A General Synthesis of Very High Specific Activity Tritiomethyl Iodide

Manouchehr Saljoughian,* Hiromi Morimoto, and Philip G. Williams
National Tritium Labeling Facility, Lawrence Berkeley Laboratory 75-123, University of California, Berkeley, CA 94720, USA

A general synthesis of highly tritiated or deuteriated methyl iodide has been elaborated. Tritiodehalogenation of trichloromethyl biphenyl-4-yl ether with tritium gas over Pd/C in ethyl acetate yields the corresponding tritiomethyl biphenyl-4-yl ether of very high specific activity. Reaction of this [^{3}H]product with hydroiodic acid hydrolyses the ether, and quantitatively liberates tritiomethyl iodide. Alkylation of a secondary amine with freshly generated tritiomethyl iodide gives a high yield, and demonstrates the utility of the reagent. Radio-HPLC analysis of the intermediate labelled ether and the amine product showed complete radiochemical purity with full retention of specific activity, and the specificity of labelling was confirmed in both products by ^{3}H NMR spectroscopy. Preparatory chemistry was performed with deuterium gas and the deuteriated reaction products, and analogous analyses performed by HPLC and ^{2}H NMR spectroscopy.

Labelled methyl groups have been used in a variety of situations to shed light on physical, biochemical and physiological processes. In order to obtain the required information, the methyl groups have been labelled with all of the available isotopes of carbon and hydrogen at one time or another. [^{14}C]Methyl labelling of methionine has been used to explore differences in metabolism of schizophrenic and normal subjects,[1] by following expired $^{14}CO_2$. Similarly, the localisation and utilisation of [^{11}C]methyl-methionine in mice and in human brains has been studied by NaI scintillation counting and with gamma cameras.[2] The action of methionine and other [^{11}C]methyl labelled drugs has been pursued by the use of positron emission tomography (PET),[3] where the label was usually incorporated through the use of $^{11}CH_3I$.[4,5] ^{13}C Labelling has recently been used in combination with proton detected ^{13}C NMR spectroscopy to give stereospecific assignments of leucine and valine methyl groups in a large protein, and to aid in characterisation of the side-chain conformation of the protein in solution.[6]

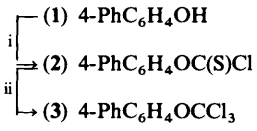
The toxicity of methionine has been followed by the administration of CD_3-methionine to rats and the use of *in vivo* ^{2}H NMR spectroscopy,[7] and the chemical specificity of the technique was demonstrated by the identification of detoxification intermediates. A host of biochemical transformations have been studied by the incorporation of chirally labelled methyl groups, *i.e.* containing H, D, and T, and exploration of the stereochemistry of the subsequent reactions.[8] In addition, receptor binding assays of neurotransmitters and their agonists[9] are made possible by the existence of tritiated ligands, some of which contain very highly labelled methyl groups.[10]

The importance of labelled methyl groups has previously led us to propose two new methods for the synthesis of monotritiomethyl iodide (up to 28.5 Ci mmol^{-1} †),[11,12] which would allow the production of the reagent in labelling facilities of almost any size. Although the singly labelled reagent is valuable, it is desirable to incorporate more radioactivity both regio- and stereo-specifically in compounds of biological interest, and thereby monitor reaction mechanisms and metabolism under extremely dilute conditions. Commercial sources of tritiomethyl iodide with close to 100% isotopic abundance of tritium exist, and rely on the conversion of tritiated methanol. However, the production of tritiated methanol involves stringent conditions,[13] and therefore is only amenable to use in commercial labelling laboratories. We now describe a new synthesis of very high specific activity methyl iodide under mild conditions.

Results and Discussion

The synthesis and use of very high specific activity methyl iodide was executed, with the following strategy: (*a*) synthesis of a trichloromethyl biphenyl-4-yl ether precursor (Scheme 1), (*b*) dehalogenation of this precursor (Scheme 2), followed by, (*c*) cleavage of the labelled biphenyl-4-yl methyl ether product, and, (*d*) reaction of the liberated methyl iodide with a substrate of interest (Scheme 3).

Synthesis of the Chlorinated Precursor.—In exploratory studies for this precursor, several compounds were prepared bearing a trichloromethyl moiety attached to heteroatoms such as O and S, *e.g.* trichloromethyl *N,N*-diphenylcarbamate, trichloromethyl *p*-fluoroanisole and trichloromethyl phenyl sulphide. None of these were found to have all the characteristics desired for preparation of a tritiomethyl iodide precursor with high molar specific activity. For example, trichloromethyl phenyl sulphide was readily synthesised and dehalogenated, but gave a highly volatile intermediate after hydrogenolysis which proved difficult to contain, and at high specific activity could pose a potential contamination hazard.



Scheme 1. *Reagents:* i, $SCCl_2$ + NaOH; ii, Cl_2 + $CHCl_3$.

