Pure & Appl. Chem., Vol. 69, No. 3, pp. 423–430, 1997.
Printed in Great Britain.

Towards chemical libraries of annonaceous acetogenins

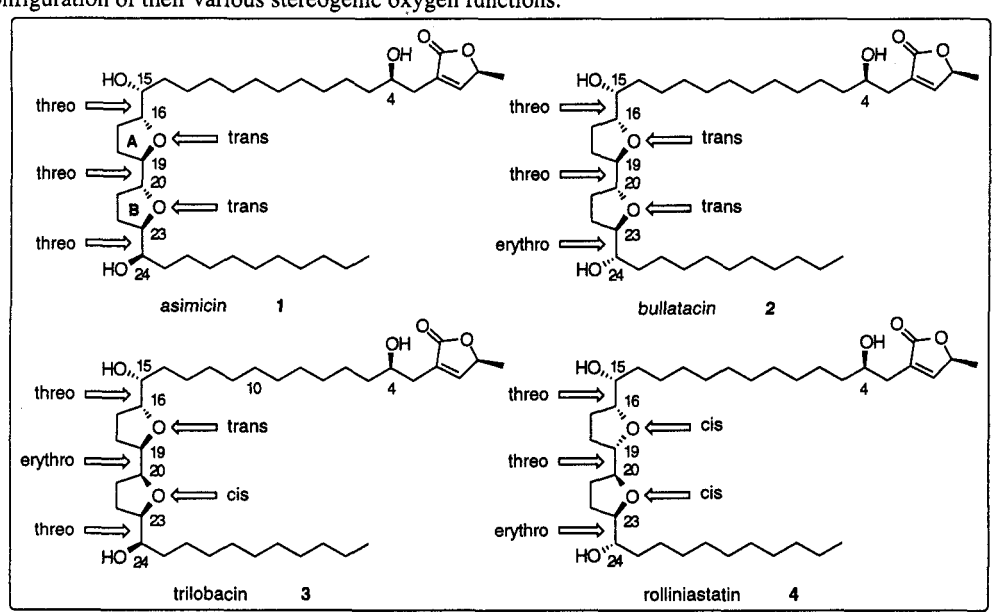
Ehud Keinan,[ab*] Anjana Sinha,[a] Ahmad Yazbak,[b] Santosh C. Sinha[a] and Subhash C. Sinha[a*]

[a]*Department of Molecular Biology and the Skaggs Institute of Chemical Biology, The Scripps Research Institute, 10550 N. Torrey Pines Road, La Jolla, CA 92037, U.S.A. and* [b]*Department of Chemistry, Technion - Israel Institute of Technology, Technion City, Haifa 32000, Israel.*

Abstract: Many of the Annonaceous acetogenins, particularly those containing a bis-THF moiety, exhibit outstanding cytotoxicity and pesticidal activity. Their remarkable structural diversity suggests that a complete chemical library of these compounds should be prepared and systematically screened. We show here several approaches to meet this synthetic challenge using the naked carbon skeleton strategy as well as a convergent synthesis. The key transformations include the Sharpless asymmetric dihydroxylation reaction, the Mitsunobu inversion of alcohols and ligand-assisted chirality transfer methods based on rhenium and vanadium oxides. These approaches provide an easy access to naturally occurring acetogenins (e.g. asimicin, bullatacin, trilobacin, rolliniastatin and solamin) as well as the non-natural isomers.

Introduction

To date, more than 230 different acetogenins have been isolated from 26 plants of the Annonaceae.[1] Many of these Annonaceous acetogenins have exhibited remarkable cytotoxic, antitumor, antimalarial, immunosuppressive, pesticidal and antifeedant activities.[2] For example, studies with human solid-tumor cell-lines show that some of the bis-THF derivatives, such as compounds **1-4** (Scheme 1), are many orders of magnitude more cytotoxic than adriamycin.[3] Interestingly, these fatty acid derivatives share very similar carbon skeletons, with their striking diversity originating mainly from the relative and absolute configuration of their various stereogenic oxygen functions.



Scheme 1: Adjacent bis-THF Annonaceous acetogenins.

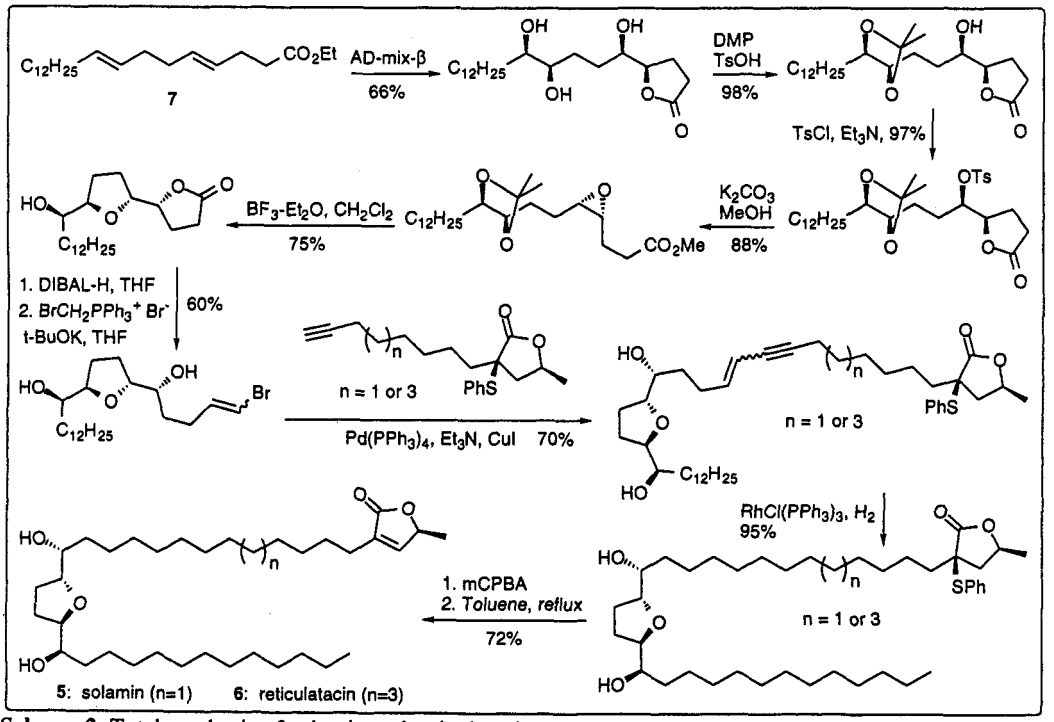
Taking in consideration that the number of plants within the Annonaceae family exceeds 2300, one may conclude that isolation and full characterization of the entire naturally occurring repertoire of the Annonaceous acetogenins will require a formidable effort. The urgent need for a comprehensive biological screening of such compounds led us to develop combinatorial synthetic approaches that will generate a complete chemical library of isomeric acetogenins.[4]

