gradient of 36–45% sucrose (w/w).

Adenylate Cyclase Assay. Adenylate cyclase activity in the purified kidney membranes was assayed under conditions similar to that described by Neuman and Schneider[17] except that the $MgCl_2$ concentration was 1 mM, 0.5 mM 3-isobutyl-1-methylxanthine was used in place of theophylline, and 0.2 mM [8-^{14}C]ATP was used as substrate. The [8-^{14}C]cAMP product was isolated by column chromatography by the procedure of White and Karr[18] with [2,8-^{3}H]cAMP as tracer.

Inhibition of Prolactin Secretion. Pituitary cells were isolated from estradiol-primed rats as previously described.[19] Prolactin release was stimulated with 10^{-7} M TRH. The effect of varying concentrations of sCT or analogues on prolactin release was measured after 3 h of incubation. Prolactin levels in the culture media were measured by a double-antibody radioimmunoassay using materials and protocols supplied by the National Pituitary Agency of the NIADDK.

Acknowledgment. We are grateful to the NIADDK for generously supplying rat prolactin radioimmunoassay reagents. This work was supported by Grant A9848 from the Natural Sciences and Engineering Research Council of Canada.

Registry No. sCT, 47931-85-1; des-Leu^{19}-sCT, 103977-62-4; des-Gln^{20}-sCT, 114504-94-8; des-Thr^{2}-sCT, 105004-95-3; des-Tyr^{22}-sCT, 78312-75-1; des-Leu^{19}, Gln^{20}-sCT, 114504-95-9; des-Leu^{19}, Gln^{20}, Thr^{21}-sCT, 114532-34-2; des-Leu^{19}, Gln^{20}, Thr^{21}, Tyr^{23}-sCT, 114504-96-0; adenylate cyclase, 9012-42-4; prolactin, 9002-62-4.

(17) Neuman, W. F.; Schneider, N. *Endocrinology* **1980**, *107*, 2082.
(18) White, A. A.; Karr, D. B. *Anal. Biochem.* **1978**, *85*, 451.
(19) Shah, G. V.; Epand, R. M.; Orlowski, R. C. *J. Endocr.* **1988**, *116*, 279.

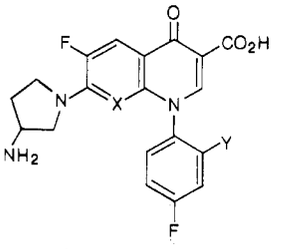
Design, Synthesis, and Properties of (4*S*)-7-(4-Amino-2-substituted-pyrrolidin-1-yl)quinolone-3-carboxylic Acids[†,1]

Terry Rosen,*[‡] Daniel T. W. Chu, Isabella M. Lico, Prabhavathi B. Fernandes, Kennan Marsh, Linus Shen, Valerie G. Cepa, and André G. Pernet

Abbott Laboratories, Abbott Park, Illinois 60064. *Received December 16, 1987*

The quinolonecarboxylic acids constitute a class of extremely potent and orally active broad-spectrum antibacterial agents. These compounds have been shown to inhibit DNA gyrase, a key enzyme in bacterial DNA replication. The 7-(3-aminopyrrolidinyl)quinolone A-60969 (1) is a particularly potent member of this class and is currently undergoing clinical evaluation. We have studied a series of enantiomerically homogeneous (4*S*)-7-(4-amino-2-substituted-pyrrolidinyl)quinolones in an effort to utilize the 2-position of the pyrrolidine moiety to improve upon the solubility and pharmacokinetic properties of this class of compounds while still maintaining potent antibacterial activity. We have found that the absolute stereochemistry at the 2-position of the pyrrolidine ring is critical to the maintenance of such activity. In this paper, we report the full details of the asymmetric synthesis and the in vitro and in vivo structure–activity relationships of this series of compounds as well as the physiochemical properties, such as water solubility and log *P*, associated with the structural modifications. We also discuss the pharmacokinetic properties of several of these compounds in mice and the pharmacokinetics of **59**, which has the best overall properties of agents in this study, in dog.

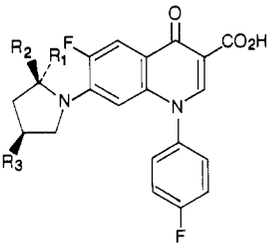
The quinolonecarboxylic acids constitute a class of extremely potent and orally active broad-spectrum antibacterial agents.[2] These compounds have been shown to inhibit DNA gyrase, a key enzyme in bacterial DNA replication.[3] The 7-(3-aminopyrrolidinyl)quinolones **1**–**3** are



1: X = N; Y = F
2: X = CH; Y = F
3: X = CH; Y = H

particularly potent members of this class; compound **1** (A-60969) is currently undergoing clinical evaluation.[4] Although these (aminopyrrolidinyl)quinolones show excellent antibacterial activity, they usually have very low solubility.

We recently reported that compound **4** is significantly more potent than its enantiomer **5**.[5] We also found that



4: $R_1 = CH_2OH$; $R_2 = R_3 = H$
5: $R_1 = R_3 = H$; $R_2 = CH_2OH$
6: $R_1 = R_2 = H$; $R_3 = NH_2$

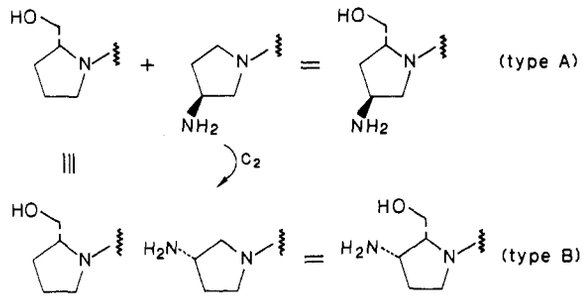
[†] This paper is dedicated to Professor E. C. Taylor on the occasion of his 65th birthday.

[‡] Current address: Pfizer, Medicinal Chemistry Department, Central Research Division, Groton, CT 06340.

(1) For a preliminary report of this work, see: Rosen, T.; Chu, D. T. W.; Lico, I.; Fernandes, P. B.; Shen, L.; Pernet, A. *Abstracts of the 27th Interscience Conference on Antimicrobial Agents and Chemotherapy*; New York, NY, October 4–7, 1987; Vol. 115, Abstr. No. 253. Rosen, T.; Chu, D. T. W.; Fesik, S. W.; Cooper, C. S.; Grief, V. J.; Fernandes, P. B.; Shen, L. L.; Pernet, A. G. *Abstracts of Papers*, 193rd National Meeting of the American Chemical Society, Denver, CO, April 5–10, 1987; American Chemical Society: Washington, DC, 1987; MEDI 66. Portions of this work will be presented as part of the International Telesymposia on Quinolones, 1988.
(2) Cornett, J. B.; Wentland, M. P. *Annu. Rep. Med. Chem.* **1986**, *21*, 139 and references therein.
(3) Cozzarelli, N. R. *Science (Washington, D.C.)* **1980**, *207*, 953.

0022-2623/88/1831-1598$01.50/0

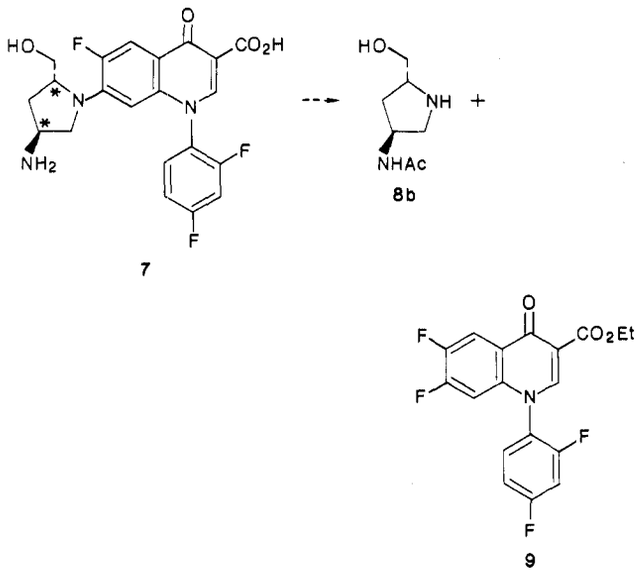
the enantiomer **6** is at least as potent as, and perhaps the active component of, the racemic mixture **3**. One may envision two ways of hybridizing the substituted pyrrolidine moieties of the active enantiomer **4** and compound **6**:



In this study, we have focused on a series of (4*S*)-4-amino-2-substituted-pyrrolidines (type A) as 7-position substituents on the quinolone nucleus. We have used the 2-position of the pyrrolidine moiety as a handle to modify the physiochemical properties of **1–3**. This work has resulted in agents that have the excellent potency associated with **1–3** but that possess substantially improved solubility and pharmacokinetic properties. In this paper, we report the full details of the synthesis, physiochemical properties, and antibacterial activity of this series of compounds as well as the in vivo efficacy and pharmacokinetic properties of several of the agents in mice. We also discuss details of the pharmacokinetics of **59**, which shows the best overall properties of the agents in this study, in dog.

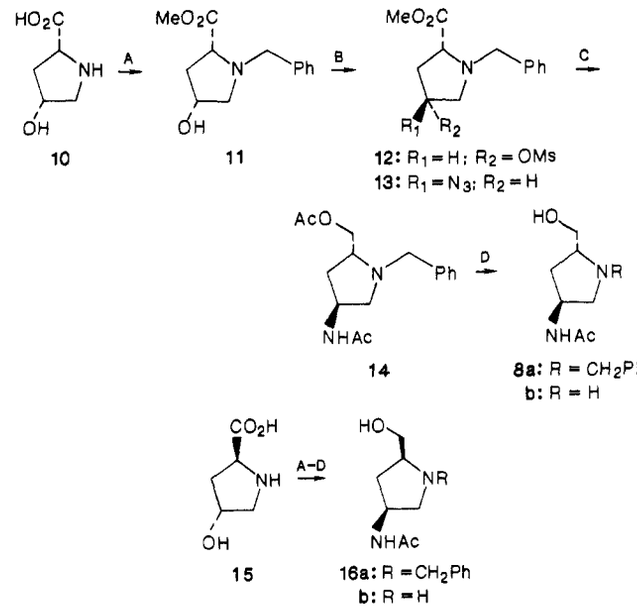
Chemistry

The basic approach to the synthesis of this series of compounds is illustrated below:

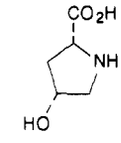


Displacement of the 7-fluorine substituent in the known ester **9** with the amine **8b** and subsequent hydrolysis of the acetamide and ester groups is expected to furnish the desired quinolone **7**. Compound **8b**, or a related derivative, was also viewed as an attractive intermediate for conversion to other 2-substituted pyrrolidine analogues. We chose *cis*-4-hydroxy-D-proline (**10**) as a chiron for **8b**. The

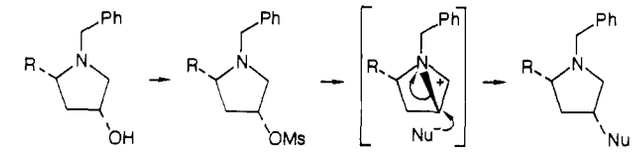
Scheme I[a]



[a] A: (1) MeOH/HCl, (2) $PhCH_2Cl$. B: (1) MsCl, Et_3N, (2) *n*-Bu_4NN_3/CH_3CN, 65 °C. C: (1) $LiAlH_4$/THF, (2) Ac_2O. D: (1) NaOMe/MeOH, (2) H_2–Pd/C, MeOH/HCl.



essential chemical concerns are conversion of the 4-hydroxyl substituent to nitrogen with inversion of configuration and reduction of the carboxyl group without epimerization. The strategy of inverting the stereochemistry at the 4-position of **10** by a sequence involving activation and subsequent S_N2 displacement is complicated by the possibility of participation by the ring nitrogen, resulting in a net retention of configuration. Such participation has been observed in 2-(halomethyl)pyrrolidines[6] and 3-chloro-1-ethylpiperidine,[7] and recently it has been proposed that such a double-inversion process may be involved in displacements on related 4-(tosyloxy)-pyrrolidines.[8]



(4) (a) Chu, D. T. W.; Fernandes, P. B.; Claiborne, A. K.; Gracey, H. E.; Pernet, A. *Abstracts of the 25th Interscience Conference on Antimicrobial Agents and Chemotherapy*; Minneapolis, MN, September 29–October 2, 1985; Vol. 113, Abstr. No. 131. (b) Stamm, J.; Vojtko, C.; Weisz, J.; Hanson, C.; Chu, D. T. W.; Fernandes, P. B. *Ibid.* **1985**; Vol. 113, Abstr. No. 132. (c) Fernandes, P. B.; Bower, R. R.; Jarvis, K.; Swanson, R.; Chu, D. T. W. *Ibid.* **1985**; Vol. 113, Abstr. No. 133.

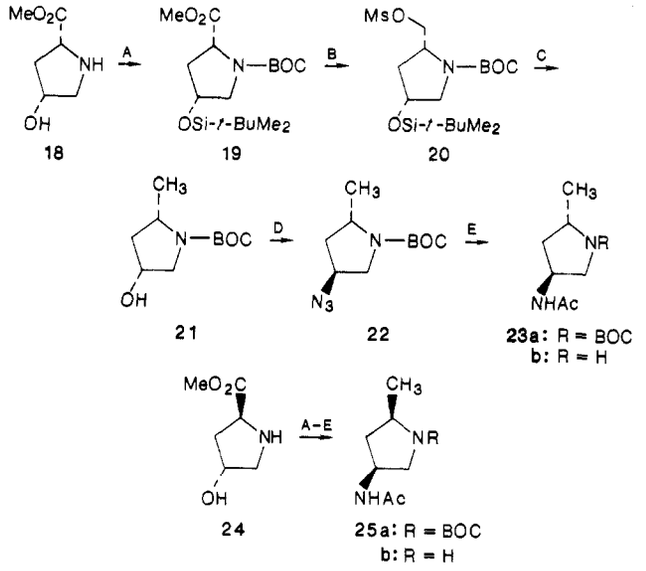
(5) Cooper, C. S.; Chu, D. T. W.; Fernandes, P. B.; Pihuleac, E.; Pernet, A. *Abstracts of the 25th Interscience Conference on Antimicrobial Agents and Chemotherapy*; Minneapolis, MN, September 29–October, 2, 1985; Vol. 113, Abstr. No. 130.

(6) (a) Fuson, R. C.; Zirkle, C. L. *J. Am. Chem. Soc.* **1948**, *70*, 2760. (b) Hammer, C. F.; Heller, S. R. *Chem. Commun.* **1966**, 919. (c) Hammer, C. F.; Ali, M. M.; Weber, J. D. *Tetrahedron* **1973**, *29*, 1767. (d) Hammer, C. F.; Weber, J. D. *Tetrahedron* **1981**, *37*, 2173.

(7) Hammer, C. F.; Heller, S. R.; Craig, J. H. *Tetrahedron* **1972**, *28*, 239.

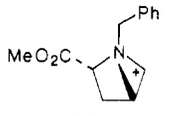
(8) Thottathil, J. K.; Moniot, J. L. *Tetrahedron Lett.* **1986**, *27*, 151.

Scheme II[a]



[a] A: (1) BOC_2O, (2) *t*-$BuMe_2SiCl$. B: (1) $LiBH_4$, (2) MsCl, Et_3N. C: (1) $LiEt_3BH$, (2) *n*-Bu_4NF. D: (1) MsCl, Et_3N, (2) *n*-Bu_4NN_3. E: (1) H_2–Pd/C, (2) Ac_2O, (3) TFA.

