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An Approach to the Site-Selective Diversification of Apoptolidin A with Peptide-Based Catalysts

Chad A. Lewis[†], **Kate E. Longcore**[‡], **Scott J. Miller**[†,*], and **Paul A. Wender**[‡,*]

[†]Department of Chemistry, Yale University, 225 Prospect Street, New Haven, Connecticut 06520-8107

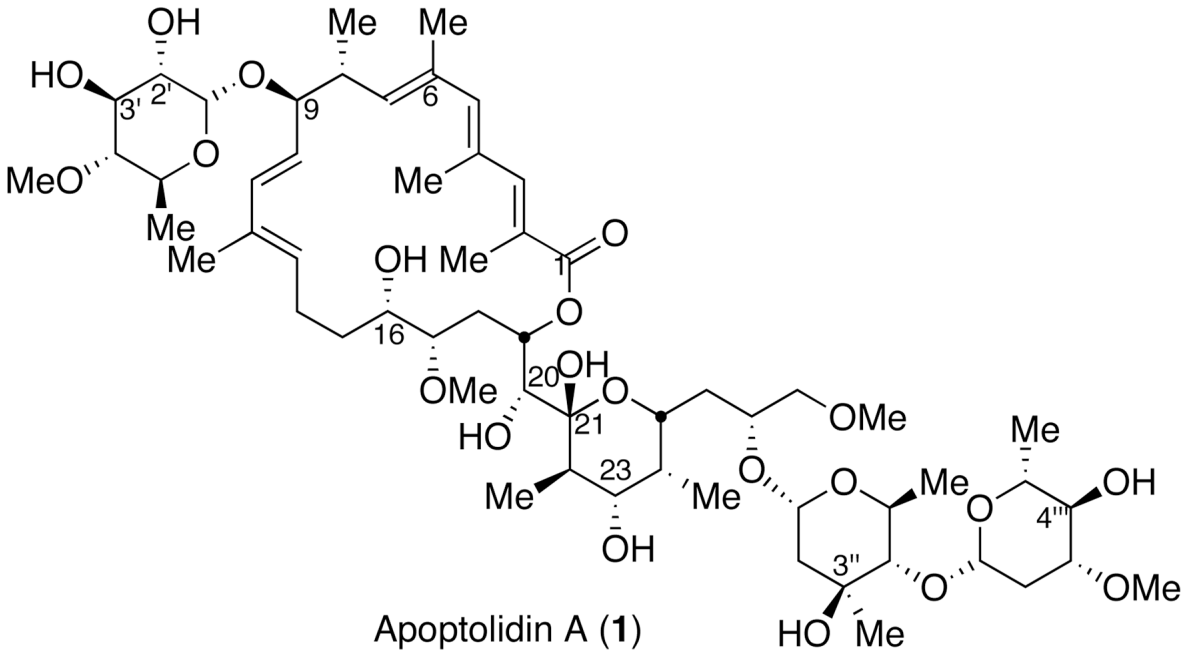
[‡]Department of Chemistry and Department of Chemical and Systems Biology, Stanford University, Stanford, California, 94305-5080

We report the application of peptide-based catalysts to the site-selective modification of apoptolidin A (**1**), an agent that displays remarkable selectivity for inducing apoptosis in E1A-transformed cell lines. Key to the approach was the development of an assay suitable for the screening of dozens of catalysts in parallel reactions that could be conducted using only microgram quantities of the starting material. Employing this assay, catalysts (e.g., **11** and ***ent*-11**) were identified that afforded unique product distributions, distinct from the product mixtures produced when a simple catalyst (*N*,*N*-dimethylaminopyridine (**10**)) was employed. Preparative reactions were then carried out with the preferred catalysts so that unique, homogeneous apoptolidin analogs could be isolated and characterized. From these studies, three new apoptolidin analogs were obtained (**12**–**14**), each differing from the other in either the location of acyl group substituents, or in the number of acetate groups appended to the natural product scaffold. Biological evaluation of the new apoptolidin analogs was then conducted using growth inhibition assays based on the H292 human lung carcinoma cell line. The new analogs exhibited activities comparable to apoptolidin A.

Apoptolidin A (**1**) was isolated by Seto and coworkers from the fermentation of the actinomycete *Nocardiopsis* sp. based on its selectivity for inducing apoptosis in E1A-transformed cells.[1] Notably, apoptolidin is at least *four orders of magnitude* more active in E1A-transformed cells than in non-transformed cells. Due to its exquisite selectivity and the medicinal potential that such selectivity holds, apoptolidin A (**1**) has been the focus of much synthetic interest,[2] and several derivatives involving modification of its hydroxy groups, deglycosylation, and cycloadduct formation have been reported.[3] Evaluation of the activity of these derivatives has provided a foundation for understanding how the structural features of apoptolidin influence its functional activity including selective induction of apoptosis in E1A-transformed 3Y1 rat fibroblasts, inhibition of isolated F_0F_1-ATPase, and growth inhibition of human-derived cancer cells. These studies have been further augmented by the isolation of apoptolidins B, C, and D.[4]

*To whom correspondence should be addressed. (S.J.M.) Tel: 203-432-9885. Fax: 203-436-4900. scott.miller@yale.edu. (P.A.W.) Tel: 650-723-0208. Fax: 650-725-0259. wenderp@stanford.edu.

