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Synthesis of 5,12-Dioxocyclam Nickel (II) Complexes Having Quinoxaline Substituents at the 6 and 13 Positions as Potential DNA Bis-Intercalating and Cleaving Agents

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Several dioxocyclams containing quinoxaline moieties, as well as their nickel(II) complexes were synthesized and studied for their ability to bind and oxidatively cleave DNA. Although no evidence for binding by intercalation was found, the ability of the Ni(II) complexes to cleave DNA in the presence of Oxone was strongly dependent on both the nature and the spatial orientation of the quinoxaline moieties, suggesting at least transient association of these complexes with DNA.

Introduction

Bis-intercalating antibiotic, antitumor agents[1,2] constitute a growing class of compounds of interest both for their biological activity as well as their mode of interaction with DNA. They are structurally related and consist of a cyclic octa- or deca-depsipeptide framework with two appended aromatic nitrogen heterocycles (quinoxaline for echinomycin and triostin, hydroxyquinaldic acids for sandramycin, luzopeptin, and thiocoraline) disposed about a 2-fold axis of symmetry. The depsipeptide is rigidified by sulfur cross-links, cross-ring hydrogen bonding, and/or *N*-methyl substituents to restrict conformational mobility.

These compounds interact with DNA by intercalating the aromatic nitrogen heterocycle moieties into the minor groove of DNA, sandwiching two DNA base pairs. Initially, it was thought that the depsipeptide backbone held the two heterocycles rigidly 11 Å apart, the required distance to span two base pairs (Figure 1). Subsequent studies indicated a higher degree of conformational variation,[3] with an X-ray structure of sandramycin having an intrachromophore (heterocycle) distance of 17–19.5 Å,[4] indicating that the conformation upon bis-intercalation is substantially different from that in the
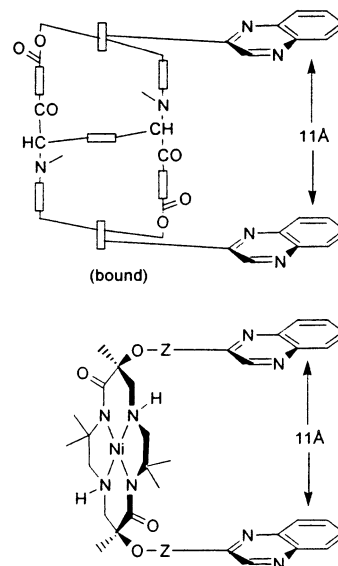

FIGURE 1. Schematic Diagram of Triostin A and Ni–Cyclam Quinox Complex.

unbound state. Elegant synthetic and binding studies[4,5] of sandramycin analogues showed that the depsipeptide backbone is responsible for most of the binding affinity (6.0 kcal/mol), with intercalation of the first heterocycle increasing binding by 3.2 kcal/mol, and the second by 1.0 kcal/mol.

The binding and cleaving of DNA by macrocyclic metal complexes is a related area of active research.[6] Most pertinent to the studies reported below are those metal

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(1) For reviews see: (a) Waring, M. J. Echinomycin and Related Quinoxaline Antibiotics. In *Molecular Aspects of Anticancer Drug–DNA Interactions*; Neidle, S.; Waring, M., Eds; *Top. Mol. Struct. Biol.*; CRC Press: Boca Raton, 1993; Vol. 1, pp 213–242. (b) Wakelin, L. P. G.; Waring, M. J. DNA Intercalating Agents. In *Comprehensive Medicinal Chemistry*; Hamisch, C., Sammes, P. G., Taylor, J. B., Eds.; Pergamon: Oxford, 1989; Vol. 2, pp 703–724.

(2) For recent biological studies, see: (a) Bailly, C.; Hamy, F.; Waring, M. J.; *Biochemistry*, **1996**, *35*, 1150–1161. (b) Fletcher, M. C.; Fox, K. R.; *Biochemistry*, **1996**, *35*, 1064–1075. (c) Mollegaard, N. E.; Bailly, C.; Waring, M. J.; Nielsen, P. E. *Biochemistry* **2000**, *39*, 9502–9507.

(3) (a) Alfredson, T. V.; Maki, A. H.; Waring, M. J.; *Biopolymers*, **1991**, *31*, 1689–1709. (b) Sheldrick, G. M.; Guy, J. J.; Kenard, O.; Rivera, V.; Waring, M. J. *J. Chem. Soc., Perkin. Trans. II*, **1984**, 1601–1605. (c) See ref 1a for summary.

(4) Boger, D. L.; Chen, J. H.; Saionz, K. W. *J. Am. Chem. Soc.* **1996**, *118*, 1629–1644.

(5) (a) Boger, D. L.; Lee, J. K. *J. Org. Chem.* **2000**, *65*, 5996–6000. (b) Boger, D. L.; Chen, J.-H.; Saionz, K. W.; Jin, Q. *Bioorg. Med. Chem.* **1998**, *6*, 85–102. (c) Boger, D. L.; Saionz, K. W. *Bioorg. Med. Chem.* **1999**, *7*, 315–321. (d) Boger, D. L.; Ichikawa, S.; Tse, W. C.; Hedrick, M. P.; Jin, Q. *J. Am. Chem. Soc.* **2001**, *123*, 561–568.

SCHEME 1

* = (±) C-2
* = (R)(S) Centro
"imine cyclam"

SCHEME 2
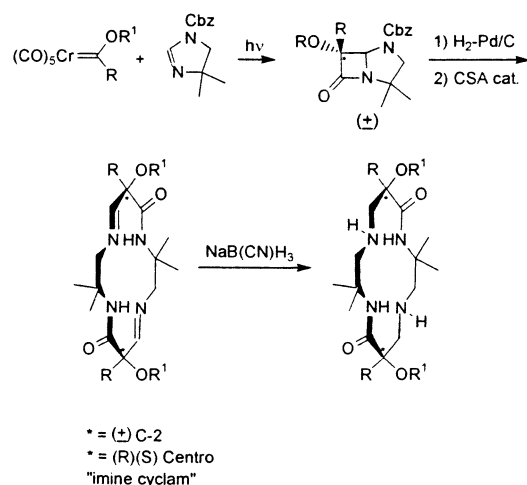
macrocycles having (appended) moieties capable of intercalating into DNA, to bind the metal complex to DNA prior to an oxidative cleavage event. These include iron-methidiumpropyl-EDTA,[7] the rhodium and ruthenium phenanthroline-based systems,[8] and metalloporphyrin systems.[6b] Considerably less common are metal complexes constituted to act as bis-intercalators for DNA, although a few are known.[9,10]

An efficient synthesis of dioxocyclams,[11] 14-membered tetrazamacrocycles having two amines and two amides as part of the ring and intermediate between cyclic peptides and cyclic polyamines, has been developed in these laboratories[12] (Scheme 1). Because a stereocenter is set in the photochemical reaction of imidazolines with chromium carbene complexes, the resulting azapenams are racemic. Dimerization of this racemic material produces two diastereoisomeric cyclams, the racemic (R)(R)/(S)(S) C-2 symmetric diastereoisomers and the achiral (R)(S) centrosymmetric diastereoisomer. Use of a chiral optically active imidazoline produces a single enantiomer of the azapenam, which, in turn produces only the C-2 symmetric cyclam as a single enantiomer.[13] An X-ray crystal structure of a C-2 nickel dioxocyclam complex[13] suggested that complexes of this sort might serve as a surrogate for the cyclic depsipeptide platform to support two quinoxaline rings with the appropriate orientation
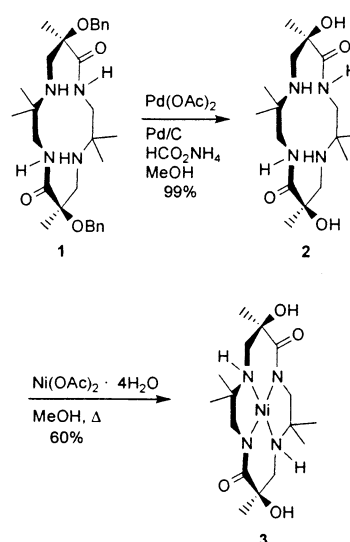
and distance for bis-intercalation into DNA (Figure 1). To assess the feasibility of this, molecular modeling studies (Molecular Simulations Inc. Discover program, CFF91 Force Field) were carried out. The ordination of the complex was taken from the X-ray structure of the (methoxy)(methyl) cyclam,[12c] and the methoxy groups were computationally replaced by quinoxaline carboxylic acid groups. Minimization of the free complex showed that the bis-quinoxaline groups preferred to be disposed roughly parallel to each other with only a minor difference in energy for interquinoxaline ring distances between 7 and 12 Å. This, coupled with the well-established ability of simple nickel-cyclam complexes to effect oxidative cleavage of oligonucleotides in the presence of oxidants[6a,14] led to the synthetic and DNA binding and cleaving studies presented below.

Results and Discussion

Synthesis of Quinoxaline-Containing Dioxocyclams. The previously reported[12a] C-2 symmetric O-benzyl dioxocyclam **1** was chosen as the initial platform for quinoxaline incorporation. Debenzylation provided the bis-tertiary alcohol **2** for coupling to quinoxaline-2-carboxylic acid chloride (Scheme 2). Because the ring secondary amines were likely to be more reactive toward acylation than the desired tertiary alcohol sites, the nickel complex **3** was prepared prior to acylation.[15]

All attempts to acylate **3** with quinoxaline-2-carboxylic acid chloride resulted in intractable mixtures of poly-acylated products, which, furthermore, did *not* contain nickel. Apparently, complexation to nickel was not suf-

(6) For reviews see: (a) Burrows, C. J.; Muller, J. G. *Chem. Rev.* **1998**, *98*, 1109−1151. (b) Pratviel, G.; Bernadou, J.; Meunier, B. *Adv. Inorg. Chem.* **1998**, *45*, 251−312. (c) Long, E. C. *Acc. Chem. Res.* **1999**, *32*, 827−836; For recent papers, see: (d) Jin, L.; Yang, P. *Microchem. J.* **1998**, *58*, 144−150. (e) Herebian, D.; Sheldrick, W. S. *J. Chem. Soc., Dalton Trans.* **2002**, 966−974.

(7) Hertzberg, R. P.; Dervan, P. B. *J. Am. Chem. Soc.* **1982**, *104*, 313−315.

(8) For a review see: Erkkila, K. E.; Odom, D. T.; Barton, J. K. *Chem. Rev.* **1999**, *99*, 2777−2795.

(9) Drexler, C.; Hosseini, M. W.; Pratviel, G.; Meunier, B. *Chem. Commun.* **1998**, 1343−1344.

