SYNTHESIS OF TRITIUM LABELLED DSP 4, A SELECTIVE NORADRENALINE NEUROTOXIN.

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SUMMARY

DSP 4 (N-(2-Chloroethyl)-N-ethyl-2-bromobenzylamine, 1) is a neurotoxin, selective for neuronal noradrenaline (NA). Tritium labelled DSP 4 (4) with a specific activity of 105 mCi/mmol was prepared. The key step in the synthesis is a reduction of the aminoester 2 with activitated sodium boro[^{3}H]hydride thus forming the alcohol 3.

Key-words: 2-chloro-[^{3}H]ethylamines, noradrenaline uptake inhibitor, tritium labelling, reduction.

INTRODUCTION

2-Chloroethylamines are important tools in the study of noradrenergic neurons (1). In the 1970's Ross and co-workers studied the chemical and pharmacological properties of the 2-chloroethylamine, DSP 4, 1, (2,3,4). It is a selective NA neurotoxin and and irreversible inhibitor of NA uptake in central noradrenergic neurons. Its mode of action involve some covalent bond formation since the effects are irreversible. To further investigate the mechanism when DSP 4 reacts with the

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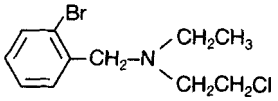
receptor, radiolabelled material was needed. This paper describes the preparation of tritium labelled DSP 4, with a specific activity of 105 mCi/mmol, suited for the studies in vitro (5).



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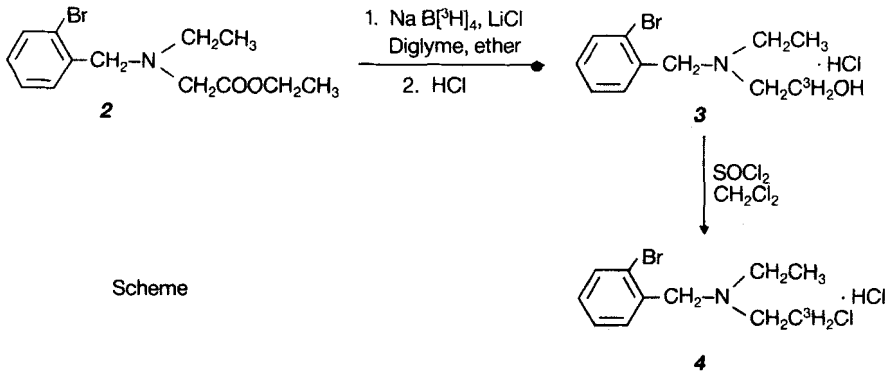
RESULTS AND DISCUSSION

The presence of a bromine atom in the phenyl ring in DSP 4 makes labelling by catalytic hydrogenation or hydrogenolysis with tritium gas impossible or extremely difficult. Therefore, we chose sodium boro-[^{3}H]hydride as the tritium source, since it is well-known that esters can be reduced by activated borohydrides. See reference 6 for a study concerning all aspects of reduction of esters with saline borohydrides. The synthetic route to DSP 4 is outlined in the Scheme. The aminoester 2, easily obtained by standard reactions, was reduced by 1 equivalent of sodium boro[^{3}H]hydride in the presence of 1 equivalent of LiCl in a 1:1 mixture of diglyme and ether, for 8 days. The desired aminoalcohol 3 was obtained in a yield of about 60%. The conditions for the reduc-



2 3 4

Scheme

tion were determined from several runs on cold material. The use of THF instead of ether as solvent gave a lower yield which is in contrast to a similar reduction of 5-carbenoxy-2-pyrrolidinone (7). Compound 3 was then treated with 8 equivalents of $SOCl_2$ using standard conditions and tritium labelled DSP 4 (4) was obtained in 78% yield. The specific activity was 105 mCi/mmol as measured by weighing.

EXPERIMENTAL

Sodium boro[^{3}H]hydride (66 Ci/mmol) was purchased from New England Nuclear. Merck aluminia 60 (0.25 mm) precoated plates were used for TLC analyses. GC analyses were performed on a Carlo Erba 4160 chromatograph equipped with a fused silica column coated with 3 µm cross-linked SE-54. ^{1}H NMR spectra were recorded at 60 MHz with a Varian EM 360 A spectrometer, using tetramethylsilane as internal standard. Radiochemical purity was determined by scanning TLC plates using a Berthold Dünnschicht-Scanner II. Radioactivity was measured with a Packard Tri-Carb 460 C liquid scintillation spectrometer.

N-(2-Bromobenzyl)-N-ethylglycine ethyl ester (2)

To a stirred mixture of N-ethyl-2-bromobenzylamine (8) (1.5 g, 7.0 mmol), K_2CO_3 (0.9 g, 7.0 mmol), and DMF (10 ml), at 120°C, was added 1.2 g (7.0 mmol) of 2-bromoethyl acetate over a 10 min period. The reaction mixture was stirred for another 60 min at 120°C, whereafter 50 ml of H_2O was added. The mixture was extracted with ether (2 x 100 ml) and the combined extracts were dried (Na_2SO_4). Evaporation of the solvent gave a crude product which was distilled at reduced pressure to give 1.0 g (48%) of compound 2. Bp 92-95°C (0.01 mm Hg) NMR ($CDCl_3$) 1.05 (t, 3H), 1.20 (t, 3H), 2.72 (q, 2H), 3.39 (s, 3H), 3.87 (s, 3H), 4.18 (q, 4H), 7.0 - 7.8 (m, 4H).

N-(2-Hydroxy-[2-^{3}H]ethyl)-N-ethyl-2-bromobenzylamine hydrochloride (3). A mixture of sodium boro[^{3}H]hydride (500 mCi, 7.5 µmol), sodium boro-hydride (0.6 mg, 15.5 µmol), LiCl (1 mg, 23 µmol), and diglyme (0.5 ml) was stirred for 60 min. Compound 2 (7 mg, 23 µmol) in ether (0.5 ml) was then added and the reaction mixture was stirred under nitrogen for 8 days. The mixture was quenched with 100 µl of acetic acid, made alkaline with Na_2CO_3, and extracted with ether (3 x 1.5 ml). The organic phase was dried over Na_2SO_4 and HCl in ether was added to the ether extract. After addition of 5.2 mg (18 µmol) of the cold analogue of 3, the ether was evaporated and the residue was recrystallized from EtOH/ether to give 9 mg of compound 3. The radiochemical purity was 83% as determined by TLC (Al_2O_3, ether/hexane 1:1).

N-(2-Chloro-[2-^{3}H]ethyl)-N-ethyl-2-bromobenzylamine hydrochloride (4). To a solution of compound 3 (9 mg, 31 µmol) in CH_2Cl_2 (0.5 ml), 20 µl (250 µmol) of $SOCl_2$ was added. The reaction mixture was stirred for 24 h and evaporated to dryness. Two recrystallizations from EtOH/ether afforded 7.8 mg (81%) of [^{3}H]DSP 4 (4). The specific activity was 105 mCi/mmol and the radiochemical purity was 90% determined by TLC (Al_2O_3, ether/hexane 1:1) and scanning.

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