Supporting Information

Antidiabetic Profiling of Veramycins, Polyketides Accessible by Biosynthesis, Chemical Synthesis, and Precursor-Directed Modification

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1. General Information – Experimental Section

NMR spectra were recorded in CD_3OD, $CDCl_3$ or $(CD_3)_2SO$ on Bruker Avance 700, 500, 400 or 300 MHz spectrometers. The 700 and 500 MHz spectrometers were equipped with a 5 mm TXI cryoprobe. COSY, HSQC, HMBC, and ROESY experiments were recorded using standard pulse programs. Chemical shifts were referenced to the solvent signals. $CDCl_3$: ^1H: 7.27 ppm, ^{13}C: 77.0 ppm; CD_3OD:

^{1}H: 3.30 ppm, ^{13}C: 49.0 ppm; $(CD_3)_2SO$: ^{1}H: 2.50 ppm, ^{13}C: 39.5 ppm. UPLC-HR-MS data were obtained on a quadrupole time of flight spectrometer (LC-QTOF maXis II, Bruker Daltronics, Bremen, Germany) using a BEH C18 (150 x 2.1 mm, 1.7 µm column, Waters, Germany) with a linear gradient of 5-95% MeCN in H_2O + 0.1% TFA at 450 µL/min in 18 min with UV detection in 205-640 nm range. Mass spectra were acquired using the ESI source in the range from 50–2000 *m/z*. HPLC for separation and purification was performed on a semi-preparative Agilent 1200 HPLC system equipped with a Synergi Fusion C18 column (250 x 25 mm, 4 µm, DAD at 220 and 254 nm). All solvents used for separation and purification were analytical grade. All chemicals and solvents/anhydrous solvents for synthesis were commercially supplied and used without further purification. A heating mantle was used as the heat source when reactions required heating. Reactions were monitored using analytical Agilent 1200 HPLC systems or by TLC.

2. Cultivation, Isolation and Structure Elucidation

Bacterial Strain: *Streptomyces sp*. ST157608 was obtained from the strain collection of Sanofi-Aventis Deutschland GmbH, Industriepark Höchst, Germany.
Cultivation and Extraction: Pre-cultures of *Streptomyces* sp. ST157608 were conducted in 0.3 L Erlenmeyer flasks with 0.1 L of pre-culture using a medium consisting of $CaCO_3$ (0.2%), solid corn steep (0.5%), glucose (1.5%), soybean meal (0.15%), and NaCl (0.5%) with an initial pH value of 7.0. Incubation was performed for 4-7 days on a rotary shaker at 180 rpm and 28 °C. Inoculation of the main fermentation with the pre-cultures was conducted in forty 2 L Erlenmeyer flasks, each containing 0.5 L of the main-culture medium consisting of glycerol (3%), $CaCO_3$ (0.5%), soybean meal (0.15%), and NaCl (0.2%) with an initial pH value of 7.2. Incubation was performed on a rotary shaker at 180 rpm and 28 °C for 7 days. Afterwards, cells and supernatants were separated by centrifugation followed by addition of a mixture of 2% Amberlite® XAD 16 and XAD 7 (1:1) to the supernatant and stirring overnight. Finally, the resin was harvested, washed with H_2O, lyophilized, extracted with MeOH, and dried under reduced pressure to yield 5 g of crude extract.
Isolation: The extracts prepared from liquid and solid fermentations were purified by semi-preparative reversed-phase HPLC eluting with a linear gradient of 45-70% MeCN/H_2O with 0.05% TFA in 30 min. The following yields were obtained: compounds **17** (1.8 mg, t_R = 10.2 min), **13** (4.4 mg, t_R = 8.2 min), **18** (0.3 mg, t_R = 12.5 min), **19** (0.1 mg, t_R = 19.6 min), **20** and **21** (1.0 mg, t_R = 15.4 min), **1** (1.2 mg, t_R = 9.3 min), **23** (0.3 mg, t_R = 13.1 min), and **22** (0.4 mg, t_R = 14.6 min).

*Veramycin A (**17**)*. colorless, amorphous powder; LC-UV [(MeCN in H_2O+0.05% TFA)] λ_{max} 221, 298 nm; NMR data see tables SI-1 and SI-2; HRMS (ESI-TOF) *m/z*: $[M+Na]^+$ calcd for $C_{22}H_{28}O_5Na$ 395.1828; found 395.1813.

*TM-123 (Veramycin B) (**13**)*. colorless, amorphous powder; LC-UV [(MeCN in H_2O+0.05% TFA)] λ_{max} 221, 298 nm; NMR data see table SI-2; HRMS (ESI-TOF) *m/z*: $[M+Na]^+$ calcd for $C_{21}H_{26}O_5Na$ 381.1672; found 381.1672.

*Veramycin C (**18**)*. colorless, amorphous powder; LC-UV [(MeCN in H_2O+0.05% TFA)] λ_{max} 222, 296 nm; NMR data see table SI-3; HRMS (ESI-TOF) *m/z*: $[M+Na]^+$ calcd for $C_{20}H_{22}O_5Na$ 365.1359; found 365.1358.

*Veramycin D (**19**)*. colorless, amorphous powder; LC-UV [(MeCN in H_2O+0.05% TFA)] λ_{max} 221, 296 nm; NMR data see table SI-4; HRMS (ESI-TOF) *m/z*: $[M+Na]^+$ calcd for $C_{22}H_{26}O_4Na$ 377.1723; found 377.1722.

*cis-Veramycin E (**20**)*. colorless, amorphous powder; LC-UV [(MeCN in H_2O+0.05% TFA)] λ_{max} 221, 294 nm; NMR data see table SI-5; HRMS (ESI-TOF) *m/z*: $[M+Na]^+$ calcd for $C_{21}H_{26}O_4Na$ 365.1723; found 365.1738.

*trans-Veramycin E (**21**)*. colorless, amorphous powder; LC-UV [(MeCN in H_2O+0.05% TFA)] λmax 222 nm; NMR data see table SI-6. NMR spectroscopic data; HRMS (ESI-TOF) *m/z*: [M+Na]+ calcd for $C_{21}H_{26}O_4Na$ 365.1723; found 365.1724.

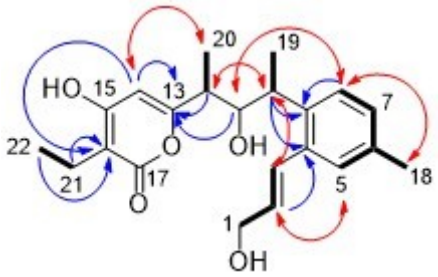
*NFAT-133 (**1**)*. colorless, amorphous powder; LC-UV [(MeCN in H_2O+0.05% TFA)] λ_{max} 221 nm; NMR data see section 8.2.2.17; HRMS (ESI-TOF) *m/z*: $[M+Na]^+$ calcd for $C_{17}H_{24}O_3Na$ 299.1617; found 299.1619.

*Veramycin F (**22**)*. colorless, amorphous powder; LC-UV [(MeCN in H_2O+0.05% TFA)] λ_{max} 222 nm; NMR data see section 8.2.2.17; HRMS (ESI-TOF) *m/z*: $[M+Na]^+$ calcd for $C_{15}H_{18}O_3Na$ 269.1148; found 269.1148.

*Veramycin G (**23**)*. colorless, amorphous powder; LC-UV [(MeCN in H_2O+0.05% TFA)] λ_{max} 222 nm; NMR data see section 8.2.2.17; HRMS (ESI-TOF) *m/z*: $[M+Na]^+$ calcd for $C_{17}H_{20}O_3Na$ 295.1304; found 295.1309.

NMR data:

Table S1: spectroscopic data (700 MHz, CD_3OD) for Veramycin A (**17**).

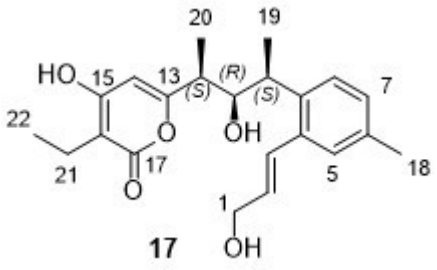


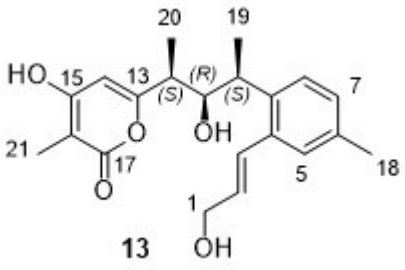
	δ_C[a]	δ_H[b] (*J* in Hz)	HMBC[c]	ROESY[d]
1	63.8	4.23 ddd (5.6, 2.7, 1.6)	2, 3	-
2	132.8	6.13 dt (15.6, 5.6)	1, 3, 4	18
3	129.5	6.86 dt (15.6, 1.6)	1, 2, 4, 5, 6, 9	10, 11, 19, 20
4	136.9	-	-	-
5	128.5	7.20 d (1.6)	3, 9, 18	2, 18
6	136.9	-	-	-
7	129.6	7.02 dd (7.9, 1.6)	8, 9, 18	14, 18
8	128.3	7.13 d (7.9)	4, 6, 7, 10	10, 11, 12, 19
9	140.2	-	-	-
10	39.8	3.07 dq (7.8, 6.9)	4, 8, 9, 11, 12, 19	3, 8, 12, 20
11	77.1	3.99 dd (7.8, 5.6)	9, 10, 12, 13, 19, 20	3, 8, 12, 19, 20
12	43.1	2.46 dq (7.0, 5.6)	11, 13, 14, 20	8, 10, 11, 14
13	167.2	-	-	-
14	101.3	5.88 s	11, 12, 13, 15, 16, 21	10, 11, 12, 19, 20, 21

15	167.6	-	-	-
16	105.3	-	-	-
17	168.6	-	-	-
18	21.1	2.26 s	6, 7	5, 7
19	18.5	1.27 d (6.9)	9, 10, 11	8, 11, 12
20	13.0	1.12 d (7.0)	11, 12, 13	10, 11, 14
21	17.3	2.36 q (7.4)	15, 16, 17, 22	-
22	12.8	1.02 t (7.4)	16, 21	-

[a]Recorded at 175 MHz; referenced to residual CD_3OD at δ 49.2 ppm. [b]Recorded at 700 MHz; referenced to residual CD_3OD at δ 3.31 ppm. [c]Proton showing HMBC correlation to indicated carbon. [d]Proton showing ROESY correlation to indicated proton.

Table S2: Comparison of the chemical shifts and the $^3J_{H\text{-}H}$ coupling constants of **17** and **13**.



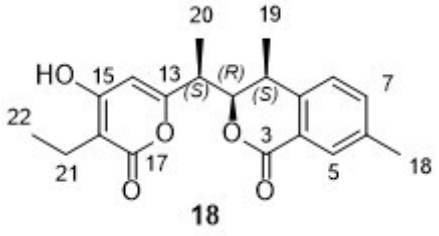


	δ_H[a] (*J* in Hz) of **17**	δ_H[a] (*J* in Hz) of **13**
1	4.23 ddd (5.6, 2.7, 1.6)	4.23 dt (5.6, 1.6)
2	6.13 dt (15.6, 5.6)	6.13 dt (15.6, 5.6)
3	6.86 dt (15.6, 1.6)	6.85 dt (15.6, 1.5)
4	-	-
5	7.20 d (1.6)	7.20 d (1.6)
6	-	-
7	7.02 dd (7.9, 1.6)	7.02 dd (8.0, 1.6)
8	7.13 d (7.9)	7.13 d (8.0)
9	-	-
10	3.07 dq (7.8, 6.9)	3.08 dq (7.7, 6.9)
11	3.99 dd (7.8, 5.6)	3.99 dd (7.7, 5.7)
12	2.46 dq (7.0, 5.6)	2.47 dq (7.0, 5.7)
13	-	-
14	5.88 s	5.91 s

15	-	-
16	-	-
17	-	-
18	2.26 s	2.27 s
19	1.27 d (6.9)	1.28 d (6.9)
20	1.12 d (7.0)	1.13 d (7.0)
21	2.36 q (7.4)	1.83 s
22	1.02 t (7.4)	-

[a]Recorded at 700 MHz; referenced to residual CD_3OD at δ 3.31 ppm.

Table S3: NMR spectroscopic data (700 MHz, CD_3OD) for Veramycin C (**18**).



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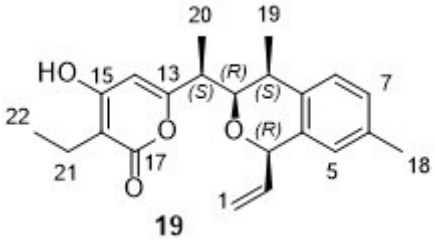
	δ_C[a]	δ_H[b] (*J* in Hz)	HMBC[c]	ROESY[d]
1	-	-	-	-
2	-	-	-	-
3	167.5	-	-	-
4	124.5	-	-	-
5	131.1	7.82 dq (1.9, 0.7)	3, 7, 9, 18	18
6	139.0	-	-	-
7	136.5	7.43 ddq (7.8, 1.9, 0.7)	5, 9, 18	11, 18
8	128.2	7.23 d (7.8)	3, 4, 5, 6, 7, 10	10, 19
9	145.0	-	-	-
10	35.6	2.83 dq (7.1, 2.5)	4, 8, 9, 19	8, 11
11	83.3	4.63 dd (10.4, 2.5)	9, 12, 19, 20	10, 14, 20
12	41.5	3.11 dq (10.4, 6.8)	10, 11, 13, 14, 20	11, 14, 19
13	163.7	-	-	-
14	102.6	6.22 s	12, 13, 15, 16, 21	11, 12, 19, 20
15	167.0	-	-	-
16	106.2	-	-	-

17	168.0	-	-	-
18	20.9	2.38 t (0.7)	5, 6, 7, 9, 19	5, 7
19	15.1	1.20 d (7.1)	9, 10	12, 14
20	16.5	1.47 d (6.8)	11, 12, 13	11, 14
21	17.4	2.43 q (7.4)	15, 16, 22	-
22	12.7	1.06 t (7.4)	16, 21	5

[a]Recorded at 175 MHz; referenced to residual CD_3OD at δ 49.2 ppm. [b]Recorded at 700 MHz; referenced to residual CD_3OD at δ 3.31 ppm. [c]Proton showing HMBC correlation to indicated carbon. [d]Proton showing ROESY correlation to indicated proton.

Table S4: NMR spectroscopic data (500 MHz, CD_3OD) for Veramycin D (**19**).



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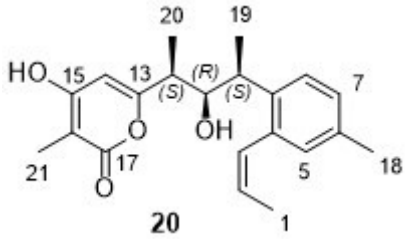
	δ_C[a]	δ_H[b] (*J* in Hz)	HMBC[c]	ROESY[d]
1	118.2	5.30 ddd (10.2, 1.8, 0.6)	3	-
		5.47 ddd (17.1, 1.8, 1.0)	3	
2	101.2	5.88 ddd (17.1, 10.2, 7.7)	4	10
3	82.0	5.16 d (7.7)	1, 6, 9	5, 11
4	167.3	-	-	-
5	127.0	6.85 br. S	9, 18	3, 18
6	136.9[e]	-	-	-
7	128.8	6.95 dd (7.7, 1.7)	6, 9, 10, 18	10, 18, 19
8	129.7	6.94 d (7.8)	6, 9, 10, 18	10, 18, 19
9	138.6[e]	-	-	-
10	35.8	2.50 qd (6.9, 2.2)	20	11
11	79.9	3.81 dd (10.2, 2.2)	19	3, 10, 20
12	42.1	2.81 dq (10.2, 6.8)	11, 14, 20	14, 19
13	165.6[e]	-	-	-
14	102.2	6.13 s	12, 13, 15, 16	11, 12, 19
15	167.5[e]	-	-	-
16	105.7[e]	-	-	-

17	168.5[e]	-	-	-
18	21.1	2.27 br. S	5, 6, 7, 8	-
19	17.5	1.18 d (6.9)	9, 10, 11	2
20	17.1	1.40 d (6.8)	11, 12, 13	11
21	17.4	2.39 q (7.4)	15, 16, 17, 22	-
22	12.8	1.04 t (7.4)	16	-

[a]Recorded at 125 MHz; referenced to residual CD_3OD at δ 49.2 ppm. [b]Recorded at 500 MHz; referenced to residual CD_3OD at δ 3.31 ppm. [c]Proton showing HMBC correlation to indicated carbon. [d]Proton showing ROESY correlation to indicated proton.

Table S5: NMR spectroscopic data (500 MHz, CD_3OD) for *cis*-Veramycin E (**20**).



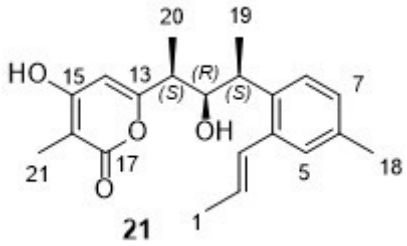
20

	δ_C[a]	δ_H[b] (*J* in Hz)	HMBC[c]	ROESY[d]
1	14.5	1.62 dd (7.0, 1.8)	2, 3, 5, 9	3, 5, 20
2	128.3	5.77 dq (11.2, 7.0)	1, 4, 5	5, 10, 20, 21
3	130.3	6.45 dq (11.2, 1.8)	5, 9	10, 11, 19, 20
4	137.0	-	-	-
5	131.3	6.90 dq (2.0, 0.6)	3, 7, 9, 18	1, 3, 18
6	136.2	-	-	-
7	129.0	7.03 ddq (8.0, 2.0, 0.6)	5, 8, 9, 18	18
8	128.0	7.18 d (8.0)	3, 4, 5, 6, 7, 9, 10	10, 11, 12, 19
9	141.1	-	-	-
10	40.0	2.96 q (7.0)	4, 8, 9, 11, 12, 19	3, 8, 12, 20
11	77.3	3.93 dd (7.0, 5.4)	9, 10, 12, 13, 19, 20	8, 12, 19, 20
12	42.7	2.47 qd (7.0, 5.4)	11, 13, 14, 20	8, 10, 11, 14, 19
13	167.1	-	-	-
14	101.1	5.95 s	11, 12, 13, 15, 16, 21	10, 11, 12, 19, 20
15	167.7	-	-	-
16	99.1	-	-	-
17	168.9	-	-	-

18	21.0	2.27 t (0.6)	5, 6, 7, 9, 10, 11, 19	1, 10, 21
19	17.7	1.23 d (7.0)	9, 10, 11	7, 8, 11, 12, 14
20	12.4	1.11 d (7.0)	11, 12, 13	1, 10, 11
21	8.2	1.84 s	14, 15, 16, 17	14

[a]Recorded at 125 MHz; referenced to residual CD_3OD at δ 49.2 ppm. [b]Recorded at 500 MHz; referenced to residual CD_3OD at δ 3.31 ppm. [c]Proton showing HMBC correlation to indicated carbon. [d]Proton showing ROESY correlation to indicated proton.

Table S6: NMR spectroscopic data (700 MHz, CD_3OD) for *trans*-Veramycin E (**21**).



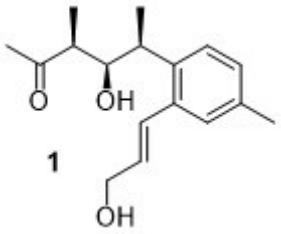
21

	δ_C[a]	δ_H[b] (*J* in Hz)	HMBC[c]	ROESY[d]
1	18.9	1.85 dd (6.5, 1.7)	2, 3, 4	-
2	129.3	5.99 dq (15.4, 6.5)	1, 4	8
3	130.2	6.59 dq (15.4, 1.7)	1, 4, 8, 9	10
4	137.8	-	-	-
5	131.4	6.91 d (1.5)	3, 7, 9, 18	-
6	136.7	-	-	-
7	128.9	6.98 dd (8.0, 1.5)	4, 6, 8, 9, 18	11, 18
8	128.3	7.11 d (8.0)	2, 3, 4, 6, 7, 9, 10, 18	2, 3, 11, 18, 19
9	139.7	-	-	-
10	39.3	3.01 q (7.0)	4, 8, 9, 11, 12, 19	3, 14, 20
11	77.3	3.93 dd (7.0, 5.5)	9, 10, 12, 13, 19, 20	8, 14, 19, 20
12	42.9	2.48 qd (7.0, 5.5)	11, 13, 20	8, 10, 11, 14, 19
13	167.1	-	-	-
14	101.0	5.92 s	12, 13, 15, 16, 21	10, 11, 12, 19, 20
15	167.7	-	-	-
16	99.1	-	-	-
17	168.9	-	-	-
18	21.2	2.25 s	6, 7, 8, 9	7, 8
19	18.1	1.26 d (7.0)	9, 10, 11	8, 11, 12

20	12.9	1.13 d (7.0)	11, 12, 13	10, 11, 14
21	8.3	1.83 s	14, 15, 16, 17	14

[a]Recorded at 175 MHz; referenced to residual CD_3OD at δ 49.2 ppm. [b]Recorded at 700 MHz; referenced to residual CD_3OD at δ 3.31 ppm. [c]Proton showing HMBC correlation to indicated carbon. [d]Proton showing ROESY correlation to indicated proton.

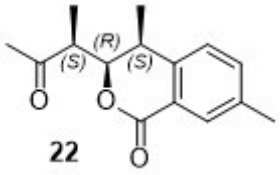
Table S7: NMR spectroscopic data (500 MHz, CD_3OD) for NFAT-133 (**1**).



	δ_C[a]	δ_H[b] (*J* in Hz)	HMBC[c]
1	63.7	4.25 dd (5.4, 1.7)	2, 3, 4, 5, 9
2	132.8	6.16 dt (15.6, 5.4)	1, 3, 4, 5, 6, 9
3	129.3	6.95 dt (15.6, 1.7)	1, 2, 4, 5, 6, 9
4	137.1	-	-
5	128.6	7.25 d (1.5)	3, 7, 9, 18
6	137.0	-	-
7	129.7	7.07 dd (8.0, 1.5)	4, 5, 6, 9, 18
8	128.0	7.14 d (8.0)	3, 4, 5, 6, 9, 10, 18, 19
9	140.2	-	-
10	39.7	3.09 dq (8.9, 6.8)	-
11	76.6	4.21 dd (8.9, 3.6)	-
12	51.2	2.36 dd (7.0, 3.6)	-
13	214.1	-	-
14	28.4	2.06 s	-
15	-	-	-
16	-	-	-
17	-	-	-
18	21.0	2.30 s	-
19	19.3	1.29 d (6.8)	-
20	9.6	0.95 d (7.0)	-
21	-	-	-

[a]Recorded at 125 MHz; referenced to residual CD_3OD at δ 49.2 ppm. [b]Recorded at 500 MHz; referenced to residual CD_3OD at δ 3.31 ppm. [c]Proton showing HMBC correlation to indicated carbon.

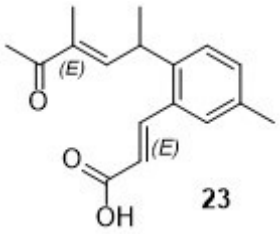
Table S8: NMR spectroscopic data (700 MHz, CD_3OD) for Veramycin F (**22**).



	δ_C[a]	δ_H[b] (*J* in Hz)	HMBC[c]	ROESY[d]
1	-	-	-	-
2	-	-	-	-
3	167.3	-	-	-
4	124.4	-	-	-
5	131.2	7.79 dq (1.9, 0.7)	3, 7, 9, 18	-
6	139.0	-	-	-
7	136.4	7.46 ddq (7.8, 1.9, 0.7)	5, 9, 18	18
8	128.4	7.29 d (7.8)	3, 4, 5, 6, 7, 9, 10	11, 12
9	144.8	-	-	-
10	34.0	3.12 dq (7.1, 2.6)	4, 8, 9, 11, 19	-
11	83.8	4.69 dd (10.6, 2.6)	9, 12, 13, 19, 20	-
12	48.5	3.15 dq (10.6, 7.1)	10, 11, 13, 20	-
13	212.4	-	-	-
14	30.2	2.30 dd (0.2)	12, 13	-
15	-	-	-	-
16	-	-	-	-
17	-	-	-	-
18	20.9	2.38 t (0.7)	4, 5, 6, 7, 8, 9	-
19	14.8	1.17 d (7.1)	9, 10, 11	-
20	12.7	1.13 d (7.1)	11, 12, 13	-
21	-	-	-	-

[a]Recorded at 175 MHz; referenced to residual CD_3OD at δ 49.2 ppm. [b]Recorded at 700 MHz; referenced to residual CD_3OD at δ 3.31 ppm. [c]Proton showing HMBC correlation to indicated carbon. [d]Proton showing ROESY correlation to indicated proton.

Table S9: NMR spectroscopic data (500 MHz, CD_3OD) for Veramycin G (**23**).



