TRITIUM LABELLING OF PSYCHOPHARMACOLOGIC AGENTS

Ouri Buchman and Michael Shimoni

Radiochemistry Department
Nuclear Research Centre-Negev
Beer-Sheva, P.O.Box 9001,Israel

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SUMMARY

Attempts to reach phenothiazine derivatives labelled with tritium at high specific activity, are described. All the syntheses are based on catalysed dehalogenation procedures. The most encouraging results are those obtained by halogenation of the desired compound followed by catalytic dehalogenation with Pd/C. The specific activities are in the range of 10,000-40,000 mCi/mmol.

INTRODUCTION

Phenothiazine derivatives have a tranquilizing effect and have been responsible for revolutionary changes in the treatment of psychiatric disorders. They have been proved to be remarkably safe agents when compared with previously used sedative and hypnotic drugs. They are the drugs of choice for relieving agitation and other symptoms characteristic of schizophrenia, such as hallucinations, delusions, and paranoid tension[1]. The phenothiazines appear to produce changes at all levels of the cerebrospinal axis and often produce the parkinsonism syndrome[2].

Structurally, these compounds have a three-ringed skeleton in which two benzene rings are linked by a sulfur and a nitrogen atom. Usual substitutions are in position 2 or 10 (nitrogen atom). All the phenothiazines used in psychiatry have a three-carbon bridge between the ring and the side-chain nitrogen atoms. The investigation of their clinical effects has improved immensely in the last decade, particularly with the development of radioimmunoassay procedures which require labelled compounds at high specific radioactivity.

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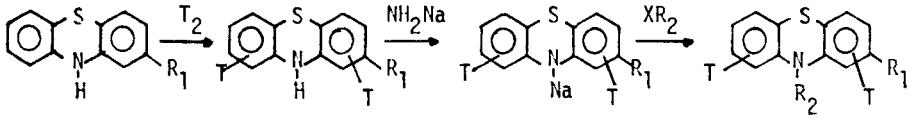
Attempts to label with tritium some of the phenothiazine derivatives provided promising results. A decarboxylation method was used for the preparation of compounds labelled in position 5[3], giving a specific activity of 120 - 160 mCi/mmol with chlorpromazine, 3-methylthiophenothiazine, thioridazine and thiethylperazine. We obtained different results when applying a non-specific labelling method, catalyzed by platinum oxide, to chlorpromazine (2,000 mCi/mmol) and to perphenazine (1,100 mCi/mmol)[4]. On the basis of this latter procedure and using palladium oxide as catalyst[5], we tritiated a series of phenothiazine derivatives under standard experimental conditions. The specific activities reached were in the range of 140 - 1,300 mCi/mmol, except in the case of phenothiazine (3,800 mCi/mmol)[6].

RESULTS AND DISCUSSION

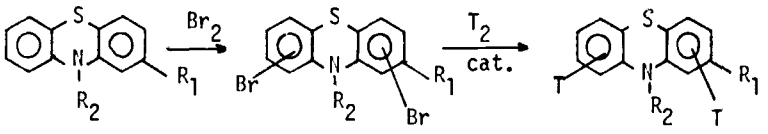
In the present work, we describe our attempts in order to raise the specific activities of the most available and potently active phenothiazine derivatives using tritium labelling. Our main interest has been focused on developing a tritiation procedure which could be applied to as large a number of compounds as possible. We were convinced that to reach increased specific activities the approach must be by direct incorporation of tritium through synthesis. Firstly, we took into consideration the possibility of performing the radioactive synthesis by adapting procedures as developed in the literature[7] and which may be summarized as in scheme I:



(I)

It seems obvious that such a scheme requires the use of high specific activity phenothiazine (R_1 = H) already in the early steps of the synthesis. We preferred to keep this option as a last alternative.

