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Synthetic Route to the GE3 Cyclodepsipeptide

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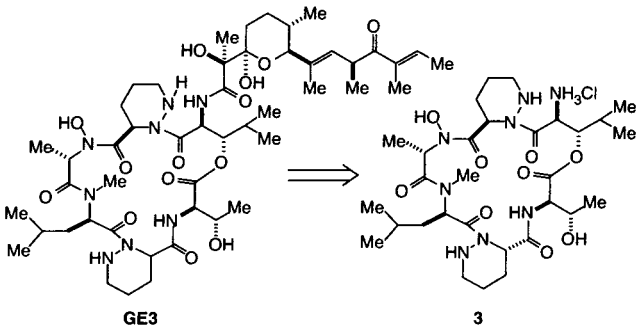
ABSTRACT



A reasonably efficient [2 + 2 + 2] fragment condensation strategy has been developed for assembling the cyclodepsipeptide sector of GE3 that involves 5–7. A Carpino HATU-mediated macrolactamization was used to close the 19-membered cyclodepsipeptide ring.

Medical interest in the antitumor agent GE3 has risen steadily ever since it was found to display potent growth inhibitory effects against solid human tumors in mice and it was suggested to have a novel mechanism of action.[1]

Specifically, a single 2 mg/kg dosage of GE3 brings about a 47% reduction in tumor size in immunocompromised mice transplanted with PSN-1 human pancreatic carcinoma after only 11 days,[1] and importantly, at this low dosage, GE3 is neither lethal nor highly toxic. GE3 is thought to act by preventing deregulated E2F/DP transcription factors from binding to target genes that encode for proteins critically involved in cancer cell division, a mechanism that has yet to be clarified and confirmed by other biology groups. In the case of A431 human lung cancer cells, this mode of action is correlated with an ability to halt cell cycle progression from the G1 to S phase. GE3 is also able to selectively inhibit cyclin A gene expression in Saos-2 cells without repressing β-actin gene expression. These combined biological effects and the current dearth of supply have made GE3[2] and its sister molecule A83586C major prizes for total synthesis.[3] In this paper, we now report a reasonably efficient synthetic route to the cyclodepsipeptide sector of GE3. We believe that it will not only expedite a future chemical synthesis of the natural product but also allow for the creation of a diverse collection of analogues. The work reported herein constitutes a further development and augmentation of our previous synthetic studies on this class.[3]

The main points of difference between GE3 and A83586C lie in the *N*-methyl D-Leu residue, which replaces the *N*-methyl-D-Ala unit in the cyclodepsipeptide core, and in

(1) 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.

(2) For a synthesis of the GE3 acyl side chain, see: Makino, K.; Henmi, Y.; Hamada, Y. *Synlett* **2002**, 613.

(3) For the first total synthesis of A83586C, see: (a) Hale, K. J.; Cai, J. *J. Chem. Soc. Chem. Comm.* **1997**, 2319. (b) Hale, K. J.; Cai, J.; Delisser, V. M. *Tetrahedron Lett.* **1996**, *37*, 9345−9348. (c) Hale, K. J.; Cai, J. *Tetrahedron Lett.* **1996**, *37*, 4233−4236. (d) Hale, K. J.; Cai, J.; Manaviazar, S.; Peak, S. A. *Tetrahedron Lett.* **1995**, *36*, 6965−6968. (e) Hale, K. J.; Delisser, V. M.; Yeh, L.-K.; Peak, S. A.; Manaviazar, S.; Bhatia, G. S. *Tetrahedron Lett.* **1994**, *35*, 7685−7688. (f) Hale, K. J.; Manaviazar, S.; Delisser, V. M. *Tetrahedron* **1994**, *50*, 9181−9188. (g) Hale, K. J.; Bhatia, G. S.; Peak, S. A.; Manaviazar, S. *Tetrahedron Lett.* **1993**, *34*, 5343−5346. (h) For a detailed account of our A83586C synthesis and the synthesis of other complex cyclodepsipeptides, see: Hale, K. J.; Bhatia, G. S.; Frigerio, M. *The Chemical Synthesis of Natural Products*; Hale, K. J., Ed.; Sheffield Academic Press: Sheffield, 2000; Chapter 12, p 349.