23

	δ_C[a]	δ_H[b] (*J* in Hz)	HMBC[c]	ROESY[d]
1	170.1	-	-	-
2	121.3	6.31 d (15.7)	1, 3, 4	5
3	144.1	8.02 d (15.7)	1, 2, 4, 5, 9	5, 10, 19, 20
4	134.2	-	-	-
5	128.4	7.42 d (1.4)	3, 4, 7, 9, 18	2, 18
6	137.9	-	-	-
7	132.5	7.23 dd (8.0, 1.6)	5, 9, 18	18, 19
8	127.9	7.31 d (8.0)	3, 4, 5, 6, 9, 10	10, 11, 19
9	142.2	-	-	-
10	36.3	4.19 dq (9.0, 7.0)	4, 8, 9, 11, 12, 19	3, 19, 20
11	149.4	6.77 dq (9.0, 1.1)	9, 10, 13, 19, 20	8, 14, 19
12	137.2	-	-	-
13	202.4	-	-	-
14	25.6	2.28 s	11, 13	10, 10
15	-	-	-	-
16	-	-	-	-
17	-	-	-	-
18	20.9	2.34 s	5, 6, 7, 8, 9	5
19	21.2	1.42 d (7.0)	9, 10, 11	3, 8, 11
20	11.4	1.79 d (1.1)	9, 11, 12, 13	3, 10
21	-	-	-	-

[a]Recorded at 125 MHz; referenced to residual CD_3OD at δ 49.2 ppm. [b]Recorded at 500 MHz; referenced to residual CD_3OD at δ 3.31 ppm. [c]Proton showing HMBC correlation to indicated carbon. [d]Proton showing ROESY correlation to indicated proton.

3. *J*-Based Configurational Analysis (*J*BCA)

In *J*BCA, the knowledge of the range of variability of the magnitude of $^{2,3}J_{H,C}$ values is required for each specific substitution pattern. It strongly depends on the electronegativity of the substituents directly linked to the stereogenic carbon centers under investigation and may be retrieved from the literature or measured in analogous model compounds with known configuration(s). The application of standard JBCA is further limited if a deviation (>10°) from purely staggered conformations is encountered and/or in cases where not a single conformation is predominant but *J* values rather represent an ensemble average of multiple-conformer equilibria.[1,2]

The determination of the relative configuration of C11/C12 of **1**, **13**, **17**, **20**, and **21** is not affected by the presence of bulky substituents. *J*-values fit well into the determination scheme for 1,2-hydroxymethyl dimethine systems[1] and the configuration is correctly predicted as *threo* – in contrast to the C10/C11 relation where the influence of the steric and electronic properties of the bulky *o*-substituted phenyl group attached to C10 cannot be neglected.

The conformational preferences of vicinal alkyl aryl compounds **SI-1**-**SI-4** that can be regarded as model systems for the C10/C11 region of **1**, **13**, **17**, **20**, and **21** (cf. Figure SI-1) have been studied in detail. Experimentally observed $^{3}J_{Ha\text{-}Hb}$ values in CD_3OD for both *erythro* and *threo* isomers of **SI-1**-**SI-4** comprise an unusual narrow range from 7.0 to 8.7 Hz.[3] For **1**, **13**, **17**, **20**, and **21**, respectively, $^{3}J_{H11\text{-}H12}$ values of 6.8-7.0 Hz were determined. At a first glance, values in the 7-9 Hz range would fit well into the *J*BCA "large" classification for the *erythro* configuration with a predominant *anti* conformation. This assumption seemed to be corroborated by ROESY interactions between H12 and the CH_3 protons at C10. However, the observed ROESY interactions comply as well with the *gauche*$^-$ conformer of the *threo* configured isomers. Since all substituents on C_a and C_b are gathered on one side the *gauche* conformations should be energetically disfavored. However, in this exceptional case, based on the experimental observations[3] and *ab initio* MO calculations not only the *anti* but also the *gauche*$^-$ conformers of the *threo* isomers and the *gauche*$^+$ conformers of the *erythro* isomers are considerably populated. The stabilization of these *gauche* conformations has been attributed to attractive OH/π interactions.[4] As a result, $^{3}J_{Ha\text{-}Hb}$ values for both *erythro* and *threo* configured pairs of diastereomers adopt intermediary values in a similar range representing the *anti*/*gauche* conformational ensembles and lead to similar ROESY effects for both configurations, too. These findings render the accurate prediction of the configuration based on the standard classification system of $^{3}J_{H\text{-}H}$ and $^{2,3}J_{C\text{-}H}$ coupling constants as "small", "medium" or "large" for conformationally flexible systems in JBCA in these particular vicinal alkyl aryl systems by experimental NMR parameters alone very difficult.

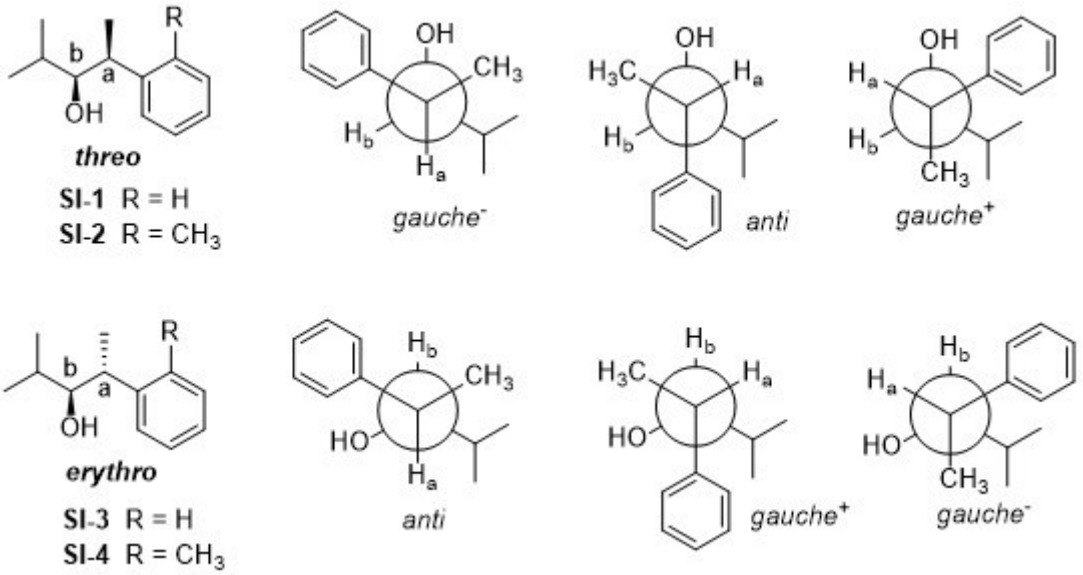


Figure S1: 2-Methyl-4-phenyl-pentan-3-ol derivatives and their possible staggered conformers. The conformer designations refer to the H_a-H_b relations.

4. Optimization of the Evans Aldol Reaction

When applying the same conditions as for the synthesis of **33** (reaction of the enolate at –78°C to 0°C in the presence of DIPEA as base) **37** was only obtained in 53% yield and the non-Evans-*anti* diastereomer **SI-5** was isolated as the major side product (31%). Careful investigation of the reaction conditions revealed that the formation of this diastereomer was more pronounced at low temperatures. In contrast to the reaction of ***epi*-28** and the (*Z*)-enolate generated from **27** the reaction with **28** represents a stereochemically mismatched case. Disfavored *syn* pentane interactions likely destabilize the closed, cyclic transition state leading to **37**[5] and the reaction may occur through an alternative, open transition state[6] yielding the non-Evans-*anti* product **SI-5** whose absolute configuration was unambiguously determined by single-crystal X-ray analysis of its derivative **SI-6** which resulted from *endo*-cleavage of the oxazolidinone ring of **SI-5** under Weinreb conditions. Therefore, optimization of the reaction conditions was required to obtain acceptable diastereoselectivity and satisfactory yields of the *syn*,*syn* product **37**. Finally, **37** was obtained in 81% yield when performing the reaction at 0°C in the presence of NEt_3.

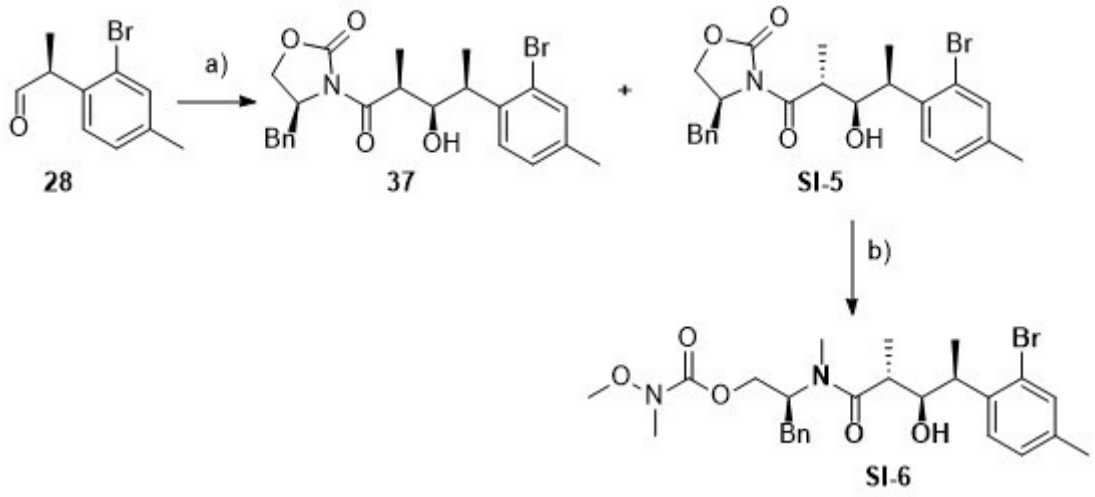


Figure S2: Reactions and conditions: a) **27**, *n*-Bu_2BOTf, DIPEA, CH_2Cl_2, 0 °C, 1 h, then **28**, –78°C to 0°C, 2 h, 53% of **37** and 31% of **SI-5**; f) $MeONHMe_3 \cdot HCl$, Me_3Al, THF, 0°C to r.t., 16 h, 12% (41% brsm).

5. Synthesis

(4*R*)-4-Benzyl-3-[2-(2-bromo-4-methyl-phenyl)acetyl]oxazolidin-2-one (30):

1. To a solution of 2-(2-bromo-4-methylphenyl)acetic acid **29** (6.87 g, 30.0 mmol) in CH_2Cl_2 DMF (12 µL, 5 mol%) and oxalyl chloride (3.09 mL, 36.0 mmol, 1.2 eq.) were added. The mixture was stirred for 2 h at r.t. and then concentrated under reduced pressure to yield 2-(2-bromo-4-methyl-phenyl)acetyl chloride (7.43 g, quant.) as a colorless oi,l which was directly used in the next step without further purification.

2. To a stirred solution of (4*R*)-4-benzyloxazolidin-2-one (5.32 g, 30.0 mmol) in THF (90 mL) *n*-BuLi (2.5 M in heptane, 13.2 mL, 33.0 mmol, 1.1 eq.) was added dropwise at –78°C. Stirring was continued at –78 °C for 20 min whereupon a solution of 2-(2-bromo-4-methyl-phenyl)acetyl chloride (7.43 g, 30.0 mmol) in THF (10 mL) was added dropwise. The mixture was stirred for 1 h at –78 °C, warmed to r.t. within 1 h, quenched with saturated aqueous NH_4Cl solution, and concentrated under reduced pressure. After the residue was taken up in MTBE, the organic layer was washed with water, dried over $MgSO_4$, and concentrated. Oxazolidinone **30** (9.92 g, 85%) was obtained as a viscous, colorless oil after flash chromatography (*n*-heptane/MTBE 85:15). **^{1}H-NMR** ($(CD_3)_2SO$), 400 MHz): 7.48 (s, 1H), 7.26 (m, 6H), 7.18 (br d, 1H, *J* = 7.7 Hz), 4.69 (m, 1H), 4.37 (m, 2H), 4.24 (m, 2H), 2.99 (m, 2H), 2.31 (s, 3H); **^{13}C-NMR** ($(CD_3)_2SO$, 100 MHz): 170.2, 153.6, 139.2, 135.2, 133.3, 131.5, 130.9, 129.4, 128.9, 128.4, 127.3, 125.0, 66.4, 55.5, 42.7, 37.8, 20.7; **HRMS** (ESI) *m/z* calcd. for $[M+H]^+$ $C_{19}H_{19}BrNO_3$: 388.0548, found: 388.0542; **Specific rotation:** $[\alpha]_D^{24}$ = – 58 (*c* = 1.1; $CHCl_3$).

(4*R*)-4-Benzyl-3-[(2*R*)-2-(2-bromo-4-methyl-phenyl)propanoyl]oxazolidin-2-one (31):
To a stirred solution of (4*R*)-4-benzyl-3-[2-(2-bromo-4-methyl-phenyl)acetyl]oxazolidin-2-one **30** (6.92 g, 17.8 mmol) in THF (70 mL) NaHMDS (2 M solution in THF, 9.80 mL, 19.6 mmol, 1.1 eq.) was added dropwise within 10 min at –60 °C. The mixture was stirred for 1 h at –50 °C to –60 °C and then cooled to –70 °C. MeI (5.55 mL, 89.1 mmol, 5.0 eq.) was added in one portion and stirring was continued for 2 h at –70 °C to –30 °C and for 2 h at –30 °C. The reaction was quenched at –30 °C by dropwise addition of acetic acid (3.1 mL) and warmed to r.t. The volatiles were removed under reduced pressure. After, the residue was taken up in toluene, the resulting solution was washed with water, dried over $MgSO_4$, and concentrated under reduced pressure. ^{1}H NMR of the crude product indicated a mixture of the 2*R* (major) and 2*S* isomers (*dr* = 9:1). Flash chromatography (*n*-heptane/MTBE 85:15) furnished **31** (5.39 g, 75%) as a colorless, viscous oil which crystallized upon trituration with Et_2O. **^{1}H-NMR** ($(CD_3)_2SO$), 400 MHz): 7.44 (s, 1H), 7.25 (m, 7H), 5.08 (q, 1H, *J* = 7.0 Hz), 4.73 (m, 1H), 4.33 (m, 1H), 4.25 (d, 1H, *J*= 2.8 Hz), 3.02 (m, 2H), 2.28 (s, 3H), 1.43 (d, 3H, *J* = 7.1 Hz); **^{1}H-NMR** ($CDCl_3$, 500 MHz): 7.40 (s, 1H), 7.26 (m, 6H), 7.12 (m, 1H), 5.27 (q, 1H, *J* = 7.0 Hz), 4.67 (td, 1H, *J* = 9.2, 4.7 Hz), 4.17 (d, 2H, *J* = 4.8 Hz), 3.33 (dd, 1H, *J* = 13.3, 2.9 Hz), 2.79 (dd, 1H, *J* = 13.2, 9.9 Hz), 2.31 (s, 3H), 1.56 (d, 3H, *J* = 7.1 Hz) ; **^{13}C-NMR** ($CDCl_3$, 125 MHz): 174.4, 152.7, 138.7, 136.5, 135.2, 133.4, 129.3, 128.9, 128.4, 127.7, 127.3, 124.6, 66.1, 55.1, 43.5, 37.9, 20.6, 17.1; **HRMS** (ESI) *m*/*z* calcd. for $[M+H]^+$ $C_{20}H_{21}BrNO_3$: 402.0705, found: 402.0701; **Specific rotation:** $[\alpha]_D^{23}$ = – 117 (*c* = 2.0; $CHCl_3$).

(2*R*)-2-(2-Bromo-4-methyl-phenyl)propan-1-ol (32):
To a stirred solution of **31** (1.25 g, 3.11 mmol) in THF (8 mL) water (2.4 mL) and $NaBH_4$ (600 mg, 15.6 mmol, 5.o eq.) were added. The mixture was stirred at r.t. for 24 h whereupon it was quenched at 0 °C by dropwise addition of a solution of 10% NaCl in 2 N HCl followed by dilution with EtOAc. The phases were separated and the aqueous phase was extracted with EtOAc. The combined organic phases were washed with saturated aqueous $NaHCO_3$ solution and brine, dried over $MgSO_4$, and concentrated. The auxiliary, (4*R*)-4-benzyloxazolidin-2-one (623 mg) precipitated upon trituration of the residue with Et_2O and was collected by filtration. Flash chromatography (*n*-heptane/EtOAc 2:1 to 100% EtOAc) of the concentrated filtrate provided **32** (974 mg, 95%) as a colorless oil and another portion of (4*R*)-4-benzyloxazolidin-2-one (169 mg; total yield: 729 mg, 99%). **^{1}H-NMR** ($(CD_3)_2SO$, 400 MHz): 7.40 (s, 1H), 7.21 (m, 1H), 7.14 (m, 1H), 4.69 (t, 1H, *J* = 5.3 Hz), 3.55 (dt, 1H, *J* = 10.5, 5.4 Hz), 3.38 (m, 1H), 3.19 (m, 1H, OH), 2.25 (s, 3H), 1.15 (d, 3H, *J* = 7.0 Hz); **^{1}H-NMR** ($CDCl_3$, 500 MHz): 7.42 (s, 1H), 7.16 (m, 1H), 7.13 (m, 1H), 3.79 (dd, 1H, *J* = 10.7, 6.5 Hz), 3.70 (dd, 1H, *J* = 10.8, 6.4 Hz), 3.48 (m, 1H), 2.32 (s, 3H), 1.28 (d, 3H, *J* = 7.0 Hz); ; **^{13}C-NMR** ($CDCl_3$, 125 MHz): 139.3, 138.0, 133.5, 128.5, 127.2, 125.0, 67.4, 40.3, 20.6, 17.1; **HRMS** (ESI) *m*/*z* calcd. for $[M+H-H_2O]^+$ $C_{10}H_{11}Br$: 211.0122, found: 211.0118; **Specific rotation:** $[\alpha]_D^{22}$ = – 7.5 (*c* = 0.8; $CHCl_3$).

(2*R*)-2-(2-bromo-4-methyl-phenyl)propanal (*epi*-28):
To a stirred solution of (*R*)-alcohol **31** (910 mg, 3.97 mmol) in CH_2Cl_2 (20 mL) $NaHCO_3$ (404 mg, 4.77 mmol, 1.2 eq.) was added, followed by DMP (2.02 g, 4.77 mmol, 1.2 eq.). The mixture was stirred at r.t. for 2 h whereupon it was quenched with a mixture of saturated $Na_2S_2O_3$ solution and brine (1:1 v/v) and extracted with CH_2Cl_2. The combined organic phases were dried over $MgSO_4$ and concentrated under reduced pressure (heating bath temperature of the rotary evaporator 30°C). Flash chromatography (*n*-heptane / EtOAc 8:1) furnished aldehyde ***epi*-28** (861 mg, 95%) as a pale yellow oil. **^{1}H-NMR** ($(CD_3)_2SO$, 400 MHz): 9.65 (s, 1 H), 7.52 (s, 1 H), 7.22 (d, 1H, *J* = 7.6 Hz), 7.12 (d, 1H, *J* = 7.7 Hz), 4.04 (q, 1H, *J* = 7.1 Hz), 2.30 (s, 3 H), 1.33 (d, 3H, *J* = 7.1 Hz); **^{1}H-NMR** ($CDCl_3$, 500 MHz): 9.73 (s, 1H), 7.49 (s, 1H), 7.15 (br d, 1H, *J* = 7.7 Hz), 7.00 (d, 1H, *J* = 7.9 Hz), 4.13 (q, 1H, *J* = 7.1 Hz), 2.34 (s, 3H), 1.42 (d, 3H, *J* = 7.1 Hz); **^{13}C-NMR** ($CDCl_3$, 125 MHz): 200.5, 139.2, 134.6, 133.8, 133.4, 128.9, 124.9, 51.6, 20.7, 14.1; **HRMS** (ESI) *m*/*z* calcd. for $[M+H]^+$ $C_{10}H_{12}BrO$: 227.0072, found: 227.0070; **Specific rotation:** $[\alpha]_D^{22}$ = – 151 (*c* = 0.9; $CHCl_3$).

(*S*)-4-Benzyl-3-((2*S*,3*R*,4*R*)-4-(2-bromo-4-methylphenyl)-3-hydroxy-2-methylpentanoyl)-oxazolidin-2-one (33):

To a stirred solution of propionyl oxazolidinone **27** (802 mg, 3.30 mmol) in CH_2Cl_2 (10 mL) Bu_2BOTf (1 M in CH_2Cl_2, 3.63 mL, 3.63 mmol, 1.1 eq.) was added dropwise at 0 °C followed by dropwise addition of DIPEA (690 µL, 3.96 mmol, 1.2 eq.). The mixture was stirred for 1 h at 0 °C and then cooled to –78°C. Aldehyde ***epi*-28** (824 mg, 3.63 mmol, 1.1 eq.) was added dropwise and stirring was continued at –78°C for 1 h and at 0 °C for an additional hour. The reaction was quenched by the addition of phosphate buffer (pH 7) followed by dropwise addition of MeOH/33% H_2O_2 (2:1 v/v, 35 mL). After stirring for 1 h at 0 °C the mixture was extracted with CH_2Cl_2. The combined organic phases were washed with saturated aqueous $NaHCO_3$ solution and brine, dried over $MgSO_4$, and concentrated under reduced pressure. The *syn*,*anti* aldol product **33** (1.18 g, 78%) was obtained as a colorless crystalline solid after flash chromatography (*n*-heptane/EtOAc 4:1 to 2:1) of the residue. **^{1}H-NMR** ($(CD_3)_2SO$, 400 MHz): 7.43 (d, 1H, *J* = 8.1 Hz), 7.26 (m, 6H), 7.09 (d, 1H, *J* = 7.7 Hz), 5.04 (d, 1H, *J* = 6.2 Hz), 4.62 (dtd, 1H, *J* = 7.8, 5.3, 2.6 Hz), 4.24 (m, 2H), 4.00 (m, 1H), 3.37 (m, 1H), 3.28 (dd, 1H, *J* = 7.2, 4.7 Hz), 2.96 (d, 2H, *J* = 5.4 Hz), 2.22 (s, 3H), 1.16 (d, 3H, *J* = 7.1 Hz), 1.12 (d, 3H, *J* = 7.0 Hz); **^{1}H-NMR** ($CDCl_3$, 500 MHz): 7.33 (m, 7H), 7.12 (br d, 1H, *J* = 7.8 Hz), 4.69 (m, 1H), 4.21 (m, 3H), 3.97 (m, 1H), 3.53 (br t, 1H, *J* = 7.1 Hz), 3.29 (dd, 1H, *J* = 13.5, 3.0 Hz), 2.80 (dd, 1H, *J* = 13.3, 9.6 Hz), 2.30 (s, 3H), 1.34 (d, 3H, *J* = 7.0 Hz), 1.26 (d, 3H, *J* = 7.1 Hz) **^{13}C-NMR** ($CDCl_3$, 125 MHz): 176.4, 152.9, 139.7, 138.0, 135.1, 133.2, 129.4, 128.9, 128.7, 128.0, 125.1, 127.4, 75.5, 66.2, 55.4, 40.6, 40.4, 37.8, 20.6, 18.2, 10.6; **HRMS** (ESI) *m*/*z* calcd. for $[M+H]^+$ $C_{23}H_{27}BrNO_4$: 460.1123, found: 460.1123; **Specific rotation:** $[\alpha]_D^{23}$ = + 21 (*c* = 0.65; $CHCl_3$).

(2*S*,3*R*,4*R*)-4-(2-Bromo-4-methyl-phenyl)-3-hydroxy-*N*-methoxy-*N*,2-dimethyl-pentanamide (*epi*-26):
To a stirred suspension of MeONMe·HCl (211 mg, 2.10 mmol, 3.0 eq.) in THF (3.5 mL) Me_3Al (2 M solution in toluene; 1.05 mL, 2.1 mmol, 3 eq.) was added dropwise at 0 °C. The mixture was stirred for 30 min at 0 °C. A solution of **33** (323 mg, 0.7 mmol) in THF (3.5 mL) was added dropwise and stirring was continued for 30 min at 0 °C and for 3 h at r.t. The reaction was quenched at 0 °C by careful addition of saturated aqueous potassium sodium tartrate solution followed by extraction with MTBE. The combined organic phases were washed with brine, dried over $MgSO_4$, and concentrated. Flash chromatography (*n*-heptane/EtOAc 3:1 to 100% EtOAc) of the residue furnished Weinreb amide ***epi*-26** (212 mg, 88%) and (4*S*)-4-benzyloxazolidin-2-one (120 mg, 97%) as colorless solids. **^{1}H-NMR** ($(CD_3)_2SO$, 400 MHz): 7.62 (d, *J* = 8.0 Hz, 1H), 7.34 (s, 1H), 7.11 (d, 1H, *J* = 8.2 Hz), 5.01 (d, 1H, *J* = 5.8 Hz), 3.87 (dt, 1H, *J* = 8.0, 5.3 Hz), 3.31 (s, 3H), 3.24 (m, 1H), 2.99 (s, 3H), 2.65 (m, 1H), 2.23 (s, 3H), 1.12 (d, 3H, *J* = 7.1 Hz), 1.04 (d, 3H, *J* = 7.0 Hz); **^{13}C-NMR** ($CDCl_3$, 125 MHz): 178.0, 140.4, 137.5, 133.2, 128.4, 127.9, 125.1, 75.1, 61.4, 40.3, 36.3, 32.0, 20.5, 18.4, 10.1; **HRMS** (ESI) *m*/*z* calcd. for $[M+H]^+$ $C_{23}H_{27}BrNO_4$: 344.0861, found: 344.0858; **Specific rotation:** $[\alpha]_D^{23}$ = – 19 (*c* = 1.0; $CHCl_3$).

