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^{18}O Assisted Analysis of a γ,δ-Epoxyketone Cyclization: Synthesis of the C16−C28 Fragment of Ammocidin D

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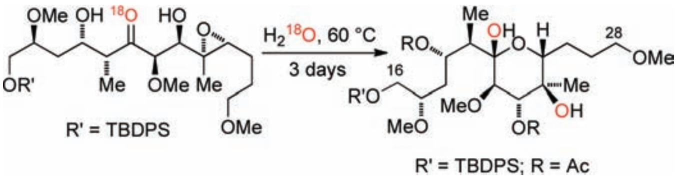
ABSTRACT



The C16−C28 fragment common to the cytotoxic macrolide ammocidin D has been prepared by a stereospecific 5-exo closure of a γ,δ-epoxyketone followed by a rearrangement to a pyran acetal. The reaction pathway was traced by ^{18}O labeling of the keto carbonyl and observation of ^{18}O induced ^{13}C shifts in the pyran acetal product. NMR data of the synthetic C16−C28 fragment compared favorably to the natural product providing support of the assigned stereochemistry.

During the course of screening extracts for apoptosis inducers in Ras-dependent Ba/F3−V12 cells, Hayakawa and co-workers identified the macrolide ammocidin A (Figure 1) from the culture broth of *Saccharothrix* sp. AJ9571.[1] In 2009, the same research group reported on the isolation and antiproliferative properties of ammocidins B−D.[2] Structurally, ammocidins A−D share a common 20-membered macrolactone and differ primarily in glycosylation at C24. For example, ammocidin A incorporates a β-D-olivomycose-β-D-digitoxose disaccharide at C24 while ammocidin D is devoid of sugars at this position (Figure 1). Only the two-dimensional structure of the common aglycone (ammocidinone) was assigned by NMR analysis with degradation by acidic methanolysis providing full assignment of the deoxy sugars by isolation of the derived methyl acetals. Hayakawa has noted that the structure of ammocidin A bears a resemblance to apoptolidin A,[3] a macrolide of fully assigned stereochemistry.[1b] Among the common structural features shared by these polyketides are a central 20-membered macrolactone, a 28-carbon seco-acid incorporating a C21−C25 cyclic acetal (Figure 1) and 6-deoxy glucose at C9. Based on comparison to apoptolidin A and analysis of NOE data, the stereochemistry of ammocidin A/D was tentatively assigned as shown in Figure 1.[4] Given the significant antiproliferative properties of ammocidins

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(1) (a) Murakami, R.; Tomikawa, T.; Shin-Ya, K.; Shinozaki, J.; Kajiura, T.; Kinoshita, T.; Miyajima, K.; Seto, H.; Hayakawa, Y. *J. Antibiot.* **2001**, *54*, 710–713. (b) Murakami, R.; Tomikawa, T.; Shin-Ya, K.; Shinozaki, J.; Kajiura, T.; Kinoshita, T.; Miyajima, A.; Seto, H.; Hayakawa, Y. *J. Antibiot.* **2001**, *54*, 714–717.

(2) Murakami, R.; Shinozaki, J.; Kajiura, T.; Kozone, I.; Takagi, M.; Shin-Ya, K.; Seto, H.; Hayakawa, Y. *J. Antibiot.* **2009**, *62*, 123–127.

(3) Hayakawa, Y.; Kim, J. W.; Adachi, H.; Shin-Ya, K.; Fujita, K.; Seto, H. *J. Am. Chem. Soc.* **1998**, *120*, 3524–3525.

(4) The relative stereochemistry between C8−C9, C16−C20, and C21−C25 remains ambiguous.

