Synthesis of Unlabelled, ^{3}H- and ^{125}I-Labelled ß-CIT and its ϖ-Fluoroalkyl Analogues ß-CIT-FE and ß-CIT-FP, Including Synthesis of Precursors

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SUMMARY

The full synthesis of the cocaine congener 2ß-carbomethoxy-3ß-(4-iodophenyl)tropane (ß-CIT) and its N-fluoroalkyl analogues, fluoroethyl- and fluoropropyl-nor-ß-CIT (ß-CIT-FE and ß-CIT-FP) starting from cocaine is described. The synthetic routes include the preparation of precursors for labelling with radionuclides such as ^{11}C, ^{18}F, ^{76}Br, ^{123}I, ^{125}I and ^{3}H. Here we also report the labelling with ^{125}I or ^{3}H for use in autoradiographic examination of human brain sections.

Key words: Dopamine transporter, ß-CIT, ß-CIT-FE, ß-CIT-FP, ^{125}I, ^{3}H

INTRODUCTION

Cocaine is a commonly abused drug which has local anesthetic properties. Numerous derivatives of cocaine have been synthesised and especially such ones that have a phenyl ring attached directly to the tropane ring system have much higher affinity to the cocaine binding sites than cocaine itself. These binding sites are now known to be monoamine transporters as dopamine, noradrenaline and serotonin transporters (1). A widely used analogue is ß-CIT (2ß-carbomethoxy-3ß-(4-iodophenyl)tropane, 8-azabicyclo[3,2,1]octane-2-carboxylic acid, 3-(4-iodophenyl)-8-methyl-methyl ester 1R-(exo, exo)) (2) which has been labelled with ^{11}C (3) or ^{123}I (2) and used for PET (4) and SPECT (2), respectively. Two newly developed analogues have an N-fluoroethyl or N-fluoropropyl group instead of the N-methyl group (ß-CIT-FE and ß-CIT-FP) and have been labelled with ^{11}C or ^{18}F for Positron Emission Tomography (PET) (5,6,7) and ^{123}I for Single Photon Emission Computed Tomography (SPECT) (8,9).

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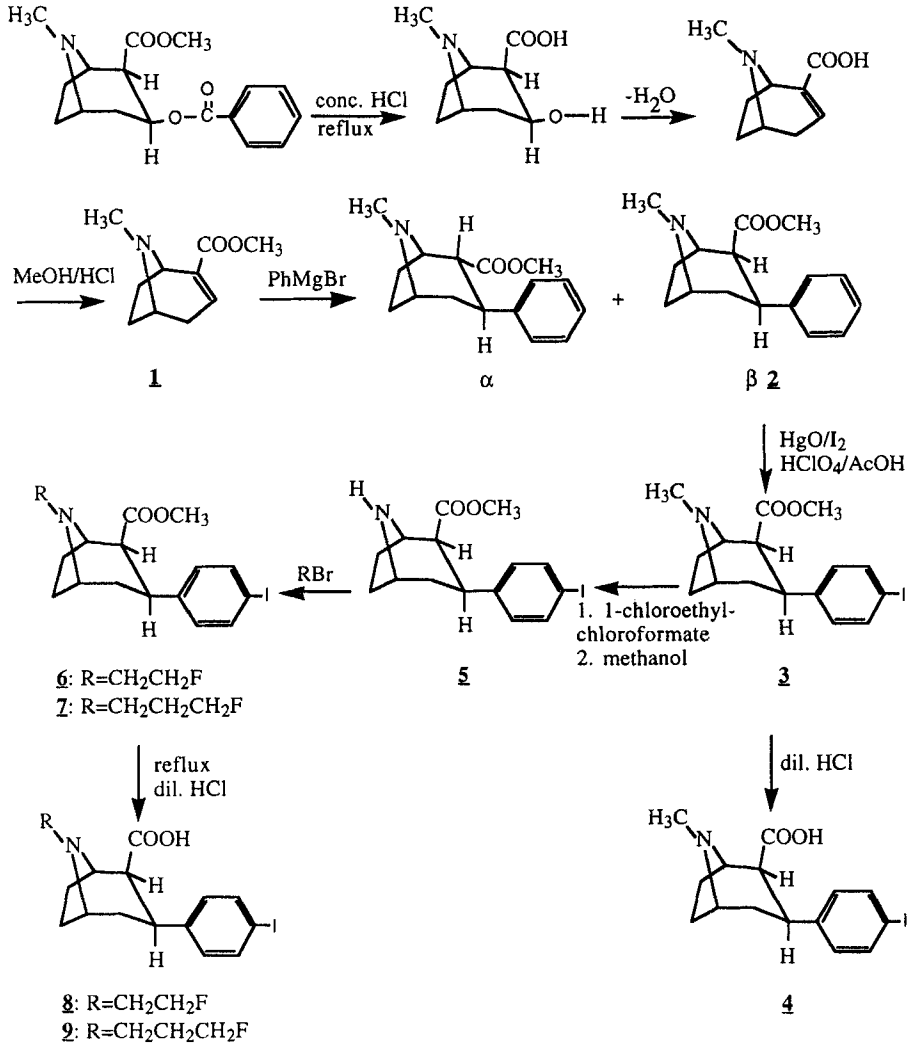
CCC 0362–4803/96/070675–11

