Pergamon

Bioorganic & Medicinal Chemistry 8 (2000) 523–532

BIOORGANIC & MEDICINAL CHEMISTRY

Synthesis of a Netropsin Conjugate of a Water-Soluble *epi*-Quinocarcin Analogue: the Importance of Stereochemistry at Nitrogen

Brad Herberich, Jack D. Scott and Robert M. Williams*

Department of Chemistry, Colorado State University, Fort Collins, CO 80523, USA

Received 9 August 1999; accepted 15 October 1999

Abstract—The efficient synthesis of a water-soluble C11a-*epi*-analogue (**6b**) of quinocarcin is described. This substance, and a netropsin amide conjugate (**8**) lack the capacity to inflict oxidative damage on DNA due to the stereoelectronic geometry of their oxazolidine nitrogen atoms. The capacity of these substances to alkylate DNA through the generation of an iminium species has been examined. Both compounds were found to be unreactive as DNA alkylating agents. The results of this study are discussed in the context of previous proposals on the mode of action of this family of antitumor alkaloids.

Introduction

Quinocarcin,[1–3] tetrazomine[4,5] and bioxalomycin[6–8] are cytotoxic oxazolidine-containing natural products obtained from various *Actinomycetes* sp. These substances have been shown to display potent antitumor activity in vitro and in vivo.[9–15] We have previously shown that the fused oxazolidine ring of these naturally occurring substances undergoes a spontaneous redox disproportionation reaction that, in the presence of molecular oxygen, results in the net reduction of dioxygen to superoxide radical anion culminating in strand scission of DNA.[16–19] During our studies on the mechanism of this novel auto redox disproportionation reaction, we postulated an unusual stereoelectronic requirement of the non-bonded electron pair on the oxazolidine nitrogen atom to adopt a *trans*- antiperiplanar geometry with respect to the adjacent oxazolidine methine hydrogen atom (**A**, Fig. 1) as an obligate structural parameter for inflicting oxidative damage on DNA and other cellular macromolecules (Fig. 1).[20,21] Experimental support for the stereoelectronic control of this reaction has been obtained and reported in the literature from this laboratory[17–21] through the synthesis of the C11a-*epi*-analogue **6a** which lacked the capacity to produce superoxide or cleave DNA. In contrast, the naturally configured analogues **5a**, **5b** and **7** retained the capacity to reduce molecular oxygen to superoxide and mediated oxidative cleavage of DNA. An X-ray structural determination on compound **5a** confirmed the geometry of the oxazolidine ring as that depicted in Figure 1.[20,21]

In 1988, Remers and co-workers reported the results of computer simulations on the covalent and non-covalent binding of quinocarcin to DNA and postulated that the nitrogen atom of the oxazolidine ring would have to undergo pyramidal inversion via a boat/twist conformational change to adopt a *trans*-antiperiplanar relationship to the oxazolidine C–O bond for ring-opening to the putative iminium species **9**, which was invoked as the electrophilic, DNA-alkylating reactive intermediate leading to the proposed adduct **10** (Fig. 2).[22] This conformational change (**1a**→**1b**) was calculated to require ~10 kcal/mol and suggested that iminium ion formation should occur readily via conformer **1b**. To further probe the stereoelectronic requirements for ring-opening of a fused oxazolidine to a putative DNA-alkylating iminium species, we have designed the C11a-*epi*-series **6** (quinocarcin numbering, Fig. 1) which disposes the non-bonded electron pair of the oxazolidine nitrogen atom *trans*-antiperiplanar to the C7–O bond.

The stereochemistry of the C11a-*epi*-system was previously secured through an X-ray structural determination

*Corresponding author. Tel.: +1–970–491–5610; fax: +1–970–491–5610; e-mail: rmw@chem.colostate.edu

0968-0896/00/$ - see front matter
PII: S0968-0896(99)00314-4

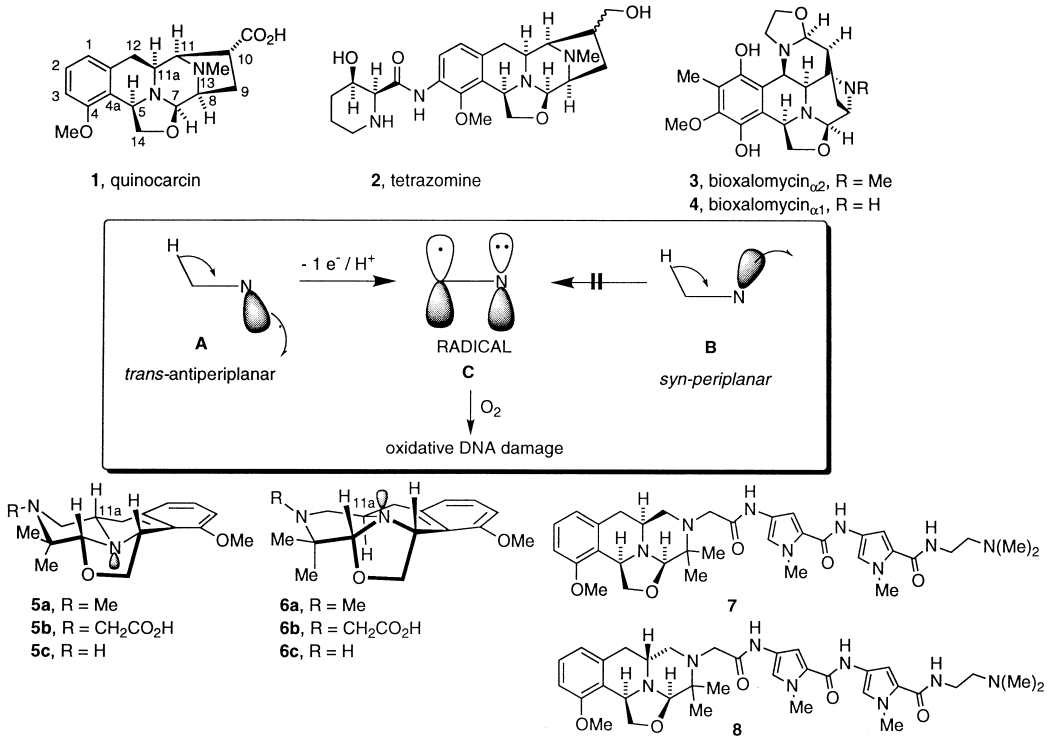


Figure 1.

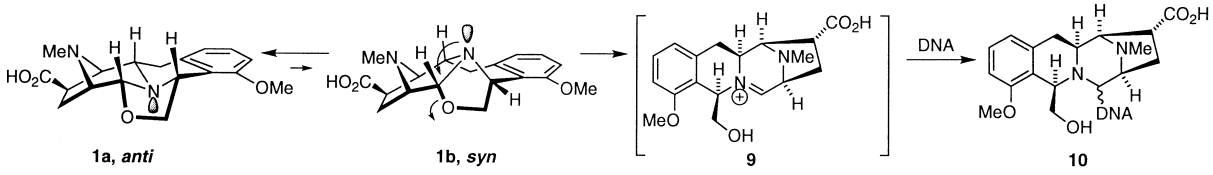


Figure 2.

on compound **6a**.[21] As discussed above, this stereoelectronic arrangement in the oxazolidine ring also renders such compounds very stable to the auto-redox disproportionation manifested in the natural series. Thus, the expectation was that the C11a-*epi*-series (**6**), should readily form an iminium ion and exhibit DNA alkylation reactivity. As discussed previously for compounds **5a** and **6a**, limited water solubility of these substances precluded an in-depth study of their interactions with DNA. The synthesis of the water-soluble analogues in the naturally configured series **5b** and **7** have been previously reported and their biochemical reactivity discussed.[19] The purpose of the present study, was to examine the stereochemically complementary analogues **6b** and **8**; the synthesis and reactivity of these substances are reported herein.

