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3-(4-(6-Fluoroalkoxy-3,4-dihydroisoquinoline-2(1*H*)-yl)cyclohexyl)-1*H*-indole-5-carbonitriles for SERT imaging: Chemical synthesis, evaluation in vitro and radiofluorination

Uta Funke *, Steffen Fischer, Achim Hiller, Matthias Scheunemann, Winnie Deuther-Conrad, Peter Brust, Jörg Steinbach

Institut für Interdisziplinäre Isotopenforschung, Permoserstr. 15, D-04318 Leipzig, Germany

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ABSTRACT

Aminocyclohexyl indoles bind with high affinity and specificity toward the serotonin transporter (SERT). Based on this structural lead, we designed fluoroalkoxydihydroisoquinoline-cyclohexyl indole carbonitriles for future application as ^{18}F-labeled tracers for SERT imaging by PET. Six compounds, three pairs of cis- and trans-isomer derivatives, respectively, were synthesized and evaluated in vitro. The chemistry of the new compounds, their affinity and specificity data, the general route to the phenolic precursor for labeling, and the successful ^{18}F-fluoroalkylation of one pair of compounds are described herein.

The serotonin transporter (SERT) regulates the extracellular serotonin concentration by the reuptake of serotonin from the synaptic cleft into the presynaptic nerve cell and consequently plays a key role in serotonergic signaling.[1,2] Alterations of SERT densities in the brain are related to several neurodegenerative and psychiatric disorders such as Parkinson's disease and depression.[1,2]

Different classes of compounds therefore were investigated for their antidepressant properties as selective serotonin reuptake inhibitors (SSRI), for example, 3-(aminocycloalkenyl)-indole-5-nitrile derivatives[3,4] and arylpiperazinyl-cyclohexyl indole derivatives.[5]

Meagher et al. presented aminocyclohex(en)yl indole carbonitriles, in particular the 6-methoxydihydroisoquinoline *cis*-cyclohexyl derivative **1** (Fig. 1), with nanomolar affinities to the SERT and moderate to high selectivities toward the serotonin 5-HT_{1A} and adrenergic α_1 receptors.[6] These promising results initiated our development of potential ^{18}F-labeled tracers for positron emission tomography (PET).

So far, for imaging and characterization of the SERT in humans only ^{11}C-labeled ligands are used, with [^{11}C]McN5652 and [^{11}C]DASB as the most widely accepted PET radiotracers.[2] The high non-specific binding of [^{11}C]McN5652 in the brain results in low signal-to-noise ratio, so that imaging of SERT in regions of moderate or low density is difficult. This shortcoming could only partially be improved by diarylsulfide-based compounds such as [^{11}C]DASB, [^{11}C]MADAM, [^{11}C]AFA, or [^{11}C]ADAM and [^{18}F]ADAM.[7]

Our intention thus was to provide a more suitable and preferentially ^{18}F-labeled SERT-radiotracer. Based on the above mentioned class of indole derivatives, in particular on the chemical lead **1**, we synthesized two new 6-fluoroalkoxydihydroisoquinoline- and four known dihydroisoquinoline-cyclohexyl[6] derivatives. Organic synthesis of the non-radioactive reference compounds, the in vitro affinities and the radiofluorination via ^{18}F-fluoroalkylation are reported herein.

Schemes 1–3 show the synthesis of the non-radioactive compounds. The first building block was synthesized by the basic condensation of the 5-cyano indole **2** with 1,4-cyclohexanedione mono-ethylene ketal **3**.[5,8,9] In this step, attention should be paid

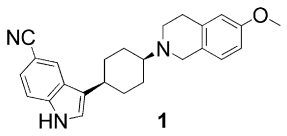

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Figure 1. Chemical lead for the development of 6-fluoroalkoxydihydroisoquinoline derivatives.

* Corresponding author. Tel.: +49 341 235 3695; fax: +49 341 235 2731.
E-mail address: funke@iif-leipzig.de (U. Funke).

