Electronic Supplementary Material (ESI) for Chemical Communications

Supporting Information

Synthetic Modification of Salinomycin:

Selective *O*-Acylation and Biological Evaluation

Björn Borgström,[a] Xiaoli Huang,[b] Martin Pošta,[a] Cecilia Hegardt,[c] Stina Oredsson,[b] and Daniel Strand*[a]

[a]*Centre for analysis and synthesis, Department of Chemistry, Lund University, Box 124, 221 00 Lund, Sweden.*

[b] *Department of Biology, Lund University.*

[c] *Department of Oncology, Clinical Sciences, Lund University.*

PART I

Index

I. General procedures

All reactions were carried out under an atmosphere of nitrogen in oven-dried glassware. Reactions were stirred using Teflon-coated magnetic stir bars. Reactions were monitored by thin layer chromatography (TLC) using aluminum-backed plates (Merck $60F_{254}$). TLC plates were visualized by UV-light (254 nm) and/or treatment with phosphomolybdic acid (PMA) (5% in EtOH), followed by gentle heating. Organic phases were dried using phase separators (International Sorbent Technology). Solvents were removed using Heidolph rotary evaporators. Products were purified by flash chromatography using silica gel 60 Å, 40 – 60 μm or an automatic chromatography system (Biotage Isolera One) using the solvent systems indicated. Optical rotation data were obtained on a Perkin Elmer Model 341 polariometer and are reported as $[\alpha]_D^T$ (*c* = grams/100 mL), where D indicates the sodium D line (589 nm) and T indicates the temperature. NMR spectra were recorded on Bruker Ultrashield 400 plus (^{1}H-NMR at 400 MHz and ^{13}C-NMR at 101 MHz) or Varian Unity Inova (^{1}H-NMR at 500 MHz and ^{13}C-NMR at 125 MHz) magnetic resonance spectrometers. Spectra were processed using MestReNova 8.1. ^{1}H-NMR are reported in chemical shifts downfield from $SiMe_4$ using the respective residual solvent peak as internal standard (chloroform = 7.26 ppm; benzene = 7.16 ppm; methanol = 4.86 ppm).[1] ^{1}H-NMR spectra are reported as follows: chemical shift (δ, ppm), multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, dd = doublet of doublets, dt = doublet of triplets, ddt = doublet of doublet of triplets, td = triplet of doublets, qd = quartet of

[1] G. R. Fulmer, A. J. M Miller, N. H. Sherden, H. E. Gottlieb, A. Nudelman, B. M. Stoltz, J. E. Bercaw and K. I. Goldberg, *Organometallics*, 2010, **29**, 2176.

doublets, m = multiplet, app. = apparent, br s = broad singlet), coupling constant(s) in Hz, and integration. Significant peaks are reported within the overlapping 2.5 – 0.5 ppm region of the ^{1}H-NMR spectra. Acidic protons (O*H*) are assumed to reside in the 2.5 – 0.5 ppm region unless listed explicitly. ^{13}C-NMR spectra are reported in chemical shifts using the respective residual solvent peak as internal standard (chloroform = 77.16 ppm; benzene = 128.06 ppm; methanol = 49.0 ppm).[1] For known compounds (references given) only ^{1}H-NMR spectra are reported. Infrared spectra were recorded on a Bruker ALPHA-P fourier transform spectrometer (FTIR) and are reported as follows: wavenumbers (cm^{-1}), description (w = weak, m = medium, s = strong, br = broad). High-resolution mass spectra (HRMS) were recorded on a Micromass Q-TOF ESI spectrometer. Salinomycin sodium was obtained from Chemtronica AB as technical grade salinomycin (~12%) and purified as described. 3-(4-Methoxyphenyl) propionic chloride was prepared following the procedure of House.[2] Anhydrous methanol, CH_2Cl_2, hexane, and dimethylformamide were collected from a dry solvent system (MB SPS-800). All other reagents and solvents were purchased from commercial suppliers and used as received.

*II. Isolation of salinomycin sodium salt (**2**) and salinomycin (**1**)*

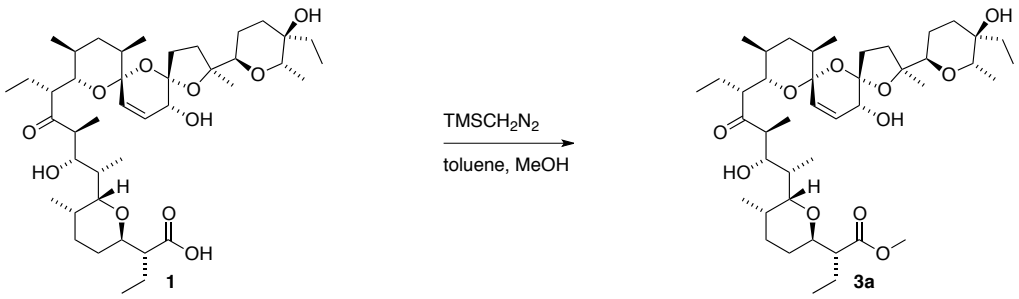
Isolation of salinomycin sodium salt (2). Granular technical grade SA (289 g, 12%) was charged in a round bottom flask and EtOAc (900 mL) as added. The resulting suspension was vigorously stirred with a mechanical stirrer for five minutes to give a dark brown suspension. The suspension was allowed to sediment for 20 minutes and then decanted. The sediment was re-extracted with EtOAc (900 mL) following the same procedure. The combined organic layers were concentrated under reduced pressure to ~1 L and washed with Na_2CO_3 (5 x 200 mL, 5% w/v aq.). The aqueous layer was back-extracted with EtOAc (900 mL). The combined organic layers were dried with $MgSO_4$ and filtered though a plug of celite. The resulting brown solution was concentrated under reduced pressure to give a brown amorphous solid (45.6 g). The solid material was re-dissolved in EtOAc (500 mL) and washed with HCl (2 x 100 mL, 0.1 M aq.) and Na_2CO_3 (2 x 200 mL, 5% v/w aq.). The organic layer was separated, dried over $MgSO_4$, filtered, and concentrated under reduced pressure to give an amorphous solid. The solid material was re-suspended in MeCN (600 mL)

[2] H. O. House and W. C. McDaniel, *J. Org. Chem.*, 1977, **42**, 2155.

and the non-soluble materials removed by filtration. The MeCN solution was then concentrated to dryness under reduced pressure. Purification by flash chromatography (25 – 50% EtOAc/petroleum ether) gave SANa (**2**) as a pale yellow amorphous solid (37.8 g), pure by ^{1}H- and ^{13}C-NMR and a single spot by TLC.

Isolation of salinomycin (1). SANa (**2**) (3.0 g, 3.9 mmol) was dissolved in EtOAc (70 mL) and washed with HCl (3 x 70 mL, 0.1 M aq.). The organic layer was separated, dried using a phase separator, and concentrated to give SA (**1**) (2.9 g, quant.) as a white amorphous solid, pure by ^{1}H- and ^{13}C-NMR and a single spot by TLC.

*III. Procedures and characterization data for compounds **3a – c**, **5a – q**, **6**, **7a/b**, **8**, **9a – c**, **10**, and **11***



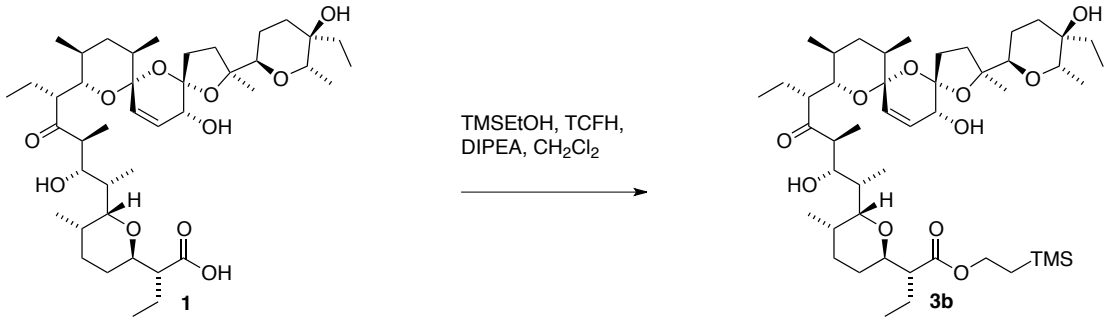
Methyl ester 3a.[3] To a stirred solution of SA (**1**) (190 mg, 0.25 mmol) in a mixture of methanol (1 mL) and toluene (2 mL), at room temperature (RT), was added TMS-diazomethane (150 µL, 0.30 mmol, 2 M in hexanes) drop-wise over 2 minutes. The resulting pale yellow solution was stirred for 2 hours, after which the volatiles were removed under reduced pressure to give a yellow crude product. Purification by flash chromatography (20 – 33% EtOAc/petroleum ether) gave ester **3a**.

Yield: 160 mg, 84%. Isolated as a white amorphous solid, >95% pure by NMR and a single spot by TLC.

^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 6.07 (dd, *J* = 10.8, 2.3 Hz, 1H), 5.97 (dd, *J* = 10.8, 1.4 Hz, 1H), 4.07 – 3.95 (m, 3H), 3.86 (s, 3H), 3.91 – 3.79 (m, 2H), 3.70 – 3.56 (m, 3H), 3.15 – 3.06 (m, 1H), 3.02 (td, *J* = 10.9, 4.4 Hz, 1H), 2.88 (d, *J* = 5.6 Hz, 1H),

[3] Known compound, see: Y. Miyazaki, H. Kinashi, N. Otake, M. Mitani and T. Yamanishi, *Agr. Biol. Chem. (Tokyo)*, 1976, **40**, 1633.

2.69 (dt, *J* = 9.9, 2.6 Hz, 1H), 2.42 (br s, 1H), 2.38 (dt, *J* = 12.5, 9.4 Hz, 1H), 2.26 – 2.12 (m, 1H), 2.09 – 0.73 (m, 39H), 1.42 (s, 3H), 1.22 (d, *J* = 7.0 Hz, 3H), 1.06 (q, *J* = 13.4, 11.9 Hz, 1H), 0.95 (d, *J* = 6.7 Hz, 3H), 0.69 (d, *J* = 6.8 Hz, 3H) ppm.



TMSEt ester 3b. To a stirred solution of SA (**1**) (2.9 g, 3.9 mmol) in CH_2Cl_2 (45 mL), at 0 °C, was added DIPEA (2.3 mL, 13 mmol) in one portion, followed by addition of TMSEtOH (3.9 mL, 27 mmol) and *N*′-tetramethyl chloroformamidinium hexafluorophosphate (TCFH) (1.2 g, 4.4 mmol) in one portion respectively. The resulting reaction mixture was allowed to reach RT overnight, then diluted with EtOAc (75 mL) and washed with HCl (50 mL, 0.1 M aq.), $NaHCO_3$ (50 mL, 0.1 M aq.), and brine (50 mL). The organic layer was separated, dried using a phase separator, and concentrated under reduced pressure to give an amorphous solid. Purification by flash chromatography (0 – 100% EtOAc/petroleum ether) gave TMSEt ester **3b**.

Yield: 2.0 g, 61%. Isolated as a white amorphous solid, >95% pure by NMR and a single spot by TLC.

R_f: 0.29 in 33% EtOAc/petroleum ether. Stains blue with PMA.

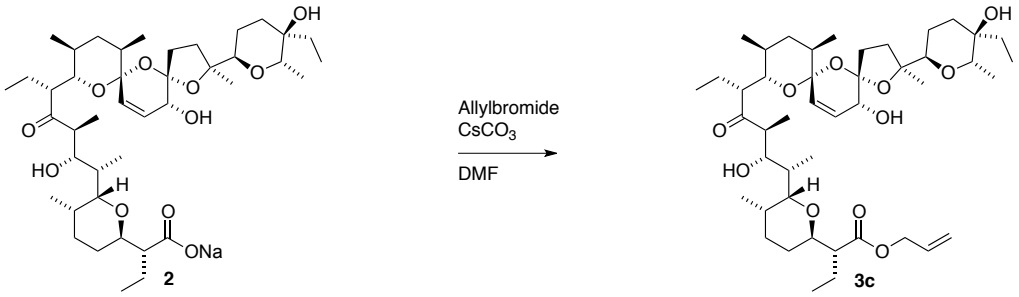
Optical rotation: $[\alpha]_D^{20}$: -39.4 (*c* = 1.0 CH_2Cl_2).

^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 6.08 (dd, *J* = 10.8, 2.3 Hz, 1H), 5.98 (dd, *J* = 10.7, 1.4 Hz, 1H), 4.52 – 4.32 (m, 2H), 4.09 – 3.95 (m, 3H), 3.88 – 3.78 (m, 2H), 3.74 – 3.63 (m, 2H), 3.55 (dd, *J* = 10.5, 2.8 Hz, 1H), 3.26 – 3.11 (m, 1H), 3.03 (d, *J* = 5.8 Hz, 1H), 2.99 (td, *J* = 11.0, 4.5 Hz, 1H), 2.72 (dt, *J* = 10.2, 2.7 Hz, 1H), 2.46 – 2.32 (m, 2H), 2.27 – 2.14 (m, 1H), 2.09 – 0.77 (m, 39H), 1.42 (s, 3H), 1.22 (d, *J* = 6.9 Hz, 3H), 0.95 (d, *J* = 6.7 Hz, 3H), 0.74 (t, *J* = 7.2 Hz, 3H), 0.68 (d, *J* = 6.8 Hz, 3H), 0.08 (s, 9H) ppm.

^{13}C-NMR ($CDCl_3$, 101 MHz) δ: 213.4, 175.8, 133.0, 121.1, 106.5, 99.2, 87.9, 80.7, 77.1, 75.1, 74.3, 71.9, 71.0, 69.4, 67.8, 63.7, 57.4, 48.8, 47.8, 40.5, 39.2, 36.7, 36.6, 33.5, 30.7, 30.5, 29.3, 28.3, 26.4, 26.0, 22.8, 22.4, 20.9, 19.9, 17.7, 17.6, 15.7, 14.7, 14.1, 13.0, 12.0, 11.2, 7.5, 6.5, -1.3 ppm.

FTIR (CH_2Cl_2 film): 3427 (s, br), 3054 (w), 2967 (s), 2941 (s), 2877 (w), 1709 (s) cm^{-1}.

HRMS-ESI *(m/z)*: $[M + Na]^+$ Calcd for $C_{47}H_{82}NaO_{11}Si$ 873.5524; Found 873.5541.



Allyl ester 3c. To a stirred solution of SANa (**2**) (1.0 g, 1.3 mmol) in DMF (10 mL), at RT, was added Cs_2CO_3 (0.84 g, 2.6 mmol) in one portion, followed addition of allyl bromide (0.17 mL, 1.9 mmol) drop-wise over 30 seconds. The resulting pale yellow reaction mixture was stirred for 21 hours, then added to brine (10 mL) and extracted with EtOAc (5 x 10 mL). The combined organic layers were dried using a phase separator and concentrated under reduced pressure to give a yellow oil. Purification by flash chromatography (25% EtOAc/petroleum ether) gave allyl ester **3c**.

Yield: 801 mg, 79%. Isolated as a white amorphous solid, >95% pure by NMR and a single spot by TLC.

R_f: 0.58 in 50% EtOAc/petroleum ether. Stains blue with PMA.

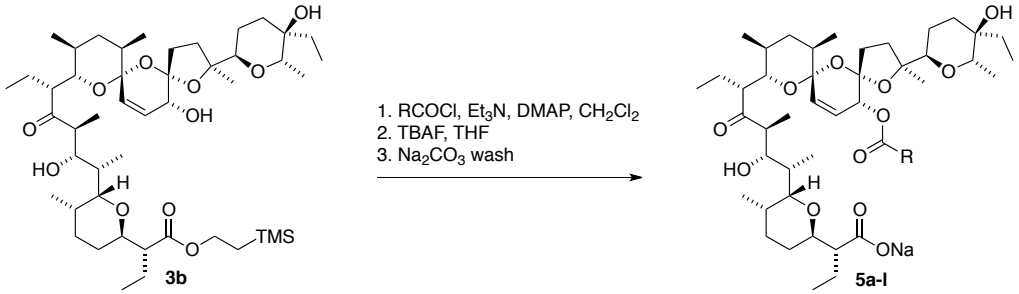
Optical rotation: $[\alpha]_D^{21}$ –46.4 (c = 0.5 CH_2Cl_2).

^{1}H–NMR ($CDCl_3$, 400 MHz) δ: 6.12 – 5.93 (m, 3H), 5.40 (dq, J = 17.3, 1.6 Hz, 1H), 5.22 (dq, J = 10.6, 1.3 Hz, 1H), 4.86 – 4.74 (m, 2H), 4.09 – 3.96 (m, 3H), 3.89 (d, J = 9.2 Hz, 1H), 3.82 (q, J = 6.9 Hz, 1H), 3.71 – 3.63 (m, 2H), 3.57 (dd, J = 10.4, 3.0 Hz, 1H), 3.21 – 3.10 (m, 1H), 3.03 (td, J = 10.9, 4.3 Hz, 1H), 2.89 (d, J = 5.6 Hz, 1H), 2.72 (dt, J = 9.9, 2.9 Hz, 1H), 2.43 (br s, 1H), 2.42 – 2.32 (m, 1H), 2.26 – 2.15 (m, 1H), 2.11 – 0.71 (m, 39H), 1.41 (s, 3H), 1.20 (d, J = 6.9 Hz, 3H), 1.05 (q, J = 13.0 Hz, 1H), 0.94 (d, J = 6.8 Hz, 3H), 0.68 (d, J = 6.8 Hz, 3H) ppm.

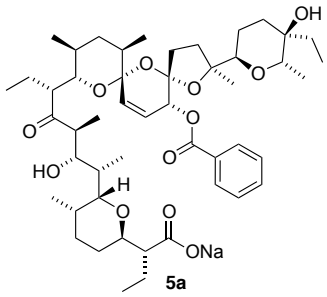
^{13}C–NMR ($CDCl_3$, 101 MHz) δ: 214.1, 175.2, 133.0, 132.6, 121.1, 118.5, 106.4, 99.2, 87.8, 80.1, 77.1, 75.0, 74.2, 71.9, 71.0, 69.6, 67.8, 65.8, 57.4, 48.7, 47.9, 40.5, 39.2, 36.7, 36.6, 33.6, 30.7, 30.5, 29.2, 28.2, 26.4, 25.9, 22.8, 22.3, 20.5, 19.8, 17.7, 15.7, 14.7, 14.1, 12.8, 12.0, 11.2, 7.5, 6.5 ppm.

FTIR (neat): 3521 (w), 3392 (s, br), 2978 (m), 1711 (s) cm^{-1}.

HRMS-ESI *(m/z)*: $[M + Na]^+$ Calcd for $C_{45}H_{74}NaO_{11}$ 813.5129; Found 813.5133.



General procedure for the preparation of esters 5a – l. To a stirred solution of alcohol **3b** (1.0 equiv.) in CH_2Cl_2, at RT, was added Et_3N (6.0 equiv.) in one portion, followed by addition of DMAP (excess) in one portion, and then acid chloride (3.0 equiv.) drop-wise over 30 seconds. The resulting pale yellow solution was stirred overnight, then diluted with EtOAc (10 mL) and washed with Na_2CO_3 (3 x 10 mL, 0.1 M aq.). The organic layer was separated, dried using a phase separator, and concentrated under reduced pressure to give a pale yellow oil. Filtration through a short plug of silica gave the intermediate ester product as clear a oil. To a stirred solution of the intermediate product in THF, at RT, was added TBAF (3.0 equiv., 1.0 M in THF) drop-wise over 30 seconds. The resulting pale yellow solution was stirred until complete consumption of starting material (typically less than one hour, TLC control), then diluted with EtOAc (10 mL) and washed with Na_2CO_3 (3 x 10 mL, 0.1 M aq.). The organic layer was separated, dried using a phase separator, and concentrated under reduced pressure. Purification by flash chromatography (EtOAc/petroleum ether) provided the deprotected product as a mixture of the acid and salt forms. The product mixture was re-dissolved in EtOAc (10 mL) and washed with Na_2CO_3 (3 x 10 mL, 0.1 M aq.). The organic layer was separated, dried using a phase separator, and concentrated several times from *n*-pentane to give C20 esters **5a – l**.



5a

Ester 5a.

Yield: 7.2 mg, 61%. Isolated as a white amorphous solid, >95% pure by NMR and a single spot by TLC.

R_f: 0.39 in 25% EtOAc/petroleum ether. UV-active and stains blue with PMA.

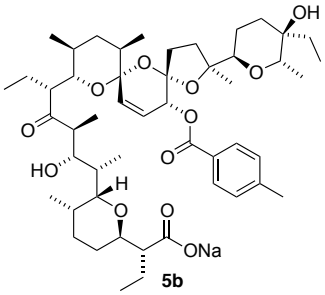
Optical rotation: $[\alpha]_D^{20}$: -41.1 (*c* = 0.2 CH_2Cl_2).

^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 8.34 (d, *J* = 7.2 Hz, 2H), 7.51 (t, *J* = 7.3 Hz, 1H), 7.43 (t, *J* = 7.5 Hz, 2H), 6.25 (dd, *J* = 10.9, 2.9 Hz, 1H), 6.03 (dd, *J* = 10.8, 1.5 Hz, 1H), 5.75 (dd, *J* = 2.6, 1.5 Hz, 1H), 5.25 (br s, 2H), 4.38 (q, *J* = 6.9 Hz, 1H), 4.31 (d, *J* = 10.6 Hz, 1H), 3.91 (dd, *J* = 10.7, 4.7 Hz, 1H), 3.69 (dd, *J* = 10.1, 2.2 Hz, 1H), 3.54 (d, *J* = 9.9 Hz, 1H), 3.37 (dd, *J* = 12.2, 2.5 Hz, 1H), 2.80 (td, *J* = 11.0, 3.1 Hz, 1H), 2.71 (dd, *J* = 11.3, 2.7 Hz, 1H), 2.68 – 2.57 (m, 1H), 2.32 (qd, *J* = 12.3, 5.8 Hz, 1H), 1.79 (s, 3H), 2.10 – 0.75 (m, 44H), 0.97 (t, *J* = 7.4 Hz, 3H), 0.68 (d, *J* = 6.9 Hz, 3H) ppm.

^{13}C-NMR ($CDCl_3$,101 MHz) δ: 216.4, 184.2, 166.9, 133.2, 131.2, 129.0, 128.7, 127.6, 124.0, 105.4, 100.4, 89.1, 76.6, 75.9, 75.7, 74.2, 71.7, 69.8, 67.6, 67.4, 55.3, 51.4, 50.4, 41.0, 39.1, 36.1, 35.2, 32.6, 32.5, 32.4, 28.3, 28.2, 28.1, 27.1, 24.1, 20.1, 19.6, 17.6, 16.2, 15.9, 14.7, 13.4, 12.6, 12.0, 10.8, 6.9, 6.8.ppm.

FTIR (CH_2Cl_2 film): 3301 (s, br), 2973 (m), 2935 (m), 2855 (w), 1715 (s), 1562 (s) cm^{-1}.

HRMS-ESI *(m/z)*: $[M + H]^+$ Calcd for $C_{49}H_{74}NaO_{12}$ 877.5078; Found 877.5106.



5b

Ester 5b.

Yield: 43.5 mg, 55%. Isolated as a white amorphous solid, >95% pure by NMR and a single spot by TLC.

R_f: 0.41 in 25% EtOAc/petroleum ether. UV-active and stains blue with PMA.

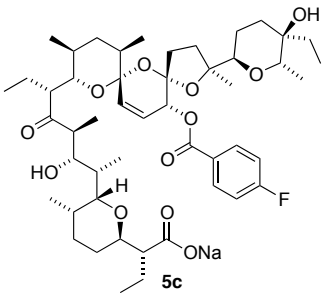
Optical rotation: $[\alpha]_D^{22}$: -13.4 (c = 0.6 CH_2Cl_2).

