

SYNTHESIS OF [¹¹C]-SARIN

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SUMMARY

[¹¹C]-acetone, prepared from ¹¹CO₂ and methyl lithium, was reduced to [¹¹C]-isopropanol. The latter reacted with methylphosphonic acid difluoride in the presence of diisopropylamine, to yield [¹¹C]-sarin.

3.4 GBq (100 mCi) of [¹¹C]-sarin may be obtained from about 55.5 GBq (1.5 Ci) of ¹¹CO₂ in 40 minutes. The product purified by HPLC, is obtained with a specific radioactivity ranging from 22.2 to 33.3 GBq (600 to 900 mCi/μmol.).

Key words : [¹¹C]-Sarin - PET - acetylcholinesterase inhibitor.

INTRODUCTION

Sarin (methylphosphonofluoridic acid isopropylester) is an extremely active cholinesterase inhibitor. This very lipophilic molecule shows a good specificity and a high affinity for the enzyme.

The use of [^{11}C]-sarin, in PET, should define the exact functional localization of the acetylcholinesterase (AChE) activity and reveal the cerebral mapping of the enzyme.

It is known that the main role determined for AChE is to terminate the excitatory action of acetylcholine after the latter has acted on the post synaptic receptor. However, other functions are apparently attributed to this enzyme, in particular concerning neuron growth during development and regeneration. The possibility of visualizing AChE sites *in vivo* would therefore be a precious tool in the study of degenerative disease (e.g. Alzheimer disease).

A synthesis of sarin has already been published (ref.1). Synthesis with carbon-14 was reported by A. Chang Sin-Ren et al.(ref.2).

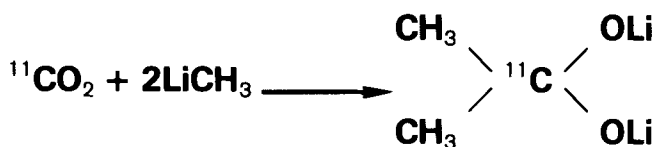
The relative ease of preparation of [^{11}C]-acetone (ref.3) and facile reduction to [^{11}C]-isopropanol (ref.4), which readily reacts with methylphosphonic acid difluoride (furnished by the Centre d'Etudes du Bouchet, France) led us to develop this sequence for the [^{11}C]-labelling of sarin. The very high specific radioactivity obtained with carbon-11 labelled molecules renders this radioisotope convenient for PET studies with [^{11}C]-sarin. It should be noted that sarin is highly toxic (DL50 for the monkey is 60-70 $\mu\text{g}/\text{kg}$).

RESULTS AND DISCUSSION

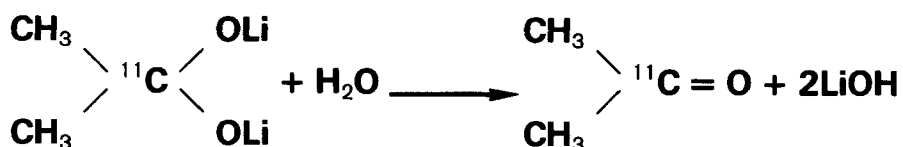
Carbon-11 is obtained by the nuclear reaction: $^{14}\text{N}(\text{p},\alpha)^{11}\text{C}$ Nitrogen (Air Liquide, purity N60) target, under 8 bar pressure, is irradiated with 20 MeV protons at 30 μA intensity for 30 mn.

The Carbon-11 so produced, combines immediately with traces of oxygen present in the target to give about 55.5 GBq (1.5 Ci) of $^{11}\text{CO}_2$. After irradiation, $^{11}\text{CO}_2$ is trapped in a small stainless

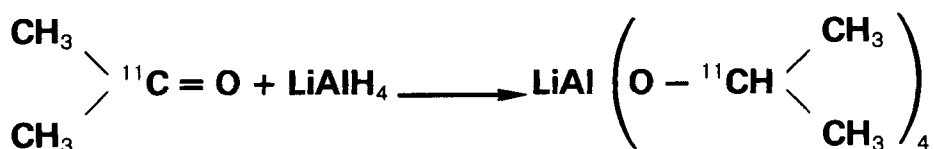
steel tube cooled to -186°C (liquid argon). On warming to room temperature the $^{11}\text{CO}_2$ released reacted with methyllithium to give the following complex.