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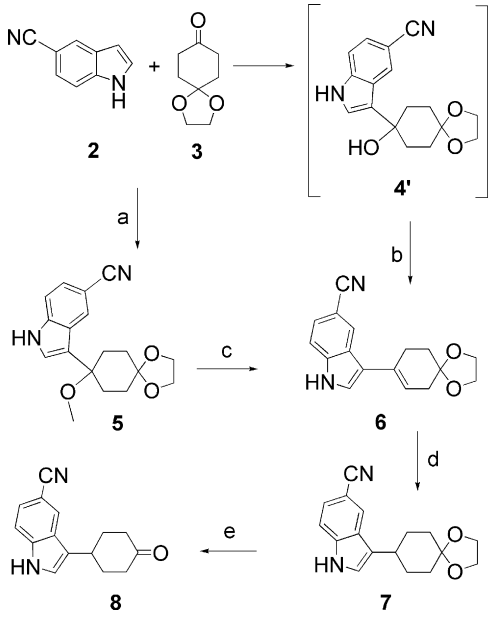
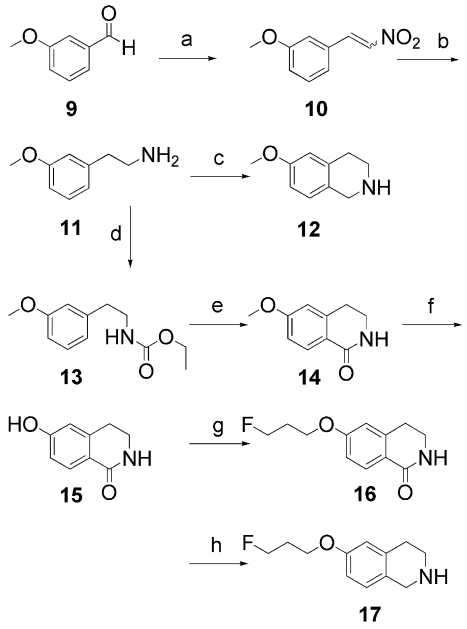


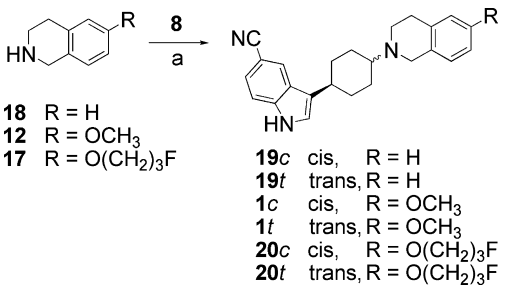
Scheme 1. Reagents and conditions: (a) KOH, MeOH, 78 °C; (b and c) KOH, MeOH, 85–90 °C (61%); (d) H_2, Pd/C, THF/MeOH, rt, 24 h (79%); (e) conc. HCl, THF, rt, 24 h (82%).



Scheme 2. Reagents and conditions: (a) CH_3NO_2, NH_4OAc, AcOH, reflux, 2–3 h (69%); (b) $LiAlH_4$, THF, CH_2Cl_2, 0 °C/rt, 3–4 h (62%); (c) HCHO, H_2O, 37% HCl, 100 °C, 4 h (92%); (d) $ClCO_2Et$, Et_3N, CH_2Cl_2, 5 °C/rt, 2 h (quant.); (e) PPA, 120–130 °C, 3 h (52%); (f) BBr_3, CH_2Cl_2, −78 °C/rt, 16–18 h (60%); (g) $I(CH_2)_3F$, K_2CO_3, MeCN, reflux, 10 h (78%); (h) $LiAlH_4$, THF, 0 °C/ reflux, 5 h (90%).

to superheated reaction temperatures (Scheme 1, b and c). Standard reaction conditions (Scheme 1, a) led to a high content of the methoxyderivative **5** as byproduct, which was isolated and identified via NMR-spectrometry.[10] Hydrogenation of **6** afforded the saturated ketal **7**, followed by acidic hydrolysis to the indolyl-cyclohexanone **8**.[5,9]

The synthesis of the tetrahydroisoquinoline building blocks (Scheme 2) started with a condensation reaction of the *m*-anisaldehyde **9** to the nitrostyrene **10**,[11] followed by reduction to the phenethylamine **11**.[12] Cyclisation via Pictet–Spengler[13] formed the 6-methoxytetrahydroisoquinoline **12**. Compound **10** was also used as educt for the synthesis of the phenethylcarbamate **13**, immediately converted to the lactam **14**.[14] The thus obtained intramolecular protecting group enabled the following fluoropropylation to the ether **16** after the demethylation[15] to the phenol **15**. Reduction of the isoquinolinone with $LiAlH_4$ provided the 6-fluoropropoxy-tetrahydroisoquinoline **17.**



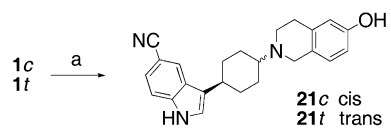
Scheme 3. Reagent and condition: (a) $NaBH(OAc)_3$, MeCN, AcOH, rt, 16–20 h. Flash chromatography: silica gel, cyclohexane:MTBE: 30% NH_3 1:2:0.1 or 1:3:0.1 (16–37% per isomer).

