evaporated to give **7** as an oil (1.61 g) which was essentially pure by TLC (silica plate, eluant 10% MeOH in CH_2Cl_2, R_f 0.8) and which was used without further purification. It was dissolved in a mixture of MeOH (70 mL) and acetic acid (30 mL) and hydrogenated in the presence of 10% Pd-C (0.4 g) at about 20 bar and 60 °C for 48 h. The mixture was cooled and filtered, and the solvent evaporated to give *S*-**27** as an oil. This was converted to *S*-**27**·HCl which was crystallized from a mixture of MeOH and EtOAc, mp 171-173 °C, yield 1.34 g (95%), $[\alpha]^{23}_D$ -10.7° (*c* 1.0, MeOH).

(*S*)-[4-[2-[[2-Hydroxy-3-(4-hydroxyphenoxy)propyl]amino]ethoxy]phenoxy]-*N*-(2-methoxyethyl)acetamide (*S*-70). A mixture of **6** (5.58 g, 15.6 mmol) and (*S*)-1-[4-(benzyloxy)phenoxy]-2,3-epoxypropane (4.0 g, 15.6 mmol; $[\alpha]^{23}_D$ +8.1° (*c* 1.03 in MeOH) [lit.[4] $[\alpha]^{20}_D$ +8.6° (*c* 1.02 in MeOH)]) in propan-2-ol (50 mL) was heated under reflux for 16 h and then the solvent was evaporated to give an oil A (8.8 g) which was essentially pure by TLC (silica plate, eluant 10% MeOH in CH_2Cl_2, R_f 0.85) and which was used without further purification. The oil A (4.7 g, 7.7 mmol), 10% Pd-C (800 mg) and ammonium formate (0.98 g, 15.5 mmol) in EtOH (200 mL) was heated at 50 °C for 2.5 h. A TLC check showed the presence of some starting material. A further amount of ammonium formate (0.5 g, 7.9 mmol) was added and heating continued for 2 h. The mixture was filtered and the solvent evaporated. The residual oil was converted to *S*-**70**·HCl, which was crystallized from a mixture of MeOH and EtOAc, mp 166-167 °C, yield 1.75 g from oil A (49%), $[\alpha]^{23}_D$ -10.5° (*c* 1.02 in MeOH).

Registry No. 1 (R^1 = OMe), 139733-51-0; 1 (R^1 = OH), 139733-52-1; **2**, 139733-53-2; **3**, 108856-98-0; **4**, 10263-66-8; **5**, 71031-03-3; **6**, 133025-88-4; **7**, 139733-54-3; **9**, 139733-55-4; **9**·HCl, 139733-56-5; **10**, 139733-57-6; (*S*)-**10**, 115656-35-4; (*S*)-**10** ester, 107332-64-9; **11**, 139733-58-7; **12**, 139733-59-8; **12**·HCl, 139733-60-1; **13**, 139733-61-2; **13**·HCl, 139733-62-3; **14**, 139733-63-4; **15**, 139733-64-5; **15**·HCl, 139733-65-6; **16**, 139733-66-7; **16**·HCl, 139733-67-8; **17**, 139733-68-9; **18**, 139733-69-0; **19**, 139733-70-3; **20**, 139733-71-4; **20**·HCl, 139733-72-5; **21**, 139733-73-6; **22**, 139733-74-7; **23**, 139733-75-8; **23**·HCl, 139733-76-9; (*S*)-**23**, 115656-45-6; **24**, 139733-77-0; **25**, 139733-78-1; **26**, 139733-79-2; **26**·HCl, 139733-80-5; **27**, 139892-81-2; **27**·HCl, 139892-82-3; (*S*)-**27**, 129689-30-1; (*S*)-**27**·HCl, 129689-28-7; (*R*)-**27**, 139733-81-6; (*R*)-**27**·HCl, 139733-82-7; (*R*)-**27** ester, 139733-51-0; **28**, 139733-83-8; **29**, 139733-84-9; **29**·HCl, 139733-85-0; **30**, 139733-86-1; **30**·2HCl, 139733-87-2; **31**, 139733-88-3; **31**·HCl, 139733-89-4; **32**, 139733-90-7; **32**·HCl, 139733-91-8; **33**, 139733-92-9; **34**, 139733-93-0; **35**, 139733-94-1; **36**, 139733-95-2; **36**·HCl, 139733-96-3; **37**, 139733-97-4; **38**, 139733-98-5; **39**, 139733-99-6; **39**·HCl, 139734-00-2; **40**, 139734-01-3; **40**·HCl, 139734-02-4; **41**, 139734-03-5; **41**·HCl, 139734-04-6; **42**, 139734-05-7; (*S,S*)-**43**, 139734-06-8; (*S,S*)-**43**·HCl, 139734-04-6; **44**, 139734-08-0; **45**, 139734-09-1; **46**, 139734-10-4; **47**, 139734-11-5; **47**·HCl, 139734-12-6; **48**, 139734-13-7; **48**·HCl, 139734-14-8; **49**, 139734-15-9; **50**, 139734-16-0; **51**, 139734-17-1; **52**, 139734-18-2; **53**, 139734-19-3; **53**·HCl, 139734-20-6; **54**, 139734-21-7; **54**·HCl, 139734-22-8; **55**, 139734-23-9; **55**·HCl, 139734-24-0; **56**, 139734-25-1; **56**·HCl, 139734-26-2; **56**·oxalate, 139734-27-3; **57**, 139734-28-4; **58**, 139734-29-5; **59**, 139734-30-8; **59**·HCl, 139734-31-9; **60**, 139734-32-0; **60**·HCl, 139734-33-1; **61**, 139734-34-2; **61**·HCl, 139734-35-3; **61** ester, 139734-36-4; **62**, 139734-37-5; **62**·HCl, 139734-38-6; **63**, 139734-39-7; **63** ester, 139734-40-0; **64**, 139734-41-1; **65**, 139734-42-2; **65** ester, 139734-43-3; **66**, 139734-44-4; **66**·HCl, 139734-45-5; **67**, 139734-46-6; **67**·HCl, 139734-47-7; **68**, 139734-48-8; **68**·HCl, 139734-49-9; **68** ester, 139734-50-2; **69**, 139734-51-3; **69** ester, 139734-52-4; **70**, 139734-53-5; **70**·HCl, 139734-54-6; (*S*)-**70**, 139892-83-4; (*S*)-**70**·HCl, 139892-84-5; $ClCH_2CON(CH_3)CH_2Ph$, 73685-56-0; $ClCH_2CON(CH_3)(CH_2)_2OCH_3$, 139734-55-7; $ClCH_2CONHC_6H_3$-2,6-$(CH_3)_2$, 1131-01-7; $ClCH_2CONHCH_2C_6H_3$-2,4-Cl_2, 56978-45-1; $ClCH_2CONHBu$-*t*, 15678-99-6; $ClCH_2CONHPh$, 587-65-5; $ClCH_2CON(CH_2CH_3)_2$, 2315-36-8; NH_2CH_3, 74-89-5; NH_2Et, 75-04-7; NH_2Pr-*n*, 107-10-8; NH_2Pr-*i*, 75-31-0; NH_2Bu-*n*, 109-73-9; NH_2Bu-*i*, 78-81-9; NH_2Bu-*s*, 13952-84-6; NH_2CH_2Bu-*t*, 5813-64-9; $NH_2(CH_2)_5CH_3$, 111-26-2; $NH_2CH_2CH{=}CH_2$, 107-11-9; NH_2Pr-*c*, 765-30-0; NH_2 C_5H_9-*c*, 1003-03-8; $NH_2(CH_2)_2OH$, 141-43-5; $NH_2(CH_2)_3OH$, 156-87-6; $NH_2CH(CH_3)CH_2OH$, 78-91-1; $NH_2C(CH_3)_2CH_2OH$, 124-68-5; $NH_2(CH_2)_2OCH_3$, 109-85-3; $NH_2(CH_2)_3OCH_3$, 5332-73-0; $NH_2CH(CH_3)CH_2OCH_3$, 37143-54-7; $NH_2(CH_2)_2NH_2$, 107-15-3; $NH_2CH_2CONH_2$, 598-41-4; NH_2CH_2Ph, 100-46-9; $NH_2CH_2C_6H_4$-*p*-CH_3, 104-84-7; $NH_2CH_2C_6H_4$-*p*-OCH_3, 2393-23-9; $NH_2CH_2C_6H_4$-*o*-Cl, 89-97-4; $NH_2CH_2C_6H_4$-*p*-Cl, 104-86-9; $NH_2(CH_2)_2Ph$, 64-04-0; (*S*)-$NH_2CH(CH_3)Ph$, 2627-86-3; $NH_2(CH_2)_2OPh$, 1758-46-9; NH_2OCH_2Ph·HCl, 2687-43-6; $NH(CH_3)_2$, 124-40-3; $NH(CH_3)(CH_2)_2OH$, 109-83-1; (*S*)-1-[4-(benzyloxy)phenoxy]-2,3-epoxypropane, 122797-04-0; 2-(aminomethyl)thiophene, 27757-85-3; *N*-chloroacetyl-2,3,4,5-tetrahydroisoxazole, 139734-56-8; *N*-chloroacetyl-1,3-dihydroisoindole, 41910-53-6; 2-(aminomethyl)furan, 617-89-0; pyrrolidine, 123-75-1; piperidine, 110-89-4; 4-hydroxypiperidine, 5382-16-1; morpholine, 110-91-8; 1-methylpiperazine, 109-01-3; 1,2,3,4-tetrahydroisoquinoline, 91-21-4; chloroacetyl chloride, 79-04-9.

Quinolone Antibacterial Agents. Synthesis and Structure-Activity Relationships of a Series of Amino Acid Prodrugs of Racemic and Chiral 7-(3-Amino-1-pyrrolidinyl)quinolones. Highly Soluble Quinolone Prodrugs with in Vivo Pseudomonas Activity

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A series of amino acid prodrugs of racemic and chiral 7-(3-amino-1-pyrrolidinyl)-6-fluoro-1,8-naphthyridine-3-carboxylic acids, 1-cyclopropyl-6,8-difluoro-3-quinolinecarboxylic acids, 1-cyclopropyl-6-fluoro-3-quinolinecarboxylic acids, and 5-amino-1-cyclopropyl-6,8-difluoro-3-quinolinecarboxylic acids have been prepared and evaluated for comparative antibacterial activity. Compounds were prepared by acylation of the 3-amino group of the pyrrolidine with common amino acids using standard peptide chemistry. This series has been compared with the parent compounds for antibacterial activity in vitro and in vivo as well as for comparative solubility. The amino acid analogues were less active in vitro, but had equal or increased efficacy in vivo. Indeed, it was proven that these compounds, which were stable to acid and base under the reaction conditions for their preparation, were rapidly cleaved in serum to give the parent quinolones. The amino acid derivatives showed a 3-70 times improved solubility when compared to the parent compounds. The most active compound of the series was [*S*-(*R**,*R**)]-7-[3-[(2-amino-1-oxopropyl)amino]-1-pyrrolidinyl]-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylic acid (PD 131112).

