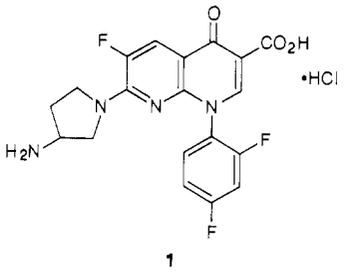
Asymmetric Synthesis and Properties of the Enantiomers of the Antibacterial Agent 7-(3-Aminopyrrolidin-1-yl)-1-(2,4-difluorophenyl)-1,4-dihydro-6-fluoro-4-oxo-1,8-naphthyridine-3-carboxylic Acid Hydrochloride

Terry Rosen,*,† Daniel T. W. Chu, Isabella M. Lico, Prabhavathi B. Fernandes, Linus Shen, Saul Borodkin, and Andrē G. Pernet

Abbott Laboratories, Abbott Park, Illinois 60064. *Received January 11, 1988*

Compound 1 [7-(3-aminopyrrolidin-1-yl)-1-(2,4-difluorophenyl)-1,4-dihydro-6-fluoro-4-oxo-1,8-naphthyridine-3-carboxylic acid hydrochloride] is a potent member of the quinolonecarboxylic acid class of antibacterial agents and is currently undergoing clinical evaluation. We have developed efficient asymmetric syntheses of the enantiomers of this agent. The *S*-(+) enantiomer **1a** is 1–2 $\log_2$ dilutions more active than the *R*-(−) enantiomer **1b** against aerobic bacteria and 1–2 or more $\log_2$ dilutions more active against anaerobic bacteria in vitro. The enantiomer **1a** shows significantly better in vivo activity in a *Pseudomonas aeruginosa* mouse protection model compared to racemic **1**. Coupled with the improved solubility profile of **1a** relative to racemic material, these features may be of practical significance from a clinical standpoint.

The quinolonecarboxylic acids constitute a class of extremely potent and orally active broad-spectrum antibacterial agents. Several structure–activity studies have resulted in the discovery of agents such as norfloxacin, ciprofloxacin, and ofloxacin, which have been shown to inhibit DNA gyrase.[2,3] A particularly potent member of this class of compounds is 7-(3-aminopyrrolidin-1-yl)-1-(2,4-difluorophenyl)-1,4-dihydro-6-fluoro-4-oxo-1,8-naphthyridine-3-carboxylic acid hydrochloride (**1**, A-60969),[1,4] which is currently undergoing evaluation in humans. In addition to its excellent activity versus Gram-positive and Gram-negative aerobes, compound **1** is the most potent member of this class of agents versus anaerobes thus far reported.[4,5]



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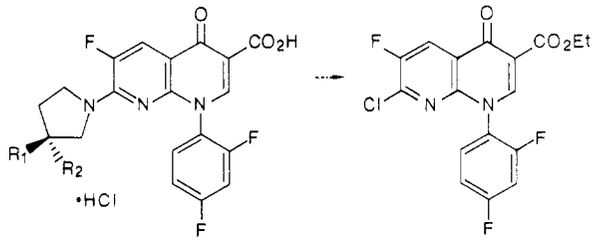
In order to evaluate potential differences in antibacterial activity as well as pharmacological, metabolic, and toxicological properties, we have developed syntheses of the enantiomers of **1**. In this paper, we report the details of the efficient synthesis and in vitro antibacterial and DNA gyrase inhibitory activities of the enantiomers of **1** as well as the in vivo comparison of the more active *S*-(+) enantiomer and the racemic mixture in mouse protection tests. We will also discuss the comparative solubility properties of racemic **1** and (*S*)-(+)-**1**.

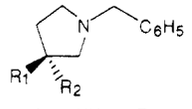
Chemistry

The synthesis of the requisite asymmetric acetamidopyrrolidine precursor **2a** is summarized in Scheme I. Asymmetric hydroboration of **4** with diisopinocamphenylborane (derived from (−)-α-pinene), as described previously by Brown,[6] affords (*R*)-*N*-benzyl-3-hydroxypyrrolidine. Activation of the secondary hydroxyl group by conversion to its methanesulfonate ester (84% yield) followed by S_N2 displacement with azide affords **7** in 87% yield. Exposure of **7** to hydrogen in the presence of a platinum catalyst results in selective reduction of the azide function. Subsequent acetylation of the derived amine affords the desired acetamide **2a** in 64% yield from **7**. Alternatively, treatment of **7** with thioacetic acid yields directly in a single step **2a**.[7]



1a: $R_1 = NH_2$; $R_2 = H$
b: $R_1 = H$; $R_2 = NH_2$

3



2a: $R_1 = NHAc$; $R_2 = H$
b: $R_1 = H$; $R_2 = NHAc$

Debenzylation of **2a** results in the displacement precursor **8**. The crude amine is used immediately; nucleophilic aromatic displacement on the known[8] naphthyridine **3** affords the coupled product **9** in 73% yield. Base hydrolysis of the ester moiety followed by acidic cleavage of

† Current address: Pfizer, Medicinal Chemistry Department, Central Research Division, Groton, CT 06340.

