Journal of Labelled Compounds and Radiopharmaceuticals
J. Labelled Cpd. Radiopharm. **43**, 137-146 (2000)

Synthesis of [^{125}I]-3β-(4-Ethyl-3-iodophenyl)nortropane-2β-carboxylic Acid Methyl Ester ([^{125}I]EINT)

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Summary

A WIN 35,065-2 analog, 3β-(4-ethyl-3-iodophenyl)nortropane-2β-carboxylic acid methyl ester (EINT), has been radiolabeled with iodine-125 by radioiododestannylation of the trimethyltin derivative, N-*tert*-butoxycarbonyl-3β-(4-ethyl-3-trimethyltinphenyl)nortropane-2β-carboxylic acid methyl ester (**2**), using carrier-free sodium iodide-125. The radiolabeling consists of a one-pot, two-step method entailing radioiododestannylation followed by nitrogen deprotection. Purification by reversed-phase HPLC gives [^{125}I]EINT in 34.2% yield with high radiochemical purity (>99%) and high specific activity (1988 mCi/μmol, 73.6 GBq/μmol, based on the specific activity of the Na^{125}I used).

Key Words: Radioiodination, iodine-125 labeling, serotonin transporter, iododestannylation, chloramine-T, [^{125}I]EINT

Introduction

Several iodine-125-labeled WIN 35,065-2 analogs have been useful in the *in vitro* pharmacological characterization of the monoamine transporters (Figure 1) (1-10). In addition, the iodine-123 analogs have proven to be highly useful single photon

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CCC 0362–4803/2000/020137–10$17.50

Received 9 June 1999
Revised 12 July 1999
Accepted 3 August 1999

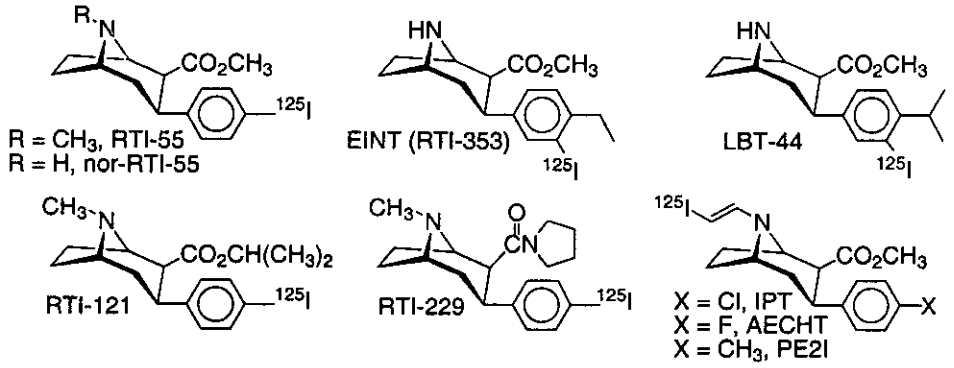


Figure 1. WIN 35,065-2 Radiotracer Analogs

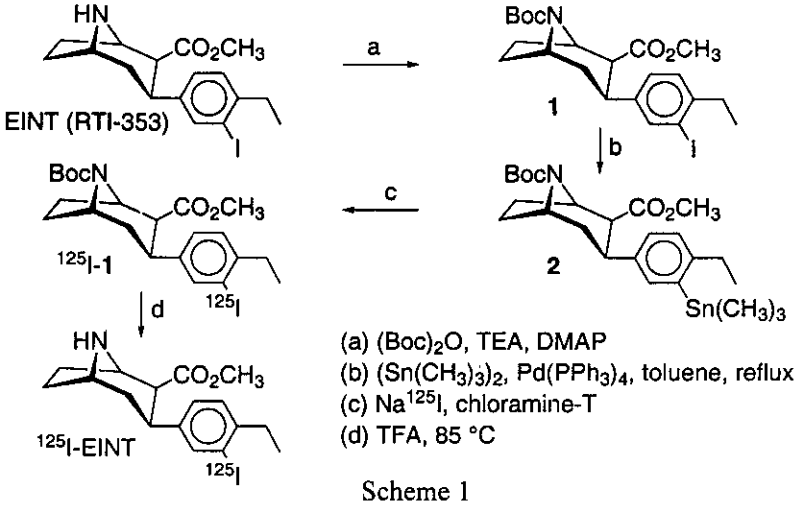
emission computed tomography (SPECT) imaging agents for *in vivo* studies (11-13). One of the first compounds studied was RTI-55 (also known as β-CIT) which was developed as a radioligand for the dopamine transporter (DAT) (14, 15). IPT, AECHT, and PE2I (Figure 1) are other iodine-labeled WIN 35,065-2-type radiotracers developed for studying the DAT. Later studies showed that RTI-55 also labeled the serotonin transporter (5-HTT) (16, 17); however, the lack of transporter selectivity limits its use for many *in vitro* and *in vivo* studies directed toward the 5-HTT.

