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A Synthetic Approach to Carbon-14 Labeled Anti-bacterial Naphthyridine and Quinolone Carboxylic Acids.

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SUMMARY

Labeled versions of (S)-clinafloxacin (1) and two naphthyridine carboxylic acid anti-bacterial compounds 2 and 3 which are currently in development were synthesized. Preparations started from hitherto unknown bromo compounds 22 and 10, from which the corresponding ^{14}C-labeled aromatic carboxylic acids 23 and 12 were generated by metal-halogen exchange followed by carboxylation reaction. Details of these preparations are given.

Key words: ^{14}C-labeled antibacterial naphthyridine and quinolone carboxylic acids.

INTRODUCTION

In the past few years, there has been a surge of interest in constructing more potent variants of fluorinated anti-bacterial naphthyridine and quinolone carboxylic acids (1). The worldwide quest for these agents, has yielded some clinically significant compounds showing enhanced potency against gram-negative and gram-positive organisms. Like nalidixic acid these compounds inhibit DNA gyrase, a crucial topoisomerase enzyme in bacterial DNA replication and cell division (2).

Our laboratories have produced some drug candidates in this class, and further development required labeled analogs for pharmacokinetics and metabolism investigations. We wanted a preparative approach that would be applicable with minor modifications, to any desired compound in the group. A strategy was developed and applied to the syntheses of 1,4-dihydro-4-oxo[4-^{14}C] versions of the antibacterial compounds: (S)-7-(3-amino-1-pyrrolidinyl)-8-chloro-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-3-quinoline carboxylic acid ((S)-clinafloxacin hydrochloride, CI-960,

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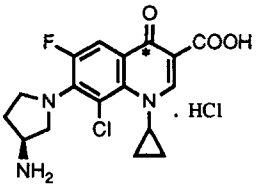
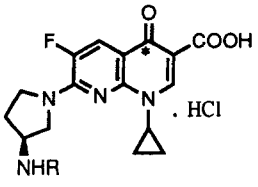
1) (3), (S)-7-(3-amino-1-pyrrolidinyl)-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylic acid (PD 131628, 2) (4), and [S-(R*, R*)]-7-[3-[(2-amino-1-oxopropyl)amino]-1-pyrrolidinyl]-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylic acid (CI-990, 3)(4).

Figure 1



(1) (S)-Clinafloxacin



(2) **PD 131628** R = H

(3) **CI-990** R = (S)-alanyl-

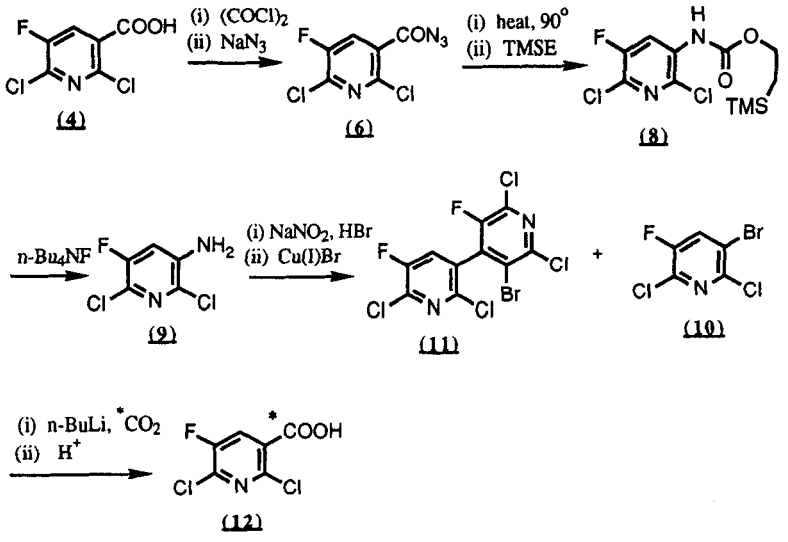
Since aromatic carboxylic acids or derivatives thereof are convenient starting materials (5) from which these antibacterial compounds could be prepared, we decided to make labeled aromatic carboxylic acids from bromo precursors by metal halogen exchange followed by carboxylation protocol using $^{14}CO_2$. From such labeled acids, labeled PD 131628 , CI-990 and (S)-clinafloxacin (CI-960) would be synthesized. First, the bromo precursors 3-bromo-2,6-dichloropyridine (10) and 1-bromo-3-chloro-2,4,5-trifluorobenzene (22) was each made from 2,6-dichloro-5-fluoropyridine-3-carboxylic acid and 1-bromo-2,4,5-trifluorobenzene.

