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Exceedingly Efficient Synthesis of (±)-Grandifloracin and Acylated Analogues

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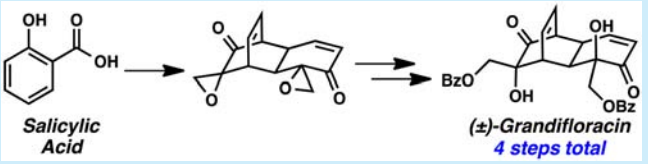
Supporting Information

ABSTRACT: A highly efficient regio- and stereoselective total synthesis of (±)-grandifloracin via a tandem dearomative epoxidation/spontaneous Diels–Alder cyclodimerization from salicylic acid in only four steps is reported. The synthetic route allows for late-stage diversification of the core structure to give ready access to analogues of this promising agent against pancreatic cancer.



Pancreatic cancer is one of the most severe forms of cancer, with a 5 year survival rate of only 3–5%. This high mortality rate has not improved during the last four decades of chemotherapeutic research.[1] To date, there has not been any report of an effective treatment against the disease, and the agents used against other types of cancer have been shown to have little to no effect on pancreatic cancer.[2] It has been hypothesized that this might be the result of pancreatic cancer cells being hypovascular and thereby able to proliferate in a nutrient-deficient and hypoxic environment.[3] The lack of viable treatments for and the severity of pancreatic cancer create a pressing need to develop new drugs to combat the disease.[4]

One means to overcome these challenges is to develop antiausterity agents. These drugs target the biochemical pathways that allow cancer cells to thrive under low nutrition, thereby reducing or halting the cancerous cells' ability to survive in hypovascular conditions without affecting the surrounding cells.[4] A recent breakthrough came in 2012 when the Awale group extracted (+)-grandifloracin from the stem of *Uvaria dac.*[5] (+)-Grandifloracin (Figure 1) was shown to be a potent

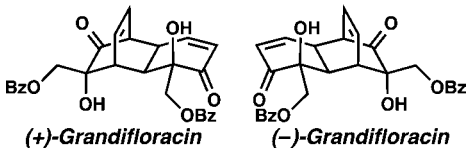


Figure 1. (+)-Grandifloracin and (−)-grandifloracin.

antiausterity agent against pancreatic cancer cell lines PANC-1 (PC_{50} 14.5 μM), PSN-1 (PC_{50} 32.6 μM), MIA PaCa-2 (PC_{50} 17.5 μM), and KLM-1 (PC_{50} 32.7 μM).[6] Interestingly, the first isolation of grandifloracin from *Uvaria grandiflora* in 1997[7] was later shown to be the (−)-enantiomer of grandifloracin.[8]

In a comprehensive study of (+)-grandifloracin, its mode of action was determined to be the induction of autophagic programmed cell death in PANC-1 cells rather than through typical apoptotic modes.[9] This evidence strongly supports the potential efficacy of antiausterity agents in the treatment of pancreatic cancer.

The first synthesis of grandifloracin in racemic form was reported in 2007 by Quideau and co-workers.[10] This synthesis hinged on a hydroxylative dearomatization of 2-hydroxybenzyl benzoate, which subsequently underwent a spontaneous Diels–Alder cyclodimerization to directly yield (±)-grandifloracin, albeit in a modest 30% yield. A subsequent asymmetric syntheses of (+)-grandifloracin by Lewis and co-workers similarly made use of a spontaneous Diels–Alder reaction of a chiral cyclohexa-2,4-dienone obtained after a five-step sequence following the microbial oxidative dearomatization of benzoic acid.[7] Notably, Toste and co-workers have also reported the synthesis of a fluorinated (−)-grandifloracin derivative,[11] utilizing an asymmetric dearomative fluorination and cyclodimerization of silyloxymethylphenol, followed by deprotection and acylation in a single step. However, they note that the corresponding benzoate ester only gave trace conversion in the fluorination step.

