

complex side chains as well as to create further structural variation at the level of two- and three-carbon side-chain substituents.

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## Pyridonecarboxylic Acids as Antibacterial Agents. 2.<sup>1</sup> 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<sup>2</sup>

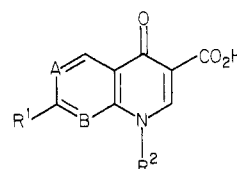
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The title compounds having nitro, amino, cyano, chloro, or fluoro as the C-6 substituent were prepared. Introduction of the chloro and cyano groups at C-6 was accomplished by the Sandmeyer reaction of 6-amino-1,8-naphthyridine derivatives 9 via their 6-diazonium salts 10. The reaction was extended to the synthesis of the 6-fluoro analogues 20, involving the Balz-Schiemann reaction of the diazonium tetrafluoroborate 19. Furthermore, a series of the 1-ethyl (24 and 27-30), 1-vinyl (35-37), 1-(2-fluoroethyl) (38 and 39), and 1-(difluoromethyl) (40) analogues of 7-substituted 6-fluoro-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylic acids was prepared. 1-Pyrrolidinyl and, particularly, N-substituted or unsubstituted 1-piperazinyl groups were introduced as the C-7 variants. As a result of this study, 1-ethyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-1,8-naphthyridine-3-carboxylic acid (24b; named enoxacin, originally AT-2266) was found to show the most broad and potent in vitro antibacterial activity, an excellent in vivo efficacy on systemic infections, and a weak acute toxicity. Structure-activity relationships of compounds with variations of substituents at C-1, C-6, and C-7 are also discussed.

In earlier papers, we had reported the synthesis and antibacterial activity of pyrido[2,3-d]pyrimidines (1),<sup>3,4</sup> 1,6- and 1,8-naphthyridines (2 and 3),<sup>1</sup> and quinolines (4),<sup>5</sup> all of which possess a 1,4-dihydro-4-oxopyridine-3-carboxylic acid moiety as a common structure. This class of compounds has increasingly attracted attention as a source of new antibacterial agents.<sup>6</sup> It has been reported that nalidixic acid (3, R<sup>1</sup> = CH<sub>3</sub>; R<sup>2</sup> = C<sub>2</sub>H<sub>5</sub>)<sup>7</sup> and oxolinic acid [4, R<sup>1</sup> = 6,7-(OCH<sub>2</sub>O), R<sup>2</sup> = C<sub>2</sub>H<sub>5</sub>]<sup>8</sup> are a specific inhibitor

Chart I<sup>a</sup>



- |                  |   |
|------------------|---|
| 1, A = B = N     | 4, A = B = CH   |
| 2, A = N; B = CH | 5, A = CR <sup>3</sup> ; B = N                                    |
| 3, A = CH; B = N | 6, A = CF; B = CH; R <sup>2</sup> = C <sub>2</sub> H <sub>5</sub> |

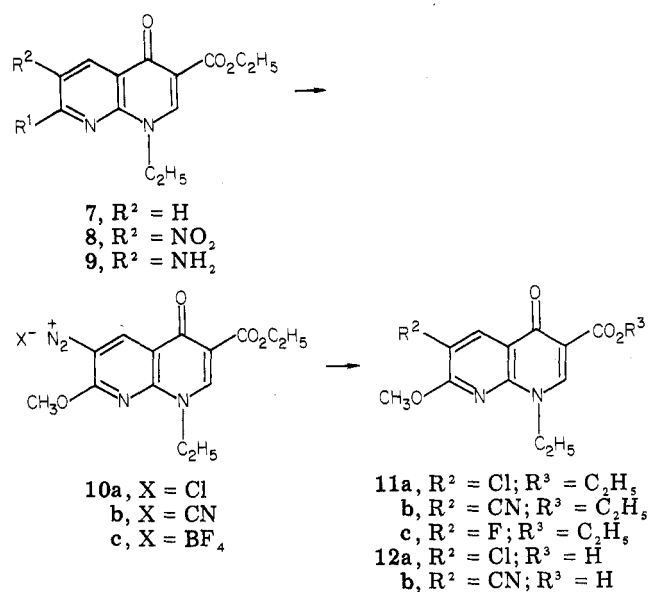
<sup>a</sup> a, R<sup>1</sup> = , b, R<sup>1</sup> = , c, R<sup>1</sup> = .

- (1) Paper 1: Hirose, T.; Mishio, S.; Matsumoto, J.; Minami, S. *Chem. Pharm. Bull.* **1982**, *30*, 2399.
- (2) This work was presented in part (a) at the 11th International Congress of Chemotherapy and 19th Interscience Conference on Antimicrobial Agents and Chemotherapy, Boston, MA, Oct 1979 (In "Current Chemotherapy and Infectious Disease"; Nelson, J. D.; Grassi, C., Eds.; American Society for Microbiology: Washington, DC, 1980; Vol. 1, p 454) and (b) at the 100th Annual Meeting of the Pharmaceutical Society of Japan, Tokyo, Apr 1980; Abstr, p 275. (c) Matsumoto, J.; Takase, Y.; Nishimura, Y. European Patent 9425, 1980; *Chem. Abstr.* **1980**, *93*, 168305x.
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- (5) Minami, S.; Matsumoto, J.; Sugita, M.; Shimizu, M.; Takase, Y. German Offen. 2362553, 1974; *Chem. Abstr.* **1974**, *81*, 105562k.
- (6) For a review, see Albrecht, R. *Prog. Drug Res.* **1977**, *21*, 9.
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of DNA synthesis in susceptible bacterial cells, and this inhibition is due to the prevention of DNA gyrase activity.<sup>9</sup> Recent findings indicate that piromidic acid (1a, R<sup>2</sup> = C<sub>2</sub>H<sub>5</sub>)<sup>3</sup> and pipemidic acid (1b, R<sup>2</sup> = C<sub>2</sub>H<sub>5</sub>)<sup>4</sup> also act by the same mechanism.<sup>10</sup>

A continuing interest in this field led us to an investigation of 1,6,7-trisubstituted 1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylic acids (5), with the C-6 substituent being either nitro, amino, chloro, or fluoro; little information about the effect of the C-6 substituent upon antibacterial activity has been available thus far.

- (9) For a review, see: Crumplin, G. C.; Midgley, J. M.; Smith, J. T. "Topics in Antibiotic Chemistry"; Sammes, P. G., Ed.; Ellis Horwood: West Sussex, England, 1980; Vol. 3, pp 11-38.
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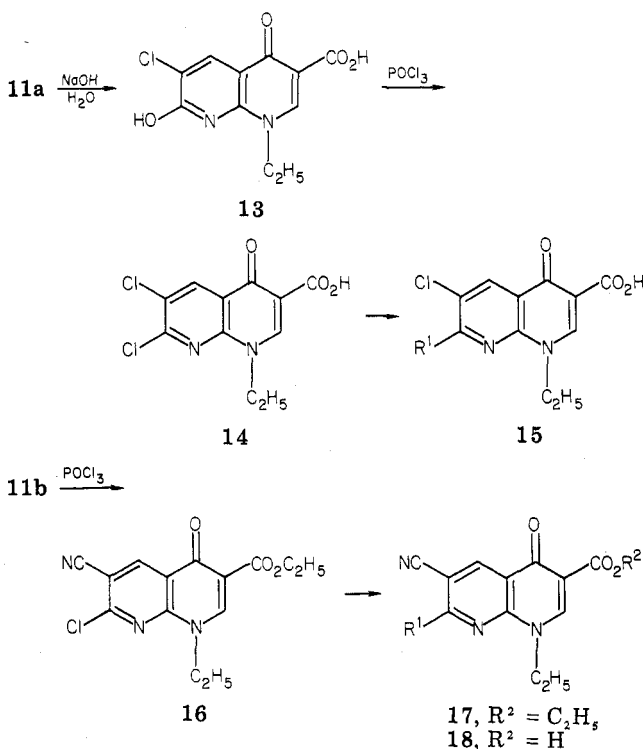
Scheme I<sup>a</sup>

<sup>a</sup> For 7-9: a, R<sup>1</sup> = CH<sub>3</sub>O; b, R<sup>1</sup> = CH<sub>3</sub>CON(CH<sub>2</sub>)<sub>4</sub>N-;  
c, R<sup>1</sup> = (CH<sub>2</sub>)<sub>4</sub>N- (except for 7).

1-Pyrrolidinyl, 1-piperazinyl, and *N*-methyl-1-piperazinyl groups were selected to be introduced at C-7 of 5 at the outset of this study on the basis of our successful development of piromidic and pipemidic acids. As a result of the present study, we found a promising candidate, 1-ethyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-1,8-naphthyridine-3-carboxylic acid (24b), as a potent new antibacterial agent. Recent papers dealing with the quinoline analogues norfloxacin (6b)<sup>11</sup> and perfloracin (6c)<sup>12</sup> prompted us to report our results on a synthesis of the 1,8-naphthyridine derivatives 5, as well as 24b, and the structure-activity relationships (SAR's) associated with variations of the C-1, C-6, and C-7 substituents of 5.

Although a number of 1,8-naphthyridine derivatives 3 without the C-6 substituent have been reported, little appears to be known about derivatives of 5 with substituents at C-6,<sup>6,13</sup> probably due to synthetic difficulties. An attractive intermediate for the synthesis of 5 would be a 6-diazonium salt of an appropriately functionalized naphthyridine, such as 10, which might be converted into the 6-cyano, 6-chloro, and 6-fluoro analogues 11 under conditions of the Sandmeyer reaction or the like as depicted in Scheme I.

**Chemistry.** The requisite diazonium salt 10 was readily prepared by diazotization of the corresponding 6-amino compound 9 via the 6-nitro analogue 8. Thus, ethyl 1-ethyl-1,4-dihydro-7-methoxy-4-oxo-1,8-naphthyridine-3-carboxylate (7a) was nitrated with a mixed acid (fuming nitric acid and concentrated sulfuric acid) to give 8a in 81% yield. Reduction of 8a with iron powder in acetic acid gave the 6-amino derivative 9a, which was then diazotized with sodium nitrite in concentrated hydrochloric acid. The resulting diazonium chloride 10a, without isolation, was successively treated with cuprous chloride, giving the 6-chloro compound 11a in 64% yield. The 6-cyano congener 11b could analogously be prepared

Scheme II<sup>a</sup>

<sup>a</sup> For 15, 17, and 18: a, R<sup>1</sup> = (CH<sub>2</sub>)<sub>4</sub>N-; b, R<sup>1</sup> = N(CH<sub>2</sub>)<sub>4</sub>-;  
c, R<sup>1</sup> = CH<sub>3</sub>-N(CH<sub>2</sub>)<sub>4</sub>-.

in good yield by diazotization of 9a with sodium nitrite in a dilute sulfuric acid, followed by successive treatment with a mixture of cuprous cyanide and potassium cyanide. The esters 11a,b were hydrolyzed to the acids 12a,b, respectively.

Compounds 11a,b served as the starting compounds for the preparation of the 6-chloro and 6-cyano derivatives 15 and 18, respectively, with a cyclic amino group at C-7 (Scheme II). Alkaline hydrolysis of 11a, followed by chlorination of 13 with phosphoryl chloride, afforded the 6,7-dichloro compound 14. Regiospecific displacement of the 7-chloro group in 14 with the selected amine was effected by refluxing in acetonitrile to give good to excellent yields of the desired compounds 15a-c; in this case, the possibility of an isomeric structure due to displacement of the 6-chloro group by the amine was excluded because the product 15a was identical with a compound that was derived through an unambiguous route involving the reaction of 12a with pyrrolidine. Treatment of the 6-cyano-7-methoxy compound 11b with phosphoryl chloride gave directly the 6-cyano-7-chloro derivative 16, which was then treated with the amines in acetonitrile to afford the 6-cyano-7-(cyclic amino) esters 17a-c. These esters were converted into the corresponding carboxylic acids 18a-c by acidic hydrolysis.

