

TRITIUM AND DEUTERIUM EXCHANGE-LABELING OF AROMATIC AND  
ORGANOMETALLIC COMPOUNDS IN HEPTAFLUOROBUTYRIC ACID

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SUMMARY

The use of heptafluorobutyric acid (HFBA) as a solvent/reagent for tritiation (or deuteration) or aromatic organic and organometallic compounds is described. Typically the substrate (0.1-1 mmole) and tritium oxide (5-25  $\mu$ l, 5 Ci/g) are dissolved in 0.3-1 ml HFBA and heated at 130-145° for 4-7 days. Aromatic compounds bearing electron-donating substituents are tritiated much more readily than those bearing electron withdrawing substituents. Substrate decomposition was minimal, and good to excellent yields of relatively pure tritiated materials are obtained simply by cooling, neutralization, and extraction. Detailed studies of deuterium exchange in HFBA-d under analogous conditions are also reported.

Key words: Tritium exchange, organometallic compounds,  $^3\text{H}$ -Imipramine

INTRODUCTION

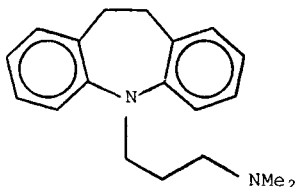
In connection with a variety of drug metabolism studies under way in our laboratory, we were interested in a simple method for tritiation and/or deuteration of a wide variety of aromatic and organo-transition-metallic compounds. Of the numerous methods<sup>1</sup> for labeling organic compounds by exchange procedures, the method of Garnett *et al.* employing a Rh catalyst in acetic acid/water (1:1) appeared attractive. Unfortunately, many of our compounds were poorly soluble in this reaction medium, and only a slight improvement in propionic acid/water (9:1) was noted. However, during these studies (Table 1, expt. 1-3) it was noted that the specific activity of the products varied in a fashion suggestive of electrophilic attack, i.e., MeO > H > NO<sub>2</sub>. We therefore decided to investigate the possibility of carrying out the desired tritium/deuterium exchange reactions directly under acidic conditions without metal catalysts. Heptafluorobutyric acid (HFBA) was chosen as a solvent/reagent system because it appeared to offer a number of advantageous properties including relatively high acidity, excellent solvent properties, a high boiling point combined with great thermal stability, ready commercial availability, and the presence of only a single exchangeable proton per

molecule. We have now carried out many deuterium and tritium exchanges on a wide variety of substrates, and find that HFBA is indeed a convenient, mild and effective reagent for this purpose.

#### RESULTS AND DISCUSSION

Inspection of the data in Table 1 indicates several key features of tritium exchange reactions in HFBA. Thus, the specific activities obtained are  $10^2$ - $10^3$  fold greater than those obtained in HOAc/H<sub>2</sub>O/RhCl<sub>3</sub> solution under comparable conditions. Secondly, the specific activities decrease as the substrates become less electron rich, as expected for electrophilic exchange process (e.g., expt. 4-8). Very good specific activities are easily obtained if the substrate has a large number of exchangeable protons, or is exceptionally electron-rich, or both (expt. 9,10).

For work involving radiolabeled compounds it is generally important to know the location of the label in the molecule. To shed light on this aspect of our tritiation reactions in HFBA, as well as to gain some insight to the mechanism of the exchange reaction and the factors which govern it, a series of deuterium exchange reactions was carried out and analyzed (Tables 2 and 3). In general, deuterium exchange in HFBA-d was more facile than in D<sub>2</sub>O/DOAC containing RhCl<sub>3</sub>, the recoveries of material were usually better, and the exchange was more selective in that a narrower distribution of deuterated species was produced. For example, as shown by the mass spectral data in Tables 2 and 3, anisole incorporated 1.87 D/molecule in HFBA-d (expt. 17), and only 0.79 D/Molecule in the rhodium reagent (expt. 16) despite a larger amount of exchangeable D being present in the latter case. Analysis of the two deuterated anisoles by nmr indicated that in HFBA-d there was a 17:1 preference for exchange at ortho/para position relative to meta positions, whereas this ratio was only 2.5:1 with the rhodium reagent.



imipramine

A similar comparison using imipramine led to essentially similar conclusions (expts. 12-15). Nmr and mass spectral studies indicated that in HFBA-d, exchange is confined to the aromatic protons. With the rhodium reagent

the majority of the exchange also occurred at the aromatic positions. No exchange occurred in  $D_2O/DOAc$  in the absence of  $RhCl_3$ . This result, combined with the broad distribution of deuterated species formed in the presence of the rhodium catalyst, is in agreement with the role of  $\pi$ -complexation by rhodium as proposed by Garnett and coworkers.<sup>2</sup> In contrast, the relatively sharp distribution of deuterated imipramine species produces in HFBA-d (Table 3, expt. 12) is more consistent with rapid electrophilic substitution of D for H at the four ring positions ortho or para to the nitrogen, together with slower exchange at the other four aromatic protons. This interpretation is also supported by nmr studies; the aromatic absorption of material deuterated in HFBA-d is a broadened but symmetrical singlet, whereas in undeuterated imipramine it is a very broad and highly skewed "singlet".