(10) (a) Önfelt, B.; Lincoln, P.; Nordén, B. *J. Am. Chem. Soc.* **2001**, *123*, 3630−3637 and references therein; (b) Gude, L.; Fernández, M.-J.; Grant, K. B.; Lorente, A. *Tetrahedron Lett.* **2002**, *43*, 4723−4727.

(11) For reviews, see: (a) Izatt, R. M.; Pawlak, K.; Bradshaw, J. S.; Bruening, R. L. *Chem. Rev.* **1995**, *95*, 2529−2586. (b) Kimura, E. *Pure Appl. Chem.* **1986**, *42*, 1461−1466.

(12) (a) Betshart, C.; Hegedus, L. S. *J. Am. Chem. Soc.* **1992**, *114*, 5010−5017. (b) Hegedus, L. S.; Moser, W. H. *J. Org. Chem.* **1994**, *55*, 7779−7784. (c) Dumas, S.; Lastra, E.; Hegedus, L. S. *J. Am. Chem. Soc.* **1995**, *117*, 3368−3379. (d) Puntener, K.; Hellman, M. D.; Kuester, E.; Hegedus, L. S. *J. Org. Chem.* **2000**, *65*, 8301−8306.

(13) Hsiao, Y.; Hegedus, L. S. *J. Org. Chem.* **1997**, *62*, 3586−3591.

(14) (a) Chen, X.; Rokita, S. E.; Burrows, C. J. *J. Am. Chem. Soc.* **1991**, *113*, 5884−5886. (b) Muller, J. G.; Chen, X.; Dadiz, A. C.; Rokita, S. E.; Burrows, C. J. *J. Am. Chem. Soc.* **1992**, *114*, 6407−6411. (c) Burrows, C. J.; Rokita, S. E. *Acc. Chem. Res.* **1994**, *27*, 295−301. (d) Shih, H.-C.; Tang, N.; Burrows, C. J.; Rokita, S. E. *J. Am. Chem. Soc.* **1998**, *120*, 3284−3288. (e) Lepentsiotis, V.; Domagala, J.; Grgic, I.; van Eldik, R.; Muller, J. G.; Burrows, C. J. *Inorg. Chem.* **1999**, *38*, 3500−3505. (f) Stuart, J. N.; Goerges, A. L.; Zaleski, J. M. *Inorg. Chem.* **2000**, *39*, 5976−5984.

(15) Protection of cyclam amines by coordination to metals has previously been reported: Patinec, V.; Gardinier, I.; Yaouanc, J.-J.; Clément, J.-C.; Handel, H.; des Abbayes, H. *Inorg. Chem. Acta* **1996**, *244*, 105−108.

SCHEME 3

SCHEME 4
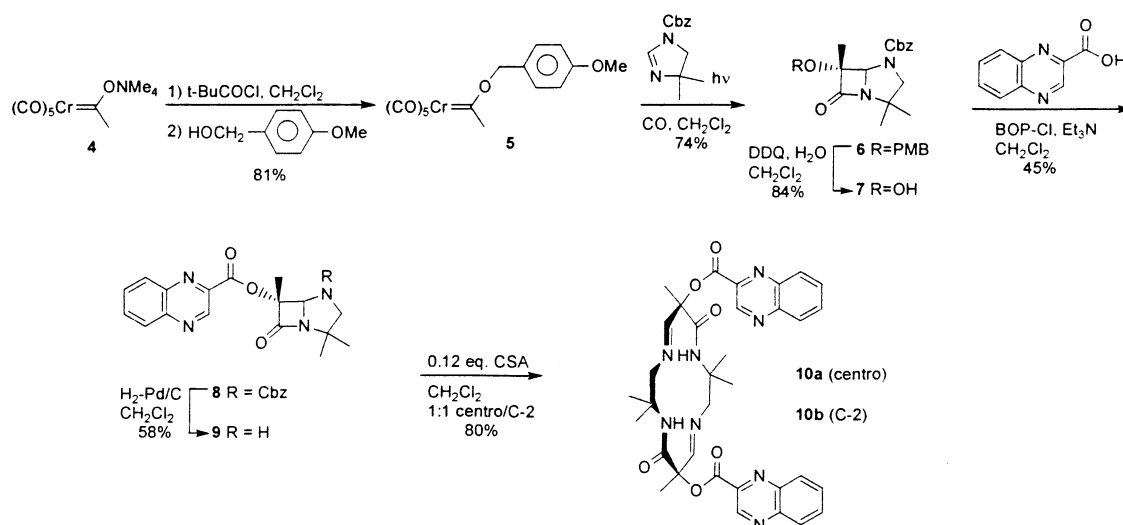
ficient to prevent acylation of the ring secondary amines. Once acylated, the complexation to nickel was weakened and demetalation occurred. Because introduction of quinoxaline into the preformed cyclam failed, introduction at an earlier stage of the synthesis was attempted (Scheme 3).

Differentially protected azapenam **6** was prepared by the photolysis of PMB carbene complex **5** with Cbz-protected dimethyl imidazoline. Oxidative removal of the PMB with DDQ proceeded in good yield, and tertiary-alcohol azapenam **7** was coupled to quinoxaline-2-carboxylic acid in modest yield using conditions developed for sterically hindered peptide coupling.[16] Reductive removal of the Cbz group followed by acid-catalyzed dimerization produced a 1:1 mixture of the centro- and C-2 symmetric cyclam imines **10a** and **10b**. Remarkably, washing the crude reaction mixtures with aqueous saturated sodium bicarbonate solution resulted in *complete* hydrolysis of the ester linkage, giving the bis-imine-bis-hydroxy cyclam corresponding to **2**. Because the inordinate lability of the ester linkage would prevent meaningful DNA binding and cleaving studies, a more robust linkage was sought (Scheme 4).

Hydroxyazapenam **7** was coupled to 2-bromomethylquinoxaline under Finkelstein conditions in excellent yield. Reductive removal of the Cbz group went smoothly (1 atm. H_2, Pd/C) and was not compromised by competing cleavage of the quinoxaline benzylic ether linkage. Attempted dimerization under standard conditions (catalytic CSA, CH_2Cl_2, 25 °C) resulted in no reaction even after 9 days. By heating the reaction mixture at 60° in a pressure tube for 20 h, dimerization occurred, giving a 2:1 ratio of the desired C-2 symmetric and the undesired centrosymmetric *imine* cyclam. Separation by flash chromatography on triethylamine-treated silica gel gave the desired C-2 symmetric imine cyclam in 50% yield. The undesired centrosymmetric diastereoisomer could not be isolated in pure form.

Reduction of the macrocyclic bis-imine proved problematic. Standard reduction conditions (NaBH₄, MeOH/
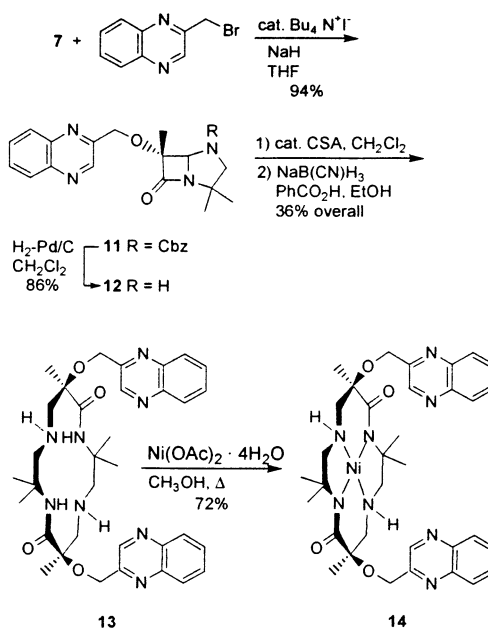
H_2O) resulted in competitive reduction of the quinoxaline moiety. Heterogeneous hydrogenation under a variety of conditions resulted in preferential reduction of the quinoxaline ring. The desired reduction was achieved by taking advantage of the large difference in basicity between the macrocyclic imine ($pK_a \approx 6$) and the quinoxaline "imine" ($pK_a \approx 0.6$). Use of sodium cyanoborohydride in ethanol in the presence of benzoic acid ($pK_a \approx 4$) to selectively protonate and activate the macrocyclic imine groups led to clean reduction, producing **13** in 72% yield for the reduction step, 36% overall yield from azapenam **12**. An X-ray crystal structure[17] confirmed that both quinoxalines were on the same face of the macrocycle. Because of the flexibility of the uncomplexed macrocycle, the quinoxaline rings are not held parallel at a distance of ~10 Å, but are somewhat closer and at an oblique angle to each other. (As noted above, the quinoxaline groups in unbound triostin A and sandra-

(16) Tung, R. D.; Rich, D. H. *J. Am. Chem Soc.* **1985**, *107*, 4342–4343.

(17) See Supporting Information for full X-ray structural data.

SCHEME 5
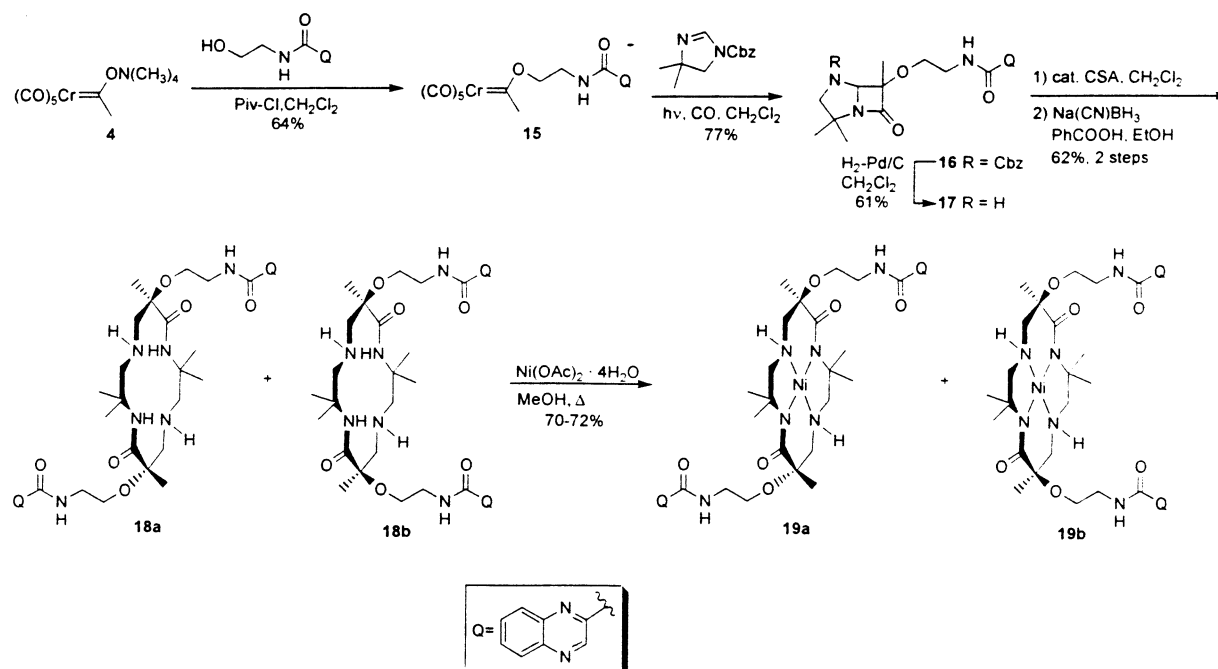
mycin are also substantially displaced from their DNA-bound distances and geometry.) Introduction of nickel(II) into **13** by heating with nickel acetate in methanol for a few minutes produced complex **14** in good yield. X-ray quality crystals of **14** could not be obtained.

