Journal of Labelled Compounds and Radiopharmaceuticals—Vol. XXXIX, No. 1

SYNTHESIS OF MULTIDRUG RESISTANCE MODULATOR LY335979 LABELED WITH DEUTERIUM AND TRITIUM

Boris A. Czeskis

Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN 46285

SUMMARY

Dideutero and ditritioisotopomers of the multidrug resistance modulator LY335979 were prepared by initial bromination of 5-hydroxyquinoline under acidic conditions followed by Mitsunobu coupling of 6,8-dibromo-5-hydroxyquinoline with (*S*)-glycidol. Opening of the resulting epoxide with dibenzosuberylpiperazine LY335995 resulted in dibromoanalog of LY335979, which was finally reductively debrominated with deuterium or tritium in the presence of palladium on carbon.

Key words: multidrug resistance modulator, LY335979, deuterium labeling, tritium labeling.

INTRODUCTION

Multiple drug resistance is a serious problem in the treatment of neoplastic diseases. Many anticancer agents such as anthracyclines, vinca alkaloids, epipodophyllotoxins, colchicine, and taxol, are not effective against tumors which possess intrinsic or acquired resistance. One mechanism for the resistance involves the overexpression of P-glycoprotein which functions as an energy-dependent drug efflux pump.[1,2] Compounds which can bind to P-glycoprotein and block its transport function, have been called multidrug resistance modulators or resistance reversal agents. Recently, a novel multidrug resistance modulator LY335979 has been identified.[3,4] It circumvented drug resistance in human, mouse, and Chinese hamster cell lines by reducing cell proliferation.[4] It was shown that *anti*-geometry

CCC 0362–4803/97/010049–10

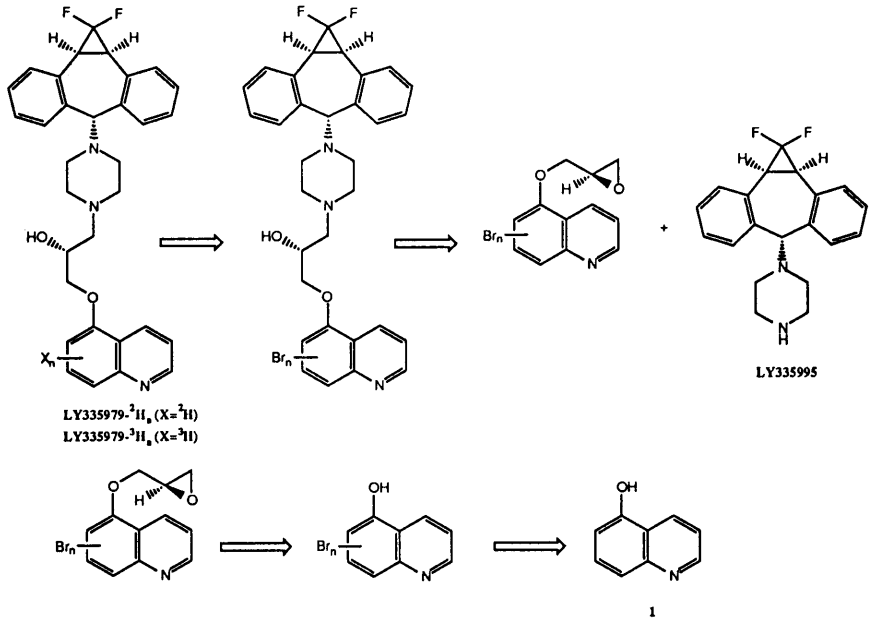
Received 29 June 1996
Revised 7 August 1996

of the dibenzosuberylpiperazine fragment and the (*R*)-configuration of secondary alcohol group are important for the potency of LY335979. This compound was synthesized by the coupling of dibenzosuberylpiperazine with epoxypropyloxyquinoline,[3] which derived from 5-hydroxyquinoline and chiral glycidyl nosylate. For drug disposition and mechanistic studies radiolabeled material of high specific activity was needed. Therefore it was necessary to find a method for the introduction of at least two tritium atoms in the molecule of LY335979. We report herein a synthesis of LY335979 labeled with deuterium and tritium.