^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 8.21 (d, J = 8.1 Hz, 2H), 7.22 (d, J = 7.9 Hz, 2H), 6.24 (dd, J = 10.8, 2.9 Hz, 1H), 6.02 (dd, J = 10.8, 1.6 Hz, 1H), 5.73 (dd, J = 2.7, 1.5 Hz, 1H), 5.27 (br s, 2H), 4.36 (q, J = 7.5, 7.1 Hz, 1H), 4.30 (d, J = 10.1 Hz, 1H), 3.92 (dd, J = 10.9, 4.6 Hz, 1H), 3.69 (dd, J = 10.1, 2.3 Hz, 1H), 3.54 (d, J = 10.0 Hz, 1H), 3.37 (dd, J = 12.1, 2.5 Hz, 1H), 2.80 (td, J = 11.0, 2.6 Hz, 1H), 2.75 – 2.65 (m, 1H), 2.69 – 2.58 (m, 1H), 2.37 (s, 3H), 2.29 (dd, J = 12.8, 5.1 Hz, 2H), 2.24 – 0.71 (m, 43H), 1.78 (s, 3H), 1.20 (d, J = 6.8 Hz, 3H), 0.68 (d, J = 6.8 Hz, 3H) ppm.

^{13}C-NMR ($CDCl_3$, 101 MHz) δ: 216.5, 184.1, 167.0, 143.8, 131.2, 129.4, 127.8, 126.3, 123.8, 105.5, 100.4, 89.1, 76.6, 75.9, 75.6, 74.1, 71.6, 69.8, 67.5, 67.4, 55.3, 51.4, 50.5, 41.0, 39.1, 36.1, 35.2, 32.6, 32.5, 32.4, 28.3, 28.1, 27.1, 24.0, 21.9, 20.1, 19.5, 17.6, 16.2, 15.9, 14.6, 13.4, 12.5, 12.0, 10.8, 6.9, 6.8, 1.2 ppm.

FTIR (CH_2Cl_2 film): 3333 (s, br), 2965 (m), 2934 (m), 2875 (w), 1714 (s), 1611 (w), 1562 (s) cm^{-1}.

HRMS-ESI *(m/z)*: $[M + H]^+$ Calcd for $C_{50}H_{76}NaO_{12}$ 891.5234; Found 891.5262.



5c

Ester 5c

Yield: 17.0 mg, 54%. Isolated as a white amorphous solid, >95% pure by NMR and a single spot by TLC.

R_f: 0.50 in 25% EtOAc/petroleum ether. UV-active and stains blue with PMA.

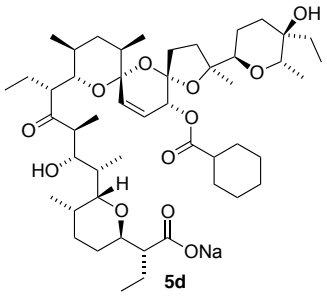
Optical rotation: $[\alpha]_D^{22}$: -20.4 (*c* = 0.6 CH_2Cl_2).

^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 8.40 – 8.33 (m, 2H), 7.09 (t, *J* = 8.7 Hz, 2H), 6.25 (dd, *J* = 10.9, 2.8 Hz, 1H), 6.01 (dd, *J* = 11.0, 1.4 Hz, 1H), 5.72 (dd, *J* = 2.7, 1.5 Hz, 1H), 5.14 (br s, 2H), 4.39 (q, *J* = 6.8 Hz, 1H), 4.30 (d, *J* = 10.3 Hz, 1H), 3.91 (dd, *J* = 11.2, 4.8 Hz, 1H), 3.67 (dd, *J* = 10.0, 2.2 Hz, 1H), 3.52 (d, *J* = 10.0 Hz, 1H), 3.37 (dd, *J* = 12.2, 2.4 Hz, 1H), 2.80 (td, *J* = 11.1, 3.1 Hz, 1H), 2.71 (dd, *J* = 11.1, 2.8 Hz, 1H), 2.68 – 2.58 (m, 1H), 2.32 – 2.18 (m, 1H), 2.08 – 0.73 (m, 44H), 1.79 (s, 3H), 0.97 (t, *J* = 7.4 Hz, 3H), 0.67 (d, *J* = 6.9 Hz, 3H) ppm.

^{13}C-NMR ($CDCl_3$, 101 MHz) δ: 216.4, 184.3, 165.9, 134.1, 134.0, 127.4, 124.1, 115.9, 115.7, 105.3, 100.3, 89.1, 76.5, 75.8, 75.7, 74.2, 71.7, 69.9, 67.6, 67.3, 55.2, 51.4, 50.4, 41.0, 39.0, 36.1, 35.2, 32.6, 32.5, 32.4, 28.44, 28.37, 28.1, 27.0, 24.1, 20.1, 19.6, 17.6, 16.2, 15.9, 14.7, 13.4, 12.6, 12.0, 10.7, 6.9, 6.8 ppm.

FTIR (CH_2Cl_2 film): 3297 (s, br), 2969 (m), 2935 (m), 2874 (w), 2857 (w), 1715 (s), 1603 (w), 1563 (s) cm^{-1}.

HRMS-ESI *(m/z)*: $[M + H]^+$ Calcd for $C_{49}H_{73}FNaO_{12}$ 895.4984; Found 895.4955.



5d

Ester 5d.

Yield: 79.0 mg, 93%. Isolated as a white amorphous solid, >95% pure by NMR and single spot on TLC.

R_f: 0.31 in 25% EtOAc/petroleum ether. Stains blue with PMA.

Optical rotation: $[\alpha]_D^{20}$ –21.4 (*c* = 1.0 CH_2Cl_2).

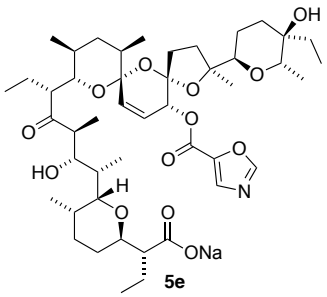
^{1}H–NMR (C_6D_6, 400 MHz) δ: 6.31 (s, 2H), 5.91 (br s, 1H), 5.58 (d, *J* = 1.3 Hz, 1H), 5.50 (br s, 1H), 4.70 (d, *J* = 10.3 Hz, 1H), 4.48 (q, *J* = 6.7 Hz, 1H), 4.33 (dd, *J* = 11.0, 5.1 Hz, 1H), 4.05 (dd, *J* = 10.0, 2.2 Hz, 1H), 3.60 (d, *J* = 10.1 Hz, 1H), 3.16 (td, *J* = 11.0, 3.2 Hz, 1H), 3.06 – 2.96 (m, 2H), 2.74 – 2.63 (m, 1H), 2.58 (dd, *J* = 11.3, 2.8 Hz, 1H), 2.50 (qd, *J* = 12.8, 4.2 Hz, 1H), 2.34 – 0.81 (m, 42H), 1.94 (s, 3H), 1.02 (d, *J*

= 6.9 Hz, 3H), 2.35 – 0.62 (m, 9H), 0.77 (t, *J* = 7.4 Hz, 3H), 0.59 (d, *J* = 6.6 Hz, 3H) ppm.

^{13}C–NMR (C_6D_6, 101 MHz) δ: 216.8, 184.1, 176.4, 127.9, 124.4, 105.4, 100.4, 89.0, 76.5, 76.3, 75.4, 74.6, 71.4, 69.8, 67.7, 67.2, 55.9, 51.1, 50.9, 42.7, 41.0, 38.7, 36.7, 35.8, 32.9, 32.6, 30.2, 29.3, 29.2, 28.6, 28.5, 27.3, 26.4, 26.1, 25.7, 24.6, 24.5, 20.5, 20.1, 17.3, 16.6, 15.8, 14.7, 13.6, 13.1, 12.0, 11.1, 7.1, 6.9 ppm.

FTIR (CH_2Cl_2 film): 3315 (s, br), 2965 (m), 2932 (s), 2873 (w), 2857 (m), 1734 (s), 1713 (s), 1563 (s) cm^{-1}.

HRMS-ESI *(m/z)*: $[M + H]^+$ Calcd for $C_{49}H_{80}NaO_{12}$ 883.5547; Found 883.5524.



5e

Ester 5e.

Yield: 23.7 mg, 46%. Isolated as a white amorphous solid, >95% pure by NMR and single spot on TLC.

R_f: 0.33 in 20% acetone/petroleum ether. UV-active and stains blue with PMA.

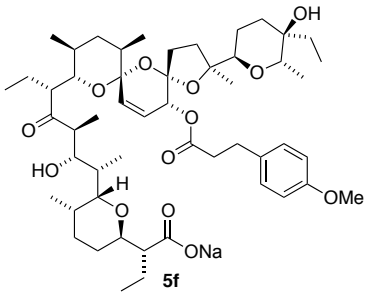
Optical rotation: $[\alpha]_D^{20}$ –22.3 (*c* = 2.4 CH_2Cl_2).

^{1}H–NMR ($CDCl_3$, 400 MHz) δ: 9.62 (d, *J* = 0.9 Hz, 1H), 7.86 (d, *J* = 0.9 Hz, 1H), 6.23 (dd, *J* = 10.9, 2.8 Hz, 1H), 5.94 (dd, *J* = 10.9, 1.5 Hz, 1H), 5.75 (dd, *J* = 2.7, 1.6 Hz, 1H), 4.99 (br s, 1H), 4.39 (q, *J* = 6.8 Hz, 1H), 4.28 (d, *J* = 9.7 Hz, 1H), 3.88 (dd, *J* = 11.2, 4.6 Hz, 1H), 3.66 (dd, *J* = 10.1, 2.2 Hz, 1H), 3.49 (d, *J* = 9.9 Hz, 1H), 3.39 (dd, *J* = 12.0, 2.4 Hz, 1H), 2.80 (td, *J* = 11.1, 3.1 Hz, 1H), 2.70 (dd, *J* = 11.0, 2.4 Hz, 1H), 2.67 – 2.57 (m, 1H), 2.50 – 0.69 (m, 49H), 1.79 (s, 3H), 0.67 (d, *J* = 6.9 Hz, 3H) ppm.

^{13}C–NMR ($CDCl_3$, 101 MHz) δ: 216.3, 184.4, 160.9, 151.4, 148.6, 131.4, 126.8, 124.4, 105.0, 100.1, 89.2, 76.4, 75.9, 75.7, 74.4, 71.6, 70.4, 67.3, 67.2, 55.2, 51.5, 50.2, 40.9, 39.0, 36.1, 35.5, 32.5, 32.4, 32.4, 29.8, 28.4, 28.1, 27.0, 24.1, 20.1, 19.6, 17.5, 16.1, 16.0, 14.6, 13.4, 12.1, 12.0, 10.7, 6.8, 6.6 ppm.

FTIR (CH_2Cl_2 film): 3273 (m, br), 2962 (m), 2932 (s), 2874 (w), 1744 (m), 1711 (m), 1562 (s) cm^{-1}.

HRMS-ESI (*m/z*): $[M + H]^+$ Calcd for $C_{46}H_{71}NNaO_{13}$ 868.4823; Found 868.4834.



5f

Ester 5f.

Yield: 14.9 mg, 24%. Isolated as a white amorphous solid, >95% pure by NMR and single spot on TLC.

R_f: 0.36 in 40% EtOAc/petroleum ether. UV-active and stains blue with PMA.

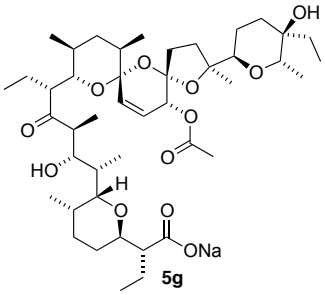
Optical rotation: $[\alpha]_D^{20}$ –23.0 (*c* = 1.5 CH_2Cl_2).

^{1}H–NMR ($CDCl_3$, 400 MHz) δ: 7.19 (d, *J* = 8.6 Hz, 2H), 6.77 (d, *J* = 8.7 Hz, 2H), 6.20 (dd, *J* = 10.9, 2.6 Hz, 1H), 5.87 (dd, *J* = 10.9, 1.6 Hz, 1H), 5.34 (dd, *J* = 2.5, 1.6 Hz, 1H), 4.39 (q, *J* = 6.8 Hz, 1H), 4.27 (d, *J* = 10.1 Hz, 1H), 3.87 (dd, *J* = 11.2, 4.4 Hz, 1H), 3.77 (s, 3H), 3.69 (dd, *J* = 10.1, 2.1 Hz, 1H), 3.45 (d, *J* = 9.9 Hz, 1H), 3.35 (dd, *J* = 11.9, 2.2 Hz, 1H), 3.04 – 2.71 (m, 6H), 2.68 (dd, *J* = 11.3, 2.6 Hz, 1H), 2.64 – 2.53 (m, 1H), 2.26 (qd, *J* = 12.8, 4.9 Hz, 1H), 2.08 – 0.73 (m, 45H), 1.74 (s, 3H), 0.71 (s, 3H), 0.67 (d, *J* = 6.9 Hz, 3H) ppm.

^{13}C–NMR ($CDCl_3$, 101 MHz) δ: 215.6, 184.1, 173.6, 158.0, 133.0, 129.7, 126.8, 124.3, 113.7, 104.7, 99.6, 88.9, 76.1, 75.8, 75.6, 74.7, 71.7, 70.0, 67.0, 66.8, 55.4, 55.0, 51.4, 50.4, 40.7, 39.0, 36.2, 36.1, 35.4, 32.6, 32.5, 29.9, 29.4, 28.3, 28.1, 27.0, 24.1, 24.0, 20.1, 19.9, 17.5, 16.2, 16.0, 14.8, 13.3, 12.4, 12.0, 10.7, 6.9, 6.7 ppm.

FTIR (CH_2Cl_2 film): 3298 (m, br), 2969 (m), 2934 (s), 2856 (m), 1738 (m), 1713 (m), 1612 (s) cm^{-1}.

HRMS-ESI (*m/z*): $[M + H]^+$ Calcd for $C_{52}H_{80}NaO_{13}$ 935.5497; Found 935.5513.



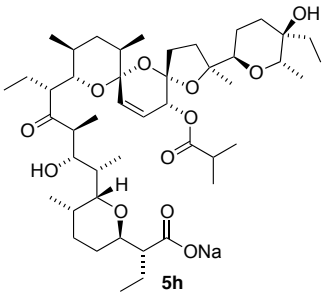
5g

Ester 5g.[3]

Yield: 32.6 mg, 78%. Isolated as a transparent film, >95% pure by NMR and a single spot on TLC.

Known compound see reference 4.

^{1}H–NMR ($CDCl_3$, 400 MHz) δ: 6.21 (dd, *J* = 10.9, 2.5 Hz, 1H), 5.86 (dd, *J* = 10.9, 1.6 Hz, 1H), 5.35 (dd, *J* = 2.4, 1.8 Hz, 1H), 4.43 (q, *J* = 6.8 Hz, 1H), 4.27 (dd, *J* = 10.4, 0.9 Hz, 1H), 3.88 (dd, *J* = 11.0, 4.8 Hz, 1H), 3.71 (dd, *J* = 10.0, 1.9 Hz, 1H), 3.44 (d, *J* = 10.0 Hz, 1H), 3.37 (dd, *J* = 12.0, 2.0 Hz, 1H), 2.85 (td, *J* = 11.0, 3.2 Hz, 1H), 2.68 (dd, *J* = 11.1, 2.5 Hz, 1H), 2.65 – 2.54 (m, 1H), 2.29 (dq, *J* = 12.6, 5.1 Hz, 1H), 2.22 (s, 3H), 2.19 – 0.80 (m, 43H), 1.75 (s, 3H), 0.76 (t, *J* = 7.5 Hz, 3H), 0.71 (d, *J* = 6.7 Hz, 3H), 0.68 (d, *J* = 7.0 Hz, 3H) ppm.

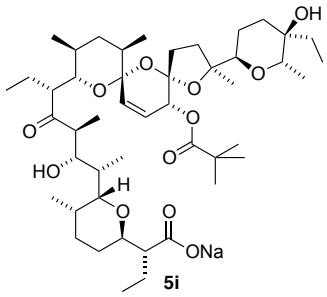


5h

Ester 5h.[3]

Yield: 48.2 mg, 83%. Isolated as a white amorphous solid, >95% pure by NMR and single spot on TLC.

^{1}H–NMR ($CDCl_3$, 400 MHz) δ: 6.18 (dd, *J* = 10.9, 2.7 Hz, 1H), 5.86 (dd, *J* = 11.0, 1.4 Hz, 1H), 5.31 (dd, *J* = 2.6, 1.5 Hz, 1H), 5.12 (br s, 2H), 4.39 (q, *J* = 6.8 Hz, 1H), 4.25 (dd, *J* = 10.3, 1.4 Hz, 1H), 3.88 (dd, *J* = 11.3, 4.4 Hz, 1H), 3.71 (dd, *J* = 10.1, 2.2 Hz, 1H), 3.46 (d, *J* = 10.0 Hz, 1H), 3.36 (dd, *J* = 12.1, 2.2 Hz, 1H), 2.93 – 2.75 (m, 2H), 2.67 (dd, *J* = 11.1, 2.8 Hz, 1H), 2.64 – 2.52 (m, 1H), 2.32 (qd, *J* = 12.4, 5.1 Hz, 1H), 2.08 – 0.69 (m, 53H), 1.74 (s, 3H), 0.66 (d, *J* = 6.9 Hz, 3H) ppm.



5i

Ester 5i.

Yield: 40.9 mg, 88%. Isolated as a white amorphous solid, >95% pure by NMR and single spot on TLC.

R_f: 0.61 in 50% EtOAc/petroleum ether. Stains blue with PMA.

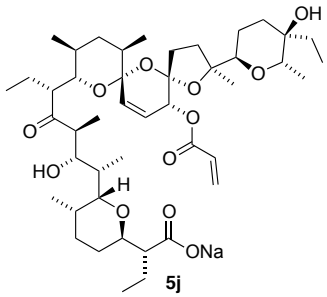
Optical rotation: $[\alpha]_D^{22}$: -25.7 (c = 0.7 CH_2Cl_2).

^{1}H–NMR ($CDCl_3$, 400 MHz) δ: 6.17 (dd, J = 10.9, 2.8 Hz, 1H), 5.87 (dd, J = 10.8, 1.5 Hz, 1H), 5.29 (dd, J = 2.8, 1.5 Hz, 1H), 5.21 (br s, 2H), 4.37 (q, J = 6.8 Hz, 1H), 4.28 (dd, J = 10.4, 1.5 Hz, 1H), 3.88 (dd, J = 11.1, 4.4 Hz, 1H), 3.72 (dd, J = 10.1, 2.2 Hz, 1H), 3.48 (d, J = 9.9 Hz, 1H), 3.38 (dd, J = 12.0, 2.2 Hz, 1H), 2.81 (td, J = 10.9, 3.3 Hz, 1H), 2.68 (dd, J = 11.0, 3.1 Hz, 1H), 2.66 – 2.55 (m, 1H), 2.34 (qd, J = 12.7, 4.7 Hz, 1H), 2.08 – 0.70 (m, 47H), 1.77 (s, 3H), 1.23 (s, 9H), 0.67 (d, J = 7.0 Hz, 3H) ppm.

^{13}C–NMR ($CDCl_3$, 101 MHz) δ: 216.1, 183.9, 179.1, 128.0, 123.7, 105.3, 100.5, 88.9, 76.1, 76.0, 75.7, 74.4, 71.6, 69.8, 68.0, 67.2, 55.1, 51.4, 50.4, 41.1, 39.1, 39.0, 36.1, 34.9, 32.55, 32.47, 32.4, 29.6, 28.3, 28.1, 27.3, 27.0, 24.2, 20.3, 20.2, 17.5, 16.3, 15.7, 14.9, 13.4, 12.6, 12.0, 10.8, 6.9, 6.6 ppm.

FTIR (CH_2Cl_2 film): 3301 (s, br) 2968 (s), 2934 (s) 1731 (s), 1714 (s), 1564 (s) cm^{-1}.

HRMS-ESI (*m/z*): $[M + H]^+$ Calcd for $C_{47}H_{78}NaO_{12}$ 857.5391; Found 857.5418.



5j

Ester 5j.

Yield: 15.3 mg, 13%. Isolated as a white amorphous solid, >95% pure by NMR and single spot on TLC.

R_f: 0.38 in 50% EtOAc/petroleum ether. Stains blue with PMA.

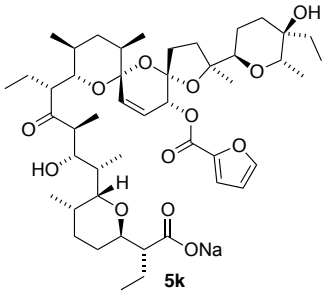
Optical rotation: $[\alpha]_D^{20}$ –20.4 (*c* = 0.5 CH_2Cl_2).

^{1}H–NMR ($CDCl_3$, 400 MHz) δ: 6.63 – 6.44 (m, 2H), 6.22 (dd, *J* = 10.9, 2.6 Hz, 1H), 5.89 (dd, *J* = 10.9, 1.6 Hz, 1H), 5.81 (dd, *J* = 8.6, 3.1 Hz, 1H), 5.47 (dd, *J* = 2.5, 1.6 Hz, 1H), 5.16 (br s, 2H), 4.39 (q, *J* = 6.7 Hz, 1H), 4.26 (d, *J* = 10.1 Hz, 1H), 3.88 (dd, *J* = 11.2, 4.5 Hz, 1H), 3.69 (dd, *J* = 10.1, 2.2 Hz, 1H), 3.47 (d, *J* = 10.0 Hz, 1H), 3.36 (dd, *J* = 12.1, 2.3 Hz, 1H), 2.82 (td, *J* = 11.1, 3.2 Hz, 1H), 2.68 (dd, *J* = 10.8, 2.8 Hz, 1H), 2.65 – 2.55 (m, 1H), 2.55 – 0.70 (m, 47H), 2.26 (qd, *J* = 12.4, 4.7 Hz, 1H), 1.75 (s, 3H), 0.67 (d, *J* = 6.9 Hz, 3H) ppm.

^{13}C–NMR ($CDCl_3$, 101 MHz) δ: 215.8, 184.0, 166.5, 132.7, 128.4, 126.8, 124.3, 104.8, 99.7, 89.0, 76.0, 75.9, 75.7, 74.7, 71.7, 70.0, 67.1, 66.8, 55.0, 51.5, 50.4, 40.7, 39.0, 36.2, 36.1, 32.5, 29.6, 28.3, 28.1, 27.0, 24.1, 22.5, 20.1, 20.0, 17.5, 16.2, 16.0, 14.9, 14.2, 13.3, 12.4, 12.0, 10.7, 6.9, 6.6 ppm.

FTIR (CH_2Cl_2 film): 3293 (s, br), 2966 (m), 2934 (s), 2874 (w), 2858 (w), 1713 (s), 1564 (s) cm^{-1}.

HRMS-ESI *(m/z)*: $[M+H]^+$ Calcd for $C_{45}H_{72}NaO_{12}$ 827.4921; Found 827.4922.



5k

Ester 5k.

Yield: 62.2 mg, 60%. Isolated as a white amorphous solid, >95% pure by NMR and single spot on TLC.

R_f: 0.33 in 25% EtOAc/petroleum ether. UV-active and stains blue with PMA.

Optical rotation: $[\alpha]_D^{20}$ –20.5 (*c* = 0.6 CH_2Cl_2).

