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A Synthetic Strategy for the Cyclodepsipeptide Core of the Antitumor Antibiotic Verucopeptin

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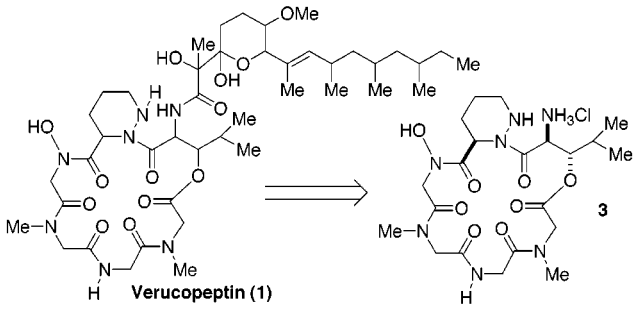
ABSTRACT



An efficient [2 + 2 + 2]-fragment condensation strategy is described for obtaining the cyclodepsipeptide core of verucopeptin. The 19-membered macrocycle was established through a Carpino HATU mediated macrolactamization, which proceeded in good yield under high-dilution conditions.

Verucopeptin is a powerful antitumor antibiotic isolated from the fermentation broths of Philippino soil microorganism *Actinomadura verrucospora*.[1] It significantly prolongs the life expectancy of mice with B16 melanoma, conferring a 162% life extension when given at the low dosage of 2 mg/kg/day. Structurally, verucopeptin belongs to the A83586C/GE3 family[2] of pyranylated cyclodepsipeptides, which also count azinothricin[3] and citropeptin[4] among their number. At present, the relative and absolute stereochemistry of verucopeptin remains unknown, notwithstanding extensive chemical and spectroscopic investigations by the Bristol-Myers[1a,b] and Concordia University[1c] groups.

It is thought that molecules of the A83586C/GE3 class function as anticancer agents by selectively preventing deregulated E2F-DP transcription factors from binding to and activating genes encoding for proteins involved in cell growth and proliferation (e.g., DNA-polymerase-α, dihydrofolate reductase, thymidine kinase, etc.).[2b] Understanding how they do this is currently a topic of great interest, as it could potentially lead to the detailed characterization of an important new drug target. In this regard, several modes of action are presently being considered. One is that members of the A83586C family are binding to individual E2F and DP proteins and preventing them from heterodimerizing. The latter is considered essential for high-affinity binding to target DNA, which in turn is a prerequisite for efficient transcriptional activation.[5] Alternatively, molecules of this class might be complexing to individual E2F-DP heterodimers and sterically blocking their interaction with various target genes. E2F/DP-drug binding could also be halting the recruitment

(1) Verucopeptin. (a) Isolation and biological properties: Nishiyama, Y.; Sugawara, K.; Tomita, K.; Yamamoto, H.; Kamei, H.; Oki, T. *J. Antibiot.* **1993**, *46*, 921. (b) Structure determination: Suguwara, K.; Toda, S.; Moriyama, T.; Konishi, M.; Oki, T. *J. Antibiot.* **1993**, *46*, 928. (c) Biosynthesis and partly revised spectroscopic assignments: Tsantrizos, Y. S.; Shen, J.; Trimble, L. A. *Tetrahedron Lett.* **1997**, *38*, 7073.

(2) (a) A83586C: Smitka, T. A.; Deeter, J. B.; Hunt, A. H.; Mertz, F. P.; Ellis, R. M.; Boeck, L. D.; Yao, R. C. *J. Antibiot.* **1988**, *41*, 726. (b) GE3: Sakai, Y.; Yoshida, T.; Tsujita, T.; Ochiai, K.; Agatsuma, T.; Saitoh, Y.; Tanaka, F.; Akiyama, T.; Akinaga, S.; Mizukami, T. *J. Antibiot.* **1997**, *50*, 659.

(3) Azinothricin: Maehr, H.; Liu, C.; Palleroni, N. J.; Smallheer, J.; Todaro, L.; Williams, T. H.; Blount, J. F. *J. Antibiot.* **1986**, *39*, 17.

