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# Facile Radiolabelling and Purification of 2β-[O-$^{11}CH_3$]-Carbomethoxy-3β-aryltropanes: Radiotracers for the Dopamine Transporter.

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## Summary

Two potent dopamine transporter ligands, 2β-[O-$^{11}CH_3$]-carbomethoxy-3β-(4-methylphenyl)tropane and its 4-chlorophenyl analogue, were synthesized by O-alkylation at the 2β-carboxy position with [$^{11}$C]-iodomethane. Separation of the [$^{11}$C]-labelled tropane from excess carboxylic acid precursor was readily achieved by semipreparative HPLC providing pure radiotracers at high specific activities (800-3000 mCi/μmole) suitable for *in vivo* PET studies. Radiosynthesis of this class of compounds by [O-$^{11}CH_3$]-methylation offers advantages over previously reported [N-$^{11}CH_3$]-methylation methods.

**Key Words: carbon-11, positron emission tomography, dopamine uptake sites, radiotracer, tropane.**

## Introduction

For both historical and practical reasons the majority of PET neuroreceptor imaging studies have concentrated on the post-synaptic binding site [1,2], but of late an increasing amount of attention has focused on pre-synaptic uptake sites. Such neuroreceptors actively participate in the transport of the neurotransmitter out of the synapse and back into the neuron, thus terminating signal transduction [3,4]. Growing

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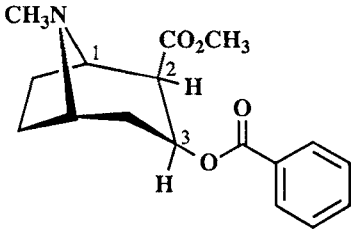
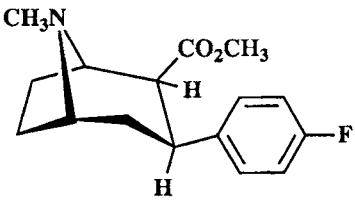
evidence that the dopamine transporter (DT) is the site of action responsible for the reinforcing properties of cocaine [5,6] has piqued interest in this system. Recently it was demonstrated that chronic cocaine exposure in rats effects significant changes (both up and down regulation) in the binding of DT selective [$^3$H]-ligands in striatum and nucleus accumbens [7,8], supporting the hypothesis that the DT plays a role in the behavioural responses to chronic cocaine addiction.

Derivatives of cocaine in which the benzoyl group on $C_3$ is replaced with an aryl group [9] are potent ligands for the DT. The most studied compound of this series, WIN 35,428 (also known as CFT), 2β-carbomethoxy-3β-(4-fluorophenyl)tropane, is several times more potent than cocaine and, of significance for *in vivo* studies, is metabolized more slowly due, in part, to the lack of the benzoyl linkage which is a primary site of cocaine metabolic cleavage [10,11]. [$^3$H]-WIN 35,428 has been used as a tool for the study of cocaine binding sites and a probe for detection of Parkinson's disease [12-14].




**Cocaine**



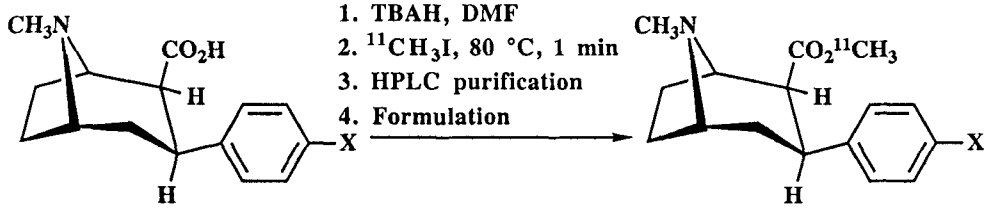

**WIN 35,428**

Recently [$^{11}$C]-WIN 35,428 has been synthesized by [N-$^{11}$CH$_3$]-methylation with [$^{11}$C]-iodomethane [15,16] and PET studies have been reported in both primates and humans [16-18]. As anticipated from *in vivo* studies in rodents [13], its imaging potential for the DT are superior to [$^{11}$C]-cocaine [19], [$^{11}$C]-nomifensine [20], or [$^{18}$F]-GBR 11319 [21], with a demonstrable high sensistivity to dopaminergic cell loss in subjects with mild cases of Parkinson's disease.