Accordingly, a procedure for the preparation of a solid, stable trichloromethyl ether as our preferred precursor for the desired species was elaborated. The synthesis (Scheme 1) involved the preparation of an *O*-ester of chlorothiocarbonic acid[14] (**2**) from biphenyl-4-ol (**1**) and thiophosgene, and chlorinolysis[15] of the ester with chlorine gas to give the required trichloromethyl biphenyl-4-yl ether (**3**) in *ca.* 50% overall yield.

Dehalogenation of the Trichloro Ether.—The nonradioactive standard for the dehalogenated product (biphenyl-4-yl methyl ether) was produced by alkylation of biphenyl-4-ol with dimethyl sulphate in basic solution, at low temperature.

† 1 Ci = 3.7 × 10^{10} Bq.

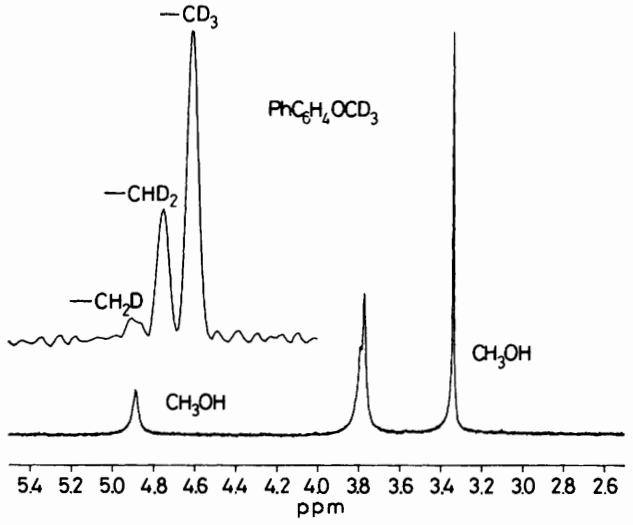


Figure 1. 46 MHz Deuterium NMR spectrum of biphenyl-4-yl [^{2}H]methyl ether in CH_3OH taken with 1 024 scans, showing the region from 2.5–5.5 ppm. The inset shows an expansion of the substrate resonances (3.68–3.90 ppm). The spectrum was acquired with 8 K points, and the FID of the expanded trace was Gaussian multiplied (LB = −1, GB = 0.20) and zero-filled to 16 K points before Fourier transformation.

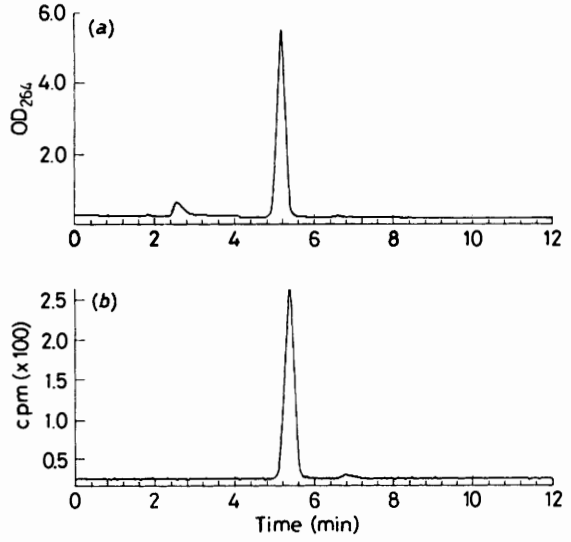
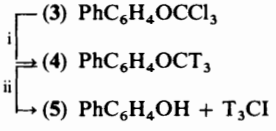


Figure 2. Radio-HPLC chromatogram of biphenyl-4-yl [^{3}H]methyl ether. (*a*) UV absorption trace (264 nm), and (*b*) radioactivity trace from the in-line solid scintillant detector. The traces are slightly offset in time since the sample passes through the UV cell before the radioactivity detector.

Table. Calculated specific activities (Ci mmol^{-1}) from mass spectral, radio-HPLC and NMR abundance of multiply labelled species.[a]

Reaction type	Mass spectral	% of Theoretical	NMR	% of Theoretical
Deuteriation reaction	69.6	80.8	67.1	77.9
Teflon reaction vessel	75.0	87.1	—	—
Superdeuteride reaction	78.4	91.0	—	—
Tritiation reaction	68.8[b]	80.0	70.2	81.5

[a] Assuming tritium has a specific radioactivity of 28.76 Ci/milliatom. [b] HPLC data.



Scheme 2. *Reagents and conditions:* i, $T_2(D_2)$ + Pd − C (30%); ii, HI + AcOH, 127 °C.

