Natural Products Synthesis

Total Synthesis of Apoptolidin**

*Hermut Wehlan, Mario Dauber, M.-Teresa Mujica Fernaud, Julia Schuppan, Rainer Mahrwald, Burkhard Ziemer, M.-Elisa Juarez Garcia, and Ulrich Koert**

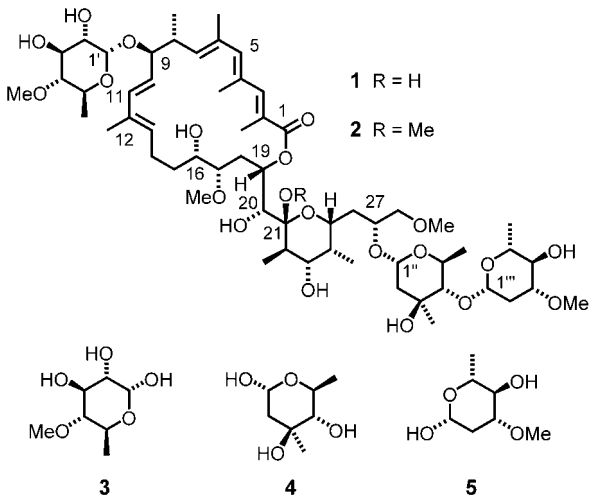
Apoptosis inducers specific for cancer cells are of interest for tumor therapy.[1] Apoptolidin (**1**), isolated from *Nocardiop-*

[*] Dipl.-Chem. H. Wehlan, M. Dauber, Dr. M.-T. Mujica Fernaud, Dr. J. Schuppan, Dipl.-Chem. M.-E. Juarez Garcia, Prof. Dr. U. Koert
Fachbereich Chemie
Philipps-Universität Marburg
Hans-Meerwein-Strasse, 35032 Marburg (Germany)
Fax: (+49) 6421-282-5677
E-mail: koert@chemie.uni-marburg.de
Priv.-Doz. Dr. R. Mahrwald, Dr. B. Ziemer
Institut für Chemie
Humboldt-Universität zu Berlin
Brook-Taylor-Strasse 2, 12489 Berlin (Germany)

[**] This work was supported by Schering AG Berlin and the Fonds der Chemischen Industrie. H.W. thanks the Schering Forschungsgesellschaft for a predoctoral fellowship. The authors thank Prof. Dr. U. Eder for his helpful support.

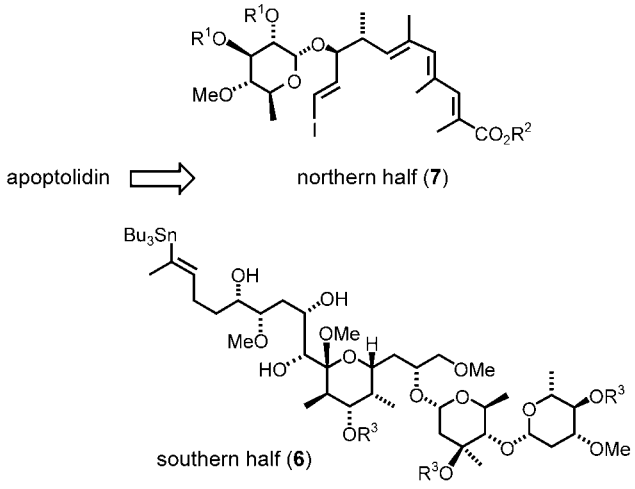
Supporting information for this article is available on the WWW under http://www.angewandte.org or from the author.