Reductive amination[16] of **8** with the appropriate tetrahydroisoquinoline components **12**, **17**, and **18** led to the SERT ligands **1**, **20**, and **19**, respectively, as isomer mixtures in a ratio of 2:3 (cis:trans). The cis- and trans-isomers were separated by liquid chromatography (Scheme 3).

The 6-methoxydihydroisoquinoline-cis- and -trans-derivatives **1***c* and **1***t* served as starting material for the synthesis of the precursors (Scheme 4) for the radiosyntheses. Cleavage of the methyl ether group using BBr_3[15] resulted in the phenolic compounds **21***c* and **21***t*, respectively.

Compounds **1***c*/**1***t*, **19***c*/**19***t*, and **20***c*/**20***t* (Scheme 3) were tested regarding their affinity and selectivity for the SERT. For human SERT, human norepinephrine transporter (hNET), and human dopamine transporter (hDAT), IC_{50} values of the test compounds were determined on HEK293 cells stably expressing these monoamine reuptake transporters using [^{3}H]paroxetine/[^{3}H]citalopram, [^{3}H]nisoxetine, and [^{3}H]WIN35,428 as the specific radioligands. r5HT$_{1A}$ affinity was determined on rat cortical membrane homogenate with [^{3}H]-8-OH-DPAT as specific radioligand.[17]

All test compounds were assayed in at least three independent experiments, each conducted in triplicate.[18] The target specific K_i values of the reference compounds **19***c*, **19***t*, **1***c*, **1***t*, and the two newly developed compounds **20***c* and **20***t* are given in Table 1. Compounds **1***c*/**1***t*, **19***c*/**19***t*, and **20***c*/**20***t* selectively bind to the hSERT in the nanomolar range. Their affinity toward the [^{3}H]citalopram-binding site is up to 140-fold higher than toward the [^{3}H]paroxetine-binding site. These results might reflect the recently reported differences in the binding sites of specific SERT ligands.[20,21] In general, the cis-isomers show a significantly higher affinity to the hSERT and a lower affinity to the hNET than the respective trans-isomers. In comparison to their trans-isomers, the alkoxy-substituted cis-isomers **1***c* and **20***c* possess remarkably lower affinity to the r5HT$_{1A}$. The K_i values for hDAT appear similar (~400 nM) for all compounds and isomers.

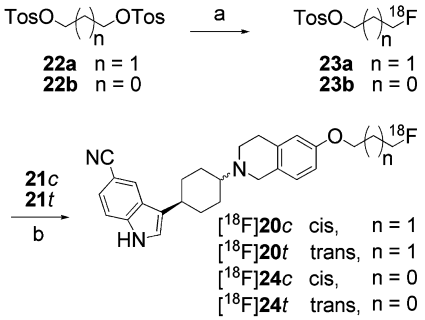


Scheme 4. Reagents and conditions: (a) BBr_3, CH_2Cl_2, −78 °C/rt; workup: aqueous $NaHCO_3$, 4 °C (42%).

Table 1
Binding affinities of reference and test compounds at hSERT, r5HT$_{1A}$, hDAT, and hNET; K_i in nM

Compound	hSERT		r5HT$_{1A}$	hNET	hDAT
	[^{3}H]citalopram	[^{3}H]paroxetine	[^{3}H]-8-OH-DPAT	[^{3}H]nisoxetine	[^{3}H]WIN35,428
19*c*	4.51 ± 1.97	189 ± 79.3	>1000[a]	1430 ± 370	424 ± 203
19*t*	90.7 ± 17.9	286 ± 84.9	2530 ± 1120	184 ± 95.5	309 ± 25.0
1*c*	4.10 ± 2.32	165 ± 67.0	13,100 (10,600, 15,600)[a]	1020 ± 188	370 ± 149
1*t*	73.7 ± 25.2	518 ± 199	267 ± 38.8	96.7 ± 23.8	462 ± 96.6
20*c*	6.16 ± 0.91	914 ± 129	5670 (7150, 4190)[a]	772 ± 217	366 ± 162
20*t*	327 ± 160	2310 ± 833	2863 ± 728	102 ± 71.4	343 ± 113
Paroxetine	0.42 ± 0.13	0.38 ± 0.12	—	—	—
Citalopram	4.38 ± 1.17	32.8 ± 8.69	—	—	—
MADAM	0.73 ± 0.37	2.03 ± 1.08	—	—	—

IC_{50} values were obtained from triplicates in each experiment, and the calculated K_i values are given as single values ($n = 1$), means and single values ($n = 2$), or means ± SD ($n > 2$).
[a] $n = 2$.