In order to introduce a fluoro group at C-6 of the 1,8-naphthyridine ring, the Balz-Schiemann reaction<sup>14</sup> was applied initially to the 6-amino-7-methoxy derivative 9a. Diazotization of 9a with sodium nitrite in 42% tetrafluoroboric acid gave an 85% yield of the diazonium tetrafluoroborate 10c, which showed a characteristic IR absorption at 2200 cm<sup>-1</sup> (ν, N≡N). However, attempts to convert the diazonium salt 10c into the fluoro compound 11c under a variety of conditions were unsuccessful.

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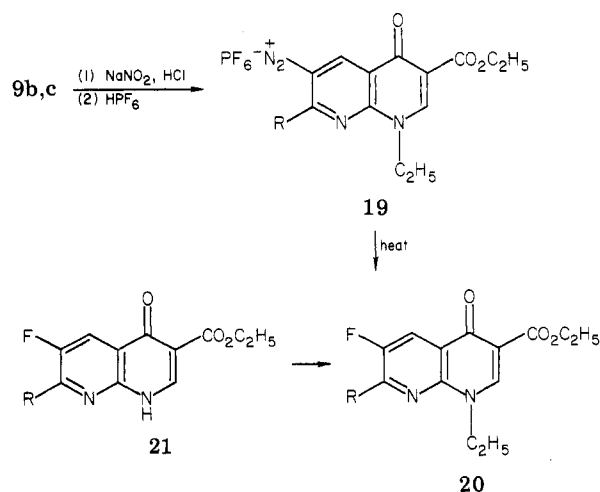
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Table I. 1-Ethyl-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylic Acid Derivatives

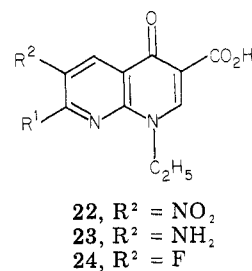
compd	mp, °C	recrystn solvent	yield, <sup>a</sup> %	synth method <sup>b</sup>	formula <sup>c</sup>
8a	224-227	EtOH	80.8	A	C <sub>14</sub> H <sub>15</sub> N <sub>3</sub> O <sub>6</sub>
8b	211-212	EtOH	89.5	A	C <sub>19</sub> H <sub>23</sub> N <sub>5</sub> O <sub>6</sub>
8c	205-207 <sup>d</sup>	EtOH	91.6	B	C <sub>17</sub> H <sub>20</sub> N <sub>4</sub> O <sub>5</sub>
9a	250-252	EtOH	79.9	C	C <sub>14</sub> H <sub>17</sub> N <sub>3</sub> O <sub>4</sub>
9b	265-270	EtOH	78.2	C	C <sub>19</sub> H <sub>25</sub> N <sub>5</sub> O <sub>4</sub>
9c	270-272 <sup>e</sup>	EtOH	37.5	C	C <sub>17</sub> H <sub>22</sub> N <sub>4</sub> O <sub>3</sub>
11a	156-157	EtOH-H <sub>2</sub> O	63.7	D	C <sub>14</sub> H <sub>15</sub> ClN <sub>2</sub> O <sub>4</sub>
11b	221-223	EtOH	66.0	E	C <sub>13</sub> H <sub>15</sub> N <sub>3</sub> O <sub>4</sub>
12a	271-275	DMF	72.3	F	C <sub>12</sub> H <sub>11</sub> ClN <sub>2</sub> O <sub>4</sub>
12b	281-284	DMF	93.0	F	C <sub>13</sub> H <sub>11</sub> N <sub>3</sub> O <sub>4</sub>
13	>300	DMF	83.3	G	C <sub>11</sub> H <sub>9</sub> ClN <sub>2</sub> O <sub>4</sub>
14	262-267 <sup>f</sup>	DMF	65.7	H	C <sub>11</sub> H <sub>8</sub> Cl <sub>2</sub> N <sub>2</sub> O <sub>3</sub>
15a	297-300 <sup>g</sup>	DMF	85.7	I	C <sub>15</sub> H <sub>16</sub> ClN <sub>3</sub> O <sub>3</sub>
			75.6	J	
15b <sup>h</sup>	246-248	AcOH/NH <sub>4</sub> OH <sup>i</sup>	82.1	I	C <sub>15</sub> H <sub>17</sub> ClN <sub>4</sub> O <sub>3</sub>
15c	232-233	DMF	77.6	I	C <sub>16</sub> H <sub>19</sub> ClN <sub>4</sub> O <sub>3</sub>
16	252-256 <sup>j</sup>	DMF	77.1	H	C <sub>14</sub> H <sub>12</sub> ClN <sub>3</sub> O <sub>3</sub>
17a	259-260	EtOH	75.3	K	C <sub>18</sub> H <sub>20</sub> N <sub>4</sub> O <sub>3</sub>
17b	151-154	DMF	51.7	K	C <sub>18</sub> H <sub>21</sub> N <sub>5</sub> O <sub>3</sub> ·1.2H <sub>2</sub> O
17c	195-196	EtOH	74.2	K	C <sub>19</sub> H <sub>23</sub> N <sub>5</sub> O <sub>3</sub>
18a	296-298	DMF	91.1	F	C <sub>16</sub> H <sub>16</sub> N <sub>4</sub> O <sub>3</sub>
18b <sup>k</sup>	242-245 dec	HCl/AcONa <sup>i</sup>	79.4	F	C <sub>16</sub> H <sub>17</sub> N <sub>5</sub> O <sub>3</sub>
18c	233-235	DMF	73.6	F	C <sub>17</sub> H <sub>19</sub> N <sub>5</sub> O <sub>3</sub>
20a	220-222	EtOH-CH <sub>2</sub> Cl <sub>2</sub>	8.2	L	C <sub>17</sub> H <sub>20</sub> FN <sub>3</sub> O <sub>3</sub>
			84.7	M	
20b	195-197	AcOEt	36.2	L	C <sub>19</sub> H <sub>23</sub> FN <sub>4</sub> O <sub>4</sub>
			89.7	M	
22a	256-258	DMF	95.7	F	C <sub>15</sub> H <sub>16</sub> N <sub>4</sub> O <sub>5</sub>
22b	234-236	DMF	80.9	N	C <sub>15</sub> H <sub>17</sub> N <sub>5</sub> O <sub>5</sub> ·2H <sub>2</sub> O
22c	219-221	EtOH-CHCl <sub>3</sub>	54.9	R	C <sub>16</sub> H <sub>19</sub> N <sub>5</sub> O <sub>5</sub> ·0.3H <sub>2</sub> O
23a	290-296 dec	NaOH/AcOH <sup>i</sup>	85.1	O	C <sub>15</sub> H <sub>18</sub> N <sub>4</sub> O <sub>3</sub>
23b	253-255	NaOH/AcOH <sup>i</sup>	29.3	N	C <sub>15</sub> H <sub>19</sub> N <sub>5</sub> O <sub>3</sub> ·2H <sub>2</sub> O
23c	253-256	EtOH	26.1	P	C <sub>16</sub> H <sub>21</sub> N <sub>5</sub> O <sub>3</sub>
24a	299-300	AcOEt-CH <sub>2</sub> Cl <sub>2</sub>	73.5	F	C <sub>15</sub> H <sub>16</sub> FN <sub>3</sub> O <sub>3</sub>
24b <sup>l</sup>	220-224	EtOH-CH <sub>2</sub> Cl <sub>2</sub>	75.3	Q	C <sub>15</sub> H <sub>17</sub> FN <sub>4</sub> O <sub>3</sub>
24c	227-230	EtOH-CH <sub>2</sub> Cl <sub>2</sub>	75.0	R	C <sub>16</sub> H <sub>19</sub> FN <sub>4</sub> O <sub>3</sub>
			89.3	T	

<sup>a</sup> Yields are of purified products and are not maximal. <sup>b</sup> Capital letters refer to the method described in the Experimental Section. <sup>c</sup> All compounds were analyzed for C, H, N, and, where present, Cl and F; analytical results were within  $\pm 0.3\%$  of the theoretical values. <sup>d</sup> Literature<sup>13a</sup> mp 200-201 °C. <sup>e</sup> Literature<sup>13a</sup> mp 259 °C. <sup>f</sup> Literature<sup>13a</sup> mp 270-273 °C. <sup>g</sup> Literature<sup>13a</sup> mp >300 °C. <sup>h</sup> HCl salt: mp 290-300 °C. Anal. (C<sub>15</sub>H<sub>17</sub>ClN<sub>4</sub>O<sub>3</sub>·HCl) C, H, Cl, N. <sup>i</sup> Purified by reprecipitation on treatment with the acid and subsequently with the base or vice versa. <sup>j</sup> Literature<sup>13b</sup> mp 243 °C. <sup>k</sup> HCl salt: mp 265-273 °C. Anal. (C<sub>16</sub>H<sub>17</sub>N<sub>5</sub>O<sub>3</sub>·HCl) C, H, Cl, N. <sup>l</sup> For salts, see the Experimental Section.

Scheme III<sup>a</sup>

<sup>a</sup> For 19-21: a, R = , b, R =

Compound 9b was then employed for the Balz-Schiemann reaction. Nitration of ethyl 7-(4-acetyl-1-piperazinyl)-1-ethyl-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylate (7b), followed by reduction of the 6-nitro compound 8b, gave the requisite amino analogue 9b.

Chart II<sup>a</sup>

<sup>a</sup> a, R<sup>1</sup> = ; b, R<sup>1</sup> = ; c, R<sup>1</sup> = .

Diazotization of 9b with sodium nitrite in 65% hexafluorophosphoric acid furnished a 94% yield of the 6-diazonium hexafluorophosphate 19b (Scheme III). When 19b was heated at 80-100 °C with *n*-heptane, the fluorination proceeded smoothly to give a 36% yield of the desired 6-fluoro compound 20b, though unsatisfactory in yield. The same conditions permitted the conversion of 7-pyrrolidinyl-6-diazonium hexafluorophosphate 19a into the 6-fluoro-7-pyrrolidinyl analogue 20a but in a very poor yield. Compounds 20a,b were alternatively prepared in good yields by the N-ethylation of 21a,b, respectively. The synthesis of 21, which is important for aspects of the large-scale preparation of 20 and its derivatives, particu-

