–	30/14
4	*tert*-amylOK	THF	−25	–	0/0
5	LiHMDS	THF	−78	–	0/0
6	LiHMDS	THF	−25	–	89/59
7	LiHMDS	MeTHF	−25	–	74/58
8	LiHMDS	DME	−25	–	35/27
9	LiHMDS	$PhCH_3$	−25	–	77/58
10	LiHMDS	THF	−25	$ZnCl_2$	92/67
11	LiHMDS	THF	−25	BF_3-OEt_2	77/42
12	LiHMDS	THF[b]	−5	$ZnCl_2$-TMEDA	99/80[f]
13	LiHMDS	THF[c]	−5	$ZnCl_2$-TMEDA	48/25
14	LiHMDS	THF[d]	−5	$ZnCl_2$-TMEDA	30/15

[a] The amide **2** was added to the base at −78 °C followed by **3** and warm up to the reaction temperature.
[b] The base was added to a mixture of **2**, **3** and 0.5 equiv. $ZnCl_2$-TMEDA at −65 °C followed by warm up to −5 °C.
[c] The base was added to a mixture of **2**, **3** and $ZnCl_2$-TMEDA at −30 °C followed by warm up to −5 °C.
[d] The base was added to a mixture of **2**, **3** and $ZnCl_2$-TMEDA at −5 °C.
[e] The yield was determined by quantitative HPLC against a standard.
[f] Isolated yield.

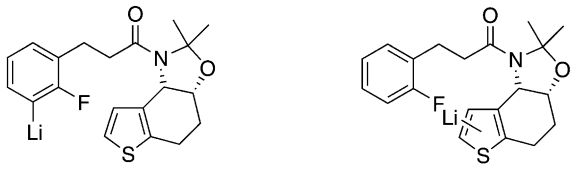


Figure 4. By-products of lithiation of **2**.

reproducible upon scale up and the reaction mixture contained a number of impurities that made purification difficult. Control experiments showed that **2** was not stable in the reaction medium upon warming above −40 to −30 °C although **3** was stable under the reaction conditions even at 0 °C overnight. Quenching experiments confirmed our hypothesis that this instability was the result of lithiation of the fluorobenzene and thiophene moiety in **2** (Figure 4) at the higher temperatures needed to open the epoxide. This competition between the decomposition of **2** and its reactivity towards **3** appears to be general in a variety of solvents (Table 4). Milder bases such as lithium hexamethyldisilazide (LiHMDS) did appear to offer some benefit although the yield was still modest and dependent on the rate of warming up of the reaction mixtures (Table 4, entries 5–9).

In order to facilitate the reaction, we sought to enhance the reactivity of the epoxide by addition of Lewis acids so that the enolate could effect the opening of **3** at lower temperatures where the former is still stable. Al- and Ti-based Lewis acids were investigated first, but no reaction was observed at low temperature while decomposition of **2** and **3** occurred upon warming of the mixture. Finally (Table 4, entries 10 and 11), addition of $ZnCl_2$ showed some improvement but it was the combination of $ZnCl_2$ with TMEDA that afforded good yield and reproducible reaction on 10-kg scale. We have utilized zinc-diamine complexes in conjunction with enolates in the past and found them to enhance significantly the reactivity of the (ester) enolate while affording enhanced stability at higher temperature.[21]

This trend appears to hold with the amide enolate of **2**. For example, control experiments showed that the enolate is stable for up to 24 h in the presence of 0.5 equivalents of $ZnCl_2$-TMEDA at −5 °C in THF. In the absence of the complex, the enolate of **2** is not stable above −30 °C as mentioned above. The best results were obtained by mixing **2** and **3** and solid $ZnCl_2$-TMEDA in THF at −65 °C followed by addition of LiHMDS. After 1 hour, the mixture was warmed to −5 °C for 16 h to achieve complete conversion and 80 % isolated yield of **23** after heptanes-ethyl acetate crystallization. Although the rate of warming up was not critical to the success of the reaction, the base did have to be added at lower temperature. If the initial addition of the base occurred at higher temperatures, inferior yields were obtained. It appeared that now **3** was decomposing and we presume that N−H deprotonation needs to occur at lower temperature to avoid side reactions.

The reaction appears to be highly diastereoselective with less than 0.5–1% of any of the undesired diastereomer detectable by HPLC analysis. The various isomers have been all synthesized independently by employing the *erythro* epoxide and/or the unnatural tyrosine isomer. The isomer at the alpha position to the amide was prepared by employing thermodynamic alkylation conditions as described above (Figure 1: III + IV and ref.[6]).