Supporting Information Available: Spectral data for new analogs including ^{1}H NMR, ^{1}H-^{1}H COSY, and tandem mass spectrometry data. This material is available free of charge via the Internet at http://pubs.acs.org.



Apoptolidin A (**1**)

Previously, the Wender group produced a number of apoptolidin A analogs through a progressive protection, functionalization, and deprotection sequence.[3d] The products obtained from this three-step sequence were tested for cell proliferation and mitochondrial F_0F_1-ATPase inhibition (Table 1).[3f] These results revealed that the structural alterations in compounds **2**–**8**, as well as isoapoptolidin (**9**), resulted in subtle and quite varied biological effects. While the stepwise functionalization of apoptolidin used to produce analogs **2**–**8** proved effective in obtaining new derivatives for biological evaluation, the site of modification of apoptolidin in these studies is controlled, and therefore limited by, the intrinsic reactivity of the starting substrate. A complementary approach to analogs would exploit catalyst controlled, site-selective functionalization of the complex natural product itself.[5] While enzymes can often be used for this purpose, application of readily available and varied small molecule catalysts offers another effective modification strategy. Peptide-based catalysts, for example, have been used for the site-selective modification of erythromycin A,[6] producing novel analogs in sufficient quantity so that biological studies could be undertaken.[7] The application of this approach to the apoptolidin scaffold is reported herein offering a one-step, organocatalytic route to new biologically active apoptolidin derivatives.

Apoptolidin presents a special challenge to a catalyst-based diversification strategy as it possesses eight hydroxy groups, six of which are secondary. Moreover, whereas a structure such as erythromycin is readily available on a multi-gram scale, apoptolidin, at the outset of this study, was available in only multi-milligram quantities. While a scaled-up fermentation could readily solve the supply issue given recent improvements in the titer of apoptolidin (ca. 150 mg/liter),[4a] we elected to screen small-molecule catalysts for selective derivatization of **1** with only ~25 milligrams of apoptolidin as the available starting material as it represents a more commonly encountered situation for which this approach would be used. In order to assess feasibility, we chose to study catalytic acyl transfer as the reaction of interest. If successful, such an approach could help to validate the application of small-molecule catalyst library methodology to the derivatization of complex natural products,

often in scarce supply, as an important avenue for study at the intersection of the fields of catalysis and natural products science.[8]

Results and Discussion

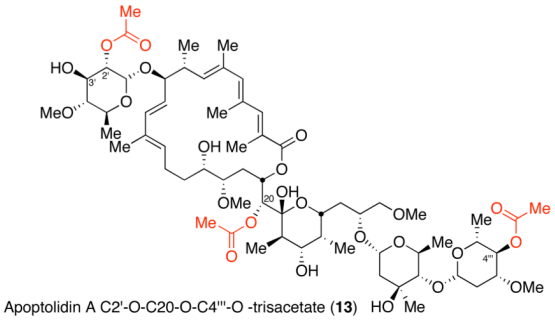
In order to allow for a sufficiently thorough study of the peptide-catalyzed acylation of apoptolidin A (**1**), an assay was developed which would consume as little of **1** as possible but deliver sufficient amounts of products for HPLC analysis. Catalytic reactions were performed using 0.02 mg apoptolidin dissolved in 30 μL of chloroform. This design, which was executed through the preparation of stock solutions, allowed for screening of dozens of structurally diverse peptide catalysts (~150–160) and required a total of only 5 mg of substrate. After quenching the reactions, the crude mixtures were analyzed by analytical reversed-phase HPLC. Reactions were defined as successful "hits" when the corresponding HPLC traces revealed a peak pattern that was significantly different from those observed with control reactions. Control reactions were defined as those that afforded the product distributions based on the inherent reactivity of the substrate under conditions of catalytic acylation mediated by *N*,*N*-dimethylaminopyridine (DMAP) and in the absence of peptide catalyst. For the functionalization of **1**, acetic anhydride was chosen as the acyl donor due to its simplicity and the availability of some "authentic" acetyl derivatives of apoptolidin.[3d] Several pre-existing peptide-catalyst libraries[9] were then examined for catalytic modification of **1**. Experiments were performed at two different levels of conversion: low conversion reactions were targeted to monoacetates of **1** that would otherwise require multistep syntheses; high conversion reactions were projected to deliver bis-, tris -or other poly(acetates) that would likewise be difficult to access by designed synthetic routes based on conventional protection strategies. Indeed, the objective was to arrive at structures that would be specific to catalysts. Follow-up and scale-up studies were therefore projected to be straightforward, provided the screening reactions proved reproducible.

For the low conversion reactions, acetic anhydride (100 equiv) and triethylamine were introduced into the reaction mixtures along with 10 mol% of peptide-based catalysts (total reaction concentration, 0.590 mM). When DMAP (**10**) was employed as the control catalyst in order to define the inherent selectivity profile for unprotected **1**, it was found that the inherent reactivities of the C2'- and C3'- hydroxy groups were roughly equivalent, providing nearly equimolar amounts of the corresponding acetates (**6**:**12** ~1:1; Figure 1a). After screening 156 catalysts, peptide **11** was found to provide a notable enhancement of production of **12**, with a product ratio of ~1:4 (**6**:**12**, Figure 1b). Importantly, this selective peptide-catalyzed reaction was readily reproduced on a larger scale (0.02 mg to 7.1 mg apoptolidin), and apoptolidin A C2'-O-acetate (**12**) was isolated. Critical to our goals, monoacetate **12** proved to be a previously unknown compound, while monoacetate **6** had been accessed previously.[3d] The structure of **12** was secured by NMR, and chemical shift perturbations were consistent with those observed for the known C2' benzoate analog (**2**).[3d] Furthermore, **12** was isolated in the quantity and purity needed for biological testing (*vide infra*).