The synthesis of the requisite displacement precursor **8b** is summarized in Scheme I.[9] Conversion of **10** to its methyl ester followed by N-benzylation provides **11** in 84% yield. Nitrogen is introduced at the 4-position by sequential methanesulfonate ester formation (89% yield) and displacement with azide (90% yield). The ^{13}C NMR spectrum of the product obtained indicates the presence of a single stereoisomer. Reduction of the ester and azide groups is accomplished with lithium aluminum hydride,[10] and acetylation of the derived amino alcohol gives **14** in 87% yield. Cleavage of the primary acetate followed by removal of the benzyl protecting group cleanly provides **8b** in quantitative yield. The cis isomer **16b** is obtained from *trans*-4-hydroxy-L-proline by the identical sequence of reactions used to convert **10** to **8b**. NOE studies on intermediates from both sequences (**10** → **8a**, **15** → **16a**) demonstrate unambiguously that the azide displacements occur to give the indicated Walden inversion products.[9] The lack of neighboring-group participation is presumably related to the large strain energy associated with the required aziridinium ion **17**; the strain energy of the bicyclo[2.1.0]pentane system has been calculated to be 56.1 kcal mol^{-1}.[11]



17

The diastereomeric 2-methylpyrrolidine displacement precursors **23b** and **25b** are synthesized as shown in Scheme II. Successive protection of the amino and hydroxyl functions in **18** affords **19** in 98% yield. The ester group is reduced with lithium borohydride (93% yield), and the resulting primary alcohol is activated by conversion to the corresponding methanesulfonate ester (**20**) (94% yield). Deoxygenation is accomplished by treatment with lithium triethylborohydride; desilylation with tetra-*n*-butylammonium fluoride affords **21** in 63% yield for the two-step sequence. The hydroxyl group is converted to the corresponding azido group with inversion of configuration in 43% yield by a procedure analogous to that in Scheme I. Reduction of the azide with hydrogen in the presence of palladium catalyst followed by acetylation of the resulting amine and removal of the *tert*-butoxycarbonyl protecting group gives the desired disubstituted pyrrolidine **23b**. The epimeric compound **25b** is obtained from **24** by an identical sequence of reactions.

The syntheses of several related pyrrolidine derivatives are summarized in Scheme III. Oxidation of **8a** employing the Swern protocol and subsequent Wittig olefination with methylenetriphenylphosphorane provide **27**. Treatment of **27** with hydrogen in the presence of a palladium catalyst results in simultaneous debenzylation and olefin reduction to give the displacement precursor **28**.

The 2-(pyrrolidinylmethyl)pyrrolidine derivative **31b** is obtained from **11** by a six-step sequence of reactions. Heating **11** with pyrrolidine affords the amide **29**. The secondary hydroxyl group in **29** is converted to the azide **30** with inversion of configuration. Lithium aluminum hydride reduction of **30** followed by acetylation gives an acetamide; subsequent debenzylation provides the displacement precursor **31b**.

The synthesis of the bicyclic amine **35** begins with **32**, an intermediate in the preparation of **25** (Scheme II). The primary hydroxyl group is converted to its methanesulfonate ester, which is displaced with azide to give **33**. Removal of the silyl protecting group in **33** and subsequent treatment with methanesulfonyl chloride gives the activated derivative **34**. Exposure of **34** to hydrogen in the presence of a palladium catalyst results in reduction of the azide function; intramolecular cyclization of the incipient amino mesylate occurs spontaneously under the reaction conditions to afford the desired amine **35**.[12]

Preparation of the methoxymethyl derivative **40b** begins with the BOC-pyrrolidine **36**. Protection of the secondary hydroxyl group followed by reduction and alkylation affords the methyl ether **37**. The BOC protecting group is removed and converted to its benzyl analogue. This manipulation was necessary because of subsequent difficulties encountered in attempting to remove the protecting group on the primary amino substituent after coupling to the quinolone nucleus (cleavage of the methyl ether occurs under strong acid conditions); the side reaction can be avoided when the primary amino group is protected with a BOC group. Hence, the benzyl on the ring nitrogen differentiates the two groups prior to displacement. The hydroxyl substituent in **38** is converted to the azide **39** by using the standard protocol. Lithium aluminum hydride reduction of the azide function followed by protection and hydrogenolysis provides the desired pyrrolidine **40b**.

The synthesis of the (fluoromethyl)pyrrolidine **44b** proved to be less straightforward. The only isolable product obtained upon treatment of **8a** with (diethylamido)sulfur trifluoride (DAST) is the ring-expanded piperidine **45** (Scheme IV). This result is not surprising considering the observations of ring expansion in 2-halomethylpyrrolidines reported earlier.[6] Presumably, the ring nitrogen displaces the activated primary substituent in the DAST reaction to generate the aziridinium species de-

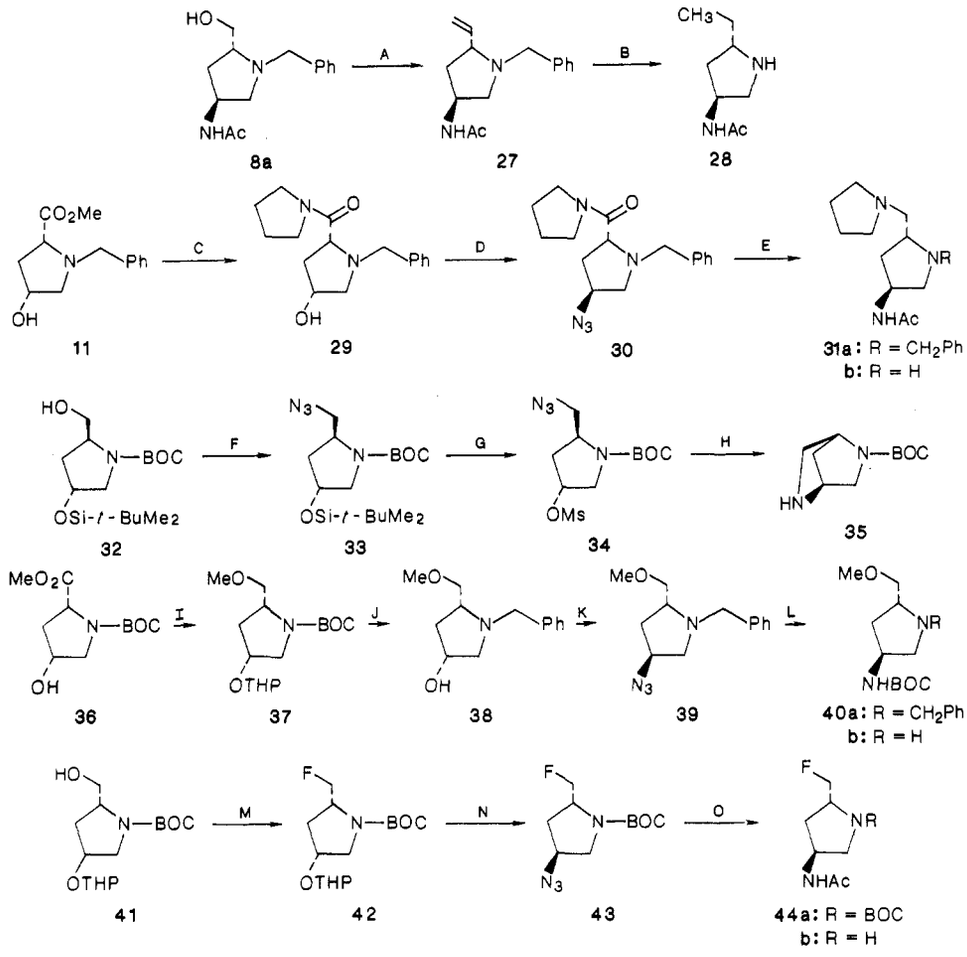
(9) For full details, see: Rosen, T.; Fesik, S. W.; Chu, D. T. W.; Pernet, A. G. *Synthesis* **1988**, 40.

(10) We have found that it is best to slowly add a THF solution of the azide to a stirring mixture of $LiAlH_4$ and THF at ca. −10 °C in order to control the exotherm. On one occasion, when the $LiAlH_4$ was added to the solution of azide, the exotherm caused a fire.

(11) Chang, S.; McNally, D.; Shary-Tehrany, S.; Hickey, M. J.; Boyd, R. H. *J. Am. Chem. Soc.* **1970**, *92*, 3109.

(12) For the synthesis of a related bicyclic derivative, see: Sépulchre, J. C.; Cléophax, J.; Hildesheim, J.; Gero, S. D.; Janot, M.-M. *C. R. Hebd. Seances Acad. Sci., Ser. C* **1969**, 849.

Scheme III[a]



[a] A: (1) $(ClCO)_2$, DMSO; Et_3N, (2) $Ph_3P{=}CH_2$. B: H_2–Pd/C, HCl/MeOH. C: pyrrolidine, 100 °C. D: (1) MsCl, Et_3N, (2) n-Bu_4NN_3. E: (1) $LiAlH_4$, THF, (2) Ac_2O, (3) H_2–Pd/C, HCl/MeOH. F: (1) MsCl, Et_3N, (2) n-Bu_4NN_3. G: (1) n-Bu_4NF, (2) MsCl, Et_3N. H: H_2, Pd/C. I: (1) dihydropyran, PPTS, (2) $LiBH_4$ (3) KOH, MeI. J: (1) HOAc, (2) TFA, (3) $PhCH_2Br$. K: (1) MsCl, Et_3N, (2) n-Bu_4NN_3/CH_3CN, 65 °C. L: (1) $LiAlH_4$, (2) BOC_2O, (3) H_2–Pd/C. M: (1) MsCl, Et_3N, (2) n-Bu_4NF. N: (1) HOAc/H_2O/THF, (2) MsCl, Et_3N, (3) n-Bu_4NN_3, O: (1) CH_2COSH, (2) TFA.

picted in Scheme IV, which is subsequently opened by fluoride ion to give the observed product **45**. The stereochemistry of **45** was not assigned and that which is depicted would be expected on the basis of mechanistic considerations. In an effort to minimize the potential of nitrogen participation, the carbamate derivative **46** was investigated as a substrate for the DAST reaction. However, treatment of **46** with DAST at −78 °C affords cleanly the cyclic carbamate **47**, resulting from participation by the BOC oxygen and subsequent loss of isobutylene. Attempted displacement of the methanesulfonate ester in **48** with fluoride ion results in the cleavage of the silyl protecting group followed by intramolecular cyclization to afford the bicyclic ether **49**. This problem was overcome by switching to a tetrahydropyranyl protecting group; treatment of the THP analogue of **48** with tetra-*n*-butylammonium fluoride in refluxing THF affords cleanly the fluoromethyl derivative **42** (Scheme III). Acidic cleavage of the THP protecting group and subsequent activation and azide displacement affords **43**. Reductive acetylation of **43**, using thiolacetic acid,[13] provides an acetamide; further treatment with trifluoroacetic acid affords the desired pyrrolidine **44b**.

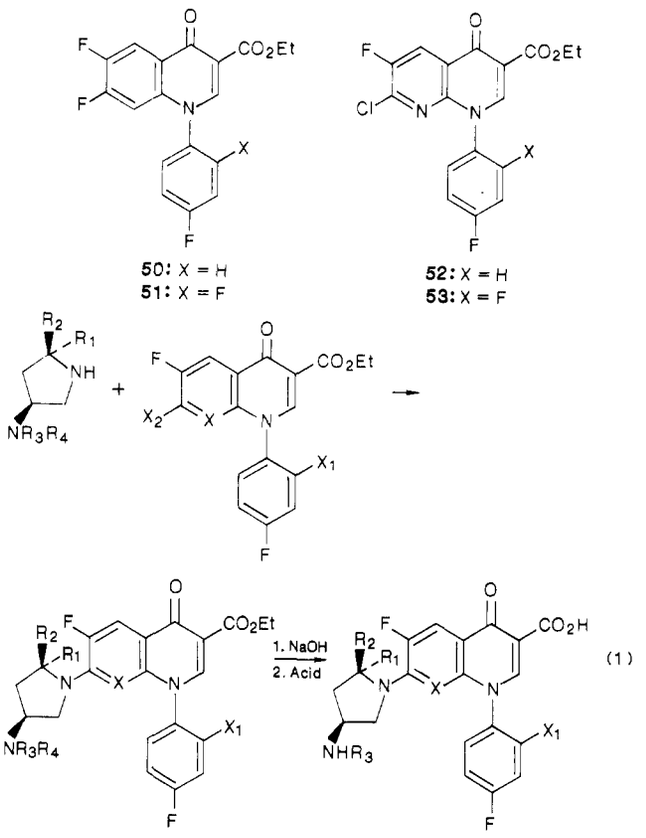
The quinolone antibacterials of this study (Table I) were prepared by displacement of halogen at the 7-position of the appropriate quinolone ester with the amines of Schemes I–III.[14] Sequential hydrolysis of the ester moiety and removal of the amine protecting group afford the desired 7-(4-amino-2-substituted-pyrrolidin-1-yl)-quinolone-3-carboxylic acid. The general route is illustrated in eq 1.

Biological Evaluation of Compounds

Compounds **7** and **54–66** were evaluated against a wide variety of organisms. The minimum inhibitory concentrations (MICs) of these agents against several representative Gram-positive and Gram-negative bacteria relative to ciprofloxacin are shown in Table II. The MICs of **1**, **2**, **4**, and **5** are shown for comparison. Compound **7**, which possesses a hydroxymethyl substituent at the 2-position of the pyrrolidine ring with the absolute stereochemistry analogous to the active antipode **4** is approximately 4 $\log_2$ dilutions more active than the epimeric de-

(13) We have found thiolacetic acid to be a generally useful and chemoselective reagent for the reductive acetylation of azides; Rosen, T.; Lico, I. M.; Chu, D. T. W. *J. Org. Chem.* **1988**, *53*, 1580.

(14) Compound **50**: Chu, D. T. W., unpublished results. Compound **51**: Chu, D. T. W.; Fernandes, P. B.; Claiborne, A. K.; Maleczka, R. E.; Klock, P.; Shen, L.; Patel, J.; Pernet, A. *Abstracts of the 26th Interscience Conference on Antimicrobial Agents and Chemotherapy*; New Orleans, LA, September 28–October 1, 1986; Vol. 114, Abstr. No. 428. Compounds **52** and **53**: Chu, D. T. W.; Fernandes, P. B.; Claiborne, A. K.; Gracey, E. H.; Pernet, A. G. *J. Med. Chem.* **1986**, *29*, 2363.

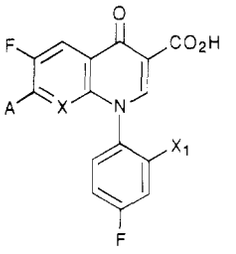


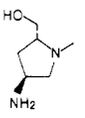
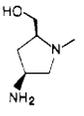
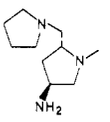
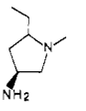
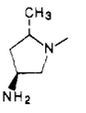
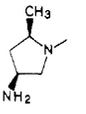
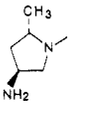
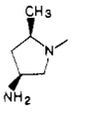
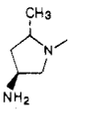
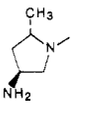
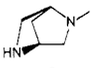
rivative (**54**). However, **7** is appreciably less active than **2**, which is unsubstituted at the 2-position of the pyrrolidine moiety. The 2-(pyrrolidinylmethyl)pyrrolidinyl derivative **55** is significantly less active than **2**, most notably against Gram-positive organisms (approximately 5 $\log_2$ dilutions less active). The 2-ethylpyrrolidinyl analogue **56** shows excellent in vitro activity, although it is somewhat less active against *Pseudomonas aeruginosa* than is desirable. The 2-methylpyrrolidinyl derivative **57** is perhaps 1 $\log_2$ dilution more active. It is important to note that the epimer of **57**, compound **58** (absolute stereochemistry at the 2-position of the pyrrolidine ring the same as **5** and **54**), shows diminished activity. This result combined with the data obtained for the pairs **4**/**5** and **7**/**54** suggests that the decreased activities of **58**, **5**, and **54** relative to **57**, **4**, and **7**, respectively, are the results of some type of a negative steric interaction, the molecular nature of which is not presently understood, which affects the ability of these quinolones to effectively inhibit the supercoiling process as opposed to a negative polar interaction (same trend for CH_2OH and CH_3). The naphthyridine analogue of **57**, compound **59**, is the most potent member of this series. This compound has been evaluated against approximately 200 strains of bacteria and is generally as potent or more potent than ciprofloxacin against all species except for *P. aeruginosa*, against which it is 1 $\log_2$ dilution less active. The related monofluorophenyl compound **62** also shows excellent antibacterial activity as do the bicylic analogues **63** and **64**.

Several of these agents were evaluated in a supercoiling inhibition assay using purified DNA gyrase isolated from *Escherichia coli*.[15] The results are shown in Table III. The data for compound **1** and ciprofloxacin are shown for comparison. The important feature of these results is the difference in activity within each of the pairs of epimers (**7**/**54**, **57**/**58**, **59**/**60**). These data indicate that the differences observed in the MICs for each of these pairs is related to inherent activity at the enzymatic level as opposed to some permeability or transport differences. It should be noted that these differences in I_{50} values cor-

(15) Otter, R.; Cozzarelli, N. R. *Methods Enzymol.* **1983**, *100B*, 171.

