TRITIUM LABELING OF POTENTIAL LIPOPHILIC MYELIN PROBES

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SUMMARY

Two potential lipophilic myelin imaging agents (1,1,2,2-tetrafluoro-1,2-diphenylethane and 1-fluoroadamantane) were tritium labeled. The most effective method employed the microwave discharge activation of tritium gas technique and resulted in specific activities of 177 mCi/mmol for 1,1,2,2-tetrafluoro-1,2-diphenylethane and 593 mCi/mmol for 1-fluoroadamantane. Using this tritiation method significant amounts of tritium-for-fluorine substitution was also observed in the labeling of 1-fluoroadamatane, resulting in nearly equivalent amounts of tritiated adamantane and fluoroadamantane.

Key Words: Tritium labeling, Exchange labeling, Microwave discharge labeling, Myelin, Positron Emission Tomography

INTRODUCTION

Although a number of neurological disorders are primarily or entirely manifested in white matter of the brain, there currently exists no clinical diagnostic procedure for the noninvasive quantitative evaluation of brain myelin structure and composition. Several clinical imaging methods under development at present offer the potential to differentially image myelin in the human brain. Tomographic nuclear magnetic resonance (NMR) scanning has been used to demonstrate brain white matter in both normal (1) and pathologic conditions (1,2). A second approach to tomographic myelin imaging involves the use of lipophilic gamma (3) or positron (4,5) emitting radiotracers which preferentially accumulate in white matter under equilibrium conditions. The current study is aimed at the tritium labeling of several potential lipophilic myelin probes to be used for in vivo animal evaluation.

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The compounds, 1,1,2,2-tetrafluoro-1,2-diphenylethane (tetrafluorobibenzyl, TFBB) and 1-fluoroadamantane (FAd), were chosen for three reasons, 1) their expected ease of labeling (6,7,8) with the short lived positron emitting isotope ^{18}F (T 1/2 110 min) using ^{18}F-F_2 (9) lend them well to positron emission tomographic (PET) studies, 2) the ^{18}F labeled compounds are likely to be biologically stable (10) and 3) both TFBB and FAd are highly lipophilic and thus would be expected to concentrate in brain myelin.

MATERIALS AND METHODS

Adamantane and 1-bromoadamantane were purchased from Aldrich Chemicals trans 1,2-difluorostilbene from PCR and Freon-11 and 2% F_2/Neon from Matheson. These were used as received from the suppliers. Silica Gel (TLC and bulk) were purchased from Merck.

The catalytic exchange tritiations were carried out by New England Nuclear Tritium Labeling Service Division. The microwave discharge activation labelings were carried out at Brookhaven National Laboratory in the Department of Chemistry. These tritiations used Metricel TCM-200 filters as the substrate support surface purchased from Gelman and tritium gas ($^3H_2$) supplied by Oak Ridge National Laboratory.

HPLC analyses were carried out on a Waters Model 6000A chromatograph equipped with a U6K injector and 440 UV absorbance monitor monitored at 254 nm. Columns used were: μBondapak C-18 (Waters) and MicroPak Si-10, CN-10 and MCH-10 (Varian). GLC analyses were carried out on a Varian 4600 GLC with the CDS-401 Data System.

Tritiated samples were counted using both a Beckman LS-II and a Packard 460 CD liquid scintillation spectrometers with internal standard calibration.

Tetrafluorobibenzyl was prepared by direct fluorination with elemental fluorine (F_2) of 1,2-difluorostilbene (-78° in Freon-11) by the method of Guzikowski, MacGregor and Fowler (6) purified by silica gel chromatography and recrystallization from ethanol. Fluoroadamantane was prepared both by direct fluorination of adamantane using the method of Alker, et al (7) and by direct fluorination with F_2 of 1-bromo-adamantane as described by Rozen and Brand (8). The latter method was preferred due to its higher yield. The fluoroadamantane was also purified by silica gel chromatography and recrystallization from ethanol. GLC purity determination (OV-101 column

at 100°C) showed the FAd to be 99.5% pure containing 0.5% residual 1-bromoadamantane.

