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ANU AIRAKSINEN

New Tropane Analogues as Potential Brain Imaging Agents

Synthesis, Conformational Study and *In vitro*/*In vivo* Evaluation

Doctoral dissertation

To be presented by permission of the Faculty of Natural and Environmental Sciences of the University of Kuopio for public examination in Auditorium L21, University of Kuopio, Friday 20th December 2002, at 12 noon

Department of Chemistry
University of Kuopio

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ABSTRACT

Phenyltropanes are cocaine analogues, which bind to the dopamine (DAT), serotonin (SERT) and norepinephrine (NET) transporters in the brain. For this reason, radiolabeled forms of these compounds have been used as diagnostic radiopharmaceuticals in the *in vivo* imaging of the monoamine reuptake sites in brain by single photon emission tomography (SPET) or positron emission tomography (PET). Phenyltropanes are also under vigorous research because they are potential agents in the treatment of cocaine abuse.

The binding affinity and selectivity of phenyltropanes for monoamine binding sites can be influenced by varying the substituents present in the tropane ring. During the last decade, numerous tropane derivatives have been studied in order to characterize which molecular structures could have improved monoamine receptor binding properties. However, the influence of substitution of the tropane ring at 6- or/and 7-position on receptor binding has remained unknown. In the present study, we developed two routes for synthesizing 6/7-substituted phenyltropanes. In addition, a novel route to 3,4-disubstituted pyrroles was developed. 6/7-Carboethoxy-2β-acetoxy-3β-phenyltropane analogues were synthesized via 1,3-dipolar cycloaddition and 6/7-methyl-2β-carbomethoxy-3β-phenyltropane analogues via the Mannich reaction. Radiolabeling with I-125 was performed for one selected compound. The effect of 6/7 substitutions on receptor binding on DAT, SERT and NET was evaluated in *in vitro* and selected compounds were tested *in vivo* in locomotor activity tests in mice. The 6- or 7-carboethoxy substituted 2β-acetoxy analogues and 3,4-disubstituted pyrroles showed no affinity in the *in vitro* evaluation. However, the 2β-carbomethoxy analogues with a methyl group at the 6- or 7-position showed increased selectivity for SERT, but decreased overall affinity for the transporters. In the *in vivo* locomotor tests, 6-methyl-2β-carbomethoxy-3β-(*p*-iodo)phenyltropane increased the locomotion of mice in a dose dependent manner, but the corresponding demethylated analogue had no apparent effect on locomotor activity.

The influence of different substituents on tropane ring conformation was examined with ^{1}H NMR spectroscopic and computational methods. The conformational analysis of selected phenyltropanes showed that the 2β, 3β-substituted analogues preferred the relatively rigid chair conformation. However, those analogues, which have carbonyls or 2α, 3α-substituents were very flexible and had different conformations within very low energy barriers.

National Library of Medicine Classification: QV 113, WN 180

Medicinal Subject Headings: tropanes / chemical synthesis; pyrroles; cocaine / analogs & derivatives; molecular conformation; diagnostic imaging; brain; crystallography, X-ray; magnetic resonance spectroscopy; motor activity; mice

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Kuopio, December 2002

Anu Airaksinen

ABBREVIATIONS

Bn	benzyl group
chloramine-T	N-chloro-p-toluenesulphonamide sodium salt
β-CIT	2β-carbomethoxy-3β-(4-iodophenyl)tropane
DAT	dopamine transporter
Et	ethyl group
HPLC	high performance liquid chromatography
IC_{50}	concentration causing 50% inhibition
Me	methyl group
NET	norepinephrine transporter
NMR	nuclear magnetic resonance
nor-β-CIT	2β-carbomethoxy-3β-(4-iodophenyl)nortropane
PET	positron emission tomography
Ph	phenyl group
rt	room temperature
SERT	serotonin transporter
SPET	single photon emission tomography
TMS	tetramethylsilane
TosMIC	tosylmethylisocyanide
Tos	tosyl group

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original publications referred to in the text by their Roman numerals:

I. **Airaksinen, A. J.; Tuppurainen, K. A.; Lötjönen, S. E.; Niemitz, M.; Yu, M.; Vepsäläinen, J.; Laatikainen, R.; Hiltunen, J.; Bergström, K. A.** Nuclear Magnetic Resonance and Molecular Orbital Study of Some Cocaine Analogues. *Tetrahedron* **1999**, *55*, 10537-10546.

II. **Airaksinen, A. J.; Ahlgren, M.; Vepsäläinen, J.** One-Pot Synthesis of 3,4-Disubstituted 1H-Pyrroles from 2-Tropanones. *J. Org. Chem.* **2002**, *67*, 5019-5021.

III. **Airaksinen, A. J.; Lipsonen, J.; Ahlgren, M.; Vainiotalo, P.; Bergström, K. A.; Laatikainen, R.; Vepsäläinen, J.** Reduction of 6/7-Substituted 3-Phenyltrop-3-en-2-ones: Stereoselectivity and Conformational Analysis of the Products. *Tetrahedron*, in press

IV. **Airaksinen, A. J.; Huotari, M.; Shvetsov, A.; Vainiotalo, P.; Männistö, P. T.; Tuomisto, L.; Bergström, K. A.; Vepsäläinen, J.** Synthesis and Biological Evaluation of 6/7-*exo*-Methyl-3β-(4-iodo)phenyltropane-2β-carboxylic Acid Methyl Esters. *Manuscript*

CONTENTS

1. INTRODUCTION

Phenyltropanes are cocaine analogues, which bind to dopamine (DAT), serotonin (SERT) and norepinephrine (NET) transporters, in the same way as their parent compound cocaine.[1-5] Phenyltropanes have been under vigorous research since the 1990's, when it was discovered, that their binding properties can be influenced by modifications of the tropane skeleton.[6] Phenyltropanes with high selectivity to the DAT have been developed, but the SERT and NET selective phenyltropanes have proved more difficult to attain. In the late 90's, a new target for the modifications was introduced. The substitution at the ethylene bridge i.e. at the 6/7-position of the tropane ring was shown to have also some influence on selectivity, but the phenomenon remained to be clarified.[7,8] In the early 2000's it was found that the conformation of the tropane ring had its own role in selective binding and new conformationally constrained SERT and NET selective compounds were developed.[9,10]

Structural analogues of the phenyltropanes have provided new tools for brain research. When labelled with a radionuclide, these compounds enabled imaging of the monoamine transporters *in vivo* with brain scanning methods, such as SPET and PET.[11] This new diagnostic possibility gave novel information about neurological and neuropsychiatric disorders e.g. Parkinson's disease[12] and social phobia.[13] However, even thought some selective phenyltropanes were developed, new, especially SERT and NET selective, imaging agents are needed.

This thesis reports the results of studies focused on the development of strategies for preparing 6/7-substituted phenyltropanes and also the elucidation of the influence of 6/7-substitution on their biological activity by using *in vitro* and *in vivo* tests. In addition, we have examined the influence of different substitutions on the tropane ring conformation with ^{1}H NMR spectroscopic and computational methods.

2. REVIEW OF THE LITERATURE

2.1. Pharmacology of phenyltropanes

2.1.1. Structure of phenyltropanes and binding to central monoamine transporters

Phenyltropanes (**1**) are cocaine analogues, which lack the benzoylester functionality present in cocaine. Instead of that moiety, they have phenyl ring directly attached to the tropane ring.[14]

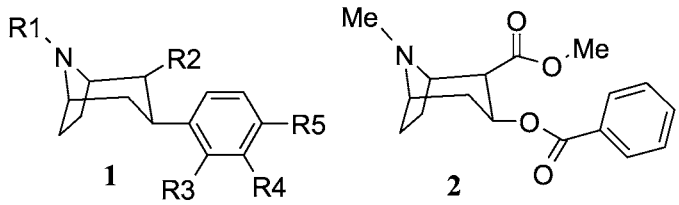
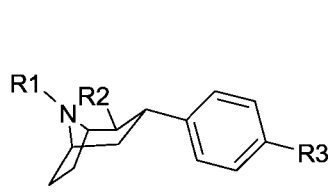


Figure 1. Basic structure of phenyltropanes (**1**) and (-)-cocaine (**2**).

Phenyltropanes, like their parent compound cocaine (**2**), bind to DAT, SERT and NET in the brain.[1-5,15] These Na^+/Cl^- dependent transporters are located presynaptically in the neuronal plasma membranes. They control the concentration of the corresponding neurotransmitters in the synaptic cleft by taking up the released transmitters rapidly back into the nerve terminals.[16] Cocaine blocks this uptake and thus increases the concentration of the neurotransmitters in the synaptic cleft.[5]

(-)-Cocaine binds enantio- and stereoselectively with almost equal affinities to all three of monoamine transporters.[17,18] However, with phenyltropanes it has been observed that modifications at substituents of the tropane ring can change the selectivity ratios of the transporters. On the basis of the structure-activity relationship data and molecular modeling studies, some basic structural features, which increase the selectivity to only a single type of the transporter have been found. In general, it has been observed that large substituents at the N-position[19-23] and at the 2-position of the tropane ring[24-30] increase DAT selectivity. Small electronegative substituents on the *m* and *p* positions of 3β-phenyl

ring are known to enhance affinity to all the transporters[31-36] and by further modifications at the phenyl ring, together with demethylation of the N-position, some SERT selective analogues have been developed.[37-39] With phenyltropanes there are some exceptions from the stereoselectivity rule, which is important for cocaine. Increased DAT selectivity has been found among analogues which have a phenyl group at the 3α-position instead of the 3β-position and which have the tropane ring in boat conformation (**3**).[40-45] Furthermore, there are some conformationally constrained tricyclic tropane derivatives (**4**) which show striking selectivity to either SERT or NET, depending on which substituents are added.[9,10] Some rigid analogues (**5**) have also been shown to have enhanced SERT selectivity,[47,46] but only when there is no substitutent at the 2-position.[48,49]



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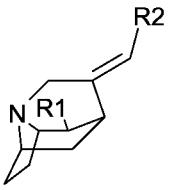

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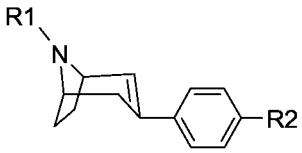


