AD NUMBER

AD-B155 291

NEW LIMITATION CHANGE

TO

Approved for Public Release;
Distribution Unlimited.

FROM

B-3

AUTHORITY

Rept. D'lmt'd per memo. dtd 28 Aug 95, Signed by Col. Gary R. Gilbert, MCMR-RMI-S (OL-Y). DCS/Info. Mgmt., Ft. Detrick, MD.

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

SUBJECT: Request Change in Distribution Statement

1. The U.S. Army Medical Research and Materiel Command has reexamined the need for the limited distribution statements on technical reports for Contract Number DAMD17-89-C-9062. Request the limited distribution statement for AD Numbers [redacted], [redacted], ADB155281, ADB164099, and ADB170085 be changed to "Approved for public release; distribution unlimited." Copies of these reports should be released to the National Technical Information Service.

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

for Gary R. Gilbert
GARY R. GILBERT
COL, MS
Deputy Chief of Staff
for Information Management

AD_____

PREPARATION OF RADIOCHEMICAL-LABELED COMPOUNDS FOR THE US ARMY DRUG DEVELOPMENT PROGRAM

ANNUAL REPORT

JOHN A. KEPLER

APRIL 18, 1991

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

SECURITY CLASSIFICATION OF THIS PAGE

REPORT DOCUMENTATION PAGE

Form Approved OMB No. 0704-0188

1a. REPORT SECURITY CLASSIFICATION: Unclassified	1b. RESTRICTIVE MARKINGS
2a. SECURITY CLASSIFICATION AUTHORITY	3. DISTRIBUTION/AVAILABILITY OF REPORT: Distribution authorized to U.S. Government agencies only; Proprietary Information, April 1991
2b. DECLASSIFICATION/DOWNGRADING SCHEDULE	
4. PERFORMING ORGANIZATION REPORT NUMBER(S)	5. MONITORING ORGANIZATION REPORT NUMBER(S)
6a. NAME OF PERFORMING ORGANIZATION: Research Triangle Institute; 6b. OFFICE SYMBOL (If applicable)	7a. NAME OF MONITORING ORGANIZATION
6c. ADDRESS (City, State, and ZIP Code): Post Office Box 12194, Research Triangle Park, NC 27709-2194	7b. ADDRESS (City, State, and ZIP Code)
8a. NAME OF FUNDING/SPONSORING ORGANIZATION: U.S. Army Medical Research & Development Command; 8b. OFFICE SYMBOL (If applicable): SGRD-RMI-S	9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER: DAMD17-89-C-9062
8c. ADDRESS (City, State, and ZIP Code): Fort Detrick, Frederick, Maryland 21702-5012	10. SOURCE OF FUNDING NUMBERS

PROGRAM ELEMENT NO.	PROJECT NO.	TASK NO.	WORK UNIT ACCESSION NO.
62300A	63002D810	AE	DA318715

11. TITLE (Include Security Classification)

(U) Preparation of Radiochemical-Labeled Compounds for the US Army Drug Development Program

12. PERSONAL AUTHOR(S)

John A. Kepler

13a. TYPE OF REPORT	13b. TIME COVERED	14. DATE OF REPORT (Year, Month, Day)	15. PAGE COUNT
Annual Report	FROM 3/23/90 TO 3/22/91	1991 April 18	45

16. SUPPLEMENTARY NOTATION

17. COSATI CODES			18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)
FIELD	GROUP	SUB-GROUP	Antimalarial; Carbon-14; Tritium, Synthesis; Radiolabel; Chemical warfare protective agents; Cholinesterase Reactivators; RA 5, 1
07	03		
06	01		

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, 1990 to March 22, 1991. 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 (USAMRDC) and to provide these compounds along with some commercially prepared compounds to investigators designated by the USAMRDC.

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

20. DISTRIBUTION/AVAILABILITY OF ABSTRACT	21. ABSTRACT SECURITY CLASSIFICATION
☐ UNCLASSIFIED/UNLIMITED ☒ SAME AS RPT ☐ DTIC USERS	Unclassified

22a. NAME OF RESPONSIBLE INDIVIDUAL	22b. TELEPHONE (Include Area Code)	22c. OFFICE SYMBOL
Mary Frances Bostian	301-663-7325	SGRD-RMI-S

DD Form 1473, JUN 86 — Previous editions are obsolete — SECURITY CLASSIFICATION OF THIS PAGE

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 this report period a sample of β-[16-^{14}C]arteether was prepared. Development work for preparing [^{2}H$_{8}$]thiodiglycol, bis([^{14}C]trifluoromethyl)disulfide, [16-^{14}C]-artemisinin, and tritium and carbon-14 labeled WR-238605 was completed. The development work for preparing carbon-14 labeled WR-242511 was started.

Foreword

During the period March 23, 1990 to March 22, 1991, 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.

Table of Contents

1.0 Summary

During this report period a sample of β-[16-^{14}C]arteether was prepared. Development work for preparing [$^{2}H_8$]thiodiglycol, bis([^{14}C]trifluoromethyl)-disulfide, [16-^{14}C]artemisinin, and tritium and carbon-14 labeled WR-238605 was completed. The development work for preparing carbon-14 labeled WR-242511 was started.

A total of eight shipments were made during this report period.

2.0 Synthesis of Labeled Compounds

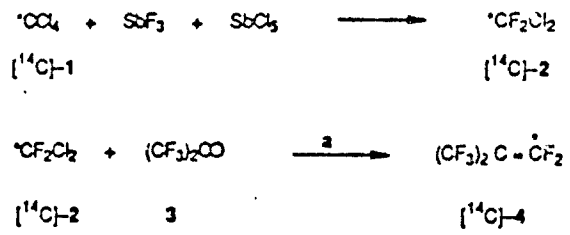
2.1 [1-^{14}C]Perflucroisobutylene ([^{14}C]-4)

The scheme being investigated for the synthesis of [1-^{14}C]perfluoroisobutylene ([^{14}C]-4) is outlined in Chart 1. The scheme is based on the reported synthesis of [^{13}C]dichlorodifluoromethane[1] ([^{13}C]-2) and gem-difluoroolefins.[2] Acetophenone (5, Chart 2) is being used as a model compound because of the toxicity and volatility of 4. Earlier studies[3] established that reaction of acetophenone and dibromodifluoromethane (7, BFM) in the presence of hexamethylphosphoroustriamide (HMPT) gave 70-80% yield of olefin 6 at ambient temperature, but when BFM was replaced with dichlorodifluoromethane (2, CFM) no 6 was formed unless the reaction was heated above 80°C. Evidence was presented[3] that the differences in yield and reaction conditions when using CFM compared to BFM was not due to the lack of formation of complex 8, (Chart 3), but its subsequent conversion to ylide 10 by HMPT.

Heating the reaction mixture will cause technical difficulties with the radiosynthesis because of the low boiling points of hexafluoroacetone (33) (-26°C) and the product, PFIB (4) (6°C). Two strategies to overcome this problem were investigated. An attempt was made to prepare BFM by modification of the method used to prepare [^{13}C]-2.[1] Reaction of carbon tetrabromide with antimony trifluoride and antimony tribromide[4] did not give any detectable BFM.

