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EFFICIENT SYNTHESIS OF ENANTIOMERICALLY PURE THIOESTER PRECURSORS OF [^{11}C]McN-5652 FROM RACEMIC McN-5652

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SUMMARY

An improved synthesis of the enantiomerically pure thioester precursors of [^{11}C](+)-McN-5652 ([^{11}C](+)-1), and [^{11}C](-)-McN5652 ([^{11}C](-)-1) starting from racemic McN-5652 ((±)-1) is described. The synthetic method includes the resolution of (±)-1 by repeated crystallization of the (+)- and (-)-di-p-toluoyltartrates yielding (+)-McN-5652 ((+)-1) and (-)-McN-5652 ((-)-1), each with > 98 % enantiomeric purity. S-Demethylation of (±)-1, (+)-1 and (-)-1, respectively was achieved by treatment with sodium amide at low temperatures (-78 °C) followed by conversion of the intermediate thiols 2 with acetyl chloride to give the corresponding thioester precursors (±)-3, (+)-3 or (-)-3. This demethylation method almost completely suppressed the isomerization of the pharmacologically active trans diastereomer into the inactive cis form. Chiral HPLC analyses confirmed that the S-demethylation proceeded without any racemization. ^{11}C-Labelling of (+)-3 or (-)-3 yields enantiomerically pure [^{11}C](+)-McN-5652 or [^{11}C](-)-McN5652, each in 22 %

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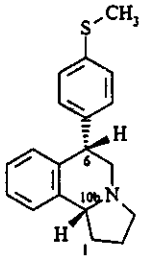
radiochemical yield (decay-corrected, related to $[^{11}C]CO_2$) and a specific radioactivity of 74 GBq/μmol (2 Ci/μmol) at the end of synthesis (EOS).

INTRODUCTION

A number of hexahydropyrroloisoquinolines are potent inhibitors of the serotonin (5-HT), dopamine and norepinephrine uptake sites. The compound McN-5652 (trans-1,2,3,5,6,10b-hexahydro-6-[4-(methylthio)phenyl]pyrrolo-[2,1-a]isoquinoline 1, Scheme 1) is an exceedingly potent and selective blocker of the serotonin uptake sites [1,2]. Structure activity investigations have shown that the inhibition is strongly dependent on the stereochemistry of this compound [1,2]. The active isomer is the trans-(+) compound ((+)-McN-5652, (+)-1) with a K_i value of 0.4 nM for *in vitro* [^{3}H]5-HT uptake inhibition in the rat cerebral cortex [3].



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Scheme 1. Structure of McN-5652 (trans-1,2,3,5,6,10b-hexahydro-6-[4-(methylthio)phenyl]-pyrrolo-[2,1-a]isoquinoline 1

As demonstrated by Suehiro et al. [4], McN-5652 labelled with ^{11}C is a suitable radiotracer for imaging serotonin uptake sites with positron emission tomography (PET). *In vivo* investigations in rodents confirm the usefulness of this tracer due to its high selectivity and specificity in binding to 5-HT uptake sites and high target-to-nontarget ratios [4]. In the baboon [5] and human brain [6], satisfactory target-to-nontarget ratios of 1.5 – 1.8 are obtained after 95 – 115 min using the biologically active (+)-enantiomer.

$[^{11}C]$(+)-McN-5652 is prepared by methylation of the normethyl compound 2 with $[^{11}C]$methyl iodide [7]. The normethyl precursor 2 was synthesized by demethylation of (+)-McN-5652 through

treatment with sodium thiomethoxide [7]. The shelf-life of the unstable thiol 2 can be prolonged by conversion into thioesters as thiol acetate 3, butyrate, and benzoate derivatives of McN-5652 [8]. (+)-McN-5652, the starting compound for the precursor synthesis, is not commercially available. The synthesis of (+)-1 is associated with a number of problems. The known preparation methods of 1 [1,9] yield a racemic product. The reported procedure [1] to resolve (±)-1 by recrystallisation of the (+)-tartrate gives no satisfactory results [10]. Furthermore, the S-demethylation with sodium thiomethoxide in DMF is accompanied by a conformational change, which reduces the portion of the desired trans diastereomer to approximately 40 % [7].

These problems are prevented by a stereoconservative synthesis utilizing the transformation of the 6-(4-bromophenyl) analogues of 1 into the corresponding trisisopropylsilyl protected thioethers [10]. However, this procedure requires time consuming preparation of the bromophenyl compound.

