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Research Article

Synthesis of [quinoline-3-^{14}C]-SSR97193 (ferroquine) from [2-^{14}C]-malonic acid

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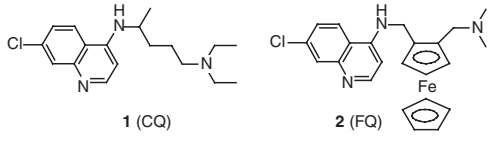
Summary

The antimalarial [quinoline-3-^{14}C]-SSR97193 (ferroquine) (**8**), an analogue of chloroquine (CQ) (**1**), was synthesized from [2-^{14}C]-malonic acid with an overall radiochemical yield of 15%. The synthetic route via [^{14}C]-Meldrum's acid (**9**) was designed to minimize the intermediacy of radiolabelled volatiles. This synthesis involves a four-step route to labelled 4,7-dichloroquinoline, which is the key intermediate for the synthesis of many analogues of CQ. Copyright © 2004 John Wiley & Sons, Ltd.

Key Words: antimalarial; carbon-14; 4,7-dichloroquinoline; ferroquine

Introduction

Increasing global resistance to the two most widely used antimalarial drugs, chloroquine (CQ) and the antifolate sulphadoxine/pyrimethamine by strains of the malaria parasite *Plasmodium falciparum* urgently demands the development of potent and inexpensive alternatives.[1] Postulation about the mechanism of action of CQ (**1**) and the basis for CQ resistance continues.[2] One approach has focussed interest on SSR97193, commonly referred to as ferroquine (FQ), (7-chloro-4-[(2-N′,N′-dimethylaminomethyl)-*N*-ferrocenylmethylamino]quinoline) (**2**), a racemate in which both enantiomers appear to be equally active.[3–5]



1 (CQ) **2** (FQ)

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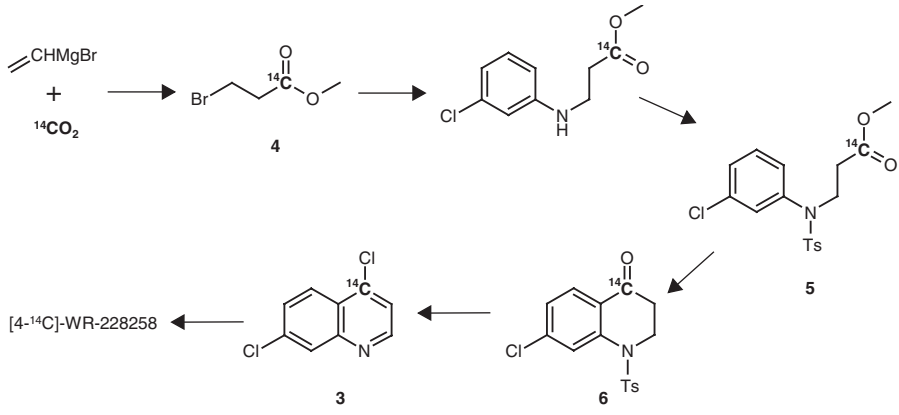
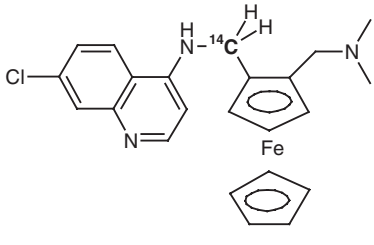


Scheme 1. Synthesis of [4-^{14}C]-WR-228258 from $^{14}CO_2$ via [4-^{14}C]-4,7-dichloroquinoline[7]

In order to perform the *in vitro* and *in vivo* metabolism and pharmacokinetic studies, a specifically labelled form of FQ was required. Whilst published isotopic labelling of FQ has been limited to deuteration,[6] a synthesis of [4-^{14}C]-4,7-dichloroquinoline (**3**) was presented by Kepler and Twine starting from [^{14}C]-carbon dioxide and proceeding via methyl [1-^{14}C]-3-bromopropionate (**4**) (Scheme 1).[7] The yield to the target molecule, WR-228258, over nine steps was 12%. Of particular note is the *N*-tosylated intermediate **5** formed prior to the cyclization to facilitate enhanced formation of the 7-chloro isomer over the 5-chloro isomer, which improves the yield of the intermediate **6** from around 50% to above 80%.[8] We considered the intermediacy of the radiolabelled volatile **4** a potential disadvantage to any route related to this approach.

