Synthesis of Caged HMG-CoA Reductase Substrates for Elucidation of Cellular Pathways

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ABSTRACT. The synthesis of photocaged substrates of the biologically important enzyme HMG-CoA reductase is reported. HMG-CoA bearing a *p*-hydroxyphenacyl (pHP) photocage moiety was synthesized in an overall yield of 14% over seven steps in addition to caged forms of mevalonate and mevaldehyde. The absorption maximum and quantum yield for decaging of the photocaged compounds is pH dependent with a λ_{max} = 330 and ϕ = 5%, respectively, at pH 9.1 but λ_{max} = 290 and ϕ = 16% at pH 6.7.

Introduction

The mevalonate pathway is a major biological pathway responsible for the synthesis of many important biomolecules such as terpenes, lipids, and steroids (Figure 1).[1] One point of major interest in this pathway is the rate-limiting step whereby HMG-CoA is converted into mevalonate via a mevaldehyde intermediate[2] catalyzed by the enzyme HMG-CoA reductase (HMGR).[3] Over many years, sophisticated experimental and computational approaches[3-4] have revealed a complex mechanism involving major structural rearrangements of the enzyme, two reduction steps, and a complex co-factor exchange event.[2] Therefore, many of the structural and chemical details of the reaction are unclear. In order to improve the understanding of this fundamental biochemical mechanism and other details of the mevalonate pathway, the development of new experimental methods is required.

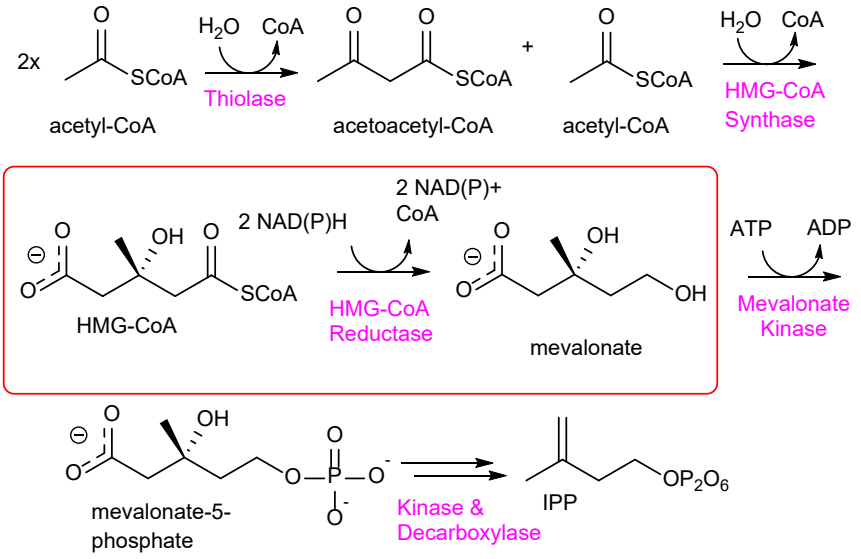


Figure 1. The mevalonate pathway.

One strategy for enzymatic pathway elucidation is modulation of activity through external triggers.[5] Such control is necessary for advanced biophysical studies such as time resolved crystallography.[6] For methods of activation, a common technique is incorporation of a photolabile "photocage" onto a relevant substrate. A photocage is a moiety that undergoes a transformation when activated by light.[7] This activation can lead to dissociation of the photocage resulting in an uncaged substrate, which becomes available to undergo normal reactions.

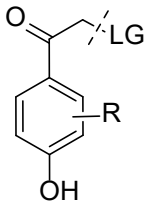


Figure 2. *para*-Hydroxyphenacyl (pHP) type photocage.

Photocages have found use in different applications including organic synthesis, polymerization, and structural biology.[6-7] Many types of photocages have been developed with a range of different photophysical properties.[7] Givens and coworkers introduced the *p*-hydroxyphenacyl (pHP) group (Figure 2) as a photocage for a variety of functional groups.[8] This cage has found applications in biochemical studies due to suitable quantum yield for cleavage of $\Phi \sim 0.20$, depending on the leaving group (LG) and an absorption maximum above 300 nm to allow for triggering with light outside of the absorption range of aromatic amino acid residues in proteins.[9]