Table II. 1-Ethyl-6-fluoro-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylic Acids Derivatives

compd	R	mp, °C	recrystn solvent	yield, <sup>a</sup> %	synth method <sup>b</sup>	formula <sup>c</sup>
27a	NH <sub>2</sub>	>300	DMF	83.3	S	C <sub>11</sub> H <sub>10</sub> FN <sub>3</sub> O <sub>3</sub>
27b	NH <sub>2</sub> CH <sub>2</sub> CH <sub>2</sub> NH	273-276	AcOH/NH <sub>4</sub> OH <sup>d</sup>	83.4	T	C <sub>13</sub> H <sub>15</sub> FN <sub>4</sub> O <sub>3</sub>
27c	3-oxo-1-piperazinyl	>300	DMF	71.4	T	C <sub>15</sub> H <sub>15</sub> FN <sub>4</sub> O <sub>4</sub>
27d	CH <sub>2</sub> (CH <sub>2</sub> CH <sub>2</sub> ) <sub>2</sub> N-	211-213	DMF	84.3	T	C <sub>16</sub> H <sub>18</sub> FN <sub>3</sub> O <sub>3</sub>
27e	O(CH <sub>2</sub> CH <sub>2</sub> ) <sub>2</sub> N-	261-263	DMF	79.6	T	C <sub>15</sub> H <sub>16</sub> FN <sub>3</sub> O <sub>4</sub>
27f	S(CH <sub>2</sub> CH <sub>2</sub> ) <sub>2</sub> N-	251-253	MeCN	83.5	T	C <sub>15</sub> H <sub>16</sub> FN <sub>3</sub> O <sub>3</sub> S
27g	homopiperazinyl <sup>e</sup>	238-240	AcOH/NH <sub>4</sub> OH <sup>d</sup>	72.7	T	C <sub>16</sub> H <sub>19</sub> FN <sub>4</sub> O <sub>3</sub>
27h	1-azepinyl	192-193	EtOH	74.0	T	C <sub>17</sub> H <sub>16</sub> FN <sub>3</sub> O <sub>3</sub>
27i	CH <sub>2</sub> (CH <sub>2</sub> CH <sub>2</sub> CH <sub>2</sub> ) <sub>2</sub> N-	188-190	EtOH	78.1	T	C <sub>18</sub> H <sub>18</sub> FN <sub>3</sub> O <sub>3</sub>
27j	Ph-N(CH <sub>2</sub> CH <sub>2</sub> ) <sub>2</sub> N-	237-239	MeCN	50.4	T	C <sub>21</sub> H <sub>21</sub> FN <sub>4</sub> O <sub>3</sub>
27k	PhCH <sub>2</sub> N(CH <sub>2</sub> CH <sub>2</sub> ) <sub>2</sub> N-	220-222	EtOH-CH <sub>2</sub> Cl <sub>2</sub>	31.7	T	C <sub>22</sub> H <sub>23</sub> FN <sub>4</sub> O <sub>3</sub>
27l	Et-N(CH <sub>2</sub> CH <sub>2</sub> ) <sub>2</sub> N-	193-194	EtOH-CH <sub>2</sub> Cl <sub>2</sub>	91.6	T	C <sub>17</sub> H <sub>21</sub> FN <sub>4</sub> O <sub>3</sub>
28a	<i>n</i> -Pr-N(CH <sub>2</sub> CH <sub>2</sub> ) <sub>2</sub> N-	208-209	EtOH-CH <sub>2</sub> Cl <sub>2</sub>	32.4	U	C <sub>18</sub> H <sub>23</sub> FN <sub>4</sub> O <sub>3</sub>
28b	<i>n</i> -Bu-N(CH <sub>2</sub> CH <sub>2</sub> ) <sub>2</sub> N-	184-185	EtOH-CH <sub>2</sub> Cl <sub>2</sub>	61.2	U	C <sub>19</sub> H <sub>25</sub> FN <sub>4</sub> O <sub>3</sub>
28c	<i>s</i> -Bu-N(CH <sub>2</sub> CH <sub>2</sub> ) <sub>2</sub> N-	213-214	EtOH-CH <sub>2</sub> Cl <sub>2</sub>	26.5	U	C <sub>19</sub> H <sub>25</sub> FN <sub>4</sub> O <sub>3</sub>
29	OHC-N(CH <sub>2</sub> CH <sub>2</sub> ) <sub>2</sub> N-	292-294	EtOH-CH <sub>2</sub> Cl <sub>2</sub>	97.5	V	C <sub>16</sub> H <sub>17</sub> FN <sub>4</sub> O <sub>4</sub>
30	CH <sub>3</sub> CON(CH <sub>2</sub> CH <sub>2</sub> ) <sub>2</sub> N-	>300	EtOH-CH <sub>2</sub> Cl <sub>2</sub>	85.6	O	C <sub>17</sub> H <sub>19</sub> FN <sub>4</sub> O <sub>4</sub>

<sup>a-c</sup> See footnotes a-c in Table I. <sup>d</sup> Purified by reprecipitation on treatment with the acid and subsequently with the base. <sup>e</sup> Hexahydro-1*H*-1,4-diazepinyl.

Table III. 1,7-Disubstituted 6-Fluoro-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylic Acid Derivatives

compd	mp, °C	recrystn solvent	yield, <sup>a</sup> %	synth method <sup>b</sup>	formula <sup>c</sup>
31	213-215	MeCN	92.9	M	C <sub>19</sub> H <sub>23</sub> FN <sub>4</sub> O <sub>5</sub>
32	168-170	AcOEt	90.3	W	C <sub>19</sub> H <sub>22</sub> ClFN <sub>4</sub> O <sub>4</sub>
33	184-185	Me <sub>2</sub> CO	79.8	M	C <sub>19</sub> H <sub>22</sub> F <sub>2</sub> N <sub>4</sub> O <sub>4</sub>
34	282-284	AcOEt-CH <sub>2</sub> Cl <sub>2</sub>	19.4	X	C <sub>18</sub> H <sub>19</sub> F <sub>2</sub> N <sub>4</sub> O <sub>4</sub>
35	264-266	MeCN	80.6	Y	C <sub>17</sub> H <sub>17</sub> FN <sub>4</sub> O <sub>4</sub>
36 <sup>d</sup>	256-260 dec	AcOH/NH <sub>4</sub> OH <sup>e</sup>	82.4	Z	C <sub>15</sub> H <sub>15</sub> FN <sub>4</sub> O <sub>3</sub>
37	205-207	MeCN	43.6	R	C <sub>16</sub> H <sub>17</sub> FN <sub>4</sub> O <sub>3</sub>
38	223-225	HCl/NH <sub>4</sub> OH <sup>e</sup>	75.4	Q	C <sub>15</sub> H <sub>16</sub> F <sub>2</sub> N <sub>4</sub> O <sub>3</sub>
39	224-226	MeCN	82.2	R	C <sub>16</sub> H <sub>18</sub> F <sub>2</sub> N <sub>4</sub> O <sub>3</sub>
40	284-286	DMF-EtOH	97.6	Q	C <sub>14</sub> H <sub>13</sub> F <sub>3</sub> N <sub>4</sub> O <sub>3</sub>

<sup>a-c</sup> See footnotes a-c in Table I. <sup>d</sup> HCl salt: mp ~290 °C dec (EtOH-H<sub>2</sub>O). Anal. (C<sub>15</sub>H<sub>15</sub>FN<sub>4</sub>O<sub>3</sub>·HCl) C, H, Cl, F, N. Methanesulfonic acid salt: mp 292-294 °C dec (EtOH-H<sub>2</sub>O). Anal. (C<sub>15</sub>H<sub>15</sub>FN<sub>4</sub>O<sub>3</sub>·CH<sub>3</sub>SO<sub>3</sub>·1/2H<sub>2</sub>O) C, H, F, N, S.

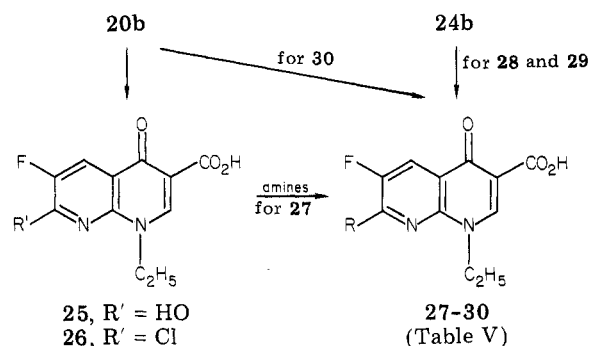
<sup>e</sup> Purified by reprecipitation on treatment with the acid and subsequently with the base.

larly 24b, will be reported elsewhere.<sup>15</sup>

The 6-fluoro esters 20a,b thus obtained were hydrolyzed under acidic conditions into the corresponding 6-fluoro carboxylic acids 24a,b. Reductive N-methylation of 24b upon treating with formaldehyde and formic acid afforded the *N*-methylpiperazinyl compound 24c. Analogously, the 6-nitro carboxylic acids 22a,b were prepared by hydrolysis of 8c,d, respectively, and 22c was derived from 22b by the reductive N-methylation. The 6-amino carboxylic acids 23a-c were obtained by reduction of the corresponding nitro compounds 22a-c.

Since compound 24b showed an excellent in vitro antibacterial activity as described in the following section, the 6-fluoro analogues substituted at C-7 by cyclic (and acyclic) amino groups other than the piperazinyl group of 24b were prepared (Scheme IV). Prolonged heating of 20b with alkali, followed by chlorination of 25 with phosphoryl chloride, afforded the 7-chloro-6-fluoro derivative 26. Upon treatment of 26 with a variety of amines, the displacement reaction proceeded quite regioselectively

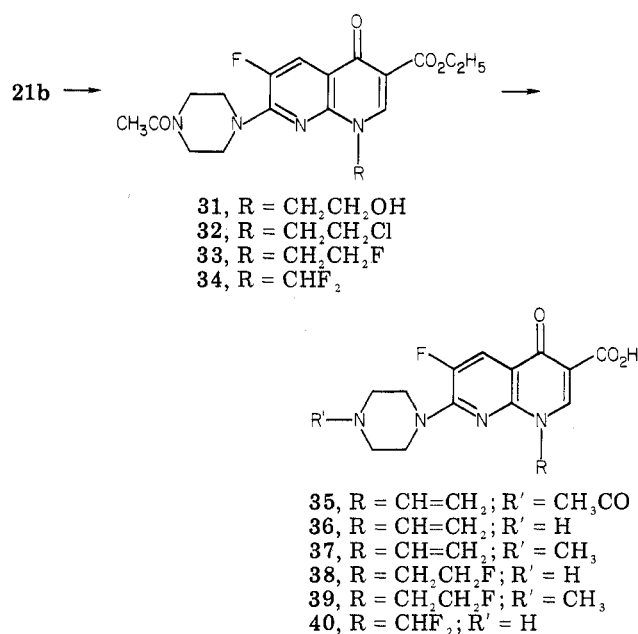
Scheme IV



at C-7 to give the desired 7-substituted 6-fluoro derivatives 27a-l. Reductive alkylation on the piperazinyl N-4 of 24b was carried out by treatment with propionaldehyde, butyraldehyde, and isobutyraldehyde in formic acid to afford the corresponding N<sup>4</sup>-alkyl derivatives 28a-c. *N*-Formyl compound 29 was prepared by treatment of 24b with formamide in formic acid. Partial hydrolysis of 20b by alkali gave the *N*<sup>4</sup>-acetyl carboxylic acid 30. Among these compounds thus prepared, 28a-c, 29, and 30 were identified as metabolites of 24b in animals tested.<sup>16</sup>

(15) Matsumoto, J.; Miyamoto, T.; Minamida, A.; Nishimura, Y.; Egawa, H.; Nishimura, H. accepted for publication in *J. Heterocycl. Chem.* See also ref 2c.

## Scheme V



Finally, the ethyl group at N-1 of **24b** and **24c** was replaced by a vinyl, fluoroethyl, or difluoromethyl group (Scheme V), because these groups seemed attractive as variants that might possibly improve the antibacterial activity of the pyridonecarboxylic acids.<sup>17</sup> Thus, treatment of **21b** with ethylenedichlorohydrin, followed by chlorination of **31** with thionyl chloride, afforded the chloroethyl derivative **32**. On treatment of **32** with potassium hydroxide in ethanol, elimination of the hydrogen chloride proceeded smoothly to give an 81% yield of the vinyl compound **35**, along with a small amount of the hydrolyzed product, 6-fluoro-1,4-dihydro-7-hydroxy-4-oxo-1-vinyl-1,8-naphthyridine-3-carboxylic acid (**41**). The N<sup>4</sup>-protective group in **35** was removed by alkaline hydrolysis to give the desired piperazinyl vinyl derivative **36**, which was subsequently heated with 37% formalin in formic acid to produce the N<sup>4</sup>-methylpiperazinyl vinyl analogue **37**. Furthermore, the fluoroethyl derivative **33** was prepared from **21b** by the reaction with 2-fluoroethyl *p*-toluenesulfonate. Subsequent acid treatment of **33** provided the deprotected acid **38**, which was converted into the N<sup>4</sup>-methylpiperazinyl analogue **39** by reductive methylation. The difluoromethyl compound **40** was prepared, though in a poor yield, by the reaction of **21b** with (difluoromethyl)carbene, formed in situ from sodium chlorodifluoroacetate, followed by deprotection of **34** with hydrochloric acid.

