Chiral DNA Gyrase Inhibitors. 3. Probing the Chiral Preference of the Active Site of DNA Gyrase. Synthesis of 10-Fluoro-6-methyl-6,7-dihydro-9-piperazinyl-2*H*-benzo[*a*]quinolizin-20-one-3-carboxylic Acid Analogues

Robert A. Fecik,[†] Pratik Devasthale,[†] Segaran Pillai,[†] Ali Keschavarz-Shokri,[†] Linus Shen,[‡] and Lester A. Mitscher*,[†]

Departments of Medicinal Chemistry and of Molecular Biosciences, University of Kansas, 4010 Malott Hall, 1251 Wescoe Hall Drive, Lawrence, Kansas 66045-7582, and Antiinfectives Research Department, Abbott Laboratories, Abbott Park, Illinois 60064

Received July 16, 2004

In pursuit of an apparent literature anomaly, *S*- and *R*-6-methyl-6,7-dihydro-2*H*-benzo[*a*]-quinolizin-2-one-3-carboxylic acids (**12** and **22**) were synthesized by an unambiguous route from optically active norephedrines, and their antibacterial potencies were measured. Against Gram-negative microorganisms and DNA gyrase a preference for *S*-absolute configuration was found whereas *R*-absolute stereochemistry was more active against Gram-positives. These results are in partial conflict with an earlier report. In an attempt to enhance potency, racemic 10-fluoro-9-piperazinyl (**35**) and related analogues were synthesized by a novel route. The latter analogues were surprisingly unimproved in potency. The implications of these findings are briefly discussed.

Introduction

The empirically driven evolution of ever more effective quinolone antiinfective agents is a powerful tribute to the power of modern medicinal chemical methodology. Nonetheless, a detailed understanding of their molecular modes of action and toxicity would be even more efficient. Unfortunately, accomplishing this presents major present challenges. These drugs are now known to operate through more than one target and target components. DNA gyrase, topoisomerase IV, and, to a lesser extent, human topoisomerase II all have significantly relevant affinities for quinolones when in the presence of target DNA. None of these enzymes have been crystallized. Further, the binding site consists of enzyme, DNA, and up to four quinolone molecules. Capturing all this in a crystal suitable for X-ray analysis will be a major accomplishment.

At present we are forced to rely instead on insights gained from analysis of the utility of specific quinolone structures in interfering with this process. The underlying assumption, however reasonable, is that all of these agents are acting in precisely the same manner.

With this backdrop in mind, in this paper we probe and examine further through synthesis of chiral ligands the best presently accepted predictive model for quinolone action, the Shen model as extended by Morressey et al. This model provides a satisfactory rationale for the very significant eudismic ratio observed when substituents attached to the quinolone nitrogen atom contain nearby chiral centers. Starting with flumequine and continuing with levofloxacin and subsequently with many more examples, it has been established that the *S*-enantiomers are very much more active in intact bacteria and at the enzyme level than the distomeric *R*-enantiomers.

There is, however, a preliminary report, albeit involving a different ring system, in which the opposite stereochemical preference is asserted. Operating on the principle that it is the exception that probes the rule and when properly interpreted can deepen our understanding, we have repeated this work and addressed this anomaly.

The absolute stereochemistry of a substituent containing a nearby chiral center attached near N-1 of rigid analogues of tricyclic quinolone antiinfective agents produces a very significant eudismic effect when the absolute chemistry is *S* in the cases of flumequine (**1**),[1] S-25930 (**2**),[1] benofloxacin (**3**),[2] and nadifloxacin (**4**).[3] The bioisosteric replacement of O for methylene as in levofloxacin (**5**)[4,5] and S-12681 (**6**),[6] as well as bioisoelectronic replacement of S for methylene as in GRB-23790 (**7**)[7] and WIN-58161 (**8**),[8] produces the same biological preference. The enantiopreference for *S*-configuration has now become orthodox as exemplified by recent development candidates pazufloxacin (T-3761) (**9**)[9] and DV-7751a (**10**)[10] and many other lead molecules. These structures are illustrated in Figure 1. This differential effect of chirality has been rationalized to be due to asymmetric stacking in a cooperative model of the molecular mode of action of these drugs.[11]

It is known also that the nature of the substituent attached to the α-carbon is significant for binding to DNA gyrase as opposed to human topoisomerase II.[12] Against the latter enzyme (associated with certain cytotoxicities), compounds with a methyl group are less active than those with a hydrogen atom or a methylene moiety at the corresponding position.

Thus, our attention was drawn to a preliminary literature report that in a different ring system (the 6-methyl-6,7-dihydro-2*H*-benzo[*a*]quinolizin-2-one-3-car-

* To whom correspondence should be addressed. Phone: (785) 864-4562. Fax: (785) 864-5326. E-mail: lmitscher@ku.edu.
[†] University of Kansas.
[‡] Abbott Laboratories.

10.1021/jm0401356 CCC: $30.25
Published on Web 01/26/2005

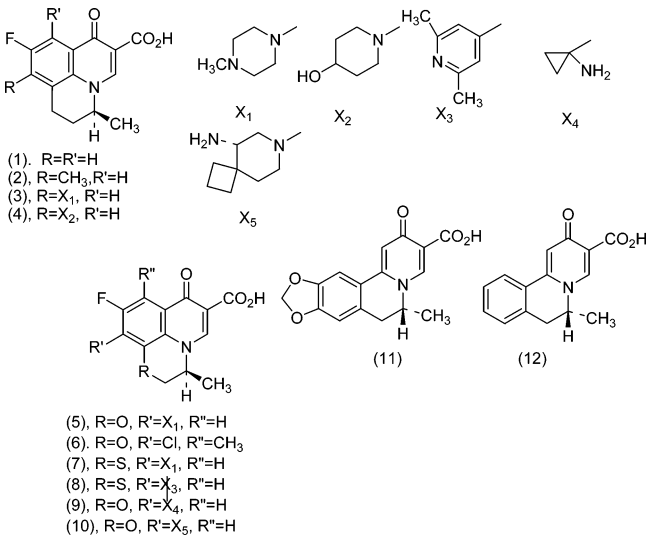


Figure 1. Structures of many of the previously described quinolone antiinfective agents possessing chiral substituents attached to N-1.

boxylic acids such as Ro-14−5319 (**11**) and its desmethylenedioxo analogue Ro-14-4299) (**12**)) the eutomer is reported to be *R*.[13,14] To advance our understanding of the structural requirements of the drug-binding pocket for quinolones, we decided to explore this seeming anomaly further by preparation of analogues using starting materials from the chiral pool so that the absolute stereochemistry of the products would be unambiguously established and additional chiral analogues could be prepared efficiently.

Chemistry

For the record, since the experimental details of the earlier work have not been published, we report herein the details of the synthesis of (***R***)-Ro-14-4299 (**12**) starting with different, optically active materials. This route (Scheme 1) proceeded without significant difficulty in our hands after only minor modifications following the briefly reported reaction sequence.[14] Because direct hydrogenolysis of the unneeded benzylic hydroxyl group of (1*S*,2*R*)-(+)-norephedrine (**13**) required rather forcing conditions, it was first diacetylated to ester amide (**14**) in 92% yield with acetic anhydride−pyridine. Hydrogenolysis of **14** then proceeded smoothly at atmospheric pressure and 80−90° C using 5% palladium−barium sulfate in 70% perchloric acid solution to produce *R*-2-acetamino-1-phenylpropane (**15**) in 72% yield. Next the *N*-acetyl group was hydrolyzed to produce chiral amine **16**, isolated in 90% yield as its hydrochloride salt. This was then formylated to amide **17** in 62% yield by heating with ethyl formate. A Bischler−Napieralski cyclization of **17** using polyphosphoric acid gave chiral 3,4-dihydroisoquinoline **18** in 65% yield as its hydrochloride salt.

