Synthesis and In Vitro Antibacterial Activity of Some 1-(Difluoromethoxyphenyl)quinolone-3-carboxylic Acids

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Received June 13, 1988, from *Infectious Disease and Molecular Biology Research, Medical Research Division, and the ‡Biomedical Research Computing Section, American Cyanamid Company, Lederle Laboratories, Pearl River, NY 10965. Accepted for publication December 28, 1988.

Abstract □ We report on the synthesis of *N*-1-phenylquinolones in which the difluoromethoxy moiety is utilized as a halogen replacement. The antibacterial activity is discussed with reference to *N*-1-halophenylquinolones.

In a previous communication,[1] we reported the use of the difluoromethoxy moiety as a potential replacement for the halogen in the 6-position of the third generation quinolone-3-carboxylic acids. In that study, we attribute the lack of activity of the difluoromethoxy moiety to steric interaction with the piperazine in the 7-position.[2] We report the utilization of this moiety as a halogen replacement in the phenyl group of 1-(substituted phenyl)quinolone-3-carboxylic acids where steric factors, especially for *p*-substituted phenyl, should be minimal.

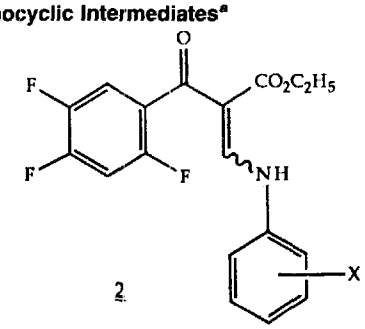
A variety of groups are reported to be halogen like,[3,4] and have behaved as such in biological profiles. However, to date, no moiety has been reported to replace the 6-fluorine, although the 6-chloro is somewhat similar in quinolones that provide the same degree of biological activity.[1,5,6] A literature reference on quinolones with a substituted phenyl at the 1-position reported that the activity of 2,4-diF ≃ 4-F > H > Cl > Br.[7]

Chemistry—The anilines, which were prepared by the difluoromethylation of the corresponding phenols with chlorodifluoromethane and then catalytic hydrogenation,[1,8] reacted smoothly with intermediates **1**[9] to generate **2** (Table I) which cyclized on treatment with base to form **3** (Table II). Intermediates **2** were in general used without purification. The reaction of **3** with piperazine or *N*-methylpiperazine generated compounds **4** (Scheme I; Table III).[7]

Results and Discussion

All of the intermediate esters and acids (**3**) were essentially devoid of antibacterial activity as judged by the MIC (μg/mL) versus appropriate bacteria. The derivatives of **4** which have the 3′,4′-difluoromethoxy group (**4e,f**) were also inactive in the in vitro assay. Compounds **4a,b**, showed modest in vitro activity against the organisms tested, with the exception of *Pseudomonas aeurginosa* and *Streptococcus faecalis* where they had poor activity. The activity of **4a,b** was considerably less than that reported for similar quinolones with an *N*-1-(2′-fluorophenyl) moiety.[7,10] In general, the unsubstituted piperazine derivatives were 2-to-4-fold more active than their *N*-methyl counterparts in vitro (i.e., **4a,b** and **4g,h**;[7,10] Tables IV and V). While the 4′-difluoromethoxy analogue **4c** had activity comparable to **4a**, the *N*-methyl derivative **4d** was, in general, more active by a log than **4c**. However, the overall activity was 4–8-fold less active than that of pefloxacin and **4h** in the same test. However, the overall activity of **4c,d** was somewhat better than a quinolone containing an *N*-1-(4′-bromophenyl) moiety and generally comparable to the *N*-1-(4′-chlorophenyl) compound.[7] Compound **4d** is 5-to-10 times more active than the compound with a *N*-1-(4′-tolyl) group.[7] The in vitro activity ratio of **4g,h** is again roughly the expected ~4-fold difference. The unexpected activity of **4d** was repeated in an in vitro test against an expanded number of organisms (Table II) as compared with **4h**. Thus, in the series of quinolones which contain an *N*-1-(4′-difluoromethoxyphenyl) moiety, the difluoromethoxy residue was halogen like when compared with 4′-Br and 4′-Cl residues, but none compared with the 4′-F residue for overall activity.