The search for the ideal quinolone antibacterial agent continues in many laboratories.[1] Such an agent will have potent activity against a broad spectrum of Gram-positive and Gram-negative aerobic and anaerobic organisms as

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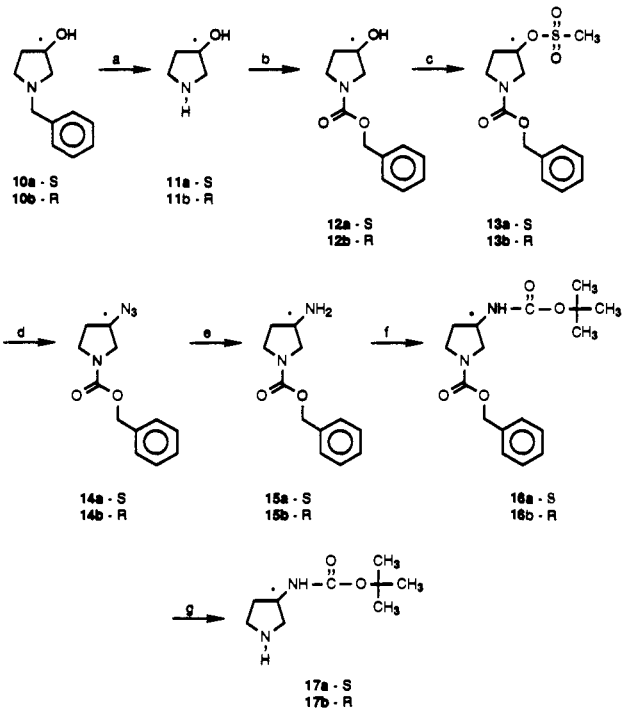
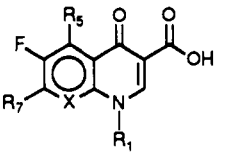
Scheme I[a]

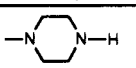
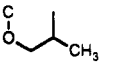
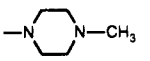
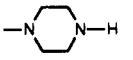
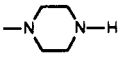
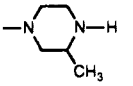
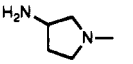
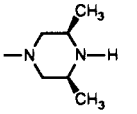
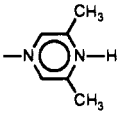
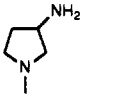


[a] (a) H_2, 20% Pd–C, MeOH; (b) CbzCl, H_2O, pH 7.0, 5 °C; (c) MsCl, pyridine, 5 °C. (d) NaN_3, DMF, 100 °C; (e) H_2, Ra–Ni, MeOH; (f) di-*tert*-butyl dicarbonate, 1.0 N NaOH, *t*-BuOH, room temperature; (g) H_2, 20% Pd–C, MeOH.

well as mycobacteria; it will have minimal adverse reactions, and have excellent aqueous solubility and oral bioavailability. The list of quinolones currently approved or being tested in man continues to increase (Table I).[2-10]

Table I. Clinically Significant or Marketed Quinolone Type Antibacterial Agents



name	X	R_1	R_5	R_7	ref
norfloxacin	CH	Et	H		2
ofloxacin			H		3
enoxacin	N	Et	H		4
ciprofloxacin	CH	c-Pr	H		5
lomefloxacin	CF	Et	H		6
tosufloxacin	N	2,4-F_2-C_6H_4	H		7
sparfloxacin	CF	c-Pr	NH_2		8
WIN-57273	CH	c-Pr	H		9
clinafloxacin	CCl	c-Pr	H		10

A great body of knowledge has been accumulated in this area which covers mechanism of action,[11] structure–activity relationships,[12] and adverse side effects.[13] This has led

(1) For a comprehensive review through 1976 see: (a) Albrecht, R. Development of antibacterial agents of the nalidixic type. *Prog. Drug Res.* **1977**, *21*, 9–104. More recent reviews include: (b) Brighty, K. E.; McGuirk, P. R. Antibacterial Agents. *Annu. Rep. Med. Chem.* **1991**, *26*, 123–131. (c) Siporin, C.; Heifetz, C. L.; Domagala, J. M. *The New Generation of Quinolones*; Marcel Dekker, Inc.: New York, 1990. (d) Bouzard, D. Recent Advances in the Chemistry of Quinolones. *Recent Progress in the Chemical Synthesis of Antibiotics*; Springer-Verlag: Heidelberg, 1990.

(2) Koga, H.; Itoh, A.; Murayama, S.; Suzue, S.; Irikura, T. Structure–Activity Relationships of Antibacterial 6,7- And 7,8-Disubstituted 1-Alkyl-1,4-dihydro-4-oxoquinoline-3-carboxylic Acids. *J. Med. Chem.* **1980**, *23*, 1358–1363.

(3) Hayakawa, I. Eur. Pt. 47005; *Chem. Abstr.* **1982**, *97*, 55821b.

(4) Matsumoto, J.; Miyamoto, T.; Minamida, A.; Nishimura, Y.; Egawa, H.; Nishimura, H. Pyridonecarboxylic Acids as Antibacterial Agents. 2. Synthesis and Structure–Activity Relationships of 1,6,7-Trisubstituted-1,4-Dihydro-4-oxo-1,8-naphthyridine-3-carboxylic Acids, Including Enoxacin, a New Antibacterial Agent. *J. Med. Chem.* **1984**, *27*, 292–301.

(5) Wise, R.; Andrews, J. M.; Edwards, L. J. In Vitro Activity of Bay 09867, a New Quinolone Derivative, Compared with those of Other Antimicrobial Agents. *Antimicrob. Agents Chemother.* **1983**, *23*, 559–564.

(6) Chin, N. X.; Novelli, A.; Neu, H. C. in Vitro Activity of Lomefloxacin (SC-4711; NY-198), a Difluoroquinolone-3-carboxylic acid, Compared with Those of Other Quinolones. *Antimicrob. Agents Chemother.* **1988**, *32*, 656–662. Wise, R.; Andrews, J. M.; Ashby, J. P.; Matthews, R. S. In Vitro Activity of Lomefloxacin, a New Quinolone Antimicrobial Agent, in Comparison with Those of Other Agents. *Antimicrob. Agents Chemother.* **1988**, *32*, 617–622.

(7) Chu, D. T. W.; Prabhavathi, B. F.; Akiyo, K. C.; Pihuleac, E.; Nordeen, C. W.; Maleczka, R. E.; Pernet, A. G. Synthesis and Structure–Activity Relationships of Novel Arylfluoroquinolone Antibacterial Agents. *J. Med. Chem.* **1985**, *28*, 1558–1564.

(8) Miyamoto, T.; Matsumoto, J.; Chiba, K.; Egawa, H.; Shibamori, K.; Minamida, A.; Nishimura, Y.; Okada, H.; Kataoka, M.; Fujita, M.; Hirose, T.; Nakano, J. Synthesis and Structure–Activity Relationships of 5-Substituted 6,8-Difluoroquinolones, Including Sparfloxacin, a New Quinolone Antibacterial Agent with Improved Potency. *J. Med. Chem.* **1990**, *33*, 1645–1656.

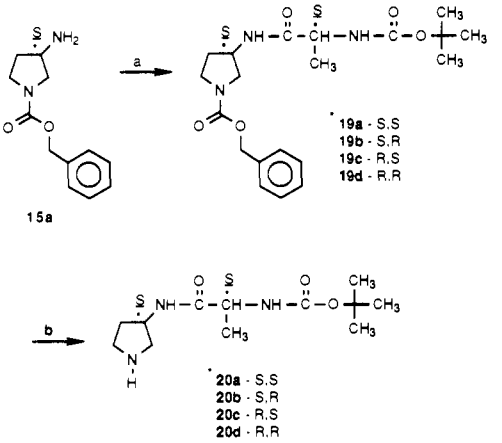
(9) Sedlock, D. M.; Dobson, R. A.; Deuel, D. M.; Lesher, G. Y.; Rake, J. B. In Vitro and In Vivo Activities of a New Quinolone, WIN 57273, Possessing Potent Activity Against Gram-Positive Bacteria. *Antimicrob. Agents Chemother.* **1990**, *34*, 568–575.

(10) (a) Norrby, S. R.; Jonsson, M. Comparative In Vitro Activity of PD 127,391, a New Fluorinated 4-Quinolone Derivative. *Antimicrob. Agents Chemother.* **1988**, *32*, 1278–1281. (b) Wise, R.; Ashby, J. P.; Andrews, J. M. In Vitro Activity of PD 127,391, an Enhanced-Spectrum Quinolone. *Antimicrob. Agents Chemother.* **1988**, *32*, 1251–1256.

(11) (a) Shen, L. L.; Mitscher, L. A.; Sharma, P. N.; O'Donnell, T. J.; Chu, D. T. W.; Cooper, C. S.; Rosen, T.; Pernet, A. G. Mechanism of Inhibition of DNA Gyrase by Quinolone Antibacterials: A Cooperative Drug-DNA Binding Model. *Biochemistry* **1989**, *28*, 3886–3894. (b) Hooper, D. C.; Wolfson, J. S. Mode of Action of the Quinolone Antimicrobial Agents: Review of Recent Information. *Rev. Infect. Dis.* **1989**, *11* (Suppl. 1), S141–S. (c) Drlica, K.; Coughlin, S. Inhibitors of DNA Gyrase. *Pharmacol. Ther.* **1989**, *44*, 107–121.

to the identification of three significant basic groups substituted at the 7-position of the quinolone ring. The great majority of the quinolones currently under development possess the 1-piperazinyl moiety at this position. However, the replacement of the piperazine ring with a 3-amino-1-pyrrolidine or 3-(aminomethyl)-1-pyrrolidine substituent, which are successful piperazine mimics, has greatly increased the Gram-positive activity of some quinolones under investigation.[14] Other important improvements brought about by this body of knowledge include the substitution of a cyclopropyl or 2,4-difluorophenyl group at the 1-position and the placement of a fluoro substituent at the 6-position. The zwitterionic character displayed by quinolones having a basic substituent in the 7-position causes them to display low water solubility (<0.2 mg/mL). Since water solubility at physiological pH is important for preclinical testing, in vivo efficacy, and parenteral formulation, we decided to investigate methods of improving solubility. The acid addition salts of ciprofloxacin (**5**) (hydrochloride) and tosufloxacin (**7**) (*p*-toluenesulfonate) still exhibit poor water solubility (2–50 mg/mL). Therefore, it was felt that chemical modification might be the more prudent way to improve water solubility. Major modifications to the parent molecule, such as hydroxylation or adding extra amines to the amino substituent of the side chain, were usually deleterious.[15] Even putting simple acyl groups on the side chain nitrogen to remove the zwitterionic character did not improve solubility or antibacterial activity, presumably because of the lost basicity of the side chain and the in vivo stability of the acyl group.[15] In a pilot study, we added a basic group to the acyl function (in the form of an alanine moiety) and similarly saw a great decrease in in vitro activity. However, upon either oral or subcutaneous dosing, good in vivo efficacy was observed. These compounds also possessed a high degree of water solubility (3–70 times when compared to the parent compounds). These initial results implied that the amino acid acyl analogues may act as soluble prodrugs for the quinolone antibacterials. In this current work, we report a series of common amino acids covalently bonded to the nitrogen of the 3-aminopyrrolidine side chain. Not only did they greatly increase the water solubility of the quinolones to which they were attached, but they were rapidly cleaved in vivo to give the parent compound with its antibacterial activity intact.

Scheme II[a,b]



[a] (a) Boc-*S*-Ala, *i*-isobutyl chloroformate, *N*-methylmorpholine, CH_2Cl_2, −20 °C; (b) H_2, 20% Pd-C, MeOH. [b] Rather than using the more complex nomenclature for two stereocenters, e.g. [S-(R*,R*)], we will designate the pyrrolidine center first (S), and the amino acid center second (S) to give (S,S) for **20a**, etc.