Table I. Structures of Compounds



no.	A	X	X_1
7		CH	F
54		CH	F
55		CH	F
56		CH	F
57		CH	F
58		CH	F
59		N	F
60		N	F
61		CH	H
62		N	H
63		CH	F
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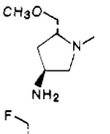
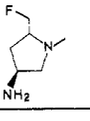
65		N	F
66		N	F

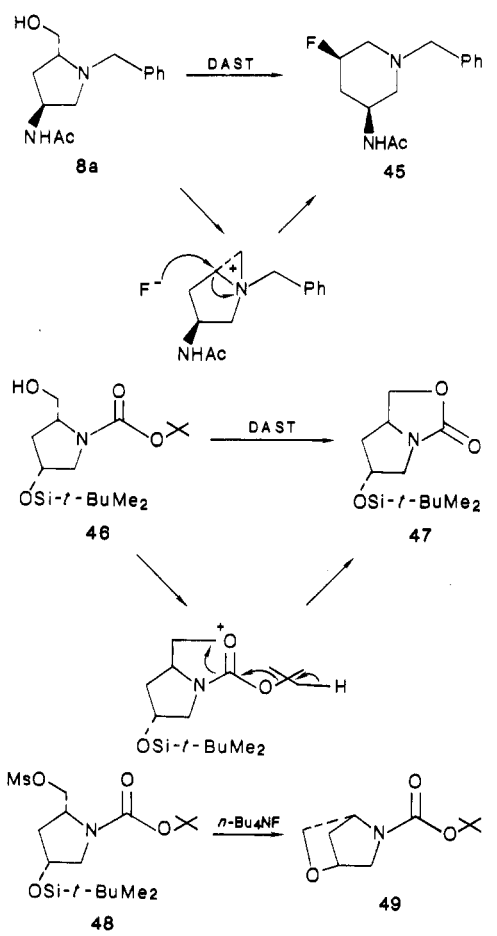
Table II. Minimum Inhibitory Concentrations of Selected Quinolones

organism	1	2	4	5	7	54	55	56	57	58	59	60	61	62	63	64	65	66	CIP
S. aureus ATCC 6538P	0.02	0.05	0.1	3.1	0.1	1.56	1.56	0.05	0.02	0.39	0.02	0.2	0.05	0.05	0.1	0.1	0.1	0.05	0.2
S. aureus CMX 686B	0.05	0.05	0.1	3.1	0.39	6.2	1.56	0.05	0.05	0.39	0.02	0.2	0.05	0.05	0.2	0.2	0.1	0.05	0.39
S. aureus A5177	0.05	0.1	0.39	3.1	0.78	6.2	3.1	0.1	0.2	0.78	0.05	0.39	0.05	0.1	0.39	0.39	0.2	0.1	0.39
S. aureus 45	0.05	0.1	0.39	3.1	1.56	12.5	3.1	0.1	0.1	0.78	0.05	0.39	0.05	0.1	0.78	0.78	0.39	0.1	0.78
S. epidermidis 3519	0.1	0.1	0.39	3.1	0.78	12.5	3.1	0.1	0.2	0.78	0.05	0.39	0.05	0.1	0.39	0.39	0.39	0.2	0.78
S. faecium ATCC 8043	0.2	0.2	3.1	>100	0.78	25	6.2	0.2	0.1	3.1	0.1	3.1	0.2	0.39	0.39	0.39	0.78	0.2	0.39
S. bovis A5169	0.78	0.78	25	100	1.56	50	25	0.2	0.2	6.2	0.2	6.2	0.2	0.78	1.56	1.56	1.56	0.78	1.56
S. agalactiae CMX 508	0.2	0.2	3.1	100	0.78	12.5	25	0.1	0.1	6.2	0.2	6.2	0.2	0.78	0.78	0.78	0.78	0.39	0.78
S. pyogenes 930	0.1	0.2	3.1	50	0.39	25	25	0.1	0.2	1.56	0.2	1.56	0.2	0.39	0.78	0.78	0.78	0.2	0.78
E. coli Juhl	0.02	0.05	0.78	50	0.78	3.1	0.78	0.1	0.05	0.78	0.02	0.39	0.02	0.05	0.05	0.05	0.2	0.1	0.02
K. pneumoniae 8045	0.01	0.05	0.78	25	0.78	3.1	0.39	0.05	0.02	0.78	0.02	0.39	0.01	0.05	0.05	0.05	0.1	0.1	0.02
P. aeruginosa 5007	0.78	1.56	3.1	>100	0.78	12.5	1.56	6.2	3.1	5.0	0.78	12.5	3.1	0.39	1.56	0.39	1.56	0.78	0.39
P. aeruginosa K799/WT	0.39	0.39	3.1	>100	0.39	12.5	1.56	0.78	0.39	6.2	0.78	3.1	0.78	0.39	0.39	0.39	1.56	0.78	0.2
Acinetobacter CMX 669	0.05	0.1	0.78	>100	6.2	25	12.5	0.2	0.1	3.1	0.1	0.78	0.1	0.1	0.39	0.39	0.78	0.39	0.39

Table III. Supercoiling Inhibition Activity of Selected Quinolones (DNA Gyrase Isolated from *E. coli* H560)

compd	I_{50}, μg/mL	compd	I_{50}, μg/mL
1	0.4	**59**	0.6
7	0.4	**60**	7.5
54	10	**66**	0.75
57	0.9	ciprofloxacin	0.9
58	18		

Scheme IV



respond quite well, qualitatively, with the differences in MIC values.

However, although the hydroxymethyl derivative **7** has an I_{50} against purified gyrase that is a factor of 2 better than its methyl analogue **57**, its activity against bacteria (MICs) is significantly lower than that of **57**. We believe that this discrepancy is related to a negative effect of the polar hydroxyl substituent in whole cells (related to transport or permeability). The fluoromethyl analogue **66** was synthesized with the intention of being isosteric with the hydroxymethyl derivative **7** yet not possessing the polar hydroxy substituent. Indeed, this derivative shows activity quite comparable to **59** against whole cells (as well as against purified gyrase). Similarly, the methoxy analogue **65** also shows improved whole cell activity when compared to **7**.

Table IV. In Vivo Activity of Selected Quinolones for *S. aureus* 10649

compd	route	ED_{50}, mg/kg per day (95% CL[a])	compd	route	ED_{50}, mg/kg per day (95% CL[a])
3	sc	0.8 (0.5–1.3)	**3**	po	6.2 (3.9–9.8)
7	sc	0.7 (0.4–1.1)	**7**	po	5.4 (2.8–10.4)
56	sc	0.8 (0.5–1.2)	**56**	po	2.4 (1.1–5.4)
57	sc	<0.5	**57**	po	3.3 (2.1–5.2)
59	sc	0.9 (0.6–1.4)	**59**	po	<2.5
61	sc	0.8 (0.5–1.2)	**61**	po	4.4 (2.8–7.0)
62	sc	2.0 (1.1–3.6)	**62**	po	5.7 (2.8–11.7)
CIP	sc	1.6 (1.0–2.5)	CIP	po	15.5 (9.9–24.1)

[a] Confidence limit.

Table V. In Vivo Activity of Selected Quinolones for *E. coli* Juhl

compd	route	ED_{50}, mg/kg per day (95% CL)	compd	route	ED_{50}, mg/kg per day (95% CL)
3	sc	1.0 (0.6–1.8)	**3**	po	5.8 (4.3–7.8)
7	sc	5.9 (3.7–9.6)	**7**	po	>16.0
56	sc	2.4 (1.4–4.2)	**56**	po	9.6 (3.2–29.0)
57	sc	0.9 (0.6–1.4)	**57**	po	3.6 (2.1–6.1)
59	sc	0.6 (0.2–1.4)	**59**	po	2.7 (1.3–5.3)
CIP	sc	0.2 (0.1–0.2)	CIP	po	1.9 (1.2–3.0)

In order to determine in vivo efficacy, several of these compounds were evaluated in mouse protection tests. Ciprofloxacin (CIP) was used as a standard. The results for compound **3** are provided for reference. The compounds were administered either orally (po) or subcutaneously (sc). The results are shown for *Staphylococcus aureus* (Table IV) and *E. coli* (Table V). Subcutaneously, against *S. aureus*, all of the agents in this study show excellent efficacy. Orally, all of the compounds shown in Table IV exhibit activity that is significantly better than ciprofloxacin. Compound **59**, the most potent member of this series in vitro, also shows the best activity in vivo. As shown in Table V, the compounds evaluated show good to excellent activity subcutaneously against *E. coli*. Orally, compound **7** does not exhibit signficant activity, and **42** shows moderate activity. Once again, **59** is the most potent member of this new series, exhibiting activity similar to or slightly less than that of ciprofloxacin.

Table VI. Aqueous Solubility of Selected Quinolones

compd	solubility, mg/mL	log *P*	compd	solubility, mg/mL	log *P*
1	0.008	−0.46	**58**	0.182	+0.03
7	0.06	−0.98	**59**	0.15	−0.20
54	0.06		**60**	0.34	−0.11
55	0.68	−0.56	CIP[a]	0.092	−1.16
57	0.053	+0.085			

[a] Solubility determined in pH 7.4 phosphate buffer solution (0.05 M).

Physiochemical and Pharmacokinetic Properties

The solubility of selected members of this study in Ringer's buffer solution and their respective octanol/water partition coefficients are shown in Table VI. It can be seen that all compounds examined exhibit improved solubility relative to the 2-unsubstituted derivative **1**. Apparently, substitution at the 2-position of the pyrrolidine ring results in a perturbation of the symmetry of this system that is associated with their intermolecular packing (high melting point, poor solubility). Thus, the introduction of the methyl substituent in compound **59** results not only in improved aqueous solubility but also better solubility in general. This point is illustrated by examination of the respective log *P* values for **1** and **59**. Although compound **59** is approximately 20 times more soluble in aqueous media than **1**, it can be seen that introduction of the methyl substituent results in the expected slight increase in log *P* (−0.46 → −0.20). Thus, if one makes the reasonable assumption that solubility in water closely parallels solubility in Ringer's buffer, then it follows from the log *P* data that there is an even greater increase in lipid (octanol) solubility in going from **1** to **59** than the corresponding increase in aqueous solubility. Thus, the 2-position substituent on the pyrrolidine ring appears to improve solubility not by changing the overall polar nature of the molecule but instead by reducing intermolecular binding.

The improved solubility properties associated with the introduction of the 2-methyl substituent on the pyrrolidine ring are manifested in greatly improved oral absorption properties. The blood levels of several of these compounds after oral administration to mice are shown in Table VII. Analogous data for ciprofloxacin are provided for comparison.

Compounds **1** and **2**, which are unsubstituted at the 2-position of the pyrrolidine moiety, were each administered orally to mice at a dose of 100 mg/kg. It can be seen that all of the 2-methyl analogues shown with the exception of **61** attain better blood levels, even when administered at only 25 mg/kg. Compound **59** exhibits an excellent pharmacokinetic profile, attaining a C_{max} of 5.9 μg/mL and still present in the serum 24 h after administration. The data can be compared to that of ciprofloxacin, which attains a C_{max} of 1.3 μg/mL and is no longer present in the serum at 6 h after administration (also administered at 25 mg/kg). It is interesting to note the epimer of **59**, compound **60**, which is inherently less active but possesses even better solubility, achieves an outstanding C_{max} of 12.4 μg/mL.

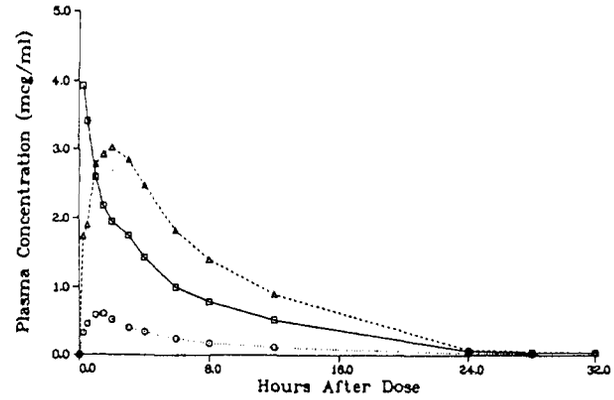


Figure 1. Mean plasma concentrations of **59** in dog after 10 mg/kg oral dose (Δ, solution, $n = 2$) and 5 mg/kg iv dose (□, $n = 2$); comparison to mean plasma concentration of **1** (○, $n = 4$) after 10 mg/kg oral dose (suspension).

Pharmacokinetic Evaluation of Compound 59 in Dog

Compound **59** was rapidly absorbed after oral administration (10 mg/kg) in each of two dogs. Peak plasma levels of parent drug of 4.0 μg/mL and 2.7 μg/mL were recorded 1 and 3 h after dosing (see Figure 1). After both po and iv dosing (5 mg/kg), plasma concentrations of parent drug declined in a biexponential manner with an average elimination half-life of 5.0 h ($n = 4$). Assuming dose proportionality between the 5 mg/kg iv dose and the 10 mg/kg po dose, 72% of the **59** dose was absorbed after oral administration. Plasma levels of **59** after oral administration are compared to plasma levels of **1** after 10 mg/kg dose in dogs in Figure 1. Due to solubility limitations, **1** was administered as a 10 mg/mL suspension. The peak plasma concentration of **59** is approximately 5 times greater than that of **1** with a similar increase in area under the plasma–time curve. Low plasma concentrations of **59** (10–50 ng/mL) were recorded 48 h after dosing. Approximately 17% of the dose of **59** was recovered in the urine after 48 h; 12% of the drug recovered was parent drug and ~5% was a base hydrolyzable conjugate—presumably the glucuronide of the carboxylic acid.

Summary of Results

This study has demonstrated that the 2-position of the pyrrolidine ring may be used to improve the solubility properties of 7-(4-aminopyrrolidinyl)quinolones. Furthermore, the (2*S*,4*S*)-4-amino-2-methylpyrrolidine substituent confers the same spectrum and level of potency

Table VII. Pharmacokinetic Properties of Selected Quinolones in Mice

compd	mg/kg po	blood level of compound[a] (μg/mL) at *t* (h)					
		0.5	1.0	2.0	3.0	6.0	24.0
1	100		2.3	1.7	1.7	0.9	
59	25	5.9	3.2	3.3	2.8	1.3	0.2
60	25	5.3	12.4	3.7	2.2	1.1	0.0
62	25	2.4	1.9	1.5	0.8	0.3	0.1
2	100		0.5	0.7	0.9	0.7	0.0
57	25	3.7	4.3	2.8	1.1	0.1	0.0
61	25	1.0	0.8	0.4	0.2	0.0	0.0
CIP	25	1.3	0.8	0.3	0.1	0.0	0.0

[a] These data were determined by a bioassay procedure (see the Experimental Section) and represent total activity present in the serum. The values for compound **59** were verified by HPLC analysis.

associated with the 2-unsubstituted derivative. However, the methyl substituent results in greatly improved pharmacokinetic properties in mouse and dog. Compound **59** exhibits the best overall properties of compounds in this study and is undergoing further biological evaluation.

Experimental Section

General Methods. Unless otherwise noted, materials were obtained from commercial suppliers and used without further purification. Melting points are uncorrected. Infrared (IR) spectra were determined with a Perkin-Elmer 683 infrared grating spectrometer or a Nicolet 60SC FT-IR. ^{1}H NMR spectra were determined on a General Electric GN-300 spectrometer operating at 300.1 MHz. ^{13}C NMR spectra were measured at 75.5 MHz with a GN-300 spectrometer. NMR spectra were determined with $CDCl_3$ as the solvent, unless otherwise noted. Chemical shifts are expressed in ppm downfield from internal tetramethylsilane. Significant ^{1}H NMR data are tabulated in the order: multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet), number of protons, coupling constant(s) in hertz. Mass spectra were obtained with a Hewlett Packard 5985A mass spectrometer or a Kratos MS-50 instrument with EI source (70 eV). Gravity column chromatography was done with Merck silica gel 60 (70–230 mesh ASTM) and flash chromatography[16] was done with Matrex silica Si (particle size: 35–70 μm). Thin-layer chromatography (TLC) was performed with Analtech silica gel GF TLC plates (250 μM), and compound visualization was effected with a 2% solution of sulfuric acid in ethanol, a 5% solution of molybdophosphoric acid in ethanol, or a 0.25% solution of ninhydrin in 1-butanol. Elemental analyses were performed by the Microanalytical Laboratory, operated by the Analytical Department, Abbott Laboratories, North Chicago, IL.

