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

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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[1] 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** (C_{12}−C_{20} fragment) with dithiane **7** (C_{21}−C_{28} 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*-butyllithium 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 C_{20} epimers, ca. 1.5:1 ratio). Attempts aimed at improving the diastereoselectivity of this reaction by changing the conditions (e.g., additives, base)[2] 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 C_{16}, C_{23}, and C_{25} hydroxyl groups with TBAF (90% yield), forming **9a** and **9b**, compounds from which the dithiane moiety was cleaved through the action of $PhI(OCOCF_3)_2$[3] to afford **10a** and **10b** (collapse of C_{25} hydroxy group onto the newly unveiled carbonyl group at C_{21}). 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 C_{20}−C_{21} regions for NMR spectroscopic analysis through preparation of cyclic carbonate derivatives. To this end, **11a** and **11b** were exposed to the action of triphosgene

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(1) Nicolaou, K. C.; Fylaktakidou, K. C.; Monenschein, H.; Li, Y.; Weyershausen, B.; Mitchell, H. J.; Wei, H.; Guntupalli, P.; Hepworth, D.; Sugita, K. *J. Am. Chem. Soc.* **2003**, *125*, 15433−15442.

(2) For examples of stereochemically controlled dithiane coupling reactions, see: (a) Nicolaou, K. C.; Baran, P. S.; Zhong, Y.-L.; Fong, K. C.; Choi, H.-S. *J. Am. Chem. Soc.* **2002**, *124*, 2190−2201. (b) Smith, A. B., III; Condon, S. M.; McCauley, J. A.; Leazer, J. L., Jr.; Leahy, J. W.; Maleczka, R. E., Jr. *J. Am. Chem. Soc.* **1997**, *119*, 947−961.

(3) Stork, G.; Zhao, K. *Tetrahedron Lett.* **1989**, *30*, 287−290.

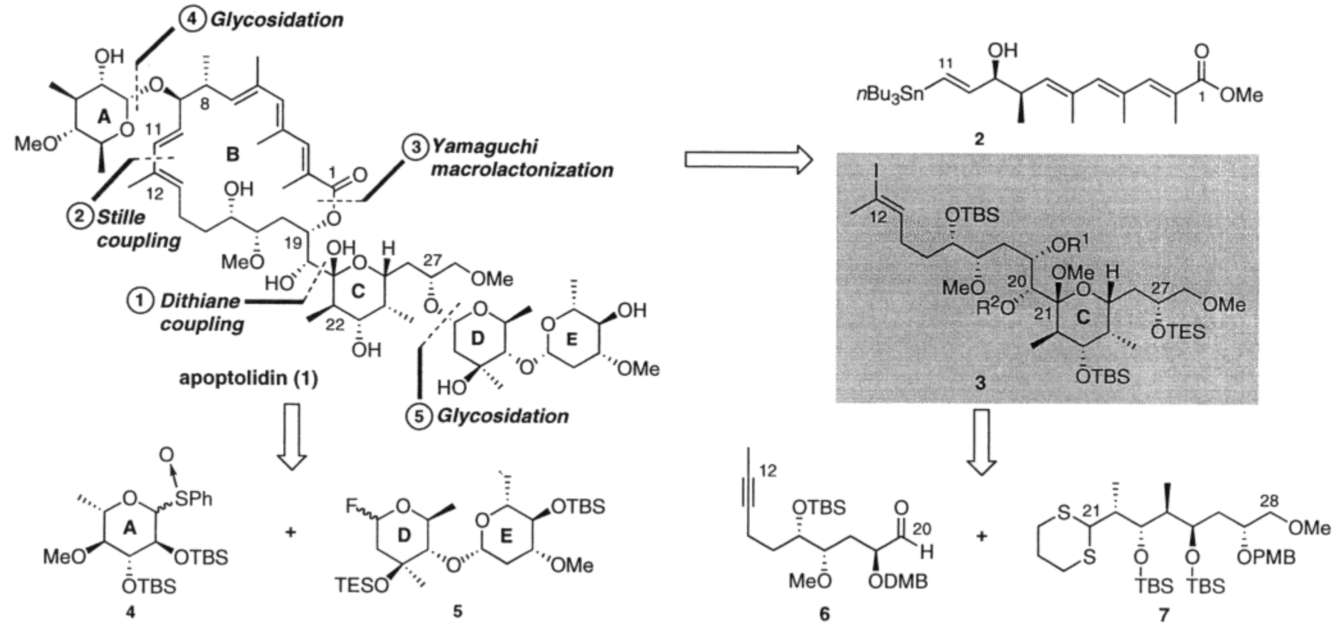
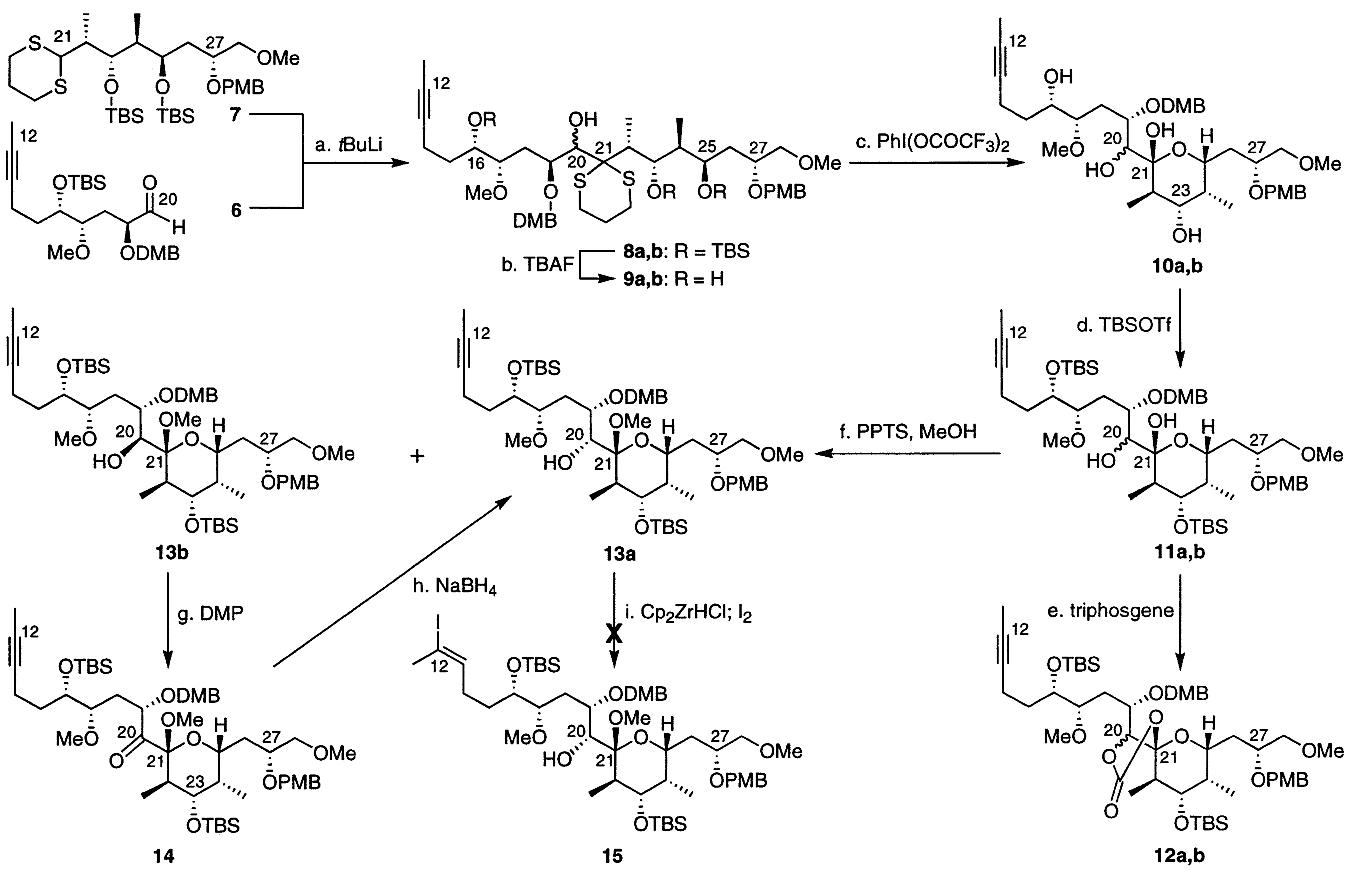


Figure 1. Brief retrosynthetic analysis of apoptolidin (**1**).

