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Synthesis of Pluraflavin A "Aglycone"

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Abstract: The "aglycone" of pluraflavin A (**2**) has been synthesized. The key features of this synthesis include a 1,3-dipolar cycloaddition between a nitrile oxide (cf. **14**) and an olefin (**22**) to yield an isoxazoline followed by subsequent conversion into the γ-pyrone of pluraflavin A. The epoxide moiety linked to the pyrone is installed prior to Diels−Alder installation of the D ring, which allows access to a number of potentially active cytotoxic intermediates en route to the final compound. The preliminary in vitro results of two such compounds are also included with the racemic title compound exhibiting cytotoxicity in the nanomolar range.

Introduction

Since Maeda et al. first described the parent compound in 1956, the pluramycin (**1**) family of antitumor antibiotics has expanded both in terms of structural diversity and biological underpinnings.[1] Studies have shown that, as a general mode of action, the members of this family containing a C_{14}/C_{15} oxirane undergo apparent sequence-selective electrophilic attack upon N_7 in guanine in the major groove of DNA.[2−6] This covalent interaction leads to DNA damage and, in time, tumor cell death.

Isolated from cultures of *Saccharothrix* sp. DSM 12931, the pluraflavins were first reported by Vértesy and co-workers in 2001 to be pluramycin-like in terms of their structure.[7] Furthermore, they showed that the epoxide-bearing isolate, pluraflavin A (**2**, Figure 1), exhibits excellent potency against a number of human tumor cell lines in the low to subnanomolar range.[7] Structurally, pluraflavins are unique in the family due the substitution pattern at C_5. This center bears a hydroxymethyl function O-linked to a 3′-*epi*-vancosamine moiety. Given the ongoing interest in our laboratory in the total synthesis and diverted total synthesis of natural products as discovery resources in oncology,[8] we naturally followed emerging developments in the pluraflavin area.

Information on the cytotoxicity of the pluramycin family has been continually updated with the isolation of new entries. By contrast, relatively few approaches to their total synthesis had been reported.[9,10] More recently, however, a number of elegant attempts in this field have been described by the groups of McDonald, Suzuki, and Tietze.[11−18] This recent work in the pluramycin field has culminated in the successful syntheses of the aglycones of altromycin and kidamycin,[12,18] both γ- and δ-indomycinones,[13,14,17,19] and episcufolin[16] through a variety of synthetic strategies. To date, no synthetic strategies toward pluraflavin A have been described. In time, our own interest in the pluraflavins matured into a research program aimed at pluraflavin A. As is our custom, our efforts tend to start with an undertaking in the total synthesis of the natural product itself. A concurrent goal is the development of an implementable synthesis plan, which also lays open the natural target molecule to levels of molecular editing which are not necessarily accessible from the natural product itself.[8] We took note that

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(1) Maeda, K.; Takeuchi, T.; Nitta, K.; Yagishita, K.; Utahara, R.; Osato, T.; Ueda, M.; Kondo, S.; Okami, Y.; Umezawa, H. *J. Antibiot.* **1956**, *9*, 75–81.
(2) Sun, D.; Hansen, M.; Clement, J. J.; Hurley, L. H. *Biochemistry* **1993**, *32*, 8068–8074.
(3) Hansen, M.; Yun, S.; Hurley, L. *Chem. Biol.* **1995**, *2*, 229–240.
(4) Murray, V.; Moore, A. G.; Matias, C.; Wickham, G. *Biochim. Biophys. Acta* **1995**, *1261*, 195–200.
(5) Prakash, A. S.; Moore, A. G.; Murray, V.; Matias, C.; McFadyen, W. D.; Wickham, G. *Chem. Biol. Interact.* **1995**, *95*, 17–28.
(6) Hansen, M.; Hurley, L. *J. Am. Chem. Soc.* **1995**, *117*, 2421–2429.
(7) Vertesy, L.; Barbone, F. P.; Cashmen, E.; Decker, H.; Ehrlich, K.; Jordan, B.; Knauf, M.; Schummer, D.; Segeth, M. P.; Wink, J.; Seibert, G. *J. Antibiot.* **2001**, *54*, 718–729.
(8) Wilson, R. M.; Danishefsky, S. J. *J. Org. Chem.* **2006**, *71*, 8329–8351.
(9) Hauser, F. M.; Rhee, R. P. *J. Am. Chem. Soc.* **1979**, *101*, 1628–1629.
(10) Uno, H.; Sakamoto, K.; Honda, E.; Ono, N. *Chem. Commun.* **1999**, 1005–1006.
(11) Fei, Z. B.; McDonald, F. E. *Org. Lett.* **2007**, *9*, 3547–3550.
(12) Fei, Z. B.; McDonald, F. E. *Org. Lett.* **2005**, *7*, 3617–3620.
(13) Hsu, D. S.; Matsumoto, T.; Suzuki, K. *Chem. Lett.* **2006**, *35*, 1016–1017.
(14) Tietze, L. F.; Singidi, R. R.; Gericke, K. M. *Org. Lett.* **2006**, *8*, 5873–5876.
(15) Tietze, L. F.; Gericke, K. M.; Singidi, R. R. *Angew. Chem., Int. Ed.* **2006**, *45*, 6990–6993.
(16) Tietze, L. F.; Gericke, K. M.; Singidi, R. R.; Schuberth, I. *Org. Biomol. Chem.* **2007**, *5*, 1191–1200.
(17) Tietze, L. F.; Singidi, R. R.; Gericke, K. M. *Chem.—Eur. J.* **2007**, *13*, 9939–9947.
(18) Fei, Z.; McDonald, F. E. *Abstracts of Papers*, 232nd ACS National Meeting, San Francisco, CA, Sept. 10−14, 2006; ORGN-717.
(19) Krohn, K.; Tran-Thien, H. T.; Vitz, J.; Vidal, A. *Eur. J. Org. Chem.* **2007**, 1905–1911.