 DOI: 10.1002/anie.200460172

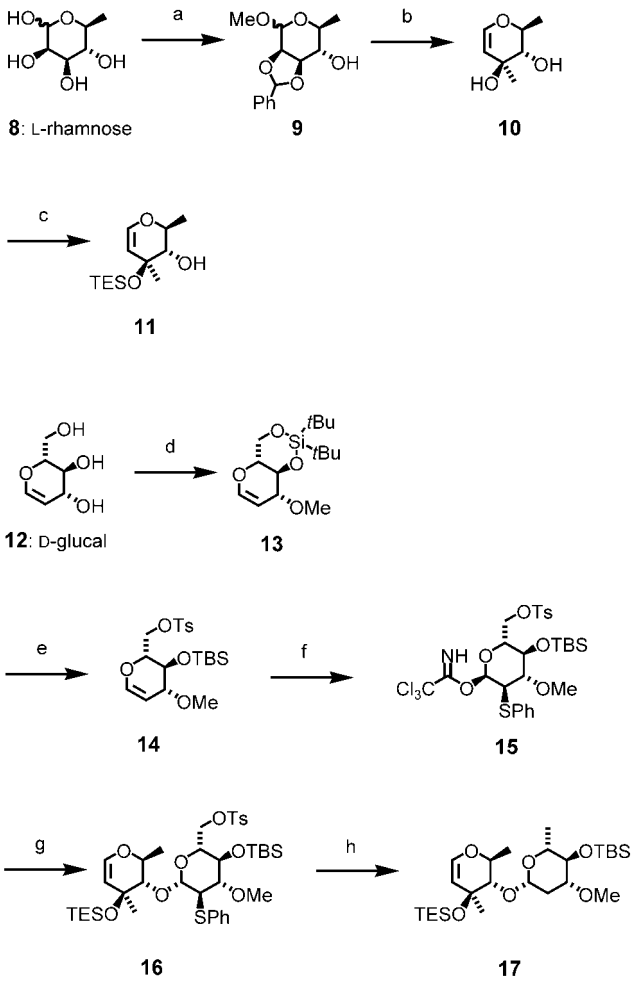


sis sp. by Hayakawa et al., selectively induces apoptosis in rat glia cells transformed with the E1A oncogene (IC_{50} = 11 ng mL^{-1}) but not in untransformed cell lines.[2,3] The apoptotic activity of **1** was correlated with its inhibition of mitochondrial F_0-F_1-ATPase.[4] Apoptolidin (**1**) is a 20-membered macrolactone with a side chain containing a cyclic six-membered hemiketal. 6-Deoxy-4-*o*-methyl-L-glucose (**3**) is attached to O9, and a disaccharide consisting of L-olivomycose (**4**) and D-oleandrose (**5**) is linked to O27. The sugar residues are a prerequisite for the potent bioactivity of **1**. Treatment of **1** with MeOH/Amberlyst-15 produces 21-*o*-methylapoptolidin (**2**).[5] Apoptolidin can rearrange into the 21-membered macrolactone isoapoptolidin by a O19→O20 acyl shift.[6] The noticeable biological activity of apoptolidin (**1**) and its structural challenges make it a prominent synthetic target.[7,8] In continuation of our synthesis of the aglycon apoptolidinone[9] we communicate here the completion of the total synthesis of apoptolidin.

Our synthetic strategy for apoptolidin (Scheme 1) is based on an early introduction of the sugar residues and a late cross-coupling of the fully glycosylated southern half **6** with the glycosylated northern half **7** followed by the macrolactonization. The starting point for the synthesis of the O27 disaccharide portion was L-rhamnose (**8**) which was transformed into the benzylidene-protected methyl acetal **9** (Scheme 2). Treatment of **9** with 6 equiv of methyllithium[10]



Scheme 1. Retrosynthetic analysis of apoptolidin.

