Published in final edited form as:

J Org Chem. 2017 April 07; 82(7): 3432–3440. doi:10.1021/acs.joc.6b02801.

Synthesis of Transition-State Inhibitors of Chorismate Utilizing Enzymes from Bromobenzene *cis*-1,2-Dihydrodiol

Xiao-Kang Zhang^{1,‡}, Feng Liu^{2,‡}, William D. Fiers², Wen-Mei Sun¹, Jun Guo¹, Zheng Liu^{1,*}, and Courtney C. Aldrich^{2,*}

¹Key Laboratory of Pesticide & Chemical Biology of Ministry of Education, College of Chemistry, Central China Normal University, 152 Luoyu Road, Wuhan, Hubei 430079, P. R. China

²Department of Medicinal Chemistry, University of Minnesota, 308 Harvard Street SE, 8-174 WDH, Minneapolis, Minnesota 55455, United States

Abstract

In order to survive in a mammalian host, *Mycobacterium tuberculosis* (*Mtb*) produces aryl-capped siderophores known as the mycobactins for iron acquisition. Salicylic acid is a key building block of the mycobactin core and is synthesized by the bifunctional enzyme MbtI, which converts chorismate into isochorismate via a S_N2'' reaction followed by further transformation into salicylate through a [3,3]-sigmatropic rearrangement. MbtI belongs to a family of chorismate-utilizing enzymes (CUEs) that have conserved topology and active site residues. The transition-state inhibitor **1** described by Bartlett, Kozlowski and co-workers is the most potent reported inhibitor to date of CUEs. Herein we disclose a concise asymmetric synthesis and the accompanying biochemical characterization of **1** along with three closely related analogues beginning from bromobenzene *cis*-1,2-dihydrodiol produced through microbial oxidation that features a series of regio- and stereoselective transformations for introduction of the C-4 hydroxy and C-6 amino substituents. The flexible synthesis enables late-stage introduction of the carboxy group and other bioisosteres at the C-1 position as well as installation of the enol-pyruvate side chain at the C-5 position.

Graphical Abstract

*Corresponding Authors: aldrich015@umn.edu; liuz1118@mail.ccnu.edu.cn.

‡X.-K. Z. and F. L. equally contributed to this work.

Supporting Information: Copies of ¹H NMR and ¹³C NMR spectra of all new compounds. This material is available free of charge via the Internet at <http://pubs.acs.org>.

INTRODUCTION

Tuberculosis (TB) is an infectious disease caused by *Mycobacterium tuberculosis* (*Mtb*), which latently infects one-third of the world's population and is responsible for an estimated two million deaths annually.¹ Drug susceptible TB is challenging to treat, compared to most other bacterial infections, and requires at least six months of combination chemotherapy using the first-line TB drugs isoniazid, rifampicin, pyrazinamide, and ethambutol that are the most effective and best tolerated TB drugs. Drug-resistant (DR) TB is associated with poor treatment outcomes, significant adverse effects, and extraordinary long treatment times spanning up to two years. Consequently, there has been a renewed interest to develop new antibacterial agents with novel modes of action that are effective against DR-TB and can shorten the duration of TB chemotherapy.²

Disruption of iron metabolism in *Mtb* represents a promising therapeutic strategy for combatting TB since iron is essential for survival and growth of *Mtb*, but is highly restricted in a mammalian host.³ In order to establish an infection and persist in a host, *Mtb* synthesizes iron-chelating siderophores called mycobactins that abstract iron from host proteins.⁴ The biosynthesis of mycobactins is performed by a mixed nonribosomal peptide synthetase-polyketide synthase (NRPS-PKS) pathway encoded by 14 genes *mbtA*–*mbtN*.⁵ The starter unit of this pathway is prepared by MbtI, a magnesium-dependent bifunctional salicylate synthase (Figure 1), which transforms chorismate into salicylate via the intermediate isochorismate.⁶ MbtI belongs to a large family of chorismate-utilizing enzymes (CUEs)⁷ that are present in plants, bacteria, fungi and apicomplexan parasites, but not in mammals. The isochorismatase activity of MbtI requires Lys205, which is postulated to nucleophilically activate a water molecule for attack at the C-6 position of chorismate, and Glu252 that is believed to polarize the C-4 hydroxy leaving group (Figure 1). The conversion of chorismate to isochorismate likely occurs via a concerted S_N2'' reaction mechanism via transition state **TS-1**, although a stepwise mechanism is also plausible. In the second reaction, which occurs in the same active site, pyruvate is eliminated from isochorismate via an intramolecular [3,3]-sigmatropic rearrangement to afford salicylic acid via bicyclic transition state **TS-2**.^{6a, 10, 11, 12}

The pioneering work of Bartlett, Kozlowski and co-workers to develop inhibitors of CUEs focused on transition-state mimics and led to the synthesis of (±)-**1**, which remains the most potent inhibitor of CUEs reported to date with an inhibition constant (*K_i*) of 53 nM against the isochorismate synthase EntC from *Escherichia coli* that catalyzes an identical first half reaction as MbtI.⁸ EntC helps synthesize 2,3-dihydroxybenzoic acid, the starter unit for the biosynthesis of the siderophore enterobactin in *E. coli*. Regioisomer (±)-**2** was approximately an order of magnitude less potent than (±)-**1** with a *K_i* of 450 nM against EntC. These data suggest more pronounced positive charge build-up at C-6 versus C-4 in **TS-1**.⁸ In complimentary work, Abell, Payne and co-workers disclosed simpler benzoic acid inhibitors with *K_i* values in the low micromolar range against a wide variety of CUEs including MbtI.⁹ Since MbtI possesses isochorismatase activity, we hypothesized that **1** would serve as an excellent template for inhibitor design and herein describe an asymmetric synthesis of **1** from readily available enantiopure bromobenzene *cis*-1*S*,2*S*-dihydrodiol **6**. Our synthesis was planned to allow late-stage introduction of groups at both C-1 and C-5, in

order to explore structure-activity relationships (SAR) at these positions. To investigate the importance of the enolpyruvyl side-chain at C-5, we conceived of analogue **3** wherein the olefin is removed altogether (Figure 2). Analogue **4** was inspired by the benzoic acid inhibitors described by Abell, Payne and co-workers,^{9b} who showed addition of a methyl group to the enolpyruvyl side chain enhanced potency by an order of magnitude. To study the SAR at C-1, we designed analogue **5** that incorporates 2,6-difluorophenol as a lipophilic carboxylic acid bioisostere.^{13,14}

RESULTS AND DISCUSSION

Synthesis of inhibitors

The racemic construction of core the cyclohexene in **1** by Bartlett, Kozlowski and co-workers was accomplished efficiently through Diels-Alder addition of a propiolate ester to a protected l-amino-1,3-butadiene.⁸ The three contiguous stereocenters in the cyclohexene skeleton were assembled through manipulations of epoxides in a regio- and stereoselective manner. In our synthetic route, enantiopure bromobenzene *cis*-1,2-dihydrodiol **6** was chosen as the starting material, which is commercially available or can be produced by fermentation of bromobenzene with *E. coli* JM 109 (pDTG601a) on a medium to large scale.¹⁵ Our decision to use **6** was inspired by the elegant and efficient syntheses of several cyclohexane based natural products including aminocyclitols and aminoinositols.¹⁶

As shown in Scheme 1, our synthesis began with the smooth conversion of **6** into the corresponding benzylidene acetal, which was subjected to epoxidation by *m*-chloroperoxybenzoic acid (*m*-CPBA) to provide bromo epoxide **7** with complete regio- and stereoselectivity.¹⁷ The optimized condition for hydride opening of epoxide **7** at the C-3 allylic position involved dropwise addition of a solution of lithium aluminum hydride (LAH) in Et₂O at room temperature over a short period of time (10 minutes) and further stirring for 10 minutes. It is noteworthy that no evidence of proto-debromination at C-1, the major product observed through the use of powdered LAH or extended reaction time (> 1 hour), was observed even on a five-gram scale under this optimized condition. Protection of the resulting crude alcohol by *p*-methoxybenzyl (PMB) chloride afforded **8** (71% over two steps). The protection of the hydroxy group at C-4 by a PMB group was not arbitrary. Our initial choice of a *t*-butyldiphenylsilyl (TBDPS) group tended to migrate from the C-4 to C-5 position during subsequent steps. Acid-hydrolysis of benzylidene **8** using *p*-toluenesulfonic acid (TsOH) gave rise to diol **9** in 86% yield. The inversion of stereoconfiguration at the C-6 position was achieved in a highly regio- and stereoselective manner by cyclic sulfite chemistry.¹⁸ Treatment of diol **9** with thionyl chloride in the presence of pyridine gave rise to a 5,6-cyclic sulfite, which formed as an epimeric (1:1) mixture of configurations at the sulfur atom. Opening of cyclic sulfite with sodium azide in DMF at room temperature afforded azido alcohol **10** in 85% yield over two steps. A small amount (12%) of regioisomer **10'** was also isolated, which likely formed from azido anion attack at the C-2 position through a S_N2' mechanism. 2D NOESY analysis of this side product elucidated the relative stereochemistry of the azide, precluding an allylic azide rearrangement for the formation of **10'** from the desired regioisomer, **10**. Staudinger reduction of azide in **10** using triphenylphosphine followed by *t*-butyloxycarbonyl (Boc)-protection of the resulting amine

provided **11** in 83% yield over two steps. The relative configurations of the three contiguous stereocenters in **11** were confirmed by the 2D NOESY spectra (see supporting information).

