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SYNTHESIS OF [^{77}Br] 5,7-DIBROMO-4-OXO-1,4-DIHYDRO-QUINOLINE-2-CARBOXYLIC ACID, A POSSIBLE SPECT TRACER FOR NMDA RECEPTOR STUDIES

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SUMMARY

The synthesis of [^{77}Br] 5,7-dibromo-4-oxo-1,4-dihydroquinoline-2-carboxylic acid is described. The labelling is performed in order to obtain a tracer for SPECT studies of the N-methyl-D-aspartate receptor in human brain. Radiosynthesis was carried out using a non-isotopic nucleophilic halogen exchange, starting from 5-iodo-7-bromo-4-oxo-1,4-dihydroquinoline-2-carboxylic acid. In order to obtain the best radiochemical yield, several parameters such as reaction temperature, reaction time, amount of precursor and amount of Cu catalyst were studied. Under the best labelling conditions, the radiochemical yield was 85 %. The product was found to be chemically and radiochemically pure as determined by HPLC.

KEY WORDS: NMDA receptor, bromide-77, copper(I) catalysed halogen exchange, [^{77}Br] 5,7-dibromokynurenic acid.

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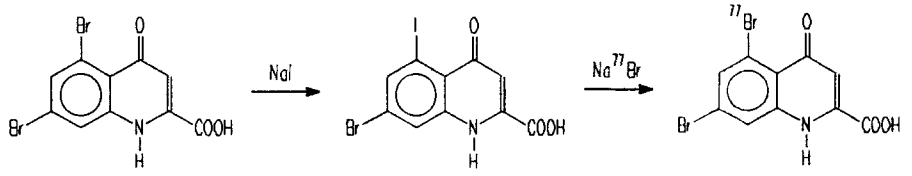
INTRODUCTION

Overactivation of Excitatory Amino Acid (EAA) receptors may be associated with several disorders of the central nervous system[1-2]. The NMDA receptor plays an important role in learning and developmental processes but also in various pathological conditions. Overactivation of the NMDA subtype of EAA receptors by abnormally high extracellular glutamate levels leads to lethal Ca^{2+} influx (via the NMDA receptor ion channel) and to neuronal death in cerebral ischemia, epilepsy and Alzheimer's disease[3-7] Since Johnson and Ascher[8] discovered that glycine potentially amplifies the depolarisation induced by NMDA in cultured cells by increasing the channel-opening frequency of the receptor, compounds which would block the effect of glycine have been sought as potentially superior NMDA antagonists. Derivatives of the non-selective EAA antagonist kynurenic acid have been evaluated for their antagonist activity at receptors sensitive to NMDA and optimisation led to some potent and selective glycine/NMDA antagonists[9], such as 5,7-dibromokynurenic acid. From these observations we decided to synthesise the bromine-77 labelled 5,7-dibromokynurenic acid as a possible SPECT tracer for the NMDA receptor. Therefore, we prepared the 5,7-dibromokynurenic acid **1** (Scheme 1) as reference material for characterisation and HPLC analysis of the tracer. Compound **1** was synthesized using a modified Conrad-Limpach method[10]. 5-Iodo-7-bromokynurenic acid **2** was used as a precursor for the tracer and was prepared using a non-isotopic nucleophilic halogen exchange in the presence of iodide, Cu(I) and an excess of reducing ($SnSO_4$ and gentisic acid) and complexing (citric acid) agents[10-12] (Scheme 1). The bromine-77 production was performed at the VUB cyclotron using the ^{75}As (α, 2n) ^{77}Br reaction[13] with As_2O_3 as target material[14]. The purification was accomplished by passage of the radioisotope through an anion-exchange column (Dowex AG 1x8)[15]. Labelling was started from the iodinated precursor **2** (Scheme 1) and performed using the same type of nucleophilic halogen exchange method. This permitted us to obtain the expected radiotracer **3**, which was separated and purified by RP-HPLC. The optimum labelling conditions are presented in the paper.