Hydrogenolysis of the trichloromethyl ether (**3**) (Scheme 2) with D_2 gas gave the corresponding trideuterio precursor (as confirmed by TLC and HPLC) which was then analysed by ^{2}H NMR spectroscopy. The spectrum (Figure 1) showed peaks for three deuteriated species with integrated intensities in the ratio CD_3:CD_2H:CDH_2 = 64.6:28.4:7.1, separated by the primary isotope effect on their chemical shifts.[16] This observation was corroborated by the mass spectrum of the sample, which contained peaks for the four species CD_3, CD_2H, CDH_2, and CH_3, with the CD_3 peak the most abundant [*m*/*z* 187 (52%), 186 (34%), 185 (11%), and 184 (3%)]. From these abundances it is possible to calculate a theoretical specific radioactivity for an analogous tritiated sample, and the results are given in the Table. When the equivalent deuteriation experiment was performed in a Teflon reaction vessel and the product was separated and analysed by mass spectrometry, the ratio of the four species was *m*/*z* 187 (66.9%), 186 (25.7%), 185 (6.3%), and 184 (1.1%). The clearly higher incorporation of deuterium yields a higher calculated specific activity (Table), and this result taken with other reactions involving catalytic hydrogenation have confirmed to us that the glass surface of reaction vessels is a contributing factor in the isotopic dilution of tritium or deuterium reactants.[17]

As a possible alternative to catalytic dehalogenation, the trichloro precursor was treated with commercial superdeuteride reagent ($LiEt_3BD$), and again a substantially higher incorporation of deuterium was observed: [*m*/*z* 187 (73%) and 186 (27%)]. The calculated specific activity for an analogous tritiation reaction would suggest a level of 91% of the theoretical activity, significantly better than either of the catalytic dehalogenation reactions (Table).

Carrier-free catalytic tritiodehalogenation of the trichloro precursor gave the tritiated ether in 32% yield and 68.8 Ci mmol^{-1} specific activity, as calculated from the radio-HPLC data (Figure 2). It should be noted that considerable cleavage of the ether linkage was observed during dehalogenation reactions, but the biphenyl-4-ol by-product was removed by the flash chromatography step of the work-up. Thus the clean HPLC trace may be reconciled with the moderate yield. The ^{3}H NMR spectrum of the tritiated ether is shown in Figure 3, and once again shows peaks for three tritiated species (CT_3, CT_2H, CTH_2) as well as *ca.* 5% of the tritium in an aromatic position. Calculation of the specific activity from the methyl signals of the tritium NMR spectrum indicated a specific activity of 70.2 Ci mmol^{-1}, which is in excellent agreement with the HPLC data.

The listing of calculated specific activities in the Table shows that the labelled methyl group always contains less than 100% tritium, and this isotopic dilution is a feature of catalytic hydrogenation and dehalogenation reactions. The level of deuterium or tritium to protium replacement is greatly enhanced by careful attention to dryness of glassware, catalyst, reagents, and solvents.

Cleavage of the Labelled Ether.—Cleavage of the ether (**4**) (Scheme 2) to generate methyl iodide was studied with a number of reagents having nucleophilic iodide ion, *e.g.* lithium iodide,[18] trimethylsilyl iodide,[19] aluminium tri-iodide,[20] and hydroiodic

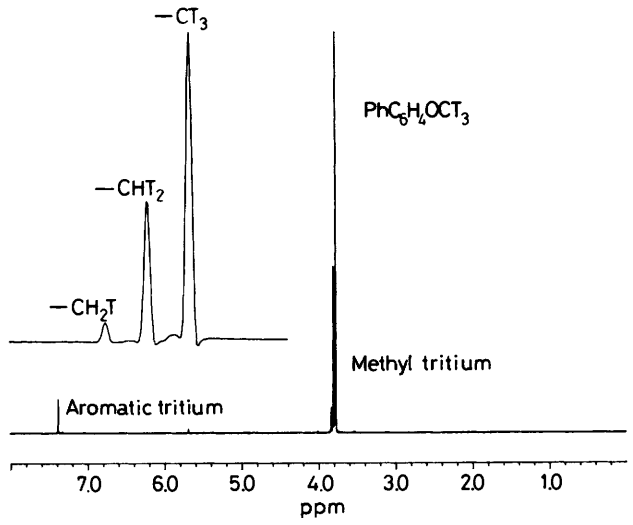
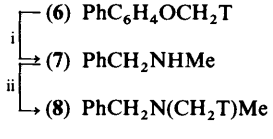


Figure 3. 320 MHz Tritium NMR spectrum of biphenyl-4-yl [^{3}H]methyl ether in CD_3OD taken with 4 096 scans, showing the region from 0–8 ppm. The inset shows an expansion of the substrate resonances (3.70–3.90 ppm). The spectrum was acquired with 8 K points, and the FID of the expanded trace was Gaussian multiplied (LB = −1, GB = 0.15) and zero-filled to 16 K points before Fourier transformation.

acid.[21] Hydroiodic acid was first used in 1861 for cleavage of an ether,[22] and is generally used as the constant-boiling aqueous solution (b.p. 127 °C/760 Torr;* *d* 1.70; 57%, 7.57M) or with a co-solvent such as acetic acid. In the latter case, the reaction mixture is heated (2–8 h) under reflux while the methyl iodide is trapped as it is formed. In the present experiments, HI in glacial acetic acid gave complete cleavage of the ether in less than 2 h at 127 °C, and 96% of the theoretical yield of biphenyl-4-ol was found in the generation bulb. HPLC analyses showed that no biphenyl-4-yl methyl ether was left unchanged, indicating 100% cleavage: from 240 mCi radioactivity placed into the bulb only 4 mCi remained after 2 h, and prior ^{3}H NMR analysis of the tritiated biphenyl-4-yl methyl ether suggests this would arise from the aromatic portion of the biphenyl-4-ol. Hence, HI was the preferred reagent for the generation of CT_3I from the labelled biphenyl-4-yl methyl ether.

