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Synthesis of non-THF analogs of acetogenin toward simplified mimics

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Abstract—Acetogenin analogs in which the bis-adjacent THF ring was replaced with an enantioselectively synthesized 1,2-cyclopentanediol bis-ether skeleton were synthesized to obtain simplified mimics, and their inhibitory effect on mitochondrial NADH–ubiquinone oxidoreductase (complex **I**) was examined. The results clearly demonstrate that the 1,2-cyclopentanediol bis-ether motif can substitute for the bis-THF ring while maintaining very potent inhibitory activity at the nanomolar level.

1. Introduction

Acetogenins isolated from the plant family *Annonaceae* have potent and diverse biological effects such as antitumor, antimalarial, pesticidal, and antifeedant activities.[1] The inhibitory effect of acetogenins on mitochondrial NADH–ubiquinone oxidoreductase (complex **I**) is of particular importance since their diverse biological activities are thought to be attributable to this effect.[1] Their structures are characterized by an α,β-unsaturated γ-lactone ring, one to three tetrahydrofuran (THF) ring(s) with flanking OH group(s), an alkyl spacer linking the γ-lactone and THF, and a long alkyl tail. These unique structural features and multiple chiral centers, especially more than four chiral centers in the THF portion, make them challenging synthetic targets. A number of total synthesis of acetogenins has been reported since 1990s.[2] However, all methods reported to date require at least about 20 reaction steps. Structural simplification while maintaining all the essential functionalities of acetogenins may reduce the task of synthesizing a variety of acetogenin mimics, which can be used to probe the structural and functional features of mitochondrial complex **I**.

Wu and co-workers have developed a series of acetogenin mimics in which the bis-adjacent THF ring and flanking OH groups were replaced with an ethylene glycol bis-ether unit and all chiral centers of the THF region were eliminated, namely 'linear mimics'.[3] These analogs with simplified structures elicited fairly potent cytotoxicity against several tumor cell lines, being nearly equipotent with adriamycin.[4] One of the analogs (i.e., AA005) was shown to inhibit mitochondrial complex **I** at a sufficiently high concentration.[3e] It is however unclear whether the potent inhibitory effect on mitochondrial complex **I** can be retained with this kind of drastic structural simplification since cytotoxicity assays have to take into consideration factors such as membrane transport, intracellular transport and metabolic inactivation. Actually, in Ref. 3a, it seems to be somewhat confusing that bullatacin, one of the most potent acetogenins so far reported,[1] showed rather lower cytotoxicity than the mimics. Furthermore, since no natural acetogenin was investigated as a control in related reports,[3b,c,d] one cannot directly compare the changes in the cytotoxicity of the mimics. It should be noted that similar structural simplification by another research group resulted in a drastic decrease in cytotoxicity.[5]

In the present study, we remarked a structural similarity between the hydroxylated bis-THF skeleton of natural acetogenins and the hydroxylated 1,2-cyclopentanediol bis-ether motif, especially the relative spatial position of four oxygen atoms as illustrated in Figure 1. To examine whether the latter can substitute for the former

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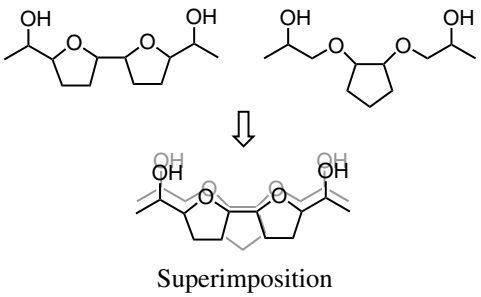


Figure 1. Superimposition of the hydroxylated bis-THF and the hydroxylated 1,2-cyclopentanediol bis-ether skeletons.