Results and Discussion

The retrosynthetic plan for the synthesis of **6b** was initially envisioned to follow a similar route to that employed in the construction of the related quinocarcin analogues **5b** and **7** which proceeded through alkylation of the secondary amine of tetracycle **5c**.[19] However, in the event, we were unable to effect the cyclization of the amino alcohol-aldehyde precursor to **6c** in synthetically useful yields and had to deploy a somewhat different strategy. Previous experience with this type of ring-closure dictated that *N*-alkylation (at N13, quinocarcin numbering) to a tertiary amine gave improved yields in the key oxazolidine-forming cyclization reaction. The *anti*-saturated acid **13** was envisioned to be readily available from the previously reported β-hydroxy ester **11**[20] via elimination to **12**, saturation of the olefin and epimerization to the kinetically favored *anti*-stereochemistry. Reduction of the acid of compound **13** to the corresponding alcohol **14**, secondary amine formation **16** and alkylation with *t*-butylbromoacetate would furnish the tertiary amino alcohol **17**. Oxidation of the alcohol to the corresponding aldehyde **18** followed by the crucial concomitant oxazolidinone hydrolysis and oxazolidine ring closure was anticipated to provide the key tetracyclic acid **6b**. Homologation of the acid of **6b** to the corresponding netropsin amide **8** using our previously established protocol[19] involving the in situ reductive acylation with **19** would complete the synthesis.

As shown in Scheme 1, conversion of **11** to the unsaturated ester **12** proceeded in excellent yield through treatment of **11** with oxalyl chloride. The incipient labile β-chloro ester was immediately treated with triethylamine at reflux temperature to provide **12**. Catalytic hydrogenation of **12** provides the corresponding saturated ethylester as a ~1:1 mixture of *syn*- : *anti*-diastereomers. Without separation this mixture was subjected to saponification with lithium hydroxide in ethanol, epimerization of the *syn*-ester to the *anti*-ester and concomitant hydrolysis of the ester to the acid ensues to yield *anti*-**13** in 72% overall yield from **12**. Next, treatment of acid **13** with oxalyl chloride provides the acid chloride which was immediately reduced with sodium borohydride to give the desired *anti*-alcohol **14** in excellent yield. Mesylation of **14** and alkylation with 2-amino-2-methyl-1-propanol (**15**) provided the desired secondary amine **16** in 74% yield for the two steps.[23]

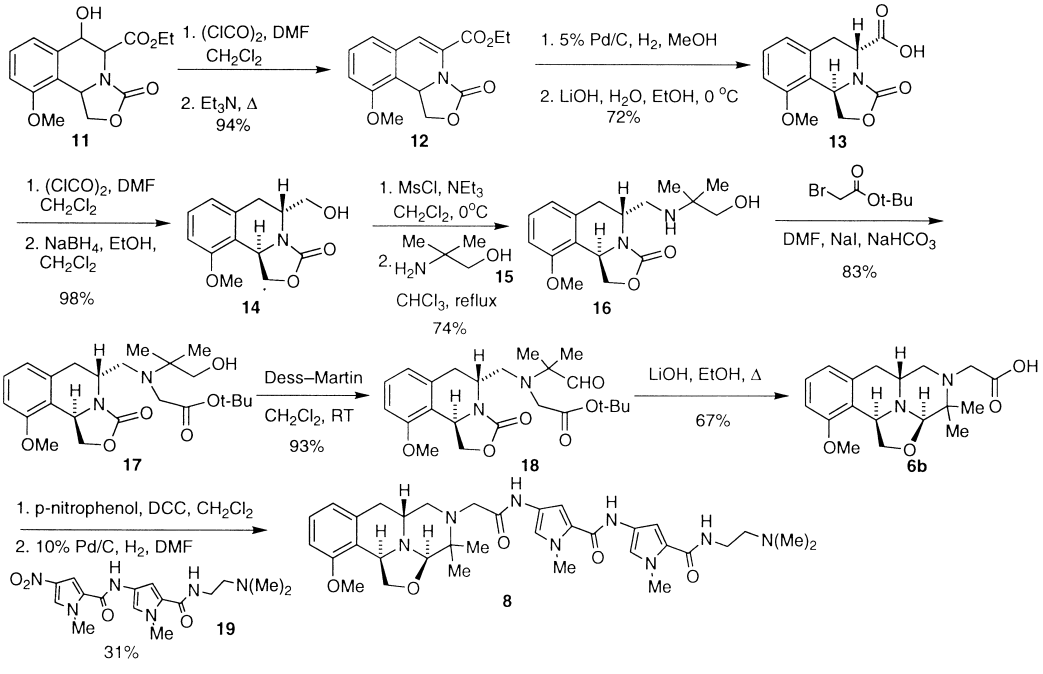
As mentioned above, we examined the conversion of **16** to **6c** by Dess–Martin oxidation to the aldehyde (57%) followed by attempted ring closure under basic conditions as previously described for **5c**.[19] However, in this case, only decomposition attended many attempts to effect the closure to **6c**. Thus, alkylation of **16** with *t*-butylbromoacetate provided the tertiary amine **17** in 83% yield without any δ-lactone formation which, we observed to occur with less hindered bromoacetate derivatives. Oxidation of the primary alcohol of **17** with Dess–Martin periodinane gave the key aldehyde **18** in 93% yield. We were very pleased to see that treatment of **18** with lithium hydroxide in hot ethanol effected the cleavage of the oxazolidinone, closure of the putative amino alcohol to the desired oxazolidine and hydrolysis of the *t*-butyl ester to the acid **6b** in 67% overall yield. It is worth mentioning that this transformation represents one of the highest yielding ring closure reactions to a fused oxazolidine ring system thus far reported in this family of alkaloids.

The final conversion of **6b** to **8** proceeded smoothly and was accomplished by transforming the acid of **6b** to the corresponding active *p*-nitrophenyl ester. Reduction of the known nitro compound **19**[24] to the corresponding primary amine and direct acylation with the *p*-nitrophenyl ester provided **8** in 31% overall yield from **6b**.

Evaluation of 6b and 8 for superoxide production

The capacity of **6b** to produce superoxide was measured by following the reduction of nitroblue tetrazolium (NBT). In the reduction process NBT is reduced to monoformazan which can be detected spectrophotometrically in the visible range. We have previously shown that this reduction is completely inhibited by the addition of superoxide dismutase (SOD).[17–19]

The assay was carried out as described previously in measuring the superoxide production of quinocarcin and analogues **5a**, **5b** and **7**.[19] Compound **6b** was added to an aerated solution of NBT (0.12 mM) in a 20 mM phosphate buffer (pH 7) such that the final concentration of **6b** was 2.0 mM. The optical absorbance was recorded over 15 min and the change in optical density was the average slope for the linear change in optical density over the reaction time. The rates of superoxide production were calculated from the molar extinction coefficient of formazan at 500 nm (12,200) and by assuming the reaction is pseudo first order in O_2. As expected based on the stereoelectronic parameters previously established via **6a**, nitroblue tetrazolium in the presence of **6b** was not reduced, demonstrating the inability of **6b** to produce superoxide. By comparison,



Scheme 1.

under the same conditions the all *syn*–analogue **5b** had a rate of superoxide production of 0.82×10^{-9} M s^{-1},[19] while a 1.0 mM solution of quinocarcin exhibits a rate of 4.2×10^{-9} M s^{-1} and a 0.1 mM solution of bioxalomycin α_2 demonstrates a rate of 3.88×10^{-8} M s^{-1}.

Evaluation of DNA cleavage and alkylation reactions

To assay the DNA alkylating abilities of **6b** and **8**, band shift assays were conducted using ^{32}P-5′-end-labeled deoxyoligonucleotides. In these experiments, solutions of the analogues and 5′-^{32}P-labeled DNA were incubated at 37 °C for 12 h. The DNA was then precipitated with ethanol and dried. The samples were suspended in water and loading dye, and loaded onto a denaturing or non-denaturing electrophoresis gel. After the gels were run, they were stored with photographic film at −80 °C for 12 h. If alkylation of the DNA occurred, the mobility of the alkylated DNA should be retarded due to the increased mass.

In the event, when **6b** was incubated under a wide range of concentrations (5 μM∼5 mM) with a number of DNA templates (templates A–C, Fig. 3) at different concentrations (0.1–10 mM), no evidence for DNA alkylation was observed. As expected from the lack of capacity of either **6b** or **8** to produce superoxide, neither substance effected the oxidative cleavage of DNA.

The netropsin conjugate **8** was incubated (at high concentrations (2.0–4.0 mM)) with DNA templates D, E and F (0.85–2.45 mM), which contained netropsin binding sequences (Fig. 4).[19] Despite many alterations of the reaction parameters, we did not detect any evidence for DNA alkylation with this substance.

