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Synthesis of Stable Isotope Labeled Chloroquine, Amodiaquine and Their Metabolites

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Anti-malaria drugs chloroquine, amodiaquine and their metabolites were stable isotope labeled with ^{13}C and ^{15}N starting from uniformly ^{13}C labeled benzene and ^{15}N labeled potassium nitrate to give M+7 isotopomers. Chloroquine, and its metabolites were prepared from 7-chloro-1,2,3,4-tetrahydroquinolin-4-one through addition to the carbonyl with the corresponding amines; and the amodiaquine and its metabolites were prepared from 4,7-dichloroquinoline in a selective aryl-halogen exchange with the corresponding amines.

Keywords: chloroquine, amodiaquine, uniformly ^{13}C labeled benzene, mass spectral standards

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Introduction

Chloroquine, discovered in 1934,[1,2] has traditionally been used to prevent and treat malaria in areas where malaria is known to be sensitive to the pharmaceutical,[3] although certain types of malaria have developed wide spread resistance to chloroquine, thus requiring patients to be given alternative approaches or additional medications. Chloroquine is on the World Health Organization's list of Essential Medicines, as the most effective and safe medicine needed in arena. In addition, its availability in a generic version makes chloroquine a readily affordable drug in developing countries.[4]

Chloroquine has also found use for treatment of amebiasis and rheumatic diseases. Significantly, it has also been studied as anti-retroviral and anti-cancer agents.[3]

Amodiaquine,[5] structurally very similar to chloroquine, has also been used for treatment of malaria where resistance to chloroquine is significant. However, there is some notable side effects with the use of amodiaquine, such as hepatotoxicity.[6] In general, antimalarial drugs of this class have shown to cause severe side effects.[7, 8] Therefore, diagnostic monitoring of chloroquine and amodiaquine and their metabolites by quantitative mass spectrometry has found recent increased attention. Importantly, stable isotope labeled reference samples have become the gold standard for facilitate accurate measurements and method development for mass spectrometry detection and quantification.[9,10]

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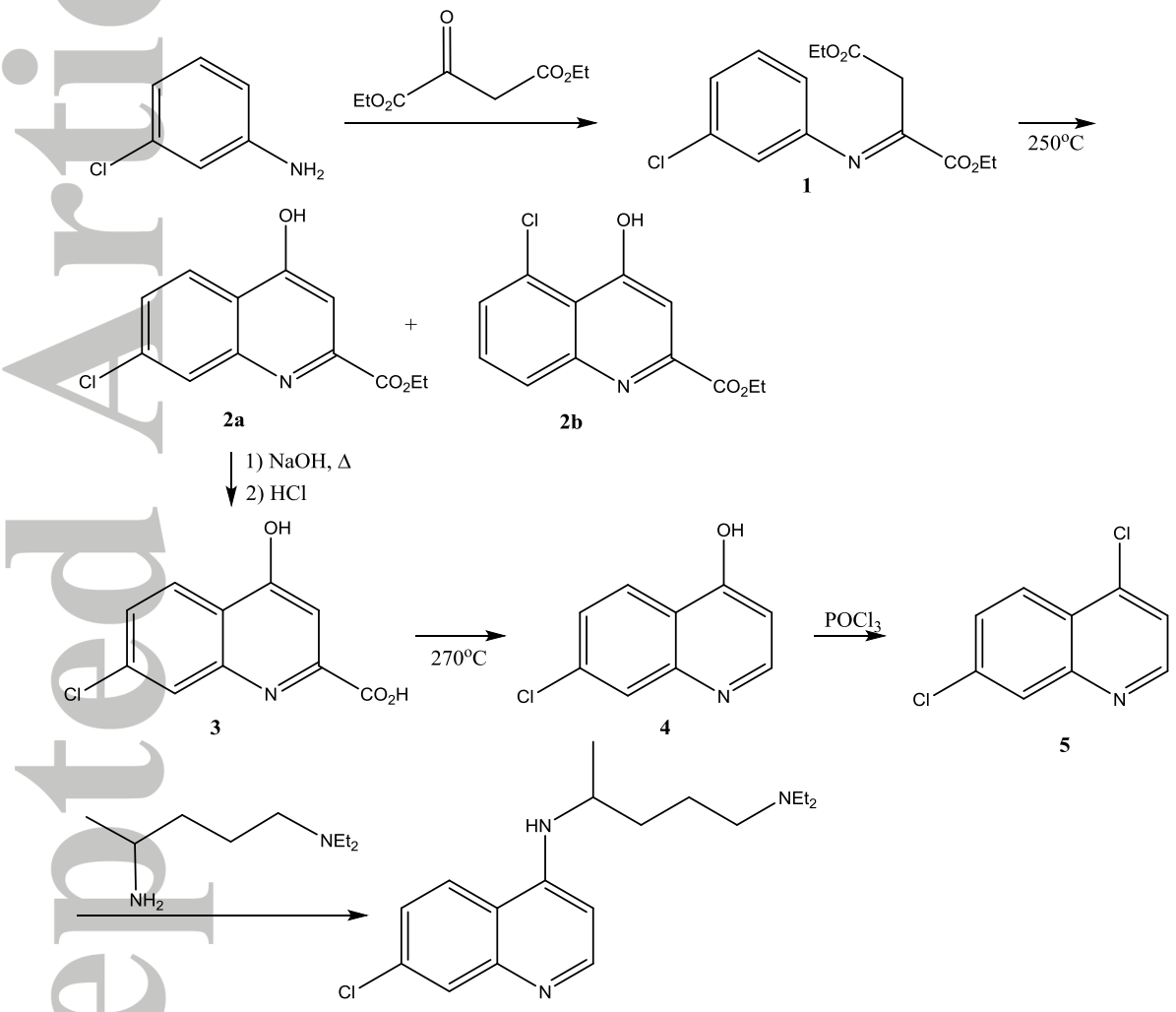
We have been interested in the synthesis of stable isotope labeled compounds since the 1980's, these include the syntheses of sulfur and nitrogen mustard metabolites and polyaromatics.[9,10] We now report on the synthesis of stable isotope labeled chloroquine, amodiaquine and their metabolic products.

Previous Synthetic Routes to Chloroquine

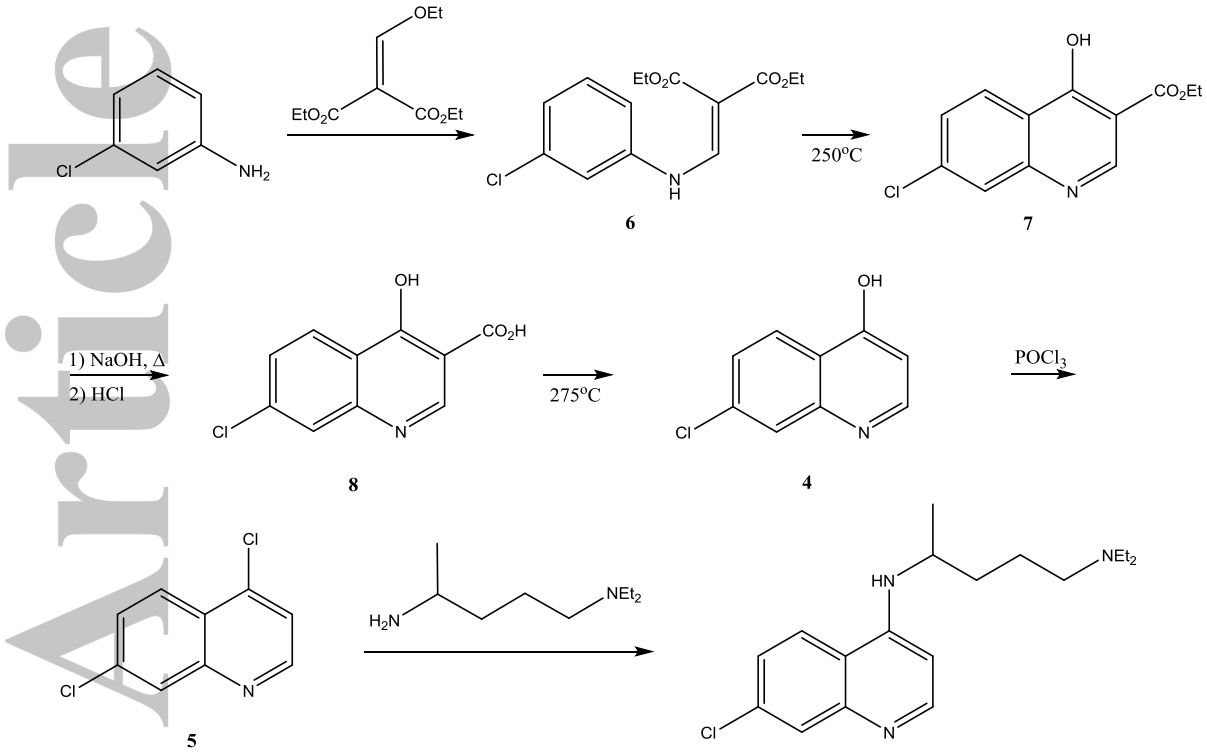
Chloroquine and related compounds are traditionally made by Conrad-Limpach synthesis,[11,12] shown in Scheme 1:



Scheme 1. Conrad-Limpach synthesis of chloroquine

Condensation of *m*-chloroaniline with diethyl oxaloacetate to give diethyl (E)-2-((3-chlorophenyl)imino)succinate **1** was followed by pyrolytic cyclization to give ethyl 7-chloro-4-hydroxyquinoline-2-carboxylate **2a** and ethyl 5-chloro-4-hydroxyquinoline-2-carboxylate **2b**. Saponification of ester **2a** to its acid 7-chloro-4-hydroxyquinoline-2-carboxylic acid **3** followed by thermal decarboxylation provided 7-chloroquinolin-4-ol **4**. Subsequent reaction of **4** with phosphorus oxychloride gave 4,7-dichloroquinoline **5**. Yields are generally high, except for the cyclization step of diethyl (E)-2-((3-chlorophenyl)imino)succinate **1** to ethyl 7-chloro-4-hydroxyquinoline-2-carboxylate **2a**, in which the annulation closes partly into the ortho position to the chloro group resulting in a mixture containing about 50% of the undesired 5-chloro isomer **2b**.

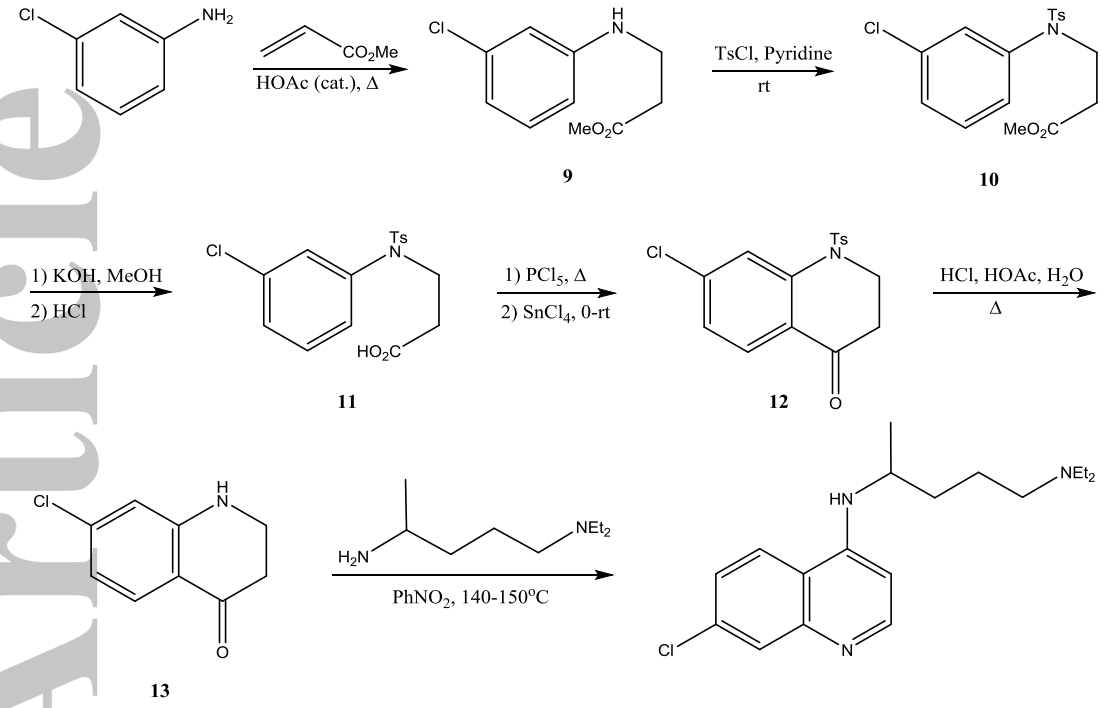
Chloroquine has also been constructed using the Gould-Jacobs approach,[13] as shown in Scheme 2:



Scheme 2. Gould-Jacobs synthesis of chloroquine

The reaction of diethyl ethoxymethylenemalonate with *m*-chloroaniline forms diethyl 2-(((3-chlorophenyl)amino)methylene)malonate **6**. This product undergoes cycloaddition in refluxing diphenyl ether to form ethyl 7-chloro-4-hydroxyquinoline-3-carboxylate **7**. The presence of other positional isomer is negligible. The saponification of ester **7** affords acid **8**, and subsequent decarboxylation gives decarboxylated product **6**. Overall, this synthesis requires several very high temperature reactions and thermal decompositions are possible.

