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Toward a Structure–Activity Relationship for Apoptolidin: Selective Functionalization of the Hydroxyl Group Array

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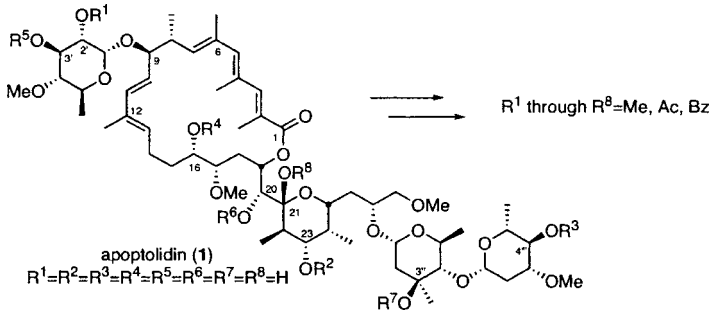
ABSTRACT



To investigate the structural basis for the exceptional selectivity and activity of apoptolidin (1), a strategy has been devised that allows for selective functionalization of seven of its eight hydroxyl groups based on progressive silyl protection, derivatization, and deprotection. The syntheses of these derivatives and their ability to inhibit F_0F_1-ATPase are reported.

Apoptolidin (**1**, Figure 1), a structurally complex macrolide isolated in 1997 by Seto and co-workers,[1] has been shown to be a highly selective inducer of apoptosis in certain cancer cell lines. Significantly, it is among the top 0.1% most selective agents screened to date in the NCI's 60 human cancer cell line panel.[2] Apoptosis, or programmed cell death, is an important mechanism in the treatment of cancer. Agents that can selectively induce apoptosis in cancer cells show great promise for therapeutic use.

Because of its novel activity and potential as an anticancer agent, apoptolidin has been the target of numerous synthetic efforts.[3] It has also been the focus of biochemical investigations, leading to the proposal that it operates through inhibition of mitochondrial F_0F_1-ATPase.[4]

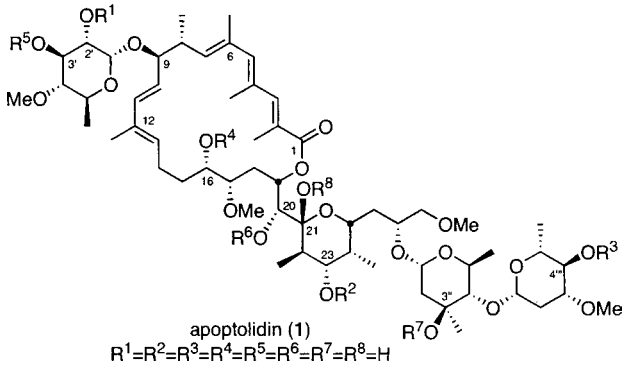


Figure 1. Structure of apoptolidin.

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(1) (a) Kim, J. W.; Adachi, H.; Shin-ya, K.; Hayakawa, Y.; Seto, H. *J. Antibiot.* **1997**, *50*, 628. (b) Hayakawa, Y.; Kim, J. W.; Adachi, H.; Shin-ya, K.; Fujita, K.; Seto, H. *J. Am. Chem. Soc.* **1998**, *120*, 3524.
(2) Developmental Therapeutics Program NCI/NIH. http://dtp.nci.nih.gov (accessed November 2002).

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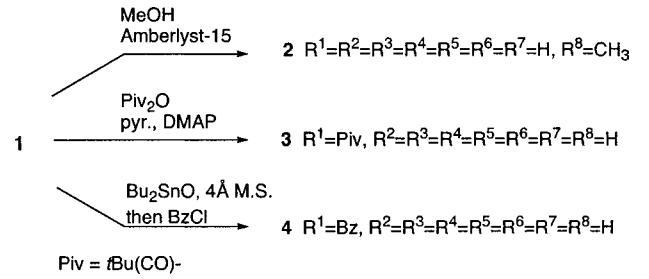
Because **1** can be obtained in substantial quantities by fermentation (109 mg/L),[1] information about its structure−activity relationship (SAR) could in principle be rapidly acquired through selective modification of its densely arrayed functionality. This information is critical to the design of simplified synthetic analogues of apoptolidin that would share its high selectivity, but would be more stable and amenable to therapeutic use. Such information is also of consequence in establishing the chemistry needed to synthetically access such analogues, as relatively little work has been reported on chemical modifications of this new structural class.[4c,5b] In this letter, we report our efforts directed at the site-selective modification of the eight hydroxyl functionalities of **1**. The hydroxyl groups were targeted for this initial study because of their potential role as pharmacophoric hydrogen bond donors and acceptors.

