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Synthetic Studies on Et-743. Assembly of the Pentacyclic Core and a Formal Total Synthesis

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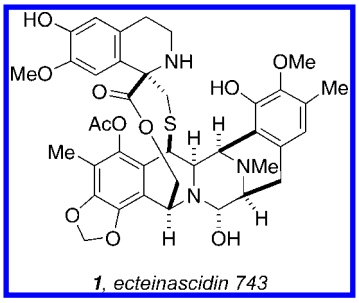
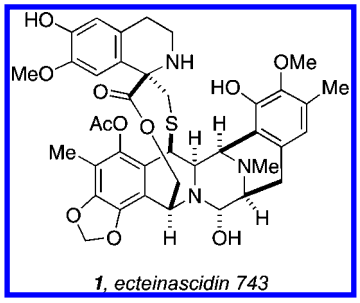


1, ecteinascidin 743

A formal total synthesis of the potent anticancer agent Et-743 is described. The tetrahydroisoquinoline core is stereoselectively constructed using a novel radical cyclization of a glyoxalimine. Further elaboration of this core rapidly accessed the pentacyclic core of Et-743, but a mixture of regiosisomers was obtained in the key Pictet−Spengler ring closure. A known advanced intermediate in the synthesis of Et-743 was intercepted, constituting a formal synthesis of the molecule.

Introduction

Members of the tetrahydroisoquinoline family of alkaloids display a wide range of biological properties such as antitumor and antimicrobial activities.[1] Of particular significance within this family is Ecteinascidin 743 (Et-743, **1**, Figure 1,) which has been demonstrated to possess extremely potent cytotoxic activity with in vitro IC_{50} values in the 0.1−1 ng/mL range in several cell lines (as a measure of RNA, DNA, and protein synthesis inhibition).[2] Et-743 is currently in phase II/III clinical trials for the treatment of ovarian, endometrial, and breast cancers and several sarcoma lines.[3]The scarcity of the natural product from marine sources renders Et-743 an important target for synthesis. Corey and co-workers reported the first total synthesis of Et-743 in 36 steps with an overall yield of 0.72%.[4a]



1, ecteinascidin 743

FIGURE 1. Ecteinascidin 743 (**1**).

A second-generation synthesis improved the overall yield to 2.04%, but still required 36 steps.[4b] Fukuyama and co-workers achieved a total synthesis of Et-743 in 50 steps and 0.56% overall yield.[5] More recently, Zhu and co-workers reported a 31 step synthesis in 1.7% overall yield.[6] Most recently, Danishefsky and co-workers reported a formal total synthesis[7]via a pentacyclic compound that intercepted a late-stage intermediate of Fukuyama's route.[5] Despite the advancements in the state-of-the-art in total synthetic approaches to Et-743, the clinical supply of this complex drug is semisynthetically derived from natural cyanosafracin B, obtained by fermentation as reported by PharmaMar.[8]

Our laboratory has been developing methodology for the assembly of tetrahydroisoquinoline natural products and has