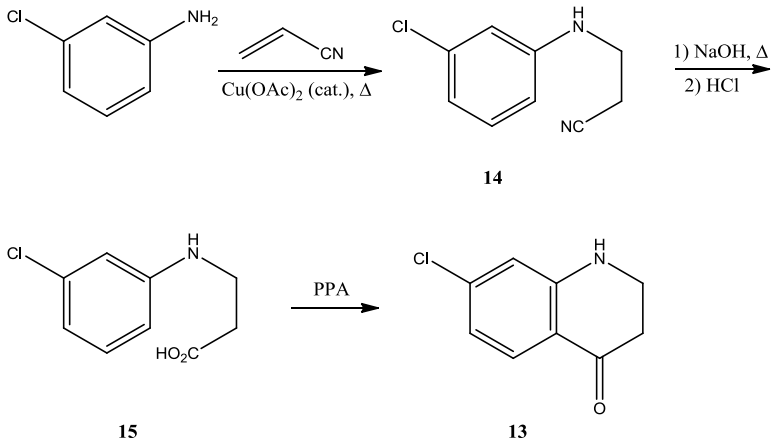
W. S. Johnson[14] improved upon synthetic methods to chloroquine as illustrated in Scheme 3:



Scheme 3. W. S. Johnson synthesis of chloroquine

m-Chloroaniline undergoes a Michael addition with methyl acrylate in the presence of catalytic amount of acetic acid to form methyl 3-((3-chlorophenyl)amino)propanoate **9**. Reaction of **9** with tosyl chloride gives rise to methyl 3-((N-(3-chlorophenyl)-4-methylphenyl)sulfonamido)propanoate **10**. Subsequent hydrolysis affords the acid **11**. Treatment of the acid **11** with phosphorus pentachloride followed by a Friedel-Crafts reaction afforded exclusively 7-chloro-1-tosyl-2, 3-dihydroquinolin-4(1H)-one **12**. Cleavage of the tosyl group from **12** affords 7-chloro-2, 3-dihydroquinolin-4(1H)-one **13**. In the presence of an oxidizing agent such as nitrobenzene, **13** reacted with 2-amino-5-diethylaminopentane to form chloroquine.

A closely related synthetic approach starts with *m*-chloroaniline and acrylonitrile as shown in Scheme 4:[15]



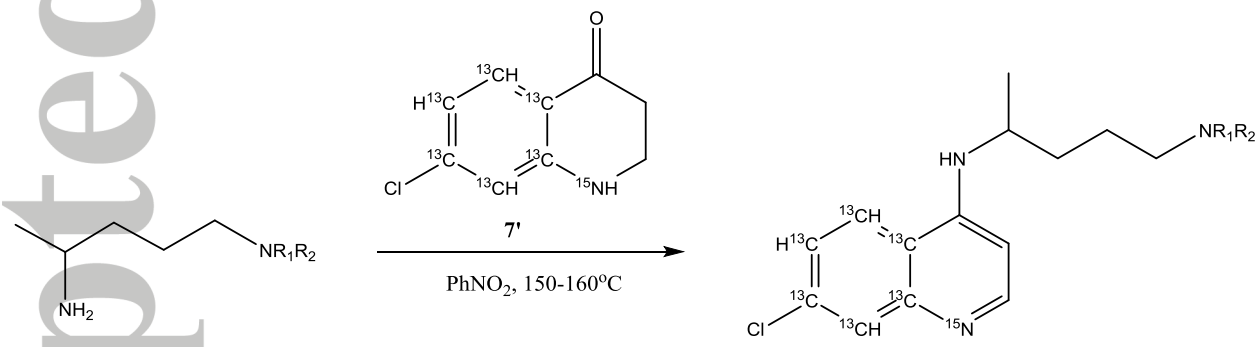
Scheme 4. Alternate to W. S. Johnson synthesis

m-Chloroaniline is cyanoethylated by heating with acrylonitrile and a catalytic amount of cupric acetate to give the Michael addition product 3-((3-chlorophenyl)amino)propanenitrile **14** in 60% yield. Upon alkaline hydrolysis **14** produces the corresponding propionic acid **15**, which is further cyclodehydrated with PPA (polyphosphoric acid) to give 7-chloro-2, 3-dihydroquinolin-4(1H)-one **13** in 50% yield. In this reaction, the unwanted 5-chloro isomer did not form.

Results and Discussion

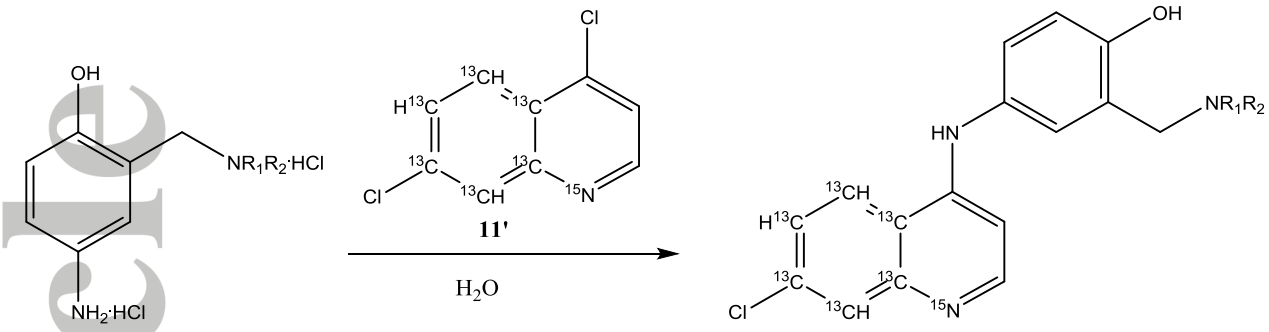
Radioactive isotope labeled chloroquine and its metabolites have been prepared for pharmacological studies. The synthesis of stable isotope labeled materials, however, has not enjoyed the same level of attention, with only syntheses of deuterated side chains reported.[16] The degradation of chloroquine and amodiaquine was studied, both of them undergo de-alkylation (de-ethylation) by P450 to form desethyl and bisdesethyl derivatives.[17] The synthesis of the dealkylated stable isotope labeled metabolites are also very scarce.[16]

We adapted W. S. Johnson's method for the synthesis of stable isotope labeled (SIL) chloroquine, amodiaquine, and their metabolites. The chloroquine series **8'**, **9'** and **10'** were prepared from 7-chloro-2,3-dihydroquinolin-4(1H)-one **7'** with the corresponding amines; while the amodiaquine series **12'**, **13'** and **14'** were prepared from 4,7-dichloroquinoline **11'** with the corresponding amines, as shown in scheme 5 and 6.



chloroquine **8'**, $R_1 = R_2 = Et$,
desethyl chloroquine **9'**, $R_1 = H$, $R_2 = Et$
bisdesethyl chloroquine **10'**, $R_1 = R_2 = H$

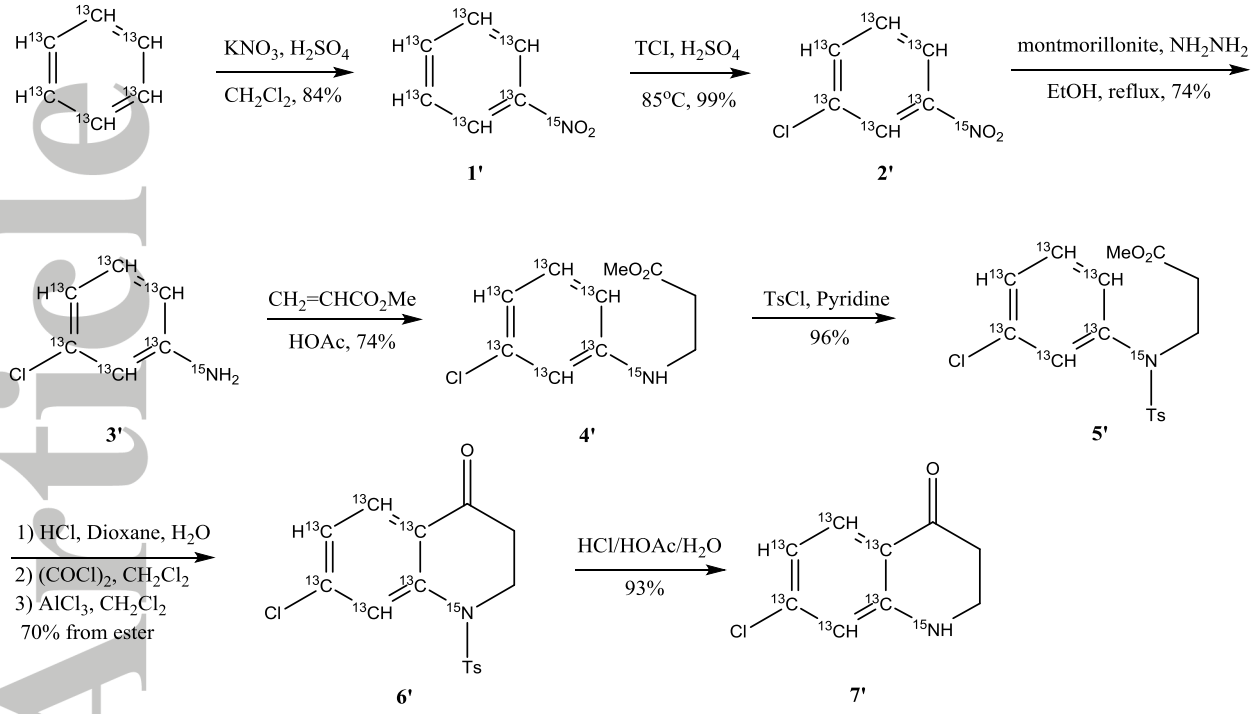
Scheme 5. Key step to produce labeled chloroquine and its metabolites



amodiaquine **12'**, $R_1 = R_2 = Et$
desethyl amodiaquine **13'**, $R_1 = Et$, $R_2 = H$
bisdesethyl amodiaquine **14'**, $R_1 = R_2 = H$

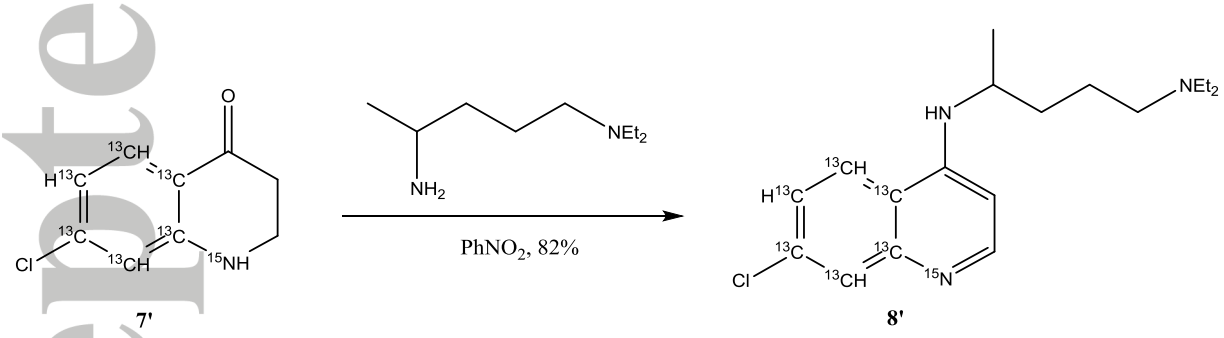
Scheme 6. Key step to produce labeled amodiaquine and its metabolites

The syntheses of precursors follows the synthetic route shortly outlined *vide infra*: Nitration of benzene-1,2,3,4,5,6-$^{13}C_6$ (Scheme 7) with $K^{15}NO_3$ and concentrated sulfuric acid affords (nitro-^{15}N)benzene-1,2,3,4,5,6-$^{13}C_6$ **1'**. Nitrobenzene reacts with trichloroisocyanuric acid (TCI) and concentrated sulfuric acid to afford 1-chloro-3-(nitro-^{15}N)benzene-1,2,3,4,5,6-$^{13}C_6$ **2'**. Reduction of 1-chloro-3-nitrobenzene **2'** is affected with hydrazine in the presence of montmorillonite to provide 3-chloroaniline-1,2,3,4,5,6-$^{13}C_6$, ^{15}N **3'**. Reaction of 3-chloroaniline **3'** with methyl acrylate in the presence of glacial acetic acid produces ethyl 3-((3-chlorophenyl-1,2,3,4,5,6-$^{13}C_6$)amino-^{15}N)propanoate **4'**. This product is then treated with *p*-toluenesulfonyl chloride to yield ethyl 3-((N-(3-chlorophenyl-1,2,3,4,5,6-$^{13}C_6$)-4-methylphenyl)sulfonamido-^{15}N)propanoate **5'**. Hydrolysis of the ester with hydrochloric acid in dioxane-water, followed by acid chloride formation using oxalyl chloride, and a subsequent Friedel-Crafts reaction affords 7-chloro-1-tosyl-1,2,3,4-tetrahydroquinoline-4a,5,6,7,8,8a-$^{13}C_6$, ^{15}N **6'**. Removal of tosyl group is achieved with acetic acid and hydrochloric acid in water at reflux to form 7-chloro-1,2,3,4-tetrahydroquinoline-4a,5,6,7,8,8a-$^{13}C_6$, ^{15}N **7'**.