Chemistry

The Boc-protected chiral (*S*)- and (*R*)-3-aminopyrrolidines **17a** and **17b** (Scheme I) were prepared from the chiral 1-benzyl-3-pyrrolidinols **10a** and **10b**, which were in turn synthesized using the procedure of Brown.[16] The benzyl protecting group was removed by catalytic hydrogenation to give the chiral 3-aminopyrrolidines **11a** and **11b**, and the pyrrolidine nitrogen was reprotected with a carbobenzyloxy group to give **12a** and **12b** to prevent aziridine formation during the subsequent steps.[17] The alcohols were converted to the mesylates **13a** and **13b** using methanesulfonyl chloride in pyridine at 5 °C. Sodium azide in dimethylformamide at 100 °C converted the mesylates into their enantiomeric azides **14a** and **14b**. Catalytic reduction of the azides with Raney nickel in methanol provided the corresponding 3-aminopyrrolidines **15a** and **15b** in excellent yield and enantiomeric purity. The 3-amino group was protected using di-*tert*-butyl dicarbonate and 1.0 N sodium hydroxide in *tert*-butyl alcohol to give the Boc derivatives **16a** and **16b**. Catalytic hydrogenation using Pearlman's catalyst[18] in methanol pro-

(12) (a) Domagala, J. M.; Hanna, L. D.; Heifetz, C. L.; Hutt, M. P.; Mich, T. F.; Sanchez, J. P.; Solomon, M. New Structure–Activity Relationships of the Quinolone Antibacterials Using the Target Enzyme. The Development and Applications of a DNA Gyrase Assay. *J. Med. Chem.* **1986**, *29*, 394–404. (b) Fernandes, P. B.; Chu, D. T. W. Structure-Activity Relationships of the Fluoroquinolones. *Antimicrob. Agents Chemother.* **1989**, *33*, 131–135. (c) Stein, G. E. The 4-Quinolone Antibiotics: Past, Present, and Future. *Pharmacotherapy (Carlisle, Mass.)* **1988**, *8*, 301–314. (d) Koga, H. Structure-Activity Relationships and Drug Design of the Pyridonecarboxylic Acid Type (Nalidixic Acid Type) Synthetic Antibacterial Agents. *Kagaku no Ryoiki, Zokan* **1982**, *136*, 177–202.

(13) (a) Ball, P. Long-Term Use of Quinolones and Their Safety. *Rev. Infect. Dis.* **1989**, *11* (Suppl. 5), S1365–S1370. (b) Christ, W.; Lehnert, T.; Ulbrich, B. Specific Toxicologic Aspects of the Quinolones. *Rev. Infect. Dis.* **1988**, *10* (Suppl. 1), S141–S146. (c) Furet, Y. X. and Pechere, J.-C. Usual and Unusual Antibacterial Effects of Quinolones. *J. Antimicrob. Chemother.* **1990**, *26* (Suppl. B), 7–15.

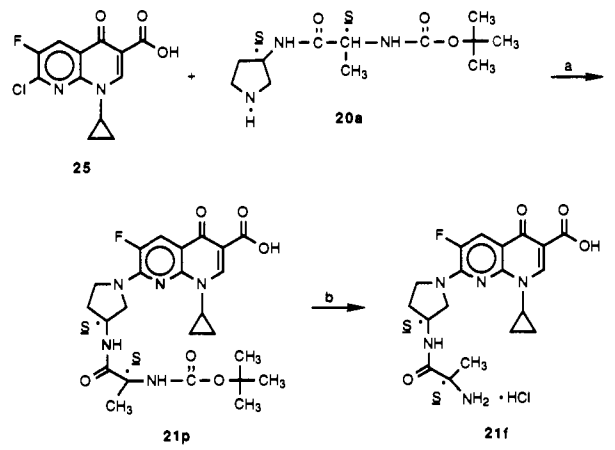
(14) (a) Reference 7. (b) Reference 10. (c) Domagala, J. M.; Heifetz, C. L.; Priebe, S.; Sanchez, J. P.; Sesnie, J. S.; Trehan, A. K. PD 131112. A Highly Soluble Quinolone Prodrug With Outstanding In Vivo Pseudomonas Activity. 30 Interscience Conference on Antimicrobial Agents and Chemotherapy, Atlanata, GA, 1990; Abstr. 375.

(15) (a) Reference 12a. (b) Culbertson, T. P.; Domagala, J. M.; Hagen, S. E.; Hutt, M. P.; Nichols, J. B.; Mich, T. F.; Sanchez, J. P.; Schroeder, M. C.; Solomon, M.; Worth, D. F. Structure-Activity Relationships of the Quinolone Antibacterials. The Nature of the C_7-Side Chain. In *International Telesymposium on Quinolones*; Fernandes, P. B., Ed.; Prous Science: Barcelona, Spain, 1989; p 47–71.

(16) Brown, H. C.; Vara Prasad, J. V. N.; Gupta, A. K. Hydroboration. 78. Reinvestigation of the Hydroboration of *N*-Benzyl-3-pyrrolines. Preparation of *N*-Benzyl-3-pyrrolidinol and (*N*-Benzyl-3-pyrrolidinyl)boronate of Very High Purity. *J. Org. Chem.* **1986**, *51*, 4296–4298.

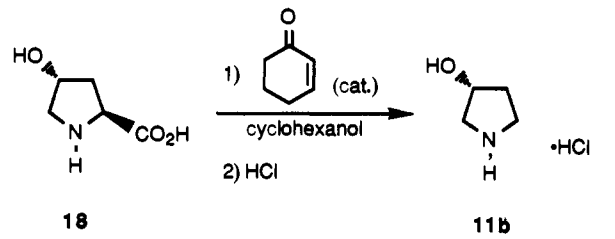
(17) (a) Hammer, C. F.; Heller, S. R.; Craig, J. H. Reactions of β-Substituted Amines-II. Nucleophilic Displacement Reactions on β-Chloro-1-ethylpiperidine. *Tetrahedron* **1972**, *28*, 239–253. (b) Fuson, R. C.; Zirkle, C. L. Ring Enlargement by Rearrangement of the 1,2-Aminochloroalkyl Group; Rearrangement of 1-Ethyl-2-chloromethylpyrrolidine to 1-Ethyl-3-chloropiperidine. *J. Am. Chem. Soc.* **1948**, *70*, 2760–2762. (c) Thottathil, J. K.; Moniot, J. L. Lithium Diphenylcuprate Reactions with 4-Tosyloxy-L-proline; An Interesting Stereochemical Outcome. A Synthesis of Trans-4-phenyl-L-proline. *Tetrahedron Lett.* **1986**, *27*, 151–154.

Scheme III[a]



[a] (a) Et_3N, CH_3CN, reflux; (b) 1.0 M HCl, EtOH, reflux.

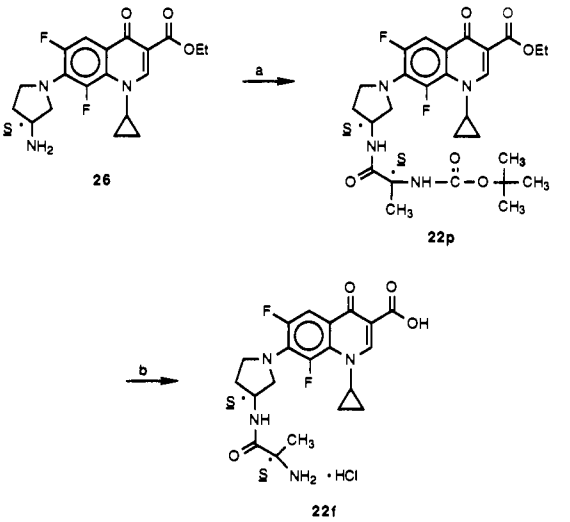
vided the chiral pyrrolidine side chains **17a** and **17b**. The (*S*)-3-aminopyrrolidine side chain could also be prepared from (*R*)-3-hydroxypyrrolidine (**11b**) produced by the decarboxylation of L-hydroxyproline (**18**)[19] and cycled through the sequence of reactions in Scheme I.



The coupling of the Boc-protected amino acids to the Cbz-protected, chiral 3-aminopyrrolidines was accomplished using the mixed anhydride in standard peptide forming reactions (Scheme II).[20] Deprotection of the pyrrolidine nitrogen was accomplished by catalytic hydrogenation as in the previous example. The chiral purity of the pyrrolidines was established by HPLC at the Cbz-protected 3-aminopyrrolidine **15**, the Boc-protected 3-aminopyrrolidine **17**, and the Boc-protected amino acid acylaminopyrrolidine **20** stages. Enantiomeric purity could be established in all of these cases, but diastereomeric purity in the amino acid acylamino compounds could not be established. However, because of the manner of their coupling to the 3-aminopyrrolidines, racemization of the amino acids was assumed to be minimal.[20]

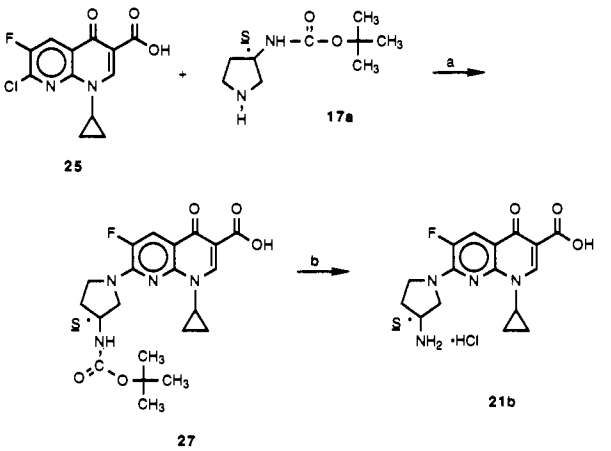
Coupling reactions of the amino acid side chains to the quinolone substrates, exemplified by the preparation of **21f** were done in refluxing acetonitrile using 1 equiv of the quinolone or naphthyridine substrate, 1.0–1.5 equiv of side chain and 3 equiv of triethylamine (Scheme III). The penultimate intermediates (**p**) were deprotected using 1.0 M hydrochloric acid in ethanol at reflux to produce part of the final series (**21e–21l, 22d, 22e, 22h, 22j, 23c–23e, 24d–24f**).

Scheme IV[a]



[a] (a) Boc-*S*-Ala, *i*-butyl chloroformate, *N*-methylmorpholine, CH_3CN, 5 °C; (b) 1.0 M HCl, EtOH, reflux.

Scheme V[a]



[a] Et_3N, CH_3OH, reflux. (b) 1.0 M HCl, EtOH, reflux.

A second amino acid coupling procedure, exemplified by the preparation of **22f**, involved reacting the mixed anhydride of the Boc-protected amino acid with the ester of the quinolone or naphthyridine in which the 7-position was already substituted with the 3-aminopyrrolidine side chain (Scheme IV). Deprotection of both the amine and ester protecting groups of the penultimate intermediate (**p**) using 1.0 M hydrochloic acid in ethanol produced the remainder of the amino acyl series (**21d, 22f, 22g, 22l, 23b, 23f**).

The preparation of the quinolones with the chiral 3-aminopyrrolidine in the 7-position was done by standard coupling reactions as exemplified by the preparation of **21b** (Scheme V). The quinolone molecule was reacted with the protected 3-aminopyrrolidine to produce the penultimate intermediate (**p**) which was deprotected using 1.0 M hydrochloric acid to produce the final products as the hydrochloride salts (**21a–21c, 22a–22c, 23a, 24a–24c**).

