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Chemistry & Drug Discovery

Supporting Information

Supporting Information

Selected NMR Data for Final Compounds

Semisynthesis and Cytotoxicity of Hypothemycin Analogues

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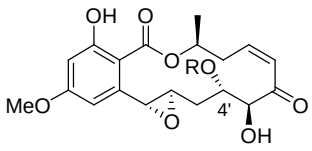
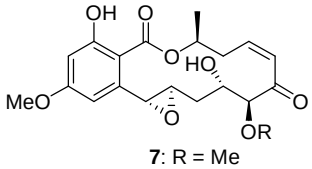
General Methods. NMR spectra were obtained using a Bruker DRX 400 spectrometer. ^{1}H NMR spectra were collected at 400 MHz, and signals are reported in parts per million (δ ppm) relative to the central solvent peak ($CHCl_3$ = 7.26 ppm; CHD_2OD = 3.30 ppm, CHD_2SOCD_3 = 2.50 ppm, CHD_2COCD_3 = 2.05 ppm). ^{13}C NMR spectra were collected at 100 MHz, and signals are reported in parts per million (δ ppm) relative to residual solvent peak ($CDCl_3$ = 77.0 ppm).



6: R = Me

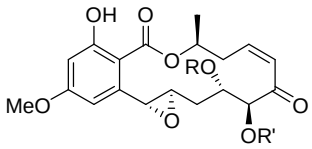
4'-*O*-Methylhypothemycin (6). ^{1}H NMR ($CDCl_3$) δ 12.07 (s, 1H), 6.42 (d, *J* = 2.5 Hz, 1H), 6.40 (d, *J* = 2.5 Hz, 1H), 6.33 (dd, *J* = 11.5, 3.0 Hz, 1H), 6.20 (td, *J* = 11.5, 2.5 Hz, 1H), 5.52 (dqd, *J* = 11.5, 6.0, 2.0 Hz, 1H), 4.77 (br s, 1H), 4.39 (d, *J* = 1.5 Hz, 1H), 3.80 (s, 3H), 3.62 (dd, *J* = 9.5, 1.5 Hz, 1H), 3.47 (s, 3H), 3.17 (dt, *J* = 17.5, 11.5 Hz, 1H), 2.78 (br d, *J* = 9.5 Hz, 1H), 2.57 (dq, *J* = 17.5, 3.0 Hz, 1H), 2.20 (dd, *J* = 15.0, 9.5 Hz, 1H), 1.43 (d, *J* = 6.0 Hz, 3H), 0.97 (dd, *J* = 15.5, 9.5 Hz, 1H). ^{13}C NMR ($CDCl_3$, partial) δ 200.0, 166.2, 165.2, 145.4, 142.5, 126.4, 103.7, 101.0, 79.4, 75.8, 73.2, 62.9, 57.5, 56.8, 55.5, 36.9, 31.9, 21.0. Chemoselectivity of methylation was assigned on the basis of HMBC correlation from H-4' (3.62 ppm) to the methyl carbon (56.8 ppm) and from the methyl proton resonance (3.47 ppm) to C-4' (79.4 ppm). Proton resonances were assigned with the aid of COSY correlation, and carbon resonances were assigned using HSQC.



7: R = Me

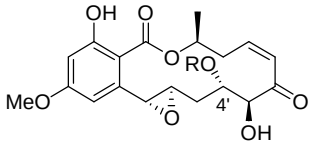
5'-*O*-Methylhypothemycin (7). ^{1}H NMR ($CDCl_3$) δ 12.08 (s, 1H), 6.42 (d, *J* = 2.5 Hz, 1H), 6.39 (d, *J* = 2.5 Hz, 1H), 6.34 (dd, *J* = 11.5, 3.0 Hz, 1H), 6.11 (td, *J* = 11.5, 2.5 Hz, 1H), 5.48 (dqd, *J* = 11.5, 6.0, 1.5 Hz, 1H), 4.41 (d, *J* = 1.5 Hz, 1H), 4.20 (d, *J* = 2.0 Hz, 1H), 4.03 (br d, *J* = 9.5 Hz, 1H), 3.79 (s, 3H), 3.48 (s, 3H), 3.18 (dt, *J* = 17.5, 11.5 Hz,

1H), 2.86 (br d, *J* = 11.5 Hz, 1H), 2.53 (br dq, *J* = 17.5, 2.5 Hz, 1H), 2.01 (dd, *J* = 15.5, 10.0 Hz, 1H), 1.42 (d, *J* = 6.0 Hz, 3H), 1.34 (dd, *J* = 15.5, 9.5 Hz, 1H).



