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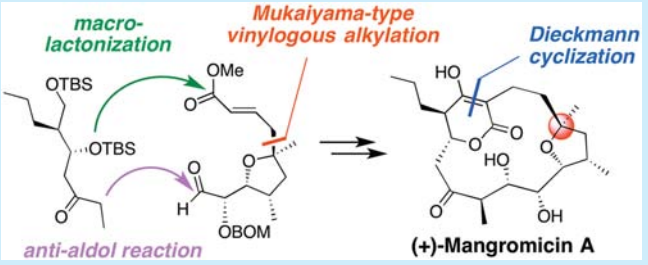
Total Synthesis and Determination of the Absolute Configuration of Naturally Occurring Mangromicin A, with Potent Antitrypanosomal Activity

Hirokazu Takada, Takeshi Yamada, Tomoyasu Hirose, Takuma Ishihara, Takuji Nakashima, Yōko Takahashi, Satoshi Ōmura,* and Toshiaki Sunazuka*

Graduate School of Infection Control Sciences and Kitasato Institute for Life Sciences, Kitasato University, 5-9-1 Shirokane, Minato-ku, Tokyo 108-8641, Japan

Supporting Information

ABSTRACT: An enantioselective total synthesis of (+)-mangromicin A has been accomplished. The tetrahydrofuran ring of mangromicin A, possessing a tetrasubstituted carbon center, was constructed by Mukaiyama-type vinylogous alkylation via a cyclic oxocarbenium intermediate derived from a γ-hydroxy ketone with ideal stereoselectivity, and the 4-hydroxydihydropyrone scaffold was generated via Dieckmann cyclization at a late stage of the total synthesis. The reliable asymmetric synthesis of (+)-mangromicin A has revealed the absolute configuration of naturally occurring mangromicin A.



Mangromicin A (**1**), B (**2**), and C (**3**) (Figure 1) were isolated from a culture broth of the rare actinomycete

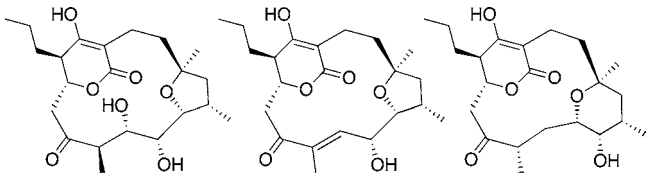


Figure 1. Structures of mangromicins.

Lechevalieria aerocolonigenes K10−0216 by our group in 2012 using a customized physicochemical screening system.[1−3] Mangromicins A and B possess a macrocyclic pentadecane framework, including a 5,6-dihydro-4-hydroxy-α-pyrone and a highly substituted tetrahydrofuran moiety containing a tetrasubstituted carbon center. The macrocyclic pentadecane framework of these novel metabolites is related to akaeolide, which was subsequently discovered by Igarashi et al. in 2013.[4] Although an X-ray crystallographic structure of mangromicin A was obtained, its absolute configuration has not been determined because the Flack parameter did not allow determination of the absolute configuration.

Mangromicins A and B were found to exhibit in vitro antitrypanosomal activity against *Trypanosoma brucei brucei* GUTat 3.1 with IC_{50} values of 2.4 and 43.4 μg/mL, respectively.[1,2] The value for **1** is similar to that of the known antitrypanosomal drugs suramin and eflornithine.[1] Furthermore, we expected that **1** probably has a different antitrypanosomal action mechanism because of its novel chemical structure. Thus, we envisioned the total synthesis and structure−activity relationship studies of mangromicin A with a view to using it as a lead compound for the development of a new antitrypanosomal drug.

In the course of isolation and structural determination analysis of the mangromicins, a 5,6-dihydro-4-hydroxy-α-pyrone moiety was found to be susceptible to oxidation conditions. This dihydropyrone moiety causes the rigid configuration of the transannulated pentadecane framework of the mangromicins. Therefore, we envisioned a late-stage installation of the dihydropyrone and its scaffold (Scheme 1). We planned to construct the macrocyclic pentadecane skeleton containing the dihydro-α-pyrone moiety via a combination of macrolactonization and Dieckmann cyclization of compound **4** at a late stage of the synthesis. The linear intermediate **4** can be obtained by anti-aldol reaction of ketone **5** and γ-alkoxy aldehyde **6**. The tetrahydrofuran ring moiety of **6** can be constructed by Mukaiyama-type vinylogous alkylation[5] in a stereoselective manner for the newly generated tetrasubstituted carbon center. The γ-hydroxy ketone **7** would be prepared from a commercially available D-mannitol derivative.

