

## Synthetic approaches and biodistribution studies of [<sup>11</sup>C]methylphenidate

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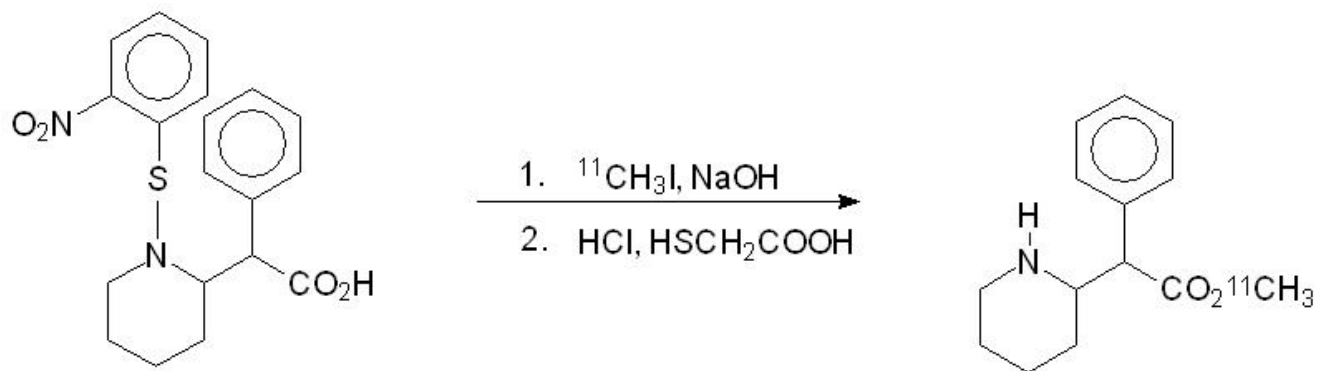
**ABSTRACT – Purpose:** The purpose of this study was a) to present a facilitated method for the preparation and workup of [<sup>11</sup>C]d-threo-methylphenidate ([<sup>11</sup>C]d-threo-MP) (a ligand that was shown to bind selectively to the presynaptic dopaminergic transporters) from [<sup>11</sup>C]methyl iodide ([<sup>11</sup>C]CH<sub>3</sub>I), b) to demonstrate that the ligand can as well be produced by an alternative labeling method employing [<sup>11</sup>C]diazomethane as the labeling agent and c) to present biodistribution data for this tracer obtained in rats. **Methods:** <sup>11</sup>C-labeling with [<sup>11</sup>C]CH<sub>3</sub>I was performed using either [d-threo-1-(2-nitrophenylsulfanyl)piperidin-2-yl]phenyl-acetic acid (d-threo-N-NPS-ritalinic acid) under addition of sodium hydroxide as base or the previously prepared sodium salt of d-threo-N-NPS-ritalinic acid. The two approaches were compared with regard to radiochemical yield and purification procedures needed in order to obtain a sufficiently pure tracer solution for human use. For the alternative reaction pathway using [<sup>11</sup>C]diazomethane as the labeling agent the reaction was performed with d-threo-N-NPS-ritalinic acid. The biodistribution of [<sup>11</sup>C]d-threo-MP was determined in rats at 5, 10 and 30 min post injection of the tracer. **Results:** The application of the sodium salt of d-threo-N-NPS-ritalinic acid as precursor resulted in higher radiochemical yields than the use of the free acid under basic conditions, the yields were 20 ± 8 % and 6 ± 3 %, respectively for the final isolated product (based on [<sup>11</sup>C]CH<sub>3</sub>I starting activity). The alternative labeling approach by

means of [<sup>11</sup>C]diazomethane as the labeling agent was demonstrated to give radiochemical yields of 76 ± 8 % (based on [<sup>11</sup>C]diazomethane starting activity, determined by HPLC analysis of the crude reaction mixture before final work-up) within shorter process times. Based on [<sup>11</sup>C]methane starting activity both approaches result in similar yields (17 % and 15 %, respectively) Biodistribution studies in rats revealed a low blood activity (0.09 % injected dose/g (% ID/g)) at 5 min post injection (p.i.), as well as a relatively high liver uptake (15.9 % ID at 30 min) compared to a lower kidney uptake (3.2 % ID at 30 min). Brain uptake was 0.9 % ID/g already 5 and 10 min p.i.. **Conclusions:** The application of the sodium salt of d-threo-N-NPS-ritalinic acid as precursor for the radiosynthesis of [<sup>11</sup>C]d-threo-MP reduces the amount of [<sup>11</sup>C]methanol formed from the reaction of [<sup>11</sup>C]CH<sub>3</sub>I with sodium hydroxide, that is added to generate the carboxylic anion of d-threo-N-NPS-ritalinic acid needed for labeling with [<sup>11</sup>C]CH<sub>3</sub>I. The purification process could be simplified (omission of one solid phase extraction step), resulting in an easily automated process for the production of the tracer. The preparation of [<sup>11</sup>C]d-threo-MP by means of [<sup>11</sup>C]diazomethane as the labeling agent appears to be an interesting alternative to the [<sup>11</sup>C]CH<sub>3</sub>I methods because of shorter overall process times and high labeling yields. Biodistribution data show a rapid extraction of the tracer from the blood pool. Tracer excretion seems to take place predominantly via the hepatic pathway since liver uptake at 30 min was considerably higher than kidney uptake. [<sup>11</sup>C]d-threo-MP exhibits a rapid and sufficiently high brain uptake in rats.