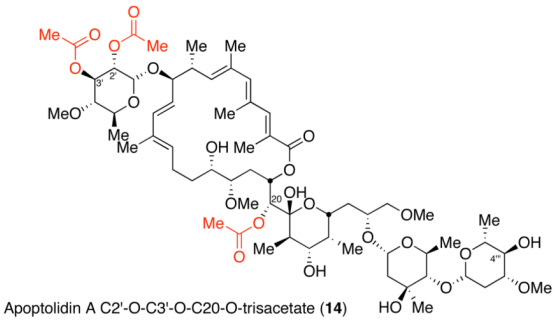
We then turned our attention to the discovery of catalysts for the selective formation of poly(acetate)-analogs of apoptolidin A. Experimentally, this study was conducted by increasing the concentration of acetic anhydride and triethylamine five-fold each. An increase in the peptide-catalyst loading to 25 mol% was also found to be beneficial for access to the bis- and tris(acetates). In comparison to the control reaction catalyzed by DMAP (**10**, Figure 2a), we found that several of the peptide catalysts offered substantial perturbation of the reactivity of the hydroxy group array of apoptolidin A. Among these, the enantiomer of catalyst **11** (***ent*-11**) provided the product distribution shown in Figure 2b.

Once again, scale-up of the ***ent*-11**-catalyzed reaction revealed good reproducibility, enabling isolation of the major peak for characterization.

In this case, the major product peak was found to be a mixture of two distinct tris(acetate) compounds, which were further separated by silica gel chromatography. The two new apoptolidin A analogs were then studied by spectroscopic techniques, including tandem mass spectrometry fragmentation and NMR spectroscopy. The characteristic mass fragmentation patterns, along with the marked downfield chemical shift of protons alpha to the newly introduced acetates relative to the parent compound,[10] allowed assignment of new structures **13** and **14** (Table 2).



Apoptolidin A C2'-O-C20-O-C4'''-O -trisacetate (**13**)



Apoptolidin A C2'-O-C3'-O-C20-O-trisacetate (**14**)

A particularly significant issue in the generation of new analogs of **1** employing any approach is the vulnerability of **1** towards rearrangement to the ring-expanded variants related to isoapoptolidin (**9**).3b,c Notably, each of the new structures obtained in this study of catalytic derivatization provides NMR spectra consistent with the parent structure of **1**, rather than that of the rearranged isomer **9**. In particular, as shown in Figure 3 (also Table 2), the C19-proton and C25-proton exhibit characteristic chemical shifts associated with each structure.

The three newly isolated apoptolidin A analogs (**12**–**14**) were tested for growth inhibitory activity in H292 human lung carcinoma cells (Table 3). As in cells treated with apoptolidin A, reduced cell numbers were observed at higher concentrations of analogs. The acetylated analogs demonstrated marginally less potent activity than apoptolidin A; however, these findings are consistent with previous observations, namely that apoptolidin A analogs tend to retain biological activity when the hydroxy group array is modestly modified (Table 1). These findings indicate that either the modifications do not interfere with the apoptolidin's interaction with its molecular target, proposed in part to be mitochondrial F_0F_1-ATP synthase,[11,3f] or that the analogs are converted back to apoptolidin, perhaps through the action of esterases, in cell-based assays.

Conclusions

Peptide-based catalysts have been used to afford differentially functionalized analogs of apoptolidin A. The screening of catalyst libraries was possible with substrate samples involving microgram quantities of the polyol natural product. The reproducibility of the results was excellent when screening experiments were scaled up in order to allow isolation and characterization of apoptolidin analogs. From these studies, three new compounds were obtained, and their structures were determined. These new natural product analogs were then evaluated in growth inhibition assays using the H292 human lung carcinoma cell line. All three analogs showed nanomolar potencies, consistent with other acylated apoptolidin analogs. Overall, these studies provide encouraging results that further demonstrate that small molecule, nonenzymatic catalysts can be useful tools in the selective modification of complex molecules, providing a potentially powerful entry into structural and functional space of relevance to natural product scaffolds. The fidelity of the experiments conducted on minute screening scale, and subsequently upon scale-up for new analog isolation, bode well for execution of related protocols in integrated studies involving natural products isolation, derivatization, biological evaluation and optimization.[7] The step-economy[12] achieved with this diversification strategy through avoidance of protecting group manipulations argues further for its use in the modification of complex molecules available in limited quantities.

