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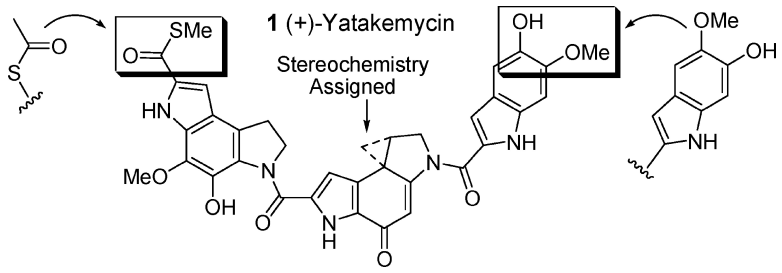
J. Am. Chem. Soc., **2004**, 126 (27), 8396-8398• DOI: 10.1021/ja0472735 • Publication Date (Web): 16 June 2004



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Total Synthesis, Structure Revision, and Absolute Configuration of (+)-Yatakemycin

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Yatakemycin (**1**),[1] isolated from the culture broth of *Streptomyces* sp. TP-AO356, represents the newest and most potent member of a class of antitumor compounds that includes CC-1065,[2] duocarmycin A,[3] and duocarmycin SA[4] (Figure 1). Each derives its biological properties through a characteristic and distinguishable DNA alkylation reaction.[5−9] The structure of (+)-yatakemycin was disclosed in 2003 as **2** on the basis of extensive spectroscopic studies.[1] As such, it represented a remarkable hybrid of the preceding natural products containing a central alkylation subunit identical to that found in duocarmycin SA, a right-hand subunit similar to that of the duocarmycins and a left-hand subunit similar to the central and right-hand subunits of CC-1065. Distinct from the preceding natural products, it represents the first naturally occurring member of this class that contains DNA-binding subunits flanking each side of the alkylation subunit. Since earlier studies established that this "sandwiched" arrangement conferred remarkable properties (enhanced DNA alkylation rate, uniquely altered alkylation selectivity, potent cytotoxic activity) unto a series of duocarmycin SA analogues,[10] we became especially interested in yatakemycin. With a sample of natural material provided by Igarashi, we recently reported yatakemycin's DNA alkylation properties, which proved to be characteristic of such a "sandwiched" compound.[5]

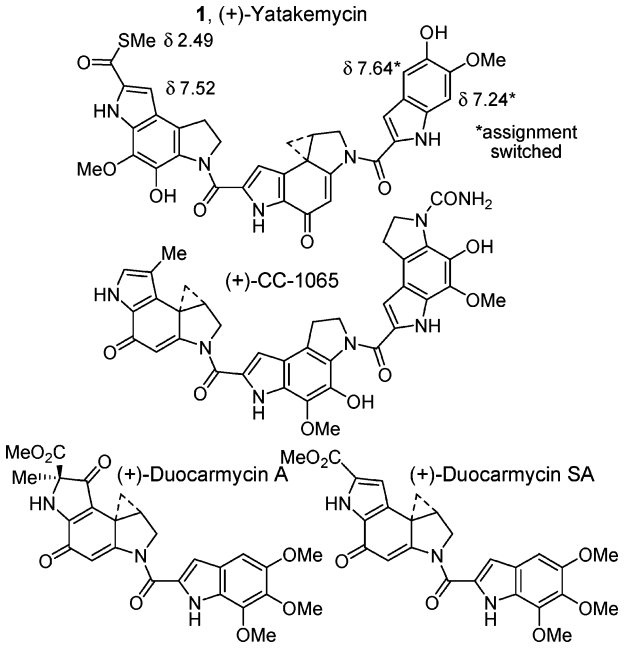


Figure 1. Natural products.

Herein we report the total synthesis of **2** and its lack of correlation with the natural product (Figure 2).[11] On the basis of spectroscopic distinctions between **2** and yatakemycin, the natural product structure was reformulated as **3**, now bearing a left-hand subunit essentially identical to the central and right-hand subunits of CC-1065. Total synthesis of this alternative structure (thiomethyl ester vs thioacetate) provided a compound nearly identical to, but still subtly distinct from, the natural product. A further reformulation of the yatakemycin structure as **1**, incorporating the alternatively substituted right-hand subunit as well as the thiomethyl ester, was confirmed by total synthesis of (+)- and *ent*-(−)-**1** in studies that also unambiguously established the absolute configuration of the natural product.