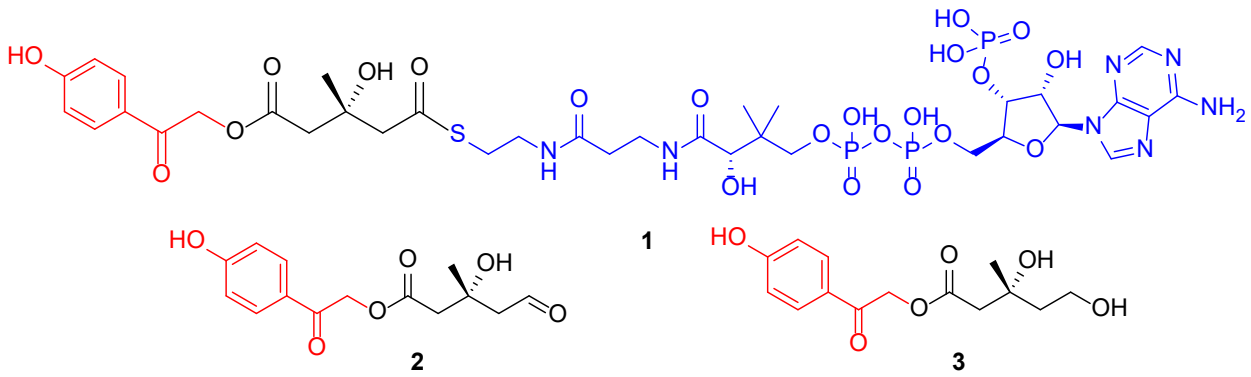
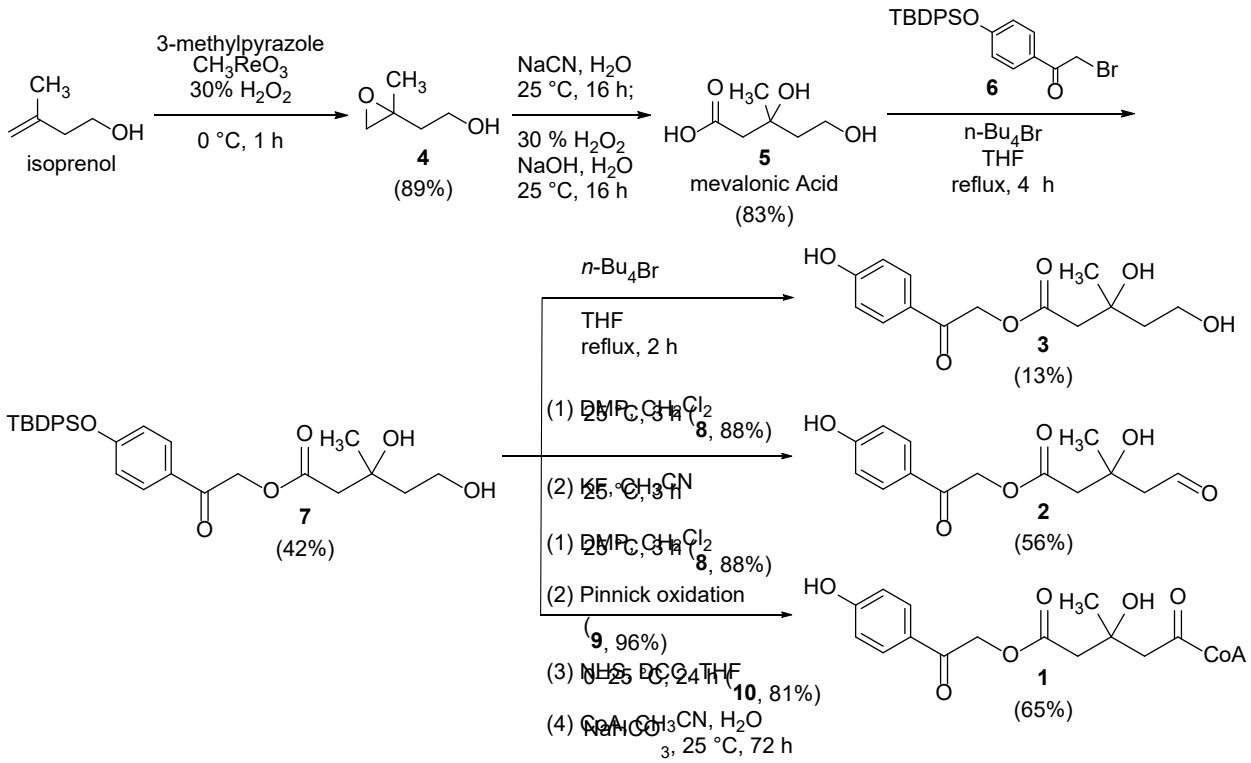


Figure 3. Caged HMGR substrates with pHP photocage in red and CoA in blue.

We designed photocaged substrate **1** (Figure 3), which we envisioned as having a pHP group ester-linked to the carboxylate of HMG-CoA. Caging of the terminal carboxylate would be expected to prohibit the normal activity of HMGR due to the necessity of the 3-hydroxy-3-methyl glutaryl (HMG) group on HMG-CoA to enter a binding pocket before the enzymatic reaction can proceed.[10] Modulation of activity would be achievable by exposing a substrate/enzyme system to light which would lead to rapid decaging and generation of active substrate. Related caged derivatives of mevaldehyde **2** and mevalonate **3**, which are also key intermediates in the mevalonate pathway and could thus be used for studies of other steps in the pathway, are additional targets as tools for elucidation of the interesting catalytic modes of these enzymes. .

Results and Discussion

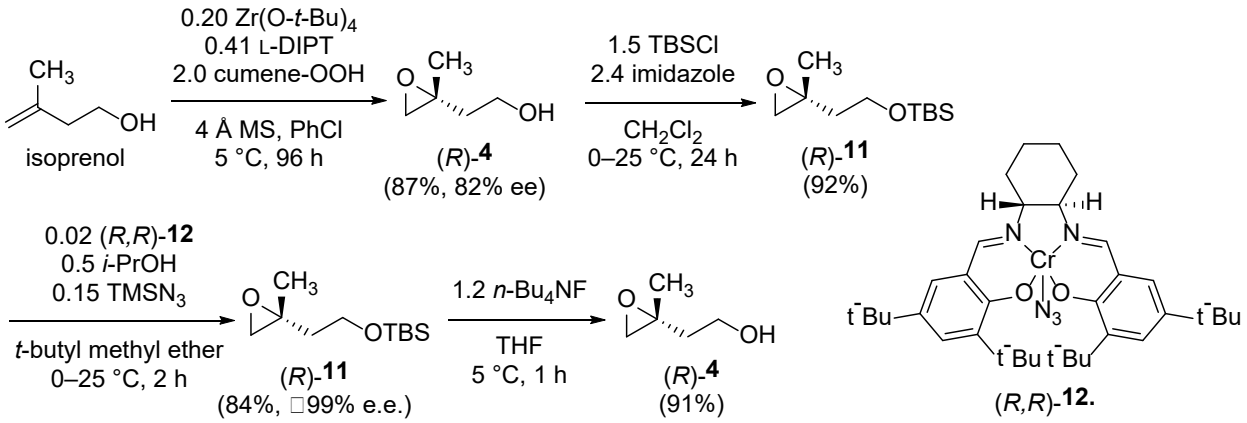


Scheme 1. Synthesis of pHP caged HMGR substrates.

Although mevalonate and derivatives are commercially available, and several previous syntheses have been reported,[11] we found that a particularly simple, expeditious source of the HMGR substrates for the present study could be realized starting with isoprenol, an inexpensive, commercially available compound (Scheme 1). Metal-catalyzed epoxidation with hydrogen peroxide leads to epoxide **4**, which upon cyanide addition and *in situ* nitrile hydrolysis gives racemic mevalonic acid **5**. The overall yield is 74% over two steps comprised of three reactions which are easily scalable to gram amounts.