DISCUSSION

After retrosynthetic analysis of LY335979, we decided to prepare its deuterated and tritiated

SCHEME 1

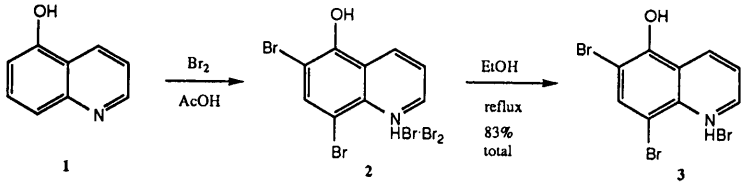


derivatives by the reductive debromination of the corresponding aromatic bromide, which could be obtained by initial bromination of 5-hydroxyquinoline (**1**), and following attachment of the necessary fragments to bromoquinolinol (Scheme 1).

There are many examples of halogenation of hydroxyquinolines in the literature.[5,6] But, to the best of our knowledge, only a few early attempts to brominate the 5-hydroxyquinoline were published, which resulted in the formation of a mixture of mono- and dibromides.[7-10]

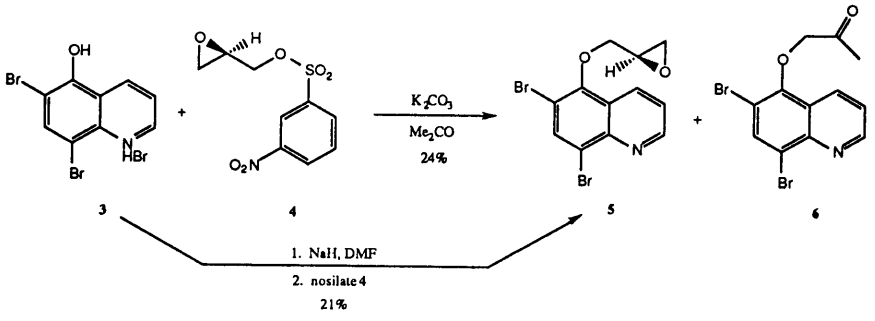
We investigated the bromination of 5-hydroxyquinoline (**1**) under several different conditions. Thus, reaction of **1** with two or three equivalents of bromine in the presence of sodium hydroxide, as it was published for hydroxypyridine,[11] appeared to be not regioselective and afforded a labile mixture of at least three compounds: dibromide, monobromide, and starting material. Different results were obtained when the bromination was performed under acidic conditions (Scheme 2). Reaction of **1** with 3 equivalents of bromine in acetic acid resulted in exclusive formation of the 6,8-dibromo derivative. This compound can be isolated as a free base if the reaction mixture is treated with aqueous sodium acetate, but the product is rather unstable and we were not able to use it in further transformations. If acetic acid is removed after completion of the reaction, quinolinium bromide perbromide **2** is quantitatively formed. It is easily decomposed into the corresponding hydrobromide (**3**) upon refluxing with ethanol.

SCHEME 2



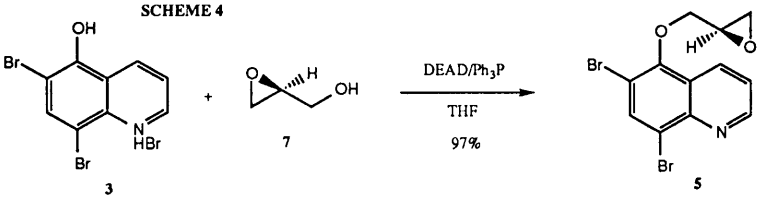
The next step included attachment of chiral glycidyl fragment to the quinolinol **3**. Reaction of **3** with nosylate **4** in the presence of potassium carbonate in acetone under conditions described earlier[3] unexpectedly gave a mixture (3:2) of epoxide **5** and ketone **6** in low yield (Scheme 3). The formation of **6** may result from the rearrangement of the epoxide **5** which