(2*S*,3*R*,4*R*)-3-Hydroxy-*N*-methoxy-*N*,2-dimethyl-4-[2-[(*E*)-3-[*tert*-butyl(dimethyl)silyl]-oxyprop-1-enyl]phenyl]pentanamide (35):
Cesium carbonate (122 mg, 0.372 mmol, 4.0 eq.), ***epi*-13** (32.0 mg, 0.093 mmol), and freshly prepared[7] *tert*-butyl-dimethyl-[(*E*)-3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)allyloxy]silane **34** (42.0 mg, 0.140 mmol, 1.5 eq.) were weighed into a screw-capped reaction tube. The vessel was evacuated and back-filled with argon (three times) whereupon a degassed mixture of THF and water (9:1, 1.5 mL) followed by XPhos Pd G4 (4.7 mg, 6 mol%) was added. The reaction tube was closed and the mixture was stirred for 3 h at 60 °C. After cooling to r.t. it was diluted with MTBE and extracted with brine. The organic phase was dried over $MgSO_4$ as well as concentrated and the residue was purified by flash chromatography (*n*-heptane/EtOAc 6:1 to 2:1) to yield 29.0 mg (71%) of the coupling product **35**. **^{1}H-NMR** ($(CD_3)_2SO$, 400 MHz): 7.44 (d, 1H, *J* = 8.1 Hz), 7.15 (s, 1H), 6.99 (d, 1H, *J* = 8.0 Hz), 6.79 (br d, 1H, *J* = 15.7 Hz), 6.05 (dt,1H, *J* = 15.6, 4.8 Hz), 4.78 (d, 1H, *J* = 5.5 Hz), 4.29 (dd, 2H, *J* = 4.7, 1.4 Hz), 3.86 (m, 1H), 3.21 (s, 3H), 3.15 (m, 1H), 2.96 (s, 3H), 2.68 (m, 1H), 2.23 (s, 3H), 1.08 (d, 3H, *J* = 7.0 Hz), 1.03 (d, 3H, *J* = 7.0 Hz), 0.90 (s, 9H), 0.08 (s, 6H); **^{1}H-NMR** (CD_3OD, 700 MHz): 7.25 (s, 1H), 7.21 (m, 1H), 7.05 (d, 1H, *J* = 8.2 Hz), 6.99 (br d, 1H, *J* = 15.7 Hz), 6.08 (dt, 1H, *J* = 15.6, 4.9 Hz), 4.35 (br d, 2H, *J* = 4.8 Hz), 4.03 (dd, 1H, *J* = 9.2, 1.8 Hz), 3.66 (s, 3H), 3.52 (br s, 1H), 3.27 (m, 1H), 3.19 (s, 3H), 3.16 (m, 1H), 2.30 (s,

3H), 1.23 (d, 3H, *J* = 7.0 Hz), 1.14 (d, 3H, *J* = 7.0 Hz), 0.94 (s, 9H), 0.11 (s, 6H); **^{13}C-NMR** (CD_3OD, 176 MHz): 178.3, 140.4, 137.8, 136.6, 132.0, 129.3, 129.1, 128.9, 128.1, 77.1, 65.2, 40.5, 37.8, 32.3, 26.5, 21.1, 20.3, 19.3, 13.9, –5.0; **HRMS** (ESI) *m*/*z* calcd. for $[M+Na]^+$ $C_{24}H_{41}NNaO_4Si$: 458.2703, found: 458.2701; **Specific rotation:** $[\alpha]_D^{22}$ = – 53 (*c* = 0.4; $CHCl_3$).

(3*S*,4*R*,5*R*)-5-[2-[(*E*)-3-[*tert*-butyl(dimethyl)silyl]oxyprop-1-enyl]phenyl]-4-hydroxy-3-methyl-hexan-2-one (36):
To a stirred solution of Weinreb amide **35** (79.0 mg, 0.18 mmol) in Et_2O (1.5 mL) MeMgBr (3 M solution in Et_2O, 150 µL, 0.45 mmol, 2.5 eq.) was added dropwise at 0 °C. The mixture was stirred at 0 °C for 3 h and was quenched with saturated aqueous NH_4Cl solution. The aqueous phase was extracted with MTBE. The combined organic phases were dried over $MgSO_4$ and concentrated. Flash chromatography (*n*-heptane/EtOAc 8:1) yielded 58.0 mg (82%) of methyl ketone **36** as a colorless, viscous oil. **^{1}H-NMR** ($(CD_3)_2SO$, 400 MHz): 7.31 (d, 1H, *J* = 8.0 Hz), 7.19 (s, 1H), 7.03 (dd, 1H, *J* = 8.0, 1.2 Hz), 6.90 (d, 1H, *J* = 15.7 Hz), 6.10 (dt, 1H, *J* = 15.7, 4.6 Hz), 4.44 (d, 1H, *J* = 6.2 Hz), 4.31 (d, 2H, *J* = 3.7 Hz), 4.13 (ddd, 1H, *J* = 8.3, 6.3, 4.1 Hz), 3.08 (quin, 1H, *J* = 7.4 Hz), 2.64 (qd, 1H, *J* = 6.9, 4.3 Hz), 2.26 (s, 3H), 2.07 (s, 3H), 1.06 (d, 3H, *J* = 7.0 Hz), 1.00 (d, 3H, *J* = 6.9 Hz), 0.91 (s, 9H), 0.10 (s, 6H); **^{1}H-NMR** ($CDCl_3$, 400 MHz): 7.21 (m, 2H), 7.08 (d, 1H, *J* = 8.0 Hz), 6.94 (d, 1H, *J* = 15.5 Hz), 6.09 (dt, 1H, *J* = 15.5, 4.7 Hz), 4.35 (d, 2H, *J* = 4.7 Hz), 4.16 (dd, 1H, *J* = 9.4, 2.4 Hz), 3.24 (m, 1H), 2.75 (qd, 1H, *J* = 7.1, 2.6 Hz), 2.31 (s, 3H), 2.21 (s, 3H), 1.22 (d, 3H, *J* = 7.1 Hz), 1.15 (d, 3H, *J* = 7.0 Hz), 0.94 (s, 9H), 0.11 (s, 6H); **^{13}C-NMR** ($CDCl_3$, 125 MHz): 212.8, 138.0, 137.1, 136.0, 131.6, 128.7, 127.7, 127.0, 125.9, 75.5, 63.9, 48.5, 36.8, 28.7, 25.9, 21.0, 18.4, 18.2, 8.5, –5.2; **HRMS** (ESI) *m*/*z* calcd. for $[M+Na]^+$ $C_{23}H_{38}NaO_3Si$: 413.2488, found: 413.2484; **Specific rotation:** $[\alpha]_D^{22}$ = – 40 (*c* = 0.42; $CHCl_3$).

(3*S*,4*R*,5*R*)-4-Hydroxy-5-[2-[(*E*)-3-hydroxyprop-1-enyl]phenyl]-3-methyl-hexan-2-one (*epi*-1):
A solution of **36** (43.0 mg, 0.11 mmol) in THF (0.5 mL) was prepared in a polyethylene reaction vessel. HF·py (70% HF, 50 µL, 17.5 eq.) was added and the mixture was stirred for 3 h at r.t. whereupon it was diluted with MTBE and quenched with satd. aqueous $NaHCO_3$ solution. The organic phase was separated, dried over $MgSO_4$, and concentrated. ***Epi*-1** (22.0 mg, 72%) was obtained after flash chromatography (*n*-heptane/EtOAc 2:1) as a as a colorless, viscous oil. **^{1}H-NMR** (CD_3OD, 500 MHz): 7.21 (d, 1H, *J* = 8.0 Hz), 7.14 (s, 1H), 6.97 (br d, 1H, *J* = 8.6 Hz), 6.83 (d, 1H, *J* = 15.7 Hz), 6.03 (dd, 1H, *J* = 15.7, 5.6 Hz), 4.13 (dd, 2H, *J* = 5.6, 1.4 Hz), 4.09 (dd, 1H, *J* = 8.0, 4.6 Hz), 3.15 (m, 1H), 2.66 (m, 1H), 2.20 (s, 3H), 1.99 (s, 3H), 1.08 (d, 3H, *J* = 7.0 Hz), 1.04 (d, 3H, *J* = 7.0 Hz); **^{13}C-NMR** (CD_3OD, 125 MHz): 214.3, 140.2, 138.0, 136.7, 132.1, 130.0, 129.5, 128.2, 128.0, 76.6, 63.9, 51.2, 38.8, 30.9, 21.1, 19.2, 10.2; **HRMS** (ESI) *m*/*z* calcd. for $[M+Na]^+$ $C_{17}H_{24}NaO_3$: 299.1623, found: 299.1617 **Specific rotation:** $[\alpha]_D^{23}$ = – 15.4 (*c* = 0.85; MeOH).

(2*S*)-2-(2-Bromo-4-methyl-phenyl)propanal (28):

a) **(4*S*)-4-Benzyl-3-[2-(2-bromo-4-methyl-phenyl)acetyl]oxazolidin-2-one:** The attachment of the auxiliary was performed via a mixed anhydride[8,9]: To a solution of 2-(2-bromo-4-methylphenyl)acetic acid **29** (11. 5 g, 50.0 mmol) in toluene (50 mL) (4*S*)-4-benzyloxazolidin-2-one (8.86 g, 50.0 mmol, 1.0 eq.) and NEt_3 (20.9 mL, 150 mmol, 3.0 eq.) were added. The mixture was stirred and heated to 90 °C. Pivaloyl chloride (7.38 mL, 60.0 mmol, 1.2 eq.) was added dropwise and stirring was continued at 90 °C for 90 min whereupon another portion of pivaloyl chloride (3.69 mL, 30.0 mmol, 0.6 eq.) was added dropwise. Stirring was continued for 3 h 30 min at 90 °C. The reaction mixture was cooled to r.t., diluted with MTBE, washed with water, 6 N HCl, 6 N NaOH, 2 N HCl, saturated aqueous $NaHCO_3$ solution, and brine. The organic phase was separated, dried over $MgSO_4$, and concentrated. Flash chromatography (*n*-heptane / MTBE 4:1 to 1:1) of the residue furnished (4*S*)-4-benzyl-3-[2-(2-bromo-4-methyl-phenyl)acetyl]oxazolidin-2-one (15.8 g , 81%) as a colorless, viscous oil. ^{1}H- and ^{13}C-NMR data were identical to the ones reported for the corresponding (*R*)-enantiomer **30**. **HRMS** (ESI) *m*/*z* calcd. for $[M+Na]^+$ $C_{19}H_{18}BrNNaO_3$: 410.0368, found: 410.0359; **Specific rotation:** $[\alpha]_D^{22}$ = + 66 (*c* = 1.06; $CHCl_3$).

b) **(4*S*)-4-Benzyl-3-[(2*S*)-2-(2-bromo-4-methyl-phenyl)propanoyl]oxazolidin-2-one:** The Evans alkylation was performed analogously as described for the (*R*) isomer **31** on a 41.3 mmol scale yielding 13.1 g (79%) of the (*S*)-alkylation product. ^{1}H- and ^{13}C-NMR data were identical to the ones reported for the corresponding (*R*)-enantiomer **31**. **HRMS** (ESI) *m*/*z* calcd. for $[M+H]^+$ $C_{20}H_{21}BrNO_3$: 402.0705, found: 402.0701; **Specific rotation:** $[\alpha]_D^{23}$ = + 125 (*c* = 1.1; $CHCl_3$).

c) **(2*S*)-2-(2-Bromo-4-methyl-phenyl)propan-1-ol**: The reductive cleavage was performed analogously as described for the (*S*) isomer **32** on a 30.6 mmol scale yielding 6.64 g (95%) of the (*S*)-alcohol. Another reaction run on a 3.95 mmol scale furnished 878 mg (97%) of the (*S*)-alcohol. ^{1}H- and ^{13}C-NMR data were identical to the ones reported for the corresponding (*R*)-alcohol **32**. **HRMS** (ESI) *m*/*z* calcd. for $[M+H-H_2O]^+$ $C_{10}H_{11}Br$: 211.0122, found: 211.0118; **Specific rotation:** $[\alpha]_D^{21}$ = + 5.5 (*c* = 1.1; $CHCl_3$).

d) **(2*S*)-2-(2-Bromo-4-methyl-phenyl)propanal (28)**: The (*S*)-alcohol was oxidized as outlined for the (*R*)-isomer furnishing 6.46 g (92%) of (*S*)-aldehyde **28** (28.2 mmol scale). ^{1}H- and ^{13}C-NMR data were identical to the ones reported for the corresponding (*R*)-enantiomer ***epi*-28**. **HRMS** (ESI) *m*/*z* calcd. for $[M+H]^+$ $C_{10}H_{12}BrO$: 227.0072, found: 227.0067; **Specific rotation:** $[\alpha]_D^{22}$ = + 166 (*c* = 1.2; $CHCl_3$).

(*S*)-4-Benzyl-3-((2*S*,3*R*,4*S*)-4-(2-bromo-4-methylphenyl)-3-hydroxy-2-methylpentanoyl)-oxazolidin-2-one (37):

To a stirred solution of propionyl oxazolidinone **27** (5.83 g, 24.0 mmol) in CH_2Cl_2 (70 mL) Bu_2BOTf (1 M in CH_2Cl_2, 26.4 mL, 26.4 mmol, 1.1 eq.) was added drop-wise at 0 °C followed by dropwise addition of DIPEA (5.02 mL, 28.8 mmol, 1.2 eq.). The mixture was stirred for 1 h at 0 °C and then cooled to –78°C. (2*S*)-2-(2-Bromo-4-methyl-phenyl)propanal **22** (5.72 g, 25.2 mmol, 1.05 eq.) was added dropwise and stirring was continued at –78°C for 1 h and at 0 °C for an additional hour. The reaction was quenched by the addition of phosphate buffer pH 7 (5 mL) followed by dropwise addition of MeOH/33% H_2O_2 (2:1 v/v, 35 mL). After stirring for 1 h at 0 °C, the organic phase was separated and the aqueous phase was extracted with CH_2Cl_2. The combined organic phases were washed with saturated aqueous $NaHCO_3$ solution and brine, dried over $MgSO_4$, and concentrated under reduced pressure. Flash chromatography (*n*-heptane/EtOAc 6:1 to 3:2) of the residue furnished the *syn*,*syn* aldol product **37** (5.90 g, 53%) and the *non*-Evans *anti* product **SI-5** (3.46 g, 31%) as colorless foams.

Optimized procedure: To a stirred solution of propionyl oxazolidinone **27** (97.0 mg, 0.400 mmol) in CH_2Cl_2 (1.2 mL) Bu_2BOTf (1 M in CH_2Cl_2, 0.440 mL, 0.440 mmol, 1.1 eq.) was added dropwise at 0 °C. The mixture was stirred for 10 min at 0 °C. NEt_3 (73.0 µL, 0.520 mmol, 1.3 eq.) was added dropwise and stirring was continued for 1 h at 0 °C whereupon a solution of (2*S*)-2-(2-bromo-4-methyl-phenyl)propanal **28** (100 mg, 0.44 mmol, 1.1 eq.) in CH_2Cl_2 (0.5 mL) was added dropwise. The mixture was stirred for 2 h at 0 °C. It was quenched with phosphate buffer pH 7 (1 mL) followed by dropwise addition of MeOH/33% H_2O_2 (2:1 v/v, 1 mL). After stirring for 1 h at 0 °C, the mixture was concentrated under reduced pressure and the residue was taken up in MTBE. The organic phase was washed with saturated aqueous $NaHCO_3$ solution and brine, was dried over $MgSO_4$, and concentrated under reduced pressure. Flash chromatography (*n*-heptane/EtOAc 6:1 to 4:1) of the residue gave the *syn*,*syn* aldol product **37** (149 mg, 81%) and the *non*-Evans *anti* product (*S*)-4-benzyl-3-((2*R*,3*R*,4*S*)-4-(2-bromo-4-methylphenyl)-3-hydroxy-2-methylpentanoyl)oxazolidin-2-one **SI-5** (2.9 mg, 1.6%) as colorless foams.

SI-5: **^{1}H-NMR** (CD_3OD, 500 MHz): 7.39 (s, 1H), 7.26 (m, 6H), 7.13 (m, 1H), 4.70 (m, 1H), 4.39 (m, 1H), 4.24 (dd, 1H, *J* = 8.9, 8.1 Hz), 4.19 (dq, 1H, *J* = 6.9, 9.0 Hz), 4.15 (dd, 1H, *J* = 8.9, 3.0 Hz), 4.02 (dd, 1H, *J* = 9.0, 3.4 Hz), 3.54 (dq, 1H, *J* = 7.1, 3.4 Hz), 3.20 (dd, 1H, *J* = 13.5, 3.3 Hz), 2.81 (dd, 1H, *J* = 13.5, 8.8 Hz), 2.28 (s, 3H), 1.29 (d, 3H, *J* = 7.1 Hz), 1.23 (d, 3H, *J* = 6.9 Hz); **^{13}C-NMR** (CD_3OD, 125 MHz): 178.0, 155.3, 141.8, 139.2, 137.2, 134.0, 130.7, 130.6, 129.8, 129.3, 128.1, 125.1, 76.7, 67.2, 56.7, 42.9, 40.6, 38.4, 20.6, 14.9, 13.3; **HRMS** (ESI) *m*/*z* calcd. for $[M+H]^+$ $C_{23}H_{27}BrNO_4$: 460.1123, found: 460.1117; **Specific rotation:** $[\alpha]_D^{22}$ = + 81 (*c* = 2.0; $CHCl_3$).

37: **^{1}H-NMR** (CD_3OD, 500 MHz): 7.36 (s, 1H), 7.25 (m, 4H), 7.13 (m, 3H), 4.39 (m, 1H), 4.18 (m, 2H), 4.12 (dd, 1H, *J* = 8.2, 6.1 Hz), 3.72 (dq, 1H, *J* = 6.9, 6.1 Hz), 3.29 (dq, 1H, *J* = 8.2, 6.9 Hz), 3.03 (dd, 1H, *J* = 13.6, 3.2 Hz), 2.89 (dd, 1H, *J* = 13.6, 8.0 Hz), 2.26 (s, 3H), 1.24 (d, 3H, *J* = 6.9 Hz), 1.17 (d, 3H, *J* = 6.9 Hz); **^{13}C-NMR** (CD_3OD, 125 MHz): 177.2, 154.9, 141.3, 139.3, 136.8, 134.0, 130.7, 130.7, 129.8, 129.6, 128.2, 125.2, 76.0, 67.6, 56.8, 44.0, 43.2, 38.1, 20.6, 19.0, 13.0; **HRMS** (ESI) *m*/*z* calcd. for $[M+H]^+$ $C_{23}H_{27}BrNO_4$: 460.1123, found: 460.1123; **Specific rotation:** $[\alpha]_D^{22}$ = + 51 (*c* = 2.0; $CHCl_3$).

(*S*)-2-((2*R*,3*R*,4*S*)-4-(2-Bromo-4-methylphenyl)-3-hydroxy-*N*,2-dimethylpentanamido)-3-phenylpropyl methoxy(methyl)carbamate (SI-6):
To a stirred suspension of MeON(H)Me·HCl (905 mg, 9.0 mmol, 3.0 eq.) in THF (15 mL) was added a solution of Me_3Al (2M in toluene, 4.5 mL, 9.0 mmol, 3.0 eq.) dropwise. The mixture was stirred for 30min at 0°C after which a solution of **SI-5** (1.38g, 3.0 mmol) in THF (15 mL) was added dropwise. Stirring was continued for 30 min at 0°C and for 16h at r.t. The mixture was carefully quenched with saturated potassium sodium tartrate solution (20 mL) and extracted with MTBE. The combined organic phases were washed with saturated aqueous $NaHCO_3$ solution and brine, dried over $MgSO_4$ and concentrated under reduced pressure. Flash chromatography (*n*-heptane/EtOAc 3:1) of the residue furnished unreacted starting material **SI-5** (981 mg, 71%) and *endo* ring-opening product **SI-6** (185 mg, 12%; 41% brsm). **^{1}H-NMR** ($(CD_3)_2SO$, 400 MHz): 7.87 (d, 1H, *J* = 8.2 Hz), 7.39 (d, 1H, *J* = 0.7 Hz), 7.23 (m, 6H), 7.12 (m, 1H), 4.79 (d, 1H, *J* = 7.2 Hz), 4.18 (m, 1H), 4.02 (dd, 1H, *J* = 10.8, 4.3 Hz), 3.87 (dd, 1H, *J* = 10.8, 6.6 Hz), 3.67 (td, 1H, *J* = 7.0, 4.9 Hz), 3.60 (s, 3H), 3.25 (dd, 1H, *J* = 6.9, 4.9 Hz), 3.05 (s, 3H), 2.82 (dd, 1H, *J* = 13.9, 6.7 Hz), 2.73 (dd, 1H, *J* = 13.9, 7.4 Hz), 2.29 (m, 1H), 2.26 (s, 3H), 1.12 (d, 3H, *J* = 7.0 Hz), 1.04 (d, 3H, *J* = 7.1 Hz); **^{13}C-NMR** ($CDCl_3$, 100 MHz): 176.1, 157.1, 140.8, 137.8, 136.9, 133.4, 129.3, 128.7, 128.6, 128.2, 126.8, 124.2, 77.2, 65.7, 61.6, 50.1, 42.6, 42.0, 37.5, 35.5, 20.5, 16.4, 16.3; **HRMS** (ESI) *m*/*z* calcd. for $[M+H]^+$ $C_{25}H_{34}BrN_2O_5$: 521.1651, found: 521.1650; **Specific rotation:** $[\alpha]_D^{22}$ = + 7.5 (*c* = 1.6; $CHCl_3$).

(2*S*,3*R*,4*S*)-4-(2-Bromo-4-methylphenyl)-3-hydroxy-*N*-methoxy-*N*,2-dimethylpentan-amide (26):
To a stirred suspension of MeON(H)Me·HCl (917 mg, 9.12 mmol, 3.0 eq.) in THF (20 mL) a solution of Me_3Al (2 M in toluene, 4.56 mL, 9.20 mmol, 3.0 eq.) was added dropwise. After stirring the mixture for 30 min at 0 °C, a solution of **37** (1.40 g, 3.04 mmol) in THF (5 mL) was added dropwise. Stirring was continued for 1 h at 0 °C and for 3 h at r.t. The mixture was carefully quenched with saturated potassium sodium tartrate solution (4 mL) and extracted with MTBE. The combined organic phases were washed with saturated aqueous $NaHCO_3$ solution and brine, dried over $MgSO_4$, and concentrated under reduced pressure. Flash chromatography (*n*-heptane/EtOAc 3:1 to 2:1) of the residue gave **26** (996 mg, 95%) as a colorless oil and (*S*)-4-benzyloxazolidin-2-one (491 mg, 91%) as a colorless solid. **^{1}H-NMR** (CD_3OD, 600 MHz): 7.39 (s, 1H), 7.24 (m, 1H), 7.13 (m, 1H), 4.00 (dd, 1H, *J* = 7.8, 4.4 Hz), 3.49 (s, 3H), 3.30 (dq, 1H, *J* = 7.8, 6.9 Hz), 3.13 (s, 3H), 2.80 (b, 1H), 2.27 (s, 3H), 1.24 (d, 3H, *J* = 6.9 Hz), 1.10 (d, 3H, *J* = 7.0 Hz); **^{13}C-NMR** (CD_3OD, 150 MHz): 178.2, 142.1, 139.2, 134.2, 129.8, 129.6, 125.1, 76.2, 61.8, 42.6, 39.6, 32.5, 20.5, 18.1, 11.6; **HRMS** (ESI) *m*/*z* calcd. for $[M+H]^+$ $C_{15}H_{23}BrNO_3$: 344.0861, found: 344.0857; **Specific rotation:** $[\alpha]_D^{22}$ = ± 0 (*c* = 8.5; $CHCl_3$).

(2*S*,3*R*,4*S*)-4-(2-((*E*)-3-((*tert*-Butyldimethylsilyl)oxy)prop-1-en-1-yl)-4-methylphenyl)-3-hydroxy-*N*-methoxy-*N*,2-dimethylpentanamide (38):
Cesium carbonate (2.61 g, 8.00 mmol, 4.0 eq.), **13** (689 mg, 2.00 mmol) and freshly prepared[7] *tert*-butyl-dimethyl-[(*E*)-3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)allyloxy]silane **34** (746 mg, 2.5 mmol, 1.25 eq.) were weighed into a screw-capped reaction tube. The vessel was evacuated and back-filled with argon (three times) after which a degassed mixture of THF and water (9:1, 1.5 mL) followed by XPhos Pd G4 (35 mg, 2 mol%) was added. The reaction tube was closed and the mixture was stirred for 2 h at 60 °C. Another portion of XPhos Pd G4 (17.5 mg, 1 mol%) was added and stirring was continued for 2 h at 60 °C. After cooling to r.t., the reaction mixture was diluted with MTBE and extracted with brine. The organic phase was dried over $MgSO_4$ and concentrated and the residue was purified by

flash chromatography (*n*-heptane/EtOAc 6:1 to 2:1) to yield 844 mg (96%) of the coupling product **38** as a brownish oil which solidified upon standing. **^{1}H NMR** (CD_3OD, 700 MHz): 7.25 (s, 1H), 7.21 (m, 1H), 7.05 (d, 1H, *J* = 8.2 Hz), 6.99 (br d, 1H, *J* = 15.7 Hz), 6.08 (dt, 1H, *J* = 15.6, 4.9 Hz), 4.35 (br d, 2H, *J* = 4.8 Hz), 4.03 (dd, 1H, *J* = 9.2, 1.8 Hz), 3.66 (s, 3H), 3.52 (br s, 1H), 3.27 (m, 1H), 3.19 (s, 3H), 3.16 (m, 1H), 2.30 (s, 3H), 1.23 (d, 3H, *J* = 7.0 Hz), 1.14 (d, 3H, *J* = 7.0 Hz), 0.94 (s, 9H), 0.11 (s, 6H); **^{13}C NMR** (CD_3OD, 176 MHz): 178.3, 140.4, 137.8, 136.6, 132.0, 129.3, 129.1, 128.9, 128.1, 77.1, 65.2, 40.5, 37.8, 32.3, 26.5, 21.1, 20.3, 19.3, 13.9, –5.0; **HRMS** (ESI) *m*/*z* calcd. for $[M+Na]^+$ $C_{24}H_{41}NNaO_4Si$: 458.2703, found: 458.2695; **Specific rotation:** $[\alpha]_D^{22}$ = + 4 (*c* = 4.0; $CHCl_3$).