Of the eight hydroxyl groups in **1**, the C-21 hydroxyl is unique being part of a cyclic hemi-ketal. Its selective derivatization was thus achieved by treatment with Amberlyst-15 sulfonic acid resin in methanol to afford ketal **2** in 80% yield (Scheme 1*)*. Of the remaining hydroxyl groups

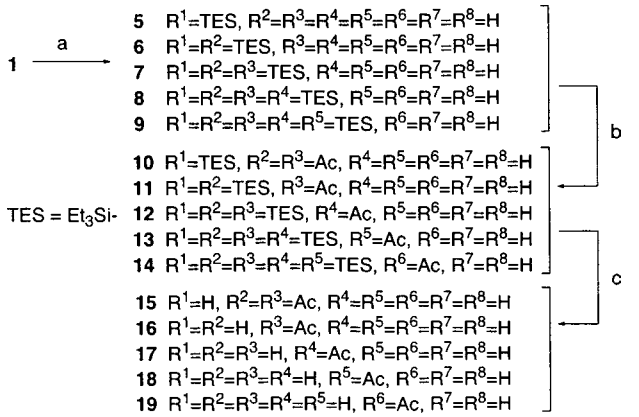
Scheme 1



present in apoptolidin, the one at C-2′ was found to be the most reactive toward acylation, and can thus be selectively esterified by using pivaloyl anhydride to give **3**. Alternatively, the C-2′/C-3′ diol can be activated by formation of the dibutylstannylidene acetal, followed by treatment with benzoyl chloride to produce exclusively the C-2′ benzoate **4**.

Most of the remaining hydroxyl groups in **1** can be selectively functionalized by employing a strategy of progressive selective protection, followed by acetylation and deprotection (Scheme 2*)*. The triethylsilyl ether protecting

Scheme 2[a]



[a] Reagents and conditions: (a) TESOTf, DCM, THF, pyr or 2,6-lut; (b) Ac_2O, DCM, pyr or TEA, DMAP; (c) HF·pyr, pyr, THF.

group proved ideal for this purpose as it is easily installed with good chemoselectivity, and can be removed under mild conditions. *tert*-Butyldimethylsilyl ethers, in contrast, can be installed only with difficulty, and were not readily removed. By altering the number of equivalents of triethylsilyl triflate used, each of the corresponding silyl ethers of apoptolidin, compounds **5**−**9**, can be selectively formed. Careful acetylation of each of these silyl ethers produces compounds **10**−**14**, respectively. For the mono-triethylsilyl ether derivative **5**, acetylation could not be completely controlled, and only the bis-acetylated derivative **10** was cleanly isolated. Finally, silyl deprotection of each derivative with buffered HF results in compounds **15**−**19**.

The location of the acetyl group in acetates **15**−**19** was determined by analysis of their proton chemical shift assignments based on 2D-NMR experiments. The downfield chemical shift of the proton geminal to the newly formed ester was used to identify the site of derivatization. Similarly, proton chemical shifts also serve to confirm that macrolide ring expansion has not occurred (vide infra).[5] The location of the silyl ethers in compounds **5**−**14** can be inferred by the identities of **15**−**19**. When analyzed by tandem mass spectrometry, apoptolidin and its derivatives fragment in a consistent way that allows for a cross-check of the NMR assignments, and thus serves as an independent confirmation of structure (Figure 2). On the basis of the fragmentation patterns of **10**−**14**, it can also be concluded that silyl transfer does not occur during the acetylation of **5**−**9**.