In this paper, we describe a route to prepare the thioester precursors (+)-3 and (-)-3 starting from racemic McN-5652 avoiding the disadvantages of the known syntheses. The preparation is based on the resolution of (±)-1 by recrystallisation with (+)- and (-)-di-p-toluoyltartaric acid and S-demethylation of 1 with sodium amide in liquid ammonia (Scheme 1).

RESULTS AND DISCUSSION

Preparation of (+)-McN-5652 and (-)-McN-5652

The parent compound McN-5652 ((±)-1) was prepared starting from 2-phenylpyrrolidine and 4-methylthiomandelic acid in a five-step synthesis as reported in [1,9]. Initially, we tried to resolve the racemic product by repeated crystallization with (+)-tartaric acid from ethanol or acetonitrile similar to [1]. This procedure gave no enrichment of any enantiomer, which confirms the results of Huang et al. [10].

As an alternative, we tested the recrystallization of (±)-1 with (+)- and (-)-di-p-toluoyltartaric acid. Unexpectedly, the initial crystallization with (+)-di-p-toluoyltartaric acid from acetonitrile yielded

the (-)-enatiomer of 1 with an enantiomeric purity of about 90 %. Optically pure (-)-1 (enantiomeric excess (ee) > 98 %) was obtained after an additional recrystallization from ethanol. The mother liquor of the initial crystallization was enriched with the (+)-enantiomer (ee > 90 %). A further crystallization of the recovered amine with (-)-di-p-toluoyltartaric acid from acetonitrile gave optically pure (+)-1 (ee > 98 %).

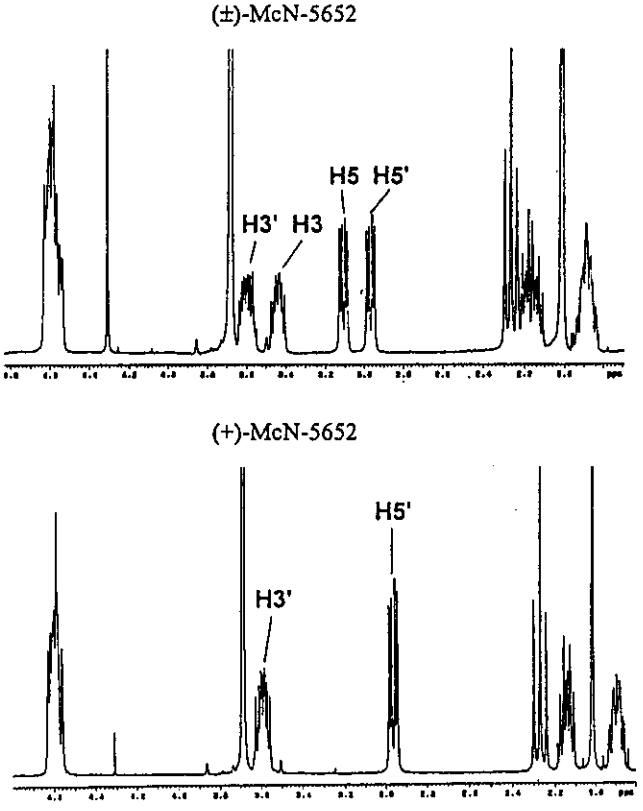


Fig. 1. ^{1}H NMR spectra of (±)-McN-5652 and (+)-McN-5652 as their (+)-MTPA salts (H3, H5 are assigned to (-)-1; H3', H5' to (+)-1)

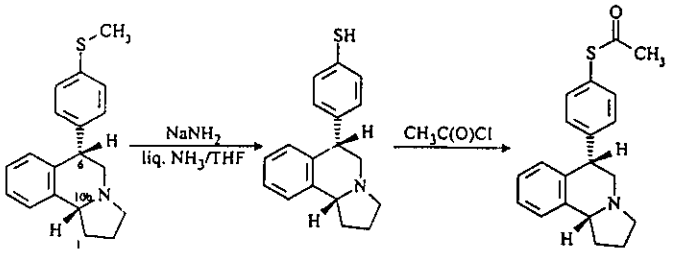
The enantiomeric purity was determined by ^{1}H-NMR of the McN-5652 enantiomers with (+)-Mosher's acid ((+)-α-methoxy-α-(trifluoro-methyl)phenylacetic acid, (+)-MTPA) [11]. Typical NMR spectra of (±)-1 and (+)-1 as their (+)-MTPA complexes are shown in Fig. 1.

