Supporting Information

Substrate Conformation Correlates with the Outcome of Hyoscyamine 6β-Hydroxylase Catalyzed Oxidation Reactions

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Experimental Procedures

S1. Materials and general notes

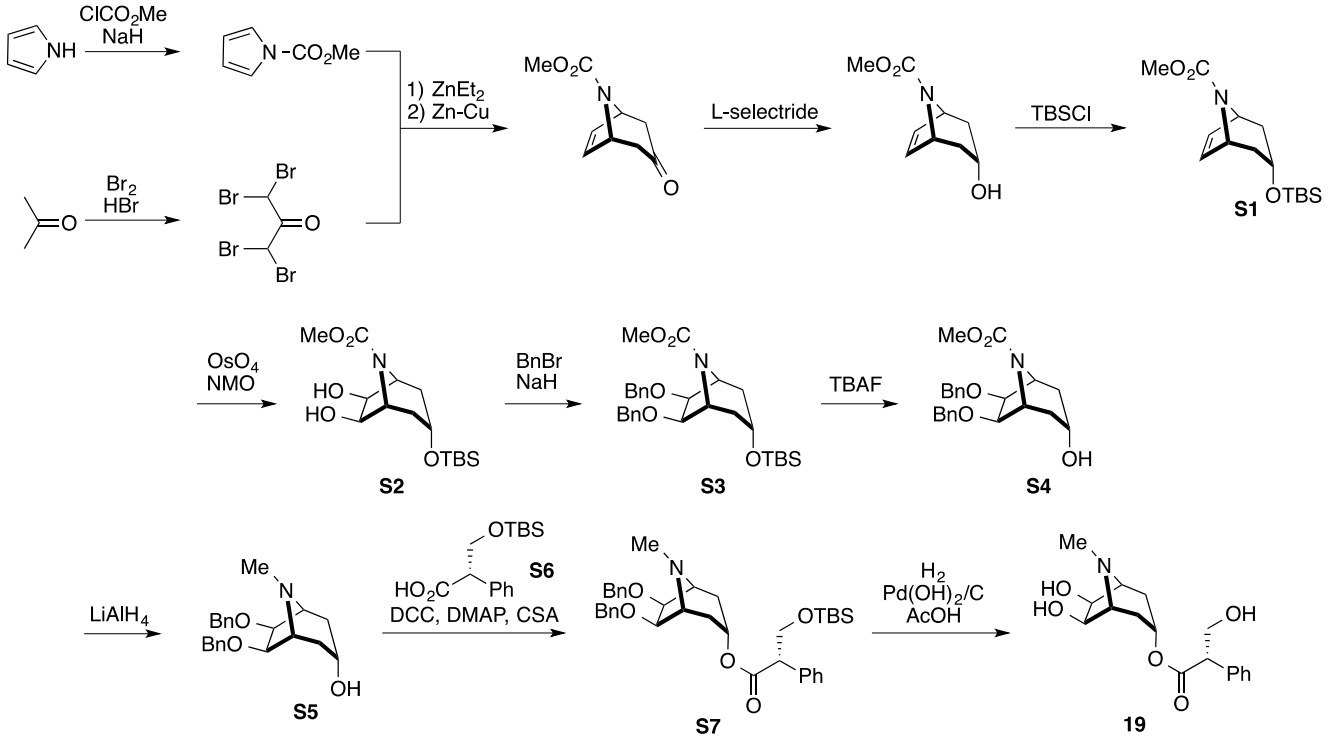
General: All chemicals and reagents were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO) or Fisher Scientific (Pittsburgh, PA) and were used without further purification unless otherwise specified. Tetrahydrofuran (THF) was distilled from sodium/benzophenone, and dichloromethane (DCM) was distilled from calcium hydride under an argon atmosphere. Oligonucleotide primers were prepared by Integrated DNA Technologies (Coralville, IA). Kits for DNA gel extraction and spin minipreps were products of Qiagen (Valencia, CA). PureLink Genomic DNA Mini Kit was acquired from Invitrogen (Carlsbad, CA). KOD DNA polymerase was purchased from Novagen (Madison, WI). Enzymes and molecular weight standards used in the cloning experiments were obtained from New England Biolabs (Ipswich, MA). Reagents for sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) were purchased from Bio-Rad (Hercules, CA). Growth medium components were acquired from Becton Dickinson (Sparks, MD). Sterile syringe filters were bought from Fisher Scientific. Amicon YM-10 ultrafiltration membranes are products of Millipore (Billerica, MA). Silica gel column chromatography was carried out using SiliaFlash P60 (230–400 mesh, Silicycle).

Bacterial Strains and Plasmids: The *h6h* clone (pMH1)[1] was kindly provided by Dr. Hashimoto from NAIST in Japan. *Escherichia coli* DH5α from Bethesda Research Laboratories (Gaithersburg, MD) was used for routine cloning procedures. The protein overexpression host *E. coli* BL21 star (DE3) was obtained from Invitrogen. Vector pET24b(+) for protein overexpression was purchased from Novagen.

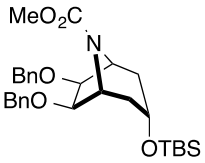
Instrumentation: Standard genetic manipulations of *E. coli* were performed as described by Sambrook and Russell.[2] DNA sequencing was performed at the core facility of the Institute of Cellular and Molecular Biology, the University of Texas at Austin. DNA concentrations were measured using a NanoDrop ND-1000 UV−vis instrument from Thermo Fisher Scientific. High-performance liquid chromatography (HPLC) was performed using a Beckman System Gold 125 Solvent Module with a 166 detector equipped with a C18 reversed-phase column (Microsorb 100-5 C18 250×4.6 mm, Agilent Technologies (Santa Clara, CA)). LC-ESI-TOFMS analysis was performed using an Agilent Technologies HPLC system equipped with a pump (G1311C), an auto sampler (G1329B), and a ToF mass spectrometer (G6230B) with an electrospray ionization (ESI) source. LCMS separations were performed using an Eclipse Plus C18 column (50 × 2.1 mm, 5 μm particle size, Zorbax guard column) at a flow rate of 0.5 or 0.4 mL/min using 0.1% formic acid in H_2O (solvent A) and acetonitrile (solvent B). The obtained LCMS data were analyzed using MassHunter software (Agilent Technologies). NMR spectra were recorded using a Varian DirectDrive 600 MHz, a Varian Inova 500 MHz or a Varian DirectDrive 400 MHz NMR spectrometer at the Nuclear Magnetic Resonance Facility at the University of Texas at Austin. Deuterated solvents were used as internal standards in the NMR spectra unless stated otherwise. Chemical shifts are reported as parts per million (ppm) relative to those of $CDCl_3$, 7.26 ppm for ^1H NMR and 77.16 ppm for ^{13}C NMR, respectively.

S2. Synthesis of hyoscyamine analogues

S2.1 Synthesis of 6β,7β-dihydroxyhyoscyamine (19)

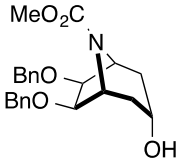


Scheme S1. Synthesis of 6β,7β-dihydroxyhyoscyamine (**19**).



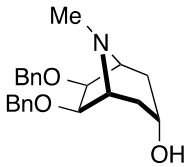
Methyl 6,7-bis(benzyloxy)-3-((*tert*-butyldimethylsilyl)oxy)-8-azabicyclo[3.2.1]octane-8-carboxylate (S3)

To a solution of **S2** (2.00 g, 6.03 mmol, synthesized as described in the literature[3,4]) in THF (40 mL), sodium hydride (60% in mineral oil, 772 mg, 19.3 mmol) was added portion-wise at 0 °C. To this mixture were then added benzyl bromide (2.15 mL, 18.1 mmol) and tetra-butylammonium iodide (110 mg, 0.3 mmol) at 0 °C. The mixture was stirred at room temperature. After 4 h, the reaction was quenched by slow addition of MeOH (10 mL) and then H_2O (50 mL). The resulting solution was extracted with ethyl acetate (3 × 50 mL), and the combined organic phase was washed with brine, dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The crude product was purified by flash chromatography on silica gel (hexanes/ethyl acetate = 4/1) to yield **S3** (3.02 g, 98%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.36–7.24 (10H, m, Ph), 4.67–4.56 (4H, m, CH_2 of Bn), 4.54 (1H, d, *J* = 6.1 Hz, H-6 or H-7), 4.49 (1H, d, *J* = 6.1 Hz, H-6 or H-7), 4.33 (1H, m, H-1 or H-5), 4.20 (1H, m, H-1 or H-5), 3.91 (1H, m, H-3), 3.68 (3H, s, NCO_2Me), 2.06 (1H, ddd, *J* = 4.2 Hz, *J* = 4.2 Hz, *J* = 14.5 Hz, H-2 or H-4), 1.92 (1H, ddd, *J* = 4.2 Hz, *J* = 4.2 Hz, *J* = 14.2 Hz, H-2 or H-4), 1.58 (2H, m, H-2, H-4), 0.70 (9H, s. Si-*t*Bu), –0.10 (3H, s. Si-Me), –0.11 (3H, s. Si-Me), 0.10 (3H, s. Si-Me). ^{13}C NMR (101 MHz, $CDCl_3$) δ 154.6, 138.3, 138.1, 128.3, 128.3, 128.1, 128.0, 127.6, 127.6, 80.8, 80.0, 72.4, 72.2, 65.0, 59.7, 59.1, 52.3, 37.3, 36.3, 25.6, 17.6, –5.3, –5.3. ESI-HRMS calcd. for $C_{29}H_{42}NO_5Si^+$ $[M+H]^+$ 512.2827, found 512.2829.



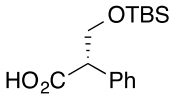
Methyl 6,7-bis(benzyloxy)-3-hydroxy-8-azabicyclo[3.2.1]octane-8-carboxylate (S4)

To a solution of **S3** (2.82 g, 5.52 mmol) in THF (13 mL), tetrabutylammonium fluoride (1 M in THF, 15.3 mL, 15.3 mmol) was added at 0 °C, then warmed to room temperature. After 48 h, the mixture was diluted with DCM and H_2O. The resulting solution was extracted with DCM (3 × 100 mL), and the combined organic phase was washed with brine, dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The crude product was purified using flash chromatography on silica gel (hexanes/ethyl acetate = 1/2) to yield **S4** (2.12 g, 97%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.35–7.22 (10H, m, Ph), 4.66–4.57 (4H, m, CH_2 of Bn), 4.56 (1H, d, *J* = 5.6 Hz, H-6 or H-7), 4.53 (1H, d, *J* = 5.6 Hz, H-6 or H-7), 4.28 (1H, m, H-1 or H-5), 4.18 (1H, m, H-1 or H-5), 3.97 (1H, t, *J* = 4.2 Hz, H-3), 3.64 (3H, s, NCO_2Me), 2.24 (1H, s, br, OH), 2.06 (1H, ddd, *J* = 4.4 Hz, *J* = 4.4 Hz, *J* = 15.0 Hz, H-2 or H-4), 1.91 (1H, ddd, *J* = 4.4 Hz, *J* = 4.4 Hz, *J* = 14.8 Hz, H-2 or H-4), 1.69 (1H, d, *J* = 15.0 Hz, H-2 or H-4), 1.66 (1H, d, *J* = 14.8 Hz, H-2 or H-4). ^{13}C NMR (101 MHz, $CDCl_3$) δ 154.7, 138.3, 138.3, 128.3, 128.3, 127.9, 127.9, 127.5, 127.5, 81.8, 81.0, 72.6, 72.5, 64.1, 59.5, 59.1, 52.3, 36.6, 35.7. ESI-HRMS calcd. for $C_{23}H_{28}NO_5^+$ $[M+H]^+$ 398.1962, found 398.1979.



6,7-Bis(benzyloxy)-8-methyl-8-azabicyclo[3.2.1]octan-3-ol (S5)

To a suspension of lithium aluminum hydride (1.30 g, 34.2 mmol) in ether (180 mL), was added a solution of **S4** (2.00 g, 5.03 mmol) in ether (5.0 mL) at 0 °C, then warmed to room temperature. After 24 h, the reaction was slowly quenched by adding H_2O at 0 °C until the organic phase changes to a clear solution. The resulting solution was filtered through Celite. The filtrate was evaporated to yield **S5** (1.37 g, 77%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.37–7.22 (10H, m, Ph), 4.70 (2H, d, *J* = 11.8 Hz, CH_2 of Bn), 4.57 (2H, d, *J* = 11.8 Hz, CH_2 of Bn), 4.56 (2H, s, H-6, H-7), 4.02 (1H, t, *J* = 4.9 Hz, H-3), 4.26 (2H, m, H-1, H-5), 2.62 (3H, s, NMe), 2.11 (2H, ddd, *J* = 4.6 Hz, *J* = 4.6 Hz, *J* = 15.1 Hz, H-2, H-4), 1.55 (2H, m, H-2, H-4). ^{13}C NMR (101 MHz, $CDCl_3$) δ 139.0, 128.2, 127.7, 127.2, 83.9, 72.8, 65.4, 64.4, 39.3, 34.7. ESI-HRMS calcd. for $C_{22}H_{28}NO_3^+$ $[M+H]^+$ 354.2064, found 354.2078.



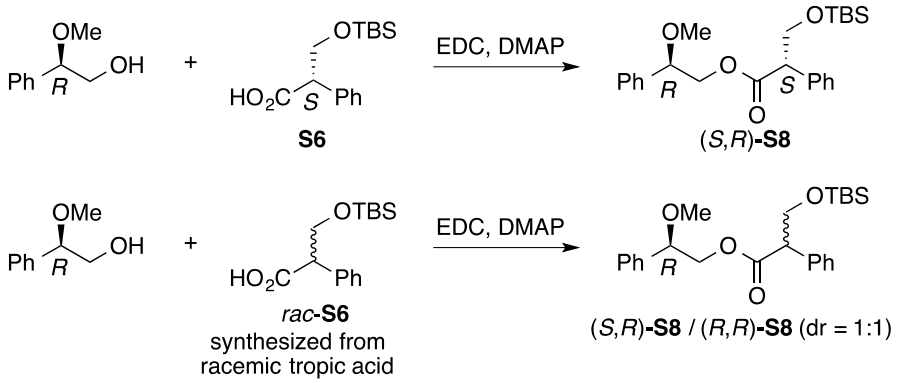
(*S*)-3-((*tert*-Butyldimethylsilyl)oxy)-2-phenylpropanoic acid (S6)

To a solution of L-tropic acid (1.86 g, 11.1 mmol, prepared as described in the literature[5]) and imidazole (2.44 g, 35.8 mmol) in DMF (18 mL), *tert*-butyldimethylchlorosilane (2.54 g, 16.8 mmol) was added at room temperature. After 22 h, the reaction mixture was diluted in ethyl acetate, added HCl (1 N, 50 mL), and stirred for 15 min. The resulting solution was extracted with ethyl acetate (3 × 50 mL), and the combined organic phase was dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The crude product was purified using flash chromatography on silica gel (hexanes/ethyl acetate = 10/1) to yield **S6** (1.80 g, 57%) as a pale yellow oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 11.12 (1H, br, COOH), 7.32–7.24 (5H, m), 4.18 (1H, dd, *J* = 9.2 Hz, *J* = 9.2 Hz), 3.84 (1H, dd, *J* = 9.2 Hz, *J* = 9.2 Hz), 3.12 (1H, dd, *J* = 9.2 Hz, *J* = 9.2 Hz), 0.91 (9H, s), 0.06 (3H, s), 0.00 (3H, s). ^{13}C NMR (101 MHz, $CDCl_3$) δ 178.8, 135.4, 128.8, 128.5, 127.9, 65.3, 54.7, 25.9, 18.3, –5.4, –5.4. ESI-HRMS calcd. for $C_{15}H_{24}O_3SiNa^+$ $[M+Na]^+$ 303.1387, found 303.1391.

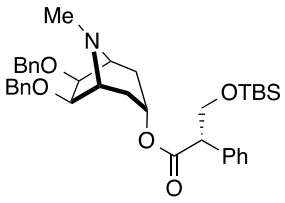
Determination of the enantiomeric purity of S6

To an ice-cold solution of (*R*)-(–)-2-methoxy-2-phenylethanol (17 mg, 0.113 mmol), 1-(3-dimethylamino-propyl)-3-ethylcarbodiimide hydrochloride (23 mg, 0.120 mmol), *N*,*N*-dimethyl-4-aminopyridine (DMAP, ca. 1 mg), in DCM (1 mL) was added **S6** (20 mg, 0.071 mmol) at 0 °C. The reaction mixture was stirred at room temperature for 10 h. TLC analysis indicated the full consumption of **S6**. H_2O (1 mL) was added to the reaction mixture, and the resulting solution was extracted with DCM (3 × 2 mL). The combined organic phase was dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The crude product was purified using flash chromatography on silica gel (hexanes/ethyl acetate = 20/1) to yield **S8** (14.8 mg, 50%) as a pale yellow oil. The diastereomeric ratio of **S8** was determined to be 96:4 (Figure S13a). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.35–7.25

(10H, m), 4.36 (1H, dd, J = 4.3 Hz, J = 7.5 Hz), 4.26–4.12 (3H, m), 3.84 (1H, dd, J = 5.6 Hz, J = 8.7 Hz), 3.78 (1H, dd, J = 5.7 Hz, J = 9.4 Hz), 3.22 (3H, s), 0.86 (9H, s), 0.02 (3H, s), 0.00 (3H, s). ^{13}C NMR (101 MHz, $CDCl_3$) δ 177.9, 143.5, 141.4, 134.1, 134.0, 133.6, 133.7, 133.0, 132.4, 87.7, 73.5, 70.8, 62.5, 60.3, 31.3, 23.7, 0.0, 0.0. ESI-HRMS calcd. for $C_{24}H_{34}O_4SiNa^+$ $[M+Na]^+$ 437.2124, found 437.2121. A racemic mixture of monosilyl protected carboxylic acid *rac*-**S6** was also prepared from commercially available racemic tropic acid. *rac*-**S6** was similarly derivatized by (*R*)-(–)-2-methoxy-2-phenylethanol into the corresponding esters and analyzed by ^{1}H NMR (Figure S13b).

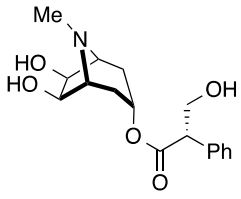


Scheme S2. Determination of the enantiomeric purity of **S6**.



6,7-Bis(benzyloxy)-8-methyl-8-azabicyclo[3.2.1]octan-3-yl (*S*)-3′-((*tert*-butyldimethylsilyl)oxy)-2′-phenylpropanoate (S7)

A mixture of **S5** (363 mg, 1.03 mmol), **S6** (409 mg, 1.55 mmol), *N*,*N*′-dicyclohexylcarbodiimide (DCC, 340 mg, 1.65 mmol), DMAP (13 mg, 0.1 mmol), and 10-camphorsulfonic acid (CSA, 71 mg, 0.3 mmol) in DCM (12 mL) was stirred at room temperature for 3 d. The resulting solution was filtered through Celite to remove white precipitates. The filtrate was loaded on a silica gel column pre-equilibrated with $CHCl_3$. The column was washed with $CHCl_3$, and the product was eluted with $CHCl_3$/MeOH =20/1. Impurities derived from DCC in the fractions containing **S7** were precipitated in a small volume of $CHCl_3$ and filtered to give a pure sample of **S7**. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.38–7.20 (15H, m, Ph), 4.98 (1H, t, J = 4.9 Hz, H-3), 4.55 (1H, d, J = 11.6 Hz, CH_2 of Bn), 4.46 (1H, d, J = 11.6 Hz, CH_2 of Bn), 4.29 (1H, d, J = 11.4 Hz, CH_2 of Bn), 4.26 (1H, d, J = 6.3 Hz, H-6 or H-7), 4.17 (1H, dd, J = 8.4 Hz, J = 9.7 Hz, H-3′), 4.12 (1H, d, J = 11.4 Hz, CH_2 of Bn), 3.80 (1H, dd, J = 5.8 Hz, J = 9.7 Hz, H-3′), 3.72 (1H, d, J = 6.3 Hz, H-6 or H-7), 3.65 (1H, dd, J = 5.8 Hz, J = 8.4 Hz, H-2′), 3.83 (1H, m, H-1 or H-5), 3.23 (1H, m, H-1 or H-5), 2.69 (3H, s, NMe), 2.35 (1H, ddd, J = 4.5 Hz, J = 4.5 Hz, J = 15.4 Hz, H-2 or H-4), 2.25 (1H, ddd, J = 4.6 Hz, J = 4.6 Hz, J = 15.4 Hz, H-2 or H-4), 1.71 (1H, d, J = 15.4 Hz, H-2 or H-4), 1.55 (1H, d, J = 15.4 Hz, H-2 or H-4). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.8, 138.4, 138.3, 136.1, 128.9, 128.3, 128.3, 128.2, 127.9, 127.8, 127.7, 127.6, 127.5, 83.0, 82.4, 77.5, 73.1, 66.9, 65.9, 65.7, 64.7, 54.9, 40.0, 32.2, 31.8, 25.8, 18.2, -5.4, -5.5. ESI-HRMS calcd. for $C_{37}H_{50}NO_5Si^+$ $[M+H]^+$ 616.3453, found 616.3472.

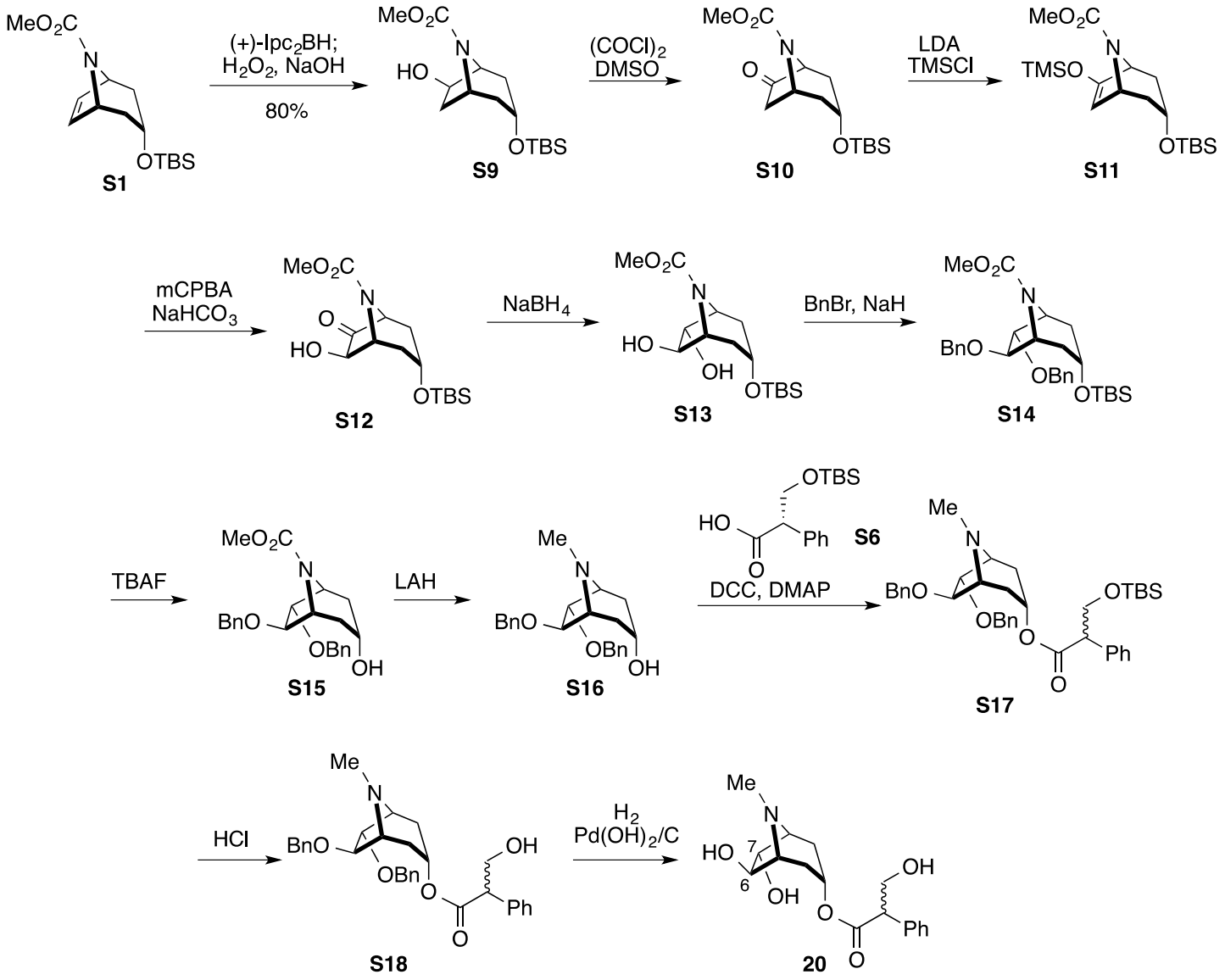


6β,7β-Dihydroxyhyosyamine (19)

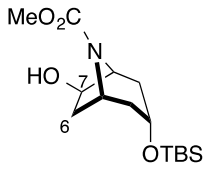
Compound **S7** (200 mg, 0.32 mmol) was dissolved in AcOH (2 mL)/MeOH (20 mL)/H_2O (20 mL) and 20% $Pd(OH)_2$/C (200 mg) was added. The reaction mixture was stirred under a hydrogen atmosphere (1 atm) for 12 h. The reaction mixture was filtered through a pad of Celite, and the filtrate was concentrated. The crude product was purified by flash chromatography on silica gel ($CHCl_3$/MeOH/30% NH_3 = 100/10/1, then 100/17/1) to yield **19** (92 mg, 88%). ^{1}H NMR ($CDCl_3$, 400 MHz, see Figure S16) δ 7.36–7.25 (5H, m, Ph), 4.99 (1H, m, H-3), 4.36 (1H, d, J = 5.9 Hz, H-6 or H-7), 4.16 (1H, m, H-3′), 3.94 (1H, m, H-6 or H-7), 3.84–3.77 (2H, m, H-2′, H-3′),

3.65 (3H, s, br, OH), 3.04 (1H, s, H-1 or H-5), 2.96 (1H, s, H-1 or H-5), 2.45 (3H, s, NMe), 2.23–2.09 (2H, m, H-2, H-4), 1.60 (1H, d, J = 15.6 Hz, H-2 or H-4), 1.39 (1H, d, J = 15.6 Hz, H-2 or H-4). ^{13}C NMR (101 MHz, $CDCl_3$, see Figure S17) δ 172.0, 135.3, 129.0, 128.1, 127.9, 73.9, 73.7, 67.5, 65.5, 65.3, 64.3, 54.5, 34.4, 26.2, 26.1. ESI-HRMS calcd. for $C_{17}H_{24}NO_5^+$ $[M+H]^+$ 322.1649, found 322.1647.

S2.2 Synthesis of 6β,7α-dihydroxyhyoscyamine



Scheme S3. Synthesis of 6β,7α-dihydroxyhyoscyamine (**20**).



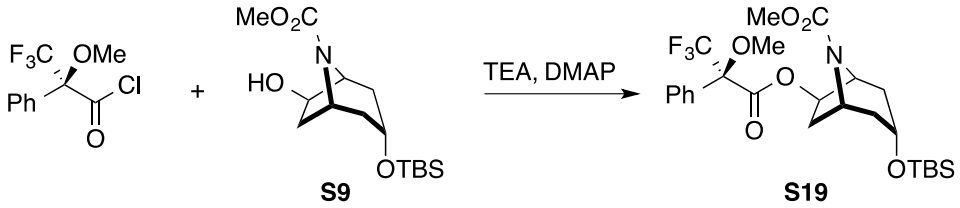
Methyl (1*S*,3*R*,5*R*,7*R*)-3-((*tert*-butyldimethylsilyl)oxy)-7-hydroxy-8-azabicyclo[3.2.1]-octane-8-carboxylate (S9)

Enantioselective hydroboration of **S1** has been previously reported.[6] To crystals of (+)-Ipc_2BH (3.90 g, 13.6 mmol, prepared as described in the literature[7]), a solution of **S1** (2.70 g, 9.08 mmol) in THF (180 mL) was added dropwise at –30 °C. After 4 h, methanol (9 mL), NaOH (3 N, 9 mL), and H_2O_2 (30%, 9 mL) were added to the reaction mixture sequentially. The resulting mixture was

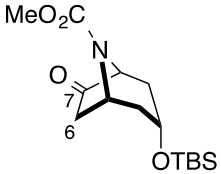
warmed to room temperature and stirred for 14 h. The resulting mixture was diluted with ethyl acetate (200 mL) and H_2O (100 mL). The aqueous solution was extracted with ethyl acetate (3 × 150 mL) and the combined organic phase was dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The crude product was purified using flash chromatography on silica gel (hexanes/ethyl acetate = 2/3) to yield **S9** (2.30 g, 80%) as a white solid. The optical purity was determined to be >99% ee by Mosher's method as described below. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.73 (1H, ddd, *J* = 2.1 Hz, *J* = 6.9 Hz, *J* = 6.9 Hz, H-7), 4.35 (1H, m, H-5), 4.04 (1H, m, H-1), 3.98 (1H, dd, *J* = 4.6 Hz, *J* = 4.6 Hz, H-3), 3.71 (3H, s, NCO_2Me), 2.86 (1H, dd, *J* = 6.9 Hz, *J* = 13.3 Hz, H-6), 1.97 (2H, m, br, H-2 and H-4), 1.73 (1H, m, H-2), 1.65 (1H, m, H-6), 1.58 (1H, m, H-4), 0.89 (9H, s, Si-*t*Bu), 0.02 (3H, s, Si-Me), 0.02 (3H, s, Si-Me). ^{13}C NMR (101 MHz, $CDCl_3$) δ 154.9, 74.9 (m), 65.1, 62.7, 53.4, 52.3, 40.9 (m), 37.8 (m), 36.6 (m), 25.7, 17.8, –5.2. ESI-HRMS calcd. for $C_{15}H_{30}NO_4Si^+$ $[M+H]^+$ 316.1939, found 316.1964.

