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Rapid Enantioselective and Diastereoconvergent Hybrid Organic/Biocatalytic Entry into the Oseltamivir Core

Virendra K. Tiwari, Douglas R. Powell, Sylvain Broussy,* and David B. Berkowitz*

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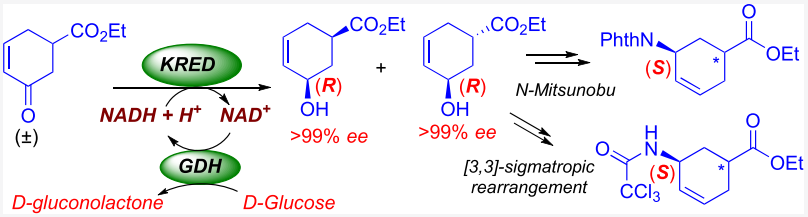

ABSTRACT: A formal synthesis of the antiviral drug (−)-oseltamivir (Tamiflu) has been accomplished starting from *m*-anisic acid via a dissolving metal or electrochemical Birch reduction. The correct absolute stereochemistry is efficiently set through enzyme-catalyzed carbonyl reduction on the resultant racemic α,β-unsaturated ketone. A screen of a broad ketoreductase (KRED) library identified several that deliver the desired allylic alcohol with nearly perfect facial selectivity at the new center for each antipodal substrate, indicating that the enzyme also is able to completely override inherent diastereomeric bias in the substrate. Conversion is complete, with D-glucose serving as the terminal hydride donor (glucose dehydrogenase). For each resulting diastereomeric secondary alcohol, O/N-interconversion is then efficiently effected either by synfacial [3,3]-sigmatropic allylic imidate rearrangement or by direct, stereoinverting N-Mitsunobu chemistry. Both stereochemical outcomes have been confirmed crystallographically. The α,β-unsaturation is then introduced via an α-phenylselenylation/oxidation/pyrolysis sequence to yield the targeted (*S*)-*N*-acyl-protected 5-amino-1,3-cyclohexadiene carboxylates, key advanced intermediates for oseltamivir pioneered by Corey (*N*-Boc) and Trost (*N*-phthalamido), respectively.

INTRODUCTION

The current pandemic due to the SARS-CoV-2 virus has highlighted the importance of developing both therapeutic agents, such as remdesivir[1] and dexamethasone,[2] in this case and prophylactic (e.g., mRNA-based vaccines) antiviral agents, in parallel. In this communication, we describe an efficient new route to the most established therapeutic agent for influenza A−C, namely, oseltamivir (Tamiflu).[3] To our knowledge, the only previously reported biocatalytic entries into the oseltamivir skeleton involve either lipase-/esterase-mediated kinetic resolution[4]/desymmetrization[5] or whole-cell-mediated microbial arene dioxygenation.[6] We report herein a hybrid chemo/biocatalytic route into oseltamivir that involves the use of an isolated enzyme to set the key stereocenter from a prochiral center with nearly perfect stereoinduction and complete conversion. This is also the first example of the use of a dehydrogenase enzyme in oseltamivir synthesis, with D-glucose serving as the stoichiometric reductant for the key stereochemically defining step. This is of particular significance given the current interest in biocatalytic routes in process chemistry, in general, and toward antivirals, in particular.[7]

Viral neuraminidase (NA) cleaves a terminal sialic acid on the host cell glycoprotein receptor to which the viral hemagglutinin (HA) is bound, thereby releasing the budding virion from the infected cell.[8] By inhibiting the viral NA enzyme, oseltamivir stabilizes the viral HA−host−receptor interaction, thereby preventing virion release and slowing the progression toward increased viral load. NA cleavage of the terminal sialic acid linked to a galactose moiety through an α-(2,3)- or an α-(2,6)-glycosidic linkage proceeds through a putative oxocarbenium ion-like TS stabilized by interaction with an enzymatic tyrosine residue (Scheme 1). The oxocarbenium ion has a $^4H_5$ half-chair geometry that maps directly onto the geometry of oseltamivir, making this an ideal TS analogue.[9] Zanamivir (Relenza), another potent NA inhibitor, displays an E_5 geometry. Oseltamivir displays much higher oral bioavailability and has been the prodrug of choice for the treatment of influenza.[10,11] Oseltamivir was developed by Gilead and marketed by Roche as Tamiflu, and the first generic version was approved by the FDA in 2016. The recent discovery that amino-piperidine-based inhibitors of the

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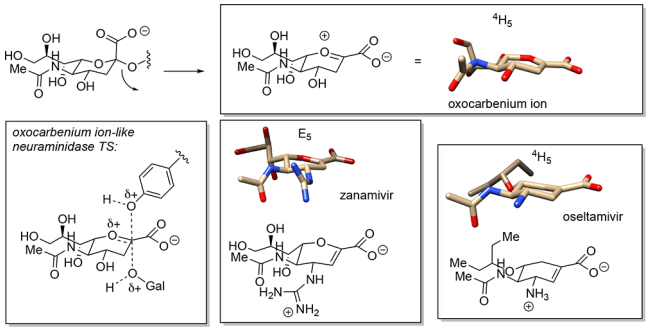
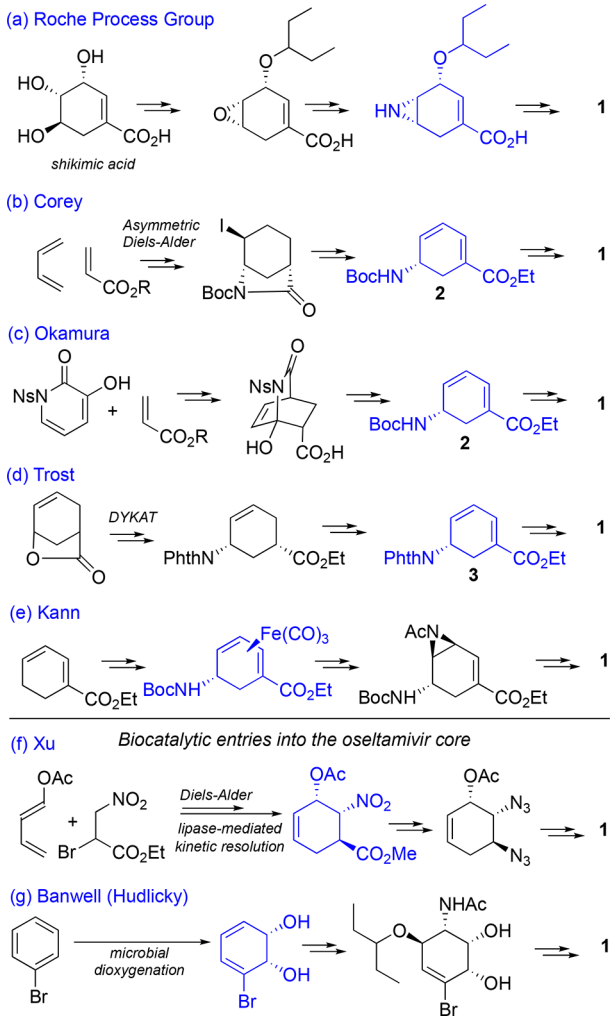
Scheme 1. Neuraminidase Reaction and Oseltamivir as the TS Analogue: Putative Oxocarbenium Ion ($^4H_5$) and Enzymatic Transition State (See 4ZJQ for the Resulting Intermediate) for Zanamivir (E_5; 1NNC) and Oseltamivir ($^4H_5$; 3CL0)



influenza virus cell entry act synergistically with oseltamivir[12] has greatly heightened interest in the drug today.

Notable synthetic entries into this scaffold are delineated in Scheme 2, highlighting key transformations in each case. In the original Roche process chemistry route, shikimic acid, a highly functionalized, stereochemistry-rich chiron is converted to a key homoallylic epoxide intermediate (Scheme 2a).[13] Corey developed a chiral oxazaborolidinium-ion-catalyzed Diels–Alder reaction-based synthesis. This route passes through a chiral cyclohexenoid intermediate that then undergoes a key iodo-lactamization reaction to give key chiral intermediate **2**, a major focus of this communication. Transformation of **2** into a cyclic *N*-acyl aziridine, followed by ring opening, furnishes **1** (Scheme 2b).[14] Okamura reported a convergent [4 + 2]-cycloaddition-based route reaction for the synthesis of **2** (Scheme 2c).[15] In the Trost synthesis of oseltamivir, palladium-catalyzed asymmetric allylic alkylation and rhodium-catalyzed dienoate aziridination lead to intermediate **3** (Scheme 2d),[16] another key target of the current study. Kann (Scheme 2e) elegantly exploited a built-in $Fe(CO)_3$-coordinated moiety to at once effect diene activation and steer facial selectivity.[17]

Scheme 2. Stereocontrolled Routes to the Oseltamivir Core

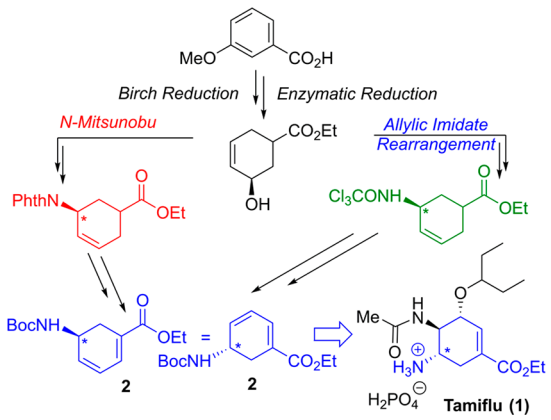


RESULTS AND DISCUSSION

Consistent with our longstanding interest in the use of enzymes as both stereoselective reporters[18] and stereoselective catalysts[19] for advancing organic synthesis, we set out to develop a new enzyme-assisted entry into the oseltamivir core. While the Tamiflu scaffold continues to draw the close attention of synthetic chemists,[20] there have been few approaches that exploit enzymatic chemistry. The Xu[4] route employs a diastereoselective Diels–Alder reaction and diazidation, followed by classic lipase-catalyzed kinetic resolution (Scheme 2f). Banwell[6d,21] (Scheme 2g) and Hudlicky[6a,22] utilize microbial arene oxidation as a key step to set the stereochemistry for their Tamiflu syntheses (Scheme 2g). To our knowledge, this report constitutes the first entry into the oseltamivir framework that exploits dehydrogenase chemistry and one that does so with essentially complete stereocontrol.