We report here the radiosynthesis of two potent analogues of WIN 35,428, namely 2β-[O-$^{11}$CH$_3$]-carbomethoxy-3β-(4-methylphenyl)tropane (**3**, aka RTI-COC-32) and 2β-[O-$^{11}$CH$_3$]-carbomethoxy-3β-(4-chlorophenyl)tropane (**4**, aka RTI-COC-31) by an alternative route, namely [O-$^{11}$CH$_3$]-methylation with [$^{11}$C]-iodomethane at the 2β-carboxy position. This approach to $^{11}$C-radiolabelling of this important class of compounds enjoys significant advantages over [N-$^{11}$CH$_3$]-methylation in terms of speed, radiochemical yields, and especially ease of purification of the radiotracers.

## Results and Discussion

2β-Carboxy-3β-(4-methylphenyl)tropane (**1**) and 2β-carboxy-3β-(4-chlorophenyl)-tropane (**2**) were prepared by literature methods from the corresponding methyl esters by hydrolysis in aqueous dioxane [22,23]. Residual starting materials, which would reduce the specific activity of the radiolabelled products, were below detection limits (< 0.01%) by high performance liquid chromatography (HPLC) analysis.




(**1**) — X = $CH_3$
(**2**) — X = Cl

(**3**) — X = $CH_3$
(**4**) — X = Cl

The radiosyntheses of [$^{11}$C]-(**3**) and [$^{11}$C]-(**4**) from (**1**) and (**2**) respectively is depicted in the Scheme above. Alkylation of the carboxylic acid anion of (**1**) or (**2**) by [$^{11}$C]-iodomethane proceeds rapidly in DMF with >90% incorporation of activity as the methyl ester within one min at 80 °C. Model reactions showed that methylation is complete after one min at ambient temperature; for radiolabelling reactions heating is required to raise the temperature rapidly since the [$^{11}$C]-iodomethane is trapped at -70 °C. After warming, the reaction mixture is quenched with HPLC buffer and the product isolated by reverse phase semi-preparative HPLC. Radiochemical yields (from [$^{11}$C]-iodomethane) of formulated, sterile and pyrogen-free products were 40-55% (not decay corrected) while specific activities were typically 800-3000 mCi/μmole (EOS). Synthesis times (from $^{11}CO_2$ production) were 19-21 min. Radiochemical purities were >99% and the product was stable for one hour after formulation with less than 1% radiolysis occurring. It has been reported that exposure to strong base can induce epimerisation at $C_2$ in cocaine analogues [9,24]. However no products corresponding to the 2-α-carbomethoxy epimers of [$^{11}$C]-(**3**) or [$^{11}$C]-(**4**) were detected (<1%) by analytical HPLC.

Previous work on radiolabelling phenyltropane analogues of cocaine with $^{11}$C have focused on introduction of the label at the N-methyl position. In addition to the

facile radiolabelling of (**3**) and (**4**), a major advantage of radiolabelling at the 2-β-carbomethoxy position with [$^{11}$C]-iodomethane is in ease of purification. Separation of nor-N-methyl precursor from product has proved onerous, requiring a separate precursor derivatization step [15] or, in another report [16], the use of very small quantities of precursor and omitting separation altogether. Under the described conditions, the labelled methyl ester is readily separated from excess carboxylic acid precursor by semi-preparative reverse phase HPLC ($\alpha$ = 6), providing a fast and efficient purification of the product. Having the label in a different position may also prove useful in the study of the metabolic fate of this important class of compounds.

Given the high separation factor it is likely that solid-phase techniques using small Sep-pak or similar cartridges would provide sufficient resolving power, eliminating the need for HPLC purification. Binding studies have shown that the N-methyl group of phenyltropanes is not essential for high affinity towards the dopamine transporter; in contrast a free carboxy group at the 2-β- position results in a large reduction in affinity compared to the 2-β-carboxy methyl ester [25]. This may reduce the need for complete separation of precursor from radiolabelled product, assuming that the precurosr possesses no further pharmacological properites.