8: R, R' = Me

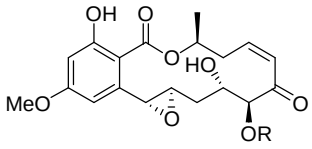
4'-,5'-*O*-Dimethylhypothemycin (8). ^{1}H NMR ($CDCl_3$) δ 12.12 (s, 1H), 6.43 (d, *J* = 2.5 Hz, 1H), 6.39 (d, *J* = 3.0 Hz, 1H), 6.33 (dd, *J* = 11.5, 3.0 Hz, 1H), 6.13 (td, *J* = 11.5, 3.0 Hz, 1H), 5.49 (dqd, *J* = 11.5, 6.0, 2.0 Hz, 1H), 4.41 (d, *J* = 2.0 Hz, 1H), 4.35 (d, *J* = 1.5 Hz, 1H), 3.79 (s, 3H), 3.62 (dd, *J* = 9.5, 1.5 Hz, 1H), 3.49 (s, 3H), 3.43 (s, 3H), 3.23 (dt, *J* = 17.5, 11.0 Hz, 1H), 2.75 (dt, *J* = 10.0, 1.5 Hz, 1H), 2.55 (dq, *J* = 17.5, 3.0 Hz, 1H), 2.23 (ddd, *J* = 15.5, 10.0, 1.0 Hz, 1H), 1.42 (d, *J* = 6.0 Hz, 3H), 1.22 (dd, *J* = 15.5, 10.0 Hz, 1H).



9: R = MOM

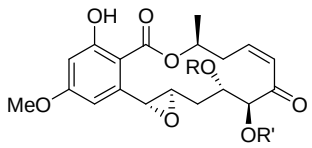
4'-*O*-Methoxymethylhypothemycin (9). ^{1}H NMR ($CDCl_3$) δ 12.10 (s, 1H), 6.35-6.39 (m, 3H), 6.19 (td, *J* = 11.5, 2.5 Hz, 1H), 5.50 (dqd, *J* = 11.5, 6.0, 2.0 Hz, 1H), 4.83 (d, *J* = 7.5 Hz, 1H), 4.75 (1H, overlapped), 4.74 (d, *J* = 7.5 Hz, 1H), 4.38 (d, *J* = 1.5 Hz, 1H), 3.97 (dd, *J* = 9.5, 1.5 Hz, 1H), 3.78 (s, 3H), 3.40 (s, 3H), 3.15 (dt, *J* = 17.5, 11.5 Hz, 1H), 2.74 (br d, *J* = 9.5 Hz, 1H), 2.56 (dq, *J* = 17.5, 2.5 Hz, 1H), 2.23 (dd, *J* = 15.5, 9.5 Hz, 1H), 1.42 (d, *J* = 6.0 Hz, 3H), 0.98 (dd, *J* = 15.5, 9.5 Hz, 1H). ^{13}C NMR ($CDCl_3$) δ 199.7,

171.2, 166.2, 165.0, 145.4, 142.3, 126.4, 103.9, 103.7, 100.8, 96.3, 78.6, 76.5, 73.2, 62.6, 57.5, 55.8, 55.4, 36.9, 32.0, 21.0. Chemoselectivity of methoxymethylation was assigned on the basis of HMBC correlation from H-4' (3.97 ppm) to the methylene MOM carbon (96.3 ppm) and from the methylene MOM proton resonances (4.83 and 4.74 ppm) to C-4' (76.5 ppm). Proton resonances were assigned with the aid of COSY correlation, and carbon resonances were assigned using HSQC.