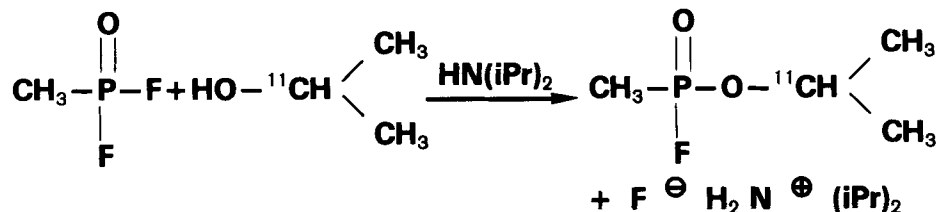
The complex is then hydrolysed and the excess methyllithium destroyed.



Reduction of the [¹¹C]-acetone with Lithium Aluminium Hydride (LAH), and hydrolytic work up, produces [¹¹C]-isopropanol.



The [¹¹C]-isopropanol reacts instantaneously with methylphosphonic acid difluoride in the presence of diisopropylamine.



- Chromatography (HPLC) of the reaction mixture reveals [¹¹C]-sarin as the major of the two radioactive products.

- This [¹¹C]-sarin is chromatographically pure, giving a

shoulder free peak when co-injected in the presence of the reference compound in HPLC.

- Retention time of isopropanol, methylphosphonic acid difluoride and sarin are respectively: 6, 9 and 15 minutes.

- A HPLC column, packed with styrene-divinylbenzene crosslinked copolymers (PRP-1), was used in order to prevent possible damage of the [^{11}C]-sarin by reaction with residual silanols, which can be present on C18 bonded silica columns.

- When methyllithium is in a too high excess, the yield of [^{11}C]-acetone decreases due to formation of [^{11}C]-tert-butanol, as has been described earlier (ref.3).

- Lithium Aluminium Hydride, used to reduce acetone in isopropanol, is solubilized in ether. The use of THF was not succesful because, even after its evaporation, traces of THF were carried into the last reaction flask and hindered the purification of sarin (the retention times of THF and sarin are the same).

- The obtained [^{11}C]-sarin is in the racemic form.

- The enzymatic inhibition was measured by Ellman's technic: sarin inhibits acetylcholine esterase in the presence of acetylthiocholine. This reaction was detected by optical absorbance at 412 nm (ref.5).

- 3.4 GBq (100 mCi) of [^{11}C]-sarin were obtained 40 minutes after the end of bombardment with a specific radioactivity ranging from 22.2 to 33.3 GBq/ μmol (600-900 mCi/ μmol).

MATERIALS AND METHODS

1) Apparatus

The apparatus was described earlier (ref.3). The reactions were prepared in cylindroconical tubes (1.5 ml) closed by chromatographic septa (Touzart et Matignon) and interconnected with teflon tubes (int. ϕ : 0.08 cm), wich were fixed onto medical needles.

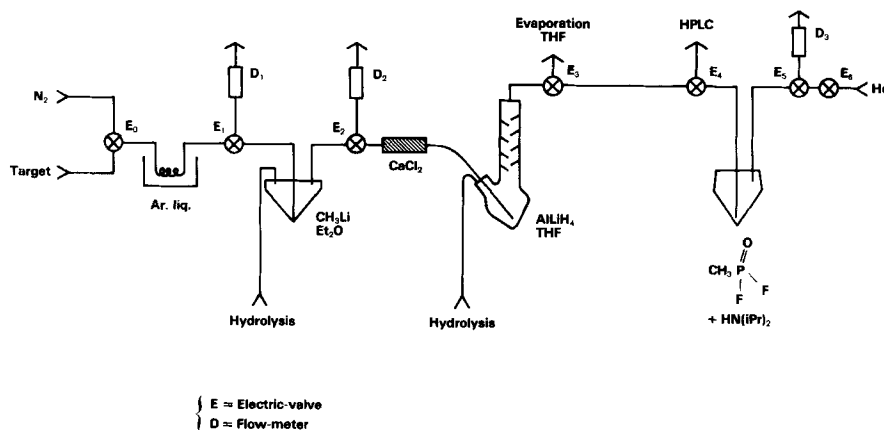
The passage of gases was controlled by compressed air-operated electrovalves.

The reaction tubes were fixed on a mobile rod and could be transferred from a cold bath (-10°C) to a hot bath (110°C).

