proton ($\delta(H)_{[RCo(Hdmg)(Ddmg)L]} - \delta(H)_{[RCo(Hdmg)_2L]}$), as well as that for the deuteron ($\delta(D)_{[RCo(Ddmg)_2L]} - \delta(D)_{[RCo(Hdmg)(Ddmg)L]}$) is about -70 ppb. The two hydrogen bonds are coupled[16] in a cooperative way: not only the hydrogen bond where deuteration occurs is weakened, but also the other. The deuteration of either one or two hydrogen bonds results in further long-range secondary isotope shifts $^n\Delta$ (n is the number of intervening bonds). The equatorial C=N carbon atoms of **1** are 64 ppb less shielded for [MeCo(Ddmg)$_2$py] than for the undeuterated complex. The same $^3\Delta$ value is observed on the side of the D bridge for [MeCo(Hdmg)(Ddmg)py], where the inequivalence of the C=N carbon atoms clearly evidences the symmetry decrease caused by deuteration of one bridge. A

Enantiopure Simple Analogues of Annonaceous Acetogenins with Remarkable Selective Cytotoxicity towards Tumor Cell Lines**

Bu-Bing Zeng, Yikang Wu, Qian Yu, Yu-Lin Wu,* Yan Li, and Xiao-Guang Chen

Annonaceous acetogenins, a relatively new class of natural products so far only found in Annonaceae, have been attracting worldwide attention in recent years because of their potent biological activities, especially as growth inhib-

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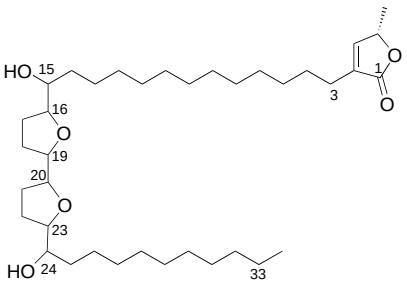
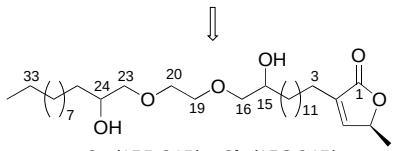
itors of certain tumor cells.[1] They have been shown to function by blocking complex I in mitochondria[2] as well as ubiquinone-linked NADH oxidase in the cells of specific tumor cell lines, including some multidrug-resistant[3] ones. These features make the acetogenins excellent leads for new antitumor agents. However, due to the scant natural resources, the substantial amounts of enantiomerically pure samples required for further biological and clinical studies appear to be attainable only by means of chemical synthesis. Unfortunately, all the total syntheses of annonaceous acetogenins which have so far appeared in the literature[4] still require more than 10 reaction steps, which makes the scale-up very difficult. Herein we wish to report a potential solution to the problem, which relies on structure simplification while maintaining all essential functionalities of the acetogenins.

It has been postulated that the antitumor activities of the acetogenins are associated with their ionophoric ability.[5] Therefore, the hydroxy and ethereal oxygen atoms in the acetogenins appear to be essential for the biological activities. The THF rings, however, do not seem to be essential. A straightforward means of structural simplification is hence to remove the ethylene bridge in the THF rings,[6] which eliminates two chiral centers and greatly simplifies the synthesis. Thus, the bis-THF annonaceous acetogenin isodesacetyluvaricin **1** and its isomers may be reduced to the linear[7] mimics **2** (Figure 1). In the latter case, four chiral



1a (15*R*,16*R*,19*R*,20*R*,23*R*,24*R*), isodesacetyluvaricin
1b (15*S*,24*R*)
1c (15*S*,24*S*)
1d (15*R*,16*R*,19*R*,20*R*,23*R*,24*S*), desacetyluvaricin

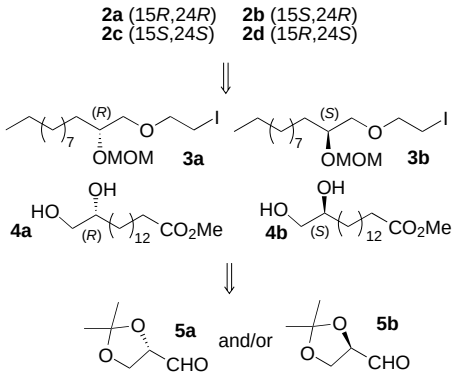


2a (15*R*,24*R*) **2b** (15*S*,24*R*)
2c (15*S*,24*S*) **2d** (15*R*,24*S*)

Figure 1. Representation of the general idea of simplifying bis-THF natural annonaceous acetogenins into linear analogues.

centers are removed from the molecule. This greatly reduces the difficulty encountered in the synthetic endeavors and thus promises easier scale-up in the future.

