Synthesis of [^{123}I]*tert*-Butyl 8-iodo-5,6-dihydro-5-methyl-6-oxo-4*H*-imidazo[1,5-a][1,4]benzodiazepine 3-carboxylate, a Potential SPECT Imaging Agent for Diazepam-Insensitive (DI) Benzodiazepine Receptors.

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SUMMARY

[^{123}I]*tert*-Butyl 8-iodo-5,6-dihydro-5-methyl-6-oxo-4*H*-imidazo [1,5-a][1,4]benzodiazepine 3-carboxylate ([^{123}I]**3**), a high affinity and selective radioligand for the diazepam insensitive (DI) benzodiazepine receptor was synthesized in 2 steps from *tert*-butyl 8-bromo-5,6-dihydro-5-methyl-6-oxo-4*H*-imidazo[1,5-a][1,4]benzodiazepine 3-carboxylate (**4**). A key step in the synthesis of this potential SPECT imaging agent utilized $(Ph_3P)_3Pd$ mediated stannylation of **4** with $(Bu_3Sn)_2$ to the corresponding 8-tributyltin derivative **5**. Iododestannylation of **5** with no carrier added [^{123}I]NaI in aqueous ethanolic CH_3CO_3H furnished [^{123}I]**3** (85%) in greater than 98% radiochemical purity following TLC purification.

Key Words: [^{123}I]*tert*-Butyl 8-iodo-5,6-dihydro-5-methyl-6-oxo-4*H*-imidazo[1,5-a][1,4]benzodiazepine 3-carboxylate ([^{123}I]**3**], High affinity, Selective, Diazepam-insensitive, SPECT, Imaging agent, Iododestannylation.

INTRODUCTION

The diazepam-insensitive subtype (DI) of benzodiazepine (BZ) receptor is pharmacologically and structurally distinct from other diazepam-sensitive (DS) BZ receptors [1]. DI BZ receptors are predominantly localized in the cerebellum [2]. Prototypic DS BZ receptor

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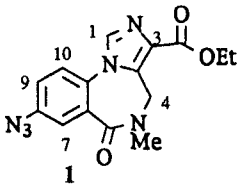
ligands such as 1,4-benzodiazepines (e.g. diazepam) and triazolobenzodiazepines exhibit very low (micromolar) binding affinity for DI and high (nanomolar) affinity for DS [3]. Since the non-selective, high affinity DI ligands Ro 15-4513 (1) and Ro 19-4603 (2) were found to antagonize the behavioral effects of ethanol [4], this site has been implicated in the behavioral and biochemical effects of this alcohol.

Due in part to the paucity of selective DI ligands, the pharmacological and physiological roles of DI have remained unclear [6]. Until recently, compound 1 (DI/DS ratio=0.6) was the most selective DI ligand described [3, 7]. This compound was selected as a target for structural manipulation since previous studies [3] indicated that changes in the ester and aromatic ring substitution were critical for high affinity binding to DI BZ receptors. We recently reported the structural requirements for high affinity and selective binding of 1 to DI and identified several compounds with both higher DI affinity and selectivity than the parent 1 [8]. Compound 3 displayed a 3-fold improved selectivity and a 2-fold improved binding affinity for DI [8].



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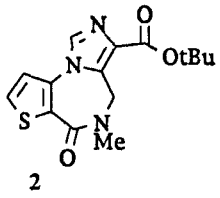

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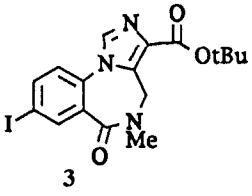


3

Positron emission tomography (PET) and single photon emission computed tomography (SPECT) have been successfully employed for the non-invasive study of central nervous system receptors such as dopaminergic [9], opiate [10] and muscarinic [11]. The utility of PET and SPECT has been reviewed exhaustively [12]. Although offering comparable resolution, SPECT offers several advantages over PET including commercial availability of ^{123}I, lower cost, and ease of adaptability to small hospitals and clinics.