Our synthesis commenced with the reduction of compound (−)-**8**, the enantiomer of which has been reported by Smith et al.,[6] with $LiBH_4$ to provide alcohol (+)-**9** in good yield (Scheme 2). Subsequently, mesylation of the resulting primary alcohol and nucleophilic displacement with NaCN afforded nitrile (+)-**10**. The obtained (+)-**10** was treated with MeLi followed by acidic hydrolysis to furnish the corresponding ketone, which was then

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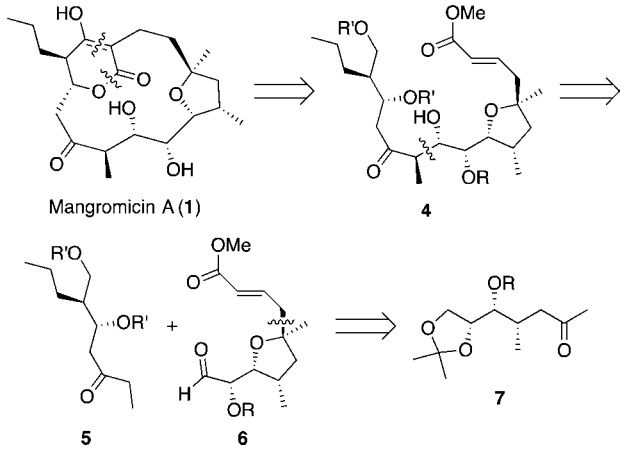
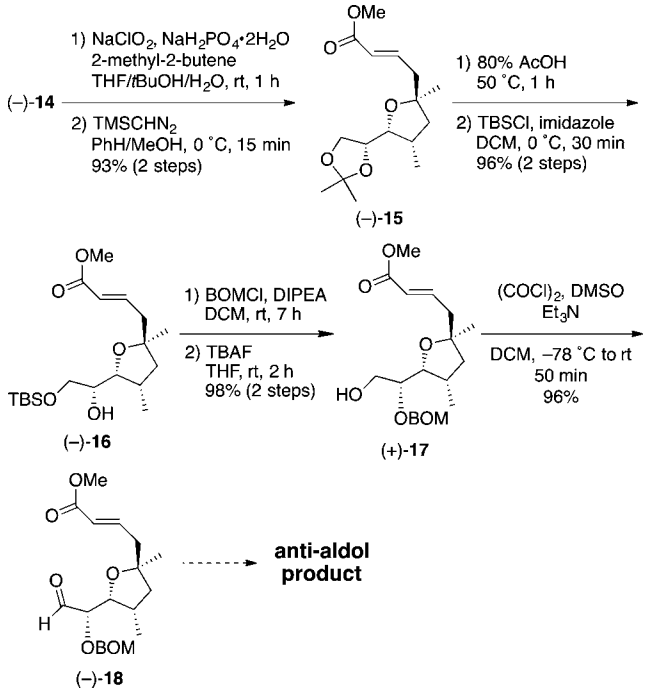
Scheme 1. Retrosynthesis of Mangromicin A (1)