Photocage incorporation was accomplished by substitution of α-brominated pHP derivative **6** with **5** in the presence of a phase transfer agent, tetra-*n*-butylammonium bromide (TBAB), to give 42% of silyl-protected pHP intermediate **7**. Exploring the identity of side products of this

reaction to determine the reason for this moderate yield revealed the presence of caged mevalonate **3** in low yields from silyl group cleavage. The hydroxy group of **7** was oxidized using Dess-Martin periodinane (DMP)[12] to give silyl-protected aldehyde **8**, which was deprotected with potassium fluoride to give caged mevaldehyde **2**. Aldehyde **8** was also employed in a Pinnick oxidation[13] to give carboxylic acid **9**. This compound was activated as the *N*-hydroxysuccinimide ester **10**. CoA coupling was accomplished in a bicarbonate buffer. Over the course of this reaction, the silyl group on the pHP cage also cleaved to give caged HMG-CoA **1** in good yield and 14% overall yield for the entire seven-step synthesis.



Scheme 2. Asymmetric epoxidation and Jacobsen epoxide enantiomeric enhancement to ≥99% e.e. (*R*)-**5**

The synthesis in Scheme 1 leads to **2** and **3** as racemic mixtures of enantiomers and to **1** as a mixture of diastereomers due to the non-enantioselective first step, which results in lack of stereocontrol of the 3-methyl-3-hydroxy stereocenter in the HMG segment. This element of stereochemistry may be controlled by performing the initial epoxidation with a zirconium catalyst and the chiral diisopropyl L-tartrate (L-DIPT) ligand,[14] which gives a high yield of up to 87% and an 82% e.e. of (*R*)-**4** (Scheme 2). Further increase of e.e. is accomplished by protection of **4** as TBS ether **11** followed by Jacobsen enantiomeric enhancement through removal of the unwanted

enantiomer by selective epoxide opening catalyzed by *(R,R)*-**12**.[15] Silyl deprotection leads to (*R*)-**4** with ≥99% e.e. as ascertained using ^{19}F NMR analysis of a Mosher acid derivative[16] (Figure S2). Although the subsequent conversion of (*R*)-**4** to (*R*)-**5** was not expected to result in any loss of configuration, the stereochemical integrity of this step was confirmed by NMR analysis of the Mosher ester derivative of (*R*)-**5** (Figure S4-6). An important feature of this synthesis is that is allows easy access to the opposite stereoisomer (*S*)-**4** which is not expected to bind to the enzymes in the mevalonate pathway and is thus an important control compound for enzymology studies of these proteins. (*S*)-**4** was synthesized with ≥99% e.e. by using D-DIPT in place of L-DIPT in the epoxidation and the (*S*,*S*) Jacobsen catalyst in place of the (*R*,*R*) system in the enhancement step as confirmed spectroscopically (Figure S7-8). Synthesis of highly stereopure (*R*)- and (*S*)-derived caged products **1**, **2** and **3** was accomplished by using the ≥99% e.e. samples of (*R*)- and (*S*)-**4**. Mevalonate product **3** was observed to be unstable in both organic and aqueous solutions due to intramolecular lactonization.[17] In contrast, photocaged HMG-CoA **1** was found to be stable with no notable degradation observed by NMR over two days at room temperature in various buffer solutions.

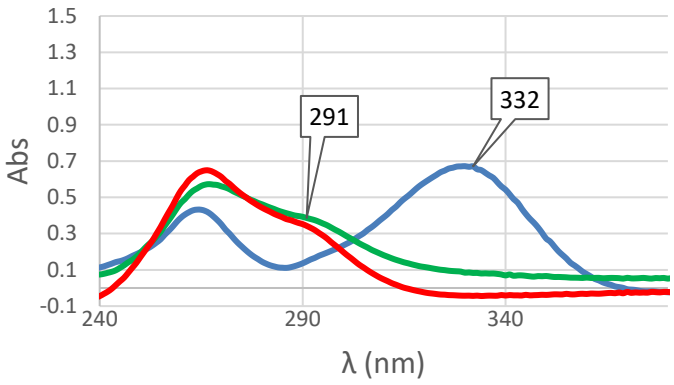


Figure 4. pH dependence of UV/Vis spectrum of **1** from pH = 9 (blue) and pH = 6.8 (green) to pH = 5.5 (red). Absorption at 266 nm corresponds to adenine.

The photophysical properties of the caged products were evaluated. For all caged systems, the λ_{max} has a pH dependency with absorption at 332 nm at pH = 9 and a shift to 291 nm upon lowering the pH to 6.8 (Figure 4.) This is consistent with previous reports of the pHP system)[7] and reflects the deprotonated and protonated state of the phenoxy moiety of the pHP photocage. The protonation state was shown to be important for the mechanism and quantum yield of the photochemical decaging reaction.[8] The quantum yield of the caged system was therefore evaluated at pH 6.7 and 9.1 using a bromopentaamminecobalt(III) bromide[18] actinometer. Upon irradiation with 300 nm wavelength light, the quantum yield for the photocleavage of **1** was found to be 16% at pH 6.7 and 5% at pH 9.1 (Figure S10-11). This dependence of the quantum yield on the pH is expected based on the different protonation states of the phenolic group at these pH values and is in line with previous reports of other pHP caged compounds.[9] The higher quantum yield at pH=6.7, which is outside the range of the maximum rate pH of *plasmodium mevalonii* HMGR,[5] suggests that the reaction could be slowed to allow an easier direct observation of the reaction using time resolved crystallography using the photocaged substrate **1**.

Conclusions

Herein, we report the synthesis of photocaged substrates of the mevalonate pathway. Access to these compounds is facilitated by efficient racemic and enantioselective syntheses of mevalonate derivatives. Incorporation of the pHP group provides photocaged HMG-CoA, mevaldehyde, and mevalonate substrates. The photophysical properties of the caged systems exhibit a pH dependency of absorbance and photocleavage quantum yields. These results provide

materials for detailed investigations of the mevalonate pathway and other related biochemical processes using a phototriggered substrate.