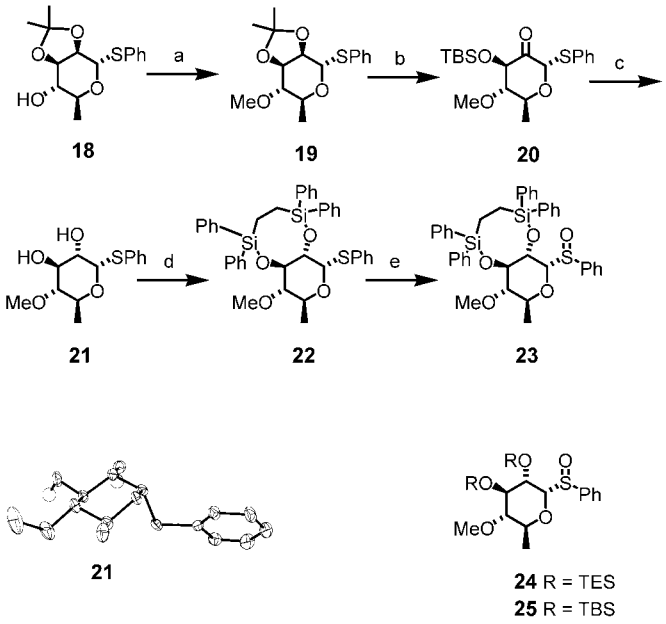


Scheme 2. a) 1. MeOH, DOWEX 50WX-8-200; 2. $PhCH(OMe)_2$, *p*TsOH, DMF 58%; b) 6 equiv MeLi, THF, 20°C, 30 h, 43%; c) 1. Ac_2O, Py, DMAP, CH_2Cl_2; 2. TESOTf, lutidine; 3. DIBAH, CH_2Cl_2, −60°C, 71%; d) 1. $tBu_2Si(OTf)_2$, lutidine, DMF/CH_2Cl_2 1:1, −50°C; 2. MeI, Ag_2O, 87%; e) 1. TBAF, THF; 2. *p*TsCl, Py, CH_2Cl_2, 0°C; 3. TBSOTf, lutidine, CH_2Cl_2, 68%; f) 1. PhSCl, CH_2Cl_2; 2. Ag_2CO_3, CH_3CN/H_2O 9:1, 87%; 3. Cl_3CCN, NaH, 91%; g) **11**, TMSOTf, Et_2O, −60→−40°C, 1 h; h) 1. NaI, DMF, 90°C, 2 h; 2. Bu_3SnH, AIBN, toluene, 100°C, 7 h, 70% for three steps. AIBN = 2,2′-azobis(isobutyronitrile), DIBAH = diisobutylaluminum hydride, DMAP = 4-dimethylaminopyridine, TES = triethylsilyl, TMS = trimethylsilyl, TBA = tetrabutylammonium.

gave via a cyclohexenone intermediate the glycal **10**, which was converted into the protected olivomycal building block **11**. D-Glucal (**12**) was 4,6-silyl-protected and methylated at O3 to give **13**. Desilylation of the latter followed by tosylation of the primary OH group and protection of the secondary alcohol function with a *tert*-butyldimethylsilyl (TBS) group gave **14**. In preparation for a β-selective glycosylation, an auxiliary SPh substituent was introduced at C2 of the D-oleandrose building block.[11] To this end **14** was treated first with PhSCl, then with Ag_2CO_3 in H_2O/CH_3CN, and the

resulting α-anomeric hemiacetal was converted into the trichloroacetimidate **15**. The TMSOTf-mediated glycosylation of **11** and **15** produced the disaccharide **16** with very high β-selectivity (>95:5). The conversion of the tosylate into an iodide and the combined reductive removal of the iodo and the thio function completed the preparation of the protected 2-deoxydisaccharide building block **17**.

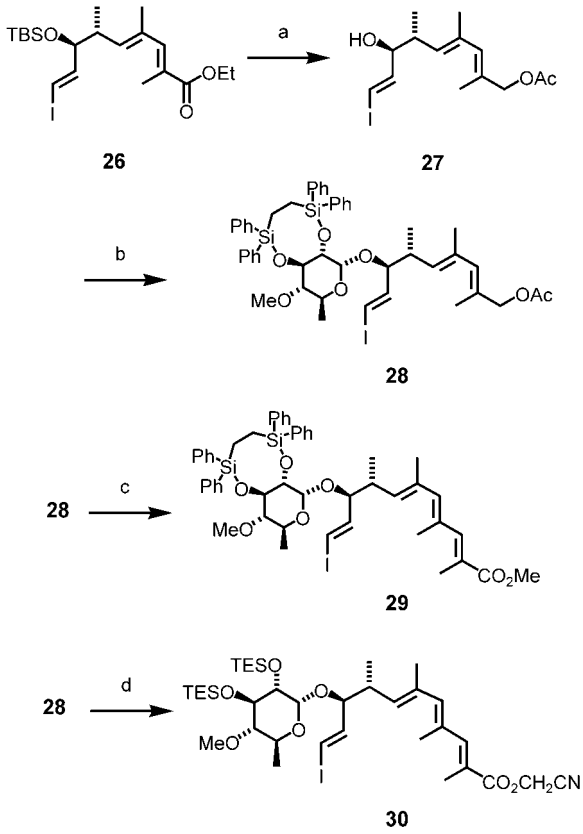
An inversion at C2 converts L-rhamnose into 6-deoxy-L-glucose. Therefore, L-rhamnose is a suitable starting material for the O9 sugar residue of apoptolidin (Scheme 3).[12] The



