

Supporting Information

Structure Revision of Poecillastrin C and the Absolute Configuration of the β -Hydroxyaspartic Acid Residue

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1. Experimental Section

General Procedures

NMR spectra were measured on a JEOL alpha 600 NMR spectrometer and referenced to solvent peaks: δ_{H} 3.30 and δ_{C} 49.0 for CD_3OD ; δ_{H} 7.24 and δ_{C} 77.0 for CDCl_3 . ESI mass spectra were measured on a JEOL JMS-T100LC mass spectrometer. LC-MS experiments were performed on a Shimadzu LC-20AD solvent delivery pump with a COSMOSIL 2.5 C_{18} -MS-II column and interfaced to a Bruker amaZon SL mass spectrometer.

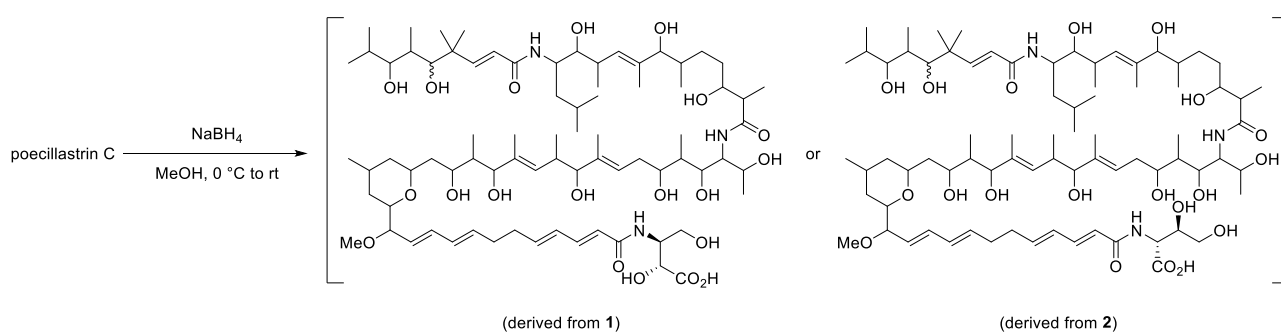
Marfey's analysis

The substrate (30–100 μg) was hydrolyzed in 6N HCl at 110 $^\circ\text{C}$ for 1–5 h. The solution was concentrated and redissolved in H_2O (100 μL). To the solution was added 1 wt% 1-fluoro-2,4-dinitrophenyl-5-L-alanineamide (L-FDAA) in acetone (100 μL) and 1M NaHCO_3 (20 μL). The reaction mixture was kept at 55 $^\circ\text{C}$ for 30 min, quenched with 2N HCl (10 μL), and analyzed by LC-MS (7% MeCN/ H_2O + 0.5% AcOH).

Isolation of poecillastrin C

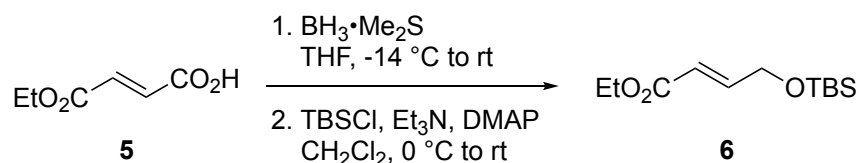
The frozen marine sponge *Poecillastra* sp. (70 g, wet weight) collected at Oshima-Shinsone was extracted with EtOH and then $\text{CHCl}_3/\text{MeOH}$ (1:1). The combined organic layer was concentrated, and partitioned between $\text{CHCl}_3/\text{H}_2\text{O}$. The H_2O layer was extracted with *n*-BuOH. The combined organic layer was concentrated and the residue was subjected to ODS flash column chromatography with stepwise elution of 30% MeCN, 70% MeCN, MeCN, and $\text{CHCl}_3/\text{MeOH}$ (1:1). The 70% MeCN fraction was further purified with RP-HPLC (COSMOSIL 5 C_{18} -AR-II, 65% MeCN + 1% AcOH) to give poecillastrin C (3.2 mg): ESIMS $[\text{M} + \text{H}]^+$ 1429

Reduction of poecillastrin C and the other congeners



To a solution of poecillastrin C (30 μg , 21 nmol) in MeOH (0.15 mL) was added NaBH_4 (0.3 mg) at 0 $^\circ\text{C}$. The reaction mixture was allowed to warm to room temperature and stirred for 4 h. The reaction mixture was concentrated to afford a crude diol, which was used for Marfey's analysis without purification: ESIMS $[\text{M} + \text{H}]^+$ 1435

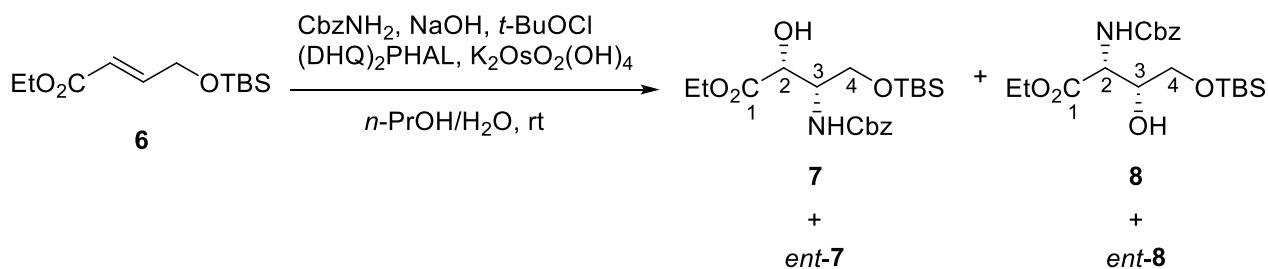
Synthesis of 6



To a solution of monoethyl fumarate (**5**, 1009 mg, 7.001 mmol) in THF (3.5 mL) was added a solution of $\text{BH}_3\cdot\text{Me}_2\text{S}$ (2.0 M in THF, 3.5 mL, 7.0 mmol) at -14°C . The reaction mixture was allowed to warm to room temperature and stirred for 12 h. The reaction mixture was quenched with 50% AcOH, concentrated, and the residual slurry was treated with saturated aqueous NaHCO_3 at 0 $^\circ\text{C}$. The resulting mixture was extracted with EtOAc. The organic layer was washed with brine, dried over MgSO_4 , and filtered. The solution was concentrated to afford a crude allyl alcohol (318 mg) as a colorless oil, which was used in the next reaction without further purification.

To a solution of the crude allyl alcohol (130 mg) in CH_2Cl_2 (2 mL) was added Et_3N (0.181 mL, 1.30 mmol), TBSCl (177 mg, 1.17 mmol) and DMAP (12 mg, 0.1 mmol) at 0 $^\circ\text{C}$. The reaction mixture was allowed to warm to room temperature and stirred for 5 h. The reaction mixture was diluted with H_2O and extracted with CH_2Cl_2 . The organic layer was washed with brine, dried over MgSO_4 , and filtered. The solution was concentrated, and the residue was purified with silica gel column chromatography (*n*-hexane/EtOAc = 8/2) to afford **6** (186 mg) as a colorless oil: ^1H NMR (600 MHz, CDCl_3) δ 0.06 (6H, s), 0.90 (9H, s), 1.27 (3H, t, 7.2 Hz), 4.18 (2H, q, 7.2 Hz), 4.31 (2H, dd, 2.1 Hz, 3.4 Hz), 6.07 (1H, dt, 15.8 Hz, 2.1 Hz), 6.97 (1H, dt, 15.8 Hz, 3.4 Hz); ESIMS $[\text{M} + \text{Na}]^+$ 267

Synthesis of 7, 8, and their enantiomers

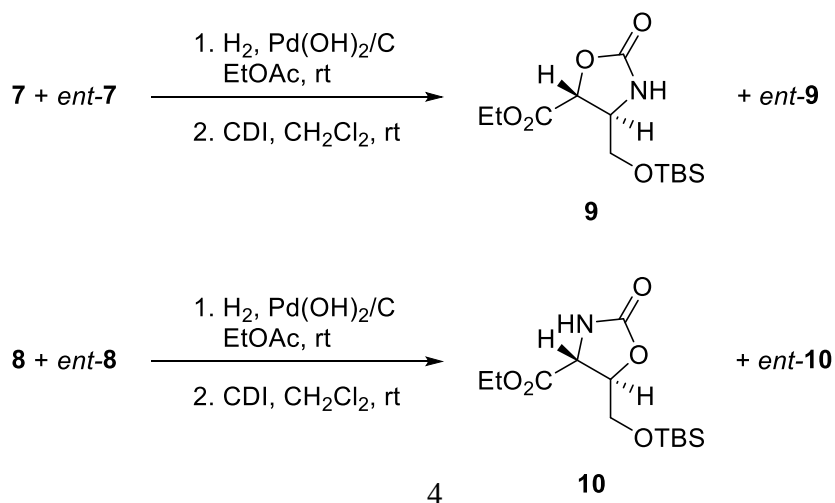


To a solution of benzyl carbamate (328 mg, 2.17 mmol) in *n*-PrOH (2 mL) was added 1.0 M NaOH (2.140 mL, 2.1 mmol) and *t*-BuOCl (0.241 mL, 2.13 mmol) at room temperature. The reaction mixture was stirred for 7 min, and to which were added a solution of (DHQ)₂PHAL (27.3 mg, 0.0350 mmol) in *n*-PrOH (0.4 mL), a solution of **6** (171 mg, 0.700 mmol) in *n*-PrOH and a solution of K₂OsO₂(OH)₄ (10.3 mg, 0.028 mmol) in H₂O (0.2 mL). After stirring for 3 h, the reaction mixture was quenched with saturated aqueous Na₂SO₃ at 0 °C and extracted with EtOAc. The organic layer was washed with brine, dried over MgSO₄, and filtered. The solution was concentrated, and a half of the residue was roughly purified with silica gel column chromatography (*n*-hexane/EtOAc = 17/3 to 7/3) to afford a crude carbamate, which was further purified with HPLC (COSMOSIL 5SL-II, *n*-hexane/*i*-PrOH = 99/1 to 197/3) to afford the mixture of **7** and *ent*-**7** (37.6 mg), and the mixture of **8** and *ent*-**8** (24.2 mg) as a colorless oil.

