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SYNTHESIS AND RADIOLABELLING OF 2β-CARBOMETHOXY-3β-(3'-IODO-4'-ISOPROPYLPHENYL) NORTROPANE AS A RADIOLIGAND FOR THE EXPLORATION OF THE SEROTONIN TRANSPORTER BY SPET.

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KEY WORDS: Cocaine derivatives, radioiodination, serotonin transporter.

SUMMARY

In order to develop a potential tool for SPET examination of the serotonin transporter (5-HTT) in the human brain, we report the synthesis and radiolabelling of 2β-carbomethoxy-3β-(3'-iodo-4'-isopropylphenyl) nortropane (LBT-44). We also report *ex vivo* autoradiography performed in the rat brain. The radiosynthesis of [^{125}I]LBT-44 was accomplished by iododestannylation of a trimethyltin precursor using [^{125}I]sodium iodide and peracetic acid or choramine-T as oxidant. After purification by reverse phase HPLC, [^{125}I]LBT-44 was obtained in a radiochemical yield higher than 75% and a radiochemical purity greater than 95%. *Ex vivo* autoradiographic studies revealed a marked accumulation of [^{125}I]LBT-44 in brain areas rich in 5-HTT whereas no accumulation was observed in the striatum, which is rich in dopamine transporters. These results are in favour of specific binding of [^{125}I]LBT-44 to 5-HTT that is required for SPET exploration in human.

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INTRODUCTION

Exploration of the human brain by Single Photon Emission Tomography (SPET) has greatly increased the last years due to successful development of new tracers labelled with single photon emitting radionuclides such as 123-iodine. Radioiodinated ligands with high affinity and specificity for the serotonin uptake sites would be of great interest for better understanding of several disease conditions. Since serotoninergic neurons degenerate in both Alzheimer's and Parkinson's disease (1, 2) and abnormalities of the serotoninergic system have been strongly linked to the pathogenesis of depression (3), imaging the serotonin transporter (5-HTT) would be a potential tool to evaluate the integrity of serotoninergic neurons in these diseases.

The cocaine analogue, 2β-carbomethoxy-3β-(4'-iodophenyl) tropane (β-CIT) has been proposed for *in vivo* imaging of the 5-HTT since it binds to this transporter (4). However, β-CIT also binds to the dopamine transporter (DAT) and this lack of specificity represents a disadvantage for the exploration of human brain tissue with moderate 5-HTT density such as the neocortex. Boja et al (5) recently evaluated the binding potency of several N-demethylated tropane derivatives to the DAT, 5-HTT and norepinephrine transporter (NET). These studies revealed that, in comparison to their N-methylated analogues, these compounds have an approximately ten-fold enhanced affinity for the 5-HTT while the increase in DAT affinity is not more than two-fold. Among these compounds, nor-β-CIT, the N-demethylated analogue of β-CIT has been labelled with 123/125-iodine and evaluated for its binding properties in monkeys and humans (6, 7). Despite an intense accumulation of nor-β-CIT in the striatum, a region rich in DAT, high and specific binding was observed in brain regions rich in 5-HTT such as the thalamus. These results are in agreement with *in vitro* results reported by Boja et al (5) and confirm that N-demethylation of tropane derivatives provides compounds with enhanced affinity for the 5-HTT.

Several structure activity relationship studies on nortropane derivatives have since been performed in order to develop new compounds with higher specificity for the 5-HTT (8).

They have shown that the affinity of these N-demethylated tropane derivatives depends on the nature of the substituent linked at the 4' position of the aromatic ring, affording in several cases compounds with high affinity and specificity for the 5-HTT, and the synthesis and biological properties of 2β-carbomethoxy-3β-(4'-ethyl-3'-iodophenyl) nortropane have been reported on this basis (9). These studies have revealed that this nortropane derivative possess a high *in vitro* affinity and specificity for the 5-HTT. Moreover, *in vivo* competition studies in rats showed that this compound inhibited the binding of [^{125}I]β-CIT in 5-HTT-rich cerebral regions such as the hypothalamus, thalamus and frontal cortex. Further to these findings, we have recently reported the preparation and PET examination of three potent N-demethylated tropane derivatives with alkyl or alkenyl substituents at the 4' position (10). However, no suitable radioiodinated tracer is to date available for the exploration of the 5-HTT by SPET.

We report here the synthesis, radiolabelling with ^{125}I and preliminary *ex vivo* binding properties of a new iodinated nortropane analogue, 2β-carbomethoxy-3β-(3'-iodo-4'-isopropylphenyl) nortropane or LBT-44.