With the key intermediate **11** in hand, we embarked on the carbonylation at the C-1 position (Scheme 2). Unfortunately, initial attempts using trichlorophenyl formate as a CO surrogate [Trichlorophenyl formate/Pd(OAc)₂/Xantphos/Et₃N]¹⁹ were unsuccessful. The carbonylation protocol reported in the synthesis of Tamiflu (bearing a striking resemblance to **1**) by Wong and coworkers²⁰ employing nickel catalysis [Ni(CO)₂(PPh₃)₂/iPr₂NEt/EtOH]²¹ failed as well, possibly due to the diminished reactivity of vinyl bromides.²² To our delight, Suzuki cross-coupling of **11** with potassium vinyltrifluoroborate afforded the diene **12** in 70% yield. Oxidative cleavage of **12** by OsO₄/NaIO₄ in the presence of 2,6-lutidine²³ gave aldehyde **13** in 65% yield without oxidation of the internal olefin.²⁴ We employed a Pinnick oxidation²⁵ of aldehyde **13** to secure the corresponding carboxylic acid, which was in turn converted to the methyl ester **14** by trimethylsilyldiazomethane (79% yield from **13**).

Previously, Bartlett and coworkers⁸ installed the enol-pyruvate side chain according to Ganem's three-step protocol: (1) Rhodium-catalyzed insertion of dimethyl diazomalonate into the OH bond at the C-5 position [N₂C(CO₂Me)₂/Rh₂(OAc)₄]; (2) alkylation with Eschenmoser's reagent [Me₂NCH₂I/Et₃N]; and (3) methylation and *in situ* fragmentation [MeI/MeCN].²⁶ To reduce the number of linear synthetic steps, we attached the enol-pyruvate side chain according to the two-step protocol developed by Abell and coworkers with a slight, but crucial modification.^{9d} Treatment of alcohol **14** with triethyl diazophosphonoacetate (**15**) and Rh₂(OAc)₄ delivered the phosphonate, which without column purification underwent Horner-Wadsworth-Emmons (HWE) reaction with paraformaldehyde under aqueous conditions (aqueous K₂CO₃/2-PrOH).²⁷ Attempts using the reported anhydrous condition and *t*-BuOK as base led to aromatization.^{9d} Simultaneous deprotection of PMB and Boc groups by trifluoroacetic acid (TFA) at 0 °C afforded diester **16**. The overall three-step yield of **16** from alcohol **14** is 33%. Finally, hydrolysis of the diester with aqueous potassium hydroxide furnished the desired inhibitor **1** as the dipotassium salt. The synthesis of **4** was achieved in an identical manner to that described for the synthesis of **1** from **14** except for the use of aqueous acetaldehyde solution instead of formaldehyde in the HWE step. Our synthetic sequence avoids the deleterious anhydrous HWE conditions displaying both high functional group compatibility and ease of operation and provides a rapid and practical access to enol-pyruvate side chain analogues.

With the completion of synthesis of **1** and **4**, we then turned our attention to **5**, a carboxylic acid bioisostere of **1** (Scheme 3). Suzuki cross-coupling of **11** with pinacol boronic ester **18** produced the desired coupled product **19** in quantitative yield. According to our established method for **1** (*vide supra*), attachment of the enol-pyruvate side chain proceeded without complication, followed by PMB group deprotection by 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) to provide **20** (22% yield from **19** over 3 steps). Hydrolysis of the ethyl ester and *t*-butyldimethylsilyl (TBS) group using aqueous sodium hydroxide, followed by removal of the Boc group by TFA gave the desired analogue **5** as the TFA salt.

Scheme 4 illustrates the synthesis of analogue **3** containing a simplified side chain. Treatment of **11** with *t*-butyl bromoacetate in the presence of tetrabutylammonium bromide

led to alkylation at C-5 position. Subsequent Suzuki cross-coupling with potassium vinyltrifluoroborate produced **21** in a two-step 92% yield. Oxidative cleavage provided aldehyde **22** in 77% yield. The desired inhibitor **2** was obtained from **22** through sodium chlorite oxidation followed by global deprotection of the PMB, Boc and *t*-butyl ester groups with aqueous TFA.

Biological evaluation

With four putative transition-state inhibitors in hand—**1**, **3**, **4**, and **5** were evaluated for enzyme inhibition against recombinant MbtI under initial velocity conditions as described previously^{12, 28} but showed <10% inhibition at 100 μ M. The lack of activity of **1** or any of the analogues against MbtI was unexpected and suggests subtle, but important structural difference between MbtI and EntC.

CONCLUSION

We have reported the first asymmetric synthesis of the transition-state inhibitor **1** along with several analogues designed to further explore the structure activity relationships of this scaffold. In comparison with the original racemic synthesis of **1**, our synthetic route employed enantiopure 3-bromobenzene *cis*-1,2-dihydrodiol **6** as a starting material. Elaboration to the key intermediate **11** containing three contiguous stereocenters was accomplished through a series of regio- and stereoselective transformations featuring cyclic sulfite chemistry for installation of an azide at C-6 and epoxidation followed by reductive opening for introduction of the alcohol at C-4. Bromocyclohexene **11** served as a versatile intermediate for modification at the C-1 position as showcased by the preparation of the 2,6-difluorophenol analogue **5**. Introduction of the terminal alkene of the enol-pyruvate side chain at the C-5 position was optimally performed using a HWE reaction under mild, aqueous conditions. The reaction enabled preparation of analogues at this position as illustrated with the preparation of **4**. Biochemical inhibition studies with MbtI demonstrate this CUE is remarkably resistant to **1** as well as analogues **3–5**. The lack of activity of **1** toward MbtI was unexpected based on the potent activity of **1** with the homologous isochorismatase EntC in *E. coli*.

EXPERIMENTAL SECTION

General Chemistry Methods

All reactions were carried out under a dry Ar atmosphere using oven-dried glassware and magnetic stirring. The solvents were dried before use as follows: THF and Et₂O were heated at reflux over sodium benzophenone ketyl; toluene was heated at reflux over sodium; CH₂Cl₂ was dried over CaH₂. Anhydrous *N,N*-diisopropylethylamine, triethylamine were used directly as purchased. Commercially available reagents were used without further purification unless otherwise noted. Aluminum TLC sheets (silica gel 60 F₂₅₄) of 0.2-mm thickness were used to monitor the reactions. The spots were visualized with short wavelength UV light or by charring after spraying with a solution prepared from one of the following solutions: phosphomolybdic acid (5.0 g) in 95% EtOH (100 mL); *p*-anisaldehyde solution (2.5 mL of *p*-anisaldehyde, 2 mL of AcOH, and 3.5 mL of conc. H₂SO₄ in 100 mL

of 95% EtOH); or ninhydrin solution (0.3 g ninhydrin in 100 ml of *n*-butanol; add 3 ml AcOH). Flash chromatography was carried out with silica gel 60 (230–400 ASTM mesh). NMR spectra were obtained on a 400-MHz or 600-MHz spectrometer. Proton chemical shifts are reported in ppm from an internal standard of residual chloroform (7.26 ppm) or methanol (3.31 ppm), and carbon chemical shifts are reported using an internal standard of residual chloroform (77.0 ppm) or methanol (49.1 ppm). Proton chemical data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad), coupling constant, integration. Chemical shifts were referenced on residual solvent peaks: CDCl₃ (δ = 7.26 ppm for ¹H NMR and 77.00 ppm for ¹³C NMR), CD₂Cl₂ (δ = 5.32 ppm for ¹H NMR and 53.84 ppm for ¹³C NMR), CD₃OD (δ = 3.31 ppm for ¹H NMR and 49.00 ppm for ¹³C NMR), D₂O (δ = 4.69 ppm for ¹H NMR). Optical rotations were measured at rt in a 1.0-dm cell. High-resolution mass spectra were acquired by electrospray ionization.

(2S,3aS,5aR,6aR,6bS)-4-Bromo-2-phenyl-3a,5a,6a,6b-tetrahydrooxireno[2',3':3,4]benzo[1,2-d][1,3]dioxole (7)—To a suspension of **6** (4.8 g, 25.1 mmol) and benzaldehyde dimethyl acetal (4.1 mL, 27.6 mmol) in anhydrous CH₂Cl₂ (100 mL) was added (1*S*)-(+)-camphor-10-sulfonic acid monohydrate (CSA·H₂O) (580 mg, 2.50 mmol) at –20 °C. After stirring at –20 °C for 2 h, at which point TLC analysis indicated that all of the starting material had been consumed, the reaction mixture was quenched with 10% aqueous NaOH (80 mL) and the separated aqueous phase was extracted with CH₂Cl₂. The combined organic fractions were then washed with brine, dried (MgSO₄) and filtered to give a clear solution that was cooled to 0 °C, treated with *m*-CPBA (30 g, 88 mmol, 50–55% pure by mass containing approximately 10% 3-chlorobenzoic acid with the balance from water) then stirred at 0 °C slowly warming to rt overnight. The ensuing mixture was treated with cold sodium metabisulfite (150 mL of a 20% w/v aqueous solution) and the separated aqueous fraction was extracted with CH₂Cl₂. The combined organic fractions were washed consecutively with saturated aqueous NaHCO₃ (be careful for the formation of CO₂, which causes pressure buildup), brine, then dried (MgSO₄), filtered and concentrated under reduced pressure to give an oily residue. Purification by flash chromatography on silica gel (10% → 15% → 20% EtOAc/hexane) provided **7** (5.35 g, 72% for two steps) as a white solid: *R*_f = 0.3 (85:15 hexane/EtOAc); [α]_D²³ +89.5 (*c* 2.00, CHCl₃); ¹H NMR (400 MHz, CDCl₃) δ 3.32–3.35 (m, 1H), 3.65 (dd, *J* = 3.6, 1.6 Hz, 1H), 4.56 (d, *J* = 7.4 Hz, 1H), 4.94 (d, *J* = 7.4 Hz, 1H), 6.01 (s, 1H), 6.53 (d, *J* = 4.3 Hz, 1H), 7.37–7.42 (m, 3H), 7.46–7.51 (m, 2H); ¹³C NMR (100 MHz, CDCl₃) δ 48.2, 48.8, 74.03, 74.05, 105.5, 126.9, 127.0, 128.1, 128.4, 129.8, 136.1; HRMS (ESI+) *m/z* calcd for C₁₃H₁₂BrO₃⁺ [*M* + *H*]⁺ 294.9964, found 294.9955 (error 3.0 ppm).