5

The influence of 6/7-substitution on the biological activity of phenyltropanes is still relatively unclear, but some general features may be noted. Substitution with large substituents, decreases the binding affinity to monoamine transporters.[7] However, phenyltropanes with a hydroxyl at the 7-position, shows increased DAT selectivity and affinity.[8] In addition, 6/7-methoxylated pseudococaine analogues have shown weak cocaine antagonism[50] and a corresponding hydroxylated phenyltropane analogue, when tested *in vivo*, has been found capable of attenuating the locomotor stimulation induced by cocaine in mice.[51] The current view is that the elevated dopamine concentration present in synaptic cleft after cocaine is the main reason for its behavioural reinforcement properties.[52,53] Cocaine and dopamine are known to have separate binding domains in the DAT,[54,55] thus it might be possible to design a drug that prevents the binding of cocaine but still allows dopamine to be transported.[56] One implication of these results is that the 6/7-substituted phenyltropanes have potential as agents to be used in the treatment of cocaine abuse.[6]

2.1.2. Psycholocomotor effects of phenyltropanes

(-)-Cocaine is a psychomotor stimulant, which increases locomotor activity in a dose-dependent manner.[57] According to studies with DAT and SERT knockout mice, DAT blockade caused by cocaine is the main mechanism for the cocaine-induced locomotion.[58-61] Phenyltropanes have also been found to increase locomotor activity and there is a good agreement between their potency for *in vitro* binding to the DAT and potency in increasing locomotion.[30,62-64]

2.1.3. Use of phenyltropanes in SPET and PET brain studies

Changes in the monoamine transporter densities are known to be involved in many neurological and psychiatric disorders, like Alzheimer's disease,[65,66] Parkinson's disease,[67,68] depression[69] and panic disorder.[70,71] Furthermore, alterations in the transporter densities have been observed to occur in social phobia[13] and alcohol abuse.[72,73]

Single photon emission tomography (SPET) and positron emission tomography (PET) are techniques which use radiolabelled tracers in the *in vivo* detection of changes in transporter densities.[11] In SPET the tracer is labelled with a γ-emitting nuclide and in PET with a β^+-emitting nuclide (Table 1).

Table 1. Most commonly used radionuclides in receptor imaging with SPET and PET, and their half times.[74]

Radionuclide	Half-time ($t_{1/2}$)	Imaging technique
^{13}N	10 min	PET
^{11}C	20 min	PET
^{18}F	110 min	PET
^{99m}Tc[a]	6.0 h	SPET
^{123}I	13 h	SPET

[a]Data from Hom *et al.* 1997.[75]

As a result of the extensive research conducted on structure–activity relationships of phenyltropanes, many potential agents for DAT and SERT imaging have been developed. However, it has been observed that the potential of a compound to bind to transporters *in vitro* does not always predict its ability to bind after *in vivo* administration.[76] Thus, the clinical applications of phenyltropanes in brain imaging have been limited.

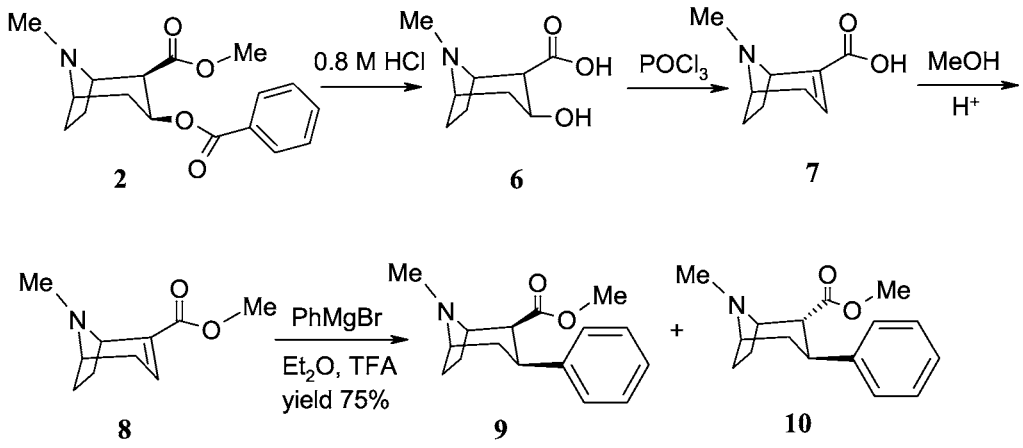
Iodine-123 labeled β-CIT (2β-carbomethoxy-3β-(4-iodophenyl)tropane) has been developed for the DAT and SERT imaging and it is in clinical use in the diagnosis of Parkinson disease with SPET.[12,77-80] Iodine-123 labeled PE2I ((E)-N-(3-iodoprop-2-enyl)-2β-carbomethoxy-3β-(4-methylphenyl)nortropane),[81] β-CIT-FP (N-(3-fluoropropyl)-2β-carbomethoxy-3β-(4-iodophenyl)nortropane)[82,83] and carbon-11 or fluorine-18 labeled β-CIT-FP[84,85] are other tracers, which have been studied as potential agents for the DAT imaging. A demethylated analogue of β-CIT, nor-β-CIT (2β-carbomethoxy-3β-(4-iodophenyl)nortropane), was developed for SERT imaging.[86,87] However, studies in rats have shown that although *in vitro* nor-β-CIT has higher affinity and selectivity to SERT, *in vivo* it has lower binding potential and slower kinetics than β-CIT.[88]

2.2. Synthesis of phenyltropanes

2.2.1. Enantioselective synthesis of phenyltropanes

An enantioselective route for the synthesis of phenyltropanes was developed by Clarke *et al.* in 1973 (Scheme 1).[14] The compounds were prepared from anhydroecgonine methyl ester (**8**), which was synthesized from (-)-cocaine in three steps: 1) ester hydrolysis, 2) dehydration and 3) esterification (Scheme 1). The phenyl group was introduced by Michaels reaction with PhMgBr to anhydroecgonine methyl ester at –20°C. The reaction produced a mixture of products with 2β- and 2α-carboxylates (**9**, **10**) being present in a ratio of 1:3. The synthetic procedure was improved by Carroll *et al.* and Meltzer *et al*,[32,34] who performed the reaction at –40°C. The ratio of the 2β- and 2α-products (**9**, **10**) was

1.6:1. The yield of the 2β-product was further increased by Xu *et al.* in 1996.[89] The reaction was carried out in CH_2Cl_2, yielding the products in the ratio of 5:1.

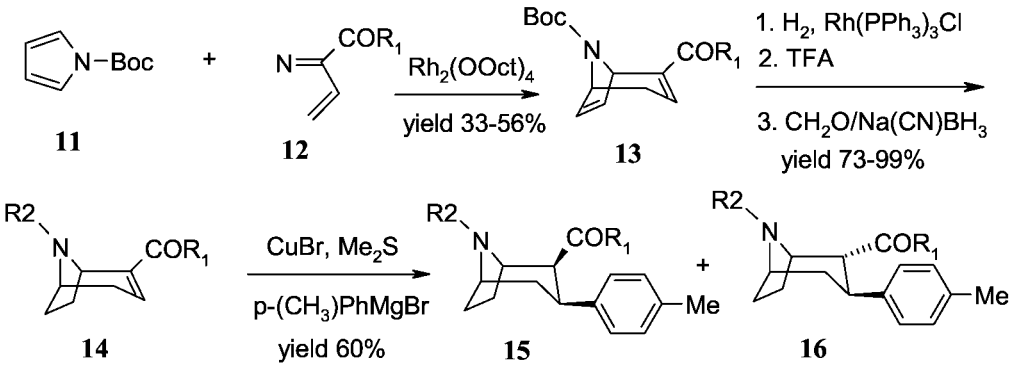


Scheme 1. Enantioselective synthesis of phenyltropanes from (-)-cocaine.[14, 32, 34]

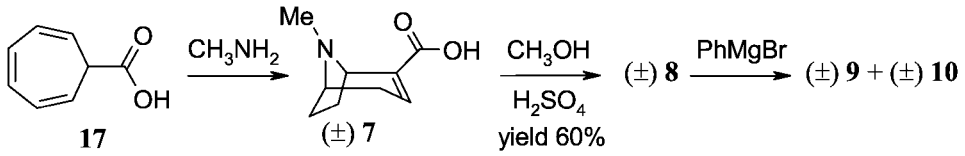
2.2.2. Synthesis of racemic phenyltropanes

The enantioselective synthesis of phenyltropanes is extremely feasible in practise, if modifications are performed in the aromatic ring. Furthermore, modifications at N- and 2-positions are easily achievable. Therefore, only a couple of routes leading to racemic mixtures have been developed. The synthesis of phenyltropanes by a tandem cyclopropanation/Cope rearrangement was presented by Davies *et al.* in 1991 (Scheme 2).[90,91] This method is based on the reaction between pyrroles (**11**) and rhodium stabilized vinylcarbenoids (**12**). However, the reaction leads easily to formation of side products.

Another approach to racemic synthesis of phenyltropanes is the reaction between 2,4,6-cycloheptatriene-7-carboxylic acid (**17**) and methylamine,[92] following the procedure of Grundmann *et al.* (Scheme 3).[93]



Scheme 2. Synthesis of racemic phenyltropanes from pyrroles and vinylcarbenoids.[90, 91]