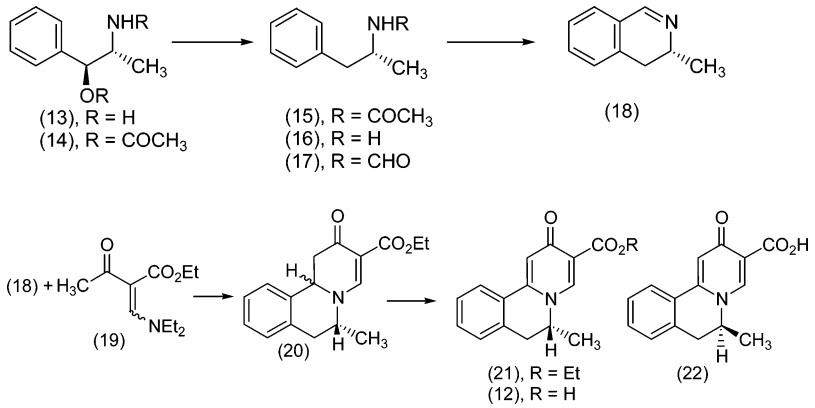
Reaction of ethyl acetoacetate with acetic anhydride and trimethyl orthoformate followed by reaction with diethylamine produced Michael acceptor diethylaminomethylene ethyl acetoacetate (**19**).[15] This cyclized to a 2:1 mixture of diastereoisomers (**20**) in 67% yield upon condensation with **18**. Oxidation of the resulting diastereoisomeric mixture with chloranil produced ester **21** in 82% yield. Sodium hydroxide hydrolysis produced the desired optically active target substance **12** ($[\alpha]_D^{24}$ +189.4° in chloroform) in 82% yield. This nine-step sequence of reactions gave overall a 10% yield.

The *S*-enantiomer **22** (Ro-14−5319) ($[\alpha]_D^{24}$ −185.1° in chloroform) was prepared with equivalent efficiency following the same route but starting with 1*R*,2*S*-(−)-norephedrine.

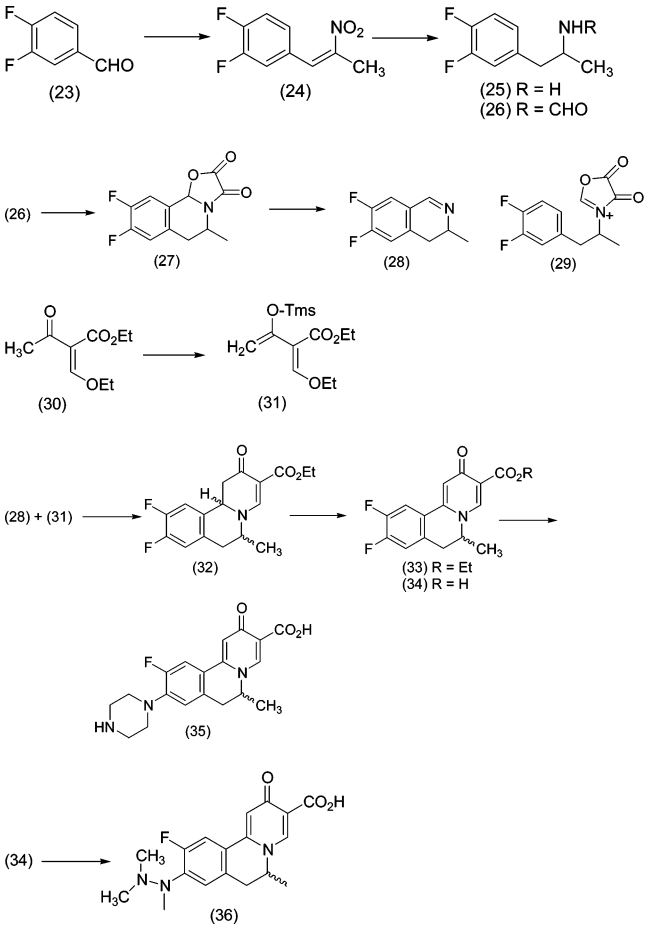
Considering the generalization based upon Pfeiffer's observation that chiral recognition most often increases in parallel with potency and/or receptor affinity[16] the synthesis of 10-fluoro-6-methyl-6,7-dihydro-9-piperazinyl-2*H*-benzo[*a*]quinolizin-20-one-3-carboxylic acid (**35**) was undertaken next (Scheme 2). The electronic contributions of the new groups to the pharmacophoric pyridine ring would be similar to those found in ciprofloxacin and levofloxacin. Synthesis of the necessary analogues by the same route as used for **12** and **22** failed due to the deactivating influences of the required fluorine-containing aromatic ring substituents in the intermediates, and a new route was therefore devised.

This route began with commercially available 3,4-difluorobenzaldehyde (**23**) and was carried out initially with racemic materials with the intention of later repeating the work with optically active materials or by resolution by any of a number of means and, in that case, identifying the stereochemistry of the eutomer and distomer by chiroptical comparisons with **12** and **22**. Thus, 3,4-difluorobenzaldehyde **23** was subjected to a Henry condensation by heating with nitroethane and ammonium acetate to produce nitro alkene **24** in 94% yield, and this in turn was converted to racemic primary amine **25** in 91% yield upon lithium aluminum hydride reduction. Formation of formamide **26** by reaction with

Scheme 1. Synthesis of *R*- and *S*-6-Methyl-6,7-dihydro-2*H*-benzo[*a*]quinolizin-2-one-3-carboxylic Acids



Scheme 2. Synthesis of 10-Fluoro-6-methyl-6,7-dihydro-9-piperazinyl-2*H*-benzo[*a*]quinolizine-20-one-3-carboxylic Acid (**35**) and Its 9-Dimethylhydrazino Analogue (**36**).



acetic formic anhydride proceeded in 100% yield. In contrast to the result when the aromatic ring contained only hydrogens, cyclization of **26** under a wide variety of standard Bischler−Napieralski conditions failed virtually completely. One presumes that the cyclization of the Ritter-type intermediate in the normally preferred manner is frustrated by decreased electronic contributions from the difluorinated aromatic ring.

Ultimately the modified Bischler−Napieralski conditions developed by Larsen et al. were found to succeed admirably despite their report that this variant gives poor results with electron deficient reactants.[17] Reaction of **26** with oxalyl chloride followed by cyclization with iron(III) chloride produced the diastereoisomeric oxalyl ester amide **27** in 85% yield for the two steps. The success of this process is likely due to the enhanced nucleophilicity of the presumed cyclic oxazolidinedione intermediate which forms a highly reactive *N*-acyliminium ion (**29**) further polarized in the presence of iron(III) chloride.

Compound **27** was immediately hydrolyzed and dehydrated with acidic methanol to give the previously elusive **28** in 73% yield. Unfortunately, however, this intermediate did not undergo the tandem Michael addition/cycloalkylation reaction with enamine **19** that had taken place previously with its nonhalogenated analogue (**18**).

Considering that the electron deficient imine moiety of dihydroisoquinoline **28** would likely be advantageous in a hetero Diels−Alder reaction and that reactions of Danishefsky's diene and related dienes with cyclic imines were known to proceed well in the synthesis of a variety of alkaloids,[18] this reaction was next attempted.

Reaction of ethyl ethoxymethyleneacetoacetate (**30**) with trimethylsilyl chloride and zinc chloride−triethylamine produced the carbethoxy analogue of the Danishefsky-type diene (**31**) in 75% yield.

When diene **31** was reacted with cyclic imine **28**, the desired dihydropyridone (**32**) was formed smoothly as a mixture of diastereoisomers that proved difficult to characterize spectroscopically, so it was aromatized promptly with chloranil to give readily characterized pyridone **33** in 47% yield for the two steps. The synthesis of the desired fluoroquinolone isomer was completed by alkaline hydrolysis to the corresponding acid (**34**) followed by a nucleophilic aromatic displacement reaction with piperazine to give the racemic target compound **35**.

The structure assigned to compound **35** is consistent with the normal outcome of analogous reactions in the well precedented benzopyridone parent series. In that case, the fluorine at position 7 is activated toward displacement over that at C-6 by the electron-withdrawing character of the carbonyl at C-4. As a result, the reaction produces products such as **1**−**10**, ciprofloxacin, norfloxacin, and a host of others. Clearly the same directing influences are expected to operate in the benzo[*a*]quinolizinone ring system of compound **34** due to the inductive effect of the analogous keto group.

For the reasons explicated in the Discussion section, analogue **36** was prepared in 64% yield by a boron difluoride-assisted nucleophilic aromatic displacement reaction with 1,1-dimethylhydrazine.

