J|A|C|S
ARTICLES
Published on Web 02/22/2002

Total Synthesis of (−)-Tetrazomine. Determination of the Stereochemistry of Tetrazomine and the Synthesis and Biological Activity of Tetrazomine Analogues

Jack D. Scott and Robert M. Williams*

Contribution from the Department of Chemistry, Colorado State University, Fort Collins, Colorado 80523

Received October 26, 2001

Abstract: The first total synthesis of the potent antitumor antibiotic (−)-tetrazomine has been accomplished. A new method for the formation of the allylic amine precursor to an azomethine ylide has been developed and exploited in an efficient [1,3]-dipolar cycloaddition to afford the key tetracyclic intermediate used in the synthesis of (−)-tetrazomine. Several analogues of tetrazomine have been synthesized and tested for antimicrobial and biochemical activity.

Introduction

The antitumor antibiotic tetrazomine **1** is a natural secondary metabolite isolated from *Saccharothrix mutabilis* subsp. *chichijimaensis* subsp. nov. by Suzuki et al.[1] Tetrazomine is a member of the tetrahydroisoquinoline family of antitumor antibiotics including ecteinascidin 743 (Et 743)[2] (**2**), bioxalomycin[3] (**3**), and quinocarcin[4] (**4**). Tetrazomine most closely resembles quinocarcin with the exception of the amino functionality at C-10′, the unusual β-hydroxy pipecolic acid moiety, and the oxidation state of C-5′. Neither the relative nor the absolute stereochemistry of tetrazomine was determined when the structure was initially reported.[1] The absolute stereochemistry of the pipecolic acid moiety has since been determined in these laboratories to be 2(*S*),3(*R*) as depicted in Figure 1.[5] The relative stereochemistry at C-5′ then remained as the only stereogenic center to be in question and was solved through a total synthesis that we recently communicated.[6]

Preliminary antitumor and antimicrobial assays of tetrazomine revealed that this substance possesses potent cytotoxicity with activity against P388 leukemia in vivo and good antimicrobial activity against both Gram-negative and Gram-positive bacteria.[1a] Tetrazomine and quinocarcin exert their cytotoxic activity through the expression of multiple mechanisms that include the mediation of oxidative damage to DNA via the reduction of molecular oxygen to superoxide by the auto-redox disproportionation of the fused oxazolidine and, possibly, DNA alkylation.[7] DNA alkylation has not been observed for tetrazomine or quinocarcin; however, alkylation has been observed with bioxalomycin α_2[8] and Et 743.[9]