### INTRODUCTION

Carbon-11 labelled d-threo-methylphenidate ([<sup>11</sup>C]d-threo-MP) has been proven to be a useful tool for the investigation of the availability of presynaptic dopaminergic neurons (dopamine transporters) in the human brain by Positron Emission Tomography (PET) (1). Methylphenidate (MP) exists in four individual isomeric forms: two pairs of diastereomers (threo- and erythro), each of which have a d- and l-enantiomer.

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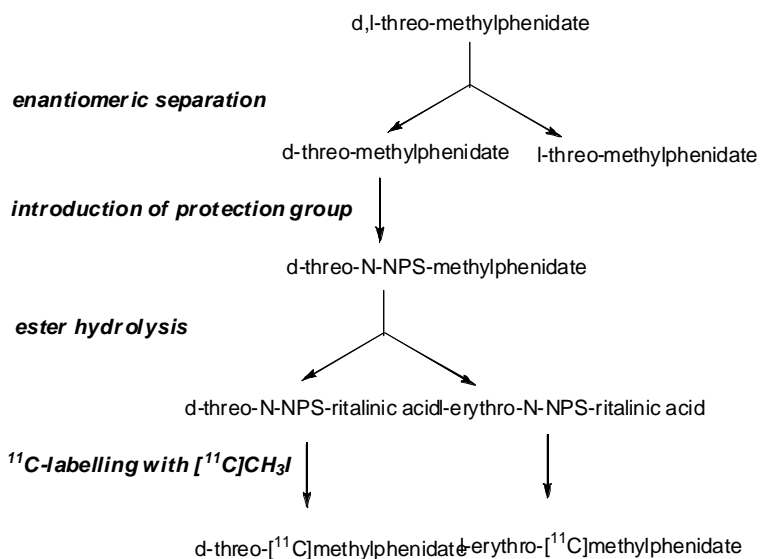
**Figure 1.** Reaction scheme of the radiosynthesis of [ $^{11}\text{C}$ ]methylphenidate.

The racemate of threo-MP in its non-radioactive form is marketed under the trade name Ritalin for the treatment of diseases associated with the dopaminergic system such as attention deficit hyperactivity disorder (ADHD) and narcolepsy. The therapeutic properties of the marketed drug substance are primarily due to the more active enantiomer d-threo-MP (2); for details of binding affinities see (3).

The radiosynthesis of [ $^{11}\text{C}$ ]d-threo-MP (and the other possible isomers) has been described for the first time by Ding et al. (4). The approach described, consists of two steps: O-methylation of N-protected

ritalinic acid with [ $^{11}\text{C}$ ]methyl iodide ([ $^{11}\text{C}$ ]CH<sub>3</sub>I) under basic conditions and subsequent deprotection (Figure 1).

The labeling procedure as described by Ding et al. (4) uses d-threo-N-NPS-ritalinic acid as precursor under addition of sodium hydroxide (NaOH) to generate the anion of the acid function for subsequent reaction with [ $^{11}\text{C}$ ]CH<sub>3</sub>I. Since NaOH may react with [ $^{11}\text{C}$ ]CH<sub>3</sub>I to form [ $^{11}\text{C}$ ]methanol, the application of the sodium salt of d-threo-N-NPS-ritalinic acid is preferable over the published method (5).



**Figure 2.** Preparation of the precursor d-threo-N-NPS-ritalinic acid for radiolabeling with carbon-11 and expected labeling products.

Here we report the preparation of the sodium salt of d-threo-N-NPS-ritalinic acid and its application in a fully automated synthesis of [ $^{11}\text{C}$ ]d-threo-MP.

In addition, radiolabeling with the highly reactive labeling precursor [ $^{11}\text{C}$ ]diazomethane and d-threo-N-NPS-ritalinic acid was also investigated. This synthetic path allows the omission of NaOH and thus avoids the formation of [ $^{11}\text{C}$ ]methanol. Moreover, it should be possible in general to realize higher specific activities of [ $^{11}\text{C}$ ]d-threo-MP due to the shorter reaction times required for labeling with [ $^{11}\text{C}$ ]diazomethane. The labeling with [ $^{11}\text{C}$ ]diazomethane is mechanistically different from the methylation with [ $^{11}\text{C}$ ]CH<sub>3</sub>I. [ $^{11}\text{C}$ ]diazomethane labels selectively functional groups that dispose of an acidic proton, such as carboxyl groups. Labeling by means of [ $^{11}\text{C}$ ]CH<sub>3</sub>I, however, requires a nucleophilic function in the molecule, such as an amine or the anion of a carboxyl group. Therefore it should in principle be possible to label ritalinic acid directly with [ $^{11}\text{C}$ ]diazomethane without the need to protect the secondary amine (see Figure 2). However, in this study we used the protected precursor d-threo-N-NPS-ritalinic acid due to the immediate availability of the precursor. Nevertheless, the principle of the labeling of [ $^{11}\text{C}$ ]d-threo-MP by means of the [ $^{11}\text{C}$ ]diazomethane method can be demonstrated by our results.