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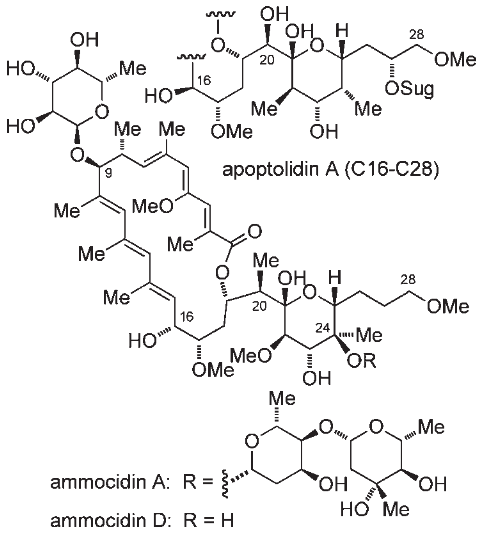


Figure 1. Tentative stereochemical assignment of ammocidin A/D.

A−D, we initiated a program directed toward the total synthesis of ammocidin D in order to assign the full structure and subsequently advance biological studies. Herein we describe synthetic and mechanistic studies leading to a stereocontrolled assembly of the C16−C28 fragment of ammocidin D.

When compared to the apoptolidins, a distinguishing structural feature of ammocidins A−D is incorporation of a tertiary alcohol at C24. The C24 hydroxyl group and substitution pattern of the pyran-acetal spanning the C21−C25 region suggested the synthetic strategy shown in Figure 2 beginning with a 5-exo opening of the neighboring methyl substituted epoxide by the C21 keto group (**I**).[5] When accompanied by hydration, the 5-exo cyclization of **I** would afford furan acetal **II** that we anticipated would isomerize to the ammocidin pyran-acetal **III**.[6]

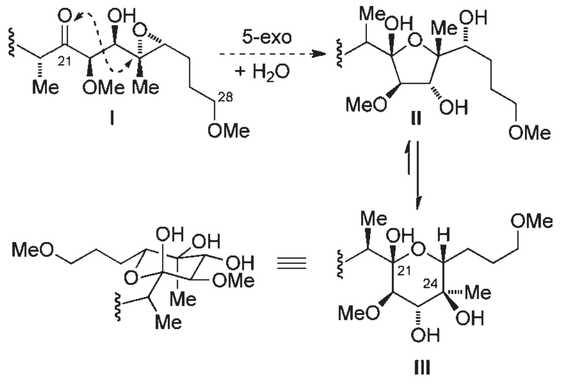
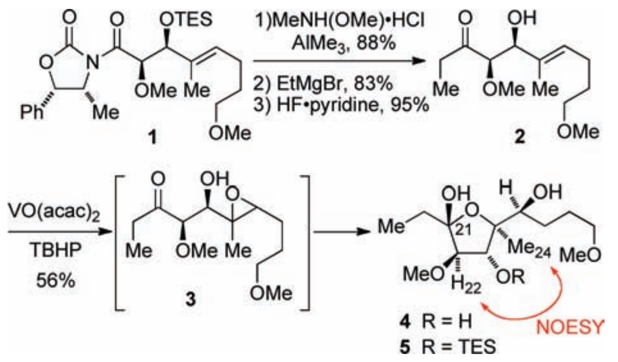


Figure 2. Synthetic strategy for acetal pyran formation.

Our first evaluation of this proposal examined cyclization of the epoxy alcohol derived from aldol adduct **2**, the C20−C28 fragment of ammocidin D (Scheme 1). Oxazolidinone **1** was obtained by an Evans asymmetric glycolate aldol starting from (*E*)-6-methoxy-2-methylhexenal, prepared in four steps from 4-methoxy methyl butanoate.[7] Conversion of **1** to a Weinreb amide followed by ethylation and removal of the TES protecting group provided allylic alcohol **2**, which was poised for a hydroxyl directed epoxidation. Our synthetic plan as shown in Figure 2 required an *anti*-2,3-epoxy alcohol; therefore we conducted a vanadium catalyzed epoxidation of **2**.[8]

Scheme 1. Unexpected Isolation of Furan Acetal **4**



Epoxidation of allylic alcohol **2** did not provide epoxide **3** but instead furan acetal **4** (Scheme 1). The structure and stereochemistry of **4** was assigned based on the combined analysis of **4** and the derived TES ether **5**. The acetal carbon (C21) of both **4** and **5** resonated at 112 ppm, approximately 12 ppm further downfield than expected for the ammocidin pyran acetal (**III**, Figure 2).[1b] Second, the observed coupling constant between H_{22} and H_{23} for both **4** and **5** was 2 Hz while the corresponding coupling constant for ammocidin D was 9.9 Hz. Further evidence for furan acetal **4** was provided by two-dimensional NMR data whereby an NOE cross-peak between H_{22} and Me_{24} was observed in the NOSY spectrum and an HMBC cross-peak between C_{21} and H_{25} was absent. Thus, acetal **4** not only possessed the incorrect ring tautomer but also inverted stereochemistry at C24 and C25 relative to that required for the ammocidins (cf. **II**, Figure 2).