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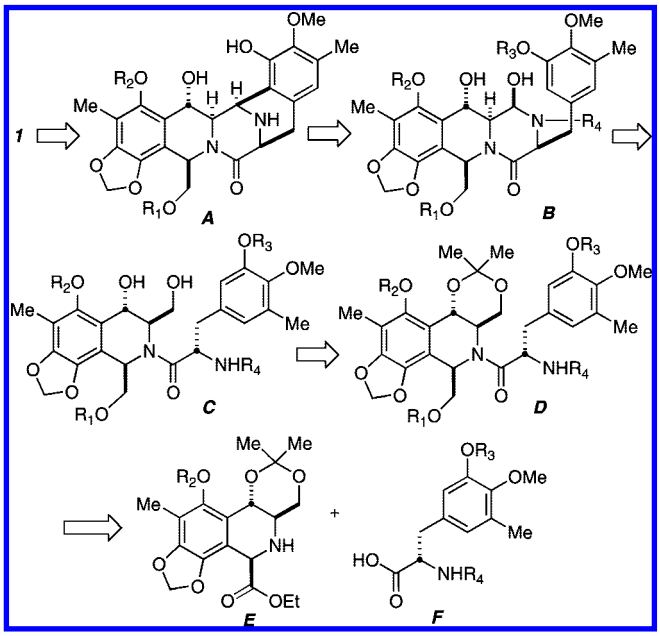
(1) Scott, J. D.; Williams, R. M. *Chem. Rev.* **2002**, *102*, 1669–1730.
(2) (a) Rinehart, K. L.; Holt, T. G.; Fregeau, N. L.; Keifer, P. A.; Wilson, G. R.; Perun, T. J.; Sakai, R.; Thompson, A. G.; Stroh, J. G.; Shield, L. S.; Seigler, D. S. *J. Nat. Prod.* **1990**, *53*, 771–792. (b) Rinehart, K. L.; Holt, T. G.; Fregeau, N. L.; Stroh, J. G.; Keifer, P. A.; Sun, F.; Li, L. H.; Martin, D. G. *J. Org. Chem.* **1990**, *55*, 4512–4515. (c) Wright, A. E.; Forleo, D. A.; Gunawardana, G. P.; Gunasekera, S. P.; Koehn, F. E.; McConnell, O. J. *J. Org. Chem.* **1990**, *55*, 4508–4512. (d) Guan, Y.; Sakai, R.; Rinehart, K. L.; Wang, A. H.-J. *J. Biomol. Struct. Dyn.* **1993**, *10*, 793–818. (e) Aune, G. J.; Furuta, T.; Pommier, Y. *Anti-Cancer Drugs* **2002**, *13*, 545–555. (f) Rinehart, K. L. *Med. Drug Rev.* **2000**, 1–27.

SCHEME 1. Synthetic Plan



reported syntheses of D,L-quinocarcinamide,[9] (−)-tetrazomine,[10] (−)-renieramycin G,[11] (−)-jorumycin,[11] and cribrostatin 4 (renieramycin H).[12] As a part of this program, we have targeted Et-743 by a convergent route that envisioned coupling of a suitably functionalized tyrosine derivative[13] with the complete tetrahydroisoquinoline core (Scheme 1.) We have successfully deployed this strategy, with the present objective of construction of pentacycle **A**, in the synthesis of (−)-renieramycin G and (−)-jorumycin.[11,12]

We have previously reported a concise and highly diastereoselective synthesis of the tetrahydroisoquinoline core of Et-743 (**E**).[14] This was achieved via an intramolecular 6-*endo* radical closure on a glyoxalimine, and the desired 1,3-*cis*-diastereomer was obtained exclusively. The synthesis of a tetrahydroisoquinoline such as **E** can be problematic because of the acid sensitivity of the benzylic hydroxyl, particularly because it is *ortho* to the phenolic hydroxyl of the aromatic ring and thus has a high propensity for *ortho*-quinonemethide formation. Herein, we report a formal total synthesis of Et-743 as part of our ongoing efforts to devise a practical and scalable synthesis of this potent antitumor antibiotic that would be amenable to the construction of analogues with anticipated potent cytotoxic activity.

Results and Discussion

The synthesis began with Borchardt's catechol **3**[15] that was regioselectively brominated to generate **4** (92% yield) (Scheme 2.) Conversion of catechol **4** to the methylenedioxy aldehyde **5** was accomplished using bromochloromethane in a sealed vessel (69% yield). Baeyer−Villiger oxidation using *m*-CPBA provided bromophenol **6** as an off-white solid following hydrolysis of the resulting formate intermediate (73% yield). Stereoselective aldol condensation of the titanium phenolate of **6** with (*R*)-Garner's aldehyde (**7**)[16] using a modification of Casiraghi's method[17] provided the *anti*-product **8** followed by allyl protection of the phenolic oxygen delivering **9** (65% yield, two steps). Subsequent hydrolysis of the oxazolidine and formation of the *trans*-acetonide (84% yield, two steps) provided **10** as an oil that cleanly underwent *N*-Boc deprotection using Ohfune's protocol[18] (76% yield) to afford free amine **11** as a stable crystalline solid. From **11**, the glyoxalimine intermediate **13** (see Scheme 3) was readily obtained by condensation with ethyl glyoxalate. Following isolation by filtration through Celite and concentration, the radical ring closure commenced with slow addition of Bu_3SnH and AIBN via syringe pump to a refluxing dilute solution of the glyoxalimine (**13**). Concentration and KF/silica chromatography[19] of the crude reaction mixture provided solid **12** as a single diastereomer (58% yield, two steps). The relative stereochemistry of **12** was secured ^{1}H NMR data and corroborated by X-ray crystallography. Examination of the crude ^{1}H NMR revealed the formation of a single diastereomer in the radical closure and exclusive 6-*endo* regioselectivity. In addition to **12** and tin impurities visible in the ^{1}H NMR spectrum, an aromatic proton arising from hydride quenching of the aryl radical revealed a ~6.6:1 ratio of **12** to reduced substrate. Slower addition rates (over 18 or 36 h) did not improve the isolated yield of **12**.

