Natural Product Analogues

Parallel Fragment Assembly Strategy Towards Multiple-Ether Mimicry of Anticancer Annonaceous Acetogenins**

*Sheng Jiang, Yan Li, Xiao-Guang Chen, Tai-Shan Hu, Yu-Lin Wu, and Zhu-Jun Yao**

The annonaceous acetogenins are naturally occurring polyketides isolated from various species of the plant genus *Annonaceae*. To date, over 400 members of this compound family have been found, most of which have been proven to exhibit high cytotoxic and antitumor activities.[1] The structural features of these acetogenins include several free hydroxy groups with various stereochemistries, variously located oxygenated functional groups, and tetrahydrofuran (THF) ring(s) along the hydrocarbon skeleton. The unique structural features and interesting biological activities of these compounds have rendered them attractive targets for the development of efficient syntheses of both the natural products and functional mimetics.[2,3] In nature, annonaceous acetogenins are believed to block oxidative pathways in complex I in mitochondria,[4] and there is evidence that some members of the family induce apoptosis in cancer cells.[5] In 1994, a group of Japanese chemists first reported NMR studies showing that annonaceous acetogenins can coordinate a calcium cation.[6] Their results prompted us to investigate the biological importance of this property and how it relates to the reported activities of these compounds. On first inspection, the THF ring(s) and flanking hydroxy groups are the structural components most likely to interact with metal cations. Over the past several years, we have successfully developed a series of annonaceous acetogenin mimetics[7–11] in which the THF rings and flanking hydroxy groups have been replaced by an ethylene glycol ether unit. These analogues with simplified structures have proved to be as active as the natural products. More interestingly, we have found that some of these analogues have much better selectivity between

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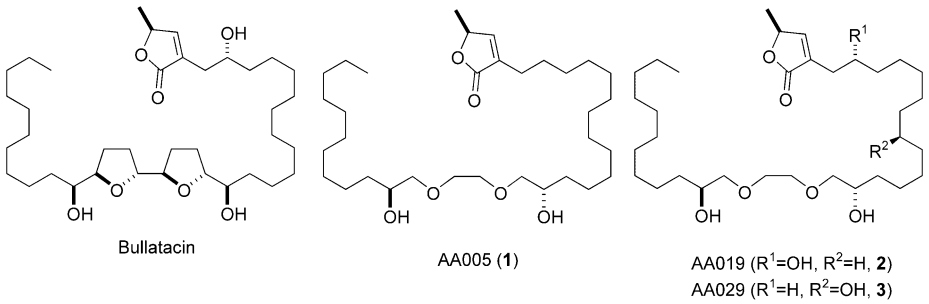
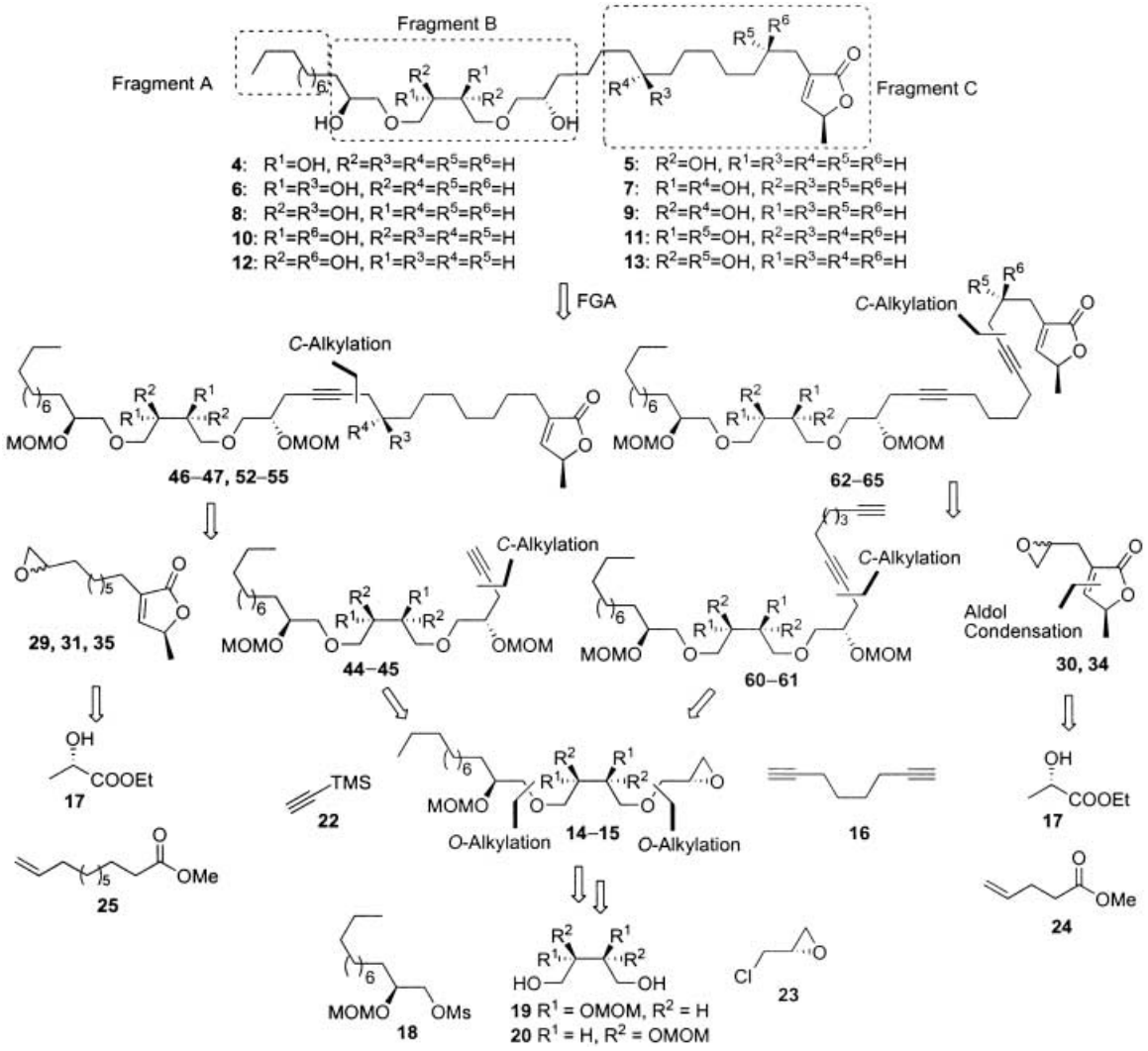
human cancer cells and normal cells.[8] Such selectivity is very difficult to achieve with the naturally occurring counterparts.