Scheme 3. Synthesis of Aldehyde (−)-18



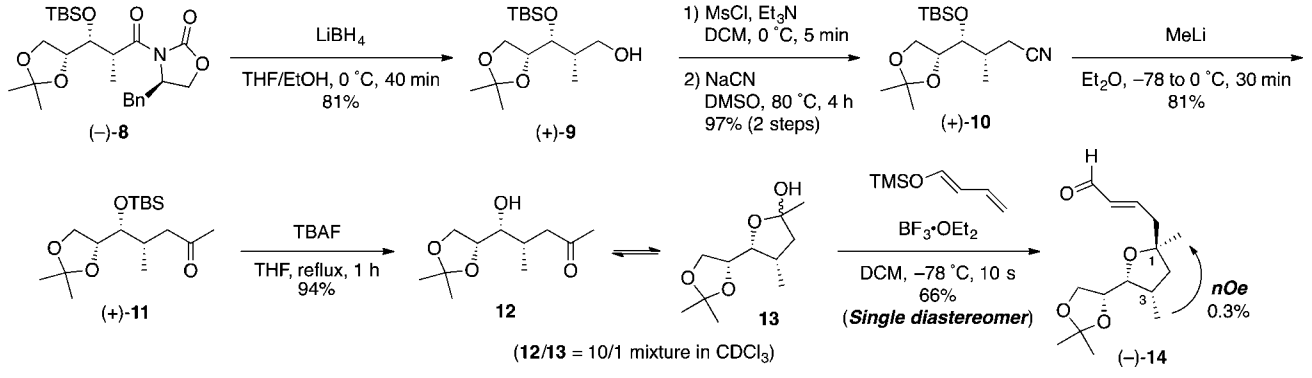
subjected to deprotection of the TBS group to yield hydroxy ketone **12** and hemiacetal **13** as an equilibrium mixture (**12**/**13** = 10/1 in $CDCl_3$). These structures were determined by the observation of both keto and acetal signals in the ^{13}C NMR spectrum. Next, our attention focused on the Mukaiyama-type vinylogous alkylation of the mixture of **12** and **13** to introduce the tetrasubstituted carbon center. The crucial furan ring formation, involving the introduction of the stereoselective tetrasubstituted carbon center, was examined under various conditions (e.g., PPTS, $TiCl_4$, $MgBr_2{\cdot}OEt_2$, and TBSOTf)[5] with 1-(trimethylsiloxy)-1,3-butadiene but without success. Eventually, the desired reaction succeeded when $BF_3{\cdot}OEt_2$ was employed as a Lewis acid and the reaction proceeded at −78 °C for 10 s in DCM. The nucleophilic substitution reaction was stereoselectively influenced from inside by steric hindrance of the methyl group at C-3 (the pseudoequatorial position). The stereochemistry was determined by observation of an NOE relationship between the C-3 and C-1 methyl groups.

With the desired tetrahydrofuran moiety possessing the tetrasubstituted carbon center in hand, the acetonide moiety of (−)-**14** was converted to the α-alkoxy aldehyde (−)-**18** for the subsequent anti-aldol reaction (Scheme 3). Pinnick oxidation of (−)-**14** produced the carboxylic acid,[7] which upon esterification yielded the desired conjugated ester (−)-**15**. Deprotection of the isopropylidene acetal followed by selective protection of the primary alcohol provided the desired silyl ether (−)-**16** in excellent yield. The resultant secondary alcohol was then protected using BOMCl, after which removal of the TBS group and Swern oxidation furnished the desired aldehyde (−)-**18** in satisfying yield.

With aldehyde (−)-**18**, the coupling partner for the anti-aldol reaction, in hand, ketone (+)-**25** was then synthesized by utilization of the Peters protocol[8] (Scheme 4). *Trans*-selective asymmetric [2 + 2] cyclocondensation of valeryl bromide and 4-pentenal (**19**) using the reported Al−salen complex **20** afforded the desired β-lactone (+)-**21** in excellent yield with high diastereo- and enantioselectivity, as per the Peters report. Opening of the lactone with $LiAlH_4$ followed by protection of the diol using TBSOTf afforded bis(silyl ether) (+)-**22**. Subsequently, allylic oxidation via SeO_2 and Dess−Martin oxidation of the secondary alcohol[9] afforded enone (+)-**24**, which upon hydrogenolysis provided the desired ethyl ketone (+)-**25**.

With both required substrates (−)-**18** and (+)-**25** in hand, we explored the next step, the key anti-aldol reaction. Collum and co-workers reported the LiHMDS/Et_3N-mediated (*E*)-selective enolization of acyclic ketones.[10] Consequently, we expected that this (*E*)-enolate would react with the suitable aldehyde to generate the desired anti-aldol adduct. Furthermore, we expected