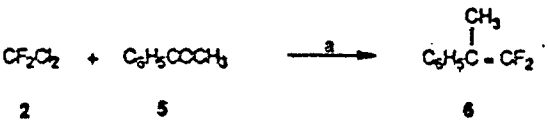
The second approach to overcome this problem was to find a catalyst that would convert salt 8 to ylide 10 at ambient temperature or below. Burton and Ishikawa et al.[2,5] have reported that metals such as zinc, mercury and cadmium promoted the reaction of the BFM•triphenylphosphine complex (9) with aldehydes and ketones. We found that zinc also promoted the reaction of the CFM•HMPT complex (8) with 5. HMPT was allowed to react with CFM (2) in N,N-dimethylacetamide (DMAc) to form 8. Acetophenone was added after stirring for 2 h and

Chart 1



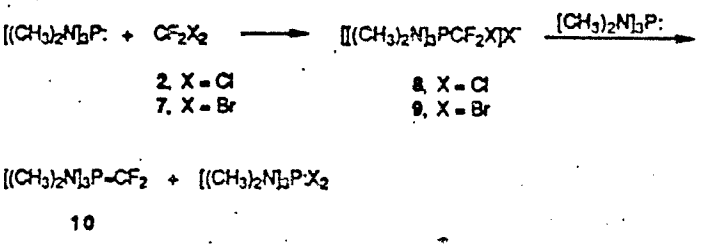
a) $[(CH_3)_2N]_3P$, triglyme

Chart 2



a) $[(CH_3)_2N]_3P$, triglyme

Chart 3



zinc was added 0.5 h later. GC analysis of the reaction mixture after stirring overnight at room temperature showed a ratio of product 6 to acetophenone of 95:5.

With an acceptable method for the conversion of 2 to 6 in hand, the entire synthetic scheme for preparing [^{14}C]-4 was tested with the acetophenone model. All of the operations were carried out on the vacuum manifold. Antimony trifluoride and antimony pentachloride were combined and placed on the manifold. Carbon tetrachloride (5 mmol), which will be the source of the carbon-14 label, was transferred to the reaction flask and the mixture was allowed to stir at 40°C for 2 h. The yield of 2 was quantitative as measured by the volume of gas generated in the vacuum line, but the purity was unknown. The 2 was allowed to react with HMPT in DMAc. After 2 h, acetophenone and dry zinc dust were added to the reaction mixture. After stirring overnight, GLC analysis showed the mixture to be 69% product 6 and 26% acetophenone.

The results of this experiment indicate that this method should be useful for preparing [^{14}C]-4, but we have not found a source for a standard sample of 4 and we have no method for its purification. There are GLC methods reported for its analysis.[6]

We suspect that [^{14}C]PFIB will rapidly polymerize because of its high reactivity coupled with the ionizing radiation associated with the radioactive label. Bayliff and Chambers[7] have reported that PFIB forms salt 11, with

$$[(CF_3)_3C]^- \ Cs^+$$

11

cesium fluoride. If PFIB can be regenerated from 11 the salt may be a suitable form for its storage and characterization. This cannot be determined however, unless a source of nonlabeled PFIB is found.

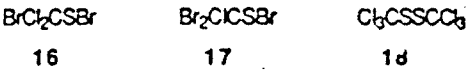
Work on this synthesis has been temporarily stopped at the request of USARMDC.

2.2 Bis([^{14}C]trifluoromethyl)disulfide ([^{14}C]-15)

Development work has continued on the synthesis of bis([^{14}C]trifluoromethyl)disulfide ([^{14}C]-15). The proposed scheme for this synthesis is outlined in Chart 4 and is based on methods[8,9] used to prepare nonlabeled 15. Earlier[3] we described modification of the reported[8] method for preparing sulfenyl chloride 13 which allowed its preparation on a small scale suitable for the radiosynthesis. Work this report period has focused on developing small scale synthesis procedures for preparing sulfenyl bromide 14 and the target compound, disulfide 15, as well as analytical and purification methods for 14 and 15.

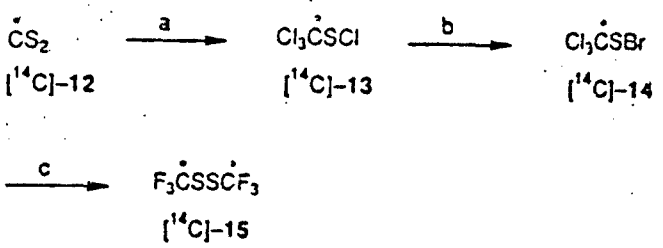
Early in the course of developing the synthesis of labeled 15, it became apparent that the lack of a good analytical method hampered progress. Three GC methods were investigated for analysis of 15; (1) 10% OV-101 on Gas Chrom Q 100/120, (2) 10% OV-210 on Chromosorp W-HP 80/100, and (3) 10% DC-200 on Chromosorp W-HP 80/120. Two of these columns, the DC-200 and the OV-101 gave satisfactory separation of 13, 14 and 15 when run with a temperature program of 20°C-190°C at 10°/min.

GC analysis of 14 prepared as described above showed that the preparation contained varying amounts (30-50%) of unknown impurities with shorter retention times than 14. When 14 was treated with fresh 48% hydrobromic acid, the amount of the impurities was greatly increased. Compounds 16 and 17 have been reported[9,10] as being formed during the preparation of 14, but were eliminated



16 17 18

Chart 4



a) Cl_2
b) HBr
c) KF

as possible candidate impurities along with disulfide 18, the photochemical decomposition product 13[11], because their boiling points are considerably higher than 14 and thus are unlikely to have retention times shorter than 14. The two major impurities were tentatively identified as chloroform and carbon tetrachloride on the basis of their GLC retention times. Attempts to purify 14 by distillation were not successful. An alternate procedure for preparing 14 was developed [HBr-HOAc (30%), -10°C, 2 hr]. The crude reaction mixture must be washed with water and sodium bicarbonate solution prior to drying over phosphorous pentoxide, but even with these extra steps the yields of 14 are high (85-90%) and the purity of the product is better than 90-95%.

With 14 prepared by the new method as starting material, the step to prepare 15 was studied using different solvents and means of addition of 14 to the reaction mixture. In our initial attempts to prepare 15 we used 18-crown-6 as reaction solvent because our early analytical methods appeared to show high yields of product. After developing a GLC method that resolves the product from the various impurities, it became apparent that the crown ether is not as good a solvent as tetramethylene sulfone (TMS) for this reaction. To date the best method for preparing 15 involves the dropwise addition of 14 over 45 min to a suspension of potassium fluoride in TMS at 165-170°C with a slow nitrogen sweep of volatile reaction products to traps cooled with liquid nitrogen. The reaction product was purified by trap (0°C) to trap (-77°C) distillation at 150 mm. GLC analysis of the headspace above the purified product shows about 80% purity while GLC analysis of a deuterochloroform solution of this material showed 96% purity. ^{13}C NMR analysis of the solution shows primarily 15 with what appears to be a small amount of hydrocarbon impurity. There are no detectable contaminants which contain a C-F bond and there is no detectable carbon disulfide. Carbon disulfide was detected in

crude samples of 15. The yield of 15 was not determined but appears to be low.