7 and *ent*-**7**: ¹H NMR (600 MHz, CDCl₃) δ 0.05 (3H, s, SiCH₃), 0.05 (3H, s, SiCH₃), 0.87 (9H, s, SiC(CH₃)₃), 1.23 (3H, t, 7.2 Hz, OCH₂CH₃), 3.63 (1H, t, 8.9 Hz, H-4), 3.73 (1H, dd, 10.0 Hz, 5.2 Hz, H-4), 4.16 (1H, m, H-3), 4.19 (2H, m, OCH₂CH₃), 4.44 (1H, brs, H-2), 5.05 (2H, s, OCH₂Ph), 5.13 (1H, d, 8.9 Hz, NH), 7.31 (5H, m, Ph); ¹³C NMR (151 MHz, CDCl₃) δ -5.6 (SiCH₃), -5.5 (SiCH₃), 14.0 (OCH₂CH₃), 18.2 (SiC(CH₃)₃), 25.8 (3C, SiC(CH₃)₃), 54.0 (C-3), 62.1 (2C, C-4, OCH₂CH₃), 66.8 (OCH₂Ph), 69.2 (C-2), 128.0 (2C, CH_{Ph}), 128.1 (CH_{Ph}), 128.5 (2C, CH_{Ph}), 136.3 (C_{Ph}), 155.8 (NHCO₂Bn), 173.6 (CO₂Et); HRESIMS calcd for C₂₀H₃₃NO₆SiNa [M + Na]⁺ 434.1975, found 434.1963.

8 and *ent*-**8**: ¹H NMR (600 MHz, CDCl₃) δ 0.04 (6H, s, SiCH₃), 0.87 (9H, s, SiC(CH₃)₃), 1.26 (3H, t, 7.2 Hz, OCH₂CH₃), 3.55 (1H, dd, 10.3 Hz, 7.6 Hz, H-4), 3.66 (1H, dd, 10.3 Hz, 4.8 Hz, H-4), 4.16 (1H, brs, H-2), 4.21 (2H, m, OCH₂CH₃), 4.38 (1H, dd, 8.9 Hz, 2.1 Hz, H-3), 5.10 (2H, s, OCH₂Ph), 5.61 (1H, d, 8.9 Hz, NH), 7.27–7.35 (5H, m, Ph); ¹³C NMR (151 MHz, CDCl₃) δ -5.5 (2C, SiCH₃), 14.1 (OCH₂CH₃), 18.2 (SiC(CH₃)₃), 25.8 (3C, SiC(CH₃)₃), 55.3 (C-2), 61.7 (OCH₂CH₃), 63.9 (C-4), 67.0 (OCH₂Ph), 71.5 (C-3), 128.0 (2C, CH_{Ph}), 128.1 (CH_{Ph}), 128.5 (2C, CH_{Ph}), 136.2 (C_{Ph}), 156.4 (NHCO₂Bn), 170.7 (CO₂Et); HRESIMS calcd for C₂₀H₃₃NO₆SiNa [M + Na]⁺ 434.1975, found 434.1963.

Synthesis of **9**, **10**, and their enantiomers



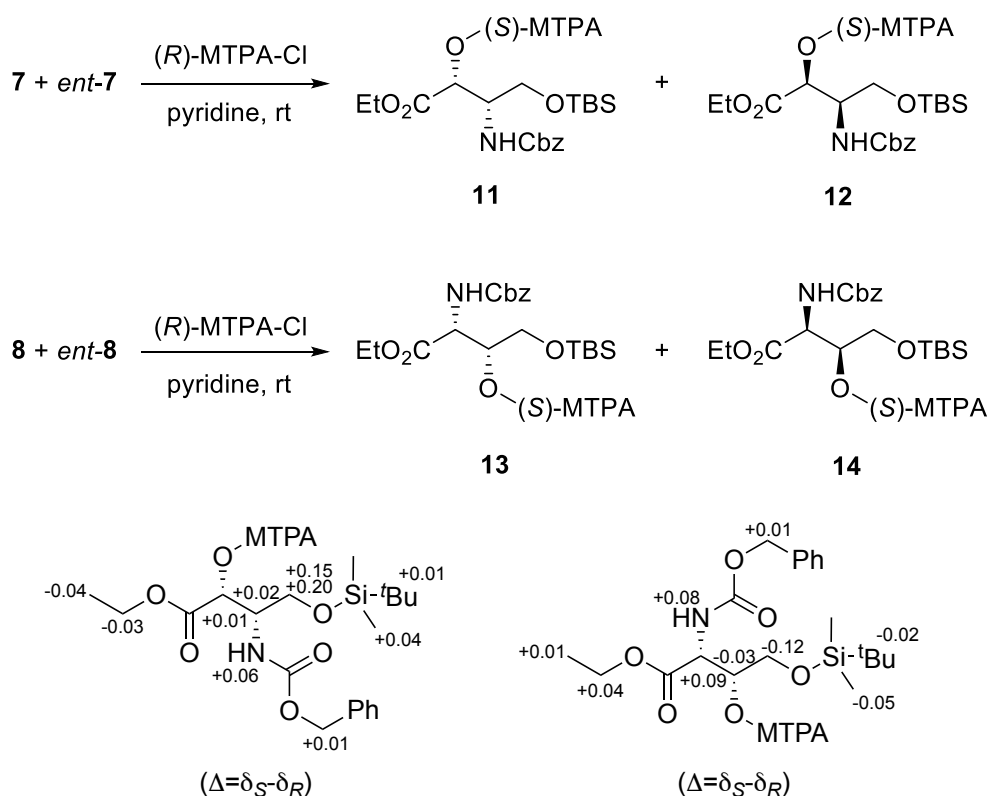
A suspension of the mixture of **7** and *ent*-**7** (5.0 mg, 0.012 mmol) and Pd(OH)₂/C (20 wt% on carbon, 0.9 mg, 0.001 mmol) in EtOAc (0.4 mL) was stirred for 16 h under H₂ atmosphere (1 atm) at room temperature. The reaction mixture was filtered and the filtrate was concentrated to afford the crude amine, which was used in the next reaction without further purification.

To a solution of the crude amine in CH₂Cl₂ (0.2 mL) was added *N,N*-carbonyldiimidazole (CDI, 3.0 mg, 0.019 mmol) at room temperature. The reaction mixture was stirred for 18 h, diluted with H₂O, and extracted with CH₂Cl₂. The organic layer was washed with brine, dried over MgSO₄, and filtered over a short pad of silica gel (*n*-hexane/EtOAc = 1/1). The solution was concentrated and the residue was purified with HPLC (COSMOSIL 5SL-II, *n*-hexane/*i*-PrOH = 49/1) to afford the mixture of **9** and *ent*-**9** (2.3 mg) as a colorless oil. Under the same procedure, the mixture of **8** and *ent*-**8** was derivatized to afford the mixture of **10** and *ent*-**10** (1.0 mg) as a colorless oil.

9 and *ent*-**9**: ¹H NMR (600 MHz, CDCl₃) δ 0.07 (6H, s, SiCH₃), 0.88 (9H, s, SiC(CH₃)₃), 1.30 (3H, t, 7.2 Hz, OCH₂CH₃), 3.66 (1H, dd, 10.5 Hz, 6.2 Hz, H-4), 3.72 (10.5 Hz, 4.5 Hz, H-4), 3.94 (1H, q, 4.8 Hz, H-3), 4.27 (2H, q, 7.2 Hz, OCH₂CH₃), 4.69 (1H, d, 4.8 Hz, H-2), 5.13 (1H, brs, NH); HRESIMS calcd for C₁₃H₂₅NO₅SiNa [M + Na]⁺ 326.1400, found 326.1412.

10 and *ent*-**10**: ¹H NMR (600 MHz, CDCl₃) δ 0.08 (6H, s, SiCH₃), 0.88 (9H, s, SiC(CH₃)₃), 1.29 (3H, t, 7.2 Hz, OCH₂CH₃), 3.78 (1H, dd, 11.5 Hz, 3.8 Hz, H-4), 3.90 (1H, dd, 11.5 Hz, 3.4 Hz, H-4), 4.25 (2H, q, 7.2 Hz, OCH₂CH₃), 4.35 (1H, d, 4.8 Hz, H-2), 4.66 (1H, ddd, 4.8 Hz, 3.8 Hz, 3.4 Hz, H-3), 5.42 (1H, brs, NH); HRESIMS calcd for C₁₃H₂₅NO₅SiNa [M + Na]⁺ 326.1400, found 326.1412.

Synthesis of 11–14



To a solution of the mixture of **7** and *ent*-**7** (5.0 mg, 0.012 mmol) in pyridine (0.2 mL) was added (*R*)-MTPA-Cl (4.6 μ L, 0.025 mmol) at room temperature. The reaction mixture was left for 19 h, quenched with *N,N*-dimethyl-1,3-propanediamine (3.1 μ L, 0.025 mmol), concentrated, and the residue was filtered over a short pad of silica gel (*n*-hexane/EtOAc = 7/3). The eluent was concentrated and the residue was purified with HPLC (COSMOSIL 5SL-II, *n*-hexane/EtOAc = 24/1) to afford (*S*)-MTPA ester **11** (1.1 mg) and **12** (2.9 mg) as a colorless oil. Under the same procedure, the mixture of **8** and *ent*-**8** was derivatized to afford **13** (2.8 mg) and **14** (0.9 mg) as a colorless oil.