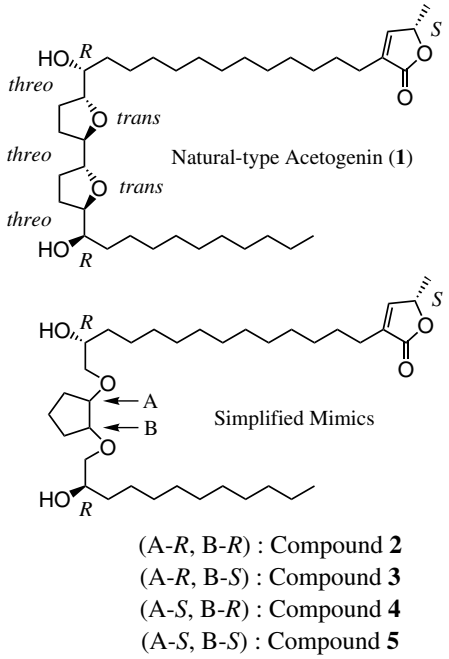


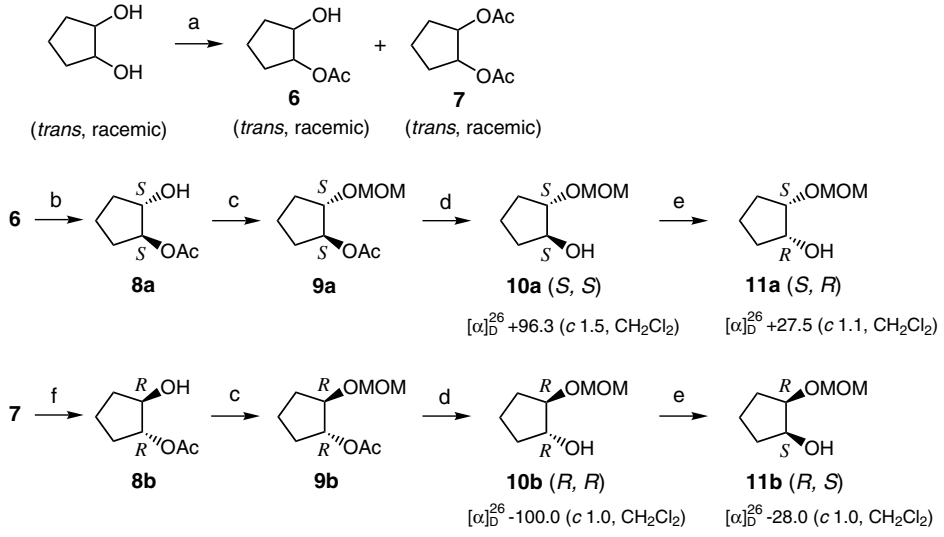
Figure 2. Structures of natural-type acetogenin and its simplified mimics synthesized in this study.

while maintaining a potent inhibitory effect on complex **I**, we synthesized acetogenin mimics, wherein the bis-THF skeleton was replaced with an enantioselectively synthesized 1,2-cyclopentanediol bis-ether unit (Fig. 2), and examined their inhibitory activities using bovine heart mitochondrial complex **I**.

2. Results and discussion

The synthetic procedures for the construction of the 1,2-cyclopentanediol bis-ether moiety are outlined in Scheme 1. (±)-*trans*-1,2-Cyclopentanediol was acetylated to give mono- and diacetates (**6** and **7**, respectively). Racemic monoacetate **6** was subjected to enantioselective hydrolysis by *Pseudomanas fluorescens* lipase (Amano AK, Wako Pure Chemical Ind., Ltd) in vinyl acetate[6] to afford compound **8a**. The optical rotation of **8a** was in good agreement with that of known sample ($[\alpha]_D^{26}$ +30.7, *c* 1.3, CH_3CN; lit.[7] $[\alpha]_D^{22}$ +29.2, *c* 1.0, CH_3CN). The optical purity was determined after conversion of **8a** into **10a**, which was prepared by MOM ether protection of **8a** and the hydrolysis of the resultant acetate **9a**.[8] Mitsunobu inversion of alcohol **10a** using diethyl azodicarboxylate (DEAD), TPP, and *p*-nitrobenzoic acid and subsequent hydrolysis provided compound **11a**.[8] The optical purities of **10a** (>98% ee) and **11a** (>98% ee) were determined by ^1H NMR analyses of the corresponding (*R*)-MTPA esters. The ^1H NMR spectra showed a clear resolution of the α-methoxy (δ 3.54 and 3.61 for **10a** and **11a**, respectively) and MOM methyl (δ 3.31 and 3.35) protons.