While we expected compounds **6b** and **8** to be incapable of causing oxidative damage to DNA based on the stereoelectronic grounds detailed above, we were somewhat surprised at the lack of reactivity of these substances toward DNA alkylation. The stereoelectronic configuration of the non-bonded electron pair on the oxazolidine nitrogen atom in **6b** and **8** is disposed *trans*-antiperiplanar to the C–O bond of the oxazolidine ring and, in accordance with the modeling predictions of Remers,[22] should facilitate ring-opening to an iminium species **20** which in principle, should be capable of forming the DNA alkylation adduct **22** (Scheme 2).

We examined an alternative, indirect probe for the capacity of this system to undergo ring-opening. Quinocarcin, when treated with NaCN under basic conditions, gives the corresponding α–aminonitrile derivative DX–52–1, which displays antitumor activity.[25–29] However,

```
Template A- 5'-TTTATTAACGTAATGCTTAATCGCAATGGGATT-3'
            3'-AAATAATTGCATTACGAATTAGCGTTACCCTAA-5'

Template B- 5'-CCCACATCACTATACACGCGCGAAATTTCTGCGTAT-3'
            3'-GGGTGTAGTGATATGAGCGCGCTTTAAAGACGCATA-5'

Template C- 5'-ATACCCACATCACGCGCGTATACCAAATTTCTCATTACT-3'
            3'-TATGGGTGTAGTGCGCGCATATGGTTTAAAGAGTAATGA-5'
```

Figure 3. DNA templates incubated with **6b**.

```
Template D- 5'-ACAGCATGTCCTACGAATTCGTAGGACATGCTGT-3'
            3'-TGTCGTACAGGATGCTTAACGATCCTGTACGACA-5'

TemplateE- 5'-GGAATTGGGCAATTGCCCAATTCC-3'
            3'-CCTTAACCCGTTAACGGGTTAAGG-5'

Template F- 5'-GGGCCCAAATTTGGGCCC-3'
            3'-CCCGGGTTTAAACCCGGG-5'
```

Figure 4. DNA templates incubated with **8**. The netropsin binding regions are in bold face.

stirring **6b** with NaCN under basic conditions[30] gave mostly recovered starting material with no detectable production of the corresponding α-aminonitrile **21**. Conversely, the exact same reaction conditions when applied to **5b** cleanly afforded the α-aminonitrile **23** (Scheme 3).[31]

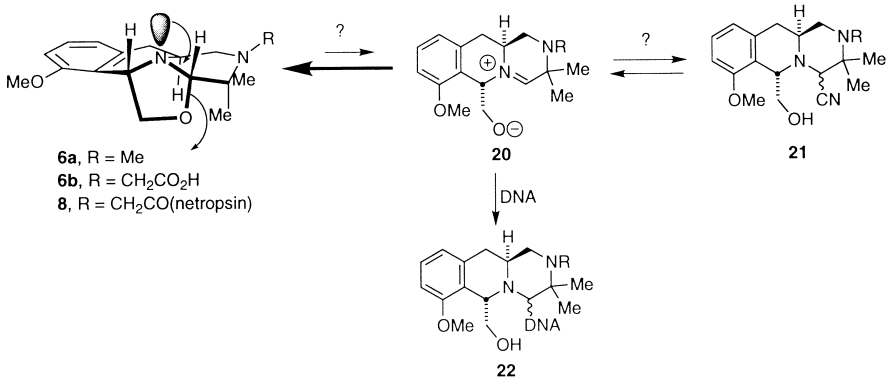
The difference in reactivity between **5b** and **6b** towards the ring-opening/cyanation reaction raises interesting questions concerning the intrinsic, relative stability and facility of ring-opening of the oxazolidine rings of these two substances. Based on stereoelectronic considerations, it is possible that the oxazolidine ring of **6b** and **8** might be destabilized by an $n\rightarrow\sigma^*$ (anomeric effect) interaction and rendered more reactive to ring-opening to the corresponding iminium species. However, comparison of the C–O bond lengths for compounds **5a** and **6a** as revealed from their respective X-ray structures, shows that both compounds have C–O bond lengths of 1.424 and 1.427Å, respectively.[20,21] An anomeric effect would typically lengthen the C–O bond which is not observed in the solid state for **6a** relative to **5a** to any significant extent.[32]

Due to the lack of reactivity of **6b** and **8**, one final derivative of **6b** was synthesized to probe a previously proposed mechanism of DNA alkylation. The reductive activation of naphthyridinomycin was proposed by Zmijewski et al., to involve formation of an incipient *ortho*-quinone methide species with participation of the phenolic residue to yield an iminium ion upon re-closure.[33,34] A similar mechanism depicted for analogues **6b** or **8** is shown in Scheme 4. In this mechanism the phenol of **24** would be deprotonated, resulting in opening of the oxazolidine ring and formation of imine **25**. The non-bonded pair of electrons on the nitrogen of the imine could then add to the electrophilic quinone methide to restore aromaticity and form iminium ion **26**, which is the proposed DNA alkylating agent.

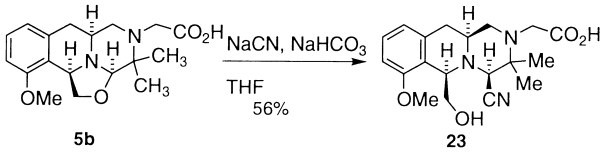
The demethylation of **6b** to produce phenol **24** was readily achieved using BBr_3 in 47% yield. To assay for DNA alkylation, phenol **24** and DNA template **A** were incubated at 37 °C in a phosphate buffer (pH 8) for 12 h and the samples were evaluated as described earlier using gel electrophoresis. Again, substance **24** demonstrated no evidence for DNA alkylation by the gel shift assay.

Conclusions

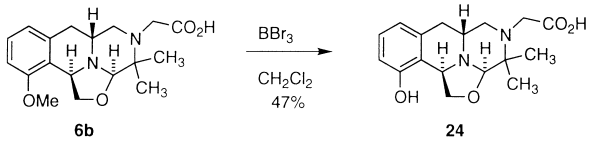
The results of this study underscore the subtle yet significant changes in chemical and biochemical reactivity



Scheme 2.



Scheme 3.



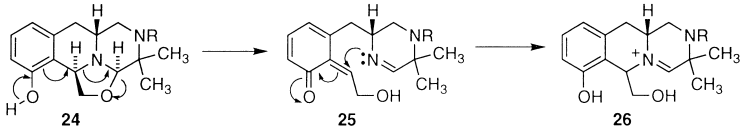
Scheme 5.

rendered by the stereoelectronic geometry of the nitrogen atom in the simple fused oxazolidine ring of this family of alkaloids. The lack of DNA alkylation products with analogues that possess the *epi*-quinocarcin stereochemistry (**6b**, **8** and **24**) may be due to steric congestion around C-7 caused by the *gem*-dimethyl groups or may be a manifestation of the intrinsic instability of the alkylated product. The proximity of the reaction center (C-7) to a *gem*-dimethyl group may make the alkylation difficult due to steric interactions. On the other hand, the bioxalomycins, which are sterically congested around C-7, both alkylate and covalently cross-link DNA.[35]

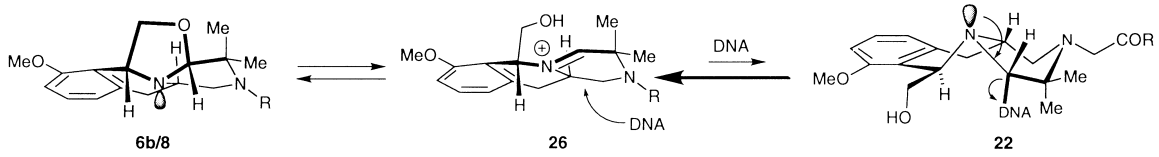
Another explanation for the absence of DNA alkylation and cyanation by the *epi*-quinocarcin analogues is illustrated in Scheme 6. If **6b**/**8** is alkylated via the ring-opened iminium species **26** from the more accessible α-face by DNA, the developing non-bonded electron pair on the nitrogen atom in the resulting product (**22**) would be expected to initially adopt an *anti*-periplanar geometry with respect to the DNA base residue due to repulsion in the transition state. Although this configuration at nitrogen (*cis*-decalin-type) is expected to be slightly less stable than the alternative configuration at nitrogen (*trans*-decalin-type),[36] if this is the geometry initially formed at nitrogen, the DNA residue can be easily expelled to give back iminium **26** and then **6b**/**8**. The relative instability of such adducts would also explain the failure to prepare α-aminonitrile product **21** from **6b**.