Besides ^{1}H NMR, the enantiomeric purity of 1 was determined by chiral HPLC analysis. Shah and Maryanoff [12] describe a HPLC method based on cellulose carbamate as stationary chiral phase. We also achieved a separation of (+)-McN-5652 and (-)-McN-5652 by using the glycopeptide teicoplanine as stationary phase. Under these conditions, the retention times of (-)-enantiomer and (+)-enantiomer of 1 were 35.8 min and 38.0 min, respectively.

Synthesis of the thioester precursors

Starting from McN-5652, the preparation of the precursor for synthesis of [^{11}C]McN-5652 consists of the S-demethylation of 1 and subsequent conversion of the intermediate 2 into the thioester 3. Pyrroloisoquinolines isomerize in the course of heating in the presence of bases [13]. For this reason, the demethylation of McN-5652 with sodium thiomethoxide at 160 °C yields a product which contains the cis and trans diastereomer of the normethyl compound 2 in a ratio of approximately 60:40 [7]. This value is independent of the initial diastereomer ratio and equivalent to the equilibrum ratio. This isomerization reduces the yield of the pharmacologically active trans diastereomer.

In order to minimize the undesired isomerization reaction, we examined the S-demethylation of racemic McN-5652 by using sodium amide (Scheme 2).



1 2 3

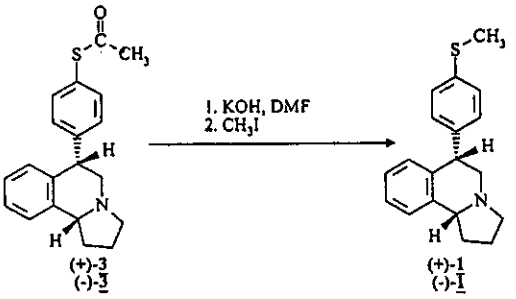
Scheme 2. Synthesis of the thioester precursor for [^{11}C]McN-5652

The reaction was carried out according to a modified literature procedure [14]. The demethylation proceeded in a mixture of liquid ammonia/THF at -78 °C within 20 min. A fourfold excess of sodium amide was necessary for a complete conversion. Under these conditions, the isomerization reaction was suppressed substantially. Less than 10 % of the trans diastereomer was converted into the cis form. Prolongation of the reaction time and a higher excess of sodium amide favoured the isomerization and led to an increased portion of the cis compound.

The intermediate thiol 2 was converted with acetyl chloride into the thioester 3 (Scheme 2). The trans diastereomer was purified either by flash chromatography or by preparative TLC.

The described demethylation method was applied to the preparation of enantiomerically pure thioester (+)-3. A sample of the (+)-thioester was converted into McN-5652 by hydrolysis of (+)-3 with potassium hydroxide and methylation with methyl iodide (Scheme 3). The product was identified by chiral HPLC analysis as optically pure (+)-1, which established that no racemization occurred during S-demethylation of (+)-1 by treatment with sodium amide.

Preparation of the (-)-thioester (-)-3 starting from enantiomeric pure (-)-1 and chiral HPLC of recovered (-)-McN-5652 gave analogous results.



(+)-3
(-)-3

(+)-1
(-)-1

Scheme 3. Conversion of thioesters 3 into McN-5652 1

Similar results were obtained when the precursor (+)-3 or (-)-3, prepared by the described method, was used for ^{11}C-labelling of (+)-McN-5652 or (-)-McN5652 in a similar procedure as reported [7,10]. The thioester precursors were hydrolyzed by treatment with potassium hydroxide. The

released thiols (+)-2 or (-)-2 were methylated with [^{11}C]methyl iodide in DMF. [^{11}C](+)-McN-5652 or [^{11}C](-)-McN-5652 was obtained with an overall radiochemical yield of 22 % (corrected for decay, related to [^{11}C]CO_2) in a preparation time of 35 min from [^{11}C]CO_2. The radiochemical purity was >98 % and the specific activity about 74 GBq/μmol (2 Ci/μmol) (EOS). The products were identified by chiral HPLC as enantiomerically pure [^{11}C](+)-1 or [^{11}C](-)-1, which confirmed that no racemization occurred during the thioester synthesis and the labelling step.

