Synthetic Studies on Quinocarcin: Total Synthesis of (±)-Quinocarcinamide via Dipole Cycloaddition of an Azomethine Ylide Generated by NBS Oxidation

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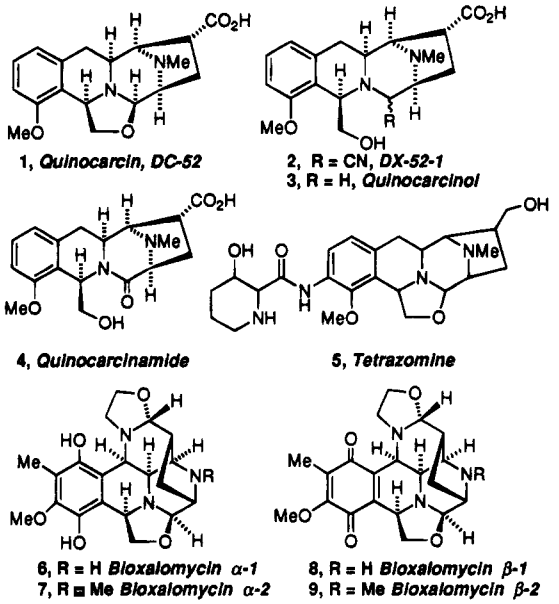
The total synthesis of (±)-quinocarcinamide and a formal total synthesis of the antitumor, antibiotic quinocarcin involving a [3 + 2] dipolar cycloaddition reaction is described. The synthesis involves as a key step an NBS oxidation of an allylic tertiary amine to an imminium ion which is converted *in situ* into an azomethine ylide. Aspects of the ylide formation and a rationale for the regio- and stereocontrol for the cycloaddition process are discussed.

Introduction

Quinocarcin (**1**) is natural secondary metabolite produced by *Streptomyces melanovinaceus*[1] and is the simplest member of the naphthyridinomycin/bioxalomycin/saframycin class of antitumor antibiotics. Quinocarcin displays weak antimicrobial activity against Gram-negative organisms and, as the citrate salt, displays promising antitumor activity against several lines of solid mammalian carcinomas including St-4 gastric carcinoma, Co-3 human colon carcinoma, human mammary carcinoma, M5076 sarcoma, B16 melanoma, and P388 leukemia.[2] The semisynthetic derivative of **1**, DX-52-1 (**2**) also retains many of the antitumor properties exhibited by **1**.[3]

Quinocarcin is coproduced with a biologically inactive reduction product, quinocarcinol (**2**), and we have shown[4] that quinocarcin spontaneously disproportionates anaerobically into quinocarcinol and an oxidation product, quinocarcinamide (**3**), which is also biologically inactive.

More recently, a new member of this class of compounds, tetrazomine (**5**), was isolated [5] from *Saccharothrix mutabilis*, and preliminary antitumor/antimicrobial assays report activity similar to that displayed by quinocarcin.[5] Tetrazomine shows good antimicrobial activity against both Gram-negative and Gram-positive organisms and activity against P388 leukemia *in vivo*. The most complex members of this class of compounds includes the bioxalomycins (**6**–**9**) which have been isolated from *Streptomyces* sp. LL-31F508 that display potent antitumor activity as well as excellent activity against methicillin-resistant *Staphylococcus aureus* (MRSA).[6] Bioxalomycin β-2 was found to be identical to naphthyridinomycin produced by *Streptomyces lusitanus* NRRL 8034; this work therefore established that the hydrated form of naphthyridinomycin, is in fact, an artifact of the isolation procedure and that the primary biosynthetic product contains both oxazolidine rings intact.



Modes of action for this class of compounds include the oxidative cleavage of DNA via the production of superoxide and the alkylation of DNA.[4] Superoxide production by quinocarcin was first reported[7] by Tomita et al., and a mechanistic explanation has now been offered.[4,8] The ability to reduce molecular oxygen appears to be linked to the capacity for these compounds to undergo redox disproportionation reactions of their oxazolidine moieties which, under anaerobic conditions, lead to the products **3** and **4** (in the case of **1**) and, under aerobic conditions, culminate in superoxide production.[5] Preliminary mode of action studies in P388 leukemia cells demonstrated that quinocarcin blocks RNA synthesis in preference to DNA and protein synthesis, although a detailed mechanistic understanding of their cytotoxic properties has not been fully elucidated.[9]

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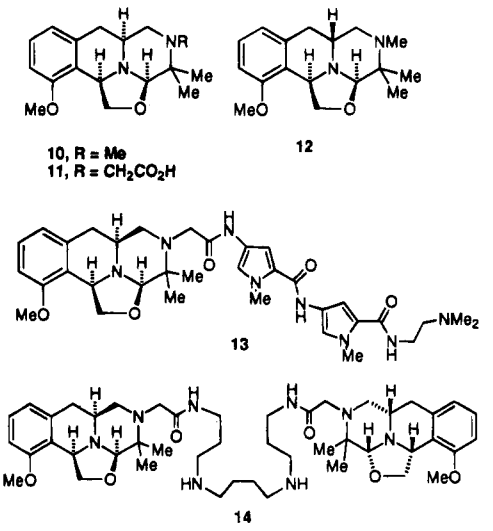
(1) Tomita, F.; Takahashi, K.; Shimizu, K. *J. Antibiot.* **1983**, *36*, 463.
(2) Fujimoto, K.; Oka, T.; Morimoto, M. *Cancer Res.* **1987**, *47*, 1516.
(3) Saito, H.; Hirata, T.; Kasai, M.; Fujimoto, K.; Ashizawa, T.; Morimoto, M.; Sato, A. *J. Med. Chem.* **1991**, *34*, 1959.
(4) Williams, R. M.; Glinka, T.; Flanagan, M. E.; Gallegos, R.; Coffman, H.; Pei, D. *J. Am. Chem. Soc.* **1992**, *114*, 733.
(5) (a) Sato, T.; Hirayama, F.; Saito, T. *J. Antibiot.* **1991**, *44*, 1367. (b) Suzuki, K.; Sato, T.; Morioka, M.; Nagai, K.; Abe, K.; Yamaguchi, H.; Saito, T.; Ohmi, Y.; Susaki, K. *J. Antibiot.* **1991**, *44*, 479.
(6) (a) Bernan, V. S.; Steinberg, D. A.; Montenegro, D. A.; Korshalla, J. D.; Maiese, W. M.; Greenstein, M. Abstracts of the 33rd ICAAC, New Orleans, LA, Oct 17-20, 1993; Abstract 570. (b) Steinberg, D. A.; Singh, M. P.; Jacobus, N. V.; Petersen, P. J.; Maiese, W. M.; Greenstein, M. Abstracts of the 33rd ICAAC, New Orleans, LA, Oct 17–20, 1993; Abstract 571. (c) Zaccardi, J.; Morton, G. O.; Ascroft, J. S.; Tsao, R.; Siegel, M.; Maiese, W. M.; Ellestad, G. A. Abstracts of the 33rd ICAAC, New Orleans, LA, Oct 17–20, 1993; Abstract 572. (d) 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.
(7) Tomita, F.; Takahashi, K.; Tamaoki, T. *J. Antibiot.* **1984**, *37*, 1268.
(8) Williams, R. M.; Flanagan, M. E.; Tippie, T. N. *Biochemistry* **1994**, *33*, 4086.