On the other hand, diacetate **7** was subjected to enantioselective hydrolysis by *P. fluorescens* lipase (Amano AK, Wako Pure Chemical Ind., Ltd) in 100 mM potassium phosphate buffer (pH 7.0)[6] at 26 °C to afford compound **8b**. The specific rotation of **8b** was in good agreement with the reported value ($[\alpha]_D^{26}$ −27.3, *c* 1.4, CH_3CN;



Scheme 1. Reagents and conditions: (a) acetic anhydride (10 equiv), $YbCl_3$, THF, rt, 1 day, **6** (53%), **7** (44%); (b) lipase Amano AK, vinyl acetate, rt, 1 day, 40%; (c) MOMCl, $(i\text{-}Pr)_2NEt$, CH_2Cl_2, rt, 1 day, 97%; (d) K_2CO_3, MeOH, 40 °C, 1 h, 89%; (e) (i) DEAD, Ph_3P, $p\text{-}NO_2C_6H_4COOH$, toluene, 80 °C, 1 h, 86%, (ii) K_2CO_3, MeOH, 40 °C, 1 h, 99%; (f) lipase Amano AK, 100 mM potassium phosphate buffer (pH 7.0), rt, 1 day, 48%.

lit.[7] $[\alpha]_D^{22}$ −28.2, *c* 1.0, CH_3CN). Compounds **10b** and **11b** were synthesized by the same procedures as described above in the enantiomerically pure form (>98% ee, from the ^1H NMR spectra of the corresponding (*R*)-MTPA esters).[8]

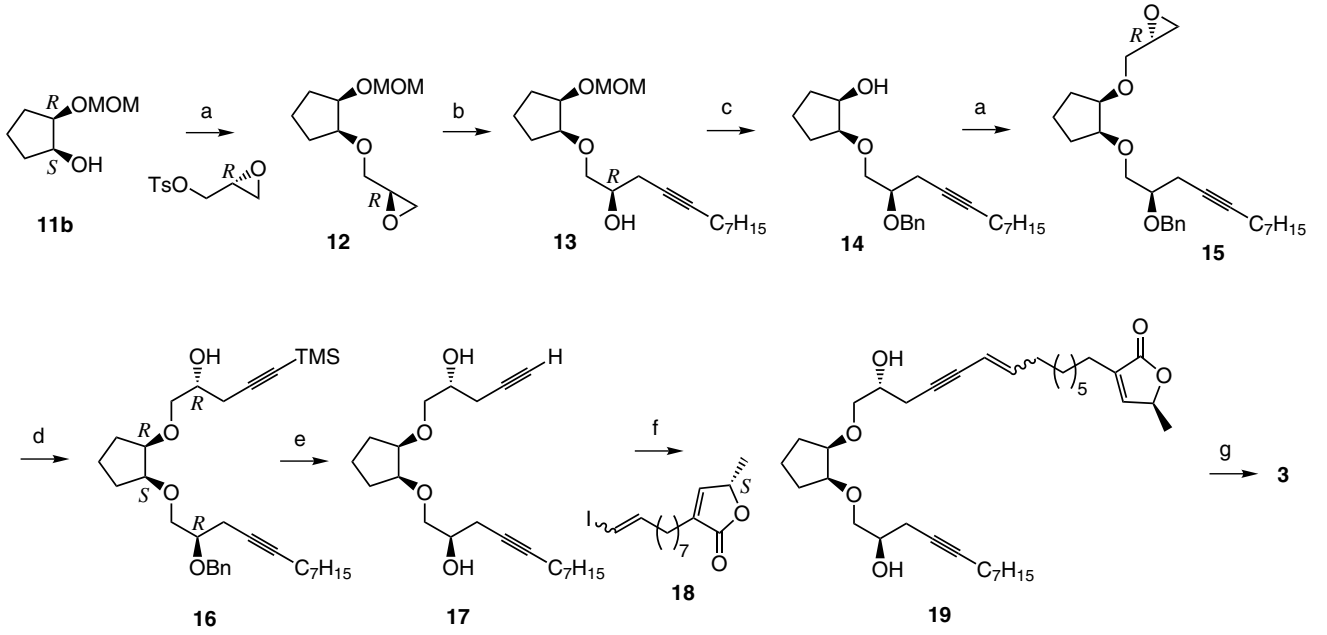
Construction of the entire acetogenin molecule was performed according to a procedure similar to that reported,[9] as outlined in Scheme 2 taking the (*R*,*S*) derivative (**11b**) as an example. The structural features other than the bis-THF ring portion were set to be same as those of the synthetic acetogenin (**1**) since the inhibitory potency of **1** is identical to that of the most potent natural acetogenins like bullatacin.[10] Reaction of the alkoxide of **11b** with (2*R*)-glycidyl tosylate gave a 97:3 mixture of the desired **12** and its epimer wherein the glycidyl configuration was inversed due to initial epoxide attack by the alkoxide followed by extrusion of tosylate.[11] These isomers could be separated by chromatography on silica gel after conversion to the benzyl derivative **14**. Opening of epoxide **12** with 1-lithio-1-nonyne in the presence of BF_3 etherate[12] provided **13**. Protection of the alcohol by benzyl ether and subsequent deprotection of MOM ether gave **14**. Reaction of the alkoxide of **14** with (2*R*)-glycidyl tosylate gave a 95:5 mixture of the desired **15** and its epimer as described above. The diastereomers were separable after conversion to the alcohol **16** that was obtained by the reaction of **15** with lithium TMS acetylide in the presence of BF_3 etherate. Deprotection of the benzyl ether of **16** was achieved by treatment with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) in CH_2Cl_2/H_2O (20:1) at room temperature without saturation of the triple bonds. Pd(0)-catalyzed coupling of alkyne **17** with vinyl iodide **18**, which was prepared as described,[13] provided enyne **19**. Since selective hydrogenation of **19** with Wilkinson's catalyst resulted in appreciable reduction of the butenolide double bond as pointed out by Marshall and Chen,[14] hydrogenation was carried out with diimide, generated in situ from tosylhydrazine,[15] to obtain **3**.[16] Compounds **2**, **4**, and **5** were synthesized by the same method starting from the corresponding enantiomerically pure alcohols.[16] All final products were purified by a preparative HPLC (Wakosil-II 5C18HG, 20 × 250 mm) to separate a trace amount of byproducts in which the butenolide double bond was reduced and/or the spacer portion is still partially unsaturated.