10: R = MOM

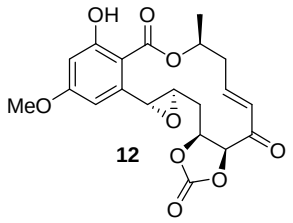
5'-*O*-Methoxymethylhypothemycin (10). ^{1}H NMR ($CDCl_3$) δ 12.11 (s, 1H), 6.42 (d, *J* = 2.5 Hz, 1H), 6.39 (d, *J* = 2.5 Hz, 1H), 6.33 (dd, *J* = 11.5, 3.0 Hz, 1H), 6.12 (td, *J* = 11.5, 2.0 Hz, 1H), 5.48 (dqd, *J* = 11.0, 6.0, 1.5 Hz, 1H), 4.82 (d, *J* = 7.0 Hz, 1H), 4.72 (d, *J* = 7.0 Hz, 1H), 4.54 (d, *J* = 1.5 Hz, 1H), 4.44 (d, *J* = 1.5 Hz, 1H), 4.07 (br d, *J* = 11.5 Hz, 1H), 3.79 (s, 3H), 3.50 (s, 3H), 3.17 (dt, *J* = 17.5, 11.5 Hz, 1H), 2.91 (br d, *J* = 9.5 Hz, 1H), 2.53 (br d, *J* = 15.5 Hz, 1H), 2.01 (dd, *J* = 15.5, 10.5 Hz, 1H), 1.42 (d, *J* = 6.0 Hz, 3H), 1.36 (dd, *J* = 15.5, 9.5 Hz, 1H).



11: R, R' = MOM

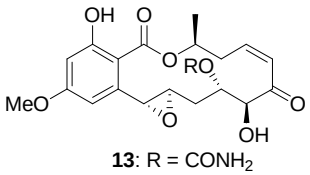
4'-,5'-*O*-Bis(methoxymethyl)hypothemycin (11). ^{1}H NMR ($CDCl_3$) δ 12.18 (s, 1H), 6.41 (d, *J* = 2.5 Hz, 1H), 6.39 (d, *J* = 2.5 Hz, 1H), 6.35 (dd, *J* = 11.5, 3.0 Hz, 1H), 6.12 (td, *J* =

11.5, 2.5 Hz, 1H), 5.48 (dqd, *J* = 11.5, 6.0, 2.0 Hz, 1H), 4.79 (d, *J* = 7.0 Hz, 1H), 4.76 (d, *J* = 7.0 Hz, 1H), 4.73 (d, *J* = 1.5 Hz, 1H), 4.70 (d, *J* = 7.0 Hz, 1H), 4.65 (d, *J* = 7.5 Hz, 1H), 4.41 (d, *J* = 1.5 Hz, 1H), 4.03 (dd, *J* = 10.0, 1.5 Hz, 1H), 3.78 (s, 3H), 3.43 (s, 3H), 3.35 (s, 3H), 3.21 (dt, *J* = 17.5, 11.5 Hz, 1H), 2.72 (dt, *J* = 10.0, 1.5 Hz, 1H), 2.52 (dq, *J* = 17.5, 2.5 Hz, 1H), 2.30 (ddd, *J* = 14.5, 10.0, 1.0 Hz, 1H), 1.41 (d, *J* = 6.0 Hz, 3H), 1.24 (dd, *J* = 16.0, 9.0 Hz, 1H).



12

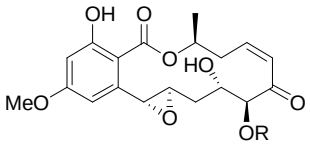
7'-8'-(*E*)-Hypothemycin-4',5'-carbonate (12). ^{1}H NMR ($CDCl_3$) δ 11.60 (s, 1H), 7.07 (dt, *J* = 16.0, 7.0 Hz, 1H), 6.45 (d, *J* = 2.5 Hz, 1H), 6.44 (d, *J* = 2.5 Hz, 1H), 6.38 (d, *J* = 16.0 Hz, 1H), 5.53 (quintetd, *J* = 6.5, 4.0 Hz, 1H), 5.37 (d, *J* = 7.5 Hz, 1H), 5.06 (td, *J* = 8.0, 3.5 Hz, 1H), 4.38 (d, *J* = 1.5 Hz, 1H), 3.81 (s, 3H), 2.87 (m, 1H), 2.63-2.72 (m, 2H), 2.39 (dt, *J* = 16.0, 3.5 Hz, 1H), 1.96 (m, 1H), 1.47 (d, *J* = 6.5 Hz, 3H).