We attempted bromination of the aromatic rings of phenothiazine derivatives with molecular bromine, by adaptation of the method used for phenothiazine[8]. The syntheses were followed by catalytic dehalogenation with tritium gas (scheme II):



(II)

In the first experiments, the exchange of the aromatic bromine with tritium was catalyzed by platinum black, which was activated by the *in situ*[4] reduction of PtO_2 with tritium gas. Data of the specific activities obtained are given in table 1. These results were disappointing and, therefore, this procedure was discontinued.

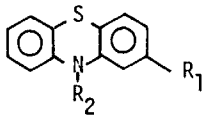
Table 1: Debromination with activated platinum catalyst.

Compound	Total tritium consumed (Ci)	Tritium used in catalyst activation (Ci)	Incorporated ^3H(mmol) / Substrate (mmol)	Spec.Act. mCi/mmol
Perphenazine	20	6	1.2	decomp.
Promethazine	16	6	0.9	400
Levomepromazine	18	6	2.0	0
Chlorpromazine	20	6	1.1	1,800

Experimental conditions: 0.1 mmol substrate in 0.5 ml methanol and 0.1 ml triethylamine, 0.05 mmol PtO_2, reaction temp.: 25°C.

In a second series of experiments, in which Pd/C was used as catalyst, the results were more encouraging. But, in spite of the incorporation of 0.75 - 2.6 tritium atoms per molecule into the crude brominated compounds, the final products were relatively poorly labelled. The specific activities were improved (table 2) by addition of a great excess of triethylamine to the bromo derivative.

Table 2: Debromination with Pd/C catalyst.



Compound	R_1	R_2	Incorporated ^3H / Substrate	Specific Activity* mCi/mmol
Phenothiazine	H	H	2.0	39,100
Promethazine	H	$CH_2CH(CH_3)N(CH_3)_2$	2.5	36,700
Promazine	H	$(CH_2)_3N(CH_3)_2$	2.0	25,600
Perazine	H	$(CH_2)_3N\langle\rangle NCH_3$	2.1	15,300

*The specific activities are given for the best results obtained with purified products.

An insoluble triethylammonium bromide salt, which precipitated from the medium (dioxane), was discarded and the tritiations were performed on the free-base compounds. This prevents the competitive hydrogen-tritium exchange in the hydrobromide salts of the phenothiazines. Comparative results are given in table 3.

In addition, the solvent effect on the efficiency of tritiation was determined with several derivatives. The results shown in table 4 were not found positive enough to decide whether an aprotic solvent such as dioxane is more effective than methanol, generally employed as the tritiation medium in our previous work[4,5]. However, due to the good solubility of the bromo derivatives in dioxane, and in order to facilite comparative experiments, all the labellings were performed in this solvent.

Table 3: Effect of triethylamine on specific activity.

Compound	Triethylamine added (ml)	Specific activity* mCi/mmol
Fluphenazine	0.1	7,500
	0.1	9,300
	0.25	18,800
Promethazine	0.1	11,800
	0.25	36,700
Trifluoroperazine	0.1	12,800
	0.25	24,000

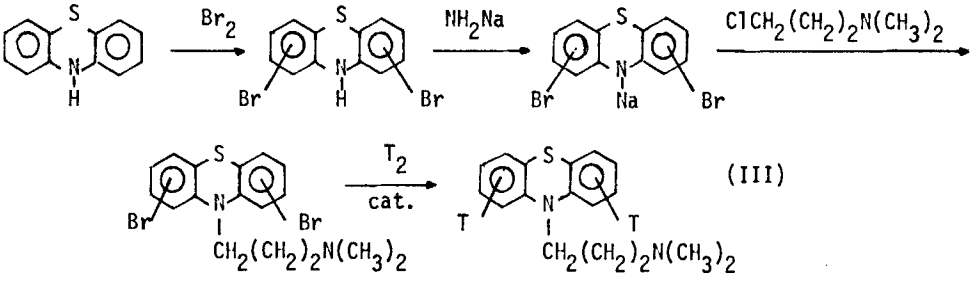
*The specific activities are given for the purified products.

