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AUTHORITY

USAMRMC ltr, 28 Aug 1995

DEPARTMENT OF THE ARMY
U.S. ARMY MEDICAL RESEARCH AND MATERIEL COMMAND
FORT DETRICK, MARYLAND 21702-5012

REPLY TO ATTENTION OF:
MCMR-RMI-S (70-1y) ERRATA AD B144445 28 Aug 95

MEMORANDUM FOR Administrator, Defense Technical Information Center, ATTN: DTIC-HDS/William Bush, Cameron Station, Building 5, Alexandria, VA 22304-6145

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2. Point of contact for this request is Ms. Judy Pawlus, DSN 343-7322.

for Pauline Rinehart
GARY R. GILBERT
COL, MS
Deputy Chief of Staff
for Information Management

AD _____

PREPARATION OF RADIOCHEMICAL-LABELED COMPOUNDS FOR THE U.S. ARMY DRUG DEVELOPMENT PROGRAM

ANNUAL REPORT

JOHN A. KEPLER

APRIL 20, 1992

Supported by

U.S. Army Medical Research and Development Command
Fort Detrick, Frederick, Maryland 21702-5012

Contract No. DAMD17-89-C-9062

Research Triangle Institute
P. O. Box 12194
Research Triangle Park, North Carolina 27709

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19. ABSTRACT (Continue on reverse if necessary and identify by block number)

This report summarizes work carried out on contract DAMD17-89-C-9062 during the period March 23, 1991 to March 22, 1992. The purpose of the work on this contract was to prepare and fully characterize radiolabeled samples of compounds which are of current interest to the U.S. Army Medical Research and Development Command (USARMDC) and to provide these compounds along with some commercially prepared compounds to investigators designated by the USARMDC.

The procedure followed for preparing the compounds involved first designing a synthetic scheme and then optimizing the individual reactions in the synthetic scheme using nonlabeled chemicals. When all of the reactions had been optimized, a tracer run was done where a small amount of the radiolabeled starting material was diluted with

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19. ABSTRACT (continued)

nonlabeled starting material, and the reaction sequence performed from beginning to end on the exact scale that was planned for the master run. Any problems which were discovered in the tracer run were worked out, and then the tracer run was repeated, if necessary, or the master run was done.

The final products were analyzed for chemical and radiochemical purity, and specific activity. Procedures used for the analyses included TLC-radio-scan, autoradiography, HPLC, UV, and NMR and mass spectrometry where required. In addition to analyzing the compounds when they were first prepared, they were also analyzed prior to shipment to approved investigators.

The labeled compounds were stored at the Research Triangle Institute and sent to investigators upon request of the Project Monitor. An up-to-date list of compounds in inventory was provided to the Project Monitor each month.

During the report period of March 23, 1991 to March 22, 1992 the syntheses of 8-[4-amino-1-methylbutyl)amino]-2,6-dimethoxy-4-methyl-5-[3-(trifluoromethyl)phenoxy]-[4-^{14}C]quinoline succinate ([^{14}C]WR-238605), 8-[(4-amino-1-methylbutyl)amino]-2,6-dimethoxy-4-methyl-5-[3-(trifluoromethyl)[6-^{3}H]phenoxy]quinoline succinate ([phenoxy-6-^{3}H]WR-238605), 8-[(4-amino-1-methylbutyl)amino]-5-(1-hexoxy)-6-methoxy-4-methyl[^{14}C]-quinoline (DL) tartrate ([4-^{14}C]WR-242511) and [16-^{14}C]artelinic acid were completed. A total of 31 mCi of [4-^{14}C]WR-238605 was prepared with specific activity of 21 mCi/mmol (37 μCi/mg), a total of 165 mCi of [phenoxy-6-^{3}H]WR-238605 was prepared with specific activity of 165 mCi/mmol (284 μCi/mg), a total of 23 mCi of [4-^{14}C]WR-242511 was prepared with specific activity of 21 mCi/mmol and a total of 2.3 mCi of [16-^{14}C]artelinic acid was prepared with specific activity of 12 mCi/mmol (29 μCi/mg). Development work was initiated on the syntheses of [^{14}C]HI-6 and [^{3}H]CEES.

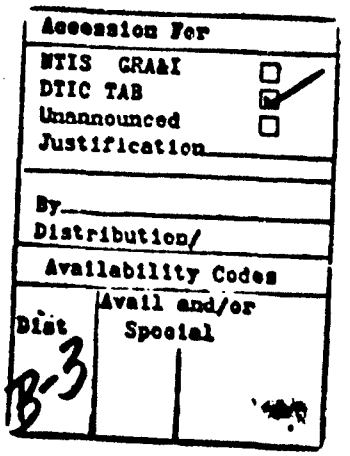
Foreword

During the period March 23, 1991 to March 22, 1992, the Research Triangle Institute worked on a project entitled "Radiochemically-Labeled Compounds Synthesis Laboratory". Dr. Robert E. Engle of Walter Reed Army Institute of Research was the Contracting Officer's Technical Representatives.

Citations of commercial organizations and trade names in this report do not constitute an official Department of the Army endorsement or approval of the products or services of these organizations.

The following conventions are used in this report in order to avoid confusion between nonlabeled and labeled compounds: (a) unless otherwise designated, a compound and the number associated with it represents a nonlabeled entity. (b) Numbers and names, including partial names of labeled compounds, will be preceded by an appropriate modifier in brackets, i.e. [^{14}C]-10 or aldehyde [^{14}C]-10, etc. Specifiers will be included when required for clarity, i.e. [1,2-^{3}H]-10, [2-^{3}H]-10, etc.

The terms HPLC-RAM and TLC-RAM are used when radioactivity monitors are used as detectors with otherwise conventional HPLC or TLC analyses.



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1.0 Summary

During the report period of March 23, 1991 to March 22, 1992 the syntheses of 8-[4-amino-1-methylbutyl)amino]-2,6-dimethoxy-4-methyl-5-[3-(trifluoromethyl)phenoxy][4-^{14}C]quinoline succinate ([^{14}C]WR-238605), 8-[(4-amino-1-methylbutyl)amino]-2,6-dimethoxy-4-methyl-5-[3-(trifluoromethyl)[6-^{3}H]-phenoxy]quinoline succinate ([phenoxy-6-^{3}H]WR-238605), 8-[(4-amino-1-methylbutyl)amino]-5-(1-hexoxy)-6-methoxy-4-methyl[^{14}C]quinoline (QL) tartrate ([4-^{14}C]WR-242511) and [16-^{14}C]artelinic acid were completed. A total of 31 mCi of [4-^{14}C]WR-238605 was prepared with specific activity of 21 mCi/mmol (37 μCi/mg), a total of 165 mCi of [phenoxy-6-^{3}H]WR-238605 was prepared with specific activity of 165 mCi/mmol (284 μCi/mg), a total of 23 mCi of [4-^{14}C]-WR-242511 was prepared with specific activity of 21 mCi/mmol and a total of 2.3 mCi of [16-^{14}C]artelinic acid was prepared with specific activity of 12 mCi/mmol (29 μCi/mg). Development work was initiated on the syntheses of [^{14}C]HI-6 and [^{3}H]CEES.

A total of nine shipments were made to investigators as designated by the Project Officer, Dr. R. R. Engle.

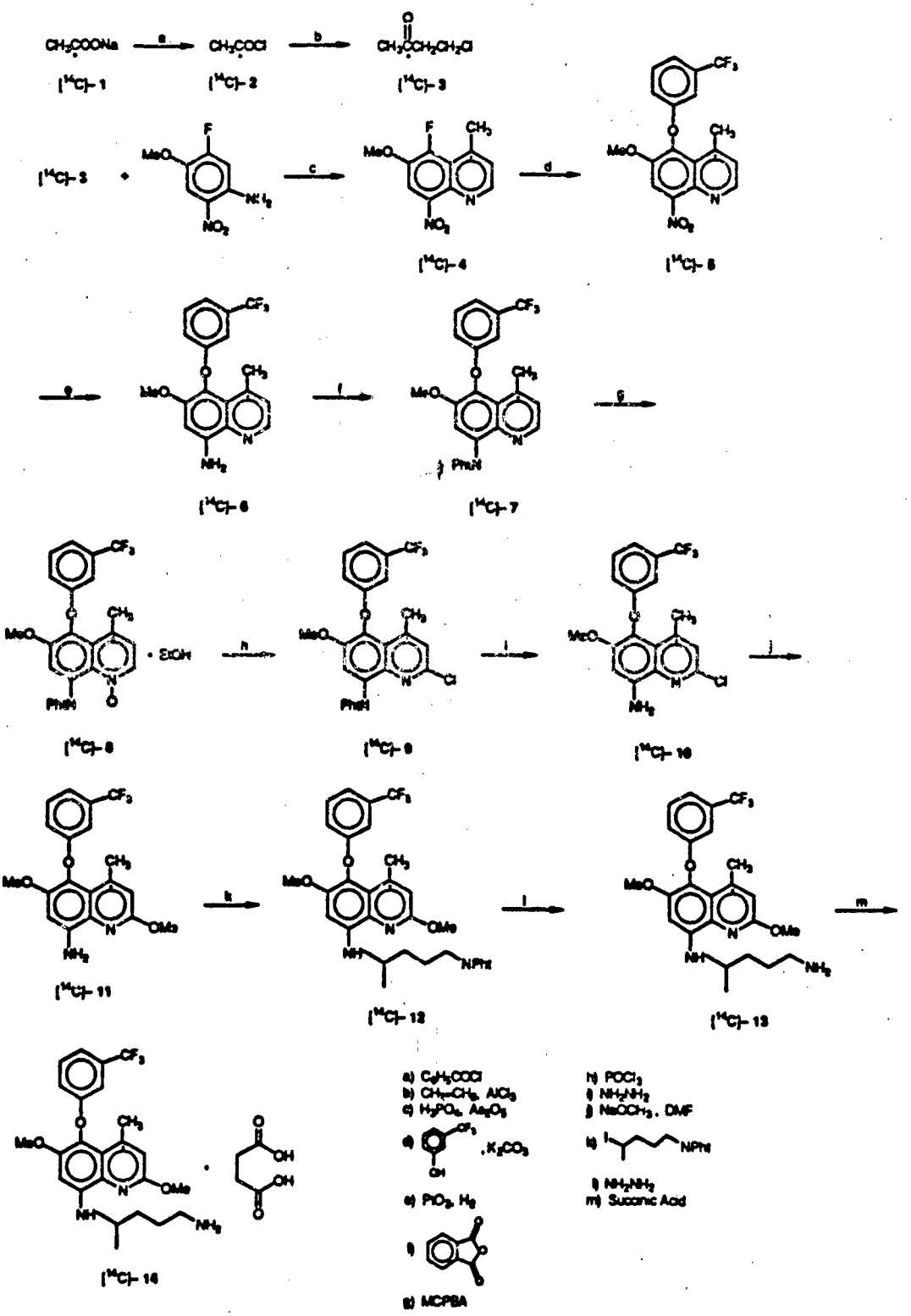
2.0 Synthesis of Labeled Compounds

2.1 WR-238605: 8-[(4-amino-1-methylbutyl)amino]-2,6-dimethoxy-4-methyl-5-[3-(trifluoromethyl)phenoxy][4-^{14}C]quinoline Succinate; [4-^{14}C]-WR-238605 ([^{14}C]-14)

The method used to prepare [4-^{14}C]WR-238605 is outlined in Chart 1. A total of 31 mCi was prepared with specific activity of 21 mCi/mmol (37 μCi/mg). This material was assigned lot no. CT-6949-61.

A large amount of quinoline [^{14}C]-4 was needed because it is an intermediate for the syntheses of both [^{14}C]WR-238605 and [^{14}C]WR-242511 (section 2.3), consequently 1 Ci of sodium [1-^{14}C]acetate([^{14}C]-1) was required as starting material. Because it is not prudent to use this much radioactivity in the early steps of this reaction sequence, it was decided to prepare [^{14}C]-4 in three batches. In the first run with 300 mCi of [^{14}C]-1, we encountered major difficulties and obtained only 10.7 mCi (3.6% radiochemical yield) of [^{14}C]-4. A repeat tracer run, which gave a 17% yield of [^{14}C]-4, did not point out any major problems with the chemistry of these steps, but indicated some changes with the mechanics of carrying out the reactions were warranted. A second batch of 300 mCi of [^{14}C]-1 was carried through the sequence and afforded a 23% radiochemical yield of [^{14}C]-4. The third batch of [^{14}C]-1 (400 mCi) gave a 28% radiochemical yield of quinoline [^{14}C]-4. The overall yield of [^{14}C]-4 was 192 mCi (19.2% radiochemical yield) from the one curie of [^{14}C]-1. A portion of this material (45.7 mCi) was set aside for the synthesis [4-^{14}C]WR-242511 (see later) and the remainder was used for the synthesis of [4-^{14}C]WR-238605. Base catalyzed reaction of [^{14}C]-4 with 3-(trifluoromethyl)phenol afforded an 88% radiochemical yield of nitroquinoline [^{14}C]-5.

Chart 1


a) C_6H_5COCl
b) $CH_2=CH_2$, $AlCl_3$
c) H_3PO_4, As_2O_5
d)
e) PtO_2, H_2
f)
g) MCPBA
h) $POCl_3$
i) NH_2NH_2
j) $NaOCH_3$, DMF
k)
l) NH_2NH_2
m) Succinic Acid

The reduction of the nitro group of [^{14}C]-5 was done in three batches, again because of the large amount of material. The first portion was reduced to the 8-amino derivative [^{14}C]-6 followed by conversion to the 8-phthalimido derivative [^{14}C]-7 to yield 48.5 mCi of product. Reduction of the second portion gave the desired [^{14}C]-6 plus approximately 20% of an unknown impurity. This mixture was treated with phthalic anhydride to form [^{14}C]-7. It appeared that the impurity also formed a phthalimido derivative, suggesting that it is a partially reduced ring system that also has an 8-amino group. This mixture of phthalimido derivatives was purified by column chromatography to yield 28.7 mCi of [^{14}C]-7. The third portion of [^{14}C]-5 was reduced and converted to [^{14}C]-7 to yield 48.8 mCi of product. The total yield of [^{14}C]-7 was 126 mCi which is an 88% radiochemical yield from nitroquinoline [^{14}C]-5.

The conversion of [^{14}C]-7 to N-oxide [^{14}C]-8 was carried out in three portions because of the variability experienced with this reaction. Treatment of a 20.3 mCi portion of [^{14}C]-7 with m-chloroperbenzoic acid (MCPBA) gave 12.3 mCi (61% yield) of [^{14}C]-8 after purification by chromatography on alumina (basic, Act III). A second portion (48.8 mCi) of [^{14}C]-7 was similarly oxidized, but gave only 14 mCi (14% yield) of [^{14}C]-8 after purification by chromatography. The lower yield was attributed to the fact that this material was allowed to stay on the column longer than the first run, and decomposed or irreversibly bound to the alumina. A test was run using three equal portions of [^{14}C]-8 and three different workup procedures: 1) a quick flush through an alumina column, 2) a slow (~ 2 h) flush through an alumina column [same size as in (1)], and 3) an extraction of the chloroform reaction mixture with 15% potassium carbonate solution. The radiochemical recoveries of these three procedures were as follows: 1) 51%, 2) 18%, and 3) 100%. It is obvious from these results that contact of [^{14}C]-8 with alumina lowered the

yield. The results of the test indicate that alumina should not be used to remove the excess MCBA from the reaction mixture. It does not explain, however, how other groups (Starks[1]) and the original literature[2] procedure were able to obtain high yields of N-oxide with the alumina procedure. The results of the test also suggests that our past experience of inconsistent results with this reaction were related to the alumina chromatography and not the quality of MCPBA. It also should be pointed out that while the radiochemical recovery is high with the extraction process, the radiochemical purity is ~ 90% because extraneous radioactive materials are not removed as with the alumina chromatography. On the basis of the above test, the third portion (56.9 mCi) of [^{14}C]-7 was converted to the N-oxide [^{14}C]-8 and worked up by the base extraction method to give 56.7 mCi of material of ~ 90% radiochemical purity. The total yield from all three runs is 83 mCi (66% yield) of [^{14}C]-8. The N-oxide [^{14}C]-8 was treated with phosphorus oxychloride to obtain the 2-chloro compound, [^{14}C]-9 in 74% radiochemical yield (61.5 mCi). The 8-amino group of [^{14}C]-9 was deprotected with hydrazine to obtain [^{14}C]-10 (61.0 mCi, 99% radiochemical yield). Displacement of the 2-chloro atom with sodium methoxide in DMF gave [^{14}C]-11 (46.6 mCi, 76% radiochemical yield). This last step was carried out using sodium methoxide prepared by the treatment of methanol with sodium hydride. This method did not give the N-formyl by-product obtained in prior syntheses where the sodium methoxide was prepared by reaction of sodium with methanol. The two methods give comparable results, but both methods have been found to be labor intensive. The sodium-methanol method required a chromatography to separate the N-formyl by-product from [^{14}C]-11 with subsequent basic hydrolysis of the by-product to give additional [^{14}C]-11. The method using sodium hydride-methanol went to ~ 85-90% completion and required chromatography to separate [^{14}C]-11 from unreacted [^{14}C]-10

which was recycled to form [^{14}C]-11. The work up of the sodium hydride method is very tedious because of formation of emulsions.

The alkylation of [^{14}C]-11 gave [^{14}C]-12 in 89% radiochemical yield (41.3 mCi) which upon treatment with hydrazine gave the free base [^{14}C]-13 in 89% radiochemical yield (36.8 mCi). Treatment of [^{14}C]-13 with succinic acid afforded, after purification, 835 mg (31 mCi) of [4-^{14}C]WR-238605 ([^{14}C]-14) with specific activity of 37 μCi/mg, 21 mCi/mmol.

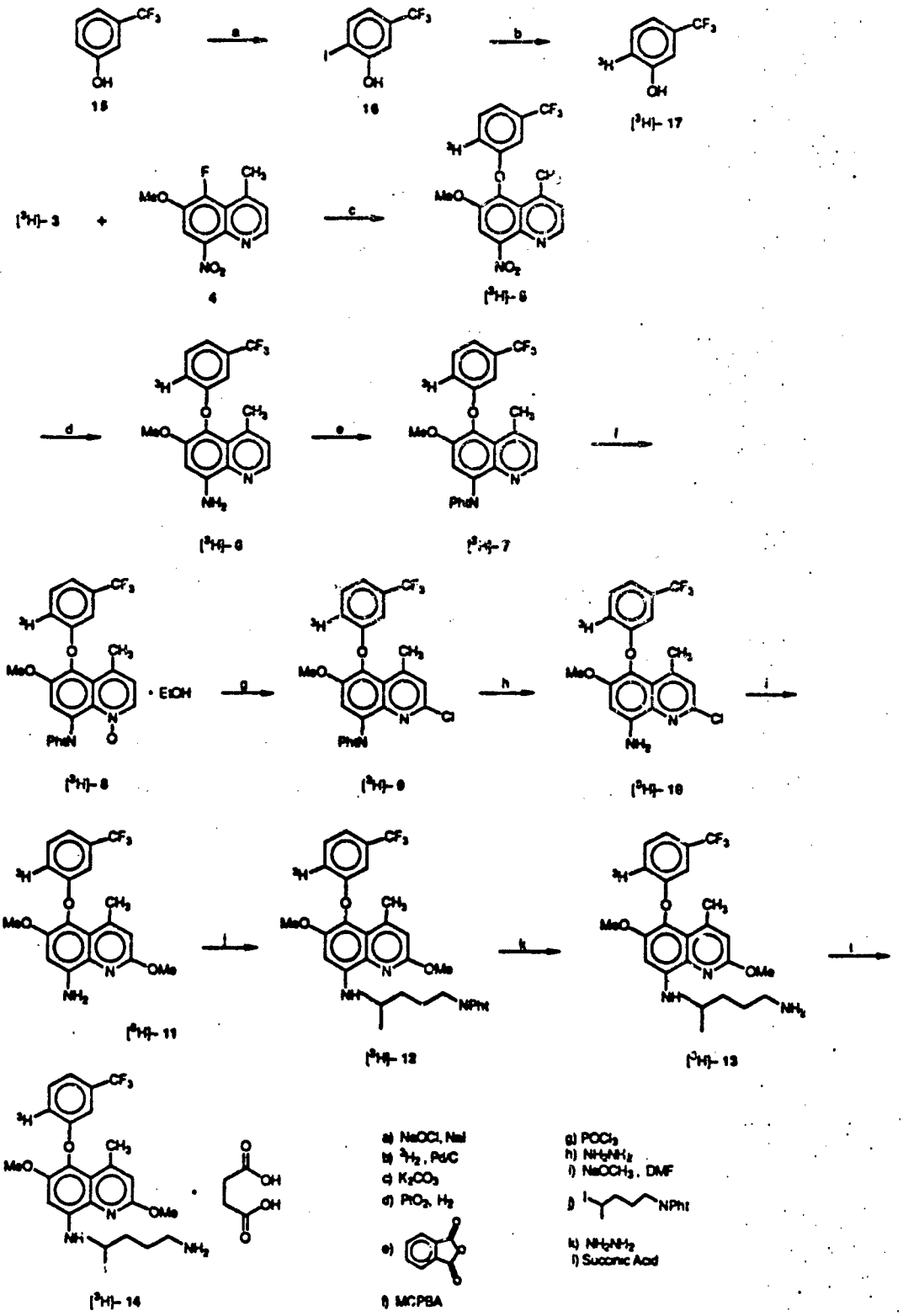
2.2 WR-238605: 8-[(4-Amino-1-methylbutyl)amino]-2,6-dimethoxy-4-methyl-5-[3-(trifluoromethyl)[6-^{3}H]phenoxy]quinoline Succinate; [phenoxy-6-^{3}H]-WR-238605 ([^{3}H]-14)

[phenoxy-6-^{3}H]WR-238605 ([^{3}H]-14) was synthesized by the scheme outlined in Chart 2. A total of 124 mCi was prepared with specific activity of 165 mCi/mmol (284 μCi/mg). This material was assigned lot no. CT-6949-145.

