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Synthesis and biological activity evaluation of 20-*epi*-salinomycin and its 20-*O*-acyl derivatives†

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20-*epi*-Salinomycin and six 20-*O*-acylated analogs were synthesized and tested for their antiproliferative activity. Both the C1-protecting group of the salinomycin and the acidity of the substituted benzoic acid are crucial to the Mitsunobu conversion. 20-*epi*-Salinomycin showed similar antiproliferative activity as salinomycin, but its 20-*O*-acylated analogs were 2–10 times more potent. In addition, the 20-*epi*-20-*O*-acylated salinomycin derivative **9d** and **9e** had better selectivities than salinomycin between cancer and neuron cell lines. The spatial configuration of the C20-hydroxyl group has little influence on the activities, but the acyl groups cause an obvious difference by producing possible effects on the stability and permeability of the salinomycin–alkali metal ions complexes.

1. Introduction

Salinomycin (**1**, Fig. 1) is a kind of polyether antibiotic isolated from *Streptomyces albus*. Up to now, it has been used in broiler batteries and other livestock as an anticoccidial drug and also fed to ruminants and pigs to improve nutrient absorption and feed efficiency.[1] A study in 2009 found salinomycin kills breast cancer stem cells (CSCs) *in vitro* and inhibits mammary tumor growth in mice, and its effect is 100 times higher than the clinically used antitumor drug taxol.[2] Salinomycin targets both CSCs and more differentiated non-CSC tumor cells in different types of human cancers, which display efficient mechanisms of resistance to cytotoxic drugs and radiation.[3] Several possible mechanisms of salinomycin were illuminated, such as induction of apoptosis and cell death, interference with ABC transporters, inhibition of oxidative phosphorylation and inhibition of the Wnt/β-catenin signaling pathway, but the exact mechanisms were still not fully elucidated.[4]

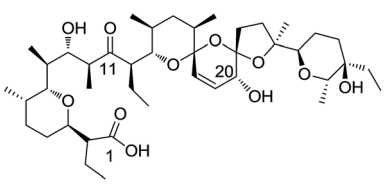
The encouraging biological activities of salinomycin have drawn increasing attentions of pharmacologists and medicinal chemists. Huczynski's group synthesized several C1-amides, C1-esters as well as C1-conjugated derivatives of salinomycin, some of which showed slightly stronger antitumor activities.[5] Daniel Strand's group synthesized several C20 hydroxyl acylated salinomycin analogs, which displayed IC_{50} values down to one fifth that of the native structure against breast cancer cells.[6] Their followed study found C20-deoxy-saliomycin reduces anticancer activity significantly, which emphasizes the importance of substitution at C20 for the activity.[7]

In our previous work, we have synthesized and tested the biological activities of 17-*epi*-salinomycin and 17,21-di-*epi*-salinomycin as well as their benzoylated derivatives. The results showed that the 17-*epi*-salinomycin and its analogue almost lost activity but 17,21-di-*epi*-salinomycin and its analogue enhanced the activity, indicating the important roles of the spatial configurations of the salinomycin.[8] To elucidate the effect of C20-hydroxyl configuration on the biological activities, herein we reported the synthesis and evaluation of anti-tumor activities of 20-*epi*-salinomycin and its 20-*O*-acyl derivatives.

2. Results and discussion

2.1 Chemistry

First of all, the carboxylic group of salinomycin was converted to methyl ester (**2**), but no product of Mitsunobu reaction was detected when *p*-nitrobenzoic acid, DIAD and Ph_3P were added to this substrate in THF, presumably because the conformation of salinomycin methyl ester **2** is unfavorable to the reaction (Scheme 1). Afterwards, salinomycin TMSEt-ester (**4**), which has



salinomycin(1)

Fig. 1 The structure of salinomycin.

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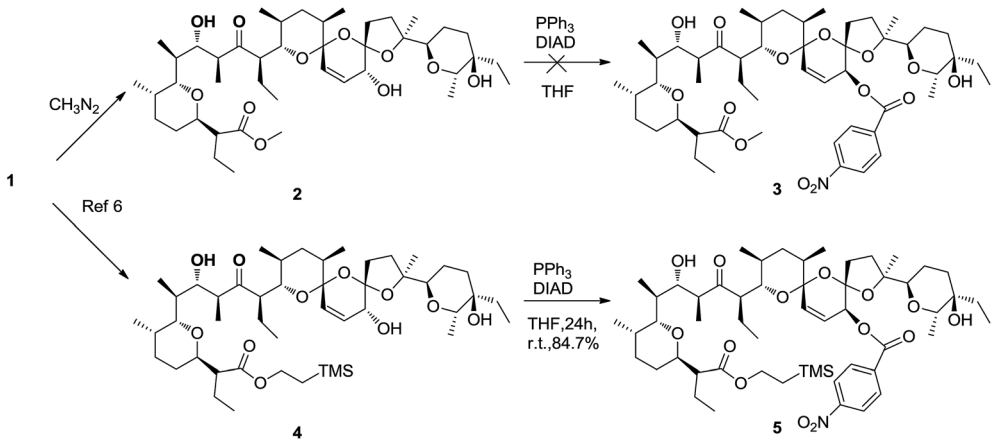