(3) (a) Zelek, L.; Yovine, A.; Etienne, B.; Jimeno, J.; Taamma, A.; Martín, C.; Spielmann, M.; Cvitkovic, E.; Misset, J. L. *Ecteinacidin-743 in Taxane/Antracycline Pretreated Advanced/Metastatic Breast Cancer Patients: Preliminary Results with the 24 h Continuous Infusion Q3 Week Schedule*; 36th Annual Meeting of the American Society of Clinical Oncology, New Orleans, May 20-23, 2000; Abstract number 592. (b) Delaloge, S.; Yovine, A.; Taamma, A.; Cottu, P.; Riofrio, M.; Raymond, E.; Brain, E.; Marty, M.; Jimeno, J.; Cvitkovic, E.; Misset, J. L. *Preliminary Evidence of Activity Of Ecteinacidin-743 (ET-743) in Heavily Pretreated Patients with Bone and Soft Tissue Sarcomas*; 36th Annual Meeting of the American Society of Clinical Oncology, New Orleans, May 20-23, 2000; Abstract number 2181. (c) Le Cesne, A.; Judson, I.; Blay, J. Y.; Radford, J.; an Oosterom, A.; Lorigan, P.; Rodenhuis, E.; Donato Di Paoula, E.; Van Glabbeke, M.; Jimeno, J.; Verweij, J. *Phase II of ET-743 in Advance Soft Tissue Sarcoma in Adult:A STBSG-EORTC Trial*; 36th Annual Meeting of the American Society of Clinical Oncology, New Orleans, May 20-23, 2000; Abstract number 2182. (d) Aune, G. J.; Furuta, T.; Pommier, Y. *Anti-Cancer Drugs* **2002**, *13*, 545–555.

(4) (a) Corey, E. J.; Gin, D. Y.; Kania, R. S. *J. Am. Chem. Soc.* **1996**, *118*, 9202–9203. (b) Martinez, E. J.; Corey, E. J. *Org. Lett.* **2000**, *2*, 993–996.

(5) Endo, A.; Yanagisawa, A.; Abe, M.; Tohma, S.; Kan, T.; Fukuyama, T. *J. Am. Chem. Soc.* **2002**, *124*, 6552–6554.

(6) Chen, J.; Chen, X.; Bois-Choussy, M.; Zhu, J. *J. Am. Chem. Soc.* **2006**, *128*, 87–89.

(7) Zheng, S.; Chan, C.; Furuuchi, T.; Wright, B. J. D.; Zhou, B.; Guo, J.; Danishefsky, S. J. *Angew. Chem., Int. Ed.* **2006**, *45*, 1754–1759.

(8) Cuevas, C.; Pérez, M.; Martín, M. J.; Chicharro, J. L.; Fernández-Rivas, C.; Flores, M.; Francesch, A.; Gallego, P.; Zarzuelo, M.; de la Calle, F.; García, J.; Polanco, C.; Rodríguez, I.; Manzanares, I. *Org. Lett.* **2000**, *2*, 2545–2548.

(9) Flanagan, M. E.; Williams, R. M. *J. Org. Chem.* **1995**, *60*, 6791–6797.