A dominant structural feature that appears in more than 40% of the known Annonaceous acetogenins, particularly in those showing the highest anti-tumor activity, is a linear ten-carbon skeleton (i.e. carbons 15-24 in **1**-**4**) that comprises two adjacent tetrahydrofuran rings flanked by two hydroxyl groups. Having six stereogenic carbinol centers, this unit alone may appear in the form of as many as 64 stereoisomers. To date, only four different diastereomers of this fragment have been identified in the naturally occurring bis-THF acetogenins. Here we present an efficient methodology to engender the complete 64-member library.

The "naked carbon skeleton" strategy

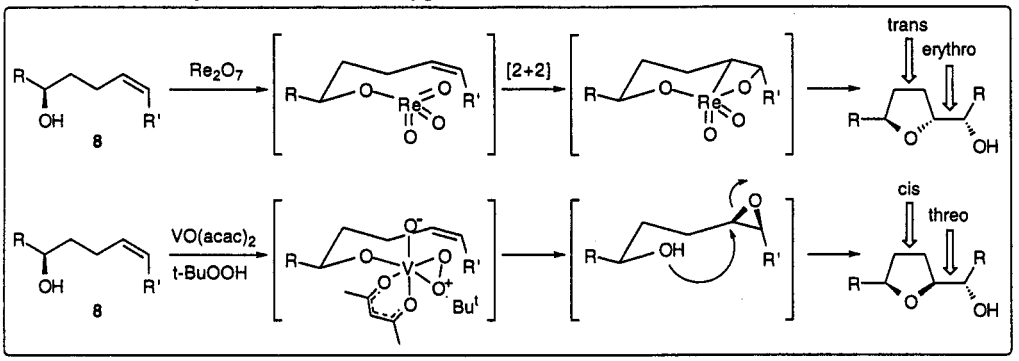
The commonly practiced approach to the synthesis of naturally occurring polyoxygenated asymmetric carbon skeletons borrows the required asymmetric centers from natural products,[5] particularly from sugar molecules.[6] Recently developed methods for the enantioselective functionalization of simple olefins, such as the Sharpless asymmetric dihydroxylation (AD) reaction,[7] offer an alternative synthetic strategy. The easy preparation of the non-functionalized ("naked") carbon skeleton of the target molecule represents the first stage of this approach. All the required stereogenic carbinol centers are then introduced onto this partially unsaturated hydrocarbon chain. We have recently demonstrated the advantages of the "naked carbon skeleton" strategy in the asymmetric total synthesis of (+)-aspicilin,[8] antibiotic A26771B,[9] the WCR pheromone,[9] as well as various Annonaceous acetogenins.[10,11,12] For example, asymmetric synthesis of the 18-membered macrolide (+)-aspicilin was achieved in 14 steps and 11% overall yield from hexadeca-1,3,15-triene. All the required stereogenic carbinol centers were introduced onto this partially unsaturated hydrocarbon chain using the AD reaction.[8]

Solamin, **5**, and reticulatacin, **6**, are two members of the mono-THF subgroup of the Annonaceous acetogenins. All four carbinol centers in the synthesis of both compounds were obtained in a single AD step, starting with ethyl (E,E)-uneicosa-4,8-dienoate, **7** (Scheme 2).[10] Ring closure of the resultant (R,R,R,R)-tetrol into a tetrahydrofuran ring with overall retention of configuration at all centers was achieved with double inversion at position 5.



Scheme 2: Total synthesis of solamin and reticulatacin.