Experimental Section

General Experimental Procedures

Proton NMR spectra were recorded on either a Varian 500 or 600 MHz spectrometer. Proton chemical shifts are reported in ppm (δ) relative to residual solvent peak (CD_3OD, 3.31 ppm). Data are reported as follows: chemical shift (multiplicity [singlet (s), doublet (d), triplet (t), quartet (q) and multiplet (m)], coupling constants [Hz], integration). NMR data were collected at 25 °C. High-resolution and tandem mass spectrometry was performed at the Stanford University Mass Spectrometry Facility. Analytical thin-layer chromatography (TLC) was performed using Silica Gel 60 Å F-254 precoated plates (0.25 mm thickness). Visualization was accomplished after staining with cerium ammonium molybdenate (CAM) solution. Flash column chromatography was performed using Silica Gel 60 Å (32–63 μm).

Preparative reversed-phase HPLC were run on a Varian Pro-Star chromatograph equipped with a single wavelength UV detector (230 nm). Analytical reversed-phase HPLC was performed on a Hewlett-Packard 1100 Series chromatograph equipped with a diode array detector (230 nm). All reactions were carried out under a nitrogen atmosphere employing oven or flame-dried glassware. All solvents were either distilled or obtained from passing through activated alumina.

Peptide Synthesis and Screens

Peptides were synthesized on solid support using commercially available Wang polystyrene resin preloaded with FMOC protected amino acid. Couplings were performed using 5 equiv. amino acid derivative, 5 equiv. HBTU, and 10 equiv. Hünig's base in DMF for 3 h. Deprotections were performed using 20% piperidine in DMF for 20 min. To minimize diketopiperazine formation, dipeptides were deprotected using 50% piperidine in DMF for 5 min. Peptides were cleaved from solid support using a mixture of MeOH:DMF:NEt_3 (9:1:1) for 5 days. The peptides were characterized by electrospray mass spectrometry and used in parallel reaction screens without further purification. Peptides of interest were purified by reversed-phase chromatography on a Biotage SP4 using C_{18} silica gel. The peptides were purified by a gradient of 55% MeOH/H_2O and increasing to 80% MeOH/H_2O over 35 column volumes.

Isolation of Apoptolidin A

Apoptolidin A was isolated as previously reported.[1a,4a] Briefly, following fermentation and centrifugation of the producing organism, *Nocardiopsis* sp., EtOAc/brine extraction provided crude material. Partial purification via silica gel column chromatography (10% MeOH/$CHCl_3$) was followed by final purification by preparative reversed-phase HPLC (Alltech Alltima™ column (length: 250 mm, ID: 22 mm) packed with C18 (10 μm) stationary phase, 30–80% CH_3CN/H_2O linear gradient in 20 min). Fractions containing apoptolidin A were lyophilized to dryness.

Growth Inhibition Assay

Cell proliferation assays using H292 cells were performed by plating cells at a density of 1000 cells/well in 100 mL RPMI supplemented with 10% fetal bovine serum, penicillin, and streptomycin in a 96-well plate. Cells were allowed to settle and adhere for 24 hours at 37 °C in a 5% CO_2 atmosphere before apoptolidin A derivative additions were performed in triplicate from 4 mM stock solutions in DMSO to give a maximum final DMSO concentration of 0.25% (10 mM to 0.5 nM in three-fold dilutions). After 48 hours, viable cell number was quantified using thiazolyl blue tetrazolium bromide.[13] Briefly, 10 mL of a 5 mg thiazolyl blue tetrazolium bromide per mL culture media was added to each well and incubated at 37 °C and 5% CO_2 for 2.5 h at which time the cells were lysed with 100 mL of a detergent solution (10% Triton × 100 in 0.1 N HCl in isopropanol). Following thorough mixing of the cells via pipette, the plates were read using a VERSAmax tunable microplate reader (Molecular Devices) with SOFTmax Pro® version 3.1.1 software. Plates were read at 570 nm and subtracted at 690 nm. EC_{50} values were calculated by fitting data with GraphPad Prism® 4 (nonlinear regression, sigmoidal dose response). The reported values are the average of three independent experiments, and the error represents the standard error of the mean.

General Catalyst Screening Procedure for the Acetylation of Apoptolidin A

A chloroform solution of Apoptolidin A (0.02 mg, 17.7 nmol/20 μL) was treated with a solution of the pre-dissolved catalyst (0.004 mg, 20 μL, 25 mol% based upon average MW of 1000). Acetic anhydride (0.166 nL in 10 μL, 100 equiv.) and triethylamine (0.244 nL in 10 μL, 100 equiv.) were then introduced. The reaction vessel was then wrapped in foil, and the reaction mixture was allowed to stir for approximately 48 h before it was quenched with MeOH (5 μL). The reaction mixture was then concentrated and subsequently redissolved in a solution CH_3CN:H_2O (1:1, 20 μL). The sample was then analyzed by reversed-phase HPLC (Waters Xterra RP 18 3.5 μm, 3.0×100 mm), eluting initially with 70% H_2O/CH_3CN, applying a gradient to 35% H_2O/CH_3CN over 60 minutes (flowrate, 0.75 mL/min). The retention time of the peptide catalyst used for the reaction was also determined independently using the same method. For higher conversion screening of apoptolidin analogues, acetic anhydride and triethylamine stoichiometries were increased to 500 equivalents and the peptide loading to 25 mol%.