We have previously demonstrated that apoptolidin is isolated along with an isomer, isoapoptolidin (**20**). Our group and the group of Sulikowski have independently established the structure of this ring-expanded isomer and have shown that it forms readily from **1** in aqueous solution or basic methanol solution (Figure 3).[5] Furthermore, isoapoptolidin

(3) (a) Schuppan, J.; Wehlan, H.; Keiper, S.; Koert, U. *Angew. Chem., Int. Ed.* **2001**, *40*, 2063. (b) Toshima, K.; Arita, T.; Kato, K.; Tanaka, D.; Matsumura, S. *Tetrahedron Lett.* **2001**, *42*, 8873. (c) Nicolaou, K. C.; Li, Y.; Fylaktakidou, K. C.; Mitchell, H. J.; Sugita, K. *Angew. Chem., Int. Ed.* **2001**, *40*, 3854. (d) Nicolaou, K. C.; Li, Y.; Fylaktakidou, K. C.; Mitchell, H. J.; Wei, H. X.; Weyershausen, B. *Angew. Chem., Int. Ed.* **2001**, *40*, 3849. (e) Schuppan, J.; Ziemer, B.; Koert, U. *Tetrahedron Lett.* **2000**, *41*, 621. (f) Sulikowski, G. A.; Jin, B. H.; Lee, W. M.; Wu, B. *Org. Lett.* **2000**, *2*, 1439. (g) Chen, Y. Z.; Fuchs, P. L. *Abstr. Papers Am. Chem. Soc.* **2002**, *224*, U246. (h) Chaudhary, K.; Crimmins, M. T. *Abstr. Papers Am. Chem. Soc.* **2002**, *224*, U246. (i) Junker, B.; Pauette, B.; Guseilla, D.; Taylor, R. E. *Abstr. Papers Am. Chem. Soc.* **2002**, *223*, B236. (j) Xu, J.; Loh, T. P. *Abstr. Papers Am. Chem. Soc.* **2000**, *219*, 816-ORGN.

(4) (a) Salomon, A. R.; Voehringer, D. W.; Herzenberg, L. A.; Khosla, C. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 14766. (b) Salomon, A. R.; Voehringer, D. W.; Herzenberg, L. A.; Khosla, C. *Chem. Biol.* **2001**, *8*, 71. (c) Salomon, A. R.; Zhang, Y.; Seto, H.; Khosla, C. *Org. Lett.* **2001**, *3*, 57.

(5) (a) Wender, P. A.; Gulledge, A. V.; Jankowski, O. D.; Seto, H. *Org. Lett.* **2002**, *4*, 3819. (b) Pennington, J. D.; Williams, H. J.; Salomon, A. R.; Sulikowski, G. A. *Org. Lett.* **2002**, *4*, 3823.

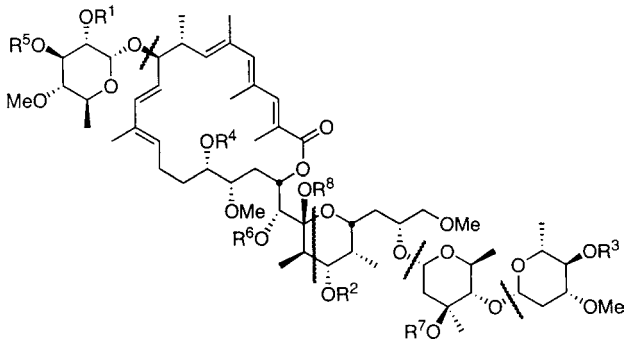


Figure 2. Some common (+)ESIMS fragmentation sites for derivatives of **1**.

is demonstrably less active than **1** in the ATPase inhibition assay (IC_{50} = 17 μM for **20** as compared with 0.7 μM for **1**). In addition to probing its role in recognition, functionalization of the C-20 alcohol was therefore explored as a way to prevent isomerization to a less active compound.

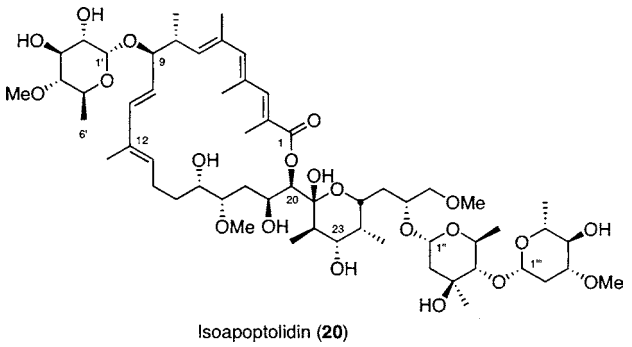
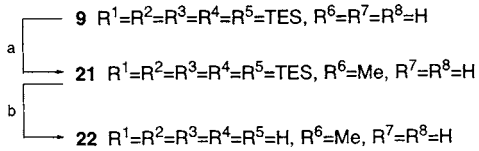


Figure 3. Structure of isoapoptolidin.

