Organic LETTERS

Letter

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Total Synthesis of GEX1Q1, Assignment of C-5 Stereoconfiguration and Evaluation of Spliceosome Inhibitory Activity

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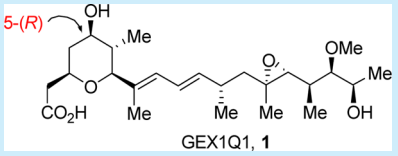
Supporting Information

ABSTRACT: An enantioselective total synthesis of GEX1Q1 has been accomplished in a convergent manner. The C-5 asymmetric center has now been assigned through synthesis. GEX1Q1 displayed slightly better spliceosome inhibitory activity over its C-5 epimer. The salient features of this synthesis include an asymmetric hetero-Diels–Alder reaction to construct the tetrahydropyran ring and a Suzuki cross-coupling to assemble the key segments.



GEX1Q1 (**1**, Figure 1) is one of the six natural products including GEX1A (herboxidiene) isolated from a culture broth of *Streptomyces* sp. by Yoshida and co-workers in 2002.[1] It has shown cytotoxicity with an IC_{50} value of 0.93 μM against human tumor cell lines in vitro.[1] GEX1Q1 contains 10 chiral centers. Nine of them have been assigned by extensive spectroscopic studies. However, the C-5 hydroxy stereochemistry had not been assigned.[1,2] The related natural product, 5-deoxy GEX1Q1, known as herboxidiene (**2**), displayed important biological properties. It showed induction of cell cycle arrest in G1 and G2/M phase in human normal fibroblast cell lines.[3] It also caused reduction of plasma cholesterol by upregulating gene expression.[4] Furthermore, it displayed potent spliceosome inhibitory activity.[5] As a consequence, there has been immense interest in total synthesis[6,7] and biological studies[8] of herboxidiene. We recently reported a convergent total synthesis[9] and spliceosome inhibitory properties[10] of herboxidiene. In our continuing interest in herboxidiene and its derivatives, we have explored the chemistry and biology of GEX1Q1. Herein, we report a convergent synthesis of GEX1Q1, assignment of the stereochemistry of the C-5 asymmetric center and evaluation of spliceosome inhibitory activity of GEX1Q1 and its C-5 epimer.

Our retrosynthetic analysis of GEX1Q1 is outlined in Figure 1. We planned to carry out Suzuki cross-coupling of vinyl iodide **3** and vinyl boronate **4** to construct GEX1Q1. A similar strategy was employed by Forsyth and co-workers[7a] and by us[9] during the synthesis of herboxidiene. The functionalized tetrahydropyran ring **3** would be synthesized from tetrahydropyranone derivative **5**. Vinyl boronate **4** was synthesized by us previously.[9] We planned to carry out an asymmetric synthesis of **5** via a hetero-Diels–Alder reaction with diene **6** and propargylaldehyde derivative **7**.

An asymmetric synthesis of tetrahydropyranone derivative **11** is shown in Scheme 1. Silyloxy diene **6** was prepared from aldehyde **8** as described by us previously.[11] An asymmetric