Two problems were encountered in the preparation of [phenoxy-6-^{3}H]WR-238605 which are believed to be related to the radiochemical stability of intermediates [^{3}H]-7 and [^{3}H]-13. During the initial master synthesis, quinoline [^{3}H]-7 with specific activity of about 730 mCi/mmol completely decomposed to a less polar compound upon standing for 48 h. This problem was overcome by converting [^{3}H]-7 to N-oxide [^{3}H]-8 immediately after its preparation. Similarly, the quinoline free base [^{14}C]-13 with specific activity of about 700 mCi/mmol nearly completely decomposed within a few hours of its preparation. This problem was overcome by reducing the specific activity of [^{3}H]-13 in the second master synthesis.

In the second master synthesis a sample of iodophenol 16, prepared by the reaction of 3-trifluoromethylphenol (15) with sodium iodide and sodium hypochlorite[3,4], was catalytically reduced with tritium and Pd/C to give 530 mCi of [^{3}H]-17. After dilution with nonlabeled 17, it was allowed to react with

Chart 2

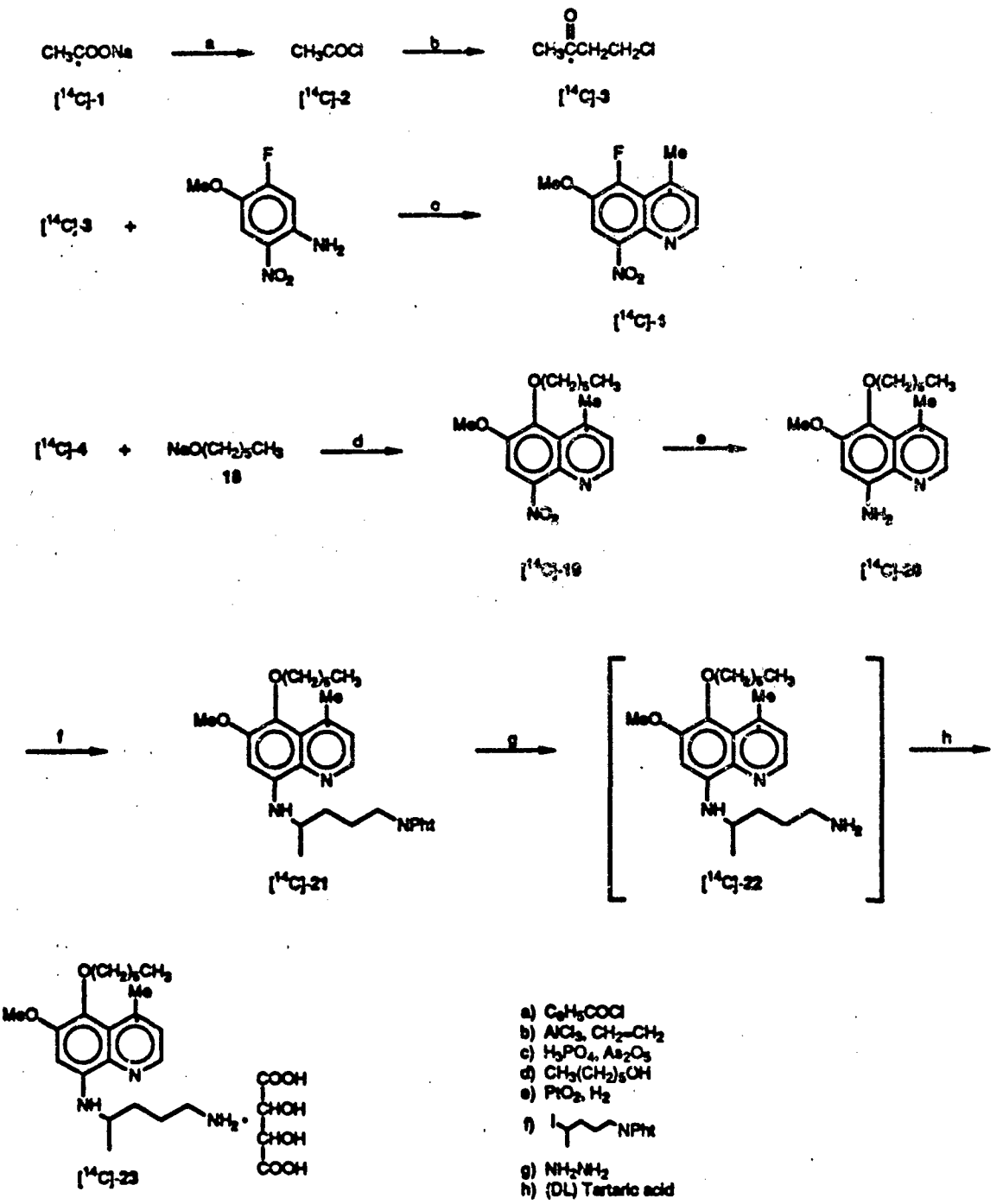

15
16
[3H]- 17
[3H]- 3
4
[3H]- 5
[3H]- 6
[3H]- 7
[3H]- 8
[3H]- 9
[3H]- 10
[3H]- 11
[3H]- 12
[3H]- 13
[3H]- 14
a) NaOCl, NaI
b) 3H2, Pd/C
c) K2CO3
d) PtO2, H2
e)
f) MCPBA
g) POCl3
h) NH2NH2
i) NaOCH3, DMF
j)
k) NH2NH2
l) Succinic Acid

fluoroquinoline 4 to give [^{3}H]-5 in 85% radiochemical yield. Catalytic reduction of nitroquinoline [^{3}H]-5 followed by immediate treatment with phthalic anhydride gave protected aminoquinoline [^{3}H]-7 in 80% radiochemical yield. This material was converted to N-oxide [^{3}H]-8 (81% radiochemical yield) which was treated with phosphorus oxychloride to obtain 2-chloroquinoline [^{3}H]-9. Treatment of [^{3}H]-9 with hydrazine removed the phthalimido protecting group to afford amine [^{3}H]-10. [^{3}H]-10 was treated with sodium methoxide to displace the 2-chloro function to give 2-methoxy quinoline [^{3}H]-11 in 52% radiochemical yield from [^{3}H]-8. Alkylation of [^{3}H]-11 with 4-iodophthalimidopentane gave [^{3}H]-12 in 84% radiochemical yield after purification by chromatography. This compound had specific activity of about 340 mCi/mmol. This sample was diluted with an equal amount of nonlabeled 13 prior to removing the phthalimide protecting group. This was done because, as mentioned above, previously when the protecting group was removed from [^{3}H]-12 which had a specific activity of about 700 mCi/mmol, the [^{3}H]-13 formed nearly completely decomposed within a few hours. It was expected that [^{3}H]-13 with specific activity of about 100 mCi/mmol would be more stable. Treatment of the mixture of [^{3}H]-12 and 13 with hydrazine followed by workup and treatment with succinic acid afforded 436 mg of [^{3}H]-14 as tan crystals with m.p. 146-148°C. The specific activity was determined to be 284 μCi/mg (165 mCi/mmol) giving a total of 124 mCi.

2.3 WR-242511: 8-[(4-Amino-1-methylbutyl)amino]-5-(1-hexoxy)-6-methoxy-4-methyl[4-^{14}C]quinoline (DL)-Tartrate; [4-^{14}C]WR-242511 ([^{14}C]-23)

The method used to prepare [4-^{14}C]WR-242511 ([^{14}C]-23) is outlined in Chart 3. A total of 23 mCi of [4-^{14}C]WR-242511 was prepared with specific activity of 20.9 mCi/mmol (39.9 μCi/mg). This material was assigned Lot no. LF-6797-73.

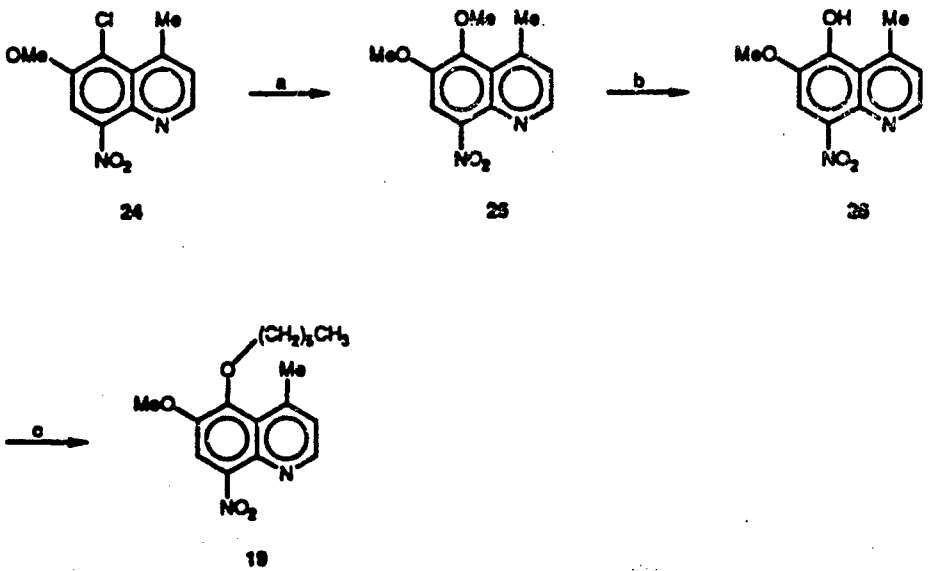
Chart 3


CH3COONa
[14C]-1
CH3COCl
[14C]-2
CH3CCH2CH2Cl
[14C]-3
[14C]-3 +
MeO
F
NH2
NO2
Me
N
[14C]-4
[14C]-19
[14C]-20
NaO(CH2)5CH3
18
O(CH2)5CH3
NPht
[14C]-21
[14C]-22
[14C]-23
COOH
CHOH
a) C6H5COCl
b) AlCl3, CH2=CH2
c) H3PO4, As2O5
d) CH3(CH2)5OH
e) PtO2, H2
f) NPht
g) NH2NH2
h) (DL) Tartaric acid

The synthetic scheme outline in Chart 3 differs from the published scheme[5] in that fluoroquinoline 4 replaces chloroquinoline 24 (Chart 4) as the intermediate for preparing hexoxyquinoline 19. This was done for several reasons: (a) we have experience in preparing [^{14}C]-4; (b) [^{14}C]-4 was required for the synthesis of [^{14}C]WR-238605 (see earlier), and economies could be made by preparing a large batch of [^{14}C]-4 and using it for both syntheses; (c) the number of steps with radioactive material to intermediate 19 is reduced; and (d) it was anticipated that the fewer steps, plus the expectation that the flourine of 4 would be more susceptible to nucleophilic displacement than the chlorine of 24, would lead to a better yield of 19.

Reaction of [^{14}C]-4 with sodium hexoxide in hexanol afforded a 92% chemical (82% radiochemical) yield of quinoline [^{14}C]-19 after purification by chromatography. [^{14}C]-19 was diluted with an equal weight of nonlabeled 19 and reduced by using platinum oxide as catalyst in a stainless steel bomb (glass liner) in a hydrogen atmosphere at 45 psi. Work-up by filtration of the reaction mixture through Celite and rinsing with THF gave quantitative chemical and radiochemical yields of amine [^{14}C]-20. [^{14}C]-20 was allowed to react with 4-iodo-1-phthalimidopentane and N,N-diisopropylethylamine in acetonitrile. The reaction was followed by TLC and additional 4-iodo-1-phthalimidopentane and N,N-diisopropylethylamine was added during the course of the reaction. The thoroughly dried crude product was purified by column chromatography to give [^{14}C]-21 in 73% radiochemical yield. Treatment of [^{14}C]-21 with hydrazine to remove the phthalimido protecting group (88% crude chemical yield) followed by treatment with a solution of DL-tartaric acid in ethanol yielded the target compound [^{14}C]-23 of 99.5% radiochemical purity in 82% radiochemical yield after crystallization from ethanol.

Chart 4



24 25 26 19

a) NaOH
b) HCl
c) KOH, $Br(CH_2)_5CH_3$

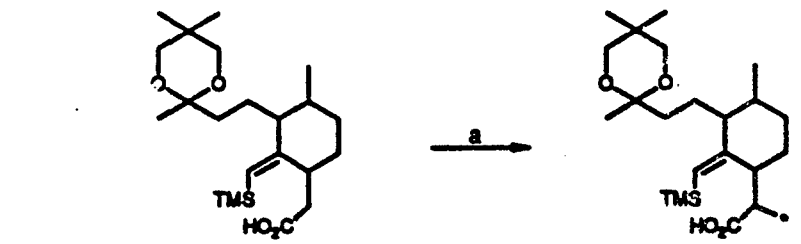
2.4 WR-255633: [16-^{14}C]Artelinic Acid ([^{14}C]-32)

The scheme used for the synthesis of [16-^{14}C]artelinic acid ([^{14}C]-32) is shown in Chart 5. The synthesis of [16- ^{14}C]artemisinin ([^{14}C]-29) follows the method of Avery.[6] A total of 2.3 mCi of material was prepared which had specific activity of 58 μCi/mg.

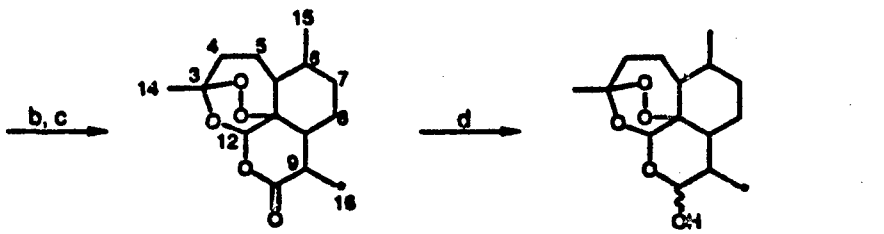
The original focus of the development work was on making the alkylation of 27[7] to give 28 reliable, especially with respect to the consumption of 27 because the separation of 27 from 28 is difficult. The usual precautions for moisture sensitive reactions were taken; thus, all equipment was oven dried, the solvent, THF, was freshly distilled from benzophenone ketyl, the diisopropylamine was distilled from calcium hydride and stored over molecular sieves under an argon atmosphere, the normality of the butyl lithium solution used to prepare the lithium diisopropylamide (LDA) was determined by titration prior to use, and the methyl iodide was distilled and stored over copper.

When these precautions are taken the alkylation of 27 was reasonably reliable giving a product ratio of 27 to 28 of about 3:97 when run in the normal way. However, when the reaction is run using the vacuum line techniques necessary for the radiosynthesis, the product ratio was erratic varying from nearly all 28 to nearly no 28. Since in using the vacuum line technique, the reaction is run under reduced pressure (the vapor pressure of THF) it was thought that the problem may be due to the large head space on the vacuum line. After reducing the head space by using a smaller reaction flask and adding an adapter, the 27:28 ratio varied in three runs as ~ 0:100, 30:70 and 15:85. Further modification of the mechanics of carrying out the reaction which involved addition of 27 to the LDA solution via a side-arm adapter in a closed system rather than by cannula, resulted in consistent 27:28 ratios of 1:9, or better. Attempts to convert unreacted 27 to 28 by realkylating the

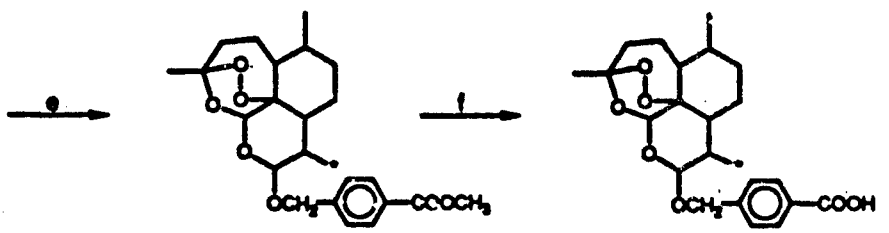
Chart 5



27 [^{14}C]-28

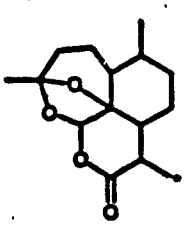


[^{14}C]-29 [^{14}C]-30



[^{14}C]-31 [^{14}C]-32

a) LDA, $\dot{C}H_3I$
b) O_2/O_3
c) BHT, SiO_2, 3 M H_2SO_4
e) $HOCH_2$—⟨⟩—$COOCH_3$
f) 5% KOH/CH_3OH



[^{14}C]-33

reaction mixture was unsatisfactory. The 1:9 ratio of 27 to 28 was considered acceptable when it was found that ozonolysis of a sample of 28 containing 12% of 27 as an impurity gave essentially the same yield (16%) of purified artemisinin (29) as did a pure sample of 28.

Further study of the alkylation was carried out, however, in an attempt to make the reaction more efficient with respect to the labeled starting material [^{14}C]methyl iodide. The alkylation of 27 is normally carried out with a 2.5 fold excess of methyl iodide.[6] Two experiments were carried out to determine if better utilization of [^{14}C]methyl iodide could be achieved by reacting the anion of 27, first with one equivalent of [^{14}C]methyl iodide followed by 1.5 equivalents of nonlabeled methyl iodide. In the first experiment, a 1:1 mixture of 28 to 27 was realized after reaction of 27 for 1 h with one equivalent of methyl iodide. After addition of the remaining 1.5 equivalent of methyl iodide, HPLC analysis showed a 95:5 ratio of 28 to 27, which is nearly identical to the results obtained when the 2.5 equivalents of methyl iodide are added in one portion. In the second experiment, a 3:1 ratio of 28 to 27 was realized 1 h after the first addition of methyl iodide. This ratio did not change after the reaction was allowed to continue for an additional 1 h. The reaction went to 95% completion upon the addition of 1.5 equivalents more of methyl iodide. The results from these two experiments indicated that the stepwise procedure had the potential for increasing the utilization of [^{14}C]methyl iodide, however when this procedure was used in a master synthesis, only 38% incorporation of the labeled methyl iodide was realized. We did not use, and do not plan to use the stepwise procedure in other preparations of [^{14}C]-28.

In a master synthesis, alkylation of 27 with 2.5 equivalents of [^{14}C]methyl iodides gave 34 mCi (85% radiochemical yield) of crude [^{14}C]-28

which was 87% radiochemically pure. Two radioactive impurities are formed in this reaction which have HPLC retention times similar to that of [^{14}C]-28. We speculate that one of these impurities is the C-9 epimer of 28, and the other is the dimethylated product.

A portion (1.12 mCi) of the crude [^{14}C]-28 was mixed with 409 mg of nonlabeled crude 28 prepared by the same method as its labeled counterpart. This material was used for a tracer study of the ozonization reaction. Purification of crude tracer [^{14}C]-28 by flash chromatography gave 226 mg (55% chemical yield) and 0.77 mCi (68% radiochemical yield) of partially purified tracer [^{14}C]-28. Purification of the master preparation of [^{14}C]-28 by this same method gave 56% chemical and 60% radiochemical recovery of purified [^{14}C]-28. Since the flash chromatography resulted in 30-40% loss of product, while only marginally improving its purity, the crude [^{14}C]-28 from a second master run was purified by filtering through a pad of silica (230-400 mesh) via aspiration. The recovery was 87%.