(10) (a) Scott, J. D.; Williams, R. M. *Angew. Chem., Int. Ed.* **2001**, *40*, 1463–1465. (b) Scott, J. D.; Williams, R. M. *J. Am. Chem. Soc.* **2002**, *124*, 2951–2956.

(11) (a) Lane, J. W.; Chen, Y.; Williams, R. M. *J. Am. Chem. Soc.* **2005**, *127*, 12684–12690. (b) Lane, J. W.; Estevez, A.; Mortara, K.; Callan, O.; Spencer, J. R.; Williams, R. M. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 3180–3183. (c) Vincent, G.; Lane, J. W.; Williams, R. M. *Tetrahedron Lett.* **2007**, *48*, 3719–

(12) (a) Vincent, G.; Williams, R. M. *Angew. Chem., Int. Ed.* **2007**, *46*, 1517–1520. (b) Vincent, G.; Chen, Y.; Lane, J. W.; Williams, R. M. *Heterocycles* **2007**, *72*, 385–398.

(13) Jin, W.; Williams, R. M. *Tetrahedron Lett.* **2003**, *44*, 4635–4639.

(14) Fishlock, D.; Williams, R. M. *Org. Lett.* **2006**, *8*, 3299–3301.

(15) Prep a red from 2,3-dimethoxytoluene according to: Shinhababu, A. K.; Ghosh, A. K.; Borchardt, R. T. *J. Med. Chem.* **1985**, *28*, 1273–1279.

(16) (*R*)-Garner's aldehyde was synthesized from D-serine according to: Garner, P.; Park, J. M. *Org. Synth.* **1992**, *70*, 18–28.

(17) Casiraghi, G.; Cornia, M.; Rassu, G. *J. Org. Chem.* **1988**, *53*, 4919–4922. In our case, sonication was not required as described in the original paper.

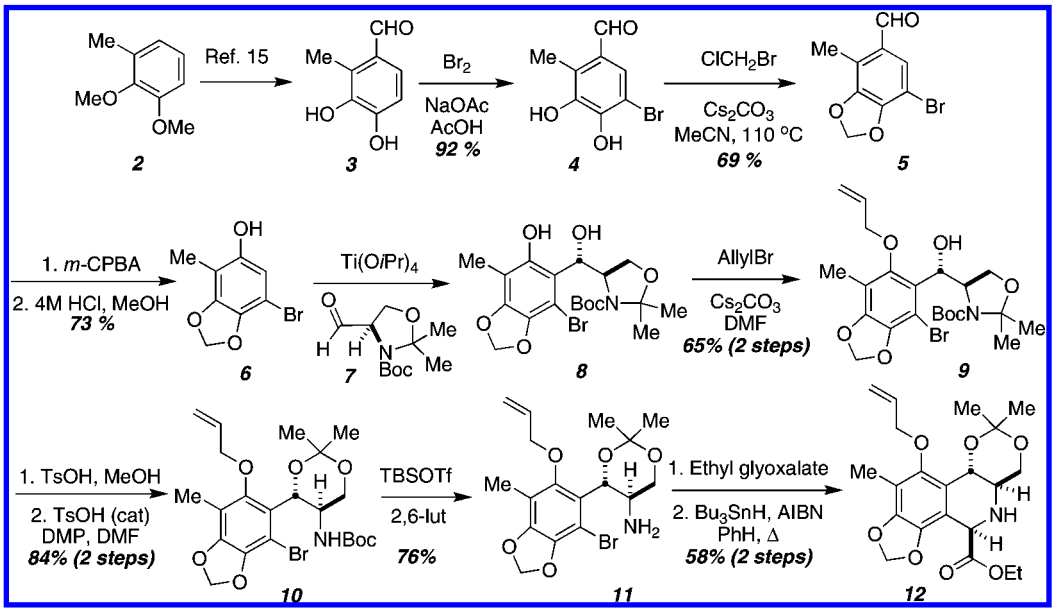
(18) Sakaitani, M.; Ohfune, Y. *J. Org. Chem.* **1990**, *55*, 870–876.