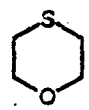
Work has been stopped on this synthesis until further notice at the request of USARMDC.

2.3 1,5-Dihydroxy-[1,1,2,2,4,4,5,5-$^{3}H_8$]-3-thiapentane; [$^{2}H_8$]thiodiglycol, ([$^{2}H_8$]-20)

The synthesis of [$^{2}H_8$]thiodiglycol ([$^{2}H_8$]-20) was accomplished by using the scheme shown below.[12,13] Reaction of [$^{2}H_4$]-19 with sodium sulfide gave

$$\underset{[^{2}H_4]\text{-}19}{BrCD_2CD_2OH} + Na_2S \longrightarrow \underset{[^{2}H_8]\text{-}20}{HOCD_2CD_2SCD_2CD_2OH}$$

a 98% yield of [$^{2}H_8$]-20 after distillation. GLC analysis of this product, however, indicated the presence of about 15% of an impurity with the same retention time as 1,4-thioxane (21). Chromatography of [$^{2}H_8$]-20 gave a sample



21

that was 98% pure by GLC analysis (peak area ratio), but with an unacceptable elemental analysis that was 0.8% low for carbon and 0.7% low for sulfur. The elemental analysis implied that the sample was wet. Vacuum distillation, however, failed to give a sample with an improved elemental analysis. GLC analysis of the distilled material showed it to be 96% pure. This compares to a commercial sample of 20 from Aldrich Chemical Co. which was 96.4% pure by GLC. The major impurity elutes as a broad peak somewhat earlier than 20 in both the commercial sample and the synthesized [$^{2}H_8$]-20 sample. The impurity

was shown not to be ethylene glycol by comparison of its GLC retention time with an authentic sample. The data on this sample is currently being evaluated by USARMDC to determine if it is sufficiently pure for its intended purpose. If the sample is not pure enough for use, further purification will be attempted.

2.4 WR-255131: β-[16-^{14}C]Arteether ([^{14}C]-24)

The master synthesis of β-[16-^{14}C]arteether, ([^{14}C]-24, Chart 5) was completed. Sodium borohydride reduction of [16-^{14}C]artemisinin ([^{14}C]-22) yielded 187 mg of [16-^{14}C]dihydroartemisinin ([^{14}C]-23) (99% radiochemical yield). [^{14}C]-23 was treated with boron trifluoride etherate which gave a 182 mg mixture of α- and β-[16-^{14}C]arteether, (81% radiochemical, 89% chemical yield).

An additional 42 mg of [^{14}C]-23 from inventory (lot # 5994-123) was treated with boron trifluoride etherate to yield a 38 mg mixture of α- and β-[16-^{14}C]arteether, (92% radiochemical, 82% chemical yield).

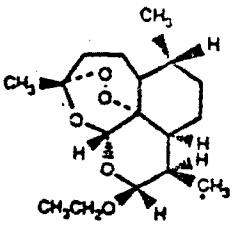
The two lots of the α- and β-[16-^{14}C]arteether mixture were combined and purified by HPLC. The mixture was dissolved in ethanol and 5 mg injections were made onto a preparative column (Rainin Dynamax, C-18, 8 μ, 21.4 x 250 mm, 85% CH_3CN-H_2O, 9.9 mL/min). Each β-[16-^{14}C]arteether fraction collected was checked for purity on an analytical system, (Altex Ultrasphere-ODS 5μ, 4.6 x 250 mm, 60% CH_3CN-H_2O, 1.2 mL/min). Impure fractions were combined and repurified. A total of 103 mg (41% chemical yield), 2.17 mCi (51% radiochemical yield) of β-[16-^{14}C]arteether of 96% radiochemical purity was obtained.

A significant amount (20%) of α-[16-^{14}C]arteether (25) is formed in the synthesis of β-[16-^{14}C]arteether. Methods of isomerizing α-arteether to β-arteether (Chart 6) were investigated with the hope of obtaining more of the

Chart 5

[^{14}C]-22

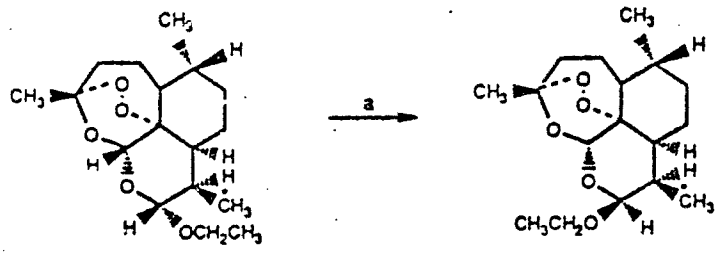
[^{14}C]-23



[^{14}C]-24

a) $NaBH_4$
b) $BF_3 \cdot OEt_2$, EtOH

Chart 6



[^{14}C]-25 [^{14}C]-24

a) $BF_3 \cdot OEt_2$, EtOH

desired β-[16-^{14}C]arteether. In a preliminary run, pure α-arteether was treated with boron trifluoride etherate in benzene-ethanol solution. After 42 h at room temperature HPLC analysis showed that a 1:1 mixture of α- and β-arteether was present along with several impurities. To determine the position of equilibrium, two experiments were run: 1) the β-isomer was treated with one equivalent of boron trifluoride etherate in an ethanol-benzene solution and 2) the α-isomer was treated with two equivalents of boron trifluoride etherate in an ethanol-benzene solution. These conditions duplicate those used in the formation of arteether from dihydroartemisinin.

The β-isomer slowly isomerized to a mixture of α- and β- isomers heavily favoring the β-isomer (94:6 after 48 h). The α-isomer slowly isomerized to the β-isomer giving a 1:1 mixture of isomers after 50 h. The reaction was monitored for a total of 140 h, and although the ratio of isomers remained relatively constant at 1:1 during this time, it appeared that the isomers were consumed to produce the major impurity, possibly dihydroartemisinin.

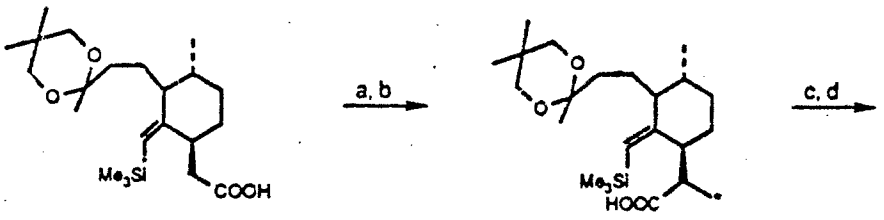
These results indicate that it is possible to isomerize α- to β-arteether but that it is not very efficient. No further work is planned in this area since the synthesis provided the amount of β-[16-^{14}C]arteether that was requested.