The strategic approach taken here is presented in Scheme 3. It was envisioned that one could rapidly arrive at a key cyclic

Scheme 3. New Hybrid Organic/Biocatalytic Approach into the Corey and Trost Intermediates



α,β-unsaturated ketone via Birch reduction of *m*-anisic acid. This would, in principle, allow for enzymatic installation of the key stereocenter through facially selective carbonyl reduction, despite the presence of a potentially confounding second stereocenter, adjacent to the ester functionality. A major challenge would therefore be to find not only a dehydrogenase active site that would exhibit high facial selectivity for such a substituted cyclohexenone system but also one that would be highly promiscuous in tolerating functionality at C5 and be

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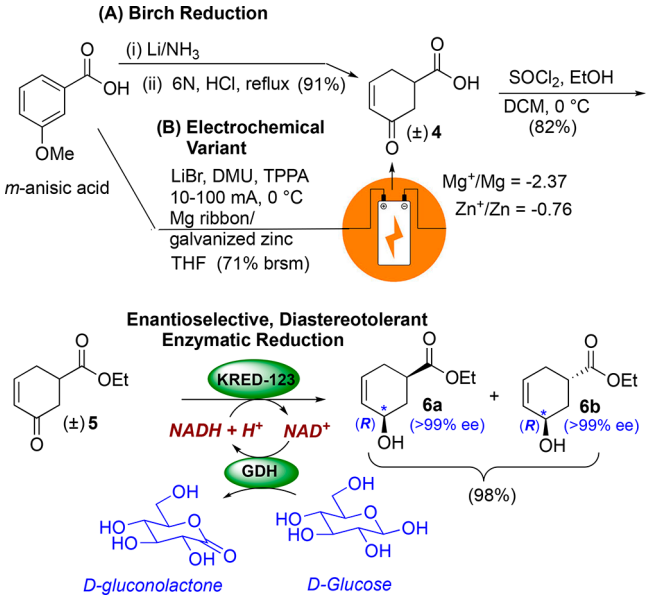
fully "stereotolerant" at that position. The next key step would be to translate the enzymatically installed C−O absolute stereochemistry into the requisite C−N stereochemistry for Tamiflu. It was envisioned that this could be achieved either by a direct stereoinvertive N-Mitsunobu transformation or via O,N-allylic transposition through stereospecific synfacial [3,3]-sigmatropic rearrangement (Scheme 3).

The initially required Birch reduction of *m*-anisic acid proceeded smoothly to give compound **4** under traditional dissolving metal conditions with Li metal serving as reductant in liquid ammonia. Optimal yields were obtained using ice (water added dropwise into liquid ammonia at −78 °C) as a proton source as opposed to EtOH−H_2O mixtures as delineated in detail in the SI (see Table S1). Under these conditions, one obtains high conversion to the desired 5-carboxy-cyclohexenone, following acid-catalyzed enol ether hydrolysis and conjugative alkene isomerization to **4** (Scheme 4A).[23] Pleasingly, the recently described[24] electrochemical

Scheme 4. Efficient Birch Reduction/KRED-Mediated Introduction of the Proper Absolute Stereochemistry



alternative to the Birch reduction also proved successful for this transformation. Utilizing Mg as an anode and a galvanized steel nail (Zn) as a cathode with dimethylurea as proton source and tris(pyrrolidino)phosphoramide as an internal overcharge protectant, one obtains the desired 5-carboxycyclohexenone without the need for the acid-catalyzed isomerization step (Scheme 4B)

Next, we set out to explore a wide array of nicotinamide-dependent dehydrogenases, for the sought-after facially selective and diastereotolerant, substituted cyclohexenone reduction. In this endeavor, we were particularly drawn to the KRED (ketoreductase) family of enzymes that have proven useful by both academic and industrial chemists.[25] In our own hands, KRED enzymes had already shown potential on both sides of the house, as catalytic reporters for our in situ enzymatic screening efforts[18c] and as chiral catalysts, in and of themselves.[19e] In fact, we are delighted to report here streamlined pathways to the key advanced intermediates for olseltamivir, **2** (Corey) and **3** (Trost), that feature KRED-catalyzed facially selective cyclohexenone reduction as the key step (Scheme 4 and Table 1; for details see Tables S2 and S3).

According to our synthetic design (Schemes 3 and 4), the desired allylic alcohol **6** would be obtained by a regioselective 1,2-cyclohexenone reduction reaction and with *R*-stereoselectivity. We had hoped to find enzymes that would treat the resident carboethoxy stereocenter at C5 in (±)-**5** as a "spectator" stereocenter and deliver a hydride to the correct face of the ketone regardless of the configuration of that pre-existing stereocenter. Toward this end, a set of 82 KRED enzymes were screened under identical conditions: 0.025 mmol of (±)-**5**, 1 mg of enzyme, 24 h, 30 °C, in the presence of glucose dehydrogenase (GDH) for NAD(P)H cofactor regeneration from D-glucose (Table 1 and SI Tables S2 and S3).

In the ideal case, a quantitative yield in combination with *R*-stereoinduction would result in a 50:50 mixture of *cis*/*trans* diastereomers, in a "diastereotolerant" or "catalyst-controlled" reduction.[26] During the screening, besides the remaining starting ketone, three products were identified by ^{1}H NMR: the desired allylic alcohol **6** in various *cis*/*trans* ratios, the saturated alcohol **7** (resulting from 1,4- and subsequent 1,2-reduction), and the saturated ketone **8** (1,4-reduction only). Product distributions for all 82 enzymes were calculated based on integration of characteristic signals on the ^{1}H NMR spectra (see Table 1 and the SI, particularly Tables S2 and S3). Among the 61 NADPH-dependent KRED enzymes, only a few gave acceptable yields of **6** together with a *cis*/*trans* ratio close to 50:50. For example, KRED-101 gave 69% yield of **6** with 48:52 *cis*/*trans* ratio, resulting in 78% and 86% ee for the *cis* and *trans* diastereomers, respectively. The enantiomeric excess was determined by chiral phase HPLC analysis on the *p*-bromobenzoate derivatives of the enzymatic reduction products vs racemic standards (see SI) obtained by Luche reduction of (±)-**5** ($NaBH_4$/$CeCl_3$; 9:1 *cis*/*trans* selectivity). NADH-dependent KRED enzymes (Table 1) generally gave better yields of 1,2-reduction and enantiomeric excess values for the **6a** and **6b** products than NADPH-dependent ones (Table 1 and see SI Table S2).

In particular, KRED-117, -123, and -126 gave complete conversion, complete regioselectivity, and nearly perfect diastereotolerance toward C5 and nearly perfect facial selectivity at the carbonyl center (>99% ee for **6a** and **6b**)! The *R*-absolute configuration was established by determination of optical rotation values of **3** and **2** and comparison with literature values (see the Experimental Section). The reaction was also achieved on a gram scale with KRED-123, giving 98% yield of combined *cis*/*trans* diastereomers **6** in 99% ee each. Moreover, KRED-123 recycling has been shown to be possible for at least three cycles, resulting in an estimated turnover number of 20 000 cycles (see Experimental Section).

The desired *R*-absolute configuration having been set, the diastereomeric mixture of enantiopure alcohols **6** was converted to building blocks **3** and **2**. A nitrogen-Mitsunobu reaction upon **6** with phthalimide and DIAD cleanly gave **9** as a 1:1 mixture of diastereomers. The desired α,β-unsaturation with respect to the carboethoxy group was then smoothly installed by ester enolate formation, α-phenylselenation, oxidation, and selenoxide elimination. Literature precedents indicate that the Mitsunobu reaction on cyclic, allylic secondary alcohols can proceed through either an S_N2- or an S_N2'-mechanism, depending mostly on the substrate and to a lesser extent on the nucleophile and the solvent. The related,

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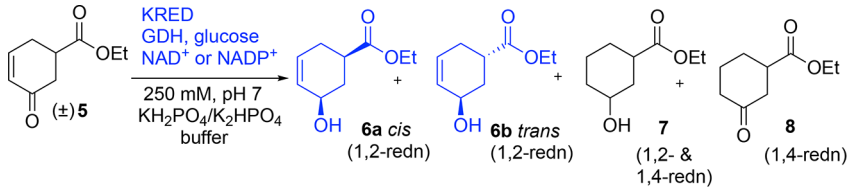
Table 1. Screening of KREDs[a]



KRED enz.	recovered SM of **5** (%)	total (%) yield[b] of **6**	ratio of **6a**/**6b** (cis/trans)[c]	% ee of **6a**/**6b**	% yield[b] of **7** (cis/trans ratio)[c]	% yield[b] of **8**
NADPH-dependent KREDs						
101[d]	0	69	48:52	~ 78 (*R*)/86 (*R*)	31 (71:28)	0
102	14	trace	-	-	58 (88:12)	28
103	6	0	-	-	19	75
104	13	60	20:80	-	27 (81:19)	0
105	61	0	-	-	6	33
106	trace	0	-	-	57 (86:14)	43
107	14	trace	-	-	80 (34:66)	6
119	0	50	66:34	~ 70 (*R*)/92 (*R*)	50 (53:47)	0
120	0	Trace	-	-	100 (50:50)	trace
134	0	22	13:87		78 (11:89)	0
135	0	33	15:85		67 (12:88)	0
136	12	6			66 (62:38)	16
137	26	trace	-	-	57 (34:66)	17
139	0	0	-	-	14	86
NADH-dependent KREDs						
101	**3**	**89**	46:54	>99 (*R*)/>99 (*R*)	**8**	**0**
104	34	53	80:20		13	0
105	25	65	80:20		10	0
107	77	8	15		15	0
117	**0**	**100**	50:50	>99 (*R*)/>99 (*R*)	**0**	**0**
118	43	48	48:52		9	0
119	36	56	>99:1		8	trace
122	81	10	9		9	0
123	**0**	**100**	50:50	>99 (*R*)/>99 (*R*)	**0**	**0**
125	83	3	-		14	0
126	**0**	**99**	49:51	>99 (*R*)/>99 (*R*)	**trace**	**0**
129	60	28	91:9		12	0

[a]Reaction conditions: 0.025 mmol of (±)-**5**, 1 mg of enzyme, 24 h, 30 °C, in the presence of glucose dehydrogenase (GDH) for NAD(P)H cofactor regeneration from D-glucose. [b]Product distribution was established by integration of characteristic signals for each compound in the crude NMR spectrum, followed by calculation of the percentage of each component. See the SI for details. [c]The *cis*/*trans* ratio is given only when accurate measurement was possible, typically for >20% product. [d]2 mg of enzyme were used.