Table I—Monocyclic Intermediates[a]

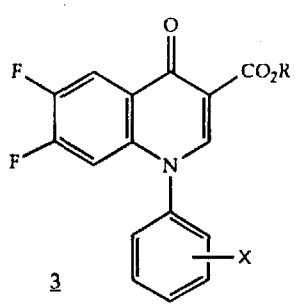


2

No.	X	Yield, %	mp, °C	Formula
2a	2-CHF_2O	54	oil	$C_{19}H_{14}F_5NO_4$
2b	4-CHF_2O	56	oil	$C_{19}H_{14}F_5NO_4$
2c	3,4-di-CHF_2O	83	oil	$C_{20}H_{14}F_7NO_5$

[a] All new compounds have supporting spectral data and were used as is.

Table II—Cyclic Intermediates[a]



3

No.	X	R	Formula	Yield, %	mp, °C
3a	2-CHF_2O	C_2H_5	$C_{19}H_{13}F_4NO_4$	88	122.5–125.5
3b	4-CHF_2O	C_2H_5	$C_{19}H_{13}F_4NO_4$	70	201–203
3c	3,4-di-CHF_2O	C_2H_5	$C_{19}H_{13}F_6NO_5$	70	154.5–156.5
3d	2-CHF_2O	H	$C_{17}H_9F_4NO_4$	91	197–199
3e	4-CHF_2O	H	$C_{17}H_9F_4NO_4$	82	270–272
3f	3,4-di-CHF_2O	H	$C_{18}H_9F_6NO_5$	83	225–228

[a] All new compounds have consistent supporting data.

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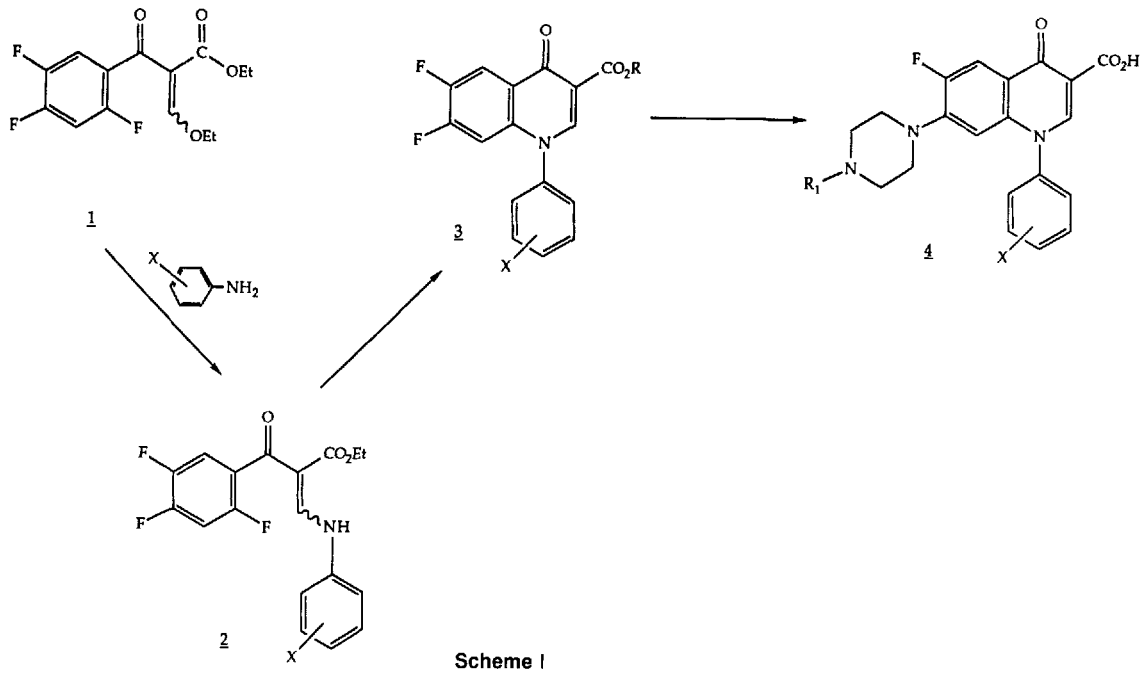