(4) Citropeptin: Hayakawa, Y.; Nakagawa, M.; Toda, Y.; Seto, H. *Agric. Biol. Chem.* **1990**, *54*, 1007.

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of "essential" transcriptional coactivators such as the CREB transcription factor.[6]

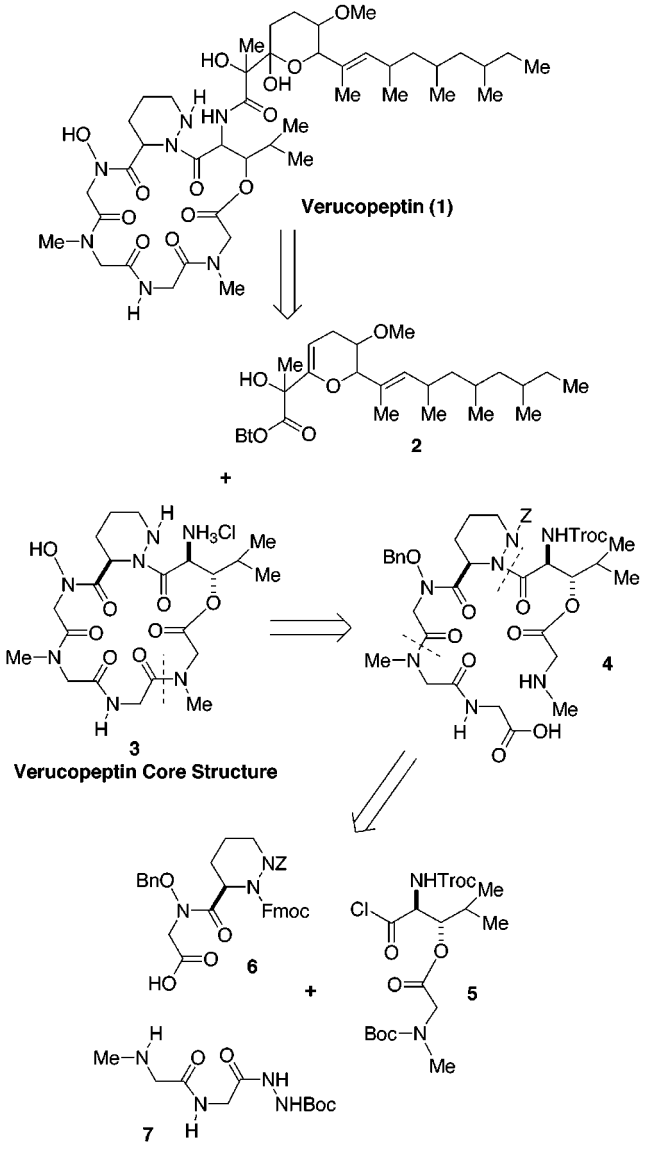
Some time ago, we initiated a total synthesis program,[7,8] on the A83586C-GE3-verucopeptin family of antitumor antibiotics, for the purpose of preparing novel analogues that could probe these mechanistic issues further. It is hoped that our analogue work will lead to the identification of several new, structurally less elaborate, E2F inhibitors with enhanced antineoplastic effects. In furtherance of these goals, we now report a synthetic strategy for the cyclodepsipeptide core of verucopeptin.

Although eight diastereomeric possibilities exist for this core region, the fact that all other family members have a (3*R*)-piperazic acid unit linked to a (2*S*,3*S*)-3-hydroxyleucine suggests that an identical sequence prevails in verucopeptin. As a consequence, we made diastereoisomer **3** the object of our initial synthetic planning (Scheme 1). Past experience with A83586C suggested that a [2 + 2 + 2]-fragment condensation between **7**, **6**, and **5** (in that order) could take us toward the cyclization precursor **4**, which could then be macrolactamised between its Gly and Sar termini without the risk of epimerization. Global deprotection would subsequently yield **3**, appropriately tailored for future chemoselective coupling to a range of activated esters or acid chlorides, which could include **2**, for a total synthesis of verucopeptin itself. Such a "biogenetically modeled" coupling strategy had previously been used to great effect during our total syntheses of A83586C[7] and 4-*epi*-A83586C,[8] and so it was anticipated that such an approach would proceed equally satisfactorily here. With this as background, we will now present details of our route to **3**.