Scheme 1 Synthesis of the 20-*epi*-20-*O*-*p*-nitrobenzoylated salinomycin EtTMS ester **5**.

a more bulky protecting group on the carboxylic group, was synthesized according to Strand's report.[6] As expected, compound **4** was converted to *p*-nitrobenzolated salinomycin TMSEt-ester (**5**) smoothly with *p*-nitrobenzoic acid, DIAD and Ph_3P in 84.7% yield. In a recently reported work, Shi also synthesized azido-salinomycin by the utilization of the Mitsunobu reaction with TMSEt-ester (**4**) as the substrate.[9] These results indicated the bulky protecting group indeed influenced the conformation of salinomycin and the reactive sites were thus exposed, leading to smooth inversion of hydroxyl configuration. Because the intramolecular hydrogen bond of C9 hydroxyl group and steric-hindrance effect of C28,[6,9] the substitution reaction proceeded on the allylic C20 hydroxyl group, which was proved by the acylation shift of the H-20 in ^{1}H NMR (Table 1).

However, benzoic acid and *p*-bromobenzoic acid could not react with salinomycin TMSEt-ester (**4**) under the similar conditions, which may be due to the difference of acidity (*p*-nitrobenzoic acid (pK_a 3.43) < *p*-bromobenzoic acid (pK_a 3.97) < benzoic acid (pK_a 4.20)). To synthesize 20-*epi*-20-*O*-acylated salinomycins, another synthetic route was designed, such as acylation of 20-*epi*-salinomycin TMSEt-ester (**6**) (Scheme 2). In fact, K_2CO_3 could hydrolyze *p*-nitrobenzoyl group of compound **5** to give key intermediate **6** without affecting C1-TMSEt-ester. Acylation reaction with anhydride or chloride afforded corresponding derivatives **7a–e** in moderate yields, which were used in the next step directly without characterization.[6] Subsequently, deprotection of compounds **5** and **7a–e** with KF in DMF at 80 °C afforded corresponding 20-*epi*-20-*O*-acyl salinomycin sodium salt **8** and **9a–e** in 40–60% yields.