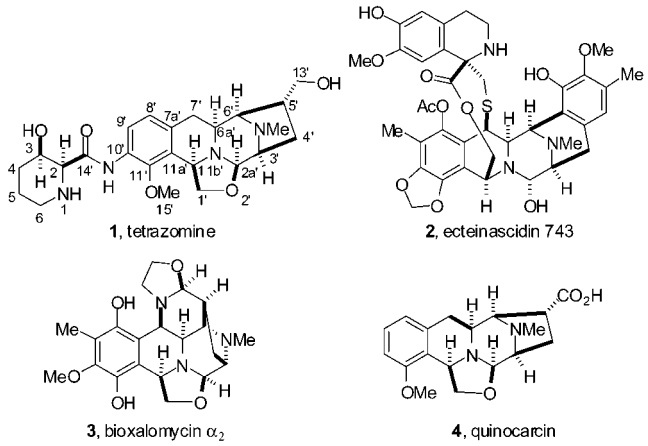


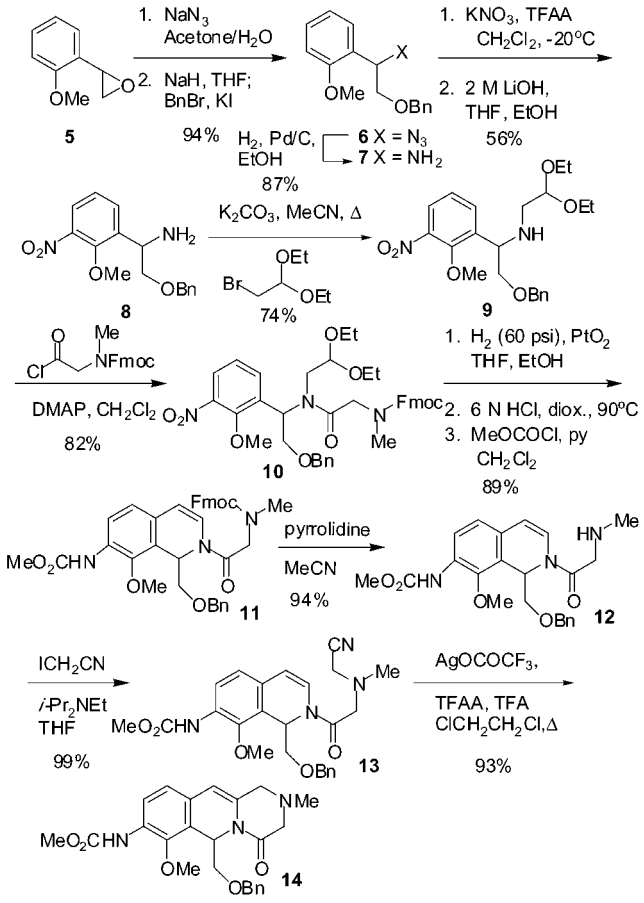
Figure 1. Structures of tetrazomine, ecteinascidin 743, bioxalomycin a2, and quinocarcin.

Prior to our communication,[6] the total synthesis of tetrazomine had not been reported in the literature[10] although two syntheses of the AB ring system of tetrazomine have been reported.[11] Ponzo and Kaufman[11a] reported a racemic synthesis followed

* To whom correspondence should be addressed. E-mail: rmw@chem.colostate.edu. FAX: (970)-491-3944.

(1) (a) Suzuki, K.; Sato, T.; Morioka, M.; Nagai, K.; Abe, K.; Yamaguchi, H.; Saito, T. *J. Antibiot.* **1991**, *44*, 479−485. (b) Sato, T.; Hirayama, F.; Saito, T. *J. Antibiot.* **1991**, *44*, 1367−1370.
(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.
(3) (a) Zaccardi, J.; Alluri, M.; Ashcroft, J.; Bernan, V.; Korshalla, J. D.; Morton, G. O.; Siegel, M.; Tsao, R.; Williams, D. R.; Maiese, W.; Ellestad, G. A. *J. Org. Chem.* **1994**, *59*, 4045−4047. (b) Bernan, V. S.; Montenegro, D. A.; Korshalla, J. D.; Maiese, W. M.; Steinberg, D. A.; Greenstein, M. *J. Antibiot.* **1994**, *47*, 1417−1424.
(4) (a) Tomita, F.; Takahashi, K.; Shimuzu, K. *J. Antibiot.* **1983**, *36*, 463−467. (b) Takahashi, K.; Tomita, F. *J. Antibiot.* **1983**, *36*, 468−470.
(5) Scott, J. D.; Tippie, T. N.; Williams, R. M. *Tetrahedron Lett.* **1998**, *39*, 3659−3662.
(6) Scott, J. D.; Williams, R. M. *Angew. Chem.* **2001**, *40*, 1463−1465.
(7) Williams, R. M.; Flanagan, M. E.; Tippie, T. N. *Biochemistry* **1994**, *33*, 4086−4092.
(8) Williams, R. M.; Herberich, B. *J. Am. Chem. Soc.* **1998**, *120*, 10272−10273.
(9) (a)Pommier, Y.; Kohlhagen, G.; Bailly, C.; Waring, M.; Mazumder, A.; Kohn, K. W. *Biochemistry* **1996**, *35*, 13303−13309. (b) Moore, R. M., II; Seaman, F. C.; Wheelhouse, R. T.; Hurley, L. H. *J. Am. Chem. Soc.* **1998**, *120*, 2490−2491.

Scheme 1



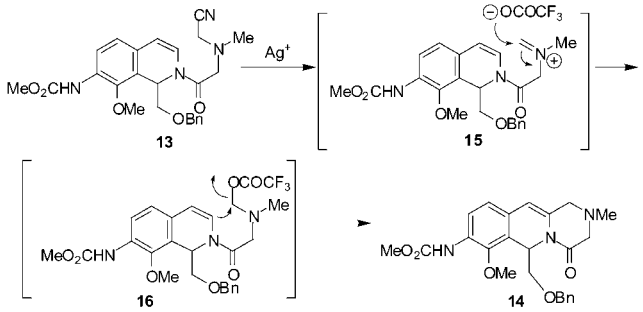
by an enantioselective synthesis by Wipf and Hopkins.[11b] Herein, the first total synthesis of (−)-tetrazomine is described along with the determination of its relative and absolute stereochemistry.[6] In addition, the chemistry developed for the total synthesis of tetrazomine was conscripted for the synthesis of several analogues of tetrazomine and their biochemical and biological activity have been examined.

