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Function of the alkyl side chains of Δlac-acetogenins in the inhibitory effect on mitochondrial complex I (NADH-ubiquinone oxidoreductase)

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Abstract—We synthesized a series of Δlac-acetogenins in which the two alkyl side chains were systematically modified, and examined their inhibitory effect on bovine heart mitochondrial complex I (NADH-ubiquinone oxidoreductase). The results revealed that the physicochemical properties of the side chains, such as the balance of hydrophobicity and the width (or bulkiness) of the chains, are important structural factors for a potent inhibitory effect of amphiphilic Δlac-acetogenins. This is probably because such properties decide the precise location of the hydrophilic bis-THF ring moiety in the enzyme embedded in the inner mitochondrial membrane.

Acetogenins isolated from the plant family Annonaceae have potent and diverse biological effects such as antitumor, antimalarial, and insecticidal activities.[1–3] Their inhibitory effect on mitochondrial NADH-ubiquinone oxidoreductase (complex I) is of particular importance since the diverse biological activities of acetogenins are thought to be attributable to this effect.[2] Their unique structural features and multiple chiral centers, especially more than four in the THF portion, make acetogenins challenging synthetic targets.[4] On the other hand, structural simplification, while maintaining all the essential functionalities of acetogenins may facilitate the task of synthesizing a variety of acetogenin mimics which may be used not only as possible chemotherapeutic agents, but also as molecular probes to investigate the functional features of mitochondrial complex I,[5,6] one of the largest known membrane protein complexes.

We recently synthesized new acetogenin mimics named Δlac-acetogenins, that consist of the hydroxylated adjacent bis-THF ring and two alkyl side chains without a α,β-unsaturated γ-lactone ring.[7,8] Some Δlac-acetogenins had strong inhibitory effect on bovine heart mitochondrial complex I at the nanomolar level despite lacking a γ-lactone ring which is a structural feature common to a large number of natural acetogenins.[1–3] Interestingly, several lines of evidence, for example, competition tests using a fluorescent ligand and the effect on superoxide production from complex I, revealed that the binding site of Δlac-acetogenins is different from that of natural acetogenins as well as ordinary complex I inhibitors such as rotenone and piericidin A.[7,8] Thus, Δlac-acetogenins were shown to be a new type of inhibitor acting at the terminal electron transfer step of complex I. Accordingly, a detailed analysis of their inhibitory action would provide valuable insights into the functional features of complex I.

To elucidate the mode of inhibition of Δlac-acetogenins, identification of the crucial structural factors required for the inhibition is highly helpful. A previous structure–activity study concerning Δlac-acetogenins indicated that the number of carbon atoms (i.e., hydrophobicity) of the alkyl side chains remarkably affects the inhibitory potency (see compounds **1**–**4**, Fig. 1).[7] The optimal length of the side chains for the inhibition is about 11 carbons, as revealed for **3**. A comparison of the inhibitory effects of compounds **3** and **5**–**7**

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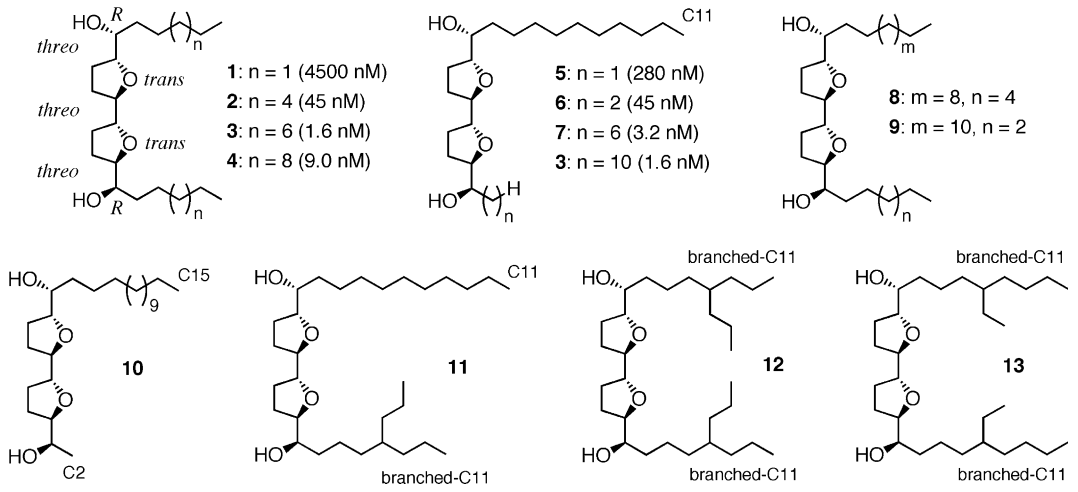