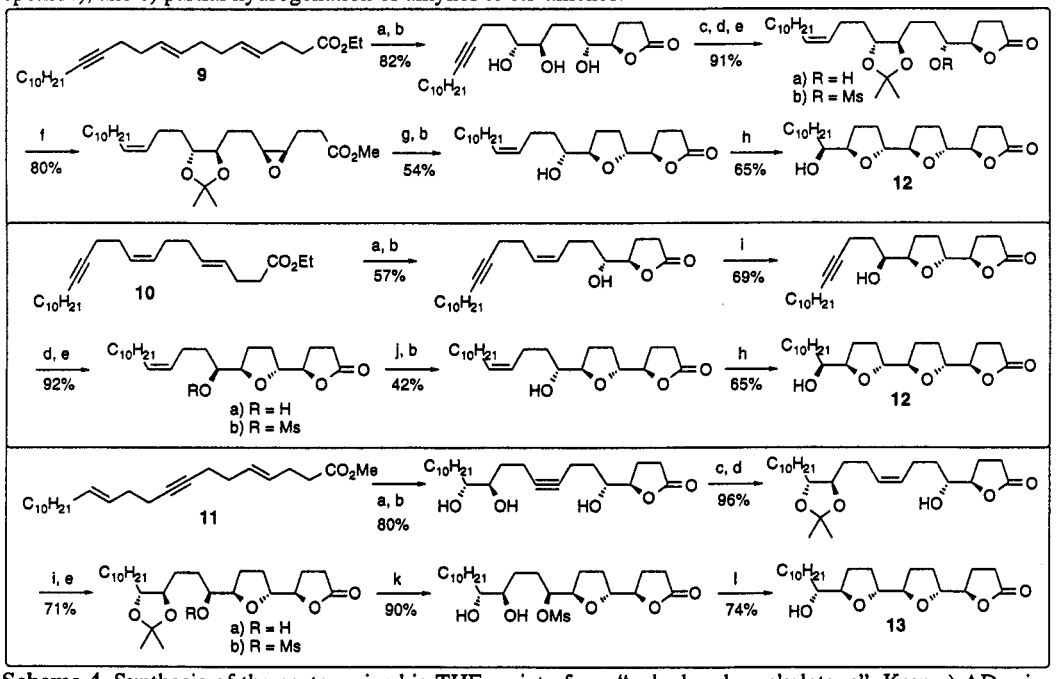
Merging the high enantioselectivity of the AD reaction with the stereospecificity of the Mitsunobu inversion of alcohols[13] together with the highly stereoselective chirality transfer reactions renders the naked carbon skeleton strategy the method of choice for asymmetric synthesis of polyoxygenated aliphatic structures directly from achiral polyenes. The proposed mechanisms of two such chirality transfer reactions leading to chiral THF derivatives are depicted in Scheme 3. The Kennedy oxidative-cyclization with Re_2O_7, produces the *trans*-THF ring with erythro stereochemistry at the two vicinal carbinol centers.[14] Conversely, the oxidative cyclization process with $VO(acac)_2$ produces the *cis*-THF ring with threo stereochemistry at the two vicinal oxygen functions.[15]



Scheme 3: Proposed mechanisms of chirality transfer via ligand-assisted alkene oxidation reactions.

We have shown, using a 4,8-dienol substrate, that two consecutive oxidative cyclizations with dirhenium heptoxide can be carried out in a single step to produce a bis-tetrahydrofuran product.[11] Yet, the reaction may be stopped after the first cyclization to give the mono-tetrahydrofuran derivative.

The synthesis of the two diastereomers, **12** and **13**, (Scheme 4) was achieved starting with a variety of non-functionalized skeletons, **9-11**,[12] and applying various combinations of the following five transformations: a) the AD reaction, b) oxidative cyclization with dirhenium heptoxide, c) Mitsunobu alcohol epimerization, d) nucleophilic ring closure onto an activated oxygen function (e.g. mesylate or epoxide), and e) partial hydrogenation of alkynes to *cis*-alkenes.



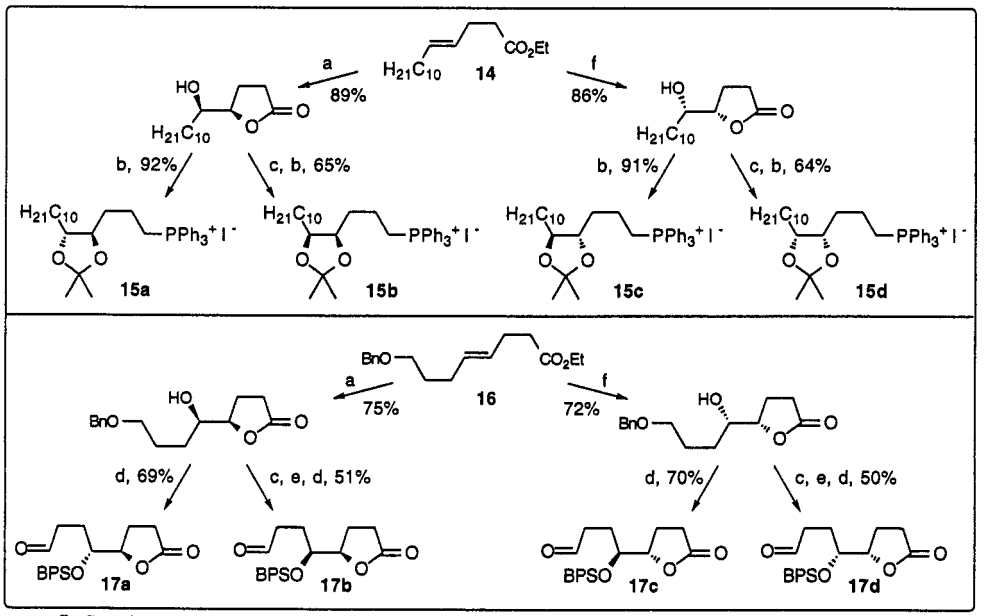
Scheme 4. Synthesis of the acetogenins bis-THF moiety from "naked carbon skeletons". Key: a) AD-mix-β, $MeSO_2NH_2$, *t*-butanol:water. b) i. aq. KOH then 3N HCl; ii. TsOH (5%), CH_2Cl_2. c) Dimethoxypropane, acetone, TsOH, (cat). d) 5% Pd/$CaCO_3$/lead, hexane/cyclohexene/Et_3N. e) MsCl, Et_3N, CH_2Cl_2. f) K_2CO_3 MeOH. g) $BF_3.Et_2O$, CH_2Cl_2. h) Re_2O_7, H_5IO_6, CH_2Cl_2. i) Re_2O_7, lutidine, CH_2Cl_2. j) Cesium propionate, DMF. k) TsOH, MeOH/H_2O (4:1). l) pyridine, 100°C.