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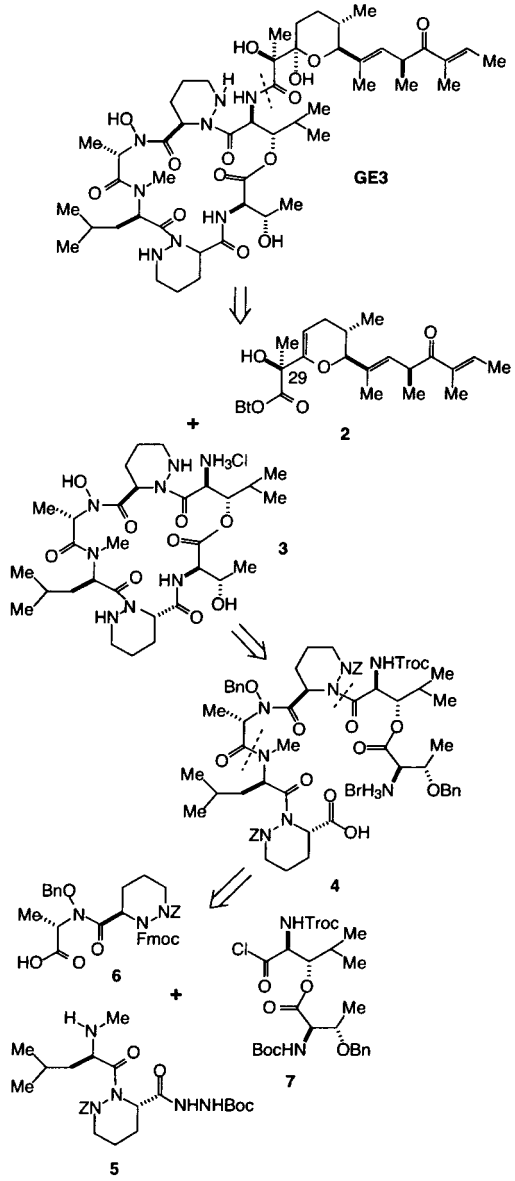
the nature of the C(29)-tertiary alcohol alkyl grouping that, in the case of GE3, is a methyl rather than an ethyl. Although these two changes might appear rather innocuous to the unitiated in this field, our most recent synthetic experiences have shown that they will almost certainly necessitate that a different synthetic strategy be developed for setting the C(29)−OH and that modified tactics be adopted for assembly of the cyclodepsipeptide.

Our overall retrosynthetic planning for GE3 (Scheme 1) is currently predicated upon the biogenetically inspired

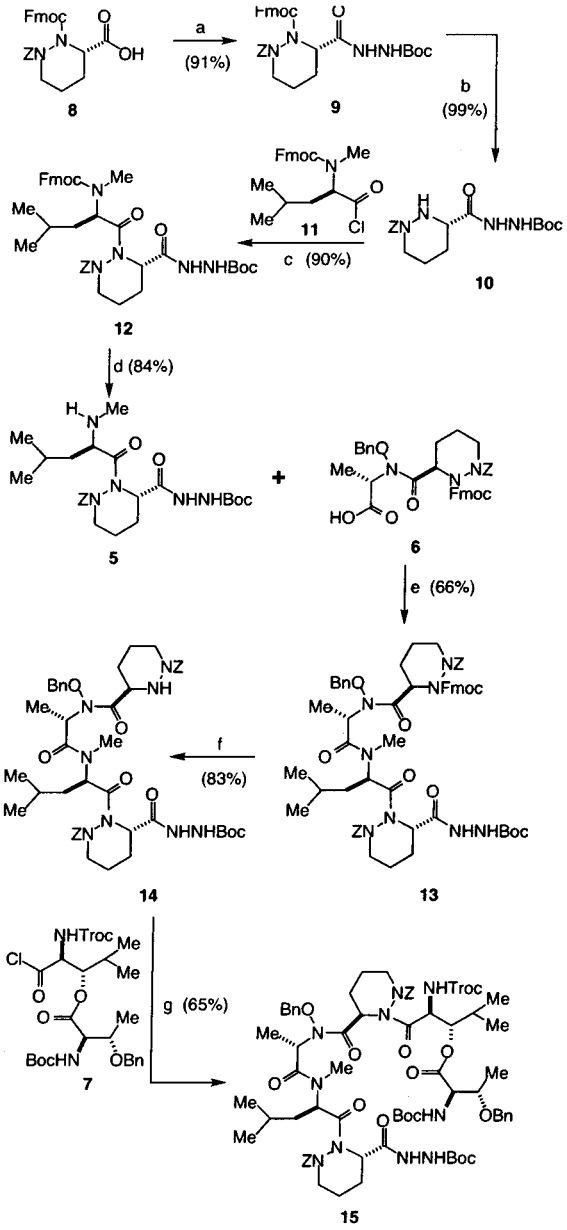
Scheme 1. Retrosynthetic Analysis of GE3



coupling of cyclodepsipeptide **3** with the activated benzotriazole ester **2**; a strategy that will deliberately avoid the use of protecting groups at the final stages of the synthesis. Our proposed pathway to the cyclodepsipeptide **3** was founded upon the successful [2 + 2 + 2] union of **5**−**7**.