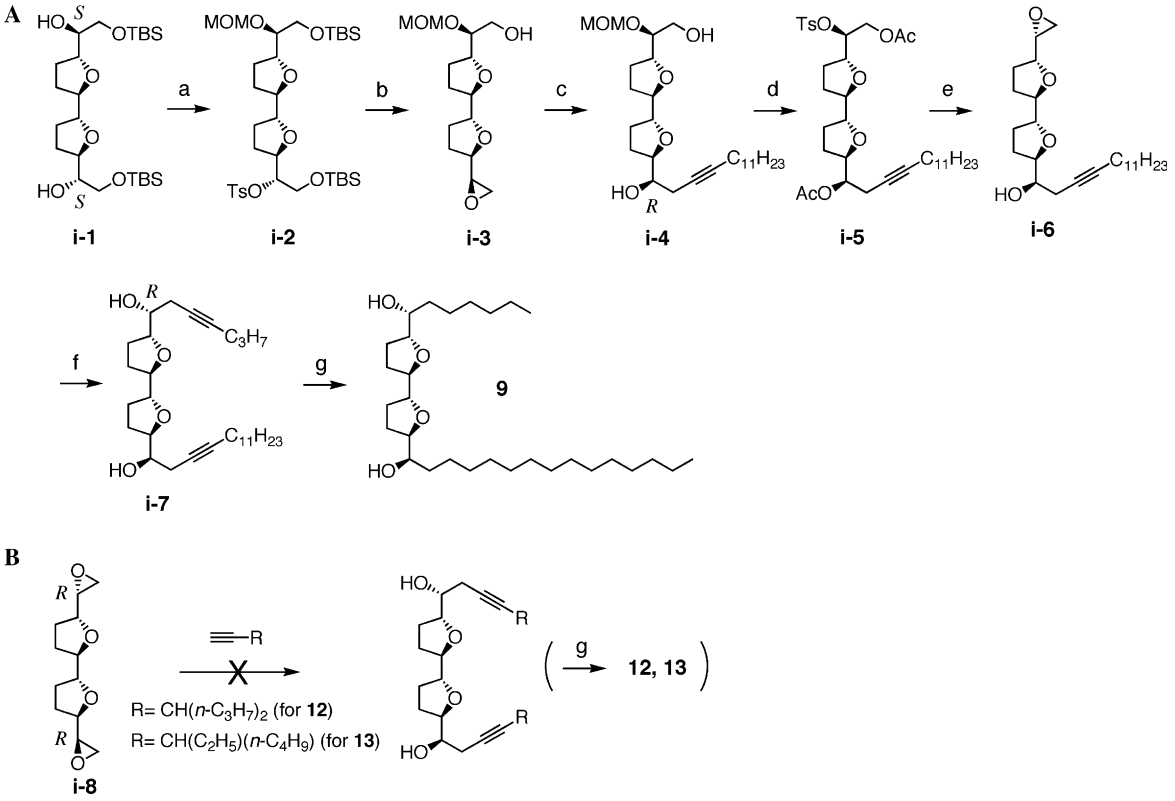
Figure 1. Structures of Δlac-acetogenin derivatives examined in this study. The molar concentrations in parentheses are the IC_{50} values determined in the previous study.[7]

suggested that the balance of hydrophobicity of the two side chains attached to the C_2-symmetric bis-THF portion is an important structural factor of these inhibitors. However, since compounds **3** and **5**–**7** largely differ in the total number of carbon atoms, it remains to be established whether the difference in inhibitory effect is actually due to an imbalance of hydrophobicity or a difference in the hydrophobicity of the entire molecule. The solution of this question is unavoidable not only to know the function of the side chains, but also to predict the function and location of the hydrophilic bis-THF ring moiety. Therefore, to solve this important question, we newly synthesized compounds **8** and **9** that possess two side chains of different lengths, but maintain a total of 22 carbons as is the case of a potent derivative **3**. In addition, to further elucidate the function of the side chains, we also synthesized a series of derivatives and evaluated their inhibitory effects on bovine heart mitochondrial complex I.