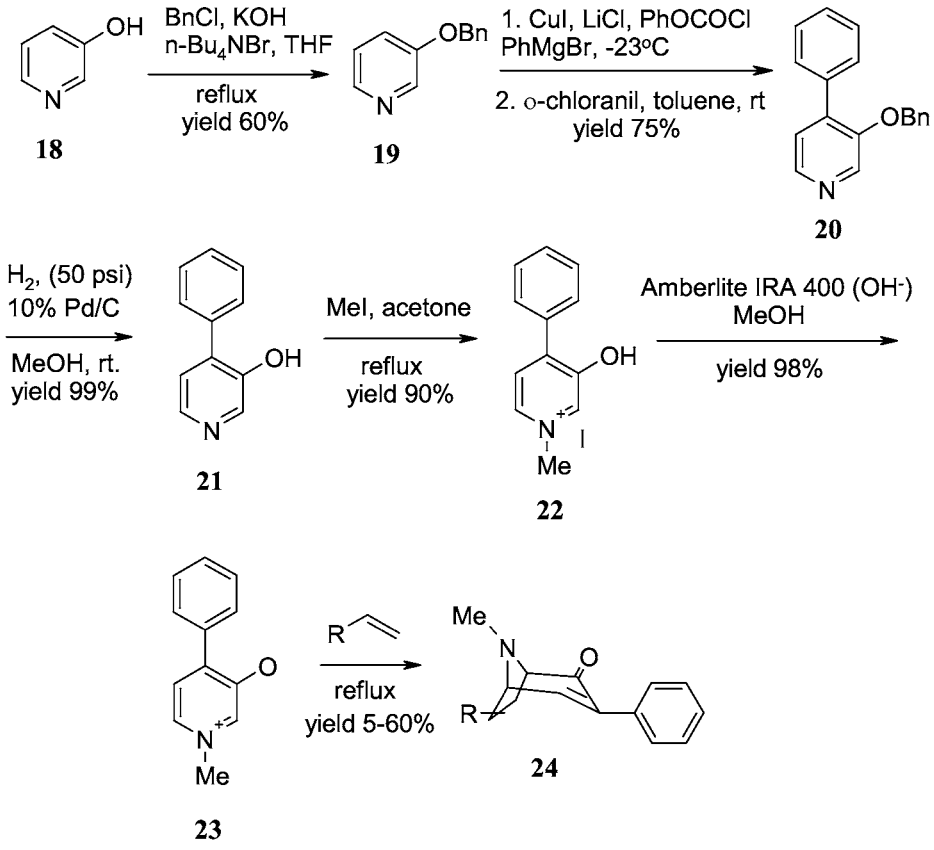
Scheme 3. Synthesis of racemic phenyltropanes from 2,4,6-cycloheptatriene-7-carboxylic acid.[91]

2.2.3. Synthesis of 6/7-substituted phenyltropanes

At the moment, there is no direct procedure for preparing 6/7-substituted phenyltropanes starting from (-)-cocaine. Therefore alternative routes have been developed. There are two main strategies for achieving the 6/7-substituted phenyltropane structures; a strategy exploiting pyridinium betaine-based dipolar cycloaddition and a method based on a Mannich type of condensation.

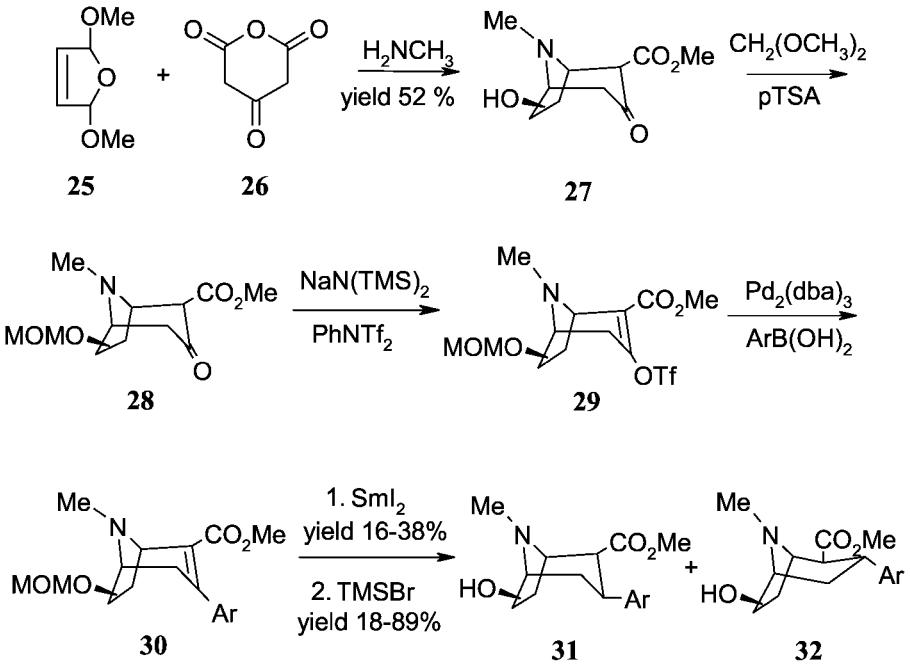
A synthesis of 6/7-substituted tropanes by 1,3-dipolar cycloaddition was introduced by Takahashi *et al.* in 1989[94,95] and the route was applied to the synthesis of a natural antiglaucoma compound, i.e. Bao Gong Teng A and its analogues.[96-101] Kozikowski *et al.* adapted the procedure for the synthesis of 6/7-substituted phenyltropenones (**24**) (Scheme

4),[102] and this was then further used in the preparation of starting materials for the synthesis of 6/7-substituted 2-alkyl-3-phenyltropanes.[103,104]



Scheme 4. Synthesis of racemic 6/7-substituted phenyltropenones by 1,3-dipolar cycloaddition.[102]

A synthetic route to tropanes via the Mannich type of condensation was developed by Willstätter *et al.* as early as 1923.[105] Originally, the route was developed for the synthesis of natural cocaine, and it was adapted for the synthesis of 6/7-substituted cocaines.[50, 106-108] The route was further modified for the synthesis of 6- or 7-hydroxy/methoxy phenyltropanes (**31**) (Scheme 5).[8, 51, 109-111]



Scheme 5. Racemic synthesis of 6/7-hydroxy phenyltropanes via the Mannich type of condensation.[8]

3. AIMS OF THE STUDY

The general purpose of the study was to develop methods for the preparation of new phenyltropane derivatives suitable for brain imaging as diagnostic radiopharmaceuticals.

The specific aims were:

1. To study substituent effects on tropane ring conformation

2. To develop synthetic methods for the preparation of 6/7-substituted phenyltropanes and to perform the initial radiosynthesis of one such compound

3. To examine the influence of 6/7-substitution on biological activity with *in vitro* and *in vivo* experiments

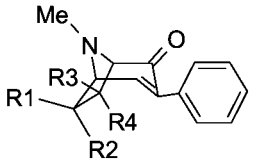
4. EXPERIMENTAL

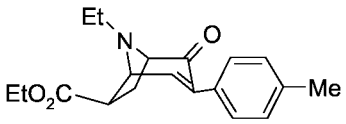
4.1. Syntheses and identification of the products

All moisture sensitive syntheses were performed under a nitrogen atmosphere with oven-dried glassware. [II - IV] In addition, solvents were dried, distilled and stored under a nitrogen atmosphere. The starting materials were purchased from commercial suppliers unless otherwise mentioned. Ecgonine[I] (**6**), β-CIT[VI] and nor-β-CIT[I] were synthesized according to Swahn *et al.* 1996 with some minor modifications.[112] Column chromatography was carried out on Kieselgel 60.[II-IV] HPLC chromatography was performed with a semi-preparative μBondapak® C18 column (Waters). ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AM 400 WB [I, II] or a Bruker Avance 500 MHz [II-IV] spectrometer in $CDCl_3$ using TMS as the internal reference.

4.1.1. Synthesis of starting materials

Starting materials not available from commercial sources were prepared by a dipolar cycloaddition from substituted pyridiumolates[II, III] and by a Mannich type of condensation from trisubstituted furans.[VI]



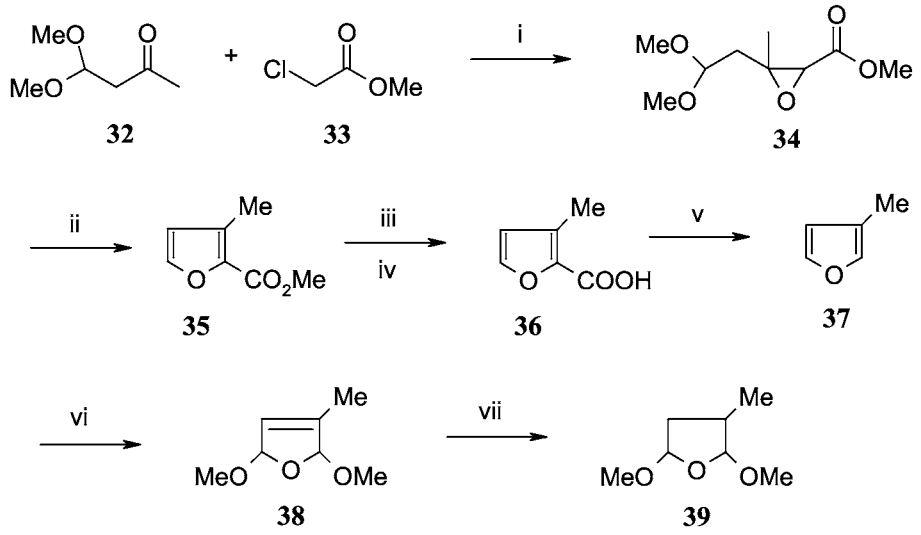


24a; R1= -CO_2Et, R2= -H, R3 = -H, R4 = -H
24b; R1= -H, R2= -H, R3 = -CO_2Et, R4 = -H
24c; R1= -H, R2= -CO_2Et, R3 = -H, R4 = -H
24d; R1= -H, R2= -H, R3 = -H, R4 = -CO_2Et

24e

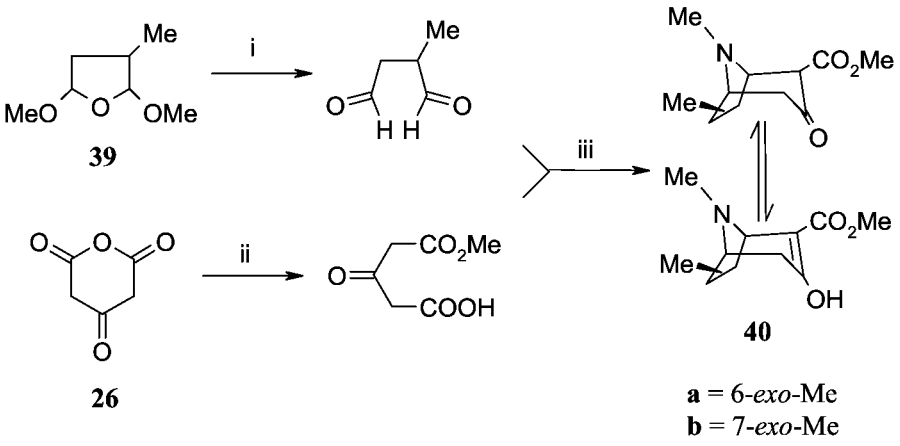
Synthesis of **24a-d** by dipolar cycloaddition was performed according to the method developed by Kozikowski *et al.* (Scheme 4, see page 20),[102] using ethylacrylate as a dienophile. Starting material **24e** was prepared by using tolylmagnesium bromide and ethyliodide instead of phenylmagnesiumbromide and methyliodide, respectively.