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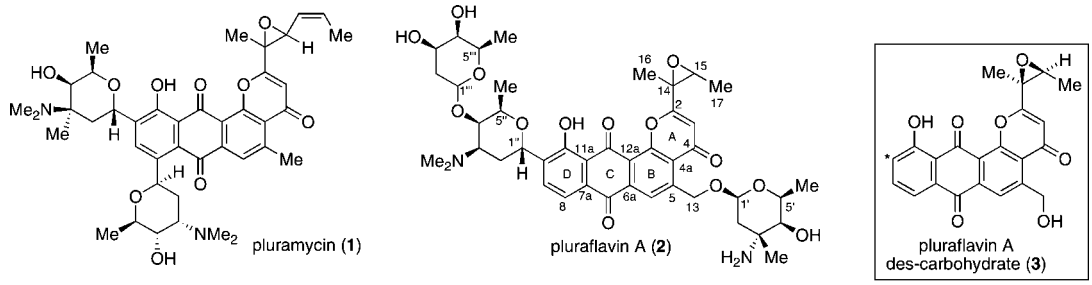
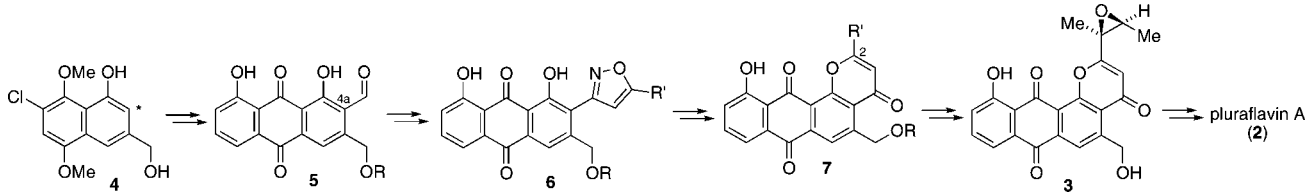


Figure 1. Structures of pluramycin (**1**), pluraflavin A (**2**), and its *des*-carbohydrate (**3**).

Scheme 1. Proposed Isoxazole-Based Approach to Pluraflavin A (**2**) from **4**[10,21]



pluraflavin A contains both C and O glycosidic linkages. We defined as our first subgoal the synthesis of **3**, corresponding to the "aglycone"[20] of pluraflavin A. This compound, or congeners thereof, would serve to inform whether useful biological function is available in the absence of glycosidic domains. If this were not the case, incorporation of handles to enable C-glycosylation at C_{10} (see asterisk, Figure 1) could be undertaken. The biological consequences of the O-glycosylation at C_{13} could also be evaluated. At the level of chemical synthesis, there was concern from the outset about possible functional group management issues which would have to be addressed in the co-installation of the C_{14}/C_{15} oxirane and the labile glycosidic bonds attaching the *epi*-vancosamine and oliose domains.