Concurrent with the above synthetic studies, an extended, amide-linked quinoxaline-cyclam system was synthesized (Scheme 5). Quinoxaline-2-carboxylic acid was coupled to ethanolamine using peptide coupling conditions (EDCI/HOBt), and the resulting amide was used to produce extended quinoxaline-amide-chromium carbene complex **15**. Photolysis with protected imidazoline produced the protected azapenam **16** which underwent reductive deprotection in fair yield to give free azapenam **17**. Dimerization again required heating in a sealed tube, and the resulting imine-cyclam was reduced under previously developed conditions to give a 1:4 mixture of the undesired centrosymmetric cyclam **18a** and the desired C-2 symmetric cyclam **18b** in 62% overall yield over two steps. These diastereoisomers were not separable by flash chromatography. However, centrosymmetric cyclam **18a** was highly crystalline and could be recrystallized away from **18b**, giving access to pure samples of each compound. Introduction of nickel under conditions developed above gave nickel cyclam complexes **19a** and **19b**. Again the centrosymmetric diastereoisomer was highly crystalline and both free ligand **18a** and nickel complex **19a** were characterized by X-ray crystallography.[17] X-ray quality crystals of **18b** and **19b** could not be obtained. In comparison to the ether-linked quinoxaline-cyclam **13**, the macrocyclic ring in **18a** is substantially flatter, with the quinoxaline folding back over the cyclam ring. Complexation flattens the ring more (Figure 2).

To avoid the production of the unwanted centrosymmetric diastereoisomer, with its attendant separation problems, the sequence in Scheme 5 was repeated starting with optically active imidazoline **20** (Scheme 6). In

contrast to Scheme 5, after dimerization and reduction, free ligand **24** resisted purification. Treatment of the crude material with nickel acetate gave a low yield of the nickel complex **23**, which was demetalated by treatment with concentrated HCl to give free ligand **24**. With compounds **13**, **14**, **18**, **19**, **23**, and **24** in hand, DNA binding and cleaving studies were initiated.

Bifunctional intercalators induce the unwinding of negatively supercoiled DNA, resulting in a gradual decrease of the agarose gel electrophoresis mobility, followed by a return to near-normal mobility as increased concentrations of intercalator induces rewinding to form positive supercoils.[18] Free cyclam ligands **2**, **13**, **18a**, **18b**, and **24**, as well as the corresponding nickel complexes **3**, **14**, **19a**, **19b**, and **23** were added to ΦX174 RFI DNA (0.25 μg in 9 μL of 50mM Tris-HCl$-$pH 8) in 1 μL of DMSO in ratios of agent/DNA ranging from 0.02 to 20. After incubation for 3h and 15h at 25 °C and 37 °C, gel electrophoresis showed *no* appreciable decrease in mobility. In contrast, echinomycin under the same conditions, even at the lowest (0.02) concentration led to complete uncoiling of DNA. Thus, neither the free cyclam ligands nor the nickel complexes are functional analogues of the bis-intercalating DNA binders. Whether they fail entirely to intercalate, or simply bind only weakly and have a high off-rate remains an open question.

Because a number of macrocyclic nickel complexes catalyze the oxidative cleavage of nucleic acids,[6a,14] the ability of nickel complexes **3**, **14**, **19a**, **19b**, and **23** as well as the nickel complex of **1** to cleave DNA under oxidative conditions was next addressed. Intact, supercoiled DNA (Form I) migrates relatively fast when subjected to gel electrophoresis. If scission (nicking) of one of the strands occurs, a slower moving, open circular form (Form II) is

(18) (a) Huang, C.-H.; Prestayko, A. W.; Crooke, S. T. *Biochemistry* **1982**, *21*, 3704−3710. (b) see ref 4 for the application of this procedure to sandramycin.

SCHEME 6
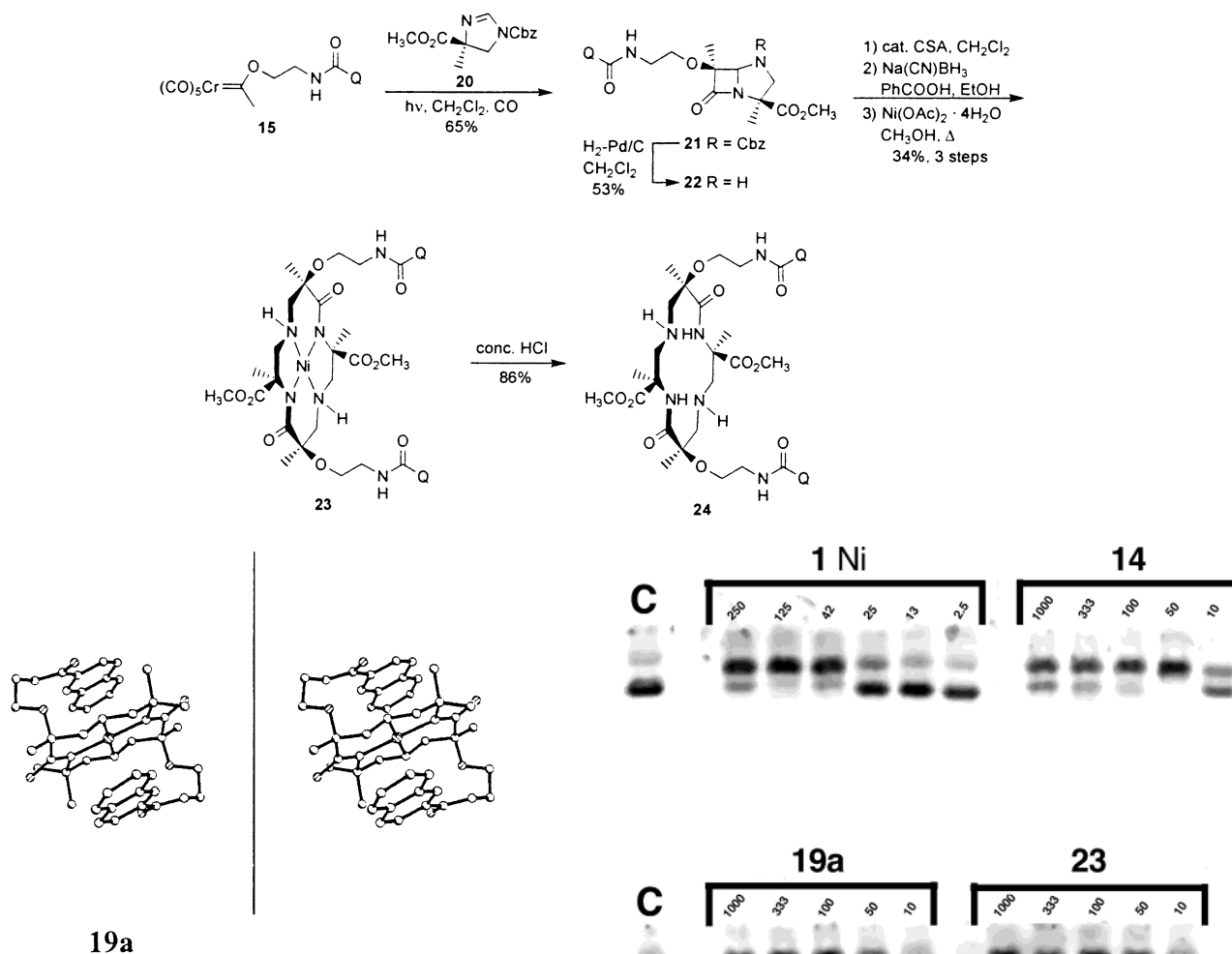
FIGURE 2. Stereoview of **19a**.

FIGURE 3. DNA Cleaving Studies. Supercoiled pB*R*322 plasmid DNA (20 μM bp) was incubated for 1 h with with 1mM Oxone, and varying concentrations of nickel complexes in aqueous solution. The control lane sample was identical save for the absence of nickel complex. The numbers at the head of each column denote μM concentration of the nickel complex.

generated. Cleavage of both strands generates a linear form (Form III), which migrates at a rate between that of Form I and Form II. Multiple strand scissions results in streaking of the lane on an agarose gel.

Supercoiled pB*R*322 plasmid DNA (20 μM bp) was incubated for 1 h with varying concentrations of the above nickel complexes (0.25−1000 μM in aqueous solution) and 1 mM Oxone and the resulting solutions were subjected to gel electrophoresis. The results (Figure 3) show that the DNA cleaving activity of these complexes is primarily a function of the appended side chains, as well as the spatial disposition, because the nickel-heterocyclic core structure is identical. The least-active nickel complex was that of the free tertiary alcohol **3**, the only complex lacking a π-aromatic system. Even at a concentration of 1 mM, substantial amounts of intact supercoiled DNA remained. The nickel complex of benzyl ether **1** was substantially more effective, resulting in complete conversion of supercoiled DNA (Form I) to open circular Form II at a concentration of 125 μM. Cis-quinoxaline ether complex **14** had similar cleaving activity. The most interesting comparison is between the extended quinoxaline amide complexes **19a**, **19b**, and **23**. *Trans*-amide complex **19a** and chiral *cis*-amide complex **23** have roughly the same DNA cleaving ability, requiring 100 μM concentrations for the complete conversion of

DNA from Form I to Form II, compared to *cis*-quinoxaline ether complex **14**, which required a 50 μM concentration. A majority of Form II remained even at 1000 μM concentrations. In marked contrast, cis-quinoxaline amide complex **19b** completely converted Form I DNA to Form II at a 50 μM concentration and resulted in multiple

strand breaks, as indicated by extensive streaking on the gel, at 100 μM concentration.