13: R = $CONH_2$

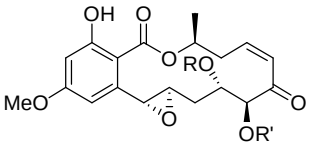
4'-Carbamoylhypothemycin (13). ^{1}H NMR ($CDCl_3$) δ 12.11 (s, 1H), 6.54 (dd, *J* = 11.5, 3.0 Hz, 1H), 6.41 (d, *J* = 2.5 Hz, 1H), 6.37 (d, *J* = 2.5 Hz, 1H), 6.24 (td, *J* = 11.5, 2.5 Hz, 1H), 5.54 (dqd, *J* = 11.0, 6.0, 2.0 Hz, 1H), 5.11 (d, *J* = 10.0 Hz, 1H), 4.77 (d, *J* = 5.0 Hz,

1H), 4.40 (d, *J* = 1.5 Hz, 1H), 3.79 (s, 3H), 3.57 (d, *J* = 5.0 Hz, 1H), 3.14 (dt, *J* = 17.5, 11.5 Hz, 1H), 2.71 (d, *J* = 10.0 Hz, 1H), 2.58 (dm, *J* = 17.5 Hz, 1H), 2.32 (dd, *J* = 15.5, 10.5 Hz, 1H), 1.43 (d, *J* = 6.0 Hz, 3H), 1.01 (d, *J* = 15.5, 9.5 Hz, 1H).



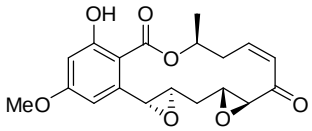
14: R = $CONH_2$

5'-Carbamoylhypothemycin (14). ^{1}H NMR ($CDCl_3$) δ 12.10 (s, 1H), 6.38-6.42 (m, 3H), 6.16 (td, *J* = 11.5, 2.5 Hz, 1H), 5.48 (dqd, *J* = 11.5, 6.0, 2.0 Hz, 1H), 5.42 (d, *J* = 2.0 Hz, 1H), 4.43 (d, *J* = 1.5 Hz, 1H), 4.22 (t, *J* = 9.0 Hz, 1H), 3.79 (s, 3H), 3.17 (dt, *J* = 17.5, 11.0 Hz, 1H), 2.89 (br d, *J* = 9.5 Hz, 1H), 2.79 (br d, *J* = 9.0 Hz, 1H), 2.08 (dd, *J* = 15.5, 10.5 Hz, 1H), 1.41 (d, *J* = 6.0 Hz, 3H), 1.36 (dd, *J* = 15.5, 10.0 Hz, 1H).



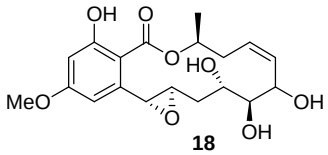
15: R, R' = $CONH_2$

4'-,5'-Dicarbamoylhypothemycin (15). ^{1}H NMR (CD_3OD) δ 6.58 (dd, *J* = 11.5, 3.0 Hz, 1H), 6.40 (d, *J* = 2.5 Hz, 1H), 6.34 (d, *J* = 2.5 Hz, 1H), 6.28 (td, *J* = 11.5, 2.5 Hz, 1H), 5.55 (dqd, *J* = 11.5, 6.0, 2.0 Hz, 1H), 5.48 (d, *J* = 1.5 Hz, 1H), 5.17 (dd, *J* = 10.0, 1.5 Hz, 1H), 4.45 (d, *J* = 1.5 Hz, 1H), 3.78 (s, 3H), 3.17 (dt, *J* = 17.0, 11.5 Hz, 1H), 2.64 (dt, *J* = 10.0, 1.5 Hz, 1H), 2.54 (dq, *J* = 17.5, 2.0 Hz, 1H), 2.31 (dd, *J* = 15.5, 10.0 Hz, 1H), 1.41 (d, *J* = 6.0 Hz, 3H), 1.31 (dd, *J* = 15.5, 10.0 Hz, 1H).



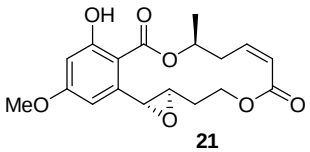
16

4'-,5'-Epoxyhypothemycin (16). ^{1}H NMR ($CDCl_3$) δ 11.45 (s, 1H), 6.44 (d, *J* = 2.5 Hz, 1H), 6.40 (d, *J* = 2.5 Hz, 1H), 6.19 (td, *J* = 12.0, 4.5 Hz, 1H), 6.05 (dd, *J* = 12.0, 1.0 Hz, 1H), 5.63 (m, 1H), 4.28 (d, *J* = 2.0 Hz, 1H), 3.80 (d, *J* = 2.0 Hz, 1H), 3.79 (s, 3H), 3.44 (br s, 1H), 2.85 (br d, *J* = 15.5 Hz, 1H), 2.80 (m, 1H), 2.75 (br d, *J* = 10.0 Hz, 1H), 2.46 (br dd, *J* = 15.5, 4.5 Hz, 1H), 1.98 (ddd, *J* = 15.5, 9.5, 1.5 Hz, 1H), 1.48 (d, *J* = 6.5 Hz, 3H).