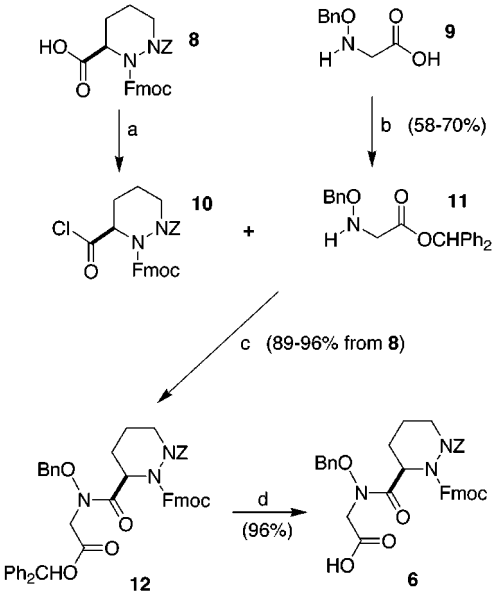
Early attention was directed at the preparation of dipeptide **6** (Scheme 2). For this, the protected hydroxamic acid **9** was needed; it was synthesized according to the method of Kolasa and Chimiak.[9] Its acid grouping was temporarily blocked as a diphenylmethyl ester,[10] and the remaining NH coupled to the known piperazic acid chloride **10** under AgCN-assisted amidation conditions.[7,8,11] Dipeptide **12** was isolated in excellent yield (89−96%). The diphenylmethyl ester grouping was then cleaved from **12** with trifluoroacetic acid (TFA) to obtain acid **6**.

Dipeptide **7** was synthesized by coupling **13** with **14** under standard DCC conditions[12] and cleaving the Z-group from **15** by catalytic hydrogenolysis (Scheme 3). A *tert*-butyl carbazate group was selected for protecting the Gly acid residue, as this circumvented diketopiperazine formation

Scheme 1. Retrosynthetic Planning for the Verucopeptin Cyclodepsipeptide Core

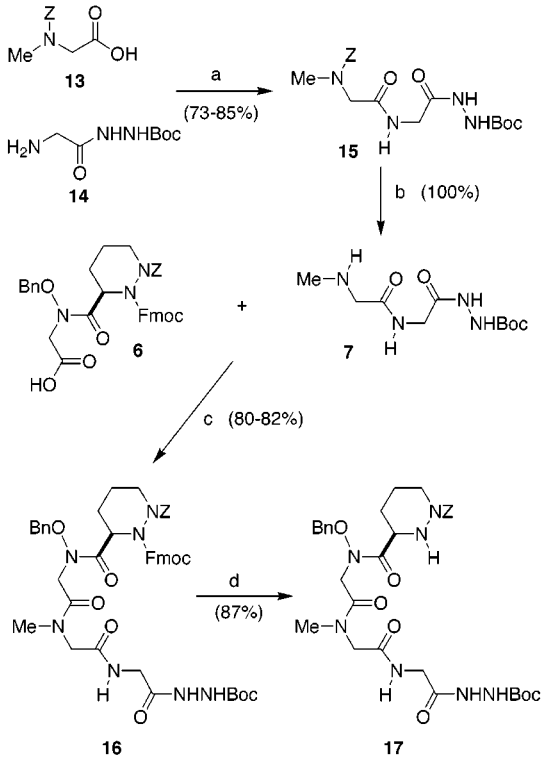


Scheme 2. Preparation of Dipeptide **6**[a]



[a] Reagents and conditions: (a) $(COCl)_2$ (20 equiv), C_6H_6 (0.55 M), rt (20 min), then warm to 50 °C, stir 1.5 h. (b) $Ph_2C{=}N_2$ (2 equiv), Me_2CO (0.4 M), rt, 24 h. (c) AgCN (1.5 equiv), C_6H_6 (0.43 M), 70 °C, 40 min. (d) CF_3CO_2H (36 equiv), PhOH (1.5 equiv), CH_2Cl_2 (0.09 M), 0 °C, 2 h.