(2*R*,4*R*)-1-Benzyl-2-carbomethoxy-4-hydroxypyrrolidine (11). Under a nitrogen atmosphere, in a 2-L round-bottom flask equipped with a magnetic stir bar and a reflux condenser were placed 400 mL of methanol. To the system was added 45.6 mL (50.3 g, 640 mmol) of acetyl chloride (addition funnel, ice bath) followed by 60 g (0.46 mol) of the amino acid **10**. The resulting solution was heated at reflux for 7–8 h and cooled to room temperature. The solution was diluted with ether, and the resulting white solid (84 g, quantitative yield), (2*R*,4*R*)-2-carbomethoxy-4-hydroxypyrrolidine hydrochloride, was collected by suction filtration, mp 121–123 °C.

Under a nitrogen atmosphere, in a 250-mL round-bottom flask equipped with a magnetic stir bar and a reflux condenser were placed 10.8 g (59.6 mmol) of the hydrochloride prepared above and 50 mL of dichloromethane. To this stirring solution were added 16.6 mL (12.0 g, 119 mmol) of triethylamine and 13.7 mL (15.1 g, 119 mmol) of benzyl chloride. The reaction mixture was heated at reflux for 6 h, 50 min. The resulting suspension was partitioned between chloroform and 1 M aqueous sodium hydroxide, the layers were separated, and the organic phase was washed with brine, dried over sodium sulfate, and concentrated with a rotary evaporator. The resulting orange liquid (20 g) was purified by flash column chromatography (500 g of silica gel) with ethyl acetate as the eluant to obtain 11.7 g (84% yield) of **11** as a very pale yellow oil: ^{1}H NMR δ 1.95 (ddd, 1 H, J = 1.4, 4.0, 14.2), 2.39 (ddd, 1 H, J = 5.8, 10.0, 14.2), 2.64 (dd, 1 H, J = 1.4, 4.0), 3.02 (ddd, 1 H, J = 1.4, 1.4, 9.9), 3.35 (dd, 1 H, J = 4.0, 10.0), 3.64 (s, 3 H), 3.72 (d, 1 H, J = 13.0), 3.88 (d, 1 H, J = 13.0), 4.26 (dddd, 1 H, J = 1.4, 1.4, 4.0, 5.8), 7.30 (m, 5 H); ^{13}C NMR δ 39.1, 51.9, 58.0, 61.6, 63.2, 70.7, 127, 128, 129, 138, 175. Anal. ($C_{13}H_{17}NO_3$) C, H, N.

(2*R*,4*R*)-1-Benzyl-2-carbomethoxy-4-[(methylsulfonyl)oxy]pyrrolidine (12). Under a nitrogen atmosphere, in a 250-mL round-bottom flask equipped with a rubber septum and a magnetic stir bar were placed 5.05 g (21.5 mmol) of alcohol **11** and 6 mL of dichloromethane. To this stirring solution, at 0 °C, was added 13.2 mL (9.55 g, 94.6 mmol) of triethylamine followed by 3.73 mL (5.41 g, 47.3 mmol) of methanesulfonyl chloride. The reaction mixture was stirred for 19 h, during which time the ice bath expired. The reaction mixture was diluted with dichloromethane and washed with saturated aqueous sodium bicarbonate, water, and brine. The dichloromethane solution was dried over sodium sulfate and concentrated with a rotary evaporator to afford 7.7 g of orange oil. This crude material was subjected to flash column chromatography (250 g of silica gel), gradually increasing the polarity of the eluant from 3:1 ether/hexanes to 5:1 ether/hexanes to obtain 6.02 g (89% yield) of the methanesulfonate ester **12** as a pale yellow viscous oil: ^{1}H NMR δ 2.34 (dddd, 1 H, J = 1.1, 2.6, 6.6, 15), 2.62 (ddd, 1 H, J = 1.5, 2.6, 5.5), 2.73 (dd, 1 H, J = 5.5, 11), 2.98 (s, 3 H), 3.26 (br d, 1 H, J = 11), 3.31 (dd, 1 H, J = 6.8, 8.8), 3.58 (d, 1 H, J = 13), 3.72 (s, 3 H), 4.03 (d, 1 H, J = 13), 5.16 (m, 1 H), 7.25 (m, 5 H); ^{13}C NMR δ 36.6, 38.7, 52.0, 57.3, 58.2, 63.2, 77.7, 127, 128, 129, 137, 173; mass spectrum, m/z 313 (parent), 254, 218, 158. Anal. ($C_{14}H_{19}NO_5S$) C, H, N.

(2*R*,4*S*)-4-Azido-1-benzyl-2-carbomethoxypyrrolidine (13). Under a nitrogen atmosphere, in a round-bottom flask equipped with a magnetic stir bar and a rubber septum were placed 5.12 g (16.4 mmol) of the methanesulfonate ester **12** and 5 mL of acetonitrile. To this stirring solution was added 11.6 g (40.9 mmol) of tetra-*n*-butylammonium azide. This stirring solution was heated at 50–60 °C for 95 min, diluted with ethyl acetate, and washed with water and brine. The combined aqueous washings were extracted with ethyl acetate, the combined organic fractions were dried over sodium sulfate, and the solvent was removed with a rotary evaporator to afford 9.54 g of an orange oil. The crude material was purified by flash column chromatography (100 g of silica gel) with 2:3 ether/hexanes as the eluant to obtain 3.83 g (90% yield) of azide **13** as a pale yellow liquid: IR ($CHCl_3$) 2105, 1770 cm^{-1}; ^{1}H NMR δ 2.16 (ddd, 1 H, J = 4.8, 8.5, 13), 2.33 (ddd, 1 H, J = 6.3, 7.4, 14), 2.53 (dd, 1 H, J = 5.1, 10), 3.30 (dd, 1 H, J = 6.4, 10), 3.56 (dd, 1 H, J = 6.7, 8.2), 3.64 (d, 1 H, J = 13), 3.67 (s, 3 H), 3.90 (d, 1 H, J = 13), 4.10 (m, 1 H), 7.25 (m, 5 H); ^{13}C NMR δ 35.8, 51.8, 57.67, 57.72, 58.9, 63.5, 127, 128, 129, 138, 173; mass spectrum, m/z 260 (parent), 201, 91. Anal. ($C_{13}H_{16}N_4O_2$) C, H, N.

(2*R*,4*S*)-4-Acetamido-2-(acetoxymethyl)-1-benzylpyrrolidine (14). Under a nitrogen atmosphere, in a round-bottom flask equipped with a magnetic stir bar and a rubber septum were placed 3.50 g (13.5 mmol) of **13** and 14 mL of tetrahydrofuran. The system was immersed in an ice/acetone bath. To the stirring solution was added 1.99 g (52.3 mmol) of lithium aluminum hydride. The resulting mixture was stirred for approximately 0.5 h, at which time a vigorous exotherm occurred (*CAUTION*),[10] and the reaction mixture solidified. To the system was added 20–25 mL of tetrahydrofuran, and the reaction mixture was stirred at room temperature for 3 h. The system was cooled in an ice/acetone bath, and a mixture of 10 mL of 1 M aqueous sodium hydroxide and 100 mL of tetrahydrofuran was added cautiously to the system. The cold bath was removed, and the reaction mixture was stirred at room temperature. Sodium sulfate was added to the system, and the mixture was stirred at room temperature. The white granular precipitate was removed by suction filtration through a pad of Celite, and the filtrate was concentrated with a rotary evaporator to afford 3.05 g of very pale yellow oil. In a round-bottom flask were placed this oil and 11 mL of pyridine. To the system were added 3.76 mL (2.72 g, 27 mmol) of triethylamine and 6.2 mL (6.77 g, 66.4 mmol) of acetic anhydride. This solution was stirred at room temperature under a nitrogen atmosphere for 7–8 h, diluted with ethyl acetate, and washed with water and brine. The combined aqueous washings were extracted with several portions of dichloromethane, the combined organic fractions were dried over sodium sulfate, and the solvent was removed with a rotary evaporator. The crude product was purified by flash column chromatography (35 g of silica gel) with ether followed by ethyl acetate as the eluant to obtain 3.41 g (87% yield) of **14** as an orange oil, which partially crystallized upon standing. This material was used in subsequent transformations without further purification. An analytical sample was prepared by a second column chromatography: mp 72–74 °C; ^{1}H NMR δ 1.75 (ddd, 1 H, J = 7.4, 8.8, 13), 1.91 (s, 3 H), 2.07 (s, 3 H), 2.12 (m, 2 H), 3.02 (m, 1 H), 3.24 (dd, 1 H, J = 6.6, 9.2), 3.48 (d, 1 H, J = 13), 4.09 (m, 2 H), 4.34 (m, 1 H), 5.34 (br, 1 H), 7.28 (m, 5 H); ^{13}C NMR δ 20.9, 23.2, 35.7, 47.4, 58.2, 59.2, 60.1, 66.0, 127, 128, 129, 138, 170, 171; mass spectrum, m/z 290 (parent), 231, 217, 91. Anal. ($C_{16}H_{22}N_2O_3$) C, H, N.

(2*R*,4*S*)-4-Acetamido-1-benzyl-2-(hydroxymethyl)pyrrolidine (8a). Under a nitrogen atmosphere, in a 1-L

(16) Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923.

round-bottom flask equipped with a magnetic stir bar and a rubber septum were placed 2.94 g (10.1 mmol) of **14** and 18 mL of methanol. To this stirring solution was added 718 mg (13.3 mmol) of sodium methoxide. The resulting solution was stirred at room temperature for 135 min, diluted with chloroform, and washed with water and brine. The combined aqueous washings were extracted with chloroform, the combined organic fractions were dried over sodium sulfate, and the solvent was removed with a rotary evaporator to obtain 2.54 g (quantitative yield) of the title alcohol as a yellow oil, which became a light tan solid upon concentration from a mixture of ether and hexanes; mp 111–113 °C. This material was used in subsequent transformations without further purification. A small portion of this material was recrystallized from ether to obtain a sample for elemental analysis: ^{1}H NMR δ 1.75 (ddd, 1 H, J = 7.3, 9.2, 13), 1.91 (s, 3 H), 2.18 (dd, 1 H, J = 8.8, 9.2), 2.13 (ddd, 1 H, J = 6.3, 8.6, 13), 2.77 (br s, 1 H), 2.95 (dddd, 1 H, J = 2.1, 3.5, 6.3, 9.2), 3.36 (dd, 1 H, J = 6.4, 9.2), 3.43 (d, 1 H, J = 13), 3.44 (dd, 1 H, J = 2.1, 11), 3.67 (dd, 1 H, J = 3.5, 13), 3.98 (d, 1 H, J = 13), 4.31 (ddddd, 1 H, J = 6.4, 6.5, 7.3, 8.6, 8.8), 5.72 (br d, 1 H, J = 6.5), 7.27 (m, 5 H); ^{13}C NMR δ 23.2, 34.8, 47.8, 58.0, 59.7, 61.7, 62.9, 127, 128, 129, 138, 170; mass spectrum, m/z 249 (parent + H), 230, 217, 158, 91. Anal. ($C_{14}H_{20}N_2O_2$) C, H, N.

Analogous procedures were used to convert *trans*-4-hydroxyl-L-proline to (2*S*,4*S*)-4-acetamido-1-benzyl-2-(hydroxymethyl)pyrrolidine. Physical and spectral data for intermediates are given below.

(2*S*,4*R*)-1-Benzyl-2-carbomethoxy-4-hydroxypyrrolidine: ^{1}H NMR δ 2.08 (ddd, 1 H, J = 3.0, 8.0, 13), 2.26 (ddd, 1 H, J = 7.3, 7.7, 13), 2.48 (dd, 1 H, J = 3.7, 10), 3.33 (dd, 1 H, J = 5.5, 10), 3.65 (dd, 1 H, J = 7.7, 8.0), 3.66 (s, 3 H), 3.69 (d, 1 H, J = 13), 3.90 (d, 1 H, J = 13), 4.46 (dddd, 1 H, J = 3.0, 3.7, 5.5, 7.3), 7.30 (m, 5 H); ^{13}C NMR δ 39.5, 51.7, 58.1, 61.1, 63.6, 70.1, 127, 128, 129, 138, 174; mass spectrum, m/z 235 (parent), 176, 91. Anal. ($C_{13}H_{17}NO_3$) C, H, N.

(2*S*,4*R*)-1-Benzyl-2-carbomethoxy-4-[(methylsulfonyl)oxy]pyrrolidine: ^{1}H NMR δ 2.42 (m, 2 H), 2.77 (dd, 1 H, J = 6.4, 11), 2.98 (s, 3 H), 3.42 (dd, 1 H, J = 6.1, 11), 3.63 (dd, 1 H, J = 7.4, 7.4), 3.67 (d, 1 H, J = 13), 3.68 (s, 3 H), 3.91 (d, 1 H, J = 13), 5.22 (m, 1 H), 7.25 (m, 5 H); ^{13}C NMR δ 36.8, 38.3, 51.9, 57.6, 58.0, 63.3, 78.9, 127, 128, 129, 137, 173; mass spectrum, m/z 313 (parent), 254, 158, 91. Anal. ($C_{14}H_{19}NO_5S$) C, H, N.

(2*S*,4*S*)-4-Azido-1-benzyl-2-carbomethoxypyrrolidine: IR ($CHCl_3$) 2110, 1735 cm^{-1}; ^{1}H NMR δ 2.14 (dddd, 1 H, J = 1.1, 2.9, 6.3, 14), 2.52 (ddd, 1 H, J = 7.7, 9.6, 14), 2.64 (dd, 1 H, J = 5.9, 10), 3.08 (dd, 1 H, J = 1.1, 10), 3.34 (dd, 1 H, J = 6.2, 9.2), 3.56 (d, 1 H, J = 13), 3.72 (s, 3 H), 3.92 (m, 1 H), 4.02 (d, 1 H, J = 13), 7.25 (m, 5 H); ^{13}C NMR δ 35.7, 52.0, 57.6, 57.9, 58.4, 63.6, 127, 128, 129, 137, 173; mass spectrum, m/z 260 (parent), 201, 91. Anal. ($C_{13}H_{16}N_4O_2$) C, H, N.

(2*S*,4*S*)-4-Acetamido-2-(acetoxymethyl)-1-benzylpyrrolidine: ^{1}H NMR δ 1.59 (dddd, 1 H, J = 1.8, 1.8, 5.8, 14), 1.94 (s, 3 H), 2.10 (s, 3 H), 2.38 (ddd, 1 H, J = 7.4, 9.6, 14), 2.74 (br d, 1 H, J = 10), 2.85 (m, 1 H), 3.38 (d, 1 H, J = 13), 4.07 (d, 1 H, J = 13), 4.13 (dd, 1 H, J = 1.6, 5.0), 4.38 (m, 1 H), 5.99 (br d, 1 H, J = 7.4), 7.28 (m, 5 H); ^{13}C NMR δ 21.4, 23.8, 36.6, 47.9, 58.8, 60.3, 61.0, 66.5, 127.6, 128.7, 129.2, 139, 169, 171; mass spectrum, m/z 291 (parent + H), 231, 217, 158, 91. Anal. ($C_{16}H_{22}N_2O_3 \cdot {}^1/_3H_2O$) C, H, N.

(2*S*,4*S*)-4-Acetamido-1-benzyl-2-(hydroxymethyl)pyrrolidine (16a): ^{1}H NMR δ 1.67 (dddd, 1 H, J = 1.4, 1.4, 5.2, 14), 1.90 (s, 3 H), 2.40 (ddd, 1 H, J = 7.8, 9.9, 14), 2.60 (dd, 1 H, J = 5.2, 10), 2.83 (dddd, 1 H, J = 1.6, 3.0, 5.2, 9.9), 2.93 (dd, 1 H, J = 1.4, 10), 3.16 (br s, 1 H), 3.42 (dd, 1 H, J = 1.6, 11), 3.47 (d, 1 H, J = 13), 3.63 (dd, 1 H, J = 3.0, 11), 3.94 (d, 1 H, J = 13), 4.35 (ddddd, 1 H, J = 1.4, 1.5, 5.2, 7.0, 7.8), 6.48 (br d, 1 H, J = 7.0), 7.27 (m, 5 H); ^{13}C NMR δ 23.3, 35.4, 47.5, 58.1, 61.0, 61.4, 63.3, 127, 128, 129, 138, 169; mass spectrum, m/z 249 (parent + H), 217, 158, 91; HRMS, calcd for $C_{14}H_{21}N_2O_2$ 249.1602, found 249.1579.

