JOC Article

A Modular Synthesis of Annonaceous Acetogenins

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A synthesis of four Annonaceous acetogenins, asiminocin, asimicin, asimin, and bullanin, by a modular approach from seven fundamental subunits, **A**−**G**, is described. The approach employs a central core aldehyde segment, **C**, to which are appended an aliphatic terminus, **A** or **B**, a spacer subunit, **D** or **E**, and a butenolide terminus, **F** or **G**. Coupling of the **A**, **B**, **D**, and **E** segments to the core aldehyde unit is effected by highly diastereoselective additions of enantiopure allylic indium or tin reagents. The butenolide termini are attached to the **ACD**, **BCE**, or **BCD** intermediates by means of a Sonogashira coupling. The design of the core, spacer, and termini subunits is such that any of the C30, C10, or C4 natural acetogenins or stereoisomers thereof could be prepared. IC_{50} values for the four aforementioned acetogenins against H-116 human colon cancer cells were found to be in the 10^{-3} to 10^{-4} μM range. The IC_{90} activities were ca. 10^{-3} μM for asimicin and asimin but only 0.1−1 μM for bullanin and asiminocin.

Introduction

Genera of the plant family Annonaceae have proven to be rich sources of a unique family of acetogenins with a wide range of bioactivities.[1] The leaves, bark, and seeds of the edible fruits of these plants have been used in folk medicine as insecticides, fungicides, antiparasitics, and a remedy for snake bite. More recently a significant number of the nearly 400 members of this family have been shown to exhibit high levels of cytotoxicity against human tumor cell lines, including those that exhibit multiple drug resistance (MDR).[2] Interestingly, normal epithelial and bone marrow cells are significantly less affected than tumor cells. Although the acetogenins are found in relatively abundant plants, they are present in only minute amounts as complex mixtures of structurally similar isomers. Thus isolation of a pure component presents a major challenge. A typical protocol yields only 10−20 mg of material from 15 kg of stem bark and then only after multiple separation steps involving partition extraction and chromatography on several different columns.[3] Final purification often requires preparative HPLC or radial chromatography. Furthermore, the products isolated are waxes or gums, thus precluding structure determination through X-ray diffraction.

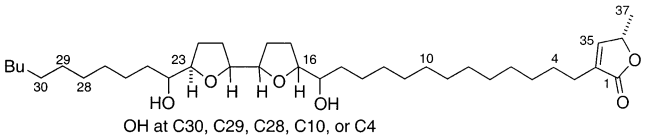

FIGURE 1. Structural features of adjacent bistetrahydrofuran Annonaceous acetogenins with high antitumor activity.

Structures of Annonaceous Acetogenins

The Annonaceous acetogenins contain an unbranched chain of 30−32 carbon atoms attached to the 3-position of (*S*)-5-methyl-(5*H*)-2-furanone. This chain typically contains 3−5 or more oxygen substituents with at least one of these located near the center embedded in a tetrahydrofuran moiety. Some acetogenins possess ketonic groups or (*Z*) double bonds in the chain, reflecting their presumed fatty acid origins. Those with the highest antitumor activity feature a core structure of two tetrahydrofuran rings connected at the α-position with flanking CHOH substituents at the α′-positions and a third OH substituent, often located at C30, C29, C28, C10, or C4 (Figure 1).[1] Structures with one, two, or three tetrahydrofuran rings are commonly found. These rings need not be adjacent. Core structures containing a tetrahydropyran ring are also known.[1] To date, the great majority of acetogenins with an adjacent bistetrahydrofuran core unit have been shown to have the (*R*) configuration at C23.

It should be noted that despite the extreme cytotoxicity reported for the aforementioned acetogenins (IC_{50}'s of 10^{-12} μg/mL in cell culture assays against human tumor

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(1) (a) Gu, Z.-M.; Zhao, G.-X.; Oberlies, N. H.; Zeng, L.; McLaughlin, J. L. In *Recent Advances in Phytochemistry*; Arnason, J. T., Mata, R., Romeo, J. T., Eds.; Plenum Press: New York, 1995; Vol. 29, pp 249−310. (b) Zeng, L.; Ye, Q.; Oberlies, N. H.; Shi, G.; Gu, Z.-M.; He, K.; McLaughlin, J. L. *Nat. Prod. Rep.* **1996**, 275. (c) Cavé, A.; Figadére, B.; Laurens, A.; Cortes, D. In *Progress in the Chemistry of Natural Products*; Herz, W., Kirby, G. W., Moore, R. E., Stegilch, W., Tamm, C., Eds.; Springer-Verelag Chemistry: New York, 1997; Vol. 70, pp 81−288.

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cell lines), studies on bullatacin[2], asimin, and several analogues[4] indicate that they exhibit only modest growth inhibition of normal cells. Hence the differential cytotoxicity is quite high. Preliminary investigations have shown that acetogenins selectively inhibit ATP synthesis in tumor cells but not in noncancerous cells. This mode of action is unique and offers a previously unexplored mechanism for cancer chemotherapy.

Background

Numerous publications on the synthesis of Annonaceous acetogenins and their possible precursors have appeared in recent years.[5−8] The sequences developed in those investigations provided adequate amounts of the final products for structure confirmation, but few, if any, could be judged both efficient and versatile. Motivated by the reported high levels of activity against human tumor cell lines and low toxicity toward normal cells, we initiated a program to develop a modular synthetic approach that could be used to prepare the most active naturally occurring structural types and analogues thereof for structure−activity evaluation.