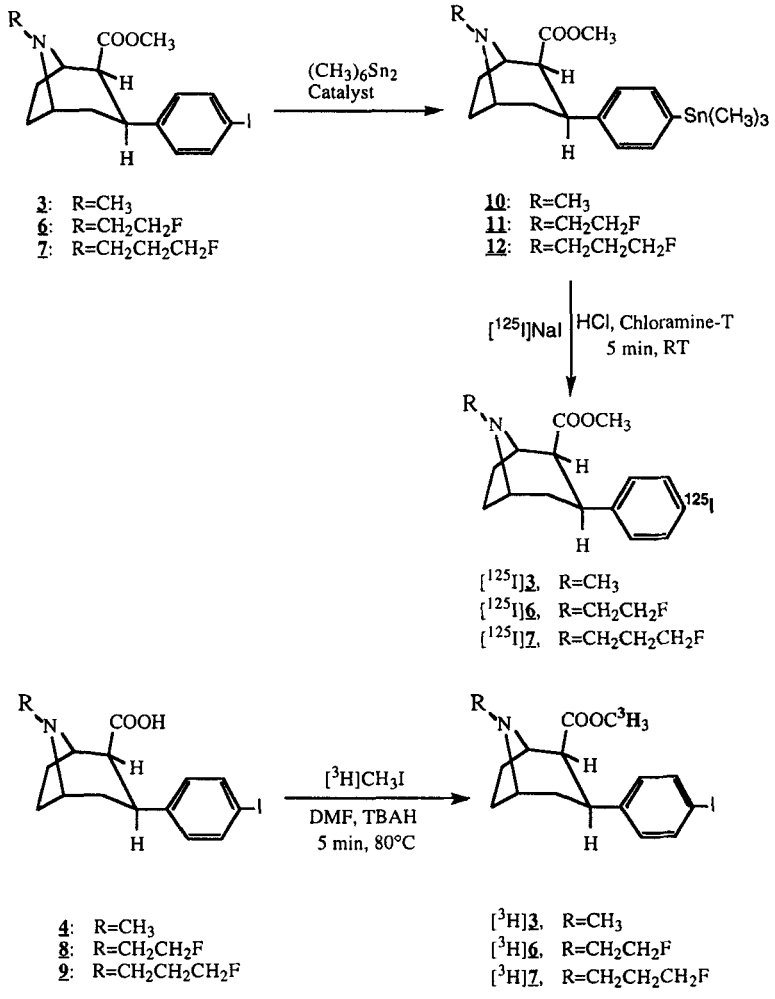
Received 28 January 1996
Revised 6 February 1996



Scheme 1

Some of the syntheses of the starting materials have been described in the literature but we feel that it could be useful to have all the steps described in detail in one single paper. The starting material is naturally occurring cocaine and all the active compounds have the same configuration at C-2 and C-3. An alternative synthetic route which gives the racemic mixture of aryltropane derivatives, starting from cycloheptatriene carboxylic acid, has been reported previously (10).

