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Stereocontrolled Synthesis of a Potential Transition-State Inhibitor of the Salicylate Synthase MbtI from *Mycobacterium tuberculosis*

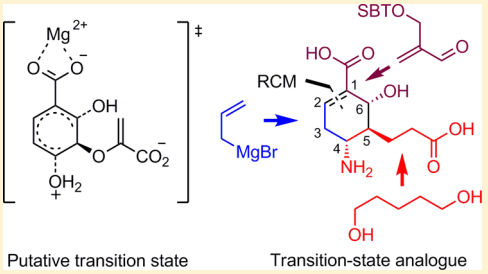
Zheng Liu,[†,‡] Feng Liu,[†] and Courtney C. Aldrich*[,†]

[†]Department of Medicinal Chemistry, University of Minnesota, 308 Harvard Street Southeast, Minneapolis, Minnesota 55455, United States

[‡]Key Laboratory of Pesticide & Chemical Biology, Ministry of Education, College of Chemistry, Central China Normal University, 152 Luoyu Road, Wuhan, Hubei 430079, China

Supporting Information

ABSTRACT: Mycobactins are small-molecule iron chelators (siderophores) produced by *Mycobacterium tuberculosis* (*Mtb*) for iron mobilization. The bifunctional salicylate synthase MbtI catalyzes the first step of mycobactin biosynthesis through the conversion of the primary metabolite chorismate into salicylic acid via isochorismate. We report the design, synthesis, and biochemical evaluation of an inhibitor based on the putative transition state (TS) for the isochorismatase partial reaction of MbtI. The inhibitor mimics the hypothesized charge buildup at C-4 of chorismate in the TS as well as C−O bond formation at C-6. Another important design element of the inhibitor is replacement of the labile pyruvate side chain in chorismate with a stable C-linked propionate isostere. We developed a stereocontrolled synthesis of the highly functionalized cyclohexene inhibitor that features an asymmetric aldol reaction using a titanium enolate, diastereoselective Grignard addition to a *tert*-butanesulfinyl aldimine, and ring closing olefin metathesis as key steps.



INTRODUCTION

Tuberculosis (TB), the leading cause of infectious disease mortality after HIV-AIDS, is due to members of the *Mycobacterium tuberculosis* (*Mtb*) complex that includes seven closely related species.[1] The emergence of multidrug and extensively drug resistant strains of *Mtb* has renewed focus on the development of antitubercular agents with novel modes of action.[2] With the exception of pyrazinamide, whose mechanism remains an enigma,[3] the antibiotics presently used in TB chemotherapy target a limited set of biochemical pathways in macromolecular and cofactor biosynthesis.[4] Iron is a required cofactor for more than 40 enzymes in *Mtb* but is highly restricted in a vertebrate host where the concentration of free iron (Fe^{2+}) is estimated to be 10^{-24} M.[5] To obtain iron, *Mtb* deploys two complementary strategies: synthesis of the mycobactin siderophores,[6] which are small-molecule iron chelators that scavenge iron from host tissues, and uptake of heme through a specialized heme receptor followed by heme degradation to release the iron.[7] The relative contribution of each mechanism for iron mobilization *in vivo* is unknown, and both may play a role during different stages of infection. *Mtb* mutants unable to produce mycobactins cannot replicate under iron-restricted conditions and are cleared *in vivo*, thereby confirming the importance of mycobactin-mediated iron acquisition in *Mtb*.[8]

The bifunctional salicylate synthase MbtI is responsible for the first committed step in mycobactin biosynthesis through the conversion of chorismate into salicylic acid, which is accomplished in two partial reactions at the same active site.[9] In the first reaction, MbtI catalyzes the interconversion of chorismate to isochorismate that has been hypothesized to proceed through a concerted S_N2'' reaction mechanism via transition state **TS1** (Figure 1).[9c,10] The isochorismatase activity of MbtI requires Lys205, which nucleophilically activates a water molecule for attack on chorismate at C-6 and Glu252 that polarizes the C-4 hydroxyl leaving group. In the second reaction, pyruvate is eliminated through an intramolecular [3,3]-sigmatropic rearrangement, formerly a retro-Ene reaction, to afford salicylic acid via bicyclic transition state **TS2** (Figure 1).[11]

MbtI represents an appealing target for the development of inhibitors of mycobactin biosynthesis because it is structurally and biochemically characterized, has no human orthologs, and is conditionally essential under iron-deficient conditions. Following the foundational studies of Abell et al. of inhibitors of chorismate-utilizing enzymes,[12a−e] Payne and co-workers were the first to disclose inhibitors of MbtI, and their most potent compound is the rationally designed substrate mimic **1** (Figure 2A) containing a 2,3-dihydroxybenzoate scaffold.[12f,g] This compound is a competitive inhibitor and possesses an inhibition constant (K_i) of approximately 10 μM. In a complementary approach employing high-throughput screening, Vasan and co-workers reported benzimidazole-2-thione **2** as a noncompetitive inhibitor with potency similar to that of **1** (Figure 2A).[13] On the basis of the modest potency of the

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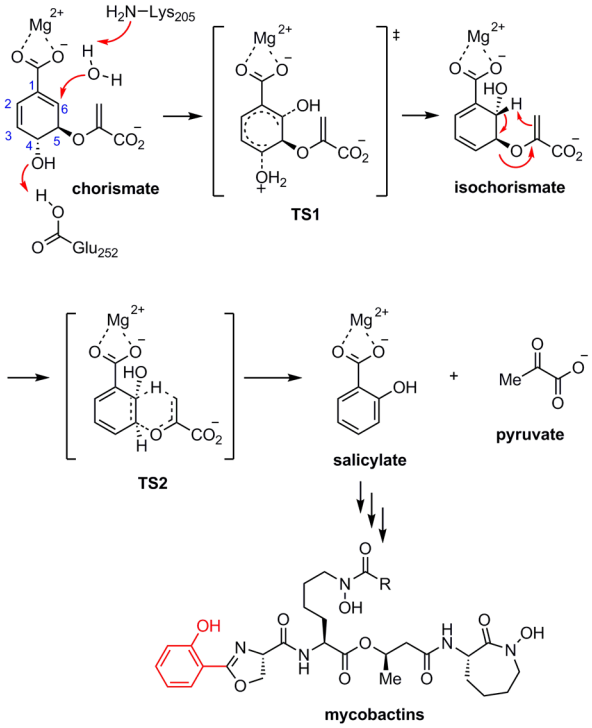

Figure 1. Conversion of chorismate to salicylate via isochorismate catalyzed by MbtI.

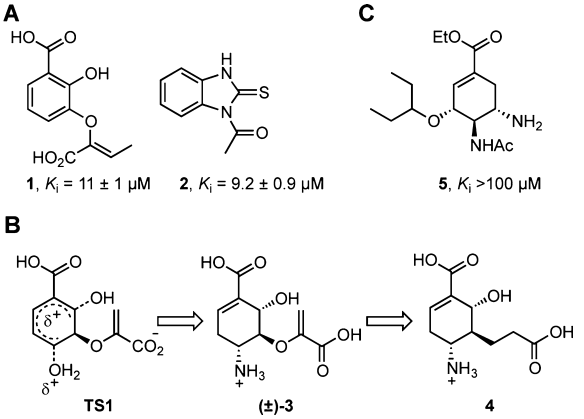

Figure 2. (A) Reported MbtI inhibitors. (B) Rationale for TS inhibitor design of **4**. (C) Oseltamivir (Tamiflu), a neuraminidase TS inhibitor. K_i values are with respect to MbtI.

described MbtI inhibitors, we chose to investigate potential transition-state (TS) inhibitors of MbtI. TS inhibitors exploit the affinity of an enzyme for the TS relative to the Michaelis complex.[14] Capturing even a small percentage of the TS binding energy with an inhibitor, in theory, allows the design of exceptionally potent inhibitors. TS strategies are less successful when the noncatalyzed rate is high because the amount of potential TS stabilization will be smaller. In the case of MbtI, the first transition state, **TS1**, is more desirable for mimicking than the second bicyclic transition state, **TS2**, because of the ease of the noncatalyzed sigmatropic rearrangement.[11,15]