Trisubstituted furanes (**39**) for the Mannich type of condensation were prepared by a five-step procedure starting from Darzens condensation of 4,4-dimethoxy-2-butanone (**32**) and methyl chloroacetate (**33**) (Scheme 6).[113-115]



Scheme 6. i) $NaOCH_3$, 3h, -10°C, overnight, rt., yield 98%; ii) 160 °C, overnight, 84%; iii) 5 M NaOH, 2h, reflux; iv) conc. HCl, 15 min., 95%; v) Cu powder, 210 °C, 2-3h, 75%; vi) Br_2, Na_2CO_3, MeOH, -40 °C, 1h, 70%; vii) Raney-Ni, H_2, 3 d, 61%.

The Mannich reaction between hydrolyzed trisubstituted furane (**39**) and β-ketoglutaric anhydride (**19**) gave racemic 6/7-methylsubstituted tropinones **40a** and **40b** after purification steps with yields of 26% and 21%, respectively (Scheme 7).



Scheme 7. i) 0.1 M HCl, 75°C, 1h; ii) MeOH, rt, 1h; iii) $MeNH_2$-HCl, NaOH, citrate buffer, rt., 3d, yield 21-26%.

4.2. Spectral analyses[I, III]

For the complete ^1H spectral analyses, the samples (5- 20 mM) were filtered and degassed using the freeze-pump-thaw technique.[I] For the compounds **41-45** and **47**, the samples were not degassed, because resolution was high enough without degassing.[III] The ^1H NMR spectra were measured at 303 K with a Bruker AM 400 WB-spectrometer[I] or with a Bruker Avance 500 MHz spectrometer at 298 K[III] in $CDCl_3$, unless otherwise mentioned, by using TMS as the internal reference. Preparation of the spectra for analysis and the analysis itself were made with PERCHit software.[116] FIDs were multiplied with sin*exp window function, Fourier transformed and base line corrected. The spectra were solved first by the integral transform method, after which the solutions were refined by the total-line shape procedure. A detailed long-range coupling analysis was performed only for β-CPT (**9**).[I] The trial signs of the couplings were adapted from the analysis of tropinone[117] (this analysis also yields, due to symmetry, the relative signs of the couplings) or presumed on the basis of general rules. The effects of the signs were then tested by total-line-shape fitting. For compounds **41-45** and **47** the signs of the couplings were ignored, except in the geminal couplings and in the cases where the sign was essential for a good fit

between the calculated and the measured spectra.[III] Dihedral angles were calculated with the Haasnoot[118] and Altona[119] equations using the graphical interface of the PERCHit software.

4.3. Computational methods

MO calculations[I] for α-CPT (**10**), β-CPT (**9**), nor-β-CIT, (-)-cocaine (**2**) and ecgonine (**6**) were performed at the semi-empirical AM1[120] and *ab initio* HF/6-31G* and HF/3-21G levels[121] (used for nor-β-CIT, since 6-31G* parameterisation for iodine is missing) employing the AMPAC (QCPE No. 506, ver. 2.1) and GAUSSIAN94 (version RevD.3) program packages[121] running on an IBM RISC/6000 320 workstation. All the geometric variables were completely optimised for each compound. Molecular dynamic calculations[I] for α-CPT (**10**), β-CPT (**9**), nor-β-CIT, (-)-cocaine (**2**) and ecgonine (**6**) and geometric optimisation[III]with molecular mechanics using MM+ force field for products **41-45** and **47** were calculated by HyperChem™ software.

4.4. X-ray crystallography [II-III]

X-ray structures were determined for compounds **40b**, **51b**, **42**, **44a** and **50a.** X-ray diffraction data were collected with a Nonius Kappa-CCD diffractometer using Mo-Kα radiation (λ= 0.71073 Å). Denzo and Scalepack programs were used for cell refinement and data reduction.[122] The structure was solved by direct methods with SHELXS 97.[123] Structure refinement was carried out with SHELXL 97.[123] All hydrogen atoms were placed on idealized positions except for hydroxyl hydrogens which were positioned from Fourier difference maps and refined with fixed thermal parameters.

4.5. Mass spectroscopy[III, IV]

Accurate mass measurements were made using a Fourier transform ion cyclotron resonance mass spectrometer, Bruker BioApex 47e (Bruker Daltonics, Billerica, USA), equipped with a 4.7 Tesla superconducting magnet, Infinity™ cell and interfaced to an electrospray ionization source. The measurements were made in the broadband mode with external calibration. The sample solution was continuously introduced into the interface sprayer by a syringe infusion pump at a flow rate of 50 μl/h under atmospheric pressure. The compounds were first dissolved into methanol at a concentration of 2 mg ml^{-1} and 2 μl of these solutions were diluted to 1 ml with methanol:acetic acid solution (100:1).

4.6. Pharmacological evaluation[VI]

All animals that were used for *in vitro* and *in vivo* experiments were from the National Laboratory Animal Center, Kuopio, Finland. All experiments were performed according to European Community guidelines for the use of experimental animals and reviewed by the Animal Ethics Committee of the University of Kuopio and approved by the local Provincial Government.

4.6.1. In vitro-evaluation of the products

The affinities (IC_{50}) for the dopamine (DAT), serotonin (SERT) and norepinephrine (NET) transporters were determined by *in vitro* competition studies using [^{3}H]-GBR-12935, [^{3}H]-paroxetine and [^{3}H]-nisoxetine, respectively, as radioligands and Kuo-Wistar rat brain regions from striatum, the combined amygdala and hippocampus regions, and cerebral cortex, respectively.[124-127] For comparison purposes, the affinity of (-)-cocaine and β-CIT were also measured. Competition studies were performed at a fixed concentration of the radioligand and a fixed range of four concentrations for the test compounds. The compounds were analyzed as racemates.

4.6.2. In vivo-evaluation of the products

The locomotor activity measurements of the test compounds in the form of water soluble hydrochlorides were performed in mice. Hydrochlorides of (-)-cocaine and β-CIT were also tested as reference compounds, in addition to physiological saline solution as a control. Injections were given intraperitoneally (i.p.) and the mice were monitored during a period of 360 minutes at 10 min intervals. Due to its short duration of action, (-)-cocaine was only monitored for 240 min. The total locomotor activity was measured by recording the movements of the heat radiation source (i.e. the mouse). Data was represented as mobility (0 to 100) during each mobility integration time (10 min). The area underneath the mobility curve (AUC) was used to estimate the effect of the test compounds ($AUC_{0\text{-}300\ min}$) on locomotor activity. The statistical significance of any effect was tested using one-way ANOVA followed by Bonferroni's modification of the Newman-Keuls test.

5. RESULTS AND DISCUSSION

5.1. Synthesis

The structures of the compounds prepared during this work are shown in Tables 2-5. All the compounds in the Tables 2, 3 and 5 were synthesized from the 6/7-carboethoxy-3-aryl-trop-3-en-2-ones (**24a-e**) and also part of the compounds shown in Table 4. Compounds **48-49** in the Table 4 were synthesized from the 6/7-methyl-2-carbomethoxytropin-3-ones (**40a-b**).

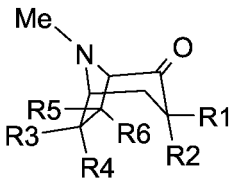


Table 2.

Comp	R1	R2	R3	R4	R5	R6	Yield
41a[a]	Ph	H	CO_2Et	H	H	H	99%
41b[a]	Ph	H	H	H	CO_2Et	H	99%
41c[a]	Ph	H	H	CO_2Et	H	H	22%
41d[a]	Ph	H	H	H	H	CO_2Et	99%
42[a, b]	OH	Ph	H	CO_2Et	H	H	12%

[a]High resolution mass measured; [b]X-ray structure determined

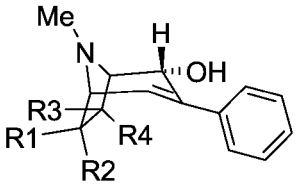


Table 3.

Compound	R1	R2	R3	R4	Yield
43a[a, b]	CO_2Et	H	H	H	24%
43b[a, b]	H	H	CO_2Et	H	32%
43c[a, b]	H	CO_2Et	H	H	38%

[a]*In vitro* affinity determined; [b]High resolution mass measured

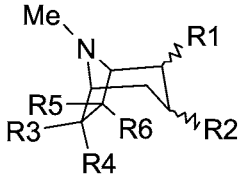


Table 4.

Compound	R1	C2	R2	C3	R3	R4	R5	R6	Yield
44a[a, b]	OH	β	Ph	β	CO_2Et	H	H	H	35%
44b[a]	OH	β	Ph	β	H	H	CO_2Et	H	48%
44c[c]	OH	β	Ph-*p*-Me	β	CO_2Et	H	H	H	61%
45a[a]	OH	α	Ph	β	CO_2Et	H	H	H	99%
45b[a]	OH	α	Ph	β	H	H	CO_2Et	H	99%
45c	OH	α	Ph	β	H	CO_2Et	H	H	58%
46a	OCOMe	β	Ph	β	CO_2Et	H	H	H	99%[d]
46b[e]	OCOMe	β	Ph	β	H	H	CO_2Et	H	85%[d]
46c[c, e]	OCOMe	β	Ph-*p*-Me	β	CO_2Et	H	H	H	15%[d]
46d[e]	OCOMe	β	Ph-*p*-I	β	CO_2Et	H	H	H	30%[d]
46e[e]	OCOMe	β	Ph-*p*-I	β	H	H	CO_2Et	H	33%[d]
47[a]	OH	α	Ph	α	H	CO_2Et	H	H	62%
48a[a, e]	CO_2Me	β	Ph	β	Me	H	H	H	41%[d]
48b[a, e]	CO_2Me	β	Ph	β	H	H	Me	H	31%[d]
49a[a, e]	CO_2Me	β	Ph-*p*-I	β	Me	H	H	H	64%[d]
49b[a, e]	CO_2Me	β	Ph-*p*-I	β	H	H	Me	H	48%[d]
49c[a, e, f]	CO_2Me	β	Ph-*p*-I	β	Me	H	H	H	19%[d]
49d[a]	CO_2Me	β	Ph-*p*-$SnMe_3$	β	Me	H	H	H	51%[d]
49e	CO_2Me	β	Ph-*p*-I^{125}	β	Me	H	H	H	34%[d]

[a]High resolution mass measured; [b]X-ray structure determined; [c]Ethyl at N-position; [d]Yield of the last step; [e]*In vitro* affinity determined; [f]Hydrogen at N-position.