Scheme 3. Route to Tetrapeptide **17**[a]

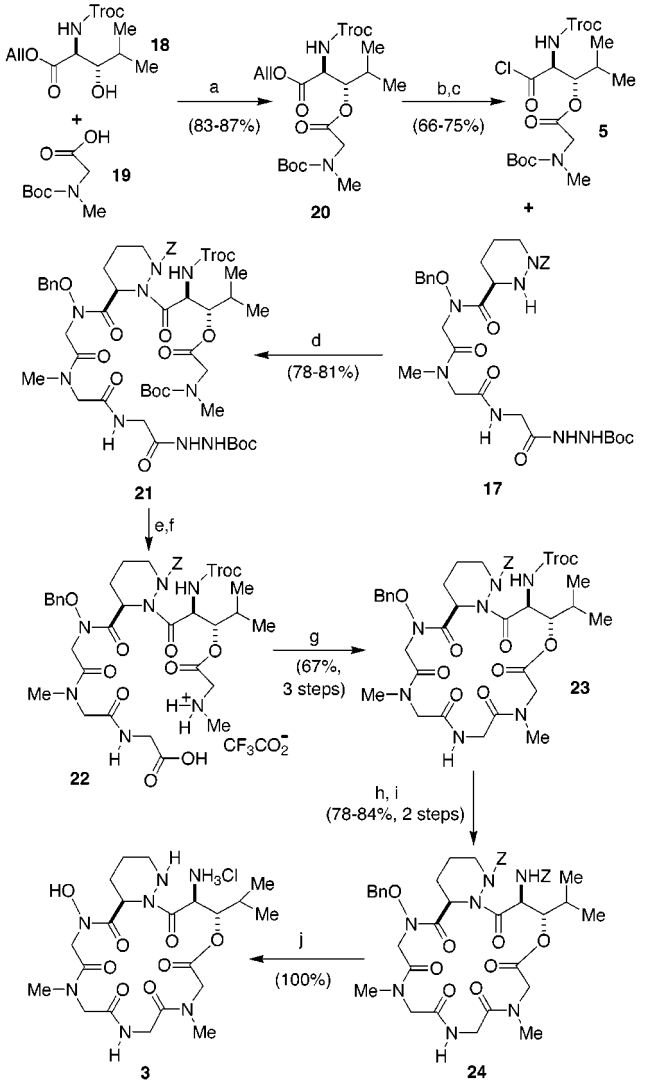


[a] Reagents and conditions: (a) DCC (1.05 equiv), DMAP (0.2 equiv), CH_2Cl_2 (0.25 M), rt, 24 h. (b) H_2, 20% $Pd(OH)_2/C$ (0.2 equiv), MeOH (0.26 M), rt, 8 h. (c) BOP-Cl (1.3 equiv), Et_3N (2 equiv), CH_2Cl_2 (0.07 M), −20 °C (20 min), then warm to 0 °C, 3 h. (d) Et_2NH (40 equiv), MeCN (0.12 M), rt, 20 min.

during hydrogenolysis of the Z-group; the latter was problematic when ester protecting groups were employed in the sequence. With **6** and **7** in hand, their union was effected with BOP-Cl[13] and Et_3N at low temperature. Tetrapeptide **17** was isolated after the Fmoc group[14] had been cleaved from **16** with diethylamine in MeCN.