but relatively sterically unencumbered, 2-cyclohexen-1-ol system reacted mostly through an S_N2 manifold with carboxylic acid nucleophiles (8−28% of non-S_N2 products detected).[27] More sterically hindered derivatives of conduritol (cyclohex-5-ene-1,2,3,4-tetra-ols) react exclusively by an S_N2 mechanism or with up to 73% S_N2'-mechanism, depending on the relative stereochemistry of the protected hydroxy groups.[28] For the S_N2'-mechanism, the participation of both oxygen atoms of the carboxylate nucleophile was postulated. The Mitsunobu reaction with nitrogen nucleophiles shows some dependence on the steric demand of the putative S_N2- vs S_N2'-transition states. The use of TsNHBoc as an N-Mitsunobu nucleophile on 2-cyclohexen-1-ol induced a decrease in the enantiomeric excess of the product, presumably through a competing S_N2'-mechanism.[29] However, the use of TsNHPMB on 4,5-bis((*tert*-butyldimethylsilyl)oxy)cyclohex-2-en-1-ol gave exclusively the product resulting from an S_N2-mechanism.[30]

In our system, due to symmetry, S_N2- and S_N2'-mechanisms would lead to the same positional isomer; only the stereochemistry could differ. The absolute stereochemistry (*S*-configuration, see below) and the very high enantiomeric excess obtained for **3** suggest that the reaction likely proceeds through an S_N2 mechanism with complete inversion of configuration. Some participation of an S_N2' pathway cannot be excluded but would have had to proceed with complete retention to give the *S*-stereoisomer (Scheme 5). The optical rotation of **3** matched that reported previously by Trost for this advanced intermediate, and the X-ray structure determination for **9b** (Scheme 5 and SI) verified the relative stereochemistry.[16b] The phthalimide protecting group of **9** was readily converted to a Boc group in two steps to give **12**, and α,β-unsaturation was introduced as above to give **2**, the Corey intermediate. Chiral HPLC analysis confirmed the preservation of ee (98%) in the O,N-interconversion, and again the optical rotation matched reported values, confirming the absolute stereochemistry.[14,20h,31]

A complementary sigmatropic rearrangement route into the Corey intermediate was pursued in parallel from enzymatic products **6a**/**6b**. This approach exploits the symmetry in this system, whereby synfacial O,N-allylic transposition upon *R*-configured alcohols **6a**/**6b** provides positionally and stereo-

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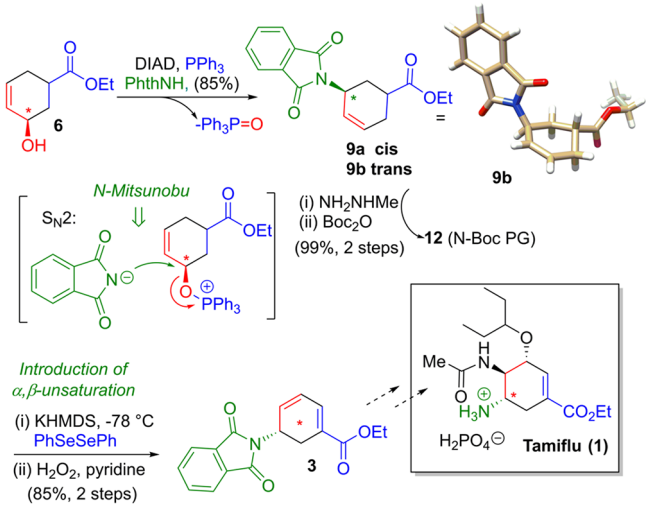
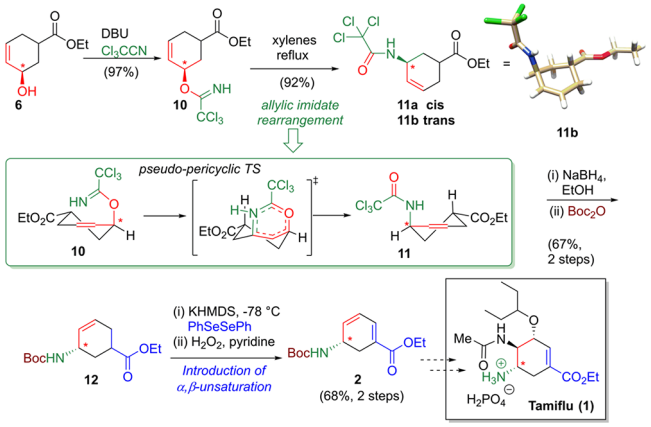
Scheme 5. Synthesis of Trost Intermediate 3 via Stereo-Inverting N-Mitsunobu Transformation



chemically equivalent *S*-amide products to those obtained through the direct displacement stereoinverting the N-Mitsunobu route (see Schemes 5 and 6). In the event, [3,3]-

Scheme 6. Access to the Corey Intermediate via Syn-Facial [3,3]-Sigmatropic Rearrangement



sigmatropic allylic imidate rearrangement[32] of trichloroacetimidate **10** proceeded efficiently, appropriately shifting the chirality from the *R*-secondary alcohol to the key *S*-trichloroacetamide **11** in 92% yield, with the absolute stereochemistry being cemented by X-ray crystal structure determination of **11b** (Scheme 6 and SI). The thermal [3,3]-sigmatropic rearrangement of allylic trichloroacetimidates[33] is known to proceed stereospecifically in a synfacial fashion on 6-membered ring systems such as cyclohexenes[30,34] and dihydropyrans.[35] DFT calculations suggest that such rearrangements typically proceed asynchronously, via a pseudopericyclic transition state (Scheme 6).[36] The rearrangement product **11** could easily be converted to the Corey intermediate **12** by amide cleavage ($NaBH_4$ and EtOH) followed by Boc protection (Boc_2O).

CONCLUSIONS

In summary, this new biocatalyst-assisted approach into the therapeutically important oseltamivir core includes the following distinguishing features: (a) use of Birch reduction (traditional or electrochemical) of a simple, inexpensive aromatic precursor to enter the needed substituted cyclohexenone scaffold, (b) use of enzymatic reduction to install absolute stereochemistry in a nearly perfect process that completely overrides inherent diastereofacial bias in the antipodal substrates (98% yield, >99% ee), and (c) complementary routes into the Corey intermediate through N-Mitsunobu or allylic imidate sigmatropic rearrangement manifolds. In particular, remarkable facial selectivity and diastereotolerance of the KRED-mediated[37] chemistry speaks to the value of hybrid synthetic organic/biocatalytic routes in support of both chemical biology and process chemistry.[7a,25b,38]

EXPERIMENTAL SECTION

I. General Procedure. Unless otherwise noted, all reactions were performed in oven-dried glassware. All air- or water-sensitive reactions were conducted under an inert atmosphere (N_2 or Ar) using oven-dried glassware. THF was distilled from sodium benzophenone ketyl. Methanol was distilled over magnesium and iodine. Dichloromethane and diisopropylamine were distilled over CaH_2. Other reagents were obtained from commercial sources and used without further purification. Reaction progress was monitored by TLC or GC-MS (HP model 5890 GC with model 5972 MS). Flash chromatography was performed using silica gel 60 (230−400 mesh). ^{1}H NMR spectra were recorded on Bruker-DRX-Avance-400 or 500 MHz instruments with chemical shifts reported relative to residual $CHCl_3$ (7.25 ppm). Proton-decoupled ^{13}C NMR spectra were acquired on Bruker-DRX-Avance-400 or 500 MHz instruments with chemical shifts reported relative to $CDCl_3$ (77.0 ppm). High-resolution mass spectra were acquired at the Nebraska Center for Mass Spectrometry (University of Nebraska). Enzymes of the KRED (ketoreductase) family were obtained from Codexis. Crystallography was performed by Douglas R. Powell at the University of Oklahoma.

II. Synthetic Procedures for the Synthesis of Ketone 5. *5-Oxocyclohex-3-ene-1-carboxylic Acid (4). A. Birch Reduction of m-Anisic Acid.* A three-necked round-bottom flask, equipped with an ammonia condenser, glass stopper, and septum, was charged with *m*-anisic acid (10.0 g, 66 mmol) and H_2O (10 mL). The condenser was filled with dry ice and acetone, and ammonia was condensed to a total volume of about 350 mL. The ammonia inlet was replaced with an Ar inlet, and a slow stream of argon was introduced above the surface. Pieces of Li metal (1.4 g, 200 mmol) were slowly added, upon stirring, over a period of 30 min. After the blue color completely disappeared (about 1 h), the condenser was removed, and ammonia was allowed to evaporate under a stream of argon. The residue was acidified to pH 1 with 1 M HCl(aq) followed by 6 M HCl(aq) and heated to reflux for 1 h using a silicon oil bath. After allowing the reaction flask to cool to rt, the product was extracted with Et_2O (5 times), and the combined organic layers were dried over Na_2SO_4 and evaporated, to give acid **4** (8.4 g, 91%) as a slightly yellow solid. ^{1}H NMR and ^{13}C{1H} NMR spectral data were consistent with those previously reported.[23] Both the use of water (ice) as a proton source and the specific addition sequence proved important in the optimization of this reaction (see SI, especially Table S1, for more details).

B. Electrochemical Reduction Reaction.[24] A Mg ribbon anode and galvanized-Zn cathode (commercial nail) were set up around a number 24-sized septum (photographs for the experimental setup are shown in SI). To an oven-dried 7-dram vial charged with a small magnetic stir bar were added *m*-anisic acid (100 mg, 0.6 mmol), 1,3-dimethylurea (DMU) (2.4 mmol), and tri(pyrrolidin-1-yl) phosphine oxide (TPPA) (4.8 mmol). Then a solution of lithium bromide (2.4 mL; 2 M in THF) was added, and the resultant reaction mixture was degassed for 5 min under Ar flow, followed by the addition of THF (15 mL) under Ar. The reaction vial was placed at 0 °C, and electrodes were connected via alligator clips to a BIO-RAD Model 3000 XI power supply. The reaction mixture was initially subjected to 10 mA constant current for 1.5 h, followed by a steady ramping up to

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20 mA for 2 h, 25 mA for 2 h, and finally 100 mA for the next 3 h. The reaction was monitored by TLC and after 9 h; the power supply was disconnected; and the reaction mixture was transferred into an RB flask. The electrodes were washed with EtOAc, and the combined organics were concentrated on a rotary evaporator. The residue was taken up in saturated sodium potassium tartrate solution/EtOAc, partitioned in a separatory funnel, and the organic layer was further washed with brine. After drying over Na_2SO_4 and concentrating under reduced pressure, the crude product was purified by SiO_2 flash column chromatography (99:1 EtOAc/CH_3OH) to give **4** (30 mg, 71% brsm) and recovered *m*-anisic acid (50 mg) (for a view of the experimental setup, see Figure S1 in the SI).