SCHEME 3



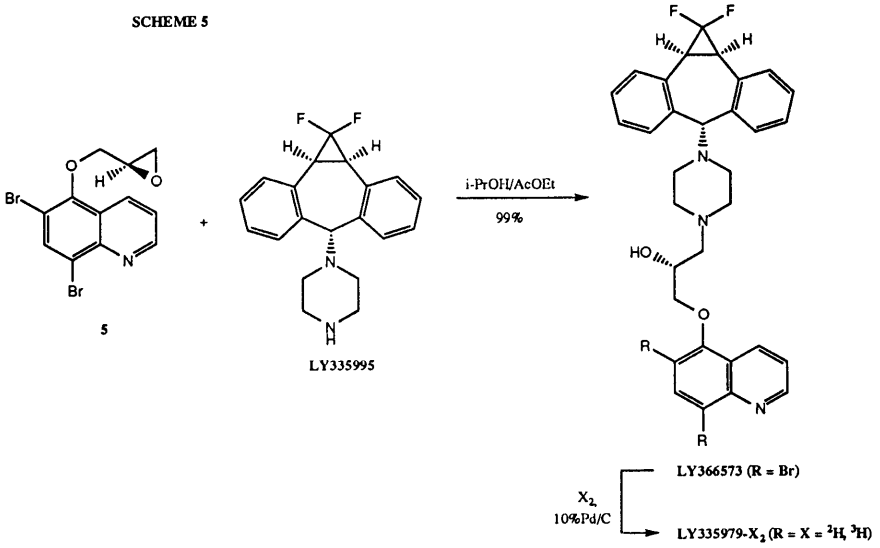
normally occurs in the presence of Lewis acids including salts of lithium and magnesium.[12-15] Condensation of the sodium phenolate generated from **3** and sodium hydride, with nosylate **4** in dimethylformamide furnished the desired product **5** in poor yield as well. The best method for the coupling appeared to be Mitsunobu reaction of hydroxyquinoline **3** with (*S*)-glycidol **7**, which gives the desired product **5** in nearly quantitative yield (Scheme 4). The triphenylphosphine-diethyl azodicarboxylate (DEAD) complex is prepared separately and added to phenol **3**, followed by an addition of the alcohol **7**. An excess of the complex is needed and probably serves as an acceptor of HBr, liberating the free base from the quinolinium salt under mild conditions (attempts to use equimolar amounts of all reagents gave a very low yield of **5**).

SCHEME 4



The next step included the epoxide opening with chiral piperazine LY335995[3] (Scheme 5). This reaction occurred smoothly when a mixture of ethyl acetate and isopropanol is used as a solvent, to give adduct LY366573 in high yield. Potentially, reductive debromination of

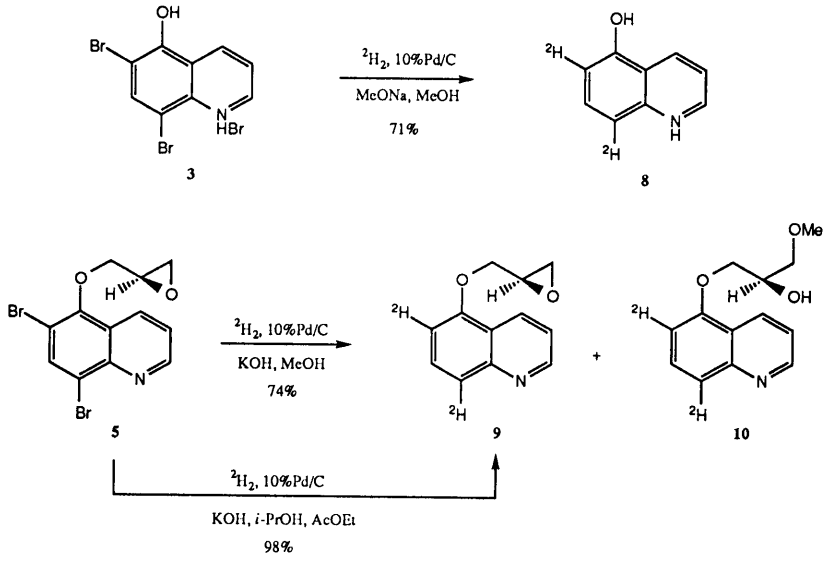
SCHEME 5



LY366573 could be a problem because 5-dibenzosuberyl group is known as a protective group for amines, and can be removed by hydrogenolysis on palladium.[16] However this group happened to be stable during deuteration under basic conditions in the presence of palladium catalyst and excess of potassium hydroxide. As a result dideuteroproduct LY335979-$^2H_2$ was obtained from dibromide LY366573 in good yield. Tritiation of dibromide LY366573 was accomplished under the same conditions to give radiolabeled product LY335979-$^3H_2$ of high radiochemical purity.[17]

