

Alteration of the Bis-tetrahydrofuran Core Stereochemistries in Asimicin Can Affect the Cytotoxicity

Subhash C. Sinha,^{*,†} Zhiyong Chen,[†] Zheng-Zheng Huang,[†] Eiko Nakamaru-Ogiso,[‡] Halina Pietraszkiwicz,[§] Matthew Edelstein,[§] and Frederick Valeriote[§]

The Skaggs Institute for Chemical Biology and the Departments of Molecular Biology, and Molecular and Experimental Medicine, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, California 92037, and Josephine Ford Cancer Center, Henry Ford Health System, Detroit, Michigan 48202

Received August 14, 2008

Abstract: A systematic analysis using 10 synthetic asimicin stereoisomers revealed that the stereochemistry of the bis-tetrahydrofuran core, including the tetrahydrofuran rings and the adjacent hydroxy functions, had significant effect on its cytotoxicity. Our findings set to rest the highly controversial perception that the stereochemistry of the tetrahydrofuran core has little effect on the activity, which is not true for its cytotoxic effect, and also reinforces the previous conclusion that asimicin is a highly potent anticancer compound.

Annonaceous adjacent bis-tetrahydrofuran (bis-THF) acetogenins, asimicin (**1.1a**) and bullatacin (**1.1b**), are highly potent cytotoxic molecules.¹ Reportedly, they are several orders of magnitude more cytotoxic than doxorubicin, in particular against multidrug resistant cell lines. Although the exact mechanism of action remains unknown, these compounds are believed to display anticancer activity by inhibiting the mitochondrial enzyme, complex I, inducing the expression of pro-apoptotic genes, and arresting cells in multiple phases, including G1 and G2/M.² On the basis of a comparison among various acetogenins and their analogues, it has been shown that the THF core, including both the THF ring and the neighboring free hydroxyl functions, are crucial structural elements for their strong complex I inhibitory effect and high anticancer activity.³ Yet, stereochemistry of the THF core is believed to have little effect on the activity of an acetogenin.¹ This would imply that all 64 stereoisomeric asimicins (**1.1–1.16(a–d)**) with dissimilar stereochemistry in the bis-THF core (Figure 1) would have identical anticancer activity. This is an unlikely assumption from a historic perspective of medicinal chemistry. Although one could cite the famous example of bullatacin being more than a billion times more potent than asimicin and trilobacin (**1.13a**) in MCF-7 cells, in spite of the fact that these molecules differ from one other at only one stereogenic center in the THF core,⁴ the results could not be corroborated in later studies.⁵ Therefore, we prepared several asimicin stereoisomers, including **1.1a–d** and **1.4a–d**, and using these compounds as well as the previously described asimicin stereoisomers, **1.6a** and **1.8a**,⁶ we determined both their anticancer activities and the complex I inhibitory effects. On the basis of the results of these studies, we report here that both the cytotoxic activity, and to a lesser extent the complex I inhibitory effect of acetogenins, do indeed depend upon the

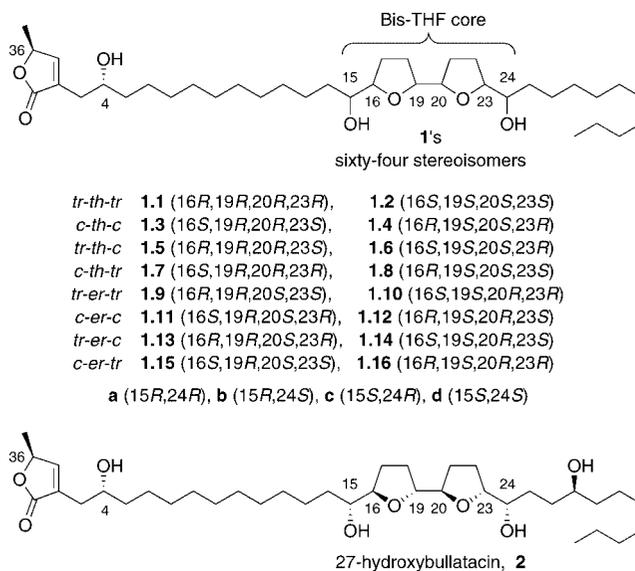


Figure 1. Structure of 64 stereoisomeric asimicins arising from six stereogenic centers of the bis-THF core and a related compound, 27-hydroxybullatacin.

stereochemistry of the THF core. In addition, it was noted that a chronic exposure of cancer cells to these compounds could be essential to achieve any therapeutic effect.