Moreover, we report biodistribution data for [ $^{11}\text{C}$ ]d-threo-MP obtained after intravenous tracer injection in rats, since until now in the literature no equivalent data were available but may be useful for the estimation of radiation dose and/or for the purpose of comparing the pharmacokinetics of [ $^{11}\text{C}$ ]d-threo-MP with other radioligands used for the assessment of the dopaminergic system with PET.

## MATERIALS AND METHODS

### Chemicals

Acetonitrile (CH<sub>3</sub>CN, for DNA synthesis), 4-nitrobenzoic acid (4-NBA) (p.a., 99%), NaOH, elemental iodine and 1 M hydrochloric acid (HCl) were purchased from Merck (Darmstadt, Germany), 1,2-dimethoxyethane (DME) (dried over molecular sieve, 99.5%), ammonium formate and N,N-dimethylformamide (DMF) (dried over molecular sieve, 99.5%) were acquired from Fluka (Buchs, Switzerland). Mercaptoacetic acid was obtained

from Aldrich (St. Louis, Missouri) and molecular sieve 4Å was obtained from Alltech (Deerfield, Illinois). Helium gas (He 6.0) and hydrogen gas (H<sub>2</sub> 6.0) were delivered by Messer Griesheim (Düsseldorf, Germany). Nickel (Ni) (Shimalite Ni 80/100) was obtained from Shimadzu (Kyoto, Japan), phosphate buffered saline (BupH™) was obtained from Pierce (Rockford, Illinois) and d,l-threo-MP was a gift from Ciba Geigy, Basel, Switzerland.

### Precursor synthesis

N-protected d-threo ritalinic acid, [d-threo-1-(2-nitrophenylsulfanyl)piperidin-2-yl]phenylacetic acid was prepared from d,l-threo-MP as described by Ding et al. (4). The sodium salt of N-protected d-threo ritalinic acid was prepared from 12 mg (32 μmol) d-threo-N-NPS-ritalinic acid and 16 μL 2 M NaOH: the mixture was dried in an argon gas stream after addition of three times 1 mL CH<sub>3</sub>CN each at 60 °C. The resulting sodium salt was redissolved in 3 mL dry CH<sub>3</sub>CN.

### Labeling with carbon-11

#### Method A: labeling with [ $^{11}\text{C}$ ]CH<sub>3</sub>I

[ $^{11}\text{C}$ ]CO<sub>2</sub> was produced at the cyclotron (PETtrace, GE Medical Systems, Uppsala, Sweden) of the PET-center of the University Tübingen. By irradiating nitrogen gas with 16.5 MeV protons  $^{11}\text{C}$ -activity was obtained as [ $^{11}\text{C}$ ]CO<sub>2</sub> via the  $^{14}\text{N}(p,\alpha)^{11}\text{C}$  nuclear reaction. Using the Mel MicroLab (GE Medical Systems, Uppsala, Sweden) [ $^{11}\text{C}$ ]CO<sub>2</sub> was trapped on molecular sieve 4 Å and converted to [ $^{11}\text{C}$ ]methane by H<sub>2</sub> in presence of a Ni catalyst. [ $^{11}\text{C}$ ]Methane was reacted with elemental iodine at 760 °C, yielding [ $^{11}\text{C}$ ]CH<sub>3</sub>I in 61% radiochemical yield (EOB) based on produced [ $^{11}\text{C}$ ]CO<sub>2</sub> within 12.5 min.

The activity was trapped in an automated system for  $^{11}\text{C}$ -methylation reactions (GE Medical Systems, Münster, Germany) at -40 °C in 400 μL CH<sub>3</sub>CN containing 2.1 mg (5.3 μmol) of the sodium salt of N-NPS-ritalinic acid. The methylation reaction occurred at 80 °C during 8 min. After cooling to 30 °C, 150 μL 1 M HCl and 150 μL of a solution of 250 μL mercaptoacetic acid in 5 mL CH<sub>3</sub>CN was added and the protecting group was removed during 3 min at room temperature. After addition of 250 μL 2 M NaOH and 650 μL of HPLC eluent (0.17 M ammonium formate buffer pH 8.5/CH<sub>3</sub>CN, 70/30, v/v) the reaction mixture was separated by

semipreparative HPLC (Partisil 10 ODS 3, 250 x 8 mm, 0.17 M ammonium formate buffer pH 8.5/CH<sub>3</sub>CN, 70/30, v/v, 8 mL/min). Under these conditions the *k'*-values were 4.7 for l-erythro-MP and 7.0 for d-threo-MP (Figure 3). The fraction containing [<sup>11</sup>C]d-threo-MP was diluted with 70 mL water and passed through a Sep Pak C-18 plus cartridge (Waters) conditioned with 10 mL ethanol and 10 mL water. The cartridge was rinsed with 10 mL water and [<sup>11</sup>C]d-threo-MP was eluted with 10 mL ethanol. The solvent was removed under reduced pressure and the residue dissolved in 4 mL phosphate buffered saline.