(19) Effective removal of tin impurities from Bu_3SnH-mediated reactions: Harrowven, D. C.; Guy, I. L. *Chem. Commun.* **2004**, 1968–1969.

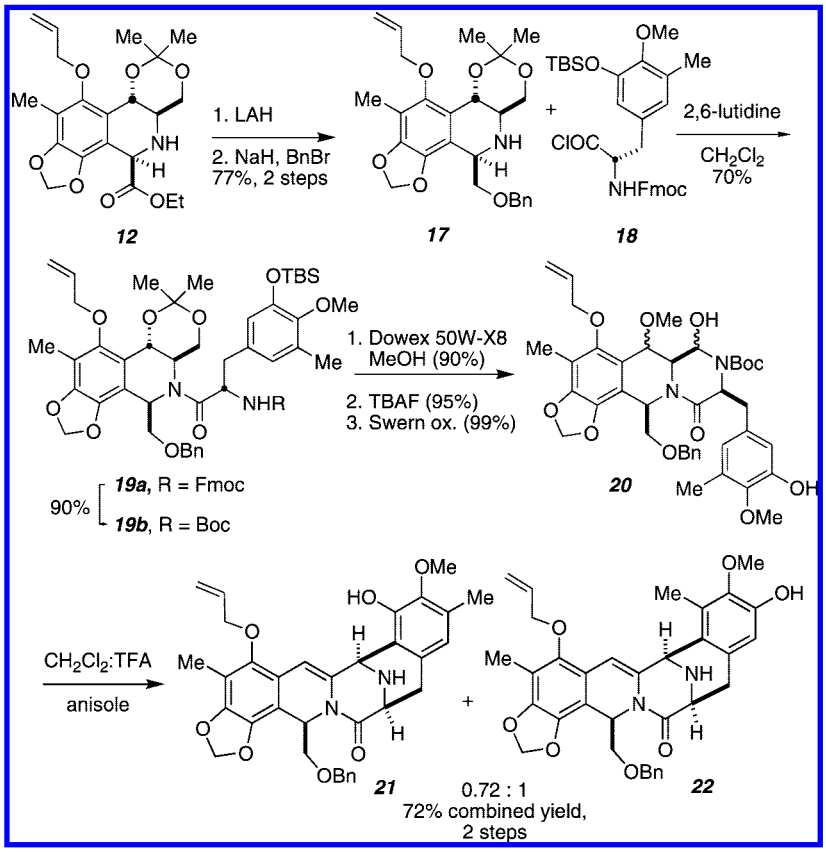
(20) (a) De Paolis, M.; Chiaroni, A.; Zhu, J. *Chem. Commun.* **2003**, 2896–2897. (b) Chen, X.; Chen, J.; De Paolis, M.; Zhu, J. *J. Org. Chem.* **2005**, *70*, 4397–4408.

(21) (a) Herberich, B.; Kinugawa, M.; Vazquez, A.; Williams, R. M. *Tetrahedron Lett.* **2001**, *42*, 543–546. (b) Jin, W.; Metobo, S.; Williams, R. M.

SCHEME 2. Tetrahydroisoquinoline Core of Et-743



SCHEME 3. Pentacycle Construction



The diastereoselectivity of this reaction stands apart from numerous Pictet−Spengler cyclizations on related substrates that provide tetrahydroisoquinolines exclusively as the 1,3-*trans*-diastereomers.[11,20,21] We qualitatively rationalize the *cis*-diastereoselectivity of this radical process using the Beckwith−Houk chairlike transition state model for intramolecular radical ring closures (Figure 2).[22] The lowest-energy chair conformation (**A**) adopted by the *trans*-acetonide of the substrate (**13**) results in both the glyoxalimine and aryl substituent being in an equatorial disposition. In this conformation, 1,3-diaxial steric effects and allylic strain interactions are minimized in the ring-forming transition state. To further examine the stereocontrol imparted by the acetonide ring, the *cis*-acetonide substrate **14** was prepared (using Casiraghi's method from the magnesium phenolate of **6**).[17] Substrate **14** resulted in a 1:1 mixture of 1,3-