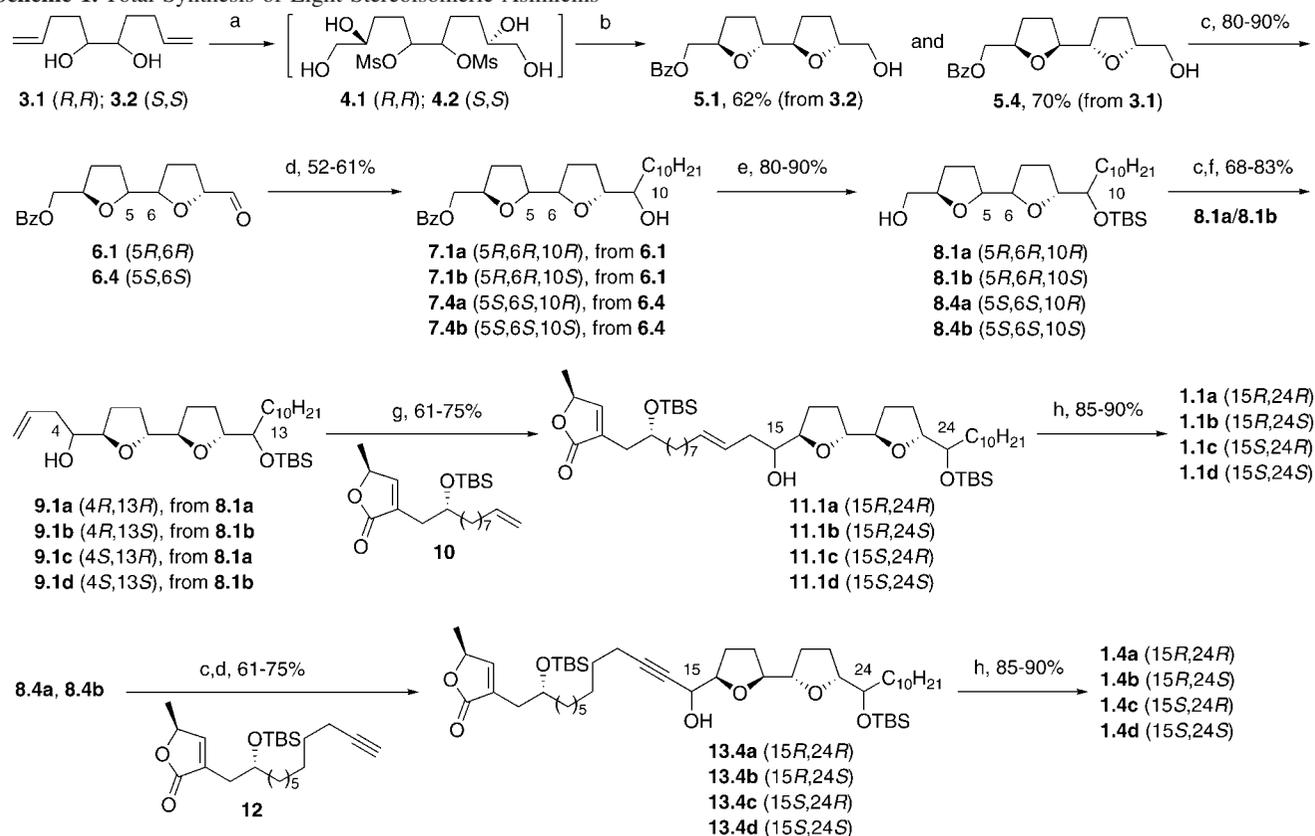
Synthesis of compounds **1.1a–d** and **1.4a–d** was achieved by starting with the bis-THF intermediates **5.1** and **5.4**, respectively, by modifying the general strategy that we recently used in the synthesis of 27-hydroxybullatacin, **2** (Figure 1).⁷ The new strategy, especially that used in the synthesis of **1.4's**, was different from any previous strategies by being more versatile, as this yielded the desired stereoisomeric asimicins rapidly using simple processes and fewer number of intermediates, steps, and chromatography.^{8,9} This strategy may also be applied to the asimicin library and analogues' syntheses using the appropriate bis-THF intermediates. Compounds **5.1** and **5.4** were prepared from **3.2** and **3.1**, via **4.2** and **4.1**, respectively, in four easy steps, including mesylation, Sharpless asymmetric dihydroxylation with AD-mix- β , Williamson-type etherification and monobenzylation. Compounds **5.1** and **5.4** were oxidized using Swern reaction, and the resulting aldehydes, **6.1** and **6.4**, were alkynylated with 1-decyne using Carreira's enantioselective alkylation¹⁰ to give both *threo* and *erythro* products. The alkynylated products were hydrogenated over Pd–C, and the resulting saturated compounds **7.1a,b** and **7.4a,b** were converted to alcohols **8.1a,b** and **8.4a,b**. We applied two alternative strategies to convert alcohol **8's** to the target compound **1's**. In one strategy, alcohols **8.1a,b** were oxidized and the resulting aldehydes were allylated using Brown's method¹¹ with (+)- and (–)-Ipc₂B-allyl, affording compound pairs, **9.1a/9.1c** (from **8.1a**) and **9.1b/9.1d** (from **8.1b**), respectively. These alkenes were cross-metathesized¹² with butenolide **10**^{7a} using Grubbs' catalyst,¹³ and the resulting products **11.1a–d** were hydrogenated and deprotected to afford **1.1a–d**, as described for compound **2**.^{7a} In an alternative approach, aldehyde products from alcohols **8.4a,b** were reacted with **12** under Carreira's asymmetric alkylation conditions using (+)- or (–)-NME and Zn(OTf)₂ to give **13.4a/13.4c** and **13.4b/13.4d**. These products were hydrogenated and deprotected as above affording compounds **1.4a–d**.