Tritium Labeling of Tetrafluorobibenzyl

Method 1. Exchange labeling was attempted by New England Nuclear Tritium Labeling Services. TFBB (27.0 mg) was dissolved in 0.4 mL of 3:1 dimethylformamide: triethylamine (very dry); to this was added 75 mg of 5% Rh/C catalyst and 25 Curies of tritiated water. The reaction mixture was stirred for 18 hours at 80°C. Labile tritium was removed in vacuo, using ethanol as solvent. After filtration from the catalyst, the product was again taken to dryness, in vacuo, and then taken up in 50 mL of ethanol. An aliquot (50 mCi) was removed and shipped from 116.27 mCi produced. This method produced no labeled TFBB as determined by TLC chromatography (silica gel, using 10% ether/90% petroleum ether). All activity on the TLC plate remained at or near the origin.

Method 2. Carried out at New England Nuclear Tritium Labeling Services. TFBB (27 mg) was dissolved in 0.3 mL of trifluoroacetic acid (very dry); to this was added 75 mg of 5% Rh/C catalyst and 25 Curies of tritiated water. The reaction mixture was stirred for 16 hours at 80°C in a sealed system. Labile tritium was removed, in vacuo, using ethanol as solvent. After filtration from the catalyst, the product was taken to dryness in vacuo, and then taken up in 50 mL of ethanol. TLC (as above) of this sample showed two peaks, TFBB (Rf = 0.41) which contained 62% of activity and a 38% impurity (Rf 0.08). TFBB prepared by this method had a calculated specific activity of 0.28 mCi/mg (72 mCi/mmol) after purification by preparative TLC on silica gel using 5% ether/95% petroleum ether. Analysis of the purified material on silica gel and alumina TLC showed the TFBB to be > 98% purity. No other peaks were observed.

Method 3. Microwave Discharge Activation of Tritium Gas (MDA). This method involves the solid phase exchange labeling of the substrate by reactive tritium species formed by passing a stream of tritium gas through a microwave discharge (2450 MHz). This method has been successfully used to label a variety of compounds and has been previously described (11). The labeling of TFBB was carried out using

a microporous membrane filter as the carrier surface for the substrate and a modified reaction vessel capable of holding multiple microporous membrane filters as detailed in reference 12. Accordingly 3 membrane filters (Gelman Metricel TCM-200 47 mm in diameter 0.20 μm pore size) were coated with a solution of TFBB (2.06 mg/200 μL hexane) and allowed to air dry. The three filters, held in a slotted teflon holder, were placed into the labeling vessel and the system was evacuated. The labeling was carried out for 6 minutes using 4 Torr (2 Ci) $^3H_2$ gas pressure. The tritium gas was activated using 30 watts (2450 MHz) of forward microwave power. The gas was circulated in the closed loop reaction system with an internal cycling pump. The substrate was not cooled during the reaction. During the 6 minutes the pressure dropped from 4.0 to 1.3 torr, which resulted in an increase in the size of the discharge plasma.

The crude TFBB, which was eluted from the filters using 10 mL of 95% ethanol contained 168.0 mCi. The ethanol was evaporated leaving 31.7 mCi of nonlabile tritium. Chromatographic purification was carried out using successive silica gel columns (10 cm x 0.9 cm) with hexane as the eluting solvent. The final purification resulted in a recovery of 4.3 mCi of ^{3}H-TFBB with a calculated specific activity (based on 6.18 mg labeled) 0.70 mCi/mg (177 mCi/mmol). Radiochemical purity of the ^{3}H-TFBB was determined by TLC (98%) and by HPLC (95.6%). HPLC analysis was carried out using a reverse phase C-18 column eluted with 70% acetonitrile/30% water (isocratic) at a flow of 1 mL/min. Samples were collected and counted. Under these conditions 95.9% of the recovered activity eluted with TFBB (11.5 min) two other minor peaks were observed accounting for 3.9% (retention time, 12.5 min) and 0.2% (retention time, 16 min) of the recovered tritium activity respectively.

Tritium Labeling of 1-Fluoroadamantane

Method 1. Microwave discharge activation of tritium gas. As described previously for TFBB, the FAd (4.0 mg in 200 μL hexane) was deposited a Metricel TCM-200 filter (47 mm diameter, 0.20 μm pore size). The single filter was exposed to the microwave-activated tritium gas stream. Tritium gas at 3.5 torr (1.75 Ci) was used with a microwave power of 30 watts for 15 minutes at ambient temperature. During

this time the pressure dropped from 3.5 to 3.0 torr. 80.0 mCi of crude ^{3}H-FAd was recovered from the filter by ethanol elution. Solvent evaporation resulted in recovery of 64 mCi of nonlabile tritium. Chromatography on silica gel (10 cm x 0.9 cm column) using hexane and 10% chloroform/90% hexane resulted in two major tritium peaks containing 14.8 mCi and 15.4 mCi. TLC on silica gel (hexane as developing solvent) showed the two peaks to be adamantane (Rf=0.75) and 1-fluoroadamantane (Rf 0.40) respectively, in a ratio of 0.96/1. This assignment was also confirmed by comparison with silica gel column chromatography of nonlabeled materials and HPLC (see below). The calculated specific activity of the ^{3}H-FAd was 3.85 mCi/mg (593 mCi/mmol). TLC and HPLC purity analyses are described below.