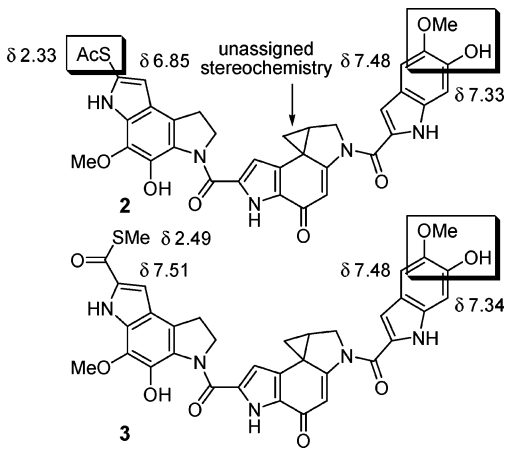


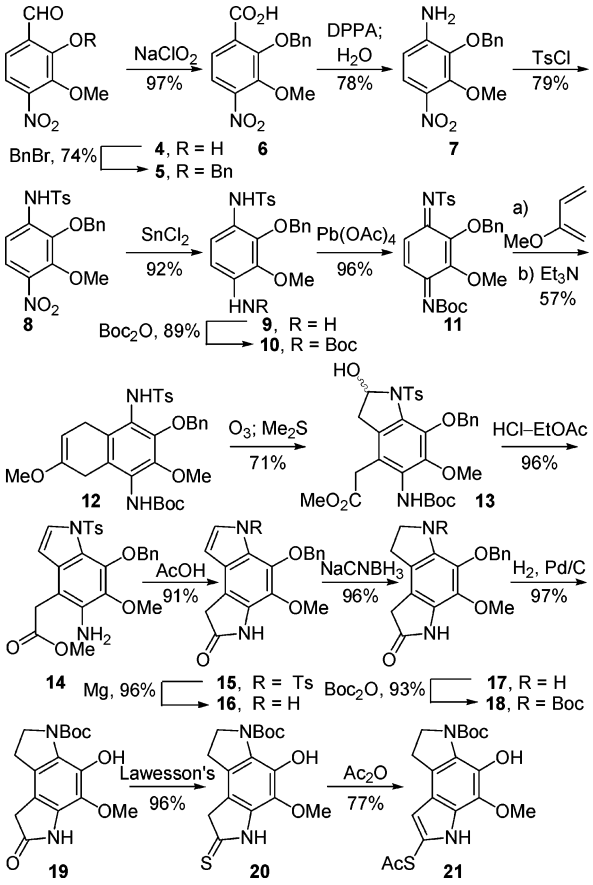
Figure 2. Original and first alternative structure.

Total Synthesis of 2. Key to the synthesis of the putative yatakemycin structure **2** was the preparation of the left-hand subunit **21** bearing the indole thioacetate. The remaining central subunit was obtained following protocols first developed in or adopted from our total synthesis of duocarmycin SA,[12,13] and the 6-hydroxy-5-methoxyindole-2-carboxylic acid was readily available in one step from commercially available material.[14]

Synthesis of the key left-hand subunit began with **4**, which was readily available from *o*-vanillin.[15] Phenol protection as the benzyl ether (1.5 equiv of BnBr, 2.0 equiv of K_2CO_3, 25 °C, 14 h, 74%) followed by aldehyde oxidation of **5** (1.2 equiv of $NaClO_2$, 25 °C, 2 h, 97%) provided **6** (Scheme 1). Subsequent Curtius rearrangement (1.05 equiv of DPPA, 3 equiv of Et_3N, 25 °C, 3 h) with water trap of the intermediate isocyanate (78% overall) provided amine **7**, which was N-tosylated (1.5 equiv of *p*-TsCl, 25 °C, 2 h, 79%) to give **8**. Nitro reduction (5 equiv of $SnCl_2 \cdot 2H_2O$, 85 °C, 0.5 h, 92%), Boc protection of amine **9** (1.2 equiv of Boc_2O, 65 °C, 14 h, 89%), and subsequent oxidation of **10** (1.05 equiv of $Pb(OAc)_4$, 25 °C, 2 h, 96%) provided the selectively activated *p*-quinonediimine **11**. The key Diels−Alder reaction of **11** with 2-methoxy-1,3-butadiene (20 equiv, 40 °C, 48 h) provided a single cycloadduct regioisomer that was treated with Et_3N in situ to effect aromatization to **12** (57% overall).[16] The cycloaddition regioselectivity is domi-