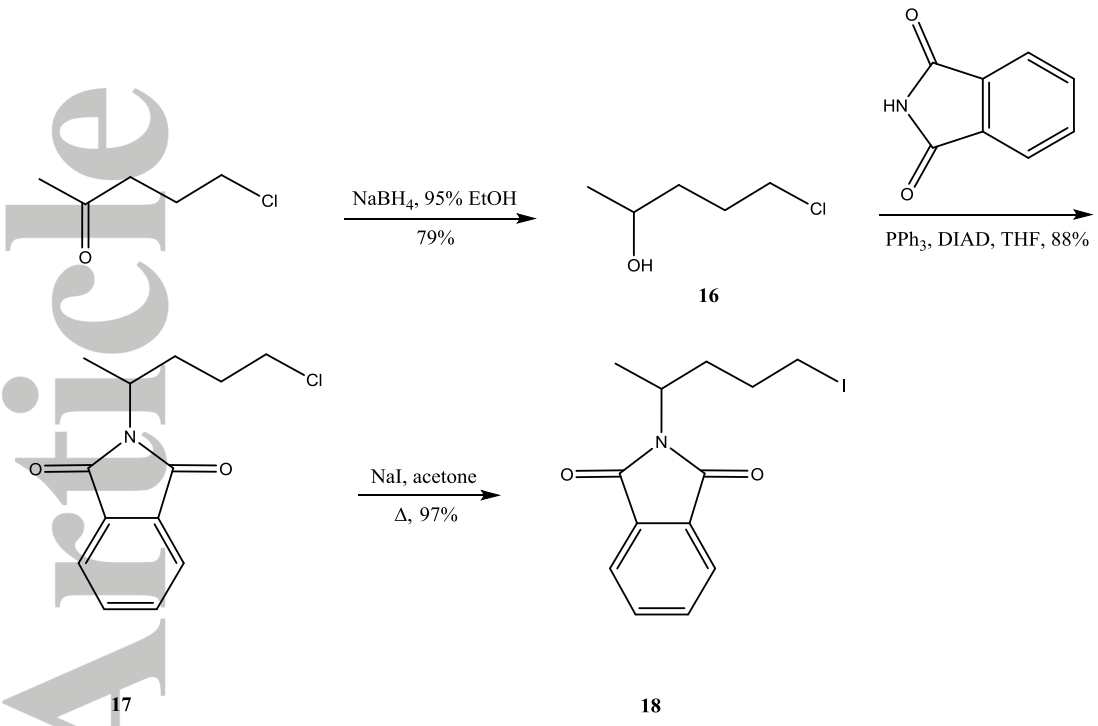
Scheme 7. Synthesis of labeled precursors for chloroquine and metabolites

[^{13}C, ^{15}N]Chloroquine **8'** is then formed in the reaction of 2,3-dihydroquinolin-4(1H)-one **7'** with 2-amino-5-diethylaminopentane in nitrobenzene at 150-160^{o}C as shown in Scheme 8.



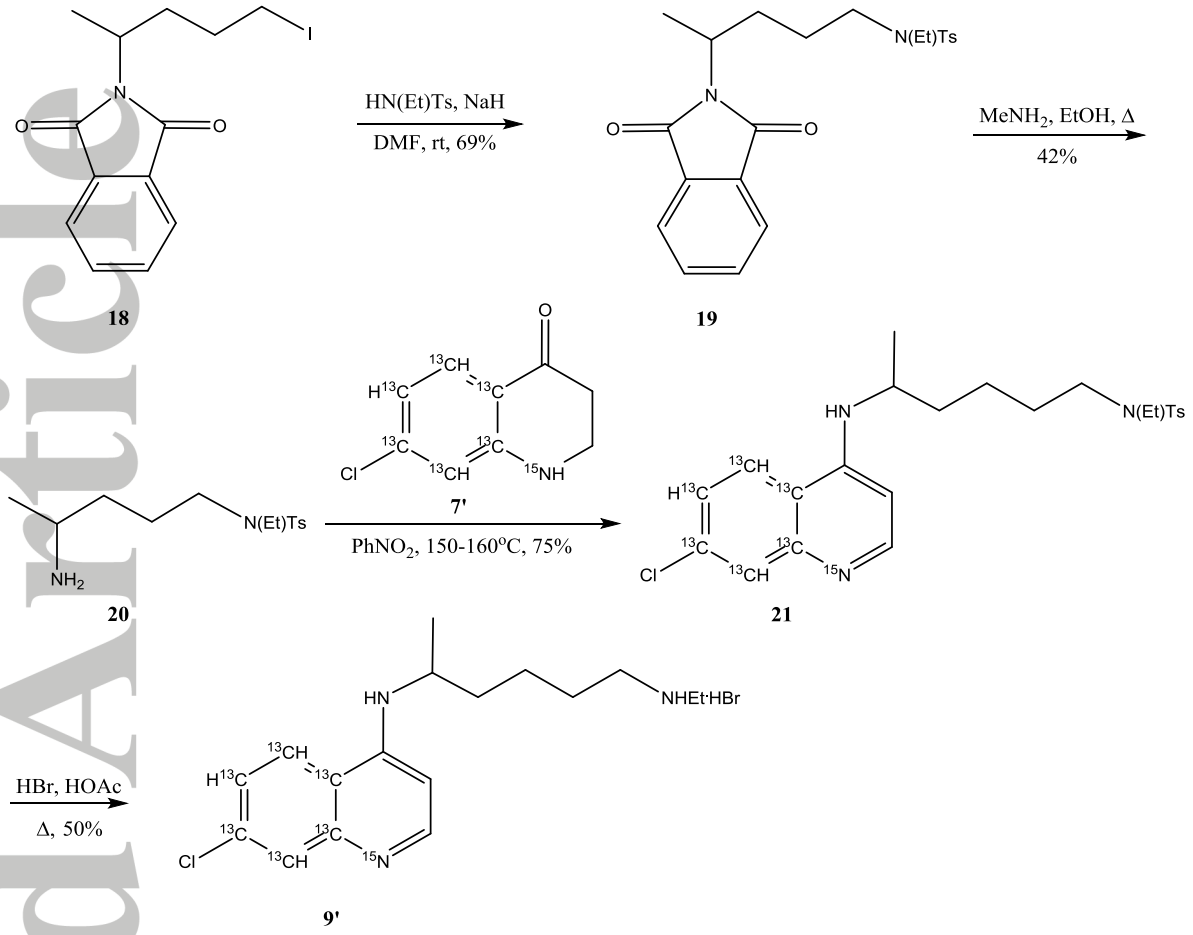
Scheme 8. Synthesis of labeled chloroquine

For the synthesis of [^{13}C,^{15}N]desethylchloroquine and [^{13}C,^{15}N]bisdesethylchloroquine, the corresponding amines need to be prepared (Scheme 9, 10 and 11). 5-chloropentane-2-ol **16** is obtained by reduction of 5-chloro-2-pentanone with sodium borohydride in 95% ethanol. The product undergoes a Mitsunobo reaction with phthalimide to form 2-(5-chloropentan-2-yl)isoindoline-1,3-dione **17**. A subsequent Finkelstein reaction with sodium iodide forms 2-(5-iodopentan-2-yl)isoindoline-1,3-dione **18**. This product serves as a common intermediate for the syntheses of the two amines **20**, **23.**



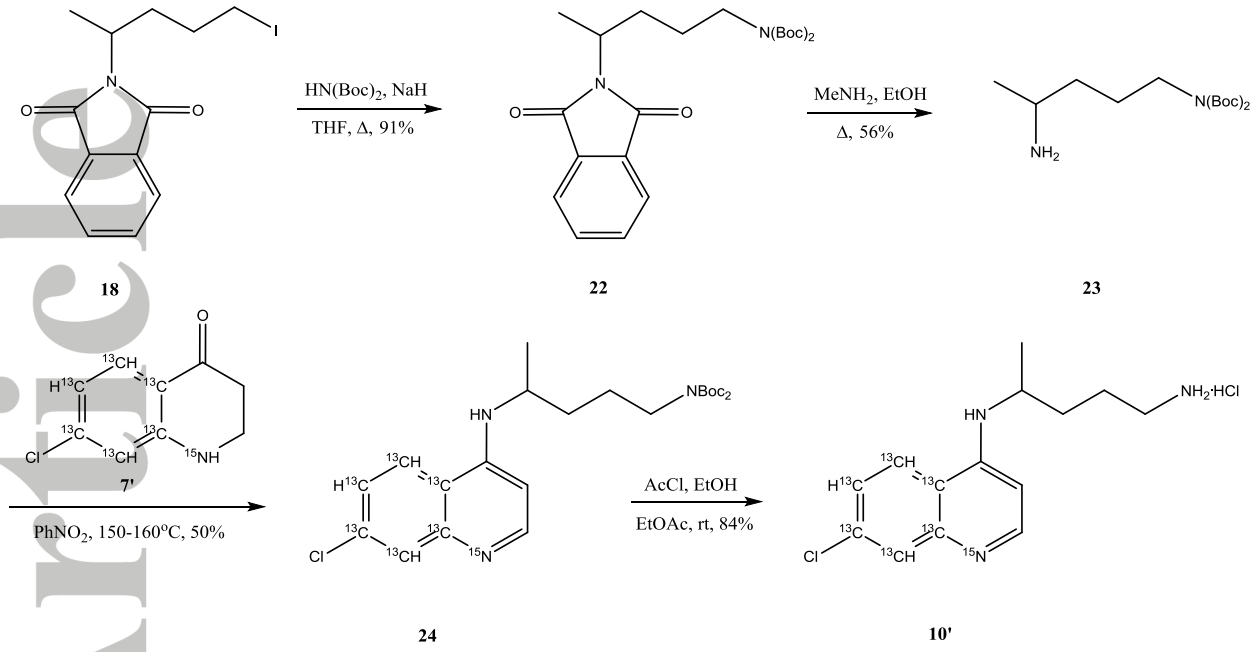
Scheme 9. Precursor synthesis for chloroquine metabolites

For the synthesis of desethylchloroquine, the reaction sequence is shown in Scheme 10, 2-(5-iodopentan-2-yl)isoindoline-1,3-dione **18** reacts with *N*-ethyl-*p*-toluenesulfonamide in the presence of sodium hydride in DMF to form N-(4-((7-chloroquinolin-4-yl)amino)pentyl)-*N*-ethyl-4-methylbenzenesulfonamide **19**. Cleavage of phthalimide group in methylamine provides N-1-ethyl-N-1-tosylpentane-1, 4-diamine **20**. Reaction of **20** with 7-chloro-2,3-dihydroquinolin-4(1H)-one **7'** in nitrobenzene affords N-(4-((7-Chloroquinolin-4-yl-4a,5,6,7,8,8a-$^{13}C_6$, ^{15}N)amino)pentyl)-N-ethyl-4-methylbenzenesulfonamide **21**. Removal of tosyl group with hydrobromic acid, acetic acid in water provides desethylchloroquine hydrobromide **9'**.