* To whom correspondence should be addressed. Phone: (858) 784-8512. Fax: (858) 784-8732. E-mail: subhash@scripps.edu.

[†] The Skaggs Institute for Chemical Biology, TSRI.

[‡] Department of Molecular and Experimental Medicine, TSRI.

[§] Josephine Ford Cancer Center.

Scheme 1. Total Synthesis of Eight Stereoisomeric Asimicins^a

^a Key: (a) (i) MsCl, Et₃N, CH₂Cl₂, 0 °C; (ii) AD-mix-β, *tert*-BuOH/H₂O, 0 °C, then Na₂SO₃. (b) (i) Pyridine, reflux; (ii) BzCl, Et₃N, CH₂Cl₂, 0 °C. (c) Oxalyl chloride, DMSO, CH₂Cl₂, 0 °C. (d) (i) Zn(OTf)₂, (+) or (−)-NME, DIPEA, toluene; (ii) H₂, Pd/C, EtOAc. (e) (i) TBSOTf, 2,6-lutidine, CH₂Cl₂; (ii) LiOH, MeOH, THF, H₂O. (f) (+) or (−)-Ipc₂B-allyl, Et₂O. (g) Grubbs catalyst [(PCy₃)₂Ru=CHPh]Cl₂, CH₂Cl₂, syringe pump. (h) (i) TsNHNH₂, AcONa, DME/H₂O, syringe pump; (ii) 5% HF, CH₂Cl₂, CH₃CN.

