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Synthesis of a tri-tritiated heterobifunctional reagent, a potential tool in photoaffinity labeling technology.

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SUMMARY

The synthesis of the radiolabelled heterobifunctional reagent N-methyl-N-chloroformyl-N'-terbutoxycarbonyl-para-phenylenediamine **A** (fig. 1) is presented. In particular, the use of tri-tritiated methyl iodide in defined reaction conditions allowed the synthesis of this probe **A** (fig. 1) with a high specific radioactivity (77 Ci/mmol).

INTRODUCTION

In the last ten years, the need for heterobifunctional reagents for the synthesis of photoaffinity labels has considerably increased. These reagents carry usually an electrophilic center so that they can be coupled to a ligand molecule and a photoactivatable group remaining unmodified in the coupling step but allowing further photolabeling experiment. The photoactivatable groups which have been described are usually arylazides, diazirines, and diazo compounds[1] and some of these reagents are commercially available. Owing the chemical unstability of aryldiazonium ions, only very few studies were made with these probes, although this chemical group has been demonstrated to be a powerful photoaffinity label[2,3].

We have recently described the synthesis of a new heterobifunctional reagent, wearing a carbamoyl chloride group as electrophilic center, and a protected diazonium as a hidden photoactivatable group[4] **B** (fig. 1).

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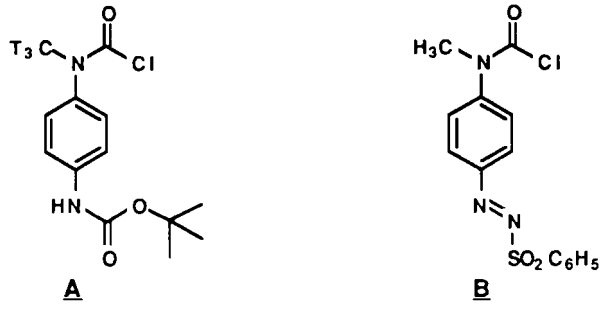
Figure 1 :



A B

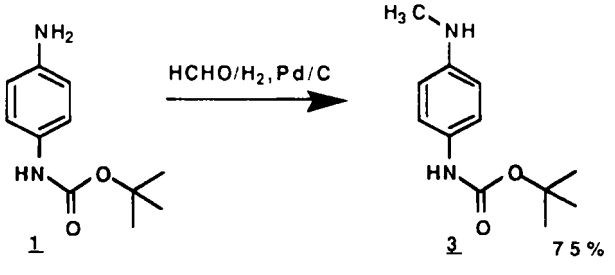
The usefulness of this reagent was demonstrated, in particular through its efficient coupling to nucleophilic functions followed by an easy deprotection to the diazonium moiety[5]. However, photoaffinity labeling experiments require the use of radiolabeled probes, for example to allow the identification of unknown receptors. In particular the use of probes with high specific radioactivity would be very advantageous for the detection of receptors present at very low concentrations, such as of hormone or neurotransmitter receptors.

In the present work we describe the synthesis of a tri-tritiated bifunctional reagent **A** (fig. 1) possessing a potential aryldiazonium moiety.

RESULTS AND DISCUSSION

Since our aim was to synthesize reagent **A** (fig. 1) with high specific radioactivity, we did not use the classical methylation reaction[5] shown in scheme 1 to introduce the radioactivity, which would allow the incorporation of only one tritium atom.

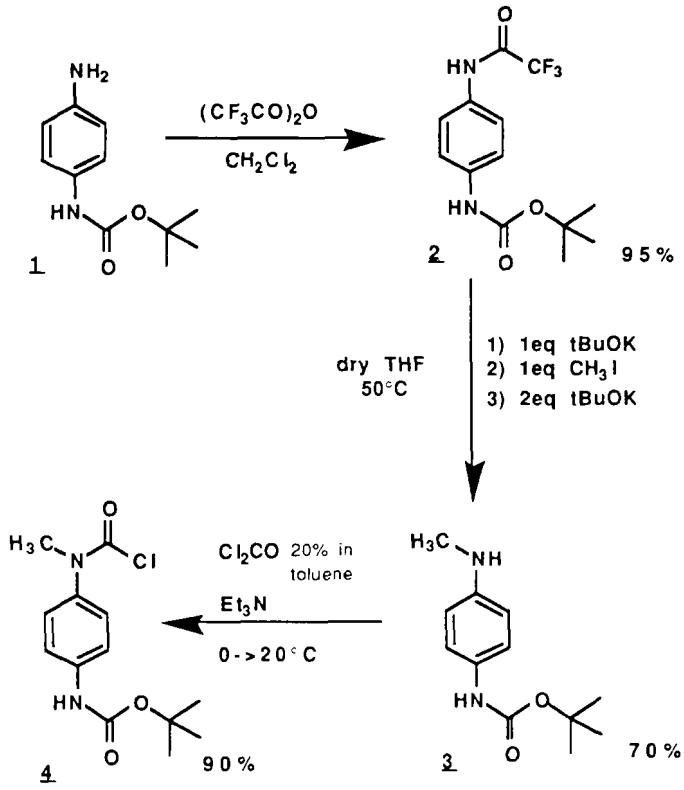
Scheme 1 :