(2S,3aS,4R,7aS)-7-Bromo-4-[(4-methoxybenzyl)oxy]-2-phenyl-3a,4,5,7a-tetrahydrobenzo[*d*][1,3]dioxole (8)—To LAH (932 mg, 24.6 mmol) was added Et₂O (25 mL) at rt. The mixture was sonicated at rt for 10 min and stored until the grey impurities settled. The clear supernatant was considered a 1 M LAH solution, which was used in the following epoxide-opening reaction. To a solution of epoxide **7** (1.36 g, 4.61 mmol) in Et₂O (40 mL) was added the freshly prepared LAH solution (11.5 mL, 11.5 mmol) dropwise at rt for 10 min. The reaction mixture was stirred at rt for an additional 10 min, then cooled down

to 0 °C, quenched by the sequential addition of 0.6 mL of H₂O (slowly added), 0.6 mL of 15% aqueous NaOH, and 1.8 mL of H₂O. The mixture was warmed up to rt, vigorously stirred for 15 min, and treated with anhydrous MgSO₄. After stirring for 15 min, the mixture was filtered through a pad of Celite. The resulting filtrate was concentrated and dried for 2 h under high vacuum to provide the crude intermediate alcohol, which was used directly in the step.

To the crude intermediate alcohol prepared above in DMF (20 mL) was added 4-methoxybenzyl chloride (940 µL, 6.92 mmol), NaH (277 mg, 60 % dispersion in mineral oil, 6.92 mmol), and TBAI (170 mg, 0.46 mmol) at 0 °C. The reaction mixture was stirred at 0 °C and allowed to slowly warm to rt overnight. The mixture was quenched with 250 mL of brine and extracted with Et₂O (3 × 300 mL). The combined organic fractions were dried (MgSO₄), filtered, and concentrated under reduced pressure. The residue was purified by flash chromatography on silica gel (10% → 15% EtOAc/hexane) to afford **8** (1.37 g, 71% for two steps) as a colorless oil: *R*_f = 0.4 (85:15 hexane/EtOAc); [α]_D²³ –22.4 (*c* 5.7, CHCl₃); ¹H NMR (400 MHz, CDCl₃) δ 2.18–2.27 (m, 1H), 2.39–2.49 (m, 1H), 3.81 (s, 3H), 4.38 (t, *J* = 6.5 Hz, 1H), 4.60 (s, 2H), 4.75 (d, *J* = 6.4 Hz, 1H), 5.97 (s, 1H), 6.17 (t, *J* = 4.2 Hz, 1H), 6.85–6.90 (m, 2H), 7.21–7.27 (m, 2H), 7.35–7.42 (m, 3H), 7.43–7.50 (m, 2H); ¹³C NMR (100 MHz, CDCl₃) δ 29.7, 55.2, 71.4, 73.1, 78.0, 78.3, 104.1, 113.8, 118.9, 127.0, 128.3, 129.4, 129.47, 129.54, 130.0, 136.8, 159.3; HRMS (ESI+) *m/z* calcd for C₂₁H₂₁BrNaO₄⁺ [*M* + Na]⁺ 439.0515, found 439.0516 (error 0.2 ppm).

(1*R*,2*S*,6*R*)-3-Bromo-6-[(4-methoxybenzyl)oxy]cyclohex-3-ene-1,2-diol (9)—To a solution of benzylidene acetal **8** (1.8 g, 4.31 mmol) in CH₂Cl₂/EtOH (60 mL, 1:5) was added TsOH·H₂O (1.06 g, 5.56 mmol) at rt. The reaction mixture was stirred for 24 h at rt before the addition of saturated aqueous NaHCO₃ (50 mL). After removal of volatile solvents, the residual aqueous phase was extracted with EtOAc (3 × 20 mL). The combined organic layers were washed with H₂O, dried (Na₂SO₄), and concentrated. The residue was purified by flash chromatography on silica gel (40% → 50% EtOAc/hexane) to afford diol **9** (1.22 g, 86%) as a white solid: *R*_f = 0.4 (1:1 hexane/EtOAc); [α]_D²³ –77.2 (*c* 5.0, CHCl₃); ¹H NMR (400 MHz, CDCl₃) δ 1.99–2.09 (m, 1H), 2.51–2.61 (m, 1H), 3.03 (s, 2H), 3.74–3.87 (m, 5H), 4.38 (d, *J* = 3.7 Hz, 1H), 4.49 (d, *J* = 11.3 Hz, 2H), 4.58 (d, *J* = 11.3 Hz, 2H), 6.04–6.08 (m, 1H), 6.84–6.91 (m, 2H), 7.21–7.28 (m, 2H); ¹³C NMR (100 MHz, CDCl₃) δ 31.9, 55.2, 71.6, 72.2, 72.3, 72.9, 113.9, 121.0, 129.46, 129.52, 129.9, 159.4; HRMS (ESI+) *m/z* calcd for C₁₄H₁₇BrNaO₄⁺ [*M* + Na]⁺ 351.0202, found 351.0204 (error 0.6 ppm).

(1*R*,2*R*,6*R*)-2-Azido-3-bromo-6-[(4-methoxybenzyl)oxy]cyclohex-3-en-1-ol (10)—Diol **9** (940 mg, 2.86 mmol) and pyridine (700 µL, 8.57 mmol) were dissolved in CH₂Cl₂ (40 mL) and cooled to 0 °C. Thionyl chloride (311 µL, 4.29 mmol) was then added dropwise at 0 °C. The resulting mixture was stirred for 20 min at 0 °C, and then filtered through a pad of silica gel, which was washed with hexane/EtOAc (1:1). The filtrate was concentrated in a rotary evaporator, and the residue was further dried under high vacuum. The intermediate cyclic sulfite was obtained as a mixture of two diastereoisomers that were used in the next step without further purification.

To a solution of the cyclic sulfite prepared above in DMF (30 mL) at rt was added sodium azide (560 mg, 8.58 mmol). The mixture was stirred for 24 h at rt. After the reaction was complete, the residue was partitioned between Et₂O (100 mL) and water (300 mL). The layers were separated, and the aqueous layer was extracted with Et₂O (2 × 100 mL). The organic extracts were combined, dried (MgSO₄), and concentrated under vacuum. Purification by flash chromatography on silica gel (25%→30% EtOAc/hexane) afforded azido alcohol **10** (859 mg, 85%) as a white solid, along with its regioisomer **10'** (126 mg, 12%) as a colorless oil. See the supporting information for determination of the stereochemistry of **10**.

Data of **10**: R_f = 0.4 (2:1 hexane/EtOAc); $[\alpha]_D^{23}$ -7.6 (c 1.6, CHCl₃); ¹H NMR (400 MHz, CDCl₃) δ 1.99–2.09 (m, 1H), 2.41–2.51 (m, 1H), 3.42 (s, 1H), 3.48–3.57 (m, 4H), 3.72–3.83 (m, 4H), 3.96–4.03 (m, 1H), 4.50 (d, J = 11.3 Hz, 2H), 4.59 (d, J = 11.3 Hz, 2H), 6.03–6.08 (m, 1H), 6.85–6.92 (m, 2H), 7.22–7.29 (m, 2H); ¹³C NMR (100 MHz, CDCl₃) δ 30.9, 55.0, 68.4, 71.2, 75.1, 75.2, 113.8, 119.2, 129.3, 129.40, 129.42, 159.2; HRMS (ESI+) m/z calcd for C₁₄H₁₇BrN₃O₃⁺ [M + H]⁺ 354.0448, found 354.0446 (error 0.6 ppm).

Data of **10'**: R_f = 0.6 (2:1 hexane/EtOAc); ¹H NMR (400 MHz, CDCl₃) δ 1.71–1.83 (m, 1H), 2.43–2.52 (m, 1H), 3.43–3.50 (m, 1H), 3.73 (s, 3H), 3.99–4.07 (m, 1H), 4.14–4.20 (m, 1H), 4.48 (d, J = 11.4 Hz, 1H), 4.64 (d, J = 11.4 Hz, 1H), 6.10–6.18 (m, 1H), 6.86–6.94 (m, 2H), 7.22–7.31 (m, 2H); ¹³C NMR (100 MHz, CDCl₃) δ 32.4, 55.3, 61.9, 71.3, 71.6, 77.2, 114.1, 123.1, 129.55, 129.58, 129.66, 134.3, 159.5; HRMS (ESI+) m/z calcd for C₁₄H₁₇BrN₃O₃⁺ [M + H]⁺ 354.0448, found 354.0447 (error 0.3 ppm).

tert-Butyl [(1*R*,5*R*,6*R*)-2-bromo-6-hydroxy-5-(4-methoxybenzyloxy)cyclohex-2-enyl]carbamate (11**)**—To azido alcohol **10** (770 mg, 2.17 mmol) in a solution of THF/H₂O (10 mL, 9:1) was added Ph₃P (741 mg, 2.83 mmol) at rt. The resulting mixture was stirred for 19 h at rt. After the reaction was complete, THF was removed under reduced pressure. The residue was partitioned between Et₂O (20 mL) and H₂O (20 mL). The layers were separated and the aqueous layer was extracted with Et₂O (1 × 20 mL). The organic extracts were combined, dried (MgSO₄), and concentrated under vacuum to afford the intermediate amino alcohol as a yellow oil that was directly used in the next step.