Synthetic Plan

Our plan was based on previous success in constructing the bistetrahydrofuran core structure through stereoselective additions of enantiomerically enriched γ-alkoxy allylic stannanes, or the related allylic indium halide analogues, to enantioenriched γ-silyloxy aldehydes, followed by tetrahydrofuran ring closure of sulfonic ester derivatives of the adducts (Figure 2).[5,6] The allylation methodology provides a versatile route to either enantiomer of the syn or anti diastereomeric adduct. Additionally it is possible to prepare enantiomeric and diastereomeric γ-silyloxy aldehyde substrates, thereby allowing reasonably direct access to any of the 64 possible bistetrahydrofuran core isomers. Of course, the efficiency of this approach would expectedly show some dependence on stereochemistry such that all isomers might not be equally accessible.

The initial goals of the present investigation were to develop a modular route to representative adjacent bistetrahydrofuran Annonaceous acetogenins with the threo, trans, threo, trans, threo core stereochemistry and

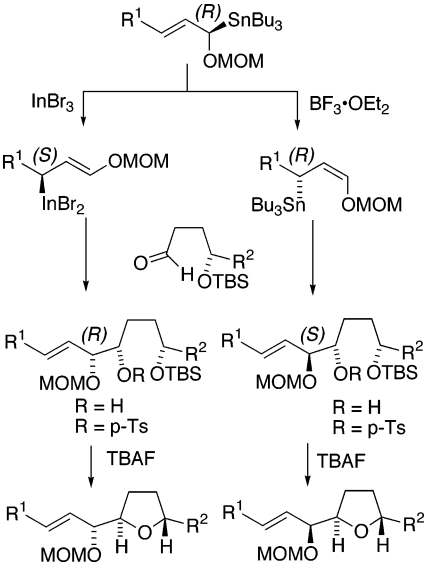

FIGURE 2. Core tetrahydrofuran assembly.

hydroxyl substituents at C30, C10, and C4 as a test of concept. Natural acetogenins with these features have been found to strongly inhibit the growth of human colon, breast, and lung cancer cells.[3] The high convergency and modular nature of the synthetic plan would provide more efficient access to these bioactive compounds, and nonnatural analogues thereof, than previous more linear routes. Following the attainment of these initial synthetic goals we would be well-positioned to interchange the various modules to produce unnatural acetogenins with the potential for improved drug properties.

The central element of our plan entails the synthesis of four major segments consisting of two pairs of allylic stannanes, equivalent to the aliphatic termini **A** and **B**, and the spacer units **D** and **E** (Figure 3). These would be joined sequentially to the dialdehyde precursor **C** by the aforementioned allylation methodology to assemble stereochemically homogeneous core precursors, which would then be cyclized to the bistetrahydrofuran core units. Sonogashira coupling[9] of the butenolide termini **F** or **G** would provide the fully elaborated acetogenin structures. The syntheses would be completed by hydrogenation of the side chain multiple bonds and deprotection of the OH functions.

We selected asiminocin, asimin, and asimicin as the initial synthetic targets. These acetogenins have been reported to exhibit inhibitory activities of $\sim 10^{-12}$ μg/mL against HT-29 human colon cancer cell lines.[3]

Segment Synthesis

Our synthesis of the aliphatic terminus **A** of asiminocin is depicted in Scheme 1. The known ester aldehyde **2**[10] was treated with dibutylzinc in the presence of the bissulfonamide catalyst **3** by the Knochel protocol[11] to afford the alcohol **4** as a 95:5 mixture (Mosher ester analysis)[12] of enantiomers in high yield. Sequential reduction and oxidation of the DPS protected derivative **5** yielded the enal **7**, which was subjected to a stannation-

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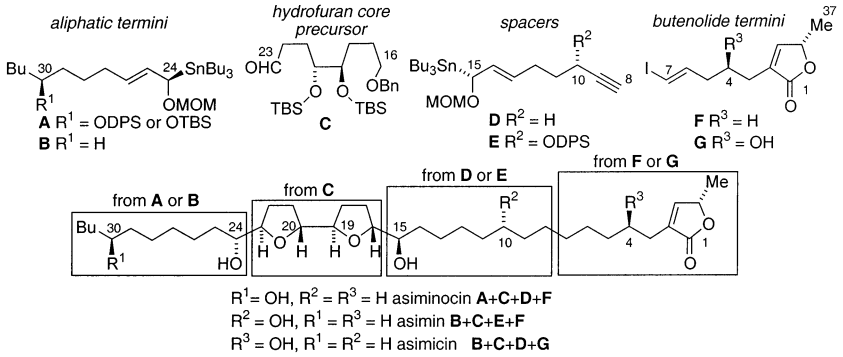
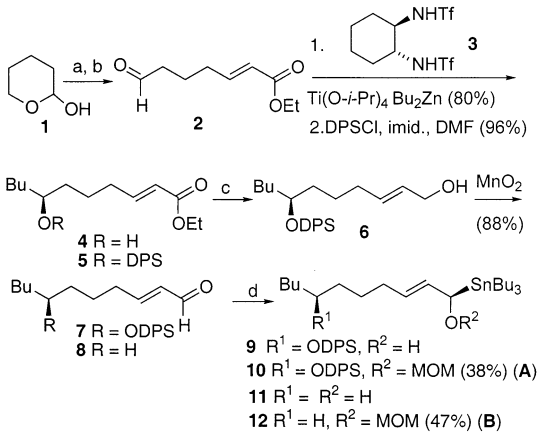


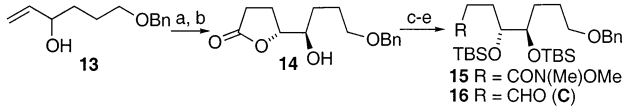
FIGURE 3. A modular synthesis of bistetrahydrofuran Annonaceous acetogenins.

