Total Synthesis of Apoptolidin: Completion of the Synthesis and Analogue Synthesis and Evaluation

K. C. Nicolaou,† Yiwei Li,† Kazuyuki Sugita,† Holger Monenschein,† Prasuna Guntupalli,† Helen J. Mitchell,† Konstantina C. Fylaktakidou,† Dionisios Vourloumis,† Paraskevi Giannakakou,‡ and Aurora O’Brate‡

Contribution from the Department of Chemistry and The Skaggs Institute for Chemical Biology, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, California 92037, Department of Chemistry and Biochemistry, University of California, San Diego, 9500 Gilman Drive, La Jolla, California 92037, and Winship Cancer Institute, Emory University School of Medicine, Atlanta, Georgia 30322

Received August 18, 2003; E-mail: kcn@scripps.edu

Abstract: The total synthesis of apoptolidin (1) is reported together with the design, synthesis, and biological evaluation of a number of analogues. The assembly of key fragments 6 and 7 to vinyl iodide 3 via dithiane coupling technology was supplemented by a second generation route to this advanced intermediate involving a Horner–Wadsworth–Emmons coupling of fragments 22 and 25. The final stages of the synthesis featured a Stille coupling between vinyl iodide 3 and vinylstannane 2, a Yamaguchi lactonization, a number of glycosidations, and final deprotection. The developed synthetic technology was applied to the construction of several analogues including 74, 75, and 77 which exhibit significant bioactivity against tumor cells.

Introduction

In the preceding paper we discussed a retrosynthetic blueprint for apoptolidin (1) and described studies that led to the construction of the proposed building blocks required for the total synthesis of this formidable synthetic target. In this article, we detail our investigations which culminated in the first total synthesis of 1 and several of its analogues.

Results and Discussion

Figure 1 depicts a brief version of the retrosynthetic blueprint for apoptolidin (1), whose more detailed analysis was presented in the preceding paper. According to this analysis, the projected strategy calls for the assembly of fragments 2 and 4–7 to the final target via a sequence involving, in order of construction, the following key steps: (a) a dithiane coupling between 6 and 7 and elaboration of the resulting intermediate to a suitable vinyl iodide partner (3); (b) a Stille coupling to join vinyl iodide 3 with vinylstannane 2; (c) glycosidation of the formed intermediate and advancement to a seco acid; (d) Yamaguchi macrolactonization and elaboration to a more advanced intermediate; (e) glycosidation to attach the final disaccharide domain; and (f) final deprotection. We will begin the discussion of the total synthesis of apoptolidin (1) with our first attempt to construct the challenging vinyl iodide 3.