Ozonolysis of tracer [^{14}C]-28 gave a 29% yield (45 mg) of [15-^{14}C]-artemisinin ([^{14}C]-29) with a chemical purity and radiochemical purity of 78% and 95% (area ratios), respectively, after flash chromatography. This material was further purified by preparative HPLC (Waters preparative RCM, 25 x 200 mm, C18, 45% H_2O-CH_3CN, 9.9 mL/min, 210 nm). Sample injection size was 15 mg. A total of 22 mg (14% chemical yield) containing 139 μCi (13% radiochemical yield) of [16-^{14}C]artemisinin ([^{14}C]-29 was realized. This sample was shown to be contaminated with [16-^{14}C]deoxyartemisinin ([^{14}C]-33, however. The presence of [^{14}C]-33 was discovered when sodium borohydride reduction of tracer [^{14}C]-29 to give [16-^{14}C]deoxyartemisinin ([^{14}C]-30) failed to give as clean a product as in the past. Investigation of the reason(s) for the failure of this reduction revealed that the reversed phase

HPLC system used to analyze and purify [^{14}C]-29 did not separate artemisinin and deoxyartemisinin. These two compounds, however, are easily separated by normal phase HPLC (Waters Resolve spherical silica, 5 μ, 3.9 x 150 mm, 10% EtOAc-Hexane, 1.2 mL/min, 33 t_R 6.22 min, 29 t_R 8.16 min). It was established later that [16-^{14}C]artemisinin ([^{14}C]-29) of purity suitable for further transformation could be obtained by careful flash chromatography alone. Ozonization of master [^{14}C]-28 afforded a 14% radiochemical yield of [^{14}C]-29 which was > 98% radiochemically pure after two flash chromatographies.

A portion of the purified [^{14}C]-29 was diluted with nonlabeled artemisinin for use in a tracer study of the synthesis of [16-^{14}C]artelinic acid ([^{14}C]-32). Sodium borohydride reduction of [^{14}C]-29 went, as expected, to give a 72% yield of [16-^{14}C]dihydroartemisinin ([^{14}C]-30). Reaction of [^{14}C]-30 with methyl 4-(hydroxymethyl)benzoate afforded methyl artelinate ([^{14}C]-31) in 72% radiochemical yield after chromatography. Alkaline hydrolysis of [^{14}C]-31 afforded a quantitative yield of tracer [16-^{14}C]-artelinic acid ([^{14}C]-32) which was 95% radiochemically pure. Recrystallization of the product from methanol-water[6] failed to improve its purity, but purification was achieved by preparative HPLC to afford a 59% radiochemical yield of product which was > 99% radiochemically pure.

The master synthesis of [16-^{14}C]artelinic acid was completed by the same method used for preparing tracer [^{14}C]-32. Sodium borohydride reduction of master [^{14}C]-29 gave a quantitative yield of crude [^{14}C]-30, which upon treatment with methyl 4-(hydroxymethyl)benzoate and boron trifluoride etherate gave ester [^{14}C]-31 in 66% radiochemical after purification by flash chromatography. The purified material was hydrolyzed and the product purified by preparative HPLC to give 2.3 mCi (66% radiochemical yield) of [16-^{14}C]-artelinic acid with specific activity of 58 μCi/mg.

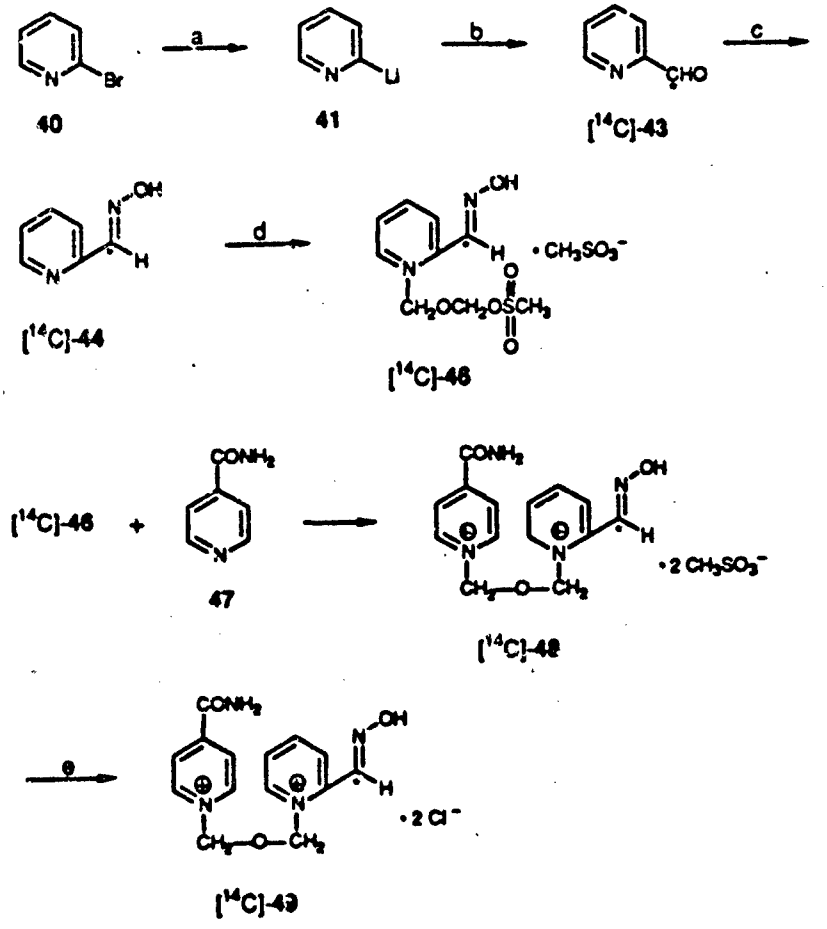
2.5 WR-249655: 1-(2-Hydroxyimino[^{14}C]methyl)-1-pyridino-3-(4-carbamoyl-1-pyridino)-2-oxapropane Dichloride ([^{14}C]HI-6, [^{14}C]-49)

The synthesis scheme for preparing [^{14}C]HI-6 is shown in Chart 6. The scheme essentially follows a recently reported[8] synthesis of [^{14}C]HI-6, but uses the modification of Starks Associates[1] which avoids the use of the carcinogenic bis(chloromethyl) ether. The starting material N-methyl[^{14}C]-formanilide is commercially available, and has been purchased.

Initial attempts to prepare pyridine-2-carboxaldehyde (43) gave only a trace yields of product. Purification of 2-bromopyridine (40) and N-methylformanilide (42) by fractional distillation gave somewhat improved yields (15%), but which were still far below the literature yields of 80%.[8] The yield was further improved by reverse addition, i.e. the solution of 2-bromopyridine was added to the solution of n-butyl lithium. These yields (40-50%), however, were still considerably below the literature yield. Interestingly, the expected side product, N-methylaniline was obtained in 92% yield which indicated that N-methylformanilide had almost completely reacted. A tracer experiment was done to determine the fate of the [^{14}C]formyl group of [^{14}C]-42.

The results of the tracer run are shown in Table 1. Two radioactive side products were revealed, one of which was purified and tentatively identified as 2-pyridine[^{14}C]methanol ([^{14}C]-50) on the basis of its ^{1}H NMR spectrum. Confirmation of this structure, however, requires further analyses. The origin material could not be isolated in a pure enough form to allow identification. The results of the tracer run indicates that reaction is complete after 3.5 h, but not after 1 h when 21% of the starting N-methyl[^{14}C]formanilide ([^{14}C]-42) remains. It is interesting that the ratio of aldehyde [^{14}C]-43 to by-product [^{14}C]-50 is relative constant prior to workup during

Chart 6



a) n-BuLi
b) $C_6H_5N(Me)\dot{C}HO$ ([^{14}C]-42)
c) NH_2OH-HCl
d) $(CH_3\overset{\overset{O}{\|}}{\underset{\underset{O}{\|}}{S}}OCH_2)_2O$ (45)
e) Dowex 1-X2 ion exchange resin, Cl^-

Table 1

Distribution of Radioactivity by TLC-RAM Analysis

Time (h)	% origin	% Side Product [^{14}C]-50 ?	% Aldehyde [^{14}C]-43	% Starting Mat. [^{14}C]-42	Comment
1	46	11	15	21	prior to workup
3.5	49	19	28	1	prior to workup
3.5	6	14	72	1	after workup[1]
3.5	9	27	63	0	after isolation of crude product[2]

1. Workup involves: a) acidification with aqueous hydrochloric acid, b) extraction with ether, c) basification with potassium carbonate, d) extraction with ether.

2. The crude product was isolated by drying the final ether extract, evaporating the solvent on the flash evaporator and drying the product under high vacuum.

the course of the reaction being 1.4 at 1 h and 1.5 at 3.5 h. Prior to work-up, nearly half of the radioactivity is at the origin. After workup, there is only 6% of origin material in the ether layer, but 33% of the recovered amount of radioactivity (23% of the starting amount of radioactivity) was in the aqueous layer and is presumably mostly origin material. The fact that the percent of [^{14}C]-43 in the ether layer is reduced from 72% to 63%, while the amount of the impurities appears to increase is believed to be due to loss of [^{14}C]-43 by volatization during drying under high vacuum. This is supported by the fact that only 70% of the starting amount of radioactivity was recovered. The distribution of radioactivity, based on the beginning amount of radioactivity, was as follows: 30% in aldehyde [^{14}C]-43, 13% in alcohol [^{14}C]-50, 4% in origin material, 23% in the aqueous waste and 30% unaccounted.

The formation of [^{14}C]-50 suggest that aldehyde 43 is under going a base catalyzed Cannizzaro dismutation reaction under the reaction conditions (see below), and that the water soluble radioactivity is due, at least in part, to [^{14}C]-51. It appears that 50 is formed at a constant rate during the reaction, since the ratio of [^{14}C]-43 to [^{14}C]-50 is similar at 1 and 3.5 h. The

43 50 51

amount of [^{14}C]-51 cannot exceed the amount of [^{14}C]-50, so much of the origin material exhibited prior to workup may be due to an intermediate that goes to aldehyde 43 during workup, or the TLC-RAM analysis could be misleading because of loss of volatile [^{14}C]-43 and [^{14}C]-50 from the TLC plate.

In summary, the tracer reaction showed that the yield of aldehyde 43 was reduced because of its volatility, and because it was consumed in a side reaction. We plan to repeat the tracer experiment taking precautions to minimize losses due to volatility. We also plan to confirm the structure of 50 and explore methods to minimize its formation.

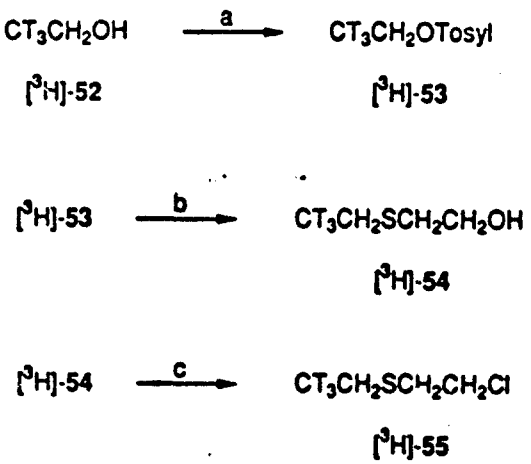
2.6 2-Chloroethyl [2-^{3}H]Ethyl Sulfide; [ethyl-2-^{3}H]CEES ([^{3}H]-55)

Preliminary investigation of the scheme outlined in Chart 7 for preparing [ethyl-2-^{3}H]CEES ([^{3}H]-55) has been started. Gas chromatography methods for following the reaction of mercaptoethanol with ethyl tosylate (53) and for the conversion of 2-hydroxyethyl ethyl sulfide (54) to CEES have been developed.

Reaction of 2-mercaptoethanol with ethyl tosylate in the presence of potassium carbonate afforded an 80% yield 54 after isolation by vacuum transfer.

At present, we are investigating the conversion of 54 to CEES by reaction with thionyl chloride by a literature method.[9]

Chart 7



a) 

b) $HSCH_2CH_2OH$, K_2CO_3, $CHCl_3$
c) $SOCl_2$

3.0 Shipments

A total of nine shipments were made to investigators as authorized by the Project Monitor during the period March 23, 1991 - March 22, 1992 (Table 2).

4.0 Inventory

A list of the compounds held in inventory March 22, 1992 by the Research Triangle Institute for the USARMDC is given in Table 3.

5.0 References

1. Procedure of Starks Associates Inc., provided by Dr. R. R. Engle.
2. Craig, C. Y.; Parashothaman, K. K. J. Org. Chem. 1970, 35, 1721.
3. Edgar, K. J.; Falling, S. N. J. Org. Chem. 1990, 55, 5287.
4. Kepler, J. A., Annual Report, Contract DAMD17-89-C-9062, April, 1991.
5. Chen, E. H.; Tanabe, K.; Sagglimo, A. J.; Nadiff, E. A. J. Med. Chem. 1987, 30, 1193.
6. Avery, M. A. Final Report on Contract DAMD17-88-C-8048, February 23, 1990.
7. The intermediate 27 was provided by Starks Associates, Inc.
8. Nicholas, C.; Madelmont, J. C.; Maurizis, J. C.; Ganigul, H.; Miyniel, J. M.; Demerseman, P.; Sentenac-Rounianou, H.; Vayre, A. J. Label. Cmpd. and Radiopharm. 1990, 28, 1375.
9. Mohler, H.; Songe, J. Helv. Chem. Acta 1940, 23, 1210.

SHIPMENTS

March 23, 1991 to March 22, 1992

WR No.	Name	Lot No.	Amount	Date	Recipient
238605	8-[(4-Amino-1-methylbutyl)amino]-2,6-dimethoxy-4-methyl-5-[(3-trifluoromethyl)phenoxy][4-^{14}C]-quinoline Succinate	CT-6639-85-1	0.68 mCi	4/04/91	Lt. Col. H. Kyle Webster Bangkok, Thailand
1544	[quinoline-3-^{14}C]Chloroquine diphosphate	6312-15	100 µCi	5/28/91	COL. Thomas Brewer WRAIR
238605	8-[(4-Amino-1-methylbutyl)amino]-2,6-dimethoxy-4-methyl-5-[(3-trifluoromethyl)phenoxy][4-^{14}C]-quinoline Succinate	CT-6639-85-1	2.04 mCi	6/18/91	Dr. David Hawkins Huntingdon Research Centre, England
6026	6-Methoxy-8-(6-diethylamino-hexylamino)lepidine-4-^{14}C Dihydrochloride	CT-5385-99-1	0.673 mCi 18.3 mg	7/24/91	Dr. Alan Buckpitt Univ. of CA at Davis Davis, CA
242511	8-[(4-Amino-1-methylbutyl)amino]-5-(1-hexoxy)-6-methoxy-4-methyl-[4-^{14}C]quinoline (DL)-tartrate	LF-6797-73	5.15 mCi 129 mg	7/23/91	Dr. David R. Hawkins England
253997	[16-^{14}C]Dihydroartemisinin	LF-7044-115	0.44 mCi 9.92 mg	2/17/92	COL. Thomas Brewer WRAIR
255663	[16-^{14}C]Artelinic Acid	LF-7044-94	1.22 mCi 42 mg	2/17/92	COL. Thomas Brewer WRAIR

Shipments (Table 2 cont.)

WR No.	Name	Lot No.	Amount	Date	Recipient
249309	[16-^{14}C]Artemisinin	LF-7044-45C	0.05 mCi 0.306 mg	2/17/92	Dr. Steven Meshnick CUNY Medical School, New York, NY
238605	8-[(4-Amino-1-methylbutyl)amino]-2,6-dimethoxy-4-methyl-5-[(3-trifluoromethyl)-[6-^{3}H]phenoxy]-quinoline Succinate	CT-6949-145	5.23 mCi 18.4 mg	3/23/92	COL. Thomas Brewer WRAIR

Table 3

RESEARCH TRIANGLE INSTITUTE
Inventory - Contract No. DAMD17-89-C-9062
April 1, 1992

WR No.	Compound	Lot No.	Origin	Specific Activity	Amount Available
1065	2-[(3-Aminopropyl)amino][1,2-^{14}C]-ethanethiol Dihydrochloride	5172-103	RTI	24.4 mCi/mmole	0.553 mCi
		5662-81-B	RTI	10.1 mCi/mmole	0.508 mCi
		5662-81-A	RTI	10.5 mCi/mmole	0.967 mCi
		5662-161	RTI	9.89 mCi/mmole	1.26 mCi
1544	[3-^{3}H]Chloroquine	154-3b*	Monsanto	1.56 mCi/mg*	10.18 mCi
+1544	[quinoline-3-^{14}C]Chloroquine Diphosphate	3612-15*	Amersham	2.62 mCi/mmole	0.1 mCi
2721	S-[2-(3-Aminopropylamino)[1,2-^{14}C]ethyl]-phosphorothioic Acid	CT-5324-181	RTI	101 µCi/mg	1.616 mCi
2823	S-[2-(5-Aminopentylamino)[1,2-^{14}C]ethyl]-phosphorothioic Acid	3612-95	RTI	33.3 µCi/mg	0.83 mCi
2975	[methoxy-^{3}H]Primaquine Diphosphate	3612-171	Monsanto	55.5 mCi/mmole*	6.01 mCi
		155b*	Monsanto	0.18 mCi/mg*	0.639 mCi
		155c*	Monsanto	0.13 mCi/mg*	4.06 mCi
2975	[1-aminopentyl-1-^{14}C]Primaquine Diphosphate	477b*	Monsanto	16.4 mCi/mmole	0.12 mCi
		477c*	Monsanto	15.8 mCi/mmole	0.60 mCi
		477d*	Monsanto	15.8 mCi/mmole	0.11 mCi
		477e*	Monsanto	1.61 mCi/mmole	0.21 mCi
+2975	[quinoline-2,4-^{14}C]Primaquine Diphosphate	2850-51-E	New England Nuclear	1.55 mCi/mmole	0.47 mCi
		2176-067	New England Nuclear	2.57 mCi/mmole	9.985 mCi
+2978	[2-^{14}C]Pyrimethamine Pamoate Hemihydrate	3612-3	RTI	1.50 mCi/mmole	0.24 mCi
+2978	[2-^{14}C]Pyrimethamine	2572-194	Amersham	14.7 mCi/mmole	0.75 mCi
		3193-158	Amersham	54 mCi/mmole	25.5 mCi

WR No.	Compound	Lot No.	Origin	Specific Activity	Amount Available
3090	2,4,7-Triamino-6-(2-methylphenyl)[7-^{14}C]-pteridine	156*	Monsanto	1.88 mCi/mmole	3.02 mCi
3091	N'-(4-Chlorophenyl-^{14}C)-N^{5}-isopropyl-diguanide Hydrochloride	321*	Monsanto	12 mCi/mmole	2.42 mCi
3689	S-[2-(3-Methylaminopropylamino)[1-^{14}C]-ethyl]phosphorothioic Acid	CT-4928-123-1	RTI	54.6 μCi/mg	2.69 mCi
		CT-4928-127-1	RTI	46.1 μCi/mg	1.47 mCi
		CT-5385-1[illegible]	RTI	51.3 μCi/mg	10.77 mCi
3863	1,4-Bis[2-(7-chloro-4-[3-^{14}C]quinolyl)]-aminopropylpiperazine	158*	Monsanto	1.19 mCi/mmole	1.87 mCi
4809	1-Methyl-4-[4-(7-chloro-4-[3-^{14}C]quinolyl-amino)benzoyl]piperazine	159*	Monsanto	0.29 mCi/mmole	0.325 mCi
5473	4,6-Diamino-1-(4-chloro[^{14}C]phenyl)-2,2-dimethyl-1,2-dihydro-s-triazine Hydrochloride	464a*	Monsanto	12.5 mCi/mmole	1.48 mCi
5677	[^{14}C]Dypnoneguanylhydrazone Hydrochloride	160*	Monsanto	0.44 mCi/mmole	0.80 mCi
5949	2,4-Diamino-5-(3,4,5-trimethoxybenzyl)-[2-^{14}C]pyrimidine	161*	Monsanto	1.35 mCi/mmole	0.09 mCi
6026	6-Methoxy-8-(6-diethylaminohexylamino)-[2,3-$^{2}H_2$]lepidine Dihydrochloride	CT-4928-79	RTI	---	517 mg
6026	6-Methoxy-8-(6-diethylaminohexylamino)-lepidine-4-^{14}C Dihydrochloride	CT-5385-99-1	RTI	16.1 mCi/mmole	3.02 mCi
		CT-5385-99-2	RTI	16.2 mCi/mmole	3.51 mCi
6241	[3-^{14}C]Atropine Sulfate Monohydrate	4869-147-3	RTI	13 mCi/mmole	1.8 mCi

