Bioorganic & Medicinal Chemistry Letters 19 (2009) 3498–3501

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

[^{68}Ga]Ga-DO$_2$A-(OBu-L-tyr)$_2$: Synthesis, ^{68}Ga-radiolabeling and in vitro studies of a novel ^{68}Ga-DO$_2$A-tyrosine conjugate as potential tumor tracer for PET

Carsten Burchardt [a,†], Patrick J. Riss [a,*,†], Frederic Zoller [a], Simone Maschauer [b], Olaf Prante [b], Torsten Kuwert [b], Frank Roesch [a]

[a] Johannes Gutenberg-University Mainz, Institute of Nuclear Chemistry, Fritz-Strassmann-Weg 2, 55128 Mainz, Germany
[b] Friedrich-Alexander University, Laboratory of Molecular Imaging, Clinic of Nuclear Medicine, Schwabachanlage 6, 91054 Erlangen, Germany

ARTICLE INFO

Article history:
Received 3 April 2009
Revised 30 April 2009
Accepted 3 May 2009
Available online 7 May 2009

Keywords:
F-98 glioblastoma cells
Gallium-68
Macrocyclic ligands
Radiopharmaceuticals
Tyrosine

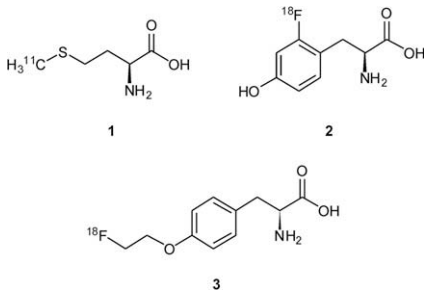
ABSTRACT

The synthesis, ^{68}Ga-labeling and in vitro study of the novel tyrosine chelate derivative [^{68}Ga]Ga-1,4,7,10-tetraazacyclododecane-1,7-diacetic acid-4,10-di-(*O*-butyl)-L-tyrosine ([^{68}Ga]Ga-DO$_2$A-(OBu-L-tyr)$_2$) as a potential tracer for imaging tumor metabolism by positron emission tomography (PET) is presented. This approach combines the biological amino acid transporter targeting properties of L-tyrosine with the outstanding availability of ^{68}Ga^{III} via the ^{68}Ge/^{68}Ga generator. In vitro studies utilizing the F98-glioblastoma cell line revealed specific uptake of [^{68}Ga]Ga-DO2A-(OBu-L-tyr)$_2$ that was comparable to that of the reference *O*-(2-[^{18}F]fluoroethyl)-L-tyrosine (FET). These promising results indicate a high potential of [^{68}Ga]Ga-DO$_2$A-(OBu-L-tyr)$_2$ for molecular imaging of tumor-driven amino acid uptake by PET.

The influx of amino acids into proliferating cells is mediated by specific transmembrane transporters, for example, the sodium-depending systems A and ASC or the sodium-independent system L.[1] To meet the need of rapidly proliferating tumors for, in particular, the non-endogenous amino acids, amino acid transport systems are overexpressed in naturally occurring neoplasms as well as in certain tumor cell lines.[2] This biochemical process can be visualized in vivo with positron emission tomography (PET), a quantitative modality of molecular imaging.[3] Various positron-emitter labeled amino acids have already been studied to probe the uptake and incorporation of amino acids into functional proteins as well as the amino acid transporter expression.[4,5] One attempt to visualize intracellular protein synthesis rate utilized (*S*)-2-amino-4-([^{11}C]methylthio)butanoic acid (**1**), isotopically labeled with carbon-11.[6] Taking the limited availability of carbon-11 in a clinical context into account, (*S*)-2-amino-3-(2-[^{18}F]fluoro-4-hydroxyphenyl) propanoic acid (**2**) was synthesized. The longer half-life of ^{18}F results in the capability to distribute the tracer to satellite PET sites. Due to the electrophilic production route, this tracer did not find systematic application.[7]

Further improvements involved (*S*)-3-(4-(2[^{18}F]-fluoroethoxy)phenyl)-2-aminopropanoic acid, FET (**3**), a compound that is not incorporated into proteins and thus solely reflects amino acid transport. It is readily available via nucleophilic fluorination.[8] The structural formulas of **1**, **2**, and **3** are shown in Scheme 1.