1 3 75 %

Therefore methyl iodide was used as shown in scheme 2

Scheme 2 :



N-terbutoxycarbonyl-para-phenylenediamine **1** was synthesized as already described[6]. Compound **2** was obtained in almost quantitative yields by N-trifluoroacetylation of **1** whith trifluoroacetic anhydride in CH_2Cl_2. Methylation of **2** whith methyl iodide in the presence of potassium terbutoxide (tBuOK) in THF gave **3** in 70% yield and the final compound **4** was obtained through classical treatment with phosgene. The use of tri-tritiated methyl iodide as radioactive source allowed the synthesis of the corresponding reagent **A** (fig. 1) with a specific radioactivity of 77 Ci / mmol (2.85 TBq / mmol). This chemical was identified to the non-radiolabeled probe by co-elution on HPLC.

The methylation reaction on the trifluoroacetanilide derivative **2** (step **2** to **3** in scheme 2) showed unexpected difficulties and required extensive set up. The unusual experimental procedure deserves some comments. The classical procedure that is, use of one equivalent of a base followed by the alkylating reagent

and final aqueous treatment did not succeed. Either a conversion to the starting amine or a N,N' dialkylation reaction was observed. A series of experimental trials led to the conclusion that using tBuOK as base, the addition of two equivalent excess of this base at 50°C, (scheme 2, step **2** to **3**) allowed a successful methylation reaction. Despite the absence of satisfactory explanations for this unusual procedure, it fulfills the desired experimental conditions for the synthesis of a radioactive chemical; that is, short reaction time in rather mild conditions, satisfactory yields and no use of a too large excess of the radioactive precursor (^{3}H-Methyl iodide).

CONCLUSION

We have described in this article the synthesis of the heterobifunctional reagent **A** (fig. 1), a tri-tritiated molecule with high specific radioactivity. This radioactive reagent can be coupled to enzymes or receptors ligands possessing a nucleophilic moiety (i.e. an amine function) and converted subsequently to the photoactivatable diazonium salt by diazotization. Alternatively, if this reaction creates problems, the reagent **A** can be first converted to the radioactive reagent **B** (fig. 1) and than coupled to the ligand molecule[7]. The potentialities of the radioactive reagent **A** is of interest for biological studies, in particular this reagent would avoid the use of iodinated derivatives using ^{125}I isotope. Radioactive photoaffinity probes using this reagent are currently synthesized.

EXPERIMENTAL SECTION

General

^{1}H-NMR spectra were recorded at 200 MHz, on a BRUCKER WP-200 SY instrument, δ are given in ppm.

Melting points were taken on a Kofler block and are uncorrected.

Mass spectra were obtained with a Finnigan spectrometer.

N-terbutoxycarbonyl-para-phenylenediamine **1**.

Compound **1** was synthesized as previously reported[6].

m.p. : 114 - 116°C

^{1}H-NMR ($CDCl_3$) : δ = 1.50 (s, 9H, terbutoxyl); 3.43 (broad s, 2H, NH_2); 6.33 (broad s, 1H, NHBoc); 6.63 (d, 2H, arom, J = 8.6 Hz); 7.13 (d, 2H, arom, J = 8.6 Hz).

N-trifluoroacetyl-N'-terbutoxycarbonyl-para-phenylenediamine **2**.

A solution of trifluoroacetic anhydride (1 ml, 7 mmol) in 20 ml of dry CH_2Cl_2

was added dropwise at room temperature, for 1 h 30, to a stirred mixture containing **1** (1.04 g, 5 mmol) and $NaHCO_3$ (1.18 g, 14 mmol) in 35 ml of dry CH_2Cl_2. Compound **2** crystallized out and thus a strong stirring was required during this addition. The reaction was monitored on thin layer chromatography (TLC, ethyl acetate/hexane, 4/6). After a complete disappearance of **1**, CH_2Cl_2 was evapored under reduced pressure, and the obtained white solid was successively washed with water (3 x 10 ml) and by a mixture of CH_2Cl_2/hexane (50/50, 3 x 10 ml). After filtration the solid was vacuum dried leading to 1.44 g (95 % yield) of product **2** obtained as a white solid which was pure enough (^{1}H-NMR, TLC) for further reactions.

m.p. : 198 - 200°C
^{1}H-NMR (CD_3COCD_3) : δ = 1.48 (s, 9H, terbutoxyl); 7.60 (m, 4H, arom).