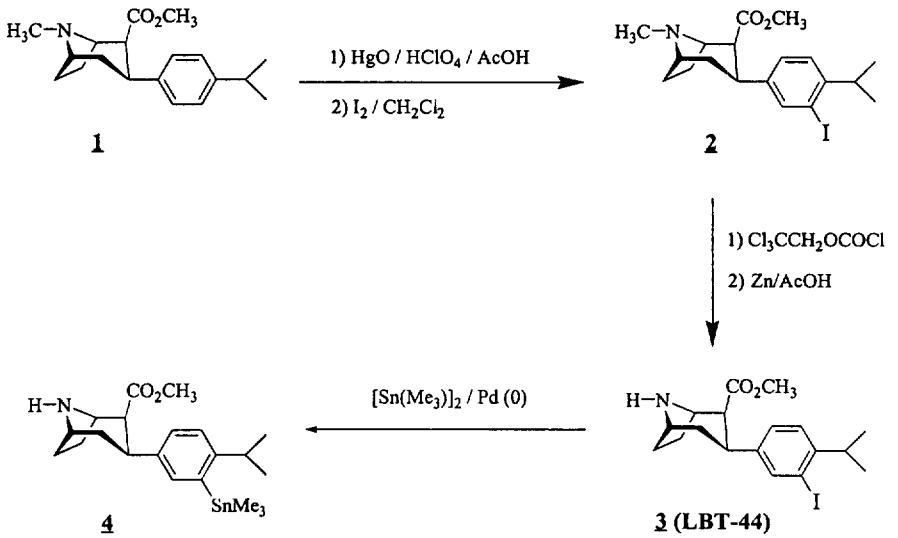
RESULTS AND DISCUSSION

Chemistry

Compound **1** (Scheme 1), prepared as previously reported (11), was the starting material for the synthesis of compound **2** by direct iodination of the aromatic ring using a mixture of yellow mercuric oxide, perchloric acid and acetic acid (5). The substitution pattern of the phenyl ring was elucidated by NMR hydrogen carbon three bonds correlation which exhibited only interaction between the isopropyl carbon and one aromatic hydrogen at 7.03 ppm (doublet). Moreover, a correlation between carbon 3 of the tropane structure and two aromatic hydrogens (dd at 7.17 ppm and d at 7.58 ppm) corroborated the structure assignments. Pure compound **2** was obtained after flash chromatography purification in a 76% yield. Nortropane **3** (LBT-44) was prepared by N-demethylation of compound **2**. This reaction involved the conversion of compound **2** to its corresponding carbamate using 2,2,2-trichloro

ethylchloroformate followed by zinc-acetic acid reduction to supply LBT-44 according to previously described procedures (11, 12). The stannyl compound 4, used as the precursor for the radioiodination, was prepared from compound 3 and hexamethylditin under palladium (0) catalysis.

Scheme 1: Synthesis of 2β-carbomethoxy-3β-(3'-iodo-4'-isopropylphenyl) nortropane 3 (LBT-44) and 2β-carbomethoxy-3β-(3'-trimethylstannyl-4'-isopropylphenyl) nortropane 4



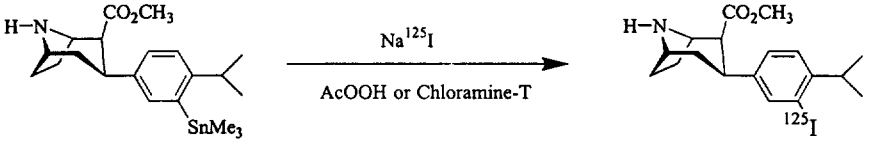
Radiochemistry

[^{125}I]LBT-44 (Scheme 2) was prepared by iododestannylation of the trimethylstannyl precursor 4 using [^{125}I]sodium iodide and peracetic acid or chloramine-T as oxidizing agent at room temperature according to a general radiolabelling method (13). The radioiodinated LBT-44 was isolated by reverse phase HPLC using MeOH / H_2O / Et_3N (85 / 15 / 0.2) as eluent and compared to its corresponding unlabelled analogue by HPLC with simultaneous UV and radioactivity detection (Figure 1). [^{125}I]LBT-44 was shown to be the expected product on the basis of the elution profile and it was obtained no-carrier-added in radiochemical yields of 75% and higher than 90% using peracetic acid and chloramine-T, respectively. For both

radiolabelling methods, [^{125}I]LBT-44 was obtained with a radiochemical purity higher than 95% and a specific activity of 2200 Ci/mmol.

