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Organocatalytic, Enantioselective Synthesis of VNI: A Robust Therapeutic Development Platform for Chagas, a Neglected Tropical Disease

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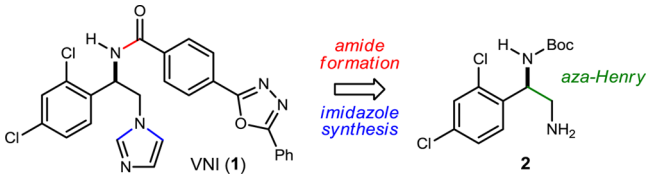
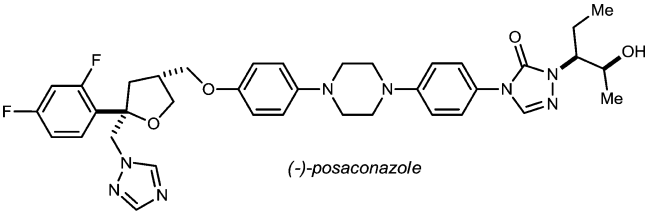
ABSTRACT



VNI is a potent inhibitor of CYP51 and was recently shown to achieve a parasitological cure of mice infected with *T. cruzi* in both acute and chronic stages of infection. *T. cruzi* is the causative parasite of Chagas disease, a neglected tropical disease. The first enantioselective chemical synthesis of VNI (at a materials cost of less than $0.10/mg) is described. Furthermore, the key enantioselective step is performed at the 10 g scale.

Chagas disease affects more than 8 million people throughout the Americas,[1] and recent evidence suggests that it is proliferating beyond these traditional geographical borders, tracking with the lengthening reach of disease vectors that favor warm climate.[2] The causative parasite of Chagas—*T. cruzi*—infects humans in two stages. The acute stage is treated with benznidazole or nifurtimox, which have been associated with low efficacy and general toxicity. There are no established treatments for the chronic form of infection, but posaconazole is currently considered the most promising therapeutic and is in phase II clinical trials. Unfortunately, it is widely regarded as unaffordable with a cost estimated at over $1000 per patient. The cost of treatment is an immediate consideration for drug development since Chagas is endemic to populations in low resource areas,[3] and mere repurposing efforts for expensive antifungal drugs such as posaconazole are likely to have little impact. There is insufficient incentive for industrial drug development, resulting in a neglected disease status for Chagas.[4−7]



Posaconazole was brought to market as an antifungal therapeutic[8] and has been shown to inhibit trypanosomal sterol 14α-demethylase (CYP51)[9−12] similarly to other

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small molecules,[13] thereby disrupting membrane formation in *T. cruzi*. CYP51 has emerged as an effective drug target since all eukaryotes rely on endogenous sterol production for membrane biogenesis, and this target has been further validated in a murine model of acute Chagas infection.[14] VNI[15] was recently discovered to inhibit sterol synthesis in *T. cruzi*,[13] as well as bind to CYP51 in a manner analogous to posaconazole.[11] A contributing factor to the high cost for posaconazole is its long and relatively inefficient synthesis;[16] however, no enantioselective synthesis of VNI has been reported. Further development of VNI, the more potent enantiomer, and its promise as a therapeutic of reasonable cost, rests in part upon the availability of a short, selective, and high yielding synthesis.[17] Herein we report the first enantioselective chemical synthesis of VNI. This preparation provided the gram-scale quantities of VNI to establish its efficacy against *T. cruzi* in a mouse model of infection, including evidence for its effectiveness against the chronic infection.[18] The preliminary studies enabled by this material also suggest that VNI toxicity is low.

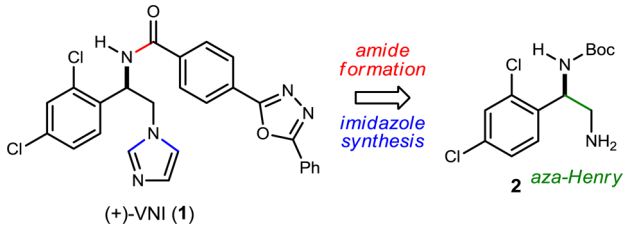
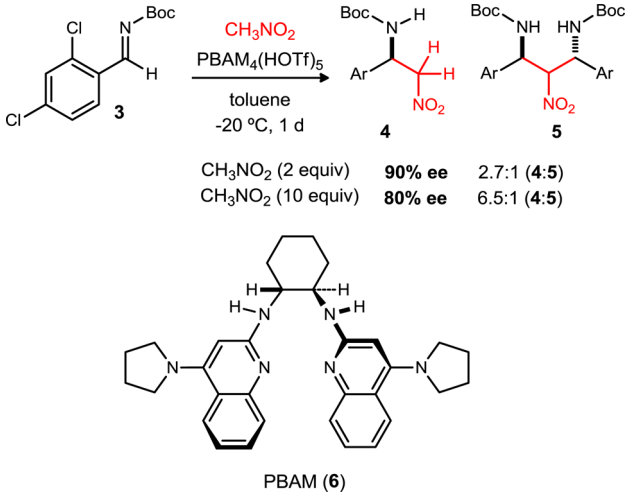


Figure 1. Retrosynthetic approach to VNI to maximize convergency and modularity.