Results and Discussion

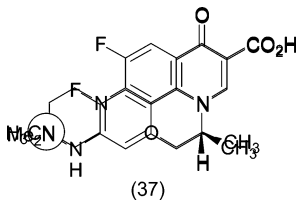
The *R*- and *S*-enantiomers **12** and **22** were tested in vitro against several Gram-positive and Gram-negative bacteria as well as against purified DNA gyrase isolated from Gram-negative *Escherichia coli* and Gram-positive *Micrococcus luteus* using standard, published, methodology.[15] The results are recorded in Table 1. In the present work these enantiomers are considerably less potent than has been reported for Ro-14-9578 enantiomers. Nevertheless the *S*-enantiomer was the more potent against the purified enzyme from *E. coli* H560 and against two strains of intact *E. coli* (SS [supersensitive] and H560 [from which the enzyme preparation was derived]. Against the Juhl strain, however, there was no enantiopreference, and against the KNK 437, strain potency was too weak to decide. Against *Pseudomonas aeruginosa*, once again the *S*-enantiomer was preferred. Thus, against intact Gram-negative microorganisms or against the purified DNA gyrase from *E. coli* the *S*-enantiomer (**22**) shows the same enantiopreference as does **1**−**10** so that **12** and **22** are not exceptional in this regard. These results conflict with those reported in the meeting abstract and poster.

The bioactivity against Gram-positive microorganisms *E. facium,* and *M. luteus* and from DNA gyrase obtained from it was too low to lead to any conclusion. On the other hand, against *S. aureus* the *R*-enantiomer was preferred in accord with the literature report for **11** and

Table 1. In Vitro Minimum Inhibitory Concentration Values (MIC, μg/mL) and Enzyme Inhibitory Concentrations for Synthetic Analogues Compared to Standard Ciprofloxacin (**38**)

microorganism	**12**	**22**	**35**	**36**	**38**
Staphylococcus aureus ATCC 6538P	25	50	25	25	0.2
Micrococcus luteus 9341	100	>100	–	–	1.56
Enterobacterium faecium ATCC 8043	>100	>100	–	–	0.78
Escherichia coli Juhl	25	25	50	25	0.02
Escherichia coli SS	6.25	1.56	–	–	0.005
Escherichia coli H560	50	25	–	–	0.01
Escherichia coli KNK 437	>100	>100	0.2	–	0.2
Pseudomonas aeruginosa	100	50	50	>25	0.05
E. coli H560 DNA gyrase (IC_{50} in μg/mL)[a]	32	20	30	–	3.1
M. luteus 934 DNA gyrase (IC_{50} in μg/mL)[a]	>250	>250	–	–	–

[a] The values reported for minimum inhibitor constants were obtained in the usual 2-fold agar dilution series manner as the average of triplicate runs. The values for the IC_{50} and CC_{50} determinations were obtained as the average of triplicate runs also using the methodology previously detailed.[25] Error bars are rarely if ever reported in the literature for such measurements; however, our experience is that the deviation is typically ±35% in our hands.



(37)

Figure 2. One putative stacking mode of ofloxacin and of 10-fluoro-6-methyl-6,7-dihydro-9-piperazinyl-2*H*-benzo[*a*]quinolizin-20-one-3-carboxylic acid.

12. It is possible that the different ring system of the benzo[*a*]quinolizines accounts for this partial departure from the prevailing pattern. Recent work demonstrating that topoisomerase IV, rather than DNA gyrase, is often the preferred target for quinolones in Gram-positives may provide a plausible rationale for this discrepancy.

The eudismic ratios measured for **12** and **22** do not exceed four against any of the bacteria and is even less against the purified enzyme. The data reported in the earlier work[14] (utilizing different bacterial strains) indicated a much higher potency and eudismic ratio (0.8−6.5 depending upon the strain of *E. coli* chosen and >62.5 against *S. aureus*). A later publication using racemic Ro-14-5319 (Ro-14−9578) confirmed that the primary target in Gram-negatives is DNA gyrase.[13] No results were reported for DNA gyrase from Gram-positives or against bacterial topoisomerase IV (that enzyme not being known at the time).

It seemed likely that the lower potencies for **12** and **22** were due to the lack of substituents in the aromatic ring in such as those generally associated with excellent activity in ordinary fluoroquinolones. Hence, substituted analogue **35** was prepared and tested. Disconcertingly, its in vitro antibacterial activity was scarcely improved.

It was initially hypothesized that the relative arrangement in space of the keto acid moieties in ring A of **12** and **22** result in placement of the distal amino group of the C-7 piperazino moiety in an incorrect place compared to that of ordinary quinolones (such as ciprofloxacin). This idea is illustrated in formula **37** (Figure 2)**.** To test this hypothesis, analogue **36** was prepared and tested as its distal amino function can occupy the same spatial relationship with respect to the keto acid moiety of typical fluoroquinolones. It was no more active than **35** making this rationalization unlikely. Upon the basis of these disappointments, synthesis of additional analogues of **12** and **22** and of the enantiomers of **35** and **36** was not undertaken.

Conclusions

Many publications support the idea that a in tricyclic quinolone analogues containing a methyl group on an asymmetrically substituted carbon attached to N-1, as with ofloxacin and its analogues, superior antimicrobial activity is obtained when the absolute stereochemistry is *S*. A single report challenges this in molecules possessing a different ring system. The results of this study do not agree with these conclusions when Gram-negative microorganisms are involved. Against Gram-positives, however, our results agree that the eutomer is *R*. Surprisingly, adornment of these new analogues with substituents associated with high potency among other quinolones failed to result in an improvement. Reorienting the distal nitrogen atom so that it can occupy the same space as that in classical quinolones also failed to result in an improvement. It seems likely then that the active site of the various enzymatic targets for these drugs differs sufficiently to distinguish between these analogues. Clearly, more work will be required to settle this issue conclusively. When a modeling consensus emerges that rationalizes the potency of fluoroquinolones at the molecular level, it should have sufficient power to rationalize these new findings.

Experimental Section

Optical rotations were recorded on a Perkin-Elmer polarimeter model 241. The concentrations for optical rotations are represented in units of g/100 mL. The biological testing was performed at Abbott laboratories. Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Infrared (IR) spectra were obtained on a Perkin-Elmer 1420 spectrophotometer. Proton (^{1}H NMR) and carbon (^{13}C NMR) nuclear magnetic resonance spectra were recorded on Varian FT-80, Varian XL-300, GE QE-300, or Bruker AM-500 spectrometers in the indicated solvents using tetramethylsilane as the internal standard, unless otherwise specified. All chemical shifts are expressed in parts per million (δ). Electron impact mass spectra (El), chemical ionization mass spectra (Cl), and high-resolution mass spectra were obtained on a Varian CH-5 or Ribermag R10-10 mass spectrometer. Microanalyses were performed using a Hewlett-Packard Model 185 CHN analyzer at the University of Kansas. Ultraviolet (UV) spectra were recorded on a Hewlett-Packard Diode Array 8450A. Flash chromatography and medium-pressure chromatography (MPLC) were performed on Merck silica gel (230−400 mesh) and gravity column chromatography on Merck silica gel (70−270 mesh). Preparative centrifugal thin-layer chromatography (radial chromatography) was performed on a Harrison Model 7924 Chromatotron using Merck silica gel 60 PF-254.

(1*R*,2*R*)-(+)-Norephedrine Diacetate (14). Ac_2O (31 mL, 0.33 mol) was added dropwise to a solution of (1*S*,2*R*)-(+)-norephedrine (**13**, 24.41 g, 0.16 mol) in pyridine (30 mL, 0.37 mol) at r.t. The reaction mixture was stirred for 24 h at r.t. after which it was poured onto about 10 g of crushed ice. Keeping the reaction mixture cool, the pH of the solution was adjusted to about 1 with concentrated HCl. The mixture was then extracted into CH_2Cl_2 (100 mL × 3). The organic layer was washed with 5% NaOH (50 mL × 3) and brine, dried (Na_2SO_4), filtered, and evaporated under reduced pressure to obtain

33.4 g (0.142 mol, 88%) of the crude diacetate. Recrystallization (CH_2Cl_2/ether/hexanes) afforded 32.5 g (86%) of colorless crystals: mp 97−98 °C,[19] R_f 0.38 (50:50-hexane:ethyl acetate); ^{1}H NMR (80 MHz, $CDCl_3$) δ 1.0 (d, J = 7.2 Hz, 3H), 1.9 (s, 3H), 2.1 (s, 3H), 4.1−4.6 (m, 1H), 5.75 (d, J = 4 Hz, 1H), 7.3 (s, 5H); IR (KBr) 3100, 1630, 1540, 1440, 1270, 1150, 1130, 650, 600 cm^{-1}; UV−vis (MeOH) λ_{max} 205 (12 048) nm; MS (El) *m/e* 236 (M^+ + 1, 0.66), 176 (4.9), 86 (99), 44 (100); $[\alpha]_D^{24}$ +79.88° (c = 0.022, $CHCl_3$); Analysis calcd for $C_{13}H_{17}NO_3$: C, 66.36; H, 7.28; N, 5.95; found: C, 66.39; H, 7.60; N, 6.18.