SCHEME 1[a]



[a] (a) $Ph_3P{=}CHCO_2Et$, THF, reflux (88%); (b) Swern (97%); (c) DIBAL-H, hexanes (97%); (d) (1) Bu_3SnLi, (2) ADD, (3) (*S*)-BINOL, EtOH, LAH, (4) MOMCl, *i*-Pr_2NEt.

SCHEME 2[a]

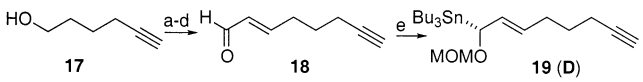


[a] (a) $(MeO)_3CMe$, $EtCO_2H$ (96%); (b) AD-mix β (99%); (c) MeONHMe·HCl, $AlMe_3$, (99%); (d) TBSCl, Im (99%); (e) DIBAL-H (99%).

oxidation-reduction-protection sequence[13] leading to the (*R*)-allylic α-OMOM stannane **10** (**A**) of high enantiomeric purity. The commercially available enal **8**[14] was carried through the same stannane sequence to give the related (*R*)-allylic α-OMOM stannane **12** (**B**) in four steps.

Our synthesis of the known core precursor **C** (**16**) was effected as previously described[6e] from allylic alcohol **13** in the five steps outlined in Scheme 2. The spacer unit **D** (**19**) was synthesized along the same lines as the stannane termini **A** and **B** starting from 5-hexyn-1-ol (**17**) as in Scheme 3.

SCHEME 3[a]



[a] (a) Swern; (b) $Ph_3P{=}CHCO_2Et$ (67%); (c) DIBAL-H (83%); (d) MnO_2; (e) (1) Bu_3SnLi, (2) ADD, (3) (*S*)-BINOL, EtOH, LAH, (4) MOMCl, *i*-Pr_2NEt.

The second spacer unit **E** (Scheme 4) was assembled from the known diepoxy diol **22**,[15] prepared by a modified procedure in four steps from 1,5-cyclooctadiene. Treatment of the derived chloride **23** with LDA effected elimination to the dialkyne diol **24**.[16] Dihydroxylation of the bis-DPS ether enediyne **25** followed by diol oxidative cleavage with lead tetraacetate and subsequent Wittig homologation yielded the conjugated ester **28**, which was subjected to successive reduction with DIBAL-H and oxidation with MnO_2 to afford enal **30**. The synthesis of the allylic stannane spacer segment **31** (**E**) was completed by application of the previously employed four-step stannylation-oxidation-reduction-protection sequence.

Our initial route to the OTBS analogue **39** of ester **28** (Scheme 5) commenced with 4-pentenol (**32**) and proceeded by a route analogous to that employed in Scheme 4. However, intermediates **33**−**37** proved somewhat volatile, resulting in diminished yields. Ultimately, this sequence failed because the ozonolysis of enyne **38** could not be achieved satisfactorily. The successful route outlined in Scheme 4 circumvented these two problems. Initially, the TBS ether was employed in this sequence, but conversion of **30** (TBS in place of DPS) to stannane **31** (TBS in place of DPS) proceeded in only 13% yield, possibly the result of TBS cleavage in the reduction step.

The two butenolide termini, segments **F** and **G**, were prepared according to Schemes 6 and 7. Both utilized the known α-SPh lactone **41**.[17] For the former, alkylation of **41** with iodide **42** and subsequent sulfoxide elimination afforded butenolide **44**. The alcohol derivative **45** was oxidized to the unstable aldehyde **46**, which was converted without purification to vinyl iodide **47** (**F**) upon Takai homologation.[18]

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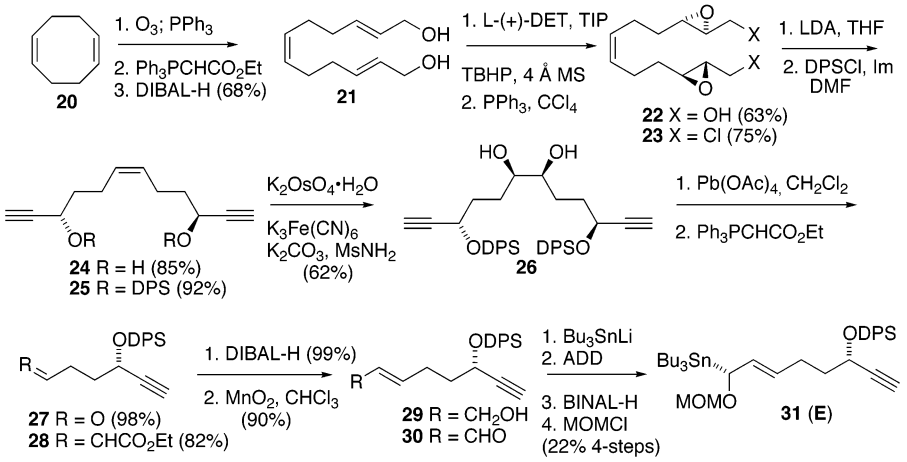
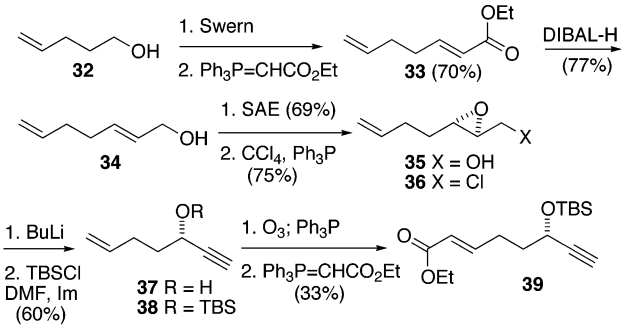
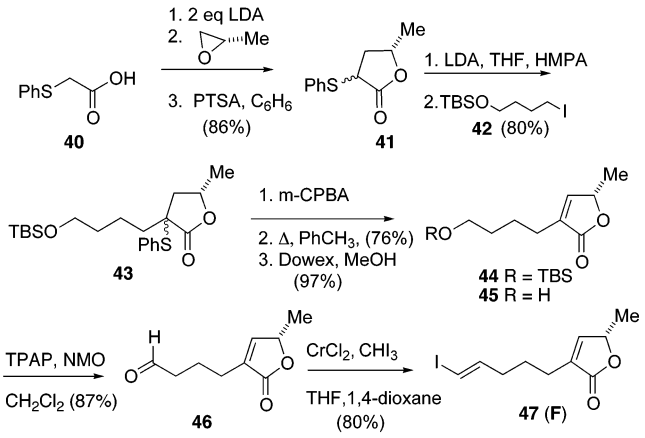
SCHEME 4