CONCLUSION

Enantiomerically pure thioester precursors for the synthesis of [^{11}C](+)- and [^{11}C](-)-McN-5652 were prepared starting from racemic McN-5652. The resolution of (±)-1 with (+)- and (-)-di-p-toluoyltartaric acid gave (+)-1 and (-)-1, each with ee > 98 %. These enantiomers were converted into the thioester precursors (+)-3 and (-)-3 separately utilizing the sodium amide S-demethylation. This preparation method prevented the undesired isomerization almost completely and was not accompanied by any racemization.

EXPERIMENTAL

Materials

All reagents and solvents were purchased from commercial suppliers and were used without purification with the following exceptions: THF was dried by distillation over sodium. N-Vinyl-2-pyrrolidinone, ethyl benzoate, bromoform and acetyl chloride were distilled before use. Ammonia (Messer-Griesheim, ultra high purity grade) was dried over calcium oxide and potassium hydroxide. Methylthiomandelic acid was prepared from 4-methylthiobenzaldehyde according to the method of Compere [15]. 2-Phenylpyrroline was obtained from N-vinyl-2-pyrrolidinone using the procedure of Jacob [16]. This compound was converted into 2-phenylpyrrolidine by reduction with dimethyl amine/borane complex (in acetic acid, 4 h, room temperature).

(±)-McN-5652 was prepared starting from 4-methylthiomandelic acid and 2-phenylpyrrolidine in a five-step synthesis using literature procedures [1,9]. Crude (±)-1 was purified by flash chromatography over silica gel 60 (ethyl acetate/hexane/methanol 5/5/1).

General Procedures

^{1}H and ^{13}C NMR spectras were recorded on a Varian INOVA 400 NMR spectrometer. The chemical shifts are expressed in ppm relative to tetramethylsilane. $CDCl_3$ or benzene-d_6 were used as solvent and internal standard. The enantiomeric purity of McN-5652 was determined by ^{1}H NMR spectra of the (+)-MTPA salts utilizing the procedure of Villani et al. [11]. The (+)-MTPA salts of 1 were prepared by dissolving (+)-MTPA and 1 in form of the free amine (in molar ratios) in benzene-d_6.

Mass spectra were obtained on a trio 2000 spectrometer (VG Organic, UK) using positive ion mode with electrospray as interface (ES +).

Optical rotations (589 nm) were obtained by using a JASCO Digital Polarimeter DIP-370.

HPLC analyses and purifications were performed with the following chromatographic systems:

Chemical and radiochemical purities were determined with a PUROSPHER RP 18 column (125 mm x 3 mm, 5 µm) eluted isocratically with acetonitrile/water (50/50) containing 0.1 M ammonium formate at a flow rate of 0.5 ml/min.

HPLC analyses of enantiomeric purity was done with a CHIROBIOTIC T column (250 mm x 4.6 mm, 5 µm) from Astec eluted isocratically with methanol/acetic acid/triethylamine (100/0.1/0.05) at a flow rate of 1 ml/min.

Semipreparative HPLC was performed with a µ-BONDAPAK C18 column (300 mm x 7.8 mm, 10 µm) eluted isocratically with acetonitrile/water (60/40) containing 0.01 M ammonium formate at a flow rate of 8 ml/min.

Resolution of racemic trans-1,2,3,5,6,10b-hexahydro-6-[4-(methylthio)phenyl]pyrrolo-[2,1-a]-isoquinoline ((±)-1)

Racemic McN-5652 as free amine (0.72 g, 2.4 mmol) was dissolved in acetonitrile (200 ml), filtered and added to a filtered solution of (+)-di-p-toluoyltartaric acid (0.94 g, 2.4 mmol) in acetonitrile (50 ml). The combined solutions were left to stand at room temperature for 24 h. The off-white crystalline solid (0.75 g) was filtered, dried, dissolved in hot absolute ethanol (20 ml), and left to stand at room temperature for 3 days to give off-white crystalls (0.56 g). The product was identified by ^{1}H NMR of the (+)-MTPA salt and chiral HPLC as (-)-McN-5652 with ee > 98 %.

A small sample of the salt was partitioned between methylene chloride and aqueous NaOH. The obtained sample of free amine was recrystallized with (-)-tartaric acid from acetonitrile. The (-)-tartrate salt showed an optical rotation of $[\alpha]^{21}_D$ -51.90 (MeOH, c 0.236).