(2*R*,4*R*)-1-(*tert*-Butoxycarbonyl)-4-[(*tert*-butyldimethylsilyl)oxy]-2-carbomethoxypyrrolidine (19). In a 2-L round-bottom flask were placed 98 g (0.54 mol) of **18**·HCl and 650 mL of dichloromethane. To this suspension was added 164 g (220 mL, 1.62 mol) of triethylamine, and the system was immersed in an ice–salt bath. To the system was added 130 g (0.59 mol) of di-*tert*-butyl dicarbonate, and the reaction mixture was stirred under nitrogen for 12 h, during which time the ice bath expired. The reaction mixture was washed with 1 M aqueous phosphoric acid and saturated aqueous sodium bicarbonate, dried (Na_2SO_4), and concentrated with a rotary evaporator. The resulting yellow oil was crystallized from hexanes to obtain 118 g (90% yield) of pure (2*R*,4*R*)-1-(*tert*-butoxycarbonyl)-2-carbomethoxy-4-hydroxypyrrolidine as a white solid: mp 74–77 °C; ^{1}H NMR δ 1.45 (2 s, 9 H), 2.09 (2 dd, 1 H, J = 6.3, 14.0), 2.34 (m, 1 H), 3.49–3.73 (m, 3 H), 3.79 (2 s, 3 H), 4.34 (m, 2 H); mass spectrum, m/z 246 (parent + H), 190. Anal. ($C_{11}H_{19}NO_5$) C, H, N.

In a 2-L round-bottom flask were placed 118 g (0.48 mol) of the (*tert*-butoxycarbonyl)pyrrolidine prepared above and 150 mL of *N*,*N*-dimethylformamide (DMF). To this stirring solution were added 68.1 g (1.0 mol) of imidazole and 80.1 g (0.53 mol) of *tert*-butylchlorodimethylsilane. The reaction mixture was stirred at room temperature under nitrogen for 1.5 h, diluted with ether, and washed with water, 1 M aqueous phosphoric acid, and saturated aqueous sodium bicarbonate. The ether solution was dried (Na_2SO_4) and concentrated with a rotary evaporator to obtain 172 g (99% yield) of **19** as a clear colorless oil: ^{1}H NMR δ 0.05 (2 s, 6 H), 0.86 (2 s, 9 H), 1.45 (2 s, 9 H), 2.08–2.36 (m, 2 H), 3.31 (m, 1 H), 3.63 (m, 1 H), 3.71 (s, 3 H), 4.35 (m, 2 H); mass spectrum, m/z 360 (parent), 304. Anal. ($C_{17}H_{33}NO_5Si$) C, H, N.

(2*R*,4*R*)-1-(*tert*-Butoxycarbonyl)-4-[(*tert*-butyldimethylsilyl)oxy]-2-(hydroxymethyl)pyrrolidine. In a 3-L three-neck round-bottom flask were placed 169 g (0.47 mol) of **19** and 300 mL of THF. The system was placed under a nitrogen atmmosphere and cooled in a ice–salt bath. To this stirring solution was added 15.6 g (0.72 mol) of lithium borohydride in 150 mL of THF dropwise through an addition funnel. The reaction mixture was stirred for 16 h, during which time the ice bath expired. The reaction mixture was diluted with ethyl acetate, and ice was added to the system. After the ice melted, the layers were separated. To the organic phase was cautiously (exothermic) added 1 M aqueous phosphoric acid. The layers were separated, and the organic phase was washed with saturated aqueous sodium bicarbonate and brine, dried (Na_2SO_4), and concentrated with a rotary evaporator to afford 146 g (93% yield) of (2*R*,4*R*)-1-(*tert*-butoxycarbonyl)-4-[(*tert*-butyldimethylsilyl)oxy]-2-(hydroxymethyl)pyrrolidine as a clear colorless oil: ^{1}H NMR δ 0.04 (s, 6 H), 0.74 (s, 9 H), 1.42 (s, 9 H), 2.25 (m, 1 H), 3.29–4.72 (complex m, 8 H); mass spectrum, m/z 332 (parent + H). Anal. ($C_{16}H_{33}NO_4Si$) C, H, N.

(2*S*,4*R*)-1-(*tert*-Butoxycarbonyl)-4-hydroxy-2-methylpyrrolidine (21). In a 1-L round-bottom flask were placed 140 g (0.42 mol) of (2*R*,4*R*)-1-(*tert*-butoxycarbonyl)-4-[(*tert*-butyldimethylsilyl)oxy]-2-(hydroxymethyl)pyrrolidine and 130 mL of dichloromethane. To the system was added 85.4 g (118 mL, 0.85 mol) of triethylamine. The system was cooled in an ice–salt bath, and 72.6 g (50 mL, 0.63 mol) of methanesulfonyl chloride was added to the mixture through an addition funnel. The reaction mixture was stirred under nitrogen for 15 h, during which time the ice bath expired. The reaction mixture was partitioned between ethyl acetate and water. The ethyl acetate solution was washed with 1 M aqueous phosphoric acid and saturated aqueous sodium bicarbonate, dried (Na_2SO_4), and concentrated with a rotary evaporator to obtain 162 g (94% yield) of **20** as a viscous yellow oil: ^{1}H NMR δ 0.05 (s, 6 H), 0.86 (s, 9 H), 1.52 (2 s, 9 H), 2.10 (br m, 2 H), 3.06 (br s, 3 H), 3.29 (br m, 1 H), 3.60 (br m, 1 H), 4.14–4.56 (complex m, 4 H); mass spectrum, m/z 410 (parent), 371, 313.

Under a nitrogen atmosphere, in a 3-L three-neck round-bottom flask were placed 80 g (0.20 mol) of **20** and 120 mL of THF. The system was cooled in an ice bath and 800 mL (0.80 mol) of 1 M lithium triethylborohydride in THF was added to the system through an addition funnel. The cold bath was removed, and the reaction mixture was stirred at room temperature for 2 h. The mixture was diluted with ethyl acetate and washed with water, 1 M aqueous phosphoric acid, saturated aqueous sodium bicarbonate, and brine. The ethyl acetate solution was dried (Na_2SO_4) and concentrated with a rotary evaporator. The resulting oil was diluted with ethyl acetate, solids were removed by suction filtration, and the filtrate was concentrated to obtain 59 g of clear yellow oil. This procedure was repeated on the same

scale to afford 64 g of additional product. The crude material was combined and used without further purification. The oil from above was placed in a 1-L round-bottom flask. To the system was added 430 mL (0.43 mol) of 1 M tetra-*n*-butylammonium fluoride in THF. This solution was stirred under nitrogen for 2.5 h, diluted with 800 mL of ethyl acetate, and washed with three 300-mL portions of water. The combined aqueous washings were extracted with four 100-mL portions of ethyl acetate. The combined organic fractions were dried (Na_2SO_4) and concentrated with a rotary evaporator to afford 117 g of yellow oil. The crude product was purified by flash chromatography with 1:2 ethyl acetate/hexanes as the eluant to obtain 49.6 g (63% yield) of pure **21** as a white solid: mp 75–78 °C; ^{1}H NMR δ 1.36 (d, 3 H, J = 6.25), 1.47 (s, 9 H), 1.67 (br m, 3 H), 2.25 (m, 1 H), 3.35 (ddd, 1 H, J = 1.3, 2.9, 12.1), 3.61 (br, 1 H), 4.42 (m, 1 H); mass spectrum, m/z 202 (parent + H). Anal. ($C_{10}H_{19}NO_3$) C, H, N.

(2*S*,4*S*)-4-Azido-1-(*tert*-butoxycarbonyl)-2-methylpyrrolidine (22). In a 1-L round-bottom flask were placed 18.3 g (91 mmol) of **21**, 30 mL of dichloromethane, and 23 g (32 mL, 0.23 mol) of triethylamine. The system was cooled in an ice bath. To the system was slowly added 20.8 g (14.4 mL, 182 mmol) of methanesulfonyl chloride. The reaction mixture was stirred under nitrogen for approximately 14 h, during which time the ice bath expired. The reaction mixture was diluted with ether, washed with 1 M aqueous phosphoric acid and saturated aqueous sodium bicarbonate, dried (Na_2SO_4), and concentrated with a rotary evaporator to obtain 24.1 g of a light red oil. This material was subjected to flash column chromatography with 1:1 ethyl acetate/hexanes as the eluant to obtain 16.1 g of oil. This oil was placed in a 1-L round-bottom flask and dissolved in 25 mL of acetonitrile. To this solution was added 18.1 g (63.5 mmol) of tetra-*n*-butylammonium azide, and the mixture was heated at 65 °C for 3 h under nitrogen. The reaction mixture was diluted with ether and the upper ether layer was washed with two 100-mL portions of saturated aqueous sodium bicarbonate and brine. The combined aqueous washings and initial lower layer were extracted with three 100-mL portions of ether. The combined organic fractions were dried (Na_2SO_4) and concentrated with a rotary evaporator. The crude material was purified by flash column chromatography with 1:4 ethyl acetate/hexanes as the eluant to obtain 8.75 g (43% yield) of pure **22** as an oil: ^{1}H NMR δ 1.25 (d, 3 H, J = 6.25), 1.47 (s, 9 H), 1.69 (m, 1 H), 2.17 (br, 1 H), 3.50 (m, 2 H), 4.05 (m, 2 H); mass spectrum, m/z 227 (parent + H), 205, 171.

(2*S*,4*S*)-4-Acetamido-1-(*tert*-butoxycarbonyl)-2-methylpyrrolidine (23a). A solution of 8.86 g (39.2 mmol) of **22** in 250 mL of methanol containing 4.2 g of 10% palladium on carbon was placed under 4 atm of hydrogen. After 1 h, the catalyst was removed by filtration through Celite, and the filtrate was concentrated with a rotary evaporator. In a 500-mL round-bottom flask were placed the crude amine and 13 mL of pyridine. To the system was added 8.0 g (11.1 mL, 79.5 mmol) of triethylamine, and the system was cooled in an ice bath. To the system was added 8.1 g (7.5 mL, 79.5 mmol) of acetic anhydride and the solution was stirred for 19 h at room temperature under nitrogen. The reaction mixture was diluted with chloroform and washed with 10% aqueous hydrochloric acid, saturated aqueous sodium bicarbonate, and brine. The chloroform solution was dried (Na_2SO_4) and concentrated with a rotary evaporator to obtain 7.47 g of (2*S*,4*S*)-4-acetamido-1-(*tert*-butoxycarbonyl)-2-methylpyrrolidine (**23a**) as a red/brown gummy solid, which was used in subsequent transformations without further purification. The purified acetamide (pale yellow solid) melts at 107–110 °C; ^{1}H NMR δ 1.23 (d, 3 H, J = 6.2), 1.46 (s, 9 H), 1.71 (s, 1 H), 1.98 (s, 3 H), 3.23 (br m, 2 H), 3.63 (br m, 1 H), 3.94 (br m, 1 H), 4.50 (dd, 1 H, J = 6.4, 12.7), 5.61 (br, 1 H); mass spectrum, m/z 243 (parent + H). Anal. ($C_{12}H_{22}N_2O_3$) C, H, N.

Analogous procedures were used to convert **24** to (2*R*,4*S*)-4-acetamido-1-(*tert*-butoxycarbonyl)-2-methylpyrrolidine. Physical and spectral data for intermediates are given below.

(2*S*,4*R*)-1-(*tert*-Butoxycarbonyl)-4-[(*tert*-butyldimethylsilyl)oxy]-2-carbomethoxypyrrolidine: ^{1}H NMR δ 0.05 (s, 6 H), 0.87 (s, 9 H), 1.45 (2 s, 9 H), 2.05 (m, 1 H), 2.19 (m, 1 H), 3.30 (m, 1 H), 3.53 (m, 1 H), 3.67 (2 s, 3 H), 4.27 (m, 1 H), 4.36 (m, 1 H); mass spectrum, m/z 360 (parent + H), 300, 258. Anal. ($C_{17}H_{33}NO_5Si$) C, H, N.

(2*S*,4*R*)-1-(*tert*-Butoxycarbonyl)-4-[(*tert*-butyldimethylsilyl)oxy]-2-(hydroxymethyl)pyrrolidine: ^{1}H NMR δ 0.05 (s, 6 H), 0.86 (s, 9 H), 1.47 (s, 9 H), 1.90 (m, 1 H), 3.27–4.25 (complex m, 7 H), 4.89 (br d, 1 H, J = 6.6); mass spectrum, m/z 332, 258. Anal. ($C_{16}H_{33}NO_4Si$) C, H, N.

(2*S*,4*R*)-1-(*tert*-Butoxycarbonyl)-4-[(*tert*-butyldimethylsilyl)oxy]-2-[[(methylsulfonyl)oxy]methyl]-pyrrolidine: ^{1}H NMR δ 0.05 (s, 6 H), 0.85 (s, 9 H), 1.44 (s, 9 H), 1.98 (m, 2 H), 2.98 (s, 3 H), 3.30 (d, 1 H, J = 3.9), 4.05–4.50 (complex m, 5 H); mass spectrum, m/z 410 (parent + H), 354, 310.

(2*R*,4*R*)-1-(*tert*-Butoxycarbonyl)-4-hydroxy-2-methylpyrrolidine: ^{1}H NMR δ 1.24 (d, 3 H, J = 7.0), 1.47 (s, 9 H), 2.40 (m, 1 H), 3.45 (m, 2 H), 3.90–4.65 (complex m, 4 H); mass spectrum, m/z 201 (parent), 186. Anal. ($C_{10}H_{19}NO_3 \cdot {}^1/_4H_2O$) C, H, N.

(2*R*,4*S*)-4-Azido-1-(*tert*-butoxycarbonyl)-2-methylpyrrolidine: ^{1}H NMR δ 1.32 (d, 3 H, J = 7.0), 1.47 (s, 9 H), 1.71 (m, 1 H), 2.32 (m, 1 H), 3.33 (m, 1 H), 3.65 (br, 1 H), 3.93 (m, 1 H), 4.10 (m, 1 H); mass spectrum, m/z 226 (parent), 211, 197, 183.

(2*R*,4*S*)-4-Acetamido-1-(*tert*-butoxycarbonyl)-2-methylpyrrolidine (25a): ^{1}H NMR δ 1.30 (d, 3 H, J = 6.25), 1.46 (s, 9 H), 1.99 (s, 3 H), 2.44 (m, 1 H), 3.09 (m, 1 H), 3.34 (m, 1 H), 3.84 (m, 2 H), 4.34 (m, 1 H), 5.64 (br d, NH, J = 6.0); mass spectrum, m/z 243 (parent + H), 227, 215, 201. Anal. ($C_{12}H_{22}N_2O_3 \cdot {}^1/_{10}H_2O$) C, H, N.

(2*R*,4*S*)-4-Acetamido-1-benzyl-2-(pyrrolidin-1-ylmethyl)pyrrolidine (31a). Under a nitrogen atmosphere, in a 25-mL round-bottom flask were placed 705 mg (3.00 mmol) of **11** and 2 mL of dichloromethane. To this stirring solution was added 1.00 mL (853 mg, 12.0 mmol) of pyrrolidine, and this solution was stirred at room temperature for 85 min. A reflux condenser was attached to the system and the mixture was heated. After 2 h, 10 min, 2.0 mL (1.7 g, 24 mmol) of pyrrolidine was added to the system, and the mixture was heated at ca. 105 °C for 3 h and concentrated with a rotary evaporator. The crude orange oil was purified by flash column chromatography (50 g of silica gel) with 8% methanol/chloroform as the eluant to obtain 650 mg of **29** as a yellow oil: ^{1}H NMR δ 1.5–1.9 (complex, 5 H), 2.26 (ddd, 1 H, J = 6, 10, 14), 2.63 (m, 1 H), 2.96 (dd, 1 H, J = 4.5, 14), 3.1–3.4 (complex, 4 H), 3.54 (m, 1 H), 3.74 (d, 1 H, J = 14), 3.97 (d, 1 H, J = 14), 4.31 (m, 1 H), 5.53 (m, 1 H), 7.3 (m, 5 H); mass spectrum, m/z 274 (parent), 256. Anal. ($C_{16}H_{22}N_2O_2 \cdot 0.5H_2O$) C, H, N.