Scheme 3. a) MeI, KOH, DMF, 0°C, 99%; b) 1. *p*TsOH, MeOH, 89%; 2. TBSCl, imidazole, DMAP, CH_2Cl_2, 91%; 3. Dess–Martin periodinane, 80%; c) 1. $NaBH_4$, MeOH, 0°C, 15 min, 97%; 2. TBAF, THF, 94%; d) $Ph_2Si(Cl)CH_2CH_2Si(Cl)Ph_2$, imidazole, DMF, 0°C, 1 h, 92%; e) *m*CPBA, CH_2Cl_2, −78→−20°C, 2 h, 92%, 2:1 mixture of epimers. *m*CPBA = *meta*-chloroperbenzoic acid.

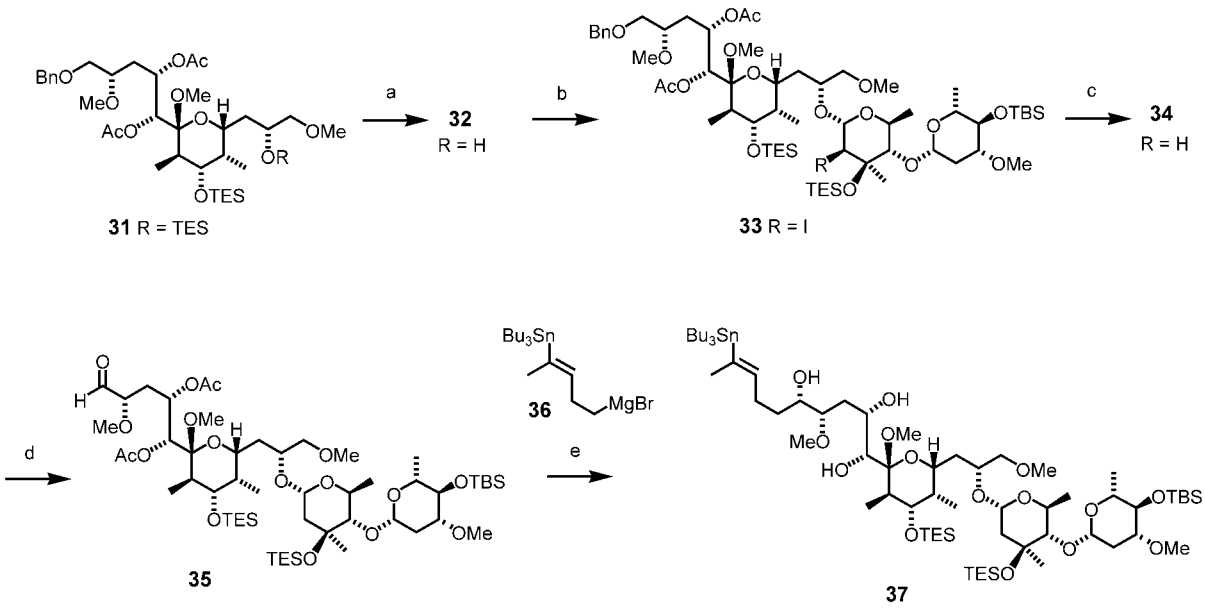
acetonide-protected L-rhamnose thioglycoside **18**[13] was O-methylated to obtain **19**. Acetonide cleavage, TBS protection of O3, and subsequent oxidation of the remaining 2-OH group provided the ketone **20**. A highly stereoselective reduction of **20** with $NaBH_4$ gave the corresponding alcohol, which was desilylated to provide the diol **21**. The relative configuration of **21** was confirmed by X-ray structure analysis (Scheme 3).[14] The following choice of the right O2/O3 L-glucose protecting groups was crucial for the successful glycosylation of the northern half. An α-selective glycosylation required a passive O2 protecting group that can be removed at the end without affecting the highly unsaturated and acid-sensitive target molecule. Silyl ethers should be the best choice. After several unsuccessful glycosylation attempts (trichloroacetimidate, glycosyl fluoride, thioglycoside activation by PhSOTf) we focused on the Kahne glycosylation by means of sulfoxide activation.[15] Initially we prepared and examined the bis(TES)- and bis(TBS)-protected sulfoxides **24** and **25**. However, these glycosyl donors gave unsatisfying glycosylation results (vide infra), which prompted us to develop a new protecting group. Treatment of diol **21** with 1,4-dichloro-1,1,4,4-tetraphenyl-1,4-disilabutane ($SIBACl_2$)[16] gave the disilyl ether **22** in 92% yield. Oxidation of **22** with *m*CPBA led to the desired SIBA-protected glycosyl sulfoxide **23**.