2.5 WR-249309: [16-^{14}C]Artemisinin ([^{14}C]-22)

Studies of the preparation of [16-^{14}C]artemisinin ([^{14}C]-22) according to the method of Avery[14] have been started (Chart 7). Initial problems with the alkylation of the dianion of 26 were traced to incomplete drying of 26 and a low titer of n-butyl lithium. Correction of these problems lead to 94% yield of 27 from alkylation of 26. Ozonization of 27 followed by treatment with acid afforded artemisinin in 37% yield after purification.

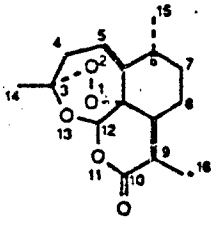
The alkylation of 26 is carried out with 2.4 equivalents of methyl iodide. This is done to insure complete conversion of 26 to 27, because at

Chart 7



26

[^{14}C]-27



[^{14}C]-22

a) 2 eq LDA
b) $^{*}CH_3I$
c) O_3, O_2
d) H_3O^+

present, we do not have a method for separating 26 and 27. The results of a tracer run suggested that radiolabeled methyl iodide could be conserved by first treating the dianion with one equivalent of [^{14}C]methyl iodide followed later by a second equivalent of nonlabeled methyl iodide. When this strategy was carried out on the master run an 80:20 (determined by ^{1}H NMR) mixture of 26 and [^{14}C]-27 was obtained. We suspect that the lack of complete reaction was caused by impurities in the purchased [^{14}C]methyl iodide since the specific activity of the product, 35 mCi/mmol, is close to the expected 32 mCi/mmol if the [^{14}C]methyl iodide was contaminated with 20% of its precursor [^{14}C]methanol.

As stated earlier, we do not have a method for separating 26 and 27, so an experiment was done to determine if a mixture of 26 and 27 could be converted to 27 without dialkylation. Accordingly, a 40:60 synthetic mixture of 26 and 27 was subjected to the alkylation conditions. The ^{1}H NMR spectrum of the isolated product was identical with that of 27 with no detectable 26 and no indication of dialkylation.

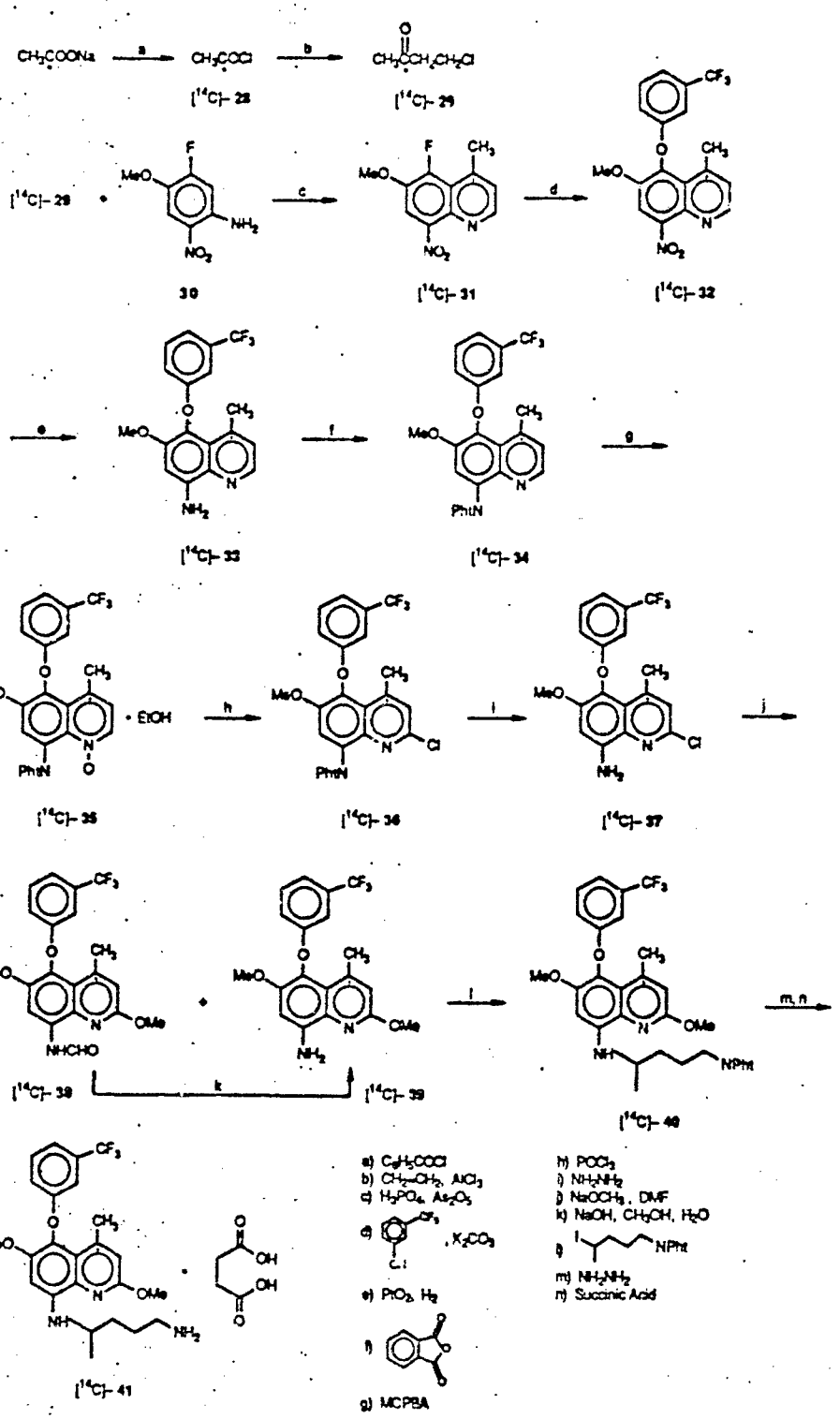
We plan to use this method to convert the 80:20 mixture of [^{14}C]-27 and 26 from the master run to give pure [^{14}C]-27. We expect the master run to be completed by the end of May, 1991.

2.6 WR-238605: 8-[(4-Amino-1-methylbutyl)amino]-2,6-dimethoxy-4-methyl-5-[3-(trifluoromethyl)phenoxy]quinoline Succinate

2.6.1 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]-41)

Work on a repeat synthesis of [4-^{14}C]WR-238605 ([^{14}C]-41) was begun this report period (Chart 8). A previous preparation of this material gave lower than expected yields of the N-oxide [^{14}C]-35 from the reaction of [^{14}C]-34

Chart 8

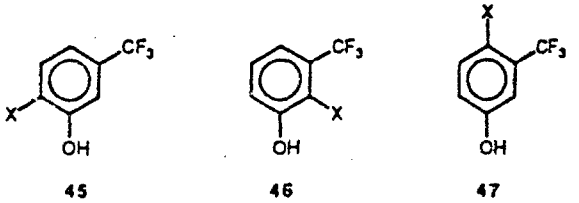

a) C_6H_5COCl
b) $CH_2{-}CH_2$, $AlCl_3$
c) H_3PO_4, As_2O_5
d) , K_2CO_3
e) PtO_2, H_2
f)
g) MCPBA
h) $POCl_3$
i) NH_2NH_2
j) $NaOCH_3$, DMF
k) NaOH, CH_3OH, H_2O
l)
m) NH_2NH_2
n) Succinic Acid

with m-chloroperbenzoic acid (MCPBA).[3] Consequently, this reaction was reinvestigated before starting the repeat synthesis. A sample of MCPBA was purified to determine if its purity was relevant to the yield of 35 from 34. Reaction of 34 with purified MCPBA gave a 71% of 35. This yield approaches our best yield of the past (76%), but the product has a low melting point. Nevertheless, it appears that the purity of the MCPBA is important to the yield of 35.