Two possible reaction pathways, outlined in Scheme 2, could account for the unexpected production of furan acetal **4** starting from vanadium catalyzed epoxidation of allylic alcohol **2**. In path A, epoxidation leads to a *syn*-2,3-epoxy alcohol (*syn*-**3**) followed by a 5-exo closure of the γ,δ-epoxyketone leading to furan acetal **4**. Path B presents a second scenario starting from the *anti*-2,3-epoxy alcohol (*anti*-**3**) followed by a 6-endo closure to intermediate pyran

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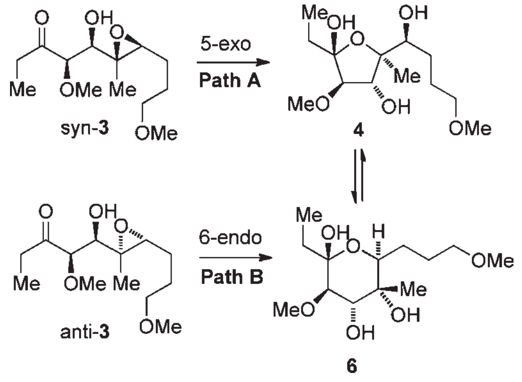
[5] (a) Morten, C. J.; Jamison, T. F. *J. Am. Chem. Soc.* **2009**, *131*, 6678–6679. (b) Morten, C. J.; Byers, J. A.; Van Dyke, A. R.; Vilotijevic, I.; Jamison, T. F. *Chem. Soc. Rev.* **2009**, *38*, 3175–3192.

[6] (a) Holland, J. M.; Lewis, M.; Nelson, A. *Angew. Chem., Int. Ed.* **2001**, *40*, 4082–4084. (b) Holland, J. M.; Lewis, M.; Nelson, A. *J. Org. Chem.* **2003**, *68*, 747–753.

[7] Martin, S. F.; Dodge, J. A.; Burgess, L. E.; Limberakis, C.; Hartmann, M. *Tetrahedron* **1996**, *52*, 3229–3246.

[8] (a) Adam, W.; Wirth, T. *Acc. Chem. Res.* **1999**, *32*, 703–710. (b) Sharpless, K. B.; Verhoeven, T. R. *Aldrichimica Acta* **1979**, *12*, 63–74.

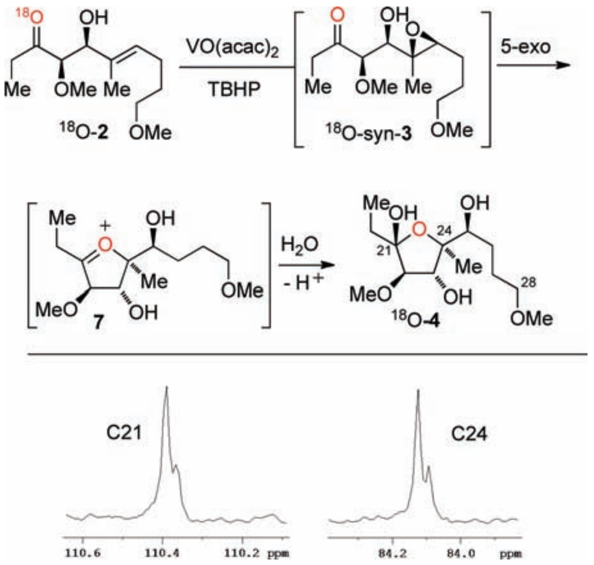
Scheme 2. Possible Routes Leading to Furan Acetal **4**