Apoptolidin A C2'-O-acetate (12)

To a solution of apoptolidin A (7.1 mg, 0.0063 mmol) in $CHCl_3$ (18 mL) was added Ac_2O (60 μL, 0.63 mmol), triethylamine (88 μL, 0.63 mmol), and catalyst **11** (2.7 mg, 0.0030 mmol). The reaction was deemed complete after 80 h as determined by analytical HPLC. Methanol (10 mL) was then added, and the reaction was concentrated. Purification by preparative HPLC provided unreacted apoptolidin A (**1**) (0.5 mg, 7%), the C2'-O-acetate **12** (1.9 mg, 26%), and the C3'-O-acetate **6**.

Apoptolidin A C2'-O-acetate (12)

^{1}H NMR (500 MHz, CD_3OD), see Table 2; HRMS *m/z* 1193.6442 (calcd for $C_{60}H_{98}O_{22}$ + Na, 1193.6447); R_f = 0.35 (9/1 $CHCl_3$/MeOH v/v).

Apoptolidin A C3'-O-acetate (6)

Spectroscopic data for monoacetate **6** matched that previously reported. See reference [3d].

Apoptolidin A C2'-O-C20-O-C4"'-O-trisacetate (13) and apoptolidin A C2'-O-C3'-O-C20-O-trisacetate (14)

Apoptolidin A (28.5 mg, 24.5 μmol) was dissolved in $CHCl_3$ (35 mL) and the peptide catalyst was added (5.7 mg, 6.3 μmol). Triethylamine (1.74 mL, 12.5 mmol) was then added and the solution allowed to stir for 10 minutes. Lastly, acetic anhydride (1.18 mL, 12.5 mmol) was added and the reaction was then covered in foil and stirred at room temperature. The reaction was monitored by sampling of aliquots that were quenched in MeOH and concentrated. After 44.5 hours, the reaction was deemed complete and was quenched with MeOH (4 mL). The reaction was concentrated and then dissolved in 1:1 CH_3CN:H_2O. The crude mixture was purified by semi-prep reverse phase HPLC (Waters XTerra column RP-18 10μm, 7.8×150 mm) and lyophilized. This material was then subjected to silica gel purification to separate the two trisacetates, **13** and **14** using 95/5 $CHCl_3$/MeOH. The fractions containing products were concentrated and a second purification by silica gel yielded the desired products **13** (3.2 mg, 10%), and **14** (0.7 mg, 2%).

Apoptolidin A C2'-O-C20-O-C4"'-O-trisacetate (13)

^{1}H NMR (600 MHz, CD_3OD), see Table 2; HRESMS *m/z* 1277.6655 (calcd for $C_{64}H_{102}O_{24}$ +Na, 1277.6659); R_f = 0.44 (9/1 $CHCl_3$/MeOH v/v).

Apoptolidin A C2'-O-C3'-O-C20-O-trisacetate (14)

^{1}H NMR (600 MHz, CD_3OD), see Table 2; HRESMS *m/z* 1277.6670 (calcd for $C_{64}H_{102}O_{24}$ +Na, 1277.6659); R_f = 0.36 (9/1 $CHCl_3$/MeOH v/v).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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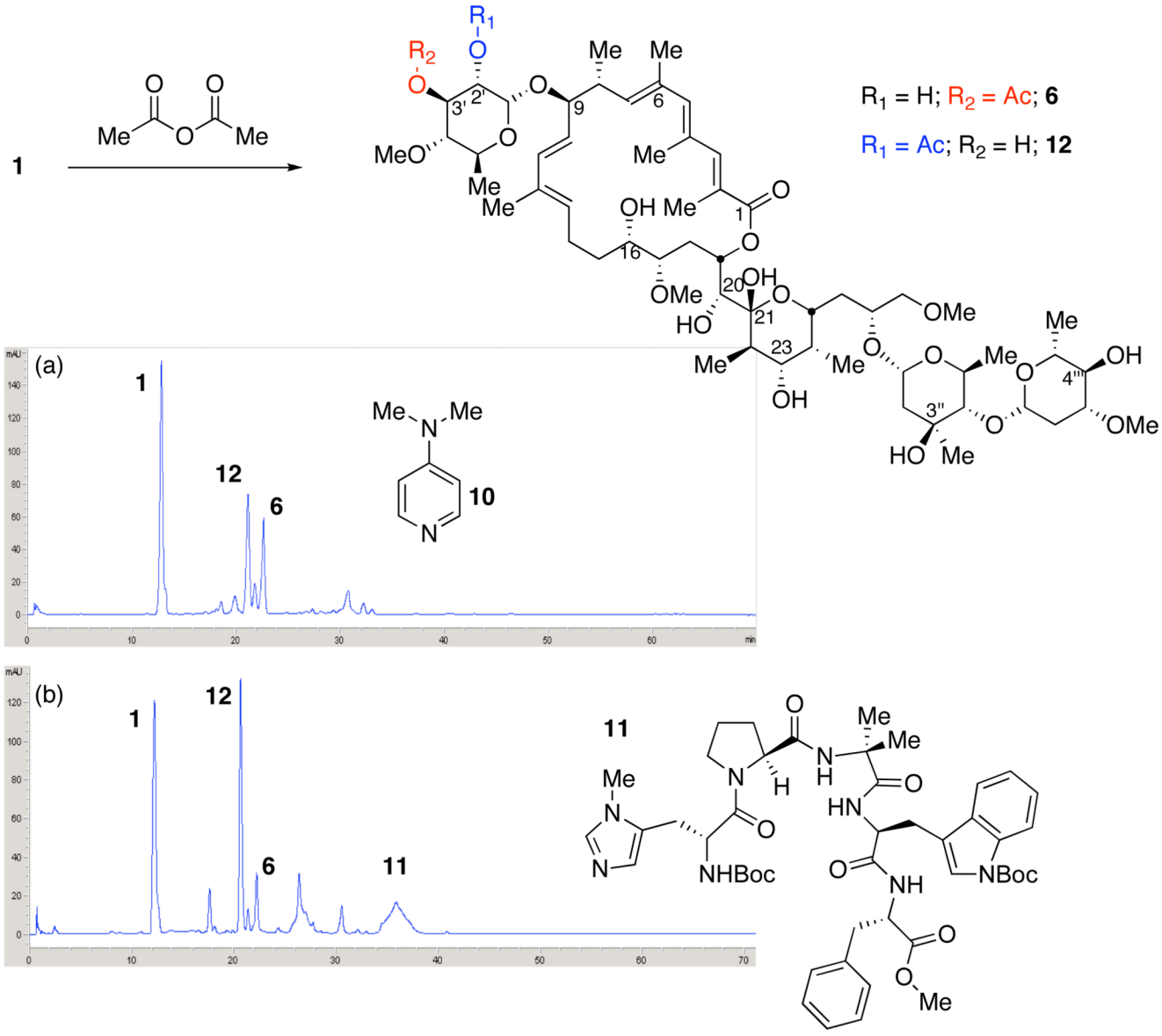


