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Synthesis of [$^{13}C_6$]primaquine

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In support of a program to identify toxic metabolites of the antimalarial, primaquine, its [$^{13}C_6$] analog was prepared from [$^{13}C_6$] anisole in seven steps.

Keywords: primaquine; carbon-13; malaria; 8-aminoquinoline

Introduction

Compounds with isotopic labels (analogs) have been widely utilized by pharmaceutical and biological scientists to study biosynthesis, absorption, distribution, metabolism, and excretion of those materials in animal and human studies.[1] Historically, the use of radiolabeled analogs was the method of choice because detection and measurement of radioactivity was easily accomplished. With the evolution of mass spectrometry (MS) and especially the combined technology of HPLC (liquid chromatography (LC)) and MS (LC/MS), the detection and quantitation of compounds labeled with stable isotopes (^{2}H and ^{13}C especially) in now easily and inexpensively accomplished.[2] Also, the high sensitivity of modern MS instruments allows detection of even trace quantities of metabolites without the inconvenience and safety issues of handling radiolabeled materials.

Primaquine, an 8-aminoquinoline, is the only drug currently available for radical cure of relapsing malaria caused by *Plasmodium vivax*.[3] This drug is also used for the prophylaxis of all types of malaria including *P. falciparum*[4] that causes the most severe form and a majority of deaths from this disease, especially among children in Africa.[5] In combination with clindamycin, this compound is also effective in prevention and treatment of *Pneumocystis* pneumonia in AIDS patients.[6] A major shortcoming of this drug is its propensity to cause methemoglobinemia and hemolysis, especially in individuals who are deficient in glucose-6-phosphate dehydrogenase.[7] Other 8-aminoquinolines such as tafenoquine, which was developed as a safer alternative to primaquine[8] and sitamaquine, a drug candidate in clinical trials for the treatment of visceral leishmaniasis, also have the same drawback.[9] There is strong evidence to indicate that intraerythrocytic generation of reactive oxygen species by redox-active metabolites of primaquine is the cause for this toxicity.[10,11] Primaquine undergoes rapid metabolism in humans and animal models, and the major metabolite, carboxyprimaquine, accounts for most of the parent drug.[12] Carboxyprimaquine is evidently not the metabolite responsible for this toxicity.[13] Toxic metabolites appear to be very reactive and formed in small quantities. Hence, their identification in complex metabolic mixtures remains a major challenge. Drug candidates labeled with stable isotopes have been effectively used to track metabolically derived compounds in complex metabolic mixtures.[1,2] Here, we describe the synthesis of [$^{13}C_6$] primaquine for use in hepatocyte, microsomal, or *in vivo* metabolism studies.

Experimental

General

[$^{13}C_6$]Anisole and other reagents were purchased from Sigma-Aldrich (St Louis, MO, USA). NMR spectra were recorded on a Varian-Mercury-plus-400 or Varian Unity-Inova-600 spectrometer (Agilent Technologies, Palo Alto, CA, USA) using $CDCl_3$ and methanol-d_4 unless otherwise stated. MS data were obtained from an Agilent Series 1100 SL equipped with an ESI source (Agilent Technologies, Palo Alto, CA, USA). Column chromatography and preparative thin-layer chromatography (TLC) were carried out using Merck silica gel 60 (230–400 mesh) (Sigma-Aldrich Corp., St Louis, MO, USA) and silica gel GF plates (20 × 20 cm, thickness 0.25 mm) (Analtech Inc., Newark, DE, USA), respectively.

Synthesis of [$^{13}C_6$]primaquine diphosphate

[$^{13}C_6$]4-Nitroanisole (**3**)