Scheme 2. Construction of the Tetrasubstituted Carbon Center



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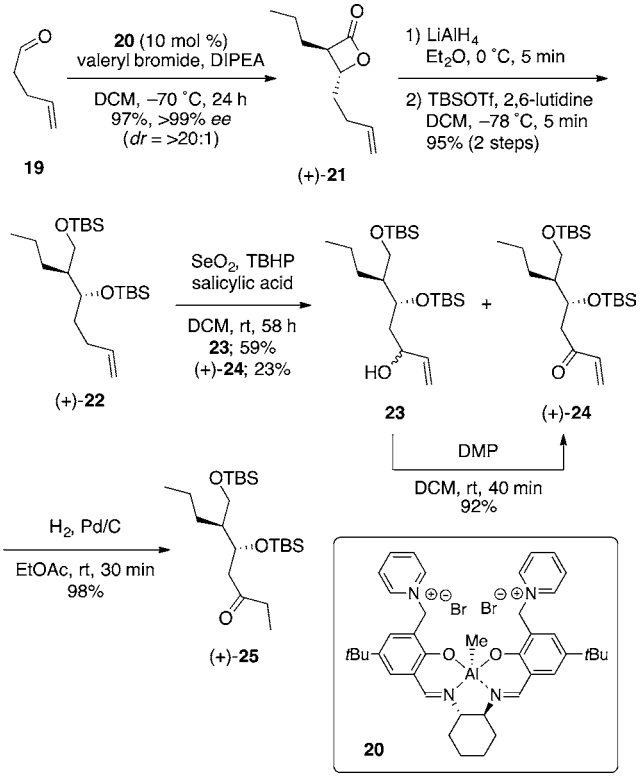
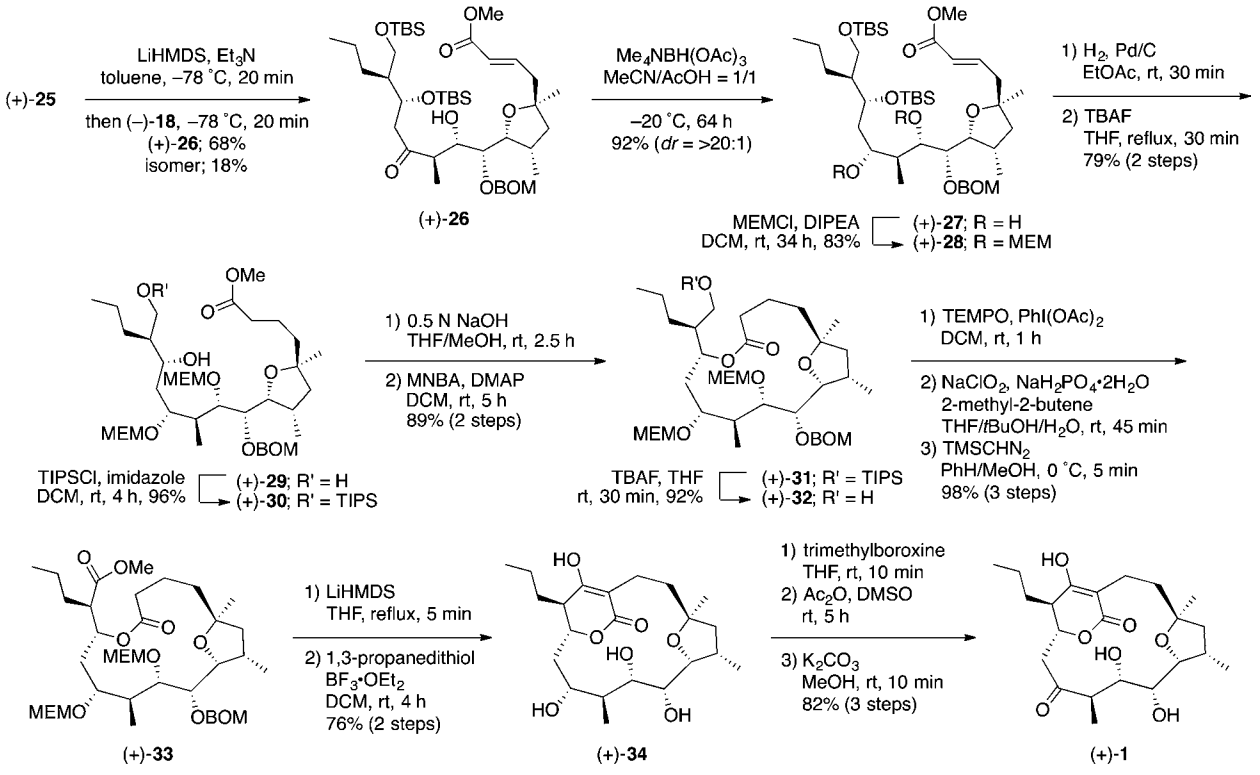
Scheme 4. Synthesis of Ethyl Ketone (+)-25