Results and Discussion

The synthesis began with the regioselective opening of the previously described[12] epoxide **5** with sodium azide (Scheme 1). The resultant primary alcohol was protected as the corresponding benzyl ether and the azide was hydrogenated to afford amine **7**. Selective nitration ortho to the methoxy group was accomplished at low temperature by using potassium nitrate and trifluoroacetic anhydride.[11a] Hydrolysis of the trifluoroacetamide afforded amine **8**.

Alkylation of the primary amine with bromoacetaldehyde diethylacetal[13] provided the secondary amine **9**, which was coupled with N-Fmoc-sarcosine acid chloride to provide amide **10** in 82% yield.

Scheme 2



Hydrogenation of the nitro group was accomplished by using platinum oxide to afford the aniline derivative necessary for acid-promoted cyclization upon the acetal.[10a,11a] Cleavage of the Fmoc group was not observed during the hydrogenation with the use of platinum oxide. The aniline was then protected as the methyl carbamate affording **11** in 89% overall yield for the three steps from **10**. Cleavage of the Fmoc group with pyrrolidine in acetonitrile afforded the secondary amine **12** in high yield. Attempts to convert the secondary amine **12** to the tricyclic substance **14** under standard Mannich conditions with formalin or paraformaldehyde afforded little or none of the desired tricyclic product and a two-step sequence was chosen. First, the amine was converted to the aminonitrile **13** by alkylation with iodoacetonitrile.

Attempted iminium ion cyclization to form the tricyclic substance **14** with $AgNO_3$ or $AgBF_4$[14] afforded none of the desired product. After extensive experimentation, it was found that treatment of **13** with silver(I)trifluoracetate in the presence of TFA and TFAA afforded the desired tricycle **14** in 93% yield. Due to the fact that this cyclization did not yield tricycle **14** when other silver salts were used, we speculate that this cyclization does not proceed through a *6-endo-trig* ring closure[15] on the "free" iminium ion **15** (Scheme 2). Alternatively, the trifluoroacetate anion appears obligatory to trap the incipient iminium ion species to form **16** that allows for the cyclization to occur via a *6-exo-tet* process.[15]

One of the key steps of our synthesis involves an azomthine ylide dipolar cycloaddition[10c,e] to form the piperizine ring. Treatment of allylic amine **14** with NBS in refluxing chloroform yielded a dark green solution of the corresponding iminium ion species that upon deprotonation with triethylamine afforded the dark blue azomethine ylide that was trapped by *tert*-butyl acrylate to afford a 5:1 mixture of separable cycloadducts **17** and **18**, respectively (Scheme 3). The diastereoselectivity observed for the dipolar cycloaddition results from approach of the acrylate from the least hindered face of the azomethine ylide. A similar bias was also observed in our quinocarcinamide synthesis.[10e]

Surprisingly, the use of methyl acrylate as the dipolarphile afforded a 1:1 mixture at C-5′ that is a consequence of poor *endo*/*exo* selectivity during the addition. By contrast, a similar dipolar cycloaddition utilized in our quinocarcin synthesis[10e] provided product only from *exo*-addition with the use of methyl acrylate.

(10) For total syntheses of quinocarcin see: (a) Danishefsky, S. J.; Harrison, P. J.; Webb, R. R.; O'Neill, B. T. *J. Am. Chem. Soc.* **1985**, *107*, 1421−1423. (b) Fukuyama, T.; Nunes, J. J. *J. Am. Chem. Soc.* **1988**, *110*, 5196−5198. (c) Garner, P.; Ho, W. B.; Shin, H. *J. Am. Chem. Soc.* **1993**, *115*, 10742−10753. (d) Katoh, T.; Kirihara, M.; Nagata, Y.; Kobayashi, Y.; Arai, K.; Minami, J.; Terashima, S. *Tetrahedron* **1994**, *50*, 6239−6258. (e) Flanagan, M. E.; Williams, R. M. *J. Org. Chem.* **1995**, *60*, 6791−6797.

(11) (a) Ponzo, V. L.; Kaufman, T. S. *J. Chem. Soc., Perkin Trans. 1* **1997**, 3131−3133. (b) Wipf, P.; Hopkins, C. R. *J. Org. Chem.* **2001**, *66*, 3133−3139.

