ORGANIC LETTERS

2013
Vol. 15, No. 12
3118–3121

Total Synthesis of Aplyronine C

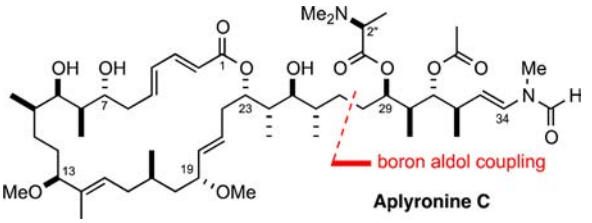
Ian Paterson,* Sarah J. Fink, Lydia Y. W. Lee, Stephen J. Atkinson, and Simon B. Blakey

University Chemical Laboratory, University of Cambridge, Lensfield Road, Cambridge CB2 1EW, U.K.

ip100@cam.ac.uk

Received May 13, 2013

ABSTRACT



A highly stereocontrolled total synthesis of the cytotoxic marine macrolide aplyronine C is described. The route exploits aldol methodology to install the requisite stereochemistry and features a crucial boron-mediated aldol coupling of an *N*-vinylformamide-bearing methyl ketone with a macrocyclic aldehyde to introduce the full side chain. The synthesis of two novel C21−C34 side chain analogs is also reported.

Actin, the most common protein in eukaryotic cells, is involved in numerous vital cellular functions including cell shape maintenance, division, locomotion, and adhesion.[1] Actin dynamics are normally tightly controlled by a number of actin-binding proteins; dysregulation has been implicated in diseases including stroke, cystic fibrosis, and cancer.[1a,2]

Aplyronines A−H[3] (Figure 1) comprise a family of actin-binding marine macrolides that can serve as small molecule mimics of actin-binding proteins. They were isolated in low yield (10^{-5}−10^{-7}% based on wet weight) from the Japanese sea hare *Aplysia kurodai* by Yamada and co-workers based on their potent cytotoxicity against HeLa-S3 cells.[4] Notably, the antiproliferative efficacy of aplyronine A (**1**) has been demonstrated *in vivo* against P388 leukemia (T/C 545%, 0.08 mg/kg) and Lewis lung carcinoma (T/C 556%, 0.04 mg/kg), leading to its identification as a promising anticancer drug candidate.[5]

Aplyronine A forms a 1:1 complex with globular actin (G-actin, K_d = 100 nM), inhibiting polymerization, and also depolymerizes fibrous actin (F-actin).[6] X-ray analysis of the actin−aplyronine A crystal structure,[7] structure−activity relationship studies,[8] and photoaffinity studies[9] have highlighted the importance of the C24−C34 tail region in the strong actin depolymerizing activity. The mechanism of cytotoxicity for these macrolides remains unelucidated. Recent work has shown that aplyronine A causes caspase-dependent apoptosis with rapid disassembly of the actin cytoskeleton and dephosphorylation of focal adhesion kinase[10] and has suggested an interaction between the aplyronine−actin complex and a secondary biomolecule such as Arp2/3.[11]

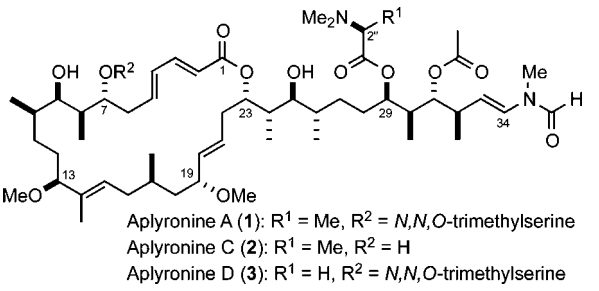


Figure 1. Structures of selected aplyronines.

(1) Reviews: (a) Allingham, J. S.; Klenchin, V. A.; Rayment, I. *Cell Mol. Life Sci.* **2006**, *63*, 2119. (b) Yeung, K.−S.; Paterson, I. *Angew. Chem., Int. Ed.* **2002**, *41*, 4632.

(2) (a) Pantaloni, D.; le Clainche, C.; Carlier, M. F. *Science* **2001**, *292*, 1502. (b) Pollard, T. D.; Borisy, G. G. *Cell* **2003**, *112*, 453.