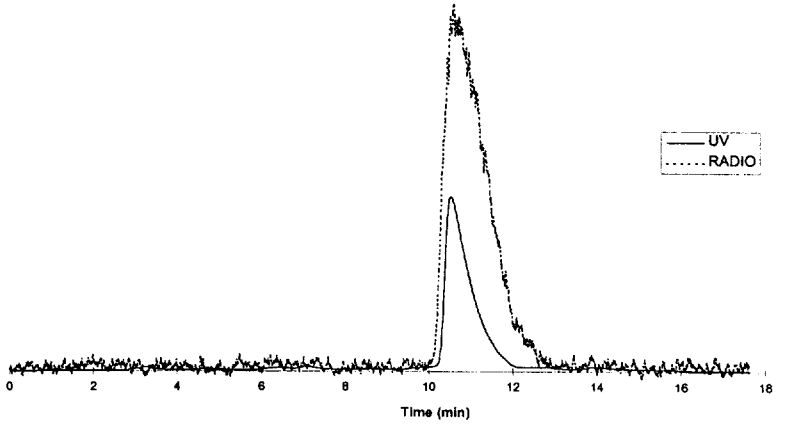
Although high radiochemical yields were obtained with both oxidizing agents, chloramine-T gave higher yield than peracetic acid. However, the use of chloramine-T requires fine control of the molar ratio of oxidant to substrate and of the reaction time, as chlorinated side products may appear, affording decreased radiolabelling yields and difficult separation from the desired iodinated compound (14).

Scheme 2: Radiosynthesis of [^{125}I]LBT-44



4 **[^{125}I]LBT-44**

Figure 1: HPLC analysis by coinjection of stable iodinated and [^{125}I]radioiodinated LBT-44 on a RP18 column using MeOH / H_2O / Et_3N (85 /15 / 0.2) as eluant.

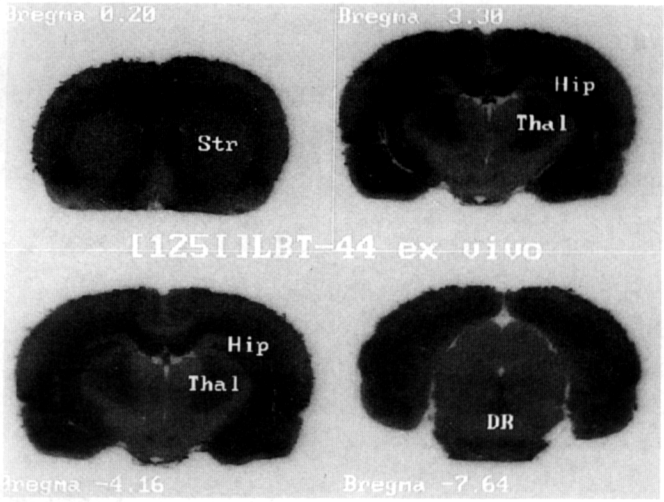


Ex vivo autoradiographic studies

Figure 2 shows the *ex vivo* biodistribution of [^{125}I]LBT-44 in coronal rat brain sections identified according to the atlas of Paxinos and Watson (15). Marked accumulation of [^{125}I]LBT-44 can be observed in several areas rich in 5-HTT such as the hippocampus (Hip), thalamus nuclei (Thal) and dorsal raphe (DR). In contrast, no fixation occurred in the striatum (Str), a brain region rich in DAT. The optical density ratios in comparison with the striatum were 1.4 for the hippocampus and 1.2 for both the thalamus and the dorsal raphe. On the basis of these observations, it can be assumed that [^{125}I]LBT-44 has poor affinity for the DAT whereas accumulation in brain regions such as the hippocampus, thalamus or dorsal raphe could be attributed to 5-HTT binding.

This paper describes the synthesis, radiolabelling and preliminary *ex vivo* autoradiographic studies of a new iodinated nortropane derivative: LBT-44. The unlabelled compound was prepared by direct iodination on the aromatic ring whereas the ^{125}I radioiodinated analogue

Figure 2: *Ex vivo* cerebral biodistribution of [^{125}I]LBT-44 in the rat brain. Note the accumulation of the product in the hippocampus (Hip), thalamus (Thal) and dorsal raphe (DR), whereas no fixation was seen in the striatum (Str).



was obtained by iododestannylation of the corresponding trimethyltin precursor with a good radiochemical yield (75% and >90%) and with a radiochemical purity greater than 95%. Preliminary *ex vivo* studies are in favour of specific binding of [^{125}I]LBT-44 to 5-HTT in the rat brain. In order to evaluate its potential use for SPET exploration of the 5-HTT, the complete *in vitro* and *in vivo* characterization of LBT-44 in rodents and non-human primates is currently being performed.