A surprising observation with all of these nickel complexes is that as the ratio of complex to Oxone approaches one, cleavage is suppressed! This is most apparent with complex **19b**, for which complex destruction of DNA was noted at 100 μM and 300 μM concentrations, but at 1mM, substantial amounts of Form II were reproducibly observed. This phenomenon was less extreme with the other nickel complexes but, again, reproducibly observable. The reason for this is not clear. It is possible that high concentrations of nickel result in decomposition or sequestering of the Oxone, reducing its ability to cleave DNA, or that two equivalents of oxidant per equivalent of nickel complex are required for cleavage of DNA.

The failure to observe any interaction of the above compounds with DNA stands in marked contrast to the strong dependence of DNA cleaving ability on both the specific side chains on the nickel macrocycle core, as well as the stereochemistry at these centers. This is most apparent in a comparison of cis and trans quinoxaline-amide complexes **19b** and **19a**. These complexes are identical in all respects except for the disposition of the two potential intercalating quinoxaline amide groups. Complex **19b**, which has the two quinoxaline moieties reasonably disposed for bis-intercalating is highly active, whereas complex **19a**, having only one quinoxaline group per face is very much less active. This implies some, at least transient, association of the complexes with DNA that is dependent on side chain structure and disposition. The existence and nature of this interaction awaits experimental verification.

In conclusion, several dioxocyclams containing quinoxaline moieties, as well as their nickel(II) complexes were synthesized and studied for their ability to bind and oxidatively cleave DNA. Although no evidence for binding by intercalation was found, the ability of the Ni(II) complexes to cleave DNA in the presence of Oxone was strongly dependent on both the nature and spatial orientation of the quinoxaline moieties, suggesting at least transient association of these complexes with DNA.

Experimental Section

General Procedures. THF was distilled from sodium-benzophenone ketyl, DMF was distilled from MgSO$_4$ and stored over 4 Å molecular sieves, CCl$_4$ was distilled from P$_2$O$_5$ and stored over 4 Å molecular sieves, CH$_2$Cl$_2$ and Et$_3$N were distilled from CaH$_2$, Et$_3$N was stored over KOH pellets. Commercially available reagents were used as received except where indicated. Unless otherwise stated, all NMR spectra (300 MHz for ^{1}H NMR and 75 MHz for ^{13}C NMR) were recorded in CDCl$_3$. Chemical shifts are given in δ ppm relative to CHCl$_3$ (δ7.27, ^{1}H) or CDCl$_3$ (δ77.23, ^{13}C). Column chromatography was performed with ICN 32−66 nm, 60 Å silica gel using flash column techniques. Mass spectra (LSIMS) were obtained using a Fisons VG AutoSpec mass spectrometer with a cesium ion gun, *m*-nitrobenzyl alcohol was used for the matrix and the resolution was set to 10 000. The following chemicals were prepared according to literature procedures: Pentacarbonyl [(methyl)- {(tetramethyl-ammonio)oxy}carbene}-chromium (0) **1**,[19] 1-(benzyloxycarbonyl)-4,4-dimethyl- Δ^2-imidazoline,[12a] (S)-N−(Benzyloxycarbonyl)-4-carbomethoxy- Δ^2-

(19) Fischer, E. O.; Maasbol, A. *Chem. Ber.* **1967**, *100*, 2445−2456.

imidazoline **20**,[13] (6S*, 13S*) 3,3,6,10,10,13-hexamethyl-6,13-bis(phenylmethoxy)-1,4,8,11-tetraazacyclotetradecane-5,12- dione **1**.[13]

Hydroxycyclam (2). Benzyl ether cyclam **1** (175 mg, 0.33 mmol), 10% Pd/C (200 mg, excess), Pd(OAc)$_2$ (200 mg, excess), and ammonium formate (600 mg, excess) were combined in a 50 mL round-bottom flask that had been purged with argon. Approximately 25 mL of methanol was slowly added under a steady stream of argon. The flask was then lowered into a 0 °C ice bath before it was allowed to stir and slowly come to room temperature. After 2−3 days at room temperature, the crude reaction mixture was filtered through Celite, and the solvent was removed in vacuo. The contents of the flask were taken up in 25 mL of dichloromethane then washed with 2 × 8 mL of saturated NaHCO$_3$. The aqueous layer was back extracted with 5 × 10 mL of dichloromethane. The combined organic fractions were dried over Na$_2$SO$_4$ before the solvent was removed *in vacuo,* producing hydroxycyclam **2** (114 mg, 99%) as a white solid: MP 194−196 °C; ^{1}H NMR (300 MHz) δ 6.78 (bs, 2H), 3.57 (d, $J = 11.4$ Hz, 2H), 3.18 (d, $J = 11.1$ Hz, 2H), 2.38 (d, $J = 11.4$ Hz, 2H), 2.19 (d, $J = 11.1$ Hz, 2H), 1.37 (s, 6H), 1.36 (s, 6H), 1.28 (s, 6H); ^{13}C NMR δ 174.4, 75.1, 58.6, 56.1, 53.3, 27.7, 25.5, 24.2; IR (neat) ν 3400, 3261, 1655, 1533 cm^{-1}; FABHRMS *m/z* 345.2502 (M + H$^+$, C$_{16}$H$_{33}$N$_4$O$_4$ requires 345.2502).

Quinoxaline Amide (Q−C(O)NHCH$_2$CH$_2$OH). Quinoxaline-2-carboxylic acid (2.2 g, 12.64 mmol) and ethanolamine (726 μL, 12.04 mmol) were stirred at 0 °C in 100 mL of dry DMF. Under a stream of argon, 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (6.5 g, 33.8 mmol) and 1-hydroxybenzotriazole (6.5 g, 48.2 mmol) were added in portions over 30 min. The reaction was allowed to slowly warm to room-temperature overnight before 250 mL of ethyl acetate was stirred in and used to transfer the crude reaction mixture to a separatory funnel. The organic layer was then washed with 3 × 75 mL of saturated NH$_4$Cl, followed by 3 × 75 mL of saturated NaHCO$_3$. Each aqueous layer was back-extracted with 5 × 35 mL of CH$_2$Cl$_2$. The combined organic fractions were dried with MgSO$_4$ before the solvent was removed in vacuo. Purification via flash chromatography (SiO$_2$, 3.5 × 15 cm, 6% *i*-PrOH:CH$_2$Cl$_2$), produced Q−C(O)NHCH$_2$CH$_2$OH (2.43 g, 93%) as an off-white solid. Alternatively, the alcohol could be recrystallized from ethyl acetate/hexane (75−81%) producing off-white crystals: MP 127−130 °C; ^{1}H NMR δ 9.69 (s, 1H), 8.39 (bs, 1H), 8.12−8.23 (m, 2H), 7.88 (m, 2H), 3.94 (m, 2H), 3.76 (m, 2H), 2.37 (bs, 1H); ^{13}C NMR δ 164.1, 143.9, 143.8, 143.2, 140.2, 131.8, 131.0, 129.7, 129.5, 62.2, 42.6; IR (neat) ν 3388, 1663 cm^{-1}; MS *m/z* 218.1 (MH$^+$). Anal. Calcd for C$_{11}$H$_{11}$N$_3$O$_2$: C, 60.82; H, 5.10; N, 19.34. Found: C, 61.02; H, 5.20; N, 19.50.

General Procedure for Preparation of Chromium Alkoxycarbene Complexes (5), (15). The tetramethylammonium carbene complex **4** (1 equiv) and the corresponding alcohol, *p*- methoxybenxyl alcohol or Q−C(O)NHCH$_2$CH$_2$OH (1.1 equiv), were dissolved in CH$_2$Cl$_2$ (25−30 mL/mmol **4**), placed under argon, then cooled to 0 °C with an ice bath. Pivaloyl chloride (1.1 equiv) was then added slowly over several minutes and the reaction was allowed to come to room-temperature overnight (~12−15 h). The crude reaction mixture was filtered through Celite before washing twice with saturated NaHCO$_3$. The aqueous layer was back-extracted with CH$_2$Cl$_2$ until no color remained in the aqueous phase (1−3 times). The organic layers were combined, dried with MgSO$_4$ then filtered before addition of silica gel (1−1.5× wt. crude material) followed by rotary evaporation. The adsorbed crude mixture was purified by flash chromatography (SiO$_2$, 5% ethyl acetate/hexane for **5**, 60% ethyl acetate/hexane for **15**). Collection of the orange band followed by solvent evaporation provided the carbene complexes as orange-red solids.

_p_-Methoxybenzyl Chromium Carbene Complex (5). Tetramethylammonium carbene complex **4** (3.07 g, 9.9 mmol), *p*-methoxybenzyl alcohol (1.36 mL, 10.9 mmol), and pivaloyl

chloride(1.34 mL, 10.9 mmol) were allowed to react according to the general procedure to give carbene complex **5** (2.87 g, 81%): MP 49 °C D; ^{1}H NMR δ 7.43 (d, J = 8.4 Hz, 2H), 7.01 (d, J = 8.7 Hz, 2H), 5.89 (bs, 2H), 3.86 (s, 3H), 2.99 (s, 3H); ^{13}C NMR δ 357.8, 223.5, 216.7, 160.6, 130.5, 126.2, 114.5, 83.4, 55.6, 26.7; IR (neat) ν 2063, 1920 cm^{-1}; MS *m/z* 357.0 (MH$^+$).

Quinoxaline Amide Carbene Complex (15). Tetramethylammonium carbene complex **4** (1.87 g, 6.05 mmol), Q$-$C(O)NHCH$_2$CH$_2$OH (1.45 g, 6.66 mmol), and pivaloyl chloride (0.82 mL, 6.66 mmol) were allowed to react according to the general procedure to give carbene complex **15** (1.68 g, 64%): MP 55 °C D; ^{1}H NMR δ 9.68 (s, 1H), 8.44 (bs, 1H), 8.10$-$8.22 (m, 2H), 7.87 (m, 2H), 5.06 (bs, 2H), 4.19 (m, 2H), 3.02 (s, 3H); ^{13}C NMR δ 361.1, 223.7, 216.7, 164.1, 144.4, 144.0, 143.1, 140.5, 132.1, 132.0, 131.3, 130.0, 129.8, 49.3, 39.0; IR (neat) ν 2063, 1921, 1678 cm^{-1}; MS *m/z* 436.1 (MH$^+$).