The versatility of this modular synthetic approach stems from the ability to choose an appropriate starting carbon framework, induction of asymmetry using the suitable AD reagent and selection of the reaction sequence. Since the molecular asymmetry in this strategy originates from the AD reaction we employ it as the first transformation in the synthetic scheme. The relative stereochemistry of all the remnant stereogenic centers is determined by the sequence of the other four reactions.

Library via a convergent synthesis

One way to achieve the library of 64 stereoisomers is to combine the above mentioned principles with the advantages of convergent synthesis. Based on retrosynthetic analysis we prepared two fragments, each containing two stereogenic centers, one is a phosphonium salt and the other is an aldehyde. One fragment contains stereogenic centers 23 and 24, while the other comprises centers 15 and 16, referring to the structures **1-4**. Since each fragment exists as four stereoisomers, their combinatorial coupling by Wittig olefination should give rise to 16 different stereoisomeric alkenes. Enantioselective dihydroxylation of the latter (in all four possible stereochemical ways) should produce the complete 64-member library.

The synthesis of phosphonium salts **15a-d** and aldehydes **17a-d** was achieved using the AD reaction with alkenes **14** and **16**, respectively (Scheme 5). In both cases, positioning of an ester function at the vicinity of a double bond allowed regioselective differentiation of the resultant hydroxyl groups via selective lactonization.[16] The Mitsunobu inversion of the free alcohol, leads to all four stereoisomers in each case.



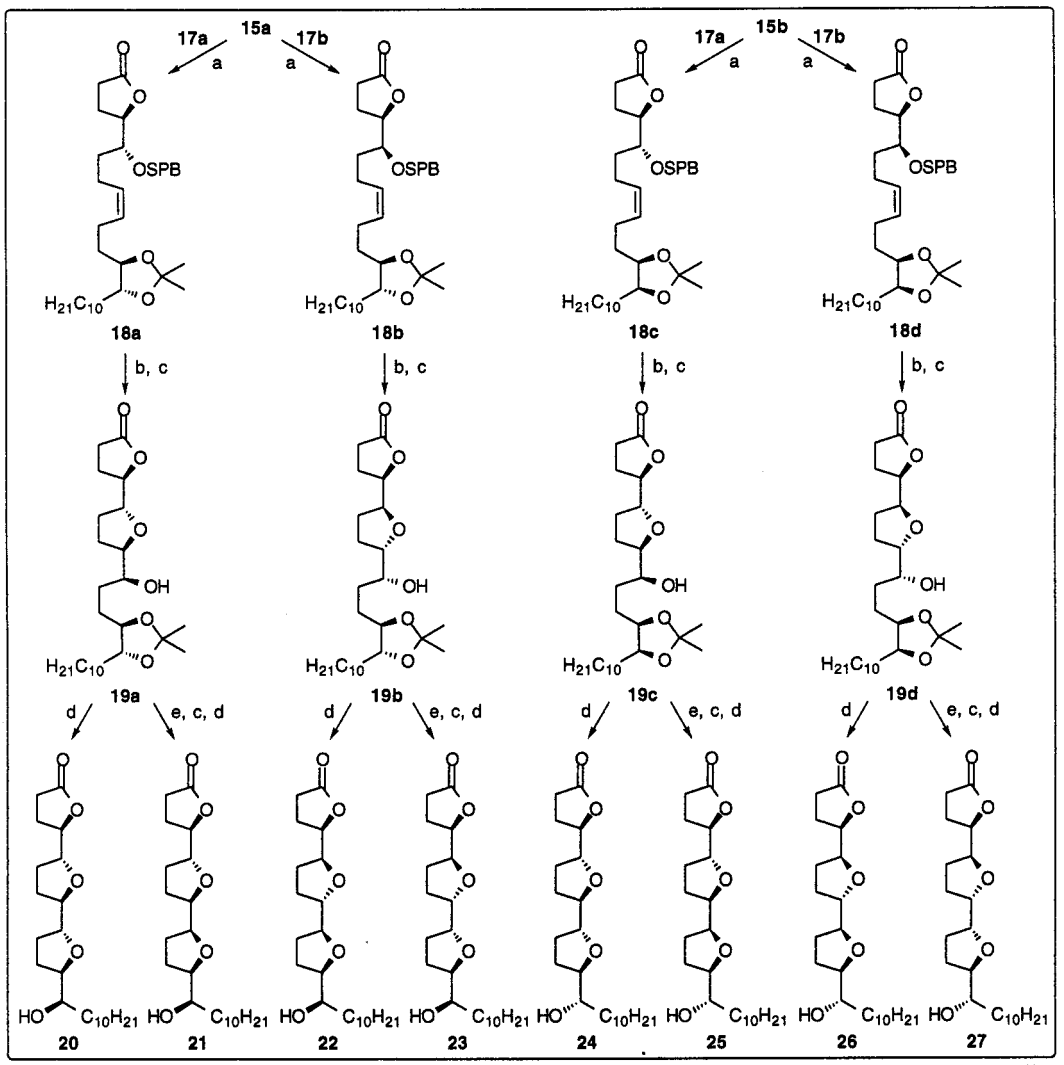
Scheme 5: Synthesis of chiral building blocks for acetogenins libraries: Key: a) i. AD-mix-β, methanesulfonamide, *t*-BuOH-H_2O. ii. aq. KOH, MeOH then 3N HCl. iii. TsOH, CH_2Cl_2. b) i. LAH, Et_2O-THF. ii. acetone, TsOH, C_6H_6. iii. I_2, PPh_3, imidazole, toluene. iv. PPh_3, $NaHCO_3$, CH_3CN, reflux. c) *p*-nitrobenzoic acid, PPh_3, DEAD, C_6H_6. d) i. BPSCl, imidazole, DMF. ii. H_2, Pd-C (10%), MeOH, propionic acid. iii. PCC, celite, CH_2Cl_2. e) i. aq. KOH, EtOH, reflux, then 3N HCl. ii. TsOH, CH_2Cl_2. f) i. AD-mix-α, methanesulfonamide, *t*-BuOH, H_2O. ii. aq. KOH, MeOH then 3N HCl. iii. TsOH, CH_2Cl_2.