## Biological Results and Discussion

The in vitro antibacterial activity of compounds prepared in the present study against representatives of Gram-positive (*Staphylococcus aureus* 209P JC-1) and Gram-negative bacteria (*Escherichia coli* NIHJ JC-2 and *Pseudomonas aeruginosa* Tsuchijima) is shown in Table IV. Minimal inhibitory concentrations (MIC's) of **3a-c** (R<sup>2</sup> = C<sub>2</sub>H<sub>5</sub>),<sup>1</sup> nalidixic acid, and pipemidic acid are included for comparison.

Table IV. In Vitro Antibacterial Activity<sup>a</sup>

compd	min inhibitory concn, µg/mL		
	<i>S. aureus</i> 209P JC-1	<i>E. coli</i> NIHJ JC-2	<i>P. aeruginosa</i> Tsuchijima
<b>3a</b>	12.5	25	>100
<b>3b</b>	25	6.25	25
<b>3c</b>	25	6.25	25
<b>15a</b>	12.5	>100	>100
<b>15b</b>	3.13	0.78	6.25
<b>15c</b>	6.25	1.56	12.5
<b>18a</b>	3.13	12.5	25
<b>18b</b>	6.25	1.56	6.25
<b>18c</b>	12.5	1.56	12.5
<b>22a</b>	25	100	>100
<b>22b</b>	6.25	6.25	25
<b>22c</b>	12.5	3.13	50
<b>23a</b>	>100	>100	>100
<b>23b</b>	>100	3.13	6.25
<b>23c</b>	25	1.56	12.5
<b>24a</b>	0.39	1.56	3.13
<b>24b</b>	0.78	0.2	0.78
<b>24c</b>	1.56	0.39	1.56
<b>27a</b>	>100	1.56	50
<b>27b</b>	25	6.25	50
<b>27c</b>	6.25	0.78	12.5
<b>27d</b>	0.78	6.25	12.5
<b>27e</b>	1.56	3.13	6.25
<b>27f</b>	1.56	3.13	12.5
<b>27g</b>	1.56	0.78	1.56
<b>27h</b>	3.13	6.25	50
<b>27i</b>	12.5	25	>100
<b>27j</b>	6.25	12.5	>100
<b>27k</b>	0.78	6.25	12.5
<b>27l</b>	0.78	0.78	3.13
<b>28a</b>	1.56	1.56	12.5
<b>28b</b>	3.13	3.13	25
<b>28c</b>	1.56	3.13	25
<b>29</b>	3.13	1.56	12.5
<b>30</b>	3.13	3.13	50
<b>36</b>	1.56	0.1	0.2
<b>37</b>	3.13	0.2	0.78
<b>38</b>	0.39	0.2	0.78
<b>39</b>	0.78	0.2	1.56
<b>40</b>	6.25	0.78	6.25
NA <sup>b</sup>	50	1.56	50
PPA <sup>c</sup>	6.25	1.56	6.25

<sup>a</sup> See the Experimental Section. <sup>b</sup> Nalidixic acid.

<sup>c</sup> Pipemidic acid.

Firstly, we wish to discuss the SAR's associated with modification of the C-6 substituent of compounds **15**, **18**, and **22-24** compared with **3a-c** (R<sup>2</sup> = C<sub>2</sub>H<sub>5</sub>). Introduction of an amino, nitro, cyano, chloro, or fluoro group at C-6 of **3a-c** influenced markedly the antibacterial activity. Thus, in a series of the pyrrolidinyl compounds (**15a**, **18a**, **22a**, **23a**, and **24a**), the fluoro and cyano groups cause an increase in activity against all the bacteria tested, whereas others result in a complete loss of activity, particularly against the Gram-negative bacteria; it is noticed that a remarkable difference between the fluoro and the chloro groups in an effect upon the activity is observed in the series **a**. With respect to the piperazinyl and N-methylpiperazinyl derivatives (i.e., series **b** and **c** of compounds **15**, **18**, and **22-24**), introduction of the C-6 substituent tends to enhance the activity against both Gram-positive and Gram-negative organisms, with a few exceptions. In both series of compounds, the activity against *S. aureus* increases in the order NH<sub>2</sub> ≤ H < NO<sub>2</sub> = CN < Cl < F, whereas the Gram-negative activity follows the sequence NO<sub>2</sub> ≤ H < NH<sub>2</sub> = CN = Cl < F, with the amino, cyano, and chloro compounds being equally active. In the **b** series of compounds, the activity against *Escherichia coli* increases in the order H = NO<sub>2</sub> < NH<sub>2</sub> < CN < Cl < F. It is of particular interest that replacement of hydrogen by

(16) Nakamura, R.; Yamaguchi, T.; Sekine, Y.; Hashimoto, M. *J. Chromatogr.* 1983, 278, 321.

(17) For example, see ref 4. Albrecht, R. *Eur. J. Med. Chem.* 1977, 12, 231. Agui, H.; Saji, I.; Nakagome, T. Japanese Patent Kokai 139 094, 1977; *Chem. Abstr.* 1978, 88, 105406g. Lee, K. T. German Offen. 2706 781, 1977; *Chem. Abstr.* 1977, 87, 201507r.

Table V. Efficacy on Systemic Infections and Acute Toxicity with Oral Administration in Mice<sup>a</sup>

compd	<i>S. aureus</i> 50774: ED <sub>50</sub> <sup>b</sup> (MIC) <sup>c</sup>	<i>E. coli</i> P-5101: ED <sub>50</sub> (MIC)	<i>P. aeruginosa</i> 12: ED <sub>50</sub> (MIC)	LD <sub>50</sub> , mg/kg po
24b	10 (0.78)	1.8 (0.1)	9.0 (0.78)	>2000
24c	4.8 (1.56)	1.2 (3.13)	10.6 (0.2)	210
36	33.4 (3.13)	1.3 (0.1)	2.4 (0.39)	>2000
37	10.5 (3.13)	1.1 (0.1)	3.7 (1.56)	354
38	11.5 (1.56)	3.0 (0.1)	27.2 (0.78)	>2000
39	1.4 (0.39)	0.52 (0.1)	4.2 (1.56)	1866
NA <sup>d</sup>	>800 (50)	29.2 (3.13)	267 (100)	1800
PPA <sup>e</sup>	215 (25)	12.8 (3.13)	70.8 (25)	>2000

<sup>a</sup> See the Experimental Section. <sup>b</sup> In milligrams per kilogram. <sup>c</sup> In micrograms per milliliter. <sup>d</sup> Nalidixic acid.  
<sup>e</sup> Pipemidic acid.

halogen, especially fluorine, at C-6 in the 1,8-naphthyridine system results in an outstanding enhancement of the activity against both Gram-positive and Gram-negative organisms. A comparison of the activity of the piperazinyl (15b, 18b, 22b, 23b, and 24b) and the *N*-methyl-1-piperazinyl analogues (15c, 18c, 22c, 23c, and 24c) with the same C-6 substituent generally found the former to be more active than the latter, except for the activity of two sets (22b vs. 22c and 24b vs. 24c) against *E. coli*. Compound 24a, bearing the pyrrolidine ring at C-7, is the most potent as far as activity against *S. aureus*, whereas 24b and 24c, bearing the piperazine ring instead, have better activity against Gram-negative organisms. Overall, the most active is compound 24b, which is about 30 times as active as 3b ( $R^2 = C_2H_5$ ) against every organism tested.

SAR's of the C-7 substituent in a series of analogues with an ethyl group at N-1 (see 24 and 27–30) were substantially comparable to those for the corresponding 5,8-dihydro-5-oxopyrido[2,3-*d*]pyrimidine-6-carboxylic acids (1,  $R^2 = C_2H_5$ ), which had previously been studied by us.<sup>3,4</sup> Thus, modification of the cyclic amino moiety at C-7 resulted in a significant decrease in activity as observed in 24a and 27c–i compared with 24b. Only compound 24a with the 1-pyrrolidinyl group, however, is more active than 24b against the Gram-positive *Staphylococcus* strain. A comparison of the activity between 24b and 27d, as well as 27g and 27h, obviously indicates that the presence of a basic NH group in the cyclic amino function is a prerequisite to optimal activity. However, if this NH group is an amide, such as in the 3-oxo-1-piperazinyl group of 27c, activity is decreased. Introduction of an alkyl, aralkyl, or aryl group at the piperazinyl N-4 of 24b (i.e., 24c, 27j–l, and 28a–c) causes a decrease in activity; in particular, the activity against Gram-negative bacteria is reduced markedly with an increase in the chain length and, hence, with an increase in the lipophilicity of the group introduced. Compounds 27a–c, 29, and 30, identified as metabolites of 24b,<sup>16</sup> are substantially less active. Of these derivatives possessing the ethyl group at N-1, no compounds superior to 24b in activity were found.

Variation of the N-1 substituent on the naphthyridine ring significantly influenced the in vitro activity. The excellent activity of the vinyl and fluoroethyl analogues (see 36–39) should be noted. The difluoromethyl group in 40, however, led to a considerable decrease in activity. In each comparison between 24b and 36, as well as 24c and 37, it is revealed that replacement of the ethyl group by a vinyl group causes an increase in the Gram-negative activity, whereas it reduces appreciably the Gram-positive activity. The fluoroethyl function, in contrast to the vinyl group, enhances the Gram-positive activity, while the Gram-negative activity is essentially unchanged (compare 24b and 24c with 38 and 39, respectively).

Selected compounds (24b,c and 36–39) were then tested on systemic infections due to *S. aureus* 50774, *E. coli*

P-5101, and *P. aeruginosa* 12, with oral administration in mice, and compared with nalidixic acid and pipemidic acid. The results are listed in Table V, which includes for reference the MIC's against the organisms employed. The median effective dose (ED<sub>50</sub>) values of these compounds are always much lower than those of the reference drugs.

With the exception of 38, the in vivo efficacy on the experimental infection due to the Gram-negative bacteria was highly increased by changing the N-1 substituent from ethyl to vinyl or fluoroethyl (see 36, 37, and 39). When the ethyl group of 24b was changed to fluoroethyl (38), however, the efficacy on the pseudomonal infection was reduced to one-third the potency of the parent compound. On the other hand, this variation at N-1 resulted in a decreased efficacy on the infection due to *S. aureus*, a Gram-positive bacterium; for example, the most effective compound, 36, on the pseudomonal infection is the least effective on the staphylococcal infection. Of much interest is compound 39, which is about 7, 3.5, and 2 times as potent as 24b on systemic infections caused by *S. aureus*, *E. coli*, and *P. aeruginosa*, respectively. However, an acute toxicity test with a single oral administration in mice revealed the most effective compound, 39, to possess a smaller value of the median lethal dose (LD<sub>50</sub>) than that of 24b, but it is comparable to the LD<sub>50</sub> of nalidixic acid, as shown in Table V. It is noteworthy that compounds 24c and 37, as well as 39, which have the *N*<sup>4</sup>-methyl group on the piperazine ring in common, exhibit more potent toxicity than the corresponding *N*<sup>4</sup>-unsubstituted compounds 24b, 36, and 38, of which every LD<sub>50</sub> is >2000 mg/kg orally in mice.