10.1021/ja0472735 CCC: $27.50

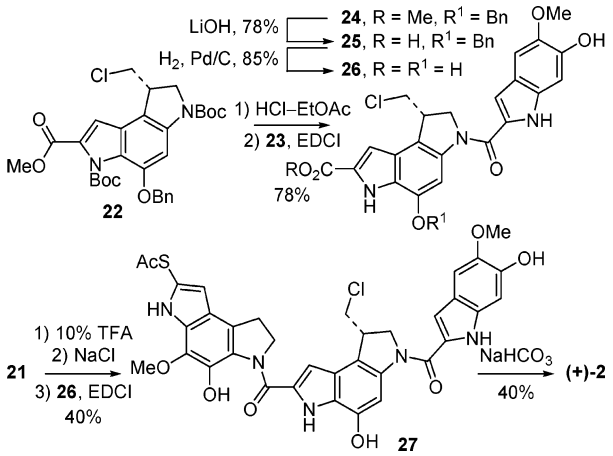
Scheme 1



nated by the stronger electron-withdrawing character of the *N*-tosylimine and set the stage for cleavage of the enol ether with release of two differentially oxidized side chains suitable for closure to an appropriately functionalized dihydropyrroloindole.[16] Thus, ozonolysis of **12** (O_3, −78 °C) followed by reductive workup (10 equiv of Me_2S, 71%) provided the labile 2-hydroxyindoline **13**, which in turn was treated with 4 N HCl−EtOAc (25 °C, 0.6 h, 96%) to induce aromatization and *N*-Boc deprotection. Further cyclization of **14** to lactam **15** was achieved upon treatment with HOAc (25 °C, 24 h, 91%). At this stage, all the necessary functionality was in place for straightforward synthesis of **21**. The indole *N*-tosyl group was reductively cleaved using Mg in MeOH (20 equiv of Mg, sonication, 25 °C, 1.5 h, 96%). Subsequent indole reduction (2.0 equiv of $NaCNBH_3$, 25 °C, 2 h, 96%) afforded **17**, which was *N*-Boc protected (2.0 equiv of Boc_2O, 65 °C, 1.5 h, 93%) and followed by benzyl ether hydrogenolysis (1 atm H_2, 10% Pd/C, 25 °C, 2 h, 97%) to afford **19**. Treatment of **19** with Lawesson's reagent (1.0 equiv, 25 °C, 1.5 h, 96%) gave the thiolactam **20**, which was selectively acylated (3 equiv of Ac_2O, 5 equiv of pyridine, 25 °C, 7 h, 77%) to provide **21**.

After considerable experimentation, a sequence for the assemblage of the subunits was developed as shown in Scheme 2. Resolution of **22** on a Chiralcel OD column provided both enantiomers cleanly (2 × 25 cm, 2% *i*-PrOH−hexanes, 7 mL/min, $\alpha = 1.22$). Boc deprotection of **22** (4 N HCl−EtOAc, 70 °C, 1 h) followed by coupling of the indoline hydrochloride salt with **23**[14] (4 equiv of EDCI, 3 equiv of $NaHCO_3$, DMF, 25 °C, 14 h, 78%) provided **24** (Scheme 2, only one enantiomer shown). Hydrolysis of the methyl ester (4 equiv of LiOH, THF−MeOH−H_2O, 25 °C, 14 h, 78%) and benzyl ether deprotection (1 atm H_2, 10% Pd/C, THF, 2 h, 85%) afforded **26**. This order of deprotections avoided competitive spirocyclization of the alkylation subunit, which in turn