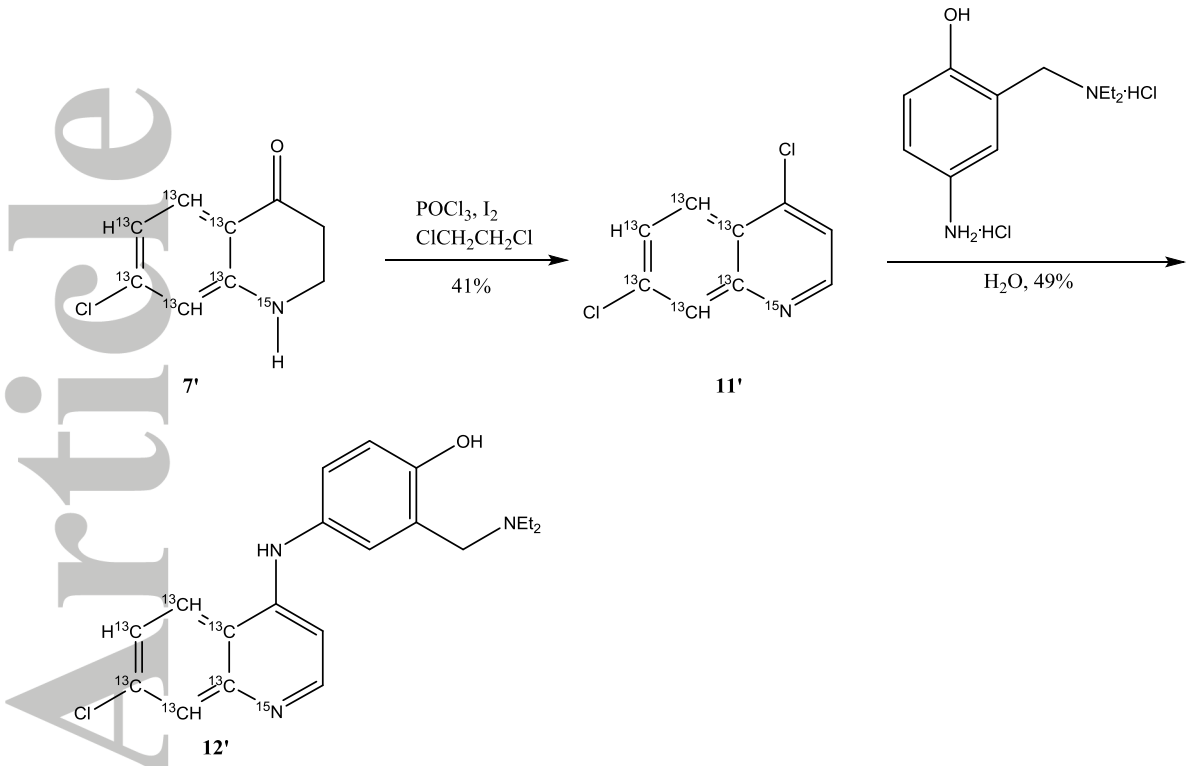
Scheme 10. Synthesis of labeled desethylchloroquine

For the synthesis of bisdesethylchloroquine, the reactions are shown in Scheme 11. Reaction of di-*t*-butyliminodicarboxylate with 2-(5-iodopentan-2-yl)isoindoline-1,3-dione **18** in the presence of sodium hydride, in THF, provides 2-(5-di-*t*-butyl-iminodicarboxypentan-2-yl)isoindoline-1,3-dione **22**. Removal of phthalimide group leads to 2-(5-di-*t*-butyl-iminodicarboxypentan-2-yl)amine **23**. Reaction of **23** with 7-chloro-2,3-dihydroquinolin-4(1H)-one **7'** in nitrobenzene then produces tert-butyl (tert-butoxycarbonyl)(4-((7-chloroquinolin-4-yl-4a,5,6,7,8,8a-$^{13}C_6$, ^{15}N)amino)pentyl)carbamate **24**. Removal of di-Boc groups using hydrogen chloride generated from acetyl chloride and ethanol finally yields labeled bisdesethylchloroquine hydrochloride **10'**.

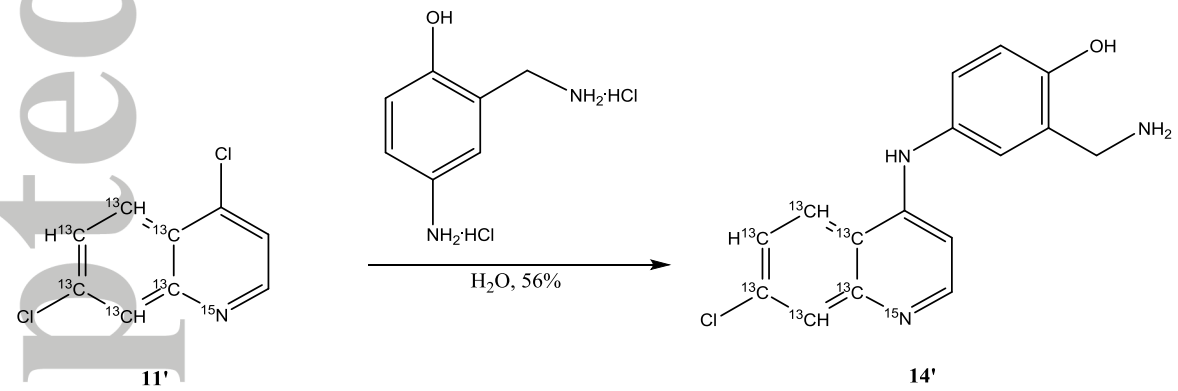


Scheme 11. Synthesis of labeled bisdesethylchloroquine

For the synthesis of [^{13}C,^{15}N]amodiaquine, [^{13}C,^{15}N]desethylamodiaquine and [^{13}C,^{15}N]bisdesethylamodiaquine, the reactions are shown in Scheme 12. 7-Chloro-2, 3-dihydroquinolin-4(1H)-one **7'** is converted to 4,7-dichloroquinoline **11'**[18], it then reacts with the corresponding amines as shown in Scheme 12 and 13 to provide [^{13}C,^{15}N]amodiaquine **12'** and [^{13}C,^{15}N]bisdesethylamodiaquine **14'**, only one regioisomer is obtained for the synthesis of bisdesethylamodiaquine.[19, 20]

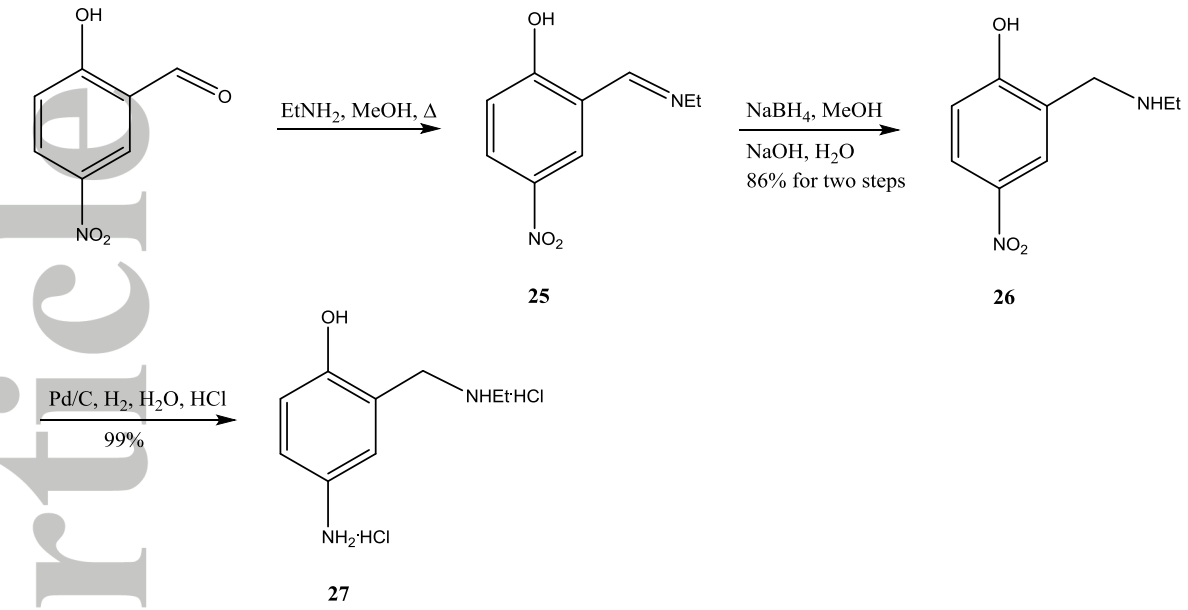


Scheme 12. Synthesis of labeled amodiaquine



Scheme 13. Synthesis of labeled bisdesethylamodiaquine

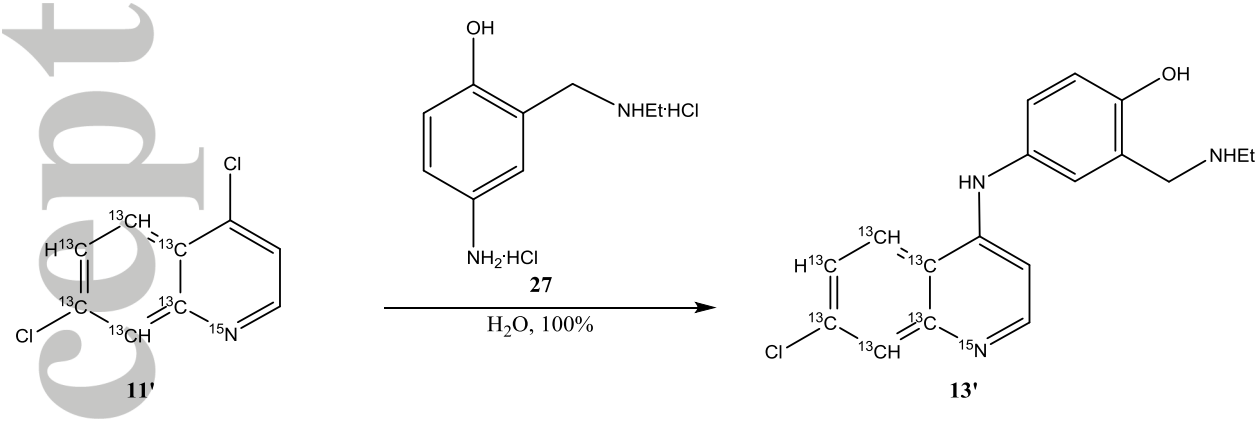
The corresponding amine precursor for desethylamodiaquine is constructed as illustrated in scheme 14.



Scheme 14. Precursor synthesis for desethylamodiaquine

2-((Ethylimino)methyl)-4-nitrophenol **25** is prepared from 2-hydroxy-5-nitrobenzaldehyde and ethylamine in refluxing methanol. The enamine is reduced by sodium borohydride in a basic solution to afford 4-amino-2-(ethylamino)methylphenol **26**, hydrogenation of nitro group by palladium on carbon provides the desired amine salt 4-amino-2-((ethylamino)methyl)phenol bishydrochloride **27**.

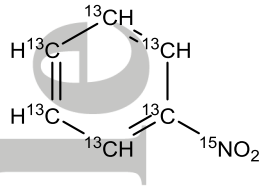
Labeled desethylamodiaquine **13'** is prepared by heating 4,7-dichloroquinoline **11'** and 4-amino-2-((ethylamino)methyl)phenol bishydrochloride **27** in water as shown in Scheme 15.[20]



Scheme 15. Synthesis of labeled desethylamodiaquine

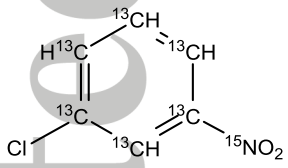
In summary, we report a concise, straightforward synthetic route to stable isotope labeled chloroquine, amodiaquine and their metabolites, with consideration to the cost of starting materials as well as atom and isotope efficiencies of the chemistry used. To our knowledge, these are the first reported syntheses of [^{13}C,^{15}N] labeled antimalarial drugs.

Experimental: General



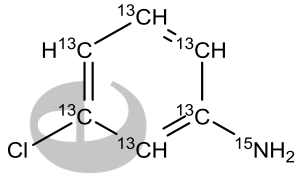
(Nitro-^{15}N)benzene-1,2,3,4,5,6-$^{13}C_6$ **1'**:

A modification of the reported procedure was used:[21] A one-neck 250 mL round bottom flask equipped with a reflux condenser was charged with benzene-1,2,3,4,5,6-$^{13}C_6$ (8.41 g, 100 mmol), potassium [^{15}N]nitrate (10.21 g, 100 mmol) and dichloromethane (60 mL) and the mixture was cooled with an ice bath. Sulfuric acid (21.32 mL) was added dropwise to the above mixture through an addition funnel. The mixture was stirred at room temperature overnight. Additional sulfuric acid (25 mL) and potassium [^{15}N]nitrate (4.08 g, 40 mmol) were added and the mixture was stirred at room temperature overnight. The reaction progress was monitored for completion by ^{13}C NMR. The layers were separated and the aqueous layer was washed with dichloromethane. The combined organic layers were washed with water, saturated sodium bicarbonate and brine, then dried over sodium sulfate. The solvent was removed by rotary evaporation and the residue was distilled under vacuum at 92-94^{o}C (15 mmHg) to provide the title product as a colorless liquid (10.99 g, 84%). ^{1}H NMR ($CDCl_3$, 300 Hz) δ 8.61-8.49 and 8.08-7.94 (bd, *J* = 167.4 Hz), 7.93-7.77 and 7.40-7.24 (bd, *J* = 163.2 Hz), 7.55-7.40 (bpent, *J* = 8.67 Hz). ^{13}C NMR ($CDCl_3$, 75 Hz) δ 148.37 (ddt, *J* = 9.73, 14.66, 67.47 Hz), 134.82 (mt, *J* = 55.40 Hz), 129.47 (mt, *J* = 56.17 Hz), 123.63 (mt, *J* = 62.28 Hz).



