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Facile Synthetic Access to and Biological Evaluation of the Macrocyclic Core of Apoptolidin

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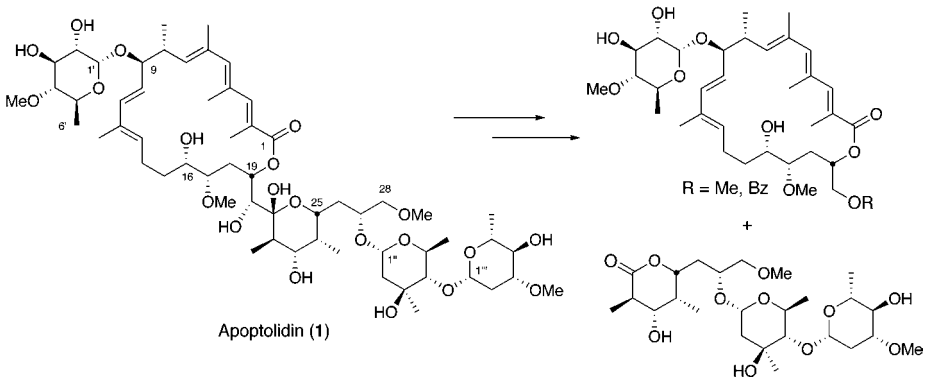
ABSTRACT



Oxidative cleavage of the C-20/C-21 bond in apoptolidin (1) provides two fragments of similar complexity, facilitating a divide-and-diversify strategy for the determination of the structural basis for apoptolidin's biological activity, the remarkably selective induction of apoptosis in sensitive cell lines. The ability of compounds derived from this cleavage to inhibit mitochondrial F_0F_1-ATPase is reported.

In 1997, Seto and co-workers isolated the 20-membered macrolide apoptolidin (**1**) in a screen for novel compounds that selectively induce apoptosis in cells transformed with the E1A oncogene.[1] Apoptolidin was subsequently found to be among the top 0.1% most selective of 37 000 compounds assayed in the National Cancer Institute's 60 cell line screen.[2] Khosla and co-workers have demonstrated that apoptolidin is an inhibitor of F_0F_1-ATPase and proposed that the inhibition of this enzyme could be the basis for the reported biological activity of **1**.[3] Because of the remarkably selective activity of **1** and its potentially novel mode of action, apoptolidin represents a promising lead compound for the treatment of cancer. The medicinal potential and structural complexity of apoptolidin has prompted considerable interest in synthetic approaches to this target.[4] A highly promising alternative approach to accessing this compound, and more importantly, analogues with clinical potential, arises from the fact that **1** can be obtained in high yield from its natural source (109 mg/L). Consequently, a key challenge for advancing this therapeutic lead is the development of methods for the selective modification of its diverse functionality, as needed to access derivatives with improved activity and stability.

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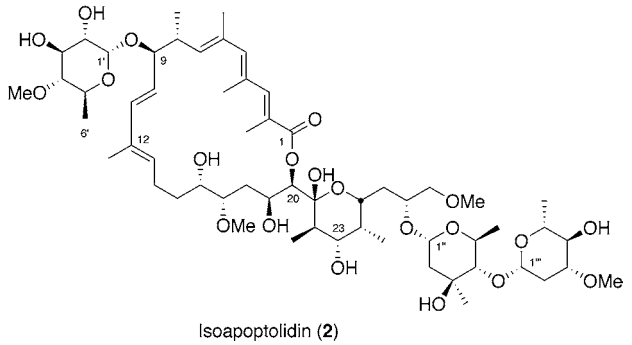
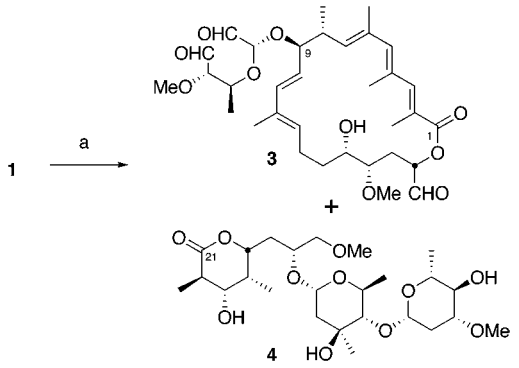


Figure 1. Structure of isoapoptolidin.

It has been shown that apoptolidin isomerizes in aqueous solution or slightly basic methanol to its ring-expanded isomer, isoapoptolidin (**2**, Figure 1).[5] This isomer is a less potent inhibitor of mitochondrial F_0F_1-ATPase and is certainly present under the conditions used in cell-based assays. As part of our efforts to elucidate the structural basis of apoptolidin's biological activity and access better and more stable leads, we previously reported a procedure by which the hydroxyl group array of apoptolidin can be selectively functionalized while keeping the core structure intact.[6] We report herein an efficient procedure for readily accessing the macrocyclic subunit of apoptolidin, thereby providing the basis for exploring the activity of this subunit, and for accessing derivatives in which this core is attached to diverse components.