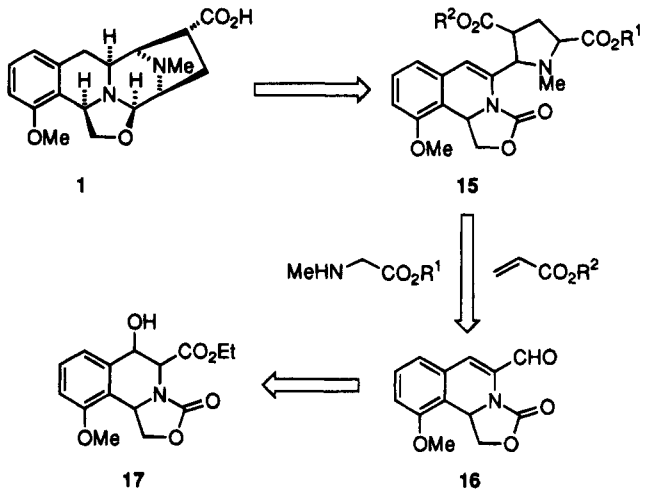
0022-3263/95/1960-6791$09.00/0

Due to the promising antibiotic and antitumor activities displayed by this class of compounds, interest in the total synthesis of the natural products and the synthesis of biologically active structural analogs has attracted considerable attention. Quinocarcinol methyl ester was synthesized by Danishefsky and co-workers in 1985.[10] Fukuyama and Nunes reported the first total synthesis of (±)-quinocarcin,[11] and more recently, two groups, Terashima and co-workers[12] and Garner and co-workers,[13] have achieved syntheses of (−)-quinocarcin. We have been involved in the synthesis of biologically active structural analogs of quinocarcin which have focused on the construction of the biologically functional oxazolidine ring.[14,15] Other noteworthy synthetic approaches to quinocarcin have also appeared in the literature,[16] and a significant amount of activity on the total synthesis of the structurally related naphthyridinomycins (i.e., bioxalomycins)[17] and saframycins[18] has been reported.

As part of our efforts to study the structural and stereoelectronic intricacies of the oxygen-dependent mechanism of DNA damage inflicted by this class of compounds, we have reported the syntheses of the tetracyclic core analogs of quinocarcin **10**−**14**.[14,15] As an extension of this synthetic methodology, we now wish to report an efficient procedure for accessing the bicyclic pyrrolidine-containing framework of these natural products which has resulted in the synthesis of (±)-quinocarcinamide (**4**). In addition, the route described herein intersects an intermediate in the total synthesis of (−)-quinocarcin reported by Garner and co-workers.[13]



Scheme 1



Results

Our initial approach to quinocarcin was directly analogous to that employed in the syntheses of **10**−**14**.[14,15] Our strategy for generating the pyrrolidine ring (Scheme 1, **15**) was to condense a sarcosine ester with the unsaturated aldehyde **16**, generating the imminium ion *in situ* where upon treatment with base would lead to the intermediate azomethine ylide. Following ylide formation, [3 + 2] cycloaddition with an appropriate acrylate ester should afford a pyrrolidine displaying the regiochemical configuration illustrated by **15**. This regiochemistry was anticipated based on the expectation that there would be preponderant negative charge density α to the sarcosyl ester moiety resulting in a larger HOMO coefficient at that center. If the proper relative stereochemistry in the pyrrolidine ring was obtained (particularly at C-8 and C-11)[19] then conversion of this substrate

(9) Jett, J. R.; Saijo, N.; Hong, W.-S.; Sasaki, Y.; Takahashi, H.; Nakano, H.; Nakagawa, K.; Sakurai, M.; Suemasu, K.; Tesada, M. *Invest. New Drugs* **1987**, *5*, 155.

(10) Danishefsky, S. J.; Harrison, P. J.; Webb, R. R.; O'Neill, B. T. *J. Am. Chem. Soc.* **1985**, *107*, 1421.

(11) Fukuyama, T.; Nunes, J. J. *J. Am. Chem. Soc.* **1988**, *110*, 5196.

(12) (a) Saito, S.; Tamura, O.; Kobayashi, Y.; Matsuda, F.; Katoh, T.; Terashima, S. *Tetrahedron* **1994**, *50*, 6193. (b) Saito, S.; Tanaka, K.; Nakatani, K.; Matsuda, F.; Katoh, T.; Terashima, S. *Tetrahedron* **1994**, *50*, 6209. (c) Katoh, T.; Nagata, Y.; Kobayashi, Y.; Arai, K.; Minami, J.; Terashima, S. *Tetrahedron* **1994**, *50*, 6221. (d) Katoh, T.; Kirihara, M.; Nagata, Y.; Kobayashi, Y.; Arai, K.; Minami, J.; Terashima, S. *Tetrahedron* **1994**, *50*, 6239. (e) Katoh, T.; Kirihara, M.; Yoshino, T.; Tamura, O.; Ikeuchi, F.; Nakatani, K.; Matsuda, F.; Yamada, K.; Gomi, K.; Ashizawa, T.; Terashima, S. *Tetrahedron* **1994**, *50*, 6259. (f) Katoh, T.; Kirihara, M.; Nagata, Y.; Kobayashi, Y.; Arai, K.; Minami, J.; Terashima, S. *Tetrahedron Lett.* **1993**, *34*, 5747. (g) Katoh, T.; Kirihara, M.; Nagata, Y.; Kobayashi, Y.; Arai, K.; Minami, J.; Terashima, S. *Tetrahedron Lett.* **1993**, *34*, 5743. (h) Katoh, T.; Kirihara, M.; Nagata, Y.; Kobayashi, Y.; Arai, K.; Minami, J.; Terashima, S. *Tetrahedron Lett.* **1993**, *34*, 5751. (i) Saito, S.; Tanaka, K.; Nakatani, K.; Matsuda, F.; Terashima, S. *Tetrahedron Lett.* **1989**, *30*, 7423. (j) Saito, S.; Matsuda, F.; Terashima, S. *Tetrahedron Lett.* **1988**, *29*, 6301.

(13) (a) Garner, P.; Ho, W. B.; Shin, H. *J. Am. Chem. Soc.* **1993**, *115*, 10742. (b) Garner, P.; Sunitha, K.; Shanthilal, T. *Tetrahedron Lett.* **1988**, *29*, 3525. (c) Garner, P.; Sunitha, K.; Ho, W-B.; Youngs, W. J.; Kennedy, V. O.; Djebli, A. *J. Org. Chem.* **1989**, *54*, 2041. (d) Garner, P.; Ho, W. B.; Grandhee, S. K.; Youngs, W. J.; Kennedy, V. O., *J. Org. Chem.* **1991**, *56*, 5893. (e) Garner, P.; Arya, F.; Ho, W. B. *J. Org. Chem.* **1990**, *55*, 412.