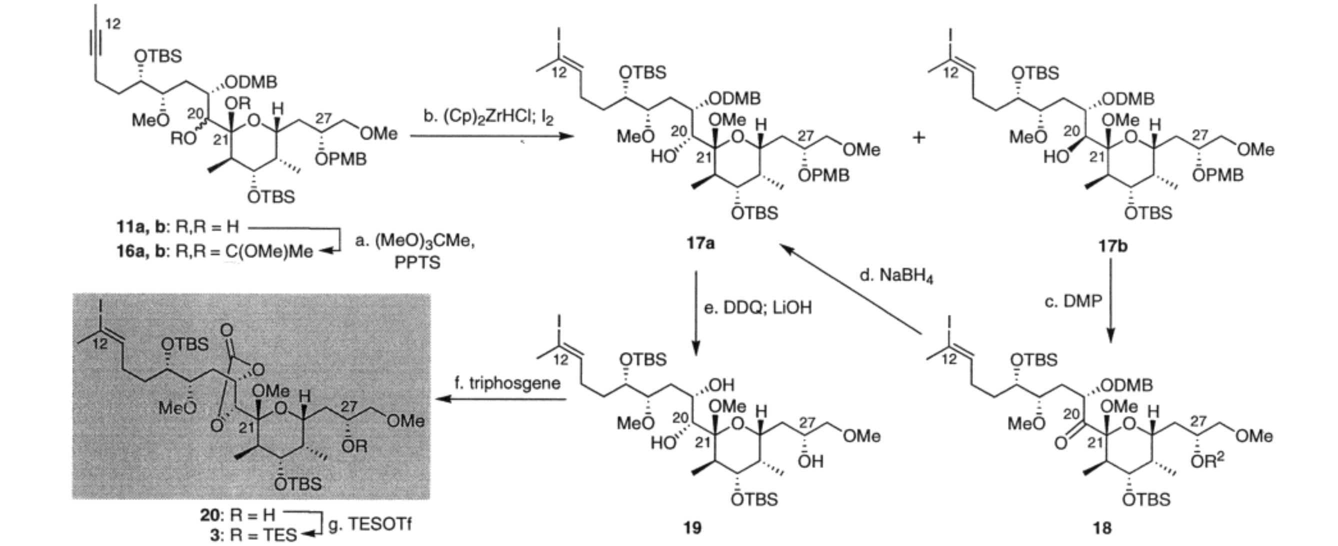
Scheme 1. Dithiane Coupling and Abortive Attempt To Synthesize Vinyl Iodide **15**[a]



[a] (a) Dithiane **7** (3.0 equiv), *t*BuLi (3.0 equiv), HMPA (16.0 equiv), THF, −78 °C, 1.5 h; then **6** (1.0 equiv), THF, −100 °C, 2 h, dr ca. 1.5:1, 96%, isomers chromatographically separated and taken through individually; (b) TBAF (6.0 equiv), THF, 25 °C, 12 h, 90%; (c) $PhI(OCOCF_3)_2$ (1.5 equiv), MeCN: phosphate buffer (pH = 7.0, 4:1), 0 °C, 10 min; (d) TBSOTf (2.5 equiv), 2,6-lutidine (5.0 equiv), CH_2Cl_2, −78 °C, 3 h, 78% over two steps; (e) triphosgene (1.5 equiv), py (30 equiv), CH_2Cl_2, −78 → 0 °C, 30 min, 88%; (f) PPTS (0.5 equiv), MeOH, 25 °C, 12 h, 95%; (g) DMP (2.0 equiv), $NaHCO_3$ (20 equiv), CH_2Cl_2, 25 °C, 1 h, 85%; (h) $NaBH_4$ (5.0 equiv), MeOH, 0 → 25 °C, 4 h, 90%; (i) $(Cp)_2ZrHCl$ (3.0 equiv), THF, 65 °C, 3 h; I_2 (3.0 equiv), THF, −25 °C, 2 min. DMP = Dess Martin periodianane; DMB = 3,4-dimethoxybenzyl; THF = tetrahydrofuran; PPTS = pyridinium *p*-toluenesulfonate; PMB = *p*-methoxybenzyl; TBS = *tert*-butyldimethylsilyl.

in the presence of pyridine,[4] furnishing carbonates **12a** and **12b** (88% yield). As shown in Figure 2, ^{1}H NMR spectroscopic analysis (NOE) of these compounds revealed the major isomer (**12a**) as the desired C_{20} (*R*) isomer.

Scheme 2. Synthesis of Vinyl Iodide **3**[a]



[a] (a) $(MeO)_3CMe$ (50 equiv), PPTS (0.1 equiv), CH_2Cl_2, 25 °C, 12 h, 95%; (b) $(Cp)_2ZrHCl$ (3.0 equiv), THF, 65 °C, 3 h; I_2 (3.0 equiv), THF, −25 °C, 2 min, ca. 6:1 ratio of regioisomers, 90%; (c) DMP (2.0 equv), $NaHCO_3$ (50 equiv), CH_2Cl_2, 25 °C, 1 h, 88%; (d) $NaBH_4$ (5.0 equiv), MeOH:ether (1:1), 0 → 25 °C, 4 h, 86%; (e) DDQ (4.0 equiv), CH_2Cl_2: phosphate buffer (pH = 7, 1:1), 0 → 25 °C, 4 h; LiOH (6.7 equiv), MeOH, 25 °C, 12 h, 85% over two steps; (f) triphosgene (1.1 equiv), py (30 equiv), CH_2Cl_2, −78 → 0 °C, 30 min, 88%; (g) TESOTf (6.0 equiv), 2,6-lutidine (12.0 equiv), CH_2Cl_2, −78 °C, 2 h, 95%. DDQ = 2,3-dichloro-5,6-dicyano-1,4-benzoquinone; TES = triethylsilyl.

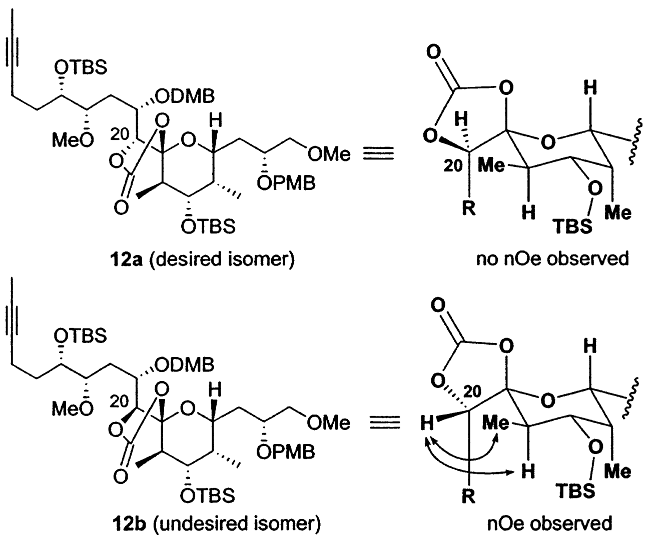


Figure 2. Stereochemical assignments of cyclic carbonates **12a** and **12b** based on H[1] NMR spectroscopic analysis.

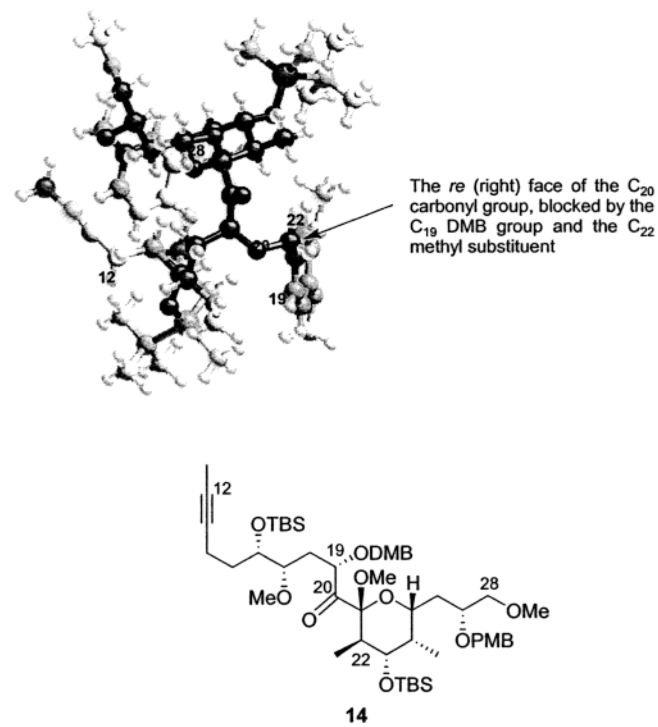


Figure 3. Ball and stick (BNS) model of ketone **14** showing the severe steric hindrance at the *re* face of the C20 stereocenter based on mixed MonteCarlo-LowMode calculation.