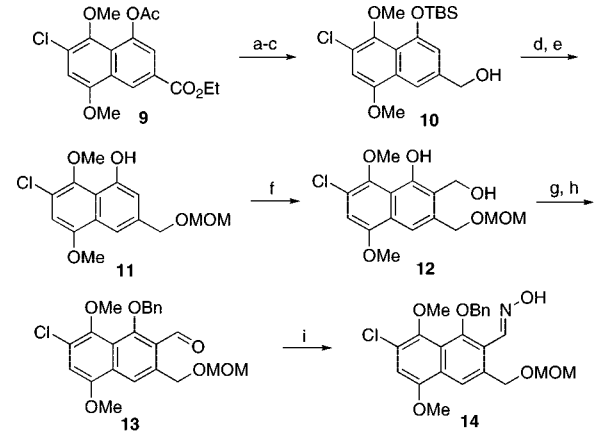
Our plan of synthesis envisioned starting with the known naphthalene derivative **4** (Scheme 1). It was further anticipated that the D ring could be fashioned from a Diels−Alder dynamic on a suitable juglone-like version of **4**. In addition, a formyl group would be introduced at C_{4a} (pluraflavin numbering, see asterisk in compound **4**). Since this center is *ortho* to an existing phenolic function, a variety of options for the overall formylation could be entertained (vida infra). At this level of planning, we leave open the question of timing between D ring introduction and C_{4a} formylation. In any case, the aldehyde at C_{4a} would be converted to an oxime which, upon elaboration to the corresponding nitrile oxide, would give rise to an isoxazole (see **5**→**6**).

It was envisioned that opening of the isoxazole, in some as-yet-unspecified manner, would set the stage for cyclization to γ-pyrone with emergence of a side chain at C_2 eventually destined to serve as the required 2,3-oxiranyl *sec*-butyl group. Below, we describe the realization of our first milestone in the project, that is, the total synthesis of **3**.

Results and Discussion

Chemistry. Our collective fascination with using isoxazoles to store valuable functionality, which is exposed through a well-directed ring cleavage reaction, dates back more than 45 years when one of us was engaged as a postdoctoral fellow at Columbia University.[21] To service the case at hand, it would of course be necessary to synthesize an appropriately functionalized isoxazole. It was with this subgoal in mind that our journey commenced.

Scheme 2. Synthesis of Oxime **14**[a]



[a] Key: (a) K_2CO_3, EtOH; (b) TBSCl, imidazole, DMF, 90% (over two steps); (c) $LiAlH_4$, THF, 92%; (d) MOMCl, $^iPr_2NEt$, CH_2Cl_2; (e) TBAF, THF, 76% (over two steps); (f) $(CH_2O)_n$, Et_2AlCl, CH_2Cl_2; (g) BnBr, $CsCO_3$, DMF/acetone (2:3), 53% (over two steps, 59% based on recovered **12**); (h) TPAP, NMO, 4 Å MS, CH_2Cl_2; (i) $NH_2OH\cdot HCl$, NaOH, EtOH/H_2O (2:1), 73% (over two steps).

The synthesis diverged from the known ester **9**, which was prepared in three steps from commercially available 2-chloro-1,4-dimethoxybenzene according to the protocol of Bloomer and co-workers (Scheme 2).[22] Transformation of **9**→**10** was accomplished in three steps as shown (vida infra). Following protection of the alcohol as its MOM ether and cleavage of the silyl protecting group, phenol **11** was in hand. Employing the hydroxymethylation conditions of Casiraghi et al., phenol **11** was converted into diol **12**.[23] Selective benzylation of the phenol, under mediation by cesium carbonate, and subsequent oxidation

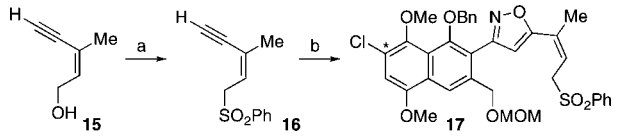
(20) Technically, the term aglycone is used to describe natural products lacking an O-glycoside, so in the case of pluraflavin A, one might also call the title compound the "*des*-carbohydrate."

(21) Stork, G.; Danishefsky, S.; Ohashi, M. *J. Am. Chem. Soc.* **1967**, *89*, 5459–5460.

(22) Bloomer, J. L.; Stagliano, K. W.; Gazzillo, J. A. *J. Org. Chem.* **1993**, *58*, 7906–7912.

(23) Bigi, F.; Casiraghi, G.; Casnati, G.; Sartori, G.; Gasparri Fava, G.; Ferrari Belicchi, M. *J. Org. Chem.* **1985**, *50*, 5018–5022.