acetal **6** and isomerization to the more stable furan acetal **4**.[9] In support of path A (cyclization by way of *syn*-**3**), epoxidation of allylic alcohol **2** with *m*-CPBA did lead to an isolable epoxide that on treatment with borontrifluoride etherate provided acetal furan **4**. Based on literature precedent, we assumed the epoxide produced in the peracid epoxidation was *syn*-**3**.[10] However, direct assignment of the *syn*/*anti* relative stereochemistry of 2,3-epoxy alcohols by NMR analysis is historically difficult.[11] In order to provide evidence for *syn*-**3** we labeled the C21 carbonyl of **2** with an ^{18}O isotope. *Epoxidation of ^{18}O-**2** followed by cyclization of the intermediate* γ,δ-epoxyketone *to furan ^{18}O-**4** would allow path A (syn-**3**) and path B (anti-**3**) to be distinguished by assignment of the ^{18}O position in furan ^{18}O-**4** based on the expected ^{18}O-induced shift of the adjacent carbon signal in the ^{13}C NMR spectrum (Scheme 2)*.[12]

The ^{18}O-labeled ketone (^{18}O-**2**) was readily prepared by stirring ketone **2** in THF using ^{18}O-labeled water and trace HCl. After 1 h, ^{18}O-**2** was isolated and determined to be $>99\%$ ^{18}O labeled.[13] Exposure of ^{18}O-**2** to VO(acac)$_2$/TBHP led to the isolation of ^{18}O-**4**. Examination of the ^{13}C NMR spectrum of ^{18}O-**4** indicated resonances corresponding to C21 and C24 carbons were accompanied by ^{18}O induced shifts (Scheme 3). The combination of assigned stereochemistry and isotope position in ^{18}O-**4** (Scheme 3) indicates *syn*-2,3-epoxy alcohol **3** underwent a 5-exo closure to **4** (path A, Scheme 2). In contrast, ^{18}O incorporation was not observed at the C25 carbon as would be expected for a 6-endo closure (path B, Scheme 2).

Scheme 3. ^{18}O-Assisted Analysis of Epoxidation−Cyclization Pathway[a]



[a] NMR analysis assisted by premixing of ^{16}O- and ^{18}O-labeled **4**.

A tentative model used to rationalize the observed proclivity for stereoselective epoxidation of aldol adduct **2** to afford γ,δ-ketoepoxide *syn*-**3** followed by a rapid cyclization starts with the preorganization of aldol adduct **2** by an intramolecular hydrogen bond (Figure 3).[14] We speculated incorporation of a second hydrogen bond by way of the C19 hydroxyl group may alter stereoselectivity to favor the *anti* epoxy alcohol or allow isolation of the *syn*-2,3-epoxy alcohol by slowing the rate of keto-epoxide cyclization (Scheme 4). In the latter case we would examine a 6-endo closure of the *syn* epoxy alcohol promoted by aqueous solvent to afford the ammocidin acetal-pyran based on the work of Jamison.[5]

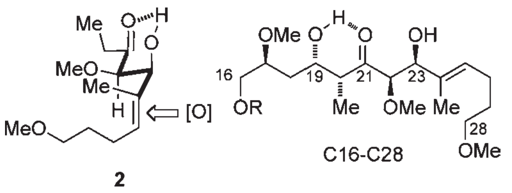


Figure 3. Assumed hydrogen bonding of C20−C28 and C16−C28 fragments.

Preparation of the C16−C28 fragment of ammocidin (Figure 3) started with a double diastereoselective Mukaiyama aldol reaction between aldehyde **8** and silyl enol ether **9**. Aldehyde **8** was prepared in five steps from (−)-malic

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[9] Merck Molecular Force Field calculations (MMFF94) estimate furan acetal **4** to be 1.3 kcal more stable then pyran acetal **6**.

[10] (a) Loh, T. P.; Feng, L. C. *Tetrahedron Lett.* **2001**, *42*, 3223–3226. (b) Smith, A. B., III; Adams, C. M.; Barbosa, S. A. L.; Degnan, A. P. *J. Am. Chem. Soc.* **2003**, *125*, 350–351. (c) Fleming, K. N.; Taylor, R. E. *Angew. Chem., Int. Ed.* **2004**, *43*, 1728–1730.