With the key intermediate **23** in hand, the synthesis of **1** proceeded uneventfully by deprotection of the BOC group and coupling to the methyl carbamate *tert*-butylglycine as described previously and is described in detail in the Experimental Section.[2]

Conclusions

In conclusion, we have succeeded in the preparation of **1**, employing a convergent approach which utilized the asymmetric reduction of the chloromethyl ketone **4** to afford the *threo* stereochemistry by using new enzymatic and asymmetric hydrogenation methodologies. Moreover, we succeeded in the efficient construction of the central core by the opening of epoxide **3** with the Zn-diamine enolate of **2** in good overall yield and excellent selectivity.

Experimental Section

All yields refer to isolated purified products unless otherwise stated. All solvents used were of normal bulk solvent purity and were used without further purification. Thorough degassing was executed as described below.

Preparation of Chloro Ketone 4

$(i\text{-Pr})_2NH$ (74.7 g, 738.4 mmol) and THF (340 g) were charged to a 1-L, 3-necked, round-bottom flask (R1) under a nitrogen atmosphere. The mixture was cooled to 0 °C and *n*-BuLi (204.0 g, 750.1 mmol) was added dropwise. The mixture was cooled at −60 to −65 °C for 1 h. Then $ClCH_2COOH$ (27.7 g, 293 mmol) was added dropwise as a solution in THF (168 g) over 75 min so that the temperature is maintained at −60 to −65 °C. The mixture was stirred at −60 to −65 °C for 2 h. To another nitrogen-inerted 2-L. 3-necked, round-bottom flask (R2), **9** (45.3 g, 117.2 mmol) and THF (252.0 g) were charged. The contents of R2 were cooled to −10 °C and treated with LiHMDS (104.0 g, 117.2 mmol) dropwise over 20 min. The mixture, in R2, was stirred for 1 h at −10 °C. The mixture in R1, was treated with the solution in R2 over 35 min maintaining the internal temperature in R1 between −5 and −10 °C. The resulting mixture in R2 was stirred for 1 h at −10 °C. Upon comple-

tion of the reaction, as judged by HPLC analysis, the reaction was quenched by adding a solution of acetic acid (117.8 g, 1.96 mol) in THF (380.5 g) over 1 h at −10 to −5 °C, followed by addition of water (200 g). The organic layer was washed with saturated $NaHCO_3$ solution (571.4 g) at 0–10 °C, followed by saturated NaCl solution (340 g) at 20–25 °C. The organic layer was concentrated to ~150 mL, isopropyl alcohol (742.9 g) was added and the mixture was distilled under reduced pressure. This sequence was repeated 3 times until GC analysis indicated that the THF was removed (<0.5% by volume). Precipitation of the product could be observed during this process. The resulting slurry was cooled to 10–20 °C and stirred for 16 h. The mixture was filtered and washed with isopropyl alcohol (30 mL). The cake was dried under vacuum at 30 °C for 17 h to afford **4** as a white solid; yield: 40.82 g (100.8 mmol). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 8.38 (d, J = 2.0 Hz, 1H), 7.79 (dd, J = 2.4 Hz, J' = 8.4 Hz, 1H), 7.50 (d, J = 8.0 Hz, 2H), 7.26 (d, J = 8.0 Hz, 2H), 5.13 (d, J = 6.4 Hz, 1H), 4.73 (d, J = 6.4 Hz, 1H), 4.26 (d, J = 16 Hz, 1H), 4.13 (dd, J = 16 Hz, 1H), 4.01 (s, 3H), 3.19 (m, 1H), 3.06 (m, 1H), 1.42 (s, 9H); ^{13}C NMR ($CDCl_3$): δ = 201.2, 163.7, 155.2, 144.9, 137.2, 136.9, 134.8, 129.8, 129.4, 127.0, 110.8, 80.5, 58.3, 53.5, 47.3, 37.1, 28.2; HR-MS: m/z = 405.1577, calcd. for $C_{21}H_{25}ClN_2O_4$ $[M+H]^+$: 405.1576.