Under a nitrogen atmosphere, in a 50-mL round-bottom flask were placed 374 mg (1.36 mmol) of **29** and 0.5 mL of dichloromethane. To this stirring solution, at 0 °C, were added 1.14 mL (824 mg, 8.16 mmol) of triethylamine and 0.32 mL (466 mg, 4.08 mmol) of methanesulfonyl chloride, and the mixture was stirred at room temperature for 8 h, 10 min. During this period, additional triethylamine (0.38 mL, 275 mg, 2.72 mmol) and methanesulfonyl chloride (0.11 mL, 160 mg, 1.40 mmol) were added to the system. The reaction mixture was diluted with dichloromethane, washed with saturated aqueous sodium bicarbonate and brine, and dried over sodium sulfate. The solvent was removed with a rotary evaporator, and the crude brown liquid was subjected to flash column chromatography (20 g of silica gel) with ethyl acetate as the eluant to obtain 390 mg of the methanesulfonate ester of **29** as a viscous yellow oil.

Under a nitrogen atmosphere, in a round-bottom flask were placed 380 mg (1.08 mmol) of the methanesulfonate ester prepared above and 0.4 mL of acetonitrile. To this stirring solution was added 764 mg (2.69 mmol) of tetra-*n*-butylammonium azide. The solution was heated at 60 °C for 80 min, diluted with ethyl acetate, washed with saturated aqueous sodium bicarbonate and brine, dried (Na_2SO_4), and concentrated with a rotary evaporator to afford 375 mg of crude **30**, which was used without further purification: ^{1}H NMR δ 1.7 (m, 2 H), 2.16 (ddd, 1 H, J = 4, 8, 13), 2.31 (ddd, 1 H, J = 6, 8, 13), 2.73 (dd, 1 H, J = 4, 10), 2.92 (m, 1 H), 3.23 (m, 1 H), 3.34–3.52 (complex, 5 H), 3.65 (dd, 1 H, J = 2, 6), 3.68 (d, 1 H, J = 13), 3.93 (d, 1 H, J = 13), 4.17 (m, 1 H), 7.29 (m, 5 H); mass spectrum, m/z 299 (parent). Under a nitrogen atmosphere in a round-bottom flask were placed 370 mg of the azide **30** and 1 mL of THF. To this stirring solution, at −78 °C, was added 160 mg (4.20 mmol) of lithium aluminum hydride. The reaction mixture was stirred at −78 °C for 5 min, and the cold

bath was removed. After ca. 2 min an exotherm occurred, and the mixture was stirred at room temperature for ca. 4 h and diluted with 4 mL of THF. To the system was added cautiously a mixture of 15 mL of THF and 1.5 mL of 1 M aqueous sodium hydroxide. The mixture was stirred at room temperature, Na_2SO_4 was added to the system, and the solids were removed by suction filtration through a pad of Celite. The filtrate was concentrated with a rotary evaporator, and the residue was dissolved in 0.84 mL of pyridine. To the system were added 0.32 mL (0.23 g, 2.26 mmol) of triethylamine and 0.52 mL (0.57 g, 5.54 mmol) of acetic anhydride, and the solution was stirred under nitrogen at room temperature for 15 h. The reaction mixture was diluted with dichloromethane, washed with saturated aqueous sodium bicarbonate and brine, dried (Na_2SO_4), and concentrated with a rotary evaporator to afford 0.32 g of the title compound as an oil; ^{1}H NMR δ 1.85–2.25 (complex, 8 H), 1.93 (s, 3 H), 2.59 (dd, 1 H, J = 9, 12), 2.78 (m, 4 H), 3.06 (m, 1 H), 3.30 (dd, 1 H, J = 7, 10), 3.47 (d, 1 H, J = 13), 4.06 (d, 1 H, J = 13), 4.36 (m, 1 H), 5.62 (m, 1 H), 7.28 (m, 5 H); mass spectrum, m/z 302 (parent + H). This crude material was used in subsequent transformations without further purification.

(2*S*,4*R*)-2-(Azidomethyl)-1-(*tert*-butoxycarbonyl)-4-[(*tert*-butyldimethylsilyl)oxy]pyrrolidine (33). In a 500-mL round-bottom flask were placed 22.2 g (67.1 mmol) of **32**, 30 mL of dichloromethane, and 22.4 mL (16.3 g, 161 mmol) of triethylamine. The system was cooled to 0 °C in an ice bath and 6.4 mL (9.2 g, 80.5 mmol) of methanesulfonyl chloride was slowly added to the system. The system was stirred under nitrogen for 14 h, during which time the ice bath expired. The reaction mixture was partitioned between ethyl acetate and water. The ethyl acetate solution was washed with 1 M aqueous phosphoric acid and saturated aqueous sodium bicarbonate, dried (Na_2SO_4), and concentrated with a rotary evaporator to obtain 25 g (91% yield) of (2*S*,4*R*)-1-(*tert*-butoxycarbonyl)-4-[(*tert*-butyldimethylsilyl)-oxy]-2-[[(methylsulfonyl)oxy]methyl]pyrrolidine as a red oil, which is characterized above. This crude material (24.9 g, 60.9 mmol) was dissolved in 20 mL of acetonitrile in a 500-mL round-bottom flask. To this solution was added 22.5 g (79.1 mmol) of tetra-*n*-butylammonium azide, and the mixture was heated at 65 °C for 3 h under nitrogen. The reaction mixture was diluted with ether and the upper ether layer was washed with saturated aqueous sodium bicarbonate and brine. The combined aqueous washings and initial lower layer were extracted once with ether. The combined organic fractions were dried (Na_2SO_4) and concentrated with a rotary evaporator to afford 19.8 g (91% yield) of crude **33** as a dark yellow oil, which was used without further purification.

(2*S*,4*R*)-2-(Azidomethyl)-1-(*tert*-butoxycarbonyl)-4-hydroxypyrrolidine. In a 500-mL round-bottom flask was placed 18 g (50.6 mmol) of the crude **33**. To the system was added 56 mL (56 mmol) of 1 M tetra-*n*-butylammonium fluoride in THF. This solution was stirred under nitrogen for 4 h, diluted with ethyl acetate, and washed with saturated aqueous sodium bicarbonate and brine. The combined aqueous washings were extracted twice with ethyl acetate. The organic fractions were combined, dried (Na_2SO_4), and concentrated with a rotary evaporator to obtain 18.1 g of yellow oil. The crude product was purified by flash chromatography with 1:1 ethyl acetate/hexanes as the eluant to obtain 11.1 g (92% yield) of pure (2*S*,4*R*)-2-(azidomethyl)-1-(*tert*-butoxycarbonyl)-4-hydroxypyrrolidine as a yellow oil, which solidified in the freezer: mp 35–38 °C; ^{1}H NMR δ 1.48 (s, 9 H), 2.05 (br m, 2 H), 3.22–4.44 (m, 7 H); mass spectrum, m/z 243, 187, 143, 130.

(2*S*,4*R*)-2-(Azidomethyl)-1-(*tert*-butoxycarbonyl)-4-[(methylsulfonyl)oxy]pyrrolidine (34). In a 500-mL round-bottom flask were placed 5.0 g (20.7 mmol) of crude (2*S*,4*R*)-2-(azidomethyl)-1-(*tert*-butoxycarbonyl)-4-hydroxypyrrolidine and 7 mL of dichloromethane. To the system was added 5.2 g (7.2 mL, 51.7 mmol) of triethylamine. The system was cooled in an ice–salt bath, and 4.7 g (3.3 mL, 41.3 mmol) of methanesulfonyl chloride was slowly added over a 10-min period. The reaction mixture was stirred under nitrogen for 28 h, during which time the ice bath expired. The reaction mixture was partitioned between ether and water. The ether solution was washed with 1 M aqueous phosphoric acid and saturated aqueous sodium bicarbonate, dried (Na_2SO_4), and concentrated with a rotary evaporator to obtain 6.14 g (92%) of **34** as a yellow oil, which was used in subsequent transformations without further purification: ^{1}H NMR δ 1.48 (s, 9 H), 2.18–2.47 (m, 2 H), 3.04 (s, 3 H), 3.24–4.15 (m, 6 H); mass spectrum, m/z 321, 282.

(1*S*,4*S*)-2-(*tert*-Butoxycarbonyl)-2,5-diazabicyclo[2.2.1]-heptane Methanesulfonic Acid Salt (35). A solution of 2.5 g (7.8 mmol) of **34** in 250 mL of methanol containing 1.0 g of 10% palladium on carbon was placed under 4 atm of hydrogen. After 1 h, the catalyst was removed by filtration through Celite and the filtrate was concentrated with a rotary evaporator to afford 1.6 g (quantitative yield) of **35** as a yellow waxy solid, which was used subsequent transformations without further purification. An analytical sample was prepared by recrystallization from chloroform–hexanes: mp 180–182 °C; ^{1}H NMR δ 1.46 (s, 9 H), 1.90 (m, 1 H), 3.00–3.66 (complex m, 6 H), 4.10–4.60 (complex m, 2 H); mass spectrum, m/z 199 (parent + H), 143. Anal. ($C_{10}H_{18}N_2O_2 \cdot {}^6/_5CH_3SO_3H$) C, H, N.

(2*R*,4*S*)-1-Benzyl-4-(*tert*-butoxycarbonylamino)-2-(methoxymethyl)pyrrolidine (40a). Under a nitrogen atmosphere, in a round-bottom flask were placed 6.03 g (21.4 mmol) of **36** and 128 mL of dichloromethane. To this solution were added 2.93 mL (32.0 mmol) of 3,4-dihydro-2*H*-pyran and 537 mg (2.1 mmol) of pyridinium *p*-toluenesulfonate (PPTS). The reaction mixture was stirred at room temperature for 3 h, diluted with 150 mL of ether, washed with brine, dried ($MgSO_4$), and concentrated to afford 6.81 g of a colorless syrup. In a round-bottom flask were placed 6.66 g (20.2 mmol) of this syrup and 20 mL of THF. To the system was added 435 mg (20 mmol) of lithium borohydride, and the reaction mixture was stirred overnight under nitrogen. To the system was added cautiously 50 mL of water, and the mixture was extracted with chloroform. The chloroform extracts were dried ($MgSO_4$) and concentrated with a rotary evaporator to obtain 6.6 g of **41** as an oil. Compound **41** (6.7 g, 22 mmol) was placed in 50 mL of DMF, and KOH (5.02 g, 89.5 mmol) and methyl iodide (2.76 mL, 44.5 mmol) were added to the system. The reaction mixture was stirred at room temperature for 1 day. During this period, additional KOH (5.02 g, 89.5 mmol) and methyl iodide (2.76 mL, 44.5 mmol) were added to the system. The reaction mixture was diluted with H_2O and extracted with three portions of chloroform. The chloroform extracts were washed with H_2O, dried ($MgSO_4$), and concentrated with a rotary evaporator. The crude product was purified by flash column chromatography with 1:4 ethyl acetate/hexanes as the eluant to obtain 5.68 g of **37** as a colorless oil: ^{1}H NMR δ 1.48 (s, 9 H), 1.5–2.2 (complex), 3.36–3.38 (2 s, 3 H), 3.4–4.0 (complex, 7 H), 4.37 (m, 1 H), 4.68 (m, 1 H); mass spectrum (DCI–NH_3), m/z 316 (parent + H), 260, 232, 176. Anal. ($C_{16}H_{29}NO_5$) C, H, N.

In a round-bottom flask were placed 5.39 g (16.7 mmol) of **37** and 300 mL of 1:1:1 H_2O/THF/acetic acid. The stirring reaction mixture was heated at 70 °C for 2.5 h and extracted with three portions of dichloromethane. These extracts were washed with three portions of saturated aqueous sodium bicarbonate, dried ($MgSO_4$), and concentrated to give an oil. To this oil were added 40 mL of dichloromethane and 10 mL of trifluoroacetic acid (TFA). The reaction mixture was stirred overnight at room temperature and concentrated to afford a brown oil, which was dissolved in CH_3CN and washed with three portions of hexanes. The acetonitrile solution was concentrated and the residue dissolved in dichloromethane, and triethylamine was added to pH 11. The mixture was concentrated to afford 2.17 g of a yellow oil. This crude oil was dissolved in dichloromethane, and 5.81 mL (41.7 mmol) of triethylamine and 2.18 mL (18.3 mmol) of benzyl bromide were added to the system. The reaction mixture was stirred under nitrogen for 2 h, diluted with 50 mL of dichloromethane, washed with 1 M aqueous HCl, dried ($MgSO_4$), and concentrated to obtain 1.64 g of **38**, which was used without further purification: ^{1}H NMR δ 1.73 (m, 1 H), 2.24 (ddd, 1 H, J = 6, 10, 16), 2.41 (dd, 1 H, J = 4, 10), 2.87 (ddd, 1 H, J = 3, 8, 10), 2.93 (dd, 1 H, J = 2, 10), 3.32 (d, 2 H, J = 4), 3.39 (s, 3 H), 3.58 (d, 1 H, J = 14), 3.98 (d, 1 H, J = 14), 4.22 (m, 1 H), 7.29 (m, 5 H); mass spectrum (DCI–NH_3), m/z 222 (parent + H). Anal. ($C_{13}H_{19}NO_2 \cdot {}^1/_6H_2O$) C, H, N.

Under a nitrogen atmosphere, in a round-bottom flask were placed 1.64 g (7.45 mmol) of **38** and 7 mL of dichloromethane. To this stirring solution, at 0 °C, was added 4.57 mL (32.7 mmol) of triethylamine followed by 1.26 mL (16.4 mmol) of methanesulfonyl chloride, and the reaction mixture was stirred at 0 °C

for 1.5 h. The mixture was diluted with 100 mL of dichloromethane, washed with H_2O, saturated aqueous sodium bicarbonate and H_2O, dried ($MgSO_4$), and concentrated to afford an oil, which was dissolved in 15 mL of acetonitrile. To this solution was added 5.63 g (19.8 mmol) of tetra-*n*-butylammonium azide, and the stirring solution was heated under nitrogen at 60 °C for 2 h. The reaction mixture was diluted with 50 mL of dichloromethane and washed with 50 mL of H_2O, dried ($MgSO_4$), and concentrated. The crude product was subjected to column chromatography with 2:3 ether/hexanes as the eluant to obtain 1.20 g of pure **39**: ^{1}H NMR δ 2.00 (m, 2 H), 2.32 (dd, 1 H, J = 7, 10), 2.97 (m, 1 H), 3.33 (dd, 1 H, J = 6, 10), 3.34 (s, 3 H), 3.42 (dd, 1 H, J = 5, 10), 3.48 (d, 1 H, J = 14), 3.97 (m, 1 H), 4.07 (d, 1 H, J = 14), 7.29 (m, 5 H); mass spectrum (DCI–NH_3), m/z 247 (parent + H). Anal. ($C_{13}H_{18}N_4O$) C, H, N.

Under a nitrogen atmosphere, in a round-bottom flask were placed 292 mg (7.37 mmol) of lithium aluminum hydride and 5 mL of THF. To this stirring solution, at −10 °C, was added dropwise a solution of 1.20 g (4.87 mmol) of the azide **39** in 5 mL of THF over a period of 15 min. The reaction mixture was allowed to stir at room temperature for 2 h and cooled to −10 °C. To the system was cautiously added 1 M aqueous NaOH. The resulting slurry was diluted with THF. To the system was added sodium sulfate, and the mixture was filtered through Celite and the filtrate was concentrated with a rotary evaporator to obtain 1.07 g of an oil. This oil was dissolved in 5 mL of dichloromethane. To this stirring solution were added 1.01 mL (7.26 mmol) of triethylamine and 1.06 g (5.25 mmol) of di-*t*-butyl dicarbonate, and the reaction mixture was stirred at room temperature under nitrogen overnight. The reaction mixture was diluted with 30 mL of dichloromethane and washed with 0.5 M aqueous phosphoric acid and H_2O, dried ($MgSO_4$), and concentrated. The crude material was purified by flash column chromatography with 2% methanol/chloroform as the eluant to obtain 1.35 g of **40a**: ^{1}H NMR δ 1.42 (s, 9 H), 1.69 (m, 1 H), 2.07 (m, 2 H), 2.90 (m, 1 H), 3.28 (m, 2 H), 3.23 (s, 3 H), 3.43 (m, 1 H), 3.47 (d, 1 H, J = 14), 4.06 (d, 1 H, J = 14), 4.08 (m, 1 H), 4.47 (m, 1 H), 7.29 (m, 5 H); mass spectrum (DCI–NH_3), m/z 321 (parent + H).