The C-20/C-21 diol functionality in **1** provides an ideal site for oxidative cleavage to produce the corresponding macrocyclic aldehyde **3** and δ-lactone **4**. Toward this end, treatment of **1** with sodium periodate in a 1:1 mixture of methanol/water provided δ-lactone **4** in excellent yield but only trace amounts of macrolide fragment **3** (Scheme 1).

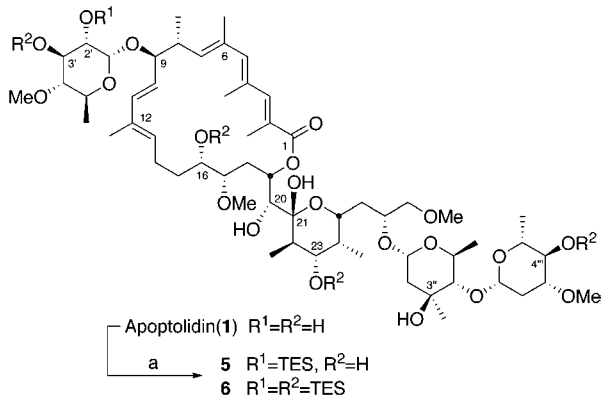
Scheme 1[a]



[a] Reagents and conditions: (a) $NaIO_4$, MeOH, H_2O (1:1) (87% **4**).

To improve access to the core macrocycle and preserve the 6-deoxy-4-*O*-methyl-L-glucose residue, protection of the C-2′ or C-3′ alcohols prior to cleavage was explored. Exposure of **1** to triethylsilyl triflate under high dilution conditions, as reported previously, gave monosilylated apoptolidin **5** in 36% yield along with recovered starting material and bis-silylated apoptolidin (Scheme 2).[6] With the C-2′

Scheme 2[a]



[a] Reagents and conditions: (a) TESOTf, DCM, THF, pyridine or 2,6-lutidine (ref 6).

alcohol suitably protected, $NaIO_4$ mediated cleavage of **5** was investigated. Unfortunately, the only product isolated from the reaction mixture in synthetically useful amounts was again δ-lactone **4**. This result suggests that the macrolide portion of apoptolidin is not stable to the relatively acidic conditions and long reaction times encountered in the aqueous sodium periodate-mediated oxidation. Attempts to perform this transformation under neutral or basic aqueous conditions resulted in no observed reaction.[7] Similarly, the use of either sodium periodate supported on silica gel[8] or

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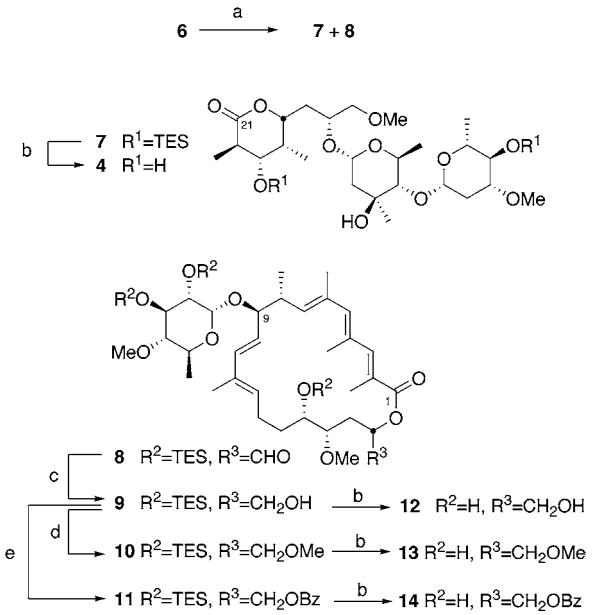
Scheme 3[a]



[a] Reagents and conditions: (a) $Pb(OAc)_4$, DCM, PhMe, TEA (87% **7**, 89% **8**); (b) HF·pyridine, pyridine, THF; (c) $NaBH_4$, THF; (d) Me_3OBF_4, 2,6-di-*tert*-butyl-4-methylpyridine, DCM (41%); (e) Bz_2O, TEA, DMAP, DCM (73%).

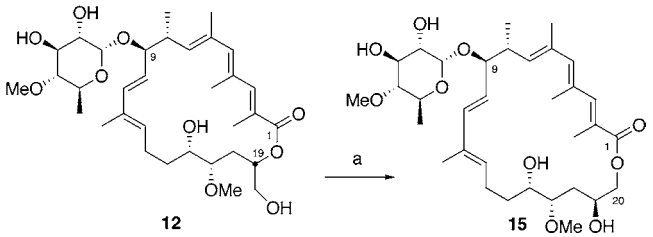
tetrabutylammonium periodate[9] in anhydrous solvents also failed to achieve the desired oxidation.