We plan one additional test reaction with magnesium monoperoxyphthalate as oxidizing agent, which has been described as a good substitute for MCPBA.[15] The synthesis of [^{14}C]WR-238605 will be started as soon as this study is complete. The current plan is to prepare a large amount of fluoroquinoline [^{14}C]-31, and use it for the synthesis of both [^{14}C]WR-238605 and [^{14}C]WR-242511 (see later).

2.6.2 8-[(4-Amino-1-methylbutyl)amino-2,6-dimethoxy-4-methyl-5-[5-(trifluoromethyl)[2-^{3}H]phenoxy]quinoline Succinate; [^{3}H]WR-238605 ([^{3}H]-41)

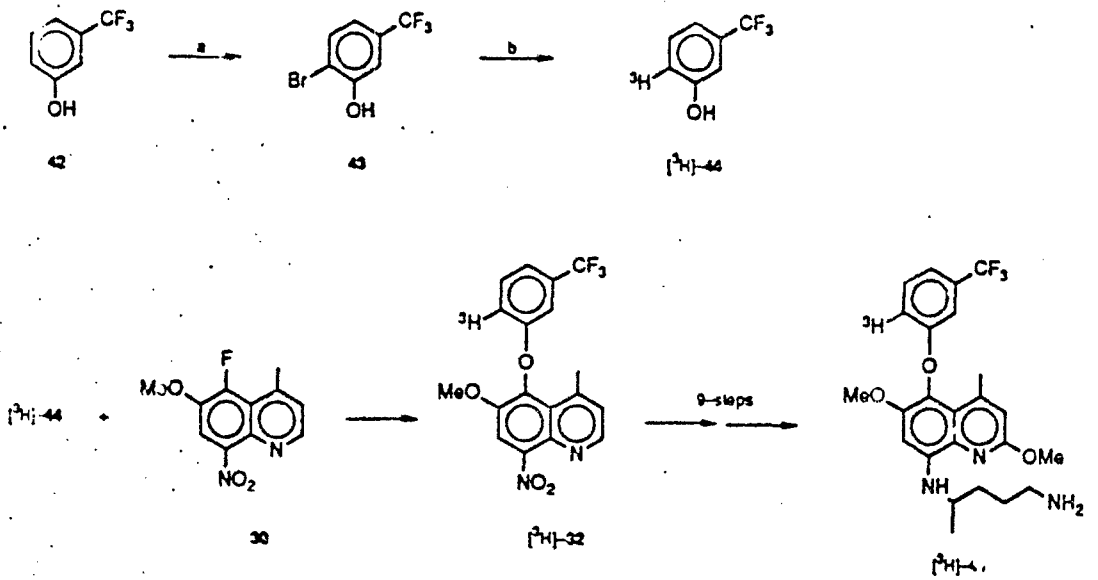
Development work for preparing [^{3}H]WR-238605 has been completed (Chart 9, Scheme I). Initially, we attempted to prepare 2-bromo-5-trifluoromethylphenol as an intermediate by using a reported procedure[16], but were able to obtain only inseparable mixtures of mono-,di-, and tribromo- compounds. Although, in theory, a mixture of halogenated phenols could be used, a monohalogenated intermediate is preferred for the radiosynthesis because it reduces the number of possible radiolabeled contaminates. Furthermore, based on steric considerations, we believed that a mono halogenated compound substituted as 45 offer advantages with selective catalytic reduction over those substituted as 46 and 47.



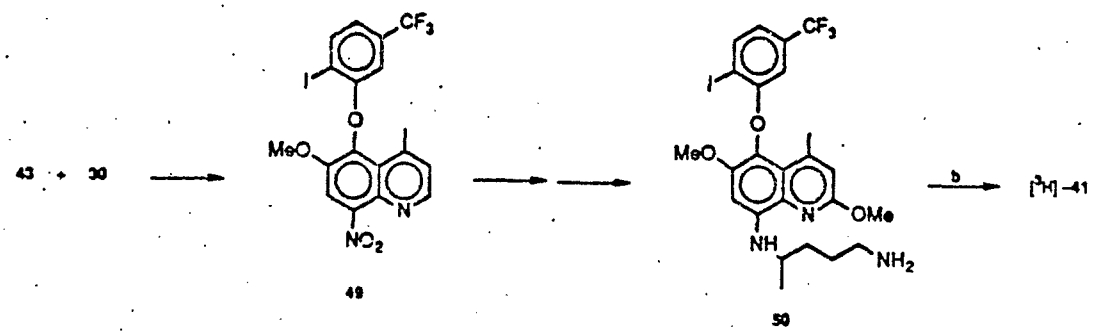
45 46 47

Chart 9

Scheme I

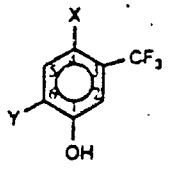


Scheme II



a) NaOCl, NaI
b) $^3H_2$, Pd/C

Edgar and Falling recently reported[17] a new method for preparing iodophenols whereby one can control the degree of iodination. Treatment of 3-(trifluoromethyl)phenol (42) with one equivalent of sodium iodide and sodium hypochlorite gave a product that was 99% pure by GLC analysis. The mass spectrum of the product showed that only one iodine had been incorporated into 42, and the ^{1}H NMR spectrum showed that the iodine was not at the 2-position (singlet at δ 7.26), but did not discriminate between 4- and 6-substitution, i.e. 43 and 48. The ^{13}C NMR spectrum indicated that the product had structure 43. A singlet at 89.9 ppm was observed for the carbon bearing iodine. Two



42 Y = H, X = H
43 Y = I, X = H
48 Y = H, X = I

quartets at 118.9 and 112 (J_{CCCF} = 4 Hz) were assigned to the 2- and 4-carbons on the basis of long range coupling with the flourine atoms. If the iodine atom had been at the 4-position, i.e. 48, then the signal at 89.9 ppm would have been a quartet.

Catalytic reductive deuteration of 43 was carried out in 0.1 N sodium hydroxide with 10% Pd/C catalyst to give a quantitative yield of [^{2}H]-44. The completion of the synthesis of [^{3}H]WR-238605 can proceed from intermediate 43 by at least two methods. The tritium label can be introduced at the beginning of the scheme (Scheme I, Chart 9) or at the last step (Scheme II, Chart 9). Although we considered Scheme II unlikely to be successful because of the sensitivity of the 2-methoxy quinoline system, we felt it worthwhile to confirm this, since Scheme II has nine fewer steps with radioactive material than Scheme I. Accordingly, the free base of WR-238605 (50) was subjected to

the catalytic reduction conditions required for reductive tritiation. Considerable decomposition of 50 was observed under these conditions, thus Scheme I rather than Scheme II will be used to prepare [^{3}H]WR-238605.