(3*S*,4*R*,5*S*)-5-(2-((*E*)-3-((*tert*-Butyldimethylsilyl)oxy)prop-1-en-1-yl)-4-methylphenyl)-4-hydroxy-3-methylhexan-2-one (39):
To a stirred solution of Weinreb amide **38** (261 mg, 0.600 mmol) in Et_2O (6 mL) MeMgBr (3 M solution in Et_2O, 0.600 mL, 1.80 mmol, 3.0 eq.) was added dropwise at 0 °C. The mixture was stirred for 2 h at 0 °C. Another portion of MeMgBr (3 M solution in Et_2O, 0.200 mL, 0.600 mmol, 1.0 eq.) was added and stirring was continued for 1 h at 0 °C and then for 1 h at r.t.. The mixture was quenched with saturated aqueous NH_4Cl solution at 0 °C and extracted with MTBE. The combined organic phases were dried over $MgSO_4$ and concentrated. Flash chromatography (*n*-heptane/EtOAc 8:1) yielded 157 mg (67%) of the methyl ketone **39** as a colorless, viscous oil. **^{1}H NMR** (CD_3OD, 500 MHz): 7.24 (s, 1H), 7.15 (d, 1H, *J* = 8.0 Hz), 7.09 (d, 1H, *J* = 8.0 Hz), 6.98 (br d, 1H, *J* = 15.5 Hz), 6.13 (dt, 1H, *J* = 15.5, 4.8 Hz), 4.39 (dd, 2H, *J* = 4.8, 1.7 Hz), 4.26 (dd, 1H, *J* = 9.2, 3.2 Hz), 3.09 (m, 1H), 2.35 (m, 1H), 2.31 (s, 3H), 2.07 (s, 3H), 1.30 (d, 3H, *J* = 6.9 Hz), 0.97 (s, 9H), 0.95 (d, 3H, J=7.0 Hz), 0.15 (s, 6H); **^{13}C NMR** (CD_3OD, 125 MHz): 214.0, 140.2, 137.2, 137.0, 132.7, 129.7, 128.6, 128.4, 127.9, 76.5, 64.9, 51.2, 39.8, 28.4, 26.4, 21.1, 19.5, 19.2, 9.4, –5.0; **HRMS** (ESI) *m*/*z* calcd. for $[M+Na]^+$ $C_{23}H_{38}NaO_3Si$: 413.2488, found: 413.2485; **Specific rotation:** $[\alpha]_D^{22}$ = – 17 (*c* = 1.0; $CHCl_3$).

(3*S*,4*R*,5*S*)-4-Hydroxy-5-(2-((*E*)-3-hydroxyprop-1-en-1-yl)-4-methylphenyl)-3-methyl-hexan-2-one (NFAT-133, 1):
A solution of **39** (89.0 mg, 0.230 mmol) in THF (0.5 mL) was prepared in a polyethylene reaction vessel. HF·py (70% HF, 30.0 µL, 5.0 eq.) was added and the mixture was stirred for 5 h at r.t. whereupon it was diluted with MTBE and quenched with saturated aqueous $NaHCO_3$ solution. The organic phase was separated, dried over $MgSO_4$ and concentrated. Flash chromatography (*n*-heptane/EtOAc 2:1 to 1:2) of the residue furnished 61 mg (97%) of NFAT-133 (**1**) as a as a colorless, viscous oil. **^{1}H NMR** (CD_3OD, 500 MHz): 7.26 (s, 1H), 7.16 (d, 1H, *J* = 8.0 Hz), 7.08 (d, 1H, *J* = 8.0 Hz), 6.96 (d, *J* = 15.6 Hz), 6.17 (dt, 1H, *J* = 15.5, 5.4 Hz), 4.27 (dd, 2H, *J* = 5.3, 1.4 Hz), 4.22 (dd, 2H, *J* = 9.0, 3.5 Hz), 3.10 (m, 3H), 2.38 (qd, 1H, *J* = 6.9, 3.6 Hz), 2.31 (s, 3H), 2.08 (s, 3H), 1.31 (d, 3H, *J* = 6.9 Hz), 0.96 (d, 3H, *J* = 7.0 Hz); **^{13}C NMR** (CD_3OD, 125 MHz): 214.0, 140.2, 137.1, 137.0, 132.7, 129.7, 129.2, 128.5, 127.9, 76.5, 63.7, 51.2, 39.7, 28.4, 21.0, 19.3, 9.6; **HRMS** (ESI) *m*/*z* calcd. for $[M+Na]^+$ $C_{17}H_{24}NaO_3$: 299.1623, found: 299.1617; **Specific rotation:** $[\alpha]_D^{22}$ = + 40 (*c* = 0.45; MeOH).

(2*S*,3*R*,4*S*)-3-((*tert*-Butyldimethylsilyl)oxy)-4-(2-((*E*)-3-((*tert*-butyldimethylsilyl)oxy)-prop-1-en-1-yl)-4-methylphenyl)-*N*-methoxy-*N*,2-dimethyl-pentanamide (40):
To a solution of alcohol **38** (484 mg, 1.11 mmol) in CH_2Cl_2 (7 mL) 2,6-lutidine (207 µL, 1.78 mmol, 1.6 eq.) was added followed by TBSOTf (383 µL, 1.67 mmol, 1.5 eq.) The mixture was stirred for 3 h at r.t. and was then concentrated under reduced pressure. Flash chromatography (*n*-heptane/EtOAc 9:1) of the residue yielded 604 mg (99%) of the bis-silyl ether **40** as a colorless, viscous oil. **^{1}H-NMR** ($(CD_3)_2SO$), 400 MHz): 7.25 (s, 1H), 7.08 (m, 2H), 6.94 (br d, 1H, *J* = 15.7 Hz), 6.15 (dt, 1H, *J* = 15.5, 4.6 Hz), 4.33 (dd, 2H, *J* = 4.5, 1.6 Hz), 4.22 (dd, 1H, *J* = 7.7, 2.9 Hz), 3.32 (s, 3H), 3.06 (m, 1H), 3.02 (s, 3H), 2.56 (dt, 1H, *J* = 3.6, 1.8 Hz), 2.25 (s, 3H), 1.15 (d, 3H, *J* = 7.0 Hz), 0.88 (m, 12H), 0.86 (m, 9H), 0.07 (m, 6H), –0.10 (s, 3H), –0.17 (s, 3H); **^{13}C NMR** ($CDCl_3$, 100 MHz): 175.5, 139.4, 136.2, 135.4, 131.4, 128.0, 127.6, 127.6, 127.1, 76.4, 64.1, 60.6, 40.0, 39.0, 32.3, 26.3, 26.0, 21.0, 18.7, 18.4, 11.2, –2.9, –3.8, –4.1, –5.1; **HRMS** (ESI) *m/z* calcd. for $[M+Na]^+$ $C_{30}H_{55}NNaO_4Si_2$: 572.3567, found: 572.3564; **Specific rotation:** $[\alpha]_D^{22}$ = + 1.0 (*c* = 5.0; $CHCl_3$).

(2*S*,3*R*,4*S*)-3-((*tert*-Butyldimethylsilyl)oxy)-4-(2-((*E*)-3-((*tert*-butyldimethylsilyl)oxy)-prop-1-en-1-yl)-4-methylphenyl)-2-methylpentanal (41):
To a solution of **40** (1.11 g, 2.01 mmol) in THF (8 mL) DIBAL-H (1.2 M in toluene, 3.35 mL, 4.02 mmol, 2 eq.) was slowly added within 10 min at –78°C. The mixture was stirred for 80 min at –78°C. Saturated potassium sodium tartrate solution (10 mL) was added dropwise and stirring was continued at –78°C for 30 min after which the mixture was warmed to 0 °C and extracted with MTBE. The combined organic phases were dried over $MgSO_4$ and concentrated (heating bath temperature of the rotary evaporator 30 °C). Flash chromatography (*n*-heptane 100% to *n*-heptane/EtOAc 4:1) yielded 954 mg (97%) of aldehyde **41** as a pale yellow oil. **^{1}H-NMR** ($CDCl_3$, 400 MHz): 9.66 (s, 1H), 7.20 (s, 1H), 7.06 (m, 2H), 6.92 (br d, 1H, *J* = 15.7 Hz), 6.09 (dt, 1H, *J* = 15.5, 4.6 Hz), 4.42 (dd, 1H, *J* = 8.4, 2.3 Hz), 4.37 (dd, 2H, *J* = 4.6, 1.8 Hz), 3.21 (quin, 1H, *J* = 7.3 Hz), 2.31 (s, 3H), 2.19 (dd, 1H, *J* = 7.0, 2.4 Hz), 1.26 (d, 3H, *J* = 6.9 Hz), 1.04 (d, 3H, *J* = 7.0 Hz), 0.95 (s, 9H), 0.88 (s, 9H), 0.12 (s, 6H), 0.00 (s, 3H), –0.05 (s, 3H); **^{13}C NMR** ($CDCl_3$, 100 MHz): 205.4, 139.0, 136.0, 135.8, 131.7, 128.5, 127.7, 126.8, 126.8, 65.4, 63.7, 50.7, 38.3, 26.1, 26.0, 21.0, 18.9, 18.4, 18.3, 7.3, –3.7, –4.4, –5.2; **HRMS** (ESI) *m*/*z* calcd. for $[M+Na]^+$ $C_{28}H_{50}NaO_3Si_2$: 513.3196, found: 513.3201; **Specific rotation:** $[\alpha]_D^{22}$ = + 31 (*c* = 1.6; $CHCl_3$).

***tert*-Butyl(((2*S*,3*S*,4*R*)-6,6-dibromo-2-(2-((*E*)-3-((*tert*-butyldimethylsilyl)oxy)prop-1-en-1-yl)-4-methylphenyl)-4-methylhex-5-en-3-yl)oxy)dimethylsilane (42):**
To a solution of aldehyde **41** (158 mg, 0.320 mmol) in CH_2Cl_2 (2.5 mL) NEt_3 (139 µL, 1.00 mmol, 3.1 eq.) was added followed by a solution of PPh_3 (405 mg, 1.54 mmol, 4.8 eq.) and CBr_4 (256 mg, 0.770 mmol, 2.4 eq.) in CH_2Cl_2 (2.5 mL) which had been freshly prepared at 0 °C. The mixture was stirred for 1 h at 0 °C after which it was concentrated under reduced pressure. The residue was purified by flash chromatography (0 to 1% EtOAc in *n*-heptane) to give dibromo-alkene **42** (171 mg, 82%) as a colorless oil. **^{1}H NMR** ($CDCl_3$, 400 MHz); 7.21 (s, 1H), 7.10 (d, 1H, *J* = 8.0 Hz), 7.03 (m, 1H), 6.91 (br d, 1H, *J* = 15.5 Hz), 6.33 (d, 1H, *J* = 9.5 Hz), 6.11 (dt, 1H, *J* = 15.5, 4.8 Hz), 4.38 (dd, 2H, *J* = 4.8, 1.7 Hz), 3.89 (dd, 1H, *J* = 8.2, 3.0 Hz), 3.12 (quin, 1H, *J* = 7.2 Hz), 2.38 (m, 1H), 2.32 (s, 3H), 1.23 (d, 3H, *J* = 6.9 Hz), 0.96 (s, 9H), 0.95 (s, 9H), 0.90 (d, 3H, *J* = 6.8 Hz), 0.13 (s, 6H), 0.08 (s, 3H), –0.04 (s, 3H); **^{13}C NMR** ($CDCl_3$, 100 MHz): 143.6, 139.1, 135.7, 135.5, 131.4, 128.3, 127.5, 127.3, 127.2, 87.5, 78.5, 63.8, 41.8, 38.5, 26.2, 26.0, 21.0, 19.3, 18.5, 18.4, 12.7, –3.5, –3.9, –5.1; **HRMS** (ESI) *m*/*z* calcd. for $[M-C_6H_{15}OSi]^+$ $C_{23}H_{35}Br_2OSi$: 513.0824, found: 513.0820; **Specific rotation:** $[\alpha]_D^{22}$ = + 9 (*c* = 0.8; $CHCl_3$).

Methyl (4*R*,5*S*,6*S*)-5-((*tert*-butyldimethylsilyl)oxy)-6-(2-((*E*)-3-((*tert*-butyldimethylsilyl)-oxy)prop-1-en-1-yl)-4-methylphenyl)-4-methylhept-2-ynoate (43):
To a solution of dibromo-alkene **42** (352 mg, 0.544 mmol) in THF (4 mL) *n*-BuLi (2.5 M in hexanes, 0.650 mL, 1.63 mmol, 3.0 eq.) was added dropwise at –78°C. The mixture was stirred for 1 h at –78°C after which methyl chloroformate (168 µL, 2.18 mmol, 4.0 eq.) was added dropwise. Stirring was continued for 1 h at –78°C. The reaction mixture was then warmed to 0 °C, quenched with saturated aqueous NH_4Cl solution and extracted with MTBE. The combined organic phases were dried over $MgSO_4$ and concentrated. Flash chromatography (1% EtOAc in *n*-heptane) of the residue provided 224 mg (76%) of ynone **43** as a colorless oil. **^{1}H NMR** ($CDCl_3$, 400 MHz): 7.21 (s, 1H), 7.04 (m, 2H), 6.90 (d, 1H, *J* = 15.6 Hz), 6.10 (dt, 1H, *J* = 15.5, 5.0 Hz), 4.37 (dd, 2H, *J* = 5.0, 1.6 Hz), 4.00 (dd, 1H, *J* = 7.5, 3.3 Hz), 3.75 (s, 3H), 3.25 (quin, 1H, *J* = 7.0 Hz), 2.59 (qd, 1H, *J* = 7.0, 3.3 Hz), 2.32 (s, 3H), 1.27 (d, 3H, *J* = 6.9 Hz), 1.12 (d, 3H, *J* = 7.0 Hz), 0.95 (s, 9H), 0.93 (s, 9H), 0.12 (m, 6H), –0.01 (s, 3H), –0.11 (s, 3H); **^{13}C NMR** ($CDCl_3$, 100 MHz): 154.2, 138.8, 135.9, 135.8, 131.7, 128.5, 127.6, 127.2, 127.0, 93.4, 89.1, 78.2, 73.9, 63.9, 52.5, 38.2, 30.3, 26.2, 26.0, 21.0, 18.5, 18.4, 18.1, 13.9, –3.5, –4.5, –5.1, –5.2; **HRMS** (ESI) *m*/*z* calcd. for $[M+Na]^+$ $C_{31}H_{52}NaO_4Si_2$: 567.3302, found: 567.3306; **Specific rotation:** $[\alpha]_D^{22}$ = + 20 (*c* = 1.2; $CHCl_3$).

***tert*-Butyl (6*R*,7*S*,8*S*,*Z*)-7-((*tert*-butyldimethylsilyl)oxy)-8-(2-((*E*)-3-((*tert*-butyldimethyl-silyl)oxy)prop-1-en-1-yl)-4-methylphenyl)-3-hydroxy-2,6-dimethylnon-2-en-4-ynoate (24):**

To a stirred solution of diisopropylamine (175 µL, 1.25 mmol, 3.0 eq.) in THF (1.5 mL) *n*-BuLi (2.5 M in hexanes, 0.500 mL, 1.25 mmol, 3.0 eq.) was added dropwise at 0 °C. The mixture was stirred for 30 min at 0 °C and was then cooled to –78 °C. *tert*-Butyl propionate **44** (187 µL, 1.25 mmol, 3.0 eq.) was added dropwise and stirring was continued for 30 min at –78 °C. LiHMDS (1 M in THF, 0.500 mL, 0.500 mmol, 1.2 eq.) was added dropwise followed by a solution of ester **43** (226 mg, 0.420 mmol) in THF (1.5 mL). The mixture was stirred for 2 h at –78°C, quenched with saturated aqueous NH_4Cl solution, and extracted with MTBE. The combined organic phases were dried over $MgSO_4$ and concentrated. Flash chromatography (2% EtOAc in *n*-heptane) of the residue furnished *β*-ketoester **24** (223 mg, 84%) as a colorless oil. **^{1}H NMR** ($CDCl_3$, 400 MHz, major tautomer (enol)): 12.26 (s, 1H, OH), 7.23 (d, 1H), 7.08 (m, 1H), 7.03 (m, 1H), 6.93 (br d, 1H, *J* = 15.6 Hz), 6.11 (br d, 1H, J = 15.6 Hz), 4.36 (dd, 2H, *J* = 5.0, 1.6 Hz), 3.98 (dd, 1H, *J* = 6.6, 4.1 Hz), 3.36 (t, 1H, *J* = 6.8 Hz), 2.74 (dd, 1H, *J* = 7.0, 4.1 Hz), 2.32 (s, 3H), 1.93 (s, 3H), 1.52 (s, 9H), 1.29 (d, 3H, *J* = 7.1 Hz), 1.15 (d, 3H, *J* = 7.0 Hz), 0.95 (s, 9H), 0.92 (s, 9H), 0.12 (m, 6H), –0.08 (m, 12H), –0.22 (s, 3H); **^{13}C NMR** ($CDCl_3$, 100 MHz, major tautomer (enol)): 172.9, 152.0, 139.2, 135.8, 135.7, 131.5, 128.4, 127.5, 127.4, 127.3, 104.3, 103.1, 81.7, 78.3, 76.4, 64.1, 38.1, 31.5, 28.2, 26.2, 26.0, 21.0, 18.4, 18.4, 17.2, 15.3, 13.5, –3.4, –4.7, –5.1, –5.1; **HRMS** (ESI) *m*/*z* calcd. for $[M+H]^+$ $C_{37}H_{63}O_5Si_2$: 643.4214, found: 643.4215; **Specific rotation:** $[\alpha]_D^{22}$ = + 13 (*c* = 1.0; $CHCl_3$).

6-((2*S*,3*R*,4*S*)-3-((*tert*-Butyldimethylsilyl)oxy)-4-(2-((*E*)-2-((*tert*-butyldimethylsilyl)-oxy)vinyl)-4-methylphenyl)pentan-2-yl)-4-hydroxy-3-methyl-2H-pyran-2-one (47):
To a stirred dispersion of *β*-ketoester **24** (104 mg, 0.162 mmol) in $MeNO_2$ (1.0 mL) HOAc (0.1 mL) was added. Stirring was continued for 15 min at r.t. after which MS4Å beads (50 mg) were added followed by SPhosAuNTf$_2$ (7.4 mg, 5 mol%). The mixture was stirred for 24 h at r.t., diluted with EtOAc, and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by flash chromatography (*n*-heptane/EtOAc 9:1 to 4:1) to furnish unreacted starting material **24** (29 mg, 28%) and 34 mg (55%, 81% brsm) of the *bis*-silylated *α*-pyrone cyclization product **47** as a colorless solid. **^{1}H NMR** (CD_3OD, 400 MHz): 7.22 (s, 1H), 7.17 (d, 1H, *J* = 7.9 Hz), 7.10 (d, 1H, *J* = 8.1 Hz), 6.98 (d, 1H, *J* = 15.6 Hz), 6.12 (dt, 1H, *J* = 15.5, 4.4 Hz), 5.89 (s, 1H), 4.42 (br d, 1H, *J* = 8.6 Hz), 4.38 (m, 2H), 3.19 (m, 1H), 2.46 (m, 1H), 2.28 (s, 3H), 1.83 (s, 3H), 1.25 (d, 3H, *J* = 6.8 Hz), 1.06 (d, 3H, *J* = 6.9 Hz), 0.96 (s, 9H), 0.90 (s, 9H), 0.13 (s, 6H), 0.00 (s, 3H), –0.30 (s, 3H); **^{13}C NMR** (CD_3OD, 100 MHz): 168.8, 167.7, 166.9, 140.2, 137.0, 137.0, 132.7, 129.8, 128.4, 128.0, 127.7, 101.7, 99.4, 78.6, 64.5, 42.0, 40.4, 26.7, 26.5, 21.1, 20.8, 19.3, 19.2, 10.4, 8.2, –3.5, –4.1, –5.1, –5.1; **HRMS** (ESI) *m*/*z* calcd. for $[M+H]^+$ $C_{33}H_{55}O_5Si_2$: 587.3588, found: 587.3582; **Specific rotation:** $[\alpha]_D^{22}$ = + 58 (*c* = 0.8; MeOH).

4-Hydroxy-6-((2*S*,3*R*,4*S*)-3-hydroxy-4-(2-((*E*)-3-hydroxyprop-1-en-1-yl)-4-methyl-phenyl)pentan-2-yl)-3-methyl-2H-pyran-2-one (TM-123, 13):
A solution of **47** (51.0 mg, 0.087 mmol) in THF (1.2 mL) was prepared in a polyethylene reaction vessel. HF·py (70% HF, 0.1 mL) was added and the mixture was stirred for 3 d at r.t. after which another portion of HF·py (70% HF, 0.1 mL) was added. Stirring was continued for 1 d at r.t. and for 3 h at 40 °C. The reaction mixture was cooled to r.t., diluted with MTBE, quenched by addition of solid $NaHCO_3$ (840 mg, 10.0 mmol), and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by flash chromatography (*n*-heptane/EtOAc 1:1 to 100% EtOAc) to give TM-123 **13** (28.0 mg, 90%) as a colorless solid. **^{1}H NMR** (CD_3OD, 400 MHz): 7.20 (d, 1H, *J* = 1.6 Hz), 7.14 (d, 1H, *J* = 7.8 Hz), 7.02 (d, 1H, *J* = 7.8 Hz), 6.84 (br d, 1H, *J* = 15.7 Hz), 6.13 (dt, 1H, *J* = 15.7, 5.8 Hz), 5.89 (s, 1H), 4.22 (d, 2H, *J* = 5.3 Hz), 3.97 (t, 1H, *J* = 6.7 Hz), 3.08 (quin, 1H, *J* = 7.0 Hz), 2.47 (quin, 1H, *J* = 6.7 Hz), 2.27 (s, 3H), 1.82 (s, 3H), 1.27 (d, 3H, *J* = 6.9 Hz), 1.13 (d, 3H, *J* = 6.8 Hz); **^{13}C NMR** (CD_3OD, 100 MHz): 169.0, 167.8, 166.9, 140.2, 136.9, 132.8, 129.6, 129.5, 128.5, 128.3, 101.1, 99.2, 77.1, 63.8, 43.1, 39.7, 21.1, 18.4, 13.1, 8.3; **HRMS** (ESI) *m*/*z* calcd. for $[M+H]^+$ $C_{21}H_{27}O_5$: 359.1859, found: 359.1854; **Specific rotation:** $[\alpha]_D^{22}$ = + 114 (*c* = 1.02; MeOH).

2-(Trimethylsilyl)ethyl butyrate (45):

To a solution of butyric acid (1.76 g, 20 mmol) and DMAP (122 mg, 5 mol%) in CH_2Cl_2 (40 mL) a solution of DCC (4.33 g, 21.0 mmol, 1.05 eq.) in CH_2Cl_2 (10 mL) was added dropwise at 0 °C. The mixture was stirred for 24 h at r.t. after which it was diluted with *n*-pentane and filtered. The filtrate was washed consecutively with saturated aqueous $NaHCO_3$ solution (3x), 1 N $KHSO_4$ solution, saturated aqueous $NaHCO_3$ solution and brine, dried ($MgSO_4$), and concentrated under reduced pressure. The residue was purified by flash filtration over a short path of silica, which was eluted with *n*-pentane (100%). Concentration of the eluate under reduced pressure provided 2-(trimethylsilyl)ethyl butyrate **45** (3.15 g, 83%) as a colorless liquid. **^{1}H NMR** ($CDCl_3$, 400 MHz); 4.18 (m, 2H), 2.29 (q, 2H, *J* = 7.4 Hz), 1.61 (m, 2H), 0.97 (m, 5H), 0.07 (s, 9H); **^{13}C NMR** ($CDCl_3$, 100 MHz): 173.8, 62.3, 36.4, 18.4, 17.3, 13.7, -1.5; **HRMS** (ESI) *m*/*z* calcd. for $[M\text{-}C_3H_7]^+$ $C_6H_{13}O_2Si$: 145.0685, found: 145.0679.