Once the identity of the correct C_{20} 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 C_{19} DMB moiety and the C_{22} methyl group leaves only the left face (*si* face) open for attack by the borohydride, leading to the observed stereoisomer. Having reached **13a** and directed all material into the desired isomer (**13a**), we then attempted to construct the required *E* vinyl iodide **15**. For this transformation, we turned to Schwartz's hydrozirconation/iodination (Cp_2-

(4) Burk, R. M.; Roof, M. B. *Tetrahedron Lett.* **1993**, *34*, 395−398.

(5) The authors thank Dr. Chenglong Li for these calculations. For details of the calculation method employed, see: Kolossváry, I.; Guida, W. C. Low Mode Search. *J. Am. Chem. Soc.* **1996**, *118*, 5011−5019.

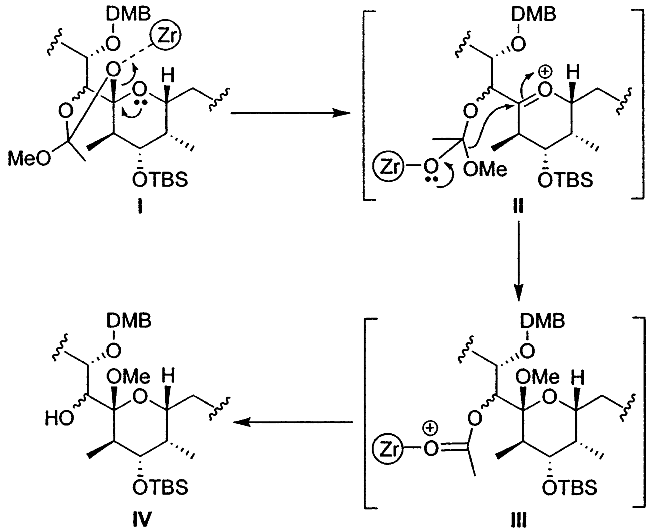


Figure 4. Postulated mechanistic rational for the Zr-mediated rupture of ortho ester **I** (**16a**,**b**) to methyl glycoside **IV** (**17a**,**b**).

$ZrHCl-I_2$)[6] 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** [$(MeO)_3CMe$, 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 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 ortho ester oxygen as in **I** initiates rupture of 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 regioisomeric 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

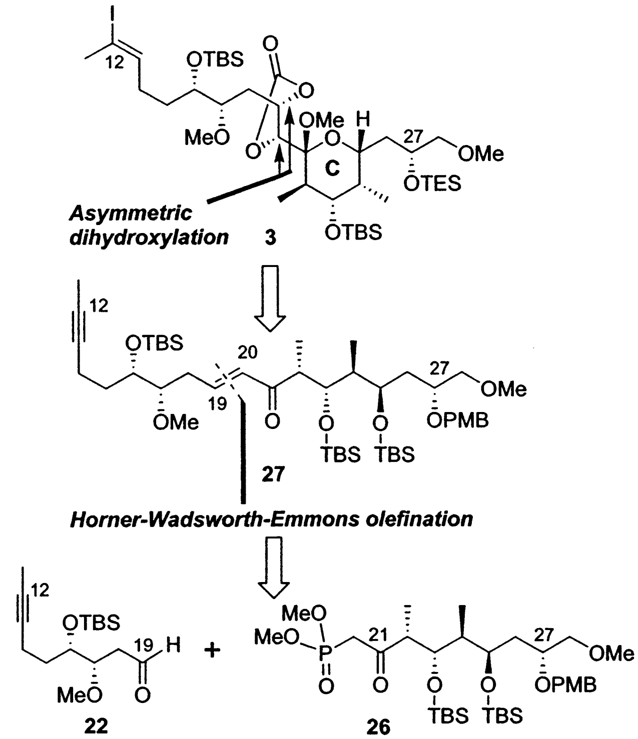


Figure 5. Second generation retrosynthetic analysis of vinyl iodide (**3**).

(TESOTf, 2,6-lutidine, 95% yield), furnished the desired $C_{12}-C_{28}$ advanced intermediate **3**.

Arriving at **3** was a milestone accomplishment in the campaign toward apoptolidin (**1**), but the overall efficiency of the charted route left considerable room for improvement. A second exploration aimed at improving this situation was, therefore, undertaken and culminated in a more concise route to this key intermediate (**3**).

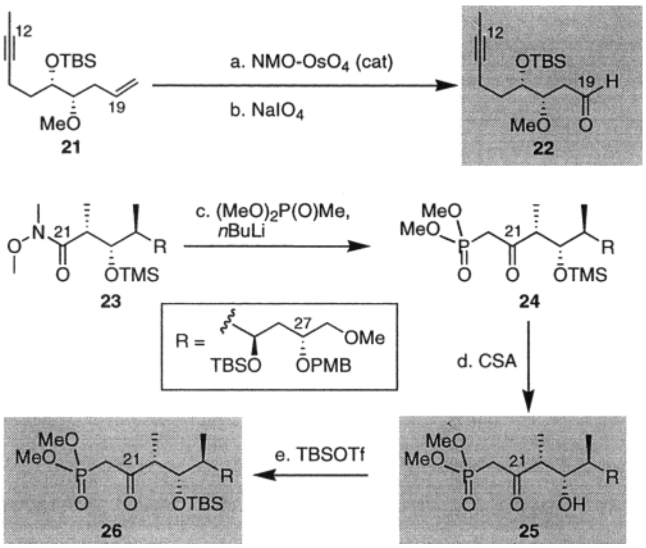
2. Second Generation Synthesis of Vinyl Iodide 3. In contemplating an alternative approach to the $C_{12}-C_{28}$ fragment **3**, the idea of securing the $C_{19}-C_{20}$ diol system from the corresponding *trans* olefin through asymmetric dihydroxylation came to mind. This analysis led, retrosynthetically, to substrate **27** upon opening ring C and generating the acetylenic moiety at C_{12}, whose disconnection, as shown in Figure 5, revealed aldehyde **22** and phosphonate **26** as potential starting points for this new endeavor.

The constructions of the newly defined building blocks **22** ($C_{12}-C_{19}$ fragment) and **26** ($C_{20}-C_{28}$ fragment) began with readily available intermediates[1] (**21** and **23**, respectively) already encountered and are shown in Scheme 3. Thus, olefinic compound **21** was subjected to dihydroxylation (OsO_4−NMO) to afford a ca. 1:1 mixture of 1,2-diols whose cleavage with sodium periodate led to **22** in 80% overall yield. For the synthesis of **26**, Weinreb amide **23** was reacted with an excess of the lithium reagent obtained from dimethyl methylphosphonate and *n*-butyllithium to afford phosphonate **24** (86% yield). The rather labile TMS group was excised from the latter compound (**24**) by treatment with CSA (97% yield) and replaced with the more robust TBS group, leading to the desired phosphonate fragment **26** (98% yield).

According to our revised plan, the newly synthesized fragments **22** and **26** were coupled through a Horner−Wadsworth−Emmons reaction.[8] Scheme 4 depicts this union and the further

(6) (a) Hart, D. W.; Blackburn, T. F.; Schwartz, J. *J. Am. Chem. Soc.* **1975**, *97*, 679−680. (b) Panek, J. S.; Hu, T. *J. Org. Chem.* **1997**, *62*, 4912−4913. (3) Nicolaou, K. C.; Bertinato, P.; Piscopio, A. D.; Chakraborty, T. K.; Minowa, N. *J. Chem. Soc., Chem. Commun.* **1993**, 619−622.
(7) Reese, C. B.; Sulston, J. E. *Proc. Chem. Soc.* **1964**, 214−215.

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



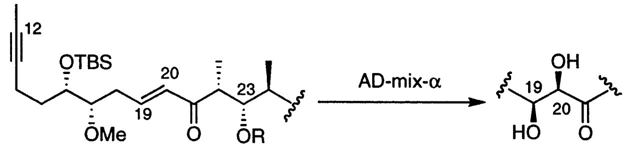
[a] (a) OsO_4 (0.05 equiv), NMO (4.0 equiv), *t*BuOH:THF:H_2O (10:2:1), 25 °C, 5 h; (b) $NaIO_4$ (3.0 equiv), *t*BuOH:THF:H_2O (20:2:1), 25 °C, 3 h, 80% over two steps; (c) $(MeO)_2P(O)Me$ (11.5 equiv), *n*BuLi (10.0 equiv), THF, −78 °C, 5 min, 86%; (d) CSA (0.1 equiv), MeOH:CH_2Cl_2 (1:10), 30 °C, 2 h, 97%; (e) TBSOTf (2.0 equiv), 2,6-lutidine (4.0 equiv), CH_2Cl_2, 0 → 25 °C, 98%. NMO = 4-methylmorpholine *N*-oxide; TMSOTf = trimethylsilyl trifluromethanesulfonate; CSA = camphorsulfonic acid.