Reduction of Chloro Ketone 4 *via* Asymmetric Hydrogenation

$[Rh(nbd)_2]BF_4$ (760 mg, 2.04 mmol) and ligand **17** (2360 mg, 2.24 mmol) were placed in a Schlenk flask, under an argon atmosphere (3 vacuum/argon cycles). Degassed 2-methyl-2-butanol (500 mL) was added and the mixture was stirred for 30 min at room temperature. (*S*)-**4** (82.5 g, 204 mmol) was placed into the 300-mL autoclave (which had passed a tightness test) under an argon atmosphere. Degassed 2-methyl-2-butanol was added (1500 mL) under argon and the solution was treated with the catalyst solution from the Schlenk flask *via* cannula into the autoclave under a slight pressure of argon. The mixture in the autoclave was flushed 3 times with 5 bar of argon and 3 times with 5 bar of hydrogen. The hydrogen pressure was set to 80 bar and the mixture was heated to 30 °C. The resulting over-pressure was adjusted down to 80 bar and the reaction mixture was allowed to stir for 40 h, at which time HPLC analysis showed full conversion. The autoclave was cooled, vented, flushed with 5 bar of argon and unloaded. The crude product mixture of **5** was crystallized from 2:1 heptane-MTBE or carried forward to the next step; yield: *ca.* 70 g (173.4 mmol)).

Enzymatic Reduction of Chloro Ketone 4

The chloro ketone **4** (30 g, 74.1 mmol) was charged under an inert atmosphere into a 1-L, 3-necked flask, followed by TEA buffer (triethanolamine solution in water, adjusted to pH 8.5–9.5 with 2 M HCl, 240 mL) and isopropyl alcohol (45 mL). The mixture was stirred for 30 min at 25–30 °C and β-NAD^+(NAD) (0.15 g) solution in TEA buffer (6 mL), KRED CDX-022 (0.6 g) solution in TEA buffer (30 mL) and TEA buffer (120 mlL) were added in sequence at 25–30 °C. The mixture was stirred for 20 h at 40–45 °C. After the reaction was finished, the mixture was extracted with MTBE (2×150 mL) at 25–30 °C. The organic layer was filtered over celite. The organic layer was concentrated to 100 mL. *n*-Heptane (240 mL) was added dropwise, while cooling to 0–5 °C, to form a suspension. The mixture was stirred for 2 h at 0~5 °C; then filtered and washed with *n*-heptane (60 mL). The cake was dried under vacuum at 45 °C for 20 h to affordd **5** as a white solid; yield: 24.6 g (14.8 mmol). ^{1}H NMR: ($CDCl_3$, 400 MHz): δ = 8.38 (d, J = 2.4 Hz, 1H), 7.79 (dd, J = 2.4 Hz, J' = 8.4 Hz, 1H), 7.48 (d, J = 8.0 Hz, 2H), 7.33 (d, J = 7.6 Hz, 2H), 6.83 (d, J = 8.4 Hz, 1H), 4.99 (d, J = 8.4 Hz, 1H), 3.99 (s, 3H), 3.94 (m, 1H), 3.81 (m, 1H), 3.59 (d, J = 6.4 Hz, 2H), 3.34 (s, 1H), 3.04 (m, 2H), 1.43 (s, 9H) 4.26 (d, J = 16 Hz, 1H), 4.13 (dd, J = 16 Hz, 1H), 4.01 (s, 3H), 3.19 (m, 1H), 3.06 (m, 1H), 1.42 (s, 9H); ^{13}C NMR ($CDCl_3$): δ = 163.5, 156.1, 144.8, 137.3, 137.0, 136.2, 129.9, 129.8, 126.8, 110.7, 79.9, 71.5, 53.9, 53.5, 47.7, 38.0, 28.3; HR-MS: m/z = 407.1721, calcd. for $C_{21}H_{27}ClN_2O_4$ $[M+H]^+$: 407.1732.

Condensation of Epoxide 3 with Amide 2

A nitrogen-inerted, 1-L, 3-necked, round-bottom flask was charged with anhydrous $ZnCl_2$ (18.9 g), TMEDA (16.2 g) and anhydrous THF (2500 g). The mixture was stirred for 1 h at 20–30 °C. A solution of **2** in THF solution (neat 158 g, 64.6 wt%, 428 mmol) was charged under nitrogen followed by cooling to −60 to −70 °C and stirring for 30 min. A solution of **3** in THF solution (neat 149 g, 64.6 wt%, 416 mmol) was charged under nitrogen at −60 to −70 °C; followed by LHMDS (1 M solution, 988 g) added dropwise at −60 to −70 °C. The mixture was stirred for 2 h at −60 to −70 °C, then 10 h at −3 to −8 °C. After the reaction was finished, it was quenched with 2 N hydrochloric acid at −3 to −8 °C to pH 7–8. Heptane (500 g) was charged and the mixture was washed with water (1000 g). The aqueous layer was extracted with THF (400 g) and heptane (100 g). The combined organic layer was concentrated to 1500 mL under vacuum below 50 °C. *n*-Heptane (300 mL) was added in portions, then the mixture was concentrated to 1500 mL under vacuum below 50 °C. Ethyl acetate (200 g) was charged and the mixture was concentrated to 600 mL under vacuum below 50 °C. The mixture was cooled to 15–20 °C slowly and stirred for 1 h. The suspension was filtered and washed with *n*-heptane (200 g). The wet cake was dried under vacuum at 40–45 °C for 12 h to yield **23** as a white solid; yield: 242 g (332.8 mmol). Due to rotomers, precise NMR assignments were not useful. The final assignment and structure confirmation occurred at the final API (**1**). HR-MS: m/z = 730.3351, calcd. for $C_{41}H_{48}FN_3O_6S$ $[M+H]^+$: 730.3321.