Determination of the enantiomeric purity of S9

A cold solution of **S9** (20 mg, 0.063 mmol), DMAP (ca. 1 mg), and triethyl amine (10 μL) in DCM (1 mL) was added to (+)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (19 mg, 0.076 mmol) at 0 °C. The reaction mixture was stirred at room temperature for 2 h. TLC analysis indicated the full consumption of **S9**. The reaction was quenched by adding water (1 mL), and the resulting solution was extracted with DCM (3 × 2 mL). The combined organic phase was dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The crude product was purified using flash chromatography on silica gel (hexanes/ethyl acetate = 5/1) to yield **S19** (30.7 mg, 92%) as a pale yellow oil. ^{1}H NMR analysis showed that **S19** is a single diastereomer as shown in Figure S14. This result indicated that the hydroboration/oxidation of **S1** gave optically pure **S9**. The corresponding diastereomeric mixture of **S19** was prepared from racemic **S9**, which was prepared from **S1** using $BH_3{\cdot}SMe_2$. ^{1}H NMR was recorded at 52 °C because **S19** existed as a mixture of two rotamers at room temperature. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.53–7.36 (5H, m, Ph), 5.79–5.56 (1H, dd, *J* = 2.0 Hz, *J* = 5.8 Hz, H-7), 4.47–4.23 (2H, m, br, H-1, H-5), 4.05 (1H, m, H-6), 3.68 (3H, s, NCO_2Me), 3.54 (3H, s, OMe), 2.82 (1H, dd, *J* = 5.8 Hz, *J* = 10.8 Hz), 2.13–1.84 (4H, m, H-2, H-2, H-4, H-6), 1.65 (1H, d, *J* = 11.4 Hz, H-4), 0.94 (9H, s, Si-*t*Bu), 0.09 (3H, s, Si-Me), 0.07 (3H, s, Si-Me). ^{19}F NMR ($CDCl_3$, 470 MHz, room temperature) δ –72.1, –72.2. ESI-HRMS calcd. for $C_{25}H_{36}F_3NO_6SiNa^+$ $[M+Na]^+$ 554.2156, found 554.2162.



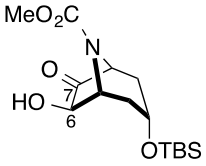
Scheme S4. Determination of the enantiomer ratio of **S9**.



Methyl (1*S*,3*R*,5*R*)-3-((*tert*-butyldimethylsilyl)oxy)-7-oxo-8-azabicyclo[3.2.1]octane-8-carboxylate (S10)

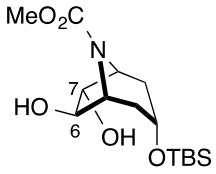
To a solution of oxalyl chloride (0.41 mL, 4.76 mmol) in DCM (8 mL), dimethyl sulfoxide (DMSO, 0.68 mL, 9.52 mmol) was added dropwise at –78 °C. After 5 min, **S9** (0.75 g, 2.38 mmol) in DCM (8 mL) was added to the reaction mixture. After 30 min, triethylamine (2.0 mL) was added to the mixture, and it was warmed to room temperature. This was followed by the addition of H_2O (20 mL), and the resulting solution was extracted with DCM (3 × 20 mL). The combined organic phase was washed with brine, dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The crude product was purified by flash chromatography on silica gel (hexanes/ethyl acetate = 6/1) to yield **S10** (0.74 g, 99%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.67 (1H, br, H-5), 4.12 (1H, m, H-3), 4.07 (1H, br, H-1), 3.73 (3H, s, NCO_2Me), 2.81 (1H, d, *J* = 17.2 Hz, H-6), 2.51 (1H, dd, *J* = 17.2 Hz, *J* = 7.6 Hz, H-6), 2.24–2.02 (2H, m, br, H-2, H-4), 1.95 (1H, ddd,

J = 14.0 Hz, *J* = 3.6 Hz, *J* = 1.6 Hz, H-2), 1.72 (1H, ddd, *J* = 14.0 Hz, *J* = 3.6 Hz, *J* = 1.6 Hz, H-4), 0.85 (9H, s, Si-*t*Bu), 0.013 (3H, s, Si-Me), 0.009 (3H, s, Si-Me). ^{13}C NMR (101 MHz, $CDCl_3$) δ 211.1, 154.1, 65.4, 59.1 (m), 52.6, 51.9 (m), 43.5 (m), 38.3 (m), 36.7 (m), 25.5, 17.7, –5.3, –5.3. ESI-HRMS calcd. for $C_{15}H_{28}NO_4Si^+$ $[M+H]^+$ 314.1782, found 314.1771.



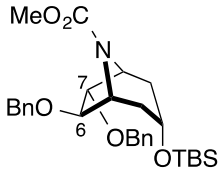
Methyl (1*S*,3*R*,5*R*,6*R*)-3-((*tert*-butyldimethylsilyl)oxy)-6-hydroxy-7-oxo-8-azabicyclo-[3.2.1]octane-8-carboxylate (S12)

To a solution of *N*,*N*-diisopropylamine (402 μL, 2.87 mmol) in THF (18 mL), was added *n*-butyl lithium (2.5 M in *n*-hexane, 1.22 mL, 3.06 mmol) at –78 °C. After 5 min, **S10** (600 mg, 1.91 mmol) in THF (6.0 mL) was added to the lithium diisopropylamide solution at –78 °C, and the mixture was stirred for 1.5 h. Trimethylsilyl chloride (485 μL, 3.82 mmol) was then added to the resulting mixture at –78°C, and it was then warmed to room temperature. Upon the completion of the reaction, 200 mM potassium phosphate buffer (pH 7.0) was added, and the resulting solution was extracted with DCM (3 × 100 mL). The combined organic phase was washed with brine, dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The resulting trimethylsilyl enol ether **S11** was unstable and was thus used in the next step without further purification. The crude intermediate was dissolved in saturated aqueous $NaHCO_3$ and *m*-chloroperbenzoic acid (70–75%, 330 mg, ca. 1.9 mmol) was slowly added at 0 °C. After 5 min, the reaction was quenched with saturated aqueous $Na_2S_2O_3$, and the resulting solution was extracted with DCM (3 × 100 mL). The combined organic phase was dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The crude product was purified by flash chromatography on silica gel (hexanes/ethyl acetate = 4/1) to yield **S12** (0.25 g, 40% from **S10**). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.47 (1H, d, *J* = 2.4 Hz, H-6), 4.42 (1H, br, s, H-5), 4.25 (1H, br, s, H-1), 4.11 (1H, m, H-3), 3.73 (3H, s, NCO_2Me), 2.55 (1H, br, s, OH), 2.22–2.05 (2H, m, H-2, H-4), 1.95 (1H, m, H-2), 1.95 (1H, m, H-4), 0.84 (9H, s, Si-*t*Bu), 0.02 (3H, s, Si-Me), 0.00 (3H, s, Si-Me). ^{13}C NMR (101 MHz, $CDCl_3$) δ 210.1, 154.9, 74.8 (m), 65.4, 59.6 (m), 58.6 (m), 52.8, 38.8 (m), 35.9 (m), 25.5, 17.8, –5.2, –5.3. ESI-HRMS calcd. for $C_{15}H_{28}NO_5Si^+$ $[M+H]^+$ 330.1731, found 330.1719.



Methyl (1*S*,3*S*,5*R*,6*R*,7*R*)-3-((*tert*-butyldimethylsilyl)oxy)-6,7-dihydroxy-8-azabicyclo-[3.2.1]octane-8-carboxylate (S13)

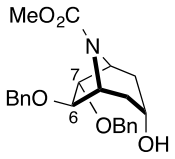
To a solution of **S12** (0.22 mg, 0.67 mmol) in MeOH (20 mL) was added $NaBH_4$ (38 mg, 1.0 mmol) at 0 °C. After 5 min, the mixture was diluted with ethyl acetate and H_2O, and the organic phase was separated. The aqueous phase was extracted with ethyl acetate (3 × 30 mL). The combined organic phase was dried over Na_2SO_4, filtered, and evaporated under reduced pressure to yield **S13** (0.22 g, 99%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.62 (1H, d, *J* = 3.1 Hz, 7-OH), 4.32 (1H, br, H-6), 4.29 (1H, br, s, H-1), 4.09 (1H, m, H-7), 4.07 (1H, m, H-3), 3.91 (1H, br, m, H-5), 3.37 (3H, s, NCO_2Me), 2.97 (1H, d, *J* = 4.2 Hz, 6-OH), 2.15–1.95 (2H, m, H-2, H-4), 1.95 (1H, d, *J* = 15.2 Hz, H-2), 1.95 (1H, d, *J* = 14.7 Hz, H-4), 0.89 (9H, s, Si-*t*Bu), 0.094 (3H, s, Si-Me), 0.090 (3H, s, Si-Me). ^{13}C NMR (101 MHz, $CDCl_3$) δ 154.8, 82.4 (m), 81.4 (m), 65.4, 60.5 (m), 56.3 (m), 52.5, 35.7 (m), 32.8 (m), 25.7, 18.0, –4.8, –5.4. ESI-HRMS calcd. for $C_{15}H_{30}NO_5Si^+$ $[M+H]^+$ 332.1888, found 332.1872.



Methyl (1*S*,3*S*,5*R*,6*R*,7*R*)-6,7-bis(benzyloxy)-3-((*tert*-butyldimethylsilyl)oxy)-8-azabi-cyclo[3.2.1]octane-8-carboxylate (S14)

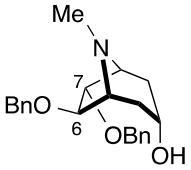
To a solution of **S13** (193 mg, 0.582 mmol) in THF (6.0 mL), sodium hydride (60% in mineral oil, 163 mg, 4.07 mmol) was added portion-wise at 0 °C, and then benzyl bromide (597 μL, 3.49 mmol) and tetrabutylammonium iodide (11 mg, 0.03 mmol) were added to the mixture at 0 °C. The mixture was stirred at room temperature. After 4 h, the reaction was quenched by slow addition of MeOH (1.0 mL) and then H_2O (10 mL). The resulting solution was extracted with ethyl acetate (3 × 30 mL), and the combined organic phase was washed with brine, dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The crude product was purified by flash chromatography on silica gel (hexanes / ethyl acetate = 6/1) to

yield **S14** (208 mg, 70%). This compound was observed as a mixture of two conformers (approximately 56:44 ratio) by NMR analysis. NMR assignments for the mixture of both conformers are shown. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.35–7.26 (10H, m, Ph), 4.63 (1H, d, *J* = 3.2 Hz, H-6), 4.61 (1H, d, *J* = 3.2 Hz, H-6), 4.06–4.43 (4H, m, CH_2 of Bn), 4.34 (1H, m, H-5), 4.21 (1H, m, H-5), 4.13 (1H, m, H-1), 4.34 (1H, m, H-1), 4.02–4.96 (3H, m, H-1, H-3, H-7), 3.68 (3H, s, NCO_2Me), 2.14 (1H, ddd, *J* = 4.2 Hz, *J* = 4.2 Hz, *J* = 14.2 Hz, H-2), 2.06–1.98 (2H, m, H-2, H-4), 1.93–1.85 (1H, m, H-4), 1.70–1.62 (1H, m, H-2), 0.76 (9H, s, Si-*t*Bu), 0.76 (9H, s, Si-*t*Bu), –0.05 (3H, s, Si-Me), –0.07 (3H, s, Si-Me). –0.08 (3H, s. Si-Me). ^{13}C NMR (101 MHz, $CDCl_3$) δ 154.5, 138.2, 138.1, 138.0, 138.0, 128.4, 128.2, 128.2, 128.0, 128.0, 127.9, 127.8, 127.7, 127.6, 127.5, 110.0, 86.2, 85.9, 84.7, 83.7, 72.1, 72.0, 71.4, 71.3, 63.7, 58.2, 57.6, 54.6, 54.2, 52.4, 52.3, 37.8, 36.9, 32.7, 32.0, 26.0, 25.7, 17.9, –4.9, –5.2, –5.2. ESI-HRMS calcd. for $C_{29}H_{42}NO_5Si^+$ $[M+H]^+$ 512.2827, found 512.2829.



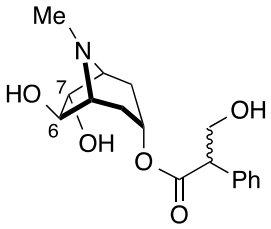
Methyl (1*S*,3*S*,5*R*,6*R*,7*R*)-6,7-bis(benzyloxy)-3-hydroxy-8-azabicyclo[3.2.1]octane-8-carboxylate (S15)

Compound **S15** was synthesized from **S14** in 99% yield according to the same procedure used in the preparation of **S4**. This compound was observed as a mixture of two conformers (approximately 55:45 ratio) by NMR analysis. NMR assignments for the mixture of both conformers are shown. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.39–7.27 (10H, m, Ph), 4.65–4.50 (3H, m, CH_2 of Bn), 4.48 (1H, m, H-1), 4.45–4.35 (3H, m, H-6, H-1, CH_2 of Bn), 4.24 (1H, m, H-5), 4.18 (1H, d, *J* = 8.9 Hz, H-7), 4.15 (1H, d, *J* = 7.3 Hz, H-7), 4.09 (1H, m, H-5), 3.97 (1H, dd, *J* = 5.0 Hz, *J* = 5.0 Hz, H-3), 3.96 (1H, dd, *J* = 5.0 Hz, *J* = 5.0 Hz, H-3), 3.69 (3H, s, NCO_2Me), 3.69 (3H, s, NCO_2Me), 2.14 (1H, ddd, *J* = 4.5 Hz, *J* = 5.1 Hz, *J* = 15.5Hz, H-2), 2.13 (1H, m, H-4), 2.09 (1H, m, H-2), 2.03–1.85 (2H, m, H-2, H-4), 1.79 (1H, br, s, OH). ^{13}C NMR (101 MHz, $CDCl_3$) δ 154.7, 154.5, 137.6, 137.5, 136.2, 136.2, 128.7, 128.5, 128.4, 128.4, 128.2, 128.1, 128.0, 127.9, 127.9, 127.8, 86.0, 85.8, 85.4, 84.9, 72.8, 72.7, 71.6, 71.4, 63.0, 57.8, 57.1, 54.5, 54.3, 52.5, 37.0, 36.1, 33.1, 32.5. ESI-HRMS calcd. for $C_{23}H_{28}NO_5^+$ $[M+H]^+$ 398.1962, found 398.1962.



(1*S*,3*S*,5*R*,6*R*,7*R*)-6,7-Bis(benzyloxy)-8-methyl-8-azabicyclo[3.2.1]octan-3-ol (S16)

Compound **S16** was synthesized from **S15** based on the same method as described for **S5** in 99% yield. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.37–7.25 (10H, m, Ph), 4.59 (1H, d, *J* = 11.5 Hz, CH_2 of Bn), 4.55 (1H, d, *J* = 10.2 Hz, CH_2 of Bn), 4.53 (1H, d, *J* = 10.2 Hz, CH_2 of Bn), 4.45 (1H, d, *J* = 11.5 Hz, CH_2 of Bn), 4.41–4.38 (2H, m, H-7, OH), 4.30 (1H, d, *J* = 6.4 Hz, H-6), 3.88 (1H, ddd, *J* = 4.8 Hz, *J* = 5.6 Hz, *J* = 5.6 Hz, H-3), 3.37 (1H, m, H-1), 3.08 (1H, m, H-5), 2.50 (3H, s, NMe), 2.22 (1H, ddd, *J* = 4.0 Hz, *J* = 5.6 Hz, *J* = 15.5 Hz, H-4), 2.12 (1H, ddd, *J* = 4.8 Hz, *J* = 4.8 Hz, *J* = 14.8 Hz, H-2), 1.68 (1H, d, *J* = 15.5 Hz, H-4), 1.60 (1H, d, *J* = 14.8 Hz, H-2). ^{13}C NMR (101 MHz, $CDCl_3$) δ 138.1, 136.7, 128.6, 128.4, 128.2, 128.1, 127.9, 127.7, 87.0, 87.0, 72.6, 71.6, 62.9, 62.4, 60.0, 36.3, 31.9, 27.9. ESI-HRMS calcd. for $C_{22}H_{28}NO_3^+$ $[M+H]^+$ 354.2064, found 354.2064.

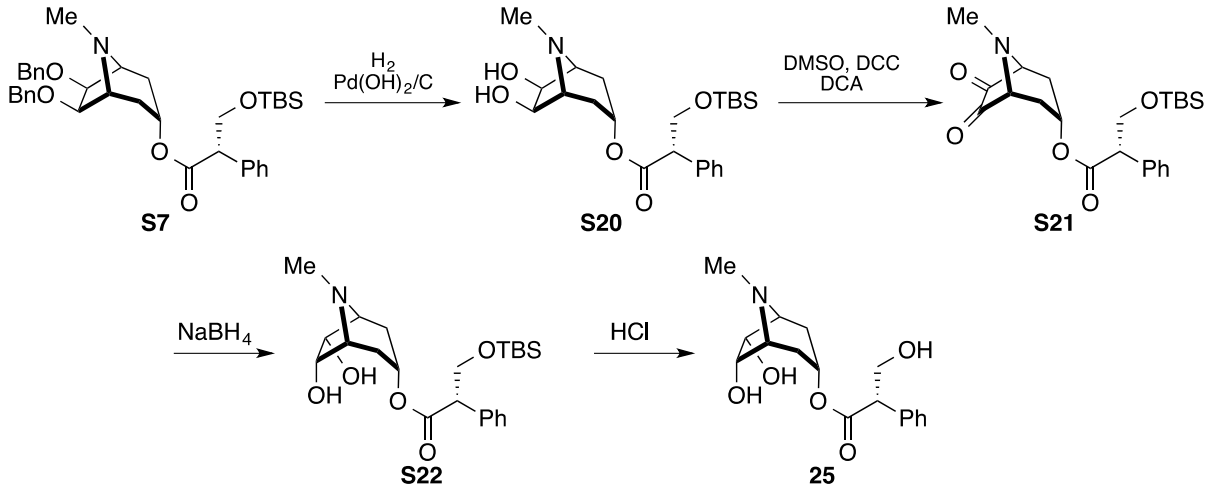


6β,7α-Dihydroxyhyoscyamine (20)

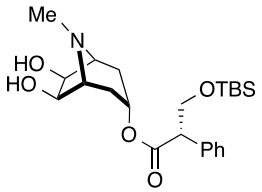
A mixture of **S16** (50.0 mg, 0.141 mmol), **S6** (57 mg, 0.215 mmol), DCC (47 mg, 0.23 mmol), and DMAP (2 mg, 0.016 mmol) in DCM (12 mL) was stirred at room temperature for 3 days. The resulting solution was filtered through Celite to remove the white precipitates. The filtrate was loaded on a silica gel column pre-equilibrated with $CHCl_3$. The column was washed with $CHCl_3$, and the product was eluted with $CHCl_3$/MeOH = 20/1. Fractions containing ester **S17** were collected and concentrated. The residue was dissolved in MeOH (5 mL) and treated with HCl (1 N, 0.3 mL) at room temperature. After 36 h, the solvent was removed under reduced pressure and the resulting residue was dissolved in $CHCl_3$. The solution was washed with saturated aqueous $NaHCO_3$. The aqueous solution was extracted with $CHCl_3$, and the combined organic phase was dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The crude product was separated by flash chromatography on silica gel (ethyl acetate/MeOH = 25/1 then $CHCl_3$/MeOH = 20/1). Factions containing

S18 were collected and concentrated. The resulting residue (28 mg) was used in the next step without further purification. The residue was dissolved in AcOH (0.2mL)/MeOH (2.0 mL)/H_2O (2.0 mL) and 20% $Pd(OH)_2/C$ (200 mg) was added. The reaction mixture was stirred under a hydrogen atmosphere (1 atm) for 12 h at room temperature. The reaction mixture was filtered through a pad of Celite, and the filtrate was concentrated. The crude product was purified by flash chromatography on silica gel ($CHCl_3$/MeOH/30% NH_3 = 100/17/1) to yield **20** (9.2 mg, 20% from **S16**) as a mixture of two inseparable diastereomers in 3 : 2 ratio due to epimerization at C2′ during the esterification reaction. Major isomer (2′*S*)-**20**: ^{1}H NMR ($CDCl_3$, 400 MHz, see Figure S18) δ 7.38–7.23 (5H, m, Ph), 5.13 (1H, dd, *J* = 5.9 Hz, *J* = 5.9 Hz, H-3), 4.36 (1H, d, *J* = 2.3 Hz, H-6), 4.31 (1H, dd, *J* = 2.3 Hz, *J* = 6.3 Hz, H-7), 4.16 (1H, dd, *J* = 10.2 Hz, *J* = 11.1 Hz, H-3′), 3.85 (1H, dd, *J* = 4.5 Hz, *J* = 10.2 Hz, H-2′), 4.16 (1H, dd, *J* = 4.5 Hz, *J* = 11.1 Hz, H-3′), 3.65 (3H, s, br, OH), 3.20 (1H, m, H-1), 3.00 (1H, s, H-5), 2.49 (3H, s, NMe), 2.31 (1H, ddd, *J* = 4.0 Hz, *J* = 5.9 Hz, *J* = 15.6 Hz, H-4), 2.18 (1H, m, H-2), 1.77 (1H, d, *J* = 16.3 Hz, H-2), 1.69 (1H, d, *J* = 15.6 Hz, H-4). ^{13}C NMR (101 MHz, $CDCl_3$, see Figure S19) δ 171.3, 135.3, 129.0, 128.4, 128.0, 82.0, 81.5, 67.0, 65.0, 63.9, 61.1, 55.3, 35.0, 27.0, 22.8. ESI-HRMS calcd. for $C_{17}H_{24}NO_5^+$ $[M+H]^+$ 322.1649, found 322.1657. Minor isomer (2′*R*)-**20**: ^{1}H NMR ($CDCl_3$, 400 MHz, see Figure S18) δ 7.38–7.23 (5H, m, Ph), 5.10 (1H, dd, *J* = 5.7 Hz, *J* = 5.7 Hz, H-3), 4.23 (1H, dd, *J* = 1.8 Hz, *J* = 6.3 Hz, H-7), 4.13 (1H, dd, *J* = 8.6 Hz, *J* = 10.8 Hz, H-3′), 3.86 (1H, m, H-3′), 3.81 (1H, m, H-2′), 3.81 (1H, m Hz, H-6), 3.65 (3H, s, br, OH), 3.20 (1H, m, H-1), 2.87 (1H, s, H-5), 2.47 (3H, s, NMe), 2.23(1H, m, H-4), 2.20 (1H, m, H-2), 1.84 (1H, d, *J* = 16.0 Hz, H-2), 1.49 (1H, d, *J* = 15.7 Hz, H-4). ^{13}C NMR (101 MHz, $CDCl_3$, see Figure S19) δ 171.7, 135.3, 129.0, 128.4, 128.0, 82.2, 81.2, 67.2, 64.9, 64.1, 61.1, 54.4, 35.1, 26.6, 22.8. ESI-HRMS calcd. for $C_{17}H_{24}NO_5^+$ $[M+H]^+$ 322.1649, found 322.1657.

S2.3 Synthesis of 6α,7α-dihydroxyhyoscyamine (25)



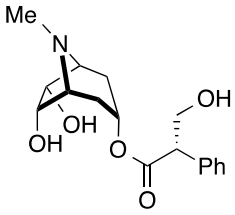
Scheme S5. Synthesis of 6β,7α-dihydroxyhyoscyamine (**25**).



6α,7α-Dihydroxy-8-methyl-8-azabicyclo[3.2.1]octan-3-yl (*S*)-3′-((*tert*-butyldimethylsilyl)oxy)-2′-phenylpropanoate (S20)

Compound **S7** (50 mg, 0.081 mmol) was dissolved in AcOH (0.01mL)/MeOH (5.0 mL) and to this solution was added 20% $Pd(OH)_2/C$ (100 mg). The reaction mixture was stirred under hydrogen atmosphere (1 atm) for 8 h. The reaction mixture was filtered through a pad of Celite, and the filtrate was concentrated. The crude product was purified by flash chromatography on silica gel ($CHCl_3$/MeOH = 10/1 then 5/1) to yield **S20** (24.8 mg, 70%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.36–7.23 (5H, m, Ph), 6.04 (s, OH), 5.00 (1H, m, H-3), 4.50 (1H, d, *J* = 6.2 Hz, H-6 or H-7), 4.16 (1H, dd, *J* = 8.8 Hz, *J* = 9.4 Hz, H-3′), 4.13 (1H, d, *J* = 6.2 Hz, H-6 or H-7), 3.80(1H, dd, *J* = 5.4 Hz, *J* = 9.4 Hz, H-3′), 3.73 (1H, dd, *J* = 5.4 Hz, *J* = 8.8 Hz, H-2′), 3.50 (1H, s, H-1 or H-5), 3.40 (1H, s, H-1 or H-5), 2.71 (3H, s, NMe),

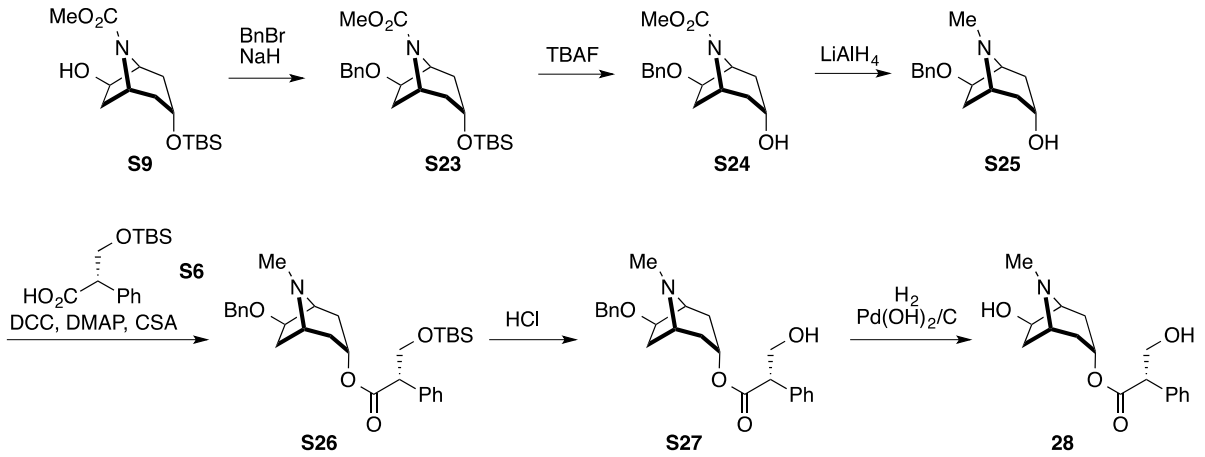
2.48–2.32 (2H, m, H-2, H-4), 1.86 (1H, d, *J* = 16.0 Hz, H-2 or H-4), 1.68 (1H, d, *J* = 16.4 Hz, H-2 or H-4), 0.84 (9H, s, Si-*t*Bu), 0.02 (3H, s, Si-Me), 0.00 (3H, s, Si-Me). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.2, 135.1, 128.9, 128.0, 127.9, 73.0, 72.9, 67.3, 67.2, 65.1, 65.1, 54.8, 36.6, 28.9, 28.7, 25.8, 18.2, –5.5, –5.5. ESI-HRMS Calcd. for $C_{23}H_{38}NO_5Si^+$ $[M+H]^+$ 436.2514, found 436.2502.



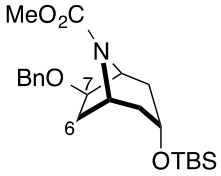
6α,7α-Dihydroxyhyoscyamine (25)

To a solution of **S20** (12 mg, 0.028 mmol) and DCC (49 mg, 0.23 mmol) in DMSO (0.3 mL), was added a solution of dichloroacetic acid (3.7 μL, 0.045 mmol) in DMSO (0.05 mL) at room temperature. After 20 h, full consumption of **S20** and formation of **S21** were confirmed by LCMS analysis. ESI-HRMS calcd. for $C_{23}H_{34}NO_5Si^+$ $[M+H]^+$ 432.2201, found 432.2200. Because of its poor stability, **S21** was not isolated. The reaction mixture was diluted with a small amount of chloroform and filtered through a pad of sand to remove white precipitates. The filtrate was evaporated to a small volume and the residual **S21** in DMSO was diluted with methanol (1 mL). The mixture was treated with $NaBH_4$ (16 mg, 0.43 mmol) at 0 °C. After 1 h, full consumption of **S21** and formation of **S22** were confirmed by LCMS analysis. ESI-HRMS calcd. for $C_{23}H_{38}NO_5Si^+$ $[M+H]^+$ 436.2514, found 436.2502. To the mixture containing **S22**, was added HCl (1 N, 0.5 mL) at room temperature. After 10 min, the reaction mixture was diluted with H_2O (approximately 5 mL) and washed with $CHCl_3$ (3 × 5 mL). The aqueous phase was lyophilized, and **25** was separated by HPLC using a semipreparative C18 column (Agilent, ZORBAX, ODS, 5 μm, 9.4 mm x 250 mm). Compound **25** was eluted isocratically with 0.1% TFA in 15% aqueous acetonitrile (flow rate: 4 mL/min) and monitored by UV absorbance at 220 nm. Fractions containing **25** were collected and lyophilized to give **25** as the TFA salt (6.0 mg, 41% from **S20**). NMR analysis of the obtained sample showed signals corresponding to an unknown isomer of **25**, which co-eluted with **25** in HPLC analysis. ^{1}H NMR (D_2O, 400 MHz) δ 7.31–7.17 (5H, m, Ph), 4.86 (1H, dd, *J* = 6.3 Hz, *J* = 6.3 Hz, H-3), 4.54 (1H, dd, *J* = 6.9 Hz, *J* = 9.0 Hz, H-6 or H-7), 4.41 (1H, dd, *J* = 6.9 Hz, *J* = 9.0 Hz, H-6 or H-7), 4.00 (1H, m, H-3′), 3.84 (1H, m, H-1 or H-5), 3.81 (1H, m, H-2′), 3.79 (1H, m, H-3′), 3.75 (1H, m, H-1 or H-5), 2.66 (3H, s, NMe), 2.35 (1H, H-2 or H-4), 2.31 (1H, H-2 or H-4), 2.20 (1H, d, *J* = 17.2 Hz, H-2 or H-4), 2.01 (1H, d, *J* = 17.0 Hz, H-2 or H-4). ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.6, 135.0, 129.1, 128.4, 128.1, 64.8, 64.8, 63.0, 63.0, 63.0, 62.2, 53.5, 39.7, 28.9, 28.6. ESI-HRMS Calcd. for $C_{17}H_{24}NO_5^+$ $[M+H]^+$ 322.1649, found 322.1629.