As part of a structure activity relationship (SAR) study directed towards development of a selective 5-HTT ligand, we found that nor-RTI-55 possessed a 10-fold greater affinity for the 5-HTT relative to the DAT (18). Bergstrom et al. prepared the radiolabeled analog of nor-RTI-55 and found it useful for studying the 5-HTT (5). In a continuation of our structure activity relationship studies of the WIN 35,065-2 class of compounds, we reported that several analogs possessing alkyl groups larger than methyl at the 4-position of the 3β-phenyl group showed increased potency and selectivity for the 5-HTT (19). In addition, we found that 3β-(3-iodophenyl)tropane-2β-carboxylic acid methyl ester showed enhanced potency for the 5-HTT relative to WIN 35,065-2 (20). Based on the SAR information that removal of the N-methyl group and addition of 4-ethyl and 3-iodo groups to the 3β-phenyl ring of WIN 35,065-2 all improved potency and selectivity for the 5-HTT, we synthesized 3β-(4-ethyl-3-iodophenyl)nortropane-2β-carboxylic acid methyl ester (EINT or RTI-353) as a potential selective ligand for the 5-HTT (21). We found that this compound possessed a K_i value of 0.063 nM for the 5-HTT (21) and was also very selective for the 5-HTT relative to the norepinephrine transporter (NET) and DAT with K_i and IC_{50} values of

90 and 329 nM, respectively (21). Since EINT contains an iodine atom replaceable by an I-123 or I-125, as well as a methyl ester for C-11 incorporation, it has potential for use as a radiotracer for characterization of the 5-HTT. In this paper we detail the radiolabeling of EINT with iodine-125. It should be noted that since our original report on the synthesis and transporter binding properties of EINT (21), Helfenbein et al. reported the radiosynthesis of an analogous compound, [^{125}I]LBT-44, which contains an isopropyl group in place of the ethyl group (6).

Results and Discussion

The synthesis of [^{125}I]EINT is shown in Scheme 1. *tert*-Butoxycarbonyl (Boc) protection of EINT with di-*tert*-butyl dicarbonate, triethylamine, and a catalytic



Scheme 1

amount of 4-(dimethylamino)pyridine provided N-*tert*-butoxycarbonyl-3β-(4-ethyl-3-iodophenyl)nortropane-2β-carboxylic acid methyl ester (**1**) in 80% yield. Conversion of **1** to the trimethyltin analog **2**, the desired radioiodination precursor, was accomplished by a palladium catalyzed stannylation with hexamethylditin in refluxing toluene. This reaction was complete in approximately 5 min. Since the presence of either iodo analog (**1** or EINT) would severely reduce the specific activity of the final radiolabeled product, the tin precursor **2** was purified by HPLC prior to radioiodination to ensure the absence of any unlabeled **1** or EINT. The extreme polarity difference among compounds **1**, **2**, and EINT suggested that it would be difficult to separate and analyze these three compounds satisfactorily by a single set of

HPLC conditions. Experiments showed that neither a Waters Nova-Pak C18 reversed-phase column (RCM, 8 mm × 10 cm, 4 μM) nor a Waters SymmetryShield RP8 reversed-phase column (3.9 × 150 mm, 5 μM) was able to provide satisfactory separation of the three compounds. Therefore, we developed two sets of conditions. A Nova-Pak C18 column was used for the analysis of **1** and **2** and a Waters SymmetryShield column for the analysis of EINT. Using the Nova-Pak C18 column eluted with 45% CH_3OH/45% CH_3CN/10% H_2O at flow rate of 1.0 mL/min gave a clean separation of **1** and **2**. Compound **2** had a $\kappa' = 9.40$ ($t_R = 11.44$ min), and **1** had a $\kappa' = 6.05$ ($t_R = 7.76$ min). A calibration curve for compound **1** was obtained by plotting the measured peak area versus the amount of sample injected. The contamination of **1** in the purified **2** sample was determined by analyzing the sample under the same conditions and calculating the content of **1** according to the linear equation and the area of **1** in the HPLC chromatogram of the purified **2**.