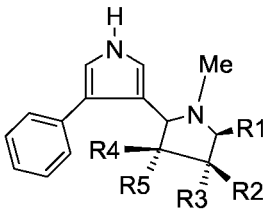


Table 5.

Compound	R1	R2	R3	R4	R5	Yield
50a[a, b]	CO_2Et	H	H	H	CO_2Et	62%
50b[a]	CO_2Et	H	CO_2Et	H	H	84%
50c[a]	CO_2Et	H	H	CO_2Et	H	32%
50d[a]	CO_2Et	CO_2Et	H	H	H	98%
50e	CO_2H	H	H	H	CO_2Et	6%

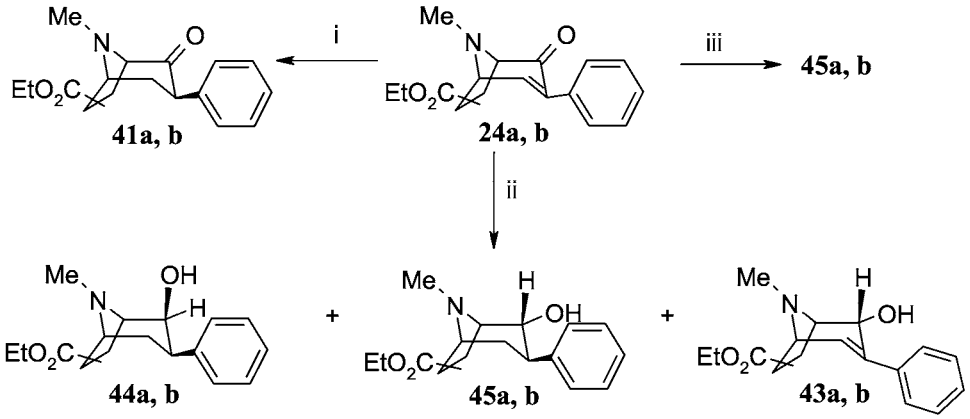
[a]*In vitro* affinity determined; [b]X-ray structure determined.

5.1.1. 6/7-Carboethoxy phenyltropanes[III]

6/7-*exo*-Substituted 3-phenyltrop-3-en-2-ones **24a** and **24b** prepared by 1,3-dipolar cycloaddition, as described on page 20, were selectively reduced to the corresponding 3β-phenyltropin-2-ones (**41a, 41b**) by catalytic hydrogenation with 5% Pd/C at room temperature for 25 minutes with quantitative yields (Table 1, Scheme 8). At elevated temperature and with a longer reaction time (17 h), the 2α-hydroxy-3β-phenyltropanes **45a** and **45b** were obtained also quantitatively (Table 3). Reduction with $NaBH_4$ gave the corresponding 2β-hydroxy-3β-phenyl analogues **44a** and **44b** with yields of 35% and 48%, respectively. In addition, two side products were formed; the 2α-hydroxyl analogue (**45a**, **45b**) and an unsaturated intermediate (**43a**, **43b**), with yields of 20-27% and 24-32%, respectively (Table 2). Compound **44c** was prepared from **31e** by $NaBH_4$ reduction with a yield of 61%.

Steric hindrance of 6-*endo*-substitution made reductions of tropinone (**24c**) more difficult and no selectivity was observed (Scheme 9). Catalytic hydrogenation for 17 hours with Pd/C gave a mixture of 3β-phenyltropin-2-one (**41c**) and a 2α-hydroxy-3β-phenyl analogue (**45c**), with yields of 22% and 58%, respectively. With a shorter reduction time

(1h) the tropinone (**41c**) was formed (yield 12%), together with an unexpected side product 3α-hydroxy-3β-phenyltropinone (**42**)(yield 20%). The side product **42** was formed only under these conditions and only for the 6-*endo*-substituted tropinone. The steric effect of the 6-*endo*-substitution was most obvious in $NaBH_4$ reduction, which gave the 2α-hydroxy-3α-phenyl analogue (**47**) as the main product with yield of 62%. The result differs from those of *exo* –substituted analogues. However, the unsaturated 2α-hydroxyl analogue (**43c**) was also formed with a yield of 38%.

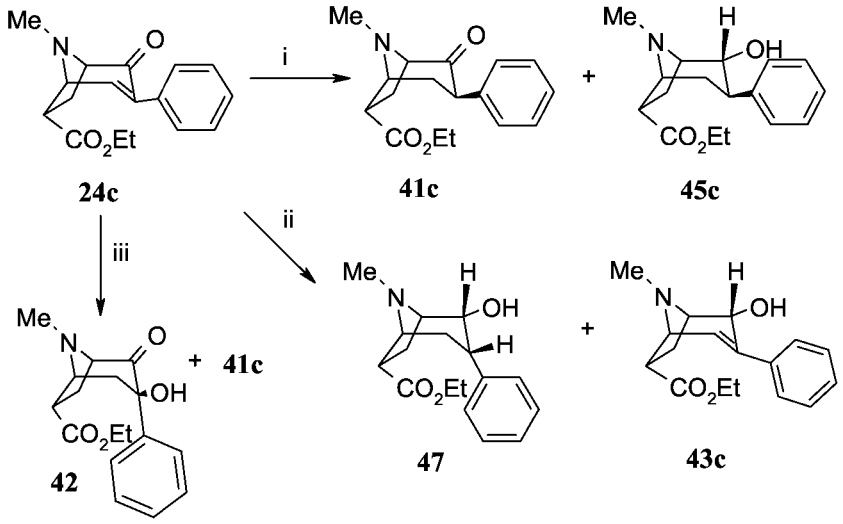


Scheme 8. i) 5% Pd/C, H_2, MeOH, rt., 25 min, yield 99%; ii) $NaBH_4$, MeOH, -30°C, 4 h, yield 24–48%; iii) 5% Pd/C, H_2, MeOH, 52 °C, 17 h, yield 99%. **a** = 6-*exo*-CO_2Et, **b** = 7-*exo*-CO_2Et.

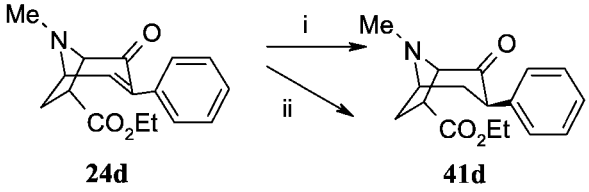
The reduction of unsaturated 7-*endo*-substituted tropinones (**24d**) produced only 3β-phenyltropinones (**41d**) with the both reducing agents (Scheme 10). Clearly, the 7-*endo*-substituent had induced such strong steric hindrance to the C(2) carbonyl group that it prevented the reduction in hydrogenation. The yield of the tropinone in $NaBH_4$ reduction was low due to degradation.

6/7-Substituted phenyltropanes with reverse ester functionality at C(2) compared to cocaine analogues (**46a-c**) were synthesized by acetic acid anhydride (Scheme 11). Yields were good for **46a** and **46b**, 99% and 85%, but clearly the larger ethyl group at nitrogen interfered with the esterification of **44c** (yield 15%). Products **46d** and **46e** were prepared

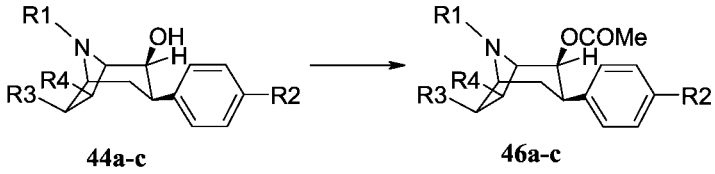
by direct iodination in a mixture of acetic acid and perchloric acid containing mercuric oxide, with yields of 30% and 33%, respectively (Scheme 12).



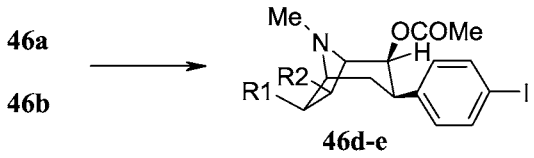
Scheme 9. i) 5% Pd/C, H_2, MeOH, 52 °C, 17 h, yield 22%; ii) $NaBH_4$, MeOH, -30°C, 4 h, 38-62%; iii) 5% Pd/C, H_2, MeOH, 52 °C, 1 h, yield 12-20%.



Scheme 10. i) 5% Pd/C, H_2, MeOH, 52 °C, 17 h, yield 99%; ii) $NaBH_4$, MeOH, -30°C, 4 h, yield 52%.



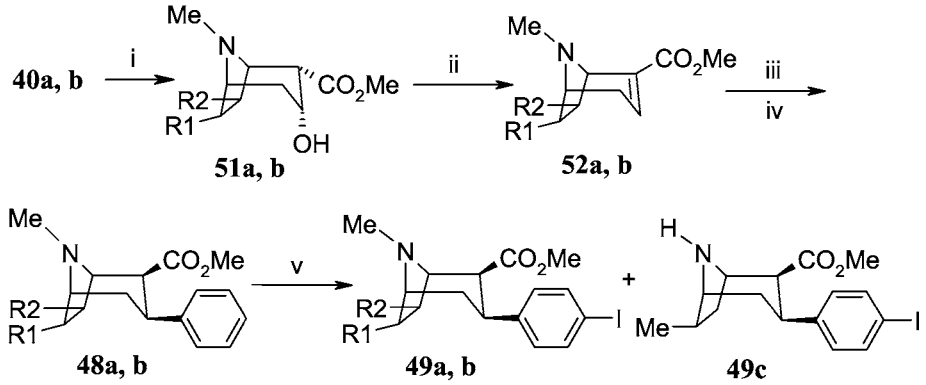
Scheme 11. Reagents and reaction conditions: acetic acid anhydride, pyridine, reflux, 4,5 h, yield 15-99%. **a**: R1= -Me, R2= -H, R3= -CO_2Et, R4= -H; **b**: R1= -Me, R2= -H, R3= -H, R4= -CO_2Et; **c**: R1= -Et, R2= -Me, R3= -CO_2Et, R4= -H



Scheme 12. Reagents and reaction conditions: HgO, I, $HClO_4$, AcOH, CH_2Cl_2, rt., overnight, yield 30-33%. **d**: R1= -CO_2Et, R2= -H; **e**: R1= -H , R2= -CO_2Et

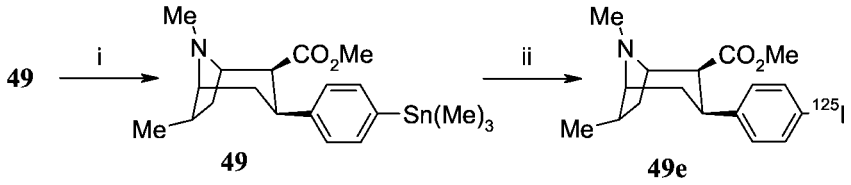
5.1.2. 6/7-Methyl phenyltropanes[VI] *and radiosynthesis of [125-I]-6-Me-β-CIT*

Tropinols **51a** (82%) and **51b** (88%) were prepared from the Mannich products **40a** and **40b** by reduction with $NaBH_4$ and converted to **52a** (38%) and **52b** (48%) by anti-elimination of water (Scheme 2). The phenyl ring was coupled to the double bond by Michael addition at -40°C under a nitrogen atmosphere, leading to the corresponding 2β, 3β-configuration of **48a** (41%) and **48b** (31%). If the reaction temperature was higher, also the Grignard product was observed. Compounds **49a** and **49b** were prepared by the direct iodination with yields of 64% and 48%, respectively. Surprisingly, the reaction also yielded the demethylated analogue **49c**, but only for the 6-*exo*-isomer (yield 19%).