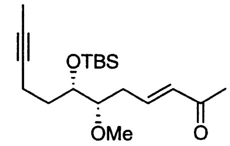
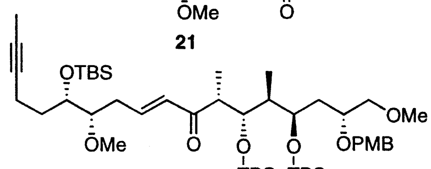
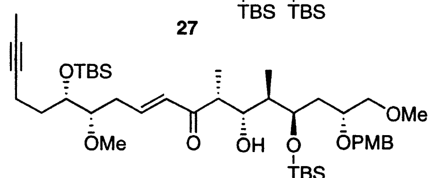
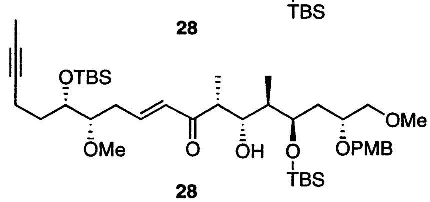
elaboration of the product to the desired vinyl iodide **3**. Thus, mixing of phosphonate **26** with activated $Ba(OH)_2$[9] in THF, followed by addition of aldehyde **22** in THF:H_2O (40:1), resulted in the formation of *trans* enone **27** in 80% yield. The mildness of this procedure is noteworthy 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 $C_{19}-C_{20}$ *syn* diol system. As shown in Scheme 4, our first attempt to use the potent Sharpless AD-mix-α[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 $C_{12}-C_{20}$ model enone **21a** exhibited considerable diastereoselectivity, leading to the expected *syn* diol (90% yield, ca. 10:1 ratio of isomers), suggesting that the $C_{12}-C_{19}$ 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 of products (desired:undesired isomers, 85% combined yield) with the free hydroxy compound (**28**). This striking 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 $C_{19}-C_{20}$ diol system **30** established, the next phase of the drive toward vinyl

Table 1. Influence of the C-23 Substituent on the Stereochemical Outcome of the Asymmetric Dihydroxylation of the C19−C20 Olefin[a]

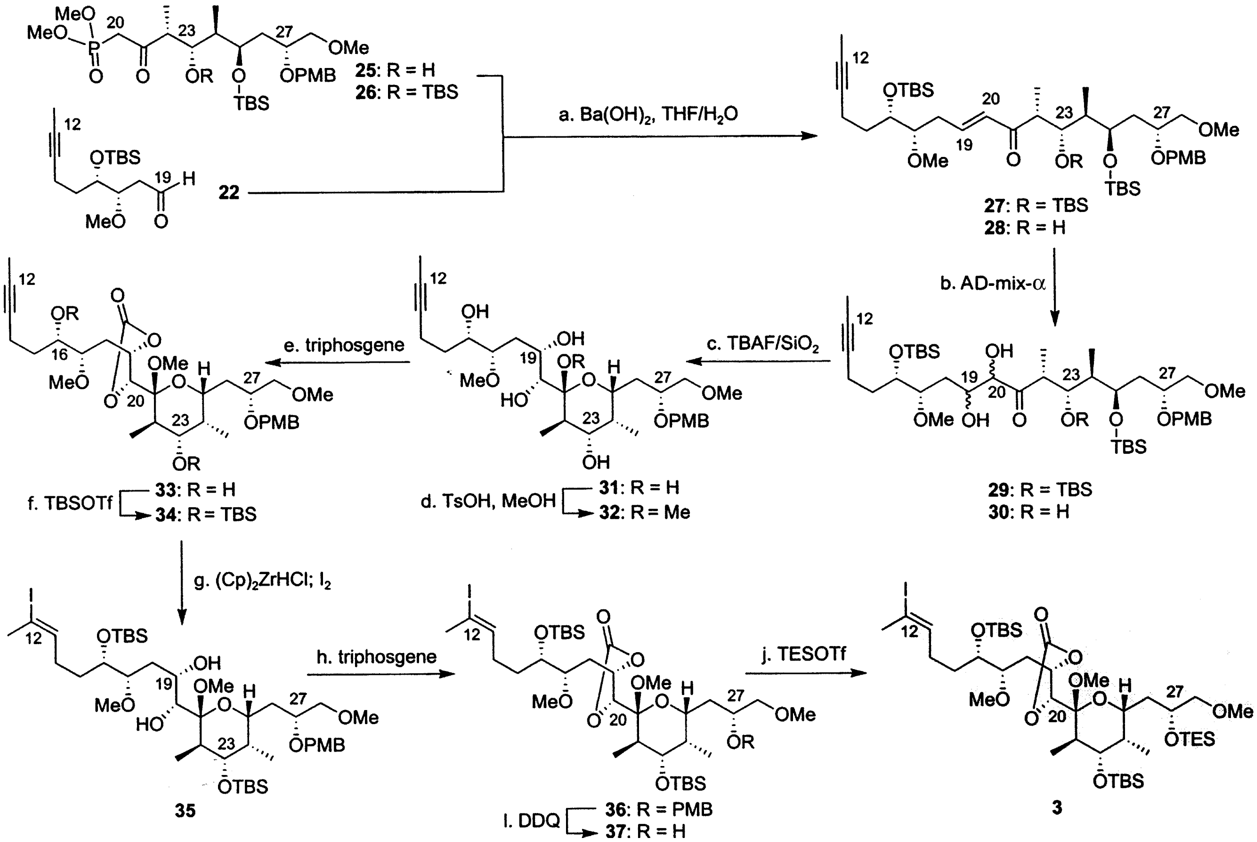


Entry	Substrate	Diastereoselectivity (C_{19}-C_{20}) *S, R* : *R, S*
1[b]	 **21**	10 : 1
2[c]	 **27**	1 : 1
3[d]	 **28**	6 : 1
4[e]	 **28**	1 : 5

[a] For reagents and conditions, see Scheme 4. [b] AD-mix-α employed, 90% yield. [c] AD-mix-α employed, 85% yield. [d] AD-mix-α employed, 85% yield. [e] AD-mix-β employed, 85% yield.

(8) For reviews of the Horner−Wadsworth−Emmons reaction, see: (a) Wadsworth, W. S., Jr. *Org. React.* **1977**, *25*, 73−254. (b) Maryanoff, B. E.; Reitz, A. B. *Chem. Rev.* **1989**, *89*, 863−927.
(9) Paterson, I.; Yeung, K. S.; Smaill, J. B. *Synlett* **1993**, 774−776. (b) Theisen, P. D.; Heathcock, C. H. *J. Org. Chem.* **1988**, *53*, 2374−2378.
(10) For review, see: Kolb, H. C.; VanNieuwenhze, M. S.; Sharpless, K. B. *Chem. Rev.* **1994**, *94*, 2483−2547. For representative examples of asymmetric dihydroxylation of electron-deficient olefins, see: (a) Bennani, Y. L.; Sharpless, K. B. *Tetrahedron Lett.* **1993**, *34*, 2079−2082. (b) Walsh, P. J.; Sharpless, K. B. *Synlett* **1993**, *8*, 605−606. (c) Nicolaou, K. C.; Li, J.; Zenke, G. *Helv. Chim. Acta* **2000**, *83*, 1977−2006.
(11) (a) Kolb, H. C.; Andersson, P. G.; Sharpless, K. B. *J. Am. Chem. Soc.* **1994**, *116*, 1278−1291. (b) Wu, Y.-D.; Wang, Y.; Houk, K. N. *J. Org. Chem.* **1992**, *57*, 1362−1369.

