Total Synthesis of 6-Deoxypladienolide D and Assessment of Splicing Inhibitory Activity in a Mutant SF3B1 Cancer Cell Line

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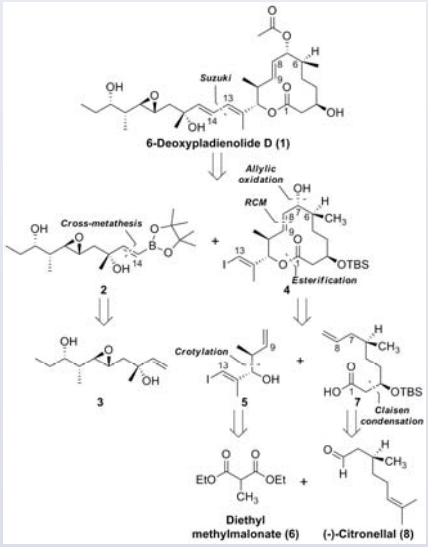
Abstract

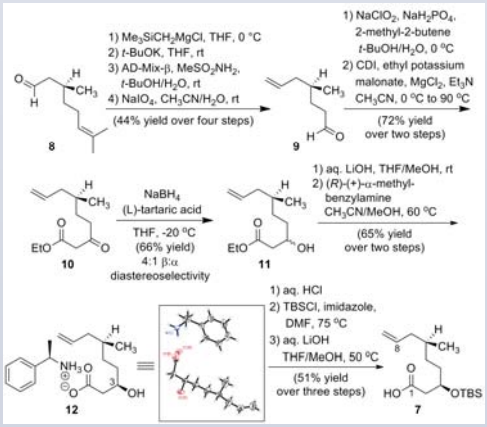
Mutations in several components of the spliceosome have been reported in various hematological malignancies and solid tumors. In particular, recurrent heterozygous mutations in SF3B1 have been identified in chronic lymphocytic leukemia, myelodysplastic syndrome, chronic myelomonocytic leukemia, uveal melanoma, breast, and pancreatic cancers. SF3B1 is a component of the U2 snRNP complex of the spliceosome and is involved in the recognition of splice sites during early spliceosomal assembly. We and others have demonstrated that mutations in SF3B1 result in neomorphic activity and trigger the production of aberrantly spliced transcripts. Thus, the discovery of small molecule modulators of SF3B1 splicing activity may have therapeutic potential in cancers harboring SF3B1 mutations.

Members of the pladienolide family of natural products have been previously shown to affect RNA splicing through modulation of the SF3b complex, and 6-deoxypladienolide D has been reported as one of the pladienolide members which exhibits sub-nanomolar growth inhibitory activity in colon cancer cells. While analogs of 6-deoxypladienolide D have been previously prepared via modification of the natural product, this semi-synthetic approach was particularly challenging due to the limited supply of 6-deoxypladienolide D and the synthetic inaccessibility to most regions of the molecule. For these reasons, coupled with the burgeoning interest to identify chemical matter able to modulate splicing in newly-identified mutant SF3B1 cancers, a total synthesis of 6-deoxypladienolide D using versatile and modular fragments was initiated.

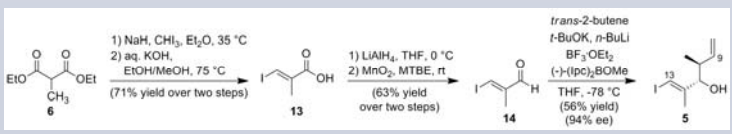
Retrosynthetic analysis



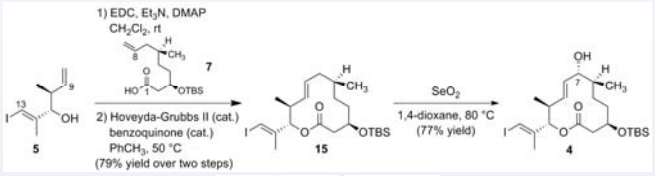
Synthesis of C1.C8 fragment

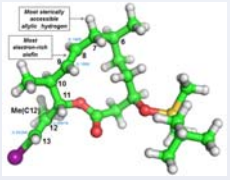


Synthesis of C9.C13 fragment

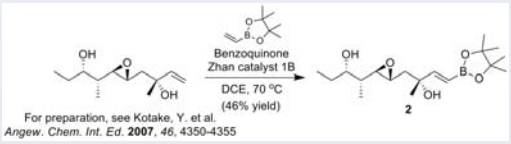


Macrocycle formation and allylic oxidation

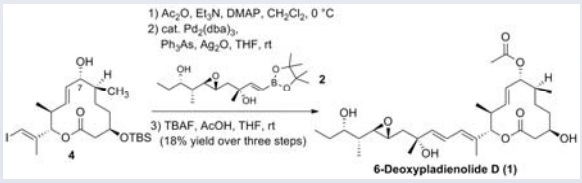




Synthesis of sidechain fragment



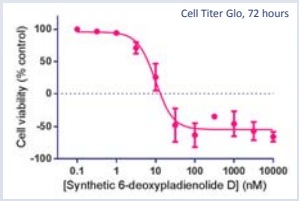
Completion of the total synthesis



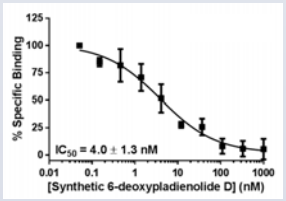
[1]H NMR and LCMS data matched the values previously reported for the biosynthesized natural product, and additional spectroscopic analysis ([13]C NMR, COSY, and HRMS) further corroborated its structure.

Biological data for 6-deoxypladienolide D

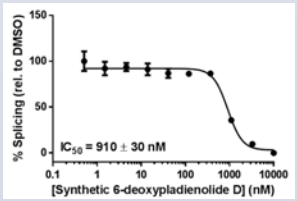
Cell viability assay (Panc 05.04 cells)



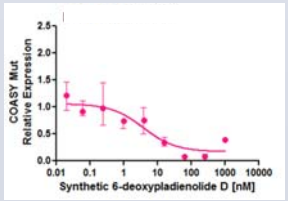
Binding assay (SF3B1^{K700E})



In vitro splicing assay (SF3B1^{K700E})



Cell PD assay (SF3B1^{K700E})



Panc 05.04 SF3B1^{Q699H,K700E} cell viability assay	GI_{50} (nM)	8.1 ± 3.2
	E_{max} (%)	-69.5 ± 11.2
Binding assay SF3B1^{K700E} (IC_{50}, nM)		4.0 ± 1.3
In vitro splicing assay SF3B1^{K700E} (IC_{50}, nM)		910 ± 30
Cell PD assay SF3B1^{K700E} COASY^{MUT} mRNA (IC_{50}, nM)		3.8 ± 2.4

Conclusions

The first total synthesis of the natural product 6-deoxypladienolide D has been achieved. Two noteworthy synthetic attributes are: 1) a late-stage allylic oxidation which proceeds with full chemo-, regio-, and diastereoselectivity and 2) the use of cost-effective starting materials and reagents to enable access to 6-deoxypladienolide D and its analogs for biological evaluation. In addition, we have found that 6-deoxypladienolide D demonstrates: 1) cellular lethality at single-digit nanomolar concentration in Panc 05.04 cells, 2) high binding affinity to the SF3b complex, 3) ability to inhibit pre-mRNA splicing, and 4) modulation of an aberrantly-spliced transcript identified in mutant SF3B1 cells.

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