Scheme 3. Synthesis of Isoxazole **17**[a]



[a] Key: (a) PPh_3, NBS, $NaSO_2Ph$, THF, 77%; (b) (i) **13**, $NH_2OH \cdot HCl$, NaOH, $^tBuOH/H_2O$ (2:1), 70 °C, 15 min; (ii) rt, $NaHCO_3$, chloramine-T; (iii) $CuSO_4 \cdot 5H_2O$, Cu (wire), alkyne **16**, 60% (over three steps from **13**).

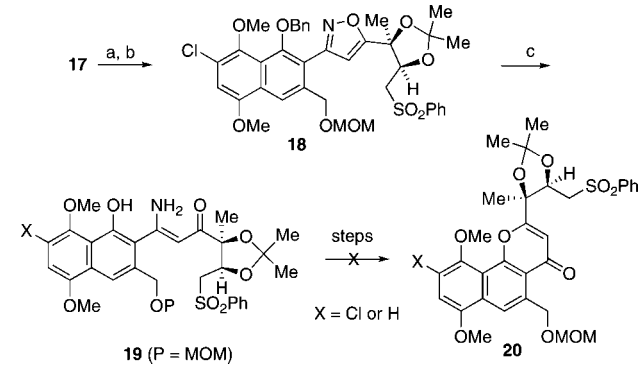
led to an aldehyde (**13**).[24] As a side note, direct formylation[25] of **11** was also possible, but the two-step protocol was more amenable to bulk preparation of this particular material. The aldehyde function was converted to the oxime, **14**, the key precursor for our projected 1,3-dipolar cycloaddition step.

Accordingly, our attention now turned to preparation of a functionalized alkyne for the synthesis of pluraflavin A. The most appropriate alkyne seemed to be 3-methylpent-3-en-1-yne. Its synthesis, both as a mixture of alkene isomers[26] as well as a pure compound, had been previously described.[27] Nevertheless, in our hands, challenges to obtaining required quantities of the desired *Z*-isomer were particularly complicated by its volatility. It was decided to add a functional group which would allow for more straightforward synthesis and handling of the required moiety. It goes without saying that the group selected for more convenient material management had to be readily removable at a later stage. With these considerations well in mind, we elected to work with a sulfone function, in particular, that of compound **16**. Its synthesis from the readily available **15**[28] using methodology developed by Murakami[29] is shown in Scheme 3. Compound **16** was then to be combined with the nitrile oxide derived from aldehyde **13**, which often requires multiple synthetic manipulations. Happily, however, the convenient conditions, reported by Fokin and co-workers, were effectively employed to convert **13** into isoxazole **17**.[30]

For ring opening of the isoxazole, our original plan contemplated Raney-Nickel-induced reductive cleavage of the N−O bond. It was also expected that the system would be subject to concurrent removal of the benzyl and the sulfone functions. While we were able to open the isoxazole ring and deprotect the phenol, in practice, removal of the sulfone was complicated by competing reduction of the aryl chloride (see asterisk on **17**) under the forcing conditions which were required (Schemes 3 and 4).

Fortunately, allylic sulfone **17** could readily be converted into acetonide **18** under a standard two-step protocol.[31] As indicated, upon heating with Raney-Nickel in ethanol, **18** did undergo the desired multistep process to yield **19** in a single laboratory operation; however, the sulfone function was not removed cleanly under these conditions. Upon exposure to higher temperatures and longer reaction times, the sulfone was partially removed; however, the desulfonylated material suffered from contamination with dechlorinated compound.

Scheme 4. Synthesis of Enamide **19** and Failed Conversion into Pyrone **20**[a]



[a] Key: (a) OsO_4 (cat.), NMO, acetone/H_2O (3:1); (b) *p*TsOH (cat.), acetone/2,2′-dimethoxypropane (1:1), 68% (over two steps); (c) Raney-Ni, EtOH, 80°C, 18 h, 42% (X = Cl), 16% (X = H).