The synthesis of the glycosylated northern half used the unsaturated ester **26**[9] from the aglycone synthesis as a common building block (Scheme 4). Ester **26** was converted into the glycosyl acceptor **27**. Initial attempts to use the

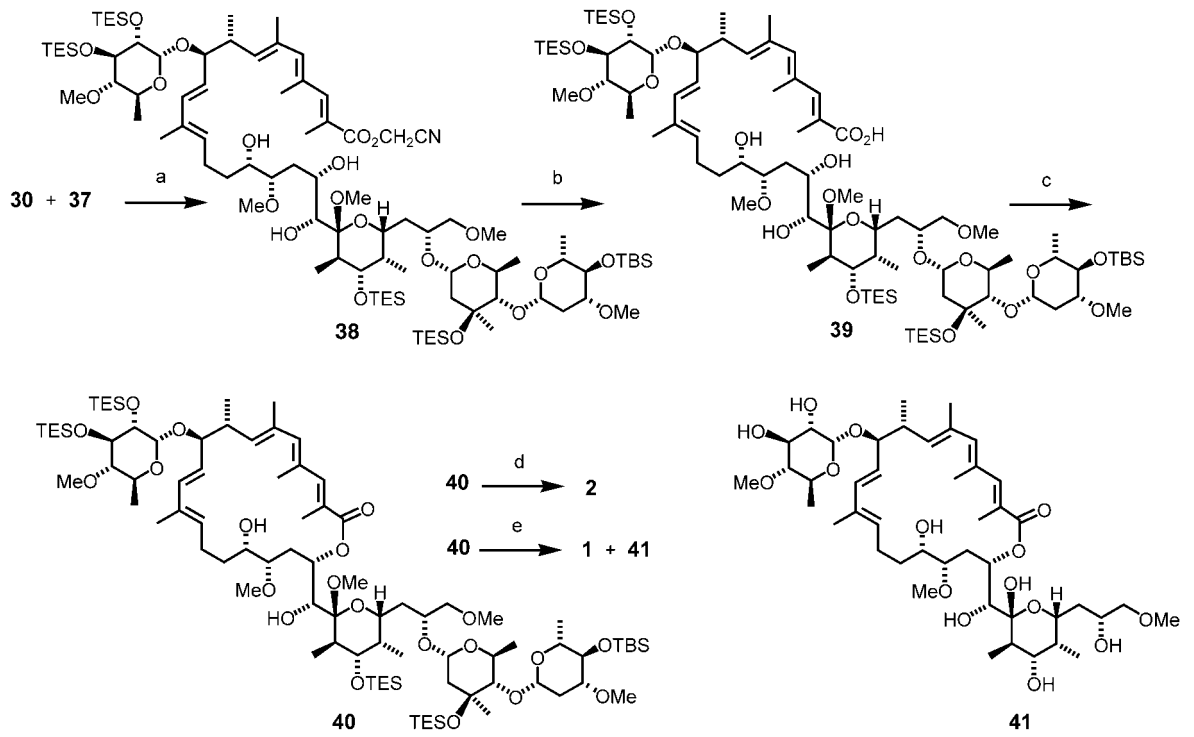


Scheme 4. a) 1. DIBAH, toluene, −78°C, 98%; 2. Ac_2O, Et_3N, DMAP, CH_2Cl_2, 0°C; 3. TBAF, THF, 0→20°C, 92%; b) 1 equiv **23**, 1.5 equiv Tf_2O, DTBMP, −80°C, 10 min; addition of **27**, −80→−35°C, 2 h; 65%, α/β = 85:15; c) 1. chromatographic separation of the anomers; 2. $LiEt_3BH$, THF, −50°C, 1 h, 75%; 3. MnO_2, CH_2Cl_2; 4. $Ph_3P{=}C(CH_3)CO_2Me$, toluene, 90°C, 44 h, 91%; d) 1. TBAF, THF, 20°C, 99%; 2. TESCl, imidazole, 20°C, 99%; 3. LiOH, THF/MeOH/H_2O 2:1:1, 0°C, 1 h, 83%; 4. MnO_2, CH_2Cl_2, chromatographic separation of the anomers, 78% α-anomer, 13% β-anomer; 5. $(EtO)_2P(O)CH(CH_3)CO_2H$, NaH, THF, 12°C, 14 h, 87%; 6. $ClCH_2CN$, Et_3N, MeCN, 20°C, 14 h, 92%. DTBMP = 2,6-di-*tert*-butyl-4-methylpyridine.

bis(TES)-protected glycosylsulfoxide **24** as a glycosyl donor failed due to loss of the TES groups under the reaction conditions (Tf_2O, DTBMP, −80→−35°C). The more stable bis(TBS)-protected glycosylsulfoxide **25** gave the desired glycoside in 50% yield but with an unacceptably low stereoselectivity (α/β = 66:33). In contrast, the reaction of the SIBA-protected glycosylsulfoxide **23** with **27** gave the expected product **28** in 65% yield with an acceptable stereoselectivity (α/β = 85:15). The final task for the comple-



Scheme 5. a) TBAF, THF, 0 °C, 90%; b) **17**, NIS, 4-Å molecular sieves, CH_2Cl_2, 0→20 °C, 72 h, 53%; anomeric ratio >95:5; c) Bu_3SnH, AIBN, toluene, 100 °C, 15 min, 96%; d) 1. H_2, Pd/C, EtOH, 90%; 2. Dess–Martin periodinane, pyridine, CH_2Cl_2, 20 °C, 1 h, 84%; e) 1. Mg, $BrCH_2CH_2Br$, Et_2O, −78 °C, 74%, ds >95:5 ; 2. KCN, MeOH, 40 °C, 16 h, 86%. NIS = *N*-iodosuccinimide.