(3) (a) Yamada, K.; Ojika, M.; Ishigaki, T.; Yoshida, Y.; Ekimoto, H.; Arakawa, M. *J. Am. Chem. Soc.* **1993**, *115*, 11020. (b) Yamada, K.; Ojika, M.; Kigoshi, H.; Suenaga, K. In *Drugs from the Sea*; Fusetani, N., Ed.; Karger: Basel, Switzerland, 2000; pp 59−73. (c) Ojika, M.; Kigoshi, H.; Suenaga, K.; Imamura, Y.; Yoshikawa, K.; Ishigaki, T.; Sakakura, A.; Mutou, T.; Yamada, K. *Tetrahedron* **2012**, *68*, 982.

(4) Aplyronine A: IC_{50} = 0.45 nM; aplyronine C: IC_{50} = 22.4 nM; aplyronine D: IC_{50} = 0.071 nM against HeLa-S3 cells (ref 3).

(5) Crews, P.; Gerwick, W. H.; Schmitz, F. J.; France, D.; Bair, K. W.; Wright, A. E.; Hallock, Y. *Pharm. Biol.* **2003**, *41*, 39.

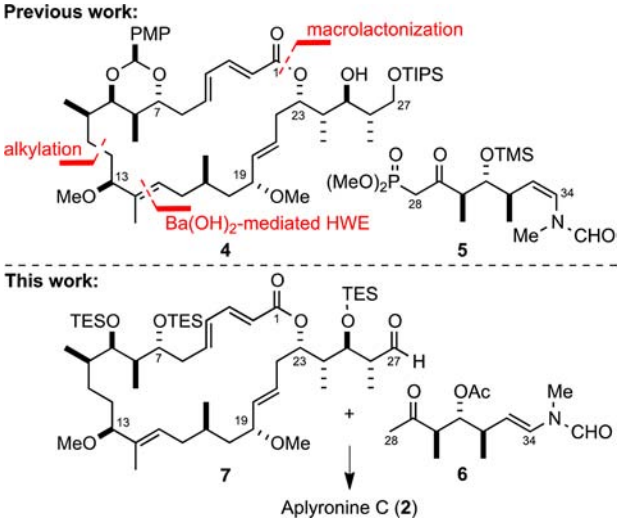
10.1021/ol401327r
Published on Web 06/03/2013

The aplyronines have elicited significant interest for their potent antitumor activities and novel actin-binding properties, as well as their unique structures.[11−16] Moreover, they hold great potential for the development of biomolecular probes and novel actin-targeting therapeutic agents. It is a testament to the severe challenge presented by these complex polyketides that, despite considerable effort from several groups,[12−16] only one prior total synthesis of aplyronines A−C has been achieved, as reported by Yamada and Kigoshi.[16a−c]

In our previous work toward the aplyronines (Scheme 1), we reported the synthesis of an advanced macrocyclic intermediate **4**[12a,b] and β-ketophosphonate **5**[12c] in pursuit of an aborted HWE fragment coupling strategy. Here, we describe the synthesis of a more suitable aldol coupling partner **6**, enabling completion of the total synthesis of aplyronine C (**2**) and providing access to some novel C21−C34 tail analogs.

Our revised strategy hinges on a key aldol fragment coupling between the C1−C27 aldehyde **7**, derived from previously synthesized macrocycle **4**, with the (*E*)-*N*-methyl-*N*-vinylformamide-bearing methyl ketone **6**. The strategic decision to incorporate the terminal (*E*)-vinylformamide into the coupling fragment was influenced by our recent total syntheses of reidispongiolide A[17] and rhizopodin,[18] and contrasts with more established approaches in which this sensitive moiety is introduced by a testing, late-stage condensation reaction with a highly functionalized C34 aldehyde.

Scheme 1. Previous Work and Revised Strategy



The synthesis of the C28−C34 methyl ketone **6** is outlined in Scheme 2. It commenced with a Sn(II)-mediated aldol reacton[19] between known (*R*)-Roche ester-derived ketone **8**[12a] and acetaldehyde to generate the all *syn* aldol adduct **9** with high yield and selectivity (97%, 15:1 *dr*).[20] A directed 1,3-*anti* reduction under Evans−Tishchenko conditions[21] set the C31 stereocenter and concomitantly capped the C29 alcohol as the ester (**10**).[22] Following silyl protection, the C29 and C33 alcohols were revealed to produce diol **11**.