RESULTS AND DISCUSSION

Sequential alkylation of activated aromatic carboxyl group with lithium dianion of ethyl hydrogen malonate, homologation of resulting β-keto ester to an enol ether, substitution with an amine, followed by intramolecular cyclization, has previously (6) been applied to the construction of naphthyridine and quinolone nuclear structures of these antibacterials. Modification of substituents thereafter yielded desired antibacterial compounds. Our present approach incorporated this sequence. We developed a method for the preparation of unknown bromo compounds 3-bromo-2,6-dichloro-5-fluoropyridine (10) and 1-bromo-3-chloro-2,4,5-trifluorobenzene (22) and converted each to the corresponding carboxylic acid. The procedure due to Poulter et al (7) was applied to 2,6-dichloro-5-fluoro-3-pyridinecarboxylic acid (4) to make the acyl chloride, then the

azide 6 which was thermally rearranged to the isocyanate 7 and treated with 2-trimethylsilyl ethanol (TMSE) to give the carbamate 8. Deprotection with n-Bu4NF in THF and repeated crystallization from hexane furnished the free 3-amino-2,6-dichloro-5-fluoropyridine (9).

Scheme 1

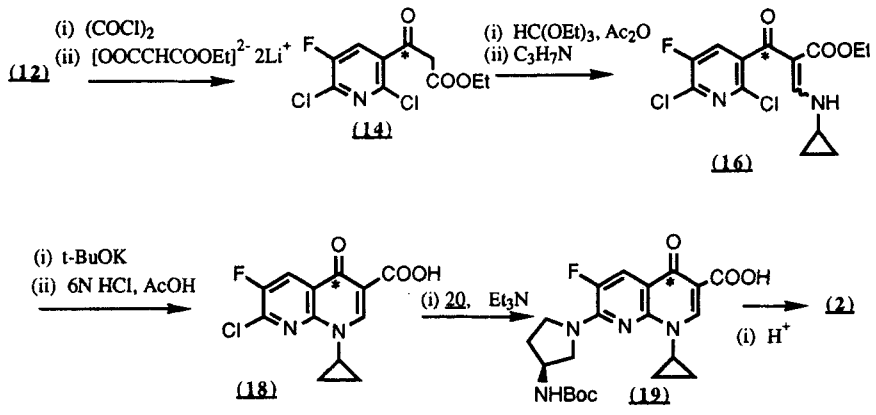


(4) (6) (8) (9) (11) (10) (12)

Sandmeyer reaction on compound 9 required a low temperature condition (-30° to -20°C) and high purity grade cuprous bromide to obtain the desired 3-bromo-2,6-dichloro-5-fluoropyridine (10). The bromo compound 10 was obtained in 89% yield as a (9:1) mixture with 3-bromo-2,2′, 6,6′–tetrachloro-5,5′–difluoro-3′–bipyridine (11), from which it was easily separated by crystallization. Using reagent grade cuprous bromide or reaction temperature higher than -20°C gave exclusively the bipyridine compound 11. There is literature precedent (8) for the observed preferential formation of bipyridine when Cu(II) is added to a diazonium salt. We believe the result obtained with reagent grade cuprous bromide is in part attributable to reagent contamination with Cu(II), and the inherent reactivity of the system.

To prepare 1-bromo-3-chloro-2,4,5-trifluorobenzene (22), commercial 1-bromo-2,4,5-trifluorobenzene (21) was deprotonated with lithium diisopropylamide (LDA) and chlorinated by the addition of hexachlorocyclopentadiene.