Scheme 6. a) 3 equiv Cu^{I} thiophenecarboxylate, NMP, 1 h, 0 °C, 89%; b) 3 equiv LiOH, THF/MeOH 3:1, 20 °C, 2 h, 88%; c) 20 equiv 2,4,6-trichlorobenzoyl chloride, 40 equiv Et_3N, THF, 6 h; toluene, 80 equiv DMAP, $c = 3 \times 10^{-4}$ M, 75%; d) HF, pyridine, THF, 20 °C, 5 d, 50%; e) H_2SiF_6 (25% in H_2O), CH_3CN, −40→−20 °C, 2 d, −10 °C, 1 d, 71% **1** and 27% **41**. NMP = *N*-methylpyrrolidone.

tion of the northern half was the introduction of the C1–C3 unsaturated ester fragment. This was accomplished using standard Wittig and Horner—Wadsworth–Emmons procedures to give the methyl ester **29** and the cyanomethyl ester **30**, respectively.

The synthesis of the glycosylated southern half made use of the bis(TES) ether **31** from the aglycone synthesis (Scheme 5).[9] Selective deprotection of the 27-*O*-TES group in **31** gave **32**, which was allowed to react with the disaccharide glycal **17** to produce the glycoconjugate **33**

with a high α-selectivity of $>95:5$.[17] Reductive removal of the auxiliary iodo function led to the benzyl ether **34**. In order to prevent side reactions with the subsequent hydrogenolysis, it was necessary to remove all tin impurities from **34** by treatment with fluoride at this stage. The corresponding primary alcohol could be oxidized to the aldehyde **35**. The final step for the completion of the southern half was the chelation-controlled addition of the Grignard reagent **36**[9] to **35** to produce the corresponding alcohol with a diastereoselectivity of 96:4. Cleavage of the two acetates led to the triol **37**.