To a solution of the crude amino alcohol prepared above in 1,4-dioxane/H₂O (13 mL, 10:3) at rt was added Boc₂O (1.18 g, 5.43 mmol) followed by Et₃N (900 μL, 6.51 mmol). The reaction mixture was stirred for 40 min at rt and concentrated under reduced pressure. Purification by flash chromatography on silica gel (15%→20%→25% EtOAc/toluene)

afforded **11** (800 mg, 86%) as a white solid: R_f = 0.5 (1:1 hexane/EtOAc); $[\alpha]_D^{23}$ +23.7 (c 2.5, CHCl₃); ¹H NMR (400 MHz, CDCl₃) δ 1.47 (s, 1H), 2.17–2.27 (m, 1H), 2.43–2.53 (m, 1H), 3.71–3.77 (m, 1H), 3.79 (s, 3H), 4.03–4.08 (m, 1H), 4.24–4.31 (m, 1H), 4.52 (s, 2H), 5.28 (d, J = 9.8 Hz, 1H), 6.03–6.08 (m, 1H), 6.85–6.90 (m, 2H), 7.24–7.29 (m, 2H); ¹³C NMR (100 MHz, CDCl₃) δ 28.3, 29.6, 55.1, 57.6, 71.2, 71.5, 74.2, 79.7, 113.7, 120.9, 128.0, 129.3, 129.8, 155.7, 159.2; HRMS (ESI+) m/z calcd for C₁₉H₂₆BrNNaO₅⁺ [M + Na]⁺ 450.0887, found 450.0888 (error 0.2 ppm).

***tert*-Butyl (1*S*,5*R*,6*R*)-6-hydroxy-5-(4-methoxybenzyloxy)-2-vinylcyclohex-2-enylcarbamate (12)**—An oven-dried vial was charged with vinyl bromide **11** (160 mg, 0.37 mmol), potassium vinyltrifluoroborate (150 mg, 1.12 mmol), and Pd(dppf)Cl₂•CH₂Cl₂ (30 mg, 0.037 mmol). The vial was capped with a rubber septum and then evacuated and backfilled with argon (this sequence was carried out twice). 3 mL of dry toluene was added via syringe through the septum, followed by the addition of EtOH (3 mL) and 2 M Na₂CO₃ (1 mL). The septum was then replaced with a Teflon screwcap and the vial was sealed. The reaction mixture was heated to 80 °C for 12 h. The reaction mixture was allowed to cool to rt, and was partitioned between EtOAc and water. Two layers were separated, and the aqueous layer was extracted with EtOAc. The organic extracts were combined and dried over anhydrous MgSO₄, and then was concentrated under vacuum to afford the residue which was purified by column chromatography on silica gel (EtOAc in hexane 30% to 35%) to afford the product **12** (98 mg, 70%) as slightly yellow oil. *R*_f = 0.7 (hexane/EtOAc 1:1); ¹H NMR (400 MHz, CDCl₃) δ 1.37 (s, 9H), 2.16 – 2.28 (m, 1H), 2.41 – 2.53 (m, 1H), 3.61 – 3.68 (m, 1H), 3.71 (s, 3H), 3.95 – 3.99 (m, 1H), 4.29 – 4.36 (m, 1H), 4.38 – 4.50 (m, 2H), 4.91 (d, *J* = 11.0 Hz, 1H), 4.98 (d, *J* = 9.6 Hz, 1H), 5.18 (d, *J* = 17.6 Hz, 1H), 5.68 (t, *J* = 4.1 Hz, 1H), 6.12 (dd, *J* = 17.6, 11.0 Hz, 1H), 6.67 – 6.82 (m, 2H), 7.07 – 7.21 (m, 2H); ¹³C NMR (100 MHz, CDCl₃) δ 27.6, 28.3, 49.3, 55.0, 69.1, 71.0, 74.6, 76.7, 77.0, 77.3, 79.0, 112.0, 113.4, 127.2, 129.0, 129.8, 133.2, 136.7, 155.4, 158.7; HRMS (ESI+) *m/z* calcd for C₂₁H₂₉NNaO₅⁺ [*M* + Na]⁺ 398.1938, found 398.1945 (error 1.8 ppm).

***tert*-Butyl [(1*S*,5*R*,6*R*)-2-formyl-6-hydroxy-5-(4-methoxybenzyloxy)cyclohex-2-enyl]carbamate (13)**—To a solution of **12** (445 mg, 1.18 mmol) in dioxane/H₂O (16 mL, 3:1) was added 2,6-lutidine (0.27 mL, 2.36 mmol), OsO₄ (2.5% in 2-methyl-2-propanol, 305 mg, 0.03 mmol) and NaIO₄ (1.01 g, 4.72 mmol). The reaction mixture was stirred at rt overnight. After the reaction was complete, H₂O (20 mL) and CH₂Cl₂ (40 mL) were added. The organic layer was separated, and the aqueous layer was extracted with CH₂Cl₂ (3 × 20 mL). The combined organic layers were washed with brine, dried (Na₂SO₄), and concentrated. Purification by flash chromatography on silica gel (50% → 55% EtOAc/hexane) afforded **13** (300 mg, 70%) as a colorless oil: *R*_f = 0.2 (1:1 hexane/EtOAc); [α]_D¹⁰ –10.7 (*c* 1.80, CHCl₃); ¹H NMR (400 MHz, CDCl₃) δ 1.37 (s, 9H), 2.45 (d, *J* = 19.8 Hz, 1H), 2.66 (d, *J* = 19.6 Hz, 1H), 3.72 (s, 3H), 4.12 (s, 1H), 4.38 (s, 1H), 4.45 (q, *J* = 10.9 Hz, 2H), 4.96 (s, 1H), 6.70–6.72 (m, 1H), 6.74–6.81 (m, 2H), 7.04–7.27 (m, 2H), 9.34 (s, 1H); ¹³C NMR (100 MHz, CDCl₃) δ 28.3, 28.4, 47.2, 55.0, 67.4, 71.1, 74.2, 79.1, 113.4, 129.1, 129.4, 137.4, 148.4, 155.1, 158.8, 192.2; HRMS (ESI+) *m/z* calcd for C₂₀H₂₈NO₆⁺ [*M* + H]⁺ 378.1911, found 378.1921 (error 2.7 ppm).

Methyl (4*R*,5*R*,6*S*)-6-(*tert*-butoxycarbonylamino)-5-hydroxy-4-(4-methoxybenzyloxy)cyclohex-1-enecarboxylate (14)—To a stirred solution containing freshly prepared aldehyde **13** (300 mg, 0.83 mmol) and 2-methyl-2-butene (1 mL) in *t*-BuOH/THF/H₂O (14 mL, 5:1:1) was slowly added sodium chlorite (971 mg, 10.8 mmol) and sodium dihydrogen phosphate (1.2 g, 9.96 mmol) in H₂O (4 mL) under 10 °C. The resultant suspension was stirred at rt for 4 h. Saturated aqueous NaHSO₃ (10 mL) was added to quench the reaction. The layers were separated, and the aqueous layer was extracted with EtOAc (3 × 20 mL). The combined organic layers were washed with brine,

dried (Na_2SO_4), and concentrated in vacuo to provide the crude carboxylic acid, which was used in the next step without further purification.

To a solution of the crude carboxylic acid prepared above in toluene/methanol (16 mL, 7:1) was added a solution of trimethylsilyldiazomethane (0.52 mL, 2.0 M in hexane, 1.04 mmol). The mixture was stirred at rt until the evolution of N_2 ceased (approximately 10 min). The solvents were removed in vacuo. Purification by flash chromatography on silica gel (45% $\rightarrow$ 50% EtOAc/hexane) afforded **14** (186 mg, 60% over two steps) as a white solid: R_f = 0.6 (1:1 hexane/EtOAc); $[\alpha]_D^{10} +1.7$ (c 1.1, CHCl_3); ^1H NMR (400 MHz, CDCl_3) δ 1.34 (s, 8H), 2.24–2.38 (m, 2H), 2.46–2.56 (m, 1H), 3.55 (s, 3H), 3.68 (s, 2H), 3.72–3.78 (m, 1H), 4.05–4.16 (m, 1H), 4.39 (q, J = 10.8 Hz, 2H), 4.51 (d, J = 10.1 Hz, 1H), 5.10 (d, J = 10.0 Hz, 1H), 6.74 (d, J = 8.3 Hz, 2H), 6.89 (s, 1H), 7.13 (d, J = 8.3 Hz, 2H); ^{13}C NMR (100 MHz, CDCl_3) δ 28.3, 28.5, 49.0, 51.6, 55.1, 68.3, 71.1, 73.6, 79.2, 103.5, 113.6, 127.6, 129.1, 129.7, 137.0, 138.7, 155.2, 158.9, 166.3; HRMS (ESI+) m/z calcd for $\text{C}_{21}\text{H}_{29}\text{NNaO}_7^+$ [$M + \text{Na}$] $^+$ 430.1836, found 430.1840 (error 0.9 ppm).

Methyl (4*R*,5*R*,6*S*)-6-amino-5-[3-ethoxy-3-oxoprop-1-en-2-yl]oxy]-4-hydroxycyclohex-1-ene-1-carboxylate (16)—An oven-dried vial was charged with alcohol **14** (150 mg, 0.37 mmol) and rhodium acetate dimer (4.4 mg, 0.01 mmol). The vial was capped with a rubber septum and then evacuated and backfilled with argon (this sequence was carried out twice). A solution of **15** (460 mg, 1.85 mmol) in toluene (4 mL) was added via syringe through the septum. The septum was then replaced with a Teflon screwcap and the vial was sealed. The reaction mixture was heated at 80 °C for 5 h. The reaction mixture was allowed to cool to rt. The reaction solution was then filtered through a short pad of Celite in a Pasteur pipette (eluting with EtOAc) and the eluent was concentrated under reduced pressure. The crude phosphonate obtained was used in the next without further purification.

K_2CO_3 (460 mg, 3.33 mmol) was dissolved in H_2O (3 mL), and the solution was cooled to rt. This aqueous solution was then added to a mixture of paraformaldehyde (111 mg, 3.70 mmol) and the crude phosphonate prepared above in 2-propanol (3 mL) at 0 °C. The reaction mixture was allowed to slowly warm to rt and stirred overnight at rt. The reaction mixture was transferred to a separatory funnel containing saturated aqueous NH_4Cl solution (5 mL). The aqueous phase was extracted with EtOAc (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 (15% $\rightarrow$ 20% EtOAc/hexane) afforded the Boc-PMB protected **16** as a colorless oil.