In order to explore the potential influence of the configuration of the hydroxy groups on the biological activities, we planned from the beginning to synthesize all four possible isomers of the simplified analogues (Figure 2) for biological

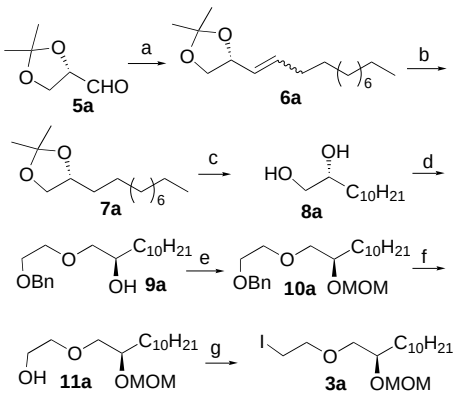


2a (15*R*,24*R*) **2b** (15*S*,24*R*)
2c (15*S*,24*S*) **2d** (15*R*,24*S*)

Figure 2. The chiral centers adjacent to the ethereal links in the targets can be attained from proper combination of **3a**, **3b**, **4a**, and **4b**, which are derived from **5a** and/or **5b** or from -lactate.

screening, using different combinations four components **3a**, **3b**, **4a**, and **4b**, which were in turn derived from one of the protected glyceraldehydes **5a** or **5b**.

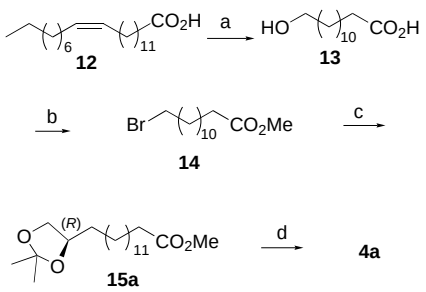
The synthesis is exemplified with preparation of **2a**, which starts with **5a** derived from -ascorbic acid following a literature procedure.[7] After chain extension[8] at the carbonyl end by Wittig reaction and hydrogenation, the acetonide **7a** was converted into diol **8a** and combined with 2-benzyloxyethyl iodide through a cyclic stannate (Scheme 1).[9] The



Scheme 1. a) $C_8H_{17}CH{=}PPh_3$, 90%; b) H_2, EtOH, Pd-C, 95%; c) HCl, MeOH; d) 1) Bu_2SnO, $CHCl_3$:MeOH (10:1), reflux, 2) CsF, ICH_2CH_2OBn, DMF, 80% over 2 steps; e) MOMCl, iPr_2NH, CH_2Cl_2, 96%; f) H_2, EtOH, Pd-C, 90%; g) I_2, imidazole, PPh_3, 82%. Bn = benzyl, MOM = methoxymethyl.

hydroxy group was masked as a MOM ether and the terminal benzyl protective group was removed by hydrogenation. The newly released primary hydroxyl functionality was then transformed into the desired iodide **3a**.

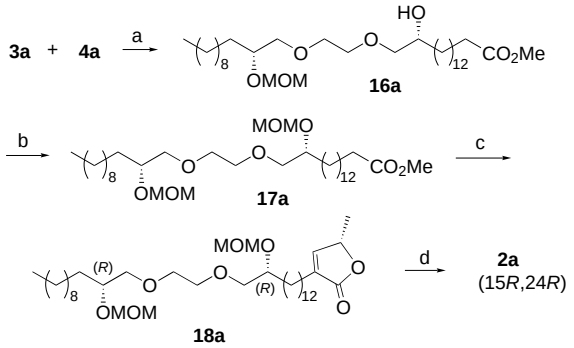
The other fragment required for the construction of target molecule **2a**, is the diol **4a**, which was prepared from *cis*-eruic acid **12** as shown in Scheme 2. The C–C double bond was first cleaved with ozone following a literature procedure[10] to give the hydroxy acid **13**, which was transformed to corresponding methyl ester in methanol in the presence of $SOCl_2$. Smooth bromination with CBr_4 in the presence of PPh_3 provided the bromo ester **14** as an immediate precursor to the corresponding phosphonium salt. In preparative runs, formation of **14**



Scheme 2. a) 1) O_3, 0–5 °C, EtOH:cyclohexane (1:5), 2) KBH_4; b) 1) MeOH, $SOCl_2$, 87% from **12**, 2) CBr_4, PPh_3, C_6H_6, 87%; c) 1) PPh_3, 2) *t*BuOK, then **5a**, 3) H_2, EtOH, Pd-C, 67% over 3 steps; d) H^+, MeOH, 91%.

could be combined with the following treatment with PPh_3 without separation, to give the desired phosphonium salt at elevated temperature in a one-pot manner. The subsequent Wittig reaction with **3a**, after hydrogenation of the C–C double bond and removal of the acetonide protective group, led to **4a**.