Oxidation of diol **11** to the corresponding keto-aldehyde required that the primary and secondary alcohols be oxidized concurrently, lest intramolecular cyclization onto the nascent aldehyde form an undesired hemiacetal. This was achieved using a double Swern oxidation.[23] Following our Wittig protocol for the synthesis of *N*-methyl-*N*-vinylformamides,[24] the ylide of phosphonium salt **12** (LiHMDS) reacted selectively with the aldehyde to introduce the *N*-vinylformamide terminus in **13**, predominantly in the (*Z*)-configuration (75% over two steps, 8:1 *Z*/*E*). Two further steps introduced the C31 acetate moiety; isomerization to (*E*)-vinylformamide **6** then proceeded smoothly in the presence of stoichiometric iodine under light-free conditions.[24] While all β-acetoxy ketone intermediates were prone to elimination and required careful handling, introduction of the C31 acetate moiety at this early stage proved crucial. Attempted isomerization of fragments bearing a C31-OTES (**13**) or -OPMB group failed to provide the desired (*E*)-vinylformamide under a variety of conditions. The C28−C34 fragment **6** was thus accessed by an efficient 10 step sequence in 60% overall yield.

(6) Saito, S.; Watabe, S.; Ozaki, H.; Kigoshi, H.; Yamada, K.; Fusetani, N.; Karaki, H. *J. Biochem. (Tokyo)* **1996**, *120*, 552.

(7) Hirata, K.; Muraoka, S.; Suenaga, K.; Kuroda, T.; Kato, K.; Tanaka, H.; Yamamoto, M.; Takata, M.; Yamada, K.; Kigoshi, H. *J. Mol. Biol.* **2006**, *356*, 945.

(8) Kigoshi, H.; Suenaga, K.; Takagi, M.; Akao, A.; Kanematsu, K.; Kamei, N.; Okugawa, Y.; Yamada, K. *Tetrahedron* **2002**, *58*, 1075.

(9) Kuroda, T.; Suenaga, K.; Sakakura, A.; Handa, T.; Okamoto, K.; Kigoshi, H. *Bioconjugate Chem.* **2006**, *17*, 524.

(10) (a) Kita, M.; Yoneda, K.; Hirayama, Y.; Yamagishi, K.; Saito, Y.; Sugiyama, Y.; Miwa, Y.; Ohno, O.; Morita, M.; Suenaga, K.; Kigoshi, H. *ChemBioChem* **2012**, *13*, 1754. (b) Ohno, O.; Morita, M.; Kitamura, K.; Teruya, T.; Yoneda, K.; Kita, M.; Kigoshi, H.; Suenaga, K. *Bioorg. Med. Chem. Lett.* **2013**, *23*, 1467.

(11) (a) Kita, M.; Hirayama, Y.; Sugiyama, M.; Kigoshi, H. *Angew. Chem., Int. Ed.* **2011**, *50*, 9871. (b) Kita, M.; Hirayama, Y.; Yamagishi, K.; Yoneda, K.; Fujisawa, R.; Kigoshi, H. *J. Am. Chem. Soc.* **2012**, *134*, 20314.

(12) (a) Paterson, I.; Cowden, C. J.; Woodrow, M. D. *Tetrahedron Lett.* **1998**, *39*, 6037. (b) Paterson, I.; Woodrow, M. D.; Cowden, C. J. *Tetrahedron Lett.* **1998**, *39*, 6041. (c) Paterson, I.; Blakey, S. B.; Cowden, C. J. *Tetrahedron Lett.* **2002**, *43*, 6005.

(13) (a) Calter, M. A.; Guo, X. *Tetrahedron* **2002**, *58*, 7093. (b) Calter, M. A.; Zhou, J. *Tetrahedron Lett.* **2004**, *45*, 4847.

(14) Marshall, J. A.; Johns, B. A. *J. Org. Chem.* **2000**, *65*, 1501.

(15) (a) El-Awa, A.; Fuchs, P. *Org. Lett.* **2006**, *8*, 2905. (b) Noshi, M. N.; El-Awa, A.; Torres, E.; Fuchs, P. L. *J. Am. Chem. Soc.* **2007**, *129*, 11242. (c) Hong, W. P.; Noshi, M. N.; El-Awa, A.; Fuchs, P. L. *Org. Lett.* **2011**, *13*, 6342.

(16) (a) Kigoshi, H.; Ojika, M.; Ishigaki, T.; Suenaga, K.; Mutou, T.; Sakakura, A.; Ogawa, T.; Yamada, K. *J. Am. Chem. Soc.* **1994**, *116*, 7443. (b) Suenaga, K.; Ishigaki, T.; Sakakura, A.; Kigoshi, H.; Yamada, K. *Tetrahedron Lett.* **1995**, *36*, 5053. (c) Kigoshi, H.; Suenaga, K.; Mutou, T.; Ishigaki, T.; Atsumi, T.; Ishiwata, H.; Sakakura, A.; Ogawa, T.; Ojika, M.; Yamada, K. *J. Org. Chem.* **1996**, *61*, 5326. (d) Kobayashi, K.; Fujii, Y.; Hayakawa, I.; Kigoshi, H. *Org. Lett.* **2011**, *13*, 900.