WR No.	Compound	Lot No.	Origin	Specific Activity	Amount Available
6570	[carbamate methyl-^{14}C]Physostigmine Salicylate	MM-5241-102-A	RTI	55 mCi/mmole	1.0 mCi
		MM-5241-102-B	RTI	17.6 mCi/mmole	1.0 mCi
6570	[2-^{14}C]Physostigmine	CT-5324-55	RTI	28.6 mCi/mmol	10.4 mCi
6570	(±)-[1 methyl-$^{2}H_3$-2,2,3,3-$^{2}H_4$]-Physostigmine Salicylate	MM-5616-131	RTI	N/A	211.0 mg
6570	[benzene ring-^{3}H]Physostigmine	TRQ-4569	Amersham	16.1 Ci/mmole	6.23 mCi
9792	4-Trifluoromethylphenyl-4'-fluorophenyl-[^{14}C]ketonehydrazone Hydrochloride	164*	Monsanto	0.365 mCi/mmole	0.08 mCi
16411	2-[(Hydroxyimino)methyl]-1-([^{14}C]methyl)-pyridinium Chloride	4929-61-A	RTI	1.9 mCi/mmole	3.66 mCi
17206	1,4-Bis(trichloromethyl)[^{3}H]benzene	165*	Monsanto	N/A	49.90 mCi
25979	1-Amidino-3-(4-nitro[^{3}H]phenyl)urea Monohydrochloride	166*	Monsanto	N/A	23.93 mCi
27799	6-{[3-(Diethylamino)[3-^{14}C]propyl]amino}-5,8-dimethoxyquinaldine β-Resorcylate	168*	Monsanto	0.41 mCi/mmole	0.03 mCi
30090	2-(3,4-Dichlorophenyl)-6,8-dichloro[2-^{14}C]-quinolyl]-4-dibutylaminomethylcarbinol Hydrochloride	169*	Monsanto	0.54 mCi/mmole	0.273 mCi
		302-4a*	Monsanto	5.43 mCi/mmole	2.78 mCi
		302-4b*	Monsanto	5.38 mCi/mmole	0.28 mCi
33063	α-(Di-n-heptylaminomethyl)-6-bromo-9-phenanthrene[^{14}C]methanol Hydrochloride	277-3b-1*	Monsanto	0.015 mCi/mg	0.75 mCi
		277-3b-2*	Monsanto	0.015 mCi/mg	0.16 mCi
		277-3b-3*	Monsanto	0.01 mCi/mg	0.76 mCi

WR No.	Compound	Lot No.	Origin	Specific Activity	Amount Available
38839	4,6-Diamino-1,2-dihydro-2,2-dimethyl-1-(3,4-dichlorobenzyloxy)-1,3-[2-^{14}C]triazine Hydrochloride	173*	Monsanto	1.10 mCi/mmole	0.928 mCi
40070	2,4-Diamino-5-piperonyl[2-^{14}C]pyrimidine	146*	Monsanto	10.6 mCi/mmole	5.85 mCi
46234	5-Chloro-2-hydroxy-N-(2-chloro-4-nitrophenyl)[ring-U-^{14}C]benzamide	5513-153-B 5513-166	RTI RTI	18.0 mCi/mmole 9.04 mCi/mmole	2.52 mCi 0.828 mCi
46234	5-Chloro-2-hydroxy-N-(2-chloro-4-nitro-[U-$^{13}C_6$]phenyl)benzamide	5662-21	RTI	N/A	161 mg
49808	2-Hydroxy-3-(8-[^{3}H]cyclohexyloctyl)-1,4-naphthoquinone	174a* 174b*	Monsanto Monsanto	38.2 mCi/mmole[#] 38.2 mCi/mmole[#]	2.15 mCi 5.47 mCi
61112	3,5-Dichloro-2,6-dimethyl-4[2,6-^{14}C]-pyridinol	342*	Monsanto	4.7 μCi/mg	2.37 mCi
74106	[^{14}C]Terephaloyldihydroxamic Acid	175*	Monsanto	2.50 mCi/mmole	4.35 mCi
77135	5-Nitrothiophene-2-[^{14}C]carboxaldehyde Valerhydrazone	176*	Monsanto	1.19 mCi/mmole	2.0 mCi
81844	1-(3,4-Dichlorophenyl)-[4-(1-ethyl-3-piperidino-3-amino)-6-methyl-2-[6-^{14}C]-pyrimidinyl]-guanidine Dihydrochloride Monohydrate	177*	Monsanto	0.96 mCi/mmole	0.29 mCi
98057	α-Dibutylaminoethyl-2,6-di(4-chlorophenyl)-4-pyridino[^{3}H]methanol Hydrochloride	178*	Monsanto	N/A	10.62 mCi
99210	4,6-Diamino-1,2-dihydro-2,2-dimethyl-1-[γ-(2,4,5-trichlorophenoxy)-propyloxy]-s-[2-^{14}C]triazine Hydrochloride	346a*	Monsanto	9.53 mCi/mmole	1.84 mCi

WR No.	Compound	Lot No.	Origin	Specific Activity	Amount Available
99662	2-(3-Dimethylaminopropylamino)-4-trichloromethyl-6-(4-trichloromethylphenyl)-s-[2,4-^{14}C]triazine	180-3a*	Monsanto	1.33 mCi/mmole	0.36 mCi
		180-3b*	Monsanto	1.33 mCi/mmole	0.36 mCi
99682	3,4-Dichloro-4-trifluoromethylbenzophenone-[^{14}C]carbonylguanylhydrazone Hydrochloride	181*	Monsanto	1.54 mCi/mmole	0.91 mCi
		181-3a*	Monsanto	1.54 mCi/mmole	0.91 mCi
122455	α-(2-Piperidyl)-3,6-bis(trifluoromethyl)-9-phenanthrene[^{3}H]methanol Hydrochloride	182*	Monsanto	0.18 mCi/mg/	1.50 mCi
		183-3a*	Monsanto	77.7 mCi/mmole/	3.02 mCi
122455	α-(2-Piperidyl)-3,6-bis(trifluoromethyl)-9-phenanthrene[^{14}C]methanol Hydrochloride	208-3	Monsanto	3.98 mCi/mmole	0.61 mCi
142490	Erythro-α-(2-piperidyl)-2,8-bis(trifluoromethyl)-4-quinoline[^{14}C]methanol Hydrochloride	187-3a*	Monsanto	12.4 mCi/mmole	0.56 mCi
		411a*	Monsanto	11.5 mCi/mmole	2.36 mCi
		411b*	Monsanto	11.5 mCi/mmole	0.23 mCi
		2572-64	RTI	10.4 mCi/mmole	0.923 mCi
		3793-133	RTI	57.8 mCi/mmole	3.28 mCi
142490	Erythro-α-(2-piperidyl)-2,8-bis(trifluoromethyl)-4-quinoline[^{14}C]methanol Methanesulfonate	433a*	Monsanto	11.6 mCi/mmole	1.51 mCi
143803	2-(Di-n-butylaminomethyl)-3,6-bis(trifluoromethyl)-9-phenanthrene[^{14}C]methanol Hydrochloride	207-2b*	Monsanto	4.34 mCi/mmole	0.82 mCi
148946	α-(Di-n-butylaminomethyl)2,6-bis(4-trifluoromethylphenyl)4-pyridine[^{14}C]-methanol Hydrochloride	256-2a*	Monsanto	3.89 mCi/mmole	0.41 mCi
		256-2b*	Monsanto	3.89 mCi/mmole	0.38 mCi
149024	1,18-Diamino-6,13-diaza-9,10-dithia-[7,8,11,12-^{14}C]octadecane Tetrahydrochloride	3612-55	RTI	13.5 mCi/mmole	2.3 mCi

WR No.	Compound	Lot No.	Origin	Specific Activity	Amount Available
151327	S-[3-(3-Methylaminopropylamino)[1-^{14}C]-propyl]phosphorothioic Acid	CT-5385 161-1	RTI	105 μCi/mg	7.56 mCi
		CT-5324-179	RTI	110 μCi/mg	2.52 mCi
158122	2,4-Diamino-6-(2-napthylsulfonyl)[2-^{14}C]-quinazoline	401-2a*	Monsanto	26.7 mCi/mmole	0.24 mCi
		401-2b*	Monsanto	26.7 mCi/mmole	1.15 mCi
159412	2,4-Diamino-6-[(3-trifluoromethylphenyl)-thio][2-^{14}C]quinazoline	289*	Monsanto	14.7 mCi/mmole	3.56 mCi
162878	2,4-Diamino-6[(3-trifluoromethylphenyl)-sulfonyl][2-^{14}C]quinazoline	298-1a*	Monsanto	14.9 mCi/mmole	1.83 mCi
		298c*	Monsanto	N/A	1.27 mCi
165533	α-(2-Di-n-butylaminoethyl)-3,6-bis(trifluoromethyl)-9-phenanthrene[^{14}C]methanol Hydrochloride	204-5*	Monsanto	4.94 mCi/mmole	1.77 mCi
165543	α-(Butylaminoethyl)-3,6-bis(trifluoromethyl)-9-phenanthrene[^{14}C]methanol Hydrochloride	242-2*	Monsanto	4.09 mCi/mmole	1.54 mCi
169626	4,6-Diacetamido-1,2-dihydro-2,2-dimethyl-1-[7-(2',4',5'-trichlorophenoxy)-propyloxy]-s-[2-^{14}C]triazine	CT-3652-93-1	RTI	16.3 mCi/mmole	8.16 mCi
171669	1,3-Dichloro-6-trifluoromethyl-9-[1-hydroxy-3-(N,N-di-n-butylamino)[1-^{14}C]propyl]phenanthrene Hydrochloride	3193-135	RTI	14.9 mCi/mmole	0.03 mCi
		3959-41	RTI	14 mCi/mmole	0.86 mCi
172435	3-Di-n-butylamino-1-[2,6-bis(trifluoromethylphenyl)-4-pyridyl][1-^{14}C]propanol Hydrochloride	348b*	Monsanto	10.2 mCi/mmole	1.34 mCi

WR No.	Compound	Lot No.	Origin	Specific Activity	Amount Available
172435	3-Di-n-butylamino-1-[2,6-bis(trifluoro methylphenyl)-4-pyridyl][1-^{14}C]propanol Methanesulfonate	2850-127	RTI	11.5 mCi/mmole	1.07 mCi
177602	Threo-α-(2-piperidyl)-2,8-bis(trifluoro-methyl)-4-quinoline[^{14}C]methanol Hydro-chloride	469a	Monsanto	13.4 mCi/mmole	3.30 mCi
		469-2a*	Monsanto	13.4 mCi/mmole	1.80 mCi
		469-3a*	Monsanto	13.4 mCi/mmole	0.197 mCi
177602	Threo-α-(2-piperidyl)-2,8-bis(trifluoro-methyl)-4-quinoline[^{14}C]methanol Methanesulfonate	434a*	Monsanto	11.9 mCi/mmole	0.95 mCi
		434b*	Monsanto	11.9 mCi/mmole	0.025 mCi
178460	1,3-Dichloro-6-trifluoromethyl-9-[1-hydroxy-3-(N-n-butylamino)[1-^{14}C]propyl]phenanthrene Hydrochloride	3793-91	RTI	16.0 mCi/mmole	1.17 mCi
180117	α-(2-Piperidyl)-2-trifluoromethyl-6-(4-trifluoromethylphenyl)-4-pyridine[^{14}C]-methanol Hydrochloride	365-2c*	Monsanto	26 μCi/mg	2.16 mCi
180117	α-(2-Piperidyl)-2-trifluoromethyl-6-(4-trifluoromethylphenyl)-4-pyridine[^{14}C]-methanol Phosphate	443-2a*	Monsanto	10.6 mCi/mmole	3.87 mCi
		443-2b*	Monsanto	10.3 mCi/mmole	2.81 mCi
		536c*	Monsanto	35 μCi/mg	2.59 mCi
180409	Threo-α-(2-piperidyl)-2-trifluoromethyl-6-(4-trifluoromethylphenyl)-4-pyridine-[^{14}C]-methanol Phosphate	536a	Monsanto	19.8 mCi/mmole	4.02 mCi
184806	2,8-Bis(trifluoromethyl)-4-(1-hydroxy-3-N-t-butylamino[1-^{14}C]propyl)quinoline Phosphate	385-2a	Monsanto	11.7 mCi/mmole	0.57 mCi
		385-2b*	Monsanto	16.5 μCi/mg	1.17 mCi
		2850-25	RTI	11.2 mCi/mmole	1.5 mCi
194965	4-[^{14}C]t-Butyl-6-t-butylaminomethyl-2-(4-chlorophenyl)phenol Phosphate	3612-151	RTI	20.9 mCi/mmole	0.65 mCi

WR No.	Compound	Lot No.	Origin	Specific Activity	Amount Available
225448	8-(4-Amino-1-methylbutylamino)-6-methoxy-5-(3-trifluoromethylphenoxy)-[4-^{14}C]quinoline Succinate	CT-2575-191	RTI	12.5 mCi/mmole	16.45 mCi
226253	Erythro-α-(2-piperidyl)-2-trifluoromethyl-6,8-dichloro-4-quinoline[^{14}C]methanol Methanesulfonate	2572-114	RTI	33 mCi/mmole	6.64 mCi
		2572-157	RTI	10 mCi/mmole	8.52 mCi
228252	4'-Chloro-5-[(7-chloro-4-[4-^{14}C]quinolyl)-amino]-3-[(1,1-dimethylethyl)amino]-methyl][1,1'-biphenyl]-2-ol Dihydrochloride	CT-3181-17-1	RTI	20.4 mCi/mmole	19.07 mCi
		CT-3181-17-2	RTI	20.2 mCi/mmole	2.08 mCi
238605	8-[(4-Amino-1-methylbutyl)amino]-2,6-dimethoxy-4-methyl-5-[(3-trifluoromethyl)-phenoxy][4-^{14}C]quinoline Succinate	CT-6949-61	RTI	21 mCi/mmole	31 mCi
238605	8-[(4-Amino-1-methylbutyl)amino]-2,6-dimethoxy-4-methyl-5-[(3-trifluoromethyl)-[6-^{3}H]phenoxy]quinoline Succinate	CT-6949-145	RTI	165 mCi/mmole	115.4 mCi
242511	8-[(4-Amino-1-methylbutyl)amino]-5-(1-hexoxy)-6-methoxy-4-methyl[4-^{14}C]-quinoline (DL)-Tartrate	LF-6797-73	RTI	20.9 mCi/mmole	17.22 mCi
249309	[16-^{14}C]Artemisinin	LF-7044-103	RTI	49.7 mCi/mmol	1.4 mCi
		LF-7044-110	RTI	46.9 mCi/mmol	1.53 mCi
		LF-7044-45C	RTI	45.5 mCi/mmol	0.074 mCi

WR No.	Compound	Lot No.	Origin	Specific Activity	Amount Available
250165	[6-^{14}C]Allopurinol Riboside	CT-3892-91	RTI	7.85 mCi/mmole	0.48 mC.
250710	[N,N-dimethylamino-$^{2}H_6$]Pyridostigmine Bromide	3959-195	RTI	----	0.4 g
250710	[2-^{14}C]Pyridostigmine Bromide	CT-4167-127	RTI	18.0 mCi/mmole	20.10 mCi
250710	[6-^{3}H]Pyridostigmine Bromide	CT-4537-81	RTI	22.5 Ci/mmole#	250.04 mCi
250710	[carbamate methyl-^{14}C]Pyridostigmine Bromide	CT-5385-67	RTI	37.6 mCi/mmole	66.96 mCi
255131	[ethyl-$^{2}H_5$]β-Arteether	5513-181-D	RTI	----	1.04 g
255131	[16-^{14}C]β-Arteether	5994-83	RTI	1.5 mCi/mmole	4 μCi
		5994-117	RTI	6.1 mCi/mmole	0.525 mCi
255663	[16-^{14}C]Artelinic acid	LF-7044-94	RTI	12.42 mCi/mmole	.93 mCi

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* Purity of these compounds have not been checked at RTI. Specific activity and amount available for shipment are those stated by the originating source and have not been confirmed at RTI.

+ WHO compounds.

Actual value of the specific activity will be less depending on length of storage due to the relatively short half life of ^{3}H.

APPENDIX

Synthesis Report

WALTER REED ARMY INSTITUTE OF RESEARCH

Contract No. DAMD17-89-C-9062

WR-238605

8-[(4-Amino-1-methylbutyl)amino]-2,6-dimethoxy-4-methyl-5-[(3-trifluoromethyl)phenoxy][4-^{14}C]quinoline Succinate

Lot No. CT-6949-61

March, 1992

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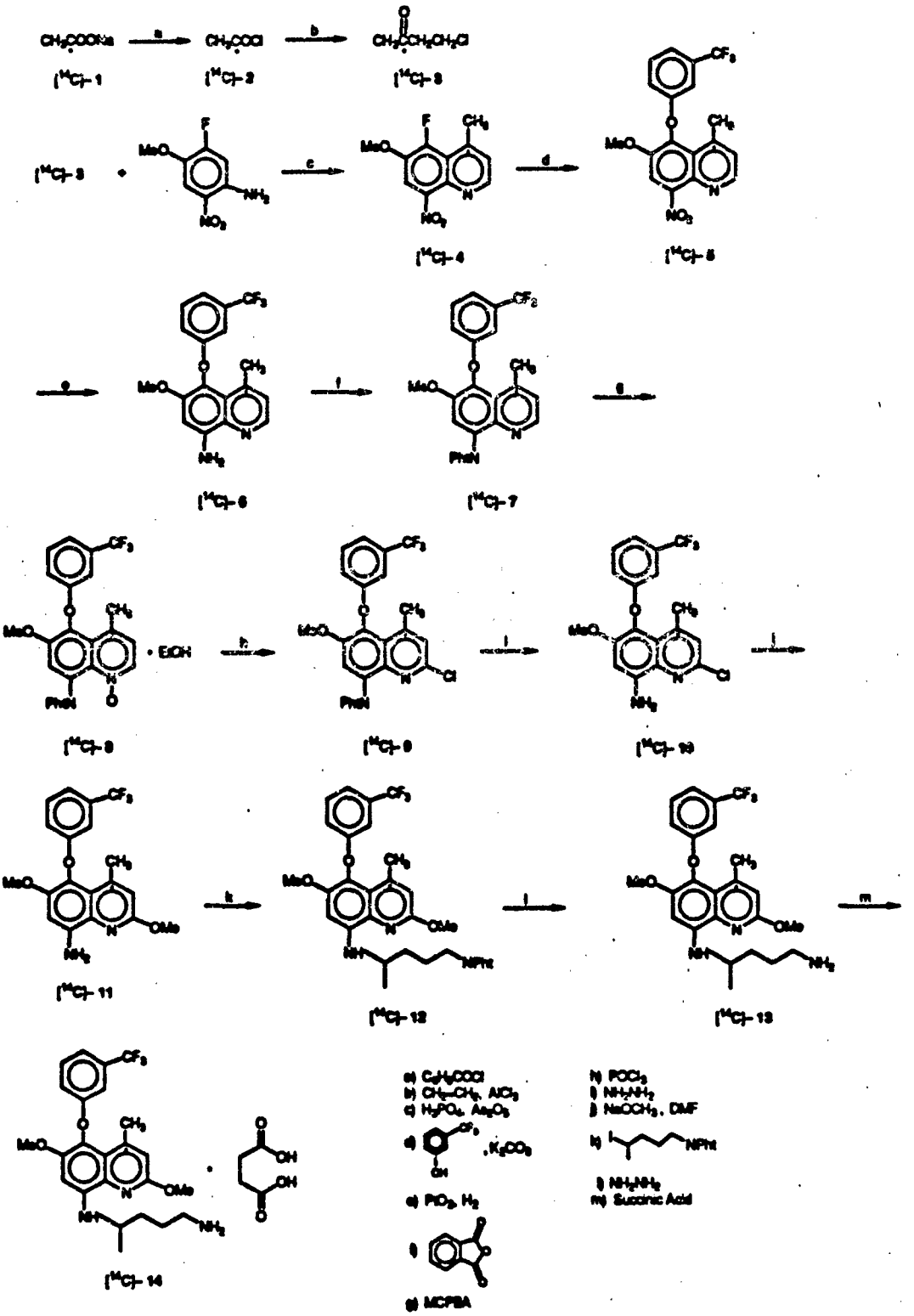
Synthesis Scheme



[1-^{14}C]Acetyl Chloride ([^{14}C]-2)

Into a 25-mL recovery flask containing a magnetic stirring bar was placed sodium [1-^{14}C]acetate (482 mCi, 659 mg, 8.04 mmol). To this was added NaOH solution (800 μL, 2.14 M). The flask was attached to the high vacuum manifold, the H_2O vacuum transferred out and the residue dried for ~ 1 h at 195°C. Note: The NaOH treatment is to neutralize any free [1-^{14}C]acetic acid that may be present. In an earlier run, we found that 300 mCi of the sodium [1-^{14}C]acetate contained 30 mCi of volatile radioactivity. The flask was allowed to cool to ambient temperature before removing it from the manifold. Benzoyl chloride (12 mL) was added to the reaction flask which was fitted with a simple Claisen distillation head connected to a vacuum distillation adapter. A drying tube containing Drierite and activated charcoal granules was attached to the side arm of the vacuum distillation adapter. A pear-shaped flask was attached to the vacuum distillation adapter as the receiver. This receiver was cooled to -30°C. The reaction flask was heated in an oil bath until the benzoyl chloride started to reflux into the bottom of the Claisen head. The reaction flask was cooled to ambient temperature and nonlabeled acetyl chloride (71 μL, 1.0 mmol) was added. The reaction mixture was heated as before. The reaction mixture was cooled and a 2nd addition of nonlabeled acetyl chloride (71 μL, 1.0 mmol) was made. The mixture was heated as before while collecting the acetyl chloride in the receiving flask at -30°C. The receiver was allowed to warm to room temperature and weighed. A total of 660 mg (84% yield) of acetyl chloride was collected. The product was used as collected in the next reaction.