As a result, compound 24b (named enoxacin, originally AT-2266) was found to possess broad and potent in vitro and in vivo antibacterial activity and weak oral acute toxicity. These findings indicate the possible use of 24b as a potent, orally administrable antibacterial agent. Enoxacin (24b) was thus selected for further biological evaluation<sup>18</sup> and is now undergoing clinical trials.

## Experimental Section

**Chemistry.** All melting points were determined on a Yanagimoto micromelting point apparatus and are uncorrected. IR spectra were recorded on a Hitachi Model 215 spectrophotometer. <sup>1</sup>H NMR spectra were taken at 60 or 100 MHz on either a Varian EM-360A or HA-100D spectrometer with Me<sub>4</sub>Si as an internal standard. Mass spectra were recorded on a Hitachi RMU-6L single-focusing mass spectrometer using the direct inlet system at 70-eV ionization potential. Elemental analysis are indicated only by symbols of the elements; analytical results were within ±0.3% of theoretical values. IR, <sup>1</sup>H NMR, and/or mass spectra

- (18) Nakamura, S.; Minami, A.; Katae, H.; Inoue, S.; Yamagishi, J.; Takase, Y.; Shimizu, M. *Antimicrob. Agents Chemother.* 1983, 23, 641. Nakamura, S.; Nakata, K.; Katae, H.; Minami, A.; Kashimoto, S.; Yamagishi, J.; Takase, Y.; Shimizu, M. *Ibid.* 1983, 23, 742.

were obtained on all compounds and were consistent with assigned structures. Organic solutions were dried over anhydrous  $\text{Na}_2\text{SO}_4$  or  $\text{MgSO}_4$ .

**Ethyl 1-Ethyl-1,4-dihydro-7-methoxy-4-oxo-1,8-naphthyridine-3-carboxylate (7a).** In a similar procedure to that described in our previous paper,<sup>1</sup> 7a was prepared from 2-amino-6-methoxypyridine by the Gould-Jacobs-type reaction of its malonate, followed by the N-ethylation of ethyl 1,4-dihydro-7-methoxy-4-oxo-1,8-naphthyridine-3-carboxylate; the details will be reported elsewhere:<sup>19</sup> mp 142.5–143 °C; IR (KBr) 1680, 1640  $\text{cm}^{-1}$ ; EIMS,  $m/z$  276 ( $\text{M}^+$ ), 240 ( $\text{M}^+ - \text{C}_2\text{H}_4\text{CO}_2$ ). Anal. ( $\text{C}_{14}\text{H}_{16}\text{N}_2\text{O}_4$ ) C, H, N.

**Ethyl 7-(4-Acetyl-1-piperazinyl)-1-ethyl-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylate (7b).** A mixture containing 5.0 g (17.8 mmol) of ethyl 7-chloro-1-ethyl-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylate,<sup>1</sup> 7.0 g (54.7 mmol) of *N*-acetylpiperazine, and 100 mL of EtOH was heated to reflux for 3 h. The reaction mixture was concentrated to dryness in vacuo. The residue was taken up in 80 mL of water, acidified with 10% HCl, and extracted with  $\text{CHCl}_3$ . The extract was washed with water and dried, and the solvent was evaporated in vacuo. The residue was crystallized from acetonitrile to give 6.5 g (98.1%) of 7b: mp 195–197 °C; IR (KBr) 1720, 1680, 1610  $\text{cm}^{-1}$ . Anal. ( $\text{C}_{19}\text{H}_{24}\text{N}_4\text{O}_4\text{H}_2\text{O}$ ) C, H, N.

**Method A. Ethyl 1-Ethyl-1,4-dihydro-7-methoxy-6-nitro-4-oxo-1,8-naphthyridine-3-carboxylate (8a).** To a stirred mixture of concentrated  $\text{HNO}_3$  (150 mL) and concentrated  $\text{H}_2\text{SO}_4$  (200 mL) was added portionwise 50 g (0.181 mol) of 7a at room temperature. After complete addition, the reaction mixture was heated at 60–70 °C for 1.5 h, poured onto 1.5 L of ice-water, and extracted with  $\text{CHCl}_3$ . The extract was washed with 7%  $\text{NaHCO}_3$ , dried, and concentrated in vacuo to give 47.0 g (80.8%) of 8a: IR (KBr) 1720, 1610, 1540, 1360  $\text{cm}^{-1}$ ; EIMS,  $m/z$  321 ( $\text{M}^+$ ), 249 ( $\text{M}^+ - \text{C}_2\text{H}_4\text{CO}_2$ ).

**Method B. Ethyl 1-Ethyl-1,4-dihydro-6-nitro-4-oxo-7-(1-pyrrolidinyl)-1,8-naphthyridine-3-carboxylate (8c).** To a stirred solution of 8a (1.0 g, 3.11 mmol) in 30 mL of acetonitrile was added 3 mL of pyrrolidine. The mixture was heated to reflux for 3 h. The solvent was evaporated in vacuo. The residue was crystallized from AcOEt to give 1.03 g (91.6%) of 8c: IR (KBr) 1720, 1680, 1540, 1350  $\text{cm}^{-1}$ .

**Method C. Ethyl 6-Amino-1-ethyl-1,4-dihydro-7-methoxy-4-oxo-1,8-naphthyridine-3-carboxylate (9a).** To a stirred solution of 8a (20 g, 62.3 mmol) in 400 mL of AcOH was added portionwise 40 g of reduced-iron powder at such a rate that the temperature did not rise over 80 °C. After complete addition, the reaction mixture was heated at 80 °C for 1 h and cooled to room temperature. After addition of EtOH (500 mL), the mixture was filtered to remove insoluble materials, and the filtrate was concentrated to dryness in vacuo. The residue was taken up in 800 mL of water and allowed to stand under ice cooling to give 14.5 g (79.7%) of 9a: IR (KBr) 3420, 3280, 2950, 1680, 1620  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{Me}_2\text{SO}-d_6$ )  $\delta$  8.63 (1 H, s, H-2), 7.62 (1 H, s, H-5), 5.47 (2 H, s,  $\text{NH}_2$ ), 4.45 and 4.23 (each 2 H, q,  $J = 7$  Hz,  $\text{NCH}_2\text{CH}_3$ ,  $\text{OCH}_2\text{CH}_3$ ), 4.07 (3 H, s,  $\text{OCH}_3$ ), 1.42 and 1.30 (each 3 H, t,  $J = 7$  Hz,  $\text{NCH}_2\text{CH}_3$ ,  $\text{OCH}_2\text{CH}_3$ ).

**3-(Ethoxycarbonyl)-1-ethyl-1,4-dihydro-7-methoxy-4-oxo-1,8-naphthyridine-6-diazonium Tetrafluoroborate (10c).** To a stirred solution of 9a (5.0 g, 17.2 mmol) in 65 mL of 42%  $\text{HBF}_4$  was added dropwise a solution of  $\text{NaNO}_2$  (1.2 g, 17.4 mmol) in 2 mL of water while the temperature was maintained at 5–10 °C. After 30 min, the precipitate was collected by filtration, washed with a mixture of  $\text{MeOH-Et}_2\text{O}$  (1:1 v/v), and crystallized from water to give 5.7 g (85.0%) of 10c: mp 148–149 °C dec; IR (KBr) 2200, 1720, 1620  $\text{cm}^{-1}$ . Anal. ( $\text{C}_{14}\text{H}_{15}\text{BF}_4\text{N}_4\text{O}_4$ ) C, H, F, N.

**Thermal Decomposition of 10c.** A mixture containing 2.0 g (5.13 mmol) of 10c and 2.0 g of anhydrous  $\text{Na}_2\text{SO}_4$  was heated at 73–85 °C for 3 h. The reaction mixture was taken up in 80 mL of EtOH- $\text{CHCl}_3$  (1:1, v/v) and filtered to remove insoluble materials. The filtrate was concentrated to dryness in vacuo. The residue was chromatographed on silica gel with  $\text{CHCl}_3$ . The solid resulting from the main fraction was recrystallized from *n*-hex-

ane-acetone to give 0.58 g (41.0%) of 7a; this was identical in all respects with an authentic specimen of 7a.

**Method D. Ethyl 6-Chloro-1-ethyl-1,4-dihydro-7-methoxy-4-oxo-1,8-naphthyridine-3-carboxylate (11a).** To a stirred solution of 9a (4.0 g, 13.7 mmol) in 50 mL of concentrated HCl was added dropwise a solution of  $\text{NaNO}_2$  (1.5 g, 21.7 mmol) in 8 mL of water over a period of 10 min at 0–3 °C. After an additional 10 min, the reaction mixture was added to a solution of cuprous chloride (2.7 g, 13.6 mmol) in 15 mL of concentrated HCl and allowed to stir at room temperature for 1 h, after which time the temperature was maintained at 65 °C for 30 min. The mixture was neutralized with 10% NaOH and extracted with  $\text{CHCl}_3$ . The extract was concentrated to dryness in vacuo; the residue was crystallized from aqueous EtOH to give 2.71 g (63.7%) of 11a: IR (KBr) 1720, 1690, 1620  $\text{cm}^{-1}$ ; EIMS,  $m/z$  310 ( $\text{M}^+$ ), 238 ( $\text{M}^+ - \text{C}_2\text{H}_4\text{CO}_2$ );  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  8.70 (1 H, s, H-5), 8.60 (1 H, s, H-2), 4.45 (4 H, q,  $J = 7$  Hz,  $\text{NCH}_2\text{CH}_3$ ,  $\text{CO}_2\text{CH}_2\text{CH}_3$ ), 4.20 (3 H, s,  $\text{OCH}_3$ ), 1.55 (3 H, t,  $J = 7$  Hz,  $\text{CO}_2\text{CH}_2\text{CH}_3$ ), 1.44 (3 H, t,  $J = 7$  Hz,  $\text{NCH}_2\text{CH}_3$ ).

**Method E. Ethyl 6-Cyano-1-ethyl-1,4-dihydro-7-methoxy-4-oxo-1,8-naphthyridine-3-carboxylate (11b).** To a suspension of 9a (10 g, 34.3 mmol) in 200 mL of water was added 5.0 g of concentrated  $\text{H}_2\text{SO}_4$ . To the reaction mixture kept at 0 °C was gradually added a solution of  $\text{NaNO}_2$  (2.6 g, 37.7 mmol) in 3 mL of water while the temperature was kept at 3–6 °C. The resulting mixture was added at room temperature to a solution of cuprous chloride (6.8 g, 38.0 mmol) and KCN (8.6 g, 132 mmol) in 160 mL of water. The reaction mixture was maintained at 80 °C for 1 h, basified with aqueous ammonia, and extracted with  $\text{CHCl}_3$ . The extract was washed with water, dried, and concentrated to dryness in vacuo. The residue was chromatographed on silical gel with  $\text{CHCl}_3$  to give 6.8 g (65.8%) of 11b: IR (KBr) 2200, 1720, 1630  $\text{cm}^{-1}$ ; EIMS,  $m/z$  301 ( $\text{M}^+$ ), 229 ( $\text{M}^+ - \text{C}_2\text{H}_4\text{CO}_2$ );  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  8.94 (1 H, s, H-5), 8.58 (1 H, s, H-2), 4.42 (4 H, q,  $J = 7$  Hz,  $\text{CO}_2\text{CH}_2\text{CH}_3$ ,  $\text{NCH}_2\text{CH}_3$ ), 1.55 (3 H, t,  $J = 7$  Hz,  $\text{CO}_2\text{CH}_2\text{CH}_3$ ), 1.45 (3 H, t,  $J = 7$  Hz,  $\text{NCH}_2\text{CH}_3$ ).