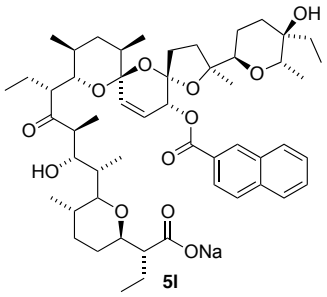
^{1}H–NMR ($CDCl_3$, 400 MHz) δ: 8.19 (dd, *J* = 3.7, 0.8 Hz, 1H), 7.56 (dd, *J* = 1.6, 0.7 Hz, 1H), 6.42 (dd, *J* = 3.7, 1.7 Hz, 1H), 6.24 (dd, *J* = 10.9, 2.8 Hz, 1H), 5.95 (dd, *J* = 10.9, 1.5 Hz, 1H), 5.70 (dd, *J* = 2.7, 1.6 Hz, 1H), 5.19 (br s, 1H), 4.35 (q, *J* = 6.8 Hz, 1H), 4.29 (dd, *J* = 10.4, 1.5 Hz, 1H), 3.89 (dd, *J* = 11.1, 4.8 Hz, 1H), 3.68 (dd, *J* = 10.1, 2.2 Hz, 1H), 3.50 (d, *J* = 10.0 Hz, 1H), 3.38 (dd, *J* = 12.1, 2.4 Hz, 1H), 2.80 (td, *J* = 11.1, 3.2 Hz, 1H), 2.70 (dd, *J* = 11.0, 2.8 Hz, 1H), 2.68 – 2.58 (m, 1H), 2.30 (qd, *J*

= 15.7, 14.2, 4.1 Hz, 1H), 2.27 – 0.71 (m, 48H), 1.78 (s, 3H), 0.67 (d, *J* = 6.9 Hz, 3H) ppm.

^{13}C–NMR ($CDCl_3$, 101 MHz) δ: 216.2, 184.2, 158.7, 147.0, 143.4, 127.0, 124.3, 121.8, 112.5, 105.2, 100.2, 89.2, 76.7, 75.8, 75.8, 74.4, 71.6, 70.0, 67.3, 67.0, 55.2, 51.5, 50.3, 40.9, 39.0, 36.1, 35.4, 32.5, 32.4, 28.4, 28.3, 28.1, 27.0, 24.1, 24.0, 20.1, 19.6, 17.6, 16.2, 16.0, 14.6, 13.4, 12.3, 12.0, 10.8, 6.9, 6.6 ppm.

FTIR (CH_2Cl_2 film): 3291 (m, br), 2973 (m), 2935 (s), 1731 (s), 1714 (m), 1567 (s) cm^{-1}.

HRMS-ESI (*m/z*): $[M + H]^+$ Calcd for $C_{47}H_{72}NaO_{13}$ 867.4871; Found 867.4896.



5l

Ester 5l.

Yield: 4.8 mg, 30%. Isolated as a white amorphous solid, >95% pure by NMR and single spot on TLC.

R_f: 0.47 in 25% EtOAc/petroleum ether. UV-active and stains blue with PMA.

Optical rotation: $[\alpha]_D^{22}$ –8.1 (*c* = 0.4 CH_2Cl_2).

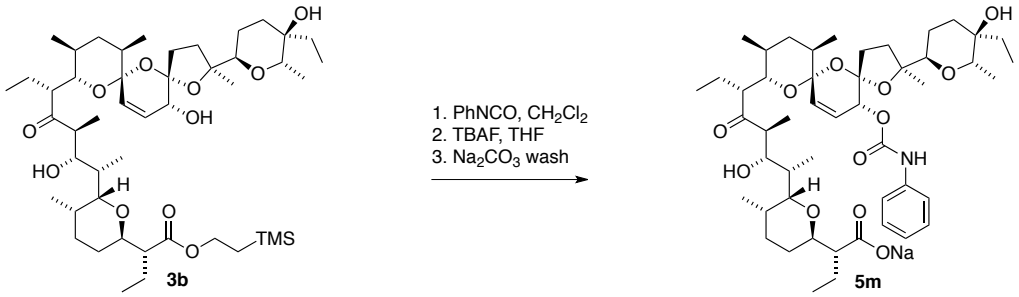
^{1}H–NMR ($CDCl_3$, 400 MHz) δ: 8.95 (s, 1H), 8.49 (d, *J* = 8.2 Hz, 1H), 8.26 (dd, *J* = 8.6, 1.8 Hz, 1H), 7.87 (d, *J* = 8.7 Hz, 1H), 7.83 (d, *J* = 8.7 Hz, 1H), 7.59 – 7.52 (m, 1H), 7.52 – 7.44 (m, 1H), 6.27 (dd, *J* = 10.9, 2.9 Hz, 1H), 6.10 (dd, *J* = 10.8, 1.5 Hz, 1H), 5.84 (dd, *J* = 2.8, 1.6 Hz, 1H), 5.60 (br s, 2H), 4.41 (q, *J* = 6.8 Hz, 1H), 4.32 (dd, *J* = 10.4, 1.6 Hz, 1H), 3.97 (dd, *J* = 11.0, 5.3 Hz, 1H), 3.71 (dd, *J* = 9.9, 2.1 Hz, 1H), 3.60 (d, *J* = 10.0 Hz, 1H), 3.38 (dd, *J* = 12.1, 2.4 Hz, 1H), 2.80 (td, *J* = 11.1, 3.1 Hz, 1H), 2.72 (dd, *J* = 11.4, 2.7 Hz, 1H), 2.70 – 2.62 (m, 1H), 2.46 (qd, *J* = 12.7, 4.8 Hz, 1H), 2.09 – 0.73 (m, 44H), 1.78 (s, 3H), 1.06 (t, *J* = 7.4 Hz, 3H), 0.69 (d, *J* = 7.0 Hz, 3H) ppm.

^{13}C–NMR ($CDCl_3$, 101 MHz) δ: 216.9, 184.1, 167.1, 135.8, 133.0, 132.3, 131.0, 128.39, 128.35, 128.1, 127.4, 126.5, 126.3, 126.1, 123.8, 105.5, 100.5, 89.0, 76.4, 76.1, 75.6, 74.0, 71.7, 70.2, 68.2, 67.5, 55.4, 51.4, 50.6, 41.1, 39.1, 36.1, 35.1, 32.7,

32.6, 32.4, 28.8, 28.2, 27.9, 27.1, 23.9, 20.2, 19.7, 17.6, 16.3, 15.9, 14.6, 13.5, 12.7, 12.0, 10.8, 7.0, 6.9 ppm.

FTIR (CH_2Cl_2 film): 3299 (s, br), 2968 (m), 2934 (m), 2874 (w), 2856 (w), 1713 (s), 1630 (w), 1562 (s) cm^{-1}.

HRMS-ESI *(m/z)*: $[M + H]^+$ Calcd for $C_{53}H_{76}NaO_{12}$ 927.5234; Found 927.5256.



Carbamate 5m. To a stirred solution of alcohol **3b** (91.6 mg, 0.11 mmol) in CH_2Cl_2 (1 mL), at RT, was added phenyl isocyanate (23 µL, 0.22 mmol) drop-wise over 30 seconds. The resulting clear solution was stirred for three days, then added to EtOAc (10 mL) and washed with $NaHCO_3$ (3 x 10 mL, 0.1 M aq.). The organic layer was separated, dried using a phase separator, and concentrated under reduced pressure. Filtration through a short plug of silica gave the intermediate carbamate product as a white amorphous solid (91.2 mg). To a stirred solution of part of the intermediate product (70.8 mg) in THF (2 mL), at RT, was added TBAF (220 µL, 0.22 mmol, 1.0 M in THF). The resulting brown solution was stirred for 2.5 hours, then diluted with EtOAc (10 mL) and washed with $NaHCO_3$ (3 x1 0 mL, 0.1 M aq.). The organic layer was separated, dried using a phase separator, and concentrated under reduced pressure. Purification by flash chromatography (6.3% EtOAc/petroleum ether) gave the deprotected product as a mixture of the acid and salt forms. The product mixture was re-dissolved in EtOAc (10 mL) and washed with Na_2CO_3 (3 x 10 mL, 0.1 M aq.). The organic layer was separated, dried using a phase separator, and concentrated several times from *n*-pentane to give carbamate **5m**.

Yield: 41.3 mg, 55%. Isolated as a white amorphous solid, >95% pure by NMR and a single spot on TLC.

R_f: 0.50 in 50% EtOAc/petroleum ether. UV-active and stains blue with PMA.

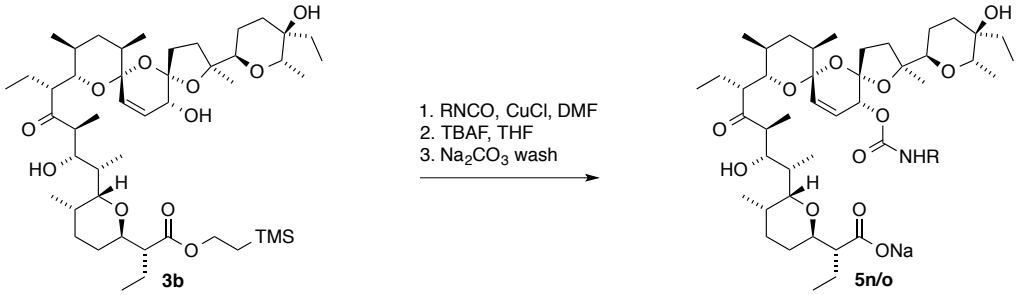
Optical rotation: $[\alpha]_D^{21}$ –21.6 (c = 0.9 CH_2Cl_2).

^{1}H–NMR ($CDCl_3$, 400 MHz) δ: 9.32 (br s, 1H), 7.55 (dd, *J* = 8.6, 1.1 Hz, 2H), 7.23 (t, *J* = 845.0, 8.4 Hz, 2H), 6.97 (t, *J* = 7.4 Hz, 1H), 6.23 (dd, *J* = 10.9, 2.4 Hz, 1H), 6.00 (dd, *J* = 10.8, 1.7 Hz, 2H), 5.40 (t, *J* = 2.1 Hz, 1H), 5.20 (d, *J* = 5.1 Hz, 1H), 4.54 (q, *J* = 6.7 Hz, 1H), 4.23 (dd, *J* = 9.8, 3.7 Hz, 1H), 3.89 (dd, *J* = 11.1, 4.9 Hz, 1H), 3.69 (dd, *J* = 10.0, 2.1 Hz, 1H), 3.54 – 3.30 (m, 2H), 2.80 (td, *J* = 11.1, 3.2 Hz, 1H), 2.69 (dd, *J* = 11.3, 2.7 Hz, 1H), 2.66 – 2.54 (m, 1H), 2.42 (qd, *J* = 12.4, 5.7 Hz, 1H), 2.15 – 0.63 (m, 41H), 1.74 (s, 3H), 1.03 (t, *J* = 7.4 Hz, 3H), 0.77 (t, *J* = 7.5 Hz, 3H), 0.73 (d, *J* = 6.6 Hz, 3H) ppm.

^{13}C–NMR ($CDCl_3$, 101 MHz) δ: 216.1, 184.2, 152.8, 139.3, 128.7, 127.1, 124.2, 122.5, 118.4, 104.7, 99.2, 88.9, 76.0, 75.4, 75.1, 74.2, 71.72, 71.66, 67.0, 66.7, 55.2, 51.1, 50.7, 40.5, 38.9, 36.9, 36.2, 33.0, 32.7, 32.5, 28.7, 28.4, 28.1, 27.0, 23.8, 20.1, 20.0, 17.4, 16.2, 16.1, 14.8, 13.3, 12.2, 12.0, 10.7, 6.91, 6.87 ppm.

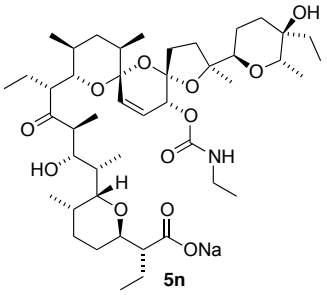
FTIR (CH_2Cl_2 film): 3254 (br, s) 2962 (s), 2932 (s), 2874 (m), 1732 (s), 1713 (s), 1602 (m), 1561 (s) cm^{-1}.

HRMS-ESI (*m/z*): $[M + H]^+$ Calcd for $C_{49}H_{75}NNaO_{12}$ 892.5187; Found 892.5216.



General procedure for the preparation of carbamates 5n and 5o. To a stirred solution of alcohol **3b** (1 equiv.) in DMF, at RT, was added isocyanate (1.5 equiv.) drop-wise over 30 seconds, followed by addition of anhydrous CuCl (catalytic, freshly ground) in one portion. The resulting green solution was stirred for two days, then diluted with EtOAc (25 mL) and washed with $NaHCO_3$ (3 x 10 mL, 0.1 M aq.). The organic layer was separated, dried using a phase separator, and concentrated under reduced pressure. Filtration through a short plug of silica gave the intermediate carbamate as a white amorphous solid. To a stirred solution of the intermediate product in THF, at RT, was added TBAF (3 equiv., 1.0 M in THF) drop-wise over 30 seconds. The resulting brown solution was stirred overnight, then diluted with EtOAc (10 mL) and washed with $NaHCO_3$ (3 x 10 mL, 0.1 M aq.). The organic layer was separated, dried using a phase separator, and concentrated under reduced pressure.

Purification by flash chromatography (13 – 75% EtOAc/petroleum ether) gave the deprotected product as a mixture of the acid and salt forms. The product mixture was re-dissolved in EtOAc (10 mL) and washed with Na_2CO_3 (3 x 10 mL, 0.1 M aq.). The organic layer was separated, dried using a phase separator, and concentrated several times from *n*-pentane to give carbamate **5n** or **5o**.



5n

Carbamate 5n.

Yield: 47.6 mg, 51%. Isolated as a white amorphous solid, >95% pure by NMR and single spot on TLC.

R_f: 0.28 in 50% EtOAc/petroleum ether. Stains blue with PMA.

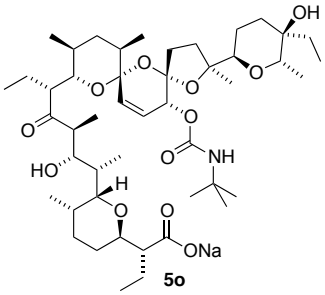
Optical rotation: $[\alpha]_D^{20}$ –17.0 (*c* = 1.5 CH_2Cl_2).

^{1}H–NMR ($CDCl_3$, 400 MHz) δ: 7.13 (dd, *J* = 6.6, 4.0 Hz, 1H), 6.17 (dd, *J* = 10.9, 2.4 Hz, 1H), 5.90 (dd, *J* = 10.9, 1.8 Hz, 1H), 5.56 (br s, 1H), 5.30 (t, *J* = 2.1 Hz, 1H), 5.06 (br s, 1H), 4.44 (q, *J* = 6.8 Hz, 1H), 4.24 (d, *J* = 10.2 Hz, 1H), 3.87 (dd, *J* = 11.2, 4.4 Hz, 1H), 3.70 (dd, *J* = 10.0, 2.2 Hz, 1H), 3.45 – 3.36 (m, 2H), 3.29 – 3.12 (m, 1H), 3.06 – 2.95 (m, 1H), 2.86 (td, *J* = 11.1, 3.2 Hz, 1H), 2.67 (dd, *J* = 11.0, 2.8 Hz, 1H), 2.63 – 2.53 (m, 1H), 2.34 (qd, *J* = 12.4, 6.0 Hz, 1H), 2.10 – 0.80 (m, 38H), 1.71 (s, 3H), 1.20 (d, *J* = 6.9 Hz, 3H), 1.07 (t, *J* = 7.3 Hz, 3H), 0.76 (t, *J* = 7.4 Hz, 3H), 0.70 (d, *J* = 6.6 Hz, 3H), 0.67 (d, *J* = 6.9 Hz, 3H) ppm.

^{13}C–NMR ($CDCl_3$, 101 MHz) δ: 215.5, 184.0, 155.6, 127.5, 123.9, 104.9, 99.1, 88.8, 75.9, 75.52, 75.45, 74.2, 71.7, 71.1, 66.9, 66.1, 55.0, 51.2, 50.5, 40.5, 38.9, 36.9, 36.2, 35.8, 32.8, 32.55, 32.50, 28.34, 28.31, 28.1, 27.0, 24.0, 20.1, 19.9, 17.4, 16.2, 16.1, 14.9, 14.7, 13.3, 12.3, 12.0, 10.7, 6.9, 6.6 ppm.

FTIR (CH_2Cl_2 film): 3285 (br, s) 2969 (m), 2935 (s), 2875 (w), 1716 (s), 1563 (s) cm^{-1}.

HRMS-ESI (*m/z*): $[M + H]^+$ Calcd for $C_{45}H_{75}NNaO_{12}$ 844.5187; Found 844.5212.



5o

Carbamate 5o.

Yield: 15.6 mg, 19%. Isolated as a white amorphous solid, >95% pure by NMR and single spot on TLC.

R_f: 0.51 in 50% EtOAc/petroleum ether. Stains blue with PMA.

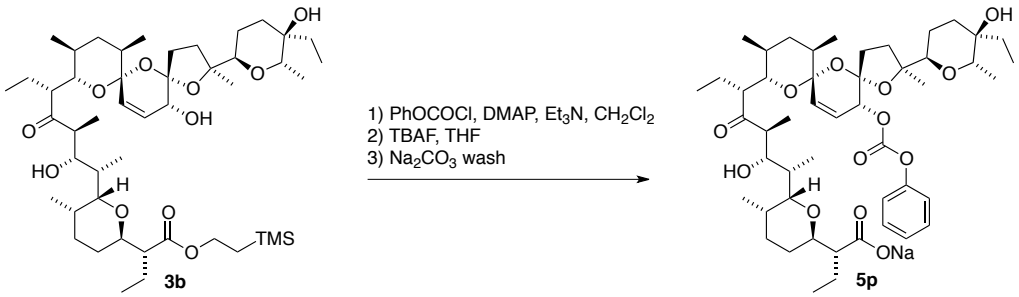
Optical rotation: $[\alpha]_D^{20}$ –15.7 (*c* = 0.5 CH_2Cl_2).

^{1}H–NMR ($CDCl_3$, 400 MHz) δ: 6.72 (br s, 1H), 6.16 (dd, *J* = 10.9, 2.6 Hz, 1H), 5.89 (dd, *J* = 10.8, 1.7 Hz, 1H), 5.34 (dd, *J* = 2.5, 1.6 Hz, 1H), 5.22 (br s, 2H), 4.46 (q, *J* = 6.8 Hz, 1H), 4.23 (dd, *J* = 10.2, 1.5 Hz, 1H), 3.89 (dd, *J* = 10.8, 4.8 Hz, 1H), 3.79 – 3.67 (m, 1H), 3.46 – 3.33 (m, 2H), 2.87 (td, *J* = 11.1, 3.3 Hz, 1H), 2.67 (dd, *J* = 10.9, 2.6 Hz, 1H), 2.64 – 2.53 (m, 1H), 2.41 – 2.28 (m, 1H), 2.09 – 0.81 (m, 38H), 1.71 (s, 3H), 1.26 (s, 9H), 0.99 (t, *J* = 7.3 Hz, 3H), 0.76 (t, *J* = 7.4 Hz, 3H), 0.69 (d, *J* = 4.3 Hz, 3H), 0.67 (d, *J* = 4.6 Hz, 3H) ppm.

^{13}C–NMR ($CDCl_3$, 101 MHz) δ: 215.7, 183.8, 154.2, 128.0, 123.6, 105.0, 99.2, 88.7, 75.9, 75.3, 75.1, 74.2, 71.7, 71.1, 66.9, 65.4, 55.1, 51.2, 50.6, 50.3, 40.6, 39.0, 36.5, 36.2, 32.8, 32.7, 32.5, 28.9, 28.4, 28.1, 27.0, 24.0, 22.8, 20.1, 19.8, 17.4, 16.3, 16.0, 14.7, 13.4, 13.1, 12.0, 10.7, 6.9, 6.7 ppm.

FTIR (CH_2Cl_2 film): 3290 (br, s) 2963 (s), 2931 (s), 2874 (m), 1719 (s), 1564 (s) cm^{-1}.

HRMS-ESI (*m/z*): $[M + H]^+$ Calcd for $C_{47}H_{79}NNaO_{12}$ 872.5500; Found 872.5529.



3b **5p**

Carbonate 5p. To a stirred solution of alcohol **3b** (271 mg, 0.32 mmol) in CH_2Cl_2 (5 mL), at RT, was added Et_3N (0.27 mL, 1.9 mmol) in one portion, followed by

addition of phenyl chloroformate (120 µL, 0.96 mmol) drop-wise over 30 seconds, and then DMAP (excess) in one portion. The resulting yellow cloudy solution was stirred overnight during which the color changed to pale red, then diluted with EtOAc (10 mL) and washed with $NaHCO_3$ (3 x 10 mL, 0.1 M aq.). The organic layer was separated, dried using a phase separator, and concentrated under reduced pressure to give a red oil. Filtration through a short plug of silica gave the intermediate carbonate product as a white amorphous powder (241 mg). To a stirred solution of part of the intermediate product (128 mg) in THF (5 mL), at RT, was added TBAF (400 µL, 0.40 mmol, 1.0 M in THF) drop-wise over 30 seconds. The resulting pale yellow solution was stirred for 1.5 hours, then diluted with EtOAc (10 mL) and washed with Na_2CO_3 (3 x 10 mL, 0.1M aq.). The organic layer was separated, dried using a phase separator, and concentrated under reduced pressure. Purification by flash chromatography (25 – 75% EtOAc/petroleum ether) gave the deprotected product as a mixture of the acid and salt forms. The product mixture was re-dissolved in EtOAc (10 mL) and washed with Na_2CO_3 (3 x 10 mL, 0.1 M aq.). The organic layer was separated, dried using a phase separator, and concentrated several times from *n*-pentane to give carbonate **5p**.

Yield: 59.2 mg, 39%. Isolated as a white amorphous solid >95% pure by NMR, and single spot on TLC.

R_f: 0.28 in 50% EtOAc/petroleum ether. UV-active and stains dark blue with PMA.

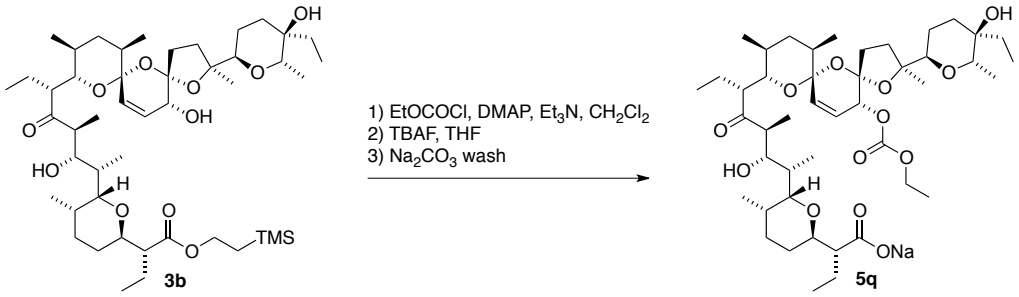
Optical rotation: $[\alpha]_D^{21}$ –12.3° (*c* = 0.80 CH_2Cl_2).

^{1}H–NMR (C_6D_6, 400 MHz) δ: 7.53 (d, *J* = 8.0 Hz, 2H), 7.09 (t, *J* = 7.9 Hz, 2H), 6.89 (t, *J* = 7.4 Hz, 1H), 6.52 (br s, 2H), 6.44 (d, *J* = 10.9 Hz, 1H), 6.35 (dd, *J* = 10.8, 2.6 Hz, 1H), 5.41 (s, 1H), 4.71 (d, *J* = 10.4 Hz, 1H), 4.53 – 4.34 (m, 2H), 4.09 (d, *J* = 9.7 Hz, 1H), 3.77 (d, *J* = 10.1 Hz, 1H), 3.16 – 3.03 (m, 2H), 2.80 – 2.68 (m, 1H), 2.68 – 2.52 (m, 2H), 2.35 – 2.17 (m, 1H), 2.13 – 0.53 (m, 46H), 1.87 (s, 3H), 1.03 (d, *J* = 6.8 Hz, 3H), ppm.