Experimental

All reagents and solvents were obtained from Sigma Aldrich or VWR International. Deionized (DI) water was used in all cases of water utilization. Flash column chromatography was performed using a Biotage Isolera Prime system with Silicycle Inc. Siliasep cartridges. ^{1}H, ^{13}C and ^{19}F NMR spectra were obtained using a Bruker AVANCE III HD 400 instrument operating at 400 MHz, 100 MHz, and 376 MHz respectively. All ^{1}H and ^{13}C data are reported in ppm (δ) relative to residual $CDCl_3$ (7.26 ppm, ^{1}H NMR; 77.23 ppm, ^{13}C NMR), CD_3OD (4.78 ppm, ^{1}H NMR; 49.15 ppm, ^{13}C NMR), and D_2O (4.79 ppm, ^{1}H NMR). Peaks are reported as br = broad signal, s = singlet, d = doublet, t = triplet, m = multiplet. Mass spectrometry measurements were conducted with electrospray ionization (ESI) using a Bruker MicrOTOF-Q II spectrometer. For infrared spectroscopy, a Jasco FT/IR-6300 instrument was used.

Compounds and Procedures:

2-(4-Hydroxyphenyl)-2-oxoethyl-(3S)-5-((2-(3-((2R)-4-(((((((2R,3S,4R,5R)-5-(6-amino-9H-purin-9-yl)-4-hydroxy-3-(phosphonooxy)-tetrahydrofuran-2-yl)-methoxy)-(hydroxy) phosphorryl)-oxy) (hydroxy) phosphoryl) oxy)-2-hydroxy-3,3-dimethylbutanamido)propanamido)ethyl)thio)-3-hydroxy-3-methyl-5-oxopentanoate (1). To a round bottom flask was added a stir bar, CoA (34 mg, 0.044 mmol), and 0.2 M $NaHCO_3$ pH 8.5 (1.2 mL). To a separate flask was added **10** (50 mg, 0.079 mmol) dissolved in acetonitrile (2 mL). The solution of **10** was transferred to the CoA solution and the reaction was stirred at 25 °C for 72 h. Acetonitrile was

removed under reduced pressure, and DI H_2O (3 mL) was added. The solution was transferred to a separatory flask and washed with ethyl acetate (2 x 5 mL). The aq mixture was concentrated under reduced pressure, and the residue was purified by loading onto a reverse phase C18 prep TLC plate (1000 μm) and using H_2O/acetonitrile 7:3 as the mobile phase to give **1** as a white wax which solidified into a white solid when exposed to MeOH (30 mg, 0.029 mmol, 65% yield). R_f = 0.7 (H_2O/acetonitrile 7:3). ^{1}H NMR (400 MHz, D_2O) δ 8.50 (s, 1H), 8.18 (s, 1H), 7.76 (m, 2H), 6.77 (m, 2H), 6.11 (d, 1H, J = 6.8 Hz), 5.40 (s, 2H), 4.75 (m, 2H), 4.55 (m, 1H), 4.23 (m, 2H), 3.98 (s, 1H), 3.81 (m, 1H), 3.49 (m, 1H), 3.42 (m, 2H), 3.35 (t, 2H, J = 6.1 Hz), 3.03 (ABq, 2H, J = 16.4 Hz), 3.01 (t, 2H, J = 6.3 Hz), 2.84 (ABq, 2H, J = 14.6 Hz), 2.40 (t, 2H, J = 6.9 Hz), 1.40 (s, 3H), 0.84 (s, 3H), 0.69 (s, 3H) ^{13}C NMR (100 MHz, D_2O) δ 200.18, 194.11, 174.92, 174.16, 172.11, 166.64, 155.78, 153.09, 149.50, 140.03, 131.29, 123.68, 118.73, 117.08, 86.83, 84.25, 74.58, 74.29, 73.83, 72.06, 70.76, 66.72, 65.98, 53.54, 45.31, 38.72, 35.64, 35.59, 29.13, 28.61, 26.84, 21.08, 18.23. HRMS (ESI/Q-TOF) m/z - negative mode: $[M-H]^-$ Calcd for $C_{35}H_{49}N_7O_{22}P_3S$. 1044.1870; Found 1044.1880.

2-(4-Hydroxyphenyl)-2-oxoethyl 3-hydroxy-3-methyl-5-oxopentanoate (2). To a round bottom flask was added **8** (100 mg, 0.19 mmol), acetonitrile (5 mL), and a stir bar, and the flask was placed under argon. To the solution was added KF (31 mg, 0.54 mmol), and the mixture was stirred at 25 °C for 3 h. The solution was concentrated under reduced pressure, and the residue was redissolved in ethyl acetate (30 mL) and transferred to a separatory funnel. The organic layer was washed with brine (3 x 30 mL), dried with $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was purified on silica using a gradient of hexane/ethyl acetate to give **2** as a clear, colorless oil (30 mg, 0.106 mmol, 56% yield). R_f = 0.1 (hexane/EtOAc 1:1). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.91 (s, 1H), 7.82 (m, 2H), 6.90 (m, 2H), 5.38 (s, 2H), 2.80 (m, 4H), 1.48 (s, 3H) ^{13}C NMR (100

MHz, $CDCl_3$) δ 202.47, 191.19, 171.14, 161.59, 130.78, 126.74, 116.09, 70.55, 66.23, 54.03, 46.13, 27.86. HRMS (ESI/Q-TOF) m/z: [M + K]+ Calcd for $C_{14}H_{16}KO_6$. 319.0578; Found 319.0627.