Here we report the full synthesis of ß-CIT, ß-CIT-FE and ß-CIT-FP starting from cocaine. The syntheses which are shown in Scheme 1 and 2, are also including preparation of precursors for labelling with radionuclides such as ^{11}C, ^{18}F, ^{76}Br, ^{125}I, ^{125}I and ^{3}H. In addition we describe the labelling of the three ß-CIT analogues (ß-CIT, ß-CIT-FE and ß-CIT-FP) with either ^{125}I or ^{3}H for use in autoradiographic examination of human brain sections.



3: R=CH_3
6: R=CH_2CH_2F
7: R=$CH_2CH_2CH_2F$

10: R=CH_3
11: R=CH_2CH_2F
12: R=$CH_2CH_2CH_2F$

[^{125}I]3, R=CH_3
[^{125}I]6, R=CH_2CH_2F
[^{125}I]7, R=$CH_2CH_2CH_2F$

4: R=CH_3
8: R=CH_2CH_2F
9: R=$CH_2CH_2CH_2F$

[^{3}H]3, R=CH_3
[^{3}H]6, R=CH_2CH_2F
[^{3}H]7, R=$CH_2CH_2CH_2F$

Scheme 2

RESULTS AND DISCUSSION

The general procedure applied in the synthetic reactions described in the present publication is the result of more than twenty years of work by different groups and starts from cocaine. The preparation of anhydroecgonine methyl ester (1, Scheme 1) is straight-forward but takes time. An alternative procedure has been described by Meltzer et al. (11). The synthesis of all analogues is based on the Grignard reaction described by Clarke et al. 1973 (12). A major improvement was introduced by Carroll et al. (13) who performed the rection at -40° which avoided disubstitution. They protonated the magnesium complex at -70° which gave a much more favourable ratio of isomers than the original procedure. Finally they did not distill their

product (2). In the early stages of our work we distilled the crude product which resulted in that the ß isomer possibly was transformed to the α isomer during the procedure. Clarke et al. (12) reported that the α isomer is the more stable one. We have used the Macintosh Chemdraw 3D program to perform an energy minimisation of the two isomers and found that this gave a total energy of 36.4 kcal/mole for the ß isomer and 29.7 kcal/mole for the α isomer. The iodination and demethylation of the phenyltropane followed the procedure by Boja et al. (14). Initially we used the iodination method by Neumeyer et al. (2) which is not described in detail and our yields were only moderate. The demethylation procedure according to Milius et al. (15) which used treatment of the trichlorocarbamate with zinc did also work.

The synthesis of the fluorinated analogues (6,7) is a simple alkylation but time-consuming which could be anticipated, especially for the fluoroethyl derivative. 2-Fluoroethyl bromide has been reported to be almost ten times less reactive than ethyl bromide (16). There are also some indications that the fluoroethyl derivative is unstable. A sample which was pure by HPLC showed the presence of several peaks after a few months in solution. In principal the fluoroethyl derivative is a nitrogen mustard even if the reactivity of fluorine as leaving group in nucleophilic substitutions is low (about 10^{-4} of the reactivity of bromine (17)).

The free acids (4,8,9) and the trimethylstannyl derivatives (10,11,12) were only prepared on a small scale (<55 mg). The tin derivatives were purified by preparative TLC as attempts to purify them by reversed phase HPLC did not give reproducible results. The acids could conveniently be purified by HPLC as the amounts needed for labelling were small. This purification was necessary as the product after hydrolysis was the hydrochloride which only gave a low yield in the labelling reactions.