(17) Paterson, I.; Ashton, K.; Britton, R.; Cecere, G.; Chouraqui, G.; Florence, G. J.; Knust, H.; Stafford, J. *Chem.—Asian J.* **2008**, *3*, 367.

(18) Dalby, S. M.; Goodwin-Tindall, J.; Paterson, I. *Angew. Chem., Int. Ed.* **2013** in press.

(19) Paterson, I.; Tillyer, R. D. *Tetrahedron Lett.* **1992**, *33*, 4233.

(20) The C29 configuration was confirmed by Mosher ester analysis; see the Supporting Information.

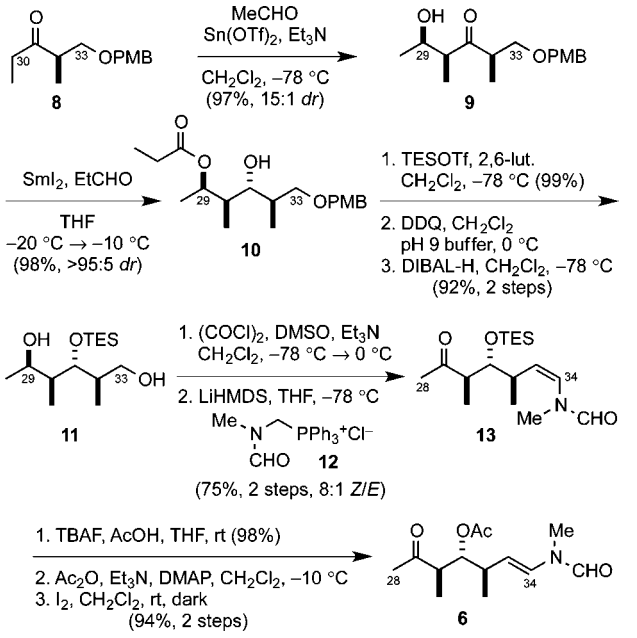
(21) Evans, D. A.; Hoveyda, A. H. *J. Am. Chem. Soc.* **1990**, *112*, 6447.

(22) The relative configuration was confirmed by NMR analysis of the corresponding acetonide; see the Supporting Information.

(23) Omura, K.; Swern, D. *Tetrahedron* **1978**, *34*, 1651.

(24) Paterson, I.; Cowden, C. J.; Rahn, V. S.; Woodrow, M. D. *Synlett* **1998**, 915.

Scheme 2. Synthesis of C28−C34 Ketone **6**

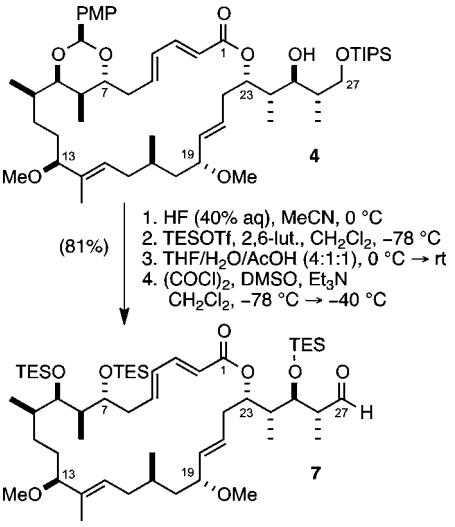


Aldehyde **7** was readily accessible in four steps from our previously reported macrocyclic intermediate **4** (Scheme 3). Accordingly, global deprotection with aqueous HF was followed by TES protection of the resultant tetraol. Selective unveiling of the C27 primary alcohol under mild conditions ($THF/H_2O/AcOH$) and subsequent oxidation provided the desired aldehyde **7**.