Scheme 13. Synthetic route to the 6/7-methyl-3-phenyltropanes. Reagents and conditions: i) $NaBH_4$, -30°C, MeOH, 3h, yield 82-88%; ii) $POCl_3$, pyridine, reflux, 1h, yield 38-48%; iii) PhMgBr, -40°C, ether, 4h; iv) –70°C, TFA, yield 31-41%; v) HgO, I, $HClO_4$, AcOH, CH_2Cl_2, rt., overnight, yield 19-64%. For all products; **a**: R1 = -Me, R2 = -H, **b**: R1 = -H, R2 = -Me.

Compound **49a** was stannylated with hexamethylditin, using Pd-tetrakis-triphenylphosphine as the catalyst with a yield of 51% (Scheme 2.). The stannylated precursor (**49d**) was iodinated (I-125) with the chloramin-T-method to give **49e** with a yield of 34%.



Scheme 14. i) Hexamethylditin, Tetrakistriphenylphosphine-Pd, Toluene, reflux, yield 51%; ii) $Na^{125}I$, chloramine-T, yield 34%.

5.1.3. Disubstituted pyrroles[II]

During attempts to modify C(2) carbonyl moiety of tropinones (**24a-d**), a synthetically very intriguing and selective one-pot route to 3,4-disubstituted pyrroles was developed. The synthesis of 3,4-disubstituted pyrroles is known to be laborious due to their propensity to react in electrophilic substitution reactions to the more electronically available α-positions of the ring.[128] In the literature, multistep synthetic routes of 3,4-disubstituted pyrroles have been reported.[129-134] However, these synthetic routes are often complicated and limited only to some substituent families. In our studies, the reaction between TosMIC,[135] the tropinones (**24a-d**) and EtONa produced 3-phenyl-4-pyrrolidinepyrroles (**50a-d**) in EtOH, at room temperature and dry conditions, with yields varying from high to moderate (98%- 32%). We did not observe any side reactions. Even more interestingly, if some moisture was present, the proline analogue **50e** was also formed. According to ^{1}H-NMR, the yield was moderate, 50%, but unfortunately, after purification a major loss occurred, leading to a poor final yield of only 6%. Apparently the main reason for the loss was the zwitterion character of the product.

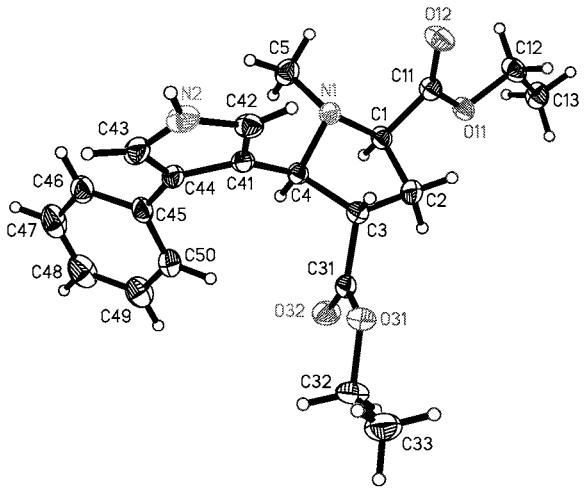


Figure 2. ORTEP representation of the X-ray crystal structure of **50a**.

The expected reaction mechanism explains the fragmentation of the tropane ring and the regiochemistry (Figure 3). The reaction is started by the 1,3-dipolar cycloaddition of the base activated nucleophilic TosMIC to the double bond, which is a Michael acceptor. After protonation, the attack of nucleophilic EtO^- to the polarized carbonyl C-2 and the leaving tendency of the tosyl group initiates an electron transfer reaction as a variation of the Grob fragmentation.[136] Bond cleavage between carbons 2 and 3 generates the pyrrole ring as a stable end product, with a phenyl ring and the disubstituted N-methylproline analogue as substituents. According to the mechanism, the reaction does not affect the chirality centers. The mechanism for the formation of the **50e** is analogous, except that the carbonyl attacking nucleophile is a hydroxyl ion.

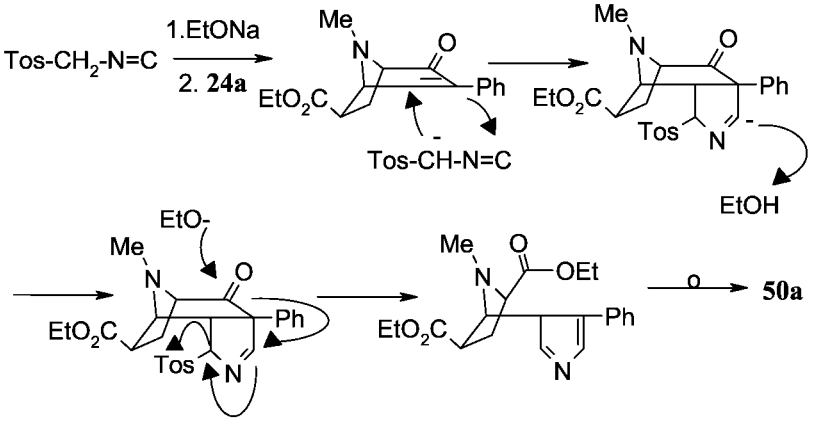


Figure 3. Expected reaction mechanism

5.2. Conformational studies

Complete ^{1}H spectral analysis was performed for α-CPT (**10**), β-CPT (**9**), nor-β-CIT, (-)-cocaine (**2**), cocaine-HCl, ecgonine-HCl (**6**) and for the products **41-45** and **47**. The most important couplings for the conformational analyses are listed in Table 6. The conformation of the tropane ring was determined based on the data of the dihedral angles from the Altona and Haasnoot equations.

5.2.1. Computational and NMR spectroscopic study of ecgonine, cocaine and some phenyltropanes[I]

The dihedral angles of α-CPT (**10**), β-CPT (**9**), nor-β-CIT, (-)-cocaine (**2**), cocaine-HCl, ecgonine-HCl (**6**) obtained by the Altona and Haasnoot equations, and angles calculated by *ab initio* HF/6-31G*-method are compared in Figure 4 and Table 7 (for cocaine-HCl only dihedral angles from empirical equations are given).

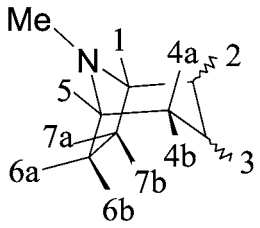


Table 6. Conformationally and configurationally the most important coupling constants (Hz) of α-CPT (**10**), β-CPT (**9**), nor-β-CIT, (-)-cocaine (**2**), ecgonine-HCl (**6**), **41-45** and **47**.[a,b]

Com.	^{3}J(1,2)	^{3}J(2,3)	^{4}J(2,4a)	^{4}J(2,4b)	^{3}J(3,4a)	^{3}J(3,4b)	^{4}J(3,5)	^{3}J(4a,5)	^{3}J(4b,5)	Conf[d]
44a	4.04	3.19	0.08	1.04	13.01	5.36	0.09	2.96	3.19	Chair[e]
44b	4.08	3.10	0.40	1.06	12.99	5.50	0.83	3.11	3.09	Chair
9	3.33	5.17	-0.56	1.32	12.90	4.87	-0.71	3.05	3.34	Chair
nor-β-CIT	2.24	5.84	-0.46	0.91	12.85	5.07	-0.81	3.13	2.56	Chair
2	3.44	5.82	-0.33	1.27	11.75	6.03	-0.57	3.08	3.33	Chair
2c	2.60	7.28	-0.57	0.98	11.77	6.25	-0.59	2.91	3.38	Chair
6c	2.58	7.05	-0.32	0.48	11.45	6.08	-0.48	3.03	3.18	Chair
45a	3.67	9.83	0.69	0.46	12.36	6.02	0.65	2.97	2.96	Chair
45b	3.77	9.77	0.14	0.03	12.57	5.97	0.59	3.05	2.83	Chair
45c	3.59	9.93	0.21	0.47	12.65	6.17	0.32	3.23	2.58	Chair
10	2.78	11.71	-0.36	-0.50	12.43	5.87	-0.60	3.02	2.95	Chair
47	8.58	5.71	1.51	0.57	6.88	12.90	0.88	9.81	2.04	Boat[e]
41a	-	-	-	-	11.92	8.02	0.94	3.63	2.23	Chair
41b	-	-	-	-	11.70	8.08	0.58	3.71	2.10	Chair
41c	-	-	-	-	11.67	8.27	0.75	3.97	1.98	Chair
41d	-	-	-	-	11.73	8.16	-0.71	3.57	2.29	Chair
43a	5.13	-	1.03	-	-	-	-	5.50	-	Half-Chair
43b	5.27	-	1.00	-	-	-	-	5.67	-	Half-Chair
43c	5.20	-	1.11	-	-	-	-	5.49	-	Half-Chair
42	-	-	-	-	-	-	-	9.40	0.94	Boat[e]

[a]The spectral analyses were performed by PERCHit software using the total line shape method. [b]The rms value (in% of the NH_3 signal = 100%) was from 0.47 to 1.79%. Standard deviations of the couplings were from 0.01 to 0.10 Hz. [c]HCl-salt. [d]Main conformation based on the ^{1}H NMR spectra. [e]Solid form configuration determined by X-ray crystallography.