In our previous work, bullatacin, a typical acetogenin and one of the most potent, was selected as a template for mimetic design.[12] The first generation of mimetics was obtained by simple modification of the THF region of the molecules and by random syntheses. Individual screening revealed that AA005 (**1**; Scheme 1) is a better agent than bullatacin against several human cancers cells,[9] such as HT-29 and HCT-8. We also recently confirmed that AA005 interacts with the same biological target as do the natural products.[10] A further investigation showed that introduction of a (4*R*)-hydroxy group into AA005 (**1**) raises the potency by up to 15 times (AA019, **2**).[11] Introduction of a (10*R*)-hydroxy group into the AA005 skeleton (AA029, **3**) had little effect on the activity.[8] To accelerate progress in the search for more potent and selective anticancer agents with such modifications and to obtain clearer information on the structure–activity relationship (SAR) of the acetogenins from these mimetics, a systematic organic synthesis strategy involving parallel fragment assembly was developed. By using this strategy, small, focused libraries could be set up efficiently (Scheme 2; these compounds are designated as second-



Scheme 1. Bullatacin and three typical mimetics **1**–**3** produced by random synthesis.



Scheme 2. General fragmentation and retrosynthetic analyses for the acetogenin mimetics **4**–**13**. FGA = functional group addition, MOM = methoxymethyl, TMS = trimethylsilyl, OMs = mesylate.