(1) A-60969 (1) is also referred to as A-61827 hydrochloride.
(2) Cornett, J. B.; Wentland, M. P. *Annu. Rep. Med. Chem.* **1986**, *21*, 139 and references therein.
(3) Fernandes, P. B.; Chu, D. T. W. *Annu. Rep. Med. Chem.* **1987**, *22*, 117 and references therein.
(4) (a) Chu, D. T. W.; Fernandes, P. B.; Claiborne, A. K.; Gracey, H. E.; Pernet, A. G. *Abstracts of the 25th Interscience Conference on Antimicrobial Agents and Chemotherapy*; Minneapolis, MN, September 29–October 2, 1985; Vol. 113, Abstract No. 131. (b) Stamm, J.; Vojtko, C.; Weisz, J.; Hanson, C.; Chu, D. T. W.; Fernandes, P. B. *Ibid.* Abstract No. 132. (c) Fernandes, P. B.; Bower, R. R.; Jarvis, K.; Swanson, R.; Chu, D. T. W. *Ibid.* Abstract No. 133.
(5) Fernandes, P. B.; Chu, D. T. W.; Swanson, R. N.; Ramer, N. R.; Hanson, C. W.; Bower, R. R.; Stamm, J. M.; Hardy, D. J. *Antimicrob. Agents Chemother.*, in press.
(6) Brown, H. C.; Vara Prasad, J. V. N.; Gupta, A. K. *J. Org. Chem.* **1986**, *51*, 4296.
(7) We have found thioacetic acid to be a convenient and highly chemoselective reagent for the reductive acetylation of azides. Rosen, T.; Lico, I. M.; Chu, D. T. W. *J. Org. Chem.* **1988**, *53*, 1580.
(8) Chu, D. T. W.; Fernandes, P. B.; Claiborne, A. K.; Gracey, E. H.; Pernet, A. G. *J. Med. Chem.* **1986**, *29*, 2363.

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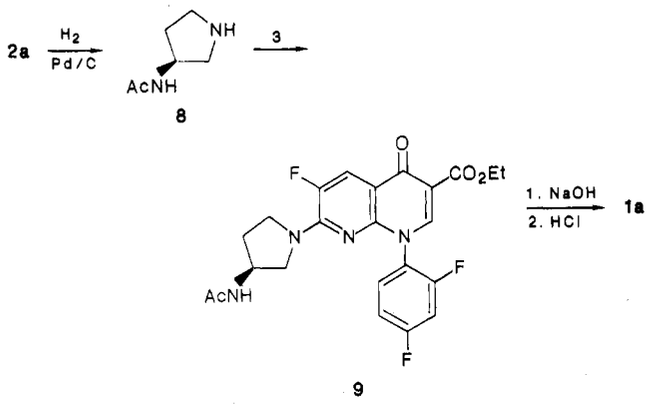
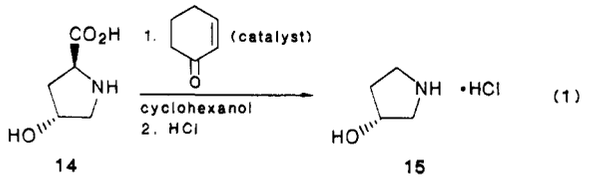


the acetamide furnishes the desired aminopyrrolidinylquinolone **1a** in 66% yield for the one-pot sequence.

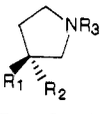
The asymmetric hydroboration of **4** is reported to proceed with complete enantiospecificity, on the basis of the optical rotation of the product alcohol.[6] We evaluated the optical purities of the amine **10** and its enantiomer **11**, derived from reduction of the corresponding azides (as in Scheme I), by conversion to their respective Mosher's[9] amides **12** and **13**. By use of this method,[10] we determined

10: R = H
12: R = (MeO)(Ph)(F_3C)CCO
11: R = H
13: R = (MeO)(Ph)(F_3C)CCO

the optical purities of **10** and **11** to be ≥97:3, although in one case the optical purity of **10** was determined to be 91:9. As a final point, there has been a recent report[11] describing a very convenient procedure for the decarboxylation of hydroxyproline (**14**) to afford (*R*)-3-hydroxypyrrolidine **15** (eq 1), which provides an excellent enantiomerically homogeneous educt for **1a**.



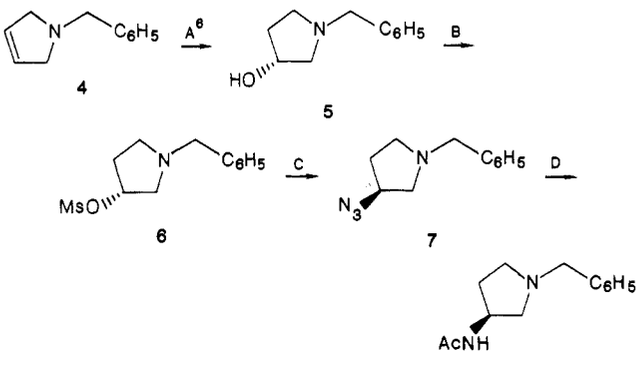
We have taken **15** and converted it to its *t*-BOC analogue **16** and carried out the identical sequence of reactions described in Scheme I for the conversion of (3*R*)-*N*-benzyl-3-hydroxypyrrolidine to **2a** in order to obtain **17**.