EXPERIMENTAL PART

Chemistry

2β-carbomethoxy-3β-(3'-iodo-4'-isopropylphenyl) tropane **2**

Compound **1** (440 mg, 1.46 mmol) was dissolved in a mixture containing 5 mL acetic acid, 1.6 mL $HClO_4$ and 310 mg yellow mercuric oxide. Iodine (1 g) in 13 mL CH_2Cl_2 and 6.5 mL acetic acid was then added dropwise. The mixture was allowed to stir at room temperature for one night, filtrated, basified with NH_4OH and extracted with CH_2Cl_2 (3 × 15 mL). The organic layers were dried and the solvents evaporated to afford a yellow wax. After flash chromatography (Et_2O / Et_3N, 95 / 5), 475 mg of pure compound **2** was obtained (76%).

^{1}H NMR : δ = 1.10 (d, 6H, ^{3}J= 6.8 Hz, $CH(C\underline{H}_3)_2$), 1.55 (m, 3H, H-4α, H-6α, H-7α), 2.07 (m, 2H, H-6β, H-7β), 2.14 (s, 3H, N-CH_3), 2.44 (td, 1H, $^3J_{4\beta,5}$= 2.7 Hz, $^2J_{4\alpha,4\beta} = {}^3J_{3,4\beta}$ = 12.2 Hz, H-4β), 2.83 (m, 2H, H-2, H-3), 3.02 (h, 1H, ^{3}J= 6.8 Hz, $C\underline{H}(CH_3)_2$), 3.40 (m, 1H, H-5), 3.43 (s, 3H, O-CH_3), 3.48 (m, 1H, H-1), 7.03 (d, 1H, ^{3}J= 8.1 Hz), 7.17 (dd, 1H, ^{3}J= 8.1 Hz, ^{4}J= 1.7 Hz), 7.58 (d, 1H, ^{4}J= 1.7 Hz).

^{13}C NMR : δ = 23.7, 25.8, 26.4, 33.7, 34.6, 38.2, 42.6, 51.7, 53.1, 62.8, 65.9, 101.5, 125.8, 128.2, 139.1, 143.3, 148.2, 172.5.

2β-carbomethoxy-3β-(3'-iodo-4'-isopropylphenyl) nortropane **3**

Compound **2** (200 mg, 0.468 mmol) was dissolved in 3 mL of 2,2,2-trichloroethylchloroformate and heated at 120°C for 2 hours. After cooling, the excess of

2,2,2-trichloroethylchloroformate was removed by distillation and the residue was dissolved in 10 mL acetic acid containing 1 g of activated zinc dust. This mixture was allowed to stir at room temperature for 4 days, filtrated, treated with NH_4OH until basified and extracted with $CHCl_3$. The organic layers were dried and the solvent evaporated. Pure compound **3** (140 mg, 72%) was isolated after flash chromatography (AcOEt / Et_3N, 10 / 1) as a yellow liquid.

^1H NMR : δ = 1.12 (d, 6H, ^3J= 6.8 Hz, $CH(C\underline{H}_3)_2$), 1.64 (m, 3H, H-4α, H-6α, H-7α), 1.98 (m, 2H, H-6β, H-7β), 2.27 (td, 1H, $^3J_{4\beta,5}$= 2.6 Hz, $^2J_{4\alpha,4\beta} = {}^3J_{3,4\beta}$ = 12.8 Hz, H-4β), 2.63 (bd, 1H, H-2), 3.05 (m, 3H, H-3, N-H, $C\underline{H}(CH_3)_2$), 3.34 (s, 3H, O-CH_3), 3.64 (m, 2H, H-1, H-5), 7.06 (s, 2H), 7.55 (s, 1H).

^{13}C NMR : δ = 23.7, 28.2, 29.6, 34.3, 35.5, 38.2, 51.5, 51.7, 54.2, 56.9, 101.6, 126.1, 128.1, 139.0, 142.5, 149.1, 174.3.

2β-carbomethoxy-3β-(3'-trimethylstannyl-4'-isopropylphenyl) nortropane **4**

To a solution under nitrogen atmosphere of compound **3** (30 mg, 0.072 mmol) in 2 mL of dry toluene was added 25 µL of hexamethylditin (0.072 mmol) and a catalytic amount of tetrakis (triphenylphosphine) palladium (0). The mixture was then heated at 110°C for 12 hours, concentrated and the crude product was purified by flash chromatography (AcOEt / Et_3N, 10 / 1) to afford pure compound **4** (20 mg, 61%) as a yellow liquid.