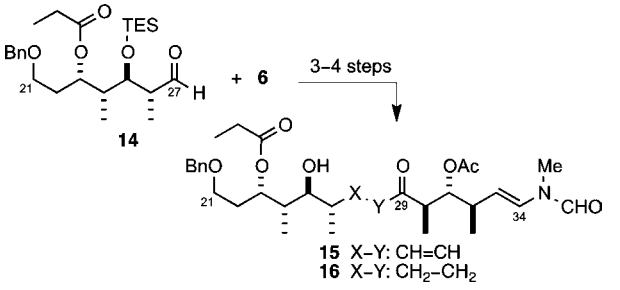
With C1−C27 aldehyde **7** and C28−C34 ketone **6** in hand, attention turned to assembly of the full aplyronine backbone.[25] Owing to the complexity of the aldehyde **7** and the acid and base sensitivity of methyl ketone **6**, especially mild conditions were required. Initially, we rehearsed this crucial fragment coupling and endgame using **14** as a truncated model for aldehyde **7**.[26] Boron-mediated aldol coupling conditions proved uniquely effective, leading to two novel C21−C34 tail analogs for the aplyronines (**15** and **16**; Scheme 4).[27]

Application of the boron aldol coupling conditions to real aldehyde **7** successfully formed the desired C27−C28 bond (Scheme 5).[17] After careful optimization, we were able to perform this delicate aldol coupling using *c*-Hex_2BCl/Et_3N for enolization of **6** at −10 °C followed by slow addition of the enolate (3 equiv) to aldehyde **7** at −78 °C. Following a mild, nonoxidative workup,

Scheme 3. Synthesis of C1−C27 Aldehyde **7**



Scheme 4. C21−C34 Side Chain Analogs



β-hydroxy ketone **17** was obtained in 61% yield with good recovery of the excess ketone **6**.

Deoxygenation at C27 was achieved using a two-step procedure:[17] the β-hydroxy ketone **17** was dehydrated with the Burgess reagent ($Et_3NSO_2NCO_2Me$)[28] to the corresponding enone, which was subsequently reduced in a 1,4-sense to ketone **18** using Stryker's reagent.[29] Under these conditions, elimination of the potentially labile β-acetoxy ketone was minimized, and complete selectivity for reduction of the enone over the sensitive $\alpha,\beta,\gamma,\delta$-unsaturated dienoate was obtained.

From this point, all that remained to obtain aplyronine C (**2**) was stereoselective reduction of the C29 ketone, installation of the appropriate C29 amino ester, and global deprotection. Screening reduction conditions on the model system[26] revealed that ketone **19** exhibits moderate inherent diastereoselectivity for the desired C29 epimer ($NaBH_4$, 2:1 *dr*; entry 1, Table 1). Attempts to enhance this selectivity using bulky reducing agents (entries 2−3) led

(25) As outlined in Scheme 1, our synthetic plan had initially anticipated forging the C27−C28 bond *via* an HWE reaction between aldehyde **7** and β-ketophosphonate **5**. While Masamune−Roush conditions (LiCl, DBU) were successful on a model system (ref 12c), attempts with real aldehyde **7** led only to β-elimination of the C25 silyl ether to give the corresponding enal.

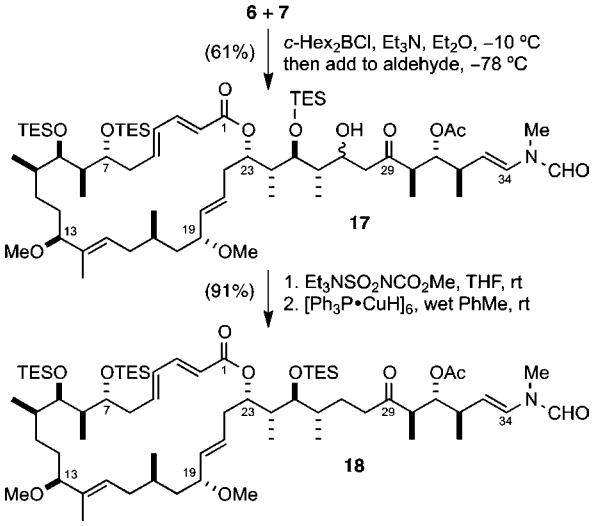
(26) Attempted formation of lithium enolates of such *N*-vinylformamide-bearing ketones resulted only in decomposition. For full details of the model fragment coupling and endgame, see the Supporting Information.

(27) Perrins, R. D.; Cecere, G.; Paterson, I.; Marriott, G. *Chem. Biol.* **2008**, *15*, 287.

(28) Burgess, E. M.; Penton, H. R.; Taylor, E. A. *J. Org. Chem.* **1973**, *38*, 26.

(29) Mahoney, W. S.; Brestensky, D. M.; Stryker, J. M. *J. Am. Chem. Soc.* **1988**, *110*, 291.