Scheme 5. Reagents and conditions: (a) [^{18}F]F$^-$, K2.2.2, K_2CO_3, MeCN, ~80 °C, 15 min; (b) K2.2.2, K_2CO_3, MeCN, ~80 °C, 10 min.

Compounds **20***c* and **20***t* were chosen as target compounds for radiolabeling with fluorine-18 via a prosthetic group in a one-pot synthesis (Scheme 5).

The labeling precursor, 1,3-bistosyloxypropane **22a** (approx. 4 mg in 1 ml MeCN), was converted to ^{18}F-labeled alkyltosylate, according to a standard procedure.[22] After the transformation of [^{18}F]fluoride into [K2.2.2.]^{18}F-carbonate complex, no-carrier-added [^{18}F]fluoropropyltosylate **23a** was obtained within 10–15 min reaction time (labeling yield [LY] 60–80%). For the second step of ^{18}F-labeling via etherification, the phenolic precursor **21***c* or **21***t* (2 mg of the corresponding hydrochloride, respectively) was activated by pretreatment with K_2CO_3 and K2.2.2 in refluxing methanol.[23] To the dried phenolate of **21***c* or **21***t*, the intermediate **23a** in MeCN was added without further purification and heated up to 80–85 °C in 10 min (LY 40–50%). The resulting mixture was purified by SPE (Sep-Pak C18 Plus cartridge) followed by semi-preparative radio-HPLC (Fig. 2).

After separation, the combined fractions of [^{18}F]**20***c* or [^{18}F]**20***t* were diluted and concentrated by SPE (RP-18 phase or alternative ion exchanger). The final products could be nearly quantitatively eluted with EtOH, MeOH, MeCN, or an aqueous buffer solution (radiochemical yield 11–22% [end of synthesis], total synthesis time 2.5 h). Radiochemical and chemical purities were determined by HPLC and TLC (⩾99%), and specific activity by analytical HPLC (50 GBq/μmol). The described procedure is potentially suitable for an automated module synthesis.

Comparable results were also achieved with the precursors **21***c* and **21***t* in the form of their free amino bases. By the same radiochemical approach, we obtained acceptable yields also for the fluoroethoxy derivatives [^{18}F]**24***c* and [^{18}F]**24***t* (Scheme 5, LY 50–60%), starting from 1,2-bistosyloxyethane **22b**.

Optimum precursor amounts for radiofluorination were determined to be 3–5 mg of **22a** or **22b**, and for etherification 2 mg of

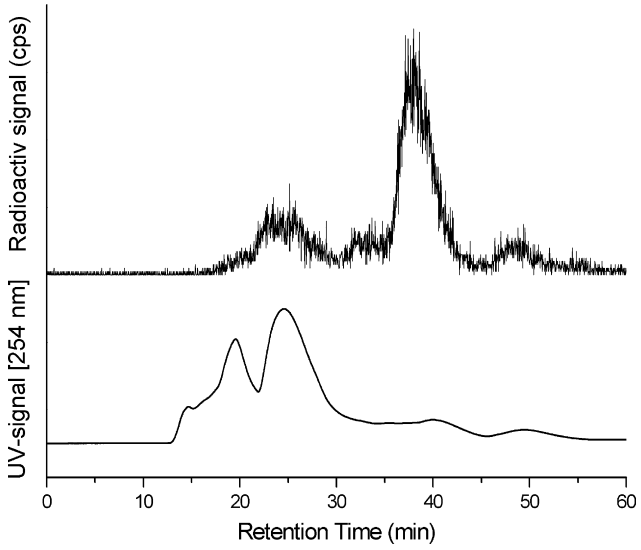


Figure 2. Semi-preparative HPL-chromatogram of [^{18}F]**20***c*, $t_R = 38$ min. Eluent: MeCN 40%, TFA 0.1%/ H_2O. Flow rate: 0.75–1.25 ml/min. Column: Multisorb RP18-7 (250 × 10 mm, 7 μm, CS Chromatography Service, Germany).