2-(Trimethylsilyl)ethyl (6*R*,7*S*,8*S*,*E*)-7-((*tert*-butyldimethylsilyl)oxy)-8-(2-((*E*)-3-((*tert*-butyl dimethylsilyl)oxy)prop-1-en-1-yl)-4-methylphenyl)-2-ethyl-3,6-dimethylnon-2-en-4-ynoate (46):
To a stirred solution of diisopropylamine (0.20 0mL, 1.45 mmol, 3.0 eq.) in THF (1.5 mL) *n*-BuLi (2.5 M in hexanes, 0.580 mL, 1.45 mmol, 3.0 eq.) was added dropwise at 0 °C. The mixture was stirred for 30 min at 0 °C and then cooled to –78 °C. 2-(Trimethylsilyl)ethyl butyrate **45** (274 mg, 1.45 mmol, 3.0 eq.) was added dropwise and stirring was continued for 30 min at –78°C. LiHMDS (1 M in THF, 0.580 mL, 0.580 mmol, 1.2 eq.) was added dropwise followed by a solution of ester **43** (264 mg, 0.480 mmol) in THF (1.5 mL). The mixture was stirred for 2 h at –78°C, quenched with saturated aqueous NH_4Cl solution, and extracted with MTBE. The combined organic phases were dried over $MgSO_4$ and concentrated. Flash chromatography (2% EtOAc in *n*-heptane) of the residue furnished *β*-ketoester **46** (321 mg, 94%) as a colorless oil. **^{1}H NMR** ($CDCl_3$, 500 MHz, major tautomer (enol)): 12.22 (s, 1H, OH), 7.23 (d, 1H), 7.08 (d, 1H), 7.04 (m, 1H), 6.92 (m, 1H), 6.12 (m, 1H), 4.36 (m, 2H), 4.28 (m, 2H), 3.96 (dd, 1H, *J* = 6.6, 4.2 Hz), 3.35 (m, 1H), 2.74 (m, 1H), 2.33 (m, 2H), 2.32 (s, 3H), 1.29 (d, 3H, *J* = 7.1 Hz), 1.15 (d, 3H, *J* = 7.0 Hz), 1.06 (m, 2H), 1.01 (m, 3H), 0.94 (s, 9H), 0.91 (s, 9H), 0.11 (m, 6H), 0.07 (m, 12H), –0.25 (s, 3H); **^{13}C NMR** ($CDCl_3$, 125 MHz, major tautomer (enol)): 173.3, 152.5, 139.0, 135.7, 135.7, 131.5, 128.4, 127.4, 127.2, 127.2, 109.6, 102.9, 78.1, 75.9, 64.1, 63.0, 38.0, 31.5, 26.1, 25.9, 21.0, 21.0, 18.4, 18.4, 17.3, 17.1, 15.1, 14.3, –1.5, –3.5, –4.8, –5.1, –5.2; **HRMS** (ESI) *m*/*z* calcd. for $[M+H]^+$ $C_{39}H_{69}O_5Si_3$: 701.4453, found: 701.4446; **Specific rotation:** $[\alpha]_D^{22}$ = + 11 (*c* = 1.0; $CHCl_3$).

6-((2*S*,3*R*,4*S*)-3-((*tert*-Butyldimethylsilyl)oxy)-4-(2-((*E*)-2-((*tert*-butyldimethylsilyl)-oxy)vinyl)-4-methylphenyl)pentan-2-yl)-3-ethyl-4-hydroxy-2H-pyran-2-one (48):
To a stirred dispersion of *β*-ketoester **46** (269 mg, 0.384 mmol) in $MeNO_2$ (2.0 mL) HOAc (0.4 mL) was added. Stirring was continued for 5 min at r.t. after which MS4Å beads (80 mg) were added followed by $SPhosAuNTf_2$ (17.6 mg, 5 mol%). The mixture was stirred for 24 h at r.t., diluted with EtOAc, and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by flash chromatography (*n*-heptane/EtOAc 9:1 to 7:3) to give 178 mg (77%) of the *bis*-silylated *α*-pyrone cyclization product **48** as a colorless solid. **^{1}H NMR** (CD_3OD, 500 MHz): 7.22 (d, 1H, *J* = 1.2 Hz), 7.18 (d, 1H, *J* = 7.9 Hz), 7.07 (dd, 1H, *J* = 7.9, 1.2 Hz), 6.99 (dt, 1H, *J* = 15.6, 1.7 Hz), 6.13 (dt, 1H, *J* = 15.6, 4.3 Hz), 5.88 (s, 1H), 4.44 (dd, 1H, *J* = 9.1, 2.3Hz), 4.39 (m, 2H), 3.19 (dq, 1H, *J* = 9.1, 6.9 Hz), 2.45 (dq, 1H, *J* = 7.0, 2.3 Hz), 2.38 (m, 2H), 2.28 (s, 3H), 1.24 (d, 3H, *J* = 6.9 Hz), 1.05 (d, 3H, *J* = 7.0 Hz), 1.01 (t, 3H, *J* = 7.4 Hz), 0.96 (s, 9H), 0.90 (s, 9H), 0.132 (s, 3H), 0.130 (s, 3H), 0.02 (s, 3H), –0.30 (s, 3H); **^{13}C NMR** (CD_3OD, 125 MHz): 168.4, 167.3, 167.1, 140.2, 137.0, 137.0, 132.7, 128.8, 128.4, 127.9, 127.7, 105.7, 101.8, 78.6, 64.5, 41.9, 40.4, 26.7,26.5, 21.1, 21.0, 19.3, 19.2, 17.3, 12.8, 10.1, –3.5, –4.1, –5.0, –5.1; **HRMS** (ESI) *m*/*z* calcd. for $[M+H]^+$ $C_{34}H_{57}O_5Si_2$: 601.3745, found: 601.3744; **Specific rotation:** $[\alpha]_D^{22}$ = + 63 (*c* = 0.6; MeOH).

3-Ethyl-4-hydroxy-6-((2*S*,3*R*,4*S*)-3-hydroxy-4-(2-((*E*)-3-hydroxyprop-1-en-1-yl)-4-methylphenyl)pentan-2-yl)-2H-pyran-2-one (Veramycin A, 17):
A solution of **48** (102 mg, 0.170 mmol) in THF (1.0 mL) was prepared in a polyethylene reaction vessel. HF·py (70% HF, 0.200 mL) was added and the mixture was stirred for 48 h at 40 °C. The reaction mixture was cooled to r.t., diluted with MTBE, quenched by addition of solid $NaHCO_3$ (840 mg, 10.0 mmol) and filtered. The filtrate was concentrated under reduced pressure and the residue was purified

by flash chromatography (*n*-heptane/EtOAc 1:2 to 100% EtOAc) to give veramycin A **17** (57.0 mg, 90%) as a colorless solid. **^{1}H NMR** (CD_3OD, 500 MHz): 7.20 (d, 1H, *J* = 1.6 Hz), 7.12 (d, 1H, *J* = 8.0 Hz), 7.01 (dd, 1H, *J* = 8.0, 1.6 Hz), 6.86 (dt, 1H, *J* = 15.6, 1.6 Hz), 6.13 (dt, 1H, *J* = 15.6, 5.6 Hz), 5.88 (s, 1H), 4.23 (m, 2H), 4.00 (dd, 1H, *J* = 7.8, 5.6 Hz), 3.07 (dq, 1H, *J* = 7.8, 6.9 Hz), 2.46 (dq, 1H, *J* = 7.0, 5.6 Hz), 2.36 (q, 2H, *J* = 7.4 Hz), 2.26 (s, 3H), 1.27 (d, 3H, *J* = 6.9 Hz), 1.12 (d, 3H, *J* = 7.0 Hz), 1.02 (t, 3H, *J* = 7.4 Hz) ; **^{13}C NMR** (CD_3OD, 125 MHz): 168.6, 167.5, 167.2, 140.2, 136.9, 136.9, 132.8, 129.6, 129.5, 128.5, 128.3, 105.3, 101.2, 77.1, 63.8, 43.1, 39.8 (b), 21.1, 18.5, 17.3, 13.0, 12.8; **HRMS** (ESI) *m*/*z* calcd. for $[M+Na]^+$ $C_{22}H_{28}NaO_5$: 395.1834, found: 395.1813; **Specific rotation:** $[\alpha]_D^{22}$ = + 128 (*c* = 0.6; MeOH).

6. Crystallographic Data Collection and Refinement Details

Diffraction data for all samples were collected at low temperatures (100K) using φ- and ω-scans on a BRUKER D8 Venture System equipped with dual IμS microfocus sources, a PHOTON100 detector and an OXFORD CRYOSYSTEMS 700 low temperature system. Mo-K_α radiation with a wavelength of 0.71073 Å, Cu-K_α radiation with a wavelength of 1.54178 Å and a collimating Quazar multilayer mirror were used. Semi-empirical absorption corrections from equivalents were applied using SADABS.[10] The structures were solved by direct methods using SHELXT[11] and refined against F^2 on all data by full-matrix least squares using SHELXL.[11] All nonhydrogen atoms were refined anisotropically N-H and O-H hydrogen atoms were located in the Fourier difference map and set to ideal distances. C-H hydrogen atoms were positioned at geometrically calculated positions and refined using a riding model. The isotropic displacement parameters of all hydrogen atoms were fixed to 1.2x or 1.5x (CH_3 and OH hydrogens) the U_{eq} value of the atoms they are linked to. All crystallographic data were deposited with the Cambridge Crystallographic Database as 2093273-2093275 and can be obtained free of charge at https://www.ccdc.cam.ac.uk/structures/.

6.1. Crystallographic data collection and refinement details for compound 33

The structure of **33** was solved in the monoclinic space group $P2_1$. The asymmetric unit contains one full molecule of **33**. The Flack parameter[12] of 0.014(4) indicated that it could be an enantiopure sample which was confirmed by statistical analysis of Bijvoet pairs.[13]

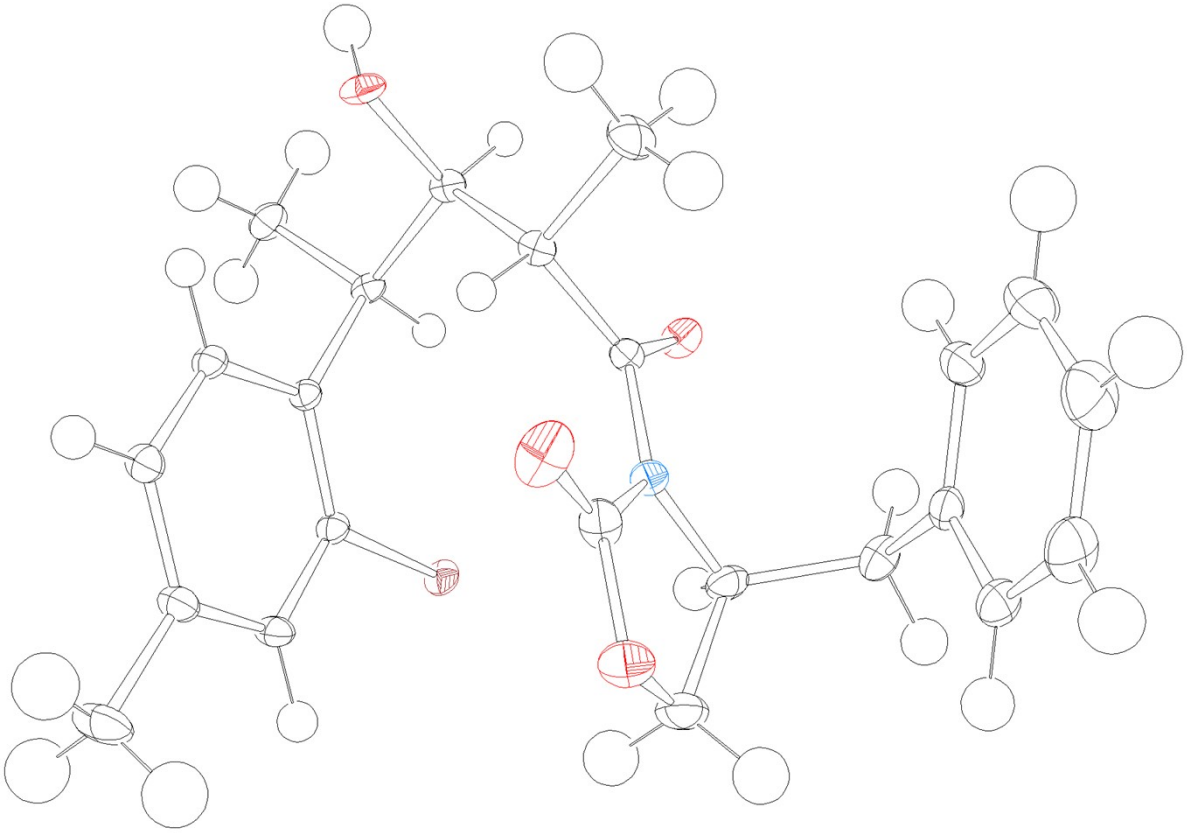


Figure S3: Molecular structure of 33. Thermal ellipsoids were set to 50% probability.

Table S10: Crystal data and structure refinement for **33**.

CCDC No	2093273
Empirical formula	C_{23} H_{26} Br N O_4
Formula weight	460.36
Temperature	100(2) K
Wavelength	0.71073 Å
Crystal system	Monoclinic
Space group	$P2_1$
Unit cell dimensions	a = 11.0574(6) Å α= 90°. b = 7.5672(4) Å β= 111.450(3)°. c = 13.7732(7) Å γ = 90°.
Volume	1072.63(10) $Å^3$
Z	2
Density (calculated)	1.425 Mg/m^3
Absorption coefficient	1.945 mm^{-1}
F(000)	476
Crystal size	0.412 x 0.092 x 0.058 mm^3
Theta range for data collection	2.957 to 35.630°.
Index ranges	–18 ≤ h ≤ 18, –12 ≤ k ≤ 12, –22 ≤ l ≤ 22
Reflections collected	140952
Independent reflections	9856 [R(int) = 0.0504]
Completeness to theta = 25.242°	99.7%
Absorption correction	Semi-empirical from equivalents
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	9856 / 2 / 268
Goodness-of-fit on F^2	1.050
Final R indices [I>2σ(I)]	R1 = 0.0225, wR2 = 0.0523
R indices (all data)	R1 = 0.0263, wR2 = 0.0535
Absolute structure parameter	0.0206(19)
Largest diff. peak and hole	0.522 and –0.357 $e.Å^{-3}$

6.2. Crystallographic data collection and refinement details for compound *epi*-26

The structure of ***epi*-26** was solved in the orthorombic space group $P2_12_12_1$. The asymmetric unit contains one full molecule. The absolute structure was confirmed with a Flack parameter of 0.018(6).

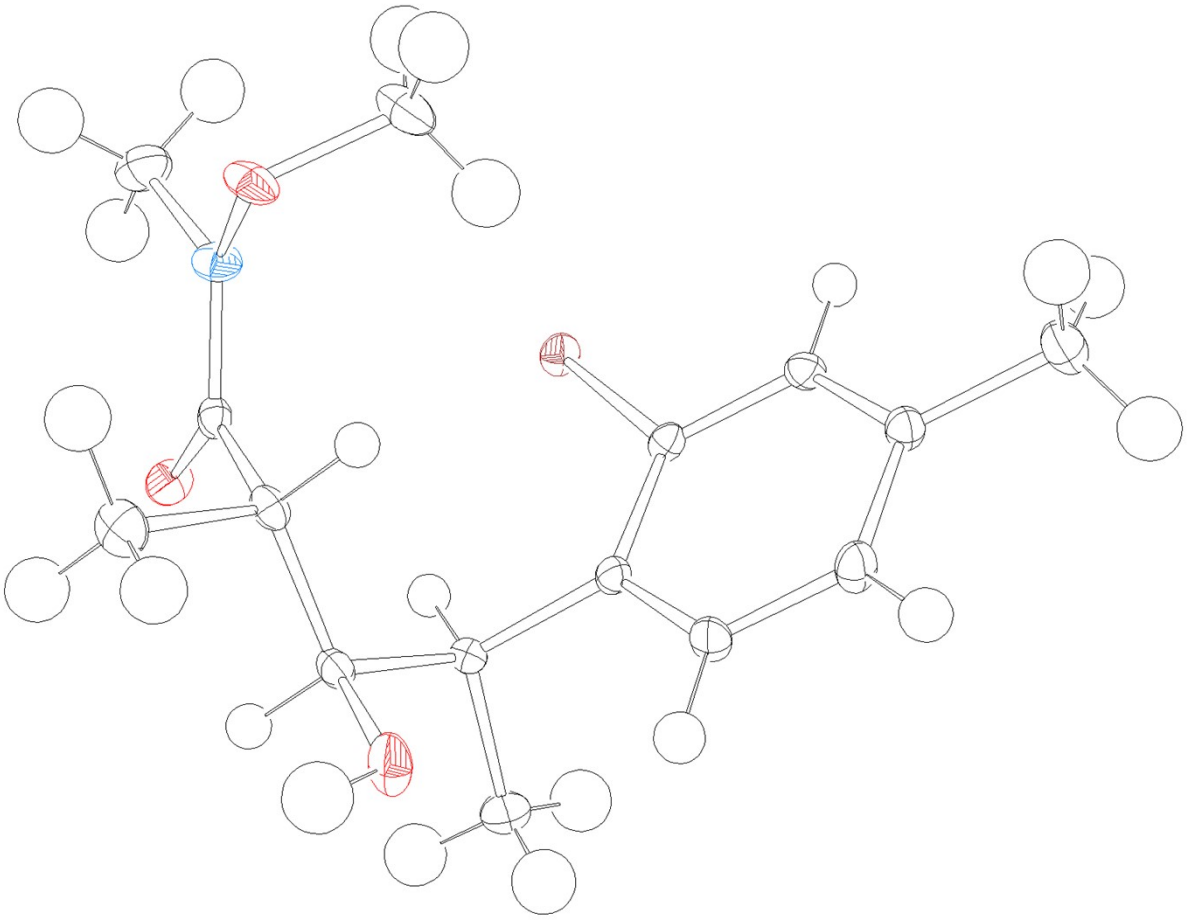


Figure S4: Molecular structure of *epi*-26. Thermal ellipsoids were set to 50% probability.

Table S11: Crystal data and structure refinement for ***epi*-26**.

CCDC No	2093274	
Empirical formula	C_{15} H_{22} Br N O_3	
Formula weight	344.24	
Temperature	100(2) K	
Wavelength	0.71073 Å	
Crystal system	Orthorhombic	
Space group	$P2_12_12_1$	
Unit cell dimensions	a = 8.1889(4) Å b = 10.6443(5) Å c = 18.0654(9) Å	α= 90°. β= 90°. γ = 90°.
Volume	1574.67(13) $Å^3$	
Z	4	

Density (calculated)	1.425 Mg/m^3
Absorption coefficient	2.617 mm^{-1}
F(000)	712
Crystal size	0.450 x 0.183 x 0.079 mm^3
Theta range for data collection	2.221 to 33.132°.
Index ranges	$-12 \le h \le 12$, $-16 \le k \le 16$, $-27 \le l \le 27$
Reflections collected	110262
Independent reflections	5998 [R(int) = 0.0684]
Completeness to theta = 25.242°	99.9%
Absorption correction	Semi-empirical from equivalents
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	5998 / 1 / 189
Goodness-of-fit on F^2	1.069
Final R indices [I>2σ(I)]	R1 = 0.0234, wR2 = 0.0503
R indices (all data)	R1 = 0.0281, wR2 = 0.0514
Absolute structure parameter	0.027(3)
Largest diff. peak and hole	0.350 and –0.545 $e.Å^{-3}$

6.3. Crystallographic data collection and refinement details for compound SI-6

The structure of **SI-6** was solved in the monoclinic space group $P2_1$. The asymmetric unit contains one full molecule of **SI-6** and the absolute structure was confirmed with a Flack parameter of 0.012(6).

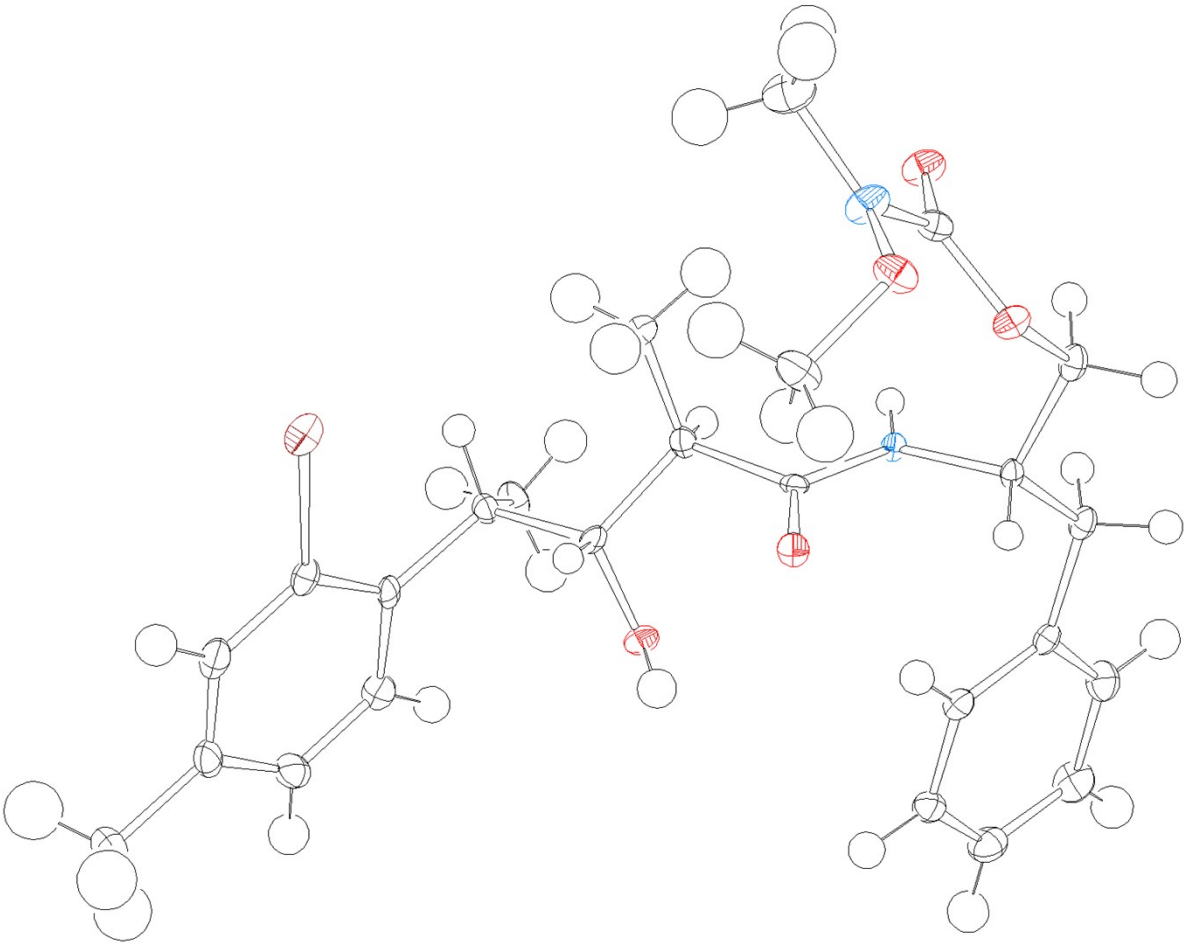


Figure S5: Molecular structure of SI-6. Thermal ellipsoids were set to 50% probability.

Table S12: Crystal data and structure refinement for **SI-6**.

CCDC No	2093275	
Empirical formula	$C_{25}\ H_{33}\ Br\ N_2\ O_5$	
Formula weight	521.44	
Temperature	100(2) K	
Wavelength	0.71073 Å	
Crystal system	Monoclinic	
Space group	$P2_1$	
Unit cell dimensions	a = 10.7555(15) Å b = 9.6510(14) Å c = 12.0461(18) Å	α= 90°. β= 92.244(5)°. γ = 90°.
Volume	1249.4(3) $Å^3$	
Z	2	
Density (calculated)	1.386 Mg/m^3	

Absorption coefficient	1.682 mm^{-1}
F(000)	544
Crystal size	0.104 x 0.020 x 0.012 mm^3
Theta range for data collection	2.491 to 27.101°.
Index ranges	–13 ≤ h ≤ 13, –12 ≤ k ≤ 12, –15 ≤ l ≤ 15
Reflections collected	39593
Independent reflections	5524 [R(int) = 0.0697]
Completeness to theta = 25.242°	99.9%
Absorption correction	Semi-empirical from equivalents
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	5524 / 3 / 309
Goodness-of-fit on F^2	1.017
Final R indices [I>2σ(I)]	R1 = 0.0291, wR2 = 0.0564
R indices (all data)	R1 = 0.0373, wR2 = 0.0586
Absolute structure parameter	0.018(4)
Largest diff. peak and hole	0.267 and –0.305 e.$Å^{-3}$

7. Biosynthesis

Streptomyces sp. ST157608 was grown in ISP2 medium and genomic DNA was isolated using the innuPREP bacterial DNA kit (Analytik Jena GmbH, Jena, Germany) and the whole genome was sequenced using Illumina sequencing technology. Assembly was performed using SPAdes. The obtained sequence was analysed using antiSMASH 4.0[14]. The most fitting candidate BGC was identified by the overall fitting number of PKS modules as well as two consecutive genes with modules consistent with the biosynthesis of the western lactone ring of the Veramycins. The putative BGC consists of 14 genes (Table S13).