The next fragment assembled was acid chloride **5**. It was prepared from the known amino acids **18** and **19** according to Scheme 4. The key steps were a DCC-DMAP mediated

Scheme 4. Route to Verucopeptin Cyclodepsipeptide **3**[a]



[a] Reagents and conditions: (a) DCC (1.1 equiv), DMAP (1 equiv), CH_2Cl_2 (0.17 M), rt, 24 h. (b) $(Ph_3P)_4Pd$ (0.1 equiv), morpholine (8.5 equiv), THF (0.17 M), rt, 30 min. (c) $(COCl)_2$ (35 equiv), C_6H_6 (0.3 M), rt, 2.5 h. (d) AgCN (1.5 equiv), C_6H_6 (0.15 M), 75−80 °C, 2−3 min. (e) CF_3CO_2H (200 equiv), CH_2Cl_2 (0.08 M), rt, 2 h. (f) NBS (2 equiv), THF/H_2O (1:1) (0.04 M), rt, 2 h. (g) Add **22** and NEM (13.5 equiv) in CH_2Cl_2 over 8 h to HATU (10 equiv) in CH_2Cl_2 at 0 °C, then warm to rt, 48 h (total final CH_2Cl_2 concentration = 0.0004 M). (h) Zn (85 equiv), AcOH/H_2O (10:1) (0.02 M), rt, 1.5 h. (i) Z-Cl (3 equiv), 10% sat. aq. $NaHCO_3$, CH_2Cl_2 (0.13 M), rt, 1 h. (j) H_2, 10% Pd/C (Aldrich wet Degussa type) (0.1 equiv), MeOH (0.01 M), HCl in MeOH (1 equiv), 24 h.

O-esterification[7a,8] to obtain **20**, a Kunz-Waldmann *O*-deallylation reaction with Pd(0) and morpholine to unmask the acid,[15] and a chlorination with excess oxalyl chloride in benzene to obtain acid chloride **5**. The chemoselective

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(8) Total synthesis of 4-*epi*-A83586C: Hale, K. J.; Cai, J.; Williams, G. *Synlett* **1998**, 149−152.

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N-acylation of **17** with **5** was effected by heating in benzene at 80 °C for 2−3 min in the presence of AgCN. Prolonging the reaction much beyond this time frame is not recommended, as it generally leads to a diminution in the yield of hexadepsipeptide **21**.

We now had to address the potentially troublesome issue of having to convert compound **21** into the hexapeptide amino acid **22**. This was accomplished nonproblematically by TFA treatment and by chemoselective oxidation of the Gly hydrazide grouping (in the presence of the Sar amine) with *N*-bromosuccinimide in aqueous THF.[16] It is presumed that the latter reaction selectively converts the acyl hydrazine into a highly reactive acyl diazene, which immediately is intercepted by the massive excess of water that is present. The success of this transformation underpinned our eventual synthesis of **3**. The macrolactamisation of **22**, to obtain **23**, was achieved in high yield using Carpino's HATU reagent,[17] under conditions of high dilution. The Troc group was now detached from **23** with Zn dust in aqueous acetic acid. To facilitate the final purification of **3**, we temporarily capped the product amine with a Z-group under classical Schotten-Baumann conditions and carefully purified **24** by SiO_2 flash chromatography. All the benzyl protecting groups were then cleaved from **24** by hydrogenolysis under mildly acidic conditions. The structure of **3** was confirmed by its high-resolution FAB mass spectrum, which contained an $[M]^+$ ion at *m*/*e* 514.2600 (calcd for $C_{21}H_{36}N_7O_8$ $[M]^+$, 514.2625), and by its IR spectrum, which showed an intense C═O stretching absorption at 1746 cm^{-1} indicative of a cyclodepsipeptide ester linkage. Additional evidence to support the assigned structure was provided by the room temperature 500 MHz ^1H NMR spectrum of **3** in CD_3OD, which clearly showed that it was a single compound, and that it existed as one conformer.

In conclusion, we have developed a highly efficient chemical pathway to molecules with the verucopeptin core structure. We anticipate that our efforts will expedite future parallel-synthesis work aimed at identifying novel new E2F-inhibitory anticancer drugs. Further work in this direction and on the synthesis of a diastereoisomer of verucopeptin will be presented in due course.

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Supporting Information Available: Low-resolution mass spectra and 500 MHz ^1H and 125 MHz ^{13}C NMR spectra of all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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