The cross-coupling of either the northern half, **29** or **30,** with the southern half **37** was possible with Cu^I thiophene-2-carboxylate in NMP (Scheme 6).[18] The methyl ester coupling product caused trouble in the subsequent methyl ester hydrolysis. We therefore focused on the cyanomethyl ester coupling product **38**. The cyanomethyl ester function in **38** could be hydrolyzed under very mild conditions (LiOH, 20 °C, 2 h) to the acid **39** without affecting the triene system or the TES protecting groups. A remarkable ring-size-selective macrolactonization of **39** produced the 20-membered lactone **40** in 75% yield. The final deprotection step required a careful examination of reagents and optimization of reaction conditions. The use of HF/pyridine in THF/pyridine at room temperature for 5 d cleaved all silyl ethers but left the methyl ketal intact and gave synthetic 21-o-methyl apoptolidin (**2**) which proved to be identical with **2** derived from natural sources ($[\alpha]_D^{22}=-76$ ($c=0.55$ in $CHCl_3$),[5] $[\alpha]_D^{22}=-67$ ($c=1.28$ in $CHCl_3$); for ^1H and ^{13}C NMR data see the Supporting Information).

We then turned our attention to the complete deprotection of **40** leading to apoptolidin (**1**). Treatment with 25% aqueous H_2SiF_6 in CH_3CN at $-40\rightarrow-10$ °C proved to be effective for cleavage of all silyl ethers[19] and noteworthy the methyl ketal. Apoptolidin (**1**) could be isolated in 71% yield by chromatographic separation on deactivated silica gel[20] with CH_2Cl_2/MeOH. In addition, the cleavage of the 27-*O*-disaccharide was observed and compound **41** was isolated in 27% yield. The physical and spectroscopic data of the synthetic apoptolidin (**1**) matched those published by Hayakawa et al. (m.p. 129–131 °C (MeOH), ref. [2]: 128–130 °C; ($[\alpha]_D^{22}=-4.4$ ($c=0.70$ in MeOH), ref. [2] $[\alpha]_D^{22}=-5.2$ ($c=1.0$ in MeOH); for ^1H and ^{13}C NMR data see the Supporting Information).

In conclusion an efficient, convergent, and stereoselective total synthesis of apoptolidin has been achieved. The distinct features of this synthesis are the early introduction of the sugar residues using a new sugar protecting group (SIBA), a Cu^I-mediated cross-coupling followed by a ring-size-selective macrolactonization, and mild deprotection conditions. This strategy should be applicable to the synthesis of apoptolidin derivatives with potential applications in apoptosis studies and tumor therapy.

Received: March 31, 2004

Keywords: cross-coupling · glycosylation · macrolides · natural products · total synthesis