S2.4 Synthesis of 7β-hydroxyhyoscyamine (28)

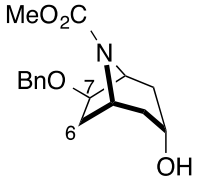


Scheme S6. Synthesis of 7β-hydroxyhyoscyamine (**28**)



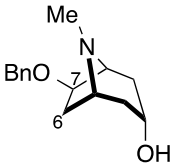
Methyl (1*S*,3*R*,5*S*,7*R*)-7-(benzyloxy)-3-((*tert*-butyldimethylsilyl)oxy)-8-azabicyclo[3.2.1]-octane-8-carboxylate (S23)

Compound **S23** was synthesized based on the same procedure as described for **S3** in 97% yield. This compound was observed as a mixture of two conformers (56:44 ratio) by NMR analysis. NMR assignments for a mixture of both conformers are shown. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.33–7.23 (5H, m, Ph), 4.55–4.41 (3H, m, CH_2 of Bn and H-7), 4.40 (1H, m, H-5), 4.32 (1H, m, H-1), 4.29 (1H, m, H-5), 4.19 (1H, m, H-1), 3.96 (1H, dd, *J* = 4.1 Hz, *J* = 4.1 Hz, H-3), 3.68 (3H, s, NCO_2Me), 3.68 (3H, s, NCO_2Me), 2.67 (1H, dd, *J* = 7.2 Hz, *J* = 13.0 Hz, H-6), 2.63 (1H, dd, *J* = 7.0 Hz, *J* = 13.0 Hz, H-6), 2.11–1.84 (3H, m, H-2, H-4, H-6), 1.64 (1H, m, H-4), 1.57 (1H, m, H-2), 0.81 (9H, s, Si-*t*Bu), 0.81 (9H, s, Si-*t*Bu), -0.01 (3H, s, Si-Me), -0.02 (3H, s, Si-Me), –0.05 (3H, s, Si-Me), –0.05 (3H, s, Si-Me). ^{13}C NMR (101 MHz, $CDCl_3$) δ 154.7, 154.5, 138.2, 138.2, 128.4, 127.8, 127.7, 127.5, 81.7, 80.9, 70.9, 70.6, 65.1, 59.0, 58.3, 53.2, 53.2, 52.2, 38.4, 38.2, 37.6, 37.5, 37.4, 36.6, 25.7, 17.7, –5.1, –5.2, –5.2, –5.2. ESI-HRMS calcd. for $C_{22}H_{36}NO_4Si^+$ $[M+H]^+$ 406.2408, found 406.2426.



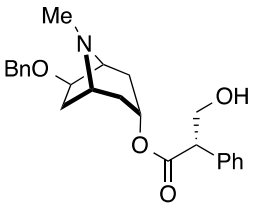
Methyl (1*S*,3*R*,5*S*,7*R*)-7-(benzyloxy)-3-hydroxy-8-azabicyclo[3.2.1]octane-8-carboxylate (S24)

Compound **S24** was similarly synthesized from **S23** as described for **S4** and was obtained in 99% yield. This compound was observed as a mixture of two conformers (approximately 56:44 ratio) by NMR analysis. NMR assignments for a mixture of both conformers are shown. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.34–7.23 (m, 5H, Ph), 4.55–4.42 (m, 3H, CH_2 of Bn and H-7), 4.39 (m, 1H, H-1). 4.32 (1H, m, H-1), 4.30 (m, 1H, H-5), 4.21 (1H, m, H-1), 4.06 (1H, dd, *J* = 4.4 Hz, *J* = 4.4 Hz, H-3), 3.68 (s, 3H, NCO_2Me), 3.67 (s, 3H, NCO_2Me), 2.67 (1H, dd, *J* = 7.2, *J* = 13.2 Hz, H-6), 2.63 (dd, 1H, *J* = 13.1 Hz, *J* = 7.0 Hz, H-7), 2.17–1.88 (3H, m, H-2, H-4, H-6), 1.88 (1H, brs, OH), 1.76 (1H, m, H-4), 1.72 (1H, m, H-4), 1.66 (1H, m, H-2). ^{13}C NMR (101 MHz, $CDCl_3$) δ 154.8, 154.6, 138.3, 138.2, 128.3, 127.7, 127.6, 127.5, 82.4, 81.6, 71.0, 70.8, 64.6, 58.7, 58.2, 52.9, 52.9, 52.3, 52.3, 38.3, 37.9, 37.6, 37.4, 36.7, 36.0. ESI-HRMS calcd. for $C_{16}H_{22}NO_4^+$ $[M+H]^+$ 292.1543, found 292.1559.



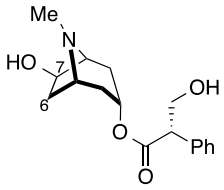
(1*S*,3*R*,5*S*,7*R*)-7-(Benzyloxy)-8-methyl-8-azabicyclo[3.2.1]octan-3-ol (S25)

Compound **S25** was synthesized from **S24** as described for the preparation of **S5**. The yield was 87%. This compound was observed as a mixture of two conformers (87:13 ratio) by NMR analysis. NMR assignments are shown only for the major isomer. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.30–7.20 (m, 5H, Ph), 4.54 (dd, *J* = 2.7 Hz, *J* = 7.2 Hz, H-7), 4.45 (d, 1H, *J* = 11.9 Hz, CH_2 of Bn), 4.45 (d, 1H, *J* = 11.9 Hz, CH_2 of Bn), 3.90 (dd, 1H, *J* = 5.1 Hz, *J* = 5.1 Hz, H-3), 3.22 (m, 1H, H-5), 3.16 (m, 1H, H-1), 2.60 (dd, 1H, *J* = 7.2 Hz, *J* = 13.2 Hz, H-6), 2.48 (s, 3H, NMe), 2.10–1.99 (m, 3H, H-4, H-2, H-6), 1.61 (d, 1H, *J* = 14.5 Hz, H-2), 1.51 (d, 1H, *J* = 14.3 Hz, H-4). ^{13}C NMR (101 MHz, $CDCl_3$) δ 138.7, 128.3, 127.5, 127.3, 84.1, 71.2, 65.4, 63.4, 60.4, 40.1, 37.3, 36.2. 36.1. ESI-HRMS calcd. for $C_{15}H_{22}NO_2^+$ $[M+H]^+$ 248.1645, found 248.1667.



7β-Benzyloxyhyoscyamine (S27)

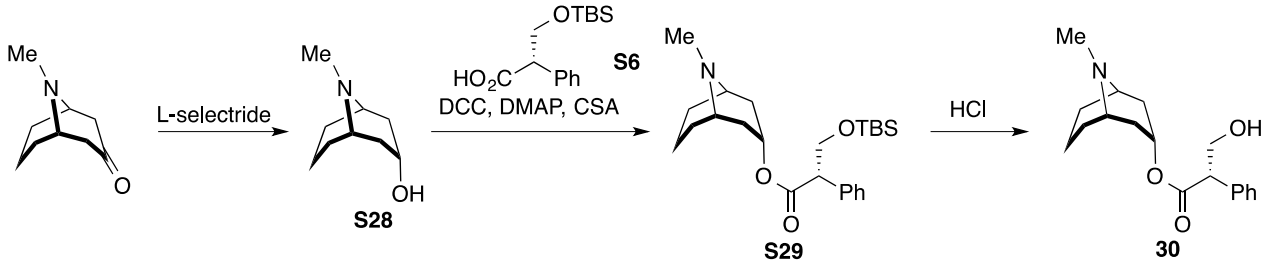
Compound **S27** was prepared from **S25** (see Scheme S6) as **S18** was prepared from **S16** (see synthesis of **20**). The yield was 43%. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.38–7.21 (10H, m, Ph), 5.04 (1H, dd, *J* = 6.5 Hz, *J* = 6.5 Hz, H-3), 4.13 (1H, dd, *J* = 10.3 Hz, *J* = 12.4 Hz, H-3′), 4.09 (1H, d, *J* = 11.7 Hz, CH_2 of Bn), 4.04 (1H, d, *J* = 11.7 Hz, CH_2 of Bn), 3.78 (1H, m, H-3′), 3.77 (1H, m, H-2′), 4.10 (1H, dd, *J* = 3.0 Hz, *J* = 7.2 Hz, H-7), 3.23 (1H, m, H-5), 3.03 (1H, m, H-1), 2.46 (3H, s, NMe), 2.22 (1H, dd, *J* = 13.5 Hz, *J* = 7.2 Hz, H-6), 2.10 (1H, m, H-4), 2.06 (1H, m, H-2), 2.01 (1H, m, H-6), 1.58 (1H, d, *J* = 15.0 Hz, H-4), 1.49 (1H, d, *J* = 15.2 Hz, H-2). ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.0, 138.8, 135.7, 129.0, 128.3, 128.2, 127.9, 127.4, 127.4, 84.1, 71.2, 68.0, 64.6, 64.1, 59.7, 54.4, 40.1, 36.0, 34.3, 33.0. ESI-HRMS Calcd. for $C_{17}H_{24}NO_4^+$ $[M+H]^+$ calc. 396.2169, found 396.2174.



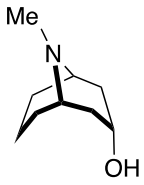
7β-Hydroxyhyoscyamine (28)

Compound **28** was synthesized based on the same method as described for **19**. The yield was 65%. Spectroscopic data of **28** were consistent with those of the natural product isolated from plants.[5] ^{1}H NMR ($CDCl_3$, 400 MHz, see Figure S20) δ 7.38–7.23 (5H, m, Ph), 5.00 (1H, dd, *J* = 5.5 Hz, *J* = 5.5 Hz, H-3), 4.16 (1H, dd, *J* = 8.3 Hz, *J* = 10.3 Hz, H-3′), 3.81 (1H, m, H-3′), 3.77 (1H, m, H-2′), 3.76 (1H, m, H-7), 3.18 (1H, m, H-5), 2.80 (1H, m, H-1), 2.78 (1H, s, br, OH), 2.42 (3H, s, NMe), 2.33 (1H, dd, *J* = 13.7 Hz, *J* = 7.3 Hz, H-6), 2.10 (1H, m, H-4), 2.03 (1H, ddd, *J* = 15.6 Hz, *J* = 5.5 Hz, *J* = 4.0 Hz, H-2), 1.75 (1H, m, H-6), 1.42 (1H, d, *J* = 15.4 Hz, H-4), 1.35 (1H, d, *J* = 15.6 Hz, H-2). ^{13}C NMR (101 MHz, $CDCl_3$, see Figure S21) δ 172.1, 135.6, 129.0, 128.1, 127.8, 75.4, 67.8, 66.5, 64.0, 58.0, 54.5, 40.2, 36.4, 30.1, 28.5. ESI-HRMS calcd. for $C_{17}H_{24}NO_4^+$ $[M+H]^+$ 306.1700, found 306.1707.

S2.5 Synthesis of 9-methyl-9-azabicyclo[3.3.1]nonan-3-yl (*S*)-3′-hydroxy-2′-phenylpropanoate (30)

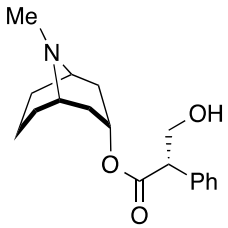


Scheme S7. Synthesis of 9-methyl-9-azabicyclo[3.3.1]nonan-3-yl (S)-3-hydroxy-2-phenylpropanoate (**30**).



9-Methyl-9-azabicyclo[3.3.1]nonan-3-ol (S28)

To a solution of pseudopelletierine (1.00 g, 6.53 mmol) in THF (50 mL), L-selectride (1 M solution in THF, 6.85 mL, 6.85 mmol) was added dropwise at –78 °C. After 3 h, the reaction was quenched with acetone (50 mL). The resulting solution was concentrated under reduced pressure. The crude product was purified by flash chromatography on silica gel ($CHCl_3$/MeOH/30% NH_3 = 100/5/1, then 80/20/1) to give **S28** (0.61 g, 60%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.16 (1H, tt, *J* = 6.9 Hz, *J* = 6.9 Hz, H-3), 2.94 (2H, m, H-1, H-5), 2.41 (3H, s, NMe), 2.40–2.32 (2H, m, H-2, H-4), 2.23 (dtt, *J* = 13.6 Hz, *J* = 5.2 Hz, *J* = 13.6 Hz, H-7), 1.92 (2H, dddd, *J* = 4.8 Hz, *J* = 4.8 Hz, *J* = 13.6 Hz, *J* = 13.6 Hz, H-6, H-8), 1.43 (1H, m, H-7), 1.32 (2H, ddd, *J* = 2.4 Hz, *J* = 6.9 Hz, *J* = 14.3 Hz, H-2, H-4), 1.13 (2H, m, H-6, H-8). Two sets of signals were observed for C-2 (C-4), C-3, C-7, and Me because **S28** exists as two conformational isomers.^{13}C NMR (101 MHz, $CDCl_3$) δ 62.6, 62.5, 51.7, 40.3, 40.3, 34.8, 34.8, 24.9, 14.3. 14.3. ESI-HRMS calcd. for $C_9H_{18}NO^+$ $[M+H]^+$ 156.1383, found 156.1391.



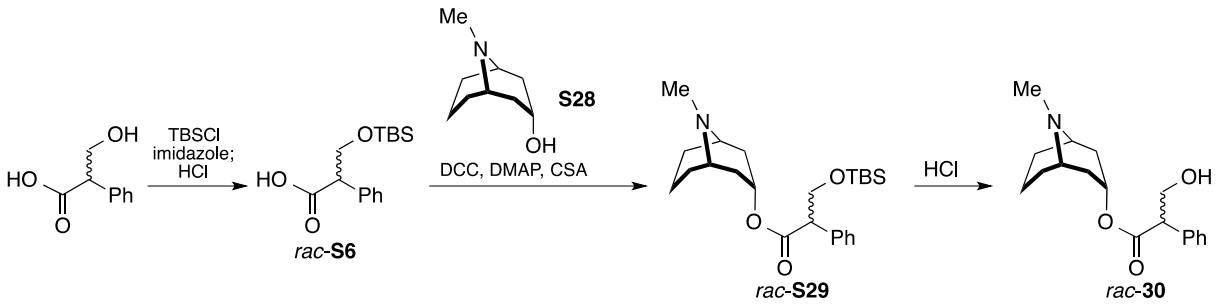
9-Methyl-9-azabicyclo[3.3.1]nonan-3-yl (*S*)-3′-hydroxy-2′-phenylpropanoate (30)

Synthesis of **30** was previously reported without NMR data.[8] A mixture of **S28** (300 mg, 1.93 mmol), **S6** (769 mg, 2.90 mmol), DCC (637 mg, 3.09 mmol), DMAP (24 mg, 0.193 mmol), CSA (134 mg, 0.579 mmol) in DCM (20 mL) was stirred at room temperature for 48 h. The resulting solution was filtered through Celite to remove the white precipitates. The filtrate was loaded on a silica gel column pre-equilibrated with $CHCl_3$. The product was eluted with $CHCl_3$/MeOH =20/1. Fractions containing **S29** (monitored by ^{1}H NMR) were combined and concentrated. The obtained residue was used in the next step without further purification. The residue was dissolved in MeOH (40 mL) and 1.0 N HCl (2.5 mL) was added. The mixture was stirred overnight at room temperature before evaporation to a small volume. The resulting acidic solution (pH 1) was diluted with H_2O (30.0 mL) and washed with $CHCl_3$ (30 mL x 3). The aqueous solution was neutralized to pH 7–8 by freshly

prepared saturated aqueous $NaHCO_3$ and then extracted with $CHCl_3$ (30 mL × 3). The combined $CHCl_3$ phase was dried over Na_2SO_4, filtered, and concentrated to give **30** (456 mg, 78% in 2 steps). ^{1}H NMR ($CDCl_3$, 400 MHz, see Figure S22) δ 7.28–7.18 (5H, m, Ph), 5.10 (1H, tt, *J* = 3.4 Hz, *J* = 7.0 Hz, H-3), 4.09 (1H, dd, *J* = 8.0 Hz, *J* = 9.6 Hz, H-3′), 3.74 (1H, m, H-2′), 3.72 (1h, m, H-3′), 2.79 (1H, m, H-5), 2.70 (1H, m, H-1), 2.33 (3H, s, NMe), 2.31 (1H, m, H-4), 2.23 (1H, ddd, *J* = 7.6 Hz, *J* = 7.6 Hz, *J* = 15.2 Hz, H-2), 1.96–1.66 (3H, m, H-6, H-7, H-8), 1.41 (1H, m, H-4), 1.19 (1H, m, H-2), 1.15 (1H, m, H-6), 1.11 (1H, m, H-7), 0.78 (1H, m, H-8). ^{13}C NMR (101 MHz, $CDCl_3$, see Figure S23) δ 172.4, 135.8, 128.7, 128.1, 127.5, 66.0, 64.2, 54.6, 51.0, 50.9, 40.5, 30.1, 30.1, 25.4, 25.3, 14.1. ESI-HRMS calcd. for $C_{18}H_{26}NO_3^+$ $[M+H]^+$ 304.1907, found 304.1897.

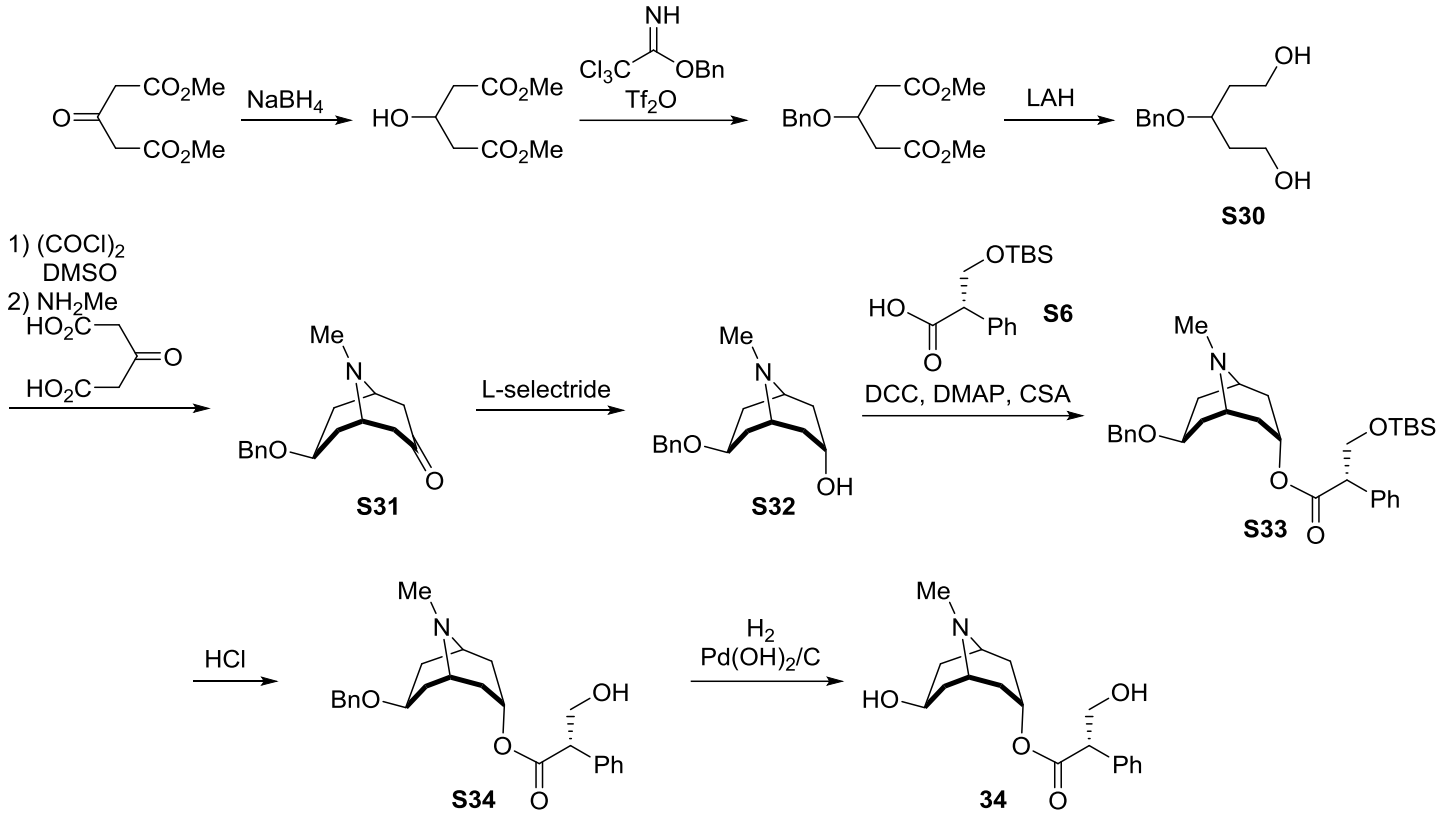
Determination of the enantiomeric purity of 30.

In order to determine the enantiomeric purity of **30**, racemic **30** (i.e., *rac*-**30**) was synthesized as described above using commercially available racemic tropic acid as the starting material (Scheme S8). The NMR and mass spectroscopic analysis of *rac*-**30** was the same as that of the optically active **30**. Baseline analytical chromatographic separation of the *R* an *S* enantiomers of *rac*-**30** was achieved using a chiral cellulose column (CHIRAL ART Cellulose-C, 5μm, 250 mm x 4.6 mm, YMC America) under the following conditions: 10% ethanol in *n*-hexane with 0.1% diethylamine (isocratic), flow rate 1 mL/min, detection at 230 nm (see Figure S15). Comparison with HPLC analysis of the optically active **30** allowed assignment of the elution time for the *S* enantiomer as 11 min with the undesired *R* enantiomer eluting at 6 min. These assignments allowed the enantiomeric purity of **30** to be assigned as 93% based on the HPLC peak integrations (see Figure S15). Formation of the undesired *R* during preparation of **30** may have been caused by epimerization during the esterification reaction in the presence of DMAP.

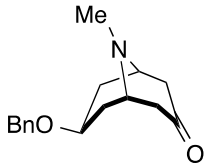


Scheme S8. Synthesis of a racemic mixture of 9-methyl-9-azabicyclo[3.3.1]nonan-3-yl-3′-hydroxy-2′-phenylpropanoate (*rac*-**30**).

S2.6. Synthesis of 7β-hydroxy-9-methyl-9-azabicyclo[3.3.1]nonan-3-yl (*S*)-3′-hydroxy-2′-phenylpropanoate (34)

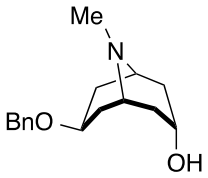


Scheme S9. Synthesis of 7β-hydroxy-9-methyl-9-azabicyclo[3.3.1]nonan-3-yl (S)-3-hydroxy-2-phenylpropanoate (**34**).



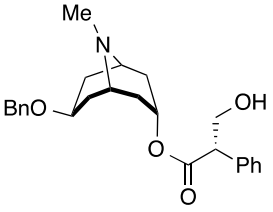
7-Exo-(benzyloxy)-9-methyl-9-azabicyclo[3.3.1]nonan-3-one (S31)

To a solution of oxalyl chloride (0.282 mL, 3.28 mmol) in DCM (8 mL), DMSO (0.311 mL, 4.37 mmol) was added dropwise at –78 °C. After 5 min, **S30** (prepared as described in the literature,[9] 0.230 g, 1.09 mmol) in DCM (4 mL) was added to the reaction mixture. After 30 min, triethylamine (2.0 mL) was added to the mixture, and stirring was continued at 0 °C for 1 h. The mixture was diluted with toluene (8 mL) and filtered to remove the white precipitates. The filtrate was concentrated under reduced pressure. To the crude dialdehyde intermediate was added H_2O (2 mL), acetone 1,3-dicarboxylic acid (153 mg, 1.09 mmol), 6 N HCl (0.20 mL), and methylamine (40% aqueous solution, 0.177 mg, 1.09 mmol). The mixture was stirred for 1.5 h at 23 °C, heated to 50 °C for 6 h and cooled to 0 °C, at which point the reaction mixture was basified to pH >9 by adding 6 N NaOH. The resulting solution was extracted with DCM (10 mL × 3). The organic phase was dried over Na_2SO_4, filtered, and concentrated. The crude product was purified by flash chromatography on silica gel ($CHCl_3$/MeOH = 50/1) to afford **S31** (59.6 mg, 21%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.37–7.26 (5H, m, Ph), 4.46 (2H, s, CH_2 of Bn), 3.62 (1H, m, H-7), 3.42 (2H, m, H-1, H-5), 2.75 (2H, dd, *J* = 6.8 Hz, *J* = 16.5 Hz, H-2, H-4), 2.61 (3H, s, NMe), 2.30 (2H, d, *J* = 11.8 Hz, H-2, H-4), 1.93–1.88 (4H, m, H-6, H-8). ^{13}C NMR (101 MHz, $CDCl_3$) δ 209.4, 138.4, 128.4, 127.7, 127.6, 70.3, 69.4, 55.7, 43.9, 40.5, 34.4. ESI-HRMS calcd. for $C_{16}H_{22}NO_2^+$ $[M+H]^+$ 260.1645, found 260.1642.



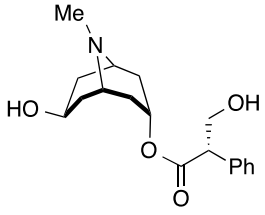
7-Exo-(benzyloxy)-9-methyl-9-azabicyclo[3.3.1]nonan-3-ol (S32)

To a solution of **S31** (60 mg, 0.23 mmol) in THF (2 mL), L-selectride (1 M solution in THF, 0.253 mL, 0.253 mmol) was added dropwise at –78 °C. After 1 h, the reaction was quenched with saturated aqueous NH_4Cl. After basifying with 6 N NaOH (pH > 10), the solution was extracted with DCM (10 mL × 3). The combined organic phase was dried over Na_2SO_4, filtered, and concentrated. The crude product was purified using flash chromatography on silica gel ($CHCl_3$/MeOH = 50/1) to afford **S32** (59.6 mg, 21%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.34–7.22 (5H, m, Ph), 4.56 (1H, m, H-7), 4.53 (2H, s, CH_2 of Bn), 4.02 (1H, m, H-3), 3.03 (2H, m, H-1, H-5), 2.42 (3H, s, NMe), 2.32 (2H, m, H-2, H-4), 1.85 (2H, ddd, *J* = 5.0 Hz, *J* = 11.0 Hz, *J* = 12.8 Hz, H-6, H-8), 1.74 (2H, dd, *J* = 6.1 Hz, *J* = 11.8 Hz, *J* = 12.8 Hz, H-6, H-8), 1.41 (2H, m, H-2, H-4). ^{13}C NMR (101 MHz, $CDCl_3$) δ 139.2, 128.3, 127.7, 127.4, 70.0, 69.8, 61.8, 52.3, 40.3, 36.4, 30.8. ESI-HRMS calcd. for $C_{16}H_{24}NO_2^+$ $[M+H]^+$ 262.1802, found 262.1804.