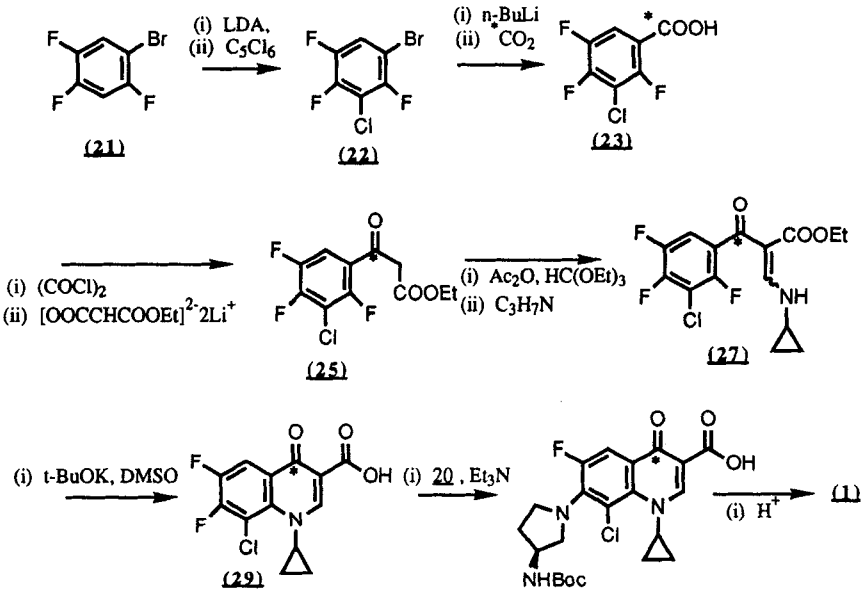
Scheme II



Compound 22 was obtained in 65% yield by distillation. The preparation of 2,6-dichloro-5-fluoro-3-pyridine-[^{14}C]carboxylic acid (12) was achieved in nearly quantitative yield by treatment of compound 10 with n-BuLi at -95°C, followed by carboxylation with $^{14}CO_2$ generated by the action of conc. H_2SO_4 on $Ba^{14}CO_3$. By the same protocol, at -78°C, 3-chloro-2,4,5-trifluoro[carboxyl-^{14}C]benzoic acid (23) was prepared. The carboxylic acids 12 and 23 were converted to their respective acid chlorides via treatment with oxalyl chloride in toluene at room temperature and neat thionyl chloride at 80°C, respectively. In our hands, alkylation of the acid chlorides 13 and 24 with the lithium dianion of ethyl hydrogen malonate provided superior access and yield of desired β-keto esters 14 and 25. Sequential transformations as in **scheme II**, yielded from 14 the 7-chloro-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-1,8-[4-^{14}C]naphthyridine-3-carboxylic acid (18), and 8-chloro-1-cyclopropyl-6,7-difluoro-1,4-dihydro-4-oxo[4-^{14}C]quinolone carboxylic acid (29) was obtained from 25 by a similar sequence.

Presumably due to activation by 4-oxo group, selective substitution at C-7 in these compounds 18, and 29 was easily accomplished by reaction with 1,1-dimethylethyl (S)-3-pyrrolidinylcarbamate (20)(4) in refluxing acetonitrile. Removal of Boc protection by treatment with HCl gas followed by crystallization gave PD 131628 (2) from compound 18, while (S)-clinafloxacin hydrochloride (1) was obtained from compound 29. Similarly, refluxing 1,1-dimethylethyl[R-(R*, S*)]-[1-methyl-2-oxo-2-(3-pyrrolidinyl)]carbamate (32)(4), in acetonitrile with 7-chloro-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-1, 8-[4-^{14}C]naphthyridine-3-carboxylic acid (18), and deprotection

Scheme III



followed by crystallization furnished CI-990 (3). Preparation of the unlabeled compound preceeded each step in the radiolabeled preparations. The unlabeled preparations permitted spectral identification of all carbon-14 labeled compounds made and measurements were in agreement with published data.

In conclusion, we have shown that the classical Curtius, Sandmeyer transformations, metal-halogen exchange and carboxylation reactions provide a rapid, facile and practical means to replace unlabeled with ^{14}C-labeled carboxylic acid group. The accessibility of labeled aromatic carboxylic acids by this means should facilitate, as in our case, the ready preparation of a variety of labeled antibacterial compounds. We believe that the added advantage of the inexpensive way of making ^{14}C-labeled precursors to these antibacterial compounds should make this approach even more attractive.

EXPERIMENTAL

General Methods.

All reactions involving the use of organolithium compounds were carried out in dried flasks and under inert atmosphere. IR spectra were recorded on a Perkin-Elmer 462 spectrometer. UV spectra were obtained on a Perkin-Elmer Lambda 3 instrument.