^{13}C–NMR (C_6D_6, 101 MHz) δ: 217.8, 183.5, 154.0, 152.2, 129.5, 127.0, 125.9, 124.8, 121.4, 104.9, 100.4, 89.2, 77.0, 76.7, 75.5, 74.3, 72.5, 71.4, 69.7, 68.0, 56.2, 51.4, 51.1, 40.9, 38.7, 36.6, 36.5, 32.8, 32.6, 32.4, 29.7, 28.7, 27.5, 27.4, 24.1, 20.6, 19.9, 17.5, 16.5, 15.9, 14.5, 13.6, 13.2, 12.0, 11.2, 7.2, 7.0 ppm.

FTIR (CH_2Cl_2 film): 3333 (s, br) 2969 (s), 2936 (s), 2875 (w), 2858 (w), 1758 (s), 1714 (s), 1563 (s) cm^{-1}.

HRMS-ESI *(m/z)*: $[M + H]^+$ Calcd for $C_{49}H_{74}NaO_{13}$ 893.5027; Found 893.5012.



Procedure for carbonate 5q. To a stirred solution of alcohol **3b** (230 mg, 0.27 mmol) in CH_2Cl_2 (5 mL), at RT, was added Et_3N (0.23 mL, 1.7 mmol), followed by addition of ethyl chloroformate (180 µL, 0.81 mmol) drop-wise over 30 seconds, and then DMAP (excess) in one portion. The resulting pale yellow solution was heated to reflux overnight, then cooled to RT, diluted with EtOAc (10 mL) and washed with $NaHCO_3$ (3 x 10 mL, 0.1 M aq.). The organic layer was separated, dried using a phase separator, and concentrated under reduced pressure to give a dark oil. Filtration through a short plug of silica gave the intermediate carbonate product as a white amorphous solid (226 mg). To a stirred solution of part of the intermediate product (110 mg) in THF (5 mL), at RT, was added TBAF (360 µL, 0.36 mmol, 1.0 M in THF) drop-wise over 30 seconds. The resulting pale yellow solution was stirred for one hour, then diluted with EtOAc (10 mL) and washed with Na_2CO_3 (3 x 10 mL, 0.1 M aq.). The organic layer was separated, dried using a phase separator, and concentrated under reduced pressure. Purification by flash chromatography (25 – 75% EtOAc/petroleum ether) gave the carbonate deprotected product as a mixture of the acid and salt forms. The product mixture was re-dissolved in EtOAc (10 mL) and washed with Na_2CO_3 (3 x 10 mL, 0.1M aq.). The organic layer was separated, dried using a phase separator, and concentrated several times from *n*-pentane to give carbonate **5q**.

Yield: 87 mg, 78%. Isolated as a white amorphous solid >95% pure by NMR, and single spot on TLC.

R_f: 0.18 in 50% EtOAc/petroleum ether. Stains dark blue with PMA.

Optical rotation: $[\alpha]_D^{21}$ –19.5 (*c* = 1.0 CH_2Cl_2).

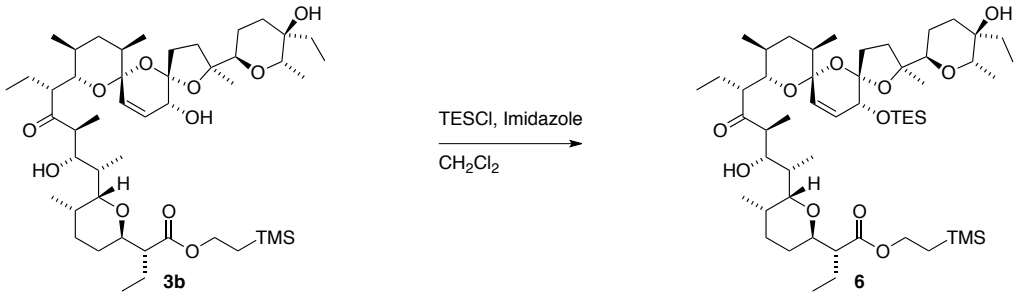
^{1}H–NMR ($CDCl_3$, 400 MHz) δ: 6.19 (dd, *J* = 10.9, 2.7 Hz, 1H), 5.96 (dd, *J* = 10.9, 1.6 Hz, 1H), 5.56 (br s, 1H), 5.15 (dd, *J* = 2.6, 1.5 Hz, 1H), 5.10 (br s, 1H), 4.29 (q, *J*

= 6.9 Hz, 1H), 4.24 (d, *J* = 10.4 Hz, 1H), 4.18 (q, *J* = 7.1 Hz, 2H), 3.89 (dd, *J* = 11.0, 4.5 Hz, 1H), 3.70 (dd, *J* = 10.1, 2.2 Hz, 1H), 3.51 (d, *J* = 10.0 Hz, 1H), 3.35 (dd, *J* = 12.2, 2.2 Hz, 1H), 2.79 (td, *J* = 11.1, 3.3 Hz, 1H), 2.69 – 2.54 (m, 2H), 2.23 (qd, *J* = 12.8, 4.5 Hz, 1H), 2.09 – 0.59 (m, 50H), 1.69 (s, 3H), 0.66 (d, *J* = 6.9 Hz, 3H) ppm.

^{13}C–NMR ($CDCl_3$, 101 MHz) δ: 216.4, 183.6, 155.0, 127.0, 124.1, 104.8, 100.0, 89.0, 76.4, 76.2, 75.6, 74.3, 71.6, 71.2, 69.7, 67.3, 64.9, 55.2, 51.5, 50.5, 40.8, 39.0, 36.0, 35.9, 32.5, 32.4, 29.6, 28.2, 27.7, 27.1, 23.8, 20.3, 19.6, 17.6, 16.2, 15.9, 14.7, 14.4, 13.4, 12.4, 12.0, 10.9, 7.0, 6.7 ppm. One carbon overlapping.

FTIR (CH_2Cl_2 film): 3270 (s, br), 2969 (m), 2935 (m), 2874 (w), 2858 (w), 1746 (s), 1714 (m), 1568 (s) cm^{-1}.

HRMS-ESI *(m/z)*: $[M + H]^+$ Calcd for $C_{45}H_{74}NaO_{13}$ 845.5027; Found 845.5001.



C20 silyl ether 6. To a stirred solution of alcohol **3b** (664 mg, 0.78 mmol) in CH_2Cl_2 (10 mL), at 0 °C, was added imidazole (153 mg, 2.3 mmol), followed by addition of triethylsilyl chloride (195 µL, 1.2 mmol) drop-wise over 30 seconds. The resulting milky white reaction mixture was stirred for 28 hours, then diluted with EtOAc, and washed with $NaHCO_3$ (3 x 10 mL, 0.1 M aq.). The organic layer was separated, dried using a phase separator, and concentrated under reduced pressure. Filtration through a short plug of silica (50% EtOAc/petroleum ether) gave silyl ether **6**.

Yield: 746 mg, 99%. Isolated as a white amorphous solid, >95% pure by NMR and single spot on TLC.

R_f: 0.66 in 25% EtOAc/petroleum ether. Stains blue with PMA.

Optical rotation: $[\alpha]_D^{22}$ –82.2 (*c* = 0.6 CH_2Cl_2).

^{1}H–NMR ($CDCl_3$, 400 MHz) δ: 6.08 (dd, *J* = 10.8, 2.5 Hz, 1H), 5.83 (dd, *J* = 10.7, 1.4 Hz, 1H), 4.53 – 4.34 (m, 2H), 4.12 (dd, *J* = 2.5, 1.4 Hz, 1H), 4.08 – 4.00 (m, 2H), 3.73 (q, *J* = 7.0 Hz, 1H), 3.68 (dd, *J* = 9.8, 1.6 Hz, 1H), 3.46 (dd, *J* = 10.5, 2.8 Hz, 1H), 3.36 (dd, *J* = 10.7, 3.3 Hz, 1H), 3.32 – 3.22 (m, 1H), 3.07 (d, *J* = 5.8 Hz, 1H),

2.99 (td, *J* = 11.0, 4.4 Hz, 1H), 2.78 (dt, *J* = 10.4, 2.8 Hz, 1H), 2.67 (br s, 1H), 2.20 – 2.10 (m, 1H), 2.10 – 0.57 (m, 64H), 1.43 (s, 3H), 0.72 (t, *J* = 7.2 Hz, 3H), 0.07 (s, 9H) ppm.

^{13}C–NMR ($CDCl_3$, 101 MHz) δ: 213.6, 176.0, 132.3, 122.0, 106.5, 99.1, 86.1, 81.2, 77.2, 75.2, 71.8, 71.6, 71.1, 69.3, 67.6, 63.8, 57.8, 48.9, 48.3, 40.6, 39.5, 36.6, 34.0, 33.9, 33.5, 30.5, 29.1, 28.2, 26.4, 22.8, 22.04, 21.98, 20.5, 19.9, 17.6, 17.5, 15.6, 14.5, 14.0, 12.9, 12.0, 11.1, 7.4, 7.0, 6.6, 5.1, -1.3 ppm.

FTIR (CH_2Cl_2 film): 3529 (s, br), 2964 (s), 2939 (s), 2877 (m), 1711 (s) cm^{-1}.

HRMS-ESI *(m/z)*: $[M + Na]^+$ Calcd for $C_{53}H_{96}NaO_{11}Si_2$ 987.6389; Found 987.6404.

Bis-TES ether 7a. To a stirred solution of diol **3a** (2.2 g, 2.8 mmol) in DMF (25 mL), at RT, was added imidazole (964 mg, 14 mmol) in one portion, followed by addition of triethylsilyl chloride (1.2 mL, 7.0 mmol) drop-wise over 30 seconds. The resulting milky white reaction mixture was stirred overnight, then diluted with EtOAc (20 mL) and washed with $NaHCO_3$ (3 x 15 mL 0.1 M aq.). The organic layer was separated, dried using a phase separator, and concentrated under reduced pressure to give the crude product. Purification by flash chromatography (1.5 – 13% EtOAc/petroleum ether) gave bis-silyl ether **7a**.

Yield: 1.5 g, 52%. Isolated as a white amorphous solid, >95% pure by NMR and single spot on TLC.

R_f: 0.27 in 6.3% EtOAc/petroleum ether. Stains blue with PMA.

Optical rotation: $[\alpha]_D^{22}$ –60.9 (*c* = 0.9 CH_2Cl_2).

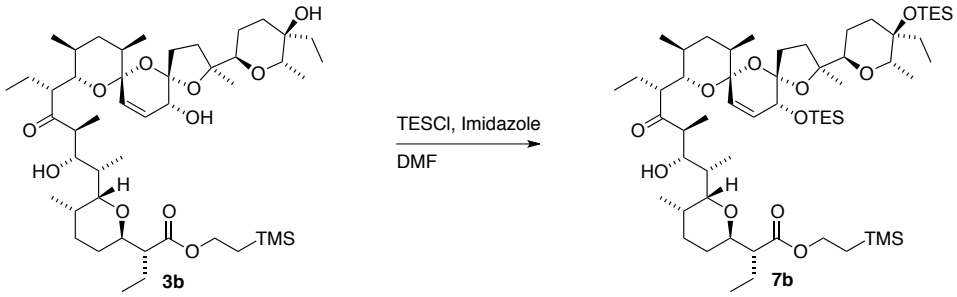
^{1}H–NMR ($CDCl_3$, 400 MHz) δ: 6.08 (dt, *J* = 10.7, 2.3 Hz, 1H), 6.06 – 5.91 (m, 1H), 5.83 (d, *J* = 9.5 Hz, 1H), 5.40 (d, *J* = 17.2 Hz, 1H), 5.22 (d, *J* = 10.4 Hz, 1H), 4.89 – 4.73 (m, 2H), 4.11 (s, 1H), 4.09 – 3.98 (m, 2H), 3.75 (q, *J* = 6.6 Hz, 1H), 3.67 (d, *J* =

9.8 Hz, 1H), 3.46 (d, *J* = 10.6 Hz, 1H), 3.36 – 3.28 (m, 2H), 3.10 – 2.99 (m, 1H), 2.89 (dd, *J* = 5.2, 2.4 Hz, 1H), 2.83 (dd, *J* = 9.6, 2.0 Hz, 1H), 2.25 – 0.47 (m, 84H) ppm.

^{13}C–NMR ($CDCl_3$, 101 MHz) δ: 214.7, 175.4, 132.6, 132.5, 122.0, 118.5, 106.5, 99.1, 86.8, 81.2, 75.13, 75.10, 72.3, 71.9, 69.5, 67.7, 65.9, 58.1, 48.6, 48.5, 40.6, 39.6, 36.6, 34.2, 34.0, 33.7, 31.8, 30.6, 28.2, 26.4, 24.0, 22.8, 22.3, 22.2, 20.8, 19.8, 17.6, 15.7, 15.6, 13.9, 12.8, 12.0, 11.1, 7.5, 7.4, 7.1, 7.0, 6.9, 5.1 ppm.

FTIR (CH_2Cl_2 film): 3539 (s br), 2954 (s), 2876 (s), 1711 (s), 1458 (s) cm^{-1}.

HRMS-ESI *(m/z)*: $[M + Na]^+$ Calcd for $C_{57}H_{102}NaO_{11}Si_2$ 1041.6858; Found 1041.6853.



Bis-TES ether 7b. To a stirred solution of diol **3b** (200 mg, 0.23 mmol) in DMF (3 mL), at RT, was added imidazole (80 mg, 0.18 mmol) in one portion, followed by addition of triethylsilyl chloride (124 µL, 0.71 mmol) drop-wise over 30 seconds. The resulting solution was stirred for 48 hours, then diluted with EtOAc (20 mL) and washed with brine (3 x 10 mL). The organic layer was separated, dried using a phase separator, and concentrated under reduced pressure. Purification by flash chromatography (6.3 — 13% EtOAc/petroleum ether) gave bis-TES ether **7b** along with minor amounts of the corresponding tri-TES ether **8**.

Yield: 159 mg, 63%. Isolated as a clear oil, >95% pure by NMR and a single spot by TLC.

R_f: 0.10 in 6.3% EtOAc/petroleum ether. Stains blue with PMA.

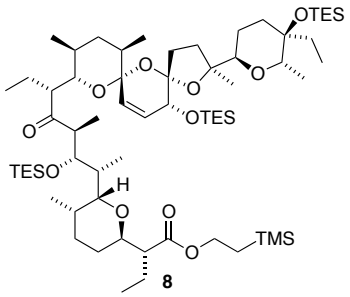
Optical rotation: $[\alpha]_D^{20}$: -61.3 (*c* = 1.0 CH_2Cl_2).

^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 6.08 (dd, *J* = 10.8, 2.5 Hz, 1H), 5.83 (dd, *J* = 10.7, 1.4 Hz, 1H), 4.53 – 4.34 (m, 2H), 4.12 (dd, *J* = 2.5, 1.4 Hz, 1H), 4.10 – 4.00 (m, 2H), 3.75 (q, *J* = 6.8 Hz, 1H), 3.68 (dd, *J* = 9.8, 2.1 Hz, 1H), 3.44 (dd, *J* = 10.5, 2.7 Hz, 1H), 3.38 – 3.27 (m, 2H), 3.05 (d, *J* = 5.8 Hz, 1H), 3.00 (td, *J* = 11.2, 4.6 Hz, 1H), 2.81 (dt, *J* = 10.4, 2.8 Hz, 1H), 2.19 – 0.41 (m, 77H), 1.43 (s, 3H), 1.13 (d, *J* = 6.9 Hz, 3H), 0.72 (t, *J* = 7.2 Hz, 3H), 0.07 (s, 9H) ppm.

^{13}C-NMR ($CDCl_3$, 101 MHz) δ: 213.8, 176.0, 132.5, 122.1, 106.5, 99.0, 86.8, 81.6, 75.18, 75.15, 72.3, 71.9, 69.3, 67.7, 63.8, 58.0, 48.9, 48.3, 40.5, 39.6, 36.6, 34.2, 34.0, 33.5, 31.8, 30.6, 28.2, 26.4, 22.8, 22.5, 22.3, 20.9, 19.9, 17.6, 17.5, 15.7, 15.6, 13.8, 12.9, 12.0, 11.1, 7.4, 7.1, 7.0, 6.9, 6.7, 5.9, 5.1, -1.3 ppm.

FTIR (CH_2Cl_2 film): 3533 (s, br), 2959 (s), 2876 (m), 1711 (s), 1459 (m) cm^{-1}.

HRMS-ESI *(m/z)*: $[M + Na]^+$ Calcd for $C_{59}H_{110}NaO_{11}Si_3$ 1101.7254; Found 1101.7279.



8

Tri-TES ether 8.

Yield: 39 mg, 14%. Isolated as a clear oil, >95% pure by NMR and a single spot by TLC.

R_f: 0.30 in 3.1% EtOAc/petroleum ether. Stains blue with PMA.

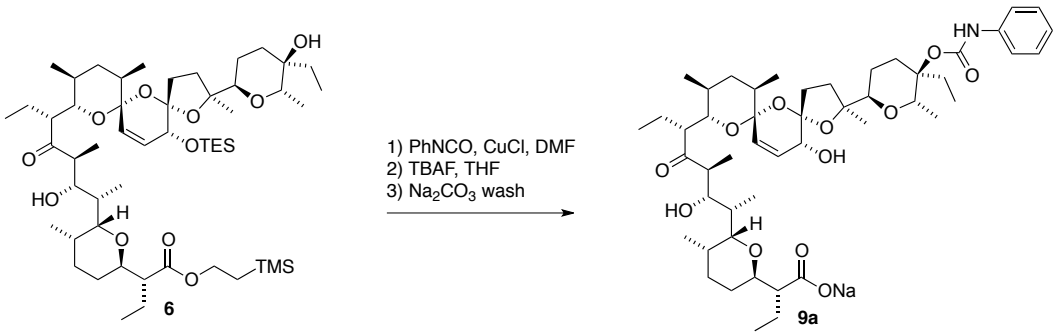
Optical rotation: $[\alpha]_D^{20}$: -52.3 (c = 0.7 CH_2Cl_2).

^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 6.05 (dd, J = 10.8, 2.5 Hz, 1H), 5.82 (dd, J = 10.7, 1.4 Hz, 1H), 4.30 – 4.13 (m, 4H), 4.11 (dd, J = 2.5, 1.4 Hz, 1H), 3.87 – 3.80 (m, 1H), 3.77 (q, J = 7.0 Hz, 1H), 3.58 (dd, J = 10.3, 3.4 Hz, 1H), 3.46 – 3.35 (m, 2H), 3.33 (dd, J = 10.7, 2.8 Hz, 1H), 2.71 (dt, J = 10.0, 2.2 Hz, 1H), 2.52 – 2.45 (m, 1H), 2.23 – 0.56 (m, 91H), 1.56 (s, 3H), 1.40 (s, 3H), 1.14 (d, J = 6.8 Hz, 3H), 0.03 (s, 9H) ppm.

^{13}C-NMR ($CDCl_3$, 101 MHz) δ: 213.4, 174.6, 132.6, 121.9, 106.5, 99.1, 86.8, 82.2, 75.7, 75.2, 73.4, 72.2, 71.7, 67.7, 62.6, 57.6, 52.8, 49.3, 40.6, 39.4, 35.5, 34.2, 34.0, 33.4, 32.0, 31.8, 30.6, 29.9, 27.1, 25.6, 22.2, 22.1, 21.3, 20.8, 18.1, 17.6, 17.1, 15.8, 15.7, 15.3, 13.5, 12.2, 10.6, 7.5, 7.4, 7.1, 7.02, 6.98, 5.5, 5.1, -1.4 ppm.

FTIR (CH_2Cl_2 film): 2955 (s), 2939 (s), 2876 (s), 1731 (s), 1715 (m), 1459 (m), 1389 (m), 1239 (s) cm^{-1}.

HRMS-ESI *(m/z)*: $[M + Na]^+$ Calcd for $C_{65}H_{124}NaO_{11}Si_4$ 1215.8118; Found 1215.8090.



Carbamate 9a. To a stirred solution of alcohol **6** (127 mg, 0.15 mmol) in DMF (3 mL), at RT, was added phenylisocyatante (200 µL, 1.53 mmol) followed by anhydrous CuCl (5 x 2 mm round beads) in one portion respectively. The resulting solution was stirred for 10 minutes during which the color changed to light green, then rapidly added to a mixture of Na_2CO_3 (10 mL, 0.1 M, aq.) and EtOAc and washed with Na_2CO_3 (3 x 10 mL, 0.1 M aq.). The organic layer was separated, dried using a phase separator, and evaporated under reduced pressure. The resulting clear oil was purified by flash chromatography (12.5 – 25% EtOAc/petroleum ether) to give impure intermediate carbamate product as a clear oil. To a stirred solution of the intermediate product in THF (5 mL), at RT, was added TBAF (100 µL, 0.10 mmol, 1 M in THF) drop-wise over 30 seconds. The resulting pale yellow reaction mixture was stirred for two hours, then diluted with EtOAc (20 mL) and washed with Na_2CO_3 (3 x 10 mL, 0.1 M aq.). The organic layer was separated, dried using a phase separator, and evaporated under reduced pressure to give clear oil. Purification by flash chromatography (2.5 – 5.0% MeOH/CH_2Cl_2) gave the deprotected product as a mixture of the acid and salt forms. The product mixture was re-dissolved in EtOAc (10 mL) and washed with Na_2CO_3 (3 x 10 mL, 0.1 M aq.). The organic layer was separated, dried using a phase separator, and concentrated several times from *n*-pentane to give carbonate **9a**.

Yield: 58 mg, 46%. Isolated as a white amorphous solid, >95% pure by NMR, and single spot on TLC.

R_f: 0.40 in 33% EtOAc/petroleum ether. Stains dark blue with PMA.

Optical rotation: $[\alpha]_D^{22}$ –42.5 (c = 1.0 CH_2Cl_2).

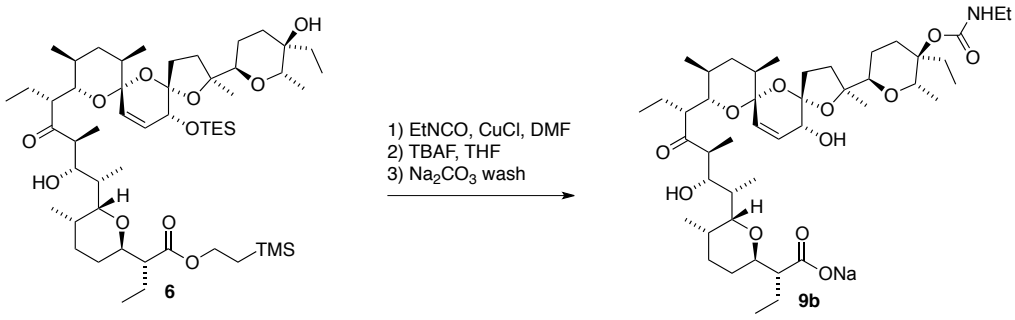
^{1}H–NMR (C_6D_6, 400 MHz) δ: 9.81 (br s, 1H), 8.11 (d, J = 7.4, 2H), 7.20 (m, 2H), 6.89 (dt, J = 7.4, 1.1, 1H), 6.17 (dd, J = 10.9, 2.3 Hz, 1H), 6.11 (d, J = 11.0 Hz, 1H), 5.49 (br s, 1H), 4.72 (q, J = 7.2 Hz, 1H), 4.63 (dd, J = 10.6, 1.7 Hz, 1H), 4.43 – 4.34

(m, 1H), 4.05 (s, 1H), 3.99 (d, *J* = 10.9 Hz, 1H), 3.90 (br s, 1H), 3.75 (d, *J* = 10.8 Hz, 1H), 3.20 – 3.01 (m, 2H), 2.95 (d, *J* = 11.5 Hz, 1H), 2.91 – 2.72 (m, 2H), 2.59 (d, *J* = 9.8 Hz, 1H), 2.53 – 0.65 (m, 31H), 1.84 (s, 3H), 1.06 (t, *J* = 7.3 Hz, 3H), 1.00 (d, *J* = 6.6 Hz, 3H), 0.79 (t, *J* = 7.3 Hz, 3H), 0.72 (d, *J* = 8.7 Hz, 3H), 0.70 (d, *J* = 6.8 Hz, 3H), 0.64 (d, *J* = 6.5 Hz, 3H) ppm.