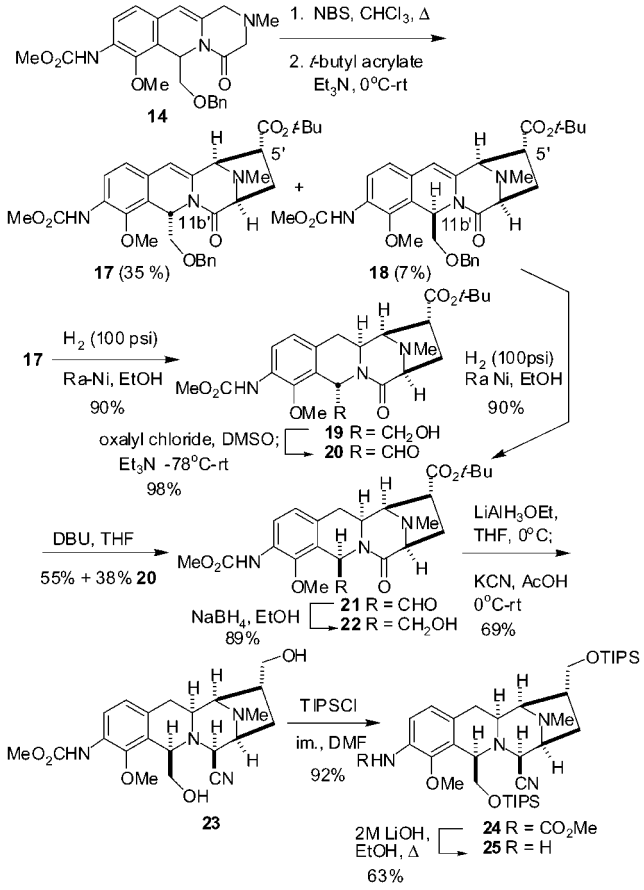
(12) Williams, R. M.; Glinka, T.; Gallegos, R.; Ehrlich, P. P.; Flanagan, M. E.; Coffman, H.; Park, G. *Tetrahedron* **1991**, *47*, 2629−2642.

(13) Zhou, B.; Edmondson, S.; Padron, J.; Danishefsky, S. J. *Tetrahedron Lett.* **2000**, *41*, 2039−2042.

(14) (a) Overman, L. E.; Jacobsen, E. J. *Tetrahedron Lett.* **1982**, *23*, 2741−2744. (b) Grierson, D. S.; Bettiol, J.-L.; Buck, I.; Husson, H.-P. *J. Org. Chem.* **1992**, *57*, 6414−6421.

(15) Baldwin, J. E. *J. Chem. Soc,. Chem. Commun.* **1976**, 734−736.

Scheme 3



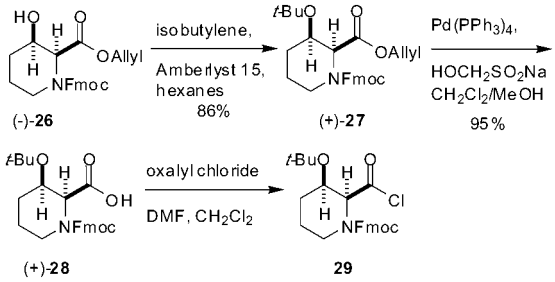
Tetracycle **18** was hydrogenated in the presence of Raney Nickel at moderate pressure to remove the benzyl group and reduce the benzylic olefin from the least-hindered face to afford **22**. The ease of reduction of the olefin was unexpected in light of literature precedent, where catalytic hydrogenation of similar benzylic olefins required much higher pressure (1000−2000 psi) and elevated temperatures.[10b,c] The major product from the cycloaddition (**17**) possessed the undesired configuration at C-11b′ as determined by ^{1}H NMR nOe analysis and an epimerization at C-11b′ was thus executed.

The benzyl ether and the olefin of **17** were reduced as above for **18** with Raney Nickel. The resultant alcohol was subjected to Swern oxidation conditions[16] to afford the corresponding aldehyde **20** that was treated with DBU to afford a 1.4:1 mixture of epimers at C-11b with the desired isomer **21** being predominant. These aldehydes were easily separated by column chromatography, allowing for recycling of the undesired epimer. Sodium borohydride reduction of the desired epimer afforded alcohol **22**.