A vector analysis on the X-ray crystal structure[21] of compound **6a** was carried out and this analysis revealed that the dihedral angle between the C–O bond of the oxazolidine and the non-bonded electron pair on the adjacent nitrogen atom in the oxazolidine ring is approximately 162° (or ~18° off normal; Fig. 5). While this angle is not an optimal 180° for a perfectly aligned *trans*-antiperiplanar disposition of the non-bonded electron pair on the nitrogen atom and the scissile C–O bond, this relatively small deviation from normal would not, a priori, be expected to preclude ring-opening of the oxazolidine. It can be assumed that the geometries of compounds **6b** and **6c** closely mimic that for **6a** which have the same stereochemistry surrounding the oxazolidine ring. Thus, although this analysis supports the notion that the oxazolidine ring of **6a**~**c** should readily suffer ring-opening to an iminium species such as **26**, the initially formed intermolecular adducts such as **21** and **22** may rapidly revert back to **6**.

As discussed above, trapping of **5b** with sodium cyanide gave the α-aminonitrile adduct **23** and the relative configuration of this substance has been assigned by ^{1}H NMR/NOE studies. Although the pyramidal configuration at nitrogen in this substance has not been assigned, it appears very reasonable that the nitrile group in this substance is in an equatorial orientation as shown in Scheme 7 and that the bridgehead nitrogen adopts a *trans*-decalin-type of geometry. If the incipient iminium species (**28**) is attacked from the β-face, *anti*- to the hydroxymethyl group, the initially formed



Scheme 4.



Scheme 6.

α-aminonitrile **29** would place the nitrile group in an axial orientation and *trans*-antiperiplanar to the bridgehead nitrogen atom and would be expected to be readily expelled regenerating **28**. The thermodynamic product **23**, would therefore result from cyanide attack *syn*- to the hydroxymethyl group from the α-face, (which is *anti*- to the methine hydrogen at C-11a) but produces the stable adduct **23**. Applying this analysis to iminium species **26**, the alternative β-face attack of cyanide is more hindered than the complementary α-face attack on **28** (in other words, *syn*- to the hydroxymethyl group) since, both the hydroxymethyl group and the C-11a methine in **26** are situated in an axial orientation rendering this a more sterically demanding approach. In addition, the β-face adduct, once readjusted to the more stable *trans*-decalin-type of conformation would situate the nitrile group axial and *trans*-antiperiplanar to the non-bonded electron pair on the bridgehead nitrogen atom and thus labile to expulsion.

The stereoelectronic geometry of the oxazolidine ring in the *natural* series may therefore be obligate for *both* the redox disproportionation manifold culminating in oxidative DNA strand scission *and* for DNA alkylation reactions. Efforts to further clarify and define these parameters are under study in these laboratories.[37]

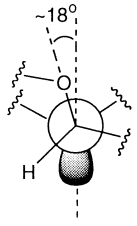
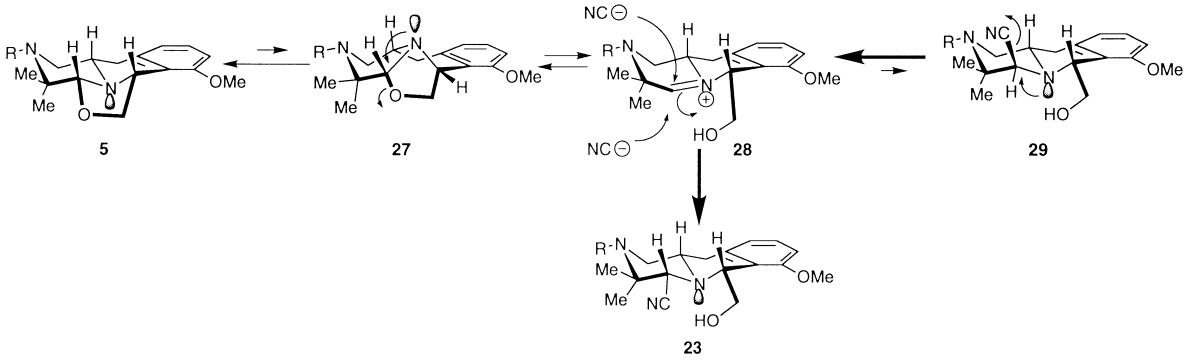


Figure 5. Dihedral angle between the non-bonded electron pair on the oxazolidine nitrogen atom and the adjacent scissile C–O bond of compound **6a** as revealed from a vector analysis of the X-ray structure.[21]

Experimental

General experimental methods

All drug concentrations were made up to 54 mM in water immediately prior to use. Deoxyoligonucleotides ('oligos') were synthesized on the Applied Biosystems 380B DNA synthesizer using standard phosphoramidite chemistry (reagents and phosphoramidites were purchased from GLEN Research). Deoxyoligonucleotides were deprotected by heating 15 h at 55 °C in NH_4OH, followed by filtering of the CPG resin and concentration of supernatant in vacuo. All oligos were purified by 20% denaturing polyacrylamide gel electrophoresis (DPAGE). Oligos of interest were 5′-end-labeled with [γ-^{32}P]ATP and T4 polynucleotide kinase (New England Biolabs). Labeled oligos were then hybridized to their corresponding blunt-ended complements in 20 mM phosphate buffer (pH 7 and 8) by heating the equimolar mixture of oligos to 80 °C for 15 min, and cooling to room temperature over 2 h. Gel-loading buffer contained 0.03% bromophenol blue and 0.03% xylene cyanole in formamide. Centrex MF 0.45 μM cellulose acetate spin filters were obtained from Schleicher & Schuell. Samples were counted on a Packard 1500 Tri–Carb liquid scintillation analyzer. Unless otherwise noted, materials were obtained from commercially available sources and used without further purification. Diethyl ether and THF were distilled from sodium benzophenone ketyl under a nitrogen atmosphere. Methylene chloride and triethylamine were distilled under a nitrogen atmosphere from calcium hydride. Dimethyl formamide was dried over 4 Å molecular sieves. The molecular sieves were activated by heating at 150 °C at 1 mm Hg for 3 h in a vacuum oven. Chromatographic separations were performed with EM Science TLC plates (silica gel 60, F254, 20×20 cm×250 μm) or with EM Science 230–400 mesh silica gel under positive air pressure. Reactions and chromatographic fractions were



Scheme 7.

monitored and analyzed with EM Science TLC plates. Visualization on TLC were achieved with ultraviolet light and heating of TLC plates submerged in a 5% solution of phosphomolybdic acid in 95% ethanol (PMA) or 2,4-dinitrophenylhydrazine in 2 M HCl (DNP) or *p*-anisaldehyde in 95% ethanol or Dragendorff solution. Melting points were determined in open capillary tubes with a Mel–Temp apparatus and are uncorrected. Infared spectra were recorded on a Perkin–Elmer 1600 series FTIR as thin films from methylene chloride and are reported as λ_{max} in wavenumbers (cm^{-1}). Elemental analyses are accurate to within 0.4% of the calculated values. Mass spectra were obtained on a 1992 Fisions VG Autospec at the Chemistry Department at Colorado State University. Nuclear magnetic resonance (NMR) spectra were acquired using a Bruker AC–300, Varian 300 or 400 spectrometer. NMR chemical shifts are given in parts per million (ppm) relative to internal $CHCl_3$, DMSO, or methanol. Proton (^1H) NMR are tabulated in the following order: multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; and m, multiplet), coupling constant in hertz, and number of protons. When appropriate, the multiplicity of a signal is denoted as 'br' to indicate that the signal was broad. All compounds without elemental analysis data were determined to have purity of at least 95% by ^1H and ^{13}C NMR.

Reductions of nitroblue tetrazolium (NBT)

Each reaction was performed in triplicate by adding an appropriate amount of drug stock solution to an aerated solution of nitroblue tetrazolium (0.12 mM) in 20 mM phosphate buffer (at pH 7) containing 1% Triton X100 detergent and the final volumes brought to 750 μL with deionized water. The optical absorbance was measured at 25 °C over a 15 min period at 500 nm (Varian DMS 80 UV–vis spectrophotometer) and the ΔOD was the average slope for the linear OD change over the reaction time. The rates for superoxide production were calculated by assuming that $[O_2]$ does not appreciably change over this time period and is in excess (zero order in oxygen). The rates were calculated from the ΔOD measurements and based on a molar extinction coefficient (ϵ_o) of 12,200 for the monoformazan product of NBT at 500 nm.