(14) (a) Williams, R. M.; Glinka, T.; Gallegos, R.; Ehrlich, P. P.; Flanagan, M. E.; Coffman, H.; Park, G. *Tetrahedron* **1991**, *47*, 2629. (b) Williams, R. M.; Ehrlich, P. P.; Zhai, W.; Hendrix, J. *J. Org. Chem.* **1987**, *52*, 2615.

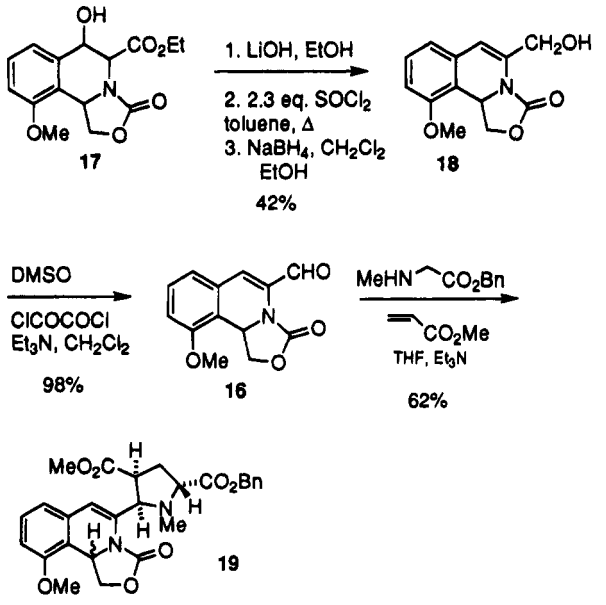
(15) Flanagan, M. E.; Rollins, S. B.; Williams, R. M. *Chem. Biol.* **1995**, 2, 147.

(16) For other synthetic approaches to quinocarcin, see: (a) Lessen, T. A.; Demko, D. M.; Weinreb, S. M. *Tetrahedron Lett.* **1990**, *31*, 2105. (b) Saito, H.; Hirata, T., *Tetrahedron Lett.* **1987**, *28*, 4065. For a related cycloaddition strategy, see: (c) Kiss, M.; Russell-Maynard, J.; Joule, J. A. *Tetrahedron Lett.* **1987**, *28*, 2187. (d) Allway, P. A.; Sutherland, J. K.; Joule, J. A. *Tetrahedron Lett.* **1990**, *31*, 4781.

(17) For synthetic approaches and total syntheses of the naphthyridinomycins, see: (a) Fukuyama, T.; Li, L.; Laird, A. A.; Frank, R. K., *J. Am. Chem. Soc.* **1987**, *109*, 1587. (b) Fukuyama, T.; Frank, R. K.; Laird, A. A. *Tetrahedron Lett.* **1985**, *26*, 2955. (c) Fukuyama, T.; Laird, A. A. *Tetrahedron Lett.* **1986**, *27*, 6173. (d) Evans, D. A.; Illig, C. R.; Saddler, J. C. *J. Am. Chem. Soc.* **1986**, *108*, 2478. (e) Evans, D. A.; Biller, S. A. *Tetrahedron Lett.* **1985**, *26*, 1907. (f) Danishefsky, S.; O'Neill, B. T.; Taniyama, E.; Vaughn, K., *Tetrahedron Lett.* **1984**, *25*, 4199. (g) Danishefsky, S.; O'Neill, B. T.; Springer, J. P. *Tetrahedron Lett.* **1984**, *25*, 4203.

(18) For syntheses of the structurally related saframycins, see: (a) Fukuyama, T., Sachleben, R. A. *J. Am. Chem. Soc.* **1982**, *104*, 4957. (b) Fukuyama, T.; Linton, S. D.; Tun, M. M. *Tetrahedron Lett.* **1990**, *31*, 5989. (c) Fukuyama, T.; Yang, L.; Ajeck, K. L.; Sachleben, R. A., *J. Am. Chem. Soc.* **1990**, *112*, 3712. (d) Kubo, A.; Saito, N.; Yamato, H.; Masubuchi, K.; Nakamura, M., *J. Org. Chem.* **1988**, *53*, 4295; (e) Saito, N.; Yamauchi, R.; Nishioka, H.; Ida, S.; Kubo, A., *J. Org. Chem.* **1989**, *54*, 5391.

Scheme 2



to quinocarcin was to be accomplished by, first, reducing the double bond, second, selectively converting the sarcosyl ester to the aldehyde oxidation state, and third, in a manner analogous to that employed in the syntheses of **10**–**14**, condense this aldehyde with the free amino alcohol forming the labile oxazolidine in the last step. It should be noted that related dipolar addition strategies were adopted by Garner[13] and Joule[16c,d] to construct the pyrrolidine ring systems of quinocarcin.

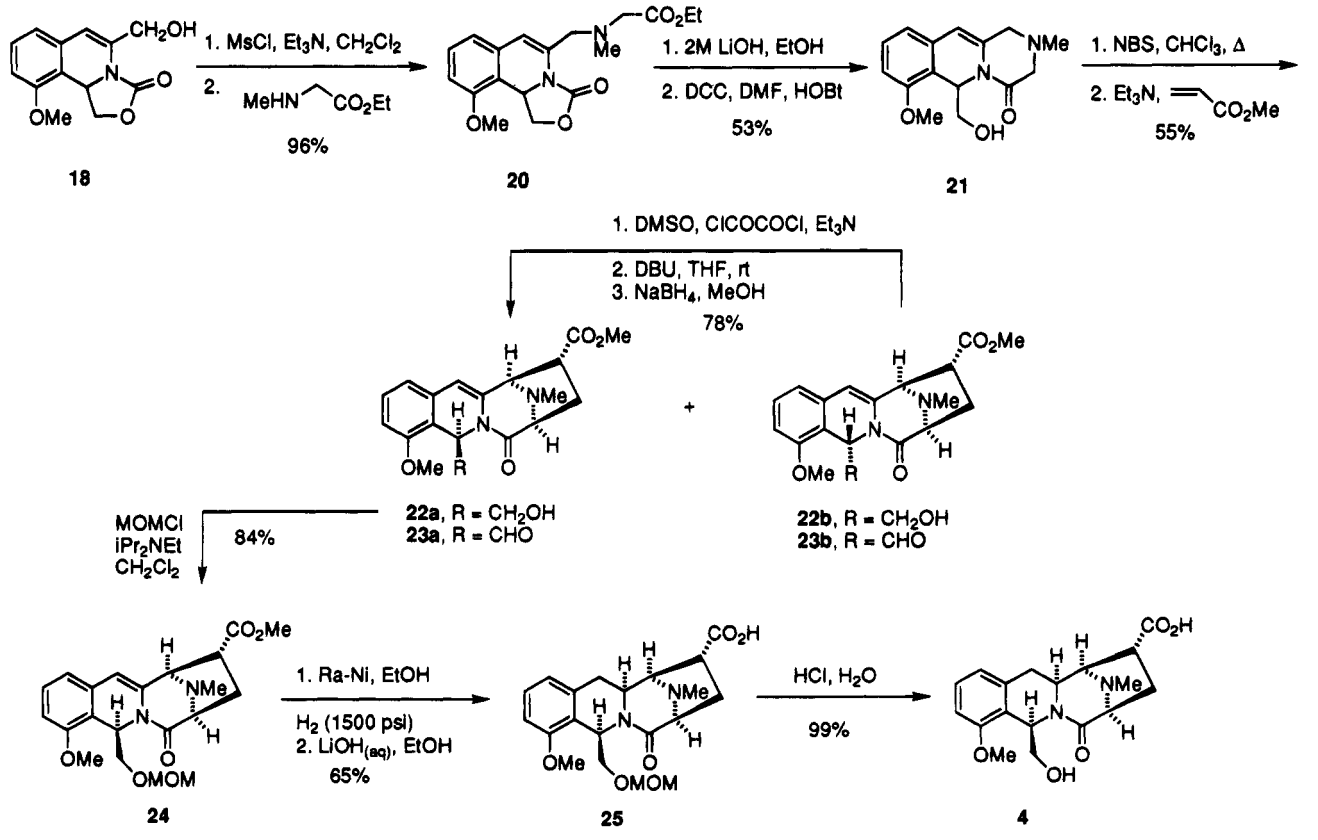
Applying this strategy (Scheme 1) to the quinocarcin system began with β-hydroxy ethyl ester **17** (prepared as a single diastereomer as previously described [14]) which was subsequently converted to the α,β-unsaturated aldehyde **16** in four chemical steps (Scheme 2).