Scheme 5. Fragment Coupling



Scheme 6. Completion of Aplyronine C (**2**)

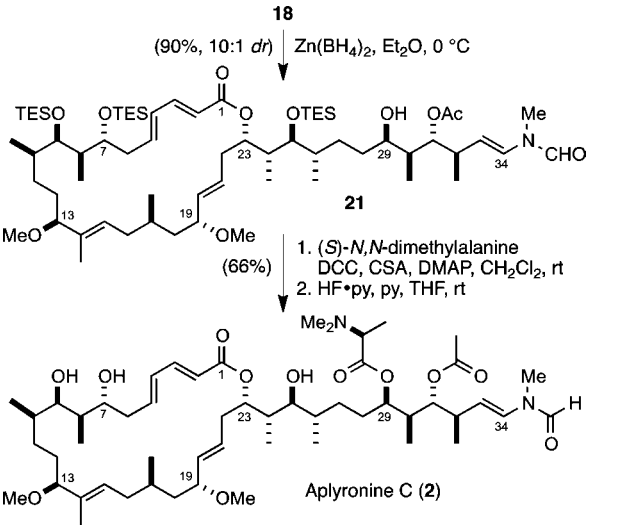
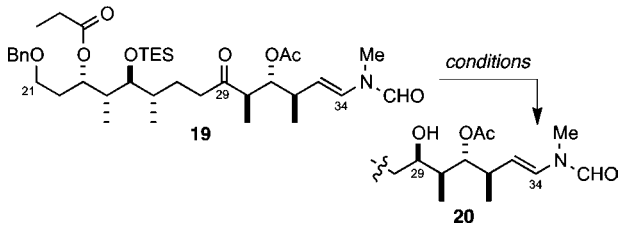


Table 1. Model Studies of C29 Reduction



entry	conditions	yield (%)	*dr*
1	$NaBH_4$, MeOH, rt	55	2:1
2	L-selectride, THF, −78 °C	–[a]	–
3	$LiAlH(Ot\text{-}Bu)_3$, THF, −10 °C → rt	–[a]	–
4	$NaBH_4$, $CeCl_3 \cdot 7H_2O$, MeOH, 0 °C	34	7:1
5	$Zn(BH_4)_2$, Et_2O, 0 °C	77	10:1

[a] Slow reaction rate, forming predominantly elimination and decomposition products.

primarily to formation of elimination-related byproducts. Luche conditions[30] (entry 4) appeared more promising, giving an improved 7:1 *dr* but a disappointingly low yield. Ultimately, $Zn(BH_4)_2$[31] was found to provide the desired alcohol **20** in 10:1 *dr* and 77% yield (entry 5).

Pleasingly, this result transferred well to the real system **18** (Scheme 6). The desired diastereomer at C29 (**21**) was obtained with good selectivity by reduction with $Zn(BH_4)_2$ (90%, 10:1 *dr*). Esterification with (*S*)-*N*,*N*-dimethylalanine under Keck conditions[32] (DCC, DMAP, CSA), as precedented by Yamada,[16a] was followed by global deprotection using HF•py and pyridine to provide (+)-aplyronine C (**2**). To our satisfaction all ^1H and ^{13}C NMR spectroscopic data for this synthetic material correlated with those reported for natural aplyronine C.[3a]

In conclusion, this highly stereocontrolled total synthesis of aplyronine C was completed in 28 steps (LLS) and 3.6% overall yield, by a route that is significantly shorter than the previous synthesis (45 steps LLS).[16b] Alcohol **21** represents an advanced common intermediate from which we can access other members of the aplyronine family. Studies toward these congeners and other novel analogs will be reported in due course.

Acknowledgment. This work was supported by the Dr. Herchel Smith Fellowship from Williams College (S.J.F.), the Croucher Foundation (L.Y.W.L.), the EPSRC (S.J.A.), and the Commonwealth Trust (S.B.B.). Mass spectrometry data were acquired at the EPSRC UK National Mass Spectrometry Facility at Swansea University.

Supporting Information Available. Experimental procedures, details of the model system, and full spectroscopic data for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

(30) (a) Luche, J. L. *J. Am. Chem. Soc.* **1978**, *100*, 2226. (b) Gemal, A. L.; Luche, J. L. *J. Am. Chem. Soc.* **1981**, *103*, 5454.
(31) Oishi, T.; Nakata, T. *Acc. Chem. Res.* **1984**, *17*, 338.
(32) Boden, E. P.; Keck, G. E. *J. Org. Chem.* **1985**, *50*, 2394.

The authors declare no competing financial interest.