11: ^1H NMR (600 MHz, CDCl_3) δ 0.03, 0.06, 0.87, 1.21, 3.43, 3.72, 4.18, 4.39, 4.89, 5.05, 5.51, 7.29–7.56; ESIMS $[\text{M} + \text{Na}]^+$ 650

12: ^1H NMR (600 MHz, CDCl_3) δ -0.02, 0.02, 0.86, 1.25, 3.23, 3.57, 4.21, 4.37, 4.83, 5.04, 5.50, 7.29–7.65; ESIMS $[\text{M} + \text{Na}]^+$ 650

13: ^1H NMR (600 MHz, CDCl_3) δ 0.00, 0.85, 1.24, 3.66, 4.16, 4.78, 5.09, 5.21, 5.61, 7.30–7.46; ESIMS $[\text{M} + \text{Na}]^+$ 650

14: ^1H NMR (600 MHz, CDCl_3) δ 0.05, 0.05, 0.87, 1.23, 3.78, 4.12, 4.69, 5.08, 5.13, 5.64, 7.29–7.50; ESIMS $[\text{M} + \text{Na}]^+$ 650

2. Marfey's analysis

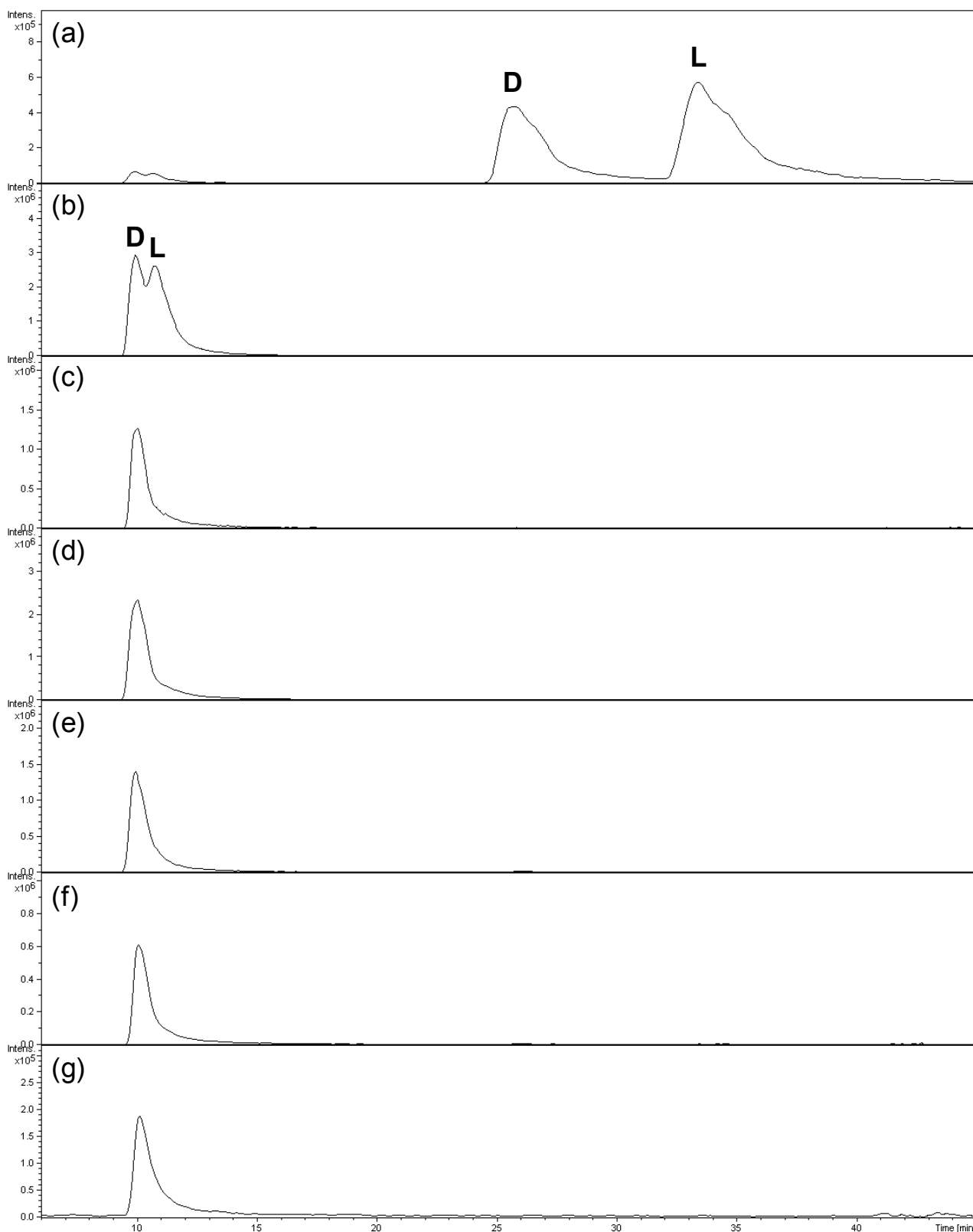


Figure S1. LC-MS chromatograms of Marfey's derivatives

(a: *erythro*- β -hydroxyaspartic acid, b: *threo*- β -hydroxyaspartic acid, c: hydrolysate of poecillastrin C, d: hydrolysate of authentic 73-deoxychondropsin A, e: hydrolysate of authentic poecillastrin B, f: hydrolysate of authentic poecillastrin C, g: hydrolysate of authentic poecillastrin D)

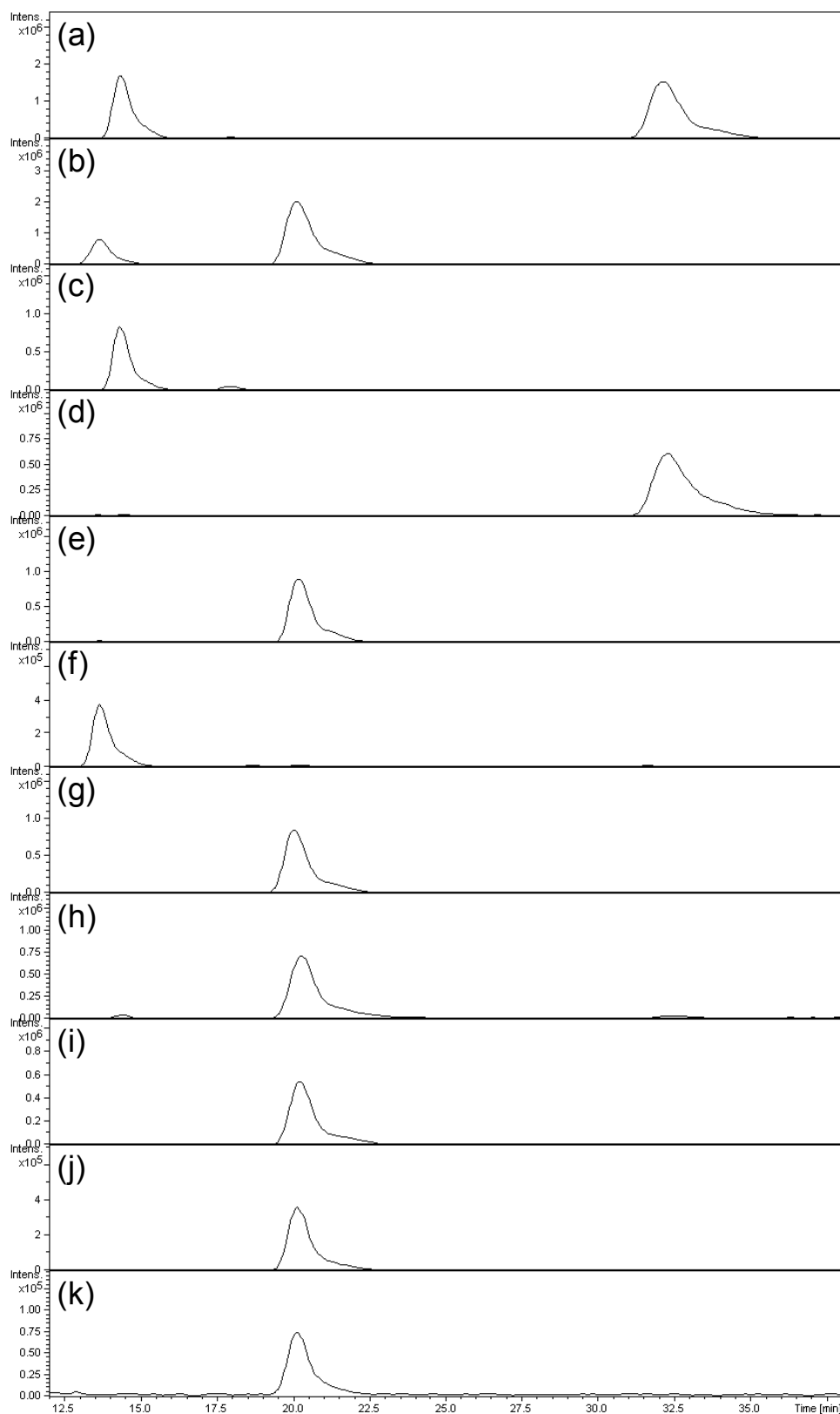


Figure S2. LC-MS chromatograms of Marfey's derivatives

acid hydrolysate of (a) **7** and *ent-7*, (b) **8** and *ent-8*, (c) **11**, (d) **12**, (e) **13**, (f) **14**, (g) reduction product of poecillastrin C, (h) reduction product of authentic 73-deoxychondropsin A, (i) reduction product of authentic poecillastrin B, (j) reduction product of authentic poecillastrin C, (k) reduction product of authentic poecillastrin D