Saponification of **17** with lithium hydroxide produced the corresponding β-hydroxy acid. This material was carried on without purification by reaction with 3 equiv of thionyl chloride in refluxing toluene which resulted in β-elimination and acid chloride formation. The intermediate α,β-unsaturated acid chloride was then reduced to allylic alcohol **18** with sodium borohydride in 42% isolated yield from **17**.

Swern oxidation of **18** afforded the α,β-unsaturated aldehyde **16** in 98% yield. The cycloaddition reaction was then carried out by reacting **16** with 5 equiv of sarcosine benzyl ester, 10 equiv of methyl acrylate, and 0.1 equiv of triethylamine. After the mixture was refluxed in THF for 24 h, a 1:1 mixture of pyrrolidine products (**19**) was isolated in 62% yield. By ^{1}H NMR homo-decoupling and NOE experiments,[20] both diastereomeric cycloadducts were determined to possess the regiochemistry and relative stereochemistry in the pyrrolidine rings illustrated in Scheme 2 which were epimeric about C-5. Consequently, only one of these compounds could possess the correct relative stereochemistry at C-11 (the non-epimerizable center) which meant that the incorrect stereochemistry was afforded at C-8 and C-10 for this substrate. Efforts to control the cycloaddition reaction by varying the ester substituents had only moderate effects on the ratio of products observed, and no new diastereomers were isolated.

In an effort to obtain the correct relative stereochemistry in the pyrrolidine ring we modified our approach by generating the dipole on a rigid, cyclic substrate. This was accomplished by first preparing tricyclic monoketopiperizine **21** (Scheme 3). From allylic alcohol **18**, reaction with methanesulfonyl chloride followed by addition of sarcosine ethyl ester afforded allylic amine **20** in 96% yield for the two steps. Saponification of the ethyl ester and deprotection of the oxazolidinone afforded the

Scheme 3

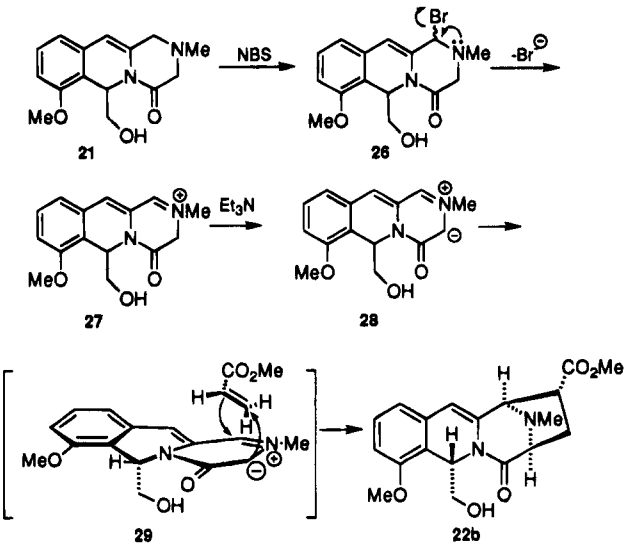


intermediate amino alcohol. This material was then carried on without purification by cyclization with DCC and *N*-hydroxybenzotriazole (HOBT) producing amide **21** in 53% isolated yield for the two steps.

A number of procedures were examined in an effort to oxidatively install a double bond in the C-ring, thereby generating the imminium precursor to the azomethine ylide. Attempts to generate the dipole via the N-oxide led to decomposition of the substrate.[21] Reaction of **21** with 1 equiv of NBS in refluxing chloroform, however, did result in formation of the dark green imminium salt. Addition of triethylamine at 0 °C to this solution resulted in a dark blue solution which, upon warming to room temperature in the presence of methyl acrylate, resulted in the formation of a 5:1 ratio of cycloadducts **22b** and **22a**, respectively (55% yield for the two steps). This ratio is presumably a manifestation of there being a preference for approach of the dipolarophile from the least hindered face. No other cycloadducts were isolated from the reaction mixture.

Compounds **22a** and **22b** turned out to be diastereomeric about C-5, and both diastereomers possessed the correct regiochemistry and *exo*-configuration from the cycloaddition. Since the desired product (**22a**) was the minor diastereomer from the cycloaddition, **22b** was subsequently efficiently epimerized to **22a** in the following manner. Amido alcohol **22b** was first subjected to Swern oxidation to give aldehyde **23b** in 94% yield. Next, **23b** was treated with 1 equiv of DBU in THF at room temperature for 3 h resulting in 80% conversion to aldehyde **23a**. The $NaBH_4$ reduction of **23a** to **22a** is a reaction that was first described in the total synthesis of quinocarcinol methyl ester by Danishefsky et al.,[10] which completed the conversion of **22b** to **22a** in 78% overall yield based on 20% recovery of **23b**. The alcohol moiety of compound **22a** was then protected by reaction with chloromethyl methyl ether and *N,N*-diisopropylethylamine affording **24** in 84% yield; this substance has been previously prepared by Garner and co-workers.[13] As previously described,[13] **24** was converted to the acid **25** by Raney-Ni (H_2, 1500 psi) reduction of the double bond followed by saponification of the methyl ester with lithium hydroxide in 65% overall yield from **24**. Finally, treatment of **25** with HCl in water resulted in essentially quantitative conversion to (±)-quinocarcinamide (**4**) which was identical by ^1H NMR and mobility on TLC to an authentic sample of quinocarcinamide obtained by the autoredox disproportionation of quinocarcin.[4] Compound **25** has been previously converted to quinocarcin[13] via partial reduction of the amide by dissolving lithium reduction and trapping the hemiaminal with cyanide to give DX-52-1 (**2**).[11,13] Quinocarcin (**1**) has been generated from DX-52-1 (**2**) under acidic or Lewis-acidic conditions.[3,11-13]

Scheme 4

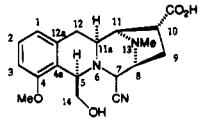


Discussion

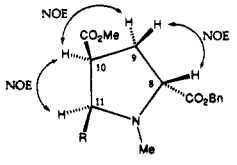
The use of NBS as an oxidant for the preparation of an azomethine ylide is to our knowledge an unrecognized usage of this reagent. However, it is possible that the ability of NBS to carry out this transformation may be peculiar to this type of substrate. Preliminary attempts to carry out the analogous cycloaddition reaction on the corresponding C-11a(12)-saturated substrate (DX-52-1 numbering[19]) have, to date, been unsuccessful. The implication of this finding is the possible requirement for the carbon undergoing bromination to be allylic. Thus, bromination presumably occurs on the allylic carbon initially forming bromide **26** which immediately suffers elimination of the bromide producing **27**. Aliquots of the reaction mixture after just 10 min revealed a new singlet in the ^1H NMR at 10.00 ppm indicative of the imminium methine. Also, the deep green color afforded is likely the result of extended conjugation upon formation of **27**.