21*c* or **21***t* (hydrochlorides), each per 1 ml reaction medium. A large excess of **22a** resulted in the formation of various byproducts during the etherification, while negligible ^{18}F-propylation was obtained probably due to direct alkylation of the phenol with **22a**.

To provide some hints on the expected brain uptake and pharmacological behavior of [^{18}F]**20***c* and [^{18}F]**20***t*, *n*-octanol–water partition coefficients (log*P*, log*D*),[24] non-specific protein binding, and chemical stability in different media under physiological conditions were determined.

Log*P* and log*D* values were calculated with ACD/LogD[25] and MarvinSketch,[26] and experimentally determined with batch exper-

Table 2
Experimental and calculated *n*-octanol–water partition coefficients of [^{18}F]fluoropropoxy derivatives

	[^{18}F]**20***c*		[^{18}F]**20***t*	
	log*P*	log*D*[a]	log*P*	log*D*[a]
ACD/log D[25]	5.41 ± 0.83	4.17	5.41 ± 0.83	4.17
MarvinSketch[26]	4.92	3.86	4.92	3.86
Extractive[27a]	—	2.47 ± 0.28	—	2.36 ± 0.15
HPLC: Multisorb[27b]	—	3.27 ± 0.52	—	3.27 ± 0.52
HPLC: Supelcosil™[27b]	—	2.54 ± 0.42	—	2.49 ± 0.41

[a] Determined values for the pH range of 7.2–7.4.

iments as well as HPLC[27] (Table 2). Experimentally, for [^{18}F]**20***c* a log*D* value of 2.47 ± 0.28 was determined by batch experiments. For [^{18}F]**20***t*, similar results with log*D* = 2.36 (± 0.15, extractive) were obtained. Within the applied pH range of 7.2–7.4, remarkably higher log*D* values of 4.17[25] and 3.86[26] were calculated for **20***c* and **20***t* (neutral form), respectively. However, the experimental values correspond to a log*P* of 2.31 ± 1.0,[25] calculated for the monoionic form, and could be caused by solvation effects in the aqueous system. Log*D* data collected by HPLC methods[24,27b] are dependent on the column systems applied but not significantly on the isomeric structure of **20**. A log*D* of 3.27 ± 0.52 for **20***c* and **20***t*, determined on the Multisorb C_{18} column, corresponds to the calculated log*D* = 3.86.[26] Results of analyses on the Supelcosil™ C_{18} column (log*D* = 2.54 ± 0.42 for **20***c*, log*D* = 2.49 ± 0.41 for **20***t*) are more consistent with the extractive data and the calculated log*D* of the monoionic form. Since log*P* values within a range of 0.9–2.5 indicate a good brain penetration of tracer compounds, we assume that [^{18}F]**20***c* and [^{18}F]**20***t* are able to cross the blood–brain barrier in a sufficient amount.

Determination of the reversible plasma protein binding of the radioligands resulted in method-dependent data. While acetonitrile precipitated only 2.7% of [^{18}F]**20***c* incubated in rat plasma,[28] ultrafiltration yielded about 50% binding of [^{18}F]**20***c* or [^{18}F]**20***t* to rat plasma protein.[29] This fraction still contains blood platelets which are known targets for SERT ligands.[2] Hence the free radiotracer fraction is about 50%, which is high enough to allow quantitative SERT imaging.[30]

No degradation was observed for [^{18}F]**20***c* and [^{18}F]**20***t* after 120 min incubation under physiological conditions[31] as determined by analytical radio-HPLC and TLC. Also under conditions more relevant for radiosyntheses such as basic conditions, and a bath temperature of 80 °C, [^{18}F]**20***c* and [^{18}F]**20***t* remained stable.[32]

In summary, radiochemical, physicochemical and pharmacological in vitro data identified the [^{18}F]fluoropropoxy derivative [^{18}F]**20***c* to be a promising radiotracer for the imaging of the SERT. Subsequently, further evaluation of [^{18}F]**20***c* will be performed to determine the radiotracer affinity and to investigate the biodistribution and the brain uptake.