A solution of HNO_3 (70%, 40 mL) and $NaNO_2$ (100 mg) was added to a stirred solution of [$^{13}C_6$]anisole (**1**) (8 g) in $CHCl_3$ (80 mL) maintaining the temperature 20–25°C. After 1 h, the reaction mixture was poured onto ice, and the organic layer was separated, washed with water, dried, and evaporated. The resulting products were chromatographed on silica gel and elution with hexanes/EtOAc 99.8:02 yielded [$^{13}C_6$]4-nitroanisole (**3**) as a pale yellow solid. (6.4 g, 57%). m.p. 50–51°C, IR (neat) ν_{max} 1588, 1495, 1328, 1259, 1172, 1104, 1019, 843, 748 cm^{-1}, HRESIMS $[M+Na]^+$ *m/z* 182.05244 (Calcd for [C[13]$_6$C[12]H_7NO_3 + Na]$^+$ 182.0705). ^{1}H-NMR (600 MHz, $CDCl_3$) δ ppm 3.88 (d, *J* = 3.6 Hz, 3H), 6.92 (d, *J* = 166.8 Hz, 2H), 8.16 (d, *J* = 169.2 Hz, 2H). ^{13}C-NMR (150 MHz, $CDCl_3$) δ ppm 56.3 (s), 113.9 (tm, *J* = 65 Hz), 125.8 (tm, *J* = 67 Hz), 141.4 (td, *J* = 68, 8.2 Hz), 164.5 (td, *J* = 66, 8.2 Hz).

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*[$^{13}C_6$]-p-*Anisidine (**4**)

Hydrazine hydrate (10 mL) was added to a stirred mixture of [$^{13}C_6$]-4-nitroanisole (6.4 g) and Pd/carbon (10%, 800 mg) in ethanol (150 mL). The reaction mixture was refluxed for 4 h and filtered through celite. The filtrate was evaporated, and the product obtained was partitioned between ethyl acetate and water. The organic layer was washed, dried, and evaporated to afford [$^{13}C_6$]-*p*-anisidine (**4**) (5.1 g, 100%). m.p. 56–58°C, IR (neat) ν_{max} 3455, 3370, 1594, 1514, 1325, 1206, 1038, 842, 740 cm^{-1}, HRESIMS $[M+H]^+$ *m/z* 130.0968 (Calcd for $[C[13]_6C[12]H_9NO+H]^+$ 130.1057). ^{1}H-NMR (600 MHz, $CDCl_3$) δ ppm 3.73 (d, *J* = 3.6 Hz, 3H), 6.62 (dm, *J* = 164 Hz, 2H), 6.74 (dm, *J* = 162 Hz, 2H). ^{13}C-NMR (150 MHz, $CDCl_3$) δ ppm 55.7 (s), 114.7 (tm, *J* = 65 Hz), 116.4 (tm, *J* = 60 Hz), 140.0 (td, *J* = 62, 9.3 Hz), 164.5 (tdt, *J* = 66, 8.2, 1.5 Hz).

*[$^{13}C_6$]4-Methoxy-2-nitroacetanilide (**5**)*

A mixture of [$^{13}C_6$]-*p*-anisidine (5.1 g), acetic acid (20 mL), and acetic anhydride (15 mL) was stirred at room temperature overnight. The reaction mixture was cooled on an ice/salt bath and fuming nitric acid (90%, 3.5 mL) was added dropwise maintaining the temperature below 10°C. After the addition, stirring was continued for 2 h. The reaction mixture was poured onto ice, and the product was filtered and dried (6.5 g, 72%). m.p. 116–118°C, IR (neat) ν_{max} 3377, 1699, 1507, 1326, 1213, 1026, 832 cm^{-1}, HRESIMS: $[M+Na]^+$ *m/z* 239.0995 (Calcd for $[C[13]_6C[12]_3H_{10}N_2O_4+Na]^+$ 239.1005). ^{1}H-NMR (600 MHz, $CDCl_3$) δ ppm 2.24 (s, 3H), 3.83 (t, *J* = 2.0 Hz, 3H), 7.21 (dm, *J* = 160 Hz, 1H), 7.64 (dm, *J* = 166 Hz, 1H), 8.61 (dm, *J* = 160 Hz, 1H), ^{13}C-NMR (150 MHz, $CDCl_3$) δ ppm 25.4 (s), 55.9 (s), 108.4 (tt, *J* = 72, 2.2 Hz), 123.3 (tm, *J* = 60 Hz), 123.8 (tm, *J* = 63 Hz) 128.5 (tm, *J* = 72 Hz), 137.0 (tm, *J* = 71 Hz), 154.9 (tm, *J* = 64 Hz).