The radioactivity was measured by four ionisation chambers : one beside the CO₂ trap, another near the tube in which acetone was reduced, the third one close to the tube in which sarin was formed and the fourth one at the chromatograph column outlet.

All this apparatus was contained in a hermetically closed shielded cell (5 cm lead).

**SCHEME OF THE APPARATUS FOR
THE [¹¹C]-SARIN SYNTHESIS**



2) Experimental

The ¹¹CO₂ formed was carried by the nitrogen flow, through a P₂O₅ trap (int. φ: 0.4 cm, L: 5cm) to retain all traces of water, and was collected in a metal loop (int. φ: 0.1 cm, L: 40 cm) cooled in liquid argon (-186°C). This operation took about 5 min and gave approximately 55.5 GBq (1.5 Ci) of ¹¹CO₂.

The loop was then removed from the liquid argon and brought to room temperature. The radioactivity was carried by a nitrogen flow (N48, 20ml/mn) into a first tube (previously oven dried) cooled to

-10°C and containing about 7 μ moles methylolithium in 100 μ l anhydrous ether (distilled over sodium, kept under argon). The transfer of radioactivity was over in less than ten seconds. The tube was introduced for a few seconds in a hot bath (110°C) in order to evaporate the ether, afterwards it was cooled to 0°C, then 100 μ l of water were added and the tube was heated again to 110°C and connected up with the second tube through an electrovalve. A nitrogen flow (20 ml/min) was used to transfer [¹¹C]-acetone in less than 4 min. A calcium chloride trap (int. ϕ :0.5 cm, L:5 cm) absorbed the water vapor carried on the gas stream.

The second tube contained 50 μ moles of LAH in 700 μ l of dry ether. The reduction of [¹¹C]-acetone was carried out during the evaporation of ether. Then 500 μ l of 0.5 N HCl solution was added and the mixture was heated to 110°C. [¹¹C]-Isopropanol was then distilled through an electrovalve into a third tube containing a mixture of methylphosphonic acid difluoride (10 μ l) and diisopropylamine (50 μ l) in DMF (100 μ l). This reaction was instantaneous.

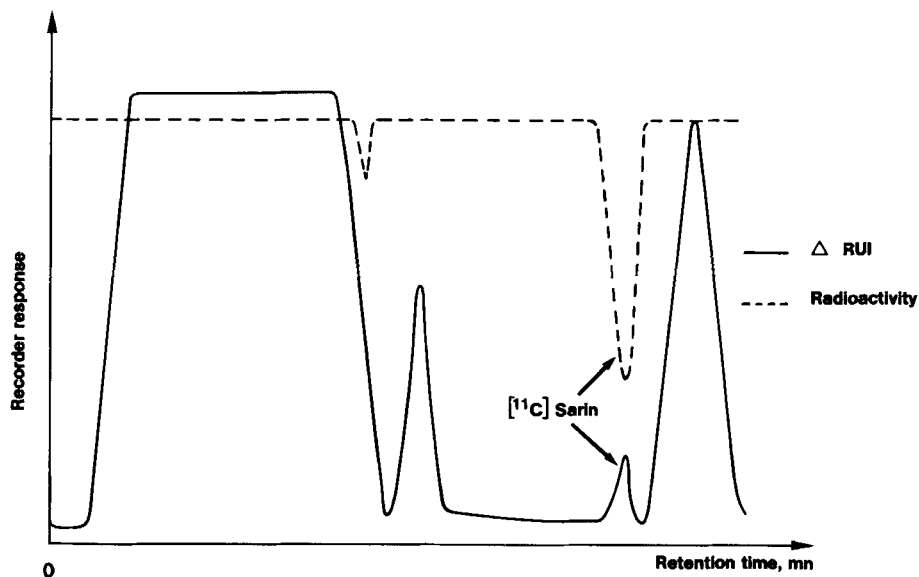
The solution was injected onto an HPLC PRP-1 column (int. ϕ :0.7cm, L:30 cm, Touzart et Matignon). The eluent used for the separation was 65% saline solution and 35% ethanol. The flow rate was 2 ml/min (Waters pump M 510).

The elution of sarin was monitored by radioactivity and refractive index, sensitivity: 64, scale factor: 0.20 (Waters detector M 410).

Specific radioactivity was measured according to previous injection of a pure standard solution of sarin.

In vivo studies are presently in progress in our laboratory.

CHROMATOGRAM OF [¹⁴C]-SARIN
SYNTHETIC MIXTURE



Acknowledgments

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