Table 4: Effect of solvent on specific activity.

Compound	Solvent	Specific activity* mCi/mmol
Fluphenazine	Methanol	13,200
	Dioxane	13,000
	Dioxane	16,500
	Dioxane	11,700
Levomepromazine	Methanol	19,400
	Dioxane	17,300
Promethazine	Methanol	11,800
	Dioxane	34,900

*The specific activities are given for the purified products.

We also attempted to improve the specific activities, following the steps as described for the synthesis of promazine in scheme III:

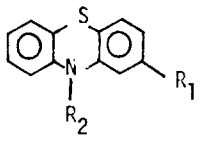


Two facts led us to try this last scheme: the high specific activity provided by catalytic debromination of bromo phenothiazine (39,000 mCi/mmol, table 2) and the success in synthesizing dihalogeno phenothiazine derivatives, starting from dichloro phenothiazine[9]. However, this procedure (scheme III) provided tritiated promazine with a specific activity of 30,200 mCi/mmol, a value not very much higher than that obtained by direct tritiation of promazine by debromination (25,600 mCi/mmol, table 2). Therefore, the use of this method of synthesis with other ring-unsubstituted phenothiazines was discontinued.

In most cases, the tritiation by catalytic debromination was applied with success on derivatives substituted in position 2 (table 5). The tritiation of thiethylperazine, containing the group $-SCH_2CH_3$, failed because of the rapid and total decomposition of the labelled compound by auto-radiolysis. Decomposition resulted even when a less efficient tritiation procedure was performed using non-specific exchange, with PdO as catalyst[5].

The behavior of the 2-chloro-substituted compounds was of particular interest (table 6). 2-Chlorphenothiazine underwent spontaneous decomposition when the tritiation was performed on the bromo derivative, while the non-specific labelling provided a purifiable radioactive molecule with a specific activity of 500 mCi/mmol[6]. With the other chloro-substituted compounds investigated, the main difficulty was in the ability to cease the incorporation of tritium, stopping the reaction when the estimated quantity of bromine atoms had reacted, while leaving the chlorine atom intact.

Table 5: Debromination of 2-substituted derivatives, catalyzed by Pd/C.

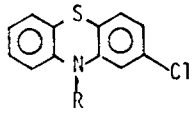


Compound	R_1	R_2	Incorporated ^3H / Substrate	Specific activity* mCi/mmol
Trifluoromethyl-phenothiazine	CF_3	H	1.8	23,300
Levomepromazine	OCH_3	$CH_2CH(CH_3)CH_2N(CH_3)_2$	2.6	17,300
Etymemazine	C_2H_5	$CH_2CH(CH_3)CH_2N(CH_3)_2$	1.5	11,500
Trifluoroperazine	CF_3	$(CH_2)_3$N⟨⟩NCH_3	1.4	24,000
Fluphenazine	CF_3	$(CH_2)_3$N⟨⟩NCH_2CH_2OH	1.9	18,800
3-Chloropropyl-2-trifluoromethyl-phenothiazine	CF_3	$(CH_2)_3Cl$	1.5	19,400
Thiethylperazine	SCH_2CH_3	$(CH_2)_3$N⟨⟩NCH_3	0.8	decomp.

*The specific activities are given for the best results obtained with purified products.

In fact, it was not possible to isolate the debromination step completely from the dechlorination one. However, an abrupt decrease in the rate of reaction was observed and considered as the end of the elimination of the bromine atoms. The results (table 6) showed that the bromo aromatic atoms, probably bonded in the positions 3, 7 and/or 9[8], are generally more reactive than chlorine in position 2.

Table 6: Debromination of 2-chloro-substituted derivatives, catalyzed by Pd/C.