Inspired by the pioneering work of Kozlowski and Bartlett et al., who reported on the synthesis of **3** as a TS inhibitor for several chorismate-utilizing enzymes,[16] we report the synthesis and characterization of cyclohexene **4** that mimics putative **TS1** through incorporation of an amino group at C-4 to resemble the charged leaving group of **TS1** (Figure 2B). Another key design element is replacement of the sigmatropically labile pyruvate side chain at C-5 with a stable C-linked propionate fragment because MbtI possesses pyruvate lyase activity, unlike the monofunctional chorismate-utilizing enzymes investigated by Kozlowski et al. Interestingly, inhibitor **4** bears a striking resemblance to Oseltamivir (trade name Tamiflu), **5** (Figure 2C), an antiviral drug developed at Gilead that is a TS inhibitor of viral neuraminidase.[17]

RESULTS AND DISCUSSION

Retrosynthetic Plan. The challenge of target molecule **4** resides in the construction of three contiguous stereocenters in the highly functionalized cyclohexene scaffold. Our first retrosynthetic plan involved disconnection of the C-5 propionate side chain to thionocarbonate **6** through a radical coupling with *tert*-butyl acrylate. This would allow us to dovetail into the elegant racemic synthesis of **7** reported by Kozlowski and co-workers (Figure 3A).[16b] We also noted the

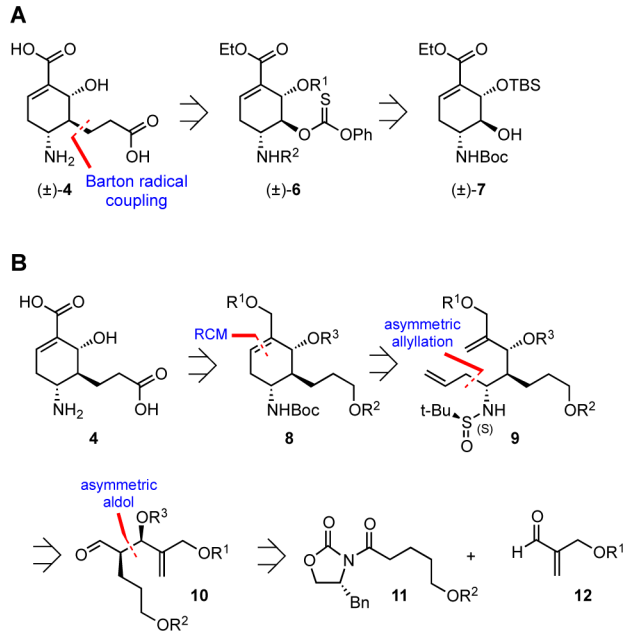

Figure 3. Retrosynthetic analysis.

structural similarity between **4** and Oseltamivir **5** (Figure 2B,C) and were inspired by the efficient ring closing metathesis (RCM) reaction reported by Yao and co-workers[18] for construction of the cyclohexene core of Oseltamivir **5**. Accordingly, as shown in Figure 3B, RCM disconnection of **8** leads to acyclic diene **9** that can be further simplified to *syn* aldol adduct **10** via an asymmetric Grignard addition employing Ellman's *N*-sulfinamide auxiliary.[19] Intermediate **10** can be readily assembled through *syn* aldol reaction between oxazolidinone **11** and aldehyde **12**. An advantage of this second approach is the ability to control stereochemistry on an acyclic precursor using contemporary methods in asymmetric synthesis.

Radical Coupling Strategy. Silyloxybutadiene **13** (Scheme 1) was prepared from crotonaldehyde in 78% yield through modification of the reported procedure[16b] using TBSOTf. The key Diels–Alder reaction between **13** and ethyl propiolate afforded cyclohexadiene in variable yields from 0 to 61% (from

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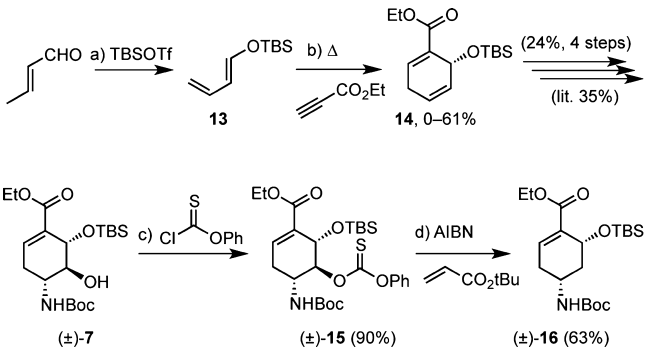
Scheme 1. Attempted Introduction of the Propionate Side Chain[a]

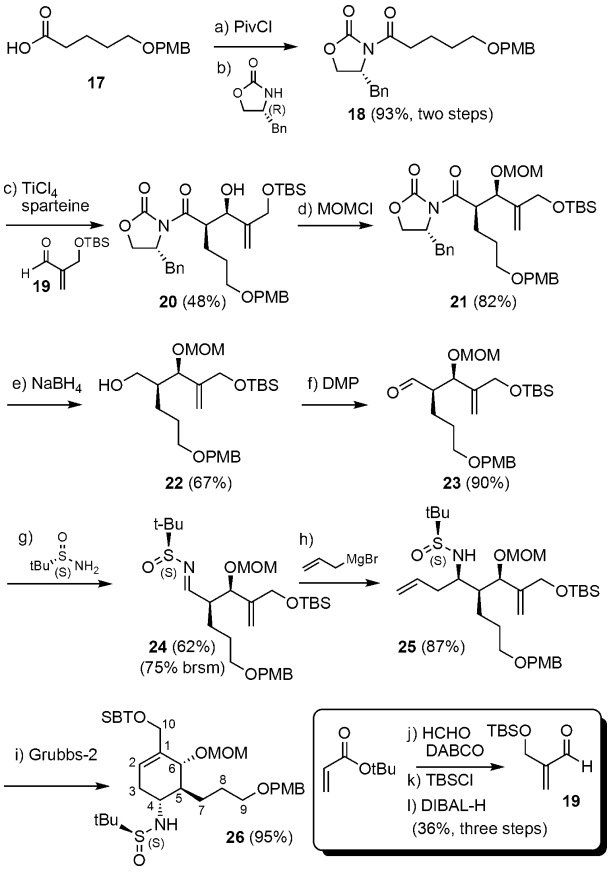


[a]Reagents and conditions: (a) TBSOTf, Et_3N, CH_2Cl_2, −20 °C → rt; (b) 2,6-di-*tert*-butyl-4-methylphenol (BHT) or methylene blue (0.05 equiv), $PhCH_3$, 65−80 °C, 6−8 days; (c) *N*-methylimidazole (3.5 equiv), CH_2Cl_2, rt, 24 h; (d) representative conditions of $(Me_3Si)_3Si$ (1.05 equiv), *tert*-butyl acrylate (10 equiv), AIBN (0.8 equiv), $PhCH_3$, reflux, 2 h.

more than a dozen attempts) with the aromatized benzoate derivative as a major byproduct even though the reaction mixture was rigorously degassed and antioxidants were employed. Notwithstanding the inconsistent yields, several grams of **14** were secured and elaborated to cyclohexene derivative (±)-**7** as described previously[16b] in four steps with an overall yield of 24% (average of 70% per step). Conversion to thionocarbonate (±)-**6** mediated by *N*-methylimidazole (NMI) proceeded in 90% yield at room temperature, whereas 4-dimethylaminopyridine (DMAP), a more conventional acylation catalyst, resulted in only low conversion (<10%).[20] Several conditions were explored to effect a radical coupling with *tert*-butyl acrylate and other radical traps;[21] however, despite considerable effort, only deoxygenated product (±)-**16** was isolated in yields of up to 63% (84% brsm) likely because of the sterically demanding environment at C-5.