3. NMR spectra

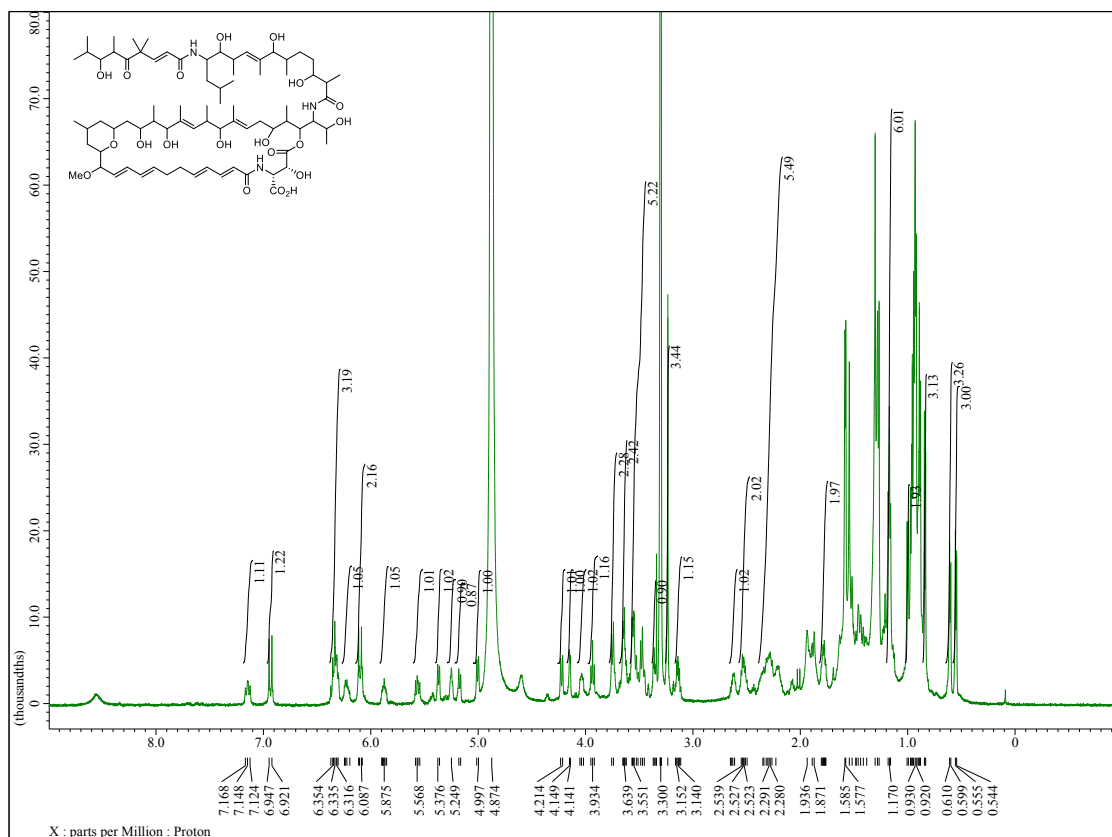


Figure S3. ^1H NMR spectrum of poecillastrin C in CD_3OD

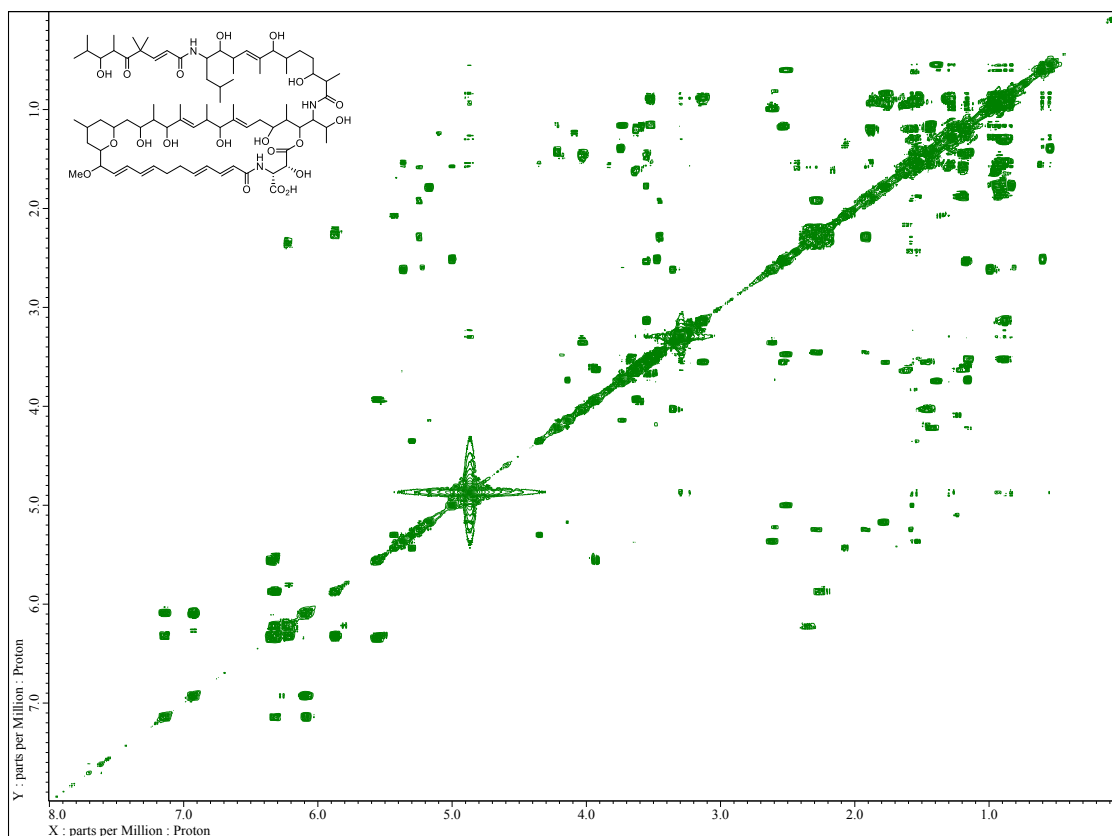


Figure S4. COSY spectrum of poecillastrin C in CD_3OD

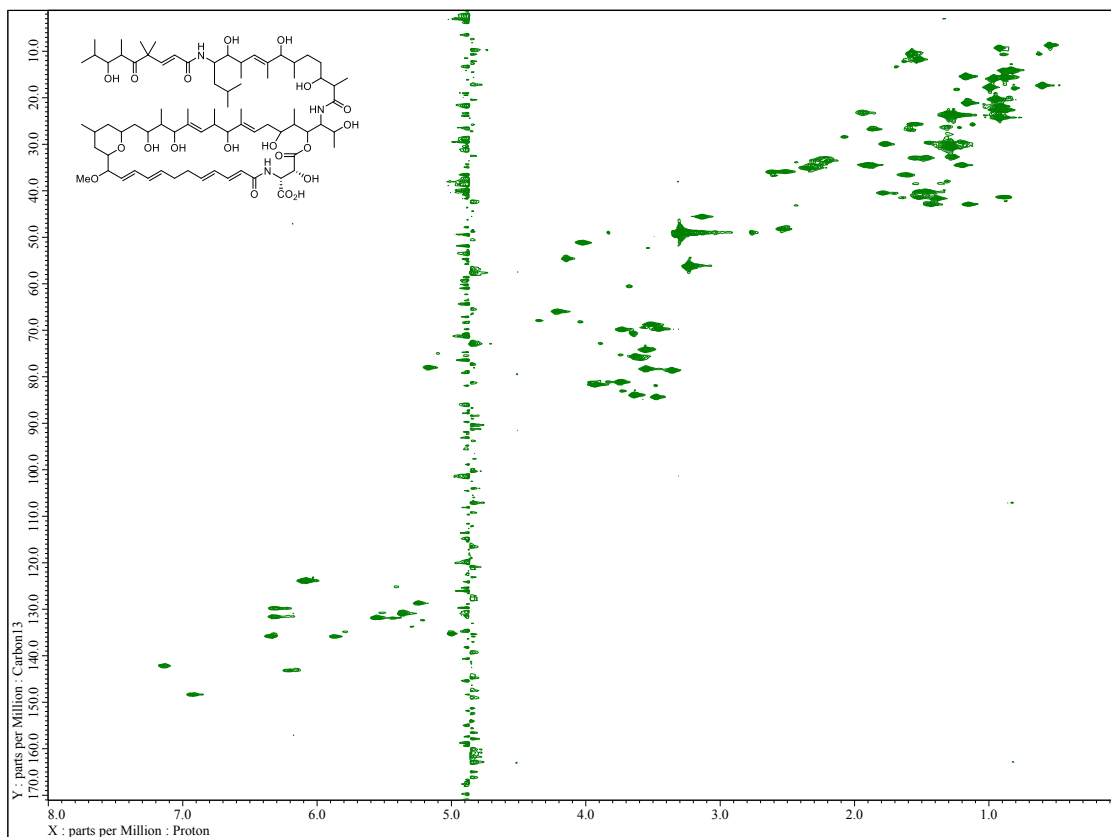