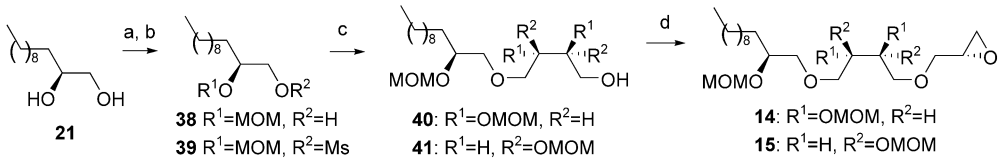
generation mimetics).[13] Herein, we report our initial results from this approach, by which we have synthesized a new class of mimetics. Several of these analogues exhibit good-to-excellent anticancer activity in the low micromolar range.

As shown in Scheme 2, a typical mimetic structure can be broken down into three major fragments A, B, and C, each of which can be synthesized individually or purchased from commercial sources. Assembly of these fragments can be achieved by convergent syntheses. By varying the combination of fragments, ten analogues of the annonaceous acetogenins were obtained by parallel assembly of prefunctionalized units (Scheme 2). The most structurally significant modification in these compounds was made in the middle region (Fragment B), where an L- or D-threotol unit was introduced. In addition, a position-shifting and configuration-variable hydroxy group was introduced into the right-hand portion of the structure (Fragment C).

A retrosynthetic analysis of **4**–**13** is shown in Scheme 2. After a standard FGA treatment of these targets, a key step involved two directional *C*-alkylations of 1,7-octadiyne **16** or trimethylsilylacetylene (**22**) with epoxides **14** or **15** and **29**, **31**, or **35**, or **30** or **34**. To form the ether bonds of the common intermediates **14** and **15**, two successive directional *O*-alkylations were used, which gave precursors **18**, **19** or **20**, and **23**. Compounds **19** and **20** could in turn be prepared easily from L- and D-tartaric acid,[14] respectively. Synthesis of terminal butenolides **30**, **31**, **34**, and **35** could be achieved by a three-step protocol that included an aldol condensation[15] and the hydrolytic kinetic resolution transformation developed by Jacobsen and co-workers.[16]

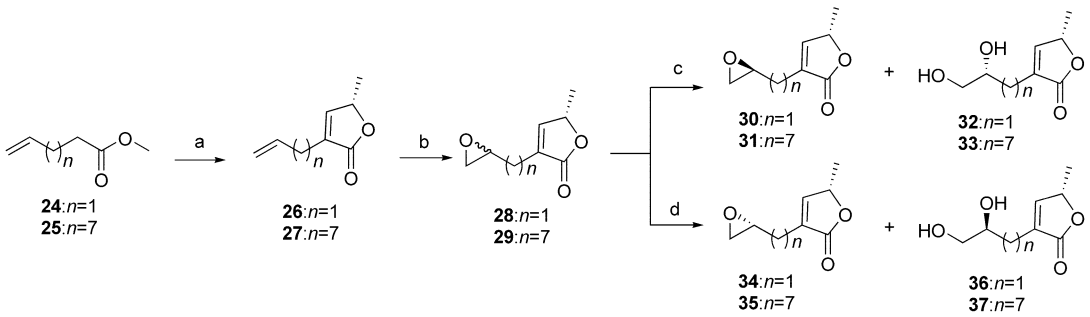
The synthesis of key intermediates **30**, **31**, **34**, and **35** is illustrated in Scheme 3. Compounds **24** and **25** can be prepared easily from the corresponding acids. Appendage of butenolide units onto **24** and **25** to form **26** and **27** involved a three-step sequence: 1) aldol reaction of **24** and **25** with freshly prepared (2*S*)-*O*-tetrahydropyranyl (*O*-THP) lactal, 2) acid-catalyzed deprotection of the *O*-THP group and in situ lactonization, and 3) elimination of a β-hydroxy group by treatment with $(CF_3CO)_2O$ and Et_3N. Regioselective epoxidation of **26** and **27** was achieved by treatment with *m*CPBA to give **28** and **29** in 86% yield. The hydrolytic kinetic resolution of Jacobsen and co-workers was applied to resolve the terminal epoxides.[16b] This reaction was performed in the presence of an (*S*,*S*)-salen–Co(OAc) complex (0.5 mol%) and H_2O (0.55 equiv) to yield **30** and **31** (43%) and diols **32** and **33** (50%) at 4°C. The diastereomeric ratio (d.r.) of **30** and of **31** (>99%) was measured by HPLC, with **28** and **29** as references.[17] Similarly, epoxides **34** and **35** were obtained with high d.r. values (>99%) in the presence of (*R*,*R*)-salen–Co(OAc) (0.5 mol%) and H_2O (0.55 equiv).[17]