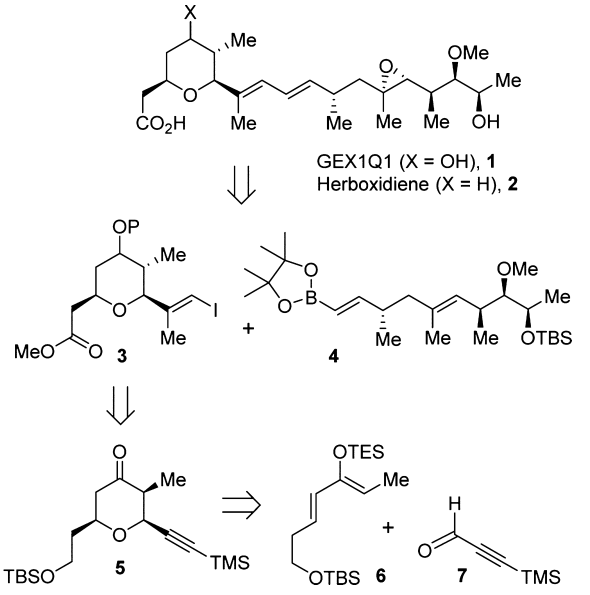


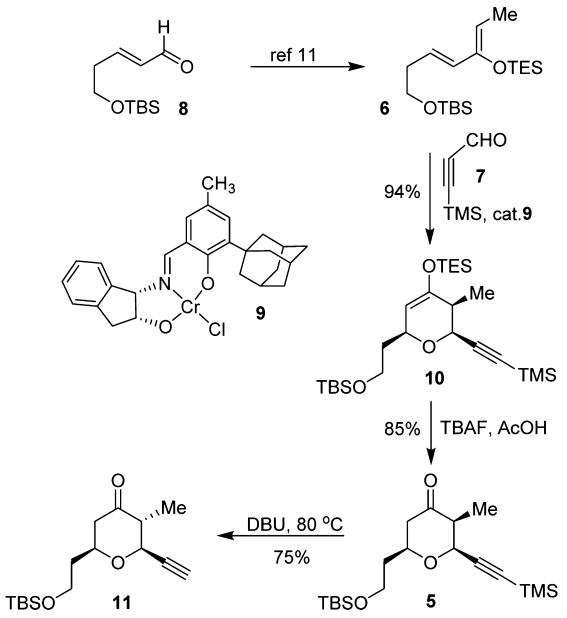
Figure 1. Retrosynthesis of GEX1Q1.

hetero-Diels–Alder reaction of diene **6** with commercially available aldehyde **7** in the presence of Jacobsen's catalyst **9**[12] (5 mol %) afforded cycloadduct **10** in 94% yield. Removal of the triethylsilyl group by treatment with tetrabutylammonium fluoride (TBAF) provided pyranone **5** in 85% yield. HPLC analysis of **5** on a chiral OD column showed enantiomeric purity of 94% ee.[13] The synthesis of segment **3** required inversion of configuration of the C-6 methyl center. This was accomplished by treatment of **5** with DBU and EtOH in toluene at 80 °C for 24 h.[14] This condition resulted in the

Received: May 9, 2014
Published: May 28, 2014

   dx.doi.org/10.1021/ol501345d | *Org. Lett.* 2014, 16, 3154–3157

Scheme 1. Synthesis of Tetrahydropyranone 11



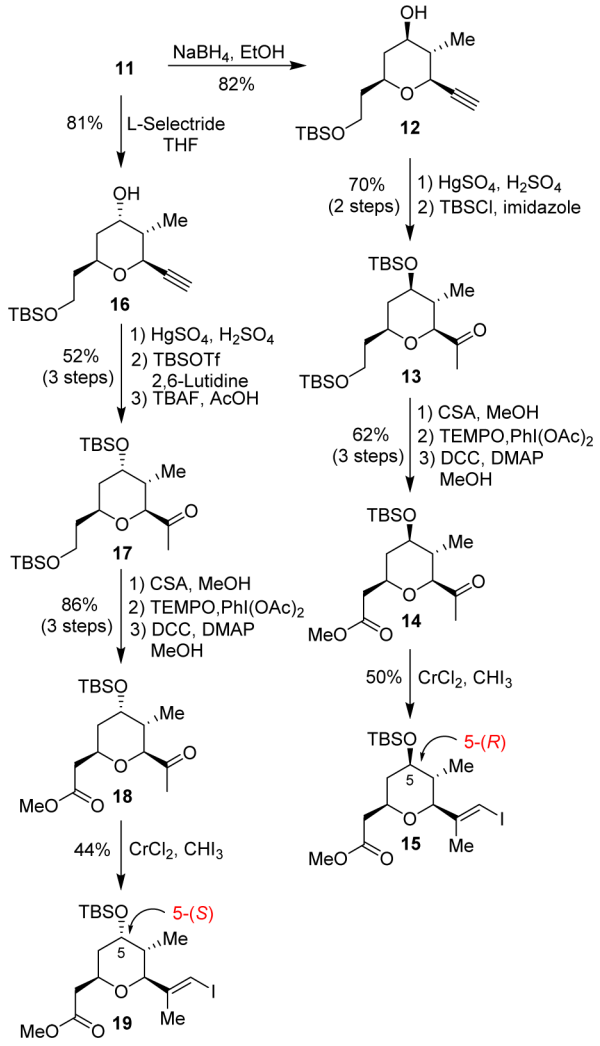
deprotection of the TMS group as well as epimerization of the C-6 methyl center. Pyranone **11** was separated by silica gel chromatography and about 10% of starting pyranone **5** was recovered. This was subjected to further epimerization to provide **11** in 75% yield after one recycle.