Figure S5. HSQC spectrum of poecillastrin C in CD₃OD

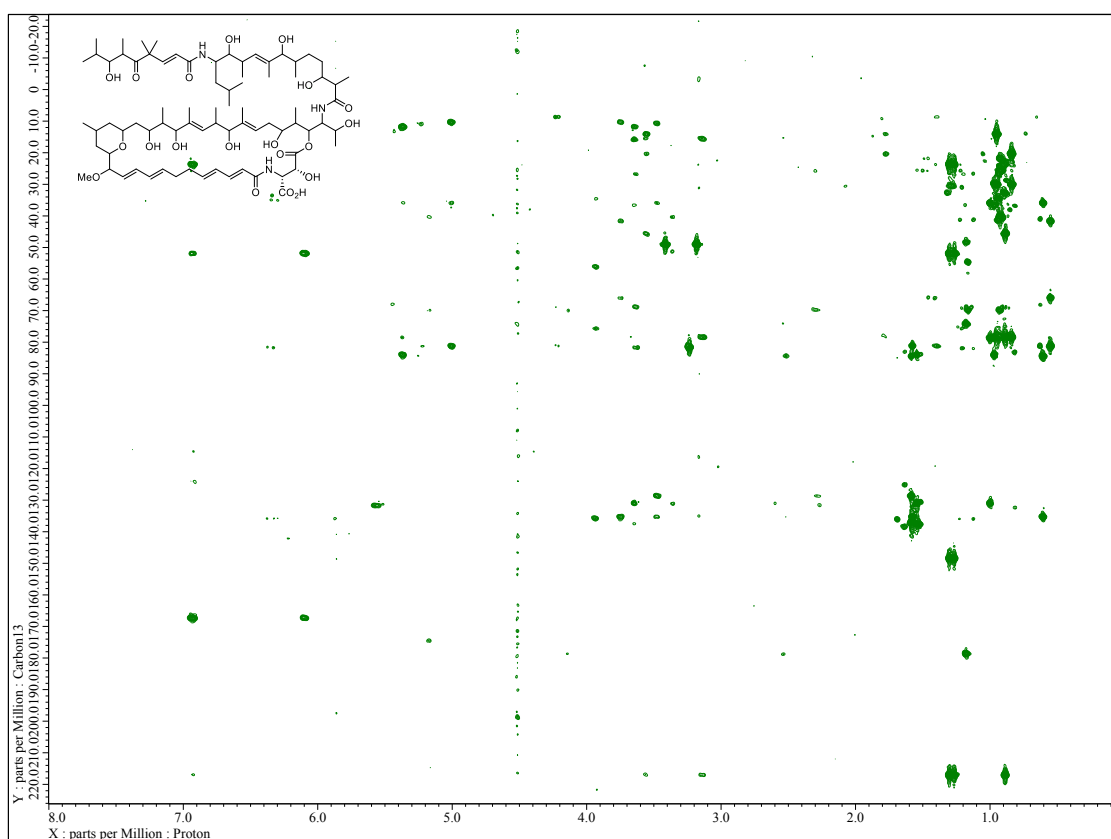


Figure S6. HMBC spectrum of poecillastrin C in CD₃OD

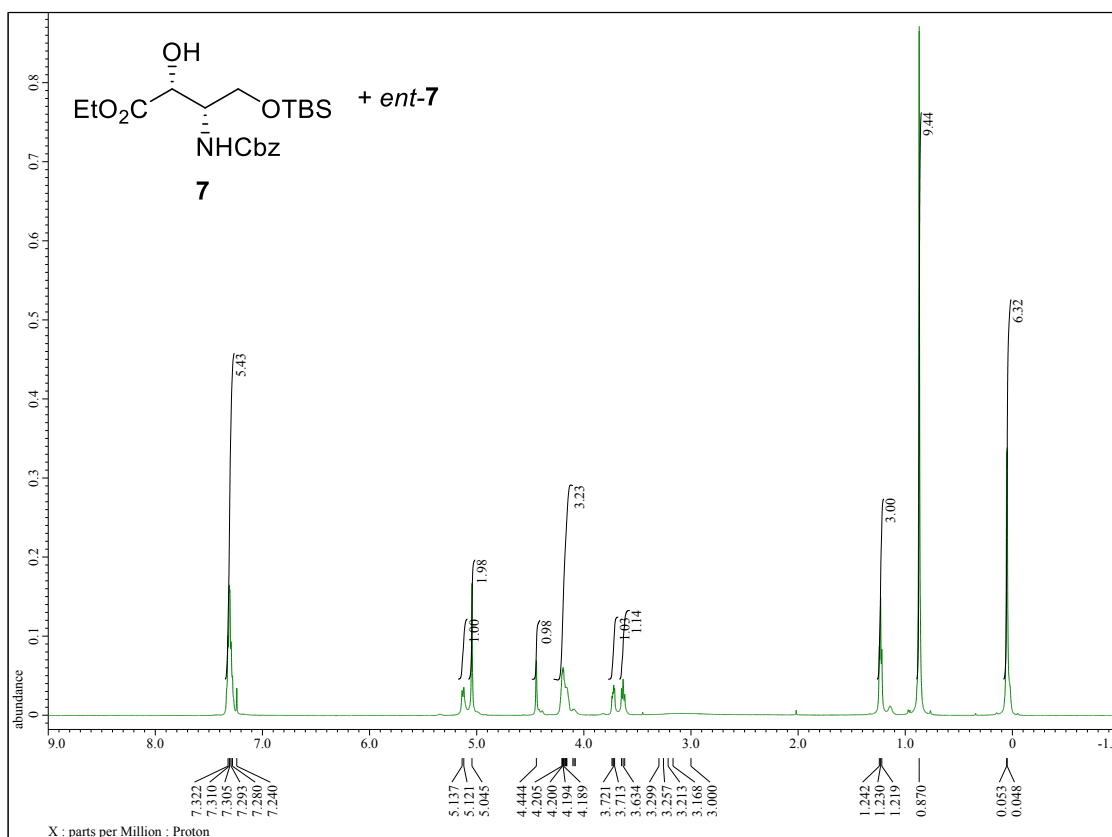


Figure S7. ^1H NMR spectrum of the mixture of **7** and *ent-7* in CDCl_3