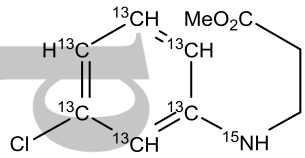
1-Chloro-3-(nitro-^{15}N)benzene-1,2,3,4,5,6-$^{13}C_6$ **2'**:

This compound was prepared following a reported procedure as outlined below:[22] A suspension of (nitro-^{15}N)benzene-1,2,3,4,5,6-$^{13}C_6$ **1'** (2.37 g, 18.2 mmol) and trichloroisocyanuric acid (1.44 g, 6.20 mmol) in sulfuric acid (7.3 mL) was heated to 80^{o}C overnight. After the addition of cold water, the resulting solution was extracted with EtOAc. The EtOAc layer was washed with water, saturated sodium bicarbonate and brine. It was then dried over sodium sulfate. The solvent was removed under reduced pressure, and the residue was purified on silica gel with 10% EtOAc/hexanes to provide 2.95 g (99%) of the title product as a light yellow liquid. ^{1}H NMR ($CDCl_3$, 300 Hz) δ 8.23 (complexed d, 1 H), 8.13 (complex d, 1 H), 7.69 (complex d, 1 H), and 7.55 (complex d, 1 H). ^{13}C NMR ($CDCl_3$, 75 Hz) δ 149.0 (complex t), 135.5 (complex t), 134.8 (complex t), 130.5 (complex t), 124.1 (complex t) and 121.9 (complex t). HRMS for $^{13}C_6H_4Cl^{15}NO_2$ calculated 163.0027 (M-H), found 163.0022.



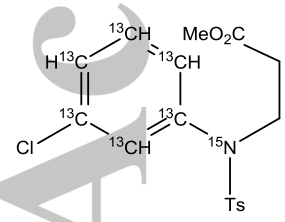
3-Chloroaniline-$^{13}C_6$, ^{15}N **3'**:

This compound was prepared following a reported procedure as outlined below:[23] To a stirred suspension of montmorillonite (5.34 g) and 1-chloro-3-(nitro-^{15}N)benzene-1,2,3,4,5,6-$^{13}C_6$ **2'** (2.936 g, 17.84 mmol) in dry ethanol (18.0 mL) was added anhydrous hydrazine (3.5 mL, 111.5 mmol) in one portion. The resulting reaction mixture was heated to reflux overnight. More hydrazine (1.0 mL, 32 mmol) was added and the reaction was heated for another night. The contents were filtered and washed with ethanol, the solvent was removed under reduced pressure and the residue was purified on silica gel with 20% EtOAc/hexanes to provide 1.787 g (74%) of the product as a light yellow liquid. ^{1}H NMR ($CDCl_3$, 300 Hz) δ 7.44-7.28 and 7.09-7.00 (m, 1 H), 7.00-6.93 and 6.91-6.74 (m, 1 H), 6.91-6.74 and 6.54-6.38 (m, 1 H), 6.54-6.38 and 6.37-6.25 (m, 1 H), and 3.76 (br d, *J* = 79 Hz). ^{13}C NMR ($CDCl_3$, 75 Hz) δ 147.8 (complex t, *J* = 62 Hz), 135.0 (complex t, *J* = 67 Hz), 130.5 (complex t, *J* = 58 Hz), 118.6 (complex t, *J* = 62 Hz) and 114.2 (complex pent, *J* = 67 Hz). HRMS for $^{13}C_6H_6Cl^{15}N$ calculated 135.0441 (M+H), found 135.0437.



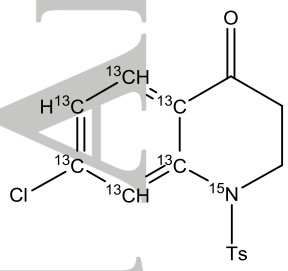
Methyl 3-((3-chlorophenyl-1,2,3,4,5,6-$^{13}C_6$)amino-^{15}N)propanoate **4'**:

This compound was prepared according to WS Johnson procedure:[14] A mixture of 3-chloroaniline-$^{13}C_6$, ^{15}N **3'** (3.349 g, 24.89 mmol), methyl acrylate (3.36 mL, 37.33 mmol), and glacial acetic acid (0.190 mL) was heated to reflux for 24 hr, GC/MS showed a complete reaction. The reaction mixture was loaded on silica gel, and purified with 20% ethyl acetate in hexanes to provide 4.06 g (74%) of the title product as a colorless liquid. ^{1}H NMR ($CDCl_3$, 300 Hz) δ 7.45-7.30 and 7.15-6.94 (m, 1 H), 6.94-6.20 (m, 1 H), 6.35-6.20 and 6.50-6.40 (m, 1 H), 6.40-6.30 and 6.30-6.20 (m, 1 H), 4.20 (br d *J* = 82 Hz, 1 H), 3.74 (s, 3 H), 3.46 (m, 2 H), 2.65 (m, 2 H). ^{13}C NMR ($CDCl_3$, 75 Hz) δ 172.9 (s, carbonyl C), 148.9 (complex t, *J* = 62 Hz), 135.3 (complex t, *J* = 68 Hz), 130.4 (complex t, *J* = 58 Hz), 117.6 (complex t, *J* = 62 Hz), 112.6 (complex t, *J* = 67 Hz), 111.5 (complex t, *J* = 62 Hz), 52.1 (s), 39.3 (dt, *J* = 9.5, 3.2 Hz), 33.7 (d, *J* = 0.9 Hz). HRMS calculated for $^{13}C_6C_4H_{12}Cl^{15}NO_2$ (M+H) 221.0809, found 221.0810.



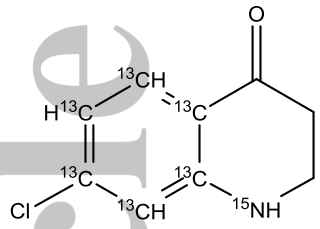
Methyl 3-((N-(3-chlorophenyl-1,2,3,4,5,6-$^{13}C_6$)-4-methylphenyl)sulfonamido-^{15}N)-propanoate **5'**:

To a solution of methyl 3-((3-chlorophenyl-1,2,3,4,5,6-$^{13}C_6$)amino-^{15}N)propanoate **4'** (4.063 g, 18.41 mmol) in pyridine (20 mL) was added tosyl chloride (3.89 g, 20.42 mmol) in one portion at room temperature. The reaction mixture was then heated to reflux for 30 minutes. The reaction was then cooled to room temperature and water was added. The mixture was extracted with ether, and the organic extracts were washed twice with 1 M HCl, water and saturated sodium bicarbonate then dried over sodium sulfate. The solvent was removed under reduced pressure and the residue was purified on silica gel with 30% ethyl acetate in hexanes to provide 5.89 g (85%) of the title product as a colorless liquid. ^{1}H NMR ($CDCl_3$, 300 Hz) δ 7.55-7.33 (bm, 1 H), 7.38 (d, *J* = 8.33 Hz, 1 H), 7.25-7.06 (bm, 1 H), 7.17 (d, *J* = 8.33 Hz), 6.99-6.78 (bm, 1 H), 6.70-6.51 (bm, 1 H), 3.70 (dt, *J* = 7.40, 3.70 Hz, 2 H), 3.51 (s, 3 H), 2.46 (dt, *J* = 7.40, 2.36 Hz, 2 H), 2.33 (s, 3 H). ^{13}C NMR ($CDCl_3$, 75 Hz) δ 171.4 (s, carbonyl C), 144.1 (s), 140.5 (complex t, *J* = 65 Hz), 134.8 (complex t, *J* = 60 Hz), 131.2-126.2 (m), 52.0 (s), 40.7 (d, *J* = 7.3 Hz), 34.1 (d, *J* = 2.6 Hz), 21.8. HRMS calculated for $^{13}C_6C_{11}H_{18}Cl^{15}NO_4S$ (M+H) 375.0898, found 375.0897.



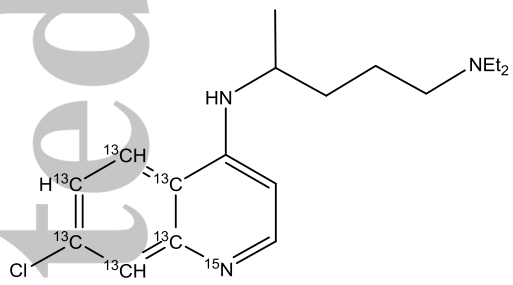
7-Chloro-1-tosyl-2,3-dihydroquinolin-4(1H)-one-4a,5,6,7,8,8a-$^{13}C_6$, ^{15}N **6'**:

A mixture of methyl 3-((N-(3-chlorophenyl-1,2,3,4,5,6-$^{13}C_6$)-4-methylphenyl)sulfonamido-^{15}N)propanoate **5'** (7.91 g, 21.10 mmol), concentrated hydrochloric acid (11.6 mL), water (35.0 mL), and dioxane (97.0 mL) was heated to reflux for 5 hours. The solution was concentrated with rotary evaporation to half of the volume, and neutralized by careful addition of solid sodium bicarbonate. The mixture was then extracted with ether to remove any unreacted starting material. The aqueous layer was acidified with concentrated hydrochloric acid and then extracted with ether. The organic extracts were dried over sodium sulfate and the solvent was removed under reduced pressure to provide the acid as gummy syrup that was used without further purification. To the crude product obtained above was added dichloromethane (10.0 mL) followed by addition of oxalyl chloride (13.72 mL, 27.43 mmol, 2.0 M in dichloromethane) and the mixture was stirred at room temperature overnight. The excess reagent and solvent were removed under reduced pressure. To this mixture was added dichloromethane (105 mL). The solution was cooled with an ice bath and aluminum chloride (3.17 g, 23.77 mmol) was added in one portion. The reaction mixture was stirred at 0^{o}C for 5-10 minutes, and allowed to warm to room temperature for 1-2 hr, or until TLC showed a complete reaction. The mixture was cooled to 0^{o}C and water was slowly added. The reaction mixture was then extracted with dichloromethane, dried over sodium sulfate and purified on silica gel with 20% ethyl acetate in hexanes to provide 5.04 g (70% from methyl 3-((N-(3-chlorophenyl-1,2,3,4,5,6-$^{13}C_6$)-4-methylphenyl)sulfonamido-^{15}N)propanoate **5'**) of the title product as colorless liquid. ^{1}H NMR ($CDCl_3$, 300 Hz) δ 8.26-8.09 (m, 1 H), 7.71-7.58 (m, 1 H), 7.62 (d, *J* = 8.50 Hz, 1 H), 7.58-7.49 (m, 1 H), 7.29 (d, *J* = 7.95 Hz), 7.05-6.90 (m, 1 H), 4.24 (dt, *J* = 6.44, 3.73 Hz, 2 H), 2.48-2.36 (m, 5 H). ^{13}C NMR ($CDCl_3$, 75 Hz) δ 191.8 (d, *J* = 51.8 Hz, carbonyl C), 145.1 (s), 143.3 (complex t, *J* = 64.1 Hz), 141.1 (complex t, *J* = 64.1 Hz), 136.5 (d, *J* = 4.15 Hz), 130.4 (s), 129.2 (complex t, *J* = 58.3 Hz), 127.0 (s), 126.0 (complex t, *J* = 60.0 Hz), 124.2 (complex t, *J* = 65.0 (s), 123.8 (dt, *J* = 61, 7.4 Hz), 46.3 (d, *J* = 5.84 Hz), 36.5 (d, *J* = 10.8 Hz), 21.8 (s).



7-Chloro-2, 3-dihydroquinolin-4(1H)-one-4a,5,6,7,8,8a-$^{13}C_6$, ^{15}N **7'**:

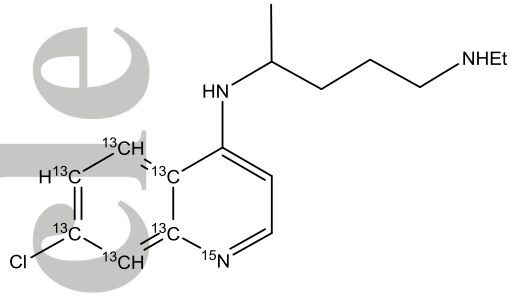
A mixture of 7-chloro-1-tosyl-2,3-dihydroquinolin-4(1H)-one-4a,5,6,7,8,8a-$^{13}C_6$, ^{15}N **6'** (5.04 g, 14.70 mol), acetic acid (50.0 mL), concentrated hydrochloric acid (50.0 mL), and water (12.5 mL) was heated to reflux for 5 hr. The mixture was diluted with water and neutralized with slow addition of solid sodium carbonate. The mixture was extracted with ether, and the organic extracts were washed with 5% potassium hydroxide, brine and dried over sodium sulfate. The solvent was removed under reduced pressure to provide 2.57 g (93%) of the title compound as a yellow solid. ^{1}H NMR ($CDCl_3$, 300 Hz) δ 8.08 (dt, *J* = 12.9, 8.33 Hz, 1 H), 8.08 (dt, *J* = 12.9, 8.33 Hz, 1 H), 7.06-6.94 (bm, 1 H), 6.50-6.39 (bm, 1 H), 4.49 (bd, *J* = 86.5 Hz, 1 H), 3.68-3.57 (m, 2 H), 3.45 (dt, *J* = 6.90, 3.45 Hz, 2 H). ^{13}C NMR ($CDCl_3$, 75 Hz) δ 192.5 (s, carbonyl C), 152.6 (complex t, *J* = 60.5 Hz), 141.4 (complex t, *J* = 66.7 Hz), 129.5 (complex t, *J* = 60 Hz), 118.8 (complex t, *J* = 60 Hz), 117.2 (apparent d, *J* = 66 Hz), 115.3 (complex t, *J* = 60 Hz), 42.3 (br d, *J* = 8.3 Hz), 38.0 (d, *J* = 12.2 Hz).