1,10b-Dihydro-10-methoxy-3*H*-oxazolo[4,3-α]isoquinolin-3-one-5-carboxylic acid ethyl ester (12). DMF (1.06 mL, 13.6 mmol) followed by oxalyl chloride (1.33 mL, 20.5 mmol) was added to a solution of **11** (4.19 g, 13.6 mmol) in 50 mL of CH_2Cl_2 at room temperature. After 30 min the solvent was stripped off and the residue dissolved in NEt_3 (30 mL) and heated at reflux for 2 h. After cooling to room temperature the reaction was diluted with Et_2O, washed with water, and dried over $MgSO_4$ to give 3.70 g (94%) of **12**. Mp 117–119 °C (recryst. Et_2O). TLC (1:1 EtOAc:hexane) R_f 0.26 (UV and PMA). ^1H NMR (300 MHz) ($CDCl_3$) δ 1.32 (t, $J=7.2$ Hz, 3H), 3.80 (s, 3H), 4.32 (dq, $J=2.5$, 7.2 Hz, 2H), 4.59 (dd, $J=8.4$, 9.3 Hz, 1H), 5.01 (dd, $J=8.7$, 9.3 Hz, 1H), 5.29 (dd, $J=8.4$, 8.7 Hz, 1H), 6.86 (dd, $J=7.8$, 8.7 Hz, 2H), 6.90 (s, 1H), 7.25 (dd, $J=7.4$, 8.7 Hz, 1H); ^{13}C NMR (75 MHz) ($CDCl_3$) δ 14.2, 53.5, 55.7, 61.8, 70.7, 112.6, 120.6, 122.1, 123.0, 127.5, 129.7, 130.3, 155.3, 155.6, 162.6. IR (NaCl, neat) 2981, 2360, 2343, 1767, 1724, 1635, 1575, 1476, 1405, 1306, 1263, 1213, 1092 cm^{-1}. Anal. calcd for $C_{15}H_{15}NO_5$: C, 62.28; H, 5.23; N, 4.84. Found: C, 62.18; H, 5.40; N, 4.89.

***trans*-1,5,6,10b-Tetrahydro-10-methoxy-3*H*-oxazolo[4,3-α]isoquinolin-3-one-5-carboxylic acid (13).** Ester **12** (5.65 g, 19.5 mmol) was dissolved in 325 mL of EtOH and 5% Pd/C (10.4 g, 4.9 mmol) was added. Hydrogen was bubbled through the solution for 5 min and the reaction was allowed to stir under an atmosphere of H_2 overnight. Argon gas was bubbled through the reaction and the mixture was filtered through a pad of Celite to give the saturated ethyl ester as a mixture of diastereomers (4.46 g, 78%). To a stirred solution of the ethyl ester (2.25 g, 8.12 mmol) in 100 mL of EtOH and 40 mL of H_2O at 0 °C was added $LiOH–H_2O$ (511 mg, 12.2 mmol). After 3 h at 0 °C the volume of the reaction was reduced by half and acidified with 1 M HCl. The reaction was extracted with ethyl acetate, dried over $NaSO_4$ and concentrated to afford acid **13** (92% yield). The acid was purified for analytical sample by recrystallization. Mp 230–232 °C (recryst. EtOAc–EtOH). ^1H NMR (300 MHz) (DMSO-d_6) δ 3.14 (d, $J=4.1$ Hz, 2H), 3.78 (s, 3H), 3.97 (t, $J=8.7$ Hz, 1H), 4.76 (dd, $J=4.1$, 4.6 Hz, 1H), 4.93 (t, $J=8.6$ Hz, 1H), 5.10 (t, $J=8.8$ Hz, 1H), 6.83 (d, $J=7.7$ Hz, 1H), 6.99 (d, $J=8.2$ Hz, 1H), 7.23 (dd, $J=8.1$, 7.9 Hz, 1H), 13.20 (bs, 1H); ^{13}C NMR (75 MHz) (DMSO-d_6) δ 29.6, 49.5, 50.1, 55.6, 69.5, 108.9, 121.3, 121.5, 128.4, 132.4, 155.7, 156.3, 171.3. Anal. calcd for $C_{13}H_{13}NO_5$: C, 59.31; H, 4.98; N, 5.32. Found: C, 59.81; H, 5.12; N, 5.31.

***trans*-1,5,6,10b-Tetrahydro-5-hydroxymethyl-10-methoxy-3*H*-oxazolo[4,3-α]isoquinolin-3-one (14).** To a solution of acid **13** (3.31 g, 12.57 mmol) in 200 mL of CH_2Cl_2 was added oxalyl chloride (1.40 mL, 22.00 mmol) and a drop of DMF. After 2 h the solvent was stripped off. The acid chloride was dissolved in 150 mL of CH_2Cl_2 and cooled to −78 °C. A slurry of $NaBH_4$ (2.38 g, 62.85 mmol) in 75 mL of EtOH was made, cooled to 0 °C, and added to the reaction. The cooling bath was removed and the reaction was allowed to warm up to room temperature. After 2 h the reaction was quenched by careful addition of 1 M HCl at 0 °C. The reaction was washed with CH_2Cl_2 and the organic phase dried over $MgSO_4$ and concentrated. The crude was purified with column chromatography (SiO_2, 3:1 EtOAc:hexane) giving 3.10 g (98%) of **14** as yellow plates. Mp 108–110 °C (recryst. EtOAc). TLC (3:1 EtOAc:hexane) R_f 0.18 (UV). ^1H NMR (300 MHz) ($CDCl_3$) δ 2.24 (t, $J=6.1$ Hz, 1H, D_2O exch.), 2.69 (d, $J=16.9$ Hz, 1H), 3.13 (dd, $J=7.0$ Hz, 16.8 Hz, 1H), 3.59–3.64 (m, 2H), 3.78 (s, 3H), 4.08 (t, $J=8.3$ Hz, 1H), 4.36 (dd, $J=7.0$, 14.1 Hz, 1H), 4.92 (dd, $J=8.5$, 8.7 Hz, 1H), 5.02 (dt, $J=8.3$, 8.5 Hz, 1H), 6.73 (dd, $J=6.0$, 8.0 Hz, 2H), 7.20 (t, $J=8.0$ Hz, 1H); ^{13}C NMR (75 MHz) ($CDCl_3$) δ 28.8, 49.5, 50.0, 55.5, 61.7, 70.2, 108.3, 122.2, 128.7, 133.4, 156.0, 158.4, 169.6. IR (NaCl, neat) 3421, 2942, 1737, 1586, 1473, 1257, 1094 cm^{-1}. Anal. calcd for $C_{13}H_{17}NO_4$: C, 62.64; H, 6.07; N, 5.62. Found: C, 62.49; H, 5.94; N, 5.66.

***trans*-1,5,6,10b-Tetrahydro-5-[(2-hydroxy-1,1-dimethylethyl)amino]methyl-10-methoxy-3*H*-oxazolo[4,3-α]-isoquinolin-3-one (16).** Methanesulfonyl chloride (116 μL, 1.5 mmol) and NEt_3 (348 μL, 2.5 mmol) were added to a solution of alcohol **14** (250 mg, 1 mmol) in 2 mL of CH_2Cl_2 at 0 °C. After 2 h the reaction was run through a short plug of silica with 10:1 CH_2Cl_2:MeOH as the eluent. The mesylate was redissolved in 3 mL of $CHCl_3$ and 2-amino-2-methyl-1-propanol (**15**) (0.95 mL, 10 mmol) was added. The reaction was allowed to reflux for 2 days. It was diluted with CH_2Cl_2, washed with $NaHCO_3$ (satd), dried over $MgSO_4$ and concentrated. The crude product was purified by column chromatography (SiO_2, 10:1 CH_2Cl_2:MeOH) affording **11** (236 mg, 70%) as slightly-yellow plates. TLC (10:1 CH_2Cl_2:MeOH) R_f 0.28 (UV and Dragendorff). Mp 111–115 °C (recryst. MeOH). ^{1}H NMR (300 MHz) ($CDCl_3$) δ 0.94 (s, 3H), 0.97 (s, 3H), 2.50 (br s, 1H), 2.51 (dd, J = 5.3, 11.7 Hz, 1H), 2.56–2.67 (m, 2H), 3.11 (dd, J = 6.8, 16.7 Hz, 1H), 3.24 (dd, J = 10.8, 17.9 Hz, 2H), 3.39 (s, 1H, D_2O exch.), 3.76 (s, 3H), 4.02 (t, J = 5.7 Hz, 1H), 4.22–4.29 (m, 1H), 4.87–4.97 (m, 2H), 6.69 (d, J = 8.0 Hz, 2H), 7.15 (t, J = 8.0 Hz, 1H); ^{13}C NMR (75 MHz) ($CDCl_3$) δ 23.6, 23.7, 30.8, 42.3, 49.0, 49.0, 53.8, 55.4, 69.1, 70.0, 108.1, 122.0, 122.1, 128.6, 133.4, 155.8, 158.3. IR (NaCl, neat) 3441, 2964, 1748, 1586, 1472, 1258, 1094 cm^{-1}. Anal. calcd for $C_{17}H_{24}N_2O_4$: C, 63.73; H, 7.55; N, 8.74. Found: C, 63.53; H, 7.46; N, 8.66.