#### **Method B: labeling with [<sup>11</sup>C]diazomethane**

[<sup>11</sup>C]Diazomethane was produced as described previously by Solbach et al. (6). To determine the radiochemical yield of [<sup>11</sup>C]d-threo-MP for the reaction of d-threo-N-NPS-ritalinic acid with [<sup>11</sup>C]diazomethane, a special experimental layout was used: the stream of He containing the online produced [<sup>11</sup>C]diazomethane was simultaneously introduced into two reactors. Reactor 1 was a sealed reaction vessel containing 1 mg (6 μmol) of 4-nitrobenzoic acid (4-NBA) in 1 mL of 1,2-dimethoxyethane at room temperature. The second reactor was an identical sealed reaction vessel (Reactivial™, Supelco, 2 mL volume) containing 1 mg of d-threo-N-NPS-ritalinic acid (2.7 μmol) in a mixture of 900 μL 1,2-dimethoxyethane (DME) and 100 μL DMF at room temperature. Esterification of 4-NBA and d-threo-N-NPS-ritalinic acid proceeded instantaneously. Removal of the protection group was achieved as described for method A: 200 μL 1 M HCl and 200 μL of a solution of 250 μL mercaptoacetic acid in 5 mL DMF were added. Within 3 minutes at room temperature the cleavage of the protecting group was finished.

Because of the quantitative reaction of [<sup>11</sup>C]diazomethane with 4-NBA (6) the yield of [<sup>11</sup>C]d-threo-MP for the reaction of [<sup>11</sup>C]diazomethane with d-threo-N-NPS-ritalinic acid could be determined in relation to the assessment of formed 4-nitrobenzoic acid[<sup>11</sup>C]methylester. For that purpose both product solutions were analyzed by radio HPLC (Partisil 10 ODS 3, 250 x 8 mm, 0.17 M ammonium formate buffer pH 8.5/CH<sub>3</sub>CN, 50/50, v/v, 4 ml/min) after completion of the reaction process. The use of the esterification reaction of 4-NBA with [<sup>11</sup>C]diazomethane to monitor the reaction is necessary for the precise

determination of the product yield, since the gas stream containing the [<sup>11</sup>C]diazomethane activity also consists of varying amounts of chlorinated by-products such as [<sup>11</sup>C]chloroform and [<sup>11</sup>C]carbon tetrachloride. A direct determination of the amount of [<sup>11</sup>C]diazomethane produced during the individual [<sup>11</sup>C]diazomethane preparation run is not possible because of the high reactivity of this labeling agent.

#### **Quality Control**

Enantiomeric purity was checked by chiral HPLC (Chiralpak AD, Daicel, 250 x 4.6 mm, hexane/isopropanol/Et<sub>2</sub>NH 980/20/1, v/v/v/, 0.84 mL/min) of the ethanolic solution eluted from the C-18 Sep Pak Plus cartridge. Radioactivity was monitored by a NaI detector and UV-absorption was measured at 220 nm (*k'*-values (retention times): d,l-erythro-MP 0.6 (5.3 min), l-threo-MP 1.1 (9.6 min), d-threo-MP 1.8 (12.7 min)).

Quality control of the final injection solution and determination of specific activity was performed by reversed phase HPLC (Partisil 10 ODS 3, 250 x 8 mm, 0.17 M ammonium formate buffer pH 8.5/CH<sub>3</sub>CN, 50/50, v/v, 4 mL/min). Radioactivity was monitored by a NaI detector and UV-absorption was measured at 220 nm (*k'*-values : d,l-erythro-MP 2.1, d,l-threo-MP 2.8).

#### **Animal experiments**

Male Sprague Dawley rats (250 g) were anesthetized by Pentobarbital (50 mg/kg) and injected with 150 μL buffered tracer solution. All rats received the same pharmacological dose of d-threo-MP, i.e. 0.2 nmol.

The solution was administered into a femoral vein. After 5, 10 and 30 min the rats were sacrificed by heart puncture. Blood, urine and tissue samples from liver, kidneys, spleen, lung, heart, muscle, fat, bone, colon, small intestine were taken, weighed and counted for activity assay. Brain tissue samples were divided into striatum, cerebellum and rest of brain. Data were related to the total radioactivity administered as percentage of injected dose and weight (% ID/g), in cases in which the total organ was taken the data were additionally related to the total organ (% ID/organ).