The synthetic routes included also preparation of many precursors for labelling with radionuclides such as ^{11}C, ^{18}F, ^{76}Br, ^{123}I, ^{125}I and ^{3}H. The free acids, ß-CIT-FE acid and ß-CIT-FP acid (8,9), were used for labelling of [^{11}C]ß-CIT-FE and [^{11}C]ß-CIT-FP in the O-methyl position from [^{11}C]methyl iodide as have been reported in detail elsewhere (5,6). [^{18}F]ß-CIT-FE and [^{18}F]ß-CIT-FP were prepared from nor-ß-CIT (5) and [^{18}F]fluoroalkyl bromides (7). The trimethylstannyl derivatives (10,11,12) were used as precursors for ^{76}Br-labelling of [^{76}Br]ß-CBT, [^{76}Br]ß-CBT-FE and [^{76}Br]ß-CBT-FP by electrophilic substitution (18,19). Furthermore, several of the synthesized compounds such as nor-ß-CIT, the free acids etc. were used as standards in metabolite studies in monkey and human plasma in connection with PET and SPECT studies (20,21).

In this paper we have extended the labelling reactions to include ^{125}I-labelling of [^{125}I]3, [^{125}I]6 and [^{125}I]7 (Scheme 2) from the corresponding trimethylstannyl derivatives (10,11,12) and [^{125}I]sodium iodide and ^{3}H-labelling of [^{3}H]3, [^{3}H]6 and [^{3}H]7, (Scheme 2) from the free acids (4,8,9) and [^{3}H]methyl iodide. The labellings proceeded in good yield (Table 1). The crude products eluted at the same retention times as authentic compounds in two different chromatographic systems with the time delay characteristic of the systems. The total radiochemical yields were 70-90% for [^{125}I]3, [^{125}I]6 and [^{125}I]7 (Scheme 2) with a specific radioactivity of 2200 Ci/mmol and 50-70% for [^{3}H]3, [^{3}H]6 and [^{3}H]7, (Scheme 2) with a specific radioactivity of 22-57 Ci/mmol. The radiochemical purity was >99% in all cases. Both the ^{3}H- and ^{125}I-labelled 3,6 and 7 were used in autoradiographic examination of human brain sections (22).

Table 1. A summary of radiochemical yields and specific radioactivities for ^{125}I- and ^{3}H-labelled ß-CIT, ß-CIT-FE and ß-CIT-FP.

Compound	^{125}I-labelled*		^{3}H-labelled		
	Radioactivity (mCi)	Yield (%)	Radioactivity (mCi)	Yield (%)	Specific radioactivity (Ci/mmol)
ß-CIT (3)	0.83	90	0.7	60	22.1
ß-CIT-FE (6)	2.44	90	1.1	52	22.4
ß-CIT-FP (7)	1.87	70	0.8	69	57.0

*The specific radioactivity of the ^{125}I-labelled compounds is assumed to be the same as for [^{125}I]sodium iodide.

EXPERIMENTAL

The radiolabelled reagents, [^{125}I]sodium iodide (2200 Ci/mmol, pH = 9-12) and [^{3}H]methyl iodide (78 Ci/mmol) in toluene (1 mL), were both obtained from Amersham Sweden AB. Phenylmagnesium bromide (3.0 M in diethyl ether) and 1-bromo-2-fluoroethane were purchased from Aldrich-Chemie GmbH & Co. KG, Steinheim, Germany and 1-bromo-3-fluoropropane from ABCR GmbH, Karlsruhe, Germany (a product of Fluorochem LTD, Derbyshire,UK). Authentic ß-CIT, ß-CIT-FE and ß-CIT-FP were obtained from RBI, Research Biochemicals International, Natick, USA. Cocaine;HCl was obtained from the Karolinska Hospital Pharmacy. Silica gel for column chromatography was Merck Kieselgel 60, 230-400 mesh and the TLC plates were Merck DC-Fertigplatten, Kieselgel 60 F_{254} 0.25 mm thick. Other chemicals were of analytical grade and used without further purification. Melting points were determined with a Reichert Thermovar melting point microscope and are uncorrected. HPLC separations were performed on Waters μ-Bondapak C18 columns (300 x 7.8 mm, 10μm). Preparative separations were performed isocratically with a Kontron 420 pump with acetonitrile-0.01M phosphoric acid (30/70 or 35/65) or acetonitrile-1% acetic acid as mobile phases and a flow of 5-6 ml/min and were monitored by UV detection in a Kontron 432 UV detector followed by a Packard Radiomatic Flo one beta series 150TR radioactivity detector with appropriate cells to detect ^{125}I or ^{3}H. The purity of the ^{125}I-labelled products was also analysed on a Kontron gradient HPLC system with a PET cell in the radioactivity detector. The specific radioactivity of the ^{3}H-labelled products was determined isocratically with HPLC by comparison with known amounts of standards.