Cold, dry TFA (0 °C) was added to the solution of the product prepared above in CH_2Cl_2 (3 mL), which was also cooled to 0 °C. After 10 min, the reaction mixture was cooled further by liquid N_2 , and the solvents were removed in vacuo. The residue was purified by flash chromatography (5% $\rightarrow$ 10% $\rightarrow$ 15% MeOH/ CHCl_3) to afford **16** (35 mg, 33% over three steps) as a white solid: R_f = 0.5 (9:1 $\text{CHCl}_3/\text{CH}_3\text{OH}$); ^1H NMR (400 MHz, D_2O) δ 1.27 (t, J = 7.2 Hz, 3H), 2.50–2.54 (m, 1H), 2.55–2.60 (m, 1H), 2.71–2.75 (m, 1H), 2.76–2.80 (m, 1H), 3.81 (s, 3H), 4.25 (q, J = 7.2 Hz, 2H), 4.32 (q, J = 4.7 Hz, 1H), 4.35 (d, J = 4.1 Hz, 1H),

4.60 (dd, $J = 6.1, 4.2$ Hz, 1H), 5.20 (d, $J = 3.6$ Hz, 1H), 5.70 (d, $J = 3.6$ Hz, 1H), 7.35–7.42 (m, 1H); ^{13}C NMR (100 MHz, D_2O) δ 13.0, 30.5, 47.1, 52.6, 62.8, 63.8, 74.6, 100.3, 122.4, 145.1, 148.7, 164.4, 166.6; HRMS (ESI+) m/z calcd for $\text{C}_{13}\text{H}_{20}\text{NO}_6^+$ $[\text{M} + \text{H}]^+$ 286.1285, found 286.1288 (error 1.1 ppm).

Potassium (4*R*,5*R*,6*S*)-6-amino-5-[(1-carboxyvinyl)oxy]-4-hydroxycyclohex-1-ene-1-carboxylate (1)—To the solution of **16** (3 mg, 0.011 mmol) in H_2O (0.1 mL) was added an aqueous solution of KOH (2.4 mg in 0.1 mL H_2O , 0.044 mmol) at 0 °C. The reaction mixture was stirred at 0 °C for 1 h, then 0.1 M HCl (0.22 mL, 0.022 mmol) was added. The resulting solution was concentrated under reduced pressure to afford a residue, which was purified by flash chromatography on silica gel (10:9:1→10:18:2 $\text{CHCl}_3/\text{MeOH}/\text{H}_2\text{O}$) to provide **1** (3 mg, 85%) as a white solid (Note: to remove the inorganic salts from the silica gel, the packed column was washed successively with MeOH followed by CHCl_3 prior to loading the crude product): $R_f = 0.2$ (10:9:1 $\text{CHCl}_3/\text{MeOH}/\text{H}_2\text{O}$); $[\alpha]_{\text{D}}^{23} -68$ (c 0.20, MeOH); ^1H and ^{13}C NMR data were identical with those reported,⁸ see the Supporting Information for the comparison. ^1H NMR (400 MHz, D_2O) δ 2.25 (ddt, $J = 19.1, 8.7, 3.0$ Hz, 1H), 2.60 (dt, $J = 18.7, 5.5$ Hz, 1H), 3.97 (td, $J = 8.8, 5.6$ Hz, 1H), 4.09 (d, $J = 7.9$ Hz, 1H), 4.17 (m, 1H), 4.80 (d, $J = 2.8$ Hz, 1H), 5.19 (d, $J = 2.8$ Hz, 1H), 6.74 (dt, $J = 5.0, 2.4$ Hz, 1H); ^{13}C NMR (100 MHz, D_2O) δ 171.8, 170.5, 155.2, 138.1, 128.0, 96.6, 79.6, 67.3, 52.3, 31.6; HRMS (ESI+) m/z calcd for $\text{C}_{10}\text{H}_{12}\text{K}_2\text{NO}_6^+$ $[\text{M} + \text{H}]^+$ 319.9933, found 319.9939 (error 1.9 ppm).

Potassium (4*R*,5*R*,6*S*)-6-amino-5-[(1-carboxyprop-1-en-1-yl)oxy]-4-hydroxycyclohex-1-ene-1-carboxylate (4)—Except for the use of acetaldehyde (40% wt in H_2O) instead of paraformaldehyde, the four-step preparation of **4** from **14** (15 mg, 0.037 mmol) was conducted in an identical manner to that described for the synthesis of **1** from **14**. Purification by flash chromatography on silica gel (10:9:1→10:18:2 $\text{CHCl}_3/\text{MeOH}/\text{H}_2\text{O}$) afforded the desired product **4** (3 mg, 25% over four steps) as a 1:1.3 mixture of isomers as a white solid. The *E/Z* isomer ratio could not be determined. Data of the mixture of isomers: $R_f = 0.2$ (10:9:1 $\text{CHCl}_3/\text{MeOH}/\text{H}_2\text{O}$); ^1H NMR (400 MHz, D_2O) δ 1.60 (d, $J = 7.1$ Hz, 3H, $\text{CH}_3\text{-CH=C}$), 1.76 (d, $J = 7.4$ Hz, 3H, $\text{CH}_3\text{-CH=C}$), 2.16–2.45 (m, 1H, H-3a, mix of two isomers), 2.55–2.76 (m, 1H, H-3b, mix of two isomers), 3.95–4.24 (m, 3H, H-4, H-5, and H-6, mix of two isomers), 5.64 (q, $J = 7.4$ Hz, 1H, $\text{CH}_3\text{-CH=C}$), 6.07 (q, $J = 7.1$ Hz, 1H, $\text{CH}_3\text{-CH=C}$), 6.80–6.87 (m, 1H, H-2), 6.88–6.95 (m, 1H, H-2); ^{13}C NMR (100 MHz, D_2O) δ 10.7 ($\text{CH}_3\text{-CH=C}$), 11.8 ($\text{CH}_3\text{-CH=C}$), 31.1 (C-3), 31.5 (C-3), 49.9 (C-6), 52.2 (C-6), 66.3 (C-4), 67.0 (C-4), 77.6 (C-5), 82.4 (C-5), 115.5 ($\text{CH}_3\text{-CH=C}$), 121.6 ($\text{CH}_3\text{-CH=C}$), 128.0* (C-1), 138.4 (C-2), 138.8 (C-2), 147.5* ($\text{CH}_3\text{-CH=C}$), 150.2* ($\text{CH}_3\text{-CH=C}$), 170.5* ($\text{CH}_3\text{-CH=C(OR)CO}_2\text{K}$), 171.6* ($\text{CH}_3\text{-CH=C(OR)CO}_2\text{K}$), 171.8* [(C-1)- CO_2K] (chemical shifts denoted with asterisks were derived from HMBC, two peaks from (C-1) and [(C-1)- CO_2K] in one isomer cannot be detected); HRMS (ESI+) m/z calcd for $\text{C}_{11}\text{H}_{14}\text{K}_2\text{NO}_6^+$ $[\text{M} + \text{H}]^+$ 334.0090, found 334.0100 (error 3.0 ppm).

***tert*-Butyl-[2,6-difluoro-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenoxy]dimethylsilane (18)**—To a solution of 4-bromo-2,6-difluorophenol (1.06 g, 5.07 mmol) in CH_2Cl_2 (20 mL) was added TBSCl (0.89 g, 5.91 mmol) and imidazole (959

mg, 14.1 mmol) at rt. After stirring for 40 min at rt, the reaction mixture was concentrated under reduced pressure. The residue was partitioned between hexane (50 mL) and water (50 mL). The layers were separated and the aqueous layer was extracted with hexane (2 × 50 mL). The organic extracts were combined, dried (MgSO₄), and concentrated under vacuum to afford 4-bromo-1-[(*tert*-butyldimethylsilyl)oxy]-2,6-difluorobenzene as a colorless oil (1.48 g, 90%) that was directly used for the next step without further purification.

An oven-dried flask was charged with Pd₂dba₃ (42 mg, 0.018 mmol), Xphos (88 mg, 0.184 mmol), bis(pinacolato)diboron (3.49 g, 13.74 mmol), and KOAc (1.35 g, 13.74 mmol). The flask was capped with a rubber septum and then evacuated and backfilled with argon (this sequence was carried out twice). A solution of 4-bromo-1-[(*tert*-butyldimethylsilyl)oxy]-2,6-difluorobenzene prepared above in 1,4-dioxane (60 mL) was added via syringe through the septum. The reaction mixture was heated at 100 °C for 40 min. After cooling to rt, the reaction mixture was filtered through a thin pad of Celite (eluting with 1:2 Et₂O/hexane), and the filtrate was concentrated under reduced pressure. The crude material was purified via flash chromatography on silica gel (5% → 10% Et₂O/hexanes → 10% EtOAc/hexane) to afford the product **18** (770 mg, 45% from 4-bromo-2,6-difluorophenol, two-step yield) as a light yellow oil: *R*_f = 0.5 (1:1 hexane/EtOAc); ¹H NMR (400 MHz, CDCl₃) δ 0.19 (s, 6H), 1.01 (s, 9H), 1.33 (s, 12H), 7.25–7.34 (m, 2H); ¹³C NMR (100 MHz, CDCl₃) δ –4.98 (t, *J* = 2.0 Hz), 18.5, 24.8, 25.5, 84.1, 117.6 (m), 135.3 (t, *J* = 15.4 Hz), 154.8 (dd, *J* = 246.6, 4.8 Hz). The carbon directly attached to the boron atom was not detected, likely due to quadrupolar relaxation.