(1*S*,2*S*)-(−)-Norepinephrine Diacetate. This compound was synthesized in 95% yield from 1*S*,2*S*-norephedrine following the same procedure. It was recrystallized from CH_2Cl_2/hexane and then ether: mp 97−98 °C. TLC, IR, NMR, and MS agreed with those of the prior specimen.

(*R*)-(+)-Amphetamine Acetate (15). 5% Pd on $BaSO_4$ (5.160 g) was dispersed in glacial HOAc (15 mL) and saturated with hydrogen at r.t. and atmospheric pressure for about 1.5 h. A mixture of (1*S*,2*R*)-(+)-norephedrine diacetate (**14**, 5.344 g, 22.74 mmol), 70% perchloric acid (5 mL), and glacial HOAc (20 mL) were added to the catalyst dispersion. The hydrogenation was carried out at 95 °C at atmospheric pressure for 4.5 h. The reaction mixture was then filtered through Celite. The filtrate was treated with saturated solution of KOH, keeping the reaction mixture cool (by adding ice) until all potassium chlorate precipitated out. The solid was filtered off. The filtrate was further basified with KOH pellets, keeping the mixture cool (using ice), to pH 10−12. The amphetamine acetate was extracted into CH_2CI_2 × 3, and the organic layer was washed with brine, dried (Na_2SO_4), filtered, and evaporated in vacuo to yield 2.866 g (71%) of amphetamine acetate as a colorless solid. 1.419 g of this product were recrystallized from ether/hexanes to yield 1.39 g of a colorless, crystalline product: mp 122−123 °C; R_f 0.42 (50:50-hexane: ethyl acetate); ^{1}H NMR (80 MHz, $CDCI_3$) δ 1.10 (d, J = 7 Hz, 3H), 1.55 (s, 1H), 1.9 (s, 3H), 2.79 (dd, J = 7, 3 Hz, 2H), 4.0−4.4 (m, 1H), 7.15−7.30 (m, 5H); IR (KBr) 3240, 3070, 2960, 1640, 1560, 1370, 1300, 1200, 1130, 770, 745, 700 cm^{-1}; UV−vis (MeOH) λ_{max} 206 (11 315) nm; MS (El) *m/e* 178 (M^+ + 1, 1.7), 118 (18.6), 91(6.5), 86 (17.4), 44 (100.00); $[\alpha]_D^{24}$ +44.9° (c = 0.2428, $CHCl_3$);[20,21] Analysis calcd for $C_{11}H_{14}N0$: C, 74.52; H, 8.52; N, 7.91; found: C, 74.20; H, 8.80; N, 7.65.

(*S*)-(−)-Amphetamine Acetate. This compound was prepared in 55% yield following the same procedure as for its enantiomer. Following recrystallization from CH_2Cl_2/hexanes, it gave mp 120−122 °C. TLC, IR, NMR, and MS agreed with those of the prior specimen.

(*R*)-(−)-Amphetamine Hydrochloride (16). *R*-(+)-Amphetamine acetate (**15**, 2.860 g, 16.16 mmol) and 44% H_2SO_4 (16 mL) were refluxed for 40 h after which the mixture was poured on ice and basified with NaOH pellets to pH 12−14. The mixture was extracted into ether × 3 and washed with 5% HCl × 3. The aqueous layer was basified with 5% NaOH solution and extracted into ether × 3. The ether solution was concentrated, and dry HCl gas was bubbled through this solution under an atmosphere of argon. Ether was evaporated in vacuo to yield 2.330 g (84% crude) of a colorless solid: mp 153−154 °C (MeOH/ether); ^{1}H NMR (80 MHz, $CDCI_3$) δ 1.15 (d, J = 7 Hz, 3H), 2.75 (d, J = 8 Hz, 2H), 3.25−3.7 (m, 1H), 7.05−7.35 (m, 5H); $[\alpha]_D^{24}$ −13.0° (c = 0.0641, H_2O).[22,23]

(*S*)-(−)-Amphetamine Hydrochloride. This was prepared as above: mp 153−155 °C following recrystallization from EtOH/ether. TLC, IR, NMR and MS agreed with those of the prior specimen.

(*R*)-(+)-*N*-Formylamphetamine (17). (*R*)-(−)-Amphetamine hydrochloride (**16**, 3.069 g, 17.9 mmol) was dissolved in EtOH (12 mL) under an argon atmosphere. Ethyl formate (16.2 mL, 200.7 mmol) and Et_3N (2.7 mL, 20.54 mmol) were added dropwise in sequence, and the mixture was refluxed for 48 h. EtOH was evaporated in vacuo and residue taken up into CH_2CI_2 (25 mL). The organic layer was washed with water and 5% HCl (30 mL × 3), dried (Na_2SO_4), filtered, and evaporated in vacuo to yield 1.806 g of crude product which was chromatographed (gravity column) using 5% 2-propanol/CH_2CI_2 as the solvent system to obtain 1.548 g (62%) of a colorless liquid. Kugelrohr distillation gave an analytical sample as a colorless, crystalline solid.: mp 49−51 °C; ^{1}H NMR (300 MHz, $CDCI_3$) δ 1.1539 (d, J = 6.66 Hz) and 1.2688 (d, J = 6.57 Hz, 3H for both), 2.64−2.90 (m, 2H), 4.24−4.44 (m, 1 H), 5.5083 and 5.6910 (both s, 1H for both), 7.10−7.36 (m, 5H), 8.04 and 8.0754 (both s, 1H for both); ^{13}C NMR (75.4 MHz, $CDCI_3$) δ 19.9533/19.9815, 42.2626, 44.9111/44.9481, 126.5560/126.8341, 128.4137/128.6525, 129.3855, 137.5160, 160.4354; $[\alpha]_D^{24}$ +21.07° (c = 0.3037, $CHCl_3$).[24] Analysis calcd for $C_{10}H_{13}$-N0: C, 73.59; H, 8.03; N, 8.58; found: C, 73.43; H, 8.37; N, 8.70.

(*S*)-(−)-*N*-Formylamphetamine. This substance was prepared as above in 50% yield and melted at 49−51 °C following recrystallization. TLC, IR, NMR, and MS agreed with those of the prior specimen.

(*R*)-3-Methyl 3,4-dihydroisoquinoline Hydrochloride (18). (*R*)-(+)-*N*-formylamphetamine (**17**, 30.5 mg, 0.19 mmol) was flushed with argon, and hot PPA (1 mL) that was preheated to 170 °C was added all at once. The reaction flask was lowered into an oil bath at 170 °C under argon. After 1.5 h, the mixture was poured on ice, concentrated HCl (5 mL) was added, and neutral components were extracted into ether (3 × 10 mL). The aqueous layer was basified with 10% NaOH and the mixture extracted into ether (3 × 10 mL). The organic layer was washed with water and brine, dried (Na_2SO_4), filtered, and evaporated in vacuo to obtain a pale yellow liquid that after radial chromatography (50% ethyl acetate−hexanes) afforded a residue. This residue was dissolved in minimum methanolic HCl and evaporated again in vacuo to yield a white solid (24 mg, 71%): mp 180−192 °C (decomp); R_f 0.20 (50:50-hexane:ethyl acetate, free base); ^{1}H NMR (300 MHz, CD_3OD) δ 1.5241 (d, J = 6.72 Hz, 3H), 3.0875 (dd, J = 17.1, 10.86 Hz, 1H), 3.3547 (dd, J = 16.62, 6.36 Hz, 2H), 4.25−4.40 (m, 1H), 7.5032 (d, J = 7.35 Hz, 1 H), 7.5674 (t, J = 7.71 Hz, 1 H), 7.8296 (t, J = 7.8 Hz, 1 H), 7.9159 (d, J = 7.83 Hz, 1 H), 9.0799 (s, 1 H); $[\alpha]D^{24}$ +2.58 (c = 0.031, H_2O).