As with the tritiations, compounds bearing only electron-withdrawing substituents exchange relatively slowly, even in HFBA-d. Thus, bromobenzene was inert in  $D_2O/DOAc/RhCl_3$ , but formed 5-10% of a  $d_1$  species in HFBA-d (expts. 19 and 20). The organometallic  $\pi$ -complex MMT also underwent slow but clean deuteration in HFBA-d (expt. 22): nmr analysis indicated a ring/methyl peak integration ratio of 3.31:3.00 ( $\delta$  4.60 and 1.94 ppm, respectively). However, electron donating groups enhanced the exchange rates and showed the expected directional effects. Thus, as shown by nmr analysis, 2-bromoaniline was converted cleanly into  $[4,6-^2H_2]$ -2-bromoaniline (cf. ref. 3). Similarly, salicylamide underwent exchange only at positions 3 and 5.

In conclusion, HFBA provides a convenient, mild and effective solvent/reagent medium for the exchange deuteration or tritiation of a wide variety of aromatic compounds and transition metal  $\pi$ -arene compounds. Except for strongly electron-deficient compounds (e.g., 2-nitrobromobenzene), quite reasonable specific activities may readily be obtained. As indicated in the following manuscript,<sup>5</sup> exchange tritiation of appropriate arylamines, followed by application of recent advances in the chemistry of aryldiazonium compounds, greatly extends the versatility of this exchange method.

Table 1. Tritium Exchange Reactions

Expt	Substrate, conditions	Temp, °C	Time	Yield (%)	Specific Activity mCi/nmole
1	100 $\mu$ l <i>o</i> -Bromoanisole in 0.2 ml Rh reagent <sup>a</sup> + 5 $\mu$ l T <sub>2</sub> O	130	5d	>90	8.5 x 10 <sup>-3</sup>
2	100 l Bromobenzene, as above <sup>b</sup>	130	5d	>90	1.9 x 10 <sup>-3</sup>
3	100 mg <i>o</i> -Bromonitrobenzene, as above	130	5d	>90	0.34 x 10 <sup>-3</sup>
4	100 mg <i>o</i> -Bromonitrobenzene in 0.5 ml HFBA + 5 $\mu$ l T <sub>2</sub> O	140	6d	90	0.04
5	100 mg <i>o</i> -Bromoanisole, as above	140	6d	95	0.87
6	100 mg Nitroaniline, as above	140	6d	93	0.60
7	400 mg <i>o</i> -Bromoaniline in 0.35 ml HFBA + 25 $\mu$ l T <sub>2</sub> O	165	5d	95	8.2
8	390 mg <i>o</i> -Toluidine in 0.4 ml HFBA + 25 $\mu$ l T <sub>2</sub> O	160	5d	89	10.7
9	169 mg Imipramine in 0.5 ml HFBA + 5 $\mu$ l T <sub>2</sub> O	115	6d	78 <sup>c</sup>	10.3
10	67 mg 2-Aminopropylferrocene in 0.5 ml CF <sub>3</sub> CO <sub>2</sub> H + 5 $\mu$ l T <sub>2</sub> O	25	7d	50 <sup>d</sup>	9.4
11	300 mg Salicylamide in 0.2 ml HFBA + 25 $\mu$ l T <sub>2</sub> O	200	4d	60 <sup>e,f</sup>	8.4

<sup>a</sup> Rh reagent is 0.02 M RhCl<sub>3</sub>·3H<sub>2</sub>O in acetic acid/water (3:1). <sup>b</sup> Reaction is not homogeneous. <sup>c</sup> % Yield of recrystallized hydrochloride. <sup>d</sup> % Yield of recrystallized succinate salt. <sup>e</sup> % Yield after chromatography and sublimation to 99.9% purity. <sup>f</sup> Stella and S. Varia, unpublished results.

Table 2. Deuterium Exchange Reactions

Expt	Substrate, conditions	Temp, °C	Time	Yield (%)	Deuterium Incorporation atoms D/molecule
12	125 mg Imipramine·HCl, 1.0 ml HFBA-d + 0.2 ml D <sub>2</sub> O	115	72 hr	79	3.62
13	125 mg Imipramine·HCl, 1.0 ml Rh reagent <sup>a</sup>	115	72 hr	45 <sup>b</sup>	2.55
14	125 mg Imipramine·HCl, 1 ml DOAc + 0.25 ml D <sub>2</sub> O	115	72 hr	79	0
15	145 mg Imipramine (free base), 1.0 ml Rh reagent <sup>a</sup>	115	6d	46	0.52
16	67 mg Anisole, 0.6 ml Rh reagent <sup>a</sup>	115	8d	50 <sup>c</sup>	0.79
17	96 mg Anisole, 0.7 ml HFBA-d	115	8d	73 <sup>c</sup>	1.87
18	300 mg o-Bromoaniline, 0.2 ml HFBA-d + 170 $\mu$ l D <sub>2</sub> O	140	10d	>80	1 <sup>d</sup>
19	204 mg Bromobenzene, 1.0 ml HFBA-d	135	86 hr	56 <sup>c</sup>	0.05-0.1
20	225 mg Bromobenzene, 1.0 ml Rh reagent <sup>a,e</sup>	135	86 hr	23 <sup>c</sup>	0
21	200 mg Salicylamide, 0.2 ml HFBA + 0.2 ml D <sub>2</sub> O	140	86 hr	70	1.13
22	345 mg MMT <sup>f</sup> , 1.2 ml HFBA-d	125	72 hr	>80	0.83