16: R_1 = H; R_2 = OH; R_3 = BOC
17: R_1 = NHAc; R_2 = H; R_3 = BOC
2a: R_1 = NHAc; R_2 = H; R_3 = CH_2Ph

In order to ensure that the azide displacement occurs with inversion of configuration even in the presence of the basic ring nitrogen of the benzylpyrrolidine moiety in Scheme I, compound **17** was converted to **2a** by sequential deblocking (TFA) and benzylation ($PhCH_2Br$), and the signs of the optical rotations of the materials obtained by the two methods were compared; there is ample precedent for the azide displacement occurring with inversion of configuration when the pyrrolidine nitrogen is protected as a carbamate group.[12] The benzylamine **2a** obtained as shown in Scheme I and the material obtained by conversion of **17** had optical rotations of $[\alpha]_D$ −19° (*c* 1.0, $CHCl_3$) and $[\alpha]_D$ −21° (*c* 1.1, $CHCl_3$), respectively, indicating that displacement on **6** occurs analogously to the carbamate derivative, with inversion of configuration. The enantiomer of **2a**, compound **2b**, prepared analogously to **2a** by utilizing the appropriate asymmetric hydroborating reagent (derived from (+)-α-pinene) in the first step of the synthetic sequence, had an optical rotation of +22° (*c* 1.3, $CHCl_3$).

Scheme I[a]



[a] (A) (+)-Ipc_2BH; (B) MsCl, Et_3N; (C) *n*-Bu_4N_3, CH_3CN; (D) CH_3COSH or (1) H_2–Pt/C, (2) Ac_2O.

Table I. Comparative Data for the in Vitro Activities of **1a** and **1b** against Aerobic Bacteria

organism	MIC, μg/mL		
	1a	**1b**	**1**
Staphylococcus aureus (ATCC 6538P)	0.02	0.05	0.02
Staphylococcus aureus (CMX 686B)	0.05	0.05	0.05
Staphylococcus aureus (A5177)	0.05	0.1	0.05
Staphylococcus aureus (45)	0.1	0.1	0.1
Staphylococcus aureus (45 RAR2)	0.05	0.1	0.1
Staphylococcus aureus (CMX 503A)	0.05	0.1	0.05
Staphylococcus aureus (CMX 553)	0.05	0.1	0.05
Staphylococcus epidermidis (3519)	0.05	0.1	0.05
Micrococcus luteus (ATCC 9341)	0.78	3.1	1.56
Micrococcus luteus (ATCC 4698)	0.78	1.56	0.78
Enterococcus faecium (ATCC 8043)	0.1	0.39	0.2
Streptococcus bovis (A5169)	0.2	0.39	0.2
Streptococcus agalactiae (CMX 508)	0.2	0.39	0.39
Streptococcus pyogenes (EES61)	0.1	0.39	0.2
Streptococcus pyogenes (930 CONST)	0.1	0.39	0.2
Streptococcus pyogenes (2548 INDUC)	0.1	0.2	0.2
Escherichia coli Juhl	0.02	0.05	0.02
Escherichia coli (SS)	0.002	0.01	0.01
Escherichia coli (DC-2)	0.2	0.39	0.2
Escherichia coli (H560)	0.01	0.05	0.02
Escherichia coli (KNK 437)	0.1	0.39	0.2
Enterobacter aerogenes (ATCC 13048)	0.05	0.2	0.1
Klebsiella pneumoniae (ATCC 8045)	0.01	0.05	0.02
Providencia stuartii (CMX 640)	1.56	1.56	1.56
Pseudomonas aeruginosa (BMH10)	0.05	0.2	0.1
Pseudomonas aeruginosa (A5007)	0.1	0.39	0.2
Pseudomonas aeruginosa (K799/WT)	0.1	0.39	0.1
Pseudomonas cepacia (296I)	3.1	6.2	6.2
Acinetobacter sp. (CMX 669)	0.05	0.1	0.05

Biological Studies

The enantiomers **1a** and **1b** were evaluated for in vitro antibacterial activity versus a variety of Gram-positive and Gram-negative bacteria. These activities are reported as

(9) Dale, J. A.; Mosher, H. S. *J. Am. Chem. Soc.* **1973**, *95*, 512.
(10) Ratios were determined by integration of the signals in the ^{19}F NMR spectrum.
(11) Hashimoto, M.; Eda, Y.; Osanai, Y.; Iwai, T.; Aoki, S. *Chem. Lett.* **1986**, 893.
(12) For additional discussion of this point, see: Rosen, T.; Fesik, S.; Chu, D. T. W.; Pernet, A. G. *Synthesis* **1988**, 40.