The mother liquors of previous crystallizations were evaporated. The salt was converted into the free amine by partitioning between aqueous NaOH and methylene chloride to give an oil (0.47 g, 1.6 mmol) which was dissolved in acetonitrile (120 ml). The filtered solution was combined with (-)-di-p-toluoyltartaric acid (0.62 g, 1.6 mmol) in acetonitrile (30 ml) and left to crystallize at ambient temperature for 1 day. The off-white solid (0.61 g) was enriched with (+)-McN-5652 (ee > 98 %, determined by ^{1}H NMR of the (+)-MTPA salt and chiral HPLC). A sample was converted into the (+)-tartrate: $[\alpha]^{21}_D$ +50.50 (MeOH, c 0.221).

(±)-trans-1,2,3,5,6,10b-hexahydro-6-[4-(acetylthio)phenyl]pyrrolo-[2,1-a]isoquinoline ((±)-3)

Sodium (70 mg, 3 mmol) was dissolved in liquid ammonia (30 ml) while cooling with acetone/dry ice. A precooled solution of 1 (0.37 g, 1.3 mmol) in dry THF (8 ml) was added to the sodium amide solution. The typical blue colour was lost immediately. A further portion of sodium (57 mg, 2.5 mmol) was added and the reaction mixture turned deep blue again. The mixture was stirred for 10 min at –78 °C. When the blue colour was stable for 10 min after the last addition of sodium

(sometimes more than two portions of sodium were necessary), excess sodium amide was decomposed with a small amount of ammonium chloride.

After complete demethylation, the cool bath was removed and the ammonia was evaporated in a nitrogen stream. Acetyl chloride (2 g, 25 mmol) was added to the resulting solution of the normethyl compound 2 while cooling with iced water. The mixture was stirred at room temperature for 15 - 20 min and then water (25 ml) was added while cooling with iced water. The thioester was extracted with dichloromethane, the organic phases were washed with water and dried with magnesium sulphate and evaporated in vacuo.

The crude product was purified by flash chromatography over silica gel 60 or preparative TLC (PSC silica gel plates Kieselgel 60/UV 254, Merck) using chloroform/ethyl acetate/ethanol (20:10:2.5) as eluent to yield a light brown oil.

Yield: 0.24 g (59 %)

MS: m/z 324 (MH^+)

^{1}H NMR (399.95 MHz, $CDCl_3$): 1.83 – 1.88 (m, 3H), 2.37 – 2.42 (m, 1H), 2.41 (s, 3H, SC(O)CH_3), 2.65 (m, 1H), 2.91 – 2.98 (m, 2H), 3.07 (m, 1H), 3.56 (m, 1H), 4.24 (t, 1H), 6.90 (d, 1H, arom), 7.09 (m, 1H, arom), 7.16 – 7.18 (m, 2H, arom),7.30 – 7.32 (m, 4H, arom)

^{13}C NMR (100.57 MHz, $CDCl_3$): 22.2, 30.1, 30.3, 45.7, 54.1, 55.9, 63.7, 125.5, 125.7, 126.3, 129.4, 129.8, 134.1, 136.6, 138.7, 147.5, 194.3

(+)-trans-1,2,3,5,6,10b-hexahydro-6-[4-(acetylthio)phenyl]pyrrolo-[2,1-a]isoquinoline ((+)-3)

The thioester (+)-3 was prepared from (+)-McN-5652 according to the procedure described above.

Yield: 53 %

(-)-trans-1,2,3,5,6,10b-hexahydro-6-[4-(acetylthio)phenyl]pyrrolo-[2,1-a]isoquinoline ((-)-3)

The (-)-enantiomer of 3 was prepared from (-)-McN-5652 as described for (±)-3

Yield: 57 %

Radiosynthesis of [^{11}C]-(+)-McN-5652 ([^{11}C](+)-1) and [^{11}C]-(-)-McN-5652 ([^{11}C](-)-1)

The precursor (+)-3 or (-)-3 (1 mg, 3 μmol) was dissolved in DMF (300 μl) containing 5M KOH (8 μl). The hydrolysis was completed after 5 min at 40 °C.

[^{11}C]Methyl iodide, prepared starting from [^{11}C]carbon dioxide according to the method of Crouzel et al. [17], was trapped in the precursor solution at –40 °C. The reaction solution was warmed to 40 °C for 2 min and diluted with 0.3 ml HPLC eluent. The HPLC purification was performed with the semipreparative HPLC method described above. The eluent containing [^{11}C](+)-1 was evaporated. The residue was dissolved subsequently in a mixture of saline and ethanol (90:10) containing 0.1 % Tween 80. The resulting solution was filtered through a 0.22 μm sterile filter. The chemical, radiochemical, and enantiomeric purity of the product was determined by the described methods.

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