Scheme 4. Second Generation Synthesis of Vinyl Iodide **3**[a]



[a] (a) $Ba(OH)_2$ (1.9 equiv), THF, 0 °C; then **22** (0.5 equiv) in THF:H_2O (40:1), 0 → 25 °C, 30 min, 80% for **27**, 68% for **28**; (b) $K_3Fe(CN)_6$ (3.5 equiv), K_2CO_3 (3.5 equiv), $NaHCO_3$ (4.5 equiv), $MeSO_2NH_2$ (1.3 equiv), $(DHQ)_2PHAL$ (0.05 equiv), OsO_4 (0.01 equiv), *t*BuOH:H_2O (1:1), 0 °C, 16 h, **29**: dr ca. 1:1, 85%; **30**: dr ca. 6:1, 83%; (c) TBAF/SiO_2 (6.0 equiv), THF, 25 °C, 16 h, 90%; (d) TsOH (catalytic), MeOH, 25 °C, 2 h; (e) triphosgene (1.5 equiv), pyridine (20 equiv), CH_2Cl_2, −78 → 0 °C, 30 min, 88% over two steps; (f) TBSOTf (3.0 equiv), 2,6-lutidine (4.0 equiv), CH_2Cl_2, 0 → 25 °C, 45 min, 96%; (g) $(Cp)_2ZrHCl$ (3.0 equiv.), THF, 65 °C, 3 h; I_2 (4.0 equiv), THF, −25 °C, 2 min, ca. 5:1 ratio of regioisomers, 90%; (h) triphosgene (1.8 equiv), pyridine (30.0 equiv), CH_2Cl_2, −78 → 0 °C, 30 min, 90%; (i) DDQ (2.0 equiv), CH_2Cl_2:H_2O pH buffer 7 (1:1), 0 → 25 °C, 1 h, 90%; (j) TESOTf (1.5 equiv), 2,6-lutidine (2.0 equiv), CH_2Cl_2, −78 °C, 99%. TsOH = toluenesulfonic acid; $(DHQ)_2PHAL$ = hydroquinine 1,4-phthalazinediyl diether.

iodide **3** could be addressed. Thus, desilylation of **30** (TBAF, silica, 90% yield) provided pentaol **31** in which the newly unveiled hydroxy group (C_{25}) engaged the C_{21} carbonyl functionality in a six-membered lactol. Of the five hydroxyl groups within **31**, the C_{21} moiety is unique as part of a hemiketal 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 (C_{16} and C_{23}) was then achieved by treatment with TBSOTf and 2,6-lutidine, leading to bis-silyl ether **34** in 96% yield. The much anticipated regioselective hydrozirconation (Cp_2ZrHCl) of **34** was followed by in situ quenching with iodine to furnish the desired *E* vinyl iodide **35**, from which the carbonate moiety had been concomitantly removed in 90% yield (ca. 5:1 regioselectivity). The cyclic carbonate was then reintroduced on **35** through the use of triphosgene and pyridine, leading to **36** (90% yield), and the PMB group was replaced by a TES group by exposure to DDQ (to afford **37**, 90% yield), followed by treatment with TESOTf−2,6-lutidine (99% yield), furnishing the targeted vinyl iodide **3**. The final exchange of the PMB group for a TES group was far from arbitrary, as control experiments had indicated that the required oxidative removal of the PMB group down the road would be harmful to the sensitive polyolefinic sites of the molecule.[12]

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 **2**[13] with vinyl iodide **3** as facilitated by $PdCl_2(MeCN)_2$ catalyst in degassed DMF[14] 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 (C_9) **38** according to the Kahne protocol[15] (activation with Tf_2O in the presence of DTBMP at −90 °C), affording glycoside **39**. The α-stereochemistry of the newly formed glycoside bond within **39** was

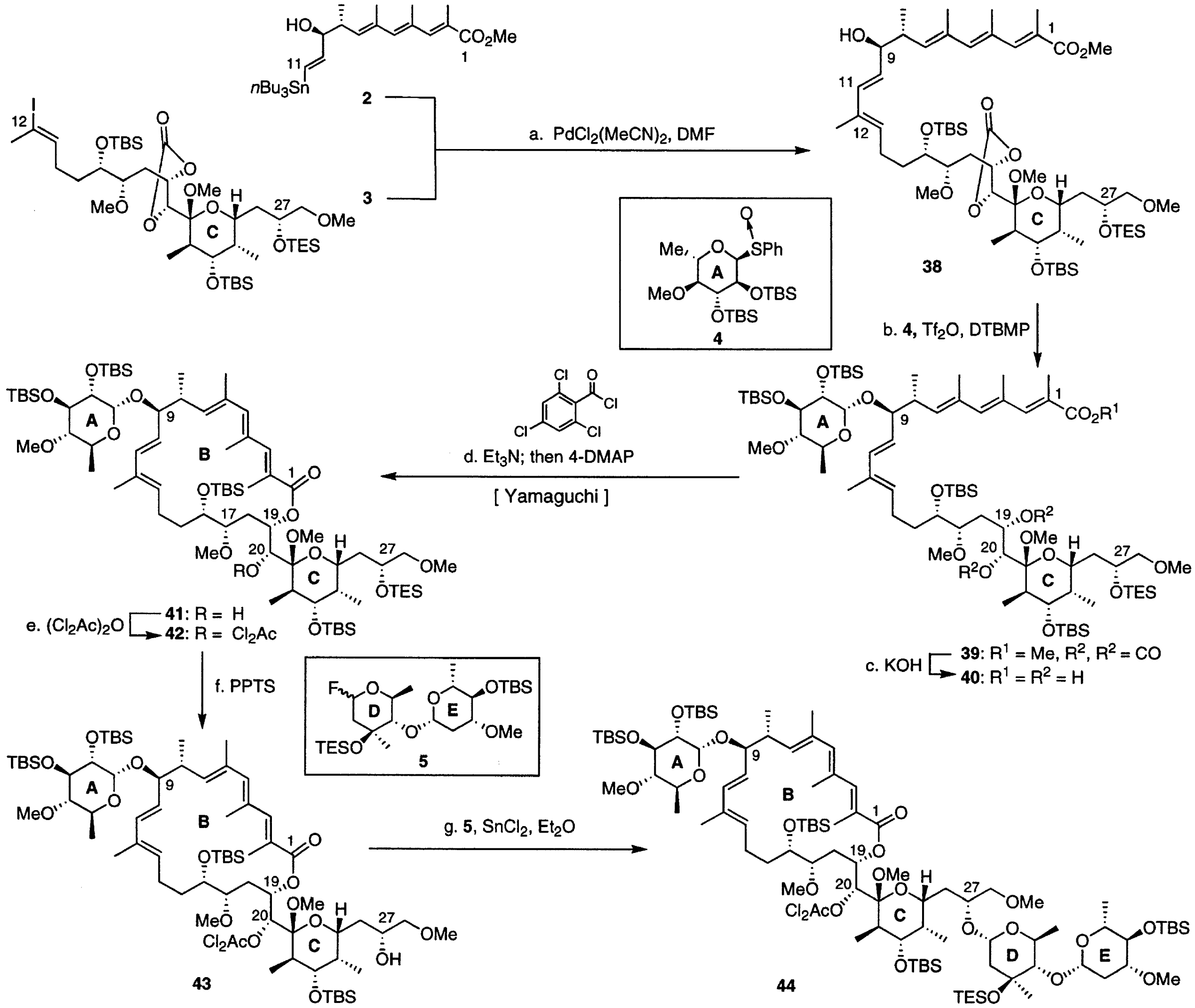
(12) In an attempted removal of the C_{27} PMB protecting group after the Stille coupling reaction (see Scheme 5), we observed as the major product the Diels−Alder cycloadduct between DDQ and the C_{11}−C_{14} diene moiety. This compound was characterized by ^{1}H NMR, IR and MS spectrometry.

(13) Attempted Stille coupling reactions on substrates in which the C_9 hydroxy group was protected (as TBS or TMS ethers or acetate) were not successful. For related observations and alternative coupling conditions, see also: Schuppan, J.; Wehlan, H.; Keiper, S.; Koert, U. *Angew. Chem., Int. Ed.* **2001**, *40*, 2063−2066.

(14) For reviews, see: (a) Stille, J. K. *Angew. Chem., Int. Ed. Engl.* **1986**, *25*, 508−524. (b) Farina, V.; Krishnamurphy, V.; Scott, W. J. *Org. React.* **1997**, *50*, 1−652. For Stille coupling reactions on related vinylstannanes with free allylic hydroxy groups, see: (c) Betzer, J.-F.; Lallemand, J.-Y.; Pancrazi, A. *Synthesis* **1998**, 522−534.

(15) Yan, L.; Kahne, D. *J. Am. Chem. Soc.* **1996**, *118*, 9239−9248.