1 **2** **3**

Scheme 1. Synthesis of the iodinated precursor, 5-iodo-7-bromokynurenic acid **2**, and of the tracer [^{77}Br]5,7-dibromokynurenic acid **3**

EXPERIMENTAL

HPLC conditions for determination of the radiochemical yields

Waters HPLC set up (6-valve injector with 100-µL loop, 590 pump), Unicam L3 UV detector at 254 nm, Mini Instruments NaI(Tl) radioactivity detector and Ankersmit two-channel recorder. Chromatographic separation is carried out on an Econosil RP C-18, 10 µm, 250 X 10 mm column using the eluent H_2O/EtOH/CF_3COOH - 67/33/0.1 at a flow rate of 7 mL/min.

Preparation of the reaction solutions

Solution A: $SnSO_4$ (420 mg), gentisic acid (308 mg) and citric acid (420 mg) were dissolved in 10 mL of a 10% acetic acid solution.

Solution B: $CuSO_4.5H_2O$ (100 mg) was dissolved in 10 mL water.

Determination of the radiochemical yield as a function of reaction time

To determine the radiochemical yield as a function of the reaction time, 1 µmol 5-iodo-7-bromokynurenic acid, dissolved in 600 µL of ethanol, was added to a mixture containing 100 µL of solution A, 25 µL of solution B and 65 µL glacial acetic acid. After addition of 10 µL bromine-77 radioisotope solution, the vial was placed in an ultrasonic bath and flushed with N_2 for 15 min. The vial was heated in a water bath for a given time. After cooling to room temperature, 100 µL of the mixture was analysed by HPLC.

Determination of the radiochemical yield as a function of catalyst concentration

The effect of the catalyst concentration on the labelling yield was investigated by addition of various amounts of solution B (0-50 μL) to a mixture of 1 μmol 5-iodo-7-bromokynurenic acid, dissolved in 600 μL ethanol, 100 μL of solution A and 65 μL glacial acetic acid. 10 μL of the radioisotope was added, the vial was placed in an ultrasonic bath, flushed with N_2 for 15 min and heated for 1 h at 60 °C. After cooling to room temperature 100 μL of the reaction mixture was analysed by HPLC.

Determination of the radiochemical yield as a function of precursor concentration

Measurement of the labelling yield as a function of precursor concentration was accomplished by addition of various amounts of precursor (0.1-10 μmol), dissolved in 600 μL of ethanol, to a mixture of 100 μL of solution A, 25 μL of solution B and 65 μL glacial acetic acid. 10 μL of radioisotope solution was added to the vial, which was placed in an ultrasonic bath, flushed with N_2 and heated at 60 °C for 1 h. Labelling efficiency was determined by analysis of 100 μL of the reaction mixture by HPLC.

Determination of the radiochemical yield as a function of reaction temperature

Influence of reaction temperature on the labelling yield was determined by adding 3 μmol precursor, dissolved in 600 μL, to a mixture of 100 μL of solution A, 25 μL of solution B and 65 μL glacial acetic acid. After adding the radioisotope, the vial was placed in an ultrasonic bath, flushed with N_2 and heated for 1 h at various temperatures. Radiochemical yields were determined by HPLC analysis of 100 μL of reaction mixture.

Tracer purification by HPLC

Waters HPLC set up (6-valve injector with 1000-μl loop, 590 pump), Unicam L3 UV detector at 254 nm, Mini Instruments NaI(Tl) radioactivity detector and Ankersmit two-channel recorder. Chromatographic separation is carried out on a Alltima RP C-18, 5 μm, 250 X 10

mm column using the eluent H_2O/EtOH/CF_3COOH - 67/33/0.1 at a flow rate of 7 ml/min. After recovery of the peak of interest, the eluent is evaporated. After rinsing the walls of the vial with 300 µl of ethanol, 9.7 mL of buffer solution (17.9 mg KH_2PO_4 and 95.3 mg $Na_2HPO_4.2H_2O$ dissolved in 10 ml water) is added with vigorous vortexing. The final solution is sterilised by means of a 0.22 µm Millipore filter.