2-(4-Hydroxyphenyl)-2-oxoethyl 3,5-dihydroxy-3-methylpentanoate (3). To a round bottom flask was added mevalonic acid **5** as a syrup (0.36 g, 1.87 mmol determined by dimethyl sulfone internal standard) along with a stir bar and dry THF (35 mL). Tetra-*n*-butylammonium bromide (1.0 g, 3.1 mmol) and bromide **6** (1.0 g, 2.2 mmol) were added to the flask, and the reaction mixture was heated under a reflux condenser to 50 °C in an oil bath for 2 h. The reaction mixture was cooled to 25 °C and filtered, and the filtrate was concentrated under reduced pressure. The mixture was redissolved in ethyl acetate (100 mL) and transferred to a separatory funnel. The solution was washed with brine (2 x 100 mL), dried with $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was loaded onto silica gel, and the product was purified with a gradient of hexane/ethyl acetate to give caged mevalonate **3** as a clear, viscous oil (68 mg, 0.24 mmol, 13% yield). R_f = 0.5 (DCM/MeOH 9:1). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.79 (m, 2H), 6.89 (m, 2H), 5.37 (s, 2H), 3.75 (t, 2H, J = 6.9 Hz), 2.70 (s, 2H), 1.90 (t, 2H, J = 7.5 Hz), 1.33 (s, 3H) ^{13}C NMR (100 MHz, $CDCl_3$) δ 194.62, 172.50, 162.09, 131.09, 125.93, 115.90, 71.29, 66.86, 57.99, 45.76, 42.68, 26.19. HRMS (ESI/Q-TOF) m/z: [M + Na]+ Calcd for $C_{14}H_{18}NaO_6$. 305.0996; Found 305.0990.

2-(2-Methyloxiran-2-yl)ethan-1-ol (4). Isoprenol (1.75 g, 20.3 mmol) was added to a round bottom flask along with a stir bar and placed under argon. The flask was placed in a 10 °C ice bath, and 3-methylpyrazole (170 mg, 2.1 mmol) was added along with methyltrioxorhenium (10 mg, 0.04 mmol). Then 30% aq H_2O_2 (2.76 mL, 24.4 mmol) was added dropwise, and the mixture was

vigorously stirred at 10 °C for 2 h. Brine (40 mL) and ethyl acetate (40 mL) were added, and the solution was transferred to a separatory funnel. The organic layer was separated and dried with $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was loaded onto a silica gel column and eluted with a gradient of hexane/ethyl acetate to give racemic epoxide **4** as a clear, colorless oil (1.85 g, 89% yield). R_f = 0.5 (100% ethyl acetate) $KMnO_4$ stain. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.68 (m, 2H), 2.75 (d, 1H, J = 4.3 Hz), 2.59 (d, 1H, J = 4.4 Hz), 2.53 (br, 1H) 1.83 (m, 2H), 1.33 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 59.32, 56.57, 53.37, 38.11, 22.00 (lit.[14] ^{1}H, ^{13}C NMR).

(*R*)-2-(2-Methyloxiran-2-yl)ethan-1-ol (4). L-Diisopropyl tartrate (0.148 g, 0.63 mmol) was added to a round bottom flask along with a stir bar and dissolved in chlorobenzene (8 mL). Activated 4Å molecular sieves (0.125 g) were added, and the vessel was flushed with argon. Zr(t-BuO)$_4$ (0.118 g, 0.31 mmol) was dissolved in chlorobenzene (2 mL) and added dropwise to the tartrate solution, and the mixture was stirred at 25 °C for 1 h. The solution was cooled in a 5 °C ice bath, and 88% cumene hydroperoxide (0.5 mL, 2.90 mmol) was added dropwise. Isoprenol (0.15 mL, 1.48 mmol) was dissolved in chlorobenzene (1 mL) and transferred to the reaction mixture dropwise. The mixture was stirred for 96 h at 5 °C after which the solution was filtered to remove the molecular sieves. The filtrate was loaded onto a silica column and eluted with a hexane/ethyl acetate gradient to give (*R*)-epoxide **4** as a clear, colorless oil (0.132g, 87% yield, 82% ee determined by (*S*)-Mosher ester derivative). NMR identical to racemic **4**.

(*S*)-2-(2-Methyloxiran-2-yl)ethan-1-ol (4). D-Diisopropyl tartrate (300 mg, 1.28 mmol) was added to a round bottom flask along with a stir bar and dissolved in chlorobenzene (5 mL). Activated 4Å molecular sieves (300 mg) were added, and the vessel was flushed with argon. Zr(t-

BuO)$_4$ (0.236 g, 0.62 mmol) was dissolved in chlorobenzene (1 mL) and added dropwise to the tartrate solution, and the mixture was stirred at 25 °C for 30 min. The solution was cooled in a 5 °C ice bath, and 88% cumene hydroperoxide (1 mL, 5.8 mmol) was added dropwise. Isoprenol (0.3 mg, 3.4 mmol) was dissolved in chlorobenzene (1 mL) and transferred to the reaction mixture dropwise. The mixture was stirred for 96 h at 5 °C after which the solution was filtered to remove the molecular sieves. The filtrate was loaded onto a silica column and eluted with a hexane/ethyl acetate gradient to give (*S*)-epoxide **4** as a clear, colorless oil (231 mg, 2.3 mmol, 66% yield, 82% ee determined by (*S*)-Mosher ester derivative). NMR identical to racemic **4**.

Mevalonic acid (5). Epoxide **4** (1.5 g, 14.6 mmol) was added to a round bottom flask containing a stir bar and cooled in a 5 °C ice bath. A separate flask was charged with sodium cyanide (0.79 g, 16.1 mmol), which was dissolved in H_2O (2 mL) and cooled in a 5 °C ice bath. The cyanide solution was then added to the epoxide flask dropwise slowly over the course of 10 min while maintaining a cold temperature of the reaction mixture. Upon complete addition, the ice bath was removed, and the mixture was stirred at 25 °C for 16 h. The solution was diluted with H_2O (25 mL) and washed with dichloromethane (2 x 20 mL) in a separatory funnel. The aq layer was filtered to remove suspended white participate, and the filtrate was subjected to reduced pressure for 5 min to remove residual DCM. The residue was transferred to a round bottom flask containing a stir bar, the pH of the solution was increased to pH 12.5 by addition of 1M aqueous NaOH, and the mixture was cooled in a 5 °C ice bath for 10 min. 30% aq H_2O_2 (20 mL, 176.5 mmol) was added dropwise, and the mixture was stirred at 25 °C for 16 hr. The mixture was transferred to a separatory funnel and washed with dichloromethane (2 x 30 mL). The aq layer was subjected to reduced pressure for 5 min to remove residual dichloromethane, and the residue was transferred to