While the linchpin Diels–Alder cyclodimerization is highly efficient in constructing the backbone of grandifloracin, its use can also be limiting for potential diversification for the study of structure–activity relationships. In each of the syntheses of the natural product, the hydroxymethyl group is protected as a benzoate ester prior to the cyclodimerization step. As such, any derivative must be obtained either by a deprotection–acylation sequence, adding extra steps to such a library synthesis, or by carrying each individual acyl group through the Diels–Alder step. This is potentially troublesome, as electronically differentiated substrates can perform with substantially varying yields.[12] As such, when planning our synthesis, we sought to carry a free hydroxymethyl group through the cyclodimerization

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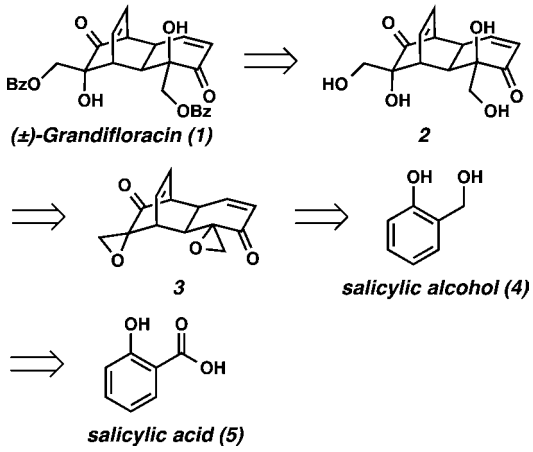
in order to facilitate subsequent derivatization for biological study of analogues of the natural product.

The aim of this project was to develop an even more efficient synthetic route to (±)-grandifloracin than previously employed that would permit late-stage diversification of a key intermediate. To achieve this, we devised a strategy (Scheme 1) in which

Scheme 1. Retrosynthesis of (±)-Grandifloracin



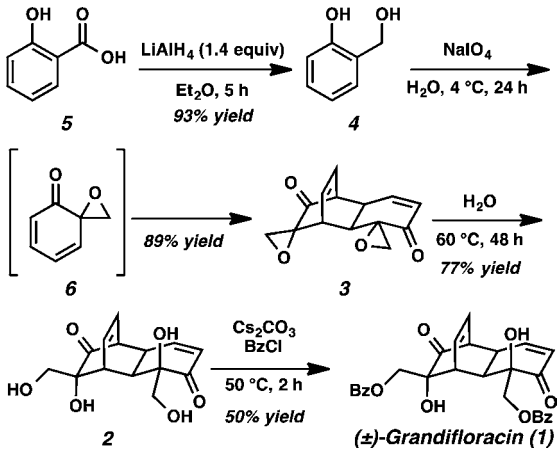
removal of the benzoyl groups of grandifloracin (**1**) yields the core structure as a diol (**2**), which would be amenable to derivatization by selective acylation of the free hydroxymethyl groups. This key tetraol was anticipated to arise from a double epoxide opening of the known bis-spiroepoxydienedione **3**.[13] This key intermediate can be readily prepared through an oxidative dearomatization of salicylic alcohol (**4**) and subsequent Diels–Alder homodimerization.

Salicylic acid (**5**) was reduced by $LiAlH_4$ to give the corresponding salicylic alcohol (**4**, 93% yield). The alcohol was then subjected to known oxidative dearomatization with sodium periodate ($NaIO_4$) in water to afford spiroepoxycyclohexadienone (**6**).[12] Diene **6** spontaneously undergoes a Diels–Alder dimerization to yield the dispiro-oxirane **3** in a remarkably stereoselective and efficient overall process (89% yield of a >10:1 mixture of diastereomers). The two epoxides were smoothly opened by gentle heating in water over 2 days to yield the core tetraol **2** (77% yield).[14] The tetraol was treated with benzoyl chloride to furnish (±)-grandifloracin (50% yield), completing the total synthesis in only four steps from salicylic acid in an overall yield of 32% (Scheme 2). Gratifyingly, we were able to identify suitable chromatographic conditions to allow separation of (+)- and (−)-grandifloracin by preparative chiral HPLC. Even after separation of the two enantiomers, the yield for each antipode is still significantly higher than that of the previously reported enantioselective total synthesis of (+)-grandifloracin.[7]

This rapid and efficient four-step synthesis of (±)-grandifloracin (**1**) was further improved by eliminating intermediate recrystallization steps (Scheme 3). Salicylic alcohol was telescoped through the synthetic route without any purification until the final compound. This resulted in a yield of 52% over the last three steps and an excellent overall 48% yield. This procedure allowed us facile access to over 200 mg of the racemic natural product and was amenable to even larger scale for the synthesis of analogues (vide infra).