Although an existing route[3] to FQ uses dimethylformamide, which is readily available radiolabelled and so would be expected to conveniently afford material radiolabelled as in **7**, the preferred site for labelling for metabolism studies was considered to be within the quinoline moiety.

By utilizing [2-^{14}C]-malonic acid as the starting material we have achieved a short synthesis to [quinoline-3-^{14}C]-FQ (**8**) avoiding the use of radiolabelled volatile intermediates (Scheme 2).



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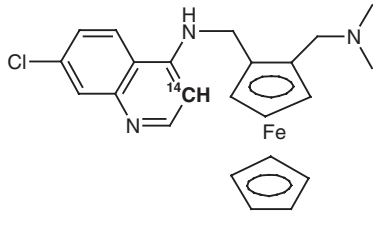

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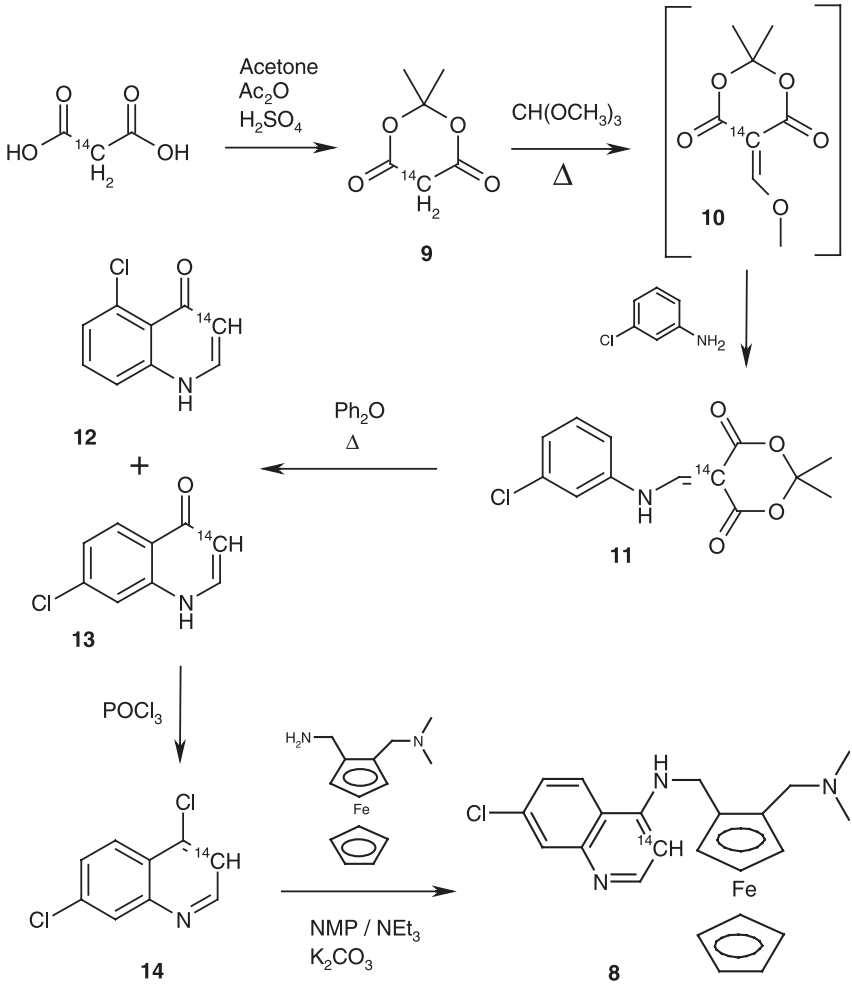

Scheme 2. Synthesis of [quinoline-3-^{14}C]-FQ (8) from [2-^{14}C]-malonic acid

Results and discussion

All route development was performed using low specific activity material (55 MBq/mmol). Formation of **9** from [2-^{14}C]-malonic acid was investigated to ascertain any benefit between either the reaction with isopropenyl acetate[9,10] or the reaction with acetone in acetic anhydride[10] (Table 1, entries 1 and 2).

