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# Bivalent polyether ionophores: Synthesis and biological evaluation of $C_2$-symmetric salinomycin dimers

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ABSTRACT

Efficient methods for the synthesis of $C_2$-symmetric dimers of salinomycin joined at either the C1 or C20 positions are reported. Similarly to the native structure, the C20-*O*-terephthalate dimer displayed activity in the low μM range against a series of cancer cell lines, while dimers joined at the C1 position were essentially inactive.



The monocarboxylic polyether ionophore salinomycin (**1**, Fig. 1) has found wide-spread commercial use in veterinary medicine as a coccidiostatic agent and non-hormonal growth promoter.[1] In 2009, this structure received additional attention as it was shown to be the most active out of 16,000 compounds in selectively targeting breast cancer stem cells (CSCs).[2,3] Used at higher concentrations, salinomycin and related polyether ionophores such as nigericin, 20-deoxy salinomycin (SY-1), and monensin have also been shown to induce apoptosis in various human cancer cell lines.[4,5]

Because of its interesting biological properties and abundant availability, several recent studies have utilized semi-synthetic approaches for the generation of analogues. In particular, chemical modification of the C1 carboxylate[6] and C20 hydroxyl group[7] have been extensively studied. Acyl derivatives of the C20 hydroxyl group exhibit enhanced basal toxicity, and significantly, also exhibit pronounced anti-CSC activity at low nM concentrations at which salinomycin itself was inactive.[8] Esterification or amide formation at the C1 position of salinomycin generally results in decreased activity, but interestingly, certain analogues, including propargyl amides, retain activity in the low μM range and show good activity against multi-drug resistant (MDR) cancer cell lines.[6a,b] More elaborate derivatives of salinomycin, including natural product conjugates, have also been described.[9]

A less explored avenue in polyether antibiotics is the synthesis and functional evaluation of multivalent structures[10]; although two dimeric salinomycin derivatives joined by amide linkers are known.[6a] The related polyether ionophore, lasalocid acid, is known to form dimeric complexes with divalent ions, which suggests the potential of this concept.[11] Very recently, it has been reported that salinomycin triazole dimers are more cytotoxic than salinomycin monomers against breast cancer cells[12]; this observation inspired us to synthesise a new series of salinomycin dimers.

Starting from the most active salinomycin derivatives in each class of C1 and C20 analogues, herein, we report the synthesis and biological evaluation of novel dimeric salinomycin analogues (Schemes 1 and 2) that display a twofold axis of symmetry (**2**–**3**, Fig. 1). *In vitro* activity evaluation towards five cancer cell lines, including the doxorubicin resistant LoVo/DX cell line, showed that the dimer joined at the C20 hydroxyl group exhibited considerable activity with $IC_{50}$ values similar to the native structure. On the other hand, dimers joined at the C1 position showed a loss of activity.

Synthetic manipulations of salinomycin are challenging as its structure is highly sensitive, not only to acidic and basic conditions,