1. Coupling of Building Blocks 6 and 7 and Synthesis of Vinyl Iodide 3.

Beyond the construction of the key building blocks described in the preceding paper, the designed strategy toward apoptolidin (1) called for the coupling of aldehyde 6 (C12–C20 fragment) with dithiane 7 (C21–C28 fragment) and elaboration to vinyl iodide 3. Scheme 1 summarizes the initial stages of this directive, whereas Scheme 2 depicts the completion of the task. Thus, lithiation of dithiane 7 with tert-butyl lithium in the presence of HMPA in THF at −78 °C followed by cooling to −100 °C and addition of aldehyde 6 resulted in the generation of coupling product 8a,b (mixture of C20 epimers, ca. 1:5:1 ratio). Attempts aimed at improving the diastereoselectivity of this reaction by changing the conditions (e.g., additives, base) failed, but since we did not know at this stage the stereochemistry of the two isomers, we opted to press on until assignment could be made. Thus, each of the chromatographically separated isomers 8a and 8b was taken through the sequence as follows. First, the TBS groups were removed from the C16, C21, and C25 hydroxyl groups with TBAF (90% yield), forming 9a and 9b, compounds from which the dithiane moiety was cleaved through the action of Phl(OOCF3)2 to afford 10a and 10b (collapse of C25 hydroxy group onto the newly unveiled carbonyl group at C23). Tetraols 10a and 10b were then converted to their bis-silylated counterparts 11a and 11b by careful exposure to 2.5 equiv of TBSOTf in dichloromethane in the presence of 2,6-lutidine at −78 °C (78% yield, two steps). At this stage, an opportunity arose to rigidify the molecules around their C20–C21 regions by control of cyclic carbonate derivatives. To this end, 11a and 11b were exposed to the action of tripheospine (2)
in the presence of pyridine, furnishing carbonates 12a and 12b (88% yield). As shown in Figure 2, 1H NMR spectroscopic analysis (NOE) of these compounds revealed the major isomer (12a) as the desired C20 (R) isomer.
Once the identity of the correct C20 stereoisomer became apparent (a series), the next objective was to advance it further and to find a way to invert the incorrect isomer (b series) so that it could be funneled back into the main pathway toward the target molecule. To accomplish these goals, 11a and 11b were separately converted to their methoxy counterparts 13a and 13b by treatment with PPTS in methanol (95% yield) and the undesired alcohol 13b was oxidized (DMP, 85% yield) to the corresponding ketone (14) whose reduction with sodium borohydride in methanol proved to be completely stereoselective, producing the desired isomer 13a in 90% yield. The exquisite stereoselectivity in favor of 13a in this reaction may be explained on steric grounds based on a MonteCarlo-LowMode model (5) (see Figure 3). Specifically, the shielding of the right face (re face) of the carbonyl group within 14 by the C19 DMB moiety and the C22 methyl group leaves only the left face (si face) open for attack by the borohydride, leading to the observed stereoisomer.

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Figure 2. Stereochemical assignments of cyclic carbonates 12a and 12b based on H1 NMR spectroscopic analysis.

Figure 3. Ball and stick (BNS) model of ketone 14 showing the severe steric hindrance at the re face of the C20 stereocenter based on mixed MonteCarlo-LowMode calculation.

ZrHCl–I$_2$ procedure, but, unfortunately, could not advance in the desired direction, observing instead extensive decomposition.

After considerable experimentation, a solution was found to circumvent this problem that involved the methyl ortho ester$^7$ of 16a and 16b as shown in Scheme 2. Prepared from C$_{20}$–C$_{21}$ diols 11a and 11b [([MeO]$_3$CMe, PPTS, 95% yield], these C$_{20}$ epimeric ortho esters were subjected to sequential hydrozirconation and iodination to afford the E vinyl iodides 17a and 17b, each accompanied by small amounts (ca. 6:1 ratio) of its regioisomeric counterpart (90% combined yield). The undesired 17b, each accompanied by small amounts (ca. 6:1 ratio) of its conformation and iodination to afford the $E$ vinyl iodides 17a and 17b, each accompanied by small amounts (ca. 6:1 ratio) of its regioisomeric counterpart (90% combined yield).

The undesired C$_{20}$ epimer 17b was converted to the correct C$_{20}$ isomer by a two-stage oxidation–reduction protocol (DMP, 88% yield; NaBH$_4$, 86% yield) via ketone 18 as described above for 13b (Scheme 1). The remarkable collapse of the ortho ester moiety of 16a and 16b during the hydrozirconation–iodination sequence to the C$_{21}$ methyl glycosides 17a and 17b is rather intriguing. This reaction required migration of the methoxy group from the ortho ester site to the C$_{21}$ anomeric position. A postulated mechanism for this unusual cascade sequence is shown in Figure 4. Thus, complexation of zirconium with the anomeric carbon–oxygen bond-forming oxonium species II. This is followed by migration of the methoxy group onto the anomeric center leading to III, which under the conditions of the reaction collapses generating a free hydroxy group at C$_{20}$ (IV) as observed in the products 17a and 17b.