***trans*-1,5,6,10b-Tetrahydro-5-[(2-hydroxy-1,1-dimethylethyl)aceticacid]amino]methyl-10-methoxyI-3*H*-oxazolo-[4,3-α]isoquinolin-3-one-*tert* butyl ester (17).** To a solution of **16** (183 mg, 0.54 mmol) in 4 mL of DMF was added $NaHCO_3$ (454 mg, 5.4 mmol), NaI (809 mg, 5.4 mmol) and *t*-butyl bromoacetate (0.88 mL, 5.4 mmol) at room temperature. After 12 h the reaction was diluted with CH_2Cl_2 and washed with water. The layers were separated and the organic layer dried over $MgSO_4$. Column chromatography (3:1 EtOAc:hexane) gave **17** (195 mg, 83%) as a yellow solid. TLC (3:1 EtOAc:hexane) R_f 0.50 (UV and Dragendorff). Mp 152–154 °C (recryst. EtOAc). ^{1}H NMR (300 MHz) ($CDCl_3$) δ 0.91 (s, 3H), 0.92 (s, 3H), 1.46 (s, 9H), 2.61 (dd, J = 6.9, 13.0 Hz, 1H), 2.76 (dd, J = 8.5, 13.0 Hz, 1H), 2.99 (d, J = 3.5 Hz, 2H), 3.05 (1/2 ABq, J = 11.6 Hz, 1H), 3.20 (1/2 ABq, J = 11.6 Hz, 1H), 3.27 (1/2 ABq, J = 18.1 Hz, 1H), 3.45 (1/2 ABq, J = 18.1 Hz, 1H), 3.73 (br s, 1H, D_2O exch.), 3.80 (s, 3H), 4.04 (dd, J = 8.0, 9.0 Hz, 1H), 4.22 (m, 1H), 4.88 (dd, J = 8.0, 9.0 Hz, 1H), 4.98 (dd, J = 8.0, 9.0 Hz, 1H), 6.72 (d, J = 8.0 Hz, 1H), 6.78 (d, J = 8.0 Hz, 1H), 7.19 (t, J = 8.0 Hz, 1H); ^{13}C NMR (75 MHz) ($CDCl_3$) δ 21.1, 23.1, 28.2, 29.4, 47.5, 49.5, 49.6, 51.7, 55.5, 59.0, 69.1, 70.1, 82.0, 108.2, 122.0, 122.7, 128.8, 133.6, 155.9, 157.9, 173.7. IR (NaCl, neat) 3466, 2976, 1755, 1587, 1473, 1258, 1152 cm^{-1}. Anal. calcd for $C_{23}H_{34}N_2O_6$: C, 63.58; H, 7.93; N, 6.45. Found: C, 63.71; H, 7.93; N, 6.22.

***trans*-1,5,6,10b-Tetrahydro-5-[(2-formyl-1,1-dimethylethyl)aceticacid]amino]methyl-10-methoxy-3*H*-oxazolo-[4,3-α]isoquinolin-3-one *tert*-butyl ester (18).** Dess–Martin periodinane (48 mg, 0.11 mmol) was added to **17** (32 mg, 0.076 mmol) in 1 mL of CH_2Cl_2. The reaction stirred for 30 min at room temperature after which a solution of 1 mL of $NaHCO_3$ (1 M) with sodium thiosulfate (273 mg) dissolved in it was added. The quenched reaction was allowed to stir for 30 min and was extracted with CH_2Cl_2. The organic phase was dried over $MgSO_4$ and concentrated to yield **18** (30 mg, 90%) as a yellow oil. TLC (3:1 EtOAc:hexane) R_f 0.65 (UV and Dragendorff). ^{1}H NMR (300 MHz) ($CDCl_3$) δ 1.03 (s, 3H), 1.04 (s, 3H), 1.45 (s, 9H), 2.61 (d, J = 7.5 Hz, 2H), 3.05 (m, 2H), 3.37 (1/2 ABq, J = 17.7 Hz, 1H), 3.44 (1/2 ABq, J = 18.0 Hz, 1H), 3.78 (s, 3H), 4.01 (t, J = 12.0 Hz, 1H), 4.33 (dd, J = 7.0, 16.0 Hz, 1H), 4.87 (dd, J = 9.0, 12.0 Hz, 2H), 6.71 (d, J = 7.0 Hz, 1H), 6.75 (d, J = 8.0 Hz, 1H), 7.18 (t, J = 8.0 Hz, 1H), 9.26 (s, 1H); ^{13}C NMR (75 MHz) ($CDCl_3$) δ 19.0, 20.3, 28.2, 29.3, 46.9, 49.5, 50.9, 51.1, 55.5, 66.4, 70.2, 81.5, 108.4, 121.8, 122.4, 129.0, 132.9, 156.0, 158.0, 171.9, 203.7. IR (NaCl, neat) 2977, 1755, 1587, 1473, 1393, 1368, 1258, 1155, 1096 cm^{-1}. HRMS (FAB) calcd. for $C_{23}H_{32}N_2O_6$ (M + H) 433.2339; found 433.2345.

4α,6α,11aβ-2-Aza-2-carboxyacetyl-1,3,4,6,11,11a-hexahydro-7-methoxy-5,4-oxazolo-3,3-dimethyl-2*H*-benzo[*b*]-quinolizine (6b). To a solution of **18** (120 mg, 0.27 mmol) in 17 mL of EtOH was added 1.66 mL of 2 M LiOH. This solution was degassed by bubbling through a stream of $N_{2(g)}$ and heated at reflux for 24 h. After cooling to room temperature, the reaction was concentrated and chromatographed (10:1 CH_2Cl_2:MeOH) to give 60 mg (67%) of **6b** as a yellow foam. TLC (10:1 CH_2Cl_2:MeOH) R_f 0.20 (UV and Dragendorff). ^{1}H NMR (300 MHz) (MeOH-d_4) δ 1.55 (s, 3H), 1.59 (s, 3H), 2.73 (m, 2H), 3.16 (m, 1H), 3.46 (m, 2H), 3.50 (1/2 ABq, J = 16.0 Hz, 1H), 3.62 (dd, J = 8.0, 9.0 Hz, 1H), 3.82 (s, 3H), 3.91 (1/2 ABq, J = 16.0 Hz, 1H), 4.26 (dd, J = 8.0, 9.0 Hz, 1H), 4.47 (s, 1H), 4.54 (t, J = 9.0 Hz, 1H), 6.78 (d, J = 8.0 Hz, 1H), 6.83 (d, J = 8.0 Hz, 1H), 7.19 (t, J = 8.0 Hz, 1H); ^{13}C NMR (75 MHz) (MeOH-d_4) δ 170.3, 158.2, 135.5, 129.2, 123.9, 121.9, 109.3, 96.5, 69.6, 62.5, 60.7, 56.1, 54.3, 53.8, 32.9, 22.5, 20.2. IR (NaCl, neat) 3381, 2953, 1651, 1472, 1383, 1340, 1261 cm^{-1}. HRMS (FAB) calcd for $C_{18}H_{25}N_2O_4$ (M + H) 333.1814; found 333.1816.