^1H NMR : δ = 0.23 (s, 9H, $^2J_{Sn\text{-}H}$= 53.7 Hz, $Sn(C\underline{H}_3)_3$), 1.16 (d, 6H, ^3J= 6.7 Hz, $CH(C\underline{H}_3)_2$), 1.65 (m, 3H, H-4α, H-6α, H-7α), 1.97 (m, 2H, H-6β, H-7β), 2.32 (td, 1H, $^3J_{4\beta,5}$= 2.7 Hz, $^2J_{4\alpha,4\beta} = {}^3J_{3,4\beta}$ = 12.5 Hz, H-4β), 2.65 (m, 3H, H-2, H-3, N-H), 3.11 (h, 1H, ^3J= 6.7 Hz, $C\underline{H}(CH_3)_2$), 3.29 (s, 3H, O-CH_3), 3.65 (m, 2H, H-1, H-5), 7.12 (m, 3H).

^{13}C NMR : δ = -8.3, 24.6, 27.5, 28.9, 33.8, 35.4, 37.2, 50.8 (2C), 53.6, 56.2, 124.4, 127.8, 134.5, 139.7, 140.8, 153.6, 174.0.

Radiochemistry

The peracetic acid method:

In a vial containing the stannyl precursor **4** (70µg), were added successively absolute ethanol (100 µL), 0.5 M H_3PO_4 aqueous solution (300 µL), [^{125}I]NaI (1 mCi, no carrier added, specific

activity 2200 Ci / mmol, Cis Bio Int, France) and 0.32% peracetic acid (200 μL). After 30 min at room temperature, the reaction was quenched with 0.5 M $Na_2S_2O_5$ aqueous solution (100 μL), basified with saturated $NaHCO_3$ aqueous solution (2 mL) and extracted with ethyl acetate (2 x 1mL). The combined organic layers were evaporated under a nitrogen stream. The radiolabelled compound was then isolated by HPLC (Beckman 331 isocratic liquid chromatograph) on a RP18 column (Kromasil, Chrompack, 250 x 4.6 mm) using MeOH / H_2O / Et_3N : 85 / 15 / 0.2 v / v / v as mobile phase (flow rate of 1 mL / min). The fraction eluted at the retention time of 10'40" was collected, passed through a C18 Sep-Pak® cartridge, eluted with ethanol (2 mL) and finally dried under a nitrogen stream. An aliquot of radioiodinated LBT-44 was coinjected with its corresponding analogue on the same HPLC system using simultaneous UV and radioactivity detectors. [^{125}I]LBT-44 was obtained in a 75% radiochemical yield, a radiochemical purity better than 95% and a specific radioactivity of 2200 Ci/mmol.

The choramine-T method:

A chloramine-T solution (60 μL, 1 mg/mL) was added to a mixture of the trimethylstannyl precursor **4** (70 μg), [^{125}I]NaI (1 mCi, no carrier added, specific activity 2200 Ci/mmol, NEN Life Sciences Products, Brussels) and aqueous hydrochloric acid (60 μL, 0.2 M). The reaction was allowed to react at room temperature for 5 min. Prior to purification on HPLC (Kontron Instruments, Isocratic HPLC Pump 420) mobile phase (500 μL) was added to the reaction mixture. The product [^{125}I]LBT-44 was isolated from unreacted precursor and radioactive impurities by injecting the crude reaction mixture in 200-250 μL portions into the reverse phase HPLC-column (Waters C-18 μ-Bondapack, 300 x 7.8 mm, 10 μm) using CH_3CN / H_3PO_4 (0.01M) : 35 / 65 as mobile phase (flow rate of 5 mL / min). The collected fractions were evaporated and the residue was dissolved in 2 mL of 70% ethanol. [^{125}I]LBT-44 was obtained in a radiochemical yield >90% with a radiochemical purity better than 95% and specific acitivity of 2200 Ci/mmol.

Ex vivo autoradiographic studies

Ex vivo autoradiographic studies were performed in male Wistar rats weighing 250g (R. Janvier, France). Rats were injected intravenously with 0.3 mL of 200 µCi of [^{125}I]LBT44, and were sacrificed 2 hours post-injection. The brains were removed, rapidly frozen on dry ice, cut in 20 µm sections at -18°C (Reichert-Jung Cryocut 1800, Leica, France) and thaw-mounted on glass slides. Sections were placed in X-ray cassettes and exposed to β-max Hyperfilms (Amersham, Buckinghamshire, UK) for 4 weeks at room temperature. Autoradiograms were developed (Kodak Lx24), fixed (Kodak AL4) and quantified using a computer imaging system (Biocom, Les Ulis, France). Absorbance values were evaluated after establishment of regions of interest.

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