It was found that incorporation of the radiolabeled amino acids into proteins is not necessarily a prerequisite for visualization of tumors in vivo by PET. Instead, successful targeting of amino acid transporters appeared to be sufficient for imaging proliferating tumor cells mainly due to enhanced amino acid transport.[9] The acceptance of the above mentioned amino acid derivative **3** by amino acid carriers indicates a principal structural tolerance among the variety of amino acid transport systems. In an extension of this concept, we hypothesize that amino acid transporters may



Scheme 1. Structural formulas of the radiolabeled amino acids L-[^{11}C-methyl]-methionine (**1**), 2-[^{18}F]fluoro-L-tyrosine (**2**) and *O*-(2-[^{18}F]fluoroethyl)-L-tyrosine (**3**).

* Corresponding author. Tel.: +49 6131 3925701.
E-mail address: riss@uni-mainz.de (P.J. Riss).
† These authors have contributed equally to this work.

0960-894X/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2009.05.001

tolerate a conjugation of even more complex structural components. This may in particular create the option to introduce chelators to the amino acids, for example, for radiolabeling with metallic radionuclides such as $^{68}Ga^{III}$, a promising positron emitter derived from the commercially available $^{68}Ge/^{68}Ga$-radionuclide generator.[10]

^{68}Ga exhibits a high positron abundance of 89% and a half-life (^{68}Ga, $t_{1/2}$ = 1.13 h) well suited for PET. The relation between mothers (^{68}Ge, $t_{1/2}$ = 270.8 d) and daughters half-life provides the possibility to elute $^{68}Ga^{III}$ as $^{68}GaCl_3$ from the generator multiple times a day. $^{68}Ge/^{68}Ga$-radionuclide generators can be used for about one year. Due to this fact this radionuclide generator system provides increased availability and significant cost reduction compared to the cyclotron-produced nuclides.

However, the challenge in preparing (radio)metal-containing amino acids with retained biological activity is to develop an appropriate synthetic strategy. In the case of many metals, covalent binding to amino acid structures is not an option. The introduction of bifunctional chelate ligands may represent an alternative approach.[11] L-Tyrosine appears to be a promising lead for the introduction of a chelating moiety. This is due to the free aromatic hydroxyl group, tolerating chemical modification as shown for FET.

In this study, we report the feasibility of generating a $^{68}Ga^{III}$-labeled tyrosine derivative that reveals specific uptake in glioblastoma cells in vitro. Various different approaches to couple targeting molecules to a macrocyclic ligand have been reported so far.[12] Herein the utility of the two ring-nitrogen atoms of 1,4,7,10-tetraazacyclododecane-1,7-diacetic acid (DO_2A) to attach two tyrosine moieties via an alkyl spacer is described for the first time. With this concept we intend to increase the intramolecular amino acid concentration of the compounds, while maintaining the overall molecular weight in a reasonable range.

The synthesis route to the precursor **9** is shown in Scheme 2. A *tert*-butyl-protected DO_2A (**7**) was synthesized in three steps starting from cyclen with an overall yield of 68%.[13] Two L-tyrosine moieties were introduced to form a bivalent DO_2A-tyrosine conjugate. To compensate potential affinity-decreasing side effects of a bulky and charged Ga^{III}-chelate, we inserted a butyl spacer unit between the hydroxyl group of L-tyrosine and a ring-nitrogen of the macrocyclic chelator.

The methylester, *tert*-butylester and BOC protective groups of **8** were subsequently cleaved employing standard reaction conditions. Purification of the resulting labeling precursor **9** was performed by HPLC, and the TFA-salts were removed using an ion-exchange resin.[14]