Chemical	Source & Catalog No.	Lot No.
Sodium [1-^{14}C]Acetate	Sigma 31,158-8	021H 9209
Benzoyl Chloride	Aldrich B1269-5	4028 LL
Acetyl Chloride	Aldrich 23,957-7	00225 MV

4-Chloro-[2-^{14}C]2-butanone ([^{14}C]-3)

Aluminum chloride (2.6 g) was weighed into a 100-mL round bottom flask containing a magnetic stirring bar. Methylene chloride (~ 60 mL) which had been dried by passing it through basic Al_2O_3 was added to the flask. The mixture was cooled to -20°C while a slow stream of ethylene was passed into the mixture via a fritted glass gas introduction tube. The [1-^{14}C]acetyl chloride (660 mg, 8.4 mmol) was dissolved in CH_2Cl_2 (5 mL) and added via syringe into the gas introduction tube. The tube was rinsed with additional CH_2Cl_2 (2 x 1 mL). Ethylene was passed into the reaction mixture fo ~ 2.25 h while keeping the mixture at -10°C. The reaction was allowed to warm to room temperature, and after stirring for 30 min was poured onto ice containing concentrated HCl (15 mL) and extracted 4 X with CH_2Cl_2. The combined CH_2Cl_2 extracts were washed with 6 N HCl, H_2O, saturated $NaHCO_3$ solution and dried over Na_2SO_4. The dried solution was counted (325 mCi) and then the CH_2Cl_2 was stripped. The residue was vacuum transferred to give 891 mg of [^{14}C-3] as a colorless liquid which was used as is in the next reaction. A residue containing 39.7 mCi did not vacuum transfer. The CH_2Cl_2 that was removed by stripping was counted and found to contain 21.6 mCi of radioactivity. This solution was carefully concentrate to a volume of ~ 5 mL and used in a separate reaction to prepare the nitroquinoline [^{14}C]-4.

Chemical	Source & Catalog No.	Lot No.
Aluminum Chloride	Fisher A-575	880667
Methylene Chloride	Fisher D-37	913251
Alumina, basic Act.I	Woelm B	1385
Ethylene	Matheson	08227
Hydrochloric Acid	Fisher A-144	FL040398

5-Fluoro-6-methoxy-4-methyl-8-nitro[4-^{14}C]quinoline ([^{14}C]-4)

To the 25-mL recovery flask containing chloroketone [^{14}C]-3 (890 mg, 8.39 mmol) prepared above, was added 3-fluoro-4-methoxy-6-nitroaniline (1562 mg, 8.39 mmol), $As_2O_5 \cdot XH_2O$ (1.45 g, 6.29 mmol) and H_3PO_4 (6 mL). The flask was fitted with a Liebig condenser (air cooled) and a nitrogen line. The mixture was heated at 72-75°C for 8 h. After the reaction mixture was cooled, nonlabeled 3 (150 mg, 140 μL) was added and the mixture heated for 5 h. The reaction mixture was cooled and a second sample of nonlabeled 3 (150 mg, 140 μL) was added and the mixture was heated for 6 h. The progress of the reaction was followed by HPLC [5 μ Spherical SiO_2, 3.9 mm x 150 mm, 93% hexane-EtOAc, 2 mL/min, UV λ 254 mμ]. The reaction was worked up by pouring onto ice containing NH_4OH (20 mL) and extracting 4X with CH_2Cl_2. The combined CH_2Cl_2 extracts were not dried but were stripped to leave a brown residue of impure [^{14}C]-4, which was dried on the vacuum manifold.

The CH_2Cl_2 solution of chloroketone [^{14}C]-3 recovered in the previous reaction was also taken through the nitroquinoline preparation sequence described above. The products from the two reactions were combined. This material was flash chromatographed on a column of SiO_2 (4 x 20 cm) using 80% CH_2Cl_2-hexane (1000 mL), 85% CH_2Cl_2-hexane (1000 mL) and then 90% CH_2Cl_2-hexane (1000 mL) as eluants and collecting fractions of 25-30 mL. The fractions were analyzed by HPLC (same system). Fractions 25-42 were combined and stripped to give 973 mg (96.9 mCi) of pure [^{14}C]-4. This material was combined with [^{14}C]-4 (32.1 mCi) from two other preparations and used in the next reaction. The impure fractions were combined and carried through the next reaction sequence separate from the pure fractions.

Chemical	Source & Catalog No.	Lot No.
3-Fluoro-4-methoxy-6-nitroaniline	WRAIR	BH 49612
Arsenic Pentoxide	Fisher A-53	790650
Phosphoric Acid (85%)	Fisher A-242	720489
Ammonium Hydroxide	Fisher A-669	860116
Methylene Chloride	Fisher D-37	913251
Silica Gel-60	E. Merch 9385-5	30283

6-Methoxy-4-methyl-8-nitro-5-(3-trifluoromethylphenoxy)[4-^{14}C]quinoline ([^{14}C]-5)

To a solution of [^{14}C]-4 (1.291 g, 5.47 mmol, 129 mCi) in acetone (30 mL) was added K_2CO_3 (3.93 g) and m-hydroxybenzotrifluoride (1.062 g). The resulting mixture was heated at 65°C for 6 h. HPLC [5 μ spherical SiO_2, 3.9 mm x 150 mm, 93% hexane-EtOAc, 2 mL/min, UV λ 254 mμ] indicated the reaction was complete. The acetone was stripped and the residue taken-up in CH_2Cl_2 and washed with H_2O. The H_2O layer was extracted 3X with CH_2Cl_2. The combined CH_2Cl_2 extracts were washed twice with 10% K_2CO_3 solution, dried over Na_2SO_4 and stripped to leave 2.06 g (127 mCi) of dirty yellow solid. The solid was mixed with a small amount of [^{14}C]-5 from a previous preparation and rinsed once with 75% hexane-CH_2Cl_2 (3 mL) and twice with hexane. The resulting solid was dried on the vacuum manifold to give 2.243 g of pale yellow crystals, mp 211-213°C (std. mp 212-213°C). This material was divided into three batches and each carried through the next reaction.

Chemical	Source & Catalog No.	Lot No.
Acetone	Fisher A-18	911267
Potassium Carbonate	Fisher P-208	905633
m-Hydroxybenzotrifluoride	Aldrich 15,603-5	01718BY
Methylene chloride	Fisher D-37	913251
Sodium Sulfate	Fisher S-421	884476

8-Amino-6-methoxy-4-methyl-5-(3-trifluoromethylphenoxy)[4-^{14}C]quinoline ([^{14}C]-6)

In a 25-mL glass-lined stainless steel bomb were placed PtO_2 (75 mg) and THF (2 mL) which had been distilled from benzophenone ketyl. The PtO_2 was treated with hydrogen at 50 psi for 20 min. The crystalline [^{14}C]-5 (773 mg, 2.04 mmol, 49 mCi) was added to the bomb. Additional THF (20 mL) was added and the resulting mixture was stirred with hydrogen at 50 psi for 16 h. TLC (SiO_2: Et_2O-NH_4OH, 30:0.25) indicated the reaction was complete. The reaction was filtered through a short column of SiO_2 (~ 3 mL) using additional THF. The resulting black solution was stripped. The residue was dissolved in a small amount of THF and filtered through a 0.45 μm Teflon filter. The solution was still black. It was stripped to leave a dark residue, 807 mg, which was immediately used in the next reaction. This reaction was repeated a total of three times with each being carried through the next reaction separately.

Chemical	Source & Catalog No.	Lot No.
Platinum(IV) Oxide	Aldrich 20,603-2	02506KT
Tetrahydrofuran	Fisher T-397	911249
Hydrogen, prep.	Matheson	J10-0448-B2
Silica Gel-60	E. Merck 9385-5	30283

6-Methoxy-4-methyl-8-phthalimido-5-(3-trifluoromethylphenoxy)[4-^{14}C]-quinoline ([^{14}C]-7)

The black residue of [^{14}C]-6 from the previous reaction was dissolved in xylene (50 mL) and phthalic anhydride (363 mg, 2.45 mmol, 20% excess) was added. This solution was heated at reflux for 5 h while collecting H_2O that formed with a Dean-Stark trap. TLC (SiO_2:Et_2O-NH_4OH, 30:0.25) indicated the reaction was complete. The reaction was poured into CH_2Cl_2 and washed thrice

with 10% KOH solution and once with H_2O. The solution was stripped (aspirator and then vacuum pump), and the residue was flash chromatographed on a column of SiO_2 (2.5 x 15 cm) using $CHCl_3$ as eluant. The pure fractions were combined and stripped to give 976 mg (48.5 mCi) of [^{14}C]-7. This reaction was run three times to obtain a total of 126 mCi of [^{14}C]-7.

Chemical	Source & Catalog No.	Lot No.
Phthalic Anhydride	Mallinckrodt 2828	MTD
Xylene	Fisher X-5	911304
Methylene Chloride	Fisher D-143	915700
Potassium Hydroxide	Mallinckrodt S-420	907057

6-Methoxy-4-methyl-8-phthalimido-5-(3-triflucromethylphenoxy)[4-^{14}C]quinoline-1-oxide Ethanolate ([^{14}C]-8)

To a solution of [^{14}C]-7 (56.9 mCi) in $CHCl_3$ (20 mL) cooled to 0°C was added a solution of m-chloroperoxybenzoic acid (MCPBA) in $CHCl_3$ (10 mL + 3 x 0.5 mL rinses). The solution was allowed to warm to room temperature and was stirred for 2 h. TLC (SiO_2: CH_2Cl_2-CH_3OH, 25:0.75) indicated ~ 30% desired product. Additional MCPBA (420 mg) was added at 0°C and the mixture allowed to warm to room temperature and stir for 16 h. TLC (same system) indicated ~ 90% product. Additional MCPBA (200 mg) was added at 0°, and the mixture was stirred for 20 h at room temperature. TLC (same system) indicated little change from the previous sample. The reaction was worked up by washing twice with 15% K_2CO_3 solution (see note below).* The $CHCl_3$ solution was dried,

*Note: During the course of running this reaction and other similar reactions to prepare 8, we have shown that both SiO_2 and Al_2O_3 should be avoided for the cleanup of the N-oxide product. This material is decomposed by chromatography on either Al_2O_3 or SiO_2. The amount of decomposition is a function of how long the 8 remains on the column.

counted (56.8 mCi) and stripped. The residue was dissolved in EtOH (20 mL) and the solution stripped to leave a pale yellow crystalline solid. The solid was combined with material (70.7 mCi) from two other preparations and used in the next reaction.

Chemical	Source & Catalog No.	Lot No.
m-Chloroperoxybenzoic Acid	Aldrich C6,270-0	6720KK
Chloroform	BJ048	BB216
Ethanol	Aaaper	91H1SV

2-Chloro-6-methoxy-4-methyl-8-phthalimido-5-(3-trifluoromethylphenoxy)-[4-^{14}C]quinoline ([^{14}C]-9)

To a solution of [^{14}C]-8 (1.698 g, 3.15 mmol, 70.7 mCi) in $CHCl_3$ (40 mL) was added $POCl_3$ (4.83 g, 31.5 mmol) dropwise over 15 min. The resulting solution was stirred at room temperature overnight. TLC (SiO_2: CH_2Cl_2-CH_3OH, 25:0.8) indicated the reaction had gone to 85% completion. Additional $POCl_3$ (600 μL) was added and the resulting solution stirred for 20 h at room temperature. TLC (same system) indicated ~ 2% of [^{14}C]-8 was still present. Additional $POCl_3$ (350 μL) was added and the resulting mixture stirred for 2 h at 60-65°C and then 16 h at room temperature. The reaction mixture was poured over ice, and the pH was adjusted to 12 with 20% NaOH. This mixture was extracted thrice with Et_2O. The combined Et_2O extracts were washed once with H_2O and once with saturated $NaHCO_3$ solution. The resulting Et_2O extract was carefully concentrated to dryness overnight with a stream of nitrogen. The residue was chromatographed on a column of SiO_2 (230-400 mesh, 25 mL) using first CH_2Cl_2 (300 mL) and then CH_2Cl_2-MeOH (25:0.8, 200 mL as eluants and collecting 8 mL fractions. The fractions (4-8) containing pure product were combined, counted and stripped to afford 1.254 g (53.5 mCi) of [^{14}C]-9.

Chemical	Source & Catalog No.	Lot No.
Chloroform	BJ049	BB216
Phosphorus Oxychloride	Aldrich 32,045-5	00830CX
Sodium Hydroxide	Fisher SS-254	874986
Ethyl Ether	Fisher E-138	921442-15
Sodium Bicarbonate	Fisher S-233	720392
Ethanol	Aaper	91H1SV

8-Amino-2-chloro-6-methoxy-4-methyl-5-(3-trifluoromethylphenoxy)[4-^{14}C]-quinoline ([^{14}C]-10)

To a suspension of [^{14}C]-9 (1.254 g, 2.45 mmol, 53.5 mCi) in EtOH (45 mL) was added a solution of NH_2NH_2 (98%) (400 µL) dissolved in EtOH (4 mL) over 5 min. The NH_2NH_2 residue was rinsed in with additional EtOH (1 mL). The resulting mixture was heated at 60-65°C for 1 h. TLC (SiO_2: 80% CH_2Cl_2-hexane) indicated the reaction was complete. This reaction was combined with a similar reaction that contained 10.7 mCi of [^{14}C]-9. The resulting mixture was stripped and the residue chromatographed on SiO_2 (56 g, 17 x 4 cm) using CH_2Cl_2 as eluant and collecting fractions of ~ 25 mL. The product was in fractions 2-5. These were combined, counted and stripped to obtain 1.061 g (61 mCi) of yellow solid.

Chemical	Source & Catalog No.	Lot No.
Ethanol	Aaper	91H1SV
Hydrazine (98%)	Aldrich 21,515-5	04306TY
Methylene Chloride	Fisher D-143	915700
Silica Gel-60	E. Merck 9385-5	30283

8-Amino-2,6-dimethoxy-4-methyl-5-(3-trifluoromethylphenoxy)[4-^{14}C]quinoline ([^{14}C]-11)

Sodium hydride (80% oil dispersion) (166 mg, 5.44 mmol) was weighed into a 50-mL flask fitted with an adapter to allow work under an inert gas (Argon).

The NaH was washed twice with hexane and then blown dry. Dimethylformamide (4.5 mL) was added followed by methyl alcohol (97 mg, 3.05 mmol, 10% excess, 123 μL). The resulting mixture was stirred for 1 h at room temperature. A solution of [^{14}C]-10 (1.061 g, 2.77 mmol, 61 mCi) in DMF (2 mL) was added. The residue of [^{14}C]-10 was rinsed in with DMF (3 x 0.5 mL). The resulting reaction mixture was stirred overnight at room temperature. TLC (SiO_2: 80% CH_2Cl_2-hexane) indicated ~ 65% [^{14}C]-11 present. The reaction mixture was heated at 40-45°C for 3 h and allowed to stand overnight at room temperature. TLC analysis (same system) indicated the reaction was 77% complete. Additional $NaOCH_3$ was prepared from NaH (66 mg) and CH_3OH (50 μL) in DMF (2 mL) and this was added to the above reaction. The reaction was then heated at 45°C for 1 h. TLC analysis (same system) showed no change from above. The reaction mixture was poured into ice water and extracted with CH_2Cl_2. An emulsion formed which was very difficult to break. Filtration through two glass fiber filter pads (at the same time) broke the emulsion. The mixture was extracted 5X with CH_2Cl_2. The combined CH_2Cl_2 extracts were stripped and the residue chromatographed on a column of SiO_2 (4 x 18 cm) using 80% CH_2Cl_2-hexane as eluant and collecting fractions of ~ 25 mL. Fractions 8-21 containing pure product were combined, counted (36.6 mCi) and stripped. Fractions 4-7 were not pure, but were combined, counted (15.1 mCi) and stripped. These impure fractions were rechromatographed on a column of SiO_2 (4 x 17 cm) using 70% CH_2Cl_2-hexane as eluant. Fractions containing pure product were combined, counted (7.9 mCi) and stripped. Fractions containing impure product were combined, counted (5.8 mCi) and stripped. This material was carried through another $NaOCH_3$ treatment. After chromatography an additional 2.1 mCi of [^{14}C]-11 were obtained. The total yield of [^{14}C]-11 was 46.6 mCi (76% radiochemical yield).

Chemical	Source & Catalog No.	Lot No.
Sodium Hydride	Aldrich 25,399-5	00804MW
Methanol	Aldrich 32,241-5	02211BY
Dimethylformamide	Aldrich 22,705-6	0341TX
Silica Gel-60	E. Merck 9385-5	30283
Methylene chloride	Fisher D-143	915700
Chloroform	Fisher C-607	911637
Chloroform	BJ048	BB216
Hexane	BJ216	BB673
Ethyl Acetate	BJ100	BB880
Methanol	A-142	911664

2,6-Dimethoxy-4-methyl-8-(4-phthalimido-1-methylbutylamine)-5-(3-trifluoromethylphenoxy)[4-^{14}C]quinoline ([^{14}C]-12)

A mixture of [^{14}C]-11 (792 mg, 2.10 mmol, 46.6 mCi), 4-iodo-1-phthalimidopentane (720 mg, 2.10 mmol) and diisopropylamine (213 mg, 2.10 mmol, 294 μL) in acetonitrile (4.2 mL) was heated at 80-85°C for 10 h. TLC (SiO_2: 80% Et_2O-Hex) indicated the reaction was ~ 43% complete. Additional alkylating agent (360 mg) and amine (147 μL) were added. The mixture was heated for 8 h at 80-85°C. TLC (same system) indicated the reaction was ~ 70% complete. Additional alkylating agent (360 mg) and amine (147 μL) were added. The mixture was heated for 8 h at 80-85°C. TLC (same system) indicated ~ 85% product and 12% [^{14}C]-11 to be present. Additional alkylating agent (180 mg) and amine (74 μL) followed by heating 10 h at 80-85°C gave a mixture of ~ 89% product and ~ 5% [^{14}C]-11. Additional alkylating agent (120 mg) and amine (50 μL) followed by heating for 8 h at 80-85°C gave a mixture of ~ 92% product and 3% [^{14}C]-11. Additional alkylating agent (80 g) and amine (34 μL) were

added and the mixture heated for 4 h at 80-85°C gave a mixture of 95% product and 3% [^{14}C]-11. Additional alkylating agent (80 mg), amine (34 μL) and CH_3CN (400 μL) were added and the mixture was heated for 5 h at 80-85°C. TLC (same system) indicated little change from the last TLC. The reaction was worked up by pouring into H_2O and extracting 4X with CH_2Cl_2. The combined CH_2Cl_2 extracts were stripped to leave 2.446 g of dark oil. This was flash chromatographed on a SiO_2 column (2.5 x 19 cm) using 75% CH_2Cl_2-hexane as eluant. The pure fractions were combined, counted (19.4 mCi) and stripped. Note: If at all possible, do not leave any [^{14}C]-11 unreacted as it is very difficult to separate this from the alkylated material.