Biological Assays

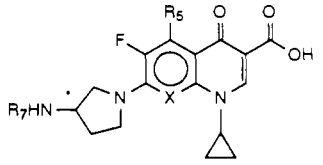
The series of 7-(3-aminopyrrolidinyl)- and 7-[(3-amino acid acylamino)pyrrolidinyl]quinolones were tested against 11 representative Gram-positive and Gram-negative organisms by using standard microtitration techniques,[21] and

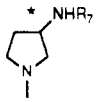
(18) (a) Pearlman, W. M. U.S. Patent 3,239,563; *Chem. Abstr.* **1966**, *64*, 15796e. (b) Pearlman, W. M. Noble Metal Hydroxides on Carbon Nonpyrophoric Dry Catalysts. *Tetrahedron Lett.* **1967**, *17*, 1663–1664. (c) Bernotas, R. C.; Cube, R. V. The Use of Pearlman's Catalyst for Selective *N*-Debenzylation in the Presence of Benzyl Ethers. *Synth. Commun.* **1990**, *20*, 1209–1212.

(19) Hashimoto, M.; Yutaka, E.; Yasutomo, O.; Toshiaki, I.; Seiichi, A. A Novel Decarboxylation of α-Amino Acids. A Facile Method of Decarboxylation by the Use of 2-Cyclohexen-1-one as a Catalyst. *Chem. Lett.* **1986**, 893–896.

(20) Meienhofer, J. Protected Amino Acids in Peptide Synthesis. In *Chemistry and Biochemistry of the Amino Acids*; Barrett, G. C., Ed.; Chapman and Hall Ltd.: London, 1985; p 297–337.

Table II. Synthetic and Physical Data of the Quinolone and Naphthyridine Antibacterials Prepared for This Study



compd	R_5	X		R_7	method of prep[a]	mp, °C	formula (anal.)[b]	purification[c]	yield,[d] %
21a	H	N	*R,S*	H	C	284–286	$C_{16}H_{17}FN_4O_3$	–	ref 22c
21b	H	N	*S*	H	C	310–312	$C_{16}H_{17}FN_4O_3 \cdot HCl \cdot H_2O$ (C,H,N,F)	trit 2-PrOH/Et_2O	87
21c	H	H	*R*	H	C	285–287	$C_{16}H_{17}FN_4O_3$ (C,H,N,F)	trit 2-PrOH	92
21d	H	N	*R,S*	*S*-Ala	B	198–200	$C_{19}H_{22}FN_5O_4 \cdot 1.5HCl \cdot 1.65H_2O$ (C,H,N,Cl)	lyophilization	62
21e	H	N	*R,S*	*R*-Ala	A	268–270	$C_{19}H_{22}FN_5O_4 \cdot 1.2HCl \cdot 2.5H_2O$ (C,H,N,Cl)	trit EtOH/Et_2O	86
21f	H	N	*S*	*S*-Ala	A	200–202	$C_{19}H_{22}FN_5O_4 \cdot 1.0HCl \cdot 1.0H_2O$ (C,H,N,F)	trit 2-PrOH/Et_2O	92
21g	H	N	*R*	*S*-Ala	A	198–200	$C_{19}H_{22}FN_5O_4 \cdot 1.0HCl \cdot 1.5H_2O$ (C,H,N,Cl,F)	trit 2-PrOH/Et_2O	97
21h	H	N	*R,S*	*S*-Phe	A	108–110	$C_{25}H_{26}FN_5O_4 \cdot 1.6HCl \cdot 3.25H_2O$ (C,H,N,Cl)	trit 2-PrOH	42
21i	H	N	*R,S*	Gly	A	157–160	$C_{18}H_{20}FN_5O_4 \cdot 1.4HCl \cdot 1.0H_2O$ (C,H,N,Cl)	trit EtOH/Et_2O	74
21j	H	N	*R,S*	*S*-Lys	A	128–130	$C_{22}H_{29}FN_6O_4 \cdot 2.0HCl \cdot 1.0H_2O$ (C,H,N,Cl)	trit EtOH/Et_2O	70
21k	H	N	*R,S*	*S*-Val	A	135	$C_{21}H_{26}FN_5O_4 \cdot 1.0HCl \cdot 2.5H_2O$ (C,H,N)	trit 2-PrOH/Et_2O	79
21l	H	N	*R,S*	*S*-c-Gln	A	289–291	$C_{21}H_{22}FN_5O_5 \cdot 2H_2O$ (C,H,N)	lyophilization	77
22a	H	CF	*R,S*	H	C	313–315	$C_{17}H_{17}F_2N_3O_3 \cdot 1.0HCl \cdot 1.25H_2O$	–	ref 22c
22b	H	CF	*S*	H	C	>300	$C_{17}H_{17}F_2N_3O_3 \cdot 1.0HCl \cdot 1.0H_2O$ (C,H,N)	trit EtOH/Et_2O	94
22c	H	CF	*R*	H	C	313–316	$C_{17}H_{17}F_2N_3O_3 \cdot 1.0HCl \cdot 1.0H_2O$ (C,H,N)	trit EtOH/Et_2O	92
22d	H	CF	*R,S*	*S*-Ala	A	200–202	$C_{20}H_{22}F_2N_4O_4 \cdot 1.1HCl \cdot 1.5H_2O$ (C,H,N,Cl,F)	trit EtOH/Et_2O	90
22e	H	CF	*R,S*	*R*-Ala	A	190–193	$C_{20}H_{22}F_2N_4O_4 \cdot 1.0HCl \cdot 2.0H_2O$ (C,H,N,Cl,F)	trit EtOH/Et_2O	69
22f	H	CF	*S*	*S*-Ala	B	127–130	$C_{20}H_{22}F_2N_4O_4 \cdot 1.5HCl \cdot 2.0H_2O$ (C,H,N,Cl,F)	trit EtOH/Et_2O	49
22g	H	CF	*R*	*S*-Ala	B	210–214	$C_{20}H_{22}F_2N_4O_4 \cdot 1.0HCl \cdot 2.25H_2O$ (C,H,N,Cl)	trit EtOH/Et_2O	76
22h	H	CF	*R,S*	*S*-Phe	A	215–219	$C_{26}H_{26}F_2N_4O_4 \cdot 1.0HCl \cdot 1.75H_2O$ (C,H,N)	trit $CHCl_3/H_2O$	66
22i	H	CF	*R,S*	Gly	B	273–274	$C_{19}H_{22}F_2N_4O_4 \cdot 1.0HCl \cdot 1.6H_2O$ (C,H,N,Cl)	lyophilization	78
22j	H	CF	*R,S*	*S*-Lys	A	190–192	$C_{23}H_{29}F_2N_5O_4 \cdot 2.0HCl \cdot 1.7H_2O$ (C,H,N,Cl)	trit EtOH/Et_2O	54
23a	H	CH	*R,S*	H	C	>300	$C_{17}H_{18}FN_3O_3 \cdot 2.0HCl \cdot 0.25H_2O$		ref 22c
23b	H	CH	*R,S*	*S*-Ala	B	217–219	$C_{20}H_{23}FN_4O_4 \cdot 1.75HCl \cdot 1.75H_2O$ (C,H,N,Cl)	trit EtOH	86
23c	H	CH	*R,S*	*R*-Ala	A	210–213	$C_{20}H_{23}FN_4O_4 \cdot 1.6HCl \cdot 1.5H_2O$ (C,H,N,Cl)	trit EtOH/Et_2O	88
23d	H	CH	*R,S*	*S*-Phe	A	197–200	$C_{26}H_{27}FN_4O_4 \cdot 1.3HCl \cdot 1.25H_2O$ (C,H,N,Cl)	trit EtOH/Et_2O	80
23e	H	CH	*R,S*	Gly	A	208–210	$C_{19}H_{21}FN_4O_4 \cdot 1.6HCl \cdot 1.5H_2O$ (C,H,N,Cl)	trit EtOH/Et_2O	90
23f	H	CH	*R,S*	*S*-Lys	B	123–125	$C_{23}H_{30}FN_5O_4 \cdot 2.2HCl \cdot 1.0H_2O$ (C,H,N,Cl)	trit EtOH/Et_2O	83
24a	NH_2	CF	*R,S*	H	C	257–259	$C_{17}H_{18}F_2N_4O_3 \cdot 1.3H_2O$	–	ref 25
24b	NH_2	CF	*S*	H	C	315–317	$C_{17}H_{18}F_2N_4O_3 \cdot 1.0HCl \cdot 1.5H_2O$ (C,H,N,Cl,F)	trit EtOH/Et_2O	95
24c	NH_2	CF	*R*	H	C	313–315	$C_{17}H_{18}F_2N_4O_3 \cdot 1.0HCl \cdot 1.75H_2O$ (C,H,N,Cl,F)	trit EtOH	94
24d	NH_2	CF	*S*	*S*-Ala	A	230–233	$C_{20}H_{23}F_2N_5O_4 \cdot 0.9HCl \cdot 1.5H_2O$ (C,H,N,Cl,F)	isoelectric pptn	83
24e	NH_2	CF	*R*	*S*-Ala	A	267–272	$C_{20}H_{23}F_2N_5O_4 \cdot 1.0HCl \cdot 0.5H_2O$ (C,H,N,Cl,F)	trit 2-PrOH	86
24f	NH_2	CF	*R,S*	Gly	A	264–266	$C_{19}H_{21}F_2N_5O_4 \cdot 1.0HCl \cdot 0.75H_2O$ (C,H,N,Cl,F)	trit EtOH/Et_2O	89

[a] Refers to the general method used and is described in the Experimental Section. [b] Symbols refer to those elements analyzed. Analyses were ±0.4% of theoretical values. [c] Trituration (trit) refers to grinding of the solids under solvent to produce a fine powder. Isoelectric precipitation refers to dissolving the solid in aqueous base, adjusting the pH to 7.2, and filtering the solid that precipitates. Lyophilization refers to dissolving the solid in aqueous hydrochloric acid, freezing the resulting solution, and removing the water from the frozen solid in vacuo. [d] Yields are those obtained from the coupling step to final product isolation, including hydrolysis, reductions, and deprotection where applicable.

their minimum inhibitory concentrations (MIC's in microgram/milliliter) were compared to standard drugs (**5**, **7**, and **8**) in multiple experiments (Table III). The compounds were also tested for their inhibition of DNA gyrase by using a previously described test.[12a,22b] The assay measured the concentration of drug (micrograms/milliliter) necessary to cleave DNA (Table III).

The in vivo potency expressed as the median protective dose (PD_{50}, mg/kg) was determined in acute systemic infections after a single dose of drug (given at time of challenge) using previously described methods.[22] The results of in vivo tests are summarized in Table IV. More extensive in vivo testing was not generally performed on compounds which did not show good activity against *Escherichia coli* and *Streptococcus pneumoniae*.

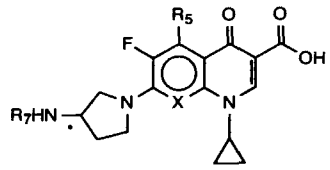
Results and Discussion

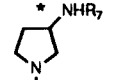
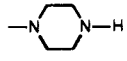
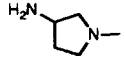
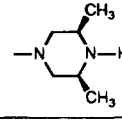
In Vitro and in Vivo Activity. By evaluating the MIC's of the Gram-negative and Gram-positive organisms (Table III), it is apparent that the amino acid derivatives, like acyl groups in general, are significantly less active than the parent drug in vitro (**21a–c**, vs **21d–l**, **22a–c** vs **22d–j**, **23a** vs **23b–f** and **24a–c** vs **24d–f**). This decrease in in vitro potency is directly attributable to a 10–50-fold reduced gyrase inhibition (Table III). However, the *R*-amino acids provided good in vitro activity against Gram-positive organisms, which may indicate an uptake by these bacteria.

(21) (a) Reference 12a. (b) Cohen, M. A.; Griffin, T. J.; Bien, P. A.; Heifetz, C. L.; Domagala, J. M. In Vitro Activity of CI-934, a Quinolone Carboxylic Acid Active Against Gram-Positive and -Negative Bacteria. *J. Med. Chem.* **1985**, *28*, 766–772.