(2*R*,4*R*)-1-(*tert*-Butoxycarbonyl)-2-(fluoromethyl)-4-(tetrahydropyran-2-yloxy)pyrrolidine (42). In a 250-mL round-bottom flask was dissolved 6.0 g (20 mmol) of **41** in 10 mL of dichloromethane. To this solution was added 4.0 g (5.6 mL, 40 mmol) of triethylamine, the reaction mixture was cooled to 0 °C with an ice–salt bath, 3.4 g (2.4 mL, 30 mmol) of methanesulfonyl chloride was added dropwise, and the reaction mixture was stirred under nitrogen for 1 h, allowing the ice bath to expire. The reaction mixture was diluted with dichloromethane, washed with 1 M aqueous phosphoric acid and saturated aqueous sodium bicarbonate, dried (Na_2SO_4), and concentrated with a rotary evaporator to obtain 7.5 g of a yellow oil. This oil was placed in a 500-mL round-bottom flask and 185 mL of 1 M tetra-*n*-butylammonium fluoride in THF was added to the system. This solution was heated under reflux under nitrogen for 1 h. The reaction mixture was concentrated with a rotary evaporator, and the residue was taken up in ethyl acetate and washed with saturated aqueous sodium bicarbonate, dried (Na_2SO_4), and concentrated. The crude material was purified by flash column chromatography (200 g of silica gel), increasing the polarity of the eluant from 1:7 ethyl acetate/hexanes to 1:4 ethyl acetate/hexanes to obtain 3.47 g (62% yield) of **42** as a pale yellow oil: ^{1}H NMR δ 1.47 (s, 9 H), 1.55 (br, 8 H), 2.05–2.20 (m, 3 H), 3.49–4.72 (complex m, 12 H); mass spectrum, m/z 304 (parent + H), 265, 248, 204. Anal. ($C_{15}H_{26}FNO_4$) C, H, F, N.

(2*R*,4*R*)-1-(*tert*-Butoxycarbonyl)-4-hydroxy-2-(fluoromethyl)pyrrolidine. In a 500-mL round-bottom flask were placed 3.4 g (11 mmol) of **42** and 70 mL of THF. To the stirring solution were added 70 mL of water and 70 mL of acetic acid. The reaction mixture was heated under nitrogen for 1.5 h. The cooled solution was made basic to pH paper with saturated aqueous sodium bicarbonate (~200 mL) and extracted with chloroform. The chloroform extracts were dried (Na_2SO_4) and concentrated with a rotary evaporator. The crude yellow oil was purified by flash column chromatography with 1:3 ethyl acetate/hexanes as the eluant to obtain a clear oil, which was crystallized from hexanes to afford 1.45g (60%) of (2*R*,4*R*)-1-(*tert*-butoxycarbonyl)-4-hydroxy-2-(fluoromethyl)pyrrolidine as a white, crystalline solid: mp 98–99 °C; ^{1}H NMR δ 1.49 (s, 9 H), 2.28 (m, 2 H), 3.41 (ddd, 1 H, J = 1.6, 1.6, 12.1), 3.58 (br, 1 H), 4.05 (m, 1 H), 4.40–4.90 (complex m, 4 H); mass spectrum, m/z 220, 181, 164. Anal. ($C_{10}H_{18}FNO_3$) C, H, N.

(2*R*,4*S*)-4-Azido-1-(*tert*-butoxycarbonyl)-2-(fluoromethyl)pyrrolidine (43). In a 100-mL round-bottom flask were placed 1.33 g (6.0 mmol) of (2*R*,4*R*)-1-(*tert*-butoxycarbonyl)-2-(fluoromethyl)-4-hydroxypyrrolidine, 2 mL of dichloromethane, and 2.1 mL (1.5 g, 15 mmol) of triethylamine. The system was cooled in an ice bath, and 1.3 g (0.95 mL, 12 mmol) of methanesulfonyl chloride was slowly added via syringe. The reaction mixture was stirred under nitrogen for approximately 14 h, during which time the ice bath expired. The reaction mixture was diluted with dichloromethane, washed with 1 M aqueous phosphoric acid, saturated aqueous sodium bicarbonate, dried (Na_2SO_4), and concentrated to a red oil with a rotary evaporator. This material was subjected to flash column chromatography with 1:1 ethyl acetate/hexanes as the eluant to obtain 1.71 g (96% yield) of the methanesulfonate ester as a red oil.

In a 100-mL round-bottom flask were placed 1.5 g (5.1 mmol) of the methanesulfonate ester prepared above and 2 mL of acetonitrile. To this solution was added 3.1 g (11 mmol) of tetra-*n*-butylammonium azide, and the mixture was heated at 65 °C for 3 h under nitrogen. The reaction mixture was diluted with ether and the upper ether layer was washed with saturated aqueous sodium bicarbonate and brine, dried (Na_2SO_4), and concentrated with a rotary evaporator. The crude material was purified by flash column chromatography (100 g of silica gel) with 1:4 ethyl acetate/hexanes as the eluant to obtain 960 mg (80% yield) of **43** as a clear colorless oil: ^{1}H NMR δ 1.47 (s, 9 H), 2.24 (m, 1 H), 3.52 (m, 2 H), 4.15 (m, 2 H), 4.30–4.90 (complex m, 3 H); mass spectrum, m/z 245 (parrent + H), 189.

(2*R*,4*S*)-4-Acetamido-1-(*tert*-butoxycarbonyl)-2-(fluoromethyl)pyrrolidine (44a). In a 100-mL round-bottom flask were placed 820 mg (3.4 mmol) of **43** and 2.13 g (2.0 mL, 28 mmol) of thiolacetic acid. The solution was stirred under nitrogen for 5 h and concentrated with a rotary evaporator with a water aspirator. The resulting red oil was subjected to flash column chromatography (100 g of silica gel), increasing the polarity of the eluant from 1:1 ethyl acetate/hexane to ethyl acetate to obtain 455 mg (54% yield) of pure (2*R*,4*S*)-4-acetamido-1-(*tert*-butoxycarbonyl)-2-(fluoromethyl)pyrrolidine as a pale yellow solid: mp 130–132 °C; ^{1}H NMR δ 1.47 (s, 9 H), 1.98 (s, 3 H), 2.34 (m, 1 H), 3.20 (br, 1 H), 3.65 (br, 1 H), 4.10 (br, 1 H), 4.34 (m, 1 H), 4.52 (m, 2 H), 5.45 (m, 1 H); mass spectrum, m/z 261 (parent + H), 161. Anal. ($C_{12}H_{21}FN_2O_3$) C, H, N.

Spectral data for isolated side products in attempts to synthesize the 2-(fluoromethyl)pyrrolidine **44** are given below.

Compound 47: ^{1}H NMR δ 0.05 (s, 6 H), 0.81 (s, 9 H), 1.55 (ddd, 1 H, J = 5.3, 10.3, 12.5), 1.97 (m, 1 H), 3.05 (dd, 1 H, J = 1.6, 12.1), 3.87 (dd, 1 H, J = 5.4, 12.2), 4.19 (m, 2 H), 4.55 (m, 2 H); mass spectrum, m/z 258 (parent + H), 217. Anal. ($C_{12}H_{23}NO_3Si$) C, H, N.

Compound 49: ^{1}H NMR δ 1.46 (s, 9 H), 1.83 (m, 2 H), 3.33 (m, 2 H), 3.84 (m, 2 H), 4.50 (m, 2 H); mass spectrum, m/z 200 (parent + H), 144. Anal. ($C_{10}H_{17}NO_3$) C, H, N.

Full experimental procedures for the preparation of **7** and **59** from the appropriate *N*-benzyl- and *N*-(*tert*-butyoxycarbonyl)-pyrrolidine precursors are provided. These conditions are representative of those used to prepare the analogues **54–58** and **61–66** unless otherwise noted. Physical and spectral data for these derivatives are also provided.

(2′*R*,4′*S*)-7-[4′-Amino-2′-(hydroxymethyl)pyrrolidinyl]-1-(2,4-difluorophenyl)-1,4-dihydro-6-fluoro-4-oxoquinoline-3-carboxylic Acid (7). In a 250-mL round-bottom flask were placed 0.46 g (1.85 mmol) of **8a** and 20 mL of 0.1 M HCl in methanol. To the system were added 8 drops of concentrated aqueous HCl and 660 mg of 5% Pd/C. The system was placed under an atmosphere of H_2 (balloon), after first flushing with nitrogen, and the reaction mixture was stirred at room temperature for 3 h, 10 min. During this period additional (150 mg) 5% Pd/C was added to the system. The mixture was diluted with methanol and filtered through a Celite pad, and the filtrate was concentrated with a rotary evaporator. The residue was dissolved in 16 mL of ethanol, and 2.2 g of Rexyn 201 basic exchange resin was added to the system. The mixture was stirred at room temperature for

2.5 h, the solids were removed by filtration through a Celite pad, and the filtrate was concentrated with a rotary evaporator to afford 250 mg of the amine **8b**, which was used without further purification. Under a nitrogen atmosphere, in a 25-mL round-bottom flask were placed 249 mg (1.58 mmol) of the amine **8b**, 0.6 mL of pyridine, and 0.19 mL (138 mg, 1.37 mmol) of triethylamine. To the system was added 500 mg (1.37 mmol) of the quinolone ester **51**, and the mixture was heated at ca. 40 °C for 2 days. During this period, additional pyridine (0.2 mL) and **51** (100 mg, 0.27 mmol) were added to the system. The reaction mixture was concentrated and subjected to flash column chromatography to afford 230 mg of adduct as a light yellow solid, mp 149–152 °C. In a 100-mL round-bottom flask were placed 197 mg (0.39 mmol) of this adduct and 3 mL of THF. To the system was added 6.4 mL (0.64 mmol) of 0.1 M aqueous NaOH, and the reaction mixture was heated at ca. 60 °C for 4.5 h and concentrated with a rotary evaporator. To the system was added 7.6 mL of 6 M aqueous HCl, and the orange solution was heated at ca. 105 °C for 17 h, 50 min and concentrated with a rotary evaporator. The residue was dissolved in H_2O, and the solution was made basic with 1 M aqueous NaOH. To this solution was added 10% aqueous NH_4Cl, and the resulting precipitate was collected by suction filtration and rinsed with H_2O, methanol, and ether. The solid was suspended in 2–3 mL of methanol and the mixture was heated to boiling and allowed to cool to room temperature. The solid was collected by filtration and rinsed with methanol and ether to afford 110 mg of an off-white solid: mp 214–217 °C; ^{1}H NMR (TFA-*d*) δ 2.72 (m, 2 H), 3.85–4.2 (complex, 4 H), 4.52 (m, 1 H), 4.89 (m, 1 H), 6.46 (m, 1 H), 7.34 (m, 2 H), 7.70 (m, 1 H), 8.29 (d, 1 H, J = 14), 9.11 (2 s, 1 H); mass spectrum (FAB), m/z 434 (parent + H). Anal. ($C_{21}H_{18}F_3N_3O_4 \cdot 0.5H_2O$) C, H, N.

(2*S*,4*S*)-4-Acetamido-2-methylpyrrolidine (23b). In a 50-mL round-bottom flask were placed 3.1 g (13 mmol) of crude **23a** and 15 g (10 mL, 130 mmol) of trifluoroacetic acid. The solution was stirred under nitrogen for 15 min and concentrated with a rotary evaporator. The residue was dissolved in 75 of methanol. To this solution was added 30 g of Rexyn 201 (OH) resin which had been rinsed with ethanol. The mixture was stirred under nitrogen for 15 min, and 10 g of additional resin was added to the mixture. The resin was removed by filtration through Celite, and the filtrate was concentrated with a rotary evaporator to obtain 2 g of crude **23b**, which was used immediately for displacement on **53**.

(2′*S*,4′*S*)-7-(4′-Amino-2′-methylpyrrolidinyl)-1-(2,4-difluorophenyl)-1,4-dihydro-6-fluoro-4-oxo-1,8-naphthyridine-3-carboxylic Acid (59). In a 100-mL round-bottom flask were placed the crude **23b** prepared above and 6 mL of pyridine. To the system were added 1.4 g (2 mL, 13.9 mmol) of triethylamine and 5.5 g (14 mmol) of **53**. The reaction mixture was heated at 65 °C for 14 h under nitrogen, and the solvent was removed with a rotary evaporator. The crude material was purified by flash column chromatography, gradually increasing the polarity of the eluant from chloroform to 10% methanol/chloroform, to obtain 4.9 g (78% yield) of displacement product. This material was dissolved in 60 mL of THF in a 1-L round-bottom flask. To the system was added 200 mL (20 mmol) of 0.1 M aqueous sodium hydroxide, and the reaction mixture was heated at 65 °C for 3 h. An additional 100 mL (10 mmol) of 0.1 M aqueous sodium hydroxide was added to the system, and the reaction mixture was heated at 65 °C for 1 h. The reaction mixture was concentrated with a rotary evaporator. To the system was added 400 mL of 6 M aqueous hydrochloric acid, and the reaction mixture was heated at 110 °C for 15 h under nitrogen. The reaction mixture was concentrated with a rotary evaporator, and the solid residue was dissolved in approximately 200 mL of water. This solution was brought to pH 7 with saturated aqueous sodium bicarbonate, and the resulting precipitate was collected by suction filtration, rinsed with water, ethanol, and ether, and dried in a vacuum oven at 40 °C to obtain 2.64 g (63% yield) of **59** as a yellow solid: mp 231–234 °C; IR (KBr) 1730, 1630 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 0.90 (br m, 3 H), 1.67 (m, 2 H), 3.5 (br m, 4 H), 7.33 (m, 1 H), 7.61 (m, 1 H), 7.80 (m, 1 H), 8.04 (d, 1 H, J = 14), 8.79, 8.81 (2 s, 1 H); mass spectrum, m/z 419 (parent + H). Anal. ($C_{20}H_{17}F_3N_4O_3 \cdot {}^1/_4H_2O$) C, H, N.

(2′*S*,4′*S*)-7-[4′-Amino-2′-(hydroxymethyl)pyrrolidinyl]-1-(2,4-difluorophenyl)-1,4-dihydro-6-fluoro-4-oxoquinoline-3-carboxylic acid hydrochloride (54): mp >240 °C; ^{1}H NMR (DMSO-d_6) δ 1.98 (m, 1 H), 2.46 (m, 1 H), 3.25–3.57 (complex, 3 H), 3.62 (m, 1 H), 3.77 (m, 1 H), 4.12 (m, 1 H), 5.99 (m, 1 H), 7.45 (m, 1 H), 7.73 (m, 1 H), 7.94 (m, 1 H), 7.95 (d, 1 H, J = 14), 8.79, 8.82 (2 s, 1 H); mass spectrum (FAB), m/z 434 (parent + H). Anal. ($C_{21}H_{18}F_3N_3O_4 \cdot HCl \cdot {}^1/_2H_2O$) C, H, N.

(2′*R*,4′*S*)-7-[4′-Amino-2′-(pyrrolidinylmethyl)-pyrrolidinyl]-1-(2,4-difluorophenyl)-1,4-dihydro-6-fluoro-4-oxoquinoline-3-carboxylic acid (55): mp ~245 °C; ^{1}H NMR δ 1.65–2.50 (complex, 12 H), 3.09 (m, 1 H), 3.76 (m, 2 H), 4.03 (m,1 H), 5.98 (m, 1 H), 7.16 (m, 2 H), 7.48 (m, 1 H), 7.98 (d, 1 H, J = 14), 8.51 (s, 1 H); mass spectrum (FAB), m/z 487 (parent + H); exact mass calcd for $C_{25}H_{26}N_4O_3F_3$ 487.1957, found 487.1961.