The coupling of **3a** and **4a** (Scheme 3) was achieved using a protocol similar to the one employed in the chain extension of **8a** above. The newly formed hydroxyl group was protected as



Scheme 3. a) 1) Bu_2SnO, $CHCl_3$:MeOH (10:1), reflux, 2) CsF, DMF, 75% over 2 steps; b) MOMCl, *i*Pr_2NH, CH_2Cl_2, 100%; c) 1) LDA, (*S*)-Me(OTHP)CHCHO, 2) 9% H_2SO_4, THF, 3) $(F_3CCO)_2O$, NEt_3, CH_2Cl_2; d) HCl, MeOH, 45–52% from **17a**. LDA = lithium diisopropylamide.

a MOM ether before the chiral center in the butenolide was introduced by an aldol condensation. After removal of the THP protective group with concurrent lactone ring closure, the hydroxyl was converted into trifluoroacetate and eliminated to give the C–C double bond. Finally, the MOM protective group was hydrolyzed using HCl in MeOH to afford the target molecule **2a**. It should be noted that in the present context, this strategy for construction of the lactone moiety after completion of the chain extension[11] gave much better yields than the previously adopted one, where the lactone was formed at early stages of the synthesis.[12]

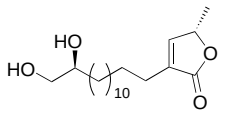
Other target molecules[13] were synthesized in a similar manner by choosing proper forms of precursors **3** and **4**. Thus, coupling of **3a** and **4b** led to **2b**, while reaction of **3b** with **4a**, and **3b** with **4b**, yielded **2c** and **2d**, respectively.

The preliminary results (Table 1) of the in vitro tests against several human solid tumor cell lines show interesting cell line selectivity. Practically no activity was found with all the four

Table 1. The preliminary results of in vitro tests (using the MTT method) against KB, A2780, HCT-8, and HT-29 human solid tumor cell lines.

Compound	EC_{50} [$\mu g\,mL^{-1}$]			
	KB	A2780	HCT-8	HT-29
2a	> 1	> 1	0.066	0.272
2b	> 1	> 1	0.097	1.12
2c	> 1	> 1	0.032	0.11
2d	> 1	> 1	0.065	7.83
19	> 1	> 1	> 1	> 1

analogues against KB and A2780 cell lines. However, impressive EC_{50} values were observed with HCT-8, which all are on the n order. Other interesting results are obtained with HT-29 cell line, different combinations of the chiral moieties show remarkable difference in the activity. Corresponding EC_{50} values for **19** are also listed in Table 1 for comparison.



19

In conclusion, we have developed a class of structurally simplified analogues of natural annonaceous acetogenins. The synthetic routes are significantly shortened. The remaining stereogenic centers are derived from the easily accessible chiral building blocks **5a** and/or **5b** or from -lactate. All four analogues show remarkable activity against the HCT-8 cell line, while better differentiation between the four analogues is found with HT-29 cell line. Further work along this line is currently in progress in our laboratory.

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[1] Reviews: a) J. K. Rupprecht, Y. H. Hui, J. L. McLaughlin, *J. Nat. Prod.* **1990**, *53*, 237; b) X. P. Fang, M. J. Rieser, Z. M. Gu, J. L. McLaughlin, *Phytochem. Anal.* **1993**, *4*, 27; c) L. Zeng, Q. Ye, N. H. Oberlies, G. E. Shi, Z. M. Gu, K. He, J. L. McLaughlin, *Nat. Prod. Rep.* **1996**, 275; d) A. Cave, B. Figadère, A. Laurens, D. Cortes, *Prog. Chem. Org. C* **1997**, *70*, 81.

[2] M. C. Gonzalez, J. R. Tormo, A. Bermejo, M. C. Zafra-polo, E. Estornell, D. Cortes, *Bioorg. Med. Chem. Lett.* **1997**, *7*, 1113, and references therein.

[3] N. H. Oberlies, V. L. Croy, M. L. Harrison, J. L. McLaughlin, *Cancer Lett.* **1997**, *115*, 73.

[4] For recent reviews, see: a) G. Cassiraghi, F. Zanardi, L. Battistini, G. Rassu, G. Aappendino, *Chemtracts: Org. Chem.* **1998**, *11*, 803; b) A. Tanaka, T. Oritani, *J. Org. Synth. Chem. Soc. Jpn.* **1997**, *55*, 877.