Table S13. Genes and predicted enzymatic function

gene	length [nt]	predicted enzymatic function	homologue/acc. nr./strain	AA identity [%]
verA	2682	LuxR type transcriptional regulator	NftA/QPP46759.1/S. pactum	94.08
verB	636	TetR type transcriptional regulator	WP_197987675/S. pactum	99.05
verC	5505	PKS	NftC/QPP46749.1/S. pactum	97.44
verD	9198	PKS	NftD/QPP46750.1/S. pactum	97.26
verE	1437	cytochrome P450	WP_197987673.1/S. pactum	99.58
verF	945	MBL fold metallo-hydrolase	WP_197987672.1/S. pactum	97.13
verG	600	carboxymuconolactone decarboxylase family protein	WP_197987671.1/S. pactum	95.48
verH	1038	methyltransferase	WP_197987670.1/S. pactum	99.13
verI	939	endo alpha-1,4 polygalactosaminidase	WP_197987669.1/S. pactum	94.79
verJ	807	transcriptional regulator	NftJ/QPP46756.1/S. pactum	95.52
verK	6483	PKS	WP_197987667.1/S. pactum	97.96
verL	10752	PKS	MBH5333909.1/S. pactum	97.66
verM	8142	PKS	WP_197987665.1/S. pactum	98.19
verN	1158	acyl-CoA dehydrogenase	WP_197987664 /S. pactum	99.48

Table S14. PKS modules with predicted and observed function.

Module	Domain	predicted	observed
1	AT	mmal	mmal
2	AT	mal	mal
	KR	active/stereo undetermined	DH domain, loss of OH
	DH	active	Active
3	AT	mmal	mmal
	KR	active/stereo B1	DH domain, loss of OH
	DH	active	active
	ER		active
4	AT	mal	mal
	KR	active/stereo B1	DH domain, loss of OH
	DH	active	active
5	AT	mmal	mmal
	KR	active/stereo B1	DH domain, loss of OH
	DH	active	active
6	AT	mmal	mmal
	KR	active/stereo unknown	active/stereo R
	DH	active	activity only observed for Veramycin G (**23**)
7	AT	mal	mal
	KR	inactive	inactive
	DH	active	reaction blocked by inactive KR7
8	AT	mmal	mmal/emal/allylmal/propargylmal
	TE	active	active

In order to corroborate the putative BGC, *verD* coding for the final modules 7 and 8 in the veramycin/panowamycin/benwamycin biosynthesis was interrupted by stable double cross over integration of the *aac(3)* apramycin resistance gene, deleting parts of the gene in the process. All PCRs were performed using Q5 polymerase (NEB) according to the manufacturer's instruction. *E. coli* strains were grown on LB agar plates supplemented with appropriate antibiotics. *Streptomyces* sp. ST157608 spores for conjugation were prepared according to "Advanced Streptomyces Genetics". Genomic DNA was isolated using the innuPREP Bacteria DNA Kit (Analytik Jena GmbH, Jena, Germany), plasmid DNA was isolated using the innuPREP Plasmid Mini Kit (Analytik Jena). For the construction of the knock out vector pLP1, (i) the backbone of pCAP03 was amplified using the primers TACGTCGCGGTGAGTTCAGG and GACCGAGATAGGGTTGAGTG, (ii) the *aac(3)* gene with the OriT of pIJ773 was amplified using the primers TGTAGGCTGGAGCTGCTTC and ATTCCGGGGATCCGTCGACC, (iii) homologous region 1 was amplified using the primers CCTGAACTCACCGCGACGTAGATCAAGGAGAACGAGCAGC and GGTCGACGGATCCCCGGAATTCGCCAGCACCATCTTGATG and (iv) homologous region 2 was amplified using the primers GAAGCAGCTCCAGCCTACAGGGATCTCCGCCTTCGGCAT and CACTCAACCCTATCTCGGTCCGCCGTGGAGTAGAACGGTA, using *Streptomyces* sp. ST157608 genomic DNA as template. All amplificates were gel purified using 1 % TAE agarose gels, the DNA was eluted from the gel using the ZYMOclean Large Fragment DNA Recovery Kit and subsequently fused using self-made isothermal assembly master mix[15] *E. coli* Top10 cells were transformed with the assembled vector using electroporation and plated on $LB_{Kan/Apra}$. Correct assembly of pLP1 was corroborated by test restriction with *Nco*I/*Xba*I. Plasmid pLP1 was subsequently transferred to *E. coli* ET12567 and conjugated to *Streptomyces* sp. ST157608 using triparental conjugation with *E. coli* ET12567 + pUB307 as helper strain following standard methodology, selecting positive transconjugands with apramycin and nalidixic acid. Triparental conjugation did not yield any transconjugands, hence it was decided to use more efficient biparental conjugation with *E. coli* ET12567 + pUZ8002 as donor strain. Therefore, the kanamycin resistance gene of pLP1 was removed by amplification of the vector using the primers TTCCACCGCCGCCTTCTATGAAAGGTTGGGCTTCGGAATCG and CATAGAAGGCGGCGGTGGAAATAAAACCGCCCAGTCTAGCTATCG and recirculating the plasmid using isothermal assembly, creating pLP2. Subsequently, *E. coli* Top10 was transformed with the assembled plasmid and selected on LB_{Apra}. Identity of the plasmid was corroborated by test restriction using *Eco*RI/*Ksp*AI/*Nco*I and the plasmid was transferred to *E. coli* ET12567 + pUZ8002. Subsequently, pLP2 was conjugated to *Streptomyces* sp. ST157608 and *Streptomyces* sp. ST104848, a second producer strain also carrying the BGC. Conjugation between the *Streptomyces* recipients and the *E. coli* ET12567 + pUZ8002 + pLP2 were carried out following standard methodology, selecting positive transconjugands with apramycin and nalidixic acid. *Streptomyces* sp. ST157608 and *Streptomyces* sp. ST104848 transconjugands were grown in $ISP2_{Apra}$ for 2 days at 30° C and 180 rpm, genomic DNA was isolated and the integration of the *aac(3)* gene as well as the absence of the vector backbone, indicating successful double crossover (and hence stable integration) were confirmed by PCR using the primers TGTAGGCTGGAGCTGCTTC and ATTCCGGGGATCCGTCGACC for the apramycin resistance cassette and TACGTCGCGGTGAGTTCAGG and CATAGAAGGCGGCGGTGGAAATAAAACCGCCCAGTCTAGCTATCG for the backbone. However, only one transconjugand colony of *Streptomyces* sp. ST157608 was confirmed to have the *aac(3)* gene integrated into the BGC by double cross over. *Streptomyces* sp. ST157608 *ΔverD* and the WT producer strain were grown in ISP2 for 3 days at 30° C and 180 rpm days and analyzed for production of Veramycin A and other derivatives by LCMS directly from the supernatant (see below).

To investigate the effect of the *verD* gene inactivation, *Streptomyces* sp. ST157608 *ΔverD* and wild type supernatants were analyzed for presence of the *m/z* corresponding to the respective molecules. In the following chromatograms the base peak chromatograms (BPC) and the respective extracted ion chromatograms (EIC) are given. The chemical formula, exact mass, as well as the predicted structure is given for each compound.

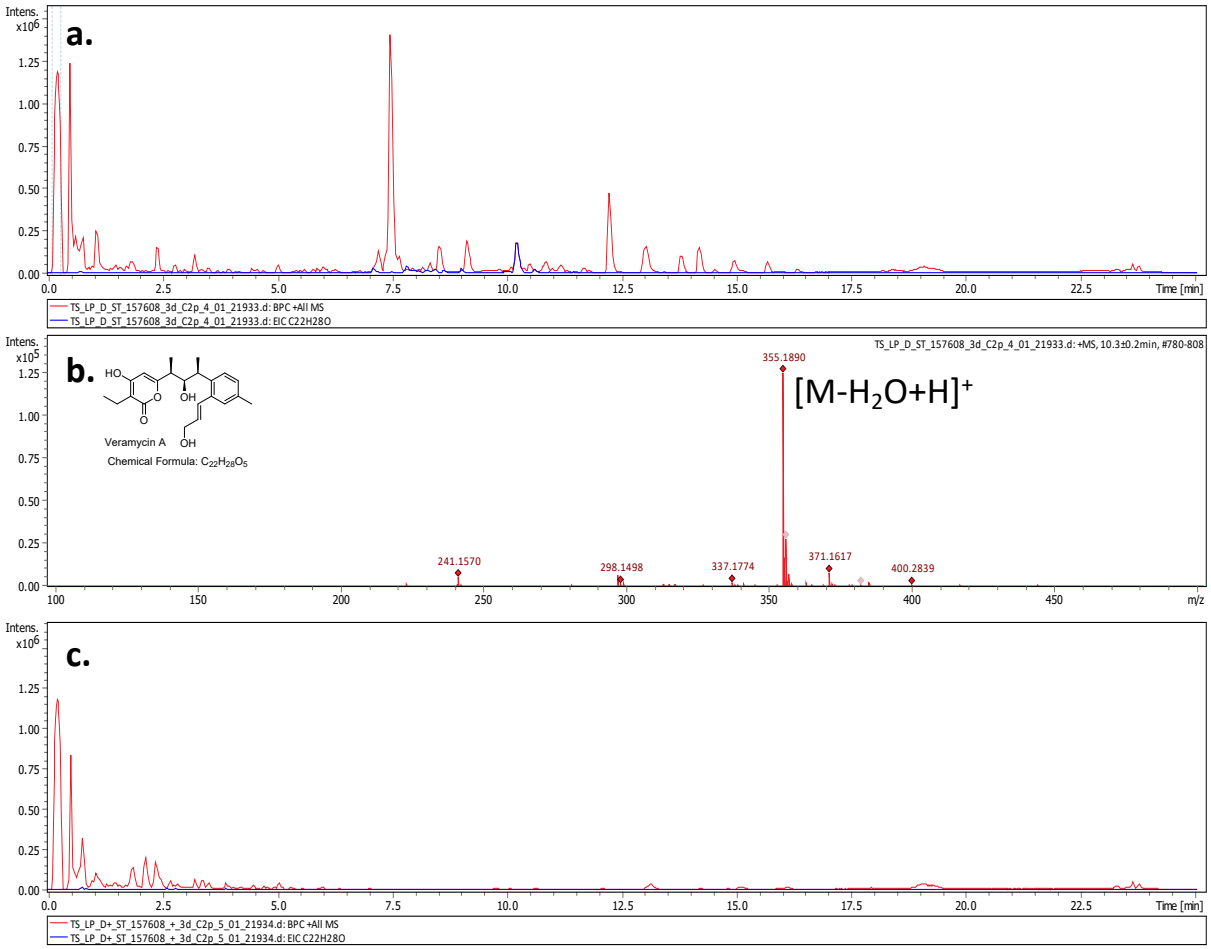


Figure S6: Overlaid BPC chromatogram (red) and EIC of molecular formula $C_{22}H_{28}O_5$ (Veramycin A), considering $[M+H]^+$, $[M-H_2O+H]^+$ and $[M+Na]^+$ adducts of the ST157608 wild type (a) and knock-out mutant (c) extracts. (b) MS spectrum of Veramycin A showing the highest signal corresponding to the $[M-H_2O+H]^+$ adduct ion.

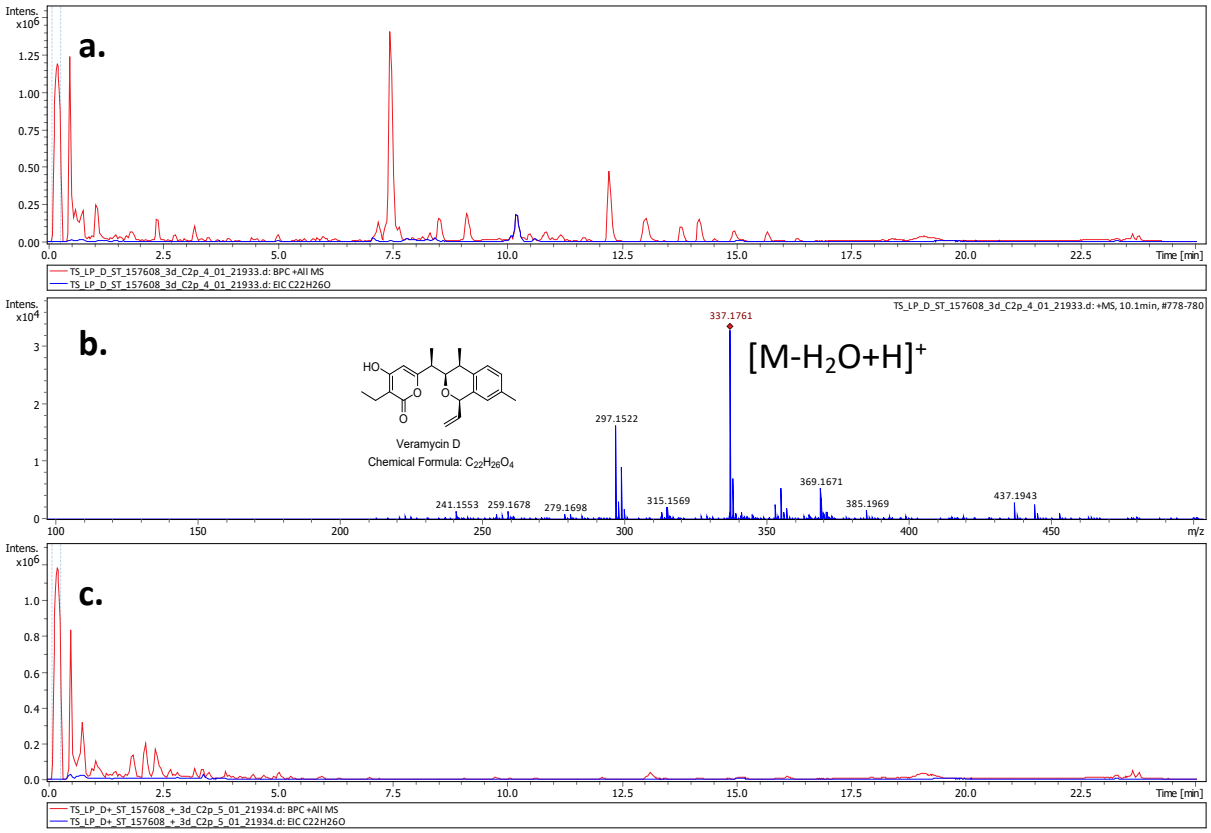


Figure S7: Overlaid BPC chromatogram (red) and EIC of molecular formula $C_{22}H_{26}O_4$ (Veramycin D), considering $[M+H]^+$, $[M-H_2O+H]^+$ and $[M+Na]^+$ adducts of the ST157608 wild type (a) and knock-out mutant (c) extracts. (b) MS spectrum of Veramycin D showing the highest signal corresponding to the $[M-H_2O+H]^+$ adduct ion.

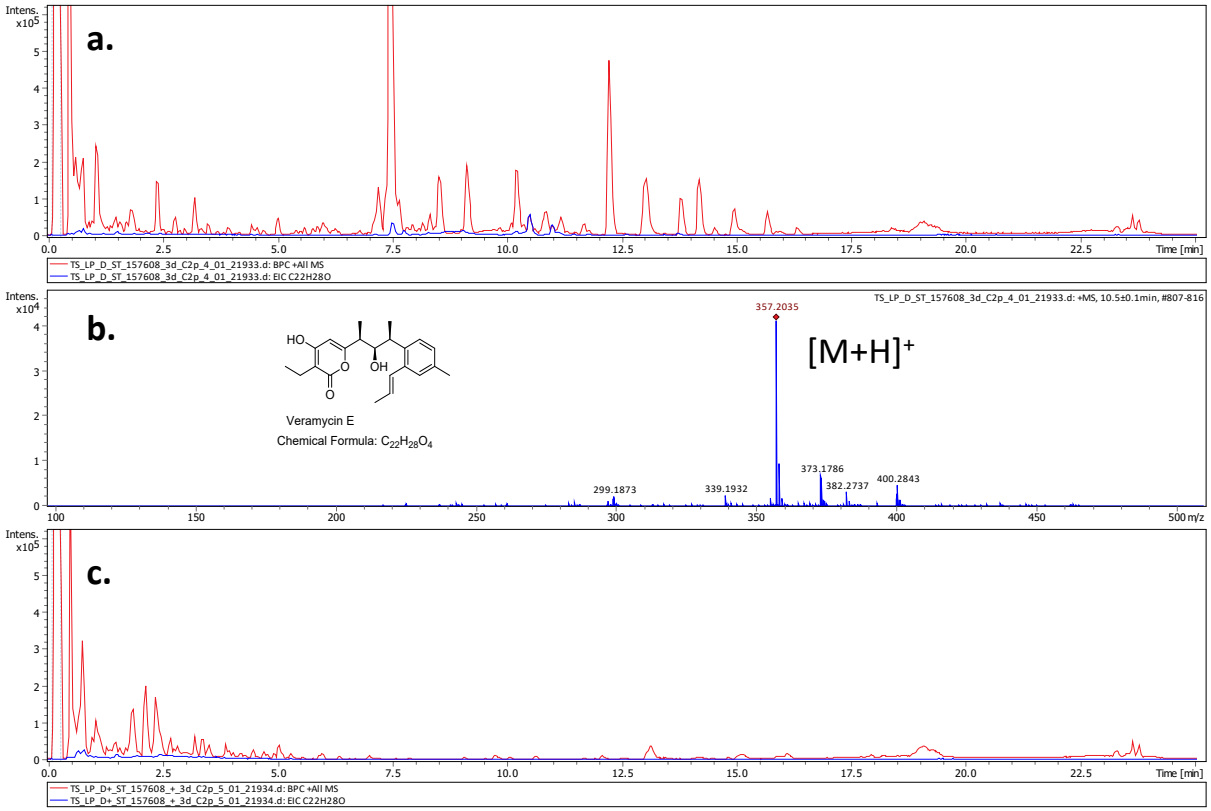


Figure S8: Overlaid BPC chromatogram (red) and EIC of molecular formula $C_{22}H_{28}O_4$ (Veramycin E), considering $[M+H]^+$, $[M-H_2O+H]^+$ and $[M+Na]^+$ adducts, of the ST157608 wild type (a) and knock-out

mutant (c) extracts. (b) MS spectrum of Veramycin E showing the highest signal corresponding to the $[M+H]^+$ adduct ion.

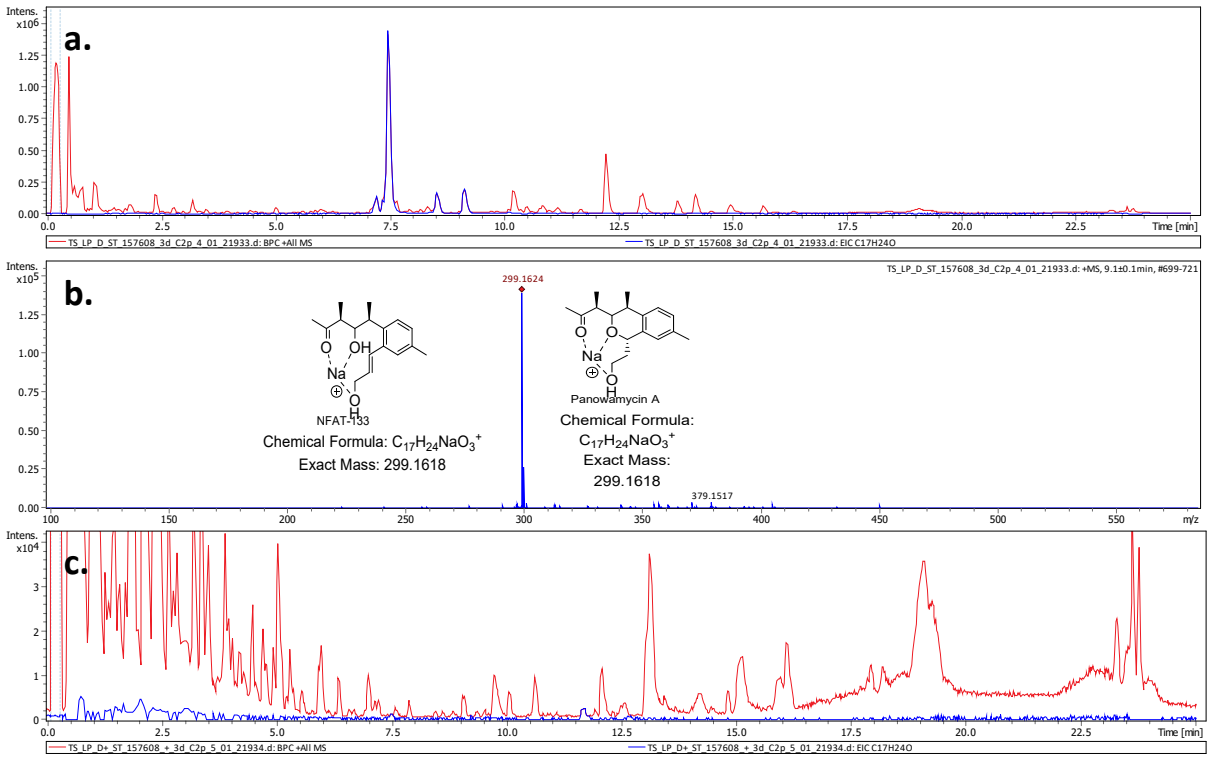


Figure S9: Overlaid BPC chromatogram (red) and EIC of molecular formula $C_{17}H_{24}O_3$ (NFAT-133 and Panowamycin A), considering $[M+H]^+$, $[M-H_2O+H]^+$ and $[M+Na]^+$ adducts, of the ST157608 wild type (a) and knock-out mutant (c) extracts. (b) MS spectrum of NFAT-133 showing the highest signal corresponding to the $[M+Na]^+$ adduct ion.

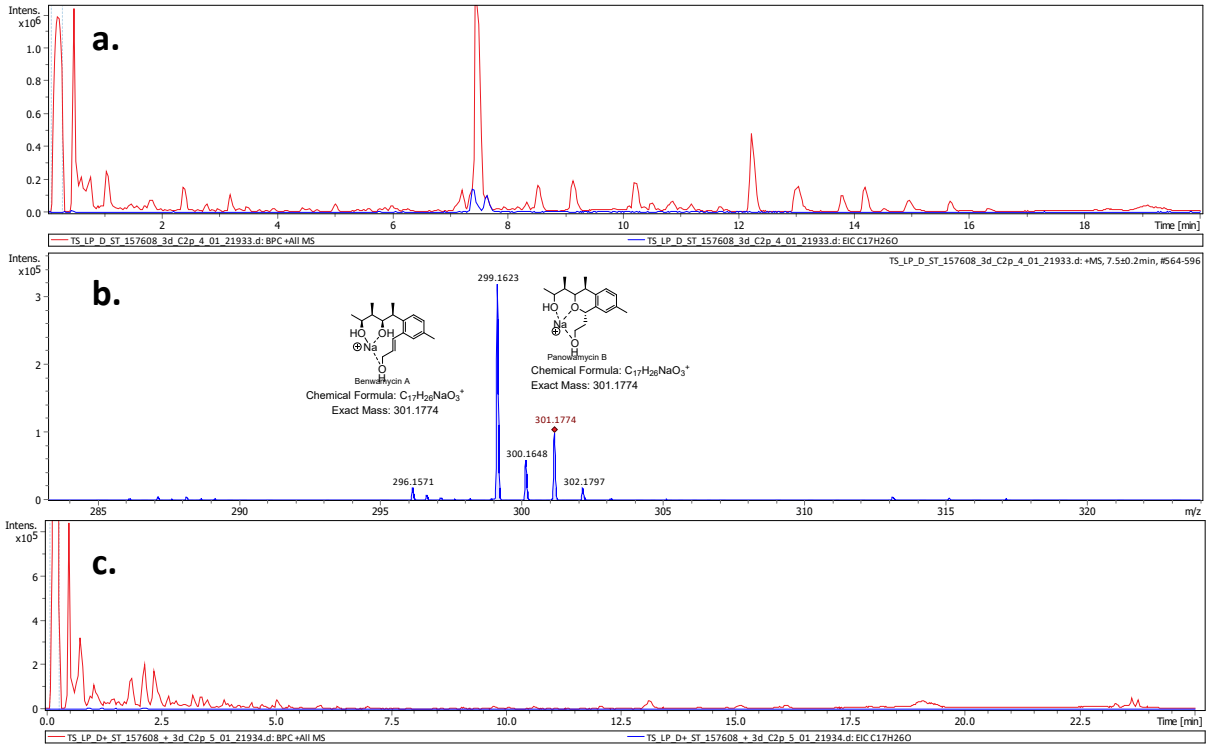


Figure S10: Overlaid BPC chromatogram (red) and EIC of molecular formula $C_{17}H_{26}O_3$ (Panowamycin B and Benwamycin A), considering $[M+H]^+$, $[M-H_2O+H]^+$ and $[M+Na]^+$ adducts, of the ST157608 wild type (a) and knock-out mutant (c) extracts. (b) MS spectrum of Panowamycin B/Benwamycin A showing the highest signal corresponding to the $[M+Na]^+$ adduct ion.