Table II. Comparative Data for the in Vitro Activities of **1a** and **1b** against Anaerobes

organism	MIC, μg/mL 1a	1b	CIP	CLIN
Bacteroides fragilis (105AT25285)	0.25	2	4	2
Bacteroides fragilis (784)	0.25	2	4	1
Bacteroides fragilis (UC-2)	0.25	2	4	0.5
Bacteroides fragilis (SFM2906A)	0.25	2	4	0.5
Bacteroides fragilis (SFM2975-7)	0.5	4	8	2
Bacteroides fragilis (SFM2929-1)	0.25	2	8	0.06
Bacteroides thetaiotaomicron (SFM2975-2)	1	4	16	0.5
Bacteroides vulgatus (792)	0.5	2	8	<=0.015
Bacteroides vulgatus (SFBC 2375)	0.25	1	8	<=0.015
Bacteroides disiens (ATCC 29426)	1	2	2	<=0.015
Bacteroides thetaiotaomicron (ATCC 29742)	0.5	8	16	4
Bacteroides thetaiotaomicron (106)	0.5	8	16	4
Bacteroides thetaiotaomicron (ATCC 29741)	0.5	8	32	8
Bacteroides melaninogenicus (ATCC 25845)	0.5	1	2	0.03
Bacteroides loescheii (ATCC 15930)	1	4	8	0.5
Bacteroides bivius (B 6140)	0.5	2	2	<=0.015
Fusobacterium nucleatum (ATCC 25586)	0.25	2	4	0.25
Fusobacterium sp. (GS-10)	0.25	2	4	0.12
Veillonella parvula (ATCC 10790)	0.25	0.5	0.25	0.06
Clostridium perfringens (104AT13124)	0.12	0.25	0.5	0.03
Clostridium perfringens (SFBC 2026)	0.12	0.25	2	0.25
Clostridium perfringens (788)	0.12	0.25	0.5	0.06
Clostridium difficile (ATCC 9689)	1	4	16	4
Clostridium difficile (ATCC 17857)	1	4	16	8
Clostridium ramosum (7)	0.5	2	8	1
Propionibacterium acnes (132)	2	1	2	0.06
Peptococcus asaccharolyticus (ATCC 14963)	0.12	0.25	2	0.06
Peptococcus magnus (ATCC 29328)	0.06	0.5	0.25	1
Peptostreptococcus sp. (TB-11)	0.25	0.5	2	0.06
Peptostreptococcus micros (ATCC 33270)	0.06	0.12	0.5	0.06
Peptostreptococcus anaerobius (ATCC 27337)	0.12	0.5	2	0.12

Table III. Supercoiling Inhibition Activity of Enantiomers of **1**

compound	I_{50}, μg/mL	compound	I_{50}, μg/mL
1a	0.16	**1**	0.4
1b	1.2	norfloxacin	1.0

minimum inhibitory concentrations (MIC, μg/mL). Representative data for the enantiomers are shown in Table I. Data for racemic **1** is provided for comparison. Similar data are also given for these compounds (Table II) versus anaerobic bacteria, with clindamycin (CLIN) as a standard. Data for ciprofloxacin (CIP) are provided for comparison. It can be seen that the *S* enantiomer **1a** is 1–2 $\log_2$ dilutions more active than the *R* enantiomer **1b**. The most notable differences are observed with streptococci, *Pseudomonas aeruginosa*, and, in particular, with many anaerobes. From a clinical standpoint, the improved activity of **1a** versus *P. aeruginosa* could prove important. This pathogen, although clinically important, tends to be one of the weaker points in the spectrum of many of the 7-fluoroquinolones.

In order to determine inherent activity, the enantiomers were assayed[13] for supercoiling inhibition activity with DNA gyrase isolated from *Escherichia coli* H560. The results are shown in Table III. Norfloxacin was run as a standard, and the result for racemic **1** is provided for comparison. It can be seen from this data that the difference in antibacterial activities between the enantiomers of **1** is related to inherent activity at the enzymatic level as opposed to differences in bacterial transport or permeability. The more active enantiomer in vitro (**1a**) and the racemic material were evaluated in mouse protection tests in order to compare in vivo potency. Mice were treated subcutaneously (sc) or orally (po) with a specific amount of the test compound divided into equal portions, which were administered at 1 and 5 h after infection. Potencies are expressed as ED_{50} values, which are given as the total dose of compound (milligrams/kilogram) required to protect 50% of the mice challenged intraperitoneally with the indicated organism. Ciprofloxacin and/or A-60969 were run as standards. The data are shown in Table IV.