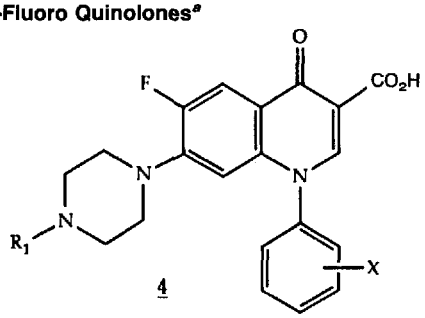
Scheme I

We performed AM1 calculations on a number of 4-substituted anilines, including the F_2CHO moiety, to determine the effect on the ring and on the NH_2. The effects could be expected to be similar to those occurring in N^1-substituted quinolones. Table VI summarizes the calculations and shows the combined charge on the 4-substituent, the ring, and the NH_2 (N plus 2H). Figure 1 graphically displays the comparison of the atomic charges versus the 4-substituent. In all cases, the data for F and F_2CHO are closely aligned. When comparing the data for effect on the NH_2, the halogens and the F_2CHO are grouped at the high end.

Experimental Section

All melting points were taken on a Mel-Temp apparatus and are uncorrected. Where analyses are reported by symbols of the elements, results were within 0.4% of the calculated values. The IR, NMR, and MS data were determined for all numbered compounds in this section and were evaluated as consistent with the indicated structures. These data are presented only where required for structural assignment (other elemental analysis data are available from the author). The IR spectra were obtained using a Perkin-Elmer 1310 IR spectrophotometer. The NMR spectra were determined with a Varian model FT-80 with Me_4Si as an internal standard. Mass spectra were obtained on Finnegan MAT model CH7 mass spectrometer.

Table III—6-Fluoro Quinolones[a]



No.	R_1	X	Yield, %	mp, °C	Formula
4a[b]	H	2-CHF_2O	50	212–214	$C_{21}H_{18}F_3N_3O_4$
4b	CH_3	2-CHF_2O	53	240–242	$C_{22}H_{20}F_3N_3O_4$
4c[c]	H	4-CHF_2O	80	221–223	$C_{21}H_{18}F_3N_3O_4$
4d	CH_3	4-CHF_2O	36	251–254	$C_{22}H_{20}F_3N_3O_4$
4e[d]	H	3,4-di-CHF_2O	72	208–212	$C_{22}H_{18}F_5N_3O_5$
4f	CH_3	3,4-di-CHF_2O	91	256–258	$C_{23}H_{20}F_5N_3O_5$
4g[d]	H	4-F	—	—	—
4h[d]	CH_3	4-F	—	—	—

[a] All new compounds have supportive spectral and analytical data. [b] C, calc. 58.2; found 57.7. [c] C, calc. 58.2, found 57.6 [d] References 10 and 11.

In Vitro Antibacterial Activity—The MIC (μg/mL) of compounds was determined by means of a standard two-fold serial dilution method using agar media.[11]

2-Difluoromethoxynitrobenzene—Chlorodifluoromethane (15 g, 0.173 mol) was passed into THF (40 mL) at 5–10 °C. Into it was added a suspension of *o*-nitrophenol (3.9 g, 0.028 mol) in 28% sodium hydroxide solution (40 mL). The ice bath was removed and the temperature of solution gradually rose to room temperature. Stirring was continued for 24 h. The reaction liquid was neutralized with conc. HCl to pH 7. The resultant was extracted with ether three times to give 3.69 g (70%) of the desired product; ^1H NMR ($CDCl_3$): 6.628 (1, t, J = 73.5 Hz, $OCHF_2$); and 7.37–7.95 ppm (4H, *m*-ArH); IR (KBr): 1536 cm^{-1}, 1355 cm^{-1} (NO_2); MS (*m/e*): 160 (M-NO)H^+.

2-Difluoromethoxyaniline—Palladium-charcoal catalyst [1%, 1.32 g (1.2 mmol)] was added to 2-fluoromethoxynitrobenzene (4.6 g, 24 mmol) in 80 mL of ethanol (5% H_2O), and the theoretical amount of hydrogen was taken up during 20 min at room temperature. The catalyst was filtered off and volatiles removed under reduced pressure to give 2.89 g (76%) of an oil; ^1H NMR ($CDCl_3$): 6.35 (1, t, J = 74.2 Hz, —$OCHF_2$); MS (*m/e*): 160 M^+.