Scheme 2

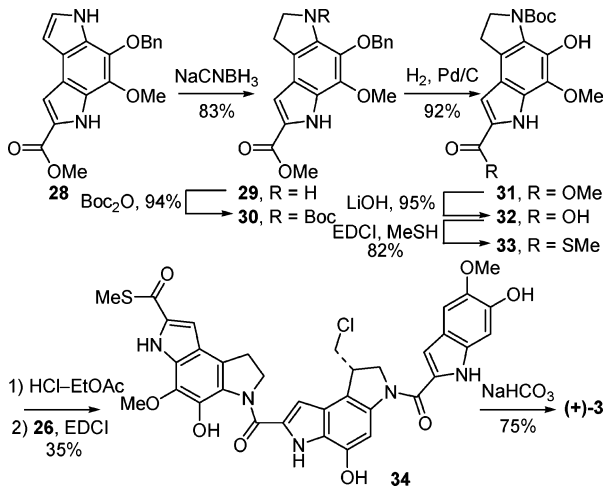


precludes effective coupling of the left-hand subunit.[10] *N*-Boc deprotection of **21** was achieved by treatment with rigorously dry 10% TFA−CH_2Cl_2 (25 °C, 0.5 h) under conditions that avoided thioacetate cleavage. Following deliberate chloride for trifluoroacetate exchange (saturated NaCl 1:1 MeOH−H_2O, evaporation, and then trituration with THF), the corresponding hydrochloride salt coupled smoothly with **26** to give **27**. From a variety of conditions screened, the most effective spirocyclization of **27** occurred upon treatment with saturated $NaHCO_3$ in 1:1:1 CH_3CN−THF−H_2O (25 °C, 1 h, 40%, unoptimized) providing each enantiomer of **2**. Alternative methods commonly employed resulted in significant (1:1:1 Et_3N−THF−H_2O) or quantitative (NaH/DMF, DBU/DMF) cleavage of the sensitive thioacetate.

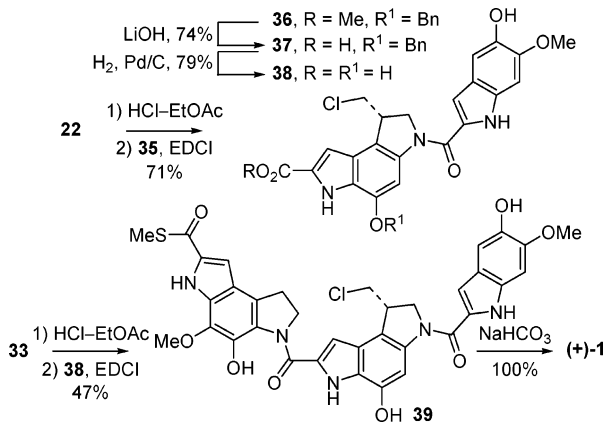
The ^1H NMR of **2** did not match that of natural yatakemycin, and the greatest discrepancies occurred in the left-hand subunit. The ^1H NMR chemical shift of the indole C3−H of natural yatakemycin is found at δ 7.52, significantly downfield of the corresponding proton in **2** at δ 6.85, and the thioacetate methyl group of **2** occurred 0.16 δ upfield of the corresponding protons in the natural material.[1] Model substrates and computer ^1H NMR predictions supported a reassignment of the C2 substituent as a thiomethyl ester versus thioacetate. The electron-withdrawing character of a C2-carbonyl would account for the downfield shift in the indole C3−H, and this reformulated structure (**3**) is more consistent with other members of the natural product family. Most notably, the left-hand subunit would now be identical to the central and right-hand subunits of CC-1065, albeit capped as a thiomethyl ester. Most diagnostically, aryl thiomethyl esters[17] have a methyl ^{13}C NMR chemical shift of roughly δ 11, matching the corresponding chemical shift reported for yatakemycin (δ 11.14), whereas aryl thioacetates[18] occur at δ 30. Thus, yatakemycin was reformulated as **3** and targeted for synthesis.

Total Synthesis of Revised Structure 3. The revised left-hand subunit was prepared enlisting a late-stage intermediate in the synthesis of the central and right-hand subunits of CC-1065.[19] Selective reduction of the more reactive indole of **28** (10 equiv of $NaCNBH_3$, HOAc, 1 h, 83%) followed by *N*-Boc protection (3 equiv of Boc_2O, THF, 65 °C, 2 h, 94%) and benzyl ether hydrogenolysis (1 atm H_2, 10% Pd/C, THF, 2 h, 92%) provided **31** (Scheme 3). Methyl ester hydrolysis (4 equiv of LiOH, THF−MeOH−H_2O, 25 °C, 14 h, 95%) provided the carboxylic acid **32** that was coupled with CH_3SH (excess, 4 equiv of EDCI, DMF, 0 °C, 2 h, 82%) to provide the key thiomethyl ester **33**. *N*-Boc deprotection (4 N HCl−EtOAc, 25 °C, 0.5 h) followed by coupling with **26** (0.7 equiv, 4 equiv of EDCI, 25 °C, 16 h, 35%, unoptimized) and spirocyclization of **34** (saturated $NaHCO_3$ 1:1:1