The regiochemical outcome of the cycloaddition reaction is presumably a manifestation of structure **28** representing the major canonical form of the azomethine ylide. As in the previous cycloaddition reaction, this is the result of negative charge being effectively stabilized on the carbon α to the amide carbonyl. Due to the cyclic nature of **28**, the cycloadducts formed are forced to have the correct relative stereochemical configuration between C-8 and C-11. As compared to the cycloaddition reaction discussed above affording **19**, which is most probably reacting through the "W" configuration of the ylide, such relative stereochemical control ambiguities do not exist for **28**. The approach of the dipolarophile is presumably governed by the conformation of the ylide (structure **29**, Scheme 4) which positions the hydroxymethyl group in a pseudoaxial orientation effectively shielding the bottom face of the ylide and favoring formation of the observed major isomer **22b**.

(19) The numbering of cycloadducts are based on the numbering for DX-52-1 (**2**) shown below.



(20) Results of NOE experiments for cycloadducts **14a** and **14b**. The additional diastereomeric center at C-5 is contained within "R".



(21) Chastanet, J.; Roussi, G. *J. Org. Chem.* **1985**, *50*, 2910. Chastanet, J.; Roussi, G. *Heterocycles* **1985**, *23*, 653. Chastanet, J.; Fathallah, H.; Negron, G.; Roussi, G. *Heterocycles* **1992**, *34*, 1565.

The methodology described herein represents an efficient method to access the bicyclic framework of quinocarcin which should be applicable to the other members of this class of antitumor antibiotics. This approach is particularly attractive because the starting isoquinoline **18** can be prepared efficiently on a large scale and all of the subsequent steps described are applicable to preparative scale chemistry. Although an epimerization is necessary in the final stages of the synthesis, this three-step procedure is efficient and benefits from a thermodynamically favored epimerization reaction. Additional applications of the chemistry described herein are currently being explored in these laboratories for synthetic access to tetrazomine, the bioxalomycins, and mechanism-based structural analogs that will be used in further exploiting the unique chemical reactivity of these oxazolidine-containing antitumor antibiotics.

Experimental Section

^{1}H NMR and ^{13}C NMR data was collected using a Bruker AC-300 (300 MHz) spectrometer, and NOE data was collected on a Bruker (500 MHz) instrument.

5-(Hydroxymethyl)-10-methoxy-3-oxo-1,10b-dihydro-5*H*-oxazolo-2,3-*a*]isoquinoline (18). To a stirred solution of **17** (prepared as described in ref 14) (11 g, 0.036 mol) dissolved in ethanol (360 mL) was added 2 M LiOH (36 mL, aqueous). The resulting mixture was stirred at 25 °C for 2 h at which time the reaction mixture was acidified to pH 5 with 1 N HCl(aq), water (100 mL) was added and the mixture was extracted (3 × 200 mL) with dichloromethane. The dichloromethane layers were combined, dried over anhydrous Na_2SO_4, and concentrated to dryness in vacuo affording 9.4 g of the corresponding β-hydroxy acid. This material was then slurried in dry toluene (375 mL), and to this mixture was added thionyl chloride (12.8 g, 0.108 mol). The resulting mixture was heated to reflux with stirring for 3 h. The reaction mixture was then cooled to room temperature and concentrated under reduced pressure. Dichloromethane (250 mL) was added and the resulting solution cooled to −78 °C. To this mixture was then added, over a 10 min period, an ice bath-cooled slurry of sodium borohydride (6.8 g, 0.18 mol) in ethanol (125 mL). The resulting mixture was warmed to room temperature, stirred for 2 h, and then cooled to 0 °C and carefully quenched with 1 N HCl(aq). The resulting mixture was then extracted with dichloromethane (2 × 200 mL), and the organic extracts were combined, dried over anhydrous Na_2SO_4, and concentrated to dryness *in vacuo*. The crude material was purified by flash chromatography (silica; 3:1 ethyl acetate/hexanes) affording **18** (oil, 3.7 g, 42% yield from **17**).

^{1}H NMR (300 MHz) ($CDCl_3$) δ TMS: 3.81 (3 H, s), 3.98 (1H, br s), 4.39 (1 H, dd, J = 6.6 , 13.6 Hz), 4.50−4.61 (1 H, m), 4.54 (1 H, dd, J = 9.2, 11.5 Hz), 5.06 (1 H, dd, J = 7.9, 9.2 Hz), 5.28 (1 H, dd, J = 7.9, 11.5 Hz), 5.93 (1 H, s), 6.73 (1 H, d, J = 7.1 Hz), 6.78 (1 H, d, J = 8.3 Hz), 7.23 (1 H, t, J = 7.6 Hz). ^{13}C NMR (74.47 MHz) ($CDCl_3$) δ: 54.8 (d), 55.4 (q), 61.6 (t), 70.6 (t), 110.0 (d), 111.7 (d), 117.5 (s), 118.4 (d), 129.1 (s), 129.5 (d), 132.0 (s), 136.0 (s), 154.9 (s). IR (NaCl): 3450, 2938, 2841, 1756, 1645, 1602, 1576, 1477, 1446 cm^{-1}. Anal. Calcd for $C_{13}H_{13}NO_4$: C, 63.15; H, 5.30; N, 5.67. Found: C, 63.40; H, 5.24; N, 5.60.

3-[[[(Ethoxycaronyl)amino]methyl]-10-methoxy-3-oxo-1,10b-dihydro-5*H*-oxazolo[2,3-*a*]-isoquinoline (20). To a stirred solution of **18** (400 mg, 1.62 mmol) dissolved in CH_2Cl_2 (14 mL) at 0 °C was added methanesulfonyl chloride (556 mg, 4.85 mmol) followed by triethylamine (820 mg, 8.10 mmol). The resulting mixture was stirred at 0 °C for 30 min, and sarcosine ethyl ester hydrochloride (1.2 g, 8.10 mmol) (Aldrich) was added. The mixure was allowed to come to room temperature and stirred for 8 h. The reaction mixture was then concentrated under reduced pressure, redissolved in ethyl acetate (25 mL), passed through a short column of silica gel (eluted with ethyl acetate), and concentrated to dryness *in vacuo* affording **20** (oil, 588 mg, 96%).