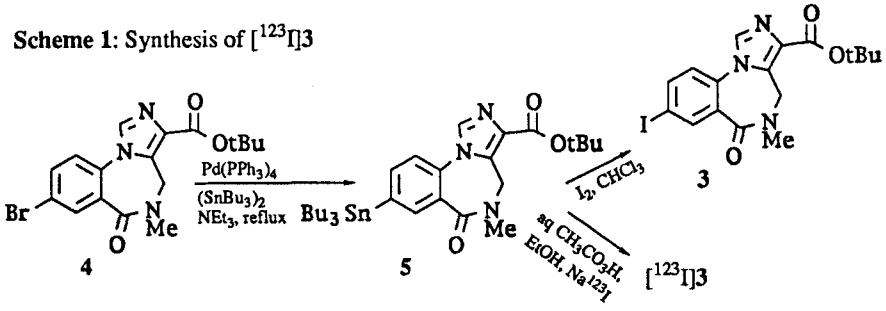
BZ receptors (DS) have been imaged using [^{123}I]Ro16-0154 [13] suggesting that it may also be possible to image DI receptors in a similar fashion. The presence of an aromatic iodo group in compound 3 identified it as an attractive target for radiolabelling with ^{123}I for potential use as a SPECT imaging agent for DI. Radioiodinated imaging agents for the DI subtype have not been previously reported. We therefore report here the synthesis of [^{123}I]3, a novel radioprobe for BZ DI receptors.

SYNTHESIS

Reaction of the aromatic bromide 4 (Scheme 1) with excess $(Bu_3Sn)_2$ in the presence of a catalytic quantity of $(Ph_3P)_4Pd$ in refluxing

triethylamine [14] afforded the organontin precursor **5** in 81% yield (taking into account the 81% unreacted **4** recovered from the reaction mixture). Treatment of **5** with excess iodine [15] in chloroform (0.1M solution) at ambient temperature furnished **3** in quantitative yield; this material proved to be identical chromatographically and spectroscopically to an authentic sample synthesized by a different route [8]. Treatment of excess **5** with no-carrier-added [^{123}I]NaI in the presence of aqueous ethanolic peracetic acid for 5 min at room temperature yielded [^{123}I]**3** in 85% radiochemical yield (Scheme 1). The [^{123}I]**3** was separated from unreacted **5** by TLC on silica gel. The material thus purified proved to be >98% radiochemically pure.

Scheme 1: Synthesis of [^{123}I]**3**



DISCUSSION

Attempts to generate **5** by via bromine-lithium exchange of **4** with n-butyllithium in THF at -78 °C (followed by quenching with tributyltin chloride) resulted in complete decomposition with no detectable **5**. The failure of this reaction is most likely a result of attack on the amide and ester carbonyl groups of **4** by n-butyllitium. A key reaction in this synthesis is therefore the $(Ph_3P)_4Pd$-induced bromine-tin exchange in the generation of **5**. This method appeared to be a good choice since it has been used to obtain the tributyltin derivative at the C-7 position of the structurally related imidazobenzodiazepine derivative, iomazenil (Ro16-0154), for which the synthesis and radioiododestannylation have been reported [14].

The low (16%) yield of **5** may be improved by chromatographic separation and recycling of the starting material **4**. The inefficient formation of **5** was not unexpected since it is known that aryl bromides react much slower with $(Bu_3Sn)_2/(Ph_3P)_4Pd$ than aryl iodides [16]. Use of higher boiling point solvents or longer reaction times [16] may help improve the yield of **5**.

Compound **5** exhibited two very characteristic broad singlets at δ 4.37 and 5.14 ppm indicating the lack of planarity of the imidazobenzodiazepine ring system. The 1:1 splitting of the singlet for *t*-Bu (1.55 and 1.65) suggests the existence of rotamers.

The high radiochemical yield of [^{123}I]**3** from **5** suggests a very efficient iododestannylation reaction using this precursor. An additional advantage of this reaction is its rapidity, coupled with the fact that no dilution of specific activity is likely. The ease of chromatographic separation of [^{123}I]**3** from unreacted **5** would prove advantageous in future SPECT studies with this radioligand.

EXPERIMENTAL

All steps involving the use of ^{123}I were first performed with unlabelled materials and the structures of the products were confirmed spectroscopically. High resolution mass spectra (HRMS) were determined using a V.G. Micromass 7070F mass spectrometer. Chromatographic separations of radioisotopes were accomplished using 250 μM analytical silica gel plates (GTLC, Macherey-Nagel, Germany). Radioactivity determinations were made using a Radcal model 4050 Radionuclide calibrator (Radcal Corporation). Radioactivity was detected on TLC plates using a Bioscan system 200 imaging scanner. [^{123}I]NaI [no carrier added generated by Xe(p, 2n) reaction] was purchased from Nordion International Inc, Vancouver, Canada and used immediately upon receipt.

tert-Butyl 8-(tributylstannyl)-5,6-dihydro-5-methyl-6-oxo-4*H*-imidazo[1,5-a][1,4]benzodiazepine 3-carboxylate (5).