[5] a) S. Sasaki, K. Muruta, H. Naito, H. Sugihara, K. Hiratani, M. Maeda, *Tetrahedron Lett.* **1995**, *36*, 5571; b) S. Sasaki, K. Muruta, H. Naito, R. Maemura, E. Kawahara, M. Maeda, *Tetrahedron* **1998**, *54*, 2401.

[6] Our efforts along this line started in the early 90's Y.-L. Wu, Z.-J. Yao and Q. Yu, **1997**, CN-A 97 1 06368.0).

[7] During the preparation of this manuscript, Grée et al. published a synthetic endeavor aimed at similar target molecules: Y. L. Huérou, J. Doyon, R. L. Grée, *J. Org. Chem.* **1999**, *64*, 6782. However, they have currently only finished construction of the two chiral centers adjacent to the ethereal links, without introduction of the butenolide moiety.

[8] Y.-F. Wang, Y.-L. Wu, *Hecheng Huaxue* **1993**, *1*, 215 (*Chem. Abstr.* **1994**, *121*, 133755p).

[9] a) N. Nagashima, M. Ohno, *Chem. Lett.* **1987**, 141; b) H.-S. Byun, E. R. Kumar, R. Bittman, *J. Org. Chem.* **1994**, *59*, 2630; c) R. Martínez-Bernhardt, P. P. Castro, G. Godjoian, C. G. Gutiérrez, *Tetrahedron* **1998**, *54*, 8919.

[10] Y.-C. Wang, F.-C. Liu, *Synth. Commun.* **1994**, *24*, 1265.

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[11] Similar strategies have been employed in the total synthesis of natural acetogenins. For example, see: a) J. Marshall, H.-J. Jiang, *J. Org. Chem.* **1998**, *63*, 7066; b) S. Hanessian, T. A. Grillo, *J. Org. Chem.* **1998**, *63*, 1049.

[12] For example, see: Q. Yu, Y.-K. Wu, Y.-L. Wu, *J. Chem. Soc. Perkin Trans. 1* **1999**, 1183, and references therein.

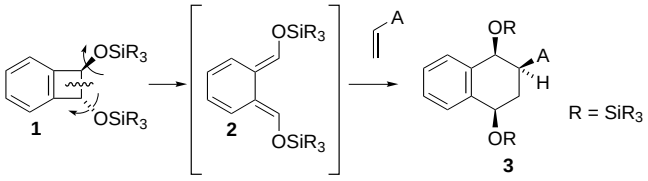
[13] ^{1}H NMR data ($CDCl_3$, 600 MHz) and optical rotations for **2a–d**. Compound **2a**: $\delta = 6.98$ (d, $J = 1.2$ Hz, 1H), 4.99 (dq, $J = 7.2$, 1.2 Hz, 1H), 3.83–3.74 (m, 2H), 3.72–3.60 (m, 4H), 3.53 (dd, $J = 9.6$, 10.2 Hz, 2H), 3.32 (dd, $J = 9.6$, 8.4 Hz, 2H), 2.26 (br.t, $J = 7.8$ Hz, 2H), 1.55 (quint, $J = 6.6$ Hz, 2H), 1.40 (d, $J = 7.2$ Hz, 3H), 1.50–1.20 (m, 40H), 0.88 (t, $J = 6.9$ Hz, 3H); $[\alpha]_D = +3.4$ ($c = 2.31$ in $CHCl_3$). Compound **2b**: $\delta = 6.98$ (br.s, 1H), 4.99 (br.q, $J = 6.6$ Hz, 1H), 3.82–3.74 (br.m, 2H), 3.72–3.60 (br.m, 4H), 3.54 (br.d, $J = 9.6$ Hz, 2H), 3.32 (br.t, $J = 9.0$ Hz, 2H), 2.26 (br.t, $J = 7.5$ Hz, 2H), 1.54 (quint, $J = 7.2$ Hz, 2H), 1.40 (d, $J = 6.6$ Hz, 3H), 1.48–1.16 (br.m, 40H), 0.88 (t, $J = 7.2$ Hz, 3H); $[\alpha]_D = -6.9$ ($c = 0.4$ in $CHCl_3$). Compound **2c**: $\delta = 6.97$ (d, $J = 1.2$ Hz, 1H), 4.98 (dq, $J = 7.2$, 1.2 Hz, 1H), 3.81–3.75 (m, 2H), 3.71–3.61 (m, 4H), 3.52 (dd, $J = 9.6$, 10.2 Hz, 2H), 3.31 (dd, $J = 9.6$, 8.4 Hz, 2H), 2.50 (br.s, 2H, OH), 2.26 (t, $J = 7.8$ Hz, 2H), 1.53 (quint, $J = 7.8$ Hz, 2H), 1.40 (d, $J = 6.6$ Hz, 3H), 1.48–1.20 (m, 40H), 0.87 (t, $J = 7.2$ Hz, 3H); $[\alpha]_D = 16.3$ ($c = 2.17$ in $CHCl_3$). Compound **2d**: $\delta = 6.98$ (d, $J = 1.2$ Hz, 1H), 4.99 (dq, $J = 1.2$, 6.6 Hz, 1H), 3.78 (m, 2H), 3.72–3.62 (m, 4H), 3.53 (dd, $J = 2.4$, 9.6 Hz, 2H), 3.32 (dd, $J = 9.6$, 8.4 Hz, 2H), 2.26 (br.t, $J = 7.8$ Hz, 2H), 2.15 (br.s, 2H, OH), 1.55 (quint, $J = 7.2$ Hz, 2H), 1.40 (d, $J = 6.6$ Hz, 3H), 1.48–1.20 (m, 40H), 0.88 (t, $J = 6.9$ Hz, 3H); $[\alpha]_D = -87.4$ ($c = 0.35$ in $CHCl_3$).