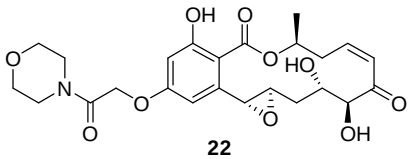
18

6'-Dihydrohypothemycin (18). ^{1}H NMR (DMSO-d_6) δ 11.99 (s, 1H), 6.44 (d, *J* = 2.5 Hz, 1H), 6.33 (d, *J* = 2.5 Hz, 1H), 5.70 (m, 1H), 5.51 (br t, *J* = 10.5 Hz, 1H), 5.39 (m, 1H), 4.71 (d, *J* = 5.5 Hz, 1H), 4.63 (d, *J* = 5.5 Hz, 1H), 4.56 (d, *J* = 4.5 Hz, 1H), 4.37 (d, *J* = 1.5 Hz, 1H), 4.29 (m, 1H), 3.76 (s, 3H), 3.67 (t, *J* = 4.0 Hz, 1H), 3.53 (m, 1H), 2.81 (br d, *J* = 10.0 Hz, 1H), 2.62-2.77 (m, 2H), 1.95 (br dd, *J* = 12.5, 9.5 Hz, 1H), 1.59 (br t, *J* = 12.0 Hz, 1H), 1.41 (d, *J* = 6.5 Hz, 3H).



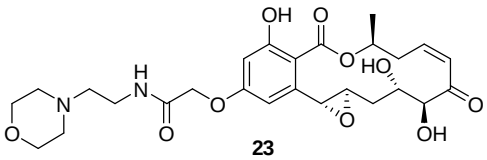
21

Hypothemycin Diester (21). ^{1}H NMR ($CDCl_3$) δ 11.88 (s, 1H), 6.46 (d, *J* = 2.5 Hz, 1H), 6.39 (d, *J* = 2.5 Hz, 1H), 6.20 (td, *J* = 12.0, 5.5 Hz, 1H), 5.78 (dd, *J* = 12.0, 1.0 Hz, 1H), 5.58 (dqd, *J* = 11.0, 6.5, 2.0 Hz, 1H), 4.65 (ddd, *J* = 13.5, 12.0, 2.0 Hz, 1H), 4.58 (d, *J* = 2.0 Hz, 1H), 4.16 (ddd, *J* = 11.5, 4.0, 3.0 Hz, 1H), 3.79 (s, 3H), 3.59 (dt, *J* = 13.5, 12.0 Hz, 1H), 2.72 (dt, *J* = 10.0, 2.0 Hz, 1H), 2.42 (dm, *J* = 15.0 Hz, 1H), 2.28 (ddt, *J* = 13.5, 5.0, 2.0 Hz, 1H), 1.88 (m, 1H), 1.47 (d, *J* = 6.0 Hz, 3H).



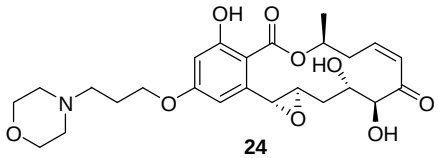
22

4-*O*-(1-(Morpholino-4-yl)ethan-1-on-2-yl)hypothemycin (22). ^{1}H NMR (CD_3OD) δ 6.45 (dd, *J* = 11.5, 3.0 Hz, 1H), 6.39 (d, *J* = 2.5 Hz, 1H), 6.37 (d, *J* = 2.5 Hz, 1H), 6.18 (td, *J* = 11.5, 2.5 Hz, 1H), 5.50 (dqd, *J* = 11.5, 6.0, 1.5 Hz, 1H), 4.83 (s, 2H), 4.57 (d, *J* = 1.5 Hz, 1H), 4.39 (d, *J* = 1.5 Hz, 1H), 4.08 (dd, *J* = 10.0, 1.5 Hz, 1H), 3.64-3.69 (m, 4H), 3.51-3.58 (m, 4H), 3.12 (dt, *J* = 17.5, 11.5 Hz, 1H), 2.81 (br d, *J* = 9.5 Hz, 1H), 2.54 (dq, *J* = 17.5, 2.0 Hz, 1H), 2.00 (dd, *J* = 15.0, 10.0 Hz, 1H), 1.41 (d, *J* = 6.0 Hz, 3H), 1.10 (dd, *J* = 15.0, 9.5 Hz, 1H).