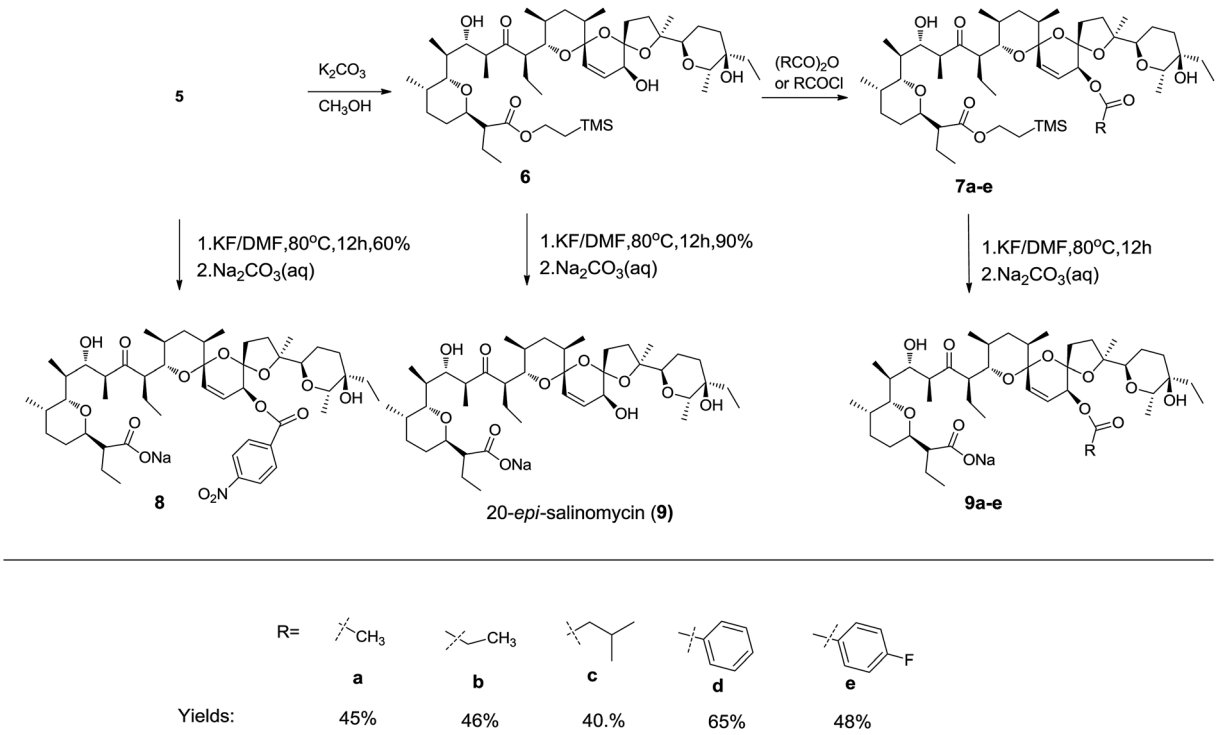
To determine the relative configuration of C20, the ^{1}H NMR spectrums of analog **9d** and its epimer 20-*O*-benzoyl salinomycin sodium salt 10[6] were compared by the chemical shifts and coupling constants of hydrogen atoms of C20 and double bond. There are significant differences between corresponding signals of compound **9d** and **10** (Table 1). H-20 has an apparent high-field displacement and a larger coupling constant than H-20′, while the H-18 and H-19 in double bond have significant low-field displacements than H-18′ and H-19′. The 'twist-boat' conformations of the compounds may give some explanations (Fig. 2): (a) the dihedral of H-19′ and H-20′ in compound **10** are nearly 90°, while that of H-19 and H-20 in compound **9d** are far less than 90°; (b) the aromatic ring is paralleled with the double bond in compound **9d**, which may lead to some deshielding effect to H-18 and H-19.

2.2 Biological activity

The antiproliferative activity of the 20-*epi*-salinomycin sodium salt and its acylated analogs were evaluated in HT-29 colorectal cancer, HGC-27 gastric cancer and triple negative MDA-MB-231 breast cancer cells using MTT assay (Table 2).[8] 20-*epi*-Salinomycin (**9**) showed similar activity to salinomycin with IC_{50} in micromole level, while most 20-*epi*-*O*-acylated analogs displayed excellent antiproliferative activities with IC_{50} values in submicromolar level. Both the simple fatty acylated and benzoylated analogs (except *p*-nitrobenzolated analog **8**) exhibited better antiproliferative activities than salinomycin and its 20-epimer. Compared with the simple fatty acylated derivatives (**9a**, **9b** and **9c**), the benzoylated analogs (**9d** and **9e**) were 10-fold more potent than salinomycin. In addition, the orientations of the acyl groups are crucial, for example, 20-*O*-benzoyl salinomycin (**10**) was 10-fold more potent than its 20-epimer **9d**.

Table 1 The comparison of ^{1}H NMR spectrums of **9d** and **10** (part)

9d	Chemical shift (coupling constant)	**10**	
H-18	6.44–6.36 ppm (m)	H-18′	6.24 ppm (d, J = 10.8 Hz)
H-19		H-19′	6.02 ppm (d, J = 10.8 Hz)
H-20	5.27 ppm (d, J = 4.8 Hz)	H-20′	5.74 ppm (s)



Scheme 2 Synthesis of the 20-*epi*-acyl salinomycin analogs.

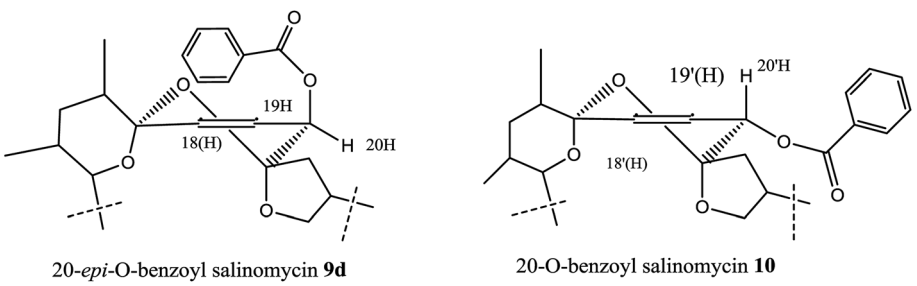
Compound **8** almost lost antiproliferative activity, which may be due to the strong electron-withdrawing effect of the nitro group.