The remaining key intermediates **14** and **15** were synthesized as outlined in Scheme 4. Diol **21** was easily prepared from D-mannitol[8] and was regioselectively protected as MOM ether **38** in 90% yield by a two-step procedure.[18] The remaining primary hydroxy group was then masked as a mesylate (**39**, 100% yield). Parallel reaction of mesylate **39** with diols **19** and **20** afforded monoalcohols **40** and **41** in 78% yield. In the presence of a phase-transfer catalyst,[19] further *O*-alkylation of alcohols **40** and **41** by treatment with *R*-(−)-epichlorohydrin **23** led to key intermediates **14** and **15**, respectively, in 78% yield.



Scheme 4. Reagents and conditions: a) 1. $HC(OCH_3)_3$, D-10-camphorsulfonic acid, CH_2Cl_2, 2. diisobutylaluminum hydride in toluene, CH_2Cl_2, 0°C, two steps: 90%. b) MsCl, Et_3N, CH_2Cl_2, 100%. c) **19** or **20** (for **40** and **41**, respectively), NaH, DMF, 130°C, 78%. d) NaOH, H_2O, Bu_4NHSO_4, (*R*)-epichlorohydrin, 78%.

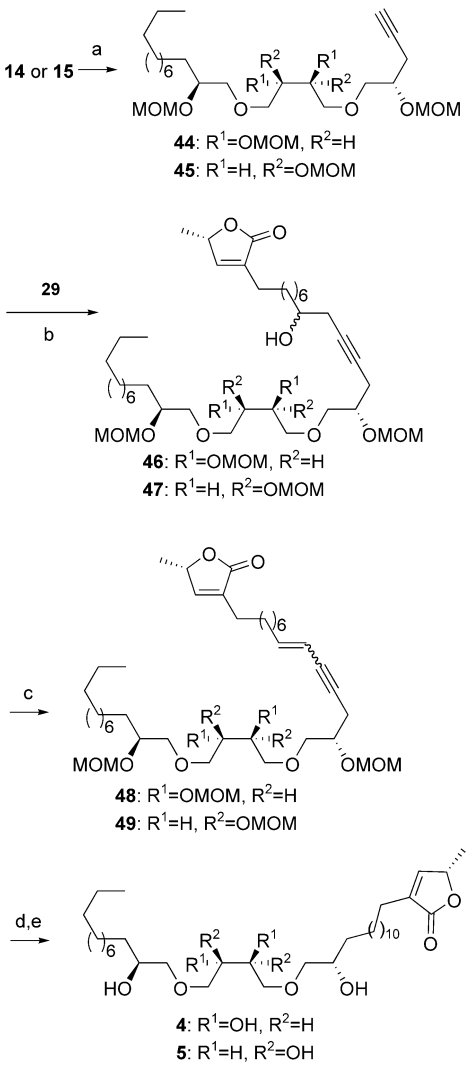
With the functionalized segments described above in hand, a series of parallel assemblies was undertaken that involved the sequential *C*-alkylation of terminal alkynes by the required epoxides. Regioselective opening of epoxides **14** and **15** by treatment with the monolithium salt of trimethylsilylacetylene, followed by protection with MOM and deprotection of the TMS group afforded the "left-hand" segments