Scheme 3



Scheme 4



THF−CH_3CN−H_2O, 25 °C, 2 h, 75%) provided both enantiomers of **3** (only one enantiomer shown), which proved to be substantially more stable and easy to handle relative to **2**. Like **2**, **3** did not correlate with yatakemycin. However, the ^{1}H NMR chemical shifts in the left-hand and central subunits of **3** matched those of an authentic sample, suggesting that this portion of the structure incorporating the reformulated thiomethyl ester was now in place. The remaining discrepancies rested with subtle perturbations in the ^{1}H NMR chemical shifts in the right-hand subunit of **3**. A reexamination of the ^{1}H NMR and HMBC data disclosed in the structure identification established that the substituent locations, but not their identity, had been defined. Computer simulations and literature comparisons indicated that the reported chemical shifts of C4−H and C7−H of the right-hand subunit may have been switched and more closely matched those of a 5-hydroxy-6-methoxyindole. Consequently, **1** was targeted for synthesis and bears this right-hand subunit substituent reformulation as well as the left-hand subunit thiomethyl ester.

Total Synthesis of (+)- and *ent*-(−)-Yatakemycin (1). *N*-Boc deprotection of each enantiomer of **22** (4 N HCl−EtOAc, 70 °C, 1 h) and coupling with 5-hydroxy-6-methoxyindole-2-carboxylic acid (**35**,[20] 4 equiv of EDCI, 3 equiv of $NaHCO_3$, DMF, 25 °C, 14 h, 71%) followed by sequential methyl ester hydrolysis of **36** (4 equiv of LiOH, THF−MeOH−H_2O, 25 °C, 14 h, 74%) and benzyl ether deprotection of **37** (1 atm H_2, 10% Pd/C, THF, 2 h, 79%) provided **38** (Scheme 4, only the natural enantiomer is shown). *N*-Boc deprotection of **33** (4 N HCl−EtOAc, 25 °C, 0.5 h), coupling with **38** (0.7 equiv, 4 equiv of EDCI, DMF, 25 °C, 16 h, 47%), and subsequent spirocyclization of **39** (saturated $NaHCO_3$ 2:1 DMF−H_2O, 25 °C, 0.7 h, 100%) provided (+)- and *ent*-(−)-**1**, which proved to be indistinguishable from natural yatakemycin (^{1}H NMR, IR, MS, TLC, HPLC). Moreover, the enantiomer depicted in Scheme 4 exhibited a strong dextrorotatory $[\alpha]_D$ (+100) matching that of the natural product (+99), establishing the absolute stereochemistry and confirming an earlier tentative assignment made in the DNA alkylation studies of **1**.[5]

Thus, the first total synthesis of yatakemycin is described in efforts that served to revise the natural product structure as **1** and to establish the absolute stereochemistry. In addition to constituting the first naturally occurring "sandwiched" member of this class of DNA alkylating compounds, its structure now incorporates an unusual thiomethyl ester, suggesting that protein conjugation may contribute to its biological properties. Such studies on **1** and its key analogues are in progress and will be reported in due course.[21]

Acknowledgment. We gratefully acknowledge the financial support of the NIH (CA41986 and CA42056) and the Skaggs Institute for Chemical Biology. We thank Professor Igarashi of the Toyama Perfectural University for authentic samples of yatakemycin. D.B.K. and M.S.T. are Skaggs Fellows.

Supporting Information Available: Full experimental details and comparison ^{1}H NMR spectra of natural and synthetic **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(20) Synthesis of 5-hydroxy-6-methoxyindole-2-carboxylic acid (**35**): 3-benzyloxy-4-methoxybenzaldehyde was condensed with $N_3CH_2CO_2Me$ (4 equiv, 4 equiv of NaOMe, MeOH, −15 °C, 3 h, then 0 °C, 24 h, 82%) and then subjected to cyclization in xylenes (130 °C, 12 h, 66%). Methyl ester hydrolysis (4 equiv of LiOH, 25 °C, 14 h, 99%) and benzyl ether deprotection (1 atm H_2, 10% Pd/C, 25 °C, 1 h, 83%) provided **35**. See: MacKenzie, A. R.; Moody, C. J.; Rees, C. W. *J. Chem. Soc., Chem. Commun.* **1983**, *22*, 1372−1373.
(21) L-1210 IC_{50} = 5 pM (indistinguishable) for both (+)- and *ent*-(−)-**1**.

JA0472735