In summary, phenyltropane analogues of cocaine can be radiolabelled with [$^{11}$C]-iodomethane in the 2-β-carbomethoxy position. This approach allows short synthesis times, high yields, and facile purification of the radiotracers. Both (**3**) and (**4**) bind more avidly, by a factor of ca. 10, to the dopamine transporter than WIN 35,428 [26]. Thus they are clearly worth investigating as potential PET radioligands.

## Experimental

DMF was stirred overnight with BaO, then distilled under reduced pressure from BaO and stored over 4 Å molecular sieves. Purification and analyses of radioactive mixtures by HPLC were performed with in-line uv (254 & 214 nm) detectors in series with a NaI crystal radioactivity detector. The HPLC columns used were either A - Waters Novapak $C_{18}$ (250 mm x 7.5 mm, 7 μ), or B - Alltech CAP $C_8$ (250 mm x 4.6 mm, 10μ). Peak areas were measured using Hewlett-Packard 3396 and Waters 746 recording integrators. Isolated radiochemical yields

were determined with a dose-calibrator (Capintec CRC-712M). Sterility and pyrogenicity testing were performed using standard procedures. Samples of the radiolabeled product prepared according to the procedure described below were determined to be sterile and pyrogen-free in all cases. 2β-Carboxy-3β-(4-methylphenyl)tropane (**1**) and 2β-carboxy-3β-(4-chlorophenyl)-tropane (**2**) were prepared by literature methods[22,23] from the corresponding methyl esters by hydrolysis in aqueous dioxane. Both carboxylic acids were recrystallized twice from aqueous acetonitrile to remove traces of starting material and gave satisfactory elemental analyses (C, H, N). The methyl esters (**3**) and (**4**) were prepared from cocaine by the method of Carroll via anhydroecgonine methyl ester [22,23].

**2β-[O-$^{11}CH_3$]-Carbomethoxy-3β-(4-methylphenyl)tropane [$^{11}$C]-(3).** [$^{11}$C]$CH_3I$, produced from $^{11}CO_2$ [27,28], was swept by a stream of nitrogen (30 ml/min) through phosphorous pentoxide and sodalime traps into a solution of 2β-carboxy-3β-(4-methylphenyl)tropane (**1**) (1.4-1.6 mg) in DMF (200 μL) containing tetrabutylammonium hydroxide (4 μL, 1M in methanol) cooled to -70 °C. Upon trapping of the maximal activity the solution was heated to 70 °C for one min, cooled, diluted with 300 μL of HPLC buffer and purified by semi-preparitive HPLC (column A, 40% $CH_3CN$:60% $H_2O$ + 0.1N $NH_4HCO_2$, 4 mL/min, 254 nm $R_{T_{product}}$ = 5 min). The desired fraction was collected, evaporated to dryness, and the residue taken up in 10 mL of sterile saline. This was passed through a sterile 0.22 μm filter (Anatop) into a sterile, pyrogen free bottle containing aqueous sodium bicarbonate (1 mL, 8.4%). The radiochemical purity and specific activity of the final solution was determined by analytical HPLC using column B; (40% $CH_3CN$:60% $H_2O$ + 0.05 N $NH_4Cl$, 4 mL/min, 214 nm $R_{T_{product}}$ = 2.5 min). Confirmation of the identity of the radiolabelled product was achieved by co-injection with authentic (**3**) using a further four different HPLC columns (Alltech $C_{18}$ Econosil, Waters $C_{18}$ Novapak, Phenomenex Ultracarb 7 and Alltech silica Econosil).

**2β-[O-$^{11}CH_3$]-Carbomethoxy-3β-(4-chlorophenyl)tropane [$^{11}$C]-(4)** was synthesized as described above for [$^{11}$C]-(**3**) from 2β-carboxy-3β-(4-chlorophenyl)tropane (**2**). Within experimental error, results were comparable.

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