General Procedure for the Photoreaction of Chromium Alkoxycarbene Complex (5) with 1-(benzyloxy-carbonyl)-4,4-dimethyl-Δ^2-imidazoline and Quinoxaline Chromium Alkoxycarbene Complex (15) with 1-(benzyloxycarbonyl)-4,4-dimethyl-Δ^2-imidazoline and (S)-N$-$(Benzyloxycarbonyl)-4-carbomethoxy-Δ^2-imidazoline (20) to Form Azapenams (6), (16), (21). The carbene complex (1 equiv), protected imidazoline (1 equiv), and CH$_2$Cl$_2$ (25$-$30 mL/mmol carbene complex, freshly distilled under argon, CaH$_2$) were combined into a dry Pyrex pressure tube. The solution was purged with argon by bubbling through a long needle for 1$-$2 h. The reactions were either irradiated with a 450 W Conrad-Hanovia 7825 medium-pressure mercury lamp at 35° C (**6** and **16**) or with 2 × 500 W halogen lamps at 70° C (**21**) under 80 psi CO pressure. The reactions were monitored by the fading of color. After 1$-$3 days, the CH$_2$Cl$_2$ was removed from the crude reaction mixture by rotary evaporation. Methanol was used to dissolve the crude material and the insoluble Cr(CO)$_6$ was then filtered and reused. After adsorbing onto silica gel (2$-$3 × wt. crude carbene complex), the resulting crude mixtures were chromatographed (SiO$_2$, 35% EtOAc/hexane for **6**, 60% EtOAc/1% triethylamine/hexane for **16** and **21**).

p-methoxybenzyl Azapenam (6). The *p*-methoxybenzyl carbene complex **5** (4.96 g, 13.9 mmol) and 1-(benzyloxycarbonyl)-4,4-dimethyl- Δ^2-imidazoline (3.23 g, 13.9 mmol) were allowed to react according to the general procedure at 35 °C to provide **6** (4.35 g, 74%) as an off- white solid: MP 67$-$70 °C; ^{1}H NMR (Cbz rotamers) δ 7.38 (m, 5H), 7.19 (d, J = 8.7 Hz, 2H), 6.86 (m, 2H), 5.13$-$5.28 (m, 3H), 4.52$-$4.73 (m, 2H), 3.81 (s, 3H), 3.72$-$3.80 (m, 1H), 3.18 (d, J = 10.2 Hz, 1H), 1.65 (bs, 3H), 1.40s, 1.32s, 1.20s (6H); ^{13}C NMR δ 173.5, 159.3, 154.1, 153.5, 135.8, 129.8, 129.4, 129.2, 128.8, 128.5, 128.2, 127.7, 113.9, 90.6, 77.7, 75.2, 74.7, 68.2, 67.8, 61.0, 60.6, 55.5, 26.2, 22.3, 15.0, 14.6; IR (neat) ν 1774, 1709 cm^{-1}; MS *m/z* 425.2 (MH$^+$). Anal. Calcd for C$_{24}$H$_{28}$N$_2$O$_5$: C, 67.91; H, 6.65; N, 6.60. Found: C, 67.78; H, 6.73; N, 6.39.

Extended Quinoxaline Azapenam (16). The quinoxaline amide carbene complex **15** (703 mg, 1.61 mmol) and 1-(benzyloxycarbonyl)-4,4-dimethyl- Δ^2-imidazoline (375 mg, 1.61 mmol) were allowed to react according to the general procedure at 35 °C to provide **16** (621 mg, 77%) as a light-yellow viscous liquid: ^{1}H NMR (Cbz rotamers) δ 9.68 (s, 1H), 8.09$-$8.38 (m, 3H), 7.87 (m, 2H), 7.34 (m, 5H), 5.06$-$5.31 (m, 3H), 3.67$-$3.97m, 3.44s, (5H), 3.16 (d, J = 10.5 Hz, 1H), 1.63s, 1.38s, 1.31s, 1.26s, 1.28s (9H); ^{13}C NMR δ 173.4, 172.9, 171.0, 163.4, 154.0, 153.3, 143.9, 143.4, 143.2, 140.3, 136.0, 135.7, 131.7, 131.6, 130.9, 130.8, 130.0, 129.7, 129.6, 128.7, 128.5, 128.4, 128.2, 128.0, 90.3, 90.2, 74.9, 74.3, 67.7, 67.6, 64.9, 64.6, 63.2, 61.4, 60.9, 60.5, 55.7, 53.6, 39.6, 38.7, 29.0, 25.9, 22.0, 21.0, 14.0, 13.9; IR (neat) ν 3400, 1774, 1712, 1678 cm^{-1}; MS *m/z* 504.2 (MH$^+$). Anal. Calcd for C$_{27}$H$_{29}$N$_5$O$_5$: C, 64.40; H, 5.80; N, 13.91. Found: C, 64.62; H, 5.90; N, 14.12.

Extended Chiral Quinoxaline Azapenam (21). The quinoxaline amide carbene complex **15** (1.67 g, 3.85 mmol) and chiral imidazoline **20** (1.07 g, 3.85 mmol) were allowed to react

according to the general procedure at 70 °C providing **21** (1.15 g, 65%) as a light-yellow viscous liquid: [a]^{23}$_D$ +21.1 (c 1.31, CHCl$_3$); ^{1}H NMR (Cbz rotamers) δ 9.61 (s, 1H), 8.03$-$8.38 (m, 3H), 7.79 (m, 2H), 7.53 (bs, 1H), 7.30 (m, 5H), 5.04$-$5.25 (m, 3H), 4.32m, 4.13d, J = 10.5 Hz (1H), 3.65$-$3.80 (m, 7H), 3.47d, J = 10.8 Hz, 3.23d, J = 11.4 Hz (1H). 1.75s, 1.49s, 1.39s, 1.35s, 1.24s (6H); ^{13}C NMR δ 172.8, 170.7, 163.2, 152.8, 143.7, 143.2, 140.1, 135.4, 135.0, 131.5, 130.7, 129.6, 129.3, 128.6, 128.5, 128.3, 128.1, 127.9, 90.5, 77.7, 76.6, 76.1, 68.2, 67.8, 66.1, 65.5, 64.9, 64.7, 58.6, 58.3, 53.2, 52.9, 52.6, 39.5, 25.7, 18.1, 14.0; IR (neat) ν 3400, 1781, 1735, 1678 cm^{-1}; FABHRMS *m/z* 548.2124 (M + H$^+$, C$_{28}$H$_{30}$N$_5$O$_7$ requires 548.2145).

Hydroxyazapenam (7). *p*-Methoxybenzyl protected azapenam **6** (3.07 g, 7.23 mmol) was dissolved in CH$_2$Cl$_2$ (70 mL). Water was added (3.5 mL) and the flask was cooled to 0 °C with an ice bath before 2,3 -dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) (1.97 g, 8.68 mmol) was added in portions over 30 min. After stirring for 4 h (monitored by analytical silica gel TLC using 50% EtOAc/hexane), the crude mixture was diluted with approximately 200 mL of CH$_2$Cl$_2$ then washed with 3 × 75 mL of saturated NaHCO$_3$. The combined aqueous layers were back-extracted with 3 × 75 mL of CH$_2$Cl$_2$. The combined organic fractions were dried over MgSO$_4$, filtered and the solvent was removed by rotary evaporation. The crude mixture was purified by flash chromatography (SiO$_2$, 3.5 × 14 cm, 500 mL 30% EtOAc/hexane, 500 mL 40% EtOAc/hexane), yielding **7** (1.85 g, 84%) as an off white solid: MP 96$-$98 °C; ^{1}H NMR (Cbz rotamers) δ 7.37 (m, 5H), 5.04$-$5.29 (m, 3H), 3.78d, J = 10.2 Hz, 3.70d, J = 10.5 Hz (1H), 3.54 (bs, 1H), 3.16 (d, J = 10.5 Hz, 1H), 1.61s, 1.36s, 1.26s, 1.19s (9H); ^{13}C NMR δ 175.3, 175.0, 154.3, 153.7, 136.0, 128.7, 128.5, 128.4, 128.0, 85.3, 85.2, 77.9, 67.9, 67.7, 61.3, 60.9, 60.7, 60.6, 26.1, 26.0, 22.2, 16.8; IR (neat) ν 3450, 1771, 1707 cm^{-1}; MS *m/z* 305.2 (MH$^+$). Anal. Calcd for C$_{16}$H$_{20}$N$_2$O$_4$: C, 63.14; H, 6.62; N, 9.20. Found: C, 62.92; H, 6.49; N, 9.03.

Carbamate-Protected Quinoxaline Ester Azapenam (8). Hydroxyazapenam **7** (844 mg, 2.77 mmol), 2-quinoxaline carboxylic acid (531 mg, 3.05 mmol), bis(2-oxo-3- oxazolidinyl)-phosphinic chloride (848 mg, 3.33 mmol), and 50 mL of freshly distilled CH$_2$Cl$_2$ (CaH$_2$) were combined into a flame-dried 100 mL round-bottom flask and brought to 0 °C under argon before triethylamine (1.39 mL, 9.98 mmol) was added dropwise. The reaction was allowed to come to room temperature slowly overnight before the crude reaction mixture was transferred to a separatory funnel then washed with 2 × 15 mL of 5% NaHCO$_3$. The combined aqueous fractions were back-extracted with 3 × 15 mL of CH$_2$Cl$_2$. The combined organic fractions were dried with MgSO$_4$ and the solvent was removed in vacuo. The crude reaction mixture was purified by flash chromatography (SiO$_2$, 3.5 × 15 cm, 40% EtOAc, hexane) providing **8** (574 mg, 45%) as a light yellow amorphous solid. Due to the extremely labile quinoxaline ester linkage, only ^{1}H NMR data was obtained: ^{1}H NMR (Cbz rotamers) δ 9.64 (m, 1H), 8.30 (d, J = 7.8 Hz, 1H), 8.22 (d, J = 7.2 Hz, 1H), 7.93 (m, 2H), 7.37 (m, 5H), 5.61bs, 5.57bs, 5.04$-$5.29m (3H), 3.89bs, 3.86bs, 3.79d, J = 10.5 Hz, 3.70 d, J = 10.5 Hz (1H), 3.41s, 3.25d, J = 10.8 Hz, 3.16d, J = 10.2 Hz (1H), 1.69s, 1.62s, 1.37s, 1.28s, 1.27s, 1.19s (9H).