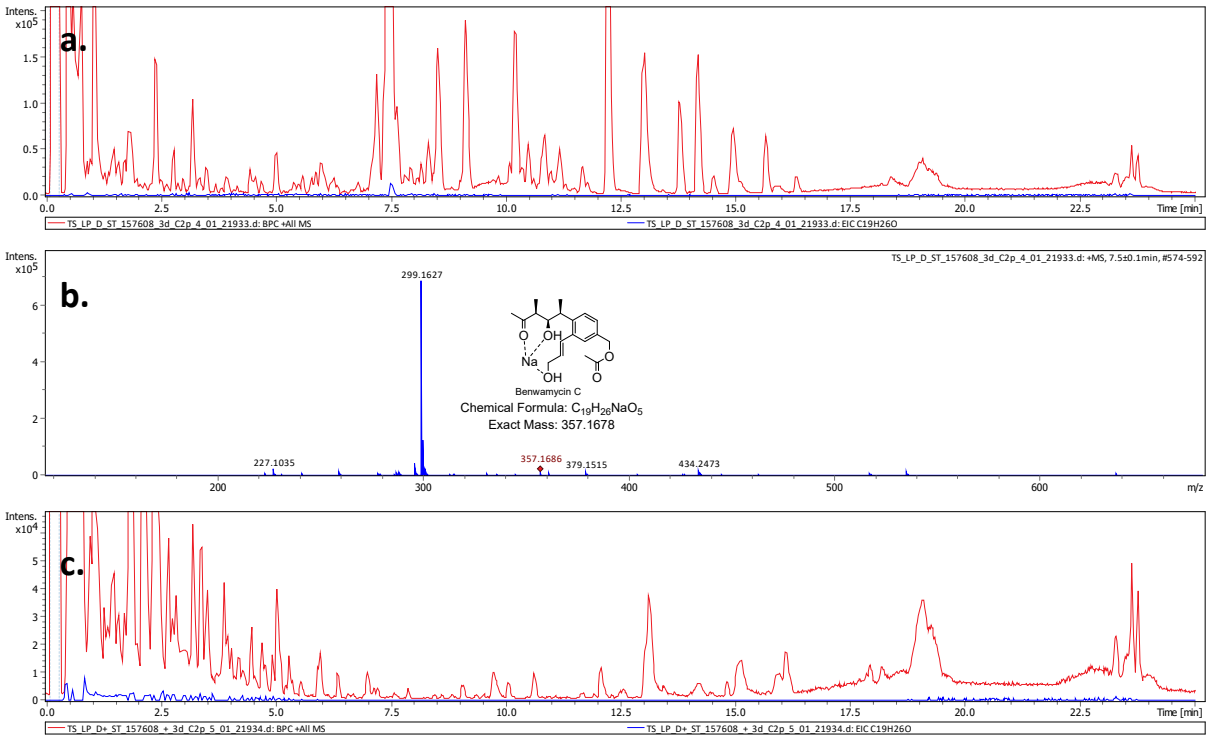


Figure S11: Overlaid BPC chromatogram (red) and EIC of molecular formula $C_{19}H_{26}O_5$ (Benwamycin C), considering $[M+H]^+$, $[M-H_2O+H]^+$ and $[M+Na]^+$ adducts, of the ST157608 wild type (a) and knock-out

mutant (c) extract. (b) MS spectrum of Benwamycin C showing the highest signal corresponding to the $[M+Na]^+$ adduct ion.

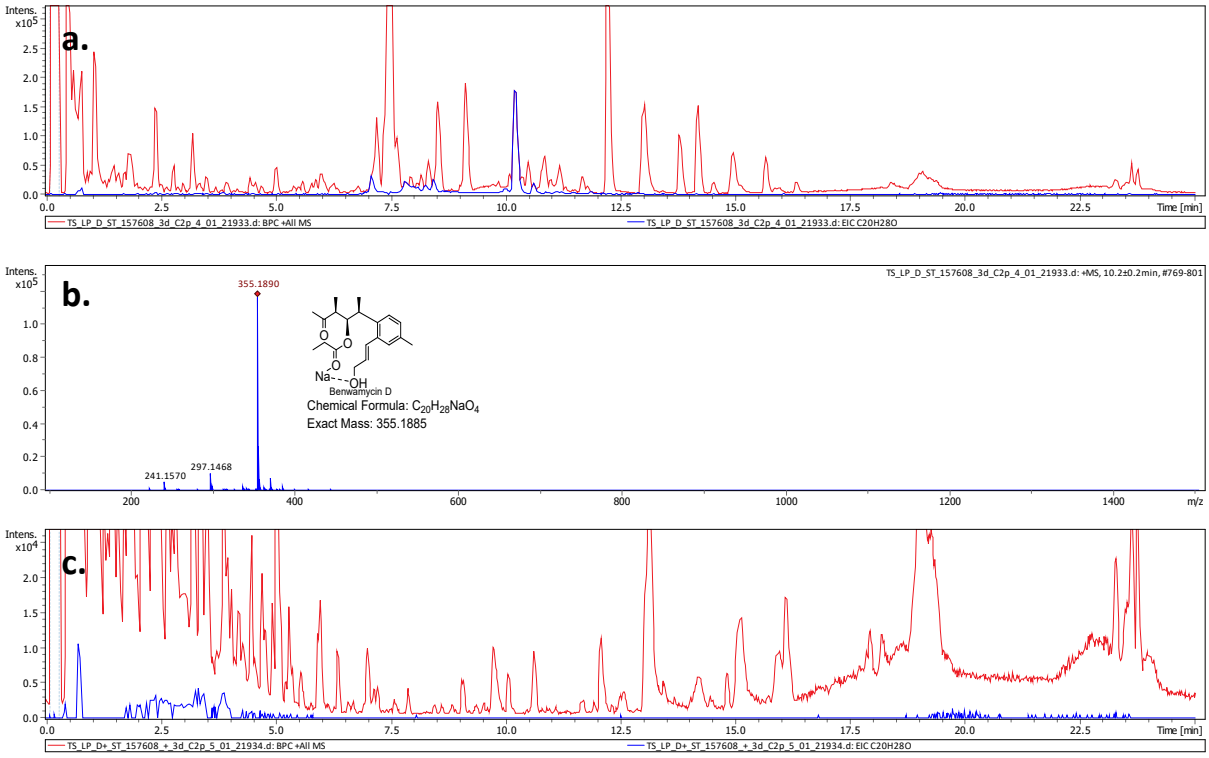


Figure S12: Overlaid BPC chromatogram (red) and EIC of molecular formula $C_{20}H_{28}O_4$ (Benwamycin D), considering $[M+H]^+$, $[M-H_2O+H]^+$ and $[M+Na]^+$ adducts, of the ST157608 wild type (a) and knock-out mutant (c) extract. (b) MS spectrum of Benwamycin D showing the highest signal corresponding to the $[M+Na]^+$ adduct ion.

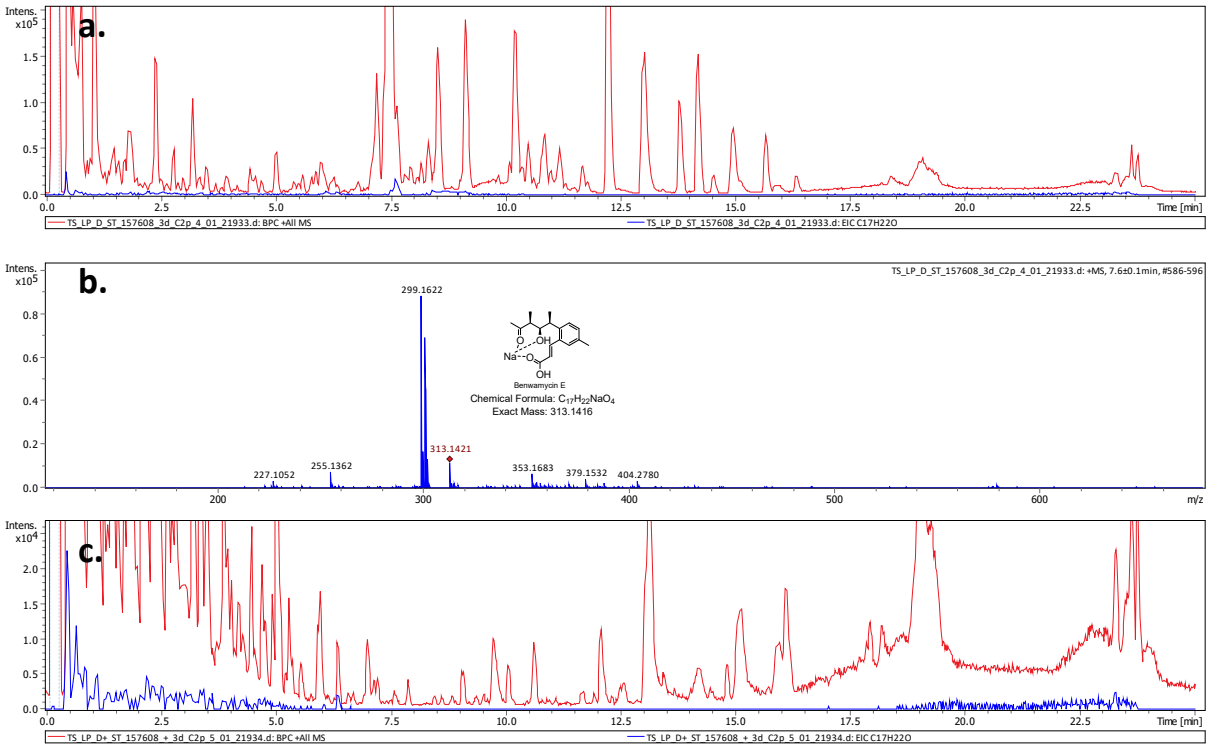


Figure S13: Overlaid BPC chromatogram (red) and EIC of molecular formula $C_{17}H_{22}O_4$ (Benwamycin E), considering $[M+H]^+$, $[M-H_2O+H]^+$ and $[M+Na]^+$ adducts, of the ST157608 wild type (a) and knock-out mutant (c) extract. (b) MS spectrum of Benwamycin E showing the highest signal corresponding to the $[M+Na]^+$ adduct ion.

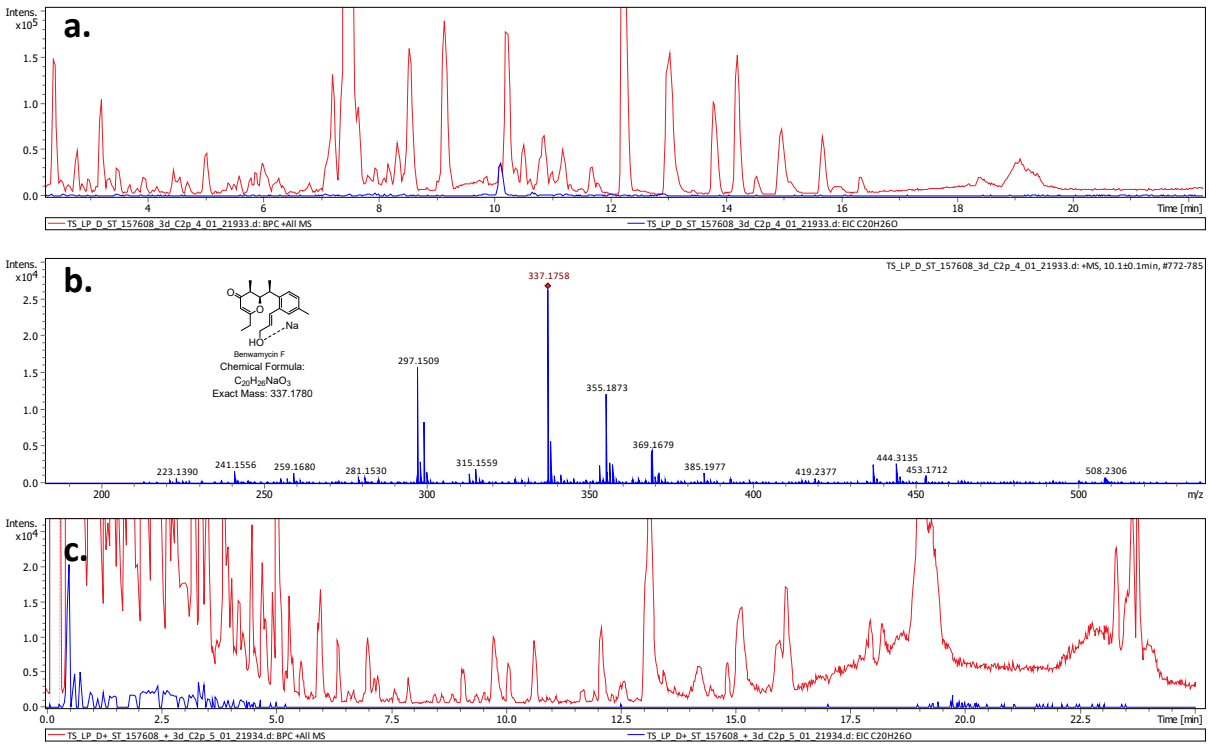


Figure S14: Overlaid BPC chromatogram (red) and EIC of molecular formula $C_{17}H_{22}O_4$ (Benwamycin F), considering $[M+H]^+$, $[M-H_2O+H]^+$ and $[M+Na]^+$ adducts, of the ST157608 wild type (a) and knock-out mutant (c) extract. (b) MS spectrum of Benwamycin F showing the highest signal corresponding to the $[M+Na]^+$ adduct ion.

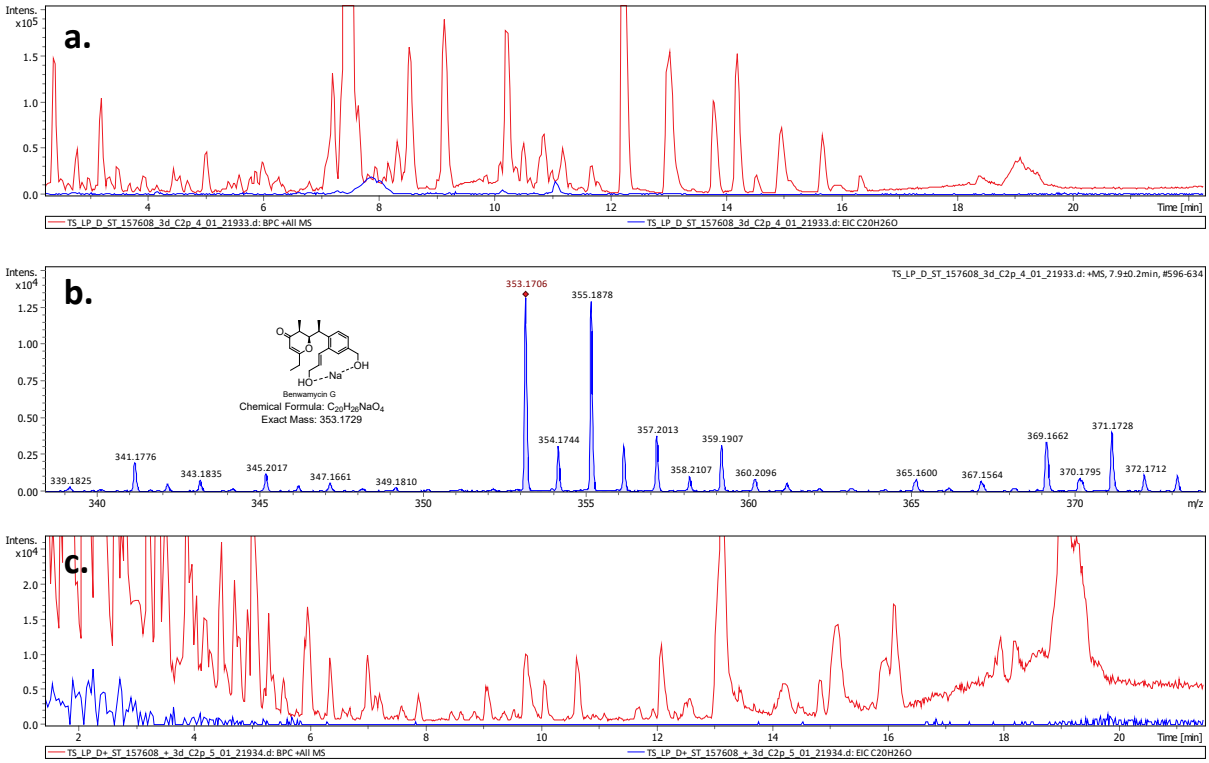


Figure S15: Overlaid BPC chromatogram (red) and EIC of molecular formula $C_{20}H_{26}O_4$ (Benwamycin G), considering $[M+H]^+$, $[M-H_2O+H]^+$ and $[M+Na]^+$ adducts, of the ST157608 wild type (a) and knock-out mutant (c) extract. (b) MS spectrum of Benwamycin G showing the highest signal corresponding to the $[M+Na]^+$ adduct ion.

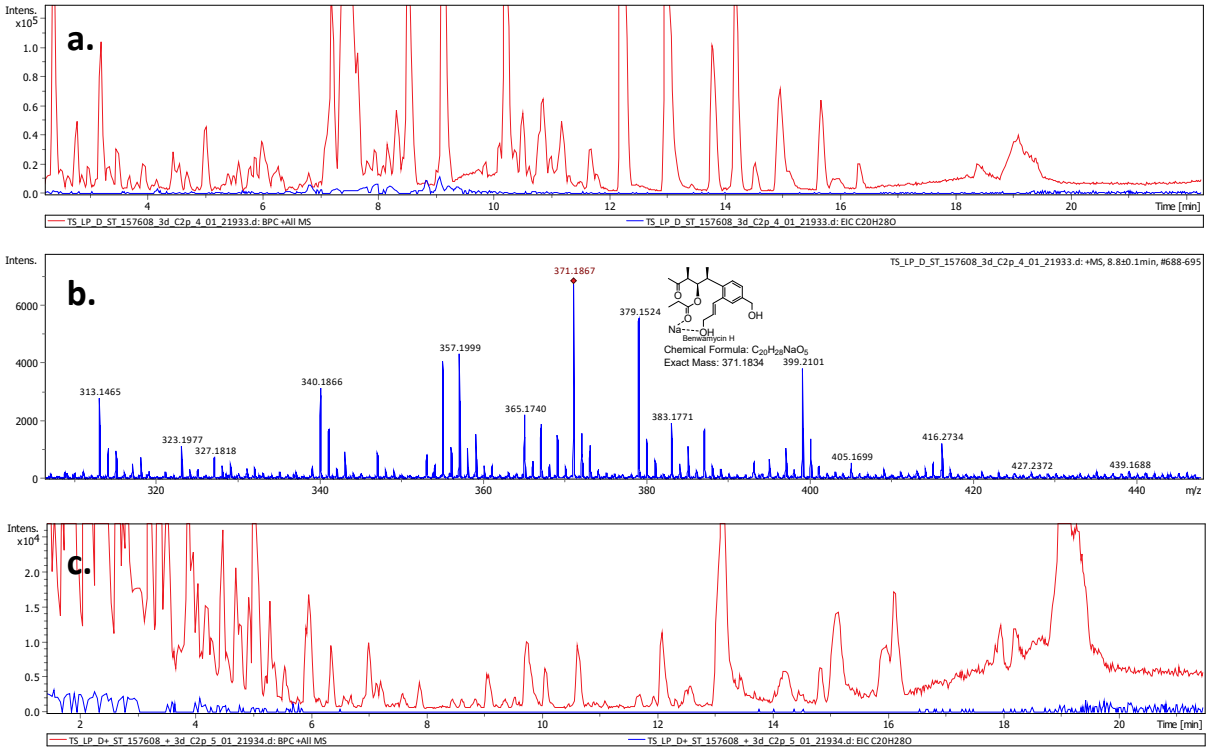


Figure S16: Overlaid BPC chromatogram (red) and EIC of molecular formula $C_{20}H_{28}O_5$ (Benwamycin H), considering $[M+H]^+$, $[M-H_2O+H]^+$ and $[M+Na]^+$ adducts, of the ST157608 wild type (a) and knock-out mutant (c) extract. (b) MS spectrum of Benwamycin H showing the highest signal corresponding to the $[M+Na]^+$ adduct ion.

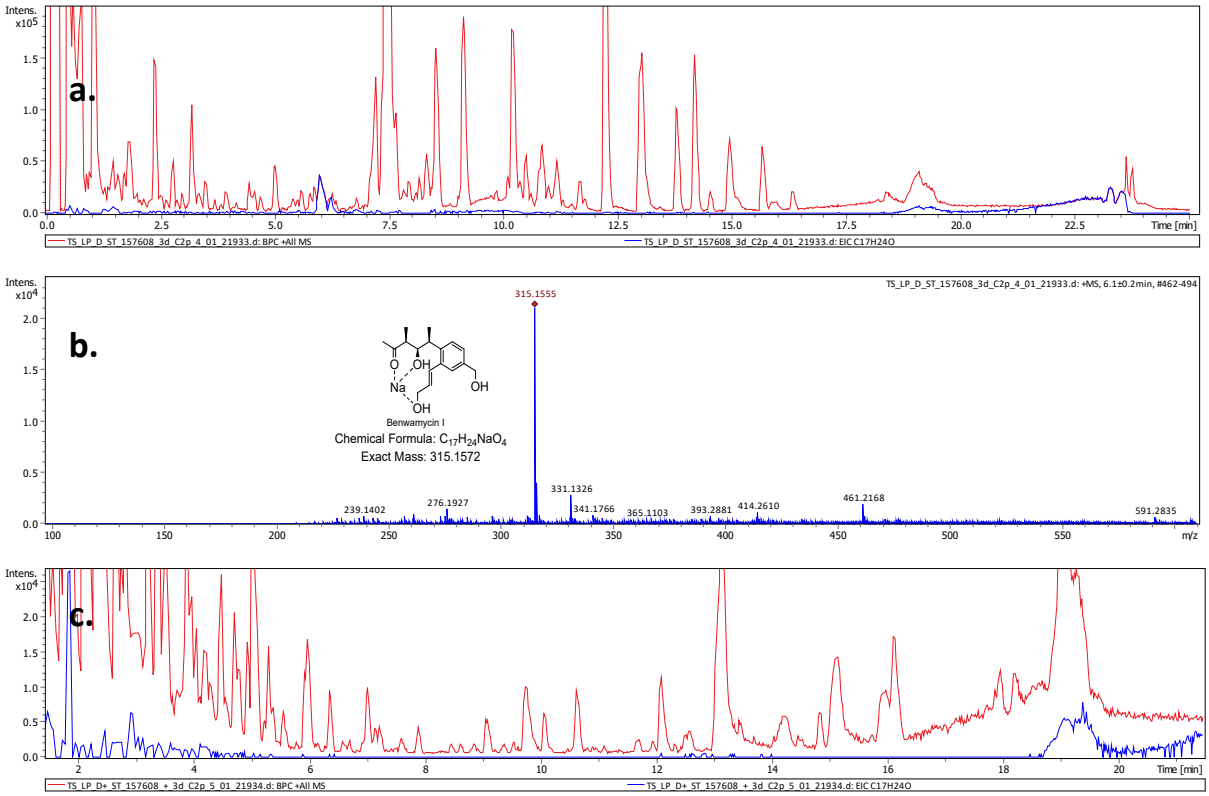


Figure S17: Overlaid BPC chromatogram (red) and EIC of molecular formula $C_{17}H_{24}O_4$ (Benwamycin I), considering $[M+H]^+$, $[M-H_2O+H]^+$ and $[M+Na]^+$ adducts, of the ST157608 wild type (a) and knock-out mutant (c) extract. (b) MS spectrum of Benwamycin I showing the highest signal corresponding to the $[M+Na]^+$ adduct ion.

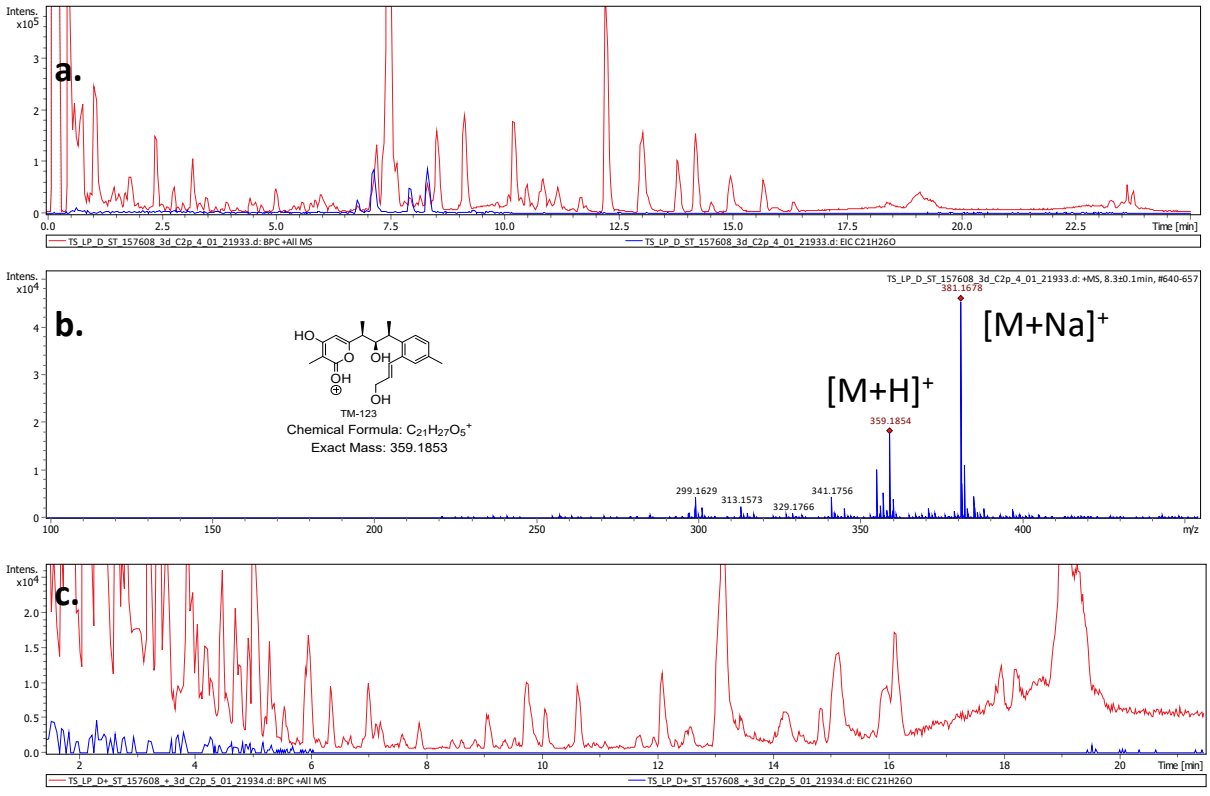


Figure S18: Overlaid BPC chromatogram (red) and EIC of molecular formula $C_{17}H_{24}O_4$ (TM-123), considering $[M+H]^+$, $[M-H_2O+H]^+$ and $[M+Na]^+$ adducts, of the ST157608 wild type (a) and knock-out mutant (c) extract. (b) MS spectrum of TM-123 showing the highest signal corresponding to the $[M+Na]^+$ adduct ion.

