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A New and Efficient Synthesis of Monotritiomethyl Iodide

Manouchehr Saljoughian , Hiromi Morimoto, Albert M. Dorsky and Henry Rapoport.
National Tritium Labeling Facility, Lawrence Berkeley Laboratory, University of California, Berkeley, CA 94720 USA.

Hendrik Andres , Sandoz Ltd, Basle, Switzerland.
Yui S. Tang and Alexander Susan. Sandoz Pharmaceuticals Corp., East Hanover, NJ 07936 USA.

Summary

A new and efficient synthesis of high specific activity TCH_2I containing one atom of tritium per molecule is reported. The precursors for this reagent, chloromethyl p-phenylbenzoate or bis-chloromethyl terephthalate, were prepared by the condensation of the corresponding acid chlorides with paraformaldehyde in the presence of zinc chloride. Tritiodehalogenation of the precursors with tritium gas and Pd-C (10%) in dimethylformamide afforded the desired tritioesters. Cleavage of the esters by lithium iodide at 180°C (SN_2 type reaction), or by HI at 140°C gave mono tritium labeled methyl iodide in more than 95% yield. The reaction of the latter with a number of amines yielded tritium labeled N-methylated products readily as was shown by Radio-HPLC. The specificity of the labeling in the final products was confirmed by tritium nmr spectroscopy.

Key Words: Monotritiomethyl Iodide, Synthesis, [^{3}H]-N-Methylation, Tritium NMR Spectroscopy.

Introduction

The need for tritium labeled compounds specifically labeled in the methyl group as a probe for biological studies has become more and more apparent. Therefore, an extensive effort has been made to find a general method for the labeling of methyl groups with high specific activity.

According to literature references, methyl groups in a wide variety of organic compounds can be labeled with tritium by several different procedures, e.g. catalysed exchange with tritium gas or tritiated water, halogenation followed by tritiodehalogenation, direct labeling by the Wilzbach method and catalytic reduction of formyl group.[1] Most notable of the methods with wider applicability are the various processes of methylation, employing tritiated methylating

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reagents.These reagents are currently derived from tritiated methanol which is available from the catalytic reduction of carbon monoxide and/or carbon dioxide with tritium gas or tritium-hydrogen mixtures (Fischer Tropsch type synthesis).[2] Subsequent conversion of this reagent into [^{3}H]methyl iodide using hydriodic acid has been reported with 100% isotopic abundance.[3] Monotritiomethyl iodide with low to moderate specific activity has previously been prepared by a number of different methods, e.g. the reaction of HTO with diazomethane and methyl formate.[4] These reactions give monotrititated methanol which, after hydrolysis with HI, yield TCH_2I. Monotritiomethyl iodide at nearly theoretical specific activity was also prepared from bis-chloromethyl ether through several steps.[5] In this method, the precursor was hydrogenolyzed and the tritiated dimethyl ether hydrolysed with hydriodic acid. All of these tritiation procedures published to date suffer from several drawbacks, such as hazardous conditions, highly toxic reagents, numerous steps and low yields.

We now report a new and efficient synthesis of high specific activity TCH_2I by preparation of chloromethyl esters as the precursors. Tritiodehalogenation of the precursors with tritium gas in the presence of Pd-C afforded the tritiated esters which were then cleaved with either LiI or HI.