The initial quantity of VNI was obtained from a nonrenewable source and without preparative details.[13] And since no preparation of VNI existed in the literature, a synthesis selective for the more potent enantiomer was needed. Key structural features of VNI include a chiral benzylic amine carbon, an amide substituent that projects a substituted biphenyl into the substrate access channel of CYP51, and an imidazole that interacts directly with the iron-heme of CYP51.[12] Figure 1 outlines a modular design for the synthesis route, invoking an enantioselective aza-Henry reaction to construct the styrenyl diamine backbone of VNI in the key step. The high degree of convergency would be reflected in a short longest linear sequence (LLS).[19]

Scheme 1. Competitive Double Addition of Imine to Nitromethane



N-Boc imine **3** was prepared using a standard procedure from 2,4-dichlorobenzaldehyde.[20] Feasibility for the key stereochemistry-determining step was first evaluated by analysis of the enantioselective addition of commercially available nitromethane to *N*-Boc imine **3** using

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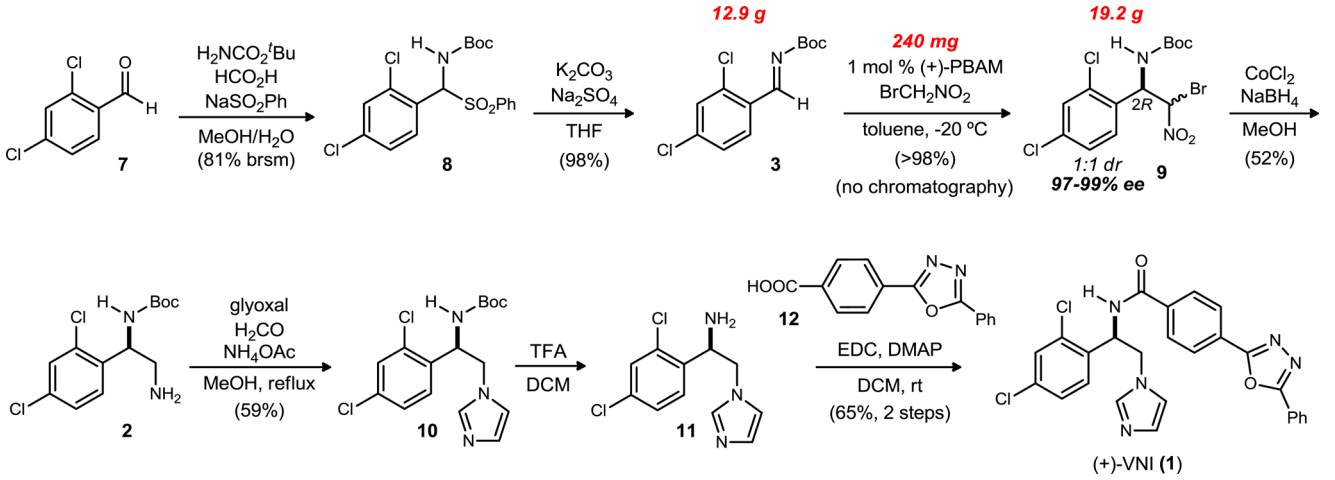
Scheme 2. Multigram-Scale Enantioselective Synthesis of VNI for Preclinical Studies



bis(amidine) catalysis (Scheme 1).[21,22] Using our first generation catalyst[23] to form this adduct would have required a higher catalyst loading and longer reaction time, as well as the use of nitromethane as solvent. To circumvent this problem, we selected a more Brønsted basic version from our second generation of catalysts for the reaction.[19,24] This approach delivered a disappointing 2.7:1 ratio of **4**:**5**, the latter resulting from an unexpectedly competitive addition of adduct **4** to a second equivalent of imine **3**. In principle, this 2:1 adduct could be minimized through the use of excess nitromethane; however, the ratio was improved only to the 6.5:1 level and at the cost of a drop in enantioselection (Scheme 1). As a result, commercially available bromonitromethane was investigated using the hypothesis that the bromine substituent would slow the second addition as a result of its size. In the event, the desired adduct (**9**) was prepared in excellent yield (90%), using only 2 equiv of bromonitromethane (Scheme 2).[25] This reaction was catalyzed by (+)-PBAM (**6**),[26] which delivered the addition product in 97–99% ee for each diastereomer. Immediate treatment of the mixture with cobalt boride formed *in situ*[27] provided the fully reduced product (amine **2**). Although adduct **9** is a mixture of diastereomers, they are homochiral at the benzylic position, thereby producing the same enantiomer after reduction to diamine **2**. Formation of the imidazole was achieved by standard condensation of amine **2** with glyoxal, formalin, and ammonium acetate.[28] Deprotection and subsequent acylation of the amine with carboxylic acid **12**[29] provided milligram quantities of the desired dextrorotatory product.