^{13}C–NMR (C_6D_6, 101 MHz) δ: 217.6, 184.4, 153.7, 140.7, 131.1, 128.9, 122.9, 122.8, 120.7, 107.3, 99.2, 87.7, 82.5, 76.9, 75.6, 75.5, 75.0, 71.3, 68.7, 66.1, 56.7, 52.0, 50.6, 41.0, 38.9, 36.9, 36.2, 34.5, 32.8, 32.7, 28.8, 28.6, 27.5, 23.6, 22.8, 21.1, 20.8, 17.6, 16.3, 16.1, 14.3, 13.9, 13.6, 13.2, 12.6, 11.8, 7.3 ppm.

FTIR (neat): 3346 (s, br), 2969 (m), 2937 (m), 1764 (s), 1715 (s), 1571(s) cm^{-1}.

HRMS-ESI *(m/z)*: $[M + H]^+$ Calcd for $C_{49}H_{75}NNaO_{12}$ 892.5187; Found 892.5181.



Carbamate 9b. To a stirred solution of alcohol **6** (116.7 mg, 0.12 mmol) in DMF (2 mL), at 0 °C, was added ethyl isocyanate (10 µL, 0.12 mmol) drop-wise over 1 minute, followed by addition of anhydrous CuCl (catalytic, freshly ground) in one portion. The resulting dark green solution was allowed to reach 6 °C over 17.5 hours, then diluted with EtOAc (20 mL) and washed with brine (3 x 20 mL). The aqueous layer was back extracted with EtOAc (20 mL). The combined organic layers were dried using a phase separator and evaporated under reduced pressure. The crude mixture was filtered through a short plug of silica (3 – 25% EtOAc/petroleum ether) to give the intermediate carbamate product (40.9 mg, R_f 0.63 in 50% EtOAc/petroleum ether) as a clear oil, together with recovered starting material (83.4 mg). To a stirred solution of the intermediate product in THF (5 mL), at RT, was added TBAF (40 µL, 0.040 mmol, 1 M in THF) drop-wise over 30 seconds. The resulting pale yellow reaction mixture was stirred for three hours, then diluted with EtOAc (20 mL) and washed with Na_2CO_3 (3 x 10 mL, 0.1 M aq.). The organic layer was separated, dried using a phase separator, and evaporated under reduced pressure

to give a clear oil. Purification by flash chromatography (2.5 – 5.0% MeOH/CH_2Cl_2) gave the deprotected product as a mixture of the acid and salt forms. The product mixture was re-dissolved in EtOAc (10 mL) and washed with Na_2CO_3 (3 x 10 mL, 0.1 M aq.). The organic layer was separated, dried using a phase separator, and concentrated several times from *n*-pentane to give carbamate **9b**.

Yield: 9.3 mg, 9% (83% based on recovered starting material). Isolated as a white amorphous solid, >95% pure by NMR, and single spot on TLC.

R_f: 0.23 in 25% EtOAc/petroleum ether. Stains dark blue with PMA.

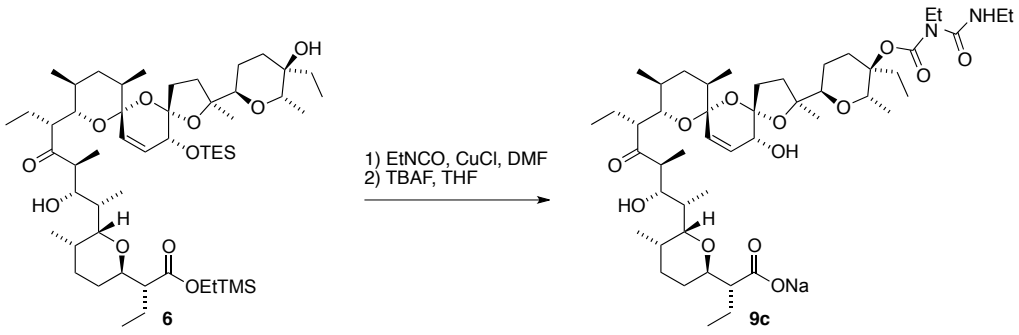
Optical rotation: $[\alpha]_D^{22}$ –11.1 (c = 0.9 CH_2Cl_2).

^{1}H–NMR (MeOD, 400 MHz) δ 6.19 (dd, J = 10.7, 2.3 Hz, 1H), 5.97 (d, J = 10.7 Hz, 1H), 4.47 (q, J = 6.9 Hz, 1H), 4.30 (d, J = 9.9 Hz, 1H), 4.05 (s, 1H), 3.95 (dd, J = 10.5, 4.2 Hz, 1H), 3.78 (d, J = 9.3 Hz, 1H), 3.71 – 3.56 (m, 2H), 3.20 – 2.99 (m, 2H), 3.00 – 2.80 (m, 3H), 2.47 (d, J = 14.0 Hz, 1H), 2.29 – 0.70 (m, 44H), 1.55 (s, 3H), 1.28 (d, J = 6.9 Hz, 3H), 1.12 (t, J = 7.1 Hz, 3H), 0.79 (d, J = 6.9 Hz, 3H), 0.74 (d, J = 6.7 Hz, 3H) ppm.

^{13}C–NMR (MeOD, 101 MHz) δ: 217.5, 183.8, 158.0, 132.7, 124.0, 107.9, 100.5, 89.2, 83.2, 78.7, 77.3, 75.5, 74.9, 72.9, 70.0, 67.7, 57.2, 52.0, 50.1, 41.7, 39.8, 37.5, 37.3, 36.2, 34.1, 32.4, 29.6, 28.4, 27.9, 27.7, 26.9, 24.3, 22.4, 21.3, 18.6, 17.9, 16.1, 15.5, 14.7, 13.7, 13.4, 13.2, 11.7, 7.6, 6.9 ppm.

FTIR (CH_2Cl_2 film): 3267 (s, br), 2968 (m), 2936 (m), 2874 (w), 1781 (s), 1745 (m), 1709 (m), 1576 (m) cm^{-1}.

HRMS-ESI *(m/z)*: $[M + H]^+$ Calcd for $C_{45}H_{75}NO_{12}Na$ 844.5187; Found 844.5170.



Allophanate 9c. To a stirred solution of alcohol **6** (121 mg, 0.13 mmol) in DMF (5 mL), at RT, was added ethyl isocyanate (15 µL, 0.19 mmol) drop-wise over 30 seconds, followed by addition of anhydrous CuCl (catalytic, freshly ground) in one portion. The resulting pale green solution was stirred for three days (NMR control)

followed by addition of additional ethyl isocyanate (30 μL, 0.38 mmol) in one portion. The resulting reaction mixture was stirred for a further 24 hours, then diluted with EtOAc (50 mL) and washed with brine (3 x 20 mL). The organic phase was separated, dried over a phase separator, and concentrated under reduced pressure. Filtration through a short plug of silica (6.1 – 25% EtOAc/petroleum ether) gave the intermediate allophanate product (144 mg) as a clear oil. To a stirred solution of a part of the intermediate product (54.5 mg) in THF (5 mL), at 0 °C, was added TBAF (131 μL, 0.13 mmol) drop-wise over 30 seconds. The resulting pale yellow solution was stirred for three hours, then diluted with EtOAc (20 mL) and washed with Na_2CO_3 (3 x 20 mL, 0.1 M, aq.). The organic layer was separated, dried using a phase separator, and concentrated under reduced pressure. Purification by flash chromatography (2.5% MeOH/CH_2Cl_2) gave allophanate **9c**.

Yield: 30.1 mg, 26%. Isolated as a white amorphous solid, >95% pure by NMR, and single spot on TLC.

R_f: 0.32 in 5% MeOH/CH_2Cl_2. Stains dark blue with PMA.

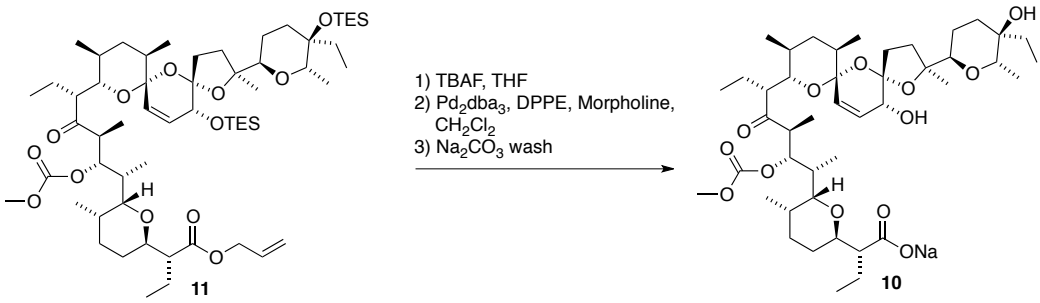
Optical rotation: $[\alpha]_D^{22}$ –30.1 (*c* = 0.9 CH_2Cl_2).

^{1}H–NMR (C_6D_6, 400 MHz) δ: 8.71 (s br, 1H), 6.37 (dd, *J* = 10.9, 1.2 Hz, 1H), 6.18 (dd, *J* = 11.0, 2.2 Hz, 1H), 4.64 (d, *J* = 10.1 Hz, 1H), 4.33 – 4.25 (m, 1H), 4.18 (s, 1H), 4.17 – 4.11 (m, 1H), 4.07 (d, *J* = 10.3 Hz, 1H), 4.04 – 3.87 (m, 3H), 3.84 (dd, *J* = 9.8, 2.3 Hz, 1H), 3.32 – 3.10 (m, 2H), 2.97 (td, *J* = 10.9, 3.5 Hz, 1H), 2.91 – 2.80 (m, 1H), 2.64 (dd, *J* = 11.1, 2.7 Hz, 1H), 2.51 – 2.39 (m, 1H), 2.39 – 2.25 (m, 2H), 2.16 – 2.06 (m, 1H), 2.00 – 0.57 (m, 43H), 1.61 (s, 3H), 0.76 (app d, *J* = 6.5 Hz, 6H), 0.69 (d, *J* = 6.8 Hz, 3H), 0.53 (t, *J* = 7.4 Hz, 3H) ppm.

^{13}C–NMR (C_6D_6, 101 MHz) δ: 216.7, 178.3, 155.3, 154.4, 133.2, 122.2, 106.9, 100.1, 89.1, 85.8, 75.2, 74.2, 73.9, 71.8, 69.1, 67.6, 56.7, 50.8, 49.7, 41.4, 39.6, 38.8, 37.1, 36.8, 35.6, 33.2, 30.3, 30.2, 28.3, 27.0, 26.7, 26.5, 26.2, 23.2, 22.6, 20.1, 18.1, 17.1, 16.0, 15.0, 14.9, 14.4, 13.6, 13.0, 12.5, 11.1, 7.0, 6.5 ppm.

FTIR (neat): 3395 (s, br), 2957 (s), 2928 (s), 2874 (m), 2856 (m), 2367 (w), 2340 (w), 1713 (s), 1680 (w), 1539 (m) cm^{-1}.

HRMS-ESI *(m/z)*: $[M + H]^+$ Calcd for $C_{48}H_{80}N_2NaO_{13}$ 915.5558; Found 915.5580.



Carbonate 10. To a stirred solution of ester **11** (184 mg, 0.17 mmol) in THF (5 mL), at RT, was added TBAF (0.51 mL, 0.51 mmol, 1.0 M in THF) drop-wise over 30 seconds. The resulting yellow solution was stirred overnight, then diluted with EtOAc (20 mL) and washed with Na_2CO_3 (3 x 10 mL, 0.1 M aq.). The organic layer was separated, dried using a phase separator, and concentrated under reduced pressure to give the intermediate carbonate product as a brown oil. To a stirred solution of the intermediate product in CH_2Cl_2 (15 mL), at RT, was added Pd_2dba_3 (29 mg, 0.032 mmol), followed by DPPE (25 mg, 0.064 mmol) and morpholine (28 µL, 0.32 mmol) in one portion respectively. The resulting yellow solution was stirred overnight, then diluted with EtOAc (20 mL) and washed with Na_2CO_3 (3 x 10 mL, 0.1 M aq.) and brine (20 mL). The organic layer was separated, dried using a phase separator, and concentrated under reduced pressure. Purification by flash chromatography (2.5 – 5% MeOH/CH_2Cl_2) gave the deprotected product as a mixture of the acid and salt form. The product mixture was re-dissolved in EtOAc (10 mL) and washed with Na_2CO_3 (3 x 10 mL, 0.1M aq.). The organic layer was separated, dried using a phase separator, and concentrated several times from *n*-pentane to give carbonate **10**.

Yield: 83.4 mg, 58%. Isolated as a white amorphous solid, >95% pure by NMR and single spot on TLC.

R_f: 0.25 in 5% MeOH/CH_2Cl_2. Stains blue with PMA.

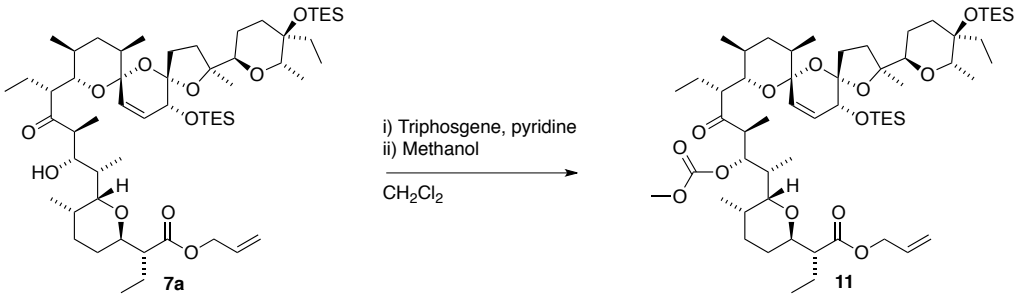
Optical rotation: $[\alpha]_D^{20}$ –63.1 (*c* = 1.2 CH_2Cl_2).

^{1}H–NMR (MeOD, 400 MHz) δ: 6.13 (dd, *J* = 10.8, 2.4 Hz, 1H), 5.92 (dd, *J* = 10.7, 1.6 Hz, 1H), 5.24 (dd, *J* = 8.8, 2.2 Hz, 1H), 3.98 (s, 1H), 3.97 – 3.90 (m, 1H), 3.81 (q, *J* = 7.1 Hz, 1H), 3.72 (s, 3H), 3.65 – 3.57 (m, 2H), 3.58 – 3.52 (m, 1H), 3.49 (dd, *J* = 10.7, 2.0 Hz, 1H), 2.87 – 2.73 (m, 2H), 2.40 – 2.24 (m, 2H), 2.03 – 0.72 (m, 42H), 1.39 (s, 3H), 1.21 (d, *J* = 7.0 Hz, 3H), 1.01 (d, *J* = 7.2 Hz, 3H), 0.70 (d, *J* = 6.8 Hz, 3H) ppm.

^{13}C–NMR (MeOD, 101 MHz) δ: 214.6, 178.4, 157.2, 133.5, 122.6, 107.6, 100.4, 89.2, 81.8, 79.8, 77.7, 75.6, 75.5, 75.2, 72.0, 69.1, 58.2, 55.2, 54.8, 50.9, 49.5, 41.6, 40.0, 37.6, 37.3, 34.5, 32.7, 31.6, 30.8, 30.4, 27.7, 26.2, 23.3, 22.8, 22.2, 17.8, 16.0, 15.3, 14.9, 14.1, 13.6, 12.0, 10.5, 6.7 ppm.

FTIR (neat): 3392 (s br), 2976 (m), 1746 (s), 1713 (s), 1560 (w, br) cm^{-1}.

HRMS-ESI *(m/z)*: $[M + H]^+$ Calcd for $C_{44}H_{72}NaO_{13}$ 831.4871; Found 831.4871.



Carbonate 11. To a stirred solution of alcohol **7a** (144 mg, 0.14 mmol) in CH_2Cl_2 (5 mL), at RT, was added pyridine (250 µL, 3.1 mmol), followed by addition of triphosgene (126 mg, 0.43 mmol) in one portion (gas evolution). The resulting clear reaction mixture was stirred for two hours during which the color turned to brown. MeOH (70 µL, 1.7 mmol) was then added drop-wise over 30 seconds. The resulting solution was stirred for three hours, then diluted with EtOAc (20 mL) and washed with $NaHCO_3$ (3 x 10 mL, 0.1 M aq.). The organic layer was separated, dried using a phase separator, and concentrated under reduced pressure. Purification by flash chromatography (3.1 – 13% EtOAc/petroleum ether) gave carbonate **11**.

Yield: 126 mg, 83%. Isolated as a white amorphous solid, >95% pure by NMR and single spot on TLC.

R_f: 0.39 in 13% EtOAc/petroleum ether. Stains blue with PMA.

Optical rotation: $[\alpha]_D^{20}$ –75.1 (*c* = 0.7 CH_2Cl_2).

^{1}H–NMR (C_6D_6, 400 MHz) δ: 6.17 – 6.04 (m, 1H), 5.98 – 5.88 (m, 2H), 5.61 (dd, *J* = 9.2, 1.6 Hz, 1H), 5.36 (dq, *J* = 17.2, 1.7 Hz, 1H), 5.09 (dq, *J* = 10.3, 1.3 Hz, 1H), 5.07 – 4.93 (m, 2H), 4.31 (d, *J* = 1.4 Hz, 1H), 4.29 – 4.20 (m, 1H), 3.96 – 3.86 (m, 2H), 3.83 (dd, *J* = 9.8, 2.8 Hz, 1H), 3.56 (dd, *J* = 10.7, 2.7 Hz, 1H), 3.50 (s, 3H), 3.49 – 3.45 (m, 1H), 3.16 – 3.00 (m, 2H), 2.39 – 0.54 (m, 75H), 1.85 (s, 3H), 1.33 (d, *J* = 7.2 Hz, 3H), 1.18 (d, *J* = 6.8 Hz, 3H) ppm.

^{13}C–NMR (C_6D_6, 101 MHz) δ: 211.6, 173.9, 156.0, 134.1, 132.6, 122.3, 117.6, 107.1, 99.6, 87.3, 81.5, 78.6, 77.0, 75.5, 74.0, 73.8, 73.0, 68.8, 65.5, 57.9, 54.2, 49.1, 48.8, 40.9, 39.9, 37.0, 35.0, 34.8, 33.9, 31.9, 30.8, 29.4, 26.9, 22.9, 22.7, 22.6, 21.5, 21.3, 18.0, 16.0, 15.9, 14.3, 13.4, 13.1, 11.7, 10.2, 7.7, 7.31, 7.26, 7.2, 5.4 ppm.

FTIR (CH_2Cl_2 film): 2955 (s), 2939 (s), 2876 (s), 1753 (s), 1737 (s), 1714 (s) cm^{-1}.

HRMS-ESI *(m/z)*: $[M + Na]^+$ Calcd for $C_{59}H_{104}NaO_{13}Si_2$ 1099.6913; Found 1099.6906.

*IV. Structural assignment of **5a–q**, **9a-c**, and **10***

Assignment of analogs acylated at the C20 position (5a – q). The acyl groups of esters **5a – l**, carbamates **5m – o**, and carbonates **5p/q** were assigned to the C20 position using diagnostic downfield shifts in the ^{1}H-NMR spectra of the C20 protons for the respective acylated structure relative to the C20 proton of the salinomycin sodium salt (**2**). In the solvent indicated:[4] C20*H* **5a – l**, ^{1}H-NMR $\Delta\delta_{SA}$ = 1.78 – 1.22 ppm; C20*H* **5m – o**, ^{1}H-NMR $\Delta\delta_{SA}$ = 1.33 – 1.22 ppm; C20*H* **5p/q**, ^{1}H-NMR $\Delta\delta_{SA}$ = 1.23 – 1.07 ppm). The ^{1}H-NMR signals of the allylic C20 protons were assigned by a $^{3}J_{HH}$ coupling to the characteristic olefinic C19 protons in the respective COSY spectra (Figure 1). For ^{1}H-NMR, ^{13}C-NMR, and partly assigned COSY spectra of **5a – q**, see pages S58 – S104.

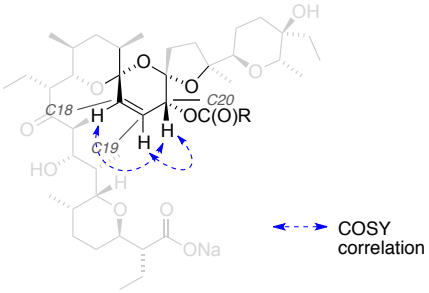


Figure 1. Diagnostic COSY correlations for analogs **5a – q**.

Assignment of carbonate 10. The acyl group of carbonate **10** was assigned to the C9 position by a diagnostic $^{3}J_{CH}$ correlation from the C9 proton (^{1}H δ: 5.24 ppm) to the carbonate carbonyl C43 (^{13}C-NMR δ: 157.2 ppm) in the HMBC spectrum (Figure 2). The C43 was assigned by a $^{3}J_{CH}$ correlation from the characteristic C44 protons (^{1}H-NMR δ: 3.72 ppm) in the HMBC spectrum. The C9 proton was assigned by a $^{3}J_{CH}$ coupling to C11 (^{13}C-NMR δ: 214.6 ppm) in the HMBC spectrum, together with a $^{3}J_{HH}$ correlation between the C9 proton and the C10 proton (^{1}H-NMR δ: 3.58 – 3.52 ppm) in the COSY spectrum. The assignment of the C10 proton was confirmed by a $^{3}J_{CH}$ coupling to C38 (^{13}C-NMR δ: 14.1 ppm) in the HMBC spectrum. For ^{1}H-NMR, ^{13}C-NMR, and partly assigned COSY and HMBC spectra of carbonate **10**, see pages S115 – S118.

[4] The ^{1}H-NMR chemical shift for the C20*H* in salinomycin sodium salt (**2**) is 4.07 ppm in $CDCl_3$, 4.25 ppm in benzene-d_6 (400 MHz).

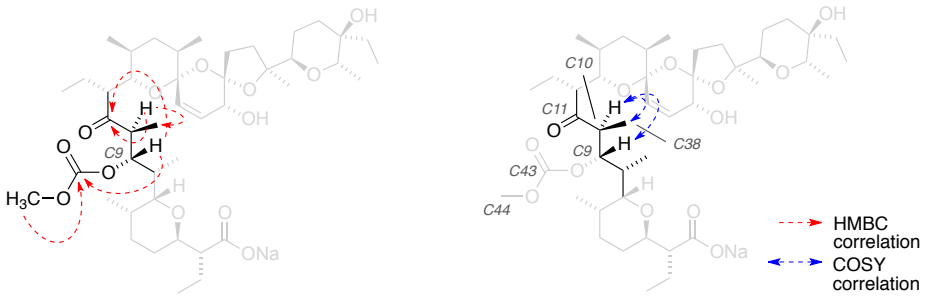


Figure 2. Diagnostic HMBC and COSY correlations for carbonate **10**.