The fractions containing product and small amounts of the starting material were combined, counted (25.8 mCi) and stripped to leave a residue. This residue contained ~ 5% of the unalkylated amine. This material was treated with the same alkylating conditions as the above described alkylation reaction. After chromatography of this second alkylation reaction, a total of 41.3 mCi of pure [^{14}C]-12 was obtained. This was used in the next reaction.

Chemical	Source & Catalog No.	Lot No.
Diisopropylamine	Aldrich 11,0019	01021 BP
4-Iodo-1-phthalimidopentane	Ash Stevens	AP-VIII-200
Acetonitrile	BJ015	BA181
Methylene chloride	D-143	915700
Silica Gel-60	E. Merck 9385-5	30283
Hexane	BJ216	BB573
Ethyl Acetate	BJ1C0	BB880

8-[(4-Amino-1-methylbutyl)amino]-2,6-dimethoxy-4-methyl-5-[(3-trifluoromethyl)]phenoxy][4-^{14}C]quinoline Succinate ([^{14}C]-14)

A solution of [^{14}C]-12 (4.3 mCi) in ethanol (20 mL) was treated with hydrazine (98%, 250 μL). The resulting solution was heated at 65-70°C for 10 h. TLC (SiO_2: CH_3OH-NH_4OH, 25:1) indicated ~ 10% of [^{14}C]-12 remaining. Additional hydrazine (50 μL) was added and the mixture heated at 65-70°C for 3 h. TLC (same system) indicated ~ 8% of [^{14}C]-12 remaining. Additional hydrazine (100 μL) was added and the mixture heated at 65-70°C for 6 h. TLC (same system) indicated ~ 2% of [^{14}C]-12 remaining. Additional hydrazine (100 μL) was added and the mixture heated at 65-70°C for 10 h. The reaction was worked up by stripping the ethanol. The resulting white solid containing some yellow oil was extracted ~ 15X with Et_2O-hexane (4:1, 150 mL total). The extract was stripped and then dried on high vacuum (0.05 Torr at 40-50°C) for 2 h to remove any remaining hydrazine. The resulting yellow oil weighed 1.060 g and contained 42 mCi of radioactivity. This material was flash chromatographed on a column of SiO_2 (4 x 15 cm) using the solvents shown in the following table.

Volume (mL)	Solvent	Fractions (~ 25-30 mL)
400	EtOAc	1 - 10
200	2.5% CH_3OH-EtOAc	11 - 17
200	5% CH_3OH-EtOAc	18 - 23
200	10% CH_3OH-EtOAc	24 - 30
200	20% CH_3OH-EtOAc	31 - 36
200	40% CH_3OH-EtOAc	37 - 42
220	50% CH_3OH-EtOAc	43 - 48
250	55% CH_3OH-EtOAc	49 - 58
300	60% CH_3OH-EtOAc	59 - 79

Fractions 24-72 were combined, counted (36.8 mCi) and stripped. The residue was dissolved in Et_2O (10 mL) filtered through a cotton plug into a 50 mL centrifuge tube and blown to dryness with a stream of N_2. The residue of brown oil weighed 890 mg. This was dissolved in Et_2O (11 mL) and treated with succinic acid (227 mg, 1.92 mmol) dissolved in CH_3OH (0.8 mL) and EtOH (2 mL). This solution was blown to dryness with a stream of N_2. A seed crystal of unlabeled WR-238605 was added and the resulting mixture was sonicated with Et_2O (15 mL) for ~ 2 min. The resulting suspension was cnetrifuged and the supernatant removed with a pipette. The tan powdery residue was rinsed twice with Et_2O (3 mL) in the same manner. All the Et_2O solutions were combined. The tan powder was dried on high vacuum manifold overnight and the combined Et_2O washes stored in the freezer overnight. A second crop of solid formed in the Et_2O wash. It was collected and rinsed as above. HPLC [Accusphere CN, 5 μ, 3.9 mm x 25 cm, CH_3OH:CH_3CN:0.01 M NH_4CO_2 (adjusted to pH 3 with formic acid), 20:50:30] indicated the two crops had the same purity. Crop 2 was slurried in Et_2O and transferred into the tube containing crop 1. The combined solid was dried on the high vacuum manifold. The mother liquor and Et_2O washes from these two crops were combined, counted and found to contain 22 mCi. This solution was blown down and converted to the free base with saturated Na_2CO_3 solution. The Na_2CO_3 solution was extracted with CH_2Cl_2. The CH_2Cl_2 extract was concentrated with a stream of N_2 and put through a small column of SiO_2 (3 mL) using EtOAc followed by a step gradient of 10, 20, 30, 40 and 60% CH_3OH in EtOAc as eluant. The fractions containing pure [^{14}C]-13 were combined and stripped. The resulting oil (~ 450 mg) was dissolved in Et_2O and filtered through a 0.45 μm Teflon filter into a 50 mL centrifuge tube. This solution was treated with succinic acid (115 mg, 0.97 mmol) dissolved in CH_3OH (0.4 mL) and EtOH (0.6 mL). The resulting

mixture was concentrated in a stream of N_2 to an oily residue. To this residue was added Et_2O (20 mL) and a seed crystal of 14 and the mixture was sonicated for ~ 2 min. The resulting tan solid was collected, washed twice with Et_2O and dried. TLC (same system) indicated the purity of this material and that of the combined first two crops were the same (> 97%). They were slurry combined with Et_2O and then dried on the vacuum manifold. An ultraviolet spectrum of the combined material indicated a chemical purity of ~ 93%. This is possibly due to the presence of excess succinic acid. The tan solid was combined with a previous preparation of [^{14}C]-14 [CT-6639-85-1, 1.9 mCi, 72 mg] and the resulting mixture recrystallized from hot isopropanol. The sample was allowed to cool in the refrigerator overnight. The first corp was collected, rinsed with Et_2O and dried on the vacuum manifold. The mother liquor combined with the Et_2O rinses was allowed to stand overnight. A second crop was collected, rinsed with Et_2O and dried. The two materials had the same melting point. They were combined by dissolving in CH_3OH, filtering through a small cotton plug and concentrated to dryness with a stream of N_2. Et_2O (20 mL) and a seed of 14 were added and this mixture was sonicated for ~ 2 min. The resulting tan solid was collected, rinsed twice with Et_2O and then dried on the vacuum manifold to give 825 mg of light tan powder, mp 145-147°C (ref. sample mp 145-148°C). This material had a UV purity of greater than 99% (λ_{268} EtOH). Radiochemical purity was determined by radio-TLC to be as follows: 99%, SiO_2[CH_3OH-NH_4OH (25:1), R_f 0.41]; 99%, SiO_2[CH_3OH-HOAc (8:1), R_f 0.56]. Radiochemical purity was also determined by HPLC as follows: 98% Accusphere CN, 5 μ, 3.9 mm x 25 cm [CH_3OH:CH_3CN:0.01 M NH_4CO_2 (adjusted to pH 3 with formic acid), 20:50:30]. This material was given Lot no. CT-6949-61.

Chemical	Source & Catalog No.	Lot No.
WR-238605	WRAIR	BN# BJ83119
Ethanol	Aaper	91H 1SV
Hydrazine (98%)	Aldrich 21,515-5	04306TV
Ethyl Ether	BJ107	BA694
Hexane	BJ216	BB673
Ethyl Acetate	BJ100	BB880-
Succinic Acid	Columbia	S-1220
Methanol	BJ230	BC024

Synthesis Report

WALTER REED ARMY INSTITUTE OF RESEARCH

Contract No. DAMD17-89-C-9062

WR-238605

8-[(4-Amino-1-methylbutyl)amino]-2,6-dimethoxy-4-methyl-5-[(3-trifluoromethyl)[6-^{3}H]phenoxy]quinoline Succinate

Lot No. CT-6949-145

March, 1992

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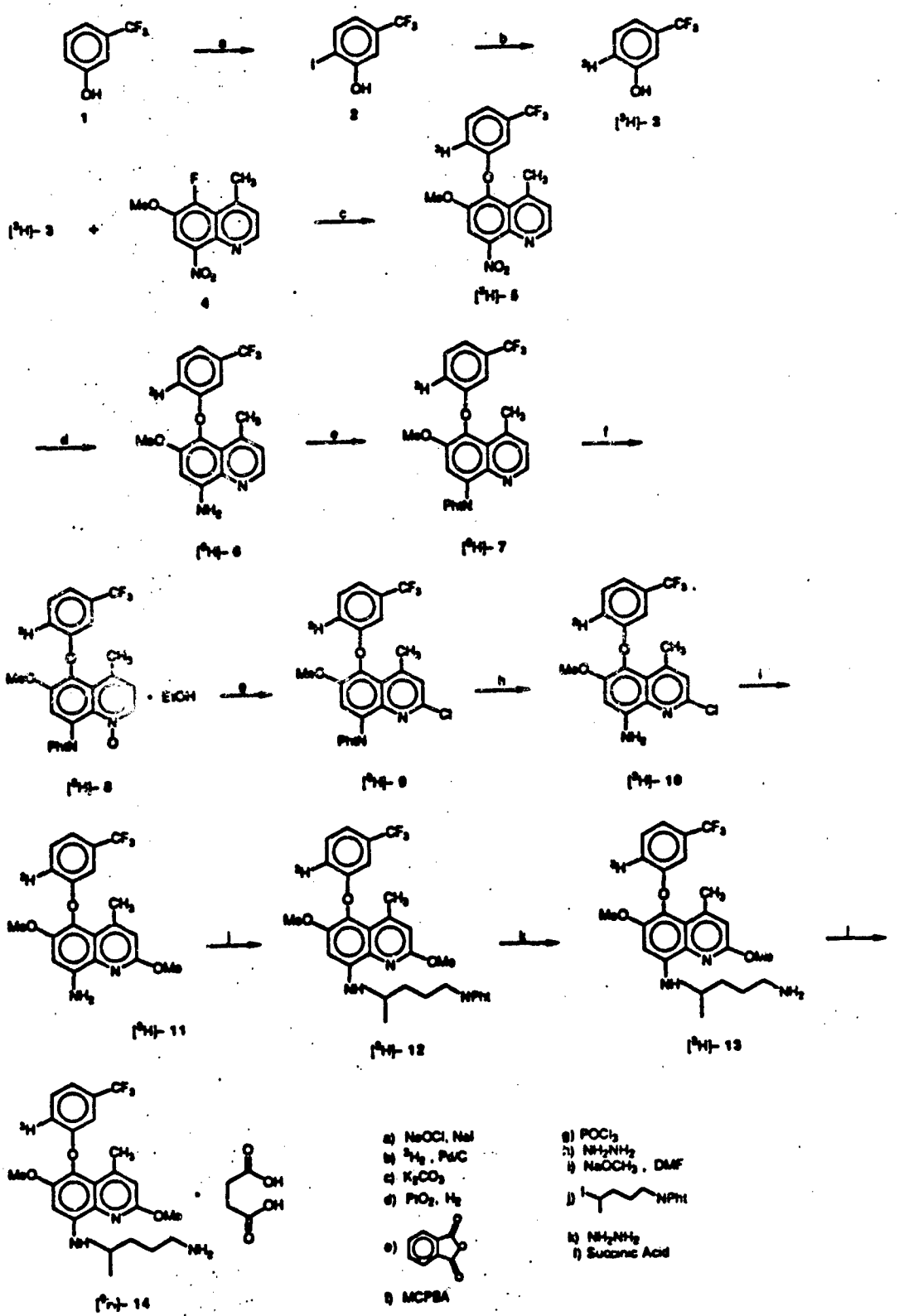
Chart 1


a) NaOCl, NaI
b) $^3H_2$, Pd/C
c) K_2CO_3
d) PtO_2, H_2
e)
f) MCPBA
g) $POCl_3$
h) NH_2NH_2
i) $NaOCH_3$, DMF
j)
k) NH_2NH_2
l) Succinic Acid

2-Iodo-5-trifluoromethylphenol (2)

A solution of 3-trifluorophenol (1) (3.0 g, 18.5 mmol) in CH_3OH (50 mL) was cooled to 0°C. To this was added a solution of potassium iodide (2.79 g, 18.6 mmol) in sodium hydroxide solution (1.85 mL, 10 N). The resulting mixture was warmed to 43°C and a solution of sodium hypochlorite (Clorox, 34.5 g) added dropwise at such a rate as to prevent the build up of a red color, i.e., the reaction mixture remained yellow. The addition was stopped when red color no longer formed when a drop of Clorox was added. The reaction was stirred at 43°C for 1 h after the Clorox addition was stopped. The reaction mixture was cooled to room temperature which caused a precipitate to form. A 10% solution of sodium thiosulfate (20 mL) was added. This caused the precipitate to dissolve to leave a clear, yellow solution. The mixture was stirred for 15 min. before adjusting the solution to pH 7 with 5% aqueous HCl. The mixture was extracted with Et_2O (2 x 75 mL) and the combined Et_2O extracts washed with saturated NaCl solution (2 x 50 mL) and dried ($MgSO_4$). The Et_2O was stripped to leave a yellow oil. GC (DC-200, 90°-210°C) indicated the product was ~ 62% pure. The oil was chromatographed on a column of SiO_2 (2.5 x 19 cm, 230-400 mesh) using 75% $CHCl_3$-hexane as eluant. The fractions containing pure product were combined and stripped to give 1.36 g (26% yield) of pale yellow crystals, mp 44-46°C.

Chemical	Source & Catalog No.	Lot No.
3-Trifluorophenol	Aldrich 15,603-5	01718BY
Sodium Iodide	Aldrich 32,245-8	06523CT
Sodium Hypochlorite (5.25% solution)	Clorox	

Reference

1. Edgar, K. J.; Falling, S. N., J. Org. Chem. 1990, 55, 5287.

3-Trifluoromethyl[6-^{3}H]phenol ([^{3}H]-3)

A mixture of 6-iodo-3-trifluoromethylphenol (15.0 mg) and Pd/C (10%, 6.4 mg) in NaOH solution (600 μL, 1.0 N) was treated with tritium gas for 4 h. The reaction mixture was filtered through Celite (0.5 mL) followed by rinsing with H_2O (3 mL). The resulting solution was not made acidic. The flask containing the solution was attached to the high vacuum manifold and the H_2O vacuum transferred from the solution. Ethanol (~ 3 mL) was vacuum transferred into the flask containing the reaction residue and the resulting solution stirred for 10 min. The EtOH was then vacuum transferred out of the reaction flask and fresh EtOH transferred in. This was done for a total of four EtOH exchanges. The residue remaining after the fourth EtOH exchange was rinsed into a 100 mL volumetric flask containing unlabeled 3-trifluoromethylphenol (243 mg, 1.50 mmol) using EtOH that contained HCl (1 mL, 1.0 N). The resulting solution was counted (530 mCi) and then stripped. The residue was rinsed with acetone (9 mL) into the reaction flask for the next reaction.

Chemical	Source & Catalog No.	Lot No.
6-Iodo-3-trifluoromethylphenol	RTI	LF-6376-1^1
Palladium on Activated Carbon (10%)	Aldrich 20,5699-9	02803 MN
Tritium	NEN	2870-005
Ethanol	Aaper	91H1SU
Acetone	A-18	911267

6-Methoxy-4-methyl-8-nitro-5-(3-trifluoromethyl[6-^{3}H]phenoxy)quinoline ([^{3}H]-5)

To the acetone solution of [^{3}H]-3 prepared above was added 5-fluoro-6-methoxy-4-methyl-8-nitroquinoline (356 mg, 1.50 mmol) and K_2CO_3 (1 g), and the resulting mixture was heated at 65°C for 6 h. TLC (SiO_2: 25% EtOAc-hexane)

indicated no [^{3}H]-3 remaining. The acetone was stripped, and the residue was dissolved in CH_2Cl_2 and washed with H_2O. The H_2O layer was then extracted 3X with CH_2Cl_2. The combined CH_2Cl_2 extracts were washed twice with 10% K_2CO_3 solution, dried over Na_2SO_4, counted (505 mCi) and stripped. The residue was dried on the vacuum manifold over the weekend to give 470 mg of yellow crystals. These were used as is in the next reaction.

Chemical	Source & Catalog No.	Lot No.
Acetone	Fisher A18	911267
Potassium Carbonate	Fisher P-208	905633
m-Hydroxybenzotrifluoride	Aldrich 15,603-5	01718 BV
Methylene Chloride	Fisher D-37	913251
Sodium Sulfate	Fisher S-421	884476

8-Amino-6-methoxy-4-methyl-5-(3-trifluoromethyl[6-^{3}H]phenoxy)quinoline ([^{3}H]-6)

In a 25-mL glass-lined stainless steel bomb was placed PtO_2 (60 mg) and THF (2 mL) which had been distilled from benzophenone ketyl. The PtO_2 was reduced with H_2 (50 psi) for 1 h. To the resulting slurry was added the solid [^{3}H]-5 prepared above and THF (15 mL) to wash in any residue of [^{3}H]-5. This mixture was treated with H_2 (50 psi) for 16 h. TLC (SiO_2: 80% Et_2O-hexane) indicated two materials present but no [^{3}H]-5 present. The mixture was treated with H_2 (50 psi) for 4 h. TLC (same system) indicated product with small amount (~ 3%) of unknown material present. The mixture was stirred with H_2 (50 psi) for 3 h. TLC (same system) indicated no change from previous sample. The reaction mixture was filtered through SiO_2 (5 mL) using additional THF. The THF solution was stripped and the residue immediately used in the next reaction.

Chemical	Source & Catalog No.	Lot No.
Platinum(IV) Oxide	Aldrich 20,603-2	02506KT
Tetrahydrofuran	Fisher T-397	911249
Hydrogen, prep.	Matheson	J10-0448-B2
Silica Gel-60	E. Merck 7734	9622428

6-Methoxy-4-methyl-8-phthalimido-5-(3-trifluoromethyl[6-^{3}H]phenoxy)quinoline ([^{3}H]-7)

The residue of [^{3}H]-6 from the previous reaction was dissolved in xylene (70 mL) and phthalic anhydride (268 mg) added. This solution was heated at reflux for 6 h while collecting the H_2O formed with a Dean-Stark trap. TLC (SiO_2:80% Et_2O-hexane) indicated the reaction was complete. The xylene was stripped using a vacuum pump and the residue dissolved in CH_2Cl_2 and washed thrice with 10% KOH and once with H_2O. The CH_2Cl_2 was stripped and the resulting residue flash chromatographed on a column at SiO_2 (2.5 x 10 cm) using EtOAc as eluant. The fractions containing pure product were combined, counted (402 mCi) and stripped to leave 708 mg of tan solid. This was used as is in the next reaction.

Chemical	Source & Catalog No.	Lot No.
Phthalic Anhydride	Mallinckrodt 2828	MTD
Xylene	Fisher X-5	911304
Methylene Chloride	Fisher D-143	915700
Potassium Hydroxide	Mallinckrodt 6984	KJTY
Sodium Sulfate	Fisher S-420	907057

6-Methoxy-4-methyl-8-phthalimido-5-(3-trifluoromethyl[6-^{3}H]phenoxy)-quinoline-1-oxide ([^{3}H]-8)

To a solution of [^{3}H]-7 (708 mg) in $CHCl_3$ (15 mL) at 0°C was added m-chloroperoxybenzoic acid (MCPBA) (361 mg, 20% excess) in $CHCl_3$ (7 mL + 2 x

0.5 mL rinses). The resulting solution was allowed to warm to room temperature and stirred for 16 h. TLC (SiO_2: CH_2Cl_2-CH_3OH, 25:0.75) indicated the reaction was ~ 46% complete. Additional MCPBA (330 mg) was added and the resulting solution stirred at 40-50°C for 6 h. TLC (same system) indicated the reaction was ~ 60% complete. Additional MCPBA (220 mg) was added and the resulting mixture stirred at room temperature for 16 h. TLC (same system) indicated ~ 67% product to be present. Additional MCPBA (100 mg) was added and the mixture stirred at 50°C for 2 h. TLC (same system) indicated ~ 71% product and ~ 9% [^{3}H]-7 present. Additional MCPBA (300 mg) was added and the mixture stirred at room temperature for 16 h. TLC (same system) indicated no change from the previous sample. The reaction solution was washed twice with 10% KOH solution and once with H_2O (see note below).* It was then counted (325 mCi) and stripped. The residue was dissolved in EtOH (20 mL) and this solution was stripped to leave 643 mg of yellow crystals. This material was used as is in the next reaction.