2-Bromomethylquinoxaline. 2-Methylquinoxaline (2.25 mL, 17.5 mmol), N$-$ bromosuccinimide (3.1 g, 17.5 mmol) that had been recrystallized from H$_2$O then dried (P$_2$O$_5$) just before use, 2,2′-azobisisobutyronitrile (57 mg, 0.35 mmol), and 50 mL of CCl$_4$ were combined in a 100 mL round-bottom flask. The reaction was allowed to stir in front of two 500 W halogen lamps at 60 °C for 1 h. The succinimide floating in the crude reaction mixture was removed by filtration using 10 mL of CCl$_4$ to rinse the flask. The remaining solvent was removed in vacuo. Flash chromatography (SiO$_2$, 3.5 × 16 cm, 30% EtOAc/hexane), yielded 2- bromomethylquinoxaline (2.22 g, 57%) as a light pink solid: MP 65$-$67 °C; ^{1}H NMR δ 9.01 (s, 1H), 8.07$-$8.15 (m, 2H), 7.81 (m, 2H), 4.72 (s, 2H); ^{13}C NMR δ 151.9, 145.5, 141.8, 141.6, 130.7, 130.5, 129.4, 31.2, 25.5; MS

m/z 223.0 (MH$^+$), 225.0 (MH$^+$). Anal. Calcd for C$_9$H$_7$N$_2$Br: C, 48.46; H, 3.16; N, 12.56. Found: C, 48.58; H, 3.32; N, 12.50.

Carbamate-Protected Quinoxaline Ether Azapenam (11). Hydroxyazapenam **7** (1.38 g, 4.54 mmol), 2-bromomethylquinoxaline (1.06 g, 4.76 mmol), tetrabutylammonium iodide (17 mg, 0.045 mmol), and 60% NaH in mineral oil (200 mg, 4.99 mmol) were combined in a 50 mL round-bottom flask. This was cooled to 0 °C under argon with an ice bath before 16 mL of THF was quickly added with a syringe. The ice bath was removed after 30 min. and the reaction was allowed to stir for 24 h at room temperature at which time it was quenched with dropwise addition of 2 mL of H$_2$O. The crude mixture was diluted to ~75 mL with diethyl ether then washed with 3 × 15 mL of H$_2$O; the aqueous layer was back-extracted with 2 × 15 mL of diethyl ether. The organics were dried over MgSO$_4$, and the solvent was removed by rotary evaporation. Purification was done with flash chromatography (SiO$_2$, 3.5 × 13 cm, 50% EtOAc/hexane), giving the protected quinoxaline azapenam **11** (1.91 g, 94%) as an off-white solid: MP 78−80° C; ^{1}H NMR (Cbz rotamers) δ 9.06 (s, 0.33H), 8.95 (s, 0.66H), 7.98−8.11 (m, 2H), 7.72 (m, 2H), 7.22−7.36 (m, 5H), 5.11−5.32 (m, 3H), 4.55 (dd, J = 4.5, 12.6 Hz, 2H), 3.79 (d, J = 10.8 Hz, 0.66H), 3.72 (d, J = 10.2 Hz, 0.33H), 3.17 (d, J = 10.8 Hz, 1 H), 1.61s, 1.46s, 1.34s, 1.18s (9H); ^{13}C NMR δ 172.8, 172.4, 153.9, 153.2, 152.3, 152.2, 144.2, 142.0, 141.4, 135.9, 135.6, 130.1, 130.0, 129.7, 129.6, 129.3, 129.0, 128.6, 128.4, 128.1, 128.0, 90.7, 74.9, 74.4, 68.1, 67.7, 61.4, 61.0, 60.4, 25.9, 22.0, 14.4, 14.1; IR (neat) ν 1774, 1713 cm^{-1}; MS m/z 447.1 (MH$^+$). Anal. Calcd for C$_{25}$H$_{26}$N$_4$O$_4$: C, 67.25; H, 5.87; N, 12.55. Found: C, 67.41; H, 6.01; N, 12.73.

General Procedure for Deprotection of the N-Cbz-azapenams (8), (11), (16), (21). The carbamate-protected azapenams were dissolved in a mixture of 5:1 CH$_2$Cl$_2$:triethylamine (10 mL/mmol Cbz-azapenam). The 10% Pd/C (55wt % Cbz-azapenam) was carefully added under a steady flow of argon. A balloon filled with H$_2$ was appended and the flask purged briefly with a needle. After the reaction stirred at room temperature for 4 h, it was filtered and most of the solvent was removed in vacuo. The yellow solid was dissolved in CH$_2$-Cl$_2$ and washed three times with saturated NaHCO$_3$ (washed two times with 5% NaHCO$_3$ for **8**). The aqueous layer was back extracted twice with CH$_2$Cl$_2$ before the organic layers were combined, dried over MgSO$_4$, and the solvent removed in vacuo. After adsorbing onto silica gel (2× wt. crude), the crude reaction mixtures were chromatographed (SiO$_2$, 75% EtOAc/ 1% triethylamine/hexane for **9**, 1% triethylamine/EtOAc for **12**, 8% i-PrOH/1%triethylamine/EtOAc for **17**, 2% i- PrOH/ 1%triethylamine/EtOAc for **24**) to give the deprotected azapenams as bright-yellow solids.

Quinoxaline Ester Azapenam (9). The protected quinoxaline ester azapenam **8** (380 mg, 0.83 mmol) was deprotected according to the general procedure to give the quinoxaline ester azapenam **9** (156 mg, 58%). Due to the extremely labile quinoxaline ester linkage, only ^{1}H NMR data was obtained: ^{1}H NMR δ 9.52 (s, 1H), 8.28 (dd, J = 1.5, 8.4 Hz, 1H), 8.19 (d, J = 7.8 Hz, 1H), 7.89 (m, 2H), 5.14 (s, 1H), 3.18 (d, J = 11.1 Hz, 1H), 2.78 (d, J = 10.8 Hz, 1H), 2.50 (bs, 1H), 1.71 (s, 3H), 1.64 (s, 3H), 1.18 (s, 3H).

Quinoxaline Ether Azapenam (12). The protected quinoxaline ether azapenam **11** (1.13 g, 2.53 mmol) was deprotected according to the general procedure to give the quinoxaline ether azapenam **12** (678 mg, 86%): MP 131−133 °C; ^{1}H NMR δ 9.06 (s, 1H), 8.11−8.15 (m, 1H), 8.05−8.08 (m, 1H), 7.78 (m, 2H), 5.10 (d, J = 12.9 Hz, 1H), 5.04 (d, J = 12.9 Hz, 1H), 4.89 (s, 1H), 3.12 (d, J = 10.8 Hz, 1 H), 2.69 (d, J = 11.4 Hz, 1H), 2.35 (bs, 1H), 1.61 (s, 3H), 1.48 (s, 3H), 1.15 (s, 3H); ^{13}C NMR δ 175.0, 152.8, 144.6, 142.2, 141.7, 130.3, 129.9, 129.5, 129.2, 90.4, 78.1, 68.2, 62.2, 61.3, 25.1, 22.0, 15.0; IR (neat) ν 3354, 1751 cm^{-1}; MS m/z 313.2 (MH$^+$). Anal. Calcd for C$_{17}$H$_{20}$N$_4$O$_2$: C, 65.37; H, 6.45; N, 17.94. Found: C, 65.49; H, 6.51; N, 18.18.

Extended Quinoxaline Azapenam (17). The protected extended quinoxaline azapenam **16** (371 mg, 0.74 mmol) was deprotected according to the general procedure to give the quinoxaline azapenam **17** (165 mg, 61%): MP 55−57 °C; ^{1}H NMR δ 9.68 (s, 1H), 8.32 (bs, 1H), 8.18 (m, 2H), 7.88 (m, 2H), 4.77 (s, 1H), 3.95 (m,2H), 3.81 (m, 2H), 3.07 (d, J = 10.8 Hz, 1H), 2.64 (d, J = 10.2 Hz, 1H), 2.29 (bs, 1H), 1.57 (s, 3H), 1.38 (s, 3H), 1.10 (s, 3H); ^{13}C NMR d174.9, 163.0, 143.5, 143.0, 139.9, 131.2, 130.4, 129.4, 129.1, 89.5, 77.0, 76.4, 64.3, 61.8, 60.8, 39.5, 24.7, 21.6, 14.6; IR (neat) ν 3345, 1752, 1671 cm^{-1}; FABHRMS m/z 370.1865 (M + H$^+$, C$_{19}$H$_{24}$N$_5$O$_3$ requires 370.1879).

Extended Chiral Quinoxaline Azapenam (22). The protected chiral quinoxaline azapenam **21** (490 mg, 0.90 mmol) was deprotected according to the general procedure to give the chiral quinoxaline azapenam **22** (195 mg, 53%): MP 61−63 °C; [a]$^{23}_D$ +107.9 (c 0.61, CHCl$_3$); ^{1}H NMR δ 9.67 (s, 1H), 8.30 (bs, 1H), 8.17 (m, 2H), 7.86 (m, 2H), 4.90 (s, 1H), 3.73−3.99 (m, 4H), 3.72 (s, 3H), 3.67 (d, J = 12.0 Hz, 1H), 2.68 (d, J = 12.0 Hz, 1H), 2.33 (bs, 1H), 1.74 (s, 3H), 1.36 (s, 3H); ^{13}C NMR δ 174.9, 172.0, 163.4, 143.8, 143.4, 140.3, 131.6, 130.8, 129.8, 129.5, 90.3, 80.1, 77.4, 66.4, 64.8, 60.8, 53.0, 39.8, 17.5, 14.9; IR (neat) ν 3349, 1761, 1738, 1673 cm^{-1}; FABHRMS m/z 414.1767 (M + H$^+$, C$_{20}$H$_{24}$N$_5$O$_5$ requires 414.1777).

General Procedure for Dimerization of Quinoxaline Azapenams (9), (12), (17), (22). The quinoxaline azapenams (1 equiv) and camphorsulfonic acid (0.12 equiv) were combined into a Pyrex pressure tube with CH$_2$Cl$_2$ (65 mL/mmol for **9** and **12**; 100 mL/mmol for **17**, **24**). The solution was heated (50 °C for **9**, 65 °C for **12**, **17**, 80 °C for **22**) for 15 h then washed three times with saturated NaHCO$_3$ (except for **9**, in which the crude reaction mixture was concentrated in vacuo then loaded directly on a flash column; SiO$_2$, 1% triethylamine/ EtOAc, producing a 1:1 mixture of **10a:10b** in 80% yield). The aqueous layer was back extracted twice with CH$_2$Cl$_2$ before the organic layers were combined, dried with Na$_2$SO$_4$, and the solvent removed in vacuo. The crude reaction mixture from the dimerization of **12** was dissolved in a minimum amount of ethyl acetate before being loaded on to a 1.5 × 10 cm silica gel column that had been slurry packed with 2% triethylamine/ EtOAc, then eluted with 1% triethylamine/EtOAc for the first 100 mL followed by 500 mL of 10% ^{i}PrOH/1% triethylamine/ EtOAc; 5 mL fractions were collected for the first ten, 20 mL fractions for the rest. Collection of the bright yellow band provided the cis imine cyclam in 50% yield; the trans cyclam was never isolated. For azapenams **17** and **22**, the crude reaction mixtures were taken directly to the reduction step without further purification.