RCM Approach. As a result of the capricious yield of cyclohexadiene **14** coupled with an inability to advance to intermediate **15**, we next focused attention on the complementary RCM route as described below because the propionate side chain is installed early in the synthesis. Synthesis began with acylation of Evans' (*R*)-phenylalanine-derived oxazolidinone with known carboxylic acid **17**[22] under mixed anhydride conditions to afford *N*-acyloxazolidinone **18** in 93% yield. The required aldehyde **19** coupling partner was prepared with a single purification step through Baylis−Hillman reaction[23] of *tert*-butyl acrylate and formaldehyde in aqueous tetrahydrofuran (THF) followed by *tert*-butyldimethylsilyl (TBS) protection and diisobutylaluminum hydride (DIBAL-H) reduction (Scheme 2, bottom inset). Initially, asymmetric boron-mediated aldol reaction[24] of **18** with aldehyde **19** using dibutylboron triflate as a Lewis acid in Et_2O provided the *syn* aldol adduct **20** in high diastereoselectivity (dr of >20:1) but only 36% yield. As an alternative, the titanium enolate of **18** was investigated according to methodology developed by Crimmins and coworkers.[25] Treatment of **18** with $TiCl_4$ and 2.5 equiv of (−)-sparteine in CH_2Cl_2 at −78 °C followed by the addition of aldehyde **19** afforded **20** (dr of >20:1) in an improved 48% yield. Protection as a methoxymethyl (MOM) ether employing CH_3OCH_2Cl and catalytic tetrabutylammonium iodide (TBAI) furnished **21**. The chiral auxiliary was then reductively removed

Scheme 2. Synthesis of Cyclohexene 26[a]



[a]Reagents and conditions: (a) PivCl, Et_3N, THF, 0 °C, 6 h; (b) (*R*)-4-(benzyl)oxazolidin-2-one, *n*-BuLi, −78 °C, 5 min, then added to the mixed anhydride, −78 °C for 30 min, 0 °C for 30 min; (c) $TiCl_4$, (−)-sparteine, CH_2Cl_2, −78 °C, 1.5 h, then **19** added, −40 °C for 3 h, −20 °C overnight; (d) CH_3OCH_2Cl, *i*-Pr_2NEt, TBAI, toluene, 90 °C, 7 h; (e) $NaBH_4$, THF/H_2O (3:1), 0 °C to rt, overnight; (f) Dess−Martin periodinane, $NaHCO_3$, CH_2Cl_2, rt, 30 min; (g) (*S*)-(−)-2-methyl-2-propanesulfinamide, anhydrous $CuSO_4$, PPTS, CH_2Cl_2, rt, 24 h; (h) allylmagnesium bromide, CH_2Cl_2, −20 °C, 2.5 h; (i) 14 mol % Grubbs second-generation catalyst, CH_2Cl_2, reflux, 14 h, then DMSO added, rt, 16 h; (j) HCHO, DABCO, THF/H_2O (1:1), reflux, 16 h; (k) TBSCl, imidazole, CH_2Cl_2, rt, 16 h; (l) DIBAL-H, CH_2Cl_2, −78 °C, 1 h.

with sodium borohydride,[26] and Dess−Martin oxidation[27] of the resultant alcohol **22** provided aldehyde **23**. Condensation with Ellman's (*S*)-(−)-*tert*-butanesulfinamide auxiliary yielded *tert*-butanesulfinyl aldimine **24**. As first reported by Xu and coworkers, the combination of pyridinium *p*-toluenesulfonate (PPTS) and anhydrous $CuSO_4$ dramatically promoted imine formation, which was necessary for sterically congested aldehyde **23**.[28] The stereocontrolled installation of the allyl group in **25** was achieved in 87% yield and excellent diastereoselectivity (dr of >20:1) by the addition of allylmagnesium bromide to **24** in CH_2Cl_2 at −78 °C.[19,29] Ring closing metathesis of **25** employing the Grubbs second-generation catalyst[30] gave cyclohexene derivative **26** in 95% yield.

The remaining synthetic operations involve oxidation of the primary alcohols at C-9 and C-10, removal of the *tert*-butanesulfinamide auxiliary, and deprotection of the MOM group at C-6 of cyclohexene **26** (Scheme 3). We initially sought

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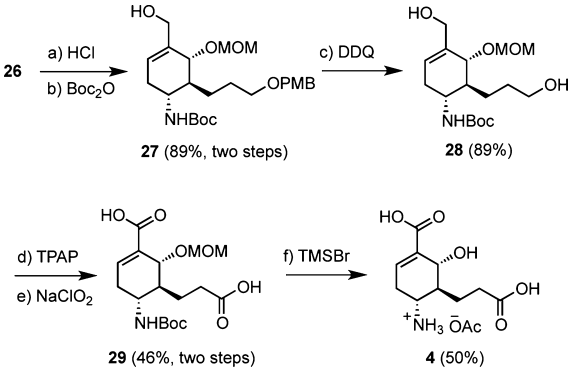
Scheme 3. Synthesis of 5[a]



[a]Reagents and conditions: (a) 4 M HCl in 1,4-dioxane, MeOH, 0 °C, 1 h; (b) Boc_2O, Et_3N, 1,4-dioxane/H_2O (2:1), rt, 1 h; (c) DDQ, CH_2Cl_2/H_2O (20:1), rt, 2 h; (d) tetrapropylammonium perruthenate, NMO, 4 Å molecular sieves, CH_2Cl_2, rt, 15 min; (e) $NaClO_2$, 2-methyl-2-butene, *t*-BuOH/THF/H_2O (5:1:1), rt, 2 h; (f) TMSBr, CH_2Cl_2, −78 °C, 1 h.

to adjust the oxidation level at C-9 and C-10 and maintain the *tert*-butanesulfinamide as a nitrogen protecting group. However, we discovered the *tert*-butanesulfinamide moiety is readily oxidized under mild oxidation conditions to a sulfonamide,[19] which undergoes facile elimination because of its axial conformation combined with the enhanced acidity of the vicinal allylic proton. Thus, the sulfinamide auxiliary was first removed along with the TBS group by treatment with 4 M HCl in a dioxane/MeOH solvent. The resultant amino alcohol was Boc-protected to afford **27** in 89% yield over two steps. Subsequent PMB deprotection with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) gave diol **28**. Direct oxidation to dicarboxylic acid **29** had a very low yield, so we employed a two-stage procedure with tetrapropylammonium perruthenate (TPAP)[31] to provide an intermediate dialdehyde that was converted to dicarboxylic acid **29** through Pinnick−Lindgren oxidation.[32] Deprotection of **29** with trimethylsilyl bromide[33] in CH_2Cl_2 at −78 °C afforded enantiopure target compound **4** as the acetate salt that was purified by silica gel chromatography employing a quaternary solvent system consisting of $CHCl_3$, MeOH, H_2O, and AcOH.

The relative configurations of three contiguous stereocenters in **4** were confirmed by the 2D NOESY studies of cyclohexene **28**. As shown in Figure 4, NOE correlation of H-4 with H-7 and H-8 indicates an *anti* relationship between H-4 and H-5. Because of NOE correlation of H-6 with H-7 and H-8 as well as

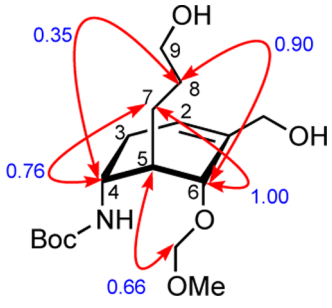


Figure 4. Key NOE correlations of **28**. The integral of the H-6/H-9 cross-peak was used as the internal calibrant (its integral was set to 1.00).

the correlation of H-5 with the CH_2 group of the MOM group at C-6, we conclude that H-5 and H-6 also have an *anti* relationship.

Biological Evaluation. Putative transition-state inhibitor **4** was evaluated for enzyme inhibition against recombinant MbtI under initial velocity conditions as described previously[13] but showed <10% inhibition at 100 μM. The modest potency of **4** clearly indicates it is a poor TS mimic. To rationalize the observed activity, we docked **4** into MbtI using the recently reported cocrystal structure of MbtI with a chorismate analogue.[34] Introduction of a CH_2 moiety in place of the C-5 oxygen atom of chorismate led to loss of a key hydrogen bond with Arg405, while the protonated C-4 amino group made a potentially repulsive electrostatic interaction with Arg405 (Figure S1 of the Supporting Information).