*Ethyl 5-Oxocyclohex-3-ene-1-carboxylate (**5**). Esterification Reaction.* Thionyl chloride ($SOCl_2$; 520 μL, 7.14 mmol) was added dropwise to 30 mL of ethanol at 0 °C. After 10 min, acid **4** (1 g, 7.14 mmol) was added, as a solid, at 0 °C, and the reaction mixture was allowed to warm to rt over 3 h. The reaction was quenched by addition of NH_4Cl (aq., sat'd), and the product was extracted with Et_2O. The organic layer was dried over Na_2SO_4 and evaporated, and the residue was purified by flash column chromatography on silica gel (20% EtOAc in hexanes) to give ester **5** as a yellow oil (980 mg, 82%). ^1H NMR and $^{13}C\{^1H\}$ NMR spectral data matched with reported data.[39] HRMS (TOF MS ESI$^+$) *m*/*z*: $[M + Na]^+$ calcd for $C_9H_{12}NaO_3$ 191.0678, found 191.0686.

*(±)-Ethyl 5-Hydroxycyclohex-3-enecarboxylate Diastereomers (**6a**/**6b**). Luche Reduction.* A solution of ketone **5** (680 mg, 4.05 mmol) and $CeCl_3{-}7H_2O$ (ceric chloride−heptahydrate; 5.42 mmol, 2.02 g) in methanol (40 mL) was stirred at 0 °C for 15 min, followed by the addition of $NaBH_4$ (5.00 mmol, 189 mg). The resulting mixture was stirred at 0 °C for 30 min and then quenched via dropwise addition of NH_4Cl (aq., saturated) solution. Following extraction with CH_2Cl_2, the organic phase was dried (Na_2SO_4) and evaporated. Flash column chromatography on silica gel (30% EtOAc in hexanes) gave **6a**/**6b** (570 mg, 86%) as a 9:1 mixture of racemic, *cis*- and *trans*-diastereomers, respectively. See below for complete spectral characterization of each diastereomer.

III. Ethyl 5-Hydroxycyclohex-3-enecarboxylate Diastereomers (6a/6b). *Reduction with a KRED Enzyme (Illustrated for the NADH-Dependent KRED-123 Enzyme).* A mixture of ketone **5** (16.8 mg, 0.1 mmol, 50 mM), KRED-123 (1 mg), dithiothreitol (0.5 mM), magnesium sulfate (2 mM), NAD^+ (1.3 mM), D-glucose (80 mM), and glucose dehydrogenase in 250 mM potassium phosphate buffer pH 7.0 (2 mL final volume) was shaken at 30 °C for 24 h. The reaction mixture was extracted with Et_2O, and the combined organics were dried over Na_2SO_4 and were evaporated to give alcohol **6a**/**6b** as a colorless oil and 1:1 mixture of *cis*/*trans* diastereomers (16 mg, 94%). The alcohols can be separated by column chromatography on silica gel (5 to 15% EtOAc in hexanes). **6a** (*cis*-diastereomer). ^1H NMR (500 MHz, $CDCl_3$): δ 5.76−5.71 (m, 2H), 4.27 (m, 1H), 4.13 (q, J = 7.0 Hz, 2H), 2.68 (ddt, J = 10.5, 7.0, 3.0, Hz, 1H), 2.27−2.21 (m, 4H), 1.71 (ddd, J = 13.0, 10.5, 8.0 Hz, 1H), 1.24 (t, J = 7.0 Hz, 3H). $^{13}C\{^1H\}$ NMR (100 MHz, $CDCl_3$): δ 175.3; 130.8, 126.9, 66.0, 60.7, 37.9, 34.2, 27.4, 14.1; $[\alpha]^{20}_D$ + 168.6° (*c* 0.2, $CHCl_3$). **6b** (*trans*-diastereomer). ^1H NMR (500 MHz, $CDCl_3$): δ 5.90−5.86 (m, 1H), 5.84−5.82 (m, 1H), 4.27 (m, 1H), 4.13 (q, J = 7.0 Hz, 2H), 2.76 (m, 1H), 2.34−2.18 (m, 3H), 2.09 (dt, J = 13.5, 3.0 Hz, 1H), 1.79 (ddd, J = 14.0, 12.5, 4.5 Hz, 1H), 1.24 (t, J = 7.0 Hz, 3H). $^{13}C\{^1H\}$ NMR (100 MHz, $CDCl_3$): δ 175.5, 130.9, 127.9, 62.9, 60.3, 34.5, 33.6, 27.3, 14.0. $[\alpha]^{20}_D$ + 11.0° (c 0.6, $CHCl_3$). HRMS (FAB, 3-NBA) *m*/*z*: $[M + Li]^+$ calcd for $C_9H_{14}O_3Li$ 177.1103, found 175.1106.

Scale-Up of Enzymatic Transformation. a. With KRED-123. Ketone **5** (1.00 g, 5.95 mmol, 100 mM) was treated for 24 h with KRED-123 (20 mg), NAD^+ (0.078 mmol, 1.3 mM), dithiothreitol (0.5 mM), magnesium sulfate (2 mM), glucose dehydrogenase (600 U), and D-glucose (9.6 mmol, 160 mM) in potassium phosphate buffer of pH 7.0 (250 mM, 60 mL final volume) with shaking at 30 °C for 24 h to give products **6a**/**6b** after workup as a 1:1 mixture of *cis*/*trans* diastereomers (990 mg, 98% yield). This product was used for the next step without further purification.

b. With KRED-117. Ketone **5** (1.00 g, 5.95 mmol, 100 mM), when treated for 36 h with KRED-117 (2 mg), NAD^+ (0.078 mmol, 1.3 mM), dithiothreitol (0.5 mM), magnesium sulfate (2 mM), glucose dehydrogenase (600 U), and D-glucose (9.6 mmol, 160 mM) in potassium phosphate buffer pH 7.0 (250 mM, 60 mL final volume) with shaking at 30 °C for 36 h gave **6a**/**6b** (678 mg, 88% yield brsm) as a 1:1 mixture of *cis*/*trans* diastereomers after workup, with 240 mg of ketone **5** being recovered. This product was used for the next step without further purification.

Enzyme Recycling. A mixture of ketone **5** (33.6 mg, 0.2 mmol, 100 mM), KRED-123 (1 mg), dithiothreitol (0.5 mM), magnesium sulfate (2 mM), NAD+ (1.3 mM), D-glucose (160 mM), and glucose dehydrogenase (20 U) in 250 mM potassium phosphate buffer of pH 7.0 (2 mL final volume) was shaken at 30 °C for 12 h. The reaction products were separated from the enzyme by filtration through a semipermeable membrane using a Centricon cell (centrifugation at 8000 rpm, 10 kDa-MW-cutoff membrane, Millipore). Filtration to a minimal volume was followed by the addition of water (500 μL) and a second centrifugation cycle. The combined filtrates were extracted with Et_2O, and the organic phase was dried (Na_2SO_4) and evaporated to give 31 mg (91%) of alcohols **6a**/**6b** (1:1 mixture of diastereomers). The solution of KRED-123 enzyme thereby recovered was resubmitted twice to the same reaction and workup conditions to give an additional 30 mg (88%, second run) and 32 mg (94%, third run) of alcohols **6a**/**6b**. The combined amount of isolated product was 93 mg (550 μmol). The enzyme molecular weight was estimated to be 36 kDa by SDS-PAGE (single band). This corresponds to ∼28 nmol of protein (1 mg) being employed here. This translates to a total turnover of approximately 20,000 substrate equivalents per enzyme active site over three cycles. Note: On a large scale (1 g) with a single cycle, a 98% yield was obtained (*vide supra*).

IV. Derivatization of Allylic Alcohol Product for HPLC Analysis (6aa and 6ba). To alcohol **6** (216 mg, 1.2 mmol) dissolved in THF (0.2 M) under nitrogen flow and at 0 °C was added NaH (52 mg, 2.16 mmol), followed by *p*-bromobenzoyl imidazole (356 mg, 1.44 mmol). The resultant reaction mixture was allowed to warm to rt and stirred for another 1 h. The reaction mixture was diluted with EtOAc washed with brine, and the aq. layer was back-extracted with EtOAc twice. The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated under the reduced pressure, then purified by flash column chromatography using EtOAc/hexane (1:9) as an eluent to give **6aa**/**6ba** (333 mg, 89%). Chiral HPLC conditions: column Chiralcel OD (250 mm length, 4.6 mm inner diameter, 5 μm, particle size), flow rate 0.8 mL/min, UV detection at 254 nm, 0.8% isopropyl alcohol/99.2% hexanes. Both *cis*- and *trans*-diastereomers were obtained with >99% ee: 15.06 min (*trans*, 5*R*), 21.91 min (*cis*, 5*R*). It was found that the mixture of diastereomers could also be separated by flash column chromatography using toluene/CH_2Cl_2 (9:1) as eluent. (**6aa**) *cis*-diastereomer, colorless oil. ^1H NMR (400 MHz, $CDCl_3$): δ 7.91 (d, J = 8.6 Hz, 2H), 7.58 (d, J = 8.6 Hz, 2H), 6.08 (ddd, J = 9.8, 5.1, 2.2 Hz, 1H), 5.94 (ddd, J = 9.9, 3.2, 1.7 Hz, 1H), 5.54 (broad, 1H), 4.19 (q, J = 7.1 Hz, 2H), 2.92−2.80 (m, 1H), 2.48 (dt, J = 18.2, 5.1 Hz, 1H), 2.37−2.18 (m, 2H), 1.97 (ddd, J = 14.3, 12.7, 4.3 Hz, 1H), 1.29 (t, J = 7.1 Hz, 3H). $^{13}C\{^1H\}$ NMR (100 MHz, $CDCl_3$): δ 174.09, 165.42, 131.67, 131.17, 129.71, 129.25, 128.09, 126.46, 70.05, 60.68, 37.83, 30.53, 27.18, 14.15. HRMS (TOF MS ESI$^+$) *m*/*z*: $[M + Na]^+$ calcd for $C_{12}H_{17}O_4NaBr$ 375.0208 (^{79}Br), 377.0187 (^{81}Br); found 375.0191(^{79}Br), 377.0197(^{81}Br). (**6ba**): ^1H NMR (400 MHz, $CDCl_3$): *trans*-diastereomer, colorless oil δ 7.91 (d, J = 8.6 Hz, 2H), 7.59 (d, J = 8.6 Hz, 2H), 5.95 (ddd, J = 9.3, 5.0, 2.5 Hz, 1H), 5.83−5.70 (m, 1H), 5.68−5.59 (m, 1H), 4.21−4.04 (m, 2H), 2.85−2.73 (m, 1H), 2.50 (ddd, J = 12.6, 5.8, 3.1 Hz, 1H), 2.42−2.30 (m, 2H), 1.94 (td, J = 12.2, 9.1 Hz, 1H), 1.24 (t, J = 7.1 Hz, 3H). $^{13}C\{^1H\}$NMR (100 MHz, $CDCl_3$) *cis*-diastereomer: δ 174.86, 165.16, 131.98, 131.66, 131.18, 129.40, 128.02, 124.20, 66.84, 60.67, 35.29, 30.68, 27.72, 14.23. HRMS (TOF MS ESI$^+$) *m*/*z*: $[M + Na]^+$ calcd for $C_{12}H_{17}O_4NaBr$ 375.0208 (^{79}Br), 377.0187 (^{81}Br); found 375.0195 (^{79}Br), 377.0201 (^{81}Br).