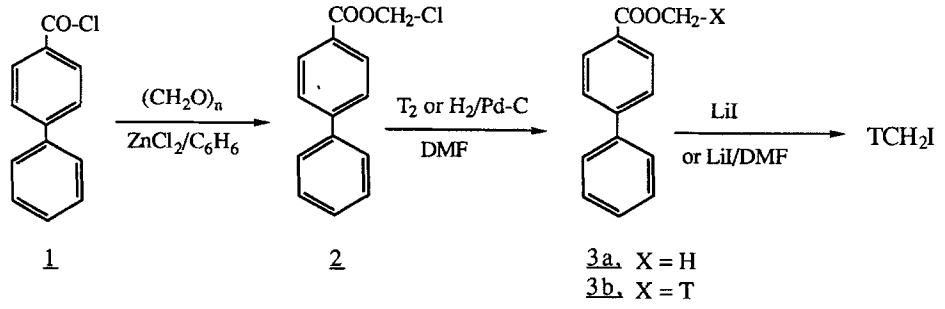
Results and Discussion

We believe the most efficient method to obtain [^{3}H]methyl iodide with specific activity of 29 Ci/mmol is through the tritiation of chloromethyl esters and subsequent hydrolysis or cleavage of the tritiomethyl esters, leading simply and directly to [^{3}H]methyl iodide.

(a) Synthesis of Chloromethyl Esters

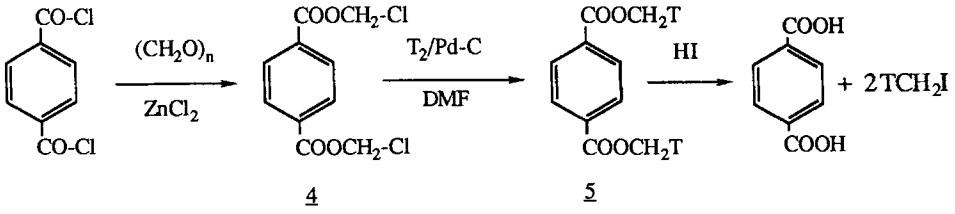
Chloromethyl benzoate (bp 115/8mmHg) was prepared by the condensation of benzoyl chloride and paraformaldehyde in the presence of fused zinc chloride.[6] Similarly the solid chloromethyl p-phenylbenzoate (mp 102^o) was prepared by the condensation of biphenylcarbonyl chloride and paraformaldehyde in the presence of fused zinc chloride in 35% yield (Scheme I). Using trioxane as the source of formaldehyde in this reaction gave slightly better yield of the desired product (38%). Preparation of bis-chloromethyl terephthalate (mp 125^o) was finally achieved in 49% yield by fusion of terephthalic acid chloride and paraformaldehyde in the presence of zinc chloride (Scheme II).

Scheme I



1 2 3a, X = H 3b, X = T

Scheme II



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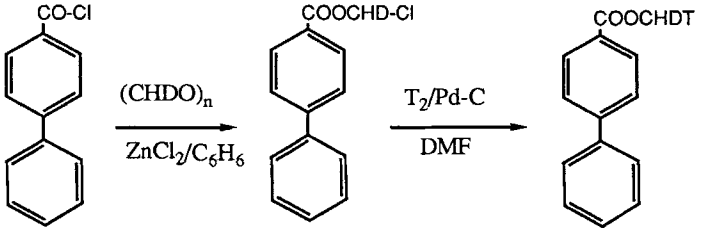
(b) Hydrogenolysis and Tritiation of Chloromethyl Esters

Hydrogenolysis of the C-Cl bond in chloromethyl benzoate was very slow and under one atmosphere of hydrogen and with various amounts of Pd/C (10%) in the presence of triethylamine, there was only 30% reduction over 48-72 hrs. In contrast, complete hydrogenolysis of the C-Cl bond of chloromethyl p-phenylbenzoate, with H_2 in the presence of Pd-C (10%) and in DMF, occurred in less than 12 hrs and greater than 95% conversion to the methyl p-phenylbenzoate as was shown by proton nmr and thin layer chromatography. These results indicate that the presence of the p-phenyl substituent in the precursor has influenced the rate of hydrogenolysis of the C-Cl bond. The reason for this enchanced reactivity is as yet not known. Catalytic tritium-halogen replacement (using carrier free tritium gas) of chloromethyl p-phenylbenzoate over Pd-C (10%), with purified DMF as the solvent gave monotritiomethyl p-phenyl benzoate (specific activity, 22 Ci/mmol) with a radiochemical purity of greater than 98% as shown by radio-HPLC. In another experiment, the reaction of [^{2}H] paraformaldehyde[7] with p-phenylbenzoyl chloride gave chloromonodeuteriomethyl p-phenylbenzoate (Scheme III). When this compound was subjected to tritium-halogen replacment, tritiodeuteriomethyl p-phenyl benzoate resulted. This compound is the precursor for the synthesis of (dl) chiral methyl iodide. By analysis of the ^{1}H coupled ^{3}H nmr spectra of these two compounds, it was possible to extract values for proton-proton geminal coupling constants of the methyl groups, which are inaccessible in any other way. The chemical shifts and coupling constants are given in the experimental section.