We examined the inhibitory activities of the acetogenin mimics synthesized in this study against bovine heart mitochondrial complex **I** according to the method described previously (Table 1).[17] All the test compounds (**2**–**5**) elicited very potent inhibition at the nanomolar level, being nearly equipotent with bullatacin. No

Table 1. Summary of the inhibitory potencies (IC_{50}) of the test compounds[a]

Compounds	IC_{50} (nM)
1	0.83 (±0.03)
2	1.9 (±0.06)
3	1.0 (±0.04)
4	1.4 (±0.04)
5	0.90 (±0.03)
Bullatacin	0.85 (±0.03)

[a] The IC_{50} value is the molar concentration needed to reduce the control NADH oxidase activity (0.60–0.65 μmol NADH/min/mg of protein) in submitochondrial particles by half. Values are means ± SD of three independent experiments.



Scheme 2. Reagents and conditions: (a) NaH, THF/DMF (1:1), rt, 1 h, then (2*R*)-glycidyl tosylate, rt, 6 h, 85%; (b) 1-nonyne, *n*-BuLi, $BF_3{\cdot}Et_2O$, THF, −78 °C, 15 min, 91%; (c) (i) BnBr, NaH, THF/DMSO (1:1), rt, 1 day, 88%, (ii) 3% AcCl (in MeOH), CH_2Cl_2, 40 °C, 3 h, 87%; (d) TMS–acetylene, *n*-BuLi, $BF_3{\cdot}Et_2O$, THF, −78 °C, 15 min, 65%; (e) (i) DDQ, CH_2Cl_2/H_2O (20:1), rt, 1 day, 70%, (ii) K_2CO_3, MeOH, rt, 1 h, 95%; (f) $Pd(Ph_3P)_4$, CuI, Et_3N, rt, 5 h, 64%; (g) $TsNHNH_2$, NaOAc, DME/H_2O (5:3), reflux, 4 h, 85%.