Table IV. Comparative in Vivo Activities[a] of Racemic **1** and the *S* Enantiomer **1a**

test organism (dose)	compound	route	ED_{50} (95% CL), mg/kg per day
S. aureus NCTC 10649 (100 × LD_{50})	**1a**	sc	0.4 (0.2–0.6)
	1	sc	0.4 (0.3–0.6)
	ciprofloxacin	sc	4.1 (2.7–6.2)
	1a	po	0.8 (0.5–1.2)
	1	po	1.0 (0.6–1.6)
	ciprofloxacin	po	15.1 (10.4–21.8)
E. coli Juhl (100 × LD_{50})	**1a**	sc	0.2 (0.1–0.3)
	1	sc	0.3 (0.2–0.4)
	ciprofloxacin	sc	0.3 (0.1–0.4)
	1a	po	0.8 (0.5–1.2)
	1	po	1.2 (0.7–1.9)
	ciprofloxacin	po	1.3 (0.8–2.0)
P. aeruginosa A5007 (1000 × LD_{50})	**1a**	sc	19.1 (12.3–29.7)
	1	sc	>25
	1a	po	11.5 (8.5–15.5)
	1	po	27.9 (17.7–44.1)

[a] Each compound was tested at three doses, with 10 animals per dose. ED_{50} is the median dose calculated from cumulative mortalities.

Table V. Comparative Solubilities of **1a** and **1** in Aqueous Media

media	solubility, μg/mL 1a	1
urine, pH 5.1	32.4	23.5
urine, pH 5.7	27.5	16.3
urine, pH 6.3	20.7	12.7
urine, pH 7.0	17.1	12.2
0.15 N sulfate, pH 5.4	8.7	6.5
0.15 N phosphate, pH 5.6	4.3	1.8
0.15 N acetate, pH 6.1	4.1	1.7
0.01 N chloride, pH 6.5	4.5	1.2
Ringer's buffer, pH 7.4	13.0	8.0

It can be seen that the antipode **1a** is not significantly more active than the racemic mixture **1** against *Staphylococcus aureus* NCTC 10649 or *E. coli* Juhl. However, the single enantiomer **1a** shows significantly improved oral efficacy in the *P. aeruginosa* A5007 infection model relative to racemic **1**. In this more "challenging" infection (note the absolute ED_{50} values compared with those in the *S. aureus* and *E. coli* models), the improved in vitro potency of **1a** manifests itself, and improved in vivo activity is observed.

Solubility Studies

In addition to potential differences in the biological properties of racemic **1** and its enantiomers, we were also interested in comparing physical properties, particularly solubility. Compound **1** has quite low solubility in aqueous media (8 μg/mL in Ringer's buffer solution). Thus, the solubilities of the *S*-enantiomer **1a** and racemic **1** were

(13) Hogberg, T.; Khanna, I.; Drake, S. D.; Mitscher, L. A.; Shen, L. L. *J. Med. Chem.* **1984**, *27*, 306.

compared in several media. Representative results are shown in Table V. It can be seen that the *S* enantiomer **1a** shows greater solubility than the racemic mixture in all media examined. It should be noted that the solubility of the *R*-enantiomer **1b** was also determined in Ringer's buffer and showed the same solubility as the *S* enantiomer **1a**, as expected.

Summary of Results

Efficient asymmetric syntheses of the enantiomers of **1** have been achieved. The two enantiomers were evaluated in vitro against several aerobic and anaerobic organisms; the *S*-(+) enantiomer **1a** is 1–2 $\log_2$ dilutions more active than the *R*-(−) enantiomer **1b** versus aerobic bacteria and 1–2 or more $\log_2$ dilutions more active against anaerobic bacteria. Similarly, **1a** is more active than **1b** in a supercoiling inhibition assay with DNA gyrase isolated from *E. coli* H560. The *S* enantiomer **1a** was compared with racemic material in mouse protection tests. In *S. aureus* and *E. coli* infection models, significant differences in efficacy are not observed between **1a** and **1**. However in a *P. aeruginosa* model, the single enantiomer is significantly more efficacious than the racemic material. These results are probably due to the greater difficulty associated with clearing the *P. aeruginosa* infection. The activities of both the single enantiomer and the racemic material are both outstanding with respect to *S. aureus* and *E. coli*, and it is difficult to distinguish differences in activity because of the extremely low MIC values involved. However, the factor of ~2-fold improvement in in vitro activity of **1a** relative to **1** manifests itself in improved efficacy in vivo in the *P. aeruginosa* model as relatively higher MIC values are involved; in this case, the blood levels attained of the compound at a given dose are less likely to be in as large of an excess compared to the MIC values as in models employing the more susceptible *S. aureus* and *E. coli* organisms.

Coupled with the increased activity of **1a**, its improved solubility profile may be of practical significance from a clinical standpoint. Further studies on the biological properties of **1a** are in progress.