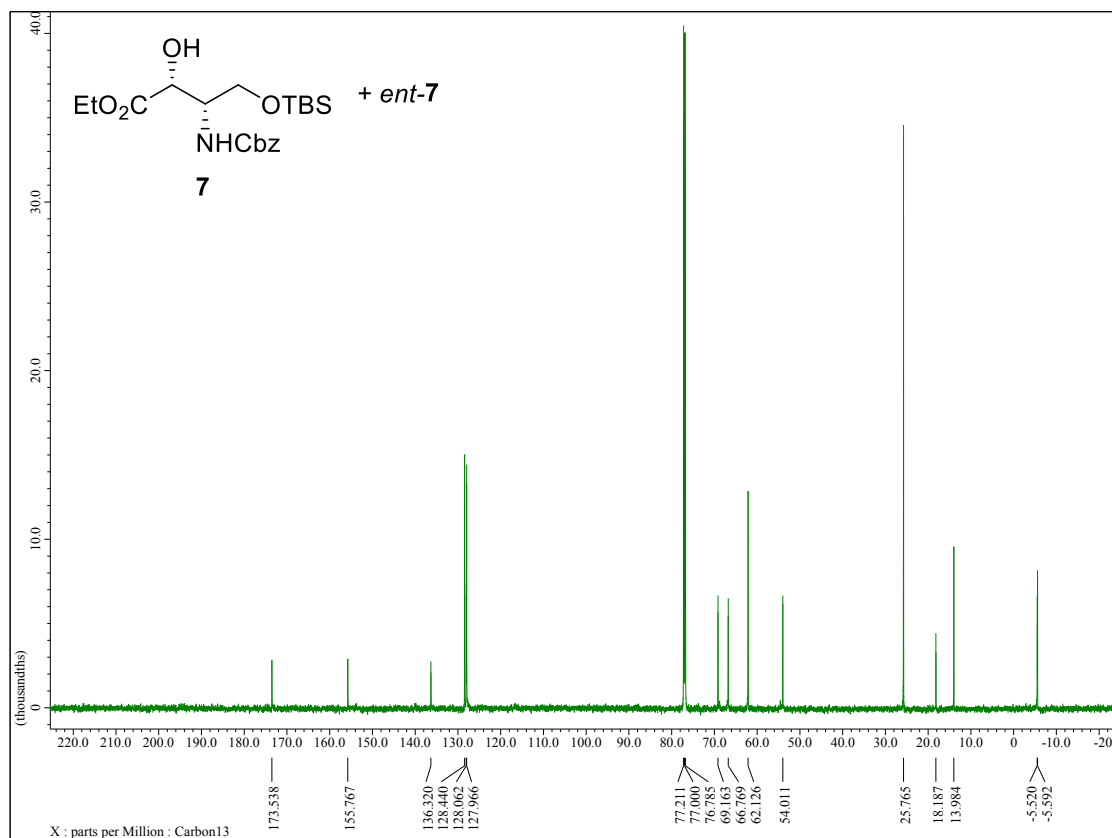
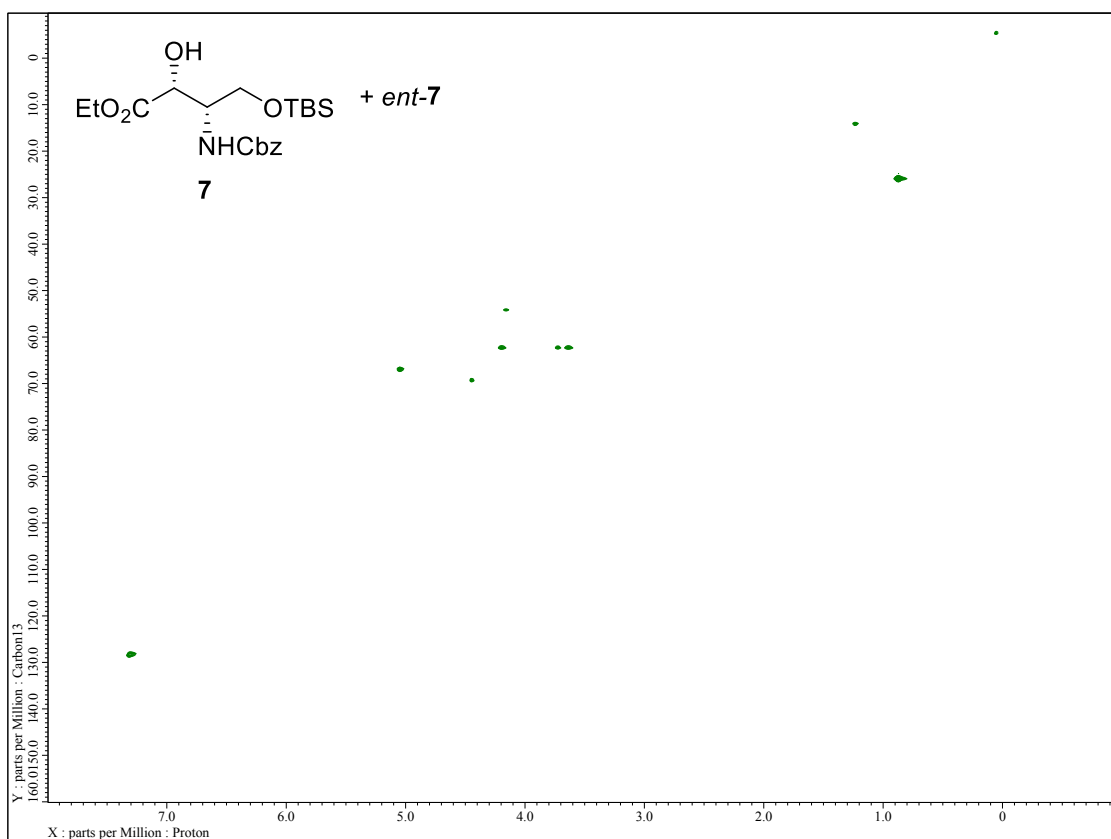
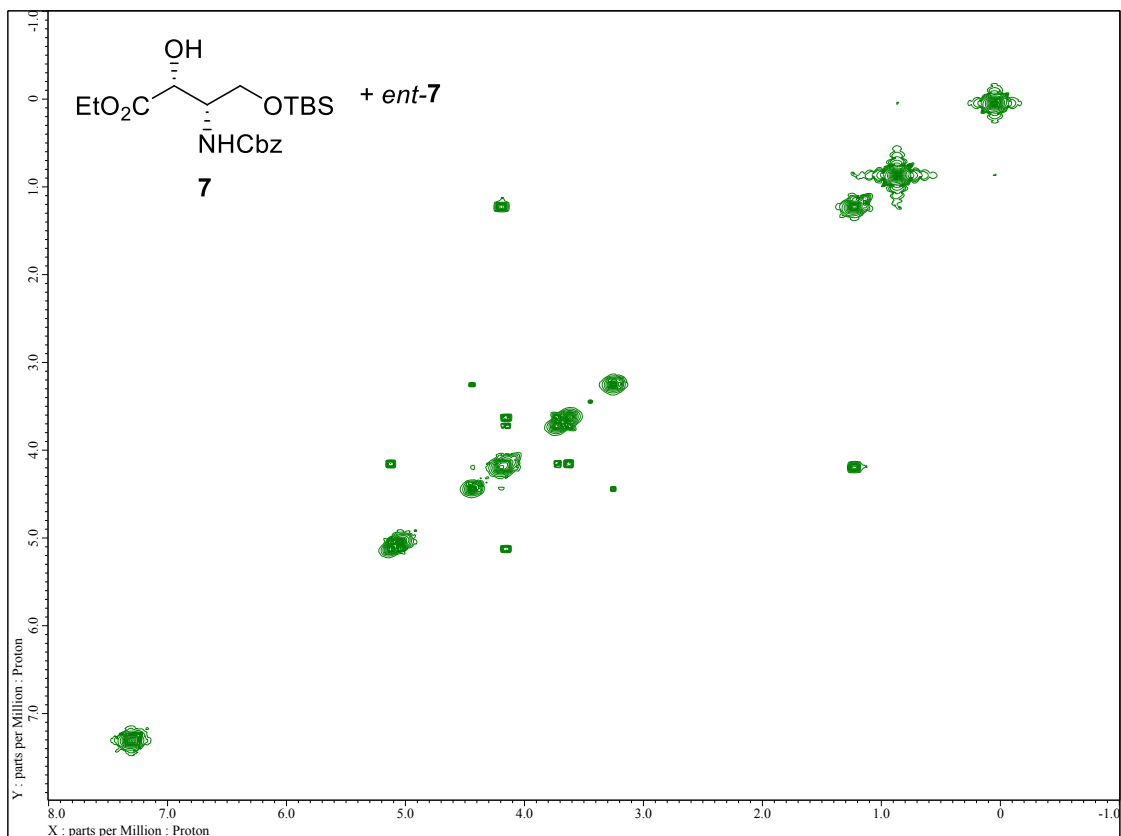
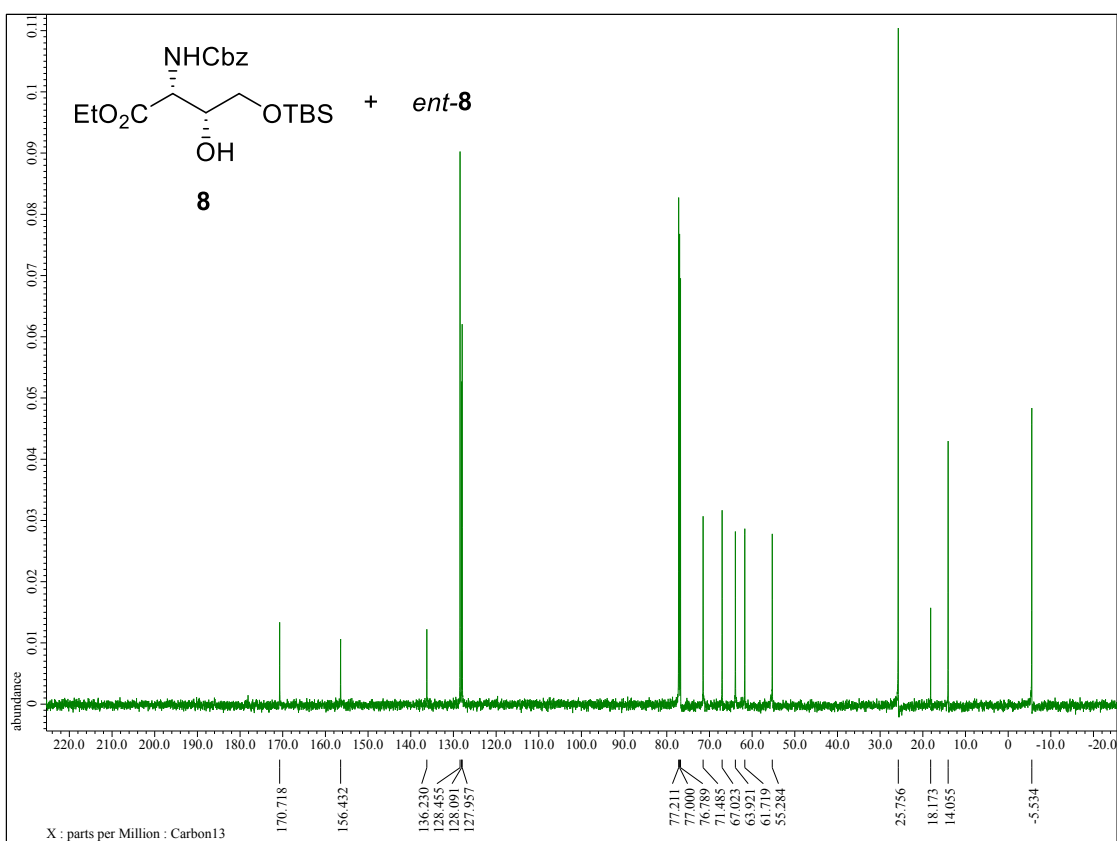
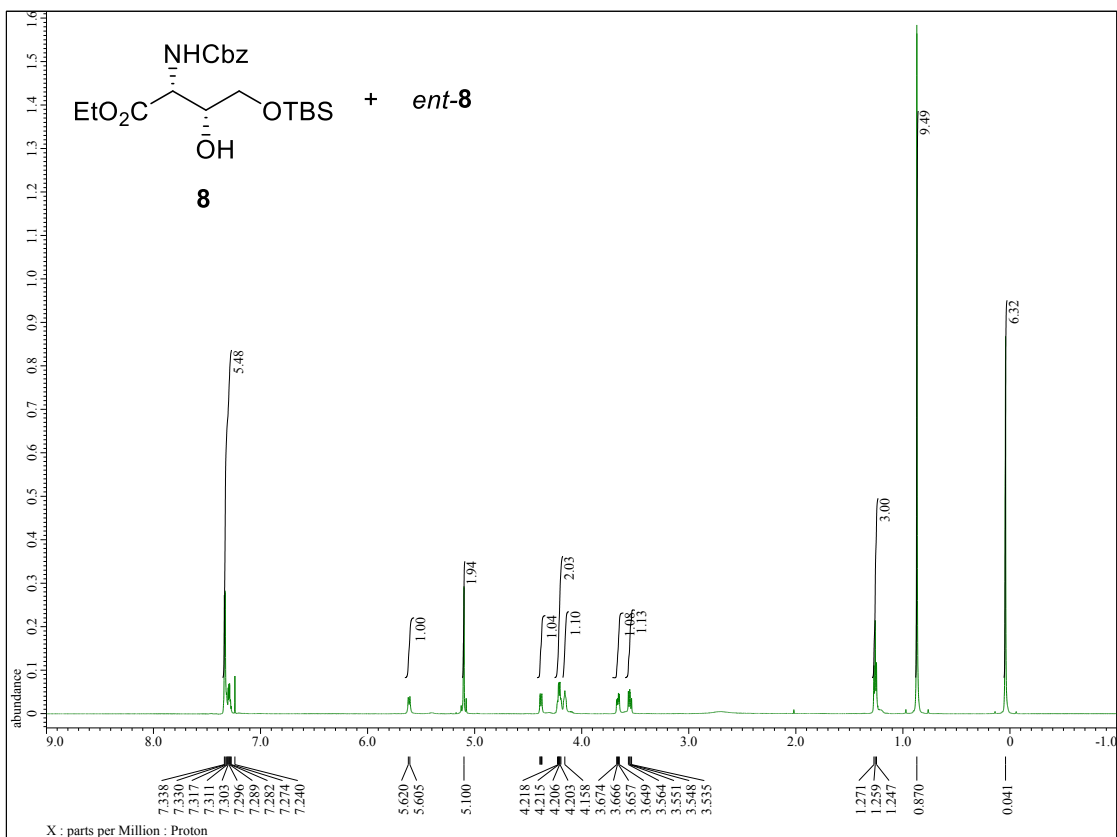
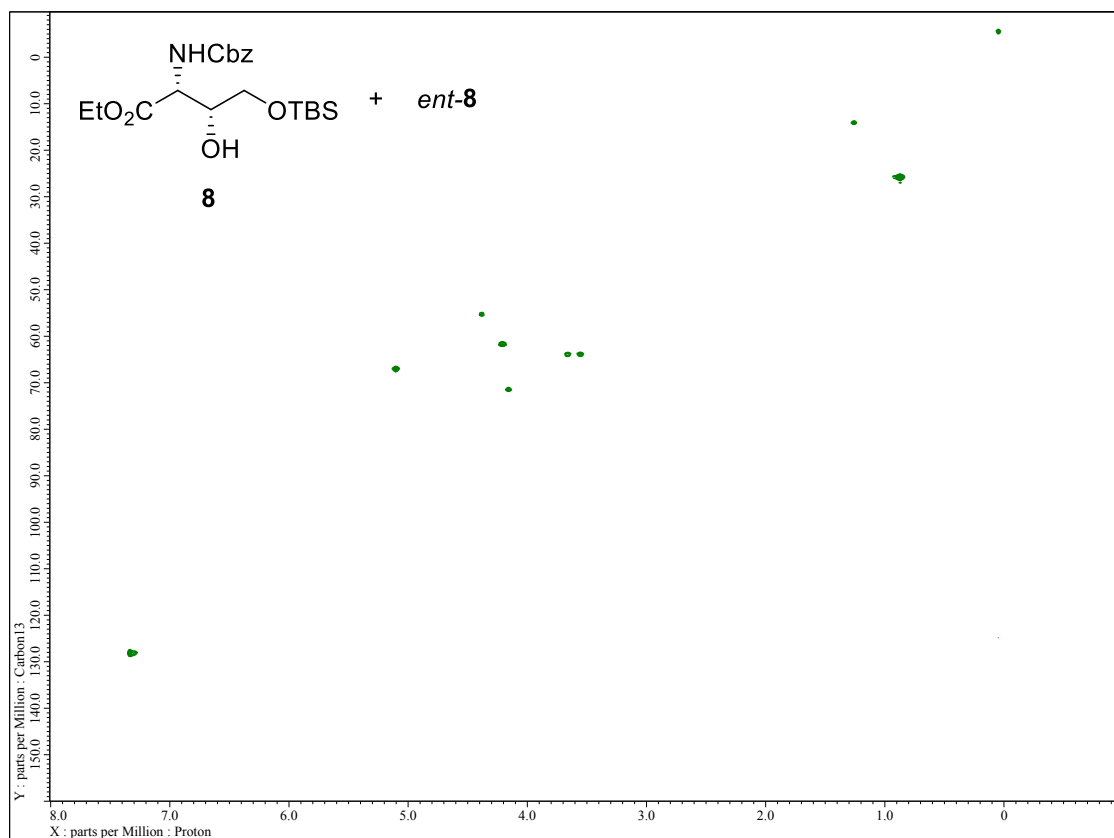
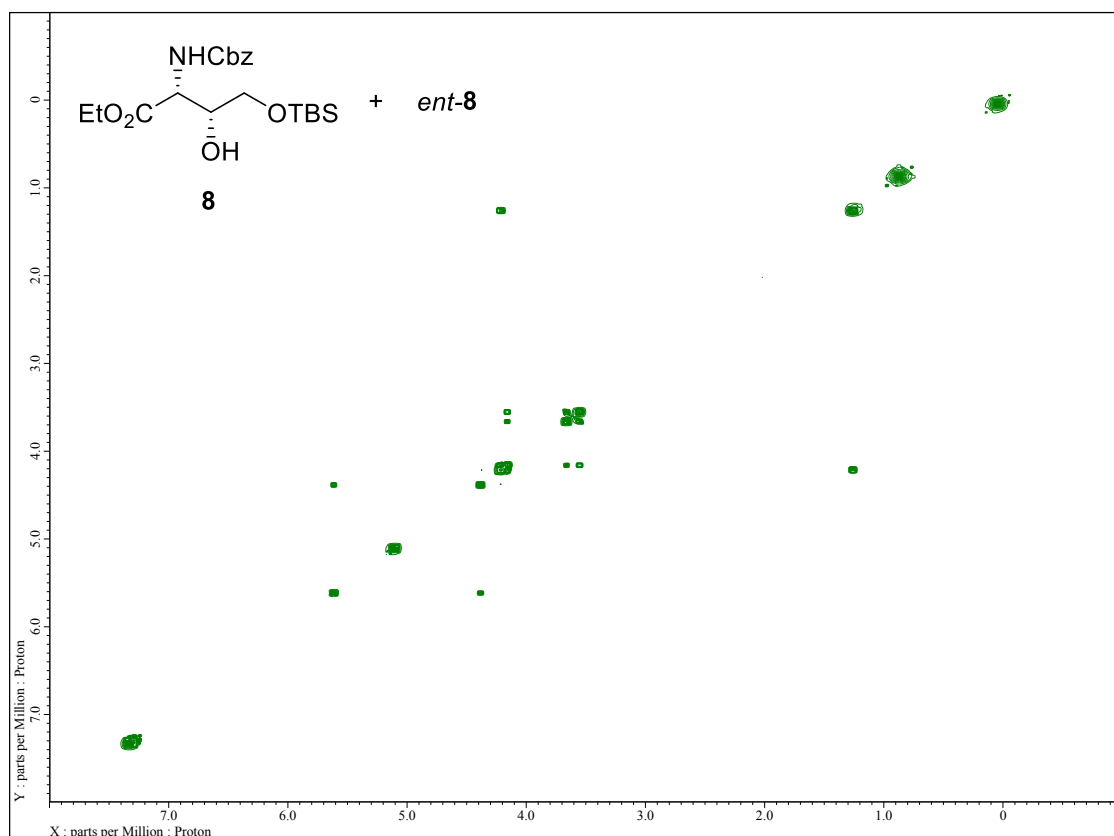