The simultaneous reduction of the *tert*-butyl ester and partial reduction of the amide were fortuitously accomplished in a single step with $LiAlH_3OEt$ in THF at 0 °C. The resultant carbinolamine was trapped with sodium cyanide under acidic conditions[17] to afford the corresponding stable aminonitrile **23**. The stereochemistry of the aminonitrile was assigned via 2D ^{1}H NMR analysis (ROSEY, TOCSY, and gDQCOSY). The relative stereochemistry of the nitrile was found to be opposite

(16) Huang, S. L.; Swern, D. *J. Org. Chem.* **1978**, *43*, 4537−4538.
(17) Martinez, E. J.; Corey, E. J. *Org. Lett.* **2000**, *2*, 993−997.

Scheme 4



to that expected based on the known stereochemistry of cyanocycline and the carbinolamine of Et 743. This raises an important question concerning the possible influence of this stereogenic center on the biological reactivity of the aminonitrile versus what has been observed with cyanocycline and Et 743 with respect to DNA alkylation. Further studies to penetrate this issue, however, are warranted. The two primary alcohols were protected as their triisopropylsilyl ethers and the methyl carbamate was hydrolyzed to afford aniline **25** that was ready to couple to a protected β-hydroxy pipecolic acid derivative.

A fully protected β-hydroxy pipecolic acid was prepared in order to couple to the free aniline as illustrated in Scheme 4. Since the amino nitrile moiety has been shown to be stable toward TFA, the secondary alcohol of the previously described amino acid **26**[18] was protected as the corresponding *tert*-butyl ether. Treatment of (−)-**26** with isobutylene in the presence of Amberlyst 15 ion-exchange resin[19] afforded the *tert*-butyl ether (+)-**27** in 85% yield. Cleavage of the allyl ester[20] was accomplished in high yield with $Pd(PPh_3)_4$ to afford **28**. Attempts at coupling the free carboxylic acid (**28**) to aniline **25** with reagents such as EDCI, BOP reagent, or BOPCl were all uniformly unsuccessful. It was thus found that acid chloride **29**, prepared from **28** with oxalyl chloride, resulted in a successful condensation with aniline **25** without epimerization.

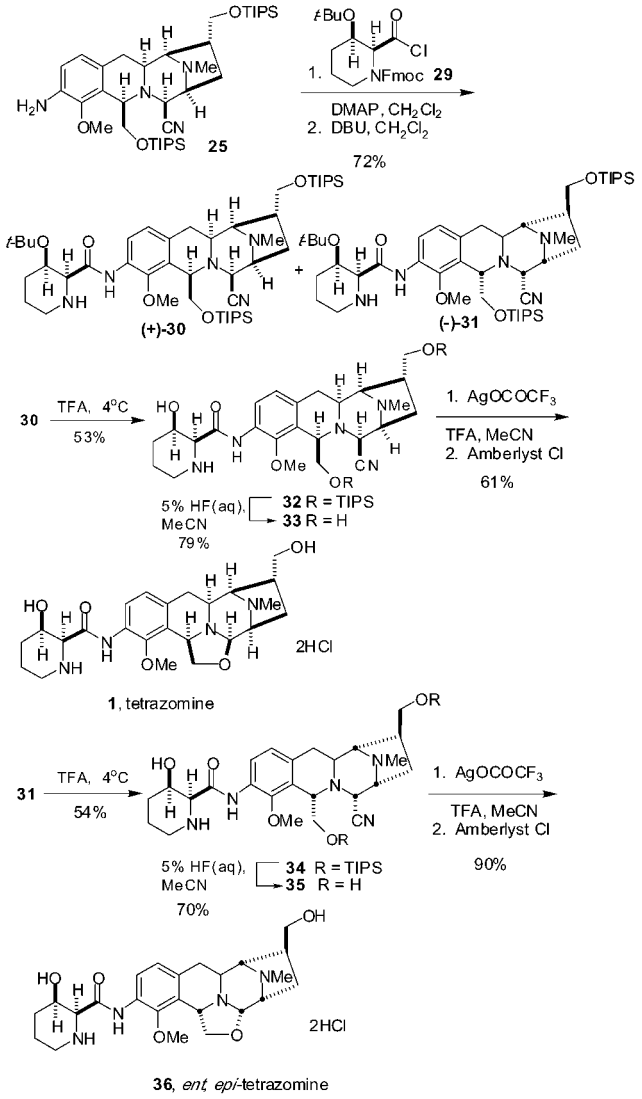
Coupling of acid chloride **29** to aniline **25** in the presence of DMAP followed by cleavage of the Fmoc group with DBU afforded the corresponding pipecolamide **30** (plus a separable diastereomer **31** constituted as the *ent*-tetrahydroisoquinoline portion; obtained as a 1:1 mixture of optically active diastereomers) (Scheme 5).