[11] (a) Mihelich, E. D. *Tetrahedron Lett.* **1979**, 4729–4732. (b) Rossiter, B. E.; Verhoeven, T. R.; Sharpless, K. B. *Tetrahedron Lett.* **1979**, 4733–4736.

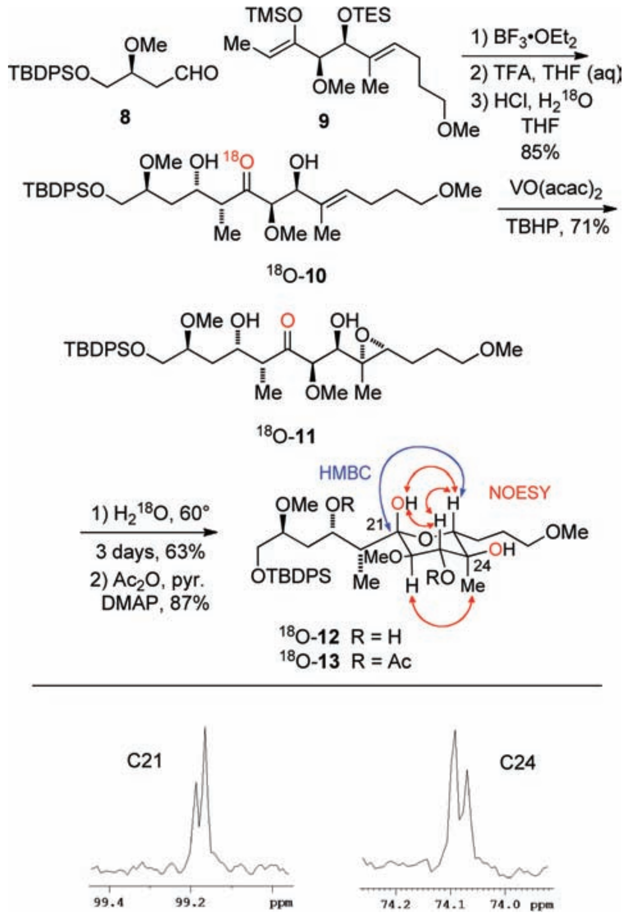
[12] (a) Gree, D.; Gree, R.; Lowinger, T. B.; Martelli, J.; Negri, J. T.; Paquette, L. A. *J. Am. Chem. Soc.* **1992**, *114*, 8841–8846. (b) Risley, J. M.; Vanetten, R. L.; Uncuta, C.; Balaban, A. T. *J. Am. Chem. Soc.* **1984**, *106*, 7836–7840. (c) Wilgis, F. P.; Neumann, T. E.; Shiner, V. J. *J. Am. Chem. Soc.* **1990**, *112*, 4435–4446.

[13] The extent of ^{18}O incorporation was determined by LC-MS.

[14] In the case of the vanadium-mediated epoxidation coordination of either the C23 hydroxyl group and/or C21 carbonyl may be considered.

[15] (a) Saito, S.; Hasegawa, T.; Inaba, M.; Nishida, R.; Fujii, T.; Nomizu, S.; Moriwake, T. *Chem. Lett.* **1984**, 1389–1392. (b) Saito, S.; Ishikawa, T.; Kuroda, A.; Koga, K.; Moriwake, T. *Tetrahedron* **1992**, *48*, 4067–4086.

Scheme 4. Preparation of Ketone ^{18}O-**11**, Cyclization to Pyran Acetal **12** and Analysis of ^{13}C NMR Spectrum of ^{18}O-**13**