Scheme 5. Synthesis of Fully Protected Apoptolidin **44**[a]



[a] (a) **2** (4.0 equiv), $PdCl_2(MeCN)_2$ (0.1 equiv), DMF, 25 °C, 15 h, 86%; (b) **4** (10.0 equiv), Tf_2O (2.5 equiv), DTBMP (10.0 equiv), Et_2O, −90 °C, 1.5 h; (c) KOH (20 equiv), dioxane:H_2O (10:1), 65 °C, 24 h; (d) 2,4,6-trichlorobenzoyl chloride (20 equiv), Et_3N (40 equiv), THF, 0 → 25 °C, 5 h; then 4-DMAP (80 equiv), toluene (0.0001 M), 25 °C, 12 h, 27% over three steps; (e) $(Cl_2Ac)_2O$ (100 equiv), py, 0 °C, 5 min, 90%; (f) PPTS (1.0 equiv), MeOH:CH_2Cl_2 (1:1), 0 °C, 1.5 h, 80%; g) **5** (2.0 equiv), $SnCl_2$ (6.6 equiv), Et_2O, 25 °C, 12 h, 70%. DTBMP = 2,6-di-*tert*-butyl-4-methylpyridine, 4-DMAP = 4-(dimethylamino)pyridine; Cl_2Ac = dichloroacetyl.

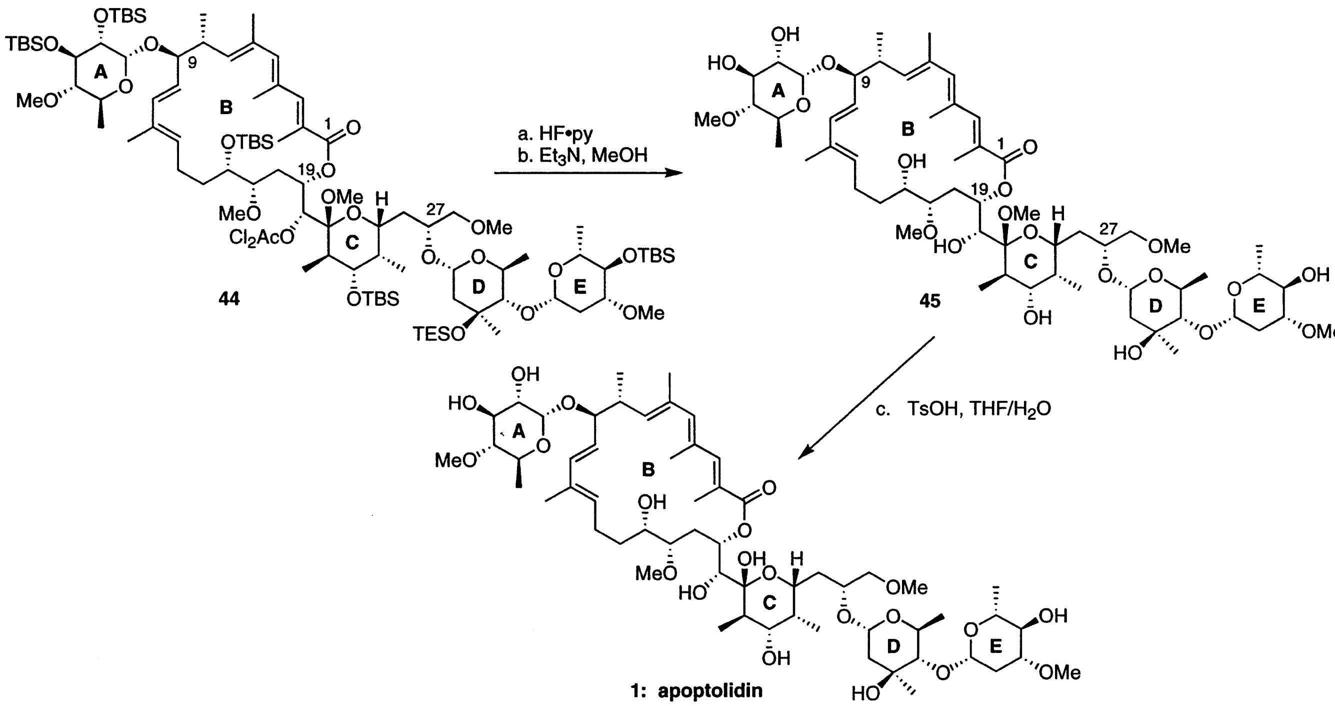
confirmed by the observed coupling constant of the anomeric proton ($J_{1,2}$ = 3.5 Hz) with its neighboring proton (H-2). In preparation for the obligatory macrolactonization reaction, conditions were sought and found for the selective cleavage of the C_1 ester group and the $C_{19}-C_{20}$ carbonate ring without damaging the sensitive TES ether at C_{27}.[16] The successful conditions involved exposure of **39** to KOH in dioxane−water (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-tichlorobenzoyl 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_{19} position as the lactone site rather then 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 (Ac_2O) in the presence of triethylamine and 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 $SnCl_2$ in ether to afford the desired α-glycoside **44** in 70% yield. The α-stereochemistry of the newly, and exclusively, formed

(16) For various conditions to hydrolyze methyl esters, see: Greene, T. W.; Wuts, P. G. M. In *Protective Groups in Organic Synthesis*, 3rd ed.; Wiley & Sons: New York, 1999; pp 384−387.

(17) (a) Haines, A. H.; Sutcliffe, E. J. *Carbohydr. Res.* **1985**, *138*, 143−147. (b) Reese, C. B.; Stewart, J. C. M.; van Boom, J. H.; de Leeuw, H. P. M.; Nagel, J.; de Rooy, J. F. M. *J. Chem. Soc., Perkin Trans. 1* **1975**, 934−936.

Scheme 6. Deprotection Steps Leading to Apoptolidin (**1**)[a]



[a] (a) HF·py (excess), THF, −25 °C, 96 h; (b) Et_3N:MeOH (1:10), 25 °C, 3.5 h, 40% over two steps; (c) TsOH (1.0 equiv), THF:H_2O (1:1), 0 °C, 2.5 h, 60%. Cl_2Ac = dichloroacetyl.

glycoside bond was evident from the relatively small coupling constant ($J_{1,2}$ = 3.0 Hz) associated with the relevant anomeric 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 isoapoptolidin[19] 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_2CO_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 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_3N, 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_3N 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

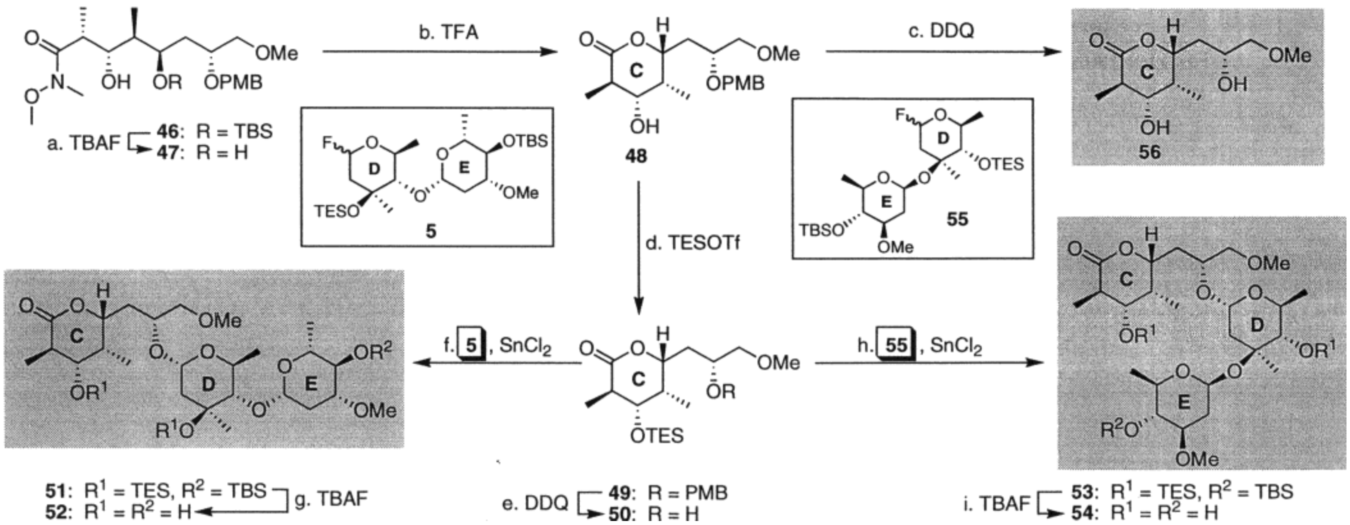
(18) For brief discussions on the stability of apoptolidin, see: (a) Salomon, A. R.; Voehringer, D. W.; Herzenberg, L. A.; Khosla, C. *Chem. Biol.* **2001**, *8*, 71−80. (b) Nicolaou, K. C.; Li, Y.; Fylaktakidou, K. C.; Mitchell, H. J.; Sugita, K. *Angew. Chem., Int. Ed.* **2001**, *40*, 3854−3857.