***tert*-Butyl {(1*S*,5*R*,6*R*)-2-[3,5-difluoro-4-(*t*-butyldimethylsiloxy)phenyl]-6-hydroxy-5-(4-methoxybenzyloxy)cyclohex-2-enyl}carbamate (**19**)**—An oven-dried vial was charged with vinyl bromide **11** (78 mg, 0.182 mmol) and Pd(dppf)Cl₂•CH₂Cl₂ (15 mg, 0.018 mmol). The vial was capped with a rubber septum and then evacuated and backfilled with argon (this sequence was carried out twice). The solution of **18** (202 mg, 0.545 mmol) in toluene (1 mL) was added via syringe through the septum, followed by the addition of EtOH (1 mL) and 2 M Na₂CO₃ (0.5 mL). The septum was then replaced with a Teflon screwcap and the vial was sealed. The reaction mixture was heated at 70–80 °C for 30–40 min. The reaction mixture was allowed to cool to rt, then partitioned between Et₂O (5 mL) and water (5 mL). The layers were separated, and the aqueous layer was extracted with Et₂O (2 × 5 mL). The organic extracts were combined and dried (MgSO₄), and concentrated under vacuum. Purification by flash chromatography on silica gel (15% → 20% → 25% EtOAc/hexane) afforded **19** (108 mg, quantitative yield) as a colorless oil: *R*_f = 0.6 (1:1 hexane/EtOAc); [α]_D²³ +41.8 (*c* 5.5, CHCl₃); ¹H NMR (400 MHz, CDCl₃) δ 0.18 (s, 6H), 1.00 (s, 9H), 1.37 (s, 9H), 2.24–2.34 (m, 1H), 2.58–2.68 (m, 1H), 3.21 (br s, 1H), 3.70–3.77 (m, 1H), 3.79 (s, 3H), 3.95–4.02 (m, 1H), 4.53–4.65 (m, 3H), 4.83 (d, *J* = 9.7 Hz, 1H), 5.91–5.96 (m, 1H), 6.83–6.93 (m, 4H), 7.24–7.30 (m, 2H); ¹³C NMR (100 MHz, CDCl₃) δ –5.03 (t, *J* = 1.7 Hz), 18.4, 25.4, 28.2, 28.9, 52.9, 55.2, 71.3, 72.7, 75.4, 79.7, 109.5 (dd, *J* = 7.2, 16.8 Hz), 113.9, 124.5, 129.4, 130.1, 131.4 (m, weak), 132.2 (m), 134.5, 154.7 (dd, *J* = 6.2, 244.3 Hz), 156.0, 159.3; HRMS (ESI+) *m/z* calcd for C₃₁H₄₄F₂NO₆Si⁺ [*M* + *H*]⁺ 592.2900, found 592.2890 (error 1.7 ppm).

tert-Butyl {(1*S*,5*R*,6*R*)-2-[3,5-difluoro-4-(*t*-butyldimethylsiloxy)phenyl]-6-(1-(ethoxycarbonyl)vinyl)-5-hydroxycyclohex-2-enyl}carbamate (20)—An oven-dried vial was charged with alcohol **19** (70 mg, 0.118 mmol) and rhodium acetate dimer (3.6 mg, 8 μ mol). The vial was capped with a rubber septum and then evacuated and backfilled with argon (this sequence was carried out twice). A solution of **15** (109 mg, 0.436 mmol) in toluene (4 mL) was added via syringe through the septum. The septum was then replaced with a Teflon screwcap and the vial was sealed. The reaction mixture was heated at 80 °C for 5 h. The reaction mixture was allowed to cool to rt. The reaction solution was then filtered through a short pad of Celite in a Pasteur pipette (eluting with EtOAc) and the eluent was concentrated under reduced pressure. The crude phosphonate was used in the next without further purification.

K₂CO₃ (830 mg, 6.00 mmol) was dissolved in water (1 mL), and the solution was cooled to rt. This aqueous solution was then added to a mixture of paraformaldehyde (50 mg, 1.66 mmol) and the crude phosphonate prepared above in 2-propanol (1 mL) at 0 °C. The reaction mixture was allowed to warm to rt and was stirred for 4 h at rt. The reaction mixture was transferred to a separatory funnel containing saturated aqueous NH₄Cl (5 mL). The aqueous phase was extracted with EtOAc (3 $\times$ 5 mL). The combined organic layers were dried (Na₂SO₄), filtered, and concentrated under reduced pressure. The residue was purified by flash chromatography on silica gel (15% $\rightarrow$ 20% EtOAc/hexane) to afford 80 mg of the PMB-protected **20** as a colorless oil.

To a solution of PMB-protected **20** prepared above (60 mg, 0.087 mmol) in CH₂Cl₂/H₂O (7.35 mL, 20:1) was added DDQ (40 mg, 0.174 mmol) at rt. The reaction was stirred for 3 h at rt, and treated with saturated aqueous NaHCO₃. The layers were separated and the aqueous layer was extracted with CH₂Cl₂. The combined organic layers were dried (Na₂SO₄), filtered, and concentrated. Purification by flash chromatography on silica gel (25% $\rightarrow$ 30% EtOAc/hexane) afforded **20** (15 mg, 30%, three steps) as a colorless oil: *R*_f = 0.3 (2:1 hexane/EtOAc); $[\alpha]_D^{23} +27.1$ (*c* 1.5, CHCl₃); ¹H NMR (400 MHz, CDCl₃) δ 0.18 (s, 6H), 1.01 (s, 9H), 1.28–1.38 (m, 12H), 2.25–2.37 (m, 1H), 2.69–2.82 (m, 1H), 3.01 (br s, 1H), 4.16–4.30 (m, 4H), 4.68–4.86 (m, 2H), 5.20–5.26 (m, 1H), 5.56–5.61 (m, 1H), 5.93–6.00 (m, 1H), 6.83–6.93 (m, 2H); ¹³C NMR (100 MHz, CDCl₃) δ –5.0, 14.1, 18.4, 25.5, 28.2, 31.4, 50.1, 61.6, 67.2, 79.7, 82.8, 100.9, 109.7 (dd, *J* = 7.1, 16.5 Hz), 124.3, 131.6 (m, weak), 132.0 (m), 134.8, 151.0, 154.7 (dd, *J* = 6.1, 244.6 Hz), 155.2, 163.8; HRMS (ESI+) *m/z* calcd for C₂₈H₄₂F₂NO₇Si⁺ [*M* + *H*]⁺ 570.2693, found 570.2699 (error 1.0 ppm).

2-[(1*R*,2*S*,6*R*)-2-Amino-3-(3,5-difluoro-4-hydroxy)phenyl-6-hydroxycyclohex-3-enyloxy]acrylic acid (5)—Aqueous NaOH (1.0 M, 0.3 mL, 0.3 mmol) was added dropwise to a solution of **20** (5 mg, 8.8 μ mol) in THF (1 mL) and H₂O (0.3 mL) at 0 °C. After stirring overnight at rt, the reaction mixture was acidified to pH 5–6, and partitioned between CH₂Cl₂ (4 mL) and water (2 mL). The layers were separated and the aqueous layer was extracted with CH₂Cl₂ (2 $\times$ 4 mL). The organic extracts were combined, dried (Na₂SO₄), then concentrated under vacuum to afford the crude carboxylic acid that was directly used for the next step.

Cold anhydrous TFA (0 °C) was added via syringe to the crude carboxylic acid prepared above, which was also cooled to 0 °C. After 15 min, the reaction mixture was cooled further to –78 °C. The TFA was removed under high vacuum via rotovap (pre-dried beforehand) when the temperature slowly increased from –78 °C to rt. The resulting residue was purified by flash chromatography on silica gel (pre-washed with MeOH followed by CHCl₃ to remove inorganic salts from the silica gel) using 65:25:4:2 CHCl₃/MeOH/H₂O/AcOH and lyophilized to afford the TFA salt of **5** (4 mg, quantitative yield) as a white powder: *R_f* = 0.4 (65:25:4:2 CHCl₃/MeOH/H₂O/AcOH); $[\alpha]_{\text{D}}^{23} +62.5$ (*c* 0.4, H₂O); ¹H NMR (400 MHz, CD₃OD) δ 2.24–2.35 (m, 1H), 2.63–2.73 (m, 1H), 3.99–4.05 (m, 1H), 4.07–4.14 (m, 1H), 4.42–4.48 (m, 1H), 5.20 (s, 1H), 5.48 (s, 1H), 5.98–6.03 (m, 1H), 6.95–7.04 (m, 2H); ¹³C NMR (176 MHz, CD₃OD) δ 33.9, 54.3, 68.6, 83.6, 103.4, 111.4 (dd, *J* = 5.2, 17.9 Hz), 129.4, 129.9, 130.8 (m, very weak), 133.2, 135.5 (t, *J* = 16.0 Hz), 154.0 (dd, 7.2, 243.1 Hz); 171.3; ¹⁹F NMR (376 MHz, CD₃OD) δ –133.9; HRMS (ESI+) *m/z* calcd for C₁₅H₁₆F₂NO₅⁺ [*M* + *H*]⁺ 328.0991, found 328.0995 (error 1.2 ppm).

***tert*-Butyl 2-[(1*R*,2*S*,6*R*)-2-(*tert*-butoxycarbonylamino)-6-(4-methoxybenzyloxy)-3-vinylcyclohex-3-enyloxy]acetate (**21**)**—To a solution of alcohol **11** (100 mg, 0.23 mmol) in toluene (2 mL) was added consecutively tetrabutylammonium bromide (8 mg, 0.023 mmol), *tert*-butyl bromoacetate (90 mg, 0.46 mmol), and 50% aqueous NaOH (0.5 mL) at 0 °C. The mixture was warmed to rt, and stirred for 18 h. The layers were separated, and the aqueous layer was extracted with Et₂O. The combined organic layers were washed with brine, dried (MgSO₄), and concentrated in vacuo. Purification by flash chromatography on silica gel (25%→30% EtOAc/hexane) afforded the desired glycolate ether (123 mg, quantitative) as a colorless oil.