The synthetic procedures of compounds **8**–**11** are outlined in Scheme 1A, taking **9** as an example. The key intermediate **i-1** was synthesized as described previously.[9,10] Treatment of **i-1** with 0.5 mol equiv of TsCl (repeated twice) and sequential MOM ether protection of the secondary hydroxy group afforded **i-2**. Desilylation of **i-2** with TBAF, and the opening of the epoxide **i-3** with the lithium acetylide, derived from 1-tridecyne, in the presence of Et_2AlCl[11] provided **i-4** (72% in two steps). After acetyl protection of both hydroxy groups, deprotection of the MOM ether and sequential treatment with TsCl afforded **i-5**. Hydrolysis of the acetyl groups gave epoxide **i-6** (80%). The opening of the epoxide **i-6** with the lithium acetylide, derived from 1-pentyne, in the presence of Et_2AlCl provided **i-7** (78%). Hydrogenation of **i-7** with 10% Pd/C afforded **9**.[12] To obtain compound **10**, the final mono-epoxide **i-6** was opened by catalytic hydrogenation with 10% Pd/C.[12] For the synthesis of **11**, 3-*n*-propyl-1-hexyne was synthesized by a Corey–Fuchs reaction[13] from 2-*n*-propyl-1-pentanal, which was prepared by Swern oxidation of commercially available 2-*n*-propyl-1-pentanol.

To synthesize **12** and **13**, we initially examined the simultaneous opening of the diepoxide **i-8**[9] with the corresponding lithium acetylide in the presence of Et_2AlCl, as shown in Scheme 1B, but the yield of the product was very poor. We attempted to improve this reaction step under various conditions; for instance, $BF_3{\cdot}Et_2O$[14] was used in place of Et_2AlCl at several molar ratios and temperatures. All cases, however, resulted in unsatisfactory results. Therefore, we synthesized **12** and **13** by the same procedures used for **9**.[12] For the synthesis of **13**, 3-ethyl-1-heptyne was prepared by a Corey–Fuchs reaction from commercially available 2-ethyl-1-hexanal.

The inhibition of complex I activity was determined by NADH oxidase assay using bovine heart submitochondrial particles.[8] Previous study indicated that the inhibitory effect of **3** (C_{11}–C_{11}) is comparable to that of bullatacin,[7] one of the most potent natural acetogenins.[15,16] The IC_{50}, that is, the molar concentration needed to halve the control NADH oxidase activity, of **3** was 1.7 (±0.08) nM with the present submitochondrial particles preparation. The IC_{50} values of compounds **8** and **9** were 7.5 (±0.39) and 34 (±6.6) nM, respectively. Taking into account the identical total number of carbon atoms on the side chains of **3** (C_{11}–C_{11}), **8** (C_{13}–C_9), and **9** (C_{15}–C_7), it is obvious that the greater the loss of the balance, the weaker the inhibitory effect becomes. Similar results were obtained for other compound sets, **10** (C_{15}–C_2, $IC_{50} = 410 \pm 23$ nM) versus **5** (C_{11}–C_2, $IC_{50} = 280 \pm 30$ nM) and **9** (C_{15}–C_7, $IC_{50} = 34 \pm 6.6$ nM) versus **7** (C_{11}–C_7, $IC_{50} = 3.2 \pm 0.30$ nM), though the total number of carbons of the former is larger than that of the latter in these pairs. These results indicate that the balance in hydrophobicity of the two side chains is an important structural factor for a potent inhibitory effect. It is thus likely that the balance decides the precise location of the hydrophilic bis-THF ring moiety, which may be located at or close to the membrane surface area of the enzyme embedded in the inner mitochondrial membrane.