Chemical	Source & Catalog No.	Lot No.
m-Chloroperoxybenzoic Acid	Aldrich C6,270-0	6720KK
Chloroform	Fisher C-606	911746
Ethanol	Aaper	91H1SU

2-Chloro-6-methoxy-4-methyl-8-phthalimido-5-(3-trifluoromethyl[6-^{3}H]-phenoxy)quinoline ([^{3}H]-9)

To a solution of [^{3}H]-9 (643 mg, 1.19 mmol, 325 mCi) dissolved in $CHCl_3$ (10 mL) was added $POCl_3$ (1.82 g, 11.9 mmol, 1.12 mL) over 30 min. The resulting solution was heated at 65°C for 1.5 h. TLC (SiO_2:CH_2Cl_2-CH_3OH,

*Note: Do not attempt to purify this material by chromatography on either Al_2O_3 or SiO_2. This leads to decomposition which is a function of how long the material stays on column.

25:0.8) indicated the reaction was complete. The reaction mixture was poured over ice and the pH adjusted to 12 with 20% NaOH. The resulting solution was extracted 4X with Et_2O. The combined Et_2O extracts were washed with H_2O, saturated $NaHCO_3$ solution, saturated NaCl solution and dried (Na_2SO_4). The dried solution was stripped to give 678 mg of solid which was used as is in next reaction.

Chemical	Source & Catalog No.	Lot No.
Chloroform	C-606	911746
Phosphorus Oxychloride	Aldrich 32,045-5	00830CX
Sodium Hydroxide	Fisher SS-254	874936
Ethyl Ether	Fisher E-138	921442-15
Sodium Bicarbonate	Fisher S-233	720392
Ethanol	Aaper	91H1SU

8-Amino-2-chloro-6-methoxy-4-methyl-5-(3-trifluoromethyl[6-^{3}H]phenoxy)-quinoline ([^{3}H]-10)

To a suspension of [^{3}H]-9 (678 mg) in EtOH (25 mL) was added over 20 min NH_2NH_2 (98%, 1.8 mL). The resulting mixture was heated at 65°C for 1.5 h. TLC (SiO_2:80% CH_2Cl_2-hexane) indicated the reaction was complete. The reaction was allowed to stir overnight at room temperature. The EtOH was stripped from the mixture and the residue was extracted ~ 20X with CH_2Cl_2. The CH_2Cl_2 extracts were filtered through a short column containing SiO_2 (1 mL) and sand (1 mL) into a 100 mL volumetric flask. This extract was counted (348 mCi) and then stripped. The residue of amine [^{3}H]-10 was immediately taken to the next reaction.

Chemical	Source & Catalog No.	Lot No.
Ethanol	Aaper	91H1SV
Hydrazine (98%)	Aldrich 21,515-5	04306TY
Methylene Chloride	Fisher D-143	915700
Silica Gel-60	E. Merck 9385-5	30283

8-Amino-2,6-dimethoxy-4-methyl-5-(3-trifluoromethyl[6-^{3}H]phenoxy)quinoline ([^{3}H]-11)

A suspension of sodium methoxide was prepared as follows: Sodium hydride (80% oil dispersion, 68 mg, 2.27 mmol) was weighed into a 50-mL flask fitted with an adapter to allow working under an inert gas (argon). The NaH was washed twice with hexane and then blown dry. Dimethylformamide (2 mL) was added followed by CH_3OH (40 mg, 1.25 mmol, 51 μL). The resulting mixture was stirred for 1 h and then the [^{3}H]-10 prepared above was added in DMF solution (1 mL plus 3 x 0.5 mL rinses). The resulting dark reaction mixture was stirred overnight at room temperature. TLC (SiO_2: 80% CH_2Cl_2-hexane) indicated an ~ 1:1 mixture of product and starting material [^{3}H]-10. The mixture was warmed at 40-45°C for 2 h. TLC (same system) now indicated ~ 60% product and 9% [^{3}H]-10. Additional $NaOCH_3$ was prepared from NaH (44 mg) and CH_3OH (41 μL) and this was transferred as a slurry in DMF to the reaction mixture. The resulting mixture was heated at 40-45°C for 2 h. TLC (same system) indicated no change from the previous sample. The reaction mixture was poured over ice and was extracted 5X with CH_2Cl_2. The combined CH_2Cl_2 extracts were washed once with H_2O, dried over Na_2SO_4 and stripped, with the last traces of DMF being removed by a vacuum pump connected to the rotary evaporator. The H_2O layer from the above extraction containing some emulsion was filtered through two stacked glass fiber filter mats topped with ~ 50 mm of Celite. The filtrate was extracted 4X with CH_2Cl_2. The combined CH_2Cl_2 extracts were washed twice with H_2O, dried (Na_2SO_4) and stripped. The residues from the two sets of CH_2Cl_2 extractions were combined and chromatographed on SiO_2 (25 g) using 80% CH_2Cl_2-hexane (75 mL), CH_2Cl_2 (25 mL), $CHCl_3$ (pentane stabilized, 25 mL), $CHCl_3$ (EtOH stabilized, 25 mL) and CH_2Cl_2-CH_3OH, 1:1 (25 mL) while collecting 25 mL fractions. Pure product was found only in fraction 2, which

was counted (168.5 mCi) and then stripped to obtain 223 mg of dirty yellow solid. This was used as is in the next reaction.

Chemical	Source & Catalog No.	Lot No.
Sodium Hydride	Aldrich 25,399-5	00804 MW
Methanol	Aldrich 32,241-5	02211 BY
Dimethylformamide	Aldrich 22,705-6	0341 TX
Silica Gel-60	E. Merck 9385-5	30293
Methylene Chloride	Fisher D-143	915700
Chloroform	Fisher C-607	911791
Chloroform	BJ 048	BB216
Hexane	BJ 216	BB673
Ethyl Acetate	BJ 100	BB880
Methanol	BJ 230	BC024

2,6-Dimethoxy-4-methyl-8-(4-phthalimido-1-methylbutylamino)-5-(3-trifluoromethyl[6-^{3}H]phenoxy)quinoline ([^{3}H]-12)

A mixture of [^{3}H]-11 (233 mg, 0.59 mmol, 169 mCi), diisopropylamine (61 mg, 0.60 mmol, 84 μL) and 4-iodo-1-phthalimidopentane (205 mg, 0.60 mmol) in CH_3CN (2 mL) was heated at 80-85°C for 12 h. TLC (SiO_2: 80% Et_2O-hexane) indicated ~ 57% product and ~ 30% [^{3}H]-11 present in the reaction mixture. Additional alkylating agent (103 mg), diisopropylamine (42 μL) and CH_3CN (0.5 mL) were added and the mixture heated at 80-85°C for 5 h. TLC (same system) indicated ~ 72% product and ~ 17% [^{3}H]-11 present in the reaction mixture. Additional alkylating agent (103 mg), amine (42 μL) and CH_3CN (0.5 mL) were added and the mixture heated at 80-85°C for 10 h. TLC (same system) indicated ~ 84% product and ~ 8% [^{3}H]-11 present. Additional alkylating agent (103 mg) and amine (42 μL) were added and the mixture heated

at 80-85°C for 10 h. TLC (same system) indicated no change from previous sample. Additional alkylating agent (206 mg), amine (84 μL) and CH_3CN (0.5 mL) were added and the mixture heated at 80-85°C for 10 h. TLC (same system) indicated no change from previous sample. The reaction mixture was poured into H_2O and extracted thrice with CH_2Cl_2. The combined CH_2Cl_2 extracts were dried (Na_2SO_4) and stripped. The residue was chromatographed on SiO_2 (15 mL) using 80% CH_2Cl_2-hexane as eluant. The fractions containing radiochemically pure product were combined and stripped to give 750 mg of yellow oil. TLC (same system) indicated this material was radiochemically pure but contained some unlabeled impurities (stained by I_2). This material was rechromatographed on a column of SiO_2 (20 x 2.5 cm) using 75% CH_2Cl_2-hexane and changing slowly to 100% CH_2Cl_2 and finally to 50% EtOAc-CH_2Cl_2 as eluants. The fractions containing pure product were combined (149 mCi) and stripped to obtain a residue of 263 mg of yellow oil (specific activity of ~ 338 mCi/mmol).

Chemical	Source & Catalog No.	Lot No.
Diiscpropylamine	Aldrich 11,0019	01021 BP
4-Iodo-1-phthalimidopentane	Ash Stevens	AP-VIII-200
Acetonitrile	BJ015	BA-181
Methylene Chloride	D-143	915700
Silica Gel-60	E. Merck 9385-5	30283
Hexane	BJ216	BB673
Ethyl Acetate	BJ100	BB880

8-[(4-Amino-1-methylbutyl)amino]-2,6-dimethoxy-4-methyl-5-[3-trifluoromethyl[6-^{3}H]phenoxy]quinoline Succinate ([^{3}H]-14)

The [^{3}H]-12 (263 mg, 0.44 mmol, 149 mCi) prepared above was mixed with unlabeled free base (13) prepared from a standard sample of WR-238605 of

(256 mg, 0.44 mmol). The resulting mixture in EtOH (35 mL) was treated with NH_2NH_2 (98%, 29 mg, 0.88 mmol, 100% excess, 28 μL). This solution was heated at 65-70°C for 2.5 h. TLC (SiO_2: CH_3OH-NH_4OH, 25:1) indicated only 8% desired product to be present. Additional NH_2NH_2 (170 μL) was added and the mixture was heated at 65-70°C for 2.5 h and then stirred overnight at room temperature. TLC (same system) indicated ~ 5% of [^{3}H]-12 still present. Additional NH_2NH_2 (50 μL) was added and the mixture heated at 65-70°C for 3 h. TLC (same system) indicated ~ 2% [^{3}H]-12 still present. Additional NH_2NH_2 (50 μL) was added and mixture stirred for 16 h at room temperature. TLC (same system) indicated no change from the previous sample. The reaction mixture was stripped to remove the EtOH. The residue was extracted ~ 12X with 80% Et_2O-hexane (~ 60 mL total). These extracts were filtered into a flask through a cotton plug covered with 1.25 cm of sand. The filtrate was stripped and the residue dissolved in EtOAc and washed thrice with H_2O. The solution was dried (Na_2SO_4) and counted (146 mCi). To this EtOAc solution of [^{3}H]-13 was added a solution of succinic acid (104 mg, 0.88 mmol) dissolved in CH_3OH (3 mL). The resulting solution was stripped to ~ 3-4 mL. The concentrated solution was filtered through a 0.45 μm Teflon filter into a 50-mL centrifuge tube and then evaporated to dryness with a stream of nitrogen. The residue was dried on the vacuum manifold for 1 h. To the resulting dark oil was added Et_2O (5 mL) and a small seed crystal of unlabeled WR-238605. This mixture was sonicated for ~ 2 min. The mixture was centrifuged and the supernatant separated from the tan solid with a pipet. The pellet was washed 3 x 4 mL of Et_2O. The solid was dried on the vacuum manifold for 16 h to give 435 mg of tan crystals, mp 146-148°C (reference sample mp 145-148°C). This material had a UV purity of 100% (λ_{268} EtOH). Radiochemical purity was determined by radio-TLC to be as follows: 97%, SiO_2[CH_3OH-NH_4OH (25:1), R_f 0.32]; 98% SiO_2[CH_3OH-HOAc (8:1),

R_f 0.56]. Radiochemical purity was also determined by HPLC as follows: 98%, Accusphere CN, 5 μ, 3.9 mm x 25 cm [CH_3OH:CH_3CN:0.01 M NH_4CO_2 (adjusted to pH 3 with formic acid), 20:50:30]. [^3H]NMR (500 mHz) indicated the presence of only a trace amount of the [5-^3H]isomer. This material was given lot no. CT-6949-145.

Chemical	Source & Catalog No.	Lot No.
WR-238605	WRAIR	BM# BJ83119
Ethanol	Aaper	91H1SU
Hydrazine (98%)	Aldrich 21,515-5	04306TV
Ethyl Ether	BJ107	BA694
Hexane	BJ216	BB673
Ethyl Acetate	BJ100	BB880
Succinic Acid	Columbia	S-1220
Methanol	BJ230	BC024

Synthesis Report

WALTER REED ARMY INSTITUTE OF RESEARCH

Contract No. DAMD17-89-C-9062

8-[(4-Amino-1-methylbutyl)amino]-5-(1-hexoxy)-6-methoxy-4-methyl[4-^{14}C]quinoline (DL)-Tartrate
[quinoline-4-^{14}C]WR-242511

Lot No. LF6797-73

July, 1991

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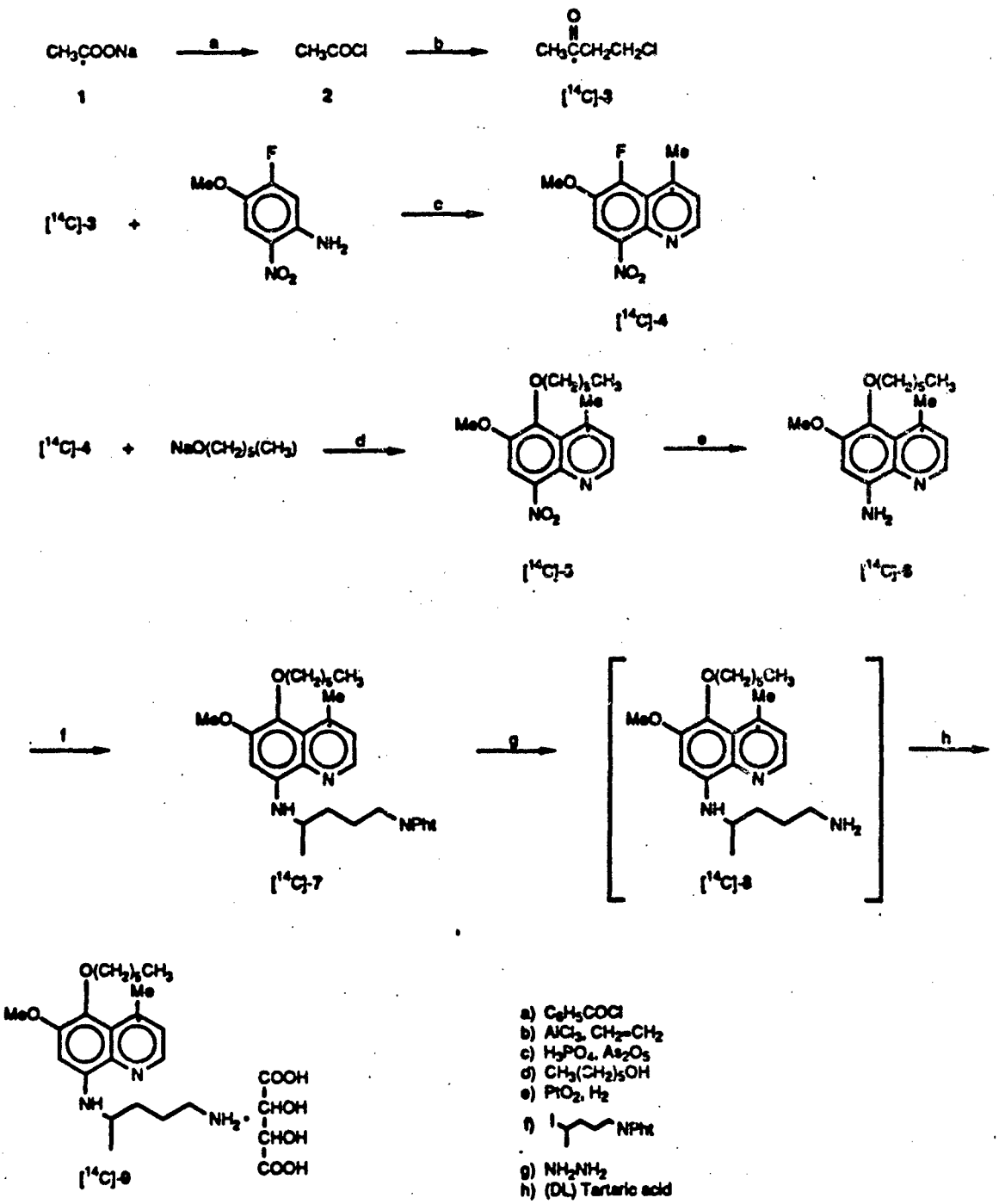
Synthetic Scheme



5-Hexoxy-6-methoxy-4-methyl-8-nitro[4-^{14}C]quinoline, ([^{14}C]-5)

To a solution of 5-fluoro-6-methoxy-4-methyl-8-nitro-[4-^{14}C]quinoline (288 mg, 0.97 mmol, 45.6 mCi, 47.6 mCi/mmol) in hexanol (9 mL) was added sodium hexoxide (51 mg Na in 5 mL of hexanol; 2.48 mL, 44 mmol/mL, 1.1 mmol). The mixture (under N_2) was immediately placed in an 80°C oil bath. After 45 min, HPLC analysis (Waters μBondapak C18, 10 μ, 90% CH_3CN-H_2O, 1 mL/min, 254 nm) indicated that the reaction was complete. The mixture was allowed to cool to room temperature. The solvent was removed on the rotary evaporator (0.5 Torr, 33°C) and the residue was dried under high vacuum (15 h). The residue was dissolved in EtOAc, filtered through Celite and concentrated to a deep red oil. The oil was purified by column chromatography (SiO_2, 5 g, 230-400 mesh, 15 mm ID) with hexane as the initial eluent (200 mL; to wash off excess hexanol) then 4% EtOAc-hexane. The fractions containing product were combined, and the solvent was removed on the rotary evaporator to give 283 mg of yellow crystals (37.5 mCi, 82% yield). This material was used in the next reaction without further purification.

Chemicals and Sources

Hexanol	Aldrich	02906 TV
Sodium	Aldrich	00228 KW
Ethyl Acetate	Burdick & Jackson	AV802
Methylene Chloride	Burdick & Jackson	AZ323
Silica Gel	E. Merck	30269

8-Amino-5-hexoxy-6-methoxy-4-methyl[4-^{14}C]quinoline, ([^{14}C]-6)

Platinum oxide (90 mg) and THF (2 mL; distilled from benzophenone ketyl) were placed in a 25 mL stainless steel bomb (glass liner) and the PtO_2 was

prereduced with hydrogen @ 45 psi for 30 min. A solution of 5-hexoxy-6-methoxy-4-methyl-8-nitro[4-^{14}C]quinoline (283 mg, 0.89 mmol, 37.53 mCi) and 5-hexoxy-6-methoxy-4-methyl-8-nitroquinoline (280 mg, 0.88 mmol) in THF (9 mL) was added to the Pt suspension and the mixture was allowed to stir for 16 h under a hydrogen atmosphere (45 psi). TLC analysis (SiO_2; CH_2Cl_2-CH_3OH, 50:1) showed the reduction to be complete. The mixture was filtered through Celite, rinsed with THF, and was concentrated to a brown oil (567 mg, 36.8 mCi, 98% yield). The product was used in the next reaction without further purification.