Figure 1.

Low conversion reactions of apoptolidin A (**1**). (a) Reversed-phase HPLC trace of the reaction mixture when **10** is employed as the catalyst. (b) Reversed-phase HPLC trace of the reaction mixture when **11** is employed as the catalyst.

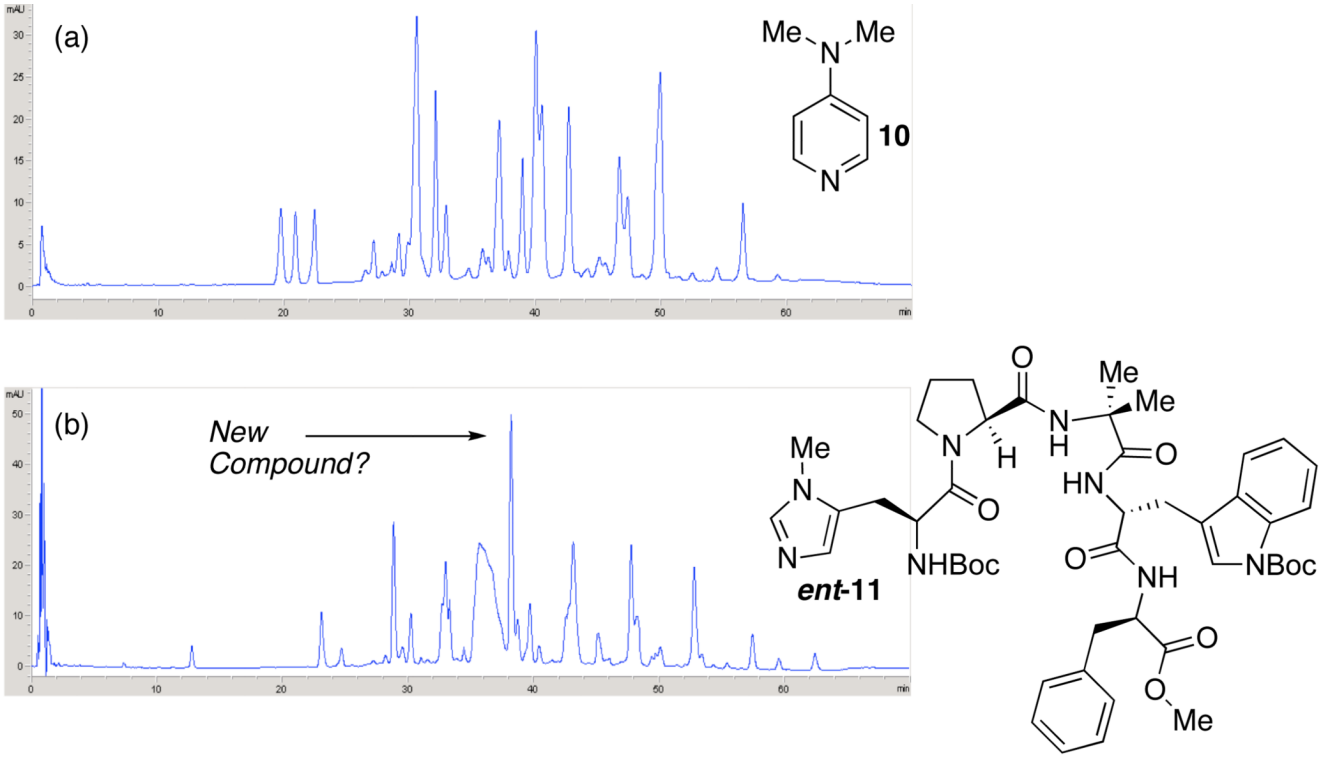
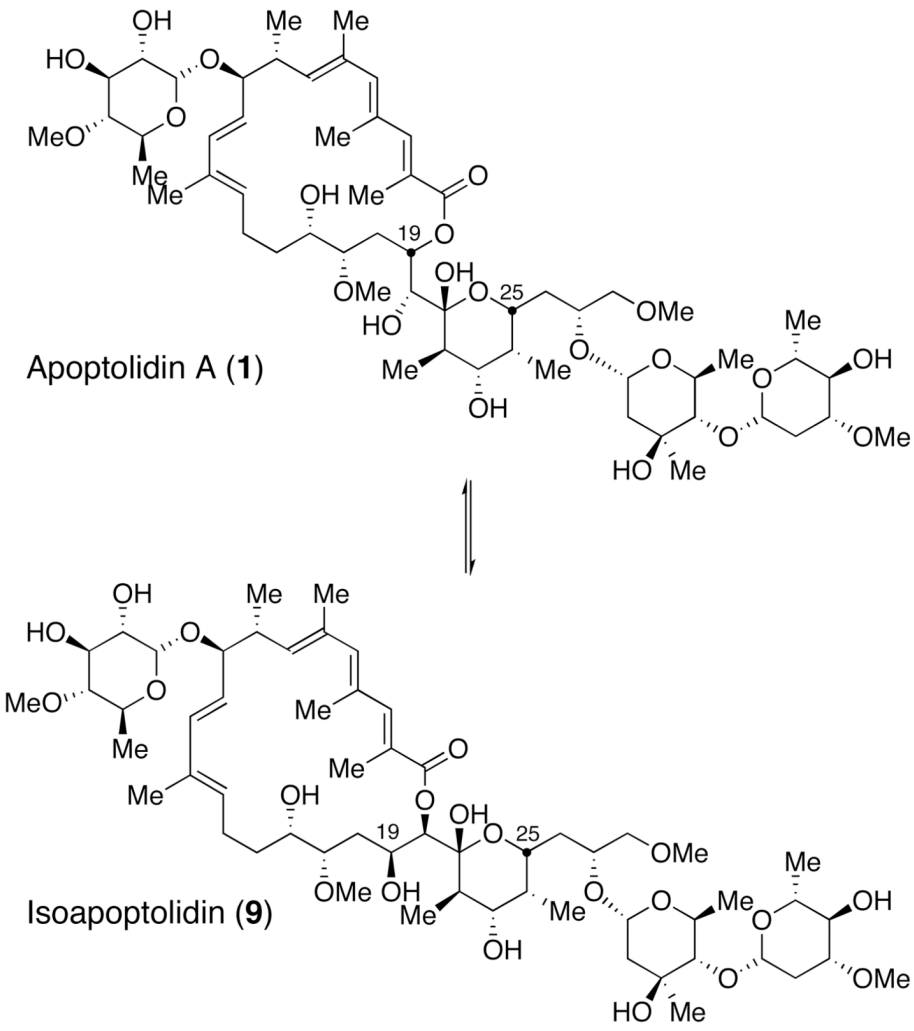