Figure S8. ^{13}C NMR spectrum of the mixture of **7** and *ent-7* in CDCl_3







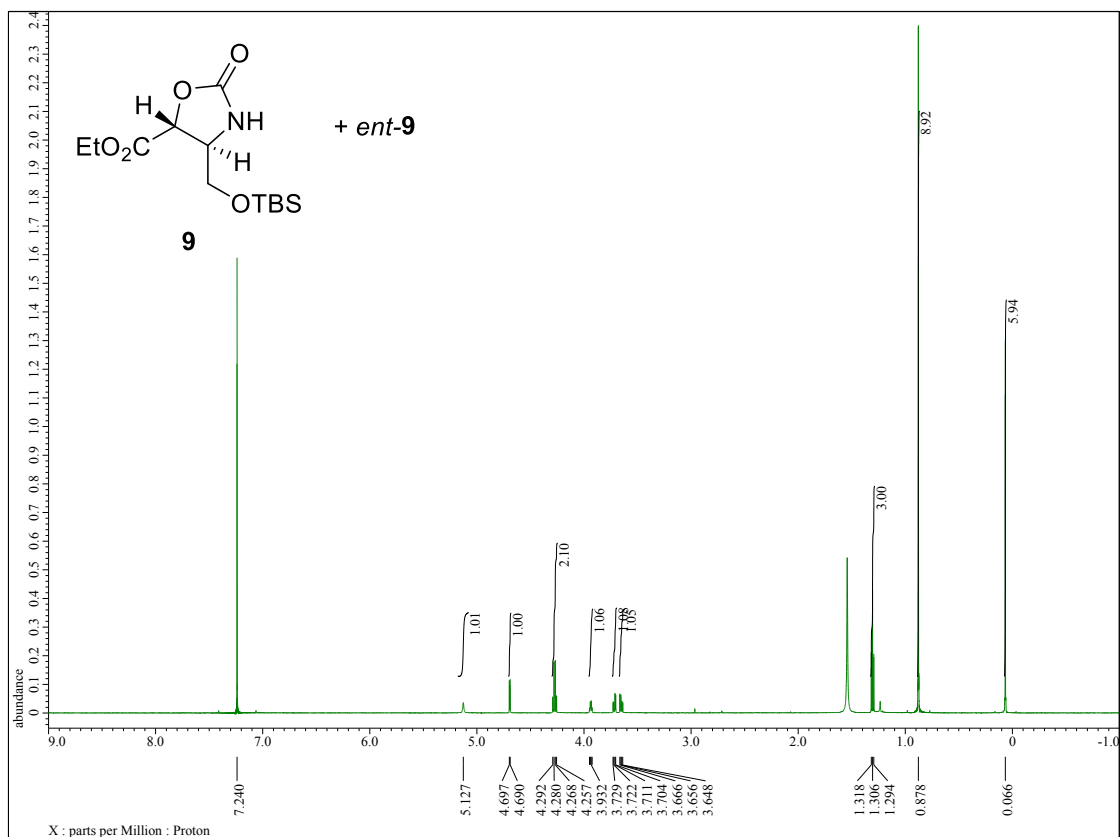


Figure S15. ^1H NMR spectrum of the mixture of **9** and *ent-9* in CDCl_3

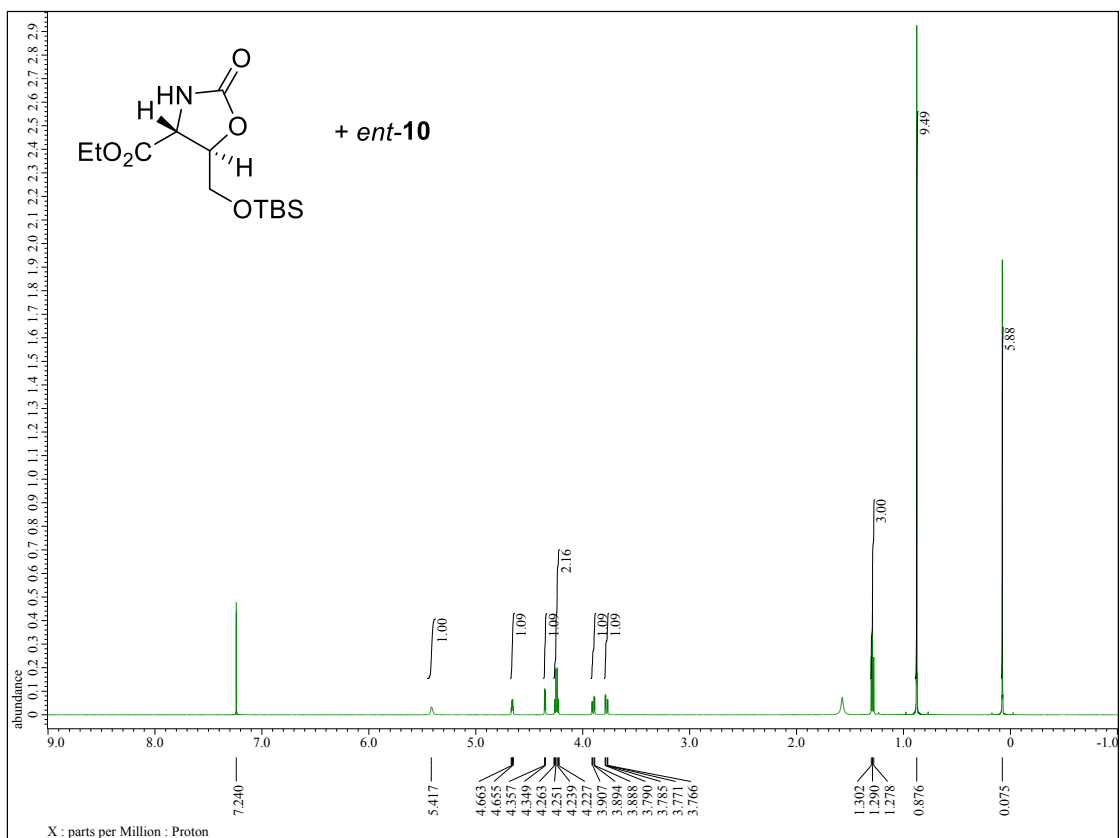


Figure S16. ^1H NMR spectrum of the mixture of **10** and *ent-10* in CDCl_3

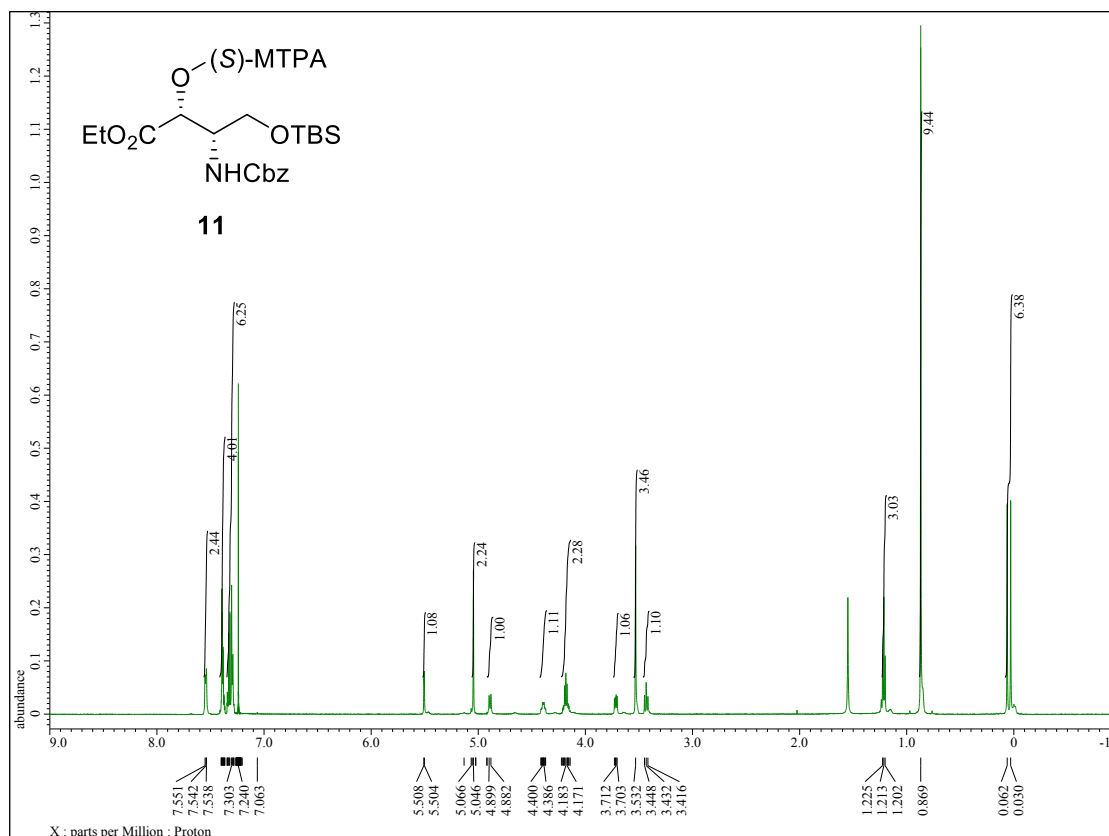