(19) (a) Wender, P. A.; Gulledge, A. V.; Jankowski, O. D.; Seto, H. *Org. Lett.* **2002**, *4*, 3819−3822. (b) Pennington, J. D.; Williams, H. J.; Salomon, A. R.; Sulikowski, G. A. *Org. Lett.* **2002**, *4*, 3823−3825.

(20) Samples of apoptolidin were kindly provided by Professor C. Khosla of Stanford University.

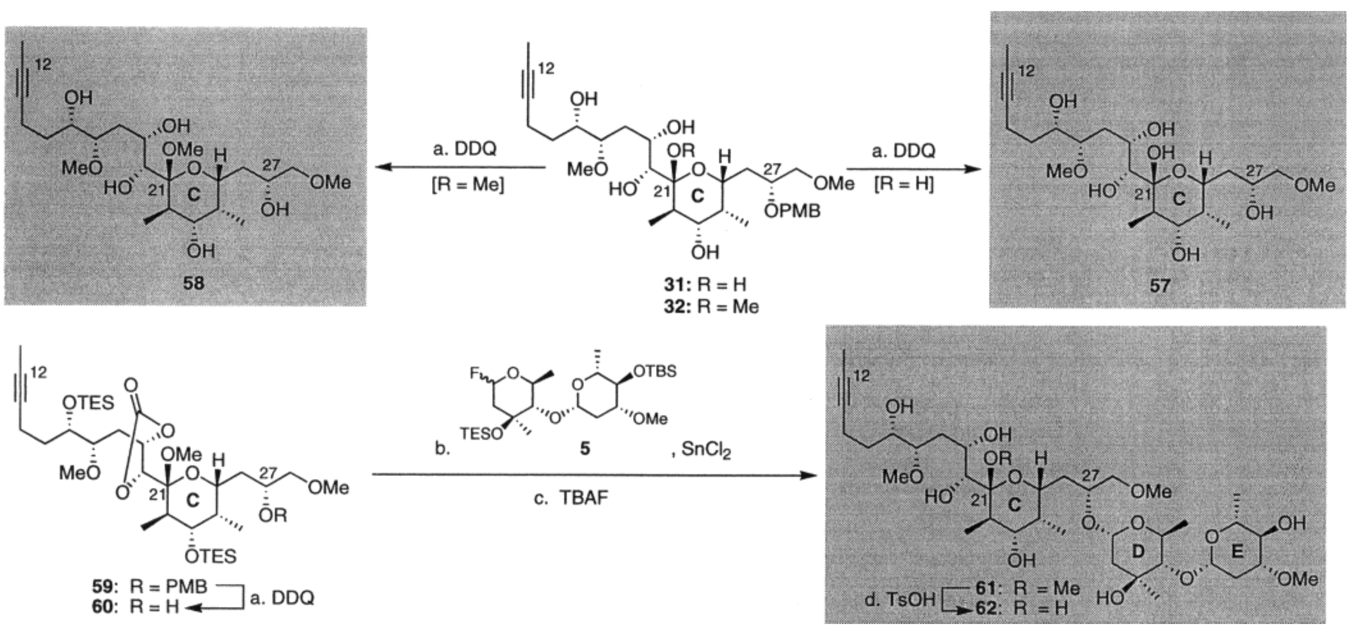
(21) Wender and co-workers reported an alternative way to prepare apoptolidin methyl glycoside from the natural product, see: Wender, P. A.; Jankowski, O. D.; Tabet, E. A.; Seto, H. *Org. Lett.* **2003**, *5*, 487−490.

Scheme 7. Construction of **C**, **D**, **E** Carbohydrate Domain Analogues **52**, **54**, and **56**[a]



[a] (a) TBAF (2.5 equiv), THF, 0 → 25 °C, 12 h, 96%; (b) TFA (1.2 equiv), THF, 0 °C, 1 h, 85%; (c) DDQ (1.5 equiv), CH_2Cl_2:phosphate buffer (pH = 7, 2:1), 0 °C, 2 h, 98%; (d) TESOTf (1.5 equiv), 2,6-lutidine (2.0 equiv), CH_2Cl_2, 0 → 25 °C, 2 h, 87%; (e) DDQ (1.5 equiv), CH_2Cl_2:phosphate buffer (pH = 7, 2:1), 0 → 25 °C, 2 h, 80%; (f) **5** (1.0 equiv), $SnCl_2$ (3.0 equiv), Et_2O, 0 → 25 °C, 4 h, 68%; (g) TBAF (6.0 equiv), THF, 0 → 25 °C, 24 h, 90%; (h) **55** (0.9 equiv), $SnCl_2$ (3.0 equiv), Et_2O, 0 → 25 °C, 2 h, 60%; (i) TBAF (6.0 equiv), THF, 0 → 25 °C, 24 h, 86%.

Scheme 8. Construction of C12−C28 Polyketide Analogues **57**, **58**, and **62**[a]



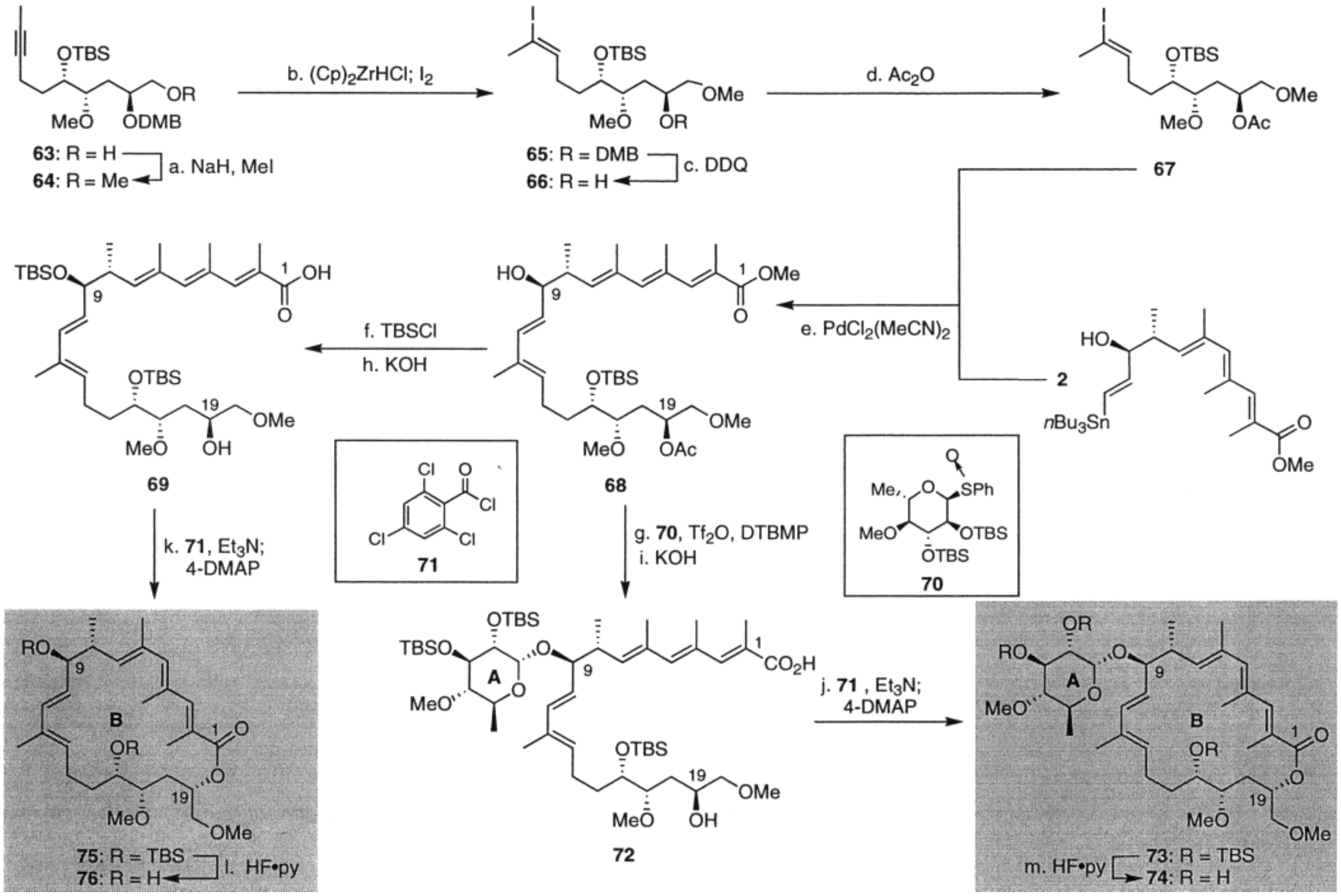
[a] (a) DDQ (1.5 equiv), CH_2Cl_2:phosphate buffer (pH = 7, 2:1), 0 °C, 2 h, 80% for **57**, 85% for **58** and 85% for **60**; (b) **5** (1.2 equiv), $SnCl_2$ (3.0 equiv), Et_2O, 0 → 25 °C, 4 h, 73%; (c) TBAF (6.0 equiv), THF, 0 → 25 °C, 24 h, 90%; (d) TsOH (0.2 equiv), THF:H_2O (2:1), 0 °C, 2.5 h, 60%.