Because of the potential lability of an acetate functionality in cell-based assays, an alternative method of derivatizing the C-20 alcohol was sought to prevent isomerization to isoapoptolidin **20**. By preventing the formation of isoapoptolidin, one could begin to investigate which isomer is primarily responsible for the desired biological response: the selective induction of apoptosis. It was found that penta-(triethylsilyl)-protected apoptolidin **9** reacted smoothly with Meerwein's salt in the presence of 2,6-di-*tert*-butyl-4-methylpyridine to produce exclusively the C-20 methyl-ether **21**. Compound **21** could subsequently be deprotected to give the apoptolidin derivative **22** (Scheme 3). The location of the methyl-ether was confirmed by observation of the strong HMBC correlation between C-20 and the C-20 methoxy protons. Attempts to perform similarly controlled methylations of compounds **5**−**8** suffered from lack of chemoselectivity.

With methyl-ether **22** in hand, we next examined its stability when dissolved in Dulbecco's phosphate-buffered

Scheme 3[a]



[a] Reagents and conditions: (a) Me_3OBF_4, DCM, 2,6-di-*tert*-butyl-4-methylpyridine; (b) HF·pyr, pyr, THF.

saline[6] solution (PBS), conditions which have been shown to promote isomerization of **1** to **20**.[5a] While ring expansion of the macrolactone cannot take place in the case of compound **22**, **22** was found to partially convert to a second isomeric compound over a period of several hours, as observed by LC-MS. The identity of this isomer of **22** is under investigation. Similarly, the C-20 acetate derivative **19** is also unstable under these conditions, producing several new compounds over a 5-h period. In short, while derivatization of **1** at C-20 blocks isomerization to the less active isoapoptolidin structure, the resulting derivatives **19** and **22** undergo other transformations. Nevertheless, these compounds convert slowly enough that their performance in the ATPase inhibition assay can provide valuable SAR information.

Preliminary evaluation of the activities of apoptolidin derivatives **2**, **4**, **15**−**19**, and **22** focused on their ability to inhibit mitochondrial F_0F_1-ATPase.[4] While the stability of acetate functionalities could be compromised in cell-based assays, this mitochondrial ATPase inhibition assay is both rapid and cell-free. Under these conditions, it is likely that the acetate derivatives will be stable to hydrolysis for the duration of the assay. To test the stability of acetates in the mitochondrial ATPase inhibition assay, compound **17** was exposed to all of the assay components for up to 24 h. The assay solution was then analyzed for hydrolysis products of **17**, using ESIMS. Even after 24 h, no hydrolysis products of **17** were detected under these conditions.

The results of the ATPase inhibition assay (Table 1) indicate that functionalization of single hydroxyl groups present in **1** does not appreciably change activity, with some derivatives somewhat more active and some less. The only sites where a statistically significant decrease in activity is observed are at C-20 and C-21. This is also the location that is most changed upon isomerization from **1** to **20**. It is not clear at present whether this decrease in activity is a result of a conformational change, isomerization, or the blocking of a critical pharmacophoric atom.

In conclusion, we have devised a systematic method for the site-selective functionalization of the individual hydroxyl groups in apoptolidin. While no strong trends emerge in the ATPase inhibition activities for the derivatives in this series, there is evidence to suggest that the C-20 and C-21 functionalities influence both the activity and stability of apoptolidin derivatives. Of great importance is the observa-

(6) Dulbecco, R.; Vogt, M. *J. Exp. Med.* **1957**, *106*, 167.

Table 1. Activity of Apoptolidin Hydroxyl Derivatives in Mitochondrial F_0F_1-ATPase Inhibition

compd	IC_{50} (μM)
apoptolidin (**1**)	0.7 ± 0.5
isoapoptolidin (**20**)	17 ± 5.0
2	2.3 ± 0.5
4	0.3 ± 0.5
15	0.4 ± 0.5
16	0.8 ± 0.5
17	0.8 ± 0.5
18	0.4 ± 0.5
19	1.1 ± 0.5
22	2.8 ± 0.5

tion that many of these derivatives possess ATPase inhibition activity similar to **1**. Derivatization of these sites could thus be used to probe mechanism and tune solubility, transport, metabolism, and chemical stability as needed for therapeutic development. The relationship of these cell-free assays to whole cell functional assays is under investigation. Further studies will be reported in due course.

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Supporting Information Available: Experimental procedures and characterization data for compounds **2**−**19**, **21**, and **22**. This material is available free of charge via the Internet at http://pubs.acs.org.

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