Scheme 1. Reagents and conditions: (a) i—TsCl (0.5 equiv), 4-DMAP, Et_3N, CH_2Cl_2, rt, 12 h, 55% (repeated twice); ii—MOMCl, $(i\text{-Pr})_2NEt$, CH_2Cl_2, rt, 15 h, 98%; (b) TBAF, THF, 50 °C, 2 h, 88%; (c) 1-tridecyne (10 equiv), *n*-BuLi (10 equiv), Et_2AlCl (10 equiv), toluene, 0 °C, 1 h, 82%; (d) i—AcCl, 4-DMAP, CH_2Cl_2, 35 °C, 2 h, 96%; ii—$BF_3{\cdot}Et_2O$, Me_2S, −20 °C, 1 h, 96%; iii—TsCl, 4-DMAP, Et_3N, CH_2Cl_2, 35 °C, 12 h, 92%; (e) K_2CO_3, MeOH, rt, 2 h, 80%; (f) 1-pentyne (10 equiv), *n*-BuLi (10 equiv), Et_2AlCl (10 equiv), toluene, 0 °C, 1 h, 78%; (g) H_2, 10% Pd/C, EtOH, rt, 12 h, 94%.

Next, to examine the effect of the shape of the side chain on the activity, we synthesized compound **11** possessing a branched side chain of 11 carbons, which is one of the bulkiest structures that can be synthesized by the procedures used for the preceding analogues. The total number of carbon atoms of the side chains in this derivative is identical to that of **3**. The IC_{50} value of **11** was 27 (±2.1) nM, indicating that the branched structure is unfavorable for the inhibition. The replacement of both side chains of **3** with the branched chain of 11 carbons resulted in a drastic decrease in activity [**12**, $IC_{50} = 1500 \pm 190$ nM]. On the basis of this result, we next synthesized compound **13** possessing an ethyl branch in both chains in anticipation of the recovery of inhibitory potency. Expectedly, **13** ($IC_{50} = 870 \pm 20$ nM) significantly recovered the inhibitory effect compared to **12**. It is therefore clear that expansion of the width of the side chain is remarkably unfavorable for the inhibitory action. This is probably because the side chains directly interact with the hydrophobic domain of complex I rather than merely partitioning into the lipid membrane phase, whereupon the enzyme recognizes the molecular shape of the side chains in a strict sense.

In conclusion, the precise location of the hydrophilic bis-THF ring moiety, which is decided by the physicochemical properties of the side chains, is critical to the inhibitory effect of amphiphilic Δlac-acetogenins. Although a high-resolution 3D structure for complex I is not available, this feature may be closely related to the specific nature of the putative binding site in complex I embedded in the inner mitochondrial membrane.