^{1}H-NMR spectra were recorded with Varian (EM 390) 90 mHz spectrometer, or a Gemini 200 mHz spectrometer. Radiochemical purity of every labeled compound was determined by tlc radiochromatogram with Bioscan 200 imaging scanner. Radiochemical counting was performed on a Parkard 574 liquid scintillation counter using Beckman Ready-Solv MP cocktail. HPLC analyses of final products were performed on a Waters Associates 600E system with on line Applied BioSystems 1000S diode array detector and either a β-RAM radioactivity detector or Radiomatic series A-200 radioactivity flow detector. Column chromatography was carried out on a Merck Kieselgel 60 (230μ).

3-Amino-2,6-dichloro-5-fluoropyridine(9)

To a suspension of 2,6-dichloro-5-fluoro-3-pyridinecarboxylic acid (20 g, 95.23 mmol) oxalyl chloride (24 mL) in anhydrous toluene (100 mL) under N_2 atmosphere was added a drop (approx. 5 μl) of anhydrous dimethylformamide (DMF). It was stirred for 1.50 h and the yellow solution was decanted into a 500 mL flask and solvent was stripped. It was azeotroped twice with toluene (50 mL) to give an oil. The oil in dry acetone (450 mL) was added dropwise to a vigorously stirring ice cold solution of NaN_3 (38.0 g, 584.5 mmol) in water (90 mL). Following complete addition, it was stirred for a further 15 min at ice-bath temperature, then diluted with hexane (800 mL), and the organic phase was separated. The aqueous phase was extracted with hexane (2X250 mL), and the combined organic extract was dried over $MgSO_4$ and evaporated *in vacuo* (caution!) to give an oil. The oil was taken up in toluene (150 mL), and after 1h at 90°, 2-trimethylsilylethanol (24 mL) was added. After stirring for 6 h at 90°C the solvent was removed, and the residue was taken up in ethyl acetate (400 mL) washed with 1N NaOH (100 mL), then brine, and dried over $MgSO_4$ to give a brown oil. The oil was dissolved in THF (250 mL), and tetrabutylammonium fluoride (1.0 M solution in THF) (28 mL) was added. After stirring for 2 h at 50°C, the solvent was removed and the product was taken up in ether (500 mL), washed with 1.0 N NaOH solution, then brine, and dried on $MgSO_4$. The solvent was stripped to give a brown solid. The solid was dissolved in hexane, bleached with charcoal and crystallized from hexane at refrigeration temperature to give free amine (12.6 g). Proton nmr (δ) 7.0, (d), 4.9, brs. exc. D_2O. MS EI+, m/z (rel. abund): M^+ 180 (100), 159 (6.40), 144 (13.27), 124 (19.91), 117 (19.19), 97 (23.34), 91 (27.61) , 82 (20.26), 70 (7.55), 62 (7.70).

3-Bromo-2,6-dichloro-5-fluoropyridine(10)

3-Amino-2,6-dichloro-5-fluoropyridine (4.11 g, 22.7 mmol) was dissolved at room temperature in 48% HBr (60 mL) and cooled to -20°C (CO_2-ethylene glycol bath). A solution of $NaNO_2$, (1.80 g, 26.1 mmol) in water (50 mL) pre-cooled to -20°C, was added dropwise in 10 min to the solution while stirring vigorously with a mechanical stirrer. After stirring for an additional 45 min, cuprous bromide (99.999%, 4.07 g, 28.4 mmol) in 60 mL of 48% HBr, pre cooled to -50°C, was added (caution!) below the surface of reaction via a long stem funnel. The yellow solid which separated in a few minutes was immediately extracted with ether (500 mL), washed with sodium bisulfite, then brine, and dried. The oil (85:15 product to dimer) obtained was crystallized twice from pet. ether at refrigeration temperature to give pure compound (6.8 g). NMR ($CDCl_3$) 7.80 (d). MS EI+ m/z M^+ 245, and other ions at 210, 164, 129, 94 and 68.

1-Bromo-3-chloro-2,4,5-trifluorobenzene (22).