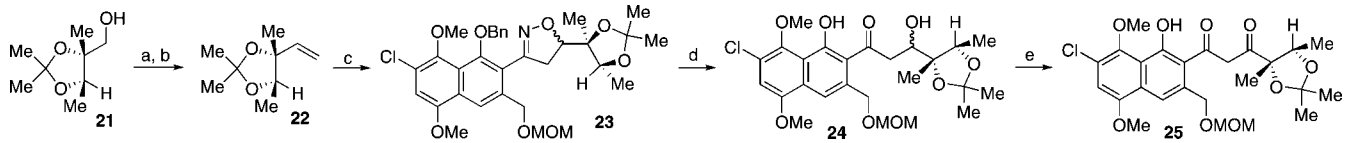
An even more persistent problem presented itself in the attempted preparation of pyrone **20**. In our hands, the hydrolysis and subsequent dehydrative cyclization required of enamide **19** (or related enamides) could not be accomplished. Despite the multitude of related examples which would have suggested that the conversion to **20** should be possible, for some reason, the precedents did not apply to our case.[32−39]

It seemed that we might be able to circumvent the challenge at hand by recourse to an isoxazoline in place of the aforedescribed isoxazole. The difficulty in the hydrolysis/cyclization of the vinylogous amide (cf. **19**→**20**) was indeed surprising, but we hoped that matters might proceed more smoothly if the product of the ring cleavage were an imine rather than the vinylogous amide arising from reduction of an isoxazole. This hypothesis was of course supported by well-known precedents for the conversion of isoxazolines into their corresponding β-hydroxy ketones.[40,41] Obviously, the original enamide (**19**) was in the oxidation state required for the synthesis of pyrone. By contrast, a formal hydroxy imine derived from reduction of an isoxazoline would require subsequent oxidation to reach our subgoal. Fortunately, this modification did, in fact, lead to the anthrapyran core of pluraflavin A (vida infra).

The known racemic alcohol **21**,[42] prepared in five steps from tiglic acid, was converted into olefin **22** by PCC oxidation followed by Wittig olefination.[43] The alkene (**22**) then underwent straightforward thermal cycloaddition with the presumed nitrile oxide derived from oxime **14**. There was obtained a ∼3:2 mixture of stereoisomers **23**.[44] According to the protocol due

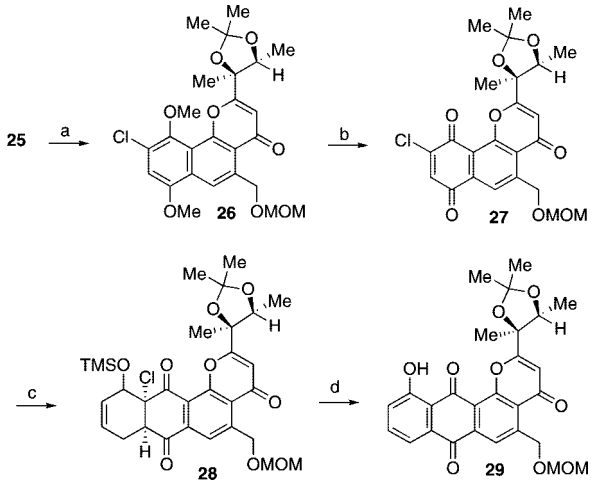
(24) Ley, S.; Norman, J.; Griffith, W.; Marsden, S. *Synthesis* **1994**, 639–666.
(25) Hofslokken, N. U.; Skattebol, L. *Acta Chem. Scand.* **1999**, *53*, 258–262.
(26) Oroshnik, W.; Mebane, A. D. *J. Am. Chem. Soc.* **1954**, *76*, 5719–5736.
(27) Biagetti, M.; Bellina, F.; Carpita, A.; Viel, S.; Mannina, L.; Rossi, R. *Eur. J. Org. Chem.* **2002**, 1063–1076.
(28) Mori, K.; Ohki, M.; Sato, A.; Matsui, M. *Tetrahedron* **1972**, *28*, 3739–3745.
(29) Murakami, T.; Furusawa, K. *Synthesis* **2002**, 479–482.
(30) Hansen, T. V.; Wu, P.; Fokin, V. V. *J. Org. Chem.* **2005**, *70*, 7761–7764.
(31) VanRheenen, V.; Kelly, R. C.; Cha, D. Y. *Tetrahedron Lett.* **1976**, *17*, 1973–1976.
(32) Zen, S.; Harada, K.; Nakamura, H.; Iitaka, Y. *Bull. Chem. Soc. Jpn.* **1988**, *61*, 2881–2884.
(33) Jones, R. C. F.; Duller, K. A. M.; Vulto, S. I. E. *J. Chem. Soc., Perkin Trans. 1* **1998**, 411–416.
(34) Saxena, R.; Singh, V.; Batra, S. *Tetrahedron* **2004**, *60*, 10311–10320.
(35) Scott, M. S.; Luckhurst, C. A.; Dixon, D. J. *Org. Lett.* **2005**, *7*, 5813–5816.
(36) Sundberg, R. J.; Pearce, B. C. *J. Org. Chem.* **1985**, *50*, 425–432.
(37) Kenar, J. A. *J. Am. Oil Chem. Soc.* **2003**, *80*, 1027–1032.
(38) Kurykin, M. A.; Vol'pin, I. M.; German, L. S. *J. Fluorine Chem.* **1996**, *80*, 9–12.
(39) Iijima, I.; Taga, N.; Miyazaki, M.; Tanaka, T.; Uzawa, J. *J. Chem. Soc., Perkin Trans. 1* **1979**, 3190–3195.
(40) Curran, D. P. *J. Am. Chem. Soc.* **1982**, *104*, 4024–4026.
(41) Kozikowski, A. P.; Adamczyk, M. *Tetrahedron Lett.* **1982**, *23*, 3123–3126.
(42) Bowden, M. C.; Patel, P.; Pattenden, G. *Tetrahedron Lett.* **1985**, *26*, 4793–4796.
(43) Wittig, G. *Chem. Ber.* **1954**, *87*, 1318–1330.
(44) Hassner, A.; Rai, K. M. L. *Synthesis* **1989**, 57–59.