Netropsin conjugate (8). Preparation of 4α,6α,11aβ-2-aza-3,3-dimethyl-1,3,4,6,11,11a-hexahydro-2-(4-nitrophenoxyacetyl)-7methoxy-5,4-oxazolo-2*H*-enzo[*b*]quinolizine. To a stirred mixture of **6b** (12.0 mg, 0.036 mmol) and *p*-nitrophenol (5.5 mg, 0.040 mmol) in 2 mL of CH_2Cl_2 at 0 °C was added 1,3-dicyclohexylcarbodiimide (8.2 mg, 0.040 mmol). The reaction stirred at 0 °C for 1 h, and was then warmed to room temperature and stirred for an additional 15 h. The reaction mixture was then concentrated under reduced pressure and purified by PTLC (10:1 CH_2Cl_2:MeOH) to afford 8.5 mg (52%) of the corresponding *p*-nitrophenylester derivative of **6b** as a yellow oil. TLC (10:1 CH_2Cl_2:MeOH) R_f 0.76 (UV and Dragendorff). ^{1}H NMR (300 MHz) ($CDCl_3$) δ 1.28 (s, 3H), 1.40 (s, 3H), 2.57 (dd, J = 2.7, 15.6 Hz, 1H), 2.71 (dd, J = 11.1, 15.6 Hz, 1H), 2.89–2.92 (m, 2H), 3.01–3.08 (m, 1H), 3.51 (1/2 ABq, J = 17.1 Hz, 1H), 3.60 (dd, J = 7.8, 8.7 Hz, 1H), 3.78 (s, 3H), 3.84 (1/2 ABq, J = 17.1 Hz, 1H), 4.15–4.20 (m, 2H), 4.44 (t, J = 8.7 Hz, 1H), 6.67 (d, J = 8.1 Hz, 1H), 6.72 (d, J = 7.5 Hz, 1H), 7.12

(dd, $J=7.5$, 8.1 Hz, 1H), 7.28 (d, $J=9.0$ Hz, 2H), 8.25 (d, $J=9.0$ Hz, 2H); ^{13}C NMR (75 MHz) ($CDCl_3$) δ 169.5, 156.7, 155.2, 145.3, 135.4, 127.3, 125.2, 123.5, 122.3, 120.7, 107.4, 97.6, 67.7, 59.4, 55.3, 55.2, 51.9, 51.7, 48.5, 32.8, 25.1, 19.3. IR (NaCl, neat) 2936, 1781, 1590, 1523, 1472, 1346, 1261, 1207, 1105, 1012, 914, 864 cm^{-1}. To a stirred solution of **19** (10.2 mg, 0.028 mmol) in 0.5 mL of DMF and degassed with argon was added 5% Pd/C (6 mg, 0.0028 mmol) and the resulting mixture saturated with hydrogen. The mixture was then stirred at room temperature under 1 atm of hydrogen for 24 h. The reaction mixture was then filtered through Celite into a solution of the *p*-nitrophenyl ester obtained above (8.5 mg, 0.029 mmol) dissolved in 0.5 mL of DMF. To the reaction was added NEt_3 (12 μL, 0.087 mmol) and the resulting solution stirred at room temperature for 7 h. The reaction mixture was then concentrated and the crude product purified by PTLC (3% concd NH_4OH in methanol) to give 5.6 mg (31%) of netropsin conjugate **8** as a yellow oil. TLC (3% NH_4OH in MeOH) R_f 0.59 (UV and Dragendorff). ^{1}H NMR (300 MHz) ($CDCl_3$) δ 1.25 (s, 3H), 1.30 (s, 3H), 2.23 (s, 6H), 2.44 (t, $J=5.7$ Hz, 2H), 2.52–2.61 (m, 2H), 2.67–2.72 (m, 1H), 2.81 (t, $J=11.3$ Hz, 1H), 2.89 (d, $J=17.4$ Hz, 1H), 2.98–3.05 (m, 1H), 3.40 (q, $J=5.7$ Hz, 2H), 3.52 (d, $J=17.4$ Hz, 1H), 3.66 (dd, $J=7.8$, 8.4 Hz, 1H), 3.80 (s, 3H), 3.88 (s, 3H), 3.90 (s, 3H), 4.20 (s, 1H), 4.24 (dd, $J=7.8$, 8.4 Hz, 1H), 4.48 (t, $J=8.7$ Hz, 1H), 6.43 (m, 1H), 6.51 (d, $J=1.8$ Hz, 1H), 6.59 (d, $J=1.8$ Hz, 1H), 6.71 (dd, $J=7.8$, 8.4 Hz, 2H), 7.09 (d, $J=1.8$ Hz, 1H), 7.10 (d, $J=1.8$ Hz, 1H), 7.14 (dd, $J=7.8$, 8.4 Hz, 1H), 7.38 (br s, 1H), 9.34 (br s, 1H); ^{13}C NMR (75 MHz) ($CDCl_3$) δ 169.1, 161.7, 158.9, 156.7, 135.3, 127.4, 123.6, 123.1, 121.2, 121.1, 120.7, 118.9, 118.6, 107.6, 103.3, 103.2, 97.4, 77.2, 68.0, 59.4, 57.9, 55.4, 55.0, 53.5, 52.7, 49.8, 45.1, 36.6, 36.5, 32.8, 24.8, 18.8. IR (NaCl, neat) 3293, 2935, 2845, 2775, 1654, 2586, 1541, 1467, 1437, 1402, 1261, 1203, 1163, 1063 cm^{-1}. HRMS (FAB) calcd for $C_{34}H_{47}N_8O_5$ (M + H) 647.3669; found 647.3677.

6α,11aβ-2-Aza-2-carboxyacetyl-1,3,4,6,11,11a-hexahydro-7-methoxy-4-cyano-3,3-dimethyl-5-hyroxyl-2*H*-benzo[*b*]quinolizine (23). A solution of **5b** (6 mg, 0.018 mmol) in 0.45 mL of 1:2 THF:$NaHCO_3$ (satd) was adjusted to pH 7 using 1 M HCl. The solution was treated with 0.5 M NaCN (0.036 mmol) and stirred for 18 h. The pH of the solution was adjusted to 6–7 using 1 M HCl and the solution was extracted with 1:1 EtOAc:THF. The organic phase was dried over Na_2SO_4 and concentrated. The crude product was purified by column chromatography (SiO_2, 100:1 EtOAc:AcOH) affording **23** (3.7mg, 56%) as a slightly-yellow foam. TLC (9:1 CH_2Cl_2:MeOH) R_f 0.36 (UV and Dragendorff). ^{1}H NMR (300 MHz) ($CDCl_3$) δ 1.36 (s, 3H), 1.38 (s, 3H), 2.64 (m, 3H, 1H is D_2O exchangeable), 2.74 (m, 2H), 2.87 (1/2 ABq, $J=18.0$ Hz, 1H), 3.10 (m, 1H), 3.52 (1/2 ABq, $J=18.0$ Hz, 1H), 3.63 (dd, $J=5.1$, 11.1 Hz, 1H), 3.79 (m, 1H), 3.84 (s, 3H), 3.95 (s, 1H), 4.24 (m, 1H), 6.72 (d, $J=8.1$Hz, 1H), 6.77 (d, $J=8.1$Hz, 1H), 7.18 (t, $J=8.1$Hz, 1H); ^{13}C NMR (75 MHz) ($CDCl_3$) δ 170.60, 155.83, 135.56, 128.07, 121.88, 120.19, 116.31, 108.79, 99.92, 66.15, 65.27, 58.95, 56.52, 54.74, 52.69, 52.15, 33.52, 26.08, 16.89. IR (NaCl, neat) 3422, 2926, 2253, 1774, 1382, 1060 cm^{-1}. HRMS (FAB) calcd for $C_{19}H_{26}N_3O_4$ (M + H) 360.1929; found 360.1923.