Ethyl α-[[[2-(difluoromethyoxy)phenyl]amino]methylene]-2,4,5-trifluoro-β-oxo-benzenepropionate ester (2a)—The 2-difluoromethoxyaniline (0.64 g, 4 mmol) in 5 mL of ethanol was added in a dropwise manner to the solution of ethyl-2,4,5-trifluorobenzoylacetate (1.1 g, 3.64 mmol) in 10 mL of ethanol, producing an exotherm. The reaction solution was stirred at room temperature for 1 h. The precipitate was removed by filtration, washed with ethanol, and dried to yield 0.82 g (54.3%), mp 100–102 °C; ^1H NMR (DMSO-d_6): δ 0.905–1.07 (3H, 2t, J = 7.0 Hz, CH_3), 4.04 (2H, q, J = 7.0 Hz, OCH_2), 7.26–7.87 (6H, m, ArH), 7.14–7.63 (1H, t, J = 7.3 Hz, $OCHF_2$), 8.52–8.71 (1H, 2d, J = 3.5 Hz, =*CH*—N—), and 11.17–12.66 ppm (1H, 2d, J = 13.5 Hz, NH); IR (KBr): 1571 cm^{-1} (ester); MS (*m/e*): 416 (M^+).

Anal.—Calc. for $C_{19}H_{14}F_5NO_4$: C, 54.9; H, 3.40; N, 3.37; F, 22.9. Found: C, 54.9; H, 3.42; N, 3.14; F, 23.2.

1-[2-(Difluoromethoxy)phenyl]-6,7-difluoro-1,4-dihydro-4-oxo-3-quinolinecarboxylic acid; ethyl ester (3a)—To **2a** (0.4 g, 1 mmol)

Table IV—Microbiology Evaluation—In Vitro Antibacterial Activity[a]

Organism	Source[b]	Minimal Inhibitory Conc., μg/mL							
		Pefloxacin	4a	4b	4c	4d	4e	4f	4g
Escherichia coli	LL	0.060	4	8	4	0.25	>128	>128	<0.008
Escherichia coli	CMC	0.060	4	16	2	0.25	>128	>128	<0.008
Serratia marcescens	K	0.120	16	64	16	4	>128	>128	0.250
Serratia marcescens	VGH	2.000	64	>128	64	16	>128	>128	1.000
Proteus rettgeri	CMC	0.120	32	64	32	2	>128	>128	0.060
Morganella morganii	CMC	0.060	16	64	16	2	>128	>128	0.060
Pseudomonas aeurginosa	LL	2.000	128	>128	16	8	>128	>128	1.000
Pseudomonas aeurginosa	VGH	1.000	128	>128	16	8	>128	>128	0.250
Staphylococcus aureus	VGH	0.500	32	64	32	2	>128	>128	0.060
Staphylococcus aureus	SMIT	0.250	8	64	4	0.50	128	>128	<0.008
Streptococcus faecalis	VGH	4.000	64	>128	32	4	128	>128	2.000
Streptococcus faecalis	UCI	4.000	64	>128	16	16	128	>128	2.000
Escherichia coli	ATCC	0.030	4	16	4	0.50	>128	>128	<0.008
Staphylococcus aureus	ATCC	1.000	16	128	16	2	>128	>128	0.120

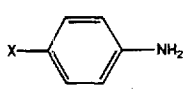
[a] Medium: Mueller–Hinton; incubation: 18 h, 35 °C; method: agar dilution. [b] Source of clinical isolate.

Table V—Microbiology Evaluation—In Vitro Antibacterial Activity[a]