a round bottom flask containing a stir bar. This solution was cooled in a 5 °C ice bath for 10 min, and the pH was lowered to pH 5.6 by dropwise addition of 1M aqueous HCl while taking care to maintain steady bubble formation. The solution was stirred at 25 °C for 16 h. The pH of the solution was increased to pH 8.5 by addition of 1M aq NaOH, and the solution was stirred for an additional 16 h at 25 °C. The water was removed under reduced pressure in a 40 °C water bath. The clear viscous/solid residue was dissolved in ethanol (20 mL) added dropwise with vigorous stirring over 10 min. The white NaCl precipitate was filtered, and the solvent was removed under reduced pressure. The residue was again dissolved in ethyl acetate (20 mL) and filtered to remove remaining NaCl, and the solvent was removed under reduced pressure. Residual ethyl acetate was removed by addition of H_2O (5 mL) to the product residue, and solvent was removed under reduced pressure in a 40 °C water bath. The resulting product residue **5** was a clear, colorless syrup containing residual water which required no further purification (2.35 g, 12.2 mmol determined by dimethyl sulfone internal standard, 83% yield). NMR as sodium mevalonate: **^{1}H NMR** (400 MHz, D_2O) δ 3.58 (t, 2H, J = 7.4 Hz), 2.21 (d, 2H, J = 2.9 Hz), 1.68 (t, 2H, J = 7.4 Hz), 1.11 (s, 3H). in agreement with reported ^{1}H-NMR.[19] **^{13}C NMR** (100 MHz, D_2O) δ 180.61, 71.10, 58.15, 48.18, 42.86, 26.30.

2-Bromo-1-(4-((*tert*-butyldiphenylsilyl)oxy)phenyl)ethan-1-one (6). A round bottom flask was charged with 4-hydroxy-acetophenone (0.20 g, 1.5 mmol) which was dissolved in DCM (10 mL). A stir bar was added, and the vessel was charged with argon. Imidazole (0.11 g, 1.6 mmol) was added, and *tert*-butyldiphenylsilyl chloride (0.41 mL, 1.6 mmol) was added dropwise. The reaction was stirred at 25 °C for 12 h. The solution was transferred to a separatory funnel and washed with brine (2 x 20 mL). The organic layer was separated, dried with anhyd $MgSO_4$, filtered and concentrated under reduced pressure. ^{1}H NMR of the crude mixture revealed the presence of

product with a major impurity. Attempts to resolve the product from the impurity were unsuccessful using flash chromatography. Instead, the reaction mixture was carried through without further purification. The crude product was transferred into a round bottom flask along with a stir bar and argon. The material was dissolved in ethyl acetate (10 mL). $CuBr_2$ (1.0 g, 4.5 mmol) was added, and the solution was refluxed for 4 h under a reflux condenser. The mixture was allowed to cool to 25 °C and filtered through celite. The filtrate was concentrated under reduced pressure to give a dark green residue, which was purified with silica gel chromatography using a gradient of hexane/ethyl acetate. Product fractions were combined, and the solvent was evaporated to give **6** as a clear, viscous oil, which crystallized as a clear, colorless crystals upon sitting at 25 °C for 12 h (0.52 g, 1.16 mmol, 77% yield). R_f = 0.5 (hexane/EtOAc 9:1). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.77 (m, 2H), 7.70 (m, 4H), 7.45 (m, 2H), 7.39 (m, 4H), 6.81 (m, 2H), 4.33 (s, 2H), 1.11 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 190.16, 160.99, 135.59, 132.12, 131.28, 130.5, 128.21, 127.51, 120.21, 31.06, 26.58, 19.68. (lit.[20] ^{1}H, ^{13}C NMR).

2-(4-((*tert*-Butyldiphenylsilyl)oxy) phenyl)-2-oxoethyl 3,5-dihydroxy-3-methyl pentanoate (7). To a round bottom flask was added mevalonic acid **5** as a syrup (0.36 g, 1.87 mmol determined by dimethyl sulfone internal standard) along with a stir bar and dry THF (35 mL). Tetra-*n*-butylammonium bromide (1.0 g, 3.1 mmol) and bromide **6** (1.0 g, 2.2 mmol) were added to the flask, and the reaction mixture was heated under a reflux condenser to 50 °C for 2 h. The reaction mixture was cooled to 25 °C and filtered, and the solution was concentrated under reduced pressure. The mixture was redissolved in ethyl acetate (100 mL) and transferred to a separatory funnel. The solution was washed with brine (2 x 100 mL), dried with $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was loaded onto silica gel, and the product was purified with a gradient of hexane/ethyl acetate to give silyl protected **7** as a clear, yellow oil (0.41

g, 0.79 mmol, 42% yield). R_f = 0.13 (hexane/ethyl acetate 1:1). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.69 (m, 6H), 7.44 (m, 2H), 7.38 (m, 4H) 6.82 (m, 2H), 5.31 (ABq, 2H, *J* = 16.1 Hz), 3.88 (m, 2H), 2.70 (ABq, 2H, *J* = 14.0 Hz), 1.86 (m, 2H), 1.39 (s, 3H), 1.11 (s, 9H) ^{13}C NMR (100 MHz, $CDCl_3$) δ 191.24, 171.73, 161.31, 135.59, 132.09, 130.52, 130.19, 128.24, 127.26, 120.37, 72.43, 66.0, 59.72, 46.17, 42.82, 27.04, 26.59, 19.68. HRMS (ESI/Q-TOF) m/z: [M + Na]+ Calcd for $C_{30}H_{36}NaO_6Si$. 543.2173; Found 543.2155.