Method 2. Direct fluorination of ^{3}H-adamantane (7). ^{3}H-Adamantane which was recovered from the MDA tritiation of FAd by ^{3}H-for-F substitution was used in this procedure as the source of ^{3}H-Ad. To 5.95 mCi of ^{3}H-Ad was added 20.5 mg (150 µmol) of "cold" adamantane in 10 mL of Freon-11. The solution was cooled to -78° and fluorine gas (2.0% in N_2, 0.8 µmol/mL) was bubbled through the solution at 30 mL/min for 7 min (168 µmol F_2). The reaction solution was evaporated to dryness with a stream of argon gas and the resulting residue (5.34 mCi) chromatographed on silica gel (10 cm x 0.9 cm) with hexane, 10% $CHCl_3$/90% hexane. Unreacted adamantane (4.9 mCi) and fluoroadamantane (375 µCi) were thus separated and collected. The two fractions were analyzed by TLC and HPLC for radiochemical purity.

TLC analysis on silica gel using hexane showed ^{3}H-adamantane to be 99% pure (Rf=0.75) with 1% remaining at the origin. ^{3}H-Fluoroadamantane (Rf=0.40) was > 98% pure with no other observed peaks.

HPLC analysis of ^{3}H-adamantane was carried out on a bonded nitrile column using 100% acetonitrile. Purity was > 98% with no other peaks observed and 103% ^{3}H recovery from the column. ^{3}H-fluoroadamantane was chromatographed on a Varian Si-10 silica gel column in 100% hexane. Purity was > 98% with no other peaks observed. Tritium recovery from the column was 112%.

Although not used in these analyses, adamantane and fluoroadamantane despite their high melting points are quite volatile and readily chromatographed by GLC

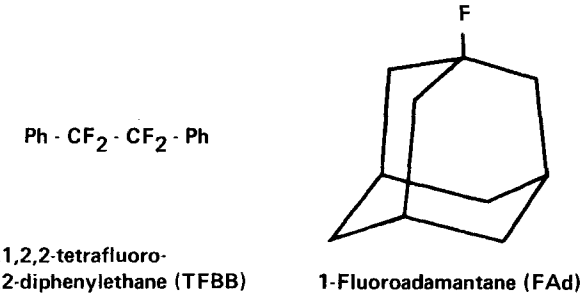
at low temperatures. Using a 5% OV-101 (50 cm x 1/8") column at 100°C and 20 cc/min helium flow rate, fluoroadamantane has a retention time of 2.3 min, adamantane 1.7 min.

Octanol/Saline Partition Coefficients (13)

Each of the above tracers (TFBB, Ad, FAd) was partitioned between octanol and aqueous saline as an index of their lipophilicity. The tritiated compound was added to tubes containing 2 mL each of octanol and phosphate buffered saline (pH 7.4), vortexed and finally centrifuged to separate the phases. Duplicate aliquots were analyzed for radioactivity by liquid scintillation counting. To detect the possible presence of contaminating impurities in the tritiated tracers the octanol phases were repartitioned against fresh saline and the determination of radioactivity in each phase repeated.

Figure

Proposed Fluorinated Myelin Agents



1,1,2,2-tetrafluoro-1,2-diphenylethane (TFBB)

1-Fluoroadamantane (FAd)

RESULTS AND DISCUSSION

Labeling of TFBB using Rh/C with a tertiary amine (triethylamine) and tritiated water was unsuccessful with this aromatic system whereas when the same catalyst was used under acid conditions (trifluoroacetic acid) moderate labeling occurred (72 mCi/mmol). In contrast, labeling using the microwave activation of tritium gas method (MDA) with the substrate deposited on a microporous membrane filter yielded a

product that was readily purified at a specific activity of 177 mCi/mmol, a further indication of the versatility of this method.