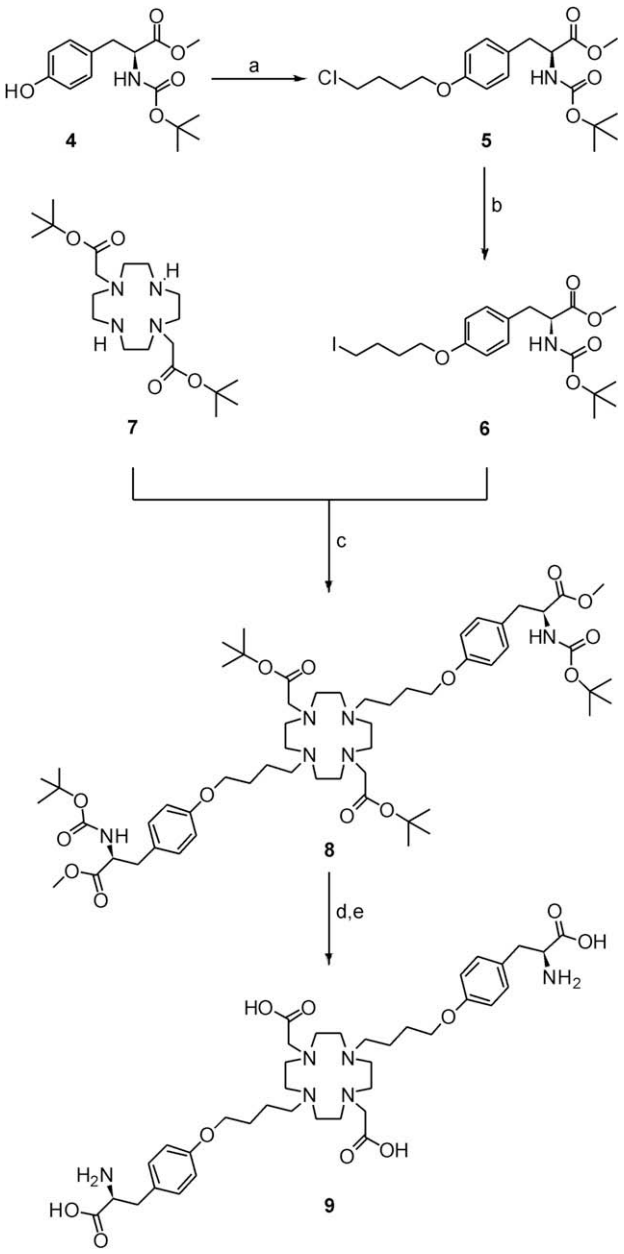
^{68}Ga-labeling of the precursor **9** was carried out in aqueous solution at 90 °C with 50 μl $^{68}Ge/^{68}Ga$-generator eluate, containing purified no carrier added $^{68}GaCl_3$.[15] The labeled product (Scheme 3) was obtained in 94 ± 2% yield after 5 min.

The time dependency of labeling yields for **10** is given in Figure 1. The radiochemical purity after solid phase extraction was greater than 98% as analyzed by radio-TLC and radio-HPLC.

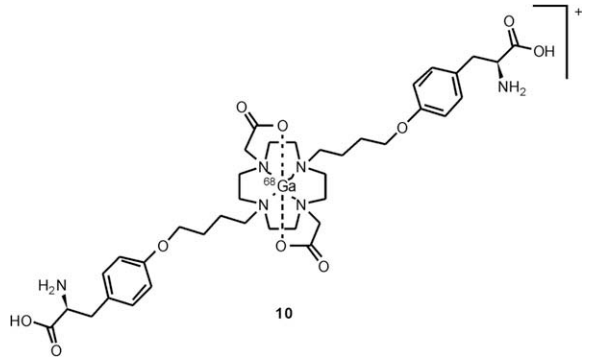
The radiolabeled product was formulated in phosphate-buffered saline (PBS) buffer (pH 7.4), and sterile-filtered prior to application. Stability of the ^{68}Ga-labeled complex **10** was proven in a diethylentriamine-penta-acetic acid (DTPA)- and an *apo*-transferrin challenge experiment at 37 °C for 2 h. The results indicate adequate stability with about 99% of ^{68}Ga-labeled DO_2A-(OBu-L-tyr)$_2$ remaining intact within this period of time (Fig. 2).

In vitro studies for the determination of specific uptake of the novel tracer **10** were performed utilizing a F98 rat glioblastoma cell line.

This in vitro model has successfully been used previously in various studies which aimed at characterizing amino acid transport of tracers.[17] The time-dependency of uptake was followed over



Scheme 2. Synthesis route to the labeling precursor **9**. Reagents and conditions: (a) 1-bromo-4-chlorobutane, K_2CO_3, acetone, reflux, 91% yield, (b) NaI, acetone, 94%, (c) *N,N*-diisopropylethylamine, MeCN, 60 °C, 42% yield, (d) 1 M NaOH/dioxane, (e) TFA, 60% yield (over two steps).