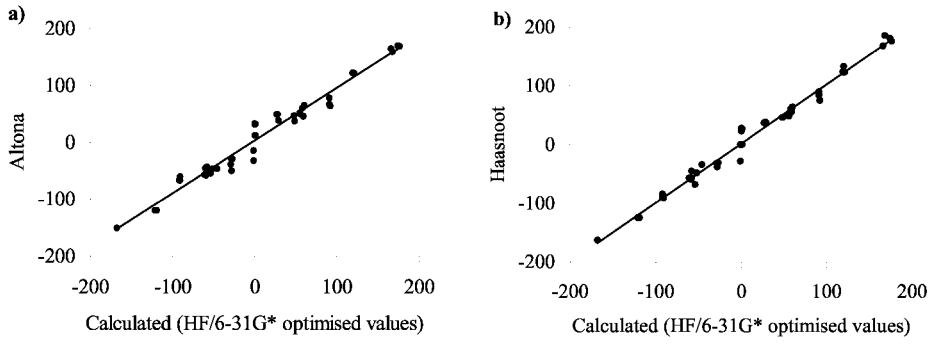


Figure 4. Dihedral angles obtained by the Altona and Haasnoot equations vs. HF/6-31G* optimised angles.

In general, the fits are good: standard deviations are 6.6° and 11.9° (r = 0.994, r = 0.984) for the Haasnoot and Altona equations, respectively, but the fits are poor when the absolute value of the dihedral angle is less than 30°. This observation can be explained by the nature of the coupling function: the value of the function is not sensitive to the dihedral angle around 0°. Also the couplings for the protons in the vicinity of the substituents give angles deviating clearly from the calculated values and from each other in different compounds (for example angles 1- 2); the finding implies that the empirical equations fail to predict small conformational variations between compounds with different substituents.

Comparison of dihedral angles indicates that the substituents do not perturb to any significant manner the remote parts of the tropane ring system. Variations in the ring are rather small with other compounds, except ecgonine. Some bending may be observed in the tropane ring chair angle, i.e. the angle between planes C(2)-C(3)-C(4) and C(1)-C(5)-C(2)-C(4) on the C(7)C(1)C(2) moiety, especially for ecgonine and nor-β-CIT. The clear bending of ecgonine is obviously due to the effect of a hydrogen bond type interaction between the hydroxyl and carboxylic acid groups. The lack of a methyl group on the nitrogen enables the slight bending of nor-β-CIT, but the direction is opposite to ecgonine. The (6)-C(7)-bridge is highly symmetrical for all of the compounds. However, the molecular dynamics calculations indicate that the bridge can make surprisingly large movements. One conformationally important observation is that the values of $^{3}J(3,4a)$ and

$^3J(3,4b)$ are close to the values predicted by the equations: which means that there is virtually no other conformation present in the system, at least not within a free energy of 9 -10 kJ/mol (assuming $N_{boat} / N_{chair} = e^{-\Delta G/RT}$).

Table 7. Dihedral angles (in deg.) calculated by the HF/6-31G*method and the Altona and Haasnoot equations.

Dihedral $Angle_{HH}$	α-CPT (**10**)[a]			β-CPT (**9**)[a]			Nor-β-CIT[a]		
	HF/6-31G	Altona	Haas-noot	HF/6-31G	Altona	Haas-noot	HF/6-31G	Altona	Haas-noot
1-2	60	65	64	-59	-58	-59	-54	-54	-68
1-7a	-28	-50	-38	-28	-30	-33	-29	-39	-32
1-7b	91	67	90	91	66	85	91	78	84
2-3	-168	-151	-163	-52	-47	-48	-58	-43	-45
3-4a	166	165	168	174	170	181	176	169	176
3-4b	48	47	46	55	51	50	56	50	48
4a-5	-58	-46	-57	-59	-46	-57	-61	-56	-57
4b-5	59	46	57	59	45	55	58	60	60
5-6a	27	49	37	28	49	38	29	39	38
5-6b	-92	-67	-90	-91	-67	-90	-91	-60	-91
6a-7a	1	12	0	-1	14	0	0	12	0
6a-7b	-120	-120	-124	-120	-120	-125	-121	-120	-125
6b-7a	120	122	133	119	122	124	120	121	123
6b-7b	0	31	23	-1	32	28	0	33	28
Dihedral $Angle_{HH}$	Ecgonine (**6**)[b]			Cocaine (**2**)[a]			Cocaine (**2**)-HCl[b]		
	HF/6-31G	Altona	Haas-noot	HF/6-31G	Altona	Haas-noot	HF/6-31G[c]	Altona	Haas-noot
1-2	-66	-55	-64	-59	-58	-58	-	-55	-64
1-7a	-27	-35	-31	-27	-29	-31	-	-35	-30
1-7b	92	78	79	92	64	75	-	77	77
2-3	-38	-32	-25	-46	-46	-34	-	-38	-25
3-4a	164	160	182	168	159	186	-	158	186
3-4b	47	48	47	49	37	46	-	36	45
4a-5	-60	-52	-57	-60	-45	-57	-	-52	-58
4b-5	59	52	56	59	45	55	-	51	55
5-6a	28	42	32	27	49	37	-	42	35
5-6b	-92	-58	-82	-92	-66	-84	-	-60	-90
6a-7a	0	15	0	1	12	0	-	15	0
6a-7b	-120	-120	-123	-119	-120	-125	-	-120	-125
6b-7a	120	122	124	121	121	123	-	123	124
6b-7b	0	28	22	1	32	27	-	28	22

[a] Angles from Altona and Haasnoot equations calculated in $CDCl_3$. [b] Angles from Altona and Haasnoot equations calculated in D_2O. [c] *Ab initio* HF/6-31G* data not available.

The substituent and the solvent effects are rather large for the HCl salts, which can be particularly well observed from geminal couplings. Protonation also has conformational effects on the ring. The negative chloride-ion at the positively charged nitrogen causes repulsion towards the carbonyl oxygen of the C(2) group and thus forces it away from the charge. This enlarges the dihedral angle, reducing $^{3}J(1,2)$ and also reduces the dihedral angle and elevates $^{3}J(3,2)$. As a result the calculated-observed differences are not so small for ecgonine. The conformational change also destroys the W-route between protons 4b and 2. Since ^{4}J couplings are very sensitive to the planarity of the pathway, a deviation from a W-type planar pathway causes a decrease in the coupling.[137] This is apparent for neutral cocaine, β-CPT and nor-β-CIT and is useful in distinguishing the α- and β-isomers.

5.2.2 .^{1}H -NMR Spectroscopic study of 6/7-carboethoxy phenyltropanes[III]

Conformational analysis of compounds **41-45** and **47** was performed using molecular mechanistic models and the Haasnoot equation for the proton couplings from ^{1}H NMR spectral analyses. The Haasnoot equation was used, because it was found to have better fit than the Altona equation between the calculated and empirical values (Figure 4.). The coupling data of compounds **44a, 44b** and **45a- 45c** correlated well with the calculated values of the chair conformation, leading to the conclusion that the conformational equilibrium is highly in favour of the chair form, likewise with the compounds discussed in the previous chapter. The unsaturated analogues **43a-43c** had $^{3}J(1,2)$ and $^{3}J(4a, 5)$ couplings of over 5 Hz, parallel to the dihedral angle, which is between the angles of boat and chair conformations, leading to the conclusion that these compounds are mainly in a half-chair conformation. The conclusion is supported by the increased $^{4}J(2,4a)$ and $^{4}J(2,7a)$ couplings, known also as W-route, due to their sensitivity to the planarity of the pathway. On the basis of modeling, we conclude that the structure is also rather rigid, probably due to the conjugation with the double bond.

Conformation analysis of products **41a-41d**, **42** and **47** suggested that they are all very flexible and different conformations are separated energetically with a very low barrier. The coupling data of tropinone compounds **41a-41d** did not correspond to any single pure

conformer, but a conformer that was between the chair and half-chair conformations. Furthermore, molecular dynamics calculations suggested that the carbon 2-4 moiety was very flexible. The structure of **42** had only few conformationally informative couplings, making the ^{1}H NMR structural analysis difficult, and thus the structure was derived from the X-ray analysis. Nonetheless, a comparison of the few couplings with the calculated results confirms that the boat conformation is the main form also in solution. The coupling data of the 2α, 3α- substituted product **47** corresponded well to the values for the boat conformation. However, the half-chair and chair conformations are energetically separated with a very low barrier.

5.3. *In vitro*-evaluation[VI]

The affinities (IC_{50}) of **43a-c**, **46b-e**, **48a-b**, **49a-c** and **50a-d** for the dopamine (DAT), serotonin (SERT) and norepinephrine (NET) transporters were determined by *in vitro* competition studies. Compounds **43a-c**, **46b-e** and **50a-d** did not show any significant specific binding. The affinities of compounds **48a-b** and **49a-c** showed that attaching a methyl group to the 6,7-bridge decreased the affinity for all of the transporters. Part of the decreased activity was clearly due to the fact that all of the tested compounds were racemates. Compounds **48a** and **48b**, which lacked an iodine on the phenyl ring, were also almost inactive, and only minor affinities for DAT were observed (Table 8). However, **49a** and **49b** had affinity for all three transporters, and **49b** showed some selectivity towards SERT, essentially through diminished affinity for DAT. For NET, the decrease was even more dramatic. Substitution at the 7-position was found to be more favorable for SERT selectivity than the 6-position, though the total affinity for SERT was also decreased. The demethylated analogue **49c** had a similar binding profile as **49b**, but with slightly better affinities.