Each diastereomer was carried on separately, through the end of the synthesis. The *tert*-butyl ether was cleaved with TFA at 4 °C followed by cleavage of the TIPS groups with HF in acetonitrile to afford diastereomers **33** and **35**. To finish the synthesis of tetrazomine, the oxazolidine ring was closed upon treatment of the amino nitrile with silver trifluoroacetate in the presence of excess TFA. Addition of Amberlyst ion-exchange resin (Cl− form) followed by filtration and lyophillization afforded tetrazomine and *ent,epi*-tetrazomine (**36**) as their di-HCl salts. After purification by reversed phase HPLC, the synthetic tetrazomine exhibited identical spectroscopic properties to that of natural tetrazomine.[21]

This series of manipulations served to confirm the relative stereochemistry of the pentacyclic tetrahydroisoquinoline core of the natural product. The absolute configuration of the

(18) Scott, J. D.; Williams, R. M. *Tetrahedron Lett.* **2000**, *41*, 8413−8416.
(19) Alexakis, A.; Gardette, M.; Colin, S. *Tetrahedron Lett.* **1988**, *29*, 2951−2954.
(20) Honda, M.; Morita, H.; Nagakura, I. *J. Org. Chem.* **1997**, *62*, 8932−8936.
(21) Provided by Yamanouchi Pharmaceutical Company.

Scheme 5



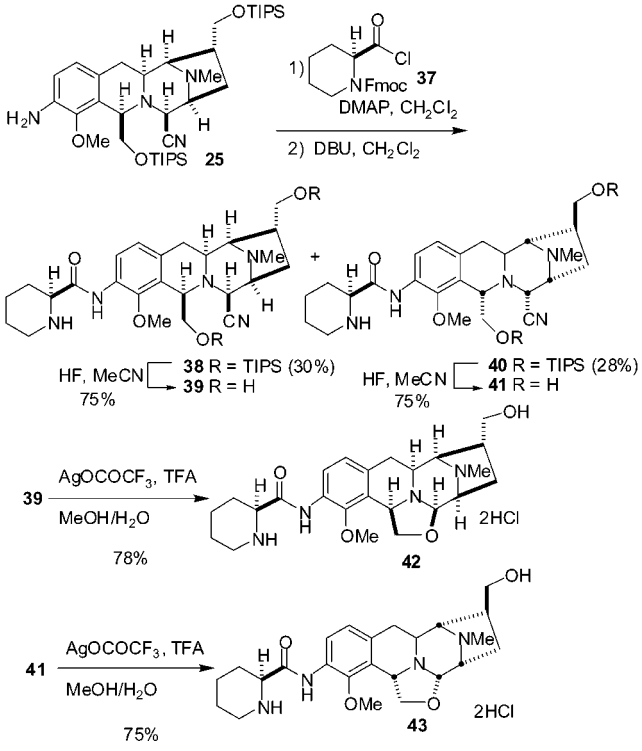
tetrahydroisoquinoline core is therefore assumed to be that depicted in structure **1** based on biosynthetic considerations since quinocarcin, bioxalomycin, and ecteinascidin all possess the same absolute configuration of the tetrahydroisoquinoline core.

In an effort to probe the biochemical and biological activity of tetrazomine, several analogues of tetrazomine were prepared as shown in Scheme 6. Aniline **25** was coupled to N-Fmoc-L-pipecolic acid chloride (**37**) followed by cleavage of the Fmoc and TIPS groups to afford the two aminonitrile diastereomers **38** and **40**. The absolute stereochemistry of the tetracyclic core of these compounds was assigned by comparison of the CD spectra of **38** and **40** to that of compound **32**. Removal of the TIPS groups with HF in acetonitrile from **38** and **40** afforded **39** and **41**, respectively. Finally, the oxazolidine rings were installed as described above for tetrazomine to afford the two deoxytetrazomine analogues **42** and **43**.