Anhydroecgonine methyl ester (1, Scheme 1) (23). Cocaine;HCl (25.1 g , 0.074 mol) was refluxed in concentrated hydrochloric acid for 20 hours. The reaction was allowed to cool to room temperature, kept at 4^{o} for a further 2 hours after which time the benzoic acid was

removed by filtration. The filtrate was concentrated in a rotatory evaporator, 80 ml of methanol was added and the solvent evaporated. The white solid residue was triturated with several portions of diethyl ether and filtered to remove traces of benzoic acid. The residue was dried at room temperature in vacuum for 40 minutes. Methanol (400 ml) was saturated with hydrogen chloride gas and the solution was added to the residue above. After standing for three days at room temperature methanol was removed and the residue was made alkaline (pH 11-12) with 4M NaOH and extracted with several portions of diethyl ether which were combined and dried over sodium sulfate. The solvent was evaporated and the residue (11 g) was distilled in vacuum at a bath temperature of 120° and 0.05 mm Hg. The distillation was performed in batches of 2 g which were absorbed on glass wool in the micro scale distillation apparatus and collected in a cup under the cold-finger condenser. The yield was 9.9 g (75%).

2ß-carbomethoxy-3ß-phenyltropane (2, ß-CPT, Scheme 1) (13). A four-necked round bottom flask was equipped with a condenser, an efficient stirrer, a low-temperature thermometer, a pressure-equalised dropping funnel and inlet and outlet for nitrogen gas. The outlet was connected to a mercury trap.The outlet of the dropping funnel was connected to a piece of teflon tubing to make it possible to place the drops more in the middle of the flask to avoid an otherwise thick precipitate on the wall of the flask. A solution of anhydroecgonine methyl ester (1, 6.1 g, 0.034 mol) in dry diethyl ether (85 ml) was dropped to a vigourously stirred solution of phenylmagnesium bromide (25 ml, 3M) in diethyl ether (540 ml) at -60° to -40° (temperature of the reaction mixture) during 0.5 hours. After the addition of the ester the temperature was kept at -40° for 2.5 hours. The temperature was lowered to below -70° and trifluoroacetic acid (8.5 g in 40 ml of diethyl ether) was added. After the addition the temperature was allowed to increase to about -5° and 200 ml of water was added in small portions.The aqueous layer was acidified to pH1 with concentrated hydrochloric acid, separated from the organic phase, made alkaline with concentrated ammonium hydroxide and extracted with diethyl ether (5x100 ml). The combined ether phases were dried over sodium sulfate, filtered and evaporated to give 7.7 g of crude product as a dark yellow oily residue which was purified by column chromatography on silica gel (100 g/2.5g crude material) in three portions with diethyl ether/triethylamine (9:1) as eluent. The collected fractions were analysed by TLC with the same eluent and the ß isomer was found at R_f = 0.8.The total yield was 3.5 g (0.014 mol, 41%). The melting point was 55-59° (lit 62-64° (12). The yield of the unwanted α isomer was 1.3 g (0.005 mol, 15%).