Scheme 5. Synthesis of Cyclization Precursor **25** via Isoxazoline **23**[a]



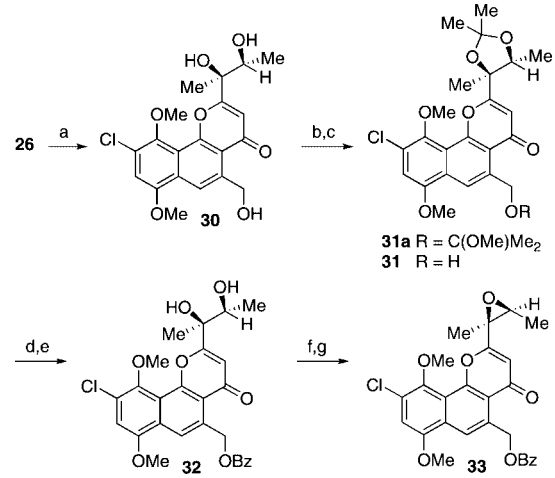
[a] Key: (a) PCC, Celite, 4 Å MS, CH_2Cl_2; (b) $H_2C{=}PPh_3$, THF, 0°C, 44% (over two steps); (c) **14**, chloramine-T, CH_2Cl_2/EtOH:H_2O (4:10:1), 81% (based on **14**); (d) Raney-Ni, $B(OH)_3$, $H_{2(g)}$, EtOH/H_2O (5:1), 68% (78% BRSM); (e) IBX, EtOAc, reflux, 84%.

Scheme 6. Cyclization of β-Diketone **25** and Subsequent Conversion into Anthrapyran Core **29**[a]



[a] Key: (a) NaOAc, AcOH, 80°C, 86%; (b) CAN, MeCN/H_2O (4:1), 0°C; (c) 1-trimethylsiloxy-1,3-butadiene, PhH, 85°C, 4 h; (d) (i) Jones' reagent, acetone, 0°C; (ii) Et_3N, CH_2Cl_2, 60% (from **26**).

Scheme 7. Synthesis of Epoxide **33**[a]



[a] Key: (a) HCl, THF/MeOH (2:1), 50°C; (b) *p*TsOH (cat.), acetone/2,2′-dimethoxypropane (1:1); (c) AcOH/H_2O (4:1), 78% (over three steps from **26**); (d) BzCl, Et_3N, CH_2Cl_2, 99%; (e) HCl, THF/MeOH (2:1), 50°C, 71%; (f) MsCl, $^iPr_2NEt$, CH_2Cl_2, 0°C; (g) K_2CO_3, MeOH/THF (1:1), 0°C, 60% (over two steps) plus 10% debenzoylated epoxide.

to Curran, the isoxazoline was converted into the desired keto alcohols, **24**.[40] Subsequent oxidation using IBX afforded β-diketone **25** in good overall yield[45] (Scheme 5).