■ CONCLUSION

We designed and synthesized an inhibitor based on the hypothetical transition state of the isochorismate partial reaction catalyzed by MbtI wherein the C-4 hydroxyl group of chorismate is protonated by Glu252, resulting in bond cleavage and concomitant C−O bond formation at C-6 due to nucleophilic activation of a water molecule by Lys205. MbtI is a bifunctional enzyme and also catalyzes pyruvate elimination via an intramolecular [3,3]-sigmatropic reaction. To prevent this potential reaction from occurring in our inhibitor, the pyruvate side chain was replaced with a stable propionate isostere. Two complementary synthetic routes to target inhibitor **4** were explored. The initial route capitalized on the beautiful chemistry developed by Bartlett and Kozlowski for the preparation of a cyclohexene intermediate (±)-**7**. Frustrated by our inability to install the propionate side chain through a radical-mediated process and the fickle yield of the key Diels−Alder reaction, we undertook a novel synthetic route to enantiopure **4**. This second approach featured an asymmetric aldol reaction of a titanium enolate, a diastereoselective Grignard addition to a *tert*-butylsulfinyl aldimine, and ring closing olefin metathesis as key steps. Enzyme inhibition studies revealed **4** is a poor TS mimic and potentially suggests an alternate TS that does not involve charge buildup at C-4 and/or a more prominent role of the C-5 oxygen atom in chorismate for binding. These studies provide a synthetic and mechanistic foundation for future efforts in the development of TS-based inhibitors of MbtI.

■ EXPERIMENTAL SECTION

General Method. All reactions were conducted under a dry Ar atmosphere using oven-dried glassware and magnetic stirring. The solvents were dried before being used as follows. THF and Et_2O were heated at reflux over sodium benzophenone ketyl; toluene was heated at reflux over sodium, and CH_2Cl_2 was dried over CaH_2. Anhydrous *N*,*N*-diisopropylethylamine and triethylamine were used directly as purchased. Commercially available reagents were used without further purification unless otherwise noted. Aluminum TLC sheets (silica gel 60 F_{254}) of 0.2 mm thickness were used to monitor the reactions. The spots were visualized with short wavelength UV light or by charring after being sprayed with a solution prepared from one of the following solutions: phosphomolybdic acid (5.0 g) in 95% EtOH (100 mL), a *p*-anisaldehyde solution (2.5 mL of *p*-anisaldehyde, 2 mL of AcOH, and 3.5 mL of concentrated H_2SO_4 in 100 mL of 95% EtOH), or a ninhydrin solution (0.3 g of ninhydrin in 100 mL of *n*-butanol, with addition of 3 mL of AcOH). Flash chromatography was conducted with silica gel 60 (230−400 ASTM mesh). NMR spectra were recorded on a 400 or 600 MHz spectrometer. Proton chemical data

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are reported as follows: chemical shift, multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad), coupling constant, and integration. Chemical shifts were referenced on residual solvent peaks: $CDCl_3$ (δ 7.26 for ^{1}H NMR and δ 77.00 for ^{13}C NMR), CD_2Cl_2 (δ 5.32 for ^{1}H NMR and δ 53.84 for ^{13}C NMR), CD_3OD (δ 3.31 for ^{1}H NMR and δ 49.00 for ^{13}C NMR), and D_2O (δ 4.79 for ^{1}H NMR). Optical rotations were measured at rt in a 1.0 dm cell. High-resolution mass spectrometry was performed on a linear trap quadrupole (LTQ) mass spectrometer with electrospray ionization and a resolution of 60000 (at m/z 400).

(*E*)-1-(*tert*-Butyldimethylsilyloxy)-1,3-butadiene (13). To a solution of freshly distilled crotonaldehyde (5.00 mL, 60.3 mmol, 1.00 equiv) in CH_2Cl_2 (70 mL) at −10 °C was added Et_3N (12.0 mL, 36.6 mmol, 1.40 equiv), and the reaction mixture was stirred for 5 min. Next, TBSOTf (15.0 mL, 65.3 mmol, 1.10 equiv) was slowly added and the reaction mixture warmed to 23 °C over 2 h and then stirred for an additional 16 h at 23 °C. The reaction mixture was partitioned between CH_2Cl_2 (75 mL) and saturated aqueous $NaHCO_3$ (75 mL), dried ($MgSO_4$), and concentrated under reduced pressure. The title compound was purified by distillation over a Vigreux column (bp 60−62 °C/11 mmHg) to afford the title compound (8.70 g, 78%) as a colorless liquid. The analytical data (^{1}H and ^{13}C NMR and HRMS) matched the reported data for this compounds prepared by an alternate procedure [reported yield of 61% using crotonaldehyde (1.0 equiv), TBSCl (1.2 equiv), Et_3N (1.2 equiv), $ZnCl_2$ (0.013 equiv), hydroquinone (0.02 equiv), and benzene at 80 °C for 24 h].[16b]

(±)-(4*R*,5*S*,6*S*)-Ethyl 4-(*tert*-Butoxycarbonylamino)-6-(*tert*-butyldimethylsilyloxy)-5-(phenoxythiocarbonyloxy)cyclohex-1-enecarboxylate (15). To a solution of alcohol (±)-7[16b] (76.0 mg, 0.180 mmol, 1.00 equiv) in CH_2Cl_2 (5 mL) at 23 °C was added phenyl thionochloroformate (50.0 μL, 0.36 mmol, 2.00 equiv) followed by *N*-methylimidazole (50.0 μL, 0.65 mmol, 3.60 equiv). The reaction mixture was stirred for 16 h at 23 °C, the reaction quenched with H_2O (30 mL), and the mixture extracted with CH_2Cl_2 (3 × 30 mL). The combined organic extracts were washed with saturated aqueous NaCl (30 mL), dried (Na_2SO_4), and concentrated *in vacuo*. Purification by flash chromatography (5:1 hexane/EtOAc) afforded the title compound (89 mg, 90%) as a white solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.16 (s, 3H), 0.30 (s, 3H), 0.90 (s, 9H), 1.33 (t, J = 7.1 Hz, 3H), 1.42 (s, 9H), 2.44−2.69 (m, 2H), 4.12−4.25 (m, 1H), 4.25−4.38 (m, 2H), 4.83 (s, 1H), 5.49−5.57 (m, 1H), 6.24 (d, J = 9.1 Hz, 1H), 7.04−7.11 (m, 3H), 7.27−7.32 (m, 1H), 7.37−7.44 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ −5.1, −4.5, 14.3, 18.0, 25.8, 28.3, 29.6, 43.9, 60.9, 63.7, 79.0, 79.5, 121.7, 126.7, 129.55, 129.64, 138.8, 153.3, 154.9, 165.9, 193.6; HRMS (ESI+) calcd for $C_{27}H_{41}NNaO_7SSi^+$ $[M + Na]^+$ m/z 574.2265, found m/z 574.2266 (error of 0.2 ppm).

(±)-(4*R*,6*R*)-Ethyl 4-(*tert*-Butoxycarbonylamino)-6-(*tert*-butyldimethylsilyloxy)cyclohex-1-enecarboxylate (16). To a solution of ester (±)-**15** (44.0 mg, 0.080 mmol, 1.00 equiv) in refluxing toluene (4 mL) was added dropwise a solution of $(Me_3Si)_3SiH$ (40 μL, 0.12 mmol, 1.55 equiv), AIBN (7 mg, 0.04 mmol, 0.50 equiv), and *tert*-butyl acrylate (20 μL, 0.12 mmol, 1.55 equiv) in toluene (2 mL). The reaction mixture was heated at reflux for 2 h. After the mixture had cooled to room temperature, the solvent was removed *in vacuo*. Purification by flash chromatography (5:1 hexane/EtOAc) afforded the title compound (20 mg, 63%) as a white solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.10 (s, 3H), 0.18 (s, 3H), 0.88 (s, 9H), 1.31 (t, J = 7.1 Hz, 3H), 1.40 (s, 9H), 1.73 (dt, J = 14.3, 3.8 Hz, 1H), 2.01−2.09 (m, 1H), 2.27−2.39 (m, 1H), 2.55 (dd, J = 20.0, 5.1 Hz, 1H), 4.07 (q, J = 4.4 Hz, 1H), 4.10−4.20 (m, 1H), 4.21−4.32 (m, 1H), 4.75−4.81 (m, 1H), 6.55 (d, J = 8.1 Hz, 1H), 6.91−6.98 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ −5.0, −4.8, 14.3, 18.0, 25.8, 28.4, 32.6, 33.9, 42.0, 60.6, 62.7, 78.6, 132.0, 138.8, 155.3, 166.3; HRMS (ESI+) calcd for $C_{20}H_{37}NNaO_5Si^+$ $[M + Na]^+$ m/z 422.2333, found m/z 422.2337 (error of 0.9 ppm).