7-Exo-(benzyloxy)-9-methyl-9-azabicyclo[3.3.1]nonan-3-yl (*S*)-3′-hydroxy-2′-phenylpropanoate (S34)

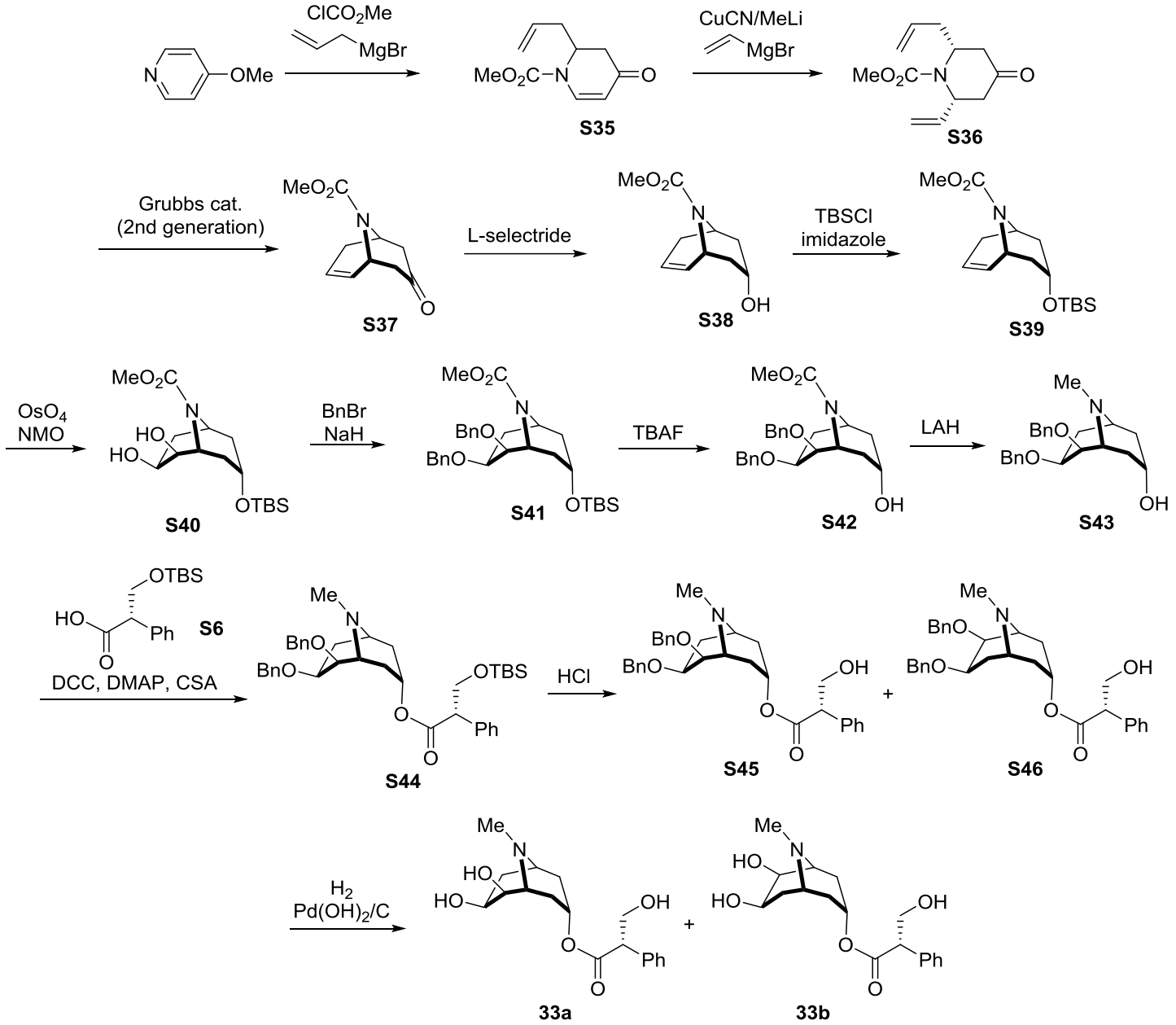
A mixture of **S32** (41.0 mg, 0.157 mmol), **S6** (63.5 mg, 0.235 mmol), DCC (51.8 mg, 0.251 mmol), DMAP (2 mg, 0.016 mmol), and CSA (11 mg, 0.047 mmol) in DCM (2 mL) was stirred at room temperature for 36 h. The resulting solution was filtered through Celite to remove the white precipitates. The filtrate was loaded on a silica gel column pre-equilibrated with $CHCl_3$. The column was washed with $CHCl_3$, and the product was eluted with $CHCl_3$/MeOH =20/1. Fractions containing the ester product **S33** were collected and concentrated. The resulting residue was dissolved in MeOH (5 mL) and treated with HCl (1 N, 0.3 mL) at room temperature. After 19 h, the reaction mixture was concentrated and partitioned between $CHCl_3$ and H_2O. The aqueous phase was neutralized with saturated aqueous $NaHCO_3$ (pH 8) and extracted with $CHCl_3$. The combined organic phase was washed with brine, dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The crude product was purified by flash chromatography on silica gel ($CHCl_3$/MeOH/30% NH_3 = 200/10/1) to yield **S34** (58.0 mg, 90%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.38–7.16 (10H, m, Ph), 5.13 (1H, tt, *J* = 2.4 Hz, *J* = 6.9 Hz, H-3), 4.24 (1H, d, *J* = 12.0 Hz, CH_2 of Bn), 4.23 (1H, d, *J* = 12.0 Hz, CH_2 of Bn), 4.13 (1H, m, H-7), 4.03 (1H, dd, *J* = 9.1 Hz, *J* = 11.1 Hz, H-3′), 3.70 (1H, dd, *J* = 4.9 Hz, *J* = 11.1 Hz, H-3′), 3.61 (1H, dd, *J* = 4.9 Hz, *J* = 9.1 Hz, H-2′), 3.03 (1H, m, H-1 or H-5), 2.96 (1H, m, H-1 or H-5), 2.51 (1H, br, OH), 2.43 (3H, s, NMe), 2.43–2.99 (2H, m, H-2 or H-4), 1.89–1.71 (3H, m, H-6, H-8, H-6 (or H-8)), 1.62 (1H, d, *J* = 15.6 Hz, H-2 or H-4), 1.47 (1H, dd, *J* = 6.4 Hz, *J* = 13.2 Hz, H-6 or H-8), 1.42 (1H, d, *J* = 15.6 Hz, H-2 or H-4). ^{13}C NMR (101 MHz, $CDCl_3$) δ172.5, 139.3, 135.3, 128.9, 128.4, 128.0, 127.8, 127.4, 127.3, 69.3, 69.2, 65.7, 64.9, 54.2, 51.8, 51.8, 40.5, 33.0, 33.0, 30.6, 30.4. ESI-HRMS calcd. for $C_{25}H_{32}NO_4^+$ $[M+H]^+$ 410.2326, found 410.2317



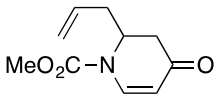
7β-Hydroxy-9-methyl-9-azabicyclo[3.3.1]nonan-3-yl (*S*)-3′-hydroxy-2′-phenylpropanoate (34)

Compound **S34** (20.8 mg, 0.0508 mmol) was dissolved in AcOH (0.2 mL)/MeOH (2.0 mL)/ H_2O (2.0 mL) and 20% $Pd(OH)_2$/C (20 mg) was added. The reaction mixture was stirred under a hydrogen atmosphere (1 atm) for 12 h at room temperature. The reaction solution was filtered through a pad of Celite, and the filtrate was concentrated to give **34** (15.0 mg, 91%). ^{1}H NMR ($CDCl_3$, 400 MHz, see Figure S24) δ 7.40–7.24 (5H, m, Ph), 5.06 (1H, m, H-3), 4.03 (1H, m, H-3′), 4.13 (1H, tt, *J* = 6.0 Hz, *J* = 10.0 Hz, H-7), 3.83– 3.75 (2H, m, H-2′, H-3′), 2.96 (1H, m, H-1 or H-5), 2.86 (1H, m, H-1 or H-5), 2.66 (2H, br, OH), 2.39 (3H, s, NMe), 2.38–2.21 (2H, m, H-2, H-4), 1.75–1.55 (3H, m, H-6, H-8, H-6 (or H-8)), 1.55 (1H, d, *J* = 15.9 Hz, H-2 or H-4), 1.37 (1H, d, *J* = 15.6 Hz, H-2 or H-4), 1.10 (1H, dd, *J* = 5.8 Hz, *J* = 13.2 Hz, H-6 or H-8). ^{13}C NMR (101 MHz, $CDCl_3$, see Figure S25) δ 172.3, 135.8, 129.0, 128.3, 127.8, 66.0, 64.3, 62.1, 54.3, 51.5, 51.4, 40.2, 34.0, 33.6, 31.3, 31.2. ESI-HRMS calcd. for $C_{18}H_{26}NO_4^+$ $[M+H]^+$ 320.1856, found 320.1848.

S2.7. Synthesis of 6β,7β-dihydroxy-9-methyl-9-azabicyclo[3.3.1]nonan-3-yl (*S*)-3′-hydroxy-2′-phenylpropanoate (33)



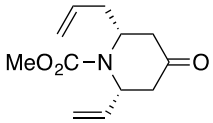
Scheme S10. Synthesis of 6β,7β-dihydroxy-9-methyl-9-azabicyclo[3.3.1]nonan-3-yl (*S*)-3′-hydroxy-2′-phenylpropanoate (**33**).



Methyl 2-allyl-4-oxo-3,4-dihydropyridine-1(2*H*)-carboxylate (S35)

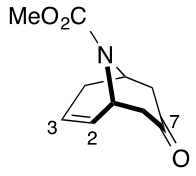
To a solution of 4-methoxypyridine (7.26 mL, 71.5 mmol) in THF (500 mL) was added methyl chloroformate (5.59 mL, 72.2 mmol) at –30 °C. After stirring for 1 h, vinylmagnesium bromide (1.0 M in THF, 75.1 mL, 75.1 mmol) was added slowly over 30 min. The mixture was warmed to 10 °C over 2 h and then poured into 10% HCl (200 mL). The mixture was stirred at room temperature for 10 min and extracted with ethyl acetate (3 × 400 mL). The combined organic phase was washed with saturated aqueous $NaHCO_3$, brine, dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The crude product was purified by flash chromatography on silica gel (hexanes / ethyl acetate = 3/1) to yield **S35** (6.55 g, 47%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.68 (1H, d, *J* = 7.7 Hz), 5.63 (1h, ddt, *J* = 16.9 Hz, *J* = 7.3 Hz, *J* = 7.3 Hz), 5.25 (1h, d, *J* = 7.7 Hz),

5.04–4.92 (2H, m), 4.54 (1H, m), 3.77 (3H, s), 2.70 (1H, dd, *J* = 6.4 Hz, *J* = 16.6 Hz), 2.41 (1H, d, *J* = 16.6 Hz), 2.37–2.21 (2H, m). ^{13}C NMR ($CDCl_3$,101 MHz) δ 192.9, 152.9, 141.7, 132.7, 119.1, 107.0, 54.1, 52.5, 39.1, 35.0. ESI-HRMS calcd. for $C_{10}H_{14}NO_3^+$ $[M+H]^+$ 196.0968, found 196.0921.



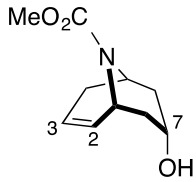
Methyl 2-allyl-4-oxo-6-vinylpiperidine-1-carboxylate (S36)

To a suspension of CuCN (2.75 g, 30.7 mmol) in THF (52 mL), was added MeLi (1.6 M in diethylether, 19.2 mL, 30.7 mmol) dropwise at –78 °C. The mixture was stirred at 0 °C for 10 min, and then cooled to –78 °C. To the resulting mixture, was added vinylmagnesium bromide (0.7 M in THF, 43.9 mL, 30.7 mmol) dropwise. After 10 min, a solution of **S35** (4.00 g, 20.5 mmol) in THF (15 mL) was added dropwise. After 6 h, the mixture was poured into a vigorously stirred mixture (9:1) of saturated aqueous NH_4Cl/NH_4OH (200 mL). After 1 h, the resulting solution was extracted with ethyl acetate (3 × 200 mL), and the combined organic phase was washed with brine, dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The crude product was purified by flash chromatography on silica gel (hexanes / ethyl acetate = 4/1) to yield **S36** (3.95 g, 86%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.90 (1H, ddd, *J* = 4.9 Hz, *J* = 10.6 Hz, *J* = 17.8 Hz), 5.65 (1H, tdd, *J* = 7.3 Hz, *J* = 8.7 Hz, *J* = 17.0 Hz), 5.21 (1H, m), 5.21–5.13 (2H, m), 5.06 (1H, m), 5.02 (1H, m), 4.56 (1H, m), 3.73 (3H, s), 2.66 (2H, m), 2.58 (1H, dd, *J* = 7.3 Hz, *J* = 15.2 Hz), 2.48–2.39 (2H, m), 2.21 (1H, ddd, *J* = 8.7 Hz, *J* = 8.7 Hz, *J* = 16.6 Hz). ^{13}C NMR ($CDCl_3$,101 MHz) δ 207.1, 156.1, 138.8, 134.1, 118.3, 116.4, 53.3, 53.0, 52.9, 42.9, 42.2, 40.7. ESI-HRMS calcd. for $C_{12}H_{18}NO_3^+$ $[M+H]^+$ 224.1281, found 224.1273.



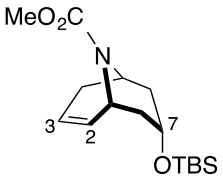
Methyl 7-oxo-9-azabicyclo[3.3.1]non-2-ene-9-carboxylate (S37)

S37 was synthesized as described in the literature with some modifications.[10] Grubbs second generation catalyst (570 mg, 0.67 mmol, 5 mol%) was added to a solution of **S36** (3.00 g, 13.4 mmol) in DCM (1.34 L) at room temperature. After 12 h, the mixture was concentrated under reduced pressure. The crude product was purified by flash chromatography on silica gel (hexanes / ethyl acetate = 2/1) to yield **S37** (2.4 g, 92%). Two sets of signals were observed for H-1, H-5, C-1, C-2, C-3, C-4, C-5, C-6, and C-8, because of a mixture of two conformational isomers. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.77–5.64 (2H, m, H-2, H-3), 5.00–4.78 (2H, m, H-1, H-5), 4.75 (3H, s, NCO_2Me), 2.67–2.52 (3H, H-4, H-6, H-8), 2.32 (1H, d, *J* = 14.6 Hz, H-8), 2.23 (1H, d, *J* = 15.8 Hz, H-6), 1.96 (1H, d, *J* = 18.1 Hz, H-4). ^{13}C NMR ($CDCl_3$,101 MHz) δ 207.8, 154.7, 128.4, 127.9, 124.6, 124.0, 52.9, 48.3, 48.1, 47.0, 47.0, 46.8, 46.5, 45.0, 44.7, 30.8, 30.5. ESI-HRMS calcd. for $C_{10}H_{14}NO_3^+$ $[M+H]^+$ 196.0968, found 196.0952.



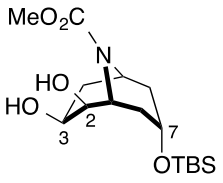
Methyl 7-hydroxy-9-azabicyclo[3.3.1]non-2-ene-9-carboxylate (S38)

L-Selectride (1.0 M solution in THF, 13.1 mL, 13.1 mmol) was added dropwise to a solution of **S37** (2.34 g, 12.0 mmol) in THF (100 mL) at –78 °C. After 1 h, the reaction was quenched with saturated aqueous NH_4Cl (100 mL). The resulting solution was extracted with ethyl acetate (3 × 100 mL), and the combined organic phase was washed with brine, dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The crude product was purified by flash chromatography on silica gel (hexanes / ethyl acetate = 2/3) to yield **S38** (1.92 g, 81%). Two sets of signals were observed for H-1, H-5, C-1, C-2, C-3, C-4, C-5, C-6, C-8, and CO because of the existence of two conformational isomers. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.15 (1H, m, H-2), 5.88 (1H, m, H-3), 4.71–4.48 (2H, m, H-1, H-5), 4.01 (1H, m, H-7), 3.68 (3H, s, NCO_2Me), 2.84 (1H, m, OH), 2.67 (1H, m, H-4), 2.21–2.09 (2H, H-4, H-6), 1.94–1.85 (3H, m, H-6, H-8, H-8). ^{13}C NMR ($CDCl_3$,101 MHz) δ 154.8, 154.7, 132.9, 132.3, 127.0, 126.5, 65.2, 52.5, 45.9, 45.3, 44.5, 43.7, 38.2, 37.8, 35.4, 34.9, 30.8, 30.4. ESI-HRMS calcd. for $C_{10}H_{16}NO_3^+$ $[M+H]^+$ 198.1125, found 198.1122.



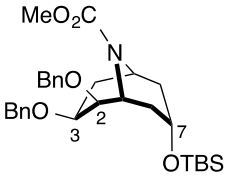
Methyl 7-((*tert*-butyldimethylsilyl)oxy)-9-azabicyclo[3.3.1]non-2-ene-9-carboxylate (S39)

To a solution of **S38** (1.90 g, 9.63 mmol) in DMF (20 mL), was added imidazole (983 mg, 14.4 mmol) and TBSCl (1.72 g, 11.6 mmol) at room temperature. After 6 h, the mixture was diluted with H_2O. The resulting solution was extracted with a mixture (4:1) of hexanes and ethyl acetate (3 × 100 mL), and the combined organic phase was washed with brine, dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The crude product was purified by flash chromatography on silica gel (hexanes / ethyl acetate = 8/1) to yield **S39** (2.59 g, 86%). **S39** exists as a mixture of two conformers based on NMR analysis. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.81 (1H, m, H-2), 5.62 (1H, m, H-3), 4.61–4.39 (2H, m, H-1, H-5), 4.03 (1H, m, H-7), 3.67 (3H, s, NCO_2Me), 2.55 (1H, m, H-4), 2.08–1.97 (2H, H-4, H-6), 1.87 (1H, m, H-8), 1.71–1.60 (2H, m, H-6, H-8), 0.83 (9H, s, Si-*t*Bu), –0.02 (6H, s, Si-Me), –0.02 (6H, s, Si-Me). Two sets of ^{13}C signals were observed for C-1, C-2, C-3, C-4, C-5, C-6, C-8, CO, OMe, and SiMe ^{13}C NMR ($CDCl_3$,101 MHz) δ 155.0, 154.9, 129.8, 129.2, 125.2, 124.6, 64.5, 52.4, 52.4, 46.1, 45.6, 44.7, 44.0, 38.7, 38.2, 35.7, 35.2, 30.7, 30.4, 25.6, 17.8, –4.97, –5.01, –5.17, –5.20. ESI-HRMS calcd. for $C_{16}H_{30}NO_3Si^+$ $[M+H]^+$ 312.1989, found 312.1987.



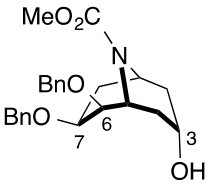
Methyl 7-((*tert*-butyldimethylsilyl)oxy)-2,3-dihydroxy-9-azabicyclo[3.3.1]nonane-9-carboxylate (S40)

To a mixture of **S39** (500 mg, 1.61 mmol) and 50% *N*-methylmorpholine (490 mg, 2.41 mmol) in a mixture of acetone and H_2O (4:1, 50 mL), was added OsO_4 (a small crystal) at room temperature. After 6 h, the reaction was quenched with saturated aqueous $NaHSO_3$ (2 mL) and was diluted with H_2O. The resulting solution was extracted with ethyl acetate (3 × 100 mL), and the combined organic phase was washed with brine, dried over Na_2SO_4, filtered, and evaporated under reduced pressure to yield **S40** (551 mg, 100%). Two sets of signals were observed for H-1, H-3, H-3, H-5, C-1, C-2, C-3, C-4, C-5, C-6, C-8, CO, and Me because **S40** exists as two conformational isomers. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.95–4.80 (1H, m, H-3), 4.55–4.31 (2H, m, H-1, H-5), 3.87–3.71 (2H, m, H-2, H-7), 3.68 (3H, s, NCO_2Me), 3.15–2.45 (2H, br, OH), 2.22–2.08 (2H, m, H-6, H-8), 1.82–1.68 (2H, m, H-4), 1.47 (2H, m, H-6, H-8), 0.86 (9H, s, Si-*t*Bu), –0.02 (6H, s, Si-Me). ^{13}C NMR ($CDCl_3$,101 MHz) δ 156.5, 156.2, 71.6, 71.5, 63.2, 63.1, 63.0, 52.9, 52.7, 51.4, 51.3, 45.6, 45.1, 36.4, 36.0, 34.5, 33.8, 33.5, 33.1, 25.7, 17.9, –4.9, –4.9. ESI-HRMS calcd. for $C_{16}H_{31}NO_5SiNa^+$ $[M+Na]^+$ 368.1864, found 368.1773.



Methyl 2,3-bis(benzyloxy)-7-((*tert*-butyldimethylsilyl)oxy)-9-azabicyclo[3.3.1]nonane-9-carboxylate (S41)

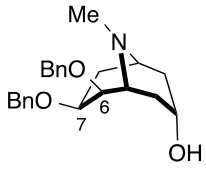
Compound **S41** was synthesized based on the same method as described for **S3**. The yield was 94%. **S41** exists as a mixture of two conformers based on NMR analysis. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.44–7.22 (10H, m, Ph), 4.85–4.41 (7H, m, H-1, H-3, H-5, CH_2 of Bn), 3.87 (1H, m, H-7), 3.70 (3H, s, NCO_2Me), 3.65 (1H, m, H-2), 3.61 (3H, s, NCO_2Me), 2.31–2.05 (3H, m, H-4, H-6, H-8), 1.76 (1H, m, H-4), 1.45 (1H, m, H-8), 2.34 (1H, m, H-6), 0.88 (9H, s, Si-*t*Bu), 0.04 (6H, s, Si-Me). ^{13}C NMR (101 MHz, $CDCl_3$) δ 156.0, 155.8, 138.9, 138.8, 138.8, 138.8, 128.3, 128.2, 128.2, 127.9, 127.4, 127.3, 127.3, 127.2, 76.3, 75.4, 71.0, 70.8, 70.7, 70.2, 70.1, 63.9, 63.8, 52.6, 52.4, 48.2, 47.0, 45.3, 45.0, 36.5, 36.1, 33.1, 32.7, 32.4, 31.9, 25.8, 18.0, 18.0, –4.8, –4.8, –4.8, –4.8. ESI-HRMS calcd. for $C_{30}H_{44}NO_{45}^+$ $[M+H]^+$ calc. 526.2983, found 526.2982.



Methyl 2,3-bis(benzyloxy)-7-hydroxy-9-azabicyclo[3.3.1]nonane-9-carboxylate (S42)

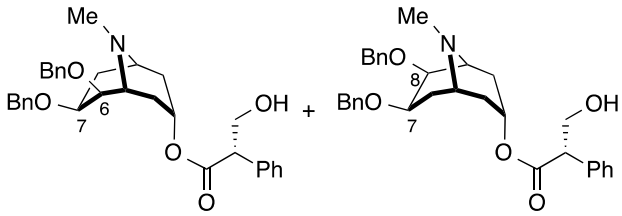
Compound **S42** was synthesized based on the same method as described for **S4**. The yield was nearly quantitative. **S42** exists as a mixture of two conformers based on NMR analysis. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.42–7.22 (10H, m, Ph), 4.85–4.39 (7H, m, H-1, H-3, H-5, CH_2 of Bn), 3.83 (1H, m, H-7), 3.68 (3H, s, NCO_2Me), 3.61–3.57 (1H, m, H-4), 3.59 (3H, s, NCO_2Me),

2.37–2.18 (2H, m, H-6, H-8), 2.15–1.95 (2H, m, H-4, OH), 1.70 (1H, m, H-4), 1.36 (1H, m, H-6), 1.24 (1H, m, H-8). ^{13}C NMR (101 MHz, $CDCl_3$) δ 156.0, 155.8, 138.7, 138.7, 138.6, 138.6, 128.3, 128.3, 128.2, 128.2, 127.9, 127.5, 127.5, 127.4, 127.3, 127.3, 76.1, 75.2, 71.0, 70.7, 70.3, 70.2, 70.1, 70.0, 63.3, 63.2, 52.7, 52.5, 47.9, 46.9, 45.2, 44.9, 35.5, 35.3, 32.5, 32.1, 32.1, 31.9. ESI-HRMS calcd. for $C_{24}H_{30}NO_5^+$ $[M+H]^+$ 412.2118, found 412.2108.



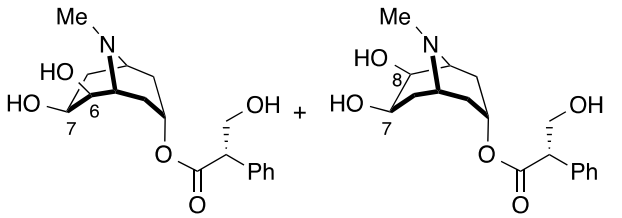
6,7-Bis(benzyloxy)-9-methyl-9-azabicyclo[3.3.1]nonan-3-ol (S43)

Compound **S43** was synthesized based on the same method as described for **S5**. The yield was 85%. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.41–7.23 (10H, m, Ph), 4.71 (2H, s, CH_2 of Bn), 4.63 (1H, ddd, *J* = 4.2 Hz, *J* = 5.2 Hz, *J* = 12.2 Hz, H-7), 4.54 (2H, s, CH_2 of Bn), 4.06 (1H, m, H-3), 3.61 (1H, m, H-6), 3.21 (1H, m, H-5), 3.12 (1H, m, H-1), 2.59 (3H, s, NMe), 2.38 (1H, m, H-2), 2.32–2.22 (2H, m, H-4, H-8), 1.60 (1H, ddd, *J* = 1.8 Hz, *J* = 5.1 Hz, *J* = 12.2 Hz, H-8), 1.28 (1H, ddd, *J* = 1.8 Hz, *J* = 5.0 Hz, *J* = 14.7 Hz, H-2), 1.10 (1H, ddd, *J* = 1.9 Hz, *J* = 5.0 Hz, *J* = 14.8 Hz, H-4). ^{13}C NMR (101 MHz, $CDCl_3$) δ139.2, 139.1, 128.2, 128.2, 127.7, 127.6, 127.3, 127.3, 78.5, 72.1, 70.9, 70.0, 62.3, 55.5, 52.2, 42.1, 34.2, 31.8, 29.3. ESI-HRMS calcd. for $C_{23}H_{30}NO_3^+$ $[M+H]^+$ calcd. 368.2220, found 368.36.



6β,7β-Bis(benzyloxy)-9-methyl-9-azabicyclo[3.3.1]nonan-3-yl (*S*)-3′-hydroxy-2′-phenylpropanoate (S45) and 7β,8β-Bis(benzyloxy)-9-methyl-9-azabicyclo[3.3.1]nonan-3-yl (*S*)-3′-hydroxy-2′-phenylpropanoate (S46)

Compound **S45** was synthesized from **S43** (see Scheme S10) as **S18** was prepared from **S16** (see synthesis of **20**). **S45** was obtained as a mixture with its diastereomer **S46** in 96% yield. Diastereomer 1: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.38–7.15 (15H, m, Ph), 5.17 (1H, m, H-3), 4.71 (2H, s, CH_2 of Bn), 4.32 (1H, d, *J* = 12.2 Hz, CH_2 of Bn), 4.30 (1H, d, *J* = 12.2 Hz, CH_2 of Bn), 4.19 (1H, m, H-7), 4.01 (1H, dd, *J* = 9.0 Hz, *J* = 2.2 Hz, H-3′), 3.72 (1H, dd, *J* = 2.6 Hz, *J* = 5.0 Hz, H-3′), 3.62 (1H, m, H-2′), 3.40 (1H, m, H-6), 3.16 (1H, m, H-5), 3.13 (1H, m, H-1), 2.59 (3H, s, NMe), 2.55–2.17 (4H, m, H-2, H-4, H-8, OH), 1.65 (1H, ddd, *J* = 1.5 Hz, *J* = 5.0 Hz, *J* = 12.3 Hz, H-8), 1.45 (1H, m, H-2), 1.09 (1H, m, H-4). Because of the presence of the two diastereomers, signals differing by less than 0.2 ppm in the ^{13}C NMR could not be assigned unambiguously, therefore assignment of the ^{13}C NMR signals to one diastereomer or the other should not be considered definitive. ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.4, 139.1, 135.4, 128.9, 128.3, 128.2, 128.0, 127.8, 127.7, 127.7, 127.3, 127.2, 127.1, 78.0, 72.2, 71.0, 69.6, 66.2, 64.8, 55.1, 54.2, 51.6, 42.1, 30.1, 29.4, 28.0. Diastereomer 2: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.38–7.15 (15H, m, Ph), 5.17 (1H, m, H-3), 4.66 (2H, s, CH_2 of Bn), 4.28 (2H, s, CH_2 of Bn), 4.24 (1H, m, H-7), 4.05 (1H, dd, *J* = 2.3 Hz, *J* = 9.1 Hz, H-3′), 3.69 (1H, dd, *J* = 2.6 Hz, *J* = 5.0 Hz, H-3′), 3.62 (1H, m, H-2′), 3.62 (1H, m, H-6), 3.21 (1H, m, H-5), 3.08 (1H, m, H-1), 2.59 (3H, s, NMe), 2.55–2.17 (4H, m, H-2, H-4, H-8, OH), 1.47 (1H, m, H-8), 1.29 (1H, m, H-4), 1.25 (1H, m, H-2). ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.4, 139.2, 135.4, 128.9, 128.3, 128.2, 128.0, 127.8, 127.8, 127.7, 127.3, 127.2, 127.1, 77.8, 72.2, 71.1, 69.6, 66.3, 64.8, 55.2, 54.2, 51.7, 42.0, 30.3, 29.4, 28.2. ESI-HRMS calcd. for $C_{32}H_{38}NO_5^+$ $[M+H]^+$ calc. 516.2744, found 516.2750.



6β,7β-Dihydroxy-9-methyl-9-azabicyclo[3.3.1]nonan-3-yl (*S*)-3′-hydroxy-2′-phenylpropanoate (33a) and 7β,8β-Dihydroxy-9-methyl-9-azabicyclo[3.3.1]nonan-3-yl (*S*)-3′-hydroxy-2′-phenylpropanoate (33b)

Compound **33** was synthesized as a diastereomeric mixture of **33a** and **33b** in 98% yield based on the same method as described for **20**. Compounds **33a** and **33b** co-elute as a single peak during HPLC analysis. NMR spectra of one of the two isomers is nearly identical to that of the enzymatically obtained **33** (see Section S4.5 for summary of

NMR properties) with small changes in chemical shift possibly caused by pH variation between the samples (see Figures S34–S36). NMR properties for the nonenzymatic product determined from the diastereomeric mixture of **33a** and **33b**: ^{1}H NMR (D_2O, 400 MHz) δ 7.38–7.23 (5H, m, Ph), 5.08 (1H, d, *J* = 7.0 Hz, H-3), 4.38 (1H, dd, *J* = 4.0 Hz, *J* = 6.2 Hz, H-7), 4.07 (1H, dd, *J* = 7.6 Hz, *J* = 10.4 Hz, H-3′), 3.79 (1H, m, H-2′), 3.72 (1H, m, H-3′), 3.54 (1H, m, H-8), 3.49 (1H, m, H-5), 3.37 (1H, m, H-1), 2.78 (3H, s, NMe), 2.64–2.46 (2H, m, H-2, H-4), 2.03 (1H, m, H-6), 1.87 (1H, d, *J* = 17.4 Hz, H-4), 1.83 (1H, m, H-6), 1.15 (1H, d, *J* = 17.8 Hz, H-2). ^{13}C NMR (D_2O, 101 MHz) δ 172.6, 134.8, 129.1, 128.1, 128.0, 69.3, 62.1, 61.5, 59.6, 59.3, 53.4, 53.2, 37.7, 31.7, 25.7, 22.7. ESI-HRMS calcd. for $C_{18}H_{26}NO_5^+$ $[M+H]^+$ calc. 336.1805, found 336.1810.

S3. Cloning and expression of *h6h*, and purification of H6H

The *h6h* gene was first amplified by polymerase chain reaction (PCR) using pMH1[1] as the template and cloned into the *Nde*I/*Xho*I site of the pET24b(+) plasmid for the expression of H6H without a His_6 tag. The desired recombinant plasmid was then used to transform *E. coli*. BL21 star (DE3). An overnight culture of *E. coli* BL21 star (DE3) transformant grown at 37 °C in LB medium supplemented with kanamycin (50 mg/mL) was used in a 200-fold dilution to inoculate 6 L of the same medium. The large cultures were grown at 15 °C to minimize the formation of inclusion bodies. When the OD_{600} reached 0.4–0.6, IPTG (isopropyl β-D-1-thiogalactopyranoside) was added to a final concentration of 0.1 mM to induce gene expression. After incubation for an additional 24 h at 15 °C, cells were harvested by centrifugation at 4 °C, washed with Tris·HCl buffer (20 mM, pH 7.5), pelleted again by centrifugation, and stored at –80 °C. The typical yield was 6 g of wet cells per liter of culture.

All purification operations were carried out at 4 °C. Thawed cells were resuspended in lysis buffer (20 mM Tris·HCl, pH 7.5, 0.1 mM DTT, 1 mM EDTA). The cell suspension was subjected to 8 × 30 s ultrasonic bursts, with a 1 min cooling interval between each blast. Cellular debris was removed by centrifugation. The protein pellet was resuspended in a minimal amount of Tris·HCl buffer (20 mM, pH 7.5) and applied to a DEAE-Sepharose CL-6B column (2.5 cm × 24 cm) pre-equilibrated with the same buffer. The elution was then continued with a linear gradient of NaCl from 80 to 240 mM in 20 mM Tris·HCl buffer, pH 7.5. The fractions containing the desired H6H protein as determined by SDS-PAGE, were pooled, concentrated by ultrafiltration on an Amicon concentrator using an YM 10 membrane, and desalted by dialyzing against 20 mM Tris·HCl buffer (pH 7.5) with 10% glycerol. The purified H6H protein was stored at –80 °C. Protein concentration was determined by NanoDrop. The SDS-PAGE gel analysis of the purified H6H is shown in Figure S1.

S4. In vitro enzyme activity assay of H6H with substrate analogues

S4.1 Typical assay conditions and procedures

A hyoscyamine analogue (1 mM) was incubated with H6H (31–68 μM), α-KG (5 mM), $FeSO_4$ (0.4 mM), sodium ascorbate (4 mM) in Tris·HCl buffer (50 mM, pH 7.4) at room temperature (100 μL total volume). Reactions were terminated by one of two different methods. Method A: two reaction volumes of acetonitrile were added to the incubation mixture and the resulting mixture was centrifuged to remove the precipitated proteins. Method B: the incubation mixture was cooled to 0 °C and filtered through an YM-10 membrane using an Amicon ultrafiltration unit to remove protein. Quenched reaction mixtures were stored at –20 °C until analysis by HPLC with UV detection and/or LCMS. For HPLC analysis, the sample was eluted isocratically using 0.1% TFA in aqueous acetonitrile. The percentage of acetonitrile varied from 13% to 25% depending on the polarity of the substrate analogue used in the assay. Elution of the products and substrate was monitored by setting the UV-detector at 220 nm. For LCMS analysis, the quenched reaction mixture (typically 5 μL) was diluted 200-fold with H_2O, filtered through a 0.2 μm PTFE membrane syringe filter (VWR international), and injected (typically 1 μL of the diluted sample) to the LCMS system equipped with an Eclipse Plus C18 column. Separation was achieved

by the gradient program described in Section S1.