Scheme III



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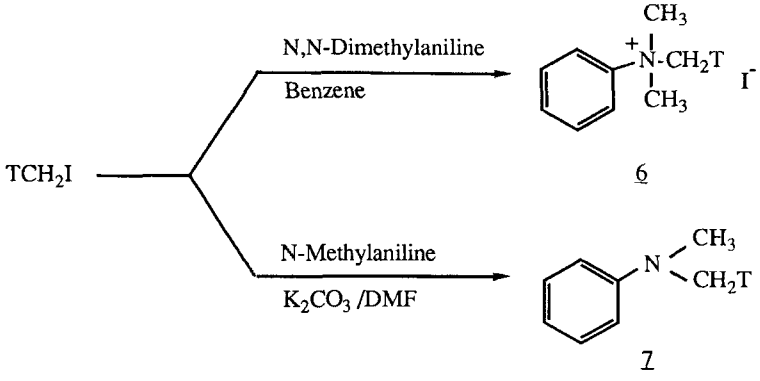
Catalytic tritium-halogen replacement of bis-chloromethyl terephthalate with carrier free tritium over Pd-C (10%) with DMF as solvent afforded di-[methyl-^{3}H]terephthalate at a specific activity of 40-52 Ci/mmol depending on the excess tritium used.The specific activity of the di-[methyl-^{3}H]terephthalate could readily be estimateded by proton NMR. The 360 MHz spectrum revealed a doublet with J_{HT}=11.7 Hz at 3.92 ppm for the CH_2T group and a small singlet at 3.94 ppm for the unlabelled methyl groups. The estimation of the specific activity was done by peak height comparison. The resulting di[methyl-^{3}H] terephthalate is slightly volatile (slow sublimation at 20^{o}C under high vacuum) and for this reason we finally chose chloromethyl p-phenyl benzoate as the preferred chloromethyl precursor.

(c) Generation and Reaction of [^{3}H]Methyl Iodide

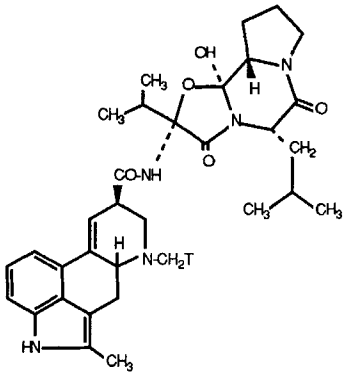
Cleavage of esters to iodides is generally achieved by treatment with hydriodic acid at 140^o C, and with di-[methyl-^{3}H]terephthlate, [^{3}H]methyl iodide was produced in 90-95% yield by HI treatment. In an alternative method, the ester was treated with lithium iodide and heated to 180^o C to generate methyl iodide in 97% yield. This reaction presumably proceeds via nucleophilic attack by iodide ion on the methyl group with concomitant displacement of carboxylate anion. Lithium iodide has previously been used for cleavage of the methyl esters to the corresponding carboxylic acids, but the generation of methyl iodide from the reaction mixture has not been the primary goal in these earlier studies.[8-12] In this procedure, the absence of solvent makes a major contribution to the simplicity of the isolation, and the only volatile product, methyl iodide, is readily vacuum transferred (350 mm, liq.N_2). One can also use DMF as the solvent, refluxing the mixture for 3 hr at 160^o C. All of these procedures generate [^{3}H]methyl iodide at high temperature.