Concentration of an acetone solution of [^{14}C]-malonic acid *in vacuo* on a small scale (2.9–3.9 mmol in a 25 ml round-bottom flask) resulted in a thin film of solid [2-^{14}C]-malonic acid being deposited around the inner surface of the flask. Subsequently, it was found that this deposit would not dissolve in the small amount of isopropenyl acetate (1.1 eq) that was used as both reactant and solvent and it was only on addition of acetic acid that significant reaction

Table 1. Formation of [1,3-dioxane-5-^{14}C]-2,2-dimethyl-[1,3]dioxane-4,6-dione (9) from [2-^{14}C]-malonic acid

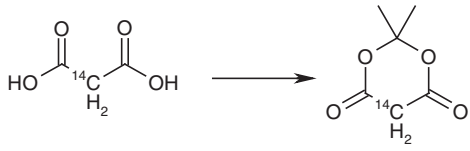

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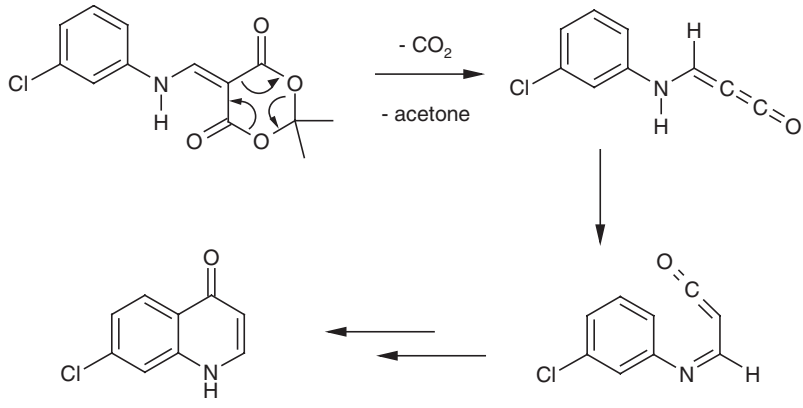
Table entry	Specific activity	Method	Time	Yield (%)	RCP (%)
1	Low	A	2 h at 0°C + 64 h at RT	53	92
2	Low	B	2 h at 0°C + 72 h at RT	68	93
3	High	C	2 h at 0°C	65	> 99

Method A: Isopropenyl acetate (1.1 eq), AcOH (10 eq), c.H_2SO_4 (1 drop).
Method B: Acetone (1.1 eq), Ac_2O (2.1 eq), c.H_2SO_4 (1 drop). Additional acetone (1.1 eq), Ac_2O (1.0 eq), c.H_2SO_4 (1 drop) added after 24 h.
Method C: Acetone (1.1 eq), Ac_2O (2.1 eq), c.H_2SO_4 (1 drop).

occurred (Table 1, entry 1). Performing the reaction in acetone avoided issues caused by the lack of solubility of the [^{14}C]-malonic acid in isopropenyl acetate and increased the yield. Purification of **9** by column chromatography was hindered by poor stability on silica, as demonstrated by 2-D TLC. A side-product of the reaction appeared to be [^{14}C]-acetic acid, which was noted on concentration *in vacuo* during work up after the reaction using methods B and C (Table 1, entries 2 and 3). The yield using high specific activity [2-^{14}C]-malonic acid (Table 1, entry 3) was similar (65%, > 99% radiochemical purity (RCP)) with reduced quantities of radiolabelled volatile materials observed, possibly due to reduced reaction time.

The synthesis of **14** from **9** proceeded using methods described by Cassis *et al.*[11] Formation of the methoxymethylene intermediate **10** in trimethyl orthoformate at 80°C was followed by room temperature addition of 3-chloroaniline. The product **11** was isolated in good yield (81%, > 99% RCP). The thermal cyclization from **11** to the isomers **12** and **13** was achieved in diphenyl ether at reflux (b.p. 259°C) for 8 min. Due to the absence of an *ortho*-substituent on the anilino moiety of **11** it had been expected that both **12** and **13** would be formed in similar amounts. In practice, the observed ratio was 2:1 in favour of the desired **13** with an isolated yield after chromatography of 55% (> 99% RCP).

The technique utilized by Kepler and Twine[7] to enhance the selectivity towards the formation of the 7-chloroquinoline, via *N*-tosylation, appeared inappropriate in our case as recent work by Walz and Sundberg[12] suggested that the unsubstituted *N*-H is essential for the decomposition of the dioxane-4,6-dione ring (Scheme 3).