significant difference was observed between the cis and trans isomers. On the basis of structure–activity studies of ordinary acetogenins possessing the hydroxylated mono- or bis-THF ring(s), we previously concluded that the stereochemistry of the hydroxylated THF ring portion does not significantly affect the inhibitory potency.[18] It should however be realized that the stereochemical difference in the bis-THF portion makes little difference in the three-dimensional structure of this moiety because of the flexibility, which was corroborated by an exhaustive conformational space search analysis.[18a] Using enantioselectively synthesized 1,2-cyclopentanediol bis-ether analogs in which the relative spatial position of the two ether oxygen atoms is almost completely fixed, the present study unambiguously supports the previous conclusion. At the same time, it was also shown that the spatial position of the two oxygen atoms slightly affects the inhibitory potency (**2** vs **5**).

In conclusion, our work for the first time has demonstrated that the hydroxylated bis-THF skeleton of natural acetogenins can be replaced with a much simpler structure while maintaining very potent inhibitory activity at the enzyme level.

Acknowledgements

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(*m*/*z*) 617.5 $[M+Na]^+$. Anal. Calcd for $C_{36}H_{66}O_6$: C, 72.68; H, 11.18. Found: C, 72.47; H, 11.01. For **4**: colorless oil. $[\alpha]_D^{24}$ +16.6 (*c* 0.10, EtOH). ^1H NMR (400 MHz, $CDCl_3$): δ 6.99 (m, 1H), 5.00 (dq, J = 1.5, 7.0 Hz, 1H), 3.82–3.76 (m, 4H), 3.72 (br s, 1H), 3.64 (dd, J = 2.4, 9.3 Hz, 1H), 3.48 (dd, J = 3.0, 10.0 Hz, 1H), 3.45 (br s, 1H), 3.43 (dd, J = 6.7, 10.0 Hz, 1H), 3.20 (dd, J = 9.3, 9.3 Hz, 1H), 2.26 (t, J = 7.3 Hz, 2H), 1.85–1.70 (m, 6H), 1.61–1.25 (m, 40H), 1.41 (d, J = 7.0 Hz, 3H), 0.88 (t, J = 6.8 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 173.93, 148.86, 134.35, 81.26, 80.41, 74.96, 73.71, 70.60, 69.93, 33.13, 32.80, 31.92, 29.70, 29.62, 29.60, 29.58, 29.52, 29.35, 29.19, 28.58, 28.46, 27.40, 25.74, 25.55, 25.18, 22.70, 19.23, 19.17, 14.14. ESI-MS (*m*/*z*) 617.5 $[M+Na]^+$. Anal. Calcd for $C_{36}H_{66}O_6$: C,72.68; H, 11.18. Found: C, 72.39; H, 11.03. For **5**: colorless oil. $[\alpha]_D^{24}$ +69.4 (*c* 0.04, EtOH). ^1H NMR (500 MHz, $CDCl_3$): δ 6.99 (m, 1H), 5.00 (dq, J = 1.5, 7.0 Hz, 1H), 3.80–3.73 (m, 4H), 3.52 (dd, J = 2.9, 9.6 Hz, 2H), 3.26 (dd, J = 8.3, 8.3 Hz, 2H), 2.52 (br s, 2H), 2.25 (t, J = 7.4 Hz, 2H), 1.95–1.90 (m, 2H), 1.59–1.53 (m, 4H), 1.45–1.26 (m, 40H), 1.41 (d, J = 7.0 Hz, 3H), 0.88 (t, J = 6.9 Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 173.94, 148.87, 134.32, 85.60, 74.00, 70.64, 33.07, 31.91, 29.72, 29.68, 29.61, 29.45, 29.52, 29.34, 29.32, 29.19, 27.39, 25.55, 25.17, 22.69, 20.81, 19.22, 14.13. ESI-MS (*m*/*z*) 617.5 $[M+Na]^+$. Anal. Calcd for $C_{36}H_{66}O_6$: C, 72.68; H, 11.18. Found: C, 72.48; H, 10.94.

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