The generated methyl iodide was transferred as liberated (no trapping) to the substrate in K_2CO_3/DMF and yielded methylated products in 70-90% yield. Thus, the reaction of tritiated methyl iodide generated from monotritiomethyl p-phenylbenzoate (specific activity 15 Ci/mmol) with N-methylaniline gave [^{3}H] N,N-dimethylaniline in 50% yield with a specific activity of 12 Ci/mmol (Scheme IV).

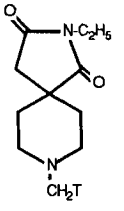
Scheme IV



The utility of the new preparation of [^{3}H]methyl iodide was further demonstrated by the [^{3}H]-N-methyl labeling of two pharmacologically active substances Sandoz RS-86 and Sandoz CBM 36-733. By this procedure [^{3}H] RS-86 was prepared at a specific activity of 20 Ci/mmol and [^{3}H] CBM 36-733 was prepared at a specific activity of 648 mCi/mmol.



Sandoz CBM 36-733



Sandoz RS-86

Conclusion

We report a new and high yielding synthesis for monotritiomethyl iodide. The labeling procedure developed in this work afforded the tritiated products in a simple manner, and application of proton and triton NMR spectroscopy was used to determine the specificity of labeling in the final products. Lithium iodide, with or without solvent (DMF), and HI have been found to be very effective reagents for the cleavage of the tritium labeled esters. The generated methyl iodide has been directly reacted with the compound desired to be N-methylated in the presence of an organic base. This technique can provide tritium labeled methyl iodide with a specific activity of milli to multicuries/mmol depending on the activity of the isotopic source and is applicable in all radiochemistry laboratories which can handle tritium gas. Hence, we report the facile preparation of TCH_2I (and potentially of TDCHI) in high chemical yield with high radiochemical purity, a generally applicable reagent for [^{3}H]-N-methylation and similar reactions.

Experimental

Tritium gas was purchased from Oak Ridge National Laboratory and contained 97.9% T_2, with the largest contaminant being DT(1.76%). All starting materials were purchased and repurified prior to use. DMF was distilled from BaO immediately prior to use. ^{1}H and ^{3}H NMR spectra were recorded in $CDCl_3$ or D_2O, on an IBM AF-300 NMR spectrometer. HPLC analyses of the precursor and [^{3}H]-N-methylated products were performed using a Waters C-18 radial pak column with a mobile phase of $MeOH/H_2O/NH_4OH$(50/50/1, 3mL/min). Mass peaks were observed by UV detection at 249 nm on a HP 1040A diode array spectrophotometer, and radioactivity measurements were made with a Berthold HPLC flow detector,using a lithium glass scintillant cell with an efficiency of ca. 0.05%. Purifications by preparative radial thin layer chromatography were performed on a Chromatotron model 7924T (Harrison Reseach, Palo Alto CA). Tritiated samples were counted with

a Packard 2002 liquid scintillation counter. Melting points were determined on an electrothermal apparatus and are uncorrected. All micro and mass spectrometric analyses were carried out by the Analytical Laboratory, College of Chemistry, University of California, Berkeley.

Chloromethyl p-Phenylbenzoate(**2**).