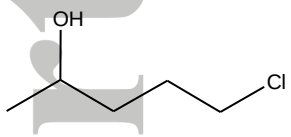
Chloroquine **8'**:

A mixture of 7-chloro-2,3-dihydroquinolin-4(1H)-one-4a,5,6,7,8,8a-$^{13}C_6$, ^{15}N **7'** (0.849 g, 4.5 mmol), 2-amino-5-diethylaminopentane (1.118 g, 7.065 mmol) and nitrobenzene (0.456 g, 3.78 mmol) was heated to 150-160^{o}C (oil temp) overnight. The solvent and excess reagents were removed by vacuum distillation at 80^{o}C/15 mmHg, and the residue was purified on silica gel with dichloromethane: methanol: ammonium hydroxide 80:20:1 to provide 1.21 g of the title compound as a colorless solid (82%), the material was further purified on HPLC (Waters, XTerra C18, 5 μ, 4.6 x 150 mm, 1mL/min, linear gradient 98/2 to 20/80 of 0.1% TFA in water/acetonitrile for 16 minutes, retention time 10.88 min) to get the product as trifluoroacetic acid salt. ^{1}H NMR (D_2O, 300 Hz).δ 8.40-8.35 and 8.00-7.90 (m, 1 H), 8.30-8.20 (m, 1 H), 8.15-8.05 and 7.60-7.50 (m, 1 H), 7.90-7.80 and 7.40-7.30 (m, 1 H), 6.85-6.70 (m, 1 H), 4.15-4.05 (m, 1 H), 3.20-3.02 (m, 6 H), 1.75 (br s, 4 H), 1.35 (d, *J* = 5.25 Hz, 3 H), 1.18 (br t, *J* = 6.81 Hz, 6 H). ^{13}C NMR ($CDCl_3$, 75 Hz) δ 154.9 (d, *J* = 57.9 Hz), 142.0 (d, *J* = 12.3 Hz), 138.9 (t, *J* = 65.1 Hz), 137.6 (dt, *J* = 10.5, 62.7 Hz), 126.7 (dt, *J* = 5.24, 59.3 Hz), 123.7 (t, *J* = 58.1 Hz), 118.5 (t, *J* = 66.3 Hz), 114.6 (dt, *J* = 6.99, 60.5 Hz), 98.5, 57.4, 51.0, 49.4, 47.1, 31.7, 20.1, 18.6, 8.0. HRMS calculated for $^{13}C_6C_{12}H_{26}Cl^{15}NN_2$ (M+H) 327.2068, found 327.2057.

Syntheses leading to desethylchloroquine ($^{13}C_6$, ^{15}N) **9'**

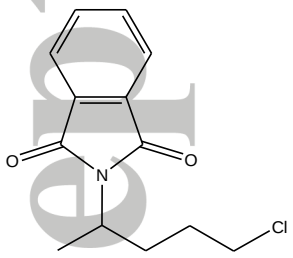


Desethylchloroquine **9'**



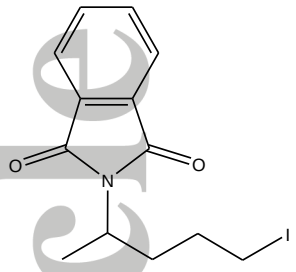
5-Chloropentan-2-ol **16**:

This compound was prepared following a reported procedure as outlined below:[24] To a stirred solution of 5-chloro-2-pentanone (12.05 g, 100 mmol) in 95% EtOH (100 mL) was added $NaBH_4$ portionwise at 0°C. The mixture was stirred at 0°C for 30 min and allowed to warm to room temperature and stir for 2 hr. The reaction mixture was cooled to 0°C and acetone (20.0 mL) was added dropwise. The solvents were removed under reduced pressure and water was added to the residue. The solution was extracted with ether and the combined extracts were dried over sodium sulfate and evaporated. The residue was distilled (bp 74-76°C/15 mmHg) to give 9.27 g (79%) of the title product as a colorless liquid. ^{1}H NMR ($CDCl_3$) δ 3.84 (sext, *J* = 6.19 Hz, 1 H), 3.58 (dt, *J* = 6.69, 0.88 Hz, 2 H), 2.01-1.76 (m, 2 H), 1.69-1.49 (m, 2 H), 1.22 (d, *J* = 6.19 Hz, 3 H). ^{13}C NMR ($CDCl_3$) δ 67.6, 45.3, 36.5, 29.1, 23.9.



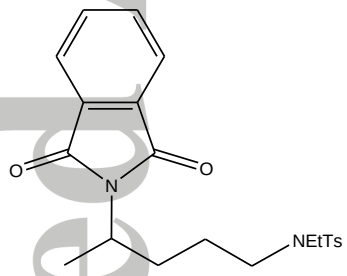
2-(5-Chloropentan-2-yl)isoindoline-1,3-dione **17**:

To a solution of 5-chloropentan-2-ol (2.69 g, 21.94 mmol), phthalimide (3.55 g, 24.14 mmol), and triphenylphosphine (6.33 g, 24.14 mmol) in THF (50 mL) was added dropwise diisopropyl azodicarboxylate (4.88 g, 24.14 mmol) at 0°C. The mixture was allowed to warm to room temperature and stirred overnight. To the reaction mixture was added silica gel and slurry was concentrated. The mixture was loaded onto a silica gel column and purified with 15% ethyl acetate in hexanes to provide the title product (4.88 g, 88%) as a clear liquid that solidified on standing. ^{1}H NMR ($CDCl_3$) δ 7.86-7.79 (m, 2 H), 7.74-7.67 (m, 2 H), 4.44-4.31 (m, 1 H), 3.53 (dt, *J* = 6.80, 1.26 Hz, 2 H), 2.30-2.15 (m, 1 H), 1.9-1.84 (m, 1 H), 1.84-1.64 (m, 2 H), 1.50 (d, *J* = 6.96 Hz, 3 H). ^{13}C NMR ($CDCl_3$) δ 168.7, 134.2, 132.2, 123.4, 46.9, 44.5, 31.2, 30.0, 19.0.



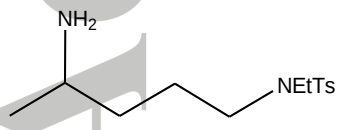
2-(5-Iodopentan-2-yl)isoindoline-1,3-dione **18**:

A mixture of 2-(5-chloropentan-2-yl)isoindoline-1,3-dione **17** (6.99 g, 27.77 mmol) and sodium iodide (6.24 g, 41.66 mmol) in acetone (75 mL) was refluxed for two days. The reaction mixture was filtered and the solvent was removed under reduced pressure. The residue was purified on silica gel with 15% ethyl acetate in hexanes to provide the title product (9.23 g, 97%) as a clear liquid. ^{1}H NMR ($CDCl_3$) δ 7.86-7.79 (m, 2 H), 7.74-7.67 (m, 2 H), 4.43-4.30 (m, 1 H), 3.17 (t, *J* = 6.96 Hz, 2 H), 2.28-2.14 (m, 1 H), 1.95-1.69 (m, 3 H), 1.49 (d, *J* = 6.96 Hz, 3 H). ^{13}C NMR ($CDCl_3$) δ 168.6, 134.2, 132.1, 123.4, 46.6, 34.8, 30.9, 19.0, 5.77.



N-(4-(1,3-Dioxoisoindolin-2-yl)pentyl)-N-ethyl-4-methylbenzenesulfonamide **19**:

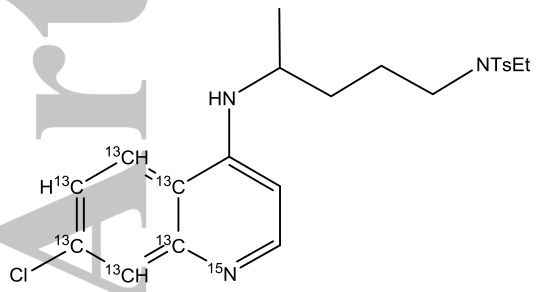
To a mixture of 2-(5-iodopentan-2-yl)isoindoline-1,3-dione (4.61 g, 13.45 mmol) **18** and *N*-ethyl-*p*-toluenesulfonamide (3.74 g, 18.78 mmol) in DMF (50.0 mL) was added sodium hydride (0.75 g, 18.77 mmol, 60% in oil) in one portion at 0^{o}C. The mixture was allowed to warm to room temperature and stirred overnight. Water was added slowly and the reaction was extracted with diethyl ether, the organic extracts were washed with 0.2 M sodium hydroxide, dried and concentrated. The residue was purified on silica gel with 35-40% ethyl acetate in hexanes to provide the title product as a clear liquid (3.86 g, 69%). ^{1}H NMR ($CDCl_3$) δ 7.85-7.79 (m, 2 H), 7.75-7.69 (m, 2 H), 7.66 (d, *J* = 8.28 Hz, 2 H), 7.24 (d, *J* = 8.28 Hz, 2 H), 4.41-4.26 (m, 1 H), 3.33-2.96 (m, 4 H), 2.38 (s, 3 H), 2.16-2.00 (m, 1 H), 1.82-1.67 (m, 1 H), 1.56-1.43 (m, 2 H), 1.49 (d, *J* = 6.96 Hz, 3 H). ^{13}C NMR ($CDCl_3$) δ 168.7, 143.2, 137.4, 134.1, 132.2, 129.8, 127.3, 123.3, 47.2, 47.1, 43.0, 30.9, 26.2, 21.7, 18.9, 14.2.



N1-Ethyl-N1-tosylpentane-1,4-diamine **20**:

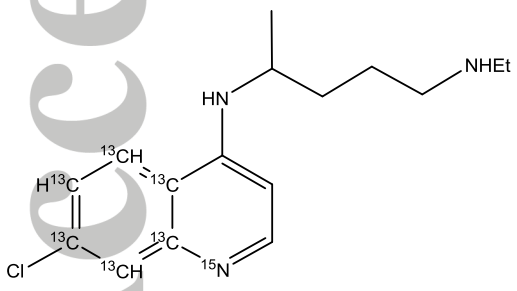
To a mixture of N-(4-(1,3-dioxoisoindolin-2-yl)pentyl)-N-ethyl-4-methylbenzenesulfonamide **19** (3.86 g, 9.32 mmol) in ethanol (38.0 mL) was added methylamine (3.48 mL, 27.97 mmol, 33% wt in

ethanol). The reaction mixture was heated at reflux overnight. The reaction was cooled to room temperature, precipitates were filtered off and washed with ethanol. The filtrate was concentrated and acidified with 1 M hydrochloric acid, then extracted with ether. The aqueous layer was then basified with sodium hydroxide and extracted with ether. The organic extracts from the basic extraction were concentrated to provide the title product (1.11 g, 42%) as a clear liquid. ^{1}H NMR ($CDCl_3$) δ 7.69 (d, *J* = 8.55 Hz, 2 H), 7.29 (d, *J* = 8.55 Hz, 2 H), 3.21 (q, *J* = 7.23 Hz, 2 H), 3.12 (dt, *J* = 7.02, 2.30 Hz, 2 H), 2.88 (sext, *J* = 6.30 Hz, 1 H), 1.68-1.48 (m, 2 H), 1.84-1.64 (m, 2 H), 1.37-1.25 (m, 2 H), 1.10 (t, *J* = 7.23 Hz, 3 H), 1.05 (d, *J* = 6.30 Hz, 3 H). ^{13}C NMR ($CDCl_3$) δ 143.2, 137.4, 129.8, 127.3, 47.8, 46.8, 42.9, 37.1, 25.9, 24.3, 21.7, 14.3. HRMS calculated for $C_{14}H_{24}N_2O_2S$ (M+H) 285.1637, found 285.1627.



N-(4-((7-Chloroquinolin-4-yl-4a,5,6,7,8,8a-$^{13}C_6$,^{15}N)amino)pentyl)-N-ethyl-4-methylbenzenesulfonamide **21**:

A mixture of N-1-ethyl-N-1-tosylpentane-1,4-diamine (0.462 g, 1.63 mmol) **20**, 7-chloro-2,3-dihydroquinolin-4(1H)-one (^{15}N, $^{13}C_6$) **7'** (0.193 g, 1.02 mmol) in nitrobenzene (0.088 mL, 0.852 mmol) was heated at 160-165^{o}C overnight. The reaction solution was loaded onto silica gel and eluted with 70% ethyl acetate in hexanes to provide 0.347 g (75%) of the title product as brown syrup. ^{1}H NMR ($CDCl_3$) δ 8.44-8.17 (bm, 1 H), 7.83-7.54 (m, 1 H), 7.65 (d, *J* = 8.39 Hz, 2 H), 7.27 (d, *J* = 8.39 Hz, 2 H), 7.33-6.98 (m, 1 H), 6.71-6.61 (m, 1 H), 6.49-6.40 (m, 1 H), 6.33-5.81 (m, 1 H), 3.90-3.76 (m, 1 H), 3.29-3.05 (m, 4 H), 2.41 (s, 3 H), 1.81-1.66 (m, 2 H), 1.39 (d, *J* = 6.91 Hz, 3 H), 1.06 (t, *J* = 7.45 Hz, 3 H). ^{13}C NMR ($CDCl_3$) δ 143.4 (t, *J* = 62.8 Hz), 137.2 (d, *J* = 65.3 Hz), 129.7, 126.3 (dt, *J* = 60.0, 5.97 Hz), 123.2 (t, *J* = 72.1 Hz), 116.0 (t, *J* = 58.9 Hz), 49.3, 47.3, 43.2, 32.4, 25.5, 21.5, 20.1, 14.0. HRMS calculated for $^{13}C_6C_{17}H_{28}Cl^{15}NN_2O_2S$ (M+H) 453.1843, found 453.1826.