Figure S17. ^1H NMR spectrum of **11** in CDCl_3

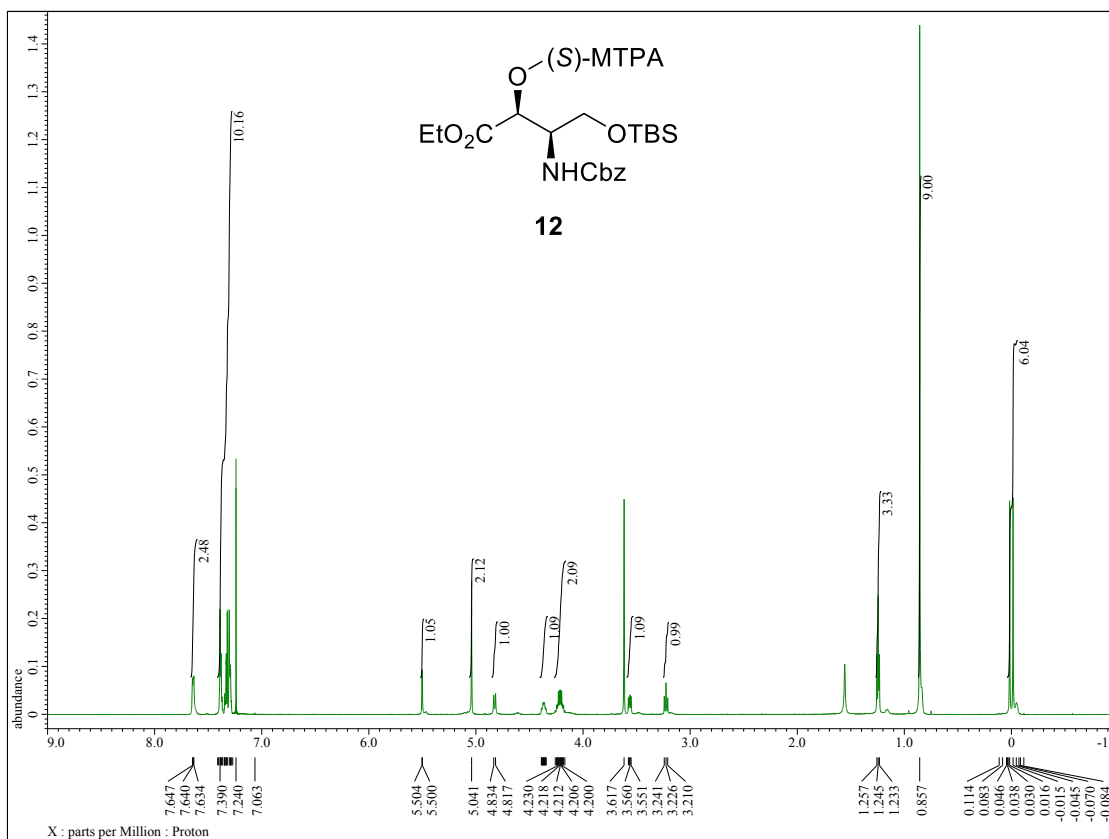


Figure S18. ^1H NMR spectrum of **12** in CDCl_3

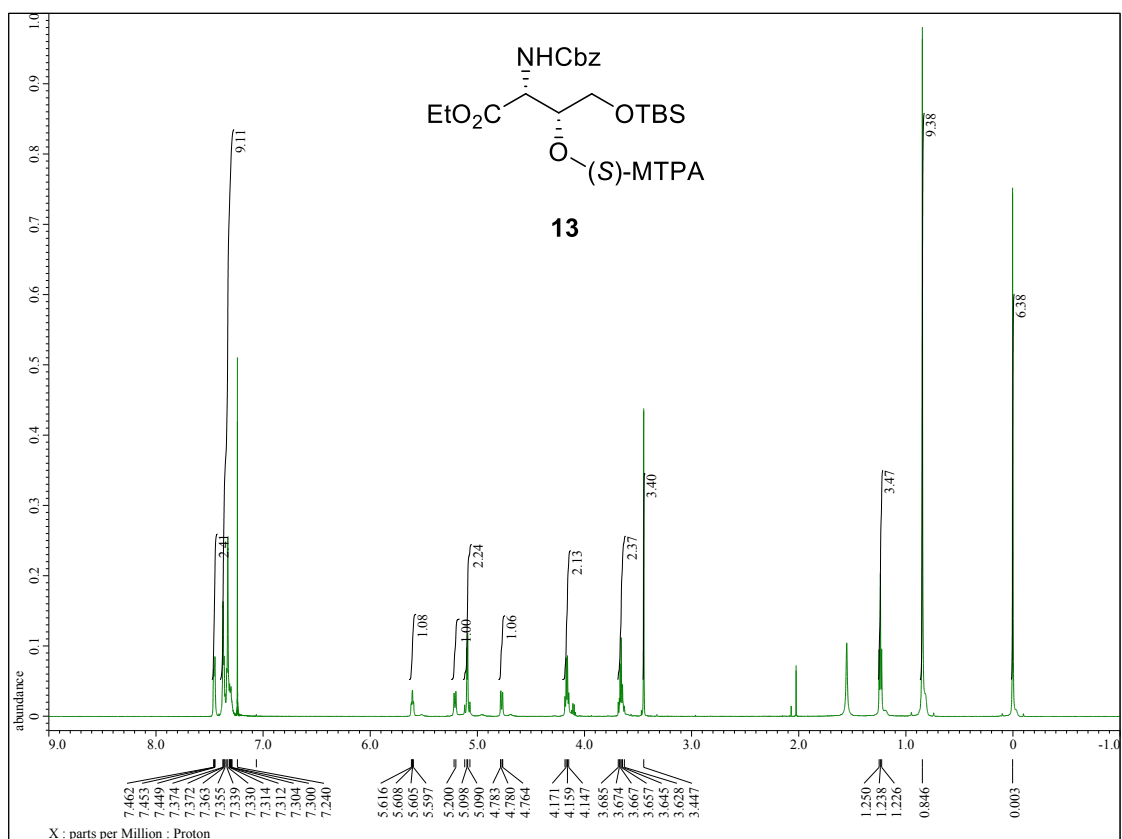


Figure S19. ^1H NMR spectrum of **13** in CDCl_3

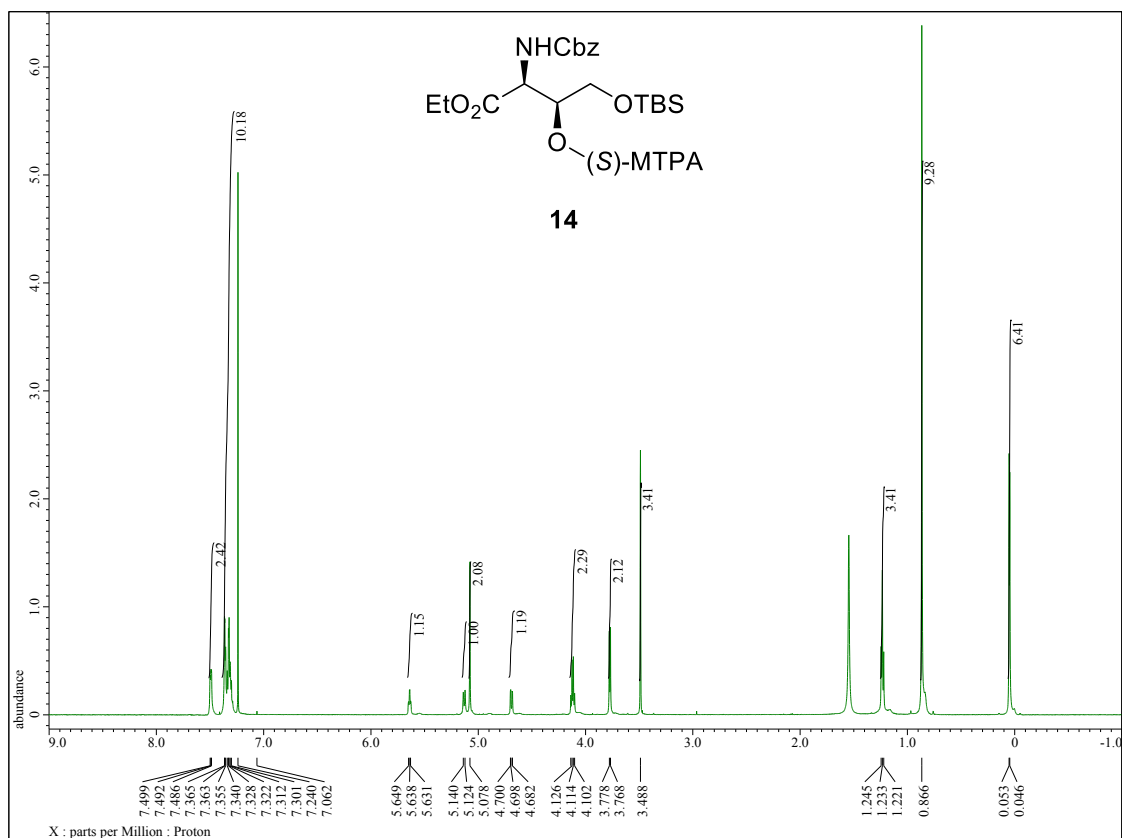


Figure S20. ^1H NMR spectrum of **14** in CDCl_3