**Method F. 6-Chloro-1-ethyl-1,4-dihydro-7-methoxy-4-oxo-1,8-naphthyridine-3-carboxylic Acid (12a).** A mixture containing 3.0 g (9.65 mmol) of 11a, 80 mL of 1 N HCl, and 20 mL of EtOH was heated at 90–95 °C for 1 h. The resulting clear solution was allowed to cool. The precipitate was filtered off, washed with EtOH, and recrystallized to give 2.3 g (72.3%) of 12a: IR (KBr) 3000, 1700, 1600  $\text{cm}^{-1}$ ; EIMS,  $m/z$  282 ( $\text{M}^+$ ), 238 ( $\text{M}^+ - \text{CO}_2$ ).

**Method G. 6-Chloro-1-ethyl-7-hydroxy-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylic Acid (13).** A stirred suspension of 11a (5.0 g, 16.1 mmol) in 50 mL of 1 N NaOH was heated at 90–95 °C for 45 min. Neutralization of the mixture with 30% AcOH afforded 4.1 g (94.7%) of 13: IR (KBr) 1705, 1650, 1620  $\text{cm}^{-1}$ .

**Method H. 6,7-Dichloro-1-ethyl-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylic Acid (14).** A mixture containing 2.7 g (10 mmol) of 13 and 15 mL of  $\text{POCl}_3$  was heated to reflux for 30 min. The excess of  $\text{POCl}_3$  was evaporated in vacuo, and the residue was poured onto ice-water to give precipitates, which were filtered off, washed with water, and recrystallized to give 1.9 g (65.7%) of 14: IR (KBr) 1720  $\text{cm}^{-1}$ ; EIMS,  $m/z$  287 ( $\text{M}^+$ ), 243 ( $\text{M}^+ - \text{CO}_2$ ).

**6-Chloro-1-ethyl-1,4-dihydro-4-oxo-7-(1-pyrrolidinyl)-1,8-naphthyridine-3-carboxylic Acid (15a).** (A) **Method I.** A mixture containing 0.50 g (1.74 mmol) of 14, 0.75 g (10.5 mmol) of pyrrolidine, and 20 mL of acetonitrile was heated to reflux for 45 min. The solvent was evaporated in vacuo, and the residue was taken up in 10 mL of water, neutralized with 30% AcOH, and extracted with  $\text{CHCl}_3$ . The extract was concentrated to dryness to give 0.48 g (85.7%) of 15a: IR (KBr) 1700, 1610  $\text{cm}^{-1}$ ; EIMS,  $m/z$  321 ( $\text{M}^+$ ), 277 ( $\text{M}^+ - \text{CO}_2$ ), 249 ( $\text{M}^+ - \text{C}_2\text{H}_4\text{CO}_2$ ).

According to this procedure, compounds 15b,c were prepared from 14 with anhyrous piperazine and *N*-methylpiperazine, respectively.

(B) **Method J.** A mixture containing 0.50 g (1.77 mmol) of 12a, 1.0 g (14.1 mmol) of pyrrolidine, and 30 mL of DMF was allowed to stir at 50–60 °C for 2 h. After the same workup as in method I, 15a (0.43 g, 75.6%) was obtained.

**Method K. Ethyl 6-Cyano-1-ethyl-1,4-dihydro-4-oxo-7-(1-piperazinyl)-1,8-naphthyridine-3-carboxylate (17b).** To a

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refluxed solution of anhydrous piperazine (2.0 g, 23.2 mmol) in 30 mL of acetonitrile was added 1.0 g (3.27 mmol) of 16 over a period of about 15 min with stirring. The reaction mixture was heated to reflux for an additional 1 h and allowed to cool. The resulting precipitate was filtered off and recrystallized to give 0.60 g (51.7%) of 17b: IR (KBr) 2200, 1710, 1620  $\text{cm}^{-1}$ ; EIMS,  $m/z$  355 ( $\text{M}^+$ ), 283 ( $\text{M}^+ - \text{C}_2\text{H}_4\text{CO}_2$ ).

In a similar manner, compounds 17a,c were prepared from 16 with pyrrolidine and *N*-methylpiperazine, respectively.

**Ethyl 7-(4-Acetyl-1-piperazinyl)-1-ethyl-6-fluoro-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylate (20b).** (A) **Method L.** To a stirred solution of 9b (1.0 g, 2.56 mmol) in 20 mL of 1 N HCl was added dropwise a solution of  $\text{NaNO}_2$  (250 mg, 3.6 mmol) in 3 mL of water, while the temperature was maintained at 0–3 °C. After the reaction mixture was stirred for 5 min, aqueous 65% HPF<sub>6</sub> was added to the mixture until precipitation was complete. The precipitate was filtered off and dried over  $\text{P}_2\text{O}_5$  in vacuo to give 1.3 g (94.2%) of the diazonium salt 19b: mp 128–131 °C dec; IR (KBr) 2180  $\text{cm}^{-1}$ . A suspension of 19b (1.0 g, 1.84 mmol) in 40 mL of *n*-heptane was heated to reflux for 15 min and allowed to cool. The resulting precipitate was collected by filtration, taken up in 30 mL of water, and extracted with  $\text{CHCl}_3$ . The extract was dried and concentrated to dryness in vacuo to afford a crude product, which was recrystallized to give 0.26 g (36.2%) of 14b: IR (KBr) 1720, 1680, 1620  $\text{cm}^{-1}$ ; EIMS,  $m/z$  390 ( $\text{M}^+$ ), 318 ( $\text{M}^+ - \text{C}_2\text{H}_4\text{CO}_2$ );  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  8.55 (1 H, s, H-2), 8.23 (1 H, d,  $J = 14$  Hz, H-5), 4.45 (2 H, q,  $J = 7$  Hz,  $\text{CO}_2\text{CH}_2\text{CH}_3$ ), 4.37 (2 H, q,  $J = 7$  Hz,  $\text{NCH}_2\text{CH}_3$ ), 3.80 (8 H, s, piperazine H), 2.18 (3 H, s,  $\text{COCH}_3$ ), 1.50 (3 H, t,  $J = 7$  Hz,  $\text{CO}_2\text{CH}_2\text{CH}_3$ ), 1.47 (3 H, t,  $J = 7$  Hz,  $\text{NCH}_2\text{CH}_3$ ).

Similarly prepared was compound 20a from 9c via 19a using cyclohexane. Diazonium salt 19a: mp 125–130 °C dec; IR (KBr) 2180  $\text{cm}^{-1}$ .

(B) **Method M.** A mixture containing 3.62 g (10 mmol) of ethyl 7-(4-acetyl-1-piperazinyl)-6-fluoro-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylate (21b),<sup>15</sup> 2.76 g (20 mmol) of  $\text{K}_2\text{CO}_3$ , and 40 mL of DMF was heated at 100 °C for 30 min with stirring. To this mixture was added 4.68 g (30 mmol) of ethyl iodide. The resulting mixture was allowed to stir at the same temperature for 3 h and filtered to remove insoluble materials. The filtrate was concentrated to dryness in vacuo. The residue was taken up in a mixture of water (30 mL) and  $\text{CHCl}_3$  (50 mL). The organic phase was separated, washed with water, dried, and chromatographed on silica gel with  $\text{CHCl}_3$  to give 3.5 g (89.7%) of 20b; this compound was identical in all respects with an authentic specimen of 20b prepared by the method L.

Also prepared according to this procedure were 20a [from 21a<sup>15</sup> with ethyl iodide (Table I)] and 31 and 33 [from 21b with ethylene bromohydrin and 2-fluoroethyl tosylate, respectively (Table III)].

**Method N.** 1-Ethyl-1,4-dihydro-6-nitro-4-oxo-7-(1-piperazinyl)-1,8-naphthyridine-3-carboxylic Acid (22b). A mixture containing 4.1 g (9.82 mmol) of 8b, 40 mL of 15% HCl, and 20 mL of EtOH was heated at 90–100 °C for 100 min with stirring and allowed to cool. The resulting precipitate (the HCl salt of 22b) was filtered off and dissolved in a minimum volume of hot water. The aqueous solution was basified with aqueous ammonia to afford precipitates, which were filtered off and recrystallized to give 2.76 g (80.9%) of 22b: IR (KBr) 1630, 1600, 1560, 1360  $\text{cm}^{-1}$ ; EIMS,  $m/z$  347 ( $\text{M}^+$ ), 303 ( $\text{M}^+ - \text{CO}_2$ ).

**Method O.** 6-Amino-1-ethyl-1,4-dihydro-4-oxo-7-(1-pyrrolidinyl)-1,8-naphthyridine-3-carboxylic Acid (23a). A stirred suspension of 9c (0.90 g, 2.72 mmol) in 20 mL of 1 N NaOH was heated at 80–90 °C for 15 min. The resulting solution was treated with charcoal and neutralized with AcOH to give 0.70 g (85.1%) of 23a: IR (KBr) 3360, 3300, 1680, 1610  $\text{cm}^{-1}$ ; EIMS,  $m/z$  302 ( $\text{M}^+$ ), 258 ( $\text{M}^+ - \text{CO}_2$ ).

**Method P.** 6-Amino-1-ethyl-1,4-dihydro-7-(4-methyl-1-piperazinyl)-4-oxo-1,8-naphthyridine-3-carboxylic Acid (23c). A mixture containing 0.50 g (1.36 mmol) of 22c, 0.10 g of 5% Pd/C, and 50 mL of EtOH was hydrogenated at 50–60 °C until the required volume of  $\text{H}_2$  had been taken up. The reaction mixture was filtered to remove the catalyst. The filtrate was concentrated to dryness in vacuo. The crystalline residue was purified by column chromatography on silica gel with  $\text{CHCl}_3$  to give 0.12 g (26.1%) of 23c: EIMS,  $m/z$  331 ( $\text{M}^+$ ), 287 ( $\text{M}^+ - \text{CO}_2$ ), 261 [ $\text{M}^+ - \text{CH}_2=\text{N}(\text{CH}_3)\text{CH}_2\text{CH}_3$ ], 217 [ $287 - \text{CH}_2\text{N}(\text{CH}_3)\text{CH}_2\text{CH}_3$ ].

**Method Q.** 1-Ethyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-1,8-naphthyridine-3-carboxylic Acid (Enoxacin, 24b). A suspension of 20b (5.0 g, 12.8 mmol) in 50 mL of 10% HCl was heated to reflux for 4 h and allowed to cool. The resulting precipitate was filtered off and washed with EtOH to give 4.15 g (91%) of 24b-HCl. This salt was dissolved in 120 mL of hot water and treated with charcoal, and, after the addition of 20% HCl (5 mL), the solution was cooled to give the pure hydrochloride of 24b, mp >300 °C. Anal. ( $\text{C}_{15}\text{H}_{17}\text{FN}_4\text{O}_3\cdot\text{HCl}$ ) C, H, Cl, F, N.

The hydrochloride was dissolved again in 20 mL of water with heating. Subsequent addition of 2.2 mL of concentrated aqueous ammonia under cooling led to precipitation of crystals, which were filtered off and washed with cold water to give 3.1 g (75.3%) of 24b: IR (KBr) 2400–2000, 1615  $\text{cm}^{-1}$ ; EIMS,  $m/z$  320 ( $\text{M}^+$ ), 276 ( $\text{M}^+ - \text{CO}_2$ ), 234 ( $\text{M}^+ - \text{CH}_2=\text{NCH}_3$ );  $^1\text{H}$  NMR ( $\text{Me}_2\text{SO}-d_6$ )  $\delta$  8.92 (1 H, s, H-2), 8.00 (1 H, d,  $J = 14$  Hz, H-5), 4.49 (2 H, q,  $J = 7$  Hz,  $\text{NCH}_2\text{CH}_3$ ), 3.76 and 2.85 (each 4 H, m, piperazine H), 1.39 (3 H, t,  $J = 7$  Hz,  $\text{NCH}_2\text{CH}_3$ ).