Intermediate 17a was then converted to triol 19 by removal of the PMB and DMB groups employing a two-step protocol. Thus, exposure of 17a to excess DDQ caused removal of the PMB group and engagement of the DMB with the nearby hydroxy group (C$_{20}$) to form, initially, the corresponding benzylidene system and, subsequently, the two regiosomeric aryl esters, which were hydrolyzed by treatment with LiOH in methanol to furnish triol 19. Finally, protection of the C$_{19}$–C$_{20}$ diol system as a cyclic carbonate (triphosgene–py, 88% yield), followed by silylation of the remaining C$_{27}$ hydroxy group.

According to our revised plan, the newly synthesized fragments 22 and 26 were coupled through a Horner–Wadsworth–Emmons reaction. Scheme 4 depicts this union and the further
elaboration of the product to the desired vinyl iodide 3. Thus, mixing of phosphonate 26 with activated Ba(OH)₂ in THF, followed by addition of aldehyde 22 in THF₂·H₂O (40:1), resulted in the formation of trans enone 27 in 80% yield. The mildness of this procedure is noteworthy as no epimerization at C 22 was observed and a variety of functional groups, including a free hydroxyl group at C 23 (i.e., 25 to 28, Scheme 4), were tolerated.

With an efficient way to generate enone substrates such as 27 and 28, we were now in a position to probe the feasibility of the asymmetric dihydroxylation reaction to form the desired C₁₉⁻C₂₀ syn diol system. As shown in Scheme 4, our first attempt to use the potent Sharpless AD-mix- 3α 10 in order to accomplish this goal failed, leading to an inseparable mixture (ca. 1:1) of the two possible isomers 29. Several modifications of this protocol aimed at improving the outcome also met with failure, forcing us into an investigation of different substrates as an alternative means to make headway along the designed pathway. It was reasoned that such substrate variations may, indeed, change the stereochemical outcome of this reaction based on the accepted mechanistic rational according to which a good stacking fit between the cinchona alkaloid chiral ligand and the olefinic substrate is important. 11 Bulky substituents, in particular, may disturb proper orientation of the substrate with regard to the required arrangement for high diastereoselectivity. It was with this hypothesis in mind that substrates 21a and 28 (see Table 1) were synthesized and subjected to asymmetric dihydroxylation, in addition to the originally tested 27. The truncated C₁₂⁻C₁₀ model enone 21a exhibited considerable diastereoselectivity, leading to the expected syn diol (90% yield, ca. 10:1 ratio of isomers), suggesting that the C₁₂⁻C₁₀ substituents have no stereocontrolling influence on the reaction. In contrast, the substituent on the C 23 oxygen exerted a strong influence on the dihydroxylation reaction switching from the random diastereoselection with the TBS derivative (27) to a satisfactory 6:1 ratio in 85% yield (see Table 1). This result is even more remarkable if we consider that the stereocontrolling element (the group on the C 23 oxygen) is situated four carbons away from the olefinic site where the reaction takes place. Dihydroxylation was also performed on enone 28, employing the opposite chiral ligand (AD-mix-β), affording the syn diol with the antipodal stereochemistry in comparable yield and diastereoselectivity. This observation pointed to the fact that the C 23 hydroxy group was essentially a bystander as far as the diastereoselectivity. It was with this hypothesis in mind that substrates 21a and 28 (see Table 1) were synthesized and subjected to asymmetric dihydroxylation, in addition to the originally tested 27. The truncated C₁₂⁻C₁₀ model enone 21a exhibited considerable diastereoselectivity, leading to the expected syn diol (90% yield, ca. 10:1 ratio of isomers), suggesting that the C₁₂⁻C₁₀ substituents have no stereocontrolling influence on the reaction. In contrast, the substituent on the C 23 oxygen exerted a strong influence on the dihydroxylation reaction switching from the random diastereoselection with the TBS derivative (27) to a satisfactory 6:1 ratio in 85% yield (see Table 1). This result is even more remarkable if we consider that the stereocontrolling element (the group on the C 23 oxygen) is situated four carbons away from the olefinic site where the reaction takes place. Dihydroxylation was also performed on enone 28, employing the opposite chiral ligand (AD-mix-β), affording the syn diol with the antipodal stereochemistry in comparable yield and diastereoselectivity. This observation pointed to the fact that the C 23 hydroxy group was essentially a bystander as far as the stereocontrol of the dihydroxylation was concerned and that it was the bulkiness of its substituent that had the decisive influence on this process.