Figure 2.

High conversion reactions of apoptolidin A (**1**). (a) Reversed-phase HPLC trace of the reaction mixture when **10** is employed as the catalyst. (b) Reversed-phase HPLC trace of the reaction mixture when ***ent*-11** is employed as the catalyst.



^{1}H NMR Comparison of Apoptolidin and Analogs

	Apoptolidin A (1) δ ppm	Isoapoptolidin (9) δ ppm	12 δ ppm	13 δ ppm	14 δ ppm
C19-H	5.32	4.41	5.29	5.47	5.47
C25-H	3.99	4.21	3.95	4.00	4.01

Figure 3.
Diagnostic apoptolidin A (**1**) and isoapoptolidin (**9**) NMR data (CD_3OD solvent)

Table 1

Assay Results with Apoptolidin Analogs (modified from ref. 3f).

Compound		GI_{50} (μM) AD12-3Y1	GI_{50} (μM) 3Y1	IC_{50} (μM) F_oF_1-ATPase
Apoptolidin A	**1**	0.0065	> 1.0	0.7
C2′-OBz	**2**	0.0036	> 1.0	0.3
C4″′-OAc, C23-OAc	**3**	0.0095	> 0.6	0.4
C4″′-OAc	**4**	0.0098	> 1.0	0.8
C16-OAc	**5**	0.056	> 1.0	0.8
C3′-OAc	**6**	0.0027	> 1.0	0.4
C20-OAc	**7**	0.011	> 1.0	1.1
C20-OMe	**8**	0.012	> 1.0	2.8
Isoapoptolidin	**9**	0.009	> 1.0	17

Table 2

NMR Spectroscopic Data (CD_3OD) for Apoptolidin A Analogs[a]