^{1}H NMR (300 MHz) ($CDCl_3$) δ TMS: 1.26 (3 H, t, J = 7.3 Hz), 2.53 (3 H, s), 3.43 (1 H, 1/2 ABq, J = 16.9 Hz), 3.54 (1H, 1/2 ABq, J = 16.9 Hz), 3.59 (1H, 1/2 ABq, J = 14.9 Hz), 3.81 (3 H, s), 4.17 (2 H, q, J = 7.1 Hz), 4.33 (1 H, 1/ABq, J = 14.9 Hz), 4.49 (1 H, dd, J = 9.2 Hz, J = 10.2 Hz), 4.98 (1 H, dd, J = 8.5 Hz, J = 8.7 Hz), 5.28 (1 H, dd, J =8.2 Hz, J = 10.7 Hz), 6.02 (1 H, s), 6.74 (2 H, t, J = 8.4 Hz), 7.22 (1 H, t, J = 7.8 Hz). ^{13}CNMR (74.47 MHz) ($CDCl_3$) δ: 14.2 (q), 41.5 (q), 54.6 (d), 55.4 (q), 56.0 (t), 57.4 (t), 60.3 (t), 69.4 (t), 109.8 (d), 112.8 (d), 118.2 (d), 119.0 (s), 129.3 (d), 132.3 (s), 134.2 (s), 154.6 (s), 154.9 (s), 171.0 (s). IR (NaCl): 2925, 2853, 1759, 1645, 1576, 1476 cm^{-1}. Anal. Calcd for $C_{18}H_{22}N_2O_5$: C, 62.42; H, 6.40; N, 8.09. Found: C, 62.25; H, 6.60; N, 7.93.

2-Aza-6-(hydroxymethyl)-7-methoxy-2-methyl-4-oxo-1,3,4,6,11,11a-tetrahydro-2*H*-benzo[*b*]quinolizine (21). To a stirred solution of **20** (252 mg, 0.666 mmol) in ethanol (33 mL) was added 2 M LiOH (aqueous, 3.3 mL). The reaction mixture was degassed by argon purge and the resulting mixture stirred at room temperature for 8 h. The solution was then cooled to 0°C, and the pH was adjusted to 5 with 6 N HCl(aq) and concentrated to dryness *in vacuo*. The dry residue was then redissolved in DMF (10 mL) and cooled to 0 °C, and to this solution was added *N*-hydroxybenzatriazole (108 mg, 0.799 mmol) followed by DCC (165 mg, 0.700 mmol). The reaction was stirred at 0 °C for 1 h and then allowed to come to room temperature and stir for an additional 18 h. After the reaction mixture was concentrated under reduced pressure at 35 °C, the residue was redissolved in CH_2Cl_2 (50 mL), filtered through celite, washed with saturated $NaHCO_3$(aq), dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The crude material was then purified by flash chromatography (silica gel; 10:1 dichloromethane/methanol, 0.5% concd NH_4OH) affording pure **21** (oil, R_f = 0.53, 97 mg, 53% yield).

^{1}H NMR (300 MHz) ($CDCl_3$) δ TMS: 2.42 (3 H, s), 3.39 (1 H, 1/2 ABq, J = 13.6 Hz), 3.48 (2 H, s), 3.55 (1 H, 1/2 ABq, J = 13.6 Hz), 3.66−3.69 (2 H, m), 3.83 (3 H, s), 5.66 (1 H, s), 6.24 (1 H, dd, J = 4.6 Hz, J = 7.0 Hz), 6.65 (1 H, d, J = 7.6 Hz), 6.74 (1 H, d, J = 8.3 Hz), 7.18 (1 H, t, J = 8.1 Hz) (note: the exchangeable proton was not observable). ^{13}C NMR (300 MHz) ($CDCl_3$) δ: 43.8 (q), 50.1 (d), 55.4 (q), 55.4 (t), 59.1 (t), 64.1 (t), 106.3 (d), 109.3 (d), 116.6 (s), 117.4 (d), 128.9 (d), 131.6 (s), 131.8 (s), 155.2 (s), 166.6 (s). IR (NaCl): 3184, 2939, 1680, 1643, 1474 cm^{-1}; HRMS (FAB) calcd for $C_{15}H_{19}N_2O_3$ (MH^+) 275.1396, found 275.1386.

Methyl (5α,8β,10β,11β)-5,7,8,9,10,11-Hexahydro-4-methoxy-5-(hydroxymethyl)-13-methyl-7-oxo-8,11-iminoazepino[1,2-*b*]isoquinoline-10-carboxylate (22a) and Methyl (5α,8α,10α,11α)-5,7,8,9,10,11-Hexahydro-4-methoxy-5-(hydroxymethyl)-13-methyl-7-oxo-8,11-iminoazepino[1,2-*b*]-isoquinoline-10-carboxylate (22b). To a stirred solution of **21** (44 mg, 0.160 mmol) in $CHCl_3$ (11 mL) was added NBS (28 mg, 0.160 mmol) and the resulting solution brought to reflux for 1 h (dark green color formed). At this time, the reaction mixture was cooled to 0 °C, and to the mixture was added methyl acrylate (687 mg, 8.00 mmol) followed by addition of a solution of triethylamine (162 mg, 1.60 mmol) in $CHCl_3$ (5 mL) over a 15 min period (dark blue color formed). The reaction mixture was then allowed to come to room temperature and stir for 1 h. The reaction was then concentrated under reduced pressure, redissolved in CH_2Cl_2, washed with saturated $NaHCO_3$, dried over anhydrous Na_2SO_4, and concentrated to dryness *in vacuo*. The crude material was then purified by PLC (silica gel; 3:1 CH_2Cl_2/THF, 0.25% concd NH_4OH), affording **22a** (oil, 5 mg, R_f = 0.38) and **22b** (oil, 27 mg, R_f = 0.24) (55% combined yield).

22a. ^{1}H NMR (300 MHz) ($CDCl_3$) δ TMS: 1.79 (1 H, br s), 2.46 (3 H, s), 2.54 (1 H, dd, J = 9.81, 13.0 Hz), 2.61−2.69 (1 H, m), 3.33 (1 H, dd, J = 6.3, 9.8 Hz), 3.59−3.65 (1 H, m), 3.70 (1 H, d, J = 6.5 Hz), 3.76 (3 H, s), 3.84 (3 H, s), 4.04 (1 H, s), 5.70 (1 H, s), 6.18 (1 H, dd, J = 4.0, 8.1 Hz), 6.68 (1 H, d, J = 7.5 Hz), 6.75 (1 H, d, J = 7.9 Hz), 7.20 (1 H, t, J = 8.0 Hz). ^{13}C NMR (74.47 MHz) ($CDCl_3$) δ: 34.9, 35.6, 47.4, 48.9, 52.4, 55.4, 64.8, 65.3, 66.9, 105.5, 109.4, 116.5, 117.7, 129.1, 131.2, 134.9, 155.2, 170.1, 173.6. IR (NaCl): 3425, 2951, 2842, 1737, 1683,

1642, 1474 cm^{-1}. HRMS (FAB): calcd for $C_{19}H_{23}N_2O_5$ (MH^+): 359.1607, found 359.1602