While numerous studies have been directed to an understanding of the activity relationships among various types of acetogenins and their analogues, either obtained from natural sources or prepared synthetically,¹⁴ no efforts have been made to elucidate the stereochemistry–activity relationships, especially in relation to the THF cores.¹⁵ Therefore, to understand this relationship, we determined the inhibitory effect of compounds **1.1a–d**, **1.4a–d**, **1.6a**, and **1.8a** on both tumor cell proliferation and the complex I activity. These complementary studies were necessary to make comparison between the two activity data sets, which previously have been found by others not to correlate for many acetogenin analogues. The complex I activity was determined using bovine heart submitochondrial particles (SMP^a),¹⁶ as described earlier.^{7a} Briefly, compound **1**'s at various concentrations were added to 15 μg of SMPs/mL containing 0.25 M sucrose, 1 mM MgCl₂, and 50 mM phosphate buffer (pH 7.5), and mixed. After 5 min equilibration of SMP with inhibitors, the reaction was started using 150 μM NADH, and progress of reaction determined spectrophotometrically at 340 nm. The results (IC₅₀ of the compounds) are shown in Table 1. All compounds possessed comparable IC₅₀ values, suggesting that the stereochemistry of both the bis-THF rings and the adjacent hydroxyl functions in stereoisomeric asimicins may have only little effect on the complex I activities.

Next, we evaluated the cytotoxic activities of compounds **1.1a–d**, **1.4a–d**, **1.6a**, and **1.8a** using murine and human cancer cell lines, including colon cells (Colon38 and H116), and lung

cells (H125). Murine and human leukemia (L1210 and CCRF-CEM) cells were used as the reference tumors of the assay. A normal granulocyte/macrophage committed progenitor cell from the marrow (CFU-GM), which represents the target for the most common chemotherapeutic toxicity in vivo, myelosuppression, were used to determine their selectivity to tumor cells vs normal cells. Such comprehensive tests allow us not only to elucidate the structure–activity relationship but also to identify compounds that could be advanced to further pharmacological studies. The anticancer activities of **1.1a–d**, **1.4a–d**, **1.6a**, and **1.8a** were determined using both the disk diffusion and cell proliferation assays, as described earlier.¹⁷

Briefly, for the disk diffusion assay, a volume of 15 μL of each sample was dropped onto a 6.5 mm filter disk and allowed to dry overnight. Cells were plated in soft agar in 60 mm tissue culture dishes, and the disk was placed close to the edge of the dish. The dishes were incubated for 7–10 days, and the zone of inhibition from the edge of the filter disk to the beginning of normal-sized colony formation was measured. The diameter of the filter disk, 6.5 mm, was arbitrarily taken as 200 units. Any excessively toxic sample at the first concentration was diluted by 1:4 or 1:10 decrements, and the experiments were repeated. At some dilution, quantifiable cytotoxicity (zone of inhibition <750 units) was invariably obtained (see Supporting Information for the data). A difference in the sensitivity of the tumor cells versus either the normal or leukemia cells to a compound, is presented in Table 2 as $HCT-116\Delta_{CEM} = n$, indicating that there was an “n” unit zone differential between the tumor (human colon HCT-116) and leukemia (human CEM). For differential values ≥250 units between solid tumor cells and either normal

^a Abbreviations: DIPEA, diisopropylethylamine; Ipc₂B-allyl, allyl diisopinocampheylborane; SMP, submitochondrial particles; NADH, nicotinamide adenine dinucleotide.

Table 1. Inhibitory Effect of the Stereoisomeric Asimicins and Their Analogues on Complex I Activity

compd	IC ₅₀ (nM)	compd	IC ₅₀ (nM)
1.1a	1.2	1.1b	2.5
1.1c	3.3	1.1d	9.0
1.4a	7.5	1.4b	3.0
1.4c	2.5	1.4d	5.0
1.6a	2.0	1.8a	3.0

Table 2. Cytotoxicity and Selectivity of Asimicin Stereoisomers Determined by Disk Diffusion Assay