Coupling of all four Wittig reagents derived from **15a-d** with the four aldehydes **17a-d** yields, in principle, 16 stereoisomeric Z-alkenes. This strategy is demonstrated here by the synthesis of four such alkenes, **18a-d** (Scheme 6). Oxidative cyclization of **18a-d** with Re_2O_7/lutidine affords the corresponding *trans*-substituted tetrahydrofurans, **19a-d**. Conversion of **19a** to the corresponding mesylate followed by acid-catalyzed acetonide-cleavage and ring closure produces the tricyclic lactone **20**. Alternatively, Mitsunobu inversion of the free alcohol's configuration within **19a** prior to its activation and ring-closure, affords lactone **21**. These two alternatives represent an additional branching point in the synthetic scheme, providing an opportunity to double the number of the final stereoisomeric products. Similarly to **20** and **21**, isomers **22** and **23** were obtained from **19b**, isomers **24-25** from **19c**, and isomers **26-27** from **19d**.

Since products **24-27** are epimers of **20-23** at the free hydroxyl position, their respective interconversion is possible via the Mitsunobu reaction. We have confirmed this alternative pathway by the synthesis of

diastereomers **24** and **25** from **20** and **21**, respectively. Altogether, by using two diastereomeric phosphonium salts, **15a-b** and two diastereomeric aldehydes, **17a-b** we have synthesized eight diastereomers of the desired tricyclic intermediates, **20-27**. Consequently, combinatorial coupling of all four phosphonium salts **15a-d** with all four aldehydes **17a-d** following the same synthetic approach should produce a library of 32 out of 64 possible stereoisomers of the bis-THF segment of the acetogenins.

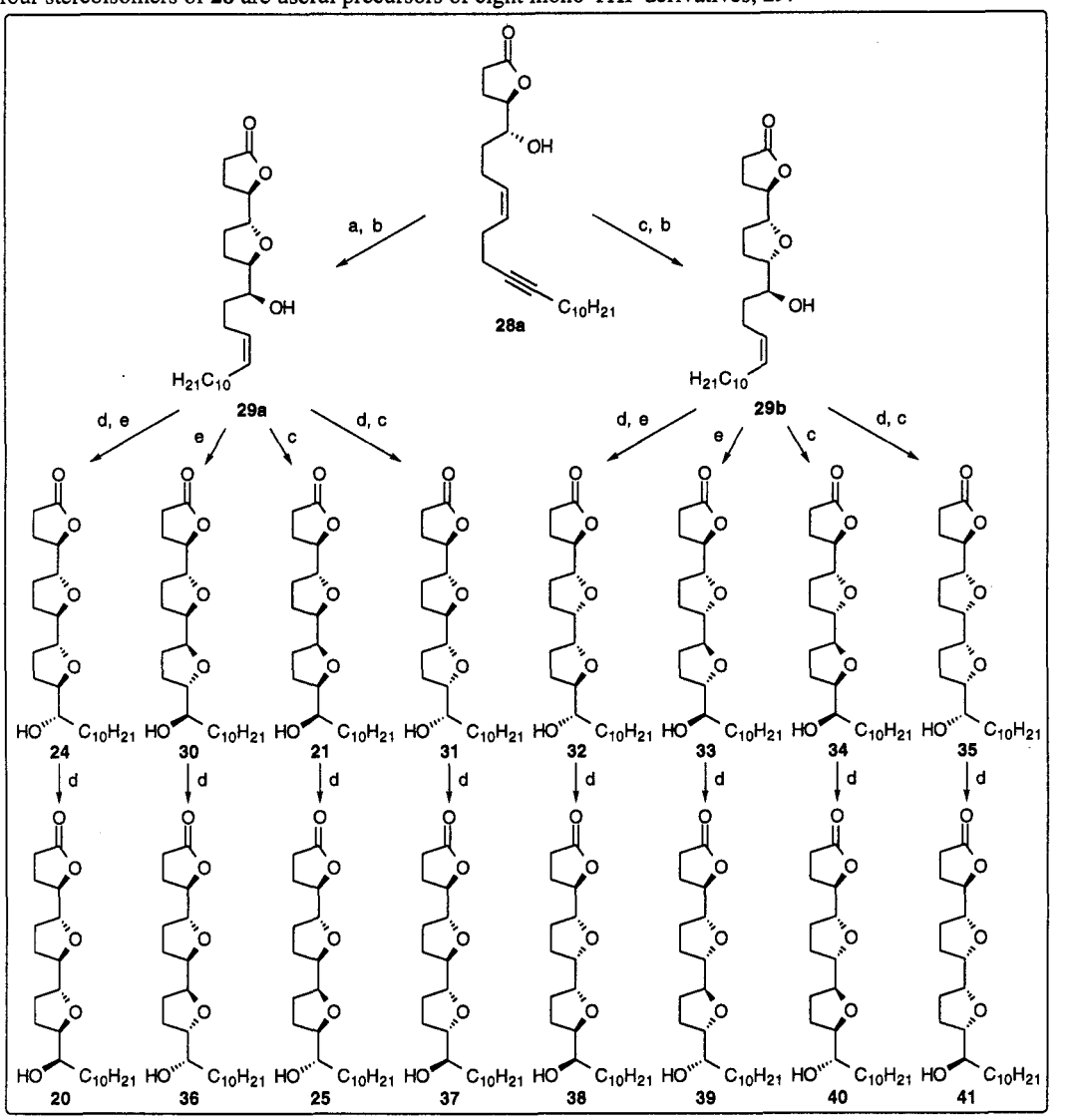
As illustrated in Scheme 6, the library of stereoisomeric intermediates which may be prepared from the 16 diastereomeric olefins **18** is limited to acetogenins with *trans* geometry at the THF ring A (as is the case with asimicin, **1**, bullatacin, **2**, and trilobacin, **3**, Scheme 1). This is the result of using Re_2O_7 in the oxidative cyclization reaction. Nevertheless, this limitation could be avoided by carrying out the oxidative cyclization reaction with $VO(acac)_2$. This reaction, which proceeds via epoxidation followed by ring closure, is known to produce *cis*-THF rings (Scheme 3).[14] Thus, the employment of $VO(acac)_2$ instead of Re_2O_7 should yield, in principle, the remaining 32 stereoisomers in which ring A has *cis* geometry (as appears in rolliniastatin, **4**). Although in simple cases this reaction is known to proceed with 80-90% stereoselectivity, with substrates **18a-d** we obtained the desired *cis*-THF product with less than 65% selectivity.