Dibromoquinoline intermediates **3** and **5** were also converted into the corresponding deuterated compounds **8** and **9** which can be used for the preparation of the labeled analogs of LY335979 (Scheme 6). Deuteration of the hydrobromide **3** took place in the presence of sodium methoxide in methanol whereas this solvent was not suitable for epoxide **5** because of its partial nucleophilic opening which furnished the mixture of epoxide **9** and methoxyketone **10** in ~2:1 ratio. This problem was avoided when an ethyl acetate - isopropanol system was used as a solvent in conjunction with potassium hydroxide as a base, to give epoxide **9** selectively in high yield.

SCHEME 6



EXPERIMENTAL

The NMR spectra were obtained on a General Electric QE-300 at 300 (^{1}H) and 75 (^{13}C) MHz. Chemical shifts are reported in parts per million (ppm) downfield from tetramethylsilane. Microanalytical, IR, UV, MS, and optical rotation data were provided by the Physical Chemistry Research Department of the Lilly Research Laboratories. Flash chromatography was performed using silica gel 60 (230-400 mesh). Unless otherwise noted, HPLC was conducted on a Hitachi instrument with a UV detector at 240 nm; Zorbax SB-C18 column (4.6 mm x 25 cm); eluting with mobile phase consisting of 50% of 0.04M (0.5%) aqueous monobasic ammonium phosphate and 50% of acetonitrile at a flow rate of 1 mL/min.

6,8-Dibromo-5-hydroxyquinoline hydrobromide, 3:

To a stirred solution of 5-hydroxyquinoline (**1**) (1.45 g, 10.0 mmol) in acetic acid (15 mL) was added a solution of bromine (1.7 mL, 33.0 mmol) in acetic acid (5 mL) dropwise over a period of 10 min. The reaction mixture was stirred for 4.5 h at room temperature, then evaporated *in vacuo* and re-evaporated three times with toluene to give 5.437 g (100%) of 2,6-dibromo-5-hydroxyquinolinium bromide perbromide **2** as a fine orange solid. This product was suspended in ethanol (150 mL), refluxed for 5 min, and then partly concentrated *in vacuo* to leave *ca.* 100 mL of a suspension, which was cooled down to 0-5°C. The precipitate was collected, washed with cold ethanol and ether, and dried *in vacuo* to give 3.188 g (83%) of dibromide **3** as a light green solid: R_f = 0.65 (hexane/ethyl acetate, 1:1); IR (KBr): 720, 750, 801, 1174, 1301, 1410, 1542, 1590, 1623, 3074, 3438 cm^{-1}; UV (EtOH) $\lambda_{max}(\varepsilon)$: 332 nm (289), 248 nm (34330); ^{1}H-NMR (CD_3OD) δ 8.07 (dd, J = 8.5, 5.5 Hz, 1H, H-3), 8.51 (s, 1H, H-7), 9.16 (d, J = 5.5 Hz, 1H, H-4), 9.40 (d, J = 8.5 Hz, 1H, H-2); MS (FD) m/z(%): 303 (M^+-HBr, 100).

Analysis calc'd for $C_9H_6Br_3NO$: C, 28.16; H, 1.58; N, 3.65; Br, 62.45. Found: C, 28.14; H, 1.62; N, 3.63; Br, 62.20.

(*R*)-6,8-Dibromo-5-(2,3-epoxyprop-1-yloxy)-quinoline, 5:

To a solution of triphenylphosphine (2.39 g, 9.1 mmol) in tetrahydrofuran (8 mL) at 0-5°C (ice bath) was added a solution of diethyl azodicarboxylate (1.59 g, 9.1 mmol) in tetrahydrofuran (2 mL) dropwise. The resulting solution was stirred at 0-5°C for 5 min and then added dropwise to a suspension of phenol **3** (1.0 g, 2.6 mmol) in tetrahydrofuran (8.0 mL) followed by an addition of a solution of (*S*)-glycidol (**7**) (0.68 g, 9.2 mmol) in tetrahydrofuran (2 mL). The reaction mixture was stirred overnight at room temperature,