(22) (a) Beckwith, A. L. J.; Schiesser, C. H. *Tetrahedron* **1985**, *41*, 3925

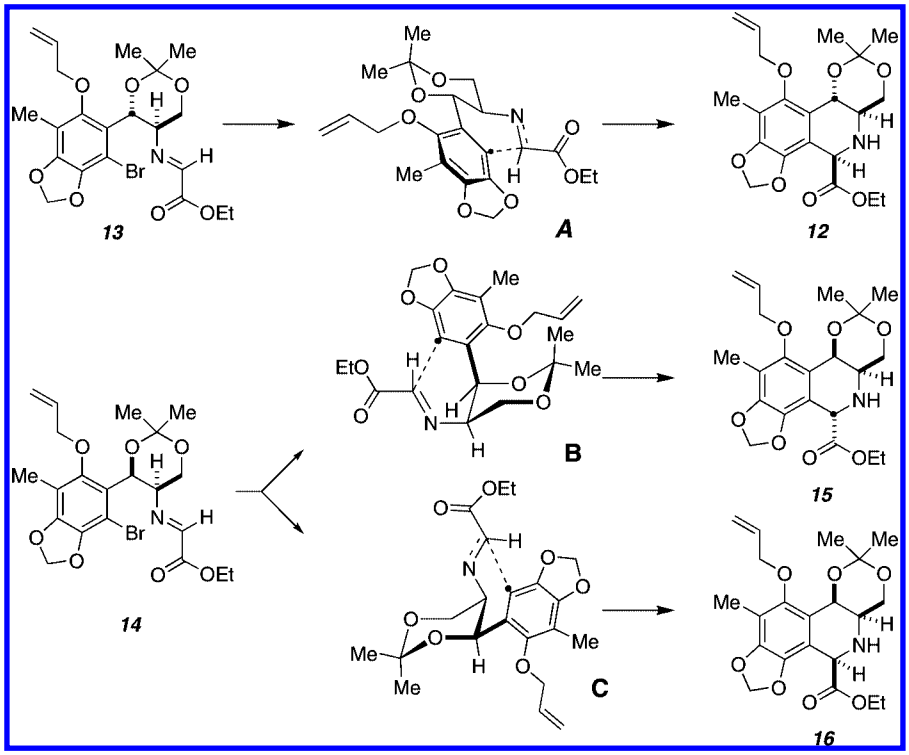


FIGURE 2. Transition state models to rationalize the observed 1,3 relative stereochemistry in the tetrahydroisoquinoline radical ring closure.

trans- and 1,3-*cis*-tetrahydroisoquinolines (**15** and **16**, both are known compounds),[20] which suggests the energy difference between transition state conformations **B** and **C** (axial aryl group versus axial glyoxalimine) is negligible.

As shown in Scheme 3, reduction of the tetrahydroisoquinoline ester (**12**)[14] with LAH, followed by immediate protection as the benzyl ether (**17**), proceeded cleanly in 77% yield over two steps. The substituted tyrosine amino acid component (**18**) has been previously reported by us, utilizing the oxazinone template technology developed in our laboratory that was benzylated with the advanced aromatic side chain.[13] Thus, acylation of the tetrahydroisoquinoline (**17**) was achieved via the *N*-Fmoc-protected amino acid chloride (**18**) to give amide **19a** without epimerization. The use of the *N*-Boc free acid with a variety of coupling agents (DCC, HOBt, HATU) all resulted in very sluggish reactions with poor isolated yields, as did the attempted use of the *N*-Boc acid fluoride.

Treatment of **19a** with diethylamine provided the free amine, which was not isolated in favor of immediate evaporation of excess base and solvent and subsequent Boc protection of the crude material. Isolation following chromatography provided compound **19b** in 90% yield. Removal of the acetonide from **19b** was accomplished using the extremely mild, albeit slow, method of stirring with Dowex 50W-X8 cationic resin in methanol. Complete deprotection took 8−12 h, but the yield was quantitative following simple filtration and concentration. Instead of providing the usual diol product, this substrate incorporated methanol at the benzylic position thus providing the methyl ether as a ∼1:1 mixture of diastereomers. Not unexpectedly, the benzylic stereogenic center loses stereochemical integrity since the methanol is incorporated via the incipient *ortho*-quinonemethide species arising from the acidic deprotection conditions.