Screening of other oxidants led to the finding that lead tetraacetate effects diol cleavage in substrates derived from **1** with the C-20/C-21 diol exposed. After some experimentation, this reagent was found to work most effectively on pentatriethylsilyl protected apoptolidin, **6**. This reaction reaches completion rapidly and under mild anhydrous conditions, thus allowing for the isolation of both δ-lactone **7** and macrocycle **8** in excellent yields (Scheme 3; 87 and 89%, respectively) on up to a 220 mg scale.

With efficient and facile access to quantities of the macrocyclic core in hand, attention was directed at evaluating the stabilities and activities of derivatives. For this purpose, the fully protected macrocyclic aldehyde **8** was smoothly reduced using sodium borohydride in THF to produce the primary alcohol **9**. It was expected that **9** would be susceptible to ring-expansion via acyl migration, as has been observed with apoptolidin.[5] Despite this possibility, **9** can be fully desilylated using buffered HF·pyridine to produce macrolide **12** with no evidence of isomerization. In contrast, when dissolved in a dilute solution of triethylamine in methanol, macrolide **12** was observed to convert entirely to its ring-expanded isomer **15** over a period of 72 h (Scheme 4).

The primary alcohol in **9** can be efficiently converted to the methyl ether or benzoate ester as a means of preventing macrolide ring expansion. Treatment of **9** with Meerwein's salt or benzoyl anhydride followed by deprotection gave the functionalized macrocycles **13** and **14**, respectively. Acyl migration was not observed under the conditions of these transformations.

Scheme 4[a]



[a] Reagents and conditions: (a) TEA, CD_3OD.

The functionalized macrolide and δ-lactone fragments were assayed for inhibitory activity against mitochondrial F_0F_1-ATPase (Table 1). While the δ-lactone fragment, **4**, has greatly reduced potency as compared with apoptolidin, macrolide fragments **12**−**15** retain significant activity in this assay. Furthermore, alcohol **12** and the corresponding methyl ether **13** both have a potency that is comparable to that of isoapoptolidin in this assay system. Benzoate derivative **14**, in contrast, is approximately two times less active than **12** and **13**. This trend parallels the slight decrease in potency that is observed when apoptolidin is functionalized at C-20, either as the acetate or the methyl ether.[6] Ring expansion of macrolide **12** to isomeric **15**, which could be viewed as functionalization at C-20, also results in a 50% decrease in potency. The assay results for compounds **12**−**15** are consistent with the reported activity of the methanolysis product **16**, as described by Khosla and co-workers (Figure 2).[3c] Compound **16** is reported to inhibit F_0F_1-ATPase with an IC_{50} of 10 μM but is 2 orders of magnitude less active in cells that are sensitive to **1**. Experiments to correlate the ability of these compounds to inhibit mitochondrial F_0F_1-ATPase with their desired biological activity, the selective induction of apoptosis, are currently underway.

Table 1. Activity of Apoptolidin Oxidative Cleavage Products[a]

compound	IC_{50} (μM)
apoptolidin (**1**)[6]	0.7 ± 0.5
isoapoptolidin (**2**)[6]	17 ± 5.0
4	190 ± 50.0
12	13 ± 5.0
13	16 ± 5.0
14	32 ± 5.0
15	34 ± 5.0

[a] Assay conducted according to the procedure described in ref 5a.

In summary, a procedure has been developed that allows for facile access to the macrolide subunit of **1** and to new derivatives of apoptolidin. The activities of these derivatives in the F_0F_1-ATPase assay have been determined. While less

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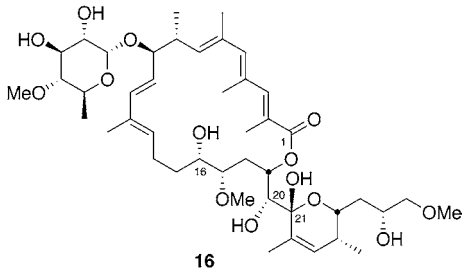

16

Figure 2. Acidic methanolysis product of **1** reported by Khosla and co-workers.

active than **1**, these derivatives do exhibit activity even though they are greatly simplified structurally. Importantly, these derivatives, readily available in terms of both speed and quantity, serve as superb advanced intermediates for accessing and evaluating the activity of diverse analogues. Further synthetic and assay studies in this and related series are in progress.

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Supporting Information Available: Experimental procedures and characterization data for compound **4** and compounds **7**−**15**. This material is available free of charge via the Internet at http://pubs.acs.org.

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