4-Biphenylcarbonyl chloride (1.4g, 6.5 mmol) was dissolved in dry, thiophene free benzene (10 mL) and paraformaldehyde (1.5g) was added. To this mixture a trace of zinc chloride was added and it was refluxed at 90°C for 1.5 hr. TLC R_f 0.6 ($CHCl_3$/Hexane-1/7) showed a product and unreacted starting material. Longer reaction time up to 24 hr did not give more product, but a by-product was formed.The reaction was then discontinued, cooled and the solvent was evaporated. The residue was flash chromatographed (CH_2Cl_2) and the product was separated from the starting material and the by-product using preparative radial thin layer chromatography : yield, 540 mg,2.17mmol (35%); mp 101-102°C; ^{1}H NMR ($CDCl_3$): δ 6 (s,2H), 7.5(m,3H),7.6(d,2H), 7.7(d,2H), 8.1(d,2H). Anal. Calcd for $C_{14}H_{11}ClO_2$: C,68.1;H,4.4; Cl, 14.4. Found: C,67.9; H,4.4; Cl,14.4.

Methyl p-Phenylbenzoate(**3a**)

A mixture of p-phenylbenzoic acid (3g, 15 mmol), absolute methanol (8g) and conc. H_2SO_4 (0.5g) were placed in a round-bottomed flask and refluxed at 70°C for 5 hr. The excess alcohol was then distilled off. The residue was poured into water (10mL) and washed with concentrated aqueous $NaHCO_3$ until all the acid was removed. The aqueous solution was extracted with $CHCl_3$ (3x15mL) and the combined organic layer dried ($MgSO_4$). The solid residue after the evaporation of $CHCl_3$ was recrystallized from ethanol (96%): yield, 2.5g, 11.8 mmol (78%); mp 112°C (lit.[13] mp 115-116°C); ^{1}H NMR ($CDCl_3$): δ 3.9(s,3H), 7.4(m,3H), 7.6(m,4H), 8.1(d,2H).

[Methyl-$^3H_1$] p-Phenylbenzoate(**3b**)

Chloromethyl p-phenylbenzoate (**2**,7.5 mg, 0.03 mmol) was dissolved in DMF(1mL) and Pd-C(10%,15 mg) was added. N,N-Diisopropylethylamine(5μL) was added and the compound was tritiated with T_2 for 12 hr.The solution was then filtered and the filtrate was freeze-dried. A white solid,**3b**, was left as the only product of the hydrogenation : yield,6 mg,0.3 mmol (95%); specific activity 22 Ci mmol^{-1};^{1}H NMR ($CDCl_3$): δ 3.9 (s,2H),7.4(m,3H),7.6(m,4H),8.1(d,2H); ^{3}H NMR (^{1}H decoupled) 3.9 (s); ^{3}H NMR (^{1}H Coupled) 3.9(t, $^2J_{HT}$ =11.7 Hz, $^2J_{HH}$ =10.9 Hz).

Bis-chloromethyl Terephthalate(**4**)

Terephthalic acid chloride (50 mmol) and paraformaldehyde (100 mmol) were fused together with a catalytic amount of zinc chloride and held at 120° C for two hours with occasional stirring. The coold reaction mixture was then extracted with CH_2Cl_2 which was evaporated and the crude product was chromatographed over silica gel using CH_2Cl_2 as eluent. Recrystallization from CH_2Cl_2 - cyclohexane afforded pure product : yield, 6.5 g (49%) m.p.120-125° C ;^{1}H NMR (C_6D_6): δ 5.27(s,4H), 7.75(s,4H).Anal. Calcd for $C_{10}H_8Cl_2O_4$: C, 45.6; H, 3 ;Cl, 27 Found: C, 45.3 ; H, 3; Cl,26.7.

Di-[methyl-$^3H_1$]Terephthalate(**5**)

Bis-chloromethyl terephthalate (**4**,14 mg, 0.05 mmol) was dissolved in DMF (0.5mL) and Pd-C (10%, 10 mg) and diisopropylethylamine (10 μL) were added. The solution was tritiated with 10 Ci of T_2 for 1 hour at room temperature. Then the mixture was frozen, the excess T_2 pumped off and the solution lyophilized. t-Butanol was added two times and lyophylized in order to remove labile tritium. The residue was flash chromatographed over silica gel using methylene chloride. The crude product (10.5 mg, 108%) was directly converted to [^{3}H] methyl iodide.^{3}H NMR ($CDCl_3$): δ 3.4 (s).