Assignment of analogs acylated at the C28 position (9a – c). The acyl groups of carbamates **9a**/**b** and allophanate **9c** were assigned to the C28 position using diagnostic downfield shifts in the ^{13}C-NMR spectra of the C28 signals for the respective acylated structure relative to the C28 signal of the salinomycin sodium salt (**2**). In the solvent indicated:[5] *C*28 **9a**, ^{13}C-NMR δ: 82.5 ppm, $\Delta\delta_{SA}$ = 10.2 ppm; *C*28 **9b**, ^{13}C-NMR δ: 83.2 ppm, $\Delta\delta_{SA}$ = 10.9 ppm; *C*28 **9c**, ^{13}C-NMR δ: 85.8 ppm, $\Delta\delta_{SA}$ = 15.6 ppm. For carbamates **9a** and **9b**, the C28 signal was assigned by a $^{2}J_{CH}$ coupling from the C29 proton (**9a**: ^{1}H δ: 4.63 ppm **9b**: ^{1}H δ: 4.47 ppm) and a $^{3}J_{CH}$ coupling from the C30 protons (**9a**: ^{1}H δ: 0.82 ppm **9b**: ^{1}H δ: 1.28 ppm) in the respective HMBC spectra (Figure 3). The assignment of the C28 signals for **9a** and **9b** were corroborated by the absence of this signal in the respective DEPT-135 spectra. The C30 protons were assigned by a $^{3}J_{HH}$ coupling to the C29 proton in the respective COSY spectra. The C29 proton was assigned by a $^{3}J_{CH}$ coupling to C25 (**9a**, ^{13}C-NMR δ: 75.6 ppm; **9b**, ^{13}C-NMR δ: 75.5 ppm) in the respective HMBC spectra. The C25 signals were assigned by a $^{3}J_{CH}$ coupling from the characteristic C33 protons (**9a**, ^{1}H-NMR δ: 1.84 ppm; **9b**, ^{1}H δ: 1.55 ppm) in the respective HMBC specta and by the positive signals in the respective DEPT-135 spectra. For allophanate **9c**, the C28 signal in the ^{13}C-NMR spectrum was assigned by a $^{2}J_{CH}$ coupling from the C29 proton (^{1}H-NMR δ: 4.33 – 4.25 ppm) in the HMBC spectrum and by the absence of this signal in the DEPT-135 spectrum. The allophanate moiety of **9c** was assigned by $^{3}J_{CH}$ couplings from the C44 protons (^{1}H δ: 4.06 – 3.77 ppm) and the C47 protons (^{1}H δ: 3.32 – 3.10 ppm) to C46 (^{13}C-NMR δ: 154.5 ppm) in the HMBC spectrum, and by couplings from the C44 protons to C43 (^{13}C-NMR δ: 155.3 ppm) in the HMBC

[5] The ^{13}C-NMR chemical shift for C28 in salinomycin sodium salt (**2**) is 72.3 ppm in methanol-d_4 and 70.2 ppm in benzene-d_6 (400 MHz).

spectrum. For ^{1}H-NMR, ^{13}C-NMR, and partly assigned DEPT-135, COSY, and HMBC spectra of **9a** – **c**, see pages S119 – S134.

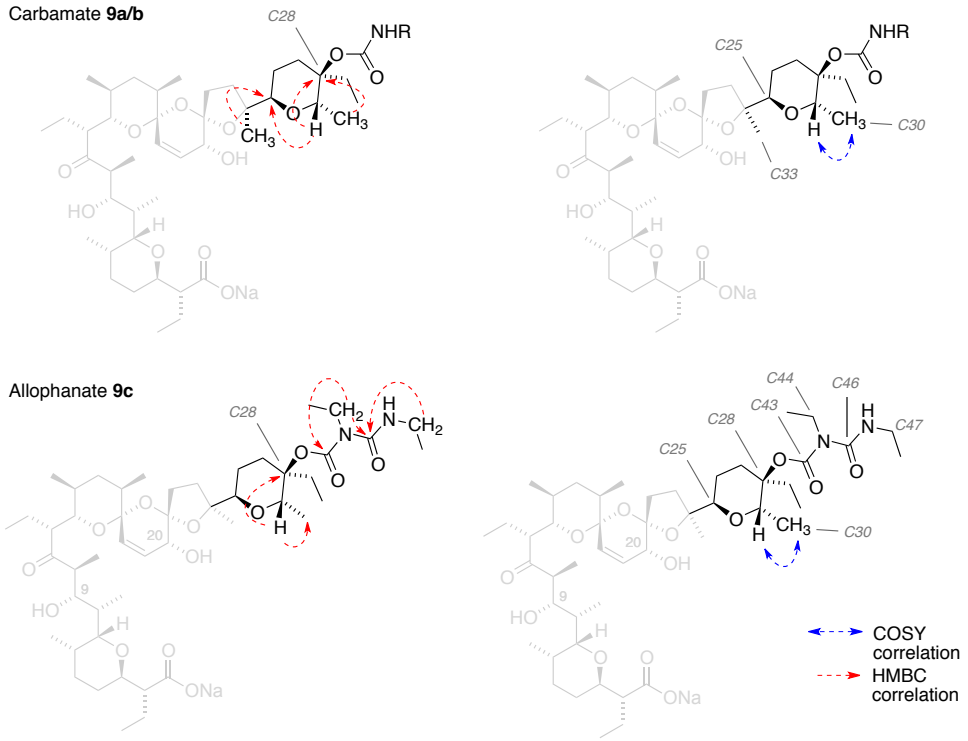


Figure 3. Diagnostic HMBC and COSY correlations for analogs **9a** – **c**.

*V. MTT methods and data for compounds **2**, **3a**, **5a – q**, **9a – c**, and **10***

Sample preparation. Samples of each analog (~50 mg) were dissolved in MeCN (1 mL) followed by addition of water (~0.5 – 1 mL). The resulting cloudy mixture was frozen in liquid nitrogen and lyophilized over night to give the analogs as fluffy white amorphous powders. The analogs were weighed to 0.01 mg precision in tarred vials. The samples were dissolved in DMSO and stored at -50 ˚C.

Cell lines and culturing conditions. The human breast carcinoma cell lines JIMT-1 (ACC589) and MCF-7 (HTB-22) were cultured at 37 ˚C in a humidified incubator with 5% CO_2 in air. The JIMT-1 cell line was purchased from the German Collection of Microorganisms and Cell Cultures (DSMZ, Braunschweig, Germany). The MCF-7 cell line was obtained from American Type Culture Collection (ATCC, Manassas, VA, USA). JIMT-1 cells were routinely cultured in Dulbecco's modified Eagle's medium/nutrient mixture Ham's F12 medium. The MCF-7 cells were maintained in RPMI 1640 medium. Both cell lines were cultured with the addition of 10% fetal calf

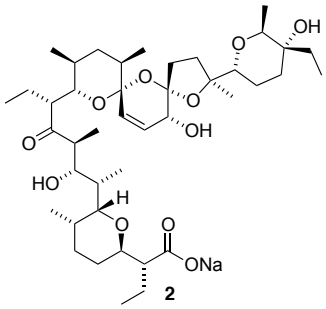
serum, nonessential amino acids (1 mmol/l), insulin (10 mg/mL), penicillin (100 U/mL), and streptomycin (100 mg/mL).

Dose-response assay. An MTT[6] assay was used to evaluate the dose-response of the compounds and for calculation of inhibitory concentration 50 (IC_{50}) as a measure of potency.[7] The compounds were dissolved in DMSO and serially diluted in PBS and used at final concentrations from 0.05 μM to 20 μM. The final DMSO concentration in the assay was 0.2% for all concentrations used. Accordingly, the control was treated with 0.2% DMSO in PBS. For the assays, cells were seeded in 96-well plates (6000 cells for MCF-7 and 5000 cells for JIMT-1 per well in 180 μL medium) and incubated for 24 hours before addition of the compounds. The effect was evaluated by an MTT assay after 72 hours of treatment. MTT solution (20 μL, 5 mg/mL in PBS) was added to each well and the 96-well plates were returned to the incubator for 1 hour. The medium was removed and the blue formazan product was dissolved by the addition of 100 μL of 100% DMSO per well. The plates were swirled gently for 10 minutes to dissolve the precipitate. Absorbance was monitored at 540 nm using a Labsystems iEMS Reader MF(Labsystems Oy, Helsinki, Finland) and the software DeltaSoft II v.4.14 (Biometallics Inc., Princeton, NJ, USA). For each compound,[8] three independent dose-response experiments were performed with six replicates in each experiment. The software program GraphPad Prism (San Diego, CA, USA) was used to plot dose-response curves.

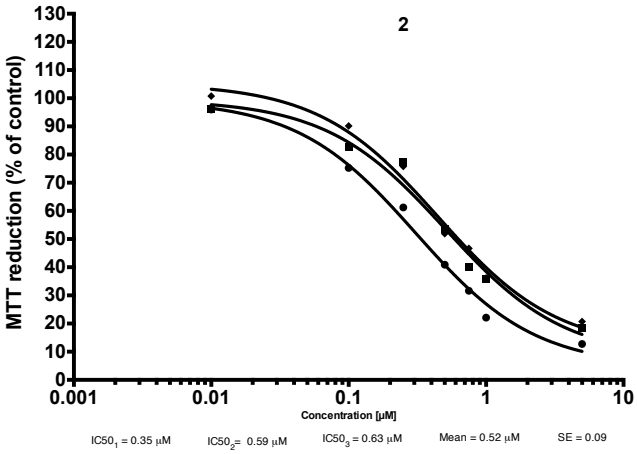
Statistical analysis. GraphPad Prism 6.0 was used for analysis of the absolute IC_{50} value for each dose-response curve of each compound, as well as for the calculation of the mean and standard error (SE).

[6] (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
[7] C. M. Holst and S. M. Oredsson, *Toxicol In Vitro,* 2005, **19**, 379.
[8] For methyl ester **3a**, n = 2 with JIMT-1 and n = 1 with MCF-7.

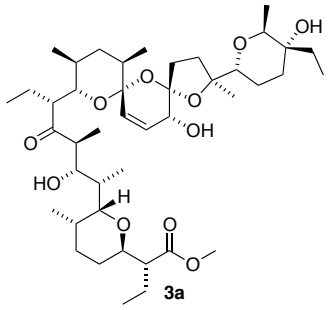
MTT dose-response curves in JIMT-1 cells.



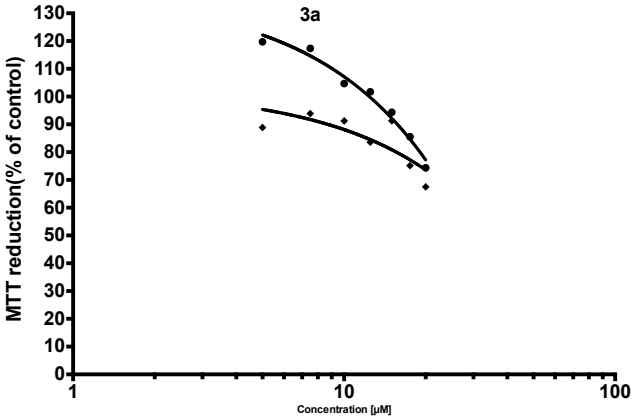
2

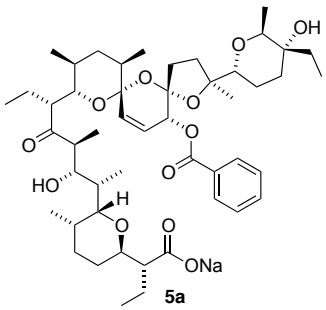


$IC50_1$ = 0.35 µM $IC50_2$= 0.59 µM $IC50_3$ = 0.63 µM Mean = 0.52 µM SE = 0.09

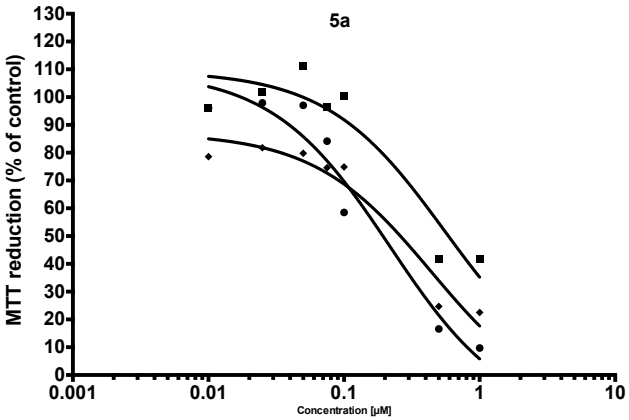


3a

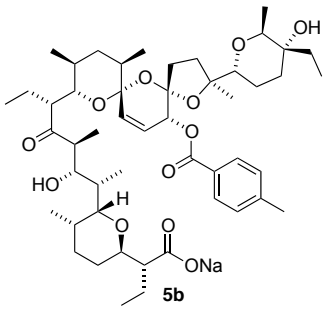




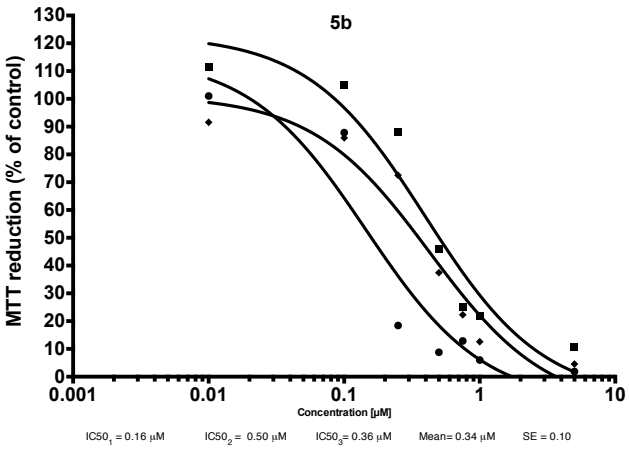
5a



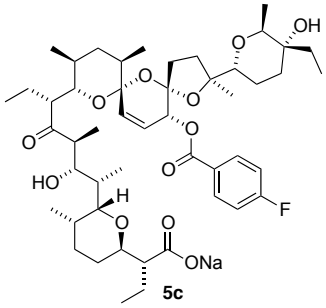
$IC50_1$ = 0.19 µM $IC50_2$ = 0.59 µM $IC50_3$ = 0.26 µM Mean = 0.35 µM SE = 0.12



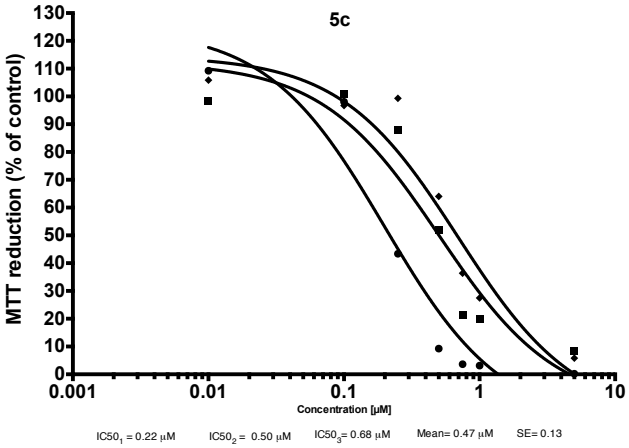
5b



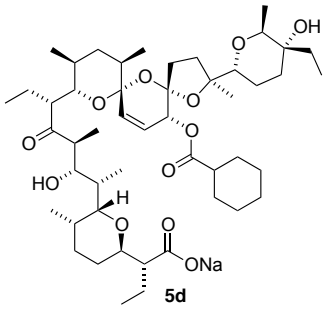
$IC50_1$ = 0.16 μM $IC50_2$ = 0.50 μM $IC50_3$= 0.36 μM Mean= 0.34 μM SE = 0.10



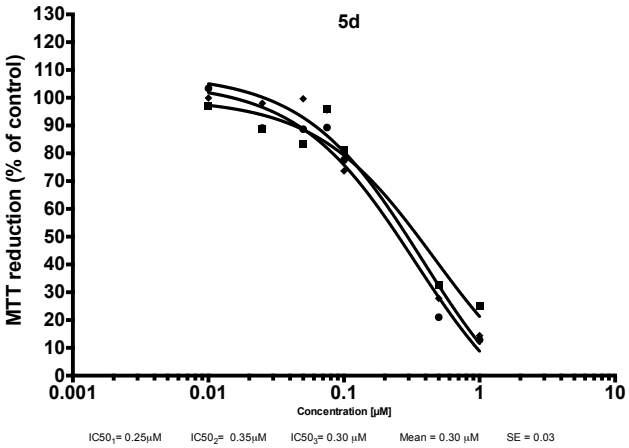
5c



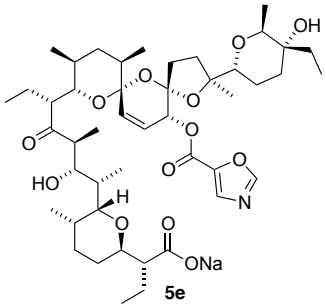
$IC50_1$ = 0.22 μM $IC50_2$ = 0.50 μM $IC50_3$= 0.68 μM Mean= 0.47 μM SE= 0.13



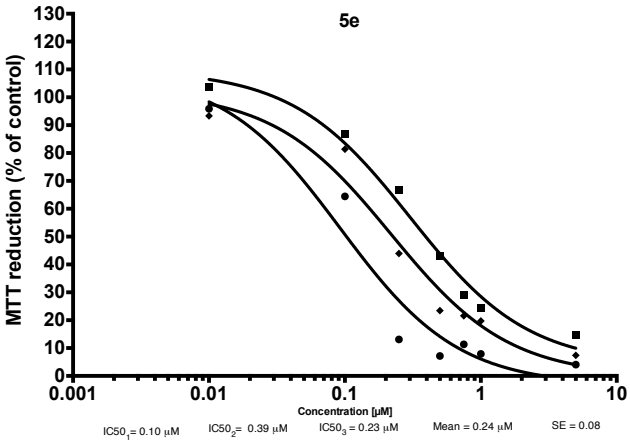
5d



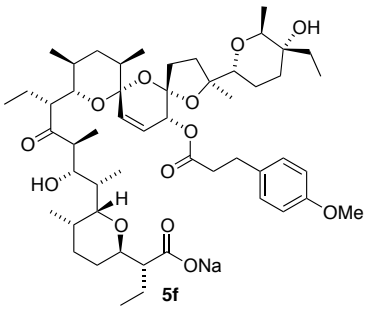
$IC50_1$= 0.25μM $IC50_2$= 0.35μM $IC50_3$= 0.30 μM Mean = 0.30 μM SE = 0.03



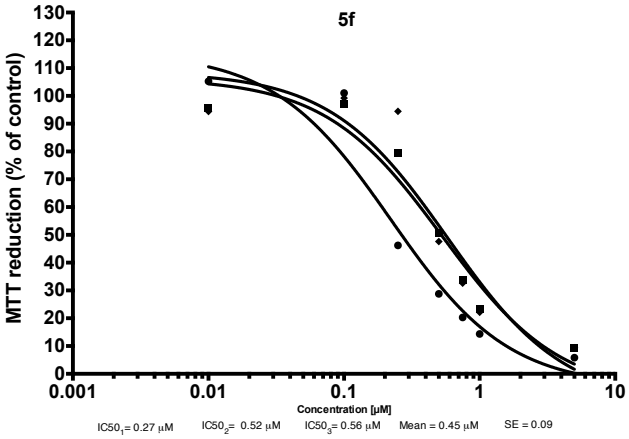
5e



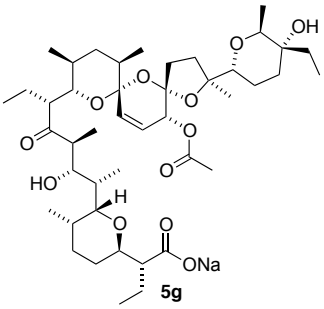
$IC50_1$= 0.10 μM $IC50_2$= 0.39 μM $IC50_3$ = 0.23 μM Mean = 0.24 μM SE = 0.08



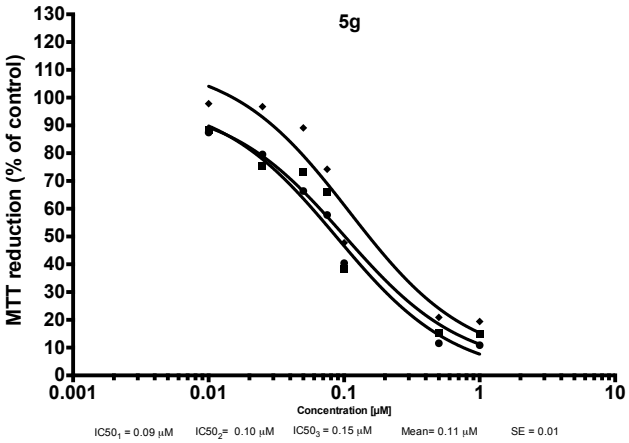
5f



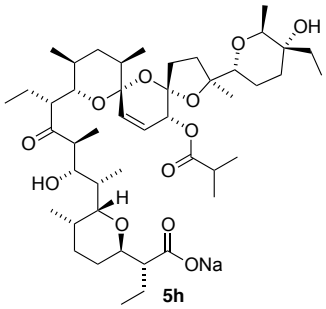
$IC50_1$ = 0.27 µM $IC50_2$ = 0.52 µM $IC50_3$ = 0.56 µM Mean = 0.45 µM SE = 0.09



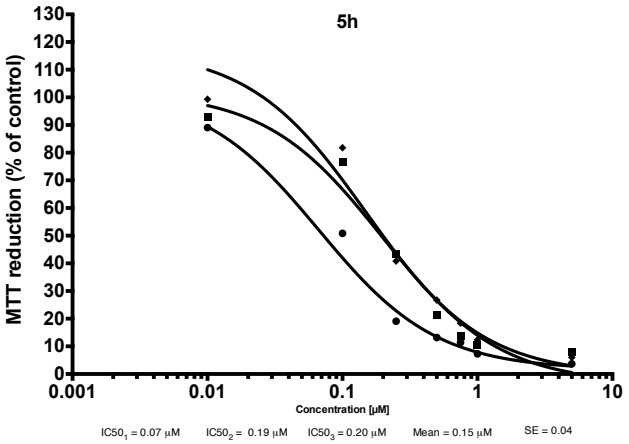
5g



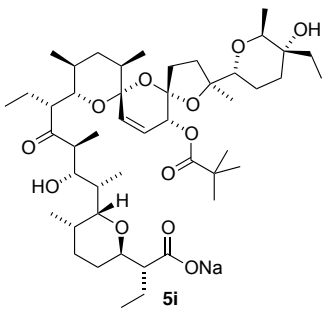
$IC50_1$ = 0.09 µM $IC50_2$ = 0.10 µM $IC50_3$ = 0.15 µM Mean= 0.11 µM SE = 0.01



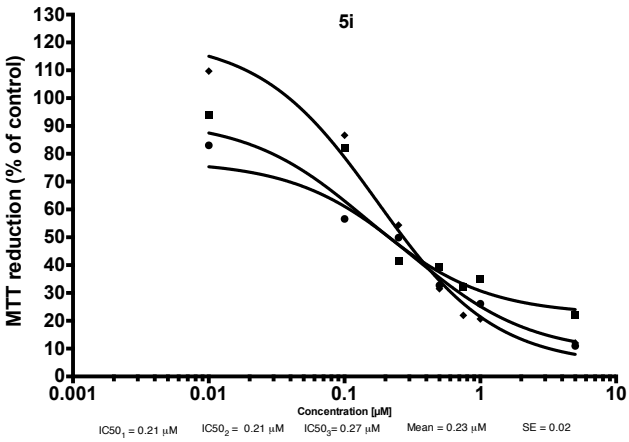
5h



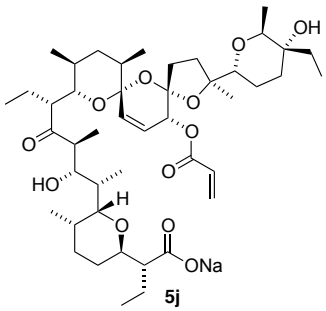
$IC50_1$ = 0.07 µM $IC50_2$ = 0.19 µM $IC50_3$ = 0.20 µM Mean = 0.15 µM SE = 0.04