The syntheses of vinyl iodides are shown in Scheme 2. To obtain the equatorial alcohol, we carried out reduction[15] of **11** with $NaBH_4$ in ethanol at 0 °C to furnish alcohol **12** in 82% isolated yield. Acetylene derivative **12** was treated with a catalytic amount of $HgSO_4$ in 3 M H_2SO_4 as described by Yates and co-workers[16] to afford the corresponding ketone. The hydroxyl group was protected as the TBS ether to provide bis-TBS derivative **13** in 70% yield over two steps. This was converted to methyl ester **14** in a three step sequence involving: (1) selective deprotection of the primary TBS group by exposure to CSA in MeOH at 0 °C for 2 h; (2) oxidation of the resulting primary alcohol with TEMPO in the presence of $PhI(OAc)_2$ in aqueous CH_2Cl_2 at 23 °C for 4 h; and (3) esterification of the resulting acid with MeOH using DCC and DMAP in CH_2Cl_2 at 23 °C for 2 h. Methyl ester **14** was then converted to vinyl iodide **15** using a procedure described by Takai and co-workers[17] by exposure to $CrCl_2$ and CHI_3 in THF at 23 °C for 15 h, providing **15** in 50% yield (brsm).

An asymmetric synthesis of tetrahydroprane derivative **19** bearing 5-(*S*)-configuration is also shown in Scheme 2. To set the 5-(*S*)-configuration with an axial alcohol, we planned to reduce tetrahydropyranone **11** with L-Selectride.[18] Treatment of **11** with L-Selectride in THF at −78 °C for 1 h afforded alcohol **16** in 81% yield. This was converted to bis-TBS ether **17** in 52% yield over three steps.[19] Compound **17** was converted to methyl ester **18** and then to vinyl iodide **19** as described for compound **15**.

Toward the synthesis of GEX1Q1, we first carried out Suzuki cross-coupling of boronate **4**[9] and vinyl iodide **15** containing the 5-(*R*)-configuration. As shown in Scheme 3, reaction of **4** and **15** in the presence of $Pd(Ph_3P)_4$ (5 mol %) and Cs_2CO_3 in THF at 55 °C for 2.5 h provided the coupling product **20** in 82% yield. Treatment of **20** with 1 M HCl in methanol at 23 °C

Scheme 2. Stereoslective Synthesis of 5-(*R*)- and 5-(*S*)-Tetrahydropyran derivatives 14 and 19