The following experiments illustrate various methods of [^{3}H]methyl iodide generation

a) Treatment of the precursor with HI.

The [^{3}H] methyl iodide was generated from its precursor **5** using the apparatus shown in Figure 1 and the following procedure:

Bis[methyl-^{3}H]terephthalate (**5**,4.84 mg, 0.0250 mmol, specific activity 40 Ci/mmol) was introduced into the reaction flask (a) followed by acetic anhydride (52 μL), hypophosphorous acid (40 μL) and HI (400 μL of a 57% aqueous solution), at 0°C. The reaction mixture was stirred at 0°C for 30 min and the temperature was allowed to reach 20°C during an additional stirring for 30 min. Next, after establishing a slow stream of nitrogen in the apparatus shown in Figure 1, the temperature was slowly increased to 125°C over a period of 10 min while the methyl iodide was generated as evidenced by the precipitate formed in the $CuSO_4$ solution. The reaction mixture was stirred at 125°C for 2 hours followed by the introduction of additional HI solution (8mL) and heating at 125°C for one more hour. Further, the trap (g) was disconnected and attached to a vacuum manifold, frozen in liquid nitrogen , its content degased at this temperature then transferred into a reaction tube containing the nor-derivative of the compounds to be tritiated, the equivalent amount of diisopropyl ethylamine and DMF. The reaction tube was then sealed under vacuum and its content stirred at an appropriate temperature needed for completion of the N-methylation. Finally, the usual isolation consisted of removal of the volatile components followed by preparative HPLC isolation and analysis of the pure reaction product.

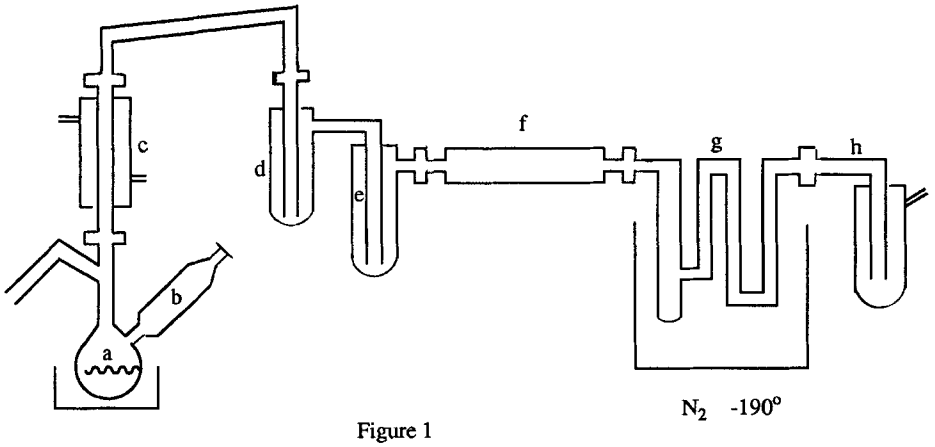


Figure 1

a-15 mL two necked reaction flask b-funnel c-condenser d-10 mL 5% ascorbic acid in water

e-15 mL 5% cupric sulfate in water f- phosphorus pentoxide g-trap h-bubbler

b) Treatment of the precursor with LiI in DMF

Methyl p-phenylbenzoate (**3a**, 106mg, 0.5mmol) was dissolved in DMF(1mL), lithium iodide(334 mg, 2.5 mmol) was added and the reaction mixture was gently heated to 140°C for 2-3 hr. At about 60 °C the lithium iodide dissolved in the DMF and the mixture became homogeneous. The extent of the reaction was monitored by TLC (CH_3CN/AcOH-99/1) and reaction was complete at this time. At the conclusion a precipitate formed which was not soluble in DMF. The mixture was then cooled to room temperature and the generated methyl iodide was vacuum transferred and trapped into a solution of N,N-dimethylaniline (44 μL) in benzene (250 μL). After heating this mixture for 30 min at 50-60°C a white precipitate was formed which was filtered to give trimethylphenylammonium iodide: yield, 88mg (58%);mp 215°C (lit.[8] mp 220°C).^{1}H NMR (D_2O): δ 3.6(s,9H), 7.7 (dm, 5H).