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V. Synthetic Procedure-Trost Intermediate via N-Mitsunobu Rxn. *(5S)-Ethyl 5-(1,3-dioxoisoindolin-2-yl)cyclohex-3-enecarboxylate Diastereomers (**9**).* To a 1:1 mixture of alcohols **6a**/**6b** (280 mg, 1.65 mmol), triphenylphosphine (694 mg, 2.65 mmol), and phthalimide (390 mg, 2.65 mmol) in THF (20 mL) at 0 °C was added, dropwise, DIAD (693 μL, 3.52 mmol). After 1 h at 0 °C and 1 h at rt, the solvent was evaporated, and the residue was purified by column chromatography on silica gel (10% EtOAc in hexanes) to give the title compound **9** (418 mg, 85%) as a white solid (1:1 mixture of *cis*/*trans* diastereomers). ^{1}H and ^{13}C{1H} NMR spectral data for the *cis*-diastereomer matched reported data.[16b] ^{1}H NMR (400 MHz, $CDCl_3$) *cis*-diastereomer: δ 7.80 (dd, *J* = 5.5, 3.0 Hz, 2H), 7.69 (dd, *J* = 5.5, 3.0 Hz, 2H), 5.93−5.89 (m, 1H), 5.56 (dm, *J* = 10.2 Hz, 1H), 5.00−4.94 (m, 1H), 4.12 (q, *J* = 7.0 Hz, 2H), 2.80−2.73 (m, 1H), 2.41−2.37 (m, 2H), 2.27 (q, *J* = 12.4 Hz, 2H), 2.20 2−15 (m, 1H), 1.18 (t, *J* = 7.5 Hz, 3H). ^{13}C {^{1}H}NMR (100 MHz, $CDCl_3$) *cis*-diastereomer: δ 174.1, 167.9, 133.9, 131.9, 128.0, 126.6, 123.2, 60.6, 47.4, 39.5, 29.1, 27.1, 14.2. *trans*-Diastereomer: δ 7.84−7.79 (m, 2H), 7.68−7.24 (m, 2H), 6.04−5.99 (m, 1H), 5.59 (dm, *J* = 10.0 Hz, 1H), 5.00−4.96 (m, 1H), 4.23−4.1 (m, 2H), 3.15−3.08 (m, 1H), 2.54−2.46 (m, 1H), 2.42−2.34 (m, 1H), 2.90 (ddd, *J* = 4.0, 6.0, 13.6 Hz, 1H), 2.17 (ddd, *J* = 13.6, 8.8, 6.4 Hz, 1H), 1.27 (t, *J* = 7.0 Hz, 3H). ^{13}C {^{1}H}NMR (100 MHz, $CDCl_3$) *trans*-diastereomer: δ 174.8, 168.2, 133.9, 131.9, 129.2, 124.5, 123.1, 60.6, 44.3, 36.7, 29.7, 26.4, 14.2. HRMS: (TOF MS ESI$^+$) *m*/*z* [M + Na]$^+$ calcd for $C_{17}H_{17}NO_4Na$ 322.1050; found 322.1042.

A reaction using pure *cis*-alcohol **6a** (purified by column chromatography) gave pure *trans*-product **9b** without epimerization: $[a]^{20}_D$ −218.4° (*c* = 0.35, $CHCl_3$).

*(S)-Ethyl 5-(1,3-Dioxoisoindolin-2-yl)cyclohexa-1,3-dienecarboxylate (**3**).* To a solution of ester **9** (200 mg, 0.67 mmol) in THF (6 mL) at −78 °C was added dropwise KHMDS (0.5 M in toluene, 2.0 mL, 1.0 mmol), and the mixture was stirred for 1 h. Solid diphenyl diselenide (251 mg, 0.8 mmol) was added at −78 °C, and the resulting solution was stirred for 3 h at the same temperature and then quenched by addition of NH_4Cl (aq, sat'd), followed by extraction with Et_2O. The organic phase was dried over Na_2SO_4 and evaporated, and the residue was purified by column chromatography on silica gel (15% EtOAc in hexanes) to give the phenyl selenide intermediate as a nearly 2:1 mixture of diastereomers (236 mg, 78%). This mixture of diastereomers (180 mg, 0.4 mmol) was then dissolved in THF (4 mL) at 0 °C, and H_2O_2 (95 μL of a 30% aqueous solution, 0.8 mmol) and pyridine (65 μL, 0.8 mmol) were added. The mixture was allowed to warm to rt over 3 h, and Et_2O was added. The organic phase was washed with $CuSO_4$ (aq., sat'd) and brine, dried over Na_2SO_4, and evaporated. The residue was purified by flash column chromatography on silica gel (15% EtOAc in hexanes) to give **3** (114 mg, 96%) as a white solid (10:1 mixture of regioisomers), and the overall yield of the two-step reaction was 85%. HRMS (TOF MS ESI$^+$) *m*/*z*: [M + Na]$^+$ calcd for $C_{17}H_{15}NO_4Na$ 320.0899; found 320.0899. ^{1}H and ^{13}C{^{1}H} NMR spectral data matched reported data,[16b] as well as the optical rotation, within experimental uncertainty: $[a]^{20}_D$ −167.5° (*c* = 2.0, $CHCl_3$), lit.: $[a]^{23}_D$ −168.2° (*c* = 2.8, $CHCl_3$). The correspondence of our optical rotation readings with that reported for this compound serves to establish the absolute stereochemistry of our compounds.

VI. Synthetic Procedure: Corey Intermediate via Imidate RR. *Ethyl (5R)-5-(2,2,2-Trichloro-1-iminoethoxy)cyclohex-3-ene-1-carboxylate (**10**).* To a solution of alcohol **6a**/**6b** (570 mg, 3.35 mmol) and DBU (601 μL, 4.02 mmol) in CH_2Cl_2 at −10 °C was added Cl_3CCN (438 μL, 4.36 mmol), and the mixture was allowed to warm to rt over 2 h. Then $NaHCO_3$ (aq., sat'd) solution was added; the product was extracted with Et_2O and CH_2Cl_2; and the organic fractions were dried (Na_2SO_4) and evaporated. The crude product was quickly purified by flash column chromatography on silica gel (30% EtOAc-hexanes) to give **10** (1.02 g, 97%) as a 1:1 mixture of *cis*/*trans* diastereomers. ^{1}H NMR (400 MHz, $CDCl_3$) *cis*-diastereomer: δ 8.31 (s, 1H), 5.94−5.89 (m, 1H), 5.79 (dt, *J* = 10.4, 2.0 Hz, 1H), 5.58−5.52 (m, 1H), 4.14 (q, *J* = 6.8 Hz, 2H), 2.77−2.68 (m, 1H), 2.56 (ddd, *J* = 12.4, 5.6, 2.4 Hz, 1H), 2.35−2.30 (m, 2H), 1.84 (dt, *J* = 4.4, 7.6 Hz, 1H), 1.25 (t, *J* = 7.2 Hz, 3H). *trans*-Diastereomer: δ 8.27 (s, 1H), 6.05−6.10 (m, 1H), 6.0−5.95 (m, 1H), 5.43−5.40 (m, 1H), 4.14 (q, *J* = 6.8 Hz, 2H), 2.87−2.79 (m, 1H), 2.45 (dt, *J* = 18.4, 4.8 Hz, 1H), 2.35−2.30 (m, 1H), 2.25−2.16 (m, 1H), 1.91−1.84 (m, 1H), 1.25 (t, *J* = 7.2 Hz, 3H). ^{13}C {^{1}H}NMR (100 MHz, $CDCl_3$) *cis*-diastereomer: δ 174.0, 162.2, 129.6, 126.0, 91.6, 74.3, 60.7, 38.0, 29.8, 27.4, 14.2. *trans*-Diastereomer: δ 175.0, 161.8, 132.5, 123.4, 91.6, 70.7, 60.6, 35.1, 29.8, 27.8, 15.6. HRMS (FAB, 3-NBA) *m*/*z*: [M + Na]$^+$ calcd for $C_{11}H_{14}NO_3Cl_3Na$ 335.9937, found 335.9931.

*Ethyl (5S)-5-(2,2,2-Trichloroacetamido)cyclohex-3-ene-1-carboxylate (**11**).* A solution of **10** (600 mg, 1.91 mmol) in 60 mL of xylene was heated using a silicon oil bath at reflux for 1 day. The solvent was evaporated, and the residue was purified by flash column chromatography on silica gel (10 to 20% EtOAc in hexanes) to give **11** (549 mg, 92%) as a white solid and 1:1 mixture of *cis*/*trans* diastereomers. The mixtures of diastereomers were separated by flash column chromatography using EtOAc:DCM (1:9) as an eluent. ^{1}H NMR (400 MHz, $CDCl_3$) *cis*-diastereomer: δ 7.29 (br. d, *J* = 6.8 Hz, 1H), 5.91−5.86 (m, 1H), 5.62 (ddd, *J* = 10.0, 4.4, 2.4 Hz, 1H), 4.59−4.52 (m, 1H), 4.14 (q, *J* = 7.2 Hz, 2H), 2.77 (dddd, *J* = 12.8, 10.0, 6.8, 3.6 Hz, 1H), 2.36−2.30 (m, 3H), 1.76 (ddd, *J* = 13.6, 9.2, 7.6 Hz, 1H), 1.24 (t, *J* = 7.2 Hz, 3H). *trans*-Diastereomer: δ 6.57 (br. d, *J* = 6.4 Hz, 1H), 6.03−5.99 (m, 1H), 5.73−5.70 (m, 1H), 4.51−4.45 (m, 1H), 4.14 (q, *J* = 7.2 Hz, 2H), 2.60−2.52 (m, 1H), 2.35−2.25 (m, 2H), 1.24 (t, *J* = 7.2 Hz, 3H), 2.13 (apt. dt, *J* = 3.2, 14.0 Hz, 1H), 1.94 (ddd, *J* = 13.6, 12.0, 4.8 Hz, 1H). ^{13}C {^{1}H} NMR (100 MHz, $CDCl_3$) *cis*-diastereomer: δ 175.1, 161.4, 129.0, 126.3, 92.7, 61.0, 46.4, 37.3, 30.3, 27.0, 14.2. *trans*-Diastereomer: δ 174.5, 161.0, 131.6, 124.4, 92.7, 60.8, 45.3, 35.5, 30.3, 27.3, 14.2. HRMS (TOF MS ESI$^+$) *m*/*z*: [M + Na]$^+$ calcd for $C_{11}H_{14}NO_3Na$ 335.9937 (^{35}Cl), 337.9925 (^{37}Cl); found 335.9935, 337.9927.