Reaction of Methyl Iodide with a Model Substrate.—A low specific activity sample of the tritiomethyl biphenyl-4-yl ether (**6**) (4.2 Ci mmol^{-1}) was treated with HI and the liberated tritiated methyl iodide was trapped in a solution of *N*-methylbenzylamine in dimethylformamide (DMF) containing *N*,*N*-diisopropylethylamine (DIEA, Scheme 3), as described above.



Scheme 3. *Reagents and conditions:* i, HI + AcOH, 127 °C, 2 h; ii, base, DMF.

Analysis of the products showed that cleavage of the ether was quantitative and the [^{3}H]-*N*,*N*-dimethylbenzylamine was isolated in 73% yield, *i.e.* 175 of the original 240 mCi was found in the product. The DMF solution in the product bulb remained basic, and no HI was detected. HPLC analyses showed that specific activity was fully retained, and positional integrity was confirmed by ^{3}H and ^{1}H NMR spectroscopy.

* 1 Torr = 133.322 Pa.

Conclusions

High specific activity methyl iodide has been synthesised and used in a simple methylation reaction. This synthetic process has a number of advantages which may make this method attractive to other radiochemists, for use in their own laboratories: *i.e.* (*a*) the ability to vary the scale as required, (*b*) the ability to store the stable, non-radioactive precursor until it is required, (*c*) choice of labelling level at the time of dehalogenation, (*d*) mild reaction conditions (temperatures and pressures) for generation of the labelled methyl iodide, and (*e*) methylation conditions which are suitable for acid labile compounds.

Other features of the synthesis include the ability to manipulate small quantities of reagent and obtain good yields, despite a reaction apparatus of relatively large volume. Throughout the investigations it was noted that the trichloro precursor is moisture sensitive, and much better yields were obtained in all reactions when particular care was taken to use dry and pure reagents *etc.* It should also be mentioned that the intermediate labelled ether (m.p. 85 °C) shows some volatility under reduced pressure (30 μmHg).

In comparison with high-pressure syntheses of methanol[13] from CO_2 and T_2 and subsequent conversion to CT_3I,[16] where the methyl group had >95% of the maximum theoretical tritium content, the product of the presently described reactions contains approximately 80%, and this level yields a sufficiently high specific radioactivity for most purposes. The dilution from 100% is thought to arise from exchangeable protons on the catalyst and glassware. The use of superdeuteride to achieve dehalogenation illustrates that higher specific activity will be attainable with the availability of supertritide or $LiAlT_4$ at 100% abundance of tritium, and this approach is currently under investigation.

Experimental

Tritium gas was purchased from Oak Ridge National Laboratories, and contained 97.9% T_2, with the largest contaminant being DT (1.76%). Deuterium gas was purchased from the Liquid Carbonic Company, and contained 99.7% D_2. Starting materials were purchased from Aldrich Chemical Co. and repurified prior to use. All melting points were determined on an electrothermal apparatus and are reported uncorrected. HPLC analysis of the precursor and the products were performed by using a Waters Associates C-18 radial pak column with a mobile phase of MeOH/H_2O (70/30, 3 ml min^{-1}). Mass peaks were observed by UV detection at 264 nm on a Hewlett Packard 1040A diode array spectrophotometer. On-line radioactivity measurements were made with a Ramona-5-LS flow detector, using a lithium glass scintillant cell with an efficiency of *ca.* 0.05%. Tritiated samples were counted in Opti-Fluor scintillant (Packard) with a Packard 1500 Tri-Carb liquid scintillation analyser. Mass spectra of deuteriated products were measured with an AEI MS-12 mass spectrometer, operating at 18 eV to minimise fragmentation. All micro and mass spectrometric analyses were carried out by the Analytical Laboratory, Chemistry Department, University of California, Berkeley.