RESULTS AND DISCUSSION

To synthesize the [^{77}Br]5,7-dibromokynurenic acid in the highest yield, conditions of radiosynthesis were optimized. Therefore some variables such as copper(I)concentration, precursor concentration, reaction temperature and reaction time were studied to obtain the optimum conditions. The optimisation was performed by changing one of the parameters while the others were kept constant.

Radiochemical yield as a function of reaction time and temperature

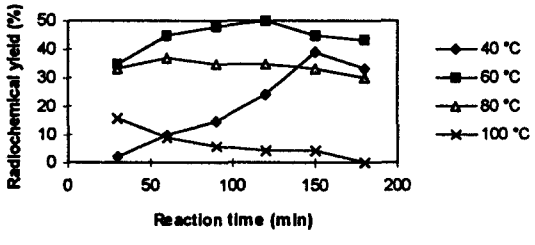


Figure 1. Radiochemical yield as a function of reaction time

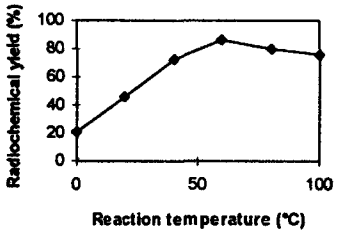


Figure 2. Radiochemical yield as a function of reaction temperature

At all temperatures, radiochemical yields decrease after a certain reaction time, after having reached a maximum (Fig. 1). The time within which this maximum is achieved depends upon the temperature. The higher the temperature, the sooner the maximum appears. This phenomenon is due to the fact that the precursor decomposes when dissolved, even at low temperatures. But decomposition increases with higher temperature. A reaction time of 1 to 2.5 h at 60 °C seems to give the best results.

The replacement of iodide by bromide seems to be a very easy reaction, because even at 0 °C we obtain about 20 % radiochemical yield. Increasing the temperature leads to a maximum labelling of 86 % at 60 °C.

Radiochemical yield as a function of copper(I) concentration

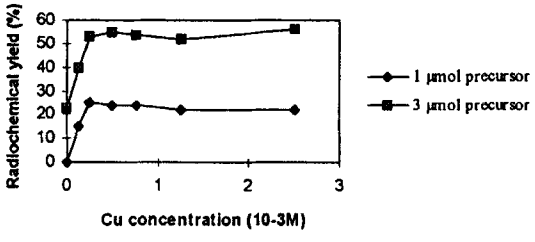


Figure 3. Radiochemical yield as a function of Cu concentration

As we expected, radiochemical yields reached a constant value at a certain concentration of catalyst. In this case, 0.25 x 10^{-3} M seems to be the concentration after which constant radiochemical yields are obtained. Using more precursor, radiochemical yields increased but also reached a constant value.

Radiochemical yield as a function of precursor concentration

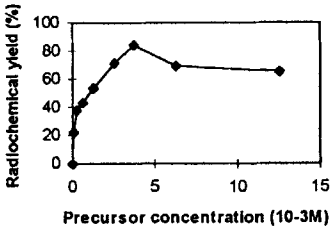


Figure 4. Radiochemical yield as a function of precursor concentration

We observed that by varying the precursor concentration, the radiochemical yield reached a maximum of 86 % at a concentration of 3.75 x 10^{-3} M. Decrease of the labelling yields with higher precursor concentrations has already been reported by Mertens et al[16]. This is probably caused by precipitation of CuI or [^{77}Br]CuBr.

It was observed that the best labelling yield for the radiobromination of 5-iodo-7-bromokynurenic acid was achieved using 1.2 mg (3 µmol) precursor and 5 µl (2.5. 10-4M) of cupric sulphate solution together with reducing and complexing agents After addition of sodium [^{77}Br] bromide the mixture must be heated for a period of 1 h at 60 °C.

CONCLUSION

This paper shows that the radiobromination of 5-iodo-7-bromokynurenic acid can be performed by a nucleophilic exchange in the presence of Cu(I) and an excess of reducing and complexing agents. Optimum reaction parameters were determined in order to obtain the [^{77}Br]5,7-dibromokynurenic acid in about 85 % yield. After HPLC purification, the tracer was obtained in a radiochemical purity > 95 %.

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