[1] L. Benitez-Bribiesca in *When Cells Die* (Eds. R. A. Lockshin, Z. Zakeri, J. L. Tilly), Wiley-Liss, New York, **1998**, pp. 453–482.
[2] J. W. Kim, H. Adachi, K. Shin-ya, Y. Hayakawa, H. Seto, *J. Antibiot.* **1997**, *50*, 628–630.
[3] Y. Hayakawa, J. W. Kim, H. Adachi, K. Shin-ya, K. Fujita, H. Seto, *J. Am. Chem. Soc.* **1998**, *120*, 3524–3525.
[4] a) A. R. Salomon, D. W. Voehringer, L. A. Herzenberg, C. Khosla, *Proc. Natl. Acad. Sci. USA* **2000**, *97*, 14766–14771; b) A. R. Salomon, D. W. Voehringer, L. A. Herzenberg, C. Khosla, *Chem. Biol.* **2001**, *8*, 71–80; c) A. R. Salomon, Y. Zhang, H. Seto, C. Khosla, *Org. Lett.* **2001**, *3*, 57–59.
[5] P. A. Wender, O. D. Jankowski, E. A. Tabet, H. Seto, *Org. Lett.* **2003**, *5*, 487–490.
[6] a) P. A. Wender, A. V. Gulledge, O. D. Jankowski, H. Seto, *Org. Lett.* **2002**, *4*, 3819–3822; b) J. D. Pennington, H. J. Williams, A. R. Salomon, G. A. Sulikowski, *Org. Lett.* **2002**, *4*, 3823–3825.
[7] Total syntheses: a) K. C. Nicolaou, Y. Li, K. C. Fylaktakidou, H. J. Mitchell, H. X. Wei, B. Weyershausen, *Angew. Chem.* **2001**, *113*, 3968–3972; *Angew. Chem. Int. Ed.* **2001**, *40*, 3849–3854; b) K. C. Nicolaou, Y. Li, K. C. Fylaktakidou, H. J. Mitchell, K. Sugita, *Angew. Chem.* **2001**, *113*, 3972–3976; *Angew. Chem. Int. Ed.* **2001**, *40*, 3854–3857; c) K. C. Nicolaou, K. C. Fylaktakidou, H. Monenschein, Y. Li, B. Weyershausen, H. J. Mitchell, H. X. Wei, P. Guntupalli, D. Hepworth, K. Sugita, *J. Am. Chem. Soc.* **2003**, *125*, 15433–15442; d) K. C. Nicolaou, Y. Li, K. Sugita, H. Monenschein, P. Guntupalli, H. J. Mitchell, K. C. Fylaktakidou, D. Vourloumis, P. Giannakakou, A. O'Brate, *J. Am. Chem. Soc.* **2003**, *125*, 15443–15454.
[8] Fragment syntheses: a) G. A. Sulikowski, W. M. Lee, B. Jin, B. Wu, *Org. Lett.* **2000**, *2*, 1439–1442; b) K. Toshima, T. Arita, D. Tanaka, S. Matsumura, *Tetrahedron Lett.* **2001**, *42*, 8873–8876; c) W. D. Paquette, R. E. Taylor, *Org. Lett.* **2004**, *6*, 103–106.
[9] a) J. Schuppan, H. Wehlan, S. Keiper, U. Koert, *Angew. Chem.* **2001**, *113*, 2125–2128; *Angew. Chem. Int. Ed.* **2001**, *40*, 2063–2066; b) J. Schuppan, B. Ziemer, *Tetrahedron Lett.* **2000**, *41*, 621–624.
[10] G. Jung, A. Klerner, *Chem. Ber.* **1981**, *114*, 740–745.
[11] a) R. Preuss, R. R. Schmidt, *Synthesis* **1988**, 694–697; b) W. R. Roush, X. F. Lin, *J. Org. Chem.* **1991**, *56*, 5740–5742.
[12] A related route to the 6-deoxy-L-glucose building block was chosen by Nicolaou et al.[7a,c]
[13] I. Bajza, A. Liptak, *Carbohydr. Res.* **1990**, *205*, 435–439.
[14] CCDC 234809 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB21EZ, UK; fax: (+44) 1223-336-033; or deposit@ccdc.cam.ac.uk).
[15] D. Kahne, S. Walker, Y. Cheng, D. VanEngen, *J. Am. Chem. Soc.* **1989**, *111*, 6881–6882.
[16] 1,4-Dichloro-1,1,4,4-tetraphenyl-1,4-disilabutane ($SIBACl_2$) was prepared by reaction of 1,2-bis(trichlorosilyl)ethane with phenylmagnesium bromide.
[17] J. Thiem, J. Elvers, *Chem. Ber.* **1980**, *113*, 3049–3057.
[18] G. D. Allred, L. S. Liebeskind, *J. Am. Chem. Soc.* **1996**, *118*, 2748–2749.
[19] A. S. Pilcher, D. K. Hill, S. J. Shimshock, R. E. Waltermire, P. DeShong, *J. Org. Chem.* **1992**, *57*, 2492–2495.
[20] Chromatotrex silicagel MB 100-40/75 from Fuji Silysia Chemical LTD.