Conversion of **25** into the desired pyrone under commonly employed acidic conditions was initially complicated by the presence of the acid labile protecting groups. Fortunately, this problem was overcome by the use of mild conditions (buffered acetic acid) to affect cyclization (Scheme 6).[46] Following this step, oxidative demethylation of **26** afforded chloroquinone **27**.[47]

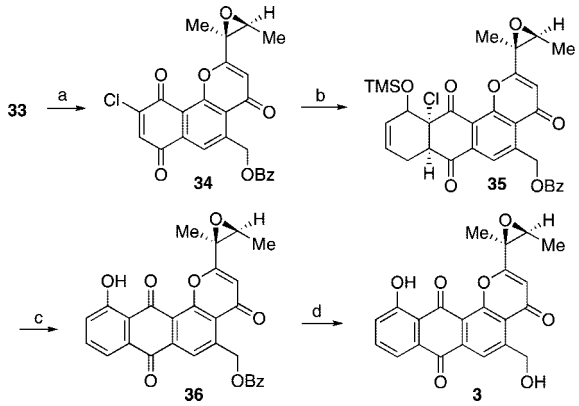
The viability of this pyrone-quinone as a dienophile was established following a Diels−Alder reaction with 1-trimethylsiloxy-1,3-butadiene to yield compound **28** as a single isomer. It was presumed, but not established, that it was derived from *endo* approach of the diene. Without purification, oxidative deprotection of the silyl ether using Jones' reagent followed by treatment with base gave, at last, **29** in good overall yield from **26**.[48,49] This sequence constituted a significant improvement relative to a previously described oxidative aromatization, both in terms of yield and ease of operation.[50]

Having achieved the synthesis of the anthrapyran core of pluraflavin A, the conversion of the protected diol into an epoxide would be required in order to reach **3**. The challenge of installing the epoxide group stems from the choice of protecting groups in our current system, which could well be avoidable in a second generation approach. Furthermore, the handling of our anthrapyran core for an extended series of manipulations employing late stage epoxide installation was complicated by poor solubility of various intermediates that made purification particularly difficult. Eventually, it was found that it would be easier to install the epoxide on the tricyclic pyrone (**26**) rather than on the anthrapyran (**29**).

Though we did have some initial success with the selective deprotection of the primary alcohol under nonaqueous conditions, the high stability of our acetonide did not lend itself to its selective deprotection with maintenance of the MOM ether. The means by which that problem was solved are detailed in Scheme 7. Heating the tricyclic pyrone **26** in methanolic acid accomplished bis-deprotection, yielding **30**. This triol was then treated under standard conditions, as described, giving rise to a mixture of **31** and the corresponding mixed ketal at C_{13} (**31a**). Fortunately, this crude product, upon exposure to the action of wet acetic acid for 10 min at room temperature, suffered conversion to **31** in high yield. The alcohol was then benzoylated under standard conditions. Following deprotection of the acetonide, diol **32** was thus obtained. Finally, **32** gave, with desired inversion at C_{15}, epoxide **33** by the usual two-step sequence without purification of the intermediate monomesylate. It should be noted that the absolute stereochemistry of the epoxide of pluraflavin A is currently unknown. In principle, either enantiomeric olefin can be fashioned by well-established dihydroxylation methods.[51,52]

(45) More, J. D.; Finney, N. S. *Org. Lett.* **2002**, *4*, 3001–3003.
(46) Fukui, K.; Matsumoto, T.; Kinoshita, T. *Bull. Chem. Soc. Jpn.* **1964**, *37*, 662–664.
(47) Jacob, P.; Callery, P. S.; Shulgin, A. T.; Castagnoli, N. *J. Org. Chem.* **1976**, *41*, 3627–3629.
(48) Bowden, K.; Heilbron, I. M.; Jones, E. R. H.; Weedon, B. C. L. *J. Chem. Soc.* **1946**, 39–45.
(49) Baker, R.; Rao, V. B.; Ravenscroft, P. D.; Swain, C. J. *Synthesis* **1983**, 572–574.
(50) Boisvert, L.; Brassard, P. *J. Org. Chem.* **1988**, *53*, 4052–4059.
(51) Kolb, H. C.; VanNieuwenhze, M. S.; Sharpless, K. B. *Chem. Rev.* **1994**, *94*, 2483–2547.

Scheme 8. Synthesis of **3**[a]



[a] Key: (a) CAN, MeCN/H_2O (6:1), 0°C; (b) 1-trimethylsiloxy-1,3-butadiene, PhH, 85°C, 4 h; (c) (i) Jones' reagent, acetone, 0°C; (ii) Et_3N, CH_2Cl_2; (d) K_2CO_3, MeOH/CH_2Cl_2 (1:1), 0°C → rt, 80% (over five steps from **33**).