2-ß-carbomethoxy-3-ß-(4-iodophenyl)tropane (3, ß-CIT, Scheme 1) (14). ß-CPT (2, 0.6 g, 0.002 mol) was dissolved in a mixture of acetic acid (7.5 ml), perchloric acid (70%, 2.5 ml) and yellow mercuric oxide (0.5g). Iodine (1.4 g) dissolved in 20 ml of dichloromethane and 10 ml of acetic acid was added in portions over 10 minutes. The mixture was stirred at room temperature overnight and the solids were separated by filtration. Water (10 ml) and dichloromethane (20 ml) were added to the filtrate. The mixture was cooled to 0° in an ice bath, made alkaline with concentrated ammonium hydroxide and the organic phase was separated. The aqueous phase was further extracted with dichloromethane (4x100 ml). The combined organic

phases were dried over sodium sulfate, filtered and evaporated to give 0.85 g (0.002 mol, 100%) of crude product as a slightly yellow oil. HPLC analysis showed that the product was nearly pure so it was used without further purification for the next step. It solidified in the freezer and had a melting point of 73-99° (lit.107-108° (14)). In subsequent experiments it was found that the product was not so pure and the yield was much higher than the theoretical one, possibly caused by mercury compounds so it was necessary to purify the crude product. The product was therefore dissolved in a small quantity of dichloromethane, diethyl ether-triethylamine 9/1 was added until a precipitate was formed which was dissolved again with dichloromethane. The resulting solution was filtered through silica Sepak cartridges until no considerable coloured ring was formed on the top the column (three times). The yield after that treatment was 81-83%.

3-β-(4-iodophenyl)tropane 2-β-carboxylic acid (4, ß-CIT acid, Scheme 1) (24). ß-CIT (3, 32.2 mg, 83.6 µmol) was heated at 100° in 4 ml of 1M hydrochloric acid for 46 hours. The solvent was evaporated and the residue was dissolved in a small quantity of water. The product was purified by repeated injections on the Bondapak column with 1% acetic acid/ 33% acetonitrile as eluent. After removal of the solvent the product was obtained in a pure state (28.7 mg, 77.3 µmol 92%). with a melting point 283-291°, decomp. (lit 318-320° (24).

2-β-carbomethoxy-3-β-(4-iodophenyl)nortropane (5, nor-ß-CIT, Scheme 1) (14). ß-CIT (3, 1.00 g, 2.6 mmol) was dissolved in dry 1,2-dichloroethane (50 ml). 1-chloroethylchloroformate (2.5 ml = 3.3 g, 21.2 mmol) was added and the mixture was refluxed under nitrogen for 1 hour.The solvents were removed and 30 ml of methanol was added. The mixture was refluxed for 30 minutes, concentrated to dryness and dissolved in dichloromethane which was washed with saturated sodium bicarbonate solution. The organic phase was dried over sodium sulfate, filtered and concentrated. The crude product (1.15 g) contained unreacted ß-CIT and was purified by chromatography (silica gel, 80 g) with diethyl ether-triethylamine (9:1) as eluent. TLC with the same system showed ß-norCIT to have an R_f of 0.15. 0.40 g of ß-norCIT was obtained (1.08 mmol, 42%) and 0.35 g of ß-CIT was recovered. The ß-norCIT had a melting point of ≈85-102° (lit. 116-117° (14)).

N-2-fluoroethyl-2-β-carbomethoxy-3β-(4-iodophenyl)nortropane (6, ß-CIT-FE, Scheme 1) (25). Nor-ß-CIT (5, 200 mg, 0.54 mmol) was dissolved in triethylamine (5 ml). 1-Bromo-2-fluoroethane (500 µl, 850 mg, 6.7 mmol) was added and the mixture was heated at 70° for two days. According to HPLC analysis the reaction was complete and the solvent was evaporated. At that moment nothing was done with the product for two months and when it was analysed again there was a lot of starting material left. Therefore the crude product was suspended in acetone (2 ml) and heated with 1-bromo-2-fluoroethane (400 µl) at 40° for 6 days when the reaction was complete. The solvent was evaporated, saturated sodium bicarbonate solution (2 ml) and two drops of concentrated ammonium hydroxide were added and the solution was extracted with five portions of diethyl ether (4 ml). After evaporation of the diethyl ether there was obtained 105.5 mg of product (0.25 mmol, 46%). The product solidified in the refrigerator

to a semichrystalline solid with melting point of 47-88° (lit. 99-101° (25)) and was sufficiently pure for use in the next reactions. When the reaction was reattempted in acetone it was found that the reaction was not complete in a week but after addition of triethylamine almost all of the starting material had disappeared after an additional day.