Compound	R	Incorporated ^3H / Substrate	Dechlorinated* compound (%)	Specif.activ.** mCi/mmol
Chlorphenothiazine	H	1.8	---	decomp.
Chlorpromazine	$(CH_2)_3N(CH_3)_2$	2.4	35	25,300
Prochlorperazine	$(CH_2)_3N\langle\rangle NCH_3$	1.2	40	3,700
Perphenazine	$(CH_2)_3N\langle\rangle NCH_2CH_2OH$	1.7	50	25,900

*Calculated from the radioscanning of the crude tritiated compound.
**The specific activities are given for the best results obtained with purified products.

Encouraging results were obtained with two of these compounds by tritiation through deiodination of the related derivatives: perphenazine (4,600 mCi/mmol) and prochlorperazine (16,100 mCi/mmol). The competition between chlorine and iodine during the halogen-tritium exchange favoured the iodine and the reaction was so fast that it left the chlorine atom unaffected. This procedure of tritiation, including the synthesis of the iodinated precursors of the chloro-substituted compounds, is still under investigation.

EXPERIMENTAL

The tritiation experiments were performed on a vacuum manifold, as previously described[10]. Ultra-violet spectra were recorded with a Perkin-Elmer UV - visible spectrophotometer, Model 402. Radiochemical and chemical purity were determined by radiochromatogram scanning of thin-layer chromatography (t.l.c.) plates on Berthold Dunnschicht Scanner II, Model LB 2722; total and specific activity were measured on Packard Tri-Carb Liquid Scintillation spectrometer, Model 3375.

Typical tritiation by debromination (Pd/C catalyst):

a) Bromination

0.1 mmol of the phenothiazine derivative, dissolved in acetic acid (salt form) or in chloroform (free base), is reacted with about 6 equivalents of molecular bromine at room temperature, in a closed vessel and under continuous stirring, during 3 - 10 days. The solvent and the unreacted bromine are removed by *in vacuo* distillation, the residue is washed twice with 5 ml methanol and the solvent is evaporated. The bromo derivative is dissolved, unde reflux, in a solution of 5 ml dioxane and 1 ml triethylamine. The triethylammonium salt is separated by centrifugation. The filtrate is evaporated and the residue is dissolved in 2 ml dioxane.

b) Tritiation

To about a quarter of the above solution is added 0.25 ml of triethylamine and 15 mg of 10% Pd/C catalyst. The solution is frozen, the reaction system is washed twice with nitrogen gas and evacuated to a residual pressure of 10^{-2}mm Hg. 20 - 40 Ci tritium gas are then transferred into the reaction vessel, developing an initial pressure of 300 - 500 mm Hg. The solution is allowed to return to room temperature and the suspension is vigorously stirred. Decrease of the pressure gives a measure of progress in the debromination with simultaneous incorporation of tritium atoms into the molecule.
With the end of the reaction, 2 - 12 Ci (0.03 - 0.2 mmol) of tritium are consumed. The reaction vessel is frozen, residual tritium is evacuated and the solvent is separated by cryo-sublimation. The catalyst-substrate mixture is washed twice with 2 ml methanol aliquots which are removed by cryo-sublimation. The substrate is then dissolved in 10 ml of methanol and filtered from the catalyst through a Millipore pre-filter giving the crude tritiated phenothiazine derivative in the free-base form. The solution is evaporated, 10 ml NaOH solution 1 N and 15 ml CCl_4 are added and vigorously shaked. The organic phase is separated, dried over $MgSO_4$, filtered and evaporated. The residue is dissolved in 10 ml of a solution of methanol:HCl (1:1). The solution is evaporated and the residual solid (phenothiazine derivative in its HCl form) is dissolved in methanol.

c) Purification and analytical control

The purification is performed by preparative t.l.c. on pre-coated silica-gel plates (2 mm, Merck) in the appropriate solvents system. The chemical and the radiochemical purity are determined by analytical t.l.c. on pre-coated silica-gel plates (0.25 mm, Merck) in three different solvents systems. The concentration of the solutions is determined by UV spectrum of the free-base in methanol. The specific activities are calculated from the radioactive concentration of the solutions, as obtained by liquid scintillation.

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