Almost all the impurities in the original sample of **2** (Figure 2) could be removed after one HPLC purification; only tiny amounts of impurities were left. The contamination of **1** in the sample of **2** was below the quantification limit of 0.0077% after two additional HPLC purifications (Figure 3).

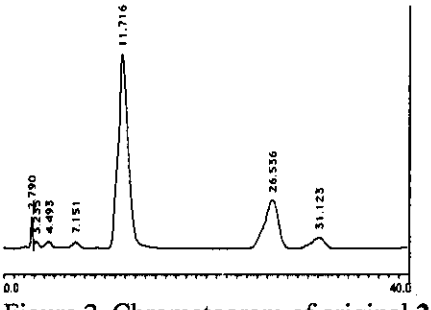


Figure 2. Chromatogram of original **2**

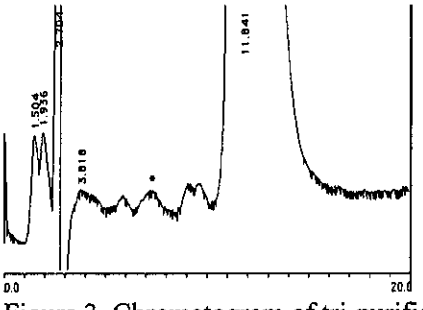


Figure 3. Chromatogram of tri-purified **2** (* marks the t_R position of **1**)

In order to further minimize the effect of compound **1**'s contamination upon the specific activity of the labeled product, a 47/1 ratio of the precursor/Na^{125}I (0.47 μmol/0.010 μmol) was used instead of 164/1 which was used in the synthesis of other tropane radioligands such as [^{125}I]RTI-229 (1). Using the 47/1 ratio, the specific activity of the labeled product was affected by less than 0.36% (0.0077% × 47), which was well below the experimental error.

In the cold synthesis of EINT, treatment of **2** with NaI and chloramine-T gave **1** which contained no detectable **2** by HPLC analysis. Removal of the Boc protecting group by treatment with trifluoroacetic acid at 85 °C for 30 min gave EINT.

Radioiododestannylation of **2** occurred in approximately one minute at room temperature with Na^{125}I and chloramine-T. The crude labeled intermediate was deprotected as described above for the cold synthesis affording [^{125}I]EINT with an overall radiochemical yield of 34.2%. The yield was relatively low compared to the radiolabeling of LBT-44 (>75%) (**6**) and RTI-229 (89%) (**1**); possibly part of the labeled Boc-EINT (**1**) was hydrolyzed or was not deprotected. The [^{125}I]EINT formed after the trifluoroacetic acid treatment was purified using a Waters SymmetryShield RP8 column eluted with 50% [CH_3OH/CH_3CN, 1:1], 50% [1% Et_3N/H_2O + HOAc to pH = 7.0] at a flow rate of 1.0 mL/min. The radiochemical purity of the labeled compound was determined to be 99.8% by HPLC with β-RAM detection (Figure 4, $\kappa' = 2.318$, $t_R = 7.17$ min) and 99.2% by TLC radioscan with the t_R and R_f, respectively, being identical to an authentic sample of EINT.

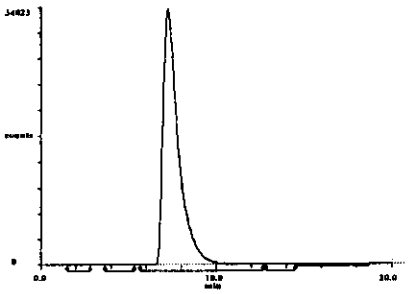


Figure 4. Chromatogram of [^{125}I]EINT (analyzed with SymmetryShield RP8 column)

The HPLC analysis of samples of the [^{125}I]EINT stored in different solutions showed that [^{125}I]EINT was more stable in ethanol/water/acetonitrile (1:1:0.1) than in 50% [CH_3OH/CH_3CN, 1:1], 50% [1% Et_3N/H_2O + HOAc to pH = 7.0] or in ethanol 100%. No detectable radiolabeled impurities were observed in a sample of [^{125}I]EINT stored at -70 °C in ethanol/water/acetonitrile (1:1:0.2) after 56 days.