## RESULTS

### *Radiochemistry*

[<sup>11</sup>C]d-threo-MP was prepared from N-protected ritalinic acid by esterification with [<sup>11</sup>C]CH<sub>3</sub>I under basic conditions (NaOH) in 6 ± 3 % (n = 3) yield, based on [<sup>11</sup>C]CH<sub>3</sub>I starting activity and corrected for decay. Alternatively, [<sup>11</sup>C]d-threo-MP was prepared from the sodium salt of N-protected ritalinic acid by esterification with [<sup>11</sup>C]CH<sub>3</sub>I in 20 ± 8 % (n = 12) yield. Overall synthesis time for both methods was 42 min including preparation of [<sup>11</sup>C]CH<sub>3</sub>I. The specific activity at EOS was 1000 Ci/mmol (37 TBq/mmol). Radiochemical purity was 98 ± 1 % and 97 ± 0.1 %, respectively.

The radiolabeling of [<sup>11</sup>C]d-threo-MP using d-threo-N-NPS-ritalinic acid and [<sup>11</sup>C]diazomethane as labeling precursor succeeded with radiochemical yields of 76 ± 8 % (n = 3) based on [<sup>11</sup>C]diazomethane starting activity and determined in solution, i.e. without complete work-up of the reaction mixture. The overall synthesis time since EOB including the production of [<sup>11</sup>C]methane (using the ProCab chemistry setup from GE Medical Systems, Uppsala, Sweden), the conversion into [<sup>11</sup>C]diazomethane, the reaction with d-threo-N-NPS-ritalinic acid and the deprotection procedure was 16 min. In order to be able to compare the radiochemical yields of [<sup>11</sup>C]d-threo-MP from both reaction pathways the calculations were also done with respect to [<sup>11</sup>C]CH<sub>4</sub> as starting activity. The resulting overall radiochemical yields of the produced [<sup>11</sup>C]d-threo-MP are similar: 15 % in case of the [<sup>11</sup>C]diazomethane labelling approach (radiochemical yield [<sup>11</sup>C]diazomethane from [<sup>11</sup>C]CH<sub>4</sub>: 20 % (6) and 76 % conversion of [<sup>11</sup>C]diazomethane into [<sup>11</sup>C]d-threo-MP) and 17 % in case of the [<sup>11</sup>C]methyl iodide labelling approach (radiochemical yield [<sup>11</sup>C]methyl iodide from [<sup>11</sup>C]CH<sub>4</sub>: 83 % (7) and 20 % conversion of [<sup>11</sup>C]methyl iodide into [<sup>11</sup>C]d-threo-MP).

The specific activity of [<sup>11</sup>C]d-threo-MP produced by this labeling approach was not determined since a fully shielded and remotely controlled production unit for the preparation of [<sup>11</sup>C]diazomethane was not available. Because of radiation protection purposes, the results described here were obtained

by bench experiments with low activities in the range of 100-1000 MBq.

### *Animal experiments*

After intravenous administration of [<sup>11</sup>C]d-threo-MP to rats the tracer activity was determined in blood, urine and various organs after 5, 10 and 30 min. The results of the biodistribution studies are shown for tissues (brain, liver, heart, kidneys, spleen, lung, muscle, bone, colon, small intestine, fat, blood, striatum and cerebellum) expressed as %ID/g in Table 1 and where applicable for the individual organs expressed as % ID/organ in Table 2.

## DISCUSSION

### *Precursor synthesis*

The preparation of the precursor [d-threo-1-(2-nitrophenylsulfanyl)-piperidin-2-yl]phenylacetic acid (d-threo-N-NPS-ritalinic acid) starts from the commercially available racemate of d- and l-threo-MP. In a first step d-threo-MP, the active enantiomer, is separated from l-threo-MP (2), followed by introduction of the protecting group and subsequent hydrolysis of the ester yielding d-threo-N-NPS-ritalinic acid.

However, the last step of precursor synthesis, the basic hydrolysis of the ester yielding the free acid, is accompanied by some isomerization, the d-threo configuration is thereby converted to the l-erythro configuration.

## RADIOLABELING

### *Labeling with [<sup>11</sup>C]CH<sub>3</sub>I*

The applied reaction procedure follows in principle the method suggested previously by Ding et al. (4) with some modifications. We were able to omit one solid phase extraction step, that was described as necessary in the original publication. It was found that the separation of threo-MP from erythro-MP could be achieved by reversed phase HPLC without a previous Sep Pak purification step if the pH value of the buffer in the HPLC eluent is adjusted to 8.5 and if the reaction mixture is adjusted to basic pH by addition of 250 µL 2 M NaOH prior to injection (Figure 3). This facilitates the automation of the

**Table 1.** Biodistribution data for [<sup>11</sup>C]d-threo-MP, values are mean of 3 experiments ± sd, expressed as % injected dose per g tissue.