*Ethyl (5S)-5-((tert-Butoxycarbonyl)amino)cyclohex-3-ene-1-carboxylate (**12**).* A solution of **9** (380 mg, 1.27 mmol) and methylhydrazine (335 μL, 6.35 mmol) in ethanol (25 mL) was heated using a silicon oil bath to reflux overnight. The solvent was then removed under vacuum, and the residue was dissolved in dichloromethane (20 mL). Diisopropylethylamine (442 μL, 2.54 mmol) and Boc_2O (416 mg, 1.91 mmol) were added, and the resulting mixture was stirred 5 h at rt. The solvent was then evaporated, and the crude product was purified by flash column chromatography on silica gel (20% EtOAc in hexanes) to give the title compound **12** (340 mg, 99%) as a 1:1 mixture of *cis*/*trans* diastereomers. ^{1}H NMR (400 MHz, $CDCl_3$) *cis*-diastereomer: δ 5.77−5.72 (m, 1H), 5.54 (br. d, *J* = 10.0 Hz, 1H), 4.57 (br. d, *J* = 7.2 Hz, 1H), 4.24 (br. s, 1H), 4.09 (q, *J* = 7.2 Hz, 2H), 2.69−2.62 (m, 1H), 2.33−2.13 (m, 3H), 1.44 (m, 1H), 1.42 (s, 9H), 4.57 (br. d, *J* = 7.2 Hz, 1H), 1.23 (t, *J* = 7.2 Hz, 3H). *trans*-diastereomer: δ 5.86−5.82 (m, 1H), 5.66 (br. d, *J* = 8.8 Hz, 1H), 4.21 (br. s, 1H), 4.13 (q, *J* = 7.2 Hz, 2H), 2.51 (apt. t, *J* = 12.0 Hz, 1H), 2.30−2.11 (m, 2H), 2.06 (br. d, *J* = 13.2 Hz, 1H), 1.79 (dt, *J* = 12.8, 4.8 Hz, 1H), 1.41 (s, 9H), 1.23 (t, *J* = 7.2 Hz, 3H). ^{13}C {^{1}H}NMR (100 MHz, $CDCl_3$) *cis*- and *trans*-diastereomers: δ 175.2, 174.8, 155.3, 154.8, 129.2, 128.9, 127.6, 126.3, 79.4, 79.3, 60.5, 46.8, 44.0, 38.5, 35.4, 32.4, 31.8, 28.4, 28.2, 27.3, 27.2, 14.2, 14.1. HRMS (FAB, 3-NBA) *m*/*z*: [M + H]$^+$ calcd for $C_{14}H_{24}NO_4$ 270.1705, found 270.1696.

To a solution of **11** (108 mg, 0.34 mmol) in ethanol at 0 °C was added sodium borohydride (51 mg, 1.36 mmol), and the reaction mixture was slowly allowed to warm to rt. Excess acetone was added, and the solvents were evaporated. The residue was dissolved in 1 mL of water and Boc_2O (148 mg, 0.68 mmol) was added. After 1 h, the product was extracted with EtOAc. The organic phase was dried over Na_2SO_4 and evaporated, and the residue was purified by column chromatography on silica gel (15% EtOAc in hexanes) to give **12** (61 mg, 67%).

*Ethyl (S)-5-((tert-Butoxycarbonyl)amino)cyclohexa-1,3-diene-1-carboxylate (**2**).* To a solution of ester **12** (236 mg, 0.88 mmol) in 10 mL of THF at −78 °C was added, dropwise, KHMDS (0.5 M in toluene, 4.5 mL, 2.25 mmol), and the mixture was stirred for 1 h. Solid diphenyl diselenide (309 mg, 0.99 mmol) was added at −78 °C, and the resulting solution was stirred for 3 h at the same temperature and then quenched by addition of NH_4Cl (aq., sat'd), followed by

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extraction with Et_2O. The organic phase was dried over Na_2SO_4 and evaporated, and the residue was purified by column chromatography on silica gel (15% EtOAc in hexanes) to give the phenyl selenide intermediate as a nearly 2:1 mixture of diastereomers (271 mg, 73%). This mixture of diastereomers (80 mg, 0.19 mmol) was then dissolved in THF (4 mL) at 0 °C, and H_2O_2 (46 μL of a 30% aqueous solution, 0.38 mmol) and pyridine (31 μL mg, 0.38 mmol) were added. The mixture was allowed to warm to rt over 3 h, and Et_2O was added. The organic phase was washed with $CuSO_4$ (aq., saturated) and brine, dried over Na_2SO_4, and evaporated. The residue contained a 2:1 mixture of regioisomers which was easily purified by flash column chromatography on silica gel (10% EtOAc in hexanes) to give the title compound **2** (31 mg, 62%) as a white solid. Chiral HPLC conditions: column Chiralcel OD (250 mm length, 4.6 mm inner diameter, 5 μm particle size), flow rate 1 mL/min, UV detection at 254 nm, 2% isopropyl alcohol/hexanes, 10.02 min (major, *S*), 11.43 (minor, *R*), 98% ee. Racemic **2** was obtained following the same procedure from racemic **6** and used as a chiral HPLC standard (see below for the HPLC chromatograms). HRMS (FAB, 3-NBA) *m/z*: $[M + Li]^+$ calcd for $C_{14}H_{21}NO_4Li$ 274.1631; found 274.1634. ^1H and ^{13}C {^1H} NMR spectral data matched reported data.[14] The optical rotation matched reported values: $[\alpha]^{20}_D$ −217.6° (*c* = 1.0, $CHCl_3$), lit. $[\alpha]^{25}_D$ −141.2° (*c* = 1.0, $CHCl_3$),[14] $[\alpha]^{20}_D$ −217° (*c* = 1.1, $CHCl_3$),[31] $[\alpha]^{20}_D$ −219.7° (*c* = 0.2, $CHCl_3$).[20h]

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.joc.1c00326.

Procedural details, KRED screening, NMR spectra, HPLC traces, and X-ray crystal structure details (PDF)

Accession Codes

CCDC 2038401−2038402 contain the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/data_request/cif, or by emailing data_request@ccdc.cam.ac.uk, or by contacting The Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033.

■ AUTHOR INFORMATION

Corresponding Authors

Sylvain Broussy − *University of Paris, CiTCoM, 8038 CNRS, U 1268 INSERM, F-75006 Paris, France;* orcid.org/0000-0003-3098-5317; Email: sylvain.broussy@u-paris.fr

David B. Berkowitz − *Department of Chemistry, University of Nebraska, Lincoln, Nebraska 68588, United States;* orcid.org/0000-0001-7550-0112; Email: dberkowitz1@unl.edu

Authors

Virendra K. Tiwari − *Department of Chemistry, University of Nebraska, Lincoln, Nebraska 68588, United States*

Douglas R. Powell − *Department of Chemistry and Biochemistry, University of Oklahoma, Norman, Oklahoma 73019, United States*

Complete contact information is available at: https://pubs.acs.org/10.1021/acs.joc.1c00326

Notes

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■ REFERENCES

(1) Eastman, R. T.; Roth, J. S.; Brimacombe, K. R.; Simeonov, A.; Shen, M.; Patnaik, S.; Hall, M. D. Remdesivir: A review of its discovery and development leading to emergency use authorization for treatment of COVID-19. *ACS Cent. Sci.* **2020**, *6*, 672−683.

(2) MacDonald, L.; Otto, T. D.; Elmesmari, A.; Tolusso, B.; Somma, D.; McSharry, C.; Gremese, E.; McInnes, I. B.; Alivernini, S.; Kurowska-Stolarska, M. COVID-19 and rheumatoid arthritis share myeloid pathogenic and resolving pathways. *bioRxiv* **2020**, 1−29.

(3) Ye, J.; Yang, X.; Xu, M.; Chan, P. K.-s.; Ma, C. Novel N-substituted oseltamivir derivatives as potent influenza neuraminidase inhibitors: Design, synthesis, biological evaluation, ADME prediction and molecular docking studies. *Eur. J. Med. Chem.* **2019**, *182*, 111635.

(4) Li, H.; Shen, S.-J.; Zhu, C.-L.; Xu, H. Enantioselective synthesis of oseltamivir phosphate (Tamiflu) via the iron-catalyzed stereoselective olefin diazidation. *J. Am. Chem. Soc.* **2018**, *140*, 10619−10626.

(5) Zutter, U.; Iding, H.; Spurr, P.; Wirz, B. New, efficient synthesis of oseltamivir phosphate (Tamiflu) via enzymatic desymmetrization of a meso-1,3-cyclohexanedicarboxylic acid diester. *J. Org. Chem.* **2008**, *73*, 4895−4902.

(6) (a) Werner, L.; Machara, A.; Hudlicky, T. Short chemoenzymic azide-free synthesis of oseltamivir (Tamiflu): Approaching the potential for process efficiency. *Adv. Synth. Catal.* **2010**, *352*, 195−200. (b) Hudlicky, T. Recent chemoenzymatic total syntheses of natural and unnatural products: Codeine, balanol, pancratistatin, and oseltamivir. *Pure Appl. Chem.* **2010**, *82*, 1785−1796. (c) Banwell, M. G.; Lehmann, A. L.; Menon, R. S.; Willis, A. C. New methods for the synthesis of certain alkaloids and terpenoids. *Pure Appl. Chem.* **2011**, *83*, 411−423. (d) Matveenko, M.; Willis, A. C.; Banwell, M. G. A chemoenzymic synthesis of the anti-influenza agent Tamiflu. *Tetrahedron Lett.* **2008**, *49*, 7018−7020.

(7) (a) Slagman, S.; Fessner, W.-D. Biocatalytic routes to anti-viral agents and their synthetic intermediates. *Chem. Soc. Rev.* **2021**, *50*, 1968. (b) Benkovics, T.; McIntosh, J. A.; Silverman, S. M.; Kong, J.; Maligres, P.; Itoh, T.; Yang, H.; Huffman, M. A.; Verma, D.; Pan, W.; Ho, H.-I.; Vroom, J.; Knight, A.; Hurtak, J.; Morris, W.; Strotman, N. A.; Murphy, G.; Maloney, K. M.; Fier, P. S. Evolving to an ideal synthesis of molnupiravir, an investigational treatment for COVID-19. *ChemRxiv* **2020**.

(8) Varghese, J. N.; McKimm-Breschkin, J. L.; Caldwell, J. B.; Kortt, A. A.; Colman, P. M. The structure of the complex between influenza virus neuraminidase and sialic acid, the viral receptor. *Proteins: Struct., Funct., Genet.* **1992**, *14*, 327−332.