The development work for preparing [^{3}H]WR-238605 is essentially complete and delivery is scheduled for September, 1991.

2.7 WR-242511: 8-[(4-amino-1-methylbutyl)amino]-5-hexoxy-6-methoxy-4-methyl[^{14}C]quinoline (D,L) Tartrate, [^{14}C]WR-242511 ([^{14}C]-55)

The scheme proposed for the synthesis of [^{14}C]WR-242511 ([^{14}C]-55) is outlined in Chart 10. The synthetic scheme differs from the published scheme[18] in that fluoroquinoline 31 replaces chloroquinoline 56 (Chart 11) as the intermediate for preparing hexoxyquinoline 51. This was done for several reasons: (a) we have experience in preparing [^{14}C]-31; (b) [^{14}C]-31 is required for the repeat synthesis of [^{14}C]WR-238605 (see earlier), and economies can be made by preparing a large batch of [^{14}C]-31 and using it for both syntheses; (c) the number of steps with radioactive material to intermediate 51 is reduced; and (d) it was anticipated that the fewer steps, plus the expectation that the flourine of 51 would be more susceptible to nucleophilic displacement than the chloride of 56, would lead to a better yield of 51.

Initial studies of the reaction of 31 with hexoxide gave low yields (35-55%) of product 51. It was determined that these low yields were due to a combination of elevated reaction temperature (~ 120°C), long reaction time, and a complicated workup procedure. This reaction gave an 87% yield of 51 when run at 80°C for 3.5 h and worked up by simply removing the solvent under vacuum and chromatographing the residue.

Conversion of 51 to aminoquinoline 52 was accomplished in 86% yield by reduction with iron fillings as reported[18] and in quantitative yield by catalytic hydrogenation with platinum oxide catalyst. The latter method will be used for the radiosynthesis, not only because of its superior yield, but

Chart 10

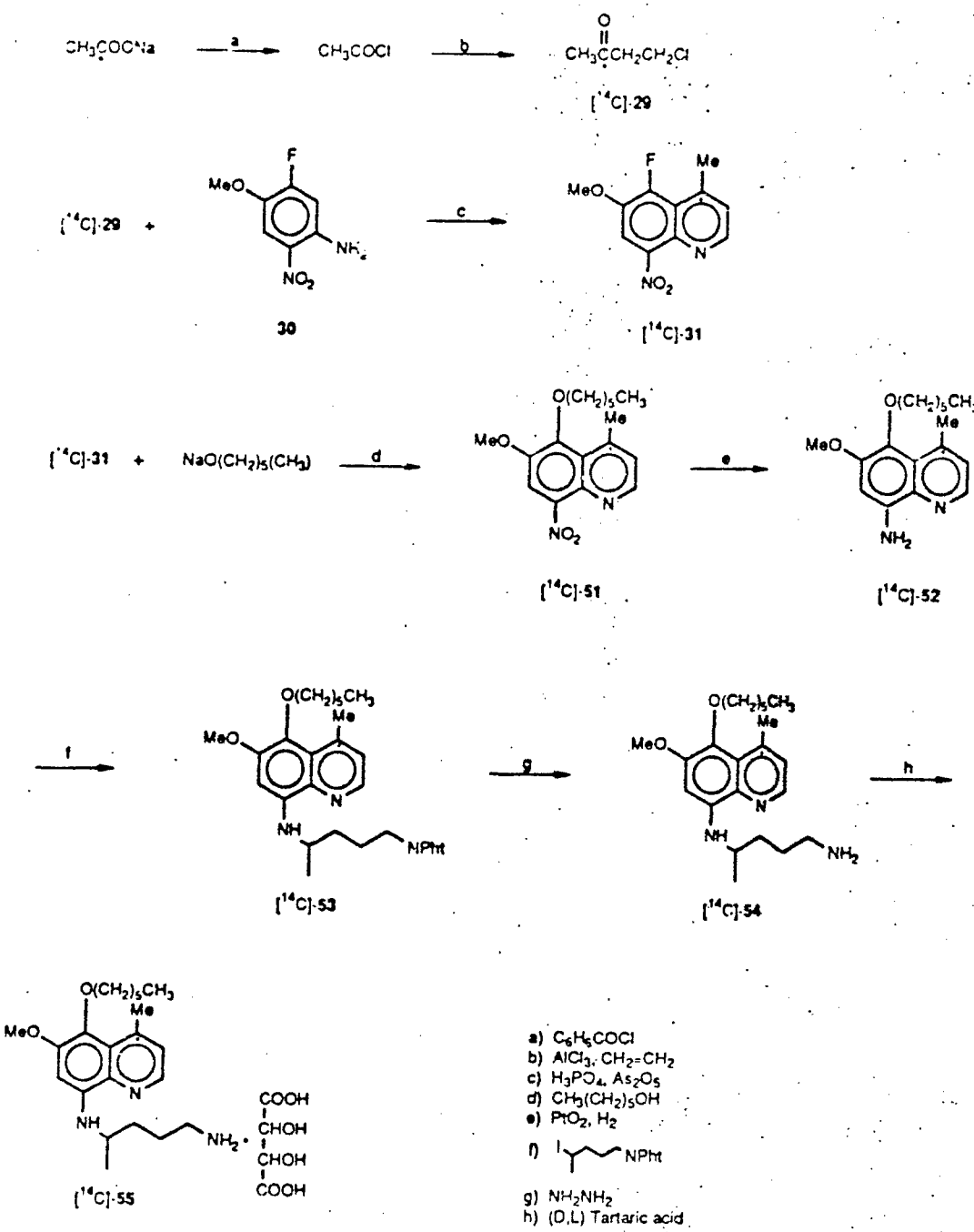
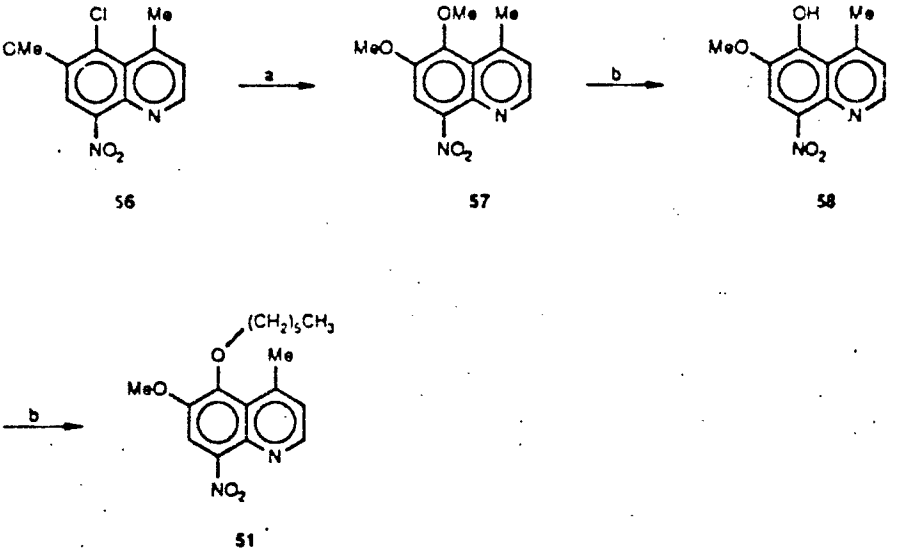