SCHEME 5

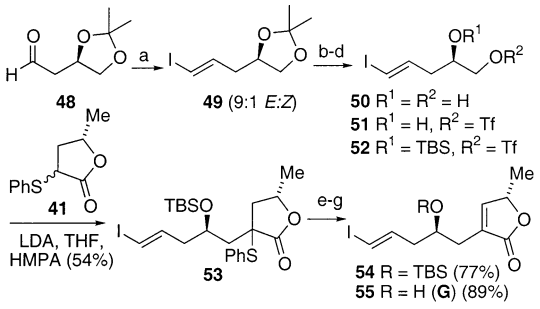


SCHEME 6



An analogous sequence[19] was employed for the synthesis of butenolide **G** (Scheme 7). However, incorporation of the eventual C4 hydroxy substituent required a somewhat lengthier sequence to prepare the alkylating agent, triflate **52**. To that end, aldehyde **48**, available in three steps from (*R*)-dimethyl malate,[20] was subjected to Takai homologation[18] affording the vinyl iodide **49**. Cleavage of the acetonide and ensuing selective conversion of the primary alcohol to the triflate and protection

SCHEME 7[a]



[a] (a) CHI_3, $CrCl_2$ (50%); (b) Dowex 50W, MeOH (93%); (c) Tf_2O, 2,6-lut; (d) TBSOTf (85%); (e) *m*-CPBA; (f) $PhCH_3$ (g) HF·py, THF.

of the secondary alcohol as the TBS ether completed the synthesis of the alkylating agent **52**.[21] The alkylation of lactone **41** was effected in 54% yield. The sulfoxide derivative underwent pyrolysis in refluxing toluene to yield the butenolide **54**, which was desilylated with HF−pyridine in THF.

Segment Coupling

The next phase of our studies was directed at the coupling of appropriate **A**−**G** intermediates and hydrogenation-deprotection of the coupling products to the selected threo, trans, threo, trans, threo core compounds, asiminocin, asimin, and asimicin, with (*R*)-C4, (*R*)-C10, and (*S*)-C30 OH substituents, as depicted in Figure 1. Core assembly for all three would employ allylindium additions to aldehyde **C** along the lines of Figure 2.

Asiminocin

The synthesis of asiminocin (**64**) proceeded according to Scheme 8. Thus, in situ transmetalation of allylic stannane **10** (**A**) with $InBr_3$ in the presence of aldehyde **16** (**C**) afforded the anti adduct **56** in 80% yield virtually free of isomeric byproducts (^{1}H NMR analysis). The double bond of the tosylate derivative **57** was reduced over Pd−C, and the intermediate alcohol was subjected

(19) This sequence closely parallels that of Mosin, B.: Maezaki, N.; Kojima, N.; Sakamoto, A.; Iwata, C.; Tanaka, T. *Org. Lett.* **2001**, *3*, 429.

(20) Saito, S.; Hasegawa, T.; Inaba, M.; Nishida, R.; Fujii, T.; Nomizu, S.; Moriwake, T. *Chem. Lett.* **1984**, 1389.

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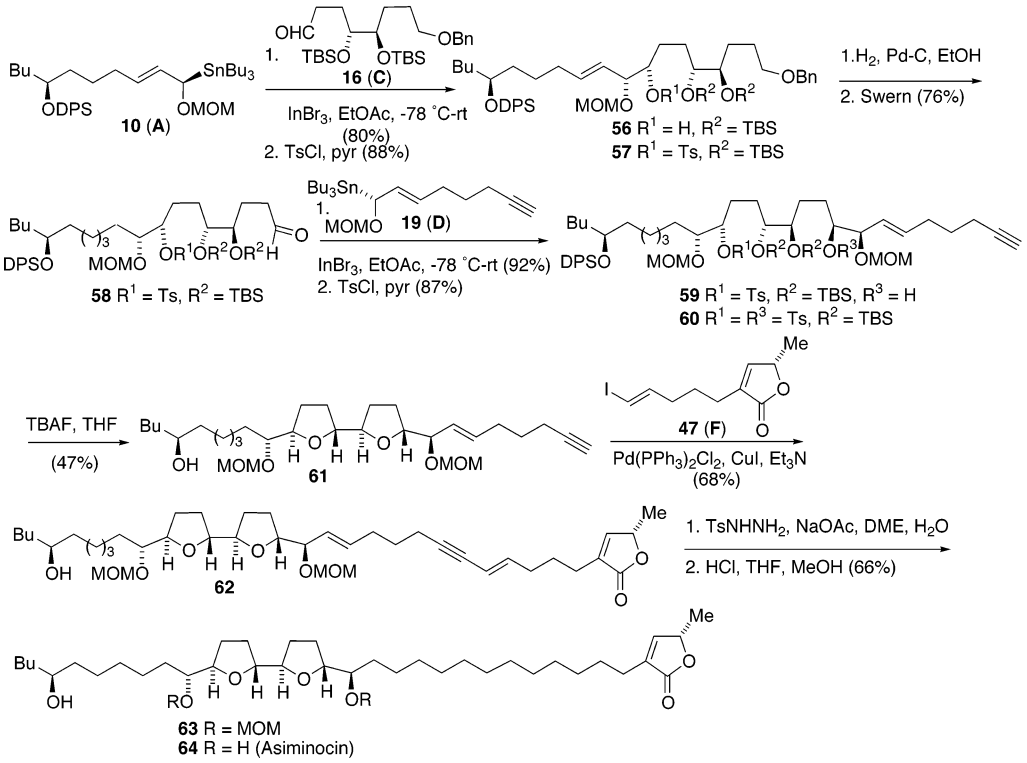
SCHEME 8