(22) (a) Miller, L. C.; Tainter, M. L. Estimation of the ED_{50} and Its Error by Means of Logarithmic-Probit Graph Paper. *Proc. Soc. Exp. Biol. Med.* **1944**, *57*, 261–264. (b) Domagala, J. M.; Heifetz, C. L.; Mich, T. F.; Nichols, J. B. 1-Ethyl-7-[3-[(ethylamino)methyl]-1-pyrrolidinyl]-6,8-difluoro-1,4-dihydro-4-oxo-3-quinolinecarboxylic Acid. A New Quinolone Antibacterial with Potent Gram-Positive Activity. *J. Med. Chem.* **1986**, *29*, 445–448. (c) Sanchez, J. P.; Domagala, J. M.; Hagen, S. E.; Heifetz, C. L.; Hutt, M. P.; Nichols, J. B.; Trehan, A. K. Quinolone Antibacterial Agents. Synthesis and Structure–Activity Relationships of 8-Substituted Quinoline-3-carboxylic Acids and 1,8-Naphthyridine-3-carboxylic Acids. *J. Med. Chem.* **1988**, *31*, 983–991.

Table III. Test Results for the Compounds in This Study



					minimum inhibitor concentrations MIC's (μg/mL)[a,b]										gyrase drug induced cleavage (μg/mL), *E. coli* H560[c,d]
					Gram-negative organisms					Gram-positive organisms					
										S. aureus					
compd	R_5	X		R_7	*E. cloac.* MA 2646	*E. coli* Vogel	*K. pneum.* MGH-2	*P. rettg* M 1771	*P. aerug.* UI-18	H-228	UC76	*E. laec.* MGH-2	*S. pneum.* SV-1	*S. pyog.* C-203	
21a	H	N	*R,S*	H	0.025	0.013	0.025	0.05	0.05	0.2	0.013	0.2	0.2	0.2	1.0
21b	H	N	*S*	H	0.013	0.006	0.013	0.025	0.1	0.05	0.013	0.1	0.05	0.05	0.25
21c	H	N	*R*	H	0.025	0.013	0.025	0.05	0.4	0.2	0.05	0.2	0.1	0.2	0.50
21d	H	N	*R,S*	*S*-Ala	0.2	0.4	0.8	1.6	3.1	3.1	0.8	1.6	0.8	0.4	0.75
21e	H	H	*R,S*	*R*-Ala	0.8	0.8	0.8	1.6	1.6	0.4	0.05	0.2	0.25	0.013	2.5
21f	H	N	*S*	*S*-Ala	0.8	0.8	1.6	3.1	3.1	3.1	0.8	1.6	0.8	0.8	5.0
21g	H	N	*R*	*S*-Ala	0.8	1.6	3.1	3.1	6.3	3.1	0.8	3.1	1.6	0.8	1.0
21h	H	N	*R,S*	*S*-Phe	0.4	0.8	3.1	6.3	6.3	3.1	0.4	0.8	0.8	1.6	–
21i	H	N	*R,S*	Gly	0.4	0.2	0.4	0.8	3.1	1.6	0.4	0.8	0.8	0.1	2.5
21j	H	N	*R,S*	*S*-Lys	12.5	12.5	25	25	>25	25	6.3	>25	>25	>25	–
21k	H	N	*R,S*	*S*-Val	0.4	0.4	1.6	1.6	3.1	1.6	0.4	1.6	1.6	1.6	–
21l	H	N	*R,S*	*S*-c-Gln	6.3	6.3	12.5	25	>25	3.1	0.8	6.3	12.5	12.5	5.0
22a	H	CF	*R,S*	H	0.025	0.013	0.025	0.05	0.05	0.025	0.006	0.05	0.05	0.05	0.1
22b	H	CF	*S*	H	0.013	0.006	0.003	0.006	0.025	0.013	0.003	0.025	0.013	0.013	0.1
22c	H	CF	*R*	H	0.006	0.013	0.013	0.025	0.1	0.05	0.006	0.05	0.025	0.05	0.50
22d	H	CF	*R,S*	*S*-Ala	0.2	0.2	0.2	0.2	0.4	0.2	0.05	0.4	0.1	0.1	1.0
22e	H	CF	*R,S*	*R*-Ala	0.4	0.2	0.4	0.8	1.6	0.05	0.006	0.025	0.013	0.006	2.5
22f	H	CF	*S*	*S*-Ala	0.1	0.1	0.2	0.2	0.1	0.1	0.025	0.1	0.1	0.1	2.5
22g	H	CF	*R*	*S*-Ala	0.2	0.4	0.8	0.8	3.1	0.8	0.2	0.8	0.4	0.4	2.5
22h	H	CF	*R,S*	*S*-Phe	1.6	0.8	1.6	3.1	25	3.1	1.6	25	>25	>25	7.5
22i	H	CF	*R,S*	Gly	0.2	0.2	0.4	0.4	1.6	0.4	0.1	0.4	0.4	0.2	5.0
22j	H	CF	*R,S*	*S*-Lys	3.1	6.3	12.5	12.5	12.5	0.4	0.2	6.3	6.3	6.3	–
23a	H	CH	*R,S*	H	0.025	0.025	0.05	0.1	0.1	0.2	0.025	0.1	0.1	0.1	0.10
23b	H	CH	*R,S*	*S*-Ala	0.8	0.4	0.8	1.6	1.6	3.1	0.4	1.6	0.4	0.4	1.0
23c	H	CH	*R,S*	*R*-Ala	0.8	0.8	0.8	1.6	1.6	0.4	0.05	0.2	0.025	0.013	1.0
23d	H	CH	*R,S*	*S*-Phe	0.8	0.8	3.1	3.1	6.3	0.8	0.2	0.4	0.4	0.4	25.0
23e	H	CH	*R,S*	Gly	0.4	0.4	0.8	1.6	1.6	3.1	0.4	1.6	0.4	0.1	2.5
23f	H	CH	*R,S*	*S*-Lys	12.5	12.5	12.5	25	25	25	3.1	>25	25	12.5	–
24a	NH_2	CF	*R,S*	H	0.006	0.006	0.006	0.025	0.05	0.013	0.006	0.025	0.006	0.025	0.25
24b	NH_2	CF	S	H	0.003	0.003	0.013	0.025	0.025	0.006	0.003	0.025	0.006	0.013	0.10
24c	NH_2	CF	*R*	H	0.013	0.013	0.025	0.05	0.02	0.025	0.006	0.05	0.003	0.025	0.75
24d	NH_2	CF	*S*	S-Ala	0.05	0.05	0.2	0.4	1.6	0.1	0.025	0.2	0.05	0.05	2.5
24e	NH_2	CF	*R*	S-Ala	0.8	0.4	0.8	1.6	3.1	0.4	0.025	0.4	0.2	0.2	5.0
24f	NH_2	CF	*R,S*	Gly	0.2	0.2	0.4	0.4	1.6	0.4	0.1	0.4	0.4	0.2	5.0
ciprofloxacin	H	CH			0.025	0.025	0.05	0.05	0.4	1.6	0.2	0.8	0.8	0.4	0.5
tosufloxacin	H	N			0.025	0.013	0.05	0.1	0.4	0.05	0.025	0.1	0.025	0.05	–
sparfloxacin	NH_2	CF			0.025	0.025	0.05	0.2	0.8	0.05	0.025	0.1	0.05	0.1	2.5

[a] Standard microdilution techniques; see ref 21a,b. [b] All values for 21a–l through 24a–f are accurate to ±50% and have been obtained from duplicate or triplicate experiments; see ref 21a,b. [c] Refers to the minimum concn. at which cleaved DNA (linear) is observed at an intensity relative to oxolinic acid at 10 μg/mL. [d] Reference 21a.

Table IV

	mouse chemotherapy PD50 (mg/kg)[a,b,c]								solubility of product HCl salt (mg/mL)			
	E. coli		*P. aerug.*		*S. pneum.*		*S. pyog.*		H_2O	HCl	pH 4	pH 7.4
compd	po	sc	po	sc	po	sc	po	sc	(distilled)	(0.1 N)	(acetate)	(phosphate)
21a	2	0.6	7	3	58	33	32	14	84	12	88	0.03
21b	0.8	0.4	4	2	19	14	19	9	100	14	101	0.05
21c	0.5	0.4	–	–	45	34	–	–	97	15	79	0.05
21d	1	0.6	7	5	28	10	15	7	>300	>350	>350	0.08
21e	22.5	5.1	–	–	>100	3	–	–	>250	189	262	0.06
21f	1.3	0.4	3	2	25	12	22	10	>350	>350	>350	0.02
21g	2	0.5	–	–	40	28	49	32	>200	>200	>200	0.07
21h	2	0.4	–	–	65	37	–	–	>200	80	>100	0.06
21i	–	–	–	–	37	10	–	–	>200	67	>200	–
21j	–	–	–	–	71	70	–	–	>300	>200	>600	0.07
21k	3	0.6	–	–	–	–	76	27	>200	>200	>200	0.08
21l	4.8	0.4	–	–	–	–	–	–	6.1	1.4	3.1	0.05
22a	1	0.2	7	3	15	6	7	2	15	3.0	2.4	0.08
22b	1	0.2	–	–	15	7	–	–	17	–	–	–
22c	0.9	0.2	–	–	14	9	–	–	11	–	–	–
22d	2	0.3	13	2	13	9	12	4	31	7.2	25	0.09
22e	31	2	–	–	>100	1	–	–	42	3.6	19.4	–
22f	1	0.4	–	–	25	9	–	–	33	5.4	19	0.07
22g	1	0.6	–	–	25	19	–	–	29	6.0	30	0.07
22h	5	1	–	–	–	–	–	–	31	7.2	21	–
22i	1	0.4	–	–	29	16	–	··	33	4.6	20	–
22j	–	–	–	–	–	–	–	–	–	–	–	–
23a	3	0.5	49	5	43	4	97	11	22	1.4	12	0.41
23b	5	0.5	–	–	>100	13	>100	5	>100	>100	>100	0.27
23c	>100	4.1	–	–	>100	3.1	–	–	>100	>100	>100	0.24
23d	5	1	–	–	>50	5	–	–	44	9.2	66	0.30
23e	6.3	1.1	–	–	>100	18	–	–	>100	>100	>100	0.22
23f	10	1	–	–	>100	12	–	–	93	69	>100	0.11
24a	2	0.4	16	4	20	9	24	6	2.4	0.1	3.2	0.08
24b	1.2	0.4	–	–	10	6.0	18	5.5	2.5	0.25	2.3	0.05
24c	1.7	0.3	–	–	15	3.5	12	7.5	2.8	0.2	2.6	0.04
24d	5.0	2.5	9.6	4	17	13	6.3	23	18.0	2.8	58	0.04
24e	2.2	0.5	–	–	18	7.7	20	6.3	21.0	3.2	71	0.08
24f	1	0.4	–	–	29	16	36	8.1	17	3.5	69	0.07
ciprofloxacin	1	0.3	15	4	260	29	180	19	42	23	90	0.10
tosufloxacin	1	0.2	6	4	14	3	5	3	0.65	0.40	0.55	0.01
sparfloxacin	1	0.4	14	7	14	13	17	17	0.09	0.02	0.03	0.09

[a] Single dose given at challenge. [b] po indicates oral administration by gavage, and sc indicates subcutaneous injection. [c] Reference 22. [d] Confidence limits for the in vivo data are ±50% of the reported values. [e] Bacterial strains cited in Table IV are the same as those used in Table III.