Experimental

Materials and Methods: EINT (RTI-353) was synthesized as previously reported (21). Chloramine-T was purchased from Aldrich Chemical Company, sodium metabisulfite from Fisher Scientific Company, and carrier-free sodium iodide-125

from Amersham International. Water with 18-megahom resistivity was obtained using a Millipore Milli-Q Plus Ultra-Pure water system. All the chemicals and solvents were used without further purification. The radio-preparative HPLC system consisted of a Beckman HPLC pump, model 110A, Waters U6K universal injector. The analytical radio-HPLC and preparative HPLC system for **2** consisted of a Rheodyne 7125 injector, Rainin HPXL pump, Rainin pressure module, Knauer variable wavelength monitor at 254 nM (for unlabeled compounds), and IN/US Systems β-RAM flow-through radioactive monitor (for labeled compounds). A Waters reversed-phase C18 Nova-Pak column (RCM, 8 mm × 10 cm, 4 μm) was used for the purification of **2**. A Waters SymmetryShield RP8 reversed-phase column (3.9 × 150 mm, 5 μm) was applied for the preparation of [^{125}I]EINT. A second column of the same type was used for the analysis of [^{125}I]EINT.

The radiochemical purity of the product was also analyzed by a radio-TLC scanner (Bioscan system 200 imaging scanner). Radio-TLC was carried out on a silica gel 60 F_{254} plate (EM Separations Technology). A γ-counter (Packard auto-gamma scintillation spectrometer 5135) was used to count the radioactivity of the labeled product. The specific activity of [^{125}I]EINT was assumed from the specific activity of the Na^{125}I used in the synthesis.

N-*tert*-Butoxycarbonyl-3β-(4-ethyl-3-iodophenyl)nortropane-2β-carboxylic acid methyl ester (1)

To a stirred solution of 200 mg (0.5 mmol) of EINT in 2.5 mL of dichloromethane under nitrogen was added 0.104 mL (0.75 mmol) of triethylamine and 142 mg (0.65 mmol) of di-*tert*-butyldicarbonate. To this solution was added a catalytic amount of 4-(dimethylamino)pyridine, and the solution was stirred overnight. The solution was diluted with 10 mL of dichloromethane and 10 mL of water. The aqueous layer was extracted 3 times with dichloromethane. The combined extracts were dried over sodium sulfate, filtered, and concentrated under reduced pressure. The crude oil was purified by flash chromatography on silica gel. Elution with 4:1 hexane/ether afforded 0.187 g (80%) of N-*tert*-butoxycarbonyl-3β-(4-ethyl-3-iodophenyl)nortropane-2β-carboxylic acid methyl ester (**1**). This material was used in the next step without further purification. ^{1}H NMR (250 MHz, $CDCl_3$): δ 7.65 (s, 1H),

7.19–7.80 (m, 2H), 1.46, 1.43 (2s, 9H, tBu). HRMS calcd for $C_{22}H_{31}INO_4$: (M+) 500.1298. Found: m/e 500.1304.

N-*tert*-Butoxycarbonyl-3β-(4-ethyl-3-trimethyltinphenyl)nortropane-2β-carboxylic acid methyl ester (2)

To a stirred solution of 121 mg (0.242 mmol) of **1** in 2.4 mL of dry toluene under nitrogen was added 198 mg (0.604 mmol) of hexamethylditin. The solution was heated to reflux. After 5 min, a black precipitate formed on the sides of the flask indicating that the reaction was complete. The solution was cooled and filtered through a plug of Celite washing with dichloromethane. The organic layer was washed with water, then concentrated under reduced pressure. The resulting oil was purified by flash chromatography on silica gel. Elution with 4:1 hexane-ether afforded 0.121 g (93%) of N-*tert*-butoxycarbonyl-3β-(4-ethyl-3-trimethyltinphenyl)nortropane-2β-carboxylic acid methyl ester (**2**).

A sample of **2** (0.87 mg in 0.2 mL methanol) was injected onto a Waters reversed-phase C18 Nova-Pak column (RCM, 8 mm × 10 cm, 4 μm) and eluted with 45% CH_3OH + 45% CH_3CN + 10% H_2O at flow rate of 1.0 mL/min. The eluent was monitored with an UV detector at 254 nM. The eluent with the largest peak at 11.7 min (between 11–13 min) was collected and the solvent evaporated using a rotary evaporator. The residue was then dried under vacuum, leaving purified **2** (0.753 mg, oil, 86.6% recovery). The sample was chromatographed twice more under the same conditions to provide the sample used for the radiolabeling. ^{1}H NMR (250 MHz, $CDCl_3$): δ 7.07 (s, 1H), 6.96 (m, 2H), 1.24, 1.22 (2s, 9H, tBu), 0.11 (t, 9H, J = 26 Hz). HRMS calcd for $C_{25}H_{40}O_4N^{116}Sn$: 534.1975. Found: m/e 534.1953 (M).