^{1}H (300 MHz), ^{2}H (46 MHz), and ^{3}H (320 MHz) NMR spectra were recorded in CD_3OD or CH_3OH, on an IBM AF-300 NMR spectrometer. Tritiated samples were made to a volume of 200 μl in Teflon tubes (Wilmad, #6005), which were then placed inside 5 mm glass NMR tubes fitted with screw-caps (Wilmad, 507-TR-8). A high quality ^{3}H band stop–^{1}H band

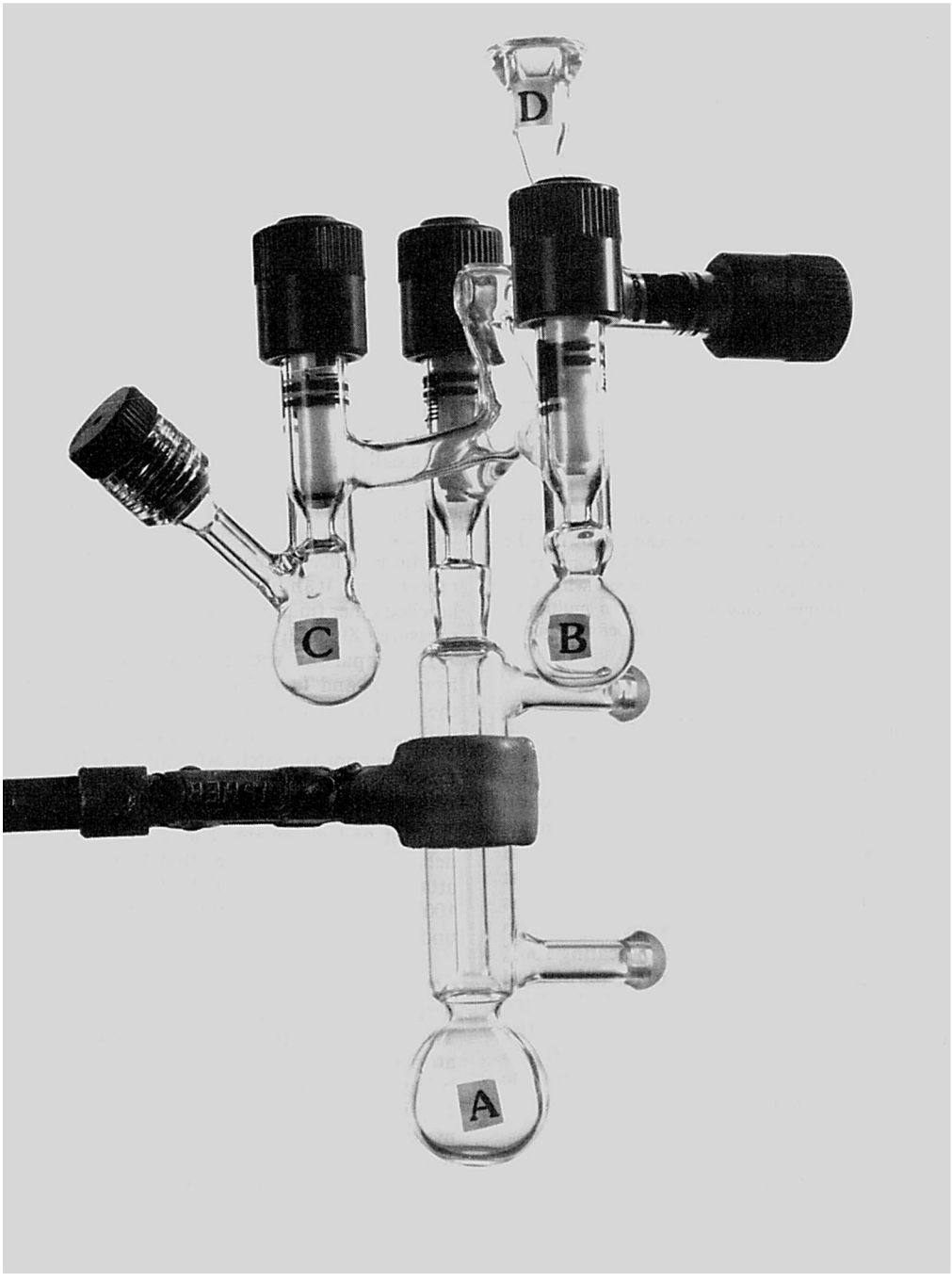


Figure 4. Reaction apparatus for methylation reactions. The whole manifold may be isolated from the vacuum line by the Teflon stopcock below connector D, and each bulb may be individually isolated. Bulb A has a microcondenser. Liquids may be removed from or added to bulb C through the side-arm tube.

pass filter (Cir-Q-Tel Inc., FBT/20-300/3-6/50-3A/3A) was placed in the proton decoupling line of the instrument, and the observe channel had an in-line ^{1}H band stop–^{3}H band pass filter. ^{3}H and ^{1}H Spectra were acquired using a $^{3}H/^{1}H$ 5 mm dual probe, over approximately 12 ppm, using a 5 s total recycle time and excitation pulses of 3.6 μs (^{3}H, 65°) and 2 μs (^{1}H, 25°). Deuteriated samples were dissolved in *ca.* 2.5 ml of CH_3OH, and spectra were acquired on a 10 mm broadband probe tuned to the ^{2}H frequency. Typical ^{2}H acquisition parameters included an observation window of approximately 11 ppm, an 8 s total recycle time and an excitation pulse of 14 μs (90°). All spectra were acquired at 297 K with the sample spinning. Referencing of tritium chemical shifts was achieved by generation of a ghost ^{3}H TMS signal from internal TMS in the ^{1}H NMR spectrum,[2,3] and deuterium spectra were referenced by assigning the CD_3 species the same chemical shift as a CT_3 signal (3.77 ppm).