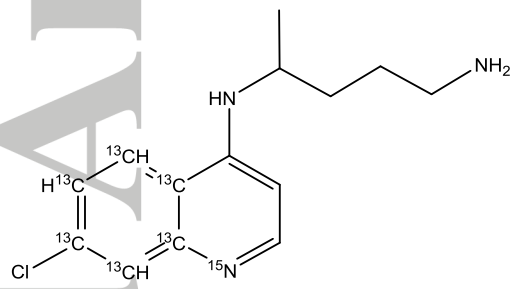


Desethylchloroquine **9'**:

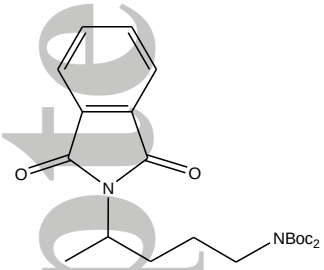
A mixture of N-(4-((7-chloroquinolin-4-yl-4a,5,6,7,8,8a-$^{13}C_6$, ^{15}N)amino)pentyl)-N-ethyl-4-methylbenzenesulfonamide **21** (0.347 g, 0.766 mmol) in 48% aqueous hydrobromic acid (2.02 mL) and acetic acid (1.13 mL) was heated to reflux overnight. The mixture was neutralized with sodium hydroxide and extracted with dichloromethane. The solvent was removed under reduced pressure

and the residue was purified on silica gel with 10-15% MeOH in CH_2Cl_2 to provide 0.114 g (50%) of the title product as a light yellow liquid. The product was then acidified with 2.0 mL of 1 M HCl and the mixture was dried under reduced pressure. The residue was purified with HPLC (Waters, XTerra , 5 μ, 4.6 x 150 mm, 1 mL/min, linear gradient, 98/2 to 20/80 of 0.1 % TFA in water/acetonitrile for 16 minutes, retention time 10.95 min). ^{1}H NMR (D_2O) δ 8.50-8.33 and 7.96-7.78 (m, 1 H), 8.22 (br t, *J* = 7.56 Hz, 1 H), 8.05 and 7.49 (m, 1 H), 7.96-7.78 and 7.53-7.44 (m, 1 H), 6.79 (q, *J* = 5.43 Hz, 1 H), 4.08 (q, *J* = 5.97 Hz, 1 H), 3.02 (q, *J* = 7.07 Hz, 4 H), 1.77 (br s, 4 H), 1.36 (d, *J* = 6.25 Hz, 3 H), 1.21 (t, *J* = 7.45 Hz, 3 H). ^{13}C NMR (D_2O) δ 156.3, 155.6, 154.9, 142.2, 142.0, 139.7 (complex t, *J* = 65.66 Hz), 138.4 (ddt, *J* = 4.70, 14.00, 63.03 Hz), 127.8 (dt, *J* = 6.30, 60.22 Hz), 124.0 ((dt, *J* = 5.03, 58.64 Hz), 119.0 (complex t, *J* = 67.16 Hz), 116.8, 115.3 (complex t, *J* = 60.8 Hz), 100.0, 46.0, 42.4, 10.3. HRMS calculated for $^{13}C_6C_{10}H_{22}Cl^{15}NN_2$ (M+H) 299.1755, found 299.1750.

Syntheses leading to bisdesethylchloroquine ($^{13}C_6$, ^{15}N) **10'**

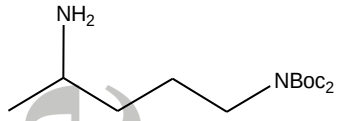


Bisdesethylchloroquine **10'**



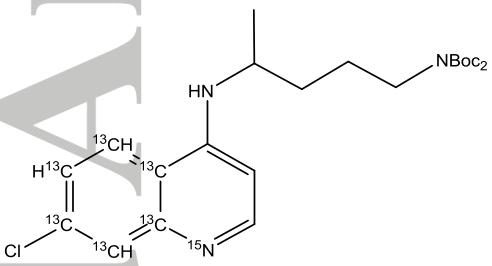
2-(5-di-tert-butyl-iminodicarboxypentan-2-yl)isoindoline-1,3-dione **22**:

To a mixture of 2-(5-iodopentan-2-yl)isoindoline-1,3-dione (4.614 g, 13.45 mmol) **18** and di-*tert*-butyl-iminodicarboxylate (4.08 g, 18.78 mmol) in THF (50.0 mL) was added sodium hydride (0.751 g, 18.78 mmol, 60% in oil) at 0°C. The mixture was allowed to warm to room temperature and was then heated to reflux overnight. The mixture was partitioned between ethyl acetate and water, and the organic extracts were washed with brine and dried over sodium sulfate. The solvent was removed under reduced pressure and the residue was purified on silica gel with 10-15% ethyl acetate in hexanes to provide 5.302 g (91%) of the title product as colorless liquid. ^{1}H NMR (D_2O) δ 7.81 (dd, *J* = Hz, 2 H), 7.70 (dd, *J* = Hz, 2 H), 4.43 (m, 1 H), 3.55 (t, *J* = 7.56 Hz, 2 H), 2.14-1.99 (m, 2 H), 1.82-1.67 (m, 2 H), 1.48 (s, 9 H), 1.45 (s, 9 H), 1.47 (d, *J* = 6.91 Hz, 3 H). ^{13}C NMR ($CDCl_3$) δ 168.4, 152.5, 133.8, 132.0, 123.1, 82.1, 47.1, 45.9, 30.9, 28.04, 28.03, 26.4, 18.7.



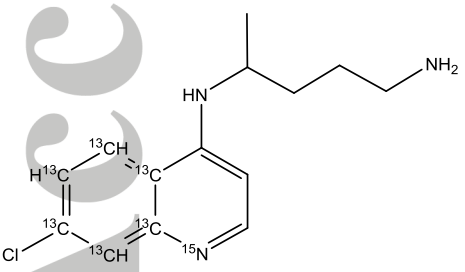
2-(5-di-tert-butyl-iminodicarboxypentan-2-yl)amine **23**:

To a solution of 2-(5-di-tert-butyl-iminodicarboxypentan-2-yl)isoindoline-1,3-dione **22** (5.30 g, 12.26 mmol) in ethanol (50.0 mL) was added methylamine (4.60 mL, 36.78 mL, 33% wt in ethanol). The reaction mixture was heated to reflux overnight. After the solution cooled to room temperature, the precipitates were filtered and washed with ethanol. The filtrate was concentrated and was purified on silica gel with 100% ethyl acetate to provide the title product (1.68 g, 56%) as a clear liquid. ^{1}H NMR ($CDCl_3$) δ 3.56 (t, *J* = 7.56 Hz, 2 H), 2.92 (sext, *J* = 6.36 Hz, 1 H), 1.61 (sext, *J* = 7.51 Hz, 2 H), 1.33 (q, *J* = 7.23 Hz, 2 H), 1.08 (d, *J* = 6.30 Hz, 3 H). ^{13}C NMR ($CDCl_3$) δ 152.9, 82.4, 46.9, 46.5, 37.1, 28.3, 26.1, 23.9. HRMS calculated for $C_{15}H_{30}N_2O_4$ (M+H) 303.2284, found 303.2291.



Tert-butyl (tert-butoxycarbonyl)(4-((7-chloroquinolin-4-yl-4a,5,6,7,8,8a-$^{13}C_6$, ^{15}N)amino)pentyl)carbamate **24**:

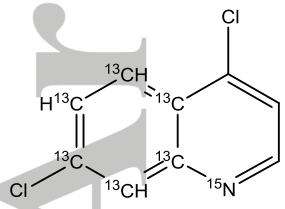
A mixture of 7-chloro-2,3-dihydroquinolin-4(1H)-one ($^{13}C_6$, ^{15}N,) **7'** (0.190 g, 1.01 mmol) and 2-(5-di-*t*-butyl-iminodicarboxypentan-2-yl)amine **23** (0.487 g, 1.61 mmol) in nitrobenzene (0.086 mL) was heated under Argon to 150-160^{o}C overnight. The solution was loaded on silica gel and eluted with 70% ethyl acetate in hexanes to provide 0.235 g (50%) of the title product. ^{1}H NMR ($CDCl_3$) δ 8.50 (m, 1 H), 8.27-8.10 and 7.73-7.55 (m, 1 H), 8.12-7.95 and 7.55-7.40 (m, 1 H), 7.55-7.40 and 7.14-6.98 (m, 1 H), 6.42 (t, *J* = 5.32 Hz, 1 H), 5.39-5.24 (m 1 H), 4.82-4.67 (m, 1 H), 3.91-3.66 (m 1 H), 3.33-3.05 (m, 2 H), 1.83-1.59 (m, 2 H), 1.47 (s, 18 H), 1.33 (d, *J* = 6.36 Hz, 3 H). ^{13}C NMR ($CDCl_3$) δ 149.3 (t, *J* = 60.9 Hz), 135.0 (t, *J* = 65.1 Hz), 128.6 (t, *J* = 65.0 Hz), 125.3 (dt, *J* = 60.3, 5.45 Hz), 121.4 (t, *J* = 58.3 Hz), 117.3 (t, *J* = 57.5 Hz), 99.2 9d, *J* = 8.43 Hz), 79.6, 48.6, 40.4, 33.3, 28.5, 20.6.



Bisdesethylchloroquine **10'**:

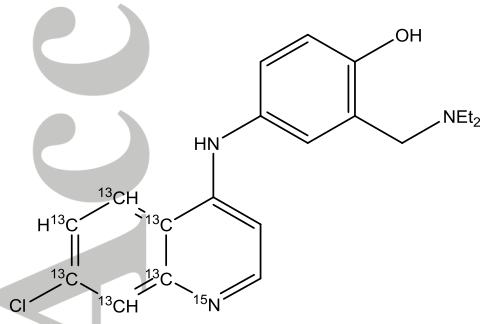
To a solution of tert-butyl (tert-butoxycarbonyl)(4-((7-chloroquinolin-4-yl-4a,5,6,7,8,8a-$^{13}C_6$, ^{15}N)amino)pentyl)carbamate **24** (0.221 g, 0.47 mmol) in ethyl acetate (6.0 mL) was first added ethanol (0.270 mL), then acetyl chloride (0.200 mL) at 0^{o}C. The reaction was allowed to warm to

room temperature and stir overnight. Water was added to dissolve the precipitate and layers were separated. The aqueous layer was washed with ethyl acetate, concentrated and the residue was purified by HPLC (Waters, Symmetry C18, 5 μ, 4.6 x 150 mm, 1 mL/min, linear gradient, 98/2 to 20/80 of 0.1 % TFA in water/acetonitrile for 16 minutes, retention time 10.75 min) to provide 0.198 g (84%) of the title product as a white solid. ^{1}H NMR (D_2O) δ 8.28-8.14 and 7.71-7.55 (m, 1 H), 8.10 (dt, *J* = 7.51, 2.80 Hz, 1 H), 7.73 and 7.17 (two br s, 1 H), 7.71-7.55 and 7.12-6.99 (m 1 H), 6.68 (q, *J* = 5.37 Hz, 1 H), 3.98 (q, *J* = 6.03 Hz, 1 H), 2.94 (t, *J* = 5.92 Hz, 2 H), 1.70 (br s, 4 H), 1.31 (d, *J* = 6.47 Hz, 3 H). ^{13}C NMR ($CDCl_3$) δ 162.8 (q, *J* = 35.8 Hz), 155.2 (d, *J* = 58.5 Hz), 141.9 (d, *J* = 13.2 Hz), 139.2 (complex t, *J* = 66.2 Hz), 137.9 (t, *J* = 62.4 Hz), 127.1 (dt, *J* = 60.5, 7.14 Hz), 123.9 (dt, *J* = 58.4, 5.19 Hz), 122.1, 118.6 (complex t, *J* = 68.3 Hz), 118.2, 114.9 (complex t, *J* = 61.6 Hz), 114.4, 110.5, 98.5, 49.6, 39.3, 31.9, 23.6, 18.7. HRMS calculated for $^{13}C_6C_8H_{18}Cl^{15}NN_2$ (M+H) 271.1442, found 271.1438.