then evaporated *in vacuo*. Flash chromatography of the residue (40% ethyl acetate in hexane) gave 904 mg (97%) of ether **5** as white crystals: mp 100-102°C (from ethanol), R_f = 0.42 (hexane/ethyl acetate, 1:1); R_t = 11 min; $[\alpha]_D^{20}$= -7.58° (c 1.05, $CHCl_3$); IR (KBr): 769, 858, 904, 1068, 1217, 1337, 1384, 1451, 1579 cm^{-1}; UV (EtOH) $\lambda_{max}(\varepsilon)$: 303 nm (5176), 241 nm (42498); ^{1}H-NMR ($CDCl_3$) δ 2.80 (m, 1H, H-3'), 2.95 (m, 1H, H-3'), 3.49 (m, 1H, H-2'), 4.01 (dd, J = 11.0, 6.4 Hz, 1H, H-1'), 4.48 (dd, J = 11.0, 2.4 Hz, 1H, H-1'), 7.55 (dd, J = 8.4, 4.2 Hz, 1H, H-3), 8.20 (s, 1H, H-7), 8.59 (d, J = 8.2 Hz, 1H, H-4), 9.06 (d, J = 3.2 Hz, 1H, H-2); MS (FD) m/z(%): 359 (M^+, 100).
Analysis calc'd for $C_{12}H_9Br_2NO_2$: C, 40.15; H, 2.53; N, 3.90; Br, 44.52. Found: C, 40.35; H, 2.73; N, 3.87; Br, 44.70.

1-[4-(*trans*-11,11-Difluorodibenzo[b,e]bicyclo[5.1.0]oct-5-yl)-piperazin-1-yl]-3-(6,8-dibromoquinolin-5-yl)oxy-(*R*)-2-propanol, LY366573:
To a suspension of epoxide **5** (500 mg, 1.3 mmol) in ethyl acetate (5 mL) and isopropanol (10 mL) was added LY335995 (475 mg, 1.455 mmol) in one portion. The reaction mixture was refluxed for 4 h, then an additional amount of LY335995 (215 mg, 0.66 mmol) was added, and refluxing was continued for 2.5 h more. The resulting mixture was evaporated *in vacuo* and subjected directly to flash chromatography (1%, then 5% of methanol in dichloromethane) to give 879 mg (99%) of adduct LY366573 as a colorless solid, R_f = 0.32 (3% methanol in dichloromethane); R_t = 16 min; $[\alpha]_D^{20}$= -8.40° (c 1.07, MeOH); IR (KBr): 741, 765, 1006, 1071, 1156, 1294, 1438, 1450, 1580, 2807, 2946, 3422 cm^{-1}; UV (EtOH) $\lambda_{max}(\varepsilon)$: 303 nm (5156), 241 nm (41071); ^{1}H-NMR ($CDCl_3$) δ 2.30-2.90 (m, 10H, CH_2N), 3.19 (d, J = 12.4 Hz, 2H, H-10, H-11 of dibenzosuberane), 3.94 (s, 1H, H-5 of dibenzosuberane), 4.00-4.30 (m, 3H, H-2, H-3), 7.00-7.40 (m, 8H, aromatic protons of dibenzosuberane), 7.52 (dd, J = 8.4, 4.3 Hz, 1H, H-3 of quinoline), 8.18 (s, 1H, H-7 of quinoline), 8.71 (d, J = 8.5 Hz, 1H, H-4 of quinoline), 9.05 (d, J = 4.4 Hz, 1H, H-2 of quinoline); MS (FD) m/z(%): 685 (M^+, 100).
Analysis calc'd for $C_{32}H_{29}Br_2F_2N_3O_2$: C, 56.08; H, 4.29; N, 6.13; Br, 23.32. Found: C, 56.13; H, 4.45; N, 5.90; Br, 23.20.