Almost all of the amino acid derivatives deliver greater in vivo efficacy than the MIC's would predict and therefore appear to be acting as prodrugs.

A significant difference in activity was also exhibited by the stereoisomers of the pyrrolidine, both in vitro and in vivo. The *S*-isomer of the 3-aminopyrrolidine side chain (**17a**), when affixed to the 7-position of the 1,8-naphthyridine **21b**, PD 131628,[23] was more active than either the racemate **21a** or the *R*-isomer **21c**. When the 6,8-difluoroquinolone was the substrate, there was a similar potency difference between the *S*-isomer **22b**, the *R*-isomer **22c**, and the racemate **22a**. This was also true for the 5-amino-6,8-difluoroquinolones **24a**, **24b**, and **24c**.

A similar difference in activities was also seen when the *R*- and *S*-amino acids were affixed to the 3-amino functionality of the pyrrolidine. A comparison of the naphthyridines, **21d** (*S*-amino acid, *R,S*-pyrrolidine) and **21e** (*R*-amino acid, *R,S*-pyrrolidine), shows an identical in vitro spectrum against Gram-negative organisms with **21e** showing a superiority against Gram-positive as previously mentioned. However, **21d** has superior in vivo efficacy when compared to **21e** (*E. coli* 1/0.6 vs 22.5/5.1; *S. pneum.* 28/10 vs 100/3). This enhanced in vivo activity is also exhibited when (*S*)-alanine is put on the (*R*)-pyrrolidine, **21g** (*E. coli* 2/0.5, *S. pneum.* 40/28) or the *S*-pyrrolidine, **21f**, PD 131112 (*E. coli* 1.3/0.4 *S. pneum.* 25/12).[24] This dominance of (*S*)- over (*R*)-alanine on the racemic 3-aminopyrrolidine can also be seen in the 6,8-difluoroquinolones, **22d** vs **22e** (*E. coli* 2/0.3 vs 31/2) and the 6-fluoro-8*H*-quinolones, **23b** vs **23c** (*E. coli* 5/0.5 vs >100/4.1).[25] This comparison demonstrates the importance of having (*S*)-alanine as opposed to (*R*)-alanine as the acylating portion of the side chain for in vivo efficacy.

A comparison of the effect of the various amino acids on the activity of the side chains, both in vitro and in vivo, shows that alanine is the superior amino acid in this series. It has already been shown that the *S*-isomer of the amino acid is most desirable.

It is also interesting to note that the least active amino acid of the series is (*S*)-lysine. The importance of having a basic group in the 7-position of the quinolone antibacterials for activity has been thoroughly demonstrated. The poor activity of the lysine analogues (**21j**, **22j**, **23f**) once again demonstrates the importance of the distance rela-

(23) Sanchez, J. P. U.S. Patent 4,916,141, 1990; *Chem. Abstr.* **1990**, *113*, 40658d.

(24) Sanchez, J. P. U.S. Patent 4,851,418, 1989; *Chem. Abstr.* 1989, *111*, 153777u.

(25) Domagala, J. M.; Hagen, S. E.; Heifetz, C. L.; Hutt, M. P.; Mich, T. F.; Sanchez, J. P.; Trehan, A. K. 7-Substituted 5-Amino-1-cyclopropyl-6,8-difluoro-1,4-dihydro-4-oxo-3-quinolinecarboxylic Acids: Synthesis and Biological Activity of a New Class of Quinolone Antibacterials. *J. Med. Chem.* 1988, *31*, 503–506.

Table V. Stability and Breakdown of PD 131112 (21f) to PD 131628 (21b)

conditions	time (h)	% cleaved to 21b
0.1 N HCl	24	0
2.0 N AcOH	24	0
0.1 N NaOH	24	1.3
MeOH, EtOH	24	0
pH 7.4	24	0
serum (dog, human, mouse)	1	>50
po or iv dosing (dog, rat, mouse)	0.25	100

tionship of the basic portion of the side chain to the rest of the molecule.

An attempt to put (*S*)-glutamine on the racemic 3-aminopyrrolidine produced the desired Boc-protected quinolone. However, during removal of the protecting group the glutamine cyclized to give **21l**. It is included in the study as an example of an acylated aminopyrrolidine which shows a decreased activity in vitro as well as in vivo.

The combination of in vitro activity, enhanced in vivo efficacy, Pseudomonas activity, solubility, gyrase activity, and bioavailability led to the choice of the naphthyridine substrate with the *S*-amino acid on the (*S*)-3-aminopyrrolidine **21f** as the most significant compound.

Solubility. In every case, the amino acid derivatives (as their hydrochloride salts) had 3–70 times improved solubility compared to their parent compounds. The most significant increase in solubility was seen in the case of the 1,8-naphthyridines which have the greatest inherent solubility. This increase in solubility was even apparent in 0.1 M hydrochloric acid where solubility is usually suppressed due to the common ion effect. The increase in solubility of the other members of the amino acid series mirrored the solubilities of their parents, that is, the smallest increase in solubility was seen in the series that had the least soluble parents. The solubilities of the amino acid derivatives were similar to those of the parent compounds at pH 7.4 due to the zwitterionic character of both sets of compounds. As expected, the stereoisomers did not show any differences in solubility.

Mechanism. Data from Tables III and IV suggest that the amino acid analogues, although stable in acid and base, are rapidly cleaved in vivo in serum to release the parent drug. Compounds such as *N*-acetyl, *N*-propionyl, or the cyclic amide **21l** which are acid and base stable and are not cleaved in vivo (serum) cannot confer either the activity or the solubility of the amino acid analogues.

Conclusions

The *S*-amino acid derivatives of potent quinolones such as **21b** greatly enhance solubility (in the form of **21f**). The amino acids are rapidly cleaved in vivo (serum) to release the parent drugs (Table V). The cleavage appears to be enzyme mediated and somewhat specific in that the *R*-amino acids are much less active in vivo. The naphthyridine **21f** was identified as the best overall compound, being cleaved to release **21b**. The prodrug combination **21f/21b** provides equal or superior activity to all the standard quinolones and is up to 400 times more soluble. Compound **21f** is even soluble in saline at >250 mg/mL and was the most active quinolone ever tested in our Pseudomonas Chemotherapy model.

Experimental Section

Instrumental Data. All melting points were determined on a Hoover capillary melting point apparatus and are uncorrected. Infrared (IR) spectra were determined in KBr on a Nicolet FTIR SX-20 instrument. Proton magnetic resonance (NMR) were recorded on either a Varian XL-200 or an IBM 100 WP100SY spectrometer. Shifts are reported in δ units relative to internal tetramethylsilane. Elemental analyses were performed on a Perkin-Elmer 240 elemental analyzer. All compounds had analytical results ±0.4% of theoretical values. All organic solutions were dried over magnesium sulfate, and all concentrations were performed in vacuo at 10–30 mmHg. Chiral HPLC was performed on the side chains and intermediates after derivization of the amino functionality with 3,5-dinitrobenzoyl chloride using a D-naphthylalanine Pirkle column (Regis) eluting with hexane/2-propanol (95:5). The final quinolones and naphthyridines were chromatographed using an Ultrasphere C18 column eluting with MeOH/0.025 $NH_4H_2PO_4$ (40:60). Optical and diastereomeric purities were accurate to 0.5%. Solubilities were determined either by HPLC areas at equilibrium or by a conventional weight volume method. All solutions were maintained for 24 h (Table IV). Stability of the amino acid quinolone derivatives was determined by HPLC. Cleavage in serum could be halted at pH 4.0 at 0–10 °C.

(*R*)-3-Hydroxy-1-pyrrolidinecarboxylic Acid, Phenylmethyl Ester (12b). A solution of 35.4 g (0.29 mol) of (*R*)-3-hydroxypyrrolidine hydrochloride (**11b**)[19] in 100 mL of water was cooled to 5 °C and neutralized to pH 7.0 by the addition of 10% sodium hydroxide using a pH meter. The resulting solution was treated dropwise with 49.0 g (0.29 mol) of carbobenzyloxy chloride maintaining the pH at 11.0 ± 0.5 by the addition of 10% sodium hyroxide. When the addition was complete, the reaction was stirred at 5 °C for 2 h and allowed to come to room temperature overnight. The reaction mixture was extracted with ethyl acetate (2 × 400 mL), and the combined extracts were washed with water, dried ($MgSO_4$), filtered, and evaporated in vacuo to give 60.1 g (95%) of **12b** as a clear colorless oil: $[\alpha]^{23}_D$ +26.2° (*c* 1.1, MeOH); NMR (DMSO-d_6) δ 1.92 (m, 2 H), 2.83 (s, 1 H), 3.47 (m, 4 H), 4.41 (m, 1 H), 5.14 (s, 2 H), 7.34 (m, 5 H); MS (*m/e* relative intensity) 222 (33, M + 1), 91 (100). Anal. ($C_{12}H_{15}NO_3$) C, H, N.

(*R*)-3-[(Methylsulfonyl)oxy]-1-pyrrolidinecarboxylic Acid, Phenylmethyl Ester (13b). A solution of 70.1 g (0.29 mol) of **12b** in 300 mL of pyridine was cooled to 5 °C and treated dropwise with 34.4 g (0.3 mol) of methanesulfonyl chloride. The reaction was stirred at 5 °C for 2 h and allowed to come to room temperature overnight. The reaction was diluted with 600 mL of ethyl acetate, the resulting precipitate was removed by filtration and washed with ethyl acetate, and the filtrate was evaporated in vacuo. The residue was partitioned between ethyl acetate and water (500 mL each) and the aqueous layer was reextracted with ethyl acetate (250 mL). The combined organic layers were washed with water (3 × 200 mL), dried ($MgSO_4$), filtered, and evaporated in vacuo to give 77.9 g (91%) of **13b** as a light yellow oil: $[\alpha]^{23}_D$ −27.3° (*c* 1.09, MeOH); NMR ($CDCl_3$) δ 2.23 (m, 2 H), 3.03 (s, 3 H), 3.65 (m, 4 H), 5.15 (s, 2 H), 5.28 (m, 1 H), 7.34 (m, 5 H); MS (*m/e* relative intensity) 300 (100, M + 1), 256 (45). Anal. ($C_{13}H_{17}NO_5S$) C, H, N.

(*S*)-3-Azido-1-pyrrolidinecarboxylic Acid, Phenylmethyl Ester (14a). A solution of 77.9 g (0.26 mol) of **13b** in 250 mL of *N*,*N*-dimethylformamide was treated with 19.5 g (0.3 mol) of finely powdered sodium azide, and the reaction mixture was heated at 100 °C for 2 h. The solvent was removed in high vacuo at 50 °C, and the residue was partitioned between ethyl acetate and water (350 mL each). The aqueous layer was reextracted with ethyl acetate (2 × 250 mL), and the combined organic layers were washed with water, dried ($MgSO_4$), filtered, and evaporated in vacuo to give 62.5 g (98%) of **14a** as a clear colorless oil: $[\alpha]^{23}_D$ +36.7° (*c* 1.09, MeOH); NMR ($CDCl_3$) δ 2.08 (m, 2 H), 3.56 (m, 4 H), 4.19 (m, 1 H), 5.17 (s, 2 H), 7.37 (m, 5 H); MS (*m/e* relative intensity): 247 (13, M + 1), 91 (100). Anal. ($C_{12}H_{14}N_4O_2$) C, H, N.