Scheme 3. Mechanism of thermal cyclization proposed by Walz and Sundberg[12]

The straightforward conversion of **13** to **14** was accomplished with phosphorus oxychloride at reflux affording the product in excellent yield (96%, >99% RCP). The final coupling with 2-(*N*,*N*-dimethylaminomethyl)-ferrocenylmethylamine was performed, as in the original synthesis,[3] in *N*-methylpyrrolidinone (NMP) at 130°C with triethylamine and potassium carbonate over 14.5 h affording [quinoline-3-^{14}C]-FQ (**8**) in fair yield (60%, >98% RCP) after purification. The RCP was then further enhanced to 99.3%, after recrystallization from dichloromethane/methanol.

Experimental

The [2-^{14}C]-malonic acid was purchased from Amersham Biosciences UK Ltd. All steps were completed at low specific activity prior to being repeated at high specific activity. High specific activity materials were characterized by comparative TLC/HPLC (to known standards) and ^{1}H-NMR for [quinoline-3-^{14}C]-FQ. Merck silica or alumina radio-TLC plates were analysed via electronic autoradiography using a Packard Instantimager. Activities were determined by liquid scintillation analysis using a Packard Tri-Carb 1900TR. Analysis by HPLC was performed using a Gilson HPLC system connected to a Packard Radiomatic 525TR flow scintillation analyser. All ^{1}H-NMR data presented, apart from that of the final product, are for low specific activity material and were run on a Bruker DRX 500.

*[1,3-dioxane-5-^{14}C]-2,2-Dimethyl-[1,3]dioxane-4,6-dione ([^{14}C]-Meldrum's acid) (**9**)*

Acetone (280 µl, 3.8 mmol) and acetic anhydride (675 µl, 7.2 mmol) were added to [2-^{14}C]-malonic acid (6992 MBq, 3.5 mmol, 2040 MBq/mmol). The solution was cooled to 0°C (ice bath) and H_2SO_4 (concentrated, **1** drop) was added. The solution was stirred under nitrogen for 2 h. Ethyl acetate (10 ml) and water

(10 ml) were added and the aqueous phase extracted to ethyl acetate (5×20 ml). The organic phases were combined, dried (over anhydrous magnesium sulphate) and filtered. The solution was concentrated *in vacuo*, then purified by column chromatography (SiO_2; ethyl acetate:dichloromethane (20:80), under pressure, 50 ml fractions). The purer fractions were combined to yield [1,3-dioxane-5-^{14}C]-2,2-dimethyl-[1,3]dioxane-4,6-dione (**9**) (4541 MBq, 65% radiochemical yield, >99% RCP). Degradation was noted on 2D TLC (SiO_2; ethyl acetate:dichloromethane (40:60)).

TLC: SiO_2; $R_f = 0.6$ (ethyl acetate:dichloromethane:acetic acid (25:70:5)).

^{1}H –NMR: ($CDCl_3$ 500 MHz) δ 1.8 (6H, s), and 3.6 (2H, s).

[1,3-dioxane-5-^{14}C]-5-[(3-Chlorophenylamino)methylene]-2,2-Dimethyl-[1,3]dioxane-4,6-dione (**11**)

Trimethyl orthoformate (12.5 ml) was added to **9** (4541 MBq, 2.3 mmol) and the mixture heated to ~80°C (oil bath) under nitrogen. The resultant solution was stirred at 80°C for 3 h. TLC (SiO_2; ethyl acetate:toluene (40:60)) indicated absence of starting material. The reaction mixture was allowed to cool to room temperature. 3-Chloroaniline (265 µl, 2.5 mmol) was added and the solution stirred under nitrogen for 1 h at room temperature. A pale yellow precipitate formed on stirring. Toluene was added until the solids dissolved. The solution was concentrated *in vacuo* and purified by column chromatography (SiO_2; ethyl acetate:toluene (5:95), gravity column, 50 ml fractions). The purer fractions were combined to yield [1,3-dioxane-5-^{14}C]-5-[(3-chlorophenylamino)methylene]-2,2-dimethyl-[1,3]dioxane-4,6-dione (**11**) (3668 MBq, 81% radiochemical yield, >99% RCP).

TLC: SiO_2; $R_f = 0.3$ (ethyl acetate:toluene (5:95)).

^{1}H –NMR: (CD_3OD 500 MHz) δ 1.71 (6 H, s), 7.29 and 7.36 (2×1 H, ddd, $J = 8.5, 2.5, 1.5$ Hz), 7.43 (1 H, t, $J = 8.5$ Hz), 7.52 (1 H, t, $J = 2$ Hz), and 8.69 (1 H, s).