S4.2 Derivatization of enzymatic products

Derivatization of hydroxyketone 23 using Ac_2O

Compound **20** was incubated with H6H as described above (100 μL total volume, 12 h). After filtration through a YM-10 membrane, the filtrate (80 μL) was subjected to HPLC analysis and eluted isocratically with a solvent of 0.1% TFA in 15% aqueous acetonitrile. The fraction containing **23** was collected and lyophilized. The resulting sample was dissolved in pyridine (0.1 mL) and treated with Ac_2O (0.1 mL) at 80 °C. After 15 min, the excess reagents were removed under reduced pressure. The residue was then dissolved in ethyl acetate (0.6 mL) and washed with saturated aqueous $NaHCO_3$ (0.5 mL). After filtration, the organic phase was analyzed by LCMS as described above (Figure S12).

Reduction of hydroxyketone 23 using $NaBH_4$

The lyophilized sample described above was dissolved in methanol (0.2 mL) and treated with $NaBH_4$ (500 mM in methanol, 5 μL) at 0 °C. After 1 h, the reaction mixture (0.25 mL) was diluted with H_2O (0.2 mL) and analyzed by LCMS after filtration (Figure S4 and S12).

S4.3 Incubation of 20 under $^{18}O_2$

All the reaction components (total 50 μL) except for $^{18}O_2$ were anaerobically mixed in a 1.5 mL tube in the glove box. The tube was placed in a 20 mL vial, which was then capped with a rubber septum. The vial was removed from the glove box and the headspace evacuated using a needle and vacuum pump. The reaction was then initiated by introducing $^{18}O_2$ (99 atom %) using a balloon. After 5 h, the incubation mixture was quenched with acetonitrile (0.1 mL). After centrifugation under aerobic atmosphere, the supernatant was analyzed by LCMS as described above. The obtained ESI-MS spectrum is shown in Figure S5a.

S4.4 Incubation of 20 in $H_2{}^{18}O$

A solution containing **20**, α-KG, $FeSO_4$, sodium ascorbate, and Tris·HCl was lyophilized and dissolved in 50 μL $H_2{}^{18}O$ (97 atom %). The reaction was initiated by mixing the solution with lyophilized H6H. After 5 h, the incubation mixture was quenched and analyzed as described above. The obtained ESI-MS spectrum is shown in Figure S5b. To further study the ^{18}O incorporation, the incubation was quenched at different time points 10–300 min (Figure S5c). Wash-out of the ^{18}O from the [^{18}O]-**23** was also investigated by monitoring of ESI-MS spectrum of **23** after 300-fold dilution with $H_2{}^{16}O$ (Figure S5d).

S4.5 Isolation and characterization of enzymatic products 31 (31a or 31b) and 33 (33a or 33b)

Compound **30** (1 mM) was incubated with H6H (68 μM), α-KG (5 mM), $FeSO_4$ (0.4 mM), sodium ascorbate (4 mM) in Tris·HCl buffer (50 mM, pH 7.4) at room temperature (20 mL total volume). The reaction was quenched by addition of acetonitrile (40 mL) and the resulting mixture was centrifuged to remove the precipitated protein. The supernatant was dried under reduced pressure. The crude products were separated by HPLC using a semipreparative C18 column (Agilent, ZORBAX, ODS, 5 μm, 9.4 mm x 250 mm). The HPLC column was eluted using 0.1% TFA in H_2O as mobile phase A and 0.1% TFA in acetonitrile as mobile phase B at a flow rate of 4 mL/min with the following gradient program: 0–2 min 0% B, 2–19 min 0–50% B, 19–20 min 85% B, 20–25 min 85–0% B, 25–30 min 0% B. Elution of the compounds was monitored by setting the UV-detector to 220 nm. Fractions containing **31** and **33** were separately collected and lyophilized. The obtained samples were analyzed

by NMR and LCMS (Figures S12, S26–S33). **31**: ^{1}H NMR (600 MHz, D_2O) δ 7.33–7.19 (5H, m, Ph), 5.00 (1H, dd, *J* = 7.1 Hz, *J* = 7.1 Hz, H-3), 4.03 (1H, dd, *J* = 7.9 Hz, *J* = 10.9 Hz, H-3′), 3.91 (1H, m, H-6), 3.85 (1H, dd, *J* = 6.5 Hz, *J* = 7.9 Hz, H-2′), 3.79 (1H, dd, *J* = 6.5 Hz, *J* = 10.9 Hz, H-3′), 3.26 (1H, m, H-1), 3.21 (1H, m, H-5), 2.71 (3H, s, NMe), 2.50 (1H, ddd, *J* = 7.5 Hz, *J* = 7.5 Hz, *J* = 17.6 Hz, H-4), 2.46 (1H, ddd, *J* = 7.0 Hz, *J* = 7.0 Hz, *J* = 17.6 Hz, H-2), 2.08 (1H, m, H-7), 1.97 (1H, m, H-8), 1.84 (1H, d, *J* = 17.6 Hz, H-4), 1.55 (1H, d, *J* = 17.6 Hz, H-2), 1.11 (1H, dd, *J* = 5.3 Hz, *J* = 15.7 Hz, H-7), 1.05 (1H, dd, *J* = 5.8 Hz, *J* = 14.6 Hz, H-8). ^{13}C NMR (151 MHz, D_2O) δ 172.9, 135.0, 129.1, 128.2, 128.1, 67.7, 62.1, 62.0, 58.9, 53.4, 53.2, 38.4, 25.7, 23.5, 23.0, 19.6. ESI-HRMS calcd *m*/*z* for $C_{18}H_{26}NO_4^+$ $[M+H]^+$: 320.1856, obsd: 320.1848. **33**: ^{1}H NMR (600 MHz, D_2O) δ 7.35–7.21 (5H, m, Ph), 5.05 (1H, dd, *J* = 7.1 Hz, *J* = 7.1 Hz, H-3), 4.35 (1H, m, H-7), 4.04 (1H, dd, *J* = 8.2 Hz, *J* = 10.9 Hz, H-3′), 3.87 (1H, m, H-6), 3.86 (1H, m, H-2′), 3.79 (1H, dd, *J* = 6.5 Hz, *J* = 10.9 Hz, H-3′), 3.42 (1H, m, H-5), 3.35 (1H, m, H-1), 2.75 (3H, s, NMe), 2.57 (1H, ddd, *J* = 7.2 Hz, *J* = 7.2 Hz, *J* = 17.2 Hz, H-4), 2.48 (1H, ddd, *J* = 6.1 Hz, *J* = 6.1 Hz, *J* = 17.6 Hz, H-2), 1.93 (1H, d, *J* = 17.2 Hz, H-4), 1.82 (1H, (1H, ddd, *J* = 5.8 Hz, *J* = 13.9 Hz, *J* = 14.6 Hz, H-8), 1.57 (1H, d, *J* = 17.6 Hz, H-2), 1.25 (1H, dd, *J* = 6.7 Hz, *J* = 14.6 Hz, H-8). ^{13}C NMR (151 MHz, D_2O) δ 172.7, 134.9, 129.3, 128.2, 128.1, 69.7, 62.2, 61.6, 59.7, 59.0, 53.4, 53.1, 37.8, 31.1, 25.8, 22.6. ESI-HRMS calcd *m*/*z* for $C_{18}H_{26}NO_5^+$ $[M+H]^+$: 336.1805, obsd: 336.1799.

S5. Gas phase computations of model systems

Gas-phase computations were performed for models of the compounds **3**, **34**, and **31a** and the associated radicals in which the *S*-tropate ester is replaced with a methoxy group. Computations were performed in two steps. First, a fast geometry optimization was performed using Hartree-Fock theory with the small 3-21G basis set. The Hartree-Fock results were then used as inputs for density functional theory geometry optimizations using the B3LYP functional and the 6-31G* basis set. Restricted computations (RB3LYP) were used for all even-electron species and unrestricted computations (UB3LYP) were used for all odd-electron species (doublet radicals). All computations were gas-phase and performed using the *Gaussian03W* software package.[11] Following DFT geometry optimization, the HO–C–C–H dihedral angles were read directly for the even-electron species. For odd-electron species, the two angles α and β were recorded as the dihedral angles between the C–O bond and the two substituents on the adjacent trigonal carbon excluding the axis of the dihedral angle (see Figure S37). The minimum dihedral angle between the C–O bond and the adjacent *p*-orbital was then calculated from

$$\theta = \left| \frac{|\beta| - |\alpha|}{2} \right|.$$

This calculation assumes that the two planes defined by the C–H and C–C substituent bonds at the trigonal carbon with the dihedral axis intersect with equal angles the plane defined by the axis of the partially occupied *p*-orbital and dihedral axis.

In the case of the substrate **3**, four model conformers (**S47a**–**S47d**) were considered based on inversion about the bridging nitrogen and rotation about the C6–O bond (see Figure S38). All optimized geometries were within 2.0 kcal/mol of each other with *exo* HO–C–C–H angles ranging from 0.4° to 3.7°. The corresponding radical conformers (**S47a-rad7**–**S47d-rad7**) differed in energy by no more than 5.0 kcal/mol with HO–C–C–*p* dihedral angles ranging from 14.1° to 21.9° (see Figure S39).

Six conformers (**S48a**–**S48f**) were considered as models for the 7-hydroxy azobicyclononane compound **34** (see Figure S40) based on different ring-flip conformations and inversion about the bridging nitrogen. Different C7–OH rotamers were considered by determining the dihedral angle with the *exo*-C–H bonds at both C6 and C8. The boat-boat ring-flip conformation was found to be much greater in energy compared to all other ring-flip conformations and excluded from the analysis. All included conformations were within 6.0 kcal/mol of each other. The *exo* HO–C–C–H dihedral angles ranged from 35.1° to 52.3°. There were 12 corresponding radical conformers for H-atom abstraction from C6 (**S48a-rad6**–**S48f-rad6**) versus C8 (**S48a-rad8**–**S48f-rad8**, see Figure S41). All optimized radicals were within 9.0 kcal/mol of each other with HO–C–C–*p* dihedral angles ranging from 10.4° to 65.6°.

Twelve conformers (**S49a**–**S49l**) were considered as models for the 6-hydroxy azabicyclononane compound **31a** based on different ring-flip conformations, inversion about the bridging nitrogen and rotation about the C6–

OH bound (see Figure S42). All optimized geometries were within 7.5 kcal/mol of each other. The *exo* HO–C–C–H dihedral angles ranged from 39.7° to 51.9°. The corresponding radical conformers for H-atom abstraction at C7 (**S49a-rad7**–**S49l-rad7**) were within 7.0 kcal/mol of each other following geometry optimization and demonstrated HO–C–C–*p* dihehdral angles ranging from 0.4° to 23.0° (see Figure S43).

S6. X-Ray crystal structure analysis

S6.1 Compound 2

Crystals of **2** grew as large colorless prisms by slow evaporation from ethanol/hexanes. The data crystal was cut from a larger crystal and had approximate dimensions 0.26 × 0.20 × 0.15 mm. The data were collected on a Rigaku AFC12 diffractometer with a Saturn 724+ CCD using a graphite monochromator with MoKα radiation (λ = 0.71073 Å). A total of 1464 frames of data were collected using ω-scans with a scan range of 0.5° and a counting time of 41 seconds per frame. The data were collected at 100 K using a Rigaku XStream Cryostream low temperature device. Details of crystal data, data collection and structure refinement are listed in Table 1. Data collection was performed using the Rigaku Americas Corporation's Crystal Clear version 1.40.[12] Unit cell refinement and data reduction were performed using Agilent Technologies CrysAlisPro V 1.171.38.46.[13] The structure was solved by direct methods using SHELXT[14] and refined by full-matrix least-squares on F^2 with anisotropic displacement parameters for the non-H atoms using SHELXL-2016/6.[15] Structure analysis utilized the programs PLATON[16] and WinGX.[17] The hydrogen atoms on carbon were calculated in ideal positions with isotropic displacement parameters set to 1.2 x Ueq of the attached atom (1.5 x Ueq for methyl hydrogen atoms). The hydrogen atom bound to O3 was located in a Δ*F* map and refined with an isotropic displacement parameter. The absolute structure was assigned by internal comparison to the known configuration at C10. The function, $\Sigma w(|F_O|^2 - |F_C|^2)^2$, was minimized, where $w = 1 / [(\sigma(F_O))^2 + (0.0298\ P)^2 + (0.5386\ P)]$ and $P = (|F_O|^2 + 2|F_C|^2) / 3$. $R_W(F^2)$ refined to 0.0654, with $R(F)$ equal to 0.0256 and a goodness of fit, S = 1.06. Definitions used for calculating $R(F)$, $R_W(F^2)$ and the goodness of fit, S, are given below.[18] The data were checked for secondary extinction effects but no correction was necessary. Neutral atom scattering factors and values used to calculate the linear absorption coefficient are from the International Tables for X-ray Crystallography (1992).[19] The ORTEP structure for compound **2** is shown in Figure S7.

S6.2 Compound 30

Crystals of **30** were obtained as clear, colorless plates by slow evaporation from DCM/hexanes using *rac*-**30**. The data crystal was cut from a larger crystal and had approximate dimensions 0.40 × 0.27 × 0.26 mm. The data were collected on a Nonius Kappa CCD diffractometer using a Bruker AXS Apex II detector and a graphite monochromator with MoKα radiation (λ = 0.71073Å). Reduced temperatures were maintained by use of an Oxford Cryosystems 700 low-temperature device. A total of 717 frames of data were collected using ω-scans with a scan range of 0.8° and a counting time of 23 seconds per frame. Details of crystal data, data collection and structure refinement are listed in Table 2. Data reduction were performed as described for **2**. The function, $\Sigma w(|F_O|^2 - |F_C|^2)^2$, was minimized, where $w = 1 / [(\sigma(F_O))^2 + (0.0595\ P)^2 + (0.2816\ P)]$ and $P = (|F_O|^2 + 2|F_C|^2) / 3$. $R_W(F^2)$ refined to 0.146, with $R(F)$ equal to 0.0545 and a goodness of fit, S = 1.01.[18] The data were checked for secondary extinction but no correction was necessary. The ORTEP structure for compound **30** (selected 2′*S* enantiomer) is shown in Figure S8.

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[18] $R_W(F^2) = \{ \Sigma w(|F_o|^2 - |F_c|^2)^2 / \Sigma w(|F_o|)^4 \}^{1/2}$ where w is the weight given each reflection. $R(F) = \Sigma (|F_o| - |F_c|) / \Sigma |F_o|\}$ for reflections with $F_o > 4(\sigma(F_o))$. $S = [\Sigma w(|F_o|^2 - |F_c|^2)^2/(n-p)]^{1/2}$, where n is the number of reflections and p is the number of refined parameters.

[19] International Tables for X-ray Crystallography, Vol. C, Tables 4.2.6.8 and 6.1.1.4, Wilson, A. J. C., editor, Kluwer Academic Press, MA, **1992**.

Supplementary Tables

Table 1. Crystal data and structure refinement for **2**.

Empirical formula	$C_{17}H_{23}NO_3$	
Formula weight	289.36	
Temperature	100(2) K	
Wavelength	0.71073 Å	
Crystal system	tetragonal	
Space group	P 43 21 2	
Unit cell dimensions	a = 9.40160(10) Å	α = 90°
	b = 9.40160(10) Å	β = 90°
	c = 34.7460(10) Å	γ = 90°
Volume	3071.20(11) Å^3	
Z	8	
Density (calculated)	1.252 Mg/m^3	
Absorption coefficient	0.085 mm^{-1}	
F(000)	1248	
Crystal size	0.260 × 0.200 × 0.150 mm^3	
Theta range for data collection	2.244 to 25.326°	
Index ranges	$-11 \le h \le 11$, $-10 \le k \le 11$, $-41 \le l \le 41$	
Reflections collected	35012	
Independent reflections	2809 [*R*(int) = 0.0893]	
Completeness to theta = 25.242°	99.7 %	
Absorption correction	None	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	2809 / 0 / 195	
Goodness-of-fit on F^2	1.062	
Final *R* indices [$I > 2\text{sigma}(I)$]	*R*1 = 0.0256, *wR*2 = 0.0651	
R indices (all data)	*R*1 = 0.0263, *wR*2 = 0.0654	
Absolute structure parameter	0.2(3)	
Extinction coefficient	n/a	
Largest diff. peak and hole	0.132 and –0.117 e.Å^{-3}	

Table 2. Crystal data and structure refinement for **30**.

Empirical formula	$C_{18}H_{25}NO_3$	
Formula weight	303.39	
Temperature	100(2) K	
Wavelength	0.71073 Å	
Crystal system	triclinic	
Space group	P -1	
Unit cell dimensions	a = 8.145(2) Å	α = 100.403(8)°
	b = 10.350(3) Å	β = 105.477(7)°
	c = 10.824(3) Å	γ = 108.860(9)°
Volume	795.8(4) Å^3	
Z	2	
Density (calculated)	1.266 Mg/m^3	
Absorption coefficient	0.085 mm^{-1}	
F(000)	328	
Crystal size	0.400 × 0.270 × 0.260 mm^3	
Theta range for data collection	2.807 to 28.882°.	
Index ranges	$-11 \le h \le 9$, $-13 \le k \le 14$, $-14 \le l \le 14$	
Reflections collected	9440	
Independent reflections	4104 [R(int) = 0.0601]	
Completeness to theta = 25.242°	99.8 %	
Absorption correction	Semi-empirical from equivalents	
Max. and min. transmission	0.7458 and 0.6431	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	4104 / 0 / 204	
Goodness-of-fit on F^2	1.012	
Final R indices [I > 2sigma(I)]	R1 = 0.0545, wR2 = 0.1301	
R indices (all data)	R1 = 0.0780, wR2 = 0.1455	
Extinction coefficient	n/a	
Largest diff. peak and hole	0.360 and –0.255 e.Å^{-3}	

Supplementary Figures

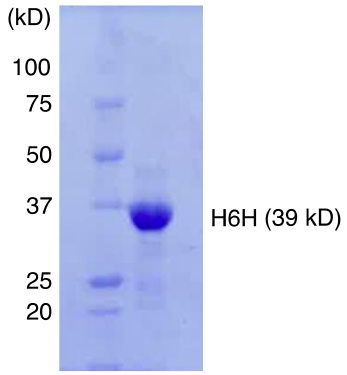


Figure S1. SDS-PAGE of purified H6H.

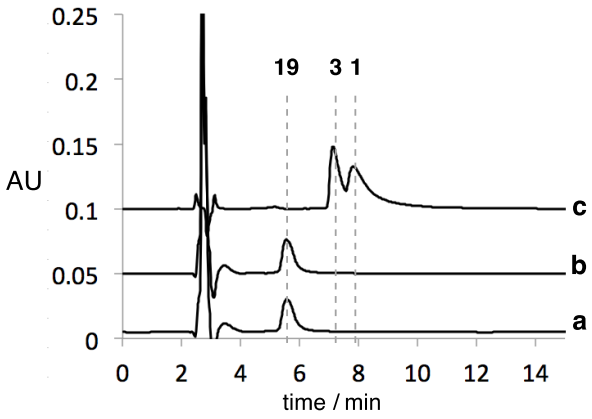


Figure S2. HPLC analysis of incubation of **19** with H6H under air in the presence of (a) α-KG or (b) succinate. Trace c shows the standard sample containing **1** and **3**.

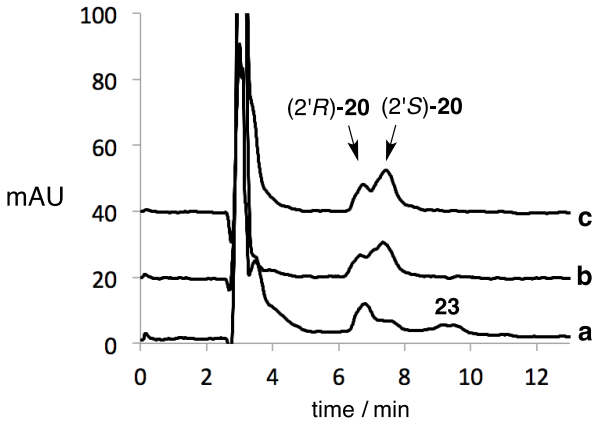


Figure S3. HPLC analysis (UV detection) following incubation of (2′S)-**20**/(2′R)-**20** (3:2 ratio) with H6H under air in the presence of (a) α-KG or (b) succinate. Trace c shows a control experiment without H6H.

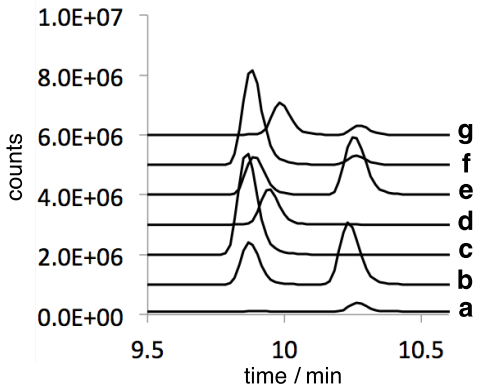
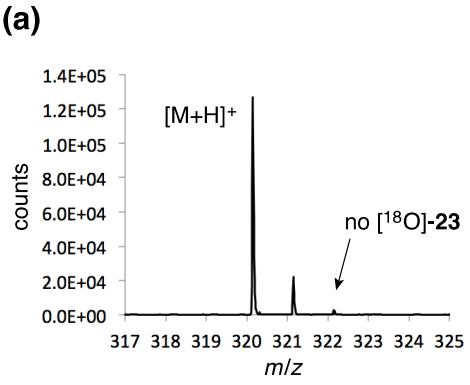
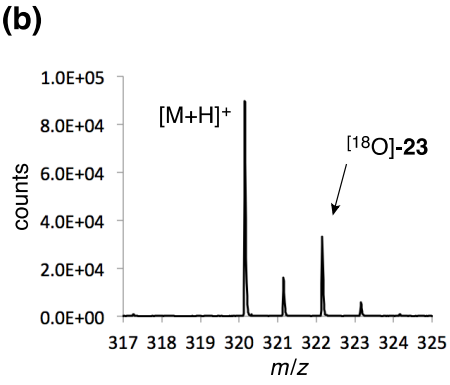


Figure S4. LCMS traces showing $NaBH_4$ reduction of the H6H reaction product from **20** (as 3:2 (2′*S*)-**20**/(2′*R*)-**20** mixture). Extracted ion chromatogram (EIC) traces corresponding to $[M+H]^+$ signals (*m*/*z* = 322.2) from each species are shown. (a) $NaBH_4$ treatment of the H6H/α-KG/air + **20** reaction product. (b) Sample of synthesized **20** showing separation of the 2′*R* (early) and 2′*S* (late) diastereomers. (c) Standard sample of **19**. (d) Standard sample of **25**. Traces e–g are co-injections of the H6H/α-KG/air + **20** product treated with $NaBH_4$ together with the standard samples **20**, **19**, and, **25**, respectively.





(c)

incubation time (min)	fraction of [^{18}O]-23 (%)
10	< 5[a]
30	< 5[a]
60	< 5[a]
120	8
300	25

[a]Acurate number could not be calculated due to small signal intensity.

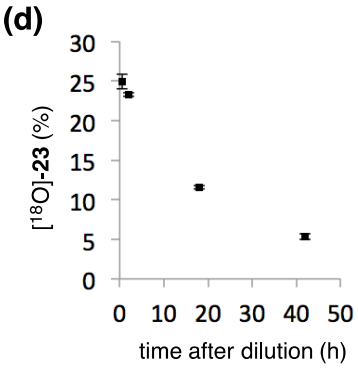


Figure S5. ^{18}O experiments with H6H/α-KG + **20** (as a 3:2 (2′*S*)-**20**/(2′*R*)-**20** mixture). (a) ESI-MS spectrum of **23** after incubation of H6H/α-KG with **20** under $^{18}O_2$ for 5 h. (b) ESI-MS spectrum of **23** after incubation of H6H with **20** in $H_2{}^{18}O$ for 5 h. (c) Incorporation of ^{18}O into **23** in the

presence of H6H and $H_2{}^{18}O$ versus time. (d) Washout of ^{18}O from [^{18}O]-**23** following dilution of the incubation mixture into $H_2{}^{16}O$.

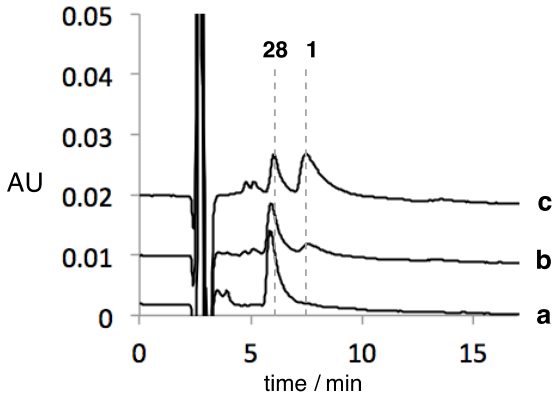


Figure S6. HPLC analysis of **28** incubated with H6H/α-KG/air for (a) 10 min or (b) 12 h. Trace c shows co-elution of the peak at 7.3 min and the standard of **1**.

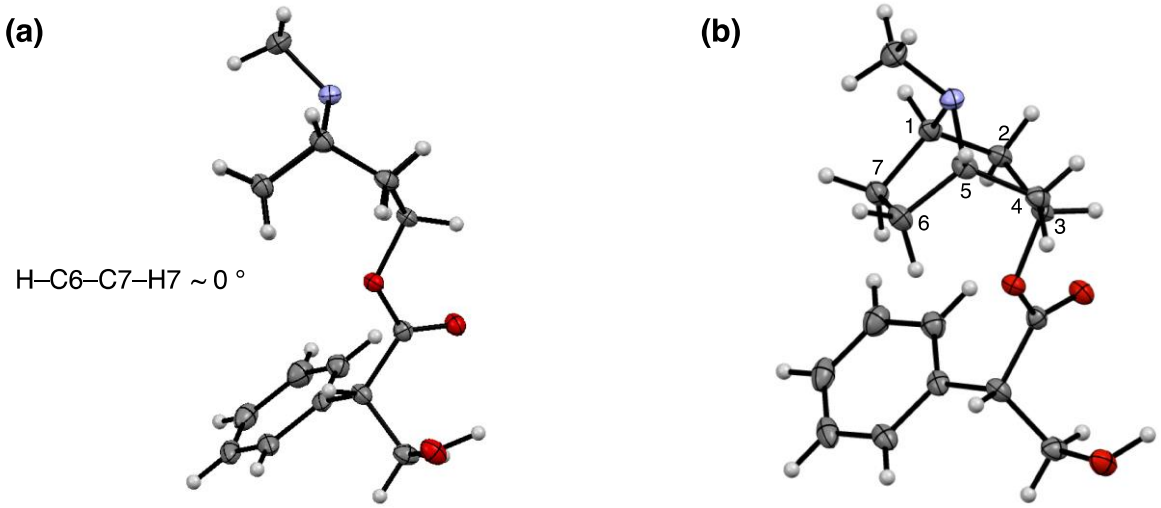


Figure S7. ORTEP structure of **2** obtained by X-ray crystallographic analysis. (a) View along C6–C7 bond corresponding to the Newman projections in Scheme 5B (b) Another view showing the envelop conformation of **2**.

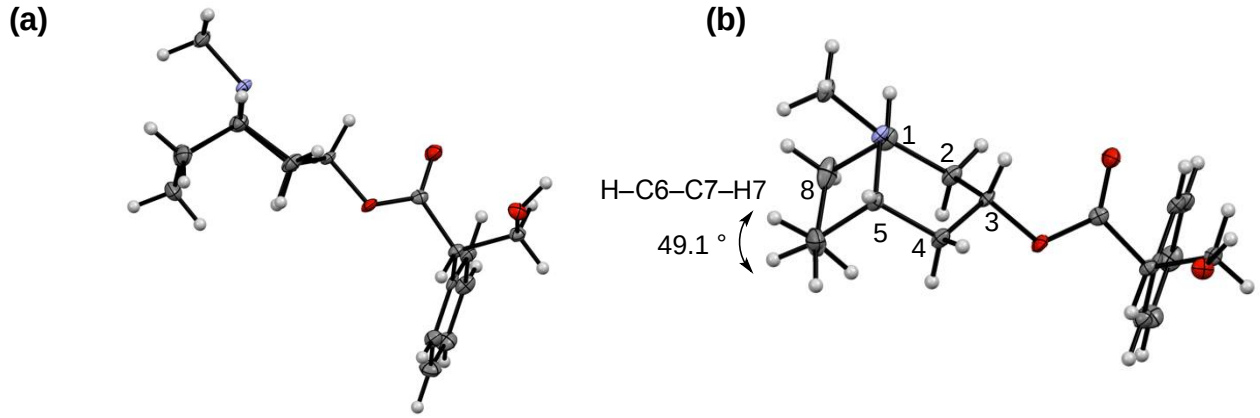


Figure S8. ORTEP structure of **30** (selected 2′*S* enantiomer) obtained by X-ray crystallographic analysis. (a) Side view. (b) View along C6–C7 bond corresponding to the Newman projections in Scheme 5B.

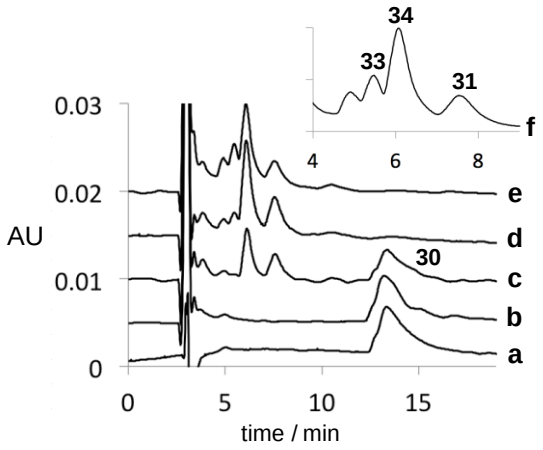


Figure S9. HPLC analysis (UV detection) following incubation of **30** with H6H and α-KG under air. (a) Standard sample of **30**, (b) control experiment without H6H (8 h), (c) **30** with H6H/α-KG/air for 10 min, (d) with H6H/α-KG/air for 1 h, (e) with H6H/α-KG/air for 8 h, (f) enlarged figure showing 4–9 min region of trace e.