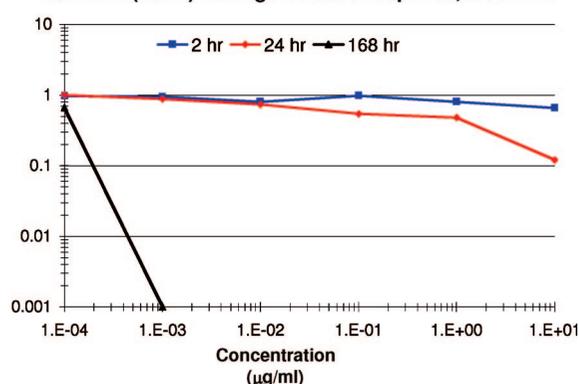
compd	$\mu\text{g}/\text{disk}$	C ₃₈ Δ L1210	C ₃₈ Δ CFU	H116 Δ CEM	H125 Δ CEM
1.1a	15			400	100
	1	500	650		
1.1b	60			550	300
	1	500	200		
1.1c	60			500	150
	3.8	550	650		
1.1d	15			450	300
	1	600	400		
1.4a	15			500	350
	1	400	100		
1.4b	15			600	600
	1	600	550		
1.4c	60			400	350
	3.8	500	550		
1.4d	15			550	400
	1	600	150		
1.6a	30			300	300
	0.3	350	0		
1.8a	30			400	400
	0.3	450	0		

Table 3. Anticancer Activity of the Stereoisomeric Asimicins to Human Colon Cancer, HCT-116

compd	1.1a	1.1b	1.1c	1.1d	1.4a	1.4b	1.4c	1.4d
IC ₅₀ (nM)	0.51	1.4	27	0.45	18	0.22	5.6	0.69

or leukemia cells defines solid tumor selective compounds in our assay and these values are bolded in Table 2. All compounds were more sensitive to Colon38 cells compared to either HCT-116 or H-125 cells by 15- to 100-fold depending upon the compound. They were also selective for Colon38 against both L1210 and CFU-GM. Selectivity was also demonstrated for all compounds for HCT-116 (and usually for H125 also) compared to CEM (Table 2). Further, some compounds were more sensitive than others to the same cell lines. For example, compounds **1.1c** and **1.4c** were 10-fold less cytotoxic to Colon38 than compounds **1.6a** and **1.8a**. Compounds **1.1a**, **1.1d**, and **1.4b** were more cytotoxic than compounds **1.1c** or **1.4c** against HCT-116 cells.

The difference in anticancer activity of various stereoisomeric asimicins was further confirmed by determining their IC₅₀ against HCT-116 cells using the cell proliferation assay. In this assay, cells were incubated with various concentration of **1.1a–d** and **1.4a–d**, and their effect on cell proliferation was determined. As shown in Table 3, several compounds possessed subnanomolar anticancer activities, but at least two compounds, **1.1c** and **1.4a**, were as much as two logs of magnitude less potent than the most potent isomer, **1.4b**. These

Asimicin (EtOH) clonogenic dose-response, HCT-116

Concentration ($\mu\text{g}/\text{ml}$)	Surviving fraction		
	2 hours	24 hours	168 hours
0.0001	0.97	1	0.66
0.001	0.94	0.88	0.001
0.01	0.8	0.73	0.001
0.1	0.98	0.54	0.001
1	0.8	0.48	0.001
10	0.66	0.12	0.001

Figure 2. In vitro therapeutic effect of asimicin to HCT-116 colon cancer cells.

results are significant because it was believed that the stereochemistry of THF rings and the hydroxy functions in the central region had no effect on their activity. Evidently, the cytotoxicity of asimicin depends highly upon the bis-THF core stereochemistry. This result also complements the previous findings that the alkyl chain and methylene bridge length, and the butenolide surrogates, all influence the anticancer activities of a bis-THF acetogenin.