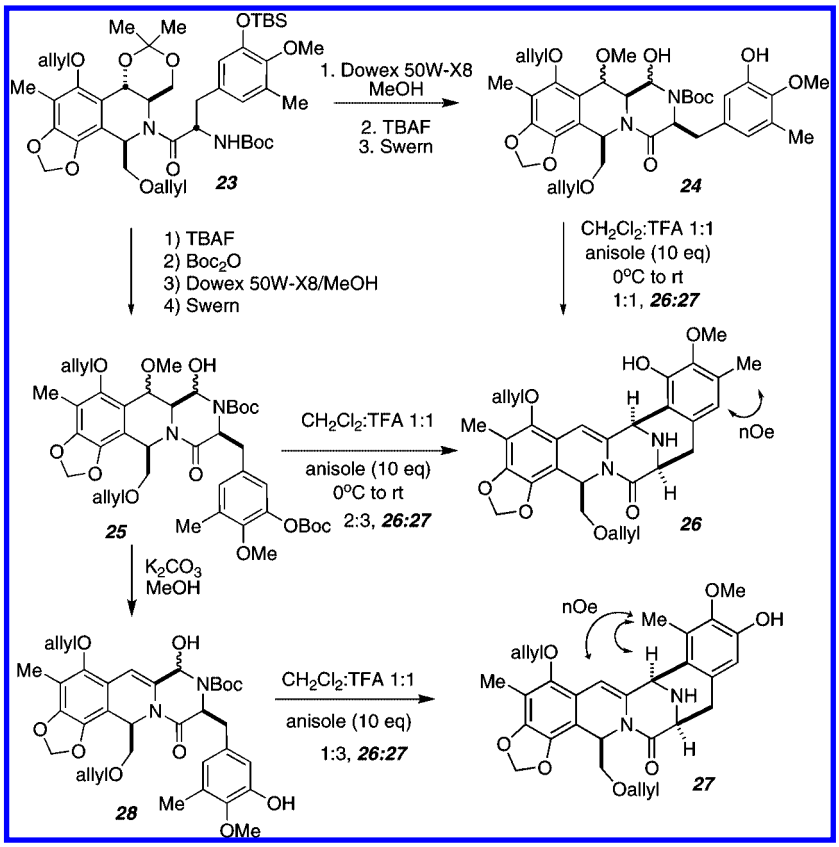
Alternatively, we found that the use of water/dichloromethane diol, but oxidation of the primary alcohol (in the presence of the free benzylic alcohol) could not, in our hands, be cleanly accomplished. The methyl ether was thus a fortuitous selective protection of the benzylic alcohol, ultimately simplifying the subsequent manipulations.

Facile deprotection of the *O*-TBS-protected phenol using TBAF was followed by oxidation of the primary alcohol using Swern conditions in high yield. This oxidation product (**20**) existed as an equilibrium mixture of the aldehyde and the corresponding hemiaminal species (illustrated) as observed by ^{1}H NMR, which was otherwise additionally complicated by amide and carbamate rotamers. The attempted oxidation using either Dess−Martin periodinane or TPAP/NMO both failed, leading to extensive decomposition. Following filtration of crude **20** through a plug of silica gel, this substance was immediately subjected to the Pictet−Spengler conditions.

The objective at this stage was to achieve the Pictet−Spengler reaction via *N*-Boc deprotection, iminium ion formation, and electrophilic aromatic substitution to provide the desired pentacyclic core of Et-743. This meant that the aromatic substitution must occur *ortho* to the free phenol, and the benzylic methyl ether must survive these conditions. Unfortunately, it had already been demonstrated above that the electron-rich aromatic ring of the tetrahydroisoquinoline component was highly sensitive to protic conditions, leading to *ortho*-quinonemethide formation.