Experimental Section

General Methods. Unless otherwise noted, materials were obtained from commercial suppliers and used without further purification. Melting points are uncorrected. NMR spectra were determined on a General Electric GN-300 spectrometer operating at 300.1 MHz. NMR spectra were determined with $CDCl_3$ as the solvent, unless otherwise noted. Chemical shifts are expressed in ppm downfield from internal tetramethylsilane. Significant ^1H NMR data are tabulated in the order: multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet), number of protons, coupling constant(s) in hertz. Mass spectra were obtained with a Hewlett-Packard 5985A mass spectrometer or a Kratos MS-50 instrument with EI source (70 eV). Flash column chromatography[14] was done with Matrex silica Si (particle size, 35–70 μm). Thin-layer chromatography (TLC) was performed with Analtech silica gel GF TLC plates (250 μM), and compound visualization was effected with a 2% solution of sulfuric acid in ethanol or a UV lamp. Optical rotations were measured in a 1-dm cell with a Perkin-Elmer Model 241 polarimeter. Elemental analyses were performed by the Microanalytical Laboratory, operated by the Analytical Department, Abbott Laboratories, North Chicago, IL.

(3*R*)-1-Benzyl-3-[(methylsulfonyl)oxy]pyrrolidine (6). In a 500-mL round-bottom flask were placed 12.65 g (71.5 mmol) of *N*-benzyl-3(*R*)-pyrrolidinol (**5**),[6] 20 mL of dichloromethane, and 25 mL (18.2 g, 180 mmol) of triethylamine. The system was cooled in an ice bath. To the system was added 11.3 mL (16.4 g, 143 mmol) of methanesulfonyl chloride, dropwise, via an addition funnel. The reaction mixture was stirred under nitrogen for 20 min at 0 °C. The ice bath was removed, and the reaction mixture was stirred at room temperature for 1.5 h. The reaction mixture was diluted with dichloromethane, washed with saturated aqueous sodium bicarbonate and brine, dried (Na_2SO_4), and concentrated with a rotary evaporator. The resulting red oil was subjected to flash column chromatography with 4:1 ethyl acetate/hexanes as the eluant to obtain 15.3 g (84% yield) of **6** as a yellow oil: ^1H NMR δ 2.10 (m, 1 H), 2.30 (m, 1 H), 2.50 (m, 1 H), 2.75–2.90 (complex m, 3 H), 3.00 (s, 3 H), 3.62 (d, 1 H, *J* = 12.6), 3.68 (d, 1 H, *J* = 13.5), 5.20 (m, 1 H), 7.30 (m, 5 H); mass spectrum, *m/z* 256 (parent + H). Anal. ($C_{12}H_{17}NO_3S \cdot {}^1/_4H_2O$) C, H, N.

(3*S*)-3-Azido-1-benzylpyrrolidine (7). In a 500-mL round-bottom flask were placed 15.3 g (60 mmol) of **6** and 20 mL of acetonitrile. To this solution was added 24 g (84 mmol) of tetra-*n*-butylammonium azide, and the mixture was heated at 65 °C for 1 h under nitrogen. The reaction mixture was diluted with ether, washed with saturated aqueous sodium bicarbonate and brine, dried (Na_2SO_4), and concentrated with a rotary evaporator. The crude material was purified by flash column chromatography with 1:4 ethyl acetate/hexanes as the eluant to obtain 10.6 g (87% yield) of **7** as a clear, yellow oil: ^1H NMR δ 1.90 (m, 1 H), 2.20 (m, 1 H), 2.46 (m, 1 H), 2.70 (complex m, 3H), 3.60 (d, 1 H, *J* = 12), 3.68 (d, 1 H, 13.5), 3.95 (m, 1 H), 7.30 (m, 5 H); mass spectrum, *m/z* 203 (parent + H). Anal.[15] ($C_{11}H_{14}N_4 \cdot {}^1/_4H_2O$) C, H, N.

(3*S*)-3-Amino-1-benzylpyrrolidine. The azide **7** (9.58 g, 47.4 mmol) was dissolved in 200 mL of methanol. To the system was added 960 mg of 5% Pt/C. The system was placed under 4 atm of hydrogen at room temperature for 4 h. The catalyst was removed by filtration through a Celite pad, and the filtrate was concentrated with a rotary evaporator to afford 7.70 g (93% yield) of the title compound as a clear, colorless oil, which was used in subsequent transformations without further purification: ^1H NMR δ 1.50 (m, 1 H), 1.61 (br s, 2 H, NH_2), 2.20 (m, 1 H), 2.31 (dd, 1 H, *J* = 4.5, 9.6), 2.46 (m, 1 H), 2.70 (m, 2 H), 3.50 (m, 1 H), 3.57 (d, 1 H, *J* = 13.5), 3.64 (d, 1 H, *J* = 13.5), 7.30 (m, 5 H); mass spectrum, *m/z* 177 (parent + H).