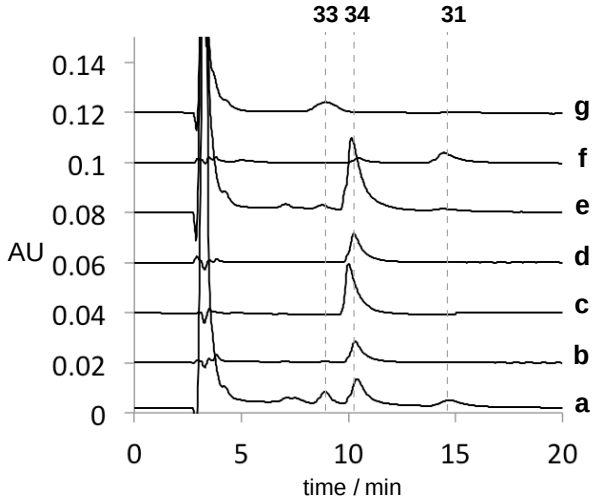


Figure S10. HPLC analysis (UV detection) of **34** and **31**. (a) Incubation of **30** with H6H/α-KG/air, (b) purified product **34**, (c) synthetic standard of **34**, (d) co-injection of purified product **34** and synthetic standard of **34**, (e) incubation of **34** with H6H/α-KG/air, (f) purified product **31**, (g) incubation of **31** with H6H/α-KG/air.

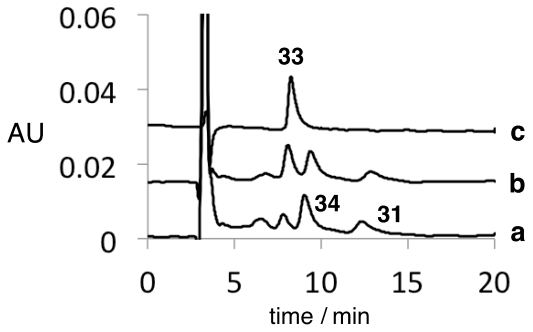


Figure S11. HPLC analysis (UV detection) of **33**. (a) Incubation of **30** with H6H and α-KG under air, (b) co-injection with synthetic standard of **33a**/**33b** mixture, (c) synthetic standard of **33a**/**33b** mixture.

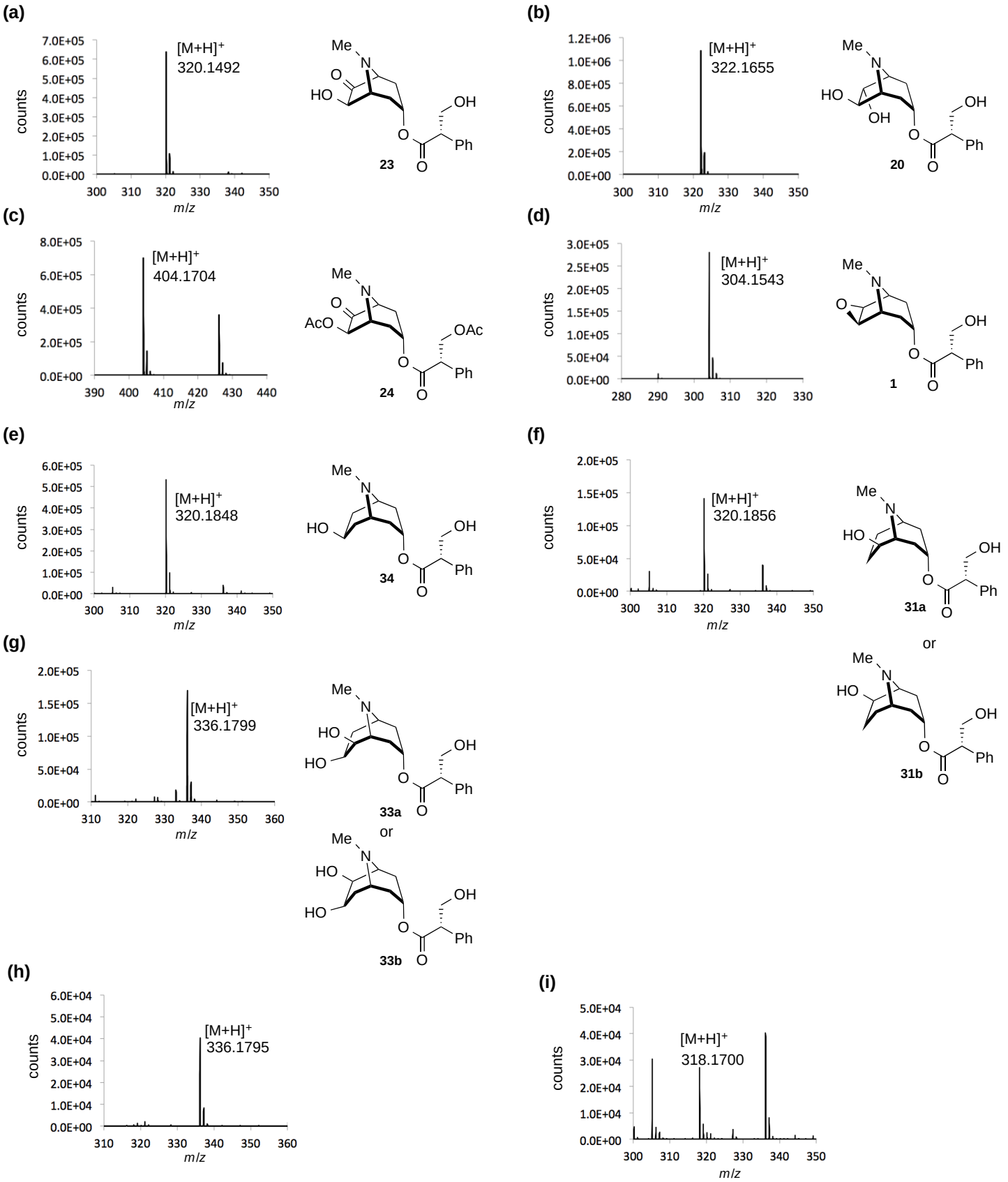


Figure S12. ESI-MS spectra of the observed enzymatic products and their derivatives. (a) **23** from **20**, (b) **20** from **23**, (c) **24** from **23**, (d) **1** from **28**, (e) **14** from **30**, (f) **31** from **30**, (g) **33** from **30**, (h) minor dihydroxylated product from reaction with **30**. (i) trace keto or epoxide product from reaction with **30**.

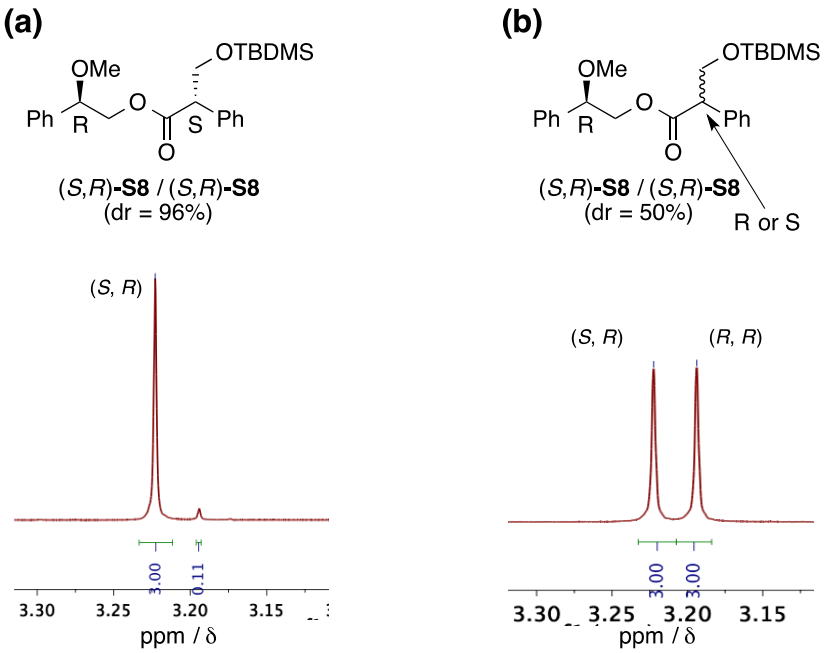


Figure S13. (a) ^{1}H NMR spectra of the ester derivative of chiral carboxylic acid (*S*)-**S6**. (b) ^{1}H NMR spectra of the ester derivative of a racemic mixture of carboxylic acid **S6**. Selected proton signals of the OMe functional group are shown.

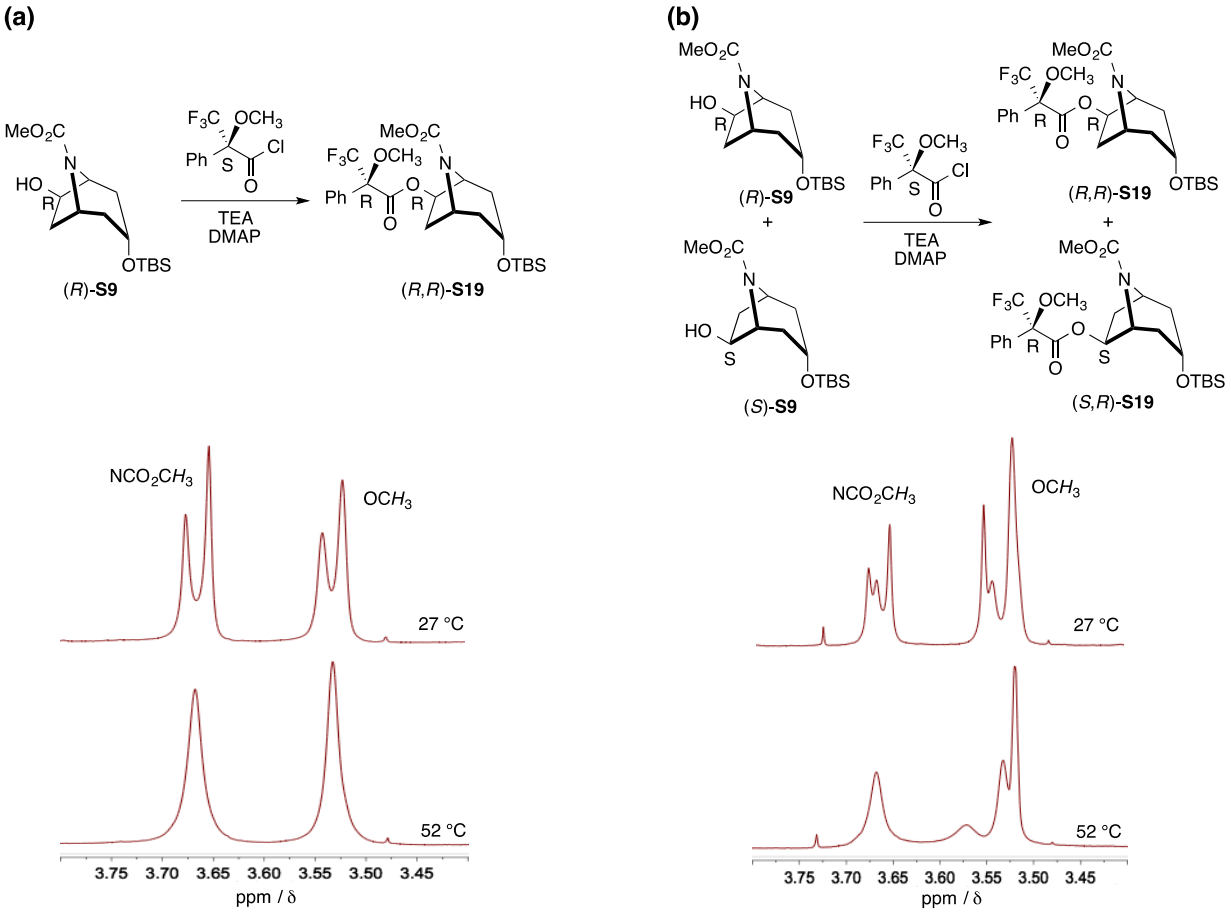


Figure S14. (a) ^{1}H NMR spectra of Mosher's ester of chiral alcohol **S9**. (b) ^{1}H NMR spectra of Mosher's ester of a racemic mixture of alcohol **S9**. Selected proton signals of NCO_2Me and OMe are shown.

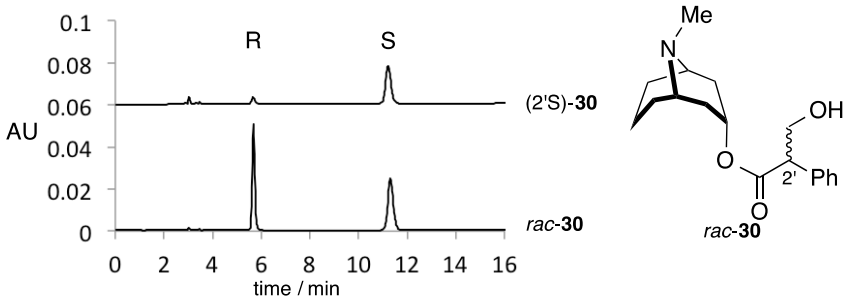


Figure S15. Determination of the enantiomeric purity of **30**.

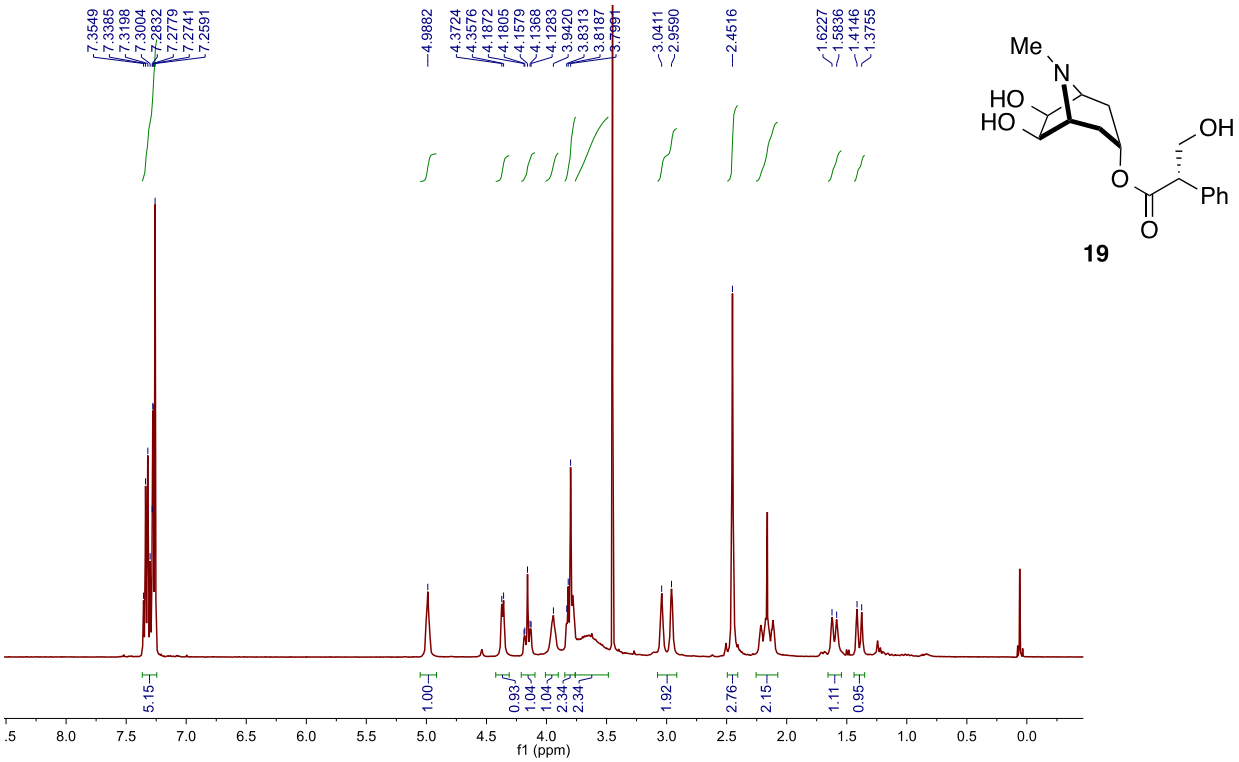


Figure S16. ^{1}H NMR of **19** (400 MHz in $CDCl_3$).

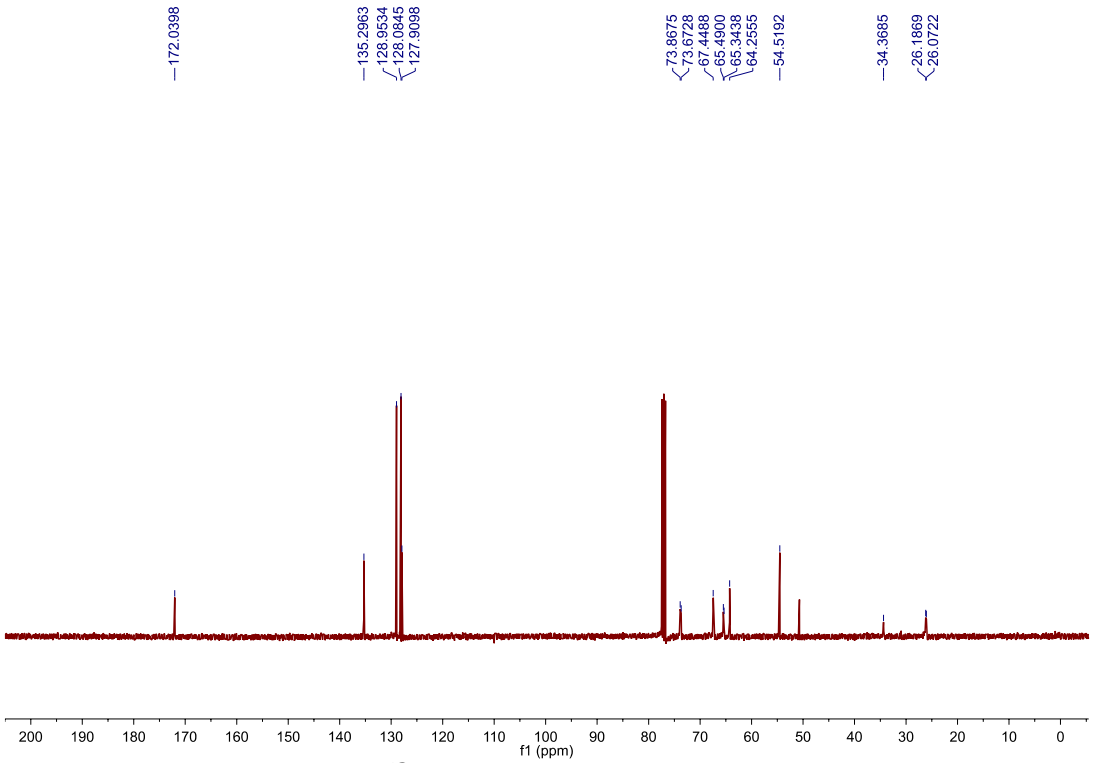


Figure S17. ^{13}C NMR of **19** (101 MHz in $CDCl_3$).

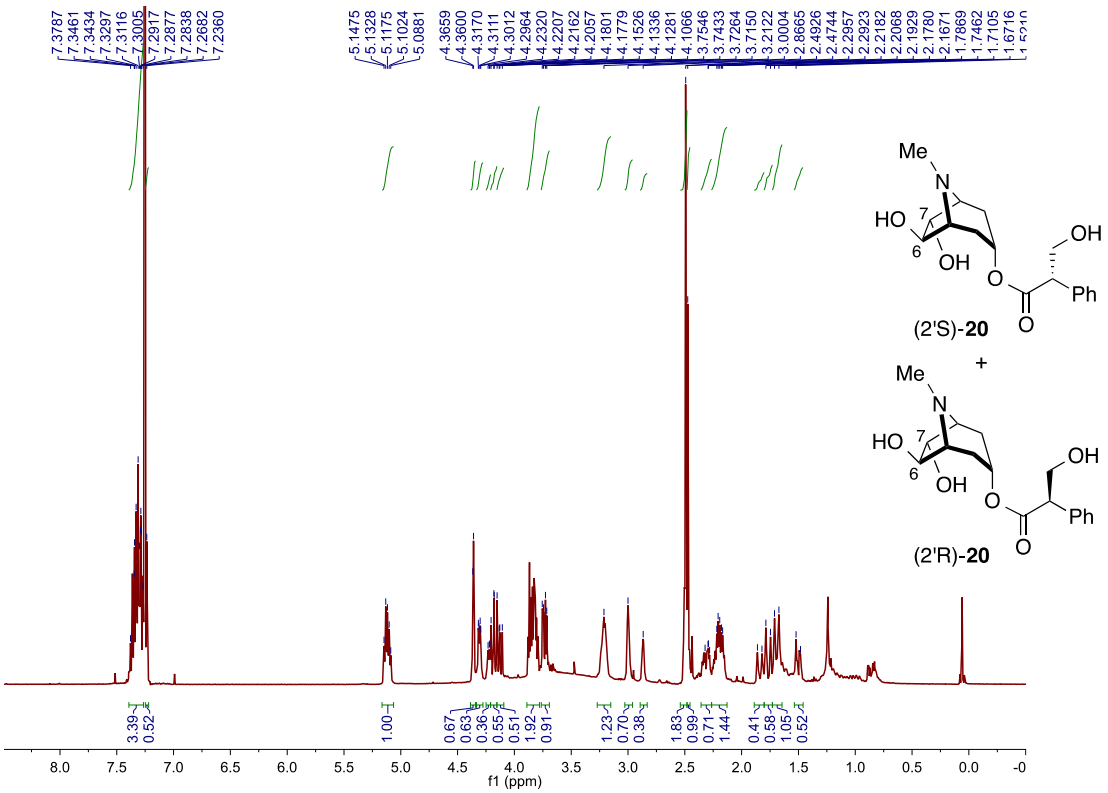


Figure S18. ^{1}H NMR of **20** (400 MHz in $CDCl_3$).

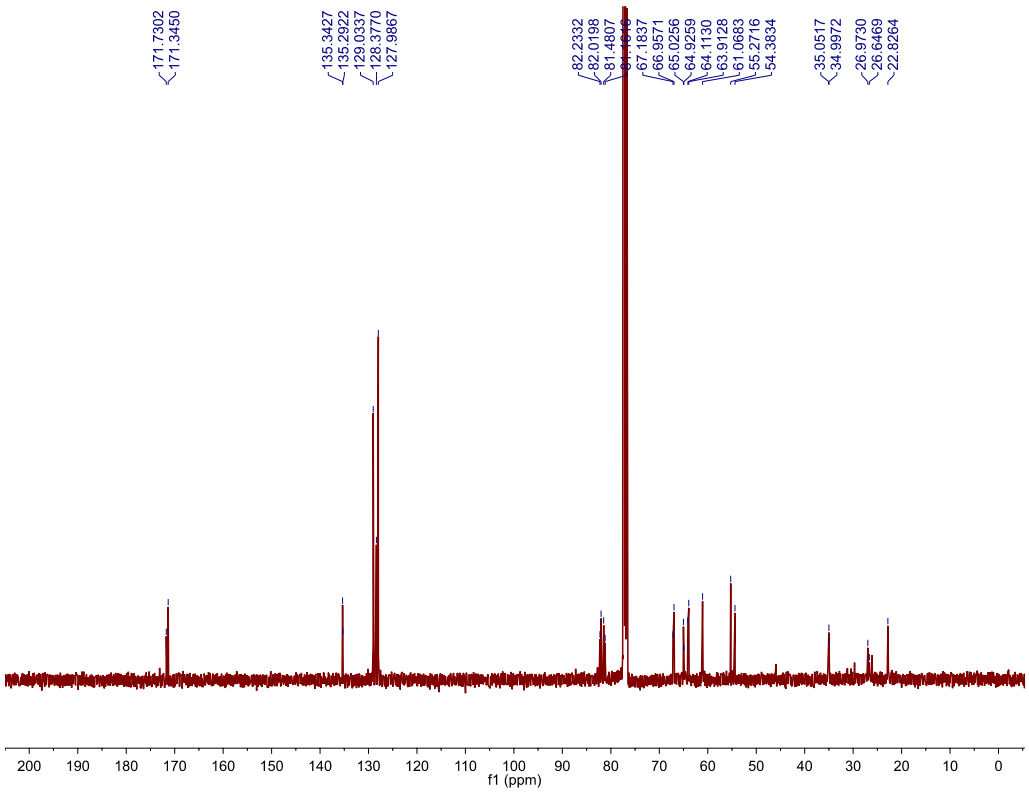


Figure S19.^{13}C NMR of **20** (101 MHz in $CDCl_3$).

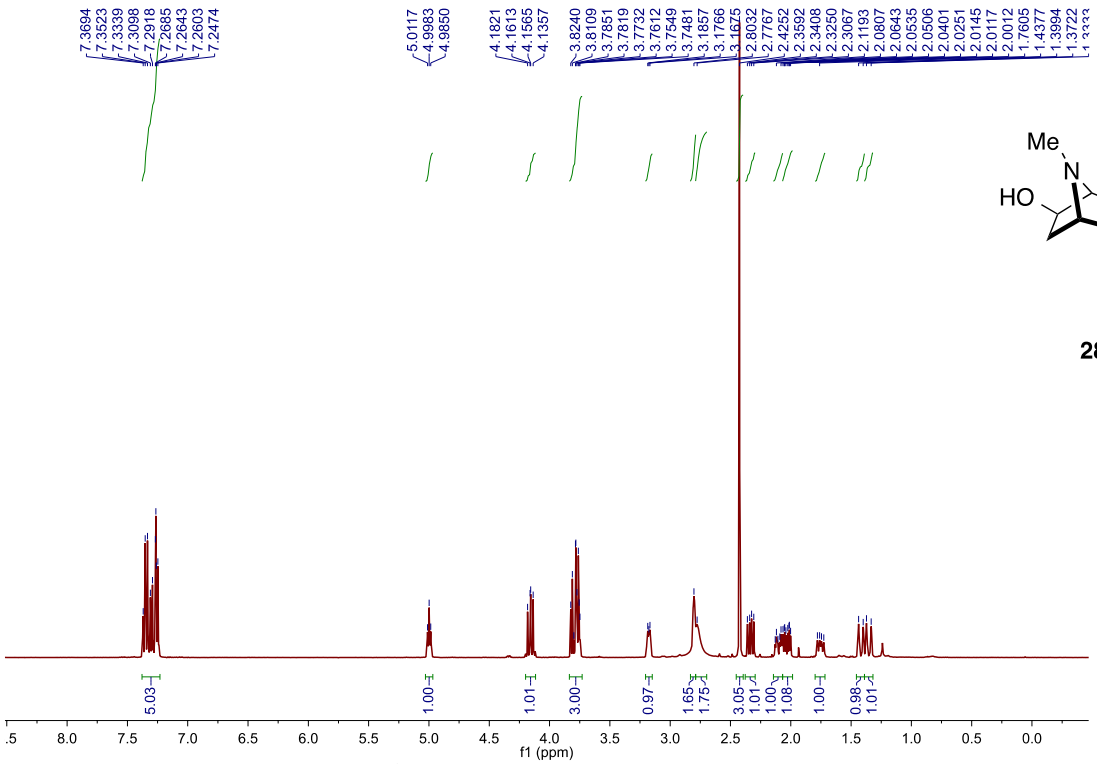


Figure S20. ^{1}H NMR of **28** (400 MHz in $CDCl_3$).

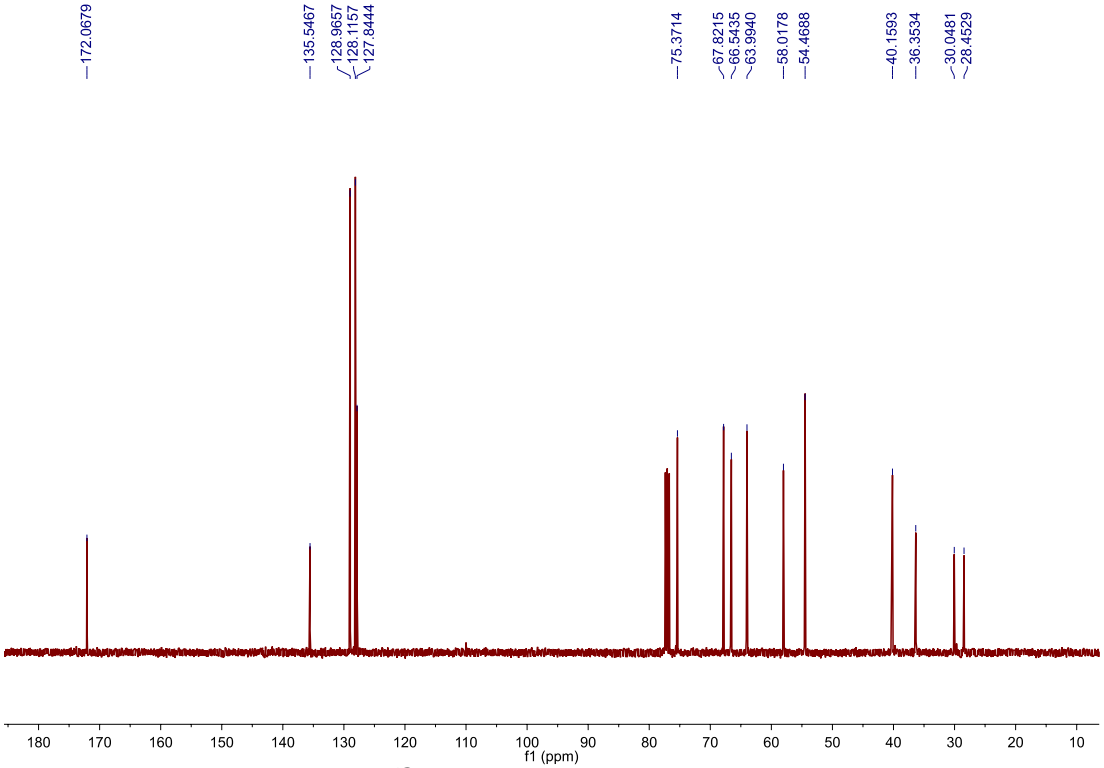


Figure S21. ^{13}C NMR of **28** (101 MHz in $CDCl_3$).