Tissue	5 min	10 min	30 min
brain	0.9 ± 0.4	0.9 ± 0.2	0.4 ± 0.1
liver	1.2 ± 0.3	1.9 ± 0.1	1.2 ± 0.6
heart	0.7 ± 0.2	0.51 ± 0.08	0.2 ± 0.1
kidneys	3.6 ± 0.8	2.1 ± 0.4	1.6 ± 0.9
spleen	1.5 ± 0.3	1.6 ± 0.2	0.9 ± 0.3
lung	4.8 ± 2.4	4.0 ± 2.1	1.3 ± 0.7
muscle	0.20 ± 0.02	0.24 ± 0.05	0.18 ± 0.05
bone	0.26 ± 0.02	0.19 ± 0.04	0.17 ± 0.01
colon	0.23 ± 0.04	0.3 ± 0.1	0.18 ± 0.04
small intestine	0.90 ± 0.10	0.6 ± 0.4	1.6 ± 0.6
fat	0.22 ± 0.16	0.16 ± 0.01	0.16 ± 0.01
urine	0.5 ± 0.3	3.1 ± 2.4	3.4 ± 0.8
blood	0.09 ± 0.04	0.07 ± 0.02	0.10 ± 0.03
striatum	1.0 ± 0.4	1.3 ± 0.4	0.7 ± 0.3
cerebellum	0.9 ± 0.3	0.9 ± 0.2	0.3 ± 0.2

**Table 2.** Biodistribution data for [<sup>11</sup>C]d-threo-MP, values are mean of 3 experiments ± sd, expressed as % injected dose per organ.

Organ	5 min	10 min	30 min
liver	12.0 ± 2.9	15.5 ± 0.4	15.9 ± 3.6
heart	0.6 ± 0.2	0.4 ± 0.1	0.2 ± 0.1
kidneys	7.7 ± 1.4	4.2 ± 1.0	3.2 ± 1.6
spleen	1.18 ± 0.04	1.0 ± 0.2	0.5 ± 0.2
lung	6.9 ± 3.3	5.3 ± 2.9	1.4 ± 0.7
small intestine	6.4 ± 1.5	4.9 ± 0.1	9.9 ± 3.7

process. By this modification we were able to use a commercially available synthesis module for the fully automated production of [<sup>11</sup>C]d-threo-MP without major alterations. By this procedure sufficient amounts of [<sup>11</sup>C]d-threo-MP could reliably be produced for clinical applications: an irradiation using 50 µA for 60 min results in about 2 to 3 GBq (EOS) of pure [<sup>11</sup>C]d threo-MP ready for injection.

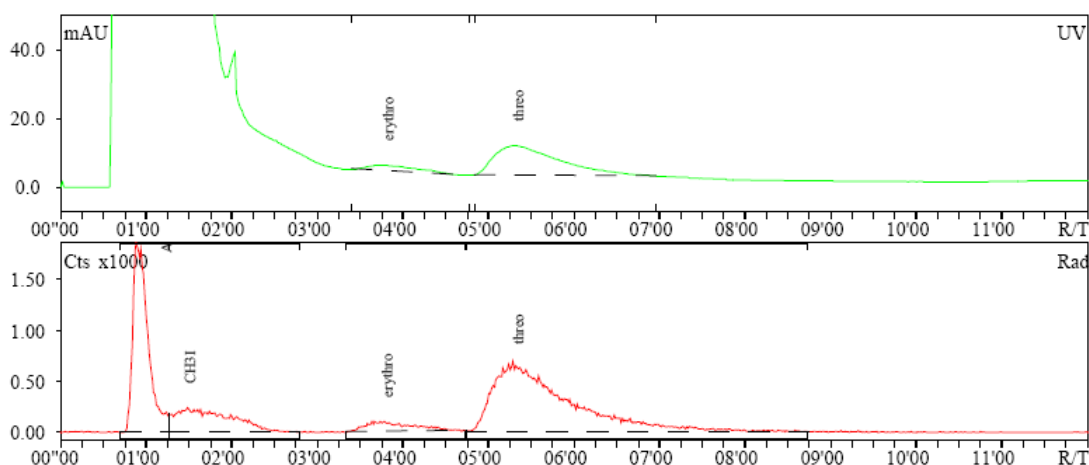
Furthermore, in our hands the use of the sodium salt of the precursor was superior to the original procedure which makes use of the free ritalinic acid and aqueous NaOH, thus producing also some amount of [<sup>11</sup>C]methanol (Table 3). Radiochemical yields were 6 ± 3 % with d-threo-N-NPS-ritalinic acid as precursor under basic conditions (NaOH). This yield is lower than initially reported by Ding et al. (4); the reasons herefore are speculative and might result from different precursor qualities or other site specific differences. Nevertheless, it could

be shown, that the use of the sodium salt of the precursor without addition of NaOH was superior to the use of free d-threo-ritalinic acid, since labeling yields were increased (20 ± 8 %).

One might expect that the formation of the sodium salt of the precursor could influence the enantiomeric purity of the final product since strongly basic conditions are applied during the preparation. Therefore, enantiomeric purity of the final product was determined by chiral HPLC and was found to exceed 98 % for both precursors used.

