

## A Mild and Convenient Method for Tritium Labelling of Activated Aromatic Compounds Using $\text{BF}_3\text{--Et}_2\text{O}$ and Tritiated Water

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A procedure for tritium exchange labelling of activated aromatic compounds using  $^3\text{H}_2\text{O}$  and  $\text{BF}_3\text{--Et}_2\text{O}$  has been developed to prepare two radio-labelled photoprobes that are difficult to obtain by conventional methods; the applicability of the method has been examined by labelling several aromatic compounds varying in activation toward electrophilic aromatic substitution.

Several methods have been developed specifically for the tritium labelling of aromatic compounds. The rapid exchange of aromatic protons with tritiated water can be accomplished using  $\text{BBr}_3$  or  $\text{EtAlCl}_2$  as catalyst, and this procedure gives high incorporations of tritium for some compounds.<sup>1,2</sup> With

this method, the initial complex formed with the aromatic ring is decomposed by the addition of tritiated water. This technique is most useful for highly activated aromatic compounds because prolonged exchange is not possible. Aluminium chloride has also been used with variable success as a

catalyst for the exchange labelling of aromatic compounds with tritiated water.<sup>3</sup> Both of the above methods require the addition of a highly reactive Lewis acid to a neat aromatic liquid and are of somewhat limited application. Existing methods for deuterium exchange labelling<sup>1,4,5</sup> are impractical for labelling with tritium on the very small scale usually encountered in radiochemical synthesis.

A simple and mild method for acid-catalysed tritium exchange labelling has been developed which uses  $\text{BF}_3\text{-Et}_2\text{O}$  as both the catalyst and solvent, and tritiated water as the source of label. Very small amounts of tritiated water and low molar ratios of  $^3\text{H}_2\text{O}$  to aromatic reactant can be employed. The procedure was used to incorporate tritium into two photoprobes (**1** and **2**) designed to label the active site of cytochrome P-450-limonene hydroxylase from mint.<sup>6</sup> A series of model aromatic compounds were also examined as reactants to test the application of this exchange labelling procedure.

For the preparation of 3,4-methylenedioxyazidobenzene **1**, *N*-acetyl-3,4-methylenedioxyaniline **3** (38 mg, 0.17 mmol) was dissolved in 1.5 ml  $\text{BF}_3\text{-Et}_2\text{O}$  (75 equiv.), and the mixture was chilled ( $-20^\circ\text{C}$ )<sup>†</sup> and transferred to a tube on ice containing 40  $\mu\text{l}$  (13 equiv.) of frozen  $^3\text{H}_2\text{O}$  (90 Ci mol<sup>-1</sup>; 1 Ci =  $3.7 \times 10^{10}$  Bq). The tube was sealed, allowed to warm to room temperature and the contents mixed gently for 3 days. Longer reaction times were not used owing to slow hydrolysis of the acetamide. The reaction mixture was then diluted with water and extracted with diethyl ether. The aqueous phase was made basic with KOH and extracted with diethyl ether, and the ether extract concentrated and subjected to TLC [silica gel G, with hexane:diethyl ether (99:1 v/v)]. The acetamide **3** so isolated was hydrolysed in 50% (aq) ethanolic KOH. The resulting ring-labelled 3,4-methylenedioxyaniline was converted to 3,4-methylenedioxyazidobenzene **1** by the method of Smith and Brown.<sup>7‡</sup> The specific activity of the purified product was  $51 \pm 3$  Ci mol<sup>-1</sup>, 57% of that of the tritium source ( $^3\text{H}_2\text{O}$  at 90 Ci mol<sup>-1</sup>).

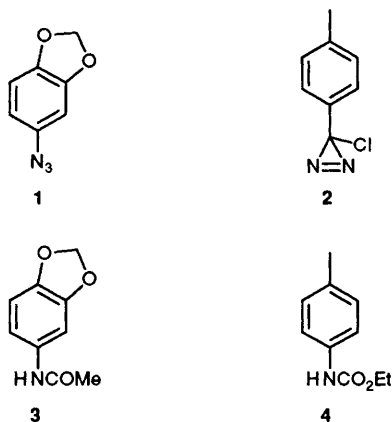
For the preparation of 3-(chloro)-3-(*p*-tolyl)diazirine **2**, the ethyl carbamate of *p*-toluidine **4** was prepared from *p*-toluidine and ethyl chloroformate using triethylamine as the catalyst. Crystalline **4** (180 mg, 1 mmol) was dissolved in 1.2 ml  $\text{BF}_3\text{-Et}_2\text{O}$  (10 equiv.); the solution was chilled to  $-20^\circ\text{C}$  and added to 50  $\mu\text{l}$  (2.8 equiv.) of frozen  $^3\text{H}_2\text{O}$  (63 Ci mol<sup>-1</sup>) on ice. The reaction mixture was sealed, allowed to stand on ice for 30 min, warmed to room temperature, and mixed gently for 3 weeks. The ring-labelled carbamate **4** was

hydrolysed without prior purification [(1 mol dm<sup>-3</sup> KOH in 50% (aq.) ethanol,  $100^\circ\text{C}$ ] and the resulting  $^3\text{H}$ -toluidine was isolated by diethyl ether extraction, partitioned into 1 mol dm<sup>-3</sup> HCl, and reextracted from the aqueous phase with diethyl ether after the addition of KOH. The  $^3\text{H}$ -toluidine (80% yield) was then converted to the  $^3\text{H}$ -chlorodiazirine **2** using literature procedures.<sup>8-10</sup> The specific activity of the purified product was 26 Ci mol<sup>-1</sup>, 41% of that of the tritium source ( $^3\text{H}_2\text{O}$  at 63 Ci mol<sup>-1</sup>).