The reaction apparatus for methylations is shown in Figure 4. The manifold consisted of three bulbs (A, B, and C), each of which could be isolated by a Teflon stopcock. The generation bulb A consisted of a heated reaction vessel (127 °C) connected to a small condenser which was kept at 40 °C during cleavage of the labelled ether. In the case of acid labile compounds, bulb B could be used to trap methyl iodide as it was generated, and any HI could be neutralised by the base (*e.g.* diisopropylethylamine) contained there. The side-arm bulb C was

used for the reaction of the substrate with methyl iodide. At the end of the cleavage reaction (methyl iodide generation) bulb A was isolated, and the methyl iodide in bulb B could then be transferred and reacted with substrate in bulb C by opening the stopcock in this arm of the apparatus.

Biphenyl-4-yl Methyl Ether (Non-radioactive Standard).—Biphenyl-4-ol (**1**) (3.6 g, 20 mmol) was added to a solution of NaOH (1.05 g, 26 mmol) in water (20 ml). Dimethyl sulphate (2.3 ml, 24 mmol) was added dropwise at 0 °C. The mixture was heated in an oil-bath at 80 °C for 2 h and then cooled. The product was filtered off, washed with 10% aqueous NaOH, then liberally with water, and drained thoroughly. Recrystallisation from benzene–methanol (8:2) gave the desired product (3.5 g, 90%), m.p. 85 °C; ^{1}H NMR ($CDCl_3$) δ 3.9 (3 H, s), 7.0 (2 H, m), and 7.3–7.6 (7 H, m).

O-*Biphenyl-4-yl Chlorothioformate* (**2**).—Thiophosgene (5.1 ml, 67 mmol) in $CHCl_3$ (40 ml) at 0 °C was treated dropwise with biphenyl-4-ol (11.9 g, 70 mmol) in NaOH (5%; 60 ml). The mixture was stirred at 0–10 °C for 1 h, after which the $CHCl_3$ layer washed with dilute HCl and water, dried, and evaporated to give the crude product (16 g, 93%). This when recrystallised from MeOH–$CHCl_3$ (2:8) gave (**2**) in high yield (15.1 g, 88%); m.p. 68–70 °C (Found: C, 62.8; H, 3.6; Cl, 14.3; S, 12.7. Calc. for $C_{13}H_9ClSO$: C, 62.9; H, 3.6; Cl, 14.1; S, 12.9%); δ($CDCl_3$) 7.4–7.6 (m).

Trichloromethyl Biphenyl-4-yl Ether (**3**).—*O*-Biphenyl-4-yl chlorothiocarbonate (**2**) (500 mg, 2 mmol) was dissolved in dry $CHCl_3$ (15 ml) and the solution was saturated with chlorine gas with simultaneous cooling in an ice-bath. TLC (hexane–chloroform 70:30) showed a new clean spot with no sign of unchanged starting material after 5 min; yield 334 mg (58%); m.p. 65 °C (Found: C, 54.3; H, 3.0; Cl, 36.8. Calc. for $C_{13}H_9OCl_3$: C, 54.5; H, 3.1; Cl, 36.7); ^{1}H NMR ($CDCl_3$) δ 7.4–7.7 (m).

Hydrogenolysis of Trichloromethyl Biphenyl-4-yl Ether.—*Deuteriodehalogenation (glass reaction vessel).* Trichloromethyl biphenyl-4-yl ether (**3**) (6 mg, 0.02 mmol) was dissolved in dry ethyl acetate (1 ml), and Pd/C catalyst (30%; 50 mg) and dry 1,8-bis(dimethylamino)naphthalene (proton sponge;[24] 50 mg, 0.23 mmol) were added. The reaction vessel was connected to the vacuum line (3 × N_2 flushing). Deuteriation was carried out under 1 atm* of deuterium gas, and the uptake of gas stopped after 2 h. The reaction vessel was then frozen in liquid nitrogen and the excess gas pumped out. The reaction mixture was brought to room temperature, N_2 was added to a pressure of 1 atm, and the reaction apparatus was disconnected from the vacuum line. The catalyst was removed by filtration, the ethyl acetate was evaporated, and the yellowish residue was dissolved in 10% $CHCl_3$ in hexane (0.5 ml) and passed through a small silica gel column. By this process, the product was separated from the proton sponge and biphenyl-4-ol to yield [$^2H_3$]methyl biphenyl-4-yl ether (**4**) (2.8 mg, 73%), m.p. 85 °C; *m/z* 187 (52%), 186 (34%), 185 (11%), and 184 (3%); ^{2}H NMR (CH_3OH, ^{1}H decoupled) δ 3.81 (CDH_2, 7.1%), 3.79 (CD_2H, 28.4%), and 3.77 (CD_3, 64.6%).