With a stereoselective entry into the desired C₁₉⁻C₂₀ diol system 30 established, the next phase of the drive toward vinyl

**Scheme 3.** Synthesis of Aldehyde 22 and β-Ketophosphonate 26a

<table>
<thead>
<tr>
<th>Entry</th>
<th>Substrate</th>
<th>Diastereoselectivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td>10 : 1</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>1 : 1</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>6 : 1</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>1 : 5</td>
</tr>
</tbody>
</table>

a For reagents and conditions, see Scheme 4. AD-mix-α employed, 90% yield. AD-mix-α employed, 85% yield. AD-mix-α employed, 85% yield. AD-mix-β employed, 85% yield.


iodide 3 could be addressed. Thus, desilylation of 30 (TBAF, silica, 90% yield) provided pentaol 31 in which the newly unveiled hydroxy group (C25) engaged the C21 carbonyl system and thus could be selectively protected as a methyl ether by the action of TsOH in methanol, leading to 32. The latter compound was then exposed to triphosgene and pyridine in dichloromethane to afford the cyclic carbonate 33 in 88% overall yield from 31. Protection of the remaining two hydroxyl groups (C16 and C23) was then achieved by treatment with TBSOTf (3.0 equiv) in THF:H2O (40:1), 0 °C, 22 h, 85% for 34; (c) K2CO3 (3.5 equiv), NaHCO3 (4.5 equiv), MeSO2NH2 (1.3 equiv), (DHQ)2PHAL (0.05 equiv), OsO4 (0.01 equiv), CH2Cl2, 60 °C, 16 h, 90%; (d) TsOH (catalytic), MeOH, 25 °C, 2 h; (e) triphosgene (1.5 equiv), pyridine (20 equiv), CH2Cl2, −78 °C → 0 °C, 30 min, 88% over two steps; (f) TBSOTf (3.0 equiv), 2,6-lutidine (4.0 equiv), CH2Cl2, 25 °C, 45 min, 96%; (g) (Cp)2ZrHCl (3.0 equiv.), THF, 65 °C, 3 h; (h) THF, −25 °C, 2 min, ca. 5:1 ratio of regioisomers, 90%; (j) triphosgene (1.8 equiv), pyridine (30.0 equiv), CH2Cl2, −78 °C → 0 °C, 30 min, 90%; (j) DDQ (2.0 equiv), CH2Cl2:H2O pH buffer 7 (1:1), 0 → −25 °C, 1 h, 90%; (k) TESOTf (1.5 equiv), 2,6-lutidine (2.0 equiv), CH2Cl2, −78 °C, 99%. TsOH = toluenesulfonic acid; (DHQ)2PHAL = hydroquinine 1,4-phthalazinediyl diether.

3. Final Stages of the Total Synthesis of Apoptolidin. The completion of the total synthesis of apoptolidin (1) from building blocks 2–5 is shown in Scheme 5. Thus, initial coupling of vinylstannane 23 with vinyl iodide 3 as facilitated by PdCl2(MeCN)2 catalyst in degassed DMF14 at ambient temperature afforded diene 38 with complete stereocontrol and in 86% yield. The next requirement was the attachment of carbohydrate unit A, an objective which was achieved by a glycosidation reaction between glycosyl donor 4 and allylic alcohol (C9) 38 according to the Kahne protocol15 (activation with Tf2O in the presence of DTBMP at −90 °C), affording glycoside 39. The α-stereochimistry of the newly formed glycoside bond within 39 was

(12) In an attempted removal of the C27 PMB protecting group after the Stille coupling reaction (see Scheme 5), we observed as the major product the Dnels−Alder cycloadduct between DDQ and the C1−C6 diene moiety. This compound was characterized by 1H NMR, IR and MS spectrometry.