Scheme 3. Structural formula of [^{68}Ga]Ga-DO_2A-(OBu-L-tyr)$_2$ (**10**).[16]

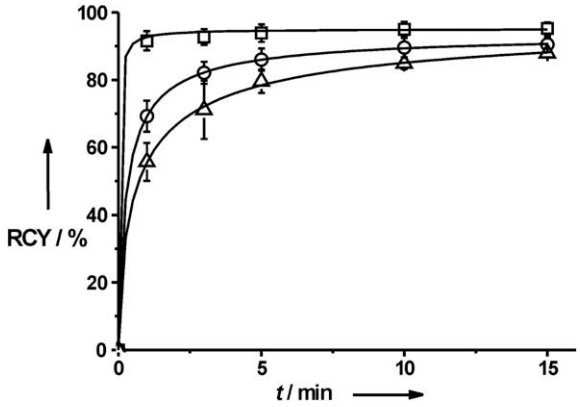


Figure 1. Radiochemical yields (RCY) of **10** by labeling of DO_2A-(OBu-L-tyr)$_2$ (**9**) with ^{68}Ga at three different temperatures (90 °C (squares), 70 °C (circles), 50 °C (triangles)) in aqueous solution. Labeling yields are given as mean values (%) ± standard deviation.

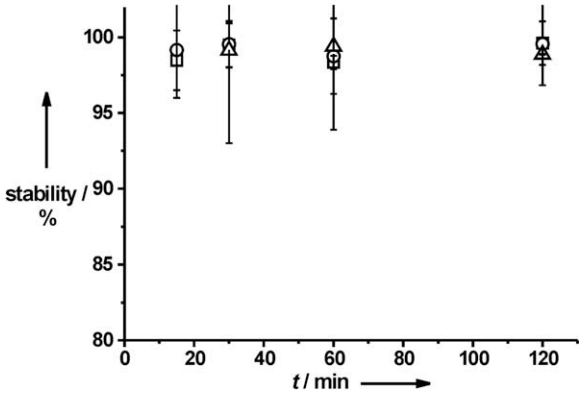


Figure 2. Challenge experiment of [^{68}Ga]Ga-DO_2A-(OBu-L-tyr)$_2$ with 10 ml of DTPA in two concentrations (1 mmol, 750-fold excess; squares; 1 μmol, 0.75 equiv, circles) and 5 ml transferrin solution (1 mmol, 375-fold excess, triangles) at 37 °C over 120 min (n = 3). Stability values were expressed as mean values of intact tracer (%) ± one standard deviation.

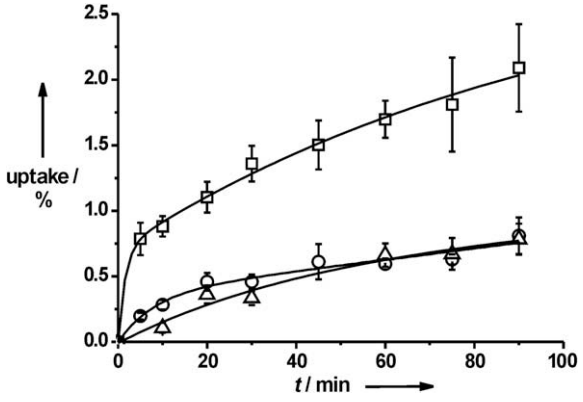


Figure 3. (a) Total uptake of **10** to F98 glioblastoma cells at 4 °C (squares, n = 6). (b) Cellular uptake of ^{68}Ga-labeled DO_2A (circles, n = 6). (c) Blocking of **10** was determined as uptake of **10** in the presence of the amino acids Trp, Ser, BCH (each 5 mM; triangles, n = 3). Uptake values were expressed as mean values (%) ± standard deviation.

90 min (Fig. 3), using the experimental methods described previously.[18]

^{68}Ga-labeled DO_2A was used to determine non-specific binding of **10** and uptake of [^{18}F]-**3** was measured as a reference for comparison. The ^{68}Ga-labeled derivative **10** showed a continuously increasing cellular uptake over 90 min. The total uptake of **10** was reduced to the level of non-specific binding in the presence of a mixture of L-tryptophan (Trp), L-serine (Ser) and 2-aminobicyclo-(2,2,1)-heptane-2-carboxylic acid (BCH).