(*S*)-3-Methyl-3,4-dihydroisoquinoline Hydrochloride. This substance was prepared in 89.8% yield by the above process, mp 180−192 °C (dec) following recrystallization from MeOH/ether. TLC, IR, NMR, and MS agreed with those of the prior specimen.

Ethyl *N*,*N*-Diethylaminomethylene Acetoacetate (19). A solution of ethyl acetoacetate (1.3 g, 10 mmol), triethylorthoformate (3 g, 20.3 mmol), and acetic anhydride (6.5 mL) were heated under argon at 110 °C for 3 h after which the mixture was evaporated under reduced pressure, azeotroped with small amounts of toluene (5 mL × 3), and dried under high vacuum for about 3 h. The crude product was redissolved in CH_2CI_2 (10 mL), and 2 mL diethylamine was added via syringe under argon. After stirring the reaction mixture for 2 h at r.t., the mixture was evaporated in vacuo and chromatographed (3:7 ethyl acetate−hexanes) to obtain 1.89 g (89%) of a dark green oil which was used as such for condensation with 3-methyl-3,4-dihydroisoquinoline: ^{1}H NMR (300 MHz, $CDCI_3$) δ 1.1 914 (br. s, 6H), 1.3219 (t, J = 7.17 Hz, 3H), 2.2938 (s, 3H), 3.33−3.41 (m, 4H), 4.2392 (q, J = 7.17 Hz, 2H), 7.6386 (s, 1H); MS (El) *m/e* 213 (M^+, 18.0), 198 (26.8), 168 (40.8), 152 (91.1), 96 (67.4), 56 (81.0), 43 (100.0).

(*R*)-Ethyl 6-Methyl-1,6,7,1b-tetrahydro-2*H*-benzo[α]-quinolizin-2-one-3-carboxylate (20). (*R*)-(+)-3-Methyldihydroisoquinoline hydrochloride (**18**, 1.129 g, 5.896 mmol), **19** (1.346 g, 6.319 mmol), and *t*-BuOH (40 mL) were refluxed under argon for 48 h after which *t*-BuOH was evaporated off under reduced pressure. The residue was taken up in CH_2Cl_2 and washed with 5% HCl (3 × 15 mL) and brine, dried (Na_2SO_4), filtered, and evaporated in vacuo to obtain a brown pasty liquid which was purified by gravity column chromatography eluting with 5% 2-propanol/ CH_2CI_2 to yield an off-white solid (1.119 g, 66.6%) as a mixture of diastereomers (1.9:1). Part of this product was recrystallized from CH_2Cl_2/ether/hexanes to afford a colorless, crystalline solid: mp 128−129 °C; R_f = 0.43 (5% *i*-PrOH−CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.2 (d, J = 3 Hz, 3H), 1.3 (t, J = 6 Hz, 3H), 1.4 (d, J = 3 Hz, 3H), 2.4−

3.0 (m, 4H), 4.2 (q, J = 6 Hz, 2H), 4.75−4.90 (m, 1 H), 7.0−7.25 (m, 4H), 8.3 (s, 1H), 8.4 (s, 1H); lR (KBr) 3020, 2960, 1650, 1590, 1570 cm^{-1}; UV−vis (MeOH) λ_{max} 206 (32 419), 244 (37 834), 307 (37 596); Analysis calcd for $C_{17}H_{19}N0_3$: C, 71.54; H, 6.72; N, 4.91; found: C, 71.24; H, 6.80; N, 5.10.

(*S*)-Ethyl 6-Methyl-1,6,7,1b-tetrahydro-2*H*-benzo[α]-quinolizin-2-one-3-carboxylate. This compound was prepared as white needles, mp 128−9 °C (from CH_2Cl_2), in 71.4% yield as above. TLC, IR, NMR, and MS agreed with those of the prior specimen.

(*R*)-Ethyl 6-Methyl-6,7-dihydro-2*H*-benzo[*a*]quinolizin-2-one-3-carboxylate (21). (*R*)-**20** (50 mg, 0.175 mmol), p-chloranil (60 mg, 0.245 mmol) and dry benzene (3 mL) were refluxed under argon for 12 h after which benzene was removed under reduced pressure and the residue chromatographed (gravity) using 5% 2-propanol−CH_2Cl_2 as the solvent system to obtain 37 mg (81%) of a paste. This was scrubbed with hexanes (3 mL) to obtain a gray powder (23 mg). A small amount of this product was recrystallized from CH_2Cl_2−hexanes to obtain colorless crystals: mp 125−127 °C; R_f = 0.19 (5% *i*-PrOH−CH_2CI_2); ^{1}H NMR (80 MHz, $CDCl_3$) δ 1.25 (t, J = 6 Hz, 3H), 1.35 (d, J = 7 Hz, 3H), 2.8 (dd, J = 16, 3 Hz, 1H), 3.35 (dd, J = 16, 5.5 Hz, 1H), 4.3 (q, J = 6 Hz, 2H), 7.0 (s, 1H), 7.15−7.80 (m, 4H), 8.2 (s, 1H); IR (KBr) 2960, 1720, 1680, 1630 cm^{-1}; UV−vis (MeOH) λ_{max} 210 (19 348), 259 (30 225); MS (El) *m/e* calcd for $C_{17}H_{17}N0_3$: 283.1208, found 283.1216; 284 (M^+ + 1, 5.43), 253 (5.28), 239 (8.43), 211 (100.00), 167 (10.17); $[\alpha]_D^{24}$ +2.53 (c 0.0103, $CHCl_3$).

(*S*)-Ethyl 6-Methyl-6,7-dihydro-2*H*-benzo[*a*]quinolizin-2-one-3-carboxylate. This substance was prepared in amorphous as above in 88% yield and was subjected directly to hydrolysis without further purification. TLC, IR, NMR, and MS agreed with those of the prior specimen.

(*R*)-6-Methyl-6,7-dihydro-2*H*-benzo[*a*]quinolizin-2-one-3-carboxylic Acid (12). (*R*)-**21** (358 mg, 1.265 mmol) was dissolved in MeOH (3 mL) and 1 N NaOH (19 mL) was added dropwise at r.t. After stirring the mixture for 8 h, it was acidified to pH 1−2 with 1 N HCl when a colorless precipitate fell out of solution. The solid was filtered under aspirator vacuum and dried overnight on the high-vacuum pump to yield 262 mg (81%) of a colorless solid. Part of this product was recrystallized from CH_2Cl_2 to obtain shiny, colorless crystals: mp 250−251 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.363 (d, J = 6.6 Hz, 3H), 2.955 (dd, J = 16, 2.7 Hz, 1H), 3.492 (dd, J = 16.2, 5.4 Hz, 1H), 4.58−4.63 (m, 1H), 7.214 (s, 1H), 7.33−7.81 (m, 4H), 8.594 (s, 1H); IR (KBr) 3690−3090, 1700, 1640 cm^{-1}; UV−vis (MeOH) λ_{max} 258 (67 030), 210 (38 954) nm; MS (El) *m/e* 256 (M^+ + 1, 1.87), 211 (100.00), 167 (24.17), 139 (17.31), 115 (37.42), 89 (16.43), 63 (25.77), 53 (37.81); $[\alpha]_D^{24}$ +189.4° (c = 0.0199, $CHCl_3$); Analysis calcd for $C_{15}H_{13}N0_3$: C, 70.56; H, 5.14; N, 5.49; found: 0, 70.60; H, 5.28; N, 5.61; $[\alpha]_D^{24}$ −185.1° (c = 0.02035, $CHCl_3$) for the *S*-isomer (prepared as above in 21% yield following extensive recrystallizations from CH_2Cl_2/hexane, mp 251−252 °C. TLC, IR, NMR, and MS agreed with those of the prior specimen).