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 C_{12}−C_{27} 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 $SnCl_2$ furnished, stereoselectively, the α-glycoside **51** (68% yield), which fully desilylated to **52** upon treatment with TBAF (90% yield). In a

(22) For the preparation of related analogues and biological studies, see: (a) Salomon, A. R.; Zhang, Y.; Seto, H.; Khosla, C. *Org. Lett.* **2001**, *3*, 57−59. (b) Wender, P. A.; Jankowski, O. D.; Tabet, E. A.; Seto, H. *Org. Lett.* **2003**, *5*, 487−490. (c) Wender, P. A.; Jankowski, O. D.; Tabet, E. A.; Seto, H. *Org. Lett.* **2003**, *5*, 2299−2302.

Scheme 9. Synthesis of Macrocyclic Analogues **74** and **76**[a]



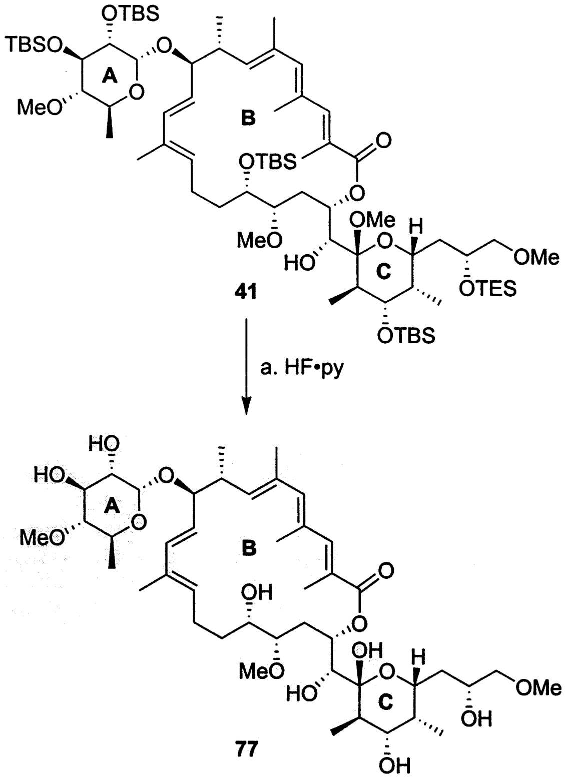
[a] (a) NaH (3.0 equiv), MeI (3.0 equiv), nBu$_4$NI (0.5 equiv), DMF, 0 → 25 °C, 12 h, 98%; (b) $(Cp)_2ZrHCl$ (2.0 equiv), THF, 65 °C, 3 h; I_2 (2.0 equiv), THF, −25 °C, 2 min, 85%; (c) DDQ (1.5 equiv), CH_2Cl_2:H_2O (18:1), 0 °C, 2 h, 90%; (d) Ac_2O (5.0 equiv), Et_3N (5.0 equiv), 4-DMAP (0.5 equiv), CH_2Cl_2, 25 °C, 12 h, 85%; (e) **2** (1.5 equiv), $PdCl_2(MeCN)_2$ (0.05 equiv), DMF, 25 °C, 15 h, 66%; (f) TBSCl (8.0 equiv), imidazole (10 equiv), DMF, 0 → 25 °C, 15 h, 81%; (g) **70** (10.0 equiv), Tf_2O (2.5 equiv), DTBMP (10.0 equiv), Et_2O, −90 °C, 1.5 h, 63%; (h) KOH (20 equiv), dioxane, 75 °C, 18 h; (i) KOH (20.0 equiv), dioxane, 75 °C, 18 h; (j) 2,4,6-trichlorobenzoyl chloride (20 equiv), Et_3N (40 equiv), THF, 0 → 25 °C, 2 h; then 4-DMAP (80 equiv), toluene (0.001 M), 70 °C, 2 h, 65%; (k) 2,4,6-trichlorobenzoyl chloride (20 equiv), Et_3N (40 equiv), THF, 0 → 25 °C, 2 h; then 4-DMAP (80 equiv), toluene (0.001 M), 65 °C, 5 h, 70%; (l) HF•py (excess), THF, −30 → 0 °C, 48 h, 80%; (m) HF•py (excess), THF, −30 → 0 °C, 48 h, 70%.

similar manner, glycosidation of **50** with disaccharide donor **55** ($SnCl_2$) 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** ($SnCl_2$, 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 macrocycle analogues **74** and **76**. Thus, methylation of the previously synthesized alcohol **63** (NaH−MeI, nBu$_4$NI, 98% yield) furnished methyl ether **64** whose hydrozirconation (Cp_2ZrHCl) 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 (Ac_2O, Et_3N, 4-DMAP, 85% yield), leading to **67**. Coupling of **67** with vinylstannane **2** proceeded smoothly under the influence of $PdCl_2(MeCN)_2$, 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 macrolactonization. The latter compound (**69**) was subjected to a modified Yamaguchi procedure (2,4,6-trichlorobenzoyl chloride, Et_3N; 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** (Tf_2O−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**.

Scheme 10. Synthesis of Advanced Apoptolidin Analogue **77**[a]



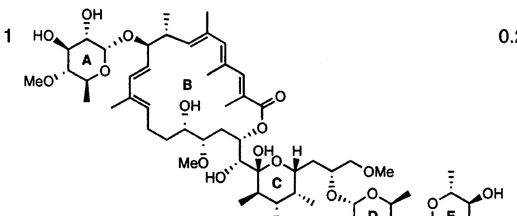
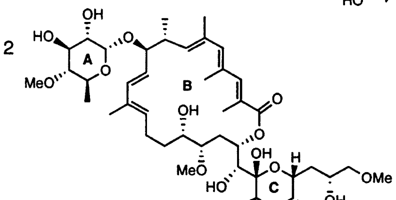
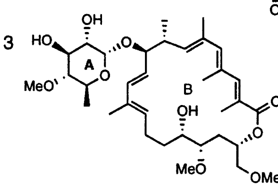
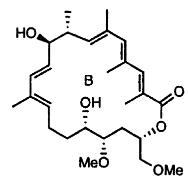
[a] (a) HF•py (excess), THF, −25 → 0 °C, 72 h, 4:1 ratio of regioisomers, 80%.

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 IC_{50} value was observed when carbohydrate **A** was attached onto the 20-membered macrolide ring at the proper position, i.e., the IC_{50} 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 (IC_{50} = 11 μM).

These observations are in line with those reported by the Khosla and Wender groups.[23] 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.

(23) (a) Salomon, A. R.; Voehringer, D. W.; Herzenberg, L. A.; Khosla, C. *Chem. Biol.* **2001**, *8*, 71−80. (b) Salomon, A. R.; Voehringer, D. W.; Herzenberg, L. A.; Khosla, C. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 14766−14771. (c) Wender, P. A.; Jankowski, O. D.; Tabet, E. A.; Seto, H. *Org. Lett.* **2003**, *5*, 2299−2302.

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

Entry	Compound	IC_{50} value (μM)
1	 **1: apoptolidin**	0.24
2	 **77**	11.5
3	 **74**	20.0
4	 **75**	45.0
5	**62**	62.0
6	**61**	74.0
7	**57**	75.0
8	**58**	100.0
9	**56**	74.0
10	**54**	62.0
11	**52**	83.0

[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.[23] IC_{50} is defined as the concentration that leads to 50% growth inhibition. IC_{50} 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 (**1**) 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 (CH_2Cl_2) were obtained by passing commercially available pre-dried, oxygen-free formulations through activated alumina columns. Yields refer to chromatographically and spectroscopically (^{1}H 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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