Thermal Intermolecular Hetero Diels–Alder Cycloadditions of Aldehydes and Imines via *o*-Quinone Dimethides**

Martin F. Hentemann, John G. Allen, and Samuel J. Danishefsky*

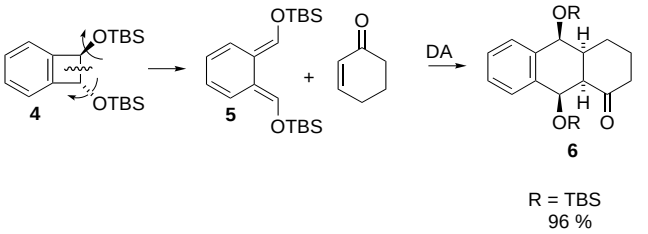
In memory of Wolfgang Oppolzer

The value of the Diels–Alder (DA) reaction in chemical synthesis can scarcely be overstated.[1] Our research group has been continuing to pursue its long-term interest in such cycloadditions, especially using suitable benzocyclobutenes.[2] In particular, we have focused on 1,2-*trans*-disubstituted disilyloxy compounds of the type **1** and have found that such systems partake in intermolecular DA cycloadditions with suitable dienophiles under remarkably mild conditions.[3] These results strongly suggest that **1** undergoes facile bond reorganization to produce *o*-quinone dimethides (5,6-dimethylenecyclohexa-1,3-diene derivatives, such as **2**), which function as dienes in DA reactions to produce products of type **3** (Scheme 1).



Scheme 1. A = activator.

While this kind of cycloaddition is well known, its primary applications have been at the intramolecular level. Thus, many of the elegant sequences that have traditionally been used to generate *o*-quinone dimethides as a prelude to cycloaddition work well only when the putative dienophile is tethered to the diene.[4] A particularly impressive illustration of our recently discovered "*trans*-1,2-bissiloxy effect" was seen in the uncatalyzed, near-quantitative formation of **6** from the cycloaddition of **4** with 2-cyclohexen-1-one (a notoriously sluggish dienophile) at 40 °C! (Scheme 2).[5] It was subsequently discovered that **4** will also effect cycloaddition with 2-cyclohexen-1-one even at ambient temperatures, presumably via **5**.



Scheme 2. TBS = *tert*-butyldimethylsilyl.

We subsequently noted that benzocyclobutene **4** exhibits thermochromic behavior, a characteristic that simplifies the monitoring of these types of reactions. Compound **4** exists as a colorless oil, either neat, or in benzene solution at temperatures below 0 °C. However, it becomes yellow upon being warmed to room temperature. This color is discharged upon recooling. The color also disappears following exposure to a dienophile. Accordingly, it seems reasonable[6] to interpret our findings in terms of a low equilibrium concentration of **5**, although in insufficient amount for detection by NMR analyses.

The initial successes with carbon dienophiles prompted us to expand the scope of our inquiry by focusing on heterodienophiles. In an extensive series of investigations in the 1980s our research group discovered and developed the first examples of diene–aldehyde and diene–imine condensations in cases where the formal heterodienophile was not especially activated.[7] However, in those studies it was necessary to take recourse to Lewis acid catalysis to accomplish such reactions with general, as opposed to specially activated, heterodieno-

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