Indeed, when substrate **20** was treated with trifluoroacetic acid in methylene chloride, it cleanly underwent the expected pentacycle formation furnishing **21** + **22** as a ∼0.72:1 *ortho:para* mixture of regioisomers in 72% combined yield. As anticipated, the benzylic methoxy group was eliminated presumably via the incipient *ortho*-quinonemethide species that forms under these conditions. In a fruitless effort to circumvent the vexing olefin formation, pentacycle formation with TFA in dry

SCHEME 4. Pictet−Spengler Regioselectivity



As part of these synthetic investigations, the intermediate **23** was prepared (in parallel with the *O*-benzyl-protected synthesis) bearing an *O*-allyl-protected hydroxymethyl at C1 of the THIQ core. This substrate was used to examine the regioselectivity of the pentacycle-forming ring closure and was utilized to acquire detailed ^{1}H NMR data, while the *O*-benzyl material **21** was carried forward in the synthesis. One interesting observation was the behavior of compound **25** containing the *O*-Boc carbonate-protected phenolic oxygen. Treatment of **25** under the same reaction conditions provided the pentacycles **26** + **27** in a 2:3 ratio of *ortho*:*para* regioisomers. The *O*-Boc carbonate would presumably be deprotected quickly under these conditions to reveal the free phenol-containing reactive species, thus resulting in a comparable regioselectivity as observed with substrate **20** (beginning with a free phenol on the aryl nucleophile moiety). Notably, however, when substrate **25** was treated with K_2CO_3/MeOH, the *O*-Boc carbonate was selectively removed (**28**) with apparent olefin formation prior to the Pictet−Spengler reaction and pentacycle formation. Treatment of **28** with TFA in dichloromethane produced the pentacycles **26** + **27** in a 1:3 ratio of *ortho*:*para* regioisomers, supporting the hypothesis that some regioselectivity in the closure might arise from an intramolecular H bond with a heteroatom at the benzylic position.[11c]

In their synthesis of renieramycin H, the Zhu group has interestingly reported control of Pictet−Spengler regioselectivity in a related system by variation of acid concentration (Scheme 4).[23] It was found in that case that lowering the concentration of methanesulfonic acid to 0.01% in CH_2Cl_2 could invert the *ortho:para* selectivity from 3.4:1 to 2:3. Furthermore, the use of acetonitrile as the solvent instead of dichloromethane favored attempt to reproduce the Zhu conditions on substrate **24** using 0.01% methanesulfonic acid in CH_2Cl_2 did not affect the regioselectivity of this reaction. The substrate was consumed to provide some material that appeared to still contain the *N*-Boc protecting group, but the ^{1}H NMR of the crude product was prohibitively complex. Subsequent treatment of this reaction crude with a TFA/anisole/CH_2Cl_2 mixture provided the pentacycles **26** + **27** with ∼1:1 regioselectivity. The same ratio is obtained if the TFA/anisole conditions are used directly on substrate **24**.

In order to redeem the synthetic utility of the olefinic products (**21** or **26**), our attention was captured by the recent formal synthesis of Et-743 reported by the Danishefsky group[7] in which the olefin (**29**, Scheme 5) underwent facile oxidation using DMDO and immediate hydride reduction delivering the benzylic alcohol **30**. With the availability of this methodology in the literature, our efforts were briefly redirected to convert our synthetic pentacycle **21** into compound **29** which would constitute a formal total synthesis of Et-743 by relay through the Danishefsky[7] and then Fukuyama[5] syntheses, respectively.

In the event, the desired pentacycle **21** (Scheme 3) was *N*-protected as the trichloroethyl carbamate (Troc), and the phenolic residue was protected as the corresponding *O*-benzyl ether in 85% yield for the two steps (Scheme 5). Removal of the *O*-allyl group under standard conditions followed by reprotection as the corresponding MOM ether provided compound **29** (56% yield for the two steps). Compound **29** perfectly matched Danishefsky's substrate by ^{1}H, ^{13}C NMR, and optical rotation, confirming the structure of compound **29**.

Since Danishefsky has previously converted[7] compound **29** into a late-stage intermediate in Fukuyama's total synthesis[5]

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