Tritium labeling of fluoroadamantane using the MDA method was quite striking, and resulted in a specific activity of nearly 0.6 Ci/mmol in the labeled parent compound. This high specific activity may be attributable to labeling at the tertiary methyne hydrogens abundant in adamantane. Selectivity for labeling at such tertiary positions using the MDA method has also been observed in amino acids (14) and in cyclic and branched alkanes (15).

In the case of 1-fluoroadamantane labeling, a second major reaction was observed, i.e. tritium-for-halogen substitution producing approximately equal amounts of ^{3}H-adamantane and ^{3}H-fluoroadamantane. This supports other observations demonstrating facile tritium halogen substitution when labeling using MDA method (16,17). This by-product can be either a contamination nuisance or desirable product yielding a specifically labeled carrier free dehalogenated tracer, depending on experimental design. With this in mind, we successfully refluorinated the ^{3}H-adamantane formed in this fashion. The yield of the ^{3}H-FAd thus produced (6%) did not represent an optimized reaction, and the unreacted ^{3}H-adamantane was easily recovered during the chromatographic purification.

Since the purpose of this study was to prepare labeled lipophilic myelin probes, a quantitative determination of the lipophilicity of these three compounds was carried out (see table below).

TABLE

Octanol-Saline Partition Coeffieients

Tracer	log P*	number of determinations
^{3}H-TFBB	5.0 ± 0.1	14
^{3}H-Ad	5.2 ± 0.1	22
^{3}H-FAd	5.7 ± 0.1	16

*Log_{10} of the octanol to phosphate buffered-saline (pH 7.4) partition coefficient (13).

It is anticipated that these compounds or structurally related ones i.e., fluorinated aryl olefins or precursors with multiple tertiary C-H bands will prove suitable as positron emitting myelin probes.

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REFERENCES

1. Doyle F.H, Pennock J.M., Orr J.S. et. al. - Lancet 11: 53 (1981)
2. Young I.R., Hall A.S., Palus C.A., et. al. - Lancet 14: 1063 (1981)
3. Frey K.A., Wieland D.M., Brown L.E., Rogers W.L. and Agranoff B.W. - Annals of Neurology 10: 214 (1981)
4. Dischino D.D., Kilbourn M.R., Raichle M.E. and Welch M.J. - J. Nucl. Med. 23: P-23 (1982) abst.
5. Dischino D.D., Wittmer S.L., Raichle M.E. and Welch M.J. - J. Labeled Comp. Radiopharm. 18: 238 (1981) abst.
6. Guzikowski A.P. - Doctoral Thesis "The Addition of Molecular Fluorine to Tolanes and Stilbenes" May 1980, University of Massachusetts, and R.R. MacGregor, J. Fowler, Brookhaven National Laboratory (personal communication)
7. Alker D., Barton D.H.R., Hesse, R.H., et al - Nouv. J. Chim. 4: 239 (1980)
8. Rozen S., Brand M. - J. Org. Chem. 46: 733 (1981)

9. Casella V., Ido T., Wolf A.P. - J. Nucl. Med. 21: 750 (1980)

10. Carbon-Fluorine Compounds. Chemistry Biochemistry And Biological Activities. A Ciba Foundation Symposium - Associated Scientific Pub. Amsterdam, 1972.

11. Hembree W.C., Ehrenkaufer R.L.E., Lieberman A. and Wolf A.P. - J. Biol. Chem. 248: 5532 (1973)

12. Ehrenkaufer R.L.E., Wolf A.P., Hembree W.C. - J. Labelled Comp. Radiopharm. 14: 271 (1978)

13. Leo A., Hansch C., Elkins D. - Chem. Rev. 71: 525 (1971)

14. Ehrenkaufer R.L.E., Hembree W.C., Lieberman S., Wolf A.P. - J. Amer. Chem. Soc. 99: 5005 (1977)

15. Gordan B.E., Peng C.T., Erwin W.R. and Lemmon R.M. - Int. J. Appl. Radiat. Isot. 33: 715 (1982)

16. RE unpublished results. The MDA labeling of toslyl leucyl chloromethylketone (TLCK) resulted in a major product which was tentatively identified as ^{3}H-TLMK (tosyl leucyl methyl ketone).

17. Peng C.T., Gordan B.E., Erwin W.R. and Lemmon R.M. - Int. J. Appl. Radiat. Isot. 33: 419 (1982)