(3*S*)-3-Acetamido-1-benzylpyrrolidine (2a). In a 500-mL round-bottom flask were placed 7.24 g (41.1 mmol) of crude 3(*S*)-amino-1-benzylpyrrolidine prepared above, 15 mL of pyridine, and 14 mL (10.4 g, 103 mmol) of triethylamine. The system was cooled in an ice bath, and 7.8 mL (8.4 g, 82.3 mmol) of acetic anhydride was added to the system. The reaction mixture was stirred at room temperature under a nitrogen atmosphere for 16 h, diluted with chloroform and washed with saturated aqueous sodium bicarbonate and brine. The chloroform layer was stirred with Norite and filtered through a Celite pad. The filtrate was dried (Na_2SO_4), and the solvent was removed with a rotary evaporator. The crude product was purified by flash column chromatography, gradually increasing the polarity of the eluant from chloroform to 10% methanol/chloroform, to obtain 6.2 g (69% yield) of **2a** as a clear, red oil: $[\alpha]^{20}_D$ −19° (*c* 1.0, $CHCl_3$). ^1H NMR δ 1.60 (m, 1 H), 1.94 (s, 3 H), 2.25 (m, 2 H), 2.53 (dd, 1 H, *J* = 6, 10.5), 2.61 (dd, 1 H, *J* = 3, 10.5), 2.85 (m, 1 H), 3.59 (s, 2 H), 4.45 (m, 1 H), 5.95 (br d, NH, *J* = 6), 7.30 (m, 5 H); mass spectrum, *m/z* 219 (parent + H). Anal. ($C_{13}H_{18}N_2O \cdot 0.15H_2O$) C, H, N.

(3′*S*)-7-(3′-Aminopyrrolidinyl)-1-(2,4-difluorophenyl)-1,4-dihydro-6-fluoro-4-oxo-1,8-naphthyridine-3-carboxylic Acid Hydrochloride (1a). Under 4 atm of hydrogen were placed 5.73 g (26.4 mmol) of **2a**, 250 mL of methanol, and 11.46 g of 20% Pd/C (wet) at room temperature for 8 h. The reaction mixture was filtered through Celite, and the filtrate was concentrated with a rotary evaporator to afford 3.6 g (quantitative yield) of **8** as a clear, yellow oil, which was used immediately for displacement on **3**. In a 250-mL round-bottom flask were placed 3.3 g (25 mmol) of crude **8** and 15 mL of pyridine. To the system were added 4 mL (2.9 g, 29 mmol) of triethylamine and 7.6 g (19.9 mmol) of **3**. The reaction mixture was stirred at 65 °C for 17 h under nitrogen, and the solvent was removed with a rotary evaporator. The crude material was purified by flash column chromatography,

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(15) Analytical data for this compound were actually obtained on the enantiomer; spectral properties of the enantiomers were identical.

gradually increasing the polarity of the eluant from chloroform to 10% methanol/chloroform to obtain 5.4 g of pure **9** and 4 g of slightly contaminated product. The impure product was subjected to a second flash column chromatography to obtain an additional 1.5 g of pure **9**; the combined yield of **9** was 6.9 g (73% yield) as a red solid: mp 240–243 °C; ^{1}H NMR δ 1.38 (t, 3 H, J = 7.5), 2.02 (s, 3 H), 3.30–3.70 (br m, 6 H), 4.36 (q, 2 H, J = 7.5), 4.55 (m, 1 H), 6.27 (br d, 1 H, J = 6), 7.05 (m, 2 H), 7.40 (m, 1 H), 7.90 (d, 1 H, J = 13.5), 8.35 (s, 1 H); mass spectrum, m/z 475 (parent + H). Anal.[15] ($C_{23}H_{21}F_3N_4O_4 \cdot {}^1/_3H_2O$) C, H, N. In a 1-L round-bottom flask were placed 5.35 g (11.3 mmol) of **9**, 100 mL of THF, and 350 mL of 0.1 M aqueous sodium hydroxide. The reaction mixture was stirred at 65 °C for 1.5 h and concentrated with a rotary evaporator. To the residue was added 400 mL of 6 M aqueous hydrochloric acid, and the reaction mixture was heated at 110 °C for 24 h under nitrogen. The reaction mixture was concentrated with a rotary evaporator, and ~70 mL of water was added to the system. This mixture was heated to boiling for approximately 5 min and chilled to 0 °C, and the precipitate was collected by suction filtration and rinsed with water, ethanol, and ether. The precipitate was recrystallized from hot ethanol, rinsed with cold ethanol and ether, and dried overnight in vacuo at 60 °C to afford 3.24 g (66% yield) of pure **1a** as a pale beige solid: mp 229–234 °C dec; $[\alpha]^{20}_D$ +14° (c 0.92, DMSO); ^{1}H NMR (DMSO-d_6) δ 2.10 (m, 2 H), 2.50 (m, 3 H), 3.30–3.40 (complex, 2 H), 3.85 (br, 2 H), 7.35 (m, 1 H), 7.60 (m, 1 H), 7.80 (m, 1 H), 8.12 (d, 1 H, J = 12), 8.83 (s, 1 H); mass spectrum, m/z 405 (parent + H). Anal. ($C_{19}H_{15}F_3N_4O_3 \cdot {}^3/_2HCl$) C, H, N.

Analogous procedures were used to convert N-benzyl-3(S)-pyrrolidinol to 3(R)-3-acetamidopyrrolidine (**2b**) and its corresponding naphthyridine analogue **1b**, $[\alpha]^{20}_D$ −15° (c 0.91, DMSO).