Cis and Trans Imine Ester Cyclams 10a, 10b: Due to the extremely labile quinoxaline ester linkage, only ^{1}H NMR and mass spec. data were obtained: ^{1}H NMR δ 9.61 (s, 2H), 9.59 (s, 2H), 9.35 (s, 2H), 8.70 (s, 2H), 8.32 (m, 4H), 8.21 (m, 4H), 8.03 (s, 2H), 7.99 (s, 2H), 7.90 (m,8H), 3.97 (d, J = 9.9 Hz, 2H), 3.72 (d, J = 12.9 Hz, 2H), 3.49 (d, J = 12.9 Hz, 2H), 3.11 (d, J = 11.7 Hz, 2H), 1.94 (s, 12H), 1.60 (s, 6H), 1.53 (s, 6H), 1.46 (s, 6H), 1.35 (s, 6H); MS m/z 653.0 (MH$^+$).

Cis Imine Ether Cyclam 12a: MP 125 °C D; ^{1}H NMR δ 9.11 (s, 4H), 7.97 (m, 4H), 7.80 (s, 2H), 7.72 (m, 4H), 7.61 (s, 2H), 5.14 (d, J = 12.6 Hz, 2H), 4.85 (d, J = 12.6 Hz, 2H), 4.07 (d, J = 11.7 Hz, 2H), 3.37 (d, J = 12.0 Hz, 2H), 1.68 (s, 6H), 1.48 (s, 6H), 1.36 (s, 6H); ^{13}C NMR δ 169.7, 166.5, 153.1, 145.0, 142.1, 141.6, 129.9, 129.8, 129.6, 129.3, 82.2, 67.8, 65.9, 54.3, 27.0, 25.3, 22.4; IR (neat) ν 3400, 1673 cm^{-1}; MS m/z 625.3 (MH$^+$). Anal. Calcd for C$_{34}$H$_{40}$N$_8$O$_4$: C, 65.37; H, 6.45; N, 17.94. Found: C, 65.51; H, 6.31; N, 17.76.

General Procedure for Reduction of Imine Cyclams to Form Cyclams (13), (18a), (18b), (24). The imine cyclams (1 equiv) and benzoic acid (2.2 equiv) were dissolved in EtOH (20 mL/mmol imine cyclam), placed under argon, then brought to −5 °C with a saltwater/ice bath. NaBH$_3$CN (2.0 equiv) was dissolved in a minimum amount of EtOH (0.5−2 mL) at room temperature then added dropwise to the reaction mixture. The

reaction was allowed to run for 1 h before slow addition of 2−5 mL of EtOAc followed by dropwise addition of 1−2 mL of 5% NaOH. After stirring for several minutes at this temperature, the contents were transferred to a separatory funnel, diluted with ethyl acetate (enough to prevent an emulsion) and washed 3 times with 5% NaOH. The aqueous layers were combined and back-extracted twice with CH_2Cl_2. After drying the combined organic layers with Na_2SO_4 the solvent was removed in vacuo. The crude reaction mixtures were dissolved in a minimum amount of CH_2Cl_2 and loaded onto a flash column (SiO_2, 90% EtOAc/1% triethylamine/hexane for **13**, 10% *i*- PrOH/1%triethylamine/EtOAc for **18a/18b**, 1% i-PrOH/ 1%triethylamine/EtOAc for **24**).

Cis Quinoxaline Ether Cyclam (13). The imine cyclam (87 mg, 0.14 mmol) was allowed to react according to the general procedure to afford **13** (63 mg, 72%) as a light yellow solid. To obtain suitable crystals for X-ray diffraction, the purified cyclam **13** (~10−15 mg) was dissolved in a minimum amount of CH_2Cl_2 then filtered through a small piece of cotton into an NMR tube (~1 cm deep). Hexane (~3−4 cm) was then very slowly layered on top. After 2−3 days, several X-ray quality crystals were harvested from the tube and X-ray data was obtained: MP 185−188 °C; ^{1}H NMR d 9.02 (s, 2H), 8.03 (m, 4H), 7.72 (m, 4H), 7.25 (bs, 2H), 4.95 (d, J = 12.6 Hz, 2H), 4.81 (d, J = 12.3 Hz, 2H), 3.55 (d, J = 11.1 Hz, 2H), 3.00 (d, J = 12.6 Hz, 2H), 2.82 (d, J = 12.6 Hz, 2H), 2.18 (d, J = 11.1 Hz, 2H), 1.46 (s, 6H), 1.37 (s, 6H), 1.17 (s, 6H); ^{13}C NMR δ 171.5, 153.2, 144.5, 142.2, 141.8, 130.5, 129.9, 129.4, 129.1, 81.6, 65.5, 56.8, 56.6, 53.3, 27.3, 25.5, 20.0; IR (neat) ν 3400, 1672 cm^{-1}; MS m/z 629.3 (MH$^+$). Anal. Calcd for $C_{34}H_{44}N_8O_4$: C, 64.95; H, 7.05; N, 17.82. Found: C, 65.04; H, 6.99; N, 17.54.

Cis and Trans Extended Amides (18a), (18b). The crude material (60 mg, 0.081 mmol) from the dimerization of **17** was allowed to react according to the general procedure to produce a 1:4 (**18a:18b**) mixture of extended amide cyclams (37 mg, 62%). After flash chromatography (necessary to separate **18a,-18b** from other unidentified impurities) the two diastereomers were separated by recrystallization (solvent diffusion): **18a,-18b** were dissolved in a minimum amount of CH_2Cl_2 (3−5 mL) then filtered through cotton into a small vial. Diethyl ether (3−5× volume CH_2Cl_2) was gently layered on top of the CH_2-Cl_2 layer which immediately formed a turbid interface that became clear after ~10 min. After 2−3 days, crystals had formed at the interface and on the bottom of the vial; the mother liquor was pipetted off and a second recrystallization was set up. After two recrystallizations, the diastereomers were completely separated (7 mg **18a**, 30 mg **18b**). X-ray diffraction showed the crystals to be the trans diastereomer.

Trans Amide 18a: MP 166−168 °C; ^{1}H NMR δ 9.66 (s, 2H), 8.42 (bs, 2H), 8.19 (m, 2H), 8.05 (m, 2H), 7.85 (m, 4H), 3.68 (m, 6H), 3.46 (m, 2H), 2.58 (d, J = 12.0 Hz, 2H), 2.50 (d, J = 11.7 Hz, 2H), 1.98 (d, J = 12.0 Hz, 2H), 1.93 (d, J = 12.3 Hz, 2H), 1.59 (bs, 2H), 1.37 (s, 6H), 1.33 (s, 6H), 1.15 (s, 6H); ^{13}C NMR δ 171.6, 163.5, 144.2, 143.6, 140.4, 131.9, 131.3, 131.1, 129.8, 79.1, 77.4, 62.8, 60.9, 57.2, 52.8, 40.0, 25.8, 23.4, 19.5; IR (neat) ν 3308, 1666, 1531 cm^{-1}; MS m/z 743.8 (MH$^+$).

Cis Amide 18b: MP 99−102 °C; ^{1}H NMR δ 9.64 (s, 2H), 8.36 (bs, 2H), 8.16 (m, 2H), 8.09 (m, 2H), 7.83 (m, 4H), 7.13 (bs, 2H), 3.60−3.74 (m, 8H), 3.40 (d, J = 11.4 Hz, 2H), 2.83 (d, J = 12.3 Hz, 2H), 2.63 (d, J = 12.3 Hz, 2H), 2.11 (d, J = 11.4 Hz, 2H), 1.37 (s, 6H), 1.31 (s, 6H), 1.22 (s, 6H); ^{13}C NMR δ 172.1, 163.9, 144.1, 144.0, 131.8, 131.1, 129.8, 129.7, 80.4, 62.2, 57.1, 56.1, 53.0, 40.2, 26.9, 25.3, 19.7; IR (neat) ν 3360, 1667, 1532 cm^{-1}; FABHRMS m/z 743.3964 (M + H$^+$, $C_{20}H_{24}N_5O_5$ requires 743.3993).

Chiral Extended Amide (24). The chiral amide imine cyclam (156 mg, 0.19 mmol) was allowed to react according to the general procedure to produce a complex mixture of products that could be partially purified by flash chromatography (95 mg, 61%). The resulting solid was subjected to the next reaction without further purification.

General Procedure for Synthesis of Nickel Complexes (3), (14), (19a), (19b), (23). In a small round-bottom flask (10−25 mL), Ni(OAc)$_2$·4H$_2$O (1.20 equiv) was mixed with 2−4 mL of methanol and gently warmed over a heat gun for several minutes to generate a slurry. The quinoxaline cyclam (1 equiv) was dissolved in 2−4 mL of methanol and then pipetted into the slurry. A reflux condenser was attached for a handle and the flask was continually warmed to boiling over a heat gun for 5−10 min (monitored by the color change from light yellow to bright pink). After the remainder of the solvent was removed in vacuo, the crude reaction mixture was dissolved in CH_2Cl_2 and washed three times with saturated NaHCO$_3$. The aqueous layer was back extracted until no more color remained in the water (3−5 times). The resulting crude mixtures were dissolved in a minimum amount of CH_2Cl_2 and loaded onto a flash column (SiO_2, 10% *i*-PrOH/1% triethylamine/EtOAc).

Hydroxycyclam Nickel Complex (3). The hydroxycyclam **2** (40 mg, 0.12 mmol) and Ni(OAc)$_2$·4H$_2$O (36 mg, 0.15 mmol) were allowed to react according to the general conditions providing nickel complex **3** (28 mg, 60%) as a bright pink solid: MP 112−115 °C; ^{1}H NMR and ^{13}C NMR indicated that the complex was isolated as a mixture of N−H isomers; spectra are included in the Supporting Information; IR (neat) ν 3184, 1574 cm^{-1}; FABHRMS m/z 401.1685 (M + H$^+$, $C_{16}H_{31}N_4NiO_4$ requires 401.1699).