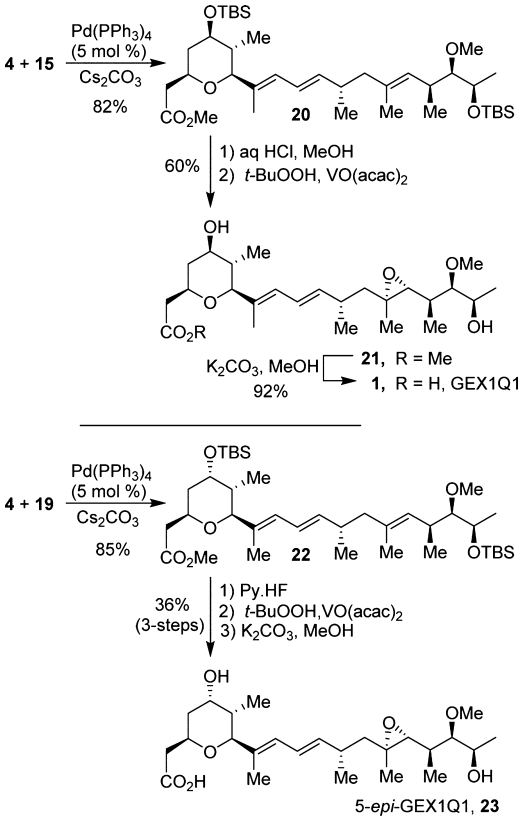


for 45 min resulted in the deprotection of TBS-ethers to furnish the corresponding diol. Exposure of the diol to a catalytic amount of $VO(acac)_2$ in the presence of *t*-BuOOH at −15 °C for 48 h afforded the epoxide **21** as a single product in 60% yield in two steps. Saponification of methyl ester **21** with K_2CO_3 in aqueous MeOH at reflux for 2 h afforded synthetic GEX1Q1 (**1**) with 5-(*R*)-hydroxy stereochemistry in 92% yield. To our delight, ^1H NMR and ^{13}C NMR data of synthetic **1** ($[\alpha]^{23}_D$ −13.8, *c* 0.12, MeOH) are identical to that reported for the natural GEX1Q1 ($[\alpha]^{20}_D$ −13.5, *c* 0.13, MeOH).[1,2]

To further compare the spectral data, as well as to evaluate the effect of the C-5 hydroxyl group stereochemistry of GEX1Q1, we synthesized the C-5-(*S*) stereoisomer of GEX1Q1. As shown, Suzuki coupling of vinyl iodide **19** with boronate **4** resulted in coupling product **22** in 85% yield. Due to steric hindrance, the deprotection of the TBS group at C-5 was sluggish under methanolic HCl conditions. However, reaction of **22** with Py.HF at 60 °C for 20 h provided the desired product in 72% yield. The resulting diol was then converted to 5-*epi*-GEX1Q1 **23** by epoxidation, followed by saponification of the methyl ester as described for GEX1Q1. Spectroscopic properties (^1H NMR and ^{13}C NMR) and optical rotation ($[\alpha]^{23}_D$ +16.9, *c* 0.13, MeOH) of compound **23** are significantly different from that of GEX1Q1.[1,2]

dx.doi.org/10.1021/ol501345d | *Org. Lett.* 2014, 16, 3154−3157

Scheme 3. Synthesis of (+)-GEX1Q1 and its C-5 Epimer



The biological properties of GEX1Q1 **1** and compound **23** were evaluated in an *in vitro* splicing system as previously described.[10] We added the compounds to splicing reactions containing a synthetic pre-mRNA substrate, ATP, and nuclear extract from HeLa cells. As previously characterized for this extract system, spliceosomes assemble on only a portion of the pre-mRNA substrate and catalyze intron removal.[10] We examined splicing activity by denaturing PAGE to separate the substrate and product mRNA, and splicing efficiency was quantified as the percent of pre-mRNA converted to mRNA. In the system, DMSO alone, which has no effect on splicing efficiency, is used as a control.[10] GEX1Q1 inhibits splicing relative to DMSO with an IC_{50} of 0.3 μM (Figure 2A and C). Compound **23** showed a slight reduction in potency with an IC_{50} of 0.8 μM. However, the difference is within the variation of splicing efficiency measured by the assay. These values are also comparable to what we have previously observed for herboxidiene.[10]

We also examined the effect of these compounds on spliceosome assembly. Spliceosomes assemble on pre-mRNA substrates via an ordered series of intermediate complexes. A subset of these complexes can be visualized by native gel analysis of the same *in vitro* splicing reactions described above. H/E and A complexes form as early intermediates that convert to B and then to C complex, at which point the splicing reaction is catalyzed and the complexes immediately disassemble. As with splicing chemistry, DMSO alone has no effect and spliceosomes assemble normally over time (Figure 2B). With increasing concentrations of GEX1Q1 (**1**) and compound **23**, spliceosome assembly halts at an A-like complex. The block in spliceosome assembly appears to be