1-[4-(*trans*-11,11-Difluorodibenzo[b,e]bicyclo[5.1.0]oct-5-yl)-piperazin-1-yl]-3-(6,8-dideuteroquinolin-5-yl)oxy-(*R*)-2-propanol, LY335979-[$^2H_2$]:

To a solution of dibromide LY366573 (50 mg, 0.073 mmol) in ethyl acetate (0.4 mL) and methanol (0.4 mL) was added a solution of potassium hydroxide (10 mg, 0.18 mmol) in methanol (0.2 mL) followed by a suspension of 10%Pd/C (7 mg) in methanol (0.2 mL). The reaction mixture was vigorously stirred under 1 atmosphere of deuterium for 1.5 h, then diluted with ethyl acetate (3 mL), filtered through a small plug of silica gel and evaporated *in vacuo* to give 36 mg (93%) of dideuteroquinoline LY335979-[$^2H_2$] as a white solid, identical to authentic LY335979 by TLC: R_f = 0.37 (3.5% methanol in dichloromethane), and HPLC: R_t = 6 min; $[\alpha]_D^{20}$= -4.82° (c 1.49, MeOH); IR (KBr): 740, 1006, 1090, 1156, 1295, 1439, 1456, 1584, 2807, 2950, 3420 cm^{-1}; UV (EtOH) $\lambda_{max}(\varepsilon)$: 309 nm (3562), 240 nm (38565); ^{1}H-NMR ($CDCl_3$) δ 2.20-2.90 (m, 10H, CH_2N), 3.19 (d, J = 12.4 Hz, 2H, H-10, H-11 of dibenzosuberane), 3.94 (s, 1H, H-5 of dibenzosuberane), 4.10-4.40 (m, 3H, H-2, H-3), 7.10-7.50 (m, 8H, aromatic protons of dibenzosuberane), 7.37 (dd, J = 8.4, 4.2 Hz, 1H, H-3 of quinoline), 7.59 (s, 1H, H-7 of quinoline), 8.57 (d, J = 8.3 Hz, 1H, H-4 of quinoline), 8.90 (d, J = 4.1 Hz, 1H, H-2 of quinoline); HRMS (FAB): calc'd for $C_{32}H_{30}D_2F_2N_3O_2$: 530.2588 Found: 530.2594.

1-[4-(*trans*-11,11-Difluorodibenzo[b,e]bicyclo[5.1.0]oct-5-yl)-piperazin-1-yl]-3-(6,8-ditritioquinolin-5-yl)oxy-(*R*)-2-propanol, Trihydrochloride Salt, LY335979-[$^3H_2$] · 3HCl:[17]

A mixture of LY366573 (10 mg, 0.0146 mmol), methanolic potassium hydroxide (0.89 *M*, 0.1 mL, 0.089 mmol) and 10%Pd/C (3.5 mg) in ethyl acetate (0.2 mL) was stirred under tritium gas (10 Ci) for 1.5 h. The catalyst was filtered and washed with ethanol. The combined filtrate was evaporated and the labile tritium was removed by repeated evaporations with ethanol to give the crude product LY335979-[$^3H_2$] (680 mCi). A portion of this product (170 mCi) was purified by HPLC (column: Prodigy ODS-2; mobile phase: methanol/water/triethylamine, 350:150:0.5), dissolved in ethanol, and treated with hydrochloric acid (3 equiv.). The resulting mixture was evaporated to give product LY335979-[$^3H_2$] · 3HCl (80 mCi), radiochemical purity 99.3% (HPLC: column: Hypersil ODS 5 μ (100 x 4.6 mm); solvent A: methanol/water/triethylamine, 100:900:10; solvent B:

methanol/triethylamine, 1000:10; gradient: 70%B to 100%B over 30 min; flow rate: 1 mL/min; UV 240 nm); specific activity 51 Ci/mmol; MS (FAB): 532.3 (M^+).

6,8-Dideutero-5-hydroxyquinoline, 8:

To a solution of dibromide **3** (100 mg, 0.26 mmol) in methanol (1.5 mL) was added sodium methoxide (70 mg, 1.3 mmol) in methanol (0.2 mL) followed by a suspension of 10%Pd/C (15 mg) in methanol (0.5 mL). The reaction mixture was vigorously stirred under 1 atmosphere of deuterium for 6 h, then diluted with ethyl acetate (6 mL), filtered through a small plug of silica gel and evaporated *in vacuo* to give 27 mg (71%) of dideuteroquinoline **8** as a white solid, identical to authentic 5-hydroxyquinoline (**1**) by TLC: R_f = 0.32 (hexane/ethyl acetate, 1:1); ^{1}H-NMR (CD_3OD) δ 7.43 (dd, J = 8.4, 4.4 Hz, 1H, H-3), 7.54 (s, 1H, H-7), 8.64 (d, J = 8.3 Hz, 1H, H-4), 8.75 (d, J = 4.4 Hz, 1H, H-2).