*[$^{13}C_6$]4-Methoxy-2-nitroaniline hydrochloride (**6**)*

A mixture of [$^{13}C_6$]4-methoxy-2-nitroacetanilide (**5**) (6.5 g) and HCl (37%, 10 mL) in ethanol (125 mL) was refluxed for 2 h. The reaction mixture was evaporated to dryness to afford [$^{13}C_6$]4-methoxy-2-nitroaniline hydrochloride (**6**) (5.6 g, 89%). m.p. 118–120°C, IR (neat) ν_{max} 3366, 2768, 2571, 1506, 1472, 1333, 1231, 1019, 803 cm^{-1}, HRESIMS: $[M+H]^+$ *m/z* 175.0876 (Calcd for $[C[13]_6C[12]H_9N_2O_3+H]^+$ 175.1033). ^{1}H-NMR (600 MHz, $CDCl_3$) δ ppm 3.87 (t, *J* = 2.0 Hz, 3H), 7.21 (dm, 158 Hz, 1H), 7.43 (dm, 164 Hz, 1H), 7.65 (dm, 166 Hz, 1H). ^{13}C-NMR (150 MHz, $CDCl_3$) δ ppm 55.8(s), 108.5 (td, *J* = 70, 2.3 Hz), 123.6 (tm, *J* = 63 Hz) 124.2 (tm, *J* = 60 Hz), 128.4 (tm, *J* = 59 Hz), 137.1 (t, *J* = 75 Hz), 155.3 (tm, *J* = 64 Hz).

*[$^{13}C_6$]6-Methoxy-8-nitroquinoline (**7**)*

A mixture of [$^{13}C_6$]4-methoxy-2-nitroaniline (**6**) (5.6 g), sulfomix[14] (45 g), water (15 mL), and glycerol (8 mL) was stirred at 130°C for 4 h under nitrogen. Additional glycerol (8 mL) was added, and the reaction was continued for a further 2 h. The reaction mixture was poured onto ice, basified with 10% aqueous NaOH, and left overnight. The precipitate was filtered, dissolved in CH_2Cl_2, and passed through a silica gel plug. The solvent was evaporated, and the residue was crystallized from methanol to afford [$^{13}C_6$]6-methoxy-8-nitroquinoline (**7**) (3.4 g, 61%). m.p. 158–160°C, IR (neat) ν_{max} 1626, 1568, 1532, 1448, 1214, 1021, 835, 785 cm^{-1}, HRESIMS: $[M+H]^+$ *m/z* 211.0995 (Calcd for $[C[13]_6C[12]_4H_8N_2O_3+H]^+$ 211.1033). ^{1}H-NMR (600 MHz, $CDCl_3$) δ ppm 3.62 (s, 3H), 7.31 (brd, *J* = 162 Hz, 1H), 7.51 (m, 1H), 7.69 (brd, *J* = 162 Hz, 1H), 8.16 (d, *J* = 8.4 Hz, 1H), 8.83 (m, 1H). ^{13}C-NMR (150 MHz, $CDCl_3$) δ ppm 56.1 (s), 109.5 (ddt, *J* = 72, 59, 4 Hz), 116.8 (tm, *J* = 69 Hz), 123.0 (s), 135.0 (ddd, *J* = 74, 56, 2 Hz), 135.2 (s), 148.3 (tm, *J* = 73 Hz), 149.6 (s), 156.0 (tm, *J* = 70 Hz).