(3*S*)-1-Benzyl-3-[(*R*)-α-methoxy-α-(trifluoromethyl)-α-phenylacetamido]pyrrolidine (12). In a 25-mL round-bottom flask under nitrogen were placed 57 mg (0.33 mmol) of 3(S)-amino-1-benzylpyrrolidine (**10**), 0.60 mL of pyridine, and 60 μL (45 mg, 0.45 mmol) of triethylamine. To the system was added 80 μL (108 mg, 0.45 mmol) of (+)-α-methoxy-α-(trifluoromethyl)phenylacetyl (MTPA) chloride[9] (derived from (R)-(+)-MTPA). The reaction mixture was stirred at room temperature under an atmosphere of nitrogen for 5 h, diluted with ether and washed with aqueous sodium bicarbonate and brine. The organic phase was dried (Na_2SO_4) and concentrated with a rotary evaporator to afford 98 mg (76% yield) of crude **12** as a yellow oil: ^{19}F NMR (trifluoroethanol, internal standard) δ 8.29 ppm; mass spectrum, m/z 393 (parent + H).

(3*R*)-1-Benzyl-3-[(*R*)-α-methoxy-α-(trifluoromethyl)-α-phenylacetamido]pyrrolidine (13): ^{19}F NMR (trifluoroethanol, internal standard) δ 8.35 ppm.

Biological Studies. The minimum inhibitory concentrations (MIC) and ED_{50} values were obtained using standard techniques as described in ref 5. The aerobic MIC values were determined on brain–heart infusion agar, and the anaerobic MIC values were determined on Wilkins–Chalgren agar. The IC_{50} values were determined by using the technique described previously.[13]

Registry No. (±)-**1**, 114636-36-1; **1a**, 114715-36-5; **1b**, 114715-37-6; **2a**, 114636-30-5; **2b**, 114636-33-8; **3**, 100491-29-0; **5**, 101930-07-8; **6**, 114715-35-4; **7**, 114636-29-2; **8**, 114636-31-6; **9**, 114636-32-7; **10**, 114715-38-7; **11**, 114715-39-8; **12**, 114636-34-9; **13**, 114636-35-0; **16**, 109431-87-0; **17**, 114636-37-2; CIP, 85721-33-1; (±)-MTPA chloride, 20445-33-4; N-benzyl-3(S)-pyrrolidinol, 101385-90-4; norfloxacin, 70458-96-7.

Imidazo[1,2-*a*]pyrimidines and Imidazo[1,2-*a*]pyrazines: The Role of Nitrogen Position in Inotropic Activity

Wayne A. Spitzer,* Frantz Victor, G. Don Pollock, and J. Scott Hayes

Eli Lilly and Company, Indianapolis, Indiana 46285. *Received May 8, 1987*

Congestive heart failure is a major medical problem for which existing medicaments have provided limited benefit. Recent new experimental drugs, including imidazo[4,5-*b*]- and imidazo[4,5-*c*]pyridines, have both inotropic and vasodilatory properties. Subtle changes in nitrogen position of these compounds have been shown to dramatically affect potency.[5] We have synthesized a series of imidazo[4,5-*b*]- and -[4,5-*c*]pyridine analogues having an imidazo nitrogen relocated at the bridgehead position. The superior inotropic activity of the [4,5-*c*]pyridines as compared to [4,5-*b*]pyridines[5] is reaffirmed by the activity of our analogues. The biological equivalence of imidazo[4,5-*b*]pyridines with imidazo[1,2-*a*]pyrimidines and imidazo[4,5-*c*]pyridines with imidazo[1,2-*a*]pyrazines is demonstrated. Further, 2-[2-methoxy-4-(methylsulfenyl)phenyl]imidazo[1,2-*a*]pyrazine and 2-[2-methoxy-4-(methylsulfonyl)phenyl]-imidazo[1,2-*a*]pyrazine are potent inotropic agents both in vitro and in vivo.

Congestive heart failure is a major cause of morbidity and mortality throughout the United States and other industrialized countries.[1] While drugs such as cardiac glycosides and β-agonists are widely used to improve the performance of the failing heart, they suffer from substantial disadvantages and limited efficacy.[2] With the advancing age of the general population, the need for more effective treatment for congestive heart failure will become even more acute, and it is appropriate, therefore, that significant effort has been directed toward this medical need as evidenced by the recent appearance of several new drugs.[3]

Some of these new drugs are characterized by the advantage of having at the same time both inotropic and vasodilatory properties.[4] Two of these compounds (AR-L115BS [sulmazole], **1c**, and LY175326 [isomazole], **2c** see Figure 2) are structurally closely related being imidazo-[4,5-*b*]- and -[4,5-*c*]pyridines, respectively. Robertson et al.[5] have recently reported a comparative study of these series of compounds. They found that for a wide range of substituents, compounds from the [4,5-*c*] series routinely show superior inotropic potency. Their study clearly illustrates that subtle changes in the position of nitrogen can play an important role in affecting biological activity.

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