1-(3,4-Difluorophenyl)-2-nitropropene (24). A solution of 3,4-difluorobenzaldehyde (**23**, 14.94 g, 105 mmol) and ammonium acetate (67.5 mmol, 5.20 g) in nitroethane (250 mL) was refluxed 3 h. After removal of the solvent under reduced pressure, the yellow residue was partitioned between CH_2Cl_2 (300 mL) and H_2O (300 mL), and the layers were separated. The aqueous layer was extracted with CH_2Cl_2 (2 × 200 mL), and the combined organic layers were washed with sat. NaCl (1 × 200 mL), dried (Na_2SO_4), filtered, and evaporated to produce a yellow oil (19.62 g, 98.5 mmol, 94% yield). Upon standing, the oil solidified to bright yellow needles that were recrystallized from EtOH to a constant melting point: mp 46−48 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.48 (s, 3H), 7.14−7.33 (m, 3H), 7.79 (s, 1H); ^{13}C NMR (75.6 MHz, $CDCl_3$) δ 14.3, 71.4, 118.5 (d, J = 18 Hz), 119.1 (d, J = 18 Hz), 127.0, 127.0, 127.1, 131.6; IR (neat) 3050, 1650, 1590, 1510, 1410, 1315, 1290, 1270, 1110 cm^{-1}; MS (CI) *m/z* 200 (M^+ + H), 184, 168, 151, 141, 133; calcd for $C_9H_9F_2NO_2$: 200.0523, found 200.0518.

1-(3,4-Difluorophenyl)-2-aminopropane (25). To a stirred suspension of lithium aluminum hydride (3 equiv, 151 mmol, 5.72 g) in THF (130 mL) under N_2 was added dropwise a solution of nitropropene **24** (10.0 g, 50.2 mmol) in THF (100 mL) with cooling by an ice bath. After being refluxed 1 h, the mixture was cooled to 0 °C and excess LAH was decomposed by the sequential addition of H_2O (20 mL), 20% NaOH (20 mL), and H_2O (40 mL), then the suspension was stirred until no gray salts remained. The white salts were removed by filtration with suction, and the filter cake was washed with Et_2O (3×). The combined filtrate and washings were washed with sat. NaCl (1 × 350 mL), dried ($MgSO_4$), filtered, and evaporated under reduced pressure to a mobile, yellow oil (7.78 g, 45.5 mmol, 91% yield): ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.11 (d, J = 4 Hz, 3H), 1.26 (br s, 2H), 2.49 (dd, J = 12, 8 Hz, 1H), 2.66 (dd, J = 8, 4 Hz, 1H), 3.14 (m, 1H), 6.85−7.15 (m, 3H); ^{13}C NMR (75.6 MHz, $CDCl_3$) δ 23.7, 45.9, 48.7, 117.4 (d, J = 17 Hz), 118.2 (d, J = 17 Hz), 125.37, 125.42, 125.44, 125.49; IR (neat) 3340, 3260, 2950, 2910, 1600, 1510, 1420, 1270 cm^{-1}; MS (CI) *m/z* 172 (M^+ + H), 141, 127, 44; calcd for $C_9H_{12}F_2N$: 172.0913, found 172.0913.

1-(3,4-Difluorophenyl)-2-(*N*-formyl)aminopropane (26). To amine **25** (2.00 g, 11.7 mmol) in Et_2O (12 mL) cooled to 0 °C was slowly added acetic formic anhydride (1.6 mL) under N_2. After complete addition, the reaction was warmed to room temperature and stirred for 18 h. The reaction mixture was diluted with Et_2O (25 mL) and washed with H_2O (1 × 20 mL) and sat. NaCl (1 × 20 mL). The ethereal layer was dried ($MgSO_4$), filtered, and evaporated under reduced pressure to a red oil (2.32 g, 11.6 mmol, 99% yield): ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.15 (d, J = 8 Hz), 1.29 (d, J = 8 Hz, 3H for both), 2.64−2.88 (m, 2H), 4.39 (m, 1H), 5.85 (br s, 1H), 6.82−7.19 (m, 3H), 8.08 (s, 1H); ^{13}C NMR (75.6 MHz, $CDCl_3$) δ 20.3, 22.2, 41.9, 43.07, 45.5, 50.4, 117.6 (d, J = 17 Hz), 118.5 (d, J = 16 Hz), 125.55, 125.60, 125.62, 125.67, 161.0, 164.4; IR (neat) 3250, 3030, 2960, 2910, 2840, 1650, 1600, 1510, 1420, 1370, 1270 cm^{-1}; MS (CI) *m/z* 200 (M^+ + H), 154, 127, 72, 44; calcd for $C_{10}H_{12}F_2NO$: 200.0887, found 200.0895.

8,9-Difluoro-6,10b-dihydro-5-methyl-5*H*-oxazolo[2,3-*a*]-isoquinoline-2,3-dione (27). To formamide **26** (28.19 g, 142 mmol) in CH_2Cl_2 (1.4 L) under N_2 was added oxalyl chloride (1.1 equiv, 156 mmol, 19.83 g, 13.9 mL) and the solution was mechanically stirred at room temperature for 30 min. The solution was cooled to −10 °C, and iron(III) chloride (1.2 equiv, 170 mmol, 27.64 g) was added. The mixture was slowly brought to room temperature and stirred for 22.5 h. 2 M HCl (1.4 L) was added to quench the reaction, and the biphasic mixture was stirred at room temperature for 1 h. After the layers were separated, the organic layer was washed with sat. NaCl (1 × 700 mL), dried (Na_2SO_4), filtered, and evaporated under reduced pressure to a dark red oil (30.46 g, 120 mmol, 85% yield): ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.49 (d, J = 4 Hz, 3H), 2.72 (dd, J = 10, 4 Hz, 1H), 3.24 (dd, J = 10, 4 Hz, 1H), 4.59 (m, 1H), 6.48 (s, 1H), 7.09 (dd, J = 7, 4 Hz, 1H), 7.35 (dd, J = 6, 4 Hz, 1H); ^{13}C NMR (75.6 MHz, $CDCl_3$) δ 18.8, 34.1, 46.4, 81.0, 114.9 (d, J = 19 Hz), 118.4 (d, J = 18 Hz), 127.50, 127.56, 129.94, 130.00, 151.7, 158.6; IR (neat) 3050, 2980, 2930, 1815, 1730, 1510, 1415, 1325, 1310 cm^{-1}; MS (CI) *m/z* 254 (M^+ + H), 182, 166, 127; calcd for $C_{12}H_{10}F_2NO_3$: 254.0629, found 254.0627.

6,7-Difluoro-3,4-dihydro-3-methylisoquinoline (28). Oxazolidinedione **27** (30.46 g, 120 mmol) in concentrated H_2SO_4−MeOH (1:19, 1.2 L) was refluxed for 20h. After cooling, the solvent was evaporated under reduced pressure. The dark red residue was partitioned between H_2O (500 mL) and EtOAc (500 mL). The organic layer was washed with 2 M HCl (2 × 250 mL). The combined aqueous and acidic washings were made basic with NH_4OH and then extracted into CH_2Cl_2 (3 × 250 mL). The combined organic extracts were washed with sat. NaCl (1 × 500 mL), dried (Na_2SO_4), filtered, and evaporated under reduced pressure to a dark yellow oil (15.79 g, 87.2 mmol, 73% yield): ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.49 (d, J = 4 Hz, 3H), 2.48 (dd, J = 10, 8 Hz, 1H), 2.75 (dd, J = 10, 4 Hz, 1H), 3.70 (m, 1H), 6.96 (dd, J = 6, 4 Hz, 1H), 7.12 (dd,

J = 8, 6 Hz, 1H), 8.20 (d, J = 2 Hz, 1H); ^{13}C NMR (75.6 MHz, $CDCl_3$) δ 18.8, 34.1, 81.0, 114.9 (d, J = 19 Hz), 118.4 (d, J = 18 Hz), 127.50, 127.56, 129.94, 130.00, 158.6; IR (neat) 3050, 2980, 2930, 1815, 1730, 1510, 1415, 1325, 1310 cm^{-1}; MS (CI) m/z 182 (M^+ + H); calcd for $C_{10}H_{10}F_2N$: 182.0781, found 182.0756.