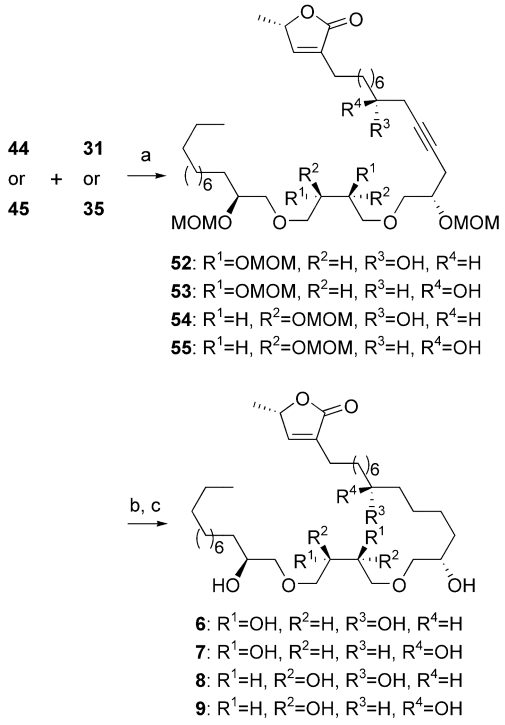
Scheme 3. Reagents and conditions: a) 1. lithium diisopropylamide, THF-hexamethyl phosphoramide, (*S*)-*O*-tetrahydropyranyl lactal, −78°C; 2. 10% H_2SO_4, THF, RT; 3. $(CF_3CO)_2O$, Et_3N, CH_2Cl_2, 60%. b) *meta*-chloroperbenzoic acid (*m*CPBA), CH_2Cl_2, 0°C, 86% yield based on 31% recovery of olefins **26**–**27**. c) (*S*,*S*)-salen–Co(OAc), H_2O, 43% yield for epoxides **30** and **31**. d) (*R*,*R*)-salen–Co(OAc), H_2O, 43% yield for epoxides **34** and **35**.

44 and **45**. Treatment of the terminal alkynes in **44** and **45** with *n*-butyllithium, followed by reaction with epoxide **29** in the presence of BF_3 etherate at −78 °C gave the fully elaborated skeletons **46** and **47** in 70% yield.[20] Elimination of the newly introduced hydroxy groups by treatment with $(CF_3CO)_2O$ and Et_3N (or MsCl and Et_3N) gave **48** and **49**. Selective reduction of the triple and double bonds in the centers of **48** and **49** was achieved by reaction with a diimide. Deprotection of the MOM ethers by treatment with boron trifluoride and dimethylsulfide gave the final products **4** and **5** (Scheme 5).[21]

Treatment of alkynes **44** and **45** with butyllithium and enantiomerically pure epoxides **31** and **35** in different combinations in the presence of BF_3 etherate at −78 °C gave the fully assembled skeletons **52**–**55**. Subsequent selective reduction of the triple bonds and cleavage of the MOM groups in **52**–**55** yielded the final products **6**–**9** (Scheme 6).



Scheme 5. Reagents and conditions: a) 1. *n*BuLi, $BF_3{\cdot}Et_2O$, THF, trimethylsilylacetylene, −78 °C; 2. iPr_2NEt, MOMCl, CH_2Cl_2, 60%. 3. Tetrabutylammonium fluoride, THF, 0 °C, 92%. b) *n*BuLi, $BF_3{\cdot}Et_2O$, THF, −78 °C, 70%. c) $(CF_3CO)_2O$, Et_3N, CH_2Cl_2, 40%, or MsCl, 1,8-diazabicyclo[5.4.0]undec-7-ene, CH_2Cl_2, 60%. d) $TsNHNH_2$, NaOAc, 1,2-dimethoxyethane (DME), reflux, 70%. e) $BF_3{\cdot}Et_2O$, Me_2S, 0 °C, 50%. Ts = tosyl.