to Swern oxidation[22] affording aldehyde **58** in 76% yield for the two steps. A second in situ transmetalation of allyllic stannane **19** (**D**) in the presence of aldehyde **58** proceeded in 92% yield. The resulting anti adduct **59** was the sole detectable product. Tosylation led to the bistosylate, which was converted to the bistetrahydrofuran **61** upon exposure to TBAF in THF for 23 h. These conditions also caused partial cleavage of the DPS ether. This cleavage was quite slow and prolonged exposure to TBAF was required, resulting in some loss of material. Conceivably, the mixture of C30 alcohol and DPS ether could be carried through the subsequent steps with final cleavage being effected in the last deprotection step. However, this option was not explored. Sonogashira coupling[9] of the alkynyl THF **61** with vinyl iodide **47** (**F**) produced the fully elaborated acetogenin framework **62**. The use of $Pd(PPh_3)_2Cl_2$ as a precatalyst proved somewhat superior to $Pd(PPh_3)_4$ (68% vs 56% yield) for this coupling reaction. Selective hydrogenation of the dienyne moieties with diimide generated from tosylhydrazide[23] afforded the octahydro product contaminated with tosylhydrazide byproducts. Hydrolysis of the MOM ethers was effected with aqueous HCl in methanolic THF to afford asiminocin (**64**), which was easily separated from the aforementioned byproducts. The rotation and spectra of the synthetic material were in close agreement with the reported values.[24]

Asimicin

A parallel sequence of reactions was employed for the synthesis of asimicin (**73**, Scheme 9). In that case the aliphatic terminus was appended to the core precursor **16** (**C**) through use of the allylic stannane **12** (**B**) and $InBr_3$. The adduct **65** (dr > 95:5 according to ^1H NMR analysis) was tosylated, debenzylated, and oxidized to aldehyde **67**. The spacer unit **19** (**D**) was transmetalated to the allylic indium bromide with $InBr_3$, and this intermediate was added to aldehyde **67** in situ. Once again the addition proceeded with high diastereoselectivity affording the anti adduct **68** (dr > 95:5). The bistosylate **69** cyclized to the elaborated bistetrahydrofuran core segment **70** upon exposure to TBAF in THF. Sonogashira coupling of the terminal alkyne of **70** with the vinylic iodide **55** (**G**) led to the fully elaborated acetogenin structure **71** which was reduced, as before, with diimide and subjected to aqueous HCl to remove the MOM ether protecting groups. The product **73** thus obtained was identified as asimicin through comparison of the NMR spectra and optical rotation.[25]

Asimin

The third target of these studies, asimin (**79**), was prepared by the sequence outlined in Scheme 10. Aldehyde **67**, previously employed in the synthesis of asimicin (Scheme 9), was treated with the allylic indium reagent derived from spacer stannane **31** (**E**) to afford the adduct **74** with high diastereoselectivity. Cyclization of the bistosylate **75** and attendant (slower) cleavage of the DPS ether with TBAF proceeded in modest yield. Extended reaction times offered no improvement and appeared to diminish the yield and purity of the isolated product. Furthermore, no significant byproducts could be identified. This situation is to be contrasted with the analogous higher yield cyclization of the bistosylate **69** in which there is no protected "remote" OH group.