2-(4-((*tert*-Butyldiphenylsilyl)oxy)phenyl)-2-oxoethyl 3-hydroxy-3-methyl-5-oxopentanoate (8). To a round bottom flask was added **7** (365 mg, 0.7 mmol), dichloromethane (5 mL), and a stir bar, and the flask was placed under argon. The solution was cooled in an ice bath at 0 °C for 5 min. Dess-Martin periodinane (440 mg, 1.0 mmol) was added, and the reaction mixture was stirred at 25 °C for 3 h. Dichloromethane (30 mL) was added, and the mixture was transferred to a separatory funnel, washed with saturated $Na_2S_2O_3$ (10 mL) and brine (3 x 50 mL), dried with $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was purified on silica using a gradient of hexane/ethyl acetate to give aldehyde **8** as a clear, viscous oil (320 mg, 0.61 mmol, 88% yield). R_f = 0.65 (hexane/EtOAc 1:1). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.89 (s, 1H), 7.69 (m, 6H), 7.39 (m, 6H), 6.80 (m, 2H), 5.31 (d, 2H, *J* = 7.8 Hz), 2.76 (m, 4H), 1.45 (s, 3H), 1.11 (s, 9H) ^{13}C NMR (100 MHz, $CDCl_3$) δ 202.38, 191.05, 171.07, 161.33, 135.58, 132.06, 130.52, 130.18, 128.24, 127.18, 120.38, 70.40, 66.19, 60.65, 54.06, 46.11, 27.81, 26.58, 19.67. HRMS (ESI/Q-TOF) m/z: [M + Na]+ Calcd for $C_{30}H_{34}NaO_6Si$. 541.2017; Found 541.1990.

5-(2-(4-((*tert*-Butyldiphenylsilyl)oxy)phenyl)-2-oxoethoxy)-3-hydroxy-3-methyl-5-oxopentanoic acid (9). To a round bottom flask was added aldehyde **8** (130 mg, 0.25 mmol), a stir bar, 2-methylbut-2-ene (0.6 mL, 5.7 mmol), *t*-BuOH (1.1 mL, 11.5 mmol), DI H_2O (0.55 mL), NaH_2PO_4 (105 mg, 0.85 mmol), and $NaClO_2$ (55 mg, 0.6 mmol). The mixture was stirred at 25 °C

for 30 min. Dichloromethane (50 mL) was added, and the mixture was transferred to a separatory funnel. The mixture was washed with brine (3 x 50 mL), dried with $MgSO_4$, filtered, and concentrated under reduced pressure to give **9** as a clear, viscous oil (129 mg, 0.24 mmol, 96% yield). R_f = 0.5 (DCM/MeOH 4:1). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.69 (m, 6H), 7.39 (m, 6H), 6.81 (m, 2H), 5.33 (ABq, 2H, J = 16.2 Hz), 2.74 (m, 4H), 1.46 (s, 3H), 1.11 (s, 9H) ^{13}C NMR (100 MHz, $CDCl_3$) δ 191.37, 173.62, 170.84, 161. 48, 135.58, 132.03, 130.54, 130.28, 128.25, 127.02, 120.42, 70.25, 66.26, 45.69, 45.44, 27.18, 26.58, 19.68. HRMS (ESI/Q-TOF) m/z: [M + Na]+ Calcd for $C_{30}H_{34}NaO_7Si$. 557.1966; Found 557.1968.

1-(2-(4-((*tert*-Butyldiphenylsilyl)oxy)phenyl)-2-oxoethyl)-5-(2,5-dioxopyrrolidin-1-yl)-3-hydroxy-3-methylpentanedioate (10). To a round bottom flask was added carboxylic acid **9** (32 mg, 0.059 mmol), dry THF (4 mL), and a stir bar, and the flask was placed under argon. The solution was cooled in an ice bath at 0 °C and charged with dicyclohexylcarbodiimide (13 mg, 0.063 mmol) and *N*-hydroxysuccinimide (8 mg, 0.07 mmol). The reaction was stirred at 25 °C for 24 h. THF was removed under reduced pressure, and the residue was redissolved in dichloromethane (3 mL). The solution was cooled in a -78 °C dry ice bath and filtered through celite. Dichloromethane (20 mL) was added and the mixture was transferred to a separatory funnel. The solution was washed with brine (2 x 20 mL), dried with $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was purified on silica using a gradient of hexane/ethyl acetate to give **10** as a clear, viscous oil (30 mg, 0.048 mmol, 81% yield). R_f = 0.75 (EtOAc 100%). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.68 (m, 6H), 7.38 (m, 6H), 6.81 (m, 2H), 5.31 (s, 2H), 3.03 (m, 4H),2.83 (m, 4H), 1.53 (s, 3H), 1.11 (s, 9H) **^{13}C NMR** (100 MHz, $CDCl_3$) δ 190.82, 170.95, 169.16, 166.27, 161.21, 135.59, 132.11, 130.50, 130.15, 128.23, 127.32, 120.34, 70.19, 66.23,

45.04, 42.92, 27.44, 26.59, 25.82, 19.67. HRMS (ESI/Q-TOF) m/z: [M + Na]+ Calcd for $C_{34}H_{37}NNaO_9Si$. 654.2130; Found 654.2109.

***tert*-Butyldimethyl(2-(2-methyloxiran-2-yl)ethoxy)silane (11).** To a round bottom flask was added epoxide **4** (390 mg, 3.8 mmol) along with a stir bar and dichloromethane (25 mL) under argon. The flask was charged with imidazole (630 mg, 9.2 mmol), and the solution was cooled in a 5 °C ice bath. To a separate vial was added *tert*-butyldimethylsilyl chloride (850 mg, 5.7 mmol) and dichloromethane(10 mL), and the solution was added dropwise to the epoxide solution. The solution was warmed to 25 °C and stirred for 24 h. Satd aq NH_4Cl (15 mL) was added, and the mixture was transferred to a separatory funnel. The aq layer was extracted with dichloromethane (3 x 10 mL), and the combined organic layers were dried with $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was loaded onto a silica column and purified with a hexane/ethyl acetate gradient to produce protected epoxide **11** as a clear, colorless oil (0.758 mg, 92% yield). R_f = 0.4 (hexane/EtOAc 9:1) $KMnO_4$ stain. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.66 (m, 2H), 2.62 (d, 1H, J = 4.9 Hz), 2.50 (d, 1H, J = 4.9 Hz), 1.78 (m, 1H), 1.62 (m, 1H), 1.28 (s, 3H)), 0.82 (s, 9H), -0.01 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 59.79, 55.57, 54.17, 39.88, 26.00, 21.66, 18.29, -5.28, -5.30. (lit.[21] ^{1}H, ^{13}C NMR).