(13) Attempted Stille coupling reactions on substrates in which the C1 hydroxy group was protected (as TBS or TMS ethers or acetate) were not successful. For related observations and alternative coupling conditions, see also: Schupper, J.; Wehlan, H.; Kesper, S.; Korti, U. Angew. Chem., Int. Ed. 2001, 40, 2063–2066.


confirmed by the observed coupling constant of the anomeric proton \((J_{1,2} = 3.5 \text{ Hz})\) with its neighboring proton (H-2). In preparation for the obligatory macrolactonization reaction, conditions were sought and found for the selective cleavage of the \(C_1\) ester group and \(C_{19}-C_{20}\) carbonate ring without damaging the sensitive TES ether at \(C_{27}\).\(^{16}\) The successful conditions involved exposure of 39 to KOH in \(\text{dioxane}:\text{H}_2\text{O} (20:1)\) at 65°C, furnishing dihydroxy carboxylic acid 40. The desired Yamaguchi macrolactonization was then brought about by treatment of 40 with 2,4,6-trichlorobenzoyl chloride in THF in the presence of triethylamine, followed by dilution of the resulting mixed anhydride in toluene containing excess 4-DMAP. Proceeding at ambient temperature, this reaction furnished the desired \(C_1-C_{19}\) lactone 41 (27% yield over three steps from diene 38) as confirmed by NMR spectroscopic analysis of 41 and its derivative, \(C_{20}\) dichloroacetate 42. Thus, COSY 1H NMR spectral data of 41 and 42 revealed the \(C_{10}\) position as the lactone site rather than the \(C_{20}\). The dichloroacetate 42, which was also proven to be an appropriate intermediate for further elaboration, was prepared, after considerable experimentation, by exposure of 41 to excess dichloroacetic anhydride in neat pyridine for 5 min, followed by flash column chromatography (90% yield). It is noteworthy that attempts to install a silyl group (TBS or TES) onto this hydroxyl group (\(C_{20}\)) failed, presumably due to the severe steric congestion, and so did standard acetylation with the anhydride (Ac2O) in the presence of 4-DMAP, the latter conditions leading to decomposition.\(^{17}\)

To complete the skeletal framework of apoptolidin (1) from 42, the disaccharide unit 5 had to be introduced at \(C_{27}\). To this end, the silyl group guarding that position was first removed (PPTS, MeOH, 80% yield) and the resulting hydroxy compound 43 was glycosidated with glycosyl donor 5 in the presence of \(\text{SnCl}_2\) in ether to afford the desired \(\alpha\)-glycoside 44 in 70% yield. The \(\alpha\)-stereochemistry of the newly, and exclusively, formed


glycoside bond was evident from the relatively small coupling constant ($J_{1,2} = 3.0$ Hz) associated with the relevant anomic proton.