A series of compounds varying in their activation toward electrophilic aromatic substitution was labelled with low specific activity  $^3\text{H}_2\text{O}$  under identical conditions. The aromatic compound (27–28  $\mu\text{mol}$ ) was dissolved in 5 ml (1450 equiv.)  $\text{BF}_3\text{-Et}_2\text{O}$  and chilled to  $-24^\circ\text{C}$ . The solution was then added to 5  $\mu\text{l}$  (10 equiv.) frozen  $^3\text{H}_2\text{O}$  (0.076 Ci mol<sup>-1</sup>) on ice. The vessel was sealed and mixed gently for 3 days at room temperature. The reaction was stopped by the addition of  $\text{H}_2\text{O}$  (1 ml) at room temperature and the mixture was extracted with diethyl ether (3  $\times$  1 ml). The extract was passed (with 1 ml rinse) over a small column of silica gel overlaid with  $\text{MgSO}_4$ . The eluant was adjusted to 5 ml and aliquots removed for liquid scintillation counting and mass determination by GLC (external calibration) to determine specific activity. The labelled *p*-cymene and xylene samples were also analysed by radio-GLC (Silar 10C column, He carrier gas,  $100^\circ\text{C}$  isothermal, Nuclear Chicago Detector). The reaction utilizing  $^2\text{H}_2\text{O}$  (Aldrich, 99 atom%) was carried out with the xylenes [24  $\mu\text{mol}$  xylenes, 5  $\mu\text{l}$   $^2\text{H}_2\text{O}$  (10 equiv.)] to determine the distribution of label among the three isomers. The isomers were separated and analysed by coupled GLC-MS (HP 5890-5985 system, RSL-150 column, He carrier gas).

The photoactive analogues of safrrole **1** and *p*-cymene **2** were synthesized as potential probes for the active site of limonene hydroxylase, a cytochrome P-450 monooxygenase from spearmint (*Mentha spicata*).<sup>6</sup> The azido-substituted methylenedioxybenzene **1** has been suggested as a general cytochrome P-450 photoprobe by Armstrong and Hollenburg who synthesized this compound in unlabelled form.<sup>11</sup> A compound similar to **2** [3-(trifluoromethyl)-3-(*m*-[<sup>125</sup>I]iodophenyl)diazirine] was used to label the active site of a rat liver cytochrome P-450.<sup>12</sup> Precursors of these two photoprobes, **3** and **4**, were readily labelled, such that the final products, **1** and **2**, were obtained at 57 and 41%, respectively, of the specific activity of the  $^3\text{H}_2\text{O}$  source. These results demonstrate that, while the system is acidic enough to effect useful exchange rates, it is sufficiently mild that the acid-sensitive reactant **3** is only slowly hydrolysed.

The effect of ring activation on exchange labelling is shown in Table 1. In the case of the xylenes, deuterium labelling indicated that <1% of the *ortho*-isomer was labelled, ca. 5% of the *para*-isomer was labelled, and the *meta*-isomer was essentially fully labelled to the extent of 1.1 deuterium per molecule. These data are consistent with the chlorination rates



**Table 1** Specific activity and tritiation (%) of selected aromatic compounds

Compound	Specific activity <sup>a/</sup> Ci mol <sup>-1</sup>	Tritiation <sup>b</sup> (%)
Chlorobenzene	0.0013	1.3
Benzene	0.0017	1.8
Toluene	0.0037	3.8
<i>p</i> -Cymene	0.0086	8.9
Xylenes	0.073	75
<i>m</i> -Xylene <sup>c</sup>	0.11	110

<sup>a</sup> The specific activity of the starting material ( $^3\text{H}_2\text{O}$ ) was 0.097 Ci mol<sup>-1</sup>. <sup>b</sup> 100% is the same specific activity as the tritium source.

<sup>c</sup> *m*-Xylene based on deuterium labelling of a mixture of xylenes where normal activating effects were observed and where > 95% of the deuterium was incorporated into the *meta*-isomer.

<sup>†</sup> More  $\text{BF}_3\text{-Et}_2\text{O}$  may be needed as **3** has limited solubility at low temperature.

<sup>‡</sup> It was necessary to make the reaction mixture basic with KOH before the normal extraction procedure.

of xylenes<sup>13</sup> and follow the normal activation and directing effects expected for an arenium ion mechanism of electrophilic aromatic substitution. In the experiments with the xylenes and *p*-cymene, no side products were detected by GLC using a flame ionization detector and no radiocontaminants were detected by radio-GLC.

The procedure described provides a relatively mild technique for <sup>3</sup>H or <sup>2</sup>H labelling of a wide range of functionalized aromatic compounds. The method uses only commercially available, reasonably stable reactants, requires no special equipment, and is easily performed in a single vessel on the microscale. Product isolation is simple and convenient, and the source of the radioactivity (<sup>3</sup>H<sub>2</sub>O) is easily separated from the labelled organic product and can be contained with minimal risk of contamination. The reaction proceeds without appreciable formation of byproducts, and even slowly reacting compounds, which are only slightly activated or deactivated, can be exchanged labelled through prolonged exposure.

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