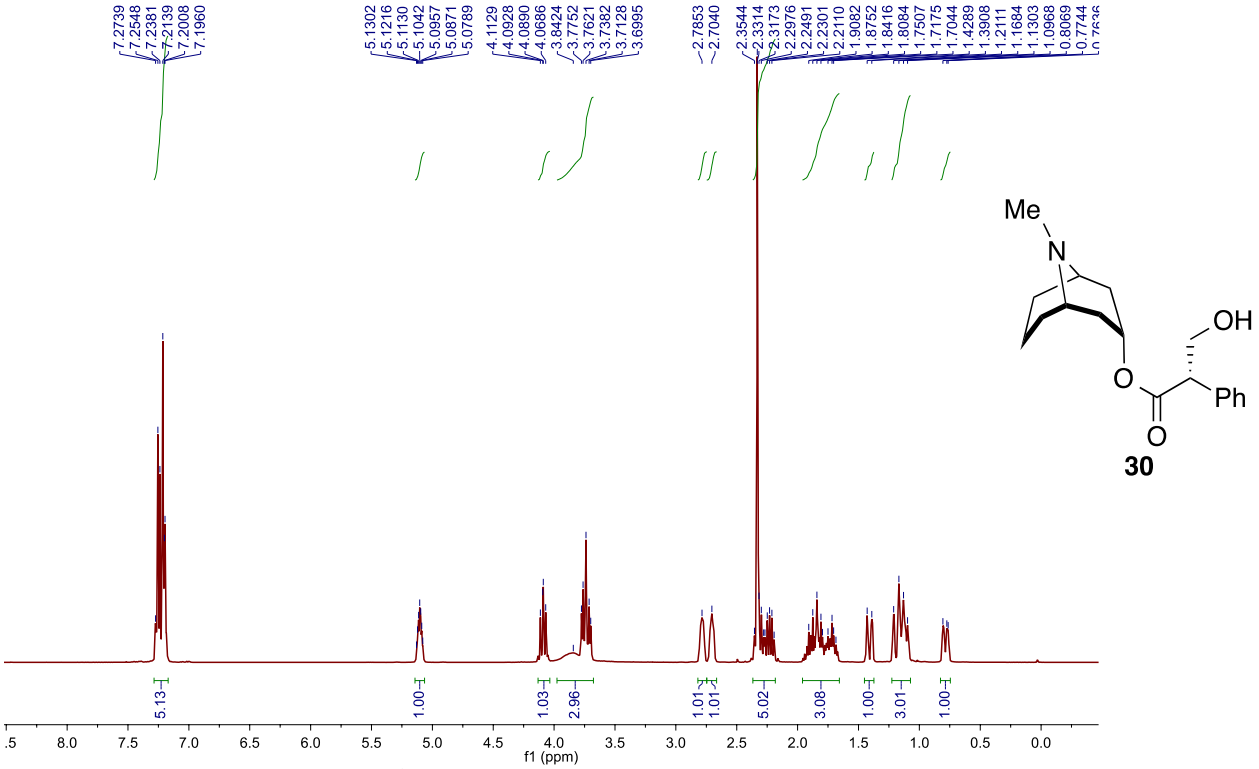


Figure S22. ^{1}H NMR of **30** (400 MHz in $CDCl_3$).

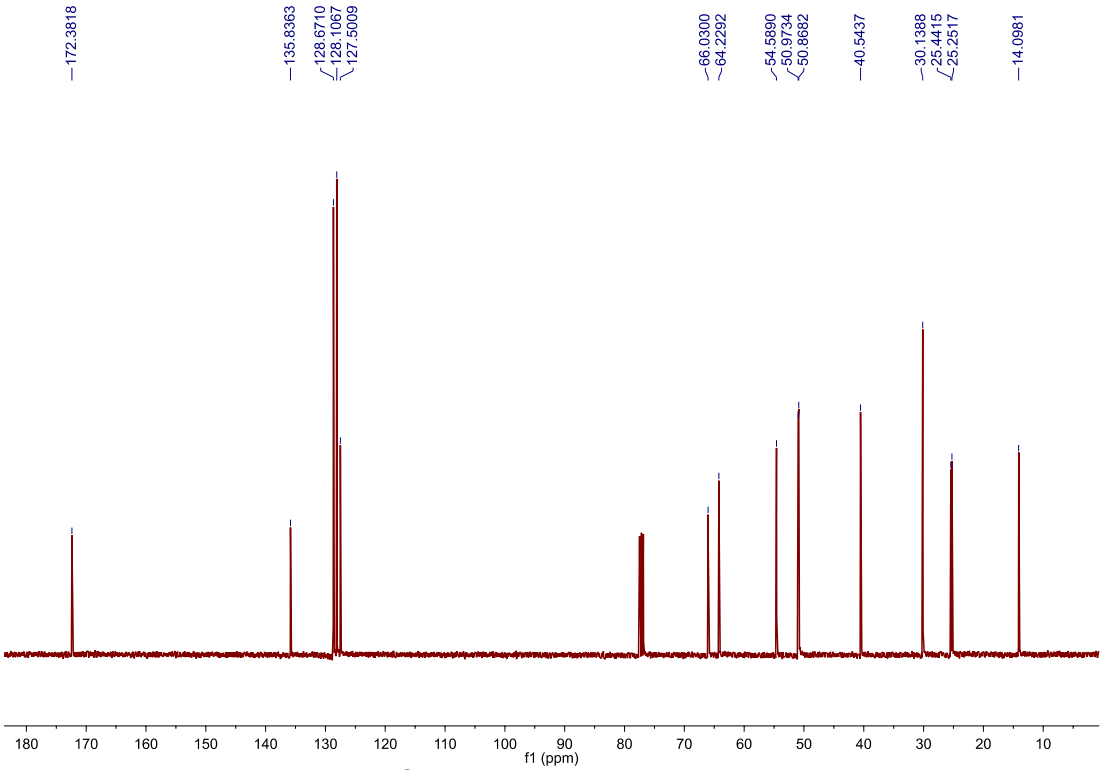


Figure S23. ^{13}C NMR of **30** (101 MHz in $CDCl_3$).

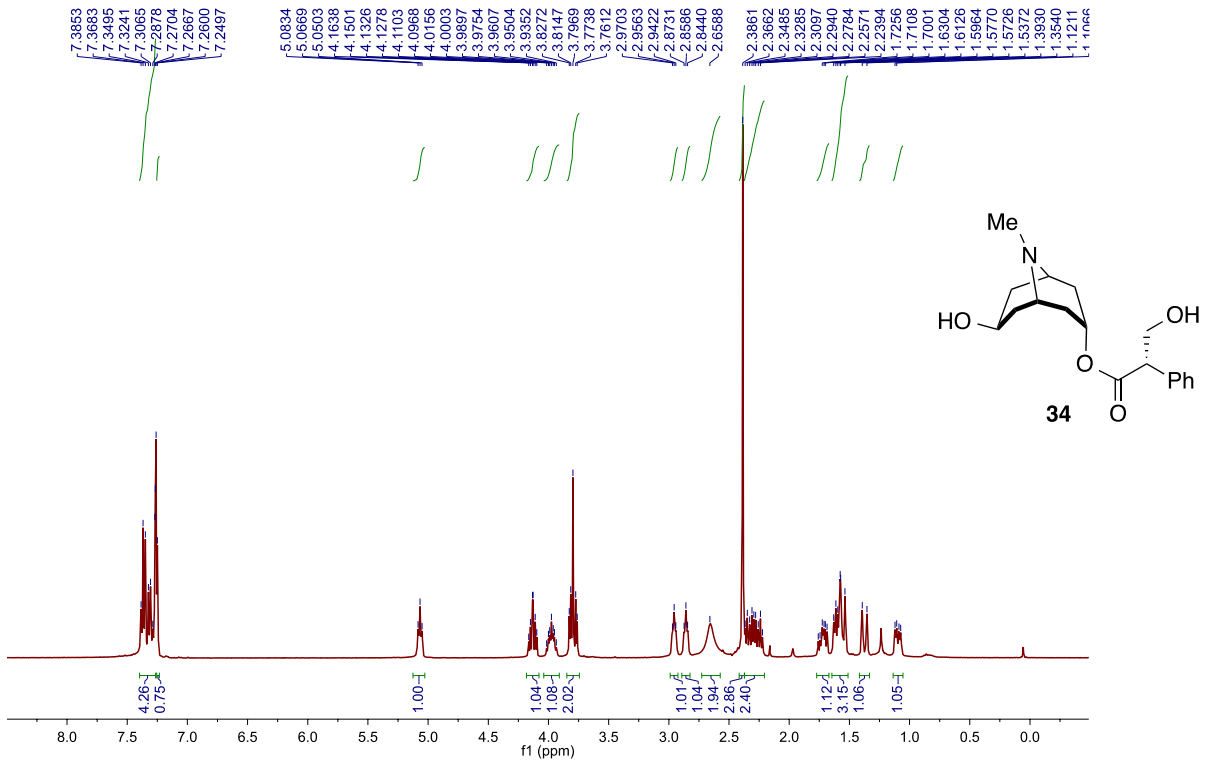


Figure S24. ^{1}H NMR of **34** (400 MHz in $CDCl_3$).

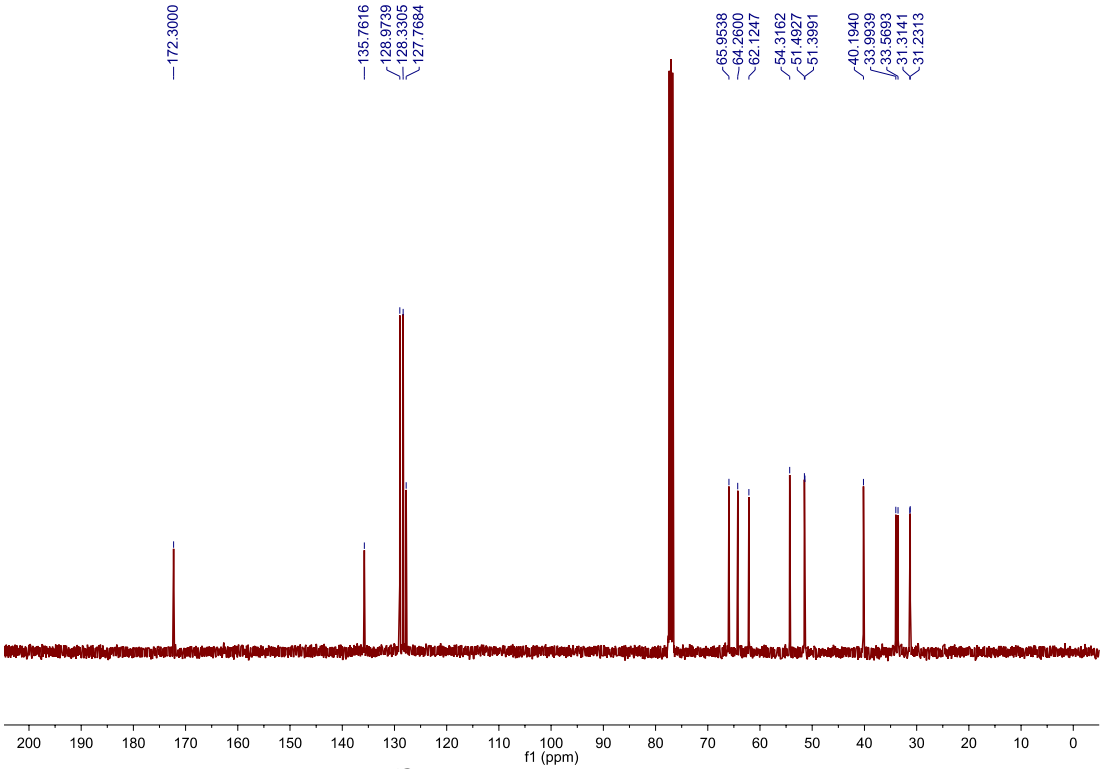


Figure S25. ^{13}C NMR of **34** (101 MHz in $CDCl_3$).

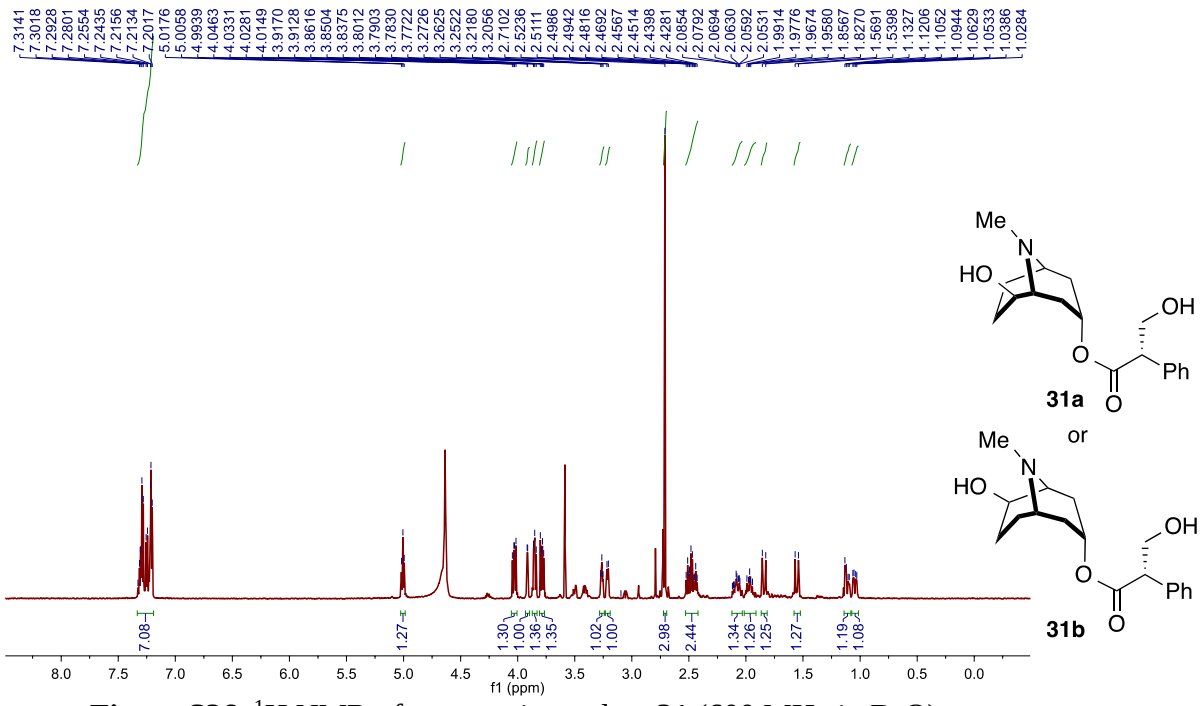


Figure S26. ^{1}H NMR of enzymatic product **31** (600 MHz in D_2O).

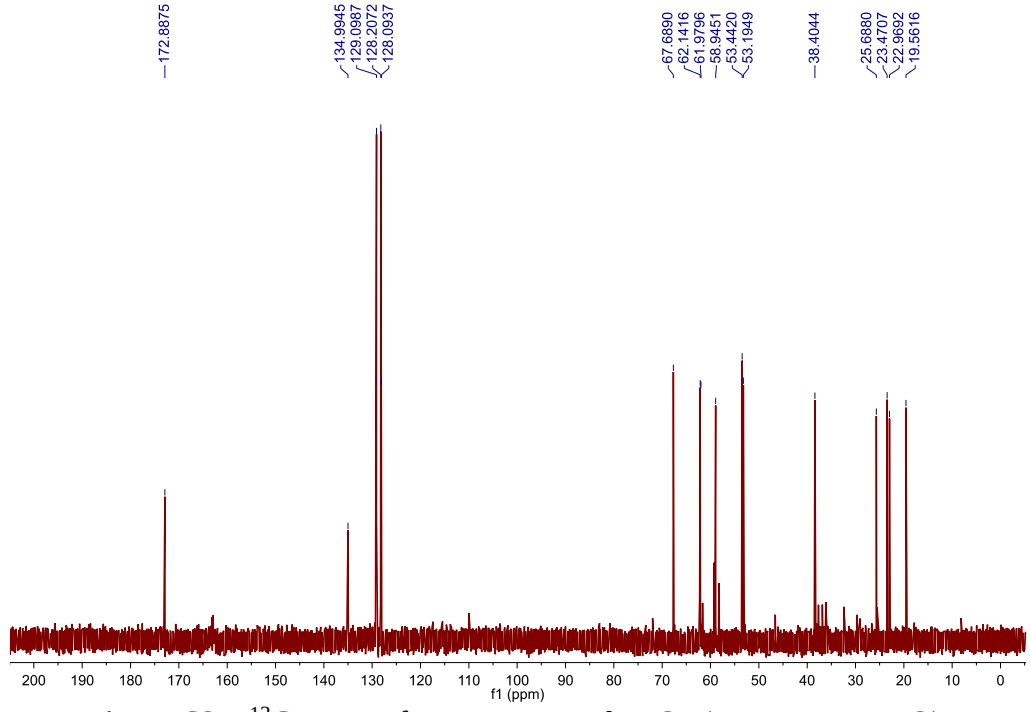


Figure S27. ^{13}C NMR of enzymatic product **31** (151 MHz in D_2O).

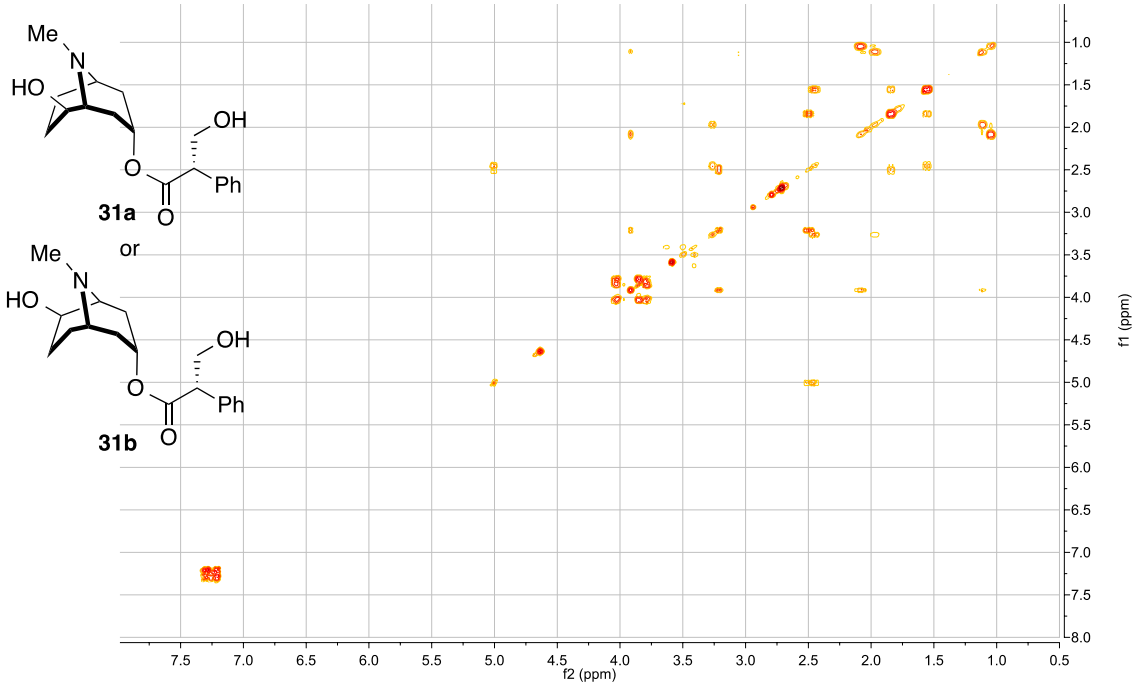


Figure S28. COSY spectrum of enzymatic product **31**.

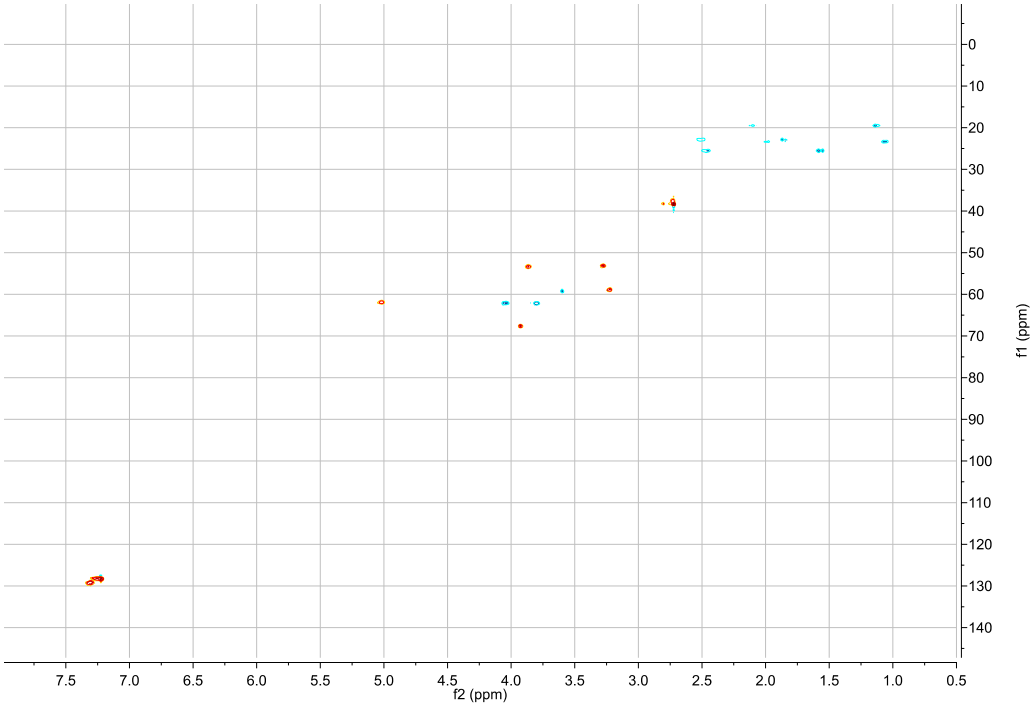


Figure S29. HSQC spectrum of enzymatic product **31**.

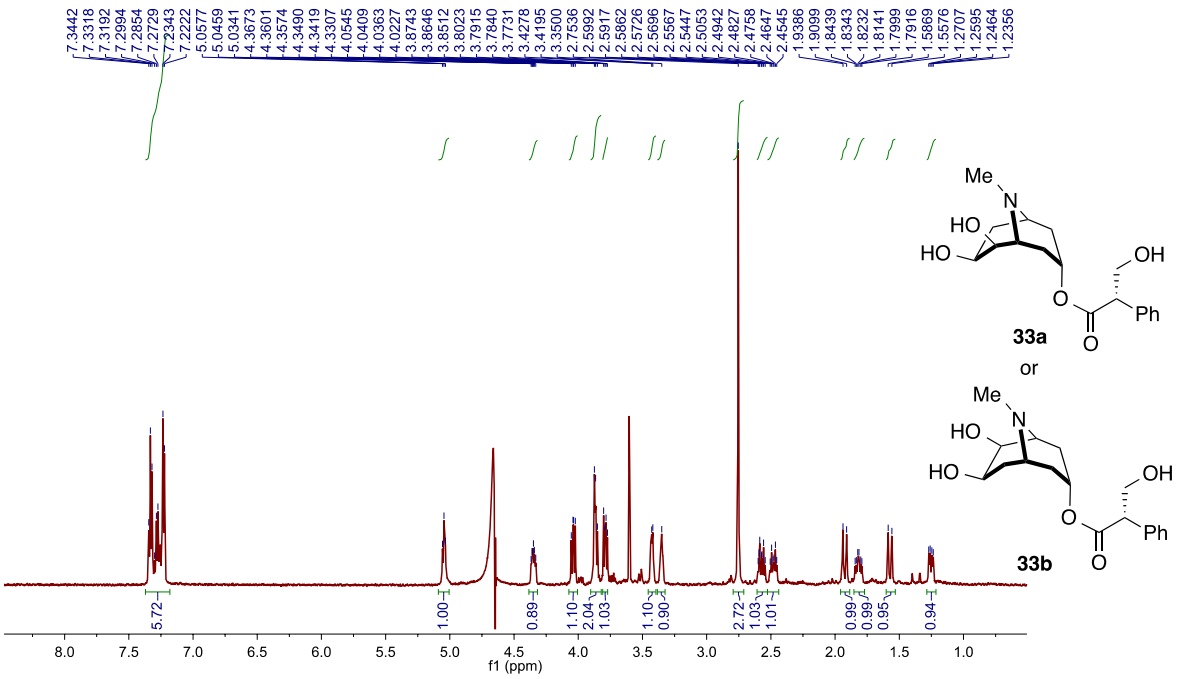


Figure S30. ^{1}H NMR of enzymatic product **33** (600 MHz in D_2O).

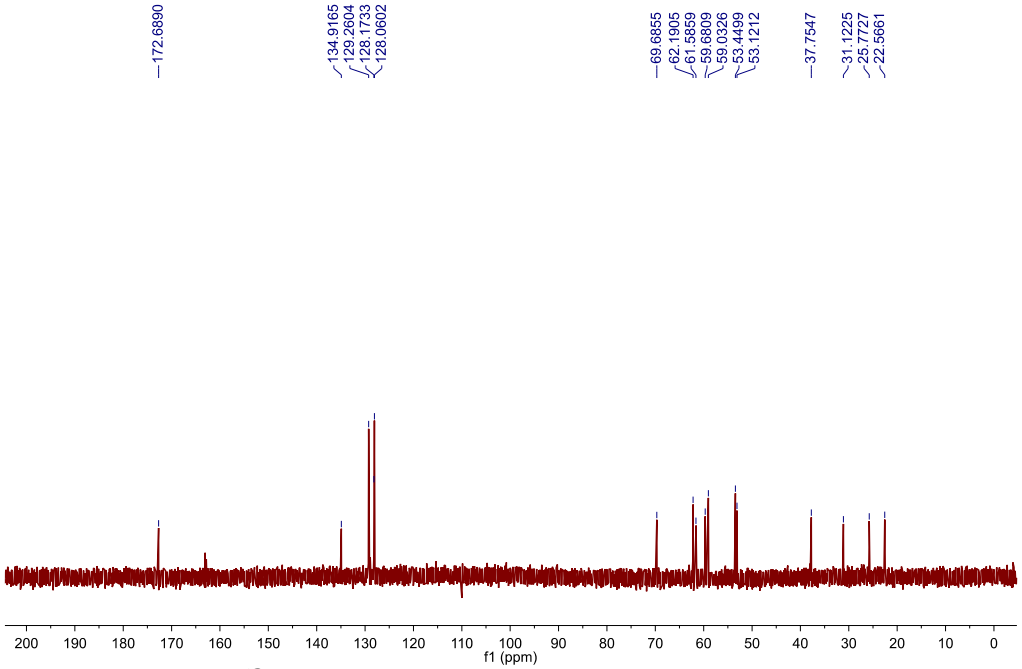


Figure S31. ^{13}C NMR of enzymatic product **33** (151 MHz in D_2O).

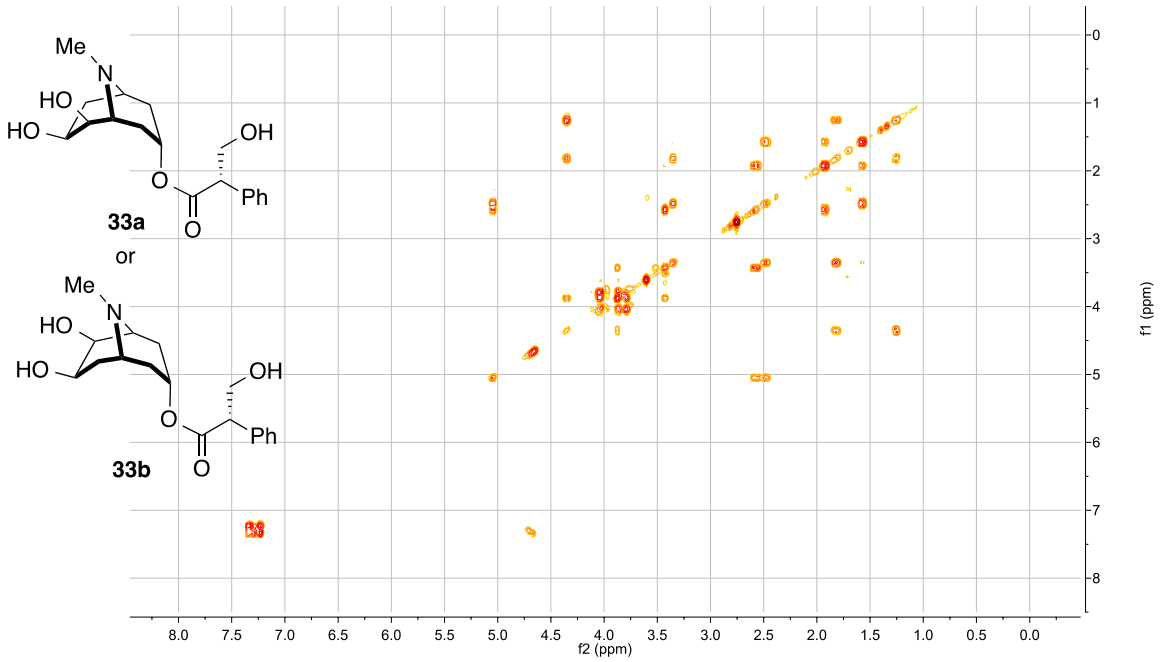


Figure S32. COSY spectrum of enzymatic product **33**.

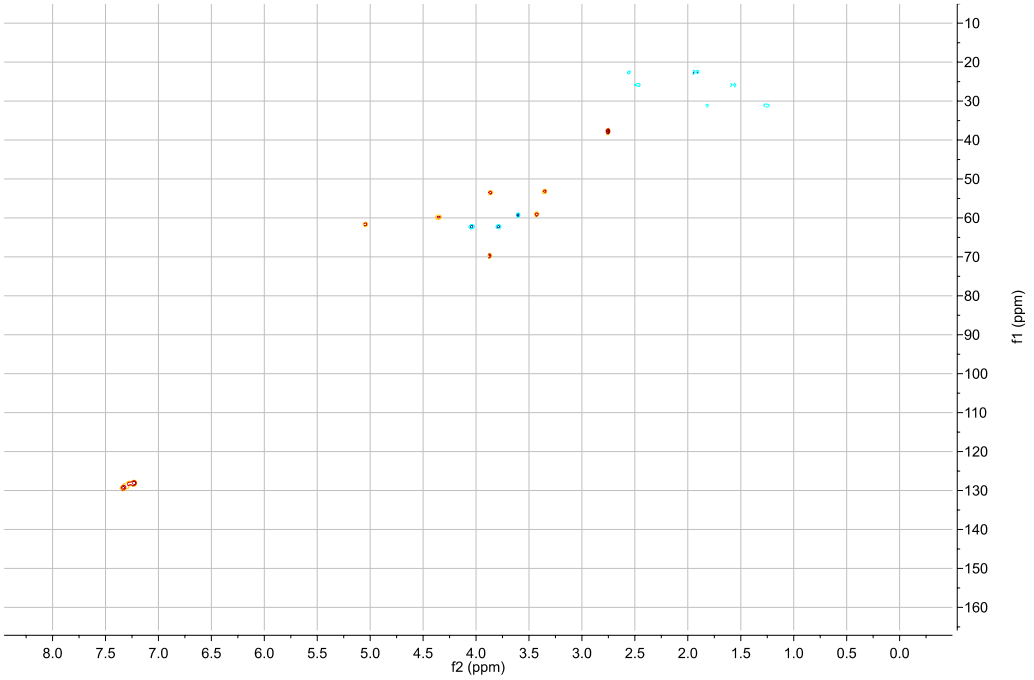


Figure S33. HSQC spectrum of enzymatic product **33**.