(*R*)-3-[5-(4-Methoxybenzyloxy)pentanoyl]-4-benzyloxazolidin-2-one (18). To a solution of acid **17**[22] (10.66 g, 44.7 mmol, 1.15 equiv) in THF (500 mL) at −78 °C was added Et_3N (10.8 mL, 77.2 mmol, 2.00 equiv) followed by pivaloyl chloride (6.00 mL, 49.2 mmol, 1.15 equiv), and the reaction mixture was warmed to 0 °C. The resulting thick white precipitate was stirred for 6 h at 0 °C and then recooled to −78 °C. In a separate flask, *n*-BuLi (2.5 M in hexane, 17.0 mL, 42.5 mmol, 1.10 equiv) was added by syringe over 5 min to a solution of (*R*)-4-benzyl-2-oxazolidinone (6.84 g, 38.6 mmol, 1.00 equiv) in THF (100 mL) at −78 °C. The oxazolidinone solution was transferred by cannula to the flask containing the mixed anhydride. The mixture was stirred for 30 min at −78 °C and 30 min at 0 °C, and then the reaction was quenched with saturated aqueous NH_4CI (200 mL). THF was removed under reduced pressure, and the aqueous layer was extracted with CH_2Cl_2 (3 × 200 mL). The combined organic layers were washed with a 10% aqueous NaOH solution, dried ($MgSO_4$), and concentrated. The crude residue was purified by flash chromatography (3:1 → 2:1 → 3:2 hexanes/EtOAc) to afford the title compound (14.2 g, 93%) as a colorless oil: $[\alpha]_D^{23}$ −37.8 (*c* 3.20, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.67−1.75 (m, 2H), 1.76−1.85 (m, 2H), 2.75 (dd, J = 13.4, 9.6 Hz, 1H), 2.89−3.05 (m, 2H), 3.29 (dd, J = 13.3, 3.2 Hz, 1H), 3.51 (t, J = 6.2 Hz, 2H), 3.80 (s, 3H), 4.14−4.19 (m, 2H), 4.45 (s, 2H), 4.66 (dddd, J = 13.3, 10.1, 7.1, 3.5 Hz, 1H), 6.87−6.92 (m, 2H), 7.19−7.23 (m, 2H), 7.25−7.37 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 21.0, 29.0, 35.1, 37.8, 55.0, 55.2, 66.1, 69.6, 72.5, 113.7, 127.2, 128.9, 129.2, 129.3, 130.6, 135.3, 153.4, 159.0, 173.0; HRMS (ESI+) calcd for $C_{23}H_{27}NNaO_5^+$ $[M + Na]^+$ m/z 420.1781, found m/z 420.1786 (error of 1.2 ppm).

2-(*tert*-Butyldimethylsilyloxymethyl)acrolein (19). To a solution of *tert*-butyl 2-(hydroxymethyl)acrylate prepared according to the reported procedure[23,35] (10.0 g, 63.2 mmol, 1.00 equiv) in CH_2Cl_2 (500 mL) at 0 °C was added imidazole (11.1 g, 165 mmol, 2.61 equiv) followed by *tert*-butyldimethylsilyl chloride (19.1 g, 126 mmol, 2.00 equiv). The reaction mixture was stirred at 23 °C overnight. After 16 h, the solvent was removed *in vacuo* to provide a colorless oil, which was dissolved in a 10:1 hexane/EtOAc solvent (220 mL). The solution was passed through a short pad of silica gel, which was washed with a hexane/EtOAc solvent (10:1). The filtrate was concentrated *in vacuo* and dried under high vacuum to afford a colorless oil, which was then used directly in the next step without further purification.

To the solution of the crude *tert*-butyl 2-(*tert*-butyldimethylsilyloxymethyl)acrylate prepared as described above in CH_2Cl_2 (300 mL) at −78 °C was added diisobutylaluminum hydride (100 mL, 1.0 M in hexane, 100 mmol) dropwise. The reaction mixture was stirred for 1 h at −78 °C, then diluted with Et_2O (300 mL), and allowed to slowly warm to 23 °C. While the reaction mixture slowly warmed to room temperature, the reaction was quenched by the sequential dropwise addition of H_2O (4 mL), a 15% aqueous NaOH solution (4 mL), and H_2O (10 mL). After warming to 23 °C, the reaction mixture was vigorously stirred for 30 min, then treated with anhydrous $MgSO_4$, and stirred for an additional 15 min. The mixture was filtered through a pad of Celite, and the resulting filtrate was concentrated *in vacuo* to afford the title compound (6.00 g, 50%, two steps) as a colorless oil, whose ^{1}H and ^{13}C NMR data agreed with the reported data for **19** prepared by an alternate synthetic route.[36]

(*R*)-4-Benzyl-3-((2*R*,3*R*)-4-(*tert*-butyldimethylsilyloxy)-methyl-2-{3-[(4-methoxybenzyl)oxy]propyl}-3-(methoxymethoxy)pent-4-enoyl)oxazolidin-2-one (21). To a solution of *N*-acyloxazolidinone **18** (3.00 g, 7.50 mmol, 1.00 equiv) in CH_2Cl_2 (100 mL) at −78 °C was added a 1.0 M solution of $TiCl_4$ in CH_2Cl_2 (8.50 mL, 8.50 mmol, 1.13 equiv) dropwise, and the solution was stirred for 10 min. (−)-Sparteine (4.30 mL, 18.8 mmol, 2.50 equiv) was added dropwise to the mixture and the solution stirred at −78 °C for 1.5 h. Freshly distilled aldehyde **19** (3.00 g, 15.0 mmol, 2.00 equiv) was then added dropwise. The reaction mixture was stirred for 1.5 h at −78 °C, 3 h at −40 °C, and 12 h at −20 °C. The reaction was quenched at −40 °C by addition of half-saturated aqueous NH_4Cl (100 mL) and the mixture quickly transferred to a separatory funnel. The organic layer was separated and the aqueous layer extracted with CH_2Cl_2 (2 × 100 mL). The combined organic layers were dried ($MgSO_4$), filtered, and concentrated. Purification of the crude residue by flash chromatography (10:1 → 8:1 → 7:1 → 6:1 PhMe/EtOAc) provided aldol adduct **20** (2.13 g, 48%) as a colorless oil, which was directly carried onto the next step.

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Chloromethyl methyl ether (1.69 g, 21.0 mmol, 5.90 equiv) was added to a solution of aldol adduct **20** (2.13 g, 3.56 mmol, 1.0 equiv), *i*-Pr_2NEt (3.66 mL, 21.0 mmol, 5.90 equiv), and Bu_4NI (131 mg, 0.36 mmol, 0.10 equiv) in PhMe (50 mL) and the mixture stirred at 90 °C for 7 h. The reaction mixture was cooled to rt and treated with saturated aqueous $NaHCO_3$ (30 mL). The layers were separated, and the aqueous layer was extracted with EtOAc (3 × 50 mL). The combined organic extracts were washed with saturated aqueous NaCl (30 mL), dried (Na_2SO_4), and concentrated. Purification by flash chromatography (4:1 → 3:1 hexane/EtOAc) afforded the title compound (1.88 g, 82%) as a colorless oil: $[\alpha]_D^{23}$ +13.5 (*c* 1.10, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 0.00 (s, 6H), 0.84 (s, 9H), 1.57−1.67 (m, 2H), 1.82−1.92 (m, 2H), 2.55 (dd, *J* = 13.2, 10.2 Hz, 1H), 3.22 (dd, *J* = 13.2, 3.0 Hz, 1H), 3.27 (s, 3H), 3.37−3.42 (m, 2H), 3.70 (s, 3H), 3.97−4.13 (m, 4H), 4.16−4.23 (m, 1H), 4.26 (d, *J* = 7.9 Hz, 1H), 4.35 (s, 2H), 4.41 (d, *J* = 6.8 Hz, 1H), 4.47 (dddd, *J* = 12.9, 9.8, 6.4, 3.0 Hz, 1H), 4.55 (d, *J* = 6.8 Hz, 1H), 5.09 (s, 1H), 5.29 (s, 1H), 6.76−6.81 (m, 2H), 7.10−7.15 (m, 2H), 7.15−7.27 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$) δ −5.6, 18.2, 25.0, 25.7, 27.0, 37.7, 46.4, 55.0, 55.7, 55.8, 62.1, 65.8, 69.7, 72.3, 77.9, 94.2, 112.8, 113.5, 127.1, 128.8, 129.1, 129.2, 130.5, 135.3, 145.8, 152.9, 158.9, 173.3; HRMS (ESI+) calcd for $C_{35}H_{51}NNaO_8Si^+$ $[M + Na]^+$ *m*/*z* 664.3276, found *m*/*z* 664.3283 (error of 1.05 ppm).