To investigate the effect of the acyl substituents present on the natural product, a small library of analogues was synthesized (Table 1). Introduction of a methyl group in the *para*, *meta*, or

Scheme 2. Total Synthesis of (±)-Grandifloracin



Scheme 3. Telescoped Synthesis of (±)-Grandifloracin

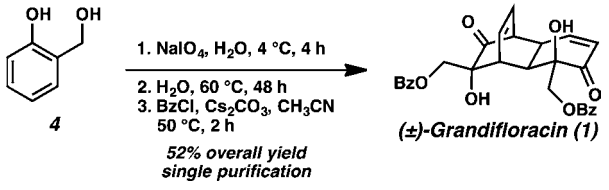
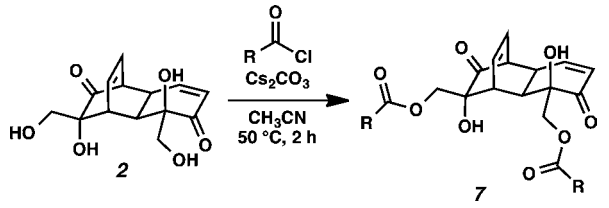
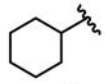


Table 1. Synthesis of a Small Library of Analogues



entry	product	R	yield [%]
1	**7a**	2-Me-C_6H_4	48
2	**7b**	3-Me-C_6H_4	51
3	**7c**	4-Me-C_6H_4	55
4	**7d**	4-OMe-C_6H_4	69
5	**7e**	4-CN-C_6H_4	25
6	**7f**		58
7	**7g**		55

ortho position (**7a–c**) of the phenyl group was well tolerated in the acylation reaction, proceeding in roughly the same yields as with benzoyl chloride. Replacement of the phenyl ring with a cyclic alkyl group (**7f–g**) slightly increased the yield of acylation. While the introduction of an electron-donating *para*-methoxy group (**7d**) substantially increased the yield of the acylation reaction, the introduction of an electron-withdrawing *para*-cyano group (**7e**) significantly lowered the yield. This trend was also observed when we attempted to synthesize the *para*-nitro analogue, which failed due to lack of reactivity. The moderate yield in the acylation step is believed to arise from competing elimination of the α-hydroxy group of the B-ring.[15]

In summary, we have completed a highly efficient synthesis of (±)-grandifloracin, which is amenable to late-stage diversification for the synthesis of analogues. In a recent study, analogue **7d**

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has been shown to have an increased antiproliferative effect compared with grandifloracin on PANC-1 and HT-29 (human colon cancer) cells, both in nutrient-rich (10% fetal bovine serum) and in nutrient-deprived conditions (0.5% fetal bovine serum).[16] This indicates that there is an incentive to develop further grandifloracin analogues for the study and treatment of pancreatic cancer.

■ ASSOCIATED CONTENT

Supporting Information

Detailed experimental procedures, IR, ^{1}H, and ^{13}C NMR spectra for all new compounds. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.5b01292.

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Notes

The authors declare no competing financial interest.

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(6) Although the PC_{50} values for (+)-grandifloracin are double digit μM, the most potent antiausterity agent known, arctigenin, is only an order of magnitude more potent in identical assays (PANC-1 assay PC_{50} ~0.51−1.7 μM). By contrast, clinical agents such as gemcitabine and paclitaxel show no activity (PC_{50} >100 μM).[3b,5]
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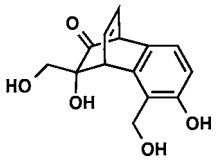

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