Our first generation approach relied on chromatography to purify adduct **9**, in addition to chromatographic purification in three subsequent steps. However, we first optimized the key enantioselective step, with respect to both catalyst loading and scale. Although decreasing the catalyst loading was favorable for enantioselection (Table 1, entries 1–3), a diminished yield was observed as the scale increased. This was partly due to issues associated with column chromatography on a large scale. Fortunately, the catalyst could be removed with straightforward filtration, delivering over 6 g of pure product (Table 1, entry 4). An almost 500-fold increase in scale from initial experiments (from 0.1 mmol scale) returned favorable results (Table 1, entry 5): filtration provided 19.2 g (nearly quantitative yield) of analytically and enantiomerically pure product using 1 mol % PBAM. The use of this highly reactive organocatalyst (> 100 turnovers) in a large scale preparation to deliver the scaffold is punctuated by the ability to recover and recycle the catalyst without loss of activity.

The overall synthesis route (Scheme 2) provided VNI analogs for evaluation with similar brevity. Among those is

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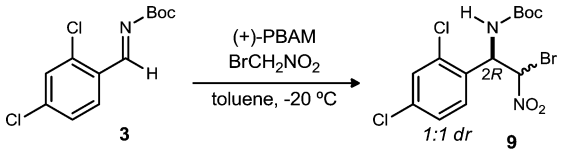
Table 1. Reaction Optimization of Key Bond Forming Step[a]



			yield[b]		
entry	cat. (mol %)	scale (mmol)	(g)	(%)	ee[c] (%)
1	10	1.0	0.37	90	95
2	5	1.0	0.37	90	96
3	2	5.8	1.99	83	96
4[d]	1.3	14.9	6.05	98	97
5[d]	**1.0**	**47.0**	**19.2**	**>98**	**98**

[a] All reactions were run at −20 °C for 2 d. [b] Yields are isolated yields. [c] Enantiomer ratios measured by HPLC using chiral stationary phase and are average of both diastereomers. [d] No column chromatography, only filtration to remove catalyst. See Supporting Information for details.

FF-VNI (**13**), prepared from the 2,4-difluoro-derivative of **9** (Figure 2). The halogenated aromatic ring is buried deep in the CYP51 pocket while the amide chain is projected into the substrate access channel. The exchange of both chlorines with fluorines within the VNI backbone resulted in a 4-fold improvement in binding to CYP51 (*T. cruzi*) relative to VNI.[17,30]

The final part of this study involved the preparation of gram quantities of VNI for evaluation in murine models of both acute and chronic Chagas infection. In this campaign, several chromatographic manipulations were targeted for replacement with filtration or active precipitation. The first three steps were completed by straightforward filtrations, and the first chromatography was applied to the borohydride reduction product **2**. The final three steps could also be completed with a single chromatographic step of the final product, aided by precipitation steps.

The route illustrated in Scheme 2 establishes that (1) the catalyzed addition has been scaled to the 20 g level using 1 mol % of the organocatalyst PBAM,[26] providing the desired product in essentially enantiomerically pure form; (2) the overall process can be used to prepare multigram quantities of VNI; and (3) nearly all steps benefit from solid intermediates, resulting in either filtration or recrystallization for most manipulations. This synthesis is therefore an immediate platform for VNI drug development, including further structure−activity relationship studies.

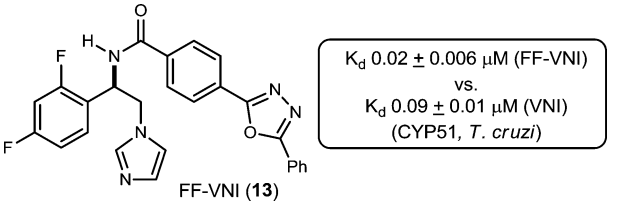


Figure 2. VNI analog preparation: FF-VNI.

In summary, VNI is prepared through total chemical synthesis using generally inexpensive materials. The salient features of the synthesis, including length (LLS = 7 steps), selectivity (>99% ee) in the key step, and nature of the intermediates (all crystalline solids) bode well for its potential scalability beyond its current multigram scale. The current materials cost of less than $0.10/mg[31] for VNI highlights the promise of a small molecule therapeutic for a disease endemic to low resource areas.[32] Furthermore, the convergency of the approach provides immediate, straightforward access to derivatives to more rapidly progress through lead optimization and into preclinical candidate selection.

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Supporting Information Available. Procedures (gram-scale) and analytical data for all new compounds, cost of materials analysis to produce VNI, and comparative analysis of posaconazole and VNI synthesis schemes. This material is available free of charge via the Internet at http://pubs.acs.org.

(30) Relative efficacy of CYP51 ligands is estimated through the use of several measurements in addition to K_d; see refs 12, 13, and 17. For this reason, VNI remains the best current lead.

(31) Unfortunately, the materials cost to produce posaconazole is unavailable, thereby preventing a direct comparison at this stage. However, the low materials cost for VNI at the gram scale bodes well for the development of an inexpensive small molecule.

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The authors declare no competing financial interest.