Organism	Source[b]	Minimal Inhibitory Conc., μg/mL			
		Pefloxacin	4d	4g	4h
Escherichia coli	MOR	0.03	0.50	0.015	0.06
Escherichia coli	VGH	0.03	0.50	0.015	0.06
Escherichia coli	CMC	0.03	1.00	0.030	0.12
Klebsiella pneumoneae	CMC	0.06	1.00	0.015	0.06
Klebsiella pneumoneae	MOR	0.12	4.00	0.060	0.12
Klebsiella pneumoneae	IO	0.12	2.00	0.060	0.25
Enterobacter cloacae	VGH	0.060	1.00	0.030	0.12
Enterobacter cloacae	K	0.03	0.50	0.015	0.06
Enterobacter cloacae	MOR	0.12	4.00	0.250	0.12
Serratia marcescens	MOR	0.12	8.00	0.060	0.12
Serratia marcescens	CMC	4.00	>16.00	2.000	16.00
Serratia marcescens	IO	0.12	16.00	0.120	1.00
Morganella morganii	VGH	0.12	8.00	0.060	0.25
Morganella morganii	CMC	0.03	2.00	0.060	0.50
Morganella morganii	MOR	0.03	2.00	0.030	0.12
Proteus rettgeri	IO	0.06	1.00	0.120	0.12
Providencia stuartii	CMC	4.00	>16.00	4.000	16.00
Citrobacter diversus	K	0.03	0.50	0.015	0.06
Pseudomonas aureus	K	4.00	16.00	1.000	4.00
Pseudomonas aureus	VGH	4.00	>16.00	2.000	16.00
Pseudomonas aureus	CMC	2.00	8.00	0.500	2.00
Staphylococcus aureus	VGH	0.12	1.00	0.120	0.12
Staphylococcus aureus	K	0.12	0.25	0.120	0.12
Staphylococcus aureus	CMC	2.00	16.00	4.000	2.00
Streptococcus faecalis	UCI	2.00	16.00		1.00
Streptococcus faecalis	VGH	2.00	16.00	2.000	4.00
Streptococcus faecalis	CMC	2.00	16.00	2.000	4.00
Escherichia coli	ATCC	0.03	0.50	0.015	0.06
Staphylococcus aureus	ATCC	0.12	1.00	0.120	0.12

[a] Medium: Mueller–Hinton; incubation: 18 h, 35 °C; method: agar dilution. [b] Source of clinical isolate.

Table VI—Atomic Charge Calculations[a,b]



X	Charge on		
	X	Ring	NH_2
F_2CHO	−0.101	+0.058	+0.041
Br	+0.036	−0.081	+0.048
H	+0.129	+0.029	+0.035
Cl	−0.040	−0.004	+0.046
F	−0.110	+0.071	+0.038
CH_3	+0.065	−0.096	+0.032
CH_3O	−0.037	+0.008	+0.031

[a] AM1 program calculations. [b] The values are the sums of the NH_2 (N + 2H), the ring atoms and Hs, and the 4-substituent.

in 15 mL of DMF was added 0.16 g (1.5 mmol) of sodium carbonate under nitrogen. The mixture was stirred at room temperature for 30 min and heated at 100 °C for 30 min. The reaction was followed by TLC (chloroform:methanol, 95:5). The reaction mixture was evaporated to dryness and ethanol was added to the residue. The yellow

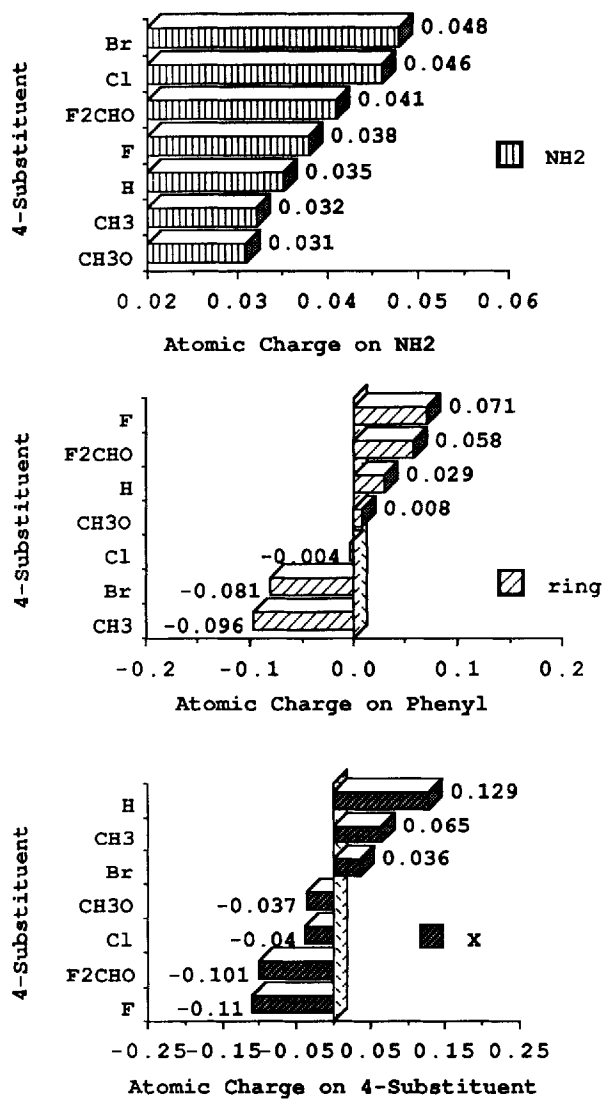


Figure 1—Comparison of the atomic charges versus the 4-substituent.