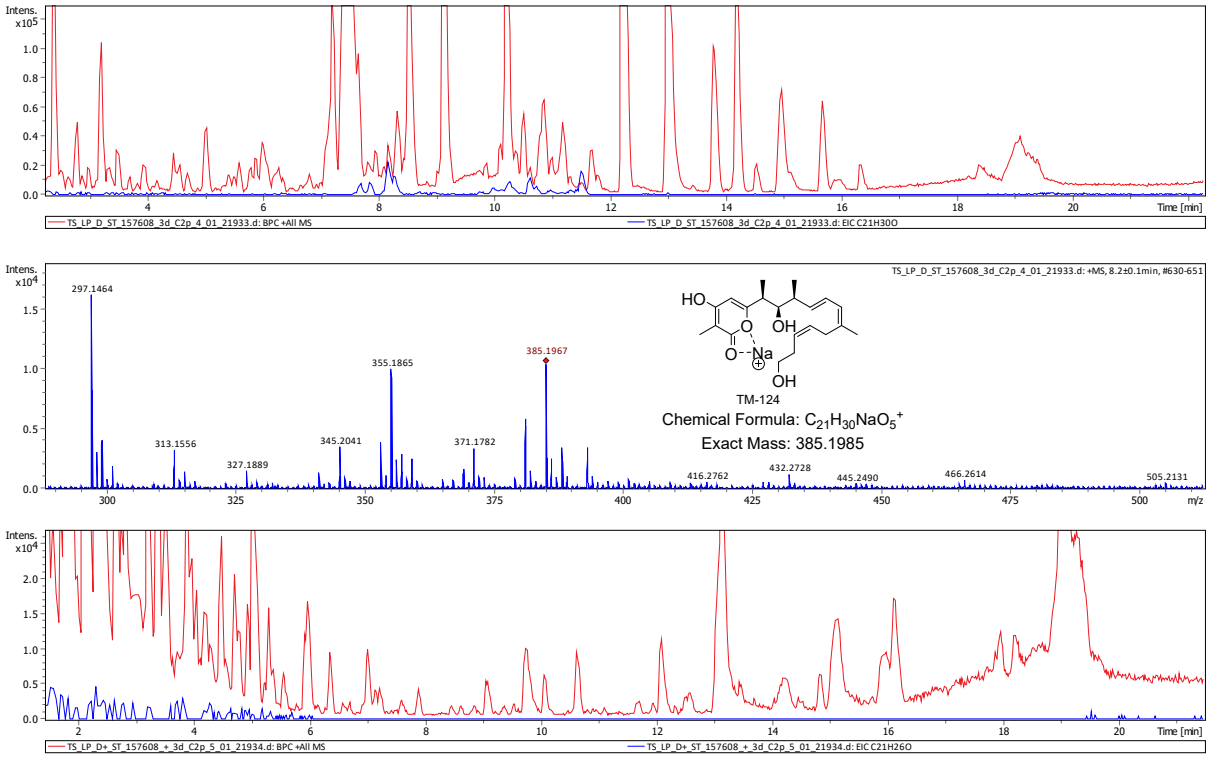


Figure S19: Overlaid BPC chromatogram (red) and EIC of molecular formula $C_{21}H_{30}O_5$ (TM-124), considering $[M+H]^+$, $[M-H_2O+H]^+$ and $[M+Na]^+$ adducts, of the ST157608 wild type (a) and knock-out mutant (c) extracts. (b) MS spectrum of TM-124 showing the highest signal corresponding to the $[M+Na]^+$ adduct ion.

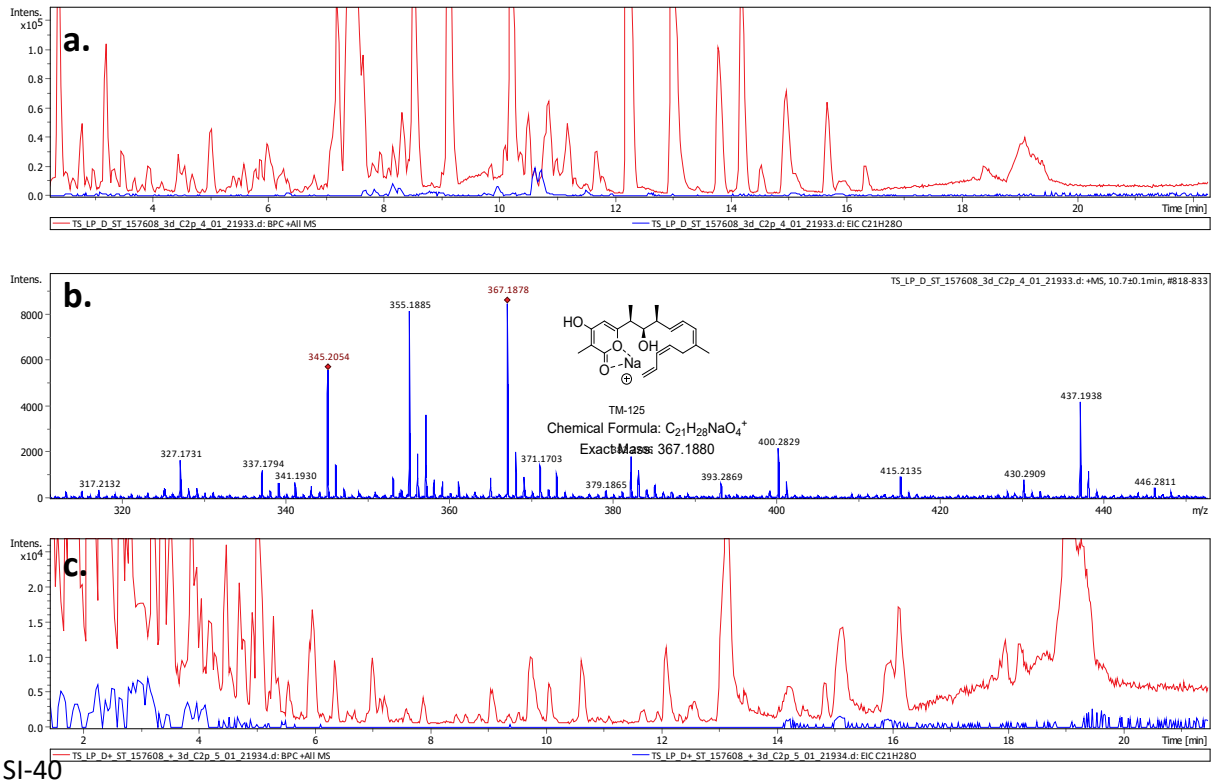


Figure S20: Overlaid BPC chromatogram (red) and EIC of molecular formula $C_{21}H_{30}O_5$ (TM-125), considering $[M+H]^+$, $[M-H_2O+H]^+$ and $[M+Na]^+$ adducts, of the ST157608 wild type (a) and knock-out mutant (c) extract. (b) MS spectrum of TM-125 showing the highest signal corresponding to the $[M+Na]^+$ adduct ion.

Feeding experiments with ^{13}C-labelled precursors. The feeding experiments were conducted with

10 mM of [1-^{13}C] acetate, [2-^{13}C] acetate, and [1-^{13}C] propionate for 4 d in 6 x 2 L Erlenmeyer flasks containing 500 mL culture media (30 g/L glycerol, 5 g/L $CaCO_3$, 15 g/L soybean meal, 2 g/L NaCl, at pH 7.5), respectively. Culture filtrate was separated from the cells by centrifugation, lyophilized, and subsequent extracted with MeOH. The obtained extracts were further purified using adsorption chromatography containing Amberlite® XAD-7 and -16 resin (1:1) eluting with a gradient starting from 30%, 50% to 100% MeOH in water. Fractions containing the polyketides were subsequently purified by size exclusion chromatography (SEC) with Sephadex LH20 and MeOH. Final purification was performed by semi-preparative RP-HPLC (Synergi® Fusion C18, 250 x 25 mm, 4 μm, DAD at 220 and 254 nm) eluting with an isocratic gradient of 35% ACN/H_2O with 0.05% TFA in 35 min to yield the labeled compounds. Incorporation rate was analyzed by ^{13}C-NMR spectroscopy.

Feeding experiments with ^{2}H-labelled precursors. The feeding experiment was conducted with deuterium-labeled [2,2,3,3,3-$^{2}H_5$]propionate, [3,3,4,4,4-$^{2}H_5$]butyrate and L-[methyl-$^{2}H_3$]-methionine. The fermentations were carried out in duplicates for 4 d in 300 mL Erlenmeyer flasks containing 50 mL culture media (30 g/L glycerol, 5 g/L $CaCO_3$, 15 g/L soybean meal, 2 g/L NaCl, at pH 7.5). The culture filtrate was separated from the cells by centrifugation, lyophilized, and subsequent extracted with MeOH. The obtained extracts were concentrated 20fold and analyzed by UPLC-HR/MS.

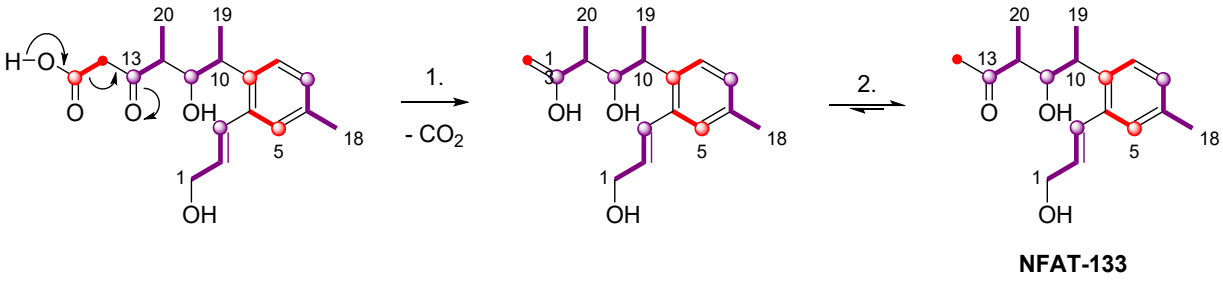


Figure S21: Decarboxylative formation of NFAT-133 (1) decarboxylation of a β-keto carboxylic acid; (2) keto-enol tautomerization. It is assumed that the reaction is catalyzed by the decarboxylase VerG.

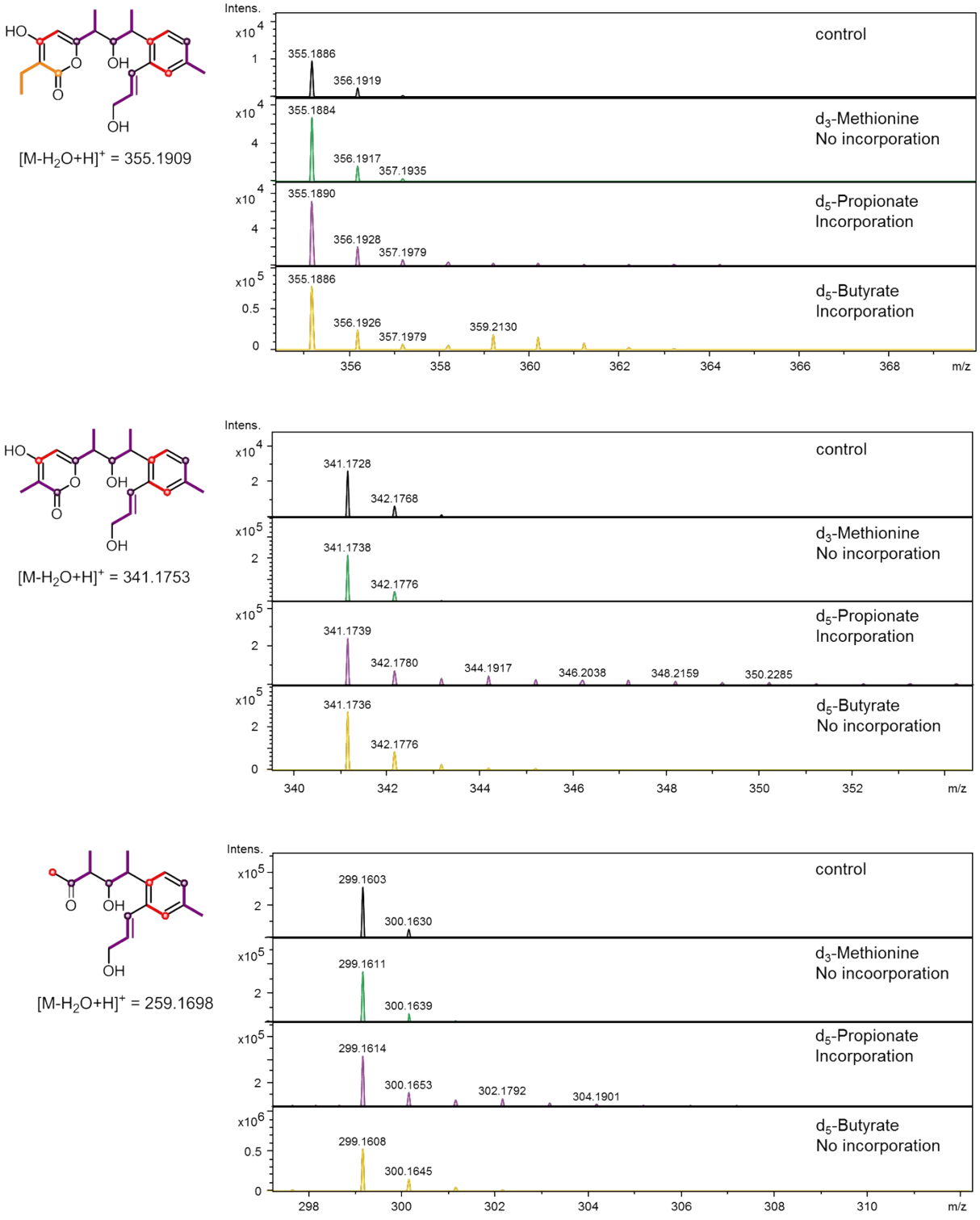


Figure S22: Mass spectra of HPLC chromatograms from the ^{2}H labeled feeding experiments for Veramycin A, B, and NFAT-133. Isotopic patterns indicate the incorporation of respective precursors.

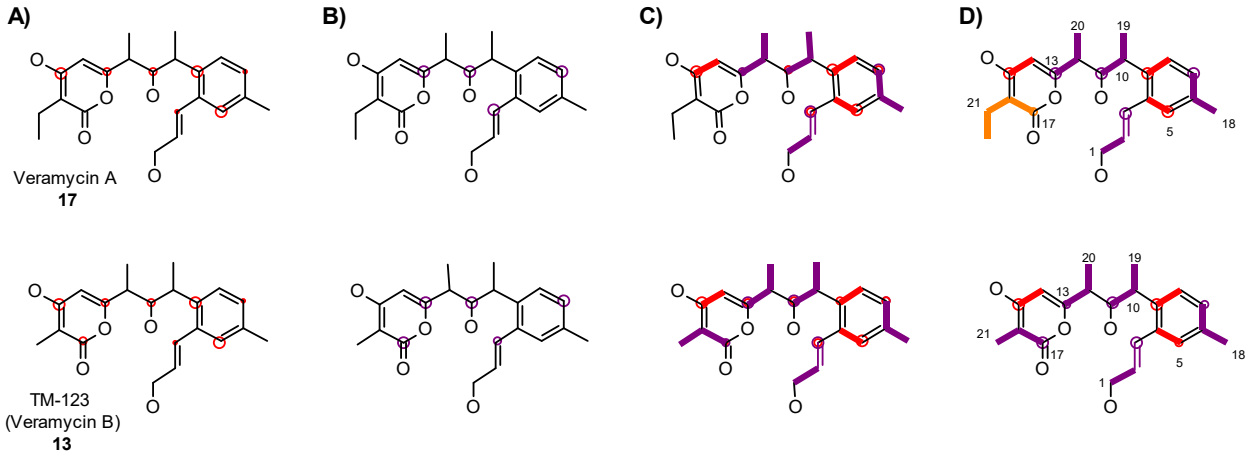


Figure S23: Identification of ^{13}C-labeled carbon atoms originate from stable isotope feeding experiment for **13** and **17**: (A) [1-^{13}C] acetate, (B) [1-^{13}C] propionate, (C) superposition of both, (D) superposition including the hypothetical result for labelled butyrate.

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8. Precursor-Directed Biosynthesis

Allyl malonic acid (**SI-7**) was commercially supplied. Propargyl- (**SI-8**), 3-chloropropyl- (**SI-9**), and isobutylmalonic acid (**SI-10**) were synthesized by saponification of their corresponding dimethyl or diethyl esters as reported.[16,17]

Pre-cultures of *Streptomyces* sp. ST157608 were conducted in 0.3 L Erlenmeyer flasks as described above. Inoculation of the main fermentation with the pre-cultures (5%) was conducted in 0.3 L Erlenmeyer flasks, containing 50 mL of the main culture medium consisting of glucose (0.4%), $CaCO_3$ (0.2%), yeast extract (0.4%), malt extract (1%), and malonic acid derivative **SI-7**, **SI-8**, **SI-9** or **SI-10** (5 M) with an initial pH value of 7.2. Incubation was performed on a rotary shaker at 180 rpm and 28 °C for 4 d. Cells and supernatants were frozen (– 50°C) and lyophilized. The residue was extracted with MeOH (40 mL, 2 h, 180 rpm, 25 °C). The extract was centrifuged (4000 rpm, 5 min) and the supernatant was filtered, concentrated under reduced pressure and re-suspended in MeOH (5 mL). Samples were taken from this extract, centrifuged, and subjected to UHPLC-MS/MS analysis on an Agilent 1290 Infinity LC equipped with a Bruker maXisII HR-QTOF-MS/MS operated in ESI$^+$ mode (column: Phenomenex UPLC ACQuity BEH C_{18} 1.7 µM, 21 x 100 mm; flow: 0.6 mL·min^{-1}; column temperature: 40 °C; gradient: 5-100% MeCN/H_2O with 0.1% TFA in 22 min). Incorporation of **SI-7** and **SI-8** was detected by the presence of the new Veramycin derivatives **49** and **50**, respectively, identified by their characteristic mass spectra (Figure S24), whereas no new derivatives corresponding to incorporation of malonic acids **SI-9** and **SI-10** could be detected.

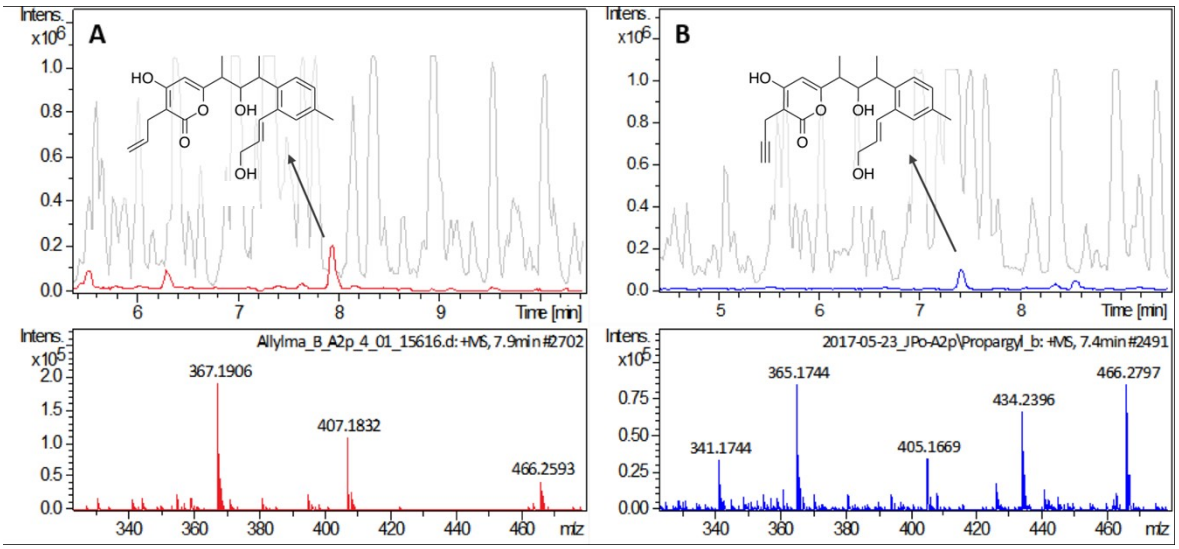

Figure S24: Basepeak chromatograms (BPC, grey) and extracted ion chromatogram (EICs colored) of allyl Veramycin **49** $[M–H_2O+H]^+$ (**A**, 7.9 min) and propargyl Veramycin **50** $[M–H_2O+H]^+$ (**B**, 7.4 min) and mass spectra of the corresponding peaks.

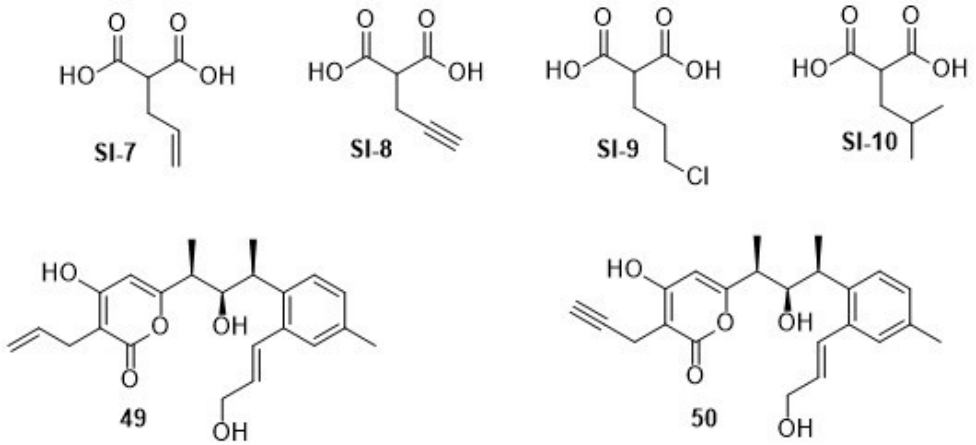

Figure S25: Structures of malonic acid derivatives employed in feeding experiments (**SI-8** – **SI-10**) and structures of allyl Veramycin **49** and propargyl Veramycin **50**.

9. Glucose Uptake Measurements in L6 Myotubes

Establishment of the L6-AS160_v2-GLUT4 cell line, cell culture conditions, and measurement of 2-deoxyglucose uptake have been described previously.[18] Briefly, cells were grown in Minimum Essential Medium alpha modification (MEMα; PAN-Biotech GmbH) supplemented with tetracycline-free 10% FCS (PAA Laboratories, Pasching, Austria), 2 µg/mL blasticidin (Life Technologies Corporation, Carlsbad, CA, USA), 0.5 µg/mL puromycin (Life Technologies Corporation), and 200 µg/mL hygromycin (Thermo Fisher Scientific). Expression of AS160_v2 protein was induced with 1 µg/mL doxycycline (Sigma-Aldrich Chemie).

After seeding and reaching confluency, L6-AS160_v2-GLUT4-myc cells were incubated in starvation medium (MEMα) 3-4 h prior to each experiment. For differentiation, the cells were grown in MEMα +

GlutaMAX supplemented with 1% horse serum (Lonza Biochemicals, Portsmouth, NH, USA) for 7 d. Cells were plated in 96-well Cytostar-T scintillating microtiter plates. After 48 h, cells were serum starved (3-4 h) and treated with various concentrations of each insulin uptake of ^{14}C-labeled 2-deoxyglucose (0.01 MBq/well; GE Healthcare, Amersham, UK) was measured. Nonspecific uptake was determined in the presence of 40 µM cytochalasin B (Thermo Fisher Scientific) and subtracted from the total uptake. Radioactive counts were measured in a Wallac 1450 MicroBeta® TriLux scintillation counter (PerkinElmer, Shelton, CT, USA) as counts per min.

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