[quinoline-3-^{14}C]-7-Chloro-1H-quinolin-4-one (**13**)

Warm diphenyl ether (liquid, 23 ml) was added to **11** (3668 MBq, 1.8 mmol) and the mixture placed under nitrogen. The mixture was heated to reflux (259°C, heating mantle) for 8 min turning from yellow to deep orange/red. The solution was allowed to cool then stirred for 16 h at room temperature. A precipitate formed. Ethanol (50 ml) was added and the solids dissolved. Analysis by TLC showed no starting material remaining. The ethanol was removed *in vacuo* and the mixture purified by column chromatography (SiO_2; methanol:toluene:triethylamine (8:87:5), gravity column, 50 ml fractions). The purer fractions were combined to yield [quinoline-3-^{14}C]-7-chloro-1*H*-quinolin-4-one (**13**) (2029 MBq, 55% radiochemical yield, >99% RCP).

TLC: SiO_2; [quinoline-3-^{14}C]-7-chloro-1*H*-quinolin-4-one (**13**) R_f = 0.14, [quinoline-3-^{14}C]-5-chloro-1*H*-quinolin-4-one (**12**) R_f = 0.08 (methanol:toluene:triethylamine (10:85:5)).

^{1}H-NMR (**13**): (CD_3OD, 500 MHz); δ 6.30 (1H, d, J = 7.5 Hz), 7.36 (1H, dd, J = 9 ~1 Hz), 7.59 (1H, d, J = 2 Hz), 7.92 (1H, d, J = 7.5 Hz), and 8.21 (1H, d).

*[quinoline-3-^{14}C]-4,7-Dichloroquinoline (**14**)*

Phosphorus oxychloride (5 ml) was added to **13** (934 MBq, 1.5 mmol, 2040 MBq/mmol) and the mixture heated to reflux (oil bath) under nitrogen for 50 min. The mixture was concentrated *in vacuo* and water (30 ml) and ethyl acetate (20 ml) added. The aqueous phase was basified to pH ~10 with ammonium hydroxide then extracted with ethyl acetate (3 × 20 ml). The organic phase was dried (over anhydrous magnesium sulphate), filtered and then concentrated *in vacuo*. This procedure was repeated with phosphorus oxychloride (10 ml) and additional **13** (2029 MBq, 1.0 mmol, 2000 MBq/mmol). The resultant materials were combined then purified by column chromatography (SiO_2; ethyl acetate:toluene:triethylamine (5:94:1) gravity column, 100 ml fractions). The purer fractions were combined to yield [quinoline-3-^{14}C]-4,7-dichloroquinoline (**14**) (2840 MBq, 96% radiochemical yield, >99% RCP).

TLC: SiO_2; R_f = 0.63 (methanol:toluene:triethylamine (10:85:5)).

^{1}H-NMR: (CD_3OD, 500 MHz); δ 7.69 (1H, d, J = 5 Hz), 7.73 (1H, dd, J = 9 ~2.5 Hz), 8.08 (1H, d, J = 2.5 Hz), 8.28 (1H, d, J = 9 Hz), and 8.79 (1H, d, J = 5 Hz).

*[quinoline-3-^{14}C]-7-Chloro-4-[(2-N′,N′-dimethylaminomethyl)-N-ferrocenylmethyl-amino]quinoline (**8**)*

Compound **14** (2735 MBq, 1.4 mmol) was dissolved in *N*-methyl pyrrolidinone with 2-(*N*,*N*-dimethylaminomethyl)ferrocenylmethylamine (520 mg, 1.5 mmol) and potassium carbonate (635 mg, 4.6 mmol) and placed under nitrogen. The mixture was heated, with stirring, to 130°C (oil bath) for 14.5 h. At room temperature chloroform (10 ml) and water (10 ml) was added to the black suspension. The organic phase was washed with ammonium hydroxide (13%, 20 ml) and the aqueous phase then extracted to chloroform (3 × 20 ml). The organic phase contained a black solid suspension, which was filtered onto celite. The filtrate was concentrated *in vacuo* and the residues dissolved in ethyl acetate (50 ml). The organic phase was washed with water (3 × 20 ml) to remove residual NMP, dried (over anhydrous magnesium sulfate), filtered, concentrated *in vacuo* and purified by column chromatography (SiO_2; ethyl acetate:toluene:triethylamine (15:85:2)) to yield the desired [quinoline-3-^{14}C]-7-chloro-4-[(2-*N*′,*N*′-dimethylaminomethyl)-*N*-ferrocenyl-methylamino]quino-

line (**8**) (1640 MBq, 60% radiochemical yield, >98% RCP). The product was recrystallized from dichloromethane/acetone (87% recovery, 99.3% RCP by HPLC). From this reaction unreacted starting material (**14**) was recovered (427 MBq, 16% of total activity).