**24b-AcOH:** mp 228–229 °C (recrystallized from EtOH). Anal. ( $\text{C}_{15}\text{H}_{17}\text{FN}_4\text{O}_3\cdot\text{C}_2\text{H}_4\text{O}_2$ ) C, H, F, N. **24b-MeSO<sub>3</sub>H:** mp >300 °C (recrystallized from EtOH-H<sub>2</sub>O). Anal. ( $\text{C}_{15}\text{H}_{17}\text{FN}_4\text{O}_3\cdot\text{CH}_3\text{O}_3\text{S}$ ) C, H, F, N, S.

**Method R.** 1-Ethyl-6-fluoro-1,4-dihydro-7-(4-methyl-1-piperazinyl)-4-oxo-1,8-naphthyridine-3-carboxylic Acid (24c). A mixture containing 6.0 g (16.8 mmol) of 24b, 12 mL of 37% formalin, and 18 mL of formic acid was heated at 120–125 °C for 17 h with stirring. The reaction mixture was concentrated to dryness in vacuo. The residue was taken up in 30 mL of water. The mixture was adjusted at first to pH 9 with 10% NaOH and subsequently to pH 7 with AcOH and then extracted with  $\text{CHCl}_3$ . The extract was washed with water, dried, and concentrated to dryness in vacuo. The residue was crystallized from  $\text{CH}_2\text{Cl}_2$ -EtOH to give 5.0 g (75%) of 24c: IR (KBr) 1710, 1630  $\text{cm}^{-1}$ ; EIMS,  $m/z$  334 ( $\text{M}^+$ ), 290 ( $\text{M}^+ - \text{CO}_2$ ), 220 [ $\text{M}^+ - \text{CH}_3\text{CH}_2\text{N}(\text{CH}_3)=\text{CH}$ ];  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  8.78 (1 H, s, H-2), 8.13 (1 H, d,  $J = 14$  Hz, H-5), 4.48 (2 H, q,  $J = 7$  Hz,  $\text{NCH}_2\text{CH}_3$ ), 3.95 (4 H, m,  $\text{CH}_2\text{NCH}_3$ ), 2.60 (4 H, m,  $\text{CH}_2\text{N}^+\text{CH}_3$ ), 2.39 (3 H, s,  $\text{NCH}_3$ ), 1.52 (3 H, t,  $J = 7$  Hz,  $\text{NCH}_2\text{CH}_3$ ), 14.2 (1 H, br s, COOH).

**1-Ethyl-6-fluoro-1,4-dihydro-7-hydroxy-4-oxo-1,8-naphthyridine-3-carboxylic Acid (25).** A suspension of 20b (7.8 g, 20 mmol) in 100 mL of 20% NaOH was heated to reflux for 43 h with stirring. After ice cooling, the precipitate (the disodium salt of 25) was filtered off and taken up in 200 mL of hot water. The aqueous solution was treated with charcoal, acidified with 20 mL of 10% HCl, while hot, and allowed to cool to give precipitates, which were filtered off and washed with water. Recrystallization from water-EtOH (1:4, v/v) gave 4.13 g (82.0%) of 25: mp >300 °C; IR (KBr) 1710  $\text{cm}^{-1}$ . Anal. ( $\text{C}_{11}\text{H}_9\text{FN}_2\text{O}_4$ ) C, H, F, N.

**7-Chloro-1-ethyl-6-fluoro-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylic Acid (26).** A mixture containing 9.5 g (37.7 mmol) of 25 and 50 mL of  $\text{POCl}_3$  was heated to reflux for 15 min. The excess of  $\text{POCl}_3$  was evaporated in vacuo, and the residue was poured into 300 mL of ice-water. After 30 min of stirring, the precipitate was filtered off and washed successively with water and acetone to give 9.3 g (91.4%) of 26: mp 265–267 °C (recrystallized from  $\text{CH}_3\text{CN}$ ); IR (KBr) 1720  $\text{cm}^{-1}$ ; EIMS,  $m/z$  270 ( $\text{M}^+$ ), 226 ( $\text{M}^+ - \text{CO}_2$ );  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  8.98 (1 H, s, H-2), 8.56 (1 H, d,  $J = 8$  Hz, H-5), 4.62 (2 H, q,  $J = 7$  Hz,  $\text{NCH}_2\text{CH}_3$ ), 1.58 (3 H, t,  $J = 7$  Hz,  $\text{NCH}_2\text{CH}_3$ ), 14.2 (1 H, s, COOH). Anal. ( $\text{C}_{11}\text{H}_8\text{ClFN}_2\text{O}_3$ ) C, H, Cl, F, N.

**Method S.** 7-Amino-1-ethyl-6-fluoro-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylic Acid (27a). To a stirred solution of 26 (1.35 g, 5 mmol) in 20 mL of DMF was added 4.8 g (50 mmol) of ammonium carbonate. The mixture was heated at 90 °C for 2 h, after which time an additional 2.4 g (25 mmol) of ammonium carbonate was added. The reaction was allowed to run for an additional 1.5 h. After the mixture was cooled, the precipitate was filtered off, washed with water, and recrystallized to give 1.1 g (87.6%) of 27a (Table II): EIMS,  $m/z$  251 ( $\text{M}^+$ ), 207 ( $\text{M}^+ - \text{CO}_2$ ).

**Method T.** 1-Ethyl-6-fluoro-1,4-dihydro-4-oxo-7-(3-oxo-1-piperazinyl)-1,8-naphthyridine-3-carboxylic Acid (27c). A mixture containing 1.5 g (5.55 mmol) of 26, 1.11 g (11.1 mmol) of 2-oxopiperazine, and 100 mL of acetonitrile was heated to reflux for 1 h and allowed to cool. The precipitate was filtered off,



washed with water, and recrystallized from DMF-EtOH (ca. 1:1, v/v) to give 1.35 g (71.4%) of **27c**: IR (KBr) 3400, 3200, 3050, 2400, 1710, 1670, 1620  $\text{cm}^{-1}$ ; EIMS,  $m/z$  334 ( $\text{M}^+$ ), 290 ( $\text{M}^+ - \text{CO}_2$ ).

**Method U.** 1-Ethyl-6-fluoro-1,4-dihydro-4-oxo-7-(4-*n*-propyl-1-piperazinyl)-1,8-naphthyridine-3-carboxylic Acid (**28a**). A mixture containing 1.0 g (3.12 mmol) of **24b**, 5 mL of propionaldehyde, and 5 mL of formic acid was heated at 90 °C for 17 h with stirring. The residue was taken up in 20 mL of 10% HCl and extracted with three 20-mL portions of  $\text{CHCl}_3$ . The combined extracts were dried and concentrated to dryness. The precipitate was recrystallized from EtOH- $\text{CH}_2\text{Cl}_2$  to give 0.02 g of **29** which was identical with its authentic specimen prepared by method V. The foregoing acidic aqueous layer was adjusted to pH 7 with 10% NaOH and extracted with three 20-mL portions of  $\text{CHCl}_3$ . The combined extracts were dried, and the solvent was evaporated. The residue was crystallized from  $\text{CH}_2\text{Cl}_2$ -EtOH (ca. 1:1, v/v) to give 0.51 g (46.5%) of **28a**.

Also prepared according to this procedure were **28b,c** (Table II) from **24b** using *n*-butyraldehyde and *sec*-butyraldehyde, respectively.

**Method V.** 1-Ethyl-6-fluoro-7-(4-formyl-1-piperazinyl)-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylic Acid (**29**). A mixture containing 5.0 g (15.6 mmol) of **24b**, 50 mL of formic acid, and 10 mL of formamide was heated to reflux for 2 h. After the mixture was cooled, the resulting precipitate was filtered off and washed with acetone to give 5.3 g (97.5%) of **29**: IR (KBr) 1720, 1670, 1625  $\text{cm}^{-1}$ .

**Method W.** Ethyl 7-(4-Acetyl-1-piperazinyl)-1-(2-chloro-ethyl)-6-fluoro-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylate (**32**). To a stirred solution of **31** (5.4 g, 13.3 mmol) in 60 mL of  $\text{CHCl}_3$  was added a solution of  $\text{SOCl}_2$  (3.17 g, 26.6 mmol) in 10 mL of  $\text{CHCl}_3$ . The mixture was heated to reflux for 30 min, cooled, mixed with 30 mL of water, and neutralized with saturated  $\text{NaHCO}_3$ . The organic layer was separated, washed with water, dried, and concentrated to dryness. The residual solid was chromatographed on silica gel with  $\text{CHCl}_3$  to give 5.1 g (90.3%) of **32**: EIMS,  $m/z$  424 ( $\text{M}^+$ ), 379 ( $\text{M}^+ - \text{C}_2\text{H}_5\text{O}$ ), 352 ( $\text{M}^+ - \text{C}_2\text{H}_4\text{CO}_2$ ); IR (KBr) 1720, 1620  $\text{cm}^{-1}$ .

**Method X.** Ethyl 7-(4-Acetyl-1-piperazinyl)-6-fluoro-1-(difluoromethyl)-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylate (**34**). To a refluxed solution of **21b** (10.8 g, 30 mmol) in 300 mL of DMF was added dropwise a solution of sodium chlorodifluoroacetate (11.4 g, 75 mmol) in 30 mL of DMF over a period of 5 min. The mixture was allowed to reflux for an additional 30 min and then cooled. The insoluble material was filtered off and washed with  $\text{CH}_2\text{Cl}_2$  to give 8.5 g (78.7% recovery) of the unchanged compound **21b**. The filtrate and the washings were combined and concentrated to dryness in vacuo. The residue was triturated with hot  $\text{CHCl}_3$ . The  $\text{CHCl}_3$  solution was washed with water and then dried over  $\text{K}_2\text{CO}_3$ , and the solvent was evaporated to leave a crude product, which was chromatographed on silica gel with  $\text{CHCl}_3$ ; the main fraction gave 2.4 g (19.4%) of **34**: IR (KBr) 1730, 1650, 1625  $\text{cm}^{-1}$ ; EIMS,  $m/z$  412 ( $\text{M}^+$ ), 340 ( $\text{M}^+ - \text{C}_2\text{H}_4\text{CO}_2$ );  $^1\text{H}$  NMR ( $\text{Me}_2\text{SO}-d_6$ )  $\delta$  8.57 (1 H, s, H-2), 7.96 (1 H, d,  $J = 14$  Hz, H-5), 8.35 (1 H, t,  $J = 59$  Hz,  $\text{CHF}_2$ ), 4.26 (2 H, q,  $J = 7$  Hz,  $\text{NCH}_2\text{CH}_3$ ), 3.81 and 3.36 (8 H, piperazine H), 2.06 (3 H, s,  $\text{COCH}_3$ ), 1.29 (3 H, t,  $J = 7$  Hz,  $\text{NCH}_2\text{CH}_3$ ).