Preparation of 1 *via* Deprotection of 23 and Condensation with (*S*)-2-(Methoxycarbonylamino)-3,3-dimethylbutanoic

A mixture of **23** (42.6 g, 58.43 mmol) and anhydrous isopropyl acetate (680 mL) were charged in an inerted round-bottom flask. HCl (45 g, 8.0 equiv., 12 M in water) was added dropwise over 30 min while cooling to 5–10 °C, and the mixture was stirred for 2 h at that temperature. Water (639 mL) was added over 1 h at 5–10 °C to form a 2-phase solution and the mixture was stirred at 50–55 °C for 8 h. After the reaction had finished, the mixture was separated at 20–25 °C and 30% NaOH solution was added dropwise to the aqueous layer over 1 h at 20–25 °C until the pH reached

8–9. The suspension was filtered and washed with water (200 g). The wet cake was dried under vacuum at 40–50 °C for 18 h to yield the free amine as a white solid which was dissolved in DMF (500 mL) and was added to a separate flask, containing (*S*)-2-(methoxycarbonylamino)-3,3-dimethylbutanoic acid (38.5 g,), HATU (77.3 g) and DMF (800 mL) and Et_3N (39.5 g). After 30 min the reaction was judged complete by HPLC analysis and water (2.5 L) was added at 25–30 °C. The suspension was stirred for 1 h at 25–30 °C and filtered and washed with water (2 L). The wet cake was suspended in ethanol (2 L) and the slurry stirred for 3 h at 20–25 °C. Then the mixture was filtered again and washed with ethanol (200 mL). The wet cake was dried under vacuum for 12 h at 40–45 °C to afford **1** as a white solid; yield: 26.97 g (35.44 mmol). ^{1}H NMR: (DMSO, 400 MHz): $\delta = 8.41$ (d, $J = 2$ Hz, 1H), 7.94 (dd, $J = 2.4$ Hz, $J' = 4.8$, 1H), 7.71 (d, $J = 8.8$ Hz, 1H), 7.62 (d, $J = 8$ Hz, 1H), 7.49 (d, $J = 8$ Hz, 2H), 7.29 (d, $J = 8.4$ Hz,, 2H), 7.25 (m, 2H), 7.15 (d, $J = 5.2$ Hz, 1H), 7.11 (m, 2H), 6.89 (d, $J = 8.8$ Hz, 1H), 6.73 (d, $J = 8$ Hz, 1H), 6.63 (d, $J = 4.8$ Hz, 1H), 4.84 (d, $J = 5.6$ Hz, 2H), 4.65 (d, $J = 4$ Hz, 1H), 3.94 (m, 2H), 3.88 (s, 3H), 3.76 (d, $J = 3.6$ Hz, 1H), 3.55 (m, 1H), 3.49 (s, 3H), 2.87 (m, 4H), 2.70 (m, 3H), 1.95 (m, 1H), 1.79 (m, 1H), 1.69 (m, 1H), 1.43 (m, 1H), 0.80 (s, 9H); ^{13}C NMR ($CDCl_3$): $\delta = 174.4$, 170.3, 163.3, 162.2, 159.8, 156.8, 144.8, 139.0, 137.7, 136.1, 135.7, 134.8, 131.7, 131.6, 130.2, 129.7, 128.5, 128.4, 127.6, 126.9, 126.3, 124.5, 122.8, 115.5, 115.3, 111.0, 69.0, 66.2, 63.2, 62.9, 55.4, 53.7, 51.8, 49.1, 43.1, 36.8, 34.1, 32.1, 29.2, 27.1, 20.7; HR-MS: $m/z = 761.3392$, Calcd for $C_{41}H_{49}FN_4O_7S$ $[M+H]^+$: 761.3379.

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