(2*S*,3*R*)-4-{[(*tert*-Butyldimethylsilyl)oxy]methyl}-2-{3-[(4-methoxybenzyl)oxy]propyl}-3-(methoxymethoxy)pent-4-en-1-ol (22). Sodium borohydride (450 mg, 11.7 mmol, 4.00 equiv) was added in one portion to a solution of oxazolidinone **21** (1.88 g, 2.93 mmol, 1.00 equiv) in 3:1 THF/H_2O solvent (60 mL) at 0 °C. The reaction mixture was allowed to warm to 23 °C and stirred overnight (~16 h). The reaction was quenched by the slow addition of saturated aqueous NH_4Cl (60 mL) and the mixture extracted with diethyl ether (3 × 50 mL). The combined organic extracts were washed with saturated aqueous NaCl, dried ($MgSO_4$), and concentrated under reduced pressure. Purification by flash chromatography (3:1 → 2:1 → 3:2 hexane/EtOAc) afforded the title compound (920 mg, 67%) as a colorless oil: $[\alpha]_D^{23}$ +60.7 (*c* 0.400, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 0.00 (s, 6H), 0.84 (s, 9H), 1.22−1.34 (m, 1H), 1.43−1.59 (m, 2H), 1.59−1.71 (m, 2H), 2.67 (br s, 1H), 3.35 (s, 3H), 3.41 (t, *J* = 6.3 Hz, 2H), 3.58−3.63 (m, 2H), 3.76 (s, 3H), 4.09 (s, 2H), 4.19 (d, *J* = 6.1 Hz, 1H), 4.39 (s, 2H), 4.48 (d, *J* = 6.5 Hz, 1H), 4.58 (d, *J* = 6.5 Hz, 1H), 5.12 (s, 1H), 5.32 (s, 1H), 6.83 (d, *J* = 8.5 Hz, 2H), 7.22 (d, *J* = 8.5 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ −5.58, −5.56, 18.2, 22.7, 25.8, 27.3, 43.1, 55.0, 55.8, 62.4, 63.2, 70.2, 72.4, 78.5, 94.6, 112.9, 113.6, 129.0, 130.5, 146.1, 159.0; HRMS (ESI+) calcd for $C_{25}H_{44}NaO_6Si^+$ $[M + Na]^+$ *m*/*z* 491.2799, found *m*/*z* 491.2806 (error of 1.4 ppm).

(2*S*,3*R*)-4-{[(*tert*-Butyldimethylsilyl)oxy]methyl}-2-{3-[(4-methoxybenzyl)oxy]propyl}-3-(methoxymethoxy)pent-4-enal (23). To a solution of alcohol **22** (920 mg, 1.96 mmol, 1.00 equiv) in CH_2Cl_2 (20 mL) at 23 °C was added solid $NaHCO_3$ (1.65 g, 19.6 mmol, 10.0 equiv) followed by Dess−Martin periodinane (1.25 g, 2.94 mmol, 1.50 equiv). The reaction mixture was stirred for 30 min at 23 °C and then the reaction quenched with a 1:1 10% aqueous $Na_2S_2O_3$/saturated aqueous $NaHCO_3$ solution (20 mL). The layers were separated, and the aqueous layer was extracted with CH_2Cl_2 (3 × 20 mL). The combined organic layers were dried ($MgSO_4$) and concentrated under reduced pressure. The resulting residue was dissolved in a minimal amount of 3:1 hexane/EtOAc solvent and filtered through a short pad of silica gel, which was rinsed with 3:1 hexane/EtOAc solvent (200 mL). The filtrate was concentrated to afford the title compound (821 mg, 90%) as a colorless oil: ^1H NMR (400 MHz, $CDCl_3$) δ 0.00 (s, 6H), 0.84 (s, 9H), 1.43−1.54 (m, 1H), 1.57−1.79 (m, 3H), 2.50 (dddd, *J* = 11.5, 8.9, 5.6, 2.7 Hz, 1H), 3.26 (s, 3H), 3.36 (t, *J* = 6.0 Hz, 2H), 3.73 (s, 3H), 4.05 (s, 2H), 4.34 (s, 2H), 4.41 (d, *J* = 6.7 Hz, 1H), 4.44 (d, *J* = 5.9 Hz, 1H), 4.56 (d, *J* = 6.8 Hz, 1H), 5.08 (s, 1H), 5.27 (s, 1H), 6.80 (d, *J* = 8.6 Hz, 2H), 7.19 (d, *J* = 8.6 Hz, 2H), 9.61 (d, *J* = 2.2 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ −5.54, −5.52, 18.2, 21.2, 25.8, 27.6, 54.6, 55.1, 55.9, 63.2, 69.7, 72.4, 75.8, 94.1, 113.6, 114.0, 129.1, 130.5, 144.8, 159.0, 203.1; HRMS (ESI+) calcd for $C_{25}H_{42}NaO_6Si^+$ $[M + Na]^+$ *m*/*z* 489.2643, found *m*/*z* 489.2647 (error of 0.8 ppm).

(*S*,*E*)-*N*-((2*S*,3*R*)-4-{[(*tert*-Butyldimethylsilyl)oxy]methyl}-2-{3-[(4-methoxybenzyl)oxy]propyl}-3-(methoxymethoxy)pent-4-en-1-ylidene)-*tert*-butylsulfinamide (24). To a solution of aldehyde **23** (820 mg, 1.76 mmol, 1.00 equiv) in CH_2Cl_2 (10 mL) were added (*S*)-(−)-*tert*-butylsulfinamide (235 mg, 1.94 mmol, 1.10 equiv), anhydrous $CuSO_4$ (562 mg, 3.52 mmol, 2.00 equiv), and pyridinium *p*-toluenesulfonate (442 mg, 1.76 mmol, 1.00 equiv). The mixture was stirred at 23 °C for 24 h and then filtered through a pad of Celite, and the filter cake was washed with CH_2Cl_2. The combined organic filtrate was washed with saturated aqueous NaCl, dried ($MgSO_4$), and concentrated. Purification by flash chromatography (3:1 → 2:1 hexane/EtOAc) afforded the title compound (616 mg, 62%, 75% brsm) as a colorless oil: $[\alpha]_D^{23}$ +165 (*c* 0.700, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 0.00 (s, 6H), 0.84 (s, 9H), 1.13 (s, 9H), 1.42−1.69 (m, 3H), 1.80−1.91 (m, 1H), 2.73−2.83 (m, 1H), 3.30 (s, 3H), 3.36 (t, *J* = 6.1 Hz, 2H), 3.72 (s, 3H), 4.06 (s, 2H), 4.24 (d, *J* = 7.1 Hz, 1H), 4.34 (s, 2H), 4.42 (d, *J* = 6.7 Hz, 1H), 4.58 (d, *J* = 6.7 Hz, 1H), 5.06 (s, 1H), 5.25 (s, 1H), 6.80 (d, *J* = 8.3 Hz, 2H), 7.17 (d, *J* = 8.3 Hz, 2H), 7.87 (d, *J* = 6.8 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ −5.71, −5.69, 18.0, 22.2, 24.2, 25.7, 27.2, 48.7, 54.9, 55.8, 56.6, 62.3, 69.4, 72.2, 77.8, 93.7, 113.5, 114.1, 128.9, 130.4, 144.6, 158.9, 169.8; HRMS (ESI+) calcd for $C_{29}H_{51}NNaO_6SSi^+$ $[M + Na]^+$ *m*/*z* 592.3099, found *m*/*z* 592.3105 (error of 1.0 ppm).