(22) Omura, K.; Swern, D. *Tetrahedron* **1978**, *34*, 1651.
(23) Hart, D. J.; Hong, W.-P.; Hsu, L.-Y. *J. Org. Chem.* **1987**, *52*, 4665.
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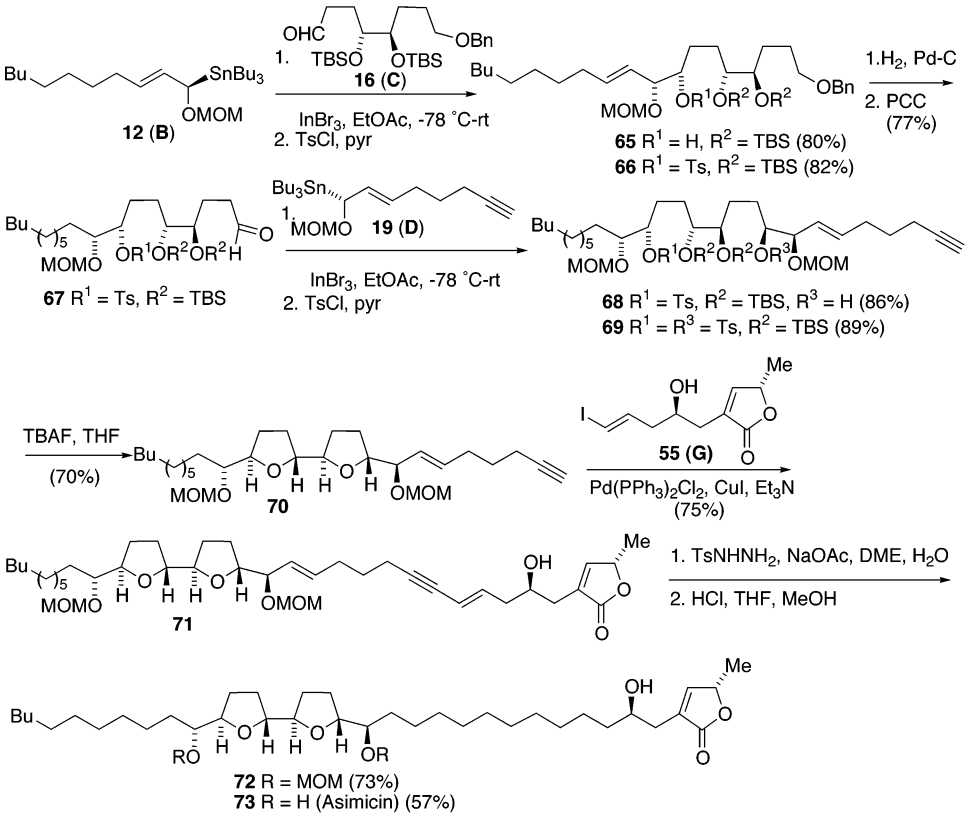
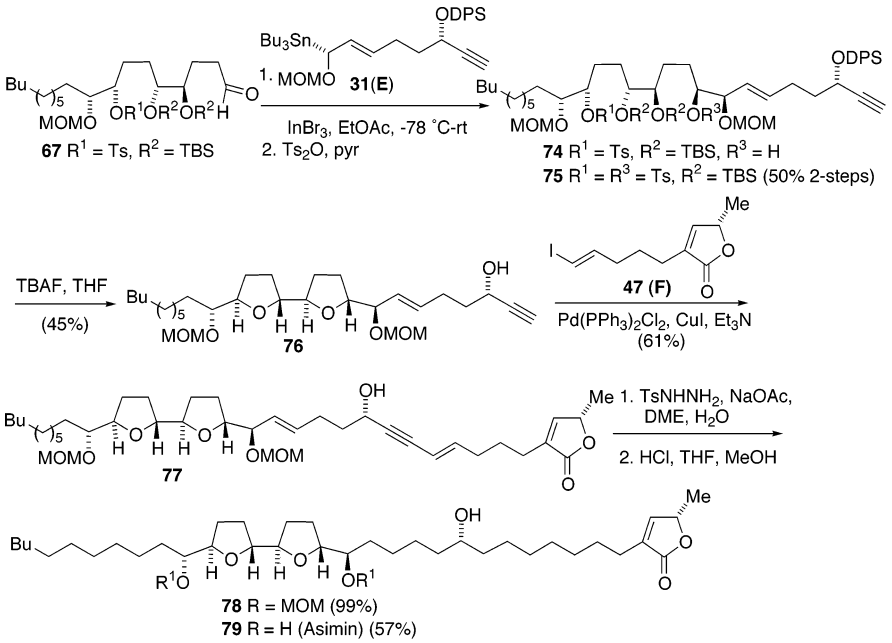
(25) Hoye, T. R.; Tan, L. S. *Tetrahedron Lett.* **1995**, *36*, 1981.

SCHEME 9



SCHEME 10

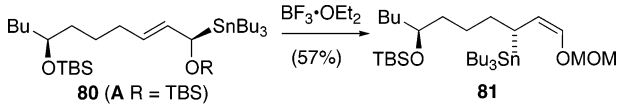


The bistetrahydrofuran **76** was coupled to the butenolide terminus **47** (**F**) by the Sonogashira methodology to afford the enyne **77** in satisfactory yield. Selective hydrogenation of this dienyne with diimide followed by acidic hydrolysis of the MOM ethers afforded asimin (**79**). The NMR spectra and rotation of this material were in close agreement with those of the natural product.[3]

Bullanin, a Core Stereoisomer of Asiminocin

As an initial step toward establishing the applicability of this modular approach to other core stereoisomers of bistetrahydrofuran acetogenins, we targeted bullanin (**91**), a C24 epimer of asiminocin.[26] As noted in Figure 2, diastereomeric homoallylic alcohol adducts can be prepared from a common α-OMOM allylic stannane through a change in reaction conditions. Transmetalation with

SCHEME 11



$InBr_3$ in situ affords erythro (anti) adducts, whereas sequential BF_3-promoted isomerization and addition leads to threo (syn) isomers. Accordingly, we could employ this latter methodology to add the aliphatic terminus **A** to the core precursor aldehyde **C** (Figure 3). In the course of our earlier studies we noted that cleavage of the DPS ether concomitant with tetrahydrofuran formation was not always complete. Accordingly, we employed the more labile TBS ether **80**, prepared from hydroxy ester **4** along the lines of Scheme 1, for the present synthesis. 1,3-Isomerization to **81** could be effected by treatment of **80** with $BF_3{\cdot}OEt_2$ (Scheme 11).

The addition of stannane **81** to aldehyde **16 (C)** afforded the syn adduct **82** diastereoselectively (dr > 95:5 according to ^1H NMR analysis) in 77% yield (Scheme 12). Isomerization of stannane **80** to **81** could also be carried out in situ followed by addition of the aldehyde. However, the isomerization proved to be relatively slow and its progress was difficult to monitor by TLC, with the result that the γ adduct **82** was sometimes contaminated with the isomeric adduct of the α stannane **80**. Hence we elected to isolate the γ stannane before addition of the aldehyde. The remainder of the synthetic sequence was identical to that described for asiminocin (Scheme 8). However, the TBAF-promoted cyclization of the TBS ether bistosylate **87** proceeded in higher yield than that of the analogous conversion of the related DPS ether **60** and cleavage of the TBS ether was complete.