22b. ^1H NMR (300 MHz) ($CDCl_3$) δ TMS: 2.10 (1 H, br s), 2.17 (1 H, dd, J = 9.9, 13.6 Hz), 2.62 (3 H, s), 2.63–2.70 (1 H, m), 2.88 (1 H, dd, J = 4.8, 10.0 Hz), 3.67–3.76 (1 H, m), 3.76 (3 H, s), 3.85 (3 H, s), 4.23 (1 H, s), 5.67 (1 H, s), 6.25 (1 H, dd, J = 4.1, 7.3 Hz), 6.67 (1 H, d, J = 7.6 Hz), 6.75 (1 H, d, J = 7.6 Hz), 7.21 (1 H, t, J = 8.0 Hz). ^{13}C NMR (74.47 MHz) ($CDCl_3$) δ: 32.11, 34.8, 50.1, 50.3, 52.5, 55.4, 64.5, 64.9, 65.7, 104.0, 109.2, 115.9, 117.2, 129.1, 132.3, 136.5, 155.3, 171.4, 173.3. IR (NaCl): 2950, 1734, 1684, 1636, 1474 cm^{-1}. Additional structural characterization was provided for this compound by the conversion into **22a**, described below.

Methyl (5α,8α,10α,11α)-5,7,8,9,10,11-Hexahydro-4-methoxy-5-formyl-13-methyl-7-oxo-8,11-iminoazepino[1,2-*b*]isoquinoline-10-carboxylate (23b). To a solution of DMSO (31 mg, 0.402 mmol) in CH_2Cl_2 (2.5 mL) and cooled to −78 °C was added oxalyl chloride (26 mg, 0.201 mmol) and the mixture stirred at −78 °C for 10 min. A solution of **22b** (36 mg, 0.100 mmol) in CH_2Cl_2 (1.0 mL) was then added and the resulting solution stirred at −78 °C for 1 h, at which time triethylamine (101 mg, 1.00 mmol) was added and the reaction mixture was allowed to warm to room temperature. The reaction was then concentrated under reduced pressure, redissolved in CH_2Cl_2 (20 mL), washed with water, dried over anhydrous Na_2SO_4, and concentrated to dryness *in vacuo* affording **23b** (oil, 34 mg, 94% yield). ^1H NMR (300 MHz) ($CDCl_3$) δ TMS: 2.22 (1 H, dd, J = 9.8, 13.5 Hz), 2.62–2.71 (1 H, m), 2.79 (3 H, s), 2.93 (1 H, dd, J = 5.0, 9.8 Hz), 3.74–3.80 (1 H, m), 3.76 (3 H, s), 3.90 (3H,s), 4.21 (1 H, s), 5.59 (1 H, s), 6.58 (1 H, s), 6.68 (1 H, d, J = 8.3 Hz), 6.80 (1 H, d, J = 7.8 Hz), 7.25 (1 H, t, J = 8.0 Hz), 9.35 (1 H, s). ^{13}C NMR (300 MHz) ($CDCl_3$) δ: 32.7, 35.0, 49.8, 52.5, 55.6, 58.1, 64.9, 65.3, 102.8, 109.1, 110.8, 117.9, 130.1, 132.1, 137.4, 155.5, 169.6, 173.2, 191.3. IR (NaCl): 2948, 2852, 1735, 1686, 1645, 1475 cm^{-1}. HRMS (FAB): calcd for $C_{19}H_{21}N_2O_5$ (MH^+) 357.1450, found 357.1451.

Epimerization of Amido Aldehyde 23b to 23a. Methyl (5α,8β,10β,11β)-5,7,8,9,10,11-Hexahydro-4-methoxy-5-formyl-13-methyl-7-oxo-8,11-iminoazepino[1,2-*b*]isoquinoline-10-carboxylate, 23a. To a stirred solution of **23b** (30 mg, 0.084 mmol) in dry THF (10 mL) was added DBU (13 mg, 0.084 mmol) and the resulting mixture stirred at room temperature for 3 h. The reaction was then dilluted with CH_2Cl_2 (25 mL), washed with saturated $NaHCO_3$(aq), dried over anhydrous Na_2SO_4, and concentrated to dryness *in vacuo*. The crude product was then purified by PTLC (silica gel; 3:1 EtOAc/hexanes) affording **23a** (oil, 22 mg, R_f = 0.41) and **23b** (oil, 6 mg, R_f = 0.50).

23a. ^1H NMR (300 MHz) ($CDCl_3$) δ TMS: 2.50 (3 H, s), 2.64–2.71 (2 H, m), 3.54 (1 H, dd, J = 6.4, 9.5 Hz), 3.73 (1 H, d, J = 6.4 Hz), 3.76 (3 H, s), 3.92 (3 H, s), 4.07 (1 H, s), 5.61 (1 H, s), 6.57 (1 H, s), 6.70 (1 H, d, J = 7.5 Hz), 6.82 (1 H, d, J = 8.3 Hz), 7.27 (1 H, t, J = 8.7 Hz), 9.29 (1 H, s). ^{13}C NMR (74.47 MHz) ($CDCl_3$) δ: 34.9, 35.6, 47.8, 52.5, 55.6, 56.5, 65.2, 66.5, 104.1, 109.3, 111.2, 118.2, 130.1, 136.0, 155.5, 168.5, 173.8, 192.1. IR (NaCl): 2951, 2842, 1734, 1687, 1647, 1475 cm^{-1}. HRMS (FAB): calcd for $C_{19}H_{21}N_2O_5$ (MH^+) 357.1450, found 357.1465.

Reduction of 23a to Amido Alcohol 22a. To a stirred solution of **22a** (14 mg, 0.039 mmol) in anhydrous MeOH (3 mL) and cooled to 0 °C was added $NaBH_4$ (6 mg, 0.157 mmol) (Baker) and the resulting mixture stirred at 0 °C for 1 h. The reaction mixture was then carfully quenched by addition of 1 N HCl(aq) until bubbling subsided. This solution was then concentrated under reduced pressure and the residue redissolved in CH_2Cl_2 (10 mL), washed with saturated $NaHCO_3$(aq), dried over anhydrous Na_2SO_4, and concentrated to dryness *in vacuo*, affording **22a** (13 mg, 94% yield). Physical data for **22a** reported above.