CH3COONa
a
CH3COCl
b
CH3CCH2CH2Cl
O
[14C]-29
[14C]-29 +
F
MeO
NH2
NO2
30
c
F
Me
MeO
N
NO2
[14C]-31
[14C]-31 + NaO(CH2)5(CH3)
d
O(CH2)5CH3
Me
MeO
N
NO2
[14C]-51
e
O(CH2)5CH3
Me
MeO
N
NH2
[14C]-52
f
O(CH2)5CH3
Me
MeO
N
NH
NPht
[14C]-53
g
O(CH2)5CH3
Me
MeO
N
NH
NH2
[14C]-54
h
O(CH2)5CH3
Me
MeO
N
NH
NH2
COOH
CHOH
CHOH
COOH
[14C]-55
a) C6H5COCl
b) AlCl3, CH2=CH2
c) H3PO4, As2O5
d) CH3(CH2)5OH
e) PtO2, H2
f) I NPht
g) NH2NH2
h) (D,L) Tartaric acid

Chart 11



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

also because of the ease of workup. Reaction of 52 with 2-iodophthalimidopentane afforded 53 in 70% yield.

All of the steps up to compound 53 now give acceptable yields for the radiosynthesis leaving only the last two reactions to be studied. The tracer synthesis of [^{14}C]WR-242511 will be started as soon as these studies are complete.

3.0 Purification

All of the impure samples of [^{14}C]WR-238605 left over from various preparations and mother liquors were combined with the inventory samples CT-4167-59-1 and CT-6120-85-1 and purified by crystallization from ethanol-ether. The yield was 310 mg (8.3 mCi) of [^{14}C]WR-238605 with specific activity of 15.7 mCi/mmol (27 μCi/mg) and 96% radiochemical purity by HPLC-RAM[19] and 97% radiochemical purity by radio-TLC.[20]

4.0 Shipments

A total of eight shipments were made to investigators as authorized by the Project Monitor during the period March 23, 1990 - March 22, 1991 (Table 1).

5.0 Inventory

A list of the compounds held in inventory March 22, 1991 by the Research Triangle Institute for the USARMDC is given in Table 2.

6.0 References

1. Andrew, L.; Willner, H.; Prochaska, F. T. J. Fluorine Chem. 1979, 13, 273.
2. Burton, D. J. J. Fluorine Chem. 1983, 23, 339 and references therein.
3. Kepler, J. A., Annual Report, Contract DAMD17-89-C-9062, April, 1990.
4. Antimony tribromide was used as a substitute for antimony pentachloride because antimony pentabromide is not a stable entity.
5. (a) Burton, D. J.; Kesling, H. S.; Naae, D. G. J. Fluorine Chem. 1981, 18, 293. (b) Hayashi, S.-I.; Nakai, T.; Ishikawa, N.; Burton, D. J.; Naae, D. G.; Kesling, H. S. Chem. Lett. 1979, 983.
6. (a) Marhevka, J. S.; Johnson, G. D.; Hagan, D. F. Anal Chem. 1982, 54, 2607. (b) Menichelli, R. P. Am. Ind. Hyg. Assoc. 1982, 43, 286.
7. Bayliff, A. E.; Chambers, R. D. J. Chem. Soc. Perk. Trans. I 1988, 201.
8. Zupancic, B. Synthesis 1975, 169.
9. Dear, A.; Gilbert, E. Synthesis 1972, 310.
10. Ospenson, S. N. U.S. Patent 2824136 (1958).
11. Prey, V.; Gutschik, E.; Berbalk, H. Monatsh. Chem. 1960, 91, 556.
12. Faber, E. M.; Miller, G. E. Organic Synthesis, Coll. Vol. II, 1943, 576.
13. Ott, D. G.; Reifield, ;M. J.; Whaley, T W. in "Synthesis and Applications of Isotopically Labelled Compounds, Proc. 2nd Int. Symposium, R. C. Muccino, Ed., Elsevier Science, Amsterdam, 1986, pg. 409.
14. Avery, M. A. Final Report on Contract DAMD17-88-C-8048, February 23, 1990.
15. Brougham, P.; Cooper, M. S.; Cummerson, D. A.; Heaney, H.; Thompson, N. Synthesis 1987, 1015.
16. McBee, E. T.; Rapkin, E. J. Am. Chem. Soc. 1951, 73, 1325.
17. Edgar, K. J.; Falling. S. N. J. Org. Chem. 1990, 55, 5287.

18. Chen, E. H.; Tanabe, K.; Sagglimo, A. J.; Nadiff, E. A. J. Med. Chem. 1987, 30, 1193.

19. J & W CN 5μm 300 x 4.6 mm column; isocratic CH_3OH-CH_3CN-0.01 M NH_4COOH (adjusted to pH 3 with 88% aq HCOOH), 20:50:30; Flow 1 mL/min; Lim, P., Report 656 on Contract DAMD17-85-C-5141, May 9, 1989.

20. Merck 5 x 20 Silica Gel 60 TLC plates; NH_4OH-CH_3OH (1:25).

Table 1

SHIPMENTS

March 23, 1990 to March 22, 1991

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-6120-43	53.2 mg	4/23/90	Cpt. Anthony Theoharides WRAIR
255131	β-[16-^{14}C]arteether	6376-28	2.17 mCi	5/30/90	Cpt. Anthony Theoharides WRAIR
1544	[quinoline-3-^{14}C]Chloroquine Diphosphate	3612-15	0.2 mCi	10/22/90	Dr. Thomas Brewer WRAIR
6026	6-Methoxy-8-(6-diethylaminohexylamino)lepidine-4-^{14}C dihydrochloride	CT-5385-99-1	0.127 mCi	12/17/90	Dr. Alan Buckpitt
6026	6-Methoxy-8-(6-diethylaminohexylamino)lepidine-4-^{14}C dihydrochloride	CT-5385-99-1	1.016 mCi	3/12/91	Dr. Alan Buckpitt
238605	8-[(4-Amino-1-lmethylbutyl)amino]-2,6-dimethoxy-4-methyl-5-[(3-trifluoromethyl)phenoxy][4-^{14}C]-quinoline Succinate	CT-6639-85-1	1.048 mCi	3/12/91	Dr. Alan Buckpitt
2975	[quinoline-2,4-^{14}C]Primaquine Diphosphate	2176-067	0.01 mCi	3/10/91	Jingdong Zhu
238605	8-[(4-Amino-1-lmethylbutyl)amino]-2,6-dimethoxy-4-methyl-5-[(3-trifluoromethyl)phenoxy][4-^{14}C]-quinoline Succinate	CT-6639-85-1	3.078 mCi	3/12/91	Dr. David Hawkins