To a solution of diisopropylamine (27.8 g, 275 mmol) in dry THF (200 mL) at -20°C was added n-BuLi (2.1 M in hexane, 121 mL, 255 mmol) and stirred for 1h under an atmosphere of nitrogen. It was added over 40 min to a solution of 2,4,5-trifluorobenzene (52.75 g, 250 mmol) in 400 mL of dry THF maintained at -78°C under an atmosphere of nitrogen. After addition was completed, it was stirred for a further 20 min and the resulting solution was added in 6 min to a solution of hexachlorocyclopentadiene (75.1 g, 275 mmol) in dry THF (200 mL) at -78°C. The solution turned green, and after 30 min the reaction was quenched by the dropwise addition of trifluoroacetic acid (45 mL); then poured onto water (1000 mL) and extracted with hexane (2X500 mL). The organic phase was washed with water, brine, dried on $MgSO_4$, and evaporated under reduced pressure to give a brown liquid. Butylated hydroxy toluene (2.0 g) was added to the brown liquid prior to distillation under reduced pressure to give (25.6 g). NMR ($CDCl_3$) δ 7.6 (m, 1H).

Ethyl 2, 6-dichloro-5-fluoro-[carbonyl-^{14}C]nicotinylacetate (14)

To a solution of 3-bromo-2, 6-dichloro-5-fluoropyridine (1.744 g, 7.12 mmol) in dry THF (35 mL), at -92°C (liq. N_2-toluene) was added n-BuLi (1.6 M, 4.45 mL) dropwise in 10 min. It was stirred at this temperature for an additional 45 min under dry N_2

atmosphere and frozen in liq nitrogen. It was transferred to a vacuum line connected to CO_2 generator. The labeled carbon dioxide was generated by the dropwise addition of conc. H_2SO_4 (10 mL) to $Ba^{14}CO_3$ (1.3932 g). The reaction vessel was evacuated at liq. N_2 temperature and $^{14}CO_2$ was admitted by suction. The liq. N_2 bath was then replaced with dry ice-acetone bath (-78°C), and the reaction was allowed to proceed overnight. It was acidified with 6 N. HCl, extracted with ether (3X50 mL), and dried. The solvent was stripped to give a brownish solid, 2,6-dichloro-5-fluoropyridine-[^{14}C]carboxylic acid (12) (1.45 g, 97%, radiochemical purity 80%). NMR ($CDCl_3$) 8.60 (d), 10.05 (brs).

2, 6-Dichloro-5-fluoropyridine-[^{14}C]carboxylic acid (1.419 g, 6.75 mmol) was suspended in dry toluene (40 mL) and excess oxalyl chloride (5.0 mL) was added, followed by a drop of dry dimethylformamide (DMF). After 2 h stirring the solvent was removed *in vacuo,* and the residue was azeotroped with toluene twice to give an oil. The oil was dissolved in dry THF (15 mL) and added dropwise to the dilithium salt of ethyl hydrogen malonate prepared as follows. Ethyl hydrogen malonate (1.56 g, 11.82 mmol, 1.75 molar equiv) and biquinoline (3.0 mg) in dry THF (32 mL) was cooled to -30°C (dry ice-ethylene glycol bath) for 45 min and n-BuLi (2.5M, 9.46 mL) was added till permanent pink coloration was observed. It was cooled to -78°C and the above solution of acid chloride in dry THF was added dropwise with stirring. It was stirred for a further 30 min after completion of addition, and then allowed to warm to room temperature. It was poured onto 2N HCl and stirred for 5 min and then extracted with ether (3X100 mL). The combined organic extract was washed with sat'd $NaHCO_3$ (2X 50 mL), water, and then brine. It was crystallized from hexane to give an inseparable mixture of keto-enol tautomers 14 (1.6335g). NMR ($CDCl_3$) δ 1.24 (tr, 3H), 1.33 (tr, 3H), 4.22 (m, 4H), 5.82 (s, 1H), 7.5 (d, 1H), 12.55 (s, 1H).

Ethyl (3-chloro-2,4,5-trifluoro-[carboxyl-^{14}C]benzoyl) acetate (25)