N-methyl-N'-terbutoxycarbonyl-para-phenylenediamine **3**.

1.22 g (4 mmol) of **2**, 0.25 ml (4 mmol) of methyl iodide and 0.45 g (4 mmol) of potassium terbutoxide (tBuOK), were dissolved in 70 ml of freshly distilled tetrahydrofuran (THF). The stirred solution was heated at 50°C for 5 min, under argon. Then a solution of dry THF (50 ml) containing 0.70 g (6,2 mmol) of tBuOK was added dropwise for 1 hour. After evaporation of the solvent under vacuum, the residue was resuspended in ethyl acetate (20 ml). The suspension was filtered off and the filtrate evaporated under reduce pressure. The resulting crude oil was then chromatographed on a silica gel column (250 g) eluted with ethyl acetate/hexane (25/75) and afforded 0.62 g of compound **3** (70 % yield) as a colourless oil which solidified very slowly.
m.p. : 49°C
^{1}H-NMR ($CDCl_3$) : δ = 1.50 (s, 9H, terbutoxyl); 2.81 (s, 3H, N-methyl); 6.26 (broad s, 1H, NHBoc); 6.57 (d, 2H, arom, J = 8.7 Hz); 7.17 (d, 2H, arom, J = 8.7 Hz).

N-methyl-N-chloroformyl-N'-terbutoxycarbonyl-para-phenylenediamine **4**.

A solution of **3** (0.444 g, 2 mmol), and triethylamine (0.5 ml, 3.6 mmol) in 18 ml of dry toluene was added dropwise to a chilled solution of phosgene in toluene (20 %, d = 0.95, 5.25 ml, 10 mmol). After the addition, the mixture was stirred for an additional 10 hours at 30°C. The reaction flask was placed under reduced pressure (30 mm Hg) to remove the excess of phosgene. The organic layer was then washed with water, dried ($MgSO_4$) and concentrated under vacuum. The resulting brown solid was recrystallized in ethyl acetate/hexane to afford 0.512 g of compound **4** (90 % yield) as a white solid.

m.p. : 137 - 138°C
^{1}H-NMR ($CDCl_3$) : δ = 1.52 (s, 9H, terbutoxyl); 3.34 (s, 3H, N-methyl); 6.42 - 6.65

(broad s, 1H, NHBoc); 7.10 (d, 2H, arom, J = 8.7 Hz); 7.37 (d, 2H, arom, J = 8.7 Hz).

N-(^{3}H-methyl)-N'-terbutoxycarbonyl-para-phenylenediamine ^{3}H-Me-**3**.

To a solution of **2** (19.5 mg, 0.064 mmol) in freshly distilled THF (0.75 ml) were successively added a solution of tBuOK (0.195 ml, 0.066 mmol) and a solution of ^{3}H-methyl iodide (5 Ci, 0.185 TBq) in THF (0.7 ml). The reaction mixture was heated at 40°C and a solution of tBuOK (0.066 mmol) in THF (0.195 ml) was added dropwise under stirring for 10 min. After evaporation of the solvent, the mixture was treated with 4 ml of water/ethyl acetate (1/1), extracted by ethyl acetate (2 x 2 ml) and the organic phases were collected and evaporated. Labile tritium atoms were eliminated by two successive evaporations with 10 ml of ethanol.

After purification by semi-preparative TLC on silica gel (ethyl acetate/hexane, 40/60), compound **3** was obtained (2 Ci, 74 GBq) with a radiochemical purity of 90 % (TLC).

N-(^{3}H-methyl)-N-chloroformyl-N'-terbutoxycarbonyl-para-phenylenediamine **A**.

To a solution of phosgene in toluene (20 %, d = 0.95, 55.5 µl, 0.107 mmol) was added dropwise at 0°C a solution of ^{3}H-Me-**3** (2 Ci, 74 GBq) in anhydrous toluene (0.27 ml) and triethylamine (6.9 µl). The mixture was stirred for 5 min at 0°C and then allowed to warm to room temperature. After evaporation of the solvent, the residue was taken up with 4 ml of water/ethyl acetate (1/1). The aqueous phase was extracted with ethyl acetate (2 x 2 ml) and the combined organic layers were evaporated to dryness. After addition of toluene (200 ml), **A** (1.09 Ci, 40.3 GBq) was obtained with a radiochemical purity superior to 98 % (TLC on silica gel, ethyl acetate/hexane - 40/60) and a specific activity of 77 Ci / mmol (2.85 TBq / mmol, MS).

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