solid was collected by filtration, washed with water, and then dried to give 0.35 g of **3a** (88%), mp 122.5–125.5 °C; ^{1}H NMR (DMSO-d_6): δ 1.26 (3H, t, J = 7 Hz, CH_3), 4.2 (2H, q, J = 7 Hz, OCH_2), 6.9–7.8 (7H, m, Ar-H), 7.25 (1H, t, J = 72.8 Hz, $OCHF_2$), and 8.52 ppm (1H, s, =*CH*—N—); IR (KBr): 1571 cm^{-1} (ester); MS (*m/e*): 396 (M^+).

Anal.—Calc. for $C_{19}H_{13}F_4NO_4$ (MW = 395.31): C, 57.3; H, 3.31; N, 3.54; F, 19.2. Found: C, 57.4; H, 3.23; N, 3.29; F, 19.0.

1-[2-(Difluoromethoxy)phenyl]-6,7-difluoro-1,4-dihydro-4-oxo-3-quinolinecarboxylic acid (3d)—A solution of **4a** (0.36 g, 0.91 mmol) in a mixture of acetic acid (2.0 mL), water (1.6 mL), and conc. H_2SO_4 (0.5 mL) was refluxed 1.5 h. The resulting suspension was cooled in an ice bath and the crystals were filtered, washed with cold water, and dried to give **3d** in a yield of 0.3 g (91%), mp 197–199 °C; ^{1}H NMR (DMSO-d_6): 7.15–8.39 (6H, ArH), 7.23 (1H, t, J = 72.7 Hz, —$OCHF_2$), 8.86 (1H, s, =*CH*—N—), and 14.48 ppm (1, s, —COOH); IR (KBr): 1725 cm^{-1} (C=O), 1615 cm^{-1} (O-C-C-C); MS (*m/e*): 368 (M^+).

Anal.—Calc. for $C_{17}H_9F_4NO_4$ (MW = 367.256): C, 55.6; H, 2.47; N, 3.81; F, 20.7. Found: C, 55.4; H, 2.38; N, 3.69; F, 20.4.

1-[2-(Difluoromethoxy)phenyl]-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinolinecarboxylic acid (4a)—A mixture of **4d** (0.18 g, 0.5 mmol) and anhydrous piperazine (0.2 g, 2.32 mmol) in 1.5 mL of dry pyridine was heated at 90 °C for 4 h. The pyridine was removed under reduced pressure and the residue was treated with methanol and ether. The solid was filtered, and then washed with H_2O and dried to give **4a** in a yield of 0.11 g (50%), mp 212–214 °C; ^{1}H NMR (DMSO-d_6): 2.76–2.93 (9H, m, piperazine —CH_2), 6.2 (1H, d, J = 7 Hz, ArH), 7.27 (1H, t, J = 72.6 Hz, $OCHF_2$), 7.5–8.0 (m, ArH), and 8.73 ppm (1H, s, =*CH*—N—); IR (KBr): 1627 (C=O); MS (*m/e*): 434 (M^+).

Anal.—Calc. for $C_{21}H_{18}F_3N_3O_4$ (MW = 433.386): C, 58.2; H, 4.19; N, 9.70; F, 13.1. Found: C, 57.7; H, 4.13; N, 9.53; F, 13.0.

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Acknowledgments

We wish to thank Dr. L. Gehrlein for the use of his spectral and analytical facilities, Dr. T. Dunne and Mr. G. Morton for NMR discussions, and Dr. M. Siegal and Mr. K. Angyal for mass spectral interpretations. We also wish to thank Ms. E. Fromwiller for the preparation of this manuscript. W. Xiao was an Industrial Postdoctoral Fellow from the Shanghai Institute of Organic Chemistry, Shanghai, China (1986–1988), and R. Krishnan was an Industrial Postdoctoral Fellow (1984–1986).