(*S*)-3-Amino-1-pyrrolidinecarboxylic Acid, Phenylmethyl Ester (15a). A solution of 61.4 g (0.25 mol) of **14a** in 900 mL of methanol was treated with 5.0 g of Raney nickel and shaken in a hydrogen atmosphere at temperatures of 21.5–25.0 °C and a pressure of 50 psi for 21.5 h. The reaction was vented and treated with an additional 5.0 g of catalyst after 19 h. The catalyst was removed by filtration through Celite, and the solvent was removed in vacuo to give 52.1 g (95%) of **15a** as a clear, colorless oil: $[\alpha]^{23}_D$ +2.8° (*c* 1.54, MeOH); NMR ($CDCl_3$) δ 1.62 (s, 2 H), 1.89 (m, 1 H), 2.06 (m, 1 H), 3.16 (m, 1 H), 3.53 (m, 4 H), 5.17 (s, 2 H), 7.35 (m, 5 H); chiral HPLC 96/4 *S*/*R*; MS (*m/e* relative

intensity) 221 (93, M + 1), 206 (34), 177 (24), 91 (100). Anal. ($C_{12}H_{16}N_2O_2$) C, H, N.

(*S*)-3-[[(1,1-Dimethylethoxy)carbonyl]amino]-1-pyrrolidinecarboxylic Acid, Phenylmethyl Ester (16a). A solution of 5.5 g (25 mmol) of **15a** in 25 mL (25 mmol) of 1.0 N sodium hydroxide and 40 mL of *tert*-butyl alcohol was treated dropwise with a solution of 5.5 g (25 mmol) of di-*tert*-butyl dicarbonate in 15 mL of *tert*-butyl alcohol. The reaction mixture was stirred at room temperature overnight, and the solvent was removed in vacuo. The residue was partitioned between ethyl acetate and water, and the layers were separated. The organic layer was reextracted with ethyl acetate, and the combined organic layers were washed with water, dried ($MgSO_4$), filtered, and evaporated in vacuo to give 6.7 g (84%) of **16a** as a white solid, mp 124–125 °C; $[\alpha]^{23}_D$ –8.73° (*c* 0.55, MeOH); NMR (DMSO-d_6) δ 1.38 (s, 9 H), 1.74 (m, 1 H), 2.50 (m, 1 H), 3.14 (m, 1 H), 3.33 (m, 2 H), 3.96 (bs, 1 H), 5.05 (s, 2 H), 7.35 (m, 5 H); MS (*m/e* relative intensity) 321 (32, M + 1), 265 (79), 221 (76). Anal. ($C_{17}H_{24}N_2O_4$) C, H, N.

(*S*)-3-Pyrrolidinylcarbamic Acid, 1,1-Dimethylethyl Ester (17a). A solution of 6.1 g (18.7 mmol) of **16a** in 100 mL of methanol was treated with 1.0 g of 20% palladium on carbon and was shaken in a hydrogen atmosphere at temperatures of 21–25 °C and pressures of 31.7–50.6 psi for 30 min. The catalyst was removed by filtering through Celite, and the solvent was removed in vacuo to give 3.3 g (95%) of **17a** as a colorless oil which solidified upon standing: $[\alpha]^{23}_D$ –22.7° (*c* 1.04, MeOH); NMR ($CDCl_3$) δ 1.44 (s, 9 H), 1.69 (m, 1 H), 2.20 (m, 1 H), 3.19 (m, 4 H), 3.39 (m, 1 H), 4.27 (bd, 1 H), 5.86 (bs, 1 H); chiral HPLC 98:2 *S/R*; MS (*m/e* relative intensity): 187 (95, M + 1), 131 (86), 69 (97). Anal. ($C_9H_{18}N_2O_2$) C, H, N.

[*S*-(*R,*R**)]-3-[[2-[[(1,1-Dimethylethoxy)carbonyl]amino]-1-oxopropyl]amino]-1-pyrrolidinecarboxylic Acid, Phenylmethyl Ester (19a).** A solution of 9.4 g (50 mmol) of (*tert*-butoxycarbonyl)-L-alanine in 150 mL of dichloromethane was treated with 5.1 g (50 mmol) of *N*-methylmorpholine at room temperature. The reaction mixture was then cooled to –20 °C and treated with 6.8 g (50 mmol) of isobutyl chloroformate. The reaction mixture was warmed to –10 °C for 5 min, recooled to –20 °C, and treated with a solution of 7.7 g (35 mmol) of **15a** in 30 mL of dichloromethane. The solution was stirred at –10 °C for 30 min and allowed to come to room temperature overnight. The reaction was washed with a 5% solution of sodium bicarbonate (2 × 100 mL) and water, dried ($MgSO_4$), filtered, and evaporated in vacuo to give 13.2 g (96%) of **19a** as a white solid: $[\alpha]^{23}_D$ –13.5° (*c* 1.5, MeOH); NMR ($CDCl_3$) δ 1.32 (d, 3 H), 1.42 (s, 9 H), 1.90 (m, 2 H), 3.55 (m, 4 H), 4.12 (m, 1 H), 4.44 (m, 1 H), 5.00 (bs, 1 H), 5.10 (s, 2 H), 6.62 (bs, 1 H), 7.34 (s, 5 H); MS (*m/e* relative intensity) 392 (10, M + 1), 336 (100), 292 (63), 258 (47), 202 (71). Anal. ($C_{20}H_{29}N_3O_5$) C, H, N.

[*S*-(*R,*R**)]-[1-Methyl-2-oxo-2-(3-pyrrolidinylamino)ethyl]carbamic Acid, 1,1-Dimethylethyl Ester (20a).** A solution of 15.7 g (40.1 mmol) of **19a** in 400 mL of methanol was treated with 1.0 g of 20% palladium on carbon and was shaken in a hydrogen atmosphere at temperatures of 24.5–25.5 °C and pressures of 48.7–51.6 psi for 3 h. The catalyst was removed by filtering through Celite, and the filtrate was evaporated in vacuo to give 10.0 g (97%) of **20a** as a colorless oil: $[\alpha]^{23}_D$ –34.3° (*c* 5.1, MeOH); NMR ($CDCl_3$) δ 1.31 (d, 3 H), 1.44 (s, 9 H), 1.76 (m, 1 H), 2.16 (m, 1 H), 2.97 (m, 2 H), 3.14 (m, 2 H), 3.84 (s, 1 H), 4.13 (m, 1 H), 4.44 (m, 1 H), 5.40 (bs, 1 H), 7.40 (bs, 1 H). The signals at 3.84, 5.40, and 7.40 could be exchanged with D_2O. Chiral HPLC 99.7% *S*; MS (*m/e* relative intensity) 258 (84, M + 1), 202 (100). Anal. ($C_{12}H_{23}N_3O_3$) C, H, N.

Preparation of Final Quinolones. General Method A: Compounds 21e–21l, 22d, 22e, 22h, 22j, 23c–23e, 24d–24f. [*S*-(*R,*R**)]-1-Cyclopropyl-7-[3-[[2-[[(1,1-dimethylethoxy)carbonyl]amino]-1-oxopropyl]amino]-1-pyrrolidinyl]-6-fluoro-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylic Acid (21p).** A suspension of 2.8 g (10 mmol) of **25**,[26] 3.9 g (15 mmol) of **20a**, 3.0 g (30 mmol) of triethylamine, and 150 mL of acetonitrile was heated at reflux for 4 h. The reaction mixture was cooled to room temperature, and the solid was removed by filtration, washed with acetonitrile, and dried in vacuo to give 4.3 g (85%) of **21p**: mp 195–197 °C; NMR (DMSO-d_6) δ 1.12 (m, 4 H), 1.19 (d, 3 H), 1.37 (s, 9 H), 1.97 (m, 1 H), 2.18 (m, 1 H), 3.65 (m, 2 H), 3.93 (m, 4 H), 4.39 (m, 1 H), 6.88 (d, 1 H), 7.94 (d, 1 H), 8.29 (d, 1 H), 8.56 (m, 1 H), 15.70 (bs, 1 H). The signals at 6.88, 8.29, and 15.70 could be exchanged with D_2O. MS (*m/e* relative intensity) 504 (100, M + 1), 430 (23). Anal. ($C_{24}H_{30}FN_5O_6$) C, H, N.

[*S*-(*R,*R**)]-7-[3-[(2-Amino-1-oxopropyl)amino]-1-pyrrolidinyl]-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylic Acid, Hydrochloride (21f).** A suspension of 1.5 g (3.0 mmol) of **21p** in a mixture of 25 mL of 1.0 M hydrochloric acid and 25 mL of ethanol was stirred at room temperature for 1 h and heated at reflux for 2 h. The solvent was removed in vacuo, the residue was triturated with 50 mL of 2-propanol/ether (1:1), and the solid was removed by filtration, washed with the above mixture and ether, and dried in vacuo to give 1.2 g (92%) of **21f**: mp 200–202 °C; NMR (DMSO-d_6) δ 1.23 (m, 4 H), 1.73 (d, 3 H), 2.07 (m, 1 H), 2.43 (m, 1 H), 3.67 (m, 6 H), 4.87 (m, 1 H), 8.01 (d, 1 H), 8.33 (s, 2 H), 9.07 (s, 1 H), 9.20 (d, 1 H), 15.81 (bs, 1 H). The signals at 8.33, 9.07, and 15.81 could be exchanged with D_2O. MS (*m/e* relative intensity) 404 (100, M + 1), 359 (15), 315 (45), 271 (88). Anal. ($C_{19}H_{22}FN_5O_4 \cdot HCl \cdot H_2O$) C, H, N.

General Method B: Compounds 21d, 22f, 22g, 22i, 23b, 23f. [*S*-(*R,*R**)]-7-[3-[(2-Amino-1-oxopropyl)amino]-1-pyrrolidinyl]-1-cyclopropyl-6,8-difluoro-1,4-dihydro-4-oxo-3-quinolinecarboxylic Acid, Sesquihydrochloride (22f).** A solution of 1.5 g (7.7 mmol) of *tert*-butoxycarbonyl-L-alanine in 15 mL of acetonitrile was cooled to 5 °C and treated with 0.8 g (7.7 mmol) of *N*-methylmorpholine. The reaction mixture was stirred at 5 °C for 5 min and cooled to –20 °C where 1.1 g (7.7 mmol) of isobutyl chloroformate was added dropwise while the temperature was maintained at –20 ± 5 °C. After the addition was complete the reaction mixture was stirred at 0 °C for 2 h and treated with a solution of 2.9 g (7.7 mmol) of ethyl (*S*)-7-(3-amino-1-pyrrolidinyl)-1-cyclopropyl-6,8-difluoro-1,4-dihydro-4-oxo-3-quinolinecarboxylate (**26p**) in 15 mL of dichloromethane. The reaction mixture was stirred at –5 °C for 2 h and allowed to come to room temperature overnight. The solvent was removed in vacuo, and the residue was dissolved in dichloromethane, washed with water, dried ($MgSO_4$), filtered, and evaporated in vacuo. The residue was dissolved in a mixture of 1.0 M hydrochloric acid (30 mL) and ethanol (20 mL) and heated to reflux for 4 h. The ethanol was allowed to evaporate, and the aqueous acid solution was heated at reflux an additional 30 min. The solution was filtered through a fiber glass pad to clarify, and the filtrate was lyophilized. The residue was triturated with ethanol and ether (50 mL, 1:1), and the solid was removed by filtration, washed with the aforementioned mixture (25 mL) and ether, and dried in vacuo to give 1.9 g (49%) of **22f**: mp 128–131 °C; $[\alpha]^{23}_D$ –32.8° (*c* 1.08, MeOH); NMR (TFA-*d*) δ 1.51 (m, 4 H), 1.83 (s, 3 H), 2.47 (m, 2 H), 4.31 (m, 6 H), 4.83 (m, 1 H), 8.12 (bs, 1 H), 9.31 (bs, 1 H); MS (*m/e* relative intensity) 421 (100, M + 1), 403 (23), 350 (50). Anal. ($C_{20}H_{22}F_2N_4O_4 \cdot 1.5HCl \cdot 2.0H_2O$) C, H, N, Cl, F.