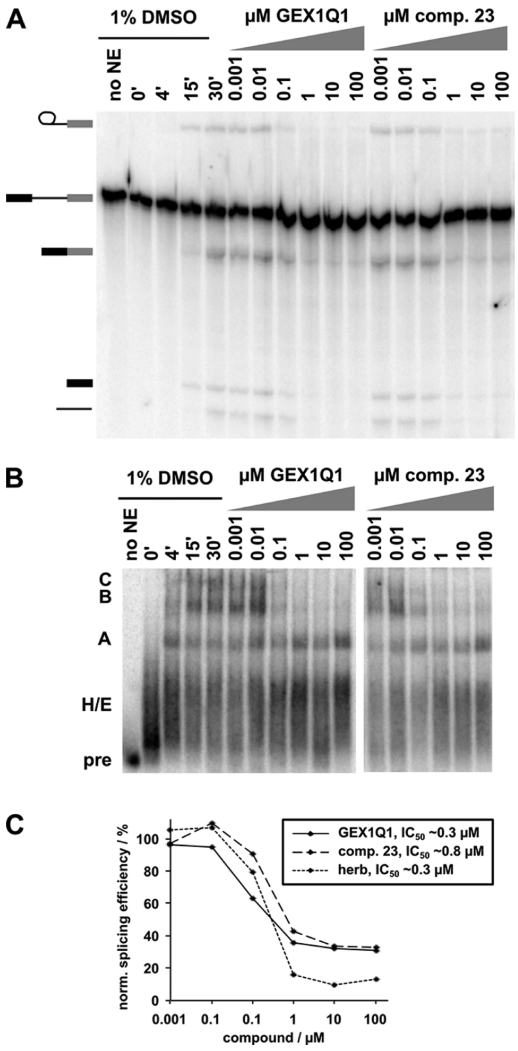


Figure 2. Impact of analogues on *in vitro* splicing. (A) Denaturing gel analysis of radiolabeled RNA isolated from splicing reactions. The first five lanes include a time course of splicing reactions in 1% DMSO followed by 30 min time points of splicing reactions incubated with indicated compound concentration. Identities of bands are schematized to the left as (from top to bottom) lariat intermediate, pre-mRNA, mRNA, 5′ exon intermediate. (B) Native gel analysis of spliceosome assembly. Aliquots of the splicing reactions described above were separated under native conditions. The identity of splicing complexes is denoted with assembly occurring in the following order: H/E → A → B → C. (C) Quantification of normalized splicing efficiency vs inhibitor concentration for the splicing reactions shown in A and B, respectively. IC_{50} refers to the concentration required to reduce *in vitro* splicing efficiency by half compared to DMSO control.

identical to that produced by herboxidiene and two other splicing inhibitors, pladienolide B and FR901464.[10]

In summary, we have accomplished a stereoselective synthesis of GEX1Q1 and assigned the C-5 hydroxyl group stereochemistry of GEX1Q1. Also, we have evaluated spliceosome inhibitory activity of GEX1Q1 and its C-5 epimer and compared their activity with herboxidiene. The synthesis features a hetero-Diels–Alder reaction to construct the tetrahydropyran ring, inversion of the C-5 asymmetric center of the resulting cycloadduct and a Suzuki coupling reaction to assemble the protected GEX1Q1. We have also probed the importance of the C-5 hydroxyl group stereochemistry of GEX1Q1 and its epimer in terms of their effect on spliceosome

dx.doi.org/10.1021/ol501345d | *Org. Lett.* 2014, 16, 3154–3157

activity. Interestingly, both GEX1Q1 and its C-5 epimer showed nearly identical potency relative to herboxidiene.[10] Therefore, the C-5 hydroxyl group stereochemistry does not significantly influence spliceosome inhibitory activity. The design and synthesis of novel herboxidiene and GEX1Q1 derivatives are in progress.

■ ASSOCIATED CONTENT

Supporting Information

General experimental procedures, characterization data for all products. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

Financial support by the National Institutes of Health and the University of California Cancer Research Coordinating Committee is gratefully acknowledged.

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