(*S*)-*N*-((4*R*,5*S*,6*R*)-7-{[(*tert*-Butyldimethylsilyl)oxy]methyl}-5-{3-[(4-methoxybenzyl)oxy]propyl}-6-(methoxymethoxy)octa-1,7-dien-4-yl)-*tert*-butylsulfinamide (25). To a solution of *tert*-butanesulfinyl imine **24** (600 mg, 1.05 mmol, 1.00 equiv) in CH_2Cl_2 (20 mL) was added allylmagnesium bromide (1.0 M in Et_2O, 3.15 mL, 3.15 mmol, 3.00 equiv) at −78 °C. The reaction mixture was warmed to −20 °C and stirred for 2.5 h at this temperature and then the reaction quenched with saturated aqueous NH_4Cl (20 mL). The layers were separated, and the aqueous layer was extracted with CH_2Cl_2 (3 × 20 mL). The combined organic layers were dried ($MgSO_4$), filtered, and concentrated. The resulting residue was dissolved in a minimal amount of Et_2O and filtered through a short pad of silica gel, which was rinsed with Et_2O. The filtrate was concentrated to provide the title compound (560 mg, 87%) as a colorless oil: $[\alpha]_D^{23}$ +80.1 (*c* 0.600, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 0.02 (s, 6H), 0.87 (s, 9H), 1.16 (s, 9H), 1.37−1.51 (m, 2H), 1.55−1.79 (m, 3H), 2.35−2.45 (m, 1H), 2.49−2.59 (m, 1H), 3.22 (d, *J* = 8.3 Hz, 1H), 3.28 (s, 3H), 3.35−3.38 (m, 3H), 3.74 (s, 3H), 3.99 (d, *J* = 15.0 Hz, 1H), 4.05 (d, *J* = 14.6 Hz, 1H), 4.16 (d, *J* = 7.3 Hz, 1H), 4.36 (s, 2H), 4.39 (d, *J* = 6.7 Hz, 1H), 4.56 (d, *J* = 6.7 Hz, 1H), 5.05−5.10 (m, 3H), 5.34 (s, 1H), 5.66−5.80 (m, 1H), 6.81 (d, *J* = 8.2 Hz, 1H), 7.19 (d, *J* = 8.2 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ −5.55, −5.52, 18.2, 22.6, 22.8, 25.7, 29.5, 38.5, 42.7, 55.0, 55.9, 56.0, 56.5, 62.1, 70.0, 72.3, 79.0, 93.9, 113.5, 113.9, 118.6, 129.0, 130.5, 134.5, 145.3, 158.9; HRMS (ESI+) calcd for $C_{32}H_{58}NO_6SSi^+$ $[M + H]^+$ *m*/*z* 612.3749, found *m*/*z* 612.3754 (error of 0.8 ppm).

(*S*)-*N*-((1*R*,5*R*,6*S*)-4-{[(*tert*-Butyldimethylsilyl)oxy]methyl}-6-{3-[(4-methoxybenzyl)oxy]propyl}-5-(methoxymethoxy)-cyclohex-3-en-1-yl)-*tert*-butylsulfinamide (26). [1,3-Bis(2,4,6-trimethylphenyl)-2-imidazolidinylidene]dichloro(phenylmethylene)-(tricyclohexylphosphine)ruthenium (13.8 mg, 0.016 mmol, 0.14 equiv) was added to a solution of diene **25** (70.0 mg, 0.114 mmol, 1.0 equiv) in CH_2Cl_2 (20 mL), and the reaction mixture was heated at reflux for 14 h. The reaction mixture was cooled to 23 °C, then treated with DMSO (1.5 mL),[37] and stirred 16 h at 23 °C. The reaction mixture was washed with saturated aqueous NaCl (50 mL), and the organic layer was dried ($MgSO_4$), filtered, and concentrated. Purification by flash chromatography (2:1 → 3:2 → 1:1 hexane/EtOAc) afforded the title compound (63 mg, 95%) as a light brown oil: ^1H NMR (400 MHz, $CDCl_3$) δ −0.01 (s, 3H), 0.00 (s, 3H), 0.85 (s, 9H), 1.11 (s, 9H), 1.16−1.29 (m, 2H), 1.54−1.70 (m, 2H), 1.99−2.07 (m, 1H), 2.23−2.34 (m, 1H), 2.48−2.59 (m, 1H), 3.26−3.46 (m, 6H), 3.73 (s, 3H), 3.92 (s, 1H), 4.02 (d, *J* = 12.8 Hz, 1H), 4.14 (d, *J* = 12.8 Hz, 1H), 4.35 (s, 2H), 4.54 (d, *J* = 6.6 Hz, 1H), 4.60−4.69 (m, 2H), 5.65 (s, 1H), 6.80 (d, *J* = 8.2 Hz, 2H), 7.17 (d, *J* = 8.2 Hz, 2H);

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^{13}C NMR (100 MHz, $CDCl_3$) δ −5.6, −5.4, 18.0, 22.5, 25.7, 26.5, 27.5, 29.2, 40.9, 53.5, 55.0, 55.5, 55.6, 64.3, 69.5, 71.6, 72.4, 95.3, 113.5, 121.5, 129.0, 130.3, 135.0, 158.9; HRMS (ESI+) calcd for $C_{30}H_{53}NNaO_6SSi^+$ $[M + Na]^+$ m/z 606.3255, found m/z 606.3252 (error of 0.5 ppm).

***tert*-Butyl ((1*R*,5*R*,6*S*)-4-(Hydroxymethyl)-6-{3-[(4-methoxybenzyl)oxy]propyl}-5-(methoxymethoxy)cyclohex-3-en-1-yl)carbamate (27).** To a solution of **26** (55 mg, 0.094 mmol, 1.0 equiv) in MeOH (2.0 mL) was added dropwise 4 M HCl in 1,4-dioxane (190 μL, 0.75 mmol, 8.0 equiv) at 0 °C. The resulting mixture was stirred at 0 °C for 1 h and then the reaction quenched with saturated aqueous $NaHCO_3$ (3 mL). Methanol was removed under reduced pressure, and the aqueous layer was treated with CH_2Cl_2 (2 mL) and 6 M aqueous NaOH (2 mL) at 0 °C. After the mixture had been stirred for 10 min at 0 °C, the layers were separated. The aqueous layer was extracted with CH_2Cl_2 (3 × 3 mL), and the combined organic layers were dried (K_2CO_3) and concentrated under reduced pressure to afford the crude amino alcohol as a viscous yellow oil, which was used directly in the next step without further purification.

To the crude amino alcohol in 1:2 H_2O/1,4-dioxane solvent (3 mL) at 23 °C was added Et_3N (26 μL, 0.19 mmol, 2.0 equiv) followed by di-*tert*-butyl dicarbonate (28 mg, 0.13 mmol, 1.4 equiv) in 1,4-dioxane (0.5 mL). The reaction mixture was stirred for 1 h at 23 °C and then partitioned between H_2O (10 mL) and CH_2Cl_2 (10 mL). The aqueous layer was extracted with CH_2Cl_2 (3 × 3 mL), and the combined organic layers were dried ($MgSO_4$), filtered, and concentrated. Purification by flash chromatography (1:1 hexane/EtOAc) afforded the title compound (39 mg, 89%) as a colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.21−1.31 (m, 2H), 1.42 (s, 9H), 1.63−1.74 (m, 2H), 1.96−2.11 (m, 2H), 2.19−2.26 (m, 2H), 3.37−3.46 (m, 5H), 3.80 (s, 3H), 3.82−3.89 (m, 1H), 3.96 (s, 1H), 4.09 (d, J = 12.0 Hz, 1H), 4.18 (d, J = 12.7 Hz, 1H), 4.40 (s, 2H), 4.64 (d, J = 6.7 Hz, 1H), 4.74 (d, J = 6.7 Hz, 1H), 5.76 (br s, 1H), 5.93 (d, J = 8.1 Hz, 1H), 6.87 (d, J = 8.5 Hz, 2H), 7.23 (d, J = 8.5 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 27.0, 27.68, 27.72, 28.4, 40.0, 46.3, 55.2, 56.1, 65.4, 69.7, 72.5, 74.0, 78.8, 96.2, 113.8, 124.6, 129.2, 130.5, 134.8, 155.4, 159.1; HRMS (ESI+) calcd for $C_{25}H_{39}NNaO_7^+$ $[M + Na]^+$ m/z 488.2619, found m/z 488.2621 (error of 0.4 ppm).