Scheme 6: Key: a) i. $KN(SiMe_3)_2$, THF, then aldehyde **7a** or **7b** in THF-HMPA. b) i. TBAF, THF. ii. Re_2O_7, lutidine, CH_2Cl_2. c) dimethoxypropane, acetone, TsOH. d) i. MsCl, Et_3N, CH_2Cl_2, ii. TsOH, MeOH-H_2O. iii. pyridine, 100 °C. e) i. *p*-nitrobenzoic acid, PPh_3, DEAD, C_6H_6, ii. aq. KOH, EtOH, reflux, then 3N HCl. iii. TsOH, CH_2Cl_2.

Library via the naked carbon skeleton approach

Since the convergent synthetic approach proceeds with satisfactory selectivity for the preparation of only 32 members of the full library we turned to the naked carbon skeleton strategy for the construction of the entire 64-member acetogenin library (Scheme 7).[17] The stereoisomeric *cis* alkenes substrates, **28a-d**, were prepared by Wittig reaction of aldehydes **17a-d** with pentadec-4-ynyltriphenylphosphorane. The advantage in using substrates **28** arises from the lack of extra coordination sites for vanadium that could interfere with the desired ligand-assisted epoxidation reaction, lowering its selectivity (as is probably the case with the more functionalized substrates **18**). Indeed, treatment of alcohol **28a** with $VO(acac)_2$/TBHP produced the *cis*-THF product **29b** with 90% selectivity, as is usually observed with simple model systems. Alternatively, treatment of the same substrate with dirhenium heptoxide afforded the *trans*-THF isomer, **29a**. These high selectivities were reproduced in the reactions of **28b** (not shown, obtained from aldehyde **17b**) with either Re_2O_7 or $VO(acac)_2$/TBHP to give **29c** and **29d** (not shown), respectively. Overall, the four stereoisomers of **28** are useful precursors of eight mono-THF derivatives, **29**.



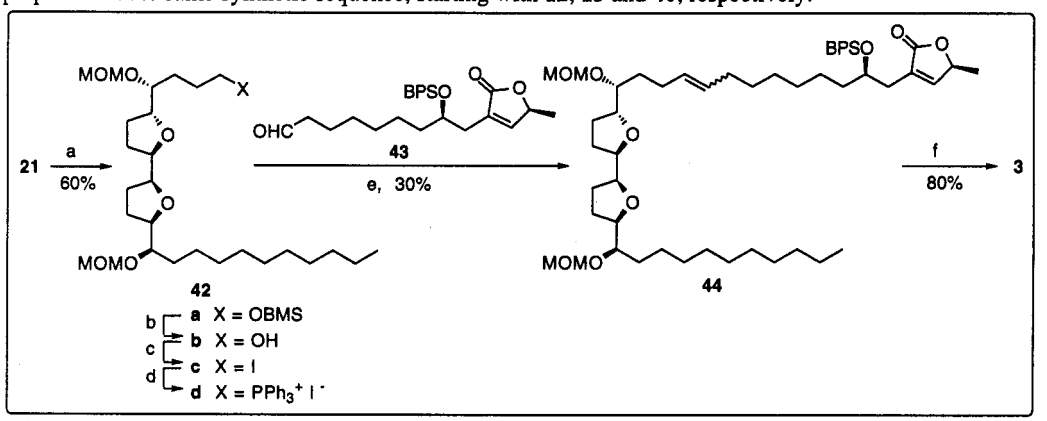
Scheme 7: a) Re_2O_7, lutidine, CH_2Cl_2, b) H_2, Pd/$CaCO_3$/Pb, hexane, Et_3N, c) $VO(acac)_2$, TBHP, CH_2Cl_2, d) i. 4-nitrobenzoic acid, PPh_3, DEAD, C_6H_6, ii. aq. KOH, MeOH, then 3N HCl, iii. TsOH, CH_2Cl_2, e) Re_2O_7, H_5IO_6, CH_2Cl_2.