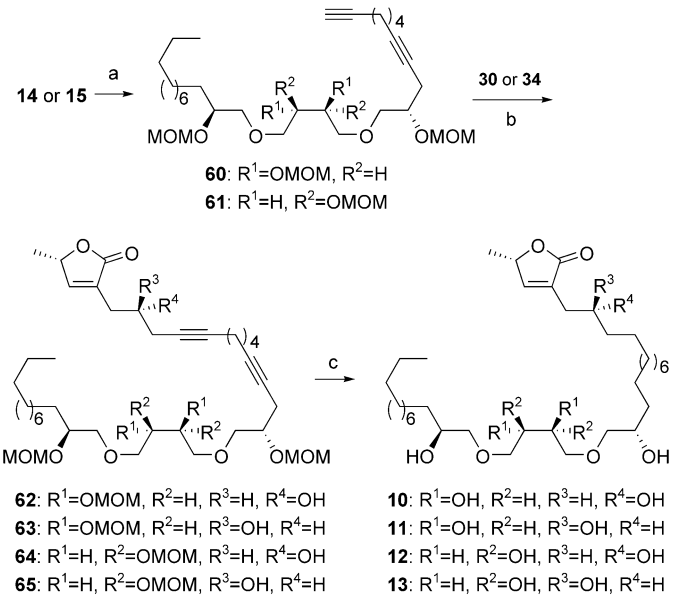


Scheme 6. Reagents and conditions: a) *n*BuLi, $BF_3{\cdot}Et_2O$, THF, −78 °C, 67%. b) $TsNHNH_2$, NaOAc, DME, reflux, 88%. c) $BF_3{\cdot}Et_2O$, Me_2S, 0 °C, 53%.

By analogy to the synthesis described above, reaction of a preformed monolithium salt solution of 1,7-octadiyne (**16**) with epoxides **14** and **15** was performed in parallel in the presence of $BF_3{\cdot}Et_2O$. The newly generated hydroxy group was protected as its MOM ether, which resulted in **60** and **61**. Parallel treatment of **60** and **61** with *n*BuLi, followed by reaction with epoxides **30** and **34** in a variety of combinations afforded **62**–**65**. The triple bonds in **62**–**65** were reduced by treatment with diimide, and deprotection of the MOM ethers afforded targets **10**–**13** (Scheme 7).

Preliminary in vitro measurements gave IC_{50} values in the low micromolar range for the synthetic mimetics (Table 1), except against the Bel-7402 cell line. Compounds **12** and **13** are more potent than **1** against KB cells. It is apparent that activity against Bel-7402 cells is decreased by the introduction of hydroxy groups into the region of the ether bonds in **1**. Such modification improved the selectivity between the KB and Bel-7402 cell lines. This result indicates that the stereochemistry in the vicinity of the ether bonds may provide important contributions to the cytotoxicity towards and selectivity between the various tumor cell lines.

In summary, a new class of multiple-ether-modified analogues (**4**–**13**) of the annonaceous acetogenins was designed based on the structures of mimetics **1** and **2**, and the analogues were synthesized enantioselectively by using a parallel fragment-assembly strategy that involved the combination of prefunctionalized units. This methodology may potentially be applicable to the synthesis of further analogues of this family, as well as other complex, focused libraries. Directional chemo- or regioselective *C*- and *O*-alkylations



Scheme 7. Reagents and conditions: a) 1. **16**, *n*BuLi, $BF_3{\cdot}Et_2O$, THF, −78 °C; 2. *i*Pr_2NEt, MOMCl, CH_2Cl_2, 66% over two steps. b) *n*BuLi, $BF_3{\cdot}Et_2O$, THF, −78 °C, 48%. c) 1. $TsNHNH_2$, NaOAc, DME, reflux; 2. $BF_3{\cdot}Et_2O$, Me_2S, 0 °C, 49% over two steps.

Table 1: Cytotoxicity data of **1**, **4**–**13**, and adriamycin in vitro.

Compound no. or name	IC_{50} (μM)			
	KB	Bel-7402	HT-29	HCT-8
1[a]	7.65	1.99	0.099	0.11
4	4.02	>10	1.84	3.49
5	13.13	>10	5.72	8.58
6	13.81	>10	7.19	5.71
7	23.30	>10	9.79	10.00
8	9.68	>10	4.56	24.46
9	21.30	>10	7.22	6.14
10	6.75	>10	3.60	3.39
11	6.38	>10	2.36	3.51
12	2.00	>10	1.75	2.00
13	2.35	4.14	1.51	3.46
adriamycin[a]	<0.01	0.95	0.055	0.11

[a] Adriamycin and compound **1** were selected as the positive controls in the screening.

served as key reactions in the synthesis of the target linear assemblies. An aldol-condensation-centered three-step procedure was utilized to form terminal γ-butenolides. The starting materials were all readily availabile and the reagents inexpensive. Biological evaluation of the products showed that introduction of hydroxy groups to the ether-bond region of the compounds results in different selectivities and potencies for different tumor cells. Most importantly, the parallel synthetic strategy presented herein may accelerate discovery of potent and selective antitumor agents. Further SAR information may be forthcoming as additional focused libraries become available.

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