Deuteriodehalogenation (Teflon reaction vessel). The above experiment was repeated in a Teflon reaction vessel of the same volume. Sample isolation and work-up as above yielded [$^2H_3$]-methyl biphenyl-4-yl ether (**4**) (2.7 mg, 70.4%), m.p. 85 °C; *m/z* 187 (66.9%), 186 (25.7%), 185 (6.3%), and 184 (1.1%).

Deuteriodehalogenation (superdeuteride reaction). Trichloromethyl biphenyl-4-yl ether (**3**) (6 mg, 0.02 mmol) was dissolved in THF (1 ml) and stirred at room temperature. Lithium triethylborodeuteride ($LiEt_3BD$, 120 μl; 1M in THF) was added and the reaction allowed to continue for 1 h, after which TLC analysis (chloroform–hexane 30:70) showed no reaction. The reaction mixture was then heated at 50 °C for a further hour, after which TLC analysis revealed the presence of the desired product (R_F 0.7) in addition to substantial material remaining at the origin. Methanol (1 ml) was added to the reaction mixture to quench unchanged superdeuteride, and the solvents subsequently evaporated. The residue was extracted with diethyl ether (5 ml), and evaporation of the ether gave a solid product (**4**): yield 1.1 mg (29%), m.p. 77 °C; *m/z* 187 (73%) and 186 (27%).

Tritiodehalogenation. Trichloromethyl biphenyl-4-yl ether (**3**) (7.5 mg, 0.026 mmol) was dissolved in ethyl acetate (1 ml) and injected into a previously dried and evacuated reaction vessel, which contained the catalyst (30% Pd/C, 50 mg) and proton sponge (50 mg, 0.23 mmol). The reaction vessel was then frozen in liquid N_2 and flushed three times with nitrogen gas. T_2 gas was then added to a pressure of 1 atm, and the reaction was continued for 2 h. The excess T_2 gas was then pumped out, and methanol (2 ml, × 3) was injected into the reaction vessel to exchange labile tritons. The volume of solvent was reduced under vacuum, and the reaction vessel was then disconnected from the vacuum line and the catalyst removed by filtration. The solvent was evaporated under a flow of nitrogen, and the residue was purified as detailed above. The solid product (**4**) was dissolved in CD_3OD (1 ml) for HPLC and NMR analyses; yield 32%; specific activity: 68.8 Ci mmol^{-1}; ^{3}H NMR (CD_3OD, ^{1}H decoupled) δ 3.83 (CTH_2, 3.8%), 3.80 (CT_2H, 30.2%), and 3.77 (CT_3, 66.0%).

Methyl Iodide from the Non-radioactive Precursor.—Biphenyl-4-yl methyl ether (100 mg, 0.54 mmol) was placed in the generation bulb and dissolved in a mixture of glacial acetic acid (1.5 ml) and hydroiodic acid (0.5 ml, 57%). The resulting mixture was stirred at 127 °C for 2 h. The methyl iodide generated was trapped in a solution of *N*-methylbenzylamine (0.15 ml) in DMF (1 ml) in the presence of DIEA (0.2 ml). After 2 h, TLC analysis (chloroform–hexane–tetrahydrofuran 60:30:10) showed complete cleavage of the ether. Subsequent HPLC analysis showed the product *N,N*-dimethylbenzylamine in 75% yield (82.5 μl); ^{1}H NMR ($CDCl_3$) δ 2.3 (6 H, s), 3.5 (2 H, s), and 7.2–7.4 (5 H, m).

[^{3}H]-N,N-*Dimethylbenzylamine* (**8**).—Biphenyl-4-yl [^{3}H]-methyl ether (**6**) (9.2 mg, 0.05 mmol; specific activity 4.2 Ci mmol^{-1}) was dissolved in glacial acetic acid (1 ml) and HI (57%; 200 μl) was added. This mixture was placed in the generation bulb of the reaction apparatus (Figure 4, bulb A). *N*-Methylbenzylamine (**7**) (10 μl, 0.077 mmol) was mixed with DMF (500 μl) and DIEA (200 μl), and added to the reaction bulb (Figure 4, bulb C). Both sides were frozen in liquid nitrogen and evacuated, then kept under approximately 1 atm of nitrogen. The generation bulb A was heated at 130 °C for 2 h, while generated tritiated methyl iodide was allowed to pass directly to the reaction side of the apparatus, (bulb C) and react with the substrate. After 2 h the heating of the generation bulb (A) was discontinued, since radio-HPLC analysis of the remaining solution showed the cleavage reaction to be complete. The reaction mixture (in bulb C) was stirred for an additional 4 h at room temperature and then a portion of this solution was analysed by radio-HPLC. Analysis showed [^{3}H]-*N,N*-dimethylbenzylamine (**8**) (5.5 μl, 73%) with a specific activity of 4.2 Ci mmol^{-1}. Further stirring of the reaction mixture at room temperature overnight resulted in a yield of 80%, although the specific activity was slightly lower. HPLC of this final product showed significant tritium eluting with the solvent front, which was presumed to be the trimethyl iodide salt. The ^{3}H NMR