Each one of the alkenes **29** can be converted to four bis-THF stereoisomers by using four different reaction conditions: For example, isomers **24**, **30**, **21** and **31** are prepared with high selectivity from **29a** using the

following four reaction conditions, respectively: a) Mitsunobu reaction followed by Re_2O_7, b) Oxidative cyclization with Re_2O_7, c) oxidative cyclization with $VO(acac)_2$/TBHP and d) Mitsunobu reaction followed by reaction with $VO(acac)_2$/TBHP. Similarly, compounds **32**, **33**, **34**, and **35** were prepared from **29b** using the same reactions: a), b), c), and d), respectively. Mitsunobu inversion of the alcohol configuration in the latter provided another set of eight stereoisomers, **20**, **25**, **36-41**. Overall, since each of the four starting materials **28a-d** can lead to 16 stereoisomers, this approach may produce the complete 64-member library of the bis-THF acetogenins.

Closing the acetogenins synthesis

With the bis-THF core in hand we could complete the synthesis of the complete 64-member library of the bis-THF acetogenins. We have demonstrated the feasibility of this synthetic approach in the preparation of the four naturally occurring acetogenins **1-4** by attachment of the butenolide fragment to the appropriate bis-THF intermediates (Scheme 8).[12,17,18] For example, $LiAlH_4$ reduction of **21** produced the corresponding triol in which the primary alcohol and the two secondary alcohols were protected as *t*-butyldimethylsilyl ether and methoxymethyl ethers, respectively, affording **42a**. The primary alcohol was then deprotected with tetrabutylammonium fluoride, converted to iodide **42c** and then transformed to the corresponding phosphonium salt **42d**. The latter was reacted with the aldehyde **43**[18] to produce the alkene **44**. Finally, homogeneous hydrogenation over Wilkinson's catalyst followed by acidic cleavage of the MOM ethers produced trilobacin, **3**.[3] Asimicin, **1**,[19] bullatacin, **2**,[20] and rolliniastatin, **4**[21] were prepared via the same synthetic sequence, starting with **12**, **13** and **40**, respectively.



Scheme 8: Key: a) i. LAH, Et_2O-THF, 0 °C to reflux, 2 h, ii. BMSCl, diisopropylethylamine, DMAP, CH_2Cl_2, rt, 12 h, then MOMCl, 12 h. b) TBAF, THF, 0 °C to rt, 1 h. c) PPh_3, I_2, imidazole, CH_2Cl_2, rt, 1 h. d) PPh_3, $NaHCO_3$, CH_3CN, 45 °C, 24 h. e) *n*-BuLi, THF, 0 °C, 0.5 h, then aldehyde **31** in THF 0 °C to rt, 1 h. f) i. $Rh(PPh_3)_3Cl$ (10%) C_6H_6, EtOH, H_2, rt, 2 h. ii. acetyl chloride, MeOH, ether, reflux, 6 h.

Conclusions

We have shown here two approaches to construct chemical libraries of Annonaceous acetogenins, particularly those belong to the bis-THF subgroup. Naturally occurring members of this family have exhibited remarkable cytotoxic and pesticidal activities. The advantages of the "naked carbon skeleton" strategy and the convergent synthesis have been demonstrated by the total synthesis of naturally occurring acetogenins (asimicin, bullatacin, trilobacin, rolliniastatin, solamin and reticulatacin) as well as the main fragment of many non-natural stereoisomers. The key transformations employed include the Sharpless asymmetric dihydroxylation reaction, the Mitsunobu inversion of alcohols and ligand-assisted chirality transfer methods based on rhenium and vanadium oxides. Systematic biological screening of the synthetic libraries will be reported in due course.

Acknowledgment: We thank the US-Israel Binational Science Foundation, the Israel Cancer Research Fund and PharMore Therapeutics Ltd. for financial support.

References

1. a) Rupprecht, J.K.; Hui, Y.-H.; McLaughlin, J.L. *J. Nat. Prod.* **53**, 237 (1990). b) Fang, X.-P.; Rieser, M.J.; Gu, Z.-M.; Zhao, G.-X.; McLaughlin, J.L. *Phytochem. Anal.* **4**, 27 (1993). c) Figadère, B. *Acc. Chem. Res.* **28**, 359 (1995). d) Koert, U. *Synthesis*, 115 (1995). d) Hoppe, R.; Scharf, H.-D. *Synthesis*, 1447 (1995). e) Zeng, L.; Zhang, Y.; McLaughlin, J.L. *Tetrahedron Lett.* **37**, 5449 (1996) and references cited therein.