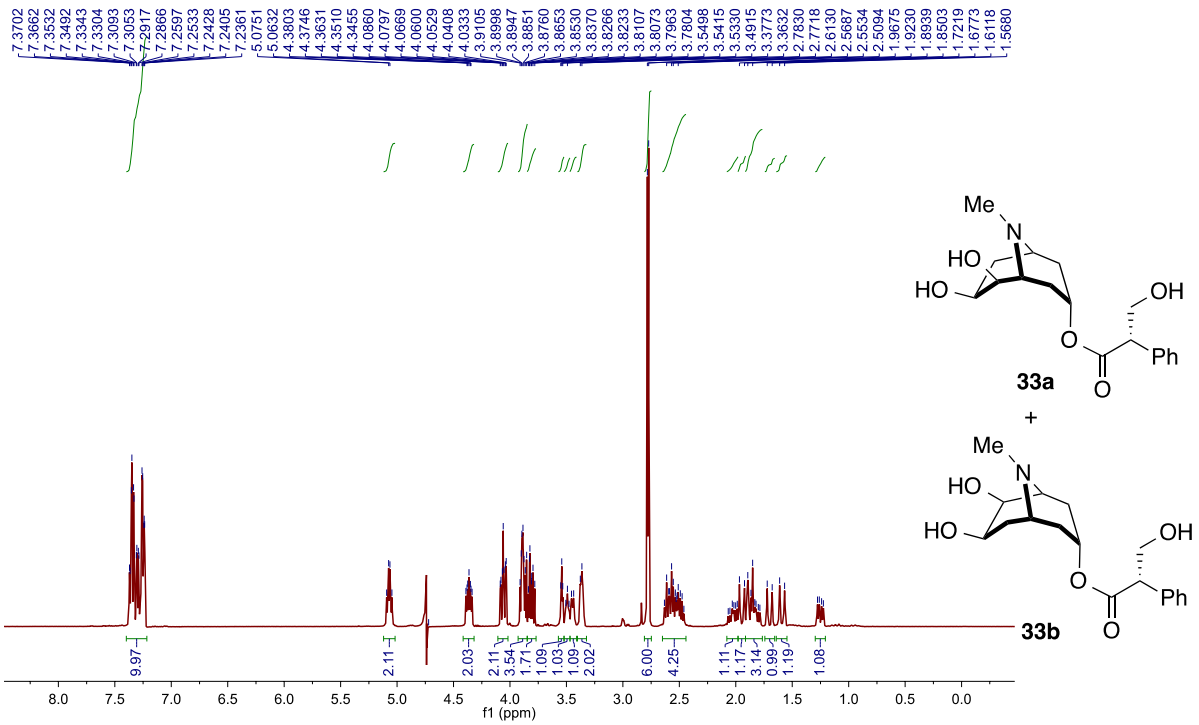


Figure S34. ^{1}H NMR of synthetic **33a**/**33b** (400 MHz in D_2O).

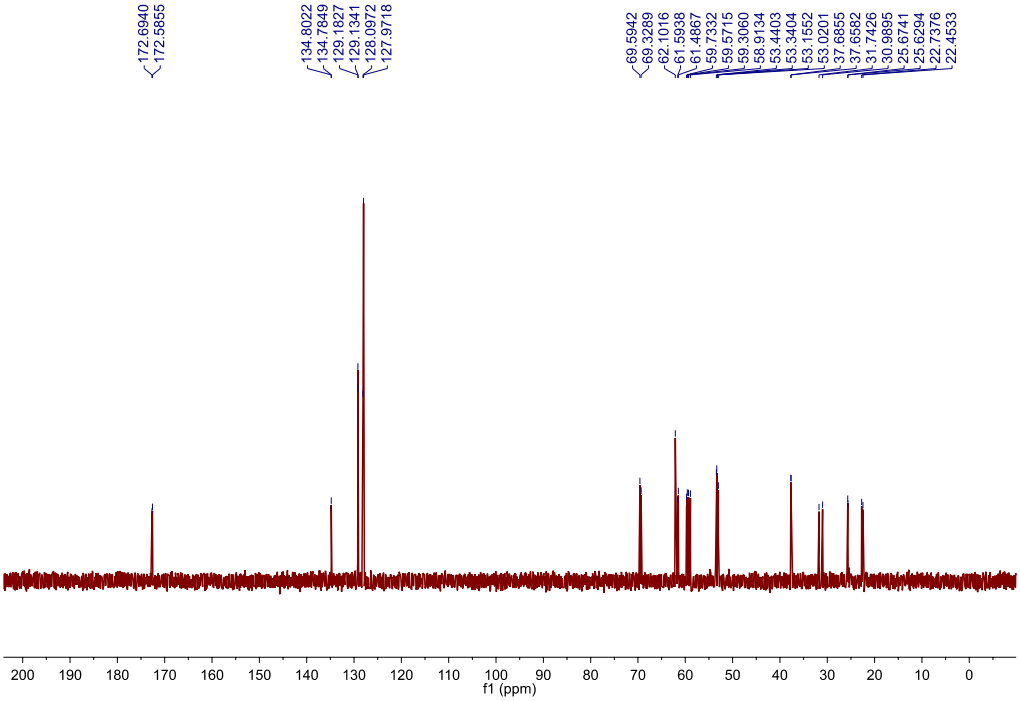


Figure S35.^{13}C NMR of synthetic **33a**/**33b** (101 MHz in D_2O).

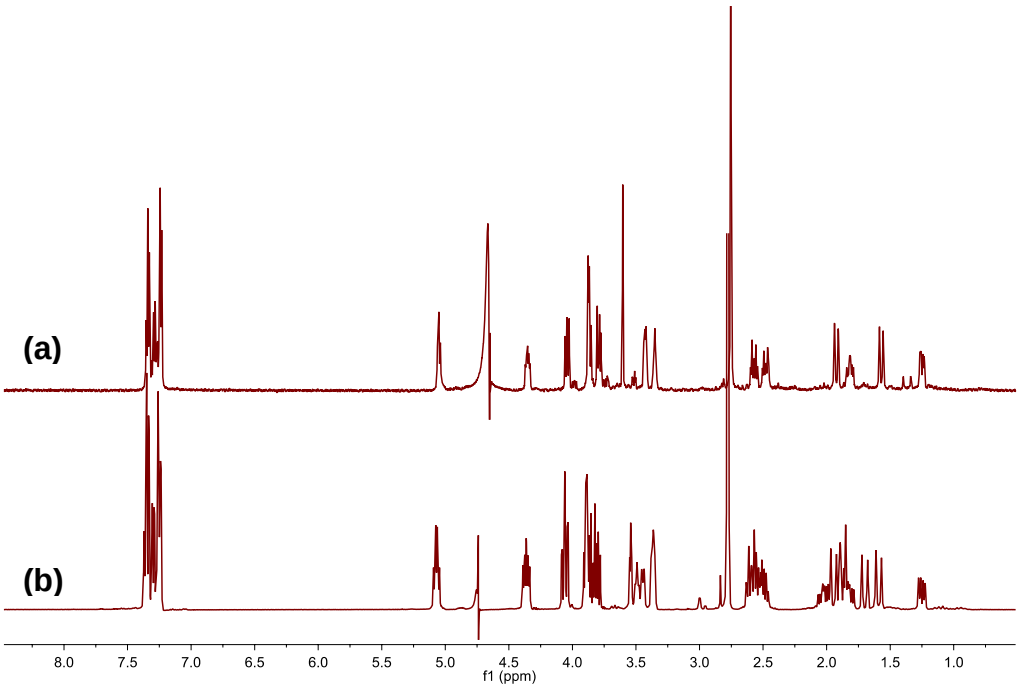


Figure S36. Overlaid ^{1}H NMR spectra of (a) enzymatic **33** (**33a** or **33b**) and (b) synthetic **33** (**33a** and **33b**).

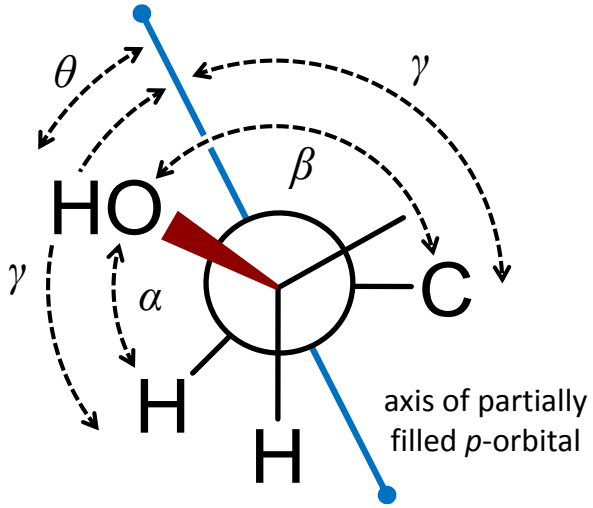
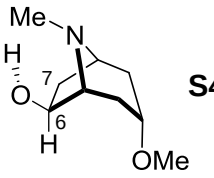
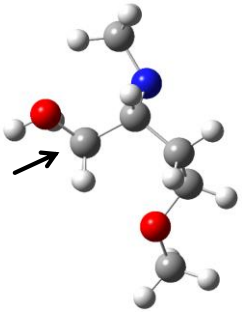


Figure S37. Schematic showing the angles and assumptions used to calculate the dihedral angle θ between the C–OH bond and the axis of the partially filled *p*-orbital and the adjacent trigonal carbon from computations of the modeled radical species.



S47a

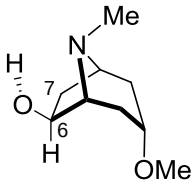


$|\theta_{exo}| = 1.3°$

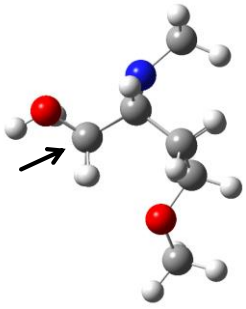
$|\theta_{endo}| = 121.4°$

$E = 0.0$ kcal/mol

view down C6-C7



S47b

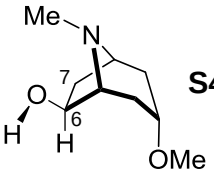


$|\theta_{exo}| = 1.8°$

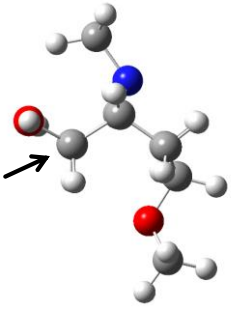
$|\theta_{endo}| = 119.0°$

$E = 2.01$ kcal/mol

view down C6-C7



S47c

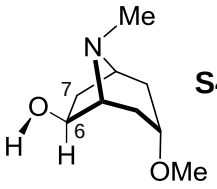


$|\theta_{exo}| = 0.4°$

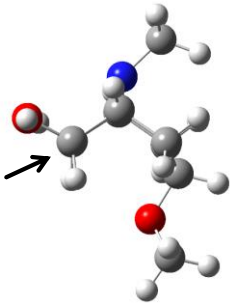
$|\theta_{endo}| = 119.1°$

$E = 0.08$ kcal/mol

view down C6-C7



S47d



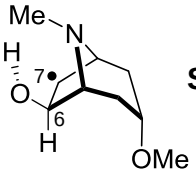
$|\theta_{exo}| = 3.7°$

$|\theta_{endo}| = 116.5°$

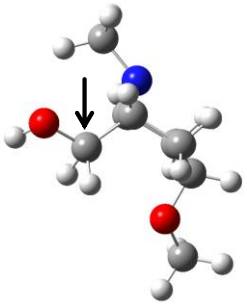
$E = 1.85$ kcalmol

view down C6-C7

Figure S38. Results of geometry optimizations (RB3LYP/6-31G*) for gas phase models of compound **3**. Energies are reported relative to the lowest energy conformer. The view down along C6–C7 bond is indicated with an arrow.



S47a-rad7



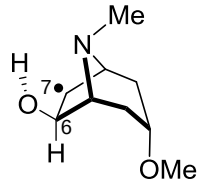
$|\alpha| = 83.6°$

$|\beta| = 111.7°$

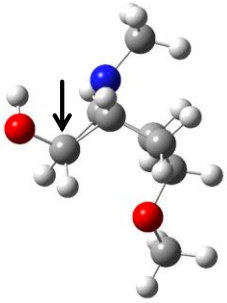
$|\theta| = 14.1°$

$E = 0.65$ kcal/mol

view down C6-C7



S47b-rad7



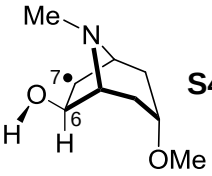
$|\alpha| = 77.0°$

$|\beta| = 110.1°$

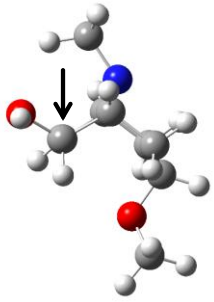
$|\theta| = 16.5°$

$E = 0.00$ kcal/mol

view down C6-C7



S47c-rad7



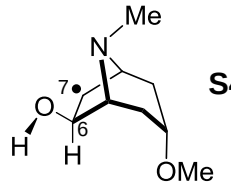
$|\alpha| = 73.3°$

$|\beta| = 117.0°$

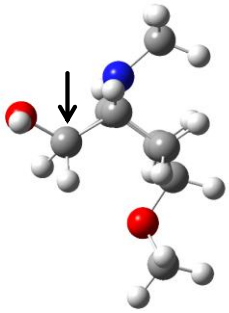
$|\theta| = 21.9°$

$E = 1.72$ kcal/mol

view down C6-C7



S47d-rad7



$|\alpha| = 74.5°$

$|\beta| = 116.1°$

$|\theta| = 20.8°$

$E = 4.87$ kcal/mol

view down C6-C7

Figure S39. Results of geometry optimizations (UB3LYP/6-31G*) for gas phase models following H-atom removal from C7 of the corresponding conformers in Figure S38. Energies are reported relative to the lowest energy radical conformer. The view down along the C6–C7 bond is indicated with an arrow.

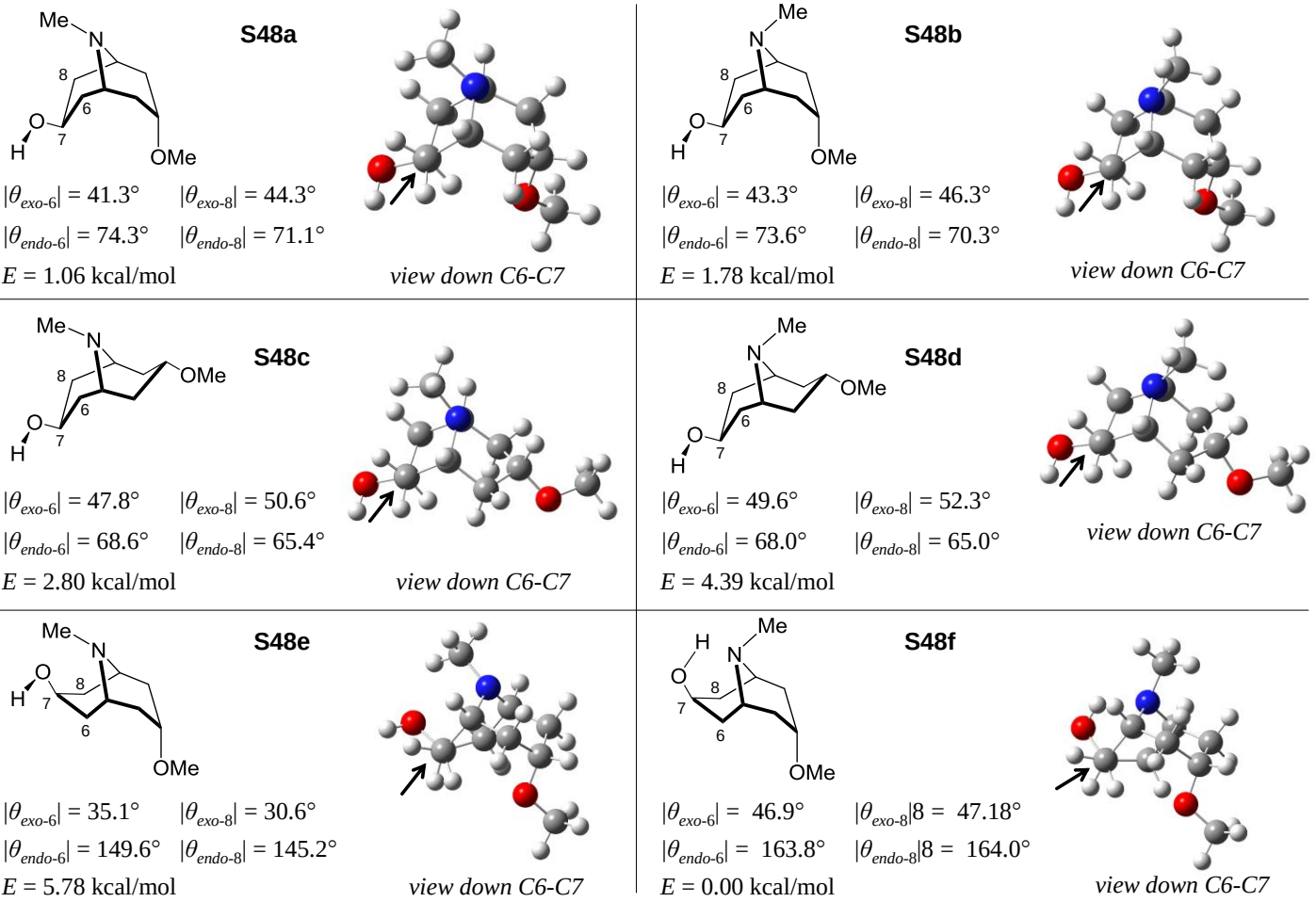


Figure S40. Results of geometry optimizations (RB3LYP/6-31G*) for gas phase models of compound **34**. Energies are reported relative to the lowest energy conformer. The view down along the C6–C7 bond is indicated with an arrow.

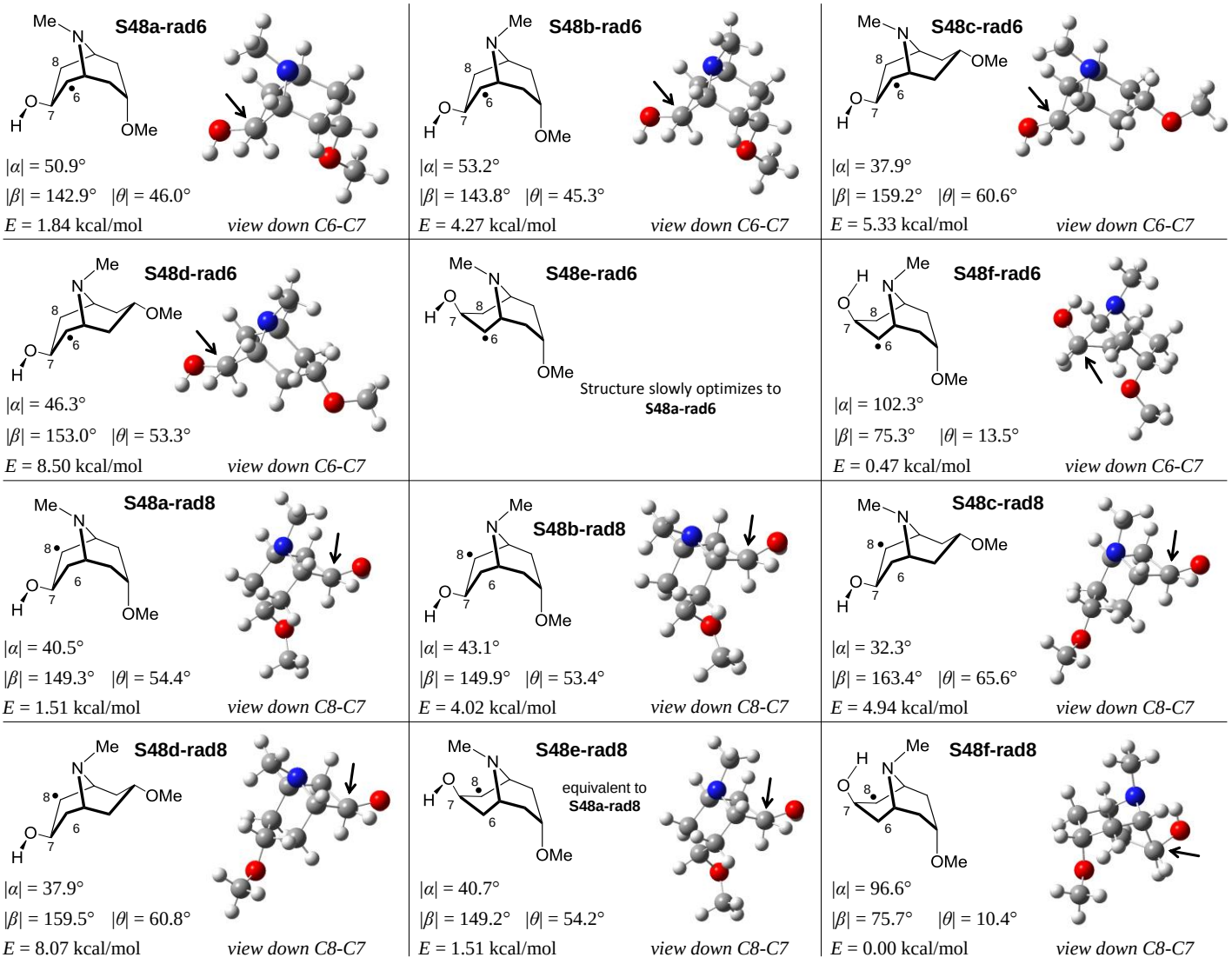


Figure S41. Results of geometry optimizations (UB3LYP/6-31G*) for gas phase models following H-atom removal from C7 of the corresponding conformers in Figure S40. Energies are reported relative to the lowest energy radical conformer. Views down along the C6–C7 or C8–C7 bond is indicated with an arrow.

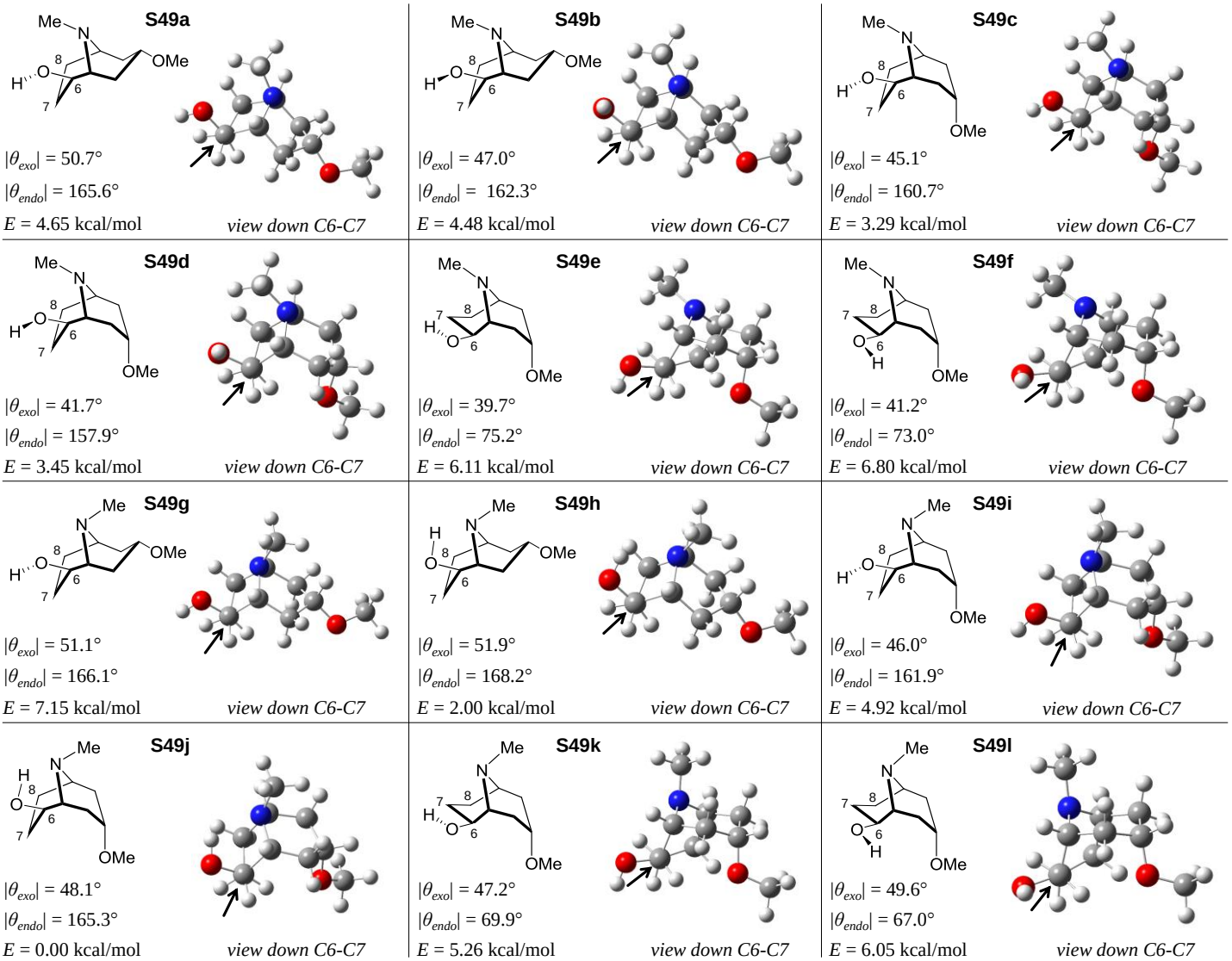


Figure S42. Results of geometry optimizations (RB3LYP/6-31G*) for gas phase models of compound **31a**. Energies are reported relative to the lowest energy conformer. The view down along the C6-C7 bond is indicated with an arrow.

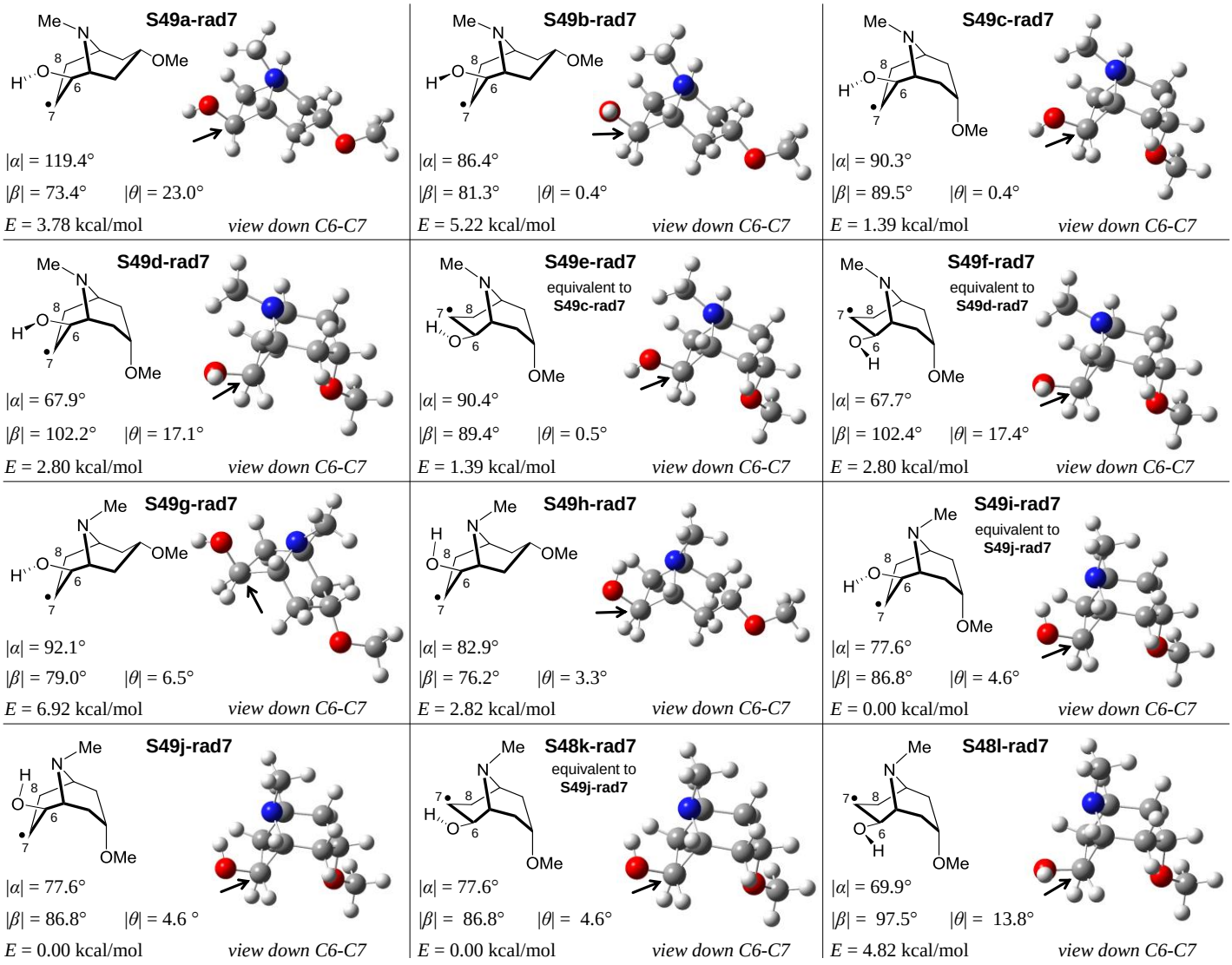


Figure S43. Results of geometry optimizations (UB3LYP/6-31G*) for gas phase models following H-atom removal from C7 of the corresponding conformers in Figure S42. Energies are reported relative to the lowest energy radical conformer. The view down along the C6–C7 bond is indicated with an arrow.