1-Bromo-3-chloro-2,4,5-trifluorobenzene (2.816 g, 11.48 mmol) in anhydrous ether (40 mL) at -78°C was treated with n-BuLi (1.6M in hexane, 7.175 mL, 11.48 mmol) added dropwise over 10 min. It was carboxylated with $^{14}CO_2$ generated from $Ba^{14}CO_3$ (2.269 g, sp. act. 13.04 mCi/mmol) and worked up as in the preceeding experiment to give 23 (2.32 g, 96%) and used as such without further purification. 3-Chloro-2,4,5-trifluoro-[carboxyl-^{14}C]benzoic acid (2.32 g) was dissolved in freshly distilled thionyl

chloride (15 mL) and heated at 90°C for 8 h under nitrogen atmosphere. It was cooled to room temperature, diluted with dry toluene (30 mL), and solvent and excess reagent were removed under reduced pressure to give an oil (acid chloride). A solution of acid chloride in THF (15 mL) was added dropwise to a lithium dianion generated from ethyl hydrogen malonate (1.825 g, 13.83 mmol), biquinoline (3.0 mg) , n-Buli (2.1M in hexane, 13.16 mL) as in preceeding experiment. Usual workup and crystallization gave inseparable keto-enol tautomers (25) (2.2 g). NMR ($CDCl_3$) δ 1.26 (tr 3H) 1.37 (tr, 3H) 3.96 (d, 2H), 4.25 (m, 2H), 5.84 (s,1H), 7.72 (m, 1H), 12.76 (s, 1H).

7-Chloro-1-cyclopropyl-1,4-dihydro-6-fluoro-4-oxo-1,8-[4-^{14}C]naphthyridine-3-carboxylic acid (18).

A solution of ethyl 2,6-dichloro-5-fluoronicotinylacetate (1.633 g, 5.83 mmol) in acetic anhydride (1.4115 g, 13.82 mmol) and triethyl orthoformate (1.296 g, 8.75 mmol) was heated under nitrogen atmosphere at 120°-130°C for 2 h, and solvent was removed under reduced pressure. The oil was taken up in anhydrous ether (30 mL), and cyclopropylamine (349.2 mg, 424 µl, 6.11 mmol) was added. After stirring overnight at room temperature, it was diluted with THF (20 mL), and t-BuOK (685 mg, 6.11 mg) was added. It was refluxed for 1h, cooled to room temperature, acidified with acetic acid, and extracted into chloroform (3X150 mL), and the combined extract was washed with water and then brine. The crude solid was taken up in a mixture of acetic acid: 3N HCl (80:20) (100 mL) and refluxed for 4 h. The solvent was stripped, and the solid was triturated with ether and filtered to give compound 18 (1.055 g). NMR ($CDCl_3$) δ 1.13 (brs, 2H), 1.35 (d, 2H), 3.80 (m, 1H), 8.70 (d, 1H), 8.90 (s, 1H), 14.0 (brs, 1H exc. D_2O).

8-Chloro-1-cyclopropyl-6,7-difluoro-1,4-dihydro-4-oxo[4-^{14}C]-3-quinoline carboxylic acid (29).

A mixture of ethyl (3-chloro-2,4,5-trifluorobenzoyl)acetate (1.825 g), acetic anhydride (1.73 mL), and triethyl orthoformate (1.87 mL) was heated at 120-130°C for 2 h, and the red solution was stirred at 60°C overnight. Solvent was removed *in vacuo* , and the residue was redissolved in dry DMSO (10 mL) followed by the addition of cyclopropylamine (495.5 µl). After stirring for 6 h t-BuOK (768 mg) was added in one portion, stirred for additional 1.5 h, acidified by dropwise addition of

acetic acid, and poured onto water. It was extracted with ethyl acetate (2X200 mL), and the combined extract was washed with water, then brine, and dried. The product crystallized from hexane to give (1.6 g). It was taken up in acetic acid:6N HCl (80:20) (35 mL) and heated at 110-120°C for 5 h. Solvent was removed under reduced pressure, and the residual solid was transferred into hot hexane with methylene chloride from which it crystallized at room temperature to give 29 (1.53 g). NMR ($CDCl_3$) δ 1.13 (brs, 2H), 1.33 (d, 2H), 4.32 (m, 1H), 8.27 (tr, 1H), 8.95 (s, 1H), 14.05 (brs, 1H).

(S)-7-(3-Amino-1-pyrrolidinyl)-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-1,8-[4-^{14}C]naphthyridine-3-carboxylic acid hydrochloride (PD 131628) (2).