Ethyl 2-Ethoxymethylene-3-trimethylsiloxy-3-butenoate (31). Fused zinc chloride (260 mg) was added to triethylamine (2.2 equiv, 139 mmol, 14.10 g, 19.4 mL) and stirred for 1 h at room temperature under N_2 until the salt was suspended in the amine. To this was added ethyl (ethoxymethylene)-acetoacetate (**30**, 11.79 g, 63.3 mmol) in benzene (14.2 mL) and then chlorotrimethylsilane (2.0 equiv, 127 mmol, 13.80 g, 16.1 mL). After 30 min, the temperature was raised to 40 °C and the reaction was stirred for 19.5 h. The mixture was filtered with suction and then run through a plug of Celite 545 with the aid of Et_2O. All remaining particulate matter was removed by two gravity filtrations through three filter paper circles, and the resulting solution was evaporated under reduced pressure to a dark red oil (12.30 g, 47.6 mmol, 75% yield): ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.10 (s, 9H), 1.01−1.15 (m, 6H), 3.93−4.08 (m, 4H), 4.14 (d, J = 2 Hz, 2H), 6.59 (s, 1H); ^{13}C NMR (75.6 MHz, $CDCl_3$) δ 0.0, 14.2, 15.2, 60.3, 70.3, 91.9, 97.6, 151.7, 153.1, 165.8; IR (neat) 2960, 2890, 1710, 1620, 1370, 1300, 1240, 1175, 1125, 1070, 1010, 840 cm^{-1}; MS (CI) m/z 259 (M^+ + H), 207, 187, 145, 119; calcd for $C_{12}H_{23}O_4Si$: 259.1368, found 259.1366.

Ethyl 9,10-Difluoro-6-methyl-1,6,7,11b-tetrahydro-2*H*-benzo[*a*]quinolizin-2-one-3-carboxylate (32). To dihydroisoquinoline **28** (5.00 g, 27.6 mmol) in CH_2Cl_2 (80 mL) at room temperature under N_2 was added trifluoroacetic acid (1.0 equiv, 27.6 mmol, 3.15 g, 2.1 mL). After 5 min, boron trifluoride diethyl etherate (1.0 equiv, 27.6 mmol, 3.92 g, 3.5 mL) was added. After another 5 min, diene **31** (1.5 equiv, 41.4 mmol, 10.70 g) was added and the mixture was stirred at room temperature for 2 h. The reaction was quenched by washing with $NaHCO_3$ (2 × 80 mL). The organic phase was separated, dried (Na_2SO_4), filtered, and evaporated under reduced pressure. The resulting dark oil was dissolved in MeOH (110 mL) and stirred over anhyd Na_2CO_3 (2.76 g) at room temperature for 1 h. The suspension was filtered and concentrated under reduced pressure, and the residue was partitioned between CH_2Cl_2 (100 mL) and H_2O (100 mL). The organic phase was dried (Na_2SO_4), filtered, and evaporated under reduced pressure to a hard, dark brown residue (8.73 g, 27.2 mmol, 99% yield) that was a complex mixture of diastereomers: ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.92−1.47 (m, 6H), 2.19−2.86 (m, 4H), 3.19−3.35 (m, 1H), 4.03−4.28 (m, 2H), 4.67−4.72 (m, 1H), 6.85−7.10 (m, 2H), 8.24 (s), 8.35 (s, 1H for both); MS (CI) m/z 322 (M^+ + H), 276, 240, 194, 182, 127.

Ethyl 9,10-Difluoro-6-methyl-6,7,-dihydro-2*H*-benzo[*a*]-quinolizin-2-one-3-carboxylate (33). Dihydropyridone **32** (0.15 g, 0.47 mmol) and tetrachloro-1,4-benzoquinone (1.5 equiv, 0.71 mmol, 170 mg) in THF (2 mL) was refluxed for 23 h. The solvent was evaporated under reduced pressure, and the crude product was purified by column chromatography (10:1, MeOH−$CHCl_3$) to give a dark brown residue (70 mg, 0.22 mmol, 47% yield): mp 134−135 °C (dec); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.24−1.42 (m, overlapping d, 1.30, J = 4 Hz, t, 1.36, J = 4 Hz, 6H), 2.82 (dd, J = 1, 10 Hz), 1H), 3.37 (dd, J = 2, 6 Hz, 1H), 4.29−4.50 (m, overlapping q, 4.29, J = 4 Hz, m, 4.43, 3H), 6.86 (s, 1H), 7.09 (dd, J = 5, 1 Hz, 1H), 7.52 (dd, J = 8, 2 Hz, 1H), 8.24 (s, 1H); ^{13}C NMR (75.6 MHz, $CDCl_3$) δ 14.4, 19.9, 33.6, 57.0, 61.1, 114.9 (d, J = 20 Hz), 118.1 (d, J = 26 Hz), 123.7, 128.9, 141.5, 146.0, 148.0, 150.1, 151.2, 153.5, 164.6, 175.8; IR (KBr) 2970, 1720, 1625, 1500 cm^{-1}; MS (CI) m/z 320 (M^+ + H), 247, 292; calcd for $C_{17}H_{16}NO_3F_2$: 320.1070, found 320.1098.

9,10-Difluoro-6-methyl-6,7,-dihydro-2*H*-benzo[*a*]quinolizin-2-one-3-carboxylic Acid (34). Ester **33** (380 mg, 1.2 mmol) was dissolved in MeOH (2.8 mL), and 1 M NaOH (18.5 mL) was added dropwise at room temperature and stirred for 5 h. The reaction was acidified to pH 1−2 with 1 M HCl, and a solid precipitated from the solution. The solid was filtered with suction and dried under vacuum pressure overnight to a light brown powder (240 mg, 0.83 mmol, 69% yield): ^{1}H NMR (300 MHz, TFA-d) δ 1.54 (d, J = 4 Hz, 3H), 3.15 (br d, J = 10 Hz, 1H), 3.65 (br d, J = 10 Hz, 1H), 5.17 (m, 1H), 7.34 (br t, J = 6 Hz, 1H), 7.78 (s, 1H), 7.88 (dd, J = 4, 2 Hz, 1H), 9.24 (s, 1H); ^{13}C NMR (125.8 MHz, TFA-d) δ 19.8, 34.1, 64.1, 112.1, 114.3, 114.6, 116.6, 118.6 (d, J = 21.9 Hz), 118.9, 121.2 (d, J = 19.0 Hz), 150.2, 152.0 (d, J = 48.7 Hz), 152.8 (d, J = 48.7 Hz), 170.7, 174.7; IR (KBr) 3390, 3000, 2940, 1685, 1610, 1490, 1410, 1290, 1250, 1190, 1140, 860 cm^{-1}; MS (CI) m/z 292 (M^+ + H), 247; calcd for $C_{15}H_{12}NO_3F_2$: 292.0769, found 292.0785.

10-Fluoro-6-methyl-6,7,-dihydro-9-piperazinyl-2*H*-benzo[*a*]quinolizin-2-one-3-carboxylic Acid (35). A mixture of difluoride **34** (50 mg, 0.17 mmol), piperazine (1.5 equiv, 0.26 mmol, 22 mg), triethylamine (3 equiv, 0.51 mmol, 52 mg), and acetonitrile (1.5 mL) was refluxed for 29.5 h. After cooling, the volatiles were removed under reduced pressure. The residue was triturated with Et_2O and filtered with suction to provide a dark brown solid (21 mg, 0.06 mmol, 35% yield): mp 216−217 °C (dec); ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.12 (d, J = 4 Hz, 3H), 2.74−2.88 (m, 9H), 3.01−3.10 (m, 1H), 4.39 (m, 1H), 6.93 (d, J = 5 Hz, 1H), 7.26 (s, 1H), 7.86 (d, J = 9 Hz, 1H), 8.76 (s, 1H); ^{13}C NMR (125.8 MHz, TFA-d) δ 20.0, 34.4, 43.6, 46.7, 48.9, 49.0, 64.2, 113.0, 121.4, 121.9, 133.6, 145.1, 150.0, 153.4, 155.8, 157.8, 166.6, 171.0, 174.3; IR (KBr) 3520, 3440, 3370, 1620, 1600 cm^{-1}; MS (CI) m/z 358 (M^+ + H), 314, 292, 129, 128, 115, 87; calcd for $C_{19}H_{21}FN_3O_3$: 358.1541, found 358.1567.