*[$^{13}C_6$]8-Amino-6-methoxyquinoline (**8**)*

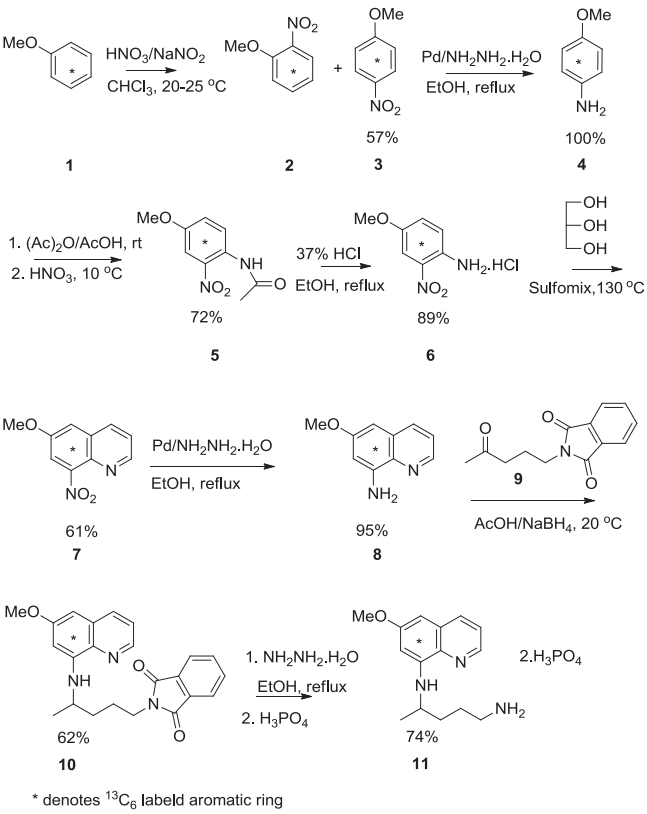
A mixture of [$^{13}C_6$]-6-methoxy-8-nitroquinoline (3.4 g), Pd/C (10%, 700 mg) and hydrazine hydrate (3 mL) in ethanol (100 mL) was refluxed for 2 h. The reaction mixture was filtered through celite, and the filtrate was evaporated. The product obtained was partitioned between water and ethyl acetate, and the organic layer was dried over Na_2SO_4 and evaporated to give [$^{13}C_6$]-8-amino-6-methoxyquinoline (**8**) (2.65 g, 95%) as a yellow gum. This was used in the next reaction without further purification.

*[$^{13}C_6$]6-Methoxy-8-(1-methyl-4-phthalimidobutylamino)quinoline (**10**)*

A mixture of [$^{13}C_6$]-8-amino-6-methoxyquinoline (**8**) (2.65 g) and 2-oxo-5-phthalimido pentane (**9**) (4.49 g) in glacial acetic acid (15 mL) was treated with $NaBH_4$ portion wise maintaining the temperature below 20°C until reaction was complete as evidenced by TLC. The reaction mixture was poured onto ice, basified with aqueous NaOH, and extracted with ethyl acetate. The organic layer was washed with water, dried, and evaporated to afford a yellow gum. This gum was chromatographed over silica gel and elution with ethyl acetate/hexanes 10:90 yielded a yellow solid that was crystallized from methanol to afford [$^{13}C_6$]-6-methoxy-8-(1-methyl-4-phthalimidobutylamino)quinoline (**10**) (3.6 g, 62%). m.p. 114–116°C, IR (neat) ν_{max} 3393, 1762,1700, 1611, 1514, 1373, 1157, 1057, 815 cm^{-1}, HRESIMS: $[M+H]^+$ *m/z* 396.2182 (Calcd for $[C[13]_6C[12]_{17}H_{23}N_3O_3+H]^+$ 396.2293). ^{1}H-NMR (600 MHz, $CDCl_3$) δ ppm 1.27 (d, *J* = 6.0 Hz, 3H), 1.64 (m, 1H), 1.72–1.87 (m, 3H), 3.64 (heptet, *J* = 6.6 Hz, 1H), 3.71 (td, 6.6, 1.8 Hz, 2H), 3.85 (s, 3H), 5.98 (d, *J* = 7.6 Hz, 1H), 6.25 (dm, *J* = 156 Hz, 1H), 6.28 (dm, *J* = 161 Hz, 1H), 7.26 (sextet, *J* = 3.9 Hz, 1H), 7.66 (m, 2H), 7.78 (m, 2H), 7.87 (dd, *J* = 8.4, 1.2 Hz, 1H), 8.48 (m, 1H). ^{13}C-NMR (150 MHz, $CDCl_3$) δ ppm 20.5 (s), 25.4 (s), 33.9 (s), 37.9 (s), 47.7 (s), 55.2 (s), 91.6 (ddt, *J* = 71, 58, 3 Hz), 96.7 (tq, *J* = 70, 3 Hz), 121.8 (s), 123.1 (s), 129.8 (tm, *J* = 57 Hz), 134.7 (s), 135.3 (ddd, *J* = 64, 57, 3 Hz), 144.2 (s), 144.9 (tm, *J* = 68 Hz), 159.3 (tm, *J* = 70 Hz), 168.4 (s).