2. 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, **29**, 249-310 (1995).
3. a) Zhao, G.-X.; Hui, Y.-H.; Rupprecht, J.K.; McLaughlin J.L.; Wood, K.V. *J. Nat. Prod.* **55**, 347 (1992). b) Zhao, G.-X.; Gu, Z.-M.; Zeng, L.; Chao, J.-F.; Kozlowski, J.F. Wood, K.V.; McLaughlin J.L. *Tetrahedron* **51**, 7149 (1995) and references cited therein.
4. For other synthetic approaches to bis-THF annonaceous acetogenins see: a) Hoye, T.R.; Ye, Z. *J. Am. Chem. Soc.* **118**, 1801 (1996). b) Hoye, T.R.; Tan, L. *Tetrahedron Lett.* **36**, 1981 (1995). c) Naito, H.; Kawahara, E.; Maruta, K.; Maeda, M.; Sasaki, S. *J.Org. Chem.* **60**, 4419 (1995). d) Koert, U.*Tetrahedron Lett.* **35**, 2517 (1994). e) Hoye, T.R.; Hanson, P.R. *Tetrahedron Lett.* **34**, 5043 (1993). f) Hoye, T.R.; Hanson, P.R.; Kovelesky, A.C.; Ocain, T.D.; Zhang, Z. *J. Amer. Chem. Soc.* **118**, 1801 (1991).
5. Seebach, D.; Hungerbühler, E. in "Modern Synthetic Methods", Scheffold, R. Ed., Salle & Sauerländer, Frankfurt/Aarau, 1980, vol. 2, 91.
6. S. Hanessian, "Total synthesis of natural products: the 'chiron' approach", Pergamon Press, Oxford (1983).
7. Kolb, H.C.; VanNieuwenhze, M.S.; Sharpless, K.B. *Chem. Rev.* **94**, 2483 (1994).
8. a) Sinha, S.C; Keinan, E. *J. Org. Chem.* **59**, 949 (1994). b) Sinha, S.C; Keinan, E. *J. Org. Chem.* (1996), submitted for publication.
9. Sinha, S.C; Sinha-Bagchi, A.; Keinan, E. *J. Org. Chem.* **58**, 7789 (1993).
10. Sinha, S.C; Keinan, E. *J. Am. Chem. Soc.* **115**, 4891 (1993).
11. Sinha, S.C; Sinha-Bagchi, A.; Keinan, E. *J. Am. Chem. Soc.* **117**, 1447 (1995).
12. Sinha, S.C.; Sinha-Bagchi, A.; Yazbak, A.; Keinan, E. *Tetrahedron Lett.* **36**, 9257 (1995).
13. Mitsunobu, O. *Synthesis* 1 (1981).
14. a) Tang, S.; Kennedy, R.M. *Tetrahedron Lett.* **33**, 3729 (1992). b) Tang, S.; Kennedy, R.M. *Tetrahedron Lett.* **33**, 5299 (1992). c) Tang, S.; Kennedy, R.M. *Tetrahedron Lett.* **33**, 5303 (1992). d) Boyce, R.S.; Kennedy, R.M. *Tetrahedron Lett.* **35**, 5133 (1994). e) McDonald, F.E.; Towne, T.B. *J. Org. Chem.* **1995**, *60*, 5750.
15. For the mechanism of vanadium catalyzed epoxidation of allylic alcohols see: a) Chong, A.O.; Sharpless, K.B. *J. Org. Chem.* **42**, 1587 (1977). For the synthesis of *cis*-THF from bishomoallylic alcohols see: b) Fukuyama, T.; Vranesic, B.; Negri, D.P.; Kishi, Y. *Tetrahedron Lett.*, 2741 (1978). c) Ujihara, K.; Shirahama, H. *Tetrahedron Lett.* **1996**, *31*, 2741 and references cited therein.
16. Monoprotection of a vicinal diol in the form of a γ-lactone has been used in the synthesis of other natural products. See: a) ref. 10-12. b) Wang, Z.-M.; Zhang, X.-L.; Sharpless, K. B.; Sinha, S. C.; Sinha-Bagchi, A.; Keinan, E. *Tetrahedron Lett.* **33**, 6407 (1992). c) Keinan, E.; Sinha, S.C.; Sinha-Bagchi, A.; Wang, Z.-M.; Zhang, X.-L.; Sharpless, K.B. *Tetrahedron Lett.* **33**, 6411 (1992). d) Sinha-Bagchi, A.; Sinha, S.C.; Keinan, E. *Tetrahedron Asymmet.* **6**, 2889 (1995).
17. Sinha, S.C.; Sinha, A.; Yazbak, A.; Sinha, S.C.; Keinan, E., manuscript in preparation.
18. Sinha, S.C.; Sinha, A.; Yazbak, A.; Keinan, E. *J. Org. Chem.,* submitted for publication.
19. Rupprecht, J.K.; Chang, C.-J.; Cassady, J.M.; McLaughlin, J.L. *Heterocycles*, **24**, 1197 (1986).
20. a) Hui, Y.-H.; Rupprecht, J.K.; Liu, Y.-M.; Anderson, J.E.; Smith, D.L.; Chang, C.-J.; McLaughlin, J.L. *J. Nat. Prod.* **52**, 463 (1989). b) Li, X.-H.; Hui, Y.-H.; Rupprecht, J.K.; Liu, Y.-M.; Wood, K.V.; Smith, D.L.; Chang, C.-J.; McLaughlin, J.L. *J. Nat. Prod.* **53**, 81 (1990).
21. Pettit, G.R.; Cragg, G.M.; Polonsky, J.; Herald, D.L.; Goswami, A.; Smith, C.R.; Moretti, C.; Schmidt, J.M.; Weisleder, D. *Can. J. Chem.* **65**, 1433 (1987).