Table 8. Inhibition of [^{3}H]-GBR-12935, [^{3}H]-paroxetine and [^{3}H]-nisoxetine binding at the dopamine, serotonin and norepinephrine transporters, respectively, in rat brain membranes.[a]

	IC_{50} (nM)			Selectivity	
Comp.	DAT	SERT	NET	DAT/SERT	NET/SERT
2	3720	2390	2950	1.6	1.2
β-CIT	21	11	45	1.9	4.1
48a	27700	NDB	NDB	-	-
48b	16900	NDB	NDB	-	-
49a	782	344	7490	2.3	21.8
49b	4470	402	13000	10.1	32.3
49c	2420	260	12000	9.3	44.4

[a]Each value is the mean of three independent experiments, each performed in triplicate. NDB = no displacement binding.

5.4. *In vivo*-locomotor studies[VI]

Compounds **49a** and **49c** where tested *in vivo* in locomotor studies, with cocaine (**2**) and β-CIT being utilized as reference compounds. Compounds **49a** and **49c** resulted in totally different behaviors. The methylated analogue **49a** increased locomotor activity in a dose dependent manner, while demethylated **49c** had no measurable influence at any tested dose (0.2, 2.0 and 20.0 mg/kg) when compared to saline treated controls. The ability of cocaine analogues to increase locomotor activity is mainly due to DAT binding.[58-60] This is probably the reason why the more SERT selective **49c** does not increase the locomotor activity at these doses. The smallest doses of **49a** at 0.1 mg/kg and 1.0 mg/kg also did not change the locomotor activity, but the dose of 10 mg/kg increased activity rapidly with a duration of 240 min (Figure 5). After the injection of **49a** at 100 mg/kg, the mice were immobile for 30 min, after which the locomotor activity increased gradually and remained elevated until the end of the observation period. The locomotor activity curves at 10 mg/kg and 100 mg/kg differed noticeably, although the difference was not apparent in the corresponding histograms of total activity counts (Figure 5). One explanation for this behavior could be that the higher dose is approaching the toxic level for this class of compounds.[57] The locomotor studies suggest that compound **49a** passes through the blood

brain barrier, but no conclusions can be drawn for compound **49c** in this regard. However, the corresponding analogue of β-CIT (i.e. nor-β-CIT) is known to pass into the brain.[87]

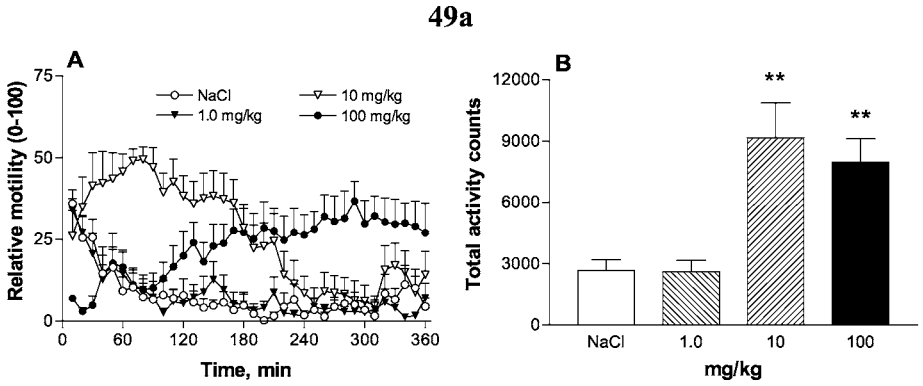


Figure 5. The time course of the effect of **49a** on locomotor activity (A) and the total activity counts (B) in mice. Statistics: **p<0.01 compared to NaCl treated control animals. Data are means ± SEM of 7-9 animals.

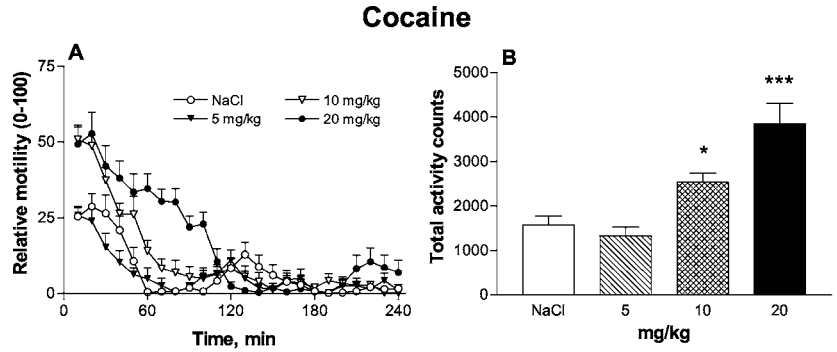


Figure 6. The time course of the effect of cocaine (**2**) on locomotor activity (A) and the total activity counts (B) in mice. Statistics: *p<0.05 and ***p<0.001 compared to saline treated control animals. Data are means ± SEM of 10 animals.

The dose of 5 mg/kg of (-)-cocaine (**2**) had no effect on locomotor activity (Figure 6). Instead, i.p. doses of 10 and 20 mg/kg increased the locomotor activity in a dose dependent manner over the time period of 90 and 120 min, respectively, with peak activity 10 to 20 min after the injection.

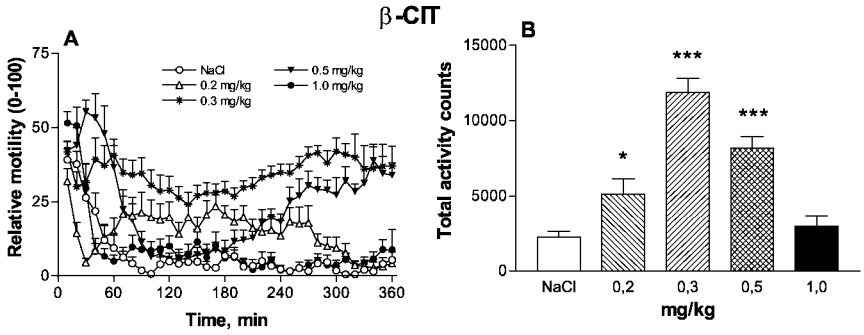


Figure 7. The time course of the effect of β-**CIT** on locomotor activity (A) and the total activity counts (B) in mice. Statistics: *p<0.05 and ***p<0.001 compared to NaCl treated control animals. Data are means ± SEM of 10 animals.

β-CIT increased locomotor activity in a dose dependent manner (Figure 7). After the highest (1.0 mg/kg) tested dose of β-CIT, the same hypoactivity effect was observed as for the highest dose of **49a**, but the activity remained low during the entire observation period. Cocaine had the shortest duration of action for increasing locomotor activity among the tested compounds (Figure 6). Compounds β-CIT and **49a** lack the 3β-benzoyl ester linkage that is present in cocaine, which probably makes them more stable towards esterases and thus prolongs their duration of action.[63]

6. SUMMARY AND CONCLUSIONS

Phenyltropanes are cocaine analogues, which bind to DAT, SERT and NET in the CNS. Radiolabelled forms of phenyltropanes are used in brain imaging with SPET or PET e.g. in the diagnosis of Parkinson's disease. In addition, they have provided novel information on the pathophysiology of social phobia, depression and alcohol abuse. Structural modifications and conformational changes in phenyltropanes are known to have an influence on binding and numerous new analogues have been developed by modifying the substituents present in the tropane skeleton. However, the influence of modifications at the 6,7-ethylene bridge on binding remains to be clarified, probably due to the challenging synthetic work needed for preparing of this class of derivatives. In the present study, two separate synthesis routes to new 6/7-substituted phenyltropanes were developed and an *in vitro*/*in vivo* evaluation of the products was performed. In addition, the analysis of solution conformation of selected phenyltropanes was made both with ^{1}H NMR spectroscopy and molecular modelling.

In order to examine the influence of the 6/7-substitution on the chemical behaviour of the phenyltropanes, stereoselective reduction of 6/7-carboethoxy-3-phenyltrop-3-en-2-ones with catalytic hydrogenation and $NaBH_4$ was studied. The position of the EtO_2C-substituent at the 6/7-bridge was found to have a decisive role in determining the products of the syntheses. The 6/7-*exo*- and 7-*endo*-substituted 3β-phenyl-2-tropanones were selectively prepared by Pd/C hydrogenation, likewise the 6/7-*exo*-substituted 2α-hydroxy-3β-phenyltropanes. However, for the 2β-hydroxy-3β-phenyl tropanes, no stereoselective route was found. The ester functionality at the 2β-position is known to be important for the biological activity of cocaine, therefore 6/7-*exo*-carboethoxy-2β-acetoxy-3β-phenyltropane analogues were prepared from the corresponding phenyltropanols and tested *in vitro* by competitive binding studies to DAT, SERT and NET. However, these analogues with the reverse ester functionality when compared to cocaine, showed no appreciable affinities. In order to find synthetic methods for modifying the 2-position of

the 6/7-cardoethoxy substituted phenyltropanes, a novel one-pot synthesis route for 3,4-disubstituted pyrroles was developed.

Phenyltropanes with a methyl group at the 6/7-position and with the same ester functionality at the 2β-position as in cocaine, were synthesized from the Mannich products and *in vitro* binding was evaluated to DAT, SERT and NET. The substitution at the 6/7-position decreased the total affinity to the monoamine transporters, but some selectivity to SERT was observed. 6-*Exo*-methyl-2β-carbomethoxy-3β-(4'-iodophenyl)nortropane was found to be the most selective derivative to SERT. This compound and its corresponding N-methylated analogue with less SERT selectivity were tested also in *in vivo* locomotor studies in mice. The results supported the *in vitro* results. In addition, the first radiolabeling of a 6/7-substituted phenyltropanes was performed with I-125 in order to test the labelling procedure for future preclinical studies. Radionuclide I-125 is suitable for *in vitro* studies e.g. autoradiography, due to its longer half-life, when compared to I-123, which is used for *in vivo* imaging with SPET. However, the labeling procedures for both nuclides are the same.

The conformational analysis of selected phenyltropanes showed that the 2β, 3β-substituted analogues prefer the relatively rigid chair conformation. However, those analogues, which have carbonyls or which have substituents at the 2α- and 3α-positions were very flexible and had several configurations within very low energy barriers.

In the literature, there are only a few studies which have examined the influence of the 6/7-substitution on chemical behaviour and biological activity of phenyltropanes. This study provides important information in that field. The results suggest that the 6/7-substitution provides new possibilities for modifying binding selectivity of tropane analogues to monoamine transporters, although no new high affinity and selective analogues were identified. In addition, the conformational analysis of the phenyltropanes with varying substituents in the tropane ring widens our knowledge of the conformational behaviour of the tropane ring.

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APPENDIX: ORIGINAL PUBLICATIONS