Finally, we determined the cytotoxicity of asimicin to HCT-116 cells at various concentrations and incubation durations. Cells were incubated with asimicin at 0.001, 0.01, 0.1, 1, and 10 $\mu\text{g}/\text{mL}$ for either 2 h, 24 h, or continuously (for 168 h), and the number of surviving clonogenic cells determined. The results are shown in Figure 2. In this analysis, 90% cell killing or 10% survival (S10) was selected for the comparison of activities. Neither 2 nor 24 h were sufficient durations of exposure even at the highest concentration (10 $\mu\text{g}/\text{mL}$) of asimicin to yield 10% survival. In contrast, for the 7-day (168 h) exposure duration, the S10 was less than 1 ng/mL. This implies that chronic exposure of asimicin is necessary for any therapeutic effect and that continuous levels of at least 1 ng/mL (in the serum) would be sufficient for a therapeutic effect.

In conclusion, by synthesizing and evaluating ten synthetic asimicin stereoisomers, we have shown that the anticancer activity of the bis-THF acetogenins depends upon the stereochemistry of the THF core, including the both THF rings and the central hydroxyl functions. A chronic exposure of tumor cells to asimicin at 1 ng/mL is predicted to be therapeutically significant in vivo. To fully understand the relationship between the anticancer activity and the bis-THF/hydroxy functions in the central region, synthesis of a complete library of all 64 stereoisomeric asimicins seems essential.

Acknowledgment. We thank the Skaggs Institute for Chemical Biology and National Institute of Health for the financial support. We are also thankful to Professor Takao Yagi for providing us the SMPs.

Supporting Information Available: Typical experimental procedure, analytical and biological data, and of ^1H and ^{13}C NMR spectra for selected compounds. This material is available free of charge via the Internet at <http://pubs.acs.org>.