While fully protected apoptolidin 44 was quite stable under refrigerator conditions, its global deprotection to apoptolidin (1) proved problematic. It was soon ascertained that the chemical sensitivity of apoptolidin itself was the main reason for this challenging task, and therefore, studies were undertaken to evaluate the stability of the natural product under a variety of conditions. These investigations led to the recognition that it was under basic conditions that apoptolidin (1) became more vulnerable to destruction rather than acidic environment, which proved more hospitable to the molecule.18 Thus, in the presence of a variety of bases, 1 converted to an isomer, recently coined isoapoptolidin19 and identified as the 21-membered macrolactone formed by migration of the acyl group from the C$_{19}$ to the C$_{20}$ hydroxyl group. This facile isomerization could be completed within 30 min in the presence of K$_2$CO$_3$ in MeOH or reach a 1:1 mixture within 36 h when exposed to triethylamine in MeOH at ambient temperature. Interestingly, this migration was also observed in neutral aqueous MeOH upon standing at ambient temperature. Apoptolidin’s behavior under acidic conditions was observed in neutral aqueous MeOH upon standing at ambient temperature. Interestingly, this migration was also observed in neutral aqueous MeOH upon standing at ambient temperature. Apoptolidin’s behavior under acidic conditions was found to be dependent on medium and temperature. For example, while exposure of 1 to TsOH in MeOH at room temperature resulted in rapid decomposition, its relative resistance to PPTS in aqueous THF allowed its recovery from the reaction medium after 4 h at 0 °C. In an effort to explore possible desilylation conditions, we screened apoptolidin (1) against a series of fluoride reagents, including TBAF, TASF, HF-Et$_3$N, HF-py, neat HF, and aqueous HF. From these investigations, it was determined that 1 was reasonably stable to HF-py in THF at relatively low temperature, and this discovery led to the fine-tuning of the conditions, ultimately providing a solution to the thorny desilylation problem of 44.

The intelligence gathering described above helped shape the final conditions that led to the liberation of apoptolidin (1) from its protected form 44. As shown in Scheme 6, these carefully controlled conditions involved sequential exposure of 44 to excess HF-py in THF at −25 °C (to remove all six silyl groups), followed by treatment with Et$_3$N in MeOH (to cleave the dichloroacetyl group, 40% yield over two steps) and final exposure to TsOH in aqueous THF (to hydrolyze the methyl glycoside, 60% yield), furnishing apoptolidin (1) via its methyl glycoside 45. The physical and spectroscopic data ($^1$H NMR, IR, UV, $[\alpha]_D$, $R_f$, HPLC and HRMS) of the synthetic apoptolidin matched those of an authentic sample.20 As further confirmation of the structure of the synthetic apoptolidin methyl glycoside 45, natural apoptolidin (1) was converted to its methyl glycoside by exposure to PPTS in MeOH.21 The physical and spectroscopic properties of these samples also matched, providing the required support for their identity.

4. Molecular Design, Chemical Synthesis, and Biological Evaluation of Apoptolidin Analogues. Having developed the technology for the construction of apoptolidin (1) and because of the molecule’s selective cytotoxicity against certain tumor cells, we decided to pursue the chemical synthesis and biological evaluation of a series of analogues. The design of these...

(20) Samples of apoptolidin were kindly provided by Professor C. Khosla of Stanford University.
compounds was aimed at probing the various domains of the molecule for biological activity as part of a structure--activity relationship (SAR) study within the apoptolidin family. Thus, the following questions were posed: (a) is the carbohydrate domain CDE of apoptolidin (1) alone capable of biological action; (b) does the polyketide site C12–C27 by itself exhibit any biological activity; and (c) could the aglycon portion of the molecule or less glycosidated structures be sufficient for biological activity?22

Scheme 7 includes the synthesis of simple carbohydrate domain mimics 52, 54, and 56 starting from the Weinreb amide intermediate 46 (whose construction was described in the preceding paper). Thus, desilylation of 46 by exposure to TBAF furnished dihydroxy compound 47 (96% yield) whose treatment with TFA resulted in the formation of lactone 48 (85% yield). The latter compound served as a common intermediate for all three targeted analogues. Thus, silylation of 48 (TESOTf-2,6-lutidine, 87% yield) followed by PMB cleavage (DDQ, 80% yield) afforded hydroxy lactone 50 via 49. Attachment of the disaccharide unit 5 onto 50 as facilitated by SnCl2 furnished, stereoselectively, the α-glycoside 51 (68% yield), which fully desilylated to 52 upon treatment with TBAF (90% yield). In a

similar manner, glycosidation of 50 with disaccharide donor 55 (SnCl₂) led to tricyclic system 53 (60% yield), deprotection of which (TBAF, 86% yield) furnished system 54. Finally, simple deprotection of the PMB-protected alcohol in 48 (DDQ) gave model system 56 in 98% yield, completing the desired collection in this series of compounds.