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17. Cells obtained from: (a) Blakely, R. hSERT-HEK293, Vanderbilt University, Nashville, USA.; (b) Boenisch, H. hNET-HEK293, University of Bonn, Germany.; (c) Storch, A. hDAT-HEK293, Technical University Dresden, Germany.
18. For hSERT, hNET, and hDAT, the IC_{50} values of the test compounds were determined on homogenates of the respective HEK293 cell line[17] versus [^{3}H]paroxetine (SERT; PerkinElmer Life Sciences; AS = 706 GBq/mmol), [^{3}H]citalopram (SERT; Amersham GE Healthcare; AS = 3121 GBq/mmol) [^{3}H]nisoxetine (NET; PerkinElmer Life Sciences; AS = 2960 GBq/mmol), and [^{3}H]WIN35,428 (DAT; PerkinElmer Life Sciences; AS = 3145 GBq/mmol). r5HT$_{1A}$ affinity was determined on rat cortical membrane homogenate versus [^{3}H]-8-OH-DPAT (Amersham GE Healthcare; AS = 8399 GBq/mmol). For binding experiments, homogenates were diluted with the respective assay buffer (hSERT and hNET: 50 mM Tris–HCl, pH 7.4, 120 mM NaCl, 5 mM KCl; hDAT: 50 mM Tris–HCl, pH 7.4, 100 mM NaCl; r5HT$_{1A}$: 50 mM Tris–HCl, pH 7.4, 4 mM $CaCl_2$, 0.1% ascorbic acid) and incubated with the respective radioligand (~working concentrations: 1 nM [^{3}H]paroxetine, 0.5 nM [^{3}H]citalopram, 0.5 nM [^{3}H]nisoxetine, 0.5 nM [^{3}H]WIN35,428, 0.3 nM [^{3}H]-8-OH-DPAT) and 6–12 concentrations of the test compounds at 21 °C for 60 min (hSERT, hNET, and r5HT$_{1A}$) or on ice for 120 min (hDAT). Non-specific binding was determined with 300 μM clomipramin (hSERT), 100 μM protriptyline (hNET), 10 μM GBR12909 (hDAT), or 10 μM serotonin hydrochloride (r5HT$_{1A}$). K_D values were obtained by homologous competition according to the described protocols: [^{3}H]paroxetine = 0.69 nM, [^{3}H]citalopram = 4.46 nM, [^{3}H]nisoxetine = 6.77 nM, and [^{3}H]WIN35,428 = 24.1 nM. K_D of [^{3}H]-8-OH-DPAT (3.1 nM on rat cortex) was taken from Newman-Tancredi et al.[19]
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27. Experimental determination of log*D*: (a) Extractive method. $n = 3$ per buffer system. Phosphate buffer: according to Sørensen, $KH_2PO_4/Na_2HPO_4{\cdot}2H_2O$, 50 mM, pH 7.2. Dulbecco buffer: Dulbecco's PBS, Thermo Fischer Scientific, USA, Product # 1890535, pH 7.2. Tris–HCl buffer: Thermo Fischer Scientific, USA, Product # BP153-500, 30 mM, pH 7.4.; (b) HPLC-method. $n \geqslant 3$ per standard or sample. Columns: Multisorb RP18-7 (250 × 4 mm, 7 μm, CS Chromatography Service, Germany), Supelcosil™ ABZ$^+$ Plus (250 × 4 mm, 5 μm, Sigma–Aldrich, Germany). Gradient: eluent A (5% MeCN + 20 mM ammonium acetate) to eluent B (80% MeCN + 20 mM ammonium acetate) in 55 min; flow rate 1 ml/min.
28. Rat plasma, freshly isolated: protein content 95 mg/ml, $V = 75$ μl. $n = 3$, two extraction steps of precipitate each, MeCN $V = 500$ μl. Incubation: 30 min, 37 °C. Centrifugation: 2000*g*, 30 min, rt.
29. Rat plasma, freshly isolated: protein content 95 mg/ml, $V = 100$–450 μl. Buffer system: Dulbecco, $V = 125$–350 μl. Incubation: 30 min, 37 °C. MPS Micropartition Ultrafiltration Device: $M_{filter} = 30$ kDa, Millipore Corporation, USA. $n = 3$, one wash each. Centrifugation: 2000*g*, 30 min, rt.
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31. Rat cortex homogenate ($V = 300$ μl, in Tris–HCl), NaCl phys. ($V = 300$ μl), Dulbecco ($V = 300$ μl), pH 7.2–7.4, respectively. One milliliter reaction vials, 37 °C, aluminum heating plate. Sampling intervals: 30 min.
32. $NaOH_{aq}$ 0.1 and 1.3 M, respectively, one milliliter reaction vials, 80 °C, aluminum heating plate. Sampling intervals: 30 min.