(*R*,*R*)-*N*,*N*′-Bis(3,5-di-*tert*-butylsalicylidene)-1,2-cyclohexanediimine chromium(III) azide ((*R*)-12). To a round bottom flask was added the chloride of (*R*,*R*)-Jacobsen's chromium catalyst (300 mg, 0.47 mmol) along with a stir bar and dry acetonitrile (50 mL). The vessel was flushed with argon. $AgClO_4$ (119 mg, 0.57 mmol) was added to a separate flask, dissolved in dry acetonitrile (25 mL) and transferred to a dropping funnel. This solution was added dropwise to the chromium solution resulting in the precipitation of silver chloride. The solution was stirred for 18

h at 25 °C. The precipitate was filtered, and the filtrate was transferred to a round bottom flask along with a stir bar and charged with argon. NaN_3 (100 mg, 1.53 mmol) was added, and the solution was stirred at 25 °C for 18 h. To the solution was added diethyl ether (100 mL), and the mixture was transferred to a separatory funnel and washed with brine (3 x 100 mL). The organic layer was dried with $MgSO_4$, filtered, and solvent removed under reduced pressure to give (*R*)-**12** as a dark brown powder (270 mg, 89% yield). IR (solid powder) v 2949, 2904, 2864, 2087, 2055, 1617, 1530, 1459, 1433, 1407, 1385, 1359, 1318, 1269, 1253, 1233, 1198, 1166, 1120, 1098, 1070, 1026, 984, 967, 926, 914, 870, 835, 812, 783, 746, 725, 665, 637, 620 cm^{-1}. (lit.[22] IR).

Jacobsen (*R*)-epoxide enantiomeric enhancement. An enantiomeric mixture of epoxide **11** (176 mg, 0.81 mmol, 82% ee (*R*)-enantiomer) was added to a round bottom flask and dissolved in *tert*-butyl methyl ether (400 µL, 3.4 mmol). A stir bar was added, and the vessel was flushed with argon. The flask was then charged with chromium catalyst (*R*)-**12** (10 mg, 0.016 mmol) and stirred at 25 °C for 5 min. The vessel was cooled in a 5 °C ice bath for 5 min, isopropyl alcohol (30 µL, 0.39 mmol) was added followed by $TMSN_3$ (15 µl, 0.12 mmol), and the mixture was stirred at 25 °C for 2 h. The crude mixture was transferred to a silica column and purified using a hexane/ethyl acetate gradient to give enriched (*R*)-epoxide **11** as a clear, colorless oil (148 mg, 84% yield, ≥99% ee determined as the (*S*)-Mosher ester derivative).

(*S*,*S*)-*N*,*N*′-Bis(3,5-di-*tert*-butylsalicylidene)-1,2-cyclohexanediimine chromium(III) azide ((*S*)-12). To a round bottom flask was added (*S*,*S*)-Jacobsen's ligand (250 mg, 0.45 mmol), a stir bar, dry THF (20 mL), and argon. $CrCl_2$ (78 mg, 0.5 mmol) was added, and the mixture was stirred for 24 h at 25 °C. The mixture was concentrated at reduced pressure, and the residue was dissolved

in ether (20 mL) and transferred to a separatory funnel. The organic layer was washed with brine (3 x 50 mL), dried with $MgSO_4$, filtered and concentrated under reduced pressure. The residue was dissolved in dry acetonitrile (20 mL) and a stir bar was added along with argon. $AgClO_4$ (120 mg, 0.57 mmol) was added to a separate flask, dissolved in dry acetonitrile (25 mL), and transferred to a dropping funnel. The $AgClO_4$ solution was added dropwise to the chromium solution resulting in the precipitation of silver chloride. The solution was stirred for 18 h at 25 °C. The precipitate was filtered, and the filtrate was transferred to a round bottom flask along with a stir bar and charged with argon. NaN_3 (101 mg, 1.5 mmol) was added, and the solution was stirred at 25 °C for 18 h. To the solution was added diethyl ether (50 mL), and the mixture was washed with brine (3 x 50 mL). The organic layer was dried with $MgSO_4$, filtered, and concentrated under reduced pressure to give (*S*)-**12** as a dark brown powder (269 mg, 0.42 mmol, 92% yield). IR identical to catalyst (*R*)-**12**.

Jacobsen (*S*)-Epoxide Resolution:

An enantiomeric mixture of epoxide **4** (305 mg, 1.4 mmol, 82% ee (*S*)-enantiomer) was added to a round bottom flask and dissolved in *tert*-butyl methyl ether (700 µL, 5.8 mmol). A stir bar was added, and the vessel was flushed with argon. The flask was charged with chromium catalyst (*S*)-**12** (18 mg, 0.028 mmol) and stirred at 25 °C for 5 min. The vessel was cooled in a 5 °C ice bath for 5 min, isopropyl alcohol (52 µL, 0.67 mmol) was added followed by $TMSN_3$ (26 µl, 0.21 mmol), and the mixture was stirred at 25 °C for 2 h. The crude mixture was transferred to a silica column and purified using a hexane/ethyl acetate gradient to give enriched (*S*)-epoxide **4** as a clear, colorless oil (249 mg, 1.15 mmol, 82% yield, ≥99% ee determined as (*S*)-Mosher ester derivative).

Silyl deprotection of epoxide 11 to epoxide 4.

To a round bottom flask was added *tert*-butyldimethylsiloxy epoxide **11** (148 mg, 0.68 mmol) along with a stir bar, and the flask was flushed with argon. The vessel was cooled in a 5 °C ice bath, and 1M tetra-*n*-butylammonium fluoride in THF (820 µL, 0.82 mmol) was added. The solution was stirred at 5 °C for 1 h, and the solvent was removed under reduced pressure. The residue was loaded onto a silica column and purified with a gradient of hexane/ethyl acetate to give epoxide **4** as a clear oil (64 mg, 91% yield). NMR identical to **4** from epoxidation.

ASSOCIATED CONTENT

Supporting Information. Spectra for characterization of all compounds discussed are available free of charge at https://pubs.acs.org

Data availability Statement The data underlying this study are available in the published article and its Supporting Information.

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