Biochemical and Biological Activity

We have previously demonstrated that tetrazomine cleaves DNA in an O_2-dependent manner[7] and that the DNA cleavage reaction is inhibited by the addition of free radical scavengers such as picolinic acid and is also inhibited by superoxide dismutase (SOD). The mechanism of DNA cleavage arises from

Scheme 6



the self-disproportionation of tetrazomine to form a carbon-centered radical that can react with molecular oxygen eventually to be expelled as superoxide radical anion. Superoxide released from the drug subsequently undergoes Fenton/Haber-Weiss redox cycling leading to the formation of various oxygen-based radicals, including hydroxyl radical, that can damage DNA.

It was further demonstrated that tetrazomine cleaves DNA in a non-sequence-specific manner with cleavage observed at every nucleotide residue, which is consistent with a freely diffusable oxidant.[7] In addition, each nucleotide residue of the tetrazomine-damaged DNA was observed as a doublet on denaturing polyacrylamide gel electrophoresis indicative of the production of both the 3′-phosphate and 3′-phosphoglycolate products. The observed product ratios of 3′-phosphate:3′-phosphoglycolate were 8:2 for tetrazomine, 6:4 for quinocarcin, and 4:6 for Fe/EDTA. It was hypothesized that the difference in ratios of the 3′-phosphate to 3′-phosphoglycolate products formed from tetrazomine or quinocarcin and Fe/EDTA might be attributed to the capacity of the drugs to noncovalently bind, thus affecting the presentation of the oxidant for hydrogen atom abstraction on the ribose−phosphate backbone. Further evidence supporting this notion was observed when DNA that was preincubated with tetrazomine and then treated with Fe/EDTA showed virtually no 3′-phosphoglycolate product formation.

It has also been postulated that quinocarcin can noncovalently bind to DNA and alkylate via an iminium ion species generated from ring-opening of the oxazolidine.[22] However, despite compelling experimental precedent for this mode of reactivity on ecteinascidin 743 and saframycin, there is no published experimental evidence for DNA alkylation by either quinocarcin or tetrazomine.

To examine the interaction of tetrazomine and analogues **33** and **36** with DNA, these compounds were incubated with a

(22) Hill, G. C.; Wunz, T. P.; Remers, W. A. *J. Comput.-Aided Mol. Des.* **1988**, *2*, 91−106.

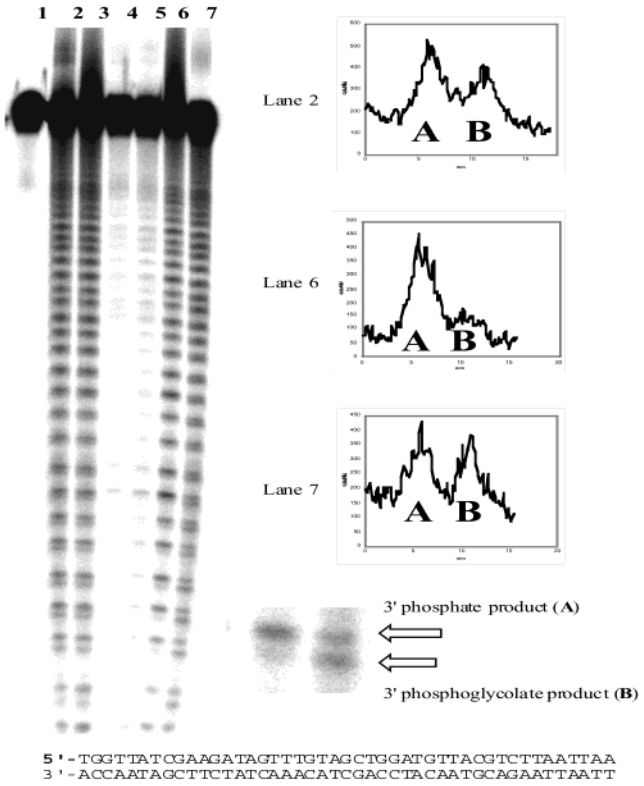


Figure 2. Lane 1: DNA (control). Lane 2: DNA + 1 mM **36**. Lane 3: DNA + 10 mM **36**. Lane 4: DNA + 1 mM **33**. Lane 5: DNA + 10 mM **33**. Lane 6: DNA + 10 mM tetrazomine. Lane 7: DNA + Fe(II)/EDTA.

synthetic ^{32}P-5′-end-labeled 45 bp duplex at pH 7 in 20 mM phosphate buffer (Figure 2). As expected, cyanotetrazominol (**33**) did not exhibit any capacity to inflict oxidative DNA damage. On the other hand, *ent,epi*-tetrazomine did mediate DNA cleavage in a non-sequence-specific manner, with a 3′-phosphate:3′-phosphoglycolate ratio of 4.5:3.5 as measured by the use of a phosphoimager.