One important caveat for the potential clinical application of salinomycin is its considerable neural toxicity.[10,11] Thus the neurotoxicities of the compounds were evaluated with neurons in cerebral cortex of E18 rats to evaluate the safety.[12] The selectivity index[5,9] values were then calculated to evaluate the potential therapeutic window of the compounds. It is an indication of a drug with efficacy against cancer cells when SI > 1.0. For selected cancer cells, salinomycin showed a low SI especially in MDA-MB-231 cells. In contrast, derivatives **9d** and **9e** had a higher SI than salinomycin.

Researchers have emphasized a significant role of the antiporter capacity of alkali metal ions rather than interaction with a specific cellular target in salinomycin and its analogs.[5,6,9,13] In addition, Boehmerle found the relationships of the antiporter capacity of salinomycin and its neurotoxicity.[10,11] Herein, our previous and this work also suggested possibly similar mechanisms of the antiproliferative activities and neurotoxicity because there were some positive correlations between the SI values in derivatives with different spatial configurations.[8] Except for 17-*epi*-salinomycin, which adopts relatively open conformation, 17,21-di-*epi*-salinomycin and 20-*epi*-salinomycin can still combine with alkali metal ions with relatively closed conformations. Although the spatial configuration of C20-hydroxyl group has little influence on the activities because it does not participate in coordination with alkali metal ions,[14] the acyl groups lead to obvious difference by producing effects on the stability and permeability of the complexes in salinomycin and its epimers.

3. Conclusion

In summary, 20-*epi*-salinomycin and a series of 20-*epi*-*O*-acyl-salinomycin derivatives were synthesized and evaluated for their antiproliferative activities as well as neurotoxicity. Both



20-*epi*-O-benzoyl salinomycin **9d** 20-O-benzoyl salinomycin **10**

Fig. 2 The 'twist-boat' conformations of compound **9d** and its epimer **10**.

Table 2 Antiproliferative activity and neurotoxicity of tested compounds *in vitro* (IC_{50} [μM][a])

Compounds	HCG-27	SI[b]	HT-29	SI	MDA-MB-231	SI[b]	Neuron cell
1	2.457 ± 0.115	2.2	1.434 ± 0.808	3.8	6.907 ± 1.310	0.8	5.490
8	>10.0	—	>10.0	—	>10.0	—	>10.0
9	2.553 ± 0.108	>0.4	2.218 ± 0.680	>0.45	6.858 ± 0.118	>0.14	>1.0
9a	0.940 ± 0.009	>1.0	0.804 ± 0.156	>1.0	1.959 ± 0.800	>0.5	>1.0
9b	0.891 ± 0.008	>1.0	0.839 ± 0.105	>1.0	1.889 ± 0.729	>0.5	>1.0
9c	0.861 ± 0.058	>1.0	0.892 ± 0.094	>1.0	2.775 ± 0.215	>0.36	>1.0
9d	0.244 ± 0.010	>4.1	0.181 ± 0.076	>5.5	0.785 ± 0.019	>1.27	>1.0
9e	0.277 ± 0.015	>3.6	0.158 ± 0.100	>6.3	0.792 ± 0.066	>1.26	>1.0
10	0.024 ± 0.001	3.2	0.026 ± 0.002	2.9	0.038 ± 0.008	2.0	0.076

[a] IC_{50} values are the mean (±SE) for 50% reduction of MTT compared to control. For all entries, $n = 3$. [b] Selectivity index (SI) of a drug is defined as the ratio of the toxic dose to the therapeutic dose (*in vitro* SI = IC_{50} neuron cell line/IC_{50} tumor cell line).

the C1-protecting group of the salinomycin and the acidity of the substituted benzoic acid are crucial to the Mitsunobu conversions. Although 20-*epi*-salinomycin showed similar antiproliferative activity as salinomycin, its 20-*O*-acylated analogs were 2–10 times more potent. In addition, the 20-*epi*-20-*O*-acylated salinomycin derivative **9d** and **9e** had better selectivities than salinomycin between cancer and neuron cell lines. These results suggest that the spatial configuration of C20-hydroxyl group has little influence on the activities, but the acyl groups cause obvious difference by producing possible effects on the stability and permeability of the salinomycin–alkali metal ions complexes.