General Method C: Compounds 21a–21c, 22a–22c, 23a, 24a–24c. (*S*)-7-(3-Amino-1-pyrrolidinyl)-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylic Acid, Monohydrochloride (21b). A near solution of 4.3 g (15 mmol) of **25**,[26] 3.5 g (19 mmol) of **17a**, 4.0 g (40 mmol) of triethylamine, and 75 mL of acetonitrile was heated at reflux for 3 h. The reaction mixture was cooled to 5 °C, and the solid was removed by filtration, washed with acetonitrile and ether, and dried in vacuo to give 6.2 g (95%) of the Boc-protected product. This solid was suspended in a solution of 1.0 M hydrochloric acid (30 mL) and ethanol (30 mL) and was heated at reflux for 3 h. The solvent was removed in vacuo, and the residue was triturated with 2-propanol/ether (20 mL each). The solid was removed by filtration, washed with the previous solvent mixture (2 × 5 mL) and ether, and dried in vacuo to give 4.8 g (87% over two steps) of **21b**: mp 310–312 °C: NMR (DMSO-d_6) δ 1.09 (m, 2 H), 1.20 (m, 2 H), 2.19 (m, 1 H), 2.33 (m, 1 H), 3.67 (m, 1 H), 3.98 (m, 5 H), 8.05 (d, 1 H), 8.44 (bs, 3 H), 8.58 (s, 1 H); chiral HPLC 98.3/1.7 *S/R*; MS (*m/e* relative intensity) 332 (25, M + 1), 288 (100). Anal. ($C_{16}H_{17}FN_4O_3 \cdot HCl \cdot 1.0H_2O$) C, H, N.

(26) Sanchez, J. P.; Mich, T. F.; Domagala, J. M.; Trehan, A. K. U.S. Patent 4,663,457, 1987; *Chem. Abstr.* **1986**, *104*, 88512t.

Registry No. **11b**, 104706-47-0; **12b**, 100858-33-1; **13b**, 122536-68-9; **14a**, 122536-70-3; **15a**, 122536-72-5; **16a**, 122536-74-7; (±)-**17**, 140629-77-2; **17a**, 122536-76-9; **17b**, 122536-77-0; **18**, 51-35-4; **19a**, 140412-80-2; **20a**, 122536-66-7; **20b**, 140412-68-6; **20c**, 122537-11-5; **20d**, 140412-67-5; **21a** (free base), 140629-71-6; **21b** (free base), 127967-03-7; **21b**·HCl, 138668-47-0; **21c** (free base), 140412-10-8; (*S,R*)-**21e** (free base), 122536-21-4; (*S,R*)-**21e**·*x*HCl, 140412-41-5; (*R,R*)-**21e** (free base), 140412-12-0; (*R,R*)-**21e**·*x*HCl, 140412-42-6; **21f** (free base), 122536-20-3; **21f**·HCl, 122536-48-5; **21g** (free base), 140412-11-9; **21g**·HCl, 122536-42-9; (*S,S*)-**21h** (free base), 122536-95-2; (*S,S*)-**21h**·*x*HCl, 140412-43-7; (*R,S*)-**21h** (free base), 122536-38-3; (*R,S*)-**21h**·*x*HCl, 140412-44-8; **21i** (free base), 140412-13-1; **21i**·*x*HCl, 140412-45-9; (*S,S*)-**21j** (free base), 140438-08-0; (*S,S*)-**21j**·2HCl, 140412-46-0; (*R,S*)-**21j** (free base), 140412-14-2; (*R,S*)-**21j**·2HCl, 140412-47-1; (*S,S*)-**21k** (free base), 140412-15-3; (*S,S*)-**21k**·HCl, 140412-48-2; (*R,S*)-**21k** (free base), 140412-16-4; (*R,S*)-**21k**·HCl, 140412-49-3; (*S,S*)-**21l** (free base), 140412-17-5; (*R,S*)-**21l** (free base), 140412-18-6; **21p**, 122536-91-8; **22a** (free base), 140629-72-7; **22a**·HCl, 140629-75-0; **22b** (free base), 133298-78-9; **22b**·HCl, 140412-50-6; **22c** (free base), 140412-19-7; **22c**·HCl, 133298-73-4; (*S,R*)-**22e** (free base), 140412-21-1; (*S,R*)-**22e**·HCl, 122536-44-1; (*R,R*)-**22e** (free base), 140412-22-2; (*R,R*)-**22e**·HCl, 122536-43-0; **22f** (free base), 122548-08-7; **22f**·3/2HCl, 140412-51-7; **22g** (free base), 140412-20-0; **22g**·HCl, 122536-36-1; (*S,S*)-**22h** (free base), 140412-23-3; (*S,S*)-**22h**·HCl, 122537-08-0; (*R,S*)-**22h** (free base), 140412-24-4; (*R,S*)-**22h**·HCl, 122536-60-1; **22i** (free base), 140412-25-5; **22i**·HCl, 140412-52-8; (*S,S*)-**22j** (free base), 140412-26-6; (*S,S*)-**22j**·2HCl, 140412-53-9; (*R,S*)-**22j** (free base), 140412-27-7; (*R,S*)-**22j**·2HCl, 140412-54-0; **23a** (free base), 140629-73-8; **23g**·2HCl, 140629-76-1; (*S,S*)-**23b** (free base), 140412-28-8; (*S,S*)-**23b**·*x*HCl, 140412-55-1; (*R,S*)-**23b** (free base), 140412-29-9; (*R,S*)-**23b**·*x*HCl, 140412-56-2; (*S,R*)-**23c** (free base), 140412-30-2; (*S,R*)-**23c**·*x*HCl, 140412-57-3; (*R,R*)-**23c** (free base), 140412-31-3; (*R,R*)-**23c**·*x*HCl, 140412-58-4; (*S,S*)-**23d** (free base), 140412-32-4; (*S,S*)-**23d**·*x*HCl, 140412-59-5; (*R,S*)-**23d** (free base), 140412-33-5; (*R,S*)-**23d**·*x*HCl, 140412-60-8; **23e** (free base), 140412-34-6; **23e**·*x*HCl, 140412-61-9; (*S,S*)-**23f** (free base), 140412-35-7; (*S,S*)-**23f**·*x*HCl, 140412-62-0; (*R,S*)-**23f** (free base), 140412-36-8; (*R,S*)-**23f**·*x*HCl, 140412-63-1; **24a** (free base), 140629-74-9; **24b** (free base), 133298-74-5; **24b**·HCl, 140412-64-2; **24c** (free base), 140412-37-9; **24c**·HCl, 140412-65-3; **24d** (free base), 140412-38-0; **24d**·HCl, 122536-56-5; **24e** (free base), 140412-39-1; **24e**·HCl, 122536-55-4; **24f** (free base), 140412-40-4; **24f**·HCl, 140412-66-4; **25**, 100361-18-0; 8-deaza-**25**, 86393-33-1; **26**, 122536-80-5; (*R*)-**26**, 122536-81-6; (±)-**26**, 140630-88-2; (±)-8-aza-**2b**, 140412-77-7; (±)-**26** (6-monofluoro derivative), 140438-09-1; 7-chloro-**26** (free acid), 140412-78-8; 5-amino-7-chloro-**26** (free acid), 140412-79-9; **27**, 127934-52-5; Boc-Ala, 15761-38-3; Boc-Gly, 4530-20-5; Boc-Lys, 13734-28-6; Boc-Phe-(*R*)-NH(3-pyrrolidinyl), 122536-86-1; Boc-Phe-(*S*)-NH(3-pyrrolidinyl), 140412-69-7; Boc-Gly-(±)-NH(3-pyrrolidinyl), 140412-70-0; Boc-Lys-(*R*)-NH-(3-pyrrolidinyl), 140412-71-1; Boc-Lys-(*S*)-NH(3-pyrrolidinyl), 140412-72-2; Boc-Val-(*R*)-NH(3-pyrrolidinyl), 140412-73-3; Boc-Val-(*S*)-NH(3-pyrrolidinyl), 140412-74-4; Boc-Gln-(*R*)-NH(3-pyrrolidinyl), 140412-75-5; Boc-Gln-(*S*)-NH(3-pyrrolidinyl), 140412-76-6.

Structure–Activity Relationships of (+)-CC-1065 Analogues in the Inhibition of Helicase-Catalyzed Unwinding of Duplex DNA†

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(+)-CC-1065 is a potent antitumor antibiotic produced by *Streptomyces zelensis*. Previous studies have shown that the potent cytotoxic and antitumor activities of (+)-CC-1065 are due to the ability of this compound to covalently modify DNA. (+)-CC-1065 reacts with duplex DNA to form a (*N*3-adenine)–DNA adduct which lies in the minor groove of DNA overlapping with a five base-pair region. As a consequence of covalent modification with (+)-CC-1065, the helix bends into the minor groove and alao undergoes winding and stiffening. In the studies described here, we have constructed templates for helicase-catalyzed unwinding of DNA that contain site-directed (+)-CC-1065 and analogue DNA adducts. Using these templates we have shown that (+)-CC-1065 and select synthetic analogues, which have different levels of cytotoxicity, all produce a significant inhibition of unwinding of a 3′-tailed oligomer duplex by helicase II when the displaced strand is covalently modified. However, the extent of helicase II inhibition is much more significant for (+)-CC-1065 and an analogue which also produced DNA winding when the winding effects are transmitted in the opposite direction to the helicase unwinding activity. This observed pattern of inhibition of helicase-catalyzed unwinding of drug-modified templates was the same for a 3′-T-tail, for different duplex region sequences, and with the *Escherichia coli rep* protein. Unexpectedly, the gel mobility of the displaced drug-modified single strand was dependent on the species of drug attached to the DNA. Last, strand displacement by helicase II coupled to primer extension by *E. coli* DNA polymerase I showed the same pattern of inhibition when the lagging strand was covalently modified. In addition, the presence of helicase II on single-stranded regions of templates caused the premature termination of primer extension by DNA polymerase. These results are discussed from the perspective that (+)-CC-1065 and its analogues have different effects on DNA structure, and these resulting structural changes in DNA molecules are related to the different in vivo biological consequences caused by these drug molecules.

Introduction

In order for DNA to serve as an efficient substrate for polymerases, the duplex DNA must be unwound. The task of unwinding DNA rapidly is carried out by a special class of enzymes called helicases.[1,2] These enzymes progress in a unidirectional or bidirectional manner through the helix utilizing energy derived from hydrolysis of nucleotide triphosphates to fuel this process. The precise mechanisms of helicase-catalyzed unwinding of DNA are not known, but in general they require a single-stranded region to which the protein initially binds. Helicase-catalyzed unwinding of DNA is important in replication, transcription, recombination, and repair.[3-5] While most in vitro studies

† Abbreviations: DDW, double-distilled water; EDTA, ethylenediaminetetraacetic acid; N, any nucleotide; pu, purine nucleotide; py, pyrimidine nucleotide; ss, single-stranded; TEMED, *N,N,N′,N′*-tetramethylethylenediamine; Tris, tris(hydroxymethyl)aminomethane.

(1) Geider, K.; Hoffmann-Berling, H. Proteins Controlling the Helical Structure of DNA. *Annu. Rev. Biochem.* **1981**, *50*, 233–260.
(2) Gefter, M. DNA Unwinding Enzymes. In *The Enzymes*; Boyer, P. D., Ed.; Academic Press: New York, 1981; Vol. 14, pp 367–372.

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