References

- (1) (a) McLaughlin, J. L. Paw paw and cancer: Annonaceous acetogenins from discovery to commercial products. *J. Nat. Prod.* **2008**, *71*, 1311–1321. (b) Bermejo, A.; Figadere, B.; Zafra-Polo, M.-C.; Barrachina, I.; Estornell, E.; Cortes, D. Acetogenins from annonaceae. Recent progress in isolation, synthesis, and mechanisms of action. *Nat. Prod. Rep.* **2005**, *22*, 269–303. (c) Alali, Q.; Liu, X.-X.; McLaughlin, J. L. Annonaceous acetogenins: recent progress. *J. Nat. Prod.* **1999**, *62*, 504–540, and references cited therein.
- (2) (a) Yuan, S.-S. F.; Chang, H.-L.; Chen, H.-W.; Kuo, F.-C.; Liaw, C.-C.; Su, J.-H.; Wu, Y.-C. Selective cytotoxicity of squamocin on T24 bladder cancer cells at the S-phase via a Bax-, Bad-, and caspase-3-related pathways. *Life Sci.* **2006**, *78*, 869–874. (b) Lu, M.-C.; Yang, S.-H.; Hwang, S.-L.; Lu, Y.-J.; Lin, Y.-H.; Wang, S.-R.; Wu, Y.-C.; Lin, S.-R. Induction of G2/M phase arrest by squamocin in chronic myeloid leukemia (K562) cells. *Life Sci.* **2006**, *78*, 2378–2383.
- (3) (a) Derbre, S.; Roue, G.; Poupon, E.; Susin, S. A.; Hocquemiller, R. The hydroxyl groups and THF rings are crucial structural elements for targeting the mitochondria, demonstration with the synthesis of fluorescent squamocin analogues. *ChemBioChem* **2005**, *6*, 979–982. (b) Abe, M.; Kenmochi, A.; Ichimaru, N.; Hamada, T.; Nishioka, T.; Miyoshi, H. Essential structural features of acetogenins: role of hydroxy groups adjacent to the bis-THF rings. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 779–782.
- (4) He, K.; Shi, G.; Zhao, G.-X.; Zeng, L.; Ye, Q.; Schwedler, J. T.; Wood, K. V.; McLaughlin, J. L. Three new adjacent bis-tetrahydrofuran acetogenins with four hydroxyl groups from *Asimina triloba*. *J. Nat. Prod.* **1996**, *59*, 1029–1034.
- (5) (a) Oberlies, N. H.; Croy, V. L.; Harrison, M. L.; McLaughlin, J. L. The Annonaceous acetogenin bullatacin is cytotoxic against multidrug-resistant human mammary adenocarcinoma cells. *Cancer Lett.* **1997**, *115*, 73–79. (b) Oberlies, N. H.; Chang, C.-J.; McLaughlin, J. L. Structure–activity relationships of diverse Annonaceous acetogenins against multidrug resistant human mammary adenocarcinoma (MCF-7/Adr) cells. *J. Med. Chem.* **1997**, *40*, 2102–2106.
- (6) Das, S.; Li, L.-S.; Abraham, S.; Chen, Z.; Sinha, S. C. A bidirectional approach to the synthesis of a complete library of adjacent-bis-THF Annonaceous acetogenins. *J. Org. Chem.* **2005**, *70*, 5922–5931.
- (7) (a) Chen, Z.; Sinha, S. C. Total synthesis of 27-hydroxy-bullatacin and its C-15 epimer, and studies on their inhibitory effect on bovine heart mitochondrial complex I functions. *Tetrahedron* **2008**, *64*, 1603–1611. Also see: (b) Tian, S. K.; Wang, Z. M.; Jiang, J. K.; Shi, M. Stereocontrolled construction of the *trans*-tetrahydrofuran units in Annonaceous acetogenins. *Tetrahedron: Asymmetry* **1999**, *10*, 2551–2562. (c) Zhao, H.; Gorman, J. S. T.; Pagenkopf, B. L. Advances in lewis acid controlled carbon–carbon bond-forming reactions enable a concise and convergent total synthesis of bullatacin. *Org. Lett.* **2006**, *8*, 4379–4382.
- (8) For the recent synthesis of the adjacent bis-THF acetogenins from this laboratory, see: (a) refs 6 and 7a. (b) Avedissian, H.; Sinha, S. C.; Yazbak, A.; Sinha, A.; Neogi, P.; Sinha, S. C.; Keinan, E. Total synthesis of asimicin and bullatacin. *J. Org. Chem.* **2000**, *65*, 6035–6051. (c) Han, H.; Sinha, M. K.; D'Souza, L. J.; Keinan, E.; Sinha, S. C. Total synthesis of 34-hydroxyasimicin and its photoactive derivative for affinity labeling of the mitochondrial Complex I. *Chem.–Eur. J.* **2004**, *10*, 2149–2158, and references cited therein.