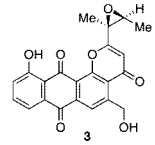
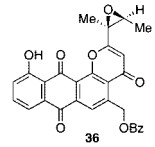
Early installation of the epoxide served two immediate functions. It simplified the handling of the sensitive anthrapyran chromophores and it allowed for straightforward access to a variety of epoxide-bearing intermediates for use in a future structure−activity relationship (SAR) study. Nevertheless, it still was necessary to demonstrate the practicality of carrying such an epoxide through a range of additional sequences (Scheme 8).

The conversion of epoxide **33** into **3** followed a sequence analogous to that previously described for the synthesis of **29** (Scheme 6). In the event, epoxide **33** was first converted to the corresponding dienophilic quinone **34**. Happily, the straightforward Diels−Alder/oxidation/aromatization protocol could then be realized without erosion of the epoxide linkage. Thus, we were able to obtain **36** in high overall yield from **33**. Finally, removal of the benzoate under standard conditions gave the target compound (**3**). Its NMR, IR, and high resolution mass spectra served independently to establish the structure shown below and were fully consistent with reported structures of this general type.[12,53] In reality, the total synthesis of **3** had proven to be more complicated than we had anticipated by naive examination of its structure.

Biology. In addition to the chemical questions described herein, a fundamental interest in the potential pharmaceutical application of this family of compounds was at the heart of this research. Accordingly, preliminary in vitro screening was conducted on a variety of intermediates as well as on **3**. The truncated results of this study are shown in Table 1.

In fact, we have conducted a rather extensive analysis of the SAR relationships encompassing a range of synthetic congeners produced in this program with respect to three cell lines shown below. A full report of the very interesting findings of this study will be provided in due course. However, suffice it to say for the moment that **3** is actually a quite active compound against each of the cell lines. Moreover, the bulk of the activity is retained where the primary alcohol function at C_{13} appears as its benzoate ester (**36**). Since that substantial potency is inherent even in the bare tetracyclic core matrix of **1**, it will be of significant interest to append carbohydrate moieties to the appropriate sites. It should thus be possible to probe the role of the glycosidic sectors (both C- and O-linked) in enhancing potency relative to **3** itself.

Table 1. Cell Growth Inhibition Against Human Lymphoblastic T-Cell Leukemia Sublines[a]

Compound	CCRF-CEM	CCRF-CEM/ Taxol®	CCRF-CEM/ VBL
 **3**	0.018±0.004	$0.031_{[1.72x]}$*	$0.026_{[1.44x]}$
 **36**	0.076±0.037	$0.119_{[1.57x]}$	$0.068_{[0.895x]}$

[a] Key: IC_{50} values are reported in micromolar. CCRF-CEM is a human lymphoblastic leukemic cell line. CCRF-CEM/Taxol and CCRF-CEM/VBL are CCRF-CEM cells that are resistant to Taxol (1184-fold resistance) and vinblastine (84-fold resistance), respectively.[54] *The numbers in brackets are fold of resistance based on the IC_{50} values compared with the parent CCRF-CEM cell lines. The dose−effect relationship data from six to seven concentrations of each drug in duplicate was analyzed with the median-effect plot through the use of a computer program.[52]

Conclusion

The synthesis of the *des*-carbohydrate of pluraflavin A has been described. The key transformation was the use of a [3 + 2] cycloaddition to afford a functionalized isoxazoline. This heterocycle was converted into the γ-pyrone of the natural product in an efficient three-step method. Installation of an epoxide followed by another cycloaddition-based sequence established the D ring of **3**. In addition, an in vitro study against three leukemic cell lines has shown that, even in the absence of any carbohydrate moieties, the anthrapyran construct is still capable of eliciting a highly potent cytotoxic effect. Future plans include the synthesis and attachment of one or more of the glycosidically bound sugars of pluraflavin A, achievement of an enantiomerically homogeneous version of **3** corresponding to the natural series, and additional pharmacological studies on this family of compounds.

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

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(52) See Supporting Information for details on the synthesis and characterization of the (−)-diol and (−)-acetonide en route to **22**.
(53) Uno, H.; Sakamoto, K.; Honda, E.; Fukuhara, K.; Ono, N.; Tanaka, J.; Sakanaka, M. *J. Chem. Soc., Perkin Trans. 1* **2001**, 229–238.
(54) Chou, T. C.; Dong, H. J.; Zhang, X. G.; Tong, W. P.; Danishefsky, S. J. *Cancer Res.* **2005**, *65*, 9445–9454.