An oven-dried vial was charged with glycolate ether prepared above (123 mg, 0.23 mmol), potassium vinyltrifluoroborate (93 mg, 0.69 mmol), and Pd(dppf)Cl₂•CH₂Cl₂ (20 mg, 0.023 mmol). The vial was capped with a rubber septum and then evacuated and backfilled with argon (this sequence was carried out twice). Toluene (1.7 mL) was added via syringe through the septum, followed by the addition of EtOH (1.7 mL) and 2 M Na₂CO₃ (1.7 mL). The septum was then replaced with a Teflon screwcap and the vial was sealed. The reaction mixture was heated at 80 °C for 4 h. The reaction mixture was allowed to cool to rt, then partitioned between EtOAc and water. The layers were separated, and the aqueous layer was extracted with EtOAc. The organic extracts were combined, dried (MgSO₄), and concentrated under vacuum. Purification by flash chromatography on silica gel (15% EtOAc/hexane) afforded the product **21** (103 mg, 92% over two steps) as a light yellow oil: *R_f* = 0.3 (5:1 hexane/EtOAc); ¹H NMR (400 MHz, CDCl₃) δ 1.44 (s, 9H), 1.47 (s, 9H), 2.36 (dd, *J* = 19.9, 4.9 Hz, 1H), 2.59 (d, *J* = 19.5 Hz, 1H), 3.79 (s, 3H), 3.80–3.84 (m, 1H), 4.00–4.08 (m, 1H), 4.16 (s, 2H), 4.41–4.54 (m, 3H), 4.97 (d, *J* = 11.0 Hz, 1H), 5.13 (d, *J* = 10.0 Hz, 1H), 5.28 (d, *J* = 17.7 Hz, 1H), 5.74–5.80 (m, 1H), 6.23 (dd, *J* = 17.7, 11.1 Hz, 1H), 6.82–6.89 (m, 2H), 7.22–7.30 (m, 2H); ¹³C NMR (100 MHz, CDCl₃) δ 27.5, 28.1, 28.4, 44.9, 55.2, 67.4, 71.2, 73.4, 76.7, 79.1, 81.6, 112.1, 113.8, 127.9, 129.3, 130.1, 133.1, 137.3, 155.4, 159.2, 169.7; HRMS (ESI+) *m/z* calcd for C₂₇H₃₉NNaO₇⁺ [*M* + *Na*]⁺ 512.2619, found 512.2620 (error 0.2 ppm).

tert-Butyl 2-[(1*R*,2*S*,6*R*)-2-(*tert*-butoxycarbonylamino)-3-formyl-6-(4-methoxybenzyloxy)cyclohex-3-enyloxy]acetate (22**)**—To a solution of **21** (65 mg, 0.13 mmol) in dioxane/H₂O (4 mL, 3:1) was added 2,6-lutidine (31 μ L, 0.27 mmol), OsO₄ (2.5% in 2-methyl-2-propanol, 30 mg, 0.003 mmol) and NaIO₄ (114 mg, 0.53 mmol). The reaction mixture was stirred at rt overnight. After the reaction was complete, H₂O (10 mL) and CH₂Cl₂ (10 mL) were added. The organic layer was separated, and the aqueous layer was extracted with CH₂Cl₂ (3 $\times$ 10 mL). The combined organic layers were washed with brine, dried (Na₂SO₄), and concentrated. The residue was purified by flash chromatography on silica gel (50% $\rightarrow$ 55% EtOAc/hexane) to afford aldehyde **22** (50 mg, 77%) as a colorless oil: R_f = 0.2 (1:1 hexane/EtOAc); ¹H NMR (400 MHz, CDCl₃) δ 1.46 (s, 9H), 1.58 (s, 9H), 2.49–2.63 (m, 1H), 2.75 (m, 1H), 3.79 (s, 3H), 3.89 (m, 1H), 4.05–4.19 (m, 3H), 4.43–4.57 (m, 2H), 4.62 (d, J = 9.8 Hz, 1H), 4.92 (d, J = 9.7 Hz, 1H), 6.83 (s, 1H), 6.86 (d, J = 8.4 Hz, 2H), 7.25 (d, J = 9.3 Hz, 2H), 9.45 (s, 1H); ¹³C NMR (100 MHz, CDCl₃) δ 28.1, 28.4, 28.6, 43.2, 55.2, 67.3, 71.5, 73.2, 75.8, 79.4, 81.8, 113.9, 129.4, 129.7, 137.7, 148.6, 155.2, 159.4, 169.6, 192.3. HRMS (ESI+) calcd for C₂₆H₃₈NO₈⁺ [M + H]⁺ 492.2592, found 492.2595 (error 0.6 ppm).

(4*R*,5*R*,6*S*)-6-Amino-5-(carboxymethoxy)-4-hydroxycyclohex-1-ene-1-carboxylic acid (3**)**—To a solution of aldehyde **22** (72 mg, 0.14 mmol) and 2-methyl-2-butene (0.6 mL) in *t*-BuOH/THF/H₂O (2 mL, 5:1:1) at 0 °C was slowly added a solution of sodium chlorite (80% *w/w* technical grade, 158 mg, 1.4 mmol) and sodium phosphate monobasic monohydrate (184 mg, 1.33 mmol) in H₂O (0.3 mL). The resulting suspension was stirred at rt for 15 h. The reaction was quenched with saturated aqueous NaHSO₃ (3 mL) at 0 °C, and the aqueous layer was extracted with CH₂Cl₂ (3 $\times$ 5 mL). The combined organic layers were dried (Na₂SO₄), filtered, and concentrated under reduced pressure. Purification by flash chromatography on silica gel (1:1 hexane/EtOAc) afforded the carboxylic acid intermediate (47 mg, 66%): ¹H NMR (400 MHz, CDCl₃) δ 1.44 (s, 9H), 1.46 (s, 9H), 2.46 (dd, J = 20.6, 5.4 Hz, 1H), 2.63 (br d, J = 20.0 Hz, 1H), 3.79 (s, 3H), 3.87 (s, 1H), 4.06 (s, 1H), 4.14 (s, 2H), 4.40–4.53 (m, 2H), 4.59 (d, J = 9.9 Hz, 1H), 5.10 (d, J = 9.7 Hz, 1H), 6.86 (d, J = 8.3 Hz, 2H), 7.13 (s, 1H), 7.20 – 7.30 (m, 2H).

To a solution of carboxylic acid intermediate prepared above (9 mg, 0.018 mmol) in CH₂Cl₂ (1 mL) were added TFA (0.3 mL) and 2 drops of H₂O. The mixture was stirred at rt overnight. The solvent was removed in vacuo. Purification by flash chromatography on silica gel (pre-washed with MeOH followed by CHCl₃ to remove inorganic salts from the silica gel) using (65:25:4:2 CHCl₃/MeOH/H₂O/AcOH) afforded the desired product **3** (4 mg, 96%) as a TFA salt. To remove the inorganic salts from the silica gel, the packed column was washed successively with MeOH followed by CHCl₃ prior to loading the crude product.

R_f = 0.3 (65:25:4:2 CHCl₃/MeOH/H₂O/AcOH); $[\alpha]_D^{23}$ –38.6 (*c* 0.088, H₂O); ¹H NMR (400 MHz, D₂O) δ 2.39 (ddt, J = 19.1, 9.3, 2.9 Hz, 1H), 2.76 (dt, J = 19.5, 5.6 Hz, 1H), 3.67 (t, J = 8.8 Hz, 1H), 4.01 (td, J = 9.4, 5.6 Hz, 1H), 4.14–4.23 (m, 1H), 4.35 (q, J = 17.2 Hz, 2H), 7.09–7.16 (m, 1H); ¹³C NMR (100 MHz, D₂O) δ 32.2, 52.1, 68.2, 69.9, 81.8, 125.7, 142.6, 168.8, 177.7; HRMS (ESI+) calcd for C₉H₁₃NNaO₆⁺ [M + Na]⁺ 254.0635, found 254.0644 (error 3.5 ppm).

MbtI Assay

The compounds **1**, **3**, **4**, and **5** were evaluated against MbtI as reported.^{12, 28} All compounds failed to show any detectable inhibition at concentrations up to 100 μ M using MbtI (0.5 μ M) and chorismate (50 μ M).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by National Institutes of Health Grant RO1 AI070219 to C.C.A., the Natural Science Foundation of China (Grant 21402058 to Z.L., 21272084 to J. G.), and the Fundamental Research Funds for the Central Universities (No. CCNU16A02001 to J. G., No. CCNU15A05017 to Z. L.).