#### **Labeling with [<sup>11</sup>C]diazomethane**

The carbon-11 labeling of d-threo-N-NPS-ritalinic acid via [<sup>11</sup>C]diazomethane for the production of [<sup>11</sup>C]d-threo-MP can be an interesting alternative to the conventional approach using [<sup>11</sup>C]CH<sub>3</sub>I as the labeling agent, provided, that an automated production of [<sup>11</sup>C]diazomethane can be realized and similar amounts can be produced. Main



**Figure 3.** Isolation of [ $^{11}\text{C}$ ]d-threo-MP from the reaction mixture by preparative HPLC (Partisil 10 ODS 3, 250 x 8 mm, 0.17 M ammonium formate buffer pH 8.5/ $\text{CH}_3\text{CN}$ , 70/30, v/v, 8 mL/min), upper channel: UV detection at 220 nm, lower channel: radioactivity.

advantages are the mild reaction conditions (room temperature, short reaction time, no supportive base needed) and the fast labeling procedure: production of [ $^{11}\text{C}$ ]diazomethane and preparation of [ $^{11}\text{C}$ ]d-threo-MP was completed within 16 min without final work-up of the reaction mixture. Labeling yields were at this point determined in solution by radio-HPLC and based on [ $^{11}\text{C}$ ]diazomethane starting activity.

In order to be able to compare the process times needed for the complete reaction procedure including semipreparative HPLC, solid phase extraction, solvent evaporation, formulation and sterile filtration for the [ $^{11}\text{C}$ ]diazomethane approach with the overall process time needed for the [ $^{11}\text{C}$ ]CH $_3\text{I}$  approach (42 min) the following estimation was done: under the assumption that the time needed for work-up is equivalent to the [ $^{11}\text{C}$ ]CH $_3\text{I}$  approach (18.5 min) an overall process time of 34.5 min would result. In principle it should

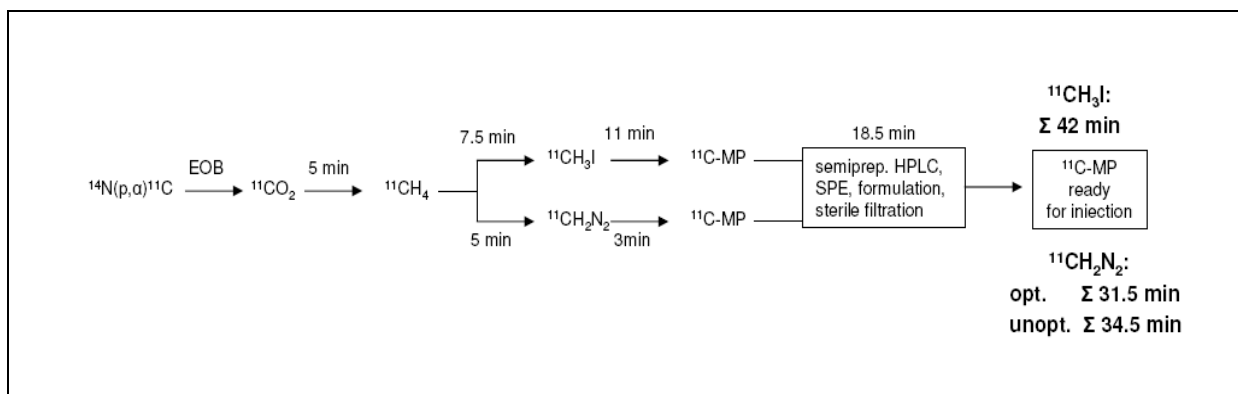
be possible to reduce the time needed for [ $^{11}\text{C}$ ]methane production to 5 min as it is realized in the [ $^{11}\text{C}$ ]CH $_3\text{I}$  production process, this would result in an optimized process time of 31.5 min (Figure 4). An effective, i.e. automated, [ $^{11}\text{C}$ ]diazomethane synthesis process will then most likely result in an improved specific activity of the labelled product [ $^{11}\text{C}$ ]d-threo-MP because of the shorter processing time.

#### ***Biodistribution Studies***

After intravenous administration of [ $^{11}\text{C}$ ]d-threo-MP to rats the tracer activity was determined in blood, urine and various tissue samples (brain, liver, heart, kidneys, spleen, lung, muscle, bone, colon, small intestine, fat, blood, striatum and cerebellum) after 5, 10 and 30 min. Results are calculated as % injected dose per g tissue (% ID/g) in Table 1 and when possible also as % injected dose per organ (% ID/organ) in Table 2.

**Table 3.** Comparison of radiochemical yields and radiochemical purity using either d-threo-N-NPS ritalinic acid with NaOH addition (column 2) or the sodium salt of d-threo-N-NPS ritalinic acid (column 3).