(9) Gloster, T. M.; Davies, G. J. Glycosidase inhibition: Assessing mimicry of the transition state. *Org. Biomol. Chem.* **2010**, *8*, 305−320.

(10) (a) Ward, P.; Small, I.; Smith, J.; Suter, P.; Dutkowski, R. Oseltamivir (Tamiflu) and its potential for use in the event of an influenza pandemic. *J. Antimicrob. Chemother.* **2005**, *55*, i5-i21. (b) Mendel, D. B.; Tai, C. Y.; Escarpe, P. A.; Li, W.; Sidwell, R. W.; Huffman, J. H.; Sweet, C.; Jakeman, K. J.; Merson, J.; Lacy, S. A.; Lew, W.; Williams, M. A.; Zhang, L.; Chen, M. S.; Bischofberger, N.; Kim, C. U. Oral administration of a prodrug of the influenza virus neuraminidase inhibitor GS-4071 protects mice and ferrets against influenza infection. *Antimicrob. Agents Chemother.* **1998**, *42*, 640−646.

(11) McMahon, A.; Martin-Loeches, I. The pharmacological management of severe influenza infection − 'existing and emerging therapies'. *Expert Rev. Clin. Pharmacol.* **2017**, *10*, 81−95.

(12) Gaisina, I. N.; Peet, N. P.; Cheng, H.; Li, P.; Du, R.; Cui, Q.; Furlong, K.; Manicassamy, B.; Caffrey, M.; Thatcher, G. R. J.; Rong, L. Optimization of 4-aminopiperidines as inhibitors of influenza a viral

entry that are synergistic with oseltamivir. *J. Med. Chem.* **2020**, *63*, 3120−3130.
(13) Rohloff, J. C.; Kent, K. M.; Postich, M. J.; Becker, M. W.; Chapman, H. H.; Kelly, D. E.; Lew, W.; Louie, M. S.; McGee, L. R.; Prisbe, E. J.; Schultze, L. M.; Yu, R. H.; Zhang, L. Practical total synthesis of the anti-influenza drug GS-4104. *J. Org. Chem.* **1998**, *63*, 4545−4550.
(14) Yeung, Y.-Y.; Hong, S.; Corey, E. J. A short enantioselective pathway for the synthesis of the anti-influenza neuramidase inhibitor oseltamivir from 1,3-butadiene and acrylic acid. *J. Am. Chem. Soc.* **2006**, *128*, 6310−6311.
(15) Kipassa, N. T.; Okamura, H.; Kina, K.; Hamada, T.; Iwagawa, T. Efficient short step synthesis of Corey's Tamiflu intermediate. *Org. Lett.* **2008**, *10*, 815−816.
(16) (a) Trost, B. M.; Zhang, T. Development of a concise synthesis of (−)-oseltamivir (Tamiflu). *Chem. - Eur. J.* **2011**, *17*, 3630. (b) Trost, B. M.; Zhang, T. A concise synthesis of (−)-oseltamivir. *Angew. Chem., Int. Ed.* **2008**, *47*, 3759−3761.
(17) Bromfield, K. M.; Graden, H.; Hagberg, D. P.; Olsson, T.; Kann, N. An iron carbonyl approach to the influenza neuraminidase inhibitor oseltamivir. *Chem. Commun.* **2007**, 3183−3185.
(18) (a) Swyka, R. A.; Berkowitz, D. B. The in situ enzymatic screening (ISES) approach to reaction discovery and catalyst identification. *Curr. Protoc. Chem. Biol.* **2017**, *9*, 285−305. (b) Malik, G.; Swyka, R. A.; Tiwari, V. K.; Fei, X.; Applegate, G. A.; Berkowitz, D. B. A thiocyanopalladation/carbocyclization transformation identified through enzymatic screening: Stereocontrolled tandem C-SCN and C-C bond formation. *Chem. Sci.* **2017**, *8*, 8050−8060. (c) Karukurichi, K. R.; Fei, X.; Swyka, R. A.; Broussy, S.; Shen, W.; Dey, S.; Roy, S. K.; Berkowitz, D. B. Mini-ISES identifies promising carbafructopyranosebased salens for asymmetric catalysis: Tuning ligand shape via the anomeric effect. *Sci. Adv.* **2015**, *1*, e1500066. (d) Ginotra, S. K.; Friest, J. A.; Berkowitz, D. B. Halocarbocyclization entry into the oxabicyclo[4.3.1]decyl exo-methylene-delta-lactone cores of linearifolin and zaluzanin A: Exploiting combinatorial catalysis. *Org. Lett.* **2012**, *14*, 968−971. (e) Friest, J. A.; Broussy, S.; Chung, W. J.; Berkowitz, D. B. Combinatorial catalysis employing a visible enzymatic beacon in real time: Synthetically versatile (pseudo)halometalation/carbocyclizations. *Angew. Chem., Int. Ed.* **2011**, *50*, 8895−8899. (f) Dey, S.; Powell, D. R.; Hu, C.; Berkowitz, D. B. Cassette in situ enzymatic screening identifies complementary chiral scaffolds for hydrolytic kinetic resolution across a range of epoxides. *Angew. Chem., Int. Ed.* **2007**, *46*, 7010−7014. (g) Dey, S.; Karukurichi, K. R.; Shen, W.; Berkowitz, D. B. Double-cuvette ISES: In situ estimation of enantioselectivity and relative rate for catalyst screening. *J. Am. Chem. Soc.* **2005**, *127*, 8610−8611. (h) Berkowitz, D. B.; Bose, M.; Choi, S. In situ enzymatic screening (ISES): A tool for catalyst discovery and reaction development. *Angew. Chem., Int. Ed.* **2002**, *41*, 1603−1607.
(19) (a) Kudalkar, G. P.; Tiwari, V. K.; Lee, J. D.; Berkowitz, D. B. A Hammett study of *Clostridium acetobutylicum* alcohol dehydrogenase (CaADH): An enzyme with remarkable substrate promiscuity and utility for organic synthesis. *Synlett* **2020**, *31*, 237−247. (b) Panigrahi, K.; Applegate, G. A.; Malik, G.; Berkowitz, D. B. Combining a *Clostridial* enzyme exhibiting unusual active site plasticity with a remarkably facile sigmatropic rearrangement: Rapid, stereocontrolled entry into densely functionalized fluorinated phosphonates for chemical biology. *J. Am. Chem. Soc.* **2015**, *137*, 3600−3609. (c) Applegate, G. A.; Cheloha, R. W.; Nelson, D. L.; Berkowitz, D. B. A new dehydrogenase from clostridium acetobutylicum for asymmetric synthesis: Dynamic reductive kinetic resolution entry into the Taxotère side chain. *Chem. Commun.* **2011**, *47*, 2420−2422. (d) Friest, J. A.; Maezato, Y.; Broussy, S.; Blum, P.; Berkowitz, D. B. Use of a robust dehydrogenase from an archael hyperthermophile in asymmetric catalysis-dynamic reductive kinetic resolution entry into (*S*)-profens. *J. Am. Chem. Soc.* **2010**, *132*, 5930−5931. (e) Broussy, S.; Cheloha, R. W.; Berkowitz, D. B. Enantioselective, ketoreductase-based entry into pharmaceutical building blocks: Ethanol as tunable nicotinamide reductant. *Org. Lett.* **2009**, *11*, 305−308. (f) Berkowitz, D. B.; Choi, S.; Maeng, J.-H. Enzyme-assisted asymmetric total synthesis of (−)-podophyllotoxin and (−)-picropodophyllin. *J. Org. Chem.* **2000**, *65*, 847−860. (g) Berkowitz, D. B.; Pumphrey, J. A.; Shen, Q. Enantiomerically enriched alpha-vinyl amino acids via lipase-mediated reverse transesterification. *Tetrahedron Lett.* **1994**, *35*, 8743−8746.
(20) (a) Chavan, S. P.; Kadam, A. L.; Shinde, S. S.; Gonnade, R. G. Furan-derived chiral bicycloaziridino lactone synthon: Collective syntheses of oseltamivir phosphate (Tamiflu), (*S*)-pipecolic acid and its 3-hydroxy derivatives. *Chem. - Asian J.* **2020**, *15*, 415−424. (b) Hayashi, Y.; Ogasawara, S. Time economical total synthesis of (−)-oseltamivir. *Org. Lett.* **2016**, *18*, 3426−3429. (c) Li, N.-G.; Shi, Z.-H.; Tang, Y.-P.; Shi, Q.-P.; Zhang, W.; Zhang, P.-X.; Dong, Z.-X.; Li, W.; Duan, J.-A. Recent progress on the total synthesis of (−)-oseltamivir phosphate (Tamiflu) for the treatment of influenza disease. *Curr. Org. Chem.* **2014**, *18*, 2125−2138. (d) Mukaiyama, T.; Ishikawa, H.; Koshino, H.; Hayashi, Y. One-pot synthesis of (−)-oseltamivir and mechanistic insights into the organocatalyzed Michael reaction. *Chem. - Eur. J.* **2013**, *19*, 17789−17800. (e) Alagiri, K.; Furutachi, M.; Yamatsugu, K.; Kumagai, N.; Watanabe, T.; Shibasaki, M. Two approaches toward the formal total synthesis of oseltamivir phosphate (Tamiflu): Catalytic enantioselective three-component reaction strategy and L-glutamic acid strategy. *J. Org. Chem.* **2013**, *78*, 4019−4026. (f) Zhu, S.; Yu, S.; Wang, Y.; Ma, D. Organocatalytic Michael addition of aldehydes to protected 2-amino-1-nitroethenes: The practical syntheses of oseltamivir (Tamiflu) and substituted 3-aminopyrrolidines. *Angew. Chem., Int. Ed.* **2010**, *49*, 4656−4960. (g) Osato, H.; Jones, I. L.; Chen, A.; Chai, C. L. L. Efficient formal synthesis of oseltamivir phosphate (Tamiflu) with inexpensive D-ribose as the starting material. *Org. Lett.* **2010**, *12*, 60−63. (h) Magano, J. Synthetic approaches to the neuraminidase inhibitors zanamivir (Relenza) and oseltamivir phosphate (Tamiflu) for the treatment of influenza. *Chem. Rev.* **2009**, *109*, 4398−4438. (i) Shie, J.-J.; Fang, J.-m.; Wong, C.-H. A concise and flexible synthesis of the potent anti-influenza agents Tamiflu and Tamiphosphor. *Angew. Chem., Int. Ed.* **2008**, *47*, 5788−5791.
(21) Matveenko, M.; Willis, A. C.; Banwell, M. G. A chemoenzymic synthesis of the anti-influenza agent Tamiflu. *Tetrahedron Lett.* **2009**, *50*, 2982.
(22) Werner, L.; Machara, A.; Sullivan, B.; Carrera, I.; Moser, M.; Adams, D. R.; Hudlicky, T.; Andraos, J. Several generations of chemoenzymatic synthesis of oseltamivir (Tamiflu): Evolution of strategy, quest for a process-quality synthesis, and evaluation of efficiency metrics. *J. Org. Chem.* **2011**, *76*, 10050−10067.
(23) (a) Biffin, M. E. C.; Moritz, A. G.; Paul, D. B. A re-examination of the sodium and liquid ammonia reduction of *m*-methoxybenzoic acid. *Aust. J. Chem.* **1972**, *25*, 1329−1334. (b) Webster, F. X.; Silverstein, R. M. Control of the birch reduction of *m*-anisic acid to produce specific 3-oxocyclohexenecarboxylic acids. *Synthesis* **1987**, *1987*, 922−924.
(24) Peters, B. K.; Rodriguez, K. X.; Reisberg, S. H.; Beil, S. B.; Hickey, D. P.; Kawamata, Y.; Collins, M.; Starr, J.; Chen, L.; Udyavara, S.; Klunder, K.; Gorey, T. J.; Anderson, S. L.; Neurock, M.; Minteer, S. D.; Baran, P. S. Scalable and safe synthetic organic electroreduction inspired by Li-ion battery chemistry. *Science* **2019**, *363*, 838−845.
(25) (a) Bornscheuer, U. T.; Huisman, G. W.; Kazlauskas, R. J.; Lutz, S.; Moore, J. C.; Robins, K. Engineering the third wave of biocatalysis. *Nature* **2012**, *485*, 185−194. (b) Moore, J. C.; Pollard, D. J.; Kosjek, B.; Devine, P. N. Advances in the enzymatic reduction of ketones. *Acc. Chem. Res.* **2007**, *40*, 1412−1419.
(26) Kosjek, B.; Tellers, D. M.; Biba, M.; Farr, R.; Moore, J. C. Biocatalytic and chemocatalytic approaches to the highly stereoselective 1,2-reduction of an α,β-unsaturated ketone. *Tetrahedron: Asymmetry* **2006**, *17*, 2798−2803.
(27) Shull, B. K.; Sakai, T.; Nichols, J. B.; Koreeda, M. Mitsunobu reaction of unbiased cyclic allylic alcohols. *J. Org. Chem.* **1997**, *62*, 8294−8303.