(2′*S*,4′*S*)-7-(4′-Amino-2′-ethylpyrrolidinyl)-1-(2,4-difluorophenyl)-1,4-dihydro-6-fluoro-4-oxoquinoline-3-carboxylic acid (56): ^{1}H NMR δ 0.74, 0.77 (2 t, 3 H, J = 7.5), 1.2–2.0 (complex), 3.09 (m, 1 H), 3.75 (m, 1 H), 5.81 (m, 1 H), 7.16 (m, 2 H), 7.47 (m, 1 H), 8.00 (d, 1 H, J = 14), 8.53 (2 s, 1 H); mass spectrum (DCI–NH_3), m/z 432 (parent + H); exact mass calcd for $C_{22}H_{21}N_3O_3F_3$ 432.1535, found 432.1555.

(2′*S*,4′*S*)-7-(4′-Amino-2′-methylpyrrolidinyl)-1-(2,4-difluorophenyl)-1,4-dihydro-6-fluoro-4-oxoquinoline-3-carboxylic acid hydrochloride (57): mp 206–210 °C; ^{1}H NMR (DMSO-d_6) δ 1.02 (2 d, 3 H, J = 6), 1.70 (m, 1 H), 1.82 (m, 1 H), 2.92–4.04 (complex, 4 H), 5.85 (m, 1 H), 7.43 (m, 1 H), 7.75 (m, 1 H), 7.88 (d, 1 H, J = 15), 7.93 (m, 1 H), 8.73, 8.76 (2 s, 1 H); mass spectrum (FAB), m/z 418 (parent + H). Anal. ($C_{21}H_{18}F_3N_3O_3 \cdot HCl$), C, H, N.

(2′*R*,4′*S*)-7-(4′-Amino-2′-methylpyrrolidinyl)-1-(2,4-difluorophenyl)-1,4-dihydro-6-fluoro-4-oxoquinoline-3-carboxylic acid (58): mp >150 °C (dec; poorly defined); ^{1}H NMR (DMSO-d_6) δ 1.08 (2 d, 3 H, J = 6), 1.37 (m, 1 H), 3.28 (m, 1 H), 3.13–3.78 (complex, 4 H), 5.88 (m, 1 H), 7.43 (m, 1 H), 7.75 (m, 1 H), 7.90 (d, 1 H, J = 15), 7.96 (m, 1 H), 8.76, 8.78 (2 s, 1 H). Anal. ($C_{21}H_{18}F_3N_3O_3 \cdot {}^1/_2HCl$) C, H, N.

(2′*R*,4′*S*)-7-(4′-Amino-2′-methylpyrrolidinyl)-1-(2,4-difluorophenyl)-1,4-dihydro-6-fluoro-4-oxo-1,8-naphthyridine-3-carboxylic acid hydrochloride (60): mp 294–296 °C dec; ^{1}H NMR (DMSO-d_6) δ 1.00 (2 d, 3 H, J = 6.0), 1.65 (m, 1 H), 2.45 (m, 1 H), 3.30–4.20 (complex m, 4 H), 7.35 (m, 1 H), 7.62 (m, 1 H), 7.84 (m, 1 H), 8.17 (2 d, 1 H, J = 12.7), 8.87 (2 s, 1 H); mass spectrum, m/z 418 (parent), 403, 386, 374. Anal. ($C_{20}H_{17}F_3N_4O_3 \cdot HCl$) C, H, N.

(2′*S*,4′*S*)-7-(4′-Amino-2′-methylpyrrolidinyl)-1-(*p*-fluorophenyl)-1,4-dihydro-6-fluoro-4-oxoquinoline-3-carboxylic acid hydrochloride (61): mp >200 °C dec (poorly defined); ^{1}H NMR (DMSO-d_6) δ 1.02 (d, 3 H, J = 6), 1.94 (m, 1 H), 2.24 (m, 1 H), 3.4–4.16 (complex, 4 H), 5.97 (d, 1 H, J = 8), 7.57 (dd, 2 H, J = 8, 8), 7.82 (dd, 2 H, J = 5, 8), 7.97 (d, 1 H, J = 14), 8.62 (s, 1 H); mass spectrum (FAB), m/z 400 (parent + H). Anal. ($C_{21}H_{19}F_2N_3O_3 \cdot HCl \cdot {}^5/_4H_2O$) C, H, N.

(2′*S*,4′*S*)-7-(4′-Amino-2′-methylpyrrolidinyl)-1,4-dihydro-6-fluoro-1-(*p*-fluorophenyl)-4-oxo-1,8-naphthyridine-3-carboxylic acid hydrochloride (62): mp >250 °C; IR (KBr) 1720, 1630 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 0.98 (br m, 3 H), 1.93 (m, 1 H), 2.18 (m, 1 H), 3.5–3.9 (complex, 4 H), 7.43 (dd, 2 H, J = 9, 9), 7.70 (dd, 2 H, J = 6, 9), 8.15 (d, 1 H, J = 14), 8.38 (br, 2 H), 8.68 (s, 1 H); mass spectrum (DCI–NH_3), m/z 401 (parent + H). Anal. ($C_{20}H_{18}N_4F_2O_3 \cdot 1.8HCl$) C, H, N.

(1′*S*,4′*S*)-7-(2′,5′-Diazabicyclo[2.2.1]heptan-2′-yl)-1-(2,4-difluorophenyl)-1,4-dihydro-6-fluoro-4-oxoquinoline-3-carboxylic acid hydrochloride (63): mp >240 °C; ^{1}H NMR (DMSO-d_6) δ 1.88 (m, 1 H), 2.18 (m, 1 H), 3.23–3.7 (complex, 4 H), 4.36 (m, 1 H), 4.73 (m, 1 H), 5.93 (m, 1 H), 7.44 (m, 1 H), 7.73 (m, 1 H), 7.82 (m, 1 H), 7.98 (2 d, 1 H, J = 14), 8.78 (2 s, 1 H); mass spectrum (FAB), m/z 416 (parent + H). Anal. ($C_{21}H_{16}F_3N_3O_3 \cdot HCl \cdot H_2O$) C, H, N.

(1′*S*,4′*S*)-7-(2′,5′-Diazabicyclo[2.2.1]heptan-2′-yl)-1-(2,4-difluorophenyl)-1,4-dihydro-6-fluoro-4-oxo-1,8-naphthyridine-3-carboxylic acid hydrochloride (64): mp >260 °C; ^{1}H NMR (DMSO-d_6) δ 1.95 (m, 2 H), 3.2–3.6 (complex m, 4 H), 4.37 (s, 1 H), 7.35 (m, 1 H), 7.61 (m, 1 H), 8.20 (2 d, 1 H, J = 12.1), 8.87 (2 s, 1 H); mass spectrum, m/z 417 (parent + H), 399, 382. Anal. ($C_{20}H_{15}F_3N_4O_3 \cdot {}^4/_3HCl$) C, H, N.

**(2′*R*,4′*S*)-7-[4′-Amino-2′-(methoxymethyl)pyrrolidinyl]-1-(2,4-difluorophenyl)-1,4-dihydro-4-oxo-1,8-naphthyridine-

3-carboxylic acid (65): mp >250 °C; ^{1}H NMR (DMSO-d_6) δ 3.2 (s, 3 H), 7.37 (m, 1 H), 7.62 (m, 1 H), 7.82 (m, 1 H), 8.18 (d, 1 H, J = 13), 8.87, 8.88 (2 s, 1 H); mass spectrum (FAB), m/z 449 (parent + H); exact mass calcd for $C_{21}H_{20}F_3N_4O_4$ 449.1436, found 449.1424.

(2′*R*,4′*S*)-7-[4′-Amino-2′-(fluoromethyl)pyrrolidinyl]-1-(2,4-difluorophenyl)-1,4-dihydro-6-fluoro-4-oxo-1,8-naphthyridine-3-carboxylic acid hydrochloride (66): mp >280 °C; ^{1}H NMR (DMSO-d_6) δ 1.75 (m, 1 H), 1.95 (m, 1 H), 3.10–4.5 (complex m, 6 H), 7.34 (m, 1 H), 7.60 (m, 1 H), 7.82 (m, 1 H), 8.13 (2 d, 1 H, J = 12.7), 8.84 (2 s, 1 H); mass spectrum, 437 (parent + H), 395, 356. Anal. ($C_{20}H_{16}F_4N_4O_3 \cdot {}^2/_3$HCl) C, H, N.

Microbiology. General Procedures. In Vitro Studies. The minimum inhibitory concentrations (MIC, μg/mL) were determined by the twofold agar dilution method on brain–heart infusion agar. The values reported are representative of those obtained versus a larger quantity of Gram-positive and Gram-negative bacteria and are shown relative to ciprofloxacin (CIP).

In Vivo Studies (Mouse Protection Tests). Mice were treated subcutaneously (sc) or orally (po) with a specific amount of the test compound divided into equal portions which were administered at 1 and 5 h after infection. Potencies are expressed as ED_{50} values, which are given as the total dose of compound (mg/kg) required to protect 50% of the mice challenged intraperitoneally with the indicated organism.

Pharmacokinetic Studies. General Procedure. Mice were administered the quantity of compound indicated orally (Table VII), as a single dose. At the specified time intervals, blood was collected from groups of five mice. All samples were assayed by a disk agar diffusion bioassay procedure. *Bacillus subtilis* 6633 or *Klebsiella pneumoniae* 10031 were used as the assay organisms, and seed agar medium no. 1 (BBL Microbiology Systems; Cockeysville, MD) was the growth medium. The plates were incubated at 32 °C for 18 h and read with an image analyzer (Optomax Inc.).

Solubility Studies. General Procedure. A known excess weight of the compound was shaken overnight with a known volume of Ringer's buffer (Ringer's buffer is a bicarbonate buffer containing sodium, potassium, calcium, and magnesium ions, initially adjusted to pH 7.5). The contents were filtered, and the clear filtrate was analyzed after appropriate dilution by HPLC (UV-absorbance detection).

Metabolism Studies. Pharmacokinetic Evaluation in Dog. Four 7–10-kg beagle dogs were fasted overnight prior to dosing until after the 12-h sampling time point; all animals were permitted free access to water. A 10 mg/mL solution of **59** (in 2 equiv of lactobionic acid) was administered by oral gavage (10 mg/kg) to two dogs and by iv bolus (5 mg/kg) to the other two animals. Fifteen sequential blood samples were obtained from each animal 0.5–48 h after dosing. Plasma was separated by centrifugation and frozen until analysis. Urine was collected from 0 to 24 and 24 to 48 h after dosing and frozen until analysis.

The HPLC method for quantitation of **59** in plasma samples was an adaptation of the method described by Granneman.[17] Compound **59** and the internal standard (compound 1 in 0.2 M phosphate buffer, pH 7.2) were extracted from plasma at neutral pH with 6 volumes of methylene chloride/ethanol (9:1). The organic layer was separated from the aqueous and evaporated to dryness with a stream of dry air at ~45 °C. Samples were reconstituted in the mobile phase. An aliquot of each urine sample was assayed as described above. An additional urine aliquot was assayed for **59** after alkaline hydrolysis (0.1 N NaOH) for 1 h at 60 °C.

Compound **59** and the internal standard, 1, were separated from plasma components on a 5 cm × 4.6 mm 3 μm Sperisorb OSD2 column (Regis Chemical Co.) with a 0.04 M H_3PO_4/0.01 M NaH_2PO_4/0.2% sodium dodecyl sulfate/0.005 M acetohydroxamic acid/40% HPLC grade acetonitrile mobile phase at a flow rate of 0.9–1.0 mL/min with UV detection of the 40-μL injection at 272 nm. Linear least squares unweighted regression analysis of the peak area ratio (**59**/1) response versus concentration of spiked plasma and urine samples was used to calculate the concentration of the samples. Plasma concentration versus time profiles of **59** were experimentally fit to exponential elimination models by using a curve stripping program; area under the plasma concentration profile was calculated by using the trapezoidal method.

Registry No. **7**, 114676-51-6; **7** (ethyl ester), 114677-03-1; **8a**, 114676-52-7; **8b**, 114676-99-2; **10**, 2584-71-6; **11**, 114676-53-8; **12**, 114676-54-9; **13**, 114676-55-0; **14**, 114676-56-1; **15**, 51-35-4; **16a**, 114676-57-2; **18**·HCl, 114676-59-4; **19**, 114760-51-9; **20**, 114676-60-7; **21**, 114676-61-8; **21** (mesylate), 114677-04-2; **22**, 113451-51-7; **23a**, 113451-55-1; **23b**, 114677-00-8; **24**, 1499-56-5; **25a**, 114676-62-9; **28**, 114676-63-0; **29**, 114676-64-1; **29** (mesylate), 114676-95-8; **30**, 114676-65-2; **31a**, 114676-66-3; **32**, 114676-67-4; **33**, 114676-68-5; **34**, 113451-54-0; **35**, 113451-59-5; **36**, 114676-69-6; **37**, 114676-70-9; **38**, 114676-71-0; **39**, 114676-72-1; **39** (amine), 114677-06-4; **40a**, 114676-73-2; **41**, 114676-74-3; **41** (mesylate), 114677-05-3; **42**, 114676-75-4; **43**, 114676-76-5; **44a**, 114676-77-6; **47**, 114676-78-7; **49**, 114676-79-8; **50**, 108138-16-5; **51**, 108138-17-6; **52**, 98105-80-7; **53**, 98105-94-3; **54**, 114676-80-1; **55**, 114691-38-2; **56**, 114676-81-2; **57**·HCl, 114676-82-3; **58**·$^1/_2$HCl, 114676-83-4; **59**, 114676-84-5; **60**·HCl, 114676-85-6; **61**·HCl, 114676-86-7; **62**·$^9/_5$HCl, 114676-87-8; **63**·HCl, 114718-07-9; **64**·$^4/_3$HCl, 114676-88-9; **65**, 114676-89-0; **66**·$^2/_3$HCl, 114676-90-3; (2*R*,4*R*)-2-carbomethoxy-4-hydroxypyrrolidine hydrochloride, 114676-47-0; *trans*-4-hydroxy-L-proline, 51-35-4; (2*S*,4*R*)-1-benzyl-2-carbomethoxy-4-hydroxypyrrolidine, 114676-48-1; (2*S*,4*R*)-1-benzyl-2-carbomethoxy-4-[(methylsulfonyl)oxy]pyrrolidine, 114676-49-2; (2*S*,4*S*)-4-azido-1-benzyl-2-carbomethoxypyrrolidine, 113451-53-9; (2*S*,4*S*)-4-acetamido-2-(acetoxymethyl)-1-benzylpyrrolidine, 114676-50-5; (2*R*,4*R*)-1-(*tert*-butoxycarbonyl)-4-[(*tert*-butyldimethylsilyl)oxy]-2-(hydroxymethyl)pyrrolidine, 114676-58-3; (2*S*,4*R*)-1-(*tert*-butoxycarbonyl)-4-[(*tert*-butyldimethylsilyl)oxy]-2-carbomethoxypyrrolidine, 114676-91-4; (2*S*,4*R*)-1-(*tert*-butoxycarbonyl)-4-[(*tert*-butyldimethylsilyl)oxy]-2-[[(methylsulfonyl)oxy]methyl]-pyrrolidine, 114676-92-5; (2*R*,4*R*)-1-(*tert*-butoxycarbonyl)-4-hydroxy-2-methylpyrrolidine, 114676-93-6; (2*R*,4*S*)-4-azido-1-(*tert*-butoxycarbonyl)-2-methylpyrrolidine, 114676-94-7; (2*S*,4*R*)-2-(azidomethyl)-1-(*tert*-butoxycarbonyl)-4-hydroxypyrrolidine, 114676-96-9; (2*R*,4*R*)-1-(*tert*-butoxycarbonyl)-4-hydroxy-2-(fluoromethyl)pyrrolidine, 114676-97-0; (2′*S*,4′*S*)-7-(4′-acetamido-2′-methylpyrrolidinyl)-1-(2,4-difluorophenyl)-1,4-dihydro-6-fluoro-4-oxoquinoline-3-carboxylic acid ethyl ester, 114677-01-9; (2*R*,4*S*)-4-amino-1-benzyl-2-(hydroxymethyl)-pyrrolidine, 114677-02-0; (2*R*,4*R*)-1-(*tert*-butoxycarbonyl)-2-(fluoromethyl)-4-[(methylsulfonyl)oxy]pyrrolidine, 114676-98-1.

(17) Granneman, G. R.; Sennello, L. T. *J. Chromatogr., Biomed. Appl.* **1987**, *413*, 199.