The synthesis of dipeptide **5** commenced from known (3*S*)-*N*(1)-Z-*N*(2)-Fmoc-piperazic acid **8** (Scheme 2), which is

Scheme 2. Synthesis of Linear Hexapeptide **15**[a]



[a] Reagents and conditions: (a) DCC (1.1 equiv), $BocNHNH_2$ (1.2 equiv), THF (0.3 M), 0 °C, 1 h, and then rt, 20 h. (b) Et_2NH (40 equiv), MeCN (0.12 M), rt, 15 min. (c) **10** (1 equiv), **11** (1 equiv), AgCN (1.5 equiv), C_6H_6 (0.3 M), 80 °C, 50 min. (d) Et_2NH (40 equiv), MeCN (0.12 M), rt, 15 min. (e) Premix acid **6** with BOP−Cl at −20 °C in CH_2Cl_2 (0.15 M), add in collidine (1.1 equiv) over 1 min, stir for 20 min, and then add solid **5** (1 equiv) followed by collidine (1.1 equiv) over 1 min, warm to rt, and stir for 16 h. (f) Et_2NH (40 equiv), MeCN (0.12 M), rt, 15 min. (g) **14** (1 equiv), **7** (1 equiv), AgCN (1.5 equiv), C_6H_6 (0.12 M), 80 °C, 3−5 min.

readily accessible through our recently introduced tandem electrophilic hydrazination/nucleophilic cyclization protocol.[3e,4] Compound **8** was initially converted to the acyl hydrazide **9** by treatment with $BocNHNH_2$, DCC, and THF over a 20 h

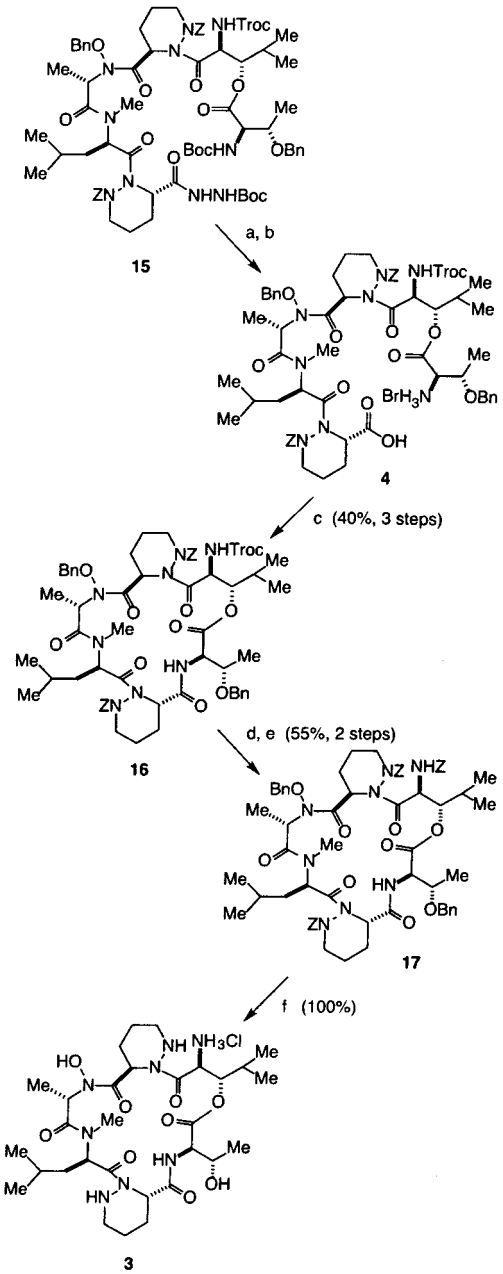
period.[5] The Fmoc group[6] was then excised from **9** with diethylamine in acetonitrile and **10** chemoselectively coupled with Fmoc-*N*-methyl-D-leucinoyl chloride **11**[7] under silver cyanide-assisted coupling conditions.[8] The latter reaction proceeded cleanly, providing the desired dipeptide **12** in 90% yield. Next, the Fmoc group was detached from **12** in 84% yield and amine **5** carefully purified by flash chromatography prior to attempting its coupling with known **6**.[3e]

A wide range of conditions were evaluated for effecting the desired amidation, but virtually all either performed badly or failed, *including the BOP−Cl/Et$_3$N system*[9] *that had previously worked so successfully in our A83586C synthesis*. After much effort, we eventually discovered that the combination of *BOP−Cl and collidine* could unite these two fragments; this regime afforded the pure tetrapeptide **13** in 66% yield.