To a solution of **4** [8] (394 mg, 1.0 mmol) in anhydrous triethylamine (10 mL) was added $(Ph_3P)_4Pd$ (115 mg, 0.1 mmol) and $(Bu_3Sn)_2$ (700 mg, 1.2 mmol) and the reaction mixture was boiled under reflux overnight. The reaction mixture was cooled, filtered and the filtrate was evaporated *in vacuo* to give a brown oil. The product mixture was separated by preparative TLC on silica gel, eluting with $CHCl_3$/MeOH (19:1) to give unreacted starting material **4** (320 mg, 81%) and **5** (92 mg, 16%) as a colorless oil: ^{1}H-NMR ($CDCl_3$) δ 8.13 (d, J=1.2 Hz, 1H, ArH^7), 7.87 (s, 1H, ArH^1), 7.70 (dd, J=1.2, 7.7 Hz, 1H, ArH^9), 7.33 (d, J=7.7 Hz, 1H, ArH^{10}), 5.14 (m, 1H, H^4), 4.37 (m, 1H, H^4), 3.26 (s, 3H, NMe), 1.65 (50%), 1.55 (50%) (s, 9H, tBu), 1.49-1.69 (m, 6H, $SnCH_2CH_2\underline{CH_2}CH_3$), 1.26-1.42 (m, 12H, $Sn\underline{CH_2CH_2}CH_2CH_3$), 0.92 (t, J=7.9 Hz, 9H, $SnCH_2CH_2CH_2\underline{CH_3}$); EIMS ($M^+$ calcd for $C_{29}H_{46}N_3O_3{}^{120}Sn$): 604.2542. Found: 604.2567.

Iododestannylation of 5 (to 3).

To a solution of **5** (18 mg, 0.03 mmol) in hydrocarbon stabilized $CHCl_3$ (2 mL) was added a solution of iodine in hydrocarbon stabilized $CHCl_3$ (2 mL of a 0.1M solution, 0.2 mmol, 6.7 equiv). TLC (1:19 MeOH/$CHCl_3$) indicated completion of the reaction after 1h at rt. The reaction mixture was treated with KF (0.5 mL of a 1.0M solution in MeOH), 5% aqueous $NaHSO_3$ (0.5 mL) and stirred for 5 min at rt. Water (5 mL) was

added and the $CHCl_3$ layer was separated and evaporated *in vacuo* to yield **3** (13 mg, quantitative) identical (^{1}H-NMR, TLC, mass spectra) to an authentic sample [8].

***tert*-Butyl 8-iodo-5,6-dihydro-5-methyl-6-oxo-4*H*-imidazo [1,5-a][1,4]benzodiazepine 3-carboxylate ([^{123}I]3).** Aqueous peracetic acid (100 µl of a 0.32% w/v solution) was added to a mixture of the tri-n-butylstannyl derivative **5** (50 µl, 0.0828 micromoles of a solution made by dissolving 1.0 mg of **5** in 1 ml of EtOH), EtOH (200 µl), and no carrier added [^{123}I]NaI (5 mCi) in a sealed vial. The reaction mixture was allowed to stand for 5 min. at r.t. and then quenched according to a previously described procedure [15] by the addition of $NaHSO_3$ (20 mg) followed by an aqueous solution of $NaHCO_3$ (25 mg in 1 mL of water) to render the mixture basic. The aqueous solution was subsequently extracted with ethyl acetate (3 x 1 ml) and the product was purified by TLC, eluting with ethyl acetate/hexanes (1:1). Analysis of the plate using a Bioscan system 200 imaging scanner indicated that the product comigrated with an authentic sample of unlabelled **3** [8]. The product was eluted from the plate with ethyl acetate, and the organic extract was evaporated under a stream of N_2. The product was redissolved in ethanol for final storage at a concentration of 1 mCi/mL and stored in the dark. The radiochemical yield was determined to be 85% after purification (>98% radiochemically pure by TLC).

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