this reaction to give a stereoselective aldol product via a chelation-controlled mechanism by the coordination of Li between the α-alkoxy group and the carbonyl moiety of aldehyde (−)-**18**. In reality, the reaction proceeded smoothly, giving the two anti-aldol products, the desired (+)-**26** and its isomer [(+)-**S9** in the Supporting Information], in 68 and 18% yield, respectively (Scheme 5). In the desired major product (+)-**26**, the stereochemistry of the hydroxy group was determined by the modified Mosher's method.[11] The stereochemistry of the α-position of the ketone was confirmed by ROESY analysis of the isopropylidene acetal compound derived from diol (+)-**27** (see the Supporting Information). The anti reduction of (+)-**26** was performed using the Evans protocol,[12] and the resulting 1,3-diol was converted to MEM ether (+)-**28**. The conjugated ester was exposed to hydrogenation conditions followed by deprotection of the TBS group, and the resultant primary alcohol was reprotected with a triisopropylsilyl (TIPS) group selectively. Subsequent hydrolysis of (+)-**30** under basic conditions produced the seco acid, which was subjected to the Shiina macrolactonization protocol[13] to yield the desired macrolactone (+)-**31** in good yield. After removal of the TIPS group, the hydroxy group was oxidized to the carboxylic acid using TEMPO and Pinnick oxidation,[7] and then the carboxylic acid was treated with $TMSCHN_2$ to afford the Dieckmann cyclization precursor (+)-**33** in excellent yield.

The crucial Dieckmann cyclization was one of the most difficult and risky steps involved in our synthesis strategy. Indeed, the application of Dieckmann cyclization for such a trans-annulated dihydropyrone formation has not previously been reported. Moreover, the expected reaction required kinetically controlled deprotonation at the α-position plus quick nucleophilic attack on the nonactivated carbonyl carbon of the ester moiety on the other side. Consequently, this transformation has never been applied in natural product syntheses. To conquer these difficulties, we examined several sets of reaction conditions. We eventually discovered a solution, namely, the dropwise addition of LiHMDS to a reflux-heated solution of (+)-**33** in

Scheme 5. Total Synthesis of (+)-Mangromicin A (1)



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THF. The reaction proceeded smoothly to afford the desired 4-hydroxydihydropyrone scaffold in excellent yield.

The next problem faced was the sensitivity of the 4-hydroxydihydropyrone moiety, which was easily oxidized to the α-pyrone, as we initially anticipated. Therefore, this dihydropyrone was immediately subjected to the next step without further purification under a nitrogen atmosphere. The MEM and BOM groups were removed under 1,3-propanedithiol and $BF_3{\cdot}OEt_2$ conditions[14] to give dihydromangromicin ((+)-**34**) in 76% yield over two steps. The generated 1,2-diol was temporarily protected with a methylboronic ester, and then the crude mixture was exposed to Albright−Goldman oxidation conditions[15] to afford acetylated mangromicin A. Fortunately, this acetylation of the 4-hydroxydihydropyrone brought about stabilization against air sensitivity and prevented the oxidation of the dihydropyrone to the α-pyrone. Finally, the acetyl group of the enol was removed by methanolysis to complete the synthesis of (+)-**1** in 82% yield over three steps.

The spectral characterizations of synthetic (+)-**1** showed a clear match with the HPLC analysis and ^{1}H NMR data of naturally occurring **1**. On the other hand, in the ^{13}C NMR analysis, some of the chemical shifts of the dihydropyrone moiety were slightly shifted (e.g., synth. 167.0 ppm, lit. 167.9 ppm at the β-position of the dihydropyrone in CD_3OD). We hypothesized that this enol formed dihydropyrone moiety was strongly affected by the measurement conditions for ^{13}C NMR analysis (e.g., concentration, temperature, etc.). Moreover, the optical rotation of the synthetic mangromicin A was opposite and inconsistent with the reported data {synth. $[\alpha]_D^{23}$ +50.5° (c = 1.00, MeOH), lit. $[\alpha]_D^{25}$ −13.6° (c = 0.10, MeOH)}.[1] Therefore, we took an accurate measurement of the optical rotation of the naturally occurring mangromicin A, isolated from the fermentation broth of *L. aerocolonigenes* K10−0216, and obtained a value of $[\alpha]_D^{25}$ +50.7° (c = 0.10, MeOH). We reasoned that the previous natural sample contained some impurity that may have provided an inaccurate value of the optical rotation. We furthermore reconfirmed the optical properties of both natural and synthetic **1** by comparison of their CD spectra, and both showed a positive Cotton effect at 254 nm (see the Supporting Information). Therefore, all of the spectral data for the synthetic compound were consistent with those for the natural compound, confirming our successful total synthesis of (+)-mangromicin A.