In an effort to investigate a preliminary structure−activity relationship with respect to the antimicrobial activities of the tetrazomine analogues, all four oxazolidine and all four aminonitrile analogues were assayed against a Gram-(+) bacteria (*Staphylococcus aureus*) and a Gram-(−) bacteria (*Klebsiella pneumoniae*) via the disk diffusion method. It was found that the deoxy compounds **42** and **43** possessed slightly better activity than either tetrazomine or compound **36** (Table 1).

The analogues that lacked an oxazolidine ring but contained the aminonitrile moiety displayed comparable antimicrobial activities to that for the oxazolidine-containing compounds (Table 2).

Conclusion

In summary, we have developed the first total synthesis of the natural antitumor antibiotic (−)-tetrazomine and have established the stereochemistry of the natural product. In particular, the relative stereochemistry at the C-5′ position, which was the only stereogenic center remaining in question following our elucidation of the relative and absolute stereochemistry of the β-hydroxypipecolic acid moiety, has now been firmly secured. The absolute stereochemistry of the tetrahydroisoquinoline core has been assigned based on biogenetic considerations relative to all other known members of this family of natural products,

Table 1. Antimicrobial Activities of Oxazolidine-Containing Analogues[a]

		zone of inhibition	
compd	amount (mg)	*Kleb* (mm)	*Staph* (mm)
1	0.2	28	12
	0.02	22	R
	0.002	10	R
36	0.12	15	R
	0.012	8	R
	0.0012	R	R
42	0.12	29	14
	0.012	21	9
	0.0012	19	R
43	0.12	24	7
	0.012	17	R
	0.0012	R	R
Penicillin G	10 units	NT	30
Streptomycin	0.01	14	NT

[a] R = resistant, NT = not tested.

Table 2. Antimicrobial Activities of Aminonitrile-Containing Analogues[a]

		zone of inhibition	
compd	amount (mg)	*Kleb* (mm)	*Staph* (mm)
33	0.12	26	12
	0.012	20	R
	0.0012	16	R
35	0.12	18	R
	0.012	13	R
	0.0012	R	R
39	0.12	27	11
	0.012	23	R
	0.0012	13	R
41	0.12	16	R
	0.012	12	R
	0.0012	R	R
Penicillin G	10 units	NT	30
Streptomycin	0.01	14	NT

[a] R = resistant, NT = not tested.

all of which possess the same absolute stereochemistry (Figure 1). The synthesis features a novel method to construct the monoketopiperazine ring fused to the dihydroisoquinoline nucleus (**13** → **14**, Schemes 1 and 2). The coupling of the optically pure β-hydroxypipecolic acid moiety (**29**) to the racemic tetrahydroisoquinoline nucleus (**25**) has permitted the simultaneous synthesis of the natural product and the *ent,epi*-tetrazomine structure (**36**). Unexpectedly, *ent,epi*-tetrazomine retained some antimicrobial activity against *Klebsiella pneumoniae*, a Gram-(−) organism, but was inactive against *Staphylococcus aureus*, a Gram-(+) organism. More surprising was the observation that *both* pipecolic acid analogues **42**, which possesses the natural stereochemistry, and **43**, which possesses the entantiomorphic tetrahydroisoquinoline nucleus, displayed antimicrobial activity against *Klebsiella pneumoniae Staphylococcus aureus*. The preparation of key aniline derivative **25** provides an intermediate that can be exploited for the attachment of other amino acid or peptide residues to this structure that may prove to be useful for enhancing the sequence-specificity of binding to and alkylating DNA. In a preliminary study exhibiting the utility of compound **25**, we have prepared pipecolic acid analogues of tetrazomine (**42** and **43**) that, somewhat surprisingly, proved to be slightly more potent antimicrobial agents than the natural product. Further studies to prepare derivatives of tetrazomine to probe the biochemistry and biology of this

important family of tetrahydroisoquinoline antitumor antibiotics are in progress in these laboratories.

Acknowledgment. This work was supported by the National Institutes of Health (Grant CA85419). We thank Yamanouchi Pharmaceutical Co. for providing the generous gift of natural tetrazomine.

Supporting Information Available: Complete experimental procedures and spectroscopic and analytical data including copies of NMR spectra (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

JA0174027