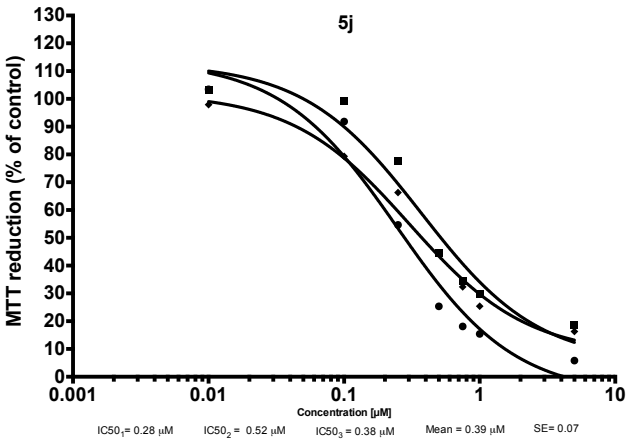
5i



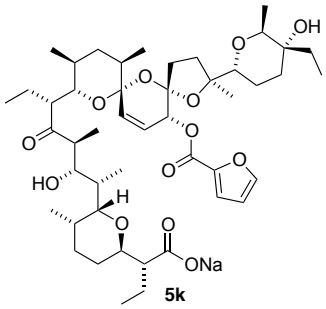
$IC50_1$ = 0.21 µM $IC50_2$ = 0.21 µM $IC50_3$ = 0.27 µM Mean = 0.23 µM SE = 0.02



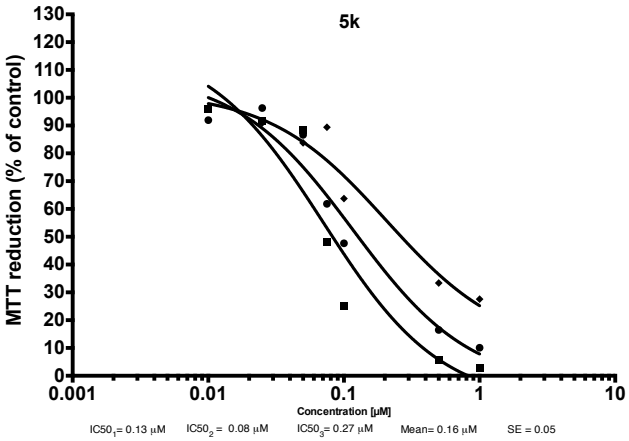
5j



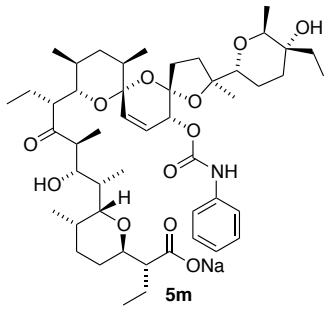
$IC50_1$= 0.28 μM $IC50_2$ = 0.52 μM $IC50_3$ = 0.38 μM Mean = 0.39 μM SE= 0.07



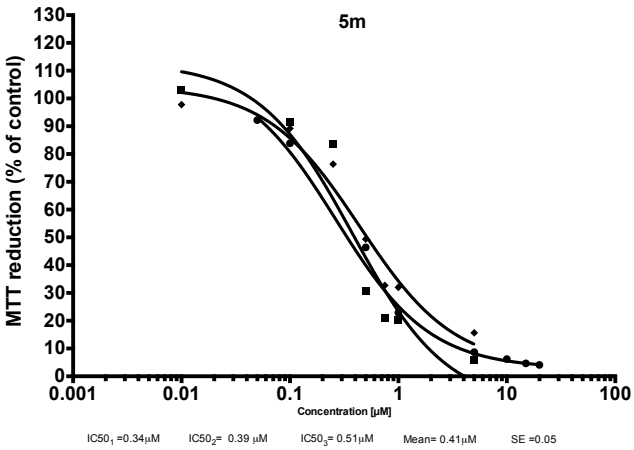
5k



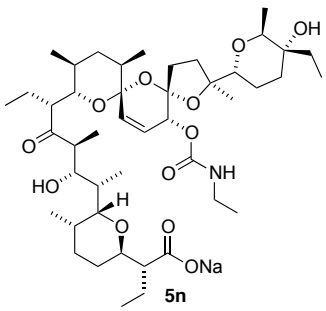
$IC50_1$= 0.13 μM $IC50_2$ = 0.08 μM $IC50_3$= 0.27 μM Mean= 0.16 μM SE = 0.05



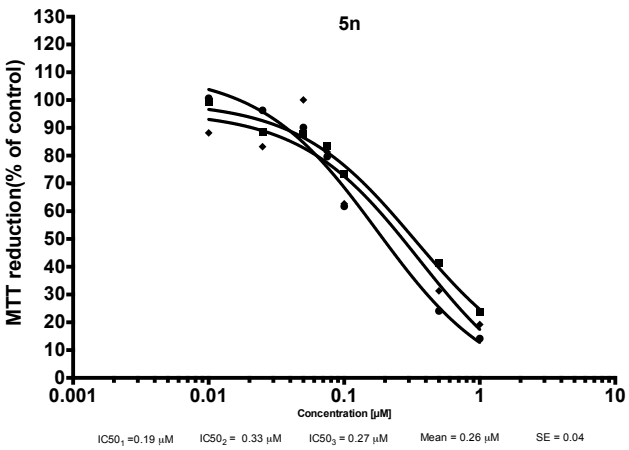
5m



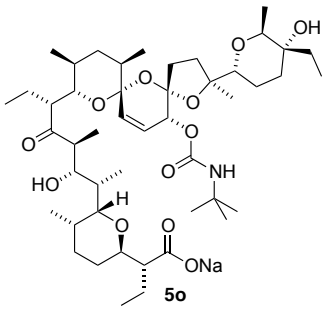
$IC50_1$ =0.34μM $IC50_2$= 0.39 μM $IC50_3$= 0.51μM Mean= 0.41μM SE =0.05



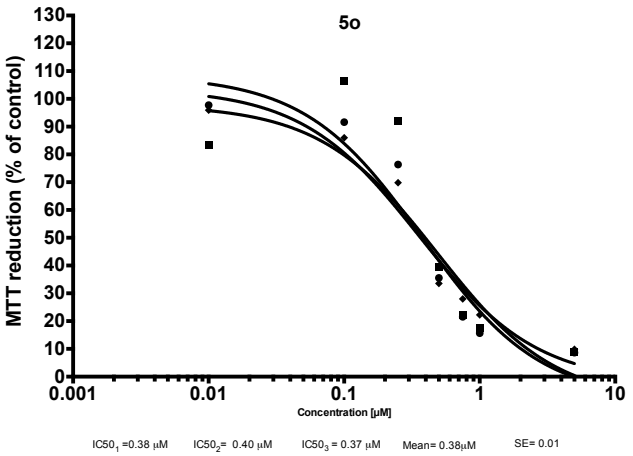
5n



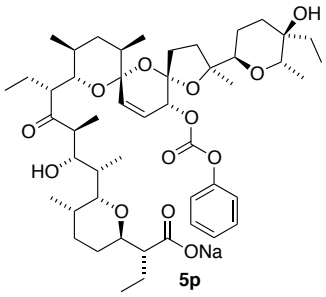
$IC50_1$ =0.19 μM $IC50_2$ = 0.33 μM $IC50_3$ = 0.27 μM Mean = 0.26 μM SE = 0.04



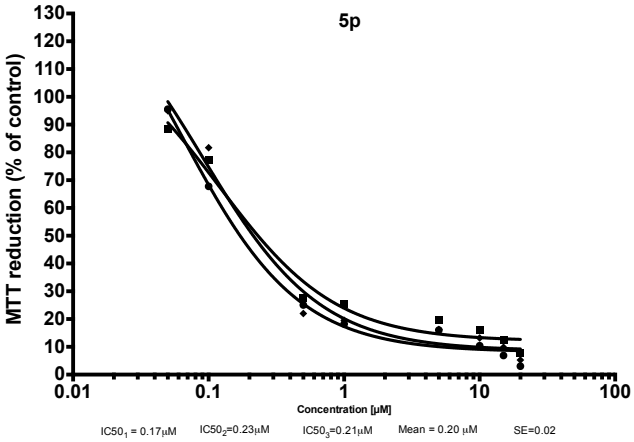
5o



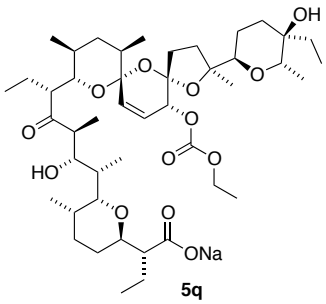
$IC50_1$ =0.38 µM $IC50_2$= 0.40 µM $IC50_3$ = 0.37 µM Mean= 0.38µM SE= 0.01



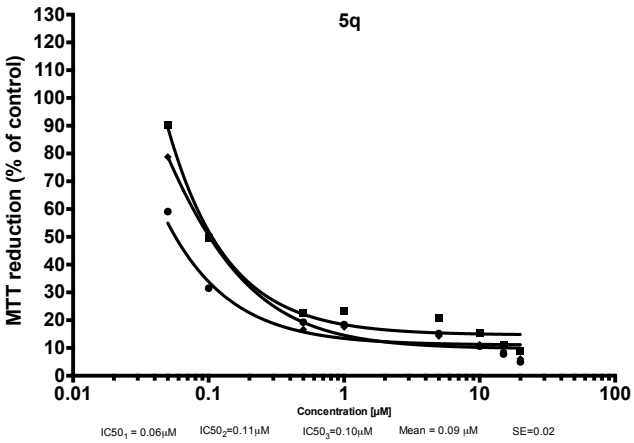
5p



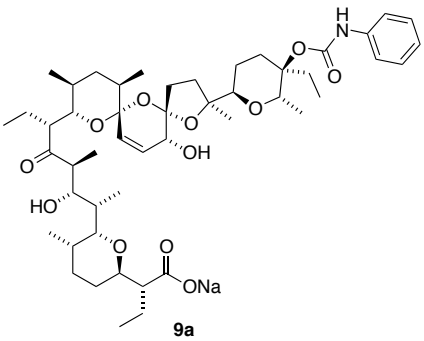
$IC50_1$ = 0.17µM $IC50_2$=0.23µM $IC50_3$=0.21µM Mean = 0.20 µM SE=0.02



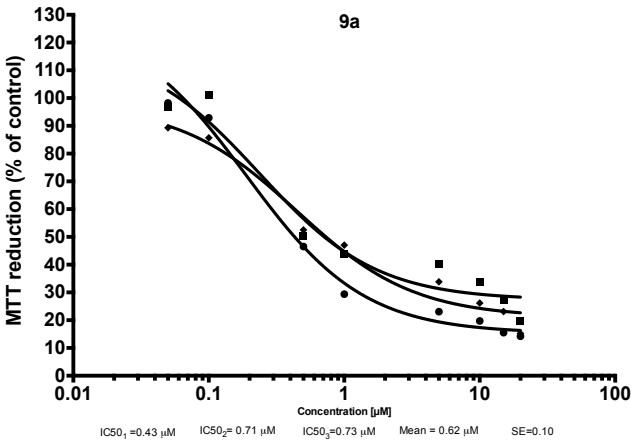
5q



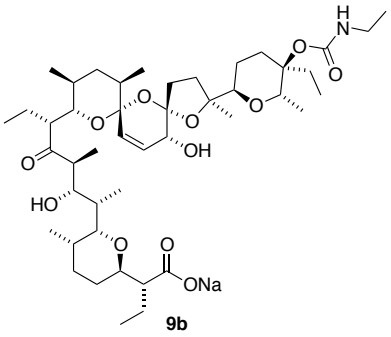
$IC50_1$ = 0.06µM $IC50_2$=0.11µM $IC50_3$=0.10µM Mean = 0.09 µM SE=0.02



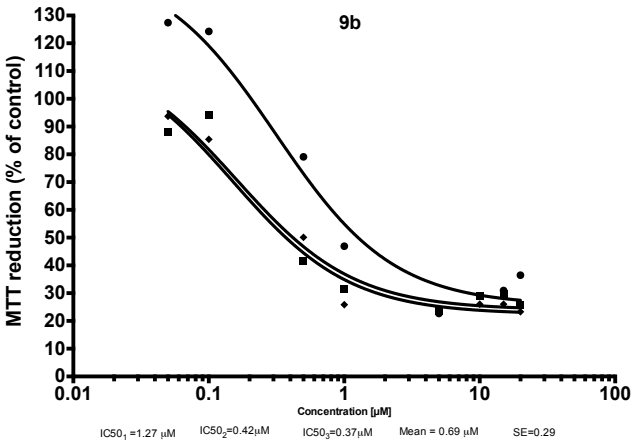
9a



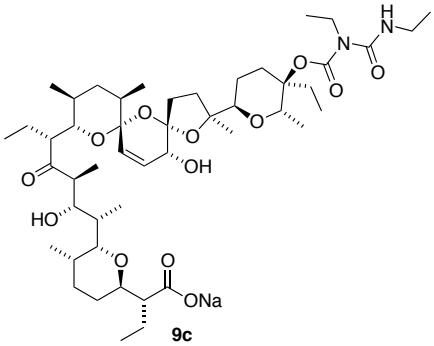
$IC50_1$ =0.43 µM $IC50_2$= 0.71 µM $IC50_3$=0.73 µM Mean = 0.62 µM SE=0.10



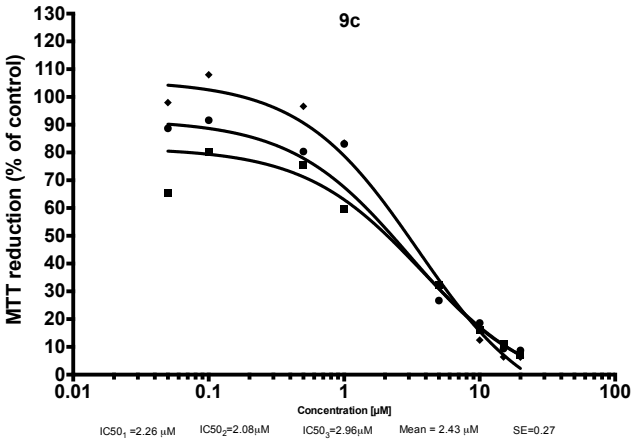
9b



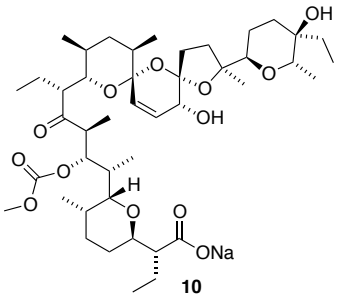
$IC50_1$ =1.27 μM $IC50_2$=0.42μM $IC50_3$=0.37μM Mean = 0.69 μM SE=0.29



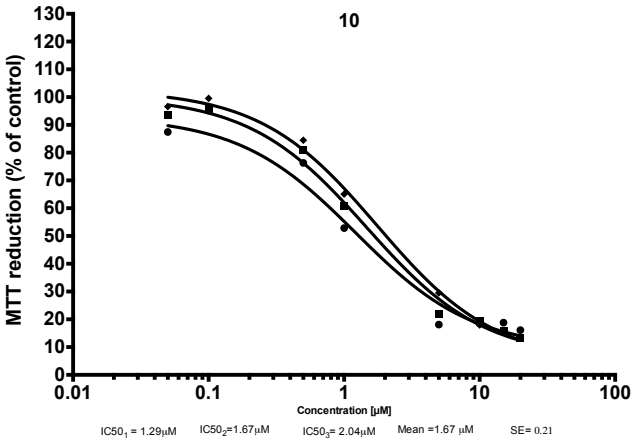
9c



$IC50_1$ =2.26 μM $IC50_2$=2.08μM $IC50_3$=2.96μM Mean = 2.43 μM SE=0.27

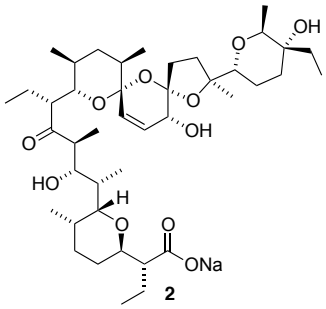


10

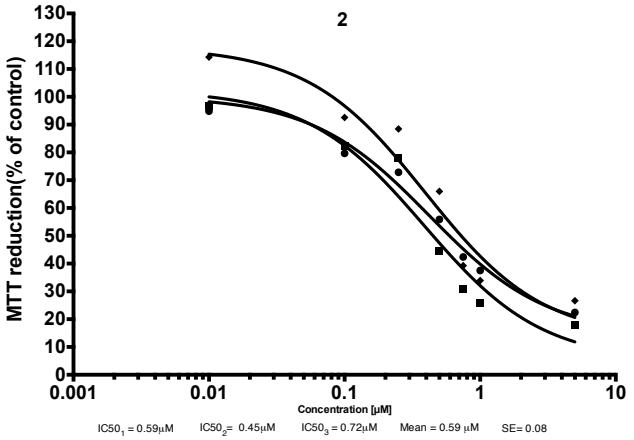


$IC50_1$ = 1.29μM $IC50_2$=1.67μM $IC50_3$= 2.04μM Mean =1.67 μM SE= 0.21

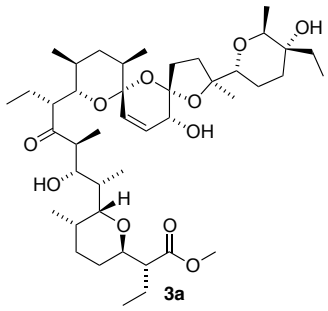
MTT dose-response curves in MCF-7 cells.



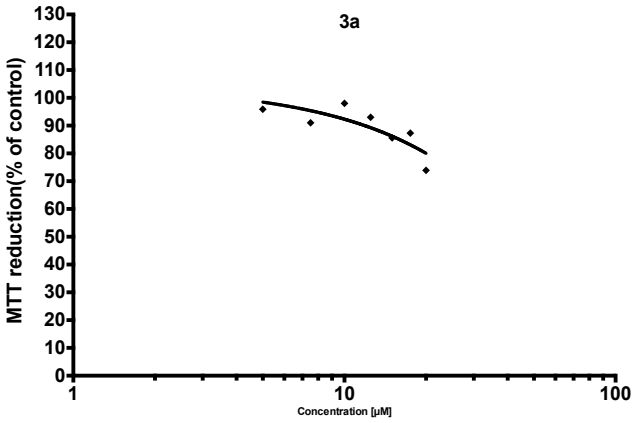
2

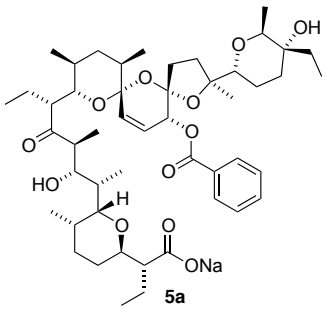


$IC50_1$ = 0.59µM $IC50_2$= 0.45µM $IC50_3$ = 0.72µM Mean = 0.59 µM SE= 0.08

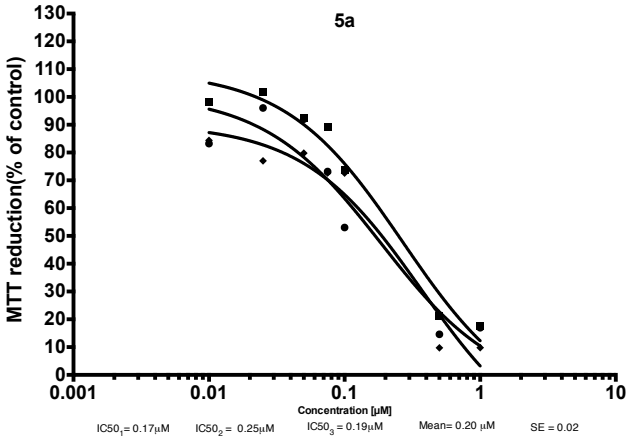


3a

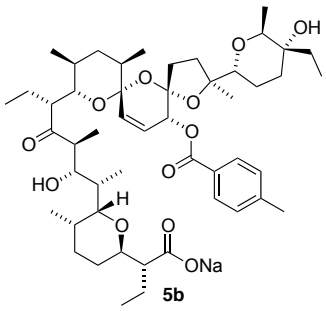




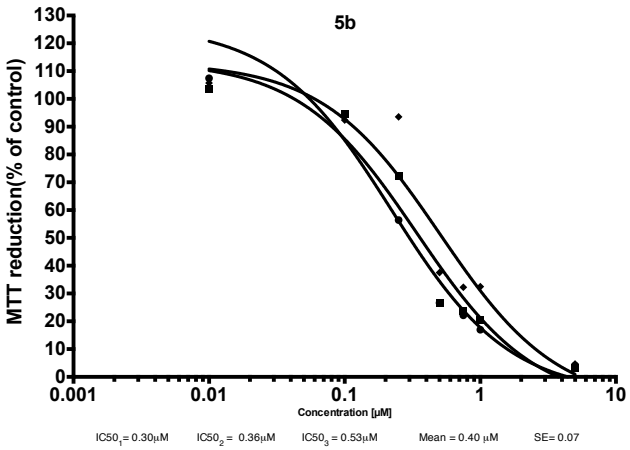
5a



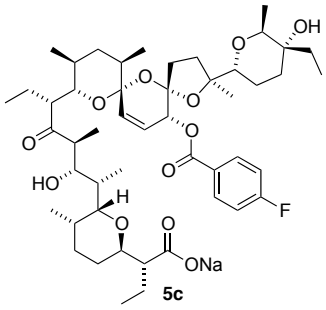
$IC50_1$= 0.17µM $IC50_2$ = 0.25µM $IC50_3$ = 0.19µM Mean= 0.20 µM SE = 0.02



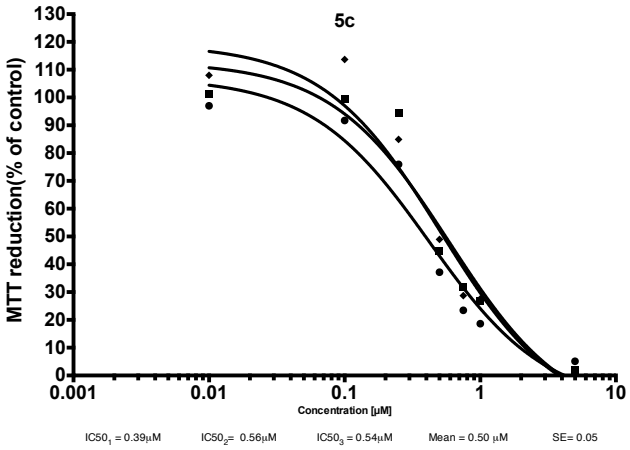
5b



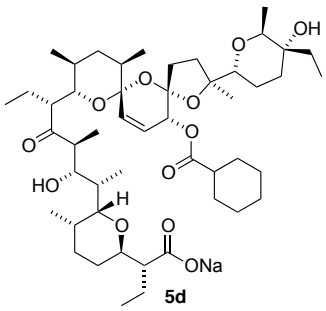
$IC50_1$ = 0.30μM $IC50_2$ = 0.36μM $IC50_3$ = 0.53μM Mean = 0.40 μM SE= 0.07



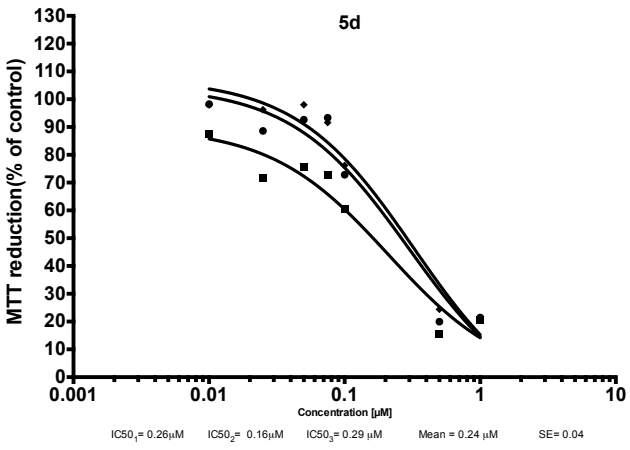
5c



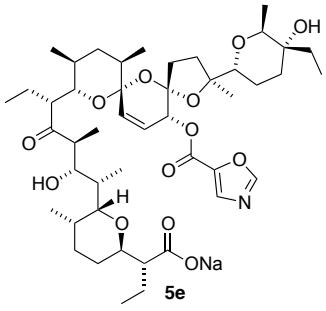
$IC50_1$ = 0.39μM $IC50_2$= 0.56μM $IC50_3$ = 0.54μM Mean = 0.50 μM SE= 0.05