The syntheses of the polyketide mimics 57, 58, 61, and 62 are summarized in Scheme 8. Thus, DDQ-induced removal of the PMB group from 31 and 32 led to targeted compounds 57 (80% yield) and 58 (85% yield), respectively. The construction of the more complex system 62 began with intermediate 59 (Scheme 8) obtained from 33 (Scheme 4) by standard silylation and proceeded through a sequence involving removal of the PMB group (DDQ, 85% yield) to afford 60, glycosidation of the latter compound with glycosyl donor 5 (SnCl₂, 73% yield), TBAF-mediated desilylation (90% yield), and final deprotection under acidic conditions (TsOH, 60% yield) of the resulting methyl glycoside 61.

Scheme 9 depicts the synthesis of the macrocyclic analogues 74 and 76. Thus, methylation of the previously synthesized alcohol 63 (NaH–Me, nBu₄NI, 98% yield) furnished methyl ether 64 whose hydroxirniconation (Cp₂ZrHCl) followed by iodine quench led to vinyl iodide 65 (85% overall yield). Exchange of the DMB group for an acetate within 65 required exposure to DDQ to generate hydroxy compound 66 (90% yield) followed by acetylation (Ac₂O, Et₃N, 4-DMAP, 85% yield), leading to 67. Coupling of 67 with vinylstannane 2 proceeded smoothly under the influence of PdCl₂ (MeCN)₂, generating polyene system 68 in 66% yield as a single stereoisomer. Silylation of the allylic alcohol within 68 (TBSCl–imidazole, 81% yield) followed by saponification with aqueous KOH in dioxane at 75°C led to seco acid 69, setting the stage for the anticipated macrocyclization. The latter compound (69) was subjected to a modified Yamaguchi procedure (2,4,6-trichlorobenzoyl chloride, Et₃N; 4-DMAP, 65°C), furnishing macrolactone 75 in 70% yield. Removal of the two silyl groups from 75 (HF-py, 80% yield) finally gave the targeted macrolide 76. In a separate branching sequence from 68, submission of allylic alcohol 68 to Kahne’s glycosidation conditions with glycosyl sulfoxide 70 (Tf₂O–DTBMP, 63% yield) afforded, after basic aqueous hydrolysis (aqueous KOH–dioxane, 75°C), glycoside seco acid 72. Yamaguchi macrolactonization (65% yield), followed by HF-py-induced desilylation (70% yield) as for the conversion of 69 to 76, led to the generation of 74 via 73. Finally, and as shown in Scheme 10, global deprotection of 41 (60% yield) afforded macrolactone system 77.
These three series of analogues were assayed for cytotoxic activity against 1A9 human ovarian carcinoma cells. Table 2 depicts the tested analogues in order of potency against these cells. Thus, it is interesting to note that while the lactone mimic analogues (entries 9–11, compounds 52, 54, and 56) and the polyketide analogues (entries 5–8, compounds 57, 58, 61, and 62) possess greatly reduced activity as compared to apoptolidin (1), the macrolide analogues (entries 2–4, compounds 74, 75, and 77) retained significant cytotoxic potency. From these results we can also infer that, although the carbohydrate and polyketide domains of the molecule by themselves do not invoke the cytotoxic action, they somehow enhance the exhibited potency of apoptolidin against tumor cells. Thus, a 2-fold increase in IC50 value was observed when carbohydrate A was attached onto the 20-membered macrolide ring at the proper position, i.e., the IC50 value of 75 was 45.0 μM, while that of 74 was at 20 μM. This trend was more apparent in analogue 77 which contains both carbohydrate A and the hemiketal ring C (IC50 = 11 μM).

These observations are in line with those reported by the Khosla and Wender groups. Furthermore, the structure–activity relationships evident from these results agree with the hypothesis put forward by Khosla, according to which the aglycone bestows biological activity while the carbohydrate side chains facilitate cellular transport of the molecule to its mitochondrial target.