Chemicals and Sources

Platinum Oxide	Aldrich	02506 KT
THF	Burdick & Jackson	AW970
Hydrogen	Matheson	J10-0175-A4
Methylene Chloride	Burdick & Jackson	AZ323
Methanol	Fisher	911129
Celite	Fisher	904725

5-Hexoxy-6-methoxy-4-methyl-8-[(1-methyl-4-phthalimidobutyl)amino][4-^{14}C]-quinoline, ([^{14}C]-7)

A mixture of 5-hexoxy-6-methoxy-4-methyl-8-amino[4-^{14}C]quinoline (567 mg, 36.8 mCi), 4-iodo-1-phthalimidopentane (755 mg, 2.17 mmol), and N,N-diisopropylethylamine (376 µL, 2.16 mmol) in CH_3CN (6 mL) was allowed to reflux under argon for 10 h. Additional 4-iodo-1-phthalimidopentane (399 mg) and N,N-diisopropylethylamine (200 uL) were added, and the mixture was allowed to reflux overnight. Radio-TLC analysis (SiO_2, 80% $CHCl_3$-EtOAc) of the reaction mixture showed 10% starting material still present. A second addition of 4-iodo-1-

phthalimidopentane (399 mg) and N,N-diisopropylethylamine (200 μL) was made, and refluxing was continued for 9 h. TLC analysis (same system) of the reaction mixture indicated 2% starting material and 94% product present. After cooling to room temperature, the reaction mixture was diluted with CH_2Cl_2 and was washed with water (20 mL). The layers were separated and the H_2O phase was extracted with CH_2Cl_2 (3 x 20 mL). The CH_2Cl_2 layers were combined and washed with H_2O (3 x 30 mL), dried with $MgSO_4$, filtered and concentrated to an oil (water aspirator, 99°C). The oil was dried on the vacuum pump (1 Torr, 25°C, 16 h). The dried oil was purified via column chromatography (oven dried SiO_2, 10 gm, 230-400 mesh, 15 mm ID) using a gradient mobile phase [20% hexane-CH_2Cl_2 (200 mL), 10% hexane-CH_2Cl_2 (150 mL), CH_2Cl_2 (200 mL), and 0.5% CH_3OH-CH_2Cl_2 until elution was complete]. The fractions were analyzed by TLC (SiO_2, 1% CH_3OH-CH_2Cl_2) and those containing pure product were combined to give 771 mg (26.7 mCi, 73% yield) of [^{14}C]-7.

Chemicals and Sources

4-Iodo-1-phthalimidopentane	Ash Stevens	BM07623
N,N-Diisopropylethylamine	Aldrich	04718 JW
Acetonitrile	Burdick & Jackson	AZ 387
Methylene Chloride	Burdick & Jackson	AZ 323
Magnesium Sulfate	J. T. Baker	337111
Hexane	Burdick & Jackson	AZ 716
Methanol	Fisher	911129
Silica Gel	E. Merck	30269

8-[(4-Amino-1-methylbutyl)amino]-5-(1-hexoxy)-6-methoxy-4-methyl[4-^{14}C]-quinoline (DL)-Tartrate; [4-^{14}C]WR-242511 ([^{14}C]-8)

5-Hexoxy-6-methoxy-4-methyl-8-[(1-methyl-4-phthalimidobutyl)amino)]-[4-^{14}C]quinoline (771 mg, 26.89 mCi) was dissolved in absolute ethanol (16 mL), and hydrazine (343 mg, 10.71 mmol) was added. The mixture was refluxed for 2 h under argon. The ppt that formed was removed by filtration and rinsed with ethanol. The filtrate was diluted with deionized water (50 mL) and concentrated (1 Torr, 25°C). The residue was dissolved in hexane (5 mL) and concentrated (water aspirator, 25°C). The residue was dissolved in CH_2Cl_2 (20 mL) and the solution was washed with 25% aq. NaOH (2 x 15 mL) and H_2O (2 x 10 mL). The aqueous layers were backwashed with CH_2Cl_2 (2 x 15 mL). The organic layers were combined and dried over potassium carbonate (1 h), treated with charcoal, filtered through Celite, and concentrated. The residual oil was dissolved in hexane (5 mL) and concentrated. The residue was dried on the vacuum pump (1 Torr, 45°C, 4 h; then 1 Torr, 25°C, 20 h) to yield 502 mg (88% crude chemical yield) of [^{14}C]-8 as an amber oil.

A hot solution of 241 mg (1.6 mmol) of (DL)-tartaric acid in absolute ethanol (20 mL) was added to a hot solution of [^{14}C]-8 in absolute ethanol (10 mL). The mixture was allowed to stir and cool to room temperature over a 3 h period, then in an ice bath for 1 h. The yellow solid was removed by filtration and rinsed with cold ethanol, diethyl ether, and petroleum ether and allowed to air dry overnight. The solid was dissolved in ethanol and treated with charcoal. The mixture was filtered through Celite with rinsing with hot ethanol. The filtrate was allowed to cool to room temperature with stirring, and placed in the freezer for 15 h. The yellow solid was collected by filtration, rinsed with cold ethanol, diethyl ether, and petroleum ether, and dried on a vacuum manifold for 16 h to give 575 mg (22.95 mCi, 85% yield

of [^{14}C]-9. The specific activity was determined to be 20.9 mCi/mmol. The chemical purity was 99.9% and the radiochemical purity was 99.5% by HPLC [Spherisorb CN, 5 μ, 4.6 x 300 mm, CH_3OH-CH_3CN-0.01 M NH_4HCO_2 (adj. to pH 3 with formic acid) 20:50:30, UV-254 and β-RAM, 1.0 mL/min]. This compound was entered into inventory as lot no. LF6797-73.

Chemicals and Sources

Absolute Ethanol	Aaper	6/18/90
Hydrazine	Aldrich	01427 KX
Hexane	Burdick & Jackson	AZ 716
Methylene Chloride	Burdick & Jackson	AZ 323
Sodium Hydroxide	E. Merck	8251
Potassium Carbonate	Fisher	893233
Charcoal	Norit	A-5711
Celite	Fisher	904725
(DL)-Tartaric Acid	Aldrich	03322 BW
Diethyl Ether	Fisher	905785-15
Petroleum Ether	Fisher	902863

Synthesis Report

WALTER REED ARMY INSTITUTE OF RESEARCH

Contract No. DAMD17-89-C-9062

[16-^{14}C]Artelinic Acid, [16-^{14}C]WR-255663

Lot No. LF-7044-94

March, 1992

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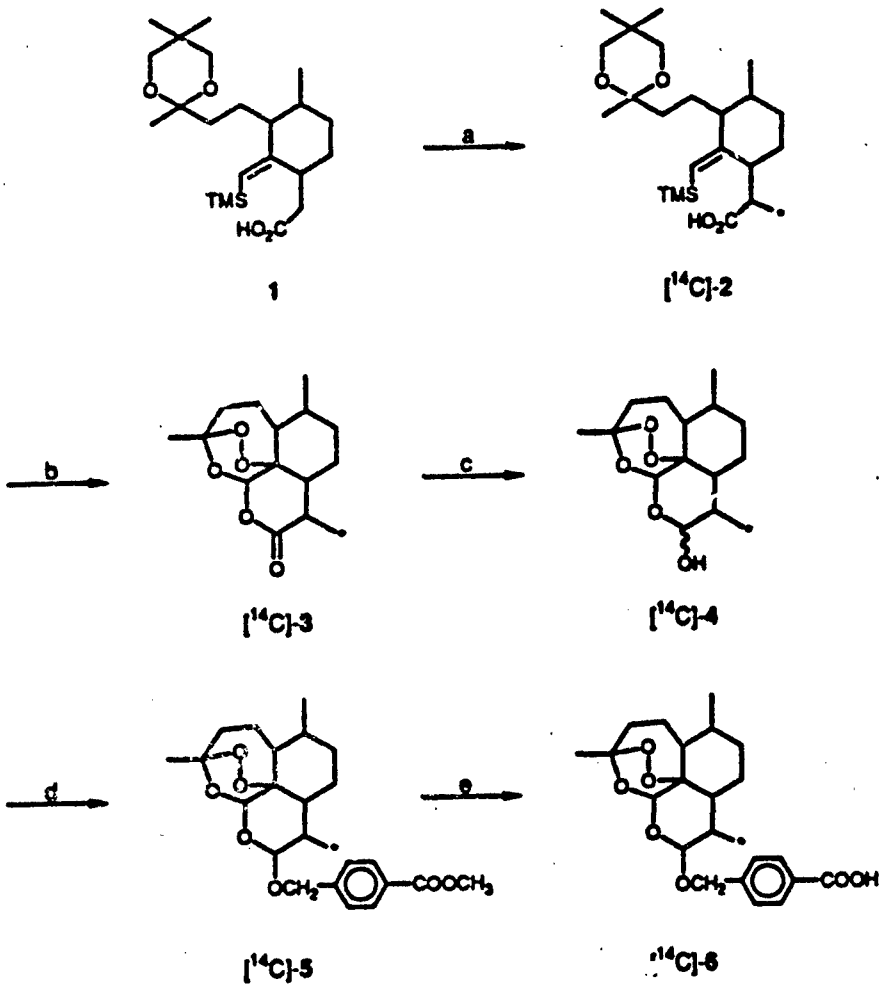

1 [^{14}C]-2

[^{14}C]-3 [^{14}C]-4

[^{14}C]-5 [^{14}C]-6

a) LDA, $\dot{C}H_3I$
b) O_2/O_3
c) $NaBH_4$
d) $HOCH_2$—⟨⟩—$COOCH_3$
e) 5% KOH/CH_3OH

2,5,5-Trimethyl-2-(2'-(4"-(1'''-carboxy[2'''-^{14}C]ethyl)-1" R-methyl-3"-trimethylsilylmethylenecyclohex-2"-yl)ethyl)-1,3-dioxane ([^{14}C]-

Note: All glassware and glass luer syringes with needles were oven dried and cooled over Drierite. Syringes were filled with N_2 before use. The starting material, (1), was predried before use by dissolving in hexane and stripping to dryness 3X, then put on the rotary evaporator under vacuum (bath 28°C) overnight.

A solution of 1 (300 mg, 0.76 mmol) in 1.5 mL of hexane was placed in an L-shaped powder addition tube (LSAT). The tube was fitted to a 3-neck flask fitted with two stopcock adapters and a magnetic stir bar. The tube is fitted so that the "L" points downward and the solution will not spill into the flask. The flask was attached to the vacuum manifold via the stopcock adapter (CSA) fitted to the central neck of the flask. The system was kept under an argon atmosphere during assembly. THF (4 mL, freshly distilled from sodium benzophenone ketyl) was added via syringe through the side arm stopcock adapter (SSA). The THF was cooled to 0°C and diisopropylamine (226 μL, 1.6 mmol, 2.2 eq.; distilled from CaH) and n-BuLi (1 mL, 1.6 M, 2.2 eq) were added via syringe through SSA. After stirring the resulting mixture for 15 min at 0°C under argon, the solution of 1 was slowly added by turning the LSAT upward. The LSAT was rinsed by cooling it with dry ice in its downward position and then transferring the condensed solvents back to the reaction flask by turning it upward. The LSAT was rinsed twice. The LSAT and SSA were replaced with stoppers. The argon flow was stopped, and the reaction flask was evacuated after being cooled with liquid nitrogen. [^{14}C]Methyl iodide (100 mCi, 55 mCi/mmol, 1.82 mmol, 2.5 eq.) was vacuum transferred to a flask containing P_2O_5 and after 5 min, was transferred to the reaction flask. The CSA was closed and the mixture allowed to stir at ambient temperature for 1 h

under the vapor pressure of THF. The volatiles were removed by vacuum transfer into a flask containing N-methylpiperidine (5 eq) to trap unreacted [^{14}C]methyl iodide for disposal. The yellow gummy residue was dissolved in 10 mL of $CHCl_3$. The solution was placed in a separatory funnel containing 10 mL of cold saturated aqueous NH_4Cl solution. The NH_4Cl solution was extracted with $CHCl_3$ (3 x 10-15 mL). The organic layers were combined and washed with saturated NaCl solution (10 mL), dried over $NaSO_4$, and filtered. The filtrate was stripped to dryness and placed under vacuum to dry. The dried residue was dissolved in 80% hexane-1% HOAc in EtOAc and flushed through a pad of silica gel (230-400 mesh) via aspiration. The pad was rinsed with 60% hexane-1% HOAc in EtOAc. Evaporation of the filtrate gave 317 mg (100% crude chemical yield) of [^{14}C]-2 which had 34.1 mCi (85% radiochemical yield) of radioactivity. This product was used in the next reaction without further purification.

Material	Lot No.	Source
1	JN13-86-1	Stark Associates
Diisopropylamine	01021 BP	Aldrich 11001-9
n-Butyl lithium	13308 JY	Aldrich 18617-1
[^{14}C]Methyl iodide	CLS-90-306-89-09	Chemsyn Science C1024
Tetrahydrofuran	911249	Fisher T397-4
Hexane	BB676	Burdick & Jackson 216-AL
N-Methylpiperdine	03116 DT	Aldrich M7260-9
Phosphorous pentoxide	02001 PX	Aldrich 21470-1
Silica gel	30283	E. Merck 9385-5
Acetic acid	FL-04-039	Fisher A386-212
Ethyl acetate	BB880	Burdick & Jackson 100-4L
Sodium sulfate	C04P8294	J. T. Baker JT3898-1
Acetyl chloride	00225 MV	Aldrich 23957-7
Methanol	BC024	Burdick & Jackson 230-4L

[16-^{14}C]Artemisinin ([^{14}C]-3)[1]

Note: Hood lights are out.

To a 250 mL two-neck flask was added [^{14}C]-2 (317 mg, 0.77 mmol, 34.1 mCi) in CH_2Cl_2 (85 mL). The second neck was fitted with a filtering flask on line to a KI trap to trap excess ozone. A slow stream of argon was introduced through a gas dispersion tube as the flask was cooled to -78°C (dry ice-isopropanol). Oxygen was passed through the flask, then ozone was allowed to bubble through the run mixture (3 min 20 sec, 70 volts, 7 psi, 0.4 L/min). The excess ozone was purged from the system with argon. While still at -78°C, the reaction mixture was treated with BHT (32 mg in 1 mL of CH_2Cl_2), SiO_2 (8.4 g, 230-400 mesh), and 3 M H_2SO_4 (2.9 mL). The resulting mixture was allowed to warm to room temperature. The flask was wrapped with foil and stirred for 16 h under argon at room temperature. The mixture was treated with $NaHCO_3$ (2.5 g) and stirred for 1 h. The mixture was filtered and rinsed with 90% CH_2Cl_2-EtOAc (100 mL) and evaporated to dryness. The crude material (251 mg, 24.98 mCi) was purified by flash chromatography (20 mm ID, Baker flash SiO_2 40 μm, 200 mL 95% hexane-EtOAc, ~ 800 mL 90% hexane-EtOAc). The fractions were analyzed by TLC (SiO_2, 60% hexane-EtOAc) and normal phase HPLC (Waters Resolve spherical silica, 5 μ, 3.9 x 150 mm, 90% hexane-EtOAc, 1.2 mL/min, 210 nm). The yield of [^{14}C]-3 was 28 mg (13% chemical yield), 4.85 mCi (14% radiochemical yield) with a purity of 99%. This material was used in the next reaction.

Material	Lot No.	Source
Butylated hydroxytoluene (BHT)	39F-0197	Sigma B-1378
Silica gel	30283	E. Merck 9385-5
Sulfuric acid	FL-04-0390	Fisher A300C-212
Sodium bicarbonate	30018023	EM Science SX0320-1

Methylene chloride	B8668	Burdick & Jackson 300-4L
Hexane	B8676	Burdick & Jackson 216-4L
Ethyl acetate	B8880	Burdick & Jackson 100-4L
Baker flash SiO_2	E37342	J. T. Baker 7024-01

[16-^{14}C]Dihydroartemisinin ([^{14}C]-4)

A solution of [^{14}C]-3 (27 mg, 4.6 mCi) in 3 mL of CH_3OH was placed in a 10-mL flask and cooled to 0°C in an ice bath. Sodium borohydride (62 mg, 1.68 mmol, 9 eq) was added to the solution and the mixture was allowed to stir for 2 h at 0°C under N_2. At 2 h, TLC (SiO_2, 60% hexane-EtOAc) analysis indicated the reaction was complete. The reaction mixture was quenched with 20% HOAc-CH_3CH (500 µL) and allowed to stir for 10 min. The mixture was stripped to a white solid which was extracted with EtOAc (3 x 7 mL). The EtOAc extracts were filtered and the filtrate stripped and dried under vacuum to give 61 mg (4.6 mCi) of crude [^{14}C]-4 as a white powder. This material was used in the next reaction without further purification.

Material	Lot No.	Source
Methanol	BC024	Burdick & Jackson 230-4L
Sodium borohydride	02320 PX	Aldrich 19807-2
Hexane	B8676	Burdick & Jackson 216-4L
Silica gel	30283	E. Merck 9385-5
Ethyl acetate	B8880	Burdick & Jackson 100-4L
Acetic acid	FL-04-0390	Fisher A38C-212

Methyl 4-(10-dihydroartemisininoxymethyl)benzoate [^{14}C]-5

To a 50-mL flask containing a suspension of 4.5 mg (0.19 mmol) of [^{14}C-4] prepared above in 6 mL of anhydrous ether was added 14.4 mg, (0.688 mmol, 3.5 eq) of methyl 4-(hydroxymethyl)benzoate and 37 µL, (0.228 mmol, 1.2 eq) of

$BF_3 \cdot Et_2O$. The solution became clear and colorless upon the addition of the $BF_3 \cdot Et_2O$. The resulting mixture was allowed to stir for 24 h at room temperature under N_2. The reaction progress was followed by TLC (SiO_2, 60% hexane-EtOAc) which showed it to be incomplete at 24 h. The mixture was allowed to continue to stir and at 43 h, TLC (same system) analysis indicated the reaction was complete. The reaction mixture was added to a separatory funnel containing 10 mL of aqueous 5% $NaHCO_3$ solution. The ether layer was further washed with 5% aqueous $NaHCO_3$ solution (2 x 10 mL) and washed with water (2 x 10 mL). The ether layer was dried over Na_2SO_4 and filtered. The filtrate was stripped to give a clear colorless glass which was purified by flash chromatography (10 mm ID, SiO_2, 230-240 mesh, 60% hexane-EtOAc). The fractions were analyzed by TLC (same system). The pure fractions were combined and evaporated to give 54 mg (3.5 mCi) of [^{14}C]-5.

Material	Lot No.	Source
Methy-p-(hydroxymethyl)benzoate	00416 PX	Aldrich 26647-7
$BF_3 \cdot Et_2O$	08823 AY	Aldrich 21660-7
Ether	921442-15	Fisher F138-4
Sodium bicarbonate	3001802	EM Science SX0320-1
Sodium sulfate	E30163	J. T. Baker JT3893-1
Silica gel	30283	E. Merck 9385-5
Hexane	BB676	Burdick & Jackson 216-4L
Ethyl acetate	BB880	Burdick & Jackson 100-4L

[16-^{14}C]Artelinic Acid ([^{14}C]-6)

To a 10-mL flask containing [^{14}C]-5 (54 mg, 0.12 mmol, 3.5 mCi) was added 5% KOH/CH_3OH (11 mL). The resulting mixture was allowed to stir at room temperature under N_2. After 4 d TLC (SiO_2, 60% hexane-EtOAc) analysis showed 4% of [^{14}C]-5 remaining. After 5 d 2% of [^{14}C]-5 remained by TLC analysis.

At 6 d TLC analysis indicated 1% of [^{14}C]-5 and the reaction was worked up. Acetic acid (82 mL, 11.5 eq) was added to the mixture and it was allowed to stir for 0.5 h. The mixture was stripped to a white solid residue which was partitioned between H_2O and ether and washed with H_2O (2 x 5 mL). The ether layer was dried over Na_2SO_4 and filtered. The filtrate was stripped and placed under high vacuum to dry. The crude product was purified by prep HPLC (Waters prep RCM μBondapak C_{18}, 10 μ, 25 x 200 mm, 65% CH_3OH-0.1 M $CH_3CO_2NH_4$ (aq), 9.9 mL/min, 235 nm). The injections were made in CH_3OH after filtration through 0.45 μm nylon filter. The injection size was 18-20 mg. The pure fractions were combined to recover 39.7 mg (2.31 mCi; 76% yield) of pure [^{14}C]artelinic acid. The product was diluted with 34.3 mg of cold artelinic acid to yield 74 mg [16-^{14}C]artelinic acid with specific activity of 12.4 mCi/mmol. The chemical purity was 99.9% and the radiochemical purity was 100% by HPLC (Waters μBondapack, C18, 10 μ, 3.9 x 300 mm, 65% CH_3OH-0.1 M $CH_3CO_2NH_4$, 1.5 mL/min, UV-235 and RAM, t_R = 9 min 24 sec, t_R std 9 min 6 sec). The product was entered in inventory as lot no. LF-7044-94.

Material	Lot No.	Source
Potassium hydroxide	897898	Fisher P250-3
Methanol	BC024	Burdick & Jackson 230-4L
Hexane	BB676	Burdick & Jackson 216-4L
Ethyl acetate	BB880	Burdick & Jackson 100-4L
Ether	921442-15	Fisher F138-4
Acetic acid	FL-04-039	Fisher A38C-212
Ammonium acetate	10115KW	Aldrich 23807-4
WR-255663	BM04131	WRAIR

Reference

1. Avery, M. A. Final Report on Contract DAMD17-88-C-8048, February 3, 1990.