10-Fluoro-6-methyl-6,7,-dihydro-9-(1,1-dimethylhydrazinyl)-2*H*-benzo[*a*]quinolizin-2-one-3-carboxylic Acid (36). A mixture of difluoride **34** (50 mg, 0.17 mmol) in 48% tetrafluoroboric acid (1 mL) was heated at 90−100° for 19 h. After being cooled to room temperature, the suspension was poured into ice−water (5 mL) and filtered with suction. The solid was washed with water and dried under vacuum pressure to give a dark brown solid (55 mg). A mixture of this intermediate boron difluoride chelate (55 mg, 0.16 mmol), 1,1-dimethylhydrazine (1.5 equiv, 0.24 mmol, 25 mg, 19 μL), triethylamine (3 equiv, 0.49 mmol, 50 mg, 69 μL), and acetonitrile (1 mL) was refluxed for 24 h. After cooling, the volatiles were removed under reduced pressure. The residue was triturated with Et_2O and filtered with suction to provide a light brown solid (34 mg, 0.10 mmol, 64% yield): ^{1}H NMR (300 MHz, TFA-d) δ 1.70 (d, J = 4 Hz, 3H), 3.28−3.90 (m, 8H), 5.33 (m, 1H), 7.43−7.56 (m, 1H), 7.93−8.38 (m, 3H); IR (KBr) 3450 (br), 3400, 1695, 1625, 1605, 1300 cm^{-1}; MS (CI) m/z 332 (M^+ + H), 329, 307, 289, 254, 232, 176, 154, 137; calcd for $C_{17}H_{19}N_3O_3F$: 332.3487, found 332.341.

Acknowledgment. The authors gratefully acknowledge partial financial support of this work by grants from the NIH-AID Institute, USA, the Kansas Health Foundation for a stipend to R.A.F., and the American Foundation for Pharmaceutical Education for a stipend to P.D.. We also thank Drs. Qun Li, Daniel T. W. Shu, Jacob Plattner, and their associates at Abbott Laboratories for some of the microbiological evaluations.

References

(1) Gerster, J. R., SR; Pecore, S. E.; Winandy, R. M.; Stern, R. M.; Landmesser, J. E.; Olsen, R. A.; Gleason, W. B. Synthesis, absolute configuration and antibacterial activity activity of 6,7-dihydro-5,8-dimethyl-9-fluoro-1-oxo-1*H*,5*H*-benzo[ij]quinolizine-2-carboxylic acid. *J. Med. Chem.* **1987**, *30*, 839−843.

(2) Ishikawa, H.; Tabusa, F.; Miyamoto, H.; Kano, M.; Ueda, H.; et al. Studies on antibacterial agents. Synthesis of substituted 6,I.;7-dihydro-1-oxo-1*H*,5*H*-benzo[i,j]quinolizine-2-carboxylic acids. *Chem. Pharm. Bull. (Tokyo)* **1989**, *37*, 2103−2108.

(3) Morita, S.; Otsubo, K.; Matsubara, J.; Ohtani, T.; Kawano, Y.; et al. Synthesis of possible metabolites of 1-cyclopropyl-1,4-dihydro-6-fluoro-5-methyl-7-(3-methyl-1-piperazinyl)- 4-oxo-3-quinolinecarboxylic acid (Grepafloxacin, OPC-17116). *Chem. Pharm. Bull. (Tokyo)* **1995**, *43*, 2246−2252.

(4) Atarashi, S.; Yokohama, S.; Yamazaki, K.; Sakano, K.; Imamura, M.; et al. Synthesis and antibacterial activities of optically active ofloxacin and its fluoromethyl derivative. *Chem. Pharm. Bull. (Tokyo)* **1987**, *35*, 1896−1902.

(5) Hayakawa, I.; Atarashi, S.; Yokohama, S.; Imamura, M.; Sakano, K.; et al. Synthesis and antibacterial activities of optically active ofloxacin. *Antimicrob. Agents Chemother.* **1986**, *29*, 163−164.
(6) Gerster, J. R., Sr.; Rustad, N. J.; Reiter, M. J.; Pecore, S. E.; Winandy, R. M.; Landmesser, J. E. *Quinolones*; J. R. Prous Science Publishers: Barcelona, 1989; p 85.
(7) Anon. European Patent 0 368 410 A2, 1995.
(8) Coughlin, S. A.; Danz, D. W.; Robinson, R. G.; Klingbeil, K. M.; Wentland, M. P.; et al. Mechanism of action and antitumor activity of (*S*)-10-(2,6-dimethyl-4-pyridinyl)-9-fluoro-3-methyl-7-oxo-2,3-dihydro-7*H*-pyridol[1,2,3-de]-[1,4]benzothiazine-6-carboxylic acid (WIN 58161). *Biochem Pharmacol* **1995**, *50*, 111−122.
(9) Fukuoka, Y.; Ikeda, Y.; Yamashiro, Y.; Takahata, M.; Todo, Y.; et al. In vitro and in vivo antibacterial activities of T-3761, a new quinolone derivative. *Antimicrob. Agents Chemother.* **1993**, *37*, 384−392.
(10) Miyadera, A.; Satoh, K.; Imura, A. Efficient synthesis of a key intermediate of DV-7751 via optical resolution or microbial reduction. *Chem. Pharm. Bull. (Tokyo)* **2000**, *48*, 563−565.
(11) Morrissey, I.; Hoshino, K.; Sato, K.; Yoshida, A.; Hayakawa, I.; et al. Mechanism of differential activities of ofloxacin enantiomers. *Antimicrob. Agents Chemother.* **1996**, *40*, 1775−1784.
(12) Sato, K.; Hoshino, K.; Tanaka, M.; Hayakawa, I.; Osada, Y.; Nishino, T.; Mitsuhashi, S. *Molecular Biology of DNA Topoisomerases and Its Application in Chemotherapy, Proc. Int. Symp.*; CRC: Boca Raton, FL, 1993; p 167.
(13) Georgopapadakou, N. H.; Dix, B. A.; Angehorn, P.; Wick, A.; Olson, G. L. Monocyclic and tricyclic analogs of quinolones: mechanism of action. *Antimicrob. Agents Chemother.* **1987**, *31*, 614−616.
(14) Georgopapadakou, N. H.; Dix, B. A.; Angehorn, P.; Wick, A.; Olson, G. L. *Program Abstr. 25th Intersci. Conf. Antimicrob. Agents Chemotherapy*; ASM: Washington, DC, 1985; pp Abstr. 129.
(15) Chu, D. T.; Fernandes, P. B.; Claiborne, A. K.; Pihuleac, E.; Nordeen, C. W.; et al. Synthesis and structure−activity relationships of novel arylfluoroquinolone antibacterial agents. *J. Med. Chem.* **1985**, *28*, 1558−1564.
(16) Lien, E. J.; Miranda, J. F.; Ariens, E. J. Quantitative structure−activity correlation of optical isomers: a molecular basis for Pfeiffer's rule. *Mol. Pharmacol.* **1976**, *12*, 598−604.
(17) Larsen, R. D.; Reamer, R. A.; Corley, E. G.; Davis, P.; Grabowski, E. J. J.; Reider, P. J.; Shinkai, E. A modified Bischler-Napieralski procedure for the synthesis of 3-aryl-3,4-dihydroisoquinolines. *J. Org. Chem.* **1991**, *56*, 6034−6038.
(18) Danishefsky, S.; Langer, M. E.; Vogel, C. On the use of the imine-diene cyclocondensation reaction in the synthesis of yohimbine congeners. *Tetrahedron Lett.* **1985**, *26*, 5983−5986.
(19) Kisfaludy, L.; Mohacsi, T.; Lowe, M.; Drexler, F. Pentafluorophenyl acetate: a new, highly selective acetylating agent. *J. Org. Chem.* **1979**, *44*, 654−656.
(20) Leonard, N. J.; Adamcik, J. A.; Djerassi, C.; Halpern, O. Transannular nitrogen−carbon interaction in cyclic aminoketones and optical rotatory dispersion. *J. Am. Chem. Soc.* **1958**, *80*, 4858−4862.
(21) Welsh, L. H.; Report on dextro racemic amphetamines. *J. Assoc. Off. Agr. Chem.* **1953**, *36*, 714−722.
(22) von Braun, J.; Friemelt, E. Abbau optisch-aktiver Carbonsaeuren mit Stickstoffswasserstoffsaeure und Schwefelsaeure. *Ber.* **1933**, *66*, 684−685.
(23) Liethe, W. Die Konfiguration der Ephedrin-basen. *Ber.* **1932**, *65*, 660−666.
(24) Cavallito, C. J.; Grey, A. P. US Patent 3,489,840, 1970.
(25) Frank, K. E.; Devasthale, P. V.; Gentry, E. J.; Ravikumar, V. T.; Keschavarz- Shokri, A.; Mitscher, L. A.; Nilius, A.; Shen, L. L.; Shawar, R.; Baker, W. R. *Combinatorial Chem., High Throughput Screening* **1998**, *1*, 73−83.

JM0401356