	1	12	13	14		1	12	13	14
position	δ_H	δ_H, mult (*J* in Hz)	δ_H, mult (*J* in Hz)	δ_H, mult (*J* in Hz)	position	δ_H	δ_H, mult (*J* in Hz)	δ_H, mult (*J* in Hz)	δ_H, mult (*J* in Hz)
3	7.41	7.38, s	7.24, s	7.24, s	1’	4.85	4.96, d (3.8)	4.96, d (3.9)	5.00, m
5	6.23	6.19, s	6.15, m	6.17, m	2’	3.44	4.53, dd (10.0, 3.9)	4.55, m	4.70, dd (10.2, 3.7)
7	5.27	5.22, d (10.0)	5.22, d (10.2)	5.22, d (9.6)	3’	3.76	3.90, dd (10.0, 9.0)	3.90, dd (10.2, 9.0)	5.41, dd (10.2, 9.6)
8	2.79	2.66, m	2.70, m	2.74, m	4’	2.76	2.81, t (9.5)	2.81, t (9.6)	3.05, t (9.6)
9	3.87	3.82, m	3.83, d (9.0)	3.87, m	5’	3.78	3.80, m	3.79, dd (8.0, 8.0)	3.88, m
10	5.26	5.05, dd (15.5, 9.0)	5.05, dd (15.6, 9.0)	5.08, dd (15.6, 8.4)	6’	1.29	1.28, d (6.0)	1.27, d (6.6)	1.30, d (6.6)
11	6.21	6.15, d (16.0)	6.16, m	6.16, m	1”	4.97	4.94, d (4.5)	5.02, m	5.01, m
13	5.71	5.66, t (7.5)	5.65, dd (7.8, 7.8)	5.64, brd t (7.8)	2”	1.96	1.93, m	1.97, m	1.96, m
14	2.50	2.47, m	2.43, m	2.43, m		1.84	1.81, m	1.83, m	1.82, m
	2.09	2.10, m	2.14, m	2.10, m	4”	3.37	3.34, m	3.35, m	3.36, m
15	1.52	1.58, m	1.54, m	1.55, m	5”	3.70	3.67, dd (10.0, 6.5)	3.74, m	3.71, m
	1.44	1.45, m	1.38, m	1.37, m	6”	1.25	1.22, d (6.5)	1.24, d (6.6)	1.23, d (6.6)
16	3.47	3.41, m	3.36, m	3.36, m	3”-Me	1.36	1.32, s	1.34, s	1.34, s
17	2.75	2.71, dd (10.0, 5.0)	2.71, m	2.69, dd (9.6, 4.5)	1”’	4.86	4.83, dd (10.0, 1.9)	4.88, m	4.83, m
18	2.20	2.16, m	2.05, m	2.04, m	2”’	2.47	2.44, ddd (12.0, 4.6, 2.0)	2.49, ddd (12.0, 5.0, 1.7)	2.44, m
	1.78	1.81, m	1.79, m	1.79, m		1.32	1.31, m	1.41, m	1.30, m
19	5.32	5.29, dt (11.5, 1.6)	5.47, dd (11.4, 4.7)	5.47, dd (11.4, 4.7)	3”’	3.21	3.17, m	3.41, m	3.19, m
20	3.57	3.53, d (1.7)	5.03, m	5.01, m	4”’	3.01	2.97, t (9.5)	4.53, m	2.97, t (9.0)

	1	12	13	14		1	12	13	14
position	**δ_H**	**δ_H, mult (*J* in Hz)**	**δ_H, mult (*J* in Hz)**	**δ_H, mult (*J* in Hz)**	**position**	**δ_H**	**δ_H, mult (*J* in Hz)**	**δ_H, mult (*J* in Hz)**	**δ_H, mult (*J* in Hz)**
22	2.08	2.05, m	1.69, dd (11.4, 6.6)	1.70, m	5”’	3.24	3.20, m	3.41, m	3.22, m
23	3.76	3.71, dd (11.0, 4.7)	3.71, m	3.71, m	6”’	1.31	1.28, d (6.0)	1.15, d (6.6)	1.27, d (6.6)
24	1.76	1.73, m	1.77, m	1.77, m	OMe	3.61	3.59, s	3.61, s	3.48, s
25	3.99	3.95, ddd (8.0, 4.1, 2.2)	4.00, m	4.01, m	OMe	3.46	3.42, s	3.36, s	3.43, s
26	1.62	1.57, m	1.52, m	1.63, m	OMe	3.40	3.36, s	3.34, s	3.37, s
	1.49	1.45, m	1.62, m	1.49, m	OMe	3.30	3.27, s	3.33, s	3.33, s
27	3.48	3.46, m	3.73, m	3.73, m	OAc	--	2.08, s	2.08, s	2.08, s
28	3.36	3.33, m	3.41, m	3.40, m	OAc	--	--	2.08, s	2.08, s
2-Me	2.14	2.11, s	2.09, s	2.09, s	OAc	--	--	2.07, s	2.01, s
4-Me	2.21	2.19, s	2.18, s	2.18, s					
6-Me	1.97	1.95, d (1.0)	1.94, s	1.96, d					
8-Me	1.17	1.13, d (6.5)	1.13, d (6.6)	1.16, d (6.6)					
12-Me	1.71	1.65, s	1.65, s	1.65, s					
22-Me	1.06	1.02, d (6.5)	1.01, d (6.6)	1.01, d (6.6)					
24-Me	0.92	0.89, d (6.5)	0.88, d (6.6)	0.88, d (6.6)					

[a] A 500 MHz NMR spectrometer was employed for analog **12**; a 600 MHz NMR spectrometer was used for analysis of analogs **13** and **14**. Apoptolidin A (**1**) data modified from ref. [1a].

Table 3

Growth Inhibition Assay of New Apoptolidin Analogs in H292 Cells.

Compound	EC_{50} (nM)
1	21 ± 4
12	60 ± 11
13	48 ± 15
14	48 ± 10