4. Experimental

4.1 Chemistry

4.1.1 General experimental information. All reactions were performed in glassware containing a Teflon coated stir bar. Solvents and chemical reagents were obtained from commercial sources and used without further purifications. ^1H and ^{13}C NMR spectra were recorded on Varian Mercury 400 MHz with $CDCl_3$ as the solvent. Chemical shifts (δ) were reported in ppm downfield from an internal TMS standard. High-resolution mass spectra were obtained in the ESI mode. Flash column chromatography on silica gel (200–300 mesh) was used for the routine purification. The column output was monitored by TLC on silica gel (100–200 mesh) precoated on glass plates (15 × 50 mm), and spots were visualized by 5% vanillin sulfuric acid/ethanol solution.

20-epi-O-p-Nitrobenzoylated salinomycin TMSEt-ester ***5****.* To a stirred solution of salinomycin TMSEt-ester **4** (1.0 g, 1.18 mmol), *p*-nitrobenzoic acid (0.5 g, 5.9 mmol) and triphenylphosphine (3.0 g, 11.8 mmol) in THF at room temperature, was slowly added the diisopropyl azodiformate (DIAD, 2.0 mL, 11.8 mmol). The mixture was stirred at room temperature for 24 hours and then concentrated under reduced pressure. The residue was purified by column chromatography (TLC, petroleum ether/EtOAc = 4 : 1, $R_f = 0.71$) to afford compound **5** (1.0 g, 1.0 mmol, 84.7%) as yellow oil.

^1H NMR (400 MHz, $CDCl_3$) δ 8.27 (d, $J = 8.2$ Hz, 1H), 8.16 (d, $J = 8.2$ Hz, 1H), 7.26 (s, 1H), 6.51 (d, $J = 10.6$ Hz, 1H), 6.33 (dd, $J = 10.0$, 5.7 Hz, 1H), 5.22 (d, $J = 5.3$ Hz, 1H), 4.55–4.32 (m, 1H), 4.12–3.99 (m, 1H), 3.76 (d, $J = 6.5$ Hz, 1H), 3.69 (d, $J = 9.6$ Hz, 1H), 3.59 (d, $J = 10.3$ Hz, 1H), 3.49 (d, $J = 7.5$ Hz, 1H), 3.24 (dd, $J = 15.1$, 7.7 Hz, 1H), 3.01 (dd, $J = 22.0$, 11.0 Hz, 1H), 2.75 (d, $J = 9.5$ Hz, 1H), 2.54 (s, 1H), 2.13 (dd, $J = 16.0$, 9.0 Hz, 2H), 1.89 (ddd, $J = 64.8$, 25.5, 13.4 Hz, 4H), 1.68–0.64 (m, 56H), 0.08 (s, 9H).

20-epi-O-p-Nitrobenzoylated salinomycin sodium salt ***8****.* To a stirred solution of the intermediate product **5** (27 mg, 0.027 mmol) in DMF, was added KF (15.6 mg, 10.0 equiv.). The resulting solution was stirred at 80 °C until complete consumption of starting material (typically 12 hours, TLC control), and then concentrated under reduced pressure. After purification by flash chromatography (TLC, petroleum ether/EtOAc = 4 : 1, $R_f = 0.28$), the product was re-dissolved in EtOAc (10 mL) and washed with Na_2CO_3 (0.1 M aq., 3 × 10 mL). The organic layer was separated, dried with Na_2SO_4, filtrated and concentrated several times from *n*-pentane to give compound **8** (15 mg, 60%) as colorless foam. ^1H NMR (400 MHz, $CDCl_3$) δ 8.31 (d, $J = 8.6$ Hz, 1H), 8.18 (d, $J = 8.7$ Hz, 1H), 6.50 (d, $J = 10.5$ Hz, 1H), 6.35 (dd, $J = 10.4$, 5.7 Hz, 1H), 5.31 (d, $J = 5.7$ Hz, 1H), 4.22–4.16 (m, 1H), 4.06–3.92 (m, 2H), 3.86 (dd, $J = 12.4$, 5.6 Hz, 1H), 3.78–3.62 (m, 3H), 2.99–2.88 (m, 1H), 2.80 (dd, $J = 9.3$, 7.6 Hz, 1H), 2.67 (d, $J = 10.1$ Hz, 1H), 2.39–0.44 (m, 62H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 215.4, 178.3, 163.8, 150.6, 135.5, 129.9, 129.1, 122.9, 105.7, 99.1, 90.3, 77.3, 75.4, 75.0, 73.8, 71.6, 71.1, 68.4, 68.3, 56.1, 49.9, 49.3, 40.2, 39.0, 37.3, 36.5, 36.0, 32.8, 31.1, 30.2, 29.7, 29.3, 28.0, 26.3, 25.9, 22.9, 22.1, 19.9, 18.0, 16.8, 16.5, 14.2, 14.1, 13.3, 13.0, 12.1, 11.0, 6.7, 6.5. HRMS-ESI (*m*/*z*): $[M + H]^+$ calcd for: $C_{49}H_{73}NNaO_{14}$, 922.4929; found: 922.4959.