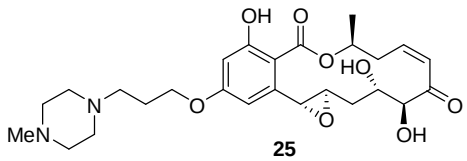
23

4-*O*-(*N*-(2-(Morpholin-4-yl)ethyl)acetamid-2-yl)hypothemycin (23). ^{1}H NMR (CD_3OD) δ 6.47 (d, *J* = 2.5 Hz, 1H), 6.45 (1H, overlapped), 6.42 (d, *J* = 2.5 Hz, 1H), 6.18 (td, *J* = 11.0, 2.5 Hz, 1H), 5.53 (dqd, *J* = 11.5, 6.0, 2.0 Hz, 1H), 4.57 (d, *J* = 1.5 Hz, 1H), 4.54 (s, 2H), 4.41 (d, *J* = 1.5 Hz, 1H), 4.06 (m, 1H), 3.64 (t, 4.5 Hz, 4H), 3.41 (t, *J* = 6.5 Hz, 2H), 3.14 (dt, *J* = 17.5, 11.5 Hz, 1H), 2.80 (br d, *J* = 10.0 Hz, 1H), 2.55 (dq, *J* = 17.5, 2.5 Hz, 1H), 2.50 (t, *J* = 6.5 Hz, 2H), 2.47 (4H, overlapped), 2.01 (dd, *J* = 14.5, 10.0 Hz, 1H), 1.42 (d, *J* = 6.0 Hz, 3H), 1.12 (dd, *J* = 15.0, 9.5 Hz, 1H).



24

4-*O*-(3-(Morpholin-4-yl)propyl)hypothemycin (24). ^{1}H NMR (acetone-d_6) δ 6.53 (dd, *J* = 11.5, 3.0 Hz, 1H), 6.41 (d, *J* = 2.5 Hz, 1H), 6.39 (d, *J* = 2.5 Hz, 1H), 6.23 (td, *J* = 11.5, 2.5 Hz, 1H), 5.55 (dqd, *J* = 11.5, 6.0, 2.0 Hz, 1H), 4.62 (d, *J* = 2.0 Hz, 1H), 4.43 (d, *J* = 2.0 Hz, 1H), 4.08-4.13 (m, 3H), 3.60 (t, *J* = 4.5 Hz, 4H), 3.14 (dt, *J* = 17.0, 11.5 Hz, 1H), 2.83 (dt, *J* = 9.5, 1.5 Hz, 1H), 2.60 (dq, *J* = 17.5, 2.5 Hz, 1H), 2.46 (t, *J* = 7.0 Hz, 2 H), 2.40 (br m, 4H), 1.99 (dd, *J* = 15.0, 10.0 Hz, 1H), 1.93 (quintet, *J* = 7.0 Hz, 2H), 1.47 (d, *J* = 6.0 Hz, 3H), 1.08 (dd, *J* = 15.0, 9.5 Hz, 1H).



25

4-*O*-(3-(4-Methylpiperazin-1-yl)propyl)hypothemycin (25). ^{1}H NMR ($CDCl_3$) δ 12.04 (s, 1H), 6.39-6.40 (s, 2H), 6.35 (dd, *J* = 11.5, 3.0 Hz, 1H), 6.19 (td, *J* = 11.5, 2.5 Hz, 1H), 5.50 (dqd, *J* = 11.5, 6.0, 2.0 Hz, 1H), 4.60 (d, *J* = 1.5 Hz, 1H), 4.39 (d, *J* = 1.5 Hz, 1H), 3.99-4.02 (m, 3H), 3.10 (dt, *J* = 17.5, 11.0 Hz, 1H), 2.90 (d, *J* = 9.5 Hz, 1H), 2.57 (dq, *J* = 17.5, 2.5 Hz, 1H), 2.42-2.54 (m, 10H), 2.29 (s, 3H), 1.99 (dd, *J* = 15.5, 9.5 Hz, 1H), 1.94 (quintet, *J* = 6.5 Hz, 2H), 1.43 (d, *J* = 6.0 Hz, 3H), 1.11 (dd, *J* = 15.5, 9.0 Hz, 1H).