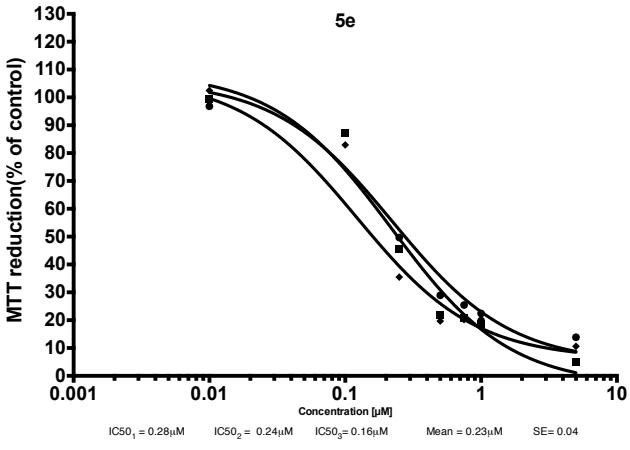
5d



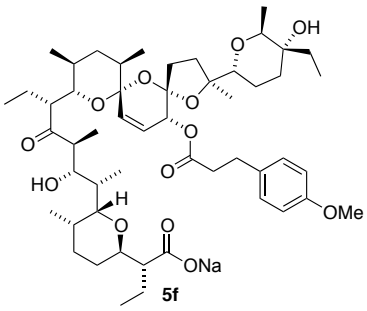
$IC50_1$= 0.26μM $IC50_2$= 0.16μM $IC50_3$= 0.29 μM Mean = 0.24 μM SE= 0.04



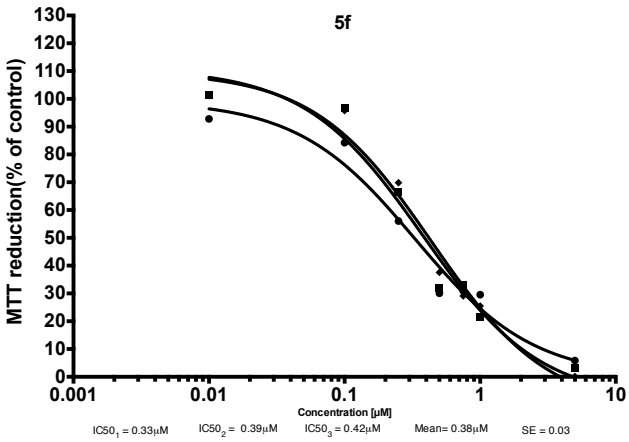
5e



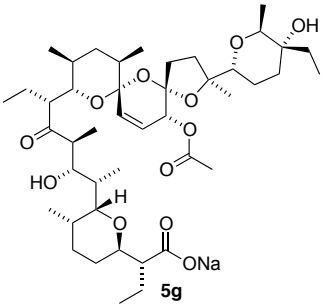
$IC50_1$ = 0.28μM $IC50_2$ = 0.24μM $IC50_3$= 0.16μM Mean = 0.23μM SE= 0.04



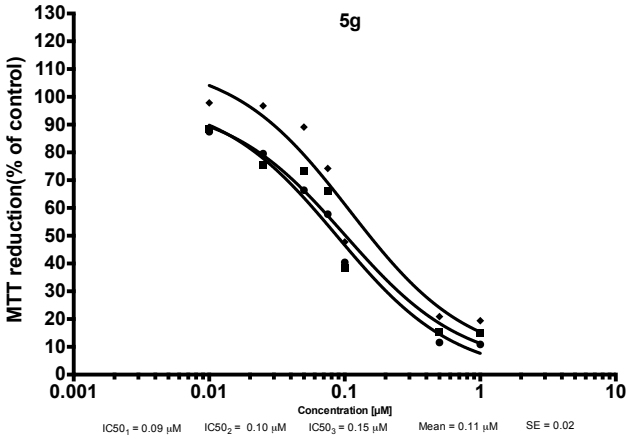
5f



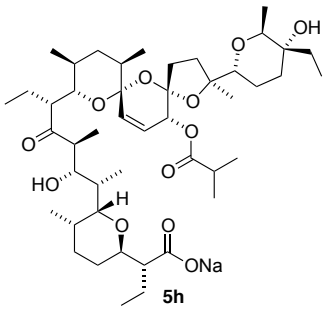
$IC50_1$ = 0.33μM $IC50_2$ = 0.39μM $IC50_3$ = 0.42μM Mean= 0.38μM SE = 0.03



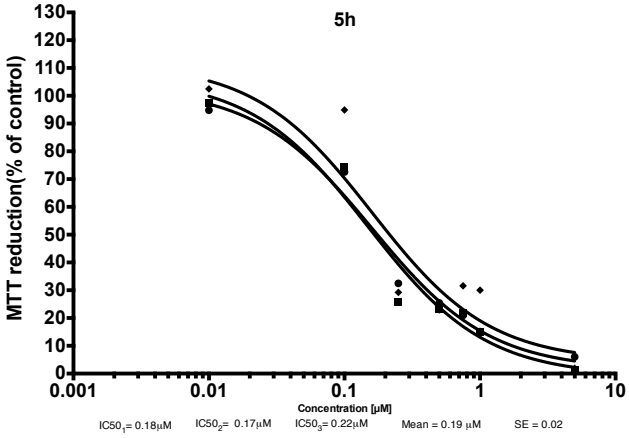
5g



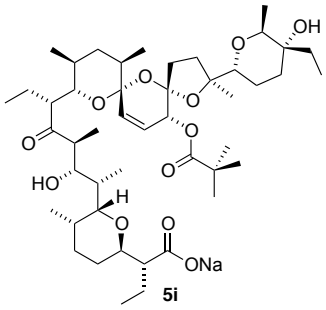
$IC50_1$ = 0.09 μM $IC50_2$ = 0.10 μM $IC50_3$ = 0.15 μM Mean = 0.11 μM SE = 0.02



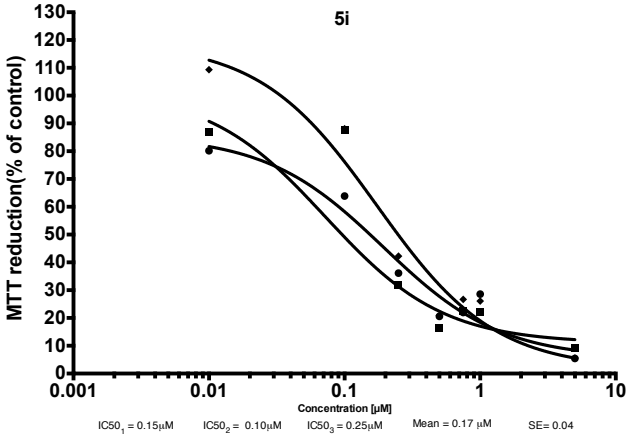
5h



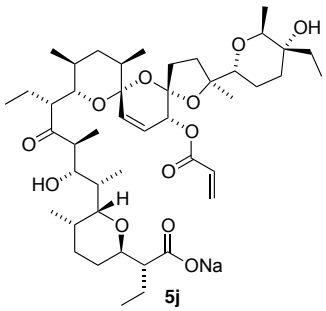
$IC50_1$= 0.18μM $IC50_2$= 0.17μM $IC50_3$= 0.22μM Mean = 0.19 μM SE = 0.02



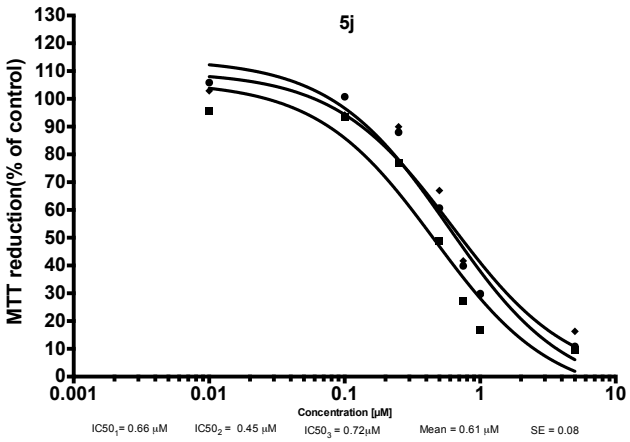
5i

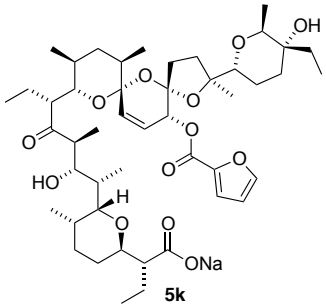


$IC50_1$ = 0.15μM $IC50_2$ = 0.10μM $IC50_3$ = 0.25μM Mean = 0.17 μM SE= 0.04

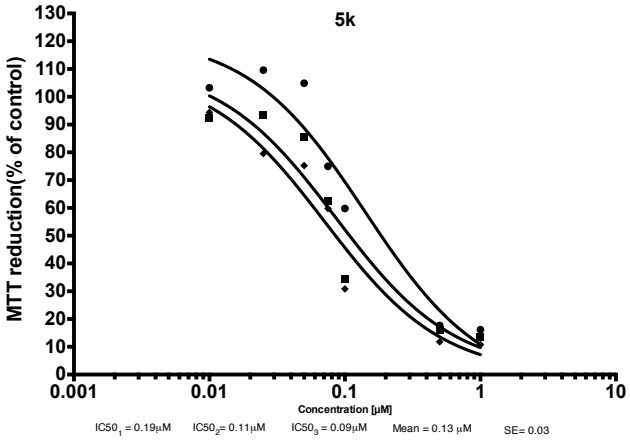

OH
O
HO
H
ONa

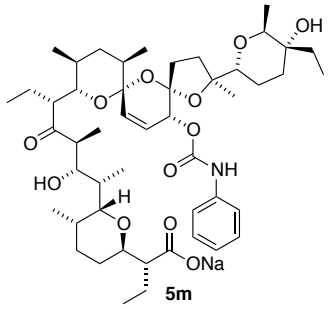
5j


5j
MTT reduction(% of control)
Concentration [μM]
IC50$_1$= 0.66 μM
IC50$_2$ = 0.45 μM
IC50$_3$ = 0.72μM
Mean = 0.61 μM
SE = 0.08

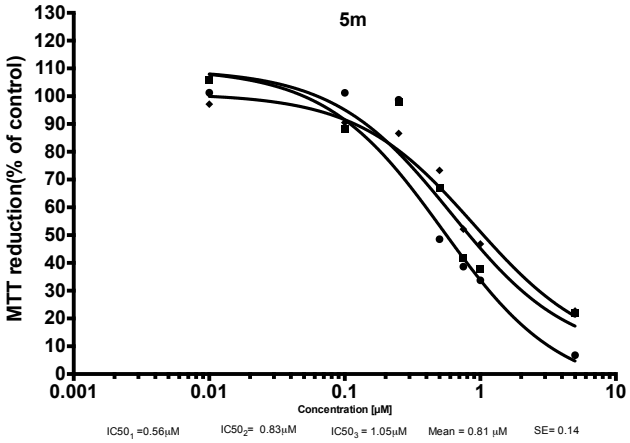

OH
O
HO
H
ONa

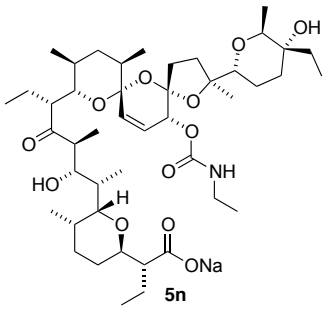
5k


5k
MTT reduction(% of control)
Concentration [μM]
IC50$_1$ = 0.19μM
IC50$_2$= 0.11μM
IC50$_3$ = 0.09μM
Mean = 0.13 μM
SE= 0.03

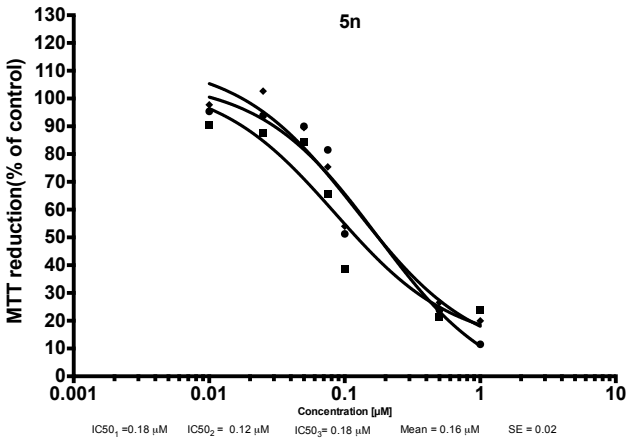

OH
O
NH
HO
H
ONa

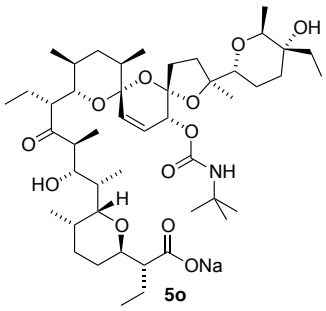
5m


5m
MTT reduction(% of control)
Concentration [μM]
IC50$_1$ =0.56μM
IC50$_2$= 0.83μM
IC50$_3$ = 1.05μM
Mean = 0.81 μM
SE= 0.14

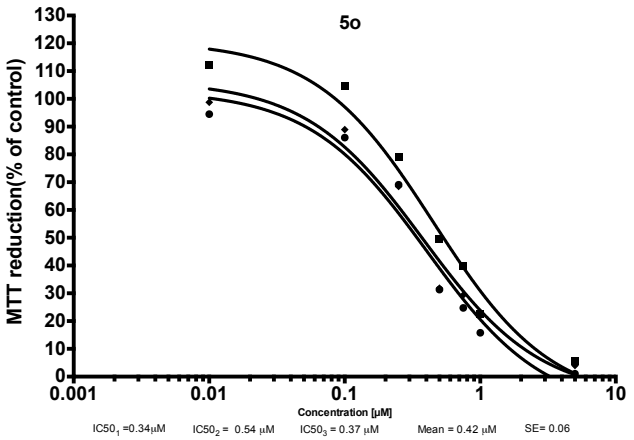

OH
O
NH
HO
H
ONa

5n

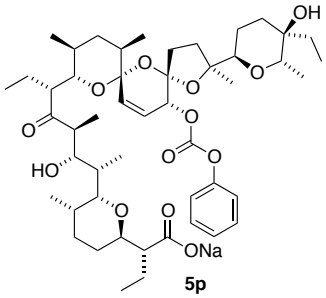

5n
MTT reduction(% of control)
Concentration [μM]
IC50$_1$ =0.18 μM
IC50$_2$ = 0.12 μM
IC50$_3$= 0.18 μM
Mean = 0.16 μM
SE = 0.02



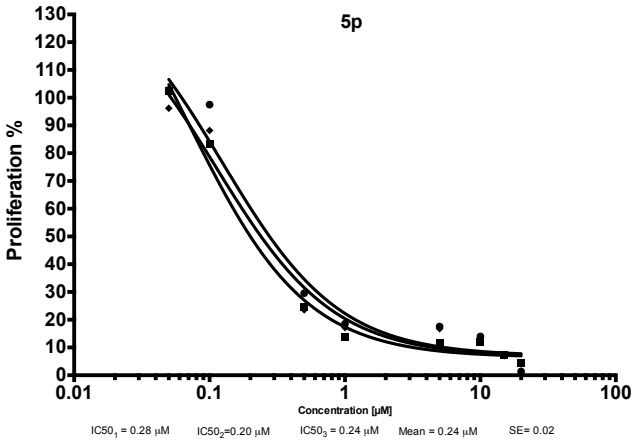
5o



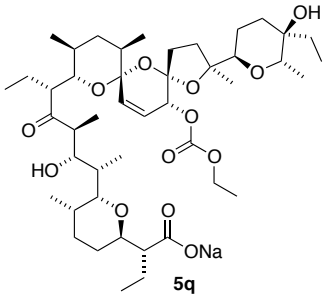
$IC50_1$ =0.34μM $IC50_2$ = 0.54 μM $IC50_3$ = 0.37 μM Mean = 0.42 μM SE= 0.06



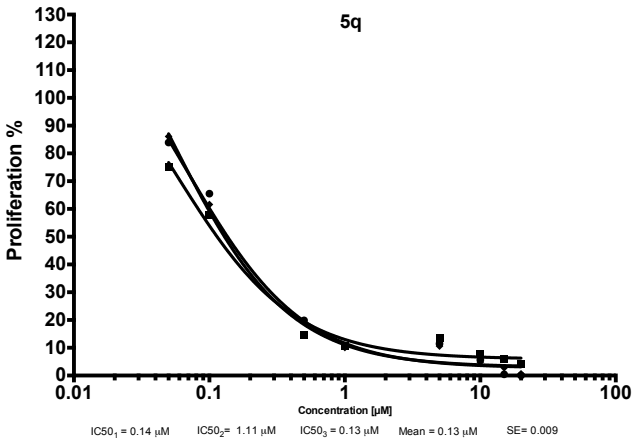
5p



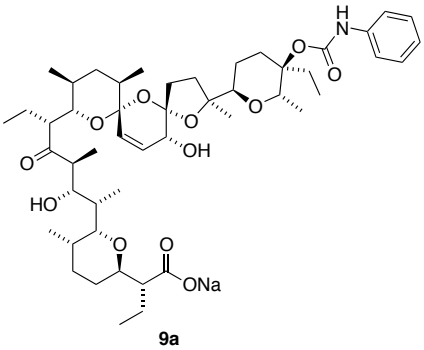
$IC50_1$ = 0.28 μM $IC50_2$=0.20 μM $IC50_3$ = 0.24 μM Mean = 0.24 μM SE= 0.02



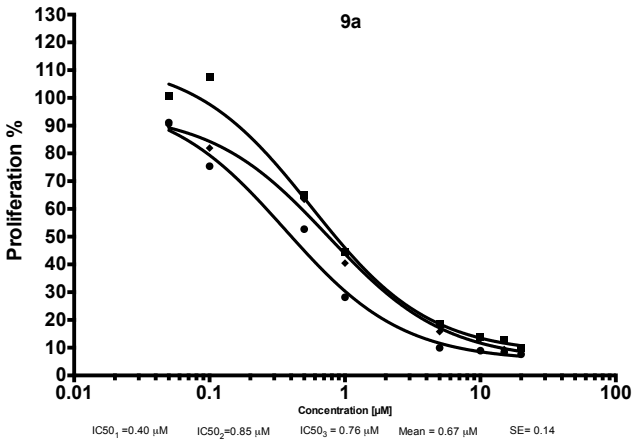
5q



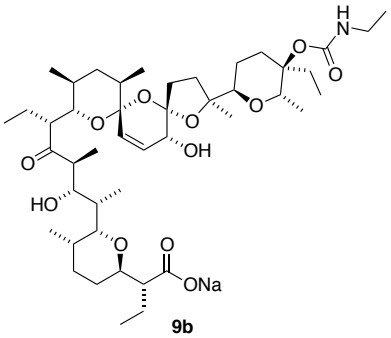
$IC50_1$ = 0.14 μM $IC50_2$= 1.11 μM $IC50_3$ = 0.13 μM Mean = 0.13 μM SE= 0.009



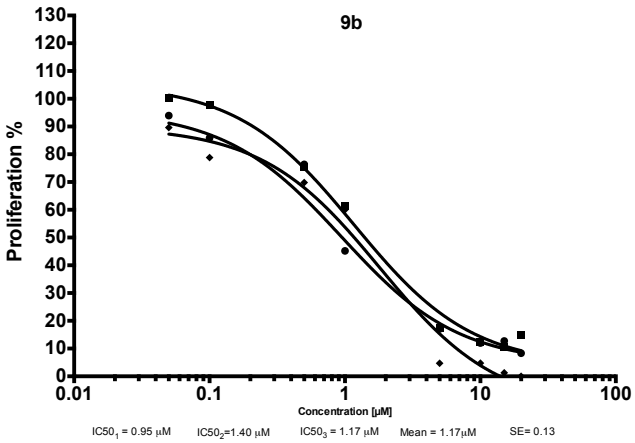
9a



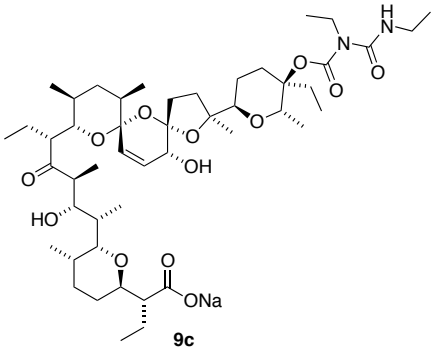
$IC50_1$ =0.40 μM $IC50_2$=0.85 μM $IC50_3$ = 0.76 μM Mean = 0.67 μM SE= 0.14



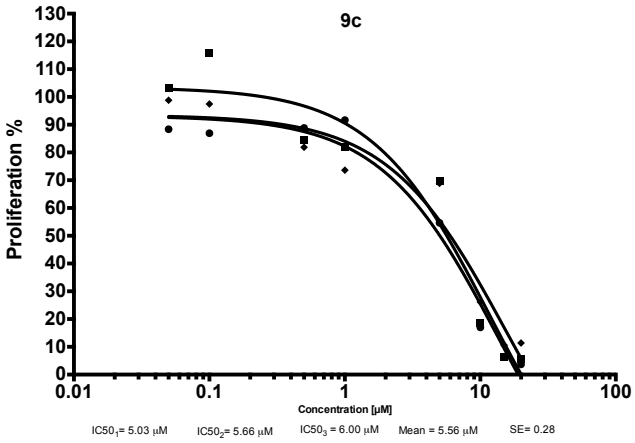
9b



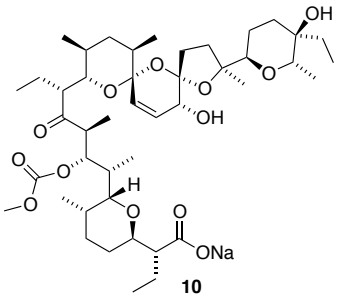
$IC50_1$ = 0.95 µM $IC50_2$=1.40 µM $IC50_3$ = 1.17 µM Mean = 1.17µM SE= 0.13



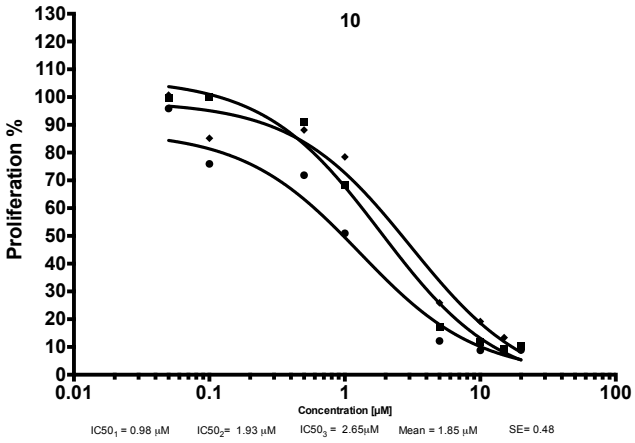
9c



$IC50_1$= 5.03 µM $IC50_2$= 5.66 µM $IC50_3$ = 6.00 µM Mean = 5.56 µM SE= 0.28



10



$IC50_1$ = 0.98 µM $IC50_2$= 1.93 µM $IC50_3$ = 2.65µM Mean = 1.85 µM SE= 0.48