***tert*-Butyl [(1*R*,5*R*,6*S*)-4-(Hydroxymethyl)-6-(3-hydroxypropyl)-5-(methoxymethoxy)cyclohex-3-en-1-yl]carbamate (28).** To a solution of **27** (40 mg, 0.086 mmol, 1.0 equiv) in 20:1 CH_2Cl_2/H_2O solvent (10 mL) was added DDQ (29 mg, 0.13 mmol, 1.5 equiv) at 0 °C. The reaction mixture was stirred for 2 h at 23 °C and then partitioned between saturated aqueous $NaHCO_3$ (10 mL) and CH_2Cl_2 (10 mL). The aqueous layer was extracted with CH_2Cl_2 (2 × 10 mL), and the combined organic layers were dried (Na_2SO_4), filtered, and concentrated. Purification by flash chromatography (Et_2O → EtOAc) afforded the title compound (23 mg, 89%) as a colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.21−1.31 (m, 2H), 1.42 (s, 9H), 1.58−1.72 (m, 2H), 2.04−2.12 (m, 1H), 2.20−2.26 (m, 2H), 2.30−2.40 (m, 2H), 3.43 (s, 3H), 3.55−3.68 (m, 2H), 3.80−3.89 (m, 1H), 3.97−4.01 (m, 1H), 4.07 (d, J = 12.4 Hz, 1H), 4.18 (d, J = 12.6 Hz, 1H), 4.67 (d, J = 6.8 Hz, 1H), 4.77 (d, J = 6.8 Hz, 1H), 5.72−5.79 (m, 1H), 5.93 (d, J = 7.5 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 26.6, 27.8, 28.4, 30.4, 39.9, 46.4, 56.1, 62.4, 65.1, 73.7, 78.9, 96.2, 124.7, 134.9, 155.5; HRMS (ESI+) calcd for $C_{17}H_{31}NNaO_6^+$ $[M + Na]^+$ m/z 368.2044, found m/z 368.2046 (error of 0.5 ppm).

(4*R*,5*S*,6*R*)-4-*tert*-(Butylcarbonyl)amino-5-(2-carboxyethyl)-6-(methoxymethoxy)cyclohex-1-enecarboxylic Acid (29). To a solution of diol **28** (23 mg, 0.076 mmol, 1.0 equiv), *N*-methylmorpholine *N*-oxide (53 mg, 0.45 mmol, 6.0 equiv), and powdered 4 Å molecular sieves (35 mg) in CH_2Cl_2 (1 mL) at 23 °C was added tetrapropylammonium perruthenate (2.0 mg, 0.0056 mmol, 0.075 equiv). The mixture was stirred for 15 min at 23 °C and then filtered through a short pad of silica gel, eluting with EtOAc. The filtrate was concentrated under reduced pressure to afford the crude dialdehyde, which was directly used in the next step without further purification.

To a solution of freshly prepared dialdehyde and 2-methyl-2-butene (0.20 mL, 1.9 mmol, 25 equiv) in 5:1:1 *tert*-butyl alcohol/THF/H_2O solvent (1 mL) at 0 °C was slowly added a solution of sodium chlorite [80% (w/w) technical grade, 52 mg, 0.46 mmol, 6.0 equiv] and sodium phosphate monobasic monohydrate (63 mg, 0.46 mmol, 6.0 equiv) in H_2O (0.3 mL). The resulting suspension was stirred at 23 °C for 2 h. The reaction was quenched with saturated aqueous $NaHSO_3$ (3 mL) at 0 °C, and the aqueous layer was extracted with CH_2Cl_2 (3 × 5 mL). The combined organic layers were dried (Na_2SO_4), filtered, and concentrated under reduced pressure. Purification by flash chromatography on silica gel[38] (210:25:4 → 180:25:4 $CHCl_3$/MeOH/AcOH) afforded the title compound (13 mg, 46% for two steps) as a colorless oil that was ∼90% pure: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.41 (s, 9H), 1.44−1.54 (m, 2H), 2.18−2.25 (m, 1H), 2.39−2.60 (m, 4H), 3.40 (s, 3H), 3.88−3.95 (m, 1H), 4.31−4.34 (m, 1H), 4.72 (d, J = 6.9 Hz, 1H), 4.86 (d, J = 6.9 Hz, 1H), 6.29 (d, J = 8.1 Hz, 1H), 7.14−7.18 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 25.0, 28.4, 29.0, 31.8, 40.1, 45.4, 56.1, 71.8, 79.3, 97.5, 128.0, 142.4, 155.5, 171.1, 178.2; HRMS (ESI−) calcd for $C_{17}H_{26}NO_8^-$ $[M - H]^-$ m/z 372.1664, found m/z 372.1668 (error of 1.1 ppm).

(4*R*,5*S*,6*R*)-4-Amino-5-(2-carboxyethyl)-6-hydroxycyclohex-1-enecarboxylic Acid Acetate Salt (4). To a solution of dicarboxylic acid **29** (13 mg, 0.035 mmol, 1.0 equiv) in CH_2Cl_2 (1 mL) was added TMSBr (40 μL, 0.30 mmol, 8.6 equiv) at −78 °C. The reaction mixture was stirred for 1 h at −78 °C and then concentrated *in vacuo* during which time the flask was warmed to 23 °C. The residue was washed with Et_2O and purified by flash chromatography on silica gel[38] (65:25:4:2 → 100:56:8:8 $CHCl_3$/MeOH/H_2O/AcOH) to afford the acetate salt of the title compound (5.0 mg, 50%) as a white solid: $[\alpha]_D^{23}$ +6.2 (*c* 0.40, H_2O); ^{1}H NMR (600 MHz, D_2O) δ 1.57−1.64 (m, 2H), 2.22−2.29 (m, 1H), 2.46 (t, J = 7.4 Hz, 2H), 2.56 (d, J = 13.5 Hz, 1H), 2.78 (d, J = 13.5 Hz, 1H), 3.68−3.74 (m, 1H), 4.55−4.59 (m, 1H), 6.84−6.88 (m, 1H); ^{13}C NMR (150 MHz, D_2O) δ 21.1 (AcOH), 24.0, 65.0, 32.7, 40.7, 46.9, 65.1, 131.8*, 134.3, 172.4*, 177.4* (AcOH), 179.6* (chemical shifts denoted with asterisks were derived from HMBC); HRMS (ESI−) calcd for $C_{10}H_{14}NO_5^-$ $[M - H]^-$ m/z 228.0877, found m/z 228.0883 (error of 2.6 ppm).

MbtI Assay. Reactions were performed under initial velocity conditions in a total volume of 50 μL at 37 °C for 30 min, and the production of salicylic acid was monitored continuously by following changes in fluorescence at 420 nm with excitation at 305 nm on a microplate reader. Assays were set up in duplicate and contained 0.5 μM MbtI in reaction buffer [100 mM Tris-HCl (pH 8.0), 1 mM $MgCl_2$, 50 μM chorismate, and 0.0025% Igepal CA-630]. A 3-fold serial dilution of inhibitor in H_2O was added to black 384-well plates coated with a nonbinding surface (Greiner). A positive control (H_2O only) and negative control (10 mM EDTA) were also included. The IC_{50} values were calculated from the Hill equation (eq 1).

$$\frac{v_i}{v_0} = \frac{1}{1 + ([I]/IC_{50})^h} \quad (1)$$

In this equation, the fractional activity (v_i/v_0) versus inhibitor concentration was fit by nonlinear regression analysis using GraphPad Prism version 6.0, where v_i is the reaction velocity at a given [I], v_0 is the reaction velocity of the DMSO control, and h is the Hill slope.

■ ASSOCIATED CONTENT

Supporting Information

Copies of ^{1}H NMR and ^{13}C NMR spectra of all new compounds and a figure illustrating a docked pose of **4** in MbtI. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.joc.5b00455.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: aldri015@umn.edu.

Notes

The authors declare no competing financial interest.

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