References

1. World Health Organization. Global tuberculosis report. 2016. http://www.who.int/tb/publications/global_report/en/
2. Koul A, Arnoult E, Lounis N, Guillemont J, Andries K. *Nature*. 2011; 469:483–490. [PubMed: 21270886]
3. (a) Tullius MV, Harmston CA, Owens CP, Chim N, Morse RP, McMath LM, Iniguez A, Kimmey JM, Sawaya MR, Whitelegge JP, Horwitz MA, Goulding CW. *Proc Natl Acad Sci U S A*. 2011; 108:5051–5056. [PubMed: 21383189] (b) Jones CM, Niederweis M. *J Bacteriol*. 2011; 193:1767–1770. [PubMed: 21296960] (c) De Voss JJ, Rutter K, Schroeder BG, Su H, Zhu Y, Barry CE 3rd. *Proc Natl Acad Sci U S A*. 2000; 97:1252–1257. [PubMed: 10655517]
4. (a) Snow GA. *Bacteriol Rev*. 1970; 34:99–125. [PubMed: 4918634] (b) Vergne F, Walz AJ, Miller M. *Nat Prod Rep*. 2000; 17:99–116. [PubMed: 10714901] (c) De Voss JJ, Rutter K, Schroeder BG, Barry CE 3rd. *J Bacteriol*. 1999; 181:4443–4451. [PubMed: 10419938]
5. (a) Quadri LE, Sello J, Keating TA, Weinreb PH, Walsh CT. *Chem Biol*. 1998; 5:631–645. [PubMed: 9831524] (b) Chavadi SS, Stirrett KL, Edupuganti UR, Vergnolle O, Sadhanandan G, Marchiano E, Martin C, Qiu WG, Soll CE, Quadri LE. *J Bacteriol*. 2011; 193:5905–5913. [PubMed: 21873494] (c) McMahon MD, Rush JS, Thomas MG. *J Bacteriol*. 2012; 194:2809–2818. [PubMed: 22447909]
6. (a) Zwahlen J, Kolappan S, Zhou R, Kisker C, Tonge PJ. *Biochemistry*. 2007; 46:954–964. [PubMed: 17240979] (b) Harrison AJ, Yu M, Gardenborg T, Middleditch M, Ramsay RJ, Baker EN, Lott JS. *J Bacteriol*. 2006; 188:6081–6091. [PubMed: 16923875]
7. (a) Kerbarh O, Bulloch EMM, Payne RJ, Sahr T, Rébeillé F, Abell C. *Biochem Soc Trans*. 2005; 33:763–766. [PubMed: 16042594] (b) Dosselaere F, Vanderleyden J. *Crit Rev Microbiol*. 2001; 27:75–131. [PubMed: 11450855] (c) Walsh CT, Liu J, Rusnak F, Sakaitani M. *Chem Rev*. 1990; 90:1105–1129.
8. Kozlowski MC, Tom NJ, Seto CT, Sefler AM, Bartlett PA. *J Am Chem Soc*. 1995; 117:2128–2140.
9. (a) Manos-Turvey A, Cergol KM, Salam NK, Bulloch EMM, Chi G, Pang A, Britton WJ, West NP, Baker EN, Lott JS, Payne RJ. *Org Biomol Chem*. 2012; 10:9223–9236. [PubMed: 23108268] (b) Manos-Turvey A, Bulloch EMM, Rutledge PJ, Baker EN, Lott JS, Payne RJ. *ChemMedChem*. 2010; 5:1067–1079. [PubMed: 20512795] (c) Payne RJ, Bulloch EMM, Kerbarh O, Abell C. *Org Biomol Chem*. 2010; 8:3534–3542. [PubMed: 20532401] (d) Payne RJ, Bulloch EMM, Toscano MM, Jones MA, Kerbarh O, Abell C. *Org Biomol Chem*. 2009; 7:2421–2429. [PubMed: 19462053] (e) Payne RJ, Bulloch EMM, Abell AD, Abell C. *Org Biomol Chem*. 2005; 3:3629–3635. [PubMed: 16211099] (f) Payne RJ, Kerbarh O, Miguel RN, Abell AD, Abell C. *Org Biomol Chem*. 2005; 3:1825–1827. [PubMed: 15889161] (g) Payne RJ, Toscano MD, Bulloch EMM, Abell AD, Abell C. *Org Biomol Chem*. 2005; 3:2271–2281. [PubMed: 16010361]

10. (a) He Z, Stigers Lavoie KD, Bartlett PA, Toney MD. *J Am Chem Soc.* 2004; 126:2378–2385. [PubMed: 14982443] (b) Meneely KM, Luo Q, Dhar P, Lamb AL. *Arch Biochem Biophys.* 2013; 538:49–56. [PubMed: 23942051]
11. (a) DeClue MS, Baldridge KK, Kast P, Hilvert D. *J Am Chem Soc.* 2006; 128:2043–2051. [PubMed: 16464106] (b) Wright SK, DeClue MS, Mandal A, Lee L, Wiest O, Cleland WW, Hilvert D. *J Am Chem Soc.* 2005; 127:12957–12964. [PubMed: 16159290] (c) DeClue MS, Baldridge KK, Kunzler DE, Kast P, Hilvert D. *J Am Chem Soc.* 2005; 127:15002–15003. [PubMed: 16248620]
12. Liu Z, Liu F, Aldrich CC. *J Org Chem.* 2015; 80:6545–6552. [PubMed: 26035083]
13. Ballatore C, Huryn DM, Smith AB. *ChemMedChem.* 2013; 8:385–395. [PubMed: 23361977]
14. Qiu J, Stevenson SH, O’Beirn MJ, Silverman RB. *J Med Chem.* 1999; 42:329–332. [PubMed: 9925739]
15. Endoma MA, Bui VP, Hansen J, Hudlicky T. *Org Process Res Dev.* 2002; 6:525–532.
16. For reviews on applications of these compounds to synthesis, see: Hudlicky T, Reed JW. *Chem Soc Rev.* 2009; 38:3117–3132. [PubMed: 19847346] Johnson RA. *Org React.* 2004; 63:117–264.
17. Matveenko M, Banwell MG, Willis AC. *Org Lett.* 2008; 10:4693–4696. [PubMed: 18816123]
18. Moon Kim B, Barry Sharpless K. *Tetrahedron Lett.* 1990; 31:4317–4320. For a review on cyclic sulfite chemistry, see: Byun H-S, He L, Bittman R. *Tetrahedron.* 2000; 56:7051–7091.
19. Ueda T, Konishi H, Manabe K. *Org Lett.* 2012; 14:5370–5373. [PubMed: 23020164]
20. Shie JJ, Fang JM, Wong CH. *Angew Chem Int Ed.* 2008; 47:5788–5791.
21. Blacker AJ, Booth RJ, Davies GM, Sutherland JK. *J Chem Soc Perkin Trans 1.* 1995; 2861–2870
22. Humphreys JL, Lowes DJ, Wesson KA, Whitehead RC. *Tetrahedron.* 2006; 62:5099–5108.
23. Yu W, Mei Y, Kang Y, Hua Z, Jin Z. *Org Lett.* 2004; 6:3217–3219. [PubMed: 15355016]
24. Hu F, Zhang YH, Yao ZJ. *Tetrahedron Lett.* 2007; 48:3511–3515.
25. Bal BS, Childers WE, Pinnick HW. *Tetrahedron.* 1981; 37:2091–2096.
26. Ganem B, Ikota N, Muralidharan VB, Wade WS, Young SD, Yukimoto Y. *J Am Chem Soc.* 1982; 104:6787–6788.
27. Liu Z, Gong Y, Byun HS, Bittman R. *New J Chem.* 2010; 34:470–475.
28. Vasan M, Neres J, Williams J, Wilson DJ, Teitelbaum AM, Remmel RP, Aldrich CC. *ChemMedChem.* 2010; 5:2079–2087. [PubMed: 21053346]

Figure 1.

Conversion of chorismate to salicylate catalyzed via isochorismate by MbtI and elaboration of salicylate to the mycobactins.

Figure 2.
Potential transition-state analogues inhibitors of MbtI **1–5**.

Scheme 1. symmetric Synthesis of Cyclohexene Skeleton^a

^aReagents and conditions: (a) benzaldehyde dimethyl acetal, CSA•H₂O, CH₂Cl₂, -20 °C, 2 h; (b) *m*-CPBA, CH₂Cl₂, 0 °C to rt, overnight; (c) 1.0 M LAH in Et₂O, Et₂O, rt, 20 min; (d) 4-methoxybenzyl chloride, NaH, TBAI, DMF, 0 °C to rt, overnight; (e) TsOH•H₂O, CH₂Cl₂/EtOH (1:5), rt, 24 h; (f) SOCl₂, pyridine, CH₂Cl₂, 0 °C, 20 min; (g) NaN₃, DMF, rt, 24 h; (h) Ph₃P, THF/H₂O (9:1), rt, 19 h; (i) Boc₂O, Et₃N, 1,4-dioxane/H₂O (10:3), rt, 40 min.

Scheme 2. Synthesis of 1 and 4^a

^aReagents and conditions: (a) Pd(dppf)Cl₂•CH₂Cl₂, potassium vinyltrifluoroborate, 2 M Na₂CO₃/toluene/EtOH (1:2:2), 70 ~ 80 °C, 15 h; (b) OsO₄, NaIO₄, 2,6-lutidine, 1,4-dioxane/H₂O (3:1), rt, 5 h; (c) NaClO₂, NaH₂PO₄, 2-methyl-2-butene, *t*-BuOH/THF/H₂O (5:1:1), 0 °C to rt, overnight; (d) TMSCHN₂, PhMe/MeOH (7:1), rt, 10 min; (e) Rh₂(OAc)₄, 15, toluene, 80 °C, 5 h; (f) paraformaldehyde for R = H, acetaldehyde solution (40% wt in H₂O) for R = CH₃, K₂CO₃, 2-PrOH/H₂O (1:1), rt, 5 h; (g) TFA/CH₂Cl₂ (1:1), 0 °C, 10 min; (h) KOH, H₂O, 0 °C, 1 h.

Scheme 3. Synthesis of analogue 5^a

^aReagents and conditions: (a) Pd(dppf)Cl₂•CH₂Cl₂, **18**, 2 M Na₂CO₃/toluene/EtOH (1:2:2), 70 ~ 80 °C, 30 ~ 40 min; (b) Rh₂(OAc)₄, **15**, toluene, 80 °C, 5 h; (c) paraformaldehyde, K₂CO₃, 2-PrOH/H₂O (1:1), 0 °C to rt, 4 h; (d) DDQ, CH₂Cl₂/H₂O (20:1), rt, 3 h; (e) 1 M NaOH, THF, 0 °C to rt, overnight; (f) anhydrous TFA, 0 °C, 15 min.

Scheme 4. Synthesis of analogue 3^a

^aReagents and conditions: (a) *t*-butyl bromoacetate, tetrabutylammonium bromide, 50% aqueous NaOH solution, PhMe, 0 °C to rt, 18 h; (b) $\text{Pd(dppf)Cl}_2 \cdot \text{CH}_2\text{Cl}_2$, potassium vinyltrifluoroborate, 2 M Na_2CO_3 /toluene/EtOH (1:1:1), 80 °C, 4 h; (c) OsO_4 , NaIO_4 , 2,6-lutidine, 1,4-dioxane/ H_2O (3:1), rt, 5 h; (d) NaClO_2 , NaH_2PO_4 , 2-methyl-2-butene, *t*-BuOH/THF/ H_2O (5:1:1), 0 °C to rt, 15 h; (e) TFA/ CH_2Cl_2 / H_2O , rt, overnight.