20-epi-Salinomycin sodium salt ***9****.* To a stirred solution of TMSEt-ester **5** (380 mg, 0.38 mmol) was added K_2CO_3 (66 mg, 0.478 mmol) in methanol (2 mL) at r.t. The resulting solution was stirred 1 h, and then concentrated under reduced pressure to give yellow oil. The residue was purified by column chromatography to afford 20-*epi*-salinomycin-TMSEt **6** (290 mg, 0.34 mmol, 89.8%) as colorless oil. To a stirred solution of the intermediate product **6** (50 mg, 0.059 mmol) in DMF, was added KF (34.1 mg, 10.0 equiv.). The resulting solution was stirred at 80 °C until complete consumption of starting material (typically 12 hours, TLC control), and then concentrated under reduced

pressure. After purification by flash chromatography (TLC, petroleum ether/EtOAc = 4 : 1, R_f = 0.15), the product was re-dissolved in EtOAc (10 mL) and washed with Na_2CO_3 (0.1 M aq., 3 × 10 mL). The organic layer was separated, dried with Na_2SO_4, filtrated and concentrated several times from *n*-pentane to give compound **9** (35 mg, 90.1%) as colorless foam.

^{1}H NMR (400 MHz, $CDCl_3$) δ 6.31 (dt, J = 21.7, 7.7 Hz, 1H), 4.35 (d, J = 4.5 Hz, 0H), 4.26 (d, J = 10.1 Hz, 1H), 4.03 (d, J = 2.4 Hz, 0H), 3.96 (dd, J = 10.2, 3.2 Hz, 0H), 3.84–3.69 (m, 2H), 3.58 (d, J = 10.1 Hz, 1H), 3.43 (d, J = 11.6 Hz, 0H), 2.86 (dd, J = 10.5, 8.4 Hz, 1H), 2.77–2.60 (m, 1H), 2.33–0.55 (m, 39H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 217.0, 184.3, 128.9, 125.6, 108.7, 99.0, 88.8, 77.3, 76.0, 75.8, 74.2, 71.4, 70.1, 68.0, 67.1, 64.9, 55.1, 51.4, 50.2, 39.7, 38.5, 35.9, 35.8, 32.9, 32.4, 32.3, 29.7, 29.3, 28.9, 28.0, 27.6, 27.0, 25.6, 23.7, 20.7, 20.2, 17.4, 16.7, 16.1, 14.5, 13.2, 12.5, 12.0, 10.9, 6.8, 6.5. HRMS-ESI (*m*/*z*): $[M + H]^+$ calcd for: $C_{42}H_{70}NaO_{11}$, 773.4816; found: 773.4846.

4.1.2 General procedure for the preparation of 20-*epi*-*O*-acylated salinomycin sodium salt 9a, 9b and 9d. To a stirred solution of alcohol **6** (0.2 mmol) in dry pyridine (2 mL) at r.t., was added DMAP (catalyst), and acid anhydride (3.0 equiv.). The resulting solution was stirred 1 h, quenched with methanol, and then concentrated under reduced pressure to give pale yellow oil. Filtration through a short plug of silica gave the intermediate **7a**, **7b** or **7d** as clear oil.[6] To a stirred solution of the intermediate product **7a**, **7b** or **7d** in DMF, was added KF (10.0 equiv.). The resulting solution was stirred at 80 °C until complete consumption of starting material (typically 12 hours, TLC control), then concentrated under reduced pressure. After purification by flash chromatography (EtOAc/petroleum ether), the product was re-dissolved in EtOAc (10 mL) and washed with Na_2CO_3 (0.1 M aq., 3 × 10 mL). The organic layer was separated, dried with Na_2SO_4, filtrated and concentrated several times from *n*-pentane to give **9a**, **9b** or **9d** as colorless foam.