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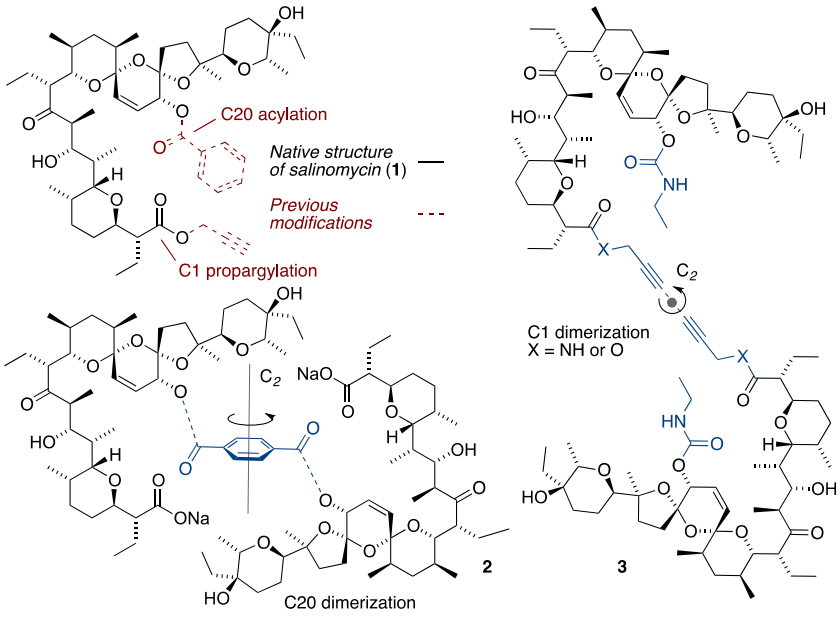
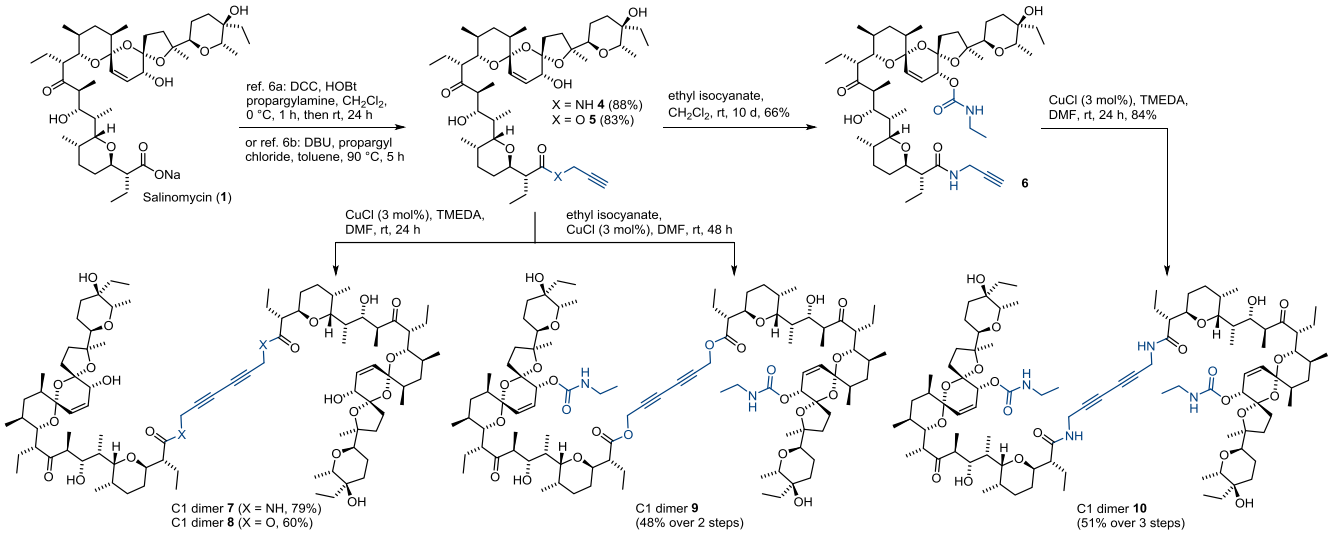



**Fig. 1.** Introduction of a twofold axis of symmetry by the dimerization of salinomycin. Dotted blue lines in **2** and **3** indicate targeted bond formation sites in the dimerization processes.




**Scheme 1.** C1 dimerization of salinomycin (**7**–**8**) and C20–*O*–acylated salinomycin (**9**–**10**).

but also to elevated temperatures.[13] However, the reported copper (I) catalysed carbamoylation of the C20 hydroxyl group suggested that Glaser-type coupling reactions of C1-propargylated derivatives would be a viable strategy to access dimeric structures. To this end, propargyl ester **5** was prepared by the reaction of salinomycin with propargyl chloride in the presence of DBU. Similarly, the previously known propargyl amide **4** was prepared in 88% yield following a reported procedure,[6a] using DCC as a coupling agent and HOBt as an activator. Gratifyingly, both derivatives were efficiently dimerized in the presence of CuCl as a catalyst under an air atmosphere[14] to form the targeted 1,3-diynes **7** and **8** (Scheme 1). In the light of the sensitivity and polyfunctionalized nature of these structures, the efficiency of the coupling reactions is noteworthy.

Acylation of the C20 hydroxyl group of salinomycin is known to enhance anti-proliferative activity, but a combination of modifications of C1 and C20 positions has not been previously explored. Both propargyl amide **4** and propargyl ester **5** were therefore exposed to ethyl isocyanate and catalytic CuCl. Using ester **5**, the copper salt catalysed both the carbamoylation reaction and the Glaser coupling to give the desired C20–*O*–ethyl carbamate C1 dimer **9**. In contrast, propargyl amide **4** gave only unidentified side-products under the same conditions. The synthesis of dimer **10** was instead accomplished by the Cu(I)-catalysed dimerization of carbamate **6**, which in turn was formed by treating **4** with ethyl isocyanate at room temperature (Scheme 1). Isolation of both dimers **9** and **10** in pure form was challenging due to the presence of small amounts of unreacted starting materials **4** and **5**, respectively. Treatment of the crude reaction mixtures with TESCl/imidazole resulted in silylation of the C20 hydroxyl groups of **4** and **5** which significantly reduced the polarity of these compounds[7c]; the dimers could then be readily separated by flash chromatography affording the desired products **9** and **10** in 48–51% yield (Scheme 1).