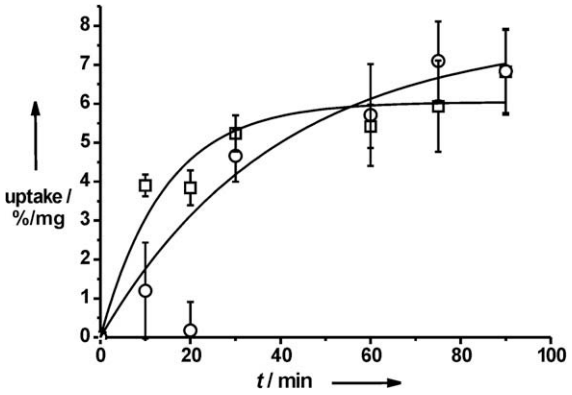


Figure 4. Comparison of the specific binding of [^{18}F]FET (**3**) (circles, n = 3) and [^{68}Ga]Ga-DO_2A-(OBu-L-tyr)$_2$ (squares n = 6) to F98 glioblastoma cells at 4 °C. Tracer uptake values were expressed as mean values related to the protein mass (%/mg) ± one standard deviation.

These results indicate specific uptake of **10** by F98 cells, presumably due to the interaction with an amino acid transporter.

In order to allow a reasonable comparison between uptake values of the novel compound **10** and [^{18}F]-**3** as a reference, the tracer uptake was normalized to the protein concentration of each sample determined by the method of Bradford.[17] As shown in Figure 4, (*S*)-3-(4-(2[^{18}F]-fluoroethoxy)phenyl)-2-aminopropanoic acid, FET (**3**) and **10** showed specific uptake to F98 rat glioblastoma cells within a similar range.

In conclusion, the presented data illustrate a novel chemical approach to synthesize a metal-containing amino acid derivative with potential for molecular imaging. This study stimulates further work capitalizing on the potential of the positron emitter ^{68}Ga or other metals for developing specific probes of amino acid metabolism in tumors. Work is in progress to use **10** for in vivo imaging of F98 glioblastoma-bearing rats by small-animal PET.

Acknowledgments

The authors want to thank the 'Fonds der chemischen Industrie' for the generous donation of chemicals and the COST D38 action for networking all over Europe.

References and notes

1. (a) Christensen, H. N. *Physiol. Rev.* **1990**, *70*, 43; (b) Souba, W. W.; Pacitti, A. J. *J. Parenter. Enteral Nutr.* **1992**, *16*, 569.
2. Miyagawa, T.; Oku, T.; Uehara, H.; Desai, R.; Beattie, B.; Tjuvajev, J.; Blasberg, R. *J. Cerebr. Blood Flow. Metab.* **1998**, *18*, 500.
3. (a) Phelps, M. E. *Proc. Nat. Acad. Sci.* **2000**, *97*, 9226; (b) Miller, P. W.; Long, N. J.; Vilar, R.; Gee, A. D. *Angew. Chem., Int. Ed.* **2008**, *47*, 8998.
4. (a) Laverman, P.; Boerman, O. C.; Corstens, F. H. M.; Oyen, W. J. G. *Eur. J. Nucl. Med. Mol. Img.* **2002**, *29*, 681; (b) Waalburg, W.; Coenen, H. H.; Crouzel, C.; Elsinga, P. H.; Langström, B.; Lemaire, C.; Meyer, G. J. *Nucl. Med. Biol.* **1992**, *19*, 227.
5. Jager, P. L.; Vaalburg, W.; Pruim, J.; de Vries, E. G. E.; Langen, K. J.; Piers, D. A. *J. Nucl. Med.* **2001**, *42*, 432.
6. (a) Langström, B.; Antoni, G.; Gullberg, P.; Halldin, C.; Malmborg, P.; Nagren, K.; Rimland, A.; Svärd, H. *J. Nucl. Med.* **1987**, *28*, 1037; (b) Ishiwata, K.; Vaalburg, W.; Elsinga, P. H.; Paans, A. M. J.; Woldring, M. G. *J. Nucl. Med.* **1988**, *29*, 1419.
7. Coenen, H. H.; Kling, P.; Stöcklin, G. *J. Nucl. Med.* **1989**, *30*, 1367.
8. (a) Wester, H. J.; Herz, M.; Weber, W.; Heiss, P.; Senekowitsch-Schmidtke, R.; Schwaiger, M.; Stöcklin, G. *J. Nucl. Med.* **1999**, *40*, 205; (b) Hamacher, K.; Coenen, H. H. *Appl. Radiat. Isot.* **2002**, *57*, 853.