*20-epi-O-Acetylated salinomycin sodium salt **9a**.* 45%, ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.35 (d, J = 10.6 Hz, 1H), 6.29 (dd, J = 10.4, 5.6 Hz, 1H), 4.96 (d, J = 5.4 Hz, 1H), 4.41–4.31 (m, 1H), 4.21 (d, J = 10.2 Hz, 1H), 4.11 (dd, J = 14.3, 7.2 Hz, 1H), 3.90 (dd, J = 10.7, 2.9 Hz, 1H), 3.70 (d, J = 9.8 Hz, 1H), 3.57 (d, J = 10.1 Hz, 1H), 3.35 (d, J = 11.8 Hz, 1H), 2.83 (t, J = 10.2 Hz, 1H), 2.68 (d, J = 10.6 Hz, 1H), 2.62 (dd, J = 8.9, 8.3 Hz, 1H), 2.29–0.43 (m, 63H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 217.0, 184.2, 169.4, 127.7, 123.9, 106.1, 99.2, 89.6, 77.2, 75.8, 75.0, 74.2, 71.4, 69.8, 67.2, 66.1, 55.2, 51.0, 50.4, 40.0, 39.0, 36.3, 35.9, 32.7, 32.3, 32.3, 27.9, 27.8, 26.9, 23.8, 22.7, 20.8, 20.5, 19.9, 17.4, 16.3, 16.1, 14.6, 13.2, 12.5, 11.8, 10.6, 6.7, 6.4. HRMS-ESI (*m*/*z*): $[M + H]^+$ calcd for: $C_{44}H_{72}NaO_{12}$, 815.4921; found: 815.5399.

*20-epi-O-Acryl salinomycin sodium salt **9b**.* 46%, ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.39 (d, J = 10.7 Hz, 1H), 6.31 (dd, J = 10.6, 5.6 Hz, 1H), 5.07 (d, J = 5.6 Hz, 1H), 4.38 (d, J = 6.5 Hz, 1H), 4.23 (d, J = 10.3 Hz, 1H), 3.93 (dd, J = 11.0, 4.3 Hz, 1H), 3.72 (d, J = 9.9 Hz, 1H), 3.60 (d, J = 9.9 Hz, 1H), 3.39 (d, J = 11.4 Hz, 1H), 2.87 (td, J = 10.9, 3.1 Hz, 1H), 2.75–2.60 (m, 2H), 2.34–2.24 (m, 2H), 2.15–0.58 (m, 65H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 217.1, 184.4, 173.0, 127.7, 123.9, 106.3, 99.2, 89.6, 75.9, 75.8, 75.1, 74.2, 71.4, 70.0, 67.3, 66.0, 55.3, 51.1, 50.3, 40.0, 38.9, 36.3, 35.9, 32.7, 32.4, 32.2, 28.8, 28.0, 27.8, 27.7, 26.9, 23.8, 20.5, 20.0, 17.5, 16.3, 16.1, 14.6, 13.2, 12.5, 11.9, 10.7, 9.1, 6.7, 6.4, 1.0. HRMS-ESI (*m*/*z*): $[M + H]^+$ calcd for: $C_{45}H_{74}NaO_{12}$, 829.5078; found: 829.5108.

*20-epi-O-Benzoylated salinomycin sodium salt **9d**.* 65%, ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.96 (d, J = 7.5 Hz, 1H), 7.52 (t, J = 7.1 Hz, 1H), 7.39 (t, J = 7.5 Hz, 1H), 6.40 (m, 6.44–6.36, 2H), 5.27 (d, J = 4.8 Hz, 1H), 4.38 (d, J = 6.5 Hz, 1H), 4.22 (d, J = 10.3 Hz, 1H), 4.11 (dd, J = 14.3, 7.1 Hz, 1H), 3.91 (d, J = 8.3 Hz, 1H), 3.72 (d, J = 9.9 Hz, 1H), 3.61 (d, J = 9.8 Hz, 1H), 3.37 (d, J = 11.6 Hz, 1H), 2.85 (t, J = 10.5 Hz, 1H), 2.76–2.59 (m, 2H), 2.33–0.41 (m, 62H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 213.8, 175.9, 171.3, 133.0, 129.7, 128.4, 123.8, 109.8, 106.5, 100.1, 99.2, 89.8, 86.5, 77.2, 75.2, 71.7, 71.5, 69.7, 68.9, 66.6, 63.7, 56.7, 48.8, 40.2, 38.9, 38.7, 36.6, 36.0, 32.5, 31.0, 30.6, 28.0, 26.8, 26.0, 22.2, 21.5, 20.1, 19.7, 18.2, 17.5, 17.4, 16.6, 16.1, 14.6, 13.7, 13.3, 13.3, 12.4, 11.9, 11.0, 6.8, 6.5. HRMS-ESI (*m*/*z*): $[M + NH_4]^+$ calcd for: $C_{49}H_{77}NNaO_{12}$, 894.5343; found: 894.5384.