Methyl (5α,8β,10β,11β)-5,7,8,9,10,11-Hexahydro-4-methoxy-5-[(methoxymethoxy)methyl]-13-methyl-7-oxo-8,11-iminoazepino[1,2-*b*]isoquinoline-10-carboxylate (24). To a stirred solution of **22a** (6 mg, 0.017 mmol) dissolved in CH_2Cl_2 (2 mL) and cooled to 0 °C was added of *N,N*-diisopropylethylamine (7 mg, 0.051 mmol) followed by chloromethyl methyl ether (3 mg, 0.043 mmol). The resulting mixture was stirred at 0 °C for 1 h at which time the reaction mixture was allowed to warm to room temperature with continued stirring for an additional 16 h. The reaction mixture was then concentrated under reduced pressure and purified by PTLC (silica gel; 3:1 EtOAc/hexanes), affording **24** (oil, 6 mg, R_f = 0.30, 84% yield). ^1H NMR (300 MHz) ($CDCl_3$) δ TMS: 2.43–2.51 (1 H, m), 2.46 (3 H, s), 2.60–2.66 (1 H, m), 3.22 (3 H, s), 3.28 (1 H, dd, J = 9.9, 6.1 Hz), 3.57–3.60 (2 H, m), 3.68 (1 H, d, J = 6.4 Hz), 3.77 (3 H, s), 3.84 (3 H, s), 4.03 (1 H, s), 4.44 (1 H, 1/2 ABq, J = 6.5 Hz), 4.64 (1 H, 1/2 ABq, J = 6.5 Hz), 5.70 (1 H, s), 6.22 (1 H, dd, J = 4.6, 6.6 Hz), 6.66 (1 H, d, J = 7.6 Hz), 6.73 (1 H, d, J = 8.3 Hz), 7.18 (1 H, t, J = 8.0 Hz). ^{13}C NMR (300 MHz) ($CDCl_3$) δ: 34.6, 35.7, 46.0, 47.4, 52.5, 55.2, 55.4, 65.5, 66.9, 67.1, 96.1, 105.7, 109.2, 117.2, 117.5, 128.8, 131.5, 134.9, 155.2, 168.8, 173.7. IR (NaCl): 2949, 1738, 1686, 1546, 1474 cm^{-1}. HRMS (FAB): calcd for $C_{21}H_{27}N_2O_6$ (MH^+) 403.1869, found 403.1872.

Methyl (5α,8β,10β,11β,11aβ)-5,7,9,10,11,11a,12-Octahydro-4-methoxy-5-[(methyoxymethoxy)methyl]-13-methyl-7-oxo-8,11-iminoazepino[1,2-*b*]isoquinoline-10-carboxylate. This procedure has been described previously by Garner et al.[13] A high pressure hydrogenation bomb was charged with compound **24** (21 mg, 0.052 mmol) dissolved in absolute EtOH (4 mL) followed by a slurry of washed W2 Raney-Ni (200 μL, Aldrich). The mixture was then subjected to high pressure hydrogenolysis (H_2, 1500 psi) for 20 h at 65 °C. After cooling, the reaction mixture was then filtered through Celite and concentrated under reduced pressure. The crude mixture was then separated by PTLC (silica gel; 3:1 EtOAc/hexanes), affording the pure methyl ester of compound **25** [13] (oil, 10 mg, R_f = 0.22, 45% yield).

^1H NMR (300 MHz) ($CDCl_3$) δ TMS: 2.29 (1 H, dd, J = 9.6, 13.1 Hz), 2.49 (3 H, s), 2.53 (1 H, dd, J = 2.3, 14.2 Hz), 2.57–2.65 (1 H, m), 2.96 (3 H, s), 3.14 (1 H, t, J = 13.5 Hz), 3.36 (1 H, dd, J = 6.8, 9.6 Hz), 3.53–3.57 (2 H, m), 3.65 (1 H, d, J = 2.6 Hz), 3.76 (3 H, s), 3.82 (3 H, s), 4.23 (1 H, dd, J = 3.0, 9.9 Hz), 4.29 (1 H, 1/2 ABq, J = 6.3 Hz), 4.42 (1 H, 1/2 ABq, J = 6.3 Hz), 5.61 (1 H, t, J = 2.4 Hz), 6.77 (2 H, t, J = 9.1 Hz), 7.18 (1 H, t, J = 7.9 Hz). ^{13}C NMR (74.47 MHz) ($CDCl_3$) δ: 32.1, 34.4, 37.0, 41.3, 49.4, 52.4, 54.3, 54.7, 55.3, 66.4, 67.1, 68.0, 96.3, 108.7, 119.5, 122.6, 127.8, 138.2, 155.7, 171.0, 174.9. IR (NaCl): 2949, 1735, 1655, 1474, 1265 cm^{-1}. HRMS (FAB): calcd for $C_{21}H_{29}N_2O_6$ (MH^+) 405.2026, found 405.2027.

(5α,8β,10β,11β,11aβ)-5,7,9,10,11,11a,12-Octahydro-4-methoxy-5-[(methyoxymethoxy)methyl]-13-methyl-7-oxo-8,11-iminoazepino[1,2-*b*]isoquinoline-10-carboxylic Acid (25). To a stirred solution of the methyl ester obtained above (10 mg, 0.025 mmol) dissolved in EtOH (0.5 mL) was added LiOH (0.8 mg,0.037 mmol) in water (20 μL). The resulting mixture was stirred at room temperature for 1.5 h at which time the solution was diluted with water (5 mL), acidified to pH 5 with 1 N HCl(aq) and the mixture extracted 3 × 10 mL with dichloromethane. The extracts were combined, dried over anhydrous Na_2SO_4, and concentrated to dryness *in vacuo*, affording 10 mg of crude product. An aliquot was purified by PTLC (silica; 8:1 chloroform/methanol) producing a pure sample of **25** (oil, R_f = 0.30). ^1H NMR (300 MHz) ($CDCl_3$) δ TMS: 2.28 (1 H, t, J = 9.8 Hz), 2.45–2.53 (2 H, m), 2.54 (3 H, s), 2.92 (3 H, s), 3.11–3.20 (1 H, m), 3.25 (1 H, dd, J = 4.8, 9.6 Hz), 3.49–3.57 (2 H, m), 3.60 (1 H, br s), 3.81 (3 H, s), 3.84 (1 H, d, J = 9.0 Hz), 4.17 (1 H, dd, J = 2.8, 10.0 Hz), 4.26 (1 H, 1/2 ABq, J = 6.3 Hz), 4.36 (1 H, 1/2 ABq, J = 6.3 Hz), 5.60 (1 H, br s), 6.67 (1 H, d, J = 7.5 Hz), 6.75 (1 H, d, J = 8.3 Hz), 7.13 (1 H, t, J = 7.9 Hz) (note: the exchangeable proton was not observable). ^{13}C NMR (74.47 MHz) (CDCl3) δ: 35.2, 49.4, 54.8, 55.3, 67.8, 96.3, 108.8, 119.5, 122.5, 128.1, 138.2, 155.7, 176.3. IR (NaCl): 3448, 2944, 1652, 1591, 1474, 1460, 1265 cm^{-1}. HRMS (FAB): calcd for $C_{20}H_{27}N_2O_6$ (MH^+): 391.1869, found 391.1877.

(±)-Quinocarcinamide (4). Compound **25** (6 mg) was dissolved in H_2O (1.0 mL) and treated with 3 drops of 1 N HCl at room temperature for 8 h. The mixture was concentrated under reduced pressure to give 5.5 mg (quant) of the HCl salt (amorphous powder). Dissolution of this substance in D_2O with the addition of ca. 1 mg of sodium carbonate

revealed by ^{1}H NMR analysis that compound **25** was quantitatively converted into (±)-quinocarcinamide (**4**). The synthetic material matched an authentic sample prepared by the autoredox disproportionation of natural quinocarcin as previously described [4] by ^{1}H NMR and TLC behavior.

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Supporting Information Available: ^{1}H NMR spectra of all new compounds and comparison spectra of synthetic and natural quinocarcinamide; experimental preparation for compounds **16**, **19** and sarcosine benzyl ester are also included (14 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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