<sup>a</sup> 0.02 M RhCl<sub>3</sub>·3H<sub>2</sub>O in 50 mole-% D<sub>2</sub>O in DOAc. <sup>b</sup> A brown precipitate formed and remained insoluble throughout the reaction and workup. <sup>c</sup> Some loss occurred with solvent removal during workup. <sup>d</sup> See discussion in text. <sup>e</sup> Bromobenzene did not dissolve completely, even at 135°C. <sup>f</sup> Methylcyclopentadienylmanganesetricarbonyl.

Table 3. Distributions of Deuterated Species Produced by Exchange Reactions

<u>Substrate</u>								
IMIPRAMINE	$d_0$	$d_1$	$d_2$	$d_3$	$d_4$	$d_5$	$d_6$	$d_7$
<u>m/e</u>	<u>280</u>	<u>281</u>	<u>282</u>	<u>283</u>	<u>284</u>	<u>285</u>	<u>286</u>	<u>287</u>
Control <sup>b</sup>	75.29	19.65	5.05					
expt 12		3.31	8.67	24.10	50.25	13.64		
expt 13	3.63	15.75	31.51	30.00	13.23	3.03	1.51	1.31
expt 15	56.77	33.28	9.94					
ANISOLE	$d_0$	$d_1$	$d_2$	$d_3$	$d_4$			
<u>m/e</u>	<u>108</u>	<u>109</u>	<u>110</u>	<u>111</u>	<u>112</u>			
Control <sup>b</sup>	92.21	7.78						
expt 16	45.88	39.03	12.07	2.89	1.83			
expt 17	6.60	26.49	41.46	23.67	1.76			

<sup>a</sup>Values reported represent the average of at least 5 scans ( $\pm$  5% RSD). The designations  $d_1$ ,  $d_2$ ... $d_n$  are nominal and have not been corrected for contributions due to the natural occurrence of  $^{13}\text{C}$ .

<sup>b</sup>Undeuterated standards.

#### EXPERIMENTAL SECTION

Tritium oxide (5 Ci/g) was obtained from New England Nuclear. Deuterium oxide, heptafluorobutyric acid and heptafluorobutyric anhydride (90%) were obtained from Aldrich. Mass spectra were determined on a Varian-MAT CH5B instrument. Specific activities of tritiated materials were determined using a Beckman LS-3133T scintillation counter and are relative to an unquenched tritium standard.

Heptafluorobutyric acid-d. Heptafluorobutyric anhydride (14.3 g, 34.8 mmole) was stirred and cooled with dry ice and  $\text{D}_2\text{O}$  (0.69 g, 34.8 mmole) was added cautiously by pipet. After addition of  $\text{D}_2\text{O}$  the mixture was allowed to warm slowly to room temperature.

Exchange reactions in HFBA. Small scale reactions were run in 13 x 100 mm culture tubes sealed with teflon lined screw caps. The tubes were placed in a 15 cm length of galvanized pipe, capped at both ends and vented by a small hole at the top end, and heated by immersion in a heating mantle filled with sand. For workup, the cooled reaction mixture was transferred by pipet into a separatory funnel containing ether or pentane and an excess of aqueous bicarbonate. After several such extractions, the combined organic layers were dried ( $\text{Na}_2\text{SO}_4$ ) and evaporated to recover the exchanged substrate. In most cases, little or no purification was necessary; in others, preparative TLC, sublimation, or acid-base extractions were used to obtain pure materials.

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2. M.R. Blake, J.L. Garnett, I.K. Gregor, W. Hannan, K. Hoa, and M.A. Long, *J. Chem. Soc. Chem. Commun.*, 930 (1975).
3. An aliquot of expt. 18 examined by nmr after 10 days showed incorporation of approximately one  $\bar{\text{D}}$ /molecule; further heating of the remaining reaction mixture for an additional 21 days yielded cleanly dideuterated material showing two one-proton singlets at  $\delta$  7.45 and 7.12 ppm in the nmr spectrum. See also A.P. Best and C.L. Wilson, *J. Chem. Soc.*, 239 (1946), and C.G. Swain, J.E. Sheats, D.G. Gorenstein and K.G. Harbison, *J. Amer. Chem. Soc.*, 97, 791 (1975).
4. The nmr of deuterated salicylamide from experiment 21 showed two broad singlets in the aromatic region at  $\delta$  7.4 and  $\delta$  7.85, and a small residual multiplet around  $\delta$  6.8 (acetone  $-\text{d}_6$ ).
5. Following paper in this journal.