4α,6α,11aβ-2-Aza-2-carboxyacetyl-1,3,4,6,11a-hexahydro-7-hydroxy-5,4-oxazolo-3,3-dimethyl-2*H*-benzo[*b*]quinolizine (24). To a solution of **6b** (10 mg, 0.030 mmol) in 0.5 mL of CH_2Cl_2 in an oven-dried three-neck flask fitted with a septum, argon line, and drying tube containing $CaCl_2$ was added BBr_3 (1.0 M soln, 180 μL, 0.18 mmol) at −78 °C. The reaction was then allowed to warm up to room temperature and stirred overnight. The reaction was quenched with water and extracted with CH_2Cl_2. After concentration the residue was chromatographed (PTLC, 10:1 CH_2Cl_2:MeOH) to give 3.3 mg of **6b** (33%) and 4.5 mg of phenol analogue **24** (47%) as a clear oil. TLC (10:1 CH_2Cl_2:MeOH) R_f 0.07 (UV and PMA). ^{1}H NMR (300 MHz) (MeOH-d_4) δ 1.55 (s, 3H), 1.59 (s, 3H), 2.69–2.73 (m, 2H), 3.14–3.24 (m, 1H), 3.35 (s, 1H), 3.40–3.52 (m, 2H), 3.64–3.73 (m, 1H), 3.85–3.94 (m, 1H), 4.32 (t, $J=8.4$ Hz, 1H), 4.49 (s, 1H), 4.56 (t, $J=8.4$ Hz, 1H), 6.65 (dd, $J=2.7$, 7.8 Hz, 2H), 7.03 (t, $J=7.8$ Hz, 1H); ^{13}C NMR (100 MHz) (MeOH-d_4) δ 174.0, 156.1, 135.5, 128.9, 122.5, 120.5, 113.5, 96.6, 69.8, 62.4, 60.9, 54.3, 53.9, 32.9, 22.6, 20.1. IR (NaCl, neat) 3409, 2926, 1640, 1466, 1380, 1277, 1160, 1123 cm^{-1}. HRMS (FAB) calcd for $C_{17}H_{23}N_2O_4$ (M + H) 319.1658; found 319.1655.

Acknowledgements

This work was supported by the National Institutes of Health (CA43969). Mass spectra were obtained on instruments supported by the NIH Grant GM49631. We are grateful to Prof. Oren P. Anderson, Colorado State University, for the vector analysis performed on the X-ray crystal structure of compound **6a**.

References and Notes

1. Tomita, F.; Takahashi, K.; Shimizu, K.-I. *J. Antibiot*. **1983**, *36*, 463.
2. Takahashi, K.; Tomita, F. *J. Antibiot*. **1983**, *36*, 468.
3. Hirayama, N.; Shirahata, K. *J. Chem. Soc. Perkin Trans. 2* **1983**, *1022*, 1705.
4. Sato, T.; Hirayama, F.; Saito, T. *J. Antibiot*. **1991**, *44*, 1367.
5. Suzuki, K.; Sato, T.; Morioka, M.; Nagai, K.; Abe, K.; Yamaguchi, H.; Saito, T.; Ohmi, Y.; Susaki, K. *J. Antibiot*. **1991**, *44*, 479.
6. 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. Bernan, V. S.; Montenegro, D. A.; Korshalla, J. D.; Maiese, W. M.; Steinberg, D. A.; Greenstein, M. *J. Antibiot*. **1994**, *47*, 1417.
8. Singh, M. P.; Peterson, J.; Jacobus, N. V.; Maiese, W. M.; Greenstein, M.; Steinberg, D. A. *Antimicrob. Agents Chemother*. **1994**, *38*, 1808.
9. For quinocarcin, see: Fujimoto, K.; Oka, T.; Morimoto, M. *Cancer Res*. **1987**, 47, 1516 and refs 10–15.
10. Jett, J. R.; Saijo, N.; Hong, W.-S.; Sasaki, Y.; Takahashi, H.; Nakano, H.; Nakagawa, K.; Sakurai, M.; Suemasu, K.; Tesada, M. *Investigational New Drugs* **1987**, *5*, 155.
11. Saito, H.; Sato, A.; Ashizawa, T.; Morimoto, M.; Hirata, T. *Chem. Pharm. Bull*. **1990**, *38*, 3202.

12. Saito, H.; Hirata, T.; Kasai, M.; Fujimoto, K.; Ashizawa, T.; Morimoto, M.; Sato, A. *J. Med. Chem.* **1991**, *34*, 1959.
13. Saito, H.; Kobayashi, S.; Uosaki, Y.; Sato, A.; Fujimoto, K.; Miyoshi, K.; Ashizawa, T.; Morimoto, M.; Hirata, T. *Chem. Pharm. Bull.* **1990**, *38*, 1278.
14. Chiang, C.-D.; Kanzawa, F.; Matsushima, Y.; Nakano, H.; Nakagawa, K.; Takahashi, H.; Terada, M.; Morinaga, S.; Tsuchiya, R.; Sasaki, Y.; Saijo, N. *J. Pharmacobio-Dyn.* **1987**, *10*, 431.
15. Plowman, J.; Dykes, D. J.; Narayanan, V. L.; Abbott, B. J.; Saito, H.; Hirata, T.; Grever, M. R. *Cancer Res.* **1995**, *55*, 862.
16. Tomita, F.; Takahashi, K.; Tamaoki, T. *J. Antibiot.* **1984**, *37*, 1268.
17. Williams, R. M.; Glinka, T.; Flanagan, M. E.; Gallegos, R.; Coffman, H.; Pei, D. *J. Am. Chem. Soc.* **1992**, *114*, 733.
18. Williams, R. M.; Flanagan; M. E.; Tippie, T. N. *Biochemistry* **1994**, *33*, 4086.
19. Flanagan, M. E.; Rollins, S. B.; Williams, R. M. *Chem. & Biol.* **1995**, *2*, 147.
20. Williams, R. M.; Glinka, T.; Gallegos, R.; Ehrlich, P. P.; Flanagan, M. E.; Coffman, H.; Park, G. *Tetrahedron* **1991**, *47*, 2629.
21. X-ray parameters for **5a** and **6a**: Anderson, K. A.; Miller, M. M.; Anderson, O. P. *Acta Cryst.* **1992**, *C48*, 561.
22. Hill, G. C.; Wunz, T. P.; Remers, W. A. *J. Computer-Aided Mol. Design* **1988**, *2*, 91.
23. The stereochemistry of this substance was confirmed by alkylating the mesylate of **14** with *N*-methyl-2-amino-2-methyl-1-propanol; the product was identical to an intermediate used in the synthesis of **6a** by ^{1}H NMR.
24. Nishiwaki, E.; Tanaka, S.; Lee, H.; Shibuya, M. *Heterocycles* **1988**, *27*, 1945.
25. For the preparation of DX-52-1 from quinocarcin, see: Saito, H.; Hirata, T. *Tetrahedron Lett.* **1987**, *28*, 4065.
26. Saito, H.; Kobayashi, S.; Uosaki, Y.; Sato, A.; Fujimoto, K.; Miyoshi, K.; Ashizawa, T.; Morimoto, M.; Hirata, T. *Chem. Pharm. Bull.* **1990**, *38*, 1278.
27. For relevant conversions of naphthyridinomycin to the corresponding α-aminonitrile derivatives, see: Zmijewski, Jr., M. J.; Goebel, M. *J. Antibiot.* **1982**, *35*, 524 and refs 28 and 29.
28. Itoh, J.; Omoto, S.; Inouye, S.; Kodama, Y.; Hisamatsu, T.; Niida, T.; Ogawa, Y. *J. Antibiot.* **1982**, *35*, 642.
29. Gould, S. J.; He, W.; Cone, M. C. *J. Nat. Prod.* **1993**, *56*, 1239.
30. Garner, P.; Ho, W. B.; Shin, H. *J. Am. Chem. Soc.* **1993**, *115*, 10742.
31. The relative stereochemistry of aminonitrile **23** was determined by ^{1}H NOE spectroscopy.
32. Deslongchamps, P. *Stereoelectronic effects in organic chemistry*. In *Organic Chemistry Series*, Pergamon: Oxford, 1983.
33. Zmijewski, Jr., M. J.; Miller-Hatch, K.; Mikolajczak, M. *Chem.-Biol. Interactions* **1985**, *52*, 361.
34. Hill, G. C.; Wunz, T. P.; MacKenzie, N. E.; Gooley, P. R.; Remers, W. A. *J. Med. Chem.* **1991**, *34*, 2079.
35. Williams, R. M.; Herberich, B. *J. Am. Chem. Soc.* **1998**, *120*, 10272.
36. Crabb, T. A.; Turner, C. H.; Newton, R. F. *Tetrahedron* **1968**, *24*, 4423.
37. The work described herein was taken in part from: Herberich, B. Ph.D. Dissertation, Colorado State University, Fort Collins, Colorado, Fall, 1999.