(a) HF-py (excess), THF, −25 → 0 °C, 72 h, 4:1 ratio of regioisomers, 80%.

Table 2. Cytotoxicity of Synthesized Apoptolidin Analogues against 1A9 Human Ovarian Carcinoma Cells

<table>
<thead>
<tr>
<th>Entry</th>
<th>Compound</th>
<th>IC50 value (μM)</th>
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<tbody>
<tr>
<td>1</td>
<td>apoptolidin</td>
<td>0.24</td>
</tr>
<tr>
<td>2</td>
<td>74</td>
<td>11.5</td>
</tr>
<tr>
<td>3</td>
<td>77</td>
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<td>9</td>
<td>56</td>
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<td>10</td>
<td>54</td>
<td>62.0</td>
</tr>
<tr>
<td>11</td>
<td>52</td>
<td>83.0</td>
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</table>

(a) The antiproliferative effects of these compounds against the 1A9 human ovarian carcinoma cells were assessed in a 72 h growth inhibition assay using the SRB (sulforhodamine-B) assay. IC50 is defined as the concentration that leads to 50% growth inhibition. IC50 values for each compound are given in μM and represent a single growth inhibition experiment.

Conclusion

A highly convergent and enantioselective total synthesis of apoptolidin (I) has been achieved. Key features of this synthesis include high levels of stereoselectivity in a number of reactions, including the crotyl boration, allyl boration, asymmetric dihydroxylation, and aldol reaction employed in order to establish the required stereocenters. Stille and Suzuki coupling reactions, dithiane coupling technology, Kahne’s sulfoxide glycosidation, and the highly efficient 1,2-thiophenyl migration/glycosidation served as the keystones for the assembly of the building blocks. The flexibility of the described strategy allows its adaptation for the generation of a wide variety of apoptolidin analogues.
and a number of them have been synthesized and tested against tumor cells, establishing a general trend for structure—activity relationships. This research could ultimately facilitate chemical biology studies in the field of apoptosis, in general, and possibly in the elucidation of the detailed mechanism of action of this novel antitumor agent.

**Experimental Section**

**General Procedures.** All reactions were carried out under an argon atmosphere with dry solvents under anhydrous conditions, unless otherwise noted. Dry tetrahydrofuran (THF), toluene, diethyl ether (ether), and methylene chloride (CH₂Cl₂) were obtained by passing commercially available pre-dried, oxygen-free formulations through activated alumina columns. Yields refer to chromatographically and spectroscopically (¹H NMR) homogeneous materials, unless otherwise stated. Reagents were purchased at the highest commercial quality and used without further purification, unless otherwise stated. Reactions were monitored by thin-layer chromatography (TLC) carried out on 0.25 mm E. Merck silica gel plates (60F-254) using UV light as visualizing agent and an ethanolic solution of phosphomolybdic acid and cerium sulfate and heat as developing agents. E. Merck silica gel (60, particle size 0.040–0.063 mm) was used for flash column chromatography. Preparative thin-layer chromatography (PTLC) separations were carried out on 0.25 or 0.50 mm E. Merck silica gel plates (60F-254), NMR spectra were recorded on Bruker DRX-600, DRX-500, AMX-500, or AMX-400 instruments and calibrated using residual undeuterated solvent as an internal reference. The following abbreviations were used to explain the multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, b = broad. IR spectra were recorded on a Perkin-Elmer 1600 series FT-IR spectrometer. Electrospray ionization mass spectrometry (ESIMS) experiments were performed on an API 100 Perkin-Elmer SCIEX single quadrupole mass spectrometer at 4000 V emitter voltage. High-resolution mass spectra (HRMS) were recorded on a VG ZAB-ZSE mass spectrometer under fast atom bombardment (FAB) conditions with NBA as the matrix or using MALDI.

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**Supporting Information Available:** Experimental procedures and compound characterization. This material is available free of charge via the Internet at http://pubs.acs.org.

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