*[$^{13}C_6$]Primaquine diphosphate (**11**)*

A mixture of [$^{13}C_6$]6-methoxy-8-(1-methyl-4-phthalimidobutylamino) quinoline (**10**) (2.0 g) and hydrazine hydrate (1 mL) in ethanol (80 mL) was refluxed for 4 h. The reaction mixture was cooled to room temperature and filtered. The filtrate was evaporated, and the residue was partitioned between ethyl acetate and aqueous NaOH (10%). The organic layer was washed with water, dried, and evaporated. The resulting gum was dissolved in ethanol (50 mL), treated with H_3PO_4 (2 mL) in ethanol (8 mL), and left overnight under stirring. The product was filtered and recrystallized from water/methanol to give [$^{13}C_6$]-primaquine



Scheme 1. Preparation of [$^{13}C_6$]primaquine.

diphosphate as orange crystals (**11**) (1.7 g, 74%). m.p. 203–205°C, IR (neat) ν_{max} 1652, 1607, 1552, 1388, 1347, 1034, 937 cm^{-1}, HRESIMS: $[M+H]^+$ *m/z* 266.2096 (Calcd for $[C[13]_6C[12]_9H_{21}N_3O+H]^+$ 266.2145). ^{1}H-NMR (600 MHz, D_2O/CD_3OD) δ ppm 1.21 (brs, 3H), 1.69 (m, 4H), 2.93 (brs, 1H), 3.56 (brs, 1H), 3.79 (s, 3H), 6.48 (dm, *J* = 160 Hz, 1H), 7.56 (d, *J* = 5.4 Hz, 1H), 8.34 (s, 1H), 8.49 (s, 1H). ^{13}C-NMR (150 MHz, $CDCl_3$) δ ppm 21.0 (s), 25.9 (s), 34.4 (s), 41.8 (s), 50.9 (s), 57.9 (s), 97.2 (m), 105.4 (tm, *J* = 70 Hz), 124.1 (s), 129.8 (t, *J* = 64 Hz), 133.6 (t, *J* =59 Hz), 142.3 (t, *J* = 67 Hz), 144.9 (s), 162.2 (t, *J* = 69 Hz).

Results and discussion

Previously, in support of studies of primaquine metabolism, our group prepared 5-deutero-, 7-deutero-, 6-methoxy-D3- and 1′-deutero-, 4′,4′-dideutero-, analogs of primaquine.[15,16] Because of the exchangeability of deuteriums at the 5- and 7-positions under acidic conditions,[17] and the possibility of removal of both the side chain and the methoxy group under metabolic conditions,[18] all of these analogs have serious limitations for metabolic studies. Because all putative toxic metabolites of primaquine contain the quinoline ring, ^{13}C labeling of the aromatic ring will afford a stable analog of six daltons higher mass, which should be retained with metabolic changes incurred in experimental systems. [$^{13}C_6$]-Primaquine diphosphate (**11**) was prepared from commercially available [$^{13}C_6$]anisole (**1**) (Scheme 1). Anisole was nitrated in a biphasic system in the presence of sodium nitrite to yield a mixture of [$^{13}C_6$]-2- (**2**) and [$^{13}C_6$]-4-nitroanisole (**3**). They were separated, and [$^{13}C_6$]-4-nitroanisole was hydrogenated to yield [$^{13}C_6$]-*p*-anisidine (**4**). [$^{13}C_6$]-*p*-Anisidine was acetylated and nitrated *in situ* to afford [$^{13}C_6$]-4-methoxy-2-nitroacetanilide (**5**). Treatment of this compound with HCl gave [$^{13}C_6$]-4-methoxy-2-nitroaniline (**6**) that was converted to [$^{13}C_6$]-6-methoxy-8-nitroquinoline (**7**) by the Skraup reaction. This product was hydrogenated, and the resulting amine (**8**) was coupled to the side chain, 2-oxo-5-phthalimido pentane (**9**), by reductive amination to give [$^{13}C_6$]-6-methoxy-8-(1-methyl-4-phthalimidobutylamino)quinoline (**10**). Deprotection by removal of the phthalimide group yielded the free amine that was treated with H_3PO_4 and crystallized from water/methanol to yield [$^{13}C_6$]-primaquine diphosphate (**11**).

The [$^{13}C_6$]-primaquine has proven to have great utility in helping to track minor metabolites of primaquine in incubates of human hepatocytes[19] and should allow new insights into the potential role of such metabolites in hematological or other toxicities of primaquine.

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Conflict of Interest

The authors did not report any conflict of interest.

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