Quantitative Analysis of 1

Compound **1** (90 μg, 0.18 μmol) was dissolved in HPLC mobile phase in a volumetric flask (25 mL). This solution (10, 20, 40, 80, and 100 μL) was analyzed under the same HPLC conditions as those used for the purification of **2**. The calibration curve was obtained by plotting the measured peak area versus the amount of the sample injected (three injections for each sample). The quantitation of **1** gave a linear equation with $R^2 = 0.9985$: $y = 80952x$, where x = the amount of **1** analyzed, nmol; y = peak area of **1**, μ V-Sec.

[^{125}I]-3β-(4-Ethyl-3-iodophenyl)nortropane-2β-carboxylic acid methyl ester ([^{125}I]EINT)

Sodium iodide-125 (1988 Ci/mmol, 73.6 GBq/μmol, 20.6 mCi in NaOH solution, 40 μL, pH 9.0) was centrifuged for 1 min at 600 rpm, then transferred to a solution of N-*tert*-butoxycarbonyl-3β-(4-ethyl-3-trimethyltinphenyl)nortropane-2β-carboxylic acid methyl ester (**2**; 252 μg, 0.47 μmol of a sample purified by HPLC) in MeOH/HOAc (100 μL, 95/5 v/v) in a Reacti-Vial (1 mL). The sodium iodide-125 vial was washed with the solution in the Reacti-vial (2 × 50 μL), and the washings were transferred back to the Reacti-vial. To this solution was added aqueous chloramine-T solution (80 μL, 10 mM, 0.8 μmol). The vial was capped, and the solution in the vial was stirred vigorously for 1 min with a Vortex. The reaction was then quenched by adding aqueous sodium metabisulfite ($Na_2S_2O_5$, 80 μL, 20 mM, 1.6 μmol) and stirring vigorously for 1 min with the Vortex. Trifluoroacetic acid (10 μL) was added to the vial, and the vial was heated at 85 °C for 30 min. The entire reaction mixture was loaded onto a Waters SymmetryShield RP8 5 μ 3.9 × 150 mm column. The Reacti-vial was washed with HPLC eluent solvent (50 μL), and the washing was loaded onto the column. The column was eluted with 50% [CH_3OH/CH_3CN, 1:1], 50% [1% Et_3N/H_2O + HOAc to pH = 7.0] at flow rate of 1.0 mL/min. The eluent was collected as 1-mL fractions, and the eluent with the largest amount of radioactivity (fraction 7) was diluted to 25 mL with ethanol as desired labeled product. HPLC analysis of the labeled product under the same conditions with a fresh column and a β-RAM radio-detector showed 99.8% purity (t_R = 7.17 min, κ' = 2.38). The radiochemical purity of the labeled product was also analyzed by TLC radioscan co-spotted with unlabeled standard EINT eluting with diethyl ether/dichloromethane/methanol/ammonium hydroxide = 30:30:10:1, indicating a purity of 99.2% (R_f = 0.42). The radioactivity of the purified labeled compound was counted by a γ-counter (Packard auto-gamma scintillation spectrometer 5135), which determined an overall radiochemical yield of 34.2%.

Stability of [^{125}I]EINT in Different Storage Solutions

[^{125}I]EINT (purified by HPLC) was stored in ethanol (100%), ethanol/water/acetonitrile (1:1:0.1), and 50% [CH_3OH/CH_3CN, 1:1], 50% [1% Et_3N/H_2O +

HOAc to pH = 7.0], respectively, under -70 °C. The radiopurity of the [^{125}I]EINT in each solution was analyzed after a certain period of time using the same HPLC conditions as for the radiopurity analysis of [^{125}I]EINT after synthesis. The radiopurity of [^{125}I]EINT stored 51 days in ethanol (100%) and 37 days in 50% [CH_3OH/CH_3CN, 1:1], 50% [1% Et_3N/H_2O + HOAc to pH = 7.0] decreased to 79.3% and 73.6%, respectively, while 96.8% of the sample kept unchanged after 56 days in ethanol/water/acetonitrile (1:1:0.1) solution.

Acknowledgment

This work was supported by the National Institute on Drug Abuse, Grant DA05477.

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