In conclusion, we achieved the enantioselective total synthesis of (+)-mangromicin A along with a determination of its absolute configuration. Our synthetic route features a Mukaiyama-type vinylogous alkylation to construct the tetrasubstituted carbon center of the tetrahydrofuran and an anti-aldol reaction to introduce all of the necessary stereocenters. Furthermore, the macrocyclic pentadecane framework, including the 4-hydroxydihydropyrone, was synthesized via Dieckmann cyclization. We are currently investigating the application of these methodologies with a view to the synthesis of other mangromicins, which hopefully will possess useful or more potent bioactive properties.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.6b03512.

Experimental procedures and compound characterization (PDF)

■ AUTHOR INFORMATION

Corresponding Authors

*omuras@insti.kitasato-u.ac.jp (S.Ō.)
*sunazuka@lisci.kitasato-u.ac.jp (T.S.)

ORCID

Toshiaki Sunazuka: 0000-0003-3452-6070

Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Nakashima, T.; Iwatsuki, M.; Ochiai, J.; Kamiya, Y.; Nagai, K.; Matsumoto, A.; Ishiyama, A.; Otoguro, K.; Shiomi, K.; Takahashi, Y.; Ōmura, S. *J. Antibiot.* **2014**, *67*, 253.

(2) Nakashima, T.; Kamiya, Y.; Iwatsuki, M.; Sato, N.; Takahashi, Y.; Ōmura, S. *J. Antibiot.* **2015**, *68*, 220.

(3) Ōmura, S.; Takahashi, Y.; Nakashima, T.; Otoguro, K.; Shiomi, K.; Iwatsuki, M.; Matsumoto, A. WO2013031239, Japan, 2013−03−07.

(4) Igarashi, Y.; Zhou, T.; Sato, S.; Matsumoto, T.; Yu, L.; Oku, N. *Org. Lett.* **2013**, *15*, 5678.

(5) Kalesse, M.; Cordes, M.; Symkenberg, G.; Lu, H.-H. *Nat. Prod. Rep.* **2014**, *31*, 563.

(6) Smith, A. B., III; Adams, C. M.; Lodise Barbosa, S. A.; Degnan, A. P. *Proc. Natl. Acad. Sci. U. S. A.* **2004**, *101*, 12042.

(7) Bal, B. S.; Childers, W. E., Jr.; Pinnick, H. W. *Tetrahedron* **1981**, *37*, 2091.

(8) Kull, T.; Peters, R. *Angew. Chem., Int. Ed.* **2008**, *47*, 5461.

(9) Dess, D. B.; Martin, J. C. *J. Org. Chem.* **1983**, *48*, 4155.

(10) Godenschwager, P. F.; Collum, D. B. *J. Am. Chem. Soc.* **2008**, *130*, 8726.

(11) Sullivan, G. R.; Dale, J. A.; Mosher, H. S. *J. Org. Chem.* **1973**, *38*, 2143.

(12) (a) Gribble, G. W.; Ferguson, D. C. *J. Chem. Soc., Chem. Commun.* **1975**, 535. (b) Saksena, A. K.; Mangiaracina, P. *Tetrahedron Lett.* **1983**, *24*, 273. (c) Nutaitis, C. F.; Gribble, G. W. *Tetrahedron Lett.* **1983**, *24*, 4287. (d) Evans, D. A.; Chapman, K. T. *Tetrahedron Lett.* **1986**, *27*, 5939. (e) Evans, D. A.; Chapman, K. T.; Carreira, E. M. *J. Am. Chem. Soc.* **1988**, *110*, 3560.

(13) Shiina, I.; Ibuka, R.; Kubota, M. *Chem. Lett.* **2002**, *31*, 286.

(14) Under any other deprotection conditions (e.g., TFA, HCl, $AlCl_3$, $TiCl_4$, BBr_3, $BF_3{\cdot}OEt_2$, etc.), the methylene acetal was formed at the 1,2-diol position.

(15) Albright, J. D.; Goldman, L. *J. Am. Chem. Soc.* **1965**, *87*, 4214.

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Org. Lett. 2017, 19, 230−233