Biological Activity

The Annonaceous acetogenins inhibit intracellular production of ATP by blocking electron transport in Complex I, a membrane-bound protein of the mitochondrial electron transport system.[27] The inhibition is thought to involve interference with ubiquinone-linked NADH oxidase in the plasma membrane. It has been proposed that the hydrophilic core of these compounds binds to the polar glyceryl end groups of the phospholipid segment of the membrane, allowing the hydrophobic chains to penetrate the bilayer and interact through the furanone terminus with the ubiquinone-dependent site in the cellular electron transport system.[28] The resultant cessation of ATP production in the cellular membrane inhibits the growth of cancer cells and leads to ultimate selective cell death.

The modular syntheses described in this report should permit access to meaningful amounts of natural acetogenins and novel analogues for biological evaluation and for structure−activity studies. As a step in this direction we were interested in measuring the cytotoxicity of the four synthetic acetogenins prepared in the present study toward H-116 human colon cancer cells. These compounds have previously been examined for antitumor activity against HT-29 colon cancer cells (Table 1).

Remarkably, it was found that the potency of the synthesized acetogenins against H-116 cells was lower than that reported for HT-29 cells by a factor of 10^8 to 10^9 (!) as measured by the IC_{50} values. The source of the apparent discrepancy between the two sets of determinations is unknown. Possibly the two cell lines possess a significant difference in susceptibility. In any event, activities in the 10^{-3} to 10^{-4} μM range exhibited by our synthesized acetogenins justify additional exploration of these compounds for cancer chemotherapy. As a comparison, 5-fluorouracil, a current chemotherapeutic agent for colon cancer,[29] shows significantly lower activity than any of the tested acetogenins toward the H-116 cell line. Of further interest are the IC_{90} results for these compounds. Although the IC_{50} values of the five acetogenins in Table 1 are comparable, both asimicin and asimin exhibit significantly lower IC_{90} levels in comparison to asiminocin and bullanin. The ratio IC_{90}/IC_{50} is a measure of the concentration response. Thus, asiminocin and bullanin, although quite active in the IC_{50} assay, show a rather shallow concentration−response plot (surviving fraction of tumor cells vs concentration of drug), reflected in the high IC_{90}/IC_{50} ratio, and would therefore be considered less likely candidates for in vivo studies.[30]

It was of interest to compare the activity of asimicin, one of the most active of the synthesized acetogenins, with its dihydro derivative **93**. This substance was prepared by hydrogenation of the butenolide **71** over Pd-on-carbon in ethanol (Scheme 13). The crude hydrogenation product (**92**) was isolated as a 1:1 mixture of diastereoisomers,[31] but following hydrolysis of the MOM ethers, it was possible to partially separate the mixture through column chromatography on silica gel resulting in a 4:1 mixture. This mixture exhibited an IC_{50} value comparable to that of the parent. However, a significantly increased drug concentration was required to reach an IC_{90} of H-116 cell growth. McLaughlin has stated that bullatacin is "much more active" than its reduction product dihydrobullatacin at the IC_{50} level.[32] However, it should be noted that rolliniastatin-1 and its dihydro derivative show comparable activity.[33] IC_{90} values were not measured for these compounds. Although it is generally conceded that the butenolide terminus plays an important role in the growth-inhibitory activity of the acetogenins,[34] this feature does not seem prerequisite to cytotoxicity in all acetogenins. Furthermore, the differences in inhibitory concentrations between the 50% and 90% levels show no obvious correlation with structure.

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SCHEME 12

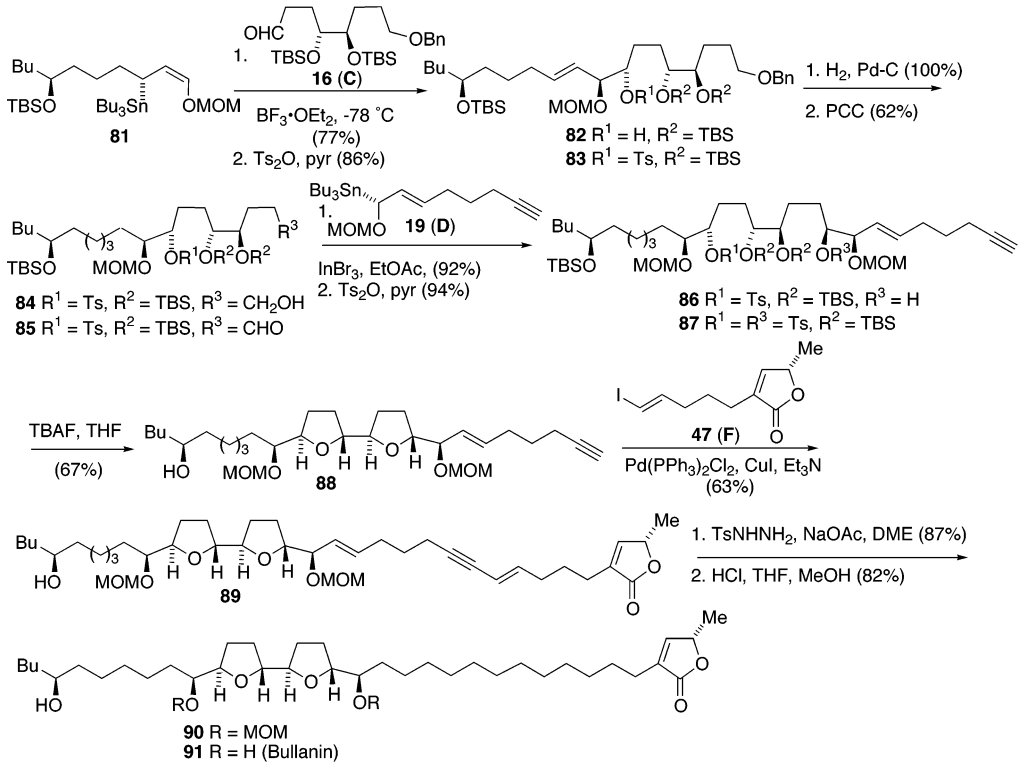
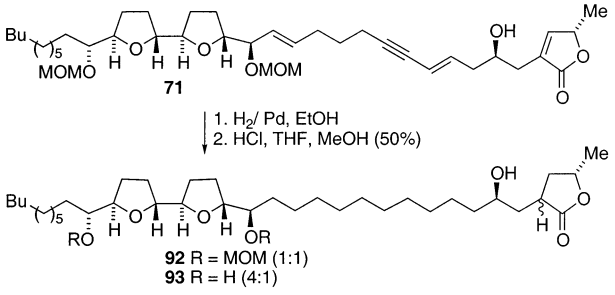