https://doi.org/10.1021/acs.joc.1c00326
J. Org. Chem. 2021, 86, 6494−6503

(28) Kwon, Y.-U.; Chung, S.-K. Facile synthetic routes to all possible enantiomeric pairs of conduritol stereoisomers via efficient enzymatic resolution of conduritol B and C derivatives. *Org. Lett.* **2001**, *3*, 3013−3016.
(29) O'Brien, P.; Rosser, C. M.; Caine, D. On the α-lithiation-rearrangement of N-toluensulfonyl aziridines: Mechanistic and synthetic aspects. *Tetrahedron* **2003**, *59*, 9779−9791.
(30) O'Brien, P.; Pilgram, C. D. Chiral lithium amide base-mediated rearrangement of *meso*-cyclohexene oxides: Asymmetric synthesis of amino- and aziridinocyclohexenols. *Org. Biomol. Chem.* **2003**, *1*, 523−534.
(31) Bromfield, K. M.; Gradén, H.; Hagberg, D. P.; Olsson, T.; Kann, N. An iron carbonyl approach to the influenza neuraminidase inhibitor oseltamivir. *Chem. Commun.* **2007**, 3183−3185.
(32) Overman, L. E. A general method for the synthesis of amines by the rearrangement of allylic trichloroacetimidates. 1,3-transposition of alcohol and amine functions. *J. Am. Chem. Soc.* **1976**, *98*, 2901−2910.
(33) Fernandes, R. A.; Kattanguru, P.; Gholap, S. P.; Chaudhari, D. A. Recent advances in the Overman rearrangement: Synthesis of natural products and valuable compounds. *Org. Biomol. Chem.* **2017**, *15*, 2672−2710.
(34) (a) Nishikawa, T.; Asai, M.; Ohyabu, N.; Isobe, M. Improved conditions for facile Overman rearrangement. *J. Org. Chem.* **1998**, *63*, 188−192. (b) Xu, C.; Liu, Z.; Wang, H.; Zhang, B.; Xiang, Z.; Hao, X.; Wang, D. Z. Rapid construction of [5−6-7] tricyclic ring skeleton of calyciphylline alkaloid daphnilongeranin B. *Org. Lett.* **2011**, *13*, 1812−1815.
(35) (a) Montero, A.; Mann, E.; Herradon, B. The Overman rearrangement in carbohydrate chemistry: Stereoselective synthesis of functionalized 3-amino-3,6-dihydro-2*H*-pyrans and incorporation in peptide derivatives. *Tetrahedron Lett.* **2005**, *46*, 401−405. (b) Ansari, A. A.; Reddy, Y. S.; Vankar, Y. D. Efficient carbon-Ferrier rearrangement on glycals mediated by ceric ammonium nitrate: Application to the synthesis of 2-deoxy-2-amino-C-glycoside. *Beilstein J. Org. Chem.* **2014**, *10*, 300−306. (c) Amann, F.; Frank, M.; Rhodes, R.; Robinson, A.; Kesselgruber, M.; Abele, S. Thermal Overman rearrangement of a glucal derivative in a tube reactor on pilot plant scale. *Org. Process Res. Dev.* **2016**, *20*, 446−451.
(36) Celebi-Ölçüm, N.; Aviyente, V.; Houk, K. N. Mechanism and selectivity of cinchona alkaloid catalyzed [1,3]-shifts of allylic trichloroacetimidates. *J. Org. Chem.* **2009**, *74*, 6944−6952.
(37) (a) Ruck, R. T.; Chen, Q.; Rivera, N.; Kong, J.; Mangion, I. K.; Tan, L.; Fleitz, F. J. Bio- and chemocatalysis for the synthesis of late stage sar-enabling intermediates for romk inhibitors and MK-7145 for the treatment of hypertension and heart failure. *Org. Process Res. Dev.* **2021**, *25*, 405. (b) Li, Z.; Wang, Z.; Wang, Y.; Wu, X.; Lu, H.; Huang, Z.; Chen, F. Substituent position-controlled stereoselectivity in enzymatic reduction of diaryl- and aryl(heteroaryl)methanones. *Adv. Synth. Catal.* **2019**, *361*, 1859−1865. (c) Xu, F.; Kosjek, B.; Cabirol, F. L.; Chen, H.; Desmond, R.; Park, J.; Gohel, A. P.; Collier, S. J.; Smith, D. J.; Liu, Z.; Janey, J. M.; Chung, J. Y. L.; Alvizo, O. Synthesis of vibegron enabled by a ketoreductase rationally designed for high pH dynamic kinetic reduction. *Angew. Chem., Int. Ed.* **2018**, *57*, 6863−6867. (d) Lauder, K.; Toscani, A.; Qi, Y.; Lim, J.; Charnock, S. J.; Korah, K.; Castagnolo, D. Photo-biocatalytic one-pot cascades for the enantioselective synthesis of 1,3-mercaptoalkanol volatile sulfur compounds. *Angew. Chem., Int. Ed.* **2018**, *57*, 5803−5807. (e) Biegasiewicz, K. F.; Cooper, S. J.; Emmanuel, M. A.; Miller, D. C.; Hyster, T. K. Catalytic promiscuity enabled by photoredox catalysis in nicotinamide-dependent oxidoreductases. *Nat. Chem.* **2018**, *10*, 770−775. (f) Staniland, S.; Adams, R. W.; McDouall, J. J. W.; Maffucci, I.; Contini, A.; Grainger, D. M.; Turner, N. J.; Clayden, J. Biocatalytic dynamic kinetic resolution for the synthesis of atropisomeric biaryl N-oxide Lewis base catalysts. *Angew. Chem., Int. Ed.* **2016**, *55*, 10755−10759. (g) Emmanuel, M. A.; Greenberg, N. R.; Oblinsky, D. G.; Hyster, T. K. Accessing non-natural reactivity by irradiating nicotinamide-dependent enzymes with light. *Nature* **2016**, *540*, 414−417. (h) Noey, E. L.; Tibrewal, N.; Jimenez-Oses, G.; Osuna, S.; Park, J.; Bond, C. M.; Cascio, D.; Liang, J.; Zhang, X.; Huisman, G. W.; Tang, Y.; Houk, K. N. Origins of stereoselectivity in evolved ketoreductases. *Proc. Natl. Acad. Sci. U. S. A.* **2015**, *112*, E7065−E7072.
(38) (a) Huffman, M. A.; Fryszkowska, A.; Alvizo, O.; Borra-Garske, M.; Campos, K. R.; Canada, K. A.; Devine, P. N.; Duan, D.; Forstater, J. H.; Grosser, S. T.; Halsey, H. M.; Hughes, G. J.; Jo, J.; Joyce, L. A.; Kolev, J. N.; Liang, J.; Maloney, K. M.; Mann, B. F.; Marshall, N. M.; McLaughlin, M.; Moore, J. C.; Murphy, G. S.; Nawrat, C. C.; Nazor, J.; Novick, S.; Patel, N. R.; Rodriguez-Granillo, A.; Robaire, S. A.; Sherer, E. C.; Truppo, M. D.; Whittaker, A. M.; Verma, D.; Xiao, L.; Xu, Y.; Yang, H. Design of an in vitro biocatalytic cascade for the manufacture of islatravir. *Science* **2019**, *366*, 1255−1259. (b) Turner, N. J.; O'Reilly, E. Biocatalytic retrosynthesis. *Nat. Chem. Biol.* **2013**, *9*, 285−288. (c) Savile, C. K.; Janey, J. M.; Mundorff, E. C.; Moore, J. C.; Tam, S.; Jarvis, W. R.; Colbeck, J. C.; Krebber, A.; Fleitz, F. J.; Brands, J.; Devine, P. N.; Huisman, G. W.; Hughes, G. J. Biocatalytic asymmetric synthesis of chiral amines from ketones applied to sitagliptin manufacture. *Science* **2010**, *329*, 305−309. (d) Bommarius, A. S.; Blum, J. K.; Abrahamson, M. J. Status of protein engineering for biocatalysts: How to design an industrially useful biocatalyst. *Curr. Opin. Chem. Biol.* **2011**, *15*, 194−200.
(39) Garnier, J.-M.; Jida, M.; Ollivier, J. Regio- and stereoselectivity in the titanium-mediated cyclopropanation of ω-alkenoic diesters: Application in the diastereoselective synthesis of pyrrolidinone. *Synlett* **2006**, *17*, 2739−2742.

https://doi.org/10.1021/acs.joc.1c00326
J. Org. Chem. 2021, 86, 6494−6503