The enormously beneficial effect of collidine upon the course of this coupling cannot be understated. Presumably, its much greater steric bulk, compared with that of Et$_3$N, helped prevent it from initiating base-catalyzed decomposition of the intermediary mixed anhydride derived from **6** and thwarted its cleavage of the Fmoc group at temperatures above 0 °C. The latter was especially problematic when Et$_3$N was employed as the base, due to the much slower rate of coupling of **5** and **6**. This was the result of the enhanced steric hindrance around the *N*-methyl D-leucine nitrogen in **5**, compared with that around its A83586C *N*-methyl-D-Ala dipeptide counterpart.[3a] Breakdown of the mixed anhydride by Et$_3$N could potentially be initiated by α-deprotonation to give a ketene intermediate or, alternatively, by enolization of the (3*R*)-Piz residue and subsequent intramolecular attack of the enolate upon the mixed anhydride. These two events (along with Fmoc deprotection) could be expected to contribute significantly to the observed decomposition when Et$_3$N is used as the base. The new BOP−Cl/collidine modification solves this thorny problem very effectively and was central to our eventual success in this venture.

After excising the Fmoc group from the (3*R*)-piperazic acid residue of **13**, the final fragment condensation was attempted with the previously prepared acid chloride **7**.[3a] The silver cyanide-mediated[8] [4 + 2] coupling of **14** with **7** was much less successful than the analogous coupling in our A83586C synthesis.[3a] After some optimization, however, a workable 65% yield of **15** was ultimately obtained.[10] At this juncture, we cleaved the two Boc groups from **15** with trifluoroacetic acid in CH_2Cl_2 (Scheme 3) and oxidized the liberated *N*-acyl hydrazine with *N*-bromosuccinimide in THF and water to obtain acid **4**.[11] A Carpino HATU-mediated cyclization[12] was then effected at a very high dilution in CH_2-

Scheme 3. Completion of the GE3 Cyclodepsipeptide **3**[a]



[a] Reagents and conditions: (a) CF_3CO_2H (200 equiv), CH_2Cl_2 (0.06 M), 0 °C, 2 h. (b) NBS (2 equiv), THF:H_2O (1:1) (0.04 M), rt, 2 h. (c) Add **4** (1 equiv) in CH_2Cl_2 (0.00086 M) to HATU (10 equiv) and NEM (13.5 equiv) in CH_2Cl_2 (0.00086 M) at 0 °C over 6 h, and then stir at 0 °C for 2 h, and at rt for 60 h. (d) Zn dust (85 equiv), AcOH:H_2O (10:1) (0.3 M), rt, 25 min. (e) BnOC(O)Cl (3 equiv), 10% aq $NaHCO_3$/CH_2Cl_2 (0.1 M), rt, 1 h. (f) H_2 (1 atm), MeOH (0.01 M), HCl (1 equiv), 10% Pd on C (0.6 g per g of **17**), rt, 16 h.

(4) Hale, K. J.; Cai, J.; Delisser, V.; Manaviazar, S.; Peak, S. A.; Bhatia, G. S.; Collins, T. C.; Jogiya, N. *Tetrahedron* **1996**, *52*, 1047.

(5) (a) BocNHNH$_2$: Boissonnas, R. A. ; Guttman, St.; Jaquenoud, P. A. *Helv. Chim. Acta* **1960**, *43*, 1349. (b) For the use of TrocNHNH2/DCC to make Troc acyl hydrazides, see: Fujii, N.; Yajima, H. *J. Chem. Soc., Perkin Trans. 1*, **1981**, 804.

(6) Carpino, L. A.; Han, G. Y. *J. Org. Chem.* **1972**, *37*, 3404.

(7) Fmoc-*N*-Methyl-D-leucine was prepared according to the method in: Freidinger, R. M.; Hinkle, J. S.; Perlow, D. S.; Arison, B. H. *J. Org. Chem.* **1983**, *48*, 77. It was converted to Fmoc-*N*-methyl-D-leucinoyl chloride by treatment with (COCl)$_2$ and C_6H_6 at rt for 2 h and used without purification after removal of the solvent and excess reagent *in vacuo*.