N-3-fluoropropyl-2-ß-carbomethoxy-3-ß-(4-iodophenyl)nortropane (7, ß-CIT-FP, Scheme 1) (25). Nor-ß-CIT (5, 320 mg, 0.86 mmol) was dissolved in triethylamine (10 ml). 1-Bromo-3-fluoropropane (500 µl, 770 mg, 5.47 mmol) was added and the mixture was heated at 70° until the reaction was complete as shown by HPLC (about 24 hours). Evaporation of the solvent resulted in 1.5 g of a slightly red solid. A part of it (1.08 g) was dissolved in dichloromethane (50 ml) which was washed twice with 50 ml of water (at pH 10 with ammonia). The combined water layers were extracted twice with dichloromethane with was combined with the first dichloromethane solution and dried. After removal of solvent the residue was dissolved in a small quantity of dichloromethane. A mixture of pentane-diethyl ether- triethylamine 20/9/1 (10 ml) was added and the solution was filtered through a silica Sep Pak cartridge which was washed twice with 5 ml of the same mixture. The solutions were combined and the solvent was removed leaving 230 mg of product (0.53 mmol, 85%). The melting point was 63-70° (lit. 82-83° (25)).

N-2-fluoroethyl-3-ß-(4-iodophenyl)nortropane-2-ß-carboxylic acid (8, ß-CIT-FE acid, Scheme 1). ß-CIT-FE (6, 28.9 mg, 69 µmol) was heated at 100° in 4 ml of 1M hydrochloric acid for 44 hours when analysis showed that 1.5% starting material remained. The residue after evaporation was purifed on the Bondapak column with 35% acetonitrile-1% acetic acid. The yield was 21 mg (51 µmol, 74%).

N-3-fluoropropyl-3-ß-(4-iodophenyl)nortropane 2-ß-carboxylic acid (9, ß-CIT-FP acid, Scheme 1). Crude 7 (420 mg corresponding to about 90 mg of pure substance) was dissolved in 2M hydrochloric acid and the solution was heated at 100° for 30 hours when HPLC analysis showed that no starting material was present. The reaction mixture was neutralised with ammonium hydroxide to pH 7 and a buffer solution (pH 7, 20 ml) was added. The solution was extracted three times with dichloromethane (50 ml) which was combined and evaporated. The yield of crude 8 was about 75 mg. A small part of this material was purified on the µ-Bondapak column with 35% acetonitrile-1% acetic acid. The UV peak corresponding to authentic reference material was collected in several runs and the combined fractions were evaporated. A small part of this product was reacted with [^{11}C]methyl iodide which gave the same product ([^{11}C]ß-CIT-FP) as treatment of the authentic substance (ß-CIT-FP acid) obtained from RBI.

2-ß-carbomethoxy-3-ß-(4-trimethylstannylphenyl)tropane (10, Scheme 2) (26). A mixture of hexamethyldistannane (60 µl, 0.28 mmol), ß-CIT (3, 80 mg, 0.21 mmol) and palladium-tetrakis-triphenylphosphine (2 mg) in dry toluen (5 ml) was refluxed under nitrogen for 4 hours. The catalyst was separated by centrifugation and the solvent was evaporated. The residue was purified by TLC (20x20 cm plate) with hexane-diethyl ether-triethylamine (10:9:1) as eluent. The desired product had an R_f = 0.38 (ß-CIT = 0.27) and the yield was 46 mg (52%).