A solution of 7-chloro-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-1,8-[4-^{14}C]naphthyridine-3-carboxylic acid, (489 mg, 1.73 mmol), 1,1-dimethylethyl (S)-3-pyrrolidinylcarbamate (322 mg 1.73 mmol) and triethylamine (289.5 µl , 2.07 mmol) in acetonitrile (30 mL) was refluxed for 30 min under an atmosphere of nitrogen. The solvent was removed under reduced pressure, and the residue was dissolved in THF (25mL) and 6N HCl (5 mL) was added. After stirring for 3 h, solvent was removed and the product was crystallized from methanol-ether to give 2 (700 mg), specific activity (12.76 mCi/mmol), TLC silica gel 60 F-254 ($CHCl_3$:EtOH:NH_4OH, 12:9:4), Rf 0.20, Radiochemical Purity 99.4% and chemical Purity 99.7%. NMR (DMSO-d_6) δ 1.09 (m, 2H), 1.20 (m, 2H), 2.19 (m, 1H), 2.33 (m, 1H), 3.67 (m, 1H), 3.98 (m, 5H), 8.05 (d, 1H), 8.44 (bs, 3H), 8.58 (s,1H).

(S)-7-(3-Amino-1-pyrrolidinyl)-8-chloro-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-[4-^{14}C]-3-quinoline carboxylic acid hydrochloride {(S)-clinafloxacin hydrochloride} (1)

A solution of 8-chloro-1-cyclopropyl-6,7-difluoro-1,4-dihydro-4-oxo[4-^{14}C]-3-quinoline carboxylic acid (1.55 g, 5.16 mmol), 1,1-dimethylethyl-(S)-pyrrolidinylcarbamate (1.03 g, 5.58 mmol), and triethylamine (1.8 mL) in anhydrous DMSO was heated at 100°C for 1h. It was poured onto cold water, extracted with ethyl acetate (2X100 mL), washed with water, and dried on Na_2SO_4. The solid product was dissolved in THF (10 mL), treated with 2.0 mL of 12N HCl, and stirred for 45 min at room temperature. It was diluted with toluene (40 mL) and then stripped to dryness. The product was dissolved in methanol and transferred into boiling isopropyl alcohol,

bleached with charcoal, concentrated, and set aside to crystallize at room temperature to yield 1 (780 mg, specific activity 12.93mCi/mmol), TLC silica gel 60F-254, (EtOH:NH_4OH; 25:5), Rf 0.17, Radiochemical Purity 99.15% and chemical purity 99+%. NMR (DMSO-d_6) δ 1.0 (brs), 1.25 (brs), 2.1 (m), 2.35 (m), 3.7-4.0 (m), 4.3(m), 7.90 (d), 8.89 (s).

[S-(R*, R*)]-7-[3-[(2-Amino-1-oxopropyl)amino]-1-pyrrolidinyl]-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-1,8-[4-^{14}C]naphthyridine-3-carboxylic acid hydrochloride (CI-990) (2)

A solution of 7-chloro-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-1,8-[4-^{14}C]naphthyridine-3-carboxylic acid (513 mg 1.81mmol), 1,1-dimethylethyl[R-(R*, S*)]-[1-methyl-2-oxo-2-(3-pyrrolidinyl)]carbamate (714 mg, 2.77mmol and triethyl amine (549 mg, 0.07 mmol) in acetonitrile (27 mL) was refluxed for 4 h and cooled to room temperature. The solid which separated was filtered, then washed with acetonitrile, and dried under high vaccum to give Boc protected CI-990 (630 mg). A suspension of the entire product (630 mg) in 1N HCl (10.5 mL) and ethanol (10.5mL) was stirred at room temperature for 1h and then heated for 2 h at reflux. It was frozen in liquid nitrogen and lyophilized. The crude solid was chromatographed on silica gel eluted with ($CHCl_3$:CH_3OH:H_2O 65:25:4), and elution was monitored by tlc. The combined fractions was evaporated *in vacuo* , and the compound was redissolved in 1N HCl, frozen and lyophilized. Crystallization from ethanol-ether gave CI-990 (445 mg), specific activity (22.14 μCi/mg), TLC silica gel 60 F-254, (EtOH:NH_4OH; 25:5), Rf 0.23, RCP 99.32%, CP 99+%. NMR (DMSO-d_6) δ 1.13 (m, 4H), 1.33 (d, 3H), 2.07 (m, 1H), 2.34 (m,1H), 3.90 (m, 6H), 4.47 (m, 1H), 8.01 (d, 1H), 8.04 (s, 2H), 8.59 (s, 1H), 8.84 (d, 1H) and 15.81 (br ,1H) .

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