(*R*)-6,8-Dideutero-5-(2,3-epoxyprop-1-yloxy)-quinoline, 9:

To a solution of dibromide **5** (100 mg, 0.28 mmol) in ethyl acetate (2 mL) was added a solution of potassium hydroxide (31 mg, 0.55 mmol) in isopropanol (0.8 mL) followed by a suspension of 10%Pd/C (15 mg) in ethyl acetate (0.3 mL). The reaction mixture was vigorously stirred under 1 atmosphere of deuterium for 1 h then diluted with ethyl acetate (6 mL), filtered through a small plug of silica gel and evaporated *in vacuo* to give 55 mg (98%) of dideuteroquinoline **9** as a white solid, identical to authentic 5-(2,3-epoxyprop-1-yloxy)-quinoline by TLC: R_f = 0.30 (hexane/ethyl acetate, 1:1); ^{1}H-NMR ($CDCl_3$) δ 2.83 (m, 1H, H-3'), 2.97 (m, 1H, H-3'), 3.48 (m, 1H, H-2'), 4.10 (dd, J = 11.0, 5.8 Hz, 1H, H-1'), 4.43 (dd, J = 11.0, 2.9 Hz, 1H, H-1'), 7.38 (dd, J = 7.8, 4.3 Hz, 1H, H-3), 7.58 (s, 1H, H-7), 8.60 (d, J = 7.4 Hz, 1H, H-4), 8.90 (d, J = 3.9 Hz, 1H, H-2).

REFERENCES

1. Endicott, J. A. and Ling, V. - *Annu. Rev. Biochem.* **58:** 137 (1989).

2. Bradley, G. and Ling, V. - *Cancer Metastasis Rev.* **13:** 223 (1994).

3. Pfister, J. R.; Makra, F.; Muehldorf, A. V.; Wu, H.; Nelson, J. T.; Cheung, P.; Bruno, N. A.; Casey, S. M.; Zutshi, N; and Slate, D. L. - *Bioorg. Med. Chem. Lett.* **5:** 2473 (1995).

4. Slate, D. L.; Bruno, N. A.; Casey, S. M.; Zutshi, N; Garvin, L. J.; Wu, H.; and Pfister, J. R. - *Anticancer Res.* **15:** 811 (1995).

5. Review: Smalley, R. K. In: *Quinolines (The Chemistry of Heterocyclic Compounds)*; Jones, G. Ed.; Wiley-Interscience: London, 1977; Vol. 32, Part 1, p. 319.

6. Review: Scriven, E. F. K. In: *Comprehensive Heterocyclic Chemistry*; Katritzky, A. R. and Rees, C. W. Eds.; Pergamon Press: Oxford, 1984, Vol. 2, p. 198.

7. Skraup, Z. H. - *Monatsh.* **3**: 531 (1882).

8. Claus, A. and Howitz, H. - *J. Prakt. Chem.* **152**: 433 (1891).

9. Claus, A. and Howitz, H. - *J. Prakt. Chem.* **160**: 532 (1895).

10. Claus, A. - *J. Prakt. Chem.* **161**: 335 (1896).

11. Koch, V. and Schnatterer, S. - *Synthesis*, 497 (1990).

12. Review: Parker, R. E. and Isaacs, N. S. - *Chem. Rev.* **59**: 737 (1959).

13. Review: Larock, R. C. *Comprehensive Organic Transformations*, VCH: New York, 1989, p. 628.

14. An, Z.; D'Aloisio, R.; and Venturello, C. - *Synthesis*, 1229 (1992), and references cited therein.

15. Sudha, R.; Narasimhan, K. M.; Saraswathy, V. G.; and Sankararaman, S. - *J. Org. Chem.* **61:** 1877 (1996), and references cited therein.

16. Pless, J. - *Helv. Chim. Acta*, **59**: 499 (1976).

17. The tritiation was conducted by A.D. Morgan at Ligand Development Services (^{3}H Custom Preparations), Amersham International plc, Cardiff Laboratories, Whitchurch, Cardiff, CF4 7YT, UK.

ACKNOWLEDGEMENT

The author is grateful to Dr. Bill Wheeler and Mark Winter for helpful discussions and suggestions.