4.1.3 General procedure for the preparation of 20-*epi*-*O*-acylated salinomycin sodium salt 9c and 9e. To a stirred solution of alcohol **6** (1.0 equiv.) in CH_2Cl_2 at r.t., was added Et_3N (6.0 equiv.) in one portion, followed by addition of DMAP, and then acid chloride (3.0 equiv.). The resulting solution was stirred overnight, then diluted with EtOAc (10 mL) and washed with Na_2CO_3 (0.1 M aq., 3 × 10 mL). The organic layer was separated, dried, and concentrated under reduced pressure to give pale yellow oil. Filtration through a short plug of silica gave the intermediate ester product **7c** and **7e** as clear oil. To a stirred solution of the intermediate product **7c** or **7e** in DMF, was added KF (10.0 equiv.). The resulting solution was stirred at 80 °C until complete consumption of starting material (typically 12 hours, TLC control), then concentrated under reduced pressure. After purification by flash chromatography (EtOAc/petroleum ether), the product mixture was re-dissolved in EtOAc (10 mL) and washed with Na_2CO_3 (0.1 M aq., 3 × 10 mL). The organic layer was separated, dried with Na_2SO_4, filtrated and concentrated several times from *n*-pentane to give **9c** or **9e** as colorless foam.

*20-epi-O-Isobutyryl salinomycin sodium salt **9c**.* 40%, ^{1}H NMR (400 MHz, chloroform-*d*) δ 6.36 (d, J = 10.8 Hz, 1H), 6.26 (dd, J = 10.8, 5.2 Hz, 1H), 5.03 (d, J = 5.4 Hz, 1H), 4.35 (d, J = 7.2 Hz, 1H), 4.20 (d, J = 10.3 Hz, 1H), 3.90 (d, J = 11.1 Hz, 1H), 3.69 (d, J = 10.1 Hz, 1H), 3.58 (d, J = 10.0 Hz, 1H), 3.36 (d, J = 11.9 Hz, 1H), 2.83 (t, J = 11.1 Hz, 1H), 2.77–2.57 (m, 2H), 2.46 (p, J = 7.3 Hz, 1H), 2.32–0.37 (m, 56H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 217.1, 184.3, 175.4, 127.6, 123.9, 106.3, 99.2, 89.6, 77.3, 75.9, 75.8, 75.0, 74.3, 71.4, 69.9, 67.3, 65.8, 55.3, 51.1, 50.4, 40.1, 38.9, 36.3, 35.9, 34.1, 32.7, 32.4, 32.3, 28.9, 28.0, 27.9, 26.9, 23.8, 20.6, 20.0, 18.9, 18.7, 17.5, 16.4, 16.1, 14.6, 13.2, 12.5, 11.9, 10.7, 6.7, 6.5. HRMS-ESI (*m*/*z*): $[M + H]^+$ calcd for: $C_{46}H_{76}NaO_{12}$, 843.5234; found: 843.5259.

*20-epi-O-p-Fluorobenzoylated salinomycin sodium salt **9e**.* 48%, ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.05–7.85 (m, 2H), 7.05 (t, J = 8.5 Hz, 2H), 6.38 (dt, J = 10.6, 8.3 Hz, 2H), 5.26 (d, J = 5.3 Hz, 1H), 4.42–4.32 (m, 1H), 4.22 (d, J = 10.3 Hz, 1H), 3.90 (dd, J = 10.6, 2.4 Hz, 1H), 3.70 (d, J = 10.0 Hz, 1H), 3.60 (d, J = 9.7 Hz, 0H), 3.37 (d, J = 11.6 Hz, 1H), 2.85 (dd, J = 10.5, 9.3 Hz, 1H), 2.75–2.53 (m, 2H), 2.28–0.43 (m, 62H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 217.3, 184.5, 164.3, 132.4, 132.3, 128.3, 124.0, 115.8, 115.6,

106.5, 99.4, 90.0, 76.2, 76.0, 75.3, 74.5, 71.6, 70.1, 67.5, 66.9, 55.5, 51.3, 50.6, 40.3, 39.1, 36.8, 36.1, 32.9, 32.6, 32.5, 28.2, 28.1, 27.1, 24.0, 20.8, 20.2, 17.7, 16.7, 16.3, 14.8, 13.4, 12.7, 12.1, 10.9, 6.9, 6.7. HRMS-ESI (m/z): $[M + H]^+$ calcd for: $C_{49}H_{73}FNaO_{12}$, 895.4984; found: 895.4984.

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