N-2-fluoroethyl-2-β-carbomethoxy-3-β-(4-trimethylstannylphenyl)nortropane (**11**, Scheme 2) (25). ß-CIT-FE (**6**, 21 mg, 50 µmol) was dissolved in toluen (1.5 ml) and nitrogen gas bubbled through the solution. Hexamethyldistannane (15 µl) and palladium tetrakis-triphenylphosphine (1.8 mg) were added. The vial was closed, flushed with nitrogen and heated at 105° for five hours.The solids were separated by centrifugation, washed with toluene and recentrifuged. The combined toluene solutions were evaporated to give a crude yield of 29.8 mg. This product was purified by TLC with hexane-diethyl ether-triethylamine (10:9:1) as eluent. A band with R_f = 0.61 was scraped and gave 13.0 mg (29 µmol, 58%) of substance. One slower and one faster band contained 4.5 mg of material.

N-3-fluoropropyl-2-β-carbomethoxy-3-β-(4-trimethylstannylphenyl)nortropane (**12**, Scheme 2) (25). A mixture of hexamethyldistannane (60 µl, 0.28 mmol), ß-CIT-FP (**7**, 88 mg, 0.21 mmol) and palladium tetrakis-triphenylphosphine (2 mg) was refluxed in toulene (5 ml) under nitrogen for 4 hours. The mixture was filtered and the toluene evaporated. The crude product was purified by TLC with hexane-diethyl ether-triethylamine as eluent. The yield was 25 mg (55 µmol, 26%).

[^{125}I]β-CIT, [^{125}I]β-CIT-FE and [^{125}I]β-CIT-FP ([^{125}I]**3**, [^{125}I]**6** and [^{125}I]**7**, Scheme 2). The trimethyltin precursor (**10**, **11** or **12**, 0.10 mg) was deposited in a 1 ml reaction vial by dissolving the weighed stock material in a known volume of diethyl ether, pipetting the calculated volume into the reaction vial and evaporation of the diethyl ether. [^{125}I]Sodium iodide (10-30 µl), hydrochloric acid (20 µl, 0.2M) and chloramine-T solution (20 µl, 1 mg/ml water) were added in that order. The vial was sealed and stirred for 5 minutes at room temperature after which time HPLC mobile phase (300 µl) was added. The solution was injected into the µ-Bondapak column which was eluted with acetonitrile-0.01M phosporic acid at a flow of 6 ml/min. The retention time of the compound was established by injection of small samples of the labelled compound and comparison with authentic compound. The percentage of acetonitrile was 30-35%, depending on compound and condition of the column and must be determined for each individual experiment. The appropriate fraction was taken and the solvent evaporated. The residue was reconstituted in 75% ethanol (2 ml) from which a sample was taken and analysed to determine the radiochemical purity.

[^{3}H]β-CIT, [^{3}H]β-CIT-FE and [^{3}H]β-CIT-FP ([^{3}H]**3**, [^{3}H]**6** and [^{3}H]**7**, Scheme 2). The free acid precursor (**4**, **8** or **9**, 0.3-0.4 mg) was transferred to a reaction vial as such if solid or in solution if the available material was not chrystalline. If it was necessary, solvent was also evaporated. Dry dimethylformamide (250 µl) and freshly prepared tetrabutylammonium hydroxide (2 µl, 0.4M, from 1.358 g of tetrabutylammonium sulfate and 0.32 g of sodium hydroxide, in 10 ml of water) were added. The required amount of [^{3}H]methyl iodide (100-150 µl) in toluene was added and the mixture was heated at 80° for 5 minutes. Most of the toluene was evaporated in a stream of nitrogen gas, mobile phase (300 µl) was added and the mixture was chromatographed as above.

Acknowledgements

The authors would like to thank Ms. Ann Eriksson, Ms. Åsa Eriksson and Ms. Louise Rudenlöv for technical assistance. This work has been performed within the COST B3 action, and was supported by grants from the Swedish Natural Research Council (K-KU 9973-307), the Swedish Medical Research Council (03560 and 09114) and Karolinska Institutet. β-CIT, nor-β-CIT, β-CIT-FE, β-CIT-FP and β-CIT-FP-acid were obtained by RBI, Research Biochemicals International, Natick, USA.

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