* 1 atm = 101 325 Pa.

spectrum (CD_3OD) of this reaction mixture showed two tritium peaks, corresponding to [^{3}H]-*N*,*N*-dimethylbenzylamine (**8**) at 2.1 ppm and [^{3}H]-*N*,*N*,*N*-trimethylbenzylammonium iodide at 3.1 ppm. ^{1}H NMR spectroscopy of the appropriate non-radioactive standards confirmed the chemical shift of the salt, which the ^{3}H NMR spectrum had shown to be present in approximately 10% yield.

Acknowledgements

This research was supported by the Biotechnology Resources Program, Division of Research Resources, National Institutes of Health under Grant P41 RR01247-06, and by the Department of Energy under Contract DE-AC03-76SF00098. Special thanks to Paul deFur for his mass spectrometry work, to Hendrik Andres for useful discussions, and to Vangie Peterson for help with the manuscript.

References

1 D. M. Israelstam, T. Sargent, N. N. Finley, H. S. Winchell, M. B. Fish, J. Motto, M. Pollycove, and A. Johnson, *J. Psychiatr. Res.*, 1970, **7**, 185.
2 D. Comar, J.-C. Cartron, M. Maziere, and C. Marazano, *Eur. J. Nucl. Med.*, 1976, **1**, 11.
3 K. Ishiwata, K. Yanai, T. Ido, Y. Miura-Kanno, and K. Kawashima, *Nucl. Med. Biol.*, 1988, **15**, 365.
4 R. F. Dannals, H. T. Ravert, A. A. Wilson, and H. N. Wagner, Jr., *Int. J. Appl. Radiat, Isot.*, 1986, **37**, 433.
5 C. Marazano, M. Maziere, G. Berger, and D. Comar, *Int. J. Appl. Radiat. Isot.*, 1977, **28**, 49.
6 D. Neri, T. Szyperski, G. Otting, H. Senn, and K. Wüthrich, *Biochemistry*, 1989, **28**, 7510.
7 R. E. London, S. A. Gabel, and A. Funk, *Biochemistry*, 1987, **26**, 7166.
8 H. G. Floss and M. D. Tsai, *Adv. Enzymol. Relat. Areas Mol. Biol.*, 1979, **50**, 243.
9 J. G. Richards and H. Mohler, *Neuropharmacology*, 1984, **23**, 233.
10 J. P. Bloxsidge, J. A. Elvidge, M. Gower, J. R. Jones, E. A. Evans, J. P. Kitcher, and D. C. Warrell, *J. Labelled Compd. Radiopharm.*, 1981, **18**, 1141.
11 M. Saljoughian, H. Morimoto, A. M. Dorsky, H. Rapoport, H. Andres, Y. S. Tang, and A. Susan, *J. Labelled Compd. Radiopharm.*, 1989, **27**, 767.
12 M. Saljoughian, H. Morimoto, and H. Rapoport, *J. Org. Chem.*, 1989, **54**, 4689.
13 D. G. Ott, V. N. Kerr, T. H. Whaley, T. Benziger, and R. K. Rohwer, *J. Labelled Compd.*, 1974, **10**, 315.
14 S. Sharma, *Synthesis*, 1978, 803.
15 N. N. Iarovenko and A. S. Vasil'eva, *J. Gen. Chem. USSR (Engl. Transl.)*, 1957, **27**, 2539.
16 J. P. Bloxsidge, J. A. Elvidge, J. R. Jones, E. A. Evans, J. P. Kitcher, and D. C. Warrell, *Org. Magn. Reson.*, 1981, **15**, 214.
17 P. G. Williams, M. Saljoughian, and H. Morimoto, unpublished data.
18 I. T. Harrison, *J. Chem. Soc., Chem. Commun.*, 1969, 616.
19 T. Morita, Y. Okamato, and H. Sakurai, *J. Chem. Soc., Chem. Commun.*, 1978, 874.
20 M. V. Bhatt and S. U. Kulkarni, *Synthesis*, 1983, 249.
21 R. L. Burwell, Jr., *Chem. Rev.*, 1954, **54**, 615.
22 A. Butlerow, *Justus Liebigs Ann. Chem.*, 1861, **118**, 325.
23 J. P. Bloxsidge, J. A. Elvidge, J. R. Jones, R. B. Mane, and M. Saljoughian, *Org. Magn. Reson.*, 1979, **12**, 574.
24 R. W. Alder, P. S. Bowman, W. R. S. Steele, and D. R. Winterman, *J. Chem. Soc., Chem. Commun.*, 1968, 723.

Paper 0/00139B
Received 9*th January* 1990
Accepted 29*th January* 1990