4,7-Dichloroquinoline-4a,5,6,7,8,8a-$^{13}C_6$, ^{15}N **11'**:

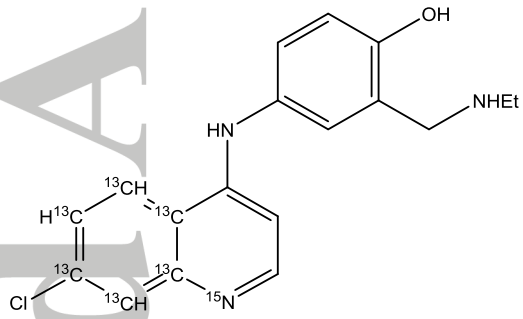
In a 3-neck 50 mL round bottom flask equipped with reflux condenser, a stirring bar and a thermometer, iodine (0.88 g, 3.45 mmol) and phosphorus oxychloride (3.76 mL, 41.07 mmol) were heated to 85-90°C for 30 min. To this solution was added 7-chloro-2,3-dihydroquinolin-4(1H)-one-4a,5,6,7,8,8a-$^{13}C_6$, ^{15}N **7'** (1.300 g, 6.90 mmol) in 1,2-dichloroethane (5.0 mL) dropwise via a cannular. The reaction was heated to 85-90°C for 2 hr, and then it was added dropwise to a mixture consisting of an aqueous solution of ammonia, ice and sodium metasulfite. The organic layer was separated and the aqueous layer was extracted with dichloromethane. The combined organic extracts were concentrated. The residue was purified on silica gel with 10% ethyl acetate/hexanes to provide the title product (0.575 g, 41%) as a white solid. ^{1}H NMR ($CDCl_3$, 300 Hz) δ 8.83-8.72 (bm, 1H), 8.58-8.35 (bm, 1H), 8.00-7.78 (bm, 1H), 7.48 (t, *J* = 4.5 Hz, 1H), 8.00-7.78 and 7.39-7.26 (bm, 1H). ^{13}C NMR ($CDCl_3$, 75 Hz) δ 192.5 (s, carbonyl C), 152.6 (complex t, *J* = 60.5 Hz), 141.4 (complex t, *J* = 66.7 Hz), 129.5 (complex t, *J* = 60 Hz), 118.8 (complex t, *J* = 60 Hz), 117.2 (apparent d, *J* = 66 Hz), 115.3 (complex t, *J* = 60 Hz), 42.3 (br d, *J* = 8.3 Hz), 38.0 (d, *J* = 12.2 Hz). HRMS calculated for $^{13}C_6C_3H_5Cl_2{}^{15}N$ (M+H) 205.0052, found 205.0046.



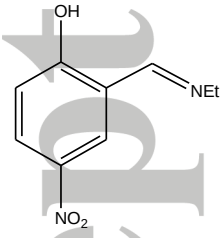
Amodiaquine **12'**:

A mixture of 4,7-Dichloroquinoline-4a,5,6,7,8,8a-$^{13}C_6$, ^{15}N **11'** (0.192 g, 0.93 mmol) and 2-((diethylamino)methyl)-4-aminophenol dihydrochloride (0.300 g, 1.12 mmol) in ethanol (3.0 mL) was heated to reflux overnight. The solvent was removed under reduced pressure, and the residue was adsorbed on silica gel and purified with 10-20% MeOH/CH_2Cl_2 to provide the crude product. This was recrystallized from ethanol to provide 200 mg (49%) of the product as a tan solid. HPLC trace (Waters, XTerra C18, 5 μ, 4.6 x 150 mm, 1 mL/Min, linear gradient, 98/2 to 40/60 of 0.1 % TFA in water/acetonitrile for 20 minutes, retention time 15.03). ^{1}H NMR (CD_3OD) δ 8.52-7.20 (m, 4 H), 7.13(dd, *J* = 0.6, 2.2 Hz, 1 H), 7.06 (d, *J* = 0.8 Hz, 1 H), 6.82 (d, *J* = 2.3 Hz), 6.62 (t, *J* = 1.4 Hz, 1 H), 3.85 (s, 2H), 2.70 (q, *J* = 1.8 Hz, 2 H), 1.16 (t, *J* = 1.8 Hz, 3 H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 157.8, 152.4, 150.2 (t, *J* = 61.6 Hz), 136.8 (t, *J* = 61.6 Hz), 132.0, 128.6-123.9 (complex m), 119.2 (t, *J* = 56.9 Hz), 117.9, 101.9, 57.0, 47.9, 11.6. HRMS calculated for $^{13}C_6C_{14}H_{22}Cl^{15}NN_2O$ (M+H) 363.1704, found 363.1719.

Syntheses leading to desethylamodiaquine ($^{13}C_6$, ^{15}N) **14'**

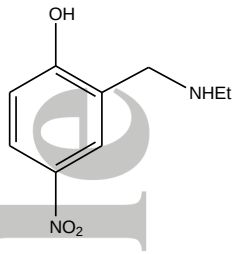


Desethylamodiaquine ($^{13}C_6$, ^{15}N) **13'**



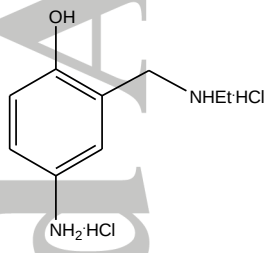
2-((E)-Ethylimino)methyl)-4-nitrophenol **25**:

The compound was prepared following the procedure below:[25] A mixture of 2-hydroxy-5-nitrobenzaldehyde (1.67 g, 10.0 mmol), ethylamine (7.0 mL, 14.0 mmol, 2 M in THF) in THF (40.0 mL) was heated to reflux for 3 h. The solvent was removed under reduced pressure and the crude material was checked by ^{1}H and ^{13}C NMR, and used without further purification. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.33 (s), 8.16 (dd, *J* = 9.25, 2.86 Hz), 6.92 (d, *J* = 9.25 Hz), 3.70 (dq, *J* = 7.31, 1.14 Hz), 1.38 (t, *J* = 7.13 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.8, 163.7, 138.6, 128.6, 127.4, 119.8, 116.5, 51.9, 15.9.



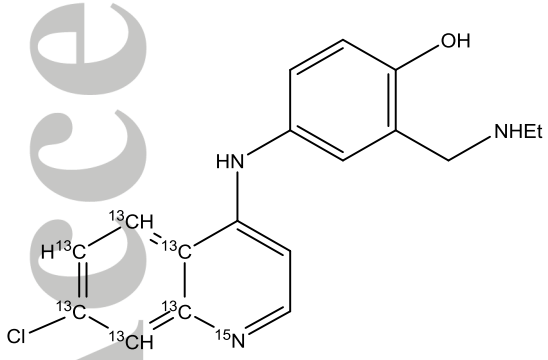
2-(Ethylamino)methyl-4-nitrophenol **26**:

2-((E)-(Ethylimino)methyl)-4-nitrophenol **25** was suspended in methanol (20 mL). To this mixture was added sodium borohydride (0.297 g, 7.85 mmol) in 0.5 mM solution of NaOH (30.0 mL) at room temperature. The reaction mixture was stirred at room temperature overnight. The solids were filtered, washed thoroughly with methanol and air-dried to provide 1.652 g (84% from 2-hydroxy-5-nitrobenzaldehyde) of the title product as a yellow solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.98 (d, *J* = 3.07 Hz), 7.86 (dd, *J* = 9.32, 3.07 Hz), 6.23 (d, *J* = 9.32 Hz), 3.94 (s), 2.84 (q, *J* = 7.32 Hz), 1.16 (t, *J* = 7.32 Hz). ^{13}C NMR (75 MHz, DMSO-d_6) δ 176.4, 130.4, 127.1, 127.0, 120.0, 118.2, 48.1, 41.1, 11.4.



4-Amino-2-((ethylamino)methyl)phenol bishydrochloride **27**:

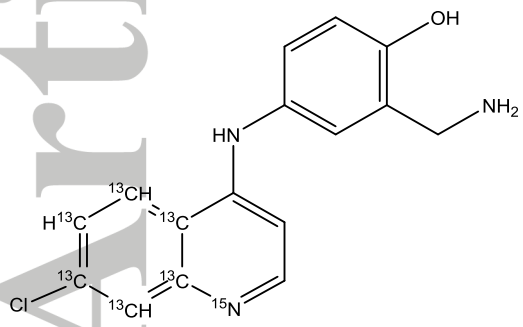
2-(Ethylamino)methyl-4-nitrophenol **26** (1.652 g, 8.42 mmol) was dissolved in 1 M HCl(18.7mL) and 10% Pd/C (0.084 g) was added. The mixture was hydrogenated at 45-50 psi at room temperature overnight. The mixture was filtered and the filtrate was concentrated and dried under vacuum to provide 1.097 g (54%) of the title product as an off-white solid. ^{1}H NMR (300 MHz, MeOH-d_6) δ 7.44 (d, *J* = 2.80 Hz), 7.31 (dd, *J* = 8.66, 2.80 Hz), 7.02 (d, *J* = 8.66 Hz), 4.20 (s), 3.09 (q, *J* = 7.34 Hz), 1.33 (t, *J* = 7.34 Hz). ^{13}C NMR (75 MHz, MeOH-d_6) δ 158.3, 127.6, 127.1, 123.5, 120.9, 117.7, 47.0, 44.1, 11.5.



Desethylamodiaquine ($^{13}C_6$, ^{15}N) **13**:

A mixture of 4,7-Dichloroquinoline-4a,5,6,7,8,8a-$^{13}C_6$, ^{15}N **11'** (0.186 g, 0.907 mmol) and 4-amino-2-((ethylamino)methyl)phenol dihydrochloride **27** (0.239 g, 0.998 mmol) in water (5.0 mL) was heated to reflux for 1 hr. The solvent was removed under reduced pressure and the residue was adsorbed

on silica gel then purified with 10% MeOH/CH_2Cl_2 to provide the crude product, which was further purified by HPLC (Waters XTerra C18, 5 μ, 4.6 x 150 mm, 1 mL/min linear gradient, 98/2-20/80 of 0.1% TFA in water/acetonitrile for 16 minutes, retention time 11.03 min) to provide 0.353 g (95%) of the title product as a white solid. ^{1}H NMR (300 MHz, D_2O) δ 8.55-8.40 and 8.13-8.04 (m, 1 H), 8.23-8.13 (m, 1 H), 8.01-7.85 (m, 1H), 7.59-7.48 and 7.43-7.27 (m, 3 H), 7.07 (d, *J* = 9.0 Hz, 1 H), 6.73 (q, *J* = 6.0 Hz, 2 H), 4.21 (s, 2 H), 3.12 (q, *J* = 9.0 Hz, 2 H), 1.28 (t, *J* = 6.0 Hz, 3 H). ^{13}C NMR (75 MHz, D_2O) δ 156.3, 155.6, 154.9, 142.2, 142.0, 139.7 (complex t, *J* = 65.6 Hz), 138.4 (ddt, *J* = 4.70, 14.0, 63.0 Hz), 128.9, 128.7, 127.8 (dt, *J* = 6.30, 60.2 Hz), 123.9 (dt, *J* = 5.03, 58.6 Hz), 119.0 (complex t, *J* = 67.2 Hz), 116.8, 115.3 (complex t, *J* = 60.8 Hz), 100.0, 46.0, 42.4, 10.3. HRMS calculated for $^{13}C_6C_{12}H_{18}Cl^{15}NN_2O$ (M+H) 335.1391, found 335.1386.



Bisdesethylamodiaquine ($^{13}C_6$, ^{15}N) **14**:

A mixture of 4,7-Dichloroquinoline-4a,5,6,7,8,8a-$^{13}C_6$, ^{15}N **11'** (0.191 g, 0.93 mmol) and 4-amino-2-(amino)methyl)phenol dihydrochloride (0.237 g, 1.12 mmol) in water (5.0 mL) was heated to reflux overnight. The solvent was removed under reduced pressure and the residue was adsorbed on silica gel then purified by FLC with 20% MeOH/CH_2Cl_2 to provide the crude product, which was further purified by HPLC (Waters XTerra, C18, 5 μ, 4.6 x 150 mm 1 mL/min for 25 minutes, linear gradient from 98/2 to 30/70 0.1% TFA in water/acetonitrile, retention time 14.03 min) to provide 0.197 g (56%) of the title product as a white solid. ^{1}H NMR (300 MHz, MeOH-d_6) δ 8.62-8.53 and 7.85-7.73 (m, 1 H), 8.37-8.25 (m, 1H), 8.16-7.85 (m, 1 H), 7.60-7.50 and 7.26-7.18 (m, 1 H), 7.35 (d, *J* = 2.52 Hz), 7.30-7.25 m, 1 H), 7.02 (d, *J* = 8.55 Hz, 1 H), 6.69 (t, *J* = 5.06 Hz, 1 H), 4.14 (s, 2 H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 155.8, 153.5, 152.8, 151.4, 149.0 (t, *J* = 58.6 Hz), 137.6 (t, *J* = 58.6 Hz), 132.4, 129.1 (d, *J* = 15 Hz), 128.0-124.0 (complex m), 122.2, 119.0 (t, *J* = 56.7 Hz), 117.5, 102.2 (d, *J* = 15 Hz), 40.7. HRMS calculated for $^{13}C_6C_{10}H_{14}Cl^{15}NN_2O$ (M+H) 307.1078, found 307.1086.

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Conflict of Interest: The authors did not report any conflict of interest.

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