9. (a) McConathy, J.; Martanello, L.; Malveaux, E. J.; Camp, V. M.; Simpson, N. E.; Simpson, C. P.; Bowers, G. D.; Zhang, Z.; Olson, J. J.; Goodman, M. M. *Nucl. Med. Biol.* **2003**, *30*, 477; (b) Shoup, T. M.; Olson, J. J.; Hoffman, J. M.; Votav, J.; Eshima, D.; Eshima, L.; Camp, V. M.; Stabin, M.; Votav, D.; Goodman, M. M. *J. Nucl. Med.* **1999**, *40*, 331.
10. (a) Roesch, F.; Knapp, F. F. In *Handbook of Nuclear Chemistry*; Vertes, A., Nagy, S., Klencsar, Z., Roesch, F., Eds.; Kluwer Academic: Dordrecht, The Netherlands, 2003; pp 81–118; (b) Fani, M.; Andre, J. P.; Maecke, H. R. *Contrast Media Mol. I.* **2008**, *3*, 67.
11. Liu, Y.; Pak, J. K.; Schmutz, P.; Bauwens, M.; Mertens, J.; Knight, H.; Alberto, R. *J. Am. Chem. Soc.* **2006**, *128*, 15996.
12. (a) De Leon-Rodriguez, L. M.; Kovacs, Z. *Bioconjugate Chem.* **2008**, *19*, 391; (b) Riss, P. J.; Kroll, C.; Nagel, V.; Roesch, F. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 5364.
13. Kovacs, Z.; Sherry, A. D. *J. Chem. Soc., Chem. Commun.* **1995**, *2*, 185.
14. Compound **9** was obtained as a white solid in an overall yield of 13% over eight steps starting from cyclene (Scheme 2). Compound **9** was purified by semi-preparative HPLC (Phenomenex® Synergy Max RP 15 × 250 mm, H_2O/MeOH 90:10, 0.01% TFA). The HPLC-purity (UV_{254}) was greater than 99%. Analytical data of **9** (TFA-salt):^{1}H NMR (600 MHz, D_2O): δ in ppm 7.08 (4H, d, J = 8.5 Hz), 6.82 (4H, d, J = 8.5 Hz), 4.04 (dd, J = 5.7, J = 7.4, 2H), 3.93 (t, J = 5.7 Hz, 4H), 3.30 (s, 4H), 3.26–3.21 (m, 8H), 3.15 (m, 4H), 3.09 (dd, J = 5.6 Hz, J = 14.6 Hz, 2H), 3.03–2.97 (m, 4H), 2.99 (dd, J = 7.4, J = 14.6, 2H) 2.93–2.85 (m, 4H), 1.73 (p, J = 7.5 Hz, 4H), 1.68 (p, J = 6.4 Hz, 4H).MS: (ESI) *m/z* (%): 380.23 (11.55) $[M+2H]^{2+}$, 380.73 (2.82), 759.46 (100) $[M+H]^+$, 760.7 (21.68), 761.48 (1.74).
15. Zhernosekov, K. P.; Filosofov, D.; Baum, R. P.; Aschoff, P.; Bihl, H.; Razbash, A. A.; Jahn, M.; Jennewein, M.; Roesch, F. *J. Nucl. Med.* **2007**, *48*, 1741.
16. *Reaction details and labeling conditions*: To 5 ml preheated aqueous solution of labeling precursor **9** (13 nmol) were added 50 μl purified ^{68}Ge/^{68}Ga-generator eluate at 90 °C. The labeling yields were monitored by radio-TLC and radio-HPLC.[15]
17. (a) Langen, K. J.; Mühlensiepen, H.; Schmieder, S.; Hamacher, K.; Broer, S.; Börner, A. R.; Schneeweis, F. H. A.; Coenen, H. H. *Nucl. Med. Biol.* **2002**, *29*, 685; (b) Langen, K. J.; Jarosch, M.; Mühlensiepen, H.; Hamacher, K.; Broer, S.; Jansen, P.; Zilles, K.; Coenen, H. H. *Nucl. Med. Biol.* **2003**, *30*, 501; (c) Pauleit, D.; Stoffels, G.; Schaden, W.; Hamacher, K.; Bauer, D.; Tellmann, L.; Herzog, H.; Bröer, S.; Coenen, H. H.; Langen, K. J. *J. Nucl. Med.* **2005**, *46*, 411.
18. Prante, O.; Bläser, D.; Maschauer, S.; Kuwert, T. *Nucl. Med. Biol.* **2007**, *34*, 305.