Table 2

RESEARCH TRIANGLE INSTITUTE
Inventory - Contract No. DAMD17-89-C-9062
April 1, 1991

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	10.5 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	0.2 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-5385-115	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	1.46 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	3.69 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	0.03 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[1] 38.2 mCi/mmole[1]	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	α-(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	RTI	105 μCi/mg	7.56 mCi
		161-1			
		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(tri-fluoromethyl)-9-phenanthrene[^{14}C]methanol Hydrochloride	204-5*	Monsanto	4.94 mCi/mmole	1.77 mCi
165543	α-(Butylaminoethyl)-3,6-bis(trifluoro-methyl)-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-[γ-(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]phenan-threne 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(trifluoro-methylphenyl)-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	13 mCi/mmole	6.54 mCi
		2572-157	RTI	10 mCi/mmole	8.52 mCi
228258	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-6639-85-1	RTI	15.7 mCi/mmole	7.05 mCi
250165	[6-^{14}C]Allopurinol Riboside	CT-3892-91	RTI	7.85 mCi/mmole	0.48 mCi
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

WR No.	Compound	Lot No.	Origin	Specific Activity	Amount Available
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

* 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

β-[16-^{14}C]Arteether, WR-255131

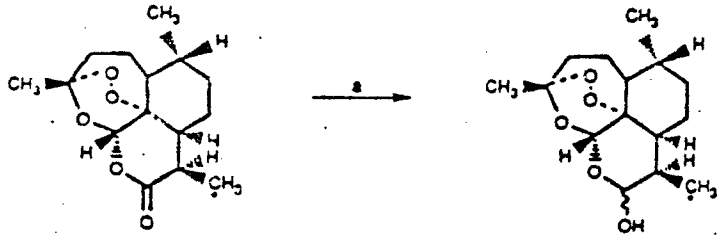
Lot No. 6376-28

June, 1990

Louise Fudala
John A. Kepler

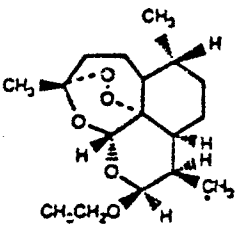
Research Triangle Institute
Post Office Box 12194
Research Triangle Park, North Carolina 27709

Synthetic Scheme



[16-^{14}C]-1

[16-^{14}C]-2



[16-^{14}C]-3

a) $NaBH_4$
b) $BF_3 \cdot OEt_2$, EtOH

Experimental

Analytical TLC were performed using E. Merck silica gel F-254 plates. Radioactive samples were counted on a Packard Tri-Carb 4000 liquid scintillation spectrometer using internal standard[1] in Ultima Gold cocktail. Developed TLC plates were scanned on a Berthold model LB 283 linear analyzer system. HPLC was done using Waters Associates Model 6000A dual pump system with a Model U6K septumless injector and a Berthold Model LB503-HDS or β-RAM Flow monitor 1B radioactivity monitors as the detector.

[16-^{14}C]Dihydroqinghaosu (2)[2]

A solution of [16-^{14}C]qinghaosu in toluene (168 mg, 0.60 mmol, 4.31 mCi) was stripped to dryness in a 50 mL recovery flask. The residue was dissolved in methanol (9 mL) and cooled to 0°C via ice bath. Sodium borohydride (180 mg, 4.89 mmol) was then added to the solution. The reaction was allowed to stir for 2 h at 0°C under N_2. Radio-TLC (SiO_2:60% hexanes-EtOAc) verified that the reaction was complete. The reaction mixture was quenched with 20% HOAc-MeOH (4 mL), and stripped to dryness yielding a white solid. This solid was extracted with EtOAc (2 x 15 mL), filtered, and the combined extracts were stripped, and dried under vacuum. A white solid was recovered (187 mg, 110% crude chemical yield, 4.25 mCi). This material was used in the next reaction.

Chemicals and Sources

[16-^{14}C]Qinghaosu	SRI International	S12524-16-2; 8939-13
Sodium Borohydride	Ventron	14121
Methanol	Fisher	A412-4
Ethyl Acetate	Fisher	E 145-4
Hexanes	Fisher	H 291-4
Acetic Acid	Fisher	A38-212

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

[16-^{14}C]Dihydroqinghaosu (187 mg, 0.66 mmol) was dissolved in benzene (9.4 mL) in a 50 mL recovery flask. Absolute EtOH (3.2 mL) was added to the solution followed by boron trifluoride etherate (81 μL, 94.1 mg, 0.66 mmol). The reaction mixture was allowed to stir at room temperature for 24 h under N_2. Radio-TLC (SiO_2:60% hexanes-EtOAc) verified the reaction was complete. The mixture was quenched with saturated NaOAc (4 mL), diluted with 20 mL of H_2O and extracted with EtOAc (2 x 20 mL). The EtOAc extracts were combined, dried with Na_2SO_4, filtered, and stripped to dryness. A gummy opaque solid (182 mg, 89% crude chemical yield) was recovered. A previously synthesized sample of [16-^{14}C]dihydroqinghaosu (lot #5994-123, WR-253997) was treated with $BF_3 \cdot OEt_2$ in a like manner to yield an additional 37.8 mg (0.73 mCi) of α,β-[16-^{14}C]arteether. These two lots were combined and purified by preparative HPLC (Rainin Dynamax C-18, 8 μ, 21.4 x 250 mm, 85% CH_3CN-H_2O, 9.9 mL/min, 224 nm) to remove α-[16-^{14}C]arteether (t_R = 11 min, 51 sec) and impurity (t_R = 23 min, 20 sec). The crude product was dissolved in absolute EtOH, and aliquotes containing approximately 5 mg of material were injected on the column. The fractions containing β-[16-^{14}C]arteether were collected (t_R = 25 min, 19 sec), stripped, and analyzed by analytical HPLC (Altex Ultrasphere-ODS, 5 μ, 4.6 x 250 mm, 60% CH_3CN-H_2O, 1.2 mL/min) using a radio-detector (cell: H130U3). Impure fractions containing significant amounts of β-[16-^{14}C]arteether were combined and repurified. All of the fractions containing pure β-[16-^{14}C]arteether were combined and stripped to give 103.3 mg (2.17 mCi, 51% radiochemical yield) of material with specific activity of 6.58 mCi/mmol. The entire lot (6376-28) was shipped to Dr. Theoharides.

Chemicals and Sources

Absolute Ethanol	Aaper	
Benzene	Burdick & Jackson	AR756
Boron Trifluoride Etherate	Aldrich	00212 CT
Sodium Acetate	Fisher	S-210
Ethyl Acetate	Fisher	E145-4
Hexanes	Fisher	H291-4
Sodium Sulfate	Fisher	S420-3
Acetonitrile	Burdick & Jackson	AX503

References

1. Radiomatic Oxi-test, ^{14}CO, Cat. No. 9001070, Batch No.1 8901, April, 1989, referred to NBS standard 4222.
2. Lin, A. J.; Klayman, D. L.; Milhous, W. J. Med. Chem. 1987, 30, 2149-2150.
3. Lin, A. J.; Lee, M.; Klayman, D. L. J. Med. Chem. 1989, 30, 1249-1252.