- (9) For the recent synthesis of the adjacent bis-THF acetogenins from other laboratories, see: (a) Huh, C. W.; Roush, W. R. Highly stereoselective and modular syntheses of 10-hydroxytrilobacin and three diastereomers via stereodivergent [3 + 2]-annulation reactions. *Org. Lett.* **2008**, *10*, 3371–3374. (b) Marshall, J. A.; Sabatini, J. J.; Valeriote, F. ABC synthesis and antitumor activity of a series of Annonaceous acetogenin analogs with a threo, trans, threo, trans, threo-bis-tetrahydrofuran core unit. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 2434–2437. (c) Natrass, G. L.; Diez, E.; McLachlan, M. M.; Dixon, D. J.; Ley, S. V. The total synthesis of the Annonaceous acetogenin 10-hydroxyasimicin. *Angew. Chem., Int. Ed.* **2005**, *44*, 580–584. (d) Hu, Y.; Brown, R. C. D. A metal-oxo mediated approach to the synthesis of 21,22-diepi-membrarollin. *Chem. Commun.* **2005**, *45*, 5636–5637. (e) Keum, G.; Hwang, C. H.; Kang, S. B.; Kim, Y.; Lee, E. Stereoselective syntheses of rolliniastatin 1, rollimembrin, and membranacin. *J. Am. Chem. Soc.* **2005**, *127*, 10396–10399.
- (10) (a) Frantz, D. E.; Fässler, R.; Carreira, E. M. Facile enantioselective synthesis of propargylic alcohols by direct addition of terminal alkynes to aldehydes. *J. Am. Chem. Soc.* **2000**, *122*, 1806–1807. (b) Kojima, N.; Maezaki, N.; Tominaga, H.; Asai, M.; Yanai, M.; Tanaka, T. Systematic construction of a monotetrahydrofuran-ring library in annonaceous acetogenins by asymmetric alkynylation and stereodivergent tetrahydrofuran-ring formation. *Chem.–Eur. J.* **2003**, *9*, 4980–4990.
- (11) Brown, H. C.; Jadhav, P. K. Asymmetric carbon–carbon bond formation via β -allyldiisopinocampheylborane. Simple synthesis of secondary homoallylic alcohols with excellent enantiomeric purities. *J. Am. Chem. Soc.* **1983**, *105*, 2092–2093.
- (12) Metathesis approach has become a highly versatile route for the synthesis of acetogenins. For the representative reports, see: (a) refs 6 and 7a. (b) Marshall, J. A.; Sabatini, J. J. An outside-in approach to adjacent bis-tetrahydrofuran Annonaceous acetogenins with C2 core symmetry. Total synthesis of asimicin and a C32 analogue. *Org. Lett.* **2006**, *8*, 3557–3560. (c) Hoye, T. R.; Eklov, B. M.; Jeon, J.; Khorooosi, M. Sequencing of three-component olefin metatheses: total synthesis of either (+)-gigantecin or (+)-14-deoxy-9-oxygigantecin. *Org. Lett.* **2006**, *8*, 3383–3386. (d) Mootoo, D. R.; Zhu, L. Total synthesis of the nonadjacent linked bis-tetrahydrofuran acetogenin bullatanocin (squamostatin C). *J. Org. Chem.* **2004**, *69*, 3154–3157, and references cited therein.
- (13) Grubbs, R. H. Olefin metathesis. *Tetrahedron* **2004**, *60*, 7117–7140.
- (14) References 5 and 12b. (b) Ye, Q.; He, K.; Oberlies, N. H.; Zeng, L.; Shi, G.; Evert, D.; McLaughlin, J. L. Longimicins A–D: Novel bioactive acetogenins from *Asimina longifolia* (Annonaceae) and structure–activity relationships of asimicin type of Annonaceous acetogenins. *J. Med. Chem.* **1996**, *39*, 1790–1796. (c) Tormo, J. R.; DePedro, N.; Royo, I.; Barrachina, I.; Zafra-Polo, M. C.; Cuadrillero, C.; Hernandez, P.; Cortes, D.; Pelaez, F. In vitro antitumor structure–activity relationships of threo/trans/threo/trans/erythro bis-tetrahydrofuranic acetogenins: Correlations with their inhibition of mitochondrial complex I. *Oncol. Res.* **2005**, *15*, 129–138, and references cited therein.
- (15) For the analogous stereoisomeric mono-THF acetogenin library, see: Curran, D. P.; Zhang, Q.; Richard, C.; Lu, H.; Gudipati, V.; Wilcox, C. S. Total synthesis of a 28-member stereoisomer library of murisolsins. *J. Am. Chem. Soc.* **2006**, *128*, 9561–9573.
- (16) SMPs are prepared as described earlier, see: Matsuno-Yagi, A.; Hatefi, Y. Studies on the mechanism of oxidative phosphorylation. *J. Biol. Chem.* **1985**, *260*, 14424–14427.
- (17) (a) Valeriote, F.; Grieshaber, C. K.; Media, J.; Pietraszkewicz, H.; Hoffmann, J.; Pan, M.; McLaughlin, S. J. Discovery and development of anticancer agents from plants. *Exp. Ther. Oncol.* **2002**, *2*, 228–236. (b) Marshall, J. A.; Pietre, A.; Paige, M. A.; Valeriote, F. A modular synthesis of Annonaceous acetogenins. *J. Org. Chem.* **2003**, *68*, 1771–1779.

JM801028C