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12. The data for compound **8**: colorless oil; $[\alpha]_D^{18}$ +1.3 (*c* 0.15, EtOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.89–3.81 (m, 4H), 3.39–3.38 (m, 2H), 2.47 (br s, 2H), 1.99–1.94 (m, 4H), 1.69–1.63 (m, 4H), 1.57–1.48 (m, 2H), 1.43–1.25 (m, 34H), 0.87 (t, J = 6.8 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 83.14 (2C), 81.77 (2C), 74.06 (2C), 33.49 (2C), 31.93, 31.89, 29.74 (2C), 29.67, 29.65, 29.62, 29.57, 29.36 (2C), 29.30 (2C), 28.97 (2C), 28.37 (2C), 25.67 (2C), 22.69, 22.68, 14.12 (2C); ESI-MS (*m*/*z*) 483.4 $[M+H]^+$. For compound **9**: colorless oil; $[\alpha]_D^{18}$ +10.9 (*c* 0.11, EtOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.90–3.81 (m, 4H), 3.41–3.36 (m, 2H), 2.44 (br s, 2H), 1.99–1.94 (m, 4H), 1.70–1.63 (m, 4H), 1.58–1.47 (m, 2H), 1.41–1.25 (m, 34H), 0.87 (t, J = 6.8 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 83.14 (2C), 81.77 (2C), 74.06 (2C), 33.49 (2C), 31.93 (2C), 31.82 (2C), 29.70 (2C), 29.40 (2C), 29.36 (2C), 28.97 (2C), 28.36 (2C), 25.67 (2C), 25.62 (2C), 22.70, 22.63, 14.12, 14.09; ESI-MS (*m*/*z*) 483.6 $[M+H]^+$. For compound **10**: colorless oil; $[\alpha]_D^{19}$ +15.4 (*c* 0.13, EtOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.89–3.81 (m, 3H), 3.81–3.75 (dt, J = 6.4, 7.5 Hz, 1H), 3.61–3.54 (dt, J = 6.7, 13.4 Hz, 1H), 3.40–3.38 (m, 1H), 2.80 (br s, 1H), 2.62 (br s, 1H), 2.00–1.94 (m, 4H), 1.67–1.47 (m, 4H), 1.41–1.25 (m, 26H), 1.13 (d, J = 6.3 Hz, 3H), 0.88 (t, J = 6.8 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 84.44, 83.10, 81.75, 81.72, 73.97, 70.41, 33.42, 31.86, 29.68 (2C), 29.63 (2C), 29.58 (2C), 29.55 (2C), 29.30, 28.95, 28.92, 28.30, 28.26, 25.61, 22.63, 18.60, 14.06; ESI-MS (*m*/*z*) 413.4 $[M+H]^+$. For compound **11**: colorless oil; $[\alpha]_D^{19}$ +28.3 (*c* 0.12, EtOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.90–3.81 (m, 4H), 3.41–3.37 (m, 2H), 2.44 (br s, 2H), 1.98–1.95 (m, 4H), 1.72–1.61 (m, 4H), 1.54–1.47 (m, 2H), 1.45–1.36 (m, 4H), 1.39–1.37 (m, 1H), 1.33–1.16 (m, 26H), 0.88 (t, J = 6.8 Hz, 3H), 0.87 (t, J = 7.0 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 83.18, 83.15, 81.78 (2C), 74.12, 74.07, 36.99, 36.06, 36.01, 33.95, 33.79, 33.49, 31.92 (2C), 29.74, 29.63 (2C), 29.34, 28.97 (2C), 28.37 (2C), 25.67, 22.79, 22.69, 19.82, 19.79, 14.53 (2C), 14.12; ESI-MS (*m*/*z*) 483.6 $[M+H]^+$. For compound **12**: colorless oil; $[\alpha]_D^{19}$+27.0 (*c* 0.20, EtOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.90–3.80 (m, 4H), 3.42–3.36 (m, 2H), 2.52 (br s, 2H), 2.02–1.90 (m, 4H), 1.72–1.60 (m, 4H), 1.54–1.46 (m, 2H), 1.45–1.18 (m, 28H), 0.87 (t, J = 7.0 Hz, 12H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 83.20 (2C), 81.79 (2C), 74.10 (2C), 36.98 (2C), 36.05 (2C), 36.00 (2C), 33.94 (2C), 33.78 (2C), 28.99 (2C), 28.37 (2C), 22.79 (2C), 19.81 (2C), 19.78 (2C), 14.54 (4C); ESI-MS (*m*/*z*) 483.4 $[M+H]^+$. For compound **13**: colorless oil; $[\alpha]_D^{20}$ +25.2 (*c* 0.19, EtOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.94–3.90 (m, 4H), 3.44–3.35 (m, 2H), 2.53 (br s, 2H), 2.02–1.91 (m, 4H), 1.78–1.60 (m, 4H), 1.58–1.51 (m, 2H), 1.51–1.10 (m, 28H), 0.88 (t, J = 6.9 Hz, 6H), 0.83 (t, J = 7.2 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 83.19 (2C), 81.79 (2C), 74.09 (2C), 38.87, 38.83, 33.93 (2C), 33.32 (2C), 32.87, 32.83, 29.00 (2C), 28.99 (2C), 28.37 (2C), 25.86, 25.79, 23.15 (2C), 22.84 (2C), 14.18 (2C), 10.89, 10.84; ESI-MS (*m*/*z*) 483.6 $[M+H]^+$.
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