TLC: SiO_2; R_f = 0.09 (ethyl acetate:toluene:triethylamine (15:85:2)), Al_2O_3; R_f = 0.22 (ethyl acetate:toluene (25:75)), Al_2O_3; R_f = 0.49 (propan-2-ol : toluene (3:97)).

^{1}H-NMR: ($CDCl_3$, 500 MHz); δ 2.20 (6H, s), 2.87 (1H, d, J = 12.5 Hz), 3.77 (1H, d, J = 12.5 Hz), 4.06 (1H, dd), 4.12 (5H, s), 4.14 (1H, dd), 4.16 (1H, dd), 4.25 (1H, dd), 4.36 (1H, d, J = 13 Hz), 6.43 (1H, d, J = 5.5 Hz), 7.25 (1H, dd), 7.59 (1H, d, J = 9 Hz), 7.63 (1H, dd), 7.89 (1H, d, J = 2 Hz) and 8.52 (1H, d, J = 5.5 Hz).

HPLC: Alltech Inertsil ODS-2, 250 × 4.6 mm, 5 µm, UV = 220 nm, flow = 1 ml/min, liquid scintillant flow = 2 ml/min, ambient temperature, eluent A: 10 mmol hexanesulfonic acid sodium salt (pH **3**, adjusted with H_3PO_4), eluent B: (20% eluent A:80% acetonitrile), t(%A) 0 (75) 35 (45) 45 (0) 50 (0) 51 (75), retention time = 21.3 min, 20 µl injection in starting eluent.

Conclusion

We have presented a short ^{14}C-labelled synthesis to the key intermediate [quinoline-3-^{14}C]-4,7-dichloroquinoline (**14**) in reasonable yield (28% over four steps) starting from [2-^{14}C]-malonic acid and utilizing Meldrum's acid (**9**) as an intermediate to minimize radiolabelled volatile intermediates. The overall yield of the synthesis of [quinoline-3-^{14}C]-FQ (**8**) was 15% after crystallization, with 99.3% RCP and a specific activity of 2014 MBq/mmol. The intermediate **14** is common to a number of compounds of interest in this therapeutic class and so it is anticipated that this approach, avoiding the use of radiolabelled volatile intermediates, might find a wider applicability.

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References

1. Ridley JR. *Nature* 2002; **415**: 686–693.
2. Stocks PA, Raynes KJ, Bray PG, Park BK, O'Neill M, Ward SA. *J Med Chem* 2002; **45**: 4975–4983.
3. Biot C, Glorian G, Maciejewski LA, Brocard JS, Domarle O, Blampain G, Millet P, Georges AJ, Abessolo H, Dive D, Lebibi J. *J Med Chem* 1997; **40**: 3715–3718.
4. Domarle O, Blampain G, Agnaniet H, Nzadiyabi T, Lebibi J, Brocard J, Maciejewski L, Biot C, Georges AJ, Millet P. *Antimicrob Agents Chemother* 1998; **42**: 540–544.

5. Delhaes L, Biot C, Berry L, Delcourt P, Maciejewski LA, Camus D, Brocard JS, Dive D. *Chem Bio Chem* 2002; **3**: 418–423.
6. Biot C, Caron S, Maciejewski LA, Brocard JS. *J Label Compd Radiopharm* 1998; **41**: 911–918.
7. Kepler JA, Twine CE. Preparation of some [4-^{14}C]-quinoline labelled antimalarial agents. Synthesis and applications of isotopically labelled compounds. In *Proceedings of an International Symposium*, Duncan WP, Susan AB (eds). Elsevier Scientific Publishing Company: Amsterdam, 1983; 207–208.
8. Johnson WS, Buell BG. *J Am Chem Soc* 1952; **74**: 4513–4516.
9. Lu PJ, Wietao P, Maitland J. *J Am Chem Soc* 1998; **120**: 8315–8318.
10. Davidson D, Bernhard SA. *J Am Chem Soc* 1949; **70**: 3426–3428.
11. Cassis R, Tapia R, Valderrama JA. *Synth Commun* 1985; **15**: 125–133.
12. Walz AJ, Sundberg RJ. *J Org Chem* 2000; **65**: 8001–8010.