(8) AgCN-mediated *N*-acylation: Durette, P. L.; Baker, F.; Barker, P. L.; Boger, J.; Bondy, S. S.; Hammond, M. L.; Lanza, T. J.; Pessalano, A.; Caldwell, C. G. *Tetrahedron Lett.* **1990**, *31*, 1237.

(9) Tung, R. D.; Rich, D. H. *J. Am. Chem. Soc.* **1985**, *107*, 4342.

(10) It is important not to prolong the heating of this reaction much beyond the 3−5 min time frame we have recommended; otherwise, decomposition ensues.

Cl_2 (0.00043 M with respect to **4**). Fortunately, this protocol closed the 19-membered cyclodepsipeptide ring reasonably successfully, the desired macrolactam **16** being isolated in 40% overall yield for the three steps from **15**. In this particular instance, the *N*-methyl D-leucine residue exerted a beneficial effect on the outcome of the reaction. It was a result that contrasted sharply with the macrolactamization in A83586C, where a 25% yield of product was obtained solely from the cyclization.[3a]

To complete the synthesis of **3**, we deprotected the Troc group of **16** with Zn dust in aqueous acetic acid[13] and temporarily capped the liberated nitrogen with a Z-group to facilitate the final purification of **17**. The latter was then cleanly deprotected by catalytic hydrogenation at atmospheric pressure over a 10% Pd on carbon catalyst in anhydrous methanol containing 1 equiv of HCl.[3a] Deprotection in an acidic medium helps prevent an *O*- to *N*-acyl shift from occurring in the β-hydroxyleucine residue.[14] The latter can be a problem when such systems are hydrogenated for protracted periods under neutral or mildly basic conditions. The final product **3** was obtained in a fairly pure condition as judged by 500 MHz ^{1}H NMR and HRMS analysis. In the HRMS, an $(M + H)^+$ ion was observed at *m*/*e* 669.3962, which correlated closely with the calculated value of *m*/*e* 669.3936 for $C_{30}H_{53}N_8O_9$ (see Supplementary Information). Proof that the lactone linkage was present was provided by the IR spectrum of **3**, which showed a strong ester C=O stretching absorption at 1741 cm^{-1}. It is our recommendation that **3** be used for subsequent couplings without any further purification.[15]

One item of note in our synthesis is the global *O*-debenzylation of **17**, which took place under much less forcing condtions than were needed for the A83586C synthesis. In that instance, the cyclodepsipeptide precursor required 24 h of hydrogenation at 75 psi before it was fully deprotected. The ease of the present deprotection and the markedly improved yield of the GE3 macrolactamization reaction clearly bode well for the development of a future commercial synthesis of this molecule. These observations, along with our findings on the [2 + 2] coupling of **5** with **6**, highlight how a change of only one amino acid residue can dramatically affect the success of a route to a linear or cyclic peptide.

In summary, a reasonably efficient synthesis of the GE3 cyclodepsipeptide has been developed. Of considerable significance is the fact that our route proceeds in 5.34% overall yield from known **8**, and its longest linear sequence is only 13 steps. The prospect of a future total synthesis of GE3 has now been brought much closer.

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Supporting Information Available: ^{1}H NMR spectra (500 MHz), ^{13}C NMR spectra (125 MHz), and HRMS data for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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(11) Cheung, H. T.; Blout, E. R. *J. Org. Chem.* **1965**, *30*, 315.

(12) Carpino, L. A. *J. Am. Chem. Soc.* **1993**, *115*, 4937.

(13) (a) Windholz, T. B.; Johnston, D. B. R. *Tetrahedron Lett.* **1967**, 2555. (b) Pfeiffer, F. R.; Cohen, S. R.; Weisbach, J. A. *J. Org. Chem.* **1969**, *34*, 2795.

(14) For the occurrence of a similar *O*- to *N*-acyl shift during removal of a *Z*-group from an acylated β-hydroxyleucine residue, see the Merck L-156,602 synthesis referred to in ref 8.

(15) Past synthetic experience in these laboratories on handling free cyclodepsipeptide hydrochlorides of the A83586C class has shown that decomposition can occur after reverse-phase chromatography with H_2O or H_2O/MeOH, when these solvents are subsequently removed on the rotary evaporator. Given that these hydrochloride salts are usually obtained in a fairly pure condition after hydrogenation, we recommend that they be used directly for subsequent couplings without any further purification.