Cis Ether Nickel Complex (14). The cis ether cyclam **13** (38 mg, 0.060 mmol) and Ni(OAc)$_2$·4H$_2$O (18 mg, 0.072 mmol) were allowed to react according to the general conditions providing nickel complex **14** (29.5 mg, 72%, all N−H isomers) as a pink solid. Flash chromatography allowed for separation of the clean N−H isomers: MP 72−75 °C; ^{1}H NMR δ 9.03 (s, 2H), 8.03 (d, J = 8.1 Hz, 2H), 7.83 (d, J = 7.8 Hz, 2H), 7.63 (m, 2H), 7.54 (m, 2H), 5.24 (d, J = 13.5 Hz, 2H), 5.03 (d, J = 13.8 Hz, 2H), 3.34 (bt, J = 12.9, 2H), 2.82 (m, 4H), 2.35 (dd, J = 2.1, 11.4 Hz, 2H), 1.95 (dd, J = 3.0, 7.2 Hz, 2H), 1.43 (s, 6H), 1.38 (s, 6H), 1.33 (s, 6H); ^{13}C NMR δ 172.9, 153.8, 144.5, 142.0, 130.2, 129.5, 129.4, 129.0, 78.0, 77.4, 66.1, 66.0, 59.1, 57.5, 24.9, 23.2, 21.0; IR (neat) ν 3400, 1567 cm^{-1}; FABHRMS m/z 685.2756 (M + H$^+$, $C_{34}H_{43}N_8NiO_4$ requires 685.2761).

Trans Extended Amide Nickel Complex (19a). The trans extended amide cyclam **18a** (10.5 mg, 0.014 mmol) and Ni(OAc)$_2$·4H$_2$O (4.5 mg, 0.017 mmol) were allowed to react according to the general conditions providing nickel complex **19a** (8.0 mg, 71%) as a pink solid: MP 135−138 °C; ^{1}H and ^{13}C NMR are included in the supplemental (N−H isomers); IR (neat) ν 3400, 1665, 1570, 1532 cm^{-1}; MS m/z 799.8 (MH$^+$).

Cis Extended Amide Nickel Complex (19b). The cis extended amide cyclam **18b** (44 mg, 0.059 mmol) and Ni(OAc)$_2$·4H$_2$O (18 mg, 0.071 mmol) were allowed to react according to the general conditions providing nickel complex **19b** (34 mg, 72%, all N−H isomers) as a pink solid. Flash chromatography allowed for separation of the clean N−H isomers: MP 96−99 °C; ^{1}H NMR δ 9.62 (s, 2H), 8.27 (bt, J = 5.6 Hz, 2H), 8.08 (d, J = 8.0, 2H), 7.97 (d, J = 8.0 Hz, 2H), 7.78 (m, 4H), 4.08 (m, 4H), 3.78−3.90 (m, 4H), 3.12 (bt, J = 12.8 Hz, 2H), 2.80 (t, J = 10.8 Hz, 2H), 2.61 (t, J = 11.6 Hz, 2H), 2.19 (d, J = 9.2 Hz, 2H), 1.90 (dd, J = 3.2, 10.8 Hz, 2H), 1.41 (s, 6H), 1.35 (s, 6H), 1.25 (s, 6H); ^{13}C NMR δ 173.1, 163.8, 144.1, 143.9, 143.6, 140.3, 131.7, 131.0, 129.7, 129.6, 77.43, 66.1, 63.3, 59.0, 57.4, 40.7, 25.2, 23.1, 20.8; IR (neat) ν 3251, 1672, 1576, 1530 cm^{-1}; FABHRMS m/z 799.3159 (M + H$^+$, $C_{34}H_{49}N_{10}NiO_6$ requires 799.3190).

Cis Extended Amide Chiral Nickel Complex (23). The partially purified extended chiral cyclam **24** (58 mg, 0.070 mmol) and Ni(OAc)$_2$·4H$_2$O (22 mg, 0.087 mmol) were allowed to react according to the general conditions providing clean nickel complex **23** (34 mg, 55%, all N− H isomers) as a pink solid. Flash chromatography allowed for the separation of the clean N−H isomers: MP 128 °C D; [a]^{23}$_D$ −362.8 (c 0.43, CHCl$_3$); ^{1}H NMR δ 9.63 (s, 2H), 8.48 (bs, 2H), 8.08 (d, J = 6.9 Hz, 2H), 7.98 (d, J = 7.2 Hz, 2H), 7.77 (m, 4H), 3.98−4.22 (m, 8H), 3.59 (s, 6H), 2.95 (t, J = 12.0 Hz, 2H), 2.26−2.42 (m, 6H),

1.49 (s, 6H), 1.26 (s, 6H); ^{13}C NMR δ 176.3, 174.6, 164.0, 144.3, 144.0, 143.9, 140.4, 131.5, 130.8, 129.8, 129.6, 77.1, 64.0, 63.3, 60.4, 56.2, 52.4, 41.0, 20.8, 20.5; IR (neat) ν 1740, 1670, 1582 cm^{-1}; FABHRMS *m/z* 885.2824 (M $-$ H$^+$, C$_{40}$H$_{47}$N$_{10}$NiO$_{10}$ requires 885.2830).

Extended Amide Chiral Cyclam (24). The chiral amide nickel complex **23** (15 mg, 0.017 mmol) was dissolved in 2 mL of CH$_2$Cl$_2$ and brought to 0 °C with an ice bath. Concentrated HCl ($\sim$37%) was dripped in with a pipet ($\sim$3$-$5 drops) until the solution turned from pink to light yellow. At this time, 5% NaOH (2 mL) was very slowly dripped into the reaction and the contents were transferred to a separatory funnel. The organic layer was diluted with 20 mL of CH$_2$Cl$_2$ before washing with 3 $\times$ 5 mL of 5% NaOH. The aqueous layer was back extracted with 2 $\times$ 8 mL of CH$_2$Cl$_2$. The combined organic layers were dried with Na$_2$SO$_4$ and the solvent removed in vacuo to provide, after purification by flash chromatography (SiO$_2$, 4% i- PrOH/1%TEA, EA), the chiral amide cyclam (12 mg, 86%) as a light yellow solid: MP 144$-$146 °C; [a]$^{23}_D$ +20.7 (c 0.15, CHCl$_3$); ^{1}H NMR δ 9.68 (s, 2H), 8.30 (bs, 2H), 8.17 (m, 4H), 8.00 (bs, 2H), 7.85 (m, 4H), 3.60$-$3.80 (m, 10H), 3.69 (s, 6H), 2.80 (t, *J* = 12.0 Hz, 3H), 2.68 (t, *J* = 12.0 Hz, 3H), 1.61 (s, 6H), 1.32 (s, 6H); ^{13}C NMR δ 174.8, 171.9, 163.9, 144.2, 144.1, 143.8, 140.6, 131.8, 130.9, 129.9, 129.7, 80.5, 61.9, 61.4, 55.9, 53.3, 53.1, 40.1, 21.4, 19.5; IR (neat) ν 3394, 1737, 1677 cm^{-1}; FABHRMS *m/z* 831.3778 (M + H$^+$, C$_{40}$H$_{51}$N$_{10}$O$_{10}$ requires 831.3790).

General Procedure for Agarose Gel Electrophoresis Bisintercalation Studies with Cyclams (13), (18a), (18b), (24), and Their Nickel Complexes (14), (19a), (19b), (23). Because of the limited solubility of some of the cyclams in water, all agents were dissolved in DMSO as either 100$\times$ or 1000$\times$ stock solutions and diluted with DMSO to the working concentrations just prior to addition of the DNA solution. The DNA solution containing 0.25 μg of supercoiled ΦX174 RFI DNA (purchased from New England Biolabs and used without further purification) in 9 μL of 50 mM Tris-HCl buffer solution (pH 8) was treated with 1 μL of agent in DMSO. The control DNA was also treated with 1 μL DMSO. The [agent] to [DNA] base pair ratios tested were 0.02, 0.04, 0.1, 0.2, 1, 5, 20. Each agent was tested under a variety of conditions: 25 °C for 3 h, 25 °C for 15 h; 37 °C for 3 h, and 37 °C for 15 h. After incubation for the given time and temperature, the reactions were quenched with 5 μL of Keller loading buffer formed by mixing Keller buffer (0.4 M Tris-HCl, 0.05 M NaOAc, 0.0125 M EDTA, pH 7.9) with glycerol (40%), sodium dodecyl sulfate (0.4%), and bromophenol blue (0.3%). The agarose gels (1%) were run at 90 V for 3 h. The gel was stained with 0.1 μg/mL ethidium bromide for 30 min, then destained by soaking in water for 15$-$20 min. The gel was then photographed under UV transillumination at 302 nm with a Polaroid 667 ISO 3000/36° using black and white film.

General Procedure for DNA Nicking Studies Using Nickel Complexes (1), (3), (14), (19a), (19b), (25). Stock solutions of the compounds to be tested were prepared in water (the nickel complexes were much more soluble in water than the free ligand cyclams) as 2.5 mM stock solutions (except **1** which was prepared as a 625 μM stock solution because of its limited solubility in water). A stock solution of supercoiled pB*R*322 plasmid DNA (purchased from New England Biolabs and used without further purification) was prepared by diluting 12.5 μL of 1000 μg/mL solution with 372 μL of Tris-EDTA (10 mM Tris-HCl and 1 mM EDTA at pH 8). This solution was stored in the $-$20 °C freezer until just before use. A nicking reaction cocktail was prepared with 84 μL of 50 μM bp pBR322, 10.5 μL of 200 mM phosphate buffer, pH 7.4, 5.25 μL of 200 mM NaCl, and 5.25 μL of water which provided 105 μL of 40 μM base pair pB*R*322. A solution of 10 mM Oxone was prepared with 5.0 mg oxone and 813 μL of water. Nicking reactions were prepared with 5 μL of DNA cocktail and 4 μL of the nickel complex in water (the control DNA was also treated with 4 mL water.) After centrifugation, the reactions were allowed to equilibrate for 15 min before addition of 1 μL of 10mM Oxone, providing a solution of 20 μM base pair supercoiled pBR322 DNA, 10 mM phosphate, 5 mM NaCl, and 1 mM oxone. After 1 h the reactions were quenched with 1 μL of 10X Ficoll loading buffer (25% Ficoll 400, 0.25% bromophenol blue, and 0.25% xylene cyanol) then loaded onto a 1% agarose gel. The gel was run at 115 V for 1 h before staining with Sybr Gold nucleic acid stain (purchased from Molecular Probes) for 2 h and scanning on a Storm Scanner 840 (Molecular Dynamics). The data were analyzed by comparing the density of the remaining form I DNA of the reactions to the control lane's form I DNA using Image Quant version 5.0 (Molecular Dynamics).

Supporting Information Available: ^{1}H and ^{13}C NMR spectra for compounds **2, 3, 5, 8, 9, 10a:10b, 14, 15, 17, 18b, 19b, 21, 22, 23,** and **24**. For compounds **13, 18a,** and **19a**, ORTEP diagrams, crystal data and structure refinement parameters, atomic coordinates, bond lengths and bond angles, anisotropic displacement coefficients, and H-atom coordinates. This material is available free of charge via the Internet at http://pubs.acs.org.

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