acid.[15] The aldol reaction between **8** and **9** proceeded with $>20{:}1$ stereoselectivity to afford *syn* aldol **10** following removal of the C23 TES ether.[16] Incorporation of ^{18}O at the keto carbonyl proceeded with 50% incorporation using conditions described earlier to provide ^{18}O-**10**.[13] Epoxidation of ^{18}O-**10** with VO(acac)$_2$-TBHP afforded a single 2,3-epoxy alcohol (^{18}O-**11**) isolable by flash chromatography, implying hydrogen bonding of the C19 hydroxyl group retarded the rate of cyclization as anticipated (Figure 3). To our delight, heating a solution of ^{18}O-**11** in ^{18}O-water at 60 °C for 3 days afforded pyran acetal ^{18}O-**12**. Following acetylation of ^{18}O-**12**, analysis of the ^{13}C NMR spectrum of diacetate ^{18}O-**13** indicated ^{18}O-incorporation at C21 and C24. Upon examination of the HMBC and NOESY spectrum of the ^{18}O-**13**, the chemical shift of the C21 carbon (99 ppm) and H$_{22}$−H$_{23}$ coupling constant (10 Hz) indicated pyran acetal **13** possessed the stereochemistry tentatively assigned to ammocidin D (Figure 2). Furthermore, the position of ^{18}O labels in ^{18}O-**13** and the product stereochemistry supported epoxidation of ^{18}O-**10** afford *anti*-2,3-epoxy alcohol (**11**) that upon heating in water yielded **12** via a 5-exo epoxide opening. However, upon heating a solution (MeOH−CH$_2$Cl$_2$) of pyran acetal **13** in the presence of catalytic PPTS complete isomerization to the corresponding furan acetal (C21: ^{13}C NMR 112 ppm) was observed indicating the latter to be thermodynamically favored.[17] This finding does not support the pathway proposed in Figure 2 but that shown in Figure 4. In this case, intermediate oxonium ion **IV** is intercepted by the C25 hydroxyl group leading to intermediate **V** and upon hydration provides pyran acetal **III**. A closely related cyclization of a γ,δ-epoxyketone has been proposed to account for the conversion of myriaporone 1 to myriaporone 2.[18]

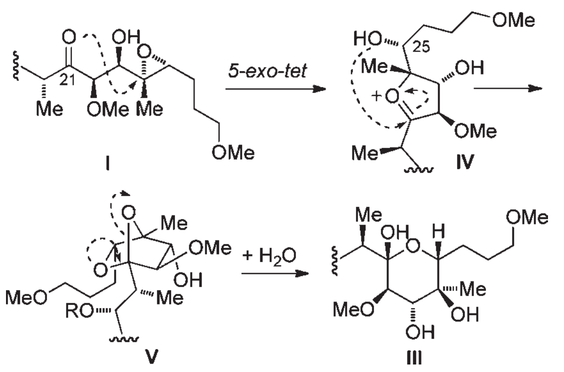


Figure 4. Revised pathway leading to acetal pyran formation.

In conclusion we have developed a synthetic route to access the C16−C28 fragment of ammocidins A−D. Comparison of the spectral data of ammocidin A−D and pyran acetal **13** (NOESY and ^{1}H−^{1}H coupling constants) support the stereochemical assignment of the C16−C28 fragment shown in Figure 1.

Acknowledgment. We thank the National Institutes of Health (CA059515). We also acknowledge Professor Richard E. Taylor (University of Notre Dame) for helpful discussions and Donald F. Stec (Vanderbilt University) for assistance with NMR data collection and analysis.

Supporting Information Available. Experimental procedures and full spectroscopic data for all new compounds. Comparison of NMR data for pyran acetal **14** to ammodicidin D. This material is available free of charge via the Internet at http://pubs.acs.org.

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(16) (a) Evans, D. A.; Yang, M. G.; Dart, M. J.; Duffy, J. L.; Kim, A. S. *J. Am. Chem. Soc.* **1995**, *117*, 9598–9599. (b) Evans, D. A.; Dart, M. J.; Duffy, J. L.; Yang, M. G. *J. Am. Chem. Soc.* **1996**, *118*, 4322–4343.

(17) In contrast heating a solution of furan acetal **4** in MeOH−CH$_2$Cl$_2$ and PPTS resulted in no change.

(18) Cheng, J.-F.; Lee, J.-S.; Sakai, R.; Jares-Erijman, E. A.; Silva, M. V.; Rinehart, K. L. *J. Nat. Prod.* **2007**, *70*, 332–336.