TABLE 1. Cytotoxicity of Annonaceous Acetogenins toward Human Colon Cancer Cells

	H-116[a]			HT-29[b]	
compound	IC_{50} (μg/mL)[c]	IC_{90} (μg/mL)[c]	IC_{90}/IC_{50}	IC_{50} (μg/mL)[c]	ref
asiminocin	5×10^{-3}	1.5	300	$<10^{-12}$	22
asimicin	5×10^{-4}	2×10^{-3}	4	$<10^{-12}$	3
dihydroasimicin	2×10^{-4}	1×10^{-1}	500		
asimin	3×10^{-4}	3×10^{-3}	10	$<10^{-12}$	3
bullanin	3×10^{-3}	1×10^{-1}	33	5×10^{-12}	24
5-fluorouracil[d]	1.5×10^{-1}	1.5	10		

[a] Present study. [b] See references. [c] 1 g/mL ≈ 1.5 M. [d] 1 g/mL ≈ 7 M.

SCHEME 13



Differential Cytotoxicity-Disk Diffussion Assays

An in vitro cell-based disk diffusion assay was employed to evaluate differential cytotoxicity of the acetogenins synthesized in this study (Table 2).[35] In this assay a 6.5-mm filter disk impregnated with a measured amount of the test compound is placed on a plate that has been seeded with either tumor or normal cells. The plates are incubated for 7 days, and a zone of colony inhibition is measured to evaluate the effective cytotoxicity of the test compound toward a given cell type. A "zone" is defined as the distance from the edge of the filter disk to the point of appearance of control-sized colonies. The diameter of the filter disk is arbitrarily taken as 200 "units" of activity. Each of the selected acetogenins was assayed against three cell types; H-116 (human colon cancer), CEM (human leukemia), and CFU-GM (human bone marrow). On the basis of past experience,[35] it has been determined that compounds exhibiting a differential activity against solid tumor cancer cells vs either leukemia or normal cells of more than 250 units are good candidates for in vivo testing and eventual progression to clinical evaluation. Accordingly, asiminocin and asimin are the most promising members of this group.

TABLE 2. Disk Diffusion Assay for Antitumor Compounds

compound	μg/disk	H-116[a]	CEM[b]	CFU-GM[c]
asiminocin	4×10^{-2}	600	300	0
asimicin	1.5×10^{-2}	450	350	300
dihydroasimicin	7.5×10^{-2}	300	250	200
asimin	3×10^{-1}	400	0	50
bullanin	1.5×10^{-2}	350	200	150
5-fluorouracil	4×10^{-2}	500	500	0

[a] Human colon cancer. [b] Human leukemia. [c] Human bone marrow.

Summary

In summary, the proposed modular synthesis approach has been reduced to practice for representative members

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of the bistetrahydrofuran Annonaceous acetogenins. The stereochemically defining steps proceed with excellent selectivity. The key bistetrahydrofuran cyclization reactions take place in ca. 50−70% yield, depending on the nature of the "remote" protected OH substituent. The relatively slow cleavage of a remote ODPS ether in the sequences leading to asiminocin (Scheme 8) and asimin (Scheme 10) required extended reaction times, which appear to lower the isolated yield of the bistetrahydrofuran products. The related cyclization reactions leading to asimicin (Scheme 9) and bullanin (Scheme 12), in which either no remote silyl ether or a labile OTBS ether was present, proceed in significantly higher yield.

The synthesized acetogenins were found to possess IC_{50} values of $\sim 10^{-2}$ to 10^{-4} μM toward H-116 solid human colon cancer cells. This value contrasts sharply with reported concentrations of $\sim 10^{-12}$ μM for the natural products vs HT-29 colon cancer cells. IC_{90} values have not previously been determined for Annonaceous acetogenins. These values were significantly higher than the IC_{50} values and, depending on the specific acetogenin, demonstrated either steep or shallow concentration−response relationships. Differential cytotoxicity, measured by a disk diffusion assay, showed that the synthesized natural acetogenins are quite selective for H-116 human colon cancer cells vs human bone marrow and leukemia cells. Future studies will explore these issues in greater detail, as the synthetic methodology delineated in this investigation should be broadly applicable to other bistetrahydrofuran acetogenins and analogues thereof.

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Supporting Information Available: Experimental procedures, ^{1}H NMR spectra of key intermediates, and selected ^{13}C NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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