Product Quality	Precursor used	
	d-threo-N-NPS ritalinic acid (+ NaOH)	sodium salt of d-threo-N-NPS ritalinic acid
Radiochemical Yield (based on [ $^{11}\text{C}$ ]CH $_3\text{I}$ starting activity)	6 $\pm$ 3 %	20 $\pm$ 8 %
Radiochemical Purity of [ $^{11}\text{C}$ ]d-threo-MP (determined by HPLC)	98 $\pm$ 1 %	97 $\pm$ 0.1 %



**Figure 4.** Comparison of the estimated process time for the complete production of [ $^{11}\text{C}$ ]d-threo-MP from [ $^{11}\text{C}$ ]diazomethane (lower part) with the realized process time for the production of [ $^{11}\text{C}$ ]d-threo-MP from [ $^{11}\text{C}$ ]CH<sub>3</sub>I (upper part).

Following injection the tracer was rapidly extracted from the blood pool resulting in a low blood activity of 0.09 % ID/g at 5 min post injection (p.i.) and remained at this level during the following period of the study. The tracer was primarily excreted via the hepatic excretion pathway (liver uptake 15.9 % ID, small intestine 9.9 % at 30 min p.i.). Renal excretion was less pronounced as indicated by a kidney uptake of 7.7 % ID at 5 min p.i. decreasing to 3.2 % ID at 30 min p.i. and a relatively low amount of tracer found in urine, ranging from 0.5 % ID/g at 5 min p.i. to 3.4 % ID/g at 30 min p.i.

Brain uptake was as high as 0.9 % ID/g within 5 and 10 min p.i.; analysis of brain substructures such as striatum and cerebellum revealed highest uptake in the striatum at 10 min p.i., whereas the activity in the cerebellum was similar to that of the total average brain activity. These findings are consistent with known distribution of dopaminergic neurons in the rat brain.

## SUMMARY

$^{11}\text{C}$ -labeling of methylphenidate was realized by three different synthetic approaches: (1) esterification of either d-threo-N-NPS-ritalinic acid with [ $^{11}\text{C}$ ]CH<sub>3</sub>I under basic conditions or (2) by the application of the sodium salt of d-threo-N-NPS-ritalinic acid as precursor (without additional base needed) or (3) by reaction of d-threo-N-NPS-ritalinic acid with [ $^{11}\text{C}$ ]diazomethane. The purification process could be simplified (omission of one solid phase extraction step), resulting in an easily automated process for the production of the tracer. The preparation of [ $^{11}\text{C}$ ]d-threo-MP by means of [ $^{11}\text{C}$ ]diazomethane as the labeling agent appears to be an alternative to the [ $^{11}\text{C}$ ]CH<sub>3</sub>I

methods because the shorter overall process times and high labeling yields result in higher specific activities of the product.

Moreover, biodistribution data of [ $^{11}\text{C}$ ]methylphenidate in rats appear to be in accordance with the expected distribution of presynaptic dopamine transporters and are valuable for calculating dosimetry for this tracer.

## REFERENCES

- [1] Volkow, N.D., Ding, Y.S., Fowler, J.S., Wang, G.J., Logan, J., Gatley, S.J., Schlyer, D.J., Pappas, N. A new PET ligand for the dopamine transporter: studies in the human brain. *J. Nucl. Med.*, 36: 2162-2168, 1995.
- [2] Patrick, K.S., Caldwell, R.W., Ferris, R.M., Breese, G.R. Pharmacology of the enantiomers of threo-methylphenidate. *J. Pharmacol. Exp. Ther.*, 241: 152-158, 1987.
- [3] Markowitz, J.S., DeVane, C.L., Pestreich, L.K., Patrick, K.S., Muniz, R. A comprehensive in vitro screening of d-, l-, and dl-threo-methylphenidate: an exploratory study. *J. Child Adolesc. Psychopharmacol.*, 16: 687-698, 2006.
- [4] Ding, Y.-S., Sugano, Y., Fowler, J., Salata, C. Synthesis of the racemate and individual enantiomers of [ $^{11}\text{C}$ ]methylphenidate for studying presynaptic dopaminergic neurons with positron emission tomography. *J. Label. Compd. Radiopharm.*, 34: 889-997, 1994.
- [5] Patt, M., Stahlschmidt, A., Blocher, A., Gündisch, D., Kovar, K.-A., Machulla, H.-J. Qualitätskontrolle und Automatisierung des Dopamintransporterliganden [ $^{11}\text{C}$ ]Methylphenidat. *Nuklearmedizin*. 37: A51, 1998.
- [6] Solbach, C., Machulla, H.-J. Production of [ $^{11}\text{C}$ ]Chloroform by direct chlorination of



[<sup>11</sup>C]methane without catalyst support for the synthesis of [<sup>11</sup>C]diazomethane. *Appl Radiat Isot.* Accepted, 2007.

[7] Larsen, P., Ulin, J., Dahlstroem, K., Jensen, M. Synthesis of [<sup>11</sup>C]iodomethane by iodination of [<sup>11</sup>C]methane. *Appl. Radiat. Isot.*, 48: 153-157, 1997.