**Method Y.** 7-(4-Acetyl-1-piperazinyl)-6-fluoro-1,4-dihydro-4-oxo-1-vinyl-1,8-naphthyridine-3-carboxylic Acid (**35**). To a hot solution of **32** (2.13 g, 5 mmol) in 15 mL of EtOH was added a solution of KOH (0.84 g, 15 mmol) in 15 mL of EtOH. The reaction mixture was heated to reflux for 2 h and allowed to cool. The precipitate was filtered off, washed with EtOH, and dissolved in 20 mL of boiling water. The aqueous solution was treated with charcoal and adjusted to pH 4-5 with 10% AcOH to give 1.45 g (80.6%) of **35**: IR (KBr) 1710  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{Me}_2\text{SO}-d_6$ )  $\delta$  8.90 (1 H, s, H-2), 8.14 (1 H, d,  $J = 13.5$  Hz, H-5), 7.83 (1 H, dd,  $J = 16$  and 9 Hz,  $\text{NCH}=\text{CH}_2$ ), 5.88 (1 H, dd,  $J = 2$  and 16 Hz,  $\text{NCH}=\text{CH}$  trans), 5.45 (1 H, dd,  $J = 2$  and 9 Hz,  $\text{NCH}=\text{CH}$  cis), 3.78 (8 H, piperazine H), 2.03 (3 H, s,  $\text{COCH}_3$ ), 14.95 (1 H, br s, COOH).

**Method Z.** 6-Fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-1-vinyl-1,8-naphthyridine-3-carboxylic Acid (**36**). A stirred suspension of **35** (4.0 g, 11.1 mmol) in 20 mL of 10% NaOH was heated to reflux for 2 h, allowed to cool, and adjusted to pH 7 with 30% AcOH. The resulting solid was filtered

off, washed with water, and taken up in ca. 40 mL of 10% AcOH with heating. The solution was adjusted to pH 9 with aqueous ammonia and cooled to give precipitates, which were dissolved in 10% AcOH; the solution was neutralized with ammonia to give 2.90 g (82.4%) of **36**: IR (KBr) 1620  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{Me}_2\text{SO}-d_6$ )  $\delta$  8.82 (1 H, s, H-2), 8.05 (1 H, d,  $J = 14$  Hz, H-5), 7.76 (1 H, dd,  $J = 16$  and 9 Hz,  $\text{NCH}=\text{CH}_2$ ), 5.82 (1 H, dd,  $J = 16$  and 2 Hz,  $\text{NCH}=\text{CH}$  trans), 5.40 (1 H, dd,  $J = 9$  and 2 Hz,  $\text{NCH}=\text{CH}$  cis), 3.75 and 2.84 (8 H, piperazine H).

The initial filtrate and the washings were combined and concentrated to dryness. Recrystallization from an ca. 1:1 EtOH- $\text{CHCl}_3$  mixture gave 0.15 g (5.4%) of 6-fluoro-1,4-dihydro-7-hydroxy-4-oxo-1-vinyl-1,8-naphthyridine-3-carboxylic acid (**41**): mp 256-259 °C; IR (KBr) 1700  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{Me}_2\text{SO}-d_6$ )  $\delta$  8.93 (1 H, s, H-2), 8.25 (1 H, d,  $J = 10$  Hz, H-5), 7.73 (1 H, dd,  $J = 16$  and 9 Hz,  $\text{NCH}=\text{CH}_2$ ), 5.93 (1 H, dd,  $J = 16$  and 2 Hz,  $\text{NCH}=\text{CH}$  trans), 5.53 (1 H, dd,  $J = 9$  and 2 Hz,  $\text{NCH}=\text{CH}$  cis), ~13.0 (br, COOH and/or OH). Anal. ( $\text{C}_{11}\text{H}_7\text{FN}_2\text{O}_4$ ) C, H, F, N.

**In Vitro Antibacterial Activity.** According to the method of Goto et al.,<sup>20</sup> the MIC (in micrograms per milliliter) was determined by the twofold agar dilution method using Mueller-Hinton agar (pH 7.4, Difco); bacterial inocula contained approximately  $10^6$  colony-forming units and the bacterial growth was observed after 20-h incubation at 37 °C.

**In Vivo Efficacy on Systemic Infections.** In vivo activity assay was carried out according to the method of Shimizu et al.<sup>21</sup> Groups of 10 or more male mice (Std-ddY,  $20 \pm 2$  g) were infected with *Staphylococcus aureus* 50774 (iv,  $5 \times 10^8$  cells), *Escherichia coli* P-5101 (ip,  $9 \times 10^6$  cells), and *Pseudomonas aeruginosa* 12 (ip,  $4 \times 10^8$  cells). The test compounds were suspended in 0.2% sodium carboxymethylcellulose and administered orally at 0- and 6-h postinfection. Survival rates were evaluated after 2 weeks for the staphylococcal infection and after 1 week for others.

**Acute Toxicity Test.** A suspension of the test compound in 0.2% carboxymethylcellulose in different concentrations was orally given to male mice (Std-ddY, four to eight in each group) at a dose of 0.1 mL/10 g of body weight. The number of dead mice was counted after 7 days, and the  $\text{LD}_{50}$  value (milligrams per kilogram) was calculated in according to the Behrens-Kaerber method.<sup>22</sup>

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**Registry No.** **3a**, 57948-69-3; **3b**, 54132-31-9; **3c**, 54132-45-5; **7a**, 76105-70-9; **7b**, 54132-41-1; **8a**, 79286-67-2; **8b**, 87939-04-6; **8c**, 65095-80-9; **9a**, 79286-68-3; **9b**, 87938-96-3; **9c**, 70186-25-3; **10c**, 79286-70-7; **11a**, 87938-89-4; **11b**, 87938-90-7; **12a**, 87938-91-8; **12b**, 87939-18-2; **13**, 87938-92-9; **14**, 70186-28-6; **15a**, 70186-29-7; **15b**, 87938-93-0; **15c**, 87938-94-1; **16**, 75064-88-9; **17a**, 87938-95-2; **17b**, 76105-58-3; **17c**, 76105-72-1; **18a**, 76105-57-2; **18b**, 87939-19-3; **18c**, 76105-73-2; **19a**, 87939-01-3; **19b**, 87938-98-5; **20a**, 87938-99-6; **20b**, 82671-13-4; **21a**, 87939-02-4; **21b**, 84233-60-3; **22a**, 87939-20-6; **22b**, 74274-57-0; **22b-HCl**, 87939-05-7; **22c**, 87939-08-0; **23a**, 87939-06-8; **23b**, 74274-56-9; **23c**, 87939-07-9; **24a**, 74274-63-8; **24b**, 74011-58-8; **24b-HCl**, 74011-81-7; **24b-AcOH**, 75167-07-6; **24b-MeSO<sub>3</sub>H**, 75167-06-5; **24c**, 74274-66-1; **25**, 87939-09-1; **25-2Na**, 87939-10-4; **26**, 79286-73-0; **27a**, 87939-11-5; **27b**, 87939-21-7; **27c**, 87939-12-6; **27d**, 74274-65-0; **27e**, 74274-64-9; **27f**, 87939-22-8; **27g**, 74274-60-5; **27h**, 87939-23-9; **27i**, 87939-24-0; **27j**, 87939-25-1; **27k**, 74274-70-7; **27l**, 74274-67-2; **28a**, 74274-68-3; **28b**, 74274-69-4; **28c**, 87939-14-8;

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29, 87939-13-7; 30, 78903-83-0; 31, 87939-03-5; 32, 87939-15-9; 33, 84209-43-8; 34, 87939-16-0; 35, 87939-17-1; 36, 74274-71-8; 36-HCl, 75167-16-7; 36-MeSO<sub>3</sub>H, 75167-17-8; 37, 74274-72-9; 38, 84209-34-7; 39, 84209-33-6; 40, 87939-26-2; 41, 84424-27-1; ethyl 1,4-dihydro-7-methoxy-4-oxo-1,8-naphthyridine-3-carboxylate, 87938-88-3; ethyl 7-chloro-1-ethyl-4-oxo-1,8-naphthyridine-3-carboxylate, 56654-05-8; *sec*-butyraldehyde, 78-84-2; *N*-methylpiperazine, 109-01-3; *N*-acetylpiperazine, 13889-98-0; pyrrolidine, 123-75-1;

piperazine, 110-85-0; ethylene bromohydrin, 540-51-2; 2-fluorethyl tosylate, 383-50-6; 2-oxopiperazine, 5625-67-2; propionaldehyde, 123-38-6; butyraldehyde, 123-72-8; sodium chlorodifluoroacetate, 1895-39-2; ethylenediamine, 107-15-3; piperidine, 110-89-4; morpholine, 110-91-8; thiomorpholine, 123-90-0; homopiperazine, 505-66-8; azepine, 12764-48-6; azocine, 1121-92-2; 1-phenylpiperazine, 92-54-6; 1-benzylpiperazine, 2759-28-6; 1-ethylpiperazine, 5308-25-8.

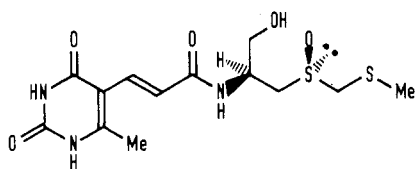
## Structure-Activity Relationships of Sparsomycin and Its Analogues. Octylsparsomycin: The First Analogue More Active than Sparsomycin

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Nine analogues of sparsomycin (1) were synthesized, and their cytostatic activity was studied in an in vitro clonogenic L1210 assay by measuring the inhibition of colony formation. The activity of an analogue, expressed as an ID<sub>50</sub> value, was compared to that of sparsomycin (Table I). Each analogue possesses not more than two structural modifications of the sparsomycin molecule 1. Comparison of the activity of 1 with that of the stereomers 2-4, having *R*<sub>C</sub>*S*<sub>S</sub>, *S*<sub>C</sub>*S*<sub>S</sub>, and *R*<sub>C</sub>*R*<sub>S</sub> chirality, respectively, shows that the *S* configuration of the chiral carbon atom is essential for an optimal activity, whereas the *R* chirality of the sulfoxide sulfur atom of sparsomycin is of importance. Study of the ID<sub>50</sub> values of the *S*-deoxo analogues 10 and 11, as well as the compounds 14 and 15 having the  $\beta$ -sulfoxide function, indicate that the presence of an oxygen atom on the  $\alpha$ -sulfur atom is essential. Isomerization of the trans double bond into the cis double bond yields isosparsomycin (16, Scheme II), which has a low activity. The cytostatic activity of sparsomycin seems to be related to its lipophilicity: octylsparsomycin (19) was shown to be three times as effective as sparsomycin.

The development of a flexible synthesis is a prerequisite for thorough studies on the biological activity and/or biochemical mechanism of the interaction of a molecule. An example, which underlines this view, is sparsomycin (1).<sup>1</sup>



1: Sparsomycin (*S*<sub>C</sub>-*R*<sub>S</sub>)

This antibiotic has been synthesized only recently.<sup>2-4</sup> Consequently, the structure-activity relationship studies of 1 that have appeared so far<sup>5-7</sup> concern analogues in which several structural parameters have been varied simultaneously, thus allowing only a limited interpretation of the results with regard to the role of the various structural fragments.

The interpretation and comparison of the available information on structure-activity relationships of sparsomycin encounters a second difficulty; the biological activity of the analogues has been determined in different systems (in vitro: KB cell culture<sup>5</sup> and cell-free ribosomal systems<sup>6</sup>; in vivo: P-388 system<sup>6</sup> and Walker 256 system<sup>7</sup>).

Sparsomycin (1) is a strong inhibitor of protein biosynthesis and has therefore attracted widespread attention. There is ample evidence<sup>8</sup> that sparsomycin has its site of interaction in the large ribosomal subunit, where it prevents peptide transfer by interfering with the peptidyl-transferase center. Sparsomycin manifests its action in intact prokaryotic cells,<sup>9</sup> eukaryotic cells<sup>10</sup> (including

transformed<sup>5,11</sup> and/or virus infected cells<sup>12</sup>), and various cell-free systems.<sup>13</sup> The behavior of sparsomycin with

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