[^{3}H]Trimethylphenylammonium Iodide(**6**)

[Methyl-^{3}H] p-phenylbenzoate(**3b**, 21mg, 0.1mmol) was dissolved in DMF (0.5mL) and heated at 140-160°C for 3 hr with lithium iodide (83 mg,0.6 mmol). The reaction was carried out as above and [^{3}H] methyl iodide was trapped as described to give **6**:15.5 mg, $5.9x10^{-2}$ mmol (64%); ^{1}H NMR (D_2O): δ 3.6(s,9H), 7.2-7.49(m,5H).^{3}H NMR δ 3.6(s); Specific Activity 172 mCi/mmol.

[^{3}H]-N,N-Dimethylaniline(**7**)

[Methyl-^{3}H] p-phenylbenzoate (**3b**, 21mg, 0.1mmol, specific activity 15 Ci/mmol) was dissolved in DMF (0.5 mL) and heated at 140-160°C for 3 hr with lithium iodide (93 mg, 0.7 mmol). The generated methyl iodide was reacted with N-methylaniline (10 μL) in the presence of K_2CO_3 (60mg). The reaction mixture was analysed by radio-HPLC and showed compound **7** in 50 % yield which was trapped in a liquid scintillation solution and counted. Specific activity 12 Ci/mmol, ^{3}H NMR ($CDCl_3$): δ 2.9 (s).

c) Treatment of the precursor with LiI.

[Methyl-^{3}H] p-Phenylbenzoate (**3b**,10mg, 0.05 mmol, specific activity=12 Ci/mmol) and lithium iodide (138 mg,1mmol) were mixed and heated at 180°C for 1 hr. The generated [^{3}H] methyl iodide was reacted with N-methylaniline (5.3 mL) in the presence of K_2CO_3 (180 mg) in DMF (200mL) for 2-3 hr. The reaction mixture was analysed by Radio-HPLC and showed compound **7** in 50% yield, specific activity 10.7 Ci/mmol.

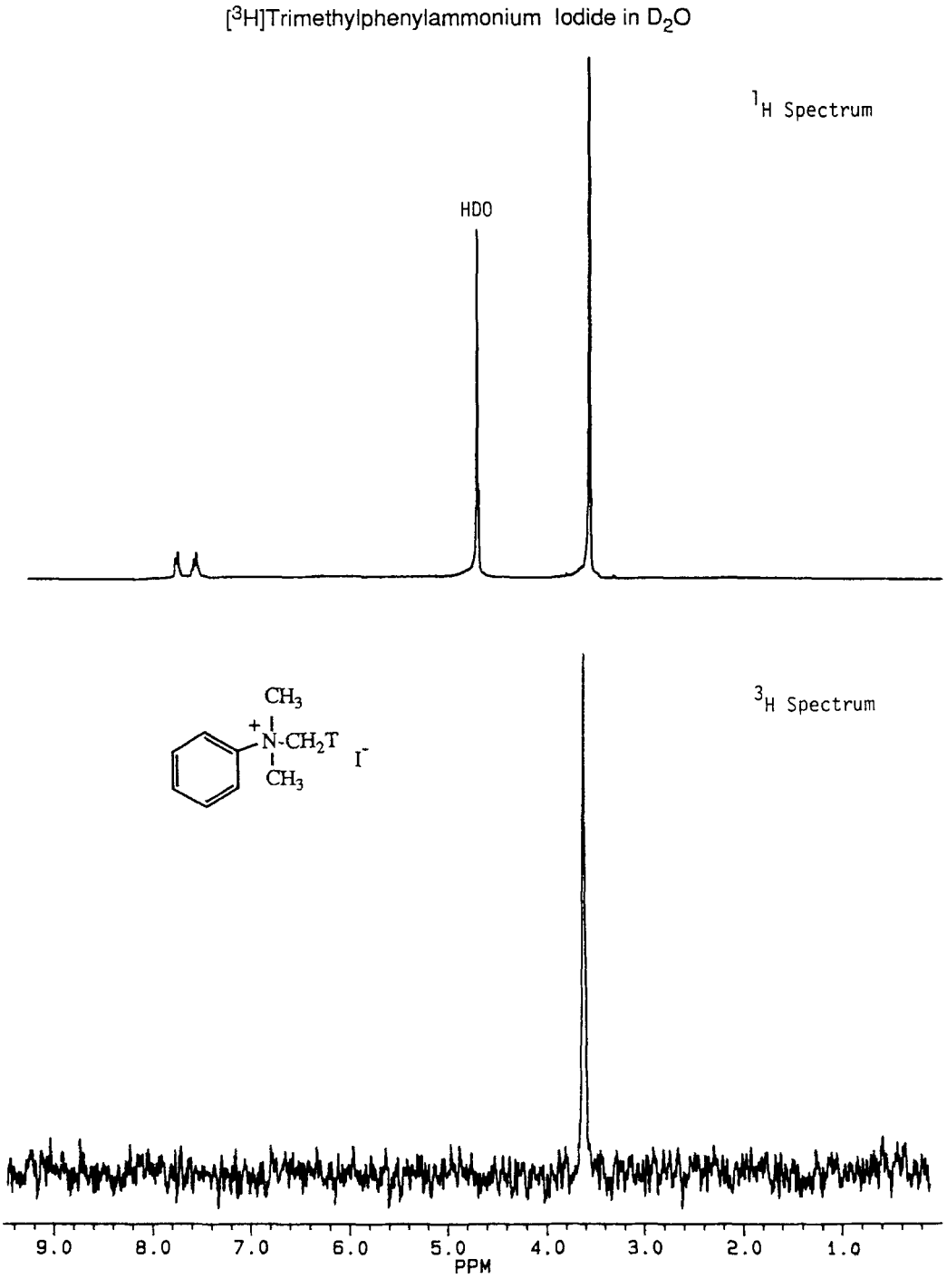
Chlorodeuteriomethyl p-Phenylbenzoate(**8**)

4-Biphenylcarbonyl chloride (120 mg, 0.56 mmol) was dissolved in dry benzene(1mL) and [^{2}H]paraformaldehyde (25 mg) was added. The chloromethyl ester formation was carried out as before and the product was purified by preparative radial thin layer chromatography to give **8**: 22mg, 0.09 mmol(16%); mp 100-102°C;^{1}H NMR ($CDCl_3$):δ 3.9 (s, 1H),7.4(m,3H),7.7(m,4H), 8.1(d,2H). ^{2}H NMR δ 3.9 (Bs,^{2}H). Ms m/z 247.

[Methyl-$^3H_1$-$^2H_1$] p-Phenylbenzoate(**9**)

Chlorodeuteriomethyl p-phenylbenzoate was tritiated under the same condition as (**3b**) to give **9**: 4.5mg, 0.23 mmol(69%); specific activity 18.6 Ci mmol^{-1}; ^{1}H NMR ($CDCl_3$): δ 3.9

(s,1H),7.4(m,3H), 7.6(m,4H), 8.1(d,2H); ^{3}H NMR δ (^{1}H decoupled) 3.9 (s); ^{3}H NMR δ (^{1}H coupled) 3.9 (d, $^{2}J_{HT}$=11.4Hz, $^{2}J_{HH}$=10.4 Hz).



Acknowledgements

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